EBS 2019

2ND EUROPEAN BIOSENSOR SYMPOSIUM 2019

FLORENCE, ITALY
FEBRUARY 18-21, 2019

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Book of Abstract

EBS 2019 is organised by
UNIFI CNR-IFAC SIOF

CONFERENCE CHAIRS
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Department of Chemistry 'Ugo Schiff' – University of Florence
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Institute of Applied Physics "Nello Carrara" (IFAC) – CNR

CONTENTS

WELCOME TO EBS2019

Distinguished Expert, Dear Friends,

We would like to welcome you in Florence in the occasion of the Second European Biosensor Symposium (EBS2019) (http://www.ebs2019.unifi.it), a biennial meeting of scientists working in the field of biosensors, bioanalysis and biorecognition, with the first edition held in Potsdam in 2017.

The four-day conference covers all the aspects ranging from the first concept of a biosensor up to the design and development of the final device, with a particular attention to new challenging applications. This with the aim of promoting the exchange of information and experiences in bioanalytics, receptor development, biointeraction, surface science and bio-sensing technology in a charming environment.

215 contributions with 5 plenary speakers, 16 keynote presentations, 64 oral talks and 130 posters will provide an exhaustive view of the research activity in the above-mentioned scientific topics.

Our hope is to create positive cross fertilization among different expertise and across generations, so as to create a stimulating environment for successful networking and support young scientists during the first steps of their scientific career.

We worked hard to increase the participation of students and young people, since we consider essential their involvement in such type of event and we think to have centered this objective with a participation of roughly 50% of students.

We would like to thank all the people who gave their valuable contribution for the conference:

- the members of the Scientific Board for their suggestions in the identification of the plenary and keynote speakers and for their evaluation of the submitted contributions;
- the members of the Local Organization Committee who took care of all the organizational aspects related to the conference, making a unique and irreplaceable work;
- the plenary and keynote speakers who accepted our invitation and will share with us their expertise;
- all the scientists who decided to report their work at the conference;
- the sponsors and the exhibitors who provided their support to the conference.

We would also like to thank the University of Florence for hosting this event in this nice environment and for providing the logistic support and the Italian Society of Optics and Photonics (SIOF) for the administrative management.

We are confident that all the participants will make the EBS2019 Conference a big success and we wish you all an enjoyable stay in Florence.

Maria Minunni

Francesco Baldini

On behalf of the organizing committee

EBS2019 COMMITTEES

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INFORMATION

Conference Secreteriat
Monday, January 18th: 11:00-18:00
Tuesday, January 19th: 08:30-13:00; 14:30-18:00
Wednesday, January 20th: 09:00-13:00; 14:30-18:00
Thursday, January 21th: 09:00-12:30

Get together cocktail
A get together cocktail is scheduled on Monday evening, January 18th. It will be served at the Symposium venue during Poster Session I from 18:00-20:00.

Social Dinner
Conference dinner is scheduled on Wednesday evening, January 20th, and it will be served at 21:00 at the "Serre Torrigiani" Via Gusciana 21- 50124 – Firenze (http://www.serretorrigiani.it). Further information on the dinner location will be given during the Symposium.

Oral presentation guidelines
Computers (Windows with PowerPoint (ppt) and Adobe Reader (pdf)) are available in all session rooms for your presentation. Files can be uploaded to the local computers during the session breaks. Speakers must arrive in their session room 15 minutes before the start of their session. Assistants are in all session rooms for technical support.
Timing:
– Plenary (40 min + 5 min questions)
– Keynote (25 min + 5 min questions)
– Oral (17 min + 3 min questions)

Poster presentation guidelines
Poster sessions are scheduled on Monday 18/02 (Poster session I, from 18:00 to 20:00), on Tuesday 19/02 (Poster session II, from 18:00 to 20:00) and on Wednesday 20/02 (Poster session III, from 18:00 to 19:30). Moreover, each poster session will be open during the afternoon coffee break.
Posters have to be attached on the morning of each poster session. The poster format is A0, 118.9 cm x 84.1 cm, portrait orientation (height > width). The assigned placement of each poster is marked on the panel with the unique poster number which appears in the final programme.
Poster fixing tools will be available on site. Authors have to take down their poster after the end of their session. Remaining posters will be collected by the organizers and discarded.

Poster talk presentation guidelines
20 posters have been selected for a short poster presentation which will be held at the beginning of each poster session. The poster presentation will be 3 minutes maximum and without questions from the audience. The presentation files (ppt or pdf) will be collected at the registration desk on the morning of each poster session.

3 BEST STUDENT POSTER AWARDS

The "Best student poster award" will be sponsored by the Open Access journal Sensors. Sensors is covered by leading indexing services, including the Science Citation Index Expanded (SCIE - Web of Science), PubMed/MEDLINE, Scopus, etc. The journal aim is to process manuscripts quickly and publish them shortly after peer-review. For further details: http://www.mdpi.com/journal/sensors.

The award will consist in:

-certificate

-monetary prize of 200€ for each of the 3 student posters

2 BEST POSTER AWARDS

The "Best poster award" will be sponsored by the journal Analytical and Bioanalytical Chemistry. Analytical and Bioanalytical Chemistry mission is the rapid publication of excellent and high-impact research articles on fundamental and applied topics of analytical and bioanalytical measurement science. Its scope is broad, and ranges from novel measurement platforms and their characterization to multidisciplinary approaches that effectively address important scientific problems. For further details: https://www.springer.com/chemistry/analytical+chemistry/journal/216.

The award will consist in:

- certificate
- voucher for Springer books worth 150€ each

BEST STUDENT ORAL AWARD

The "Best student oral award" will be sponsored by the Italian Society of Optics and Photonics (SIOF) (http://www.siof-ottica.it). SIOF was founded in 1991 with the aim of promoting research and development in optics and photonics and to coordinate these activities throughout the country. It therefore proposes to take care of the dissemination of knowledge related to these disciplines and to the scientific and technological sectors connected to them. SIOF is the national "branch" of the European Optical Society (EOS, www.myeos.org), with the aim of organizing numerous events that include national and international conferences as well as schools and workshops related to the themes of optics and of photonics. The award will be assigned to the best oral presentation given by a student.

The award will consist in:

- certificate
- monetary prize of 300€

SYMPOSIUM VENUE

The Symposium will take place at:
Centro Didattico Morgagni - CDM, Viale G.B. Morgagni 40-44

The Centro Didattico Morgagni is easy reachable by tram T1 line (direction "Careggi", stop at "Morgagni Università")

CONFERENCE PROGRAMME

Monday, February 18				
11:00-13:30	**Registration**			
13:30-13:45	**Opening**			
13:45-14:30	**PL I**	**W. Knoll**	*Bio-Sensing: Optical or by Electronics?*	
	Materials, methods and devices for biosensing I		*Biosensing for healthcare I*	
14:50-15:20	**KN1**	**E. Climent**, W. Wan, M. Weller, K. Rurack *Novel specific bio-gated hybrid materials and its integration into versatile platforms for advanced sensing applications*	**KN2**	**G. Jobst** *Biosensors in real settings*
15:20-15:40	**OC1**	**M. Riedel**, F. Lisdat *Light-directed wiring of PQQ glucose dehydrogenase to inverse opal TiO_2 architectures*	**OC2**	G. Breveglieri, E. D'Aversa, P. Pellegatti, G. Guerra, L.C. Cosenza, A. Finotti, R. Gambari, **M. Borgatti** *A novel and efficient protocol for Surface Plasmon Resonance based detection of four β-thalassemia point mutations in blood samples and salivary swabs*
15:40-16:00	**OC3**	**S. Dantism**, D. Röhlen, T. Wagner, P. Wagner, M.J. Schöning *Monitoring of the metabolic activity of different bacteria by means of a differential LAPS*	**OC4**	F. Teng, **M. Libera** *Miniature biosensors for multiplexed infection diagnostics*
16:00-16:30	**Coffee Break/Poster Session I**			
16:30-17:00	**KN3**	**M. Holzinger** *Improved immunosensing using low dimensional carbon*	**KN4**	**P.B. Luppa** *Point-of-Care Testing – How to manage the stretch between clinical needs and technological opportunities*
17:00-17:20	**OC5**	**H. Ashiba**, Y. Nakaya, H. Sato, Y. Aida, M. Fujimaki *Detection of proteins expressed by in vitro translation using external force-assisted biosensor*	**OC6**	A. Sguassero, Á. Artiga, C. Morasso, R. Ramirez Jimenez, R. Martín Rapún, R. Mancuso, S. Agostini, A. Hernis, A. Abols, A. Linē, A. Gualerzi, S. Picciolini, M. Bedoni, M. Rovaris, F. Gramatica, J. M. de la Fuente, **R. Vanna** *Development of a sequence-independent and enzyme-free approach for the detection of multiple microRNAs using a single nanostructured enhancer of SPRi*
17:20-17:40	**OC7**	**C. Wang**, S. Otto, M. Dorn, K. Heinze, U. Resch-Genger *Ratiometric Luminescent TOP Nanosensors for Simultaneously Measuring Temperature, Oxygen, and pH at a Single Excitation Wavelength*	**OC8**	**L. Lehniger**, S. Pahlow, S. Hentschel, K. Weber, J. Popp *Particle-based sample preparation strategies for the detection of relevant pneumonia pathogens*
17:40-18:00	**OC9**	**Z. Farka**, M. J. Mickert, A. Hlaváček, U. Kostiv, V. Poláchová, M. Pastucha, H. H. Gorris, P. Skládal *Single-molecule immunoassays based on upconversion nanoparticles for detection of cancer markers and bacteria*	**OC10**	**D. Calabria**, M. Zangheri, M. Mirasoli, C. Caliceti, A. Quintavalla, M. Lombardo, C. Trombini, P. Simoni, A. Roda *A smartphone-based thermochemi-luminescent biosensor for valproic acid detection in blood and saliva*
18:00-20:00	**Poster Session I + Get together cocktail**			

Tuesday, February 19				
09:00-09:45	**PL II**	**A.P.F. Turner**	*Reflections on the past, present and future of biosensing*	
	Electrochemical biosensing I		*Engineered, bio- and, synthetic receptors I*	
10:00-10:30	**KN5**	**B. Piro**, V. Noël, G. Mattana, S. Reisberg *From electrochemical biosensors to water-gated transistors*	**KN6**	**M.C. Moreno Bondi**, R.Peltomaa, B.Glahn-Martínez, E.BenitoPeña, R.Barderas, F.Amaro, G.Orellana *Advances in the quest for new selective recognition elements for optical biosensors using phage display techniques*
10:30-10:50	**OC11**	L.E. Delle, **V. Pachauri**, A. Vlandas, M. Riedel, X.T. Vu, P. Wagner, R. Thoelen, F. Lisdat, S. Ingebrandt *Nanometer scale multi-electrode arrays for biosensing*	**OC12**	**H. Adamson**, D.C. Tomlinson, C. Walti, M.J. Mcpherson, L.J.C. Jeuken *Re-engineering an enzyme switch as a generic platform for rapid biomolecule*
10:50-11:10	**OC13**	P. Bollella, S. Sharma, A.E.G. Cass, **R. Antiochia** *Minimally-invasive Microneedle-based Biosensor Array for Simultaneous Lactate and Glucose Monitoring in Artificial Interstitial Fluid*	**OC14**	X. Chen, A. J. Gross, S. Cosnier, **F. Giroud** *Enzymatic Biofuel Cells as Self-powered Sensing Devices*
11:10-11:40	**Coffee Break**			
	Electrochemical biosensing I (ctn)		*Optical biosensing I*	
11:40-12:00	**OC15**	**A. Ruff**, S. Teanphonkrang, S. Janke, A. Ernst, P. Chaiyen, J. Sucharitakul, W. Suginta, P. Khunkaewla, W. Schuhmann, A. Schulte *Allosteric activation of an enzyme embedded in a redox polymer for tuned amperometric biosensing*	**OC16**	**X. Li**, H. Altug *Decoding the cell monologue: label-free nanobiosensor enables real-time analysis of live cell secretion*
12:00-12:20	**OC17**	**C. Griesche**, S. R. Nugen, A. J. Baeumner *Electrochemical detection of E. coli utilizing genetically modified bacteriophages*	**OC18**	**N.S. Lynn**, T. Špringer, J. Slabý, B. Špačková, M. Gráfová, M.L. Ermini, J. Homola *Nanoplasmonic biosensing: consideration of analyte transport*
12:20-12:40	**OC19**	**L. Gorton**, P. Bollella, K. Kano, Y. Hibino, R. Antiochia *Fructose biosensors based on direct electron transfer between fructose dehydrogenase and electrodes*	**OC20**	**M.G. Manera**, A. Colombelli, M. Cesaria, D. Lospinoso, S. Scarano, M. Minunni, R. Rella *Tunable nanoplasmonic functional transducers: novel insights for health applications*
12:40-13:00	**OC21**	N. A. Abdelshafi, J. Bell, K. Rurack, **R. Schneider** *Microfluidic electrochemical lab-on-chip immunosensor for ultrasensitive analysis of cocaine in water, saliva, and urine*	**OC22**	**C. Desmet**, K. Vindas, R. Alvarado Meza, P. Garrigue, S. Arbault, N. Sojic, T. Leichle, A. Buhot, Y. Roupioz, L. Leroy, E. Engel *Multiparametric sensing on micro-structured optical fibres by surface plasmon resonance for multiple applications*
13:00-14:30	**Lunch**			
14:30-15:15	**PL III**	**J. Homola**	*Plasmonic biosensors for molecular diagnostics*	
	Materials, methods and devices for biosensing II		*Optical biosensing II*	
15:30-16:00	**KN7**	**K. De Wael** *Bio-inspired laser-induced electrochemical sensing strategies*	**KN8**	**G. Proll** *A combination of imaging based direct optical biosensing and microarray applications – where are the benefits?*

16:00-16:20	**OC23**	F. Schenk, J. Hutterer, P. Weber, A. Dietzel, **G. Gauglitz** *Paper-based strips for Anywhere analytics*	**OC24**	**A. Nabok**, A.M. Al-Jawdah, H. Abu-Ali, G. Catanante, J-L. Marty *Label-free detection of mycotoxins with optical planar waveguide polarization interferometry aptasensor*
16:20-16:40	**OC25**	N. Bagheri, **S. Cinti**, R. Massoud, D. Moscone, F. Arduini *A 96-well wax printed Prussian Blue paper for the visual determination of cholinesterase activity in serum*	**OC26**	**N. Nechaeva**, I. Boginskaya, I. Kurochkin *Glycated blood proteins detection by surface-enhanced Raman spectroscopy*
16:40-17:00	**Coffee Break/Poster Session II**			
17:00-17:20	**OC27**	**K.-H. Feller**, M. Büttner *Oncology meets microfluidic - development of a Lab-on-Chip chemosensitivity assay for primary cancer cells*	**OC28**	**M. Prante**, C. Ude, M. Große, L. Raddatz, U. Krings, G.T. John, S. Belkin, T. Scheper *A portable biosensor for 2,4-dinitrotoluene vapors*
17:20-17:40	**OC29**	**M. Agostini**, G. Greco, M. Cecchini *Ultra-High-Frequency Surface-Acoustic-Wave microfluidics and biosensors*	**OC30**	L. De Stefano, **I. Rea**, R. Moretta, M. Terracciano, G. Piccialli, R. Schettino. N. Borbone, G. Oliviero *Hybrid graphene oxide-porous silicon biosensor for early diagnosis of Sudden Death Syndrome*
17:40-18:00	**OC31**	**J. Cao**, B.P. Chaill, S. Schneider, A. Groß, M. Kastl, J.M. Köhler *Droplet-based microfluidic system with integrated multi-sensor technology for microtoxicological studies*	**OC32**	**T. Steinwedel**, J.C. König, D. Solle, P. Lindner, I. de Vries, T. Hentrop, M. Findeis, G. T. John, T. Scheper, S. Beutel *Development and characterisation of a new fluorescence sensor for online monitoring of bioprocesses*
18:00-20:00	**Poster Session II**			

Wednesday, February 20				
09:15-10:00	**PL IV**	**L. Torsi**, E. Macchia, K. Manoli, B. Holzer, C.Di Franco, M. Ghittorelli, F.Torricelli, D. Alberga, G.F.Mangiatordi, G. Palazzo, G.Scamarcio	*Single-molecule, label-free bio-detection with a wide-field transistor*	
	Materials, methods and devices for biosensing III		*Biosensing for healthcare II*	
10:15-10:35	**KN9**	**P. Lieberzeit**, S. Chunta, A. Strallhofer, W. Naklua, R. Suedee *Surface imprinting for label-free detection of biospecies*	**KN10**	**M.-P. Marco** *Micro & Nano(bio)technology for Diagnostics: Changes and Challenges*
10:35-10:55	**OC33**	**N. Ahmad**, B. Colak, M.J. Gibbs, D. Zhang, J. E. Gautrot, M. Watkinson, R.C. Becer, S. Krause *Collagenase Biosensor Based on Degradation of Peptide Cross- Linked Poly(ethylene glycol) Hydrogel Films Using a Quartz Crystal Microbalance*	**OC34**	**S. Vasudevan**, J. Kajtez, A-I. Bunea, N. B. Larsen, A. Gonzalez-Ramos, M. Kokaia, T.R. Moreno, A. Martínez-Serrano, A. Heiskanen, S. S. Keller, J. Emnéus *Opto-electrical carbon fiber for real-time optical stimulation and electrochemical detection of dopamine exocytosis*
10:55-11:15	**OC35**	**S. Scarano**, P. Palladino, A. Brittoli, E. Pascale, F. Torrini, V. Baldoneschi, M. Minunni *POLYDOPAMINE: a smart polymer for biosensing*	**OC36**	**S. Tombelli**, C. Trono, S. Berneschi, A. Giannetti, C. Berrettoni, R. Bernini, I.A. Grimaldi, G. Testa, G. Persichetti, G. Orellana, A.B. Descalzo, F. Salis, C. Gartner, G. Porro, G. Quarto, M. Berner, M. Schubert, M.T. O'Connell, P. Freitas, P. Luppa, H. Bittersohl, G. Gauglitz, U. Hilbig, K. Freudenberger, F. Baldini *A novel POCT optical device for the detection of immunosuppressants in transplanted patients*
11:15-11:40	**Coffee Break**			
	Electrochemical biosensing II		*Optical biosensing III*	
11:40-12:00	**OC37**	S.K.K. Galagedera, **G.-U. Flechsig** *Interaction of DNA with small molecules studied by millisecond-resolved EQCM and voltammetric isotope effects*	**OC38**	**M. Janik**, M. Koba, A. Celebańska, W.J. Bock, M. Śmietana *Micro-cavity in-line Mach-Zehnder interferometer for small-volume label-free biosensing: concept verification by thin Al2O3 film deposition*
12:00-12:20	**OC39**	**J. Tkac**, T. Bertók, L. Lorencova, E. Jane, M. Hires, F. Květoň, A. Blsakova, S. Hroncekova, V. Gajdosova *Cancer diagnostics using glycan recognition by the electrochemical biosensors with design controlled at nanoscale*	**OC40**	C.A. Mandon, **C. Marquette** *4D printing: chemiluminescent printed biosensors*
12:20-12:40	**OC41**	**V. Serafin**, G. Martínez-García, E. Martínez-Periñán, A. Valverde, F.N. Comba, M. Garranzo-Asensio, R. Barderas, S. Campuzano, P. Yáñez-Sedeño, J.M. Pingarrón *Integrated electrochemical immunosensor for sensitive determination of IL-13 receptor α2 in paraffined-embedded tumor tissues using MWCNTs/GQDs hybrid nanocarriers*	**OC42**	S. Fossati, S. Hageneder, N. Sanchez, D. Hafner, C. Thanner, M. Hiltunen, J. Hiltunen, **J. Dostalek** *Plasmonically amplified fluorescence biosensors with scaled up produced chips*

12:40-13:00	**OC43**	H. Zhang, **T. Vöpel**, N. Plumeré *An enzymatic oxygen scavenger for oxidase-based bioelectrochemical processes - Case study of an oxygen-interference free glucose biosensor*	**OC44**	**R. Rogosic**, J.W. Lowdon, B. Heidt, E. Steen Redeker, H. Diliën, K. Eersels, T.J. Cleij, B. van Grinsven *A disposable multi-drug test based on a dye displacement assay in molecularly imprinted polymers*
13:00-14:30	**Lunch**			
	Materials, methods and devices for biosensing IV		*Engineered, bio- and, synthetic receptors II*	
14:30-15:00	**KN11**	C. Liedert, M. Hiltunen, L. Hakalahti, S.Aikio, S.Uusitalo, O.-H.Huttunen, J.Hiitola-Keinänen, S. Fossati, S. Hageneder, J. Dostalek, **J. Hiltunen** *Roll-to-roll printed biosensors*	**KN12**	A. Díaz-Fernández, R. Lorenzo-Gómez, R. Miranda-Castro, N. de-los-Santos-Álvarez, **M.J. Lobo-Castañón** *Aptamer based electrochemical sensors for the analysis of tumor biomarkers*
15:30-15:50	**OC45**	**M. Smolka**, A. Haase, A. Proksch, E. Gonzalez, P. Toren, D. Nees, S. Ruttloff, L. Kuna, C. Leiner, B. Stadlober, J. Hesse, B. Hierschläger, M. Sonnleitner, S. Hemanth, J. Kafka, M. Lohse, M. Thesen, M. Horn, W. Weigel, N. Briz, G. Bijelic *Roll-to-roll imprinting and microarray spotting of biosensors*	**OC46**	**F. Bottari**, E. Daems, A.M. de Vries, P. Van Wielendaele, S. Trashin, R. Blust, F. Sobott, J.C. Martins, A. Madder, K. De Wael *The affinity between aptamers and low molecular weight compounds: a cautionary tale*
15:50-16:10	**OC47**	J. Lin, S. Daboss, D. Blaimer, **C. Kranz** *Pulse Deposition of Functional Polydopamine Films*	**OC48**	**E. Del Grosso**, G. Ragazzon, L. J. Prins, F. Ricci *Fuel-responsive allosteric DNA-based aptamers for the transient release of ATP and cocaine*
16:10-16:30	**OC49**	**X. Knigge**, Ch. Wenger, F. F. Bier, R. Hölzel *Dielectrophoretic Immobilization of single biomolecules*	**OC50**	A. Sett, L. Zara, E. Dausse, **J.-J. Toulmé** *Light-up aptasensors for the detection of biomarkers*
16:30-16:50	**Coffee Break/Poster Session III**			
16:50-17:20	**KN13**	S. Byrnes, T.C. Chang, T. Huynh, A. Astashkina, B.H. Weigl, **K.P. Nichols** *Digital Assays: There's Plenty of Room in the Middle*	**KN14**	**F. Ricci** *DNA-based nanodevices for diagnostic applications*
17:20-17:40	**OC51**	**E. Mendoza**, A. Neumann, Y. Kuznetsova, S.R.J. Brueck, J. Edwards *Advances Towards the Development of an Electrophoretic Plasmonic Nanopore Biochip Genome Sequencer*	**OC52**	**M. Heinelt**, G. Nöll, Q. Su, S. Vogt *Does DNA hybridization at surfaces follow the Langmuir model of adsorption?*
17:40-18:00	**OC53**	**F. Della Pelle**, S. Gaggiotti, M. Mascini, D. Compagnone *Gas sensors array equipped with Hairpin DNA traps for food quality and production process evaluation*	**OC54**	**S. Di Masi**, C. Malitesta *Ion imprinted electrosynthesised polymers for copper(II) detection*
18:00-19:30	**Poster Session III**			

Thursday, February 21				
09:00-09:45	**PL V**	**D. Cialla-May**, K. Weber, J. Popp	*Label-free SERS in biological and biomedical applications*	
	Materials, methods and devices for biosensing V		*Biosensing for healthcare III*	
10:00-10:30	**KN15**	J. Pallu, C. Rabin, G. Creste, M. Branca, B. Limoges, **F. Mavré** *Molecular sensing through the triggering of autocatalytic reactions: towards exponential signal amplifications in bioelectroanalytical methods*	**KN16**	P. Ertl, **M. Rothbauer** *Implantable Biosensors, Smart Implants & Self-powered Sensing Solutions*
10:30-10:50	**OC55**	N. Tran, M. Paliouras, P. Mohammadyousef, M. Trifiro, **A. Kirk** *Real-time fluorophore-free optical monitoring of ultrafast DNA amplification for qPCR*	**OC56**	M. Cretich, **D. Brambilla**, A. Romanato, M. Odinolfi, E. Chiodi, S. Descroix, A. Yalcin Ozkumur, M.S. Unlu, M. Chiari *Microarray based platform for extracellular vesicles imaging and phenotyping*
10:50-11:10	**OC57**	**V.A. Bragina**, S.L. Znoyko, A.V. Orlov, M.P. Nikitin, P.I. Nikitin *Highly sensitive immunomagnetic biosensing platform for rapid quantitative detection of thyroid-stimulating hormone*	**OC58**	N. Farhoudi, H.-Y. Leu, J. Magda, F. Solzbacher, **C. F. Reiche** *Sensing of biomedical analytes based on resonant absorption of ultrasound in smart hydrogel microstructures*
11:10-11:40	**Coffee Break**			
	Engineered, bio- and, synthetic receptors III		*Optical biosensing IV*	
11:40-12:00	**OC59**	K. Bartold, A. Pietrzyk-Le, T.P. Huynh,Z. Iskierko, M. Sosnowska, K. Noworyta, W. Lisowski, F. Sannicolò, S. Cauteruccio, E. Licandro, F. D'Souza, **W. Kutner** *Molecularly imprinted polymer for hexakis(2,2'-bithien-5-yl) DNA analog formation aiming at single-nucleotide-polymorphism detection*	**OC60**	**A. Sinibaldi**, V. Montaño-Machado, N. Danz, P. Munzert, F. Chiavaioli, F. Michelotti, D. Mantovani *Real-time study of biomolecular coatings by means of Bloch surface wave biosensors*
12:00-12:20	**OC61**	**A. Yarman**, K.J. Jetzschmann, U. Wollenberger, Frieder W. Scheller *Electrochemical MIPs for Protein Sensing Using Three Levels of Template*	**OC62**	R. D'Agata, S. Korom, A. Rozzi, M. Allegretti, P. Giacomini, R. Corradini, **G. Spoto** *Plasmonic detection of oncogenic DNA in liquid biopsy samples*
12:20-12:40	**OC63**	**A. Zanut**, A. Porchetta, G. Valenti, M. Marcaccio, F. Ricci, F. Paolucci *Electrochemiluminescent DNA sensor for the detection of specific DNA sequences*	**OC64**	**T. Allsop**, C. Mou, R. Neal, S. Scarano, D. Nagel, S. Tombelli, K. Kalli, C. Wang, M. Minunni, D.J. Webb, J. Ana-Castonon *Ultra-sensitive aptasensors based upon single-stage opto-plasmonic sensing platform*
12:40-13:30	**Awards and Closing Ceremony**			

PLENARY SPEAKERS

Dana Cialla-May

Jiri Homola

Wolfgang Knoll

Luisa Torsi

Anthony P.F. Turner

Bio-Sensing: Optical or by Electronics?

Wolfgang Knoll

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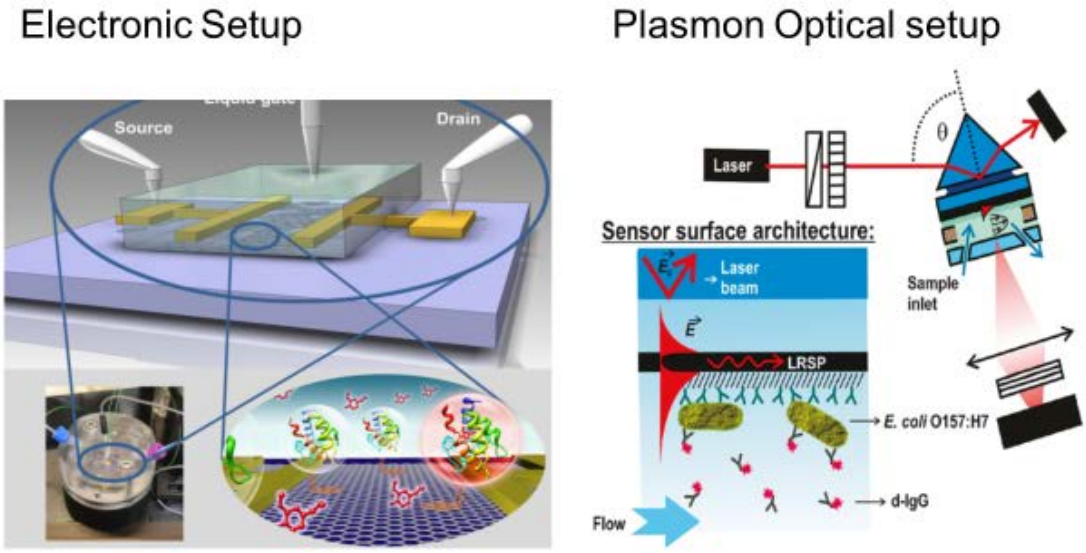
The race in Protein, DNA, or small molecule diagnostics between optical detection principles (fluorescence, surface plasmons, optical waveguides, etc.) and electrical/ electrochemical/electronic concepts is not decided yet. Both scientific communities continue to offer solutions for fast, multiplexed, simple and cheap detection of peptides, proteins, oligonucleotides, PCR amplicons, small molecules like odorants, etc. Most likely, the competition will never see a single winner that meets all needs because the different practical formats and boundary conditions for applications, as well as, market requirements may ask for specific and unique solutions that could be better achieved in one case by optics and in another situation by electronics.
Along these lines, we will briefly review the state of the art of both categories of diagnostics and will present a number of examples of what has been demonstrated for the sensitive detection of DNA by monitoring surface hybridization reactions of target strands binding from the analyte solution to surface-attached capture oligonucleotides. Other examples concern the quantitative monitoring of proteins, e.g., antibodies binding directly to the surface-immobilized antigen, or the detection of small analytes, e.g., odorant molecules recognized by odorant binding proteins immobilized on the transducer surface. A particular emphasis will be put on the physico-chemical principles of these surface recognition and binding (or dissociation) reactions in order to be able to develop criteria of how to optimize sensitivity, selectivity, etc.



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Reflections on the past, present and future of biosensing

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Since their inception as the “enzyme electrode”, first described by Clark and Lyons in 1962, biosensors have blossomed into a huge and diverse portfolio of measurement solutions that are now straining conventional definitions. Amperometric biosensors have developed into a $11b business and benefited tens of millions of people with diabetes worldwide. Surface plasmon resonance (SPR)-based biosensors have had an enormous impact on pharmaceutical discovery and life science research, while the hugely successful lateral-flow device, that brought immunoassay into the homes of millions of people, is now being instrumented to create a new generation of biosensors. The recent convergence of thinking around the escalating cost of delivering healthcare, the opportunities offered by mobile heath and the demand for more personalised medicine has stimulated enthusiasm for solutions based on biosensing. In addition, new areas have evolved where biosensors are recognised to have a pivotal role, such as in robotic surgery, tissue engineering and the production of biologics. These drivers have spurred on innovations in the area, since the direct molecular information that biosensors can deliver is essential to higher level algorithms for personalised management of health, artificial intelligence and control of biochemical systems. And all the time, the scalability of the novel materials and designs proposed must be borne carefully in mind if we are truly aiming to meet the burgeoning demand for worldwide distribution of decentralised and personalised sensing.

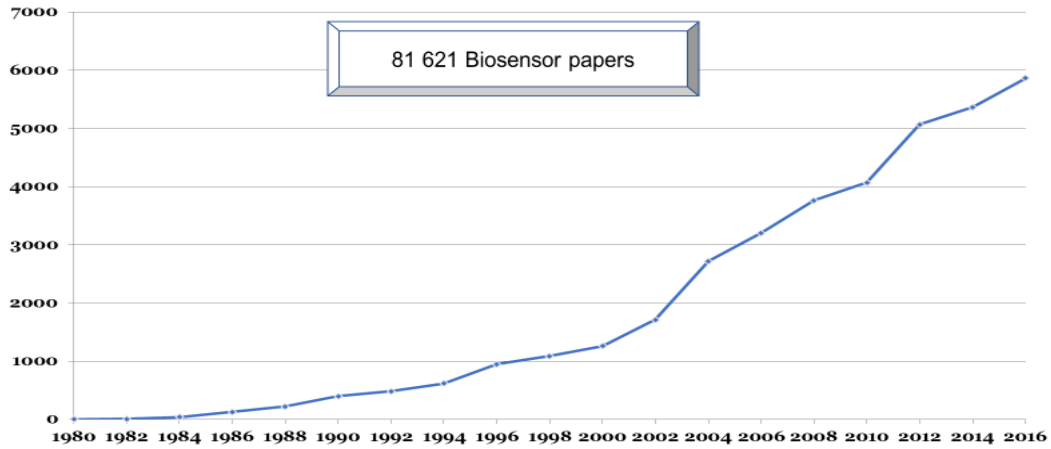

Figure 1.
Biosensor publications: 2 papers on Biosensors identified in 1980, 78 papers in 1985 and 5,933 in 2017. [Scopus “Biosensor*” Accessed 11 Sep 2018].

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Plasmonic biosensors for molecular diagnostics

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Technologies for rapid and sensitive detection of biomolecules are needed in numerous important sectors, such as environmental monitoring, food safety and medicine. Optical affinity biosensors based on surface plasmons represent the most advanced and mature optical label-free biosensor technology. Surface plasmon resonance (SPR) biosensors have become an important tool for investigation of biomolecular interactions and have been also increasingly applied to detection of chemical and biological species [1]. In recent years, the sensing potential of surface plasmons supported by various kinds of nanostructures have been also studied and numerous sensor platforms based on plasmonic modes supported by metallic nanoparticles or their arrays have been developed [2].
In this lecture we discuss selected recent advances in the development of plasmonic biosensors and report on selected results of research into plasmonic biosensors in the Optical Biosensors research group at the Institute of Photonics and Electronics. This is highly multidisciplinary research and the lecture will cover a broad variety of topics. We shall discuss advances in the design of plasmonic nanostructures and describe multiple-beam interference lithography that allows fabrication of defect-free periodic nanostructures across large areas. We shall introduce optical platforms for plasmonic biosensors, including high-performance plasmonic sensors for highly parallelized measurements and compact optical platforms for applications in the field. Transport of target molecules in microfluidic systems of plasmonic biosensors will be also discussed and routes to improving performance of nanoplasmonic biosensors will be outlined [3]. Advances in the development of functional coatings based on polymer brushes with improved resistance to fouling from complex biological media will be also discussed. In addition, selected examples of medical applications of plasmonic biosensors will be given. In particular, these will include monitoring progression of Myelodysplastic syndromes based on analysis of protein interactions and investigation of prognostic role of pregnancy associated plasma protein A2 in hemodialysis patients.

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Single molecule detection of markers with a label-free bio-electronic sensor

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Label-free single-molecule detection has been achieved so far by funnelling a large number of ligands into a sequence of single-binding events with few recognition elements host on nanometric transducers. Such approaches are inherently unable to sense a cue in a bulk milieu. Conceptualizing cells' ability to sense at the physical limit by means of highly-packed recognition elements, a millimetric sized field-effect-transistor is used to detect a single molecule. To this end, the gate is bio-functionalized with a self-assembled-monolayer of trillions of capturing anti-Immunoglobulin-G and is endowed with a hydrogen-bonding network enabling cooperative-interactions. The selective and label-free single-molecule IgG detection is strikingly demonstrated in diluted saliva while 15 IgGs are assayed in whole serum. The suggested sensing mechanism triggered by the affinity binding event, involves a work-function change that is assumed to propagate in the gating-field through the electrostatic hydrogen-bonding network. The proposed immunoassay platform is general and can revolutionize the current approach to protein detection.

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Label-free SERS in biological and biomedical applications

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Powerful detection schemes in bioanalytics are associated with the requirements for molecular specificity, high sensitivity and fast detection times. Surface enhanced Raman spectroscopy (SERS) is known to meet those requirements and the strong capability of this method in bioanalytical detection schemes is due to the enhancement of the molecular specific Raman fingerprint by 6 to 8 orders of magnitude employing plasmonic active nanostructures. [1] Various SERS detection schemes are available, e.g. label-free or direct SERS, SERS labels or tags for immune assays as well as molecular sensors to detect small molecules or ions. Within this presentation a detailed overview about label-free SERS approaches [2] are given allowing for applications in biology or biomedicine. Employing label-free SERS (i.e. the molecules of interest are interacting with the metallic surface of the SERS substrate), two different carotenoids (lycopene and β-carotene) are investigated in tomato extract samples. [3] Here, a training model was created from lycopene/β-carotene mixtures to achieve the estimation of the lycopene and β-carotene percentage in real extracts. The obtained results were compared with HPLC measurements and a good agreement was found for most of the samples illustrating the potential of SERS in bioanalytics. In combination with SERS, microfluidic systems (Lab-on-a-chip, LOC) are developed to allow for high-throughput measurements and reproducible measuring conditions. As an example, the broad spectrum antibiotic levofloxacin is characterized within simulated urine mimicking a complex biological matrix employing LOC-SERS. [4] First, different parameters such as matrix complexity, aggregation time and matrix dilution on the overall SERS signal is investigated. Within the second part of this study, levofloxacin is spiked in human urine and the quantitative analysis is achieved down to a root means square error of prediction (RMSEP) between 0.058 and 0.16 mM for the different investigated urine samples. To summarize, due to the complex composition of real biological and medical samples (e.g. salts, degradation products, metabolic endproducts, proteins, water, etc.), a number of challenges and limitations occurs: (i) salts can cause aggregation of the SERS active colloidal nanoparticles as well as the degeneration of nanostructure surfaces; (ii) degradation products or metabolic endproducts show a strong affinity toward the metallic surface and/or are highly concentrated; (iii) proteins will form a corona on metallic nanoparticles. Thus, in label-free SERS approaches the analyte molecule needs to show a high affinity toward the metallic surface and clean up processes are required when the background signal is dominating the SERS response.

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Acknowledgement: Funding of the research projects “JBCI 2.0” (03IPT513Y) within the framework “InnoProfile Transfer – Unternehmen Region“, “EXASENS” (13N13856) and “InfectoGnostics” (13GW0096F) by the Federal Ministry of Education and Research, Germany is gratefully acknowledged.

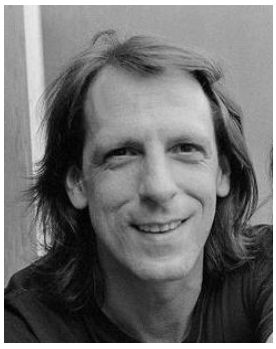
KEYNOTE SPEAKERS

Estela Climent Terol

Karolien De Wael

Peter Ertl

Jussi Hiltunen



Michael Holzinger

Gerhard Jobst

Peter Lieberzeit

Peter P. Luppa

María J. Lobo-Castañón

M.-Pilar Marco

Francois Mavré

Maria C. Moreno-Bondi

Kevin P. Nichols

Benoit Piro

Günther Prol

Francesco Ricci

Novel specific bio-gated hybrid materials and their integration into versatile platforms for advanced sensing applications

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Methods for the rapid and sensitive detection of target analytes are gaining importance in medical diagnostics and environmental monitoring, in the security, occupational health and safety as well as food sectors. Among all of the methods employed for rapid tests, lateral flow assays (LFAs) are the most commonly used, and hundreds of test kits based on this technique are available on the market. A major drawback is that most of these capture agents either indicate the analyte only indirectly, and in most cases a second binding agent able to bind directly or indirectly to the analytes is necessary (e.g., a secondary labeled antibody). Furthermore, in certain cases in which the (ultra)trace detection of an analyte is required, the traditional approach of a certain number of probe molecules being conjugated to a particular support is not sufficient. Therefore, novel concepts implementing steps of effective signal amplification are urgently required.
Keeping in mind these limitations, we thought that the sensitivity of these systems should be improvable through employment of gated reporter molecule-releasing hybrid nanoparticle materials on novel lateral flow devices. On one hand, the gated sensor material can produce a massive signal amplification, by releasing many reporter molecules only after chemical recognition of a few analyte molecules has taken place in an independent and separate step at the pore openings. On the other hand, the employment of tailored capture materials for the selective interaction with the released reporter molecules in a second arbitrary zone on the strip allows to concentrate or focus the latter for more efficient detection or to create selective multi-spot detection zones, which renders the simultaneous detection of several reporter molecules at the same time in multiplexed detection of various analytes possible.
For that purpose, we have prepared several stimuli-responsive materials for small-molecule sensing based on specific interactions between biomolecules such as antibodies with the corresponding analytes for the detection of certain explosives. In order to prepare these bio-capped materials, we have selected silica mesoporous nanoparticles (MSNs) as inorganic support due to their unique properties such as defined void structure, high inner surface area and flexible functionalization chemistry. These MSNs are loaded with a brightly fluorescent indicator dye, and the external surface is subsequently functionalized with suitable molecules able to interact with antibodies, efficiently inhibiting dye release. The opening protocol and delivery of the entrapped dye is reminiscent of a displacement reaction involving the presence of the target analyte, producing a displacement of the biomolecule and allowing the detection of the target analyte.
The presentation discusses general aspects of system design as well as analytical performance and highlights the integration into a lateral-flow assay, showing as an example the determination of the explosives TATP, TNT and PETN with fluorescence readout, in single-substance and multiplexing modes.

References:

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Biosensors for real settings

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Introduction

While it is easy to cite an example for almost all fields of application out of the numerous different biosensors published in the literature, biosensors useful for operation in real settings are rare. Most progress was achieved in medical application, namely glucose sensing for diabetes management. Followed by a clearly lower technology readiness level in mainly red biotechnology application as part of the process control.
Numerous much desired applications are not realized because of still inadequate biosensors performance. Namely with respect to unmet storage, use life, size, sterility, and analytes requirements.

Background

The medical field certainly splits into diabetes and non-diabetes applications. The newest innovation cycle in diabetes care targets long-term implantation of continuous glucose monitors (CGM). Senseonics Eversense® device, FDA cleared for 90 days of use, manages the crucial foreign body reaction by suppressing it. Different strategies will be required to obtain long term implants for relevant durations justifying the severe surgery for implantation *and* explantation. As necessarily a wireless device, miniaturization of the sensor+reader assembly has limits. In parallel less and less invasive CGMs emerge. Still true convenience requires a cheap replace, calibration free, and most relevant a short time to on-line readings after application.
Non-diabetes applications are mainly found in Blood Gas Analyzers (BGA) and in intensive care units (ICU). BGAs offering glucose, lactate, and creatinine right at the point-of-care, even in hand held devices. Missing at this panel of analytes of immediate influence on treatment is pyruvate. In intensive care the desire for lactate and glucose monitoring has a long history. Applications mainly differ by the blood access, basically creating assembly constraints for the biosensors.

Parameters important in Real Settings

Starting with the most challenging application in medical application, *in vivo* biosensor specifications will be presented with form factor, dynamic range, response time, run-in time, storage conditions, miniaturization, longevity, biocompatibility, and sterilization possibility as being the most important features. Ex vivo monitoring of blood lactate on a flying bird is an extreme example of a miniaturized auto-sampling and auto-calibrating analyzer. Such devices allow the imagination of body-attached auto-analyzers. In case of *ex vivo* biosensors sample size volume is an additional need. As an example, the first of its kind glucose/ lactate monitor from central blood for the ICU and the micro- analyzer of Jobst Technology will be presented, along with pyruvate sensor performance and life time metrics for glucose and lactate sensors.

Improved immunosensing using low dimensional carbon

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The outstanding properties of nanostructured carbon such as carbon nanotubes or graphene made them a widely used material as electronic or electrochemical transducer in biosensor devices. In particular, carbon nanotubes (CNTs) possess the outstanding combination of nanowire morphology, biocompatibility and electronic properties. Furthermore, their ease and well-documented organic functionalization brings new properties to nanostructured electrodes [1-2] such as specific docking sites for biomolecules. Moreover, CNT films exhibits a high electroactive surface area due to the natural formation of highly porous three-dimensional networks, suitable for the anchoring of a high amount of bioreceptor units, leading consequently to high sensitivities. Furthermore, the controlled formation of porous structures with reproducible identic morphology could be obtained by an optimized filtration and transfer process leading to reliable high performing label free impedimetric immunosensors [3-4]
Since several years, monolayer graphene and related 2D carbon materials are shown as promising alternative to CNTs. The presented examples will show some interesting properties of monolayer graphene for SPR biosensors [5] while CNTs remains the material of choice for 3D matrices as receptor-transducer interface for electrochemical biosensor devices .

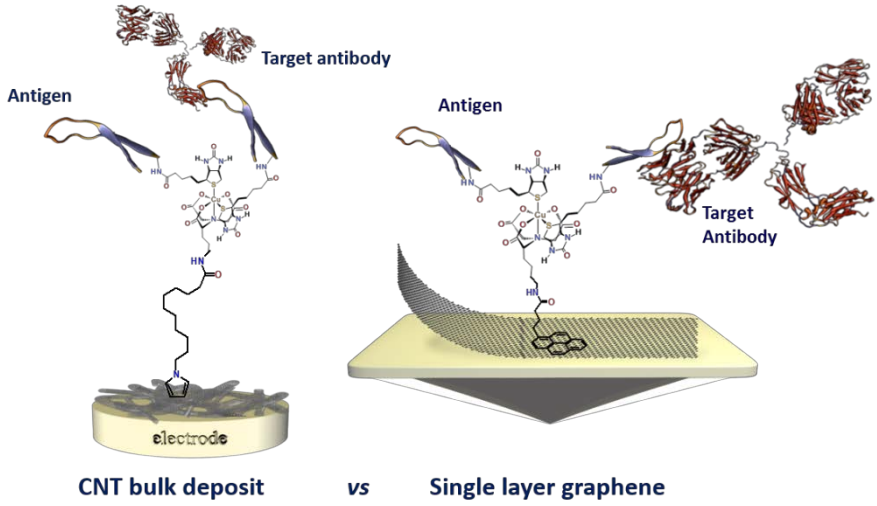

Figure 1. Illustrations of immunosensor setups using low dimensional carbon materials

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Point-of-Care Testing – How to manage the stretch between clinical needs and technological opportunities

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The presentation defines the medical needs for POCT applications against the background of important healthcare challenges and portrays the different analytical principles. Innovative nanoparticle-based techniques and alternative biological recognition elements are examples for the precipitous development of novel analytical tools for POCT applications.
As emerging application fields, the lecture will present the innovative molecular-biological methods for nucleic acid detection of infectious agents that are conceived to compete traditional microbiological diagnostics. For diabetic patients, an unprecedented beneficial opportunity will be the new possibilities of the continuous monitoring technology. Here too, the latest POCT developments will be pointed out.
Biography: Peter B. Luppa is a Professor of Clinical Chemistry at the TU München. He heads the central laboratory and blood bank in the Institute of Clinical Chemistry and Pathobiochemistry at the Klinikum rechts der Isar, the academic hospital of the TU.
He acts also as POCT coordinator for the entire hospital and chairs the working group POCT within the German Society for Clinical Chemistry and Laboratory Medicine (DGKL). He is also editor of the first German textbook on POCT, the third edition (German and English) being published in 2017.
Dr. Luppa runs a biosensor research lab and published 130 original peer-reviewed articles (listed in Pubmed, as of December 2018).

From Electrochemical Biosensors to Water-Gated Transistors

B. Piro, V. Noël, G. Mattana, S. Reisberg

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The field of conventional electrochemical biosensors (e.g. potentiometric, amperometric or impedimetric) is very rich. However, until now, biosensors have not invaded the market. This may be due to a still too high production cost, at least for most everyday life applications. With the coming of the Web of Things, i.e., connected objects implementing sensors, along with a growing need for healthcare devices, biosensors may at last fulfill their promises. For this, they must be better interfaced with electronics; transistors as biosensing components may be the way to achieve this objective. A transistor is composed of a semiconducting material with three or four terminals for connection to an external circuit. A voltage or current applied to one pair of the transistor's terminals ("gate" and "body") is amplified into changes in current through another pair of terminals ("source" and "drain"). In the past few years, field-effect transistors have been intensively investigated for biosensing applications, because of their natural integration into portable electronic devices, but also because the field effect is capacitance-related, and this capacitance is known to be very sensitive to surface changes. For example, the presence of guest molecules over one of the gate/dielectric or dielectric/semiconductor interface would result in a shift of the conductance of the semiconductor. However, the main drawback with conventional silicon-based transistors is the high cost of silicon microlithography operated in clean room, which is prohibitive for disposable sensors. Organic thin-film transistors (OTFTs) will probably never compete with inorganic ones in terms of carrier mobility and operating frequency, but they can be processed at low cost, for example by use of printing techniques, and can be easily chemically modified to adjust their properties, which is decisive for biosensors onto which the recognition elements have to be attached. In the field of organic transistors, electrochemical processes have long been confined to OECTs (Organic ElectroChemical Transistors) or ISOFET (Ion-Sensitive Organic Field-Effect Transistors). However, Electrolyte-Gated OFETs have recently emerged, where the classical dielectric of an OFET is replaced by water [1,2]. Polarization of the gate causes formation of an electrical double layer (EDL) at both the gate/water and semiconductor/water interfaces, causing accumulation of carriers in the semiconductor, thus a drain current (I_D) increase. Due to the extreme thinness of the EDLs, operating potentials are of a few hundreds of mV only, i.e. fully compatible with aqueous media. Therefore, biochemical processes occurring at these interfaces, such as molecular recognition of a target molecule onto an immobilized receptor, can be transduced and, most importantly, amplified, into a drain current variation. This paves the way for applications in the field of biosensors [3]. In this presentation, I will focus on different methods able to biofunctionalize the semiconductor interface, which is challenging because covalent or even non-covalent functionalization of semiconductors led to significant degradation of charge carriers' mobility. For applications such as immunosensing or enzymatic sensing, I will demonstrate that gate modification, which could be easier, is also pertinent and open the way to a wide variety of applications.

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Advances in the quest for new selective recognition elements for optical biosensors using phage display techniques

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The search of new recognition elements for biosensor development is of utmost importance as they have a great impact on the sensitivity and specificity of the device. Epitope-mimicking peptides, or mimotopes, are a very attractive alternative to overcome some of the limitations of competitive immunoassays. Mimotopes bind to the same antibody paratope as the antigen and elicit a similar antibody response, and therefore they can be used to replace the synthetically labeled antigens in applications where the conjugation of the target to a carrier molecule is challenging, or it can cause toxicity to the user. Several *in vitro* display technologies have been reported for screening antibody and peptide libraries which in essence they mimic the natural *in vivo* process of antibody production; however, but by far the most widely spread technique is phage display [1]. We have recently explored different phage display libraries, including domain antibody, single-chain fragment variable (scFv) as wells as peptide libraries, to discover new recognition elements for biosensor development. Novel binders for different target analytes including mycotoxins and immunosuppressant drugs were selected from domain antibody and scFv repertoires [2], whereas a 12-mer peptide library was used to identify mimotopes for their application as hapten-conjugates in competitive immunoassays for mycotoxin analysis.
Several approaches for mycotoxin analysis using the phage-displayed binders the will be reported in this presentation: 1) Development of a novel microarray-based immunoassay for fumonisin B_1 analysis, with a detection limit of 11.1 ng mL^{-1} and a dynamic range from 17.3 to 79.6 ng mL^{-1}, based on the synthetic peptide originating from a phage display library [3]. 2) Construction and application of a recombinant fusion protein of the fumonisin B_1 mimotope and a fluorescent protein to the development of a homogeneous fluorescence quenching immunoassay based on gold nanoparticles (AuNPs) for the detection of the toxin without the need for a labeled secondary antibody [4]. The approaches reported herein demonstrates the applicability of epitope mimics to sensitive immunoassays.

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Bio-inspired laser-induced electrochemical sensing strategies

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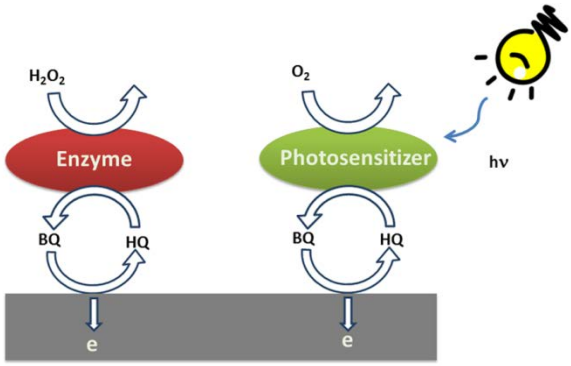
I present a sensing strategy inspired by the advantages of enzymes and photoelectrochemical sensing, namely the integration of aerobic photocatalysis and electrochemical analysis. The photosensitizer, a bioinspired perfluorinated Zn phthalocyanine, generates singlet-oxygen from air under visible light illumination and oxidizes analytes, yielding electrochemically-detectable products while resisting the oxidizing species it produces. Compared with enzymatic detection methods, the proposed strategy uses air instead of internally added reactive reagents, features intrinsic baseline correction via on/off light switching and shows C-F bonds-type enhanced stability. It also affords selectivity imparted by the catalytic process and nano-level detection, such as 20nM amoxicillin in ml sample volumes. [1]



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A combination of imaging based direct optical biosensing and microarray applications – where are the benefits?

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Microarrays offer a well-established bioanalytical format for high throughput studies of many different kinds of biomolecules. In the field of peptide [1] and protein microarrays, great progress has been achieved in immobilizing large numbers of native target molecules like recombinant human proteins or peptide libraries to microarray surfaces. With an increased content at high quality, protein and peptide microarrays offer new possibilities in proteomics and pharmaceutical research and development. At the same time, label-free sensing has evolved from high sophisticated, but low throughput to powerful multiplexed screening tools. The combination of these advancements opens a new field of bio-analytics.
Major expectations of this fruitful technology combination are e.g. a new powerful toolbox in basic proteomics research and an accelerated lead characterization process of biologics. Both application areas would benefit from a much higher data quality and depth of information in a test environment which is much closer to native conditions compared to other state of the art label-based technologies. The key of success is now combining the most suitable and powerful methods from protein arrays and imaging-based label-free detection technologies into new system solutions.
The talk will summarize technological and practical aspects of available transduction principles with imaging capabilities. Considering the unique possibilities label-free read out can provide in terms of applicable assay formats and in providing kinetic and thermodynamic information, different applications will be discussed highlighting the benefit from this combination but also identifying current bottlenecks and limitations.

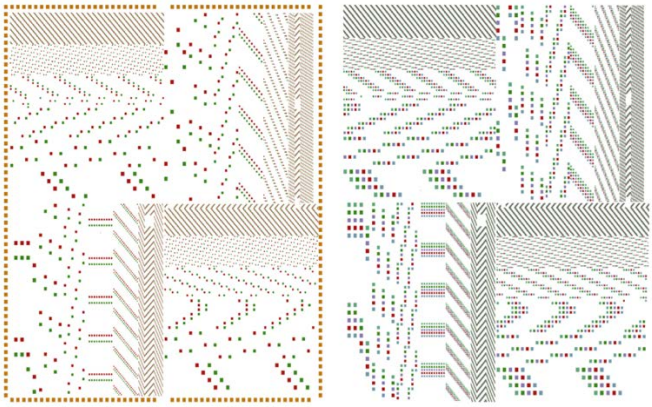

Figure 1. Label-free read out of peptide microarrays with spot sizes from 7,4 – 111 µm [1]

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Surface imprinting for label-free detection of biospecies

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Molecular imprinting has developed and matured into a technique that allows for generating biomimetic recognition properties in man-made materials [1]. Highly useful for small, molecular analytes, its applications to biological systems remains a challenge. Among several others, surface imprinting of biological analytes [2] is continuously proving itself a feasible strategy to address those challenges. Given their nature, surface MIPs are especially interesting when aiming at developing sensors or assays for biological targets.
A wide range of analytes has so far been tested successfully: for instance, surface-imprinted polymethacrylate copolymers turned out feasible for detecting LDL [3] and HDL [4] in the respective clinical ranges. Compared to standard clinical assays, the sensor system does not require extensive centrifugation steps, but is useful detect the respective lipoproteins directly in blood serum. This reduces detection time to some 15 minutes and allows for rapidly testing these species. Furthermore, the analytical result in this case can be obtained within one step, whereas current clinical tests require detecting differently bound cholesterol and subtracting signals from one another.
Larger analytes are of course also accessible: for instance, we succeeded in imprinting native – i.e. non-activated – thrombocytes by a stamp approach. The main challenge in this case is to avoid activating the cells. Quartz crystal microbalances coated with such MIP thin films respond to thrombocyte concentrations and are thus inherently feasible to detect them in blood serum. On top of that, they also function as platelet function tests: Sensor signals for samples containing the same amount of platelets turend out to depend on the amount of aspirin/acetyl salicylic acid the cells had been previously exposed to.
Surface imprinting is not limited to direct sensing, but can also be utilized in assay formats [5]: one example are MIPs for dopaminergic receptors: They are not interesting as an analyte per se, because they are usually bound to membranes of neural cells. However, they bind receptor molecules exposed to antagonists to a lesser extent than uninhibited ones. Hence they are useful for instance for pharmacological binding studies with the potential to reduce the need for animal testing.

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Micro & nano(bio)technology for diagnostics: chances and challenges

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Diagnostic has dramatically changed since the days of “house calls”, relying primarily on physical examination, to nowadays in which a wide portfolio of biochemical analytical tests is available, particularly in central laboratories. However, technological advances occurred in the last decades, point towards a revolutionary transformation of this sector. Emerging knowledge and developments in biosensors, microfluidic, bioanalytical platforms or lab-on-a-chip technologies have opened the doors to innovative diagnostic strategies. Thus, nowadays technology is expanding the number of diagnostic tests that can reach beyond the walls of centralized laboratories to the point of care (POC). Patients already benefit from the rapid detection of c-TnI in emergency rooms, and many practitioners use CRP PoC tests to early diagnose disease related inflammatory processes. PoC technologies enables quick medical decisions, as the diseases can be diagnosed at a very early stage, leading to improved health outcomes for patients by enabling the early start of treatment. In parallel, high-throughput measurement technologies for biomolecules have enabled unprecedented views of biological systems at the molecular level (genome, transcriptome, proteome or metabolome profiles). The combination of all these data could allow defining molecular signatures defining the health status or predicting clinical phenotypes related to disease risk and progression or response to therapeutic drugs and their physiological toxicity. However, despite some notable exceptions, PoC technologies still face a lot of challenges derived from poor standardization and lack of quality assurance. On the other hand, the clinical validity of molecular signatures has not been sufficiently demonstrated. The bioanalytical format used and the biosensor technology employed are some of the most critical components responsible for the analytical performance and reliability of the test. There is a need to improve these schemes improving knowledge in microfluidics, lab-on-a-chip technologies, system integration, device automation, and signal readout. Moreover, multiplexation analysis poses important challenges derived from the need to simultaneously measure distinct sets of biomolecular features on a reproducible and reliable manner. Besides for genomics, microarrays for peptide, protein or small molecule analysis still represent a big challenge and have not been sufficient standardized as for DNA microarray. Likewise, the presence of distinct biomarkers at different concentration ranges prevent from analyzing all these sets in a single run. In this communication, all these aspects will be discussed and examples of technological alternatives to circumvent these limitations in the clinical diagnostic field will be presented.

Roll-to-roll printed biosensors

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Bio-microsystems developed for sensor usage contain typically microfluidic layer performing sample handling and electrical or photonic elements for actual detection. The manufacture includes commonly several sequential steps covering micro- and nanostructuring of fluidic and optical features, printing of conductive and dielectric layers followed by the biochemical surface functionalization. Finally, the assembly step is required to integrate functionalities on a single functional platform. High-volume printing methods, such as roll-to-roll (R2R) printing and replication (Fig. 1a), are routinely used as part of the production. Still, only few concepts have been transferred successfully into scaled up fabrication of integrated microsystems (Fig. 1b,c) because of the lack of high volume production. In addition to commercialization of designs, also academic researchers benefit from the high-volume sensor fabrication when the number of sensor devices is not limiting the assessment of the concepts, designs and associated testing protocols. Here, we present a process to upscale the fabrication of integrated opto-fluidic and electrochemical sensors for biosensing applications. Figs. 1d,e,f show R2R processed waveguide sensors, surface-enhanced-Raman-spectroscopic (SERS) sensors and microfluidics, respectively [1,2,3].

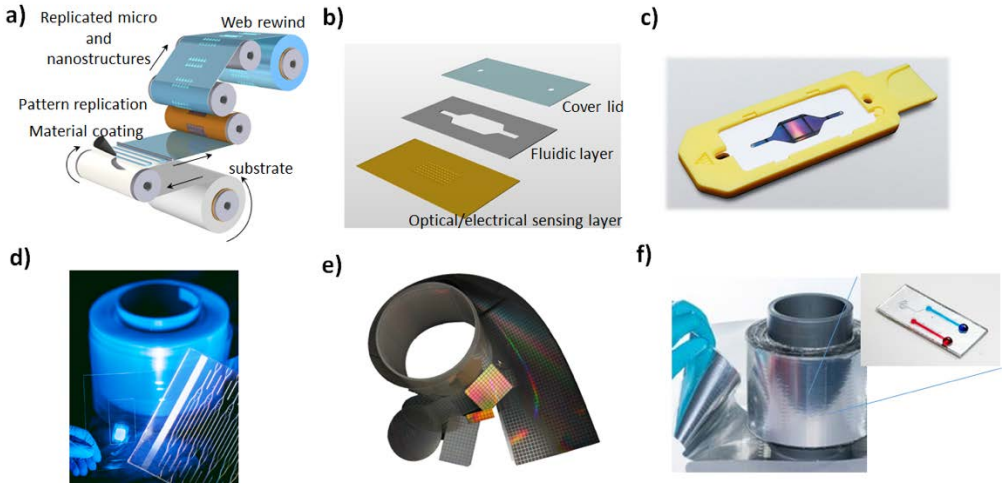

Figure 1. a) Roll-to-roll replication of surface micro- and nano-structures. b) Configuration of integrated sensor comprising layers for sensing and fluidic handling. c) Photograph from an integrated opto-fluidic biosensor. d) Roll of single-mode waveguide sensors. e) Roll of SERS sensors. f) Roll of PDMS microfluidics.

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Aptamer based electrochemical sensors for the analysis of tumor biomarkers

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Cancer is a major public health problem, constituting the second leading cause of death globally. According to WHO, cancer is responsible for 9.6 million deaths in 2018, which corresponds to almost one in six deaths worldwide. One of the keys for reducing cancer mortality is its early diagnosis, as cancer, when identified early, is more likely to respond to effective treatment, resulting in a greater probability of surviving as well as less expensive treatment. Investigations on early detection of cancer should address in parallel two important issues: the identification of selective tumor biomarkers and the development of effective tests to clinically validate them. Enzyme-linked immunosorbent assays are the gold standard method for biomarkers detection. However, new receptors with improved stability are in demand. Aptamers, also referred as chemical antibodies, are synthetic oligonucleotides capable of recognizing, and therefore binding, to almost any type of target. They have surfaced as promising alternatives to natural ones for the detection of tumor biomarkers.

At the University of Oviedo, from the Electroanalysis research group, we are working in the *in vitro* selection of aptamers by the SELEX (systematic evolution of ligands by exponential enrichment) technology, and the coupling of aptamers to electrochemical transducers, to obtain aptasensors able to selectively detect different tumor biomarkers. These aptasensors may be useful in the development of new strategies for improving the cancer early diagnosis and subsequent treatment. Herein, I will give an overview of our work in the development of variants of the SELEX process for the selection of aptamers [1], and the variety of aptasensor designs we developed. The detection schemes we propose involve the use of different amplification strategies to achieve the low LOD required, which include isothermal nucleic acid amplifications. We have focussed our attention on applications that are currently problematic to antibodies, such as the detection of the glycosylation site of aberrant glycoproteins identified as reliable cancer biomarkers [2]. The presentation will highlight the challenges in the detection of these targets and current developments for using aptasensors to solve this particular analytical problem, describing the principles underlying the aptasensor response, their merits and limitations.

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Digital Assays – There's Plenty of Room in the Middle

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In digital assays, targets are partitioned into separate compartments that are processed to give ON/OFF signals (usually with amplification) depending on whether the compartments have zero or a single target entity. Digital assays have several significant advantages over their analog counterparts: absolute quantification without calibration; reduced impacts of inhibitors; and the possibility of faster time to result.
Typically, microfluidic devices to enable digital assays are considered to require precisely controlled volumes since variation in compartment volumes causes biases in concentration estimates. Previously developed methods to infer results of digital assays with polydisperse volumes assume that the compartment volumes follow truncated normal (Gaussian) distributions or gamma distributions. These methods provide accurate results when the volumes follow the specified distributions, or when the standard deviations are small. However, in systems where compartments are made by a simple method, such as vortexing, the volume distribution does not have a known analytical form and spans orders of magnitude. To enable more possibilities in device design, we derived a method [1] to accurately calculate target concentrations from raw results when the compartment volume may vary and may not follow known parametrically described distributions.
We have demonstrated proof of principle using our statistical approach to perform digital PCR [1], digital bacterial culture [2], and digital immunoassays using only a simple system where the droplets are generated in an Eppendorf tube on a vortexer and counted on a microscope. While an Eppendorf tube and microscope is not an ideal form for an integrated product, it demonstrates that there may be a wide variety of new digital assay instruments with reduced cost or increased capabilities that are enabled through straightforward statistical corrections, since the design space for polydisperse digital assay systems is largely unexplored.

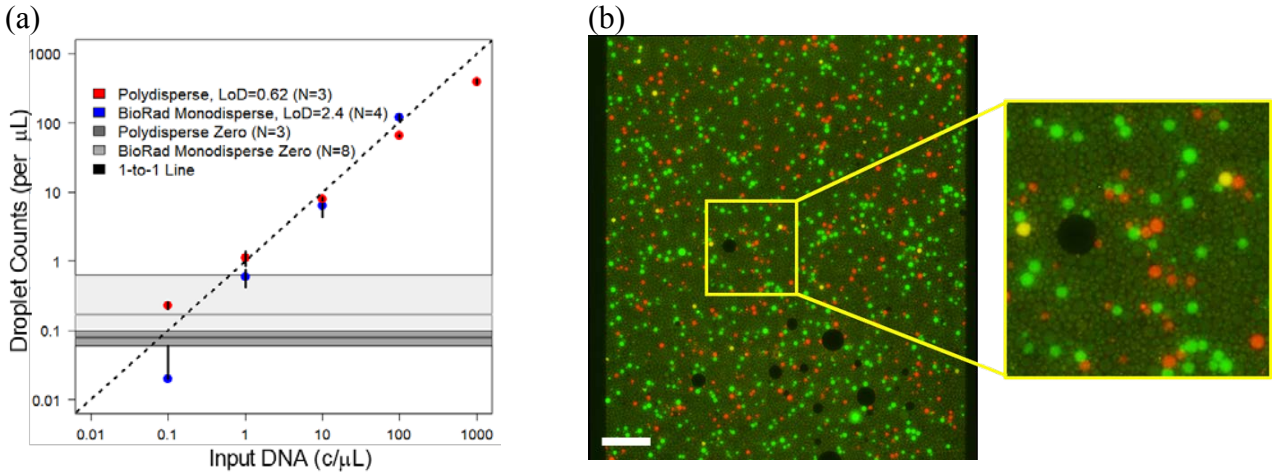

Figure 1. a) . Single-plexed ddPCR in our polydisperse droplet system compared to the commercially available BioRad ddPCR. [1] Both show the expected number of positive droplets based on known input concentrations, though the polydisperse system has a larger dynamic range than the BioRad system. (b) Multiplexing in ddPCR

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DNA-based nanodevices for diagnostic applications

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DNA nanotechnology uses DNA (or nucleic acids) as a versatile material to rationally engineer tools and molecular devices that can find a multitude of different applications (e.g., in-vivo and in-vitro diagnostics, drug delivery, genetic circuits etc.).

During this presentation I will show how to exploit the “designability” of DNA to fabricate nature-inspired DNA-based nanoswitches and nanodevices that are specifically designed to undergo a conformational change (switch) upon binding to a specific input (i.e. target). This input-triggered conformational change can be used for diagnostic, drug-delivery or synthetic-biology applications.

Molecular sensing through the triggering of autocatalytic reactions: towards exponential signal amplifications in bio-electroanalytical methods.

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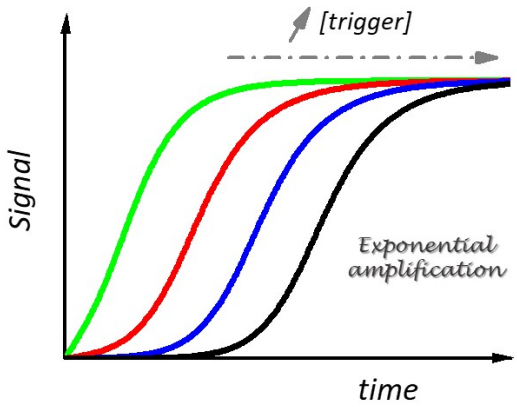
Biomolecular diagnostic requires high level of selectivity and sensitivity to provide relevant information about the pathological state of a patient. For instance, PCR is a gold standard bioanalytical technique based on exponential target amplification, in which the targeted sequence catalyses its own production through a temperature-dependent autocatalytic reaction scheme. Such autocatalytic reactions are of great analytical interest (indeed, PCR allows the selective detection of only few copies per samples). Other autocatalytic reactions have been developed for the isothermal detection of nucleic acids but they again all rely on the abundant bio-molecular machinery available for DNA replication (polymerase, nuclease, ligase,…). Unfortunately, such universal tools are not available for targets such as proteins or small organic/inorganic molecules. Alternative strategies must therefore be developed in order to take advantage of autocatalytic reactions in the detections of such targets.[1-3] In this talk, I will present our ongoing research on new autocatalytic reaction schemes for exponential molecular amplification. In particular, we explore systems based on the cross-activation between the catalytic hydrogen peroxide-triggered release of a quinonic compounds and the catalytic hydrogen peroxide production through quinonic redox cycling in presence of molecular oxygen and an appropriate reducing agent.



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Implantable Biosensors and Lab-on-a-Chip Systems

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Implantable biosensors and lab-on-a-chip systems are considered next generation diagnostic tools with great potential in the diagnosis, monitoring, management and treatment of a variety of disease conditions. For instance, implantable biosensors and smart implants can provide continuous data on the levels of a target analyte and changes in concentration over time, thus allowing the identification of trends, prediction of therapy outcomes and selection of appropriate intervention strategies. Alternatively, lab-on-a-chip technologies are used in precision medicine as early pre-screening tool to detect disease markers and monitor target analytes.
In light of the benefits of miniaturization, automation and integration my research group at TUW is developing implantable biosensors and lab-on-a-chip systems containing integrated fluid handling, degassing, actuators and various biosensing strategies. In course of the presentation three miniaturized diagnostic platforms will be presented including (1) an implantable biosensor for heart transplant rejection monitoring, (2) a smart implant capable of monitoring bone healing at the titanium implant-tissue biointerface in vivo, and (3) a self-powered lab-on-a-chip for blood analysis.

