Abstracts of oral presentations

Sunday, 18.15

The Human Factor in Scientific Thinking: The Illusions That We Live By

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The talk is based on a book published in 2015[1] and takes a candid look at science from an unusual perspective, attempting to offer some insights into how psychological factors secretly infiltrate its fabric. The common notion that science is the world of objective rationality based on methodological rigor, stringent logic, irrefutable experimental proofs, and unbiased peer reviewing and testing, is an idealistic myth which all too often dissents from what "scientific truth" *is* in reality, and from how scientists truly „function" in practice. Actually, our scientific thinking is influenced by deep-rooted human ("anthropic") factors of which we are not normally aware of. Some of these lead to what we call Mental Traps, which are the hidden sources of mistaken or misleading inferences, a phenomenon we refer to as the illusion of understanding, and mass misconceptions about apparently well-established "scientific truths". By their very nature, Mental Traps affect even the smartest, most knowledgeable, and most attentive scientists. However, by understanding the essence of the Traps one can develop the enlightening faculty of detecting and avoiding them both in one's own and in others' thoughts. It is this mental aptitude/attitude of being keenly conscious about our human nature during scientific thinking which is captured in the phrase "anthropic awareness" in the title of the aforementioned book. In the talk I address the concept of "anthropic awareness" as a kind of philosophy, I outline a simple metaphoric model of human thinking that clarifies the reasons behind the Mental Traps, and l discuss some of the Traps. Real-life case-studies involving Mental Traps in both the theory of NMR and in structure determination by NMR and MS will be mentioned in some detail. Although some prior knowledge about NMR (and perhaps MS) is an advantage, it is not necessary for the understanding of the presentation. In fact, the case studies will serve more or less as a pretext for demonstrating that "anthropic awareness" has a general relevance throughout all of natural sciences, and is useful not only in our everyday professional lives as researchers, but also in our nonprofessional everyday lives.

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Monday, 9.00

An Artificial Enzyme: How Nanoconfinement Allows the Selective Electrochemical Detection of Glucose Directly in Whole Blood

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Nanoparticles that catalyse biochemically relevant reactions have been promoted as alternative enzymes. The application of such artificial enzymes is severely restricted by poor selectivity in biological fluids; mainly because the reactions occur at active sites on the exterior surface of the nanoparticle. Enzymes in contrast typically have their active sites down a nanoconfined substrate channel where the reaction occurs in different solution conditions to bulk solution which aids in achieving selectivity for the substrate. Herein we mimic the same three-dimensional structure of enzymes in nanoparticles to allow selective reactions in biological fluids. This is achieved using a gold nanoparticle coated in a conducting mesoporous carbon shell where isolated nanochannels lead to the gold surface. We show that we can detect glucose in whole blood with no interference from other species. This is achieved by electrochemically pulsing the artificial enzymes to generate the locally required alkalinity for an effective electrocatalytic reaction in the nanochannels, as well as expelling fouling agents that would otherwise passivate the electrocatalytic reaction. The artificial enzymes are shown to be capable of detecting glucose in biological fluids, without loss of signal, for several months. This study shows how nanoconfinement in nanoparticles can be exploited to potentially allow a broad range of species to be selectively detected in biological fluids with stability that can exceed that of enzymes.

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T.M. Benedetti, S.V. Sommerville, J. Wordsworth, Y. Yamamoto, W. Schuhmann, R.D. Tilley, J.J. Gooding*, An artificial enzyme: How nanoconfinement allows the selective electrochemical detection of glucose directly in whole blood, Adv. Funct. Mater, https://doi.org/10.1002/adfm.202400322

Monday, 9.30

Biosensors Without Frontiers: A Regenerable Supply Chain for PCR in Low Resource Countries

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The dynamics of infectious disease (ID) require fast accurate diagnosis for effective management and treatment. In low and middle income countries (LMICs). syndromic or presumptive actions are followed, where positive cases may go undetected in the community, or mistreated due to wrong diagnosis. Without affordable, accessible diagnostics, this undermines effective clinical decision-making and infectious disease containment.

In-vitro diagnostics (IVDs) better facilitate management of patient pathways and potentially improve cost-effectiveness, but in LMICs, where the disease burden is highest and the cost barrier is limiting, IVD biosensors are often not accessible. A significant limitation for the gold standard PCR test, for example, also comes from the dependence on global supply chains. This vulnerability was highlighted during the Covid-19 pandemic, when countries normally supplying LMICs imposed restrictions on supplies, compromising testing.

Despite the better accuracy and detection limits for PCR, compared with other methods, like lateral flow tests (LFTs), the routine implementation of PCRs in LMICs remains prohibited by cold chain storage requirements and the high cost of biorecognition and biocatalytic materials required. Thus, the more cost effective but less efficacious LFT immunoassay remains common practice.

Opening the door for lower cost regenerable biorecognition systems [1} and integration in a gold standard PCR platform, this study expands on our previous work, to produce a nucleic acid amplification test (NAAT) for low resource settings. The design focus is on local production and regeneration. Like our earlier LAMP-BST polymerase [2], an innovative TGP-labelled fused Taq polymerase, incorporating a silaffin peptide is reported. However, unlike LAMP_BST, exceptionally, TGP-Tag is able to sustain heating to 90ºC without loss of fluorescence intensity of the TGP,

This has been demonstrated in a nucleic acid diagnostic for malaria and for dengue. The impact of primer dimers is discussed and primer design optimization. Additionally, based on this and our previous work, the engineering and use of the principle of affinity self-packing silica fusion enzymes is presented in providing dNTPs for PCR [3]. A fully integrated local production and regeneration demonstrator for infectious disease testing and containment triaging is presented.

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[2] D. Seevaratnam, F. Ansah, Y. Aniweh, G. A. Awandare, and E. A. H. Hall, Analysis and validation of silica-immobilised BST polymerase in loop-mediated isothermal amplification (LAMP) for malaria diagnosis, Anal Bioanal Chem, **2022**, *414*, 6309–6326.

[3] A. R. Bird, J. C. Molloy, and E. A. H. Hall, Biocatalytic synthesis of 2′-deoxynucleotide 5′-triphosphates from bacterial genomic DNA: Proof of principle, Biotechnol Bioeng, **2023**. *120*, 1531–1544.

Monday, 10.00

Magneto-Controlled Sensors for Potentiometric Sensing of Extracellular Alkaline Phosphatase

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Dissolved organic phosphorus (DOP) constitutes an important component of the dissolved phosphorus pool and serves as a primary phosphate source in the phosphorus-depleted marine regions. Alkaline phosphatase (ALP) enables the remineralization of various marine DOP into dissolved inorganic phosphorus [1]. Therefore, extracellular ALP activity, whether attached to the biological surface or released into the surrounding solution, is a valuable proxy for assessing DOP utilization. However, there is currently no method designed for specifically detecting extracellular ALP.

Potentiometric magneto-controlled method was developed by our group, which enables direct sensing of hydrophilic bioreceptors such as peptides and aptamers and was used for the identification of multiple bacteria, or small molecules in biological and environmental samples [2-3]. Here, this magneto-controlled sensing platform can be designed for extracellular ALP detection. Peptide (GGGGGFFFpYpYEEE) with ALP-responsive self-assembly ability was modified on the surface of MBs. The ALP catalysis mediated formation of the self-assembled peptide synchronously influences the dispersity and surface changes of MBs. The potential change can be directly measured via a magneto-controlled potentiometric method.

References:

[1] A. Srivastava, D. E. M. Saavedra, B. Thomson, J. A. L. García, Z. H. Zhao, W. M. Patrick, G. J. Herndl, F. Baltar, Enzyme promiscuity in natural environments: alkaline phosphatase in the ocean. The ISME Journal **2021**, *15*, 3375-3383.

[2] J. S. Mou, J. W. Ding, W. Qin, Deep learning-enhanced potentiometric aptasensing with magneto-controlled sensors. Angewandte Chemie International Edition **2023**, *62*, e202210513.

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Monday, 10.20

Signal Enhancement in Electrochemiluminescence Immunoassays through the Freely Diffusing $[Ir(sppy)_3]^{3-}$ Complex and its Application in Biological Imaging

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Electrochemiluminescence (ECL) is a light emission process driven by electrochemical reactions, widely applied for bioanalysis due to its precise spatiotemporal resolution, high sensitivity and reproducibility, along with minimal background noise. Commercial ECL assays typically rely on a conventional ECL system featuring tripropylamine (TPrA) as a coreactant and $[Ru(bpy)_3]^{2+}$ as a luminophore. These assays often utilize a bead-based sandwich design, where target biomarkers are trapped between a magnetic bead coated with a capture antibody and a detection antibody labelled with the $[Ru(bpy)_3]^{2+}$ emitter. In our study, we achieved a remarkable 70.9-fold enhancement in $[Ru(bpy)_3]^{2+}$ ECL in bead-based immunoassays by introducing the redox-active $[Ir(sppy)_3]^{3-}$ complex into the TPrA-containing solution.[1, 2] We monitored the ECL response of individual polystyrene beads and explored the potential mechanistic pathways contributing to the observed enhancement in the ECL signal. Additionally, we extended the application of this ECL system to enable bimodal cell imaging. This involved capturing simultaneous positive ECL (PECL) and shadow ECL (SECL) images of single cells, resulting from the concurrent red luminescence of $[Ru(bpy)_3]^{2+}$ used for membrane labelling (light-emitting object on a dark background - PECL) and green luminescence of $[Ir(sppy)_3]^{3-}$ in solution (non-emissive object shadowing the background luminescence - SECL).[3] ECL emissions of the two luminophores were then spectrally and spatially resolved, yielding two micrographs of the same cells, where PECL shows the distribution of the $[Ru(bpy)_3]^{2+}$ labels attached to the cellular membrane, and SECL reflects the local diffusional hindrance of the ECL reagents by each cell. In prospect, this approach benefits from amplified signals that can substantially reduce the detection limits in ECL (immuno)sensing. Moreover, when applied to ECL imaging, it provides deeper insight into observed (biological) systems.

