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13. ABSTRACT (Maximum 200 words) Approximately 25 scientists from around the world participated in a workshop aimed at discussing molecular interactions at marine interfaces from two perspectives: the chemistry of adhesive polymers and the biology of structures that serve as attachment organs of marine organisms. Detailed presentations of each area was complemented by joint discussions of the two perspectives in order to achieve interaction on the research programs supported by ONR.				
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September 26, 1994

To Whom It May Concern:

SUBJECT: N00014-93-1-0491

Enclosed is my report of a workshop held at The University of North Carolina on April 20-22, 1993, entitled "Molecular Interactions at Marine Interfaces". Included is a Report Documentation Page (Form 298) and a copy of the Workshop Proceedings.

If I can be of any further assistance, do not hesitate to contact me.

Sincerely yours,

Ralph S. Quatrano
John N. Couch Professor and Chair

RSQ:sk

cc: Mr. Scott Blackwood
Contracts and Grants
UNC-CH

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Molecular Interactions at Marine Interfaces

Friday Center, University of North Carolina
April 20-22, 1993

Molecular Interactions at Marine Interfaces (MIMI)

Friday Center, University of North Carolina

April 20-22, 1993

Tuesday April 20th

8:30 Hal Guard & Randall Alberte: Welcome and Program Overview

Minimally Adhesive Polymers

- | | | |
|-------|---------------------|---|
| 9:00 | Ken Wynne | <i>Minimally Adhesive Polymers - Overview</i> |
| 9:30 | Joseph Gardella | <i>Surface Chemistry of Multicomponent Polymers for Potential Non-Fouling Coatings: Air and Metal Interfacial Issues</i> |
| 10:00 | Toby Chapman | <i>Segmented Polyurethanes</i> |
| 10:30 | <i>Coffee Break</i> | |
| 11:00 | Chris Ober | <i>Self-Healing, Low Energy Polymer Surfaces using Block Copolymers</i> |
| 11:30 | Aslam Malik | <i>Future Direction in Oxatane-derived Polymers</i> |
| 12:00 | <i>Lunch</i> | |
| 1:00 | Jim Griffith | <i>Synthesis and Evaluation of Fluoroepoxy Resins Cured with Amine-terminated Polydimethylsiloxane Oligomers for Use in Marine Environments</i> |
| 1:30 | Kurt Baum | <i>Polyurethanes Based on Hybrid Fluorocarbon Polysiloxanes</i> |

Bioadhesive Surface Interactions

- | | | |
|------|--------------------------|--|
| 2:00 | John Rabolt | <i>Characterization of Polymer Surface Interactions by Spectroscopic Techniques</i> |
| 2:30 | Tom McCarthy | <i>Chemical Control of Adsorption at Polymer-Aqueous Solution Interfaces</i> |
| 3:00 | <i>Coffee Break</i> | |
| 3:30 | Georges Belfort | <i>Direct Measurements of the Intermolecular Forces between Polysaccharide Exopolymers from Marine Bacteria and solid Substrates</i> |
| 4:00 | Dave Hartsough | <i>Development of Theoretical Tools for Studying Carbohydrate Structure, Dynamics and Sugar-Surface Interactions</i> |
| 4:30 | Open Discussion | |
| 5:30 | <i>Wine & Cheese</i> | |

Wednesday April 21st

- 8:30 Dave White *Effect of Polysaccharide Surface Structure on Microbial Attachment and Biofilm Formation*
- 9:00 Michael Sinnott *Structure of Extracellular Polysaccharides of *Pseudomonas atlantica**
- 9:30 Herb Waite *Interfacial Culprits: Targeting Proteins of Byssal Adhesion*
- 10:00 Ralph Quatrano *Presence and Role of a Vitronectin-Like Protein in Adhesion of Fucus Embryos to Marine Substrates*
- 10:30 **Coffee Break**
- 11:00 Kyle Hoagland & Michael Gretz *Biochemistry of Fouling Marine Diatom Adhesives and the Effects of Substrate Preconditioning on Adhesion*
- 11:30 Gill Geesey *Characterization of Molecular Interactions between "Conditioned" Polymer Surfaces and Microbial Biofilm Matrix Polysaccharides*
- 12:00 **Lunch**
- 1:00 David Kirchman *Molecular Characterization of Specific Attachment by a Marine Bacterium*
- 1:30 Amanda Goodman *Genetic Responses of Bacteria in Biofilms*
- 2:00 Steve Snyder *Navy Biofouling Programs*
- 2:30 **Coffee Break**
- 3:00 Working Session: Emerging Research Directions for MIMI Program
- 5:30 **Wine & Cheese**

