Biomaterial Interfaces
Room 101A - Session BI+MI-WeM

Biosensors and Diagnostics

Moderators: Daniel Graham, University of Washington, Tobias Weidner, Max Planck Institute for Polymer Research, Germany

8:00am **BI+MI-WeM1 Bacteriophage-Derived Surfaces for the Targeting of Pathogenic Bacteria*, Stephane Evoy,*** University of Alberta, Canada

Bacterial pathogens cause a high level of morbidity and mortality, specifically for infants, young children, elderly and immunocompromised individuals. Antibodies have been exploited as molecular probes in order to impart specificity to bacterial biosensing platforms. Antibodies however suffer from degradation and reliability issues. The high specificity of phages offers a potent alternative for the targetting of pathogens. More specifically, recombinant phage receptor binding proteins (RBPs) responsible for phage-host specificity can be used as biological probes and present numerous advantages over the use of whole phage.

We successfully coupled phage RBP Gp047 from phage NCTC12673 onto magnetic beads. These beads were then employed for the extraction of Campylobacter cells from food matrices. Recovery rates were greater than 80% in samples spiked with as low as 10^2 cfu mL^{-1} of cells. Phage lysins have also been employed as capturing probes. We coupled recombinant lysin Gp10 from the mycobacteriophage L5 onto magnetic Dynabeads 280 for the capture of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) cells from complex media. The study employed skim cow milk spiked with MAP cells, skim milk spiked with both MAP and *Escherichia coli* cells and Middlebrook 7H9 medium spiked with MAP cells as test matrices. The functionalized beads were incubated with the spiked sample, separated, cleaned, and subjected to DNA extraction. The resulting solution was analyzed by real time PCR. The entire test was completed within 24 hours. The capture process significantly increased the PCR sensitivity and demonstrated high specificity towards MAP cells.

Further, we demonstrated the use of cysteine-tagged P22 phage RBPs on gold surface for the specific SPR detection of *Salmonella enterica serovar Typhimurium*. These results demonstrate that N-teminus Cys tagged proteins capture bacteria efficiently compared to the C-terminus Cys tagged protein due to preferential orientations. Finally, micromechanical devices have also been proposed for the detection and enumeration of bacteria. We designed and developed a microresonator array optimized for such detection. This large array-based design offers a large total area for the capture of cells, while maintaining the ability to detect the attachment of a single cell anywhere on the array. The devices were functionalized with phage GST-Gp48 tail-spike proteins to impart specificity of detection. We successfully employed these arrays for the specific detection of *Campylobacter jejuni* from clean buffer. The devices did not show any sensitivity to *Escherichia coli* bacteria confirming the specificity of detection .

8:20am **BI+MI-WeM2 Biomolecule Sensing at Attogram Levels via Nanophotonic-Optomechanical Resonators*, Anandram Venkatasubramanian,*** University of Alberta, Canada*; V.T.K. Sauer, S.K. Roy,* National Institute of Nanotechnology, Canada*; D. Wishart, W.K. Hiebert,* University of Alberta, Canada

The Gas chromatography (GC) – Mass spectrometry (MS) system is the industry benchmark in chemical analysis. However the need for complex instrument such as the ionizer makes the Mass spectrometry unsuitable for portable detection applications. Recent demonstrations with nano-optomechanical (NOMS) resonators at atmospheric pressure show they are promising for portable GCs, matching the mass detection limits of NEMS sensors in only the first generation. Owing to their superior displacement sensitivity compared to NEMS, NOMS may have competitive advantages going forward. In this regard, a free space interferometry system was used for NOMS sensing of biomolecules. The primary motivation to develop sensors for portable applications is to develop point of care diagnostic devices for health monitoring. As the state of our health is a product of our interactions with our environment, metabolomics is useful in health monitoring. Among the different human biofluids, urine is “favoured” due to their precise potrayal of metabolic breakdown products, sterility and easy to obtain large volumes. Hence we have demonstrated multiple component (5 +) biomolecule detection from derivatized human urine metabolomes (HUM) as they elute from the GC. Derivatized HUMs such as ethyl malonic acid (EMA) were tested as a single component sample to obtain the limit of detection. From the results it was observed that the minimum detectable mass was about 20 attograms with a concentration threshold of 25 µM with EMA, which is in the normal physiological range in human adults. T o the best knowledge of the authors, this is the first time a NOMS based gas sensor has been used in conjunction with a gas chromatographic system and has demonstrated physiological range of detection of biomolecules.

