



# BIOSENSOR 2017

1<sup>st</sup> European & 10<sup>th</sup> German  
BioSensor Symposium



20-23 March 2017 • Potsdam • Germany

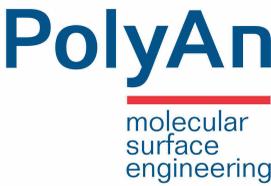
## Program and Book of Abstracts



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# Exhibitors and supporting organizations



## Table of contents

Welcome	5
Committee / Organization	6
Imprint	7
Exhibitors & supporting organizations	8
Lecture program overview	10
Poster program overview	19
Plenary lectures (PL1 – PL4)	31
Evening lecture	36
Keynote lectures (KN1 – KN16)	37
Oral presentations (O1 – O76)	53
Poster presentations (P1 – P94)	129
List of registered authors	227
Venue overview	233



## Welcome

Dear Participants,

the 1<sup>st</sup> European Biosensor Symposium is held together with the 10<sup>th</sup> German Biosensor Symposium, a biennial meeting of scientists working in the field of bioanalysis and bio-recognition. The symposium addresses all fields of scientific activities where biosensors are involved and is meant to support young scientists during the first steps of their scientific career. The original idea of the symposium, when it started in 1999 in Munich, was to create a platform beside the big and mainly commercially driven international events, which were increasingly expensive and gave little chance to present to those, who work at the bench. Preparing the 10<sup>th</sup> meeting in a series we anticipated, that what has started as a national platform was already attractive for participants from the neighboring countries and we realized that national symposia are no longer up to date in a coalescing Europe in the year 2017. Science is international and the language of science is understood everywhere, where people are more interested in facts than fictions. Biosensors are tools for measurement and quantitative analysis. Quantitative biology, still in its infancy, needs such tools to build the interface between the analogue living world and the digital world of data, data management and computing. However, there are many more applications for Biosensors, and even more new findings, how molecules work or may be employed.

You, the participants of the symposium, brought together a great variety of topics and new research, the scientific committee and the local organizers compiled from all these proposals the most interesting program. Nearly hundred presentations and a similar number of poster presentations have to be discussed, we hope you will enjoy the density of bright ideas!

The conference will be accompanied by an industrial exhibition, where companies will present their technologies, products and latest developments as well as their service in the field of bioanalysis and related technologies and research.

We invite you to take the advantage of this scientific event, which offers plenty of opportunities for extensive discussions, to establish new contacts and to strengthen existing relationships after the oral presentations, during the poster sessions, while visiting the exhibition or at the social events.

Frank F. Bier  
on behalf of the organizing committee

## Committee / Organization

### Scientific board

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## Local organization

Frank Bier (Chair),	Potsdam/DE
Ulla Wollenberger (Co-Chair),	Potsdam/DE
Carsten Hille,	Potsdam/DE
Fred Lisdat,	Wildau/DE
Frieder W. Scheller,	Potsdam/DE

## Local Organizer

UP Transfer GmbH an der Universität Potsdam  
Am Neuen Palais 10  
14469 Potsdam/DE

## Imprint

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Carsten Hille

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March 20, 2017 (online version)

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## **Exhibitors & supporting organizations**

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[www.bioelectrochemical-soc.org](http://www.bioelectrochemical-soc.org)

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[www.bst-biosensor.de](http://www.bst-biosensor.de)

C3 Prozess- und Analysetechnik GmbH, Haar, Germany  
[www.c3-analysetechnik.de](http://www.c3-analysetechnik.de)

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[www.diagnostiknet-bb.de](http://www.diagnostiknet-bb.de)

Dynamic Biosensors GmbH, Martinsried/Planegg, Germany  
[www.dynamic-biosensors.com](http://www.dynamic-biosensors.com)

Fonds der Chemischen Industrie im Verband der Chemischen Industrie  
e.V., Frankfurt/Main, Germany  
[www.vci.de/fonds](http://www.vci.de/fonds)

Fraunhofer-Institut für Zelltherapie und Immunologie, Institutsteil  
Bioanalytik und Bioprozesse, Potsdam-Golm, Germany  
[www.izi.fraunhofer.de](http://www.izi.fraunhofer.de)

IMMS Institut für Mikroelektronik- und Mechatronik-Systeme  
gemeinnützige GmbH, Ilmenau, Germany  
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Springer Verlag, Heidelberg, Germany  
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## Lecture program overview

### Session topics overview:

NANO: Nanotechnology, surface engineering and bioelectronics

AA: Advances in applications

TIF: Technologies for innovative formats

CB: Cell biosensors

BB: Bioengineered and biomimetic recognition elements

Monday, March 20		
11:00 am - 1:30 pm	Registration and poster mounting	
1:30 - 1:45 pm	Opening ceremony	
1:45 - 2:30 pm	<b>Plenary lecture (Room H03)</b> Chair: F. Bier <b>Jürgen Popp</b> , Friedrich Schiller University Jena, Germany (PL1) <i>Biophotonics and biomedical spectroscopy</i>	
	<b>NANO - Biophotonics (Room H02)</b> Chair: T. Bocklitz	<b>AA - Biosensors for biomarkers (Room H03)</b> Chair: M. Schöning
2.45 - 3:15 pm	<b>Keynote lecture</b> <b>Janina Kneipp</b> , Humboldt Universität zu Berlin, Germany (KN1) <i>One- and two-photon excited surface-enhanced Raman scattering for biosensing</i>	<b>Keynote lecture</b> <b>Petr Skládal</b> , Masaryk University, Czech Republic (KN2) <i>Immunosensing of pathogens using piezoelectric, electrochemical and surface plasmon resonance based transducers</i>
3:15 - 3:30 pm	<b>Jean-Emmanuel Clément</b> , University Bourgogne Franche-Comté, France (O1) <i>Temporal fluctuations of single protein by dynamical SERS</i>	<b>Victoria Shumyantseva</b> , Russian Federation (O8) <i>Electrochemical methods in biochemical and biomedical investigations</i>
3:30 - 3:45 pm	<b>Gilbert Nöll</b> , University of Siegen, Germany (O2) <i>New strategies for the label-free optical detection of oligonucleotides or antibodies with surface plasmon fluorescence spectroscopy</i>	<b>Richard Burkitt</b> , Leeds Beckett University, United Kingdom (O9) <i>Film and electrical signal optimisation for carbon pad printed electrodes to detect ferro/ferricyanide redox probes and pathogenically relevant pyocyanin</i>
3:45 - 4:45 pm	Coffee break + Poster viewing	

	<b>NANO - Bioelectronics (Room H02)</b> Chair: L. Gorton	<b>AA - Biosensors for biomarkers (Room H03)</b> Chair: A. Baeumner
4:45 - 5:00 pm	<b>Emmanuel Suraniti</b> , Max Planck Institute for Intelligent Systems, Germany (O3) <i>On-chip enzymatic micro biofuel cell-powered integrated circuit</i>	<b>Marco Giannetto</b> , University of Parma, Italy (O10) <i>Competitive amperometric immunosensor for determination of p53 protein in urine with carbon nanotubes/gold nanoparticles screen printed electrodes: a rapid and noninvasive screening tool for early diagnosis of bladder carcinoma</i>
5:00 - 5:15 pm	<b>Stefan Belicky</b> , Slovak Academy of Sciences, Slovak Republic (O4) <i>Electrochemical lectin biosensor: A perspective approach to prostate cancer diagnostics</i>	<b>Sevinc Kurbanoglu</b> , Ankara University, Turkey (O11) <i>Electrochemical nanobiosensor for the tyrosinase inhibition via phosphodiesterase type 5 inhibitor: sildenafil</i>
5:15 - 5:30 pm	<b>Kai Stieger</b> , Technical University of Applied Sciences Wildau, Germany (O5) <i>Effective strategies for the integration of photosystem I into photobioelectrodes by means of cytochrome c</i>	<b>Wilfried Weigel</b> , Scienion AG Berlin, Germany (O12) <i>Biofunctionalization as key step in biosensor applications – From R&amp;D to highthroughput manufacturing</i>
5:30 - 5:45 pm	<b>Paolo Bollella</b> , Sapienza University of Rome, Italy (O6) <i>Self-powered and portable biosensor based on CtCDH C291Y deposited onto AuNPs-carbon electrodes for glucose detection</i>	<b>Verónica Serafín</b> , University Complutense of Madrid, Spain (O13) <i>Novel electrochemical immunoplatfoms for the determination of emerging cardiac biomarkers</i>
5:45 - 6:00 pm	<b>Felix Julian Kröner</b> , TU Dresden, Germany (O7) <i>Biophysical analysis of Cas9 – DNA interactions and enzymatic activity with the switchSENSE biosensor platform</i>	<b>Agata Kowalczyk</b> , University of Warsaw, Poland (O14) <i>DNA hybridization biosensor based on changes of volume phase transition temperature of hydrogel</i>
6:15 - 7:00 pm	<b>Evening lecture (Room H03)</b> Chair: G. Gauglitz <b>Wolfgang Schuhmann</b> , Ruhr-Universität Bochum, Germany <i>From reagentless biosensors to biofuel cells and photobiovoltaics</i>	
7:00 - 9:00 pm	Poster viewing + Exhibition + Get together	

<b>Tuesday, March 21</b>		
9:00 - 9:45 am	<b>Plenary lecture (Room H03)</b> Chair: U. Wollenberger <b>Philip N. Bartlett</b> , University of Southampton, United Kingdom (PL2) <i>DNA detection and discrimination using electrochemical SERS</i>	
	<b>TIF - Structures (Room H02)</b> Chair: J. Emneus	<b>TIF - Modeling (Room H03)</b> Chair: T. Bachmann
10:00 - 10:30 am	<b>Keynote lecture</b> <b>Stephan S. Keller</b> , Technical University of Denmark (KN3) <i>Pillars, beams, strings and scaffolds - Sensing with micro- and nanofabricated structures</i>	<b>Keynote lecture</b> <b>Thomas Bocklitz</b> , Friedrich Schiller University Jena, Germany (KN6) <i>Investigation and construction of analysis routines for Raman-effect related data</i>
10.30 - 10:45 am	<b>Subhajt Guha</b> , IHP, Germany (O15) <i>CMOS compatible THz biosensing platform based on Germanium plasmonic antennas</i>	<b>Mihaela Puiu</b> , University of Bucharest, Romania (O30) <i>Uni – and multivariate data analysis for predicting the sensor output during the specific binding of large analytes to functionalized gold surfaces of plasmonic and acoustic devices</i>
10:45 - 11:00 am	<b>Stefan Rödiger</b> , BTU Cottbus-Senftenberg, Germany (O16) <i>Simultaneous multiparametric analysis of biomarkers on combined microbead-cell arrays</i>	<b>Inês I. Ramos</b> , Universidade do Porto, Portugal (O31) <i>Development of automated immunosensing methods for clinical and environmental analysis using lab-on-valve platforms</i>
11:00 - 11:30 am	Coffee break	
	<b>NANO - Biophotonics (Room H02)</b> Chair: R. Schaasfort	<b>AA - Imaging and sensing (Room H03)</b> Chair: C. Hille
11:30 - 11:45 am	<b>Natalia Nechaeva</b> , Institute of Biochemical Physics RAS, Russian Federation (O17) <i>New SERS-substrate for cholinesterase detection</i>	<b>Elmar Schmäzlín</b> , Leibniz-Institut für Astrophysik Potsdam, Germany (O32), <i>Raman imaging of skin samples with integral field spectroscopy</i>
11:45 am - 12:00 pm	<b>André Dathe</b> , Leibniz-Institute of Photonic Technology, Germany (O18) <i>Electrically-driven plasmons in hybrid nanostructures</i>	<b>Michael Mayer</b> , Universität Regensburg, Germany (O33) <i>Multiple detection electrochemiluminescence (ECL) for biosensing</i>

12:00 - 12:15 pm	<b>Sven Schulze</b> , University of Potsdam, Germany (O19) <i>Development of a label free fiber optical biosensor based on etched fiber Bragg grating technique</i>	<b>Hoa Thi Hoang</b> , University of Potsdam, Germany (O34) <i>FRET-based immunoassay using anti-TAMRA-antibodies coupling to liposomes and a DBD dye derivative</i>
12:15 - 12:30 pm	<b>Sebastian Beyer</b> , Bundesanstalt für Materialforschung und -prüfung, Germany (O20) <i>Colloidal metalorganic frameworks as novel biofunctional nanoparticles for immunoassay applications</i>	<b>Michael Schäferling</b> , Bundesanstalt für Materialforschung und -prüfung, Germany (O35) <i>Sensing and imaging of intracellular pH using photon upconversion based nanoprobes</i>
12:30 - 12:45 pm	<b>Claudia Pacholski</b> , University of Potsdam, Germany (O21) <i>Optical sensors based on periodic hole arrays in metallic films</i>	<b>Shavkat Nizamov</b> , BTU Cottbus-Senftenberg, Germany (O36) <i>Advanced wide-field surface plasmon microscopy of single adsorbing nanoparticles: analytics in complex biological media and electrochemical characterisation</i>
12.45 - 2.30 pm	Lunch + Exhibition <b>Poster session 1: NANO + TIF</b> (Authors present 1:30 pm – 2:30 pm)	
	<b>NANO - Surfaces (Room H02)</b> Chair: A. Lesch	<b>TIF - Pathogens (Room H03)</b> Chair: G. Proll
2:30 - 3:00 pm	<b>Keynote lecture</b> <b>Simona Scarano</b> , University of Florence, Italy (KN4) <i>Composite plasmonic nanomaterials for cheap, versatile and smart LSPR-based (bio)sensing applications: recent advances</i>	<b>Keynote lecture</b> <b>Rudolf J. Schneider</b> , Bundesanstalt für Materialforschung und -prüfung, Germany (KN7) <i>Fluorescence polarization immunoassays - Comparison of platforms</i>
3:00 - 3:15 pm	<b>Nenad Gajovic-Eichelmann</b> , Fraunhofer IZI-BB, Germany (O22) <i>Peptide decorated electropolymer films for biosensors: Comparison of different strategies for oriented peptide immobilization</i>	<b>Catharina Kober</b> , Technical University of Munich, Germany (O37) <i>Culture-independent serotyping of <i>L. pneumophila</i> in water and urine samples</i>
3:15 - 3:30 pm	<b>Nithiya Nirmalanathan</b> , Bundesanstalt für Materialforschung und -prüfung, Germany (O23) <i>Photometric methods for the quantification of functional groups on particle surfaces</i>	<b>Gunther Becher</b> , BecherConsult, Germany (O38) <i>MCC-IMS spectral analyses of headspaces from bacterial cultures for rapid identification of bacterial infections</i>

3:30 - 3:45 pm	<b>Jonas Bemetz</b> , Technical University of Munich, Germany (O24) <i>A low-cost minimal-step preparation method for mass production of antibody microarrays from polycarbonate</i>	<b>Gregory Dame</b> , Brandenburg Medical School Theodor Fontane, Germany (O39) <i>Point of care diagnostics for rapid detection of infectious diseases in water</i>
3:45 - 4:00 pm	<b>Ralf Schneider</b> , Bundesanstalt für Materialforschung und -prüfung, Germany (O25) <i>Investigation and control of protein adsorption through plasmonic interaction of fluorophore labelled proteins like BSA and metal nanoparticles</i>	<b>Brian P. Cahill</b> , Institut für Bioprozess- und Analysenmesstechnik, Heilbad Heiligenstadt, Germany (O40) <i>Dose response screening of C. vaccinii using impedimetric sensing of microfluidic droplets</i>
4:00 - 4:30 pm	Coffee break	
	<b>NANO - Bioelectronics (Room H02)</b> Chair: P. Skládal	<b>TIF - Bioelectronics (Room H03)</b> Chair: M. Minunni
4:30 - 5:00 pm	<b>Keynote lecture</b> <b>Karolien De Wael</b> , University of Antwerp, Belgium (KN5) <i>Molecular photosensitizers: emerging (bio)analytical sensing tools</i>	<b>Keynote lecture</b> <b>Patrick Wagner</b> , University of Leuven, Belgium (KN8) <i>Bio- and biomimetic sensors based on impedance spectroscopy and thermal boundary effects</i>
5:00 - 5:15 pm	<b>Gabriel Bruno Kopiec</b> , Ruhr-University Bochum, Germany (O26) <i>Electrochemical phosphate sensor based on two bioelectrocatalytic and one electrocatalytic oxidation cascade with "on-demand" 4 to 6 electrons per analyte molecule</i>	<b>Edyta Matysiak-Brynda</b> , University of Warsaw, Poland (O41) <i>Novel transferrin immunosensor based on its paramagnetic properties</i>
5:15 - 5:30 pm	<b>Peter Munyao Ndangili</b> , University of the Western Cape, South Africa (O27) <i>Electrochemical signaling of 5-enolpyruvylshikimate-3-phosphate synthase GMO plant biomarker</i>	<b>Marc Riedel</b> , Technical University of Applied Sciences Wildau, Germany (O42) <i>Connecting quantum dots with enzymes: Mediator-based approaches for the light-directed detection of glucose and fructose</i>
5:30 - 5:45 pm	<b>Eva-Maria Laux</b> , Fraunhofer IZI-BB, Germany (O28) <i>Detection of dielectrophoretically accumulated bacteria at nanoelectrode arrays by surface enhanced Raman spectroscopy</i>	<b>Susana Barreda-García</b> , Universidad de Oviedo, Spain (O43) <i>A robust indium-tin-oxide platform for the electrochemical detection of Salmonella</i>

5:45 - 6:00 pm	<b>Susana Campuzano</b> , Universidad Complutense de Madrid, Spain (O29) <i>Electrochemical sensing of biomarkers for early detection of cancer using molecular biosensors</i>	<b>Holger Schulze</b> , The University of Edinburgh, United Kingdom (O44) <i>Electrochemical biosensor platform for rapid antimicrobial resistance testing at point-of-care</i>
6:00 - 7:30 pm	Poster viewing + Exhibition	

## Wednesday, March 22

9:00 - 9:45 am	<b>Plenary lecture (Room H03)</b> Chair: F. Lisdat <b>Hubert Girault</b> , EPFL Valais Wallis, Switzerland (PL3) <i>Bio-SECM of cells and tissues</i>	
	<b>CB - Bioelectronics (Room H02)</b> Chair: A. Le Goff	<b>NANO - DNA and polyelectrolytes (Room H03)</b> Chair: F. Lisdat
10:00 - 10:30 am	<b>Keynote lecture</b> <b>Lo Gorton</b> , Lund University, Sweden (KN9) <i>Electrochemical study of the extracellular electron transfer of wild type and mutants of <i>Enterococcus faecalis</i> to electrodes</i>	<b>Keynote lecture</b> <b>Gerd-Uwe Flechsig</b> , University at Albany, SUNY, United States of America (KN11) <i>Redox-induced switching of DNA-layers observed on a millisecond timescale by means of electrochemical quartz crystal microbalance</i>
10:30 - 10:45 am	<b>Jan Oberländer</b> , FH Aachen, Germany (O45) <i>Functionalized spore-based biosensor to evaluate gaseous sterilization processes</i>	<b>Lucian Rotariu</b> , University of Bucharest, Romania (O55) <i>New strategies for dehydrogenase biosensors based on nanostructured polyelectrolytes composite materials</i>
10:45 - 11:00 am	<b>Falk Harnisch</b> , Helmholtz-Centre for Environmental Research - UFZ, Germany (O46) <i>Electroactive biofilms as recognition element for anaerobic digestion: Sensing of volatile fatty acids (VFAs)</i>	<b>Rebeca Miranda-Castro</b> , Universidad de Oviedo, Spain (O57) <i>Direct electrochemical detection of waterborne pathogen's 16S rRNA using thioaromatic-based oligonucleotide monolayers</i>
11:00 - 11:30 am	Coffee break	

	<b>CB - 3D (Room H02)</b> Chair: J. Wegener	<b>NANO - Applications (Room H03)</b> Chair: S. Scarano
11:30 - 11:45 am	<b>Claudia Caviglia</b> , Technical University of Denmark, Denmark (O47) <i>Three-dimensional sensing scaffold for bone cells studies</i>	<b>Larisa V. Sigolaeva</b> , M.V. Lomonosov Moscow State University, Russian Federation (O58) <i>Nanosized polymeric films for design of advanced biosensor systems</i>
11:45 am - 12:00 pm	<b>Karl-Heinz Feller</b> , Ernst-Abbe-Hochschule Jena, Germany (O48) <i>Towards 3D cell cultures as an alternative for irritation tests</i>	<b>Sven Ingebrandt</b> , University of Applied Sciences Kaiserslautern, Germany (O59) <i>Silicon nanowire biosensor platform to electronically sense biomolecules in physiological buffer concentration</i>
12:00 - 12:15 pm	<b>Gerardo Abbandonato</b> , NEST, Scuola Normale Superiore and Istituto Nanoscienze - CNR, Italy (O49) <i>Dual probe for biological dynamic processes</i>	<b>Jan Tkac</b> , Slovak Academy of Sciences, Slovak Republic (O60) <i>Nanoscale controlled architecture of biosensors for glycan recognition applied in diagnostics</i>
12:15 - 12:30 pm	<b>Philipp U. Bastian</b> , University of Potsdam, Germany (O50) <i>Multiplexed targeting of cells and tissue – novel surface-modulated upconversion nanoparticles for biosensing and -imaging</i>	<b>Rene Welden</b> , FH Aachen, Germany (O61) <i>Light-addressable lab-on-a-chip-based analysis platform</i>
12:30 - 12:45 pm	<b>Judith Anthea Stolwijk</b> , University of Regensburg, Germany (O51) <i>Tuning the potential of whole-cell impedance assays to assess the activity of pathway-biased GPCR ligands</i>	<b>Uwe Schröder</b> , Technische Universität Braunschweig, Germany (O76) <i>Material and structural aspects of microbial biofilm electrodes</i>
12.45 - 2:30 pm	Lunch + Exhibition <b>Poster session 2: AA + CB + BB</b> (Authors present 1:30 pm – 2:30 pm)	
	<b>CB - Diagnostics (Room H02)</b> Chair: A. Yarman	<b>NANO - Surfaces (Room H03)</b> Chair: N. Plumeré
2:30 - 3:00 pm	<b>Keynote lecture</b> <b>Andreas Lesch</b> , EPFL Valais Wallis, Switzerland (KN10) <i>Portable POC diagnostics using inkjet printed sensor plates</i>	<b>Keynote lecture</b> <b>Adrian Ruff</b> , Ruhr-Universität Bochum, Germany (KN12) <i>Design strategies for (redox-)polymers for the immobilization of enzymes on electrode surfaces</i>

3:00 - 3:15 pm	<b>Marga C. Lensen</b> , Technische Universität Berlin, Germany (O52) <i>Unique micro- and nano-patterns of gold nanoparticles on PEG-based hydrogels</i>	<b>Biljana Mitrova</b> , University of Potsdam, Germany (O62) <i>Effect of the molybdenum coordination sphere in TMAO reductase revealed by direct bioelectrocatalysis</i>
3:15 - 3:30 pm	<b>Alice Soldà</b> , University of Bologna, Italy (O53) <i>Development of enzyme-based microsensors for ex vivo analyses</i>	<b>Anna-Lena Riegel</b> , Karlsruhe Institute of Technology, Germany (O63) <i>Impact of shearing and drying on the catalytic activity of enzyme for biosensor applications</i>
3:30 - 3:45 pm	<b>Ada-Ioana Bunea</b> , Technical University of Denmark, Denmark (O54) <i>Carbon coated optical fibre for dopamine detection from cells</i>	<b>Paul Kavanagh</b> , Queens University Belfast, United Kingdom (O64) <i>Mediated enzyme electrodes for electrochemical biosensing at low overpotentials</i>
3:45 - 4:15 pm		
Coffee break		
4:15 - 7:00 pm		
<b>Social event</b> (Visit of Sanssouci Palace with bus transfer – registration necessary)		
7:30 pm		
<b>Conference dinner</b> (Campus Griebnitzsee, Building 6, Canteen)		

Thursday, March 23		
9:00 - 9:45 am	<b>Plenary lecture (Room H03)</b> Chair: F. Scheller <b>Karsten Haupt</b> , Compiègne University of Technology, France (PL4) <i>Synthetic antibody mimics and nanomaterials for biosensing</i>	
	<b>BB - MIPs (Room H02)</b> Chair: F. Scheller	<b>AA - SPR (Room H03)</b> Chair: U. Resch-Genger
10:00 - 10:30 am	<b>Keynote lecture</b> <b>Aysu Yarman</b> , Turkish-German University, Turkey (KN13) <i>Enzymes for MIPs &amp; MIPs for enzymes</i>	<b>Keynote lecture</b> <b>Richard Schasfoort</b> , University of Twente, Netherlands (KN15) <i>Overview of 25 years of commercial label free biomolecular interaction analysis</i>
10.30 - 10:45 am	<b>Maciej Cieplak</b> , Polish Academy of Sciences, Poland (O65) <i>Semi-covalent imprinting for selective protein sensing at a femtomolar concentration level</i>	<b>Daniel Stern</b> , Robert Koch-Institut, Germany (O71) <i>Simultaneous differentiation and quantification of ricin and agglutinin by an antibody-sandwich surface plasmon resonance sensor</i>

10:45 - 11:00 am	<b>Katharina J. Jetzschmann</b> , University Potsdam, Germany (O66) <i>Subunit-imprinting of the multi-domain cytochrome P450 BM3</i>	<b>Vitali Scherbahn</b> , BTU Cottbus-Senftenberg, Germany (O72) <i>Application of wide field surface plasmon microscopy for investigation of biological micro- and nano-objects</i>
11:00 - 11:30 am	Coffee break	
	<b>BB - MIPs and aptamers (Room H02)</b> Chair: P. Wagner	<b>NANO - Bioelectronics (Room H03)</b> Chair: I. Kurochkin
11:30 am - 12:00 pm	<b>Keynote lecture</b> <b>Marcus Menger</b> , Fraunhofer IZI-BB, Germany (KN14) <i>Aptamers as specific recognition elements in biosensors</i>	<b>Keynote lecture</b> <b>Alan Le Goff</b> , Université Grenoble Alpes, France (KN16) <i>Functionalized nanomaterials for oxygen reduction by multicopper oxidases: control of enzyme orientation and electron transfer pathway</i>
12:00 - 12:15 pm	<b>Giampaolo Zuccheri</b> , University of Bologna, Italy (O67) <i>Self-assembled functional DNA nanostructures as intracellular biosensors in single live human cells</i>	<b>Magdalena Murawska</b> , University of Bordeaux, France (O73) <i>Designing highly organized porous electrodes for miniaturized biofuel cells</i>
12:15 - 12:30 pm	<b>Alvaro Garcia-Cruz</b> , Polish Academy of Sciences, Poland (O68) <i>N-nitrosamine toxin determination in processed meat using a conducting thiosalenCo(III) molecularly imprinted polymer</i>	<b>Andrew James Gross</b> , Université Grenoble Alpes, France (O74) <i>Redox-embedded buckypapers for portable glucose sensing and biofuel cell applications</i>
12:30 - 12:45 pm	<b>Huangxian Ju</b> , Nanjing University, China (O69) <i>Biorecognition for selective biosensing of intracellular biomolecules</i>	<b>Felipe Conzuelo</b> , Ruhr-Universität Bochum, Germany (O75) <i>Simultaneous collection of H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub> for the evaluation of light-induced stress of PS1 photocathodes by means of SPECM</i>
12:45 - 1:00 pm	<b>Patricia Weber</b> , University of Tübingen, Germany (O70) <i>In-line monitoring of antibiotics in fermentation processes with a biomimetic optical sensor</i>	
1:15 - 1:45 pm	<p style="text-align: center;"><b>Closing ceremony</b> <b>Poster prizes + Announcement of 2<sup>nd</sup> EBS</b></p>	

## Poster program overview

<b>Poster session 1 – Tuesday, March 21</b>	
<b>Authors present 1:30-2:30pm</b>	
<b>P1</b>	<b>Gas diffusion biocathode for oxygen reduction based on direct electron transfer between carbon nanotubes and laccase</b> Martin HÄMMERLE, Karin HILGERT, Ralf MOOS University of Bayreuth, Germany
<b>P2</b>	<b>Supramolecular biocatalyst electrodes: A potential-controlled reaction switch for dual-analyte detection</b> Sven Christian FEIFEL (1), Andreas KAPP (1), Roland LUDWIG (2), Fred LISDAT (1) 1: University of Applied Sciences Wildau, Germany; 2: University of Nat. Resources & Life Sciences, Vienna, Austria
<b>P3</b>	<b>Polymer-enzyme interaction as basis for the construction of bioelectrocatalytic sensing electrodes</b> David SARAULI (1), Daniel SCHÄFER (1), B. SCHULZ (2), S. LEIMKÜHLER (2), D. FATTAKHOVA-ROHLFING (3), Fred LISDAT (1) 1: Technical University of Applied Sciences Wildau, Germany; 2: University of Potsdam, Germany; 3: University of Munich (LMU), Germany
<b>P4</b>	<b>Performance characteristic of a CNT-based enzymatic glucose/oxygen biofuel cell in physiological liquids</b> Gero GÖBEL (1), Jennifer MUNDHENK (1), Matias LARA BELTRAM (1), Thorsten HEINLEIN (2), Jörg SCHNEIDER (2), Fred LISDAT (1) 1: University of Applied Sciences Wildau, Germany; 2: Technical University Darmstadt, Germany
<b>P5</b>	<b>Carboxylated graphene as a sensing material for electrochemical uranyl ion detection</b> Robert ZIÓŁKOWSKI (1), Łukasz GÓRSKI (1), Agnieszka BALA (1), Elżbieta MALINOWSKA (1,2) 1: Warsaw University of Technology, Warsaw, Poland; 2: CEZAMAT PW, Warsaw, Poland
<b>P6</b>	<b>Quenching of Graphene quantum dots fluorescence by alkaline phosphatase activity in presence of hydroquinone diphosphate</b> Marta NEVES, María Begoña GONZÁLEZ GARCÍA, David HERNÁNDEZ SANTOS, Pablo FANJUL BOLADO DropSens S.L., Spain
<b>P7</b>	<b>Immobilization and Detection of single Nanoobjects on Nanoelectrodes</b> Xenia KNIGGE (1), Christian WENGER (2), Frank F. BIER (1), Ralph HÖLZEL (1) 1: Fraunhofer Institute for Cell Therapy and Immunology IZI, Branch Bioanalysis and Bioprocesses, Potsdam (IZI-BB), Germany; 2: IHP GmbH - Leibniz Institute for Innovative Microelectronics, Frankfurt (Oder), Germany

<b>P8</b>	<p><b>Self-assembly of alpha lipoic acid monolayers on Germanium surfaces for THz biosensing applications</b></p> <p>Marcin KAZMIERCZAK (1), Jördis MITZLOFF (1), Julia FLESCHE (2), Changjiang YOU (2), Jacob PIEHLER (2), Subhajit GUHA (1), Thomas SCHRÖDER (1)</p> <p>1: IHP GmbH, Germany; 2: University of Osnabrück, Germany</p>
<b>P9</b>	<p><b>Carbon Nanotube-based Photoelectrodes based on assembled Photosystem I</b></p> <p>Dimitri CIORNII (1), Sven C. FEIFEL (1), Kai R. STIEGER (1), Heiko LOKSTEIN (2), Mahdi HEJAZI (3), Athina ZOUNI (3), Fred LISDAT (1)</p> <p>1 Technical University of Applied Sciences Wildau; 2 Charles University in Prague, Czech Republic; 3 Humboldt University of Berlin, Germany</p>
<b>P10</b>	<p><b>DNA self-assembly for signal enhancement in nucleic acids biosensors</b></p> <p>Andrea MITI (1), Giampaolo ZUCCHERI (1,2,3)</p> <p>1: University of Bologna, Italy; 2: S3 Center of the NanoScience Institute, Italian Research Council (CNR); 3: Italian National Interuniversity Consortium of Materials Science and Technology</p>
<b>P11</b>	<p><b>Sulfonated polythiophenes for the electrochemical coupling of glucose dehydrogenase</b></p> <p>Giovanni FUSCO (1), Gero GÖBEL (2), Gabriele FAVERO (3), Franco MAZZEI (3), Fred LISDAT (2)</p> <p>1: University of Rome, Italy; 2: University of Applied Sciences Wildau, Germany.; 3: Sapienza - University of Rome, Italy</p>
<b>P12</b>	<p><b>Electrically controlled Michael addition: addressing of covalent immobilization of biological receptors</b></p> <p>Arwa LAROSSI (1,2), Asma HAMMAMI (1), Noureddine RAOUAFI (1), Vladimir MIRSKY (2)</p> <p>1: Campus Universitaire de Tunis El-Manar, Tunisia.; 2: Brandenburgische Technische Universität Cottbus-Senftenberg, Germany</p>
<b>P13</b>	<p><b>A new nanocomposite-based gelling oligopeptide for biosensors development</b></p> <p>Giovanni FUSCO (1,2), Luciano GALANTINI (2), Andrea ZERILLO (1), Riccarda ANTIOCHIA (1), Gabriele FAVERO (2), Laura CHRONOPOULOU (2), Cleofe PALOCCI (2), Franco MAZZEI (1)</p> <p>1,2: Sapienza University of Rome, Italy</p>
<b>P14</b>	<p><b>Nanodiscs: a novel technology for functional reconstitution and characterization of membrane proteins</b></p> <p>Stefanie MAK, Michael SCHMALENBERG, Peter B. LUPPA, Ruoyu SUN</p> <p>Klinikum rechts der Isar der TU München, Munich, Germany</p>
<b>P15</b>	<p><b>A Polymeric Nanosensor for Sensing of Broad pH Changes in Biofilm as Tool for the Investigation of Microbial Induced Corrosion</b></p> <p>Harald Rune TSCHICHE, Katrin HOFFMANN, Sebastian RADUNZ, Karin SCHWIBBERT, Janin SAMEITH, Jörg TOEPEL, Ute RESCH-GENGER</p> <p>Bundesanstalt für Materialforschung und-prüfung, Germany</p>

<b>P16</b>	<p><b>Biosensor based on the inhibition of the acetylcholinesterase activity and gold nanoparticles for the determination of pesticides in aqueous medium</b></p> <p>Kelvin QUESADA (1), Roy ZAMORA (1), Esteban AVENDAÑO (2), Oscar ROJAS-CARRILLO (3), Ricardo STARBIRD (1)</p> <p>1: Costa Rica Institute of Technology, Costa Rica; 2: University of Costa Rica, Costa Rica; 3: National University, Costa Rica</p>
<b>P17</b>	<p><b>Nanofunctionalized interfaces for SPR based immunosensors</b></p> <p>Sunita KUMBHAT</p> <p>Jai Narain Vyas University, Jodhpur, India</p>
<b>P18</b>	<p><b>Magnetic Nanocomposites for Rapid Biosensing of Staphylococcal Enterotoxins (SET) in Complex Food Matrices</b></p> <p>Angelika Maria NISTLER (1), Brigitte DORNER (2), Reinhard NIESSNER (1), Michael SEIDEL (1)</p> <p>1: Analytical Chemistry, TUM; 2: Robert Koch Institute, Berlin</p>
<b>P19</b>	<p><b>Using DNA origami nanostructures for amplifying signals generated in microbead-based assay for microRNA detection</b></p> <p>Youngeun CHOI (1,3,4), Stefan RÖDIGER (2), Ilko BALD (1,3,4)</p> <p>1: University of Potsdam, Germany; 2: Brandenburg University of Technology Cottbus-Senftenberg, Germany; 3: BAM Federal Institute for Materials Research and Testing, Germany; 4: SALSA School of Analytical Sciences Adlershof, Humboldt University of Berlin, Germany</p>
<b>P20</b>	<p><b>Substrate recycling principles for the detection of adrenaline to support adrenal vein sampling</b></p> <p>Denise MOLINNUS (1,2), Larissa KÄVER (1), Petra SIEGERT (1), Holger S. WILLENBERG (3), Fred LISDAT (4), Arshak POGHOSSIAN (1,5), Michael KEUSGEN (2), Michael J. SCHÖNING (1,5)</p> <p>1: FH Aachen, Campus Jülich, Germany; 2: Philipps-University Marburg, Germany; 3: Rostock University Medical Center, Germany; 4: Technical University of Applied Sciences Wildau, Germany; 5: Forschungszentrum Jülich GmbH, Germany</p>
<b>P21</b>	<p><b>Dielectric spectroscopy of bovine serum albumin up to 110 GHz</b></p> <p>Eva-Maria LAUX, Jessica GIBBONS, Elena ERMILOVA, Frank F. BIER, Ralph HÖLZEL</p> <p>Fraunhofer Institute for Cell Therapy and Immunology, Germany</p>
<b>P22</b>	<p><b>AC electrically functionalised sensor array for influenza virus detection</b></p> <p>Sandra STANKE (1), Christian WENGER (2), Frank F. BIER (1), Ralph HÖLZEL (1)</p> <p>1: Fraunhofer Institute for Cell Therapy and Immunology, Germany; 2: IHP GmbH - Leibniz Institute for Innovative Microelectronics</p>
<b>P23</b>	<p><b>Comparison of Biotin Binding Proteins for PCR Applications</b></p> <p>Carsten SCHMIDT (1), Claudia LIEBSCH (1), Christian SCHRÖDER (1), Werner LEHMANN (2), Peter SCHIERACK (1), Stefan RÖDIGER (1)</p> <p>1: BTU Cottbus-Senftenberg, Germany; 2: Attomol GmbH, Germany</p>
<b>P24</b>	<p><b>Blister-Actuated Laser Induced Forward Transfer as a novel approach for immobilisation of bio-active detection materials</b></p> <p>Lars HECHT (1), Korbinian RAGER (1), Patricia WEBER (2), Andreas DIETZEL (1)</p> <p>1: Technische Universität Braunschweig, Germany; 2: Eberhard Karls Universität Tübingen, Germany</p>

<b>P25</b>	<b>Layered spray-coated nanostructured and enzyme modified electrodes for utilisation in self-powered biosensor units</b> Tim BOBROWSKI, Sabine ALSAOUB, Kirill SLIOZBERG, Adrian RUFF, Wolfgang SCHUHMANN Ruhr-Universität Bochum, Germany
<b>P26</b>	<b>From insights into the potential-pulse assisted surface modification to fast and reproducible multi-probe DNA chip preparation</b> Daliborka JAMBREC (1), Yasin Uğur KAYRAN (1), Felipe CONZUELO (1), Arturo ESTRADA-VARGAS (2), Wolfgang SCHUHMANN (1) 1: Ruhr University Bochum, Germany; 2: Universidad de Guadalajara, México
<b>P27</b>	<b>Carbon nanoparticles as colorimetric labels in on-chip immunoassays</b> Leena MATTSSON, Selma GOGALIC, Sara DOPPLER, Ursula SAUER, Claudia PREININGER AIT Austrian Institute of Technology, Austria
<b>P28</b>	<b>SERS characterization of metal ions-induced dimerization of p-Aminothiophenol on gold nanoparticles</b> Zhiyang ZHANG (1,2), Virginia MERK (1) Ulrich PANNE (1,2), Merwe BUURMAN (2), Janina KNEIPP (1,2) 1: Humboldt-Universität zu Berlin, Germany; 2: BAM Federal Institute for Materials Research and Testing, Germany
<b>P29</b>	<b>DNA immobilization strategy and hybridization kinetic measurement on gold SPRi sensorchip</b> László Ferenc SIMON, E. Róbert GYURCSÁNYI Budapest University of Technology and Economics, Hungary
<b>P30</b>	<b>Peptide-modified nanoporous gold membranes for potentiometric sensing of copper(II) ions</b> Soma PAPP, Róbert E. GYURCSÁNYI Budapest University of Technology, Hungary
<b>P31</b>	<b>High-throughput R2R production of disposable, low-cost electrodes for EIS, biosensors and electrochemical immunoassays</b> Thorsten KNOLL (1), Nenad GAJOVIC-EICHELMANN (2), Thomas VELTEN (1) 1: Fraunhofer IBMT, Germany; 2: Fraunhofer IZI-BB, Germany
<b>P32</b>	<b>UV/Vis-Spectroelectrochemical Investigation of Cellobiose Dehydrogenases (CDHs) from different ascomycetes</b> Vogt STEPHAN, Gilbert NÖLL University of Siegen, Germany
<b>P33</b>	<b>Batteryless pH and Lactate sensing</b> Salzitsa ANASTASOVA, Henry IP, Guang-Zhong YANG Imperial College, United Kingdom
<b>P34</b>	<b>Development of a phosphorescence sensor based on surface molecularly imprinted Mn-doped ZnS quantum dots for selective recognition of cefdinir</b> Hasan BASAN, Hüma YILMAZ University of Gazi Faculty of Pharmacy, Turkey
<b>P35</b>	<b>Paper-based detection of C-reactive protein for point-of-care diagnostics</b> Jennifer Tabea ADAM, Patricia WEBER, Günter GAUGLITZ Eberhard Karls Universität Tübingen, Germany

<b>P36</b>	<p><b>Responsive polymer electrodes – Influence of temperature, pH and peptide binding</b></p> <p>Artur FANDRICH (1), Daniel SCHÄFER (1), E. WISCHERHOFF (2), B. SCHULZ (3), A. LASCHEWSKY (2), F. LISDAT (1)</p> <p>1: Technical University of Applied Sciences Wildau, Germany; 2: Fraunhofer Institute for Applied Polymer Research, Germany; 3: University of Potsdam, Germany</p>
<b>P37</b>	<p><b>Synthesis of Xylitol-stabilized gold nanoparticles: a quantitative and sensitive method for xylitol detection in oral fluid by means of colorimetric assay</b></p> <p>Simona SCARANO, Maria MINUNNI</p> <p>University of Florence, Italy</p>
<b>P38</b>	<p><b>Hyperspectral imaging of plasmon resonances for biosensing</b></p> <p>David ZOPF, Jacqueline JATSCHKA, Sophie THAMM, André DATHE, Andrea CSÁKI, Wolfgang FRITZSCHE, Ondrej STRANIK</p> <p>Leibniz Institute of Photonic Technology, Germany</p>
<b>P39</b>	<p><b>Introduction to wide field surface plasmon microscopy of nano- and microparticles: features benchmarking, limitations, and bioanalytical applications</b></p> <p>Shavkat NIZAMOV, Vitali SCHERBAHN, Vladimir MIRSKY</p> <p>Brandenburgische Technische Universität Cottbus - Senftenberg, Germany</p>
<b>P40</b>	<p><b>A piezoelectric single spot cell printing technique in the picoliter range for different mammalian cell types</b></p> <p>Michelle BIERMANN (1), Christian WARMT (2), Jörg HENKEL (2), Jacqueline FRANKE (2), Frank BIER (2)</p> <p>1: University of Applied Science, Berlin, Germany; 2: Fraunhofer Institute for Cell Therapy and Immunology IZI, Branch Bioanalysis and Bioprocesses (IZI-BB), Germany</p>
<b>P41</b>	<p><b>Application of scanning photoelectrochemical microscopy for the characterization of photosynthetic enzyme complexes</b></p> <p>Fangyuan ZHAO (1), Felipe CONZUELO (1), Volker HARTMANN (2), Huaiguang LI (3), Marc M. NOWACZYK (2), Matthias RÖGNER (2), Nicolas PLUMERÉ (3), Wolfgang LUBITZ (4), Wolfgang SCHUHMANN (1)</p> <p>1: Ruhr-Universität Bochum, Germany; 2: Ruhr-Universität Bochum, Germany; 3: Ruhr-Universität Bochum, Germany; 4: Max Planck Institut für Chemische Energiekonversion, Germany</p>
<b>P42</b>	<p><b>Development of microarray-based assays for the detection of circular RNAs in clinical research</b></p> <p>Christian WARMT (1), Henry MEMCZAK (2), Sebastian MEMCZAK (3), Jörg HENKEL (1), Nikolaus RAJEWSKY (3), Frank BIER (1,2)</p> <p>1: Fraunhofer IZI-BB, Germany; 2: University of Potsdam, Germany; 3: Max Delbrück Center for Molecular Medicine, Germany</p>

<b>P43</b>	<p><b>Dye-stained lifetime-encoded polymer microbeads for time-resolved flow cytometry application</b>  Daniel KAGE (1), Katrin HOFFMANN (1), Marc WITTKAMP (2), Jens AMESKAMP (2), Wolfgang GÖHDE (2), Thomas THIELE (3), Uwe SCHEDLER (3), Ute RESCH-GENGER (1)  1: Bundesanstalt für Materialforschung und -prüfung (BAM), Germany; 2: Quantum Analysis GmbH, Germany; 3: PolyAn GmbH, Germany</p>
<b>P44</b>	<p><b>Highly-integrated Lab-on-a-chip System for Multiparameter Analysis</b>  Harald PETER, Julia WIENKE, Frank F. BIER  Fraunhofer Institute for Cell Therapy and Immunology, Branch Bioanalytics and Bioprocesses (IZI-BB), Germany</p>
<b>P45</b>	<p><b>Lab-on-a-chip proteomic assays for psychiatric disorders</b>  Harald PETER (1), Julia WIENKE (1), Paul C GUEST (2), Nikitas BISTOLAS (1), Frank F. BIER (1)  1: Fraunhofer Institute for Cell Therapy and Immunology, Branch Bioanalytics and Bioprocesses (IZI-BB), Germany; 2: University of Campinas, Brazil</p>
<b>P46</b>	<p><b>Multifunction paper-based analytical device for bacterial cultivation and determination of nitrite</b>  Julaluk NOIPHUNG (1), Wanida LAIWATTANAPAISAL (2)  1: Chulalongkorn University, Thailand; 2: Chulalongkorn University, Thailand</p>
<b>P47</b>	<p><b>Stability monitoring of casein layer on hydrophilic SiO<sub>2</sub> surface using Quartz Crystal Microbalance with Dissipation</b>  Marek TATARKO (1), Ilia IVANOV (2), Tibor HIANIK (1)  1: Comenius University in Bratislava, Slovak Republic; 2: Oak Ridge National Laboratory, USA</p>
<b>P48</b>	<p><b>Polyelectrolyte microcapsules based sandwich assay: A more sensitive tool to commercial beads for the detection of proteins and nucleic acids</b>  Sujit Kumar VERMA, Tatiana A KOLESNIKOVA, Sebastian SPRINGER  Jacobs University Bremen, Germany</p>

<b>Poster session 2 – Wednesday, March 22</b>	
<b>Authors present 1:30-2:30pm</b>	
<b>P49</b>	<b>Piezoelectric immunosensing sensing for detecting of the Alzheimer Tau protein biomarker</b> Dujuan LI (1,2), Samuele LISI (2), Simona SCARANO (2), Maria MINUNNI (2) 1: Hangzhou Dianzi University, China; 2: Università degli Studi di Firenze, Italy
<b>P50</b>	<b>A wash-free, multiplex microbead assay for determination of emerging bioactive compounds in wastewater</b> Peter CARL (1,2), Dominik SARMA (1,2), Knut RURACK (1), Rudolf J. SCHNEIDER (1,3) 1: Bundesanstalt für Materialforschung und –prüfung (BAM), Germany; 2: Humboldt Universität zu Berlin, Germany; 3: Technische Universität Berlin, Germany
<b>P51</b>	<b>Liposome-based ECL Detection Strategies for Microfluidic Biosensors</b> Christian GRIESCHE, Michael MAYER, Axel DUERKOP, Antje J. BAEUMNER University of Regensburg, Germany
<b>P52</b>	<b>Therapeutic drug monitoring of immunosuppressants in a novel laser induced fluorescence based miniaturized setup</b> Urs HILBIG (1), Kathrin FREUDENBERGER (1), Marcel BERNER (2), Markus SCHUBERT (2), Julia STÄB (1), Günter GAUGLITZ (1) 1: University of Tübingen, Germany; 2: University of Stuttgart, Germany
<b>P53</b>	<b>Fabrication of Biosensors Based on Chemically Modified Colloidal AFM Probes</b> Sven DABOSS, Jing LIN, Peter KNITTEL, Christine KRANZ Ulm University, Germany
<b>P54</b>	<b>Development of a quick test for screening Bisphenol A from polymer materials</b> Anna RAYSAN, Rudolf J. SCHNEIDER Bundesanstalt für Materialforschung und -prüfung (BAM), Germany
<b>P55</b>	<b>Covalent anchoring of human transferrin to carbon-encapsulated iron nanoparticles in presence of magnetic field as a way of preservation of its conformational integrity and electroactivity</b> Agata KOWALCZYK (1), Edyta MATYSIAK-BRYNDA (1), Michal BYSTRZEJEWSKI (1), Duncan S. SUTHERLAND (2), Zbigniew STOJEK (1), Anna M. NOWICKA (1) 1: University of Warsaw, Poland; 2: iNANO, Aarhus University, Denmark
<b>P56</b>	<b>Detection of diclofenac molecules by means of a plasmonic sensor substrate</b> Nadja STEINKE (1), Marisa RIO (2), Roland WUCHRER (1), Thomas HÄRTLING (1,2) 1: Fraunhofer IKTS, Germany; 2: Technische Universität Dresden, Germany
<b>P57</b>	<b>Development of multiplexed binding inhibition assays for the screening of food contaminants</b> Konstanze GIER, Claudia PREININGER, Ursula SAUER Austrian Institute of Technology, Austria
<b>P58</b>	<b>Rapid Prototyping using 3-D-Printer Technology for Development of Breath-Test-Analysers</b> Gunther BECHER (1), Stefan DIETZE (1), Jens LEHMANN (2), Jörg HENKEL (2), Hans-Jürgen SMITH (3), Harold SMEETS (3) 1: BecherConsult, Germany; 2: Fraunhofer IZI; 3: CareFusion GmbH

<b>P59</b>	<p><b>New application for an old concept: A disposable optical biosensor for long-term continuous glucose monitoring in bioreactors</b></p> <p>Mircea TRIC (1), Mario LEDERLE (1), Philipp WIEDEMANN (1), Stefan WOELFL (2), Tobias WERNER (1)</p> <p>1: Mannheim University of Applied Sciences, Germany; 2: University of Heidelberg, Germany</p>
<b>P60</b>	<p><b>Study of biological barriers by electrochemistry: intestine covered electrode</b></p> <p>Grzegorz DEBOWSKI, Kinga HUSZNO, Javier SOTRES, Tautgirdas RUZGAS</p> <p>Malmö University, Sweden</p>
<b>P61</b>	<p><b>Detection of the metabolic activity of <i>Saccharomyces cerevisiae</i> using impedance spectroscopy</b></p> <p>Juliane POSSECKARDT (1), Christine SCHIRMER (1), Wolfgang FICHTNER (1), Michael MERTIG (1,2)</p> <p>1: Kurt-Schwabe-Institut Meinsberg e.V., Germany; 2: Technische Universität Dresden, Germany.</p>
<b>P62</b>	<p><b>Microfluidic system for the dielectrophoretic enrichment, characterization and manipulation of suspended cells</b></p> <p>Katharin SCHIEKE (1), Danny ECHTERMEYER (1), Steffen HOWITZ (2), Dieter FRENSE (1), Dieter BECKMANN (1)</p> <p>1: Institut für Bioprocess- und Analysenmesstechnik e.V., Germany; 2: GeSiM mbH, Germany</p>
<b>P63</b>	<p><b>Simple approach to pKA-tunable BODIPY-based fluorescent pH sensors</b></p> <p>Sebastian RADUNZ, Harald Rune TSCHICHE, Ute RESCH-GENGER</p> <p>BAM - Bundesanstalt für Materialforschung und -prüfung, Germany</p>
<b>P64</b>	<p><b>3D pyrolytic carbon microelectrodes for electrochemical sensing</b></p> <p>Suhith HEMANTH, Claudia CAVIGLIA, Yasmin Mohamed HASSAN, Jenny EMNÉUS, Stephan Sylvest KELLER</p> <p>DTU Nanotech, Technical University of Denmark, Denmark</p>
<b>P65</b>	<p><b>Integration of Radio-Frequency Permittivity Sensors into Microwell Plates for Cell Concentration Measurements</b></p> <p>Raphael Marius MGELADSE (1), Farabi Ibne JAMAL (1), Brigitte BURCKHARDT (2), Peter NEUBAUER (2), Mario BIRKHOLZ (1,2), Dietmar KISSINGER (1,3), Jan WESSEL (1)</p> <p>1: IHP, Germany; 2: Technische Universität Berlin, Germany; 3: Technische Universität Berlin, Germany</p>
<b>P66</b>	<p><b>Improving the sensitivity of ATP biosensors</b></p> <p>Jing LIN (1), Oliver KUCHLER (1), Wolfgang SCHUHMANN (2), Christine KRANZ (1)</p> <p>1: Ulm University, Germany; 2: Universität Bochum, Germany</p>
<b>P67</b>	<p><b>Development of a mobile monitoring system based on potentiometric and amperometric biosensors for evaluations in biogas processes</b></p> <p>Shahriar DANTISM (1,2), Johanna PILAS (1,4), Desiree RÖHLEN (1), Thorsten SELMER (1), Michael KEUSGEN (4), Patrick WAGNER (2), Torsten WAGNER (1,3), Michael Josef SCHÖNING (1,3)</p> <p>1: FH Aachen, Germany; 2: KU Leuven, Belgium; 3: Research Centre Jülich GmbH, Germany; 4: Philipps-University Marburg, Germany</p>

