Biomaterial Interfaces
Room: 23 - Session BI+SS+AS-TuM

Biomolecules at Interfaces
Moderator: P. Kingshott, Swinburne University of Technology, Australia

8:20am **BI+SS+AS-TuM2 Computer Simulation of Water-Mediated Adhesion between Organic Surfaces**, *A.J. Pertsin, **M.H. Grunze***, University of Heidelberg, Germany

The adhesive forces operating between various surfaces in aqueous media are of interest in many areas ranging from biology to electronics. This refers, in particular, to surfaces formed by self-assembled monolayers (SAMs) on solid substrates to modify the surface-sensitive properties of the latter. Another important example is provided by supported lipid bilayers, where the water-mediated bilayer-substrate adhesion determines the stability of the system. The present study is concerned with surfaces formed by a hydrophobic methyl-terminated SAM (C-SAM), a hydrophilic carboxyl-terminated SAM (hereafter, O-SAM), and a phosphatidylethanolamine (PE) bilayer. The surface-water-surface system was treated as an open one using the grand canonical Monte Carlo technique. The free energies of adhesion were evaluated by integration of simulated pressure-distance relations. For SAMs, both symmetric and asymmetric confinements were considered, as formed by like and unlike SAMs, respectively. As the confinement was increased, water confined by the C-SAMs experienced capillary evaporation. As a consequence, the adhesion energy was mainly determined by the direct interaction between bare C-SAMs. In the asymmetric SAM system, an incomplete capillary evaporation was observed, with the number of water molecules dropped by more than an order of magnitude. The remaining water molecules were all adsorbed on the O-SAM, while the C-SAM was separated from the rest of the system by a thin vapor layer. The calculated free energies of adhesion were in acceptable agreement with available experimental data. Unlike the SAM systems involving the hydrophobic C-SAM, the PE/water/C-SAM system did not experience capillary evaporation up to the highest confinements tried. A likely reason is a high molecular-level "roughness" of the PE/water interface due to a deep penetration of water in the PE bilayer. The pressure-distance dependence showed a slightly repulsive region with a depth comparable with the statistical uncertainty in pressure. By contrast, the pressure-distance curve of the PE/water/O-SAM system showed a well-defined minimum with a depth of about 0.7 kbar. The integration of this curve resulted in an adhesion free energy of 19±3 mJ/m^2, close to the value obtained for the O-SAM/water/O-SAM and O-SAM/water/C-SAM systems (~25 mJ/m^2).

8:40am **BI+SS+AS-TuM3 Adsorption from Saliva - Properties of Adsorbed Layers and Comparison with Other Systems**, ***T. Arnebrant**, L. Lindh, J. Sotres*, Malmö University, Sweden **INVITED**

Adsorbed salivary protein layers will cover soft and hard surfaces in the oral cavity, where they fulfill a protective function influencing adhesion and wear, and also surfaces of devices exposed to saliva. Properties of salivary films will depend on the characteristics of the surface on which they are formed as well as solution conditions (salt, pH) and will affect surface properties such as wettability and charge. Moreover, normal and lateral forces between surfaces bearing salivary films will be distinctly different than for bare surfaces. Such changes in surface properties and interactions may be relevant not only for events at oral interfaces but also for the operation of monitoring or sampling devices immersed in or exposed to saliva. Here, we show how a combined characterisation of these systems through different surface techniques provides important information on the role of this body fluid which is not available through more common chemical or biochemical approaches. The presentation will describe adsorption characteristics of salivary proteins from the total secretion as well as for purified fractions including single protein preparations. Influence by surface properties and ambient (solution) conditions will be outlined. Data on structure of salivary films as obtained by *in situ* ellipsometry, QCM-D and neutron reflectivity will be reported. Furthermore, SFA and AFM measurements of DLVO, steric, adhesive and frictional forces between surfaces bearing salivary films will be discussed. A new method for estimating the strength of salivary films based on simultaneous recording of roughness and friction data from AFM will also be described.

