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Quantitative Biological Surface Science: Challenges and Recent Advances

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ABSTRACT Biological surface science is a broad, interdisciplinary subfield of surface science, where properties and processes at biological and synthetic surfaces and interfaces are investigated, and where biofunctional surfaces are fabricated. The need to study and to understand biological surfaces and interfaces in liquid environments provides sizable challenges as well as fascinating opportunities. Here, we report on recent progress in biological surface science that was described within the program assembled by the Biomaterial Interface Division of the Science and Technology of Materials, Interfaces and Processes (www.avs.org) during their 55th International Symposium and Exhibition held in Boston, October 19—24, 2008. The selected examples show that the rapid progress in nanoscience and nanotechnology, hand-in-hand with theory and simulation, provides increasingly sophisticated methods and tools to unravel the mechanisms and details of complex processes at biological surfaces and in-depth understanding of biomolecular surface interactions.

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The area of biological surface science has experienced rapid and substantial growth over the past several decades and has been established as a complex and rich area. Research in this area has implications in fundamental questions of surface science, ranging from the function and mechanism of cell membranes to the structure of water, as well as in numerous applications, ranging from catalysis to biosensors. Here, we present a brief history of surface science leading up to the development of biological surface science and comment on some of the most recent results in this area, presented at the Science and Technology of Materials, Interfaces and Processes 55th International Symposium and Exhibition in October 2008.[1]

A Historical Perspective on the Development of Biological Surface Science. Modern surface science emerged in the 1960s with a main focus on solid state and electronic materials; it matured during the 1970s and 1980s and has diversified and developed in subsequent decades and today encompasses a broad range of technological and scientific areas. Although surfaces and surface processes have long been studied (*e.g.*, by Langmuir and other scientists), it was only when ultrahigh vacuum (UHV) systems became readily available, and with them a plethora of surface analytical tools (*e.g.*, low-energy electron diffraction, X-ray photoelectron spectroscopy, Auger electron spectroscopy, high-resolution electron energy loss spectroscopy, secondary ion mass spectrometry), that the field developed rapidly and began to provide quantitative surface characterization down to the atomic level. In essence, the initial driving forces of surface science were a combination of advances in solid state physics, molecular physics, physical chemistry, and the emergence of analytical instrumentation, as schematically depicted in Figure 1. Early on, challenges in understanding what the experimental tools were measuring were tackled by both new theoretical developments and experimentation. As the field advanced, understanding surface atomic and electronic structure and the nature of the chemisorption bond were extremely important for the continued development of basic surface science. The growth of surface science, sketched in Figure 1 and described in the caption, can be visualized as a generic S-curve of development and progress *versus* time.

As the understanding of surfaces deepened and more sophisticated surface analytical tools became available (*e.g.*, Fourier transform infrared spectroscopy, scanning probe microscopes, synchrotron-based spectroscopies, and so forth), the "simpler" questions of surface science could be answered and questions relating to more complex surfaces, including oxide, polymer, and eventually liquid surfaces, could be addressed quantitatively. In particular, the notoriously complex processes at interfaces between (bio)materials in contact with biomolecules and biological substances could be tackled. This latter area developed into the distinct field of *biological surface science*, a broad interdisciplinary area where properties and processes at interfaces between synthetic materials and biological environments are investigated

