Biomaterial Interfaces
Room 210D - Session BI-MoA

Protein-Surface Interactions
Moderator: P. Cremer, Texas A&M University

2:00pm **BI-MoA1 Thermodynamic and Kinetic Control of Protein Adsorption on Surfaces with Grafted Polymers**, ***I. Szleifer***, Purdue University **INVITED**

Grafted polymer layers modify the effective interactions between proteins and surfaces resulting in a change of the ability of the proteins to adsorb on the surface. Grafted polymers have a strong effect on both the equilibrium amount of proteins` adsorbed as well as in the kinetics of adsorption. In this talk we will review some of our understanding of the molecular parameters that determine the ability of polymer layers to reduce protein adsorption. We will present a theoretical approach that enables the study of both the kinetics and thermodynamics of adsorption. The predictions of the theory are in excellent quantitative agreement with experimental observations for the adsorption of proteins on surfaces with grafted (short and long) polyethylene oxide. We will show the role of polymer chain length and surface coverage on both the equilibrium adsorption isotherms and the kinetics of protein adsorption. For example, we find that for fixed polymer surface coverage there is a polymer molecular weight above which the equilibrium adsorption becomes independent of polymer chain length. However, the kinetics of protein adsorption depends very strongly on polymer molecular weight under all conditions. The time dependent adsorption is a very complex process due to the changes in the effective surface-protein interactions as the adsorption process progresses. Namely, the changes in the structure of the polymer layer as the proteins adsorb result in large changes in the kinetic process. We will show under what conditions the predicted equilibrium amount of protein adsorbed is finite, however, the time scale for adsorption is so slow that the layer completely prevents protein adsorption for practical purposes. Finally, we will show how chemical modifications of the polymer layer can be used to manipulate the amount, structure and time scale for adsorption and desorption of the proteins from the modified surface.

2:40pm **BI-MoA3 Approach Towards Protein Adsorption, Desorption and Exchange**, ***M. Halter***, ETH Zurich, Switzerland; *G. Szöllösi, I. Derényi,* Eötvös University, Hungary; *J. Vörös,* ETH Zurich, Switzerland

When an artificial object is introduced into a biological environment, its surface is covered almost instantly with a protein layer. Being such a crucial issue for any biological application, the processes involved in protein adsorption, desorption and exchange are still not fully understood. Many controversial theories about the reversibility or irreversibility of protein adsorption, whether an adsorbed layer is static or forming a dynamic equilibrium and other puzzles and paradoxes exist. We present a realistic model for protein adsorption that can adequately describe the observed experimental data, such as irreversibility, history dependence, or the Vroman effect. A novel instrument, the Single Channel Grating Coupler, was used to provide new insight into protein behavior at interfaces. It is a planar waveguide technique that uses the evanescent field generated by an incoupled laser beam. Fluorescently labeled proteins within this field are excited and emit a fluorescent signal. The major advantages of this instrument are its high sensitivity (lower detection limit < 10 fmol/cm@super 2@) and the possibility to measure protein exchange by varying the labeled to unlabeled protein ratio in a solution. In situ measurements of interfacial exchange reactions provide sufficient data to develop a sophisticated protein adsorption model. It assumes that each protein molecule has several different conformations in the adsorbed state with different footprint sizes and binding energies, separated by energy barriers. Numerical simulations of large numbers of proteins - supplemented by analytical calculations - allow us to reproduce the experimental data and identify the conformations of proteins. Such a model will hopefully lead to a better understanding of protein behavior at interfaces. Beyond this, knowledge of the processes involved will help to tune the important parameters to build up and control adsorbed protein layers as desired for specific applications.

