Nanometer-scale Science and Technology
Room 101D - Session NS+BI-ThA

Applied Nanoscale Microscopy Techniques/Biomaterial Interfaces – New Advances

Moderators: Stephanie Allen, The University of Nottingham, UK, Leonidas Ocola, Argonne National Laboratory

2:40pm **NS+BI-ThA2 Advancing the Development of Nanocrystal Emitters *via* Advanced Electron Microscopy Techniques*, James McBride**, K.R. Reid, S.J. Rosenthal,* Vanderbilt University

The key tool for the characterization of nanoparticles has long been transmission electron microscopy. This technique can provide the size, shape, crystal structure and chemical composition of a nanocrystal. Aberration-corrected Z-STEM has enabled the visualization of the true core/shell structure of colloidal quantum dots, accelerating their commercial development.[1] Through dynamic STEM movies we have visualized the beam-induced motion of the surface atoms of nanocrystals and learned about the instability of the atomic structure of ultrasmall nanocrystals and the surface/sub-surface of large nanocrystals.[2] However, Z-contrast can be difficult to directly interpret due to the choice in shell material or uncertainty of the 3D morphology of large, thick-shelled quantum dots. Advancements in the detector design for performing STEM energy dispersive spectroscopy mapping (STEM-EDS) have greatly facilitated the chemical imaging of nanocrystals, enabling rapid identification of their chemical structure before significant beam damage occurs. With this technological advance, we have obtained the chemical composition of an individual nanocrystal and directly correlated to its individual photophysics using our recently developed correlation technique.[3] The unique combination of optical, structural and chemical information allowed us to determine the origin of the low quantum yield plaguing non-blinking CdSe/CdS quantum dots.[4] Further, STEM-EDS imaging will be presented showing development of InP/CdS and Zn_3N_2 nanocrystals. Included in the presentation will be specifics on sample preparation and the choice of beam current/spatial resolution and sample damage.

1. McBride, J.; Treadway, J.; Feldman, L.C.; Pennycook, S.J.; Rosenthal, S.J. Structural Basis for Near Unity Quantum Yield Core/Shell Nanocrystals *Nano Lett.***2006,** *6* (7), 1496-1501.

2. McBride, J.R.; Pennycook, T.J.; Pennycook, S.J.; Rosenthal, S.J. The Possibility and Implications of Dynamic Nanoparticle Surfaces *ACS Nano***2013,** *7* (10), 8358-8365.

3. Orfield, N.J.; McBride, J.R.; Keene, J.D.; Davis, L.M.; Rosenthal, S.J. Correlation of Atomic Structure and Photoluminescence of the Same Quantum Dot: Pinpointing Surface and Internal Defects That Inhibit Photoluminescence *ACS Nano***2015,** *9* (1), 831-839.

4. Orfield, N.J.; McBride, J.R.; Wang, F.; Buck, M.R.; Keene, J.D.; Reid, K.R.; Htoon, H.; Hollingsworth, J.A.; Rosenthal, S.J. Quantum Yield Heterogeneity among Single Nonblinking Quantum Dots Revealed by Atomic Structure-Quantum Optics Correlation *ACS Nano***2016,** *10* (2), 1960-1968.

3:00pm **NS+BI-ThA3 Demonstration of Electron Mirror for Quantum Electron Microscopy*, Navid Abedzadeh**, C.S. Kim, R.G. Hobbs, K.K. Berggren,* MIT

Electron mirrors have been used in electron microscopy techniques such as low-energy electron microscopy, mirror-corrected scanning electron microscopy and photoemission electron microscopy due to their ability to introduce chromatic and spherical aberrations of arbitrary sign. More recently, a design for a quantum electron microscope (QEM), an imaging approach based on interaction-free measurement, was proposed that could take advantage of an electron mirror whose surface was patterned with a topographic grating. This grating would produce a periodically varying potential close to its surface when a voltage was applied. As a result, the grating would diffract an incident electron plane wave, presenting an opportunity to develop a low-loss electron beam splitter. The diffracted beams produced by such a beam splitter could be used to probe a sample within an electron cavity to achieve an interaction-free measurement. An electron cavity could be formed when another electron mirror is placed slightly behind the back focal plane of the grating mirror. If a sample were placed inside this cavity, repeated weak interactions with the reflected/diffracted electron beam can be used to image the sample while keeping beam-induced sample damage arbitrarily low. The approach outlined here will be used to characterize diffraction from the patterned mirror surface. Demonstration of electron diffraction from a patterned surface in a FESEM will represent a significant advancement toward the demonstration of a QEM system.