<b>P68</b>	<p><b>Early detection of cancer diseases using DNA aptamers</b>  Alexandra POTURNAYOVA (1,2), Lenka BABELOVA (1), Maja SNEJDARKOVA (1),  Monika BURIKOVA (3,1), Jozef BIZIK (3), Tibor HIANIK (2)  1: Slovak Academy of Sciences, Slovakia; 2: FMFI Comenius University, Slovakia; 3:  Slovak Academy of Sciences, Slovakia</p>
<b>P69</b>	<p><b>An optical imaging system capable of monitoring oxygen consumption and oxygen gradients within live tumor spheroids</b>  Carina SCHMITTEIN (1), Robert Johannes MEIER (2), Christina HUPF (1), Joachim WEGENER (1)  1: University of Regensburg, Germany; 2: PreSens GmbH, Germany</p>
<b>P70</b>	<p><b>Nano- and Micro-Patterns of PEG-based Hydrogels with Gold Nanoparticles for Biosensor Applications</b>  Cigdem YESILDAG, Zhenfang ZHANG, Marga Cornelia LENSEN  TU Berlin, Germany</p>
<b>P71</b>	<p><b>Multimodal, Impedance-Based Monitoring of Chemosensitivity Assays for Tumor Cells Using a Lab-on-a-Chip Platform</b>  Stefanie MICHAELIS (1), Michael SKIBA (1), Melanie BÜTTNER (2), Bernd BÜTTNER (2),  Karl-Heinz FELLER (2), Joachim WEGENER (1)  1: University of Regensburg, Germany; 2: Ernst-Abbe-University of Applied Sciences  Jena, Germany</p>
<b>P72</b>	<p><b>Engineered bioinspired hydrogel for the real-time monitoring of cell-growth by mirosensor technology</b>  Anayancy OSORIO-MADRAZO (1), Gerald URBAN (1), Peter FRATZL (2), Laurent DAVID (4),  Alexandra MONTEMBault (4), Christian GORZELANNY (3), Laurent HEUX (5), Yael POLITI (2)  1: University of Freiburg, Germany; 2: Max Planck Institute of Colloids and Interfaces,  Germany; 3: Université Claude Bernard Lyon, France; 4: University of Heidelberg,  Germany; 5: Université Grenoble Alpes, France</p>
<b>P73</b>	<p><b>A novel biosensor platform for inflammation analysis - assessment of platform feasibility</b>  Julia METZNER (1), K. LUCKERT (1), R. MOOS (2), M. HÄMMERLE (2)  1: University Bayreuth, Germany; 2: University of Bayreuth, Germany</p>
<b>P74</b>	<p><b>Heme-peptides on Transparent Electrodes</b>  Bettina NEUMANN (1), Patrycja KIELB (2), Anna FISCHER (3), Arne THOMAS (2),  Matthias SSHWALBE (4), Frieder W. SCHELLER (1), Inez M. WEIDINGER (2), Ulla WOLLENBERGER (1)  1: University Potsdam, Germany; 2: Technical University Berlin, Germany; 3:  University Freiburg, Germany; 4: Humboldt University Berlin, Germany</p>
<b>P75</b>	<p><b>Integration of platelet membrane antigens into nanodiscs for the detection of antibodies associated with autoimmune thrombocytopenia</b>  Stefanie MAK, Ruoyu SUN, Michael SCHMALENBERG, Peter B. LUPPA  Klinikum rechts der Isar der TU München, Germany</p>

<b>P76</b>	<p><b>Optimizing specificity and reproducibility of a biomimetic SPR-biosensor for acquired anti-factor VIII</b></p> <p>Christine SCHÖNMANN (1), Michael SCHMALENBERG (1), Peter LUPPA (1), Michael SPANNAGL (2)</p> <p>1: Klinikum rechts der Isar, TUM, Germany; 2: Klinikum der Universität München, LMU, Germany</p>
<b>P77</b>	<p><b>An aptamer-based biosensor for doxorubicin by means of electrochemical impedance spectroscopy</b></p> <p>Nicole BAHNER (1), Marcus MENGER (2), Katharin SCHIEKE (1), Peggy REICH (1), Dieter FRENSE (1), Dieter BECKMANN (1)</p> <p>1: Institut für Bioprocess- und Analysenmesstechnik e.V., Germany; 2: Fraunhofer Institute for Cell Therapy and Immunology (IZI-BB)</p>
<b>P78</b>	<p><b>Electrochemical biosensor based on CYP17A1 for screening of potential anticancer drugs</b></p> <p>Rami MASAMREKH (1,2), Alexey KUZIKOV (1,2), Vladimir KOSTIN (1), Maria ZAVIALOVA (1), Alexander MISHARIN (1), Alexander ARCHAKOV (1,2), Victoria SHUMYANTSEVA (1,2)</p> <p>1: Institute of Biomedical Chemistry (IBMC), Russian Federation; 2: Pirogov Russian National Research Medical University (RNRMU), Russian Federation</p>
<b>P79</b>	<p><b>First results and challenges for a transferrin MIP</b></p> <p>Xiaorong ZHANG (1), Aysu YARMAN (2), Katharina JETZSCHMANN (1), Ulla WOLLENBERGER (1), Frieder SCHELLER (1)</p> <p>1: Universitaet Potsdam, Germany; 2: Türkisch-Deutsche Universität, Türkei</p>
<b>P80</b>	<p><b>Molecularly-imprinted polymer on paper-based scaffold for cotinine microextraction</b></p> <p>Nutcha LARPANT, Yaneenart SUWANWONG, Wanida LAIWATTANAPAISAL</p> <p>Chulalongkorn University, Thailand</p>
<b>P81</b>	<p><b>Binding of Staphylococcus aureus cells to aptamer-modified gold electrodes measured with impedance</b></p> <p>Peggy REICH, Dieter BECKMANN</p> <p>Institut für Bioprocess- und Analysenmesstechnik e.V. Heilbad Heiligenstadt, Germany</p>
<b>P82</b>	<p><b>Miniaturisation of an Electrochemical Peptide-Based Biosensor for the Detection of Trypsin</b></p> <p>Ahmet UCAR (1), Eva GONZÁLEZ-FERNÁNDEZ (2), Matteo STADERINI (2), Nicolaos AVLONITIS (2), Alan F. MURRAY (1), Andrew R. MOUNT (2), Mark BRADLEY (2)</p> <p>1: The University of Edinburgh, United Kingdom; 2: The University of Edinburgh, United Kingdom</p>
<b>P83</b>	<p><b>Molecularly imprinted polymer chemosensor for selective determination of N-nitroso-L-proline in food products of animal origin</b></p> <p>Patrycja LACH (1), Piyush Sindhu SHARMA (1), Karolina GOLEBIEWSKA (1), Maciej CIEPLAK (1), Francis D'SOUZA (2), Włodzimierz KUTNER (1,3)</p> <p>1: Polish Academy of Sciences, Poland; 2: University of North Texas, USA; 3: Cardinal Stefan Wyszyński University in Warsaw, Poland</p>

<b>P84</b>	<b>A New Flow Cell for Pencil Graphite Electrode and Its Using Biosensing of Glucose in FIA System</b> Didem GIRAY DILGIN (1), Yusuf DILGIN (2) 1,2: Çanakkale Onsekiz Mart University, Turkey
<b>P85</b>	<b>Flow Injection Analysis of H<sub>2</sub>O<sub>2</sub> Using Pd Nanoparticles Modified Pencil Graphite Electrode</b> Serkan KARAKAYA, Yusuf DILGIN Çanakkale Onsekiz Mart University, Turkey
<b>P86</b>	<b>Development of acoustic sensor for detection chymotrypsin activity at surfaces</b> Ivan PIOVARCI (1), Alexandra POTURNAYOVA (1,2), Janos NAGY (3), Reka SAROK (3), Attila HUCKER (3), Katalin SZABO (3), Tibor HIANIK (1) 1: FMFI UK, Slovakia; 2, Slovak Academy of Sciences, Slovakia 3: Hungarian Dairy Research Institute, Hungary
<b>P87</b>	<b>Non-enzymatic ethanol sensor based on a nanostructured disposable screen-printed electrode</b> Sandro HAUG (1), P. Fanjul BOLADO (2), M.M.P.S. NEVES (2), M. B. GONZÁLEZ-GARCÍA (2), D. HERNÁNDEZ SANTOS (2) 1: Deutsche Metrohm GmbH & Co. KG, Germany; 2DropSens, S.L., Spain
<b>P88</b>	<b>Development of artificial receptor for computationally simulated disease biomarkers for sensing application</b> Z. ALTINTAS (1), A. TAKIDEN (1), B. SCHMID (1), M.A. MROGINSKI (1), R. SCHOMÄCKER (1), P. HILDEBRANDT (1), R. SUSSMUTH (1), U. WOLLENBERGER (2), F.W. SCHELLER (2) 1: Technical University of Berlin, Germany; 2: University of Potsdam, Germany
<b>P89</b>	<b>Virtual sensor array consisting of a single sensor element with variable affinity: an application for analysis of fish freshness</b> Yulia EFREMENKO, Vladimir M. MIRSKY Brandenburgische Technische Universität Cottbus-Senftenberg
<b>P90</b>	<b>Lab-on-a-chip based on electrochemical biosensors for malolactic fermentation monitoring of red wines</b> P. GIMÉNEZ-GÓMEZ (1), M. GUTIÉRREZ-CAPITÁN (1), F. CAPDEVILA (2), A. PUIG-PUJOL (2), C. FERNÁNDEZ-SÁNCHEZ (1), C. JIMÉNEZ-JORQUERA (1) 1: Institute of Microelectronics of Barcelona IMB-CNM, Spain; 2: Institut Català de la Vinya i el Vi (IRTA-INCAVI), Spain
<b>P91</b>	<b>Electrochemical biosensors based on DNA and DNA analogues</b> Agnieszka BALA (1), Łukasz GÓRSKI (1), Elżbieta MALINOWSKA (1,2) 1: Warsaw University of Technology, Warsaw, Poland; 2: CEZAMAT PW, Warsaw, Poland
<b>P92</b>	<b>Influenza matrix protein (M1) Binding Interactions with lipid membrane: An SPR study</b> Dahmani ISMAIL (1), Nadine JUNGnick (2), Santiago DI LELLA(2), Andreas HERRMANN(2), Salvatore CHIANTIA(1) 1: University of Potsdam, Germany; 2: Humboldt-Universität zu Berlin, Germany

<b>P93</b>	<p><b>Detection of dielectrophoretically accumulated bacteria at nanoelectrode arrays by surface enhanced Raman spectroscopy</b></p> <p>Eva-Maria LAUX (1), Aristides DOCOSLIS (2), Christian WENGER (3), Frank F. BIER (1), Ralph HÖLZEL (1)</p> <p>1: Fraunhofer IZI-BB, Potsdam, Germany; 2: Queen's University, Kingston, Canada; 3: IHP GmbH – Leibniz Institute for Innovative Microelectronics, Frankfurt (Oder), Germany</p>
<b>P94</b>	<p><b>Detection of tuberculosis DNA by means of polyelectrolyte modified fieldeffect based capacitive semiconductor sensors</b></p> <p>Thomas S. BRONDER (1,2), Arshak POGHOSSIAN (1,2), Max-Philipp JESSING (1), Sabrina SCHEJA (1), Michael KEUSGEN (3), Michael J. SCHÖNING (1,2)</p> <p>1: FH Aachen, Germany; 2: Peter Grünberg Institute, Forschungszentrum Jülich GmbH, Germany; 3: Philipps University Marburg, Germany</p>

# Plenary lectures

## New diagnosis and therapy procedures by utilizing advanced optical multi-contrast and multi-parameter spectroscopic sensors

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To better understand, treat or even cure diseases, an urging demand for new diagnostic methods providing a differential diagnosis and allow for accurate and early treatment exists. In this regard, spectroscopic methods offer the great advantage of obtaining molecular information directly from the examined sample (e.g. cells, tissue) in a label-free manner to provide a clinician with adequate support in the form of clinically-relevant information. One particularly efficient method in this regard is Raman spectroscopy to monitor intrinsic molecular vibrations distinct for every molecule.

Within this contribution we will show, that Raman spectroscopy holds great promise as point-of-care approach for a fast identification of pathogens and the determination of their antibiotic resistances, which is crucial for patient's survival [1-3]. Within the second part, it will be shown that the combination of Raman approaches with other spectroscopic technologies provides a diagnostic tool to potentially solve challenges currently faced by clinical pathology. We will among others demonstrate how the combination of CARS (coherent anti-Stokes Raman scattering), SHG (second harmonic generation) and two-photon excited autofluorescence (TPEF) enables the characterization of the morphochemistry of frozen section biopsy specimens [4-6].

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## DNA Detection and Discrimination using Electrochemical SERS

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The development of sensors for the detection of pathogen-specific DNA, including relevant species/strain level discrimination, is critical in molecular diagnostics with major impacts in areas such as bioterrorism and food safety. In this lecture we will describe the use electrochemically driven denaturation assays (*E*-melting) monitored by SERS to detect and discriminate DNA. The method relies on the high surface enhancement at nanostructured gold electrode surfaces to give very sensitive detection of immobilized dsDNA and to follow the denaturation of the dsDNA as the electrode is swept to negative potentials [1-4].

Using this technique we can discriminate short tandem repeats (STRs) [5], single nucleotide polymorphisms (SNPs) that distinguish DNA amplicons generated from bacterial DNA [6], and discriminate strains within genetically highly similar bacterial DNA using amplicons containing Variable Number Tandem Repeats (VNTRs) [7].

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## Bio-SECM of cells and tissues

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Scanning Electrochemical Microscopy (SECM) is a technique where a microelectrode is scanned over a surface to probe its electrochemical reactivity. To image biological cells and tissues by SECM is quite challenging as topological effects may affect the electrochemical response. To address this issue, we have developed soft SECM probes [1,3] that can be used in a soft contact mode for constant distance mode scanning without altering the sample.

Moreover, soft probes can also be used in a push pull mode to modify the cells environment by delivering and aspirating solutions with adjustable effective micro-volumes containing reagents and other effectors [4].

We have used soft-probe SECM to map the prognostic indicator tyrosinase (TyR) in non-metastatic and metastatic melanoma. The electrochemical readout of the TyR distribution was carried out using an enzyme labeled antibody oxidizing the redox probe tetramethylbenzidine that can be easily detected at the SECM tip. Soft-probe SECM can overcome the limitations of optical methods and opens unprecedented possibilities for improved diagnosis and understanding of the spatial distribution of TyR in different melanoma stages [5]. We shall also show how we have used banana skins as a model system to develop the present imaging strategy.

In a second part, we shall present how SECM can be used for the electrochemical imaging of dynamic topographical and metabolic changes in alive adherent mammalian cells. Extracting intracellular information by SECM is difficult, since it requires redox species travelling across the cell membrane. Cell fixation by formaldehyde and permeabilization approaches have been developed and compared. Cell fixation was found not to damage lipid membranes allowing SECM investigation of cell topography or the passive transport of redox mediators into the cells.

Additional permeabilization of the cell membrane after fixation gives access to the intracellular content. Coupling of SECM with immunoassay strategies for the detection of TyR biomarkers was used to image adherent melanoma cells. [6]

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## Biomimicry at the molecular level: Micro and nanofabricated molecularly imprinted polymers as recognition elements for chemical sensors

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Biomimicry aims at reproducing artificially essential properties of biological systems in order to exploit natural mechanisms or materials for direct applications in technology, in particular materials science. At the molecular level, one example is molecularly imprinted polymers (MIPs), also known as 'plastic antibodies'. MIPs are synthetic receptors that specifically recognize molecular targets [1]. They are highly cross-linked polymers that are synthesized through the polymerization of monomers bearing suitable functional groups, in the presence of the target molecule acting as a molecular template. This templating induces three-dimensional binding sites in the cross-linked polymer network that are complementary to the template in terms of size, shape and chemical functionality. The plastic antibody can then recognize and bind its target with an affinity and selectivity similar to a biological antibody.

We present new approaches for the synthesis of MIPs by controlled/living radical polymerization and spatially controlled localized photopolymerization. This allows for example to obtain protein-size, soluble MIP nanogels with a homogeneous size distribution [2]. They show specific binding of their targets, small organic molecules or proteins [3], with a nanomolar affinity and a good selectivity. Since MIPs are compatible with standard micro and nanofabrication techniques, they can also be obtained in any other physical form, and at the same time interfaced with other materials including transducers. The use of these functional nanomaterials for chemical and biosensing [4-8], and for cell and tissue imaging [9] will be discussed.

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## FROM REAGENTLESS BIOSENSORS TO BIOFUEL CELLS AND PHOTOBIOVOLTAICS

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Coupling of biocatalytic redox reactions with electrode surfaces is on the one hand the basis for the design of targeted biosensors, the electrochemical readout of DNA assays and on the other hand the basis for harvesting energy using biomolecules as recognition elements and catalysts. Due to the fact that the redox centers are often deeply buried within the protein structure of suitable enzymes, wiring of the enzyme integrated redox sites to the electrode surface is of utmost importance.

The presentation is hence focusing on recent developments on:

1. Wiring of enzymes using designed redox polymers
2. Protection of hydrogenases against oxygen and high-potential deactivation using redox-polymer based protection shields
3. Design of biofuel cells with increased open-circuit voltage
4. Self-powered biosensors and instrument-free substrate determination
5. Improving the power output of biofuel cells based on biosupercapacitors
6. Wiring photosystem 1 and photosystem 2 to electrodes using redox polymers for the design of biophotovoltaic cells

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# Keynote lectures

## One- and two-photon excited surface-enhanced Raman scattering for biosensing

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In SERS, inelastic (Raman) scattering of light from molecules, characteristic of specific molecular vibrations, is enhanced by many orders of magnitude, since the local optical fields of noble metal nanostructures lead to an enhancement of both the excitation and the scattered light. In this talk, SERS will be discussed in the context of sensing applications complex environments. The two-photon analogue of SERS, surface-enhanced hyper Raman scattering (SEHRS) shares most of its properties, but also has further characteristics. They include complementary spectroscopic information resulting from different selection rules. In practical spectroscopy, this can translate to advantages, including a high selectivity when probing molecule-surface interactions, the possibility to probe molecule at low concentrations due to high enhancement, and the advantages that come with excitation in the near-infrared. In this review, we give examples of the wealth of vibrational spectroscopic information that can be obtained by combining SERS and SEHRS.

The electromagnetic enhancement in SERS enhancement depends on the properties of the nanomaterials that are used as sensors. This, and the fact that the enhancement factor can vary depending on its interaction with analyte molecules can have serious implications for the application of nanoparticles as SERS sensors in analytical tasks. Possible approaches for the characterization of the nanostructures, in order to understand and predict their performance in enhanced spectroscopies, are demonstrated. This has been achieved combining information about the SERS characteristics with spatially resolved nanoaggregate quantification and ultrastructural characterization [1, 2]

Specifically, we will discuss plasmonic composite nanostructures that can be inserted into endosomes of cells. In live cell experiments, such nanoparticles are transferred into the cell, and depending on their size and surface properties, can be directed to different cellular compartments. They act as nanoprobess, informing us about chemical parameters, specifically the composition in the close proximity of the nanoparticles, or local pH. [3, 4]

SERS and other types of plasmon-supported sensing can also be achieved with nanostructured surfaces. New types of coated surfaces with unprecedented optical properties [5], together with combined one- and two-photon mapping approaches can lead to new possibilities in sensing as well.

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## Immunosensing of pathogens using piezoelectric, electrochemical and surface plasmon resonance based transducers

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Rapid detection of low levels of bacteria remains challenging for point-of-care clinical diagnosis, food testing and environmental screening. Our research focused on *Salmonella enterica* var. Typhimurium, which belongs to leading agents of gastrointestinal diseases; humans become infected particularly by consumption of contaminated food such as meat, eggs and unpasteurized dairy products. A label-free immunosensing employed screen-printed sensors combined with electrochemical impedance spectroscopy [1], piezoelectric quartz crystal microbalance (QCM) and surface plasmon resonance (SPR / Biacore, BioNavis). Specific antibody was covalently immobilized either directly to gold (cysteamine monolayer and glutaraldehyde) or through carboxymethylated dextran layers (EDC/NHS activation). These approaches provided limits of detection at 1000 CFU/mL within 10-20 min, with negligible interference from other bacteria. To improve sensitivity, signal enhancement was carried out using labelling of the captured microbes with secondary antibody-peroxidase conjugate. Afterwards, substrate mixture consisting of H<sub>2</sub>O<sub>2</sub> and 4-chloro-1-naphthol formed insoluble benzo-4-chlorocyclohexadienone which precipitated on the sensing surface and provided excellent signal amplification [2]. In this way, 100 CFU/ml of *S. Typhimurium* were detectable [3]. Several variants of immunosensors were compared, including evaluation on milk samples. Different ways for sample treatment (combinations of heat and sonication) were tested and their impact on the performance was evaluated. Atomic force microscopy was used to study the effect of the treatment on the cell shape and to confirm the specific binding of microbes to the sensing surface, cells were visible even on the rough screen-printed surfaces. AFM images and height profiles also helped to quantify effect of the precipitating product generated by the enzyme label on the improved performance of immunosensors.

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## Pillars, beams, strings and scaffolds - Sensing with micro- and nanofabricated structures

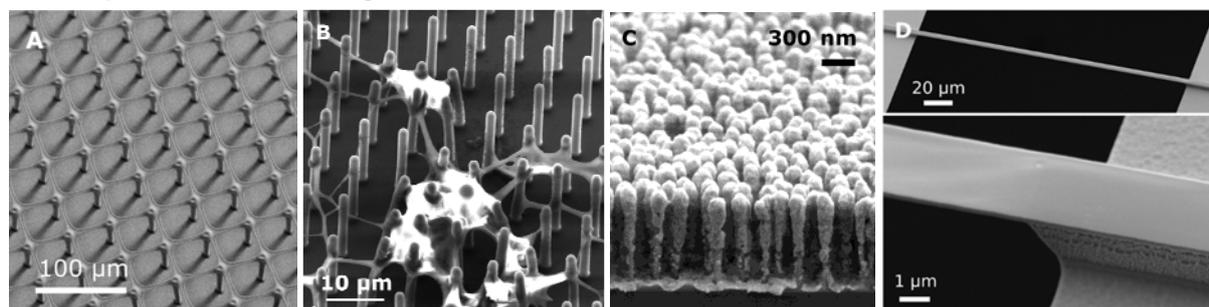
**Stephan Sylvest Keller**

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Highly optimized micro- and nanostructures such as nanopillars, beams, strings and scaffolds fabricated with cleanroom based methods allow to advance the state of the art in bioelectrochemistry, nanomechanical sensing and SERS.

In the first part of the lecture, I will focus on 3D carbon microelectrodes (3DCMEs) for electrochemical sensing and monitoring of cells. In bioelectrochemical applications, cells are typically incubated on 2D electrodes. The planar electrode geometry provides low sensitivity and poorly mimics the natural environment of the cells. We address these limitations by the development of 3D carbon microelectrodes [1], providing both a 3D environment for cellular growth as well as the possibility for 3D electroanalysis in the cell culture. These smart cells scaffolds have been used for electrochemical monitoring of dopamine release from human neural stem cells (hNSCs) [2] and alkaline phosphatase released from osteoblasts.

In the second part, I will present recent highlights of the sensor activities in “The Danish National Research Foundation and Villum Foundation’s Center for Intelligent Drug delivery and sensing Using microcontainers and Nanomechanics (IDUN)” headed by Prof. Anja Boisen at DTU Nanotech ([www.idun.dtu.dk](http://www.idun.dtu.dk)). In IDUN, metal-coated nanopillars are used as highly optimized SERS substrates [3], microcantilever beams serve as transducers for detection of biomolecules [4] and string-based resonators are employed for nanomechanical IR spectroscopy with nanoparticles and organic compounds [5]. The general trends in the center are integrated sample pretreatment and analysis using DVD based microfluidics, development of multi-sensor platforms and implementation of big data treatment.



*Figure 1: A: Suspended carbon microelectrodes; B: hNSCs on carbon micropillar arrays; C: metal-coated Si nanopillar SERS substrate; D: SiN string nanoresonator*

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## Composite plasmonic nanomaterials for cheap, versatile and smart LSPR-based (bio)sensing applications: recent advances

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Plasmonic nanomaterials (PNs) display exciting optical properties and tunability, and during last decade their use has been pushed toward innovative and creative applications, combining the design of cheap and smart detection strategies to impressive analytical performances. Nowadays there is a huge availability of metallic/semi-metallic nanostructures of different figure of merit (FOM) on the market. Alternatively, their simple fabrication can be achieved by home-made synthesis, via metal salts reduction, or by nanolithography-based techniques. As consequence, a plethora of optical substrates for sensitive and selective spectrophotometric measurements based on Localized Surface Plasmon Resonance (LSPR). For gold and silver nanoparticles, their plasmon absorption bands are generally located in the visible region, making them particularly suitable for many (bio)sensing applications. Recently, the fabrication of plasmonic composite substrates for LSPR-based (bio)sensing has gained great attention and, in particular, cheap and versatile polymeric substrates for the *in situ* growth of metal nanoparticles (mNPs) has been reported.

Here we present recent advancements and results on the coupling of two highly versatile and low cost polymers, i.e. polydimethylsiloxane (PDMS) and polydopamine (PDA), with nanoplasmonic materials for LSPR-based (bio)sensing. The obtainable plasmonic nanocomposite materials display exciting features in terms of scalability to stable, cheap and portable devices for point-of-care tests.

**Thomas Bocklitz and Jürgen Popp**

### **Investigation and construction of analysis routines for Raman-effect related data**

In the last years, Raman spectroscopic tools such as confocal Raman imaging, surface enhanced Raman spectroscopy (SERS) and coherent anti-Stokes Raman spectroscopy (CARS) demonstrated their unique potential as biomedical diagnostic or analytical tools. This potential arises from the fact that molecular vibrations within the sample are probed and these vibrations are molecule specific, thus a characteristic “molecular fingerprint” of the sample under investigation can be determined. These molecular fingerprints can be utilized to determine pathological changes within biological samples or to determine concentration estimates of certain substances. As the spectral signatures of pathological changes within biological samples and the spectral contribution of low-concentrated molecules are tiny, statistical and chemometrical treatment of the data is required.

To construct statistical models, which are stable and robust, the computational procedures have to be constructed wisely. Here we report about our recent studies on the pre-processing and analysis procedures applied for biomedical Raman spectra, Lab-on-a-chip-SERS-spectra and the CARS images. In detail we investigated all necessary correction procedures for the three techniques. Overall, we aim a fully automatic data pipeline including all pre-processing, correction and analysis steps to establish a robust analysis. Such an automatic pipeline would result in statistical models, which are stable and robust against corrupting effects and systematic variations of the device. Additionally, an automatic data pipeline allows using the full potential of vibrational Raman spectroscopy and related techniques for biomedical applications and chemical analytics.

## Fluorescence Polarization Immunoassays – Comparison of Platforms

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Immunoassays are in the majority of formats heterogeneous assays with one of the commodities – antibody or antigen – immobilized on a solid substrate or sometimes to nanoparticles or beads. All these formats require a washing step in order to separate bound from non-bound species before generating the measurement signal. Measuring fluorescence polarization is a method to distinguish between a fluorescent macromolecule and a low-molecular weight fluorophore. After irradiation of the sample with polarized light, a macromolecule like e.g. an antibody, will emit still highly polarized light (high polarization, resp. small difference ( $\Delta$ ) in degree of polarization to the background). In a fluorescence polarization immunoassay, the fluorescence of an analyte surrogate (a fluorescence “tracer”) is followed. Depolarization is high and so the measured polarization is low. When antibody is added, the tracer is bound, depolarization decreases and the  $\Delta$  in polarization is high. Increasing amounts of analyte more and more impede the tracer being bound by the tracer and thus the  $\Delta$  decreases, the typical sigmoidal relationship is obtained (Fig. 1). We dispose of 5 polarization platforms reading in plate mode, strip mode and cuvette mode. One is able to register polarization changes with time and so allows for kinetic measurements another one is hand-held. We have been comparing FPIA formats before [1-4] and now a comprehensive view on sensitivities, the importance of fast vs. slow binding kinetics as well as the choice of format can be presented.

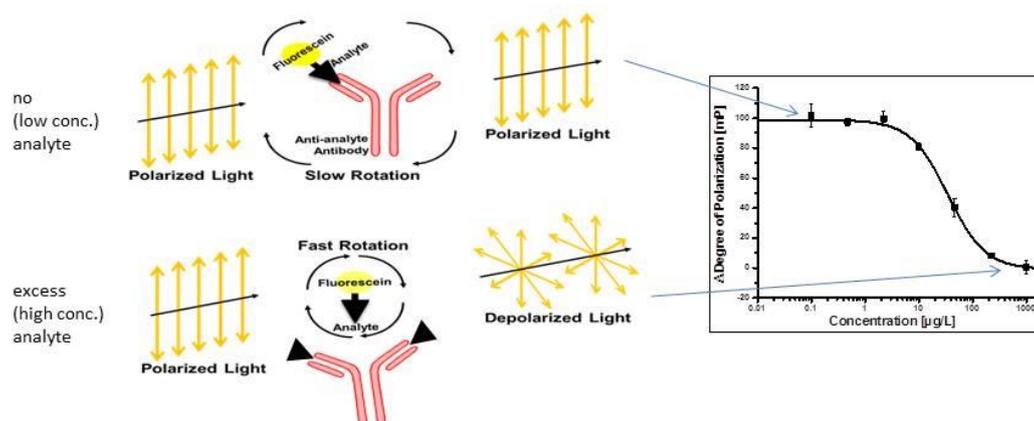


Fig. 1: Fluorescence Polarization Immunoassay: basic principles & calibration.

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## Bio- and Biomimetic Sensors Based on Impedance Spectroscopy and Thermal Boundary Effects

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In 2012, we found that the thermal denaturation of double-stranded DNA, immobilized on a sensor chip, goes along with a significant, persistent increase of the thermal boundary resistance  $R_{th}$  at the solid-to-liquid interface [1]. A qualitative explanation is the increase of surface coverage upon the transition from rigid, upright ds-DNA fragments to soft and irregularly coiled ss-DNA. This observation formed the basis for a new biosensing technique, coined as “heat-transfer method HTM”. HTM allows determining the DNA melting temperature and can be used to detect single-nucleotide polymorphisms in a label-free way. Studying exons of the PAH gene, we could show that the concept remains valid for DNA fragments with up to 100 base pairs. HTM measurements require in principle not much more than a controllable heat source and two thermocouples, one underneath the sensor chip and one above in the supernatant liquid. Conceptually, HTM is related to impedance spectroscopy in the sense that electrical currents are replaced by a thermal current. A potential advantage is therefore seen in the fact that HTM is not limited to electrically conducting liquids and chip materials [2].

In the meantime, the HTM technique was transferred to several other bio-analytical applications and, wherever possible, performed in parallel with non-Faradaic impedance spectroscopy: In case of protein detection (the peanut allergen Ara h1) with aptamer receptors, both HTM and impedance spectroscopy reach detection limits in the nanomolar range [3]. The same holds for the detection of neurotransmitters and inflammation markers such as serotonin and histamine for which molecularly imprinted polymers (MIPs) are versatile synthetic receptors [4]. Furthermore, using the fact that cell membranes are good thermal insulators, we prepared surface-imprinted polyurethane layers for the selective, HTM-based detection of various cancer-cell lines and bacterial strains [5, 6]. Current research focusses on understanding these thermal boundary effects from a fundamental point of view by comparing thermal transport mechanisms in solids, liquids, and soft-matter interlayers.

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## Electrochemical Study of the Extracellular Electron Transfer of Wild Type and Mutants of *Enterococcus faecalis* to Electrodes

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There are only a few electroactive bacteria, which are able to transfer electrons from the cell metabolism to an electrode surface directly. For most bacteria mediators are necessary and only some of the extracellular electron transfer (ET) mechanisms have been studied in depth. We have previously shown that flexible Os<sup>2+/+</sup>-redox polymers can communicate with viable bacterial cells and facilitate ET to electrodes [1,2]. The aim of this study was to electrochemically investigate the role of each component of the respiratory chain of the Gram-positive *Enterococcus faecalis* and accordingly find out the mechanism of the extracellular ET from the cells to the electrode via both Os<sup>2+/+</sup> and quinone redox polymers.

*E. faecalis* is a facultative anaerobe and aerobic respiration depends on the presence of heme, which serves as a cofactor for cytoplasmic catalase and membrane bound cytochrome *bd* oxidase. *E. faecalis* does not require heme to grow and lacks the genes for its synthesis but is able to take up heme or its analogues from the environment. When the cells are supplied with heme, a minimal respiratory chain is built up, including several NADH dehydrogenases, a demethylmenaquinol pool in the membrane and the heme-dependent cytochrome *bd* oxidase.

The wild type as well as three mutant strains of *E. faecalis* with different mutations within the ET chain were investigated using cyclic voltammetry and chronoamperometry under flow injection conditions and different experimental and culture conditions to identify possible ways of the cell-redox polymer-electrode communication.

This work was financially supported by the European Commission (project "BIOENERGY" FP7-PEOPLE-2013-ITN-607793) and the Swedish Research Council (project 2014-5908).

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## Portable POC Diagnostics using Inkjet Printed Sensor Plates

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Portable point-of-care (POC) diagnostics experiences a renaissance. On the one hand, physicians in developing and emerging countries, in particular when far away from economic and medical centres, require low-cost, rapid, and reliable systems to investigate potential diseases of their patients. On the other hand, diagnostics in developed countries have been centralised, which enables an in depth analysis of a large number of samples, but which in many cases can be linked to unreasonably high costs, delays and unnecessary accumulation of data never reaching the patients. Portable sensing platforms with reliable performance, for instance based on electroanalysis, could make a potential contribution for improving the healthcare in both areas.

The employed electrode materials and their properties play a key role for the performance of electroanalytical platforms. Sensor fabrication faces challenges in all developmental steps from the design of prototypes to the industrial production level. Screen-printing and photolithography are well-established technologies, but due to the requirements of masks the rapid design and testing of electrode shapes and materials can be cumbersome. Inkjet printing is an alternative digital, mask-less material deposition technique, where picoliter ink droplets are jetted on demand through hundreds of parallel nozzles resulting in highly reproducible patterns with micrometer resolution.

In this lecture, we present the large-scale fabrication of flexible sensors composed of up to eight parallel carbon nanotube working and counter electrodes, silver/silver chloride quasi-reference electrodes and insulating polymeric materials to define the electrode areas through fully automated multi-layer inkjet printing. These electrode chips are characterized by superior electrochemical performance and reproducibility. We demonstrate the successful application of our printed platforms in an in-house made portable multi-channel potentiostat for a broad range of biological samples including the detection of antioxidants in blood and of immunoassays for a selection of medically and environmentally relevant analytes.

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## Redox-induced switching of DNA-layers observed on a millisecond timescale by means of electrochemical quartz crystal microbalance

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We used an electrochemical quartz crystal microbalance (EQCM) to study the transient effects of potential pulse modulation upon DNA strands in presence of hexammine ruthenium(III) (RuHex) and hexammine cobalt(III) (CoHex) ions. RuHex has been used as an electroactive marker to determine surface coverage of DNA on gold electrodes, interacting with 3 phosphate groups of the DNA backbone. Some other authors have doubts about the applicability of this stoichiometry on electrode surfaces.

Our goal was to confirm the coulometric method by independent EQCM technique. However, what we found was an unexpected large frequency response of the DNA in presence of RuHex (Fig. 1) and CoHex. The large frequency change of ca. 30 Hz could not be explained by a loss or gain of RuHex ions or counter anions, even if considering their hydrate shells. The effect occurred only when a proper potential jump around the redox potential of RuHex was applied. We then studied CoHex and found that more than 30 seconds were needed to reach a new stable frequency in contrast to only 240 ms in case of RuHex. Without RuHex and CoHex, a very small frequency response occurred within 80 ms. Probable explanation considers the flexibility and viscoelasticity of the DNA-SAM influenced by the interaction with the different complex ions and switched upon application of proper moderate potentials.

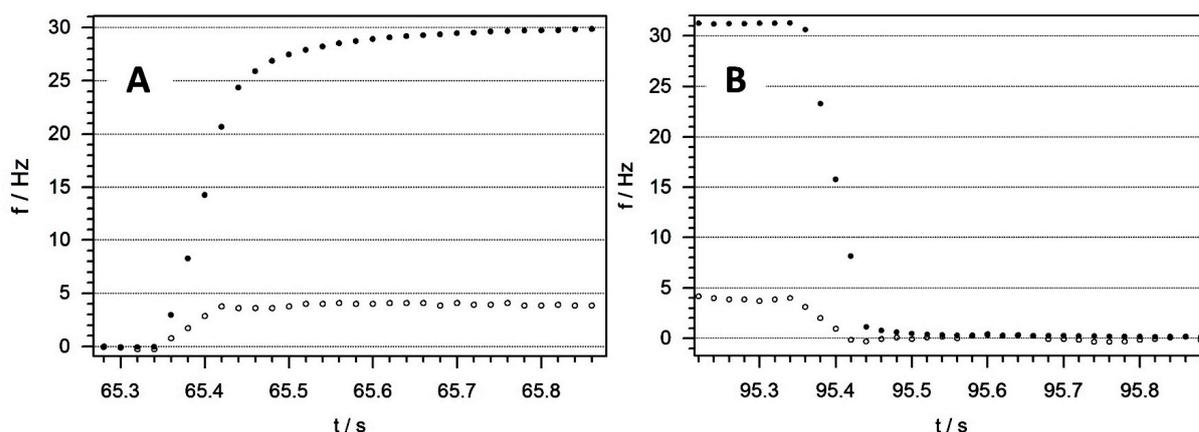


Fig. 1: EQCM frequency transients recorded with ssDNA/mercaptohexanol SAM in presence of (black circles) and without (open circles) hexammine ruthenium(III) chloride upon potential jump from -0.35 V to 0.1 V vs. Ag/AgCl (A) and back (B).

## Design strategies for (redox-)polymers for the immobilization of enzymes on electrode surfaces

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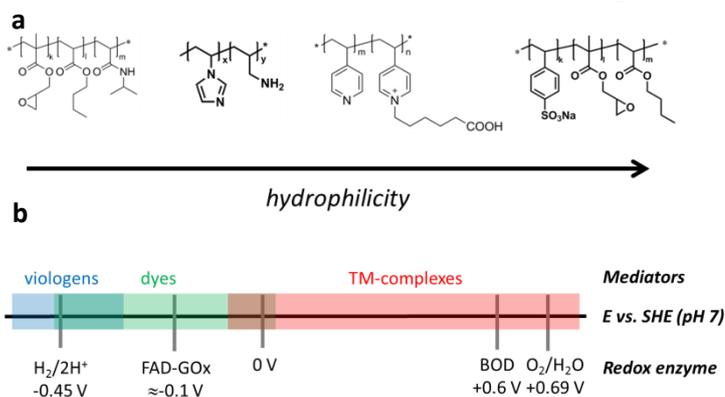
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The development of novel polymers bearing functional groups or specific redox mediators is of particular interest for the immobilization of biological recognition elements on electrode surfaces. The polymers exhibit hydrogel properties and hence provide a solvated environment for embedded enzymes. Furthermore, by integration of electron-transfer relays, i.e. covalently bound redox mediators, electrical wiring of redox-enzymes becomes possible.

We report on the targeted synthesis of novel polymers for the immobilization of enzymes, e.g. *glucose oxidase*, *cellobiose dehydrogenase*, *alcohol dehydrogenase*, *bilirubin oxidase*, photosystem

2 or *hydrogenases*. We demonstrate, that the backbone properties (e.g. hydrophilicity, see a) can be adapted to the properties of a specific enzyme and that the potential of the mediator/polymer can be fine-tuned by altering the nature (b) or structure of the redox mediator or by varying binding strategy. The polymer-enzyme electrodes were used in various biosensors for the detection of glucose, lactose, aldehydes or alcohols and as bioanodes and biocathodes in self powered devices. Moreover, we demonstrate that polymer multilayer structures can be employed to protect sensitive enzymes against damage from O<sub>2</sub> or to prevent co-reaction of interfering substances.



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## Enzymes for MIPs & MIPs for Enzymes

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In this talk innovative applications of different enzymes for the preparation of molecularly imprinted polymers (MIPs) and the development of MIPs for enzymes will be presented:

i) Recently as a supplement of the well established chemical polymerisation and electropolymerisation the enzyme-initiated polymerisation has been introduced by the group Karsten Haupt [1].

ii) In the next step of MIP preparation Proteinase K has been applied for the removal of several protein targets (Cytochrome c, Myoglobin, Concanavalin A) [2].

iii) MIPs have been coupled with enzymes in order to enhance the analytical performance of biomimetic sensors. Enzyme-labelled “tracers” have been used in analogy to competitive immunoassays also in MIP sensors and the signal has been amplified by enzymatic recycling of the redox marker ferricyanide using Horseradish Peroxidase (HRP) [3,4].

iv) Enzymatic pretreatment of the analyte can generate a product which is indicated at a lower electrode potential or a “non-target” substance is converted into a target analog. Following this concept, paracetamol was pretreated with tyrosinase and phenacetin with an esterase [5]. Furthermore, a surface architecture which comprises a substrate-converting HRP layer on top of a product-imprinted electrode for the analgesic drug aminopyrine allowed specific recognition and the elimination of interferences by ascorbic acid and uric acid [6].

v) The last examples are MIPs for macromolecules the copper enzyme tyrosinase, the tetrameric enzyme acetylcholine esterase (AChE) and the Hexameric Tyrosine-coordinated Heme Protein (HTHP) [7,8]. They integrate specific recognition with signal generation and allow regeneration of the recognition layer.

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Aptamers as specific recognition elements in biosensors

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Aptamers, Aptasensors, in vitro selection, SELEX, nucleic acids

Aptamers are short, single-stranded nucleic acids (DNA or RNA) and can use as high specific recognition elements for a broad range of target molecules. Numerous aptamer properties, but particularly the reproducible production of aptamers including chemical modifications and the ability of refolding in an active binding form, enable the use of aptamers in many different biosensor systems. The results are a new class of biosensors called Aptasensors.

## Overview of 26 years of commercial label free biomolecular interaction analysis

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Since the company Pharmacia Biosensor launched in 1990 the “Biacore” for label-free and real-time analysis of biomolecular interactions the number of publications that include data collected from commercial biosensors increased to more than 20.000 articles till 2015 (Pubmed data). Many more companies started to develop instruments enabling the direct and label free sensing of specific biomolecular binding events. Among the still “golden” standard of Surface Plasmon Resonance [1,2] other physical principles were exploited to monitor biomolecular interactions which aim to be more sensitive or are in higher through-put.

In 2015 several commercial instruments are available, meeting the demands of biotech and pharmaceutical companies with still the Biacore (now GE-Healthcare) in the basis and other instruments having more analyte throughput (e.g. Octet, Fortebio, Pall Life Sciences) or instruments developed for high multiplexing biomolecular interactions (e.g. IBIS MX96, IBIS Technologies, Mass-2, Sierra Sensors, XelPlex, Horiba Scientific etc.).

Failures of developments and potential successful commercial instruments will be reviewed in terms of benefits and features, showing that the former “one size fits all” approach is not anymore valid to meet the demands of biotech and pharmaceutical companies in the quality screening of potential therapeutic drug candidates.

With improved experimental design including SPR imaging instruments and advanced data analysis methods, high-quality data of biomolecular interaction phenomena can be obtained. These data promise additional insights into not only affinity of biomolecular pairs but also into the mechanisms of molecular binding events, which will be important for function,- regulatory protein interaction studies in order to unravel the exciting processes in living species.

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## Functionalized nanomaterials for oxygen reduction by multicopper oxidases: control of enzyme orientation and electron transfer pathway

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MCOs possess a set of two copper-containing centres: a surface located T1 centre containing one Cu<sup>II</sup> ion, responsible for the oxidation of substrates and the successive electron transfers to a buried trinuclear T2/T3 centre (TNC) at which oxygen is reduced. When attached to an electrode, these enzymes are able to realize the four-electron reduction of dioxygen into water at low overpotential. This electroenzymatic reaction finds many applications, in particular in enzymatic fuel cells and sensors. We have recently developed different types of nanomaterials which combine many advantages for MCO wiring: excellent conductivity, high specific surface, versatile functionalization techniques and strong interactions with redox enzymes. We have especially investigated the covalent and non-covalent functionalization of carbon-based nanomaterials and gold nanoparticles<sup>[1-3]</sup> for the rational immobilization and direct wiring of MCOs from different sources. We have also developed the design of self-assembled protein nanowires for mediated electron transfer and enzyme entrapment<sup>[4]</sup>. These nanomaterials are able to favor heterogeneous electron transfer to the enzyme either by favoring specific orientations of the enzyme or provide novel self-assembled nanostructured redox bioassembly.

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2. Lalaoui, N.; Holzinger, M.; Le Goff, A.; Cosnier, S. Diazonium Functionalisation of Carbon Nanotubes for Specific Orientation of Multicopper Oxidases: Controlling Electron Entry Points and Oxygen Diffusion to the Enzyme. *Chem. Eur. J.* **2016**, *22* (30), 10494–10500.
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# Oral presentations

## Temporal Fluctuations of single protein by dynamics SERS

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Protein can be viewed as molecular machine that controls our vital cellular functions. Indeed, many misfolded proteins have been related to the origin of neurodegenerative diseases such as Alzheimer. Single molecule spectroscopy has already been proved to be sensitive and selective biochemical sensors. However, monitoring the folding dynamics of protein remains a technological breakthrough.

We propose an advanced optical nanobiosensor [1,2] based on Surface Enhanced Raman Spectroscopy combining plasmonic and microfluidic chips to monitor the protein folding at the single protein level. Due to the high selectivity and sensitivity of our biosensor, we are able to probe the complex behavior of these macromolecules. Statistical analysis of spectra fluctuating [3,4] both in intensity and wavenumber is a crucial task to extract new information at the single protein level.[4]. The label free detection of proteins illustrates how the decoding of the spectra fluctuations can be used to monitor with time [6] the changes in the degree of freedom and conformations adopted by proteins.

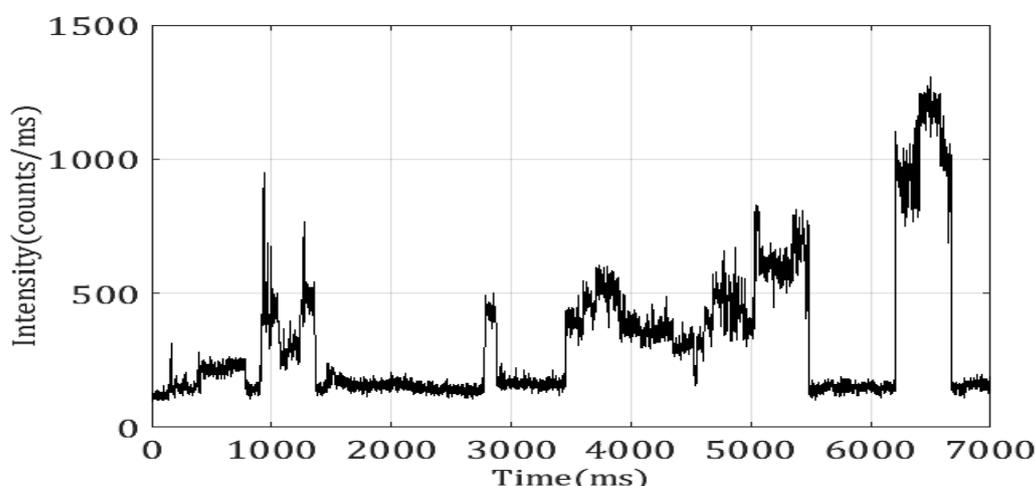


Fig. 1: Step profile of single macromolecule detection recorded every ms

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## New Strategies for the Label-Free Optical Detection of Oligonucleotides or Antibodies with Surface Plasmon Fluorescence Spectroscopy

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Molecular beacons (MBs) are DNA hairpin structures comprising a single-stranded loop with a sequence complementary to the target as well as a double-stranded stem region equipped with a fluorophore and a quencher in close proximity. Upon hybridization with target the stem opens, and fluorophore and quencher become separated. The fluorescence is switched on. MBs have been immobilized on solid substrates, and in some studies the substrate itself has been employed as quencher. As an alternative to optical detection the MB concept has been adopted for electrochemical sensors replacing the fluorophore by a redox probe. After hybridization with target, the distance between redox probe and gold electrode will change. Depending on the design, the distance between redox probe and Au electrode may increase or decrease upon hybridization, leading to a decrease or increase in current.<sup>[1-5]</sup> Recently, we have introduced surface plasmon fluorescence spectroscopy (SPFS) as a new optical readout method for surface bound MBs, which use the gold surface as the quenching unit.<sup>[6-7]</sup> Our SPFS-MB sensors comprise a low detection limit, a fast response time, and they can be reused several times. To further decrease the detection limit of our SPFS-MB sensors we need to increase the difference in fluorescence intensity between the open and the closed state. As the fluorescence in the closed state is mainly background fluorescence caused by fluorophores, which are not completely quenched, the most efficient way to diminish the background fluorescence is to remove the fluorophore. For this purpose the detection strategy has to be changed. We will present new strategies for the label-free optical detection of specific oligonucleotides and antibodies with SPFS.

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## On-Chip Enzymatic Micro Biofuel Cell-Powered Integrated Circuit

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*In situ* energy harvesting is a major limitation for the development of implantable biomedical digital devices. Considerable efforts have been made to improve Glucose/O<sub>2</sub> Enzymatic Biofuel Cells (EBFCs), able to generate electrical power in the physiological environment, in order to match the demands of biomedical electronics [1]. A key advance was the development of redox hydrogels. They permit efficient connection of high amounts of oxidoreductases to electrodes, leading to easily miniaturizable BFCs with power densities in the range of 100  $\mu\text{W}\cdot\text{cm}^{-2}$  suitable for application [2]. However, their open circuit potentials still fall below the requirements of conventional electronics. On the other hand, the power and voltage demands have decreased thanks to tremendous progress in low-power electronics [3], rendering devices compatible with the performance of EBFCs.

Here we demonstrate that common data processing functions such as binary counting can be powered by a glucose/O<sub>2</sub> micro-EBFC directly integrated on an Application Specific Integrated Circuit (ASIC) at the die level. An EBFC based on Glucose Oxidase and Bilirubin Oxidase hydrogels immobilized on 400  $\mu\text{m}$  diameter electrodes was designed on the Si chip of the 4-bit ripple-counter. The system could be continuously powered during several hours from a single 50  $\mu\text{L}$  droplet of aerated 5mM glucose solution.

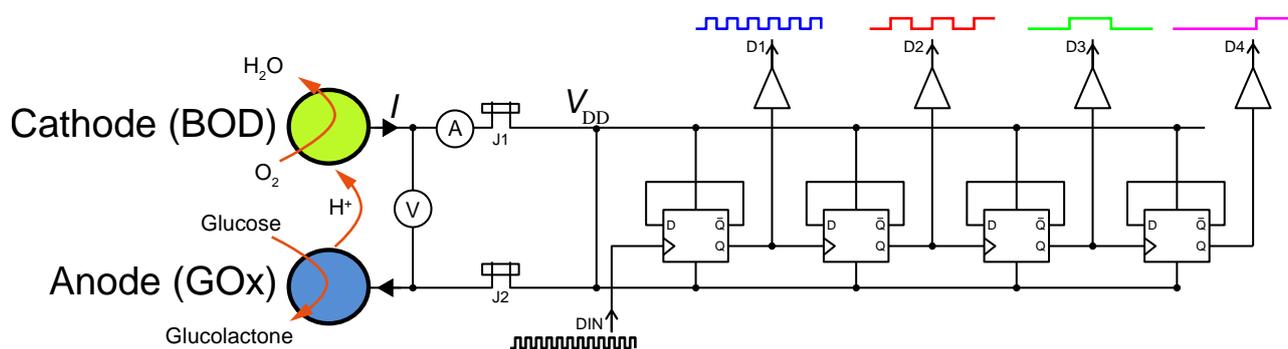


Fig. 1: Schematic of a binary counting function powered by an integrated enzymatic microbiofuel cell.

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## Electrochemical Lectin Biosensor: A Perspective Approach to Prostate Cancer Diagnostics

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Prostate cancer (PCa) is the most common cancer disease and the third leading cause of cancer deaths in men (EU-27). Being often asymptomatic is making early diagnostic a major challenge. Gold standard for PCa diagnostics is determination the concentration of PCa biomarker – prostate specific antigen (PSA) in blood serum, however, wide diagnostic grey zone and high rate of false positive and negative results require biopsy and further examination for a reliable diagnosis.

Glycosylation is one of the most common post-translational modifications and a significant attention is paid to protein glycosylation and its variations as a consequence of various diseases, including cancer. Glycans are oligosaccharide chains present on the surface of proteins, lipids or even whole cells. They often reflect physiological or pathological state of the tissue or even whole organism.

We focused primarily on the terminal sialic acid (N-Acetylneuraminic acid – Neu5Ac), as aberrant changes in sialylation are associated with malignancy. Neu5Ac can be linked to underlying galactose via  $\alpha$ 2,6 or  $\alpha$ 2,3 glycosidic linkage. While  $\alpha$ 2,6 sialylation is commonly found in human tissues,  $\alpha$ 2,3 sialylation is almost exclusively associated with cancer [1]. The structure of PSA glycan from a healthy donor was determined by MALDI-TOF and the linkage orientation was identified by chemical derivatization of terminal Neu5Ac and MALDI-TOF analysis [2]. Prepared PSA-specific immunosensor was used for detection of glycosylation changes by means of interaction with lectins – proteins that are highly specific for saccharidic structures. SNA lectin is specific to Neu5Ac linked via  $\alpha$ 2,6 linkage, therefore it only interacts with PSA isolated from a healthy donor. Contrary to this, MAA lectin interacts with PSA from PCa patient as it recognises  $\alpha$ 2,3-linked Neu5Ac. These alterations were monitored by electrochemical impedance spectroscopy (EIS) [3]. Results were verified against MALDI-TOF acquired data and this approach proved to be a potential replacement for current laborious methods for glycan analysis, when it would be possible to clearly distinguish between protein from a cancerous and non-cancerous tissue while eliminating expensive and robust instrumentation.

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## Effective strategies for the integration of photosystem I into photobioelectrodes by means of cytochrome c

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The development of light-harvesting electrodes based on biological components from photosynthesis gains substantial interest in the last years. Owing to this, we have constructed various photobioelectrodes based on photosystem I, exploiting the interactions of this light-to-charge separating super-complex with the small redox protein cytochrome c (cyt c). Cyt c act here as artificial biological scaffold molecule and as conductive wiring agent; thus various strategies of electrode construction have been developed, including monolayer<sup>[1]</sup>, multilayer<sup>[2]</sup> and 3D electrode<sup>[3]</sup> designs. Herein, we report the evolution in electrode performance and efficiency, but also show limitations and how they can be overcome. With these systems, the photocurrent density could be improved by more than two orders of magnitude, while reaching one of the best quantum efficiencies reported for photobioelectrodes so far.

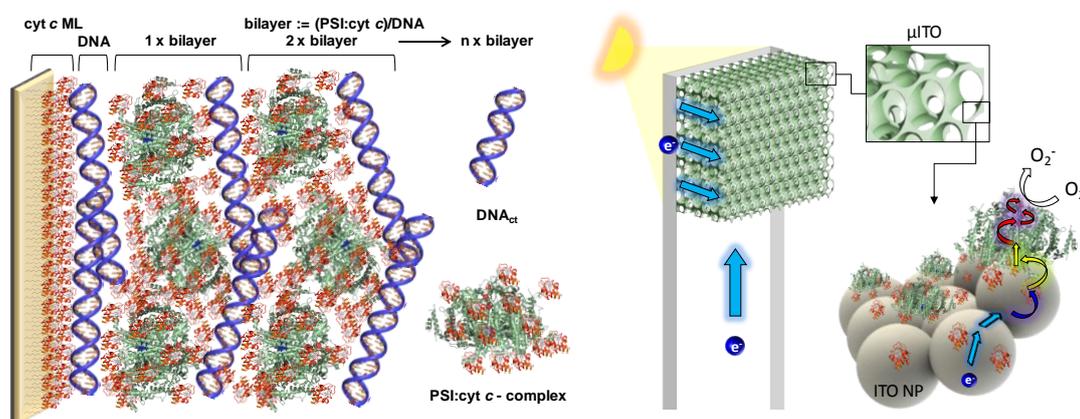


Fig. 1: **(Left)** Photobioelectrode construction based on a multilayer approach applying DNA as negatively charged polyelectrolyte. **(Right)** Photobioelectrode construction based on a 3D electrode approach using mesoporous indium tin oxide ( $\mu$ ITO).