References:

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3. S. Knežević, E. Kerr, B. Goudeau, G. Valenti, F. Paolucci, P. Francis, F. Kanoufi, N. Sojic, Bimodal Electrochemiluminescence Microscopy of Single Cells, Anal. Chem. **2023**, *18*, 7372-7378

Monday, 11.10

Nanoparticle-Imprinted Matrix: The Effect of the Nanoparticle Interface

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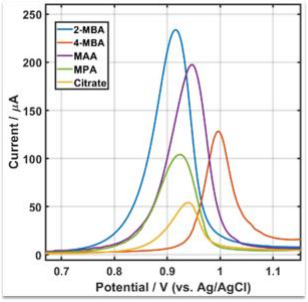
During the last few years, we have developed a new approach that aims to detect nanoparticles (NPs) selectively. The new approach is termed NAnoparticle Imprinted Matrices (NAIM) [1, 2] and is analogous to the well-known concept of molecularly imprinted polymers (MIP) in which the molecular analyte is imprinted in a polymer by polymerization of proper monomers with which it chemically associates. The removal of the template forms complementary cavities capable of selective recognition of the analyte. The NAIM approach works so well that we can detect NPs that differ in size, structure and also in their interfacial properties.

This contribution will focus on the role of the NP interface, which comprises in many cases a capping agent. We will demonstrate the ability to design systems where NPs bearing identical core shells, yet different capping agents, can be selectively detected. Then, we will present a recent study, focusing on the effect of the capping agent on the electrochemical peak oxidation potentials of the NPs. The peak potential difference between gold NPs stabilized by various ligands, such as 2- and 4-mercaptobenzoic acid, can be as high as 71 mV, which is substantial in energetic terms. A detailed study supported by DFT calculations aimed to determine the source of this interesting effect. The DFT simulations of the ligand adsorption modes on Au surfaces were used to calculate the redox potentials through the thermodynamic cycle method. The DFT results of the peak potential shift were in good agreement with the experimental results for a few ligands; however, showed some discrepancy, which was attributed to kinetic effects. We will wrap everything up by discussing the future of the NAIM approach and its applicability as a sensing method for NPs as well as for molecular species.



Ligand Type	2-MBA	4-MBA	citrate	MPA	MAA
E^0/mV	0.920±0.007	0.991±0.007	0.962±0.003	0.940±0.010	0.946±0.001

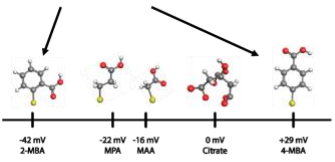


Figure 1: The effect of the capping agents on the NPs oxidation peak potentials.

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Monday, 11.40

Microscale Surface Ion Conduction for Point-of-Care Diagnostics

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Sensors that leverage the influence of a binding event on charge transport, such as field-effect transistors and nanoporous membranes, are among the most sensitive because they translate localized binding into a change in a system-scale property. However, fabrication and custom functionalization of these sensors is not trivial, and their integration with protocols that pre-enrich target species and facilitate their transport to the binding site is an active area of research. In this presentation, we demonstrate that ion concentration polarization (ICP) in the presence of fluid flow drives stable focusing and efficient capture of charged analytes within a bed of probe-modified microbeads embedded in a microfluidic channel. A key finding is that ion conduction along the surface of the beads is a significant contributor to current through the packed bed under an applied voltage bias. Therefore, hybridization of a charged analyte to the bead surface leads to a shift in the slope of the current-voltage curve.[1] This approach is versatile in that a wide range of analytes (small molecules, DNA, RNA, proteins, virus particles) can be detected electrically, in the absence of a label (Figure 1).[1,2] Further, this plug-and-play sensor uses off-the-shelf microbeads and simple electronics, making it advantageous for point-of-need (PON) testing. As a proof-of-concept, we sense virus particles in whole saliva. Finally, we explore the distinct advantages of this microporous sensor relative to nanoscale pores - namely, its amenability to sensing microscale targets (e.g., bacteria) in high ionic strength biofluids using robust molecularly imprinted polymer (MIP) coatings.

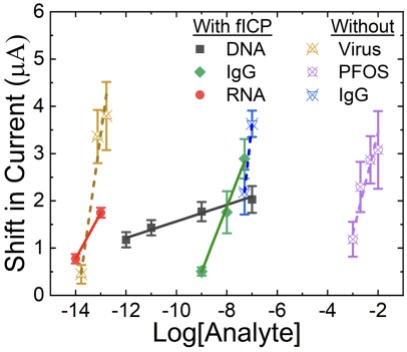

Figure 1. Response of the microscale surface ion conduction (μSIC) sensor for several classes of analyte both with (closed markers) and without (open) electrokinetic pre-enrichment.

References:

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Monday, 12.10

Microfluidic Dual Mode Electrochemical/Colorimetric Sensor Array for Simultaneous Screening of Electrolytes

Tugba Ozer

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Electrolyte analysis for early diagnosis for various diseases is growing interest. In this work, the first device combined potentiometric ion-selective electrodes (ISE) with distance-based colorimetric device for simultaneous analysis of K^+, Na^+ and Cl^- ions is presented at the point of care (POC). This hybrid sensing device was designed in a two-dimensional configuration using screen-printed K^+-ISE, Na^+-ISE and a paper-based distance-based (dPAD) colorimetric detection of Cl^-. While K^+ and Na^+ quantification were performed with the use of a lab-made low-cost (<$25) Wi-Fi supported potentiometer and its customized smartphone application while Cl^- ions were quantified an instrument-free. The total analysis time was found to be 5 min once the sample was introduced to microfluidic device. The potentiometric response of K^+-ISE was obtained as 54.14 ± 3.94 mV/per decade in the linear concentration range of 0.1 mM to 100 mM with an LOD of 0.05 mM whereas the Na^+-ISE had a response of 55.08 ± 1.15 mV/per decade in the linear concentration range of 5 mM to 1 M with an LOD of 1.36 mM. The dual mode-ISE-dPAD was applied to simultaneously detect K^+, Na^+, and Cl^- ions in spiked human pooled urine. The recovery values were found to be 90–108%, 94–105%, and 90–96%, respectively, for K^+, Na^+, and Cl^- ions. The developed assay provides low-cost, sensitive, simultaneous and rapid analysis of K^+, Na^+, and Cl^- ions on a single device for biological samples at POC. The sensing device holds great promise for real-time assessment of the physiological health conditions at-home tests especially during pandemic situations due to its simplicity, portability, and noninvasive manner of its sampling. In addition, the single-use device is not subject to biofouling and could be readily used without the need of pre-conditioning step. This dual-mode device could be easily adapted for other clinically or environmentally important ions in the future.

Figure 1. Schematic illustration of simultaneous detection on the ion-selective electrode (ISE) and Cl^- detection on the distance-based paper device (dPAD).

References:

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Monday, 16.00

Understanding Fundamental Limits and Opportunities of Liquid Junction-Free Reference Electrodes

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Realising of miniature electrochemical sensing systems require thoughtful integration of a reliable reference element to achieve precision measurements. Recent engineering efforts for wearable sensors have mainly focused on creating a polymeric diffusion barrier for hydrophilic electrolytes, but this mimics how traditional junction-based reference electrodes function.

This talk will focus on self-referencing principles that are potentially attractive for real-world application. The first approach is based on the cathodic conversion of silver iodide to iodide, yielding a defined quantity of iodide near the electrode that is used to stabilize the observed potential during a subsequent zero current pulse [1]. We are working with CSEM (Landquart) in a collaborative project to bring this principle to a microfabricated format.

The second approach focuses on the use of electrolytes of moderate to high lipophilicity doped into a polymeric membrane so that they can stabilize the potential upon partitioning into the sample. Fundamental theory and experiments are combined to draw a picture of the limitations and opportunities for this class of reference electrodes. At the lower lipophilicity scale, the reference element will fail because of ion-exchange with lipophilic samples ions. This characteristic is studied with sandwich membranes to give partition constants of the dissociated electrolyte as well as ion pairing constants, while selectivity data give information on ion-exchange selectivity [2]. When this limit is important, the operational lifetime of the reference element owing to spontaneous leaching into the sample is also of significant concern. Lifetime considerations allow one to predict required minimum partition constants that will help to find structures that are more adequate than what is available today.

To study the upper end of the lipophilicity scale, the lipophilic electrolyte ETH 500 will also be discussed. It is known that a membrane containing only ETH 500 as active ingredient will show a stable potential, independent of sample concentration, when in contact with hydrophilic solution electrolytes. The cause of this seemingly constant potential will be identified. It is shown why in membranes containing an additional ionophore and with solutions containing ions of higher lipophilicities, ETH 500 does no longer induce a constant potential. This study results in recommendations to obtain inert membrane salts of high purity and optimal lipophilicity.

References:

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Monday, 16.30

Ion-Selective Electrodes: Selectivity Coefficients for Interfering Ions of the Opposite Charge Sign

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The upper detection limit of a polymeric-membrane ion-selective electrode (ISE) is limited by Donnan failure, that is, the transfer of primary ions along with interfering ions of the opposite charge sign (commonly referred to as counter ions) from the sample into the sensing membrane. While Donnan failure is well understood, it is often difficult to compare upper detection limits for ISEs described by different sources. The majority of publications on ISEs describe Donnan failure for one type of counter ion only, making it impossible for end users to predict the interference of different counter ions from such data. Moreover, linear ranges for ISEs based on different ionophores cannot be compared to one another when Donnan failure was reported for different counter ions. To this end, we introduce here selectivity coefficients, $K_{I,X}^{\mathrm{potX}}$, for interfering ions of the opposite charge sign. Using this concept, the primary ion activity at which Donnan failure occurs can be readily predicted from measured $K_{I,X}^{\mathrm{potX}}$ values using the uncomplicated expression $a_{\mathrm{X}}^{z_I/z_x} \,/\, K_{I,X}^{\mathrm{potX}}$. Consistent with the intuition that ISE users have developed over decades for conventional selectivity coefficients, large $K_{\mathrm{I,X}}^{\mathrm{potX}}$ values are characteristic for counter ions that interfere strongly. We also show that for ISEs with an ionophore that bind the primary ion with a *n*:1 stoichiometry, $K_{I,X}^{\mathrm{potX}}$ is proportional to the stability constant of the primary ion complexes, and for both ionophore-based and ionophore-free ion-exchanger ISEs, $K_{I,X}^{\mathrm{potX}}$ is proportional to $K_{I_nX_m}^{\ -1/z_X}$, where z_{X} is the charge of the counter ion and $K_{I_nX_m}$ is the equilibrium constant describing the distribution of the primary ion and counter ion between the sample and the sensing membrane.