3:20pm **NS+BI-ThA4 Nanoscale Chemical Imaging by Photo-induced Force Microscopy*, Ryan Murdick**,* Molecular Vista

Nanoscale Chemical Imaging with Photo-induced Force Microscopy

Sung Park

Molecular Vista, Inc.

Infrared Photo-induced Force Microscopy (IR PiFM) is based on an atomic force microscopy (AFM) platform that is coupled to a widely tunable mid-IR laser. PiFM measures the dipole induced at or near the surface of a sample by an excitation light source by detecting the dipole-dipole force that exists between the induced dipole in the sample and the mirror image dipole in the metallic AFM tip. This interaction is strongly affected by the optical absorption spectrum of the sample, thereby providing a significant spectral contrast mechanism which can be used to differentiate between chemical species. Due to its AFM heritage, PiFM acquires both the topography and spectral images concurrently and naturally provides information on the relationship between local chemistry and topology. Due to the steep dipole-dipole force dependence on the tip-sample gap distance, PiFM spectral images have spatial resolution approaching the topographic resolution of AFM, demonstrating sub 10 nm spatial resolution on a variety of samples. PiFM spectral images surpass spectral images that are generated via other techniques such as scanning transmission X-ray microscopy (based on synchrotron source), micro confocal Raman microscopy, and electron microscopes, both in spatial resolution and chemical specificity. The breadth of the capabilities of PiFM will be highlighted by presenting data on various organic, inorganic, and low dimensional materials. By enabling imaging at the nm-scale with chemical specificity, PiFM provides a powerful new analytical method for deepening our understanding of nanomaterials and facilitating technological applications of such materials.

Bio: Sung Park is the CEO of Molecular Vista, which he co-founded with Prof. Kumar Wickramasinghe (UC Irvine, formerly of IBM) in 2011 to provide research and industrial tools for rapid and nanoscale imaging with chemical identification. Sung has 25 years of experience of industrial R&D, engineering, marketing and sales, and operations. Sung co-founded Park Scientific Instruments (PSI), which was one of the first commercial companies to develop and sell scanning tunneling microscopes (STM) and atomic force microscopes (AFM); PSI was acquired by Thermo Instruments in 1997, by which point PSI had sold upwards of 1,000 instruments to customers worldwide. Prior to founding Park Scientific Instruments, Sung worked as a post-doc at IBM Watson Research Center. Sung earned a Ph.D. in Applied Physics from Stanford University and BA in Physics from Pomona College.

4:00pm **NS+BI-ThA6 Strong Coupling of Localized Surface Plasmon Resonances to Light-Harvesting Complexes from Plants and Bacteria*, A. Tsargorodska, M. Cartron, C. Vasilev,*** University of Sheffield, UK*; G. Kodali,* University of Pennsylvania*; J. Baumberg,* University of Cambridge, UK*; PL. Dutton,* University of Pennsylvania*; CN. Hunter,* University of Sheffield, UK*; P. Torma,* University of Aalto*; **Graham Leggett**,* University of Sheffield, UK

Plants and bacteria harvest solar energy with extraordinary efficiency. In chloroplasts, the quantum efficiency, defined as the fraction of captured photons that goes on to cause charge separation, is estimated to be ca. 90%. The mechanisms by which such extraordinary efficiencies are realised have been the subject of intense interest. We have explored the potential offered by plasmonic techniques for the investigation of biological light harvesting complexes. Macroscopically extended arrays of gold nanostructures are fabricated by interferometric exposure of an alkylthiolate SAM on gold, enabling the fabrication of macroscopically extended arrays of gold nanostructures in a rapid, simple process. After annealing, these structures yield strong localized surface plasmon resonances (LSPRs). In contrast to the behaviour observed for most proteins, the LSPRs are split when light-harvesting membrane proteins from purple bacteria and plants are attached to the gold nanostructures, yielding pronounced changes in their extinction spectra. The splitting is large, and is different for mutant proteins containing different pigment molecules, indicating that it is sensitive to the electronic structures of the membrane proteins. The splitting is attributed to strong coupling between the LSPRs and excitons in the light-harvesting complexes. The splitting is suggestive of an asymmetric Fano-type resonance, and the plasmon-exciton coupling has been modelled with coupled harmonic oscillators. The

