Monday Morning, October 18, 2010

Marine Biofouling Topical Conference
Room: Navajo - Session MB+BI-MoM

Understanding Marine Biofouling
Moderator: S. Zauscher, Duke University

8:40am **MB+BI-MoM2 Colloidal Theories of Bacterial Attachment as Applied to Marine Bacteria: A Necessary Revision?**, ***L.K. Ista***, University of New Mexico, *G.P. Lopez*, Duke University

The majority of our knowledge of bacterial attachment and subsequent biofilm formation has been gleaned from studies on human pathogens and commensal bacteria with specialized attachment mechanisms and attachment substrata. In contrast many marine microorganisms have a variety of substratum choices, with the added challenge of new types of introduced substrata (boats, piers, pilings) as possible biofilm supports. Maintaining the genetic information needed to produce specific attachment mechanisms for each possible substratum would be maladaptive; it is very likely that marine bacteria exploit their colloid-like size and rely on colloidal interactions to drive attachment. Thus, colloidal models of bacterial attachment are of particular interest to understanding marine microbial attachment. Current models of bacterial attachment are useful for describing some bacterial attachment, but cannot predict attachment behavior in most cases. In this work we examine 3 basic assumptions of the preeminent model used for bacterial attachment, the Lewis Acid Base (LAB) model proposed by van Oss. We used gold-alkanethilate self-assembled monolayers (SAMs) and three marine bacteria to test these assumptions. The first assumption is that apolar interactions include both London dispersion (induced dipole/induced dipole) interactions and those based on fixed dipoles. Using apolar contact angle liquids of either purely London dispersion or London dispersion and dipole/induced dipole interactions we calculated the apolar component of the surface tensions of SAMs and bacteria and observed differences in the estimation of the apolar surface tension and, thus, the total surface tension on polar surfaces. The second assumption is that the Lewis acid and base components of H_{2O} surface tension are equal, which frequently leads to overestimation of Lewis basicity. We calculated surface tensions of bacteria and SAM surfaces with both LAB values and those based on solvatometric hydrogen bonding calculations and observed that the latter gave more reasonable estimation of the free energy of attachment. The third assumption is that interactions can be correlated with average surface energy for the cell. Both our observations and those in the literature have led us to believe this is untrue. We present scanning electron microscopy data that demonstrate that different parts of bacterial cells are in contact with the surface of different SAMs and that SAMs on nanoparticles can identify specific regions of heterogeneity on bacterial cell surfaces. Based on our results we propose modifications to the LAB model that may make it more able to model and predict marine bacterial attachment.

9:00am **MB+BI-MoM3 Reversible Adhesion in Barnacle Cyprids: the Weak Link in Surface Colonisation?**, *N. Aldred*, Newcastle Univ., UK, *I.Y. Phang*, MESA+ Institute for Nanotech. and Dutch Polymer Inst., Netherlands, *T. Ekblad, O. Andersson, B. Liedberg*, Linköping Univ., Sweden, *G.J. Vancso*, MESA+ Institute for Nanotech. and Dutch Polymer Inst., Netherlands, ***A.S. Clare***, Newcastle Univ., UK **INVITED**

Reversible or temporary adhesion allows barnacle cyprids to explore surfaces before they commit to permanent settlement. As such this scarcely studied system is an obvious point of attack for fouling control. The remarkable paired antennules of the cyprid bear attachment discs; hairy adhesive structures that enable the cyprid to walk over surfaces in a stilt-like fashion while resisting detachment by hydrodynamic forces. A proteinaceous secretion at the surface of the disc has been postulated to function as a 'temporary adhesive'. Here evidence is presented that suggests the antennular secretion is related to the adult settlement pheromone – the settlement-inducing protein complex – and that it functions as a reversible adhesive, but in a hybrid wet/dry adhesive system somewhat akin to insect reversible adhesion. While a complete characterisation of the antennular secretion remains an aim, the application of surface analytical techniques (atomic force microscopy and imaging surface plasmon resonance) and direct measures of cyprid behaviour, go some way to providing a mechanistic understanding of why cyprids settle at low rates on certain surfaces, which can be applied to future developments in antifouling technology.

