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

Protein Solid-Surface Interactions I

Moderator: G.U. Lee, Naval Research Laboratory

2:00pm **BI-MoA1 Formation of 2D Crystals of Proteins on Solid-Supported Lipid Bilayers Followed by AFM**, ***A. Brisson,*** University of Groningen, The Netherlands, Netherlands; *I. Reviakine, W. Bergsma-Schutter, N. Govorukhina, S. Mazeres,* University of Groningen, The Netherlands

The possibility of assembling macromolecules or particles in a controlled manner on solid surfaces constitutes a central issue in the emerging field of nanotechnology. Methods for ordering proteins as 2D crystals or helical arrays at the level of lipid surfaces have received particular attention in molecular structure determination by electron crystallography. One of these methods, called the lipid layer crystallization method, uses the specific interaction between a protein and a ligand coupled to a l ipid molecule incorporated in a lipid monolayer at the air-water interface.@footnote 1@,@footnote2@,@footnote 3@ Our objective has been twofold: 1) to extend this strategy to the case of solid-liquid interfaces; 2) to develop an in situ method for following the formation of prot ein 2D crystals. Using Atomic Force Microscopy, both processes of formation of lipid bilayers on solid supports (SPB), and of protein 2D crystallization on SPB could be revealed in real time, in a natural aqueous environment, at sub-molecular resolution.@footnote 4@,@footnote 5@ Results will be presented on the crystallization of annexin V, an inhibitor of blood coagulation which binds specifically to negatively charged lipids, and will be compared to Electron Microscopy data of 2D crystals formed on lipid monolayers. This novel experimental approach offers exciting opportunities in basic science for investigating crystallization processes and provides an adequate technology for fabricating protein-containing biofunctional surfaces. @FootnoteText@ @footnote 1@Uzgiris, E.E. Kornberg, R.D., Nature 301 (1983) 125. @footnote 2@Brisson A. et al., J. Crystal Growth 196 (1999) 456. @footnote 3@Brisson, A. et al., in Crystallization of Proteins and Nucleic Acids, A Practical Approach, Oxford Univ. Pres (in press). @footnote 4@Reviakine, I., Bergsma-Schutter, W. Brisson, A., J. Struct. Biol. 121 (1998) 356. @footnote 5@Reviakine, I. Brisson, A., (submitted). .

2:20pm **BI-MoA2 Light Activated Affinity Micropatterning of Proteins**, ***A. Chilkoti,*** *Z.-P. Yang, W. Frey,* Duke University; *T. Oliver,* Food and Drug Administration

Biomolecular patterning has diverse applications, which range from modulation of cell-substrate interactions in biomaterials and tissue engineering, to the fabrication of multianalyte biosensors, clinical assays, and genomic arrays. Motivated by these applications, we have developed a method to micropattern proteins on well-defined gold substrates, which we term light-activated affinity micropatterning of proteins (LAMP). LAMP is a multi-step patterning process: first, a gold substrate is functionalized with a binary mixture of 11-mercaptoundecanol and 16-mercaptohexadecanoic acid to provide a non-fouling, reactive self-assembled monolayer (SAM) template on gold. Next, the carboxylic acid end groups in the binary SAM are coupled to methyl @alpha@-nitropiperonyloxycarbonyl biotin succinimidyl ester (caged biotin ester) through a diamine linker. Deprotection of caged biotin by spatially-defined uv illumination at 350-360 nm reconstitutes biotin in the illuminated region, and subsequent incubation with streptavidin results in selective binding of streptavidin to regions that were previously deprotected. We have investigated and optimized LAMP by contact angle goniometry, ellipsometry, surface plasmon resonance, and X-ray photoelectron spectroscopy to maximize ligand density and pattern contrast. Micropatterning of streptavidin and an anti-biotin monoclonal antibody has been demonstrated with a spatial resolution of ~5 microns by imaging ellipsometry and confocal light microscopy of fluorophore-derivatized proteins. LAMP can be further extended to allow spatially-resolved micropatterning of multiple biomolecules by repeated cycles of spatially-defined deprotection, streptavidin incubation, followed by binding of the biotinylated moiety of interest.

