## Biomaterial Interfaces Group
## Room 613/614 - Session BI-TuM

### Protein Solid-Surface Interactions II
**Moderator:** J.L. Brash, McMaster University

8:20am **BI-TuM1 Functionality of a Model Protein at Nanostructured Surfaces**, ***D.S. Sutherland***, Chalmers and Gothenburg University, Sweden; *M. Broberg, H. Nygren,* Gothenburg University, Sweden; *B. Kasemo,* Chalmers and Gothenburg University, Sweden

Nanofabricated surfaces can be used to study the influence of surface topographic features on the behaviour of a model protein. Colloidal lithography was used with simple lift-off steps to create surfaces with defined nanotopography. The functionality of equal-quantities of fibrinogen molecules bound at surfaces containing pits of 40nm diameter or 110nm diameter was probed. The kinetics of binding of unactivated platelets to these surfaces from a static albumin-containing buffer was used as a measure of the functionality/conformation of the fibrinogen molecules. Two sets of samples were prepared, one where the surfaces were chemically homogeneous (titanium oxide) and one where the surfaces additionally contained nanodomains of gold coated in a methyl-terminated self-assembled monolayer at the base of the pits. Platelet binding on fibrinogen adsorbed at flat surfaces and surfaces with 110nm diameter pits showed relatively low binding rates which were not significantly different than that found at control surfaces (with no adsorbed fibrinogen). By contrast platelet binding on fibrinogen adsorbed at surfaces containing 40nm diameter pits gave significantly higher binding rates than both other test surfaces and control surfaces. For these samples with 40nm diameter pits similar results were obtained for both surfaces with homogeneous chemistry and for chemically nanodomained surfaces. These results are interpreted to mean that fibrinogen molecules bound at surfaces with 40nm diameter pits have altered conformation or orientation (compared to flat surfaces or surfaces with larger pits) to make available platelet-binding sites. These pits are smaller that the reported characteristic size of fibrinogen molecules (46-55nm length) and it appears that the effect is the result of topography rather than surface chemistry. These model experiments indicate that the conformation/orientation of individual protein molecules can be influenced by like-sized surface features.

8:40am **BI-TuM2 Hierarchical Ordering of Proteins at Interfaces with a Nanoscale Surface Topography**, ***V. Vogel***, *L. Smith, T. Nguyen, J.R. Dennis,* University of Washington

Elucidating mechanisms by which to control the ordering of proteins at interfaces is of fundamental importance in bioengineering and biotechnology. Whereas major progress has been made recently in stabilizing proteins at interfaces in their native states, and in controlling their orientation, much less is known how to promote their spontaneous self-assembly into a structurally well controlled supramolecular architecture. Here we discuss that nanoscale topographic surface features with ridges the size of natural ECM fibrils have a pronounced impact on protein adsorption, and on the spatial alignment of human dermal fibroblasts and cell-deposited collagen fibrils. Furthermore, by elevating cells above the surface such that they deposit collagen through a porous membrane onto the nanoscale ridges without being in physical contact with the surface, the role of the cells has been separated from the role of topography in collagen type VI deposition and fibrillogenesis. Insight into the mechanisms by which synthetic surfaces manipulate the hierarchical organization of ECM fibrils will be crucial in the rational design of the surface topography of biomaterials and of scaffolds for tissue engineering.

9:00am **BI-TuM3 Protein Adsorption on Solid Surfaces : From Static to Dynamic Properties**, ***P. Schaaf***, Institut Charles Sadron, France **INVITED**

Adsorption processes of proteins on solid surfaces have been investigated over many years but, due to their complexity, it is still difficult to predict their behavior. Indeed, proteins are highly structured polyelectrolytes, polyamphiphiles, which, in addition, are often only marginally stable. While interacting with a solid surface, they often change their structure and parallelly increase their anchoring to the surface. The influence of different parameters entering in these adsorption processes, such as the substrate charge, the substrate hydrophobicity and the protein stability, will be discussed. The dynamics of these processes will, in particular, be mentionned. It will appear that the time scales entering in adsorption processes range from tens of hours down to milliseconds. While the characteristic interaction time needed for fibrinogen, a plasma protein, to bind to a silica surface appears to be of the order of 50ms, the exchange ability of a ribonuclease molecule adsorbed on a titanium oxyde surface changes with a characteristic time of the order of 10 hours. Structural changes are observed in the adsorbed layer over similar time scales. Recent results obtained in this field, in particular by Infrared Spectroscopy and by Atomic Force Microscopy, will be presented

9:40am **BI-TuM5 Protein Adsorption Kinetics: Particle Model and Optical Experiment**, *M.A. Brusatori, C. Calonder,* ***P.R. Van Tassel***, Wayne State University

The adsorption of proteins at the liquid-solid interface is a phenomenon of great importance in bioseparations, biocatalysis, and materials biocompatibility. Adsorption is often accompanied by a surface-induced transition in either internal conformation or molecular orientation. Recently, Van Tassel, et al, presented a model in which the adsorption/transition process is modeled as the sequential surface placement of spreading disks. In this talk, we present a derivation of analytical expressions for the adsorption and spreading probabilities, whose use greatly simplyfies the form of the kinetic equations for this model, using the scaled particle theory (SPT). We also present new experimental data of fibrinogen adsorption onto SiTiO2 and dextran-coated SiTiO2 using optical waveguide lightmode spectroscopy (OWLS). We show that the SPT spreading disk model can accurately predict these and other experimental data as well as those from computer simulation.