Monday, 17.00

Blood Gas Competitive Landscape

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Blood gas analyzers are used to measure various parameters, including blood gas (pO_2, pCO_2 and pH), electrolytes (Na, K, Ca, Cl, Mg), metabolites (glucose, lactate, creatinine, BUN) and Cooximetry (total hemoglobin and fractions) in a whole blood sample. They are widely utilized for the detection of oxygen or carbon dioxide exchange, the acid-base balance, metabolic disorders, and the measurement of renal and cardiac functions. They also determine abnormal electrolyte levels and assist in identifying diabetes, sepsis, and blood vessel hemorrhage.

In recent years, blood gas analyzers have gained immense popularity across clinical diagnostic labs and critical care units due to their ease of use, cost-effectiveness, accuracy, and fast results. The rising integration of these analyzers with laboratory information systems and electronic medical records has helped to streamline the workflow and enable effective monitoring of patient health. The global blood gas market size reached $2.2 Billion in 2023 and is expected to grow by 5 to 6% in the next 5 years. Key players based on market size are Radiometer, Werfen, Abbot, Siemens, and Roche.

The operating principles behind measurement technology have largely remained unchanged, even though the size of analyzers and sensors has decreased remarkably. Integration of measurement technologies in disposable cartridges have reduced the daily burden of maintenance and quality management and enabled healthcare professionals to focus on patient care. This presentation provides an overview of the analyzer operation and principles behind sensor technology.

Monday, 17.30

Coulometric ISEs: A Critical Overview

Johan Bobacka[1], Naela Delmo[1,2], Tingting Han[1,3], Elisa Hupa[1], Ulriika Mattinen[1,2], Zekra Mousavi[1], Tomasz Sokalski[1], Ville Yrjänä[1]

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In 2015, a new coulometric transduction method for solid-contact ion-selective electrodes was introduced [1]. The initial goal was to amplify the analytical signal and improve the precision of ion-selective electrodes (ISEs) [1, 2]. The coulometric method was found effective for the detection of small (0.1 %) changes in ion activity (concentration) [3]. The coulometric method was developed further by others to account for diffusion polarization of the ion-selective membrane (ISM) [4], to utilize an electronic capacitor instead of the capacitance of the solid contact [5], to apply the method for novel ISE materials [6], and to avoid polarization of the ISM by connecting the ISE as the reference electrode [7]. In this presentation, the coulometric transduction method for ISEs is critically discussed in light of recent achievements by us and others [8].

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Monday, 18.00

Potentiometric Biosensors Based on Surface Blocking of Ion Fluxes

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Tremendous fundamental advances over the past decades have led to dramatic improvements in the performance of polymeric membrane ion-selective electrodes (ISEs). In recent years, with the introduction of new materials and emerging techniques, the applications of these sensors have been largely expanded and versatile potentiometric biosensors for small molecules, DNA, proteins, bacteria and toxicities have been developed [1]. It has been found out that the ion fluxes across the ISE membrane can be modulated via surface blockage induced by a biorecognition layer, which offers the exciting possibility for label-free potentiometric biosensing [2-6]. In this technique, the biorecognition binding events occurring at the surface of the ISE membrane can alter the inward/outward ion fluxes at the membrane/sample solution interface. The potential change induced by a biological target involved in the surface blockage could be used as an analytical signal for bioanalyte quantification.

In this presentation, several new potentiometric biosensing strategies based on the surface blocking principle will be presented. A potentiometric biosensing protocol based on the delayed Nernstian response will be described. The surface recognition interactions between a surface phage MS2 and its host bacterium *E. coli H* could impede the diffusion of the butyrylcholine cations from the aqueous phase to the sensing membrane to reach the Nernstian response. The delayed potential response can be used to measure the concentration of *E. coli H*. In addition, a surface recognition reaction between a surface epitope-imprinted polymer and SARS-CoV-2 virus can be used to block the fluxes of protamine from the sample solution to the sensing membrane. Sensitive and selective potentiometric sensing of SARS-CoV-2 virus with a detection limit of 68 CFU/mL could be obtained. A novel chonopotentiometric sensing strategy for dual-biomarker detection will also be discussed, which is based on the combination of stimulus-responsive molecularly imprinted polymer modified nanopores and the ISE. The ion fluxes of acetylcholine galvanostatically imposed to the ISE membrane surface can be blocked by the recognition reaction between the target biomarker in the sample solution and the stimulus-responsive MIP receptor in the nanopores, thus causing a potential change. Using alpha fetoprotein (AFP) and prostate-specific antigen (PSA) as model biomarkers, the proposed sensor offers the detection limits of 0.17 and 0.42 ng/mL for AFP and PSA, respectively.

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Monday, 18.30

Reliable Sensing with Field-Deployed "Unreliable" Sensors: How Physics, Information Theory, and Fake-News Algorithm Make Robust Sensing Possible

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The rapid rise in field-deployed sensors in agriculture, water-quality monitoring, automotive, healthcare, renewable energy, and industrial automation, is essential to address the emerging grand challenges of food, energy, water, environment, etc., for the full-earth scenario [1]. The field-deployed sensors do not live in air-conditioned rooms/laboratories but must grapple with uncontrolled (and potentially extreme) fluctuations in environmental temperature, humidity, biofouling, and radiation. Learning to sustainably and predictably deploy these systems would require a foundational rethinking of electronic sensing.

In this regard, Potentiometric ion-selective electrodes (ISEs) have broad applications in personalized healthcare, agriculture, oil/gas exploration, etc. However, temperature fluctuation, sensor drift, and power constraints make reliable sensing with wearable and implantable ISE sensors difficult, especially for low-cost sensors fabricated by the roll-to-roll (R2R) process. To obtain the desired accuracy, we borrow three fundamental concepts from electro-chemistry, communication theory, and computer science. First, we use the Nernst principle to determine the self-temperature from the voltage difference of a pair of sensors [2]. Second, inspired by reliable data transmission over a noisy communication channel by incorporating redundancy, we measure the same quantity (i.e., analyte concentration) with multiple sensors. We estimate the true signal by aggregating the output of the sensors based on their credibility, a technique originally developed for "truth discovery" in fake news detection algorithms. [3] Finally, we use a temperature-supervised monitoring method to detect the drift of the sensor during operation. [4]

We demonstrate the effectiveness of our approach using three testbeds: (1) under controlled temperature variation in the lab, (2) under natural temperature variation in a greenhouse, and (3) in the field to monitor nitrate activity of an agricultural site. Using the estimated signal, we develop an on-the-fly drift-correction method to make unreliable sensors reliable by correcting any systematic drifts during operation. Our approach determines solution pH within 0.09 pH for more than three months by detecting and correcting the gradual drift of pH sensors as a function of gamma-ray irradiation. In the field study, we validated our methodology by measuring nitrate levels in an agricultural field onsite over 22 days within 0.06 mM of a high-precision laboratory-based sensor. Moreover, by restricting wireless transmission to high-credible sensors, we achieve near-perfect information transfer at a fraction of the energy cost. The high-precision sensing with low-cost sensors at reduced transmission cost will pave the way for pervasive in-field sensing with electrochemical sensors.

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Tuesday, 9.00

Nanopore-Based Potentiometric Sensing

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During the long history of potentiometric ion-selective electrodes (ISEs) efforts towards their extreme miniaturization are much less obvious than in case of voltammetric electrodes. That is mainly because the decrease in size of ionophore-based membranes amplifies most of their deficiencies which can be, however, well contained with the routinely used sizes and constructions, e.g. high resistance, leaching of active components [1]. The micropipet type electrode construction dominantly used for miniaturized ISEs raised further difficulties in terms of fragility, limited lifetime and rather difficult preparation and handling. Still, the use of miniaturized ISEs could not be avoided in the early physiological ion measurements aiming to monitor intracellular and extracellular ion activities and much later as probes in imaging and profiling methods. However, it would be much to gain from a new miniaturization approach that avoids the leaching of all active components and the plasticizer while providing a more robust electrode construction. Earlier we proposed the use of chemically modified nanopores to generate ion-selective solid-state ion channels [2, 3]. Coupled with potentiometric transduction this proved to enable beside the assessment of small inorganic ions, the selective measurement of polyions, e.g. nucleic acids [4], as well as the use of hydrophilic natural ion complexing compounds that are incompatible with the conventional polymeric membrane matrices. However, these results were achieved with multipore membranes such as gold coated track-etch polycarbonate membranes.

This presentation will largely focus on chemically modified single nanopores for potentiometric sensing. These are based on nanofabricated gold nanopores with diameters as low as 12 nm and with their internal wall chemically modified by self-assembly of functional thiol derivates. The single nanopore construction further enables a better understanding of the mechanistic aspects that include beside selective ion-sensing the development of “ideally” non-selective nanopore-based sensors [5].

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Tuesday, 9.30

Electroosmotic Flow-Enhanced Ion Selectivity of Surface-Charged Nanopores Filled with a Dense Electrolyte

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Nanopores hold great promise for sensor applications, particularly for detecting DNA, RNA, proteins, and ions. A nanopore's surface charge crucially affects the ion distributions within, especially due to its high surface-to-volume ratio. As the charged pore attracts counter ions and repels co-ions, nanopores are charge selective: when subject to an electric field, counter ions will carry the majority of the current through the pore [1]. Selectivity is supposed to decrease with increasing salt concentrations, but latest technologies enabling the fabrication of sub-10 nm nanopores necessitates considering these effects [2].