Thursday April 22nd

- 9:00 Working Session: Research Collaborations & New Approaches
- 10:30 **Coffee Break**
- 11:00 Plan for next investigators' meeting
- 11:30 Adjourn (lunch on your own)

Surface Chemistry of Multicomponent Polymers for Potential Non-Fouling Coatings: Air and Metal Interfacial Issues

Joseph A. Gardella

Studies underway in our laboratory funded by ONR MIMI include the quantitative surface characterization of various polymers at the air and metal interface. Studies include the synthesis of surface modifications meant to promote and prevent adhesion where appropriate for marine coating applications. Analysis utilizes various electron and optical spectroscopic, microscopic, mass spectrometry and contact angle measurements of the surface and interfacial composition, where appropriate.

Two projects will be described in this presentation. Studies of polyurethane-siloxane systems¹ (including segmented copolymers and homopolymer-copolymer blends) have been pursued, with the general goal of providing a quantitative surface and in depth analysis of the amount of surface excess of siloxane chain as a function of segment length, bulk composition and processing treatments (i.e. solvents casting, annealing, etc.). A critical feature of these results is the understanding of the factors which govern the surface excess of siloxane, and these studies complement a variety of other systems examined in this laboratory², allowing conclusions about the nature of interactions between components in a multicomponent polymer which control the resulting surface excess of a lower energy component. Quantitative surface analysis of these systems will be presented.

The second project presented will focus on the surface modification of fluoropolymers to promote and control metal adhesion. In combination with researchers at the Naval Research Laboratory, we have combined electroless metal deposition from covalently bound precursors with the controlled lithographic modification of FEP teflon³, allowing a simple three step metal deposition with strong adhesion⁴. This process requires no high cost treatments, consisting of low wattage plasma treatments and aqueous based metallization chemistry. At a fundamental level, this work shows the value of covalently bound functional group precursors to promoting strong metal adhesion at the interface of a low surface energy polymer.

Finally, future work in the development of new surface analytical methods will be described. One major goal of this ONR sponsored program is to make available these technologies to all MIMI researchers.

1. T. Ho, X. Chen, J. A. Gardella, Jr. and K. J. Wynne, work in progress.

2. X. Chen, H. F. Lee and J. A. Gardella, Jr. "The Effects of Structure and Annealing on the Surface Composition of Multi-block Copolymers of Bisphenol A Polycarbonate and Poly (Dimethyl Siloxane)." *Macromolecules*, submitted Dec 1992.

3. US Patent #4,946,903 "Modified Fluorinated Polymer Compositions Having Increased Surface Energy Properties" T. G. Vargo and J. A. Gardella, Jr., Co-Inventors. D. J. Hook, T. G. Vargo, J. A. Gardella, Jr., K. S. Litwiler and E. V. Bright "Silanization of Radio Frequency Glow Discharge Modified Expanded Poly (tetrafluoroethylene) (ePTFE) Using Aminopropyltriethoxy Silane (APTES)," *Langmuir*, 1991, 7, 142-51. T. G. Vargo, P. M. Thompson, L. J. Crenner, R. F. Valentini, P. Aebischer, D. J. Hook and J. A. Gardella, Jr. "Monolayer Chemical Lithography and Characterization of Fluoropolymer Films," *Langmuir*, 1992, 8, 130-134.

4. T. G. Vargo, J. A. Gardella, Jr., and J. M. Calvert, "Electroless Deposition of Metals onto Fluoropolymeric Substrates," *Journal of the American Chemical Society*, in preparation.

Segmented Polyurethanes

Toby M. Chapman

Segmented polyurethanes have been prepared with special soft blocks consisting of N-alkylated polyamides and polyurethanes. If the alkyl substituents are fluorinated, there is a profound effect on the surface contact angle with water. Depending upon the method of measurement, contact angles as high as 105-111° have been measured. We are also preparing amphiphilic dendrite polymers with fluorinated exteriors which we hope will position themselves and anchor at the air interface of a polyether polyurethane.

