Monday Morning, October 15, 2007

Marine Biofouling Topical Conference

Room: 609 - Session MB+BI-MoM

Biological Interactions at the Marine Interface

Moderator: M. Grunze, University of Heidelberg, Germany

8:00am **MB+BI-MoM1 Surfaces and Signals: Dissecting Surface Properties for Controlling the Settlement and Adhesion of Algae**, ***J.A. Callow****, M.E. Callow*, University of Birmingham, UK **INVITED**

All man-made structures in marine or freshwater environments suffer from the problem of 'biofouling'- the unwanted growth of bacteria (as biofilms), algae (diatoms and seaweeds) and invertebrates (e.g. barnacles, tube-worms). Most current, commercial antifouling strategies use biocide-containing coatings, which are subject to increasingly stringent environmental restrictions. Alternative coating technologies that do not require biocides either invoke 'deterrence' to prevent organisms from sticking in the first place, or the 'non-stick' or 'foul-release' principle to facilitate the detachment of adhered organisms under moderate shear stress. The search for 'environmentally-friendly' solutions has stimulated basic research efforts in an attempt to understand which interfacial properties (e.g. roughness, wettability, charge, friction, elasticity) are important in influencing the adhesion of fouling organisms. This has been facilitated by the advent of novel technologies, such as various forms of lithography, and self-assembly, which enable the production of test surfaces with systematic variations in structure and properties at the micro- and nano-scales. Motile marine organisms such as spores of Ulva, are highly selective in their choice of a suitable surface for settlement. Therefore, one approach to developing practical coatings based upon 'deterrence' is to try to destabilize the surface cue-sensing mechanisms of the organism. The aim of this presentation is to illustrate how spores of the green alga Ulva, and the diatom, Navicula respond to surfaces presenting a range of well-characterised interfacial properties. Examples will be taken from collaborations developed within the ONR Marine Coatings programme and the EC Framework 6 Integrated Project 'AMBIO' (Advanced Nanostructured Surfaces for the Control of Biofouling).

8:40am **MB+BI-MoM3 Cationic Peptide SAMs for Biofouling Studies**, ***B. Liedberg****, P. Nygren, M. Östblom, Y. Zhou, T. Ederth*, Linköping University, Sweden **INVITED**

Positively charged peptides have been synthesized in our laboratory with the purpose of investigating their anti-biofouling potential. The peptides contain a common leading sequence of $Cys(Gly)_2$ where the cysteine residue offers an convenient handle for oriented attachment to gold. The functional part of the peptides (7-mers) consists of alternating ArgTyr, LysTyr, LysTyrGly as well as mixed layers of ArgTyr and a filling $Cys(Gly)_3$ peptide. The peptide layers have been prepared from aqueous solutions and were characterized with respect to layer thickness, conformation and long-term stability in artificial seawater (ASW) using null ellipsometry and infrared spectroscopy. The peptide layers were also examined with respect to settlement of Ulva linza zoospores. The functional ArgTyr, LysTyr, LysTyrGly peptides displayed enhanced settlement as compared to reference surfaces/coatings. Moreover, the settlement on the ArgTyr layer was greatly increased as compared to the other peptide layers, and the amount of settled spores increased with increasing fraction of the ArgTyr. Most importanty, however, the Ulva spores seemed to settle in a side-on conformation on the ArgTyr layer with their flagella (swimming arms) intact. This mode of settlement has not been observed before. Pre-incubation of the Ulva spores in a solution containing the ArgTyr peptide also influenced the subsequent attachment of the spores to polystyrene in a concentration dependent manner. The ArgTyr peptides in solution seemed to stimulate the settlement up to a concentration of ca. 20 µM above which the number of settled spores started to fall. Microscopic examination of the remaining spore solution after settlement suggests that this is due to an increase in spore death. Thus, the Arg residues in the ArgTyr peptide are believed to convey a membrane-associating effect which seems to have a deterring effect upon settling organisms. Complementary experiments on diatoms (a cell surrounded by a silica shell) revealed that these species settle in a non-selective manner on the different peptides. In addition, ongoing experiments using QCM to study vesicle adsorption onto the peptide layers give support to the suggested peptide-membrane association meachanism that resulted in an abnormal, side-on, attachment of Ulva spores. Interestingly, recent preliminary experiments using barnacle cyprids revealed marginal settlement on the ArgTyr peptide layer.

9:20am **MB+BI-MoM5 Mechanics of Barnacle Glue Surfaces and Relation to Foul Release**, ***G. Walker***, University of Toronto, Canada **INVITED**

Recent results concerning the mechanics of barnacle release will be discussed. The fracture mechanics of a whole barnacle will be examined in the context of nanoscale defect formation that is predicted by theory and structures that are observed in the natural cements.