We studied ion conduction of a 1.6 nm diameter nanopore separating two reservoirs filled with 900 mM KCl. Our molecular dynamics simulations revealed an increasing pore conductance with surface charge density, aligning with theoretical predictions. Upon individually analyzing the conductance of counter ions and co-ions, the conductance of counter ions steadily increased with surface charge density, whereas that of co-ions exhibited a contrary trend, decreasing to nearly zero at -40 mC/m^2. At such high concentrations, ion repulsion alone could not fully account for the observed high charge selectivity. Further investigation uncovered an electroosmotic flow driven by counter ions, explaining the observed reduction in co-ion conductance. This finding indicates that electroosmotic flow can make pores charge selective, even for parameter settings where charge selectivity is not expected.

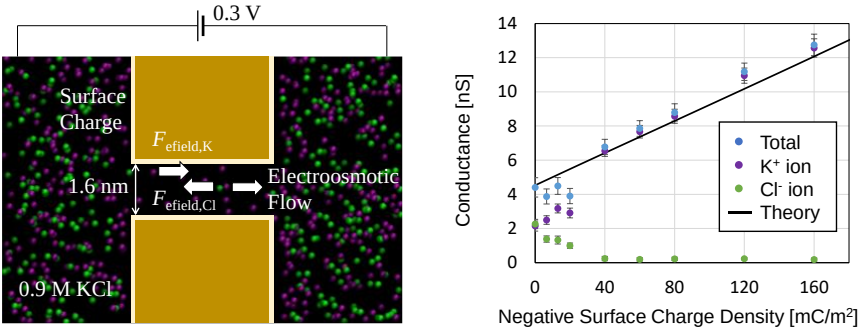

Figure: (left) schematic image of the setup for molecular dynamics simulations (right) nanopore conductance against negative surface charge

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Tuesday, 9.50

A Nanoelectrochemical Approach to Understanding Curious Chemistry in Multiphase Microenvironments

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Over the past 10 years, several reports have emerged demonstrating chemistry depends on the reaction volume in which it is occurring. This surprising finding has been observed across fields of measurement, from fluorimetry to mass spectrometry. This talk will detail our group's efforts in developing new nanoelectrochemical tools to probe curious chemistry in sub-femtoliter volumes [1]. We will answer the question: Are biochemical reactions as studied in beakers representative of reactions presently occurring within our cells? [2] In the pursuit of an answer, we will show that the famous Michaelis-Menten equation requires modification in confined volumes. We will demonstrate that electroactive molecules are spontaneously produced in suspended droplets. [3] The previous work [1-3] is performed on aqueous nanodroplets suspended in a non-aqueous phase. However, many studies detailing microdroplet curious chemistry come from droplets suspended in air, where the liquid|air boundary is the dominant interface (as opposed to the water|organic boundary). Electrochemistry in air is rather difficult. This difficulty comes from the strict requirement of needing two electrodes to make an electrochemical measurement. We will go on a journey of discovery through developing electrochemical measurement tools to probe reactivity in a bubble wall. [4] We have recently demonstrated that this experiment allows the electrochemical interrogation of single micrometer-sized liquid aerosol droplets, where a majority of the interface is the liquid|air boundary. [5] This interface accelerates enzyme reactions beyond the water|organic interface, a truth of nature that has broad implications on the genesis and propagation of life.

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Tuesday, 10.20

Droplet Nanopore Microchip for Low-volume Biomarkers Sensing

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Biomarkers are present in various metabolism processes, necessitating precise and meticulous analysis.[1] Given the need for label-free, sensitive, and real-time, biological nanopore has been applied for single biomarker sensing.[2] However, the detection of low-volume biomarkers poses challenges due to their low concentrations in dilute buffer solutions. Here, a droplet nanopore microchip is designed for high-throughput, low-volume single biomarker detection at the sub-microliter scale. To prove our concept, the platform not only enables multichannel recording but also lowers the detection limit for oligonucleotides and active peptides such as Angiotensin II, to 51 pg and 42 pg, respectively, within a 0.4 μL droplet. This advancement reduces the sample volume from the conventional millilitre to the sub-microliter, enabling biomarker detection at the picogram level. Such a leap forward in detection capability positions this platform as a promising candidate for point-of-care testing of single biomarker using nanopore, while substantially minimizing the need for sample dilution.

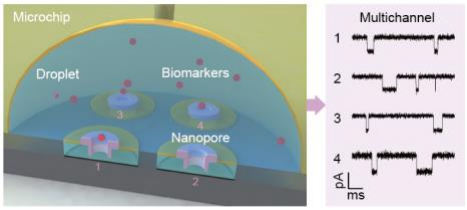

Figure 1. Multichannel and low-volume biomarkers sensing microchip

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Tuesday, 11.10

Capturing and Assaying Clinically Relevant Targets

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The analysis of cargo proteins in exosome subpopulations has considerable value in diagnostics but a translatable impact has been limited by lengthy or complex exosome extraction protocols. Currently, there is no blood test in clinical practice that can either predict risk or reliably distinguish Parkinson's disease (PD) from atypical parkinsonian syndromes. Alpha-Synuclein is present in peripheral fluids but its concentration in blood is strongly influenced by red blood cells. The egress of α-synuclein in neuronally derived exosomes predates the clinical presentation of PD, offering a means of developing a predictive or prognostic test. We have developed tools that support the clean extraction of neuronal exosomes from blood using magnetically activated polymer coated nanoparticles in low volume microfluidic configurations. The controlled lysis of these captured exosomes is followed by the electrochemical quantification of internal markers. A single antibody enzyme amplified shotgun method supports this down to very low levels with sample-to- answer times of ~60 min. Application to real patient samples enables a differentiation not only between PD patients and age matched controls but also between controls and at pre-symptomatic, at-risk patients, *years before diagnosis*.

The integration of capture nanoparticles and 3D printed fluidics also supports the assaying of cancer relevant antibodies. As a tumor-suppressing protein, p53 plays a crucial role in preventing cancer development. In response to the pathological overexpression of this antigen in tumors, the prevalence of anti-p53 antibodies increases in serum, in a manner quantitatively indicative of cancer progression. I will present an electrochemical approach that supports ultrasensitive and highly selective anti-p53 autoantibody quantification without the use of an immuno-modified electrode. We specifically employ antigen-mimicking and antibody-capturing peptide-coated magnetic nanoparticles, along with an AC magnetic field-promoted sample mixing, prior to the presentation of Fab-captured targets to simple lectin-modified sensors. The subfemtomolar assays are highly selective and support quantification from serum-spiked samples within minutes.

Tuesday, 11.40

CRISPR/Cas Assays Integrated into Paper-Based Analytical Devices

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During the past few years, there has been a rapid growth in the development of analytical assays making use of the trans-cleavage activity of the CRISPR/Cas nuclease enzyme system due to their sensitivity, specificity, and simplicity [1]. On the other hand, paper-based analytical devices (PADs) have gained a lot of attention as platforms potentially suitable for point-of-care testing (POCT) applications [2]. Despite both approaches having multiple advantages, their combination into fully integrated POCT devices has rarely been reported. In most combinations of CRISPR/Cas technology with PADs, the latter is simply used for a final signal detection step, for example in the form of a lateral flow test-strip, while otherwise the assay is performed in the liquid phase in tubes.

This presentation will begin with showing that the two technologies can be successfully combined into fully integrated PADs. At first, we have investigated the storage stability of CRISPR/Cas assay-related reagents on various types of filter paper substrates in the absence or presence of known enzyme stabilizers (e.g., disaccharides). It was found that the trans-cleavage activity of the nuclease system is maintained for over 80 days when storing the devices with all necessary reagents in dried state on filter papers at -20˚C.

As a proof-of-concept for a practical assay of clinical relevance, a fully integrated PAD for the highly sensitive quantitative determination of the hepatitis B virus surface antigen (HBsAg) is presented. In this case, CRISPR/Cas in combination with a biotin/streptavidin-linked activator DNA network [3] was used as an enzyme labeling system for an origami-type fluorescence immunoassay performed on the paper platform. The developed assay achieved a limit of detection in the order of 30 pg/mL in undiluted spiked porcine blood plasma samples, which is below the LOD of a conventional ELISA performed in a microplate format. For possible future POCT application, it is also shown that the system can be applied for HBsAg determination within 75 min in 12 µL of undiluted spiked whole blood with signal readout on a portable smartphone setup. The only user operation required except for sample application are three steps of buffer deposition, in addition to paper flap folding and unfolding. The presented results demonstrate that the CRISPR/Cas system is a promising tool for use in the development of highly sensitive paper-based assays.

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Tuesday, 12.10

Corrective Protocol to Predict Interference Free Sensor Response of Paper-based Solution Sampling Coupled with Heavy Metal Sensitive Ion-Selective Electrodes

Grzegorz Lisak[1,2], Mingpeng Yang[1,3,4], Rochelle Silva[1], Ke Zhao[1,2], Ruiyu Ding[1], Jit Loong Cyrus Foo[1], Liya Ge[1], Canwei Mao[1]

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Paper-based microfluidics combined with potentiometric measurement has emerged as an attractive approach for detecting various chemical moieties.[1] Detection of heavy metal ions, using paper substrates as solution sampling and solution delivery system to the potentiometric electrodes remains challenging despite number of physio-chemical modifications have been recently introduced.[2] This study, therefore, quantitatively investigates the adsorption of heavy metal ions on the paper substrates during paper-based potentiometric measurements and explains the super-Nernstian response through heavy metal ion adsorption rates at the paper substrates. Finally, potentiometric measurement corrective protocol was established for the paper-based *EMF* obtained empirically to predict interference free sensor response (Fig. 1). Furthermore, the ion adsorption data was also recorded for mixed metal ion solutions to understand competitive primary ion adsorption and establish corrective measures to predict interference free sensor response out of paper-based sample solution potentiometry. The report also emphasizes of importance of solvent (tetrahydrofuran, THF) used for membrane preparation which was found to influence the electrode stability due to the residue of impurities in the membrane matrix from the autooxidation of THF.

