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

Characterization of Biomaterial Interfaces

Moderator: M. Grunze, University of Heidelberg

2:00pm **BI-TuA1 Surface Characterization of Biomaterials with Protein Layers**, ***H.J. Griesser***, CSIRO Australia, Australia **INVITED**

In the fabrication and interfacial analysis of novel biomaterials and their biological interactions, vacuum-based methods occupy a prominent role. Much research centers on the fabrication of "hybrid" biomaterials, which comprise a synthetic carrier material and an immobilized layer of biologically active molecules. Low temperature gas plasma methods are well suited to the attachment of reactive chemical groups onto polymers. Alternatively, plasma polymer interlayers can be used to provide reactive surface groups for the covalent interfacial immobilization of proteins. Vacuum-based surface analysis techniques characterize the surface properties of a material and increasingly are applied to the study of interfacial interactions with biological molecules. In this talk I will present examples of recent work on the immobilization of proteins and synthetic peptides on polymers via plasma polymer interfacial bonding layers. Detailed, multitechnique characterization of surface derivatizations and protein immobilizations is essential since proteins can adsorb and thereby mimic an intended covalent immobilization. Intended attachments are first modelled using derivatization reactions, and the surface density of reactive groups is thus determined. MALDI mass spectrometry is uniquely suited to the detection of adsorbed biomolecules at amounts much below monolayer coverage, and this method has been used to distinguish between covalent and adsorptive immobilizations. MALDI-MS is also eminently suited to the study of which proteins adsorb from complex, multicomponent media. For instance, the ways in which different surface chemistries of contact lenses influence which proteins adsorb onto lenses worn by human volunteers, has been characterized by MALDI-MS, and this information is being used for the guided design of improved coatings. Finally, I will discuss how AFM in the force mode provides complementary information to vacuum-based analysis methods.

2:40pm **BI-TuA3 Surface-plasmon Field-enhanced Fluorescence Spectroscopy and -Microscopy for the Evaluation of the Hybridization Reaction of Oligonucleotides**, ***W. Knoll***, Max-Planck-Institut für Polymerforschung, Germany and Stanford Univ., Germany; *D. Kambhampati, T. Liebermann, T. Neumann,* Max-Planck-Institut für Polymerforschung, Germany

Surface plasmon spectroscopy (SPS) is widely used as a surface-sensitive technique to characterize thin film architectures, or to monitor kinetic processes like biorecognition and binding events or photo-reactions in these layers. We describe an extension of the method combining the field-enhancements obtainable at the resonant excitation of surface plasmons with fluorescence detection schemes. Controlling the balance between the evanescent character of the surface mode and the energy (Förster) transfer between the chromophores and (the acceptor states of) the metal substrate sensitivity enhancements of more than 2 orders of magnitude compared to SPS can be achieved (though not label-free). We demonstrate the potential of this mode of operation for the quantitative evaluation of hybridization reactions between surface-immobilized probe oligonucleotides (15-mers) and complements from solution. It is shown that a simple Langmuir adsorption/desorption model describes the experimental results. Single base mismatches can account for a decrease in the equilibrium constant by two orders of magnitude, a second mismatch can give a reduction by another 3 orders. A further extension that will be introduced is the simultaneous observation of several hybridization/dehybridization reactions on a 3 x 3 matrix of 9 different sensor spots by fluorescence microscopy.

3:00pm **BI-TuA4 Characterization of Supported Biomimetic Films Using Broadband Vibrationally Resonant Sum-Frequency Generation**, ***K.A. Briggman***, *T. Petralli-Mallow, L.J. Richter, A.L. Plant, J.C. Stephenson,* National Institute of Standards and Technology