10:20am **MB+BI-MoM8 Understanding Biofouling Mechanisms In Situ: Molecular Level Studies on Polymer Surface Structures in Water and Polymer-Protein Interactions**, ***Z. Chen***, University of Michigan **INVITED**

Biofouling, the growth of barnacles, seaweeds, tubeworms and other marine organisms on the hulls of ocean-going vessels, causes many problems for the US government including extra financial burdens, excessive consumption of energy, and contamination of the environment. Minimally adhesive polymers or polymers from which foulants can be easily removed are being developed as coatings for use in the marine environment. The possibility to use materials with biocides for marine anti-biofouling purposes have also been explored recently. In our group, a second-order nonlinear vibrational spectroscopic technique, sum frequency generation (SFG) vibrational spectroscopy, has been applied to study surface structures of polymer materials which may be developed as fouling control/release coatings in water, to study interactions between polymer surfaces and biological molecules in situ, and to study how biocides which are being developed for marine anti-biofouling purposes interact with cell membranes. Other techniques such as attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR), quartz crystal microbalance (QCM), atomic force microscope (AFM), and contact angle goniometer have been used as supplemental tools in such studies. The polymers which have been studied include model polymers such as polymethacrylates, model poly (dimethyl siloxane) (PDMS), commercial PDMS samples, as well as newly developed anti-biofouling polymer coatings. We elucidated that different polymer surfaces exhibit varied restructuring behaviors in water. To understand polymer surfaces in aqueous environments, it is necessary to investigate them in situ. By examining interfacial protein structures, we showed that different polymer surfaces mediate differed molecular interactions with adhesive proteins of marine organisms. Detailed structural information of proteins at the polymer/protein solution interface can be deduced. In addition, we elucidated the molecular interactions between biocides or polymers containing biocides and cell membranes. Such studies provide molecular level information regarding surface - biological molecule interactions, aiding in the design of coatings with improved anti-biofouling property.

11:00am **MB+BI-MoM10 Surface Tension and Bacterial Attachment Revisited: The Effect of Components of Interfacial Tension**, ***L.K. Ista****, B.P. Andrzejewski, K. Artyushkova, D.N. Petsev, G.P. Lopez*, The University of New Mexico

Bacterial adhesion to surfaces is controlled by the relative strengths of interfacial tensions: those between the bacterium and the surface, the bacterium and the liquid and the surface and the liquid. Models of precisely which properties of the substratum and bacteria most profoundly and predictably affect the attachment of bacteria to a sold surface, as well as methods for accurately and predictively measuring these interfacial tensions, abound, but, to date, none is, in and of itself, sufficient to accurately predict bacterial attachement. A well defined and characterized series of self-assembled monolayers (SAMs) of ω-terminated alkanethiolates on gold were used to systematically explore the effect of the various surface energetic components (e.g. polar, non-polar, electron donating and accepting) of solid substrata on the attachment of the marine bacterium, Cobetia marina. The surface energy of the SAM surfaces was calculated from contact angle data obtained from several different solvents and using different models for calculation, to determine which of these methods most accurately predicted bacterial adhesion. It was discovered that a combination of factors, specifically, the hydrophobicity and Lewis acidity of a surface, promoted the greatest adhesion to these surfaces. A model for adhesion of C. marina has been derived using this measurement. The general applicability of this model to organisms of different surface compositions and environmental niches has been explored. General and specific trends for bacterial adhesion will be discussed.

11:20am **MB+BI-MoM11 Interface Chemistry and Mechanics of Barnacles**, *G.H. Dickinson,* Duke University Marine Laboratory*, D. Ramsay, J.N. Russell, Jr.,* U.S. Naval Research Laboratory*, D. Rittschof,* Duke University Marine Laboratory*, **K.J. Wahl***, U.S. Naval Research Laboratory

Barnacles adhere to all kinds of surfaces in the ocean. These animals settle as larval cyprids, and attach by exuding a protenaceous adhesive. Only recently, with the study of soft and transparent release coatings, has the investigation of the adhesive mechanisms of the barnacle base plate become accessible. We are employing a combination of optical and mechanical spectroscopy to understand the near surface properties of barnacles. Here we report on studies of Amphibalanus amphitrite (little striped barnacle) using a combination of micro-Raman and Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) spectroscopies, Atomic Force Microscopy (AFM), and micromechanical compression testing to investigate base plate chemistry and mechanics. For barnacles grown on release surfaces, two base plate phenotypes are found - those with a 'hard,' thin adhesive morphology, and those with 'gummy' or compliant adhesive morphology. Micro-Raman, ATR-FTIR, and AFM spectroscopies show significant differences in the protein structure and mineralization of hard and gummy. ATR-FTIR spectroscopy of hard and gummy barnacles, with and without seawater present, showed strong time-dependent responses during reattachment. Mechanical differences between hard and gummy base plates were examined using a custom instrumented flat-punch mechanical tester. The base plate mechanical properties of composite moduli are of order 2 to 5 GPa, with statistically lower modulus for gummy barnacles. The overall flexibility of the barnacle base plate was similar for both hard and gummy barnacles, due to compensating morphologies. Release properties will be discussed in relation to base plate morphology and flexibility.