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## Self-Powered and Portable Biosensor Based on CtCDH C291Y Deposited onto AuNPs-Carbon Electrodes for Glucose Detection

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The electrical wiring of redox enzymes with electrode supports is one of the main issues to develop biosensors, electrobiocatalyzed chemical transformations, and biofuel cell elements [1]. For this purpose, nanomaterials (e.g., metal nanoparticles) provide a lot of advantages compared to macroelectrodes, such as enhancement of mass transport, catalysis, active surface area and control on the electrode conductive microenvironment. These features are mainly required to realize an efficient and stable enzymatic biofuel cell (EFC). The derived electrical power from EFCs is usually ranging in the order of microwatts, making the system questionable as a source of energy, especially compared to fuel cells. However, EFC configuration consisting of two electrodes may act as a self-powered biosensor device since the open-circuit voltage of the system (OCV) depends on the fuel concentration [2].

In this work AuNPs were directly electrodeposited onto a glassy carbon electrode (GCE) in order to increase the electroactive area ( $A_{EA}$ ), electron transfer rate constant ( $k^0$ ) and the roughness factor ( $\rho$ ) [3]. The so modified electrodes were used with *Corynascus thermophilus* cellobiose dehydrogenase C291Y (CtCDH 291Y) to realize the biofuel cell bioanode [4], using glucose as fuel, and with *Myrothecium verrucaria* bilirubin oxidase (MvBOD) to realize the biofuel cell biocathode, using O<sub>2</sub> as oxidant. A complete characterization of the EFCs by evaluating several parameters such as OCV, power output and life-time stability was carried out. The EFCs was miniaturized by using graphite screen-printed electrodes, further modified as reported above. Finally, the self-powered biosensor was tested for glucose detection in real samples (e.g. blood) [5].

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## Biophysical Analysis of Cas9 – DNA Interactions and Enzymatic Activity with the switchSENSE Biosensor Platform

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The analysis of the biophysical properties of Cas9 – DNA interactions (binding kinetics, affinity, endonuclease activity) is of fundamental importance for the development of new CRISPR/Cas9 applications. So far, the majority of published data is based on electro mobility shift assays. Interest in sensing technologies like SPR or BLI systems has developed. However, these conventional approaches suffer from inherent limitations and are prone to measurement artifacts.

Here we present binding and enzymatic measurements with switchSENSE, a novel type of biosensor that utilizes electro-switchable DNA layers. The system combines fluorescence measurements a dye with molecular dynamics measurements. For the latter, DNA strands of interest are driven to oscillate on the surface of a microelectrode by alternating electric potentials, while changes in their speed upon binding of a protein are monitored in real-time. Compared to SPR and BLI this system runs with very low ligand densities, which reduces artifacts such as mass transport limitation.

We present an assay to extract binding kinetic rate constants of Cas9 and dCas9 as well as their cutting efficiency in a single experiment on target and off-target DNA. In particular, we elucidate caveats in the measurements of very fast association rates  $k_{ON} > 1E6 \text{ M}^{-1}\text{s}^{-1}$  as well as very slow dissociation rates  $k_{OFF} < 1E-5 \text{ s}^{-1}$ , and show examples that expose the importance of controlling the ligand density on the biosensor surface in order to avoid measurement artifacts.

Additionally we present a standardized workflow which allows efficient screening of sequence-variations with by hybridization of unlabeled target DNAs to a standard anchor DNA. We compare different mismatch influences on binding of gRNA programmed Cas9 to immobilized DNA sequences and discuss effects on the association and dissociation rate constants, respectively.

Moreover, the enzymatic activity of Cas9 is measured in real time. The concomitant analysis of two fluorescence labels allows us to obtain a mechanistic understanding of endonuclease and strand release processes.

## Electrochemical methods in biochemical and biomedical investigations

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Cytochromes P450 (CYPs) are membrane-bound, heme-containing terminal oxidases that are crucial for the metabolic activation or inactivation of xenobiotics. CYPs are ubiquitous and are available in all forms of life from bacteria to mammals. Investigation of the catalytic activity of isolated cytochromes from the P450 superfamily requires obligatory presence of redox partners and electron donors (NADPH). Electrochemically driven CYP systems execute the dual function: substitute partner proteins and serve as a source of electrons for catalysis. Electrochemical CYP systems were applied to studying the catalytic activity, drug metabolism profiling, searching of substrate/inhibitor potential of newly synthesized chemicals, modulating of coupling efficiency, drug/drug interactions, the stoichiometry and the thermodynamics of the catalytic cycle. Electrocatalytic activity of mammalian cytochromes P450 2B4, 1A2, 3A4, 51b1 (sterol-14 $\alpha$ -demethylase *Mycobacterium tuberculosis* CYP51b1), 11A1 (P450<sub>scc</sub>), 2C9, 2D6, 17A1, and bacterial BM3, 260A1, 109C1, 109C2 and CYP109D1 were studied using different types of modified electrodes. Substrate specificity of CYP109C1, CYP109C2, CYP109D1 and CYP260A1 from the myxobacterium *Sorangium cellulosum* So ce56 were studied by cyclic voltammetry while entrapped in DDAB film on the surface of a screen-printed graphite electrode. The CYP260A1 enzyme from the myxobacterium *Sorangium cellulosum* So ce56 demonstrated electrocatalytic properties towards oxygen and C-19 steroid (androstenedione), and CYP109D1 demonstrated electrocatalytic properties towards myristic acid and  $\alpha$ -ionone. CYP109D1-dependent electrocatalytic hydroxylation of the myristic acid was confirmed by mass-spectrometry after electrolysis at controlled working electrode potential. The drug/drug interactions for mammalian CYP3A4, CYP2C9 and CYP2D6 were studied for diclofenac, erythromycin, testosterone, metabolic antioxidant preparations such as ethoxidol (2-ethyl-6-methyl-3-hydroxypyridine malate), mexidol (2-ethyl-6-methyl-3-hydroxypyridine succinate), cytochrome c, L-carnitine, lipoic acid, taurine, antihypoxant mildronate. These findings provide data for future clinical risk prediction studies – especially for those devoted to the interaction of drugs with antioxidants and antihypoxants. Electrochemically driven CYP reactions may have practically relevant providing a useful tool for drug assay studies.

Electrochemical biosensors for heme protein myoglobin were developed based on antibodies or molecularly imprinted polymers for diagnostics of acute myocardial infarction.

This investigation was performed within the framework of the Program for Basic Research of Russian State Academy of Sciences for 2013-2020.

## Film and electrical signal optimisation for carbon pad printed electrodes to detect Ferro/Ferricyanide redox probes and pathogenically relevant Pyocyanin

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Pad Printed Electrodes (PPE) were utilised for the detection of the *Pseudomonas Aeruginosa* quorum sensing molecule Pyocyanin. The ultimate aim is to incorporate the sensor into a smart wound dressing capable of early and rapid detection of infection [1]. Preliminary tests on film thickness and electrode preparation method are evaluated by comparisons between ferricyanide and Pyocyanin, using square wave voltammetry and CV measurements on prepared and commercial electrodes.

A common approach for inexpensive PPE is to utilise a porous film of carbon black both as an electrical contact and sensing surface. The surface saturation of a charge neutral phenazine such as Pyocyanin appears to impede charge flow along the electrode surface resulting in a distributed contact resistance, having implications for medical electrochemical sensors based on porous films.

The signal for Pyocyanin was controlled by rate limiting de-sorption of reduced leukopyocyanin at higher CV scan rates/faster SWV frequencies. Volcano type correlations gave maxima in a) reaction kinetics ( $k_s$ ) and b) SWV concentration dependent sensitivity between 15 and 40 micron thick films.

Evidence for the transition from diffusive to surface controlled kinetics at shorter pulse times is drawn from (i), anodic/cathodic peak height ratios (ii) a significant increase in the signal at the Quasireversible maxima (QRM) of the substrate, (iii) intercepts of integrated charge vs  $f$ (scan rate) plots. The thin film behaviour is not seen with Ferri/Ferrocyanide, though both are quasi-reversible reactions.

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## **Competitive amperometric immunosensor for determination of p53 protein in urine with carbon nanotubes/gold nanoparticles screen printed electrodes: a rapid and noninvasive screening tool for early diagnosis of bladder carcinoma.**

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p53 is an effective transcription factor, responding to diverse cellular stresses to regulate expression of target genes, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism [1]. Particularly, p53 is primarily involved in the inhibition of uncontrolled cell proliferation, upon activation and binding to specific DNA sequences. Concerning the Bladder Transitional Cell Carcinoma (BTCC), the findings of molecular genetic and clinical studies evidenced as abnormal p53 protein accumulation in the epithelial cells may result in its overexpression at level of extracellular fluids, with particular relevance for urine [2], indicating p53 as a valuable protein biomarker. In this study we report the development and validation of a novel disposable competitive amperometric immunosensor [3] for the determination of p53 at picomolar levels, based on gold nanoparticles/carbon nanotubes modified screen-printed carbon electrodes (CNT/GNP SPCEs) directly functionalized with p53 protein. The assay protocol requires the use of single anti-p53 mouse monoclonal antibody, (DO-7 clone) able to recognize both wildtype and mutated p53. Performance of the new competitive immunodevice are comparable with the majority of the sandwich immunosensors for determination of differently mutated p53 forms, despite these methods involve very complex and time/cost expensive nanostructured architectures. The immunosensor and the protocol of the electrochemical immunoassay were optimized by means of experimental design procedures. The device was successfully validated for the determination of p53 in untreated and undiluted urine samples, showing a limit of detection of 17 pM and a limit of quantification of 123 pM. The developed competitive immunosensor was proved as simple, reliable and analytically robust diagnostic tool, valuable for implementation of screening and follow-up programs for urological malignancies.

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## Electrochemical Nanobiosensor for the Tyrosinase Inhibition via phosphodiesterase type 5 inhibitor: Sildenafil

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Tyrosinase, (Tyr, EC 1.14.18.1 belongs to the oxidoreductase class, which contains copper as a cofactor in the structure, is extensively found in nature. It is a multifunctional copper-containing enzyme that catalyzes two distinct reactions of melanin synthesis: a hydroxylation of monophenols to *o*-diphenols (monophenolase activity) and an oxidation of *o*-diphenols to *o*-quinones (diphenolase activity), both using molecular oxygen. *O*-quinone is the product of catechol oxidation through Tyr catecholase activity that can be followed with amperometry. Compact systems with the ability to convert the biochemical reaction into an analytical, measurable, visible, signal are called biosensors. Biosensors produce a signal depending on the concentration of a specific analyte or group of analytes [1-3].

In this study, tyrosinase immobilization was achieved on the screen printed platform of carbon nanotubes, thionine and iridium oxide nanoparticles.

In the first part, biosensor response was characterized towards catechol, which is a well-known substrate of tyrosinase. To optimize the amperometric catechol response different number of scans of thionine (5-30 CVs), different amount of IrOx NPs (1-7  $\mu$ L) and Tyr (2-10  $\mu$ L) were studied. Scanning electron images were obtained to follow the surface changes by carbon nanotubes polythionine, Iridium oxide nanoparticles and tyrosinase. Under optimized conditions, Sildenafil's inhibition property was followed via chelating copper at the active site of tyrosinase. The Sildenafil detection using inhibition was characterized in the range between 0.2  $\mu$ M and 15  $\mu$ M and found very sensitive with low limit of detection 0.004  $\mu$ M and limit of quantitation value of 0.012  $\mu$ M for the inhibition pathway of chelating copper at the active site of tyrosinase.

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## **Biofunctionalization as Key Step in Biosensor Applications – From R&D to Highthroughput Manufacturing**

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Array based analytics have evolved into powerful tools for high-throughput multiplex analysis of a variety of classes of substrates as DNA, proteins, peptides, glycans, the detection of small molecule and screening of polymer properties. Further development of this technology focuses on new components and methods as surface functionalized substrates, probe deposition techniques, strategies of probe immobilization, target preparation and incubation as well as label and label free detection methods to improve sensitivity, reproducibility and to minimize material consumption. The transfer of these systems from open R&D platforms using e.g. microscopic slides to cartridge systems using fully integrated microfluidic chips or biosensors is another goal to enable fully automated diagnostics in the clinical laboratory and Point of Care applications.

A key step in the development of such systems is the biofunctionalization that consists of chemical functionalization of the supports to introduce reactive moieties, deposition of the probes and the subsequent immobilization reaction. All steps of this workflow widely depends on the design of the biosensors as the material and requirements related to the detection technology. Printing of probes on biosensors requires highly exactly spotting at predefined sensor elements based on optical detection of fiducials. The sensors are imbedded in microfluidic chips made of special polymer materials and of complex geometries. They can carry regions of different wettabilities and surface functionality designed for fluidic function, reagent storage and capture probe immobilization.

We will present recent results of our picoliter printing technology for reagent deposition on biosensors and into microfluidic cartridges. The presentation will also focus on a new hydrogel immobilization technology that allows immobilization of capture probes on the sensor elements without prior surface functionalization. Application examples for detection of microRNAs using CMOS and microfluidic chips will be presented.

## Novel electrochemical immunoplatforms for the determination of emerging cardiac biomarkers

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Heart failure (HF) has a great impact on health systems and society as a whole because of its incidence, prevalence, and high mortality rate. The number of admissions due to HF has quadrupled in the last 30 years. Therefore, prognostic biomarkers are needed to improve the management of the HF epidemic.

AXL is a receptor tyrosine kinase which has attracted recent attention as a potential player in the progression of HF and with HF events at short term follow. It has been found that protein concentration in serum (sAXL) is elevated in HF patients compared to controls, establishing a cut-off value of 71 ng mL<sup>-1</sup>. All these findings highlight the relevance of sAXL determination and the interest to develop simple, low cost, fast and easy to methodologies easily integrated in POC devices. Within this context, we have developed, for the first time, three different strategies for the amperometric determination of sAXL: an integrated amperometric immunoplatform, and, two magnetoimmunosensors based on the of micro and nano magnetic carriers. In all the cases sandwich formats involving covalent immobilization of the capture antibody (antiAXL) onto activated carboxylic groups and labeling of the capture antigen with a biotinylated detector antibody (Biotin-anti-AXL) and a streptavidin HRP (HRP-Strept) conjugate were used. Amperometric detection at disposable screen-printed carbon electrodes (SPCEs, at -0.20 V vs. the Ag pseudo-reference electrode) was used to monitor the affinity reactions extension, upon addition of hydroquinone (HQ) as electron transfer mediator and H<sub>2</sub>O<sub>2</sub> as the enzyme substrate.

After optimizing all the different variables affecting the preparation and performance of the the three immunosensing approaches their analytical performance was characterized and their practical applicability successfully demonstrated by the analysis of real human serum samples. The promising analytical performance exhibited by these new amperometric immunosensors of simple operation, disposable SPCE format and possibility to use pocket-size electrochemical transducers makes them very attractive alternatives to commonly used ELISAs for the development of automated POC systems for on-site determination of this powerful biomarker associated with HF.

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## DNA hybridization biosensor based on changes of volume phase transition temperature of hydrogel

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DNA biosensors belong to the group of chemical sensors which transform the chemical or biochemical recognition of a specific DNA sequence into analytically useful information. For this purpose various tags are usually employed to facilitate the detection of the DNA hybridization process. On the other hand, hybridization process detection can be based on changes in the physical parameters, as a consequence of the hybridization process, of either DNA or the matrix in which the DNA strands are immobilized. A good example of this kind of matrix is a polymeric hydrogel.

Here we propose a simple biosensing platform which involves the application of thermoresponsive hydrogel (p(NIPA-co-AA)) for detection of target DNA sequences. For this aim the hydrogel based on poly(N-isopropylacrylamide) decorated with carboxylic groups was modified with H<sub>2</sub>N-ssDNA via the amide bond. The detection of target DNA sequences was achieved successfully by monitoring the volume phase transition temperature (VPTT). It has been found that the dependence between VPTT and the concentration of the target complementary DNA is linear in concentration range from 10<sup>-12</sup> to 10<sup>-6</sup> M. The proposed DNA detection method is highly sensitive and of good reproducibility. The detection limit obtained (1.7 pM) is a substantial improvement over DNA labeled-with-tag biosensor, because the detection is based on VPTT. Circular dichroism (CD), inductively coupled plasma mass spectrometry with laser ablation (LA-ICP-MS) and quartz crystal microbalance (QCM) measurements proved that the hybridization process took place in the hydrogel matrix without any limitations.

## CMOS compatible THz biosensing platform based on Germanium plasmonic antennas

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Plasmon resonance based biosensors are considered as one of the most sensitive methods for detection of changes of the order of a single biomolecule, due to extremely high enhancement of the electromagnetic field. Establishing future biosensing platforms for real life applications calls for the integration of such plasmonic sensors on semiconductor foundry processes. This work demonstrates plasmon based resonant Germanium (Ge) antennas fabricated on standard BiCMOS technology operating at THz frequency range for biosensing; the frequency range is extremely interesting for applications like detection of conformational changes in proteins. With maximum frequency of transistors in present CMOS/BiCMOS technology reaching 0.5 THz to 0.8 THz [2], the proposed Ge plasmonic antennas, operating at 0.5 THz – 0.7 THz, also offer a true integrated platform for future devices, where the THz source and the detector circuits can be designed on the same platform as that of the sensors. We explore bow-tie Ge antenna configuration resonant at 0.57 THz (Fig.1). Another advantage of semiconductor plasmonic sensor with respect to established metal plasmonic sensor is the property of tunability of the plasmon resonance frequency based on the carrier concentration or doping of the semiconductor [3]. Therefore, the proposed Ge plasmonic antennas provide an integrated biosensing platform, where the operating frequency of the sensors can be tailored according to the application by tuning the carrier concentration in the structures.

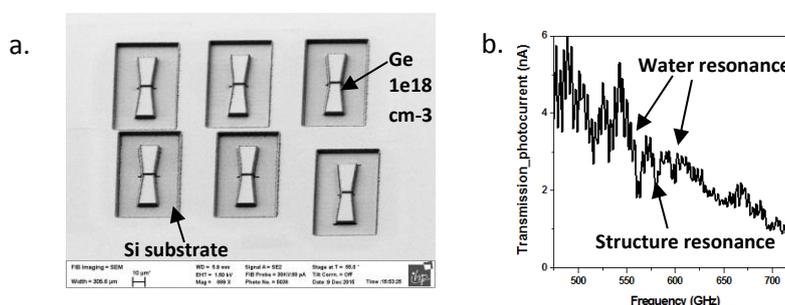


Fig. 1: a. SEM images of Ge bowtie antenna structure; b the resonant frequency is 0.5 THz

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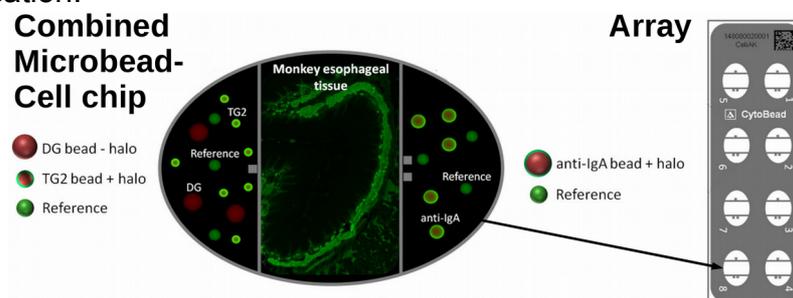
## Simultaneous Multiparametric Analysis of Biomarkers on Combined Microbead-Cell Arrays

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For disease diagnosis, two-tier approaches are recommended. Such tests start with a sensitive detection (e.g., on cells) and continue with a second specific assay (e.g., ELISA). This workflow is laborious, hard to standardize, consumes high amounts of sample material and allows a moderate sample through-put only. Previously, we have developed a fully automatic imaging platform [1], for the multiparametric detection and quantification of biomarkers (proteins, nucleic acids). Biomolecules are detected on the surface of encoded microbeads, cells or in solution. To overcome drawbacks mentioned above we implemented a unique two-tier serological diagnosis reaction environment [2]. We combined classical autoantibody (AAb) analysis on cells with multiplex detection of AAb by a planar multiplex microbead immunoassay (Figure 1). This creates the basis for simultaneous multiplex autoantibody screening and confirmatory testing [3]. We integrated our fully automatic image analysis pipeline to assess AAb quantities on microbeads and fluorescence patterns of cells/tissues both on quantitative and qualitative levels. This was shown for (I) antinuclear antibodies (seven-plex) and (II) celiac disease-specific IgA antibodies and total IgA. Both arrays were comparable with classical testing by single-parameter assays (two-tier approach). The CytoBead is the first technology for the clinical assessment of combined autoantibody screening and confirmatory testing. Our technology is applicable for the determination of various multiparametric quantifications of molecular interactions. Currently, DNA-origami and aptamers are implemented for signal amplification.



*Fig. 1: Magnification of a combined AKLIDES CytoBead assay for the simultaneous analysis of biomarkers on microbeads and cells [3].*

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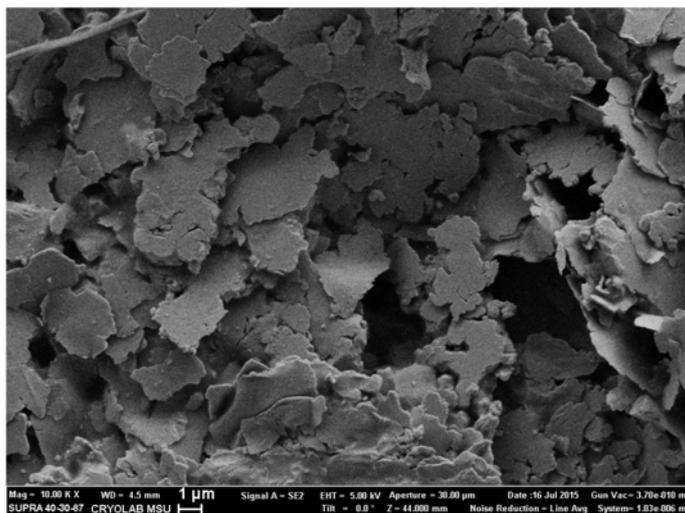
## New SERS-substrate for Cholinesterase Detection

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Human cholinesterases have important physiological functions but its activity can be reduced due to some diseases, pathologies and intoxications [1-3]. Since thiocholine (TCh) is a hydrolysis product of human cholinesterases, its accumulation can represent enzymatic status in organism. Therefore the determination of thiocholine with high accuracy is of great importance for clinical diagnostics.

The new silver SERS-substrate for thiocholine was discovered, characterized and explored. It has roughened lamellar structure (Fig. 1) that caused increasing of Raman signal from analyte. This new SERS-substrate can be used for submicromolar thiocholine detection and for cholinesterase activity detection. The spectra of substrates (acetylthiocholine and butyrylthiocholine) and the product of enzymatic hydrolysis (TCh) were obtained; calibration curves for enzymes were constructed. This technique allows determination of cholinesterase activity both in the buffer and the blood.



*Fig. 1: The typical SEM-image of the SERS-substrate*

### References:

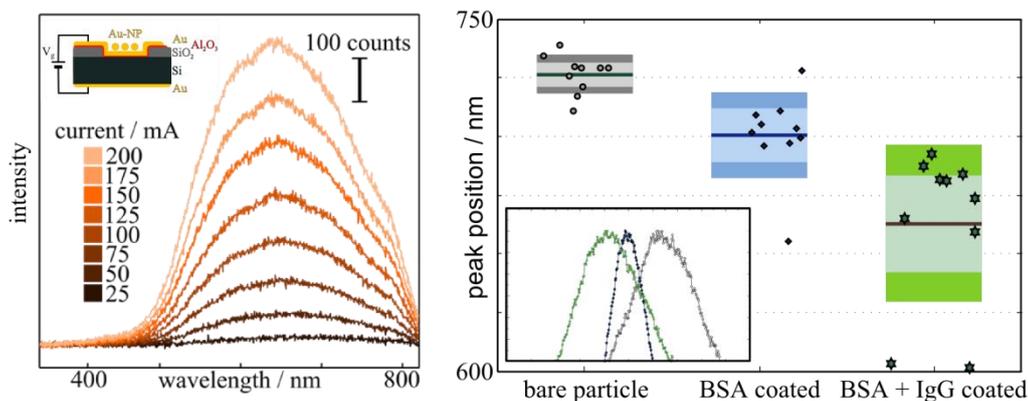
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## Electrically-driven Plasmons in Hybrid Nanostructures

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Plasmon-based sensors are excellent tools for a label-free detection of small biomolecules. An interesting group of such sensors are plasmonic nanorulers that rely on the plasmon hybridization upon modification of their morphology to sense nanoscale distances. Sensor geometries based on the interaction of plasmons in a flat metallic layer together with metal nanoparticles inherit unique advantages but need a special optical excitation configuration that is not easy to miniaturize. Herein, the concept of plasmon excitation by direct, electrically induced generation of surface plasmons based on the quantum shot noise of tunneling currents can be utilized [1]. An electron tunneling junction consisting of a metal–dielectric–semiconductor heterostructure is directly incorporated into the nanoruler basic geometry. With the application of voltage on this modified nanoruler, the plasmon modes are directly excited without any additional optical component as a light source. This electrically driven nanoruler possesses similar properties as an optically excited one and the sensing capabilities such as for the detection biomolecular binding events are comparable [2]. This new sensing principle could widen the palette of highly miniaturized, integrated plasmonic sensors compatible with monolithic integrated circuits.



*Fig. 1: Light emission by inelastic tunneling in metal-insulator-semiconductor stacks is combined with plasmon coupling of noble metal nanostructures (left). The dependency of the gap-mode resonance frequency on the separation can be utilized for sensing of proteins (right).*

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## Development of a label free fiber optical biosensor based on etched fiber Bragg grating technique

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Optical biosensors are analytical devices that integrate a biological recognition element with a physical transducer, whereby the interaction between a target molecule and the recognition element is translated into a measurable optical signal. They are a powerful alternative to conventional analytical techniques due to their high specificity, sensitivity, multiplexing capability, small size and cost effectiveness. Thus, these devices offer diverse applications for example in the field of biomedical diagnostics and process control. For fiber Bragg grating (FBG)-based optical biosensors, the sensing capability is directly encoded into the reflected Bragg wavelength. Interaction of the target molecule near the fiber surface changes the surrounding refractive index (SRI), resulting in an easily measurable shift in the reflected Bragg wavelength.

Here, we present results of the development of an optical biosensor based on etched FBGs (eFBGs). Gratings with Bragg wavelengths in a spectral range of  $\lambda_B = 1520\text{-}1570$  nm could be created by inscribing periods of refractive index modulations into the core of a standard single-mode fiber using a fs-pulsed Ti:Sa laser system working at  $\lambda = 800$  nm. While FBG-based fibers can intrinsically provide access to physical parameters such as temperature and strain, the fibers have to be chemically modified to create a sensitivity to SRI changes. Thus, FBG-fibers were etched with hydrofluoric acid to remove the cladding. Since the etching process could vary between fiber batches, the resulting sensitivity of eFBG-fibers was evaluated using solutions of different refractive indices ( $\Delta\lambda_B/\Delta n$ ). Then, the selectivity of eFBG-fibers was guaranteed by coating the fibers with antibody or aptamer molecules as recognition elements, in which several experimental strategies have been evaluated. Finally, the performance of the eFBG-based bioassay was investigated by detecting different analytes such as C-reactive protein as well-known biomarker.

## Colloidal Metalorganic Frameworks as Novel Biofunctional Nanoparticles for Immunoassay Applications

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Metal-organic framework (MOF) colloids have unique features that render them ideal signalling agents for realizing advanced immunoassay-based detection systems. MOFs are porous coordination polymers of metal nodes and organic linkers. The pore size of MOFs can be engineered and tailored to allow specific host (MOF) and guest (analyte) interactions. The particle sizes of the colloidal MOF can be tailored by employing methods from colloidal chemistry in wet synthesis. The adaption of established Layer-by-Layer polyelectrolyte coating protocols [1] allows equipping colloidal MOF particles with a nanometer thin polyelectrolyte membrane. This polyelectrolyte membrane serves as an interface for antibody binding. These biofunctional MOF nanoparticles have shown a strong immuno-binding that is sufficient for solid state immunoassays.

Our current research addresses the design of luminescence encoded colloidal particle libraries by adjusting the ratios of e.g. Terbium (green) and Europium (red) metal nodes in mixed lanthanide based MOF-76. These mixed lanthanide MOF-76 particles are envisioned to allow multiplexed immuno-detection of endocrine disruptors such as bisphenol A.

In addition we investigate the detection of analytes that do not allow the production of antibodies due to their inherent properties. Such “difficult analytes” have a strong hydrophobicity or are very small or highly toxic molecules. One example is the common plasticizer dioctylphthalate that is also a potent endocrine disruptor. MOF colloids can address this issue by specific host (MOF) : guest (analyte) interactions that result in analyte-specific colour change or exciplex-based fluorescence emission. Our overall aim is to develop methodologies that allow parallel sensing of two endocrine disruptors (e.g. bisphenol A & phthalates) by simultaneous immuno-detection and MOF:analyte specific interactions.

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## Optical Sensors Based on Periodic Hole Arrays in Metallic Films

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Periodic hole arrays in metallic films show an extraordinary transmission of light (EOT) which is based on the excitation of surface plasmon resonance (SPR) through grating coupling. These nanostructures are attractive candidates for developing tailor-made optical sensors for several reasons: 1. Sensors relying on SPR respond sensitively to refractive index changes at the metal/dielectric interface. 2. The geometrical layout of these sensors dictates their optical response facilitating the rational design and realization of highly sensitive sensor platforms. 3. EOT sensors can be operated in transmission mode enabling simple miniaturization and multiplexing. 4. Moreover, these nanostructures can be utilized for surface enhanced spectroscopy (e.g. SERS or SEIRA).[1]

Most often periodic hole arrays in metallic films are fabricated using top-down methods such as photolithography, e-beam lithography, or focused ion beam milling. In our contribution we will present a truly chemical approach to these nanostructures by a combination of colloidal lithography and electroless deposition of gold.[2] By carefully adjusting the production process we are able to prepare not only highly periodic hole arrays in gold films but also site-selectively place gold nanoparticles or gold-coated hydrogel colloids into their holes.[3,4] The sensitivity of the resulting sensor platforms to refractive index changes is significantly increased demonstrating the power of bottom-up strategies for fabricating optical sensors on large areas.

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## Peptide decorated electropolymer films for biosensors: Comparison of different strategies for oriented peptide immobilization

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Linear peptides are used as recognition elements in various sensors and arrays. Covalent coupling chemistries, affinity binding of biotinylated peptides to immobilized streptavidin and self-assembled peptide monolayers on gold surfaces belong to the most widely applied strategies for peptide immobilization. Electropolymerization offers unique opportunities to modify conductive surfaces and miniature electrodes with thin polymer films in a very short time (seconds to minutes). Villiers et al. have electropolymerized pyrrole-derivatized peptides on gold surfaces and monitored the binding of protein targets to peptide microarrays by SPR imaging [1]. This approach is chemically rather demanding as it requires covalent modification of each peptide with a pyrrole monomer and a non-standard, electro-spotting microarrayer. We have explored and compared alternative coupling chemistries which rely on biotin as a standard, commercially available peptide end-group: Biotinylated peptides were spotted onto streptavidin loaded, electropolymerized films (variant 1). Alternatively, biotinylated peptides were pre-incubated with streptavidin in solution, and the resulting peptide-streptavidin conjugates were entrapped in the growing film during electropolymerization (variant 2). A negatively charged, electropolymerized ProDOT/PSS surface was incubated with polycationic PEI, and streptavidin was then adsorbed to this surface (variant 3). Biotinylated peptide was spotted onto this surface (variant 3). Finally, biotin-modified BSA was entrapped in an electropolymer film and pre-assembled peptide-streptavidin conjugates were spotted onto this surface (variant 4). The peptide binding capacity of each surface variant was evaluated by fluorescence imaging of a biotin-peptide-TAMRA conjugate. Moreover, the accessibility of the immobilized peptides for binding by protein targets was assessed by subsequent incubation of each peptide decorated surface variant with a TAMRA-binding monoclonal antibody [2]. Marked differences in peptide concentrations and accessibility to the antibody were noticed for the four model surfaces.

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## Photometric Methods for the Quantification of Functional Groups on Particle Surfaces

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Polymer nanoparticles are of increasing importance for a wide range of applications in the material and life sciences. This includes their application as carriers for e.g., analyte-responsive ligands for DNA sequencing platforms, drugs as well as dye molecules for use as multichromophoric reporters for signal enhancement in optical assays or the fabrication of nanosensors and targeted probes in bioimaging studies.[1]

Application-relevant properties of nanometer- and micrometer-sized particles (NP) include their size (and size distribution), colloidal stability, biocompatibility, and ease of subsequent functionalization, e.g., with linkers, sensor molecules, and targeting ligands. In this respect, the knowledge of the chemical nature, the total number of surface groups and the number of groups accessible for subsequent coupling reactions with differently sized optical labels or biomolecules is mandatory. This requires robust, reliable and validated methods, which can be employed for the characterization of a broad variety of particle systems independent of their optical properties, i.e., scattering or the presence of encoding dyes, and can be preferably performed specifically, sensitively, and fast with inexpensive equipment.[2]

Particularly attractive methods are here straightforward colorimetric, and fluorometric assays. In this respect, we studied a variety of conventional labels for optical readout, utilizing e.g., a change in intensity and/or color of absorption and/or emission.[3] While in common assays, most reporters are measured directly at the particle surface, which can easily lead to signal distortions by scattering and encoding dyes, we focus on the development of cleavable and multimodal labels. These labels are detectable both bound at the particle surface and after cleavage of a linker unit in the supernatant with different analytical methods like fluorometry together with elemental analysis, ICP-OES or ICP-MS for straightforward method validation by method comparison. Here, we present our newly-synthesized cleavable labels and their application for photometric quantification of amino, thiol and carboxy surface groups on different types of nanomaterials and compare the results obtained from surface group analysis relying on conventional labels.

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## A low-cost minimal-step preparation method for mass production of antibody microarrays from polycarbonate

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Antibody microarrays are a fast and efficient method for the multiplex detection of analytes in the bioanalytical field. Glass is often used as a substrate for antibody immobilization. However, several time-consuming wet-chemical surface modification steps are necessary to allow covalent immobilization of antibodies. This results in high labour costs and large generation of organic solvent waste. Polymers provide possibilities for antibody immobilization through functional groups in their backbone but their hydrophobic nature makes it difficult to suppress unspecific binding. Most common plasma-, or UV-, as well as wet-chemical treatments are used to increase hydrophilicity.

Here a method for fast and cost-efficient preparation of antibody microarray chips from polycarbonate sheets is presented. The analysis chips were cut to their final format from a polycarbonate sheet using a digital cutting plotter. The sheet was not completely cut through, in this way multiple chips are handled at a time during the whole preparation process. The polycarbonate surface was then functionalized by screen-printing a viscous paste of a chemically modified low-cost polyetheramine on the polycarbonate substrate. After heating and washing with water a highly hydrophilic surface was obtained. Antibodies were directly spotted via micro-contact printing. The chemical activation reagents for antibody surface immobilization were included in the spotting solution, therefore a separate surface activation step and thus blocking was not necessary. After incubation, the sheets were broken apart into individual chips and assembled with a flow-cell from double-sided tape. The spots were washed and the flowcell filled with a conservation solution for storage.

For surface characterization horseradish peroxidase (HRP) antibodies were spotted on the surface and analyzed using the chemiluminescence readout system MCR-R with HRP and luminol/H<sub>2</sub>O<sub>2</sub> as reporter system. Low background chemiluminescence signals resulted in signal-to-noise ratios of over 500. Also, tests were conducted by immobilizing seven monoclonal antibodies against *Legionella pneumophila* SG 1. A chemiluminescence sandwich microarray immunoassay with heat-inactivated legionella was performed. High signal-to-noise ratios could be achieved, indicating the surface effectively prevents unspecific binding of legionella. The presented preparation method for microarrays make it a viable choice for low-cost production not only on a lab-scale, but also shows great potential for an easy integration into an automated production process.

## Investigation and control of protein adsorption through plasmonic interaction of fluorophore labelled proteins like BSA and metal nanoparticles

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Upon interaction of nanomaterials like noble metal nanoparticles (NPs) with biological systems like body fluids such as serum, a protein corona is formed.<sup>[1]</sup> This reversibly bound layer of proteins controls the transport of the NPs and their subsequent interaction with biological components.<sup>[2]</sup> The plasmonic properties of noble metal NPs like Au and Ag can considerably affect the fluorescence properties of fluorophores in their vicinity, i.e., within a near field distance. Depending on the chemical composition, size and shape of these noble metal NPs, the spectral properties of the dye, and the particle-fluorophore distance, the fluorescence is quenched or in some cases enhanced.<sup>[3,4,5]</sup> This can be monitored by fluorescence intensity and lifetime measurements, with the latter effect being accompanied by an increase in fluorescence intensity and reduction in fluorescence lifetime due to an increase in radiative rate constant. We utilized these effects to study and manipulate noble metal NP-protein interaction exemplarily for fluorophore-labeled bovine serum albumin (BSA) modified e.g. by succinylation, amination and the introduction of thiol groups, resulting in different binding affinities of the proteins.<sup>[6]</sup> Our results show that the fluorescent corona allows monitoring of the interaction of our accordingly protein-functionalized particles with biological model systems like solutions containing different amounts of various proteins. This can be eventually used for further in vitro and in vivo studies to assess the uptake, digestion, and excretion of surface functionalized noble metal NPs.

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## Electrochemical phosphate sensor based on two bioelectrocatalytic and one electrocatalytic oxidation cascade with "on-demand" 4 to 6 electrons per analyte molecule

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The *on-site* quantification of crop nutrients such as nitrate and phosphate are of considerable importance in agriculture [1]. Phosphate rock supplies are being depleted and phosphate run-off contributes to water eutrophication, which emphasizes the need to control precisely the input of phosphorous-based fertilizers on crops [2].  $\text{PO}_4^{3-}$  determination methods often involve use of strong acids, heavy metals and organic dyes [3] which are not compatible with field measurements. In this work, we introduce a green alternative method based on enzymatic phosphate analysis. Electrochemical sensing of the redox silent analyte  $\text{PO}_4^{3-}$  is achieved by the purine nucleoside phosphorylase (PNP) catalyzed reaction of inosine and phosphate to produce hypoxanthine which is subsequently oxidized by xanthine oxidase (XOx), first to xanthine and then to uric acid. While the enzymatic process produces 4 electrons for each phosphate molecule present in the sample, the additional uric acid oxidation at the underlying electrode further enhances the catalytic current by at least two additional electrons per analyte molecule. The latter process is particularly beneficial to compensate for the competing oxidation of the analyte by  $\text{O}_2$ , especially at low phosphate concentrations. Both PNP and XOx are integrated in an Os-complex modified polymer with a redox potential significantly below the onset potential for uric acid oxidation. Hence, the selection of the operation potential for  $\text{PO}_4^{3-}$  sensing opens the possibility to select either the low potential 4 electron oxidation or the high potential - high sensitivity 6 electron oxidation.

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## Electrochemical signaling of 5-enolpyruvylshikimate-3-phosphate synthase GMO plant biomarker

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Differential pulse and square wave voltammetric (DPV and SWV) techniques were used for electrochemical detection of oligonucleotide sequences related to 5-enolpyruvylshikimate-3-phosphate synthase (CP4 epsps), a common vector gene in glyphosate resistant transgenic plants. Hybridization reactions were monitored by following the guanine oxidation signal on a 3-mercaptopropionic acid-capped ZnSe quantum dot-modified gold electrode. Using a probe oligonucleotide sequence with three guanine bases (NH<sub>2</sub>-5'-CCC ACC GGT CCT TCA TGT TC-3'), its immobilization on the quantum dot modified gold electrode was successfully monitored and optimized. The electrochemical signal resulting from oxidation of nine guanine bases in the target oligonucleotide sequence (5'-GAA CAT GAA GGA CCG GTG GG-3') was found to increase with increasing concentration of hybridized target oligonucleotide sequence. The results were compared with electrochemical impedance spectroscopic (EIS) technique. EIS gave a low detection limit (1.32 nM) but its linear range was found to be narrow and occurring at high concentrations of the target analyte (100 – 150 nM). Of the two voltammetric techniques used however, SWV presented lower detection limit (1.17 nM) and higher sensitivity (43.15 nA/nM) in relation to DPV (4.36 nM and 25.88 nA/nM respectively), and could be chosen for CP4 epsps determination at low concentration level.

**Keywords:** Zinc selenide, quantum dots, guanine oxidation, transgenic plants, 5-enolpyruvylshikimate-3-phosphate synthase (CP4 epsps).

## Detection of dielectrophoretically accumulated bacteria at nanoelectrode arrays by surface enhanced Raman spectroscopy

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Electrode arrays are used for the structured collection of *E. coli* bacteria at individual nanoelectrodes (500 nm diameter). Bacteria are attracted to the electrodes under the influence of an inhomogeneous electric AC field. Dielectrophoresis, which is the dominant effect of the electric field applied here, causes a movement of the bacteria towards the electrodes and hence a concentration effect. Cells of *E. coli* are captured out of suspensions; both temporal and permanent adhesion of cells is achieved depending on electric field parameters.

Bacteria are detected by surface enhanced Raman spectroscopy (SERS), where the surface enhancement is introduced by the addition of silver nanoparticles (AgNPs, 50 nm diameter). Four different strategies for AgNP decoration of the electrodes, with and without electric fields, are compared. While all strategies produce sufficiently strong signals for single bacteria detection, the strongest signal enhancement is obtained by pre-incubation of *E. coli* with AgNPs.

The use of nanoelectrode arrays with thousands of electrodes offers rapid analyses of large numbers of individual pathogens. The combination of a selective and accelerated target capture method (dielectrophoresis) with a sensitive signal transduction technique (SERS) is promising for the detection of pathogens like bacteria or virus particles. On-line detection in a flow-through system and specific identification of pathogens from their spectra are envisaged for future perspectives.



Fig. 1: Successive dielectrophoretic accumulation of silver nanoparticles (AgNPs) and of bacteria at nanoelectrode arrays, and bacteria detection by surface enhanced Raman spectroscopy (SERS).

## Electrochemical sensing of biomarkers for early detection of cancer using molecular biosensors

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Nowadays, it is widely accepted that the prompt diagnostic of a certain disease is a key factor in the patient survival. Among the many diseases affecting mankind, cancer is of great relevance due to its high incidence, prevalence and mortality worldwide. Knowledge about cancer biomarkers has increased tremendously during the last years providing great opportunities for improving the management of cancer patients by enhancing the efficiency of detection and efficacy of therapy. During the last years, the demand of efficient, simple and disposable devices with short response times, easy-to-use, low-cost, and suitable for their mass production and to perform decentralized, routine and reliable analysis of cancer biomarkers has increased in the medical diagnosis field.

Within this context, different strategies implying the development of electrochemical platforms for the sensitive, selective and rapid biosensing of single or multiplexed cancer-related biomarkers of different molecular (genetic, regulatory and functional) levels related with breast and colorectal cancer will be discussed in this lecture. In particular, novel sensing platforms for determination of miRNAs [1, 2] and autoantibodies [3] against tumor associated antigens will be treated. The developed methodologies, based on the appropriate use and coupling of novel bioreceptors, functionalized magnetic microcarriers, attractive bioassays formats and electrochemical disposable transducers, allow the accurate determination of the target analytes at clinically relevant levels in challenging biological samples: human serum, and human tissues (both fresh and FFPE).

It is expected that these single- or multiplexed platforms will play a critical role in molecular diagnostics by providing early and rapid cancer diagnosis but decreasing the device costs, shortening the assay time and using simpler protocols which make them suitable alternatives in the implementation of user-friendly and affordable devices, particularly feasible to perform routine determinations in clinical and basic research settings. Moreover, the developed methodologies can easily be extended to the determination of other biomarkers of relevance in other types of cancer or major diseases.

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## Uni – and multivariate data analysis for predicting the sensor output during the specific binding of large analytes to functionalized gold surfaces of plasmonic and acoustic devices

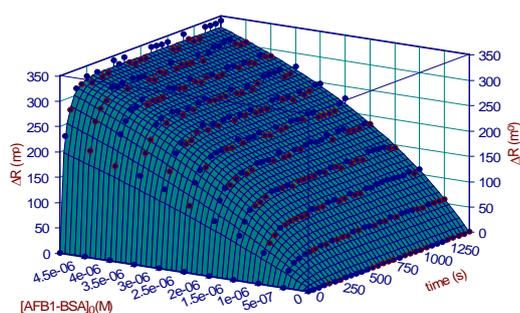
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Surface Plasmon Resonance (SPR) is a versatile technique widely applied in pharmaceutical, environmental and biological sciences. SPR based sensors provide excellent platforms for label-free and real-time investigation of biomolecular interactions such as antibody-antigen [1], enzyme-inhibitor [2] or nucleic acid hybridization [3]. The detection principle of the SPR sensors lies in the changes in the refractive index due to the mass changes near the surface [4]. Surface acoustic wave (SAW) sensors are recently developed as alternative to SPR devices, owing their enhanced sensitivity to changes in mass, density and viscosity near the surface [5]. We report here uni- and multivariate data analysis performed onto extended kinetic plots of the biointeraction pairs: acetylcholinesterase (AChE)/bioconjugates of aflatoxin B1 (AFB1) to protein carriers (peroxidase and BSA) and immobilized anti-AFB1 monoclonal antibody/AFB1-protein carriers, in order to predict the SPR and SAW output during the binding assay. Non-linear and multiple non-linear regression analysis performed on the experimental data provided 3D plots depicting the time-



evolution of the sensor response as a function of analyte concentration (Fig.1). The developed “calibration” surfaces exploit the transient periods of the extended kinetic curves, prior equilibrium, creating a “fingerprint” for each analyte at considerably shortened time frame compared to the conventional 2D calibration plots.

*Fig. 1: Calibration surface for the multivalent ligand AFB1-BSA to immobilized AChE*

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## Development of automated immunosensing methods for clinical and environmental analysis using lab-on-valve platforms

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The lab-on-valve (LOV) is a mesofluidic platform that has been recently exploited for the automation and miniaturization of bioanalytical assays [1], resorting namely to molecular recognition schemes based on immunosensing. Due to its high versatility for reagent accommodation, it is possible to establish immunoassays under several formats (eg. direct competitive ELISA, sandwich ELISA or even label-free immunoaffinity chromatography). For instance, the LOV has been used as a manifold for UV-vis micro-Bead Injection Spectroscopy ( $\mu$ -BIS) [2], a technique that involves the quantification of the target analyte by direct measurement on the surface of a solid phase capable of retaining the target analyte by molecular recognition.

The  $\mu$ -BIS-LOV strategy affords several analytical advantages, namely short time-to-result intervals (3 to 15 min), low sample volume (1-20  $\mu$ L), automated solution handling and washing steps, downscaling of reagents' consumption, low-cost analysis and little generation of waste. Additionally, the solid support is renewed before each determination, minimizing surface fouling, cross-contamination issues and functional group deactivation. No sample clean-up steps are required because interferences are separated from the target analyte upon quantification mediated by a molecular recognition element attached to the micro-bead column. The portability of the LOV device makes it compatible with point-of-care testing.

To our knowledge, this technique has been mainly employed for the evaluation and optimization of bioaffinity processes, but its potential for clinical and environmental analysis remains underexploited. Hence, in this communication, different immunosensing strategies using the LOV platform will be addressed, namely the determination of autoimmune IgG in human serum, and the assessment of drug (carbamazepine) levels in wastewater samples.

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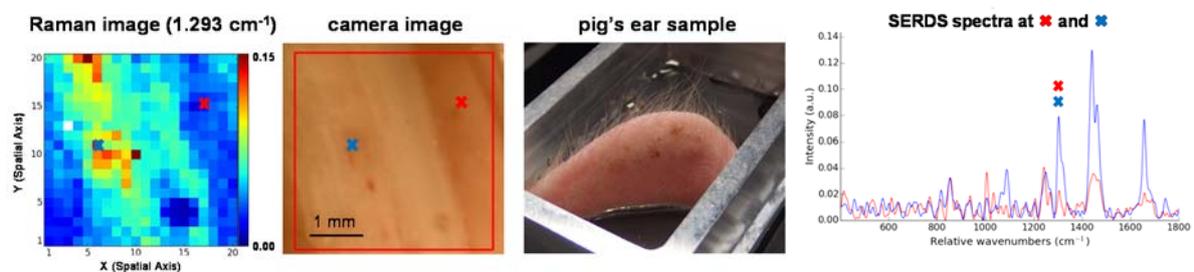
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## Raman Imaging of Skin Samples with Integral Field Spectroscopy

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Step-by-step scanning of a sample is time-consuming but still the state-of-the-art in Raman spectroscopy. Even for a few 100 image points the measurement time may add up to minutes or hours. A radical decrease in time is achieved by applying integral field spectroscopy (IFS). This method was initially developed to save scarce and expensive observing time at astronomical observatories. It combines spectroscopy and imaging capabilities in only one instrument able to provide the entire spectral and spatial information within one single exposure without any scanning procedure. Basic principle is to split the image into many discrete objects that are rearranged in one row at the input of a multiplex spectrograph. In line with a technology transfer project, IFS has been applied to medical diagnostics using Raman spectroscopy with regard to identify cancerous tissue. Our IFS system allows to record up to 400 spatially resolved Raman spectra of 1 cm<sup>2</sup> skin area samples within some seconds, which is some orders of magnitudes faster in comparison to commercial one-channel Raman microscopes.



*Fig. 1: Left: Raman and camera images of a cut pig's ear. Right: Underlying SERDS (Shifted Excitation Raman Differential Spectroscopy) spectra at the positions **x** and **x**.*

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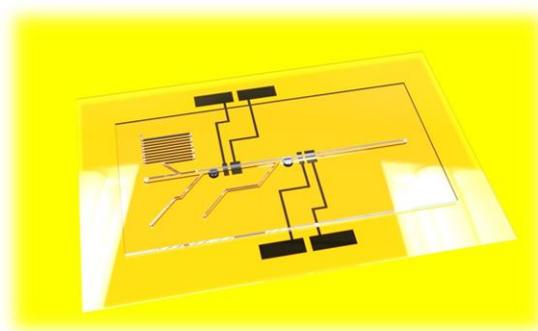
## Multiple detection electrochemiluminescence (ECL) for biosensing

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Research is presented, focusing on a novel microfluidic biosensor for the detection of pathogenic organisms. Electrochemiluminescence (ECL) is a highly sensitive analytical technique that combines advantages from electrochemical and luminescence principles, i.e. requiring simple hardware and offering lowest limits of detection as no background signal occurs. Here, we studied the possibility of dual detection based on commercially available ECL reagents and realizing it in a simple polymer-based microfluidic system. Luminol and Tris-(2,2'-bipyridyl)ruthenium(II) chloride ( $\text{Ru}(\text{bpy})_3^{2+}$ ) were used as ECL compounds, ITO and gold as electrode material. It was found that luminol-based ECL provided the same low limits of detection on gold and ITO electrodes around  $20 \pm 1$  nmol/L. For  $\text{Ru}(\text{bpy})_3^{2+}$  ECL, the same limit of detection was obtained for ITO electrodes, but only 40 nmol/L on gold electrodes. Different surface modifications were tested for enhancement of ECL signals and long-time electrode stability on ITO electrodes. In order to further lower the possible limits of detection when analyzing DNA molecules, liposomes were synthesized to entrap either of the ECL markers. Ruthenium liposomes were shown to be highly stable over more than a year at 4 °C. However, luminol could not be entrapped within the inner cavity of the liposomes. Therefore, a more hydrophilic derivative was synthesized, meta-carboxy-modified luminol that resulted again in stable luminescent liposomes. Currently, both ECL-liposome systems are under investigation for the detection of pathogen DNA sequences within the microfluidic device.



*Fig. 1: microfluidic setup*

## **FRET-based immunoassay using anti-TAMRA-antibodies coupling to liposomes and a DBD dye derivative**

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For the development of highly sensitive detection methods, immunoassays have been proven as indispensable tools in order to detect bioanalytes at very low concentration in biological samples. To improve the detection sensitivity of immunoassays, incorporation of an additional signal amplification step is required. Various signal amplification strategies have been applied which normally utilize enzymatic reactions and/or nanomaterials including nanoparticles or liposomes. In particular, liposomes are very attractive materials, because they can be easily prepared with a narrow size distribution, and accompanied by a large amount of signalling molecules leading to high sensitivity in immunoassays.

In this study, a Förster resonance energy transfer (FRET)-based assay has been developed. In this novel FRET system, the unique fluorescence properties of [1,3]dioxolo[4,5-f]-[1,3]benzodioxole (DBD)-based fluorophores (e.g., long fluorescence lifetime (~16ns), large Stokes shift (~100 nm), high photostability, and high quantum yield (>0.56)), its high hydrophobicity for efficient anchoring in liposomes, and a maleimide bioreactive group are combined to form a novel probe for the coupling anti-TAMRA-Antibodies (DC7-Ab) to liposomes. In a proof-of-concept binding between the antibody and Rhodamine 6G (R6G) was monitored. Therefore, steady-state and time-resolved fluorescence measurements (e.g., fluorescence depolarization) in combination with single-molecule fluorescence spectroscopy (fluorescence correlation spectroscopy, FCS) were used to monitor the binding interaction either between DC7-Ab and liposome or between DC7-Ab and R6G. Based on the fluorescence depolarization and the respective rotational correlation times, and the alteration in the diffusion coefficient (determined by FCS) the binding of the DC7-Ab to the liposomes was studied. Moreover, in addition to ensemble FRET data, single pair FRET experiments using pulsed interleaved excitation are used to characterize in detail the binding on a single molecule level avoiding averaging out effects.

This study demonstrates an easy, fast, and high sensitive alternative assay to detect biological analytes (such as protein, DNA...) by using rhodamine labeling of compounds instead of generating specific antibodies against each single biological analytes in combination with a smart signal amplification based on liposomes.

## Sensing and imaging of intracellular pH using photon upconversion based nanoprobos

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Sensing of intracellular pH is of particular interest in biomedical research since structure and function of biomolecules strongly depend on the concentration of protons in their environment. We have functionalized photon upconversion nanoparticles (UCNPs) with pH responsive dyes to achieve nanoprobos for intracellular pH determination. The sensing mechanism is based on a resonance energy transfer (UC-RET) from the 550 nm emission of hexagonal nanocrystals of NaYF<sub>4</sub>: Yb<sup>3+</sup>, Er<sup>3+</sup> to the pH-sensitive fluorophore pHrodo<sup>TM</sup> Red.<sup>[1]</sup> The nanocrystals were coated with thin shells of aminosilane or highly branched polyethylenimine (PEI) with several nanometer layer thickness for coupling of the pH indicator.