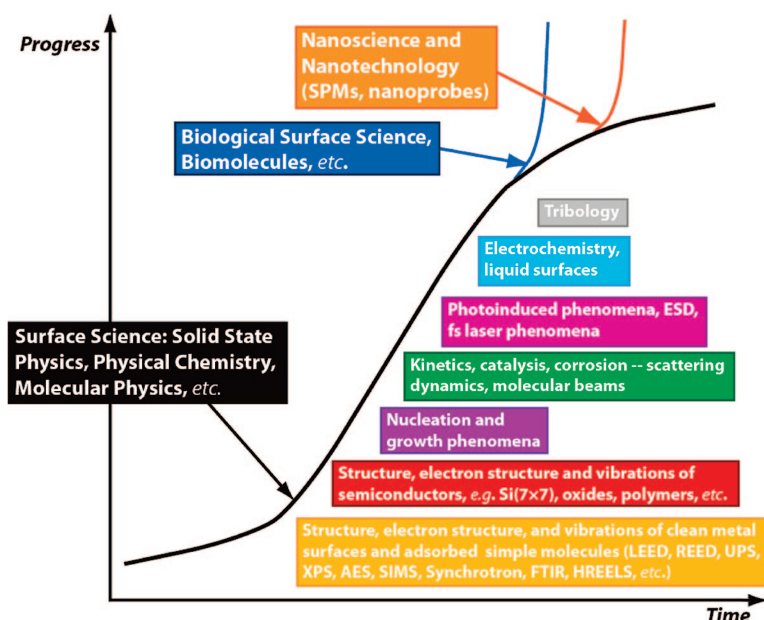

Figure 1. Development of the field of surface science can be represented qualitatively by an S-curve, with progress as a function of time. Initially, the field was characterized by method development, problem formulation, and overall slow maturity, followed by a period of vigorous growth spurred by the emergence of increasingly advanced experimental and theoretical methods, eventually to reach a high degree of maturity in the basic subareas of surface science. As the arsenal of tools and the associated knowledge base developed, new surface science subareas also developed, each giving rise to a new S-curve, with the emergence of method development and problem formulation.

and biofunctional surfaces are fabricated.[2,3] At one end of the complexity spectrum, the scientific challenge lies in mapping out the structures, bonding, dynamics, and kinetics of biomolecules at surfaces in a manner similar to what has been done for simple molecules on solid surfaces during the past three decades in surface science. At the other end of the spectrum, studies address how biofunctional surfaces can be designed to enable sensors used for disease diagnostics and to mediate cell-surface interactions related to implants and tissue engineering.

Nanoscience and nanotechnology only appear as their own surface science field relatively late on the S-curve in Figure 1. However, these fields were already present implicitly relatively early in many areas of surface science, such as catalysis and thin film technology. For some time, progress in catalysis has been impeded by the inability to translate results obtained from single crystals and macroscopic surfaces studied in ultrahigh vacuum (UHV) to the conditions occurring in practical catalysis with supported nanoparticles in high-pressure reactors as the active substances; this problem has been coined in the catalysis field as the “pressure gap” or “structure/material gap.” The pressure gap challenge has been solved by the invention of scanning probe microscopes (such as the scanning tunneling microscope, STM, and the atomic force miscrocope, AFM) that enable the systematic and direct study and comparison of nanostructured and single-crystal surfaces. The later “take-off” of nanoscience and nanotechnology as its own field (depicted in Figure 1) essentially coincides with the invention of scanning probe microscopes.[4]

Biological surface science today faces a similar challenge as catalysis did two decades ago. Here the pressure gap from catalysis is replaced by the “gap” between surface studies in UHV and studies in aqueous environments, both *in vitro* and *in vivo*. This difficult issue, however, also provides fascinating opportunities. For instance, the rapid progress in nanoscience and nanotechnology provides increasingly sophisticated methods and tools for biologists to unravel difficult problems and provides sophisticated new means to reach in-depth understanding of biomolecular surface interactions. The recent progress in this interdisciplinary area of biosurface science was at the heart of the program assembled by the Biomaterial Interface Division of the AVS for their annual technical meeting held in Boston this year. In the following pages, we provide some selected highlights from the presentations.

DNA Nucleobase-Pairing Studied Using STM. One illustration of an innovative approach to close the existing gap between UHV and liquid studies was presented by Flemming Besenbacher from the Interdisciplinary Nanoscience Center (iNANO) at Aarhus University in Denmark.[5] Together with his co-workers, Besenbacher uses the capability of the STM to explore the atomic-scale properties of materials on the molecular level and to observe directly individual DNA nucleobase-pairing.[6,7] This work provides new and deep insights into how nucleobases interact with each other. The study was done at the solid−liquid interface by co-adsorption of the cDNA bases guanine (G) and cytosine (C) on a highly oriented pyrolytic graphite (HOPG) surface from 1-octanol solvent. The high-resolution STM observations showed a well-ordered co-adsorption