Marine Biofouling Topical Conference

Room: 609 - Session MB+BI-MoA

Control of Marine Bioadhesion

Moderator: G.P. Lopez, University of New Mexico

2:00pm **MB+BI-MoA1 Engineered Polymer Coatings for Foul-Release Applications**, ***J. Genzer***, *A.E. Özçam, K. Efimenko*, North Carolina State University **INVITED**

Marine biofouling is a serious and complex problem resulting in losses of operating efficiency of ships. Current coating technologies derived from copper- and tin-based compounds are being banned because of detrimental effects on marine environment. Hence there is need for developing efficient marine coatings that would possess no ecological concern. In addition to various chemical approaches, surface topography has also been shown to be important for mechanical defense against biofouling. For instance, Hoipkemeirer-Wilson and coworkers reported that topographically corrugated surfaces are capable of reducing biofouling. The degree to which fouling was reduced was found to depend on the dimensions of the geometrical protrusions as well as the chemistry of the surfaces. Because biofouling includes a very diverse range of various species, whose sizes span several orders of magnitude, one single topographical pattern will not likely perform as an effective antifouling surface. Rather, surface corrugations having multiple length scales acting in parallel should be used in designing effective antifouling surfaces. We have developed a method leading to substrate comprising hierarchically wrinkled (H-wrinkled) topographies. These specimens were prepared by first uniaxially stretching poly(dimethylsiloxane) films, exposing them to ultraviolet/ozone (UVO) radiation for extended periods of time (30-60 minutes), and releasing the strain. After the strain was removed from the specimens the surface skin buckled perpendicularly to the direction of the strain. A detailed analysis of the buckled surface uncovered the presence of hierarchical buckling patterns; buckles with smaller wavelengths (and amplitude) rested parallel to and within larger buckles, forming a nested structure. At least 5 distinct buckle generations (G) were detected with their wavelengths ranging from tens of nanometers to a fraction of a millimeter. The method for producing coatings with H-wrinkled topographies may represent a convenient platform for designing foul-release surfaces. Our recent sea water immersion experiments involving testing over an extended time period indicated that these coating are far superior to flat coatings. We will discuss how the H-wrinkle topographies can be applied to make coatings from just about any type of material and offer methodologies for preparing amphiphilic foul-release coatings from commercially available materials. While more work still needs to be done, the initial observations suggest that the H-wrinkled coatings may represent a new and promising platform for fabricating efficient foul-release marine coatings.

2:40pm **MB+BI-MoA3 Nanoscopically-resolved Amphiphilic Surface Features as Non-toxic, Treacherous Terrain to Inhibit Marine Biofouling**, ***K.L. Wooley***, Washington University in Saint Louis **INVITED**

This presentation will detail amphiphilic nanostructured material systems, constructed from a general methodology that involves the kinetic, in situ crosslinking of thermodynamically-driven phase segregated states of polymer assemblies. Macroscopic crosslinked networks composed of amphiphilic nanodomains presented on the surface and dispersed throughout the material are obtained by crosslinking of the assemblies in bulk samples. Of particular interest for these materials are the complex surface topographies and morphologies that allow for the materials to exhibit antifouling characteristics. The crosslinked macroscopic networks have been focused upon compositions that include hyperbranched fluoropolymers and linear poly(ethylene glycol)s, although the compositional profiles are being expanded. The domains or channels that are present throughout the samples offer interesting opportunities for the packaging and release of guest molecules. The nanoscale dimensions and large interfacial surface areas provide for high loading capacities within uniform host environments, but then also promote the release of these guests at significantly reduced temperatures. The uptake and release of guests from hydrophobic vs. hydrophilic, of varying compositions, structures, and sizes, will be discussed. Most recently, unique mechanical properties have also been observed, and these data will also be presented.

