Applied Surface Science Division
Room: 102 - Session AS+BI+NS-WeM

Advances in Scanning Probe Microscopy
Moderator: S.A. Allen, The University of Nottingham, UK

8:00am **AS+BI+NS-WeM1 High-Speed Atomic Force Microscopy for Filming Biomolecular Processes**, ***T. Ando***, Kanazawa University, Japan **INVITED**

Vital phenomena are engendered through the dynamic activity of biological molecules. Therefore, observing the dynamic behavior of biological molecules in action at high spatiotemporal resolution is essential for elucidating the mechanism underlying the biological phenomena. The dynamic biomolecular processes are now widely studied using single-molecule fluorescence microscopy. However, the fluorescently labeled biological molecules themselves are invisible in the observations even using super-resolution fluorescence microscopy. The structure of biological molecules has been studied using x-ray crystallography, NMR, electron microscopy, and atomic force microscopy (AFM) but the obtained structures are essentially static. Thus, the simultaneous assessment of structure and dynamics is infeasible. To overcome this long-standing problem and make it possible to simultaneously record the structure and dynamics of biological molecules, we have been developing high-speed AFM for more than 15 years and at last it is now coming of age. Various AFM devices and control techniques were optimized or invented for high-speed scanning and low-invasive imaging. As a result, the imaging rate now reaches 10-30 frames/s for the scan range 250 ×250 nm^2, 100 scan lines, and the spatial frequency of a sample surface corrugation 0.1/nm [Prog. Surf. Sci. **83**, 337-437 (2008)]. Remarkably, even delicate protein-protein interactions are not disturbed by the tip-sample contact. With this capacity of high-speed AFM, some biological processes are successfully captured on video, such as walking myosin V molecules along actin filaments [Nature **468**, 72-76 (2010)], photo-activated structural changes in bacteriorhodpsin [Nat. Nanotechnol. **5**, 208-212 (2010)], and cooperative GroEL-GroES interactions. The high-resolution movies not only provide corroborative 'visual evidence' for previously speculated or demonstrated molecular behaviors but also reveal more detailed behaviors of the molecules, leading to a comprehensive understanding of how they operate. Thus, the high-speed AFM imaging of functioning biological molecules has the potential to transform the fields of structural biology and single-molecule biology.

8:40am **AS+BI+NS-WeM3 Integrated Imaging: Probing Molecular Interactions by Correlated Atomic Force Microscopy Approaches**, ***C. Yip***, University of Toronto, Canada **INVITED**

The development of powerful single molecule functional imaging tools has been critical to our understanding of molecular dynamics and structure-function relationships in (bio)molecular systems. Our lab's focus on the design, implementation, and application of coupled imaging and spectroscopy is providing intriguing insights into the mechanisms of membrane disruption, receptor oligomerization, and protein-membrane interactions. We have devised several correlative approaches based on the integration of in situ atomic force microscopy with fluorescence and vibrational spectroscopies for extracting the orientation, conformation, and association dynamics of membrane-associated proteins in model membranes and in live cells. Some of the key challenges and opportunities afforded these new tools will be discussed.

9:20am **AS+BI+NS-WeM5 Visible Light Emission from Fluorescent Proteins on Silver Substrate Induced by Tunneling Electrons**, ***T. Yamada***, RIKEN, Japan, *T. Iwaya, S. Matsunaga, M. Kawai*, The University of Tokyo, Japan

We detected the characteristic visible light emission from fluorescent protein molecules deposited on metallic silver (Ag) upon injection of tunneling electrons generated by a standard scanning tunneling microscope (STM) in ambient condition. A series of fluorescent proteins originating from jellyfish or coral, nowadays engineered to generate various colors of fluorescence by gene technology, is characterized with a β-barrel structure insulating the chromophore electronically from the surrounding. We purchased green, yellow, red and infrared fluorescent proteins (GFP, YFP, RFP, HcRed, molecular diameter ≈ 5 nm), deposited on a bare Ag surface, and used a Ag tip set on a STM setup to obtain images and to generate fluorescence. Light from the gap was collected by an optical fiber and introduced to a grating spectrometer with a liquid N_2-cooled CCD detector. On bare Ag surfaces, visible light was detected with the STM bias voltage within ±1.8 V in a modestly moisturized N_2 atmosphere. The spectra were unstable in general, indicating light emission upon excitation of local plasmon [1], which depends on the changeable geometry of Ag tip. The wavelength onset of emitted light was equivalent to the STM bias voltage within ±3.0 V, obeying the law of quantum energy conservation. The fluorescent proteins were dissolved in pure water, drop-cast on the Ag substrate and air-dried to form multilayers. STM images mostly showed flat terraces with steps composed of the protein molecules. Within a 200nm x 200nm scanning area, the light emission spectra apparently involved the characteristic fluorescence peaks of proteins (GFP = 540 nm (2.30 eV), YFP = 550 nm (2.25 eV), RFP = 650 nm (1.91 eV), HcRed = 660 nm (1.88 eV)) over a background of weakened Ag plasmon spectrum. The same experiment with Au tips and Au(111) substrates was with almost no detection for the characteristic fluorescence of all the proteins. For clean Au(111), although visible light was detected, the above-mentioned plasmon energy conservation stood for the bias voltage only within ±1.9 V. The maximum energy of local plasmon on Au(111) is too small to excite the florescent proteins electronically. The characteristic fluorescence from proteins is considered aided by the plasmon excitation of the Ag substrate. The protein β-barrel structure reserves the lifetime of excited state towards light emission, insulating electronically from the metallic substrate against the radiationless de-excitation process of the present surface-adsorbate system.