Supported organic films have received considerable attention as model biological membranes, as well as biomolecular templates for the development of biomimetic devices. A complete characterization of these biomimetic films requires the application of in-situ techniques, capable of probing fully hydrated systems. We have been exploring the potential of broadband vibrationally resonant sum frequency generation (SFG) as an in-situ probe for the study of hybrid bilayer membranes (HBMs). Our novel broadband approach@footnote 1@ provides a complete SFG spectrum over a window of several hundred wavenumbers, combining interface sensitivity and molecular specificity with the advantages of short acquisition times and no need for wavelength tuning. We have acquired vibrational SFG spectra of a variety of supported biomolecular compounds, including phospholipids in HBMs. A discussion of the preparation and stability of the HBMs as examined by SFG will be presented. @FootnoteText@ @footnote 1@Vibrationally resolved sum-frequency generation with broad-bandwidth infrared pulses, Opt. Lett. 23 1594 (1998).

3:20pm **BI-TuA5 Biosensors in Biomaterials Research**, ***K.I. Lundström***, Linköpings Universitet, Sweden **INVITED**

There are several surface physical tools, which are used to study the interaction between biomaterials and tissue. Many of them require special sample preparation and can not be used to follow the kinetics of interaction at the biomaterial surface. They can thus not be considered for in vivo applications either. Biosensor technologies developed for the monitoring of biomolecular interactions and utilizing (surface) physical phenomena as the detection principle should, however, be suitable for kinetic studies both in vivo ant in vitro. In this contribution some of the biosensing technologies for biomolecular interactions with and at surfaces are described, with special attention to surface plasmon resonance instrumentation and quartz crystal microbalances. It is concluded that by modifying the surface of such biosensors it is possible to study several important phenomena related to biomaterials and biocompability. Furthermore it is concluded that biosensors can be used also to monitor parameters outside the biomaterial itself, such as coagulation factors, inflammatory mediators etc. Biosensors for in vivo studies of biomaterials are also touched upon. The present use of biosensors for biomaterials related research is reviewed. A few examples from studies of e.g. plasminogen bleeding surfaces, complement activation and blood coagulation at surfaces are given. Finally some future possibilities of surface oriented biosensors for biomaterial research are speculated upon. This includes for the elucidation of the behavior of (single) cells adsorbed on or interacting with biomaterial surfaces.

4:00pm **BI-TuA7 Direct Probing of the Surface Ultrastructure and Molecular Interactions of Living Microbial Cells with Atomic Force Microscopy**, ***Y.F. Dufrene***, *C.J.P. Boonaert, P.G. Rouxhet,* Universite Catholique de Louvain, Belgium

Understanding biointerfacial phenomena such as cell aggregation and cell adhesion requires knowledge of the surface structure and physico-chemical properties of living cells with a nanometer scale resolution. In this work, atomic force microscopy (AFM) was used to determine, in physiological conditions, the ultrastructure and molecular interactions at the surface of living spores of Phanerochaete chrysosporium and their changes during germination. Cell immobilization was achieved by mechanical trapping in porous membranes. High-resolution images recorded on dormant spores showed that the surface was uniformly covered with a regular pattern of rodlets. These structures were several hundreds nm in length and had a periodicity of about 10 nm, in excellent agreement with freeze-etching characterization. Force-distance curves recorded between a silicon nitride probe and the spore surface showed no adhesion forces upon retraction. Dramatic changes of cell surface ultrastructure and molecular interactions occurred during germination. Germinating spores had a very smooth surface, partially covered with granular structures which were the residues of the rodlet layer. Force-distance curves recorded on smooth areas showed strong adhesion forces. These are attributed to binding of polysaccharides, which have been detected by X-ray photoelectron spectroscopy (XPS) and considered to be responsible for spore aggregation. The approach presented here offers new possibilities for probing the local surface properties of prokaryotic, animal and plant cells in the native state.