10:00am **BI-TuM6 Probing Immobilized Protein Peptide Architectures**, ***S.J.B. Tendler***, *M.M. Stevens, W.C. Chan, M.C. Davies, C.J. Roberts, P.M. Williams, S. Allen,* University of Nottingham, U.K.

The ability to control the assembly of molecular architecture at the nanometre scale is an important research goal. Complex molecular assemblies can be designed and constructed to have applications in several bio-analytical fields, for example, as key components in devices such as biosensors and affinity-based chromatographic supports. We have demonstrated the creation of a higher order molecular assembly which consists of a bis-biotinylated peptidic spacer between two streptavidin molecules. This molecular architecture exploits the strong affinity between streptavidin and biotin to promote self-assembly. Surface plasmon resonance has enabled us to monitor the construction of the multilayer in real time. Atomic force microscopy has been utilized to measure adhesion forces between biotinylated bovine serum albumin functionalized probes and the surface at each stage of the multilayer assembly. This facilitated the determination of surface functionality and associated mechanical properties at each of these stages. An increase in the elasticity of the system was observed once the multilayer was created. It is postulated that unraveling of an alpha-helical component in the conformation of the peptide before rupture of the streptavidin-biotin link may contribute to the increase in molecular elasticity of the multilayer. We have also demonstrated through a trifluoroethanol titration monitored by circular dichroism that variations in the solvent can affect the secondary structure of the peptide linker and hence its mechanical properties. These observations have wide implications for protein immobilization in terms of the precise control of distances of active layers, steric surface barriers, underlying surface forces and hence biological functionality.

10:20am **BI-TuM7 New Platform Technology for the Investigation of Initial Interaction of Adsorption and Cross Linking of Strong Adhesives at Solid Surfaces**, ***H. Elwing***, *F. Hook,* Goteborg University, Sweden

The contacting area between an implanted biomaterial and the surrounding tissue is of critical importance for the functional success of the biomaterial. We try to develop tissue "glues" and we get our biomimic inspiration from marine organisms. Several marine animals and plants living at hard rocks, or man made material have developed successful glues or adhesives for contacting the hard surface. There must be at least two conditions fulfilled for strong adhesion of marine organisms to a flat solid surface. Firstly there must be sufficient strength of molecular adhesion at the liquid/solid interface. Secondly it is required that the adhered molecules are cross-linked at the surface and into the tissue of the organism. We have concentrated our effort to understand more about the cross-linking mechanisms. Unfortunately there are few methods available for measuring cross-linking of biopolymers in real time, which have made research difficult. Consequently, as a first step we have developed a methodological combination of surface plasmon resonance (SPR) and Quarts chrystal microbalance (QCM-D) for simplified analysis of adsorption and cross-linking of marine adhesives, such as mussel adhesive proteins and Barnacle cement, adsorbed as monolayers on flat solid surfaces.

10:40am **BI-TuM8 Molecular Orientation Distributions in Submonolayer Films Corresponding to Quasi-Reversible Electron Transfer Behavior**, *S.S. Saavedra,* ***R.T. Robertson****, N.R. Armstrong, S. Mendes,* University of Arizona

The relationship between the molecular orientation distribution and the electron transfer behavior of immobilized redox proteins has been investigated through the use of novel waveguide-based spectroelectrochemical methods. The ability to probe the electron transfer behavior which corresponds to a particular molecular orientation will provide significant insight into the fundamental electron transfer processes that occur in physiological systems. The orientation geometry is obtained using an electroactive integrated optical waveguide (EA-IOW) format coupled with electroactive total internal reflection fluorescence (EA-TIRF). Orientation distributions of horse heart cytochrome c corresponding to quasi-reversible electron transfer have been characterized and will be discussed here.