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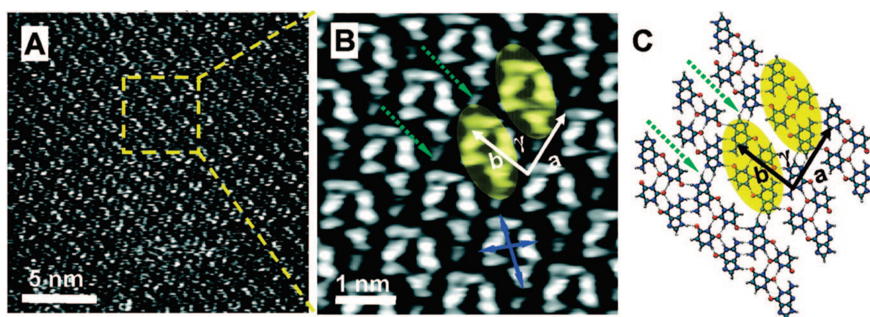

Figure 2. High-resolution STM images of guanine−uracil (G−U) base pairs at the 1-octanol/graphite interface. (A) Large-scale STM image, (B) zoom-in image of the yellow area indicated in A. (C) Molecular structure proposed by *ab initio* DFT calculations. The cyclic G−U structures are indicated by yellow ovals, their size by blue arrows, and the unit cell lattice vectors are indicated. Green arrows indicate the hydrogen bonds between the cyclic G−U structures along unit cell vector *a*. Reproduced from ref 7. Copyright 2008 American Chemical Society.

structure, attributed to rows formed from Watson−Crick G−C pairs, which was distinctly different from the structures observed for the individual G and C components.[6] An even more biologically relevant case is the nucleobase-pairing between G and uracil (U) since in nature this nucleobase-pairing is responsible for the Wobble configuration, which is fundamental to the ability of RNA to translate the genetic code and also contains suitable sites for recognition by proteins and other RNAs.[8] The G−U conformation also provides recognition signals for autoregulation of protein synthesis and has the ability to bind divalent metal ions, which is important for RNA catalysis. In experiments similar to those described by Xu *et al.*,[6] and supported by *ab initio* density functional theory (DFT) calculations, Besenbacher and co-workers have been able to show that, upon mixing of G and U molecules, a self-assembled nanoscale patterned supramolecular structure consisting of cyclic G−U elementary blocks is formed.[7] The primary structure formed, which was resolved with atomic resolution as shown in Figure 2, is stabilized by strong hydrogen bonding along the chains and relatively weaker bonding between the chains.

Besenbacher stressed the possibility of utilizing these types of structures in catalysis, particularly in connection with targeting metal ions; he even speculated about the possible role of these nanoscale supramolecular structures during the first self-catalyzing steps in the origin of life. In order to address this exciting question and also for these structures to be fully utilized as nanoscale building blocks for applications in catalysis and biotechnology, a key challenge remains: understanding of the role of water in these self-assembly processes. Improvements that make it possible to perform these studies in the presence of water are needed.

Structure of Water at Interfaces: Myth or Reality? Indeed, the nature of water at interfaces is critically important for many surface and interfacial properties and represents one of the knowledge gaps that needs extensive attention in the future, irrespective of whether nanoscale materials are the focus. For example, the role of water molecules at biomolecule-resistant (inert) surfaces remains unclear, despite evidence that they are required in essentially all biointerface-related applications. Michael Grunze from Heidelberg University in Germany addressed this issue by providing convincing, quantitative evidence that the recurring interpretation of experimental data as *ordered* structures of interfacial water at room temperature on soft organic surfaces is likely not correct. Oriented binding of water molecules to a hydrogen bond donor or acceptor group, as observed (for example) in vibrational spectroscopies, does not necessarily imply translational symmetry or "structure" as (for example) in bulk ice. Furthermore, most computer simulations employing state-of-the-art models for water reveal that the orientational order parameter decays with the second hydration shell. To revisit the question of "Structure of Water at Interfaces: Myth or Reality?" (the title of Grunze's presentation) has significant merit. A brief survey of the literature reveals that there is little experimental or modeling evidence for a sharp boundary that can be described as a 2D interface between an organic phase (*e.g.*, lipid membrane, self-assembled monolayer, polymer surface) and water. Even in the absence of water penetration into the film, as is the case for hydrophobic surfaces, the interphase is characterized by a water density gradient extending into the bulk liquid.[9−11] Although the density of water at this interface is less than bulk water, it cannot be associated with a crystalline ice phase because of the lack of translational and orientational order. Preferential orientation, that is, the constraints on rotational motion in the interphase, does give rise to a dichroism in the water vibrational bands and strong signals in sum-frequency generation (SFG) spectra.[12] So what then is the rationale for the extraordinary ability of some hydrated organic surfaces to resist protein, cell, and bacterial adhesion?[13] If it is not orientationally structured or "ice-like" water, what is it? At least for oligo(ethylene oxide)-terminated SAMs, it becomes clear that the ordering is based on the strong hydration energy of the oligomers. Depending on the molecular conformation of the oligo(ethylene oxide) terminal groups—which is a function of film density—water can form double hydrogen bridge bonds