The nanoprobos are calibrated by ratiometric dual wavelength readout at 550 nm (reference signal) and 590 nm (sensor signal) and visualized using a scanning confocal fluorescence microscope with 980 nm excitation wavelength. It was found that PEI coating enables a higher coupling of indicator molecules on the particle surface, better signal to reference ratios in ratiometric readout and an improved cellular uptake compared to the aminosilane coated particles due to a more positive zeta potential. We studied the cellular uptake efficacy of the nanoprobos and determined to which type of compartment, lysosomes, endosomes or cytosol, the probes are targeted to by measuring the pH of their microenvironment. An *in situ* control was performed in live cells by a treatment with nigericin, whereby the pH of all intracellular compartments is set at extracellular level.

Finally, we will show new strategies for the preparation of UCNP-dye conjugates with improved UC-RET efficiency to achieve higher acceptor (sensor) emission.

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## Advanced wide-field surface plasmon microscopy of single adsorbing nanoparticles: analytics in complex biological media and electrochemical characterisation.

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Detection and characterisation of nanoparticles (NPs), especially in complex biological media, still represent a major challenge for existing analytical methods. In the present work we describe a label-free, real time and cost-effective analytical method for detection of NPs with very low concentrations in real samples of consumer products. In second part, we also address the problem of electrochemical characterisation of single NPs.

Proposed methods are based on the wide-field surface plasmon microscopy (SPM)[1]. The large field of view of our SPM setup ( $\sim 1.3\text{mm}^2$ ) allows the detection of single adsorbing nanoparticles up to the hundreds of thousands. The minimal detectable size of NP is  $\sim 15\text{-}20$  nm for the Au NPs,  $\sim 40\text{-}50$  nm for polystyrene NPs. The detection rate is proportional to a volume concentration of NPs over several orders of magnitude concentration range: typically  $10^6\text{-}10^{10}$  NPs/ml [2,3]. This range can be further improved tenfold.

For the detection of NPs in complex media the influence of matrix effects should be considered. To effectively discriminate images of NPs from image perturbations caused by the matrix components, we propose to use the template matching [2]. First, the characteristic SPM images of nanoparticles (templates) are collected in aqueous suspensions. Then the detection of nanoparticles in complex environment using template matching is performed. Using this approach, the detection and characterisation of various NPs in consumer products like sunscreen cream, mineral water, juices, and wines was shown at sub-ppb level ( $\sim 100$  pg/mL).

Characterisation of detected NPs can be done by their images, but, unfortunately, the size/material can not be resolved directly. For determination of chemical composition of single NPs separately, SPM can be assisted by electrochemical analysis [3]. In this case, the gold sensor surface is used both for plasmon microscopy and as a working electrode. Applying the linear sweep of potential to this electrode, adsorbed NPs can be subjected to electrochemical dissolution whose potential characterises its material.

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## Culture-independent serotyping of *L. pneumophila* in water and urine samples

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Increasing numbers of legionellosis outbreaks within the last years have shown that a fast and sensitive detection method for *Legionellae* in water (e.g. surface water, drinking water, process water) and patient's urine is still needed. The cultivation, which takes 10 days, is still the gold standard for the detection of *Legionella*, though only a general detection of *Legionella* spp. is possible. In case of an epidemic outbreak a rapid assignment from the infected patients to the outbreak source is fundamental. For this reason a chemiluminescence sandwich microarray immunoassay (CL-SMIA) for the detection and serotyping of *Legionella pneumophila* was established on the microarray analysis platform MCR-R. A rapid and multiplexed detection method for serotyping of all serogroups and subtypes of *L. pneumophila* within only 40 min will be possible with a panel of 23 sensitive and selective monoclonal antibodies. A threshold value for every monoclonal antibody will be defined for characterisation of all immobilized components. With help of a binary code, which declares '1' above and '0' below the threshold value, a particular and unique pattern will be defined for every serogroup and subtype of *L. pneumophila*. In implication a pattern definition can be realised to directly and rapidly compare patient's urine samples with environmental water samples that are assumed to be the outbreak source. As the CL-SMIA is used for water samples as well as patients' urine samples an application in clinical diagnostics and environmental hygiene of the health authorities is possible. First results with 5 monoclonal antibodies have shown that *L. pneumophila* SG 1 ST Bellingham can be identified unambiguously, other subtypes in groups. In a proof-of-principle study it was shown that a monoclonal subtyping can be done in urine. In the future the application has to be verified with environmental water samples and water of condensation recooling plants.

## MCC-IMS Spectral Analyses of Headspaces from Bacterial Cultures for Rapid Identification of Bacterial Infections

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### Introduction:

The Ion Mobility Spectrometry (IMS) is a highly sensitive analysis method for detection of volatile organic compounds (VOCs) in gaseous samples in ppb to ppt-ranges. The study was aimed to check if the differentiation of germs is possible by IMS spectra analyses due to emission of VOC's. In contrast to decidedly sensors IMS is capable to differentiate a couple of different markers simultaneously.

### Methods:

The headspaces of fluidic bacterial cultures were analysed using MCC-IMS, a combination of a multi capillary column with an ion mobility spectrometer. 7 different germs were compared. The peaks were determined and compared by means of cluster analysis-based software. The classification has been carried out with a leave-one-out cross validation and support vector machine.

### Results:

In total up to about 400 clusters were identified in each sample of bacterial headspaces. In Head spaces, approximately 40 appeared to be suitable for differentiation.

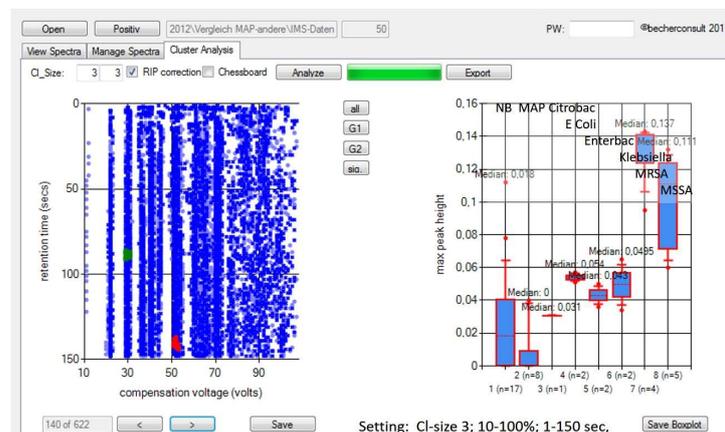


Fig. 1: Using Cluster 140 MRSA and MSSA (right bars) were differentiated from all other germs

### Conclusions:

The distinction of different species of bacteria with 100% specificity is possible. The system will be a future screening device for infectious diseases as well as other metabolic disorders, with multi-marker-sensitivity in contrast to decidedly sensors.

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## Point of need testing for rapid detection of infectious diseases in water

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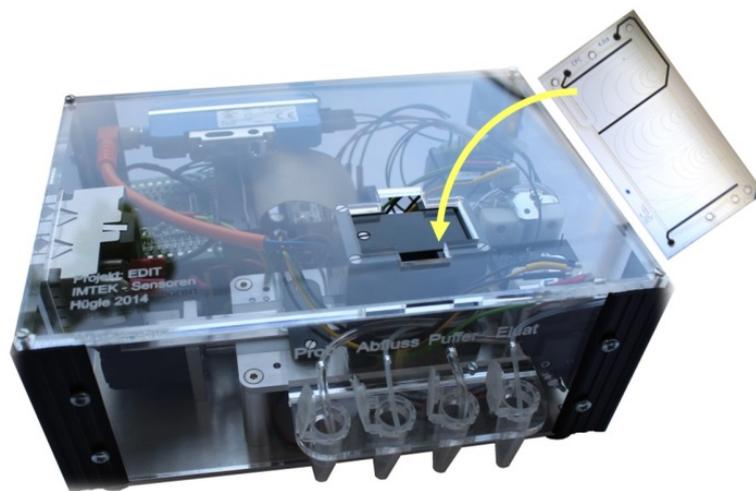
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In recent years, fast diagnostics by point-of-need-testing (PONT) have gained more importance. Previous works have demonstrated the feasibility of the lab-in-a-suitcase concept for the detection of emerging pathogens such as the H7N9 virus [1].

To expand on this concept, we present a novel lab-on-a-chip approach for rapid molecular detection of viruses, bacteriophages and bacteria.

The system combines free-flow electrophoretic (FFE) enrichment, thermoelectric lysis and gel electrophoretic purification of the released nucleic acids. Within the context of the EDIT project, the scope of the system was expanded towards automated hygiene monitoring of drinking water at the point-of-need [2]. First results were obtained for the detection of *E. coli* and bacteriophage  $\Phi$ X174 as fecal indicator organisms using rapid recombinase polymerase amplification (RPA) as well as quantitative PCR.



*Fig. 1: Automated Lab-on-chip demonstrator for sample pretreatment which implements sample concentration, lysis of sample and nucleic acid purification in a single step.*

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## Dose Response Screening of *c. vaccinii* using Impedimetric Sensing of Microfluidic Droplets

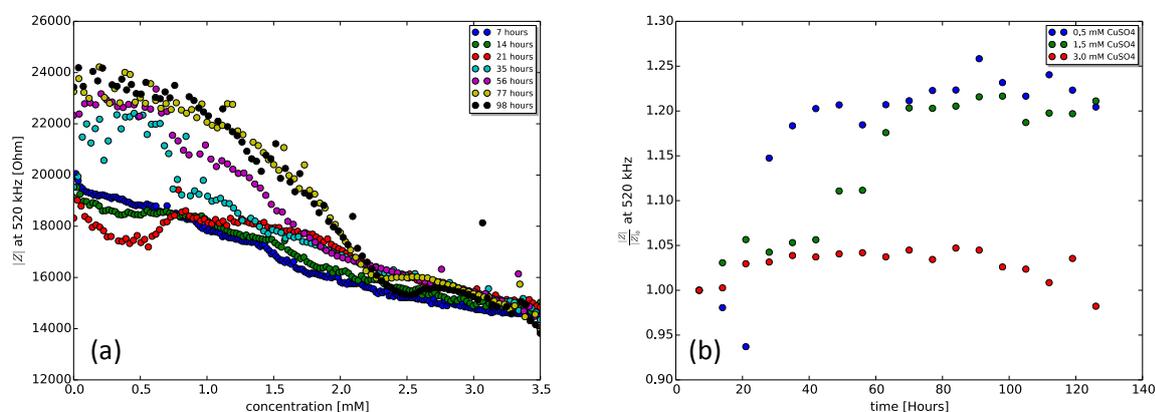
**Brian P. Cahill<sup>1</sup>, Jialan Cao<sup>2</sup>, Karippai Nobu<sup>1</sup>, Steffen Schneider<sup>2</sup>, Stefan Wiedemeier<sup>1</sup>, Thomas Nacke<sup>1</sup>, Gunter Gastrock<sup>1</sup>, J. Michael Köhler<sup>2</sup>**

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Toxicology is the scientific study of how chemicals adversely affect organisms. The use of microfluidic droplets to implement highly-resolved dose-response screening opens a new era of micro-toxicological studies. The response of the bacterium *Chromobacterium vaccinii* to the presence of controlled amounts of  $\text{CuSO}_4$  was measured using micro flow-through impedance spectroscopy. The growth of bacteria affects the resistive characteristics of the medium due to the metabolic conversion of nutrients into metabolites. Figure 1a shows three distinct areas: (i) At concentrations up to approximately 0.8 mM  $\text{CuSO}_4$ , the impedance initially decreases before increasing rapidly. (ii) Between 0.8 and 2.25 mM  $\text{CuSO}_4$ , the impedance increases slowly indicative of bacterial metabolism under stressed conditions. (iii) Above 2.25 mM  $\text{CuSO}_4$ , the impedance stays stable indicative of inhibited metabolism. Figure 1b shows bacterial growth kinetics at 3 different  $\text{CuSO}_4$  concentrations.

This work describes the development and use of an electrochemical impedance spectroscopy sensing unit developed by the authors for highly resolved dose response studies that analyze the metabolic behavior kinetics of bacteria. This facilitates high-throughput toxicological studies and the estimation of the effective concentration necessary to impede bacterial growth in a simple, miniaturized and robust platform.



**Fig. 1:** (a) Impedance modulus at 520 kHz plotted as a function of  $\text{CuSO}_4$  concentration. Each curve was measured at the time indicated in the legend. (b) Normalised impedance modulus at 520 kHz as a function of time. Each curve was measured at the  $\text{CuSO}_4$  concentration indicated in the legend.

## Novel transferrin immunosensor based on its paramagnetic properties

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Transferrin is a well-established biomarker in different human pathologies and the human recombinant transferrin is clinically applied in many diseases as thalassemia, ischemia-reperfusion injury, bacterial infections and diabetes. Therefore, it is suggested that a direct assay of this glycoprotein in the blood and cells could have a potential diagnostic value in the measurement of erythron activity and the body iron deposits.

Here we present a novel electrochemical immunosensor based on carbon-encapsulated iron nanoparticles (CEINs) decorated with monoclonal antibodies recognizing human transferrin (Tf) for detection of this glycoprotein in blood samples. The stable layer of CEINs containing carboxylic groups conjugated with the monoclonal antibody ensured good electric contact for direct electron exchange between transferrin and the electrode surface. The interaction of the immobilized antibody with transferrin (at various concentration) was monitored via various responses, i.e.: voltammetric (reduction current of Tf), gravimetric (amplitude of frequency oscillations) and impedance spectroscopy (charge transfer resistance). For the determination of transferrin in blood samples the best procedure is the gravimetric one that gave the highest sensitivity. The obtained results show excellent linear response in the Tf concentration range from  $5 \cdot 10^{-7}$  to  $5 \cdot 10^{-2}$  g·dL<sup>-1</sup> ( $5 \cdot 10^{-3}$  - 50  $\mu\text{g} \cdot \text{mL}^{-1}$ ) with the detection limit of ca.  $12.0 \pm 1.8$ ,  $15.0 \pm 2.4$  and  $24.0 \pm 5.2$  ng·dL<sup>-1</sup> for gravimetric, impedance and voltammetric analysis, respectively. The functionality of the electrochemical immunosensor has been demonstrated during the analysis of the Tf level in rat blood samples.

## Connecting Quantum Dots with enzymes: Mediator-based approaches for the light-directed detection of glucose and fructose

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During the last decade light-controlled devices have turned into the focus of research with respect to their analytical performance. This study reports on light-controlled detection schemes of sugars (glucose, fructose).

In the focus are CdSe/ZnS quantum dots (QDs) which can be attached to an electrode allowing light-induced charge carrier generation and subsequently electron transfer reactions between the electrode and the nanoparticles. The quantum dots can not only exchange electrons with the electrode, but can also interact with donor or acceptor compounds in solution, providing access to the construction of signal chains starting from an analyte molecule in solution and to a read-out by spatially focused illumination.<sup>1</sup> CdSe/ZnS QDs are chemically synthesized and immobilised via a dithiol linker on gold electrodes. Stable and well-defined photocurrents have been found over a wide potential range. This provides the basis for the combination of the QD electrodes with biochemical reactions. The functionality of the prepared QD electrodes have been investigated by using small redox molecules such as ferrocyanide and ferrocene carboxylic acid in solution resulting in a concentration-dependent increase of the photocurrent. This gives access to the construction of mediator based light-controllable signal chains. For example PQQ-dependent glucose dehydrogenase and fructose dehydrogenase have been coupled with QD electrodes for photoelectrochemical glucose and fructose detection. Finally, it is also shown that by covalent coupling of ferrocenecarboxylic acid to (PQQ)GDH conditions can be found to assemble the whole system on top of a QD electrode for biosensorial glucose detection.

These results provide the basis for light-switchable biosensing and bioelectronic applications, but also open the way for self-driven point-of-care systems by combination with solar cell approaches.

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## A ROBUST INDIUM-TIN-OXIDE PLATFORM FOR THE ELECTROCHEMICAL DETECTION OF *SALMONELLA*

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There is an increasing demand for sensitive, accurate and easy-to-use methods for the detection of pathogens in very diverse areas, such as clinical diagnosis, environmental monitoring and food safety. Among pathogens of interest, *Salmonella* spp constitute one of the major causes of foodborne diseases in humans worldwide. Monitoring the presence of *Salmonella* in food at all stages in the “farm to fork” production chain is the only way for preventing foodborne outbreaks. However, the long turnaround time of existing conventional standard culture methods for the detection of pathogens from food often leads to low productivity and reduced analytical flexibility of testing laboratories.

To overcome these drawbacks, we propose the use of an isothermal amplification method that allows the amplification of a DNA sequence, specific from the pathogen, takes place at a constant temperature. Therefore, in combination with hybridization assays, the development of simplified and miniaturized instrumentation at an effective-cost, most suited to applications in low-resource settings, can be envisioned. As a detection platform, Indium Tin Oxide (ITO) surfaces allow dual detection (optical and electrochemical) of the interest sequence.

To demonstrate the potential of indium-tin-oxide films as a platform for miniaturized DNA detection devices, herein we evaluated the thermal and the storage stability of the sensing phase designed for the electrochemical detection of a specific genome sequence of the *bipA* Gene of *Salmonella*. The amplification process is carried out with short oligonucleotides flanking this region, which act as primers. Next, amplicons are directly entrapped onto the modified ITO-electrode. To perform the electrochemical detection, we use a sandwich format with fluorescein-labeled reporter probe that interacts with anti-fluorescein-ALP conjugate. Enzyme transforms 1-naphthyl phosphate in 1-naphthol, which is detected by differential pulse voltammetry (DPV). The sensing phase can be stored without loss of sensitivity for over six months and is stable at high temperatures, so amplification process can be carried out on its surface. Currently work is focused on the implementation of the whole assay (target DNA amplification and detection) in an integrated platform.

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## Electrochemical biosensor platform for rapid antimicrobial resistance testing at point-of-care

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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, including at the United Nations General Assembly in September 2016. The recently published review on antimicrobial resistance chaired by Lord O'Neill has predicted that AMR will cause an additional 10 million deaths per year and a loss of up to US\$100 trillion from global GDP by 2050. Our ability to treat infections that once were believed to be under control is now at risk and a 'post-antibiotic era' is becoming a real possibility for the 21st century. Rapid diagnostic tests are urgently needed to enhance the rational use of antibiotics, tailor individual patient treatment and management in human and veterinary medicine, facilitate surveillance, allow the development and use of narrow spectrum antibiotics and improve drug-specific companion diagnostics.

Here, we report the successful development of an electrochemical biosensor platform based on electrochemical impedance spectroscopy (EIS) for label-free molecular diagnostics covering a wide range of targets from small molecules over proteins to different types of nucleic acids [1-3]. Nucleic acid targets which have been successfully detected with the EIS platform range from short artificial targets over PCR products directed against several antibiotic resistance genes to genomic DNA and intact 16S ribosomal RNA for direct amplification-free bacterial species identification. Besides EIS assays on gold screen-printed electrodes, recent work on the manufacturing, characterisation, and probe functionalisation of low-cost carbon screen-printed electrodes will also be presented [4]. These EIS based biosensors provide a highly suitable portfolio for the development of molecular diagnostic tests which can be performed at point-of-care.

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## Functionalized spore-based biosensor to evaluate gaseous sterilization processes

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Aseptic packaging has become a significant process for industrial packaging of medical devices, pharmaceuticals as well as food products. A key process within the aseptic process chain is represented by the package sterilization, in which vaporized hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) has become the sterilization medium of choice [1]. The sterilization efficiency is commonly evaluated by laborious microbiological challenge tests. Recently, a sensor has been introduced allowing a rapid process control [2]. The impedimetric sensor is designed to capture the modifications of immobilized resilient test microorganisms. The resulting sensor signal is correlated to the sterilization efficiency. The immobilization of bacterial spores has been enhanced by surface functionalization with organosilanes (e.g., APTES, GPTMS).

In the present study, *Bacillus atrophaeus* DSM 675 spores will be immobilized on functionalized biosensors. The interaction between hydrogen peroxide vapor and the surface functionalization will be characterized as well as the interaction towards the immobilized spores. However, the sterilization effect of vaporized hydrogen peroxide is not well understood, the sensor measurements will reveal details of the spore modification, *i.e.*, the impedance change is related to structural modifications of the resilient microorganisms, which attributed to changes in the conductivity and permittivity of the spores. The sensor-based evaluation will be supported by surface characterization with atomic force microscopy (AFM) and scanning electron microscopy (SEM).

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## Electroactive biofilms as recognition element for anaerobic digestion: Sensing of volatile fatty acids (VFAs)

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Volatile fatty acids (vfa) like acetate are highly process-relevant in anaerobic digestion (AD) and are measured off-line with gas- or liquid chromatography which is time consuming and cost intensive. To establish a real time process control especially for demand driven biogas production and increased process automation, we propose a microbial electrochemical sensor for on-line measurement of acetate and other vfa. The recognition element (receptor) of the sensor is based on a mixed species *Geobacter* sp. dominated anodic biofilm. The corresponding transducer consists of a carbon electrode that allows the implementation of different electrochemical measurement techniques like chronoamperometry and cyclic voltammetry.

The basic sensor parameters were characterized using a flow cell setup. The sensor is suitable for measuring acetate within a range of 0.5 – 5 mmol L<sup>-1</sup> with a measurement resolution of 0.25 – 1 mmol L<sup>-1</sup> acetate depending on the provided concentration and flow rate [1]. Proof of concept experiments in stirred AD reactors (10 L) revealed a biosensor signal similar to a typical intraday vfa concentration profile. The sensor showed cross sensitivity towards propionate and butyrate that can be described as a baseline sum signal (0.040 ± 0.008 mA cm<sup>-2</sup>) irrespective of the applied concentration [2]. The sensor also revealed biphasic response behavior towards dynamic changes in acetate concentration shown to be strongly dependent on prior exposure to low acetate concentrations [2]. Features that promote a future application of the biosensor, e.g. the low estimated costs as well as the self-sustainability of the living recognition element, will be critically discussed.

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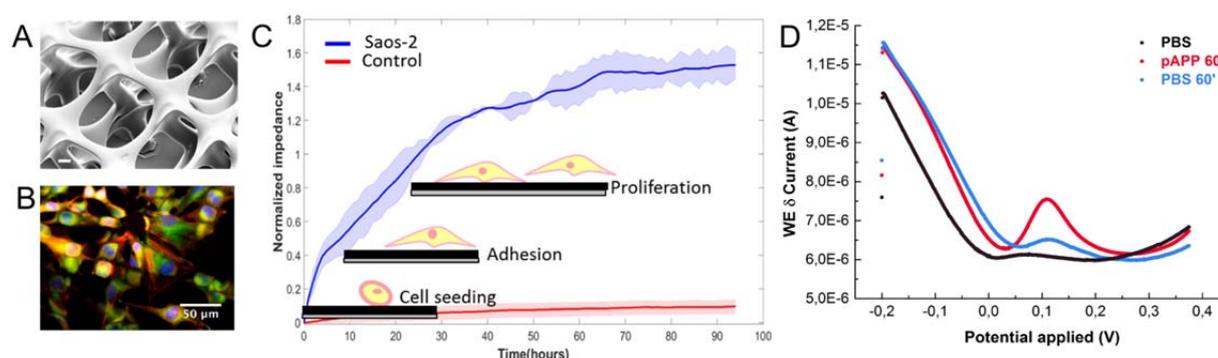
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## Three-dimensional sensing scaffold for bone cells studies

**Claudia Caviglia,<sup>1</sup> Suhith Hemanth,<sup>1</sup> Yasmin Mohamed Hassan,<sup>1</sup> Gaetano Panagia<sup>1</sup> and Stephan Sylvest Keller<sup>1</sup>**

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Conductive three-dimensional (3D) scaffolds offer the possibility for in-situ electrical sensing while providing a more in-vivo like microenvironment for real-time monitoring of cell population processes [1]. This study aims at developing a 3D in vitro sensing system for bone cell studies. 3D carbon electrodes (3dCEs) are used as both scaffold and sensor simultaneously [2] (Fig. 1A,B). The 3dCEs will provide: 1) optimal properties to support bone cell adhesion, migration and proliferation, e.g. stiffness, porosity and mass transport and 2) electric conductivity to electrochemically monitor in situ and real time bone cell fate and responses to external stimuli. The carbon electrodes have been designed and fabricated with the C-MEMS technique [3], where a patterned polymer template is heated to temperatures above 900 °C in an inert atmosphere to produce pyrolytic carbon electrodes. Impedance-based assays have been performed to monitor in real time Saos-2 cells adhesion, proliferation and differentiation (Fig. 1C). Moreover, an electrochemical assay for the quantification of the differentiation marker Alkaline Phosphatase (ALP) was implemented using square wave voltammetry (SWV) on the *in-vitro* cell culture. (Fig. 1D) Standard colorimetric assays to determine viability and ALP activity were performed in parallel under the same conditions.



*Fig. 1: A) SEM image of 3dCEs fabricated on top of the working electrode; B) confocal microscopy image of Saos-2 cultured onto the 3dCEs; C) impedance profile of Saos-2 cells grown on 2D carbon electrodes; D) SWVs on Saos-2 cells cultured on the 3dCEs. SWV were taken in PBS before (black curve) and after (blue curve) 60 minutes incubation with 5mM p- aminophenyl phosphate (pAPP) (red curve).*

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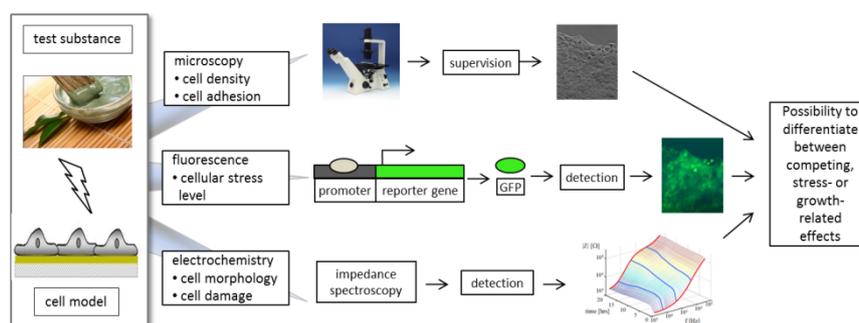
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## Towards 3D cell cultures as an alternative for irritation tests

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The development of a cell-based sensor chip for the investigation of physiological properties of a substance towards keratinocytes is reported. Our method differs from others via the innovative combination of optical and electrochemical detection sites on chip. This paper presents a new method for the complex description of cellular effects to investigate the toxic potential of substances to a human skin model in a real time, sensitive and high throughput manner (Figure 1). HaCaT keratinocytes were transfected with a green fluorescence protein (GFP) derived from a cytotoxic response promoter and serve as intelligent sensor cells. The used optical set up realizes the monitoring of cell culture as well as the fluorescence intensity ([1],[2]). The development of 3D cell cultures with matrigel scaffold and hepatocyte cell line even more increases the relevance of the sensor towards the human skin or the behavior of the human organ. This is clearly shown in experiments comparing the toxic behavior of nanoparticles in 2D- and 3D- environment [3,4].



*Figure 1: Complex description of cellular effects on the Lab-on-a-Chip via microscopical supervision of cell culture, detection of stress-induced GFP expression combined with electrochemical readout for quantification of cell morphology*

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## Dual Probe for biological dynamic processes

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A fluorescent probe structurally similar to the GFP chromophore (**Ge1**) is demonstrated to report at the same time on both the static dielectric constant ( $\epsilon$ ) and the viscosity ( $\eta$ ) of the local environment [1]. These two physicochemical properties are imaged by the probe in an *independent* way through two optical parameters (intensity and lifetime), thereby allowing for concentration-free universal calibrations (ratiometric approach). By means of time resolved measurements, we demonstrated that this photophysical behavior is due to the excited state decay pathway through two emissive states with similar dipole moments but different responses to the viscosity.

Such a probe straightforwardly yielded the polarity and the viscosity maps of several organelles in living cells [2] and showed to be easily bio-conjugable. **Ge1** has been functionalized with Cell Penetrating Peptides (CPP) engineered for drug delivery and helped revealing the interactions with the endosomal membrane during the internalization process [3].

Moreover, **Ge1** has been functionalized with a phospholipid moiety to obtain a membrane-targeted biosensor, named **Ge1L**. The lipid-assisted membrane insertion strongly reduces the rotational degrees of the fluorescent unit, which becomes very sensitive to the local liquid crystalline order. More specifically, **Ge1L** exhibit different lifetimes in liquid ordered ( $L_o$ ) and disordered ( $L_d$ ) phases of the bilayer, and the phase quantification can be simply accomplished by the phasor analysis. **Ge1L** revealed to be a useful tool to monitor the dynamic membrane remodeling after the activation of a ionic channel.

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## Multiplexed targeting of cells and tissue – novel surface-modulated upconversion nanoparticles for biosensing and -imaging

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Optical techniques for in-vitro and in-vivo analysis of biological systems (e.g. tissue or blood) are challenged by the complexity of the sample matrix. Major issues of optical techniques are the strong absorption and scattering properties of biological samples. These limiting factors can be discriminated by the use of Lanthanide-based frequency upconverting nanoparticles (UCNP). The UCNPs allow to address the bio-optical window of tissues by using an excitation at  $800 \text{ nm} < \lambda_{\text{ex}} < 980 \text{ nm}$  and emission at  $600 \text{ nm} < \lambda_{\text{em}} < 850 \text{ nm}$ . In this bio-optical window a high light penetration depth can be realized. The luminescence kinetics of Ln(III)-ions range in the millisecond timescale. Accordingly, autofluorescence of biological probes can be discriminated by time-gated detection. Additionally, the UCNPs show a low cytotoxicity and high photostability in comparison to organic dyes and quantum dots.

The UCNPs are surface-modified to obtain water solubility and to equip the tracers (UCNPs) with specific recognition elements enabling bioanalysis and -imaging under physiological conditions. The surface modification aims at sensing applications and is tailored like a “tool box” granting a variable detection system for e.g. non-invasive temperature mapping in cells or cancer targeting and therapy. The synthesis of UCNPs has advanced to a mature state, but the generation of stable water soluble UCNP with high biocompatibility and flexible surface functionalization needs further development. The phase transfer is realized by interlinked polymers to avoid ligand exchange reactions and to encapsulate natively hydrophobic UCNPs.

The first results on the road to encapsulated UCNPs with multiple surface modifications for the integration of biological relevant target molecules are shown. As detection key parameters, the optical properties of the unmodified UCNPs and the modified UCNPs foreseen to be used in biological applications are presented and the influence such as of different surface modifications is discussed.

## Tuning the Potential of Whole-Cell Impedance Assays to Assess the Activity of Pathway-Biased GPCR Ligands

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Whole-cell impedance measurements have evolved as a valuable tool to assess the activity of ligands for G-protein coupled receptors (GPCRs), which belong to the most important drug targets in the treatment of a multitude of human diseases.

Depending on the cell type and the receptor's conformation(s) stabilized by the ligand, GPCRs can couple to different downstream pathways. Recognizing this concept has become extremely important for the development of effective, low side-effect drugs and explains the increasing popularity of label-free, so-called holistic assays [1]. Label-free impedance assays measure the integrated response to all activated signaling pathway(s) that evoke changes in cell morphology, instead of quantifying only one pathway-specific event as obtained by most biochemical assays (e.g. Ca<sup>2+</sup>, cAMP). In addition, impedance assays are capable of detecting GPCR activity at endogenous receptor levels of non-transfected and primary cells in a non-invasive manner with high temporal resolution.

However, impedance response profiles to GPCR agonists are not well enough understood yet to assign general rules for identifying respective pathway activities, to unravel the time-dependent sequence of signaling events or to quantify their individual contributions. A deconvolution of such impedance signals not only asks for the use of signaling pathway modulators and supplementary biochemical assays, but first of all requires substantiated understanding of the very basic experimental parameters that influence signal profile and intensity of the assay.

Our Electric Cell-Substrate Impedance Sensing (ECIS) [2] data show that cell type-specific morphological parameters significantly influence the impedance signal upon GPCR stimulation. Signal direction and magnitude depend on the signaling pathway being activated as well as on the cell type. We also illustrate how proper selection of experimental parameters, including monitoring frequency, can improve assay stability and sensitivity as well as data interpretation [3].

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## Unique Micro- and Nano-Patterns of Gold Nanoparticles on PEG-based Hydrogels

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One of our research priorities concern the micro- and nano-patterning of biomaterials. We have fabricated micropatterns of chemistry, topography and elasticity on poly(ethylene glycol) (PEG) hydrogels to control cell adhesion and migration. Recently, we have also discovered that the presence of gold nanoparticles (Au NPs) at the surface of intrinsically anti-adhesive PEG-based hydrogels render the biointerfaces remarkably cell adhesive.

Besides controlling cellular behavior at biointerfaces, our unique nanocomposites of Au NPs and PEG-hydrogels have demonstrated to be exceptionally useful for Surface Enhanced Raman Spectroscopy (SERS). The signal enhancement emanating from probe molecules bound to Au NPs on these interfaces is one or more orders of magnitude larger than from the similar structures on solid substrates such as silicon.

In this contribution, several, versatile micro- and nano-patterning strategies of Au NPs and PEG-hydrogels will be reviewed. The cell-adhesive properties of Au NPs-PEG hydrogel nanocomposites will be highlighted and the remarkable SERS-ability will be discussed in view of (single protein or cell) biosensing applications.

## Development of enzyme-based microsensors for *ex vivo* analyses

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Francesco Paolucci,<sup>1</sup> and Alice Soldà<sup>1</sup>

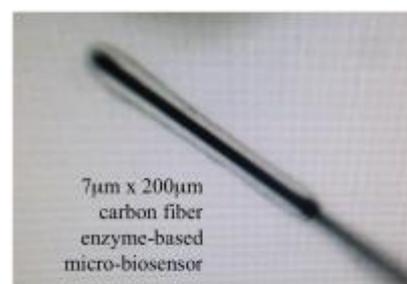
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Nowadays real-time quantitative measurements of many proteins, metabolites and cancer biomarkers, are feasible by the use of biosensors for *in vitro* and *in/ex vivo* studies [1]. Although, strategies on biosensor design are rapidly increasing, enzymatic biosensors remain a large and important field of study. Enzymes achieve molecular recognition of the substrate based on structural complementarity and exploit an analyte-dependent step to convert an electrochemically inert substrate to an active product, often revealing high selectivity, sensitivity, time scale and information content. Even if, amperometric biosensors have been around since the early 1960s [2-3], it is the production of very tiny micro-electrode biosensors that offers great utility for studying chemical signalling for *in vitro* (at single cell level) and *in/ex vivo* studies. These microelectrode biosensors have the advantage of offering a better spatial and temporal resolution and they are considerably less invasive than other electrode (i.e. microdialysis electrodes) [4]. The miniaturization of biosensors is challenging, as they need to be both extremely small and highly sensitive. In fact, smaller is the sensor, smaller is the electrode surface area, and consequently the recorded amperometric signal diminishes [1]. The requirement in miniaturizing biosensors is to maintain a signal to noise ratio that enables effective and sensitive detection of the analyte. The tiny sensing surface of the sensor has to be coated with a high density of enzymes in their native active conformation. Furthermore, for *ex vivo* analyses, it is mandatory to control the selectivity of the signal and the mechanical strength of the sensor itself, which has to enter in tissues without damaging.

*In this work, we present the development of small, robust and highly active micro-electrode enzymatic biosensors for detecting metabolites, such as glucose, lactate and ATP from single living cells to tissues analysis.*



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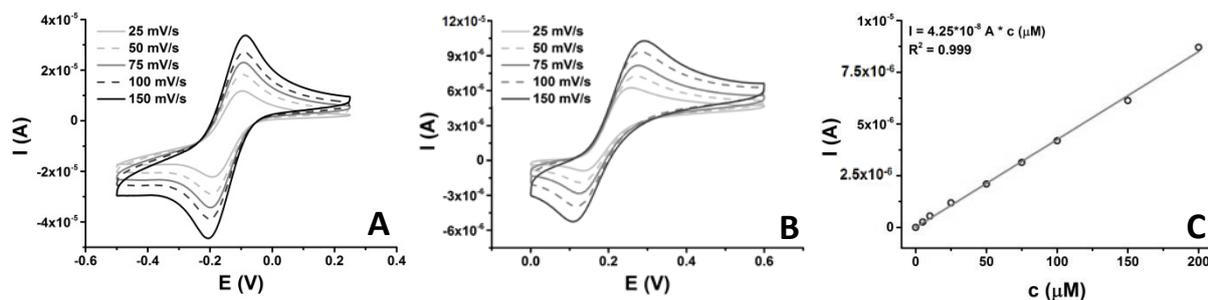
## Carbon coated optical fibre for dopamine detection from cells

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Our group is working on the development of a carbon coated optical waveguide as a potential future type of optoelectric implant for patients with e.g. Parkinson's disease. The device would allow, at the same time, controlled release of dopamine from optogenetically modified cells – upon selective light stimulation – and electrochemical monitoring of local dopamine concentrations released, in a direct feedback loop.

We have previously shown that pyrolytic carbon enhances the differentiation of human neural stem cells into dopaminergic neurons [1]. Here, we convert the polyimide coating of a quartz-based optical fibre into pyrolytic carbon to obtain a combined optical waveguide and electrode. Our preliminary test shows no changes in the optical properties of the fibre after pyrolysis. The carbon fibre electrodes show good reproducibility and electrochemical behaviour when tested with standard redox couples ( $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$  and  $[Ru(NH_3)_6]Cl_2/[Ru(NH_3)_6]Cl_3$ ) (Fig. 1A) and dopamine (Fig. 1B). The peak current for dopamine oxidation shows a linear response up to 200  $\mu M$  with a limit of detection below 5  $\mu M$  (Fig. 1C). Thus, the electrodes can potentially be used as cell culture substrates and to detect dopamine released from dopaminergic neurons grown on the carbon surface.



**Fig. 1: Electrochemistry measurements: Cyclic voltammograms at different scan rates obtained with 1 mM  $[Ru(NH_3)_6]Cl_2/[Ru(NH_3)_6]Cl_3$  (A) and 200  $\mu M$  dopamine (B). Calibration curve for dopamine detection using the intensity of the oxidation peak from cyclic voltammetry (C). All measurements used Ag|AgCl as reference electrode and a Pt wire as counter electrode.**

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\* The device is currently being patented, application number EP16176841

## New strategies for dehydrogenase biosensors based on nanostructured polyelectrolytes composite materials

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One of the most studied redox system in biological systems is based on nicotinamide adenine dinucleotide that serves as coenzyme for more than 300 enzyme reactions catalyzed by dehydrogenases. Electrochemical detection of the reduced form of this coenzyme (NADH) represents the key point in developing the dehydrogenase-based biosensors. Direct oxidation of NADH at the surface of classic electrodes requires a high overpotential and it is followed by the fouling of the electrode surface. Polyelectrolytes have proved to be efficient in detection of NADH [1] and represent a very convenient alternative for dispersing other electrode materials such as carbon nanomaterials and/or metal nanoparticles. Reduced graphene oxide has a good conductivity, a large specific area but it has a reduced dispersability due to a reduced amount of oxygen functionalities. Noble metal nanoparticles are of a great interest for electrochemical applications due to their excellent conductivity and high specific area. Polyelectrolytes can be a very attractive way to prepare stable and homogenous composite materials based on RGO and metal nanoparticles [2, 3].

This work reports the use of poly(allylamine) (PAH), electrochemically reduced graphene oxide (ERGO) and gold nanoparticles (AuNPs) for preparing composite materials used in NADH sensing. PAH was successfully used for dispersion of graphene oxide and act as a stabilizer of the ERGO and AuNPs at the surface of working electrode. Raman spectroscopy allowed us to characterize the interactions between the components while SEM revealed the specific morphology of the composites.

Electrochemical studies confirmed an enhanced electrocatalytical effect of the ERGO-PAH and AuNPs-ERGO-PAH at a lower overvoltage with high sensitivity. A specific sensitivity of  $131.0 \pm 1.2 \mu\text{A}/\text{mM}\cdot\text{cm}^2$  was achieved for an electrode modified with AuNPs-ERGO-PAH with a detection limit of  $3 \mu\text{M}$  NADH. The analytical performances of the nanostructured polymer composite NADH sensors represent a promising starting point for development of dehydrogenase-based biosensors.

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## Direct electrochemical detection of waterborne pathogen's 16S rRNA using thioaromatic-based oligonucleotide monolayers

**R. Miranda-Castro, R. Sánchez-Salcedo, B. Suárez-Álvarez, N. de-los-Santos-Álvarez, A. J. Miranda-Ordieres, M. J. Lobo-Castañón**

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Genosensing technology has mostly based on mixed self-assembled monolayers (SAMs) of thiol-modified oligonucleotides and alkanethiols on gold surfaces [1]. However, the typical backfilling approach, which incorporates the alkanethiol in a second step, gives rise to a heterogeneous distribution of oligonucleotide probes on the surface, negatively affecting to both hybridization efficiency and sensing surface stability [2].

Herein, we have compared different approaches involving SAMs of aromatic thiols (*p*-mercaptobenzoic acid and *p*-aminothiophenol) to yield DNA sensing layers for sequence-specific detection of target oligonucleotides. These monolayers were evaluated, through a sandwich-like format assay, with a target-containing solution in the presence of a signaling sequence (fluorescein-modified oligonucleotide). After surface hybridization, the binding of anti-fluorescein Fab fragment conjugated to peroxidase permits the generation of the redox reporter (oxidized tetramethylbenzidine), which produces a readily measurable chronoamperometric signal.

The developed platforms exhibit increased sensitivity with a detection limit lower than 50 pM and storage stability of months. Likewise, they are suitable for targeting native 16S rRNA of *Legionella pneumophila* from cellular lysates, enabling the detection of the pathogenic microorganism without previous amplification [3].

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## Nanosized polymeric films for design of advanced biosensor systems

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Nanosized polymer films can be constructed via alternative electrostatic adsorption of polyelectrolytes. The adsorption is to a great extent determined by the state and structure of initial surface and/or the pre-adsorbed layers. The same rule is apparently valid for electrostatic adsorption of biomolecules used for their immobilization. This is specifically important for a design and fabrication of bioanalytical devices, e.g., biosensors, where the amount of biomolecules incorporated and the strength of its binding influence on basic characteristics, such as activity and operation stability, of these bioanalytical surfaces. To demonstrate this, different fabrication regimes and properties of polymer/biomolecule thin films adsorbed onto conductive substrates were examined. The films were formed via two-steps, sequential adsorption of a polymeric component followed by the biomolecule adsorption under different pH/salt composition and temperature regimes. Strong/weak linear polyelectrolytes, amphiphilic ionogenic diblock copolymers or microgels were considered as polymeric components to be interacted with biomolecules, like enzymes or hemeproteins. Monoenzymatic systems with uniformly distributed biomaterial or bienzymatic systems with spatially separated enzymes are discussed. Specific applications to the field of biosensors are discussed.

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## Silicon nanowire biosensor platform to electronically sense biomolecules in physiological buffer concentration

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Silicon field-effect transistors with dimensions from micro- to nanoscale are widely used in the field of biosensors. Since the first introduction of ion-sensitive field-effect transistors (ISFETs) [1], they have been applied to many different applications in the field of biosensors. Very often the assays utilized enzymatic reactions. In these cases secondary responses like surface-near pH changes upon enzymatic conversion of analyte molecules were recorded. In general, the pH-sensitivity is the primary response of ISFETs, since the typically-used gate oxides are sensitive to protonation and de-protonation. The standard transducer principle for detection of biomolecules with ISFETs is potentiometry, where changes in surface potential at the liquid-solid interface are sensed and related to changes in source-drain currents of the devices. In affinity-based binding assays this method is preferentially used to detect small, charged biomolecules.

In the last decade various kinds of nanowire sensors were introduced – most of the time silicon nanowires (SiNWs). In many of the articles in this field, conductance changes of the wires upon biomolecule binding were displayed. In many publications remarkable sensitivities were reported. However, the potentiometric readout principle is highly prone to side effects originating from temperature, pH and ionic strength changes in the test liquid.

In our project we aim to combine various sensor inputs to calibrate and compensate for these side effects and to work out the real concentration of analyte. As a first step we combine potentiometric and impedimetric readout [2] for our SiNW platform. It was described earlier that the impedimetric approach at elevated frequencies offers a sensing region, which is further extended into the electrolyte solution as compared to the potentiometric readout [3, 4].

Our sensor devices contain in addition pH reference channels and structures for a temperature sensor. For precise readout of the biomolecular binding events with SiNW sensors, the methods of potentiometry and impedimetry can be combined. Both readout principles can be combined in a multivariate data analysis scheme to extract the biomolecule concentrations of a test assay. Biomolecules of different size such as DNA and proteins can be distinguished and detected by our approach.

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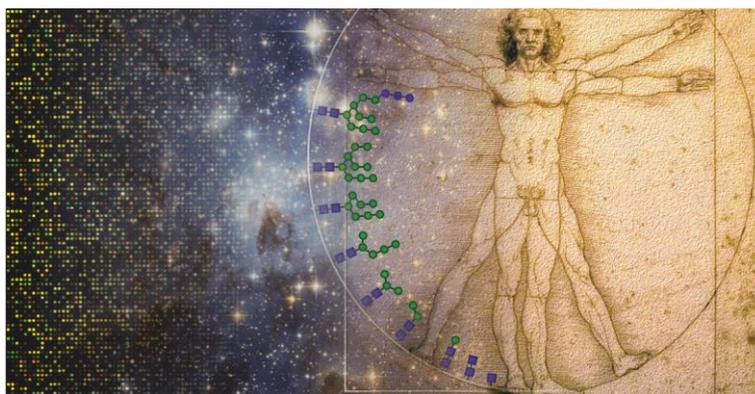
## Nanoscale controlled architecture of biosensors for glycan recognition applied in diagnostics

**Jan Tkáč, Tomáš Bertók, Ľudmila Kluková, András Hushegyi, Dominika Pihíková, Štefan Belický**

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Glycomics as a hot research field emerged to complement enormous effort in the field of genomics and proteomics to better understand pathological processes and to provide novel diagnostic tools for early stage identification of diseases. Glycans (complex carbohydrates) are attached to 70-80% of proteins in a human body and this is why analysis of protein-linked glycans is of high priority to fulfil this aim. Current state-of-the-art tool for glycan analysis is mass spectrometry (MS) combined with chromatographic and/or electrophoretic instrumentation. MS analysis of some glycan changes associated with cancer is quite challenging due to long analysis time, extensive sample pre-treatment/ derivatization and data interpretation is required by skilled operators.

This is why advanced analytical approaches are needed for advancements in the field of glycomics and diagnostics [1]. In this presentation we show that impedimetric biosensors constructed with interfacial layers controlled at nanoscale can detect glycans down to a single molecule level (i.e. aM level) [2]. The biosensors were extensively optimised in a way to resist non-specific interactions for analysis of complex samples i.e. serum from patients having various diseases (rheumatoid arthritis, systemic sclerosis, prostate cancer, leukaemia), lysates of isolated human cells (leukaemia) and even with intact leukemic cell lines and flu viruses.



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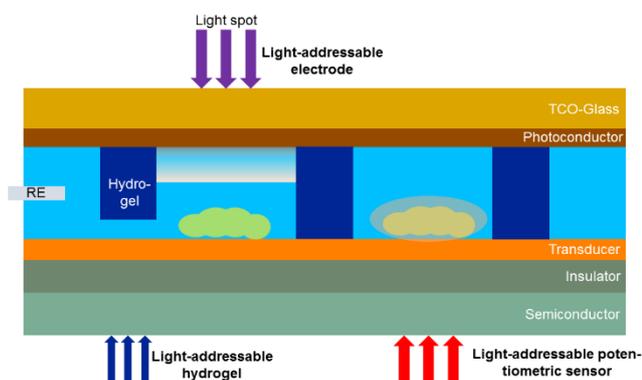
## Light-addressable lab-on-a-chip-based analysis platform

**R. Welden<sup>1</sup>, L. Breuer<sup>1</sup>, B. Schneider<sup>1</sup>, M.J. Schöning<sup>1,2</sup>, T. Wagner<sup>1,2</sup>**

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A lab-on-a-chip system with integrated microfluidics represents a miniaturized measurement system. Current products are often relatively inflexible and cost-intensive in manufacturing, due to the rigid assembly of integrated sensors and actuators. Recently, a number of technologies were introduced where specific functions can be activated or deactivated by means of a light beam. Therefore, the aim of this work is the development of an analysis platform based on a lab-on-a-chip system, which combines synergetically different light-addressable technologies. A possible light-addressable lab-on-a-chip setup is shown in Figure 1. Measurement chambers can be formed by light-addressable hydrogel-based valves. When the hydrogel is illuminated by a specific wavelength, it will collapse and open different microfluidic pathways [1]. In addition, the cell activity can be observed by a light-addressable potentiometric sensor, for example, through the determination of the pH value change [2]. A light-addressable electrode enables the manipulation inside a measurement chamber (e.g., changes of the pH value of the medium by means of electrolysis) [3]. Furthermore, light-sensitive cells could be used to trigger certain metabolic pathways. Due to the fact that all of these technologies can be activated by a programmable light source, they function spatial-independent. A system based on those technologies would show a high flexibility and low production costs.



*Fig. 1: Different possibilities for light-addressable technologies in a lab-on-a-chip system, for e.g. light-addressable hydrogels as micro valves, light-addressable electrodes for manipulation of the system and light-addressable potentiometric sensors for the detection of the metabolic activity of cells.*

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## Effect of the molybdenum coordination sphere in TMAO reductase revealed by direct bioelectrocatalysis

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TMAO reductase from *E. coli* is a mononuclear molybdenum enzyme with bis-molybdopterin guanine dinucleotide cofactor (bis-MGD) as its only prosthetic group. It is involved in the catalysis of several N-oxides, with the main substrate being trimethylamine-N-oxide (TMAO)[1]. Under anaerobic conditions TMAO reductase reduces TMAO to trimethylamine (TMA) which causes the characteristic fishy smell of stale fish [2]. From the crystal structure of homologues TMAO reductase from *S. massilia* it was determined that the coordination sphere of the molybdenum contained four dithiolene sulfurs, one serine-oxygen ligand and a terminal oxygen ligand[3]. With an *in vitro* reconstitution assay, the apoTMAO reductase was reconstituted with *in vitro* synthesized bis-MGD and different variants with changed molybdenum coordination sphere were produced in contrast to the as-isolated wild type enzyme.

To study whether the changes in the molybdenum first ligation sphere has an impact on the potential of the active center, first the direct bioelectrocatalysis of TMAO as a substrate was tested. For this purpose, the reconstituted TMAO reductases were immobilized on a carbon electrode surface, embedded in a surfactant film. Even though we could not acquire a direct non-catalytic signal from the active center, the direct bioelectrocatalysis was still possible and we could follow potential shifts as the ligation of the molybdenum was changing. The on-set potential was shifted to different values opposite to the as-isolated wild type TMAO reductase. The successful communication of the immobilized enzyme with the electrode surface and the possibility to follow ligation dependent shifts in the on-set potential would allow to test the bioelectrocatalysis of several different N-oxides, as substrates by the immobilized enzyme. Comparison between the bioelectrocatalytic activities of the different reconstituted TMAO reductase towards different substrates as compared to wild type enzyme would shed more light on the effect of the first sphere ligation in the active site.

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## Impact of Shearing and Drying on the Catalytic Activity of Enzyme for Biosensor Applications

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Biosensors offer a unique operating system, the so called “induced-fit” concept, which might constitute a key to innovative and pioneering technologies. The integration of a bio-active receptor molecule into a sensor allows the quantification of a specific substance. Biosensors are featured by good selectivity, high sensitivity, uncomplicated handling, and easy sample preparation.

However, their industrial scale production remains a great challenge. Various parameters have a significant impact on the functionality of the biosensor during the coating and drying process, thereby easily leading to a loss of the catalytic activity of the enzyme, and thus the main sensing component. When preparing biosensors by film coating from the liquid phase, these factors range from the formulation step, in which composition, pH, and shearing play an important role, to the coating and drying step, which is characterized by the employment of elevated temperatures or additives that are used to improve coating results.

In particular, this research addresses the impact of shear forces that induce mechanical stress on the structure of the enzyme and occur during biosensor-ink processing, as well as the influence of drying, a mandatory step when manufacturing biosensor coatings. Therefore, the catalytic activity of a highly active enzyme, FAD-dependent glucose dehydrogenase, was assessed in solution and coated films by a procedure especially developed for this purpose – with regard to the mentioned impacts. The obtained results are put in context with the coating process and the main challenges will be pointed out when processing biosensors.

### **Acknowledgement:**

This project is part of the research network “Molecular Interaction Engineering“ of the Helmholtz Association and is financially supported by the Federal Ministry of Education and Research (contract number 031A095B).

## Mediated enzyme electrodes for electrochemical biosensing at low overpotentials.

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Mediated enzyme electrodes comprise an oxidoreductase enzyme(s) 'wired' at an electrode surface using a redox mediator [1]. The redox mediator, normally a small artificial electroactive molecule, penetrates the enzyme structure and electrically connects the normally insulated enzyme co-factor to the electrode via an electron hopping ET mechanism. Polarisation of the electrode in the presence of an analyte, i.e. a substrate of the enzyme, generates a concentration dependent current. However, a large potential difference between the redox mediator and the enzyme, whilst providing a favorable thermodynamic gradient for electron transfer, requires a large overpotential to generate a detectable signal, which may drive additional, interfering, reactions in a complex sample matrix. Large overpotentials can be minimized by utilising redox mediators whose redox potentials are close to that of the enzyme co-factor whilst maintaining a high catalytic turnover of substrate. Here, we present a theoretical approach, based on Density Functional Theory (DFT), for the rational design of metal complexes that possess desirable electrochemical characteristics to act as redox mediators for specific enzymes, such as glucose oxidase. Specifically we report on the performance of enzyme electrodes incorporating several novel osmium bipyridyl complexes, designed using this approach, which display promising catalytic properties. For example, an enzyme electrode consisting of laccase co-immobilised with one such complex displayed an onset potential of ca. +0.5 V vs. Ag/AgCl for the electrocatalytic reduction of oxygen at pH 5. This presentation will describe how this approach may be extended to aid the design of other mediated enzyme electrode based biosensors.

