Wednesday Morning, October 22, 2008

Biomaterial Interfaces
Room: 202 - Session BI+NC-WeM

Quantitative Nanoscale Sensing and Single Molecule Techniques

Moderator: B. Liedberg, Linköping University, Sweden

8:00am **BI+NC-WeM1 Studying Single Molecules on Living Cells**, ***D. Klenerman***, Cambridge University, UK **INVITED**

One major challenge in biology is to understand how the individual molecules and complexes of the cell are organised and interact to form a functional living cell. To address this problem new biophysical tools are needed that are capable of studying single molecules in complexes both in the test-tube and on or in living cells. To determine the oligomerisation state of proteins we have used two colour single molecule coincidence detection based on the excitation of two distinct fluorophore labels on proteins with two lasers focussed to the same spot.[1] This method requires no prior knowledge of the structure of any complex formed or control of fluorophore position on the molecule. We show that this method can be used to characterise the protein oligomers formed during protein misfolding, ultimately resulting in amyloid fibril formation, and can distinguish between protein monomers and dimers on the cell surface.[2] Working together with Professor Yuri Korchev at Imperial College, we have developed a method for functional nanoscale mapping of the cell surface that is based on a scanned nanopipette. This allows high resolution, non-contact imaging of the soft and responsive cell surface using the ion current that flows between an electrode in the nanopipette and bath for distance feedback control.[3] Recently we have made a major advance in the resolution of the topographic images, by scanning with fine quartz pipettes, so we can directly visualise protein complexes on the surface of live cells.[4] The pipette can also be use to perform local nanoscale assays on the cell surface so as to perform single channel recording[5] or apply pressure to probe the mechanical properties. We have also combined high resolution topographic imaging with simultaneous recording of the fluorescence from the cell surface.[6] In addition the pipette can be used for controlled voltage driven delivery and deposition of biomolecules down to the single molecule level[3] and this is being used to probe the structure of the cell membrane using single molecule fluorescence tracking.

References
[1]. Anal.Chem. 78, 7707-7715 (2006).
[2]. PNAS 104,17662-17667 (2007)
[3]. Phys. Chem. Chem. Phys. 7 , 2859-2866 (2005)
[4]. Angewandte Chemie-International Edition 45, 2212-2216 (2006)
[5]. Biophysical Journal 83, 3296-3303 (2002).
[6]. PNAS 99 , 16018-16023 (2002)

8:40am **BI+NC-WeM3 Theory of Single Molecule Characterization using Random Telegraph Signals**, ***S. Vasudevan***, *K. Walczak, A.W. Ghosh*, University of Virginia

The future of nanoelectronics will depend not only on the capability to engineer 'smart' materials, but also on the ability to exploit new quantum phenomena that emerge at submicroscopic length scales. Molecular electronics has often been advocated as an ideal successor to silicon-based, complementary metal oxide semiconductor technology (CMOS). But its development has been thwarted by problems like poor gatability and low mobilities. Therefore we need to explore hybrid devices that do not compete with CMOS, but instead add novel functionalities by exploiting properties that are unique to molecules, such as their tendency to function as strongly correlated systems. Thus we need to account for more complex effects than usual semiclassical theory provides. In this paper we develop a theory for a new class of electronic devices that exploit correlated quantum scattering in a transistor channel. In these devices, molecules attach to the surface of a transistor channel; the chemical bonding leads to the transfer of charge and spectral weight between the molecule and the silicon surface. The overlap of molecular and silicon wavefunctions serves to passivate existing surface states as well as to create new localized molecular trap levels inside the silicon band-gap. At resonance driven by a gate, the traps are stochastically filled and emptied by the channel electrons, blocking and unblocking the channel. The resulting two-state random telegraph signal (RTS) can be used to locate the trap position both spectrally as well as spatially. This allows us to characterize and detect molecular species through unique nano-'barcodes'. The effect is enhanced in modern nanodevices as they can be fabricated practically defect free with near ballistic levels of operation. In contrast with ChemFETs, where one detects a single threshold shift for a specific molecule, here we get an entire spectral nano-'barcode' that can be compared against a compilation of theoretical responses to characterize and sense a molecular species. Since these devices operate by modulating surface properties of transistors, we call them 'SurfFETs'. The significant advantage of such SurfFETs is their exclusive detection of only molecules that overlap significantly with the channel to cause a transfer of states. This means that this electronic detection scheme is selective and inherently avoids false positives- clearly an advantageous feature for detection of molecules.