Self-Healing, Low Energy Polymer Surface Using Block Copolymers

C. K. O'er and E. J. Kramer

Self-healing low energy polymer surfaces are being produced by doping higher surface energy homopolymers with block copolymers containing one block which is miscible with the homopolymer and another which contains either siloxane or fluorinated moieties. Model block copolymers have been synthesized by first producing a block copolymer of polystyrene and 1,2-isoprene by anionic polymerization. Hydrosilylation has been used to couple siloxanes and appropriately modified fluorinated alkanes to the vinyl groups on the block copolymer. The surface excess and segment density of the block copolymer as it segregates to the surface of mixtures of the block copolymer and polystyrene is being measured by forward recoil spectrometry (FRES) and RBS. Measurements of the surface adhesion energy (JKR technique) is being correlated with measurements of the contact angle of water on the surface to establish segregation behavior to, and energy of, the resulting polymer surfaces.

Future Direction in Oxatane-derived Polymers

Aslam Malik

No abstract available.

Synthesis and Evaluation of Fluoroepoxy Resins Cured with Amine-terminated Polydimethylsiloxane Oligomers for Use in Marine Environments

Ann E. Mera and James R. Griffith

Various difunctional and trifunctional fluoroepoxy resins have been cured with two amine-terminated polydimethylsiloxane oligomers of different molecular weights. The cured films have been characterized to determine glass transition temperatures, water contact angles, equilibrium water absorptions, and surface adhesivity. For each property the effects of the relative amounts of silicon and fluorine in the cured resin are discussed along with compatibility and reaction behavior of the starting materials. The cured resins are being evaluated for their suitability as products with exceptional merit for use in marine environments.

Polyurethanes Based on Hybrid Fluorocarbon Polysiloxanes

Kurt Baum

The Objective of this program is to prepare and characterize polyurethanes based on diols with hybrid fluorocarbon-polysiloxane backbones. α,ω -Diiodoperfluoroalkanes are reacted with ethylene, and the insertion products are treated with base to give α,ω -diolefins, $\text{CH}_2=\text{CH}-\text{R}_1-\text{CH}=\text{CH}_2$. Hydrosilylation with chlorodimethylsilane gives the chlorosilanes, $\text{ClSi}(\text{CH}_3)_2\text{CH}_2-\text{CH}-\text{R}_1-\text{CH}-\text{SHSi}(\text{CH}_3)_2\text{Cl}$. Hydrolysis gives the corresponding silanols, $\text{HOSi}(\text{CH}_3)_2\text{CH}_2-\text{CH}-\text{R}_1-\text{CH}-\text{CHSi}(\text{CH}_3)_2\text{OH}$. Polysiloxane formation in the presence of 3-trifluoroacetoxypropyl chlorodimethylsilane as an end-cap gives $\text{CF}_3\text{CO}_2-(\text{CH}_2)_3-\text{Si}(\text{CH}_3)_2[\text{O}-\text{Si}(\text{CH}_3)_2\text{CH}_2\text{CH}_2-\text{R}_1-\text{CH}_2\text{CH}_2\text{Si}(\text{CH}_3)_2]_n\text{O}(\text{CH}_2)_3-\text{O}_2\text{CCF}_3$. Ester hydrolysis gives the corresponding diol, $\text{HO}-(\text{CH}_2)_3-\text{Si}(\text{CH}_3)_2[\text{O}-\text{Si}(\text{CH}_3)_2\text{CH}_2\text{CH}_2-\text{R}_1-\text{CH}_2\text{CH}_2\text{Si}(\text{CH}_3)_2]_n\text{O}(\text{CH}_2)_3-\text{OH}$. The use of acetate rather than trifluoroacetate end groups requires hydrolysis condition severe enough to reorganize siloxane units. Elastomeric polyurethanes were obtained with hexamethylene diisocyanate with glass transition temperatures of -50°C .

Characterization of Polymer Surface Interactions by Spectroscopic Techniques

John Rabolt

Polarized FTIR spectroscopy has proven to be an extremely useful technique for the study of molecular topography on surfaces. The sensitivity of this technique has provided the ability to study molecules and polymers in films whose thickness is on the order of 1.5-3.0 nm. The information obtained can be used to assess the extent of orientation and order in thin films and how this changes when a surface is placed in aggressive environments. Some of the opportunities which this technique provides for the study of protein/surface interactions will be highlighted.