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## Semi-covalent imprinting for selective protein sensing at a femtomolar concentration level

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A. Kuhn,<sup>2,\*</sup> F. D'Souza,<sup>3,\*</sup> and W. Kutner<sup>1,4,\*</sup>**

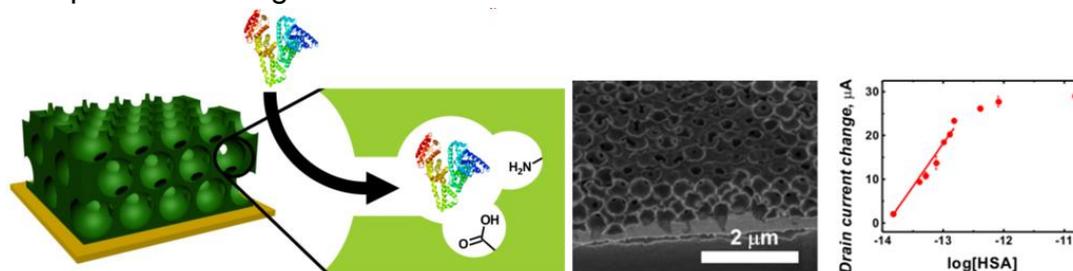
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Protein imprinting is challenging mainly because of their large size and conformation susceptibility to experimental conditions. In case of non-covalent imprinting, it is very difficult to estimate which and how many sites on surface of the protein template molecule are accessible for binding. To overcome this drawback, we introduced semi-covalent protein imprinting.[1] That is, we devised and fabricated a conducting molecularly imprinted polymer (MIP) based on bis(2,2'-bithien-5-yl)methane for human serum albumin (HSA) determination. A very high imprinting factor (IF > 20) and selectivity of the devised chemosensor proved that we the MIP featured molecular cavities of well-defined structure and high affinity to HSA. This success encouraged us to improve this approach even further. For that, we prepared a new artificial receptor material in the form of a thin macroporous MIP film with an unprecedented combined hierarchical nanostructure, used as the template, at three different size scale levels.[2] Introduction of this nanostructure resulted in extraordinary properties of this material. That is, its very high selectivity was accompanied by high sensitivity and detectability at an impressive femtomolar concentration level. These molecular recognition parameters were among the best reported in literature not only for MIP chemosensors but in the field of bio- and chemosensors in general. In order to achieve this high recognition performance, we have simultaneously applied a combination of three different approaches, namely protein semi-covalent imprinting, surface imprinting, and inverse opals structuring.



*Fig. 1: Macroporous MIP-HSA film for EG-FET determination of HSA.*

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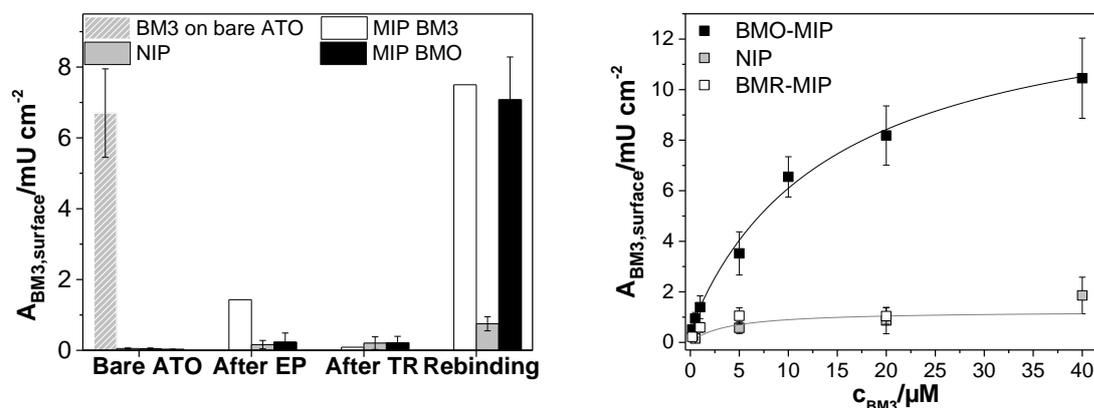
## Subunit-Imprinting of the Multidomain Cytochrome P450 BM3

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Cytochromes P450 monooxygenases use two electrons from NAD(P)H for insertion of one atom from atmospheric dioxygen into inert C-H bonds while the second is being reduced to water [1]. As a multi-domain self-sufficient electron transport system the flavocytochrome P450 BM3 is an ideal model for the general study of P450 systems [2]. Molecularly imprinted polymers (MIPs) are artificial receptor materials that are prepared by polymerization of functional monomers in the presence of a template molecule and subsequent removal of it, resulting in cavities that are complementary to the template in shape, size and functionality [3].

Uniform binding cavities for BM3 in the MIP were created by oriented immobilization of the oxygenase domain (BMO) template prior to electrochemical poly-scopoletin MIP formation. This was achieved by the interaction of their N-terminal His<sub>6</sub>-tags with antimony doped tin oxide (ATO) covered transparent electrodes [4].



**Figure 1: Left** - Characterization of the MIPs for BM3 and BMO as well as the NIP by NADPH-dependent reduction of cytochrome *c* (change in absorbance at 550 nm) after electropolymerization (EP), template removal (TR) and rebinding of BM3. **Right** - Concentration dependent rebinding of BM3 to a BMO-MIP and NIP as well as to a BMR-imprinted MIP film.

Removal and rebinding were followed by enzymatic activity (see figure 1) as well as by ferricyanide-permeation. Concentration dependent BM3-rebinding showed 6 times higher surface activity on the BMO-MIP as on the on the BMR-MIP or the non-imprinted control polymer (NIP). The MIP acts as a shape specific filter, which suppresses BM3-rebinding via the His<sub>6</sub>-tag to the BMR-MIP covered electrode. The role of the affinity tag for the recognition was evaluated in rebinding experiments by application of the holo-enzyme subsequent to thrombin digestion.

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## Self-assembled functional DNA Nanostructures as intracellular biosensors in single live human cells

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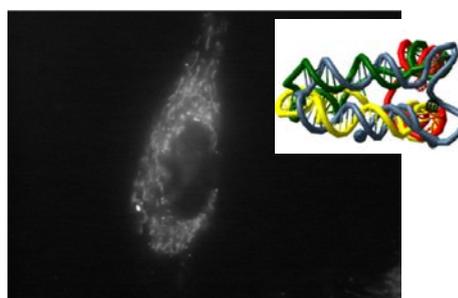
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In the last decade, *in vivo* studies have revealed that even subtle differences in size, concentration of components, or cell-cycle stage, make genetically-identical cells in a population respond differently to the same stimulus. In order to characterize such complexity of behavior and shed more light on the functioning and communication amongst cells, researchers are developing strategies to study single live cells in a population.

Self-assembled DNA nanostructures can be introduced in live cells, even without the aid of transfecting agents or by altering the membrane permeability otherwise. Recently, we have worked on the methods to design and prepare DNA-based fluorescent tetrahedral nanostructures, to deliver them to live cells and characterize such cells with fluorescence microscopy [3]. We have designed two types of sensitive DNA nanostructures (nanobiosensors). These are variation on the DNA tetrahedron motif introduced by the Turberfield group. Sensing of the desired target leads to a significant conformational change in the nanostructure that can be visualized (in solution or in the cells) via the spectroscopic properties of a FRET pair.

For example, by exploiting and tuning the properties of an intramolecular CT-motif triple helix, we designed and tested a nanostructure that can undergo a conformational transition driven by a change in pH, in a physiologically-relevant range. We showed the functioning of such functional nanostructures and their internalization in live cultured cells.



*Fig. 1: a molecular model of a DNA tetrahedral nanostructure in a compact state, after analyte recognition and a live glioblastoma cell with internalized nanostructures*

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## ***N*-nitrosamine toxin determination in processed meat using a conducting thiosalenCo(III) molecularly imprinted polymer (MIP)**

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Hazardous carcinogenic *N*-nitrosamines (NAs) contaminate several processed meat food products [1]. Herein, we propose their selective determination using a chemosensor with an organometallic molecularly imprinted polymer, MIP, [2,3] as the recognition unit, in order to bring this determination into a highly efficient, sensitive, and cost-effective level. This unit was prepared by electropolymerization of a thiosalenCo(III) complex (CS) in the presence of NA. Interactions between different NAs and CS were examined by UV-vis spectroscopy, and then stability constants of the resulting complexes determined. These constants directly correlated with free Gibbs energy gain corresponding to formation of these complexes, calculated using the density functional theory at the B3LYP/3-21G(\*) level. We confirmed the presence and absence of the NA template in the MIP film with FTIR and XPS, and characterized the film morphology with AFM. In order to demonstrate the MIP chemosensor applicability, we determined *N*-nitroso-thiazolidine-4-carboxylic acid (NT4A) in extracted smoked ham samples by using DPV and EIS. The chemosensor sensitivity and limit of detection was 7.05  $\mu\text{A } \mu\text{M}^{-1}$  and 46 nM (23.1  $\mu\text{g/kg}$ ), respectively. The latter is comparable to that reported using the HPLC method (18.4  $\mu\text{g/kg}$ ) [4].

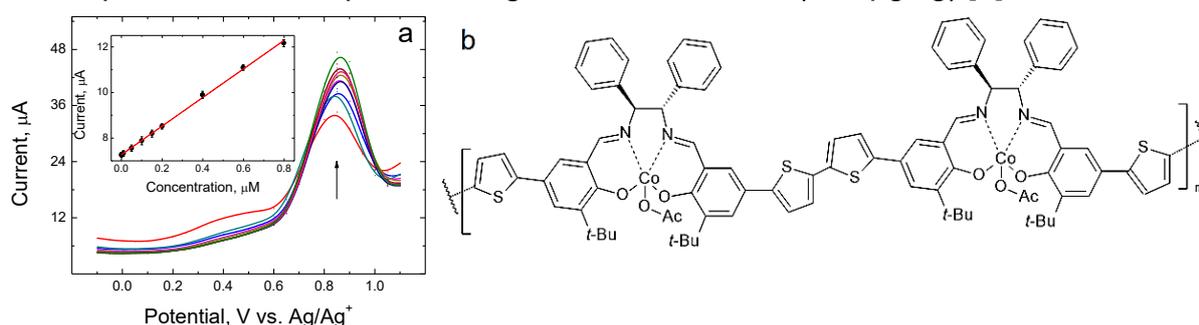


Fig 1. (a) DPV curves for 0.1 M (nBu<sub>4</sub>N)BF<sub>4</sub> of acetonitrile extracts of smoked ham, spiked with (1) 0, (2) 0.002, (3) 0.001, (4) 0.052, (5) 0.104, (6) 0.156, (7) 0.208, (8) 0.416, (9) 0.624 and (10) 0.833  $\mu\text{M}$  NT4A. Inset is the calibration plot of the normalized DPV peak current against the NT4A concentration. (b) Structural formula of the CS.

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## Biorecognition for Selective Biosensing of Intracellular Biomolecules

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Cellular functional biomolecules have been regarded as attractive targets for biomedical research, molecular diagnostics and disease therapy. Our recent efforts have been devoted to in situ analysis and highly selective detection of various cellular functional biomolecules and precise near-infrared cancer therapy. This talk will introduce our research results in bioimaging of cellular functional biomolecules, including electrochemical, chemiluminescent, scanometric, fluorescence, Raman and mass spectroscopic imaging for detection and in situ analysis of these molecules, such as glycans and protein-specific glycans on living cell surface [1], intracellular microRNA [2], sialyltransferase [3], telomerase [4], ATP and caspases [5]. Some nanoprobe designed for real-time targeted imaging and precise near-infrared therapy against cancer are also discussed [6]. The MALDI-MS patterning of caspase activities and its potential for drug resistance evaluation will be reported [7]. A recent work designs a structure switchable “lock-smart key” for cell-subtype specific siRNA delivery [8].

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## In-line monitoring of antibiotics in fermentation processes with a biomimetic optical sensor

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Fermentation processes as biotechnological method for industrial production processes are an expanding economic sector and have already found a variety of applications in the industry. Examples for such fermentation processes are production of vaccines, fine chemicals, drugs, and additives like amino acids and vitamins. In order to obtain optimum product yields, process parameters have to be monitored online and adjusted accordingly. To achieve this, a precise analytics of process-relevant parameters is a precondition. A distinction is made between off-line, at-line, and in-line process analytics [1].

Within the project BioMIP, an optical sensor for in-line process analysis within fermentation processes will be developed. The aim is to determine the concentration of products like penicillin G directly within a fermenter. The requirements of such a sensor system are very high. Because of aggressive and complex sample medium, the corresponding sensor has to show a high degree of robustness, has to be sterilizable, and has to be relatively inert to matrix effects of the fermentation medium. To achieve this, molecularly imprinted polymer particles (MIP) are used as sensitive layer. The interactions between the polymer particle and the target analyte can be monitored using the contact free and time-resolved method 1- $\lambda$ -Reflectometric Interference Sensing (1- $\lambda$ -RIfS).

The measuring technology [2] is designed as a multiple spot (MIP and NIP) device, which can be triggered and the different spots can be read out in parallel. Therefore it is possible to eliminate unspecific signals of polymer particles.

Reproducible and concentration dependent measurements are already performed with such an optical sensor for the analyte penicillin G. A further step in direction to the establishment of a working sensor is the measurement of samples in real media.

This work was supported by Land Baden-Württemberg project BioMIP-Biomimetischer Sensor zur inline Prozessanalytik (FKZ 7533-711.6-5A).

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## Simultaneous differentiation and quantification of ricin and agglutinin by an antibody-sandwich surface plasmon resonance sensor

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Ricin is one of the most toxic plant toxins known. Its accessibility and relative ease of preparation makes it a potential agent for criminal or bio-terrorist attacks. Detection of ricin from unknown samples requires differentiation of ricin from the highly homologous *Ricinus communis* agglutinin which is currently not feasible using immunological methods. Here we have developed a simple and sensitive surface plasmon resonance (SPR) sensing system for rapid differentiation between ricin and agglutinin done in real time. Both lectins were quantified in a sandwich immunoassay-like setting by capturing with a cross-reactive antibody (R109) binding to both proteins while differentiating by injection of a ricin-specific antibody (R18) in a subsequent enhancement step. The SPR-assay was reproducible and sensitive for different *R. communis* cultivars, showing no false positive results when other lectins were tested. Quantification and differentiation of both molecules was also demonstrated from a crude castor bean extract and complex matrices. For the first time, we have demonstrated how the closely related lectins can be discerned and quantified in a single assay based on immunological methods. This novel approach delivers crucial information regarding the composition, purity, concentration, and toxicity of suspicious samples containing ricin in less than 30 minutes. Furthermore, we show how enhancement injections during SPR-measurements can be used to determine the ratio of two related proteins independently of the actual protein concentration by comparing normalized enhancement response levels [1].

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## Application of wide field surface plasmon microscopy for investigation of biological micro- and nano-objects

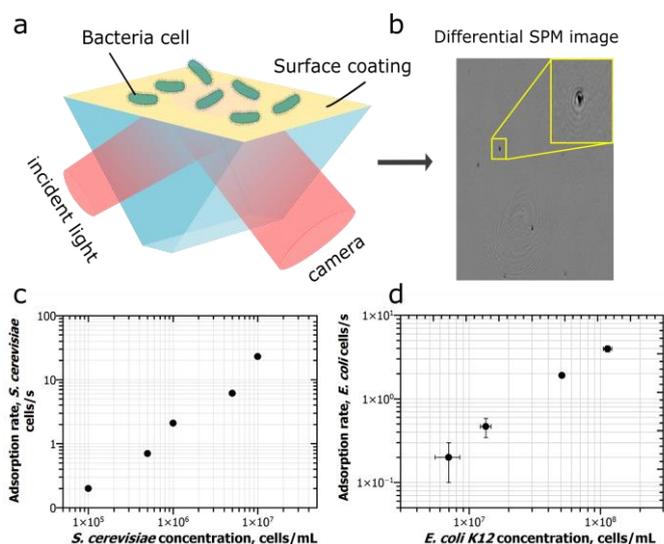
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Biological nano- and micro-species belong to our everyday life. In many cases, these species may also possess a dangerous impact on human and animal health. Therefore, fast, cost-efficient, and direct methods for detection, investigation and ultra-sensitive quantification of biological micro- and nano-objects are required. In this work, we present a new approach for detection, visualization, and quantification of model microorganisms. The approach was tested using *E. coli* K12, *S. cerevisiae* and biological nano-vesicles – exosomes.

The method is based on surface plasmon microscopy (SPM) enabling ultra-sensitive detection and visualization of small local changes of refractive index near the sensor surface [1]. Examples of its application for detection of bacterial and yeast cells are shown in Fig. 1. Surface coatings with different hydrophobicity and electrostatic properties were applied to vary the affinity properties of the biological objects. Concentration dependencies were studied. Taking into account a reasonable detection rate as one nano-/micro-object per 10 s, we obtain the limit of detection of  $10^5$ - $10^6$  species/ml. Further modification of experimental set-up may lead to a detection limit of  $\sim 10^3$  -  $10^4$  species/ml within 1-2 min without pre-concentration. Notably, our detection method is very stable against impurities and can be applied in very complex media [2].



**Fig. 1** (a) Schematic set-up for detection of bacteria cells using surface plasmon microscopy (SPM) (b) Real-time differential SPM image visualizing single bacteria cells on coated surface and a zoomed-in SPM signal of a single *E. Coli* K12 cell (inset). Adsorption rate of (c) *S. Cerevisiae* and (d) *E. Coli* K12 in dependence to the concentration.

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doi: 10.1021/acs.analchem.6b02878

## Designing highly organized porous electrodes for miniaturized Biofuel Cells

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The increasing demand on small-dimension portable and implantable devices has led to the development of new technologies to elaborate new electrode materials and methods of immobilization of bio components. In order to enhance the performance of biofuel cells (BFC) we propose highly organized porous material (providing high surface area) which allow the efficient hosting of specifically tailored enzymes without impeding the diffusion of substrate molecules thus leading to design of miniaturized implantable devices [1-3]. To improve lifetime and stability of BFC we are using different methods of immobilization of enzymes based on direct electron transfer (DET), mediated electron transfer (MET) as well as biocompatible hydrogels (GNF) [4] as an outer protective layer of the bioelectrode [5]. In preliminary experiments, bilirubin oxidase was covalently immobilized on macroporous and bare gold electrodes. The bioelectrocatalytic reduction of O<sub>2</sub> was significantly higher for the macroporous gold electrodes. (Fig.1A). Also coverage of the modified macroporous gold electrode with the GNF hyedrogel (-) improved the stability of the bioelectrode in comparison to uncoated one (---)(Fig.2)

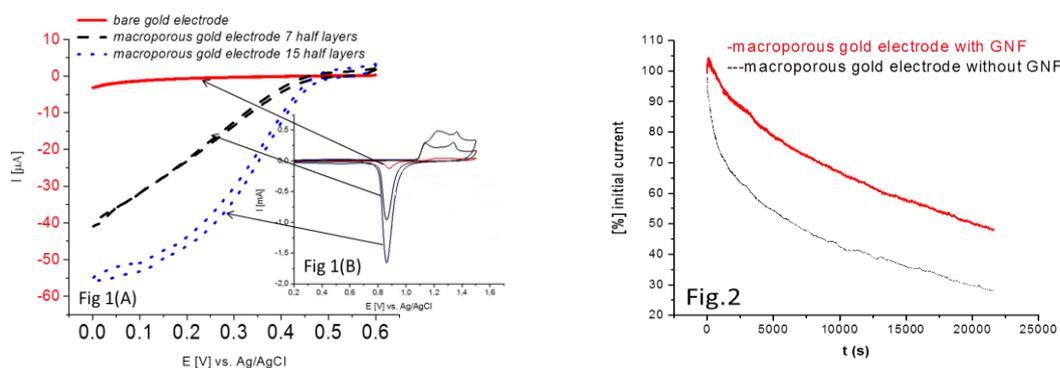


Fig. 1(A): Cyclic voltammograms obtained in oxygen saturated PB buffer (pH=7.2) at  $5\text{mV}\cdot\text{s}^{-1}$  Fig. 1(B) Electrochemical characterization of all gold electrodes. Fig. 2 Relative change of the bioelectrocatalytic current with respect to the initial current of the modified electrodes without GNF (---) and with GNF (-) in oxygen saturated phosphate buffer pH 7.2 during 6h of continuous operation at +0.1

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## Redox-Embedded Buckypapers for Portable Glucose Sensing and Biofuel Cell Applications

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The electrical wiring of redox enzymes with electrodes is of fundamental importance for the development of amperometric biosensors and biofuel cell devices<sup>[1]</sup>. Through parallel development of these elements a future generation of “clean energy” chemical sensors powered from ecological sources such as biological fluids and environmental waters may be envisioned. Hybrid self-powered sensors can even be developed for chemical detection combined with environmental remediation<sup>[2]</sup>. In this work we have constructed redox-molecule embedded multi-walled carbon nanotube buckypaper electrodes for electrical wiring of enzymes. The bioelectrodes were explored for reduction of O<sub>2</sub> and for glucose oxidation via direct and mediated electron transfer reactions, respectively. The development of “free-standing” paper electrodes with enhanced mechanical, chemical and electrocatalytic properties will be described with the ultimate goal of transforming the into wearable formats<sup>[3]</sup>. Recent innovations including a high-power glucose/O<sub>2</sub> biofuel cell will be discussed.

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## Simultaneous Collection of H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub> for the Evaluation of Light-Induced Stress of PS1 Photocathodes by Means of SPECM

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To date, several reports have shown the design of advanced architectures and engineered electron transfer chains for an efficient coupling of photosynthetic proteins with electrode surfaces, that are even capable of outperform the electron transfer rates of natural photosynthesis [1,2]. However, light-induced damage of the photosystems induces degradation of the protein with a consequent drop in activity over time, therefore substantially limiting the applicability of biohybrid devices. Although photosystem 2 (PS2) has been considered the primary target for photoinhibition, in isolated systems PS1 is similarly susceptible to light stress. Particularly, when methyl viologen is used as electron acceptor, the fast mediated electron transfer to oxygen causes the generation of superoxide anion radicals, hydroxyl radicals and hydrogen peroxide, that may lead to photodamage of PS1.

We present a new analytical methodology for the investigation of light-induced stress processes at PS1-based photocathodes that may be involved in photosystem degradation. By using dual Pt-microdisk electrodes in a scanning photoelectrochemical microscopy (SPECM) set-up, H<sub>2</sub>O<sub>2</sub> generated during the light-induced reaction was monitored by detecting the oxidation current for H<sub>2</sub>O<sub>2</sub> at one of the Pt disks. Concomitantly, oxygen consumption was followed by detecting the oxygen reduction current at the other disk. The studied biodevice was a previously reported highly efficient assembly of PS1 integrated within an Os-complex modified redox polymer acting as a conducting matrix for the transfer of electrons from the electrode surface to the PS1 moieties. The use of methyl viologen as redox mediator in solution for the collection of electrons at PS1 is evaluated. With the proposed set-up we are able to evaluate simultaneously the photocurrent generated by the PS1-based photocathode and to collect H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub> under localized irradiation conditions, thus allowing further insights in light-induced stress processes at isolated PS1.

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## Material and Structural Aspects of Microbial Biofilm Electrodes

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The great majority of microbial electrochemical technologies utilize electrochemically active bacterial biofilms as the electrocatalytic unit – a concept that has great potential towards a technical realization. Thereby, the performance of the respective biofilm electrodes decisively depends on structural and the material properties of the underlying electrode (the *substratum*). During the recent decade, especially 3D-electrode structures have been studied intensively and new structures are proposed regularly. In this presentation, the role of microscopic and macroscopic electrode structures for the short-term and long-term performance of 3D-biofilm electrodes will be elucidated. It will be demonstrated that the relevance of the dimensions of an electrode structuring depends on dimension and the properties of the biocatalytic unit (i.e., the microbial biofilm) attached to the electrode surface.

It will further be demonstrated that, surprisingly, copper as metal that is generally considered to be antimicrobial, represents a promising electrode materials for biofilm electrodes. It allows developing scalable, high performance 3D-polymer-copper electrode structures.

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# Poster presentations

## Gas diffusion biocathode for oxygen reduction based on direct electron transfer between carbon nanotubes and laccase

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Enzymatic fuel cells found increased interest in recent years. In comparison to other fuel cells neither precious metal catalysts nor expensive membranes are needed, and they can be operated at room temperature [1]. However, various issues such as limited stability or current density have to be addressed in order to improve their performance. For the oxygen reduction reaction at the biocathode based on multicopper oxidases such as bilirubin oxidase or laccase, gas diffusion electrodes have been proposed in order to avoid solubility and diffusion limitations of oxygen [2-4].

In this context, we present the design of a gas diffusion biocathode based on laccase from *Trametes versicolor*. The enzyme is adsorbed on polymer (e.g. PEG 3000) modified multi-walled carbon nanotubes enabling a direct electron transfer from the enzyme to the electrode. The onset potential of the catalytic current starts at approx. 0.65 V vs. Ag/AgCl. The current density under potentiostatic control is approx. 50  $\mu\text{A}/\text{cm}^2$  @ 0.4 V vs. Ag/AgCl in pure oxygen atmosphere (Fig. 1). The response time is quite fast, reaching a steady state within a few seconds. Further results will be presented addressing the characteristics of the biocathode as well as modifications of its design.

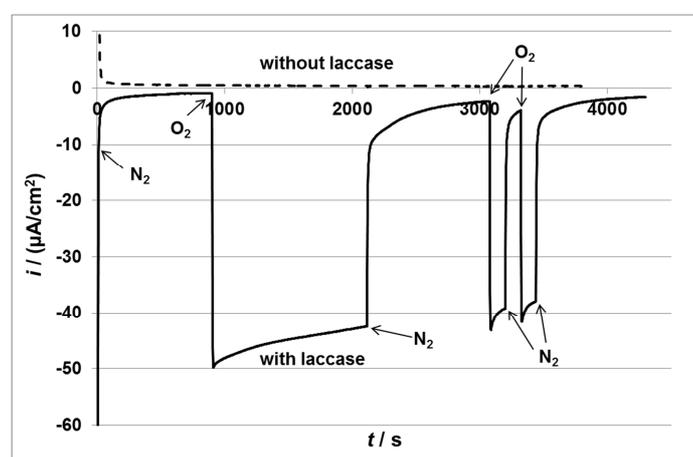


Fig. 1: Amperometric response of the gas diffusion biocathode with and without laccase to nitrogen and oxygen gas @0.4 V vs. Ag/AgCl in 0.1 M citrate buffer pH 4.5.

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## Supramolecular biocatalyst electrodes: A potential-controlled reaction switch for dual-analyte detection

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Biochemical pathways and signal chains are characterized by an effective coupling of individual reaction steps resulting in a rather high specificity in signal transduction. Additionally such highly complex systems allow a switching between different reaction cascades, depending on the presence of a certain molecule. Apparently, these efficient biological principles have been adopted for the construction of artificial signal chains.[1] In such a way different protein functionalities can be coupled to multiple electrochemical detection schemes, ensuring signal generation in the presence of individual substances.[2]

Here we report on a novel system, which allows an activity-switch between two different enzymes co-immobilized in an associated supramolecular matrix. This supramolecular network is formed by embedding two different biocatalysts - the multi-copper enzyme laccase (Lac), the multi-domain enzyme cellobiose dehydrogenase (CDH) - and the redox protein cytochrome *c* (cyt *c*) in an artificial matrix composed of carboxy-modified silica nanoparticles[3] allowing the immobilization of all the components in a layer-by-layer fashion.

Within this layered architecture two enzymes have been connected to the electrode via cytochrome *c*. Since the activity of the enzymes is controlled by the delivery or withdrawal of electrons the redox state of cyt *c* has been used for switching the activity of the biocatalysts on and off. The switchable reaction cascades for Lac and CDH are functioning in a non-separated matrix without disturbing the reaction of the other catalyst, allowing lactose and oxygen sensing, respectively.[4]

The approach is expected to open the way for the development of multiplex biosensors, and also represents a significant advance in mimicking of biological electron transfer cascades.

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## Polymer-enzyme interaction as basis for the construction of bioelectrocatalytic sensing electrodes

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Polymers can provide a suitable immobilization matrix for enzymes, but they can also be used to wire proteins with electrodes. Mainly redox polymers with defined redox centres and conducting polymers with electronic conductivity are applied. With focus on the 2<sup>nd</sup> approach we have studied the interaction of sulfonated polyanilines with dehydrogenase enzymes. Besides (PQQ)glucose dehydrogenase<sup>1,2</sup> and fructose dehydrogenase<sup>3</sup> recently xanthine dehydrogenase (XDH) has been studied.

XDH is a complex enzyme comprising four redox active cofactors: a mononuclear, five-coordinated molybdenum center, two [2Fe-2S] clusters, and a flavin adenine dinucleotide. For the study a copolymer PMSA1 (poly(2-methoxyaniline-5-sulfonic acid)-co-aniline has been synthesized and characterized. The polymer-enzyme reaction has been studied in solution with UV/Vis and cyclic voltammetry indicating that electron transfer from the substrate-reduced enzyme to the polymer is feasible.

The bioelectrocatalytic behavior has been investigated in dependence on the solution pH at ITO electrodes. Two processes can be found: First, a polymer supported enzyme electrocatalysis and second, a product-mediated enzyme communication with the electrode. The polymer supported bioelectrocatalytic process starts already at rather low pH and becomes inefficient at basic pH values – mainly because of decrease in conductivity of the polymer.

Based on the electron exchange capabilities between XDH and the polymer different enzyme electrodes are constructed. Thus, enzymes have been fixed on planar ITO electrodes by means of a polymer layer. These enzyme electrodes exhibit well-defined, substrate-induced bioelectrocatalytic currents starting at potentials below 0V vs Ag/AgCl. The efficiency can be enhanced when macroporous ITO electrodes with pore sizes of about 300nm are used. The pore structure allow incorporation of high amounts of polymer and enzyme. A current enhancement of at least one order of magnitude is found compared to flat ITO.

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## Performance characteristic of a CNT-based enzymatic glucose/oxygen biofuel cell in physiological liquids

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The performance parameters of enzymatic biofuel cells in physiological liquids is not only influenced by the electrode design but it depends also on substances in these media which can be directly oxidized or reduced at the anode and/or cathode or affect the efficiency of biocatalysis.

For investigation of these effects a biofuel based on carbon nanotubes (CNTs) is examined in different human body liquids. The CNT-anode is modified with a sulfonated polyaniline film and covalently coupled pyrroloquinoline quinone dependent glucose dehydrogenase (PQQ-GDH) from *Acinetobacter calcoaceticus*. The cathode construction uses bilirubin oxidase (BOD) from *Myrothecium verrucaria* which is bound to the PQQ-modified CNT-electrode. The resulting glucose/oxygen biofuel cell can achieve a maximum power output of more than 100  $\mu\text{W}/\text{cm}^2$  [1].

The determination of the performance characteristic in urine and saliva reveal a significant lower maximum power density compared to an EBFC in 5 mM glucose containing buffer. In urine only 12 percent and in saliva 18 percent can be achieved. The open cell potential of about 710 mV in buffer solution with 5 mM glucose is decreased to 400 mV in urine and to 665 mV in saliva.

The impact of potential interfering substances in physiological liquids on anode and cathode is elucidated by cyclic voltammetry measurements in human urine and saliva and as well as in the presence of ascorbic acid, uric acid and urea.

For both body liquids the very low glucose concentrations can be identified as one reason for the low power output. In addition the decreased cell potential causes the performance loss which is more pronounced in urine and mainly attributed to interference effects at the cathode. In saliva a diminished biochemical catalysis contributes to the lower anode performance.

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## Carboxylated graphene as a sensing material for electrochemical uranyl ion detection

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Since its discovery, graphene draws great attention of scientists all over the world and still is one of the most intensively studied materials [1]. To date the possible graphene utilization was investigated in many fields of applications [2-4] and e.g. as sorbent for heavy metal ions [5-8]. To make the sorption possible in each of above mentioned case, the graphene or graphene oxide was modified with e.g. silver nanoparticles [8], magnetic particles formed from Fe(II) and Fe(III) [9], EDTA- and amine-functionalized [5] or chitosan/FeOOH nanocomposite [7]. The graphene composites possess also strong affinity to uranyl ion [10]. Since the specific interactions of this 2D carbon material with different chemical species was confirmed, it was also used as a sensing material in analytical chemistry [11].

The present study is focused on the application of graphene modified with carboxylic groups in the development of electrochemical sensor for uranyl ion. Its concentration was determined with the use of voltammetric techniques by comparison of current change of redox indicator hexaammineruthenium(III) chloride (Fig. 1). The prepared sensor demonstrated linear response within the range of  $5 \times 10^{-8}$  to  $5 \times 10^{-6}$  mol L<sup>-1</sup> and significant selectivity towards UO<sub>2</sub><sup>2+</sup> ions over other examined cations (e.g. Cd<sup>2+</sup>, Ca<sup>2+</sup>, Sr<sup>2+</sup>, Co<sup>2+</sup>, Mg<sup>2+</sup>, Pb<sup>2+</sup> and for Fe<sup>3+</sup>).

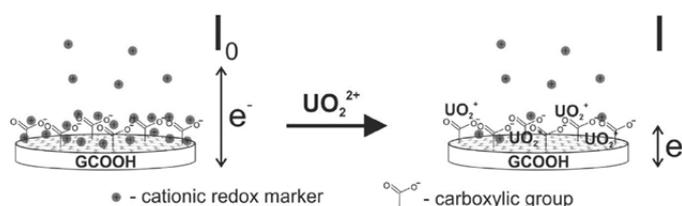


Fig. 1: The mechanism of the sensor response generation.

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## Quenching of Graphene quantum dots fluorescence by alkaline phosphatase activity in presence of hydroquinone diphosphate

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Graphene quantum dots (GQDs) are a kind of 0D material that exhibit extraordinary optical and electronic properties due to their quantum confinement and edge effects. GQDs are characterized by their chemical inertness, low toxicity, high biocompatibility, high fluorescent activity and excellent photostability [1]. Within the wide field of applications where GQDs can be used, the development of photoluminescent biosensing devices is attracting a large amount of attention.

On the other hand, alkaline phosphatase (ALP) is the most commonly used enzyme for biosensors development. Among the several ALP substrates already reported, there is hydroquinone diphosphate (HQDP) [2]. HQDP hydrolysis, catalyzed by ALP, generates the electrochemically active hydroquinone (HQ). In addition, in presence of dissolved oxygen in solution, GQDs can catalyze the oxidation of HQ to *p*-benzoquinone (Q), which, in turn, quenches the fluorescence of GQDs [3].

Herein, we present a simple and sensitive proof-of-concept, for the development of enzymatic sensors based on ALP, with luminescent detection. Therefore, GQDs were employed as a fluorescent probe, where the optical response was related with the catalytic ALP activity towards HQDP. The quenching of GQDs photoluminescence as a result of Q production, from the oxidation of HQ, enzymatically generated, was carefully evaluated assessing aspects as sensitivity, limits of detection and reproducibility and the analytical figures of merit were obtained.

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## Immobilization and Detection of single Nanoobjects on Nanoelectrodes

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The immobilization of a bioreceptor is a key element in the construction of a biosensor. Here the problem of immobilization is approached by the use of dielectrophoresis (DEP). DEP is a phenomenon in which a dipole is induced in a polarizable particle in an inhomogeneous AC electric field. By the right choice of frequency, this particle can be moved without damage and can be immobilized [1,2]. An alignment of the biomolecules can be advantageous to get a strong signal. This is possible to be achieved with DEP [3]. By the immobilization of individual addressable biomolecules, it would be possible to show explicitly where the signal has been generated. By selecting appropriate dimensions of electrodes compared to the molecules this singling with DEP is possible [4]. However, the problem arises to make any statistical statements. This problem can be solved by the use of a regular microarray consisting of many thousands of vertical Si- or W-based nanoelectrodes [5]. So it is possible to do single-object-investigations with good statistics. Here we demonstrate the successful immobilization of fluorescently labeled nanospheres as individual objects on electrodes of an array as a model system. Such proof of immobilization and singling is done via fluorescence microscopy and SEM measurements. Also by using the AFM a detection is possible [6], but significantly more time consuming. Using different electrode diameters (500nm to about 1nm) and object sizes (2µm to 20nm) a size ratio of 1:2 was discovered, for which singling occurs.

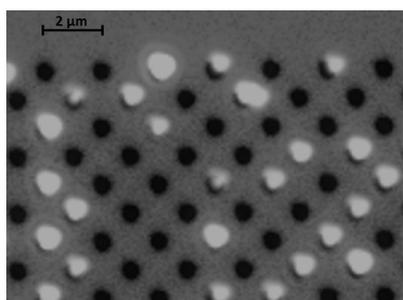


Fig. 1: Superposition of fluorescence and dark field-image of an electrode array with immobilized fluorescent nanospheres (100nm diameter).

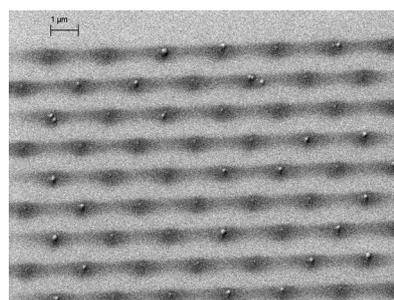


Fig. 2: SEM image of the same area without any further sample preparations. Electrodes with no, a single or two nanospheres can be clearly distinguished.

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## Self-assembly of alpha lipoic acid monolayers on Germanium surfaces for THz biosensing applications

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Lab-on-chip biosensing that harnesses the power of plasmonic resonance phenomenon is seen as a promising approach in the detection of molecular transformations for molecules at very low-concentrations (<nM) [1]. Two aspects of the biosensor device design are of key importance when operation in THz frequency range is considered: electromagnetic field enhancement in the sensor area and efficient entrapment of biomolecules in its vicinity [2]. This work focuses on the latter and demonstrates formation of self-assembled monolayers (SAM) of alpha lipoic acid on Germanium (Ge) surfaces using a wet-chemistry approach, with ethanol and water used as solvents in the incubation mixture [3]. Such SAM is the basis for formation of a biofunctional layer, composed of reactive carboxyl moieties, by stably and selectively binding to Ge through strong Ge-S bonds. It is thus able to be used for capturing of biochemical species like proteins in electromagnetic “hot-spots” of the sensor. XPS characterization of the self-assembly structures was performed to investigate dependency of the SAM quality on incubation’s mixture pH and to study oxidation of the underlying Ge surface during storage under well-defined ambient conditions as well as in buffer solutions. The observed stability of the SAMs both under ambient condition and in buffers confirms their applicability in Ge-based THz biosensors, thus allowing taking advantage of excellent electrical properties of Ge (tunability and conductivity).

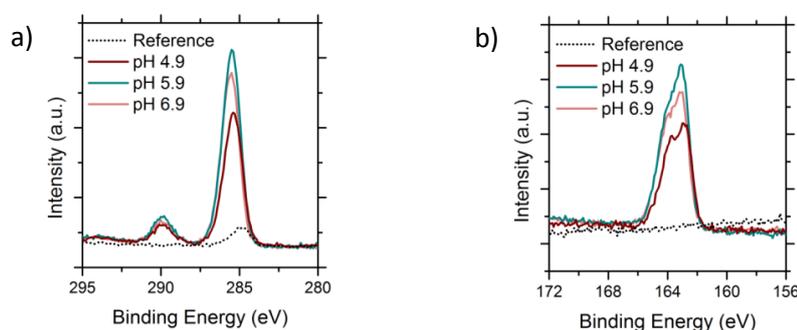


Fig. 1: XPS spectra of C1s (a) and S2p (b) regions for alpha lipoic acid self-assembled structures on Ge(001)

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## Carbon Nanotube-based Photoelectrodes based on assembled Photosystem I

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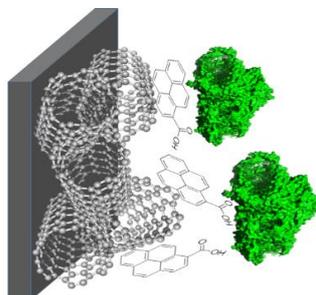
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Artificial systems exploiting the features of natural photosynthesis are increasingly becoming a focus of current research. Particularly the two photosystems (PS) of the oxygenic photosynthesis have attracted the attention of researchers to build up new solar energy-converting systems.[1]

To date a couple of approaches for assembly of photosystem I (PSI) on gold surfaces,[2], graphene,[3] and other surfaces were successfully implemented. In this contention we have been using highly conductive carbon nanotubes as suitable surface for PSI attachment. For this purpose MWNT have been assembled to a glassy carbon electrode and then modified with a  $\pi$ -system (carboxypyrene) for binding the PSI via a covalent bond. In our study we performed photochronoamperometry, photoaction spectra and stability assessment of this hybrid photobioelectrode. The current direction is mainly cathodic pointing to a connection of the luminal site of the PSI to the electrode. We could enhance the photocurrent output upon addition of the electron scavenger methyl viologen ( $MV^{2+}$ ). The system showed reasonable stability in photocurrent generation at continuous illumination.



*Fig. 1: Schematic representation of the biohybrid electrode (PSI-Photosystem I in green, carboxypyrene, carbon nanotubes in black)*

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## DNA self-assembly for signal enhancement in nucleic acids biosensors

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Some of the possible perspective advantages of the uptake of nucleic acids biosensor technology are already within reach, still, the sometimes limited sensitivity can seriously inhibit the application of biosensor-based methods when these could be useful towards detection of nucleic acids variants present only at a very low concentration.

We have adapted and succeeded at using the hybridization chain reaction (HCR)[1] towards enhancing the signal due to the specific recognition and binding of soluble nucleic acids to a surface-bound probe. The enhancement strategy consists in a triggered supramolecular polymerization of DNA sequences or nanostructures at the location of specific nucleic acids recognition. We have showed that the method can be used towards the detection of an arbitrary DNA target through proper design of the sequences of the components[2]. Preliminary experimental evidence shows a significant enhancement of the signal, which could prove useful in some applications. We also proved that HCR can have single-nucleotide sensitivity for the detection and signal enhancement.

We have recently worked at the extension of HCR towards the detection of circulating miRNA targets, biomarkers of considerable interest for diagnostics. We have showed that HCR reagents can be designed to distinguish between closely-related miRNA targets, as it would be needed in diagnostics. Modifications of the HCR design can yield hyperbranched or target-recycling assembly and thus significantly increase the detection signal. We have preliminary results on a branched HCR design that could be compatible with surface-bound biosensors implementation (electrochemical, fluorescence, luminescence, SPR).

We showed sensitivity to single-nucleotide mismatch and proved that the sensing strategy could be applicable to the detection of nucleic acids from pathogens or of circulating miRNA.

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## Sulfonated polythiophenes for the electrochemical coupling of glucose dehydrogenase.

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Glucose dehydrogenases (GDHs) have been extensively studied for biosensors and biofuel cells development thanks to the peculiar oxygen insensitivity and the possibility to directly transfer the electrons to modified electrodes. For this purposes sulfonated polyanilines were used to enhance the connection between PQQ dependent glucose dehydrogenase (PQQ-GDH) and different electrode surfaces (nanostructured carbons [1,2] or macroporous ITO [3]). Sulfonate groups make the polymer self-doped and help the positively charged PQQ-GDH to correctly orient onto the modified electrode.

In this work, sulfonated polythiophenes were evaluated and used for the first time (at the best of our knowledge) to directly connect GDH with electrodes. Polymer films were electrosynthesized onto CNTs modified gold electrodes in acetonitrile solution containing 3-thiopheneacetic acid (ThCH<sub>2</sub>CO<sub>2</sub>H), 3-methoxythiophene (ThOCH<sub>3</sub>) and 2-thiophenesulfonic acid (ThSO<sub>3</sub><sup>-</sup>) using a pulsed deposition method. The modified electrodes were electrochemically and morphologically characterized. Several parameters were considered in the pulsed potentiostatic electrosynthesis: oxidation potential, time periods and number of pulses. Concentrations of the monomers and ratios between them were optimized by evaluating the effect in the catalytic activity towards glucose of the bound PQQ-GDH. The electrodeposition of sulfonated polythiophenes provides a surface for direct bioelectrocatalysis of PQQ-GDH. The starting potential for the catalyzed glucose oxidation is below 0 V (vs. Ag/AgCl).

The obtained 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 the build up of biosensors and biofuel cells.

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## Electrically controlled Michael addition: addressing of covalent immobilization of biological receptors

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A new technology of electrical addressing of covalent immobilization of biomolecules to the electrodes is reported. It is based on Michael addition of thiol group of biomolecules to the  $\alpha,\beta$ -unsaturated carbonyl groups of benzoquinone. This “click” reaction was tested with immobilization of a number of thiolated compounds onto the simplest array consisting of two gold electrodes coated by a self-assembled monolayer of benzoquinone-terminated hexylthiol. Other thiol links providing effective electrochemistry and strong binding will be discussed too. Electrically controlled binding of hexanethiol, ferrocenylhexylthiol, human serum albumin and thiol-terminated single-stranded DNA (ssDNA) was studied. The binding was detected using cyclic voltammetry, X-ray photoelectron spectroscopy and surface plasmon resonance. It was demonstrated that the reaction requires an oxidized state of the benzoquinone moiety; this can be reached by applying of a moderate anodic potential to the electrode. Surface plasmon resonance measurements demonstrated that thiol-modified ssDNA, immobilized by this technique, binds complementary synthetic oligonucleotides or PCR-amplified fragments. The developed technology of electrical addressing of covalent immobilization can be applied for fabrication of sensor arrays.

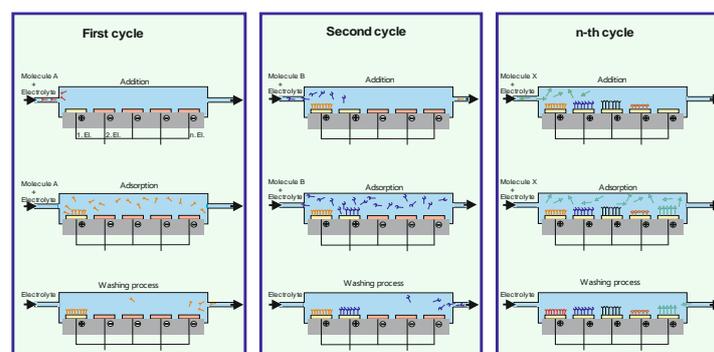


Fig. 1: Electrically addressable immobilization of different thiols (A, B, C, . . . , X) onto electrode array.

## A new nanocomposite-based gelling oligopeptide for biosensors development

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Redox proteins immobilization on electrode surfaces is one of the most important steps to be considered in the development of optimized electron transfer-based biosensors. In this work, we developed a new nanocomposite material by synthesizing a gelling tripeptide composed of Fluorenylmethyloxycarbonyl-triphenylalanine (FmocPhe3, HG) through lipase-catalyzed reaction, to be used as a new immobilization support for proteins; the gel was modified with genipin as a crosslinker in order to modulate the chemico-physical features of such hydrogel. In the crosslinked hydrogel structure gold nanoparticles (AuNPs) were embedded to enhance electron transfer efficiency. To verify the characteristics of this new nanocomposite material as enzymatic support, we evaluated the main bioelectrochemical properties and stability of *Trametes versicolor* Laccase (TvL) physically entrapped into the hydrogel structure, spread onto the graphite-based screen printed electrode surface, either in the presence or in the absence of gold nanoparticles (AuNPs). We thoroughly characterized the rheological and chemico-physical properties of the obtained materials as a function of their genipin content and we compared them with those of non-crosslinked ones in order to assess if genipin-mediated crosslinking could provide attractive features to the hydrogels. The results obtained show a significative increase of biosensors performances by modifying graphite-based electrodes with the crosslinked hydrogel nanocomposite material. That entails a reduction of the overall costs of analysis, suggesting the possible use of this polymer in the development of nanocomposite-based electrochemical biosensors.

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## Nanodiscs: a novel technology for functional reconstitution and characterization of membrane proteins

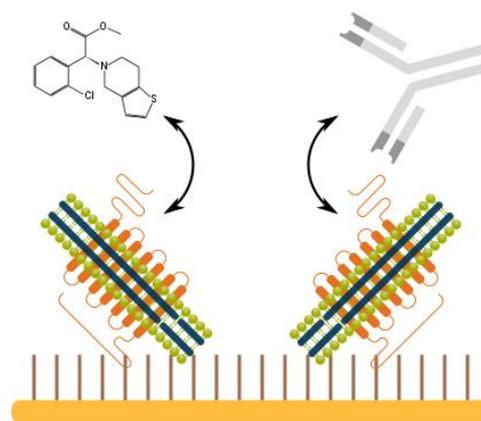
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Nanodiscs are self-assembled bilayers of phospholipids, stabilized by synthetic, amphipathic helical membrane scaffold protein [1]. Due to the native-like lipid environment, reconstitution of isolated membrane proteins into nanodiscs enables the stability and functionality of membrane proteins in solution. These reconstituted membrane proteins can be characterized by standardized methods which were developed for soluble proteins and are accessible for binding partners from both intracellular and extracellular side.

The aim of our research is to isolate membrane proteins directly from human cell lines or other model systems and reconstitute the protein of interest into nanodiscs without the addition of external lipids. We established this novel concept with erythrocyte membrane and could successfully insert different integral and peripheral membrane proteins into nanodiscs.

This novel technology is a valuable tool for diagnostic applications such as in autoimmune diseases and immune oncology. The transfer of the protein of interest with correct folding and full functionality from cell membrane into nanodiscs enables innovative possibilities of cell-free assay formats in the future.



*Fig. 1: Schematic illustration of possible interaction assays with nanodisc technology. The in nanodisc reconstituted membrane protein is immobilized on the sensor surface. Interaction between inserted receptor and small molecules (left) or antibodies (right) could be determined and analyzed with diverse methods.*

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## **A Polymeric Nanosensor for Sensing of Broad pH Changes in Biofilm as Tool for the Investigation of Microbial Induced Corrosion**

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One of today's major problems in many technical plants as well as fuel tanks is Microbial induced corrosion (MIC), leading to considerable damage and huge financial losses. Successful prevention of MIC requires the localization of first signs of corrosion as well as the identification of factors influencing the corrosion process. [1] Hence, there is a growing need for sensitive and preferably inexpensive tools that enable the early detection of MIC. Of utmost importance are methods, which provide spatially and time-resolved information and allow the determination of corrosion rates at sites of interest for possible prevention of MIC. [2]

### **RESULTS AND DISCUSSION**

Biofilm formation can lead to changes in pH, oxygen, and chloride concentration as well as to the release of certain metal ions like Fe(II) and Mn(II) depending on the chemical composition of the metal surface involved. Hence, optical methods in synergistic combination with suitable reporters or probes enabling the detection of these analytes and monitoring their changes can be used for MIC detection. Here, we propose to utilize fluorescent polymeric nanosensors for MIC detection in different biofilms via the determination of changes of the local pH value. Such nanosensors provide several advantages for imaging applications such as intracellular pH measurements with a high analyte sensitivity and ratiometric sensing by labeling and/or doping with a combination of a multitude of analyte-responsive and analyte-inert dye molecules. [3] Furthermore they can be utilized as carriers for bacteria-specific ligands (e.g., antibodies or lectins) for a more specific targeted approach. In this respect, different polymer architectures will be studied to identify an optimal candidate in terms of imaging performance in conjunction with several classes of pH-responsive fluorescent dyes like cyanines, BODIPYs, and xanthenes, exploiting different mechanism of signal generation such as photoinduced electron transfer or protonation-induced changes in the spectral position of absorption and emission spectra. In first studies, a polystyrene-based nanosensor utilizing different fluorescein derivatives (i.e., chlorinated as well as non-chlorinated versions) as pH-sensitive dye and Nile Red as an inert reference dye was synthesized, by embedding Nile Red into the polymer structure following a literature procedure [4] and conjugating a

fluorescein derivative to amine groups at the particle surface using a straightforward labeling protocol. Here, we present first studies regarding the potential of this new nanosensor for ratiometric fluorescence measurements of pH in *Escherichia Coli* embedded in Agarose gel. Next steps will include the utilization of different brominated BODIPY derivatives offering a tailor-made pKa value.

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## Biosensor based on the inhibition of the acetylcholinesterase activity and gold nanoparticles for the determination of pesticides in aqueous medium

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The enzymatic inhibition has been widely study to quantify organophosphorus compounds like insecticides [1,2,3,4]. In this study we have fixed the acetylcholinesterase (AChE) enzyme using poly(vinyl alcohol) (PVA) styrylpyridinium (SbQ) on an electrode coated with Poly(3,4-ethylenedioxythiophene) and gold nanoparticles (AuNPs) [5] by electrodeposition [5]. The electrochemical signal was recorded and it is associated to the AChE enzyme activity product (thiocholine). The electrode was characterized by scanning electron microscopy (SEM), Raman spectroscopy, Fourier transform infrared spectroscopy (FTIR) and cyclic voltammetry (CV) measurements. The enzyme activity of AChE enzyme attached to the PVA matrix was confirmed using Ellman's reagent and cyclic voltammetry (CV) measurements. The ability of the PEDOT-AuNP biosensor to measure concentrations of the organophosphate pesticide was demonstrated by monitoring the inhibition of the hydrolysis of acetylthiocholine. This straightforward strategy is potentially valuable for the development of new devices for the sensitive detection of pesticides in aqueous medium.

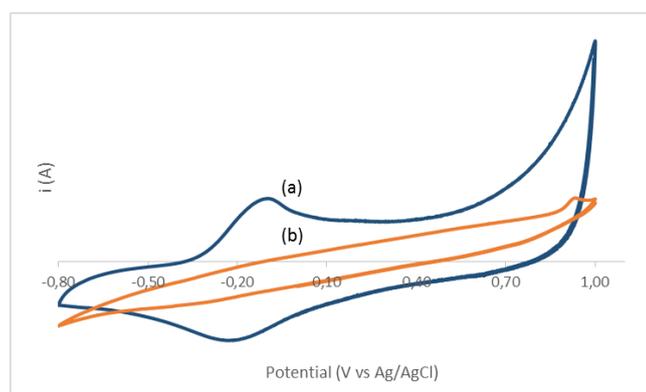


Fig. 1: Cyclic voltammogram of (a) Acetylcholinesterase fixed on the PVA-PEDOT electrode and (b) irreversible oxidation of the thiocholine (enzyme activity product) on gold electrode in 0.1M phosphate buffer, pH 7, Scan rate: 100 mV/s. Initial scan direction: positive.

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## Nanofunctionalized interfaces for SPR based immunosensors

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Surface Plasmon Resonance (SPR) based immunosensors is an ever growing area of research in the development of high-performance diagnostic devices for point-of-care analysis. Immunosensor based on antibody-antigen binding is a promising method for detection of disease biomarkers, food pathogens, explosives and environmental contaminants due high sensitivity, selectivity and feasibility for miniaturization.

Despite active research around the world in development of biosensors for various applications is growing rapidly, most of them have the limitations with respect to portability and for real-time applications. One of the major challenges in biosensors development is the fabrication of immunosensor by the immobilization of ligands/biomolecules on the transducer surface. Self-assembly method is one the most promising route for simple and highly effective immobilization of biomolecules. Self-assembled monolayers offer several attractive features such as bare minimum resources, miniaturisation is easy, high degree of order and control, mimicking the cellular microenvironment and chemical stability for high-performance applications. The functionalization of gold surfaces by organic molecules containing free anchor groups such as thiols, disulphides, amines and silanes allows tremendous flexibility for functionalization with respect to their terminal groups such as hydrophilicity, hydrophobicity and chain length.