9:40am **MB+BI-MoM5 Chemical Insights on How Shellfish Stick**, *J.D. White, C.R. Matos-Perez, J.R. Burkett, T.W. McCarthy*, ***J.W. Wilker***, Purdue University **INVITED**

Since the very first mariners traversed ocean waters, hulls have become encrusted with fouling organisms such as barnacles, oysters, tube worms, and algae. Antifouling coatings can prevent the resulting loss of vessel performance, but at a cost. Current coatings release toxins into the water, thereby killing the foulers as well as other species. Rather that destroying marine life, stopping bioadhesion processes may provide a benign means of antifouling. Consequently we have been seeking detailed knowledge of how shellfish attach themselves to surfaces. The resulting insights can be used to develop mechanism-based antifouling coatings for inhibiting the production of bioadhesives. Our characterization efforts have focused on the intractable glues and cements of mussels, barnacles, and oysters. A fruitful approach has been to work simultaneously with synthetic peptide models, extracted adhesive proteins, and material produced by the animals. Perspectives from each class of experiments can be complimentary and used to build pictures of how the animals generate their adhesives. Themes in marine bioadhesion are beginning to emerge as well as evidence for unique aspects within each system. Cross-linking of proteins plays a prominent role in curing the glues. Inorganic reactions and related oxidative chemistry also contribute to formation of the materials. Here we will present a summary of our latest findings on how shellfish stick.

10:40am **MB+BI-MoM8 Investigation of Early Marine Biofouling Events on Model Organic and Polymeric Surfaces**, ***G.P. Lopez***, Duke University **INVITED**

Marine biofouling -the accumulation of unwanted biomass on solid structures- is of major concern to maritime pursuits. Biofouling can not only decrease performance of deployed marine equipment, such as ships or oil rigs, but can also result in the transport of invasive species between ports of call. The problem of biofouling is at first a problem of microbial interaction with the water-solid interface; bacteria and diatoms themselves can form detrimental biofilms and can further enable the settlement of macrofoulers. This talk will present studies that seek to shed light on underlying chemical factors that lead to the initial attachment of metabolically homogenous populations of model marine bacterial populations to well-defined organic and polymeric surfaces. It will also present studies of the use of stimuli-responsive surfaces to allow release of attached marine biofilms.

11:20am **MB+BI-MoM10 The Promise of Fouling Deterrence as a Natural Marine Antifouling Strategy**, ***A.S. Mount***, Clemson University **INVITED**

Marine biofouling is the unwanted accumulation of bacteria, algae, plants and marine animals on submerged structures including ships. Unfortunately, man's attempts to develop effective antifouling coatings have had deleterious effects on marine life and a less toxic deterrent to cuprous oxide based paints are needed. Larval marine invertebrates have highly developed sensory organs which investigate surfaces prior to settlement, attachment and metamorphosis. We investigated this tactile chemical sense as a potential natural antifouling strategy by covalently linking the neuroendocrine hormone noradrenaline (NA) to poly(hydroxyethylmethacrylate) and to poly(methacrylic acid) polymer surfaces. NA was selected since it is well established that the soluble form it inhibits larval settlement in mollusks, barnacles, bryozoans and annelid tube worms, all of which are major macrofoulers. The NA conjugate polymer surfaces induced oyster cellular apoptosis when compared to negative controls and also deter the settlement of barnacle and oyster larvae. Fouling deterrence is promising strategy in that only treated surfaces would deter biofouling thus eliminating the need to release of any toxic substances into the oceans.