3:00pm **BI-MoA4 Mixology of Protein Solutions and the Vroman Effect**, ***A. Krishnan***, *C.A. Siedlecki, E.A. Vogler,* Pennsylvania State University

Mixing rules stipulating both concentration and distribution of proteins adsorbed to the liquid-vapor(LV) interphase from multi-component aqueous solutions are derived from a relatively straightforward protein-adsorption model. Accordingly, proteins compete for space within an interphase separating bulk-vapor and bulk-solution phases on a weight, not molar, concentration basis. This results in an equilibrium weight-fraction distribution within the interphase that is identical to bulk solution. However, the absolute interphase concentration of any particular protein adsorbing from an m-component solution is 1/mth that adsorbed from a pure, single-component solution of that protein.Applied to adsorption from complex biological fluids such as blood plasma and serum, mixing rules suggest that there is no energetic reason to expect selective adsorption of any particular protein from the mixture. Thus, dilute members of the plasma proteome are overwhelmed at the hydrophobic LV surface by the thirty classical plasma proteins occupying the first-five decades of physiological concentration. Mixing rules rationalize the experimental observations that(i)concentration-dependent liquid-vapor interfacial tension of blood plasma and serum cannot be confidently resolved,even though serum is substantially depleted of coagulable proteins(e.g. fibrinogen) and(ii) of plasma is startlingly similar to that of purified protein constituents. Adsorption-kinetics studies of human albumin (66.3 kDa) and IgM (1000 kDa) binary mixtures revealed that relatively sluggish IgM molecules displace faster-moving albumin molecules adsorbing to the LV surface. This Vroman-effect-like process leads to an equilibrium reflecting the linear combination of w/v concentrations at the surface predicted by theory. Thus, the Vroman effect is interpreted as a natural outcome of protein reorganization to achieve an equilibrium interphase composition dictated by a firm set of mixing rules.

3:20pm **BI-MoA5 Molecular Dynamics Simulation of the @gamma@ Chain Fragment of Fibrinogen on Functionalized SAM Surfaces**, *M. Agashe, S.J. Stuart,* ***R.A. Latour***, Clemson University

Protein adsorption to biomaterials surfaces is a primary governing factor of biocompatibility. While much has been learned, the molecular mechanisms involved in adsorption behavior are not understood. Empirical force field based molecular simulation methods provide an excellent approach to theoretically investigate the molecular behavior of proteins as they adsorb to surfaces. In this research, molecular dynamics simulations were conducted to investigate the adsorption behavior of a 30kDa C-terminus fragment of the @gamma@ chain of fibrinogen (Fg) as a function of surface chemistry. Simulations were conducted using the GROMACS program and force field. The surfaces were modeled to represent Au-alkanethiol self-assembled monolayers (SAMs) with 5 surface functionalities: CH3, OH, NH2, COOH, and PEG. The model system consisted of Fg in saline (explicit water with Na+ and Cl- ions) positioned over a SAM surface. Systems were contained within a 105Å x 107Å base x 80Å high simulation cell with periodic boundary conditions. 5ns simulations were performed and the effects of adsorption on Fg were analyzed. The results predict that only minor changes in Fg conformation occur during this time frame, however, Fg was observed to undergo large surface-dependent rotational and translational motions, suggesting faster kinetics for reorientation than unfolding. Although contingent on the accuracy of the GROMACS force field, which has not yet been validated for this application, these results have profound implications for surface design because they suggest that surface chemistry should be an effective means to control the orientation of adsorbed proteins.

3:40pm **BI-MoA6 The Effects of Adsorbed Proteins on the Performance of Biomedical and Biotechnological Devices**, ***T.A. Horbett***, University of Washington **INVITED**