3:40pm **MB+BI-MoA6 Basic Surface Properties and Their Influence on the Adhesion of Marine Organisms**, ***A. Rosenhahn***, University of Heidelberg, Germany **INVITED**

The prevention of biofouling is a major challenge for all man made objects which are in long term contact with seawater. In order to systematically develop non toxic coatings, a fundamental understanding of basic surface properties that inhibit or encourage settlement of marine inhabitants is required. Together with biological partner groups within the EU IP AMBIO[1] we investigate the influence of surface properties such as wetting, charge or morphology on the adhesion and on the removal properties for different marine organisms. To tune wetting and chemical surface properties, self assembly is used as highly versatile technique. For preparation of well defined micro- and nanomorphologies, different lithography and multilayer approaches are used. The interaction of different marine inhabitants with these surfaces will be discussed and compared to general protein resistive properties. Although one main focus of this work is inhibition of settlement, also release properties are tested as important measure of adhesion strength. Apart from established ways of evaluating anti fouling properties, we use digital in-line holography as new tool to study and compare the exploration of different surfaces by swimming marine organisms.[2] Following the original idea of D. Gabor,[3] coherent scattering of radiation can be used to record scattering patterns which contain three dimensional information about investigated objects due to the presence of a reference wave. Holography therefore allows the investigation of three dimensional processes e.g. by tracking particles in real time with sub¬micrometer resolution.[4] We use this novel technique to visualize and analyze the motion and exploration behavior of swimming marine organisms towards surfaces with systematically changing properties. The goal of these three dimensional tracking experiments is to gain a more detailed understanding about surface sensing and the early attachment stages of marine organisms.

[1] Ambio : Advanced Nanostructured Surfaces for the control of biofouling, FP6 EU integrated project, http://www.AMBIO.bham.ac.uk/

[2] M. Heydt, A. Rosenhahn, M. Grunze, M. Pettit, M.E. Callow, J.A. Callow, The Jornal of Adhesion, in press

[3] D. Gabor, Nature 1948, 161, 777

[4] W.Xu, M.H. Jericho, H.J. Kreuzer, Opt. Lett. 2003, 28(3), 164

4:20pm **MB+BI-MoA8 Bioresponse to Engineered Topographies**, ***A.B. Brennan***, University of Florida **INVITED**

This study examines hierarchical combinations in polymers that have been used to produce engineered surfaces, which elicit micro-topographical and chemical cues in biological systems. Nature provides complex chemical forms of polymers that are manipulated through both conformational and configurational forms to yield specific functions. Our recent studies have been focused on the design of polymeric surfaces that can be used as models in the study of biological adhesion mechanisms. The recent expansion of bioengineering has increased our need for better models of cellular adhesion and chemical manipulation of surfaces. A process commonly referred to as contact guidance has been shown to modulate cell shape and function in a variety of cell types. Control of endothelial cell (EC) shape using micropatterned chemical substrates is shown in numerous studies by influencing cell adhesion to proteins, which selectively adsorb to the chemical micropatterns. This presentation will focus on the polymer structures that we have been developing for a topographically modified surface with a range of surface energies and bulk modulus values developed through nanostructural modifications on larger microstructures. In this model, we have been able to study the interactions of the biological-induced factors with the polymer chemistry.

5:00pm **MB+BI-MoA10 Development of Environmentally Benign and Durable Nonfouling Marine Coatings**, ***S. Jiang***, University of Washington

Biofouling on ship hulls and other marine surfaces has become a global environmental and economic issue. Traditionally, the best antifouling coating is TBT (tributyltin)-based paint. Due to increased environmental concern, TBT antifouling coatings have been restricted. Non-toxic, fouling-release coatings based on silicone or fluorinated compounds are under development. These coatings are only effective on vessels moving at high speeds. Currently, we are developing environmentally benign, durable, and cost-effective nonfouling coatings, to which marine microorganisms can not attach, as the next-generation marine coatings. In this work, zwitterionic-based materials will be shown to be effective against various marine microorganisms in laboratory and field tests. We have demonstrated for the first time that poly(sulfobetaine methacrylate) [p(SBMA)] and poly(carboxybetaine methacrylate) [p(CBMA)] based materials and coatings are superlow biofouling. In addition, we have explored various approaches to apply p(SBMA) or p(CBMA) materials onto surfaces and developed several noncoatings for marine applications. Laboratory tests confirmed the excellent performance of sulfobetaine (SB)-based coatings against marine microorganisms (Ulva spores and barnacle cypris larvae). Recently, we developed SB-based paints and spray-coated them onto surfaces covered with an epoxy primer. Initial field tests of these panels clearly demonstrated that our coatings effectively deferred the settlement of hard foulants. Because of their excellent stability and high effectiveness at preventing microorganisms from adhering to surfaces, SB and carboxybetaine-based materials are excellent candidates for marine coatings. The objective of our work is to create products that will effectively defer biofouling under static conditions over a long period of time.