2:40pm **BI-MoA3 Oriented Protein Binding via Silane-SAMs Using His-tagged Proteins**, *C. Hoffmann,* University of Stuttgart, Germany; *H. Brunner,* ***G.E.M. Tovar,*** Fraunhofer Institute for Interfacial Engineering and Biotechnology, Germany

Oriented immobilization of proteins is a promising way of retaining protein activity on artificial surfaces.@footnote 1@ Modern protein engineering has already developed suitable tags for this purpose. Surface engineering is now required to provide complementary surfaces. We present experimental data on surface functionalization of silicon oxide surfaces using silane SAMs and the subsequent oriented binding of his-tagged proteins. We synthesized nitrilo-triacetic acid (NTA) silanes for direct attachment to silicon oxide surfaces, and NTA derivatives@footnote 2,3,4@ reactive to surfaces functionalized using epoxy terminated silanes. The stepwise surface functionalization and the binding capacitiy of the NTA surfaces for histidine peptides and his-tagged proteins has been investigated using XPS, MALDI-TOF-MS, contact angle measurements and in situ-ellipsometry. @FootnoteText@ @footnote 1@ Rao, S.V., Anderson, K.W., Bachas, L.G., Mikrochim. Acta, 1998, 128, 127-143. @footnote 2@ Dietrich, C., Schmitt, L., Tampe, R., Proc. Natl. Acad. Sci. USA, 1995, 92, 9014-9018. @footnote 3@ Sigal, G.B., Bamdad, C., Barberis, A., Strominger, J., Whitesides, G.M., Anal. Chem., 1996, 68, 490-497. @footnote 4@ Liley, M., Keller, T.A., Duschl, C., Vogel, H., Langmuir, 1997, 13, 4190-4192.

3:00pm **BI-MoA4 Complement Activation by IgM Immobilized on Methylated Silicon**, ***P. Tengvall,*** Linkoping University, Sweden; *A. Askendal,* Linkoping University; *K.I. Lundström,* Linkoping University, Sweden

The activation of the complement system by IgM immobilized on methylated silicon was studied by ellipsometry/antibody techniques. The IgM coated surfaces were incubated in normal human- and depleated sera at 37Â°C up to 90 minutes, and the deposited amounts of characteristic proteins probed by antibodies. The results indicate, surprisingly, that the so prepared model surface activates the human complement in a manner independent of calcium, although via the classical pathway.

3:20pm **BI-MoA5 Theoretical and Experimental Studies of the Interaction of Water with Oligo (ethylene glycol) Terminated Self Assembled Monolayers**, *R. Wang, H.J. Kreuzer,* Dalhousie University, Canada; *A.J. Pertsin,* ***M. Grunze,*** University of Heidelberg, Germany

The interaction of water with poly(ethylene glycol) and oligo(ethylene glycol) moieties in alkanethiolate SAMs is strongly dependent on the molecular conformation, lateral density and external electrical fields. In this contribution we will present ab initio quantum mechanical results and Monte Carlo Simulations on the ethylene glycol / water interaction and correlate them with vibrational sum frequency generation spectroscopic results of the OEG / water interaction,@footnote 1@ force distance measurements between neutral and charged AFM tips and OEG SAMs of different density,@footnote 2@ and single molecule force spectroscopy.@footnote 3@ @FootnoteText@ @footnote 1@M. Zolk, M. Buck, F. Eisert, M. Grunze, in preparation @footnote 2@K. Feldman, G. Habhner, N.D. Spencer, P. Harder, M. Grunze, JACS, submitted @footnote 3@F. Oesterheld and H. Gaub, New Journal of Physics 1 (1999), 6.1-6.11 3.

3:40pm **BI-MoA6 Protein Resistant Surfaces Based on Reactions of Thiol-Terminated Polyethylene Oxides with Gold**, ***J.L. Brash,*** *Y.J. Du,* McMaster University, Canada

