Biomaterials
Room 102 - Session BI+SS-WeM

Biological Interface & Surface Science
Moderator: C. Wöll, Ruhr-University Bochum, Germany

8:20am **BI+SS-WeM1 Hybridization Reactions between Surface Attached Oligonucleotides and Complements from Solution**, ***W. Knoll***, *D. Kambhampati, T. Neumann, M. Chen,* Max-Planck-Institut für Polymerforschung, Germany **INVITED**

The quantitative evaluation of hybridization reactions between surface-attached 15mer oligonucleotides and their complements from solution will be described. Reaction kinetics, as well as equilibrium binding studies are conducted in order to reveal the association/dissociation mechanism. Different strategies to prepare the interfacial probe layers are tested and compared to each other: These are 1) direct coupling of the catcher oligonucleotides to a gold substrate by thiol groups, 2) a streptavidin monolayer-based coupling scheme via biotinylated probe oligos, 3) a similar approach but based on a commercial dextran-streptavidin structure, 4) 15mers attached to polymer brushes grown by a "grafting-from" approach, and finally 5) a layer prepared by electropolymerization of hydroxyphenol-derivatized oligonucleotides. In cases where surface plasmon spectroscopy was not sensitive enough for label-free detection of the hybridization we employed our recently developed surface-plasmon field-enhanced fluorescence spectroscopy. Different versions based on having either the catcher strand labeled, or the target, or both (e.g., for energy transfer studies) will be discussed. Parameters that are studied include the effect of temperature, ionic strength, mismatch (number of bases, position) length of complement, charge density (DNA versus PNA) etc.

9:00am **BI+SS-WeM3 Functionalization of Metal-Oxide-Based Biomaterials and Biosensor Surfaces by Molecular Self-Asssembly Processes**, ***M. Textor***, *S. Tosatti, M. Zwahlen, S. Finken, J.A Hubbell, G. Haehner,* Swiss Federal Institute of Technology (ETH), Switzerland

Modifications of metal oxide surfaces based on spontaneous adsorption of alkane phosphates and polycationic copolymers and subsequent film formation are shown to be potentially very useful for designing the chemical and biochemical properties of metallic implants and optical biosensors. Alkane phosphates were found to self-assemble on a number of transition metal surfaces such as titanium oxide, tantalum oxide and niobium oxide through direct coordination of the phosphate head group to high-valency metal cations. The chemical and structural properties of the adlayers were investigated using XPS, ToF-SIMS and NEXAFS. Introducing terminal functionalities other than methyl, e.g. hydroxy, amine or oligo(ethylene oxide) groups, allows one to tailor physico-chemical properties such as wettability, surface charge or the strength of protein-surface interactions. A second class of molecular assembly systems, PEG-grafted polycationic copolymers, spontaneously form monomolecular adlayers on negatively charged metal oxide surfaces, imposing high resistance towards biomolecule adsorption. Through further functionalization of the PEG-chains with biotin or peptide moieties, specific interactions of the treated oxide surface with streptavidin or with cell receptors can be induced while preserving the low degree of non-specific events. w-functionalized alkane phosphate SAMs, as well as peptide-modified PEG-grafted copolymers have been applied to both smooth and rough titanium surfaces to produce model surfaces for the study of fibroblast and osteoblast cell-surface interactions, with independent control of surface topography and chemistry. Furthermore, the two novel molecular assembly systems are shown to have a substantial potential for the reproducible and cost-effective modification of chips in optical-waveguide-based bioaffinity sensing of proteins and of DNA/RNA, including their application to microarray-type sensor surfaces.