Chemical Control of Adsorption at Polymer-Aqueous Solution Interfaces

Thomas J. McCarthy

This talk will describe chemical strategies to study and control the process of adsorption of polymers (in particular, polysaccharides and model proteins) from aqueous solution to the solution - organic polymer interface. Our research involves both (1) controlling the structure of the solid polymer surface through the introduction and further chemistry of versatile organic functional groups (chemically resistant polymers are used as substrates) at the surface and (2) controlling the solution and interface behavior of the absorbing polymer through chemical modification and solution property control. The talk will describe our particular areas of expertise and the physical-organic tools that we can use to control interactions at marine interfaces and pinpoint the areas in which collaborative assistance could benefit our efforts. Specific examples of surface modification and polymer adsorption will be discussed and our strategies to control polysaccharide adsorption will be outlined.

M_w	M_w
400	3000
200	7
100	4
50	

7000 FTIR = polylysine
water
[water, "sea"]

**Direct Measurements of the Intermolecular Forces Between Polysaccharide
Exopolymers from marine Bacteria and Solid Substrates**
Georges Belfort

The kinetics and equilibrium adsorption of an exopolysaccharide (CB15A RsaA EPS), extracted from the fresh water bacteria *Caulobacter crescentus*, was measured for several model hydrophobic and hydrophilic surfaces. Changes in advancing contact angle resulting from adsorption onto these surfaces were determined. Using the surface force apparatus, we plan to measure the intermolecular forces (force-distance profiles) and concentration-distance profiles (from refractive index) between model EPS from marine and fresh water bacteria and various solid substrates such as inorganic mica and polymer films with different surface chemistries under simulated marine conditions. Various theories will be used to predict the concentration-distance profiles and tested against these measurements.

**Development of Theoretical Tools for Studying Carbohydrate
Structure, Dynamics and Sugar-Surface Interactions**
Kenneth M. Merz Jr. and David S. Hartsough

Our long term goal is to develop and exploit theoretical techniques to understand carbohydrate dynamics, structure and function, and the interaction of saccharides with surfaces. Currently there are no potential functions available that model intramolecular and intermolecular carbohydrate interactions for any generalized sequence. Furthermore, structural information concerning surface-carbohydrate interactions is lacking and therefore information that we can obtain using computer simulation techniques will be of great value in understanding these interactions at the molecular level. In an effort to increase our understanding of these systems, we propose the development of a general molecular mechanical potential function which accurately describes intramolecular and intermolecular interactions of carbohydrates. Such a force field will enable us to model any arbitrary polysaccharide in a solution environment and to investigate in detail its dynamic properties over time. We also propose using the newly developed force field to study the interactions and forces between individual saccharide monomers and model surfaces in an aqueous medium of high ionic strength.

Effect of Polysaccharide Surface Structure on Microbial Attachment and Biofilm Formation
David C. White and A. Sonesson

We utilize a flow-through system in which the colonization by bacteria of flat coupons in controlled laminar flow can be monitored non-destructively by measurement of fluorescence of tryptophane in the proteins. Activity of the *Vibrio harveyi* or genetically engineered *Pseudomonas* in which the activity of specific operons can be monitored by their bioluminescence. Preliminary tests indicated coating coupons with uronic acid polysaccharides increased adhesion and biofilm formation over stainless steel. Changes in the chemistry of the polymer-microbe interactions will be monitored with the attenuated total reflectance/Fourier transforming infrared spectrometer as the film forms on germanium crystals. Polymers from genetically manipulated bacteria induce differential biofilm formation. *Pseudomonas* with the bioluminescent *lux* gene cassette with the promoter for *algD* allow testing role of uronic acid polymers in adhesion and give indications of gene action in the biofilm.

Structure of Extracellular Polysaccharides of *Pseudomonas Atlantica*

Michael L. Sinnott

The title organism is known to change from mucoid to non-mucoid forms as a consequence of a genome rearrangement. We are investigating the structures of the polysaccharides produced by the mucoid form of the organism. It produces both a neutral and an acidic polysaccharide. Carbohydrate analysis, and various NMR techniques (DQF-COSY, ^{13}C - ^1H heteronuclear COSY, NOESY, polarization transfer) have revealed that the acidic polysaccharide has a heptasaccharide repeat of five rhamnose units, a β -ribofuranose unit and β -glucopyranose unit, with the acidity probably due to pyruvate groups. A rhamnotriose unit is labile, and its cleavage from the main chain results in precipitation of polysaccharide. A provisional structure will be presented.