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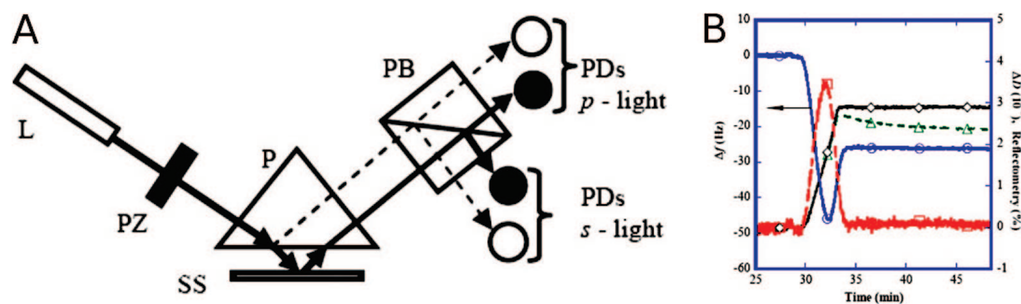


Figure 3. (A) Schematic representation of a combined reflectometry and QCM-D setup: light source (L), polarizer (PZ), prism (P), sensor surface (SS), polarizing beamsplitter (PB), and beam splitters (PDs). Compared to the conventional reflectometric setup (solid lines), the four-detector design also measures the reflection of an additional beam (dashed lines). (B) Changes in frequency (blue) and energy dissipation (red) upon addition of lipid vesicles to a silicon dioxide coated QCM-D sensor. Also shown is reflectometry data (black) measured on the QCM-D sensor as described in ref 19. Reproduced with permission from ref 19. Copyright 2008 American Institute of Physics.

with two neighboring oxygen atoms in the oligomer, which exceed the bonding energy of bulk water by about 75% (*ca*. 35 *versus* 21 kJ/mol, respectively).[14] This strongly bound hydration water cannot be displaced by proteins (or even glycoprotein adhesives excreted by marine organisms[15]), thus preventing irreversible binding to the surface. Therefore, it is *thermodynamics* and not *structure*that matters in "inert" surfaces.

The Quartz Crystal Microbalance with Dissipation Monitoring Technique for Studies of Supported Lipid Bilayers and Coupled Water. Responsible for a multitude of functions, the cell membrane is an excellent example of a surface that is inert to nonspecific biomolecular interactions. For survival, a living cell is critically dependent on molecules, ions, and signals being continuously transported across its membrane, mediated, and controlled by specialized membrane-spanning proteins embedded in an environment composed of different types of lipids. Indeed, the majority of these specific tasks rely on molecular recognition events, but through evolution, the lipids that make up the membrane in which the membrane proteins reside have evolved to exhibit very low nonspecific affinity for unrelated biomolecules. To explore and to make practical use of this important property, as well as for in-depth studies of the biophysical properties of the cell membrane and its specific functions, much work has focused on various cell membrane mimics. In this reductionist approach, cell membrane mimics supported on solid surfaces have played a very important role, in particular, because these model systems have enabled studies of the biophysical properties of the cell membrane using a large arsenal of analytical tools, many of which originated from surface science, such as scanning probe techniques, surface plasmon resonance (SPR), time-of-flight secondary ion mass spectrometry (TOF-SIMS), and so forth. A recently developed technique that has proven valuable for studies of supported lipid bilayers is the quartz crystal microbalance with dissipation (QCM-D) monitoring, which was developed by Bengt Kasemo and his team at Chalmers University of Technology in the mid-90s.[16] Besides measurements of bound mass, which is provided from changes in the resonance frequency, *f*, of the piezoelectric sensor resonator, the QCM-D technique also provides structural information of biomolecular films *via* changes in the damping, *D*, of the crystal. In this way, adsorbed lipid vesicles, which induce high damping, can be easily distinguished from planar supported membranes, which induce little or no detectable damping,[17] as illustrated in Figure 3.