ORAL PRESENTATION ABSTRACTS

Light-directed wiring of PQQ glucose dehydrogenase to inverse opal TiO_2 architectures

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The design of light-driven biohybrid systems by coupling of enzymatic reactions to light-sensitive entities has attracted much attention during the last decade with respect to their analytical performance, but also for the production of electric power from light and enzymatic substrates.[1–3]
This study reports on a light-controlled detection scheme for glucose, in which sulfonated polyanilines (PMSA) and PQQ glucose dehydrogenase (PQQ-GDH) have been integrated into inverse opal TiO_2 electrodes (IO-TiO_2).[4] Here, PMSA provides two functionalities, i.e. visible light sensitivity and electrical wiring of PQQ-GDH to IO-TiO_2 electrodes. In detail, the combination of PMSA and TiO_2 allows for a light-induced charge carrier generation within the polymer followed by a charge carrier separation at the PMSA/TiO_2 interface, which is accompanied by an electron injection into the conduction band of TiO_2. PQQ-GDH provides catalytic activity for the glucose oxidation and supplies the light-driven reaction with electrons, resulting in enhanced photoelectrochemical signals.
IO-TiO_2 electrodes have been prepared by a template approach, resulting in a macroporous structure with pores of around 650 nm and allow for a stable and functional assembly of PMSA and PQQ-GDH. The functional construction of the IO-TiO_2|PMSA|PQQ-GDH electrode can be confirmed by the generation of an anodic photocurrent, which follows the optical properties of the polymer and increases with increasing glucose concentrations. The influence of the TiO_2 electrode morphology on the glucose dependent signal response of the biohybrid system has been analysed by comparing the inverse opal architecture with flat and nanostructured TiO_2 electrodes. Here, the IO-TiO_2 electrodes give rise to an about 25-fold higher signal response as compared to the flat and nanostructured films, which can be attributed to the higher polymer and enzyme loading within the inverse opal films. The final IO-TiO_2|PMSA|PQQ-GDH electrode reaches maximum photocurrents of about 45 µA cm^{-2} and allows for a light-driven substrate oxidation starting at a quite negative potential of -0.315 V vs. Ag/AgCl (1 M KCl). Thus, the onset potential is more negative than the redox potential of the enzyme and demonstrates the beneficial combination of TiO_2 as electrode material, PMSA as light sensitive entity and wiring agent, and PQQ-GDH as biocatalyst for the light-driven conversion of glucose.

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A novel and efficient protocol for Surface Plasmon Resonance based detection of four β-thalassemia point mutations in blood samples and salivary swabs

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Optical affinity biosensors based on Surface Plasmon Resonance (SPR), such as the Biacore™ X100 instrument, are widely used to study biomolecular interactions in real-time with high sensitivity and specificity, and can be employed for the identification of single point mutations causing genetic diseases, such as thalassemia [1] and cystic fibrosis [2]. The aim of this study was to verify whether the Biacore™ X100 biosensor could be used for the real-time detection of point mutations affecting the human β-globin gene and responsible of β-thalassemia, a genetic blood disorder associated with absence (β^0) or reduction (β^+) of adult hemoglobin (HbA) and severe anemia. In particular we analyzed the four most frequent β-thalassemia point mutations in the Mediterranean area: $\beta^0$39, β^0IVSI-1, β^+IVSI-110 and β^+IVSI-6. We used a novel SPR-based interaction format, by immobilization on sensor chips of two oligonucleotide probes, one complementary to the normal sequence and the other to the mutated one. Then we injected asymmetric PCR product targets obtained from genomic DNA of analyzed subjects of different genotypes for each mutation, including healthy individuals, heterozygous β-thalassemia carriers and homozygous β-thalassemia patients. For the development of the diagnostic approach genomic DNA samples were obtained from blood samples or salivary swabs of 71 total subjects. In all cases the genotypic identification was achieved [3]. The results obtained allow proposing a new SPR-based protocol for β-thalassemia single point mutations by using blood samples and salivary swabs as a source of genomic DNA. The same approach has been used for the molecular detection of the HbS point mutation responsible for sickle cell disease (SCD), another hemoglobinopathy characterized by strong anemia and vessel occlusion, fully confirming applicability of the developed protocol to other genetic diseases (supported by AIFA, Wellcome-Trust and by EU projects ULTRAPLACAD and THALAMOSS).

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Monitoring of the metabolic activity of different bacteria by means of a differential LAPS system

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Light-addressable potentiometric sensors (LAPS) belong to the family of field-effect-based potentiometric sensors, which are capable to monitor analyte concentration variations in a spatially resolved way. In this context, a LAPS measuring system represents a promising tool to acquire a chemical image visualizing different concentrations of (bio-)chemical species in aqueous solutions, for instance, in cellular metabolism investigations. By means of the planar sensor surface of LAPS, a simple fixation of measurement chambers can be carried out to perform simultaneous and multivariate analysis of various cell suspensions. In this work, 3D-printed photo-polymer-based (PP-ABS) multi-chambers were combined with LAPS chips to determine the metabolic activity of *Escherichia coli* K12, *Lactobacillus brevis* (DSM 20054), and *Corynebacterium glutamicum* (DSM 20300) after glucose uptake, as exemplarily shown in Fig. 1. Differential measurements were taken into consideration to compensate unwanted external influences such as pH value variations of the medium and sensor signal drifts. In order to improve the technical performance of the LAPS system, a novel FPGA-controlled illumination unit was applied consisting of 16 small-sized tunable infrared laser-diode modules (LDMs). Furthermore, a salt-bridge chamber was engineered and implemented to the LAPS measuring system providing multi-analyte measurements with a single Ag/AgCl reference electrode [1].

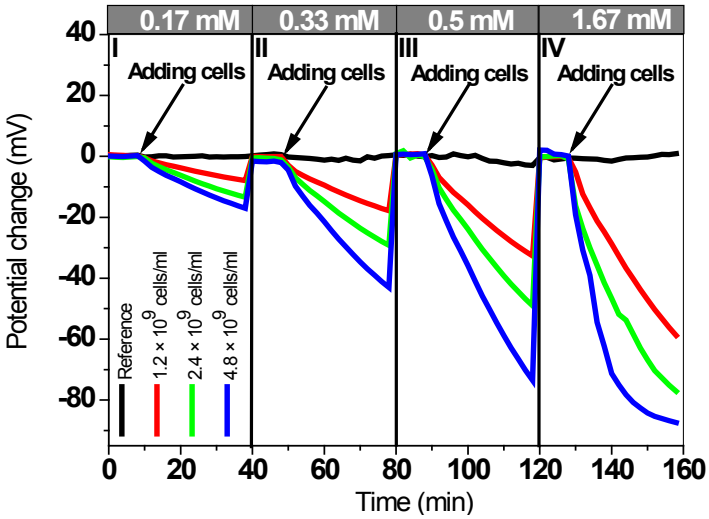

Figure 1. Four-chamber differential LAPS measurement with varying cell number of *C. glutamicum* (1.2×10^9, 2.4×10^9, 4.8×10^9 cells) and varying glucose concentration (0.17 mM, 0.33 mM, 0.5 mM, 1.67 mM). Potential changes of four successive measurements with an ascending row of glucose concentrations. Black line: reference sensor side without cells; red, green and blue lines: active sensor sides with cells.

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Miniature biosensors for multiplexed infection diagnostics

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Molecular diagnostic (MDx) sensors can detect infection and determine the infecting species to the level of an individual strain in times as short as one hour. However, the fundamental complexity of current MDx sensors has hindered their widespread clinical use in point-of-care (PoC) applications involving extensive multiplexing. We are exploring a new platform for MDx sensing with the aim of performing highly multiplexed assays isothermally in a single, small reaction chamber appropriate for PoC applications. Our approach takes advantage of electron-beam surface-patterned microgels. Because of the nature of electron beam, each sensing spot can be as small as hundreds of nanometers. (Fig. 1A) In contrast to traditional microarray spotting directly onto a solid substrate, tethering to microgels maintains oligonucleotides in a highly hydrated and conformational unconstrained state, and we have previously shown that microgel-tethered, self-reporting molecular-beacon (MB) detection probes can achieve signal-to-background levels comparable to those characteristic of untethered MB probes in solution [1]. We can furthermore amplify the signal from the individual microgels by co-tethering polystyrene microspheres, which act as spherical lenses (Fig.1B) [2]. We have combined the self-reporting detection with liquid-phase NASBA amplification in a bloodstream-infection model to achieve femtomolar sensitivity [3]. Our current efforts center on tethering NASBA amplification primers to individual microgels alongside the MB detection probes [4]. Solid-phase amplification removes the primers from solution and opens the opportunity for extensive multiplexing, since primer-dimer formation is eliminated. We are developing an expandable multiplexed respiratory virus assay and exploring novel ways to achieve highly multiplexed solid-phase amplification with femtomolar sensitivity.

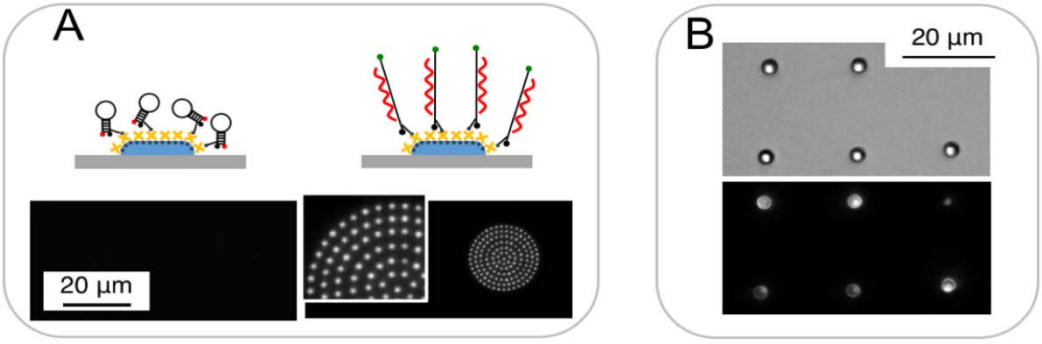

Figure 1. **(A)** Molecular–beacon probes (left) are tethered to streptavidin-activated PEG-B microgels. They fluoresce (right) when hybridized to complementary DNA. **(B)** The fluorescence signal from microgel-tethered molecular beacons is amplified by co-tethered microspheres: (top: bright-field image; bottom: fluorescence image).

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Detection of proteins expressed by *in vitro* translation using external force-assisted biosensor

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In vitro translation (IVT) is a method of synthesizing proteins from genes, which are used for various biological studies. The IVT system can also be used for detection of genes such as infectious viruses and bacteria. Thus, highly sensitive detection of proteins expressed by IVT is of use in biological experiments and biosensing.
We developed the external force-assisted near-field illumination (EFANI) biosensor as an extremely sensitive immunosensor [1,2]. In this study, the EFANI biosensor was integrated with an IVT system to develop an evaluation system for the proteins synthesized with IVT. A schematic diagram of EFANI biosensor is shown in Fig. 1(a). The sensor uses two kinds of immunobeads as labels: scatterer beads and magnetic beads. A target substance is bound with both scatterer and magnetic beads by immunoreaction. The conjugates of target and both beads are observed by camera under magnetic force application and detected as "moving signals" on the observed images. Herein, we employed green fluorescent protein (GFP) as a model target for proof-of-concept. Gold nanoparticles with a diameter of 60 nm and magnetic beads with a diameter of 50 nm were used with immobilizing anti-GFP antibodies. A solution of DNA of GFP was injected into an IVT solution (PURExpress, GeneFrontier). The incubated solution was serially diluted and mixed with the gold nanoparticles and magnetic beads, and then applied to the EFANI biosensor. Figure 1(b) is an image of a sample containing 10^3 molecules/mL of synthesized GFP acquired before magnetic force application. Figure 1(c) is an image of moving signals of the sample, which is a merged image of ones serially acquired for 35 s after the magnetic force application. Paths of moving signals are shown as bright lines towards the magnet. The number of moving signals in Fig. 1(c) is 8 counts, whereas that of a sample without synthesized GFP (negative control) was 1.3±1.3 counts (N = 4). Therefore, protein molecules expressed by IVT were detected using the EFANI biosensor. We further conducted detection of nucleoprotein of influenza virus from RNA using the developed system.

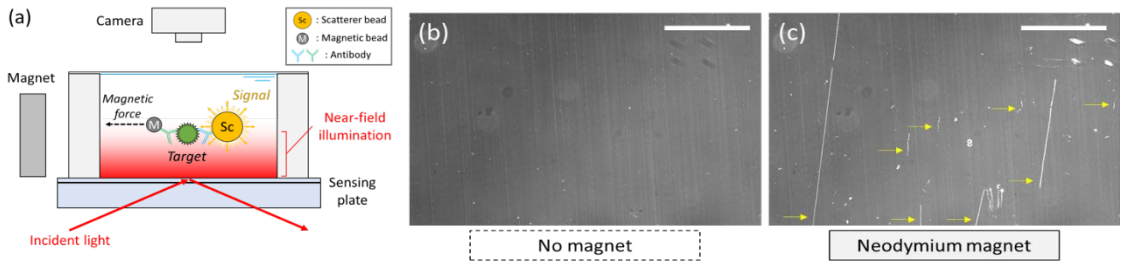

Figure 1. a) Schematic diagram of a detection system of EFANI biosensor. Observed images of GFP detection b) before and c) after magnetic force application. Yellow arrows indicate signals moved towards the magnet. Scale bar: 500 μm.

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Development of a sequence-independent and enzyme-free approach for the detection of multiple microRNAs using a single nanostructured enhancer of SPRi

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Here we purpose a simple approach for the simultaneous detection of multiple microRNAs (miRNAs) using a single nanostructured reagent as surface plasmon resonance imaging (SPRi) enhancer and without using enzymatic reactions, sequence specific enhancers or multiple enhancing steps as normally reported in similar studies. The strategy involves the preparation and optimization of neutravidin-coated gold nanospheres (nGNSs) functionalized with a previously biotinylated antibody (Ab) against DNA/RNA hybrids [1]. The Ab guarantees the recognition of any miRNA sequence adsorbed on a surface properly functionalized with different DNA probes; at the same time, gold nanoparticles permit to detect this interaction thus producing enough SPRi signal even at a low ligand concentration. The final assay allowed the simultaneous detection of four miRNAs with a limit of detection (LOD) of up to 0.5 pM (equal to 275 attomoles in 500 µL) by performing a single enhancing injection. The proposed strategy shows good signal specificity and permits to discriminate mutated sequences much better than non-enhanced SPRi. Finally, the method properly works in real samples (total RNA extracted from blood) as demonstrated by the detection of four miRNAs related to multiple sclerosis used as case study. This proof-of-concept confirms that the approach provides the possibility to detect a theoretically unlimited number of miRNAs using a simple protocol and an easily prepared enhancing reagent, and may further facilitate the development of affordable multiplexing miRNA screening for clinical purposes.

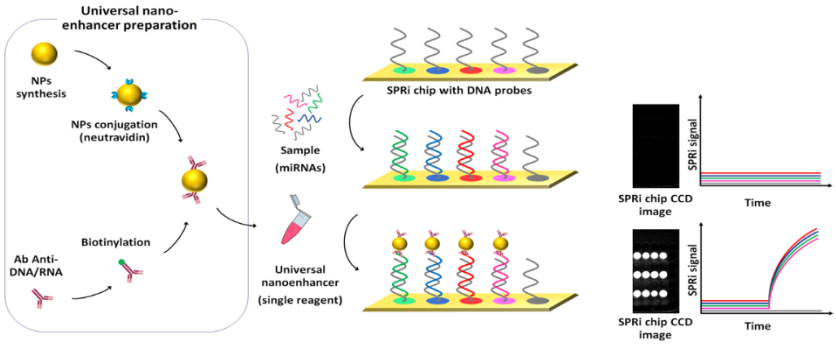

Figure 1. Schematic representation of the strategy used for the simple and simultaneous detection of multiple miRNAs using the same nanoenhancer.

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Ratiometric Luminescent TOP Nanosensors for Simultaneously Measuring Temperature, Oxygen, and pH at a Single Excitation Wavelength

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Two nanosensors for simultaneous optical measurements of temperature ("T"), oxygen ("O"), and pH ("P") have been designed. These "TOP" nanosensors are based on 100 nm-sized silica-coated polystyrene nanoparticles (PS-NPs) doped with the near infrared emissive oxygen- and temperature-sensitive chromium(III) complex ($[Cr(ddpd)_2][BPh_4]_3$ $CrBPh_4$)[1][2] and an inert reference fluorescence dye (Nile Red NR or 5,10,15,20-tetrakis-(pentafluorophenyl) porphyrin TFPP) and are covalently labeled with the pH-sensitive fluorophore fluorescein isothiocyanate (FITC). These emitters can be excited at the same wavelength and reveal distinguishable emission spectra suitable for ratiometric intensity-based and time-resolved studies in the visible and near infrared spectral region. The core-shell nanostructure of these sensors reveals high colloidal stability in various aqueous media. Studies in PBS buffer solutions and in a model body liquid demonstrate the applicability of the TOP nanosensors for optically detecting the three bioanalytically and biologically relevant analytes temperature, oxygen and pH simultaneously at the same position.

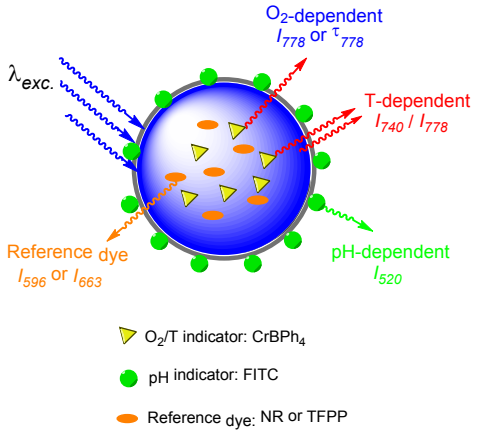

Figure 1. Luminescent multianalyte nanosensor model. O_2 / T indicator $CrBPh_4$ complex and reference dye (NR or TFPP) are encapsulated in the 100 nm PS-NPs, which are coated with a thin silica-shell. pH-responsive dye is covalently bound to particle surface for protonation and deprotonation.

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Particle-based sample preparation strategies for the detection of relevant pneumonia pathogens

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Pneumonia is one of the most common infectious diseases worldwide with high morbidity and mortality, especially for patients with a weakened immune system. Currently, the gold standard for diagnosing pneumonia is the microbiological cultivation of the pathogens along with an X-ray examination of the lung and a blood cell count [1]. Unfortunately, this approach is very time consuming and delayed initiation of the treatment reduces a successful therapy. Therefore, there is an urgent unmet medical need for fast and highly specific assays for diagnosing pneumonia.
In the current study we developed various strategies for a convenient enrichment and subsequent detection of eight clinically relevant pneumonia pathogens. In doing so, we cover the entire analysis chain starting from sample preparation up to the reliable identification of the pathogens.
The individual steps are described in detail below. Bacteria were isolated from complex samples with various amine-modified particles exploiting pH-dependent electrostatic interactions between the pathogens and the functionalized particle surface. Following this, an amplification of the target DNA as well as subsequent stringent array-based hybridization with specific complementary capture probes were performed. Finally, results were visualized by an enzyme induced silver nanoparticle deposition, providing stable endpoint signals and consequently an easy detection possibility by naked eye. The assay was optimized and successfully investigated on real sputum samples from patients.
To sum up, this novel approach enables reliable identification of pathogens within three hours and thus opens up the potential for a diagnostic method.

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Single-molecule immunoassays based on upconversion nanoparticles for detection of cancer markers and bacteria

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Single-molecule immunoassays have recently attracted wide attention due to the ability to identify and detect individual analyte molecules, which provides the ultimate sensitivity in bioanalytical applications. Popular labels for single-molecule assays are enzymes in confined volumes, however, the signal generation is time consuming, enzymes have limited stability and the supporting chips are often costly. In recent years, many techniques based on nanomaterials as detection labels have emerged, that can be used to overcome the disadvantages of enzymes and improve the assay properties [1].
Photon-upconversion nanoparticles (UCNPs) are excellent labels for single-molecule immunoassays because they emit shorter-wavelength light under near-infrared excitation (anti-Stokes emission), which limits autofluorescence and light scattering. These unique photoluminescent features enable the detection of UCNPs at the single nanoparticle level by conventional wide-field epiluminescence microscopy [2].
We have synthesized streptavidin-conjugated polyethylene glycol-coated UCNPs and applied them as labels for the detection of the cancer biomarker prostate specific antigen (PSA). The individual sandwich immunocomplexes were counted using a wide-field microscope equipped with a 980 nm laser excitation source (Figure 1A). The noise-surpassing digital readout (particle counting) resulted in 20× higher sensitivity than the analog readout (emitted light intensity) and allowed to reach a detection limit of 800 aM (23 fg mL^{-1}) in 25% serum (Figure 1B) [3].
The streptavidin-conjugated UCNPs are also suitable as labels for detection of pathogenic bacteria. We have developed an assay based on in-house prepared antibodies for the detection of *Melissococcus plutonius*, causative agent of European foulbrood. The assay provided detection limit of 10^4 CFU mL^{-1} and its practical relevance was demonstrated by the analysis of real samples of honeybees and larvae.

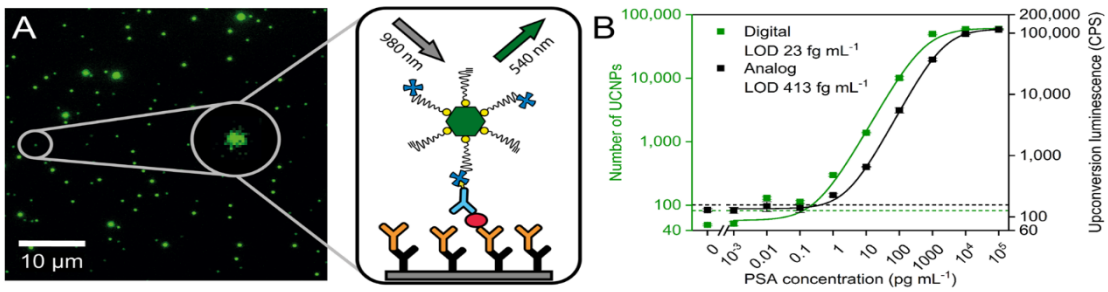

Figure 1. A) Upconversion microscopy image of microtiter plate after specific capture of 100 pg mL^{-1} of PSA with schematic representation of the immunocomplex. B) Calibration curve.

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A smartphone-based thermochemiluminescent biosensor for valproic acid detection in blood and saliva

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With the rise of precision medicine, there is a growing need for companion diagnostics to monitor the drug efficacy and tailor the therapeutic dose for each patient. Recently, smartphones have been proposed as suitable platforms for developing ready-to-use biosensors usable by everyone at the point of need, providing rapid and on site analysis with high assay detectability. So, the development of biosensor is suitable for providing indication about the individual drug absorption and bioavailability and therefore enabling optimization of the drug administration regimen. Herein, we propose for the first time a smartphone-based immunosensor employing a paper-based format and thermochemiluminescence (TCL) detection, optimized for valproic acid (VPA), that was a fatty acid with anticonvulsant properties used in the treatment of epilepsy. TCL is a chemical luminescence phenomenon in which photons are emitted upon thermally-induced fragmentation of a suitable molecule, with production of a moiety in its singlet electronically excited state [1][2]. Its peculiar characteristics make it a suitable detection principle for smartphone's-based biosensing, thanks to its high detectability and reagent-less nature of the measurement. A vertical flow immunoassay (VFIA) format was exploited to develop a rapid and one-step competitive immunoassay for VPA detection, employing silica nanoparticles doped with a TCL 1,2-dioxetane derivative as a label. The VFIA sensor is a stack of paper-based layers, in which reagents are stored in a stable form, therefore the immunoassay protocol could be completed in a very short time after the addition of the sample. Suitable accessories were developed using the 3D printing technology in order to integrate the analytical assay to the smartphone which was exploited as a multi-tasking tool, providing a power source for the heat shock required to trigger the TCL reaction and a sensitive camera for emitted photons measurement. The developed biosensor allowed to quantify VPA in blood and saliva, with limits of detection (4 and 0.05 μg mL^{-1} respectively) and dynamic ranges (4-300 and 0.05-20 μg mL^{-1}) suitable for therapeutic monitoring purposes.

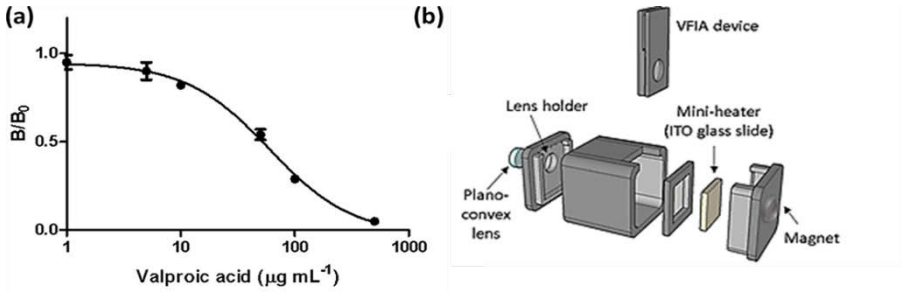

Figure 1. (a) Calibration curves obtained in blood. (b) Scheme of the integrated TCL-VFIA device.

[1] A. Roda et al, *Anal. Chem.* 84(22), 9913-9919 (2012).
[2] M. Di Fusco et al, *Anal. Bioanal. Chem.* 407(6), 1567-1576 (2015).

Nanometer scale multi-electrode arrays for biosensing

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Continuous progress in miniaturization of electrodes as transducers provides a promising route towards realization of compact and high-sensitivity biosensor platforms.[1] In this contribution, we present the realization of ultra-small, interdigitated electrode arrays of metal over silicon and glass substrates by combining micro- and nanofabrication processes such as nanoimprint lithography and subsequent photolithography. Sensor chips containing interdigitated electrodes (NEA-IDEs) of gold measuring 14 µm in length and 600 nm in width with 600 nm spacing were fabricated at 4-inch scale. The fabrication process involved nanoimprint lithography, wet-etching, metal evaporation and nano lift-off steps followed by optical lithography for metal contact lines and passivation layers. An optimized process-flow resulted in high-quality NEA-IDEs with reliable electrochemical behaviour as inferred from voltammetry and impedimetric analysis.[2] In a proof-of-concept bioassay, to demonstrate the applicability of the NEA-IDEs for biosensing, the nanostructures were modified with short DNA molecules as recognition elements for monitoring hybridization of analyte DNA via impedance spectroscopy. Stable impedance signals could be obtained using a redox ferri-/ferrocyanide system. After hybridization with complementary analyte DNA, the sensors showed an enhancement in the charge transfer resistance. Experiments with different analyte DNA concentrations demonstrate a dynamic detection range of 1–100 nM for the presented sensor configuration. With the advantages of NEA-IDE structures being individually readable and small enough to integrated into typical microfluidic channels, our platform is expected to give further boost for realization of miniaturized lab-on-a-chip biosensor devices with multiplexing capabilities.[3]

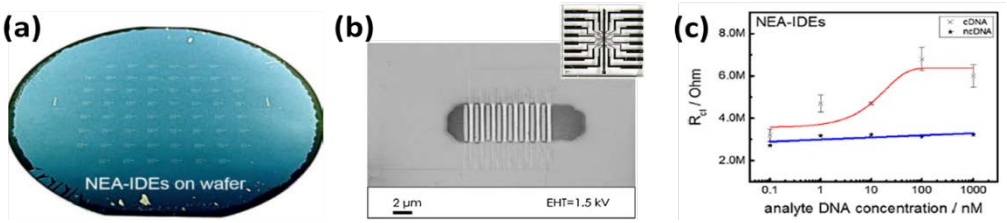

Figure 1. a) Wafer-scale fabrication of NEA-IDE platform using combined nanoimprint and photolithography techniques, b) photograph showing a chips measuring 7 x 7 mm^2 at the end of the fabrication process and SEM of NEA-IDEs, c) Electrical biosensor characteristics of the NEA-IDEs shown as dose-response graphs.

[1] V. Pachauri and S. Ingebrandt, *Assays in Biochemistry*, 60, 1, 81-90 (2016)
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[3] L.E. Delle et al. *Biosens. Bioelectron.*, *102*, 574-581 (2018)

Re-engineering an Enzyme Switch as a Generic Platform for Rapid Biomolecule Sensing

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Diagnostic assays are vital across a broad range of sectors, notably environmental, food safety and healthcare. For many applications there is a desire to move away from laboratory testing and towards rapid diagnostics, at the point of care or in the field, to accelerate test results and decision making. This drive towards rapid diagnostics requires robust underpinning technology, which can provide the sensitivity and specificity of a laboratory assay but quickly, simply and cheaply. We seek to develop a generic platform that can be used for numerous targets with little optimisation.

The ELISA has long been a gold standard of diagnostic assays but the requirement for multiple incubations and wash steps precludes their direct use in rapid diagnostics. Lateral flow tests successfully incorporate the steps of an ELISA into a strip for rapid diagnostics, but suffer from low sensitivity and qualitative results. Other techniques, such as bead based assays, surface plasmon resonance and quartz crystal microbalance, often require unsuitably cumbersome or expensive equipment. One solution to achieve rapid, simple, one-step detection is to integrate target recognition and signal generation into "active" sensing technology. Current technologies have certain drawbacks; fluorescence modulation lacks amplification for improved sensitivity, allosteric enzymes require extensive redesign from target to target and split enzymes can suffer from instability and poor activity recovery.

A promising "active" antibody sensor has been reported, based on an enzyme (β-lactamase) tethered to its inhibitor protein (BLIP) by a linker containing epitopes for antibody recognition.[1] Antibody binding pulls the inhibitor apart from the enzyme, switching on enzymatic activity and enabling amplified detection of antibody concentration by a substrate colour change. Here, we describe the protein engineering strategy taken to transform this antibody sensor into a generic platform for simple (one-step), rapid (< 20 minutes) and sensitive (pM) detection of a range of biomolecules, including proteins, virus particles and antibodies (Figure 1). For an important inflammation and infection biomarker, human C-reactive protein, we compare our enzyme-switch sensor with a commercially available ELISA kit, and confirm its suitability for point-of care testing. For a plant virus target, cow pea mosaic virus, we observe rapid detection of infected leaf samples, proving the platform's utility for in the field diagnostics. The broad applicability, simplicity, sensitivity and speed of this enzyme-switch, makes it a promising generic platform for rapid diagnostics.

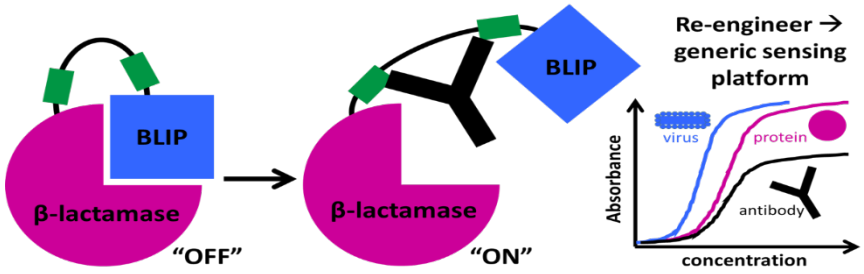

Figure 1. Schematic of enzyme switch platform and output

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Minimally-invasive Microneedle-based Biosensor Array for Simultaneous Lactate and Glucose Monitoring in Artificial Interstitial Fluid

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Microneedle arrays for minimally invasive continuous sensing in the dermal interstitial fluid (ISF) have been demonstrated in both amperometric [1,2] and potentiometric [3] modes, however there are no publication where microneedle arrays have been shown to function as second generation biosensors [4].
Here we report the first mediated pain free microneedle-based biosensor array for the continuous and simultaneous monitoring of lactate and glucose in artificial interstitial fluid (ISF). The gold surface of the microneedles has been modified by electrodeposition of Au-multiwalled carbon nanotubes (MWCNTs) and successively by electropolymerization of the redox mediator, methylene blue (MB). Functionalization of the Au-MWCNTs/polyMB platform with the lactate oxidase (LOX) enzyme (working electrode 1) and with the FAD-Glucose dehydrogenase (FADGDH) enzyme (working electrode 2) enabled the continuous monitoring of lactate and glucose in the artificial ISF. The lactate biosensor exhibited a high sensitivity (797.4 ± 38.1 $\mu A\ cm^{-2}\ mM^{-1}$), a good linear range (10-100 µM) with a detection limit of 3 µM. The performances of the glucose biosensor were also good with a sensitivity of 405.2 ± 24.1 $\mu A\ cm^{-2}\ mM^{-1}$, a linear range between 0.05 and 5 mM and a detection limit of 7 µM. The biosensor array was tested to detect the amount of lactate generated after 100 minutes of cycling exercise (12 mM) and of glucose after a normal meal for a healthy patient (10 mM).
The results reveal that the new microneedles-based biosensor array holds interesting promise for the development of wearable real-time monitoring devices to be used in sport medicine and clinical care.

[1] A.E.G. Cass, S. Sharma, Microneedle Enzyme Sensor Arrays for Continuous In Vivo Monitoring. Meth. Enzymol., 589 (2017) 413-427. http://doi.org/10.1016/bs.mie.2017.02.002
[2] S. Sharma, Z. Huang, M. Rogers, M. Boutelle, A.E.G. Cass, Evaluation of a minimally invasive glucose biosensor for continuous tissue monitoring. Anal. Bioanal. Chem. 408(29) (2016) 8427-8435. http://doi.org/10.1007/s00216-016-9961-6
[3] T.M. Rawson, S. Sharma, P. Georgiou, A. Holmes, A.E.G. Cass, D. O'Hare, Towards a minimally invasive device for beta-lactam monitoring in humans. Electrochem. Comm. 82 (2017) 1-5. http://doi.org/10.1016/j.elecom.2017.07.011
[4] L. Ventrelli, L.M. Strambini, G. Barillaro, Microneedles for transdermal biosensing: current picture and future direction, adv. Healthcare Mater., 4 (2015) 2606-2640. http://doi.org/10.1002/adhm.201500450

Enzymatic Biofuel Cells as Self-powered Sensing Devices

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With the increasing use of portable electronics and small medical devices the clean production of electrical energy from renewable resources has received recently intensive attention from many research groups. A way to achieve this goal is the use of biological catalysts such as oxidoreductase enzymes to catalyze the oxidation of renewable fuel. They harvest and convert the chemical energy stored within these fuels into electrical energy. Due to their high specificity for their substrates, research in bioelectrocatalysis is beneficial not only for the biofuel cell technology but also find applications in biosensing or bioelectrosynthesis devices.

In this presentation, we will present different strategies for the fabrication of functionalized carbon nanomaterial (CNTs) and their use to conceive redox active electrodes.[1] Functionalization of carbon material is accomplished via π-π interactions of electron rich species. Furthermore, when analyzing the protein active site structure, rationalization of the redox assemblies offers simplification of electrode fabrication.[2,3] In these cases, interactions between the enzyme and the substrate-like molecules (similar to host-guest interactions) enable preferential orientation of the oxidoreductase onto the electrode surface to afford efficient direct electron transfer or mediated electron transfer. These different strategies and the use of the selectivity of the oxidoreductases allow us for the fabrication of membrane-less enzymatic biofuel cells (EFCs). Finally, these EFCs can be applied for the detection of specific inhibitors (Figure 1).

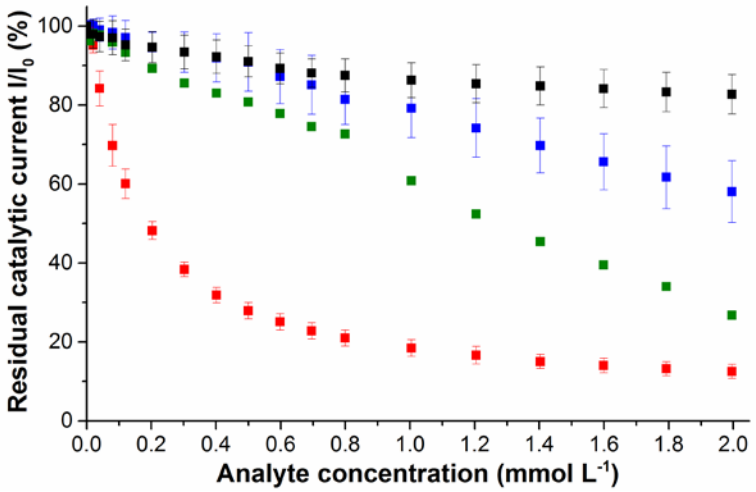

Figure 1. Calibration curve of the FAD-GDH based biosensor for three different possible enzyme inhibitors (red, blue, green) in buffer pH 7.0 containing 100 mmol L^{-1} glucose and 200 µmol L^{-1} redox mediator. The black curve accounts for dilution effect.

[1] A.J. Gross, X. Chen, F. Giroud, C. Abreu, A. Le Goff, M. Holzinger, S. Cosnier, *ACS Catal.*, 7, 4408-4416 (2017).
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Allosteric activation of an enzyme embedded in a redox polymer for tuned amperometric biosensing

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The use of allosteric enzymes has received much attention in various research areas such as drug design and the development of protein switches or biosensing in recent years. The activity of such enzymes is modulated by the binding of an allosteric effector such as specific ligands, ions, or proteins, thus modulation the substrate conversion rate of the protein which can be directly monitored via the signal amplitude of a sensors, e.g. the current output if the enzyme is electrically wired to an electrochemical transducer.
In this contribution we present a novel approach for tuned biosensing of the co-factor NADH and the urinary diseases biomarker para-hydroxy phenylacetate (p-HPA) by the allosteric modulation of the C1 reductase component of the p-HPA hydroxylase from *Acinetobacter (A.) baumannii* component. The C1 component oxidises the co-factor NADH and transfers the electron to the C2 monooxygenase component in the hydroxylase. NADH oxidation activity of the C1 protein can be modulated by the urinary metabolite p-HPA. For tuned biosensing the C1 reductase was electrically wired in a mediated electron transfer regime by deliberately designed Os-complex modified polymers with adjusted redox potentials. In the presence of the allosteric modulator p-HPA a 1.5fold increase in sensitivity for NADH detection was observed. Moreover, pre-incubation of the polymer/enzyme electrodes in p-HPA solutions allowed for the fabrication of a reagentless sensor device with enhanced sensitivity. [1] The reverse process, i.e, the detection of p-HPA by providing a constant NADH concentration that generates a current amplitude that is modulated by the addition of the urinary biomarker was also possible. Our results demonstrate that allosteric modulation can indeed be used for enhanced biosensing even when the biorecognition element is embedded in a redox polymer matrix. Moreover, the sensor is able to detect the biomarker p-HPA in artificial urine in a straightforward amperometric detection mode. No pre-treatment of the sample is required which is crucial for the fabrication of point-of-care devices.

[1] S. Teanphonkrang, S. Janke, P. Chaiyen, J. Sucharitakul, W. Suginta, P. Khunkaewla, W. Schuhmann, A. Ruff, A. Schulte, *Anal. Chem.*, 90, 5703-5711 (2018).

Decoding the cell monologue: label-free nanobiosensor enables real-time analysis of live cell secretion

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Unraveling the dynamic cell signaling profiles requires the implementation of a real-time monitoring methodology for precise analysis. The current fluorescent and colorimetric-based techniques hamper such applications as they are semi-quantitative, require time-consuming steps for molecular labeling, and provide 'snapshot' endpoint readouts that dramatically compromise the temporal resolution of the analysis. Here, we present a nanoplasmonic biosensor based on periodically patterned gold nanohole arrays for long-term and real-time monitoring of cell secretory activity without using external labels. The gold nanohole arrays facilitate a unique optical phenomenon, the so-called extraordinary optical transmission (EOT), which enables ultra-sensitive and high-throughput biomolecular detection. The biosensor can be integrated with the state-of-the-art microfluidics system that provides well-controlled liquid handling and live cell culture conditions for long-term monitoring. We primarily demonstrated the real-time monitoring of vascular endothelial growth factor (VEGF) directly secreted by microfluidic-cultured cancer cells for over 10 hours, while preserving good cell viability. Nonetheless, the recent studies have revealed the significant heterogeneity of cellular functionalities, urging us to advance the label-free analysis resolution towards single-cell level. An innovative label-free optofluidic nanoplasmonic biosensor is therefore introduced for single-cell analysis in real time. We incorporate a novel design of a multifunctional microfluidic system with small volume microchamber and regulation channels for reliable monitoring of cytokine secretion from individual cells. Different profiles of interleukin-2 (IL-2) secretion are detected and distinguished from single lymphoma cells. The sensor configuration combined with optical spectroscopic imaging further allows us to determine the spatial single-cell secretion fingerprints in real time. This innovative biosensor system is anticipated to be a powerful tool to probe live cell signaling at multiple levels for basic and clinical research, disease diagnostics and novel therapy development.

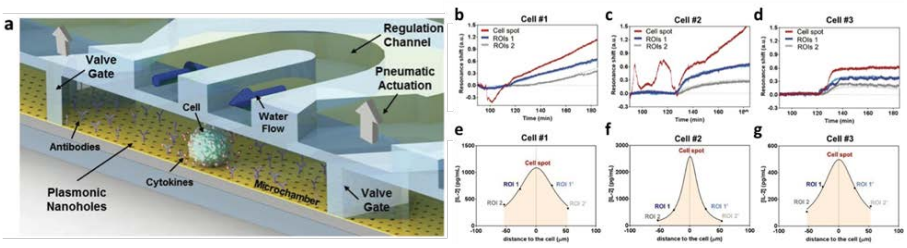

Figure 1. (**a**) A schematic view of the integrated nanoplasmonic biosensor. (**b-g**) Independent measurements of IL-2 secretion from three distinct lymphoma cells, with monitoring the cell spot ROI and surrounding ROIs simultaneously.

[1] X. Li, M. Soler, C. I. Özdemir, A. Belushkin, F. Yesilköy, H. Altug, Lab Chip 2017, 17, 2208.

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Electrochemical detection of *E. coli* utilizing genetically modified bacteriophages

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Rapid detection of bacteria in food and beverages is an important factor for public health. Utilizing reporter bacteriophages as biorecognition elements offers several advantages for the sensitive and selective detection of bacteria. We have recently demonstrated the advantages of using bacteriophages as biorecognition elements as they are highly specific for their host organism, are able to distinguish between viable and non-viable bacteria cells and can readily be genetically engineered. Upon infection of their host, the reporter enzymes encoded within the bacteriophage genome are expressed by the infected bacteria cells. In this study a genetically modified T7 bacteriophage was used for the detection of *E. coli* in aqueous samples. The bacteriophage was genetically engineered to express alkaline phosphatase. Furthermore, the enzyme was previously engineered for increased enzymatic activity and was fused to carbohydrate binding modules specific for cellulose. This modified alkaline phosphatase was hence expressed and released upon bacteriophage infection of *E. coli* following the lytic infection cycle and could be captured and concentrated in cellulose filters. The quantity of reporter enzymes was proportional to the number of *E. coli* cells present in the sample and was determined electrochemically using screen-printed carbon electrodes (SPCE). The enzymatic reaction using *p*-nitrophenyl phosphate was performed at pH10 following a buffer exchange to pH7.2 for the electrochemical detection by differential pulse voltammetry (DPV). The electrochemical detection was optimized in objective of reaction time, substrate concentration and sample volume. Compared to the United States Environmental Protection Agency's (EPA) method requiring 24 hours the reported assay requires a short analysis time of only 3 h, consisting of a bacteriophage infection period (1.5 h) and the electrochemical assay (1.5 h). So far a lower limit of detection (LOD) of 10^4 CFU/mL *E. coli* was obtained using a 1 mL sample. Current research focuses on further optimization of the assay principle towards analysis time, LOD and reproducibility. Also, automation for larger sample volume processing is of great interest.

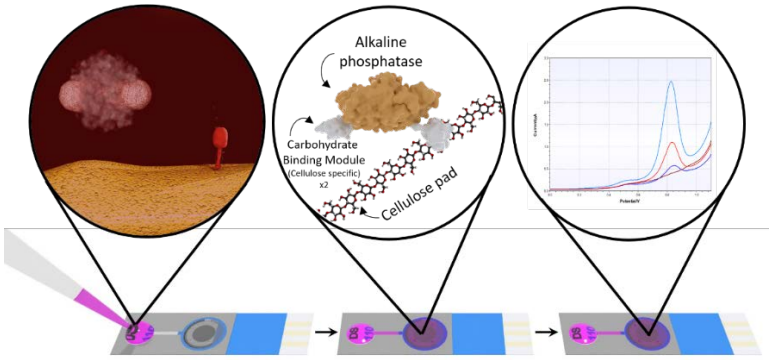

Figure 1. Assay schematic of the electrochemical detection of *E. coli* utilizing genetically modified bacteriophages. Electrochemical detected *p*-nitrophenol is generated by bacteriophage expressed alkaline phosphatase being captured with a cellulose pad.

[1] R. Arts, I. den Hartog, S. E. Zijlema, V. Thijssen, S. H. E. van der Beelen, M. Merkx, *Anal. Chem.*, 88, 4525-4532 (2016).
[2] K. Yamada, H. Shibata, K. Suzuki, D. Citterio, *Lab Chip*, 17, 1206-1249 (2017).

Nanoplasmonic biosensing: consideration of analyte transport

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Interest in the use of affinity biosensors based on plasmonic nanostructures has increased dramatically in recent years. These nanoplasmonic biosensors present a promising alternative approach to their surface plasmon resonance (SPR) predecessors, yet are unique in that their sensing surfaces have more complex architectures, often composed of localized regions of high sensitivity (e.g., individual photonic nanoparticles) dispersed along a dielectric substrate having little to no sensitivity. Under optimal conditions such that analyte interacts only with the sensitive regions (with the remaining regions passivated), the rate of analyte capture, and thus the overall sensing performance, will have a strong dependence on the architecture of a nanoplasmonic substrate. Discussion of this dependence remains rare in the photonic literature, where focus has typically been towards the optimization of a nanostructure in terms of its optical characteristics, for example, in its sensitivity to refractive index changes.
In this presentation we will discuss how changes to a nanoplasmonic architecture affect both optical performance and analyte transport. We will present experimental data concerning a wide range of plasmonic substrates, including variation of their base photonic element (nanorods, nanodisks, micro- and nanowires) as well as the packing density of such elements. We will show that, despite the large differences in optical characteristics between substrates, the characteristics of analyte transport follow relatively simple scaling trends (Figure 1), primarily dependent on the fill fraction of sensitive regions. Furthermore, we will show that these trends can be predicted beforehand with fundamental knowledge of the plasmonic architecture.

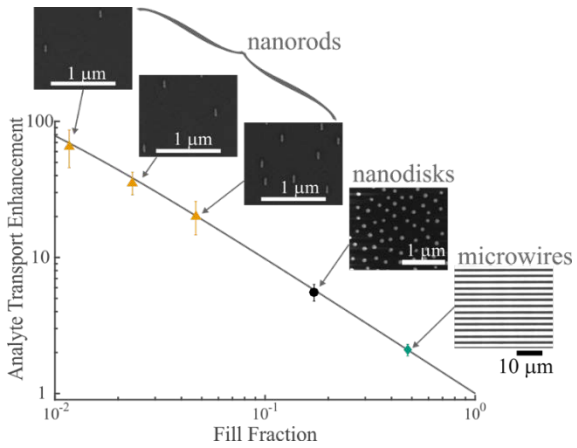

Figure 1. Analyte transport scales with the fill fraction of sensitive regions in a simple fashion.

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[2] A. M. Derfus et al., *Nano Lett.*, 4(1), 11-18 (2004).
[3] Q. Shabir et al., *Silicon*, 1-11 (2017).

Fructose biosensors based on direct electron transfer between fructose dehydrogenase and electrodes

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Fructose dehydrogenase (FDH, EC 1.1.99.11) is a bacterial membrane bound flavocytochrome oxidoreductase, which has been widely employed to develop direct electron transfer (DET) and mediated electron transfer (MET) based electrode platforms [1]. Most commonly FDH from *Gluconobacter japonicus* has been studied. FDH is a heterotrimer consisting of a catalytic dehydrogenase domain, DH_{FDH} (subunit I), where D-(−)-fructose oxidation occurs at the covalently flavin cofactor (FAD), followed by an internal electron transfer (IET) to the cytochrome domain (CYT_{FDH}, subunit II), which contains three heme *c* moieties coordinated by the enzyme scaffold, and a subunit III, which is not involved in the ET process.

We have recently investigated [2-4] how to optimize orientation and the DET reaction between FDH and electrodes. In [2] we report on the influence of pH and monovalent/divalent cations on the catalytic current response, the IET, and the structure of FDH by using amperometry, spectrophotometry, and circular dichroism. Amperometric measurements were performed on graphite electrodes, onto which FDH was adsorbed and the effect on the response current to fructose was investigated when varying the pH and the concentrations of divalent/monovalent cations. In the presence of 10 mM $CaCl_2$, a current increase of up to ≈ 240% was observed, probably due to an intra-complexation reaction between Ca^{2+} and the aspartate/glutamate residues found at the interface between the DH_{FDH} and CYT_{FDH}. In [3] we present a new method to electrodeposit highly mesoporous gold onto a polycrystalline solid gold electrode without any template. The electrodeposition is carried out by first cycling the electrode potential between +0.8 and 0 V in 10 mM $HAuCl_4$ with 2.5 M NH_4Cl and then applying a negative potential for the production of H_2 bubbles at the electrode surface. This mesoporous gold electrode was modified with 4-mercaptophenol onto which FDH was orientated to optimize the DET reaction.

In [4] an efficient DET reaction was also achieved between FDH and anthracene-modified single-walled carbon nanotubes (SWCNT) deposited onto a glassy-carbon electrode. The SWCNTs were activated *in situ* with a diazonium salt synthesized through the reaction of 2-aminoanthracene with $NaNO_2$ in 0.5 M HCl for 5 min at 0° C. After the *in situ* reaction, the 2-aminoanthracene diazonium salt was electrodeposited by running cyclic voltammograms from +1000 to −1000 mV. The anthracene-SWCNT-modified GCE was further incubated in an FDH solution to allow FDH to adsorb. Cyclic voltammograms of the FDH-modified electrode revealed two couples of redox waves ascribed to the heme c_1 and heme c_3 of the CYT_{FDH}. In the presence of 10 mM fructose two catalytic waves could clearly be seen and were correlated with two heme *c*:s, with a maximum current density of 485±21 μA cm^{-2} at 0.4 V at a sweep rate of 10 mV s^{-1}.

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[2] P. Bollella, Y. Hibino, K. Kano, L. Gorton, R. Antiochia, *Anal. Bioanal. Chem.*, 410, 3253-3264 (2018).
[3] P. Bollella, Y. Hibino, K. Kano, L. Gorton, R. Antiochia, *Anal. Chem.,* 90, 12131-12136 (2018).
[4] P. Bollella, Y. Hibino, K. Kano, L. Gorton, R. Antiochia, *ACS Catal.,* 8, 10279-10289 (2018).

Tunable nanoplasmonic functional transducers: novel insights for health applications

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Planar distributions of plasmonic nanoantennae are realized onto transparent substrates with tailored optical and sensing functionalities. Different fabrication methods based on low cost lithographic techniques, allowing the formation of large area complex plasmonic systems with variable shapes and distributions, are used to this purpose [1,2].
The optical and morphological properties of the fabricated metal nanoantennae are investigated by extinction measurements in the Visible-IR spectra, by atomic force microscopy and correlated to information predicted by numerical models based on finite element analysis. Several key parameters are explored for sensitivity optimization, namely metal, shape, size, main interparticle distance, as well as formation of ordered and disordered two-dimensional assembly of nanoantennae. Different distribution of electric field are thus generated onto the plasmonic transducers upon light illumination, mainly due to localized and/or propagating surfaces plasmon modes, leading to diverse surface and bulk refractive index sensitivities. This will allow tailoring optical properties of nanoantennae to functional abilities required by the investigated sensing assay. Far- and near-field optical properties are discussed in view of their application as highly sensitive transducers in plasmonic nanosensors of interest in health monitoring.
Owing to the clear advantages proposed by this high-throughput method, the study pave the way for the realization of highly sensitive optical devices with potential biosensing applications.

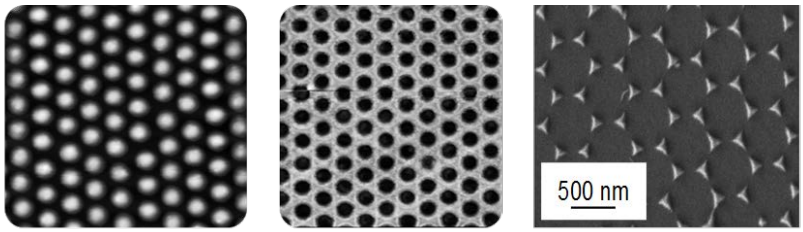

Figure 1. Some examples of ordered two-dimensional assembly of metal nanostructures to be investigated as highly sensitive plasmonic transducers

[1] P. Colson, C. Henrist, R. Cloots, *Journal of Nano- materials*, vol 2013, Article ID 948510.
[2] A. J. Haes and R. P. Van Duyne, *Anal. Bioanal. Chem.,* vol. 379, no. 7–8, pp. 920–930, (2004).

Microfluidic electrochemical lab-on-chip immunosensor for ultrasensitive analysis of cocaine in water, saliva, and urine

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The presence of anthropogenic small organic molecules in surface water is becoming more and more a societal problem, as well as a challenge for scientists to invent new analytical tools for reliable on-site analysis. A broad diversity of molecules is included in this category, originating from various human uses: pesticides, prescription and over-the-counter pharmaceuticals as well as illicit drugs like cannabinoids, heroin, or cocaine. A modular approach for the detection of such molecules, taking cocaine as a model, was followed, integrating an electrochemical ELISA [1] performed on antibody-grafted magnetic beads in a hybrid lab-on-chip system consisting of a tubing part for mixing/incubation steps [2], and a PDMS/PTFE chip [3], containing a screen-printed electrode for cyclic voltammetry measurements. After optimization of the system, a linear response of the sensor versus the logarithm of the cocaine concentration was obtained with a limit of detection of 0.15 ng/L. Within an overall assay time of 30 minutes, concentrations down to 1 ng/L could be reliably determined in water, saliva, and urine [4].

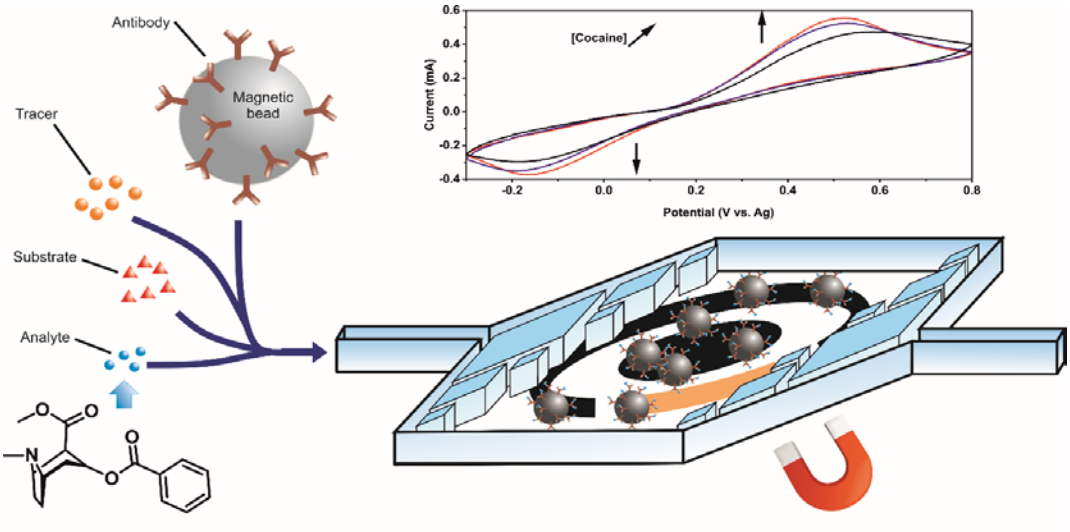

Figure 1. Antibodies immobilized on magnetic beads (MB) are injected into tubing chip together with enzyme tracer and analyte (sample). After mixing, MBs are captured on a screen-printed electrode and substrate injected. Inset: Increasing concentrations produce higher currents in cyclic voltammetry measurements

[1] N.A. Abdelshafi, U. Panne, R.J. Schneider, *Talanta*, 165, 619-624 (2017).
[2] J. Bell, E. Climent, M. Hecht, M. Buurman, K. Rurack, *ACS Sens.*, 1(4), 334-338 (2016).
[3] P. Ashokkumar, J. Bell, M. Buurman, K. Rurack, *Sens. Actuators B*, 256, 609-615 (2018).
[4] N.A. Abdelshafi, J. Bell, K. Rurack, R.J. Schneider, *Drug. Test. Anal.*, (2018), in press.

Multiparametric sensing on microstructured optical fibres by surface plasmon resonance for multiple applications

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While optical fibres are mostly known for their use in telecommunication, their development as fiberscope has opened opportunities for a wide range of sectors, to examine difficult-to-reach areas (human body, inside of machines…). For instance, optical fibres have been used in medicine for imaging purpose by endoscopy for almost 70 years, enabling to precisely target an organ and to move inside the body. In the meantime, research on microarrays and biosensing technologies has grown tremendously, driven by their potential in a broad range of applications, from *in vitro* diagnosis to environmental monitoring. Gathering together these technologies to build a biosensor on fibre would make possible targeted bioanalysis, representing a real asset in numerous cases like monitoring organ-specific biomarkers or bacterial proliferation in food production lines.
In this contribution, we will present new developments of a “lab-on-fibre” which takes advantage of the targeting possibilities of an optical fibre bundle, together with structural and molecular modifications giving it the properties of a biosensor (figure 1a). Our approach is based on microstructured optical fibre bundles composed of 6000 individual fibres (figure 1b) which, when appropriately etched and gold coated, exhibit specific plasmonic properties. Thanks to the surface plasmon resonance (SPR) phenomenon occurring at the bundle apex, biomolecular interactions can be measured through the plasmon coupling induced variation, which results in changes of the retro-reflected light intensity (figure 1c). Each of the 6000 fibres of the bundle representing individual SPR sensors, the bundle can be functionalised by multiple probes to monitor different targets (biomolecules, cells, bacteria etc.). This tool, adapted for multiplexed, real time and label free molecular analysis, would then provide a great advance for *in vivo* diagnosis or more generally *in situ* detection.

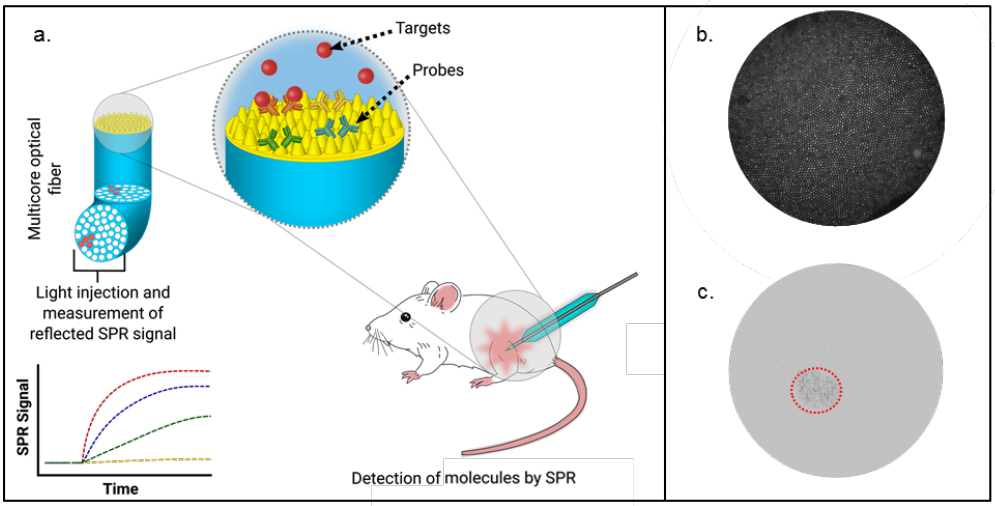

Figure 1. a. Scheme of the general principle of the lab-on-fibre for SPR detection. b. SPR image of the optical fibre bundle. c. Differential image corresponding to a molecular event at the surface.

Paper-based strips for Anywhere analytics

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In recent years the objective of performing simple, quantitative measurements in various POCT applications (Point-of-Care Testing) induced the use of lateral flow assays on paper-based strips. These quick tests allow to determine critical states of diseases at an early stage and provide a simple way to control the patient's condition e.g. after surgery. Accordingly, these strips are subsumed under the heading Anywhere Care [1] recently. Comparable to the simple pregnancy test, such strips can monitor disease markers in a simple and cost-effective manner. Labelled systems and direct optics are used to convert the binding of the analyte to the recognition element into a measurable readout – applying labels such as fluorescence or colorimetric based techniques. The use of metal nanoparticles such as gold simplifies the detection process, as the presence of the analyte leads to a red discoloration at the test-spot on the lateral flow strip. This colour change can be detected by eye for simple positive/negative decisions (pregnancy test) while the use of a digital camera or a smart phone and image processing algorithms allow the determination of exact analyte concentrations. Laser nano structuring of the strips allows even multi-channel approaches for multi-analyte detection or for on-chip reproducible measurements [2]. As an example, the measurement of salmonella (pathogens affecting humans and animals) is provided - especially with view to controlling large herds, an out-of-laboratory first control is an interesting application of such strips. Results of on-chips reproducible measurements and determinations of 2 serovars of salmonella in parallel on one chip will be discussed.
In recent years, therapeutic drug monitoring has become of high interest. In particular most psychotropic drugs work in a very small therapeutic window. This leads to the need for monitoring the level of the patient's blood titer to ensure that the patient is supplied with a sufficient amount of drugs while side effects caused by overdosing are minimized. Amitriptyline will be discussed as an example of a colorimetric test using simple non-structured strips. These contain an analyte pad where 200 µl of amitriptyline are injected. On the conjugate pad an anti-amitriptyline antibody gold nanoparticle conjugate is placed and on the test-spot a derivative of amitriptyline is immobilized. After 5 minutes the analyte passes the conjugate pad, blocks according to its concentration the conjugate, which moves further to the test-spot giving inverse proportional to amitriptyline concentration a signal (binding inhibition test scheme). By eye 100 ng/l and 1 µg/l can be discriminated. By evaluating the RGB values of the test-spot in comparison to the background amitriptyline in the sample was quantified in a concentration range between 100 ng/l and 100 µg/l. These values fit to the therapeutic window and allow the measurement in blood. Furthermore, laser structuring of the strips enables internal calibration and lower LODs which are interesting to measure psychotropic drugs as pollutants in water which could be e.g. a typical application of citizen science developments in recent years.

[1] G. Gauglitz, *Anal. Bioanal. Chem.*, 410, 1-3 (2018).
[2] F. Schenk, P. Weber, J. Vogler, L. Hecht, A. Dietzel, G. Gauglitz, *Anal. Bioanal. Chem.*, 410, 863-868 (2018).

Acknowledgement: Part of this work was funded by the BMWI within the project "Papierbasierte Low Cost Sensorik – Von der Mikrofabrikation bis zur Evaluation", IGF-Vorhaben, Nr. 18148 N/3.

Label-free detection of mycotoxins with optical planar waveguide polarization interferometry aptasensor

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A novel biosensor based on optical planar waveguide (OPW) operating as polarization interferometer (PI) [1] was utilized in this work for detection of mycotoxins, e.g. aflatoxin B1, and ochratoxin A, in direct assay with specific aptamers. The key element of the experimental set-up is the $SiO_2/Si_3N_4/SiO_2$ planar waveguide having a large differences in refractive indices of the Si_3N_4 core (n=2) and SiO_2 cladding (n=1.46) and therefore a steep angle (~47°) of propagation and large number of reflections (~500 per mm). In the OPW PI set-up shown schematically as inset in Fig. 1, the 630 nm circular polarized light from a laser diode is coupled into the waveguide via slant edge and collected on the other side (via a polarizer) with CCD camera; the reaction cell attached to the sensing window in the waveguide allows the study of biochemical reactions. Such experimental set-up can detect a phase shift between p- and s- components of polarized light developed on course of molecular binding. The refractive index sensitivity of the recently upgraded OPW PI set-up was evaluated as 8700 rad./RIU which is comparable with the existing MZ interferometer-based biosensors. Series of biosensing tests of detection of aflatoxin b1 (AFT b1) and ochratoxin A (OTA) were performed with OPW PI sensor having specific aptamers (from Microsynth, Switzerland) immobilized on the Si_3N_4 surface in the sensing window following the protocol described in [2]. Typical multi-periodic output signal caused by binding of 0.01 ng/ml of AFT b1 to its specific aptamer is shown in Fig. 1a. The corresponding phase shift values were calculated from such data and summarized as concentration dependence in Fig. 1b. The achieved LDL for AFT b1 is of 1pg/ml, a remarkable achievement for direct assay format with unlabelled aptamers. Similar results were obtained for detection OTA. Future work focuses on improvement of data acquisition and scaling down the experimental set-up towards hand-held bio-sensors capable of point-of-need detection of toxins on ppt concentration range.

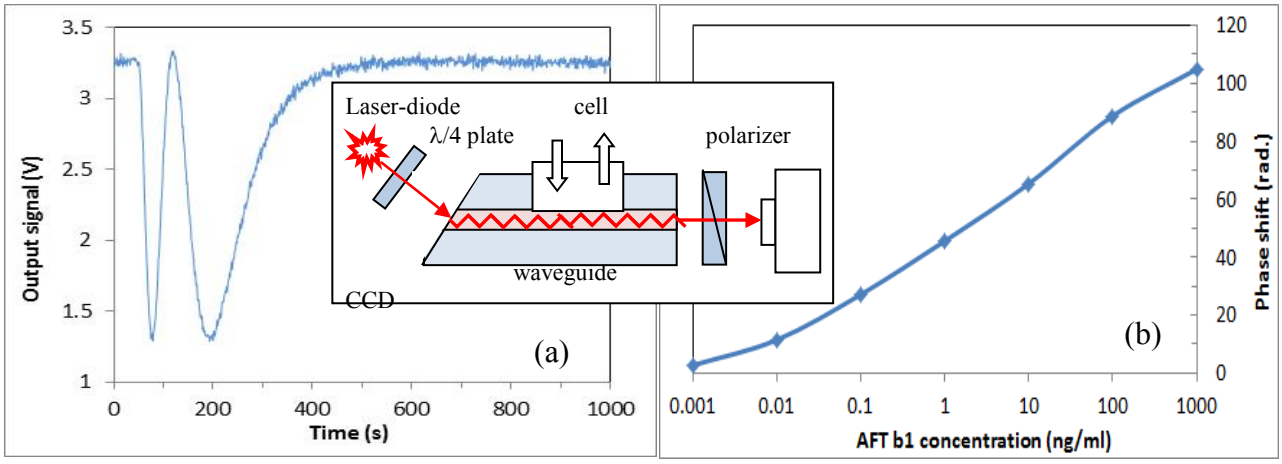

Figure 1. OWG PI experimental set-up (inset), a) typical response waveform on binding 0.01 ng/ml AFT b1 to aptamer, b) concentration dependence of PI sensor responses.

[1] A. Nabok, A.M. Al-Jawdah, A.Tsargorodska, *Sens. & Act. B,* 247, 975–980 (2017).
[2] A. Al-Jawdah, A. Nabok, et al., *Biosens. & Bioelectron., (*2018) *(in press).*

A 96-well wax printed prussian blue paper for the visual determination of cholinesterase activity in serum

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The search for analytical strategies to ensuring clinical monitoring, without recurring the use of laboratory and skilled personnel, is highly required. However, the development of brand-new Point-of-Care devices cannot overlook the successful combination among manufacture methodologies, functional (smart) materials, and transduction mechanisms. In particular, the era of sustainability we belong in, strictly requires user-friendly and low-cost approaches, adaptable in low-resource contexts, i.e. developing world. As the case of study, the detection of butyrylcholinesterase enzyme (BChE) activity in serum has been considered. Its activity gives indication regarding oxidative stress and inflammation: many diseases such as Alzheimer and diabetes are often associated with changes of its activity. Although, many approaches have been reported, both colorimetric and electrochemical, they might appear not very reasonable for developing world analysis: the color of sample limits the application of colorimetric-based approach, while the need for potentiostat can represent an issue for what concern the use of electroanalysis. Despite all the approaches reported to date, we decided to exploit chromatographic papers in order to realize a novel analytical tool. The porous structure of paper allowed both to reagentlessly synthesize Prussian Blue Nanoparticle starting from its precursors and to load the enzymatic substrate necessary to evaluate the enzyme's activity. The use of wax printing allowed fabricating a 96-well platform for multi-measure purposes. Briefly, when BChE converts the substrate (butyrylthiocholine) into butyric acid and thiocholine, the latter is oxidized by Prussian Blue which turns in its reduced colorless form, known as Prussian White. The observed fading is proportional to the presence of the by-produced thiocholine, and thus to the enzymatic activity. However, the structure of paper reveals its importance in filtering complex and colored matrices, going beyond some issues associated with colorimetric methods. Herein, the experimental procedure for Prussian Blue synthesis has been finely optimized (paper type, precursor concentration, precursor volume) and the detection of BChE activity has been carried out linearly in the 2-15 U/mL range with a detection limit down to 0.8 U/mL. Recovery tests were performed by spiking serum with different levels of BChE, obtaining percentage recoveries between 99-113%. The accuracy of the multi-wells Prussian Blue-based platform was evaluated in clinical samples received from Tor Vergata Hospital patients: a satisfactory correlation has been obtained in comparison with the gold standard method (XXX) adopted in routine clinical analyses.

Glycated Blood Proteins Detection by Surface-Enhanced Raman Spectroscopy

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New silver SERS-substrates were obtained, characterized and applied for glycated proteins selective determination analysis using phenylboronic acid. The surface of the substrates has heterogeneous flaked structure with silver particles different size: 300-400 nm fractions form flat plates 2 μm size. This chaotic structure with rough edges encourages local enhancement of electromagnetic field which stimulates a gain of Raman signal of analyte. The surface was modified with 4-mercaptophenyl boronic acid (4-mPBA).
Number of saccharides was taken as a model compounds for the glycated proteins selective determination. It contains cis-diol groups that form six-membered ethers with boronic acid. The bands of sugars and glycated proteins are difficult to visualize. Therefore the main differences in the SERS-spectra of sugar treated SERS-substrate and non-treated one are manifested in the bands 999, 1016, 1072 and 1590 cm^{-1}, which refer to the vibrations of the phenylboronic acid. Multivariate data analysis helps us solving this problem. Principal component analysis shows that the clouds of points corresponding to sugars are separated from the cloud of control points (Figure 1). It proves that silver SERS-substrates modified by 4-mPBA make it possible to separate the sugar spectra from the buffer spectra.
Moreover, these SERS-substrates can help with untrivial task to discriminate two similar proteins with minor differences. Glycation means a lot in terms of whole organism e.g. diabetes mellitus. But one cannot discriminate glycated and non glycated protein with high accuracy. New silver SERS-substrates modified by 4-mPBA allow quantitative determination of glycated albumin in human plasma (Figure 2).