In this method, no modification of the paper substrates is necessary before actual potentiometric measurements and with the attention on the solvent effect from THF. Findings in this work allow to predict sensor response based on the paper-based sample solution sampling potentiometric measurement, offering a quantitative explanation for the super-Nernstian response of paper-based potentiometric measurements and provide simple solution resigning form the need of modifying paper substrate for measurements of heavy metal ions.

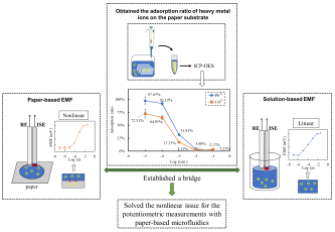

Fig. 1. Corrective protocol to predict interference free sensor response of paper-based solution sampling coupled with heavy metal sensitive ion-selective electrodes.

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Tuesday, 16.00

Nanopore-Based Sensors for Chemical, Polymer, DNA, & Protein Analysis

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We pioneered a method for using nanometer-scale pores, formed by protein ion channels reconstituted in cell membrane mimics, for the accurate determination of the identities and physical-chemical properties of single molecules [1]. The technique has been applied to detecting and quantifying ions [2,3], discriminating between polymers based on their sizes [4,5,6], and sequencing DNA [7,8,9]. We will discuss what made the method possible, and show how it is being developed for identifying proteins [10,11] and their multifarious variants [12].

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Tuesday, 16.30

Stereo-Selective Nanopore for Single-Molecule Sensing

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Proteins intricately govern diverse physiological processes, prompting extensive investigations that specifically concentrate on the single protein detection through the use of biological nanopores.[1] Despite their sensitivity, biological nanopores still face challenges in generating effective ionic current responses for heterogeneously charged peptides, compared to the more consistent responses observed with uniformly charged polynucleotides. Here, we designed an electrostatically asymmetric nanopore, orchestrating dynamic non-covalent interactions to steer peptide sidechains into specific orientations.[2] Distinct ionic current fluctuations in the stereo- and regiodefined nanopore allows near-perfect identification of single-peptide stereoisomers with an accuracy close to 100%. Through the establishment of a single-molecule frequency fingerprint spectral library, employing a mathematical model for qualitative and spectral analysis of non-covalent interaction networks inside a nanopore, we could anticipate that the confined interactions may be classified, predicted, and characterized for each amino acid, which holds the potential for achieving single protein sequencing.[3] Furthermore, a multichannel electrochemical instrumentation that seamlessly integrates a microelectrode array with amplifiers on the circuit board was designed for high-throughput, high-bandwidth, and ultralow-noise single-peptide sensing.[4] This work provides a reliable strategy to enhance the nanopore sensitivity of single-peptide analysis, facilitating more precise sensing of pathogenic mutations for the advancement of effective diagnosis and treatment.

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Tuesday, 17.10

Solutions to Overcome Competitive Displacement of Oligonucleotides from Nucleic acid-based Electrochemical Sensors in Biofluids

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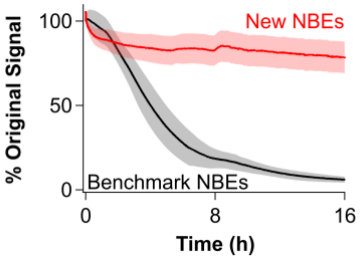
Nucleic acid-based electrochemical sensors (NBEs) allow continuous molecular monitoring in vivo.[1] These sensors rely on the sequence-specific affinity and folding dynamics of oligonucleotides for molecular recognition and signaling. NBEs are typically made of electrically conducting gold surfaces coated with self-assembled monolayers of a mixture of blocking alkylthiols and functional alkylthiol-modified oligos. The oligos are additionally modified with a redox reporter at their distal end to allow transfer of electrons to the underlying gold electrode. Although sufficiently robust for continuous, multihour sensing of small molecules, peptides, and proteins in biological fluids both in vitro and in vivo, NBEs decay over periods longer than 12 hours of continuous operation if unprotected. My laboratory invested the past five years digging deep to understand the mechanisms underlaying NBE sensor decay in biofluids, as a framework to later develop solutions that eliminate such degradation pathways. In this presentation, I will discuss the various studies we completed that led to our current understanding of NBEs decay. Additionally, I will present a biofluid mimetic that can be leveraged to specifically study competitive displacement of oligonucleotides from NBEs, a critical degradation pathway. Finally, I will demonstrate three solutions that, if combined, drastically mitigate competitive displacement to allow stable, multiday NBE sensing in vivo.



Overcoming competitive displacement of NBE monolayers in biofluids. We developed a biofluid mimetic able to fully degrade NBE baseline signal within 8 h. Leveraging this solution, we explored different strategies to mitigate competitive displacement of oligonucleotides from NBE surfaces. This presentation will discuss three of those strategies which, when combined, result in order-of-magnitude improvements in NBE stability in biofluids. The data show the average of 8 sensors plus standard deviation (shades) for protected (black) vs unprotected (red) sensors. The y axis corresponds to the relative current of the sensors extracted as the peak current of square wave voltammograms measured every 7 s for 16 h. The voltammograms were processed in batch using SACMES,[2] a Python script previously published by my laboratory.

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Tuesday, 17.30

Studying Redox-Labelled DNA SAMs Using EIS and In-Situ Fluorescence Imaging

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Redox-labelled DNA SAMs have been studied for use in biosensing applications configured for detection of nucleic acids using ssDNA SAMs, or as aptamers to sense therapeutics or small molecules.[1–4] The rate of electron transfer was found to be sensitive to the configuration of the DNA, or the mobility of the DNA in the SAM. Preparation of these sensor surfaces must control the DNA surface density to ensure efficient hybridization as well as to provide large enough changes in the k_et for sensitive detection.[3] This requires that the redox label has some access to the electrode surface. Therefore control over the average coverage and preparing a uniform distribution of the DNA SAM is important. Typically, measurements are done using SWV at a fixed frequency and the decrease or increase in the signal is strongly dependent on the change in k_et upon binding. The presence of multiple local environments on the DNA SAM modified surface can influence the average k_et measured when using SWV, influencing the analysis. In this work, methods for distinguishing the presence of multiple redox environments experienced by the redox-labelled DNA SAM were developed using EIS and redox moderated fluorescence imaging on well defined single crystal bead electrodes.[5] Changes in the EIS and fluorescence due to the hybridization of ssDNA SAMs with targets in solution were compared to the SWV measurements. The existence of more than one redox environment resulted in multiple redox rates when measured using EIS and observed using fluorescence imaging. High coverage fully hybridized dsDNA SAMs were also studied using this methodology, revealing a number of different redox environments for the redox label which depended on the assembly conditions. In many cases, the results were best described by a distribution of redox rates that was estimated using EIS and analysis based on estimating the distribution function of relaxation times. [6,7] This fundamental study of the DNA SAM modified electrode interface using EIS and highlights the utility of in-situ spectroelectrochemical approaches for characterizing the interfacial characteristics of the complex DNA SAM interface.

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Tuesday, 18.00

A Novel Approach to Determine the Surface Coverage of Affinity Ligands by SPRi

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One of the crucial stages in biosensor development involves characterizing potential recognition molecules to achieve selective and efficient target recognition, but it is also important to consider that the effectiveness also relies on the surface coverage of ligands. For the purpose of characterization, the high-throughput techniques, such as surface plasmon resonance imaging (SPRi) offer a powerful means to monitor up to hundreds of biomolecular interactions simultaneously, label free and real-time to determine the binding capacity of the ligand layers and characterize the kinetics as well. However, the widespread of the SPRi technique is limited by the fact that the surface coverage of the ligands, which can affects both the binding capacity and kinetics, is not known due to the off-line microspotting used for the SPRi biochip preparation. We were interested to develop an SPRi method to determine the surface coverage of different affinity ligands one-step and label-free.

We have previously developed specific methods for the determination of the surface coverage of nucleic acid strands e.g. PNAs[1], DNAs[2] and aptamers[3], but these methods were not applicable to protein-type recognition molecules, e.g. antibodies. To address this, our research focuses on the development of a novel universal method in which we have developed a label-free approach to precisely determine the surface coverage of different ligands. The determination is based on the shift of the resonance curve that can be measured on the recognition spots, which is proportional to the number of molecules immobilized on the surface. Our results contribute to more accurate determination of the surface density of the ligands and kinetic parameters as well.

Acknowledgment

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Wednesday, 9.00

Chemical GPS – How Optical Sensors Can Help Navigate the Microbial World.

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The Global Positioning System (GPS) has not only virtually eliminated the possibility of getting lost but has also enabled the tracking and locating of objects on a macroscopic scale. However, such precision is lacking in the microscopic realm. Despite acknowledging the presence of steep gradients and frequent fluctuations in the microscopic world, tools for identifying hotspots and monitoring chemical changes in both space and time remain elusive.

Optical sensors, also known as optodes, offer a promising solution for visualizing chemical alterations in two or even three dimensions. They hold the potential to generate maps facilitating navigation within the microbial domain. In this presentation, I will delve into our recent breakthroughs in this domain, showcasing how optode-based chemical imaging can inform and guide destructive sampling procedures. For instance, many sequencing-based methodologies yield ample amounts of data but often lack insights into the underlying chemical conditions that shaped the data obtained. The fusion of optical sensing with sequencing techniques proves to be a potent combination in addressing this gap.

Moreover, chemical imaging can steer other point measurements towards greater efficacy. As an illustration, we've devised an approach that integrates 2D optode-based imaging with microsensor measurements to accurately quantify greenhouse gas production hotspots.