Marine Biofouling Topical Conference
Room: Navajo - Session MB+BI+AS-MoA

Preventing & Characterizing Marine Biofouling
Moderator: G.P. Lopez, Duke University

2:00pm **MB+BI+AS-MoA1 Zwitterionic Polymers for Non-Fouling Coatings**, ***G. Tew***, University of Massachusetts Amherst **INVITED**

Biofouling remains a challenging problem for various fields ranging from biomedical applications and marine coatings technology, to water purification, transport, and storage systems. To date, the most widely employed protein repellent materials are poly(ethylene glycol) (PEG) or oligo(ethylene glycol) (OEG) based. Even though PEG shows excellent nonfouling character, it has low stability in the presence of oxygen and transition metal ions found in most biochemical solutions, which pushed the field to search for more robust non-fouling materials. Having hygroscopic nature similar to PEG as well as a biomimetic character, arising from their structural similarity to the head groups of lipids comprising cell membranes, zwitterions such as 2-methacryloyloxyethyl phosphorylcholine (MPC) and more recently carboxy/sulfobetaines have also been investigated as protein resistant materials. These materials concentrate only on hydrophilic modification of the substrates. However, the real biological environment is populated by different species, which have different attachment mechanisms; some prefer to adhere more on hydrophilic surfaces whereas others prefer more hydrophobic substrates. The solution to this problem has been investigated by engineering surfaces that reconstruct depending on the environment they are being exposed to, which has been found to be relatively easy to obtain with amphiphilic materials. However, these approaches are either still not sufficient to inhibit bioadhesion by themselves or they suffer from complex or labor intensive coatings preparation conditions. In this work, we are introducing a new polymeric system which carries dual functionality at the repeat unit level, a zwitterionic functionality coupled with an alkyl moiety that can be varied to adjust the amphiphilicity of the overall system. The alkyl group is varied to include PEG based, hydrocarbon, and fluorinated chains. Using these ring-opening metathesis polymerization (ROMP) based zwitterionic polymers as the foundation for non-fouling coatings, we are trying to understand what role the overall hydrophilicity/amphiphilicity of the materials play in fouling prevention.

2:40pm **MB+BI+AS-MoA3 Resistance of Saccharide-Terminated Alkylthiol Self-Assembled Monolayers to Protein Adsorption and Marine Biofouling**, ***T. Ederth***, *T. Fyrner, T. Ekblad, M. Hederos, H.-H. Lee, A. Mangone, P. Konradsson, C.-X. Du*, Linköping University, Sweden, *M.E. Pettitt, M.E. Callow, J.A. Callow*, University of Birmingham, UK, *S.L. Conlan, A.S. Clare*, University of Newcastle, UK, *F. D'Souza, G.T. Donnelly, A. Bruin, P.R. Willemsen*, TNO Science and Industry, The Netherlands, *B.G. Liedberg*, Linköping University, Sweden

The protein resistance of galactoside-terminated alkanethiol self-assembled monolayers (SAMs) can be tuned by partial methylation of the terminating saccharides, and has a non-trivial dependence on the degree of methylation [1], and for other mono- and oligosaccharide-terminated SAMs, protein resistance may vary considerably with small changes in sugar structure. We have used such mono- and oligosaccharide-terminated SAMs in a series of assays using marine fouling organisms as biological model systems, representing common micro- and macrofoulers. We investigate to what extent protein resistance properties are related to effective prevention of fouling by the marine model organisms, and discuss the results in terms of physicochemical properties of the SAMs.

[1] Hederos, M.; Konradsson, P.; Liedberg, B., *Langmuir* **2005,** 21(7), 2971-2980. DOI: 10.1021/la047203b

3:00pm **MB+BI+AS-MoA4 Influence of the Characteristics of a Mineral Coating on its Ability to Resist to the Biofouling**, ***T.H. Tran***, Ecole Nationale Supérieure des Mines de Saint Etienne, France

3:40pm **MB+BI+AS-MoA6 Influence of Physicochemical Surface Properties on the Settlement of Biofouling Microorganisms**, ***A. Rosenhahn***, Karlsruhe Institute of Technology, Germany **INVITED**