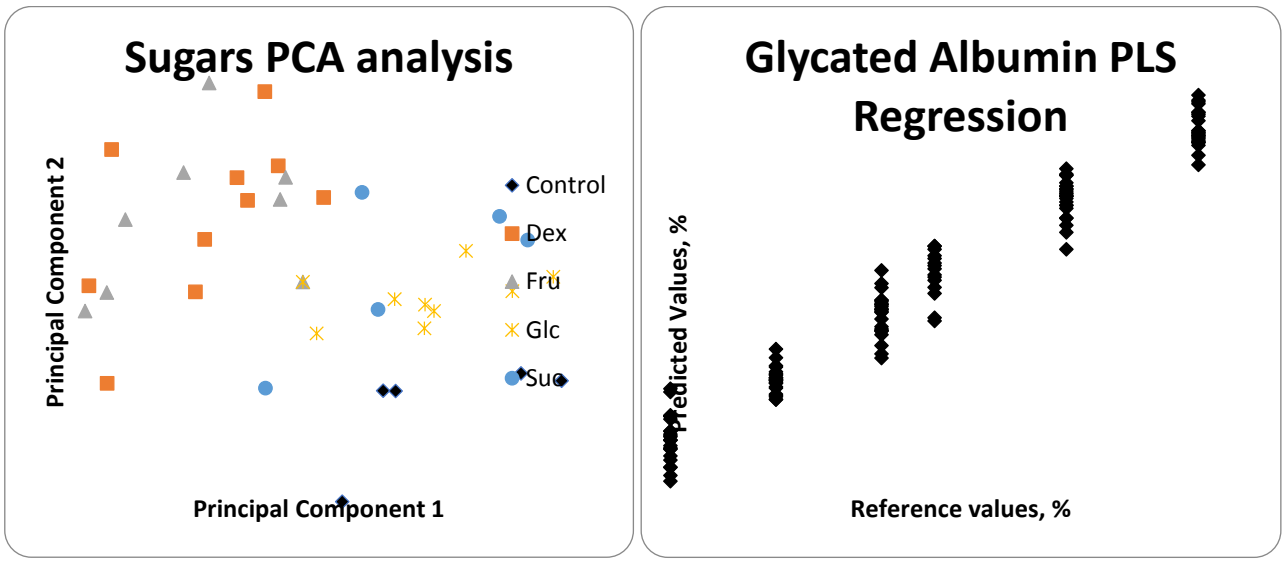

Figure 1. a) PCA discrimination of different sugars and control on the SERS-substrate. (b) Calibration curve of the glycated human albumin in the plasma on the SERS-substrate.

Oncology meets microfluidic - development of a Lab-on-Chip chemosensitivity assay for primary cancer cells

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A microfluidic chemosensitivity assay for the parallel investigation of different chemotherapeutics with focus on lung cancer by means of isolated primary cancer cells has been developed. The evaluation process is a two-step one. At first the tissue slices were prepared and immunohisto-chemically stained with different biomarkers to determine the type of lung cancer. In the second step the primary lung cancer cells were cultivated and directly grown on the microfluidic chip system [1,2] where the chemotherapeutics flow over the cells together with the cell culture medium. The evaluation of the cell vitality can be done with real time monitoring via an impede-metric analysis (ECIS) of the cell form and integrity. By means of the developed µ-TAS system a personalized chemosensitivity assay for therapy optimization was developed to identify invalid medication and/or the probability that resistance against other substances develop is reduced.

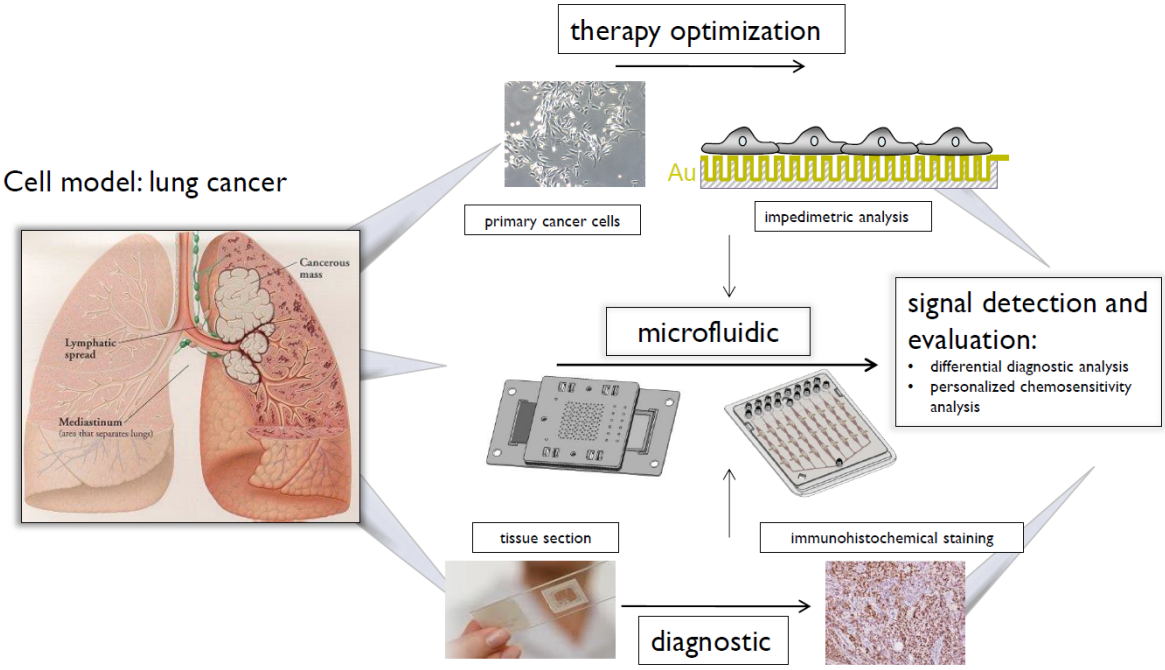

Figure 1. Chemosensitivity assay for therapy optimization and diagnostic of lung cancer

[1] M. Büttner, B. Büttner, A. Pflieger, S. Kunath, P. Fix, J. Sänger, K.-H. Feller et al., Design of experiment to study tissue disaggregation and cultivation process based on lung cancer, Journal of cellular biochemistry, in preparation
[2] M. Büttner, B. Büttner, A. Pflieger, J. Sänger, K.-H. Feller, Tumor-on-a-Chip: A microfluidic chemosensitivity assay, J. Clinical Oncology, in preparation.

A portable biosensor for 2,4-dinitrotoluene vapors

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2) Institute of Food Chemistry, Leibniz University of Hannover
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Buried explosive material e.g. landmines represent a severe issue for human safety all over the world. The majority of victims are civilians – nearly half of them are children. In addition to their direct danger to humans and animals, most explosives consist of environmentally hazardous chemicals like 2,4,6-trinitrotoluene (TNT), carcinogenic 2,4-dinitrotoluene (2,4-DNT) and related compounds. Vapors leaking from buried landmines offer a detection marker for landmines presenting an option to detect landmines without relying on metal detection. The primary explosive found in landmines is TNT. However, the TNT headspace concentration above buried landmines is extremely low and not suitable for detection purposes. A more volatile compound is 2,4-dinitrotoluene (DNT) which is an impurity and byproduct of common TNT synthesis. We report on the construction of a wireless, handy and cost effective 2,4-dinitrotoluene biosensor combining recombinant bioluminescent bacterial bioreporter cells and a compact, portable optical detection device. This biosensor could serve as a potential alternative to the current detection technique. It is shown that the used bioreporter system is heavily dependent on oxygen. Detection of 49 ppb gaseous 2,4-DNT was successful and sets the stage for further research.

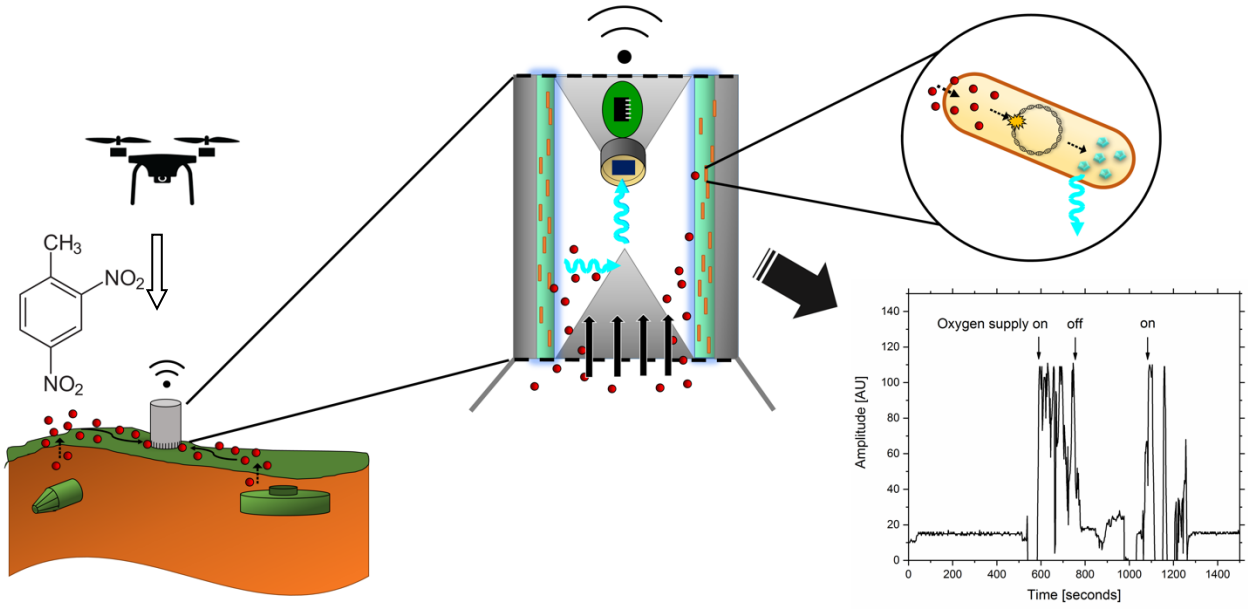

Figure 1. Proposed working principle of the biosensor. Drones drop the sensor on a contaminated field where Luciferase production in immobilized *E. coli* cells is triggered by 2,4-DNT vapors. Emitted bioluminescence is then focused onto a photodiode using two reflectors.

Ultra-high-frequency surface-acoustic-wave microfluidics and biosensors

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Rayleigh Surface acoustic waves (SAWs) are an effective means to interact with fluids and activate a number of microfluidic phenomena, such us mixing, droplet actuation, microparticle manipulation, nebulization and micropumping, to name but a few. Moreover, in a different configuration, SAWs can also be exploited for sensing applications. The SAW operation frequency is a fundamental parameter that sets the relevant length-scales of the device, and that can importantly limit the miniaturization and portability of the devices, as well as multiplexing operation and the integration of many operational blocks on the same chip. Here, I discuss the relevant length scales in sub-nanometer amplitude SAW-driven acoustic streaming and illustrate the absence of any physical limitations beyond fabrication capabilities preventing the downscaling of SAW-driven internal streaming to nanoliter microreactors and beyond[1]. GHz-SAWs are experimentally shown to importantly allow the reduction of droplet volume in digital microfluidics, and of the microchannel size in case of acoustic counterflow[2]. Finally, GHz-SAW acoustic resonators are fabricated and proposed for biosensing applications (Fig. 1), demonstrating biomolecular detection in liquid after drying with a limit of detection far better than that of standard commercial gravimetric sensors (*i.e.*, quartz-crystal-microbalances)[3].

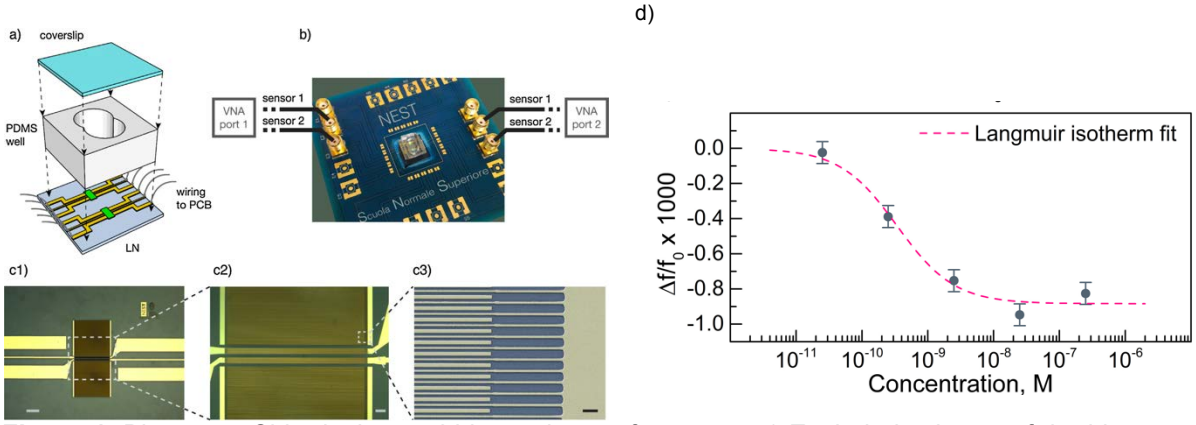

Figure 1. Biosensor Chip design and biosensing performance. a) Exploded scheme of the biosensor chip. b) Photo of the mounted biosensor chip with external connections for electrical measurements. c1) and c2) Representative optical microscope images of a resonator. Scale bars are 200 um (c1) and 40 um (c2). c3) Representative scanning electron microscopy detail of a Positive Negative Reflector (scale bar is 2 um). (d) Biosensor performance characterization: resonance shifts for different streptavidin concentrations. The sensor was functionalized with biotin-PEG-SH.

[1] RJ. Shilton, V. Mattoli, M. Travagliati, M. Agostini, A. Desii, F. Beltram, M. Cecchini, *Advanced Functional Materials* 25 (37), 5895-5901 (2015)
[2] RJ. Shilton, M. Travagliati, F. Beltram, M. Cecchini. *Advanced Materials* 26 (29), 4941-4946 (2014)
[3] M. Agostini, G. Greco, M. Cecchini. *Sensors and Actuators B: Chemical*, 254, 1–7 (2018)

Hybrid graphene oxide-porous silicon biosensor for early diagnosis of sudden death syndrome

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2) Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Via S. Pansini 5, 80131 Napoli, Italy
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Graphene oxide (GO) is the oxidized counterpart of graphene, characterized by oxygen-containing functional groups in the form of epoxy, hydroxyl and carboxyl acid groups on both the basal planes and edges [1]. Several strategies were published to functionalize GO. In particular, carboxylic acid groups on the GO sheets can be used as reactant sites for immobilization of several biological molecules [2]. Furthermore, GO exhibits a broad photoluminescence (PL) emission from 500 to 900 nm, which was proposed for the development of a new class of optoelectronic devices [3]. Unfortunately, the PL of a thin layer of GO nanosheets is too weak, mainly due to the oxygen-functional groups producing non-radiative recombination between their electrons and holes presents in sp2 clusters. A valid strategy to enhance the light generation from GO is based on its infiltration into large surface area substrate, and PSi is an optimal candidate for this task. PSi is a nanostructured material produced by electrochemical anodization of doped crystalline silicon. Due to its sponge-like morphology, characterized by a high specific surface area, PSi is an ideal transducer for the development of several kinds of biosensors [4]. In recent papers, hybrid devices constituted by GO electrostatically immobilized on amino-modified porous silicon were described. In particular, monolayer and multi-layered structures were used to infiltrate GO nanosheets by spin-coating. The enhancement and the modulation of the PL signal emitted from GO adsorbed on the hybrid structures were highlighted, while these phenomena were not observed in the case of GO on crystalline flat silicon [5]. In this work, a chemical procedure to covalently bound GO to PSi surface is developed in order to realize a stable hybrid device for biosensing purposes. The GO-PSi hybrid device is covalently conjugated to a peptide nucleic acid (PNA) sequence able to recognize the DNA mutation associated with Sudden Death Syndrome (SDS). The device is a multiparametric optical biosensor whose operating mechanism is based on the changes of PSi reflectance and GO photoluminescence.

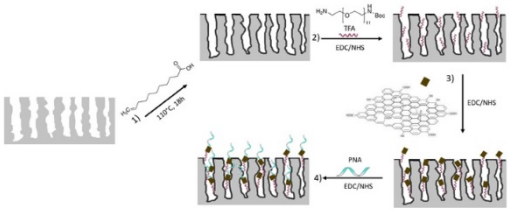

Figure 1. Functionalization scheme. *Reaction 1*: hydrosilylation process of PSi using undecylenic acid. *Reaction 2*: PEGylation process of PSi by EDC/NHS and deprotection of NH-BOC by TFA treatment. *Reaction 3*: Immobilization of GO by EDC/NHS. *Reaction 4:* immobilization of PNA probe.

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Droplet-based microfluidic system with integrated multi-sensor technology for microtoxicological studies

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Toxicology is concerned with the response of organisms to chemical stimulus. The use of microfluidic droplets to implement highly-resolved dose-response screening opens a new era of micro-toxicological studies. Here, an automated compact droplet-based microfluidic system with integrated microflow-though multi-sensor was developed for dynamic cultivation and fast generation of highly-resolved dose-response functions.
To evaluate the toxicity a time-resolved miniaturized optical double endpoint detection unit using a microflow-through fluorimeter and photometer and micro flow-through impedance spectroscopy were used. The optical sensors were applied to distinguish the changes on the endogenous cellular fluorescence signal and the cell density. The impedance measurement provides information about metabolic activity, as the growth of bacteria affects the resistive characteristics of the medium due to the metabolic conversion of nutrients into metabolites. The growth response of the bacteria *Chromobacterium vaccinii* cultivated inside 500 nL droplet against dosages of $CuSO_4$ was measured using the miniaturized sensor system.
This work shows that a micro flow-through optical- and electrochemical impedance sensing unit is very useful for highly time- and concentration-resolved dose response studies that analyze the growth and metabolic behavior kinetics of bacteria. This facilitates high-throughput toxicological studies and the estimation of the effective concentration necessary to impede bacterial growth and supplies detailed information on the effect of sub-lethal effector concentrations in a simple, miniaturized and robust platform.

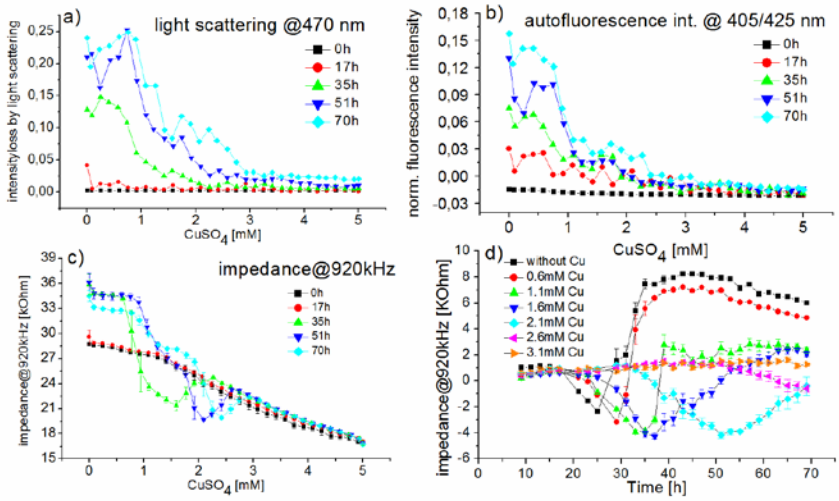

Figure 1. Time and Concentration-resolved dose response of *C. vaccinii* against $CuSO_4$. (a) autofluorescence intensity (b) photometric signal (c) Impedance modulus at 920 kHz. (d) Normalized impedance modulus at 920 kHz as a function of time.

Development and characterisation of a new fluorescence sensor for online monitoring of bioprocesses

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To meet the high regulatory and quality requirements of the food and drug administration (FDA) for the production of pharmaceuticals, the pharmaceutical and biotechnological companies are encouraged to set up innovative tools to better understand their processes and to ensure batch-to-batch reproducibility. Therefore on-line measurements of bioprocesses are becoming increasingly important.
Fluorescence spectroscopy is a highly sensitive and non-invasive technique for identification of characteristic process states or for the on-line monitoring of substrate and product concentrations. Nevertheless fluorescence sensors are mainly used in academic studies and aren´t well implemented in monitoring of industrial productions. In this work, we present a newly developed function model of a fluorescence sensor that facilitates the analysis of fluorescence measurements. The setup of the sensor was miniaturised and realised without any moveable part to be robust enough for application in technical environments. It was constructed to measure only the three most important biologic fluorophores (tryptophan, NADH and FAD/FMN), which results in a significant data reduction compared to a conventional 2D-Fluorescence spectrometer. The sensor performance was evaluated by calibration curves and selectivity tests. The measuring ranges were determined as 0.5–50 µmol*L^{-1} for NADH and 0.0025–7.5 µmol*L^{-1} for BSA and riboflavin. Online monitoring of batch cultivations of wild-type *Escherichia coli* K1 in a 10L bioreactor scale were performed. The data sets were analysed using principal component analysis and partial least square regression. The recorded fluorescence data were successfully used to predict the biomass of an independent cultivation (RMSEP 4.6%). Further possible applications, e.g. the monitoring of eukaryotic cultivations and enzymatic reactions, are currently under investigation.

Figure 1. a) Fluorescence data of an *E. Coli* K1 cultivation in comparison to offline data with a commercially available 2D-Fluorescence spectrometer. (b) Predicted biomass by PLS model and offline biomass measurements.

Collagenase biosensor based on degradation of peptide cross-linked poly(ethylene glycol) hydrogel films using a quartz crystal microbalance

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Collagenase levels were previously reported to be higher in synovial fluid of rheumatoid arthritis (RA) and osteoarthritis (OS) patients, and correlated with inflammation in acute RA and chronic pathology of OS [1]. Detection of this class of enzyme using the degradable hydrogel film format is promising as a point-of-care device for disease monitoring. Poly(ethylene glycol) (PEG) is a hydrophilic polymer and has been reported to have anti-fouling properties [2]. The use of dendritic architecture of PEG as biosensor material is promising as it has more active sites for cross-linking and low non-specific binding. The cross-linking of 4-arm PEG norbornene with specific peptide sequences is likely to offer good sensor responses and high specificity based on the peptide cross-linker used. In this study, the peptide cross-linker, GCRDVPMS↓MRGGDRCG was used to cross-link 4-arm PEG norbornene (MW 20,000) to detect collagenase. The hydrogel was deposited on gold-coated quartz crystals and their degradation in the presence of collagenase was monitored using a Quartz Crystal Microbalance (QCM). The biosensor was shown to respond to concentrations between 2 nM to 2000 nM with a lower detection limit of 2 nM.

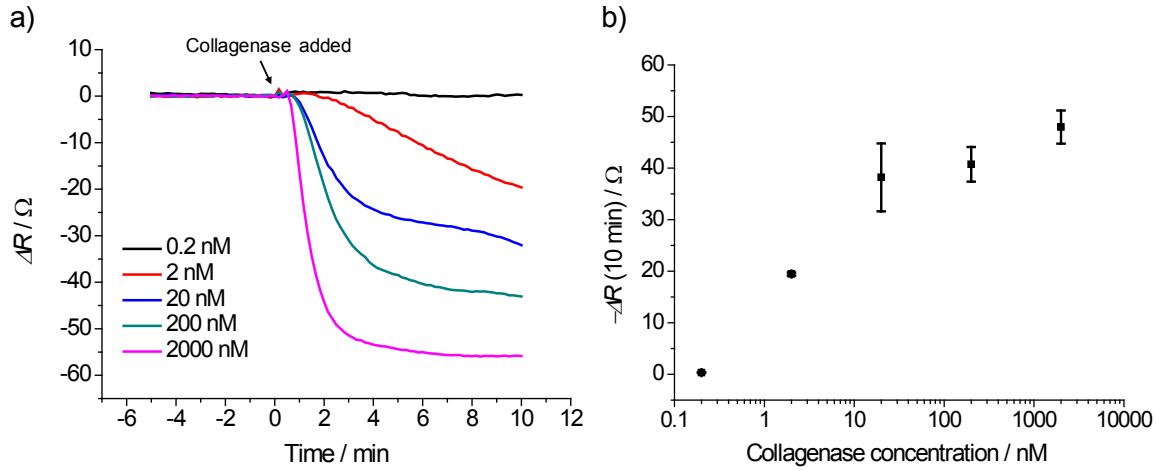

Figure 1. (a) QCM response, *ΔR* of 100% GCRDVPMS↓MRGGDRCG cross-linked PEG after addition of 0.2 nM, 2 nM, 20 nM, 200 nM and 2000 nM at t = 0. (b) Concentration dependence of the QCM response, *ΔR*, measured for 10 minutes after the addition of collagenase.

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Opto-electrical carbon fiber for real-time optical stimulation and electrochemical detection of dopamine exocytosis

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Parkinson's disease (PD) is characterized by the degeneration of dopaminergic neurons in the midbrain. The most effective therapy for the treatment of PD is levodopa. However, it leads to the development of motor complications [1]. Continuous delivery of dopamine has shown to reduce the risks associated with chronic motor complications [2]. In this work we describe a leaky opto-electrical carbon fiber as a potential neural implant for continuous supply and real-time modulation of dopamine in striatum using stem cells, optogenetics and electrochemistry. Pyrolysis of the protective polymer buffer layer on a commercial optical fiber leads to the formation of a pyrolytic carbon layer around the optical fiber; a carbon material that has proved to be biocompatible and to enhance the differentiation of human neural stem cells (hNSCs) into dopaminergic neurons [3]. The carbon coated optical fiber was furthermore made leaky by introducing pores in the carbon layer using laser micro-ablation. As seen in Figure 1a, the guided light through the carbon coated optical fiber leaks out from the pores. Through optogenetic modification of hNSCs with Channelrhodopsin (ChR-2) (a light sensitive ion channel), we were able to generate light controllable cells. Figure 1b shows optogenetically modified hNSCs cultured and differentiated on the leaky opto-electrical carbon fiber *in vitro*. Figure 1c shows the initial amperometric results obtained, first from chemical stimulation (potassium-induced depolarization), following optical stimulation using blue light. The peak heights from optical stimulation is a direct measure of dopamine exocytosis from the cells. This further proves the presence of a large number of optogenetically modified dopaminergic neurons. Moreover, only optogenetically modified cells respond to optical stimulation leading to smaller current peaks compared to chemical stimulation that activates every dopaminergic neuron in the population. These initial results provide the first proof of concept for real-time optical stimulation and electrochemical detection of dopamine exocytosis from dopaminergic neurons cultured on an opto-electrical carbon fiber. Further studies of immunocytochemistry and extensive electrochemistry are needed to quantitatively asses the dopamine release and its potential use for PD therapy.

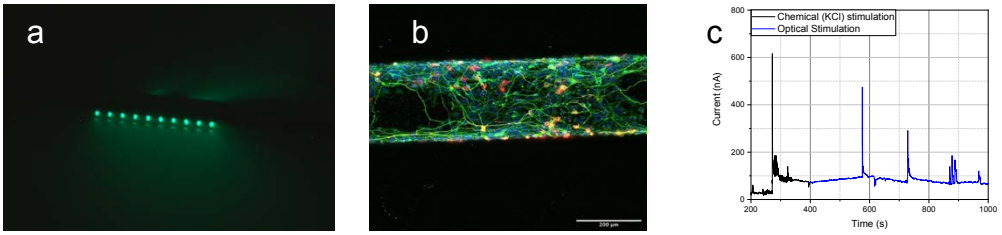

Figure 1. a) Light leaking from carbon optical fiber illuminates surrounding fluorescent nanobeads. b) Immunohistochemistry of optogenetically modified hNSCs cultured on the fiber (Red: Tyrosine hydroxylase, Green: beta-tubulin, Blue: Nuclei). c) Amperometric results showing chemical and optical stimulation.

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Polydopamine: a smart polymer for bio-sensing

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Despite the several important roles in biology, nowadays the vast research outcomes from dopamine studies is due to its chemical reactivity. This self-assembling functional monomer has been adopted for surface coating at nanometric scale as polydopamine (PDA) [1], and reduction potential of catechol moiety has been employed to produce metal nanoparticles in situ without other reducing agents or metallic seeds [2]. Recently, we have devoted our study to both coating and redox proprieties of this biocompatible nanomaterial [3-5]. In detail, we have shown that dopamine can be used to create a molecularly imprinted polymer (MIP); a robust and inexpensive alternative to naturally occurring receptors; via dopamine-analyte co-polymerization, which generates cavities complementary to the original molecule in terms of dimension, shape, and noncovalent interactions. We have applied this strategy to describe the first example of epitope-imprinted biosensor for cardiac biomarker troponin T via SPR on PDA-modified gold surface [3]. Moreover, we have reported for the first time that the growth of gold plasmonic nanoparticles (AuNPs) onto optically transparent PDA surface of disposable UV–Vis cuvettes is modulated by polymer thickness [4]. The absorbance displayed by these cuvettes change in intensity in dependence of the filling medium but presents a fixed plasmon wavelength maximum. We have used this peculiar responsiveness to evaluate the total amount of fermenting sugars in beer wort [4]. Finally, we have studied by means of UV-vis spectroscopy the catalytic activity of AuNPs spontaneously grown on PDA by using the reduction of nitrophenol to aminophenol in presence of $NaBH_4$ as catalytic model reaction. We discovered the key role of AuNPs in conferring chemical resistance to the nanocomposite material during the catalytic reaction, and we achieved the effective quantification of this pesticide and fungicide degradation product in human urine [5].

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A novel POCT optical device for the detection of immunosuppressants in transplanted patients

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Over the past 20 years, there has been a growing demand from physicians for devices able to carry out rapid and reliable measurements of chemical and biochemical parameters near the patient's bed. These devices should allow the rapid formulation of a reliable diagnosis and/or the quick choice of the most appropriate therapy, avoiding the use of centralized laboratory analyses, which implies to wait for the results a period ranging from a few hours up to, sometimes, a whole day. These are the so-called Point of Care Testing (POCT) devices that are becoming essential to the analysis of all those pathologies, where a rapid medical intervention is crucial to patient life. Optical sensing and optical biochips can definitely play an important role in the development of POCT instrumentation. Due to their miniaturization, low cost and large-scale potential automation, their use can lead to a more efficient analysis than the equipment currently available in the clinical laboratories. A novel point of care testing (POCT) optical device for the detection of immunosuppressants in transplanted patients was designed and tested, with the body interface constituted by an intravascular microdialysis catheter (MicroEye®), which provides the dialysate as clinical sample. The work was undertaken in the framework of the EU project NANODEM (NANOphotonic DEvice for Multiple therapeutic drug monitoring). The benefit of this device will be an optimized dosage of the therapeutic drugs to support patient management in a clinical environment. In particular, the system will accurately measure the patient blood drug free fraction, which is considered the active fraction in terms of both drug effect and toxicity. In order to reach the low limit of detection required by the clinicians and enable the detection of the therapeutic *drug free fraction*, a heterogeneous binding inhibition immunoassay has been developed with the use of antibody-coated fluorescent magnetic nanoparticles. Calibration curves for cyclosporine A (CyA) and mycophenolic acid (MPA) in dialysis perfusate (20% Lipofundin) were obtained with limit of detection for CyA and MPA of 0.48 ng/mL and 0.79 ng/mL, respectively. In addition, real clinical Lipofundin-based microdialysate samples, each containing CyA and MPA, were tested and results were compared with a novel liquid chromatography–tandem mass spectrometry (LC-MS/MS) method that was developed within the course of the NANODEM project at the Institute of Clinical Chemistry and Pathobiochemistry, Klinikum rechts der Isar der TU München.

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Interaction of DNA with small molecules studied by millisecond-resolved EQCM and voltammetric isotope effects

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This contribution reports on our latest research regarding redox-switching of the viscoelasticity of DNA layers observed on the millisecond time scale [1], as well as very large deuterium isotope effects observed in voltammetric measurements of certain redox-active small molecules that travel through the DNA layer on gold electrodes. To the best of our knowledge, DNA-SAM conjugated with hexammine cobalt(III) (CoHex) shows the largest isotope effect that has been reported so far (k_H/k_D = 2400) [2]. Hexammine ruthenium(III) (RuHex) does not show this isotope effect. We discuss a related thin-layer cell model [3] that we have used to explain the observed voltammetric behavior. RuHex has been described to cover the negatively charged DNA strand and provide considerable conductivity. Therefore, the most relevant difference between CoHex and RuHex may be that in order to transfer electrons, the former has to enter the DNA layer, while the latter does not. This situation is illustrated in Fig. 1A&B.
Models to describe the mechanisms of very large kinetic isotope effects of up to 730 are based on hydrogen tunneling [4]. We also ponder alternatives considering entropic effects of chelate formation and recently discovered water superstructures around DNA strands.
Both the millisecond-resolved EQCM and the voltammetric deuterium isotope effects may provide new opportunities to investigate interactions of small molecules with DNA, which could be important in drug screening and studies of DNA damage by toxic chemicals.

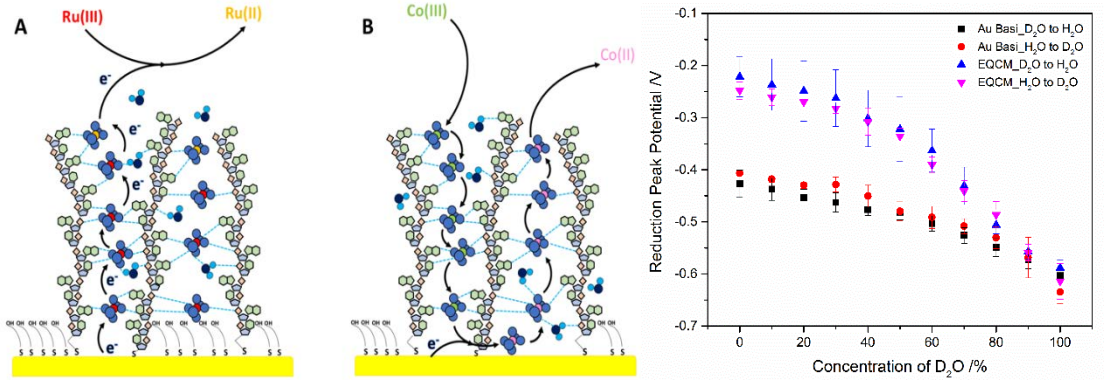

Figure 1. **Left:** charge transfer mechanisms in ssDNA-SAMs conjugated with hexammine metal complexes at reductive potentials: A) electron hopping enabled by RuHex and B) diffusion of redox-active CoHex through the SAM. **Right:** voltammetric isotope effect upon CoHex reduction peak potential [2].

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Micro-cavity in-line Mach-Zehnder interferometer for small-volume label-free biosensing: concept verification by thin Al_2O_3 film deposition

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This work presents for the first time the capability for monitoring growth of a bio-layer using a microcavity in-line Mach-Zehnder interferometer (μIMZI). Due to high sensitivity and capability for investigating sub-nanoliter volumes of liquids, the μIMZI is a perfect solution for medical diagnostics applications. The sensor response to thin high-refractive-index (high-RI) aluminum oxide (Al_2O_3) layer deposition. Next, obtained results were compared to the exposition of the functionalized μIMZI to different concentrations of alive bacteria.
A cylindrical hole with diameter *d* = 54 μm was micromachined with a femtosecond laser in a single-mode optical fiber forming μIMZI sensing structure as described in [1]. A set of μIMZIs was coated by atomic layer deposition (ALD) with a high-RI (Al_2O_3) and followed by slow chemical etching using 10 mM NaOH [2]. Change in thickness of the Al_2O_3 could be seen by monitoring μIMZI's spectral response. Next, the inner surface of another micro-cavity was functionalized, incubated with bacteriophages, immersed in different concentrations of live bacteria solution, and compared to changes in Al_2O_3 layer thickness.
At the beginning of the etching, the transmission minimum shifts towards longer wavelengths (Fig. 2 a)). When the thickness of the film decreases, a shift towards shorter wavelengths is observed. Response to bacterial biofilm formation (Fig. 2 b)) shows a good match with the response to the change in Al_2O_3 film thickness, i.e., the sensor responded to thicker layers comparably as to the higher concentration of bacteria. It is worth noting that the biofilm growth is inverse to the Al_2O_3 etching, what was indicated by arrows on Figs 2 a) and 2 b).

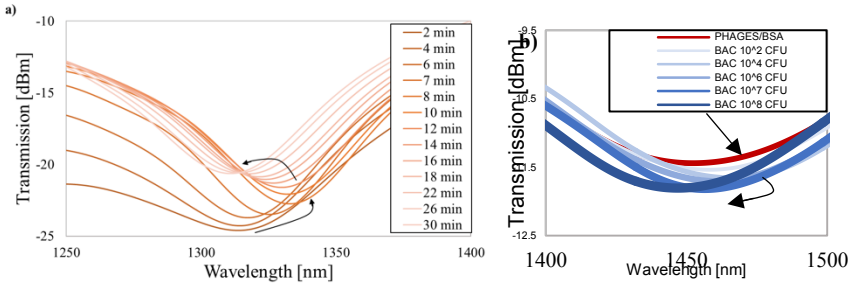

Fig. 2 a) The evolution of the optical spectrum with Al_2O_3 film etching process, recorded for a water-filled cavity. b) The optical response after incubation with different concentrations of bacteria and extensive washing.

We have successfully fabricated highly sensitive μIMZI and using Al_2O_3 film for the first time simulated the biofilm formation. The response to changes in the film thickness was compared to the response to the different concentration of alive bacteria. The results prove the capability of the μIMZI for label-free biosensing applications.

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Cancer diagnostics using glycan recognition by the electrochemical biosensors with design controlled at nanoscale

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Analysis of glycans (complex carbohydrates attached to protein or lipids) is increasingly important for disease diagnostics including various types of cancer. The main reason behind this statement is the fact that genomic/proteomic profiles cannot be applied to fully understand many pathological processes. Glycans are part of 70% of intracellular proteins with 80% of glycan present on membrane proteins making every single cell in our body heavily glycosylated. Thus, glycoproteins can be effectively applied as disease biomarkers. Traditional and novel sophisticated instrumental tools based on mass spectrometry, chromatography, electrophoresis can be quite time consuming, expensive and not sensitive enough to analyze glycans with high performance [1].
This is why novel and sophisticated bioanalytical approaches are needed for advancements in the field of glycomics and diagnostics [2]. In this contribution we will describe an alternative to mass spectrometry-based analysis of glycans by the use of lectins (glycan-binding proteins) for development of various types of electrochemical biosensors. Application of lectins allows to analyze glycans of the glycoproteins directly on an intact protein backbone. There is no need to release glycans from the proteins, what significantly simplifies assay procedure. The electrochemical biosensors constructed with interfacial layers controlled at nanoscale can detect glycans down to a single molecule level (i.e. aM level) and some of the approaches developed by us are still the most sensitive for particular analytes [2]. The biosensors were extensively optimized to resist non-specific interactions allowing to work with complex samples such as human serum from patients having various cancer diseases. The potential of electrochemistry in glycomics and biomedicine will be presented here and was recently reviewed by us in Chemical Reviews [3]. In more details electrochemical approaches for diagnostics of prostate and breast cancer will be provided using lectin-based glycoprofiling of prostate-specific antigen (PSA) for diagnostics of prostate cancer [4] and human epidermal growth factor receptor 2 (HER 2) for diagnostics of breast cancer [5].
The last part of the presentation will show application of glycan biosensors with an immobilized Tn antigen to detect antibodies. Two different approaches were applied for immobilization of a small glycan either directly to a modified surface (2D biosensor) or to a layer of human serum albumin formed on the surface (3D biosensor) and these two approaches are compared [6].

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4D printing: chemiluminescent printed biosensors

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Additive manufacturing provides a new way to develop smart functional devices thanks to a complexification of shapes and materials which cannot be found in traditional manufacturing processes. By combining progresses in 3D printing technologies and materials developments, biotechnologists find in this new field a cornucopia of possibilities to develop innovative tools and concepts for diagnosis, microfluidic, biochip development and point-of-care testing. Our group have been working on this approach for the last 3 years, digging deeply into the 3D printing technologies and ink formulation to achieve complex 3D objects with new capabilities. Such objects, called 4D printed objects can have several biochemical properties like catalysis, biomolecular recognition and biological interactions abilities.
Polyethylene-glycol (PEG) photopolymerized hydrogels and their intrinsically structure demonstrated several advantages for that purpose, such as porous architectures with highly interconnected pores, biocompatibility but also compatibility with 3D printing processes and formulation flexibility, easily adapted each application. Using these printable hydrogels, we had demonstrated that Digital Light Processing (DLP) 3D printing was a possible way to achieve millimetre size chemiluminescent biosensors through the incorporation of peroxidase and glucose oxidase during the printing process [1, 2]. Motivated by the size reduction of the obtained objects down to the micron scale and the study of the expected positive impact of this size reduction on the obtained chemiluminescent signals, we have been working on the printing of these PEG hydrogels through 2-photon polymerization (2PP) [3]. This 3D laser writing technology is indeed able to print a large variety of photopolymers with submicronic resolutions, typically down to 250 nm, thanks to the use of non-linear optical properties. Examples of successful prints of these micron-size chemiluminescent biosensors will be presented together with their performances. A special attention will be given to the analysis of the printed 4D object size and shape on the obtained analytical properties.

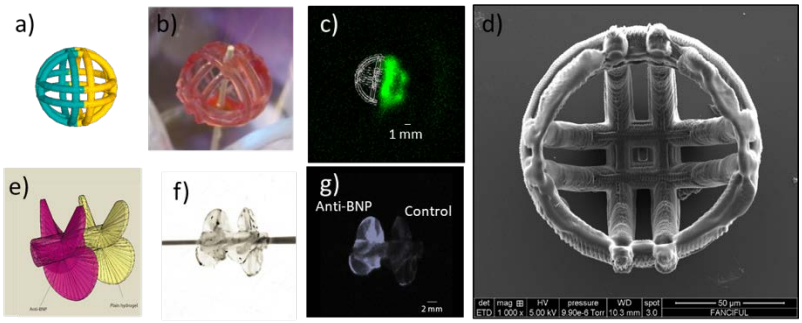

Figure 1. Examples of printed smart materials. a) STL file of fanciful ball enzymatic biosensor b) centimeter size printed using DLP technology, and c) corresponding chimiluminescent signal d) micron size fabricated using 2PP printing (SEM image). e), f) and f) blade shape immunosensor.

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Integrated electrochemical immunosensor for sensitive determination of IL-13 receptor α2 in paraffined-embedded tumor tissues using MWCNTs/GQDs hybrid nanocarriers

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Currently, accurate determination of emerging biomarkers of metastatic cancer in minimally invasive samples using simple and rapid methods, compatible with application at different settings, is highly demanded for improving cancer outcomes. Many tumors are characterized by the overproduction of a range of immunosuppressive cells and cytokines. IL-13 is a pleiotropic immune regulatory cytokine sharing many properties with IL-4, recent findings have demonstrated that highly metastatic cells expressed increased levels of the immunosuppressive cytokines interleukin (IL)-4 and IL-13 in addition to increased surface expression of the high affinity IL-13 receptor α2 (IL-13Rα2), suggesting that IL-13Rα2 mediates IL-13 effects in colorectal cancer cells [1] [2].
In order to overcome the limitations of available methods for the determination of IL-13Rα2 (ELISA and dot blot analysis) which are laborious, time-consuming and only applicable in centralized settings, the establishment of fast, simple and cost-effective methods for the sensitive and accurate determination of IL-13Rα2 in clinically relevant samples such as metastatic cancer cells or tissues will be of great relevance for early diagnosis of metastatic processes.
Within this context electrochemical immunosensors are particularly attractive because of their low cost, compatibility with miniaturization and high sensitivity particularly in connection with the use of nanomaterials as electrode surface modifiers, as nanocarriers of enzyme labels and as electrochemical nanotracers.
In this work we describe the development of a electrochemical sandwich immunosensor for determination of the IL-13Rα2 based on the use of an hybrid nanomaterial composed of multiwalled carbon nanotubes (MWCNTs) and graphene quantum dots (GQDs) as nanocarriers of detector antibody and HRP molecules. The biotinylated capture antibody is immobilized onto streptavidin-modified screen-printed electrodes through grafting with p-aminobezoic acid (p-ABA) and further activation using EDC/Sulfo-NHS chemistry. Using amperometric detection with the system H_2O_2/hydroquinone (HQ), the immunosensing platform exhibits a linear calibration plot ranging from 2.7 and 10 ng mL^{-1} IL-13Rα2, a LOD of 0.8 ng mL^{-1}, an excellent selectivity and successful applicability to the determination of the target receptor directly in small amounts of raw cellular lysates and extracts from paraffin-embedded tumor tissues.

The financial support of projects: Ministry of Economy and Competitivity CTQ2015-70023-R and CTQ2015-64402-C2-1-R (MINECO Research Projects) and S2013/MT-3029 (NANOAVANSENS Program from the Comunidad de Madrid) are gratefully acknowledged. R.B. acknowledges the financial support of the PI17CIII/00045 grant from the AES-ISCIII program.

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Plasmonically amplified fluorescence biosensors with scaled up produced chips

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Metallic nanostructures with tailored plasmonic characteristics rapidly find their application in the amplification of weak optical spectroscopy signals including Raman scattering, infrared absorption, and fluorescence [1]. Among these, fluorescence represents (arguably) mostly spread method for the readout of heterogeneous assays with ligand biomolecules attached to solid surface of a sensor chip. Plasmonic nanostructures with tuned spectral characteristics allow for the enhancement of fluorescence signal emitted from fluorophore labels by a factor up to 10^3 [2]. However, these structures are typically prepared by techniques such as electron beam lithography, which is not suitable for cost efficient scaled up production of chips and are not compatible with regular fluorescence readers. We report on the implementation of UV-nanoimprint lithography and roll-to-roll plasmonic structures that provide the enhancement of detected fluorescence intensity of 300 and which translates to the improvement of limit of detection by similar factor when applied to immunoassays. These structures were deployed to bottom of sensing wells in a microtiter plate format and to disposable polymer sensor chips with arrays of sensing spots and integrated microfluidic device (see Figure.1 below). For a developed two-resonant structure tuned for the amplification of low quantum yield emitter Alexa Fluor 790, strong plasmonic amplification occurring in the close proximity to the sensor surface allows measuring kinetics of affinity binding and reach femtomolar limit of detection.

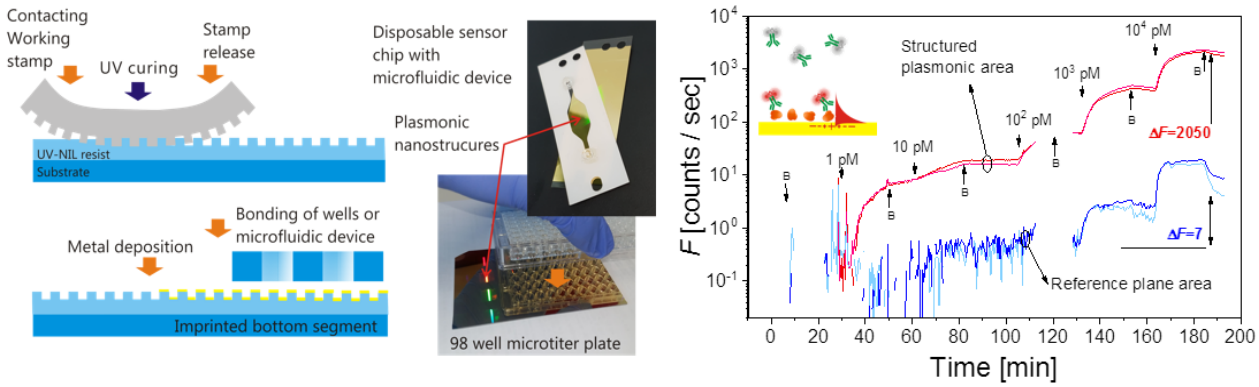

Figure 1. Schematics of UV-NIL preparation of plasmonic sensor chips (left) and example of enhanced readout of fluorescence immunoassay (right).

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An enzymatic oxygen scavenger for oxidase-based bioelectrochemical processes - Case study of an oxygen-interference free glucose biosensor

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Virtually all second-generation oxidase-based biosensor systems suffer from O_2 interference. O_2 from ambient air leads to competitive oxidation reactions with the redox mediator or with the oxidase resulting in a lower catalytic current for analyte oxidation (Figure 1) and thus in erroneous sensor readings.
We developed an O_2 scavenger system based on short-chain primary alcohols as reducing agents and an alcohol oxidase as the catalyst that specifically suppresses this interfering pathway by selectively removing O_2 while leaving the electron transfer chain for current generation unaffected. As case study we present a glucose sensor test strip which incorporates this biochemical method of O_2 removal. This system is able to efficiently remove O_2 in small volume sensors which are compatible with point-of-use applications and thus led to reliable glucose read-outs which is critical for diabetic patients. The method is in principle applicable to any measurements suffering from O_2 interferences which is a quasi-universal issue in point-of-use electrochemical sensing.

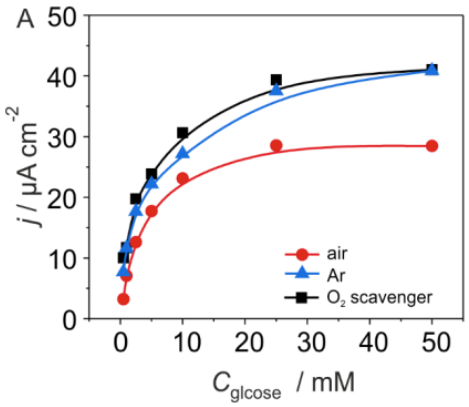

Figure 1. Calibration curves of a glucose biosensor at different experimental conditions (under air, Ar and oxygen scavenger). All experiments were performed in phosphate buffer (100 mM, pH 7.5). Fc(MeOH)2: 100 µM, GOx: 15 U/mL.

A disposable multi-drug test based on a dye displacement assay in molecularly imprinted polymers

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Over the past few decades authorities have been struggling to keep up with the development and spread of so-called new psychoactive substances (NPS). The formulation of NPS changes rapidly to avoid legislation but despite minor changes to a share core structure, they remain every inch as dangerous as their illicit counterparts, leading to drug-related overdose and permanent damage to the central nervous system [1]. To overcome this problem, we have developed a low-cost colour test for the detection of the NPS 2-methoxiphenidine (2-MXP) [2]. The test is based on a competitive binding assay that exploits the specificity of molecularly imprinted polymers (MIPs). Upon optimization of the bulk imprinting protocol, MIPs were extracted and loaded with malachite green. Incubating the dye-loaded MIPs with diarylethylamines will lead to displacement of the dye into the resounding medium, leading to a coloured filtrate upon removal of the MIP particles using a syringe filter. This colour change is absent when the dye-loaded MIPs were incubated with two legal drug compounds and two common adulteries (see Figure 1a). This indicates that it is possible to dissolve an unknown powder and incubate the resulting solution with the dye-loaded MIPs to optically determine the presence of diarylethylamines. The response can be easily quantified by simple absorbance spectroscopy. In order to further illustrate the commercial applicability of the sensor, MIPs were made for three additional illegal drugs: ketamine, amphetamine and phencyclidine (PCP). To enable simultaneous detection of these compounds, a device was constructed with a mutual inlet that is connected to four chambers containing the different MIPs. The device was characterized using the different targets and analogue molecules before benchmarking in street samples. The presence of any of the compounds in an unknown powder will lead to a colour change in the respective chamber outlet which can be picked up by *e.g.* a cell phone camera.

Figure 1. a) SDC test for 2-MXP. When incubating a dye-loaded 2-MXP MIP with two legal pharmacological compounds or two common adulterants, no color change is observed. Incubation with 2-MXP leads to dye displacement and coloring of the filtrate b) schematic illustration of the multi drug sensing device.

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Roll-to-roll imprinting and microarray spotting of biosensors

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Roll-to-roll (R2R) production is applied in many fields ranging from print industry over organic electronics to organic photovoltaics. Whenever the demanded materials can be applied in printing, coating or imprinting processes, the switch to continuous large area polymer, paper or even glass substrates brings a big increase in throughput, decrease of production cost and simplification of substrate handling. Due to the same reasons, this technology is also in focus of recent research on biosensor and lab-on-a-chip technology [1-2].
In this study, a foil based biosensor for chemiluminescence based DNA quick tests is presented (see figure 1a). The sensor microchannels were produced by R2R imprinting (ultraviolet light assisted nano imprint lithography (UV NIL) [3]). This shows the successful transfer of the chip concept from classic injection molding to R2R based production. In addition, R2R produced microstructures for optical signal enhancement where implemented on the bottom side of the chip. With these structures, optical signals which are generated inside of the microfluidic channels can be coupled out of the chip with higher efficiency (reduced losses due to internal reflections). In consequence, the signal strength which can be measured with a detector below the chip is increased. In the presented chip concept, these microstructures need to be produced on thin foil substrates (short distance between chemiluminescence source and detector is essential) and can therefore not be produced in standard production techniques like injection molding. Hence, the introduction of R2R imprinting offers novel opportunities in optical signal harvesting.
In a next step, the implementation of DNA printing of the biosensor is also transferred to a R2R process. A novel R2R Microarray Spotter will be presented, which allows high resolution printing of multiplexed biomolecule arrays on R2R imprinted biosensor microstructures. This technology enables future high throughput production of biosensor chips.

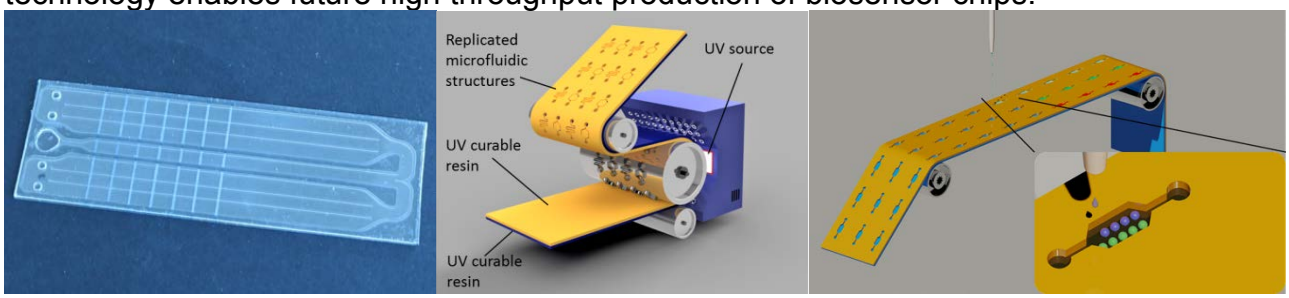

Figure 1. Left: Foil based biosensor with R2R imprinted microchannels. Center: Schematics of R2R imprinting. Right: Schematics of R2R Microarray Spotting

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The affinity between aptamers and low molecular weight compounds: a cautionary tale

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Since their discovery, DNA aptamers were regarded as the turning point for chemical and biochemical sensing for real sample analysis; however up to now their promises are far from be fulfilled. More specifically, aptamers for small molecules pose a challenge both in the selection and characterization step. The lack of a universally accepted and reliable quality control protocol for the characterization of aptamer performances coupled with the observation of inconsistent data sets in literature, prompted us to address the issue comparing different analytical methodologies to validate (or disprove) the binding capabilities of aptamers sequences. We choose three aptamers for ampicillin (β-Lactam antibiotic) which represent a relevant analytical target. The three sequences (AMP 4, AMP 17, AMP 18) reported for the first time by Song et al. [1] and used in many other publications thereof [2], were tested with three different instrumental techniques to assess their Kd and their binding mechanism in homogeneous solutions. Coupling the thermodynamic data obtained with isothermal titration calorimetry (ITC) with the structural information on the binding event given by Native Electron spray Ionization Mass spectrometry (Native ESI-MS) and ^{1}H-NMR it was possible to verify that the three sequences do not show any specific binding with the target ampicillin. Moreover the same set of experiments was repeated with another well-characterized aptamer against cocaine to successfully validate our analytical approach.The colorimetric gold nanoparticles (AuNPs) assay used in the original paper was replicated and the influence of the AuNPs on the binding event was verified, repeating the experiment in presence of AuNPs both with ITC and ^{1}H-NMR. Also in this case the aptamers showed no sign of specific binding to their target. The take-home message is that cautious is advised when selecting and characterizing new aptamers sequence or using reported sequences. A proper validation protocol to verify the binding affinity, as suggested in our approach, should be followed before using the sequences in different applications.

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Pulse deposition of functional polydopamine films

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Polydopamine (PDA), a synthetic eumelanin polymer is highly interesting in biomedically relevant research areas e.g., as adhesive coatings for cell immobilization, as immobilization matrix for enzymes or as biomimetic electron gates for artificial photosynthesis due to its functional groups[1,2]. PDA can either be deposited via a chemical process at basic pH higher than 7.4[3] or by electrochemical deposition such as cyclic voltammetry[4], which allows improved control of the surface morphology of the deposited PDA film. In this contribution, we report the deposition of PDA using pulsed electrochemical deposition techniques, in order to control film uniformity and thickness. In a first set of experiments, microspots of PDA were deposited via scanning electrochemical microscopy (SECM) in direct mode[5] using the substrate as working electrode and the microelectrode as the counter electrode. The deposition process was controlled by a multi-potential step approach and the formed PDA microspots were characterized in respect to the film morphology, film thickness and the electron transfer properties. Furthermore, conductive colloidal AFM-SECM probes[6] with a spherical electrode can be modified with PDA. By applying potential to such modified probes, the redox properties of the polymer can be switched (e.g., quinone/phenolic moieties), which will influence the adhesion properties of the polymer. Such probes are highly suitable for single cell force spectroscopy measurements based on the switchable properties of the polymer film. First results towards such PDA-modified conductive colloidal probes for cell measurements will be presented.

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Fuel-responsive allosteric DNA-based aptamers for the transient release of ATP and cocaine

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Allostery is generally considered as a thermodynamic equilibrium phenomenon. In contrast to this, we show here that this mechanism offers a key strategy to rationally design out-of-equilibrium synthetic devices. We demonstrate this by engineering allosteric DNA-based nanodevices for the transient load and release of small organic molecules. To demonstrate the versatility and generality of our approach we have employed two model DNA-based aptamers that bind ATP and cocaine through a target-induced conformational change. We have rationally re-engineered these aptamers so that their affinity towards their specific target is controlled by a DNA sequence acting as an allosteric inhibitor. The use of an enzyme that specifically cleaves the inhibitor only when it is bound to the aptamer generates a transient allosteric control that leads to the temporal release of ATP or cocaine from the aptamers. Our approach confirms how the programmability and predictability of nucleic acids make synthetic DNA/RNA the perfect candidate material to re-engineer synthetic receptors that can undergo chemical fuel-triggered release of different kinds of small molecule cargoes (ATP and cocaine) and to rationally design non-equilibrium systems. Moreover, our study illustrates the potential of transient allosteric regulation as a tool to control the functions of synthetic dissipative devices.

Dielectrophoretic Immobilization of single Biomolecules

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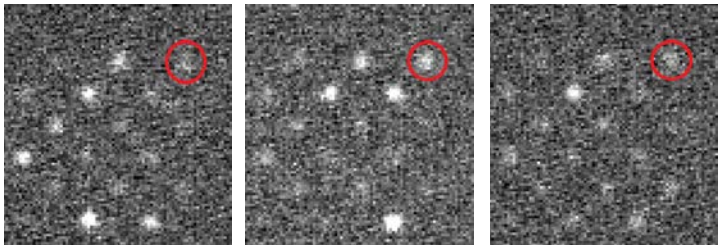
A key element in the construction of a biosensor is the immobilization of the bioreceptor. In common biosensors the bioreceptor molecules are immobilized as many randomly oriented objects on one transducer, leading to a comparatively low ensemble signal. By the use of dielectrophoresis (DEP) for immobilization, the signal could be amplified, as DEP leads to an orientation of biomolecules [1]. DEP is a phenomenon in which a dipole is induced in a polarizable particle by an inhomogeneous (AC) electric field. By the right choice of voltage and frequency, this particle can be moved without damage and can be immobilized [2]. It was shown that enzyme activity and the antibody binding function is preserved after the use of DEP [3,4]. But still, the signal that is produced is an ensemble signal, due to the combined measurement of all analytes. By the immobilization of individual addressable biomolecules, it would be possible to show explicitly where a signal has been generated. Using nanospheres as a model system, it has been shown that by the selection of appropriate dimensions and shapes of electrodes compared to the objects' size an immobilization of single objects can be ensured by DEP [5]. Using different electrode diameters and object sizes, the immobilization of exactly one single particle at each electrode tip is achieved for electrode tip diameters with half the particle size [6]. By the use of a regular microarray consisting of many thousands of vertical silicon- or tungsten-based nano-electrodes it is even possible to make statistical statements on small numbers of objects, as they allow many thousands of experiments in parallel.
These results have been transferred to the successful DEP-immobilization of autofluorescent R-PE proteins as individual biomolecules on nano-electrode arrays. The proof of immobilization and singling is done via fluorescence microscopy in combination with a histogram-method, showing the blinking of several molecules, immobilized as few or singles on the electrodes of the array. In this way the ensemble averaging of the analyte signal can be avoided, but still a statistically large sample size is investigated.



a) b) c)

Figure 1. Blinking of the fluorescence of immobilized single R-PE molecules after a) 0.5s, b) 10s and c) 30s.

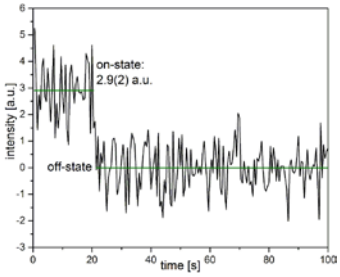


Figure 2. Trajectory of fluorescence over time, showing on- and off state.

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Light-up aptasensors for the detection of biomarkers.

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Some fluorogenic dyes (MG = malachite green, DFHBI = Z-4-(3,5-difluoro-4-hydroxybenzylidene)-2-methyl-1- (2,2,2-trifluoroethyl)-1H-imidazol-5(4H)-one, TO = thiazole orange) and their cognate RNA aptamers have been developed which induce fluorescence upon formation of an aptamer-dye complex. These "light-up" aptamers can be deliberately modified as structure-switching entities. Such bio-molecular switches allow real-time molecular sensing in complex environments.
To this end we rationally converted the MG specific aptamer into light-up Malaswitches taking advantage of kissing interactions *i.e.* Watson-Crick base pairing between two complementary loops of RNA. Using various combinations of RNA-RNA kissing motifs we designed Malaswitches for monitoring the fate of biomarkers. We first applied this strategy to the detection of human let7b miRNA precursors. Sequences complementary to the loop of let7b pre-miRNA were introduced into the apical loop of the previously identified MG aptamer whereas the stem was destabilized to form strong ternary MG-Malaswitch-pre-miR complex whereas the binary MG-Malaswitch one was not stable. The formation of the ternary complex results in a 60 fold fluorescence enhancement. The Malaswitch-MG combination quantitatively and specifically signals the presence of let7b miRNA precursors. Other Malaswitches were engineered allowing the detection of another pre-miR whose production is unbalanced in Duchenne Muscular Dystrophy.
This highly versatile approach might further lead to the development of "light up aptasensors" for the detection of biomolecules or contaminants (pesticides, drug residues, etc…).

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Advances towards the development of an electrophoretic plasmonic nanopore biochip genome sequencer

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Complete sequencing of the human genome is this millen- nium's discovery goal.[1] Rapid advances in DNA sequencing is ushering in the era of personalized genomics to the point that every individual will have access to the complete DNA sequence of their genome for a modest cost Our collaborating group is in the process of developing a novel nucleic acid (DNA, RNA) sequencing technology based on the use of an innovative electrophoretic plasmonic nanopore (EPIGeneS) biochip design system used to control the translocation rate of DNA molecules through a fractal array of plasmonic nanopores to enable massively parallel optical recognition DNA sequencing. The EPIGeneS biochip uses nanofluidic conduits to transport geometrically-conformed DNA single molecules though a fractal nanopore "trapping" barrier structure composed of silica nanopar- ticles positioned within the fluid delivery path of the chip's nanofluidic channels. Electrophoretic trans- port of the DNA molecules through the barrier's nanometer-scaled pores enables the translocation control of DNA single molecules facilitating the Raman spectroscopy detection and measurement of the DNA bases' spectral signatures as they traverse within the fractal-dimensionality nanopore barrier structures. To enhance the spectroscopy detection and measurement of the DNA nucleobase signatures, the EPIGeneS biochip uses plasmonic structures by coating the surface of the barrier silica nanoparticles with metallic layers to enable the use of surface enhanced Raman spectroscopy imaging detection tech- niques. This paper describes preliminary results obtained by our group for the development and usage of the EPIGeneS biochip platform for the investigation of the DNA translocation control through silica nano- pore barrier structures, and the Raman spectroscopy read-out of DNA base signatures during elec- trophoretic transport through the fractal nanopore barrier structures.

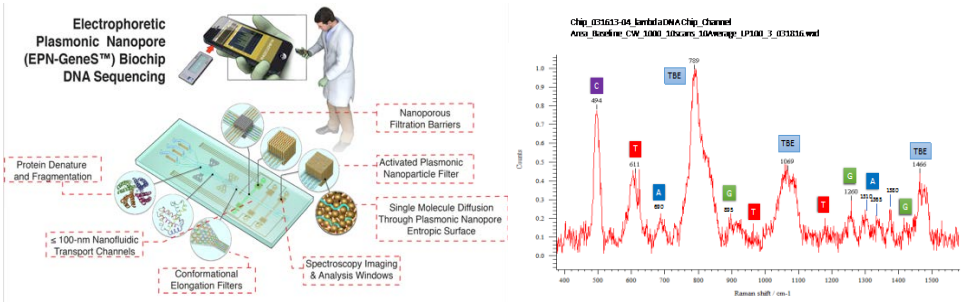

Figure 1. Raman spectra of DNA molecules translocating through nanopore barrier structure of EPIGeneS biochip.

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Does DNA hybridization at surfaces follow the Langmuir model of adsorption?

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DNA hybridization at surfaces is the key reaction in many DNA biosensors. These sensors are used to trace a specific target sequence *via* hybridization with a complementary capture probe DNA strand grafted to a surface. Most commonly the capture probe is chemisorbed to a gold surface by one or more thiol groups. Signal transduction can be achieved *e.g.* by electrochemistry, surface plasmon resonance (SPR), surface plasmon fluorescence spectroscopy (SPFS), ellipsometry, or quartz crystal microbalance (QCM) measurements. For many biosensing applications it is assumed that DNA hybridization at the surfaces follows the *Langmuir* model of adsorption. However, in only a few cases distinct experiments were carried out to prove this assumption. When we developed surface bound molecular beacons (MBs) in order to trace specific oligonucleotide sequences at surfaces *via* SPFS we recognized that after hybridization with target almost none of the target molecules can be released by intense rinsing with the same buffer solution that was used also during the hybridization event.[1-2] Also when we monitored DNA hybridization at surfaces by SPR, SPFS, or QCM we were not able to release a significant amount of target molecules by rinsing with buffer solution.[3-5] If in our experiments DNA hybridization/dehybridization can be described by the *Langmuir* model, the value for k_{off} has to be very low.
To prove whether DNA hybridization can be described by the *Langmuir* model we have carried out a detailed QCM study of the hybridization of target DNA (20 bases) with surface-grafted complementary capture probe DNA. As a result we will show which conditions must be fulfilled for DNA hybridization/dehybridization to follow the *Langmuir* model.
To show the feasibility of our approach we also investigated the binding/unbinding of the apododecin variant DtE at flavin-terminated DNA monolayers, for which *Langmuir* behavior has been reported, if multi-ligand binding can be avoided.[6-7]

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Gas sensors array equipped with Hairpin DNA traps for food quality and production process evaluation

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Nowadays, the attention given to food quality has increased both for producers and for consumers, stimulating the necessity of rapid quality control from production to market. Electronic noses allow a fast assessment of headspace, a quantitative representation or signature of a gas employing cheap sensors which can be easily functionalized. Thus, this system results easily integrable, even online, in production processes. In the last years, the use of electronic noses is rapidly expanding and there have been notable achievements relevant to the food industry [1]. On the other hand, currently, the biosensors' scene has moved towards the use of nanomaterials as 'sensing platforms' features enhancers, together whit a 'bio-element' replacement by bio-mimetic/bio-inspired strategies (e.g. MIP, aptamers, etc.). In the past decade, DNA was extensively used in sensors design, fabrication, characterization, and application providing new impulses to analytical research [2]. Very few gas sensors proposed DNA as functional material [3]. In order to explore the DNA-based recognition property in gas sensors, a rationally designed array of gas sensors was employed to analyze patterns of volatile organic compounds (VOCs) in food. To achieve this goal, gold nanoparticles (AuNPs) were used to immobilise hairpin DNA and were deposited on quartz crystal microbalances (QCM). The hpDNA employed had loops tailored to bind, with different affinities, various classes of VOCs [4]. The hpDNA loops sequences were firstly studied in-silico, and the relative binding affinities against different VOCs, belonging to relevant chemical classes, were evaluated. Seven different sequences (and then, hpDNAs) were then selected to realize a sensor array. The array was able to discriminate VOCs, on the basis of molecular weight and functional groups. The electronic nose, equipped with these sensors, has been used to monitor the evolution of headspace in carrot samples stored at different temperature. The e-nose ability to monitoring the different aroma patterns generated in the analyzed samples has been confirmed with GC-MS/SPME analysis. In conclusions the e-nose equipped with hpDNA-based sensors appears very promising to analyze food aroma pattern and return, in real-time, information on of foods quality and production process.

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Ion imprinted electrosynthesised polymers for copper(II) detection

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Molecular imprinting describes the process of creating a molecular mould in a polymeric network. Such mould is therefore specific for the target molecule imprinted in the first place. The size of these target molecules spans from large entities (such as whole cells or proteins) to drugs, and even smaller entities (e.g. ions). In the latter case, the artificial material is generally called ion-imprinted polymers (IIPs). Ion imprinting technology leads to the formation of IIP, which present some advantages compared to the traditional MIPs, such as the compatibility with aqueous media [1,2]. Thus, they can effectively be applied to identify, monitor, and remove the target ions in water environment. The IIPs characteristics can be tuned depending on the method of synthesis, which can be carried out either chemically or electrochemically. The formation of the imprinted cavities, which are complementary to the target, is achieved by using an appropriate mixture of functional monomers combined with ligands and crosslinkers. Therefore, the obtained imprinted materials could be successfully employed for different applications, such as resin in SPE techniques (IIP SPE) or as highly sensitive recognition elements in the development of biomimetic sensors (IIP sensors). For the development of a sensor, in general, the electrochemical synthesis of the ion imprinted polymers is preferred, which has the advantage of forming the imprinted polymer directly onto the sensor, thus avoiding time-consuming coupling steps. Nevertheless, few works report the electrochemical synthesis of ion imprinted polymers and their subsequent application as sensor for metal ions detection (for a review see e.g. ref. 3).
In this work, we report the synthesis, characterisation and subsequent application of an electrosynthesised IIPs for the electrochemical detection of copper(II) in water. The synthesis of IIP and non-imprinted polymer (NIP) was carried out by applying a cyclic voltammetry on a surface of screen-printed electrodes in the presence of the functional monomer p-phenylenediamine. Then, the morphology of IIPs film was studied by using surface electron microscopy (SEM) analysis. Square wave anodic stripping voltammetry (SWASV) was performed for the electrochemical detection of copper(II) in water. The limit of detection of the sensor was found to be in nM range of copper (II) concentration. The cross reactivity of the IIP against other interfering ions is also discussed. Furthermore, the shelf and working life of the sensor is reported, by evaluating the responses in a day and after the storage in buffer solution.