model yields good fits to the experimental spectra. It indicates that in light harvesting complexes 1 and 2 (LH1/2) from purple bacteria, coupling to the carotenoid *S2* state dominates, with a strength of ~ 0.2 eV. However, in a carotenoid-free mutant of LH1 the LSPR couples with a strength of ~ 0.1 eV to the bacteriochlorophyll Q_x transition, which has a smaller transition dipole moment than do the carotenoids. The coupling varies with the square root of the surface coverage of the protein, consistent with strong coupling theory. Strong coupling was also observed for self-assembling polypeptide maquettes that contain only chlorins. However, it was not observed for monolayers of bacteriochlorophyll, indicating that strong plasmon-exciton coupling is sensitive to the specific presentation of the pigment molecules.

4:20pm **NS+BI-ThA7 Microfluidic Device For Aptamer-Based Cancer Cell Capture And Genetic Mutation Detection**, ***Sarah Reinholt***, *H.G. Craighead,* Cornell University

Genetic mutations in cancer cells are not only fundamental to the disease, but can also have tremendous impact on the efficacy of treatment. Identification of specific key mutations in a timely and cost-effective way would allow clinicians to better prescribe the most effective treatment options. Here, we present a novel microfluidic device as a platform for specifically capturing cancer cells and isolating their genomic DNA (gDNA) for specific amplification and sequence analysis. To filter out rare cancer cells from a complex mixture containing a diversity of cells, nucleic acid aptamers that specifically bind to cancer cells are immobilized within a microchannel containing micropillars to increase capture efficiency. The captured cells are then lysed and the gDNA is isolated via physical entanglement within a secondary micropillar array. This type of isolation allows the gDNA to be retained within the channel, and enables multiple consecutive rounds of isothermal amplification in which different individual genes are amplified separately. The amplified gene samples undergo sequencing, and the resulting sequence information is compared against the known wildtype gene to identify any mutations. Cervical and ovarian cancer cells have been initially tested for mutations in the *TP53* gene using this technology. This approach offers a way to monitor multiple genetic mutations in the same small population of cells, which is beneficial given the wide diversity in cancer cells, and requires very few cells to be extracted from the patient sample. With this capability for genetic monitoring, precision medicine should be more accessible for the treatment of cancer.

4:40pm **NS+BI-ThA8 Molecular Processes in an Electrochemical Clozapine Sensor**, ***Thomas Winkler***, University of Maryland, College Park; *S.L. Brady,* East Carolina University; *E. Kim,* University of Maryland, College Park; *H. Ben-Yoav,* Ben-Gurion University of the Negev, Israel; *D.L. Kelly,* University of Maryland, Baltimore; *G.F. Payne, R. Ghodssi,* University of Maryland, College Park

Selectivity presents a crucial challenge in electrochemical sensing. One example is schizophrenia treatment monitoring of the redox-active antipsychotic clozapine (CLZ). To accurately assess efficacy, differentiation from its metabolite *N*-desmethylclozapine (NDMC) – similar in structure and redox potential – is critical. Here, we leverage biomaterials integration to study, and effect changes in, diffusion and electron transfer kinetics of these compounds. A key finding in our present work is differing dynamics between CLZ and NDMC once we interface the electrodes with chitosan-based biomaterial films. These additional dimensions of redox information can thus enable selective sensing of largely analogous small molecules.

Our study utilizes gold working electrodes either bare, coated with chitosan, or with our previously demonstrated redox cycling system (RCS). In the RCS, electrodeposited chitosan serves as a matrix to immobilize electroactive catechol near the electrode *via* electrografting. Small redox species diffuse through the film for oxidation at the electrode; the nearby catechol enables subsequent reduction of the analyte, establishing a signal-amplifying redox cycle. We execute cyclic voltammetry at 1m—10V/s sweep rates with CLZ, NDMC, or the model redox couple 1,1′-ferrocenedimethanol (FC).