11:00am **BI-TuM9 Surface Orientation of Peptides with @alpha@-helix and @beta@-sheet Secondary Structures on Fluorocarbon Substrates**, ***L. Gamble****, J.R. Long, P.S. Stayton,* University of Washington*; D.A. Fischer,* National Institute of Standards and Technology*; D.G. Castner,* University of Washington

The orientation of surface bound proteins can have a significant effect of their function. To aid with the interpretation of Near edge X-ray absorption fine structure (NEXAFS) spectra from adsorbed protein films we haved studied short, well-defined peptide "standards." NEXAFS is a surface sensitive technique that has been used to determine the orientation of polymers and self-assembled monolayers. Here NEXAFS is used to determine the surface orientation of short peptides chains designed to adsorb in @alpha@-helical and @beta@-sheet conformations on hydrophobic surfaces. The N K-edge spectra show an orientation dependence of the N1s to @pi@* peak between the 90° and 20° incident x-ray angles for both peptides adsorbed onto highly-ordered poly(tetrafluoroethylene) (PTFE) surfaces. The results indicate that the @beta@-sheet peptide is adsorbed with the peptide backbone "parallel" to the substrate, while the @alpha@-helix adsorbes with the helical axis parallel to the substrate. Spectra of the O K-edge support these results. The lack of orientational dependence seen for these same peptides adsorbed onto a disordered fluoropolymer surface containing different types of fluorocarbon species indicates the degree of substrate order and/or the type of surface functional groups play a key role in determining the degree of ordering in the adsorbed peptides. NEXAFS spectra were also used to distinguish between the secondary structures of the two peptides. Preliminary NEXAFS results from adsorbed protein films show that orientataion of the peptide backbone is only observed for non-gobular proteins such as fibrogen and fibronectin. Gobular proteins such as albumin do not exhibit any preferrential orientation, even on highly-ordered substrates.

11:20am **BI-TuM10 A Multi-Parameter QCM Technique for Investigations of Protein and Surface Interactions**, ***F. Höök****,* Chalmers Univ. of Tech. and Göteborg Univ., Sweden*.; M. Rodahl,* Q-Sense AB, Sweden*; B. Kasemo,* Chalmers University of Technology, Sweden

Protein molecules in contact with solid, non-biological materials, is a situation of broad scientific interest and technological importance, and there is a growing need for new tools to study these interactions. For instance, if the influence from the surface is large enough, the conformational-free-energy minimum for a protein attached on a surface might correspond to a conformation that differs from that of the native protein. It is thus likely that a protein-surface interaction might affect the conformation and hence the function of the proteins. We have developed a sensor system based on the traditional quartz crystal micro balance (QCM) technique, but where both the resonant frequency (f) and the energy dissipation (D) are measured simultaneously for a non-driven (freely oscillating) sensor crystal. This offers a possibility to investigate changes in the viscoelastic properties of adsorbed proteins in real time, which are further directly related to the conformation of the adsorbed proteins. Examples of how this type of measurements contributes with such information are presented using examples of: (i) Hemoglobin (Hb) adsorbed with and without the ligand carbon monoxide, which is known to slightly effect the conformation and stability against denaturation of Hb in solution. (ii) Antibody-antigen reactions, where we emphasize the added value from this type of multi-parameter analysis for immuno-sensing or of recognition events in general. (iii) Adsorption and enzymatic induced cross-linking of a mussel adhesive protein. We also demonstrate how additional information about these and similar types of measurements are obtained by simultaneously also measure at different frequencies, since different conformational states in some situations respond differently at different frequencies. We also demonstrate the importance of multi-parameter analysis in order to be able theoretically treat the QCM response upon adsorption of non-rigid biomolecules.

11:40am **BI-TuM11 Probing the Oxidation of Amine Modified Surfaces by MALDI Mass Spectrometry**, ***G.R. Kinsel****, R.B. Timmons, A.K. Walker, Y. Wu,* University of Texas, Arlington

The oxidation of amine modified surfaces, produced by pulsed RF plasma polymerization of allyl amine, can lead to substantial changes in the interaction of these surfaces with peptides and proteins in solution. Initial studies, using matrix assisted laser desorption / ionization (MALDI) mass spectrometry to characterize surface-peptide retention affinity, suggest that theses changes result from the acquisition of significant acidic character by the surface during the oxidation process. We have undertaken a variety of studies designed to characterize the surface chemical changes resulting from exposure of amine modified surfaces to air and to quantitate the impact of these changes on the peptide retention affinity. In these studies amine modified surfaces were exposed to pure oxygen and pure carbon dioxide environments. Time dependent changes in surface chemistry were monitored by FTIR spectroscopy and global compositional changes in surface chemistry were monitored by X-ray photoelectron spectroscopy. Subsequently, surface-peptide retention affinities were determined as a function of solution ionic strength and surface oxidation by using MALDI mass spectrometry. In addition, MALDI mass spectrometry was used to directly characterize oxidative changes in low duty cycle allyl amine polymer films to gain insight into the nature of the chemical modifications occurring in these polymer films. The results of these studies provide unique insight into the specific chemical changes and stability / reactivity of these surface modified materials.

# Author Index

## Bold page numbers indicate presenter