9:00am **BI+NC-WeM4 Magnetic Tweezers Measurement of the Bond Lifetime-Force Behavior of the IgG-Protein A Specific Molecular Interaction**, *H. Shang*, MagSense Life Sciences, ***G. Lee***, University College Dublin, Ireland

The bond lifetime-force behavior of the immunoglobulin G (IgG)-protein A interaction has been studied with magnetic tweezers to characterize the physical properties of the bond under nonequilibrium conditions. Superparamagnetic microparticles were developed that have a high and uniform magnetization to simultaneously apply a piconewton scale tensile force to many thousands of IgG-protein A bonds. A strong and a weak slip bond were detected with an effective bond length that is characteristic of short-range, stiff intermolecular interactions. These bonds are attributed to the interaction of protein A with the constant region (Fc) and heavy chain variable domain (VH) of IgG, respectively. The IgG-VH interaction appears to be one of the weakest specific molecular interactions that has been identified with a single molecule force measurement technique. This study demonstrates that magnetic tweezers can be used to rapidly characterize very weak biomolecular interactions as well as strong biomolecular interactions with a high degree of accuracy.

9:20am **BI+NC-WeM5 Elasticity Mapping of Pore Suspending Native Cell Membranes**, ***A. Janshoff***, Institute of Physical Chemistry, Germany

The mechanics of cellular membranes is governed by a non-equilibrium composite framework composed of semiflexible filamentous cytoskeleton and extracellular matrix proteins linked to a lipid bilayer. Non-local elasticity information of native cell membranes has so far been gathered by micropipette suction and rheological whole cell experiments. Locally confined measurements were conducted by using membrane-attached beads pulled by laser tweezers and by atomic force microscopy of entire cells. As yet, local mechanical information (elasticity maps) of isolated cellular membranes, such as basolateral membranes of endo- and epithelial cells, are however, not available. Here, we introduce a novel approach that allows the mapping of mechanical properties of native freestanding cellular membranes on a nanometer length scale. Basolateral membranes of polar epithelial MDCK II cells, prepared on a highly ordered porous substrate, were locally indented with the aim to unravel how the cytoskeleton and extracellular matrix (ECM) affects the viscoelasticity of such native membranes on a predefined length scale. We found a strong relation between the density and cross-linking of actin filaments and membrane stiffness.

9:40am **BI+NC-WeM6 Size Measurement of Targeted Nanoparticle Delivery Systems**, ***N. Farkas***, *J.A. Dagata, V.A. Hackley*, National Institute of Standards and Technology, *K.F. Pirollo, E.H. Chang*, Georgetown University Medical Center

The mean size and size distribution of a targeted nanoparticle delivery system (NDS) strongly influences the intrinsic stability and functionality of this molecular complex, affects its performance as a systemic drug delivery platform, and ultimately determines its efficacy towards early detection and treatment of cancer. Since its components undergo significant reorganization during multiple stages of self-assembly, it is essential to monitor size and stability of the complex throughout NDS formulation. Furthermore, reproducible and quantitative size measurement of individual entities, not only average properties of the entire population, is needed to assure potency and manufacturability of a specific formulation prior to entering clinical trials. Scanning probe microscopy (SPM) is capable of providing both high-resolution imaging of intact NDS immobilized on a substrate under fluid conditions and statistically meaningful, number-weighted averaged data for the complex. This presentation describes robust sample preparation methods and statistical image analysis of targeted liposome-based NDS with encapsulated therapeutic and diagnostic agents.[1] We present detailed examples of how variations in NDS formulation impact the size and stability of complexes with various payloads. These measurements are then compared with mean particle size distributions obtained by dynamic light scattering (DLS). SPM-based size distribution measurement technique in combination with DLS offers quantitative means of assessing size and stability, optimizing of formulation during drug development, and quality control during manufacturing of NDS.

[1] J. A. Dagata, N. Farkas, C. L. Dennis, R. D. Shull, V. A. Hackley, C. Yang, K. F. Pirollo, and E. H. Chang, Physical characterization methods for iron-oxide contrast agents encapsulated within a targeted liposome-based delivery system, Nanotechnology, in press.