With bare gold, both CLZ and NDMC exhibit similar (R^2=0.99) drastic increases in peak separation even at 0.5V/s, indicating slow electron transfer kinetics, in contrast to FC (Nernstian up to 3V/s). With both chitosan and the RCS we find that similarity broken. For diffusion, the coefficients D reveal two regimes in chitosan: dominance of bulk solution below 10mV/s (values match those from bare gold and theory), and diffusion inside the film becoming limiting at higher scan rates. This is reflected in *D* decreasing by 1.9× for FC, 17× for CLZ, and 31× for NDMC. The sharp difference between FC and the other larger two suggests a size-restriction phenomenon. The consistently 2× lower *D* for NDMC over the similarly sized CLZ points to possible electrostatic effects. With the RCS, signal amplification translates into apparent *D* increases – 9× over bare for FC, 5× for CLZ, and 3× for NMDC. Only at high scan rates does *D* decrease toward the chitosan-only value as true diffusion asserts dominance.

In conclusion, our results demonstrate the intricate interplay between biomaterials, biomolecules, and electrochemistry. They reveal intriguing distinguishing characteristics of CLZ from both the largely analogous NDMC as well as the model FC. This opens up avenues of utilizing diffusion and kinetics information to enhance selectivity in electrochemical sensing.

5:00pm **NS+BI-ThA9 Quantitative Quartz Crystal Microbalance Measurements across Transients Produced by Switching Fluid Properties**, *V. Mugnaini,* ***Dmitri Petrovykh***, International Iberian Nanotechnology Laboratory, Portugal

We systematically investigated Quartz Crystal Microbalance with Dissipation (QCM-D) measurements in aqueous solutions of model strong electrolytes that are commonly used in experiments with biological surfaces. In particular, we examined the quantitative behavior of both frequency and dissipation responses in transitions between two different aqueous solutions.

The abrupt changes in the QCM-D responses upon such transitions are sometimes referred to as “jumps” associated with switching the bulk properties of the fluid flowing through the QCM-D cell. Switching between fluids of different compositions may be important in a variety of QCM-D measurements for biointerfaces, e.g., when switching between a baseline/rinsing solution and a measurement solution, or switching between optimal buffers used for probe immobilization and for biorecognition steps [1-3]. In specialized quantitative biointerface measurements, such as measuring stabilities of DNA hybrids [2-3], switching among different solutions multiple times actually provides the basis for the measurement.

In typical QCM-D measurements, the baseline is reset after a “bulk jump”, so the data are typically only quantified between any transients, but not across them, i.e., quantification is carried out for a constant fluid composition, but not between different fluids. By considering the underlying viscoelastic formalism [4-5], we demonstrate in a series of systematic measurements for solutions of strong electrolytes that the QCM-D responses upon switching between different solutions can be quantitatively predicted and exhibit interesting scaling behavior. Classical theory of the viscosity of electrolyte solutions provides additional insight into correlations between the results measured for different salts.

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[2] S. M. Schreiner, D. F. Shudy, A. L. Hatch, A. Opdahl, L. J. Whitman, D. Y. Petrovykh, Anal. Chem. 82, 2803 (2010)

[3] S. M. Schreiner, A. L. Hatch, D. F. Shudy, D. R. Howard, C. Howell, J. Zhao, P. Koelsch, M. Zharnikov, D. Y. Petrovykh, A. Opdahl, Anal. Chem. 83, 4288 (2011)

[4] K. K. Kanazawa, J. G. Gordon II, Anal. Chim. Acta, 175, 99 (1985)

[5] M. Rodahl, F. Höök, A. Krozer, P. Brzezinski, B. Kasemo, Rev. Sci. Instrum. 66, 3924 (1995)

5:20pm **NS+BI-ThA10 ToF-SIMS/XPS Characterization of Frozen-Hydrated Hydrogels**, ***Michael Taylor***, *M.R. Alexander,* The University of Nottingham, UK; *M. Zelzer,* National Physical Laboratory, UK