When manmade surfaces are immersed into the ocean, biofouling rapidly occurs. To support the outphase of toxic coating formulations from the market we derive design rules for environmental benign alternatives. Therefore we study the interaction of biofouling organisms such as zoospores of the green seaweed Ulva linza with well defined surfaces and disentangle the influence of wetting, hydration, morphology, and charge. The obtained results are discussed in the context of time depending formation of conditioning layers. Especially because of its motility, the settlement step of Ulva is highly selective and crucial in their life cycle. A detailed investigation of the relevant phases of approach, exploration and eventually settlement is desired but challenging due to the quick, three dimensional swimming motions of spores. Digital in-line holography is suited for this application as time lapse holograms recorded with a single detector provide the 3D position of microorganisms with high accuracy and at a high frame rate. From such 4D tracking data, the sensitive response of spores and their interaction with surfaces has been studied. Statistical analysis of the motion pattern occurrence, velocity distributions and turning motions on surfaces with different chemical termination can be correlated with the accumulated biomass. By this we obtain quantitative access to the interaction between single spores and surfaces.

4:20pm **MB+BI+AS-MoA8 Interfacial Spectroscopy: *In situ* Approaches to Understand Sticky Contacts**, ***K.J. Wahl***, *D.E. Barlow, R.K. Everett, C.M. Spillmann*, Naval Research Laboratory, *G.H. Dickinson, B. Orihuela, D. Rittschof*, Duke University Marine Laboratory **INVITED**

5:00pm **MB+BI+AS-MoA10 Solid State Circular Dichroism of Insoluble Bioadhesive Films: Determining Protein Secondary Structure by Concentration Independent Analysis**, ***D.E. Barlow***, *J.L. Kulp, K.J. Wahl*, U.S. Naval Research Laboratory

Far-UV circular dichroism (CD) is a valuable method for estimating protein structure components. Analysis of protein CD spectra typically requires deconvolution to resolve overlapping bands and standard methods require that the concentration and pathlength of the sample are accurately known. While this is usually not an issue for the solution state, it is sometimes desirable or a necessity to analyze samples as solid films, complicating deconvolution. Barnacle cement is one example of a proteinaceous bioadhesive that is insoluble by standard biochemical methods and of inconsistent thickness in the native state. To analyze such samples by CD, we have applied g-factor analysis,[1] where the CD spectra are normalized by absorption spectra. This has been demonstrated as a valid, concentration independent deconvolution method, but so far has not been widely used. We will present protein secondary structure estimation results of barnacle cement films as determined by g-factor analysis and show how these results compare with those obtained by infrared spectroscopy. Potential issues and further applicability of solid state CD for bioadhesion studies will be discussed.

[1] McPhie, P. *Anal. Biochem.* **2001**, *293*, 109.

5:20pm **MB+BI+AS-MoA11 Dissipative Microbalance (QCM-D) Studies of Interfacial Processes at the Nanoscale**, ***M.A. Poggi***, Biolin Scientific

Currently there are many technologies that can study the bulk properties of nanoparticles in solution (such as light scattering) as well as experimental methods that allow one to visualize particles (microscopy or fluorescence). However, there are few technologies that can provide real-time in-situ information regarding how nanoparticles interact with other molecules or materials. Recently we have been using the quartz crystal microbalance with dissipation monitoring technology (QCM-D) to quantify the interaction of particles with surfaces and other materials (biological and organic). We will first present recently published results that address the effect of stagnant and dynamic motion of chemically modified nanoparticles on their adsorption onto silica surfaces. We were able to follow the real-time assembly (in liquid) of these chemically-modified particles. By simultaneously quantifying the changes in surface mass and viscoelasticity during the adsorption process, we were subsequently able to model the adsorption characteristics of these nanoparticles. We will also discuss recent advances that have been made in regards to using QCM-D to follow the assembly of biological nanoparticles (such as cells, viruses and lipids) and polyelectrolytes and touch upon recent electrochemical work that we have been using to study electroactive processes at interfaces.

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