Biomaterial Interfaces Group
Room 613/614 - Session BI-WeA

Biology at the Nanoscale
Moderator: H.G. Craighead, Cornell University

2:00pm **BI-WeA1 Forces and Mechanisms of Self-Assembly: Non-Equilibrium and Transient Effects**, ***J.N. Israelachvili***, University of California, Santa Barbara, US **INVITED**

Recent experimental and theoretical work has provided new insights into the intermolecular forces and mechanisms involved in the self-assembly of biological structures such as protein complexes, vesicle aggregates and structured biological materials. It appears that many biological structures can only be formed sequentially in space and time, whereby different interactions and processes occur in different regions of space and at different times. The final structure or 'state' is therefore not the thermodynamically equilibrium state, but a 'transient' structure that nevertheless performs its allotted function at optimum efficiency - be it the opening of an adhesion site or conduction pore (with a lifetime of microseconds or milliseconds), a site-specific binding protein (with a lifetime of milliseconds or seconds), a drug-delivery carrier (with a lifetime of days), or a biomaterial with a lifetime of years. Examples will be given to illustrate the generality of such systems, and the implications of non-equilibrium and transient effects to self-assembly in vivo and in vitro will be discussed.

2:40pm **BI-WeA3 Pulling Protein Networks in 2D**, ***G. Baneyx***, *V. Vogel,* University of Washington

Fibronectin (Fn), an adhesion protein with multiple recognition sites, mediates cell attachment to synthetic and biological surfaces. In solution, Fn 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, Fn assembles into detergent insoluble fibers on the surface of cells, and these matrices are considered to be the main functional form of the protein. The cell-mediated assembly of Fn into fibrillar matrices is a complex, multistep process that is still incompletely understood. This is due to the chemical complexity of the extracellular matrix, as well as a lack of experimental control over the molecular interactions and dynamic events. We will discuss how Fn fibril assembly into extended two-dimensional networks can be induced by adsorbing Fn from a physiological buffer solution to a dipalmitoylphosphatidylcholine (DPPC) monolayer. A sequential model for the assembly pathway is proposed. Striking similarities are found between the properties of these Fn fibrils assembled underneath DPPC monolayers and those found on cellular surfaces, as well as between the respective sequential assembly pathways. Spontaneous Fn fibril assembly underneath DPPC monolayers can now serve as a well controlled model system to study how different parameters such as the deletion of key Fn sequences, as well as alteration of solution conditions and the presence of other proteins, affect the molecular assembly pathway.

3:00pm **BI-WeA4 Separation of Long DNA in a Microfabricated Channel with Submicron Constrictions**, ***J. Han***, *H.G. Craighead,* Cornell University

Microfabricated fluid channels with submicron constrictions were used to separate long DNA molecules according to their sizes. The channels were fabricated on Si substrate by photolithography and reactive ion etching techniques, followed by an anodic bonding to make a sealed channel. These channels have alternating thick and thin regions, which allows long DNA molecules to relax during the electrophoretic motion.@footnote 1@ Separation was achieved by the difference in the probability for DNA molecules to escape these 'entropic traps'. Many fluorescence-labeled DNA molecules were collected at the first entropic trap, and launched simultaneously to form a band of DNA molecules. After traveling the channel, each DNA species formed a separated band, due to the mobility difference in the channel. The traveling time of the DNA bands through the channel were measured by monitoring the fluorescence intensity at the end of the channel. Several long DNA species (35~160kbps) were separated as bands in this way, typically within 30 minutes, which is significantly lower than pulsed field methods. Several structural parameters, such as the depth or the length of the thin and thick region, were varied to study the effect on the DNA mobility and the length range of molecules that a given device can separate. Once these relevant parameters are characterized, this device could be a fast way to separate DNA molecules and other polymers. @FootnoteText@ @footnote 1@ J. Han and H. G. Craighead, J. Vac. Sci. Tech. A, in publication (1999)