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Real-time fluorophore-free optical monitoring of ultrafast DNA amplification for qPCR

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Since its invention in 1983 the polymerase chain reaction (PCR) method of DNA amplification has emerged as a vital tool in clinical diagnostics, biomedical research, forensic identification, environmental sensing and many other areas. The PCR method determines whether there is a match between sample DNA and a short length of template DNA. However, current commercial PCR machines typically take over an hour to deliver a result and are also bulky and power consuming. We have recently demonstrated an efficient laser-heated thermocycling system that can complete 30 thermocycles in clinically relevant sample volumes (20 □L) in under a minute [1, 2]. However, amplification is only one part of the challenge. In order to deliver a useful result at the end of the PCR process it is necessary to determine whether amplification has occurred. Many commercial instruments make use of fluorophores such as SYBR Green which intercalate into double stranded DNA (dsDNA) or may use more sophisticated fluorescently tagged primers to permit measurement in real-time (also referred to as quantitative or qPCR). However, the incorporation of fluorophores and the sensitive photodetectors that are required to measure fluorescence add to cost and complexity of PCR systems. An alternative approach is to employ the natural difference in absorption in the ultraviolet (UV) spectrum between dsDNA, single stranded DNA and isolated nucleotides (hyperchromicity). The latter have a relatively high absorption at 260 nm due to their heterocyclic rings. Bonding of nucleotides into DNA reduces this absorption. In order to test this, the transmittance of a 1mW 260 nm UV LED through a transparent plastic PCR tube was measured during the elongation stage of each cycle of an optically heated PCR process. To prevent UV damage to the DNA, the LED was switched on for only 30 ms per cycle. The results are shown in Figure 1 for a 40 cycle (in 7 minutes) process, demonstrating that the method can reliably detect starting copy numbers as low as 100 copies per 20 □L and so is potentially useful for clinical diagnostics. To our knowledge this is the first demonstration of fluorophore-free ultrafast qPCR.

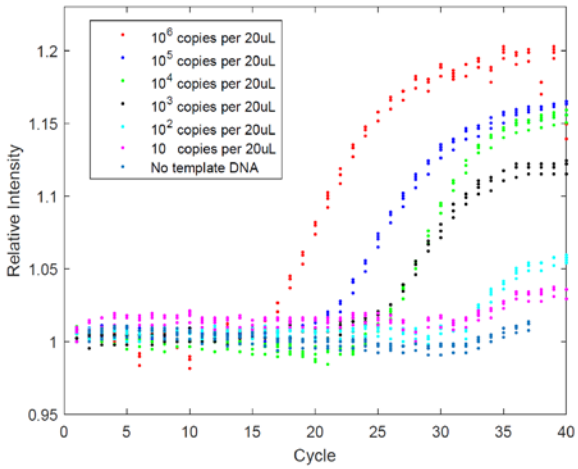

Fig 1. Relative intensity at 260 nm during thermocycling for different starting copy numbers. Total cycle time was 7 minutes.

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Microarray based platform for extracellular vesicles imaging and phenotyping

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Extracellular vesicles (EVs) are cell-derived membranous structures ubiquitously found in all biological fluids that have been only recently unrevealed as fundamental players in intercellular communication. Accordingly, recent years have witnessed an ever increasing interest in EV for their unprecedented potential towards theragnostic applications for many pathologies including cancer, neurological disorders and infectious diseases. Standard approaches to characterize EV are usually either low-throughput, laborious or based on sophisticated equipment not applicable to clinical routines. The major technical challenge in EV analysis for clinical applications is to detect disease-specific EVs in the presence of the many more bio-nanoparticles that are derived from normal cells. Another considerable biological hurdle is the heterogeneity of EVs population which could significantly affect the reproducibility of the analysis. The clinical breakthrough that EVs may represent in healthcare industry will not be realized until new enabling technologies will be developed, combining robustness, accuracy, and selectivity of analysis. Here we present the efforts towards the realization of a complete on-chip integrated platform for isolation, detection and analysis of EVs. The platform is based on immune-based enrichment coupled to EV detection by enhanced contrast in scattering signal from particles captured on a silicon layered microarray using the Interferometric Reflectance Imaging Sensor (IRIS). Arrays are prepared on special silicon substrates coated by functional polymers enabling chemoselective immobilization of DNA, antibody and peptide probes for capturing and phenotyping of vesicles followed by digital counting and fluorescence detection.

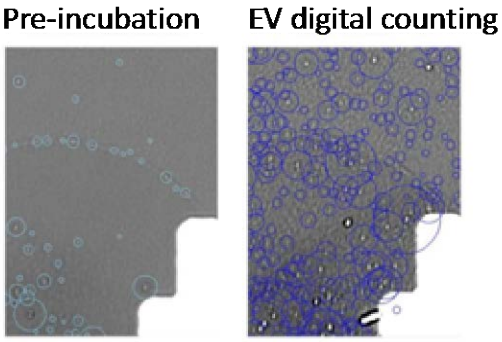

Figure 1. EV capturing and detection on CD9 antibody spot

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[2] This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 766466. INDEX

Highly sensitive immunomagnetic biosensing platform for rapid quantitative detection of thyroid-stimulating hormone

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The development of highly sensitive biosensors based on nanomaterials is an important task for biomedical research, clinical applications, food analysis, pathogen detection, etc. Using magnetic nanoparticles (MNP) as labels of unique physicochemical properties is a promising approach for increasing the sensitivity of quantitative measurements of low concentrations of bioactive agents (proteins, hormones, antibodies). Here we present a rapid high-sensitive biosensing platform based on magnetic nanolabels for quantitative detection in serum samples of a protein marker that indicates pathology of the thyroid gland, namely, thyroid-stimulating hormone (TSH). The platform combines the advantages of lateral flow technology with highly sensitive quantification of MNP over the whole volume of nitrocellulose membranes. The MNP are recorded by their nonlinear magnetization at a combinatorial frequency by a portable reader that offers the detection limit of 0.4 ng of magnetic nanolabels in an extremely wide 7-order linear dynamic range [1]. The optimal antibodies and procedure of MNP conjugation were selected by monitoring of binding kinetics with label-free interferometric biosensors [2] modified for recording of multiplex molecular interactions. The developed platform has demonstrated the limit of TSH detection in human serum of 0.017 mIU/L in a wide dynamic range of more than 3 orders of magnitude (Fig. 1) at the assay time < 30 min. The entire range of possible variations of TSH concentration in human serum is covered. Thus, the platform is promising for diagnostics of both extreme cases of TSH concentrations during hypo- and hyperthyroidism diseases.

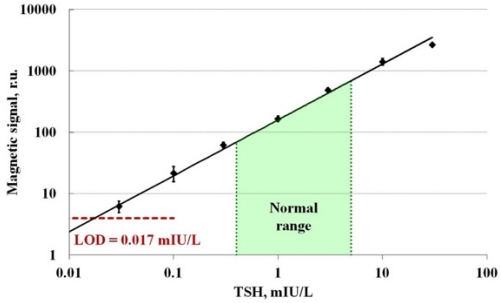

Figure 1. Calibration curve of the biosensor for detection of thyroid-stimulating hormone in human serum.

The developed biosensing platform is the first magnetic lateral flow test with sensitivity of third generation of analytical methods for TSH detection. At the same time, it retains all the advantages of immunochromatography, i.e., rapidity, simplicity and ease of handling. It can be used for on-site *in vitro* diagnostics and monitoring of socially important diseases, of food safety, etc.

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Sensing of biomedical analytes based on resonant absorption of ultrasound in smart hydrogel microstructures

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Stimuli-responsive hydrogels are hydrophilic polymer networks which can be tailored to react to changes in the concentration of the biomedical analyte in their environment by exhibiting a volume-phase transition, e.g. by swelling or shrinking [1,2]. By converting the volume change of these biocompatible smart materials into an electrical signal it is possible to create biomedical sensor devices. However, such devices are not widely employed due to the challenges associated with reliable and sensitive detection of the swelling state. This is especially true for biomedical environments and fully implantable sensors in mind. Medical ultrasound imaging is a commonly employed non-invasive technique to visualize internal structures of the body. We are developing an implantable smart hydrogel sensing platform that utilizes medical ultrasound waves for a non-invasive readout. Corresponding sensing devices consist of a regular arrangement of micromechanical resonators made from smart hydrogel that can be probed with medical ultrasound after implantation. Incident waves are strongly absorbed when their frequency matches a resonance frequency of the resonators (resonant absorption). In contrast, almost no absorption occurs if this condition is not met. Any change in the swelling state of the smart hydrogel, such as one induced by a variation of the analyte concentration, will alter the dimensions of the resonators along with a corresponding shift of their resonance frequency. By using the ultrasound to track this frequency shift, the analyte concentration can be monitored. This functional principle is based on the geometric structure of the hydrogel only. Therefore, a high level of biocompatibility of the implantable device is possible as no other materials, such as micro- and particles as well as other contrast enhancing agents, are present. Furthermore, the principle is compatible with any smart hydrogel ensuring the applicability to many different biomedical analytes. Here we will present simulation results to guide device design, a low-cost molding technique for device fabrication as well as proof-of-principle measurements with corresponding devices.

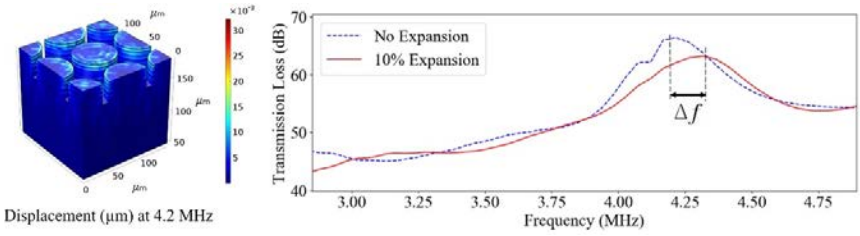

Figure 1. Simulation of the smart hydrogel microresonators with ultrasound readout: (left) finite element model for the simulation with the displacement results at a frequency of 4.2MHz indicated, (right) simulation result showing the shift in the maximum absorption frequency in response to an expansion of the structure.

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Molecularly imprinted polymer for hexakis(2,2'-bithien-5-yl) DNA analog formation aiming at single-nucleotide-polymorphism detection

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We developed a simple, inexpensive, rapid, and label free procedure of single-nucleotide-polymorphism (SNP) detection using robust chemosensors with piezomicrogravimetric, SPR, and capacitive impedimetry (CI) signal transduction [1]. With these chemosensors, we selectively detected a genetically relevant TATAAA (A – adenine, T – thymine) oligodeoxyribonucleotide under FIA conditions. For that, we used a molecularly imprinted polymer (MIP) with electrochemically synthesized probes of hexameric 2,2′-bithien-5-yl DNA analogs discriminating single purine-nucleobase mismatch at room temperature. With DFT modeling, synthetic procedures developed, and ITC quantification, we devised and synthesized A- or T-substituted 2,2′-bithien-5-yl functional monomers capable of Watson-Crick nucleobase pairing with the TATAAA template or its peptide nucleic acid (PNA) analog. Characterized by spectroscopic techniques, molecular cavities in the MIP exposed the ordered nucleobases on the 2,2'-bithien-5-yl polymeric backbone of the TTTATA hexamer probe designed to hybridize the complementary TATAAA template. Hence, an artificial TATAAA promoter was formed in the MIP. The artificial TTTATA hybridized the natural TATAAA with the complex stability constant, $K_s^{\text{TTTATA-TATAAA}} \approx 10^6$ M^{-1}, i.e., as high as that characteristic for longer-chain DNA-PNA hybrids. The CI chemosensor LOD was 5 nM. The MIP chemosensor sensitivity to the TATAAA analyte was by over 4 times higher than that of the non-imprinted polymer (NIP).

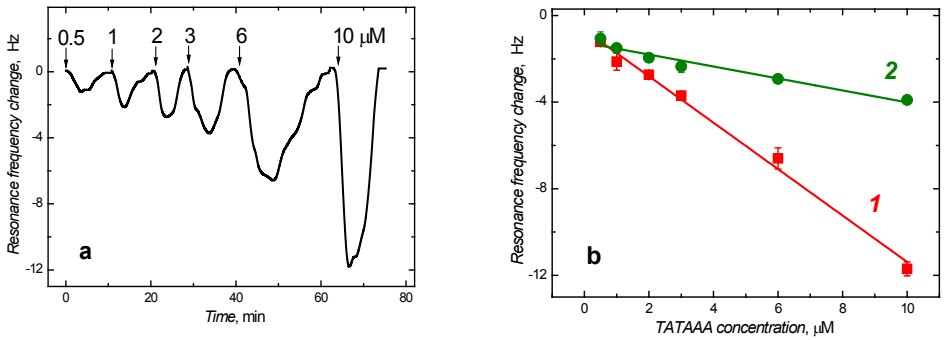

Figure 1. (a) Resonance frequency change with time for repetitive FIA injections (vertical arrows) of TATAAA of the concentration indicated at each peak for the MIP-TATAAA-film-coated Au-QCR. (b) Calibration plots for TATAAA on the (*1*) TATAAA-extracted MIP and (*2*) NIP films. The flow rate of the PBS (pH = 7.4) carrier solution was 30 μL min^{-1}.

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Real-time study of biomolecular coatings by means of Bloch surface wave biosensors

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In the present work, a combined label-free and fluorescence surface optical technique is used to quantify the mass deposited in binary biomolecular coatings. These coatings were constituted by fibronectin (FN), to stimulate endothelialization, and phosphorylcholine (PRC), for its hemocompatibility, which are two properties of relevance for cardiovascular applications [1,2]. One-dimensional photonic crystals sustaining a Bloch surface wave (BSW) are used to characterize different FN/PRC coatings deposited by a combination of adsorption (A) and grafting (G) processes. In particular, the label-free results permitted to quantitatively assess the mass deposited in FN adsorbed (185 ng/cm^2) and grafted (160 ng/cm^2). PRC binding to grafted FN coatings is also quantified, showing a coverage as low as 10 and 12 ng/cm^2 for adsorbed and grafted PRC, respectively [3]. The data obtained by the surface optical technique are complemented by water contact angle and X-ray photoelectron spectroscopy (XPS) analyses. The results are in accordance with those obtained previously by qualitative and semi-quantitative techniques (XPS, time-of-flight secondary ion mass spectrometry) on several substrates (PTFE and stainless steel) [4], making this approach a promising technique for the characterization of binary coatings containing low-molecular weight molecules with relevant properties for biomaterial applications.

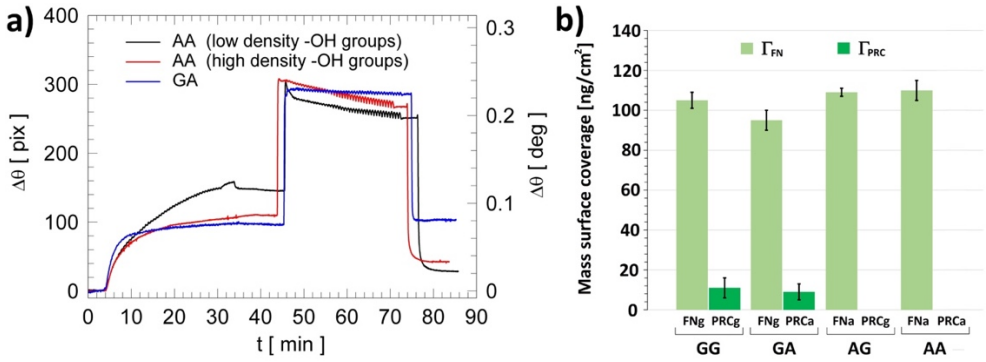

Figure 1. a) Temporal dependency of the BSW resonance recorded during the FN and PRC injection and recirculation for different adsorption (A) and grafting (G) combinations. (b) Mass surface coverages for all coating combinations.

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Electrochemical MIPs for protein sensing using three levels of template

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Specific molecular recognition is a key feature of biological processes. In order to substitute biomacromolecules by “biomimetic recognition elements”, fully synthetic receptors so-called molecularly imprinted polymers (MIPs) have been developed. Molecular imprinting is a method, pioneered by Wulff and Mosbach to create plastic antibodies or plastibodies by the polymerization of functional monomers with or without cross-linkers in the presence of target analyte (template). Subsequent removal of the template leads to the formation of binding cavities which mimics size, shape and functionality of the template[1,2]. Although MIPs for low molecular-weight substances have been successfully prepared, it is still challenging for biomacromolecules like proteins. Here we present MIP-based sensors for proteins on three structural levels of the target protein: (i) An artificial peptide tag as a target: We electrosynthesized a MIP for the Strep-Tag II peptide with the aim of reversible binding of Strep-tagged proteins to the MIP-covered electrode surface. The Cys-extended peptide W S H P Q F E K was chemisorbed on the gold electrode prior to electropolymerization of scopoletin. Binding and electrochemical removal of the Strep-Tag peptide could be demonstrated by using ferricyanide as a redox marker. The current decreased linearly up to 6 nM and reached saturation at 10 nM upon the peptide binding. Furthermore, tagged proteins could also successfully bind to the peptide-imprinted layer. (ii) A domain of the protein as a target: Both the separated domains and the holo-Cytochrome P450 BM3 have been bound prior polymer deposition via an N-terminal engineered his_6-anchor to the electrode surface. Rebinding after template removal was evaluated by quantifying the suppression of the diffusive permeability of the signal for ferricyanide and by the NADH-dependent reduction of cytochrome c by the reductase domain (BMR). The holoenzyme P450 BM3 was ca. 5.5 times more effectively recognized by the film imprinted with the oxidase domain as compared to the BMR-MIP or the non-imprinted polymer (NIP). The his_6-tagged P450 BM3 binds (30 percent) stronger which shows the additive effect of the interaction with the MIP and the binding to the electrode[3]. (i) Holoprotein as a target: A MIP-sensor for the copper containing tyrosinase was prepared by electropolymerizing o-phenylenediamine in the presence of the target protein from mushroom. The template was removed by alkaline solution. The measuring signal was generated either by measuring the formation of the oxidation product by the target enzyme or by evaluation of the permeability of the redox marker ferricyanide. The MIP-sensor has a linear measuring range up to 50 nM of tyrosinase with a limit of detection of 3.97 nM and shows good discrimination towards bovine serum albumin and cytochrome c[4].

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Plasmonic detection of oncogenic DNA in liquid biopsy samples

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Standard clinical protocols for the evaluation of oncogenic DNA mutations are based on tissue biopsy, which consists of sampling cells from the human body by puncturing organs with the use of a small-gauge needle. This procedure constitutes a significant barrier for an easy and frequent monitoring of cancer patients and is subject to limitations including the difficulty in accounting for tumor cells heterogeneity. In liquid biopsy, biological fluids are instead sampled to monitor the level of cancer biomarkers available in biological fluids such as peripheral blood and blood-derived products such as plasma and serum. The detection of nucleic acid biomarkers for cancer diagnosis and patient follow-up based on liquid biopsy represents an important challenge for current biosensing platforms. Most of the biosensors used to detect nucleic acids exploit the enzymatic amplification of sequences to be identified to achieve the needed level of sensitivity. The amplification step introduces constraints and drawbacks in the assays. For instance, PCR suffers from artefacts generated by sample contamination and recombination between homologous regions of DNA.
Efforts have been made to identify innovative PCR-free protocols for DNA detection. Most of such protocols exploit strategies for signal amplification based on the use of enzymes or metallic nanostructures. In particular, gold nanoparticles have been used to achieve the ultrasensitive detection of DNA.
Possibilities offered by nanoparticle-enhanced surface plasmon resonance imaging (SPRI) in the detection of non-amplified human genomic DNA and DNA freely circulating in human blood will be discussed in the context of applications to cancer diagnosis based on liquid biopsy.
The characteristics of functionalized nanoparticles useful for the effective nanoparticle-enhanced SPRI detection of DNA will be discussed with specific attention to streptavidin-coated gold nanoparticles. In particular, emphasis will be given to the role played by biotinylated oligonucleotides in the stabilization/destabilization of the streptavidin-capped nanoparticle dispersions.
The presented work is part of the ULTRAPLACAD Horizon 2020 project (project n. 633937).

Electrochemiluminescent DNA sensor for the detection of specific DNA sequences

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Electrochemiluminescence (ECL) is a powerful transduction technique that has rapidly gained importance as a sensitive and selective transduction technique in analytical science gathering the advantages of the electrochemical sensitivity and the spatial resolution.[1]
Methods for the detection of specific DNA sequences have attracted significant attention due to possible applications in different fields such clinic diagnostics, food safety, environmental pollution analysis, and forensic identification. DNA sensors, the electrochemical equivalent of molecular beacons, appear to be a promising tool to detect oligonucleotides. [2,3]
In this work, an electrochemiluminescent DNA (ECL-DNA) sensor was investigated. The system is comprised of a luminophore attached to a DNA "stemloop" probe by crosslink chemistry, which is immobilized on a gold electrode via self-assembled monolayer chemistry. ECL is generated according to the "oxidative-reduction" strategy using Tripropylamine (TPA) as co-reactant and Ru(bpy)32+ as luminophore. When the DNA target sequence we observed a variation DNA signaling arises due binding-induced changes in the conformation of the stem-loop probe.
Here, effect of probe density on the electrode surface, hybridization signal suppression were investigated.

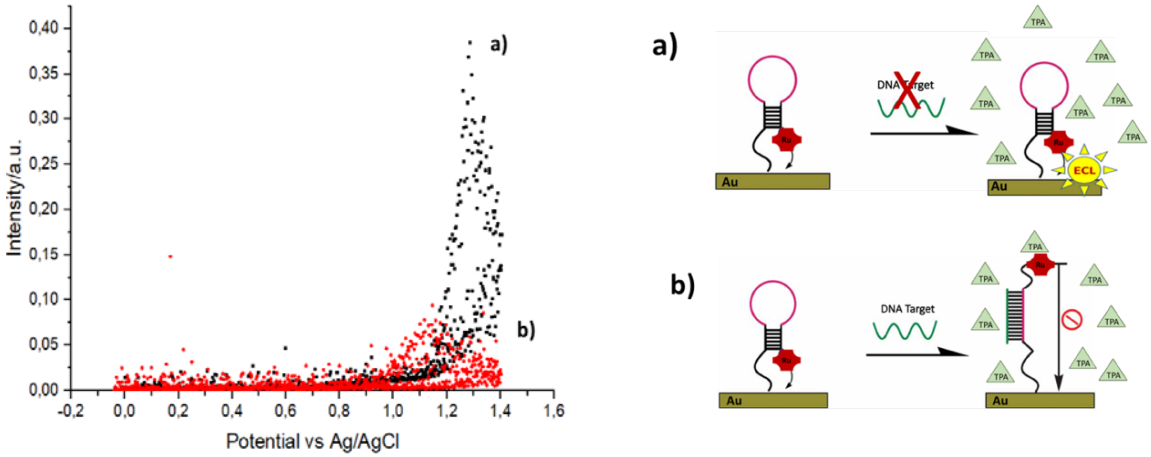

Figure 1. ECL response /left) and schematic rappresentation (right) of the DNA sensor using target sequences a) without and b) with the addition of complementary DNA target.

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Ultra-sensitive aptasensors based upon single-stage opto-plasmonic sensing platform

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To date, there are a small number of generic operating modes for the surface plasmons, these being localised, damped, short-range and long-range [1]. Here we add to that small number of generic operational modes of surface plasmons and significantly increase their sensitivity when combined with aptamers, yielding some of the lowest detection limits for an enzyme (thrombin, molecule size of 37.4 kDa) with a limit of detection of 10 aM [2] within a volume of 2mm^3. Chemical selectivity of the biosensor was assisted using complementary and non-complementary DNA sequences similar to those obtained with thrombin [2]. Furthermore, this opto-plasmonic platform has been used to detect bishpenol A in solution, yielding a limit of detection of 330±70 aM [3], without the use of enhancement techniques and by direct detection. Moreover, bisphenol A has a low molecular weight (228 Da) and is a target usually detectable only by indirect detection strategies. These detection performances relate to ultra-high spectral sensitivities of the opto-plasmonic platform ranging from 10^4 to in excess of 10^5 nm/RIU [4]. These sensitivities can be explained by the enhanced interaction lengths originating from coupled localised infrared surface plasmons on a low dimensional nano-patterned material consisting of an array of gold nano-antennae with a total array length of 2.3 cm, Fig. 1. This structure creates a "superstructure surface plasmon", spanning the entire nano-patterned material yielding the above performances [4]. This is a new sensing paradigm for small molecule detection and also paves the way for developing a portable system for in-situ agricultural measurements capable of retrieving data on a substance of major concern at ultra-low concentrations that is only achieveable from a laboratory environment at present.

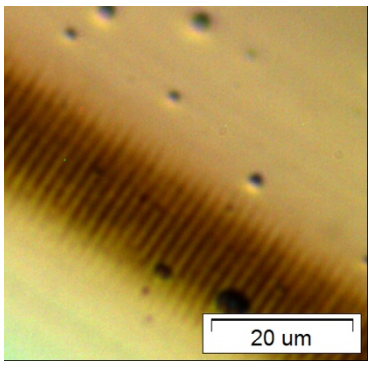

Figure 1. A visible microscope image of opto-plasmonic platform

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POSTER ABSTRACTS

Novel RT-PCR based assay for the fast detection of circRNAs

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Circular RNAs (circRNA) belong to the species of exonic, endogenic and covalently closed RNA molecules which are highly stable and conserved.[1][2][3] Until now, it seems that circRNAs are present in various organisms ranging from archaea to animals.[1] The tissue specific expression and the potential of binding miRNAs and RNA-binding proteins[1] suggests an important regulatory function of circRNAs. Therefore, they are a potential new class of biomarkers for a number of diseases, for example Alzheimer disease and some types of cancer.[2][4] Today`s methods to identify circRNAs are mainly based on types of high-throughput RNA sequencing.[2][3] This is time-consuming, cost-intensive and therefore not an option for a quantitative and qualitative high-throughput screening of circRNAs as biomarkers for clinical research in the future. Microarray technology by contrast is an outstanding tool in the field of bioanalysis and diagnostics for a simultaneous, high-throughput analysis of several thousands of analytes per sample[5] that can be used for a wide range of biomolecules. Unfortunately, today`s microarray based circRNA detection systems are time consuming, containing a lot of preparation steps[4] and are therefore only usable in the field of basic research and clinical studies in addition to RNA sequencing but are not suitable for fast and easy Point-of-Care (PoC) and in-vitro Diagnostic (ivD) applications. Our aim is to develop a novel, inexpensive on-Chip assay for the rapid and easy-to-use detection of circRNAs in different types of samples (cells, blood etc.). This assay will be the basis for new applications in the field of clinical and basic research (PoC and ivD applications) which overcomes the limitations of high-throughput RNA sequencing methods and other existing detection systems. First results, after optimizing the basic assay steps, demonstrate that a good signal to noise ratio is possible with our own labeling and detection system (figure 1). This represents a solid basis for combining all steps into one simple assay for a reliable detection of known circRNAs.

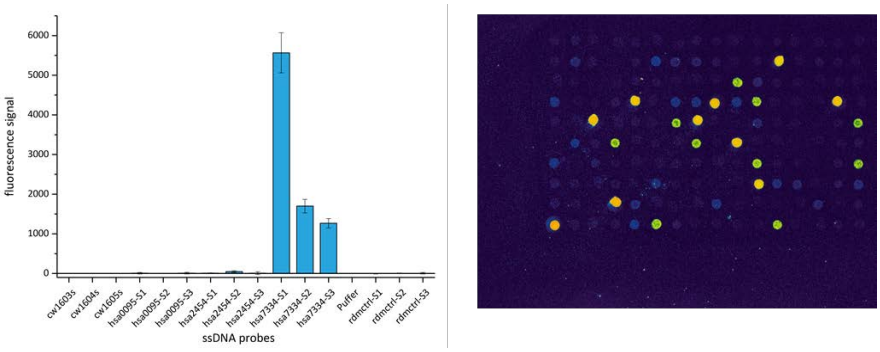

Figure 1. Microarray based detection of circRNAs (hsa7334) with different types of immobilized probes.

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Immunosensor surface functionalization by simple photochemical immobilization technique (PIT): A spectroscopic demonstration

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Surface functionalization is a key step in biosensing and antibodies (Abs) occupy a key role in bio-recognition thanks to their superior specificity. When gold is the interacting surface, the recently introduced Photochemical Immobilization Technique (PIT) has shown to be quick, easy-to-use and very effective in tethering Abs oriented upright. The molecular base of PIT relies on the selective photo-reduction of the disulphide bridges in cys−cys/trp triads in IgGs, which in turn leads to the production of reduced SH groups with strong affinity towards noble metals. The selectivity of PIT arises from the presence of only 12 triads not all of them effective in yielding thiols.

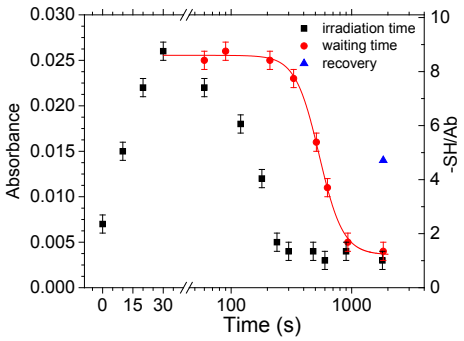

Figure 1. Ellman's assay to assess the amount of reduced thiols in solution. The black squared points refer to continuous irradiation and show an optimum at 30 s. The red circle points refer to a solution irradiated for 30 s and followed in time. The reduced thiols are active for more than 200 s. The blue triangle corresponds to a new irradiation of a solution for which the waiting time is 2000 s.

We demonstrate (Figure 1) that by irradiating Abs in solution for only 30 s with UV light from an amalgam type lamp (≈1 W/cm^2 on the sample), the Abs are "activated" and approximately 7 thiols are produced (3.5 disulphide bridge open). Once opened, SHs keep their properties for at least 200 s (Figure 1 red curve), a time long enough to convey Abs onto a gold surface on which they bind upright. The position of SH has been determined by mass spectrometry, which also confirms that only 8 thiols per Ab are reduced by UV and 4 of them (i.e. 2 disulphide bridges) are not exposed to the solvent lowering to two the number of bridges available for PIT (Figure 2). Moreover, SERS highlights that the signal from UV-treated Abs is much stronger than that coming from Abs physisorbed, thereby demonstrating higher proximity of Abs with the surface when they are UV-activated.

Figure 2. Left panel: The circles highlights the position of the two triads effective in yielding reduced thiols. Right panel: Position of Ab forming an angle φ with the surface.

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Bacteria detection using microarrays processed by Surface Plasmon Resonance imaging

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Microbiological analyses are run every day worldwide to ensure food safety. In this domain, the main challenge remains the fast specific detection of known pathogens, often present among significant amounts of irrelevant -and safe- bacterial strains. The standard microbiological protocol for bacterial detection in agronomic fields remains the microbial culture on selective media, which may take days to identify the pathogen and specifically confirm its presence in the sample. The delay is mainly due to the requirement of an enrichment phase allowing bacterial amplification before running the characterization assay. This time lag is a real bottleneck for setting up more performing and cheaper assays.
Besides food safety, similar issues are also shared for the diagnosis of bacterial infections, leading to sepsis. The main differences are the fact that samples (mostly blood) from healthy patients are sterile and that, in case of sepsis, only few bacteria might be present in 20-50 millilitres of blood. But once again, the time lap required for bacteria detection/identification is crucial. Thus, there is a strong need to develop new techniques to identify pathogenic bacteria in a shorter time.
To achieve this goal, Surface Plasmon Resonance imaging (SPRi) technology has been successfully used for the specific detection of bacterial populations growing on microarrays functionalized with antibodies targeting bacterial strains [1] (*Listeria, Salmonella, E. coli, Cronobacter,* etc). This strategy of simultaneous bacterial growth monitoring and bacterial strain specific detection enabled the detection of only few bacteria per milliltre, within few hours. Interestingly, the time-delay is directly linked to the bacterial concentration of the analysed sample and allowed quantitative assessment of the initial concentration by comparison to calibration experiments [2]. Our quantitative results are consistent with the expected doubling time of bacteria reported in the literature. More recently, we also engineered peptide microarrays for the universal detection of bacteria (presence/absence), without any prerequisite on the identity of the contaminating bacteria. Interestingly, this peptic based probing strategy gave access to new insights regarding bacteria/surfaces interactions. All these aspects will be presented in this talk, along with recent references published in the literature.

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[2] Bouguelia et al., Lab Chip. 2013 Oct 21;13(20):4024-32. doi: 10.1039/c3lc50473e

Lateral flow assay for meat authentication with visual detection

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Meat adulteration is a major concern worldwide. It usually involves the substitution of a meat product of good quality with a meat of different animal origin with lower quality, and lower cost, for economical profit. Therefore, meat authentication tests are required for protecting consumers and producers from fraud and for public health safety issues. As the production of meat and meat-based products has an extraordinary increase, nowadays, fast, reliable, inexpensive and simple analytical methods, especially biosensors, are of great demand. In this work, a lateral flow assay has been developed for meat authenticity tests. The assay is strip-based and exploits gold nanoparticles that enable visual detection by naked eye. The whole protocol includes DNA isolation from different meat samples, amplification of species-specific DNA sequences by Polymerase Chain Reaction (PCR) and detection/identification of the PCR products by the lateral flow assay. The assay has been applied for the identification of four animal species: horse, pork, beef and sheep. The detection is rapid and is visualized within 25-30 min upon the completion of the amplification reaction. The assay offers low cost, reduced analysis time, very good detectability, specificity and reproducibility. As low as 0.01% of horse and 0.02% of pork DNA were detectable in binary mixtures of horse in beef and of pork in sheep, respectively, by the proposed lateral flow device.

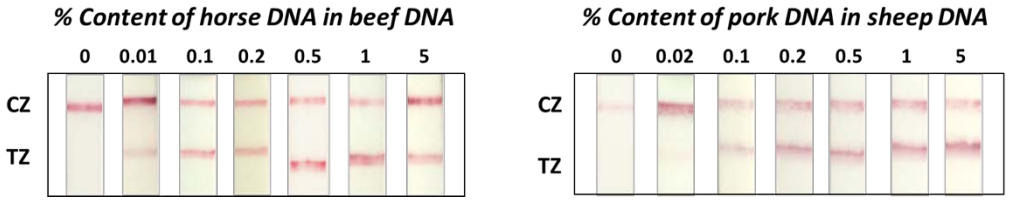

Figure 1. Detection of horse and pork meat in beef and sheep binary meat mixtures, respectively, in different % content (0.01 to 5%).

We acknowledge support of this work by the project “Research Infrastructure on Food Bioprocessing Development and Innovation Exploitation – Food Innovation RI” (MIS 5027222), which is implemented under the Action “Reinforcement of the Research and Innovation Infrastructure”, funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund).

A new smartphone-based biosensor for colorectal-cancer screening: chemiluminescent lateral flow immunoassay for fecal hemoglobin detection

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Colorectal cancer is the second leading cause of malignant death and the participation rate to screening programs based on invasive endoscopic diagnostic tests is very low. As an alternative, non-invasive stool testing based on the detection of fecal occult blood represents a valid approach for a rapid screening. However, the widespread guaiac-based test (GFOBT) is affected by a variety of interferences, thus frequently yielding false-negative and false-positive results and requiring the patient to follow a specific pre-test diet. The use of an immunoassay for detecting hemoglobin in stools could overcome these limitations. Lateral Flow Immunoassay (LFIA) is a technology currently widely applied in resource-poor or non-laboratory environments (point-of-care, POC) that is based on ready-to-use strips of cellulose-based materials containing dry reagents that are activated upon fluid sample application. The conventional colloidal gold-based LFIAs are available mostly for qualitative analyses of rather abundant analytes, however using enzymes as tracers, coupled with chemiluminescence (CL) detection, it is possible to obtain quantitative information and reach high detectability [1]. Herein, we report the development of a simple, rapid and accurate biosensor based on a CL-LFIA method applied for quantitative detection of hemoglobin in stool samples, using the smartphone BSI-CMOS photocamera as a light detector [1,2]. The biosensor is based on a competitive immunoassay using peroxidase (HRP)-labeled anti-hemoglobin antibody, which is detected, upon adding the luminol/enhancer/hydrogen peroxide-based CL substrate, by means of a smartphone camera for digital imaging and a specific application for data handling. Using a 3D printer, simple accessories were developed to turn the smartphone into a biosensing device. The developed method is simple and fast (15-min total assay time) and it allows to detect even small traces of hemoglobin in fecal samples, down to 4 pmol. When compared with the conventional GFOBT the assay is able to detect lower concentration of blood allowing an early diagnosis. This biosensor could be very useful for frequent self-screening providing a very effective tool for colorectal cancer prevention.

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Testing coastal bathing water and freshwater using a novel hand-held optical biosensor for detection of faecal pollution

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To achieve active management of bathing areas and to reduce risk associated with the presence of faecal pollution, tests capable of rapid *on-site* assessment of microbiological water quality are required. ColiSense technology can achieve results in 75 min and has *on-site* capabilities, successfully addressing these requirements. The technology encompasses a continuous fluorometric method for measuring GUS activity[1], a sample preparation protocol for the recovery of *E. coli* from environmental samples followed by GUS extraction[2] (Fig. 1) and a portable miniaturised fluorometer with incubation capabilities for GUS detection[3].
ColiSense was tested with seawater and freshwater samples. Seawater samples were collected and analysed from 11 designated bathing sites and 2 non-designated bathing sites. From the 125 samples, 108 samples had *E. coli* levels lower than 500, while 17 samples had *E. coli* levels higher than 500. A good correlation was found between ColiSense response and *E. coli* levels (R^2=0.85, N=125). Using this relationship *E. coli* levels can be estimated from unknown samples. GUS activity thresholds equivalent to the HSE action thresholds were computed. Using these thresholds, 96.8 % out of the 125 samples analysed were correctly classified as being above or below 500 *E. coli* by the ColiSense. For freshwater assessment, 4 rivers were sampled over a 1-month period. This study compared measurement of GUS activity with standard metabolic methods. This work shows that ColiSense, a hand-held biosensor, can successfully be applied to both fresh and saline water as an early warning by providing results in less than 1 hour from sample collection.

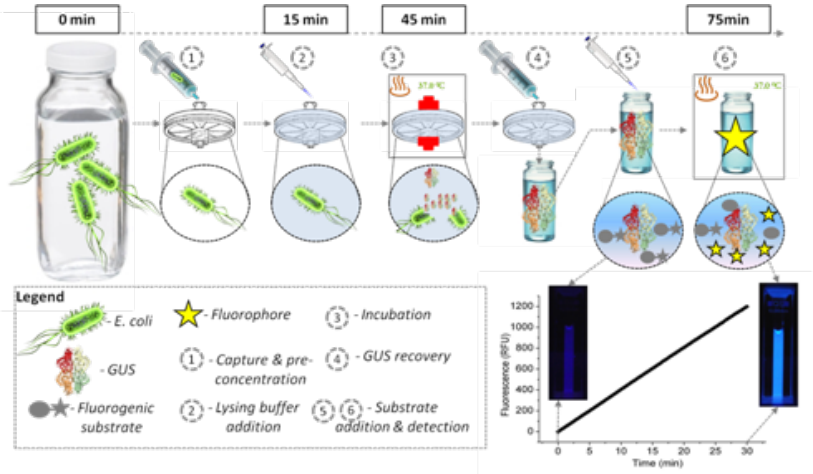

Figure 1. Graphical representation of the developed protocol for detecting *E. coli* in environmental waters using the marker enzyme GUS

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Multi-Parametric Surface Plasmon Resonance (MP-SPR) in biosensor development

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Optical methods such as Surface Plasmon Resonance have been widely used in development of biosensing approaches. SPR-based assays offer a label-free and real-time measurements that can be applied to monitor a large variety of biomolecular interactions including DNA, carbohydrates or proteins. While traditional SPR set-up enables studies within 150nm layers, Multi-Parametric Surface Plasmon Resonance overcomes such limitation which extends its applicability to functional polymers (*e.g.* MIPs), nanoparticles and living cells. Indeed, MP-SPR combines wide angular scanning and multiple wavelengths providing a unique possibility to assess coatings and surface interactions from Ångströms up to microns. Therefore, it opens to development of a large variety of biosensing assays with final applications in diagnostics, food safety or environmental control. Unique optical configuration of technology proposed by BioNavis enables not only measurement of interactions with molecules or particles but also characterization of layer properties which might be of great interest in assay development. Moreover, the technique offers the possibility to assess real samples such as blood serum, milk or waste water. Thanks to treatment of full curve SPR data, it can discriminate the specific binding directly from complex samples. The performances of MP-SPR technique are demonstrated in development of biosensing approaches such as: assessment of protein concentration in baby milk formula, detection of bacteria in food samples [1], characterization of biomimetic lipid layers [2] and exosomes [3], detection of cancer cells [4] or viruses as potential vaccines [5] as well as assessment of drug interactions with biosensor-cultured cells [6]. Assays based on MP-SPR prove to be accurate and sensitive with good correlation to reference methods such as QC or microscopy. The technology aims at new horizons in development of biosensors for clinical applications (disease markers), border controls (toxins), agriculture and food safety (GMO, contaminants) and environmental safety (microbes, antibiotic residues).

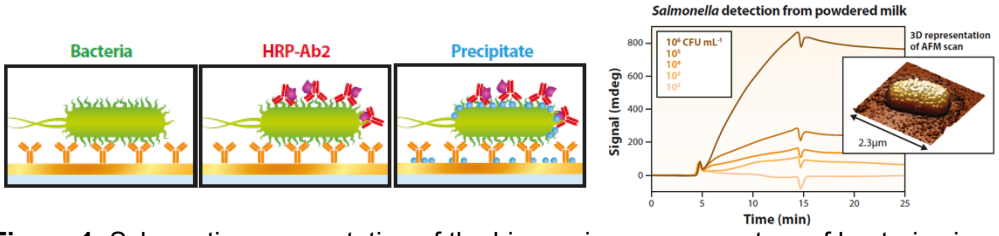

Figure 1. Schematic representation of the biosensing assay: capture of bacteria via specific antibodies followed by bio-catalyzed precipitation for signal enhancement (left). MP-SPR detection of *Salmonella* from powdered milk sample (right).

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Electrooxidative detection and quantification of dsDNA based on long-life dispersions of MWCNTs *via* poly(ionic liquid)s and amphiphilic diblock copolymers

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This work highlights the use of imidazolium-based poly(ionic liquid)s (PILs) and amphiphilic diblock copolymers as efficient dispersants of carbon nanomaterials, in particular multi-walled carbon nanotubes (MWCNTs). With these polymeric dispersants, highly stable fine aqueous dispersions of MWCNTs can be easily prepared and applied for efficient modification of screen-printed electrodes (SPEs). Such a surface modification of SPEs remarkably accelerates the electron transfer rate and increases the electroactive surface area through combining in a synergetic manner specific features of MWCNTs, such as strong adsorptive property and high specific surface area, with the advantages of PILs or amphiphilic diblock copolymers (DBC), such as high dispersing power. We further demonstrate that the resultant SPE/(MWCNT/PIL) and SPE/(MWCNT/DBC) constructs can be beneficially utilized for direct electrochemical analysis of double stranded DNA (dsDNA), specifically via the direct electrooxidation of guanine and adenine bases in salmon testes double-stranded DNA chosen as a model system. The linear ranges for the determination of dsDNA correspond to 5–500 μg/mL for oxidative peak of guanine and 0.5–50 μg/mL for oxidative peak of adenine, thereby making direct electrochemical dsDNA detection with the use of the SPE/(MWCNT/PIL) constructs a good alternative to currently applied spectral and fluorescent techniques. The SPE/(MWCNT/PIL) and SPE/(MWCNT/DBC) constructs can sense a point mutation in the 12-bases single-stranded DNA fragments. The influence of drugs onto the DNA electroanalytical characteristics was investigated.

Acknowledgements. This work was supported by Russian Science Foundation (RSF, project no. 18-44-04011) and the Deutsche Forschungsgemeinschaft (DFG, SCHA1640/18-1) within the joint RSF-DFG grant.

Optical detection of Listeria by functionalized F-doped ZnO

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Rapid detection of target bacteria is crucial issue in many areas of social interest to prevent harmful contamination for humans and the environment. Conventional methods for determining the bacteria contamination involve several steps such as pre-enrichment, selective enrichment, biochemical screening and serological confirmation, resulting money and time consuming. The large bandgap (3.3 eV), visible light transparency, availability, and multiple techniques for thin film deposition (chemical vapor deposition, sputtering, molecular beam epitaxy, and so on, make zinc oxide (ZnO) a versatile semiconductor extensively used in different applications [1]. Solar cells, piezoelectric actuators, thin film transistors, optical and electrical biosensors are just a few examples of technological returns of ZnO. Different kinds of doping were applied to ZnO, aiming at improving physical properties but preserving its transparency. Interesting results were obtained doping ZnO with fluorine (F) atoms, supposed to occupy oxygen vacancies, which are the most effective defects in ZnO [2]. A number of morphologies of nanocrystalline ZnO, such as nanoparticles, nanowires, nanosheets, nanorods, nanotubes, and nanoflowers were successfully prepared by various synthetic methods. Nowadays, the hydrothermal method is considered the most convenient synthetic procedure because of its low cost, mild experimental conditions and environmental friendliness. In this work, ammonium hydrogen fluoride, NH_4FHF, was used as fluorinating agent for the preparation of F-doped ZnO powders by hydrothermal synthesis. Undoped and F-doped ZnO powders were deposited on silicon pieces during synthesis and properly functionalized with biomolecules for detection of harmful organisms. Preliminary morphological and optical characterizations of samples were performed. Investigations regarding application of supports in biosensing are currently in progress.

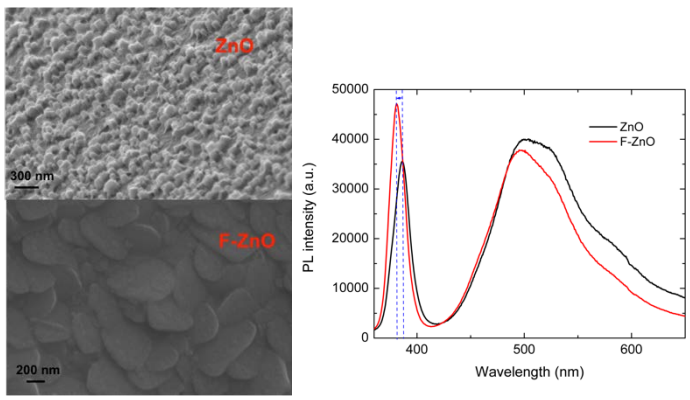

Figure 1. a) SEM images of ZnO and F-ZnO films deposited on silicon. (b) PL spectra of ZnO and F-ZnO films deposited on silicon.

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Production and characterization of enzyme nanoarrays assisted by alternating electric fields

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Dielectrophoresis (DEP) is the force acting on a polarizable particle caused by an inhomogeneous electric field. The force depends on the volume of the particle, the square of the gradient of the electric field and other factors [1]. Despite their small volume, biomolecules like antibodies [2] or enzyme molecules can be manipulated by DEP and immobilized on nanoelectrode arrays [3, 4] and even trapped as single molecules, when sufficiently high field gradients are achieved [5]. In this work, regularly arranged, vertical nanoelectrode arrays with different electrode tip sizes from 500 nm down to a few nm are used to create high field gradients. The electrodes are cylinder or cone shaped and made of tungsten or silicon. Several thousands of electrodes are placed in the same array and can be switched on simultaneously, allowing to perform thousands of experiments in parallel. Horseradish peroxidase (HRP) can be immobilized on these 500 nm tungsten cylinder electrodes and remains active [3]. Also, the deterministic singling of particles on this kind of electrodes was shown, provided that the particles are about twice the size of the electrode tips [6]. In this work, the HRP-nanoarrays obtained by dielectrophoresis are characterized and will be optimised in order to achieve deterministic singling of active HRP molecules on the nanoelectrodes. This kind of nanoarray would be an interesting tool for biosensors and single enzyme molecule studies.

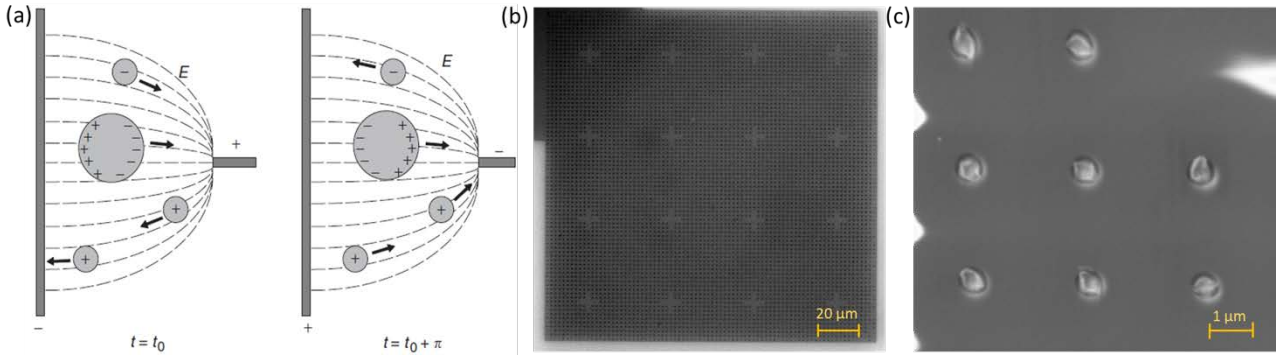

Figure 1. (a) Forces acting on a neutral polarizable particle in an inhomogeneous electric field [1]. (b) Microscope image of an array of >6000 tungsten cylinder electrodes. (c) Electron micrograph of HRP-deposits on 500 nm tungsten cylinder electrodes.

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[5] R. Hölzel, N. Calander, Z. Chiragwandi, M. Willander, F. F. Bier, *Phys. Rev. Lett.* 95, 128102 (2005)
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Electrochemical deposition of polythiophene from an aqueous solution for enzyme electrode preparation

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Conducting polymers are a valuable group of polymers since they can not only be used for a stable immobilization of enzymes but can also help to establish electrical communication with electrodes and thus become part of the transducer system [1, 2]. Here, polythiophene copolymers have been applied as modifier for a carbon electrode in order to allow the fixation of PQQ-dependent glucose dehydrogenase (PQQ-GDH) and to improve the direct electrical connection of the biocatalyst with the electrode.
Polymer films have been electrosynthesized from an aqueous solution onto carbon nanotubes from mixtures of 3-thiopheneacetic acid ($ThCH_2CO_2H$) and 3-methoxythiophene ($ThOCH_3$) using a potentiostatic pulse method. The deposition of the polythiophene occurred without the need of surfactants in the deposition mixture. The polymer significantly improves the bioelectrocatalysis of PQQ-GDH: catalytic currents already start at a potential of -0.2 V vs. Ag/AgCl. Thus, well-defined glucose detection at 0 V vs. Ag/AgCl with high current density is feasible. Compared to polythiophene films prepared in organic solvents the properties could be significantly improved [3].
Several parameters of the electrodeposition method have been studied to optimize the current output after enzyme coupling to the polymer electrode. The prepared polymer deposit has been carefully characterized to verify the polymerisation reaction. Here SEM, EDX, FT-IR, UV/Vis, XPS and Raman have been applied.
The response of the enzyme-polymer electrode towards increasing glucose concentrations shows a dynamic range extending from 1 µM to 2 mM. The low applied potential helps minimizing interferences from substances such as uric and ascorbic acid [4].
It can be also demonstrated that the enzyme electrode can be coupled to a BOD- and carbon nanotube-based cathode for building a biofuel cell. The promising results suggest a further investigation of this kind of polymers and, in particular, the study of the interaction with other enzymes in order to employ them in constructing hybrid systems for biosensing or bioenergetics.

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AC electrokinetic immobilisation of nanoparticles and proteins

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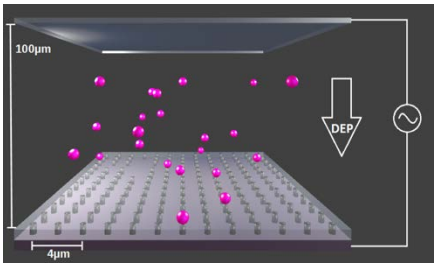
AC electrokinetic phenomena like dielectrophoresis (DEP) and AC electroosmosis have been applied to the spatial manipulation of biological cells over many years. They are increasingly exploited for the separation and immobilisation of nanoparticles and molecules in micro- and nanoelectrode systems. Especially the dielectrophoretic immobilisation of antibodies and enzymes on electrodes is important for a well controlled functionalisation of sensors.
With proteins being about three orders of magnitude smaller than cells, both the size dependence of DEP and that of thermal motion call for much higher field gradients when targeting molecules. Such an increase can be achieved by increasing the voltages applied, which, however, leads to heating and additional fluid flow that interfere with DEP action. A better approach is to use electrodes with sizes and curvatures reaching those of the target molecules. Therefore, we have developed different electrode types: Interdigitated electrodes with gaps below 1 µm, planar triangular electrodes with distances of around 100 nm, and regular arrays comprising up to 1 million pin-like electrodes with tip diameters reaching the size of proteins (< 10 nm). Successful immobilisation is demonstrated for polystyrene nanospheres, single molecules of the autofluorescent protein R-phycoerythrin [1], for horseradish peroxidase [2] and for antibodies [3]. Fluorescence microscopy shows that protein function is preserved in the course of DEP immobilisation. Localisation is controlled by scanning force, scanning electron and optical microscopy. Fluorescence polarisation microscopy reveals the immobilisation of the autofluorescent eGFP in a properly aligned manner and allows to determine the orientation of the protein's fluorescing subunit in relation to the whole molecule [4]. Nanoparticles are immobilised as singles on each electrode tip following the array's regular arrangement [5]. Latest results of the AC electrokinetic immobilisation of protein molecules as singles will also be presented.

a) 

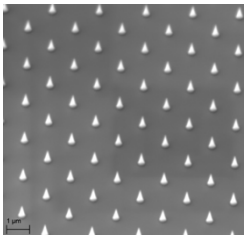
b) 

Figure 1. a) Electrode arrangement for dielectrophoretic immobilisation. b) Scannig electron microscopy of a regular array of sharp cone electrodes (bar 1 µm).

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[3] S. Otto, U. Kaletta, F.F. Bier, Ch. Wenger, R. Hölzel, *Lab Chip*, **14**, 998-1004 (2014).
[4] E.-M. Laux, X. Knigge, F.F. Bier, Ch. Wenger, R. Hölzel, *Small*, **12**, 1514-1520 (2016).
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Polymer-based hybrid materials for advanced (bio)sensing applications

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The homogeneous integration of different nanoobjects within man-made electrochemical devices is a crucial aspect when engineering (bio)analytical devices. Generally, integration efficiency of nanomaterials determines the quality of an analytical electrochemical device, its sensitivity, and eventually its market competitiveness. Furthermore, electronic conductivity of the overall ensemble can be ensured by preparing thin-film coatings, thereby allowing efficient electron transfer and facilitating electrode reactions. In particular, carbon nanomaterials (carbon nanotubes, graphene oxide, etc.) have been widely incorporated to improve layer conductivity and increase the efficiency of electron exchange. These materials in general are highly hydrophobic, rendering it rather challenging to prepare stable dispersions for an easy and reproducible modification of electrode surfaces. It is therefore evident that a certain compatibilizer is required, which on the one hand ensures homogeneous distribution of the carbon nanomaterials in aqueous media or within thin-film composite materials and, on the other hand, can serve as a non-destructive matrix for the subsequent embedment of (bio)molecules.
Both these challenges can be solved using amphiphilic polymers like poly(ionic liquid)s or diblock copolymers, the latter featuring one hydrophobic and one hydrophilic, often ionic (charged or chargeable), block. Thereby, the hydrophobic segment(s) of such amphiphilic polymers can adhere to the surface of carbon nanomaterials, while the hydrophilic moieties ensure their dispersability in aqueous media. The overall hydrophilic-hydrophobic balance of the considered amphiphilic polymers can be fine-tuned and such materials thus represent a versatile platform for the fabrication of hybrid materials.
This work reports examples of solubilization of carbon nanomaterials in aqueous media, using poly(ionic liquid)s or amphiphilic ionic/non-ionic diblock copolymers of different structure. Beneficial surface modification of electrodes by fine aqueous dispersions of carbon nanomaterials provides a considerable improvement of (bio)sensor performance. Examples of advanced direct electrochemical analysis of low molecular weight substances (drugs) and biomolecules (nucleic acids and hemoproteins) are considered.

Acknowledgements. This work was supported by the Russian Science Foundation (RSF, project no. 18-44-04011) and the Deutsche Forschungsgemeinschaft (DFG, SCHA1640/18-1) within the joint RSF-DFG grant.

Enzyme immobilized on functionalized gold nanoparticles for heavy metals sensing

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Heavy metal contamination in water and other ecosystems is one of the major environmental issues. The measurement of alteration in the structure or function of a biological macromolecule can represent an effective tool for detecting the presence of bioavailable pollutants and for screening the toxicity of environmental samples. Nowadays, optical devices sensitive to both inorganic and organic pollutants are subject of study and research in view of their application in those sites where a continuous monitoring is required in the biomedical or environmental or industrial field. Application of gold nanoparticles (AuNPs) in the sensor elements have shown promising results due to their tunable optical properties which greatly depend on their large surface-to-volume ration, high reactivity, high degree of functionalization and size-dependent properties. Biosensors represent one of areas for which the use of nanoparticles is growing. Indeed, biosensors are analytical tools whose effectiveness is highly dependent on the accuracy of the measurement and thus on its reproducibility. The use of gold nanoparticles to allow an amplification of the signal transduction for many techniques and to provide surface nanostructuration [1]. Among these techniques, the surface plasmon resonance (SPR) is the one that attracted most intensive research. It is an optical phenomenon arising from the interaction between an electromagnetic wave and the conduction electrons in a metal and it is used for probing and characterizing physicochemical changes of thin films on metal surface [2]. For example, the binding event between the recognition element and the analyte can alter physicochemical properties of transducer AuNPs, such as plasmon resonance absorption, conductivity, etc., that in turn can generate a detectable response signal [3]. Usually, the anchoring of gold nanoparticles on solid substrates is carried out using self-assembled monolayer of organic molecules (i.e organosilane), whose terminal functional groups interact electrostatically and chemically with the nanoparticles [1]. Aminopropyltriethoxysilane (APTES) is one of the most used organosilane agents for the preparation of amine-terminated surfaces. The presence of three hydrolysable ethoxy groups ensure a robust anchoring of the silane to the surface (silanization step), whereas $–NH_2$ end groups from the aminopropyl groups remains available to immobilize AuNPs through electrostatic interaction. Aim of this work was the realization of an optical biosensor based on enzyme inhibition for the detection of pollutants in environmental samples. The dynamic monitoring of the presence of chemical pollutants (responsible for the enzymatic inhibition) could be carried out by means of an optical investigation in the visible spectral range by monitoring the variation of the plasmonic peak typical of gold nanoparticles immobilised onto glass substrates [3,4]. In this work, we report the development of metal (gold) nanoparticles chemically anchored by means of appropriate surface treatments onto glass substrates. In particular, glass substrates have been modified by using aminopropyltriethoxysilane (APTES). An optimized stabilization process through the use of heat treatment [5] and deposition of polydopamine (PDA) [6], is presented. This system was used as platform for enzyme immobilization and heavy metals sensing application. All preparation steps have been monitored by UV-Vis absorption spectroscopy and X-ray photoelectron spectroscopy (XPS).

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A graphenic pH sensor on paper

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pH measurement is one of the most common laboratory analysis. Nowadays, the fast development of remote health monitoring systems is raising the demand for reliable and wearable pH sensors free from the fragility and dimensions of classical glass electrodes [1]. Many pH sensors fabricated on plastic, silicon or ceramic substrate have been reported [2]. Here we show a graphenic pH sensor fabricated on filter paper, which may represent an interesting alternative support for potentiometric devices due to the easy combination with microfluidic circuits.
A wax protective layer was printed onto filter paper using a Xerox ColorQube 8580 printer. Conductive tracks were drawn using a pencil and a pH sensitive layer consisting of graphene oxide was deposited by drop casting. A silver/silver chloride reference electrode was integrated in the device by brushing a suitable paste. The open circuit potential existing between the pH sensitive electrode and the reference electrode was measured by a potentiostat / galvanostat (Palmsens 4).
The sensor was calibrated with certified reference solutions in the range (pH 4-10) and showed a sensitivity of -52 mV/pH and a reproducibility of 12 % of the fabrication process. Bias was ± 0.1 pH units at pH 4.0, but increased up to ± 0.3 pH units at pH 10.0 due to the degradation of the support at high pH values.
The sensor was tested in complex matrices like human plasma and sea water; comparison with values obtained with a calibrated glass electrode showed deviations of ± 0.1 pH and ± 0.2 pH units for plasma and sea water respectively.
The reported fabrication technique could be applied for the development of cheap potentiometric devices using paper as support. The porous structure of paper makes this material suitable for the entrapment of enzymes and the fabrication of biosensors. The combination of such devices with microfluidic circuits onto the same paper support would be a logical step forward towards wearable sensor systems.

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Monitoring of microbial growth with a graphene-based sensor

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Chronic wounds such as diabetic foot ulcers (DFUs) and venous leg ulcers (VLUs) are problematic. not only for their impact on patient´s quality of life, but also for the marked increase in treatment costs. A variety of processes takes place during wound healing to allow the re- epithelization of the skin, but in certain cases healing mechanisms fail, extending the healing time and increasing the risk of localized infections, most commonly associated to *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus* spp.
Factors such as pH and temperature among others have been associated with wound healing. For example, the pH value in a wound was found to decrease during healing. During the SWAN-iCare (Smart wearable and autonomous negative pressure device for wound monitoring and therapy) project, wearable pH sensors based on graphenic materials have been developed and proven to be able to measure pH in human exudate as an indicator of wound healing [1]. However, it is also important to consider that colonization of wounds by microorganisms may also have an effect in pH value due to the accumulation of metabolism byproducts.
In this regard, graphene based potentiometric sensors were tested in the presence of bacterial cell growth of of *P. aeruginosa* and *S. aureus*. Four working electrodes (WE) coated with graphene oxide as the sensing material and one reference electrode (RE) Ag/AgCl were screen-printed on a thin and flexible polyethylene terephthalate (PET) substrate together with silver conducting tracks. Changes in the open circuit potential between WEs and RE were monitored together with the bacterial growth (expressed as the optical density, OD) at 600 nm) in liquid culture media, and pH.
In *P. aeruginosa* and *S. aureus*, pH (measured using the sensor) of the culture media did not match the value registered using the reference pH meter (glass electrode), but interestingly it did follow the growth curve trend generated by *P. aeruginosa* ($r=0.99$; $P<0.05$). On the other hand, for *S. aureus* not the pH (glass electrode) nor the bacterial growth influenced the sensor response, which remained stable over time.
Complementary studies need to be performed to understand the behavior of these systems in the presence of different bacterial strains. However, it is important to underline that the results described here offer the possibility to use this sensor for the monitoring of *P. aeruginosa* bacterial growth. To this end, the effect of factors such as culture media, among others should also be investigated in further detail.

[1] *P. Salvo, N. Calisi, B. Melai, C. Paoletti, T. Lomonaco, R. Fuoco, A. Paolicchi, B. Cortigiani, M. Mannini, A. Caneschi, V. Dini, M. Romanelli, F. Di Francesco, Biosensors & Bioelectronics 91 (2017) 870-877.*

Measuring multivalent interactions between influenza A and peptides using electronically switchable DNA nanolevers

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Influenza viruses pose a significant threat to public health every year. Not only can an influenza infection be fatal, but it can also have a grave effect on the economy as millions of workers fall ill every year [1]. Influenza is a mostly spherical virus with a diameter of about 120 nm and is part of the family of the Orthomyxoviridae [2]. The principle surface protein is Hemagglutinin (HA), which initiates the attachment to the host cell. It binds multivalently to sialic acid residues of cellular receptors of the host cell. [3] Peptides PeB and PeB^{GF} have been derived from antibodies to target HA on the surface of influenza virus H3N2 (A/Aichi/2/1968). [4] These peptides will be used to study the effect of multivalency in the binding process. Interactions between the influenza virus and the peptides are measured by using oscillating DNA nanolevers in the DRX^2 (Dynamic Biosensors). [5] In this method, the peptide is coupled to the end of the nanolever. The other end of the DNA is coupled to a gold surface. Since the DNA is negatively charged, an alternating voltage can induce an oscillation of the nanolever. The light signal of a fluorescent dye, attached to the DNA, is utilized to measure the oscillation dynamics. When the dye is close to the gold electrode, the fluorescence is quenched. The amplitude and /or dynamic of the oscillation of the DNA changes due to binding of a molecule, for example the virus. [6,7] Using a controlled number of DNA nanolevers on the electrode surface, the influence of multivalent binding can be determined. Furthermore multivalency can be quantified when attaching peptides in a controlled distance to the nanolevers.

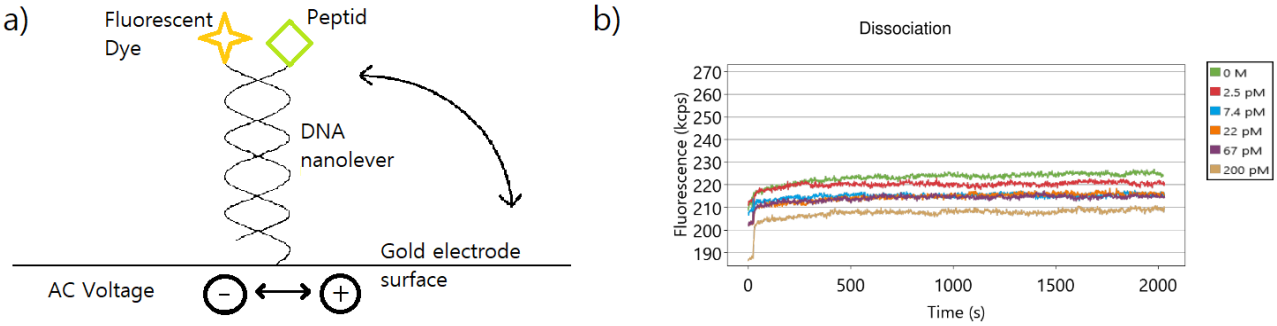

Figure 1. a) General principle of DNA nanolever biosensor: DNA nanolevers oscillate due to alternating voltage. (b) Dissociation of influenza virus H3N2 in variable concentrations from antibodies measured with DRX^2.

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Stable aqueous dispersions of carbon nanotubes via polydehydroalanine-based copolymers: preparation and application for surface modification and design of electrochemical biosensors

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Carbon nanomaterials (carbon nanotubes, graphene oxide, etc.) have been widely exploited for engineering of electrochemical (bio)analytical devices to improve their performance, remarkably increasing conductivity and efficiency of electron transfer. As such materials are typically poorly dispersible and their dispersions are not stable, it is rather challenging to apply them for an easy and reproducible modification of electrode surfaces.
This work summarizes a comprehensive approach to preparation of nanocomposite biosensor coatings containing carbon nanomaterials (MWCNTs), which are uniformly distributed within a polymer matrix. For such hybrid nanomaterials, a novel polymeric component, polystyrene-*block*-polydehydroalanine diblock copolymer (PS-*b*-PDha) bearing a hydrophobic polystyrene (PS) segment, and a hydrophilic polyzwitterionic polydehydroalanine (PDha) segment was used. PDha itself as well as PDha-based copolymers represent a promising class of polymeric materials with wide range of applications, in particular, for surface modification/functionalization and biocompatible coatings.
The considered nanocomposite (PS-*b*-PDha@MWCNTs) materials are fine aqueous dispersions (inks), which are stable over long time. Further, they can easily be drop-casted onto electrode surfaces, e.g., screen-printed electrodes. Depending on the pH, the dispersed hybrid PS-*b*-PDha@MWCNTs particles can exhibit positive or negative net charges and thus can provide the electrostatic binding of correspondingly charged biomolecules to the modified electrode surfaces.
A rationally designed nanocomposite biosensor based on a combination of amphiphilic polyzwitterionic PS-*b*-PDha diblock copolymers and carbon nanomaterials allows for optimization of such materials for a specific analytical task. Beneficial surface modification of electrodes by fine aqueous PS-*b*-PDha@MWCNTs dispersions provides a considerable improvement of biosensor performance. Examples of advanced direct electrochemical analysis of biomolecules (e.g., hemoproteins) are reported. The strategy presented herein represents an original, profitable and promising approach towards highly specific and sensitive biosensor coatings.

Acknowledgements. This work was supported by the Russian Science Foundation (RSF, project no. 18-44-04011) and the Deutsche Forschungsgemeinschaft (DFG, SCHA1640/18-1) within the joint RSF-DFG grant.

Direct electron transfer from glucose dehydrogenase to single sheet graphene electrode

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Continuous glucose monitoring (CGM) is an emerging technology that can provide a more complete picture of the diabetes patient's glucose levels. Amperometric blood glucose tests typically require redox mediators to facilitate charge transfer from the enzyme to the electrode, that are not ideal in CGM settings because of their potential toxicity or long-term stability issues. Direct electron transfer (DET) would eliminate this need and has therefore attracted substantial interest. However, most DETs studies so far have used glucose oxidase leading to controversial results because the oxygen dependency may be misinterpreted as DET. Here, we overcome this challenge by using an oxygen-insensitive glucose dehydrogenase. The biosensor strongly responded to glucose even without a redox mediator, implying direct electron transfer (Fig. 1 A). Control measurements on different surfaces further confirm that the response is enzyme-specific. To enable direct electron transfer, the enzyme was immobilized on the surface of high-quality single-layer graphene electrodes via short pyrene linkers (<1 nm) (Fig. 1 B). The activity of immobilized enzymes was confirmed by glucose measurements with relatively unexplored redox mediator - nitrosoaniline, and the influence of a most potent interferent in blood, ascorbic acid, was assessed (Fig. 1 C). This is the first demonstration of DET from an oxygen insensitive enzyme to single-layer graphene, highlighting the potential of such devices for applications in CGM.

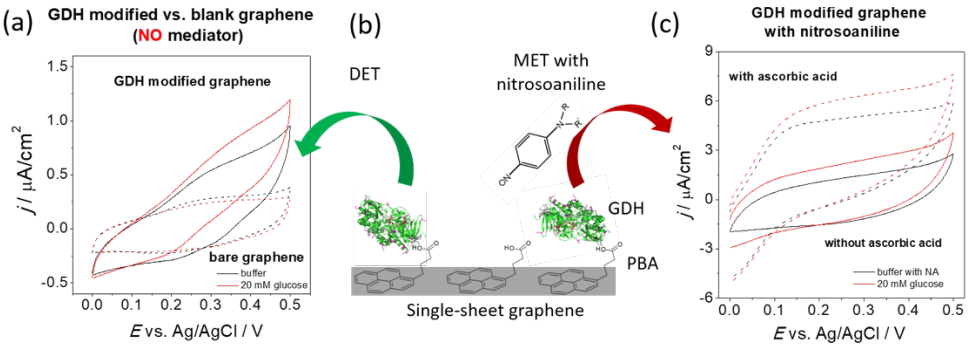

Figure 1. (a) Direct electron transfer from glucose dehydrogenase (GDH) to graphene electrode (bare graphene – dashed line) (b) Graphene surface modification scheme (c) Mediated electron transfer using nitrosoaniline derivative in absence (solid) and presence (dashed line) of ascorbic acid.

