Thursday Morning, October 5, 2000

Biomaterial Interfaces
Room 202 - Session BI+NS-ThM

Nanoscale Biology
Moderator: J.J. Hickman, Clemson University

8:20am **BI+NS-ThM1 Engineering Life into Nanofabricated Systems*, C.D. Montemagno,*** Cornell University **INVITED**

Scientists and engineers have anticipated the potential benefits of integrating engineered devices to living systems at the molecular level for many years. Hybrid systems can potentially possess many of the essential properties of life such as the abilities to "intelligently" self-assemble, repair, and evolve. We will present the results of our efforts to incorporate biological energy transduction processes and cell signaling pathways into engineered nanofabricated devices. In particular, we will illustrate our strategy for fueling, controlling and integrating a F1-ATPase biomolecular motor with a NEMS to create an engineered hybrid device. Included in the presentation will be the initial results of our efforts to develop and demonstrate an integrated F1-ATPase powered NEMS device that is fueled by light-driven ATP production. ATP is synthesized from light using artificial liposomes comprised of reconstituted FoF1-ATP synthase and bacteriorhodopsin. Subsequently, the ATP provides energy to power a recombinant, thermostable F1-ATPase biomolecular motor that is coupled to a NEMS device. We will also present our technique for integrating nanomechanical structures to biomolecular motors with a precision d 40 nm. This work capitalizes on a core feature of living systems: the capability of transforming diverse sources of energy into a generic energy currency that can be universally used. The integration of a synthetic photosynthetic system with NEMS establishes a new mechanism for fueling the next generation of nanoelectromechanical devices. Light is used to produce ATP from ADP and P, the ATP is used by the F1-ATPase biomolecular motor to produce work with ADP and P as waste products. Ultimately, we anticipate that this chemically closed system will be used to pump fluids, open and close microvalves, provide locomotion, generate electricity, and make way for "Smart Dust" applications such as long-lived microscopic intelligence and environmental sensors.

9:00am **BI+NS-ThM3 Powering Molecular Shuttles through an Artificial Photosynthetic System*, V. Vogel, H. Hess,*** University of Washington*; K. Jardine,* Arizona State University*; J. Clemmens,* University of Washington*; T.A. Moore, A.L. Moore, A. Primak,* Arizona State University*; J. Howard,* University of Washington*; D. Gust,* Arizona State University

The ultimate goal for bioengineers is to be able to engineer systems on a nanoscale as perfect as nature does in cells. Great progress has been made in recent years in biochemistry and biophysics, supplying us with information about the construction principles as well as the details of many cellular subsystems. This information is matched by recent advances in nanotechnology, allowing control of the arrangement of biomolecules on a sub-micron scale. From an engineering point of view the construction of artificial systems, performing different tasks related to the cellular environment, becomes possible. Examples of this approach are the construction of artificial photosystems,@footnote 1,2@ consisting of vesicles doped with antenna molecules, proton pumps and the enzyme ATPase, and the construction of "molecular shuttles",@footnote 3,4@ microtubules moved by motor proteins on a patterned surface. The arising challenge is to combine these subsystems into a larger, more complex system with extended functionality. Here we present a proof-of-principle experiment demonstrating the integration of a transport systems (the "molecular shuttles") with a system providing chemical energy from light (the above mentioned artificial photosystem). In the integrated system we can therefore nonintrusively control the motion of the microtubules through light. The experimental setup consists of a flow cell mounted on an epi-fluorescence optical microscope and illuminated by a laser diode. The surface of the flow cell was patterned with parallel grooves spaced between 30 nm and 1 um apart by shear-deposition of a teflon film@footnote 5@. The motor protein kinesin@footnote 6@ adsorbed preferentially along the grooves providing "tracks" for the motion of the microtubules. The microtubules were fluorescently labeled and bound to the motor proteins in the absence of ATP. The ATP-generating vesicles floated freely in the buffer solution. Illumination of the sample with light absorbed by the vesicles as followed by motion of the microtubules. The motion was mainly directed along the direction of shear of the underlying teflon film. This experiment thus demonstrated that in an integrated system, multiple self-assembled entities cooperate functionally all the way from light harvesting through charge separation across a lipid membrane and ATP-synthesis driven by a proton gradient to ATP-fueled conformational changes of kinesin leading to directed motion of microtubules on uniaxially aligned kinesin tracks. @FootnoteText@ @footnote 1@ Gust, D., T.A. Moore, and A.L. Moore, Mimicking bacterial photosynthesis. Pure & Appl. Chem., 1998. 70(11): p. 2189-2200. @footnote 2@ Steinberg-Yfrach, G., et al., Light-driven production of ATP catalysed by F0F1-ATP synthase in an artificial photosynthetic membrane. Nature, 1998. 392(6675): p. 479-82. @footnote 3@ Dennis, J.R., J. Howard, and V. Vogel, Molecular shuttles: directing the motion of microtubules on nanoscale kinesin tracks. Nanotechnology, 1999: p. 232-236. @footnote 4@ Service, R.F., Borrowing from biology to power the petite: nanotechnology researchers are harvesting molecular motors from cells in hopes of using them to drive nano-scale devices. Science, 1999. 283: p. 27-28. @footnote 5@ Wittmann, J.C. and P. Smith, Highly oriented thin films of poly(tetrafluoroethylene) as a substrate for oriented growth of materials. Nature, 1991. 352: p. 414-417. @footnote 6@ Howard, J., A.J. Hudspeth, and R.D. Vale, Movement of microtubules by single kinesin molecules. Nature, 1989. 342: p. 154-158.