Work envisaged for the immediate future involves firming up this structure, and determining the structure of the neutral polysaccharide. In the medium term, questions to be addressed involve the solution conformations of the hydrophobic anionic polysaccharide and the hydrophilic neutral polysaccharide, with reference to their adhesion properties, and the mechanisms of biosynthesis of both polymers.

Interfacial Culprits: Targeting Proteins of Byssal Adhesion

Herbert Waite

The adhesive holdfasts of marine macrofouling organisms contain a consortium of molecules that are endowed collectively with the enviable property of attaching the animals to a wide variety of surface types. To date, nothing but speculation has been advanced to account for the unusual stability and versatility of marine holdfasts. Even in mussel byssus, the most scrutinized of holdfasts, all models regarding the distribution of the 5 major byssal proteins in the vicinity of the interface remain largely speculative. Although some of the proteins are in fact extraordinarily sticky, there is no *direct* evidence implicating any of these at the interface. Mussels oblige the study of the plaque/substratum interface by voluntarily depositing byssal adhesive plaques onto any available substrata. This ingenuous opportunism can be exploited by offering mussels dialysis membranes or other materials with macromolecular porosity. Since such membranes are permeable to discrete ranges of molecular dimensions, the molecules at the interface can be preferentially targeted by reacting fouled emersed membranes on their *nonfouled* sides with various chemicals used for modifying proteins. Modification should be limited only to those proteins or parts of proteins accessible by diffusion from the unfouled side. Targeted peptides will be recovered from partial hydrolysates by conventional HPLC purification. Peptide sequences will be used to generate specific antibodies to isolate soluble precursors.

Presence and Role of a Vitronectin-Like Protein in Adhesion of *Fucus* Embryos to Marine Substrates

Ralph S. Quatrano

Zygotes of the brown alga *Fucus* provide a model system study the basic mechanisms involved in the generation of zygote asymmetry and directional transport of unique cytoplasmic components to the resulting daughter cells. Since the rhizoid cell of the two-celled embryo attaches the developing embryo to the substratum, this system can also be used to study the process of adhesion of this alga to marine substrates.

The rhizoid cell is highly polar and directionally transports (via Golgi vesicles) macromolecules that are secreted into the elongating cell wall or extracellular matrix (ECM). Previous results indicate that a highly sulfated fucan glycoprotein (F2) is locally deposited in the ECM of this elongating tip. If F2 is prevented from being enzymatically sulfated (met-embryos), normal two-celled embryos are formed by F2 is not localized in the rhizoid tip and the embryos do not adhere. Recent results have identified a vitronectin-like (Vnl) molecule with the same pattern of distribution as F2 in the *Fucus* two-celled embryo. Polyclonal antibodies to human vitronectin recognize a glycoprotein in extracts of zygotes and two-celled embryos of *Fucus* with a molecular weight (62-65 kDa) similar to human vitronectin (Vn). The specificity of the immuno-cross-reactivity is further demonstrated using controls of monospecific and non-immune antibodies, and competition by purified Vn. The *Fucus* Vnl molecule can also be isolated by procedures established for mammalian Vn; glass bead and heparin affinity chromatography. Immunolocalization and subcellular fractionation results demonstrate that Vnl is first localized in the cytoplasm of the zygote and later in the ECM of the elongating rhizoid tip. Met-embryos do not exhibit this localized ECM distribution of Vnl and do not adhere to the substratum (similar to the results with F2). In a functional assay, adhesion of *Fucus* embryos is prevented in the presence of the Vn antibody, suggesting that the Vnl plays a role in adhesion as in animal systems. Current work is focused on isolating gene sequences that code for the Vnl protein from marine algae as a first step in understanding the molecular basis of adhesion.