Abbreviations:

DET – direct electron transfer, GDH – FAD-dependent glucose dehydrogenase, MET – mediated electron transfer, NA – nitrosoaniline derivative, PBA – 1-pyrenebutyric acid

M.S. Filipiak et al. *Biosens. Bioelectron. (submitted)*

Calibration of High-Frequency Impedance Spectroscopy Measurements with Nanocapacitor Arrays

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High frequency impedance spectroscopy (HFIS) biosensors based on nano-electrode arrays (NEA) demonstrated the capability to overcome the screening limits set by the Electrical Double Layer (EDL), thus enabling label-free detection and imaging of analytes far above the sensor surface [1,2]. In order to achieve quantitatively accurate results, a precise understanding and modeling of the signal transduction chain is necessary. With reference to the CMOS array platform in [1], capacitance is measured by CBCM. Hence, the nanoelectrodes are alternatively charged and discharged by two switch transistors (Fig.1, a), which are activated by non-overlapping clocks with typically 1 ns floating time between the two phases. The column readout circuits integrate and average over multiple cycles the charging current to obtain a capacitance information. The output signal is interpreted in terms of a switching capacitance (C_{SW}), modeled by charge-pump analysis of an equivalent C-RC circuit excited by a square wave (EDL capacitance C_S in series to a parallel R_EC_E representing the bulk electrolyte [1]; C_S, R_E and C_E are extracted with the biosensor simulator ENBIOS [3]), good agreement is obtained between experiments and simulations over a broad range of frequencies and electrolyte salt concentrations [1]. *Residual discrepancies, however, require explanation and this is the main contribution of our abstract.* To this end, we firstly, consider the role of leakage currents (I_{LEAK}) in the sensor cell (due to subthreshold conduction of the inactive switch). The leakage current implies overestimating the column current I_M (and hence the capacitance). Due to the large number of cells connected on each column, a value as large as 20pA is estimated for I_{LEAK}, and measurements are corrected by compensating for it. Then, we consider the voltage waveforms at the nanoelectrode, as obtained by Spice simulations with Predictive Technology Models (PTM) of the sensor cell readout circuit (Fig.1 (b) for a 10mM electrolyte). Charge repartition between the nanoelectrode's node and C_{GS}/C_{GD} capacitance of the switching transistors during the float time distorts the otherwise square-waveform. For electrolytes with high salt concentration this effect is mitigated (due to the larger load capacitance). To account for this effect, we extract the harmonic content of the waveform by Fourier expansion of the waveform (Fig.1, b). Then, ENBIOS simulations at all harmonic frequencies are used to reconstruct the capacitance response to the actual waveform (C_F). Fig.1 (c) compares experiments (corrected for leakage) and simulations (C_{SW} or C_F). The impact of leakage is modest, whereas C_F exhibits an improved agreement with experiments at high frequency, where waveform glitches are more relevant. These corrections highlight the importance of leakage and harmonic content of the input waveforms to achieve quantitatively accurate interpretation of NEA HFIS biosensor experiments. Further work is necessary to extend these results to electrolytes with physiological salinity.

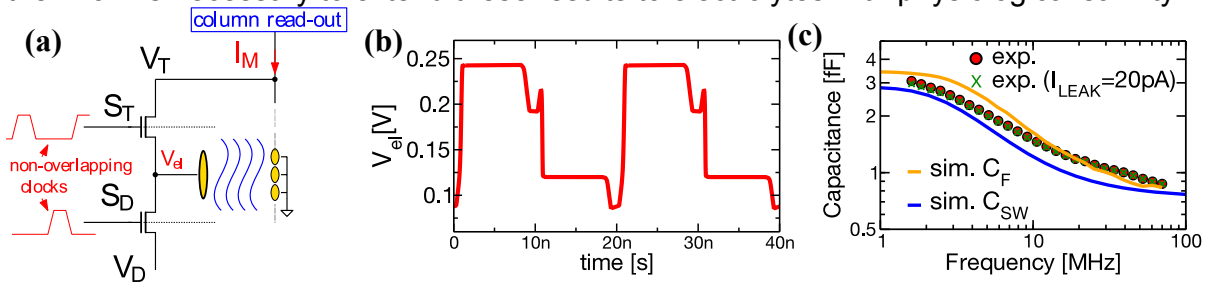

Figure 1. (a): schematic of a sensor cell. (b): voltage waveform at the electrode (NaCl 10 mM load) at 50 MHz. (c): comparison of experiments (also corrected for I_{LEAK}) and simulations (C_{SW} and C_F) in NaCl 10mM.

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Portable surface plasmon resonance (SPR) for real-time monitoring of marine obligate hydrocarbon degrading bacteria

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Environmental monitoring related to oil and gas exploration activities demands innovative technology in remote marine environments such as the high north (e.g. Arctic region). Timely delivery of critical information regarding oil spill events in such remote marine environments is critical but remains challenging as manpower and logistic are constrained. To overcome this, unmanned systems capable of remote sensing and rapid feedback for decision are needed. ESP (Environmental Sample Processor, MBARI, USA), a cutting-edge autonomous genomic platform is an example of technology addressing these challenges [1]. Changes in microbial signatures can be used to “sense” the occurrence of oil in water. The goal of our project is to utilize surface plasmon resonance (SPR), an analytical device integrated in 3G ESP, to detect and quantify hydrocarbon degrading bacteria in seawater for tracking of oil spill events. The suitcase-size SPR instrument used here is technically identical to the one integrated in the 3G ESP and is developed at the University of Washington in collaboration with MBARI [2]. The instrument was tested for the first time for the detection of oligonucleotide sequences derived from the 16S rRNA gene of *Oleispira antarctica* RB-8, an obligate microbial indicator species of marine oil contamination. We used morpholino oligomer-functionalized sensor surfaces for hybridization and streptavidin coated magnetic nanoparticles in combination with biotin-labelled probes for signal amplification [3]. Our experiments so far indicated that a simple coating procedure and a relatively cost-efficient magnetic-bead-based signal amplification is providing robust SPR based nucleic acid sensing down to 0.5 nM of a 45-nucleotide long oligo target (7.2 ng/ml) over repeated (85) sample injections. Currently we are conducting experiments with longer targets (up to 477 bp) that mimic rRNA but are still easy to handle for use on SPR. Further, total RNA extracted from *Oleispira antarctica* culture will be used in consecutive trials. The final part of the method development will include experiments where seawater will be amended with varying concentrations of *Oleispira* cells, total RNA will be extracted and detected using protocols mimicking the 3G ESP. By doing so, the developed methodology will be ready for a real-world-test during a field deployment of an ESP device.

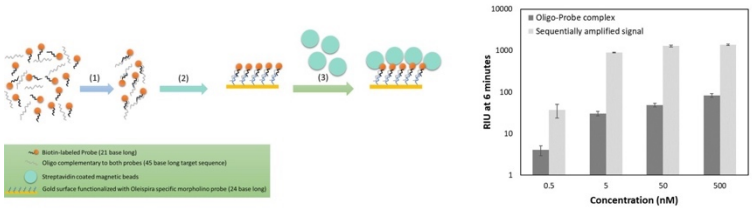

Figure 1. Sequential amplification strategy (left) and refractive index units (RIU) recorded at 6 min for various concentrations of the 45-nucleotide long oligo target and constant 50 µl bead stocks (right).

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Surface acoustic wave(SAW)- surface plasmon resonance (SPR) microfluidic biosensor for real-time monitoring and enhancement of chemical functionalization of gold films

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Surface biochemical functionalization is a fundamental process that is widely applied in many fields to add new functions, features, or capabilities to a material's surface. In particular, gold films are a very common surface coating for biosensors. A variety of functionalization strategies for gold films are currently available but their optimization (e.g. improved antifouling or bio-recognition element orientation) typically requires complex or expensive strategies. Here we present a surface acoustic wave (SAW)-driven surface plasmon resonance (SPR) microfluidic sensor which enables the realtime monitoring of biotin functionalization efficiency in the presence of SAW-induced streaming. We characterized and optimized the fluid dynamics inside the microchannel and monitored the heating with an IR camera. We demonstrated that in the case of 50-MHz 800-pm-amplitude SAW-induced streaming, the functionalization efficiency of the gold film is either improved or faster than in the case without SAWs. The technology here proposed can be easily applied to a wide variety of biological systems (e.g., proteins, nucleic acids) and devices (e.g., sensors, devices for cell cultures).

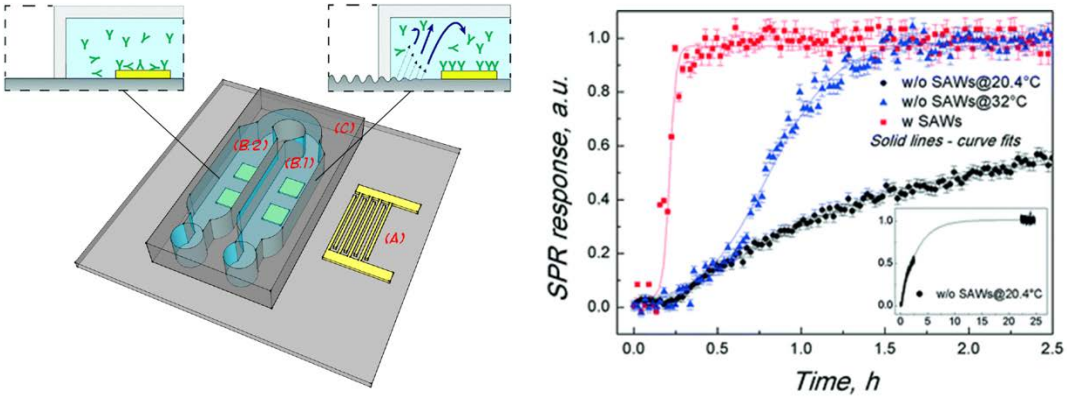

Figure 1. On the left, Schematic of the SAW-enhanced SPR chip characterized by an interdigital transducer (IDT) for SAW excitation (A), four SPR sensing areas (B), and a polydimethylsiloxane (PDMS) microchannel (C). On the right, SPR responses. Comparison between biotin adsorption kinetics during 3 hours of incubation obtained under the three experimental conditions: w/o SAWs@20.4 °C (full black dots); w/o SAWs@32 °C (full blue triangles); and with SAWs (full red squares).

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Detection of diclofenac in wastewater by genetically engineered yeast cells enclosed in a single-use flow cell

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Detection of pharmaceutical residues in wastewater becomes increasingly important. Among others, this task can be accomplished with genetically modified yeast cells which produce a fluorescent protein in presence of the analyte [1]. Recently we reported on the design, assembly and performance of a novel PDMS flow-through device with immobilized genetically modified yeast cells that generate a fluorescent signal upon stimulation with diclofenac whose intensity is then detected by fluorescence microscopy [2]. In both cell culture medium and standardized synthetic wastewater, we were able to measure diclofenac concentrations in a range from 10 to 100 µM. As particularly interesting feature, we could show that only the biologically active fraction of diclofenac is detected [2].
Here, we present a novel flow cell design. We developed a single-use flow cell based on the use of polymeric foils instead of PDMS to enclose the genetically modified yeast cells (Fig. 1a). The flow cell can be produced very cost-efficiently by roll-to-roll processing. Furthermore, a miniaturized portable spectrometric unit has been developed for the read out of the fluorescence signal.

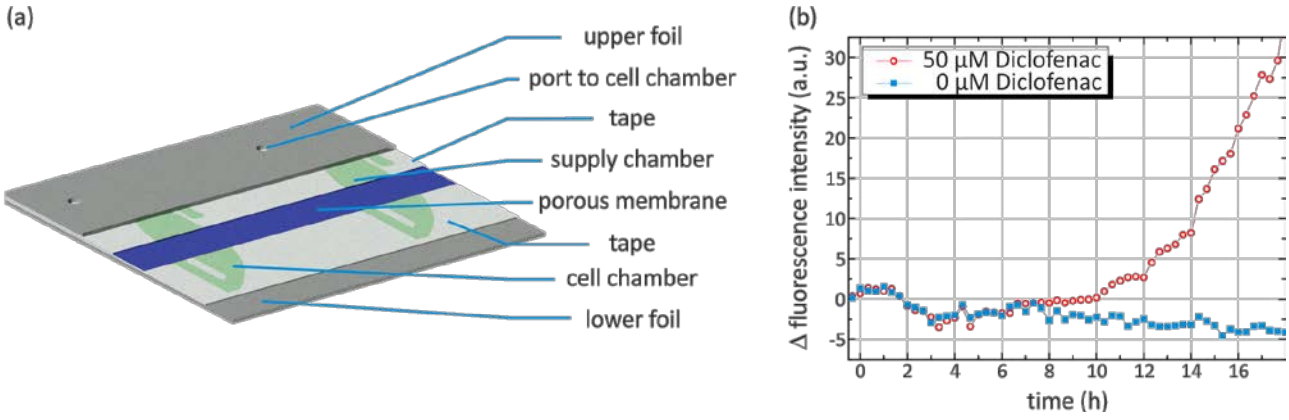

Figure 1. a) Schematic viewgraph of the single-use flow cell. (b) Time course of the fluorescence intensity in the single-use flow cell monitored via the spectrometric detection unit.

[1] A. Schuller, G. Rödel, K. Ostermann, *Sensors*, 17, 1506-1522 (2017).
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Combined optical tweezers and atomic force microscope device to create the ultimate nano-force toolbox

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In recent years, optical tweezers have generated remarkable interest in research areas other than fundamental physics, including biochemistry, biology, and medicine. The ability to gain insight into the mechanical processes and miniscule forces underlying protein folding, DNA-ligand interactions, cell adhesion, and cell mechanics opens a broad range of new applications.
Many biological and biophysical experiments benefit from a significant increase in spatial and temporal control over sample dynamics. Optical traps can be used to manipulate or hold sample components with the ability of precise force application. With motorized stages, three axis piezo driven sample scanners and multiple traps, up to 14 degrees of freedom for positioning, force application and measurement are available. While the force range of typical AFM measurements spans tens of pN (10^{-12} N) to tens of nN (10^{-9} N) with the highest resolution in the z direction, Optical Tweezers can apply and detect forces as low as 0.5 pN in three dimensions. This allows force measurements over four orders of magnitudes in the same sample.
We have combined the exceptional surface force measurement and imaging capabilities of AFM with the ability of optical tweezers to apply and measure smallest forces in 3D.
The unique combination of 3D positioning, detection, and manipulation provided by Optical Tweezers and the high-resolution imaging and surface property characterization of AFM opens up a whole new spectrum of applications.

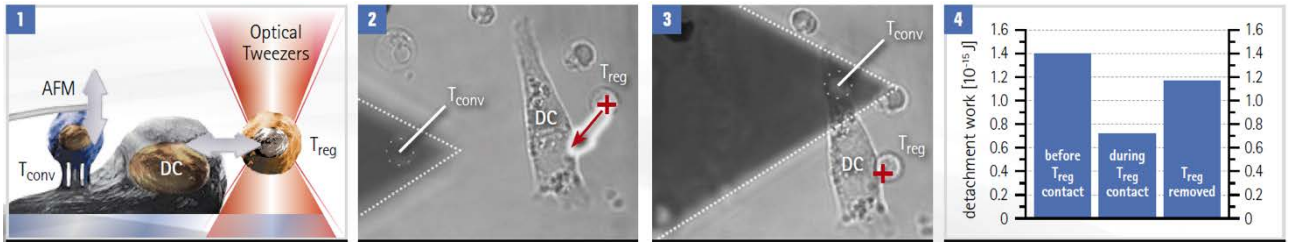

Figure 1. 1) Adhesion experiment with dendritic cells (DC) and conventional T-cells (T_{conv}). The T_{conv} is attached to a tipless cantilever, then approached to the surface-bound DC. The cantilever is pulled up and the adhesion forces are measured. A regulatory T-cell (T_{reg}) is attached to and removed from the DC with optical tweezers to test its influence on the binding strength. 2) and 3) Measurement setup. The optical trap (red cross) moves the T_{reg} while adhesion measurements are performed with a cantilever-attached T_{conv}. 4) Detachment work measured for the three situations. Treg attachment reduces DC-T_{conv} interactions. After the T_{reg} is removed, the adhesion level is almost restored. Sample courtesy of Yan Shi, University of Calgary / Tsinghua University, Beijing. The results of the original experiment designed by Yan Shi et al. (publication in print).

One electrolyte based glucose/oxygen electric power biodevice with an operating voltage exceeding 1.24 Volt

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Rapid technological developments in recent years have opened up many possibilities to construct personal electronic/electromechanical devices, including those for biomedical applications. However, such devices need to be powered by efficient electric power sources [1] that, in the case of implanted biomedical devices [2], also need to be long-lasting, safe, stable, and biocompatible. While fuel cells have the potential to address this problem, and have been researched for several decades, one of their main drawbacks is a low operating voltage, which is thermodynamically limited to 1.24 V in the case of glucose/oxygen devices [3]. Here we report a proof-of-principle demonstration of a high-voltage biodevice that is capable of generating sustained electric power at voltage, which exceeds the thermodynamic limit. Due to the interplays between faradaic and non-faradaic electrochemical processes, as well as between ionic and electronic conductivities, the biodevice, consisting of several self-charging biosupercapacitors connected in series and operating in the same electrolyte in a pulse mode, provides apparently unreachable open-circuit and operating voltages. Owning to redox enzymes, biocatalysts par excellence, individual biosupercapacitors are self-charged, and after that, the complete biodevice is externally discharged. Our results demonstrate proof-of-principle operation of a high-voltage biological power source working in an authentic human blood stream, which we anticipate to be a starting point for the design of high performance implanted power sources.

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Tag-specific affinity purification of recombinant proteins by using molecularly imprinted polymers

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Purification of recombinant fusion proteins by tag-ligand strategies has contributed to the growing application of these biomacromolecules in therapeutics, diagnostic, industrial settings or academic research [1]. The FLAG® epitope (DYKDDDDK) is a small peptide employed for the purification of recombinant proteins such as immunoglobulins, cytokines and gene regulatory proteins. It provides superior purity and recoveries of fused target proteins than the broadly applied poly-histidine tag. However, purification requires using anti-FLAG® antibody resins, the high cost and non-reusability of which restrict widespread use [2,3]. To overcome this limitation, we report herein the development of molecularly imprinted polymers (MIPs) [4,5] selective to the FLAG® tag and their application to the purification of recombinant proteins from crude cell extracts. The polymers were prepared by the epitope imprinting approach using a four amino acids peptide, DYKD, including part of the FLAG® sequence as template molecule. Polymer composition was optimized using a combinatorial approach to select the functional monomer (FM) and the cross-linker (CL) yielding the best selectivity for FLAG®-tag and DYKD-tag recognition. The optimized polymer was prepared using *N*-(2-aminoethyl)methacrylamide hydrochloride (EAMA) as functional monomer, ethylene glycol dimethacrylate as cross-linker (molar ratio T:FM:CL, 0.5:3:20) and dimethylformamide as porogen.
The imprinted resin thus obtained were packed in SPE cartridges and used to purify mCherry proteins tagged with either FLAG® or DYKD epitopes from crude cell lysates. Both mCherry variants were highly efficiently purified (R ≥ 95%, RSD ≤ 15%, n = 3) and impurities removed. Protein purification has been confirmed by gel electrophoresis (SDS-PAGE), Western Blotting and matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF/TOF MS). Unlike existing antibody-based resins, the proposed tag-imprinting strategy provides a general method for meeting the growing demand for efficient, inexpensive, versatile materials for protein labelling and purification [6].

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A Sensor for furfural detection in water based on MIPs combined with a novel SPR platform

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In this work a novel surface plasmon resonance (SPR) platform [1] has been tested to monitor the interaction between a molecularly imprinted polymer (MIP) and a small molecule as the substrate (furfural). SPR chemical sensors and biosensors have been shown to be able to play an important role in numerous fields [2]. Figure 1(a) shows the proposed SPR sensor configuration with a MIP receptor in contact to the gold film (sensing area). The optical sensor platform is based on a slab waveguide (a layer of PMMA of size 1cm x 1cm x 0.5mm) with a thin gold film on the top surface (60nm thick) inserted in a special holder, designed to produce the plasmonic resonance at the gold-dielectric interface [1]. During the measurements, as shown in Fig. 1a, a white light source is introduced in the PMMA slab waveguide by a trench illuminated with a plastic optical fiber (POF) of 1 mm in diameter and, kept at the end of the PMMA slab at 90° with respect to the trench, by another POF to carry the output light to a spectrometer. In this configuration, the trench has been used because a large incident angle is required for SPR excitation. The experimental setup is composed of a light source, a halogen lamp (HL–2000–LL, Ocean Optics) exhibiting a wavelength emission range from 360 nm to 1700 nm, the SPR sensor system and a spectrometer (FLAME-S-VIS-NIR-ES, Ocean Optics) connected to a computer [1]. The spectrum analyzer detection range is from 350 nm to 1023 nm. As proof of principle, a selective MIP was considered as the receptor for furfural (furan-2-carbaldehyde, 2-FAL) and the possibility of using the device obtained for detection of 2-FAL in aqueous media was investigated. The MIP is deposited on the gold film of the removable chip (slab waveguide) by a spin coater machine. The obtained results have shown the good performance in terms of selectivity, sensitivity and limit of detection (LOD) of this novel approach (the LOD is about 0.03 ppm). Figure 1(b) shows the variation of the resonance wavelength with respect to the blank (Δλ, nm) versus 2-FAL concentration (ppm), in a semi-log scale, and the fitting by the Hill equation, which is satisfactory. Each experimental point (black square) is the average of 5 subsequent measurements and the error bars are the respective standard deviations.

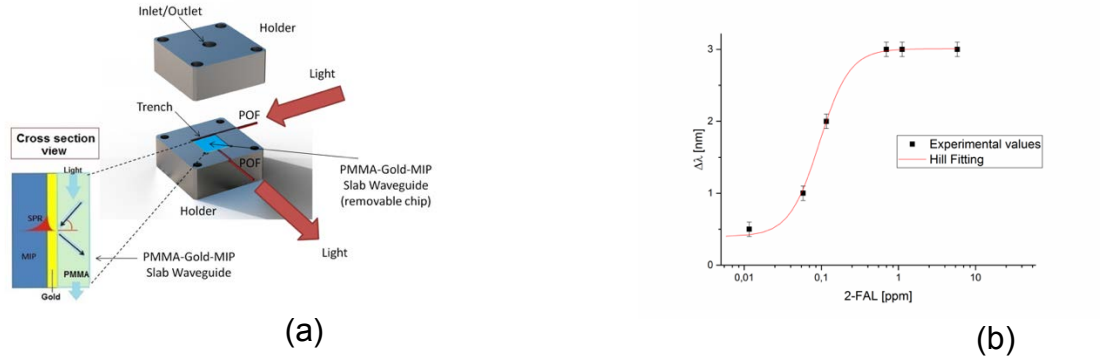

Figure 1. (a) Chemical SPR sensor outline. (b) Resonance wavelength variation (with respect to blank 0ppm) versus 2-FAL concentrations, in semi-logarithmic axes, for SPR-Slab-MIP, with the Hill fitting of data.

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An SPR biosensor in POFs to detect sepsis biomarkers

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A plastic optical fibre (POF) biosensor based on surface plasmon resonance for the detection of C-reactive protein (CRP) in serum is presented. The biosensor has been integrated into an ad-hoc developed thermo-stabilized microfluidic system allowing to avoid any thermal and/or mechanical fluctuation and to maintain the best stable conditions during the measurements.
CRP is an important protein biomarker: high levels of CRP in serum are observed after trauma, tissue necrosis, infection, and myocardial infarction and are associated with an increased risk of cardiovascular diseases [1]. In the case of infection and acute inflammatory events, such as sepsis, CRP concentration may increase up to 1000-fold, whereas its concentration in healthy human serum is lower than 1 mg L^{-1}.
The feasibility and integration of the SPR-POF sensor platform with the ad-hoc developed microfluidic system were evaluated performing a model IgG/anti-IgG assay in buffer and in serum [2]. Afterwards the biosensor was applied to the detection of CRP in serum. A dynamic signal range of 4.3 nm, a working range of 0.006–70 mg L^{-1} and a LOD of 0.009 mg L^{-1} were achieved [3]. These results are among the best compared to other SPR-based biosensors for CRP detection, especially considering that they were achieved in a real and complex medium, i.e. serum. Since the sensor performances are those requested for many clinical purposes, the whole biosensing platform could well address the continuous request of the physicians for high sensitive, easy to realize, real-time, label-free, portable and low cost sensing platforms for future lab-on-a-chip applications.

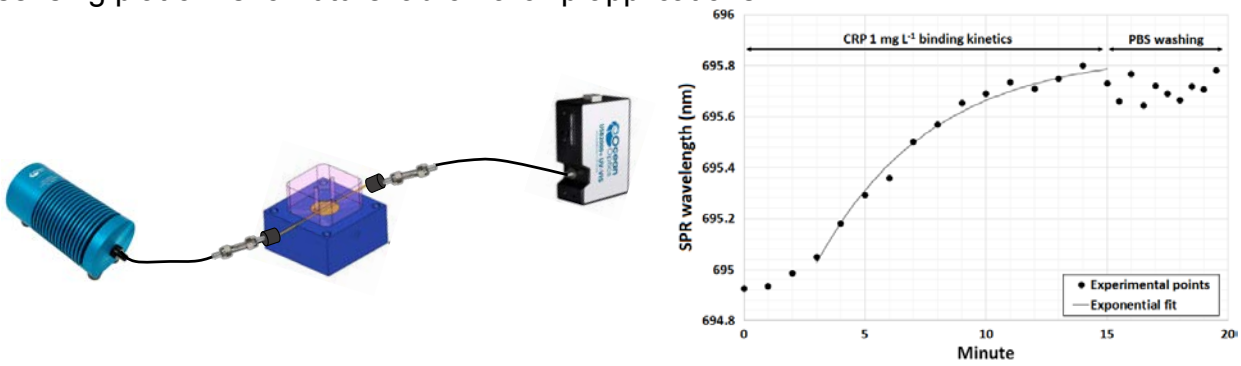

Figure 1. a) Scheme of the biosensor platform with the optical setup and the thermo-stabilized home-made flow cell. (b) Example of the binding interaction of the specific antigen (1 mg L^{-1} CRP) to the immobilized anti-CRP antibody.

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Platinum black-modified microelectrodes for biomedically relevant hydrogen peroxide detection

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It has been long known that hydrogen peroxide (H_2O_2) as an endogenous reactive oxygen species (ROS) belongs to a group of destructive molecules that can lead to protein oxidation, lipid peroxidation, and DNA damage. Whereas elevated ROS production leads to oxidative stress, it was also recently shown that at lower physiological levels, H_2O_2 acts as an intracellular signaling molecule regulating kinase-driven pathways[1]. As the involvement and concentration levels of H_2O_2 at the cellular level are not fully understood, its localized detection at a cellular level is of particular importance. Fluorescent redox-sensitive dyes are frequently used for the detection of H_2O_2 at the cellular level. H_2O_2 can also be detected electrochemically, e.g. at anodic potential (0.6 V vs. Ag/AgCl). However, such high oxidation potential can be affected by co-oxidizable substances such as ascorbic acid, catecholamine etc., which may be present in biological samples[2].

In this contribution, we discuss strategies of electrochemical H_2O_2 detection avoiding such interference problems based on electrocatalytically modified electrodes. As the measurements are targeted at the cellular level or at low sample volumes, microelectrodes and conductive colloidal AFM-SECM probes[3] will be used as electrochemical transducer for the modification with Platinum black or Prussian Blue (PB) showing enhanced sensitivity for H_2O_2 detection. Platinum black modified surfaces can be obtained by chrono-amperometry at -0.06 V vs. Ag/AgCl, reducing hydrogen hexachloroplatinate in the presence of lead acetate[4]. Using Platinum black modified electrodes, detection and quantification of H_2O_2 can be carried out at +0.3 V vs. Ag/AgCl. In addition, Prussian Blue (PB) modified electrodes allow the reduction of H_2O_2 at 0.0 V vs. Ag/AgCl and show significant higher activity in H_2O_2 reduction and 1000 times higher electrochemical rate constants[5,6]. After a thorough characterization of the developed microsensors and first applications will be shown at granulocytes and peripheral blood mononuclear cells. With the modified conductive colloidal probes we target towards highly localized mappings of H_2O_2.

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H/D isotope effects on the interaction of small molecules with self-assembled monolayers of DNA on gold electrodes

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An electrochemical quartz crystal microbalance (EQCM) was employed to study the interactions of hexammine ruthenium(III) (RuHex) and hexammine cobalt(III) (CoHex) with a mixed self-assembled monolayer of single-stranded DNA & 6-mercapto-1-hexanol (ssDNA/MCH SAM) immobilized on gold electrodes [1]. We report the largest kinetic isotope effect (k_H/k_D = 2400) at room temperature (25 °C) where a maximum shift of -400 mV was seen for the reduction peak potential in CoHex when the buffer medium was switched to deuterated water (D_2O) from normal water (H_2O) [2]. Formation of these mixed SAMs using a similar protocol proposed by Steel et.al is shown to produce heterogeneous surface packing densities with unequal distribution and aggregate formation of ssDNA and MCH over the gold surface [3]. Here by using a modified immobilization protocol that produced a low surface density mixed-SAM with a fewer aggregates we observed a notable decrease (by ≥10 Hz) in the frequency response in the presence of both RuHex and CoHex (Fig. 1). CoHex is known to strongly influence the structure of DNA forming intermolecular bridges through guanine bases while RuHex interacts through negatively charged phosphate backbone. Therefore, the most relevant difference between CoHex and RuHex may be that in order to transfer electrons, the former has to enter the DNA layer, while the latter does not. We believe that the observed differences in frequency response values are due to the influence of H/D kinetic isotope effect on the hydrogen bonds involved.

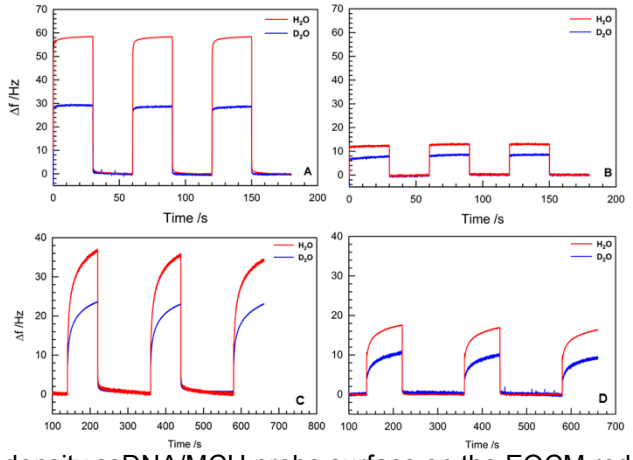

Figure 1. Effect of high-density ssDNA/MCH probe surface on the EQCM redox switching response of 50 µM **A)** RuHex **C)** CoHex and low-density ssDNA/MCH probe effect with 50 µM **B)** RuHex **D)** CoHex. Measurements were carried out in 10 mM Tris buffer made with H_2O (Red) and D_2O (Blue), pH 7.5.

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Microorganisms and biomimetics-based biosensors for environmental monitoring

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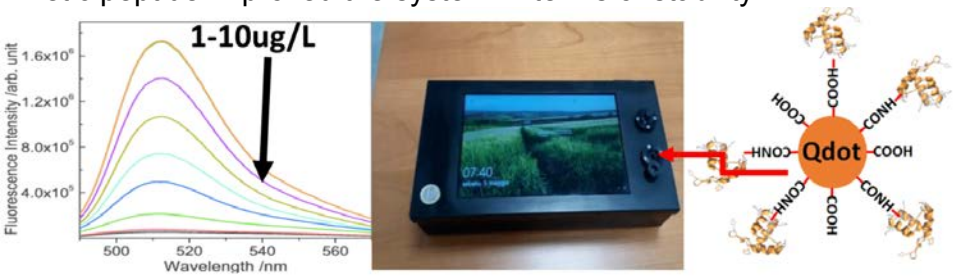
Biosensors, thanks to their fast, portable, and cost-effective features, may find a number of specific niche applications among analytical technologies currently competing for this expanding market. Because of the large number of compounds that need to be monitored in water, easy-use sensors are essential. Biosensor Srl develops both specific biomediators and miniature transducer systems. One interest is on microalgae now widely used as relevant biological indicators in the field of environmental impact studies. Owing to their ubiquity, short life cycles, easiness of culture and high sensitivity to a numbe of pollutants, these organisms are frequently utilized on ecotoxicological screening of contaminated water. Microalgae are especially sensitive in a short time of 10 min to all those pollutants that work as inhibitors at the photosystem II (PSII) level, such as heavy metals and half of the pesticides present in the market. Among the wide range of microalgae species, which have been employed to develop biosensor technology, *Chlamydomonas reinhardtii* possess a number of features that suite perfectly the requirements of an early warning environmental biosensor. It is a grass organism, easily cultivable having 8 hours doubling time and it can grow with or without carbon source, besides, it is easily transformable, and all 3 genomes are sequenced. Taking advantage of these features, optic/amperometric biosensors based on microalgal *C. reinhardtii* have been developed in our laboratory, able to detect pesticides in the environment. Recent efforts have focused on increasing the stability and selectivity of PSII from microalgae for the detection of different subclasses of pollutants. Beyond these scientific achievements, nowadays the market needs highly specific and precise in situ measurement devices able to collect and send the data in real-time for periods of months without maintenance under multi-stressors. These devices demand more robust algal biomediators. Thus, the challenge is the preservation of the algal photosynthetic functionality when integrated with electronic components or operated under fluctuating environmental conditions. To this end *C. reinhardtii* mutants able to quench $_1O^2$ and other ROS, were integrated into a newly developed miniature and portable device, to measure and collect PSII fluorescence induction data in real-time for long periods. Several photosynthetic pollutants were detected within 10 min in concentrations between ng/L-µg/L and the different algae species tested showed diverse pesticide sensitivities. Always towards to increase the biomediator performance, biomimetic peptides of the photosynthetic D1 binding niche of the microalgae *C. reinhardtii* were developed by chemical synthesis. Standing out among the others, the biomimetic mutant peptide, D1pepS268C, bound to specific quantum dots, showed high ability to mimic the microalga in binding pesticides. Replacement of whole microalgae cells by mimetic peptide improved the system in terms of stability.



Scheme 1. Schematic representation of covalent coupling of biomimetic D1 peptide to carboxyl quantum dots by carbodiimide method and small fluorescence transducer device.

A new approach also allows the integration of the biomediators with innovative stretchable printed electrodes-based electrochemical biosensor as a wearable point-of-use screening tool for toxicity environmental analyses.

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Reactive oxygen species generated on titania impregnated with horseradish peroxidase as a source for the detection of phenolic compounds

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Titanium dioxide (TiO_2) is a unique material for biosensing applications due to its capability of hosting enzymes [1, 2]. For the first time we show that TiO_2 can accumulate reactive oxygen species (ROS) under daylight irradiation or by a short pre-treatment by hydrogen peroxide (H_2O_2), both supporting the catalytic cycle of horseradish peroxidase (HRP) without the need of hydrogen peroxide (H_2O_2) to be present in the solution. We found that a short pre-treatment by H_2O_2 of carbon SPEs modified by TiO_2 impregnated by HRP (0.75 nmol per 1 mg of TiO_2) results in accumulation of approximately 14 nmol per 1 mg TiO_2 oxidizing species that cannot be washed off in following multiple washing steps. This material can serve as oxidizing agent for HRP. The pre-treated HRP|TiO_2|SPE demonstrated high sensitivity and fast response to hydroquinone, aminophenol, phenol, catechol, caffeic acid, and other phenols. The calibration curve for 4-aminophenol (4-AP) was linear in the range from 0.05 to 1 μM with a sensitivity of 2.7 ± 1.0 A M^{-1} cm^{-2} (average ± SD of three different electrodes) with a limit of detection (LOD) of 24 nM. For the electrodes that were not pre-treated, the amperometric response to 4-AP was linear in the concentration range between 0.05 to 2 μM in flow-injection analysis (FIA) mode, no H_2O_2 was added to the analyte solution. The sensitivity was 0.51 A M^{-1} cm^{-2} and the LOD 26 nM. Electron paramagnetic resonance spectroscopy indicates the presence of ROS on titania which, in interaction with HRP, initiate the electrocatalysis towards phenolic compounds. The proposed sensor design opens new opportunities for the detection of phenolic traces by HRP-based electrochemical biosensors, yet in a more simple and more sensitive way.

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The nanocoating material: a key element for development of D-shaped fiber-based label-free biosensors

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It has been proved recently that femtomolar detection of IgGs in serum can be attained by tracking the lossy mode resonance (LMR) generated with nanocoated D-shaped fibers [1]. Here the importance of the selection of the nanocoating material is addressed. In Fig. 1, the utilization of indium tin oxide (ITO) leads to important drifts, whereas no drift is distinguished when tin oxide (SnO_2) is used. In view of this, SnO2 nanocoating is the material selected for obtaining the experimental results of Fig. 2, where IgG in concentrations from 100 ngr/L to 10 mgr/l is detected.

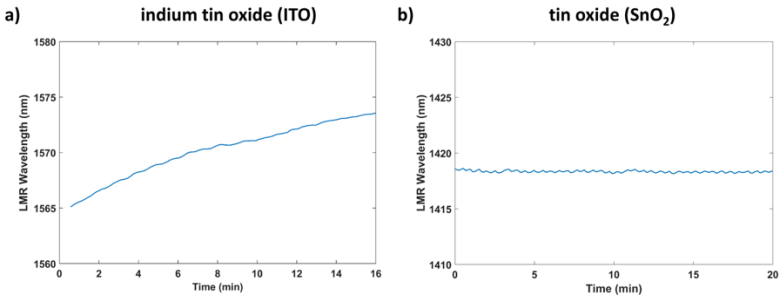

Figure 1. LMR wavelength drift in PBS for: a) ITO-coated D-shaped SMF; (b) SnO_2-coated D-shaped SMF

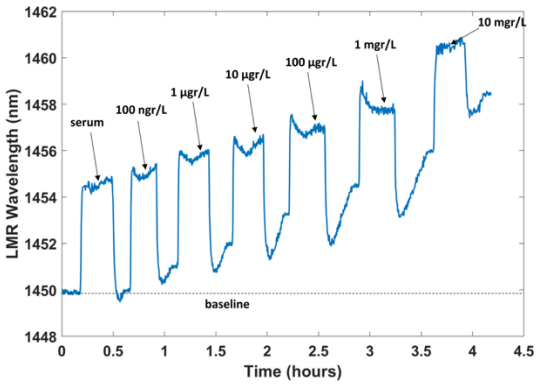

Figure 2. Sensorgram obtained with SnO_2-coated D-shaped SMF

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Controlled orientation of photosystem 1 monolayers for the fabrication of photoelectrochemical devices with anisotropic electron flow

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Photosystem 1 (PS1), one of the two key protein complexes involved in oxygenic photosynthesis, is capable of generating high-energy electrons upon the absorption of visible light. Owing to the high quantum efficiency, abundance, and robustness of PS1, this natural photodiode is an attractive building block for the fabrication of photoelectrochemical cells for energy conversion, biophotovoltaic devices for the generation of electrical power, and photosensors. After light-induced charge separation at the special chlorophyll pair P_{700} in PS1 the excited electron is internally transferred through a series of cofactors finally reaching the terminal iron-sulfur cluster F_B at the opposite end of the protein complex. The considerably large voltage difference between the two opposite redox sites at PS1 of about 1 V translates into a substantial driving force for recombination processes and the re-oxidation of reduced charge carriers at the electrode surface, thus cancelling out part of the generated photocurrent [1,2]. Therefore, one of the major challenges in the development of PS1-based devices constitutes the fabrication of stable and well-defined structures able to provide a unidirectional electron flow. Taking advantage of the amphiphilic nature of the isolated protein complex we present the effective modification of electrode surfaces with a preferred protein orientation, thus enabling an anisotropic electron transfer. The hydrophilic ends at PS1 bearing the redox centers P_{700}^+ and F_B^- adopt a specific orientation either being solubilized in water or facing air, while the hydrophobic regions stack together enabling the formation of a stable monolayer. PS1 protein complexes isolated from the cyanobacterium *T. elongatus* were spread onto the air-water interface and transferred to a gold surface serving as electrode substrate by means of the Langmuir-Blodgett technique. PS1-based photocathodes assembled using this approach proved to be an efficient means for the fabrication of functional devices enabling the effective extraction of photoelectrons.

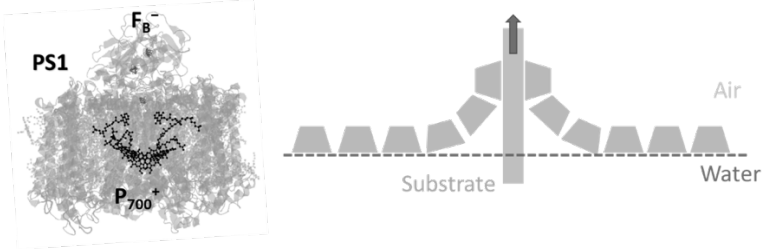

Figure 1. Schematic illustration of the two redox sites located at opposite ends of PS1 and the Langmuir-Blodgett transfer of a PS1 monolayer with a particular orientation at the water-air interface

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Electrochemical platforms for eDNA monitoring

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For the monitoring of marine species and the management of natural or commercial resources, the analysis of water for environmental DNA (eDNA) has great potential but needs to be validated in different environmental conditions. The primary objective of this project is to evaluate eDNA and eRNA for the detection and quantification of planktonic stages of the sea lice (*Lepeophtheiris salmonis*), a parasite causing a number of environmental challenges for the Norwegian aquaculture production of Atlantic salmon. Two different original approaches are herein presented for this application.
In a first approach, thiolated DNA capture probes are immobilized onto disposable gold-nanostructured carbon surfaces. The DNA is extracted from water samples, amplified and then hybridized with the capture probe and biotinylated signaling probes. The resulting biotinylated hybrids were then incubated with streptavidin–alkaline phosphatase and exposed to the enzymatic substrate α-naphthyl phosphate. The product of the enzymatic reaction was electrochemically monitored.
In a further approach, the capture probes are immobilized on streptavidin-coated paramagnetic beads in a sandwich assay format.
The results of laboratory analysis of marine water samples containing sea lice and collected in situ close by a salmon fish farm are reported together with their validation using standard RT-PCR analysis.

Selectively photo-induced immobilization of antibodies into micro-bubble resonators for immunoassays

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In order to selectively perform immunoassays into the optical micro-bubble resonator (OMBR) a spatially selective photo – chemical procedure able to bind bio-recognition elements (BRE) only in correspondence of the OMBR inner surface was performed. The procedure adopted is reported in Fig. 1. Shortly, the internal wall was silanized by 5% 3-Aminopropyl(diethoxy)methylsilane and a photo-activable cross linker (Sulfo-NHS-LC-Diazirine) was used to selectively immobilize the antibodies only onto the internal walls of the OMBR. A metal mask was necessary for the UV selective illumination of the OMBR. As proof of concept tests, the immunoassay between IgG/anti-IgG$^{Alexa\ Fluo488}$ was performed and moreover results on regeneration procedures were obtained.
For the OMBRs fabrication, slightly pressurized silica capillaries were used. For this purpose, a modified fusion splicer was used, where the electrodes were moved outside the instrument and placed in a U shaped holder that could rotate by 360° by means of a step motor [1]. The pressurized capillary was put on an XY stage and kept in a vertical position, corresponding to the rotation vertical axis of the U holder, between the two metal electrodes. During the localized arc discharge event, the silica capillary melts and, under the internal pressure, increased its radial dimension along the axial direction. In this way a hollow core shell, with a spheroidal shape, occurred: the so-called OMBR. OMBRs with some hundreds microns in diameter (from 550 μm to 650 μm) and wall thickness comprised between 2 μm and 4 μm have been successfully fabricated [2].

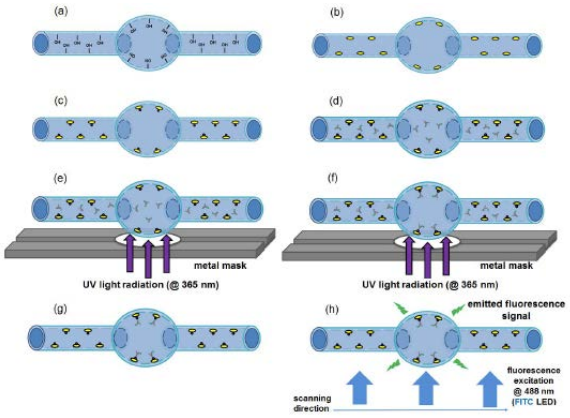

Figure 1. A sketch of the photo-chemical process able to bind selectively the fluorescent labelled bioreceptors only in correspondence of the OMBR inner surface.

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Electrodeposition of chitosan hydrogel on microelectrode for biosensor applications

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The use of biocompatible materials such as the hydrogels of polysaccharide chitosan (CHI) is of particular interest for the immobilization of enzymes on microelectrode surface for biosensor applications. In this work, we aimed the electrodeposition of chitosan hydrogel sheaths of controlled microstructure and thickness on the surface of microelectrodes. The hydrogel was deposited on microsensor chips with thin-film platinum electrodes. The first step was to precisely control the thickness and the diameter of the CHI hydrogel on the surface of the electrode. For our study we used a weakly acidic aqueous CHI solution in which we added a stoichiometric amount of acetic acid. Normally, CHI is soluble in water at $3<pH<6$. Out of this pH range, the initially soluble protonated chitosan is neutralized yielding hydrogels due to coagulation/precipitation.
We used the method of cathodic electrodeposition to achieve the CHI gelation on noble metal electrodes. During the process of molecular hydrogen formation, the pH is locally increasing, which leads to gelation near the electrode. This allow the following reaction to occur:

$Chit\text{-}NH_3^+ + HO^- \rightarrow Chit\text{-}NH_2 + H_2O$
The $Chit\text{-}NH_2$ is not soluble in water anymore and stay deposited as hydrogel at the surface of the microelectrode.
The influence of time, CHI concentration and current-density on the d and thickness growth profiles of the chitosan hydrogel were investigated. Four different CHI concentrations (0.5 - 2 wt.%) were considered in the study. It allowed us to precisely characterize the electrodeposition. The presented process allows electrochemically activated CHI membrane deposition as post-processing step on any noble metal thin-film electrodes.

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Gold thin-film based photoelectrochemical DNA-sensors

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Sputtered thin-film gold electrodes are promising as a substrate for the development of DNA based sensors due to their inherent properties such as reproducibility, high sensitivity, and low cost, leading to the possibility of using them as a disposable biosensor platform. However, critical aspects such as preparation and characterization of gold surface, evaluation of immobilized DNA probes onto gold substrates and control of their reproducibility are often dismissed, though these parameters directly affect the characteristics of the sensor. Recently, we developed a novel strategy for DNA sensing based on molecular photosensitizers generating singlet oxygen and a DNA probe attached to BASi gold disk electrodes [1]. In the current work, we evaluated the applicability of commercially available sputtered thin-film gold electrode structures that consist of three planar gold electrodes and can be used for the analysis in a drop of few microliters. These electrodes were characterized in comparison to gold disk electrodes that are typically used as a model support for DNA sensors. Three surface pre-treatment methods were compared and the optimal non-tedious pre-treatment procedure using a drop cell connector (with only 15 uL of reagent) was chosen. Different methods including electrochemical approaches for immobilizing oligonucleotide probes on the gold surface were performed with the aim to minimize/eliminate non-specific adsorption of the oligonucleotides on the gold, creating a better defined electrode surface. Surface densities of the immobilized probes through different approaches was determined using chronocoulometry with ruthenium hexaamine. Furthermore, important parameters for photoelectrochemical detection such as pH of the buffer, potential and laser power were optimised by focusing on a pheophorbide a, a well-known photosensitizer modified electrode.

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Detection of toxic microalgae in the marine environment by colorimetric and electrochemical DNA-based bioanalytical systems

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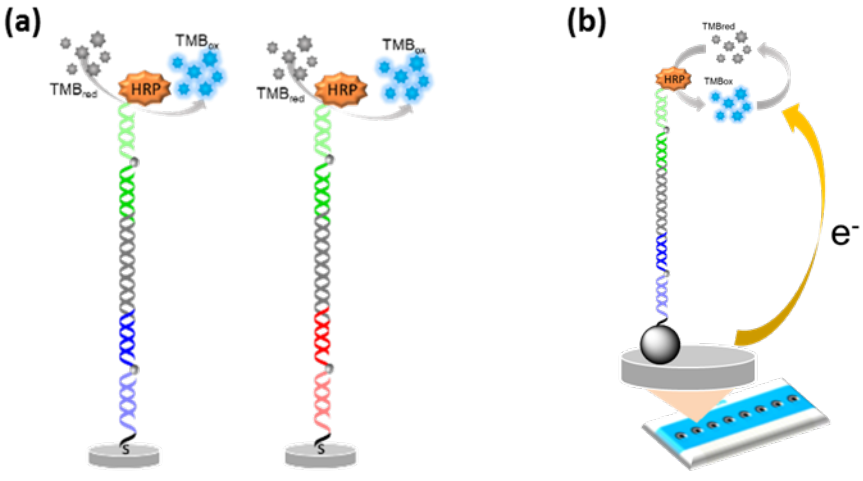
Harmful algal blooms (HABs) are natural phenomena whose frequency, intensity and geographical extent have increased during recent years. In the Mediterranean Sea, *Ostreospis* blooms are a serious concern due to their direct impact on human health and the environment. Current microalgae monitoring is performed via optical microscopy, which is time-consuming and not able to differentiate among *Ostreopsis* species. Therefore, rapid, species-specific and easy-to-use detection tools are highly needed.
In this work, we report the development of two innovative DNA-based assays: a colorimetric assay for *O.* cf. *ovata* and *O.* cf. *siamensis* and an electrochemical assay for *O.* cf. *ovata*. Both assays exploited isothermal recombinase polymerase amplification (RPA). Species-specific primers were designed to render an amplicon with single stranded DNA tails for its subsequent colorimetric and electrochemical detection via a sandwich hybridisation assay. For the colorimetric assay, thiolated capture probes were immobilized on maleimide-modified microtitre plates, whereas for the electrochemical assay the thiolated capture probe was immobilized on maleimide-modified magnetic beads, which were subsequently captured on a magnetized electrode. Following hybridisation of the RPA amplicon, colorimetric and electrochemical detection was achieved via addition of an HRP-conjugated reporter probe.
Both systems showed high specificity for the target species and calibration curves were successfully constructed using genomic DNA, achieving limits of detection of 10-14 pg/μL. The assays were applied to the analysis of planktonic and benthic environmental samples from different sites of the Catalan coast. Significant correlations were obtained with qPCR analysis and light microscopy counts, demonstrating the reliability of the developed systems. The approaches show great promise to improve understanding, prediction, management and mitigation of HABs.

Figure 1. Schematic representation of the approaches: (a) colorimetric assay; (b) electrochemical assay.



SERS bio-sensing in ion-exchanged glass microrods

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Surface-enhanced Raman spectroscopy (SERS) is a well-established technique able to detect a wide variety of chemical substances and/or biological molecules due to its capability of acquiring the unique vibrational information associated with them (i.e.: their spectral fingerprint) in different chemical and/or bio-chemical matrixes. Usually, the well-known enhancement effect for SERS is associated with the presence of metallic nanoparticles (NPs) at the substrate surface. Different chemical or physical deposition processes have been proposed to equip the substrate surface with a NPs layer. The former approaches are low cost but lack of reusability and stability. The latter strategies are expensive, time consuming and require special equipment that complicate the fabrication process.

Here, we demonstrate the possibility to obtain stable and reusable SERS platforms by low-cost silver-sodium ion-exchange process in soda-lime glass microrods. These microrods were obtained by cutting the tip of the ion-exchanged soda-lime fibre. Their final size were around few millimetersin length and one hundred microns in diameter. The ion-exchange and thermal annealing post-process parameters were optimized in order to guarantee the presence of embedded silver NPs on the surface of the glass microrods, avoiding the use of any chemical etching. The subsequent Atomic Force Microscopy (AFM) analysis confirmed the presence of these silver NPs with size of tens of nm. As preliminary bioassay test on such SERS platforms, a DNA hibridization assay was performed. In particular, the DNA-sequence [5'(ATTO647N)-CGACGGAGAAAGGGCTGCCACGXCG(BBQ)-3' X=C6-dT Thio] (1 □M) was immobilized on the silver NP via the thiol group and subsequently made react with the target sequence [5'-CCCCTGCCTGGCAGCCCTTTCTCAAGGACC-3'] (1 □M). SERS experiments confirm the presence of the DNA-sequence on the NPs embedded on the microrods surface, and its ability to interact with the target sequence was monitored by following the variations in the principal Raman spectral bands correlated to the oligonucleotides chain. These results are preparatory for the development of a new class of SERS fiber probes, based on multi-component glass oxides and suitable to host metallic NPs by ion-exchange process.

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Molecularly Imprinted Polymers for Determination of Chosen Food Toxins

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High temperature frying, broiling, grilling and especially smoking protein providing food products, such as meat, fish, poultry, eggs, and cheese generates in these products toxins, namely heteroaromatic compounds, amines, nitrosamines, etc. Continuous exposure to low doses of these toxins causes several chronic diseases, serious hormonal dysfunctions, and cancer. One of the trace amines, tyramine, may also cause unwanted interactions with antidepressant monoamine oxidase (MAO) inhibitors causing so called “Cheese reaction”. Currently used procedures for determination of these toxins in food matrices are either expensive or tedious and time-consuming. Therefore, fast, inexpensive, simple, and reliable determination procedures, without need of separation of these toxins, in the protein food matrices are in demand. Molecularly imprinted polymers (MIPs) are excellent examples of bio-mimicking recognition materials. Therefore, they have found numerous applications in selective chemosensing. Within this project, we have applied tyramine imprinted polythiophene films as a selective recognition units for devising electrochemical sensors. MIP tyramine-film coated electrodes were sensitive and selective with respect to tyramine. The linear dynamic concentration range of the devised chemosensor was 260 μM to 2,6 mM tyramine and imprinting factor was, IF = 6.9.

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Low-loss all-dielectric SERS substrates

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Dielectric nanostructures offer an extensive range of properties, suitable for spectroscopy and biosensing applications [1]. Even-though the research field, such as surface enhanced Raman spectroscopy (SERS), is heavily dominated by plasmonics, high-index dielectric nanostructures have lower intrinsic losses and, thus, are heated less by the irradiation of the excitation source [2]. At the same time, high-index dielectric nanostructures enable high field enhancement, thus opening new and interesting possibilities in SERS, especially in the case of metal-reactive and temperature-sensitive samples. Recently, we have proposed a SERS substrate based on an array of silicon pillars in a dimer configuration [3], with an average enhancement factor comparable to plasmonic structures. We introduced the use of double-enhancement emerging not only from the dimers, but also from their periodic arrangement. By employing a surface lattice resonance at the excitation wavelength and a local field enhancement at the wavelengths range of the expected Raman signal, as shown in Figure 1b, one may obtain a unique field enhancement profile, which can be adjusted to a specific test sample in SERS measurements. In this work, we will present steps towards an all-dielectric system by replacing the previously employed metallic mirror with a high-index dielectric layer, see Figure 1a, or a stack of them, forming a distributed Bragg reflector. In addition, we will discuss fabrication techniques, as well as measurement issues and opportunities. We will overview the recent development of dielectric-based SERS substrates and will present our solution for a low-loss all-dielectric SERS substrate.

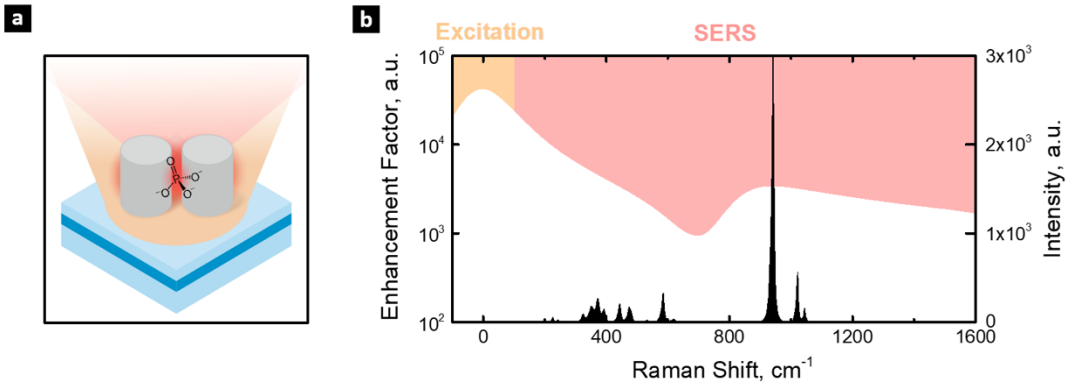

Figure 1. (a) Enhancement factor (white contour) in respect of Raman signal of lithium phosphate (black contour) [4], with excitation at 785 nm and expected SERS spectral region highlighted. (b) Graphic illustration of a unit-cell of a periodic array of silicon dimers on dielectric substrate.

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Polymer deposition on nanoporous silicon surface functionalized with his-tag-Rab7 protein. Toward protein surface imprinting for optical sensing applications

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Molecular imprinting is a universal concept to generate materials with "molecular memory" by performing a polymerization of suitable functional monomers in the presence of a target molecule acting as a template. The subsequent removal of the template creates recognition sites in the molecularly imprinted polymer (MIP) that can selectively rebind the target [1]. Although this concept proved to be successful in preparing MIPs for small molecules (~200–1200 Da), several specific problems can arise if the target is a protein [2] as the classical imprinting methodologies fail to address the peculiarities of protein targets. Due to their fragility, irreversible conformational changes may occur during polymerization; moreover, the large size of the proteins makes them difficult to remove from, or rebind to a highly cross-linked polymeric network. Among the approaches introduced to overcome these difficulties, surface imprinting emerged as the main strategy for macromolecular imprinting [2]. This approach restricts the formation of imprinted binding sites to the surface of a polymer or to a very thin polymer layer with thickness comparable to the size of the protein template. Moreover, the immobilization of the protein through a self-assembled anchor layer offers additional advantages over polymerization from a protein-monomer mixture in terms of generating uniformly accessible binding sites. Herein, we propose a new method to prepare Rab7 protein oriented surface-imprinted nanoporous silicon photonic crystals (PhCs). Nanoporous silicon PhCs are prepared by controlled electrochemical etching of silicon and subjected to thermal oxidation generating SiO_2 to aid surface modification [3]. A his-tag is used as the anchor to facilitate the protein immobilization, by exploiting a 3-step protocol functionalization of PhC surface involving i) SiO_2 silanization with glycidoxypropyltrimethoxysilane (GLYMO) preliminarly reacted with iminodiacetic acid (IDA); ii) Ni^{2+} attachment by complexation with IDA; iii) Rab7 anchoring due to strong interaction between histidine residues and Ni^{2+}. Subsequent polypyrrole chemical deposition on Rab-7 functionalized PhCs is performed under different experimental conditions. Rab7 is used as target molecule as it is a small GTPase belonging to the Rab family with a key role on different cellular pathways [4]: it is fundamental for lysosomal biogenesis, positioning and functions, and for trafficking/degradation of several signaling receptors. Furthermore, Rab7 has specific functions in neurons. Each step of PhC functionalization is monitored by Visible reflectance spectroscopy. Fourier transform of each spectrum affords a peak proportional to effective optical thickness (EOT) of the porous layer [3], which is used as analytical signal for monitoring successful functionalization event. The developed protocol for anchoring his-tag Rab7 protein followed by polymer deposition represents the preliminary step of Rab7 imprinting process, which involves subsequent protein removal by EDTA and rebinding tests monitoring the optical behavior of MIP on PhC, including its imprinting efficiency and selectivity.

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Carbon nanofibers for electrochemical sensing fabricated by novel laser-induced carbonization of electrospun polyimide

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Carbon nanofibers (CNFs) are a class of material that gained significance due to their superior properties including excellent electrical and thermal conductivity, high aspect ratio and low percolation. This makes them a popular candidate for electrochemical sensors. The two main approaches to generate CNFs are chemical vapor deposition (CVD) and thermal treatment of carbon precursor nanofibers. However, the drawbacks of those methods are the high price and low yield in case of CVD and the laborious steps for thermal treatment, which include shortening, drop-casting and the use of adverse binding agents.[1][2] In contrast, we prepared high quality CNFs in a facile manner by electrospinning polyimide nanofiber mats on indium tin oxide coated polyethylene terephthalate (ITO-PET) with subsequent carbonization by the means of a commercial CO_2 laser. This lead to a 3D structure of laser-induced carbon nanofibers (LCNFs), where any desired electrode geometry can be generated in a fast way, making the process suitable for mass production. Successful carbonization was confirmed by FTIR and Raman spectroscopy, their high porosity and a large surface area was observed by electron microscopy. These LCNFs demonstrated superior electroanalytical performance when compared to other carbon electrodes. Furthermore, by simply doping the spinning solution with a metal acetylacetonate non-enzymatic sensing abilities of LCNF can be implemented. Thus, during the lasing process the respective acetylacetonate salt is transferred into a metal oxide which acts as a nanocatalyst.[3] The influence of the metal complex content on the spinnability, laser scribing (e.g. laser power and speed) and electrochemical behaviour were hence examined. It was found that introducing a certain amount of metal salt resulted in more homogenous electrospun nanofiber morphology and uniform LCNF at scribing due to enhanced heat dissipation. They were then applied for electrochemical biomolecule detection such as NADH or dopamine outperforming commercially available gold or screen-printed carbon electrodes in terms of sensitivity. Future studies will investigate their application toward breath analysis, wound healing studies and cell-based biomedical research.

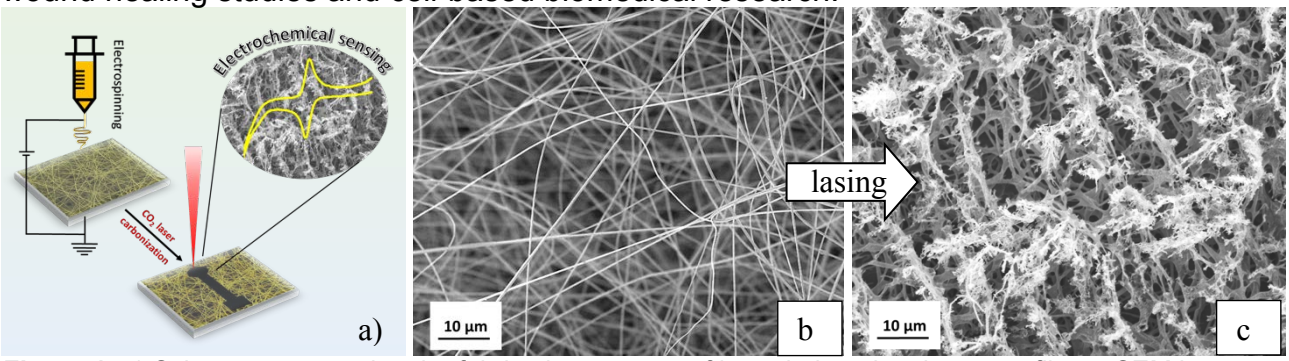

Figure 1. a) Scheme representing the fabrication process of laser-induced carbon nanofibers. SEM images of b) polyimide nanofibers and c) LCNF generated by laser scribing.

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Electrochemical discrimination of dopamine and related catecholamines at fluorine doped tin oxide

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Dopamine and its derivatives are involved in numerous signal transduction processes in brain, central and peripheral nervous system. Despite various other detection methods catecholamines are predestined for electrochemical sensing because of their redox properties. Based on their advantageous sensing characteristics carbon-derived materials are frequently used as sensor electrodes for dopamine determination. Dopamine metabolites and interfering substances in physiological samples often superimpose the dopamine signal at these electrodes by their oxidation currents. Furthermore, the formation of polymerization products of the oxidized dopamine causes a successive reduction of the sensor sensitivity. Hence, the electrochemistry of dopamine, its metabolites and common interferents is examined via differential pulse voltammetry (DPV) at fluorine doped tin oxide (FTO) based electrodes. These measurements reveal a well pronounced current peak for dopamine at +320 mV vs. Ag/AgCl. Compared to SWCNT/carbon electrodes, for the dopamine precursor L-dopa a significantly lower oxidation current is obtained, the degradation product methoxytyramine causes nearly no current signal. Additional investigations have been directed to common interferents such as ascorbic or uric acid, which can be found often in physiological samples. Both interfering substances generate nearly no oxidation signal at the FTO electrode, even though concentrations up to 1 mM are added. One disadvantage of numerous electrode materials is the loss of signal intensity at consecutive dopamine measurements. In contrast to the repeated dopamine detection at SWCNT/carbon electrodes via DPV no decrease of the dopamine oxidation current can be observed during consecutive DPV measurements at FTO (1). Considering the good linearity and high stability of the dopamine signal and the discrimination capability between different catecholamines and versus interferents, FTO facilitates the development of reusable dopamine sensors and the integration of dopamine detection in continuously working detection systems.

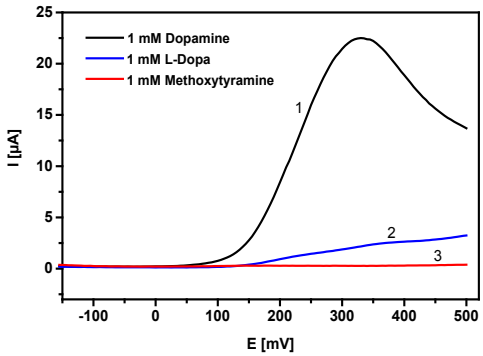

Figure 1. DPV-signal of 1 mM dopamine (1), 1 mM L-Dopa (2) and 1 mM methoxytyramine (3) in 20 mM potassium phosphate (pH 7.2) at FTO electrodes.

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Versatile electroanalytical bioplatforms for the determination of cancer-related DNA 5-methyl- and 5-hydroxymethyl-cytosines both at global and gene-specific levels

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Methylation is an important epigenetic DNA modification governing gene expression that may serve as an important indicator for the initiation and progression of cancer [1]. Apart from the modification of cytosine (C) to 5-methylcytosine (5-mC), the most studied epigenetic alteration, the Ten-Eleven Translocation (TET) enzymes mediate sequential oxidation of 5-mC to 5-hydroxymethylcytosine (5-hmC).
Locus-specific DNA hypermethylation of relevant tumor suppressor genes at the promoter region leads to their silencing and have been proposed as prognostic and diagnostic cancer biomarkers [2]. Unlike locus-specific changes, alterations of these both epimarks (5-mC and 5-hmC) at the global level are rapidly gaining prominence to recognized subsets of cancer patients with distinct clinical outcomes. The available techniques for mapping of DNA methylation (direct chemical identification, endonuclease digestion, bisulphite conversion, and affinity enrichment) require expensive equipment, long analysis times and highly skilled operators, and are unable to distinguish between 5-mC and 5-hmC.
This communication will discuss the development of versatile electrochemical biosensing platforms for the direct and PCR-independent evaluation of the presence of 5-mC or 5-hmC both at global and gene-specific levels. The implemented strategies rely on the use and coupling of functionalized magnetic beads (MBs), antibodies against the target methylated cytosines, specific DNA probes and amperometric detection at screen-printed carbon electrodes (SPCEs) [3]. The developed methodologies provide sensitive and selective determination of the target methylated DNAs in 45-90 min with a great reproducibility throughout the whole protocol. In addition, the simultaneous detection of the same or different types of cytosine methylations both at global level and in different regions/loci of the same gene or in different genes has been demonstrated to be feasible. The application of these methodologies to the determination of global methylations in genomic DNA extracted from paraffin-embedded tissues from colorectal cancer patients, and of specific methylations in the promoter region of two relevant tumor suppressor genes (*RASSF1A* and *MGMT*) in genomic DNA extracted from colorectal cells and directly in serum samples from cancer patients will also be shown.

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A flexible mechanical bending concept for smart hydrogel-based biosensing

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Smart hydrogels are three dimensional crosslinked polymer networks which react to an environmental stimulus (e.g. temperature, pH, concentration of a target analyte) by a change of their swelling state [1]. They are excellent candidates for biomedical sensing applications due to their inherent biocompatibility and the potential of tailoring them for a desired target analyte [2]. However, their current use for this purpose is limited due to the challenge of reliable, robust and sensitive detection of the smart hydrogel's swelling state, especially in a biomedical context. We have recently developed a mechanical bending sensor platform which addresses this challenge by employing a polyimide thin film with embedded metallic lead structure. The smart hydrogel is placed on one side of this sensing structure. Swelling or shrinking of the hydrogel alters the bending of the sensing structure, thereby changing the electrical impedance of the embedded metal traces. Upon sensor calibration, the impedance change can be related to the target analyte concentration. This concept has the advantages of: (i) being independent of the target analyte as the sensing structure can be equipped with any type of smart hydrogel, (ii) biocompatibility, (iii) geometric flexibility allowing for adaption to many different geometries and spatial requirements and restrictions and (iv) potential for wireless read-out as the sensing principle is based on electrical impedance change [3]. Having demonstrated the viability of the concept in proof-of-principle experiments, our current work is focused on optimizing the sensing structure with respect to maximizing the bending response and consequently the electrical signal while at the same time tailoring the smart hydrogel for a fast response. Our target application is the incorporation of the sensor into a standard catheter as depicted in figure 1. The thin film sensor will be small enough to not obstruct the catheter, hence, the catheter can be used as before but with added sensing capability for target analytes in blood. These can include drugs and medication as well as endogenous analytes, such as glucose or thrombin.

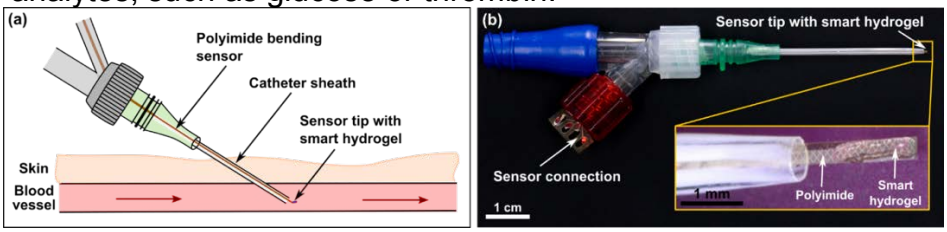

Figure 1. Concept for a sensing catheter: (a) Sketch of in-situ placement of catheter equipped with smart hydrogel sensor, (b) mock-up device with incorporated sensor structure.

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Florian Solzbacher declares financial interest in Blackrock LCC and Sentiomed, Inc. Jules Magda declares financial interest in Applied Biosensors LLC. Funded by the Joe W. and Dorothy Dorsett Brown and the Olive Tupper Foundations.

Colloidal nanoparticle modified graphene-based electrochemical platforms for small RNA determination

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Nowadays, graphene-modified electrodes and their application to electrochemical sensing have attracted enormous interest. The main advantages are a large potential window, fast heterogeneous electron transfer kinetics for various analytes and the involvement of additional “chemical features” in binding analytes (e.g. aromatic structures strongly connecting graphene via π−π stacking interactions). The decoration of graphene nanoflakes with Au nanoparticles (NPs) allows in certain cases the efficient improvement of the device sensitivity, thanks to the enhancement of the electron transfer kinetics, or the capability of catalytic reactions with certain analytes of biological interest (e.g. hydrogen peroxide). DNA sensing is an interesting application of the Au NP/graphene hybrids. The behaviour of a novel AuNPs-graphene nanostructure was, herein, investigated towards electrochemical sensing of human metabolites [1], and towards the development of a DNA-based biosensor (genosensor). An *in situ* colloidal chemistry approach has enabled to achieve a high density and very uniform coverage of the reduced graphene oxide nanoflakes with the Au NPs. The novel colloidal hybrid nanostructures result processable from solution, and, thus, suited for the modification of Screen-Printed Carbon Electrodes (SPCEs) towards original sensing platforms. The novel electrochemical platform was evaluated for the development of a genosensor for the detection of miRNAs, a family of small, non-coding RNAs that are considered important clinical biomarker candidates for many diseases, including cancer [2]. In particular, the DNA capture probes were immobilized onto the electrode surfaces, and afterwards, hybridized with the target biotinylated miRNA. The biosensing platform was then incubated with a streptavidin-enzyme-conjugate and exposed to a proper substrate. The product of the enzymatic reaction was electrochemically monitored by DPV, and a LOD of 0.7 pM (7 amol in 10 µL), with an average percentage standard deviation (RSD) of 13%, in a range of 1 – 5000 pM, was achieved. Spiked serum samples were also analyzed.