3:20pm **BI-WeA5 Engineered Nanostructures to Control Microtubule Motion Along Kinesin Tracks**, ***J.S. Clemmens***, *J.R. Dennis, J. Howard, V. Vogel,* University of Washington

Motor proteins such as kinesin have evolved to transport molecules over long distances along microtubules within cells. The objective of this study is to engineer molecular tracks of motor proteins to direct the motion of microtubules on nanoengineered synthetic surfaces. We have demonstrated that kinesin moves microtubules parallel to nanoscale ridges of shear-deposited poly(tetrafluoroethylene) (PTFE) films,@footnote 1@ presumably due to preferential adsorption of kinesin along specific topographical features. Additionally, we have observed that other proteins adsorb similarly to shear-deposited PTFE films. We aim to elucidate the molecular mechanisms of these phenomena in order to delineate design principles for engineering tracks of kinesin. To accomplish this aim, surfaces have been fabricated with well-defined nanoscale pits and grooves and systematically tested for their ability to preferentially adsorb proteins or motors from solution. Once important topographical features are identified, tracks following specified paths can be engineered. This is the first step in making molecular shuttles that can move, load, and unload cargo between user-controlled locations and against concentration gradients. In the future molecular shuttles may form the basis of transporting molecular cargo through synthetic matrices. @FootnoteText@ @footnote 1@Dennis, JR et al. "Molecular shuttles: directed motion of microtubules along kinesin tracks" Nanotechnology, in press. (1999)

3:40pm **BI-WeA6 Force and Compliance Spectroscopy of Single Peptide Molecule**, *M.A. Lantz, S.P. Jarvis,* ***H. Tokumoto***, JRCAT, Japan*; T. Martynski, T. Kusumi, C. Nakamura, J. Miyake,* NAIR, Japan

An exciting application of AFM to biology is to measure forces required to stretch and unfold individual molecule. This technique looks very promising for studying molecular structure. However, this work has been applied so far to large proteins with complex structures resulting from a variety of bonding mechanisms. This complexity makes the interpretation of the experimental results difficult. Hydrogen bonding plays a major role in the formation of the secondary and tertiary structures of polypeptides from which proteins are composed. Even though, the detailed energy landscapes involved in the formation of these structures are not well understood. Here we demonstrate a new experimental technique for performing single molecule AFM force spectroscopy on significantly smaller molecules than those previously reported. We have used this technique to study the mechanical properties of the synthetic peptide cystein3-lysine30-cystein, which we designed specifically to study hydrogen bonding. Under the experimental conditions used, the peptide adopts the a-helix structure as a result of hydrogen bonding within the molecule. Force-displacement experiments were used to measure the force (approximately 200 pN) required to stretch single peptides from the helical state into a linear chain and the measured force versus peptide elongation was used to calculate the work done in breaking the hydrogen bonds. The average experimental value of the hydrogen bond energy (20.2 kJ/mol) is in good agreement with reported theoretical calculations. In addition, we directly measured the stiffness of the molecule during elongation and found to vary from approximately 0.005 to 0.012 N/m.

4:00pm **BI-WeA7 Single Molecule Force Spectroscopy by AFM, Nanomechanics Meets Molecular Biology**, *K. Tolksdorf, M. Grandbois, M. Rief, H. Clausen-Schaumann,* ***H.E. Gaub***, Ludwig-Maximilians-Universität München, Germany **INVITED**