10:40am **BI+NC-WeM9 Biophotonics: Resonant Detection of Single Molecules**, ***A.M. Armani***, University of Southern California **INVITED**

For many biological and chemical experiments, a sensor must have high sensitivity, high specificity, and fast response time. There are many technologies which are able to achieve one or two of these three requirements, but many still face fundamental sensitivity or response limitations. Silica optical resonators are able to overcome these limitations because of the high quality factor (Q).[1],[2] In their application as a single molecule sensor, the sensitivity is derived from the long photon lifetime inside the microcavity, and the specificity is derived from functionalization of the silica surface. During the initial series of label-free detection experiments, pure Interleukin-2 (IL-2) solutions were injected into the volume surrounding the microtoroid. The microtoroid successfully detected step-like shifts in resonance wavelength from individual IL-2 molecules binding. Additional experiments have shown that even in the more complex environment of serum individual binding events of IL-2 are still resolved.[3] Therefore, this single molecule sensor will enable research in new areas of biophysics and cell biology. Acknowledgements: The author would like to thank Prof. Richard Flagan, Prof. Scott Fraser, and Dr. Rajan Kulkarni at the California Institute of Technology. A.M. Armani is supported by the Provost's Initiative for Biomedical Nanoscience and the WiSE Program at the University of Southern California.

[1] A. M. Armani, D. K. Armani, B. Min, K. J. Vahala, and S. M. Spillane, Applied Physics Letters, vol. 87, pp. 151118, 2005.

[2] Armani, D. K., Kippenberg, T. J., Spillane, S. M. & Vahala, K. J. Ultra-high-Q toroid microcavity on a chip. Nature 421, 925-928 (2003).

[3] A. M. Armani, R. P. Kulkarni, S. E. Fraser, R. C. Flagan, and K. J. Vahala, Science, vol. 317, pp. 783 (2007).

11:20am **BI+NC-WeM11 Single-Molecule Detection and Mismatch Discrimination of Unlabeled DNA Targets**, ***M. Gunnarsson***, Chalmers Institute of Technology, Sweden, *P. Jönsson*, *J. Tegenfeldt*, Lund University, Sweden, *F. Höök*, Chalmers University of Technology, Sweden

Ultrasensitive biological sensors for low-abundant DNA and protein detection have emerged as an important tool for improving biomedical diagnostics, drug discovery, forensic analysis, but also advanced bioanalytical assays in fundamental research. We report on a single-molecule readout scheme based on total internal reflection fluorescence microscopy (TIRFM) demonstrating a detection limit in the low fM regime for short (30 mer) unlabeled DNA strands. Detection of DNA targets is accomplished by mediating the binding of suspended fluorescently labeled DNA-modified small unilamellar vesicles (~100 nm in diameter) to a DNA-modified substrate by unlabeled complementary single-stranded DNA. On top of rapid and sensitive detection, the technique is also shown capable of extracting kinetic data from statistics of the residence time of the binding reaction in equilibrium, i.e. without following neither the rate of binding upon injection nor release upon rinsing. The potential of this feature is demonstrated by discriminating a single mismatch from a fully complementary 30-mer DNA target.[1] The proposed detection scheme is particularly appealing due to the simplicity of the sensor, which relies on self-assembly principles and conventional TIRFM. In contrast to most other single-molecule detection schemes the imaging mode also offers possibilities for multiple spots to be measured simultaneously in an array-based design. The proposed sensor holds particular promise in cases when information about binding kinetics is valuable, such as in single nucleotide polymorphism (SNP) diagnostics.

[1] Gunnarsson, A., et al., Single-molecule detection and mismatch discrimination of unlabeled DNA targets. Nano Letters. 8(1): p. 183-188, 2008.

11:40am **BI+NC-WeM12 Optical Fiber Microarrays for Single Molecule Detection**, ***H.H. Gorris***, *D.R. Walt*, Tufts University

Optical fiber microarrays have been employed for the detection of single enzyme molecules. Single enzyme molecules were enclosed with fluorogenic substrate in an array of 50,000 individually addressable microchambers etched into a glass optical fiber bundle. The large array size provided excellent statistics. The substrate turnover in the microchambers was monitored with epifluorescence microscopy. We have observed a broad distribution of discrete turnover rates of single ß-galactosidase molecules that can be attributed to different enzyme conformations. When a slow-binding inhibitor was added to single ß-galactosidase molecules inhibited and active states of ß-galactosidase could be clearly distinguished. With a pre-steady-state experiment, we demonstrated the stochastic character of inhibitor release, which obeys first-order kinetics. Under steady-state conditions, the quantitative detection of substrate turnover changes over long time periods revealed repeated inhibitor binding and release events, which are accompanied by conformational changes of the enzyme's catalytic site. We proved that the rate constants of inhibitor release and binding derived from stochastic changes in the substrate turnover are consistent with bulk-reaction kinetics. Furthermore, we have applied the optical fiber microarray to the detection of single horseradish peroxidase molecules. These monomeric enzyme molecules exhibit a narrower distribution of turnover rates than the tetrameric ß-galactosidase, which could be explained by the number of catalytic sites involved in substrate turnover.

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