Tuesday Afternoon, November 3, 1998

Biomaterial Interfaces Group
Room 326 - Session BI+AS+MM+NS+SS-TuA

Nanoscale to Mesocale Biomaterial Structures

Moderator: M.J. Tarlov, National Institute of Standards and Technology

2:00pm **BI+AS+MM+NS+SS-TuA1 Self-Assembly of a Multidomain Protein: Fibronectin at Lipid Model Interfaces**, ***V. Vogel***, *G. Baneyx,* University of Washington **INVITED**

Fibronectin, an adhesion protein with multiple recognition sites, mediates cell attachment to synthetic and biological surfaces. In solution, fibronectin exists in a globular state where most of its recognition sites are buried in the protein core. Surface adsorption induces conformational changes in the protein that expose many of these sites. Furthermore, it is known that on the surface of cells fibronectin assembles into detergent insoluble fibers, which are considered to be the main functional form of the protein. Fibronectin is hence a prime example of a protein with multiple recognition sites that can be regulated through environmental control. Unfortunately, the molecular pathways of activation and self-assembly are still poorly understood. We have recently found that fibronectin can self-assemble into fibrillar networks at receptor-free phospholipid monolayer interfaces under physiological conditions. This is a crucial observation since the paradigm in biology is that fibril assembly of fibronectin is mediated by membrane-bound receptor molecules. Availability of a simplified model system allows investigation of the molecular pathways by which appropriate surfaces can activate fibronectin and facilitate self-assembly.

2:40pm **BI+AS+MM+NS+SS-TuA3 Nanofabricated Substrates for Probing Single Biomolecules by Surface Enhanced Raman Scattering**, ***S. Petronis***, *L.K. Hedberg, H. Xu, M. Käll, B. Kasemo,* Chalmers Univ. of Technology and Univ. of Gothenborg, Sweden

The effect of Raman scattering enhancement when coherent laser light interacts with molecules attached to rough surfaces and microscopic metal domains has been known for more than two decades and is called Surface Enhanced Raman Scattering (SERS). The intensity of the Raman signals for such molecules is frequently enhanced by a factor 10@super 5@-10@super 6@ at best.@footnote 1,2@ However recently much larger enhancement factors, in the range 10@super 14@-10@super 15@, have been observed for molecules adsorbed on colloidal silver particles of specific dimensions.@footnote 3,4@ This giant enhancement allows the recording of vibrational spectra from a single molecule for the first time, instead of the ensemble averaged spectra from many molecules, which are normally obtained in optical spectroscopies. Here we report on an attempt to use nanolithography to fabricate structures of silver in the size range 100 - 200 nm and having different shapes in order to explore the size and geometry dependence of the SERS effect. Microfabricated structures which give the highest enhancement could be used for probing different biomolecules and perhaps designing a biosensor. SERS active substrates were prepared as arrays of silver particles on a Si wafer. Within each array the silver particles had a constant shape, size and separation. Three particle shapes (circular, triangular and square), two particle sizes (100 nm and 200 nm), and five different particle separations (10, 50, 100, 150 and 200 nm) were produced by electron beam lithography with a double-layer resist system and "lift-off" procedure. A reference area of uniformly deposited Ag film mimicked an infinite silver surface. The final structures and the chemical composition of the silver particles were characterized by Scanning Electron Microscopy (SEM) and Auger electron spectroscopy (AES), respectively. Preliminary Raman scattering experiments have been performed on the dye-molecule Rhodamin 6G adsorbed on the nanofabricated substrates. A giant enhancement of the Raman signal was observed on all patterns, but not on the Ag film or the Si surface. @FootnoteText@ @footnote 1@M.Moskovits, Rev. of Mod. Phys., vol. 57, No 3, 1985, pp 783-826 @footnote 2@A.G.Mal'shukov, Phys. Rep., vol 194, Nos 5&6, 1990, pp 343-349 @footnote 3@K.Kneip et al., Phys. Rev. Lett., vol. 78, No 9, 1997, pp1667-1670 @footnote 4@S.Nie, S.R. Emory, Science, vol. 275, No 21, 1997, pp 1102-1106

3:00pm **BI+AS+MM+NS+SS-TuA4 Nanostructured Surfaces for Biorecognition - A Novel Templating Approach**, ***H. Shi***, *B.D. Ratner,* University of Washington