Recent developments in AFM-instrumentation allow the manipulation of single molecules and measurements of intermolecular as well as intramolecular forces. We took advantage of the high spatial resolution of the AFM and developed mechanical experiments with single macromolecules. An overview on this novel kind of spectroscopy will be given and applications in the field of polymer and life sciences will be highlighted: receptor ligand interactions were measured in single molecular pairs. Lateral distributions of interaction partners on samples were mapped in a chemical imaging mode. The length distribution of individual grafted polymers at surfaces was imaged. Individual polymers and proteins that were anchored on a gold surface were picked up with the AFM tip and stretched, their viscolelasticity and yield strength was measured. Proteins were reversibly unfolded and the conformation forces were determined at the level of single secondary structure elements. DNA double strands were stretched and unzipped. A model was developed based on elastically coupled two-level systems that allows the description of basic features of

the experimental results. @FootnoteText@ Rief, M.; Oesterhelt, F.; Heymann, B.; Gaub, H. E.Science 1997, 275, 1295-1298. Rief, M.; Gautel, M.; Oesterhelt, F.; Fernandez, J. M.; Gaub, H. E. Science 1997, 276, 1109-1112. M. Rief, H. Clausen-Schaumann , H.E. Gaub, Nature Struct. Biol. (1999), in press M. Grandbois, M. Beyer, M. Rief, H. Clausen-Schaumann ,H.E. Gaub, (1999),Science in press

4:40pm **BI-WeA9 Characterization of Antibody-Antigen Interaction Forces With Magnetic Tweezers**, *S. Metzger,* Geocenters*; M. Stevens,* Naval Research Laboratory*; G. Sagvolden,* University of Oslo, Norway*; C. Yanavich,* Nova Research*; M. Natesan,* GeoCenters*;* ***G.U. Lee****,* Naval Research Laboratory

Living systems have developed the capacity to use molecular interactions to control structure and function. In order to understand the molecular mechanism of these interactions, the force between and within individual molecules has been directly measured using microscopic techniques such as AFM and optical tweezers. We describe a technique for directly measuring intermolecular interactions using magnetic force. This technique allows intermolecular forces to be studied over an extended range of force (10@super-15@ to 10@super-15@ Newton) and time (1 ms to 10 sec). We will demonstrate the principles of this technique by measuring the force and time required to rupture antibody-antigen bonds.

5:00pm **BI-WeA10 Probing the Nano-environments of Peptides on Solid Surfaces by Advanced Secondary Ion Mass Spectrometry***, T. Schenkel,* Lawrence Livermore National Laboratory*; K.J. Wu,* Charles Evans & Associates*; A.V. Barnes, M.W. Newman, J.W. McDonald, A.V. Hamza,* Lawrence Livermore National Laboratory

The interaction and bonding of peptides and proteins in the solid phase and on solid surfaces is of central importance in biotechnological research. Embedding of analyte molecules in special matrix solutions (such as 2,5 dihydroxybenzoic acid) has been shown to produce enormous increases in yields of intact molecules both for laser and single charged ion induced ablation. The mechanisms responsible for this enhancement are however not well understood. Both the binding of matrix and analyte molecules in the solid and collisional ion formation processes have been suggested to play important roles. We have investigated the effect of sodium and potassium impurities on secondary ion emission from gramicidin S by time-of-flight secondary ion mass spectrometry (TOF-SIMS) with highly charged projectiles.@footnote 1@ Highly charged ions like Xe@super44+@ or Au@super69+@ increase secondary ion yields by over two orders of magnitude as compared to singly charged ions. Each highly charged projectile emits secondaries from an area of only about 10 nm^2. Analysis of coincidences among secondary ions detected following the impact of a single projectiles allows for the characterization of the nano-environment of e. g. a peptide molecule in a matrix solution. For the gramicidin S, we found that emission of Na@super+@ and K@super+@ ions was strongly correlated with emission of sodium and potassium adduct ions, [M+Na]@super+@, [M+K]@super+@. This correlation indicates the nestling of sodium impurities around peptide molecules. In our presentation we will discuss the potential of coincidence analysis in TOF-SIMS for the probing of nano-environments on surfaces of biomaterials. @FootnoteText@@footnote 1@A. V. Hamza et al., J. Vac. Sc. Technol. A 17, 303 (1999) Acknowledgement: This work was performed under the auspices of the U. S. Department of Energy by Lawrence Livermore National Laboratory under contract No. W-7405-ENG-48.

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