8:40am **BI+MI-WeM3 Hole-mask Colloidal Lithography Method to Fabricate Chiral Metal-Nanoparticles for Plasmon Enhanced CD Measurements*, Gunnar Klös,*** Aarhus University, Denmark*; D.S. Sutherland,* Aarhus, Denmark

Hole-mask colloidal lithography is a well studied method [1] for a reliable high throughput fabrication of metal nanoparticles (NP). The plasmonic resonances and their electromagnetic near field dependencies of such NPs are widely used as bio-sensors , e. g. for high sensitivity refractive index sensing [2]. Rather recently it was also shown that the near field interactions of planar plasmonic chiral NPs can be used for sensitive chiroptical measurements of very dilute amounts of chiral material [3], allowing structural characterization of even small amounts of many biomolecules. So far the fabrication of such chiral NPs is based on time-consuming techniques such as e-beam lithography [4].

Here, I present a novel method for the fabrication of chiral Au nanocrescents based on a modified version of hole-mask colloidal lithography. This reliable and efficient method utilizes the shrinking of the hole due to the material evaporated through it, adding an additional parameter to the control over the shape of the resulting NP.

The method allows the fabrication of nanocrescents with an outer diameter of 100nm-200nm that show plasmonic responses similar to previous Au structures [2]. Furthermore, when analyzed with circular polarized light, they show a considerable circular dichroism response.

Hence, this fabrication method is a promising technique for the time- and cost-efficient production of sensitive biosensors for the structural analysis of chiral materials.

[1] Bochenkov, V. E. and Sutherland, D. S. ” *NanoLett*, Vol. 13, 1216-1220, 2013.

[2] Guerreiro, J. R. L. et al. *ACS nano*, Vol. 8, No. 8, 7958–7967, 2014.

[3] Hendry, E. et al. *NNANO*, Vol. 5, 783-786, 2010.

[4] Hentschel, M. et al. *NanoLett*, Vol. 12, 2542-2547, 2012.

9:00am **BI+MI-WeM4 Neuraminidase Assay using Glycan-Functionalized Graphene Field-Effect Transistors*, Kaho Kamada,*** *T. Ono, Y. Kanai,* Osaka University, Japan*; Y. Ohno,* Tokushima University, Japan*; K. Maehashi,* Tokyo University of Agriculture and Technology, Japan*; K. Inoue,* Osaka University, Japan*; Y. Watanabe,* Kyoto Prefectural University of Medicine, Japan*; T. Kawahara, Y. Suzuki,* Chubu University, Japan*; S. Nakakita,* Kagawa University, Japan*; K. Matsumoto,* Osaka University, Japan

A lot of the anti-influenza virus drugs such as Tamiflu® and Relenza® prevent the viruses from infecting to the next cell. Influenza viruses enter the host cells of the throat and trachea by binding to the host cell’s surface receptor molecules which contains sialic acid. After the proliferation into the cell, the viruses cleave the sialo oligosaccharides by the action of the enzyme neuraminidase (NA), and propagated viruses are detached from the cells on the infection to the next cell. Therefore, it is possible to suppress the chain of propagation of virus by inhibiting the NA. Currently, the evaluation of antiviral drugs has been conducted mainly using cultured cells, there are problems in accuracy and quantitative property. In addition, it is difficult to evaluate the mechanism of reaction. Therefore we aim to build a useful new biological model platform for drug evaluation and drug discovery research. We modified sialoglycoprotein chain on the graphene surface, and fabricated the glycan-functionalized Graphene Field-Effect Transistors (G-FET), which reproduce cell surface environment on the graphene. The reaction behavior of the virus is highly detected as the current by the G-FET. So we can quantitatively evaluate drug reaction by the physical indicators. As a first step here, we electrically measured NA reaction by the glycan-functionalized G-FET.