9:20am **BI+SS-WeM4 Buildup Mechanism for Hyaluronic Acid/polysine Films onto a Solid Interface**, *C. Picart, Ph. Lavalle, F.J.G. Cuisinier,* INSERM U424, France; *P. Schaaf,* Institut Charles Sadron (CNRS) Strasbourg, France; ***J.C. Voegel***, INSERM U424, France

The formation of a new kind of biocompatible films based on Poly-L-Lysine and Hyaluronic Acid (PLL/HA) by alternate deposition of PLL and HA was investigated. It is shown that the driving force of the buildup process appears, as for "convential" polyelectrolyte multilayer systems, to be the alternate overcompensation of the surface charge after each PLL and HA deposition. The construction of (PLL/HA) films appears to take place over two buildup regimes. The first regime is characterized by the formation of isolated islands dispersed on the surface and which grow both by addition of new polyelectrolytes on their top and by mutual coalescence of the islands. The second regime sets in once a continuous film is formed at the after the 8th bilayer deposition in our working conditions. QCM measurements at different frequencies evidences a viscoelastic behavior of the films which have a shear viscosity of the order of 0.1 Pa.s. During this second regime the mass of the multilayer film increases in an exponential rather than in a linear way. This exponential growth is explained the diffusion of free PLL chains into the interior of the film when it is brought in contact with a PLL solution and by the diffusion out of the film of a fraction of these free chain followed by their interactions with HA chains at the outer limit of the multilayer when the film is further brought in contact with a HA solution. The diffusion of free PLL chains into the film is also found to be accompanied by an expulsion of water out of the film. This new kind of biocompatible film incorporating a natural polymer of the extracellular matrix and a widely used polypeptide makes it a potential candidate for cell-targeted action and for the coating of different types of surfaces, such as implants or capsules, in order to mimic a natural extracellular gel.

9:40am **BI+SS-WeM5 Orientational Effects and Surface Free Energies in the Amino Acids Adsorption Process onto Silicon-based Surfaces**, *G.L. Gambino,* ***C. Satriano***, *G. Marletta,* University of Catania, Italy

The present paper deals with the study of the adsorption process of Lysine (Lys) and Cysteine (Cys) from aqueous solutions as a function of the substrate structure and solutions pH. The substrate effect has been studied for three silicon-based substrates, i.e. silicon dioxide, poly(hydroxymethyl)siloxane (PHMS) and oxygen plasma-treated PHMS. The pH role on the adsorption process has been investigated by performing the incubations in the amino acid solutions both at their isoelectric pH and at the physiological pH, i.e. pH = 7.4. The in situ characterization of the substrates-amino acids molecules interaction was performed by means of the Quartz Crystal Microbalance with Dissipation (QCM-D) technique. On the other hand, ex-situ measurements were performed by means of Angular Resolved X-Ray Photoelectron Spectroscopy (ARXPS) and Contact Angle (CA) measurements. In particular, ARXPS, by varying the sampling depth from @tdA@10 nm to @tdA@2 nm, allowed to elaborate a coverage model, while the CA technique, by using three test liquids, allowed to calculate the surface free energies and their relative dispersive and acid-base contributions. QCM-D data shows that Lys and Cys strongly adsorb onto the plasma-treated PHMS while on the untreated PHMS surfaces the adsorption of both amino acids does not occur. ARXPS measurements indicate that the adsorbed molecules exhibit a preferential orientation respect to the plane of the surface, however no uniform coverage is obtained for any kind of substrate. Finally, the CA measurements indicate that the polar component of the surface free energy is directly related to both the amount and the orientation of the adsorbed amino acid molecules.

10:00am **BI+SS-WeM6 Surface-bound Liposomes for Biomedical Applications**, *P. Vermette,* CSIRO, Australia; *E. Gagnon,* Universite Laval, Canada; *L. Meagher,* CSIRO, Australia; *D. Dunstan,* Melbourne University, Australia; ***H.J. Griesser***, CSIRO, Australia; *C. Doillon,* Universite Laval, Canada

