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G. Fragneto

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Biological Physics near Surfaces/Interfaces: a Perspective

G. Fragneto[a]

Institut Laue-Langevin, 6 rue Jules Horowitz, BP 156, 38042 Grenoble, France

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Abstract. Biological physics at interfaces is an expanding field of research where a wide variety of disciplines fruitfully cross-link. The high degree of complexity of the involved systems requires the concerted application of many different and complementary techniques. Future success relies both upon development of new instrumentation and a better interaction of material scientists, physicists, molecular biologists and chemists.

PACS. 68.47.Pe Langmuir-Blodgett films on solids; polymers on surfaces; biological molecules on surfaces – 68.43.-h Chemisorption/physisorption: adsorbates on surfaces – 87.85.jc Electrical, thermal, and mechanical properties of biological matter – 87.15.-v Biomolecules: structure and physical properties

Introduction

Biological systems near interfaces represent one of the most dynamic and expanding fields in science and technology and have been at the centre of recent major scientific and technological advances. This happened thanks to a greater understanding of molecular biology mechanisms in general and biological membrane functions in particular from research activities in very different areas ranging from physics to materials science and engineering, to chemistry, molecular biology, bioinformatics and medicine. The need for a multidisciplinary approach has become apparent and it is the key for future success. The definition of biological interfaces, or bio-interfaces, is very broad. They include the surfaces of cells and organelles within cells where many biological mechanisms happen as well as artificial mimics of biological surfaces or adsorption of biological molecules on solid or liquid substrates. The study of biological interfaces requires the understanding of interfacial phenomena and the use of ideas and techniques developed in surface science for the study of basic molecular features. In fact, the application to biological systems of principles from the physical chemistry of interfaces, well developed for studies of simple inorganic or synthetic molecules, has revolutionized our understanding of molecular recognition interactions.

Research on adsorption phenomena is now routinely applied to biological systems and includes concepts of forces operating across interfaces, electrical double layers at charged interfaces or the structure of water layers adjacent to bio-interfaces. On the other hand, the adsorption or adhesion to a surface of a bio-molecule in most cases induces changes in its physico-chemical properties and affects its biological activity. For this reason, the presence of interfaces plays an important role in food processing, medicine, environmental science and biotechnology.

The main approach in bio-interfacial science involves preparation and characterisation of functional surfaces for specific interactions with bio-systems, *in vivo* and *in vitro*, and studies of the molecular and kinetic processes occurring at such interfaces, ranging from small molecule and bio-molecular interactions, to cell adhesion, differentiation and tissue formation at the interface. When surface science started as a research field on its own, about forty years ago, very little was known even about the simplest model systems and the experimental and theoretical tools were very limited. For simple systems the field has advanced enormously since: total energies, electronic and atomic structures, and the lattice dynamics of many surfaces are known in great detail. Important factors have been the development of new experimental probes and theoretical methods as well as advanced preparation of well-defined model systems. These developments have allowed a vast and systematic theoretical and experimental investigation of simple model systems and paved the way for a semi-quantitative and/or conceptual understanding of a number of complex systems. Nowadays, research in the field spans a wide range of subjects including bio-mimetic surface platforms, bio-membrane and supra-molecular materials, nanotechnology, controlling cellular responses by designed surfaces with switchable properties, stem cells, cellular and molecular biomechanics, neural networks, optical, magnetic and mechanical detection systems with single-molecule sensitivity, bio-arrays and DNA, lipids, peptides, proteins, enzymes at interfaces [1,2].

[a] e-mail: fragneto@ill.fr

One of the major goals of today research in biophysics is the understanding of the interplay of single "nanomachines" in a complex assembly like, for example, a cell membrane. In the last few years, new tools have been developed for the study of single proteins or DNA molecules in complex assemblies including the patch-clamp technique, single-molecule spectroscopy and nanoscopic force spectroscopy. Molecular force spectroscopy has opened up new possibilities for the study of mechanical properties of single molecules. Nonetheless, biological function is determined not only by single-molecular events but by the physical properties of the assembly. For example, the physics of cell adhesion is not only controlled by specific forces between cell surface receptors but also by a number of generic forces, the elasticity of the membrane and the chemical potential of the molecules of the glycocalix of cells [3].