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An origami paper-based lab-on-a-chip for precision medicine in Alzheimer disease

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The rapidly aging of the population has increased the number of patients affected by several pathologies correlated to the aging, including the neurodegenerative disorders such as Alzheimer and Parkinson diseases. Consequently, the last decades have seen an increase of higher mortality as well as morbidity rates and healthcare costs for treatment, hospitalization and care assistance for this type of disorders. To this regard, the cost for managing patients affected by neurodegenerative disorders has been estimated to be approximately € 130 billion/year [1].
The recent trends of precision medicine has boosted the personalization of medical care in several field including neurological diseases, thanks to new diagnostics and therapeutics developed and discovered, respectively [2].
In this overall scenario, we propose a novel paper-based lab-on-a-chip for to deliver a cost-effective and easy to use sensing tool for a customised administration of drugs for Alzheimer disease. Among several drugs, we have designed the device for evaluating the efficiency of alkaloid compounds (e.g. Physostigmine), used for Alzheimer disease treatment. These compounds are employed being able to inhibit in reversible way the cholinesterase enzyme. Since the activity of cholinesterase is different among the patients, the administration of the customised amount of drug can improve the treatment and the quality of patient life, avoiding side effects due to the overdosage.
Herein, we propose a paper-based device to measure the cholinesterase activity in blood for evaluating the efficiency of Physostigmine inhibition. In detail, we exploited office paper to print the electrode and Vivid^{TM} Plasma Separation membrane to threat the blood sample as well as to load the reagents needed for the measurement, delivering a reagent free analytical tool. For cholinesterase activity measurement, butyrylthiocholine was used as enzymatic substrate and the by-product thiocholine was detected by using an office-paper screen-printed electrode modified with Carbon Black and Prussian Blue nanocomposite [3]. The use of this nanocomposite relays on its capability to electrocatalyse the oxidation of thiocholine allowing its detection at low applied potential (+300 mV vs. Ag/AgCl pseudoreference), without any fouling problem occurring at bare electrodes. For enzymatic measurement, several parameters have been optimised such as the concentration of substrate (i.e. 100 mM) and time of reaction (6 min), taking into account the sensitivity and the repeatability of the analysis. The calibration curve obtained in standard solution gave a linearity between 2 and 12 U/mL with a sensitivity of 0.246 ± 0.009 μA mL/U. The preliminary results obtained measuring the cholinesterase activity in whole blood will be reported.

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Identification of microorganisms by Fourier-transform infrared spectroscopy

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The need for novel techniques of rapid identification of pathogenic microorganisms arises from the massive spread of drug-resistant nosocomial strains and the emergence of centers for biohazard control. Fourier-transform infrared (FTIR) spectroscopy is a promising alternative to mass spectrometry as it is cost-effective, fast and suitable for field use. The aim of this work was to propose an algorithm for the identification of microorganisms in pure cultures based on the analysis of their FTIR spectra. The algorithm is based on the PCA-LDA approach: combination of Principal Component Analysis and Linear Discriminant Analysis. Unlike its analogues described in the literature, the algorithm is capable of identifying bacteria regardless of the culture medium or growth phase. The training sample included the most prevalent causative agents of infections and sepsis in humans: *Staphylococcus aureus* (n = 67), *Enterococcus faecalis* (n = 10), *Enterococcus faecium* (n = 10), *Klebsiella pneumoniae* (n = 10), *Escherichia coli* (n = 10), *Serratia marcescens* (n = 10), *Enterobacter cloacae* (n = 10), *Acinetobacter baumannii* (n = 10), *Pseudomonas aeruginosa* (n = 10), and *Candida albicans* (n = 10). The model we built successfully passed a series of blind tests involving clinical isolates of 10 methicillin-resistant (MRSA) and 10 methicillin-sensitive (MSSA) Staphylococcus aureus strains as well as pair mixes of these cultures with clinical isolates of *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae.* In addition, an R-code of one-step PCA-LDA implementation is provided. The one-step approach allows using initial wavenumbers as predictors and presenting the importance of each wavenumber for the identification, rather than difficultly interpretable principal components used by default.

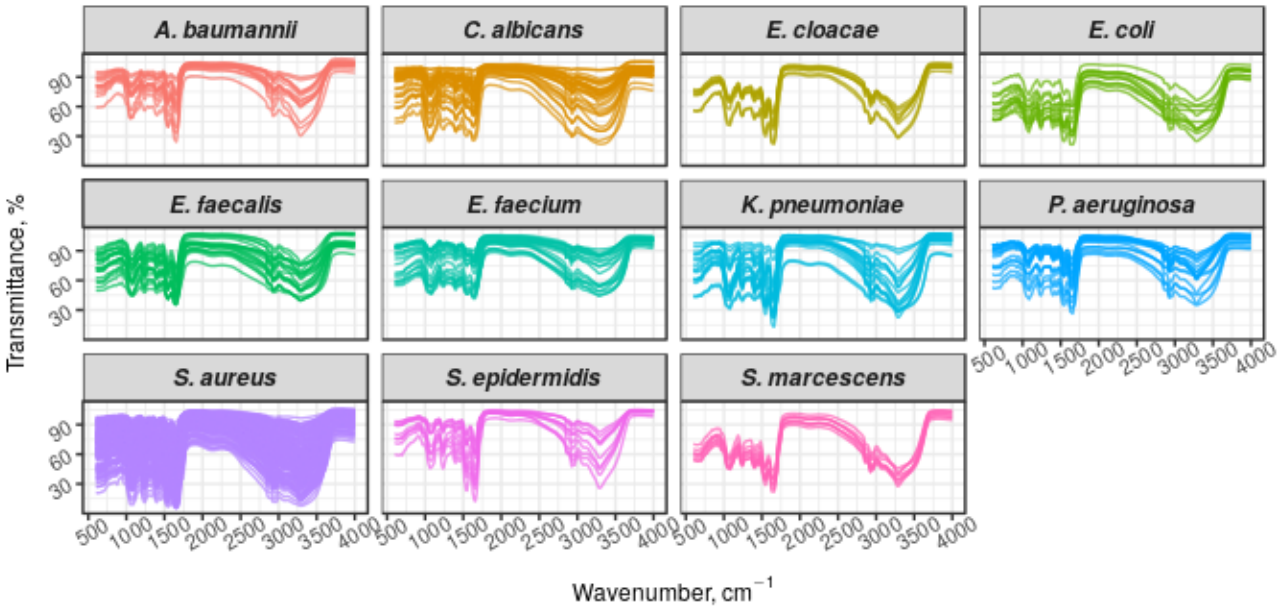

Figure 1. Initial FTIR spectra of agents of infections and sepsis in humans used for S. aureus identification.

Optimisation of a biocathode for O_2 reduction based on multi-walled carbon nanotubes and laccase

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In a recent article, Mano and de Poulpiquet reviewed O_2 reduction in enzymatic fuel cells based on multicopper oxidases such as bilirubin oxidase or laccase [1]. Miscellaneous electrode configurations based on these enzymes have already been presented in the literature encompassing direct or mediated electron transfer, various immobilisation matrices, and diverse electrode materials and structures.
In this context, we present the **optimisation** of a biocathode for O_2 reduction with respect to the **composition of the immobilisation matrix**. The enzyme was immobilised on a glassy carbon electrode by crosslinking. No mediator was employed, and a **direct electron transfer** between multi-walled carbon nanotubes (MWCNT) and laccase was established. The MWCNTs were dispersed with chitosan by ultrasound treatment. The immobilisation matrix contained a mixture of **MWCNT**, **laccase** from *Trametes versicolor*, and poly(ethylene glycol) diglycidyl ether as **crosslinker**. The total amount of the immobilisation mixture (equivalent to the film thickness) as well as the portions of the individual components were varied.
The performance of the biocathode was tested in buffer solution purged with nitrogen and pure oxygen, respectively, while a potential of 0.4 V vs. Ag/AgCl was applied (Fig.1).

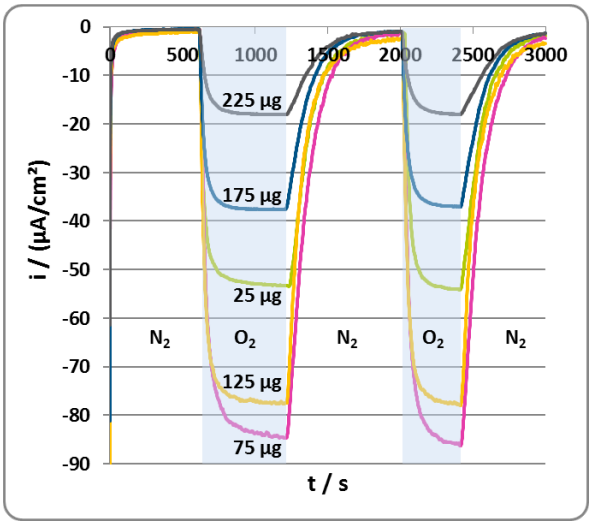

Figure 1. Amperometric response of the biocathode to nitrogen and pure oxygen @0.4 V vs. Ag/AgCl in 0.1 M citrate buffer pH 4.5. Variation of the amount of laccase, whereas the portions of MWCNT and crosslinker are constant.

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Conductive imprinted polymers for the direct electrochemical detection of β-lactam antibiotics: the case of cefquinome

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Biomimetic materials, such as molecularly imprinted polymers (MIPs), answer perfectly the demand of tuneable electrode modifiers, required for the design of electrochemical biomimetic sensors for the selective detection of emerging contaminants, such as β-lactam antibiotics. MIPs can be directly integrated with the electrode surface by electropolymerization. If the electropolymerization gives rise to conductive polymers a direct electrochemical detection of an electroactive analyte should be possible. The selectivity provided by the key-lock mechanism of MIPs cavities will add up to the specific electrochemical signal of the target leading to highly selective sensors. As a proof-of-concept for the realisation of a biomimetic sensor array for β-lactam detection in milk, a MIP-sensor for cefquinome (CFQ) detection was designed. CFQ is a fourth generation cephalosporin with a specific electrochemical signal related to its 2-amino-5-thyazolyl acetamido substituent. The selection of the monomer for CFQ-MIPs, namely 4-aminobenzoic acid (4-ABA), was based on a rational design screening of electropolymerizable monomers performed with Sybyl 7.3 software package. A broad electropolymerization study was carried out to map the pH-dependence in relation to the electrochemical properties: conductive poly(4-ABA) can be obtained at pH 1 and 7, while for intermediate pH values the polymer results to be isolating. Aiming to exploit a direct electrochemical detection, CFQ MIPs were synthesized by electropolymerization at pH 1 (0.1 M sulphuric acid) performing seven consecutive voltammetric cycles (scan rate 50 mV/s), with a monomer:target ratio of 5:1 on multi-walled carbon nanotubes graphite screen printed electrodes. Under optimum conditions, the lower cefquinome concentration detectable on the modified electrodes was 50 nM in 0.1 M PB pH 2, not far away from the CFQ maximum residue limit in milk of 38 nM established by the EU.

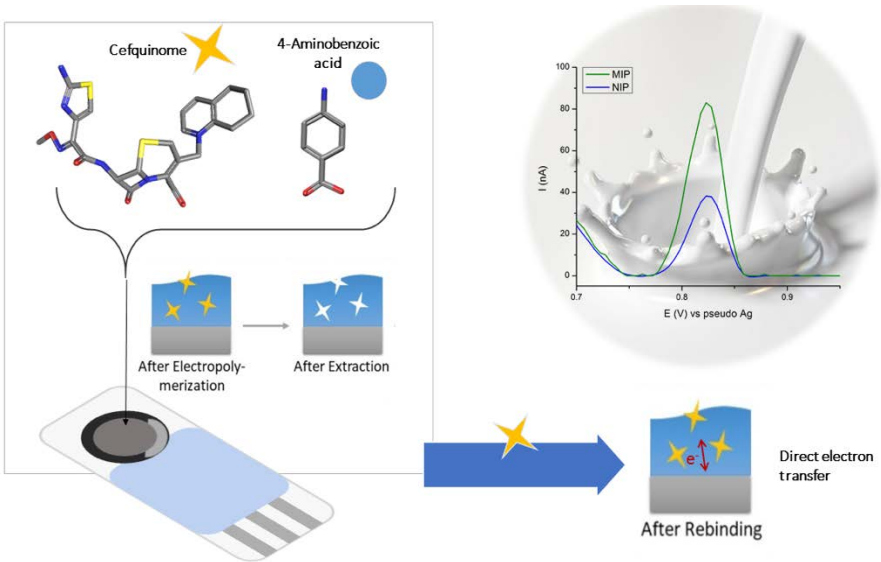

Figure 1. Graphical abstract: Graphite screen-printed electrodes modification with molecularly imprinted polymers followed by template-target removal (left side) and the target semi-quantitative determination (right side).

Study on hybridization of abasic DNA to gold-fixed capture probe

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DNA consist of a sugar-phosphate backbone which is linked to bases giving its unique structure and function. However, external environmental factors such as radiation or genotoxic agents can result in a hydrolytic cleavage of the glycosidic bonds leaving behind deoxyribose residues in the DNA strand without bases. Such DNA strands with at least one missing base are called abasic DNA, which can trigger lethal effects on the organism if not fixed. This has increased the medical demands for easy and quick nucleic acid detection devices, allowing the discrimination of abasic sites. Previous approaches have often exploited the labelling of the abasic site for application in optical assays.[1] However, since the labelling process is time consuming and costly, the development of label-free approaches based on electrochemical methods have gained great interest for the construction of DNA sensors.
Electrochemical impedance spectroscopy has already shown the potential for label-free determination of DNA hybridization.[2] Here, major efforts are focussing on the sensitivity, single base pair mismatches and the influence of the target DNA length and recognition sequence position,[3–5] while the influence of abasic sites within the target on the impedimetric signal behaviour haven't paid much attention yet.[6] Therefore, we have investigated the binding behaviour of three different 25mer abasic strands at 25mer probe DNA modified electrodes and compared the results with the hybridization signal of fullmatch target DNA. After hybridization, the chosen ssDNA introduce either an abasic site exposed to the solution ($abasic_{sol}$), in the middle ($abasic_{mid}$) or near to the electrode ($abasic_{el}$). The sensor consists of short thiol-modified ssDNA, which is attached to a gold chip electrode with a short mercapto-alkanol layer to prevent unspecific interactions between the DNA and the gold surface. The impedimetric measurements are performed in the presence of the redox system ferri-/ferrocyanide and show an increase in charge transfer resistance (R_{ct}) upon hybridization of fullmatch target ssDNA. Also for all abasic ssDNAs an increased R_{ct} is found after hybridization, demonstrating the successful formation of dsDNA. However, none of the abasic ssDNA reaches the same maximum impedance change upon hybridization as compared to the fullmatch ssDNA. The maximum response of $abasic_{el}$ and $abasic_{mid}$ ssDNA have been found to be about 40 % smaller than the fullmatch ssDNA. $Abasic_{sol}$ ssDNA reaches even only 30 % of the fullmatch ssDNA signal, indicating a different hybridization efficiency of the abasic ssDNAs or the formation of different DNA structures on the surface. The results suggests that impedance spectroscopy give rise to the label-free discrimination between abasic and fullmatch ssDNA, but also seems to allow a rough estimation of the abasic site position within the target strand. To better understand of the effects of abasic sites on the impedance behaviour, SPR experiments have been performed giving information about the hybridization efficiency of the different abasic ssDNA targets.

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Study of the effect of gold nanoparticles on PEDOT modified electrodes for electrochemical biosensing of thiol compounds using continuous flow system.

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Chemical modification of electrodes by nanostructured materials (i.e. gold nanoparticles) have been widely studied in recent years due to their unique electrochemical properties and biocompatibility [1]. Besides, the poly(3,4-ethylenedioxythiophene) has been broadly used in electrodes modifications due to its high electrical conductivity, charge injection capabilities, low impedance and biocompatibility, moreover, PEDOT allows the immobilization of biostructures like enzymes, which is of high significance in biosensors and biomedical devices [2, 3]. Therefore, electrochemical techniques as Electrochemical Impedance Spectroscopy (EIS), Square Wave Voltammetry (SWV) and Cyclic Voltammetry (CV) have been applied in order to study the electrochemical behaviour of gold nanoparticles-PEDOT modified electrodes in the detection of thiol compounds, namely n-Acetyl Cysteine in a continuous flow microfluidics system. Microfluidic systems enhance the flow transport, the mixing rate of reagents, the sensitivity due to the diffusion phenomena, meanwhile is reduced the sample volume [4, 5]. The electrode was characterized by Raman Spectroscopy, Transmission Electron Microscopy (TEM) and Electrochemical Atomic Force Microscopy (EC-AFM), allowing to understand the arrangement of the nanoparticles at the surface along with its electrochemical activity at nanoscale. Overall, the unique properties of the obtained coating are of high significance for the biosensing of thiol components in biosensors devices.

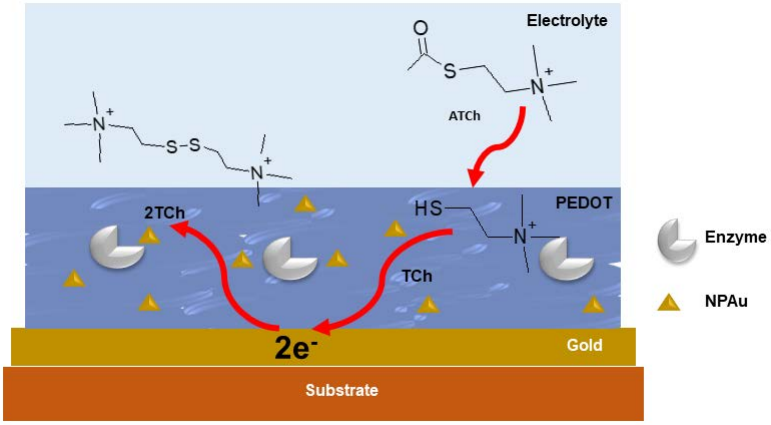

Figure 1. Gold Nanoparticles-PEDOT modified electrode.

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An enzymatic biosensor for real-time monitoring of urea during dialysis

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Cost and reliability represent major determinants for introducing new technologies in clinical settings. In this paper, we present the fabrication, testing and validation of cheap and disposable sensor exploiting a combination of graphene oxide and urease to measure parameters such as pH and urea. The possible application of these sensors for monitoring patients undergoing dialysis is discussed. A patient at the fifth stage of chronic kidney disease usually needs to be dialysed or a kidney transplantation. The adequacy of dialysis can be assessed by the dimensionless parameter Kt/V that depends on the urea concentration in blood.
A graphene oxide dispersion in water was prepared by a modified Hummers' method and deposited onto a graphite working electrodes previously screen printed on a PET film. A solution containing urease was then drop cast on one of the activated (EDC/NHS) working electrodes. The open circuit potential existing between each working electrode and a reference silver/silver chloride electrode was measured by a potentionstat/galvanostat (Palmsens 4).
The pH sensor was calibrated by certified buffer solutions, whereas solutions at different urea concentrations in the range (25 - 500 μM) were used for the urea sensor. This latter exploits the graphene oxide sensitivity to pH to detect the ammonia produced upon the reaction of urea with urease. In-vitro validation with model solutions established accuracies of ±0.2 pH units (range 3-10 pH units) for the pH biosensor, whereas the urea biosensor's intra-day and inter-day precisions in model solutions were 5 and 10% respectively. The biosensor showed a detection limit of 25 μM. The biosensor capability to monitor the efficiency of dialysis was tested with plasma samples collected from five patients undergoing this treatment. Biosensor measurements were in good agreement with values supplied from the analytical chemistry laboratory, with an average deviation of about 6 ± 3%.
Biosensor was able to follow the trend of urea concentration over time according to the equation:

$$C = e^{-\frac{Kt}{V}+const}.$$

The combination of a pH sensitive material and enzymes catalysing reactions producing acidic or alkaline species can be exploited to develop large arrays of sensors. The simple and reproducible fabrication procedure is suitable for scaling at an industrial level, to obtain cheap enough sensors to be disposed after single use.

Development of an impedimetric aptasensor for TNF-α detection

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Deregulated levels of tumour necrosis factor alpha (TNF-α), a pro-inflammatory cytokine, is associated to the progression of a variety of pathological processes, such as sepsis, heart failure, rheumatoid arthritis, diabetes, chronic wounds among others, leading to recognize TNF-α as an important biomarker. Nowadays, its identification and quantification is mainly performed by conventional techniques such as ELISA, which are costly, present a limited shelf-life, require skilled personnel and appropriate instrumentation. So, there is the clinical need for the development of new techniques. Here we describe the development of an aptamer-based impedimetric biosensor for measuring TNF-α that combines the specificity provided by DNA aptamers and the sensitivity of Electrochemical Impedance Spectroscopy (EIS), i.e. a label-free, low-cost, non-disruptive method.
Two DNA aptamers (VR11 and VR20), which mimic the inhibitory action of an anti- TNF-α antibody, selected by Systematic Evolution of Ligands by EXponential enrichment (SELEX), were immobilized by a thiol group on a gold electrode surface. A TNF-α solution in PBS (Phosphate Buffered Saline) was incubated in presence of the aptamer-based biosensor and electrochemical impedance measurements were performed by using a potensiostat/galvanostat PalmSens 4.
Preliminary data demonstrate that this sensor is able to specifically recognize TNF-α in the range of 20-100 pg/mL, suggesting its applicability for the monitoring of pathological conditions such as heart failure. Future studies will focus on the optimization of sensor performances and its application to biological samples.

Influence of a non-ionic surfactant on the biotin-streptavidin binding on gold surfaces

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Biotin-Streptavidin is well known for its strong non-covalent interaction and often used as a model system to characterize biosensors. When embedding the biotin-streptavidin system in a digital microfluidic (DMF) platform, which is based on the electrowetting effect, biofouling of the proteins appears to the hydrophobic Teflon surface of the device. A common technique to avoid biofouling is the addition of surfactants like Pluronic F-68 [1, 2].
Our overall goal is the development of a DMF platform with integrated electrical impedance spectroscopy (EIS) sensors. Here, the influence of Pluronic F-68 on the binding of the biomolecules to gold electrodes is of pivotal interest. All proteins were dissolved in phosphate buffered saline (PBS, pH 7.4). As a reference, a protocol has been used binding a SAM (a thiolated biotin, 0.5 mM) to the gold surface, blocking the uncovered surface with bovine serum albumin (BSA, 2 mg/ml), binding streptavidin (STR, 0.01 mg/ml) to the biotinylated surface and finally a biotinylated horseradish peroxidase (POD, 1:40,000 in PBS). Each reagent was incubated for 30 minutes. For analysis, 3,3′,5,5′-tetramethylbenzidine (TMB) was added as a substrate for the POD. 0.05% w/v Pluronic F-68 was added to the SAM and protein solutions and is marked with a small “p”. The last reaction was stopped by hydrochloric acid (3M) after 3 minutes. The colour change was detected using a photometer at 450 nm. Negative controls were carried out skipping one layer. Figure 1a) shows the absorption of oxidised TMB for positive and negative control pointing out that the build-up of the protein stack is not affected by Pluronic F-68.Droplet movement in DMF platforms is based on contact angle modulation by applying an electric field [3]. Thus, the effect of 0.05% w/v Pluronic F-68 in the PBS buffer solution has been studied by contact angle measurements on a Teflon surface in air. Figure 1b) illustrates the decrease of the contact angle from 119.1° (PBS) to 101.1° (PBSp) which is still sufficient for DMF platforms and droplet movement due to contact angle modulation.

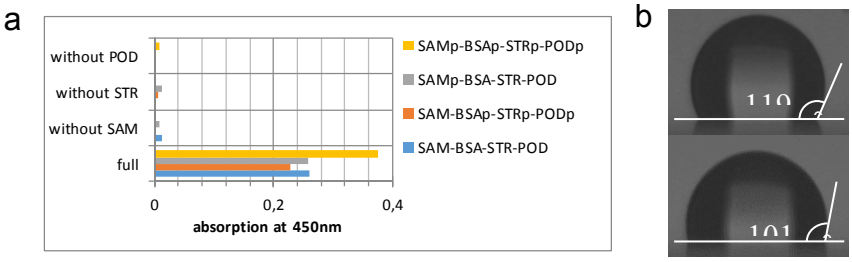

Figure 1. (a) Adsorption of TMB at 450 nm of positive and negative control of the biomolecule stack. (b) Decrease of contact angle of a PBS buffer solution droplet due to addition of Pluronic F-68 from 119.1° (PBS) to 101.1° (PBSp).

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Urea-sensitive biosensors based on stimuli-responsive hydrogels and the functionalized enzyme urease.

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Especially in biomedical analytics, the specific detection of very low analyte concentrations is indispensable for therapy and diagnostics. Enzymes like glucose oxidase for the detection of the blood sugar level are of crucial importance for the development of new miniaturized lab-on-chip sensor systems.
Stimuli-responsive hydrogels are three-dimensional polymer networks that can swell or deswell in dependence of a certain stimulus. By selecting of appropriate monomers during the polymerization, different kinds of hydrogels can be synthesized which depend on particular quantities such as pH, salt or temperature [1]. The resulting swelling pressure can be transformed into a measurable output voltage by using piezoresistive pressure sensors. Here, the swelling deflects the bending plate of the pressure sensor and, hence, deforms the piezoresistors integrated in the plate. This leads to a change in resistance due to the piezoresistive effect, which can be translated into a change in output voltage via a Wheatstone bridge circuit. Based on this concept, numerous hydrogel-based sensors have already been developed, e.g. for the detection of pH value [2], ethanol concentration [3] or glucose concentration [4].
Hydrogels can be easily functionalized by incorporating enzymes. This makes such hydrogel-based sensors particularly interesting for the construction of hydrogel-based biosensors. This contribution presents a new kind of stimuli-responsive hydrogel based on pH-sensitive hydrogels and functionalized by hydrolase urease for the detection of urea. The enzymatic reaction of the immobilized urease leads to an alkaline pH change [5], which results in a swelling of the pH-sensitive hydrogel. Based on this hydrogel system, even small urea concentrations can be detected with a high sensitivity and substrate specificity to other urea derivatives. The contribution will introduce the operational principle, will explain the enzyme-based functionalization of the hydrogel and will show experimental results of this novel piezoresistive biosensors for the detection of urea.

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Electrochemical biosensor for TMAO detection with a chimeric enzyme

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Trimethylamine *N*-oxide (TMAO) is an organic end-product originating from the degradation of dietary trimethylamine containing compounds like choline, carnitine and lecithin. In recent years this molecule has emerged as a potential biomarker for several disease states.
In the presented project, we report the development of the first enzyme-based electrochemical biosensor system for determination of trimethylamine *N*-oxide (TMAO). It employs an active chimeric variant of the TMAO reductase enzyme TorA. Such design of recognition molecules represents a generic approach for new biosensors.
The TMAO reductase TorA is a molybdoenzyme found in marine and most enterobacteria that catalyzes specifically the reduction of TMAO to trimethylamine. The chimeric TorA named TorA-FDH corresponds to the apoform of TorA from *Escherichia coli* reconstituted with the molybdenum cofactor from formate dehydrogenase (FDH). TorA and TorA-FDH were immobilized on the surface of a carbon electrode and protected with a dialysis membrane. The biosensor can be operated at an applied potential of -0.8 $V_{Ag/AgCl\ (1M\ KCl)}$ under ambient air conditions thanks to an incorporated biocatalytic deoxygenation system. In the presence of TMAO the cathodic current generation due to the reduction of a low potential mediator for effective regeneration of the enzyme is recorded and evaluated. A comparison between the biosensors utilizing either the wild type enzyme TorA or the chimeric enzyme TorA-FDH revealed a much higher sensitivity for the biosensor with immobilized TorA-FDH. This biosensor exhibits a sensitivity of 14.16 nA/µM TMAO in a useful measuring range of 2-110 µM almost similar for TMAO in buffer and in spiked serum samples. The serum measurements show that a detection of TMAO in human serum can be performed without interferences from the complex serum matrix. The short-term usage stability is also quite good. The sensor can operate over prolonged daily measurements. The described enzyme-based biosensor represents a promising approach for the future determination of TMAO levels in human blood samples.

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Simulation and optimization of a screen-printed electrode for chronoamperometric detection of biogenic amines

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In the past few years, a probable relation of the consumption of biogenic amines in food and certain symptoms like headache and diarrhea has been reported. Due to the lack of a reliable detection method, clinical studies are difficult to perform. This study aims to develop a quick sensor for the detection of biogenic amines in food. A high promising approach is the chronoamperometric measurement. It is based on the chemical oxidation or reduction of the requested analyte at an electrode. After application of voltage between two electrodes, the measured current changes by the oxidation or reduction of the analyte. This change can be measured by a high sensitive potentiostat (Figure 1). The amount of the generated current can be used to conclude on the original concentration of biogenic amine [1, 2]. In comparison to commercially available animal diamine oxidase (DAO), enzymes isolated from plants promise higher activity [3]. In our project, new DAOs are isolated from plant seedlings (peas, maize) and characterized. These new enzymes are used to oxidize the biogenic amine. Simultaneously, hydrogen peroxide is produced (Figure 1). Furthermore, a simulation study is carried out to get a better understanding of the chemical process and to optimize the electrode for chronoamperometric detection of hydrogen peroxide. Michaelis Menten kinetics is used to compute the amount of oxidized TMB after reduction of hydrogen peroxide (Figure 2). Additionally, a 3D model is generated in Comsol Multiphysics. Diffusion of the species is taken into account to simulate the accumulation of the detected oxidized substrate at the working and counter electrodes. The effect of electrode material and geometry on the sensitivity of the method is investigated. The results of this computational study can be used to optimize the chronoamperometric measurement setup.

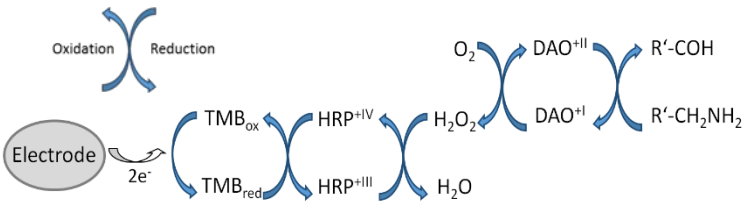

Figure 1. A redox cascade is used for the indirect detection of biogenic amines.

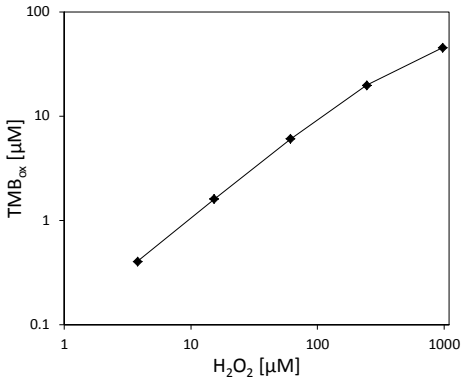

Figure 2. Generated TMB_{ox} after 5 minutes in dependency on the initial H_2O_2 concentration).

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SPR sensing in light diffusing fibers

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Surface plasmon resonance (SPR) is probably one of the most suitable methods for label-free and real-time detection of biological/biochemical binding reactions [1, 2]. Compared to traditional prism-based design, fiber-optic SPR probes are useful for remote sensing, continuous analysis and in situ monitoring. Numerous versions have been introduced, in an attempt to optimize the sensitivity of the sensor platform [3-6]. Herein we demonstrate a novel fiber-optic SPR sensor using a light diffusing optical fiber as the sensing element. The advantage of the proposed configuration is that SPR probe fabrication only requires metallization of the fiber cladding surface, i.e. no additional step is required to expose the core. Light diffusing fibers (LDF) are designed with light scattering centers in the core of the fiber, providing very efficient scattering of light through the sides of the optical fiber along its length [7]. When a thin metal film is deposited on the fiber cladding surface, and in presence of an aqueous medium in contact with the metal film, some of the scattered light rays reaching the fiber surface satisfy the phase matching condition for SPR excitation, thus leading to a decrease of the transmitted light at certain wavelengths. When the refractive index of the aqueous medium changes, the resonance wavelength changes as well. The chosen LDF (Fibrance® by Corning®) has a core diameter of 170 μm, a cladding diameter of 230 μm, and a numerical aperture > 0.5. The SPR probe consisted of a 2 cm length of LDF fixed over a microscopic slide, with the upper surface sputter-coated with a 60-nm thick gold film. The sensing probe was coupled to the white light emitted by a tungsten halogen light source (HL2000, Ocean Optics), while the transmitted light was collected by a spectrophotometer, with a detection range from 350 to 1000 nm (USB4000 VIR-NIR, Ocean Optics) (Fig. 1a) and results in the typical spectral resonance SPR-dip [5,6]. Figure 1(b) shows the experimentally obtained SPR transmission spectra, normalized to the spectrum recorded with air as the surrounding medium, for different water-glycerine solutions with refractive index ranging from 1.332 to 1.396. From quadratic fitting (Fig. 1c), we observe that the sensitivity of the SPR-dip wavelength to refractive index ranges from ≈ 1500 to ≈ 4000 nm/RIU in the analysed range [8].

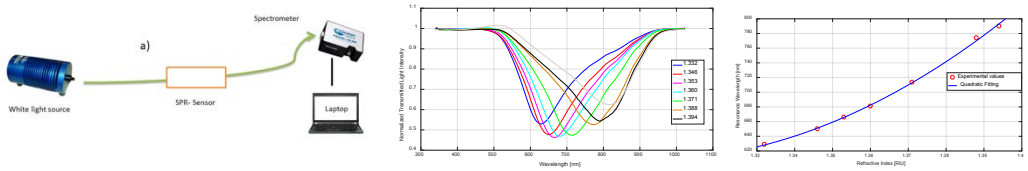

Figure 1. a) Sketch of the experimental setup. b) SPR transmission spectra, normalized to the air spectrum, for different refractive index of the aqueous medium. c) SPR wavelength as a function of the outer refractive index.

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Novel amperometric genosensor for determination of non-amplified GM soy DNA: a comparison of carbon nanotubes and glassy carbon as electrode substrates for immobilization of PNA probes

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Nucleic acid-based biosensors (genosensors) have received great attention in the past decade, being nucleic acids promising molecular probes due to the specificity for base pairing. In a research program dealing with the development of innovative sensors as analytical tools for assessing food safety [1], we combined performance of Peptide Nucleic Acids (PNA) probes with the enhancing properties of single-walled carbon nanotubes embedded on screen printed electrodes (SWCNT-SPEs). A comparison of the responses with analogous non-nanostructured glassy carbon platforms (GC-SPEs) was performed. A PNA-based Capture Probe (CP), with sequence complimentary to a 20-mer portion of "Roundup Ready" transgenic Soy DNA, was covalently immobilized on the electrode surface, exploiting the carboxylic group natively present on CNTs or electrochemically generated on GC-SPEs. A signalling PNA probe (SP), complementary to a different portion of the target DNA and bearing a biotin tag, was used in combination with a streptavidin-alkaline phosphatase conjugate to generate a three-probe sandwich leading to signals increasing with the target DNA concentration. In order to address the occurrence of nonspecific interactions between carbon substrates and the oligonucleotide probes [2], a blocking step with pyrene was introduced, which drastically increased the S/N ratio. The best performance was obtained on SWCNT-SPEs showing linearity in the 250 pM-1.75 nM range, with LOD and LOQ of 71 and 256 pM, respectively. By contrast, a linear response in the 1-10 nM range with LOD and LOQ values of 430 pM and 1.43 nM were obtained on GC-SPEs, thus confirming the enhancing properties of CNTs in the signal transduction leading to a 4-fold increase in sensitivity. Method validation on genomic DNA extracts from Soy flour containing variable percentages of genetically modified (GM) Soy was carried out, proving the discrimination capability of the genosensor towards GM material at trace levels.

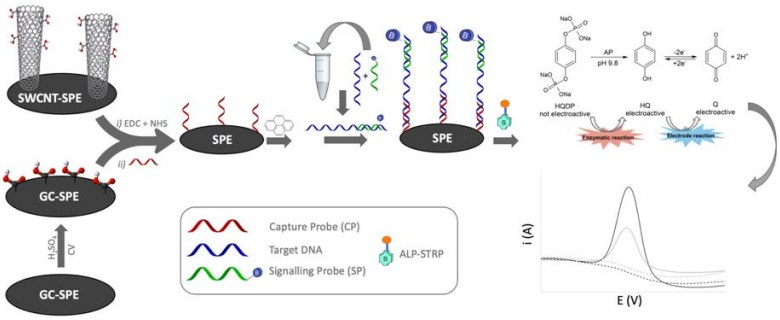

Figure 1. Scheme of the genosensor setup and read-out

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Development and characterization of a cellular biosensor for the screening of globin gene inducers useful in β-thalassemia

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The screening of molecules able to induce the production of fetal or adult hemoglobin in erythroid cells isolated from β-thalassemia patients is very important for the identification of compounds of interest for the pharmacological therapy of β-thalassemia, a genetic disease characterized by insufficient production of β-globin chains of adult hemoglobin (HbA) and severe anemia. In particular the stimulation of human γ-globin gene expression and increase of production of fetal hemoglobin (HbF) is considered a potential therapeutic approach both in β-thalassemia [1] and in sickle cell disease (SCD). In this study we report the development and characterization of a cellular biosensor for the identification of inducers of γ- and β-globin gene promoters. To this aim we first produced a vector containing green (EGFP) and red (RFP) fluorescence protein genes under the control of human γ-globin and β-globin gene promoters, respectively. Human K562 cells were used to obtain stable transfectants, where the transcriptional activity of globin promoters is proportional to the fluorescence signal produced by the respective protein [2], assessed by FACS (fluorescence activated cell sorting) analysis. The cellular model was then validated by treatment with hydroxyurea and sodium butyrate, two compounds known for their HbF inducing effects on thalassemic patients. Similar results were obtained after the administration of other compounds known for their stimulating effects on γ-globin gene in vitro and in vivo, such as mithramycin and ara-C, and new HbF inducers, demonstrating that our experimental system is suitable for a preliminary and fast screening of high numbers of compounds potentially active on the transcription of γ-globin and β-globin genes, possibly useful for β-thalassemia therapy. A good correlation was demonstrated between the results obtained using the EGFP/RFP clones and experiments performed on erythroid precursor cells from β-thalassemic patients (supported by AIFA, Wellcome-Trust and by EU projects ULTRAPLACAD and THALAMOSS).

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Biacore™ 8K and Biacore 8K+: Hardware that supports the need for efficiency in drug discovery

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Biacore 8K and Biacore 8K+ efficiently deliver high quality binding data while meeting tomorrow's challenges in small molecule and biotherapeutic screening and characterization. The eight-needle, high-sensitivity SPR systems rapidly provide kinetics, affinity and concentration data, shortening time to results by up to eight times compared to single-needle systems. The blend of system flexibility and throughput reduces the experimental cycle time, even for complex targets and new drug formats such as bispecific antibodies, providing a single solution for interaction analysis in both screening and characterization for small molecule and biotherapeutic discovery. The systems are well suited for analysis of samples from the smallest fragments to large multidomain proteins, even in crude matrices. Biacore 8K can host up to four microtiter plates and Biacore 8K+ up to twelve microtiter plates in a temperature-controlled sample and reagent compartment, ensuring that the integrity of samples is maintained. The option to queue multiple methods enables longer unattended runs with the possibility to load plates while the system is still running. Backed up with an intuitive evaluation software, rapid analysis of several runs can be made in one go, using either pre-set or user-defined evaluation methods. This poster introduces the key hardware features of Biacore 8K and Biacore 8K+.

Kinetic and concentration analysis in all directions with Biacore™ 8K and Biacore 8K+

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With the introduction of Biacore 8K and Biacore 8K+, new possibilities on how to perform kinetic and concentration analysis opens. This eight-needle high-sensitivity SPR system not only shortens time to results by up to eight times compared to single-needle systems, it also allows for kinetic and concentration analysis in parallel fashions. For instance, the new 2D Kinetics functionality offers detailed kinetics from only one sample cycle, thereby significantly reducing assay development time. For concentration measurements, the use of a parallel calibration curve provides shorter run times, which results in data with very good precision enhanced by interchannel normalization. This poster presents the high flexibility in assay set-up offered with Biacore 8K and Biacore 8K+ when determining binding rate constants, and how concentration analysis can be performed using calibration curves for each channel separately (serial approach) or with a calibration curve common for all channels (parallel approach).

Use of phospholipase A activity for detection of pathogenic bacteria *in vitro*

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Pathogenic bacteria use different methods in order to induce virulence or to survive in environmental niches. For example, *Legionella pneumophila*, the causative agent of the severe pneumonia Legionnaires' disease, secretes a variety of phospholipases, which contribute to bacterial propagation and development of disease symptoms [1]. This study aims to develop a quick sensor system for the detection of *L. pneumophila* using its phospholipase A activity. With these enzymes the bacterium is capable of cleaving added phospholipids *in vitro*, thus releasing free fatty acids that can be quantified via the NEFA-HR(2)-kit® (Fujifilm WAKO Chemicals, Neuss, Germany) [2]: The released non-esterified fatty acids are converted to Acyl-CoA and via several catalytic steps hydrogen peroxide (H_2O_2) is released. This yields in the presence of peroxidase and two dyes a blue purple pigment, whose absorbance can be measured at 550 nm. In our study, overnight cultures of different bacterial species were lysed and incubated with phospholipids (dipalmitoylphosphatidylcholine (DPPC) or dipalmitoylphosphatidylglycerol (DPPG)) at 37°C for up to 22 hours. Our results show, that DPPC is cleaved more efficiently than DPPG over time (Figure 1A), however, when comparing different aquatic bacteria, DPPG is cleaved more specifically by *L. pneumophila*. Out of 17 different bacteria, only *L. pneumophila and Acinetobacter baumannii* showed positive results after 22 h incubation time. *L. moravica* and *L. cherrii*, the two non-pathogenic *Legionella* species did not release any free fatty acids (Figure 1B). Therefore, the phospholipase system could be used for specific detection of pathogenic bacteria in water samples, and after integration into a biosensor model, for online monitoring of water devices in rural, hospital or private environments.

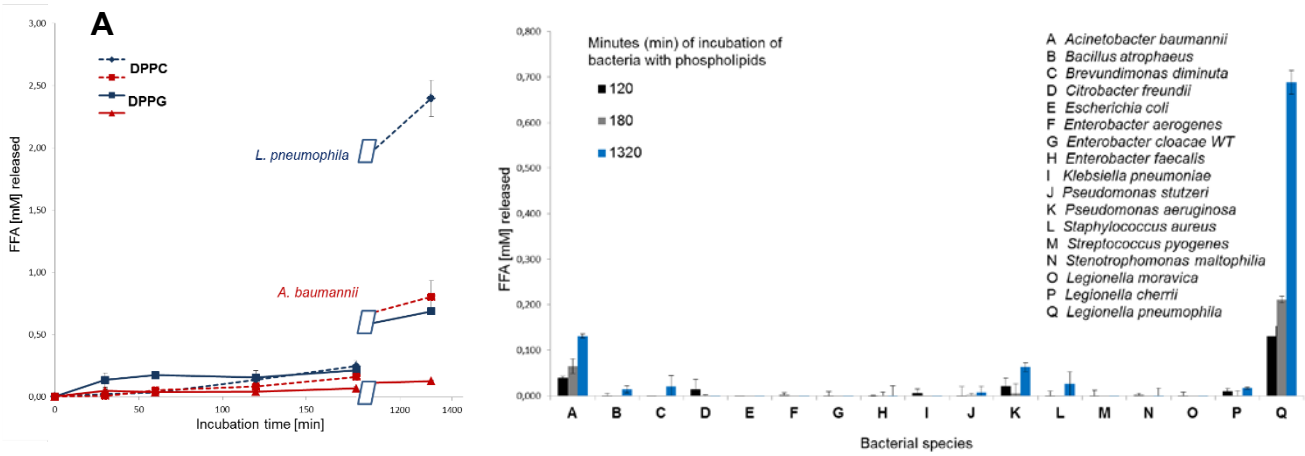

Figure 1: (A) Released fatty acids from DPPG and DPPC after 22 h. (B) From 17 tested water-borne bacteria *L. pneumophila* showed the highest release of free fatty acids cleaved from DPPG.

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Set up and application of an analytical protocol to control in real time the cleaning of painted surfaces of historical and artistical interest through “smart” devices

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Cleaning of painted surfaces of artistical and historical interest is one of the most critical step of every restoration workshop: it is an irreversible and invasive operation that must be carried out maximising its selectivity. Only the degradation patinas must be removed, minimising the impact on the materials that constitute the original work of art. To this purpose an optimal cleaning action must be as more as possible gradual and controllable by the conservator [1]. In order to satisfy these requirements, in the last decades, many chemical gels tailored for the cleaning of painted surfaces, have been set up and optimised [2], allowing an improvement of the cleaning performances respect to traditional physical gels.
On the other hand, one of the limits that still exist especially for the cleaning of easel paintings is the absence of a rigorous analytical protocol for the analysis in real time of the materials extracted during the cleaning. Then, the set up of a new rigorous analytical protocol to monitor the cleaning is mandatory. The attention has been focused on some innovative aqueous Highly Viscous Polymeric Dispersions (HVPDs) composed by polyvinyl alcohol covalently crosslinked by borax, that have been successfully used in the last years for the removal of degradation patinas from the surface of different kinds of works of art [3, 4].
The most important target of this work is to develop a method for the qualitative and semiquantitative fast monitoring of the materials extracted by the HVPDs based on the use of common smart devices like mobile phones and tablets. To this purpose plasmonic-based approaches will be presented.
In that way, the cleaning con be stopped immediately when the presence of materials appertaining to the original work of art is detected into the HVPD that was in contact with the surface to be cleaned.

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The BLI technology to measure protein, antibodies and small molecules interactions

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Bio-Layer Interferometry (BLI) is a label-free technology for measuring biomolecular interactions. It is an optical analytical technique that analyses the interference pattern of white light reflected from two surfaces: a layer of immobilized protein on the biosensor tip, and an internal reference layer (Figure 1a). Any change in the number of molecules bound to the biosensor tip causes a shift in the interference pattern that can be measured in real-time (Figures 1a and 1b). The binding between a ligand immobilized on the biosensor tip surface and an analyte in solution produces an increase in optical thickness at the biosensor tip, which results in a wavelength shift, Δλ (Figure 1c), which is a direct measure of the change in thickness of the biological layer. Interactions are measured in real time, providing the ability to monitor binding specificity, rates of association and dissociation, or concentration, with precision and accuracy.

Only molecules binding to or dissociating from the biosensor can shift the interference pattern and generate a response profile on the Octet® or BLItz® systems. Unbound molecules, changes in the refractive index of the surrounding medium, or changes in flow rate do not affect the interference pattern. This is a unique characteristic of BLI and extends its capability to perform analysis in crude samples and can be used in applications for protein:protein binding, biomolecules quantitation, affinity, and kinetics.

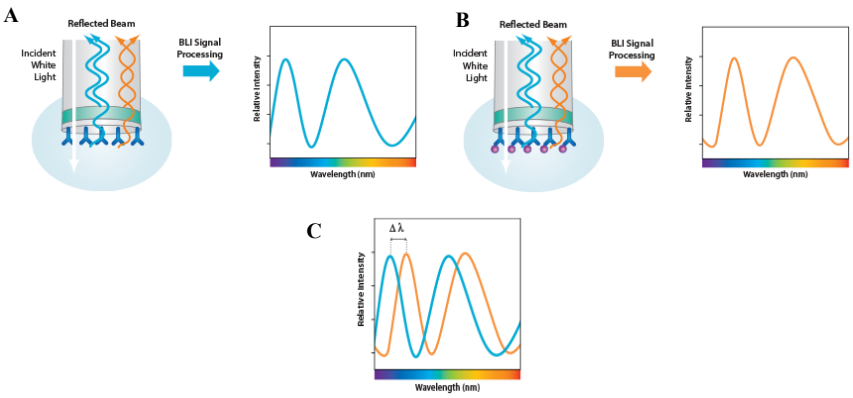

Figure 1. Working principle of BLI technology.

Colorimetric determination of total protein content in serum based on the polydopamine/protein adsorption competition on microplates

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Current methods for quantitative estimate of protein content show several limitations [1-4]. For example, the direct spectrophotometric quantitation based on the extinction coefficient of the protein at 280 nm is suitable for a purified protein only, suffering from the absorbance of any other macromolecule and for light scattering, and the sensitivity is strictly protein dependent as product of the content of aromatic amino acids. Analogously, the colorimetric assays based on copper reduction by peptide bonds and subsequent complex formation with bicinchoninic acid, or oxidative reaction with the Folin–Ciocalteau reagent (Lowry assay) are limited by the presence of several compounds like peptides, thiols and reducing sugars that lead to overestimate the protein content [2, 3]. In this context, here we introduce a facile analytical method for estimation of total protein content, even in complex matrix like human serum, based on the analysis of polydopamine film formation in presence of proteins, exploiting the competition between PDA and macromolecules for surface binding. The surface coating has been examined by using a microplate reader taking the advantage of the PDA absorbance in the visible region, obtaining new insights into the modelling of polydopamine deposition and polymer/protein adsorption competition, helpful for rational development of imprinted biosensors, and potentially offering a with broad applications ranging from diagnostic tools in medicine to food analysis. The isothermal adsorption of polydopamine on polystyrene surface of multi-well plate displays a Langmuir-shaped curve that allows the determination of the parameters of polymer film formation useful for any analytical assay depending on the surface coating, like the molecular imprinting and the optical and acoustic evanescent sensing [5, 6]. Furthermore, this model has been implemented to take in account the unprecedented competitive adsorption of biological macromolecules, and the observed sigmoidal relationship between the response and the analyte concentration results typical of calibration curves. The method here presented meets the basic criteria of optimal bioanalytical assay design and should be useful for (automated) protein determination in clinical proteomics and food analysis [1, 2, 4].

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Monitoring of fermentation processes using an enzyme-based biosensor array

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In this study, an electrochemical biosensor array for the simultaneous detection of four different analytes was developed. For the determination of ethanol, formate, D- and L-lactate, a bi-enzymatic detection principle is used with a specific NAD^+-dependent dehydrogenase in combination with a diaphorase. Enzymes were immobilized by chemical cross-linking with glutaraldehyde on the platinum biosensor chip (14×14 mm^2), which comprises five individual working electrodes (each with a diameter of 2 mm). One working electrode serves as a blank signal and is functionalized only with inert bovine serum albumin (BSA). Measurements are performed in a conventional three-electrode configuration with a miniaturized Ag/AgCl reference electrode at an applied working potential of +300 mV. For facile application of the biosensor array, a portable measurement set-up was constructed to realize on-site monitoring of complex samples [1,2]. The biosensor array can be used for the measurement of organic acids in real samples, such as fermentation sludge from biogas plants and different silage samples (maize and sugar cane). Figure 1a) shows exemplarily the amperometric measurement signal of the biosensor array for detection of ethanol, formate, D- and L-lactate in fermentation broth of a biogas reactor. As presented in Figure 1b), comparison with a conventional reference technique demonstrates a promising potential for rapid quantification of several analytes in complex media.

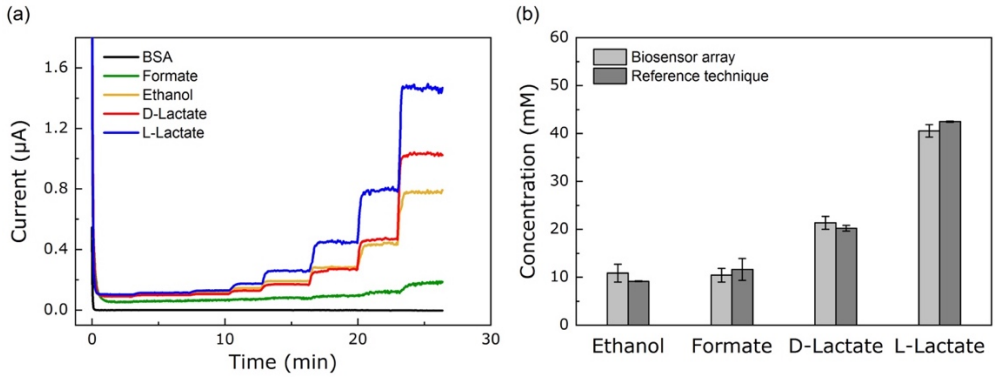

Figure 1. a) Amperometric response of the biosensor array to successive addition of fermentation broth spiked with 10 mM ethanol, formate, D- and L-lactate. (b) Comparison of the results obtained by the biosensor array and a conventional photometric technique.

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Sensitive Detection Using Inkjet Printed Sensor Plates

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Despite considerable research on biosensors, few studies have addressed the gap between the development of sensitive assays for multiple targets and their realization as inexpensive hand-held tools. In this context, we describe the use of inkjet printing technology as a simple and low-cost manufacturing process for the fabrication of biosensor arrays. In this study, we demonstrate the printing of conductive feedlines and insulating layers directly onto flexible substrates. Hereby, the nanoparticles are first printed in order to pattern two closely spaced microelectrodes for use in electrochemical sensing. The biosensor's detection capability is based on redox cycling amplification, whereby an electroactive species undergoes both, reversible oxidation and reduction between two electrodes that are independently biased. The diffusion of electroactive molecules is hindered by the affinity binding between a biomolecule and its specific target, resulting in a decrease in the faradic current in relation to the target's concentration. Therefore, the target molecules can be detected without any additional labelling steps. The example presented in this study comprises sensitive DNA detection through carbon nanoparticle ink printing, as illustrated in Fig.1. Hereby, the sensor system's anodic and cathodic electrodes were printed 1 µm apart, which led to a 4.8 factor amplification of the signal compared to measurements using the same electrode configuration yet without redox cycling. sensor system optimization, while sensor performance with regard to its response to the target analyte and a non-specific binding assessment was evaluated.

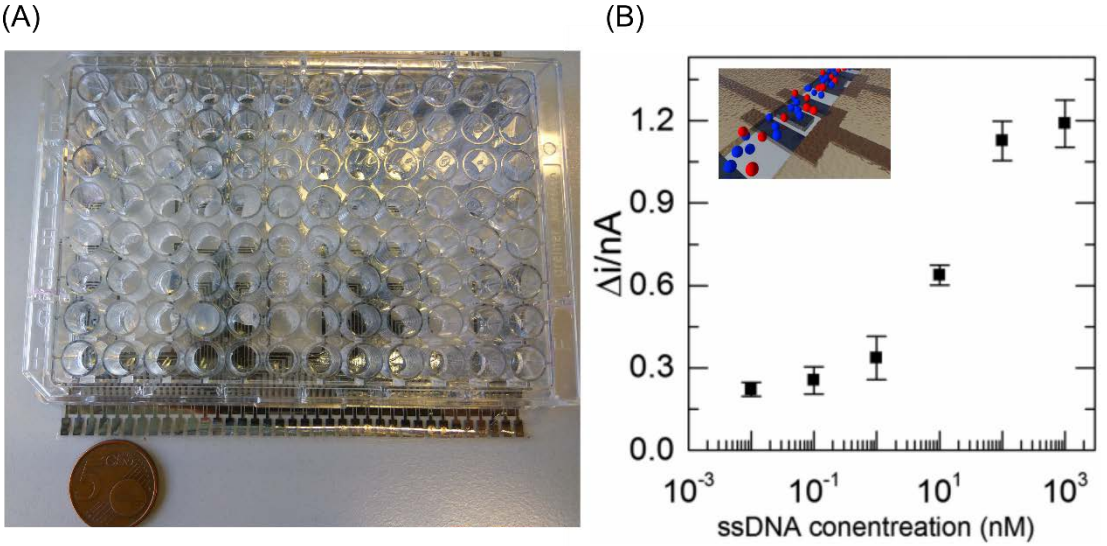

Figure 1. (A) Photograph of inkjet-printed sensor arrays in a 96-well microplate. (B) Calibration curve of redox-cycling sensors for ssDNA detection, inset: Schematic illustration of the device comprising center and outer electrodes.

Peroxidase - silver nanoparticle interaction

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Due to antimicrobial activity of silver, understanding of silver nanoparticle (AgNP) interaction with different biological materials such as proteins, lipids, cellular organelles and entire cells is of high importance. Antimicrobial activity of AgNPs involves several mechanisms, which are dependent on the specific environmental conditions including the presence of surface active molecules such as chloride, citrate, and biopolymers, e.g., proteins. The multitude of the reported mechanisms relies, however, on the release of Ag+ ion from AgNPs in course of their oxidation. At concentrations higher that 1 mM, H_2O_2 acts as an effective oxidant (Fig. 1, left). In this context, we have asked if horseradish peroxidase (HRP) can accelerate the oxidation of AgNPs. HRP has been shown to be able to establish direct electron transfer (DET) between gold and carbon based nanomaterials [1, 2]. However, to the best of our knowledge, DET between the HRP and AgNPs has not been convincingly demonstrated. In our recent experiments, we found that HRP can accelerate AgNP oxidation by H_2O_2, Fig. 1, right. In this presentation we will discuss AgNP oxidation by H_2O_2 including the acceleration of the reaction due to DET between the HRP and the AgNPs. We found that these reactions can be exploited as a basis for construction of sensors and biosensors. The use of AgNPs for the design of wireless sensors and biosensors will be discussed.

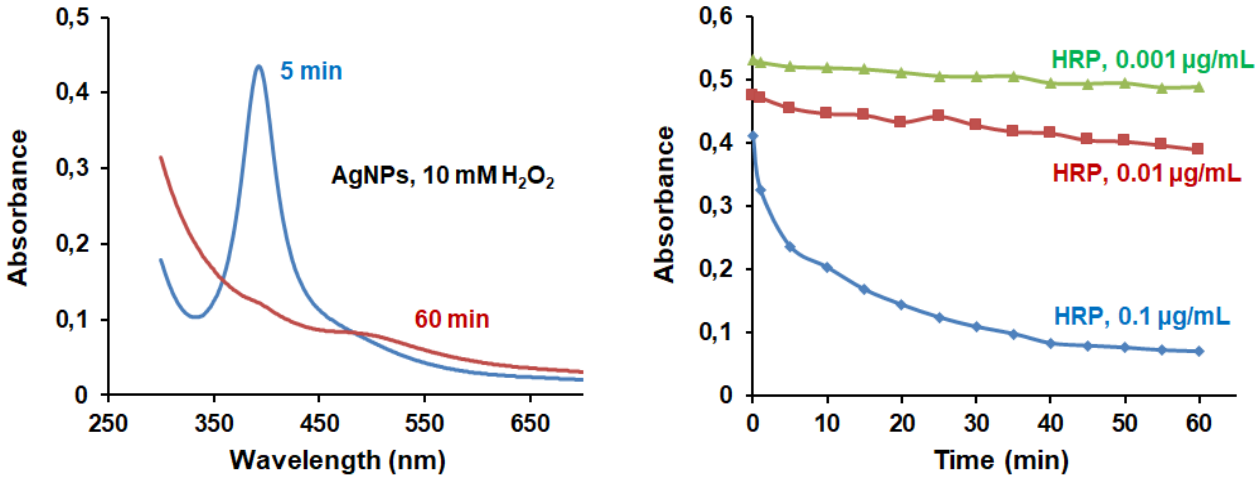

Figure 1. (Left) Absorbace of AgNP dispersion in 10 times diluted PBS, pH 7.4, room temperature (23°C). At time zero, 10 mM of H_2O_2 was added into the solution. (Right) Change of absorbance at 395 nm of AgNP dispersion in 10 time diluted PBS containing 0.1 mM H_2O_2 after addition of different concentrations of HRP.

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Novel bioluminescent biosensor based on split luciferase for real-time detection of androgenic activity in living cells

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Living cells used as sensing systems have proved to be valuable for prediction of the physiological response to drugs, chemicals, and samples in complex matrices, whose toxic effects and specific biological activity can be evaluated in an easy and straightforward manner. Thanks to their easy implementation into high-throughput screenings and integration into portable analytical platforms, cell biosensors are versatile tools for bioanalytical applications, including drug screening and environmental monitoring. Since altered protein–protein interactions (PPI) may result in pathological cellular processes, one of the major targets of the drug discovery pipeline is the investigation of protein networks through the development of reporter protein complementation assays. In this work a new biosensor for real-time monitoring of homo- dimerization of human Androgen Receptor (hAR) based on split complementation of Nanoluc was developed. NanoLuc luciferase is the smallest (only 19KDa) and brightest BL protein commercially available (150-fold brighter than firefly luciferase); moreover, thanks to the absence of post-translational modifications, Nanoluc can be genetically fused to target proteins without modifying its structure and function. The proposed biosensor is constituted by human embryonic kidney cells (HEK293) genetically engineered to express a novel splitted NanoLuc Luciferase (NanoBiT) composed by two chimeric halves: a large domain fused to hAR (LgBit-hAR) and a small domain fused to hAR (hAR-SmBit) under the control of constitutive promoter. Cells immobilized in a cartridge were treated with Testosterone, selected as model analyte (concentration range 0.01-100nM). The binding to hAR receptors able the homodimerization of the chimeric protein and the translocation into the nucleus. After 30 min of incubation time, the addition of the substrate produces BL signals proportional to the activation level of the receptors. Portable CCD camera (ATIK 11000) was employed for quantitative detection of androgen receptor dimerization. In optimized conditions, the developed biosensor is able to obtain a rapid response (30 min) with a Limit of Detection (LOD) of $3.7 \pm 0.2 \times 10^{-11}$ M and an EC50 of $8.6 \pm 0.6 \times 10^{-10}$ M, demonstrating the suitability for analysing molecules with androgenic activity, including new drugs or endocrine disrupting chemicals. This performance was obtained with real-time measurements using a nonlytic assay format providing quantitative data on protein interaction dynamics under relevant physiological conditions. Benefiting from the small size and bright luminescence of NanoLuc, it provides detection at low intracellular concentrations with minimal steric interference on appended target proteins.

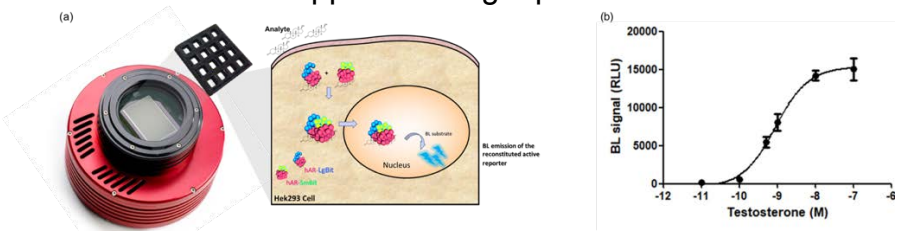

Figure 1. (a) Schematic mammalian cell-based Androgen biosensor. (b) Testosterone dose-response curve obtained with the splitted Nanoluc biosensor.

Spectroelectrochemical investigation of Cholesterol Oxidase from *Streptomyces lividans* at different pH values

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Cholesterol oxidases (ChOxs, EC 1.1.3.6*)* are monomeric flavoenzymes. After glucose oxidase ChOxs are the second most frequently used enzymes in clinical diagnostics.[1] ChOxs catalyze the oxidation of cholesterol to 5-cholesten-3-one and its subsequent isomerization to 4-cholesten-3-one.[2] There are two different types of ChOx, one with a covalently linked flavin adenine dinucleotide (FAD) cofactor and one with a strongly but non-covalently bound FAD cofactor.[3] ChOx from *Streptomyces lividans* (*Sl*ChOx) with a molar mass of about 58 kDa contains a non-covalently linked FAD-cofactor. For an application in electrochemical biosensors it is important to know details of the redox process and the redox potentials. Therefore *Sl*ChOx was investigated by spectroelectrochemistry between pH 5 and pH 9. For the measurements the enzyme was reduced stepwise in the presence of different one and two electron redox mediators. As the mediator methylene blue contributes not significantly to the spectral changes of the enzyme between 350 nm and 500 nm, for each pH value also one measurement with methylene blue only was performed. In the measurements a stable flavosemiquinone radical anion was detected. The formation of a stable flavosemiquinone radical anion was reported previously for different types of ChOxs. Based on global analysis the first (Fl(quinone)/Fl(semiquinone radical anion)) and second redox potential (Fl(semiquinone radical anion)/Fl(hydroquinone anion)) of *Sl*ChOx were determined between pH 5 and pH 9. Also the pH dependent midpoint potential was detected. The pH dependency of the redox potentials will be discussed and compared with those published previously for other types of ChOx[4,5] and further flavoenzymes, which also comprise stable semiquionone radicals.[6,7]

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Electrochemical impedance spectroscopy diagnostic platform to tackle antimicrobial resistance

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Antimicrobial Resistance (AMR) is a major threat to healthcare systems and societies on a global scale and is discussed at the highest policy levels leading to the launch of a global as well as a series of national action plans. If not tackled, the UK independent Review on AMR calculated that AMR could cause an additional 10 million deaths per year and a loss of up to US$100 trillion from global GDP by 2050. It is expected that AMR will have a wide impact on the UN Sustainable Development Goals and in 2017 the Interagency Coordination Group on Antimicrobial Resistance was formed to coordinate global action against AMR. Rapid diagnostics is needed to help reducing this threat but current methods are too slow and lack information depth to enable tailored therapy decisions. To overcome this limitation molecular tools are developed for rapid in vitro diagnostics. In an ideal scenario such devices would be available at point of care to make therapy decisions at the site of the patient possible. Here, we report the successful development of an electrochemical biosensor platform based on electrochemical impedance spectroscopy (EIS) for label-free molecular diagnostics at point of care covering a wide range of targets from small molecules over proteins to different types of nucleic acids. Nucleic acid targets which have been successfully detected with the EIS platform range from synthetic targets over PCR products derived from several antibiotic resistance genes, genomic DNA and ribosomal RNA for direct amplification-free bacterial species identification to host biomarkers for infection detection. The intrinsic properties of the developed nucleic acid test enabled direct amplification-free diagnosis of bacterial pathogens or alternatively has also been combined with an upstream PCR step. We have successfully developed EIS tests for methicillin resistance *in S. aureus* (MRSA) detection both with prior PCR amplification and PCR-free with *mecA* gene specific peptide nucleic acid (PNA) probes. For *mecA* PCR product detection we achieved a limit of detection (LOD) of 10 pM.[1] We have also demonstrated direct label- and amplification-free detection of plasmids, harbouring the gene encoding for the New Delhi metallo-beta-lactamase (*blaNDM-1*) which causes carbapenem resistance in enterobacteria (CRE)[2]. For bacterial pathogen detection we have developed a label- and PCR amplification-free EIS assay and demonstrated it for *E. coli* with a LOD of 50 pM, excellent sensitivity (~ 500 CFU/mL) was achieved by the use of PNA probes specific to *E. coli* ribosomal RNA.[3]**Errore. Il segnalibro non è definito.** To identify biomarkers of infection and to demonstrate that EIS can be used for protein and antigen detection, we developed an EIS-Assay for TREM-1 using anti-TREM-1 antibody functionalised electrodes. We successfully demonstrated the detection of TREM-1 directly from wound fluid with a limit of detection of 100 pg/mL (3.3 pM).

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Nanomaterial-based electrochemical sensing strategies for cell lines oxidative stress evaluation and for bio-compounds detection in food

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In this work, different electrochemical sensors based on nanomaterials aimed to assess cell oxidative stress and bioactive compounds in food are proposed. The realization of these sensors represents the first step, in the development of an integrated lab-on-chip microfluidic platform able to study the antioxidant protection action of food components towards oxidative stressors, to be applied in cell cultures. Carbon Black (CB) - Prussian Blue (PB) based electrodes for H_2O_2 sensing in Parkinson's disease in vitro model were developed together with a transition metal dichalcogenides (TMDs) based hybrid sensor for class-selective evaluation of polyphenols. Among, different analytical strategies, electrochemical sensors are very appealing for their simplicity, speed, sensitivity, miniaturization, and cost-effectiveness. Indeed, nanostructured sensors allow improving the sensitivity and selectivity providing larger surface area and faster electron transfers in comparison with their bulk counterparts. Direct sensing of H_2O_2 in classical metal electrodes such as platinum or gold is possible, however, it suffers from poor selectivity due to the high overpotentials needed. The selection of a proper nanomaterial and catalyst can overcome this drawback. Thus, because of the nanomaterial feature of the CB and the catalytic activity of the PB, towards the H_2O_2 reduction, good analytical performances (linear range 0.2 and 1000 µM, LOD 0.01 µM) have been obtained allowing selective H_2O_2 detection, at very low potentials (-0.05 V) in neuroblastoma SH-SY5Y cell line [2]. For the evaluation of the bioactive compounds in food, a new hybrid nanomaterial based on CB and molybdenum disulfide (MoS_2) has been employed. In this case, the CB was hybridized with the MoS_2, that is a transition metal dichalcogenide (TMD). The TMDs possess a layered structure, that could be easily processed to form a 2D 'graphene-like' nano-structure, using liquid phase exfoliation in appropriate solvents. However, TMDs properties have not yet been widely and deeply studied for (bio)sensing purposes [3]. In the proposed sensor, the CB-MoS_2 combination exhibited an improved electroanalytical (improved charge-transfer ability low charge-transfer resistance high electrical conductivity and enhanced electrocatalysis) and analytical (high sensitivity, and low fouling) performances, in comparison with sensors realized with the individual nanomaterials (CB and MoS_2 alone). Moreover, an exceptional ability to assess o-diphenols and catechins has been proved, and the electrode fouling, a typical drawback with these compounds in electrochemical analysis, was negligible. These features have been successfully exploited for the determination of polyphenols in olive oil and chocolate samples. In both applications, the nanomaterial ability to support the analysis of bio-compounds in biological and food samples have been demonstrated. Furthermore, the basis for the realization of an integrated device, able to evaluate the antioxidant protection action of food components towards oxidative stressors, have been laid.

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The application of porous silver surfaces and nanoparticles in virus detection and identification via SERS

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The application of several silver SERS [1] substrates in direct label-free virus detection and identification was studied. One such SERS substrate was produced using electron-beam physical vapor deposition method (EB-PVD). By tuning the temperature during the deposition process it was possible to manufacture porous SERS-active surfaces with varying pore sizes. The possibility of using such silver substrates for virus detection was demonstrated by testing them against 4 viral species – 2 animal viruses, rabbit myxomatosis virus (MYXV) and canine distemper virus (CDV), and 2 plant viruses, tobacco mosaic virus (TMV) and potato virus X (PVX) [2]. The specific SERS spectra of all 4 viruses were successfully observed and collected (figure 1a). The spectral data was then processed and subjected to principal component analysis (PCA) and linear discriminant analysis (LDA) in order to evaluate the discrimination between groups (figure 1b). A classification model was composed and validated. Using the classification model 100% accurate discrimination between viruses was achieved. The parts of spectral data that contributed the most to discrimination model were analyzed and compared to manual peak assignment in order to confirm that achieved discrimination was based on actual differences in virus structure between tested species.
SERS-active silver nanoparticles [3] were also used for detection and discrimination between MYXV and CDV. Because most viruses are negatively charged under physiological conditions, normal negatively charged silver colloids are ill-suited for virus detection. It was demonstrated that application of positively charged nanoparticles or positively charged aggregation inducing reagents like spermin could remedy that problem. The use of direct label-free SERS measurements for virus detection was thus shown to be possible and worthy of further study.

