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.

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