Lastly, I will highlight the strides we've made in developing chemical imaging systems suitable for in-situ applications. Until now, chemical imaging has predominantly been confined to laboratory settings, offering a limited perspective on natural environments. To address this, we've engineered a fully autonomous system capable of enduring harsh conditions for prolonged durations, providing insights beneath the surface.

Wednesday, 9.30

New Synthetic Receptors and Polymer Matrices for Electrochemical and Optical Ion Sensors

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Ion-selective chemical sensors, including ion-selective electrodes and optical sensors, have been successfully applied in biomedicine, environmental monitoring and other fields. With the advancement of science and technology, ion sensors have also been gradually applied to various frontier fields, such as wearable sensors. In these sensors, electrode membrane materials, including ionophores, ion exchangers, and polymer matrix materials, are particularly important. The design of new membrane materials is crucial for regulating and optimizing the selectivity, detection limit and biocompatibility of ion sensors.

In this report, we are going to discuss the research progress of our group in ionophores and membrane materials in recent years. We have obtained some macrocyclic molecules, tripodal receptor molecules, and signal transduction molecules through rational design and facile organic synthesis, which have exhibited promising performance in ion sensors. In addition, we found that regulating the hydrophilicity or hydrophobicity of the sensing membrane could be crucial for the electrode response. Based on this, we are going to demonstrate an electrochemical sensor that can detect the total salinity in fresh water, and it is expected to be further applied to the field of sustainable energy utilization.

Wednesday, 10.00

Water-Soluble Acidochromic Dyes Lipophilized with Quaternary Ammonium Cations as Tunable Chromoionophores for Polymeric Optodes

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Lipophilic hydrogen chromoionophores used in bulk optodes are mainly derivatives of acid-base indicators lipophilized by introducing long-chain radicals. The latter is a challenging task due to the complex structure of the parent compounds and the intricacy of product purification. This leads to a limited choice of available chromoionophores, and, in turn, of their acidities. An alternative approach to enhance the lipophilicity of water-soluble acidochromes is converting them into an ion pair with a lipophilic counterion [1].

We report here a systematic study of the optical properties of lipophilized acidochromic dyes and their behavior in polymeric sensor matrix. Lipophilic ion pairs of bromophenol blue (BPB), bromothymol blue (BTB) and thymol blue (TB) and quaternary ammonium cations were synthesized, and their optical properties in PVC–DOS phase were investigated (Fig. 1A). The effect of the nature of acidochrome and counterion on the lipophilicity and acidity of the resulting ion pair was evaluated (Fig. 1B). The partition coefficients of the obtained dyes and their acidity constants in the polymeric phase were estimated. The pH- and Cl^-- response of the optodes containing synthesized ion pairs was studied and analyzed in terms of the theoretical model described in [2] (Fig. 1C).

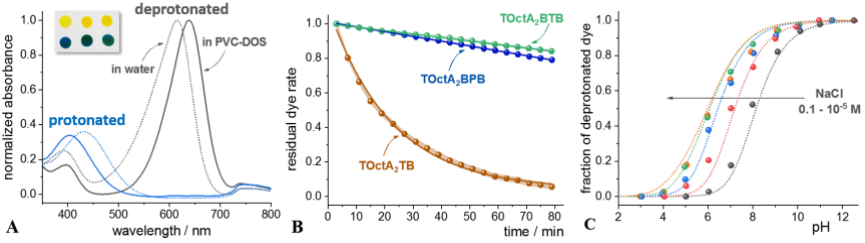

Fig. 1. A: UV-Vis spectra of BTB in water and its ion pair with tetraoctylammonium in PVC–DOS film. B: leaching of $TOctA^+$ ion pair with various acidochromic anions (deprotonated form) from the polymeric phase upon contact with aqueous solution. C: response of optodes containing BTB-$TOctA^+$ ion pair as a chromoionophore. Lines: approximation with model proposed in [2].

The ease of preparation makes lipophilic acidochromic ion pairs a viable alternative to conventional chromoionophores, and a wide variety of available water-soluble precursor indicators allows obtaining chromoionophores with tunable acidity. In addition, the response of the obtained sensors is less prone to cross-sensitivity towards electrolyte activity in the solution (Fig. 1C) compared to conventional optodes, which opens prospects for their further investigation as sensors for individual ionic activity.

This work was supported by the Russian Science Foundation, grant number 20-73-10033.

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Wednesday, 10.40

Antifouling Approaches for Polymeric Membrane Ion-Selective Electrodes in Environmental Waters

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Nowadays, polymeric membrane ion-selective electrodes (ISEs) have been employed in clinical diagnosis and environmental monitoring [1]. In recent years, such electrodes play an important role in real-time and accurate analysis of some environmental parameters, such as pH, the concentrations of Ca^{2+}, NH_4^+, and carbonate, etc. [2-4]. In many cases of practical relevance, however, when these sensors are applied in complex samples, they usually suffer from problems of biofouling which refers to the undesirable attachment and further growth of microorganisms on the surface of the membrane (Fig. 1). Such biofouling may shorten the sensor lifetime and cause the measurement errors by perturbing the analyte mass transfer to the sensing interface and/or changing the electrode selectivity.

In this presentation, our group's recent work on improvement of antifouling properties of polymeric membrane ISEs will be reported. Various antifouling approaches, such as surface modifications of a hydrophilic polydopamine layer [5], a zwitterionic polymer poly(sulfobetaine methacrylate) [6], antibacterial Ag nanoparticles, an UV photocatalytic self-cleaning coating [7] and a sunlight-driven self-cleaning paint, and release of biocide agents [8] have been developed. It can be expected that these antifouling approaches may further promote the applications of ISEs for real clinical and environmental samples.

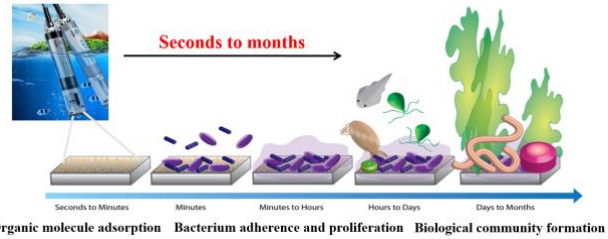

Fig. 1. Possible fouling processes of polymeric membrane ISEs in environmental waters [9].

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Wednesday, 11.00

Roles and Benefits of Physics-based and Machine Learning Modeling Tools for Environmental Sensing Using ISE Arrays

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The promise of ion-selective electrode (ISE) arrays has been touted now for several decades, yet application of these approaches for measurement of nutrient, typically minority, species in environmental waters has lagged. Promising first steps [1] rely heavily on a large number of representative water samples with which to train data-driven (machine learning (ML)) models. Creating environmentally representative samples in the lab is time consuming; further "representativeness" is defined by application and can vary significantly. Therefore, this work poses the question: to what extent might physics-based models of the sensors themselves be leveraged to generate synthetic data for training of data-driven models? i.e., can the same goals be achieved with a small amount of lab work augmented by large numbers of physically accurate synthetically generated datapoints? If so, what is the right balance, and what are the best models to use?

To tackle these questions we prepared a 10-element ISE array with the aim to (1) be responsive to the major and minor (N-nutrient) ions in fresh surface waters and (2) have varied membrane selectivity properties. ISEs with H^+, Na^+, K^+, NH_4^+, Mg^{2+}, Ca^{2+}, Cu^{2+}, Cl^-, NO_3^-, ionophores and one with no ionophore were fabricated. Environmentally-representative experimental training data (n>300) were collected in the lab.

Physically accurate synthetic data samples were created by (1) approximating the physical/chemical parameters (e.g., selectivity coefficients, E^0, and the limit of detection values) for each sensor by a global optimization algorithm that minimized the squared difference between the experimental data and Nikolsky-Eisenman equations describing the array and (2) using the optimized parameters to generate synthetic datapoints for physically representative, lognormally distributed pseudo-random ion concentrations.

Machine learning models with good ability to represent non-linear relationships and train with small datasets (random forests, support vector regression, artificial neural networks, symbolic regression) were trained to estimate ion concentrations under 3 different conditions: (1) lab data only, (2) varying mixture of lab/synthetic data with same size dataset as lab data, (3) varying mixtures of lab/synthetic data with increasing dataset size. ISE dataset used was pruned to result in minimum-viable array size achieving acceptable results. We found that augmenting experimental data with physically simulated synthetic data helped in building more robust ML models. In particular we found that generalizability of the ML models to unseen data was improved when synthetic data were incorporated in the training as long as synthetic data were generated for similar chemical background and concentrations as the target application. We hypothesize that embedding physical laws in the form of Nikolsky-Eisenman equation into the ML training results in enhancing the information content of the training data and reducing experimental lab work required to parameterize models for ISE arrays for new applications.

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Wednesday, 11.20

A Critical Comparison of Machine Learning Techniques for Interpreting Results from Sensor Data

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Accurately predicting result from unseen data, instead of merely memorizing training examples, is critical in machine learning. This is particularly important in the field of chemical sensors, where the ability to accurately predict the chemical properties or concentration levels of unknown samples is crucial, especially guaranteeing that algorithms will not generate non-physical results in the face of unseen input data. Here, the efficiency of various machine learning algorithms is examined using synthetic nonlinear sensor array data, underscoring the significance of model interpretability and generalization in ensuring meaningful results. Classical machine learning techniques, e.g., linear models, support vector machines (SVMs), *k*-nearest neighbors (*k*-NNs), decision trees, and ensemble methods are compared to symbolic regression models and deep neural networks (DNNs). Comparing novel methods to established ones in the field of machine learning is essential to ensure their reliability and robustness.