Injectable liposomes, in particular PEG-coated liposomes, are well known in the pharmaceutical industry for drug delivery. However, much of the drug never reaches the intended target site. We have developed methods for binding liposomes onto surfaces of biomedical devices for controlled local delivery of drugs adjacent to implanted biomedical devices. In this way we aim to reduce drug amounts and wastage, and control the local host response to the implant, a response which with most current biomaterials typically is dominated by fibrous tissue encapsulation. We have produced liposomes with encapsulated drugs and model substances, characterized them in terms of size and release performance, and bound them onto polymeric surfaces. PEGylated phospholipid liposomes were produced by extrusion through polycarbonate membranes of various pore sizes. The diameters of the liposomes were characterized by photon correlation spectroscopy. For binding liposomes, polymer surfaces were coated with streptavidin, which was used for affinity capture of biotinylated PEGylated liposomes. Streptavidin was covalently bound onto polymer surfaces via an amine plasma (glow discharge) polymer interlayer and a layer of polyacrylic acid, onto whose carboxylate groups the streptavidin was attached by carbodiimide chemistry. Detailed surface analyses were used to characterize and verify each step in the fabrication of the liposome coated surfaces. To test the in vivo efficacy of liposome coated biomaterials, an

angiogenesis promoting drug was encapsulated and the liposomes attached. Both in vitro and in vivo there occurred markedly enhanced angiogenesis. Another way of using the same concept may be to implant streptavidin coated biomedical implants and then inject drug-loaded liposomes. Experiments are currently underway to investigate whether circulating biotinylated liposomes can thus be enriched at an implant surface by affinity capture with surface-immobilized streptavidin.

10:20am **BI+SS-WeM7 Thiol-Reactive Surfaces for the Specific Immobilization of Biomolecules**, ***R.L. Cicero***, *D. Martin, S. McManus-Munoz, C.E.J. Dentinger, P. Kernen, P. Wagner,* Zyomyx Inc.

We present multi-component self-assembled monolayers on Au(111) surfaces that extend thiol-reactive groups and chemical moieties known to resist non-specific adsorption of proteins. Particular attention will be paid to the structural characterization of these surfaces using X-ray photoelectron spectroscopy, reflection absorption infrared spectroscopy and ellipsometry. We also investigate the effect surface density of thiol-reactive groups presented at the solid-liquid interface has with respect to homogeneity, efficiency and specificity of immobilizing thiol-containing biomolecules to these surfaces. Several methods for detecting and quantifying biomolecule immobilization are used including surface plasmon resonance spectrometry, radiometry and fluorimetry.

10:40am **BI+SS-WeM8 Control and Detection of Surface Immobilized DNA Interactions Using Electrostatically Assisted Surface Plasmon Resonance**, ***R.J. Heaton***, *A.W. Peterson, L.K. Wolf,* Boston University*; R.M. Georgiadis,* Boston University, usa

Using Surface Plasmon Resonance (SPR) spectroscopy, we demonstrate that the formation of an immobilized DNA film on gold can be controlled by non-Faradaic electrostatic charging. Furthermore, electrostatic charging can be used to enhance interactions between the immobilized probe film and target oligonucleotides in solution. By simply adjusting the potential across the surface thiol-modified single stranded oligonucleotide films can be formed with good reproducibility in coverage. The application of an attractive potential can speed up and maximize the hybridization of complementary and mismatched oligonucleotides to the probe film. We demonstrate the efficacy of this technology with application to both single area and array-mode SPR.

11:00am **BI+SS-WeM9 Non-Uniform Mixing in Fluid Surfaces**, ***J.S. Hovis***, *S.G. Boxer,* Stanford University

Cell membranes are two-dimensional heterogeneous fluid surfaces comprised of lipids, proteins, and carbohydrates. Understanding their organization at the molecular level is of critical importance for understanding cellular function. One of the key features, of the cell membrane is it's fluidity, which precludes long range order. However, due to the heterogeneous nature of the system it is possible that non-uniform mixing occurs, resulting in the local enhancement of certain membrane components. We will present results from our studies designed to probe for one type of domain, termed 'lipid rafts', using model membranes. In particular, we use supported lipid bilayers that are partitioned; the partitioning enables us to spatially contain the membrane components. By applying an electric field in the plane of the bilayer we can rearrange the membrane components in the partitioned regions. Sphingolipids and cholesterol, the major components of lipid rafts are electrically neutral and will not reorganize in response to a field; however, GM@sub 1@, a minor component, will as it has a net negative charge. Using epi-fluorescence microscopy we monitored the resultant electric field induced reorganization of the membrane components. Our results indicate that the reorganization of the GM@sub 1@ induces a reorganization of the sphingolipids and cholesterol. However, this reorganization does not appear to be concerted, suggesting that the rafts are not long-lived structures. That is, there is an increased propensity for certain components to be in close proximity to one another, but due to the fluid nature of the lipid bilayer, individual components are not in close proximity for long. This work will hopefully provide additional insight into understanding how non-uniform mixing occurs in these fluid surfaces and what the functional consequences are.