Materials that specifically recognize proteins may find a variety of applications in separations, sensors and medical materials. Molecular imprinting provides an intriguing approach to plastic antibodies against small molecules, but the use of proteins as templates has been less successful in making protein recognition materials. In this study, nanostructured surfaces with tailored protein-binding cavities are prepared by an imprinting technique based on RF-plasma deposition of organic thin films. A polysaccharide-like surface with protein-imprinted nanopits allows only the template protein to fill the pits, and to bind strongly, because the nanopits are complementary to the template protein in shape and in the distribution of functional groups. The bound protein in its pit is prevented from exchange with protein in the solution due to a strong binding and steric hindrance, while the non-template protein that is weakly adsorbed on the surface is displaceable. Atomic force microscopy (AFM) and transmission electron microscopy (TEM) showed that nanometer-sized pits, in the shape of imprinted proteins, were created on the surfaces of our protein-imprinted polymer films. Imprinting fidelity was confirmed by AFM analysis of imprints of monodisperse colloidal gold nanoparticles. Electron spectroscopy for chemical analysis (ESCA) and time-of-flight secondary ion mass spectrometry (TOF-SIMS) indicated that template proteins were washed off the surfaces of protein imprints while sugar molecules were covalently incorporated. Radiolabeled -protein adsorption showed that a protein imprint recognized its template protein from a binary mixture with a high specificity. This study illustrates a novel templating strategy for biological molecules that can be exploited for fabrication of biorecognition materials.

3:20pm **BI+AS+MM+NS+SS-TuA5 Sensing and Analyzing Single Molecular Interactions with Microfabricated Devices@footnote 1@**, *J.-B.D. Green,* ***G.U. Lee***, Naval Research Laboratory **INVITED**

There is an intense effort to create new tools for manipulating and characterizing single macromolecules because of the power that these techniques can bring to the analysis of biological macromolecules. Due to the high force and displacement sensitivity of the atomic force microscope (AFM) it has been used to measure inter- and intramolecular forces between model ligand-receptors, i.e., streptavidin-biotin, complimentary strands of DNA, and biologically relevant supra-molecular structures, i.e. titin. With the success of these measurements, there are efforts to obtain even more detailed force measurements and to establish these techniques in the biotechnology laboratory. Our efforts focus on: 1. Designing force transducers with force (10@super -12@N), time (10@super -5@s) and spatial (10@super -9@m) resolutions that push the thermal noise envelope. 2. Developing immobilization strategies that produce more reliable force measurements. We will discuss two new microfabricated devices under development in our laboratory. The first microfabricated apparatus offers an excellent platform for detailed measurements of intermolecular interactions and possibly even analysis of combinatorial arrays. The second is an ultra-sensitive detector based on piezoresistive force transduction and magnetic microparticles. The future of these and similar devices will be considered. @FootnoteText@ @footnote 1@This work has been conducted in collaboration with Alexey Novoradovsky, Jonah Harley, Mohan Natesan, Steven Metzger, David Baselt, and Richard Colton.

4:00pm **BI+AS+MM+NS+SS-TuA7 Nanomechanical Properties of Cellular Components Determined by Interfacial Force Microscopy**, ***P.R. Norton***, *K de Jong, J.F. Graham, N.O. Petersen,* University of Western Ontario, Canada

The cell membrane is the contact surface between the cell's internal environment and the outside world. Increasingly it is recognized the there is strong active coupling between mechanical properties and cellular functions in properties such as locomotion and adhesion and in cytoskeletal diseases such as muscular dystrophy.@footnote1@ There is therefore an urgent need to understand the mechanical properties of cells and cellular subcomponents at length scales << 1µm. We will describe our initial experiments to achieve this goal. We have used three different imaging techniques in our investigation of the nanomechanical properties of larynx cells. First, immunofluorescent labelling was used to permit visualization of specific cell components in the confocal microscope, for example to determine whether the cell nucleus was removed in a shearing process. The same cell was then imaged in the atomic force microscope (AFM), permitting identification of components involved in motion such as microspikes. The nanomechanical properties of cells were then studied by nanoindentation using the interfacial force microscope (IFM).@footnote2@ While we have not yet succeeded in imaging and measuring the same cell used in the confocal and atomic force microscopies, we have demonstrated the feasibility of our approach and have obtained quantitative force-distance curves on different regions of a single cell fixed in paraformaldehyde, sodium periodate and lysine, which cross-links the proteins. From these data we can derive the elastic modulus, hardness etc of the specific region of the cell. The modulus of such a cell was ~ 3GPa, comparable to a soft polymer. Similar measurements are planned on unfixed cells. @FootnoteText@

@footnote1@Chen, C.S., et al. Science 276, 1425 (1997) @footnote2@Warren, O.L., et al. Physics in Canada 54, 122 (1998)