Classical machine learning methods gave mixed results, suggesting that these techniques might not be as effective in capturing the underlying structure in complex sensor array datasets. In line with recent advancements in theoretical machine learning, overparameterized DNN models exhibit exceptional accuracy and generalization when sufficiently large datasets with thousands of sensor readings are available. However, it is important to acknowledge that DNNs are suboptimal choices in real-world sensor applications where only small datasets may be available for training. In contrast, symbolic regression models excel at predicting concentrations of unknown samples when dealing with datasets containing only a few dozen to a few hundred training examples. Moreover, symbolic regression generates human-readable mathematical expressions – a critical feature in sensor fields such as healthcare or environmental monitoring where comprehending model predictions is paramount. Importantly, the analysis shows that even using the best performing machine learning techniques, combining sensors with diverse selectivity patterns does not guarantee sufficient accuracy in complex systems. In fact, accurate predictions for ions without highly selective sensors at lower concentration levels remain elusive using sensor arrays.

As more research groups utilize the advantages of machine learning techniques to enhance the accuracy of their analytical methods, it is important to emphasize the limitations of relying on neural networks in chemical sensing applications where training data are often scarce. Instead, the use of symbolic regression models is recommended in such scenarios, as they offer both superior accuracy on smaller datasets and valuable insights into underlying mechanisms through interpretable mathematical expressions.

Thursday, 9.30

Enhanced Electrocatalytic Performance of Metal Nanoparticles Heterodimer Modified Carbon Film Electrodes

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Heterodimers are well-known nanostructures consisting of two different metallic NPs partially contacting each other through a small interfacial area. We proposed synthesis of a highly oriented metallic heterodimer array based on a selective electrodeposition onto a metal nanoparticle-embedded carbon film.[1] Since the overpotential of metal deposition on the carbon film is larger than that on the metal NPs, the second metal NPs can be attached on the embedded NPs surface, when we carefully choose the metal deposition potential as shown in Fig. 1(a). Nickel oxyhydroxide (NiOOH), which is formed by electrochemical oxidation of nickel hydroxide ($Ni(OH)_2$) in alkaline solution, is known as a good electrocatalyst toward oxidation of sugars. Fig, 1(b) and (c) compares the electrochemical NiOOH formation reaction of NiNPs modified on PdNPs embedded carbon film NiNPs directly modified on the carbon film. When the potential scan rate is 1 mV/s, the oxidation and reduction peaks are similar at both electrodes, which correspond to the electrochemical oxidation and reduction of $Ni(OH)_2$ and NiOOH(Fig.1(b)). However, redox peak currents become broader at the NiNPs modifed on carbon film at the scan rate of 20 mV/s (Fig.1(c)). In contrast, the sharp peak currents and small potential shifts are still observed at the Ni@Pd heterodimer embedded carbon indicating that $Ni(OH)_2$ on Ni@ PdNPs heterodimer exhibits a much greater rate of NiOOH formation reaction. The Ni@Pd heterodimer embedded carbon film shows significant negative shift of glucose oxidation (0.113 V) and increased current compared with those at NiNPs on the carbon film. This indicates that the electrocatalytic oxidation for sugars is drastically improved by fabricating heterodimer structure.

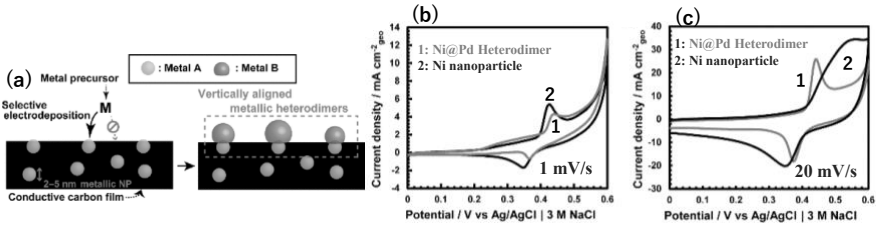

Fig.1 Fabrication of heterodimer embedded carbon film.(a), Comparison of redox Reaction of Ni@Pd heterodimer embedded carbon film and NiNPs mofified carbon film at the scan rate of 1 mV/s (b) and 20 mV/s (c).

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Thursday, 9.50

Unique Electrochemical Detection Properties of Monolayer Graphene Electrodes

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Monolayer graphene (MG) has attracted many interests due to its excellent physical and chemical properties. In this paper, we introduce our recent research on the unique electrochemical detection properties using MG electrodes.

1) Promoting ferricyanide/ferrocyanide ($[Fe(CN)_6]^{3-/4-}$) redox reaction by adding pyridine-type nitrogen (pyri-N) containing molecules [1].

Doping hetero atoms to carbon electrodes significantly changes their electrochemical properties, especially, pyri-N in the carbon skeleton promotes redox reactions. Instead of doping, we tried to change the electrochemical properties of MG just by adding pyridine-type nitrogen-containing molecules into sample solutions. Cyclic voltammograms (CV) of 1 mM $[Fe(CN)_6]^{3-/4-}$ in 0.1 M phosphate buffer solution (pH 7.4). After adding dipyrido[3,2-a:2',3'-c]phenazine (DPPZ), a significant increase in the reduction reaction current and a shift in the reaction initiation potential were observed compared to before addition. On the other hand, when we used dibenzo[a,c]phenazine (DBPZ), almost no change in current value was observed before and after addition. The results showed that a promoting effect was obtained by adding pyri-N-containing DPPZ to the sample solution, but not when pyri-N-free DBPZ were added. Furthermore, since this effect was not observed in the glassy carbon electrode, this effect can be a unique to MG electrodes.

2) Promoting effect of potassium ions (K^+) in detection of anionic species [2].
Ab initio study shows an attractive electrostatic force works between K^+ and the π-electron-rich graphene surface. We found that when K^+ are present in the sample solution, The promoter effect of K^+ in the detection of the anionic species, such as $[IrCl_6]^{-2/-3}$ as well as ascorbic acid and uric acid, was confirmed. Since it was not observed with graphite basal plane electrodes, it is a characteristic behavior of MG.

3) Redox cycling properties of microgap MG electrodes [3].

For high-sensitive electrochemical detection, a method of amplifying the redox cycle current using a microgap electrode is useful. We fabricated a microgap MG electrode by placing two MG surfaces facing each other via a 20 μm spacer made of double-sided tapes. We then measured a dual-mode CV of (ferrocenylmethyl) trimethylammonium bromide $[FcTAB]^{0/+}$ (1 mM in 1 M KCl). At high sweep speeds (>0.05 V/s), a typical CV curve was obtained at the generator electrode, and a limiting current was observed at the collector electrode. At slow sweep speeds (<0.01 V/s), limiting currents were observed in both the generator and collector electrodes. The capture rate calculated from the ratio of limiting current values was almost 100% regardless of the sweep speed. This is thought to be because the concentration of $[FcTAB]^{0/+}$ diffused between the electrodes became constant in the bulk solution confined within a microscopic region of 20 μm.

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Thursday, 10.10

Ion Sensing by TEMPO-Redox-Modulated Fluorescence of Optical Probes in Thin Films

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TEMPO belongs to a class of stable aminoxyl radicals with unique redox properties. It undergoes reversible one-electron oxidation to the corresponding oxoammonium species, which can be controlled by voltammetric techniques. This process is the driving force of the phase-transfer mechanism we propose for optical ion visualization. Its lipophilic derivative, 1-oxyl-2,2,6,6-tetramethylpiperidin-4-yl stearate (lip-TEMPO), has been shown to successfully mediate the transfer of cations into thin layer ion-selective membranes on solid contact electrodes [1, 2]. For the first time, the ion transfer mediated by a redox probe also enables the turn-on and turn-off of the optical signal from molecular probes in thin films. In the presence of a neutral hydrogen ion-selective chromoionophore, which acts as an indicator (Ind), the transfer of protons within the organic and aqueous phase controls the protonation ($IndH^+$) and deprotonation (Ind) of this H^+-selective carrier. During an anodic scan, lip-TEMPO is oxidized to lip-$TEMPO^+$ and starts to form ion pairs with the cation exchanger (R^-) present in the membrane, resulting in the transfer of protons from the thin film to the aqueous solution to maintain electroneutrality in the organic phase (Figure 1a). The reverse cathodic sweep extracts protons into the membrane to balance the charge of the cation exchanger released by reducing lip-$TEMPO^+$ to the electrically neutral radical form. The ion-selective membrane responds to the hydrogen ion concentration in the aqueous phase and therefore buffer solutions of different pH result in ion transfer waves at different potentials (Figure 1b). Such lipophilic pH indicators have different optical responses in their protonated and unprotonated form. Therefore, the electrochemically controlled ion transfer process can also be monitored based on the optical signal from the ion-selective membrane, which can be measured from real-time images using an optical microscope (Figure 1c).

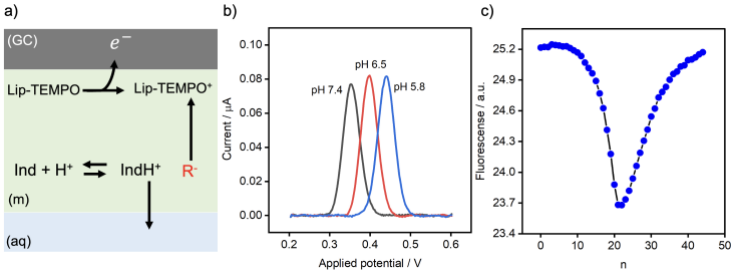

Figure 1. (a) Ion transfer mechanism, (b) Anodic scans at different pHs using a H^+-selective electrode, and (c) Fluorescence signal of the H^+-selective membrane during a voltammetric cycle, where each *n* is an image registered with the optical microscope.