11:20am **BI+SS-WeM10 Vesicle to Supported Bilayer Transformation Kinetics; Influence from Vesicle Size, Temperature and Surface Support**, ***E. Reimhult***, *K. Dimitrievski, V.P. Zhdanov, F. Höök, B. Kasemo,* Chalmers University of Technology, Sweden

Supported phospholipid bilayers (SPB) on solid surfaces are biologically functional components of high current interest, e.g., for biosensors, tissue engineering, and basic science (Sackman, Science 271:43 (1996)). We investigate how the kinetics of vesicle to bilayer transformation on SiO@sub 2@ depend on vesicle size using small Extruded Unilamellar Vesicles (EUV; diameter~30-200 nm) and Small sonicated Unilamellar Vesicles (SUV; diameter~25 nm)) and temperature (T~5 to 30°C).). The experimental results are complemented by computer modeling and MC simulations. Our results reveal weak but significant vesicle size-dependent kinetics. The rate and completeness of the vesicle-to-bilayer transformation is strongly dependent on temperature and the vesicle-to-bilayer formation on SiO@sub 2@ can under certain circumstances be completely inhibited at low temperatures. In addition, the vesicle-surface interaction was investigated for various surfaces, including oxidized Au, Pt and Ti, which all demonstrate adsorption of vesicles in an intact state independent of vesicle size and temperature. The obtained results extend our previous studies at constant vesicle size and temperature (Keller et al, Phys Rev B 61: (3) 2291 (2000)) and constitute a platform that will significantly improve the possibility to control the process on µmm-nm fabricated surfaces, from which more complex functional supported bio-membranes are constructed.

11:40am **BI+SS-WeM11 Functional Tethered Lipid Membranes on Gold**, ***K. Bender***, Stanford University

A solid supported, biomimetic lipid bilayer was formed on a gold substrate by adsorbing lipid vesicles on a self-assembled monolayer (SAM) consisting of thiol-lipopeptides mixed with thiol-peptides. The membrane was bound to the surface by the thiol-lipopeptides, the thiol-peptides being used to change the surface concentration of thiol-lipopeptide and hence control membrane fluidity. The lipid bilayer was formed by fusion of L-@alpha@-Phoshatidylcholine (eggPC) liposomes onto the mixed thiol-lipopeptide / thiol-peptide SAM. The free lipids replenish the tethered lipid layer and also form the second layer to complete the bilayer. A functionalized lipid bilayer was formed by incorporating H@super +@-ATP-synthase (extracted and purified from spinach chloroplastes) with the eggPC. This enzyme is a membrane integral protein that can synthesize or hydrolyze adenosine triphosphate (ATP) from or to adenosine diphosphate (ADP) and in doing so pumps H@super +@ through the bilayer. Impedance spectroscopy measurements demonstrated that the enzyme had not lost its biological functionality and was still active. The formation of the lipid bilayer was detected by using surface plasmon resonance spectroscopy (SPS). Finally Annexin V, a pore forming protein, was immobilised on a lipid bilayer by using Ca@super 2+@-ions to bind (by chelation) the negatively charged parts of the protein to the negatively charged lipids (1,2-Dimyristoyl-sn-Glycero-3-Phoshatidylserin) in the bilayer. Annexin V as a non-integral protein and its function as a passive ion transporter through the lipid bilayer was used for comparison of the active ion transporter H@super +@-ATP-synthase. The same techniques as described above were used to observe the formation and activity of this system.

Author Index

Bold page numbers indicate presenter