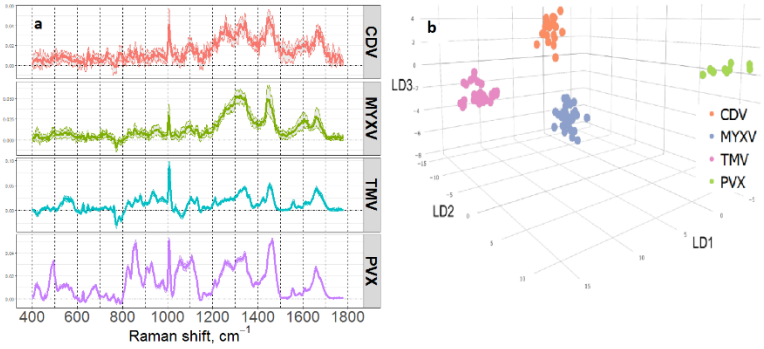

Figure 1. a) SERS spectra of viruses on porous silver surface, average ± standard deviation. (b) Visual representation of PCA-LDA analysis results using projections of spectral data on linear discriminants.

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Biophotoelectrochemistry for biosensing

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Photosynthetic pigment proteins are the most abundant and sustainable solar energy converters on Earth, evidenced by their >3-billion-year reign and diverse byproducts which we still rely on today. At the heart of photosynthesis lie reaction centers, which convert an absorbed photon into a charge separation at near unity quantum efficiency, with power conversion efficiencies up to 37%. Tapping into this process directly, however, is cumbersome, and requires specialized methods. The topic of my poster will concern the various strategies one must undertake to efficiently transduce the solar energy into electric or chemical energy using photosynthetic reactions, redox polymers and electrode architectures strategies. Specifically, I will discuss my previous works using purple bacterial reaction centers (R. *sphaeroides*) as a model system for increasing photocurrents in biophotocathode configurations (Fig 1.). By methodically increasing the substrate surface area and protein loading, we were able to increase photocurrent densities from 27 ± 5 µA cm^{-2} on a smooth silver surface to 166 ± 13 µA cm^{-2} using rough silver (18.5 % to 8% SNR)[1]. Next, we further increased this to 2100 ± 150 µA cm^{-2} (7.1% SNR) utilizing the recently reported Inverse-Opal mesoporous ITO substrates, with a estimated >600x surface area increase. Conversely, utilizing the RC in conjunction with osmium based redox polymers on a flat substrate has brought the SNR up to even higher values of 535 ± 30 µA cm^{-2} (5.65% SNR), despite the slight decrease in the peak photocurrent relative to the porous ITO. We attribute this to the irreproducible nature/complexity of fabricating the meso-ITO substrates. Overall, the various strategies to increase photocurrents typically translated to imporved signal-to-noise ratios, which, combined with our previous work revealing the bacterial RC biophotocathode to act as a biosensor for herbicides[2], translates to improved biosensing.

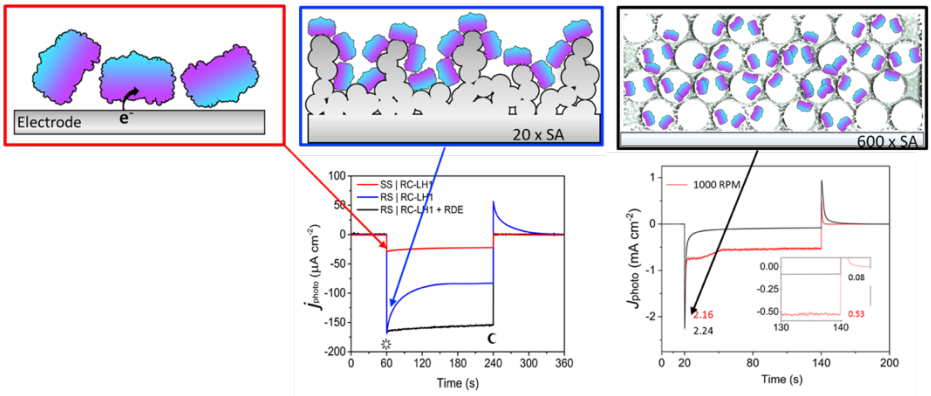

Figure 1. a) Strategies for increasing protein loading and resultant photocurrent response. Cytochrome c served as the electron donor, RC-LH1 is depicted with donor side in magenta and acceptor side in blue.

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SPR-based studies of the binding efficiency of LYAR protein to the (+25 G→A) ^{A}γ-globin gene sequences mutated in β-thalassemia

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Transcription factors regulating γ-globin gene transcription are known to interact with several elements of the γ-globin gene promoter. Interestingly, several of them (MYB, BCL11A) are strong repressors of γ-globin gene transcription. This issue is of great interest since the conclusions reached predict that the possible inhibition of these repressors might lead to the activation of γ-globin gene expression and production of fetal hemoglobin (HbF) in erythroid cells. Increased HbF has been firmly demonstrated to ameliorate the clinical severity of β-thalassemia and sickle-cell anemia patients. LYAR (Ly-1 antibody reactive clone) protein is a novel repressor of γ-globin gene transcription, which binds to a region corresponding to the γ-globin gene 5'-UTR. One of the possible effects of the ^{A}γ-(+25 G->A) mutation in β-thalassemia is a decrease of the LYAR binding efficiency to its 5'-GGTTAT-3' binding site [1,2]. The present SPR-based study was undertaken to determine whether the β-thalassemia mutations at this ^{A}γ-globin gene region alter the binding to the target DNA sequences by LYAR and LYAR-complexes by performing a BIAcore analysis. SPR-based experiments support the concept that LYAR binds to the mutated site present in β-thalassemia (5'-GAG<u>A</u>TTATCA-3', mutation underlined) with lower efficiency. This is expected to favor high level of transcription of γ-globin gene. Accordingly, the screening of β-thalassemia patients carrying this mutation would facilitate the identification of patients expected to respond efficiently to HbF inducers, such as sirolimus. This might be important in clinical trials in the step of recruitment of patients and in the development of personalized protocols in the field of precision medicine for β-thalassemia. Fully in agreement, the expression of LYAR decreases following treatment of erythroid cells (including precursor cells from β-thalassemia patients) with HbF inducers (supported by AIFA, Wellcome-Trust and by EU projects ULTRAPLACAD and THALAMOSS).

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Fluorescence optical prototype based on waveguide absorption filters for multi-assay sepsis detection

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The aim of the work is the development of an optical POCT instrument based on a multi-assay fluorescence system able to strongly reduce the response time in sepsis diagnosis.
Sepsis is considered the main cause of mortality in intensive care. For such a pathology, a timely diagnosis is essential, since it has been shown that each hour of delay in the administration of an effective pharmacological treatment increases the mortality rate of 7%. Therefore, the advent of a point of care testing (POCT) platform for sepsis is highly requested by physicians.
The POCT device is based on a polymer chip constituted by thirteen optical waveguides with embedded the detection micro-channels [1], each one devoted to the detection of a single biomarker, and thirteen waveguide absorption filters capable of maximizing the fluorescence signal collection thanks to its high numerical aperture [2]. A suitable chemistry is implemented on the micro-channels surface in order to create a sensing layer containing the capture antibody. In terms of sepsis biomarkers, the attention was focused on C-reactive protein and soluble urokinase plasminogen activator receptor.
A diffractive optical element, illuminated by a laser diode emitting at 635 nm, generates 13 parallel lines (each one for each microchannel of the chip). Fluorescence from the immune-reactions which take place within each microchannel is collected by the optical waveguides and detected by the array of thirteen coloured glass absorbing filters, directly facing the sensitive part of a CCD camera.
The absorption filter is designed to work as an optical waveguide in order to increase the optical path and, consequently, the absorption of the excitation light. A comparison of the performances of interference and absorption filters, with particular emphasis on the angular dependence of the spectral features, is reported. The failure of the interference filter for incidence angles greater than 15° and the validity of the absorbing waveguide filter for large incidence angle is experimentally demonstrated.

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Silica-titania thin film coated over-coupled long period fibre gratings for bio-sensing applications

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Optical fibre long period gratings (LPGs) have been proposed as a promising tool for label-free biosensors. The application of over coupled long period fibre gratings for the detection of small changes of the surrounding refractive index is described. The chemical overlay, which increases the refractive index (RI) sensitivity of the sensor, consists of a sol−gel-based titania−silica thin film, deposited along the sensing portion of the fibre by means of the dip-coating technique. The annealing post-process of these materials on glass substrates is conducted at high temperatures, ranging from 450 °C to 600 °C, in order to make it possible to sinter the sol-gel. The over-coupled LPG has been manufactured in such a way that the resonant band retains good visibility (close to the maximum coupling condition) in the transmission spectrum even after the high thermal sintering of the coating material [1].
The optimization of a high RI overlay deposited over the fibre for the adhesion of biological species, and to allow the over-coupled LPG to work in modal transition when the surrounding environment was a biological solution (RI ~ 1.334), is described.
Three different batches of over-coupled LPG were produced using different withdrawal speeds and sol viscosities. By carefully tuning both the overlay thickness during the deep coating process and the RI of the sol-gel material during its preparation, it was possible to bring the sensor into the so-called transition mode working region, thus maximizing the sensing performance in terms of surrounding refractive index changes. LPGs were characterized as optical refractometers in the RI range of interest for a bio-sensing application (RI from 1.33 to 1.34) and, after the functionalization of the fibre surface using a methacrylic acid/methacrylate copolymer, an antibody/antigen (IgG/anti-IgG) assay was carried out to assess the performance of sol−gel based titania−silica-coated LPG as biosensor.

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Plasmonic spot-on Raman sensor for biomolecules

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In an attempt to produce a SERS-active and low cost sensor for label free detection of trace amounts and small volumes of biomolecule samples, silver nanowires with high aspect ratio were implemented in a flow-through self assembly process on hydrophobic PTFE membrane. This bottom up assembly was followed by spot arrays design and fabrication through laser patterning of the silver nanostructured surface, leading to the formation of circular spots of defined size aimed at both concentrating microliter volumes of aqueous solution droplets and enhancing the Raman signal of micromolar amounts of biomolecules.
The spot arrays so produced were efficiently tested as SERS sensors *via* direct detection, i.e. by direct measurement of the analyte molecular Raman fingerprint, with several proteins and biomarkers of neurodegenerative diseases and they showed excellent reproducibility and sensitivity. The specificity of the detection system is intrinsic in the Raman spectroscopic signal, which enables a structural characterization of the biomolecules while a rapid and effective detection is perfomed. The silver spot arrays can also represent a versatile label free SERS platform for indirect biomolecular detection, thanks to the simple and easy functionalization of the silver spots by thiolated small molecules and receptors.

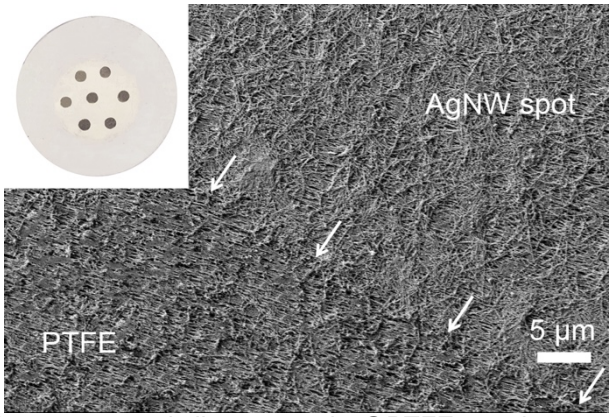

Figure 1. SEM image of laser patterned silver nanowire@PTFE membrane at the edge of a silver spot (indicated by the arrows). Inset: image of a laser-patterned produced substrate.

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Towards a possible technique to remove foxing from artefacts

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The iron ions present in the ink or in the metallic supports can influence the cellulosic material bringing it to a more accentuated phenomenon of aging.
The main cellulose degradation pathways are the acid hydrolysis of glycosidic bonds and oxidation[1][2]. Low pH values can lead to cellulose depolymerization, even at room temperature. It is well known that during the preparation of iron-gall inks, gallic acid, formed by hydrolysis of tannins extracted from gall-nuts, reacts with iron(II) sulfate (i.e., vitriol, as reported in old recipes) to give a pyrogallate complex of iron(III) and sulfuric acid. Iron-gall ink was commonly used in ancient manuscripts that are contaminated by acids, especially in the capital letters, where a high amount of ink was used. At the same time, several paper opera were supported on metal plate, contaminating the paper artwork over time. At the same time, transition-metal ions usually catalyze cellulose oxidation through a free radical mechanism known as the Fenton reaction, which involves iron ions.[3]
Macroscopically, oxidation phenomena lead to the appearance of reddish brown spots on the surface (foxing). This phenomenon induces paper weakening and perforations in the matrix[4].
The aim of this research work is to remove the iron (III) present on the red spot of paper artworks using a chelating system (based on calixarene moieties) and follow the removal procedure by electrochemical tool, based on screen printed electrodes (SPEs). For this work, the 6-calixarene was chosen, a highly versatile chelating agent, able to work in different experimental conditions. The electrochemical technique, used for this study, was the cyclic voltammetry, working a range of potential of – 05 and -0.5 V with a scan rate of 50 mV/s. We report the preliminary results about the “capture” of iron (III) present on “ad hoc” laboratory paper samples.

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Origami multiple paper-based electrochemical biosensors for pesticide detection in Brassicaceae plant waste

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Organic wastes are typically by-products of farming, industrial or municipal activities and include animal manures, crop residues, food processing wastes, municipal biosolids and wastes from some industries. Possible uses of organic wastes include use as fertilizer and soil amendment, energy recovery (heat, liquid fuels, electricity), and production of chemicals (volatile organic acids, ammonium products, alcohols). Utilization of various organic wastes in agriculture depends on several factors, including the characteristics of the waste such as nutrient and heavy metal content, energy value, odor generated by the waste, availability and transportation costs, benefits to agriculture, and regulatory considerations [1].
Among the organic waste used in agriculture, Brassicaceae family plays an important role. This family is a great source of oil and protein of plant origin for animal and human nutrition and act as biofumigant to control nematodes and other soil borne pathogens through the biofumigant process. It also can also improve the soil structure and increase fertilizer elements such as nitrogen, phosphate, potassium which are important requirement for plant growth [2].
Although utilization of organic waste in agriculture has many benefits, several factors remain to address such as the safety of the waste which significantly limits its re-use. In the context of safety, the presence of pesticides in vegetables is a crucial issue being largely used at worldwide level for improving the food production.
Herein, we report a three-dimensional origami paper-based device based on enzyme-inhibition biosensors for the detection of several classes of pesticides in Brassicaceae plant waste.
This device was composed by two different office paper-based screen-printed electrodes and multiple filter paper-based pads to load enzymes and enzymatic substrates, for delivering a reagent free device. The versatile analysis of different pesticides was carried by folding and unfolding the filter paper-based structure, without any addition of reagents and any sample treatment (i.e. dilution, filtration, pH adjustament) [3].
The paper-based platform has demonstrated the capability to detect paraoxon, 2,4-dichlorophenoxyacetic acid, and atrazine at ppb level in standard solutions by exploiting the capability of these different types of pesticides (i.e. organophosphorus insecticides, phenoxy-acid herbicides, and triazine herbicide) to inhibit butyrylcholinesterase, alkaline phosphatase, and tyrosinase, respectively [4].
The preliminary results for the detection of pesticides in Brassicaceae plant waste will be reported demonstrating the capability of this sensing tool in this complex matrix.

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A wearable origami-like paper-based electrochemical biosensor for sulfur mustard detection in liquid and gas phase

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Mustard agents (MAs) were introduced during the First World War as weapons of mass destruction principally employed in an impure aerosolised form spread into air. These family of compounds includes sulfur-based agents (bis(2-chloroethyl)sulfide, also known as sulfur mustard or Yperite), and nitrogen-based agents (bis(2-chloroethyl)ethylamine and bis(2-chloroethyl)methylamine, also known as HN1 and HN2, respectively). MAs belong to the group of vesicants, because they are associated to blistering effects upon skin exposure or inhalation [1]. Though MAs use was banned as well as for the most of CWAs, the possible employment of MAs during conflicts or terrorist attacks represents still nowadays a risk of extreme concern for many countries, shedding lights on the importance to improve the detection strategies that are currently applied for MAs monitoring [2]. In this regard, we have recently developed cost-effective and miniaturised screen-printed electrodes (SPEs) realised on polyester [3] and office paper support [4] for the amperometric bioassay of sulfur mustard (SM) and MAs simulants by exploiting MAs inhibitory activity toward the reaction of choline oxidase enzyme [5]. Basing on the principle of this bioassay, in the present work we realised a wearable and reagent-free biosensor, suitable for the on-site detection of MAs both in liquid and gas phase, to provide a fast alarming system that could play a role of utmost importance within the security field. The electrodes were screen-printed onto a filter paper support, which allowed to exploit the porosity of paper to pre-load all the needed reagents into the cellulose network, and hence to realise an origami-like and reagent-free device. Mustard agent detection was carried out by monitoring their inhibitory effects toward the choline oxidase enzyme, through the amperometric measurement of the enzymatic by-product hydrogen peroxide. A carbon black/Prussian blue nanocomposite was used as a bulk-modifier of the conductive graphite ink used as working electrode, allowing for the electrocatalysis of the hydrogen peroxide reduction. After having verified the detecting capability toward a mustard agent simulant, the applicability of the resulting origami-like biosensor was demonstrated for the rapid and real-time detection of real sulfur mustard, obtaining limits of detection equal to 1 mM and 0.019 g/m^3 min for liquid and gas phase, respectively.

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Chemiluminescence emission anisotropy real time detection by means of optical fibre radial light collection system

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Chemiluminescence is an optical detection principle increasingly employed for developing biosensors and miniaturized analytical devices [1]. The light emitted by chemiluminescence derives from an exergonic chemical reaction yielding an intermediate in its singlet excited state, which undergoes radiative decay. In many bioanalytical applications, the chemiluminescence reaction is catalysed by an enzyme that is employed as a label upon its biospecific capture on a solid surface (e.g., glass, polymer, paper).
The anisotropic emission of electric dipoles (excited fluorophores emitter) when the distance from a dielectric interface is small or comparable with the emitted wavelength is a well-known phenomenon [2]. This means that the presence of an emitter in the proximity of a solid/liquid interface implies the existence of preferential directions in the emission of the radiation, and disregarding this behaviour can give rise to inefficient photon collection. While the phenomenon is well-known for fluorescence emitters, it has never been investigated for chemiluminescence phenomena, in which, although the enzyme is immobilized on a solid surface, the intermediate products of the reaction can diffuse in the solution prior to the photon emission.
In this work a setup for the real time detection of the chemiluminescence emission anisotropy is presented. A PMMA semi-cylinder, accommodating a crown of plastic multimode optical fibres (placed at fixed angles, each one at 15° from the next one) was used to collect the chemiluminescence signal in different directions. A PMMA slide (thickness = 1 mm), set on the top of the, was used as support for the immobilization of the enzyme horseradish peroxidase that was then detected upon addition of a luminol/peroxide/enhancer chemiluminescence cocktail. For this purpose, the slide was functionalized, by dip coating process, with Eudragit ® L100 and activated with EDC – NHS. The optical fibres outputs were fixed on a suitable plastic support (a 4 × 3 holes matrix) working as interface between the collecting system (the PMMA semi-cylinder with fibres) and a CCD imaging detector built from a thermoelectrically cooled MZ-2PRO CCD camera (MagZero, Pordenone, Italy) equipped for lensless contact imaging with a round fiber optic taper (25/11 mm size, Edmund Optics, Barrington, NJ) placed in contact with the CCD sensor [3]. The imaging system measured the chemiluminescence intensity guided by every fibre was independently and simultaneously measured. With this set-up, the phenomenon of anisotropy was measured for the first time for a chemiluminescence emission catalysed by an enzyme immobilized on a solid phase. These findings open new perspectives in the development of miniaturized analytical devices based on chemiluminescence detection, since photon collection efficiency can be maximized by properly taking into account emission anisotropy.

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A rapid colorimetric enzyme-based biosensor

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Conducting polymer polyaniline is highly sensitive to pH changes in its microenvironment and its conductivity is known to increase by 8–10 orders of magnitude on doping with acids. This is due to the transition of its emeraldine salt (ES) form to emeraldine base (EB), which is also accompanied by colour change from green to blue [1]. However, its insolubility in common solvents, which results in difficult process abilities, has restricted its applications. To improve the polymer solubility, copolymerization of aniline with aniline derivatives was proposed.
In this work, we present a colorimetric hydrogen peroxide biosensor one spot utilizing the unique colorimetric properties of the polyaniline and polyanthranilic composite film coupled with horseradish peroxidase enzyme. The horseradish peroxidase enzyme is immobilized on the composite polymer by adsorption and, in the presence of its substrate, catalyzes a reversible redox color change in the host polymer. In addition, glucose biosensors were realized co-immobilizing glucose oxidase (GOx) on the PANI/PAA film and applied to colorimetric glucose detections. The biosensors developed were studied and optimized in relation to different experimental parameters by using microplate reader. Moreover, a free of charge software application was used to enable easy and clear display of the sensors' response indicating remarkable changes in the optical features. It has been demonstrated by these studies that the colorimetric biosensor is promising as quick, simple and disposable test for hydrogen peroxide detection in various applications.

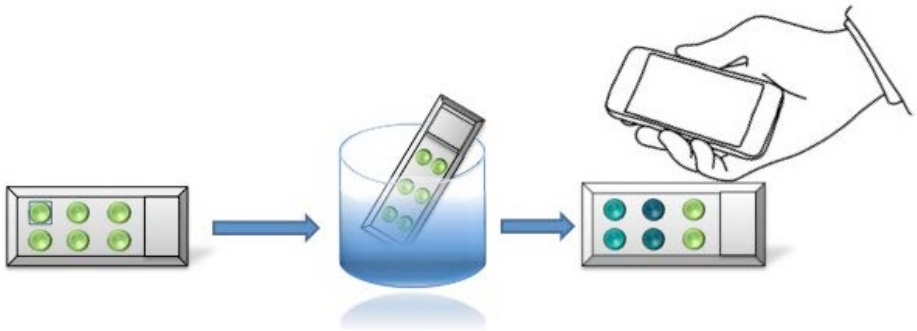

Figure 1. a) Schematic representation of the enzyme-based poly(aniline-anthranilic) composite film modified strip

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Top-down fabricated reduced graphene oxide thin-films as functional layer for surface plasmon based biosensing

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Surface Plasmon resonance (SPR) based approaches are recognized across different disciplines for studying molecular interactions at surfaces in highly sensitive, label-free manner. More recently, graphene as a two-dimensional (2D) material, has been used as an ultrathin dielectric on Au availing versatile dielectric and electrical properties and potential to tune SPR responses. Here, integration of alternative and parallel molecular detection strategies combined with SPR enable additional information for biomolecular interactions at surfaces.[1] SPR based approaches are therefore moving beyond nanometer thick Au films as typical sensor platforms and combining thin film dielectrics on top in order to produce long-range surface plasmons and improve SPR biosensor performance.[2] In this work, we demonstrate a new SPR platform, which makes use of graphene oxide (GO) flakes as building blocks for realization of a dielectric thin-film. The GO flakes were exfoliated in a unique LTEDS method followed with thin-film preparation and using lithography processes for the fabrication of rGO based SPR sensor chips in a in a top-down manner.[3] The SPR chips with rGO thin films as a dielectric, as well as a layer for immobilization of biofunctional layers, were deployed for studying the molecular binding between prostate-specific antigens (PSA), a biomarker for prostate cancer, and Concanavalin A, a protein molecule specific to PSA. Advantages such as robust immobilization of biomolecules onto rGO surfaces by using an elaborate surface chemistry approach, possibility of tuning the dielectric parameters by controlling the material composition and application of electrical field were explored. Our results confirm that the average intensity of the resulting surface plasmon and the signal-to-noise ratio on an rGO-SPR platform were tuneable and up to 3.03 times higher than that of the GO based SPR platform. Our rGO layers are expected to serve as a future workhorse for alternative SPR biosensor applications.

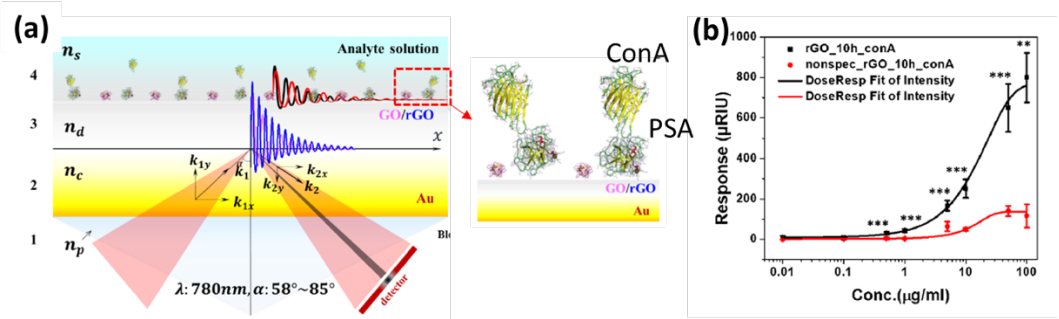

Figure 1. a) Schematic of a typical SPR biosensor platform with an additional layer of rGO film, biofunctional layer shown as zoom-in cartoon made of PSA as the receptor biomolecules and Concanavalin A as analytes, (b) SPR detection of ConA shown in the sensorgram.

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[3] X. Lu et al., *Adv. Mater. Tech.* 3, 4, 1700318 (2018)

Dual HCR-based amplification triggered by triple helix probe for the detection of microRNAs

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Hybridization Chain Reaction (HCR) is an isothermal self-assembling reaction involving in general a pair of DNA hairpins able to form a double stranded nanostructure in presence of a specific sequence showing high potentiality for biosensing [1]. We designed hybridization chain reactions specific for different microRNA targets and tested it in solution, confirming the suitability in the detection of different miRNAs, and we are now implementing the system for electrochemical detection on a custom-made device (Fig.1a). In order to improve HCR based detection we designed in parallel a system involving a triple helix probe able to control the amplification. Trials to employ triple stranded DNA in biosensing have been done reaching good sensitivity and selectivity [2]. Moreover, different recognition elements can be included in the probe on a same single-stranded sequence, allowing in principle a universal assay strategy [3]. In literature, the usage of only one of the involved oligonucleotides for signal amplification and transduction is generally reported. We herein propose the use of both the triple helix forming strands as this would give the chance to combine different transduction approaches leading to a stronger and more reliable detection. For this reason, we designed and characterized a triple helix DNA probe in which both the sequences hidden in the stem would be available for subsequent signal amplification and transduction after target recognition (Fig.1b). To simply test the feasibility of our system to induce a double response triggered by the target, we designed HCRs triggered by both the oligonucleotides involved in the triple helix. In our peculiar design, the distinct HCR products can interact with each other forming bigger nanostructures (Fig. 1c). The aim is now to adapt the strategy for on surface detection and compare it to our previous HCR based strategy on electrochemical detection.

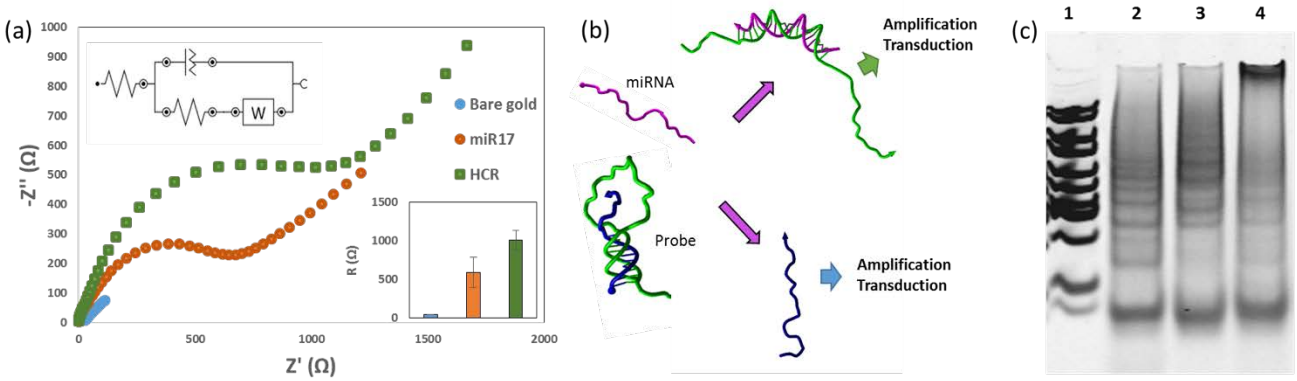

Figure 1. a) Preliminary Electrochemical impedance spectroscopy data showing the increase of DNA amount after HCR; b) Scheme of triple helix probe and splitting of the signal transduction-amplification; c) Electrophoresis gel showing individual HCR products (lane 2 and 3) and higher molecular weight product due to their interaction.

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[3] Zheng, J., et al. Analytical Chemistry, 2014. 86(4): p. 2205-221.

Enzyme Inhibitor based Capacitive biosensors for Matrix-metalloproteinase quantification in wound fluids

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One obstacle in the treatment of chronic wound patients is the lack of reliable quantification of the status of wound tissue. Hence the choice of the best suited medical treatment is difficult and innovative new sensor technologies are needed in the public health sector. Matrix metalloproteinases (MMPs) are a group of Zinc containing enzymes that catalyze the hydrolysis of damaged tissue, but also attack healthy tissue if over expressed, as in chronic wounds. Consequently, it is desirable to investigate a possible methodology for their detection and monitoring. In recent times several sensor systems utilizing Antibodies as recognition element were presented and for MMP also the approach of using the enzymes hydrolytic activity of peptides is frequently reported as the recognition event in MMP-biosensors. Our approach is different as it involves the chemical synthesis of recognition elements with enzyme inhibitors as design blueprint [1]. This project involves the synthesis of the probes, their attachment to a gold surface via self-assembled monolayers [2] as well as the buildup of a flow cell for data acquisition (Figure 1). The usage of a physisorbed enzyme inhibitors in SPR based detection schemes of MMP-2 has been reported only recently[3], but a covalently bound inhibitors for capacitive determination of protein levels in biological fluids has not been reported so far. In order to relate the sensor response to the affinity thermodynamics and for further development in the direction of a sensor array we investigated the binding events in more detail with FTSA and see how the sensor response might scale with the binding affinity of the compounds towards MMP-2/9 as target.

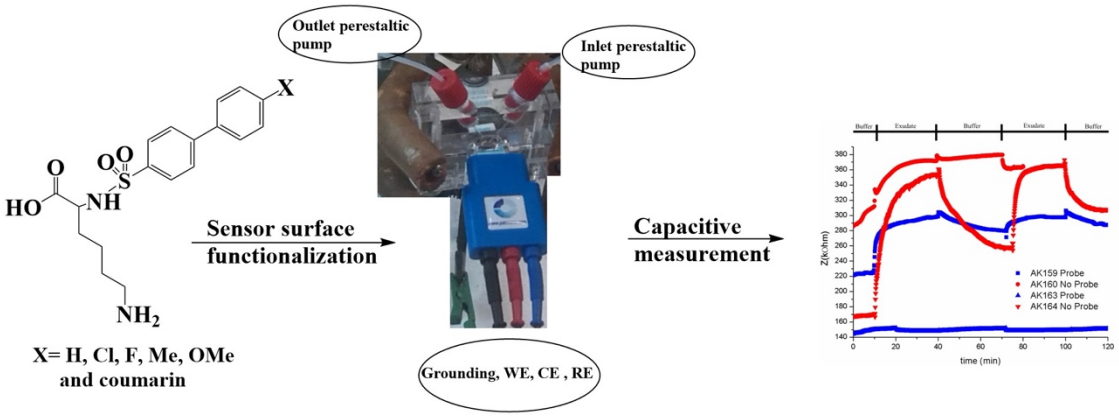

Figure 1: Sensor project line-up, from probe selection and synthesis, to test setup design to data acquisition.

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Simultaneous Detection and Quantification of DNA and Protein Biomarkers of Cardiovascular Diseases in a Microfluidic Microbead Chip

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Cardiovascular diseases are a major cause of adult mortality in the western world, leading to a high burden on the health care system. Lifestyle, genetic disposition, the lack of clearly defined risk assessment criteria and high error diagnoses directly contribute to this. The rapid and simultaneous detection of biomarkers is necessary. We integrated three cardiovascular disease biomarkers, c-reactive protein (CRP), brain natriuretic peptide (BNP) and cell free mitochondrial DNA (cfmDNA) in a microfluidic microbead chip (bi.flow Systems GmbH) that is aimed as point-of-care technology. The protein based biomarkers CRP and BNP are covalently bound on microbead surfaces and detected by specific antibody binding (fluorescent labelled). The DNA based biomarker cfmDNA was trapped by a complementary capture probe on the microbead surface. Presence of cfmDNA was verified by a complementary fluorescence labelled probe. Differently coupled microbeads were immobilized in a flow cell on the microfluidic chip and analyzed with our *VideoScan* technology (end-point and real-time) [1,2]. The detection was tested in different buffers to find the optimal reaction environment for all biomarkers. All three biomarkers, CRP, BNP and cfmDNA, were successfully detected both in independent reactions and simultaneously. Antibody detection had signal-to-noise-ratios (SNR = 10 $\log_{10}$ (signal/noise) [dB]) and relative fluorescence intensity (refMFI) of 9 dB and 0.8 for BNP as well as 14 dB and 2.8 refMFI for CRP could be achieved. The DNA based biomarker cfmDNA was detected with a SNR of 20 dB and a refMFI signal of 0.7 in presence of 1ng/µl cfmDNA. Kinetic experiments indicate that 50% of the fluorescence intensity is achieved within 7 minutes. In addition to assay progress, we developed the open source *digilogger*, which can be applied for data evaluation, visualization, classification and machine learning.

Figure 1. Overview of microfluidic chip platform (left) with the *digilogger* data analysis software (right).

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An SPRi-based biosensor for the detection and characterization of different neuronal and glial populations of circulating extracellular vesicles

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The use of extracellular vesicles (EVs) for diagnostic and disease monitoring purposes is becoming particularly appealing, considering that the pathological status greatly affects the EVs content. Moreover, brain-derived EVs present in blood plasma could be seen as a direct read-out of the condition of the brain and can thus be studied as peripheral biomarkers of neurological disorders [1]. Inspired by remarkable development of plasmonic biosensors, we have designed an antibody array using Surface Plasmon Resonance imaging (SPRi) with the aims to detect CNS-derived EVs present in human plasma and to characterize them according to the presence and the relative amount of membrane molecules involved in pathological processes.
EVs were isolated from human plasma by size-exclusion chromatography and characterized by nanoparticles tracking analysis, transmission electron microscopy, western blot and a colorimetric test. The SPRi array was optimized for the detection of EVs subpopulations, by using a suitable surface chemistry and specific antibodies for each class of vesicle to be detected.
With this strategy we demonstrated the possibility to simultaneously distinguish EVs derived specifically from neurons, microglia, astrocytes and oligodendrocytes. Moreover, the presence and relative amount of another membrane constituent (GM1) were evaluated, showing a different composition of EVs according to their cellular origin [2], and a difference in the amount of this molecule on vesicles coming from healthy subjects and Alzheimer patients.
In conclusion, SPRi can be used to discriminate different populations of circulating EVs and to perform their concomitant characterization. Thanks to these properties, the optimized SPRi biosensor demonstrated to be an effective platform for the possible use of EVs as clinical biomarkers of Alzheimer's disease.

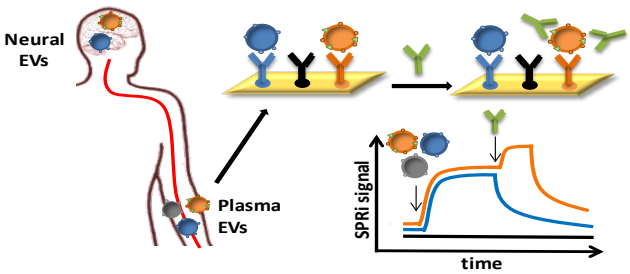

Figure 1. Schematic representation of the SPRi-based biosensor for the simultaneous detection and analysis of different neural subpopulations of human plasma EVs

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Nanodiscs incorporating functional beta-1 adrenergic receptors as novel diagnostic approach for autoimmune dilated cardiomyopathy

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Dilated cardiomyopathy (DCM) is a common cause of heart failure with a prevalence of 1:2500. Among genetic, metabolic and toxic factors inducing DCM, the presence of disease-driving anti-β1-adrenergic receptor (β1AR) autoantibodies was estimated as 25% to 75% in DCM patients. However, these autoantibodies could also be found in 10% of healthy controls. The association of anti-β1AR autoantibodies with the disease progression was proven by different analytical approaches and in vivo animal testing. Studies have shown that the removal of anti-β1AR antibodies as well as the interruption of the antibody-antigen interaction leads to a prolonged improvement of heart function in patients.

To apply treatments targeting the autoantibody-β1AR interaction, the presence and impact of anti-β1AR autoantibodies in DCM patients must be examined. Until now, a reliable, standardizable diagnostic method is still not available. Here, we present a novel in vitro diagnostic assay to support the diagnosis of anti-β1AR autoantibody-induced DCM.

To enable the presentation of the native 3-dimensional conformational epitope, the β1AR was overexpressed in human HEK293 cells and reconstituted into nanodiscs. Nanodiscs are round slides of phospholipid bilayers stabilized by membrane scaffold proteins, which provide a native-like membrane environment for the incorporation of the correctly folded β1AR and stabilize it in aqueous solution for measurements with standard routine techniques [1]. With this approach, an immunoassay in was developed and optimized for the detection of anti-β1AR autoantibodies. A surface-plasmon resonance (SPR) biosensor was used to determine both affinity and kinetic parameters for β1AR-binding antibodies.

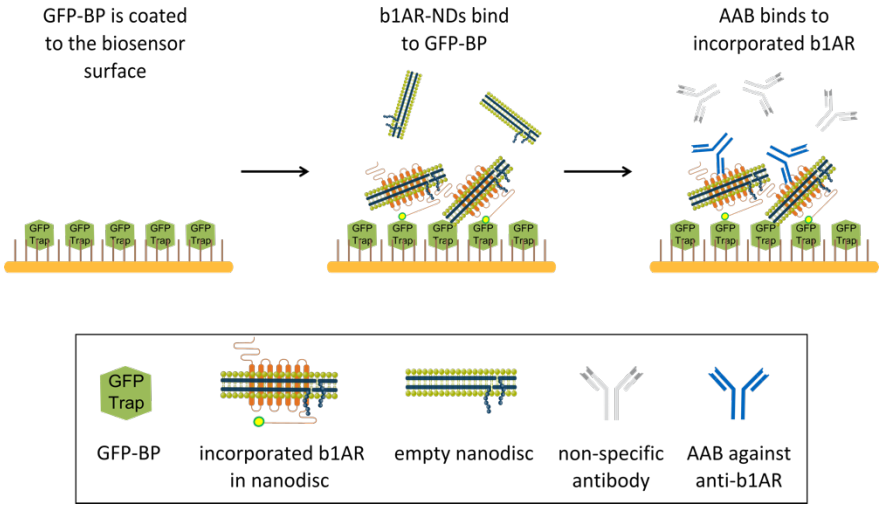

Figure 1. Binding of antibodies to β1AR-YFP NDs captured on the sensor chip via GFP-Trap. Schematic illustration of the standard SPR setup.

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Label-free Aptasensing Platform for Electrochemical Detection of Interleukin-6

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Interleukin-6 (IL-6) is a multifunctional cytokine that influences the activity of cancer cells. It is a glycoprotein which consists of 184 amino acids and has molecular weight of 26 kDa [1]. Furthermore, IL-6 is one of the major cytokines which is found at high concentrations and known to be dysregulated in cancer. Its overexpression has been reported in almost all types of tumors. The strong association between inflammation and cancer is reflected by the high IL-6 levels in the tumor microenvironment. It is involved in tumor genesis, tumor growth, and malignant differentiation of cancer cells, microenvironment, immunomodulation and metabolism [2]. A highly sensitive and selective aptasensor for quantitative detection of IL-6 was elaborated by using a glassy carbon electrode modified with p-aminobenzoic acid, p-aminothiophenol and gold nanoparticles. A thio-terminated aptamer specific for IL-6 was immobilized on the surface of the modified electrode via the formation of gold-sulphur bonds. This DNA sequence was then used as a detection probe to capture the target protein at the biosensor surface allowing label-free detection by electrochemical impedance spectroscopy. The developed aptasensor showed a good linear response from 10 pg mL^{-1} to 100 ng mL^{-1} with a detection limit of 3.3 pg mL^{-1}, within the range of physiological concentration of protein. The biosensor exhibited high selectivity and has been successfully used to detect IL-6 in blood serum collected from patients suffering of colorectal cancer, with excellent recoveries after the addition of known amount of the target.

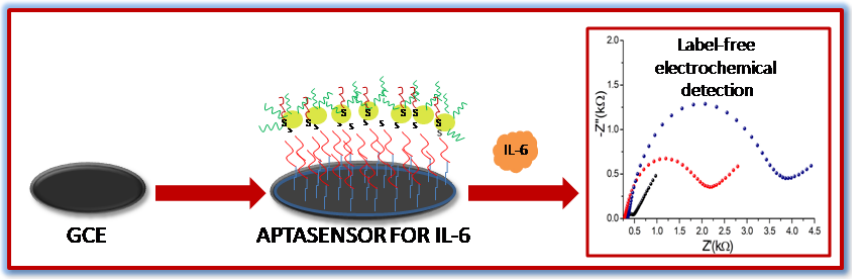

Figure 1. Schematic representation of the aptasensor elaboration protocol and testing.

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New electrochemical magnetoimmunosensor for interleukin-6 quantification in human serum

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Interleukin 6 (IL6) is a polypeptide cytokine with a structure consisting of 4 large Helix-type and a small Helix-type strand. IL6 stimulates haematopoietic cells, has an extremely important role in the immune system, and has a high responsibility in regulating the endocrine and nervous system, favoring cell growth and immunoglobulin production. The production of IL6 is regulated by several physiological factors, including diet, exercise and stress. Several clinical trials have demonstrated IL6 involvement in a number of pathological processes and play an important role in many diseases such as: rheumatoid arthritis, acute infections, *lupus erythematosus*, AIDS, transplant and is overexpressed in a series of cancers. Considering these aspects, the importance of monitoring the concentrations of IL6 in biological fluids is of significant interest in biomedical field. This task can be considered quite difficult if one considers the rather low physiological plasma concentrations of IL6 of about 6 pg mL^{-1} [1]. The development of a very sensitive electrochemical label free magnetoimmunosensor based on planar graphite-screen printed electrodes (GSPE) as transducers for the detection and quantification of IL6 proteins in human serum is described here. The immunosensor has been produced by immobilizing G-protein modified MBs functionalized with anti-IL6 antibody (anti-IL6 AB) at the surface of the GSPE using an external magnetic field. The employment of anti-IL6 AB had a double role: to ensure the formation of the antibody-antigen complex capable to capture the biomarker of interest and additionally, to provide a good selectivity for IL6 protein when the structural platform operates in complex biological matrices. A supplementary step was performed in order to block the unoccupied sites of the magnetic nanoparticles with the anti-IL6 AB but also to accomplish the formation of the antigen-antibody complex in the presence of the target molecule. All the experimental parameters involved in the elaboration and testing protocol were optimized. A linear calibration plot between the charge transfer resistance and the logarithmic concentration of IL6 was achieved in the 1 pg mL-1 to 1 µg mL-1 range. A limit of quantification of 1 pg mL-1 and a detection limit of 0.3 pg mL-1 were obtained. The optimized magnetoimmunosensor showed an excellent selectivity against some potentially interfering proteins and has been successfully applied for the determination of target protein in human serum, proving its clinical relevance [2].

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Acknowledgement
The authors are grateful for the financial support from the Romanian National Authority for Scientific Research and Innovation, CNCS/CCCDI-UEFISCDI; project number PN-III-P1-1.2-PCCDI-2017-0407/ 39PCCDI/2018 (INTELMAT), within PNCDI III.

Evolving immune-sensing on peptide microarrays: application to Zika virus diagnostics

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Protein microarrays have not yet fully realized their potential in becoming a standard method of analysis to identify protein-protein interactions. This can be ascribed to issues related to the expression and purification of a large number of proteins, to the need of oriented immobilization, to poor surface binding density, and to denaturation at the interface. Most of these challenges can be overcome by the use of arrays of peptides since these molecules can be rationally designed to recapitulate some of the structures and functions of proteins being, at the same time, much more stable and easy to manipulate. However, peptide microarrays are sometimes limited in their use by low specificity in complex biological samples and scarce immunoreactivity, especially when the epitopes involved are made by non-continuous linear sequences.
Here we present a pipeline enabling the rapid delivery of immunoassays for different diagnostics contexts which expands the current limits of peptide-based serodiagnosis. Our strategy starts from the use of computational tools for accurate immune-reactive peptide design; exploit chemo-selective strategies for optimal probes presentation on silicon sensing surfaces by the use of clickable polymeric coatings and finally generate peptide chips for fluorescence microarrays. We will show how the rigorous control of probe design, orientation and surface density enabled by our platform positively impacts the diagnostic accuracy of antibody detection in serum. Furthermore, we will show a novel strategy to increase immunoreactivity in the context of Zika virus diagnosis by functional mimicking of discontinuous epitopes of NS1 protein enabled by click chemistry-based peptide immobilization on silicon microarrays surfaces.

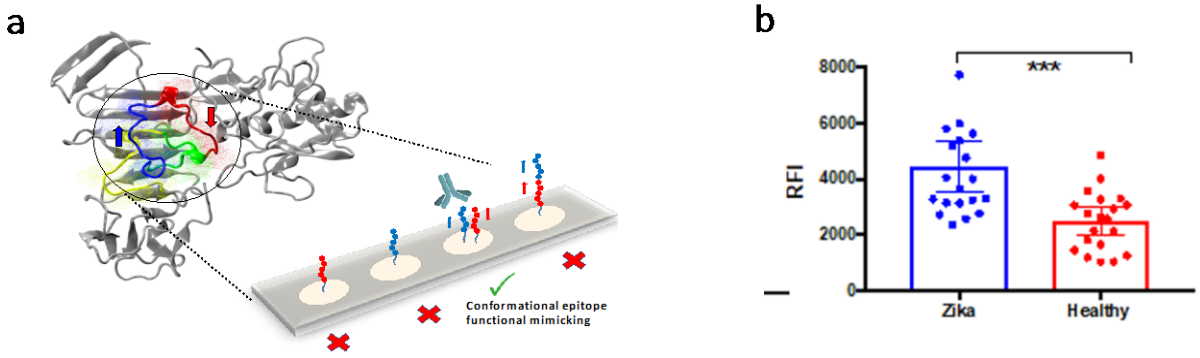

Figure 1. a) Strategy for functional mimicking of discontinuos epitopes of NS1 protein. (b) Analysis of Zika patients and healthy controls

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Analysis of KRAS, NRAS and BRAF mutational profile by combination of in-tube hybridization and universal tag-microarray in tumor tissue and plasma of colorectal cancer patients

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Microarray technology fails in detecting point mutations present in a small fraction of cells from heterogeneous tissue samples or in plasma in a background of wild-type cell-free circulating tumor DNA (ctDNA). The aim of this study is to overcome the lack of sensitivity and specificity of current microarray approaches introducing a rapid and sensitive microarray-based assay for the multiplex detection of minority mutations of oncogenes (*KRAS*, *NRAS* and *BRAF*) with relevant diagnostics implications in tissue biopsies and plasma samples in metastatic colorectal cancer patients. In our approach, either wild-type or mutated PCR fragments are hybridized in solution, in a temperature gradient, with a set of reporters with a 5' domain, complementary to the target sequences and a 3' domain complementary to a surface immobilized probe. Upon specific hybridization in solution, which occurs specifically thanks to the temperature gradients, wild-type and mutated samples are captured at specific location on the surface by hybridization of the 3' reporter domain with its complementary immobilized probe sequence. The most common mutations in *KRAS*, *NRAS* and *BRAF* genes were detected in less than 90 minutes in tissue biopsies and plasma samples of metastatic colorectal cancer patients. Moreover, the method was able to reveal mutant alleles representing less than 0,3% of total DNA. We demonstrated detection limits superior to those provided by many current technologies in the detection of *RAS* and *BRAF* gene superfamily mutations, a level of sensitivity compatible with the analysis of cell free circulating tumor DNA in liquid biopsy.

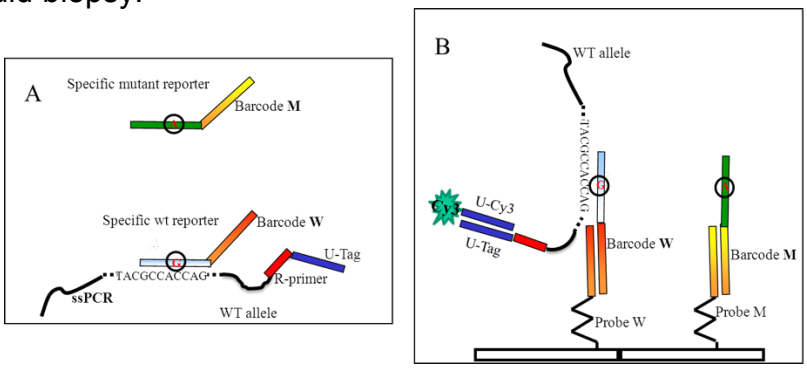

Figure 1. (A) The specific wild-type reporter hybridizes in solution with the ssPCR. (B) Different barcodes in the reporter sequences direct the ssPCRs to different positions on the array revealed when the U-tag sequence of the ssPCR binds the Universal-Cy3.

Innovative tools for *point-of-care* diagnostics and *on-field* assays

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The large progress in Lab-On-Chip (LOC) technologies and micro total analysis systems enabled the development of highly flexible instruments for a prompt diagnosis and control of diseases, monitoring of environmental threats, toxins and allergens from food and agriculture products. Here we describe the development of a multipurpose biochip with integrated microfluidic and sensing components. Specifically, the layout consists of various sensing areas, each one including an array of transducers (gold interdigitated electrodes), to which microfluidic channels are assembled and used for the delivery of functionalization and sample solutions into the chambers. Starting from this integrated platform, we optimized an extensive portfolio of robust, miniaturized and reliable biosensing tools based on innovative transduction systems. We realized Electrochemical Impedance Spectroscopy (EIS) based biosensors for the ultrasensitive, point-of-care detection of tumor biomarkers (for prostate and pancreatic cancers) [1, 2], toxins from environmental samples [3] and food contaminants [4, 5]. Our EIS platform has also been used for the near-the-bed evaluation of multiple lower genital tract pathogens (*Candida a.*, *Streptococcus a.*, *Chlamidia t.* from vaginal fluid) providing clinicians a cost and time-saving alternative tool for routinely analysis [6]. More recently, our EIS platform has also been integrated in a lab-on-chip for *X. fastidiosa* detection from olive tree leaves samples, giving advantages of portability, low-costs and ease of use compared to conventional diagnostic methods [7]. We also developed an innovative platform based on electrosynthesized Molecularly Imprinted Polymers (MIP), providing an enhanced sensitivity of small molecules with respect to standard methods [8]. Finally, we explored the high potential of Radio Frequency (RF) methods also to develop sensors and actuators based on Surface Acoustic Waves (SAW) [9, 10] to obtain robust biosensing methods for the detection of small molecules or rare cells. One of the latest challenges deals with the realization of a lab-on-chip for semen assessment, aiming to provide an useful and easy-to-use tool among the Assisted Reproductive Technologies (ARTs) for both sperm analysis and selection.

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Electrochemical biosensors: promising tools for multiplexed determination of emerging cardiovascular biomarkers

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Cardiovascular diseases are a major cause of death among adults worldwide, the number of admissions due to heart failure having quadrupled within the last 30 years. Several biomarkers have been associated with increased relative risk for cardiovascular events and their utility has expanded from constituting a mere aid in the diagnosis of disease to being important in predicting risk and prognosis [1]. However, some cardiac markers can also work as some types of cancer detectors and most cardiovascular diseases have more than one marker associated with their incidence. Due to this limited specificity, the measurement of a single cardiac biomarker is usually not enough to diagnose heart problems. Consequently, simultaneous detection of several cardiac biomarkers is attracting much attention to provide a more accurate diagnostic tool and enhance significantly the predictive value for the risk factor [2]. Furthermore, multiplex detection is also advantageous because it offers higher sample throughput, simplified analytical procedures, less sample consumption, reduced turnaround times, improved test efficiency, and a more reasonable cost compared to traditional in-parallel single-analyte assays [3]. In this context, electrochemical biosensors can be envisaged as an easy rapid and robust alternative to classical methods of analysis for clinical biomarkers being also capable of multianalyte testing and useful to facilitate point-of-care devices (POC). Amperometric sandwich immunoassay strategies using screen-printed carbon electrodes and/or arrays as substrates and micro-magnetic beads as solid supports to perform the immunological reactions were developed for the simultaneous detection tyrosine kinase receptor (AXL), lipoprotein A (LpA) and amino terminal pro-B-type natriuretic peptide (NT-proBNP) considered as very promising emerging biomarkers for cardiac risk prediction. The developed MBs-based immunosensing platforms exhibited a very attractive analytical performance in terms of sensitivity, selectivity and stability. The developed methodologies were sensitive enough to detect the analytes taking into account the established cut-off values in serum samples (71 ng mL^{-1} [4], 300 mg L^{-1} [5] and 1,000 ng mL^{-1} [3] respectively) to discriminate heart failure patients from healthy individuals, thus demonstrating the suitability of the method for clinical practice. Moreover, after minimal sample treatment (just a dilution with buffer), the results obtained in human sera samples for real patients were in good agreement with those provided by a conventional ELISA kit. Preliminary results on the development of a multiplexed platform will also be shown.

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[4] V. Serafín, R. M. Torrente-Rodríguez, M. Batlle, P. García de Frutos, S. Campuzano, P. Yáñez-Sedeño, J.M. Pingarrón. Microchim Acta 184, 4251–4258 (2017).
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Peptide-based electrochemical biosensor for detection of protease activity in cancer diagnosis

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Proteases are important in multiple processes that are carried out in normal cells. In living systems, a balance between proteases and their anti-proteases occur, thus its unbalance leads to many diseases like cancer. Recent studies have shown that they are involved in progression, and tumor growth both at primary and metastatic sites [1]. Trypsin, one of the typical well-known serine proteases, plays an important role in multiple physiological processes (e.g. food digestion, blood coagulation, fibrinolysis, and control of blood pressure) and also in a wide range of important pathological processes e.g. atherosclerosis, inflammation and cancer proliferation, invasion and metastasis [2]. It has been found that trypsin expression is increased in human cancer cells of the ovary, prostate, lung, stomach, colon, and others. Trypsin has potential as a prognosticator, and patients with trypsin-positive colorectal cancers have shorter overall and disease-free survival than patients with trypsin-negative [3]. In this work, we propose an electrochemical peptide-based biosensor for trypsin determination. The implemented methodology, involves the use of a short peptidic sequence, dually labelled with fluorescein isothiocyanate (FITC) and biotin through which it is immobilized onto neutravidin-modified magnetic beads (MBs). After incubation with HRP-anti-FITC Fab fragments, modified MBs are magnetically captured at the surface of a screen-printed carbon electrode (SPCE), and amperometric detection is performed using hydroquinone (HQ) as electron transfer mediator and H_2O_2 as the enzyme substrate. A typical "on−off" change of the amperometric signal is observed in the presence of trypsin as a consequence of the peptide cleavage and release of the FITC-labelled peptide fragment from the MBs surface (Figure 1). Once all the manufacturing steps of this peptide-based biosensor optimized and its analytical characteristics evaluated, it will be applied to the analysis of clinical samples.

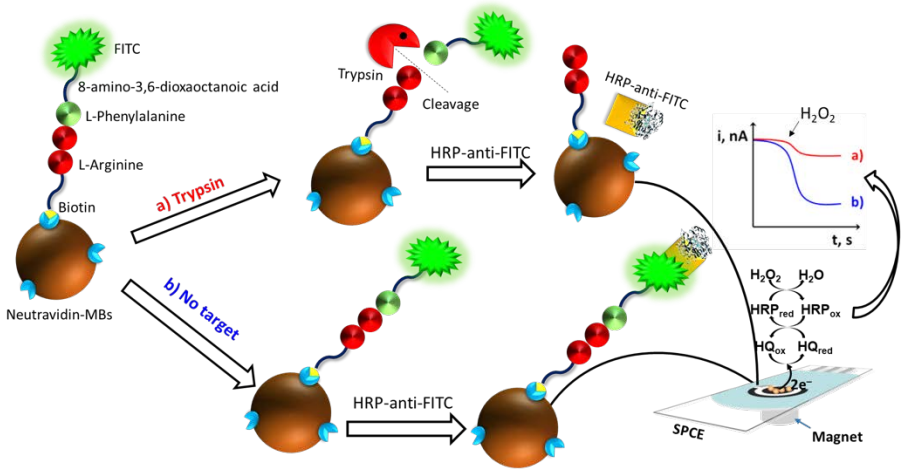

Figure 1. Schematic representation of the biosensor developed for trypsin determination by using peptide-MBs and HRP-anti-FITC Fab fragments labelling.

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$NdFeO_3$ as a new electrocatalytic material for electrochemical monitoring of dopamine

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In recent years, novel electrocatalytic materials have been proposed for developing more sensitive, simple, stable, and low-cost nonenzymatic electrochemical sensors. In this regard, in previous works [1,2] we reported the promising characteristics of various perovskite nanomaterials as electrocatalytic modifiers for screen printed carbon electrode (SPCE). Perovskite ferrite oxides exhibits fascinating properties for electrochemical sensing due to the ability of the perovskite structure to accommodate different metallic ions. Here, we reported a study in developing an electrochemical dopamine sensor (DA), an important brain neurotransmitter, based on $NdFeO_3$. $NdFeO_3$ nanoparticles, of approximately 28 nm in size, were synthesized by a simple thermal treatment method [3] as follows: metal nitrates with equal molar ratios were added to poly(vinyl alcohol (PVP) solution while the temperature of solution was kept at 90 ˚C for 2 hours. The mixed solution was placed in oven (100 ˚C) for 24h. The solid product formed was crushed, ground in a mortar to form a soft powder and finally annealed at 700˚C (in air for 3 h) for decomposing organic compounds and favor particle crystallization (Fig 1a). Perovskite nanopowders were largely characterized by complementary physical and chemical techniques. SPCE electrodes modified with $NdFeO_3$ nanoparticles were fabricated (inset Fig. 1a) and tested by cyclic voltammetry. The modified electrode exhibited a pair of redox peaks (Fig. 1b) in which the anodic peak potential is significantly shifted to lower potential compared to bare SPCE, indicating a strong promoting effect toward the electrochemical oxidation of dopamine. Moreover, the peak current of $NdFeO_3$-SPCE sensor is more than 2 times higher than of bare SPCE (Fig. 1c). demonstrating high sensitivity to DA, good linear range from 0.1 μM to 500 μM and low detection limit. The modified sensor, operating in amperometric mode at the potential of 0.2 V, exhibited rapid response and anti-interference ability from uric acid. In summary, our strategy opens an alternative horizon for novel perovskite based electrochemical sensors, which could be sensitive, easy to use and inexpensive for biomedical, food and environmental analysis applications.

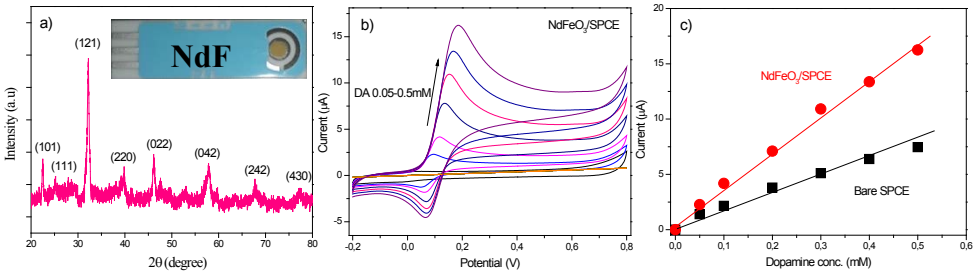

Figure 1. a) XRD of $NdFeO_3$ nanoparticles. Inset: picture of the modified sensor; b) CV responses to different DA concentrations in PBS (pH 7.0) of $NdFeO_3$-modified SPCE electrode; (c) Comparison of the calibration curves obtained with SPCE and $NdFeO_3$-SPCE electrodes.

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Innovative micro-opto-fluidic sensing platform based on rectangular glass micro-capillaries for refractive index detection

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In recent years, micro-opto-fluidic sensors have gained an increasing importance for measuring refractive index (RI) and/or concentration of solutions [1]. These sensing platforms allow label-free detection on extremely small volumes of the sample, with remote, non-contact, non-invasive optical readout [2]. The core of the microfluidic platform presented in this work consists of rectangular glass micro-capillaries, low cost devices commercially available in several formats. In order to have a better insight in the working principle of the biosensor, the capillary has been modelled as a sequence of three Fabry-Perot etalons (glass wall – channel – glass wall); the reflection and transmission coefficients at each interface have been calculated using Fresnel equations [5] as a function of the wavelength in the near infrared region. The instrumental configuration used to carry out the experimental measurements is shown in Figure 1(a). The light source is a broadband ASE source, such as a superluminescent light emitting diode (SLED) with a Gaussian emission spectrum centred at a wavelength λ = 1.55 μm. The output beam passes through an optical isolator followed by a 2x2 coupler and it is orthogonally shined on the flat surface of the capillary. The transmitted (T) and the back reflected (R) optical power are collected by two lenses and directed towards the optical spectrum analyzer. The signals are acquired and, after normalization to a reference spectrum, they are employed to compute the T/R ratio. Filling of the micro-channel is possible just by capillary force, while the sample is discarded using a peristaltic pump. The biosensor has been tested in glucose-water dilutions in concentration from 0 to 16.5 %, resulting in nominal RI values from 1.3154 to 1.3393 RIU. Figure 1(b) reports the T/R experimental spectra for a micro-capillary with thickness of the glass walls of 21 μm and channel depth of 30 μm. The tubing behaves as a resonator and the experimental spectra exhibit sharp maxima: their wavelength position shifts towards higher values when the sample RI increases. By fitting the maxima position as a function of the RI, it is possible to retrieve the linear calibration curves. Defining the sensitivity of the biosensor as the slope $S = d\lambda_{MAX}/dn_{fluid}$, values up to 484.5 nm/RIU have been found. The experimental results are in good agreement with the theoretical analysis. In the presentation, both theoretical analyses as well as experimental results will be illustrated, demonstrating the potential of this low-cost platform for sensing.

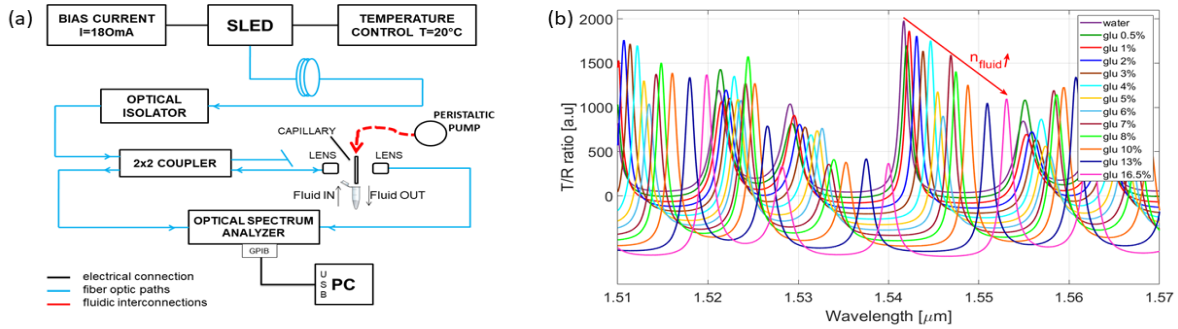

Figure 1. a) Block diagram of the all-fiber optical setup employed in the experiments. (b) Experimental spectra (shown vertically shifted of 60 a.u. with respect to each other for a better visualization).

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Integration of the platelet antigens GPIIb/IIIa and GPIb/IX into lipid bilayer nanodiscs for the detection of autoimmune thrombocytopenia

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In autoimmune thrombocytopenia (AITP), platelets are destroyed by autoantibodies against different platelet membrane antigens, mostly against glycoprotein IIb/IIIa complex (GPIIb/IIIa) and glycoprotein Ib/IX complex (GPIb/IX). Although tests for patient anti-platelet antibodies are currently available, they have low sensitivities and a negative result does not necessarily rule out AITP. With the novel nanodisc (ND) technology, a sensitive and specific test for anti-platelet antibodies can be developed. NDs are self-assembled bilayers stabilized by synthetic membrane scaffold proteins, into which membrane proteins can be integrated in a native and functional form. Our aim is the detection of patient anti-platelet antibodies by SPR using NDs incorporating platelet membrane proteins. The reconstitution of membrane proteins in NDs allows for correct protein folding and a native lipid environment, thus increasing diagnostic performance of the test.

The platelet membrane proteins GPIIb/IIIa and GPIb/IX were isolated from over-expressing HEK cell lines. As common protocols for ND preparation are costly in terms of labour and time, including membrane isolation, ultracentrifugation and long incubation steps, a simplified generation protocol was designed and tested [1]. Resulting NDs were examined with regard to size, shape and membrane protein incorporation. NDs were immobilized on an SPR gold chip and the different incorporated glycoproteins could be verified by monoclonal antibody binding. With this set-up, serum samples from patients with autoantibodies could be differentiated from healthy controls.

An encouraging retrospective study with serum samples from AITP patients is currently under way in cooperation with coagulation centre specialized in AITP.

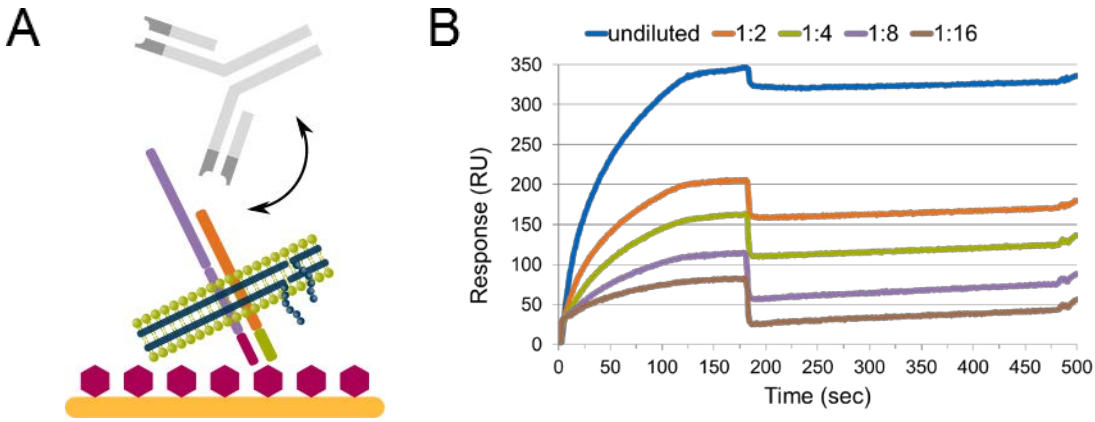

Figure 1. A: Schematic illustration of SPR assay with ND technology. B: SPR sensorgrams of nanodisc immobilization in different dilutions onto SAM gold chip.

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Development of a new biosensor for increasing the specificity and selectivity to detect anti-factor VIII antibodies from acquired haemophilia a patients in human plasma

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Haemophilia A is a coagulation disorder disease. Patients with this disease have a deficiency of coagulation factor VIII (FVIII). A lack of FVIII is manifested by life-threatening haemorrhages. Bleeding can occur after an injury or spontaneously.
Commercial therapies treat haemophilia patients with FVIII substitution preparations. Due to the high frequency and duration of the therapy, up to 15-20 % of the patient develop antibodies against the FVIII. These antibodies inhibit FVIII and the patient has a high probability to suffer from serious spontaneous bleeding. This can cause a life-threatening situation.
In order to ensure the best therapy, it is of great importance to detect as well inhibitory as non-inhibitory antibodies that any bleeding tendency does not even occur.
In previous work, a diagnostic procedure for SPR was developed [1]. This biosensor is based on a hydrogel polymer brush matrix with terminal carboxyl groups. On the activated carboxyl groups, the ligand (FVIII) binds covalent. The patient antibody against FVIII binds on the ligand and generates the measuring signal. With a novel strategy, we want to improve the cutoff-level between the inhibitory and non-inhibitory antibodies in affected patients. The biosensor surface is stabilized by the use of polymers and the immobilization mechanisms for FVIII (full length or B domain-deleted) are improved (See Fig. 1A) in order to increase selectivity and specificity of the SPR biosensor (See Fig. 1B). Measurements with authentic patient samples are under way.

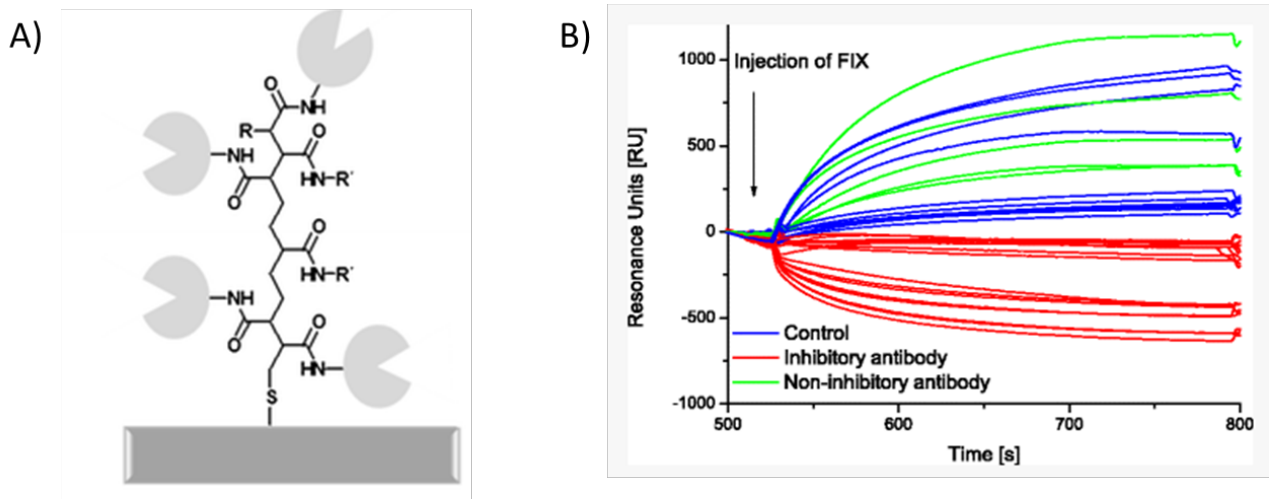

Figure 1. a) Covalent binding of FVIII on a biosensor with a functionalized polymer brushes (b) Binding curve of inhibitory, non-inhibitory and control samples (without antibodies). Figure modified according to Kocot et al. [1].

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Novel approach to optimized antibiotic therapy through patient-tailored antibiotics dosing

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Antimicrobial drug resistance is one of the main healthcare challenges and one of the biggest threats to global health. Moreover, antimicrobial drug resistances make treatment more difficult and overusing or misusing of antibiotics make resistances develop even faster. Additionally, current antibiotic treatment is frequently prone to inappropriate dosing. Therapeutic drug monitoring (TDM) by means of Point-of-Care Testing (POCT) of antibiotic concentrations in blood is a promising avenue to overcome treatment failure in infectious diseases.
The methodology of the planned interdisciplinary project is based on intravenous microdialysis and chip-based surface-enhanced Raman spectroscopy. Devoted to critically ill patients, different population groups in a multicentre clinical study will be assessed and continuous monitoring of antimicrobials will be performed in a single or multiplexing mode up to 24 h. Sophisticated pharmacokinetic/pharmacodynamic analysis of drug resorption, distribution, and metabolisation/ elimination will facilitate optimized drug therapy.
The presented approach enables fast information for clinicians to facilitate rapid decision-making for the individual patient to optimize antibiotic dosing. Consequently, the proposed POCT of antibiotic concentrations in the patient will beneficially impact therapy duration, length of stay in the hospital, and healthcare costs.