9:20am **BI+NS-ThM4 Unbinding Process of Adsorbed Proteins under External Stress Studied by AFM Force Spectroscopy*, C. Gergely, J. Voegel,*** INSERM, France*; P. Schaaf,* Institut Charles Sadron (CNRS) Strasbourg, France*; B. Senger,* INSERM, France*; J.K.H. Horber,* EMBL Heidelberg, Germany*; J. Hemmerle,* INSERM, France

We report the study of the unbinding process under a force load f of adsorbed proteins (fibrinogen) on a solid surface (hydrophilic silica) by means of AFM force spectroscopy. By varying the loading rate r, defined by f=r.t, t being the time, we find that, as for specific interactions, the mean rupture force increases with r. This unbinding process is analysed in the framework of the widely used Bell model. Thus typical dissociation rate at zero force entering in the model lies between 0.02 and 0.6 1/s. Each measured rupture is characterized by a force f0 which appears to be quantized in integer multiples of 180-200 pN.

9:40am **BI+NS-ThM5 Single-Molecule Protein-Ligand Bond-Rupture Forces Measured Using the Poisson Atomic Force Method*, Y.-S. Lo, Y.J. Zhu, J.D. McBride, T.P. Beebe, Jr.,*** University of Utah **INVITED**

It is known that bond strength is a dynamic property that is dependent upon the force loading rate applied during the rupturing of a bond. For biotin-avidin and biotin-streptavidin systems, dynamic force spectra, which are plots of bond strength vs. ln(loading rate), have been acquired in a recent biomembrane force probe (BFP) study [Merkel et al., Nature 397 (1999) 50] at force loading rates in the range of 0.05 to 60,000 pN/s. In the present study, the dynamic force spectrum of the biotin-streptavidin bond strength in solution was extended from loading rates of ~10@super 5@ to ~10@super 9@ pN/s with the atomic force microscope (AFM). The Poisson AFM statistical analysis method was applied to extract the magnitude of individual bond-rupture forces and non-specific long-range interactions from the AFM force-distance curve measurements. In addition, surface characterization methods for the analysis of protein-coated surfaces and AFM tips, both imaging and spectroscopic x-ray photoelectron spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (TOF-SIMS) will be discussed. The AFM bond strengths were found to scale linearly with the logarithm of the loading rate in two regimes with two different slopes, consistent with the view that multiple energy barriers are present along the unbinding coordinate of the biotin-streptavidin complex. In contrast, the non-specific interactions, which can be separately measured and characterized apart from the specific bond-rupture forces in this method, did not exhibit a measurable dependence on loading rate. The dynamic force spectra acquired here with the AFM combined well with BFP measurements by others, and demonstrated that unbinding forces measured by different techniques are in agreement and can be used together to obtain a dynamic force spectrum covering 11 orders of magnitude in loading rate.

10:40am **BI+NS-ThM8 Measuring the Mechanical Properties of Soft Samples by Atomic Force Microscopy*, M. Radmacher,*** Universit@um a@t G@um o@ttingen, Germany **INVITED**

The Atomic Force Microscope combines in a very unique way a very high sensitivity in detecting and applying forces (of up to a few 10's of piconewton), a high accuracy in positioning a sharp tip relativ to the sample in all three dimensions (of up to a few Angstrom), and the possibility to be operated under physiological conditions. This combination allows experiments not possible before, particularly in the field of biophysics and soft materials. One example is the mapping of mechanical properties with high spatial resolution of polymeric films and living cells. In living cells it is

possible to probe the mechanics during dynamic processes like cell migration and cell division.

11:20am **BI+NS-ThM10 Protein Adsorption and Monocyte Activation on Ge Nanopyramids***, **B. M@um u@ller,*** ETH Z@um u@rich, Switzerland*; M. Riedel,* ProBioGen, Germany*; R. Hofer, E. Wintermantel,* ETH Z@um u@rich, Switzerland

The performance of an implant material depends crucially on its surface architecture or morphology. The significance of topographic features with micrometer size on cell shape and function has been clearly demonstrated. The power of features on the nanometer scale is still under discussion. In order to get an insight into the responds of monocytes onto a well-defined substrate nanostructure, we have grown germanium nanopyramids on Si(100) in a natural way by epitaxial growth, i.e. without any lithographic technique. The density of the pyramids (hut and dome cluster) is adjusted varying the substrate temperature during deposition. The morphology of the oxidized pyramids is quantified by ex situ atomic force microscopy. To characterize the nanostructure roughness further, contact angles of water under dynamic conditions are measured in comparison with the bare Si wafer and flat Ge films on Si. The receding angles show a significant increase with pyramid density. The amount of the selected proteins g-globulin and albumin adsorbed on the nanostructures is determined spectroscopically with labeled proteins. It raises with pyramid density. The impact of nanostructuring on the biological activity of adsorbed g-globulin is addressed by immunosorption with an anti-g-globulin antibody. These data reveal that the amount of active g-globulin does not scale with the adsorbed one. Nanoscale roughness even decreases the activity. The in vitro assays with monocytes that allow studying specific aspects of inflammatory reactions of the body - an important aspect of the biocompatibility, are based on the monocyte-like cell line U937. After 5 days in vitro, the cell performance is characterized microscopically and by the secreted cytokines IL-1b, IL-1ra and TNF-a. For the CVD grown samples, a roughness increase leads to reduced cytokine expression. Consequently, implants with nanoscale roughness gives rise to less inflammatory reactions.

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