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Thursday, 11.00

Real-Time Viscoelastic Cytometry: High Throughput Optical and Mechanical Phenotyping of Liquid and Solid Biopsies

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Flow cytometry is a ubiquitous analytical technique for enumerating heterogeneous cellular populations. Because of its quantitative nature and the fact that cells may be probed at throughputs of many thousands per second, flow cytometry is considered the gold standard technique for measuring the physical and chemical properties of a large cellular populations. Although flow cytometry has proved useful in a range of clinical applications, almost all flow cytometers require unacceptably large sample/reagent volumes and do not provide spatially resolved information from within each cell. To address these issues, imaging flow cytometry (IFC) aims to combine the advantages of optical microscopy and flow cytometry to allow the for high-throughput imaging of cells within flowing environments [1]. In principle, IFC engenders enormous enhancements in information content but is attended by numerous technological challenges. We have been interested in leveraging the capabilities of microfluidic systems to manipulate, process and order micron-sized objects in a controlled and high-throughput manner. We have recently developed chip-based imaging flow cytometers that leverage either inertial or viscoelastic forces to perform ultra-high-throughput imaging of cells. [2,3] Using such systems, we are able to analyze cellular populations at rates approaching 500,000 cells/s. More recently we have developed microfluidic platforms able to measure the mechanical properties of cells at rates of up to 100,000 cells per second. Here, fluid elasticity is used to focus and deform cells. We have used this platform for a range of studies, including cell phenotyping, cytoskeletal drug analysis and identification of malignant lymphocytes in peripheral blood samples. We expect that these platforms will open up new opportunities in the assessment of blood disorders, mechanical phenotyping of rare cells and sensitive diagnostic applications [4].

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Thursday, 11.30

Electrochemical Sensors for Liquid Biopsies

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Liquid biopsies have the potential to change how we approach the detection and treatment of cancer by providing earlier and, often, personalized cancer detection. Liquid biopsies also have the potential to provide actionable results earlier than imaging because molecular changes are apparent before physical changes. One of the major targets of liquid biopsies is cell free DNA (cfDNA) and a subset of cfDNA, circulating tumor DNA (ctDNA). A large number of methods have been developed to quantify cfDNA and ctDNA using techniques like PCR and next generation sequencing (NGS). Many of these methods are either approved for use or are in clinical trials and provide an exceptional resource for cancer detection. However, in all of these cases, the methods require a priori knowledge of the DNA sequence. Tracking cancer progression is currently done using imaging methods, but this requires large, expensive instrumentation, operated and interpreted by skilled individuals in complex laboratory settings. Multiple clinical studies have found that circulating tumor DNA (ctDNA) correlates with tumor progression and precedes imaging progression. To effectively treat patients with methods personalized to their response, an accessible tool is needed to quickly determine treatment efficacy and improve long-term outcomes. We have recently discovered that circulating tumor DNA adsorbs differentially relative to DNA from health patients as a result of differences in surface chemistry. The sensing fundamentals will be shown as well as results from clinical samples. Finally, we will discuss use of microfluidics for sample preparation as a key step towards generating a point-of-care diagnostic.

Thursday, 12.00

Optical Sensing of Ions and Metabolites Based on Liquid-Liquid Extraction

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An appropriately formulated water-immiscible liquid can exhibit an optical response upon extraction of an analyte or a reaction product derived from an analyte in an aqueous sample. We have developed two platforms to use these liquids for extraction-based chemical sensing. The first one is pressure-driven droplet microfluidics, in which sub-nanoliter-sized oil segments are continuously generated for chemical analysis of sub-nanoliter-sized aqueous samples. By using a flow of preloaded arrays of sensing oils, this platform allows for continuous monitoring of multiple analytes and may serve as a bedside monitor for critical care analytes in hospitals. The second one is push-pull fluidics in a liquid channel attached to a stepper motor, which facilitates extraction between a microliter-sized oil segment and a microliter-sized aqueous sample. This portable and affordable platform is suitable for the diagnosis and management of chronic diseases in patients' homes and small clinics.

The water-immiscible liquid is doped with hydrophobic sensing chemicals used in ion-selective optodes for ion sensing under equilibrium or exhaustive mode. The liquid is doped with molecular probes or dispersed enzyme particles to detect metabolites such as glucose and lactate. Unlike traditional optical sensors, these fluidic platforms enable the tracking of optical signals of the sensor phase, the sample phase, and the interface independently. Using the fluorescence or absorbance of the sensor phase, chemical analyses of complicated samples including whole blood have been achieved without optical interference from the samples.

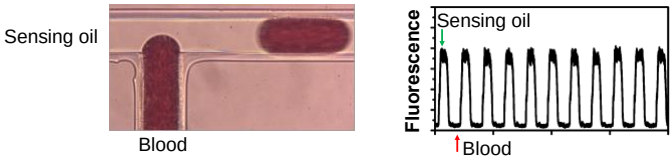

Figure 1. Blood analysis in a T-junction microfluidic chip. The laser-induced fluorescence intensity of the oil segment indicates the electrolyte concentration in the blood.

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Thursday, 16.00

The 15 Years from Fluorescent dyes to Commercial Optical Chemosensors

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From a starting point in fluorescent molecules[1], we have developed optical sensor systems for pH[2] and DO[3]. Having slowly learned that a chemosensor is much more than a responsive molecule[4,5], we went on to develop hardware[6], software[7], test protocols, and production protocols. The results—after years of work—is a sensor material that is used in products on the market and in development. As sensor scientist, this path is not one we wish to revisit. We will much rather focus on developing responsive molecules and materials[8], which has led us down a different path. In this talk, I will take you through the steps of optical sensor development from idea to product, and reveal some of the directions we are going *i* to make sensors fun again, and *ii* to make sensors for more complex analytes.

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Thursday, 16.20

In Situ pH Modulation for Enhanced Chemical Sensing of pH-Dependent Analytes

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pH is a key parameter affecting numerous chemical and biological processes, one of them being the protonation state and therefore the speciation of pH-dependent analytes such as dissolved inorganic carbon (DIC), total dissolved sulfide, and total ammonia nitrogen. However, as these analytes encompass more than one species (e.g. DIC = CO_2 + HCO_3^- + CO_3^{2-}) their determination is challenging, usually requiring complex sample pre-treatment or multi-sensor measurements.

To address these challenges, we integrate diffusion-based or electrochemical techniques to alter the sample pH close to optical or electrochemical sensors to shift the pH-dependent equilibrium of the analyte of interest to a single, measurable form. Figure 1 shows an example of this principle, integrating a polyaniline-based proton pump with a planar CO_2 optode. Initially, the optode response reflects the local concentrations of free CO_2 in the sample. Upon application of a mild potential, polyaniline releases protons from its backbone resulting in a localized decrease of the sample pH. This pH shift results in a conversion of DIC to CO_2, which in turn can be measured by the optode.[1]

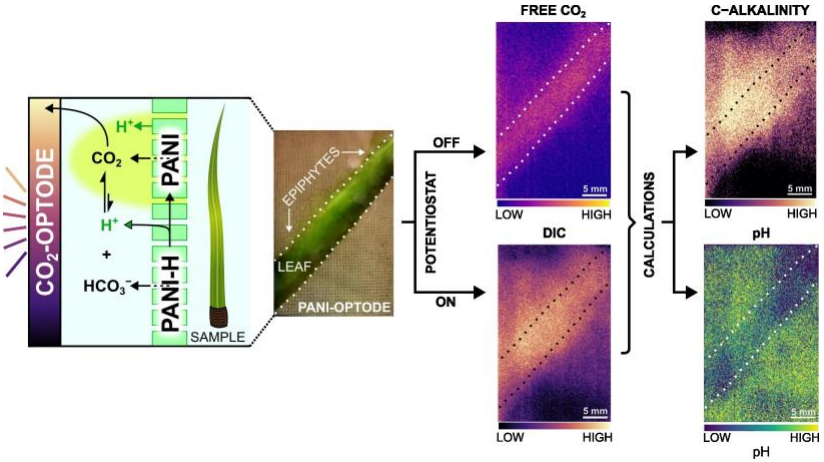

Figure 1. Measurement of free CO_2 and DIC during dark respiration of an epiphyte covered freshwater plant leaf (*V. Spiralis*), using the polyaniline-CO_2 optode tandem.

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Thursday, 16.40

The Way to advanced 2D Chemical Imaging with Optodes: from the Determination of Ionic Species to Multiparameter Measurements

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Chemical gradients and uneven distribution of analytes are prevalent phenomena in both natural and artificial systems. Optical sensors, so-called optodes, are used to obtain real-time images of two or more parameters simultaneously. While optodes have recently gained traction for O_2 and pH imaging in environmental monitoring, there is an evident gap in visualizing ionic species distribution and performing 2D multiparameter measurements with optodes [1]. In this contribution, we want to demonstrate the value of such experiments and show examples of how current state-of-the-art in these domains can be advanced.

The first project illustrates the 2D real-time NH4+ visualization approach using optodes in both planar and nanoparticle designs [2]. We explored absorbance and fluorescence readouts. A proof-of-concept showcased a biological application for identifying urease-producing pathogens (*Bacillus subtilis*) without relying on pH changes (Fig. 1). Using this optode can help to understand better the N cycle in agricultural, marine, or biological aspects.

In the second project, we utilized the dual emission of a porphyrin-based indicator with two different luminescent signals (thermally activated delayed fluorescence and phosphorescence) for dual O_2 and temperature 2D visualization. The monitoring was performed in soil with the incorporated water pipe system, through which hot water was circulated to mimic an underground heat distribution system (Fig. 2). This brings a major advance toward more routine optodes practice for varying analytical and environmental tasks.

We believe that these examples not only showcase the needs but also highlight the prospects of more advanced imaging with optodes.

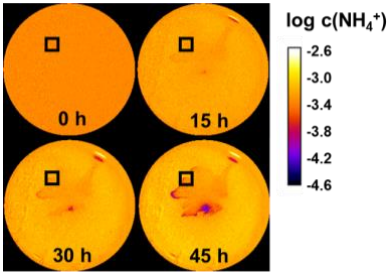

Fig. 1. Time-lapse NH_4^+ imaging of agar gel containing optode nanoparticles and inoculated with *Bacillus subtilis.*

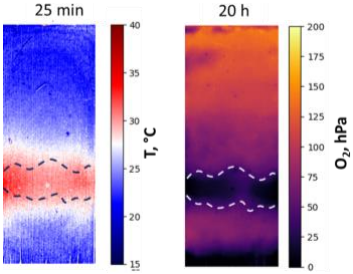

Fig. 2. 2D visualization of oxygen concentration and thermal imaging of heat transfer in soil with the hot water pipe.

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