This ability to distinguish different structural phases of adsorbed lipids on surfaces is the primary reason the technique has been so extensively used in studies of both supported lipid membranes and tethered lipid vesicles. In Kasemo's presentation at the AVS meeting, which was part of an honorary session for his contribution to the field of surface science in biotechnology, catalysis, and energy, the latest developments of the QCM-D technique were presented. The depth of information provided from combined *f* and *D* measurements at multiple harmonics (which is critical in order to translate the measured response into physically meaningful parameters such as film viscosity and elasticity) was illustrated by several examples, mainly from the area of adsorbed lipids. Kasemo also showed how the technique can be combined with simultaneous measurements using both SPR,[18] reflectometry,[19] and nanoplasmonics.[20,21] All of these techniques provide information about the molecular mass of the adsorbed entities, and since QCM-D measures coupled water in addition to the molecular mass, these types of combinations of different techniques provide information about changes in the amount of water bound to the analyzed film (see Figure 4). As stressed above, this is key information in the design of biofunctional interfaces. In combination with nanoplasmonic sensors, it also provides an improved means to quantify the plasmonic response in terms of coupled mass.[20,21] Kasemo also presented how the technique is now combined with electrochemistry and impedance spectroscopy. This, in turn, points toward the use of QCM-D for studies not only of the formation of supported lipid bilayers but also for membrane−protein mediated translocation of ions across the membrane, which is, as mentioned above, the principal biological function of the cell membrane.

Tethered Lipid Membranes Formed on Ultra-Flat Gold Substrates. Although lipid vesicles bind and spontaneously reorganize into continuous supported lipid membranes when exposed to silicon dioxide and mica surfaces, the insulating properties of these substrates make them less suitable for studies of charge translocation events. In addition, the close contact between the substrate and the lower leaflet of the supported

Although many supported or tethered bilayer architectures look acceptable when studied by structural techniques (*e.g.*, SPR, AFM, QCM-D), they often contain a significant level of structural defects generating nonspecific channels for the translocation of ions across the bilayer, thus screening any specific effect of particular membrane–protein mediated ion translocation.

membrane complicates functional integration of membrane-spanning proteins. In recent years, significant efforts have thus been focused on the design of various types of model membrane architectures on electrically conductive surfaces better suited for charge translocation measurements.[22] Wolfgang Knoll (Max Planck Institute for Polymer Research in Mainz, Germany) stressed the importance not only of appropriately designed tethers, but also of the smoothness of the substrate in order to produce supported lipid bilayers with sufficiently high electrical resistance for single-ion channel recording. The basic architecture of the model membrane system developed by his team is based on lipids covalently attached to the gold substrate *via* flexible spacers, giving this system high mechanical and chemical stability.[23] Knoll emphasized that, although many supported or tethered bilayer architectures look acceptable when studied by structural techniques (*e.g.*, SPR, AFM, QCM-D), they often contain a significant level of structural defects generating nonspecific channels for the translocation of ions across the bilayer, thus screening any specific effect of particular membrane–protein mediated ion translocation. In summary, Knoll claimed that besides the ultraflat gold substrate, the reason for his group's success relies on an optimized molecular architecture of the self-assembling lipid molecule, with a lipoic acid anchor group, a short ethylene oxide unit as the spacer, and a lipid analogue with two phytanoyl chains that improve the fluidic and, hence, sealing character of the hydrophobic core. He also emphasized that the mica template stripping method used to produce smooth gold films as large as several square centimeters makes this system attractive even when considering the needs for processing tethered membranes in large quantities for commercial biosensor applications.[24]