G-FET was produced by evaporating the electrodes on graphene obtained by the exfoliation method. 1-Pirenbutan acid succinimidyl ester as a linker, was modified human sialoglycoprotein chain having a modified amino group on the graphene channel. After dropping the NA on it, we measured time course of the neuraminidase reaction monitored by the graphene-FET.

When dropping the NA, the current value is decreased exponentially. This is because the sialic acid negatively charged was disconnected from the

sugar chain, and the hole carriers induced on graphene were decreased. The rate of decrease in current value with neuraminidase dropping is in good agreement with the activity value obtained by the absorption method (A NA molecule cuts the 1.7 of molecules per second). This shows that the rate constant obtained from electrically measurement by G-FET reflects the enzyme reaction rate.

This study has received the support of JST・CREST.

9:20am **BI+MI-WeM5 Surface-sensitive Imaging of Supported Membranes and Single Lipid Vesicles for Medical Applications***, **Fredrik Höök,*** Chalmers University of Technology, Sweden **INVITED**

Measurements of ligand-binding events to membrane-protein receptors in a near-natural environment display an opportunity in mechanistic studies of membrane receptors. Furthermore, the residence time of drug-target interactions is being increasingly recognized as a key parameter in evaluating drug efficacy, but is hampered by the technical challenge to perform such studies for membrane proteins. However, with membrane proteins embedded in nanoscale lipid vesicles and detection methods with single molecule sensitivity, such information can be gained in a broad dynamic range, as requested in both drug-screening and diagnostic applications. A diverse set of tools with single-nanoparticle sensitivity is now available, to which we recently contributed a concept that enables simultaneous fluorescent and scattering-based label-free imaging of thousands of surface-bound nanoscale entities [Agnarsson B et al., ACS Nano, 2015]. The principle is based on the use of lipid vesicles as enhancer elements in optical waveguide based fluorescence and label-free evanescent-wave scattering microscopy, making the concept compatible with analysis of both water-soluble and cell-membrane bound receptors. The concept is currently evaluated as a diagnostic assay for biomarker detection and in drug-screening applications, previously explored by us using conventional total internal reflection fluorescence (TIRF) microscopy [Gunnarsson et al., Anal Chem (2015)]. The use of scattering microscopy in the context of single-enzyme detection in complex biological fluids will be presented, with focus on single-molecule biomarker detection in cerebrospinal fluid from individuals suffering from Alzheimer's disease [Angew Chemie, 2015]. A new means to utilize the two-dimensional fluidity of supported cell-membrane derived lipid bilayers in microfluidic designs for nanoparticle size determination and sorting applications will also be presented [Simonnson et al., JACS, 2011 and Pace et al., Anal Chem, 2015].

11:20am **BI+MI-WeM11 Non-invasive Thermal Sensing using Thermographic Phosphors***, **Firouzeh Sabri,*** University of Memphis*; S. Allison,* EMCO*; P. Parajuli,* University of Memphis

F. Sabri[1], S. W. Allison[2], and P. Parajuli[1]

1. University of Memphis, Department of Physics and Materials Science

2. Emerging Measurements Co.

Thermal measurements involving thermographic phosphors, whether in the form of powder, crystal, or glass, continues to be of interest for a wide range of applications and temperature ranges. The investigation of phosphor-doped polymer films is a promising avenue for thermometry applications. Phosphor thermometry has been investigated recently for non invasive thermal assessment of biological and biomedical surfaces. For thermographic phosphors to be useful for biomedical applications they must first be encapsulated in a biocompatible, biostable, and transparent "host" that would allow optical access to the embedded phosphors. The work here demonstrates the feasibility of thin film thermographic phosphor-based thermometry where La_2O_2S:Eu particles have been embedded in a clear silicone encapsulant at different concentrations. The composite materials were prepared by means of spin-coating technology and the effect of spin speed and spin time on the thickness and distribution of the powder was investigated. . In order to improve the thermal conductivity of the composite material, a layer of carbon has been incorporated into the multilayer structure. The results presented will compare the excitation-emission behavior of the composite materials mentioned above with the properties of pure powder, at various temperatures. The effect of the tensile properties of the composite material on the excitation/ emission behavior of the materials will also be discussed. Measurements were conducted at low temperatures and at elevated temperatures and the decay characteristics were investigated as a function of temperature.