4:20pm **BI-TuA8 Contact Mechanical Properties of Confined NIPAM Films at the Biomaterial Interfaces**, ***R. Luginbuehl***, *M.D. Garrison, Y.V. Pan, R.M. Overney, B.D. Ratner,* University of Washington

Smart polymeric materials, which change their structural properties upon stimulation, are of highest interest for industrial applications in surface coating and printing, sensor technology, biotechnology, medicine, and biomaterial research. Progress in precision engineered surfaces for biosensor applications strongly depend on appropriate techniques to analyze surfaces at the micro and nanometer level. Recently, considerable research effort has focused on the investigation of co-polymers and grafted polymers containing N-isopropylacrylamide (NIPAM). These polymers can

be engineered to undergo thermally induced structural and mechanical phase transitions around 32 ÂºC, which is drawn by hydrophobic forces and hydrogen bonding. The structural phase transition is accompanied by a change in volume, and therefore a change in mechanical properties, as well as a change in the surface free energy. We carried out scanning force microscope (SFM) investigations on surface confined NIPAM films. Thin films (thickness < 10 nm) were obtained by polymerization on selected substrates. Novel SFM techniques permit the observation of the transition behavior at the nanometer scale. Photolithographically patterned thin films were used to isolate changes in the polymer structure relative to a reference substrate. Contact mechanical properties, volume transition, and the interfacial energy were monitored as a function of the system temperature. The introduced SFM technique offers a unique combination of microscopy with spectroscopic analysis of surface interactions and local subsurface structural properties.

4:40pm **BI-TuA9 Novel Biomaterials through Tailoring of Solid Surfaces***, **J. Rühe,*** Max-Planck-Institute for Polymer Research, Germany

The modification of materials by monolayers of polymers, which are covalently attached to the surface of the substrate, is a very attractive way to improve the properties of solids in bio-oriented applications. We describe several new pathways for the synthesis of surface-attached ultrathin polymer films, which carry functional groups relevant for biological or biomedical applications. The polymer molecules are either grown at the surface of the substrate in situ by using self-assembled monolayers of initiators or preformed polymers are (photo-)chemically attached to the material, which is to be modified. Additionally, the formation of ultrathin, patterned networks of functional polymers will be described. Examples for groups contained in the monolayers are peptide moieties, which could act as cell recognition sites and DNA fragments for biochip applications. The characterization of the monolayers, especially the swelling of the layers in an aqueous environment, will be described.

5:00pm **BI-TuA10 Photoisomerization and Photo-induced Alignment of Ordered Polymer Ultrathin Films Containing DNA and Polypeptide Layers: Possibilities for Optobioelectronic Substrates***, **R.C. Advincula,*** University of Alabama at Birmingham, US*; Y. Wang, E. Fells, E. Wallace,* University of Alabama at Birmingham

Alternate polyelectrolyte deposition (APD) is a relatively new technique for fabricating multilayer ultrathin polymer films. Since the polymers are adsorbed from solution, it opens up the possibility for incorporating biological macromolecules such as proteins, or nucleic acids in the active site that may be interesting for biosensing or biocompatibility issues. In the case of DNA molecules, selectivity arises from the interaction with various specific DNA reagents such as intercalators and DNA-complementary (hybridization) interactions. The multilayer complex films are also good model systems to investigate interaction between polynucleotides and polypeptides. The advantage of ultrathin film geometries in substrate supported systems is that they allow direct structural analysis using X-ray, FT-IR, SPS, etc. on a number of substrates, e.g. ITO-, Gold- coated glass, Si, etc. In this work, we report the formation of highly ordered ultrathin films containing DNA and/ or Polylysine/ Azobenzene dye multilayers fabricated using the alternate polyelectrolyte deposition (APD) approach. An important modification is the incorporation of photoisomerizable azobenzene dyes in the films to explore the possibility of using these films for some optobioelectronic applications. The photoisomerization of the dyes were investigated with respect to thickness, irradiation parameters, pair combinations, etc. The formation of polypeptide-dye multilayer complexes resulted in photo-induced circular and linear dichroism. This was investigated using polarized UV-vis spectroscopy, ATR, and SPS configurations. The conformation of the polypeptide and the DNA was investigated by FT-IR. In-situ adsorption experiments were investigated using ellipsometry and QCM.

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