References:

[1] F. Rossel, M. Pivetta, W.-D. Schneider, *Surf. Sci. Rep.* **65**, 129 (2010).

9:40am **AS+BI+NS-WeM6 Characterization of Peptide Nanotubes by Atomic Force Microscopy**, ***J.L. Remmert***, *M.C. Vasudev*, Air Force Research Laboratory, *L. Eliad, E. Gazit*, Tel Aviv University, Israel, *T.J. Bunning, R.R. Naik, A.A. Voevodin*, Air Force Research Laboratory

This work investigates the properties of aromatic dipeptides, which are of interest due to their ability to self-assemble into nanotubes and nanowires. Peptide nanotubes have been used to template inorganic materials[1] and construct nanochannels in microfluidic devices[2]. The mechanical, thermal, and electronic transport properties of these nano-structures are desired to evaluate their potential use for biomolecular electronics[3] and other applications. Atomic Force Microscopy (AFM) offers multiple modes to interrogate the response of discrete nanotubes. For instance, AFM with dry sample heating has established the thermal stability of peptide nanotubes up to 100 °C[4] with a spring constant of 160 N/m at room temperature[5]. A separate study targeting a single nanowire bridging two electrodes revealed semiconductor characteristics under repeated bias cycling[6]. We have similarly sampled detached nanotubes among peptide bundles and vertically aligned 3D arrays. Peptide nanotubes were synthesized by either Plasma Enhanced Chemical Vapor Deposition (PECVD) or solvent phase growth in 1, 1, 1, 3, 3, 3 Hexafluoroisopropanol (HFP), using approaches similar to that described by Reches et al[1]. The nanotubes were observed by SEM to vary between 85-100 nm in diameter and up to 50 μm in length. Sample density was controlled by suspension and dilution in various solvents, including HFP and water, prior to deposition on a variety of substrates. AFM studies have revealed details of the tubular outer shell with tapping and electrostatic force modes (EFM), while also probing the mechanical integrity and thermal response to localized tip-side heating.

[1]M. Reches, E. Gazit, "Casting Metal Nanowires within Discrete Self-Assembled Peptide Nanotubes", Science 300 625 (2003)

[2]N. Sopher, Z. Abrams, M. Reches, E. Gazit, Y. Hanein, "Integrating peptide nanotubes in micro-fabrication processes", J Micromech Microeng 17 2360 (2007)

[3]V. Dinca, E. Kasotakis, J. Catherine, A. Mourka, A. Ranella, A. Ovsianikov, B. Chichkov, M. Farsari, A. Mitraki, C. Fotakis, "Directed Three-Dimensional Patterning of Self-Assembled Peptide Fibrils", Nano Lett 8 538 (2008)

[4]V. Sedman, L. Adler-Abramovich, S. Allen, E. Gazit, S. Tendler, "Direct Observation of the Release of Phenylalanine from Diphenylalanine Nanotubes", J Am Chem Soc 128 6903 (2006)

[5]N. Kol, L. Adler-Abramovich, D. Barlam, R. Shneck, E. Gazit, I. Rousso, "Self-Assembled Peptide Nanotubes Are Uniquely Rigid Bioinspired Supramolecular Structures", Nano Lett 5 1343 (2005)

[6]J. Lee, I. Yoon, J. Kim, H. Ihee, B. Kim, C. Park, "Self-Assembly of Semiconducting Photoluminescent Peptide Nanowires in the Vapor Phase", Angew Chem Int Edit 50 1164 (2011)

10:40am **AS+BI+NS-WeM9 Determination of Molecular Polarization at Protein-Electrode Interfaces with Combined Optical, Transport, and Dielectric Scanning Probe Microscopy**, ***X. Chen***, *K. Kathan-Galipeau, B.M. Discher, D.A. Bonnell*, University of Pennsylvania

Bio-molecule integrated electronic devices are of great interest recently. For such systems to be designed and fabricated, the optoelectronic properties of protein molecules in ambient environment must be understood at a fundamental level. Here we demonstrate a new scanning probe based technique: torsional resonance nanoimpedence microscopy (TR-NIM), which simultaneously probes transport and dielectric properties in conjunction with optical excitation. To make a controlled interface, we start by designing a peptide molecule with ability to control protein/electrode interface interactions, as well as incorporation of several different optically active cofactors, and we successfully patterned peptides on HOPG substrates. Using TR-NIM electronic transport and the effect of optical absorption on dielectric polarizability in oriented peptide single or multiple molecular layers is determined. This approach enables quantitative comparisons of the change in polarization volume between the ground state and excited state in both single and multiple molecular layers.