Electrically Insulating Membranes Formed Across Nanoscale Apertures. Traditionally, membrane–protein mediated charge translocation events have been measured using patch-clamp techniques, which are ideally suited for studies of whole cells, or using so-called artificial (black) lipid membranes typically spanning micrometer- to millimeter-scale apertures, thus providing large liquid reservoirs on both sides of the membrane. With black lipid membranes (BLMs), single-channel recording is relatively easily achieved, but the fragile nature of BLMs makes them less suited for practical applications, such as high-throughput screening of drugs directed against ion channels, for example. The approach taken by Knoll and many others[22] has focused on tethered membranes for improved stability and robustness. While promising, this was challenged in an exciting presentation by Claudia Steinem. Together with her co-workers at Göttingen University in Germany, she has developed a platform that combines the advantages of supported lipid membranes with the need for an even larger second aqueous compartment to investigate complex integral membrane proteins with bulky water-soluble domains. This was accomplished using porous alumina substrates with highly ordered pores 60 nm in diameter, functionalized with lipid bilayers spanning the pores (Figure 4). Initially, the membranes were deposited by applying a lipid–solvent droplet on one side of a porous substrate coated with gold and modified with a hydrophobic self-assembled monolayer. In this way, high membrane resistance compatible with single-channel recordings of ion channels was proven for many systems, including the complex outer membrane protein OmpF of *Escherichia coli*.[25] Although stable for significantly longer times than traditional BLMs, they are not stable indefinitely, but display a slow increase in membrane conductance over several days and sometimes longer. By investigating slightly larger pore sizes displaying very similar electrical properties, fluorescence imaging was used to attribute the slow conductance increase to independent rupture of single membranes or membrane patches covering the pores.[26] However, in contrast to the

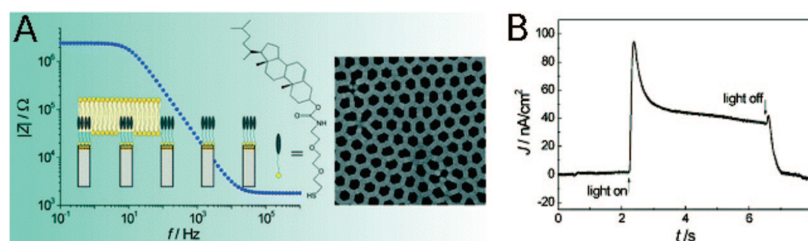


Figure 4. (A) Solvent-free lipid membranes formed on porous alumina through vesicle rupture. (B) Proton pump activity of bacteriorhodopsin upon light illumination. During illumination, a stationary current is detected, which drops to zero after switching off the light source. Reproduced with permission from ref 27. Copyright 2008 The Royal Society of Chemistry.

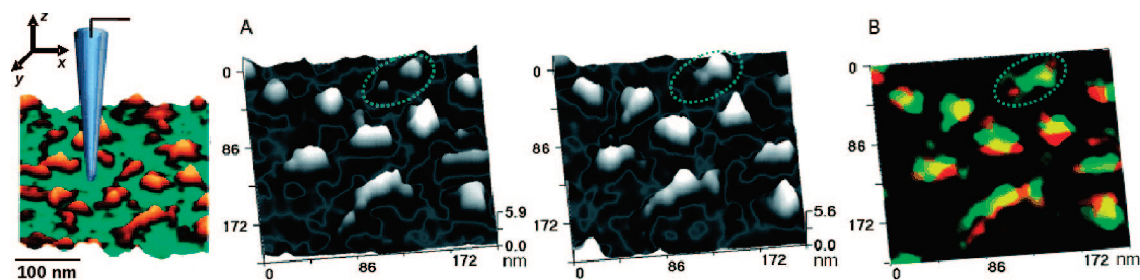


Figure 5. (A) Topographical images taken 10 min apart of an area of a cell membrane (area exhibiting aggregation is marked with dashed lines). To facilitate visualization of temporal changes, artificial color was applied to both images, the earlier image in red and the subsequent image in green. (B) In the overlay image, yellow indicates those places where the position and shape of the proteins did not change. Reproduced with permission from ref 31. Copyright 2006 Wiley-VCH Verlag GmbH & Co. KGaA.

tethered lipid membranes, but analogous to traditional BLMs, the nanoscale BLMs contain solvent traces, which may influence the function of the analyzed systems. Steinem and her team also overcame this limitation recently by succeeding in self-assembly of pore-spanning membranes *via* vesicle adsorption and subsequent rupture into a continuous planar membrane covering the majority of holes, as schematically illustrated in Figure 5.[27]

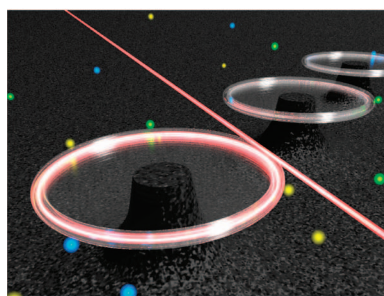
This was accomplished by adjusting the sizes of the vesicles to be sufficiently larger than the pore diameter of 60 nm and by modifying the gold film on top of the alumina substrate with (cholesterylpolyethylenoxy)thiols. As shown in Figure 4B, the proton pump bacteriorhodopsin could also be functionally incorporated into these pore-suspended membranes. However, it should be noted that there is still room for further improvement. For instance, in this case, the membrane resistance and long-time stability are lower than those obtained with the lipid–solvent painting method, and the membrane formation process is significantly slower than on silicon dioxide, for example (see above). In addition to the smoothness of the gold film, the actual curvature at the edge of the pore must be taken into consideration, and new means to assemble planar membranes across nanoscale features may be needed.