Gold is a relatively inert metal but has strong specific interactions with sulfur functions, particularly thiol. Thiols that contain bioinert or bioactive moieties (eg bioactive peptides containing a terminal cysteine) can thus be chemisorbed to gold films to provide correspondingly bioinert or bioactive surfaces. High surface densities of ligands should be possible due to the abundance of thiol binding sites in the surface. Thiolated gold surfaces thus have great potential in the design of biocompatible materials. We have used this approach in the development of a variety of surfaces based on:(1) suppression of non-specific protein adsorption, (2) selective/exclusive adsorption of a target protein from the contacting biological fluid. In the present work, surfaces have been developed consisting of HS-polyethylene oxides (HS-PEO, expected to minimize non-specific protein adsorption) chemisorbed to gold films deposited on polyurethane or silicon. PEOs of varying MW and architecture (linear, star) were reacted with mercaptoacetic acid to attach terminal thiol groups. HS-polypropylene oxides (HS-PPO) were used as controls, not expected to reduce protein adsorption. Chemisorption of the PEOs and PPOs to gold was verified by XPS. The adsorption of fibrinogen and albumin from buffer and plasma was studied by radioiodination methods. It was found that: (a) protein adsorption is low on all the modified surfaces and decreases with increasing molecular weight of the PEO, (b) with MeO as the terminal

group at the free end of PEO, the suppression of protein adsorption is less than when OH is the terminal group, (c) chemisorbed PPO suppresses the adsorption of fibrinogen and albumin but not as effectively as PEO. In ongoing work we are investigating surfaces with heparin attached to the free chain end of chemisorbed PEO.

4:00pm **BI-MoA7 Protein Adsorption on Mixed Polyethylene Glycol/Methyl Terminated Alkane Thiol Self-Assembled Monolayers**, ***T. Kim***, *K.D. Hauch, J.W. Rogers, Jr.,* University of Washington

Polyethylene glycol(PEG) functionality has been used in many settings to resist protein adsorption.@footnote 1@ In this work, PEG and methyl terminated alkane thiols were used to form mixed self-assembled monolayers(SAM) on gold.@footnote 2@ These surfaces were used to study fibrinogen(Fg) adsorption. The molar percent of PEG thiol in the assembly solution was varied from 0-100% to vary the amount of PEG thiol on the surface. The mixed SAM's were examined with XPS. The atomic composition of the surfaces assembled from pure methyl thiol and pure PEG thiol agreed with expected values. An increase in ether bonds was seen as the PEG thiol ratio in the assembly solution was increased indicating an increase of PEG thiol in the mixed monolayer. The adsorption of I-125 labeled human Fg from dilute plasma (2 hr, 37 degrees Celsius) was measured. Fg adsorption on the SAM's ranged from 11-70 ng/cm^2, significantly lower than the control, polytetrafluorinated ethylene. The pure PEG thiol monolayer adsorbed 12 times less Fg than the control and 7 times less than the pure methyl SAM. We have demonstrated that the surface composition of the SAM is influenced by the composition of the assembly solution. Increased PEG thiol in the SAM reduced Fg adsorption with the pure PEG monolayer exhibiting the lowest values. Future work will include further surface characterization of the SAM's, adsorption studies with other proteins, and examination of choline or saccharide terminated thiols. @FootnoteText@ @footnote 1@ Lopez, G.P., et. al. Glow discharge plasma deposition of tetraethylene glycol dimethyl ether for fouling-resistant biomaterial surfaces. J. Biomed. Mater. Res. 1992, 26, 415-439 @footnote 2@ Ulman, A. Formation and Structure of Self-Assembled Monolayers, Chem. Rev. 1996, 96, 1533-1554

4:20pm **BI-MoA8 Properties of Oligo(ethylene glycol)- Terminated Alkanethiolates on Gold: Influence of Lateral Hydrogen Bonding**, ***R. Valiokas***, *S. Svedhem, S. Svensson, M. Östblom,* Linköping University, Sweden*; U. Gelius,* Uppsala University, Sweden*; B. Liedberg,* Linköping University, Sweden

An exhaustive study on properties of a class of oligo(ethylene glycol) (OEG)-terminated alkanethiolates on gold is presented. Contact angle goniometry, ellipsometry, infrared reflection-absorption spectroscopy, and X-ray photoelectron spectroscopy indicate formation of highly ordered self-assembled monolayers (SAMs), and the compounds containing amide moieties display lateral hydrogen bonding. The conformation of the OEG portion is shown to depend on the oligomer chain length and the lateral interactions, as evidenced by a different phase behavior of analogous OEG compounds which contain no amide group, as well as by using mixed SAMs of shorter and longer OEG chains. The assembly kinetics is found to be important in order to fine-tune the dominating phase of the SAMs. Furthermore, the thermal properties of the SAMs are investigated and temperature-dependent phase behavior is used to understand the nature of the conformational transitions of the OEG portion in relation to hydrogen bonding. Thus, the specifically introduced lateral interactions are shown to be not only a useful tool to improve the order and stability of the SAMs, but also to control conformational/functional properties of terminal groups which in this case are oligomers. Possible further modifications of such OEG SAMs and their bio-oriented applications are discussed.