Here, we have demonstrated the fabrication of immunosensors based on self-assembly method. The SAM functionalized surfaces have been explored for detection of clinically relevant molecules such as dengue, dopamine and metal ions with proper functionalization. The optimization of the self-assembled surfaces, biomolecule immobilization and the blocking of the free active groups has been performed by using SPR and electrochemical techniques. The results are compared with physically immobilized bio-molecules with respect to sensitivity and stability. The results showed good performance of the SAM interfaces for highly sensitive and selective detection of analytes down to ppb level with good reproducibility and storage stability. The results suggest that the SAM based immunosensor interfaces has promising applications in point of care analysis.

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## Magnetic Nanocomposites for Rapid Biosensing of Staphylococcal Enterotoxins (SET) in Complex Food Matrices

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Staphylococcal enterotoxins (SET) are one of the most common causes of acute food poisoning, accounting for numerous foodborne-disease outbreaks all over the world. To ensure a fast surveillance and response to foodborne-disease outbreaks, a rapid method is required which enables a sensitive quantification of proteotoxins in large volumes of complex food matrices. The inline-coupling of a facile immunomagnetic separation step based on nanocomposites with specifically tailored magnetic and morphological characteristics to a sensitive microarray analysis is a promising tool. A novel synthesis approach for the fabrication of superparamagnetic raspberry-shaped iron oxide/silica nanocomposites was developed and fully characterized by various techniques (TEM, SEM, SQUID magnetometry, Raman and Mössbauer spectroscopy). After the functionalization of the nanocomposites with specific detection antibodies (Ab) for Staphylococcal Enterotoxin B (SEB), immunomagnetic separation (IMS) of proteotoxins directly in large volumes of food matrix is feasible. For a sensitive quantification of proteotoxins, IMS was inline-coupled with a chemiluminescence sandwich microarray immunoassay (CL-SMIA). The flow-based SMIA can be performed as shown in Fig.1. Nanocomposites exhibited high magnetization, superparamagnetism and long-term stability over at least six months, which make them excellent candidates for bioseparation applications. An efficient immunomagnetic separation was performed in 100 mL milk. A SMIA for the detection of SEB in milk was established on the flow-based microarray platform MCR 3 with a detection limit of 0.08  $\mu\text{g/L}$ . In a proof-of-concept study it was shown that SEB in milk could be successfully detected by the IMS coupled to CL-SMIA. In future, a screening of SEA, SEC, SED and SEE is planned.

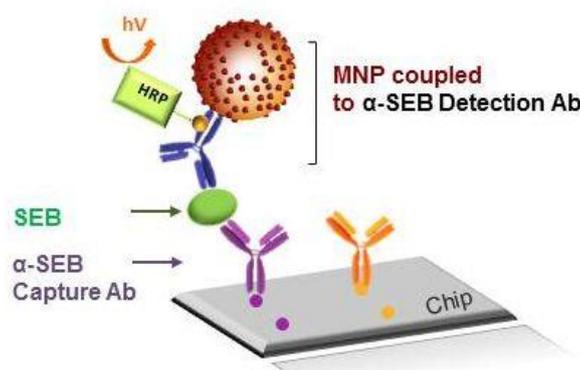


Fig. 1: Schematic scheme of magnetic nanocomposite-based SMIA

## Using DNA origami nanostructures for amplifying signals generated in microbead-based assay for microRNA detection

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microRNA (miRNA) molecules are a fairly recently discovered class of non-coding RNAs that play a key role in the regulation of gene expression. They have been regarded as promising diagnostic biomarkers as well as potential targets for new anti-cancer drugs. Unfortunately understanding their full function and behaviour has been limited largely due to the extremely low copy numbers in a cell [1]. Therefore it is critical to develop a method for ultrasensitive detection of these miRNAs. Microbead-based assays provide a good platform, offering high throughput applicability with smaller sample consumption [2]. In order to amplify the signal generated by the microbead-based assay, DNA origami nanostructures have been used. These nanostructures can have multiple dye molecules per structure as well as a DNA sequence to hybridize to the target. These modifications can be done in a controlled manner [3], thus providing a label that is not only very bright but one that can be used for quantification of the target sequence. Figure 1 shows a schematic representation of the assay.

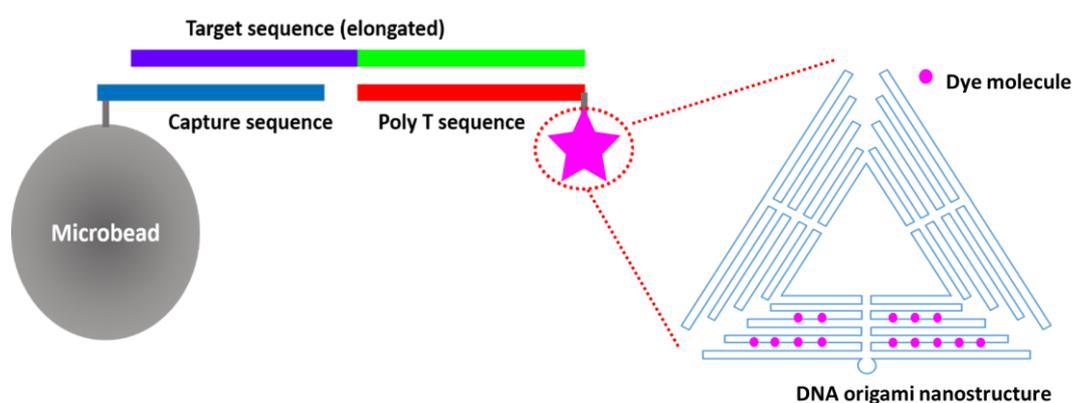


Figure 1 Schematic representation of the microbead-based assay using DNA origami nanostructure

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## Substrate recycling principles for the detection of adrenaline to support adrenal vein sampling

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Adrenal vein sampling (AVS) is the most frequently applied diagnosis method for determining primary aldosteronismus. However, it is difficult to place the catheter tip within the adrenal veins' during AVS. Therefore, adrenal veins adrenaline concentrations can be measured to confirm successful catheterization of the adrenal veins, since there is a gradient of the adrenaline concentration between adrenal blood and peripheral blood.

In this contribution, two types of high-sensitive adrenaline biosensors using substrate recycling principles have been realized. The first sensor type is based on a galvanic oxygen sensor, using the substrate-recycling principle with two enzymes: a laccase for the oxidation of adrenaline to adrenochrome and a pyrroloquinoline quinone (PQQ)-dependent glucose dehydrogenase (GDH) for the reduction of adrenochrome to adrenaline [1]. The second sensor type is based on a thin-film-sensor with an immobilized PQQ-GDH on its surface, using the bioelectrocatalysis as measuring principle [2]. Adrenaline is oxidized at an applied potential of 450 mV to adrenochrome. Afterwards, a reduction of adrenochrome was catalyzed by the enzyme GDH.

With both sensor types, a lower detection limit of 1 nM adrenaline has been achieved in both buffer solution and Ringer's solution at blood-corresponding pH value of pH 7.4. The possibility of an application of the developed biosensors for the adrenaline detection during the adrenal vein sampling procedure could open new prospects in the field of medical diagnostics.

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## Dielectric spectroscopy of bovine serum albumin up to 110 GHz

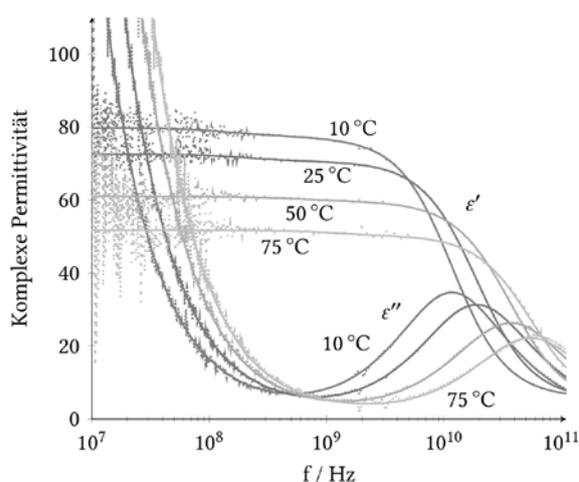
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For a rational design of biosensors that are based on electromagnetic fields detailed knowledge of the electrical properties of biological substances is indispensable. Above that, by investigating these properties in response to parameters like temperature, concentration and pH, information about molecular structures and interactions of, e.g., proteins and nucleic acids can be assessed.

In the present setup, the open end of a coaxial probe is immersed into the sample solution in a standard 1.5 ml reaction tube. Amplitude and frequency of the reflected portion of an incoming radio-frequency signal are determined with the help of a vector network analyser covering the frequency range between 10 MHz and 110 GHz. From this, dielectric permittivity  $\epsilon'$  and loss  $\epsilon''$  are calculated [1]. The sample's temperature is controlled from well below 10°C to above 80°C [2].

Dielectric properties of aqueous solutions of bovine serum albumin (BSA) are presented at protein concentrations ranging from 10 mg/ml to 800 mg/ml. Gelation is monitored, as is thermal denaturation around 60°C. The action of the denaturing chaotropic agents guanidine hydrochloride and urea is studied as well as that of the surfactant sodium dodecyl sulfate (SDS).



*Fig. 1: Permittivity  $\epsilon'$  and dielectric loss  $\epsilon''$  of aqueous BSA solutions between 10°C and 75°C (dotted lines). Solid lines show sum-of-least-squares fits to the experimental data.*

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## AC electrically functionalised sensor array for influenza virus detection

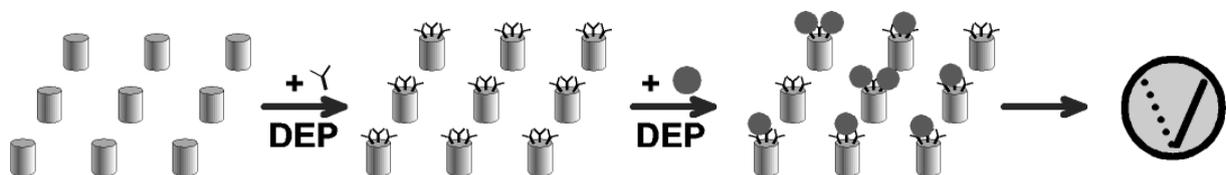
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The principal components of a biosensor typically comprise the element that recognizes the analyte, a transducer and the signal processing electronics. Integration of these stages into a single silicon chip results in a number of advantages as compared to separate units: reduced size, improved reliability, better noise rejection, and cost reduction by mass production. Moreover, the simultaneous acquisition of several sample parameters using sensor arrays becomes feasible.

However, a main obstacle for a miniaturised multi-parameter sensor lies in the individually controlled functionalisation of each sensing sub-structure. In the present work this is overcome by using inhomogeneous AC electric fields produced by arrays of sub-micrometer electrodes. These fields attract macromolecules like antibodies towards the electrode surface by dielectrophoretic forces and finally lead to a permanent immobilisation without further chemical modifications [1, 2]. Since these electrodes are addressed individually, each can be functionalised with a different antibody. Each electrode is part of an on-chip resonant circuit, whose frequency changes with surface coverage of the electrode [3] and, hence, serves as a measure of the amount of analyte bound to the antibody. For the detection of influenza virus particles dielectrophoretic attraction is also utilised for locally concentrating these particles at the sensor surface.



*Fig. 1. Immobilisation and detection scheme: Antibodies are immobilised by dielectrophoresis. Virus particles are attracted towards the electrodes and specifically bound by the antibodies. Resulting capacitance changes are monitored as a measure of virus concentration.*

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## Comparison of Biotin Binding Proteins for PCR Applications

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The streptavidin-biotin bond is well known for its high association constant and its high temperature stability. Therefore it is often used for immobilizing biomolecules onto solid surfaces. Streptavidin is even useable for nucleic acid detection and quantification under PCR conditions. In this study we compared different streptavidin homologs (SaH) including neutravidin (NA), core streptavidin (CS), traptavidin (TA) and monomeric streptavidin (mSA) to address the question: Are these SaHs differently or equally suitable for PCR applications? It is important that the proteins have high temperature stabilities and that a highest possible fraction of the bound oligonucleotides is accessible for directed hybridization. All biomolecular interactions were analyzed with our VideoScan imaging platform [1] (Figure 1), that allows monitoring the surface fluorescence of microbeads time- and temperature-resolved. Hybridization efficiencies were realized via FRET by use of complementary oligonucleotides labeled with quencher and dye molecules. We found high hybridization efficiencies of around 95 % independent of the SaHs. Thermostability was investigated by monitoring the dissociation of bound mono- or bis-biotinylated oligonucleotides, when temperature was gradually increased from 40 °C to 95 °C. If mono-biotinylated oligonucleotides were used, we found stabilities in the order: TA>Sa~NA>CS>>mSA. In case of bis-biotinylated oligonucleotides the SaHs had similar high stabilities, however, with mSA as an exception (Figure 1).

We are confident that our results are a valuable source for researchers, who develop novel biosensors and devices of routine and point-of-care diagnostics.

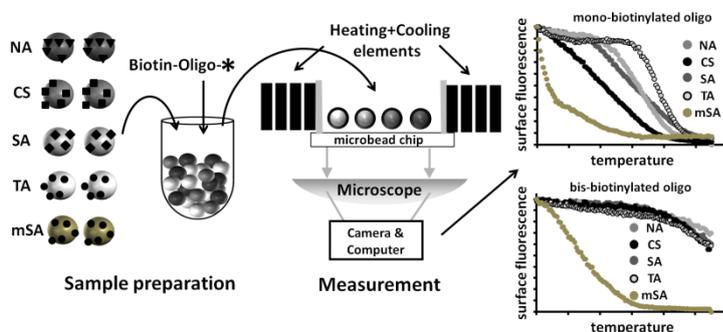


Fig. 1: Principle of measurement. Dye/Size-encoded microbead populations coupled with different SaHs were loaded with biotinylated and fluorescence (\*)-labelled oligonucleotides as described by us [1]. Microbeads were pooled and transferred into the measurement

device. Here, the microbead-chip was photographed using fluorescent light, while the chip was exposed to increasing temperatures. Imaging processing software counts microbeads, assigns them to a population and determines their mean surface fluorescence.

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## Blister-Actuated Laser Induced Forward Transfer as a novel approach for immobilisation of bio-active detection materials

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Laser Induced Forward Transfer (LIFT) is a flexible digital printing process for maskless, selective material and pattern transfer, which is particularly suited for the transfer of solids, materials exhibiting a high-viscosity as well as those whose transfer with other processes such as inkjet printing is not feasible.

Here we present a blister actuated LIFT method for the transfer of bio-active material. A schematic overview of the process is shown in Fig.1. This variant of the LIFT process uses a laser pulse to ablate a shallow part of the intermediate polyimide layer. This traps the expanding volume of gaseous ablation products

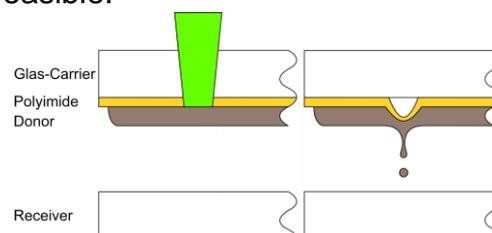


Fig. 1: Schematic overview of the blister-actuated LIFT process.

between the polyimide layer and the glass carrier thus creating a blister, whose rapid emergence generates the transfer impulse for a droplet of the subjacent material [1]. The influence of several parameters on the transfer process was investigated. In particular, the layer thicknesses of the donor material and of the polyimide, the laser pulse energy, the viscosity of the donor and the distance between the donor and receiver are of great importance. The correlation of the pulse energy on the diameter of the transferred droplets is displayed in Fig. 2., which shows that the diameter and volume of the transferred droplet can be easily controlled by varying the energy of the laser pulse.

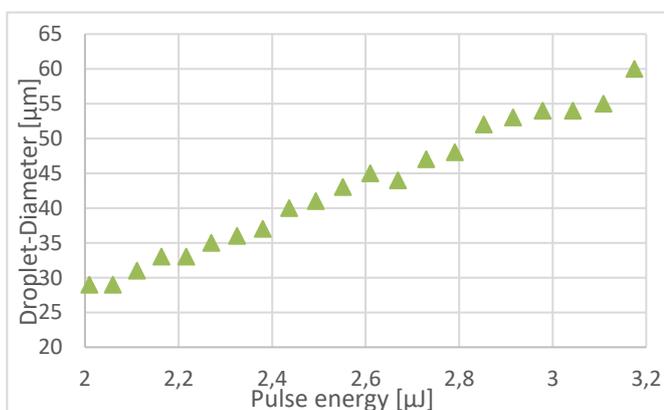


Fig. 2: Diameter of the transferred droplets on a glass receiver in relation to the laser pulse energy (Laser wavelength 515 nm, Pulse duration 230 fs)

Furthermore, the feasibility of the process for the transfer of detection antibodies was evaluated by LIFT printing and immobilizing detection antibodies for a quantitative CRP-Assay on laser structured nitrocellulose membranes which is developed in the scope of the IGF-project "Papier-basierte Low-Cost Sensorik.- Von der Mikrofabrikation bis zur Evaluation".

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## Layered spray-coated nanostructured and enzyme modified electrodes for utilisation in self-powered biosensor units

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The utilisation of conductive nanoparticles (NP) such as indium tin oxide (ITO) and antimony doped tin oxide (ATO) for electrode surface structuring is a well-known approach in electrochemical systems [1]. Primarily, surface nanostructuring leads to a higher surface area and an enlarged interfacial area between electrolyte and electrode surface. In the perspective of using such electrodes in enzymatic biofuel cells or biosensors, the increased interfacial area results in a concomitant higher amount of immobilised enzyme, e.g. in productive direct electron transfer regime leading to potentially enhanced measurable currents and lower detection limits for the analyte in question. Furthermore, nanoparticle-modified electrodes exhibit high capacitances which make them interesting for self-powered biosensors or biofuel cells with integrated charge-storing capacitors [2].

We propose an improved system for the modification of ITO electrodes with semiconductor NP with potential applications for electrochemical biosensors and biofuel cells. For nanoparticle deposition, we developed a spray-coating system which allows us to automatically and precisely cover the flat ITO electrode surfaces with homogeneous layers of nanoparticles. Subsequently, the ITO/ATO NP nanostructured electrodes were modified with a top layer of glucose oxidase (GOx), embedded in a redox polymer.

Electrochemical investigation of the nanostructured and enzyme modified electrodes was performed in phosphate buffered saline showing high catalytic activities after adding increasing concentrations of glucose. The high capacitance of the nanostructured electrodes makes them suitable for storing the energy generated during glucose conversion, similarly to the processes as described recently in a Nernstian biosupercapacitor [3]. This opens the possibility to use the developed electrodes not just as sensing units but also for self-powered biosensors. Together with a suitable (bio)cathode the development of such an autonomously working glucose biosensor is demonstrated utilising sprayed ITO/ATO nanostructured and layered electrodes. Moreover, due to their high optical transparency, these electrodes may additionally be applied in charge storing (bio)solar cells, as suggested recently [4].

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## From insights into the potential-pulse assisted surface modification to fast and reproducible multi-probe DNA chip preparation

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Future point-of-care devices have to link high-quality performance with speed, simplicity and low production costs<sup>1</sup>. Production of DNA modified chips using base-by-base DNA synthesis and spotting techniques have numerous advantages. Nevertheless, the need for expensive equipment and complex chemical synthesis of protected bases in case of base-by-base synthesis or special working conditions coupled with positioning issues of the spotting technique leave space for further improvement.

We present the preparation of DNA chips for detection of multiple DNA sequences that eliminates the need for local precise positioning or chemical pretreatment of probe DNA sequences. During the modification with different DNA probes the whole 32-electrode chip is exposed simultaneously to each solution. Electrodes are modified using Au-S chemistry and potential-assisted surface modification procedure<sup>2,3</sup>. The procedure is based on ion stirring created by fast switching between carefully selected potentials, positive and negative from the potential of zero charge of the DNA-modified Au surface during the modification. Ion stirring leads to significantly accelerated immobilization of both charged and uncharged molecules, such as DNA and thiol derivatives. Using this protocol we can reproducibly control the surface modification in a very short time. Both DNA immobilization with desired coverage and subsequent thiol passivation are achieved in the range of seconds to minutes. In addition to the very efficient surface passivation, fast potential pulse-assisted surface cleaning used before the modification of the electrode of interest, prevents cross-talk between electrodes.

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## Carbon nanoparticles as colorimetric labels in on-chip immunoassays

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The present study demonstrates the modification of carbon black and its usage as a detection label in colorimetric immunoassays in a microarray format. We show the feasibility using a binding inhibition assay for histamine, a marker of food freshness and quality and sandwich immunoassays for the detection of protein markers IL8, DCN and VEGF associated with bladder cancer. The modified carbon nanoparticles (CNPs) were characterized by determining functional surface groups, elemental composition, size, surface charge and dispersion stability to investigate the influence of modification on the assay performance with the overall goal to optimize assay conditions and enhance signal strength. Carbon nanoparticles (CNPs) were oxidized and further modified with silanes with different functional end groups (thiol, epoxy, amine) 3-glycidoxypropyltrimethoxysilane being the most effective modification. Additionally, 1 pyrenebutanoic acid succinimidyl ester (PSE) was tested as conjugation agent allowing semiquantitative read-out by naked eye over a broad histamine range (0-600 µg/mL), similar to just oxidized CNPs. The dispersion stability was enhanced with acrylic polymer Atlox and dispersion agent xanthan. With oxidized CNPs read-out could be done by naked eye; otherwise an office scanner was needed for detection. Colorimetric detection of histamine and protein markers using carbon nanoparticles was finally evaluated with fluorescence detection and conventional colorimetric detection using Au nanoparticles and HRP as labels.

### **Acknowledgements**

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## SERS characterization of metal ions-induced dimerization of p-Aminothiophenol on gold nanoparticles

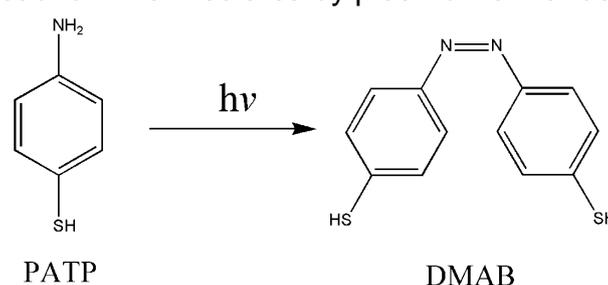
Zhiyang Zhang,<sup>1,2</sup> Virginia Merk,<sup>1</sup> Ulrich Panne,<sup>1,2</sup> Merwe Buurman,<sup>2</sup> and Janina Kneipp<sup>1,2</sup>

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Dimercaptoazobenzene (DMAB) can be formed from p-Aminothiophenol (PATP) on noble metal nanoparticles by a reaction that can be influenced by localized surface plasmons.[1] Many reaction parameters were shown to influence the pathway of DMAB formation. However, the mechanism of DMAB formation is still unclear.

The mechanism of DMAB formation is studied by surface enhanced Raman scattering (SERS). It is very interesting to delineate effects due to properties of the reacting molecular species and those related to the surface properties of the plasmonic nanostructures. Currently, we achieve this e.g., by studying the roles of metal ions in the formation of DMAB on gold nanoparticles under different parameters (such as pH, laser power). Our experiments were conducted with the usage of nanoparticle solutions and immobilized nanoparticles.[2] We perform this work, since we aim at figuring out the mechanism of plasmonic catalysis and controlling the reaction conditions precisely, exploiting the multifunctional properties of different plasmonic nanoparticles.[3] In our poster, we will show and discuss SERS data obtained in experiments at varying reaction conditions, specifically in the presence of different metal ions. Our results have implications for future sensitive detection of different reaction intermediates by plasmon-enhanced spectroscopy.



*Fig. 1: Photo-induced dimerization of PATP*

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## DNA immobilization strategy and hybridization kinetic measurement on gold SPRi sensorchip

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We were interested to develop surface plasmon resonance imaging (SPRi) chips for a panel of microRNA strands of diagnostic relevance. However, microspotting of terminal thiol labelled DNA onto gold needs careful optimization to overcome problems related with the nonspecific surface adsorption of DNA strands [1] as well as to establish optimal surface densities for subsequent hybridization with microRNAs [2]. Here we show that the sensitivity and selectivity of the hybridization assay as well as the kinetic parameters of the hybridization may vary in a large extent depending on the DNA probe immobilization. While most optimization of end point based hybridization assays are considering the hybridization efficiency as dominant criteria, here, by taking advantage of the kinetic information provided in high throughput manner by SPRi we are able to show comprehensive data relating all analytical performance parameters to the microspotting conditions. The study involved microspotting of DNA probes both as thiol labelled single-stranded DNA (ssDNA) and in prehybridized form with a complementary DNA (phDNA). The latter was found to self-regulate the optimal surface density of DNA probes at sufficiently high spotting concentrations after removal of the prehybridized complementary strands, i.e., activation of the DNA probes. In contrast in case of spotting ssDNA probes the hybridization efficiency for microRNA showed a sharp maximum as function of the probe concentration. While the optimal probe concentration may seem the one corresponding to the maximal signal the kinetic analysis reveals that the binding strength is much decreased ( $K_d$  increased).

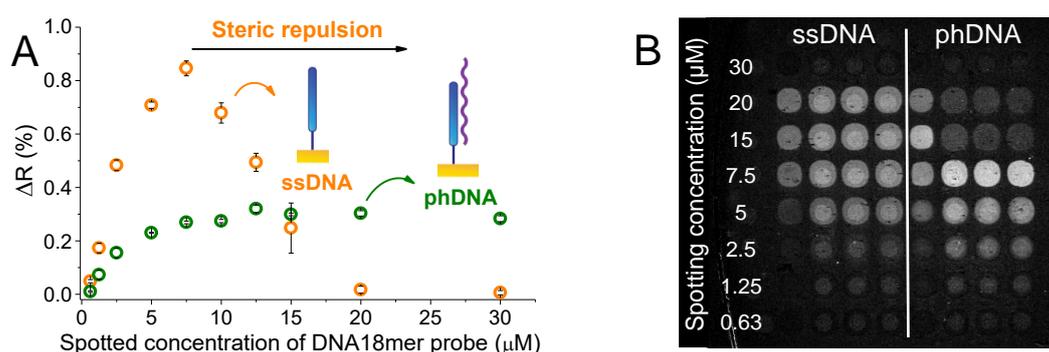


Fig. 1: **A** SPR response of DNA spots to microRNA. The DNA immobilization form indicated on the graph. **B** Typical SPR image of DNA spots immobilized in different concentration ( $\mu\text{M}$ ) and form (ssDNA or phDNA).

### Acknowledgment

This work was supported by the Lendület program of the Hungarian Academy of Sciences (LP2013-63).

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## Peptide-modified nanoporous gold membranes for potentiometric sensing of copper(II) ions

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Ion-selective electrodes (ISEs) are widely used for selective measurement of ion activities in clinical and environmental analysis [1]. Most often these electrodes take advantage of the excellent selectivity of a highly lipophilic ionophore that is incorporated in hydrophobic plasticized polymer membrane. While there is large range of natural ligands with excellent ion-selectivities, the hydrophobic membrane matrix impedes the use of such hydrophilic complexing agents. Therefore, we propose a new membrane construction that is based on the use of gold nanoporous membranes with average pore diameter of 10 nm to which thiol or disulfide functionalized compounds, regardless of their hydrophobicity can be linked through the formation of self-assembled monolayers. We have shown earlier that such nanoporous membranes modified with a mixed monolayer of an ionophore, cation-exchanger and a hydrophobic compound can form the basis of silver ion-selective electrodes with excellent selectivities[2]. Here we show that the concept can be extended to hydrophilic ligands as demonstrated by using a hydrophilic peptide, Gly-Gly-His, known to bind Cu with considerable selectivity [3]. This tripeptide is further linked to a cysteine that provides the thiol groups for the immobilization onto the gold surface. After optimizing the ratio of the thiol-labeled components, the membranes were inserted in conventional Philips electrode bodies to construct liquid contact ISEs. The potentiometric investigation revealed Nernstian response for copper(II) ions in the  $10^{-3}$  to  $10^{-5}$  M region, with submicromolar limit of detection (ca.  $10^{-7}$  M). The selectivity of the peptide modified nanoporous membrane ISEs matched that of the state of the art ionophore-based copper(II) ion selective electrodes for the most relevant cations. Furthermore, as all active components are covalently bound to the nanoporous support, the proposed peptide-based nanopores are expected to exhibit longer lifetime than the conventional plasticized polymeric membrane based ISEs, from which the active components continuously leach during use[4].

### Acknowledgment

This work was supported by the Lendület program of the Hungarian Academy of Sciences (LP2013-63).

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## High-throughput R2R production of disposable, low-cost electrodes for EIS, biosensors and electrochemical immunoassays

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Automated mass production of disposable electrodes at low-cost has been key to the market success of electrochemical glucose sensors for the diabetes home testing market. This fact notwithstanding, low-cost, quality electrodes for low-volume applications and research, are still an unmet need. Current commercial electrodes for biosensor research are produced with screen-printing technology or thin-film technology in a variety of geometries and materials, at prices starting from approx. 2 € per piece. For many applications, these devices are too expensive and cannot compete with e.g. lateral flow immunoassay technology (total production costs between 10 and 40 Cent per unit).

Reel-to-reel (R2R) production workflows can be easily scaled-up and integrated into mass-production lines. They could be one route to low-cost, mass-production of (bio)sensors in the future. In this work we demonstrate a simple two-electrode design that was printed on polymer sheets in a R2R process on a high-throughput rotogravure printing machine. Printing speeds of 10 m/min and 25 m/min have been investigated. Electrode thickness is mainly determined by the cell depth of the gravure printing cylinder. Electrode thicknesses of several micrometers have been achieved for a cell depth in the range of 60-80  $\mu\text{m}$ . Both the working and combined counter/pseudoreference electrodes consist of a graphene-based ink.

This simple design proved to be useful for different applications: As an electrode for electrochemical impedance spectroscopy (EIS) on live cells in a cell culture vessel as well as the detection system for an electrochemical HbA1c immunoassay using the homogeneous redox quenching immunoassay format [1]. For the former application, EIS was successfully used for online monitoring the degree of confluency of different cell types. For the latter application, freeze-dried immunoassay reagents were integrated with the electrode chip in a rectangular channel compartment. Electrochemical performance data of the graphene electrodes in EIS and immunoassay application are presented. Future improvements including the direct printing of electrodes and biomaterials in one workflow using a two-ink R2R rotogravure printing machine and a novel, fast-drying ink formulation are discussed.

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## UV/Vis-Spectroelectrochemical Investigation of Cellobiose Dehydrogenases (CDHs) from different ascomycetes

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In our lab we are using a homemade spectroelectrochemistry setup in order to determine the redox potential of different oxidoreductases which are of interest for applications in electrical biosensors and biofuel cells. In the past we have determined the redox potentials of glucose oxidase from *Aspergillus niger* at different pH (Vogt et al., Anal. Chem., 86, 15, 7530-7535, 2014).

Currently we are investigating the redox potentials of other important enzymes such as cellobiose dehydrogenases from ascomycetes. These CDHs catalyze the oxidation of glucose and are able to undergo direct electron transfer at different electrode materials. We will present details of our spectroelectrochemical measurements performed with different CDHs at several pH values. In order to ensure electrochemical equilibrium, the composition of the mediator has to be carefully optimized and will thus be discussed in detail. Due to the LabView routine controlling the setup, long periods for the establishment of electrochemical equilibrium can be chosen and thus, measurements with low concentrations of mediator mixtures may be performed. Currently we are working on the pH-dependent investigation of CDH from *Corynascus thermophiles* between pH 4.5 and pH 9.

Depending on the progress of our research, we may also present the results of spectroelectrochemical measurements performed with other enzymes.

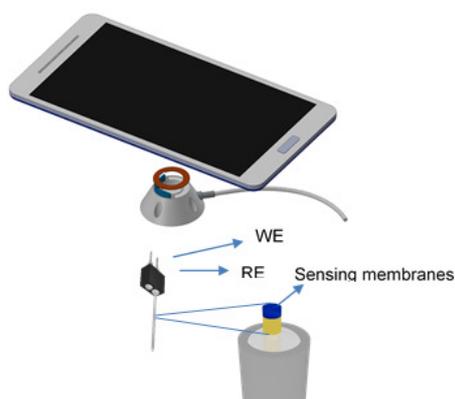
## Batteryless pH and Lactate sensing

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Due to technological maturity and recent proliferation of interdisciplinary research, a plethora of biochemical sensing platforms with wireless connectivity has been developed. This results in promising deployment opportunities in areas of point-of-care diagnosis and data capture for sports analytics.

We present a miniaturised, batteryless readout system suitable for biosensor interfacing. Sensor readings can be carried out with smartphones and tablets equipped with Near Field Communication (NFC) hardware, supporting remote powering of the sensor interfacing electronics and data readout. The development of micro-sized chemical sensing system that employs simultaneous detection of pH and lactate was used to demonstrate the effective operation of the batteryless device. In vitro sensor calibration and validation have been carried out and presented in this paper.

The electronic readout system is modularized with circuits divided between three PCBs to facilitate flexible deployment in a range of miniaturised embodiments. Here we demonstrate a cap-shaped embodiment for the electronics with needle-based pH and lactate sensing. The pH and lactate sensors were electrochemically stable throughout our experiments and demonstrate good linearity in the biologically relevant ranges.



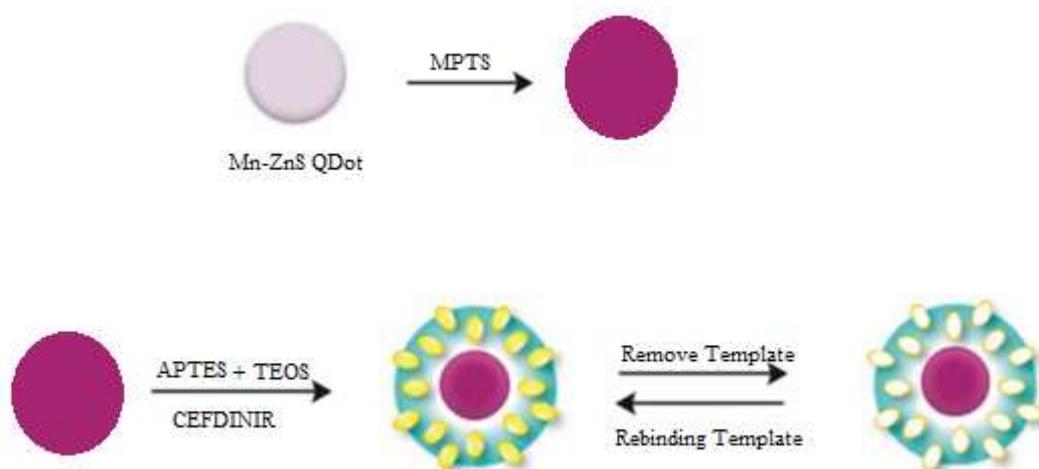
*Fig. 1. Construction of the device.*

## DEVELOPMENT OF A PHOSPHORESCENCE SENSOR BASED ON SURFACE MOLECULARLY IMPRINTED Mn-DOPPED ZnS QUANTUM DOTS FOR SELECTIVE RECOGNITION OF CEFDINIR

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Molecular imprinting has been shown to be a useful technique for the formation of recognition sites on a three-dimensional polymeric network instead of antibodies. The gold standard of generating sensors based on quantum dots (QDs) is a novel, reproducible, selective, and currently developing area. Several experiments were performed for synthesizing molecularly imprinted polymers (MIPs) and QD composites for which the high sensitivity of luminescent QD is combined with the high selectivity of MIP. The Mn-doped ZnS QDs (Mn:ZnS QDs@MIP) having much more significance thanks to their phosphorescence property. In this work, a novel phosphorescence sensor based on surface molecularly imprinted Mn-doped ZnS QDs has been developed for the determination of Cefdinir (CEF). CEF is a semi-synthetic, broad spectrum, third generation cephalosporin antibiotic [1]. It is used for the treatment of upper and lower respiratory tract infections. CEF, 3-aminopropyltrimethoxysilane (APTES) and tetraethoxysilane (TEOS) were used as a target molecule, functional monomer, and cross-linker, respectively. The developed phosphorescence sensor was successfully applied to the determination of CEF in various media.



**Figure 1.** Schematic illustration of Mn:ZnS QDs@MIP.

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## Paper-based detection of C-reactive protein for point-of-care diagnostics

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Rapid treatment of diseases often is essential for saving lives. But this is only possible if an early diagnosis can be ensured. That's why analytical devices like lateral flow assay (LFA) which can be used for point-of-care testing (POCT) are so important. For this reason, research is currently focused on their development. On the basis of low costs and simple handling, LFAs are suitable as rapid tests also in developing countries.

One of the best known examples for POCT is the pregnancy test which demonstrates a high application potential of rapid tests [1]. But there are also other rapid tests like the LFA for the detection of troponin I as myocardial infarction marker [2]. C-reactive protein (CRP) is present in blood and acts as unspecific marker for inflammatory diseases. Depending on the concentration of CRP it is possible to distinguish between bacterial or viral infections.

Therefore, the aim of the presented work is the quantitative detection of C-reactive protein, by using microfluidic paper-based analytical devices ( $\mu$ PADs). The detection antibodies are labeled with gold-nanoparticles so that signals can be read out from integrated cameras in smartphones.

Our results will demonstrate that semiquantitative detection of CRP is possible on  $\mu$ PADs. Thereby, the so called "Hook effect", which is responsible for false negative results, was considered why three different recognition structures (IgG Antibody, CRP Antigen and Antibody to CRP) were spotted in series. By correlation of signal intensity and saturation of these three spots a statement shall be made about the concentration of CRP in the sample [3]. As an example, Fig 1 shows the result of a triple measurement of the lateral flow assay for a CRP concentration of 1  $\mu$ g/mL in the sample.

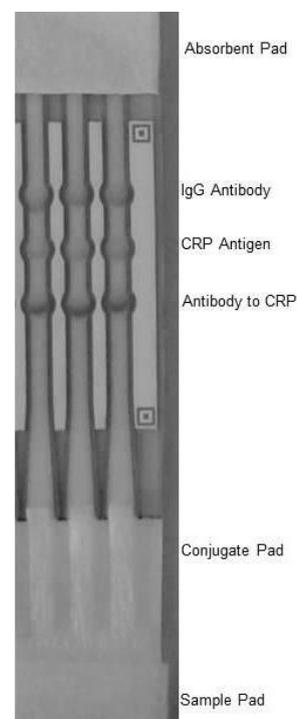


Fig. 1: Result of the lateral flow assay with a CRP concentration of 1  $\mu$ g/mL in the sample

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## Responsive polymer electrodes – Influence of temperature, pH and peptide binding

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This study deals with a thermally responsive, polymer-based electrode. Such systems are interesting with respect to the design of switchable reaction schemes on sensing electrodes. The key component is a surface-attached, temperature-responsive poly(oligoethylene glycol) methacrylate (poly(OEGMA)) type polymer containing photoreactive benzophenone- and carboxy group- side chains. Turbidimetry measurements have been applied to investigate the responsive behavior of the polymer in aqueous media. For polymer fixation on gold electrodes chemisorption of the photoreactive 2-(dicyclohexylphosphino)benzophenone (DPBP) and subsequent photocrosslinking has been used. The electrochemical behavior of the resulting polymer electrode has been investigated in buffered  $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$  solutions at room temperature and under temperature variation by cyclic voltammetry (CV). The CV experiments demonstrate that with raising temperature structural changes of the polymer layer occur. This can be mainly seen from the maximum current values. Repeated heating/cooling cycles analyzed by CV measurements and pH changes analyzed by quartz crystal microbalance with dissipation monitoring (QCM-D) reveal the reversible nature of the restructuring process.

The immobilized films are further modified by covalent binding of two small biomolecules - a hydrophobic peptide (HA-TAG) and a more hydrophilic one (FLAG-TAG). These attached components influence the hydrophobicity of the layer in a different way - the resulting change of the temperature behavior has been studied voltammetrically. It can be clearly concluded that the hydrophobic peptide has a more pronounced effect on the temperature-induced voltammetric current changes. Thus, the study confirms a previous investigation which shows that a hydrophilic antibody binding only slightly shifts the responsive behaviour [1]. For a sensorial application this means that significant changes of the film can be expected when a bimolecular recognition reaction changes significantly the hydrophilicity-hydrophobicity balance.

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## Synthesis of Xylitol-stabilized gold nanoparticles: a quantitative and sensitive method for xylitol detection in oral fluid by means of colorimetric assay

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Here we present the controlled synthesis of xylitol-stabilized gold nanoparticles (AuNPs) for the sensitive and reproducible detection of xylitol in oral fluid. The optical detection is achieved by following the early stage kinetic formation of gold nanoparticles at fixed wavelength by spectrophotometry. In fact, Xylitol in alkaline solution is able to induce the fast reduction of Au(III), which leads to highly stabilized and spherical AuNPs suitable for colorimetric assays.

Xylitol is an assessed anticariogenic agent, safe and effective in controlling dental caries [1]. Therefore, the detection of minimal salivary levels of this polyol along time is crucial to set up effective administration [2].

Traditionally, the quantitative detection of xylitol in saliva/oral fluid is carried out by commercially available enzymatic tests, cost and time expensive.

The sensitivity of the developed approach is comparable to enzymatic test, but allows to get quantitative results in minutes, in a cheap and green manner by recording absorbance intensity at 520 nm. The coefficient of variation is ~7% within the calibration range, i.e. 10-1 mg/mL, with an estimated detection limit of ~80 mg/L both in standard samples and in real oral fluid.

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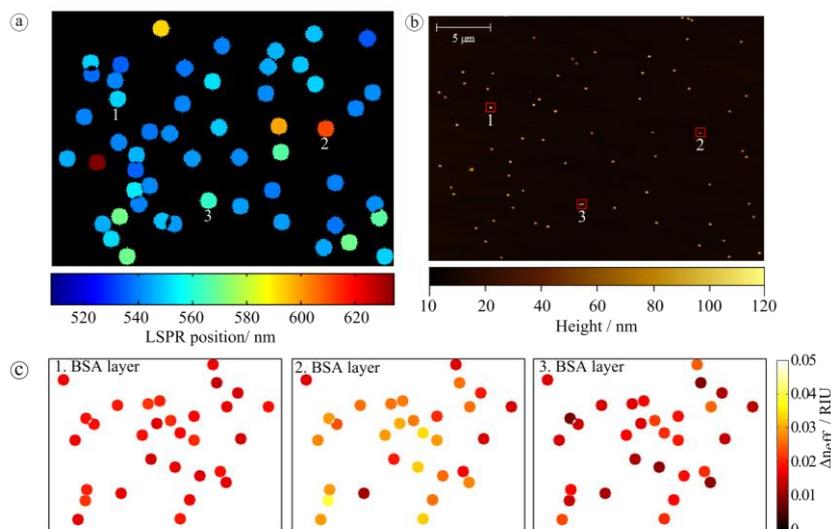
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## Hyperspectral imaging of plasmon resonances for biosensing

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We present a Fourier transform imaging spectrometer for the parallel read-out of sensors based on noble metallic nanoparticles supporting localized surface plasmons, which are oscillations of the particles' conduction electrons and can be excited by a free propagating light beam. The localized surface plasmon resonance (LSPR) frequency, which depends on the nanoparticles' size, shape, material and the local dielectric environment, manifests itself as a peak in the extinction and scattering spectrum of the particle. The sensor principle is based on the sensitivity of the scattering/extinction spectra upon changes of the local refractive index around the nanoparticle, such changes will be induced upon binding of molecules. The spectroscopy of metal nanoparticles shows great potential for label-free sensing. In combination with a microfluidic system our hyper-spectral imaging system allows the full spectroscopic characterization of many individual nanoparticles simultaneously. We experimentally quantified (incorporating atomic force microscopy as well) the correlation between geometry, position of plasmon resonance and sensitivity of the particles. We were able to follow the adsorption of protein layers and determined their spatial inhomogeneity with the help of hyperspectral imaging of single nanoparticles.



*Fig. 1: (a) Lateral distribution of the elected particle ensemble, each particle is color coded according to its LSPR peak wavelength. (b) AFM-image of the region of interest shown in a). (c) False color image displaying the change of the effective refractive index on 30 single nanoparticles induced by the successive adsorption of BSA layers [1].*

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## Introduction to wide field surface plasmon microscopy of nano- and microparticles: features benchmarking, limitations, and bioanalytical applications.

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Detection of nano- and microparticles is an important task for chemical analytics, food industry, biotechnology, environmental monitoring and for many other fields of science and industry. For this purpose, a method based on the detection and analysis of minute signals of surface plasmon resonance images due to adsorption of single nanoparticles was developed. This new technology allows one a real-time detection of interaction of single nano- and microparticles with sensor surface. Adsorption of each nanoparticle leads to characteristic diffraction image whose intensity depends on the size and chemical composition of the particle. The adsorption rate characterizes volume concentration of nano- or microparticles. Large monitored surface area of sensor surface enables a high dynamic range of counting and to a correspondingly high dynamic range in the concentration scale. Depending on the type of particles and experimental conditions, the detection limit for aqueous samples can be below 1000 nanoparticles per microliter. Stable analysis of nanoparticles in very complex environment (fruit juice, cosmetic formulations) was demonstrated.

The method can be applied for ultrasensitive detection and analysis of nano- and microparticles of biological (bacteria, viruses, endosomes), biotechnological, (liposomes, protein nanoparticles for drug delivery) or technical origin. Applications in biosensing and bioanalytics will be discussed.

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## A piezoelectric single spot cell printing technique in the picoliter range for different mammalian cell types

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The piezoelectric, contactless printing method in the picoliter range can be used in complex and innovative research areas such as bioprinting and tissue engineering [1].

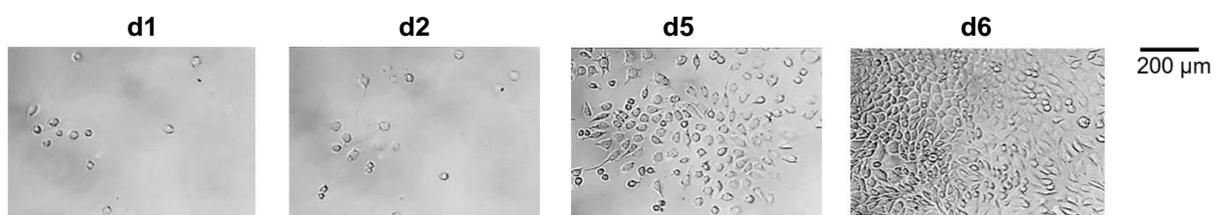
The feasibility of printing and positioning of different types of mammalian cells was successfully proven in several studies [2]. Thus, the piezoelectric, contactless printing technology obtains a high potential to overcome current limitations in the field of bioprinting in order to construct biosynthetic, implantable organs or tissue constructs.

In this research, a commercially available automated non-contact dispensing system (Spotter) SciFLEXARRAYER SX from Scienion was used for the piezoelectric printing of monocytes (U-937) and fibroblasts (L-929). An assay was developed, which allows the evaluation of the cell viability within printed single spots on targeted surfaces.

The results of the assay showed that the monocytes obtained a survival rate of 96 % after printing them onto different types of surfaces. In contrast, the fibroblasts showed a much smaller; very fluctuating survival rate in the same assay.

Therefore, the cell proliferation of the adherent fibroblasts (L-929) in a printed single spot was observed for several days (Fig.1). It was shown that the cells survived the printing and positioning process. The cells attached to the surface of the culture chambers and proliferated during the whole observation period.

The results of this research can possibly be applied to the vast field of bioprinting, tissue engineering or the field of cell-based biosensors.



*Fig. 1: Cell proliferation of piezoelectrically printed fibroblasts (L-929) in a co-culture chamber. (d1-d6: days after printing)*

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## Application of scanning photoelectrochemical microscopy for the characterization of photosynthetic enzyme complexes

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Scanning electrochemical microscopy (SECM) is an important scanning probe technique used for the study of electrochemical properties of a surface. The further demand of investigating more materials and properties that are of fundamental interest in electrochemistry and other fields requires the development of new methodologies on the basis of SECM principles to surmount the limits of the technique in addressing a range of complex and current problems.

The requirements for the analysis of photoactive materials promote the integration of a light source into a conventional SECM set-up. Variation of electrochemical properties of the analyzed photoactive materials is thus visualized by the SECM tip as a function of surface location over the scanned substrates. However, irradiation of the entire sample generates a photocurrent based on the averaged properties of the entire surface area, making it impossible to address information of particular regions of a sample and/or localized changes<sup>1,2</sup>. Moreover, global illumination over a relatively long time required for the measurement is generally associated with problems like degradation or photocorrosion of the analyzed samples<sup>2</sup>. Based on these limitations, a scanning photoelectrochemical microscopy (SPECM) set-up was implemented allowing localized interrogation of photoactive samples e.g. for the analysis of layers of photosynthetic enzyme complexes on suitable electrodes. For this, the SECM tip, which is positioned in close distance to the interrogated surface area, is used simultaneously as electrochemical probe and as a source for irradiation. Examples of the applicability of this powerful technique will be shown, ranging from the local collection of evolved oxygen at illuminated photosystem 2 (PS2) layers to the evaluation of surfaces modified with photosystem 1 (PS1) and PS1-Pt nanoparticle complexes for light-induced hydrogen evolution<sup>3</sup>.

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## Development of microarray-based assays for the detection of circular RNAs in clinical research

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Circular RNAs (circRNA) represent an upcoming promising class of biomolecules for biological and clinical research. This new species of exonic, endogenic and covalently closed RNA is highly stable, oftentimes conserved and can be the dominant product of a given genomic locus<sup>[1-3]</sup>. By now, it was shown that circRNAs are present in various organisms ranging from archaea to higher animals including mice and humans<sup>[1-4]</sup>. The tissue specific expression and the potential of binding miRNAs and RNA-binding proteins suggests an important regulatory function of circRNAs<sup>[1,5]</sup>. Because of the remarkable stability of circRNAs, they represent a potential new, promising class of biomarkers for different diseases, for example Alzheimer's disease<sup>[2]</sup>. So far the identification and quantification of circRNAs is mainly based on different types of high-throughput RNA sequencing. This approach however is time-consuming, cost-intensive and requires highly specific expertise and is therefore not an option for a quantitative and qualitative high-throughput screening of circRNAs as biomarkers in clinical research.

Microarray technology in contrast is a well-established, easy to use tool in the field of bioanalysis and diagnostics.

Its main advantage is the applicability to high-throughput screening and the ability to detect many different parameters at the same time, without the need of extensive use of analyte material. Above that, usage of microarrays in lab-on-chip systems gives the opportunity for high-throughput screening of complex circRNAs signatures.

The primary goal of our research is the development of a microarray based assay for the detection of circular RNAs, allowing a straight-forward use in the field of clinical and basic research. In contrast to the basic research, circRNAs are largely unknown in clinical research not least because of the elaborate, not easy to use sequencing based detection methods. Our microarray based system will solve this problem via special designed, splice site specific analysis. This assay will be the basis for the development of cost-effective, rapid and easy to use in-vitro diagnostic tools.

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## Dye-stained lifetime-encoded polymer microbeads for time-resolved flow cytometry application

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Flow cytometry is a standard analytical tool for biological research and in medical applications [1]. There are different requirements triggering recent device and method development depending on the desired field of application [1]. One trend is governed by the need for an increasing number of simultaneously detectable codes, i.e., fluorescent labels. The other one focuses on cost-effective methods and development of miniaturized, portable devices.

Fluorophore encoding is usually based on spectral encoding [2]. However, this approach is hampered by, e.g., spectral crosstalk [3]. Additionally, the sensitivity of fluorescence intensity measurements to fluctuations in excitation light intensity and dye concentration limits the achievable number of detection channels [3]. Moreover, spectral multiplexing typically requires several costly excitation light sources. Lifetime multiplexing and the discrimination between different encoding fluorophores and carrier beads based on their fluorescence decay kinetics could present an innovative alternative [2-4]. Encoded beads, i.e., beads with lifetime codes corresponding to the surface chemistry, have been employed to evaluate the feasibility of this approach with a custom designed flow cytometer equipped with a pulsed light source and a fast detector for time-resolved measurements in a flow.

In a first step, we used steady state and time-resolved photoluminescence measurements for the spectroscopic characterization of micrometer-sized dye-stained PMMA beads. Subsequently, the potential use of these microbeads for flow cytometry applications was analyzed with a prototype flow cytometer with lifetime detection.

With our proof-of-concept studies, we could demonstrate that lifetime discrimination and simultaneous readout of a ligand fluorescence signal for analyte quantification is feasible with a set of dye-stained polymer microbeads at single wavelength excitation. These studies are expected to pave the road for new applications of fluorescence lifetime multiplexing in time-domain flow cytometry and bead-based assays in general.

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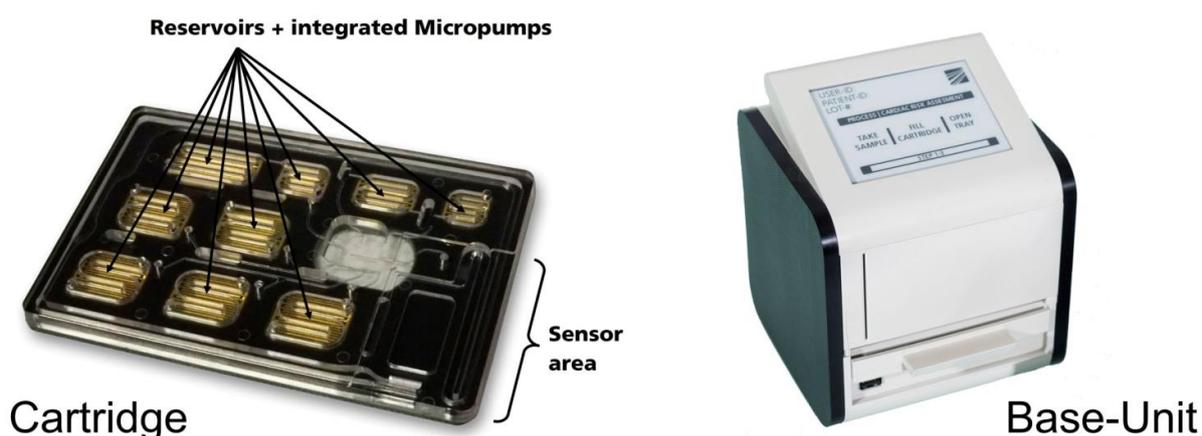
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## Highly-integrated Lab-on-a-chip System for Multiparameter Analysis

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Lab-on-a-chip multiplex assays allow a rapid identification of multiple parameters in an automated manner. Here we describe a lab-based preparation followed by a rapid and fully automated DNA microarray hybridization and readout in less than 10 minutes using the Fraunhofer in vitro diagnostics (IVD) platform to enable rapid identification of bacterial species and detection of antibiotic resistance. The use of DNA microarrays allows a fast adaptation of new biomarkers enabling the identification of different genes as well as single-nucleotide-polymorphisms (SNPs) within these genes. In this protocol we describe a DNA microarray developed for identification of *Staphylococcus aureus* and the *mecA* and *mecC* resistance genes<sup>[1]</sup>.