In this context, the presentation by Curtis Frank and Nam Joon Cho of Stanford University deserves attention; they have succeeded in transforming nonruptured vesicles adsorbed on both pure gold and titanium dioxide into a homogeneous supported lipid bilayer by the addition of an amphipathic viral peptide.[28] Equally important for the future progress in the field are improved nanoscale fabrication schemes as well as new tools to investigate the chemical and topographical composition of both modified and unmodified substrates.

Non-Invasive Imaging of Proteins in Membranes of Living Cells by High-Resolution Scanning Ion Conductance Microscopy. In the context of unraveling structural features of these types of soft matter, a significant contribution to the development of scanning probe techniques was presented by David Klenerman of Cambridge University in the UK. He presented how his team has used scanning ion conductance microscopy (SICM)[29] for noncontact imaging of the temporal dynamics of protein complexes in the plasma membrane of living cells with a resolution on the order of 10 nm. Even though the most developed version of liquid-phase AFM can image proteins on hard substrates and has also been applied successfully for imaging living cells, it has thus far not been possible to achieve molecular resolution on the surface of living cells. This is due to difficulties in controlling the probe-sample separation over the soft cell surfaces. The SICM technique provides noncontact imaging of native cell surfaces by maintaining the distance at the pipet inner radius (~10 nm) by controlling the ion current flow between an electrode in the pipet and a bath electrode for feedback control. Figure 5A illustrates the dynamics observed of protein clusters at the surface of a spermatozoon taken at 10 min intervals.[30] In Figure 5B, red and green areas correspond to mobility changes, while yellow areas correspond to immobile protein clusters. Note that the majority of proteins remained largely unchanged (yellow color in the overlay images), a result indicating that the observed dynamics are not a measurement artifact.

Klenerman also presented how the same nanopipette-based method can be used to control delivery of individual fluorophore-labeled probes reliably to a defined position on the cell surface, followed by single-molecule fluorescence tracking.[31] In this way, Klenerman and co-workers were able to release one probe to predefined regions on the membrane, which, when combined with total internal reflection fluorescence (TIRF) microscopy, enabled analysis of the diffusional properties of different subregions of both the cell membrane and probe for the presence of diffusion barriers. Andreas Janshoff of Göttingen University also demonstrated how this invasive technique was applicable to analyzing the topography of pore-suspended membranes described by Steinem (see above).

Label-Free, Single-Protein Detection. Although the work from Klenerman's group constitutes a major contribution to the field and illustrates nicely how advancements in nanotechnology already contribute to unraveling of central biological questions, real-time detection of protein–protein interactions on the level of single molecules without the introduction of fluorescent dyes remained, until recently, a dream. A breakthrough in this respect was achieved by Andrea Armani while completing her postdoctoral research at Caltech.[32] Armani, now a professor at The University of Southern California, described the development of a highly sensitive optical sensor based on an ultrahigh quality (Q) factor ($Q > 10^8$) whispering-gallery mi-



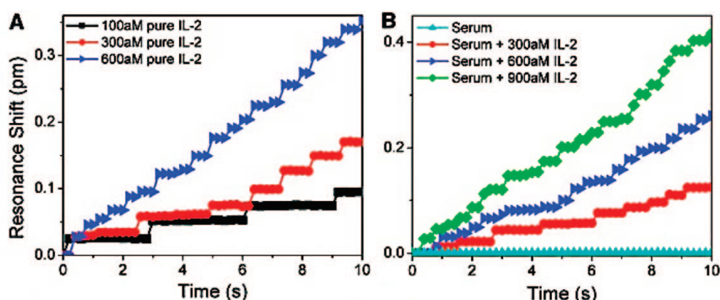


Figure 6. (Left) Schematic illustration of silica microtoroids (diameter ~80 μm) to which light is coupled *via* a tapered optical fiber. (Right) The position of the resonance wavelength as a function of time at three different interleukin-2 concentrations in (A) buffer and (B) fetal serum. Also shown in (B) is the case of pure serum (light blue trace). As protein molecules bind to the surface, the resonant wavelength position shifts, creating the steps seen. Left panel courtesy of Andrea Armani/The University of Southern California. Right panels reproduced with permission from ref 32. Copyright 2007 AAAS.