4:20pm **BI+AS+MM+NS+SS-TuA8 Unbinding Force of NTA-M@super 2+@--Histidine Complexes. The His-Tag Immobilization Force***, J.G. Forbes, P. Yim,* University of Maryland, College Park

A sequence of six or more histidines will bind tightly to a Cu, Ni, or Co complex. The compound typically used to immobilized the metal is N-(5-amino-1-carboxypentyl)iminodiacetic acid (NTA). Most proteins will not bind to the complex unless there is a sequence of histidines, which is readily added using recombinant DNA techniques. The histidine tag may be removed from the metal complex with a high concentration of imidazole or by protonating the histidines at a pH below 6. We have studied the the unbinding strength of this interaction with the atomic force microscope (AFM). To perform this measurement, we have functionalized silicon nitride AFM tips with NTA-M@super 2+@. A glass slide was coated with recombinant DNAse I with a his-tag on the C-terminus. Unbinding force measurements were made in phosphate buffered saline (PBS) to reduce electrostatic interactions. We find that the unbinding force for the NTA-M@super 2+@/His-tag interaction to be ca. 85~pN for each of the metal complexes. Interestingly, 0.5~M imidazole does not remove the interaction, but only changes the distribution of the measured forces. This is a result of the non-equilibrium condition of the tip being forced into the protein coated surface. The interaction is almost completely removed by lowering the pH to 5.0 where the histidines are protonated and can no longer coordinate with the nickel. The remaining interaction forces are due to the histidines which are exposed when the tip presses into the surface. These results provide a quantitative measurement of mechanical strength of binding of proteins to surfaces functionalized with NTA-M@super 2+@.

4:40pm **BI+AS+MM+NS+SS-TuA9 Sieving of DNA Molecules in Nanofluidic Channel***, J. Han, H.G. Craighead,* Cornell University

Entropic trapping and sieving effect of long DNA molecules was studied in variable thickness nanofluidic channels. We used photolithography and etching techniques to define fluid channels on Si wafers, and anodic bonding method to seal the channel with a thin pyrex glass coverslip. The channel consists of alternating regions with two different channel thicknesses(~100nm and 1.6μm). We studied electrophoretic motion of lambda phage DNA in this channel by epi-fluorescence microscopy. Since the radius of gyration of a typical long DNA molecule is larger than the smaller gap of the channel, the shallow part of the channel can be an entropic barrier for DNA motion.Therefore, DNA molecules were retarded when they entered into the thin region from the thick region. We measured the mobility of DNA molecules in these channels and observed that below a certain electric field, mobility of DNA molecule decreased to near zero drastically, showing that DNA molecules be entropically trapped and sieved. The threshold electric field was mainly dependent on the geometry of channel(e.g. gap size) and the length of DNA driven. This suggests a new type of separation device for DNA and other polymers.

5:00pm **BI+AS+MM+NS+SS-TuA10 Detection of Molecular Ion and Quantification of Pentapeptide on Plasma Hydroxylated Fluoropolymer by Time of Flight Secondary Ion Mass Spectrometry***, J.A. Gardella, L.M. Sun,* State University of New York, Buffalo

Abstract: Poly(hexafluoropropylene-co-tetrafluoroethylene) (FEP) was modified by a hydrogen/methanol radio frequency glow discharge plasma. Time of Flight Secondary Ion Mass Spectrometry (TOF-SIMS) was employed to characterize the modified FEP surface and three pentapetides (YGGFM, YGGFL, YIGSR) which were microsyringe deposited on the modified FEP film. New fragments of OH (CF@sub 2@)n in negative ion SIMS of the modified FEP film indicated that -OH functional group had been incorporated on the FEP surface after plasma treatment. In the positive ion SIMS of three pentapeptides on the hydroxylated FEP film, protonated molecular ions were dominant signals from the peptides whereas not many fragments were observed either from the peptides or the impurity. Sodium and potassium adduct molecular ions were detected as well as oxidized protonated molecular ion of YGGFM in the positive ion SIMS spectrum. Negative ion SIMS of YGGFL yielded a deprotonated molecular ion. The mixture of these three pentapetides was also studied by TOF-SIMS. The relative intensity of protonated molecular ions of YGGFL, YGGFM and YIGSR showed the possibility of quantification on the hydroxylated fluoropolymer by TOF-SIMS. As a study of substrate effects, TOF-SIMS spectra of these peptides on oxidized Ag substrate were recorded. Comparing SIMS results of pentapetides on Ag and on modified FEP film, fewer fragments occurred from the FEP film than that from the Ag substrate. A substrate like the FEP fluoropolymer might be beneficial for the quantification of peptides because of the intensity of parentlike species in SIMS measurement.

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