**Fig. 1:** Fraunhofer IVD platform for fully automated DNA microarray hybridization and analysis<sup>[2]</sup>. **(Left)** Lab-on-a-chip cartridge (size: 60 x 40 mm) with 9 reservoirs and integrated micropumps, microfluidic channels, thermal control elements, electronics and a sensor area for microarrays with up to 400 spots<sup>[3]</sup>. **(Right)** Base-unit (size: 14 x 14 x 14 cm) for control, readout and data analysis of the lab-on-a-chip cartridge. Results can either be transferred to a computer or analyzed directly and presented on the display.

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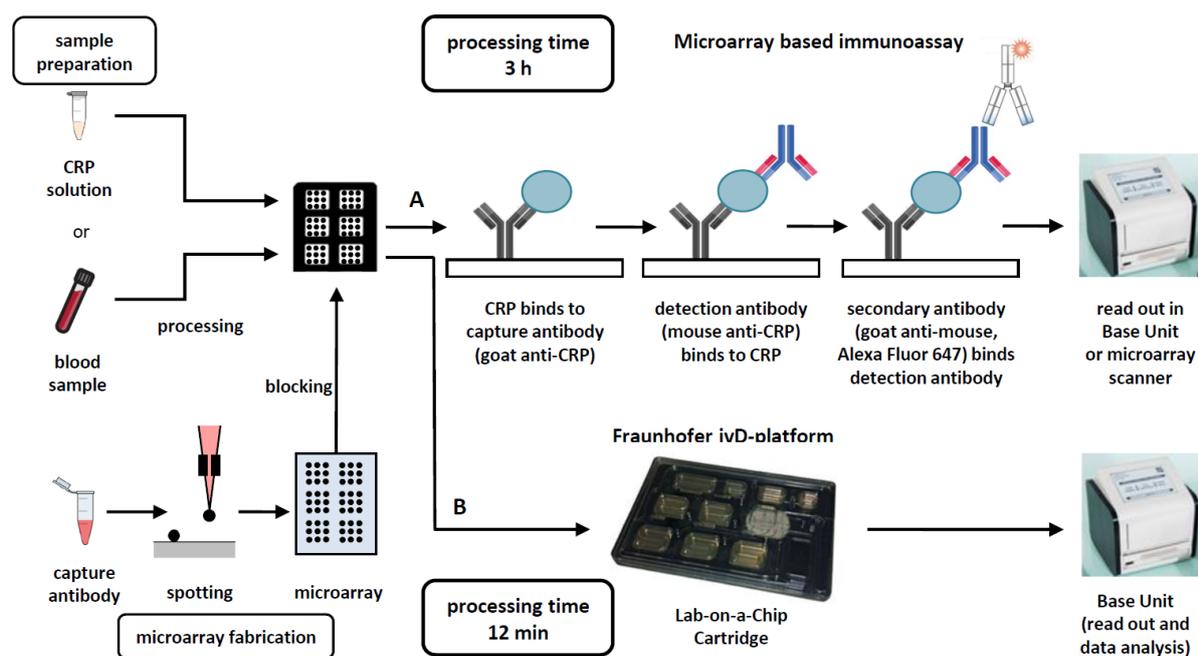
## Lab-on-a-chip proteomic assays for psychiatric disorders

**Harald Peter<sup>1</sup>, Julia Wienke<sup>1</sup>, Paul C. Guest<sup>2</sup>, Nikitas Bistolas<sup>1</sup>, Frank F. Bier<sup>1</sup>**

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Lab-on-a-chip assays allow rapid identification of multiple parameters on an automated user-friendly platform. Here we describe a fully automated multiplex immunoassay and readout in less than 15 minutes using the Fraunhofer in vitro diagnostics (ivD) platform to enable inexpensive point-of-care profiling of sera or a single drop of blood from patients with various diseases such as psychiatric disorders<sup>[1]</sup>.



**Fig. 1** Schematic of a microarray based immunoassay for the detection of CRP, including sample preparation and microarray fabrication. **A)** Laboratory based microarray protocol, using glass slides as support for the microarray, followed by several washing steps and a read out on a microarray scanner or the Fraunhofer ivd-platform Base Unit (3 hours processing time). **B)** Fully automated lab-on-a-chip based immunoassay allowing a detection of CRP in blood directly after 12 min. All antibodies and washing solutions are pumped automatically over the microarray within the cartridge followed by a read out and data analysis within the base unit.

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## Multifunction paper-based analytical device for bacterial cultivation and determination of nitrite

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Point-of-care testing (POCT) for uropathogen detection and chemical screening provides great benefits for diagnosis of urinary tract infections (UTIs), especially in the developing world. Generally, common microorganism in both uncomplicated and complicated UTIs is *Escherichia coli*. The goal of this work was to develop a portable and inexpensive paper-based analytical device (PAD) for simultaneous cultivation of bacteria and rapid biochemical test for nitrite. Nitrite test was included in the device to confirm the presenting of gram negative bacteria and it was determined by Griess reaction. The PAD was fabricated by using a wax printing technique to create a pattern on Whatman No.1 filter paper, which was then combined with a cotton sheet for supporting bacterial growth. The linear range for nitrite detection was in the range of 0.05-1.6 mg/dL ( $r^2 = 0.989$ ). The coefficients of variations (CVs) were 9.11% and 8.53 % (n=20) when the assay was performed at nitrite concentration of 0.5 and 1 mg/dL, respectively. Scanning electron microscope (SEM) analysis was used to assure the growth of bacterial cells on the PAD and results demonstrated that the bacteria can grow and form a cluster on cellulose fibers within 2 hours. Enzyme  $\beta$ -glucuronidase specifically produced by *E. coli* catalysed the conversion of a colorless substance, 5-bromo-4-chloro-3-indolyl- $\beta$ -D-glucuronide sodium salt (XG), that was pre-immobilized on the PAD, into a blue pigment. Based on the intensity of the blue pigment, the proposed devices were able to quantify the bacteria in the range of  $10^4$ - $10^7$  CFU/mL within 6 hours. Moreover, the identification of pathogen using this device offered the selectivity to only *E. coli* because *Proteus mirabilis* and *Staphylococcus saprophyticus* at concentrations  $> 10^5$  CFU/mL could not produce the blue colonies on the proposed PAD. In conclusion, the paper-based analytical device for UTI screening provides a rapid, cost-effective diagnostic method for remote areas.

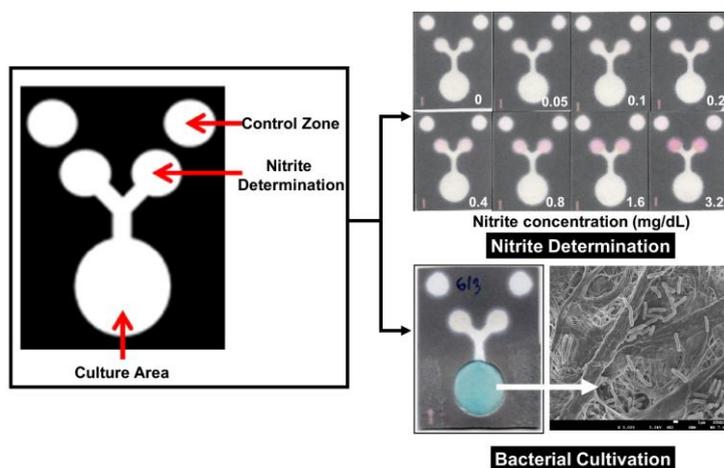


Fig.1: The paper-based analytical device for bacterial cultivation and quantification of nitrite

## Stability monitoring of casein layer on hydrophilic SiO<sub>2</sub> surface using Quartz Crystal Microbalance with Dissipation

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There exists an increasing interest in understanding the effects of milk proteins on the texture and flavour of dairy products. Alpha, beta and kappa caseins are most milk protein group are present in the micelle-like particles. Each of caseins is thought to carry specific function in stabilisation of casein micelles. Our research was concentrated on the understanding of dynamics of self-assembly of beta and kappa caseins and the stability of casein layers on hydrophilic SiO<sub>2</sub> surface using 5 MHz quartz crystal microbalance with the dissipation mode (QCM-D). The surface of SiO<sub>2</sub>/Au/Cr on 14 mm AT-cut quartz crystal was cleaned with a series of solvents and activated with oxygen plasma, rendering the surface hydrophilic. The oscillation of the crystal were stabilized around 5 MHz at 25°C under 50 µl/min flow of degassed PBS buffer. Then the solution was switch to flow of degassed 2.5 µg/min of casein in PBS buffer, stabilized at pH 7.4. The kinetics of self-assembly of casein on activated SiO<sub>2</sub> were monitored by following changes in value of fundamental, 3<sup>d</sup>, 5<sup>th</sup> 7<sup>th</sup> and 11<sup>th</sup> harmonics at 5, 15, 35 and 55 MHz respectively). Stabilization of fundamental frequency signalling the completion of the casein self-assembly was used as a marker for switching to the second flow of PBS buffer to remove loosely held casein molecules/micelles from the surface. On the next, test stage, we switch the flow to the test solution of PBS buffer stabilized at pH = 4.0, 6.0, 8.0 and 9.0 or 0.1 nM, 1 nM and 10 nM of protease. The stability of the casein layer would be disturbed as pH change or through protein cleaving peptide bonds. After frequency stabilization, the QCM was washed by flowing PBS buffer over it. The effect of trypsin, thrombin and plasmin proteases on the stability of casein layer was investigated. We demonstrated possibility to reliably detect presence of plasmin, naturally occurring milk protease, at the levels of <2 nM. This is comparable to the state of the art analytical techniques for plasmin detection. The tested approach presents a low cost alternative for the measurement of plasmin concentration, which serves as a measure of quality of milk and projected quality of dairy products .

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## **Polyelectrolyte microcapsules based sandwich assay: A more sensitive tool to commercial beads for the detection of proteins and nucleic acids.**

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A challenging and fascinating task is the development of non-invasive techniques for monitoring different biotechnological processes with the help of a novel tool that specifically detects intermediate or final products. Polyelectrolyte microcapsules produced by Layer-by-Layer assembly can serve as a universal platform for detecting various biomarkers, since their surface chemistry can be adjusted, making them suitable for a great variety of applications in biochemistry and cell biology. In this study, we focus on the development of a microcapsule-based novel single bead assay that uses flow cytometry as an optical readout. To produce a universal bio-sensing tool, the microcapsule surface is modified with detector molecules (e.g. antibodies, oligonucleotides etc.), allowing for specific recognition of an analyte. Here, we introduce new type of microcapsules modified with functional protein A and streptavidin. As a first example, the ability of detecting the disease biomarker beta-2 microglobulin in the fM to pM concentration range with the help of protein A-coated microcapsules is demonstrated. Protein A has high affinity for the F<sub>c</sub> domain of antibodies, thus resulting in their oriented immobilization on the microcapsule surface, which substantially increases probe sensitivity. In the second example, streptavidin-coated microcapsules are used for the detection of nucleic acids in the nM concentration range. Streptavidin binds specifically and tightly to biotinylated oligonucleotides, thus serving as a perfect platform for the detection of nucleic acids. In comparison with commercially available micrometer-sized beads made of polystyrene, our protein A-coated microcapsules detect the disease biomarker beta-2 microglobulin with a 500-fold higher sensitivity. Our assay allows rapid quantitative analyte measurement while providing high sensitivity and selectivity at very small sample quantities.

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## **Piezoelectric immunosensing sensing for detecting of the Alzheimer Tau protein biomarker**

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Human Tau protein detection is emerging recently because its role as one of the validated biomarker in the Alzheimer's disease (AD) diagnosis. The biological sample used for biomarker analysis is generally Cerebrospinal Fluid (CSF).

In this work, quartz crystal balance (QCM) immunosensor was developed for the rapid detection of human Tau protein (isoform 2N4R) both in buffer and artificial cerebrospinal fluid (aCSF) through direct and sandwich assay. Monoclonal antibody (clone 39E10) recognizing tau protein in its middle domain (amino acids 189-195) was immobilized on the gold surface of the QCM crystal as the primary antibody through self-assembled monolayer (SAM) of 11-Mercaptoundecanoic acid (MUA). Human Tau proteins were then captured by the immobilized antibodies, resulting in a change in the frequency. Monoclonal antibody (clone Tau 12) recognizing N-terminus of tau protein was used as secondary antibody to amplify the signal of the binding reaction between primary antibody and Tau protein. The QCM immunosensor could detect Tau protein in aCSF at the concentration of 50 nM through direct assay in 15 minutes and 25 nM through sandwich assay in 30 minutes, respectively with a the detection limit of 250 nM and 50 nM in aCSF respectively in direct and in sandwich assays.

This research demonstrated that piezoelectric-based sensing could be an interesting alternative approach for rapid detection of human tau protein in clinical samples. Future research is focused on the signal amplification methods to further improve system sensitivity to meet needs of practical clinical diagnosis

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## A wash-free, multiplex microbead assay for determination of emerging bioactive compounds in wastewater.

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Pollutants of low molecular weight, such as drug residues, are in the focus of water quality assessment: some of them, like carbamazepine are only partially degraded in wastewater treatment plants. [1] Thus, these pollutants can serve as marker substances for elimination efficiencies. Monitoring water quality demands for selective, high-throughput and multi-target analytical methods. Immunoassays, such as ELISA, offer the possibility to be highly sensitive and selective due to the specific recognition by and high affinity of target molecules to antibodies (Abs). Batch-wise processing in microtiter plates allows for the necessary high-throughput, however only a single analyte can be determined within one measurement.

To overcome these disadvantages, we developed a four-plex microbead-based flow cytometric assay, which is adaptable for the microtiter plate format. The modular and self-prepared bead support consists of polystyrene-core/silica-shell particles. [2] While, the polystyrene core is used for encoding, by introducing different amounts of fluorescent dyes, the silica shell creates a solid support for the immunoassay: The target analytes, three drugs, carbamazepine, diclofenac and caffeine and the fecal marker isolithocholic acid are coupled covalently to the surface via NHS chemistry to amino groups on the surface.

For determination of the pollutants, a mixture of specific Abs is incubated with the samples, to bind competitively on the “anchor” molecules on the surface of the beads or the analyte in solution. Bound antibodies are then visualized via fluorescent dye-labelled secondary Abs. Flow-cytometry allows for decoding of the beads and signal read-out, without washing the system. In order to decrease non-specific binding, we investigated different types of surface modifications, finding, that a PEG-based surface is suitable to support our immunoassay format. For maximum sensitivity, a design-of-experiment approach was chosen for optimization of the assay parameters. The resulting immunoassay is appropriate to quantify the pollutants in the low µg/L-range.

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## Liposome-based ECL Detection Strategies for Microfluidic Biosensors

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Liposomes have long been used as bioanalytical signal amplification system by entrapping marker molecules in their inner cavity. Here we are investigating liposomes modified with an electrochemiluminescent signal reagent,  $\text{Ru}(\text{bpy})_3^{2+}$ , on their outer surface as this will enable multimodal analysis and faster detection strategies.

Small liposomes with a mean hydrodynamic diameter of 142 nm, have been synthesized from phospholipids and cholesterol using a reverse phase evaporation method and characterized using dynamic light scattering and ICP-OES. A NHS-ester of  $\text{Ru}(\text{bpy})_3^{2+}$  was synthesized and covalently coupled to the  $\text{NH}_2$  groups on the liposomal surface. The synthesized liposomes contained a total amount of 4.5  $\mu\text{mol/L}$  of the signal reagent resulting in an average number of only 11 ruthenium molecules per single liposome. As the achieved coupling efficiency is only 10.5% more effort is currently put toward the optimization of this process in order to generate liposomes with varying numbers of ruthenium complexes bound and study optimal conditions.

Furthermore, a variety of assay strategies are investigated to best evaluate the capability of these tagged liposomes to perform ECL reactions on electrode surfaces. This includes the direct immobilization of liposomes on electrodes, as well as the lysis of liposomes on the electrode surface. Also, their ECL properties are compared to  $\text{Ru}(\text{bpy})_3^{2+}$  encapsulating liposomes and PAMAM dendrimers (polyamido amine) of generation 1.0 and 5.0, labeled with the same ECL marker, as they afford a similar enhancement strategy but are significantly smaller, 1.5 nm and 5.4 nm, respectively.

## Therapeutic drug monitoring of immunosuppressants in a novel laser induced fluorescence based miniaturized setup

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After a new organ (kidney, liver or heart) is transplanted, the patient has to receive immunosuppressive drugs to avoid an organ rejection. The drugs have a narrow therapeutic range, thereby their levels need to be monitored by therapeutic drug monitoring. During the first 2-3 days after a transplantation, the current state-of-the-art is to draw blood samples before each administration of the drug. The measurements are performed in a central laboratory of the hospital by UPLC-MS/MS or LC-MS/MS. The dosage of the drug is adapted accordingly to the found blood level. This procedure is not sufficient in some cases and a better outcome of the patient can be obtained, if hourly measurements were performed.

To be able to perform hourly measurements, a point-of-care testing (POCT) device is being developed in the European project NANODEM (NANOphotonic DEvice for Multiple therapeutic drug monitoring). The levels of the free fraction of the immunosuppressive drugs in the patient's blood will be detected. A microdialysis catheter collects the samples. Fluorescence based immunoassays are used to reach the aimed very low limits of detection. A robust, small and integrated device with a miniaturized disposable microfluidic chip is developed to meet Point-of-care Testing requirements. The novel laser induced fluorescence-based setup, several miniaturization and optimization steps are shown. Characterizations and immunoassay measurements within the measurement setup are presented.

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## Fabrication of Biosensors Based on Chemically Modified Colloidal AFM Probes

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Interaction forces between a single colloid attached to an atomic force microscopy (AFM) cantilever and a substrate can be investigated with colloidal probe AFM already developed more than 20 years ago<sup>[1]</sup>. Numerous examples using these kind of probes for different applications e.g., to probe mechanical properties of cells<sup>[2]</sup> or to probe adhesion and friction forces<sup>[3]</sup> can be found in literature. Our group recently introduced a new type of AFM- scanning electrochemical microscopy (SECM) probe bearing a conductive colloid<sup>[4]</sup>. Due to the spherical electrode located at the end of a tipless AFM cantilever characterized by a fairly low spring constant ( $k \approx 1$  N/m), the probe offers both high force control and accurate positioning at samples such as single live cells. In addition, the conductive colloid can be readily modified by electrodeposition.

Here we present a novel concept for miniaturized biosensors based on an AFM cantilever with a chemically modified gold colloid. The colloid can be modified by electrodeposition with an enzyme-containing layer using e.g., benzoxazine-based polymers<sup>[5]</sup>. Given the large surface area of such a spherical electrode, it is expected that the achieved sensitivity of the colloidal-based microbiosensor is significantly improved in comparison to disc microelectrodes with similar diameters, but at the same time providing improved lateral resolution.

For the “proof-of-principle” measurements presented in this contribution, spherical gold colloids with a diameter of 5 microns were used. The colloids were platinized prior to the modification with the enzyme layer. Glucose and ATP were chosen as analytes due to their high relevance for biomedical research. The deposition was optimized in terms of deposition cycles and the obtained miniaturized biosensors were characterized in respect to sensitivity and linear range. First experiments towards measurements at live cells will also be presented.

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## Development of a Quick Test to Screen for Bisphenol A Release from Polymer Materials

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Bisphenol A (BPA) is a common industrial chemical that is widely used as a monomer or additive in the production of polycarbonate plastics and other polymeric materials. The primary way of exposure to BPA for most people is assumed to be a direct contamination of food and water through use of containers, bottles, and other plastic storage. BPA has been identified as an important endocrine disrupting compound (EDC). It can interfere with hormonal activities like biosynthesis, metabolism and elimination of natural blood-borne hormones. Moreover, BPA can originate environmental problems [1]. A spectrum of methods have been developed with the aim of determining BPA in water. Immunoassays appear to comply with sensitivity, rapidity, and low cost requirements for effective monitoring [2].

In this work a comparative analysis was conducted of the effect of temperature and the choice of buffers, as well as the substrate at various stages of an ELISA (enzyme-linked immunosorbent assay). The method has been optimized and has shown good repeatability and sensitivity. The results obtained from ELISA will be used as the basis for the development of a Lateral Flow ImmunoAssay (LFIA).

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**Covalent anchoring of human transferrin to carbon-encapsulated iron nanoparticles in presence of magnetic field as a way of preservation of its conformational integrity and electroactivity**

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Any realistic biomedical application of magnetic nanoparticles requires that they maintain their specific ability to recognize and bind the selected molecular targets (e.g. tumor receptors, pathogens, antigens and proteins) without destroying the biological activities of the targets. It is known that the contact of transferrin with SPIONs leads to irreversible changes in the protein structure and finally to metal ions release. The application of carbon-encapsulated iron nanoparticles eliminates this problem.

The covalent anchoring of human transferrin (Tf) to carbon-encapsulated iron magnetic nanoparticles functionalized with carboxylic groups (Fe@C-COOH Nps) in the presence of a magnetic field, results in its conformational integrity and electroactivity. We demonstrated that it is possible to attach, without changing pH, more than one single layer of transferrin to the Fe@C-COOH Nps. This is a very rare phenomenon in the case of proteins. We proved, using various experimental techniques, that the proposed methodology does not lead to release of iron from Tf molecules, what was the major problem in other approaches. We believe that this finding opens new possibilities in drug delivery systems and medical diagnostics.

## Detection of diclofenac molecules by means of a plasmonic sensor substrate

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Beyond classical pollutants, the presence of pharmaceuticals is increasingly attracting attention as they have been detected in remarkable concentrations in surface waters and sewage treatment plants effluents as well as in lower concentrations in ground and drinking water [1]. Amongst human pharmaceuticals, the nonsteroidal anti-inflammatory drug diclofenac represents one of the most prescribed and likewise most frequently detected compound [1]. Thus, it can be considered as a guidance substance for the presence of pharmaceuticals within the aquatic environment. The amounts found are not acute toxic to humans yet. However, recent studies have shown that long-term exposure of relatively low concentrations of diclofenac lead to organ damage and general impairment of fish health [2]. Experts agree that an excess of pharmaceuticals will have a negative long-term impact of the aquatic and terrestrial environment. Current detection methods, e.g. GC-MS/MS or LC-MS/MS, are carried out by sampling being time consuming and costly. Hence, monitoring pharmaceuticals reliably, small and sensitive sensors allowing continuous monitoring in complex matrices are necessary.

By means of a classical surface plasmon resonance (SPR) sensor we are able to detect diclofenac concentrations in the range of less than 5 µg/L using an indirect, competitive assay with a monoclonal anti-diclofenac antibody [3]. However, classical SPR sensors are solely used in the laboratory environment owing to their bulky optics and massive construction. Here we present the current development of a sensor system for the detection of diclofenac in real-time by means of a nanostructured plasmonic sensor substrate and a photocurrent-based interrogation unit based on a wavelength-sensitive photodiode [4]. The dispensation with use of a spectrometer allows a compact sensor design for the implementation in waste water treatment process. Initial experiments were carried out to demonstrate the sufficient sensitivity of the sensor substrate structured by the use of Nanoimprint Lithography. In further experiments we will estimate how sensitive the interrogation unit has to be designed to analyse low concentrated diclofenac samples. Furthermore, nanostructured sensor substrates will be optimised concerning reproducibility.

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## Development of multiplexed binding inhibition assays for the screening of food contaminants

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Residues of bisphenol A (BPA), nonylphenol, atrazine and 2.4-D arrive in food via food contact material, by application of pesticides in agriculture or by bioaccumulation in the food chain. These chemicals belong to the group of endocrine active substances which are able to interfere with the endocrine system of organisms. Thus there is a strong need for a sensitive detection platform measuring several analytes at the same time and in different matrices. We present a biochip using biological and artificial recognition elements for the rapid screening of endocrine disrupters in food. The chemicals are small molecules and difficult to immobilize because of only few reactive functional groups. Different immobilization strategies and functionalized surfaces were tested to bind the molecules efficiently. Preliminary experiments in SciPOLY3D from Scienion AG resulted in a successful immobilization of 3 mg/ml BPA. This SciPOLY3D hydrogel enables covalent and stable immobilization of the molecule in a short time. First binding inhibition assays in buffer indicate a detection limit of 3 ng/mL and high inter slide reproducibility. In order to further increase sensitivity in complex matrices alternative labels and recognition elements are discussed.

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## Rapid Prototyping using 3-D-Printer Technology for Development of Breath-Test-Analysers

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### Introduction

For medical device technologies, there is the need for more cheap and smaller devices. The question is to bring technology into the Homecare-field which until now is expensive and therefore based in hospitals and medical practices only.

### Methods

Based on the knowledge of clinical devices for single breath tests for expired nitric oxide, there was the question for a multi-use handheld device with possible connection to data logger like PC, Tablet or as stand-alone device.

### Results

By 3-D Printing within a short time it was possible to test variants of devices. Especially the sample tubes, airflow channels, valves, flow resistances were tested for functionality in real settings together with electrochemical sensor and electronic part.



*Fig. 1) Examples of three different housing from 3-D-Printing*

### Conclusion

The device is functional suitable for testing of exhaled NO, a marker of airway inflammation, in ppb-range. The device is ready for use in Homecare-applications as Self-testing device.

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## **New application for an old concept: A disposable optical biosensor for long-term continuous glucose monitoring in bioreactors**

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During a bioprocess, physicochemical parameters such as pH, temperature and dissolved oxygen (DO) are typically monitored and controlled continuously in order to achieve high cell densities and to maximize product yield. However, real-time measurements of nutrients like glucose remain a challenge.

We present data of a biosensor that was designed and optimized for cell culture applications in disposable bioreactors with a long functional stability and minimal influence of perturbations. We demonstrated that the sensor is sterilisable and applicable for an advanced process control during mammalian cell cultivation without biofouling. The biosensor is based on a commercially available optical oxygen sensor (PreSens GmbH, Germany), coated with a glucose oxidase (GOx) containing layer. The enzyme layer is covered by a perforated hydrophilic membrane, rendering the sensor diffusion limited. By means of optimizing the enzyme layer composition and the diffusion membrane, we were able to generate sensors with varying dynamic ranges (1-40 mM glucose) and decent response times (~20 min until 95 % equilibrium) suitable for cell culture purposes.

We implemented the glucose sensors for one week in 125 mL cell culture flasks and demonstrated a tight control of glucose levels in a fed-batch culture, verified by periodical offline measurements. Before inoculating the flasks with cells, one point calibrations were performed with a defined initial glucose concentration. In addition to experimental characterization of the biosensor, numerical simulations were performed, which enabled insights into the diffusion rate and the concentration profile of the deactivating enzymatic by-product hydrogen peroxide within the biosensor. The simulations were carried out by a one-dimensional biosensor model that, allowing the determination of the theoretical glucose consumption rate of the biosensor for different glucose concentrations in the sample.

The sensor described here, allows an advanced process control, which cannot only help to improve batch to batch consistency, but also increase the product quality and yield. Since glucose is a major nutrient for mammalian cells, its tight regulation during cell culture will support the optimization of bioprocesses.

## Study of biological barriers by electrochemistry: intestine covered electrode

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Biological barriers, e.g., skin and intestinal epithelium, are vitally important as they define interactions of organisms including humans with environment. Knowing properties of these barriers is helpful in optimizing drug delivery and developing noninvasive bioanalysis methods. Additionally, the barriers are constantly challenged by, e.g., microbes and toxins, and thus they comprise powerful antioxidant, immune and repair systems. Recently, we have developed an in-vitro setup which allows us to assess a number of barrier properties. E.g., permeability of barriers by drugs or polyphenols from cosmetic formulations. The setup consists of a skin membrane covered electrode [1, 2]. In this presentation a similar setup is discussed which comprises an intestine covered electrode. The intestine covered electrode was used to investigate catalase activity present in this barrier. It was found that intestine contains a high activity of catalase enzyme. This in-vitro setup was used to study the interaction of intestine catalase with polyphenols, plant extracts, and other antioxidant containing solutions. These experiments and the data indicate that the proposed setup might present an important electrochemical, in-vitro approach to study inflammation relevant processes on tissue level.

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## Detection of the metabolic activity of *Saccharomyces cerevisiae* using impedance spectroscopy

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A major challenge in environmental monitoring is the detection of pharmaceutical residues in municipal wastewater. Whole-cell sensing systems may employ genetically engineered organisms containing biospecific recognition elements to detect the analyte by - for example - producing fluorescent proteins. These systems are able to monitor the bioavailability of the analyte specifically, rapidly and sensitively. However, for the correct interpretation of the output signal, vitality of the yeast cells has to be evaluated.

By means of impedance spectroscopy, we are able to discriminate between viable and dead cells. To this aim, living and heat-killed yeast cells (*Saccharomyces cerevisiae* (S.c.) BY4741  $\Delta$ bar1  $\Delta$ tyr1::GPD-tRFP) were cultivated on interdigitated platinum thick-film electrodes in nutrient solution. Sedimentation of living as well as dead cells on the electrode surfaces can be observed at a frequency of 1 MHz, because sedimented cells act as a resistance parallel to the medium at this frequency. Moreover, since living yeast cells are able to bud, proliferation appears as increased sedimentation. In contrast, the metabolic activity of living cells can be followed at low frequencies as 0.01 Hz due to changes of double-layer capacitance of the electrodes. The same experiments were also performed with yeast cells immobilised in agar, where sedimentation cannot take place. Here, changes in impedance were only observed at 0.01 Hz, representing metabolic activity of living cells. Interestingly, the impedance increases with time and with augmenting yeast concentration for living cells free in medium as well as immobilised in agar.

In conclusion, impedance spectroscopy is a powerful tool for discriminating live and dead yeast cells, and therefore, it may serve as internal reference method for evaluating fluorescent signals in biological whole-cell sensors.

### Acknowledgements

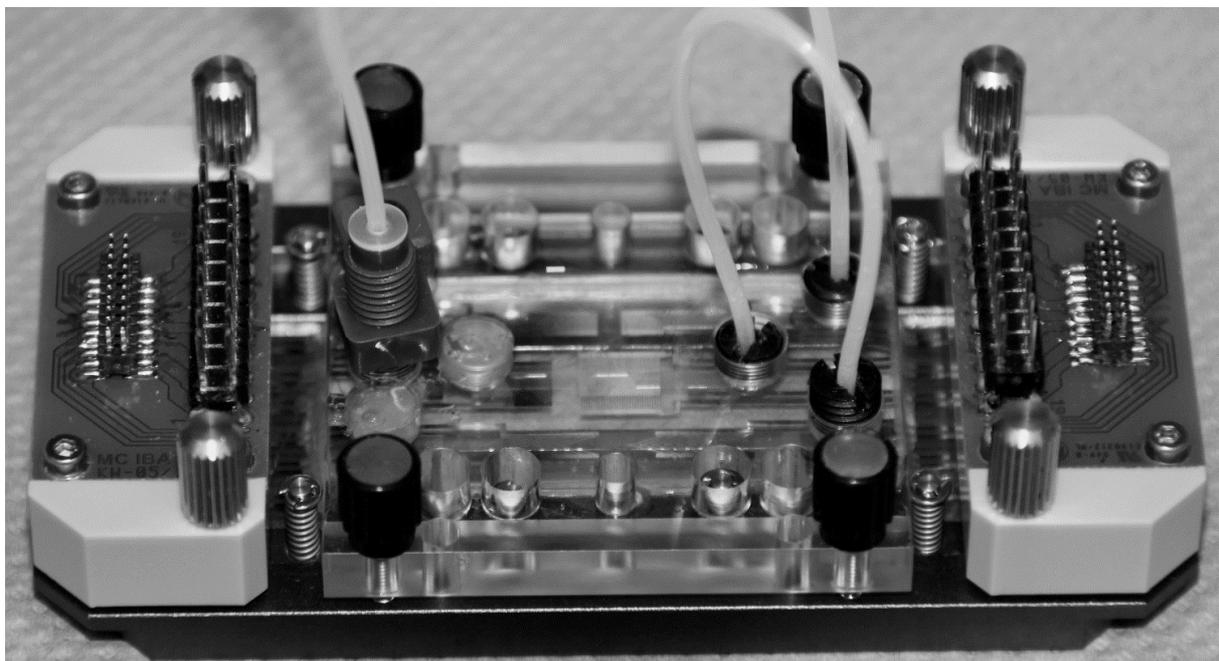
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## Microfluidic system for the dielectrophoretic enrichment, characterization and manipulation of suspended cells

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Special electrode structures in the size of a microscopic slide have been developed for the investigation of suspended cells. The electrodes have dimensions of micro- and macro-range and allow the deconvolution of the passive-electric signals of the cells (polarization of the cell membranes) from those of the surrounding medium. Figure 1 shows the complete microfluidic system in a MicCell chamber. On the electrodes, a transparent fluidic structure with three channels is located, so that the behaviour of the suspended cells in the electric field can be observed under a microscope. In the three channels, 60  $\mu\text{m}$  height and 2 mm wideness, there were interdigital electrodes with dimensions of 20  $\mu\text{m}$  for measurement of the conductivity. Between two of these electrode pairs, electrodes for the dielectrophoretic manipulation of the cells were located. The structures were fingers, castle-walls, and IDEs with dimension and distances down to 5  $\mu\text{m}$ .



*Fig. 1: Microfluidic cell with the microelectrodes, connectors, and tubes.*

A total of twelve different electrodes were integrated for dielectrophoretic enrichment, destruction of the cell membranes by pulsation, and impedimetric measurement of conductivity changes due to the destructions. The microfluidic system presented here can be applied e.g. in the field of single cell biosensors, where suspended cells are caught by the electrodes, exposed to active substances, and then examined by a microscope or by Raman spectroscopy or by electrical impedance spectroscopy.

## Simple approach to $pK_A$ -tunable BODIPY-based fluorescent pH sensors

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### Introduction

Determination of pH-values is amongst the most commonly performed analytical measurements in the material and particularly the life sciences as pH presents a very important parameter for material corrosion and a biomarker for medical diagnostics, molecular imaging, and cell biology due to its many roles in enzymatic and tissue activities inside cells like proliferation, apoptosis, multidrug resistance, and endocytosis.[1, 2] Electrochemical methods using potentiometric glass pH electrodes or ion-sensitive field-effect transistors (ISFET) are well-established and provide accurate results within a short time range, yet they are not suited e.g., for noninvasive measurements in vitro and in vivo. A straightforward alternative present optical sensors and probes, so-called pH optodes were established, which offer many advantages like a very high sensitivity and contactless measurements. The basic prerequisite for an optical pH measurement is a pH sensitive indicator dye, which shows a distinct optical behavior associated to its protonated and deprotonated form regarding the intensity and/or spectral position of its absorption and fluorescence or its fluorescence lifetime.[3] The operating range of a pH sensitive dye is, however, limited by its  $pK_A$ -value. Tuning the  $pK_A$  of dyes without affecting their spectral properties and their fluorescence quantum yield and fluorescence lifetime can be particularly beneficial for the design of versatile pH probes, suitable for a broad range of applications requiring the signaling of strongly different pH values and read out with the same inexpensive miniaturized instrumentation.

### Results

We report here the synthesis and spectroscopic characterization of BODIPY-based optical pH sensors in aqueous-organic mixed solvent with tunable  $pK_A$ -values at least from 5 to by modifying the substitution pattern of the meso-aryl substituent without significant shifts in absorption and emission. All BODIPY compounds are simply accessible in a maximum two-step synthesis and enable straightforward post-functionalization, e.g. for bio-conjugation.[4]

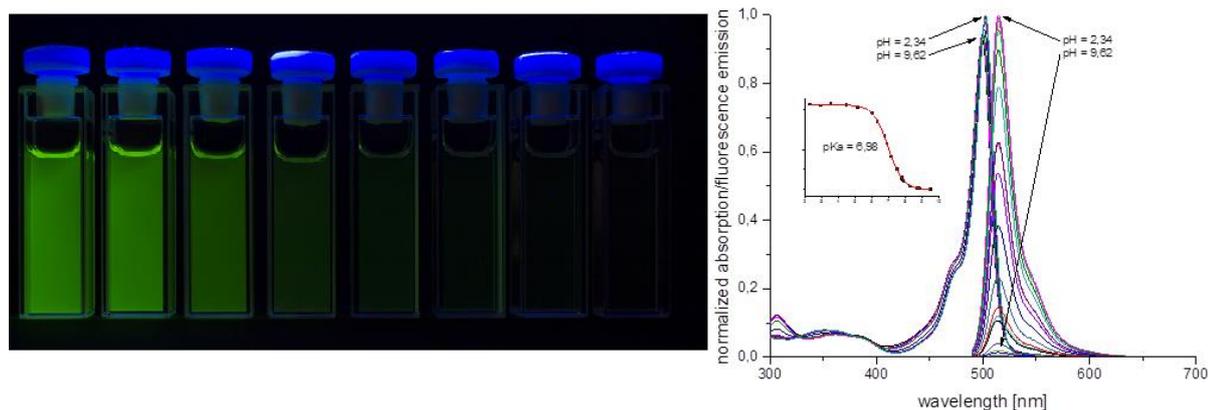


Figure 1: Fluorescence ON/OFF switch in dependence of the pH for a representatively shown BODIPY dye.

All compounds have high molar absorption coefficients ( $> 70,000 \text{ M}^{-1} \text{ cm}^{-1}$ ), high fluorescence quantum yields ( $> 50 \%$ , up to  $90 \%$ ) and, within this BODIPY series, minimum spectral shifts in absorption and emission, despite their different substitution pattern. pH-dependent fluorescence ON/OFF-switching occurs, summarized in Figure 1 exemplarily for dye SR1, caused by photoinduced electron transfer (PET) (see Figure 1). Two BODIPY dyes show near neutral and neutral  $\text{pK}_\text{A}$ -values of 6.17 and 6.98, respectively, which enable pH-measurements in biological samples. Moreover, first studies with different polymers typically used as typical matrices for the fabrication of sensor films reveal the absence of dye leaking upon protonation which presents a major problem for many indicator dyes.

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### 3D pyrolytic carbon microelectrodes for electrochemical sensing

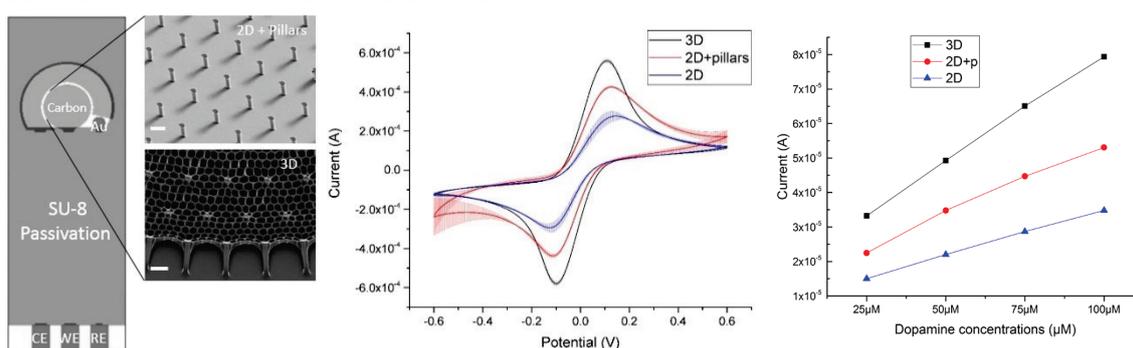
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Carbon materials have several attractive characteristics as microelectrodes for electrochemical sensing, such as wide potential window, good electrochemical activity, chemical stability, and ease in surface functionalization [1]. With the most common carbon microfabrication techniques the sensitivity is limited due to two-dimensional (2D) electrodes. In the C-MEMS method a patterned polymer template is converted into pyrolytic carbon at high temperature ( $\sim 900^\circ\text{C}$ ) in inert atmosphere ( $\text{N}_2$ ) [2]. The simple and cost-effective approach allows the fabrication of 3D carbon microelectrodes (3DCMEs) for sensor applications or as 3D cell scaffolds with the possibility for electrochemical real-time monitoring of cell populations [3].

Here, we present the fabrication and characterization of multi-layered 3DCMEs for electrochemical sensing. An optimized UV photolithography and pyrolysis process with the negative tone photoresist SU-8 was developed. 3DCMEs were fabricated on the working electrode of a microelectrode chip with a three electrode configuration (Fig. 1, left). The electrodes were characterized with cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The 3D electrode configuration shows a higher peak current in CVs (Fig. 1, center) and lower impedance in EIS. As a proof of concept for biosensing, square wave voltammetry was used for detection of dopamine (Fig. 1, right). The peak current for 3DCMEs was almost three times larger than for 2DCMEs. At present, the 3DCMEs are evaluated for real-time monitoring of dopamine released from human neural stem cells.



*Fig. 1: Left: Microelectrode chip design with three electrode configuration. SEM images of carbon micropillars (2D+pillars) and suspended grids (3D); Cyclic voltammetry with 10mM ferri-ferrocyanide redox probe (center) and dopamine detection with square wave voltammetry (right) with different electrode configurations*

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## Integration of Radio-Frequency Permittivity Sensors into Microwell Plates for Cell Concentration Measurements

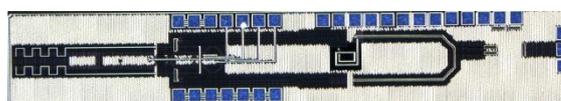
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The integration of two 32 GHz permittivity sensing chips, fabricated in a 0.13  $\mu\text{m}$  SiGe BiCMOS technology, into microwell plates on a printed circuit board (PCB) is presented. The microchip displayed in Fig. 1 has a total size of



*Fig. 1: Chip-photo (3.5 mm x 0.6 mm)*

2.1mm<sup>2</sup>, consumes 127mW of power and features on-chip DC readout. The sensing circuit is a resonator based oscillator, which results in permittivity dependent output frequencies. In the next step a frequency discriminator converts the frequency into a corresponding DC voltage [1]. The setup allows monitoring the two sensors DC outputs, indicating properties of materials under test (MUT). Furthermore, the sensor bias points can be controlled for calibration purposes. Utilizing two identical sensors allows for simultaneous reference measurements using calibration fluids. The complete setup, consisting of the chips on a PCB and a microwell plate made of polycarbonate, is shown in Fig. 2. To bring the MUT in contact with the sensing area, two holes are drilled underneath into the PCB. The Chips were mounted using flip-chip technology. The electronic components are isolated from MUTs with a bio-compatible adhesive, creating a hermetic environment. The sensors were tested with salt water solutions in different concentrations and compared to analytic results applying equations from [2]. The presented device is intended to measuring cell concentrations of bioprocess media.



*Fig. 2: Top (left) – and bottom (right) view of the complete setup*

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## Improving the sensitivity of ATP microbiosensors

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The reproducible immobilization of enzymes is a crucial step in fabricating enzymatic biosensors. Within the last years, our research focus is targeted towards the detection of adenosine-5`-triphosphate [1,2], which is considered as one of the most important autocrine and paracrine signaling molecule [3]. In order to detect ATP at biologically relevant entities such as live cells in response to chemical or mechanical stimulation, robust microbiosensors for ATP with high sensitivity and selectivity are required. The detection of ATP is based on a competitive reaction involving glucose oxidase and hexokinase as enzymatic compounds, which is providing selectivity.

With this contribution, we present approaches to improve the sensitivity of ATP microbiosensors. Recently, Andronescu et al. introduced an immobilization scheme based on water-soluble benzoxazine as immobilization matrix for enzymes [4]. We adapted this approach for the immobilization of hexokinase and glucose oxidase at micro-sized electrodes. The obtained microbiosensors are characterized by excellent mechanical stability, but also a significantly improved sensitivity in comparison to sensors using electrophoretic paint as immobilization matrix. In addition, we could show that the sensitivity of the sensors could be further enhanced and their long-term stability improved, when platinum nanoparticles were deposited at the microelectrodes prior to the immobilization of the enzymes. Characterization of the microbiosensors in terms of sensitivity as well as their long-term stability will be presented.

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## Development of a mobile monitoring system based on potentiometric and amperometric biosensors for evaluations in biogas processes

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The production of biogas by anaerobic digestion of organic material, like corn, manure or waste from food industry is an attractive alternative to fossil fuels. However, for an efficient conversion of biomass to methane the monitoring of biogas plants is crucial. In this regard, novel measurement devices are required, which enable the rapid analysis of important key parameters in the biogas reactor in order to detect process disturbances. In this context, a measurement system based on a light-addressable potentiometric sensor (LAPS) combined with 3D-printed multi-chambers was developed to be able to perform differential as well as simultaneous cell-based measurements, as presented in Fig. 1A. Undesirable variations during the measurements, like sensor drift and external pH changes of the medium can be compensated in this way, whereas changes in the metabolic activity of microorganisms can be monitored by the LAPS. As a test microorganism, *Escherichia coli* (*E. coli*) K12 has been used to study its influence towards nutrition components such as glucose. Process imbalances are also indicated by the accumulation of acidic compounds in the biogas fermentation broth. Therefore, an additional enzyme-based sensor system has been developed. For the quantification of alcohols, formate, D-/L-lactate, acetate and further fatty acids, specific enzymes are immobilized, which enable the amperometric detection. The sensor layout is presented in Fig. 1B.

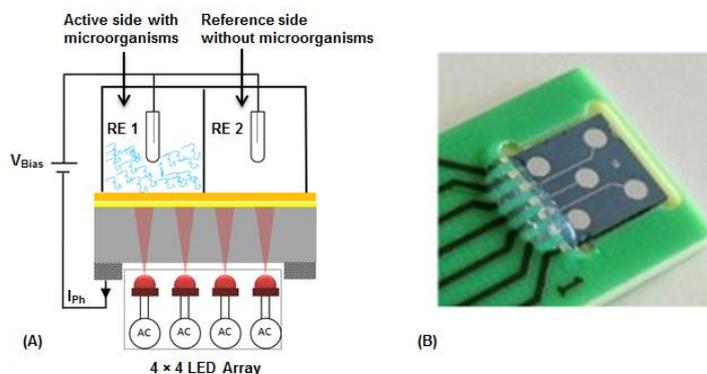


Fig. 1 (A) LAPS chip (20 mm × 20 mm) in differential set-up combined with 3D-printed multi-chambers, RE: reference electrode,  $I_{ph}$ : photocurrent,  $V_{Bias}$ : bias voltage, LED: light-emitting diode. (B) Multi-parameter sensor chip (10 mm × 10 mm) for the simultaneous detection of various organic fatty acids.

## Early detection of cancer diseases using DNA aptamers

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Cancer diseases are among the most common cause of death in the last decades. The success in cancer therapy depends on early diagnosis of this disease. Therefore it is important to search for effective diagnostics methods which could provide high sensitivity and specificity. Important progress in molecular recognition is based on DNA/RNA aptamers that selectively bind to crucial cancer markers. Aptamers are single-stranded oligonucleotides that in a solution fold into 3D structure containing binding site for selected molecules, such as oncomarkers at the surface of the cell. Thanks to the aptamers unique structure they were applied as therapeutic, diagnostic and analytical tools, transport systems for targeted drug delivery. Conjugation of highly specific aptamers with nanoparticles creates nanomaterial which could be used not only as new imaging tool, but also for quantification and amplification of oncomarker detection, for example in blood samples.

We used sgc8 aptamer that is specific to tyrosine kinase 7 (PTK7) receptors of acute lymphoblastic leukemia cells (ALL). We applied acoustic thickness shear mode (TSM) method for detection of the ALL and cancer markers. Sgc8 aptamers were immobilized at the surface of the piezocrystal. The flow of cells resulted in specific binding to the aptamers and changes in resonant frequency and motional resistance. Gold nanoparticles modified by sgc8 aptamers allowed amplification of cell recognition with limit of detection 500 cell/ml. We used also atomic force microscopy (AFM) and a single molecule force spectroscopy (SMFS) for determination of the forces between aptamers and cancer markers as well as for detection of morphological changes of the cancer cells.

### **Acknowledgments:**

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## **An optical imaging system capable of monitoring oxygen consumption and oxygen gradients within live tumor spheroids**

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Cancer cell spheroids have gained huge attention in fundamental tumour biomedicine as they are considered as a more realistic *in vitro* model of aberrant tissue compared to 2D cell monolayers. Since spheroids are not vascularized and diffusion from the periphery to the centre is limited, those cells residing in the spheroid centre suffer from a reduced nutrient supply (e.g. oxygen) towards their centre. Current techniques to measure the oxygenation in live 3D cell cultures like, for instance, Clark-microelectrodes or staining for hypoxia markers still have their individual drawbacks such as oxygen consumption by the sensor system itself or a time consuming sample preparation. Optical oxygen sensors are capable of overcoming these drawbacks. However, indicator dyes - as molecules or integrated in sensor nanoparticles - easily bleach and generate cytotoxic singlet oxygen. Particle-based sensors additionally interfere with oxygen diffusion through the spheroids and thereby alter the original gradients.

We have developed a compact microscopy-based imaging system based on planar optical oxygen sensor foils that the spheroids attach to so that spatial and temporal monitoring of oxygen levels and gradients becomes possible. The planar, biocompatible sensor films carry oxygen-sensitive luminophores and an oxygen insensitive reference dye. The ratiometric signals from the sensor films are read out in a non-contact way and thus avoid contamination of the sample. The short distance between the substrate and the basal cell membrane of about 50-100 nm enables measurement of oxygen concentration in direct proximity of the cells. The system can be completely incorporated into a normal cell culture incubator and enables long time monitoring of cells under stable growth conditions.

In our studies, we use MCF-7 spheroids as 3D tissue models to show the potential of this technique. Once applied, the spheroids settle down, adhere to the oxygen sensitive culture substrate forming a hemispherical structure. The recorded 2D oxygen image thus displays the spatial oxygen distribution of a cross-section through the middle of the spheroid. We used this new technique in proof of principle experiments to study the influence of model toxins upon respiration of spheroids.

This new tool to study 3D tissue models provides an experimental basis for pharmacological studies on the influence of drugs or toxins upon respiration, oxygen gradients, viability and drug efficacy.

## Nano- and Micro-Patterns of PEG-based Hydrogels with Gold Nanoparticles for Biosensor Applications

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Poly(ethylene glycol) (PEG) hydrogels are non-toxic, non-fouling, non-immunogenic and offer an ideal, inert background for studying specific biointeractions. [1] Although PEG-hydrogels are inert towards biomolecular interactions, surface modifications on hydrogels enable attachments of biomolecules or whole cells on their surface.

Gold nanoparticles (Au NPs) are little toxic, can be synthesized with different sizes, shapes and surface functionalities and have unique size and shape dependent optical properties like the surface plasmon resonance effects in the visible range of the electromagnetic spectrum. [2] These particularities make them suitable materials for biosensor devices via localized surface plasmon resonance (LSPR) spectroscopy and surface enhanced Raman scattering (SERS). [3,4] Recently selective and controlled cell adhesion on Au NPs coated PEG-based hydrogels has been discovered [5], which offers besides the molecular biosensors a possibility for cell-based biosensor devices.

For this purpose, in the present work PEG – Au NPs composites with defined pattern sizes (lines or rectangles) are investigated. First of all Au NPs with different sizes and shapes are synthesized and nano- or micro-patterned on PEG-based hydrogels using soft lithographic processes like Fill Molding In Capillaries (FIMIC) [6], reactive Micro Contact Printing (r- $\mu$ CP), wet Micro Contact Deprinting (wet  $\mu$ -CdP) and nano-patterning by using wrinkled PDMS stamps [7]. We can also tailor the crosslinking densities, swelling degrees, hydrophilicities, chemical functionalities and elasticities of the PEG-hydrogels using linear or star shaped (8PEG) PEG-precursors with acrylate or vinyl sulfone end groups and crosslink them via UV-curing or Michael type addition reactions, respectively. [8]

The resulting nano- and micro-patterned nanocomposite biomaterials are investigated in cell culture using mouse fibroblast cells (L929). Cells adhere selectively to and spread on the Au NPs-patterned area while the regions of pure PEG-hydrogel are effectively avoided by the cells. This strategy allows selective adhesion with high precision and offers a promising platform for fabricating single cell sensing devices.

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## Multimodal, Impedance-Based Monitoring of Chemosensitivity Assays for Tumor Cells Using a Lab-on-a-Chip Platform

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Cancer is a highly heterogeneous disease whose causes, pathogenesis, metastatic potential and response to treatment can be very different among individuals even for the same type of cancer [1]. In order to improve both, therapy and the quality of life for cancer patients, personalized medicine aims to test biopsy material of a tumor *ex vivo* with respect to its sensitivity for a given chemotherapy before the patient gets involved directly. This approach is scientifically and technically rather challenging, as all testing has to be performed fast and on a rather small cell population with a maximum of reliability.

Here, we present the development of a microfluidic sensor platform as low volume perfusion culture dish for tumor cells with all technical components to perform a multimodal, impedance-based monitoring of chemosensitivity assays with medium throughput [2]. Within the microfluidic channels the tumor cells will be exposed to a given drug candidate and changes in cell vitality will be recorded non-invasively and in real time by impedance measurements (electric cell-substrate impedance sensing, ECIS) [3]. The impedimetric assays are based on planar gold-film electrodes established on the bottom of the microfluidic chip by photolithography.

This study shows an impedimetric characterization of the cell-free microfluidic platform with respect to electrode parameters, device operation and flow features. Furthermore, a sequence of impedimetric cell-based assays was developed (yet in off-chip assays) to assess key parameters of cell vitality both, before and after application of chemotherapeutic agents. Impedance-based assays were applied to monitor (i) the acute cytotoxicity of cytostatics for a confluent cell layer as well as (ii) the ability of cells to migrate laterally and (iii) to proliferate in presence of selected cytostatics. The time- and dose-dependent answer of the tumor cells provides a first line of evidences for a tailored cancer therapy with reduced side effects. The experiments are based on the well-established lung carcinoma cell line A549.

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### Engineered bioinspired hydrogel for the real-time monitoring of cell-growth by mirobiosensor technology

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Traditional tests to determine the effect of external components on cell growth are cumbersome. Standard tests have for principle the detection of cell numbers by measuring the optical density, which are not appropriate for turbid and colored samples, and are for parallelization not feasible. They are time-consuming methods that only allow endpoint detection and no real-time monitoring of cell growth. Conductivity-based microsensors overcome these problems. Parameters like cell shape change, conductivity change inside and outside the cell and disturbances in the cell membrane could be monitored by means of the electrical method. In this work, bioinspired hydrogels are proposed to get information on the initial stage of cell migration with single cell resolution. A microfluidic device integrated with electrical cell-substrate impedance sensing (ECIS) allows the monitoring in real-time of cell adhesion and spreading in the proposed 3D hydrogel matrices. The sensor chip can capture single cells on microelectrode arrays in 2D or 3D cell culture. The new microsensor set-up which the proposed matrices mimic the extracellular matrix of the intervertebral disc (IVD) tissue is promising for the real-time monitoring of IVD cells behavior in 3D cell cultures.