Over the last decade the beneficial properties of hydrogels as artificial cell culture supports have been extensively investigated[1]. Certain synthetic hydrogels have been proposed to be similar in composition and structure to the native extracellular matrix of the stem cell niche, their *in vivo* cell habitat, which is a powerful component in controlling stem cell fate[2]. The stem cell differentiation pathway taken is influenced by a number of factors. When culturing cells within or upon hydrogels this choice can be strongly dependent on the underlying 3D hydrogel chemistry which strongly influences hydrogel-cell interactions[3]. The interrelationship between hydrogel chemistry and that of biomolecules in controlling cellular response ideally requires analysis methods to characterise the chemistry without labels and often in 3D. Time-of-flight secondary ion mass spectrometry (ToF SIMS) has the potential to be utilised for through thickness characterisation of hydrogels. The frozen-hydrated sample format is well suited to minimise changes associated with dehydration or the chemical complexity of ‘fixation’, a challenging aspect in vacuum analysis conditions[4]. Frost formation can occur in the ambient atmosphere

preventing ready depth profiling of the frozen hydrogels[5]. We develop a simple method to remove this frost by blowing with gas prior to entry into the instrument which is shown to produce remarkably good profiles on a poly(2-hydroxyethyl methacrylate) (pHEMA) hydrogel film where a model protein, lysozyme, is incorporated to demonstrate how biomolecule distribution within hydrogels can be determined. A comparison of lysozyme incorporation is made between the situation where the protein is present in the polymer dip coating solution and lysozyme is a component of the incubation medium. It is shown that protonated water clusters $H(H_2O)_n^+$ where n=5-11 that are indicative of ice are detected through the entire thickness of the pHEMA and the lysozyme distribution through the pHEMA hydrogel films can be determined using the intensity of characteristic fragment secondary ions. We also expand the developed methodology to X-Ray Photoelectron Spectroscopy (XPS) for through thickness analysis of the similar pHEMA / lysozyme hydrogels, and show that lysozyme distribution can be quantitatively mapped in hydrogels.

5:40pm **NS+BI-ThA11 GCIB-SIMS for Studying Bacterial Surfaces***, **John Stephen Fletcher**, P. Wehrli,* University of Gothenburg, Sweden*; A. Farewell,* University of Gothenburg, Seweden*; T.B. Angerer, J. Gottfries,* University of Gothenburg, Sweden

For many years ToF-SIMS has shown the promise of delivering new information of direct relevance to biological research. However, inadequacies in the ability to generate intact molecular ion species and then detect them with precise mass resolution and accuracy have held the technique back. Recent advances in ToF-SIMS, through the implementation of gas cluster ion beams (GCIBs) coupled to non-conventional MS systems, now permit the analysis of higher mass species from native, underivatised, biological specimen i.e. intact bacterial cells. Being able to characterise and understand changes in bacterial biochemistry as a result of environmental, biological or pharmacological stress is critical to address the global challenge of antibiotic resistance. For example, *E. coli* is able to rapidly adjust the biophysical properties of its membrane phospholipids to adapt to environmental challenges including starvation stress. Here, these membrane lipid modifications were investigated in glucose starved *E. coli* cultures and compared to a D*rel*AD*spo*T ($ppGpp^0$) mutant strain of *E. coli,* deficient in the stringent response, by means of time-of-flight secondary ion mass spectrometry (ToF-SIMS Cultures in stationary phase were found to exhibit a radically different lipid composition as compared to cultures in exponential growth phase. Wild-type *E. coli* reacted upon carbon starvation by lipid modifications including elongation, cyclopropanation and increased cardiolipin formation. Observations are consistent with variants of cardiolipins (CL), phosphatidylglycerols (PG), phosphatidylethanolamines (PE), phosphatidic acids (PA), and fatty acids. Notably, despite having a proteomic profile and a gene expression profile somewhat similar to the wild-type during growth, the $ppGpp^0$ mutant *E. coli* strain was found to exhibit modified phospholipids corresponding to unsaturated analogues of those found in the wild-type. We concluded that the $ppGpp^0$ mutant reacts upon starvation stress by elongation and desaturation of fatty acyl chains, implying that only the last step of the lipid modification, the cyclopropanation, is under stringent control. These observations suggest alternative stress response mechanisms and illustrate the role of the RelA and SpoT enzymes in the biosynthetic pathway underlying these lipid modifications.

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