crocavity. Typically, the sensitivity of optical microcavities of this type depends linearly on *Q*. In this case, the wavelength shifts of the light coupled with the whispering-gallery mode produced by protein binding events are several orders of magnitude too small to sense the contrast in interfacial refractive index induced by single protein-binding events. Verified through a series of measurements, Armani and her team demonstrate how microcavities with sufficiently high *Q* factors can be utilized to produce a sensitivity that scales with the square of *Q*, and in this way, they show that single-molecule sensitivities of *unlabeled* protein molecules can indeed be accomplished.

In addition to the direct effect on the optical path length of the microcavity upon a local change in refractive index, the high circulating intensities within the resonator locally heat molecules adsorbed to the whispering gallery. This thermo-optic detection mechanism was verified by performing a series of single-molecule detection experiments using molecules of varying absorption cross sections. Furthermore, by modifying the microcavities with antibodies against interleukin-2 (IL-2), detection of IL-2 (a cytokine released in response to immune system activation to extrinsic and intrinsic stimuli) at subattomolar concentrations in serum was demonstrated (Figure 6B), thereby establishing the microcavity as a sensitive and versatile transducer, which is key to future quantitative biological surface science and also has potential within diagnostics. Although data recorded over short (10 s) intervals were presented, it is interesting to note that no nonspecific binding was detected (Figure 6B). In single-molecule studies, this is particularly important and highlights again the need for inert surfaces for these types of techniques to be broadly applicable.

It should be noted that TIRF microscopy, utilized by Klenerman in analyzing the diffusivity of fluorescent probes bound to the cell membrane,[31] can also be used to detect unlabeled targets using lipid vesicles as enhancer elements,[33] as presented in a contributed talk by Anders Gunnarsson from Chalmers University in Sweden (one of the young investigator award winners). In combination with fluctuation analysis,[34] this enables the complete determination of kinetic constants from an analysis in equilibrium. It also circumvents the need for following the rate of binding and dissociation upon rinsing, which has thus far been the dominant method for determining binding constants using label-free surface analytical tools, such as SPR, QCM, *etc*. Combining analysis in imaging mode with single-protein sensitivity in the complete absence of labels as presented by Armani points toward a new era in the analysis of biomolecular interactions, in general, and for protein−protein interaction analysis and diagnostics, in particular. Another field of great relevance is nanoplasmonics, where recent progress in the understanding of plasmon coupling was presented by Anne Lazarides (Duke University), using DNA hybridization controlled assembly of nanoscale silver and gold nanoparticles.[35]

FUTURE CHALLENGES AND PROSPECTS

Biological surface science is a rapidly developing area, accelerated by progress in nanoscience and nanotechnology. As complex problems are identified and become more distinct and sophisticated, experimental tools become more readily available, quantitative descriptions of processes at biological surfaces and interfaces at the nanoscale can be realized. This development in biological surface science goes hand-in-hand with advances in theory and simulation, ranging from first principles calculations of model systems and

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molecular dynamics simulations to more phenomenological Monte Carlo simulations. As illustrated above, progress in the field is driven by both basic science research and by applications. In the former, the focus is the desire to understand the details of, for example, protein interactions with surfaces, lipid−surface and lipid−lipid interactions, and the structure, dynamics, and role of water and solvated ions in these interactions. Application-driven developments arise from the need to create new products and the desire to improve existing materials, where biointerfaces play important roles. Examples include medical implants, sensors, and biochips for medical diagnostics and drug discovery, smart nanodrug encapsulations, and scaffolds for stem cell and tissue engineering.

There is a strong and robust feedback loop between the fundamental and the application-driven development of the field as evident in the examples above, and by the many talks in the Biomaterial Interfaces Division symposium at the Fall 2008 AVS technical meeting in Boston. It is noteworthy that this development is truly interdisciplinary and occurs in parallel, with cross-fertilization and contributions from many other fields ranging from surface science and nanoscience and nanotechnology, to organic chemistry, biochemistry, molecular biology, and medicine.

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