Proteins are abundant in biological fluids, readily adsorb to most devices that contact such fluids, and often adversely affect the performance of the device. For example, adsorbed proteins are believed to lower the biocompatibility of implants in the body, non-specific adsorption of antibodies interferes in many solid phase immunoassays, and protein adsorption to the walls of microfluidic devices can cause analyte loss and/or reductions in separation efficiency. In this presentation, I will first give a series of examples illustrating the role of adsorbed proteins in device related problems. A brief review of the major mechanisms of protein adsorption affecting device performance will be given, namely variations in affinity of proteins for surfaces and differences in the ability of adsorbed cell adhesion proteins to support cell adhesion ("molecular potency"). Alterations in molecular potency have often been ascribed to denaturation of adsorbed proteins. However, studies from Norde's lab have shown that adsorbed proteins that exhibit no thermal unfolding enthalpy, and thus appear to be completely denatured, actually retain considerable structure, so these important findings will be presented. In many situations, reducing cell adhesion to a surface is desirable, but ways to accomplish this are not always clear. Towards that end, studies in my lab of platelet and monocyte

adhesion to adsorbed fibrinogen have suggested biomaterial design criteria to reduce cell adhesion that are based on the concepts of reducing molecular potency or affinity. Adsorbed proteins sometimes have low molecular potency, but the properties of the surface which cause it are unclear, so it is currently difficult to apply this criteria in designing new surfaces. In contrast, the other design criteria, a need for ultralow fibrinogen affinity surfaces, has been used to make better surfaces, as will be illustrated with glow discharge deposited tetraglyme, and with polyurethanes with added PEO.

4:20pm **BI-MoA8 The Surface Analysis and Quantification of the Electro-sprayed Fibronectin on Biocompatible Materials***, **M.J. Wang**,* Queen Mary University of London, UK, United Kingdom*; D.A. Lee, M.D. Paine, D.L. Bader, J.P.W. Stark,* Queen Mary University of London, UK

Electrospray is employed as a novel technique to incorporate biomolecules on substrates because it provides the possibility of soft landing the biomolecules. The interactions between biomolecules and substrates are, first of all, examined by surface analysis techniques such as atomic force microscopy (AFM) and Fourier Transform Infrared (FTIR) to identify the efficiency of electrospray and examine the morphology of the biomolecules. Moreover, the immunofluorescent methods provide the possibilities of gaining both quantitative and qualitative information. Fibronectin (FN) was chosen as the target molecule to be sprayed due to its functionalities such as promoting proliferation, differentiation of cells, also to promote the cell-cell and cell-substratum adhesion. Silicon wafer and medical grade stainless steel are chosen as target substrates due to their surface energy and biocompatibility. Both FTIR and AFM analysis show the effective landing of fibronectin via electrospray. The landed fibronectin shows the characteristic peaks of amide I and amide II compositions of fibronectin. Moreover, the linear relationship for the concentration of fibronectin versus the intensity of characteristic peaks of fibronectin by FTIR indicates that FTIR could serve as a semi-quantitative technique for examining the fibronectin. On the other hand, the AFM can detect the existence of Fibronectin up to the single molecule scale. The image shows the double strains of FN which is similar as the morphology of FN found in reference (Ref). Moreover, by immunofluorescence analysis, the efficiency of using electrospray to deposit FN on the substrates can be identified. And a quantitative assessment of the biomolecules on the substrates can be provided. These results provide potential possibilities of patterning array and assemblies of tissue which could be applied in the drug discovery and biosensors fields.

4:40pm **BI-MoA9 The Effect of Surface Structure and Functionality on Conformation of Surface-Adsorbed Fibrinogen***, **C.L. Berrie**, J.E. Headrick, K.L. Marchin, S. Phung,* University of Kansas

The interactions of the plasma protein fibrinogen with surfaces have been studied using atomic force microscopy (AFM). Specifically, well-characterized model substrates have been used to investigate the effect of surface chemistry and structure on the adsorption of fibrinogen. Dramatic differences in the average size and shape of fibrinogen molecules adsorbed to hydrophobic and hydrophilic substrates have been observed. These changes can be readily seen in AFM images of individual molecules with sub-molecular resolution. The differences have been quantified and correlated with the surface chemistry. In addition, new methods for patterning nanostructured substrates for use in these experiments have been investigated as well as methods for chemically functionalizing AFM probe tips in order to obtain information beyond topography. Adsorption of fibrinogen on nanostructured thin films and the effects of ionic strength and pH of the solution will also be discussed.

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