Biochemistry of Fouling Marine Diatom Adhesives and the Effects of Substrate Preconditioning on Adhesion

Kyle Hoagland and Michael R. Gretz

As a prelude to investigations of the physiology and molecular mechanisms of biosynthesis of extracellular polymeric substances (EPS) involved in adhesion during diatom fouling, we are conducting detailed chemical studies on diatom adhesives. We have isolated and mass cultured three diatoms commonly involved in biofouling, the marine stalk-forming diatom *Achnanthes longipes*, the freshwater stalked diatom *Cymbella cistula* (for structure/function comparisons), and the capsule-forming diatom *Amphora coffeaeformis*. Purified stalk and capsule EPS has been isolated from these cultures in large quantities, fractionated and characterized by standard techniques for monosaccharide and methylation analysis. Charged polymers isolated from stalks of *Achnanthes* differed from those of the freshwater species *Cymbella* and contain both uronic acid residues and sulfate-substituted galactose and xylose units. Residual material after water extraction of *Achnanthes* stalks yielded 3- and 4-linked glucan.

Preliminary analyses of *Amphora* capsular EPS have shown the presence of fucose, galactose, glucose, mannose and xylose residues with complex linkage patterns. In addition, we are conducting culture experiments on the effects of substrate preconditioning on diatom adhesion, including the influence of surface-associated organic matter and bacteria on diatom attachment, using both uniform organic coatings and natural collections of organic films from marine locations. These typical organic films will also be characterized chemically to provide data necessary to begin modeling the diatom-substrate interaction at the molecular level.

Characterization of Molecular Interactions Between "Conditioned" Polymer Surfaces and Microbial Biofilm Matrix Polysaccharides

Gill Geesey

Molecular interactions responsible for the adhesion of microbial biofilms to low energy polymers surfaces in seawater environment are being investigated through NMR and FT-IR spectroscopy of model surfaces, conditioning films and biofilm matrix exopolysaccharides. Silica and germanium surfaces are being coated with *n*-octadecyltrichlorosilane (OTS) derivatives to impart defined hydrophobic or hydrophilic properties to the substratum. The OTS-modified germanium substratum surface properties are being evaluated by contact angle measurements and attenuated total reflectance Fourier transform infrared spectrometry (ATR/FT-IR). The decapeptide repeating sequence in the polyphenolic protein from the byssal thread of *Mytilus edulis* is being synthesized and its structure and molecular interactions at the OTS-derivatized surface characterized by complementary cross polarization magic angle spinning ¹³C solid state nuclear magnetic resonance spectroscopy (CP/MAS NMR) and (ATR/FT-IR). Current efforts are focused on synthesis of L-Dopa and 3-OH proline precursors, side chain protection, and Fmoc amino group protection for polypeptide synthesis and peptide purification.

Molecular Characterization of Specific Attachment by a Marine Bacterium

David Kirchman

Many studies have examined the specific attachment mechanisms of pathogenic bacteria. In contrast, little information is available about specific attachment by nonpathogenic bacteria. We have been examining how the marine bacterium *Vibrio harveyi* attaches to chitin, which is one of the most abundant compounds in nature. Our previous ONR work suggests that this bacterium uses a 53 kDa peptide to mediate initial attachment. This peptide and another at 40 kDa are recovered from chitin after exposure to membrane extracts. The 53 kDa peptide (but not the 40 kDa) is overproduced by a transposon-generated mutant which exhibits higher levels of attachment than the wild type. Later stages of attachment appear to be facilitated by other chitin-binding peptides. In the next phase of our work, we want to explore the roles of these chitin-binding peptides in attachment. We hope to obtain *E. coli* clones that properly express the 53 kDa peptide. Additional mutants will be screened in order to obtain *V. harveyi* cells deficient in the 53 kDa and other chitin-binding peptides. These molecular approaches should definitively prove the roles of these peptides in attachment and allow us to begin to examine their regulation.

Genetic Responses of Bacteria in Biofilms
A. E. Goodman, M. L. Angles and K. C. Marshall

There is growing evidence that the physiology of bacteria in a planktonic form is very different to that of bacteria existing at a surface, whether loosely or firmly attached to the surface. The techniques of molecular genetics are allowing us to unravel the modulation and control of the genetic switches that must be occurring to enable bacteria to adapt their physiology to changing environmental conditions at surfaces. Biofilms provide an ideal environment for bacteria to engage in gene shuffling, by gene transfer mechanisms, and so become genetically adapted more rapidly to the environment confronting them. The specific questions that we shall address in this paper are:

1. Can a bacterium respond actively to a solid surface?
2. Do cells respond actively to changing conditions within biofilms?
3. Within the biofilm does gene transfer occur?

Knowledge about the genetic regulation of such processes will give us a better understanding of the mechanisms of biofilm formation and cell-cell interactions within natural microbial communities.

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