Marine Biofouling Topical Conference

Room: 4C - Session MB+BI-TuP

Marine Biofouling Poster Session

MB+BI-TuP1 Contact Angle Analysis for Barnacle Adhesives, ***E.R. Holm***, *R.A. Brizzolara*, Naval Surface Warfare Center, Carderock Division

The key step in the accumulation of biofouling on immersed surfaces is the permanent attachment of fouling organisms. Patterns of attachment and adhesion of biofouling, in response to surface properties, vary both among and within species. This variation may be mediated by interactions between surfaces and biological adhesives. We have been studying this interaction for the barnacle, Balanus amphitrite. Our initial approach addresses the propensity of barnacle adhesive to wet modified glass surfaces and commercially-available and experimental silicone fouling-release coatings. Glass surfaces were modified with various organosilane coatings to produce a range of water wettabilities. We verified attachment of organosilane to glass with XPS. We have been quantifying wetting by barnacle adhesive as contact angle. Preliminary results, for modified glass surfaces, indicated that for hydrophilic surfaces, contact angles for adhesive were equivalent to that for water. As surfaces became more hydrophobic, however, contact angles for barnacle adhesive became more variable than those for water. Individual measurements were occasionally substantially lower than typical water contact angles. Differences in contact angle among adhesive samples may be related to the protein content of the adhesive, which varies strongly among individual barnacles. Results will be related to attachment of larval barnacles and adhesion of adult barnacles. Funded by the NSWC Carderock Division In-House Laboratory Independent Research Program.

MB+BI-TuP2 Poly(ethylene glycol)-based Anti-biofouling Surfaces, ***T. Ekblad***, *G. Bergström, C.-X. Du, T. Ederth, B. Liedberg*, Linköping University, Sweden

This work describes the fabrication, characterization and biological evaluation of homogeneous and patterned hydrogel films, used as model coatings in anti-fouling experiments. The work is a part of an EC-initiative on Advanced Nanostructured Surfaces for the Control of Biofouling, AMBIO. The hydrogels consist of poly(ethylene glycol)-containing methacrylate monomers that are UV-grafted onto solid supports, e.g. silanized glass. The physical and chemical properties of these films have been studied using ellipsometry, FT-IR, AFM and a range of other surface characterization techniques. A key property of the hydrogels is that they appear to be resistant to protein adsorption from complex biofluids, including plasma and serum.[1] These observations encouraged us to test the hydrogels as anti-fouling surfaces. Hydrogels, ca. 30 nm thick, were prepared and evaluated in settlement and removal assays using a range of organisms, including barnacle cyprids of the species Balanus amphitrite, Ulva linza zoospores, Navicula diatoms and the three bacteria species Cobetia marina, Marinobacter hydrocarbonoclasticus and Pseudomonas fluorescens. It is clear from the results that the hydrogel surfaces display excellent antifouling properties. All tested organisms displayed significantly reduced settlement compared to reference coatings. The removal of settled organisms generally appeared to be less affected by the surface coating. Though the relationship is not yet confirmed, this study demonstrates that a surface with low protein adsorption also can have advantageous anti-biofouling properties. The broad-spectrum effect of the hydrogel coating does undoubtedly imply that the selected poly(ethylene glycol) chemistry acts on a fundamental stage in the settlement process of biologically diverse organisms. This stage may be the adsorption of biomolecules from glues released by the settling organisms.

[1] A. Larsson, T. Ekblad, O. Andersson, B. Liedberg, Biomacromolecules 2007, 8, 287-295.

MB+BI-TuP3 The Dynamics of Two Species of Megabalanus (Crustacea: Cirripedia: Balanidae) by a Cellular Automata Model, ***M. Apolinário***, PETROBRAS, Brazil, *A. Racco*, LNCC, Brazil

The Cellular Automata (CA) model was used in a computational simulation between an introduced species in Brazilian, Rio de Janeiro State's waters Megabalanus coccopoma and a cryptogenic species Megabalanus tintinnabulum (Crustacea: Cirripedia: Balanidae), obtaining a time series where M. tintinnabulum firstly occurs alone and then it interacts with the entrance of M. coccopoma in the system. The simulation also gets data about spatial distribution of both species and column formation, representing the specimens' assessment one above the other, as it occurs at the natural environment. The results show that total recruitment of each species within the maximum height of the columns is important for the predominance of the introduced species in comparison with the cryptogenic one. The comparison between CA model and natural distribution of both species shows that CA represents significantly the interaction between both species of barnacles in the studied area.

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