11:00am **AS+BI+NS-WeM10 Scanning Local Capacitance Measurements with High Spatial and Dielectric Resolution**, ***M.J. Brukman***, *S. Nanayakkara, D.A. Bonnell*, University of Pennsylvania

Spatial variation of dielectric properties often dictates the behavior of devices ranging

from field effect transistors to memory devices to organic electronics, yet dielectric

properties are rarely characterized locally. We present methods of analyzing 2nd

harmonic-based local capacitance measurements achieved through non-contact atomic

force microscopy. Unlike contact-based methods, this technique preserves tip

shape and allows the same probe to realize high-resolution topographic imaging and

scanning surface potential imaging. We present an improved analysis of the electrical

fields between tip and sample, yielding high sensitivity to the capacitance-induced

frequency shift.

The techniques are applied to thin-film strontium titanate and mixed-phase self-

assembled monolayers to illustrate application to high dielectric constant hard materials

and lower dielectric constant organic films. Conversion from frequency shift signal to

dielectric constant κ is demonstrated on both samples, with sub-5 nm spatial resolution

and dielectric constant resolution between 0.25 and 1.

11:20am **AS+BI+NS-WeM11 Parallel Momentum Conservation of Hot Electrons across a Metal Semiconductor Interface**, ***J.J. Garramone***, *J. Abel, R. Balsano, V.P. LaBella*, College of Nanoscale Science and Engineering, the University at Albany-SUNY

Parallel momentum of electrons is a conserved quantity as the electron traverses a barrier between two materials which lead to refraction like effects in the electrons trajectories. Ballistic electron emission microscopy (BEEM) is a scanning tunneling microscopy (STM) based technique that injects hot electrons (E>EF) into a metal-semiconductor Schottky diode[1]. A small fraction of these electrons will traverse the metal with little to no scattering and make it into the semiconductor and counted as BEEM current. This makes it an ideal technique to study parallel momentum conservation. However, direct observation of this effect has been rather elusive. To observe this effect the dependence of the attenuation length with hot electron energy of Ag on both the Si(001) and Si(111) substrates has been measured.

Samples consisted of nanometer thick Ag films that were deposited on HF cleaned Si(001) and Si(111) wafers and capped with 10 nm Au to prevent oxidation of the films. Attenuation lengths were extracted by measuring the BEEM current as a function of the metal overlayer thickness. The dependence of the attenuation length with tip bias (electron energy) displayed a sharp increase as the energy approached the Schottky barrier height for the Si(001) substrates and a slight decrease for the Si(111) substrates. This contrast is a direct result of parallel momentum conservation and the lack of zero parallel momentum states at the Si(111) interface when compared to the Si(001) interface. Additional insight into the relative contribution of both elastic and inelastic scattering can be obtained by fitting the data to a Fermi liquid based model.

[1] L. D. Bell, et al., Phys. Rev. Lett. **61** 2368 (1988).

11:40am **AS+BI+NS-WeM12 High Resolution Scanning Probe Imaging of 2D-Supramolecular Networks on Au(111), Graphite and Molybdenite**, ***V.V. Korolkov***, *S. Allen, C.J. Roberts, S.J.B. Tendler*, The University of Nottingham, UK

Chemical decoration of surfaces with various molecules and supramolecular structures has been a major strategy for introducing new properties to both organic and inorganic materials. Amongst these properties are wettability, biocompatibility, sensing properties, catalytic activity, optical properties and adhesion. Most of methods for surface modification include molecules binding to the surface via stable chemical bond. Recently methods have been developed to modify atomically flat surfaces with periodical porous molecular structures, termed 2D-supramolecular networks. The networks are commonly composed of two types of molecules serving different functions e.g. joints and ribbons. Such 2D-structures bring forward a unique surface property - a spatially controlled adsorption with almost single molecule precision.

Most networks reported in the literature have been studied using UHV STM on metal substrates and, to a lesser extent, on HOPG and non-conductive substrates. Here we present a study, utilizing both ambient STM and AFM, of 3,4,9,10-perylenetetracarboxylic diimide (PTCDI) - melamine networks deposited on Au(111), HOPG and MoS_2 substrates. AFM imaging was performed using PeakForce Tapping AFM (Bruker Inc.) and Torsion Resonance (TR)-AFM. Both STM and AFM were able to resolve a clear periodical network structure for all substrates after exposure to a solution of PTCDI and melamine molecules in dimethylformamide at 373K. AFM images show that the network forms a monolayer on both HOPG and molybdenite substrates, and also that most of the HOPG surface is covered with network structure, with some minor defects. In contrast the Au(111) surface was mostly covered with network multilayers as suggested both by TR-AFM and STM. AFM also revealed that the network structure on HOPG and molybdenite remains intact for several hours in the ambient and can be stored in N_2-ambient for up to ~24h.

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