References: Protein Adsorption in the Oral Environment, Arnebrant T, In Biopolymers at Interfaces 2nd ed. (M. Malmsten Ed.) Marcel Dekker, 2003, pp 811-856

Friction force spectroscopy as a Tool to Study the Strength and Structure of Salivary Films. Sotres J., Liselott L., Arnebrant T. 2011. *Langmuir, 27 (2011), 13692-13700.*

9:20am **BI+SS+AS-TuM5 An Atomic Force Microscopy Based Method for the Determination of Protein Stability**, *O. Croad,* Univeristy of Nottingham, UK*, S. Rigby-Singleton,* Molecular Profiles Ltd., UK*, C.J. Roberts, D.J. Scott, P.M. Williams, **S. Allen***, University of Nottingham, UK

A method for the early detection of instability and aggregation propensity of proteins and other biological macromolecules would be valuable for the rapid development of novel biopharmaceutical formulations. The aim of this study was to investigate the potential of atomic force microscopy (AFM) based adhesion force measurements to meet this need. We report the first key step in demonstrating this approach; a clear relationship between how frequently an AFM probe adheres to a protein coated surface and the fraction of unfolded proteins on that surface. Instability and subsequently protein denaturation are commonly linked with protein aggregation, and hence formulation failure. It was found that for the protein bovine serum albumin (BSA), the adhesion between AFM tips and protein-coated samples occurred much more frequently as either the concentration of a denaturant or temperature was gradually increased. We compared this behaviour with fluorescence based studies of the BSA unfolding in solution. Both methods provided us with almost identical ΔG values of stability and 50% unfolding ($[D]^{50\%}$) values. The data demonstrates for the first time, an AFM based method for protein stability determination. Interestingly, the method also appears to be a good reporter of the protein solution behaviour. With further development this approach could be utilized to screen for instability and aggregation propensity of a given protein therapeutic, in a range of conditions. The ultimate aim is to create a robust technique that can be performed rapidly and routinely.

9:40am **BI+SS+AS-TuM6 Von Willebrand Factor A1 Domain Structure and Function Changes on Surfaces**, *E. Tronic, W. Thomas, **D.G. Castner***, University of Washington

The clotting protein von Willebrand Factor (VWF) binds to platelet receptor glycoprotein 1ba (GP1ba) when VWF is activated, such as when VWF is exposed to a surface or is under high shear. However, the mechanism of surface activation is not known. This study characterizes function and adsorption behavior of the VWF A1 domain, which contains the GP1ba binding site. Surfaces tested are glass, polystyrene, and tissue culture polystyrene. Highest VWF A1- GP1ba binding is observed when A1 is adsorbed onto polystyrene, as measured by platelet rolling velocity in a parallel plate flow chamber assay. X-ray photoelectron spectroscopy (XPS) showed comparable A1 amounts are present on each surface, suggesting functional differences were not explained by differences in surface coverage. A1 surface structure was investigated using ELISA, time-of-flight secondary ion mass spectrometry (ToF-SIMS) and near-edge x-ray absorption fine structure (NEXAFS). Using monoclonal antibodies binding to a nonlinear epitope within A1, ELISA showed lower antibody binding for A1 adsorbed to polystyrene than to glass or tissue culture polystyrene. ToF-SIMS was used to identify differences in amino acid exposure, and NEXAFS showed different amide backbone ordering on the three surfaces. These studies demonstrate that the surface dependence of A1 function is likely due to differences in adsorbed surface orientation and/or conformation. This is an important consideration in *in vitro* models, where A1 is typically immobilized onto synthetic surfaces, and is also of interest for blood-contacting biomaterials. Additional studies have been done on A1 and two A1 mutants adsorbed on collagen coated tissue culture polystyrene. One mutant exhibits similar ELISA and ToF-SIMS results to the wild type A1, while the other mutant exhibits differences. This indicates that mutations in A1 can affect the conformation/orientation changes that result from A1 adsorption onto collagen.

10:40am **BI+SS+AS-TuM9 Combining Catalysis and Self-Assembly: Towards Evolvable Soft Matter**, ***R. Ulijn***, University of Strathclyde, UK **INVITED**

Molecular networks are key to the adaptiveness of biological systems and it would be very useful if this concept could be introduced into simple man-made functional materials, which could adapt to changing environments. In biology, adaptiveness (as a consequence of evolution) is achieved through a combination of catalysis, self-assembly, molecular recognition and compartmentalisation. These individual molecular processes are closely linked, a situation which may be achieved in laboratory based systems by sharing of building blocks between these individual processes, thereby giving rise to networked systems that are highly responsive and adaptive to changing external conditions. We have made the first steps towards developing evolvable materials, and will present progress in (i)

structure/function relationships in peptide self-assembly, (ii) development of catalytic peptides, (iii) self-selecting peptide libraries achieved by combining fully reversible amino acid exchange in self-assembing peptide systems. The overall aim of this area is to produce laboratory made molecular materials that incorporate the above features and are able to adapt and change their properties in response to external environmental changes. Potential applications in biomaterials science will be discussed.