11:40am **BI+MI-WeM12 Imaging Time-of-Flight Secondary Ion Mass Spectrometry to Characterize Tumor Progression and Regression***, **Lara Gamble,*** *B.M. Bluestein, D.J. Graham,* University of Washington*; F. Morrish, D. Hockenbery,* Fred Hutchinson Cancer Research Center

The tumor microenvironment has been associated with regulating tumor cell growth, metastatic potential, and chemotherapeutic drug resistance. However, very few techniques are capable of directly probing the tumor microenvironment on the micron scale. A new perspective is required to interpret and characterize this complex environment. Using imaging time-of-flight secondary ion mass spectrometry (ToF-SIMS) and a mouse model with Myc-dependent inducible and regressible pancreatic β-cell neoplasia, it is possible to relate changes in the composition and distribution of metabolic related molecules with tumor development. Myc, one of the most frequently deregulated oncogenes in human cancers, contributes to tumorigenesis through various mechanisms, including the deregulation of cell proliferation and growth, protein and mitochondrial biogenesis, and metabolic alterations. Pancreatic tissues were harvested and frozen in optimal cutting temperature (OCT) at 6 days post Myc induction. 4 µm cryosections were serially cut, with one used for H&E staining, one for ToF-SIMS analysis, and another for immunohistochemistry. ToF-SIMS data was acquired on an IONTOF TOF V with pulsed 25 keV Bi_3^+ ion beam. Principal component analysis (PCA) of ToF-SIMS image data separated regions of tumor cells from stroma within the first principal component and revealed subtle differences in chemistry between the tumor and surrounding tissue. ToF-SIMS data suggests a preferential uptake of fatty acids 18:3, and 18:2 within the tumor. The tumor also shows an increased localization of sphingomyelin fragments and vitamin E compared to the surrounding tissue. PCA was also applied to selected tumor regions to spatially and chemically analyze within the tumor and compare chemistries between different tumor sizes. Distinct chemical differences were identified between control and 6 day Myc activated β-cell islet tissues using multivariate analysis techniques. The results from C14:0 and phosphatidylcholine fragments present within the tumor are suggestive of *de novo* fatty acid synthesis. This work further demonstrates the high resolution capability of ToF-SIMS as the data clearly reveals intra-tumor chemical heterogeneity as localized high intensity regions, but histologic correlations are needed to discern the purpose and function of these structures.

12:00pm **BI+MI-WeM13 $SrI_2(Eu^{2+})$Gamma Camera for SPECT Imaging in Medical Applications***, **LaNell Williams,*** *M. Groza, E. Rowe, J. Butler,* Fisk University*; T. Peterson,* Vanderbilt University*; A. Burger,* Fisk University

The detection of gamma rays for nuclear imaging has become increasingly important in designing non-invasive imaging tools for biological research and modeling. Although imaging techniques such as computed tomography (CT) and Positron Emission Tomography (PET) have been previously used, and improved spatial resolution and sensitivity continue to be an issue. Thus, improvements in these detection devices are needed to create better images for more accurate modeling in research [Cressey, 2011]. Scintillators such as Cesium Iodide (CsI), and Sodium Iodide (NaI) have been used for many imaging techniques for their ease of growth, energy resolution, and overall effectiveness as a gamma ray detectors. In more recent studies, Strontium Iodide doped with Europium ($SrI_2(Eu^{2+})$) has shown to be a promising scintillator compared to NaI and CsI. Because of's $SrI_2(Eu^{2+})$ improved energy resolution (~2.7%), fast decay time (~1.2 µs) and light yield (110,000 photons/MeV), it is an ideal replacement for technologies that have used previously been made with NaI and CsI. [Cherepy, 2008]. In addition, $SrI_2(Eu^{2+})$ also has an emission centered around 420 nm making it an ideal scintillator to be used with silicon photomultipliers that provide lower energy consumption than the standard photomultiplier tube. The improved energy resolution of $SrI_2(Eu^{2+})$ in a gamma camera will result in an promising detector for nuclear imaging.

Author Index

Bold page numbers indicate presenter