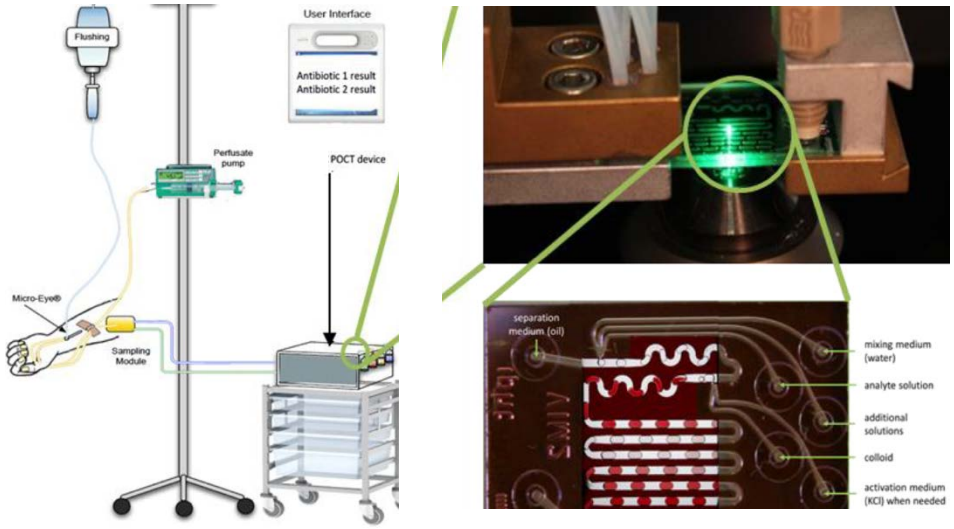

Figure 1: TDM-POCT device – concept and methodology.

Polymethylmethacrylate nanoparticles promote endocytosis of a survivin molecular beacon as theranostic agent in human cancer cells

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One of the main goals of cancer nanomedicine is the development of effective drug delivery systems. Among the optical nanoprobes, molecular beacons (MBs) have been proposed as theranostic agents that conjugate the sensing of specific mRNA with the silencing activity [1]. MBs are oligonucleotide sequences generating a fluorescent signal when they hybridize with their target mRNA. Although MBs have such a promising function in the field of cancer diagnosis and therapy, their poor stability toward nucleases and low intracellular uptake hinder their practical application. Polymeric nanoparticles (NPs) have been exploited as nanocarriers for their stability, handiness, tunable properties and biocompatibility [2]. In this context, we demonstrated the carrier ability of polymethylmethacrylate nanoparticles (PMMA-NPs) in A549 cells by confocal microscopy. After protocol optimization, a hardly detectable fluorescent signal was obtained after incubation of the cells with the MB alone (1.90 ± 0.40 fluorescent spots per cell with a mean area of 1.04 ± 0.20 μm^2), while bright fluorescent spots inside the cells were evident by using the MB loaded onto the PMMA-NPs (27.50 ± 2.30 fluorescent spots per cell with a mean area of 2.35 ± 0.16 μm^2). These results demonstrate the ability of the PMMA-NPs to promote the survivin-MB internalization, suggesting that this complex might represent a promising strategy for intracellular sensing and for the reduction of cancer cell proliferation [3].
Furthermore, experiments with Alexa Fluor 647 Dextran™ as marker for endocytosis showed that in the presence of nanoparticles the number of endocytic vesicles per cell doubled and their mean size significantly ($p<0.001$) increased, indicating a promotion of endocytosis. By using LysoTracker™ Deep Red, as marker of lysosomes, we found that nanoparticles co-localize with lysosomes. Moreover, a cellular release of nanoparticles detected in the culture medium, suggested a role of lysosomal exocytosis in nanoparticle elimination.
By using ER-Tracker Green™, as marker of the Endoplasmic Reticulum (ET), we observed the MB fluorescence in proximity of the labelled Endoplasmic Reticulum; it was indicative that the specific opening of the MB occurs in proximity of its target mRNA.

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Acknowledgements
This study was supported by the National CNR Flagship Project NANOMAX ENCODER. and by the National project “Improving therapy for breast cancer and melanoma by transcriptome-methylome profiling, integrative network inference, and design of novel theranostic tools” funded by the Istituto Toscano Tumori (ITT).

Fiber-based planar antennas for biosensing and diagnostics

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Traditional methods of pathogen determination delay treatment and also increase the recovery period for the patient. The ability to deliver critical data for decision makers in a timely manner hence makes high-performance diagnostic automation a future key component of the healthcare system. The biggest advantage of optical probes is the ability to detect low quantities of target molecules without direct contact to the sample. Although fluorescence-based approaches are available and sufficiently sensitive, they often lack the necessary flexibility for being integrated into lab-on-a-chip systems. Nanophotonics-based sensing promises to build on the advantages of optical sensing, while overcoming its limitations by providing a high sensitivity, specificity, dynamic range, as well as the possibility for easy integration into simple and affordable devices.
The project FASPEC (Fiber-based planar antennas for biosensing and diagnostics) aims at developing and prototyping a high-performance fluorescence-based molecular assay for in-vitro diagnostics that integrates lab-on-a-chip and optical readout functionalities within a single, fully automated platform. The key biophotonics innovation of the project is the replacement of the bulk optics used for collecting the fluorescence signal with a suitably designed optofluidic chip. The latter shall function as an *optical antenna* [1] to direct fluorescence towards the sensor head, hence enhancing the sensitivity of the fluorescence-based assay by orders of magnitude. Application-specific lab-on-a-chip systems equipped with our high-throughput and ultrasensitive detection scheme have been envisioned. In particular, the prototype design and validation will be focused on sepsis, which is a common hospitalization disease with mortality rates that range from 20% to 80%.

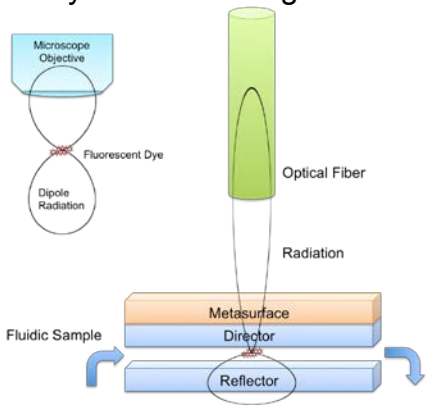

Figure 1. Collection strategy based on the concept of a planar optical antenna. The antenna architecture is made of a reflector, a director and an optional metasurface to further control the emission pattern. The narrow radiation cone is efficiently collected by an optical fiber. Inset: conventional operation of single-molecule fluorescence microscopy.

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Novel digital image analysis of FISH stained bacteria

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Introduction. The adhesion of bacteria is a widespread phenomenon and a crucial step in infection and colonization of hosts. Bacterial adhesion to organic material like heart valves or non-organic material like prostheses causes high risk for chronic infections. In order to determine the presence of bacteria adhered to different materials, the determination of attached bacteria species is of high interest.
Objectives. The aim was to develop a tool to recognize and count bacterial cells stained by Fluorescence *in situ* hybridization (FISH) using automated digital image VideoScan technology (Figure 1) (Rödiger et al. 2013). Thereby, infection-adhesion assays should be carried out to infect human tumor cell lines and porcine tissue sections. We intended to apply different algorithms enabling the determination of cells within diverse sizes of bacterial colonies.
Material & Methods. Using specific mathematical tools, we developed a novel algorithm for the detailed analysis of bacterial cells adherent to mammalian cells (HEp-2) and porcine tissue sections. Infections of hosts were carried out using cell line infection-adhesion assay and novel tissue infection-adhesion assay technique. Specific eubacterial FISH probe (EUB338 Atto647N) and species-specific *E.coli* probe (ECO453 Atto647N) were used to stain bacteria. DAPI staining was done for host analysis.
Results. Performing infection-adhesion assays for cell lines and tissue sections we were able to infect hosts with enteropathogenic *E. coli* and enteroaggregative *E. coli*. Thereby, a novel software algorithm was used to count FISH stained bacteria even in colonies. Compared to standardized technologies like lysis assay or propidium iodide staining, the FISH analysis showed higher sensitivity for the determination of bacterial cells.
Conclusion. Automated digital image analysis of FISH stained bacteria is a promising alternative for time-consuming manual methods.

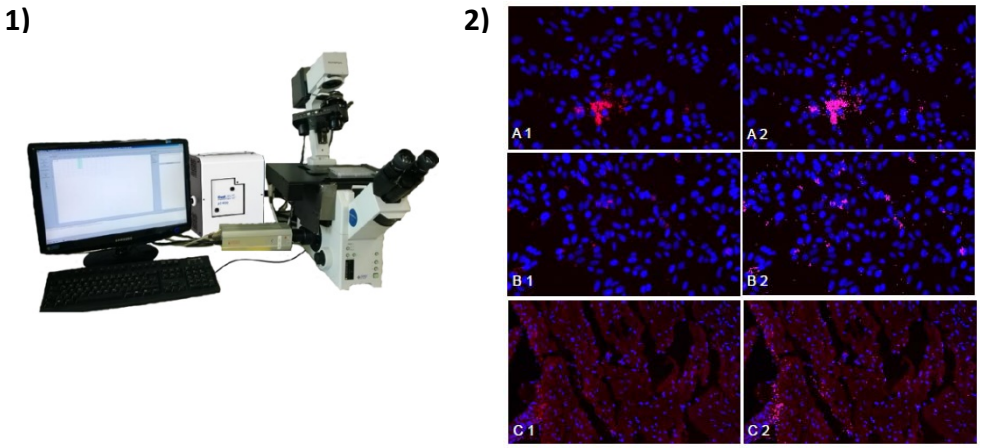

Figure 1. Automatic modular fluorescence microscope.

Figure 2. Evaluation software to detect and count bacteria adhered to cells (A, B) and porcine tissue (C).

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An aptamer-based fluorescent biosensor for insulin detection

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Biosensors for the specific, sensitive and rapid detection of analytes play an important role in healthcare diagnostics, biochemical applications, food safety, and environmental monitoring. Although several sensing concepts and devices have been developed, many longstanding challenges associated with establishing low-cost, easy-to-use, specificity, miniaturisation and reliable biosensor platforms remain largely unmet. Aptamers are single-stranded nucleic acids that have high affinity and specificity towards a wide range of target molecules. Aptamers offer exciting possibilities for enhancing sensitivity, lowering detection limits and improving stability of biosensor applications. In this paper, a 30-mer DNA hairpin aptamer with a G-quartet structure and specific for detecting insulin is described. Presence of insulin causes changes of aptamer fluorescence and upon excitation at FAM absorption band (492 nm) in the physiological buffer, fluorescence maximum was observed at 516 nm. Advantages of this biosensor were high affinity and selectivity of aptamer toward its target and unique properties including high stability and sensitivity. The limit of detection was 10 nM of insulin. This approach is simple and enables insulin to be detected rapidly and cost-effectively. Assay parameters are discussed, advantages and drawbacks are reviewed, and future immobilisation techniques are outlined.

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Analytical model describes the effect of poly-ethylene glycol on ionic screening of analyte charges in transistor-based immunosensing

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The screening of analyte charges by ions has been a major limitation of transistor-based immunosensing in physiological solutions. Recently, it has been reported that the co-immobilization of poly-ethylene glycol (PEG) with the recognition molecule has improved sensor responses upon analyte binding more than three times[1–4]. However, there is currently no analytical model available to explain this empirical effect. Existing analytical models used to describe the potential shifts at different analyte concentrations are based on the Langmuir binding of analyte [5]. We identified the receptor density, capacitance, analyte charge and dissociation constant as critical parameters that affect potential shifts. Based on our experimental data, only the analyte charge can account for a substantial signal enhancement, whereas other parameters are marginally affected by PEG. To capture the effect of PEG on the analyte charge, we introduce a pre-factor, the detectable charge q_{det}, which represents the portion of analyte charges actually detectable by the sensor (Figure 1). We propose a new analytical model to calculate the detectable charge as a function of the analyte size, the ionic strength, the binding position of the analyte with respect to the recognition molecule and the thickness of the surface layer. The model can quantitatively describe the PEG-induced signal enhancement (Figure 1 C) and can be used to recommend the choice of PEG size according to the analyte and recognition molecule used. Additionally, we include the competition of ions and the analyte for binding to the recognition molecule and the sensor surface (Figure 2). This extended model more accurately describes the concentration-dependent sensor responses than the traditional Langmuir binding model.

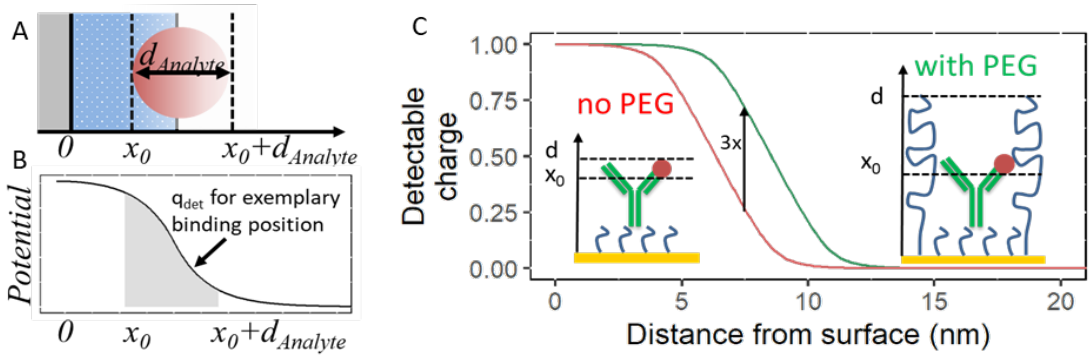

Figure 2: A) Schematic representation of the detectable charge dependent on the distance of analyte from surface. Because of their size ($d_{Analyte}$), protein analytes cannot be assumed as point charges. The analyte can theoretically bind at any position with respect to the electrode surface. Its position is described with x0 marking the side of analyte facing towards the surface and $x_0+d_{Analyte}$ marking the side of analyte facing away from the surface. B) The surface potential drops with distance from the surface following a sigmoidal decay, depending on the length of surface layer d and ionic strength. The detectable charge q_{det} is defined to be the integral of the potential distribution in the bounds of the analyte position (shaded area under the curve). In C) q_{det} is plotted as function of possible binding position with respect to the electrode surface for a surface without PEG (red) and a surface with PEG (green). With PEG, a threefold higher detectable charge can be achieved when the analyte binds at the expected distance away from the surface.

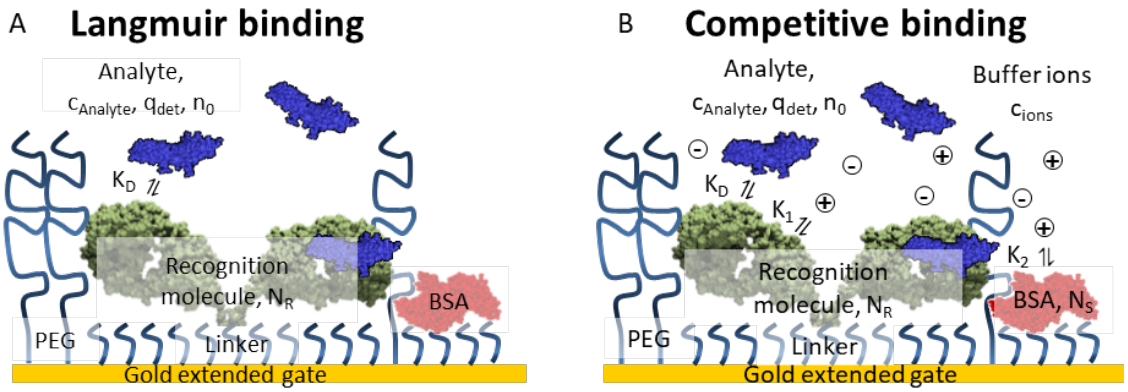

Figure 3: A) Schematic representation of the parameters considered in the model for potential shift based on Langmuir binding. The analyte has the bulk concentration $c_{Analyte}$, the total charge n_0 and the detectable charge q_{det} and is bound recognition molecule of surface density N_R with dissociation constant K_D. B) Schematic representation of the parameters considered in the model for potential shift based on competitive binding. Additionally to the parameters in A, we also consider the unspecific binding of ions from the buffer with bulk concentration c_{Ions} to the recognition molecule with dissociation constant K_1 and to the remaining surface groups of density N_S with dissociation constant K_2.

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Non-invasive electrochemical detection of melanoma in skin

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Melanoma is the most dangerous form of skin cancer with annually nearly 100'000 new incidences in Europe. Melanoma develops at the basal layer of the epidermis from where it grows first radially and then vertically. If detected early, *i.e.*, during the radial growth phase, the chances to cure by minor surgery are high. At later stages, when the tumour has reached the dermis, the 10-year survival rate drops below 40% [1]. The state-of-the-art screening tool for melanoma is dermatoscopy, operated by dermatologists inside their offices. They look for irregular shapes, colours and growth indications. However, a melanoma needs to be confirmed by a pathologist who looks at excised and sliced tissue sections applying immunohistochemistry (IHC). With this method, melanoma-associated antigens, such as Melan-A, S100 proteins or HBM-45, are labelled with an immuno-complex using a primary antibody against the antigen. A secondary antibody with peroxidase is conjugated to the primary antibody to generate locally colour changes in a solution containing a chromogenic peroxidase substrate. The obtained information on antigen expression and distribution pattern enable the classification of the progression stage of melanoma. We have recently shown electrochemical imaging with soft microelectrodes for recording maps of the distribution of the melanoma-associated antigen tyrosinase (TYR) through the adaption of immunolabelling from IHC [2]. Using electro-active substrates of the peroxidase labels enabled to record electrochemically TYR maps with micrometre resolution. Major advantages of this approach include that electrochemical imaging is not affected by optical interferences, such as colour artefacts from skin pigments or auto-fluorescence [3]. Nevertheless, all investigated tissue samples, including sections and tumour blocks, were obtained by invasive biopsy and the samples were prepared using standard fixation and permeabilisation protocols to provide access to the intracellular proteins [2],[4]. Herein, we present our newest approach of 1.) removing and collecting cells from the outermost layer of the skin, *i.e.*, the *stratum corneum*, using adhesive patches, 2.) immunolabelling of melanoma antigens, such as TYR, with peroxidases and 3.) transferring the entire patch into an electrochemical cell for Soft-Probe-Scanning Electrochemical Microscopy (SECM) imaging [5]. The soft probes are ideal tools to scan bent and flexible substrates such as an adhesive patch with collected organic material, as the soft probes brush in a gentle contact mode over the sample without inducing any damage or removal of the material from the adhesive layer. We will demonstrate the successful detection of melanoma antigens, such as TYR, in tape-collected melanoma cells that were grown with high confluency in Petri dishes. Afterwards, we show the detection of melanoma on layers of skin cells that were removed and collected from the back of melanoma mice. We believe that this approach could find attraction by dermatologists who wish to reduce the number of unnecessary excisions.

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Magnetic particles sensing: a novel dynamic approach

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Nowadays there is a growing interest in Lab-On-Chip for biomedical applications as fast and compact low-cost platforms for the detection of different analytes dispersed in a fluid (for example human blood). Among the various transduction approaches, the combination of magnetic nanoparticles (nps) with magnetoresistive sensors have been demonstrated to exhibit higher sensitivity with respect to standard fluorescence methods [1, 2].
An innovative dynamic sensing method for this kind of particles, based on magnetoresistive transduction, is under development through the ongoing European Research project MADIA (MAgnetic DIagnostic Assay for neurodegenerative diseases), in order to obtain a Lab-On-Chip platform able to perform early diagnosis of Parkinson's and Alzheimer's disease [3].
Here we report, in first instance, about the fabrication and characterization of Giant Magneto Resistance (GMR) and Planar Hall Effect (PHE) multilayers, as suitable candidate transducers to be implemented in the final device. To evaluate the capability to detect nps, we first put micron-sized droplet containing nps on the sensors surface by a Nanoscience Bioforce and compared the signal before and after dropping, in order to measure the effect of the particles' magnetic moment on the sensors.
Then, as further step towards the final device, we integrated the fluidics made in SU8 and a peristaltic pump and carried out the in-flow characterization for PHE sensors. We chose the series of structures with the aim to have also a tunable magnetic excitation directly on the chip, avoiding the need of external devices, in compliance with the Lab On Chip aim.

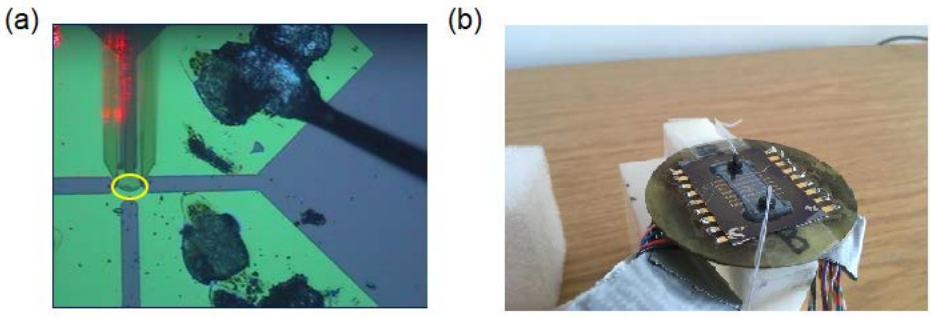

Figure 1. a) Micron sized droplet deposition. (b) Prototype sensors array bonded with fluidic module.

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Glial fibrillary acidic protein (GFAP) detection in serum: a comparison between three functionalization strategies

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Glial Fibrillary Acidic Protein (GFAP) is a promising biomarker for severe brain-related pathologies in humans. The interest on GFAP as a biomarker is related to two main aspects. First, the importance of the pathologies that can be addressed: severe traumatic injuries, intracerebral hemorrhage, glioblastoma multiforme (GBM) cancer with or without blood-brain-barrier disruption. Second, the possibility of detecting this biomarker in circulating blood. Indeed, there is a growing interest of the scientific community in designing strategies that aim at detecting GFAP with high sensitivity and selectivity [1]. In the last years an increasing number of works have demonstrated biosensors able to detect this protein, exploiting various types of surface functionalizations and probe molecules [2]. In the presented work, we compare three different functionalization strategies and antibodies (Figure 1) for GFAP detection both in clean buffers and in fetal bovine serum (FBS). The experiments are performed with gold-plated quartz-microbalance (QCM) sensors. The first strategy exploits a polyethylene glycol (PEG) layer for antifouling (F1 strategy) and two different antibodies against two different epitopes (F1a and F1b). The second strategy is based on the protein-G capability of binding and orienting the anti-GFAP antibody (F2 strategy). In the third, the anti-GFAP is split in two parts with a reducing agent and the gold surface is directly covered with the reduced antibody (F3 strategy). The capability of these functionalizations in detecting GFAP and the selectivity of the detection process are addressed. Experiments show two different regimes depending on the GFAP concentration. Below 220 nM, F3 demonstrate significant detection capabilities ($P<0.05$) with respect to the control. For concentrations equal to or higher than 220 nM, F1a showed the best performance. Finally, we perform the sensor calibration with these functionalizations using FBS as buffer, evaluating also the selectivity of the detection process. It is worth noting that the investigated functionalizations are designed for a gold surface, which is one of the most common type of sensing interface owing to its optical properties, material stability, and ease of fabrication, patterning, and functionalization [3]. This project has been supported by the GLIOMICS project of Regione Toscana.

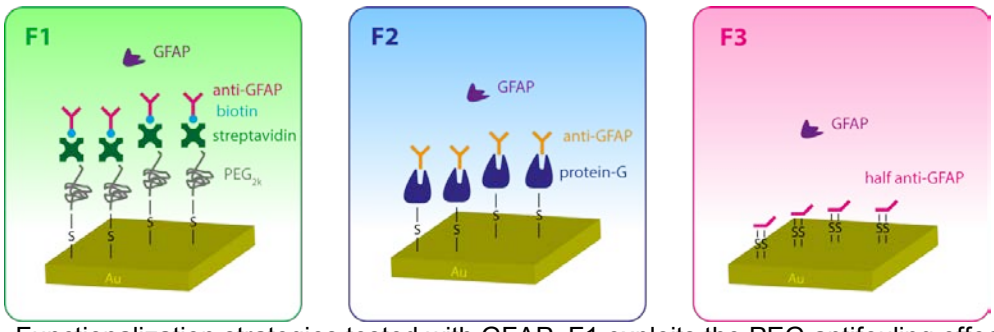

Figure 1. Functionalization strategies tested with GFAP. F1 exploits the PEG antifouling effect, F2 the protein-G binding and orientation of the antibody and F3 the high surface-coverage of the reduced antibody.

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Development of a fluorescent two-component glucose sensing system for chemosensitivity testing on primary tumor cells

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Glucose is the primary energy source of human cells and its consumption can act as an indicator for cellular metabolism and vitality. Therefore a sensing system relying on glucose as ubiquitous molecule in cell culture provides an effective method for cultivation control and sensitivity studies in lab-on-a-chip-systems without the need for additional components with susceptibility to interferences.
In the present study an effective glucose sensor has been synthesized utilizing a two-component system [1]. The receptor consists of a viologen quencher substituted with a boronic acid moiety for saccharide binding, see figure 1 (a). The second component constitutes an anionic fluorophore whose fluorescence intensity is quenched by the receptor. Upon glucose binding to the boronic acid moiety a boronate ester with negative charge is formed. This results in a reduction in quenching efficiency and therefore an increase in fluorescence intensity.
Different sensor designs were synthesized and the quenching characteristics for different anionic dyes as well as the glucose binding were studied. With adjustment of the ratio between quencher and fluorophore and choice of fluorophore the dynamic range of the sensor system has been adapted for differing applications to support a variety of cell culture media with varying glucose concentrations.
The power of the sensor was successfully demonstrated by the chemosensitivity testing on primary tumor cells to detect patient specific in-/efficiencies of cytostatic drug treatments. As figure 1 (b) shows, the developed sensor is capable to detect alterations of cellular metabolism at sub lethal toxin concentrations, thereby presenting an entirely new alternative to common in vitro vitality tests. Furthermore interference problems with assay components that are commonly noted for conventional vitality assays [2] get eliminated with the developed sensor design. Additional advantages of the presented sensor are its short response time, continuous, real time and non-invasive monitoring, which make it a versatile tool for application in lab-on-a-chip-systems.

(a) (b)

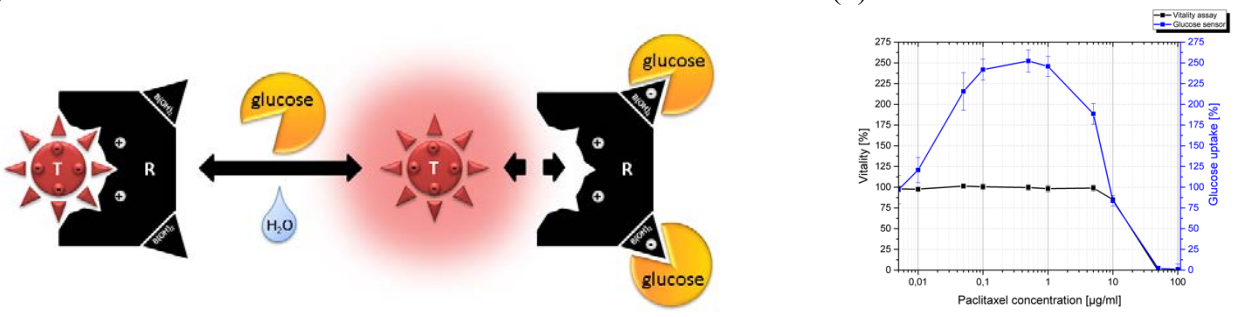

Figure 1. (a) Principle of the glucose sensor. T = transducer: anionic fluorophore in quenched non-fluorescent and bright fluorescent state; R = receptor: quencher with boronic acid moieties. (b) Comparison of conventional vitality assay (Resazurin assay) with the developed glucose sensor.

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“Heart-on-chip” for disease models and drug testing

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Cardiomyocytes (CM) adhered on the microelectrode array (MEA) as a support and electric transducer were coupled to cantilever serving as a nanomechanical sensor from the atomic force microscope (AFM) system. This combination allowed real time synchronous recording of CMs cluster beating; information on beating force (mechanocardiogram, MCG) and the triggering electric events was obtained, thus elucidating interactions associated with many heart pathologies. Such "heart-on-chip" biosensor represents a convenient tool for drug testing and disease modelling. Two variants of human pluripotent stem cells, WT human embryonic stem cell line CCTL14 and dystrophin deficient human induced pluripotent stem cell line reprogrammed from fibroblasts of a patient affected by Duchenne muscular dystrophy (DMD) were differentiated to CMs and employed with the AFM/MEA platform for diseased CMs’ drug response testing and DMD characterization. The dependence of cardiac parameters on extracellular Ca^{2+} concentration was studied; the differential evaluation helped to understand the observed effects despite the involved biological sample variability. The β-adrenergic stimulation was performed with isoproterenol to assess the ionotropic and chronotropic cell line-dependent features. The antagonist trials were carried using verapamil. For the first time, a distinctive beating-force relation for DMD cardiomyocytes was measured on the 3D cardiac in vitro model. An alternative system with CMs combined with a simple piezoelectric quartz crystal resonator was evaluated, too.

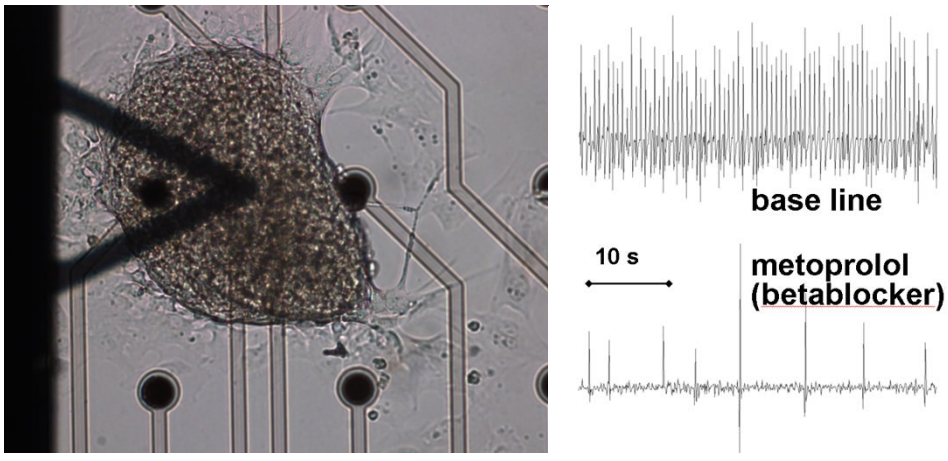

Figure 1. Cluster of cardiomyocytes attached on the microelectrode chip, the AFM triangular cantilever is contacting the top of the cluster (left). Mechanocardiograms for beating CMs and the effect of addition of betablocker (right).

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Electrochemical immunosensor for detection of diphtheria toxoid

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The need to develop an effective tool to quickly obtain information about the infection with the virulent strain *Corynebacterium diphtheriae* (producing diphtheria toxin) is important because the safety which has been achieved in relation to diphtheria as a result of preventive vaccination is only apparent. Resistance acquired artificially (using a toxoid) is not long-lasting and after about 19 years may not protect against disease. This is of particular importance in the face of a fairly poor state of the European population immunity to diphtheria where the level of antibody below the protective limit was observed among 44% of the Russian population, 40% Norwegian and 24% German. Moreover the intensifying anti-vaccination movements in many countries led to e.g. death of 6-year-old unvaccinated boy, who has never left the country, as a result of infection with the virulent strain of Corynebacterium diphtheriae in Spain in June of 2015.
Currently, the preliminary diagnosis of diphtheria is based on the occurrence of nonspecific clinical marks (sore throat, weakness, fever over 38° C). Therefore, the laboratory diagnosis needs to be conducted for confirmation. Due to the excessive cultivation time (about 48 hours) the initial diagnosis must be revealed after about 12 hours on the basis of the microscopic image. The disease develops from 24-48 hours to 5 days and without administration of diphtheria antitoxin, which is not easily accessible in Europe, it usually leads to death.
This work is dedicasted to an electrochemical biosensor developement dedicated to diphtheria toxoid detection in the analyzed sample. The use of electrodes containing receptor layers composed of antibodies (Ab) enabled the determination of diphtheria toxoid in the broad concentration range. The receptor Ab were immobilized at the gold working electrode with the use of biotn-strptavidin interaction. The toxoid mechanism of detection was based on enzymatic reaction of the horseradish peroxidase attached to the secondary antibody. The immunosensor was optimized in the point of view of highly nonspecific proteins interactions with the electrode. Ionic and non-ionic surfactants were investigated as the proteins removal during the receptor layer preparartion and detection steps. Electrode surface nonoocupied by receptors were blocek by different electrode fillers (alkanethiols of different lenght, 6-mercapto-1-hexanol or dithiocarbamate) and proteins. As was shown the nonspecific interactions were almost eliminated by application of specific dithiocarbamate with susbsequent application of bovine serum albumin. The developed electrochemical immunosensor allowed for higly selective detection of diphtheria toxoid with low detection limit.

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Paper-based DNA biosensor for visual detection of milk adulteration

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Dairy products are vulnerable to adulteration with milk derived from different animal-species of lower cost and thus of lower nutritional value. Some adulterants, such as bovine milk, may contain serious allergens or other hazard ingredients. For these reasons, controls are of great demand to protect consumers from economical fraud, as well as from health risks. This project reports the development of an easy, rapid, specific and sensitive DNA-based biosensor for milk authenticity tests of dairy products. DNA was isolated form dairy products, such as milk and yogurt, purchased from local stores. The isolated DNA was subjected to Polymerase Chain Reaction (PCR) to amplify animal-specific DNA sequences. The PCR products were then detected by the DNA biosensor. The detection was accomplished by the accumulation of gold nanoparticles at the test zone of the biosensor forming a visual red spot in the presence of target DNA. A second red spot was formed at the control zone to confirm the proper function of the sensor. The biosensor was applied for the detection of bovine species in binary mixtures of cow milk in sheep's and goat's yogurt, respectively. The proposed method offered very good detectability, as we were able detect as low as 3.1 fmol of PCR product specific to the three-animal species and 0.01% content of bovine milk in binary yogurt mixtures.

Acknowledgements

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Multifunctional pyrolytic carbon scaffolds for stem cell research

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Stem cells have the ability to both self-renew and differentiate into specialized cells in response to appropriate signals. Cell replacement therapy using stem cells is one future strategy for Parkinson's disease. Sophisticated micro/nano surfaces/structures have been employed as one way towards better understanding of cell to cell signalling mechanisms. In the presence of such structures, cells are continuously subjected to mechanical forces that influence cell division, gene expression, migration, morphogenesis and adhesion [1]. Pyrolytic carbon has been used as a tissue engineering scaffold in biosensing and life science applications due to its ability to be patterned as well as multifunctional nature i.e. conductivity, biocompatibility and mechanical support [2,3].
This work presents the microfabrication and evaluation of 2D (flat) and 3D pyrolytic carbon surfaces/scaffolds (ordered and unstructured micronanograss, Figure 1 top panel) serving both as a support for in *vitro* differentiation of human neural stem cells (hNSCs) and as an electrochemical sensor for the subsequent amperometric detection of potassium stimulated dopamine exocytosis. 3D topographies were microfabricated to guide the differentiation of hNSCs (Figure 1b & c top panel). 3D structures are believed to better mimic the *in vivo* environment by providing mechanical and structural support to enhance stem cells growth and differentiation. Our preliminary investigations show that 3D carbon micro/nanostructures (Figure 1b & c, bottom panel) enhance neurogenesis and maturation of hNSCs into dopaminergic neurons. In Figure 1d it can also be observed that the dopamine peak currents are significantly higher for the 3D structures.

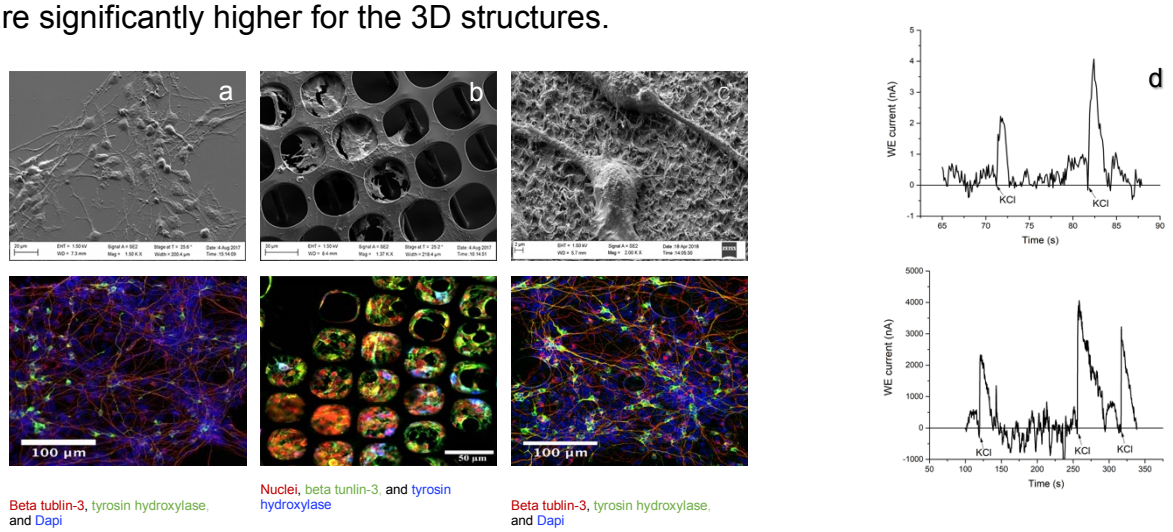

Figure 1. Scanning electron microscopic images (top panel) and fluorescence micrographs (bottom panel) of hNSCs differentiated on: a) 2D flat, b) ordered 3D, and c) unstructured micronanograss respectively, while, d) shows amperometric current signals for potassium stimulated dopamine exocytosis from hNSCs cultured on 2D flat (top panel) and 3D ordered (bottom panel) carbon structures, respectively.

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Spore-based biosensors as a rapid method for sterilization evaluation in aseptic filling machines

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Gaseous hydrogen peroxide is one of the most used sterilants for packaging in aseptic filling machines [1]. The standard methods utilized for the validation of sterilization procedures are biological indicators, which are test systems containing microorganisms resistant to a specific sterilization process. Endospores are commonly used as a test microorganism due to their high resistance against several parameters such as high temperatures, UV radiation, desiccation and several chemicals. To validate a sterilization method under investigation, the spores are exposed to the sterilant, the logarithmic kill rate is determined and at the end, the sterilization can be determined. The main drawback of these methods is their tedious physical work and their slow response; the results can be obtained at the earliest after 24 hours. As a result, novel sensor methods have been previously introduced [2] to overcome these issues. In this work, a spore-based biosensor is presented to evaluate the viability of the spores and determine gaseous hydrogen peroxide concentrations.

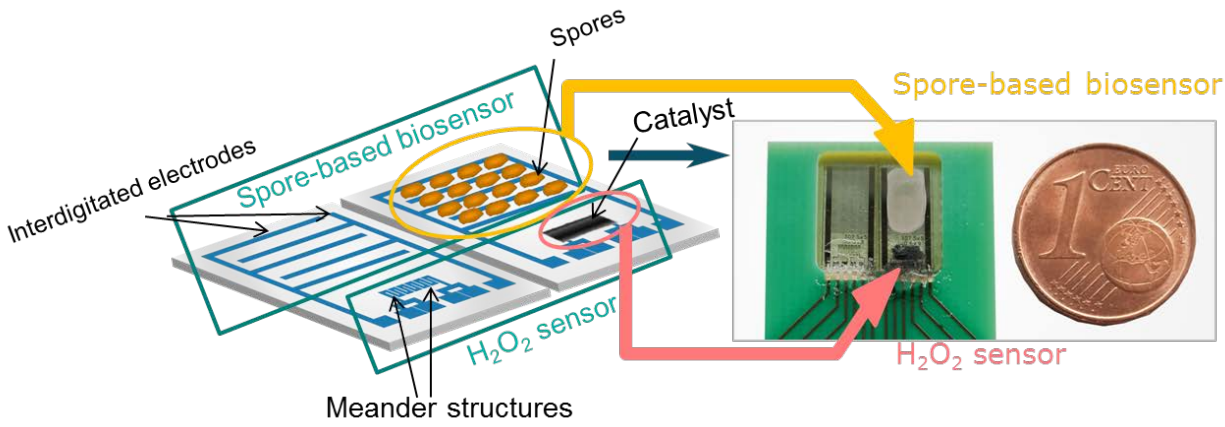

Figure 1. a) Main elements of a spore-based biosensor.

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Capacitive field-effect biosensor with acetoin reductase for acetoin detection

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Acetoin and diacetyl are key parameters during fermentation process of e.g., alcoholic beverages (beer or wine) and their buttery-like taste can influence the quality of the final product. Therefore, the monitoring of acetoin/diacetyl during fermentation processes is essential and may avoid unnecessary maturation time. In this work, a capacitive field-effect biosensor modified with a novel enzyme acetoin reductase (from *B. clausii*) for the detection of acetoin/diacetyl is presented [1,2]. Acetoin reductase (butane-2,3-diol dehydrogenase) catalyzes the reduction of acetoin/diacetyl to butane-2,3-diol, resulting in a local pH change at the sensor surface, which is detected by the pH-sensitive Al/p-Si/SiO_2/Ta_2O_5 transducer structure. With this newly developed biosensor, acetoin concentrations in the range of 10 µM to 100 µM can be determined (Fig. 1) and an average acetoin sensitivity of 65 mV/dec for the concentration range from 10 µM to 90 µM is achieved. Additionaly, preliminary measurements of acetoin in white wine have been successfully performed.

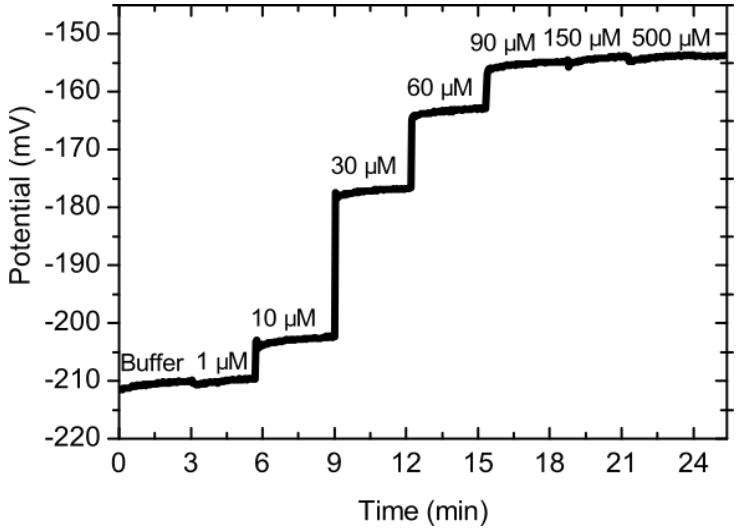

Figure 1. Dynamic response curve of the acetoin biosensor measured in buffer solution with different acetoin concentrations between 1 µM and 500 µM.

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Self-powered smart patch for sweat conductivity measurement

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Self-powered devices have gain an important attention in the recent years because of the necessity of powering the whole sensors that will surround us in the coming years, the so-called Internet of Things (IoT)[1], as an alternative to the common batteries.
In this work, we present a self-powered patch for sweat conductivity measurement, focused in the screening Cystic Fibrosis.
The core of the patch consists on a paper battery-sensor, composed of a Mg anode and a AgCl cathode, whose power output directly depends on the conductivity of the sample introduced in the battery, which acts as the electrolyte. Then, with a simple electronic circuit and two electrochromic displays, the result is shown to the user, as shown in Figure 1a. The first display, the Control Display, turns on at all the conductivities since it is used to check that the device is working properly. Then the second display, the Test Display, turns on only when the threshold conductivity corresponding to a non-healthy sample, i.e. a Cystic Fibrosis sample, is detected.
The device has been implemented combining different printed electronic techniques such as screen-printing for the electrodes and the electrochromic displays and inkjet printing for the printed circuit. Finally, all the components were hybridized to build the device.
The self-powered smart patch was tested with artificial sweat samples, at two different conductivities, at the threshold conductivity that indicates the presence of Cystic Fibrosis and at -20% of the threshold value to accomplish with the Protocol for Evaluation of Qualitative Test Performances (EP12-P) dictated by the Clinical and Laboratory Standards Institute. The device was able to separate the two populations of conductivities except in one case that a non-healthy samples was classified as a healthy sample, as shown in Figure 1b.
To conclude, we have achieved the development of a self-powered device that analyzes a liquid sample and depending on its conductivity, classifies it in two levels. Even the screening of Cystic Fibrosis is the target focused during this work; it can be modified to detect other levels of conductivity.

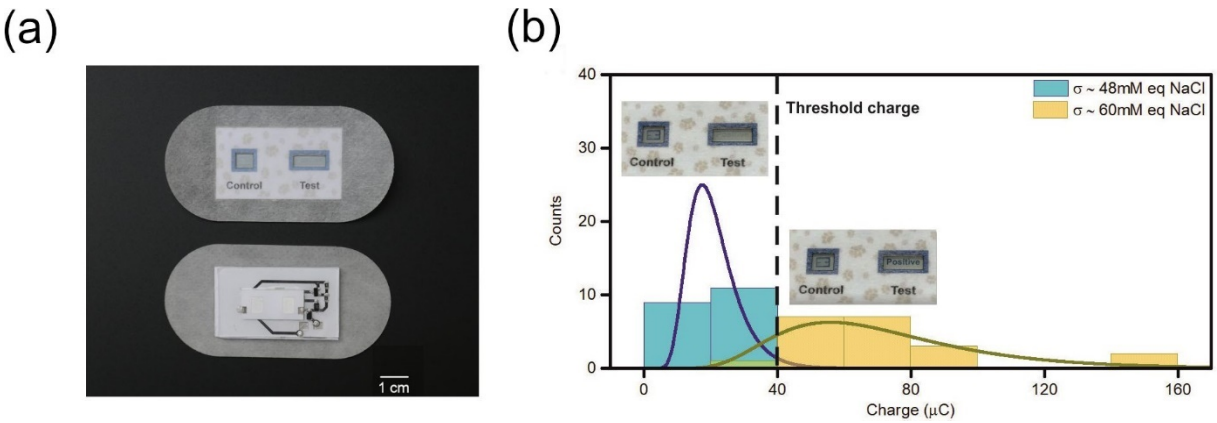

Figure 1. a) Self-powered smart patch. (b) Patch validation at healthy and non-healthy condition

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New antifouling, mixed-charge peptide-poly-L-lysine polymers for DNA detection in human plasma

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Biosensors and biomedical platforms require antifouling surfaces to prevent the non-specific adsorption of proteins or cells, and detect biological targets, such as cancer biomarkers, circulating in complex natural media (e.g., blood plasma or serum) [1]. Accurate quantification of nonspecific protein adsorption plays a key-role for the evaluation of the antifouling activity [2]. Here, we present an innovative, rapid and simple method for the synthesis of antifouling polymers by the coupling of a cationic polyelectrolyte, such as poly-L-lysine (PLL) polymer, and an anionic oligopeptide. The poly-L-lysine (PLL) backbone has been modified with different percentages (y%) of maleimide-NHS ester chains (PLL-mal(y%), from 13% to 26%), in order to achieve control over the charge distribution of PLL-coated surfaces. The anionic oligopeptide structure (CEEEEE) included one cysteine (C) and five glutamic acid (E) units, which has been attached to the PLL-mal(y%) polymers through the thiol–maleimide Michael-type addition. Contact angle and PM-IRRAS data confirmed monolayer formation of the modified PLLs. Antifouling properties of peptide-PLL surfaces have been evaluated in adsorption studies using quartz crystal microbalance with dissipation (QCM-D), and surface plasmon resonance imaging (SPRI) techniques. PLL-mal(26%)-CEEEEE exhibited excellent antifouling property in single-protein solutions, and relevant low-fouling capacity in human plasma samples. Preliminary results have been also obtained after the analysis of cancer biomarkers in diluted human plasma samples by improving the performance of SPRI biosensor with the combined use of neutral peptide nucleic acids as molecular probes, PLL-mal(y%)-CEEEEE as the antifouling layer, and gold nanoparticles for the amplification of plasmonic signal. A good discrimination of circulating tumor DNA sequences at attomolar concentration has been achieved in the bloodstream. The new PLL-mal(y%)-CEEEEE polymer offers a prominent resistance to protein fouling in complex media compared to conventional non-fouling materials, and allows to have a critical control over the biosensing interfaces by creating mixed monolayers for medical diagnostics.
The Horizon 2020 Health project ''ULTRAPLACAD'' (no. 633937) is acknowledged for financial support and the University of Twente for the research stay.

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Engineering hydrogel microparticles for immunoassay

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Hydrogel microparticles have been widely used as a versatile material platform for bead-based assays due to their hydrophilic, biocompatible and highly flexible chemical and physical properties. Since the mesh size of the polymeric network can be tuned, any type of biomolecule such as oligonucleotides, peptides and antibodies, can be easily integrated within the gel.[1,2] In order to perform an immunoenzymatic assay into hydrogels it is necessary to increase the mesh size of the network, avoiding non-specific interactions between gel and biomolecules. This can be achieved with a proper engineerization of the microparticles, choosing an anti-fouling material and using either a porogen [3] or a specific crosslinker. Here we present the synthesis, assisted by droplet microfluidic, and the characterization of engineered PEG-based microparticles with tuneable mesh size and reactive groups along the network, useful for further functionalization with biomolecules. Microfluidics offers a high-throughput platform for synthesizing uniform and monodisperse polymeric microspheres in one step[4]. We used a T-junction glass chip to produce 70-80 µm diameter hydrogel microparticles. In order to characterize our particles, water swelling and fluorescent probes diffusion have been analysed. In particular, preliminary results about the use of a cleavable crosslinker in the hydrogels synthesis show a 42% increment in particles radius and a partition coefficient for the antibodies diffusion of 67%, as a result of the increased mesh size. In conclusion, we successfully obtained hydrogel microparticles with tuneable mesh size and that can be easily functionalized to perform competitive or sandwich immunoassays.

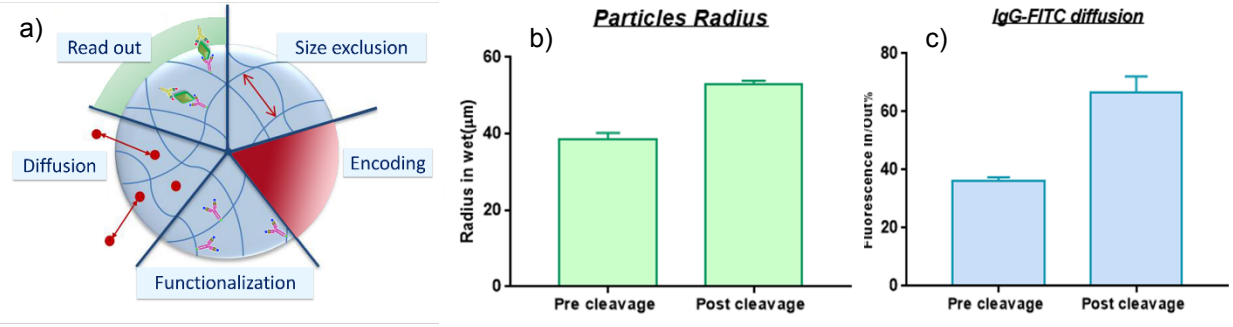

Figure 1. a) Hydrogel microparticle properties. b) Increase in particle radius as a result of the cleavage reaction c) Partition coefficient of a fluorescent probe (IgG-FITC) diffusion inside the hydrogel microparticles.

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A method for monitoring and investigation of myelodysplastic syndromes progression based on analysis of glycoprotein interactions

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Myelodysplastic syndromes (MDS) are a heterogeneous group of hematological malignancies. MDS are associated with protein's postranslational modifications that are different from protein modifications in healthy population. In particular, protein glycosylation (such as fucosylation and sialylation) varies significantly for tumor cells compared to normal cells and has significant impact on disease states and progression [1]. As there is a high risk of progression of MDS into acute myeloid leukemia (AML), understanding of biological processes associated with MDS presents an important research goal.
We report a novel method for diagnosis of MDS based on the investigation of plasma glycoprotein profile altered due to this disease. In contrast to the conventional methods that are based on detection of biomarkers via biorecognition molecules (e.g. antibodies), the reported method relies on measuring interactions between selected proteins and patients' blood plasma without targeting any specific biomolecules or interactions.
The reported method combines a protein chip comprised of lectins that specifically interact with sugar moieties and a surface plasmon resonance (SPR) biosensor that allows observing these interactions in the real-time and label-free manner. Single-surface referencing approach was employed to address biological variability of blood plasma samples and to improve precision of the measurements [2].
We used a protein array containing six selected lectins having specific binding affinities for different sugar moieties and monitored interactions of these lectins with blood plasma samples obtained from MDS patients (three MDS subgroups and MDS progressed into AML) and control samples from healthy donors. We demonstrate the ability of the proposed method to discriminate between MDS patients and healthy donors as well as among different MDS subgroups. While this work demonstrates potential of this method for diagnosis of MDS, it can be readily adopted to a broad range of diseases that affect populations or interactions of glycoproteins.

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Surface Grafted Molecularly Imprinted Polymers for the Detection of the New Psychoactive Substance 2-methoxphenidine

J.W. Lowdon[1], R. Rogosic[1], Benjamin Heidt[1], E. Steen Redeker[1], H. Diliën[1], B. van Grinsven[1], T.J. Cleij[1], K. Eersels[1]

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Molecularly imprinted polymers (MIPs) have been extensively studied as synthetic recognition elements for possible incorporation into biosensing devices. Highly selective MIPs, varying from micron-sized beads in bulk polymerization to nanoparticles made by more advanced reaction mechanisms, have been synthesized for a wide variety of targets. They have been combined with various thermal, optical and electrochemical readout platforms. Although these devices show a lot of potential in terms of specificity and sensitivity, one of the major obstacles to overcome in terms of creating a commercial application is achieving a uniform and reproducible coverage of particles on the surface. In most cases, an adhesive layer is used to immobilize the MIP beads on a measurement chip [1].

Direct surface grafting of MIPs on substrates could offer an elegant solution to this problem. In this study, aluminium substrates were hydroxylated and subsequent silylation allowed for the introduction of functionality for direct polymer grafting on the surface. MIP layers were grown for the new psychoactive substance known as 2-methoxphenidine. The protocol was optimized and rebinding was analysed using an in-house developed thermal resistance analyser. The results of this study show that the reproducibility of the sensor increases and an improved limit-of-detection was demonstrated in comparison to results obtained with previously optimized MIP particles [2] that were immobilized by stamping them into a semi-cured adhesive layer (see Figure 1). These results illustrate the potential of direct surface grafting in creating more reproducible and sensitive MIP sensor layers for sample analysis with biomimetic sensors.

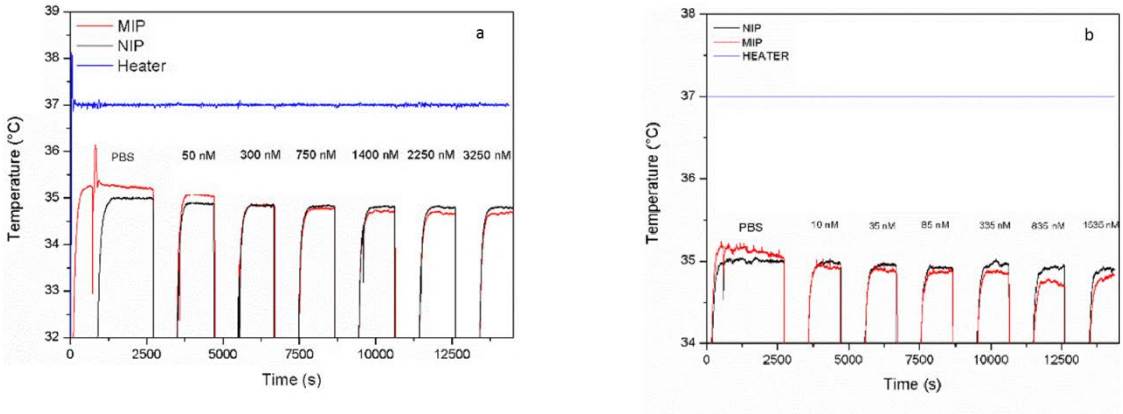

Figure 1. a) Results obtained with bulk MIP particles immobilized by means of stamping into an adhesive layer, leading to a LoD of 100 nM for 2-MXP b) A LoD of 12 nM can be obtained with surface grafted polymers for 2-MXP.

[1] K. Eersels, *et al. Nutrients*, 10, 751 (1998).
[2] J.W. Lowdon, *et al. Analyst, 143,* 2002 (2018).

ELONA-based approaches for the antibody-free detection of Troponin T, the key biomarker of acute myocardial infarction

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Currently, cardiac troponins (I or T) are the analytes of choice for the diagnosis of acute myocardial infarction, thanks to their enhanced sensitivity and specificity with respect to traditional cardiac enzymes, such as creatine kinase and myoglobin [1-3]. Many efforts are now devoted to the design and synthesis of innovative and effective biomimetic receptors alternative to classic antibodies (still used in clinical protocols at the triage stage of emergency) for the development of a new generation of portable and ultra-sensitive tests for the early diagnosis of Troponin T and I [4].
In this framework, we are exploring the perspective use of molecularly imprinted polymers (MIPs) [5] and aptamers, both by biosensors-based and bioanalytical strategies. In this work we present the first example of the use of a couple of new aptamers able to bind Troponin T. We first characterized the aptamers by a reference optical transduction, i.e. Surface Plasmon Resonance (SPR), and then explored the development of a new Enzyme Linked OligoNucleotide Assay (ELONA) test for Troponin T detection. The ELISA-like approach gives several advantages in terms of number of processed samples (up to 96/assay) and ease of signal reading [6]. Different detection strategies were investigated, both by direct and indirect detection, giving encouraging results. Moreover, the optical signal may be based on traditional enzymatic reactions with chromogenic substrates or by using nanoplasmonics [7].

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[2] A.S. Jaffe, J. Ravkilde, R. Roberts, U. Naslund, F.S. Apple, M. Galvani, H. Katus, *Circulation,* 102, 1216–1220 (2000).
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On-Chip real-time multiple displacement amplification of DNA

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DNA amplification is usually performed by polymerase chain reaction (PCR), which requires thermal cycling of the DNA mixture with accurate transition times between different temperatures. Isothermal amplification reactions overcome this issue, making the whole process simpler and less energy-consuming, and therefore attractive for development of portable, battery-operated instruments. Multiple displacement amplification (MDA) is an isothermal amplification technique carried out at 30 °C, which starting from 1-10 DNA copies, can produce 20-30 µg (in 100 µL) of DNA [1]. In this work, a lab-on-a-chip (LoC) system suitable for on-chip real-time monitoring of MDA is presented. It is based on the real-time detection of a fluorophore which acts as DNA intercalating dye. A microfluidic chip made of cyclic olefin copolymer (COC) is coupled to a System-on-Glass (SoG). In the microfluidic chip the MDA reaction is executed, whereas all the optoelectronic devices (amorphous silicon temperature and photo-sensors, interferential filter) and actuators (heaters) needed to control and monitor the reaction in real-time are integrated into the SoG. The SoG is connected to the electronic board to control the temperature of the heaters, and to a custom-made read-out circuitry for acquiring the photosensor currents [2] (Figure 1a). For performing the on-chip amplification, the dye and the MDA reagents were premixed off-chip and subsequently pipetted into the COC chip. The thermal protocol was set to a total run time of 2 hours at 30 °C. Figure 1b reports the on-chip real-time monitoring of the MDA reaction (amplification curves). Data show an increase of the photocurrent as function of time for different initial dsDNA concentrations and that after 20 minutes the photocurrent values are linearly dependent on the initial DNA concentration. Gel electrophoresis analysis revealed more amplified product of on-chip amplification with respect to a standard off-chip amplification.

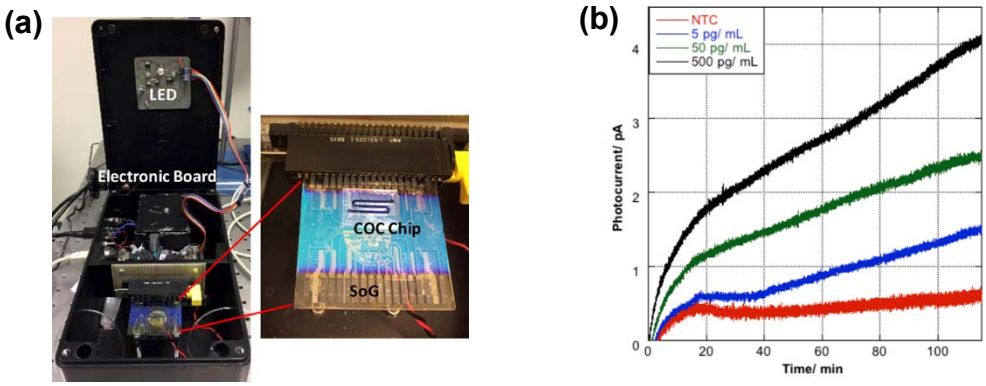

Figure 1. a) Picture of the whole developed system. b) Amplification curves of the MDA reaction.

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[2] Petrucci et al., *Biosens. Bioelectron*, 93, 315-321 (2017).

Investigation on stainless-steel stents fabricated using projection lithography and wet etching

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As a low invasive medical treatment for aneurysm and other diseases of blood vessels, insertion of stent grafts is being watched with keen interest. A stent graft is generally composed of a metal-net stent and artificial blood vessel tissue. Because it is necessary to secure the passage of blood at the diseased part, both strength and elasticity are required for the metal stent buried in the graft. For this reason, superior net structures and easy and highly precise fabrication methods of them are vigorously researched. It is supposed that most of the stents for the practical use at present are fabricated by cutting out the net structures from thin metal pipes using a laser beam. However, it is worried that the time for cutting out complicated net structures precisely become very long, and created net-structure edges are roughened caused by heat and adhesion of scattered metal fragments. In particular, it is worried that the roughness, protrusions, and points of mesh ends hart or stick in the tissues of internal organ and blood vessels. To clear such apprehensions, the authors have developed a new fabrication method of metal stents with smooth net lines using lithography and wet etching. In the new method, pipes of stainless steel SUS 304 with a diameter of 2 mm, a thickness of 50 μm, and a length of 50 mm were used, and negative resist PMER N-CA3000 PM was coated in 20-mm areas from tips in a thickness of 5 μm. The stent patterns were printed using a specified synchronized scan-exposure lithography system that was developed for this purpose in the past research [1]. In the system, stent patterns prepared on a flat film reticle were replicated on a SUS pipe coated with the resist by synchronously scanning the reticle linearly and rotating the pipe. After stent patterns were replicated on a SUS pipe, the pipe was etched in an aqueous solution of ferric chloride. Thus, a primitive model of stent was fabricated, as shown in Fig. 1. Widths of net mesh were approximately 250 μm, and the stent length was 15 mm. Next, it was considered that mechanical properties such as strength, fragility, elasticity, endurance should be investigated early not to take the wrong direction by adopting the new method. For this reason, indispensable strength of stent for securing the blood path was discussed. Blood vessels are swelled when the blood pressure is high, and taken in when the blood pressure is low. Therefore, stent should endure the differential pressure repetition of the maximum and minimum pressures. The differential pressure of the maximum and minimum blood pressures is 50-100 mmHg. For this reason, crushing force given on the stent with a cross section of 2×15 mm^2 is estimated 30 mm^2× (100/760) ×0.1013 MPa=0.40N. However, when the extended primary stent was pushed, as shown in Fig. 2, deformation of a stent for the pushing force of 0.40N was considerably small as 0.4 mm. It was found that the newly developed fabrication method of stents was promising judging from the precise structure and appropriate strength. This work was partially supported by Research Institute for Science and Technology of Tokyo Denki University, Grant Number Q17T-01, and Grant from JI Engineering.

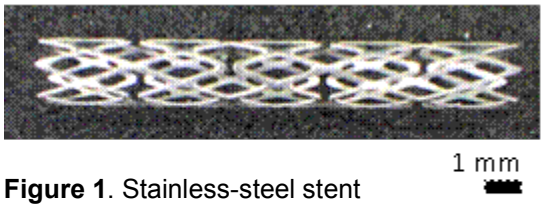

Figure 1. Stainless-steel stent fabricated using lithography and wet etching.

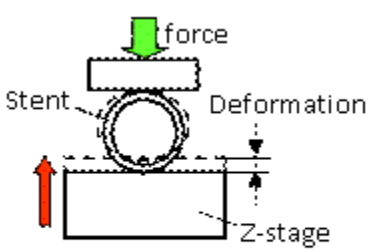

Figure 2. Experiment for investigating stent strength.

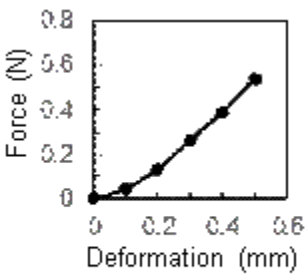

Figure 3. Deformation characteristics of stent.

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Lateral Flow ImmunoAssays for the detection of bisphenol A emissions from plastic materials

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Bisphenol A (BPA) is a chemical that has been used in the production of plastics for more than 40 years. BPA released from bottles made of polycarbonates has been identified as a potential endocrine disrupting substance. Comprehensive research has been undertaken to test and verify its effect on animals and human beings [1].
Many methods have been proposed for the determination of BPA, in particular, gas and liquid chromatography. The main disadvantages of such approaches are the high cost of the equipment, the significant duration of an analysis, and difficulties in screening a large number of samples. Lateral Flow ImmunoAssay (LFIA) attributes are high throughput, high specificity and sensitivity, as well as low cost and simplicity [2, 3].
An LFIA test strip was developed. This LFIA is based on anti-BPA antibodies (from mouse) conjugated with gold nanoparticles as the marker by passive absorption and covalent coupling, deposited in an elaborate pad. To form control and test zones, anti-mouse antibody and a BVA-BSA conjugate were spotted on the nitrocellulose membrane, respectively.
Negative samples are revealed by red lines both in the test and control zones, respectively, whereas positive samples produce a single red line only in the control zone. The measurement range is 0.05 – 23 µg/L, visually detected by the naked eye within 5 minutes. In addition, the intensities at the test line can be read by dedicated lateral flow readers (opTrilyzer® and Cube by opTricon, Berlin) for a more precise and documented determination.

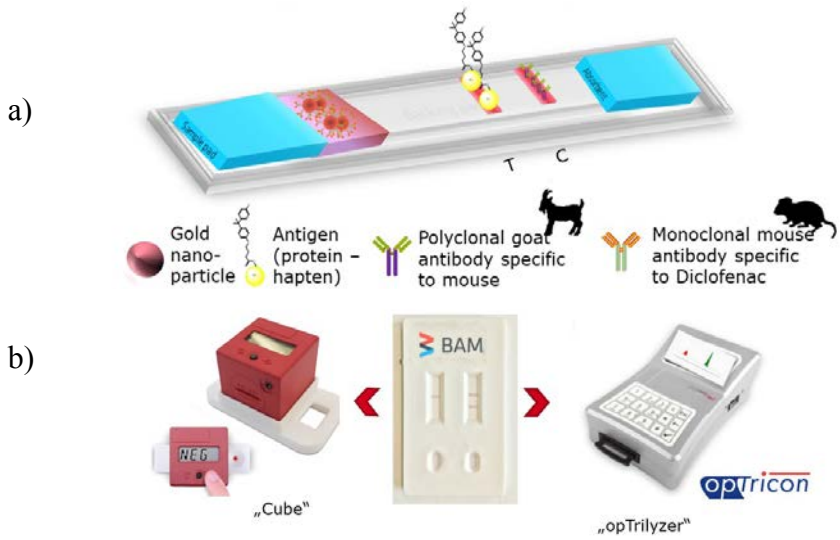

Figure 1. a) Principle of LFIA based on the competition of free BPA from the sample and immobilized BPA (BVA-BSA protein conjugate) for binding to anti-BPA antibodies grafted on gold nanoparticles; (b) LFIA strips mounted in cassette to read in commercial scanners

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High-density ZnO nanowires on Printed Circuit Boards for wearable sensors and heaters

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Quasi one-dimensional Nanowires (NWs) offer several advantages for applications in energy harvesting, sensors [1] and electronic skins [2]. However, the integration of high-quality NWs onto complete Printed Circuit Board (PCB), which is the standard substrate to assemble electronic components, is very complex. Among quasi-1D nanostructures, Zinc oxide (ZnO) NWs can be synthesized in aqueous solution at low cost and also have remarkable properties, including piezoelectricity, pyroelectricity and semiconductivity [4]. However, conventional wet-chemical methods for growing ZnO NWs on metal surfaces suffer from low reproducibility [5], and only allow the synthesis of short, low-density and low aspect-ratio NWs, which hampers applications in electronics devices. We have recently presented a low-temperature (<90°C), green-chemistry approach to integrate ZnO NWs into electronic systems on printed circuit boards (PCBs) [6]. We firstly pre-deposited on the PCB substrate a MnOOH layer at optimized concentrations to increase the NWs density. Afterwards, we demonstrated by numerical simulation that the ligand ethylenediamine, at the isoelectric point of the ZnO NWs tips, can effectively control – at 1:1 stoichiometric ratio with zinc – both speciation and supersaturation of zinc in the nutrient solution. In this regard, we employed ethanolamine (a safer precursor) for in-situ producing ethylenediamine thanks to a zinc-catalysed amination reaction of ethanolamine by ammonia. The combination of ethylenediamine, which permit to thin the NWs, with chloride ions, which concurrently stabilize their top faces, permitted us to produce ZnO NWs with untapered tips, at the highest reported densities (> 8/μm^2), length (>15 μm), and aspect-ratio (> 200) for flexible substrates. These favourable characteristics allow to add the top electrode, package the NWs, and connect to the electronic interface. We demonstrate a proof of concept by a flexible PCB heater with maximum power densities (up to 20 W/cm^2) and an ultrasensitive wearable heartbeat detector composed of ZnO piezotronic pressure sensor and electronics on a single flexible PCB. Our findings are a fundamental step towards the co-integration of high-quality NWs-based devices on PCBs.

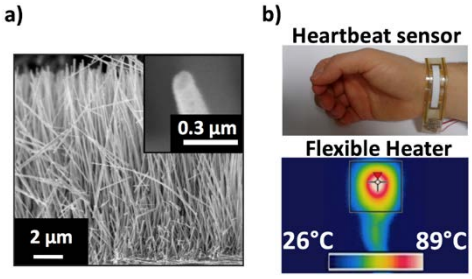

Figure 1. (a) SEM image of ZnO NWs on PCBs. Inset shows a single NW tip with regular hexagonal face. (b) Proof-of-concept applications: heartbeat detector (top), Flexible heater (bottom).

[1] Z. Fan et al., *PNAS*, 105, 11066–11070 (2008).
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