11:20am **BI+SS+AS-TuM11 Bio/Nano Interfaces of De Novo Design: Small Proteins with Large Potential**, ***M.G. Ryadnov***, National Physical Laboratory, UK

Our ability to manipulate function at interfaces in native and near-native environments is critical for the fabrication of nanostructured materials and devices. Biomolecular self-assembly lends itself to robust bio-nano systems. However, exact construction strategies to enable desired applications stumble upon the lack of control over self-assembly processes. De novo peptide design provides a saving solution to this.[1] Small proteins can be designed to deliver functions that are otherwise accessible only to macromolecular subcellular complexes. Examples include gene delivery systems,[2] fibrillar microscopic structures for tissue repair[3] and responsive antimicrobial agents[4]. A key factor in all such designs is their structural and functional relevance to native self-assembling structures, be these viruses, extracellular matrices or host defence systems. Thus, this is our ability to construct such materials at will that advances the development of efficient bio/nano-interface technologies.[5] **References** 1. Ryadnov, M. G. (2012) Prescriptive peptide design. In ***Amino acids, peptide and proteins.*** (Farkas, E. & Ryadnov, M. G., eds.) SPR, RSC Publishing (2012), v.37. 2. Lamarre, B., Ravi, J. & Ryadnov, M. G. (2011) GeT peptides: a single-domain approach to gene delivery. ***Chem. Commun.***, 47, 9045-9047. 3. Bella, A., Ray, S., Shaw, M. & Ryadnov, M. G. (2012) Arbitrary self-assembly of peptide extracellular microscopic matrices. ***Angew. Chem. Int. Ed.***, 51, 428-431. 4. Ryadnov, M. G., Mukamolova, G. V., Hawrani, A. S., Spencer, J. & Platt, R. (2009) RE-coil: an antimicrobial peptide regulator. ***Angew. Chem. Int. Ed.*** 48, 9676-9679. 5. Ryadnov, M. G. (2009) ***Bionanodesign****: Following the Nature's touch.* RSC Publishing, 250 pp.

11:40am **BI+SS+AS-TuM12 Application of CD and SRCD Techniques to the Study of Protein/Nanoparticle Complexes**, ***G. Ceccone****, S. Laera, L. Calzolai, D. Gilliland,* EC-JRC-IHCP, Italy*, R. Hussein, G. Siligardi,* Diamond Light Source, UK*, F. Rossi*, EC-JRC-IHCP, Italy

Nanotechnology is having a large impact in very different scientific fields and the use of nanotechnology-based materials is not just limited to research laboratories, but has already been applied in several industrial sectors and into real products as disparate as medical diagnostic tools, drug delivery systems, cosmetics, and consumer products.

In particular, engineered nanoparticles (ENPs) are used in different applications such as cosmetics, food and medicine and currently more than 600 products containing nanomaterials are already on the market[1,2,3]. At the same time there is a growing public concern about the safety of ENPs since it has been demonstrated that those intended for industrial and medical applications could cause adverse effects in mammalians or aquatic organisms by specific mechanisms depending on their physical chemical properties[4]. However, the interaction of nanomaterials with complex matrices is far to be understood. In fact, although it is now increasingly accepted that the surface of nanoparticles in a biological environment is modified by the so called "protein corona"[5,6], the importance of the detailed structure of the adsorbed protein-solution interfaces is still not much addressed in the nanotoxicology literature[7] .

In this work, we report the use of Circular Dichroism (CD) and Synchrotron Radiation Circular Dichroism (SRCD) to detect changes in the secondary structure and stability of different classes of proteins interacting with nanoparticles. In particular, we show that by using the SRCD we can detect structural changes of proteins in the nanomolar concentration range when they form protein-nanoparticle complexes[8]. Furthermore, the adsorption of protein on NP modifies their melting point in a composition and size dependent manner, indicating once more that the protein corona formation is strongly depending on the nanoparticles physico-chemical properties. For instance, while the presence of Au NPs do not influence the thermal unfolding process of human serum albumin (HSA), a significant decrease of the HSA melting temperature (about 6ºC) is observed in presence of Ag NPs.

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