4:40pm **BI-MoA9 1-Thiaoligo(Ethylene Oxide) SAMs: Biomimetic Matrix Structure Tuned via Hydrophilic Spacer Length & Packing Density**, ***T. Petralli-Mallow***, *D.J. Vanderah, C.W. Meuse, A.L. Plant,* National Institute of Standards and Technology

Interest in reconstituting transmembrane proteins into supported cell membrane mimics has led to development of novel tethering molecules. In order to introduce a flexible and fluid hydrophilic region at the proximal side of a supported lipid bilayer, oligo(ethylene oxide) moieties have been used as spacers between the sulfur and the alkane chain of alkanethiols. Our group has previously shown that the ethylene oxide moiety adopts a 7/2 helical conformation in self-assembled monolayers (SAM)s of both 1-thiahexa(ethylene oxide) (HS(EO)@sub 6@) decane and HS(EO)@sub 6@ octadecane on gold, indicating that the alkane chain is not the driving force for the helical conformation. Ethylene oxide structure may be controlled by packing density. For example, infrared analysis of mixed monolayers of HS(EO)@sub 6@-decane and phospholipids transferred from the air/water interface indicate that at low packing densities the EO region is disordered, but at higher packing densities the EO segment can assume the helical structure. For a series of decane-terminated 1-thiaoligo(ethylene oxide) SAMs with varying lengths of ethylene oxide spacers, infrared spectroscopy and sum frequency generation indicate that the conformation of the ethyleneoxide moieties may be controlled by their length. The conformation of the ethylene oxide segment is an extended all-trans chain in HS(EO)@sub 4@ SAM, a 7/2 helix in HS(EO)@sub 5-7@ SAM, and a less ordered conformation in the HS(EO)@sub 8@ SAM. The ability to control order via spacer length and packing suggests that HS(EO)@sub n@ SAMs may be successful platforms for biomimetic materials incorporating transmembrane proteins.

5:00pm **BI-MoA10 Reduction of Protein Adsorption on Polyethylene Glycol Covered Silica Surfaces**, ***N.A. Alcantar***, *T.L. Kuhl,* University of California at Santa Barbara*; E.S. Aydil,* University of California at Santa barbara*; J.N. Israelachvili,* University of California at Santa Barbara, US

Over the last decades, a large fraction of the scientific community has been dedicated to developing synthetic materials that can be used as implants or replacements for bones, organs, joints, tissues, skin, etc. These artificial materials must not only accomplish a specific function, but also be inert in the biological environment to which they are exposed. In general, the ability of a surface to reject proteins is a parameter used for determining its biocompatibility. Surfaces covered with polyethylene glycol (chemically (PEG, OH-(CH@sub 2@- CH@sub 2@-O)@sub n@-H) have been shown to be biocompatible as PEG enhances nonimmunogenecity, nonantigenicity and protein rejection. In order to produce a generic biocompatible surface coating, we have developed a direct method for grafting PEG onto amorphous activated silica surfaces or films. We first deposited an amorphous silica film by plasma enhanced chemical vapor deposition from SiH@sub 4@ and O@sub 2@ gases, which provides the flexibility to coat diverse materials with different shapes. These silica films were then activated by exposure to water plasma, thus increasing the number of hydroxyl groups on the surface. The silanols (Si-OH) on the resulting surface chemically react with the hydroxyl end of the PEG chain forming an ester bond, Si-O-C. The surface reaction was monitored using Attenuated Total Reflection Fourier Transform Infrared spectroscopy. Two representative fluorophore-labeled proteins were used in this study because of its relatively abundance in the blood stream. Measurements of protein absorption by fluorescence microscopy showed that the PEG coated surfaces significantly inhibit protein adsorption.

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