## A novel biosensor platform for inflammation analysis - assessment of platform feasibility

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Biosensors are - amongst others - suitable for applications in medicine, environmental analysis, food monitoring, and industrial processes due to their miniaturization potential and their specific detection principles. For medical purposes, different commercial biosensors are available on the market. Furthermore, several sensor platforms are described in the scientific literature. Antibody-based biosensor platforms, for example, offer the opportunity to diagnose the inflammatory status [1, 2].

Hence, aim of this work is to develop an electrochemical immunosensor platform for inflammation analysis allowing for protein detection in the lower concentration range (pg/ml). Furthermore, an innovative measurement scenario is proposed to address analysis challenges such as parallelization or time-consuming workflows of standard techniques, such as enzyme-linked immunosorbent assays (ELISA). Here, results are shown for the investigation of different sensor concepts for the detection of IL-13 as model analyte. All the different sensor concepts comprise the same electrochemical detection principle, but are based on different electrode materials or support materials onto which the capture antibodies are immobilized.

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## Heme-Peptides on Transparent Electrodes

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Catalysts for the efficient activation of peroxide and molecular oxygen are of high interest for applications such as biosensors, bioconversion and biofuel devices. In this respect, biomimetic and bioengineered catalysts are attractive tools as they combine catalytic efficiency with robustness in various conditions. Heme-peptides are valuable tools in mimicking the catalysis of natural heme peroxidases. Their smaller size and simpler design allow high surface concentrations and even analyses in organic solvents.

Well-known examples for nature-derived heme-peptides are Microperoxidases (MP), short fragments of cytochrome *c* generated by enzymatic digestion which can catalyze the reduction of hydrogen peroxide and oxidation of a variety of substrates analogous to peroxidases.[1] Microperoxidase-11, a heme-undecapeptide, was immobilized on mesoporous antimony tin oxide (mpATO) thin film electrodes [2] using a positively charged binding promotor and its spectroscopical, electrochemical and catalytic properties were analysed. Spectral characteristics in the visible region indicate the formation of six-coordinated low-spin species which was confirmed by Resonance Raman measurements. These also demonstrated that MP-11 adopts the same conformation in solution in presence of the binding promotor. MP-11 on mpATO is further able to catalyse the cathodic reduction of hydrogen peroxide. The onset potential of catalysis is with almost +500 mV much higher than the formal potential of the Fe<sup>2+/3+</sup> transition indicating that the enzymatic reaction proceeds via a Compound I like intermediate analogous to native peroxidases [3].

These findings show that mpATO is a suitable host material for adsorbing the heme-peptide in its native state. The same immobilization strategy can be applied for the characterization of other enzymatically active heme-peptides. Using the natural enzyme horseradish peroxidase as the starting point other catalytic structures will be created by means of different bioengineering techniques in order to design new efficient catalysts.

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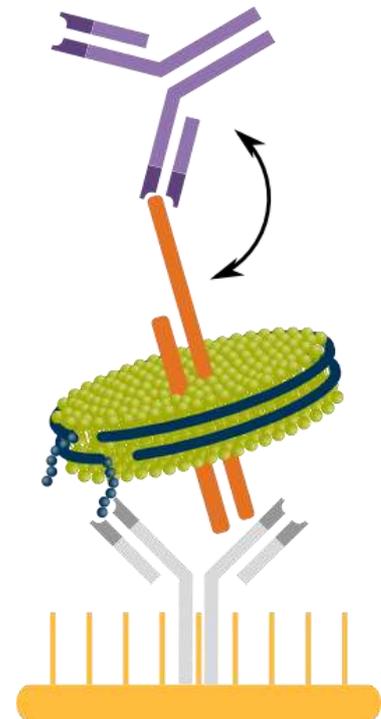
## Integration of platelet membrane antigens into nanodiscs for the detection of antibodies associated with autoimmune thrombocytopenia

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Currently, diagnosis of autoimmune thrombocytopenia (AITP) is a process of exclusion. In most cases, an autoimmune disorder can merely be assumed after other causes of a low platelet count have been excluded [1]. Although tests for patient anti-platelet antibodies are available, they have low sensitivities and a negative result does not necessarily rule out ITP. 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 functional form [2]. Our aim is the detection of patient anti-platelet antibodies using platelet antigens incorporated into NDs immobilized onto biosensor surfaces. The reconstitution of membrane proteins in NDs allows for correct protein folding and a native lipid environment, thus increasing diagnostic performance of future tests.

Platelet surface antigens associated with AITP were expressed in HEK 293 cells and incorporated into NDs. As common protocols for ND preparation are costly in terms of labor and time, they are not easily applicable in a diagnostic context. For this reason, different simplified generation protocols were designed and tested. The resulting NDs were examined by size exclusion chromatography (SEC) and western blot (WB) and found to be similar to those prepared according to standard methods. Membrane proteins expressed in HEK 293 cells and from human platelets freshly isolated from whole blood were examined by surface plasmon resonance (SPR) spectroscopy in order to ensure correct antibody binding. Preliminary experiments showed promising results concerning the use of nanodiscs in combination with biosensor surfaces. The next steps will be the examination of different sensor surfaces and immobilization techniques in order to find the best assay format for detection of anti-platelet antibodies from patient samples.



*Fig. 1: Membrane proteins reconstituted into NDs are immobilized on a biosensor via the 6xHis-tag. Interactions between inserted receptor and auto-antibodies are detected by SPR.*

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## Optimizing specificity and reproducibility of a biomimetic SPR-biosensor for acquired anti-factor VIII

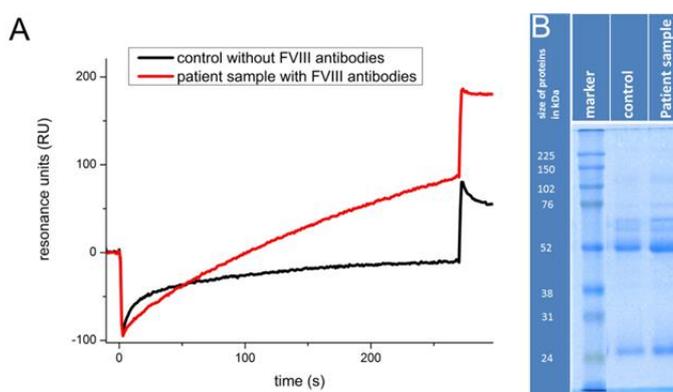
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Inhibitor haemophilia is often caused by the presence of anti-factor VIII (FVIII) antibodies. A high sensitivity SPR biosensor detecting FVIII auto- and alloantibodies and discriminating their inhibitory properties was established previously [1]. The high sensitivity of the SPR-system requires stringent antibody purification while maintaining high antibody activity for optimal results. Our aim of was to further improve sensitivity, specificity and reproducibility of the assay in order to optimize the generic workflow for the complete removal of all contaminants while maintaining maximum antibody activity. For isolation of immunoglobulins from plasma, different methods were tested: Protein A/G columns, Melon Gel, Protein A magnetic beads and columns with immobilized anti-human-immunoglobulin-antibodies. The purification efficiency was assessed by subsequent determination of total protein content and immunoglobulin concentration. Quality and purity of the preparation was assessed by SDS-Page followed by the analysis on the SPR biosensor using HC1500 Chips from Xantec with immobilized, recombinant Full-length FVIII. Isolation of immunoglobulins using agarose columns with immobilized polyvalent anti-human-immunoglobulin-antibodies is a promising approach due to its high specificity. Although the yield is low compared to protein A/G, the sensitive SPR-method can detect the purified antibodies, allowing a clear distinction between patient and control samples (Fig.1). The Isolation from FVIII antibodies with anti-human-IG-antibodies and subsequent analysis on the SPR biosensor is a promising method to detect anti-FVIII in patient samples. Further research on this method could pave the way to an application of the SPR biosensor in patient care. This could improve and accelerate the diagnosis and thus the time to therapy from haemophilia patients with anti-FVIII antibodies.

[1] C. Kocot, et al., *Ana. Bioanal Chem* 2015;**407**:5685-5693.



*Fig. 1 A: The negative sample shows very low unspecific binding after the optimized purification step. B: Using the anti-human immunoglobulin column, most of the other proteins can be completely eliminated from the samples.*

## An aptamer-based biosensor for doxorubicin by means of electrochemical impedance spectroscopy

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The contamination of municipal waste water with drug residues is an increasingly serious environmental problem. In sewage treatment plants these substances cannot be completely disintegrated, thus reach ground and drinking water and contaminate the environment. Therefore, there is a need for devices to monitor the effluent of sewage treatment.

Aptamers are single-stranded nucleic acids that bind target molecules highly specific and highly affine with their three-dimensional structure. Because of their similar affinity, but higher stability, they represent a potential alternative for antibodies. Doxorubicin is an antibiotic used as chemotherapeutic agent in cancer therapy. In the investigation presented here it was used as an example for drug residue. An aptamer for daunorubicin [1] with high sensitivity for anthracyclines was used as receptor for the biosensor developed in this work.

The strategies of immobilization as well as binding were optimized on the basis of quartz crystal microbalance and chronocoulometry. Electrical impedance spectroscopy was chosen as measuring method, because it is possible to analyse the aptamer-target-binding very sensitive in a direct way without further labelling (Fig. 1).

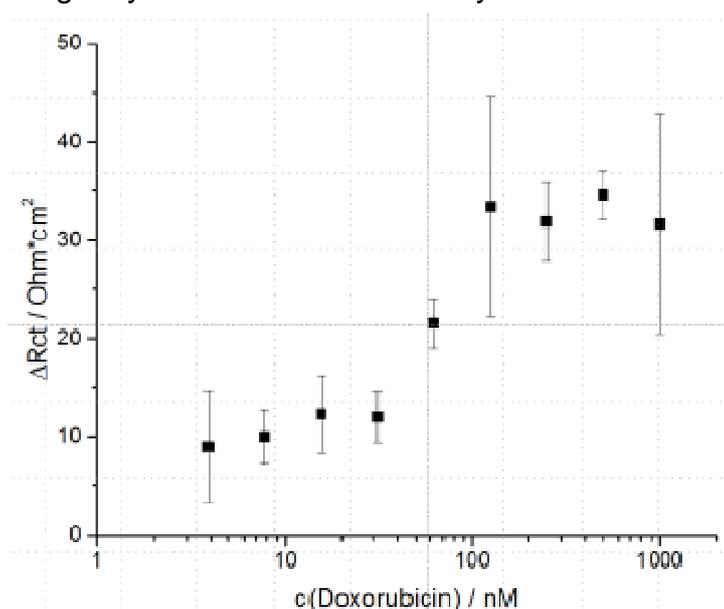


Fig. 1: Binding curve of doxorubicin.

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## Electrochemical biosensor based on CYP17A1 for screening of potential anticancer drugs

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Cytochrome P450 17A1 (CYP17A1) is a key heme-containing androgen-synthesizing enzyme, which catalyzes 17 $\alpha$ -hydroxylase and 17(20)-lyase reactions towards pregnenolone and progesterone. Reducing levels of androgens in the body is an effective way for treatment of prostate cancer, therefore, CYP17A1 is the molecular target for potential antitumor drugs. Electrochemical systems based on recombinant CYP isoenzymes are highly sensitive biosensors for analysis of catalytic activity and substrate-inhibitory potential of these enzymes. We used recombinant CYP17A1, obtained from the Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus. Newly synthesized oxazoliny derivatives of [17(20)*E*]-pregnene were tested as potential inhibitors of CYP17A1, which was immobilized on the working surface (2 mm diameter) of a graphite planar electrodes formed by screen printing by didodecyldimethylammonium bromide. Electrochemical analysis was carried out by cyclic voltammetry and amperometric titration by substrate pregnenolone. Registering catalytic current in the presence of potential inhibitors were calculated inhibition constants of tested oxazoliny derivatives of [17(20)*E*]-pregnene. The kinetic parameters of CYP17A1 towards pregnenolone were calculated based on the analysis of electrochemical characteristics. The conversion of pregnenolone and progesterone in accordance with the natural catalytic cycle of CYP17A1 has been shown by means of mass spectrometric analysis of the products of CYP17A1-dependent electrocatalytic reactions. The obtained biosensor was used for the screening of inhibitory activity of oxazoliny derivatives of [17(20)*E*]-pregnene [1, 2]. Among the tested oxazoliny derivatives of [17(20)*E*]-pregnene there were identified potent inhibitors of the catalytic activity of CYP17A1.

Acknowledgements: the work was supported by the Program for Basic Research of Russian State Academy of Sciences for 2013-2020.

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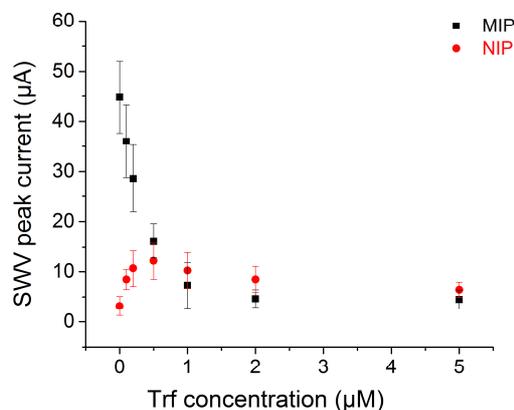
## First results and challenges for a transferrin MIP

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Transferrin (Trf) is an iron binding glycoprotein consisting of two homologous domains with a molecular weight of around 80 kDa [1]. In human serum the Trf concentration is approximately 30  $\mu\text{M}$ . Carbohydrate-deficient transferrin serves as a biomarker of alcohol abuse [2]. In this work, a non-conducting molecularly imprinted polymer (MIP) was prepared for Trf recognition using surface imprinting [3]. The functional monomer is the electropolymerizable scopoletin [4]. The MIP was deposited as a thin film on an Au electrode by oxidative potentiodynamic polymerization. The template rebinding and removal from the MIP film was detected by cyclic voltammetry (CV) and square wave voltammetry (SWV) using ferricyanide as redox marker. The linear measuring range was from 0.1 to 0.5  $\mu\text{M}$  Trf (Fig.1). The specificity of MIP was tested by using human serum albumin (HSA). It is found that the measuring signal of 5  $\mu\text{M}$  HSA was close to that of 1  $\mu\text{M}$  Trf. Several approaches for improving the specificity like influence of ionic strength, anodic desorption of the protein and SAM-MIP architectures will be presented.



**Fig. 1:** Trf concentration dependences for the MIP (black squares) and NIP (red circles) measured by SWV in 100 mM KCl solution containing 5 mM ferricyanide and 5 mM ferrocyanide. Voltammetric conditions: 50 mV pulse amplitude, 3 mV step potential, 10 Hz Frequency.

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## Molecularly-imprinted polymer on paper-based scaffold for cotinine microextraction

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Commercially available sorbent materials for solid-phase microextraction are widely used in analytical laboratories; however, non-selective binding is the major problem for sample analysis. In this study, sample collection and microextraction of cotinine, the predominant metabolite of nicotine, were proposed by using molecularly imprinted polymers (MIPs) on a paper-based scaffold. The principle of this analytical method is based on non-covalent interaction between the target analyte and a functional monomer in polymer matrix. Cotinine selective MIP was bulk polymerized and fiberglass papers were used as paper-scaffolds for cotinine MIP immobilization by using 0.5% agarose gel. By using the cotinine MIP modified paper scaffold, the linear range of cotinine detection was between 4-500  $\mu\text{g mL}^{-1}$  ( $r^2 = 0.994$ ). The ability of the MIP-paper scaffold to adsorb cotinine is 1.5-2 folds higher than non-imprinted-polymer-scaffold. The kinetic adsorption test of the MIP-paper scaffold was investigated during 15 min to 2 h and the results indicated that the maximum adsorption was obtained at 1 hour. In conclusion, the MIP-paper scaffold is very promising for facile and simple on-site sampling of cotinine and can be used for assessment of tobacco smoke exposure.

**Keywords:** Molecularly imprinted polymer, solid-phase microextraction, paper-based scaffold, cotinine

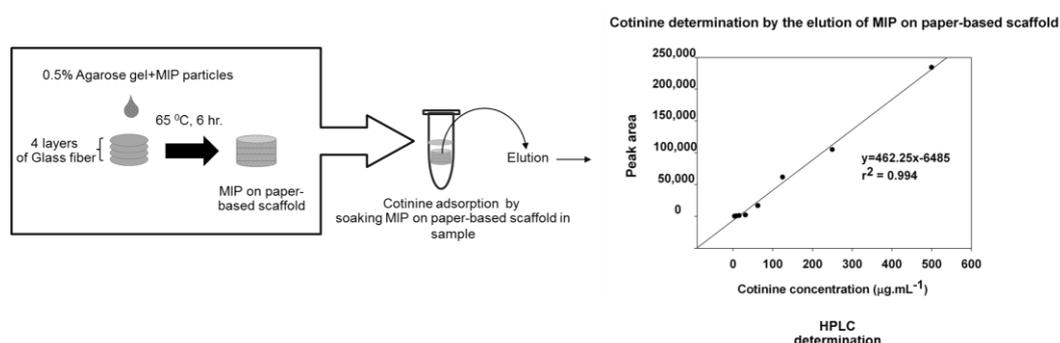


Fig1. Schematic description of cotinine determination by using molecularly-imprinted polymer on paper-based scaffold for sample preparation

## Binding of *Staphylococcus aureus* cells to aptamer-modified gold electrodes measured with impedance

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A thiol modified protein A-binding aptamer (PAA) [1] was co-immobilized with mercaptohexanol on a gold electrode via self-assembly. Mediated by the ferri-/ferrocyanide ions in the buffer solution a charge-transfer with the electrode occurs due to defects close to the aptamer. Upon binding of *Staphylococcus aureus* via the surface-bound protein A to the immobilized aptamer (Fig. 1 left), these defects are covered by the bound molecule and charge-transfer is inhibited, which can be measured by impedance increase. Impedance spectroscopy was performed using a 3-electrode flow-through chamber. By fitting the impedance spectra to an equivalent circuit, the charge-transfer resistor  $R_{ct}$  was determined as the only parameter changing significantly with the concentration of *S. aureus*. By fitting the binding curve (Fig. 1 right) to the Hill equation an apparent dissociation constant  $K_d$  of 200 cells per ml was determined. As a negative control, the binding of  $10^8$  cells per ml of *S. epidermidis* and *E. coli* K12 on aptamer modified electrodes was measured to be  $50 \pm 36 \Omega \cdot \text{cm}^2$ . Also the binding of *S. aureus* to blank electrodes, mercaptohexanol-modified electrodes or to pool-oligonucleotide modified surfaces was significantly lower than for the PAA modified surface.

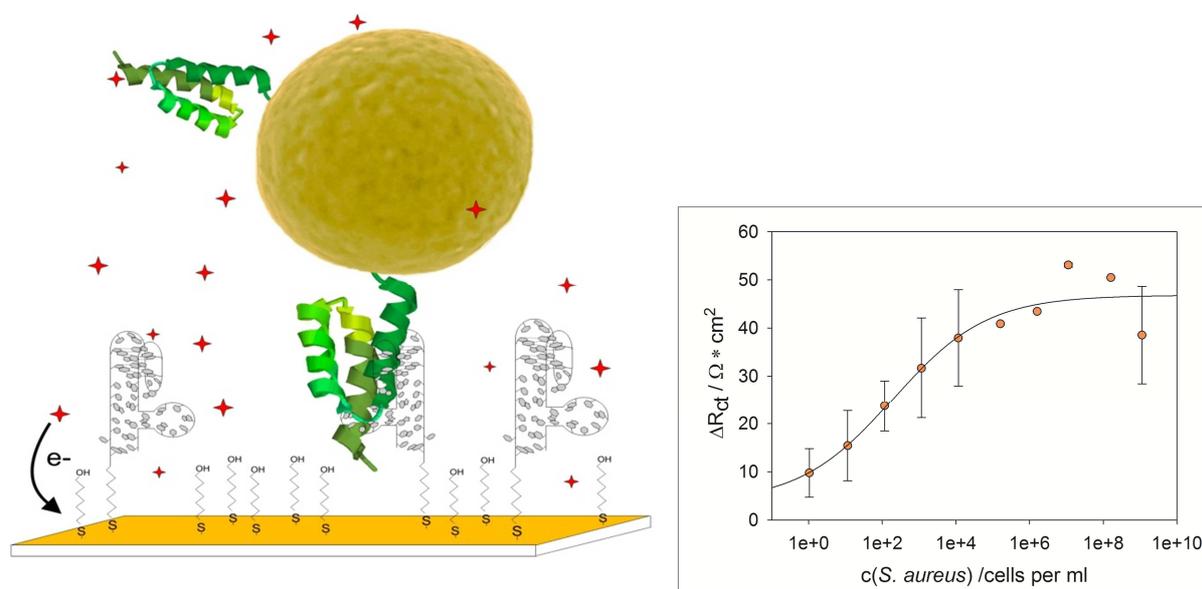


Fig. 1: **left:** schematic presentation of the binding of *S. aureus* cells with surface-bound protein A (green) to immobilized aptamers (grey) on gold surface (orange) measured via inhibition of charge-transfer mediated by ferri-/ferrocyanide (red) **right:** binding curve with Hill fit (black line)

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## Miniaturisation of an Electrochemical Peptide-Based Biosensor for the Detection of Trypsin

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The development of electrochemical peptide-based biosensors for the detection of proteases is attracting interest as proteolytic enzymes are known to be responsible for many physiological conditions [1]. We have previously established a macroelectrode sensor system for the detection of the protease trypsin [2]. Miniaturisation of such biosensing platforms is crucial to applications where implantable sensors are desirable. Furthermore, using microelectrodes could offer various advantages over macroelectrodes (e.g. decreased size, shorter response times, improved mass transport, higher signal-to-noise ratio giving higher sensitivity) [3]. In this context, we report the characterisation of an electrochemical biosensor for the detection of the protease trypsin employing self-assembled monolayer (SAM)-modified platinum microelectrodes of 25 µm diameter. The sensing probe is designed with methylene blue as the redox tag, a thiol-containing moiety as an anchor and a trypsin-specific peptide sequence (phenylalanine - arginine - arginine). Square wave voltammetry measurements have been carried out to monitor resulting changes in the peak current due to the specific proteolytic cleavage of these SAM-based redox-tagged peptides by trypsin. The construction of the sensing system has been optimised and the performance of the prepared sensors evaluated in terms of response to trypsin and assessment of non-specific binding. These microelectrode results will be compared to the previously obtained data using macroelectrodes.

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**Molecularly imprinted polymer chemosensor  
for selective determination of *N*-nitroso-L-proline  
in food products of animal origin**

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Too high temperature frying, grilling, or boiling of protein providing food products of animal origin generates toxic heteroaromatic amines and nitrosamines in these products [1], which might cause chronic diseases, for example serious hormonal dysfunctions, and even cancer [2]. We devised and fabricated a chemical sensor for selective determination of the *N*-nitroso-L-proline (Pro-NO) target toxin [3]. As the recognition unit in this chemosensor, we used thiophene polymer molecularly imprinted (MIP) with Pro-NO. By electrochemical polymerization, we deposited an MIP-(Pro-NO) films on the Pt disk electrode surface, and on the gold electrode deposited on either a quartz crystal resonator or a glass slide. After template extraction from MIP-(Pro-NO) with NaOH, we selected for signal transduction and tested piezomicrogravimetric (electrochemical quartz crystal microbalance, EQCM) and electrochemical (differential pulse voltammetry, DPV, electrochemical impedance spectroscopy, EIS) techniques proving this chemosensor high sensitivity and selectivity with respect to different interferences. To confirm that detectability of our chemosensor is sufficient for real food sample analysis, we determined Pro-NO in grilled pork neck samples. Successful determination of Pro-NO in these samples indicates that the MIP-(Pro-NO) chemosensor is a promising tool for this toxin determination in food samples of animal origin.

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## A New Flow Cell for Pencil Graphite Electrode and Its Using Biosensing of Glucose in FIA System

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Recently, pencil graphite has found great attention as an electrode material in the construction of electrochemical sensors and biosensor [1-3]. When compared with the other carbon based electrodes, PGEs have the same advantages such as the high electrochemical reactivity, commercial availability, good mechanical rigidity, disposability, lower cost and the ease of modification [1-3]. Another useful approach in electrochemical sensors and biosensors is the usage of Flow Injection Analysis (FIA) with Chemically Modified Electrodes (CMEs) in electrochemical techniques (2). Recently our research group focused on the construction of electrochemical biosensors in FIA system using PGE and a new constructed flow cell for PGE [3, 4].

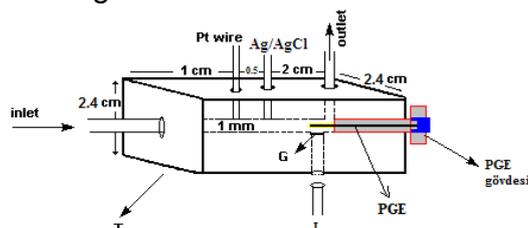


Fig.1. Schematic diagram of pelectrochemical flow cell for PGE, T: Teflon block, L: light source, G: quartz window [4]

An important application area of (CMEs) is construction of biosensors for some biologically important compounds that depend on  $\text{NAD}^+/\text{NADH}$  redox couple and dehydrogenase enzymes (1). This study reports i) the electropolymerization of a phenothiazine dyes, Methylene blue (MB) and Azure A, onto a PAMAM adsorbed pencil graphite electrode (PGE), ii) immobilization of glucose dehydrogenase (GDH) onto poly-MB/PAMAM/PGE or poly-AzA/PAMAM/PGE by crosslinking with glutaraldehyde, iii) electrocatalytic oxidation of NADH at Azine type dyes modified PGE, and iv) biosensing of glucose using GDH/poly-MB/PAMAM/GCE or GDH/poly-AzA/PAMAM/GCE in FIA system. The proposed study offers a disposable, low cost, selective and sensitive electrochemical glucose biosensor in FIA system for the first time.

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## Flow Injection Analysis of H<sub>2</sub>O<sub>2</sub> Using Pd Nanoparticles Modified Pencil Graphite Electrode

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A Pd nanoparticle modified Pencil Graphite Electrode (PGE) was proposed for the electrocatalytic oxidation and non-enzymatic determination of H<sub>2</sub>O<sub>2</sub> in Flow Injection Analysis (FIA) system. Pd nanoparticles (PdNPs) electrochemically deposited on pretreated PGE (p.PGE) surface by recording cyclic voltammograms of 1.0 mM of PdCl<sub>2</sub> solution in 0.10 M KCl at scan rate of 25 mV/s for 30 cycles. Surface morphologies of p.PGE and modified electrode (PdNPs/p.PGE) were examined by recording their Scanning Electron Microscope (SEM) images which indicate PdNPs were successfully decorated on p.PGE. Electrochemical oxidation of H<sub>2</sub>O<sub>2</sub> was investigated by recording cyclic voltammograms of p.PGE and also PdNPs/p.PGE in pH 7.0 Phosphate buffer solution contains 0.10 M KCl at scan rate of 50 mV/s. Cyclic voltammograms show that the peak potential of H<sub>2</sub>O<sub>2</sub> oxidation shifts from about +700 mV at bare p.PGE to +150 mV at PdNPs/p.PGE vs. Ag/AgCl /KCl (sat.) electrode. On the other hand reduction peak of H<sub>2</sub>O<sub>2</sub> shifts from -450 mV at bare p.PGE to -100 mV at modified electrode. Moreover, the peak currents at PdNPs/p.PGE increases about 10-15 times compared to P.PGE. This result shows that PdNPs/p.PGE exhibits a good electrocatalytic activity towards oxidation and reduction of H<sub>2</sub>O<sub>2</sub>. Then, FI amperometric analysis of H<sub>2</sub>O<sub>2</sub> was performed under optimized conditions using a new homemade electrochemical flow cell which was constructed for PGE. Thus disposable, low cost and simple modified electrode was prepared by electrodeposition of PdNPs onto surface of PGE and the electrochemical H<sub>2</sub>O<sub>2</sub> sensor was fabricated dependent on excellent electrocatalytic activity of PdNPs/PGE towards H<sub>2</sub>O<sub>2</sub>. PdNPs and PtNPs modified PGEs have already been reported for the sensitive, low cost and simple detection of H<sub>2</sub>O<sub>2</sub> [1, 2]. However, the novel statement of this study is the using of these useful modified electrodes for detection of H<sub>2</sub>O<sub>2</sub> in FIA system using a new home made flow cell which was constructed for PGE. The proposed electrode was successfully applied to determination of H<sub>2</sub>O<sub>2</sub> in real samples.

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## Development of acoustic sensor for detection chymotrypsin activity at surfaces

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Casein protein makes up about 80% of bovine milk. There are four main types of casein:  $\alpha$ -s1,  $\alpha$ -s2,  $\beta$  and  $\kappa$ . In milk, casein forms micelles that are stabilized by calcium phosphate. They are of porous structure which allows water to flow into the core of the micelle. The surface of the  $\beta$  casein micelle is hydrophobic and terminated by  $\kappa$  casein. Degradation of casein micelles in milk are tied to the processes of milk getting sour or cheese making. Proteases like plasmin and trypsin are part of this process and therefore it is interesting to analyze protease activity in milk or during the process of casein cleaving. In order to study this process we used acoustic biosensor based on Quartz Crystal Microbalance (QCM). This method measures the change of the resonant frequency of the quartz crystal by deposition of mass on the crystal or change of the medium. Using the self assembled monolayer (SAM) of mercaptoundecanoic acid (MUA) we can activate the surface of the gold electrode on the quartz crystal. This monolayer is then able to bind casein - the substrate that can be cleaved by proteases like plasmin or chymotrypsin. This results in measurable change in resonant frequency which roughly translates to the mass of the casein bound to the surface and casein cleaved away by protease interaction. This measurement allows us to observe the cleaving of casein by different proteases. In addition the experimental set-up allowed us for change of the medium, therefore it is possible to measure cleaving of casein in different conditions, for example using different pH. We performed experiments using QCM on the cleaving of  $\beta$  casein by chymotrypsin. Chymotrypsin was detected in the range of concentrations 1 pM – 20 nM. Limit of detection of this acoustic transducer was 1 pM. At this concentration approx 6% of casein layer was cleaved (this corresponds to frequency increase by approx. 9 Hz). This method can be used also for study of the activity of other proteases, such as plasmin. Topography of casein layers and its changes following chymotrypsin cleavage was also studied by atomic force microscopy.

### **Acknowledgments:**

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### **Non-enzymatic ethanol sensor based on a nanostructured disposable screen-printed electrode**

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Herein, a simple and fast method for the electrocatalytic detection of ethanol using disposable screen-printed carbon electrodes (SPCEs) modified with platinum nanoparticles (PtNPs) is presented. The catalytic properties of PtNPs are employed in the oxidation of ethanol and the electrochemical results obtained revealed that PtNPs can effectively enhance the electron transfer between the analyte of interest and the electrode. Moreover, the content of ethanol is assayed in different alcoholic beverages (beer and wine). Additionally, an alcohol-free beer is also analyzed. The results obtained corroborated the applicability of the developed sensor as a trustful analytical screening tool. The obtained results are in accordance with the tolerances for indication of alcoholic strength by volume in the labeling of alcoholic beverages allowed by EU laws [1].

[1] European Commission: Commission Directive 87/250/EEC on the indication of alcoholic strength by volume in the labelling of alcoholic beverages for sale to the ultimate consumer. Off J Europ Comm 1987,L113:57-58.

## Development of artificial receptor for computationally simulated disease biomarkers for sensing application

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The behaviour of biological molecules in aqueous environments plays important role for their mechanism of action and molecular interaction. This also effects the successful implementation of a biosensor for the detection of target molecules as the possible conformational changes lead to decrease or mask sensor signal. Hence, this work aims to simulate the behaviour of neuron specific enolase, a lung cancer biomarker, in water and buffer environments. The protein was initially divided into the clusters and epitope cluster analysis was applied. Six alternative epitopes were then selected from outside part of the protein as  $\alpha$ -helices. Each epitope was simulated using molecular dynamics and the stability of the epitopes were expressed as root-mean-square deviation of atomic positions (RMSD) and secondary structure analysis. Based on the computational results, two most stabile epitopes were selected to be synthesised in the laboratory. Original and cysteine modified epitopes were synthesised to be utilized in target detection and surface imprinting using electropolymerization, respectively. Each step of imprinting process and sensor assay were analysed using cyclic voltammetry and square wave voltammetry to obtain the results. Imprinting was successfully performed on a quartz crystal microbalance (QCM) chip by adsorbing the modified epitope on gold sensor via cysteine-gold interaction and then electropolymerizing the scopoletin on the surface as the monomer. The template molecule was removed from the polymer by employing multistep potential later on leaving the imprinting cavities behind for the rebinding assays. The sensing of entire protein via epitope imprinting will be further investigated in buffer and serum samples for the detection of lung cancer.

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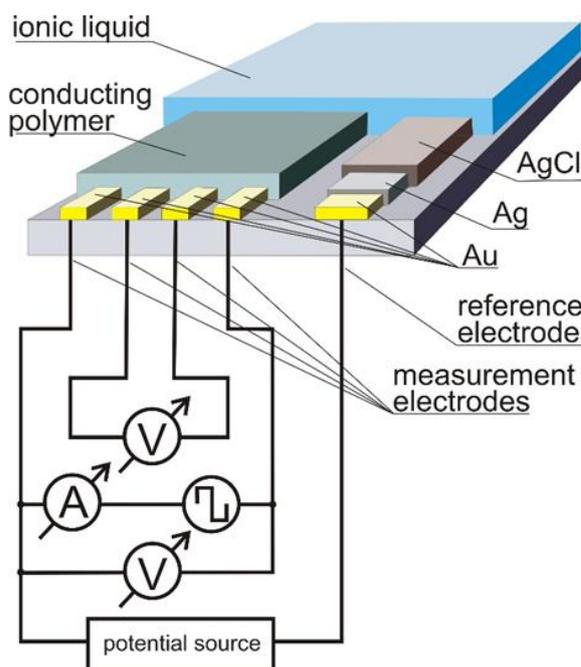
## Virtual sensor array consisting of a single sensor element with variable affinity: an application for analysis of fish freshness

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### Abstract

A concept of virtual sensor array based on electrically controlled variation of affinity properties of the receptor layer was realized on the base of integrated electrochemical chemotransistor containing polyaniline as the receptor layer. Electrical control of the redox-state of polyaniline was performed in five-electrode configuration containing four electrodes for conductivity measurements and Ag/AgCl reference electrode integrated on the same glass chip. Ionic liquid provides electrical connection between the reference electrode and chemosensitive material. Conductivity measurements demonstrated potential controlled electrochemical conversions of the receptor material between different redox-states. Binding of trimethylamine at three different potentials, corresponding to these states was studied. The results showed that both kinetic and equilibrium binding properties of the receptor are controlled by electrical potential thus providing a possibility to form a virtual sensor array using only a single sensing element. The concept was applied for monitoring of fish headspace. Using three characteristics of the sensor response measured at three different redox states of the same sensor material, we have obtained signals from a virtual sensor array consisting of nine chemosensitive elements. The sensor displays systematic changes of its nine signals during fish degradation.



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## Lab-on-a-chip based on electrochemical biosensors for malolactic fermentation monitoring of red wines

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The concentration of the L-malic and the L-lactic acids during the malolactic fermentation (MLF) process in red wines is strongly related to the quality of the final wine. Nowadays, monitoring of these analytes is carried out off-line in laboratories using traditional and tedious methods. Then, the control of the MLF process is not carried out in time. In order to solve that, a new lab-on-a-chip (LOC) device for the real-time monitoring of the MLF in field is reported in this work. The LOC is fabricated with poly-methyl-methacrylate (PMMA) and contains two electrochemical biosensors fabricated with microelectronic technology. A silicon chip of 9 mm x 11 mm defining an electrochemical cell with four platinum electrodes is used as transducer (Figure 1A). The surface of two platinum electrodes is electromodified with a three-dimensional polypyrrole membrane entrapping the main chemical reagents involved in the bienzymatic reaction used for the acids determination [1, 2]. The chip is assembled in the PMMA cell defining a volume of 20  $\mu$ l and allowing the automatic input and output of the wine samples (Figure 1B). The electroanalytical response of the biosensors (linear range, limit of detection, sensitivity and working stability during 30 days) meets the requirements of the proposed application. Finally, the lab-on-a-chip was applied for the monitoring of both acids in wine samples collected during the MLF of several red wines, showing an excellent agreement with the results obtained using the colorimetric standard method.

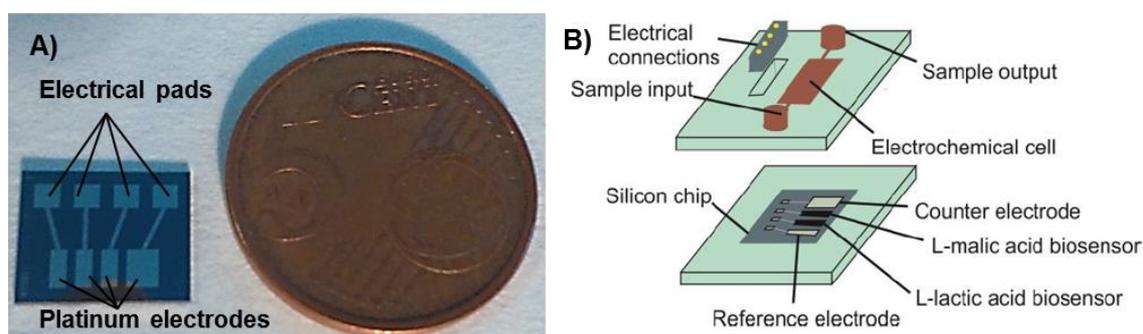


Figure 1: Scheme of the lab-on-a-chip device for the monitoring of the MLF

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## Electrochemical biosensors based on DNA and DNA analogues

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Among various groups of biosensors, sensors with receptor layers containing nucleic acids (most notably DNA) are gaining significant attention. DNA-based biosensors are typically used for the determination of specific nucleic acid sequences, which are important for diagnosis of certain genetic and infectious diseases. However, DNA sensors can be also used for the determination of other analytes, including metal ions. Despite that, the lack of resistance to restriction enzymes prevents the use of these sensors in in vivo measurements. Moreover, due to their reduced stability under environmental conditions, as well as non-specific interactions, the usefulness of DNA biosensors can be limited. To mitigate these problems, analogues of nucleic acids can be used [1]. These compounds are structurally similar to natural DNA, with alternative backbones. The most useful and popular nucleic acid analogues are peptide nucleic acid (PNA) and phosphorothioate oligonucleotide (PTO).

Herein, we present a novel electrochemical biosensors for the determination of mercury using analogues of nucleic acid as receptor layer. In case of PTO, the presence of sulfur atom in the backbone of modified nucleotide allows for strong interaction with  $\text{Hg}^{2+}$  [2]. The optimized sensor distinguishes itself with high selectivity towards mercury ions and lower detection limit of  $2.34 \cdot 10^{-11} \text{ mol} \cdot \text{L}^{-1}$ . For tested PTO sequence, it was possible to determine the linear response in the  $\text{Hg}^{2+}$  concentration range of  $10^{-11} - 10^{-7} \text{ mol} \cdot \text{L}^{-1}$ . In turn, PNA contains glycine units linked via peptide bond instead of sugar-phosphate backbone, therefore its interaction with molecules like proteins or metal ions is possible. In the proposed system [3], the AQMS current difference increases proportionally within the  $\text{Hg}^{2+}$  concentration range  $5 \cdot 10^{-8}$  to  $10^{-6} \text{ mol} \cdot \text{L}^{-1}$ , while for higher concentrations, the saturation of PNA layer with  $\text{Hg}^{2+}$  is observed. The optimized sensor distinguishes itself with high selectivity toward  $\text{Hg}^{2+}$  and lower detection limit of  $3.33 \cdot 10^{-9} \text{ mol} \cdot \text{L}^{-1}$ .

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## Influenza matrix protein (M1) binding interactions with lipid membrane: An SPR study

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Influenza virus is a respiratory pathogen that causes both seasonal and pandemic infections in humans and other hosts. The matrix protein M1 forms a shell underlying the lipid envelope of the virus and enclosing the genetic material. M1 orchestrates the process of new virion formation by binding to the inner leaflet of the plasma membrane of infected cells. Furthermore, it bridges together the viral genome and the viral spike proteins. Since M1-lipid interaction is a fundamental step in this process, we used Surface Plasmon Resonance (SPR) to measure membrane binding of M1. Also, we analyzed different M1 constructs, including the N-terminal domain (NM1), the C-terminal domain (CM1) and M1 lacking the polybasic sequence (M1m). To mimic the inner leaflet of the virus infected cell, we used a lipid monolayer model membrane composed of DOPS-DOPC 70:30 molar ratio. Our results showed that M1 and NM1 bound efficiently to DOPS-DOPC membranes, with a remarkably low dissociation rate. M1m displayed a weaker binding to the model membrane. This result shows that the PBS plays an important role in determining M1 affinity to lipids. The association of M1 with lipid membranes was affected by pH and high salt concentration, pointing at a role of electrostatic interactions. The binding of CM1 was below the limit of detection, indicating that the N-terminal domain is needed for M1-lipid interaction. These results were also correlated with a structural analysis of the different protein constructs carried on via Molecular Dynamics simulations and Circular Dichroism.

## Detection of dielectrophoretically accumulated bacteria at nanoelectrode arrays by surface enhanced Raman spectroscopy

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Electrode arrays are used for the structured collection of *E. coli* bacteria at individual nanoelectrodes (500 nm diameter). Bacteria are attracted to the electrodes under the influence of an inhomogeneous electric AC field. Dielectrophoresis, which is the dominant effect of the electric field applied here, causes a movement of the bacteria towards the electrodes and hence a concentration effect. Cells of *E. coli* are captured out of suspensions; both temporal and permanent adhesion of cells is achieved depending on electric field parameters.

Bacteria are detected by surface enhanced Raman spectroscopy (SERS), where the surface enhancement is introduced by the addition of silver nanoparticles (AgNPs, 50 nm diameter). Four different strategies for AgNP decoration of the electrodes, with and without electric fields, are compared. While all strategies produce sufficiently strong signals for single bacteria detection, the strongest signal enhancement is obtained by pre-incubation of *E. coli* with AgNPs.

The use of nanoelectrode arrays with thousands of electrodes offers rapid analyses of large numbers of individual pathogens. The combination of a selective and accelerated target capture method (dielectrophoresis) with a sensitive signal transduction technique (SERS) is promising for the detection of pathogens like bacteria or virus particles. On-line detection in a flow-through system and specific identification of pathogens from their spectra are envisaged for future perspectives.



Fig. 1: Successive dielectrophoretic accumulation of silver nanoparticles (AgNPs) and of bacteria at nanoelectrode arrays, and bacteria detection by surface enhanced Raman spectroscopy (SERS).

## Detection of tuberculosis DNA by means of polyelectrolyte modified field-effect based capacitive semiconductor sensors

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In this study, field-effect based capacitive electrolyte-insulator-semiconductor (EIS) sensors have been used to detect target deoxyribonucleic acid (DNA) with a genomic sequence of *mycobacteria tuberculosis*. Two different strategies were applied for the electrical detection of DNA molecules with the tuberculosis sequence: For the first method, the sensor surface was functionalized with a bilayer of polyelectrolyte (PAH, Poly(allylamine hydrochloride))/probe single-stranded DNA (ssDNA) followed by an on-chip hybridization of target complementary DNA (cDNA). For the second method, the hybridization reaction between probe ssDNA and target cDNA to double-stranded DNA (dsDNA) take place in-solution following by an adsorption process of the formed dsDNA molecules onto the polyelectrolyte-modified EIS sensor. In both methods, DNA molecules bind to the functionalized chip surface. As an example, the adsorption of PAH, the immobilization of probe ssDNA as well as the target DNA hybridization can be monitored dynamically by means of the field-effect sensor (see Fig. 1).

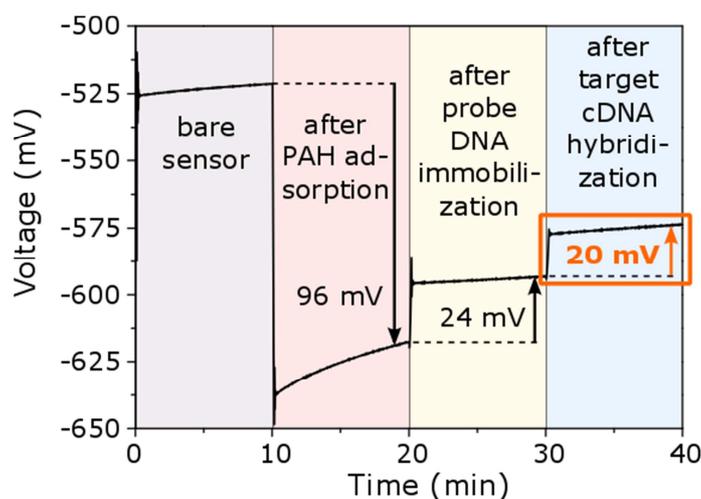


Fig. 1: Electrochemical response signal of the EIS sensor before and after sensor modification with PAH, probe ssDNA and after the successful hybridization of target cDNA.

# **List of registered authors**

Abbandonato, Gerardo	O49
Adam, Jennifer	P35
Altintas, Zeynep	P88
Baeumner, Antje J.	O33, P51
Bala, Agnieszka	P5, P91
Barreda-García, Susana	O43
Bartlett, Philip N.	PL2
Basan, Hasan	P34
Bastian, Philipp U.	O50
Becher, Gunther	O38, P58
Beckmann, Dieter	P62, P77, P81
Belicky, Stefan	O4, O60
Bemetz, Jonas	O24
Beyer, Sebastian	O20
Bianchi, Maria Vittoria	O10
Bier, Frank	O22, O28, P7, P21, P22, P40, P42, P44, P45, P93
Biermann, Michelle	P40
Bobrowski, Tim	P25
Bocklitz, Thomas	KN6
Bollella, Paolo	O6
Bunea, Ada-Ioana	O54
Burkitt, Richard	O9
Cahill, Brian P.	O40
Campuzano, Susana	O13, O29
Carl, Peter	O31, P50
Caviglia, Claudia	O47, P64
Choi, Youngeun	P19
Cieplak, Maciej	O65, P83
Ciornii, Dmitri	O5, P9
Clément, Jean-Emmanuel	O1
Conzuelo, Felipe	KN12, O75, P26, P41
Daboss, Sven	P53
Dame, Gregory	O39
Dantism, Shahriar	P67
Dathe, André	O18, P38
de Wael, Karolien	KN5
Dilgin, Yusuf	P84, P85
Emnéus, Jenny	O54, P64
Fanjul-Bolado, Pablo	P6, P87
Feifel, Sven Christian	O5, P2, P9
Feller, Karl-Heinz	O48, P71
Flechsigt, Gerd-Uwe	O56
Frense, Dieter	P62, P77
Fusco, Giovanni	P11, P13
Gajovic-Eichelmann, Nenad	O22, P31

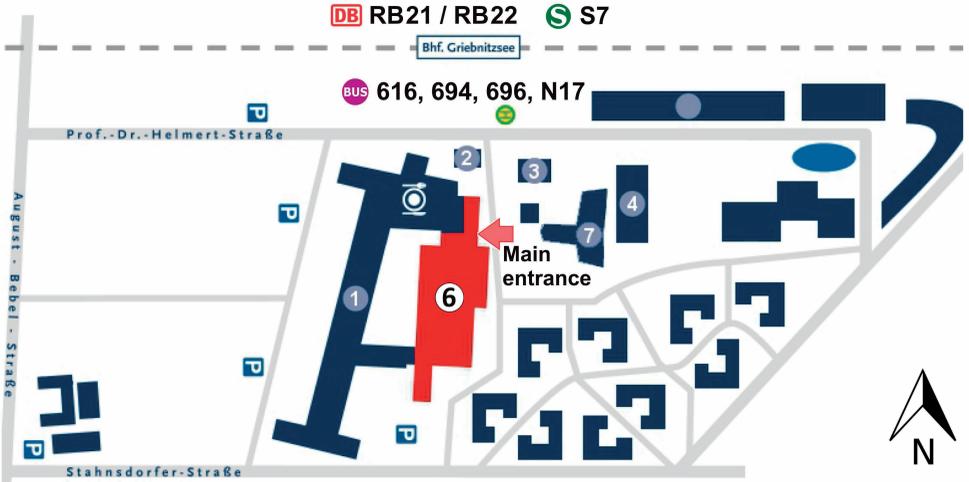
Garcia-Cruz, Alvaro	O68
Giannetto, Marco	O10
Gier, Konstanze	P57
Giménez-Gómez, Pablo	P90
Girault, Hubert	PL3
Giray Dilgin, Didem	P84
Göbel, Gero	P4, P11
Gorton, Lo	KN9, O6
Griesche, Christian	P51
Gross, Andrew James	O74
Grossmann, Kai	O16
Guha, Subhajit	O15, P8
Hämmerle, Martin	P1, P73
Harnisch, Falk	O46
Haug, Sandro	P87
Haupt, Karsten	PL4
Hecht, Lars	P24
Hianik, Tibor	P47, P68, P86
Hilbig, Urs	P52
Hildebrandt, P.	P88
Hille, Carsten	O19
Hoang, Hoa Thi	O34
Hölzel, Ralph	P21, P22, P93
Ingebrandt, Sven	O59
Ismail, Dahmani	P92
Ivanova, Salzitsa	P33
Jambrec, Daliborka	P26
Jetzschmann, Katharina J.	KN13, O66, P79
Ju, Huangxian	O69
Kage, Daniel	P43
Karakaya, Serkan	P85
Kavanagh, Paul	O64
Kazmierczak, Marcin	O15, P8
Keller, Stephan Sylvest	KN3, O47, O54, P64
Kneipp, Janina	KN1, P28
Knigge, Xenia	P7
Kober, Catharina	O24, O37
Kopiec, Gabriel Bruno	O26
Kowalczyk, Agata	O14, P55
Kröner, Felix Julian	O7
Kumbhat, Sunita	P17
Kurbanoglu, Sevinc	O11
Kurochkin, Ilja	O17
Lach, Patrycja	P83
Laiwattanapaisal, Wanida	P46, P80
Laroussi, Arwa	P12

Larpant, Nutcha	P80
Laux, Eva-Maria	O28, P21, P93
Le Goff, Alan	KN16
Lederle, Mario	P59
Lensen, Marga C.	O52, P70
Lesch, Andreas	KN10
Li, Dajuan	P49
Lin, Jing	P53, P66
Lisdat, Fred	O5, O42, O71, P2, P3, P4, P11, P20, P36
Liu, Songqin	O28
Luckert, K.	P73
Luppa, Peter	P14, P75, P76
Mak, Stefanie	P14, P75
Masamrekh, Rami	P78
Matysiak-Brynda, Edyta	O41, P55
Mayer, Michael	O33, O45, P51
Mazzei, Franco	O6, P11, P13
Memczak, Henry	P42
Menger, Marcus	KN14, P77
Metzner, Julia	P73
Mgeladse, Raphael Marius	P65
Michaelis, Stefanie	P71
Minunni, Maria	P37, P49
Miranda-Castro, Rebeca	O43, O57
Mirsky, Vladimir	O36, O72, P12, P39, P89
Mitrova, Biljana	O62
Molinnus, Denise	P20
Moos, R.	P73
Moralejo, Benito	O32
Mroginski, M. A.	P88
Murawska, Magdalena	O73
Nacak, Selma	O50
Ndangili, Peter Munyao	O27
Nechaeva, Natalia	O17
Neumann, Bettina	P74
Neumeier, Michael	O33
Neves, Marta	P6, P87
Nirmalanathan, Nithiya	O23
Nistler, Angelika Maria	P18
Nizamov, Shavkat	O36, O72, P39
Nöll, Gilbert	O2, P32
Nowicka, Anna M.	O14, O41, P55
Oberländer, Jan	O45
Osorio-Madrado, Anayancy	P72
Pacholski, Claudia	O21

Papp, Soma	P30
Peter, Harald	P44, P45
Plumeré, Nicolas	O26, O75, P41
Popp, Jürgen	PL1, KN6
Posseckardt, Juliane	P61
Poturnayova, Alexandra	P68, P86
Preininger, Claudia	P27, P57
Puiu, Mihaela	O30
Radunz, Sebastian	P15, P63
Ramos, Inês I.	O31
Raysyan, Anna	P54
Reich, Peggy	P77, P81
Riedel, Marc	O42
Riegel, Anna-Lena	O63
Rödiger, Stefan	O16, P19, P23
Roggenbuck, Dirk	O16
Rojas-Carrillo, Oscar	P16
Rotariu, Lucian	O55
Ruff, Adrian	KN12, O26, O75, P25
Ruzgas, Tautgirdas	P60
Sarauli, David	P3
Scarano, Simona	KN4, P37, P49
Schäfer, Daniel	P3, P36
Schäferling, Michael	O35
Schasfoort, Richard	KN15
Scheller, F. W.	O66, P74, P79, P88
Scherbahn, Vitali	O36, O72, P39
Schierack, Peter	O16
Schmalenberg, Michael	P14, P75, P76
Schmälzlin, Elmar	O32
Schmid, B.	P88
Schmidt, Carsten	P23
Schmittlein, Carina	P69
Schneider, Ralf	O25
Schneider, Rudolf J.	KN7, O20, O31, P50, P54
Schomäcker, R.	P88
Schöning, Michael Josef	O45, O61, P20, P67, P94
Schönmann, Christine	P76
Schröder, Uwe	O76
Schuhmann, Wolfgang	EL, O26, O75, P25, P26, P41, P66
Schulze, Holger	O44
Schulze, Sven	O19
Seidel, Michael	O24, O37, P18
Serafín, Verónica	O13
Shumyantseva, Victoria	O8, P78

Sigolaeva, Larisa V.	O58
Simon, László Ferenc	P29
Skládal, Petr	KN2
Soldà, Alice	O53
Stanke, Sandra	P22
Starbird, Ricardo	P16
Steinke, Nadja	P56
Stern, Daniel	O71
Stieger, Kai	O5, P9
Stojek, Zbigniew	P55
Stolwijk, Judith Anthea	O51
Sun, Ruoyu	P14, P75
Suraniti, Emmanuel	O3
Sussmuth, R.	P88
Takiden, A.	P88
Tatarko, Marek	P47
Tkac, Jan	O4, O60
Tschiche, Harald Rune	P15, P63
Ucar, Ahmet	P82
Verma, Sujit Kumar	P48
Wagner, Patrick	KN8, P67
Warmt, Christian	P40, P42
Weber, Patricia	O70, P24, P35
Wegener, Joachim	O51, P69, P71
Weigel, Wilfried	O12
Welden, Rene	O61
Werner, Tobias	P59
Wollenberger, Ulla	O62, O66, P74, P79, P88
Yarman, Aysu	KN13, O66, P79
Yesildag, Cigdem	P70
Yilmaz, Hüma	P34
Zhang, Xiaorong	P79
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## Building 6:

