# Thursday Morning, November 12, 2009

**Applied Surface Science**
**Room: K - Session AS2+BI-ThM**

## Scanning Probe Studies of Biological Materials

**Moderator:** I.S. Gilmore, National Physical Laboratory, UK, S. Zauscher, Duke University

8:00am **AS2+BI-ThM1 Beyond the Optical Resolution in Living Cell: Biomedical Applications of Scanning Ion Conductance Microscopy**, *Y.E. Korchev*, Imperial College London, UK, ***S. Allen***, The University of Nottingham, UK **INVITED**

Molecular Biology has advanced we know much about the individual molecular components that make up living cells down to the level of the individual atoms. The challenge, however, is to fully understand the functional integration of these components. This requires determining how the molecular machines that make up a living cell are organized and interact together not at the atomic length scale but on a nm scale. To do this we need to develop and applying nanoscale techniques for the visualisation and quantification of cell machinery in real-time and on living cells. This will lead to detailed, quantitative models of sub-cellular structures and molecular complexes under different conditions for both normal and diseased cells. This approach ultimately requires the development of novel biophysical methods. We have recently pioneered the development of an array of new and powerful biophysical tools based on Scanning Ion Conductance Microscopy that allow quantitative measurements and non-invasive functional imaging of single protein molecules in living cells. Scanning ion conductance microscopy and a battery of associated innovative methods are unique among current imaging techniques, not only in spatial resolution of living and functioning cells, but also in the rich combination of imaging with other functional and dynamical interrogation methods. These methods, crucially, will facilitate the study of integrated nano-behaviour in living cells in health and disease.

8:40am **AS2+BI-ThM3 Nanometer Scale Patterning of Biomolecules using Near-Field Optical Methods**, ***G.J. Leggett***, University of Sheffield, UK

The integration of top-down (lithographic) and bottom-up (synthetic) methods remains one of the outstanding challenges in molecular nanoscience. There are no established tools for the manipulation of surface chemical structure in the length range from ca. 100 nm to the dimensions of a single biomacromolecule. Scanning near-field photolithography (SNP), in which a scanning near-field optical microscope coupled to a UV laser is employed as a light source, may be used to execute specific molecular transformations with a spatial resolution of a few tens of nm and, at best, 9 nm (ca. $\lambda/30$). Several strategies will be described for the patterning of proteins and nucleic acids on metal and oxide surfaces. We demonstrate two approaches. First, photodegradation of oligo(ethylene glycol) (OEG) functionalised surfaces provides a simple route to the covalent attachment of proteins to non-fouling surfaces. Photodegradation of OEG-terminated thiols provides a simple, one-step route to the conversion of a protein-resistant surface to a protein-binding one. Similar methodologies may be extended to oxide surfaces, through the formation of OEG-functionalised siloxane films. Photodegradation yields aldehyde functionalities, to which nitrilo triacetic acid (NTA) functionalised amines may be coupled, and subsequently dertivatised, following nickel complexation, with histidine-tagged proteins, providing a facile route to the site-specific immobilisation of proteins on glass. Second, siloxane monolayers offer fruitful opportunities for the incorporation of synthetic chemical methods into nanolithography. Halogenated monolayers may be converted to aldehydes or to carboxylic acids, by controlling the exposure. Alternatively, 2-nitrophenylpropyloxycarbonyl (NPPOC) protected aminosiloxane monolayers on glass may be selectively deprotected by SNP, yielding amine groups for further functionalisation with very high spatial resolution. The synthetic flexibility and versatility of photochemical methods, when combined with near-field methods for control of exposure, offers enormous potential for integrating top-down and bottom-up methods.

9:00am **AS2+BI-ThM4 Nanomechanics of Glycopeptide Resistant Superbugs and Superdrugs**, ***J. Ndieyira***, Uni. College London, UK and Jomo Kenyatta Univ. of Agriculture and Tech., Kenya, *A. Donoso Barrera, M. Vogtli,* Uni. College London, UK, *M. Sushko,* Uni. College London, UK and PNNL, *D. Zhou,* Univ. of Leeds, UK, *M. Cooper,* The Univ. of Queensland, Australia, *C. Abell,* Univ. of Cambridge, UK, *T. Strunz, G. Aeppli, R. McKendry*, Uni. College London, UK

The discovery of penicillin in 1928 marked the beginning of a remarkable new era of antibiotic 'wonder drugs', saving millions of lives across the world. However the widespread and often indiscriminate use of antibiotics has fuelled the alarming growth of antibiotic resistant superbugs, including methicillin resistant Staphylococcus aureus (MRSA) and vancomycin resistant Enterococci (VRE). To remain one step ahead of the superbugs, there is now an urgent need to develop new antibiotics and yet the drug pipeline is severely limited. We recently reported the nanomechanical detection of vancomycin-cell wall peptide interactions on cantilever arrays and discriminated between vancomycin-sensitive and vancomycin-resistant phenotypes.[1] In this talk I will present our new work which exploits this technology for the search of new superdrugs active against VRE. We have investigated a series of vancomycin derivatives and detect a dramatic enhancement in surface binding affinities compared to homogeneous solution measurements. We identify a glycopeptide which binds 11,000 more strongly to resistant peptide analogues. Our findings reveal fundamental new insights into the mechanism of antibiotic bindings to a model bacterial cell wall peptides using nanosensors, which not only has important implications on the design of new devices with significantly improved antibiotic detection sensitivity but will also impact on our understanding of the mode of action of antibiotics on intact bacteria. These findings highlight the potential of BioMEMs devices for application in pharmaceutical industry and will accelerate the discovery of new antibiotics.

[1] 'Nanomechanical detection of antibiotic mucopeptide binding in a model for superbug drug resistance.' Ndieyira, W.N, Watari, M., Donoso-Barrera, A., Batchelor, M., Zhou, D., Vogtli, M., Bactchelor, M., Cooper, M., Strunz, T., Abell, C.A., Rayment, T., Aeppli, G. & McKendry R.A. *Nature Nanotechnology* **3**, 691 - 696 (2008). Also featured in Nature Nanotechnology News and Views, BBC, New Scientist, Physics Today, Chemistry World, UK T&I.

9:20am **AS2+BI-ThM5 Single Biomolecule Force Spectroscopy Measurements; the Importance of Controlled Surface Chemistry**, *S. Allen,* The University of Nottingham, UK, ***S. Zauscher***, Duke University

Over the past decade, considerable interest has focussed on the ability of atomic force microscopy (AFM) and related techniques to record forces on or between single biological molecules. Many elegant examples are evident within the literature where such approaches have been employed, for example, for studies of force induced protein and RNA unfolding processes, and the dissociation of a wide-range of biomolecular complexes, including those involved in cell adhesion. Despite these elegant examples, and the considerable advantages of performing measurements at the single molecule level, such measurements are still far from routine. Indeed, even in the most experienced hands the experiments can suffer various problems which can arise from poor control of the chemistries employed for biomolecular surface attachment e.g. such as non-specific binding, difficulties in single molecule pickup and variability between experiments.

This presentation will provide an overview of the approaches we have employed in recent studies to address such issues. This for example, will include our studies of the mechanical properties of long molecules of DNA (>1000 base pairs) in which we have demonstrated the advantages of the addition specific terminal functionalities for surface attachment [1]. The advantages will be highlighted through our investigations of the effects on DNA mechanical properties of a range of different DNA binding molecules (e.g. drugs and proteins involved in DNA replication [1,2]). For studies of the dissociation of biomolecular complexes, we will highlight the advantages of an alternative approach, in which we have exploited the unique properties of dendron functionalized surfaces. Developed in collaboration with the group of Professor J.W. Park (Pohang University of Science and Technology (POSTECH), we have recently employed such surfaces for studies of DNA hybridization [3], and interactions between intracellular signal transduction proteins [4].

[1] W. Zhang, R. Barbagallo, C. Madden, C.J. Roberts, A. Woolford, S. Allen. (2005) *Nanotechnology* 16 2325-2333

[2] W. Zhang, C. Machon, A. Orta, N. Phillips, C.J. Roberts, S. Allen, P. Soultanas (2008) *Journal of Molecular Biology* 377, 706-714

[3] J. Yung, B.J. Hong, W. Zhang, S.J.B. Tendler, P.M. Williams, S. Allen and J.W. Park (2007). *Journal of the American Chemical Society*, 129(30), 9349-9355.

[4] I.H. Kim, H.Y. Lee, H.D. Lee, Y.J. Jung, S.J.B. Tendler, P.M. Williams, S. Allen, S.H. Ryu, and J.W. Park (2009) *Analytical Chemistry* 81(9), 3276-3284

9:40am **AS2+BI-ThM6 Nanoarrayed Biomolecular Recognition followed by AFM**, ***P. Lisboa***, *L. Sirghi, A. Valsesia, P. Colpo, F. Rossi*, JRC-European Commission, Italy

The use of nanoarrayed surfaces in the field of biomolecular recognition is very promising for the improvement of bio-detection performance. Atomic Force microscope (AFM) is widely used to produce and characterize nanoarrayed surfaces and to carry out studies in the biological field. This work presents the study and characterization by AFM measurements of the fabrication steps of nanoarrayed surfaces based on organothiols (carboxylic and Polyethylene oxide) and the study of an immunoassay performed on these surfaces. The immunoassay was based on the bio-recognition between Human IgG/anti-Human IgG.

The nanoarray was fabricated by plasma colloidal lithography following the procedure developed in our lab. AFM studies of the process of nanoarray fabrication showed that during lithographic process, the etching step is crucial for the final the characteristics of the surface and that the process originates a good chemical nano-contrast.

The AFM analysis of the bio-interaction was performed after Human IgG immobilisation and anti-Human IgG recognition steps. The nanoarray was incubated with Human IgG solution resulting in an increase of height on the nano areas. The AFM image demonstrates that IgG molecules are adsorbed mainly on the border between the two organothiols. The preferential disposition of proteins on the borders of the two different materials with hydrophobic and hydrophilic groups was already reported and associated to the fact that the proteins tend to adsorb where they can find better accommodation. In our case, this effect can be explained by the fact that having the carboxylic spots with hydrophilic character the IgG hydrophobic groups are better accommodated on the border between the two materials, leading to higher adsorption on the boundaries. After the Human IgG incubation, the surface was blocked with BSA and the following step consisted in the immobilisation of anti-Human IgG. After this step, a height increase on the border of the COOH nano area is detected by AFM. The increase is about the double of the initial with Human IgG. This is an indication that the anti Human IgG binds specifically with the Human IgG already on the surface. The distribution on the borders of the nano-area can explain the better efficiency of the nano-patterns in biomolecular recognition as already described on different studies. The preferential proteins immobilisation on the nano areas boundaries seems to improve the binding efficiency of the immobilised Human IgG bio-detector by a better availability of the binding sites on the surface and reduction of steric hindrance.

10:40am **AS2+BI-ThM9 Deciphering Nanoscale Interactions: Artificial Neural Networks and Scanning Probe Microscopy**, *S. Jesse, M.P. Nikiforov, O. Ovchinnikov,* ***S.V. Kalinin***, Oak Ridge National Laboratory

Scanning Probe Microscopy techniques provide a wealth of information on nanoscale interactions. The rapid emergence of spectroscopic imaging techniques in which the response to local force, bias, or temperature is measured at each spatial location necessitates the development of data interpretation and visualization techniques for 3- or higher dimensional data sets.

In this presentation, we summarize recent advances in applications of neural network based artificial intelligence methods in scanning probe microscopy. The examples will include biological identification based on the dynamic of the electromechanical response, direct mapping of dynamic disorder in ferroelectric relaxors, and reconstruction of random bond-random field Ising model parameters in ferroelectric capacitors. The future prospects for smart multispectral SPMs are discussed.

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11:00am **AS2+BI-ThM10 Scanning Probe and Differential Interference Contrast Imaging Methods for Studying Adhesion-Induced Tension and Membrane Fluctuation of Red Blood Cells**, ***N. Farkas***, *H. Kang,* National Institute of Standards and Technology, *F. Tokumasu,* NIAID, NIH, *J. Hwang, J.A. Dagata*, National Institute of Standards and Technology

Red blood cell (RBC) membrane fluctuation mediated by cooperative relationship between its cytoskeleton and lipid bilayer plays an important role in protein dynamics that is indicative of structural-functional properties of healthy or diseased RBCs. Probing of this characteristic membrane behavior requires dynamic interrogation of RBCs under physiological conditions by high-resolution, noninvasive microscopy techniques for which RBCs are required to be immobilized on a substrate while maintaining their viability. Therefore, detailed understanding of the adhesion process and its consequence on RBC shape and dynamic membrane response is critical. In the present study, we demonstrate our ability to engineer substrates with tunable surface zeta potential (SZP) for precise control of RBC adhesion. Specifically, 10 nm gold nanoparticles are adsorbed on poly-L-lysine coated cover slips as a compliant layer to locally modify the non-specific interaction between RBC membrane and substrate. By combining scanning probe microscope (SPM) and differential interference contrast (DIC) imaging techniques we develop a quantitative measurement methodology to investigate the relationship between attachment strength, RBC morphology, cell vibration and membrane fluctuation on these charge and topographically modulated substrates. Adhesion-induced tensing of the RBC membrane on modified substrates leads to changes in cell shape and functionality as determined by SPM force-volume and DIC monitoring of membrane dynamics. The substrate preparation and measurement methods presented here provide a feasible platform to obtain structure-function relationships of viable RBCs under physiological conditions and with that allow us to investigate dynamic behavior of RBCs and their response to diseases.

11:20am **AS2+BI-ThM11 Effect of Different Cations on the Nanomechanical Response of a Model Phospholipid Membrane : A Force Spectroscopy Study**, ***L. Redondo-Morata***, *G. Oncins, F. Sanz*, University of Barcelona, Spain

Understanding the effect of mechanical stress on biological membranes is of fundamental importance in biology since cells are known to naturally perform their function under the effect of a complex combination of forces. The chemical composition of such membranes is the ultimate responsible for determining the cellular scaffold, closely related to its function. Nevertheless, there is another factor that has been widely discussed in theoretical works but never experimentally tested in an accurate way, which is the presence of ions and their nature (radius and charge) on the stability of the bilayers. Micro-scale assays have revealed a wealth of information regarding the overall membrane mechanical resistance. Nonetheless, the diversity in the chemical composition of such membranes makes it difficult to individually probe the mechanical contribution of every particular membrane component. Here we use force spectroscopy to quantitatively characterize the nanomechanical resistance of supported lipid bilayers as a function of ionic strength and the composition of the electrolyte thanks to a reliable molecular fingerprint that reveals itself as a repetitive jump in the approaching force curve, hallmark of bilayer rupture. By systematically testing a set of bilayers composed of different phospholipids immersed in electrolytes composed of a variety of monovalent and divalent cations, we first show that the cationic radius, its charge density and the hydration number have an independent and important contribution to the overall bilayer mechanical stability. This work opens up avenues for characterizing the membrane (nano)mechanical stability and to assess the effect of different ions in the structure of the bilayers in an experimental and reproducible way.

# Authors Index

## Bold page numbers indicate the presenter