Techniques for the study of bio-interfaces

The characterization and analysis of bio-interfaces represent a challenge. Performing measurements on these few nanometre thick, soft, visco-elastic and dynamic systems is close to the limits of the available tools and methods. It is important to understand the physics involved in the characterization tools to be able to extract the correct interpretation from the analysis of the measured properties. There are two major categories of classical measurement techniques, based on their principle of operation and they are: optical and mechanical techniques. Optical techniques use intensity loss, polarization or reflection angle change of light to measure thickness, dielectric constant change or mass of surface-deposited thin layers. Examples are *Ellipsometry or Surface Plasmon Resonance (SPR).* Mechanical techniques use probes like static or oscillating tips, balls, cylinders, to measure morphology as well as other mechanical properties like elasticity, viscosity, surface energetics of thin layers. These techniques include *Quartz Crystal Microbalance (QCM), Surface Force Apparatus (SFA), Atomic Force Microscopy (AFM).* Newer techniques include the use of electromagnetic radiation as the scattering of X-rays and neutrons.

Enormous progress has been made in recent years in developing techniques for single-molecule detection as well as techniques allowing probing great areas of detection.

Single-molecule fluorescence spectroscopy is exploited to probe the intra-molecular dynamics, conformations and function of several biologically relevant molecules and processes. Far-field optical nanoscopy has allowed overcoming the resolution limiting effect. It is a lens-based fluorescence microscopy technique that features a resolving power on the nanoscale. Perspectives of this technique based on Stimulated Emission Depletion (STED) microscopy in which the fluorescence ability of a die is switched off by stimulated emission, is to reach a "molecular" resolution [4]. Recently, a method has been developed based on a *scanning nanopipette* that allows robust, high-resolution, non-contact imaging of living cells, down to the level of individual protein complexes. It can also be used to probe function by performing nanoscale assays such as locally deliver controlled amounts of reagents or performing single-ion channel recording. It has been possible to watch the details of biological processes taking place on the surface of living cells, including viral entry and probe the structure of the cell membrane [5]. *Single molecule dynamic force spectroscopy with AFM* allows the investigation of the physical mechanisms of specific, structure-related intermolecular binding. Specific molecular forces, elasticities, kinetic reaction rate constants (lifetimes) and the energy landscape of the molecular binding potential are quantitatively measured on a single-molecule level on isolated but functional complexes and recently on membrane-bound cellular receptors of a living cell [6]. *High resolution AFM* at low temperature (liquid-nitrogen temperature) is under development. This technique relies on the fact that polymers (such as proteins or DNA) become stiff as the temperature is lowered, so that an increase in resolution for the AFM is expected.

Neutron and X-ray reflectometry are emerging techniques for the study of bio-interfaces. The wavelength of neutron and X-ray beams being of the order of the tenth of a nanometer, these are ideal tools for the structural and dynamical characterization of many biological systems at interfaces (the thickness of cell membranes is $\sim$ 5 nm). The specific properties of the neutrons make them suitable probes for investigations in soft-matter and biology. Biological membrane components, like most soft materials, are rich in hydrogen and neutrons have the unique capability of being scattered differently from hydrogen and deuterium. It is possible to accentuate or annihilate the scattering from individual parts of a macromolecular complex and, for example, by specific deuterium labeling it is possible to measure bilayer conformational changes and organization both in the perpendicular and lateral directions. As the deuteration of proteins is becoming an active field of research, the use of fully deuterated or partially deuterated proteins has opened up new possibilities in the study of lipid protein interactions or protein structures at lipid surfaces (during, for example, events of fusion or cellular recognition). Neutrons interact weakly with atomic nuclei, therefore they are highly penetrating so that samples in complex sample environments can be probed and *in situ* measurements from buried interfaces are possible. Neutron energies range from the meV to the eV, comparable to the energies of atomic and electronic processes. This allows for the study of dynamic properties like translations, vibrations, rotations, lattice modes, exhibited by molecules [7].

Synchrotron radiation (SR), after proving invaluable for the high-resolution structural characterization of proteins, is emerging as a powerful tool also for the characterization of bio-interfaces. Thanks to the high energy and flux, SR reflectometry provides unsurpassed resolution for structural characterization perpendicular to an interface [8]. Grazing Incidence Diffraction (GID) allows determining 2-D structures down to atomic resolution [9].

GISAXS probes sizes down to 30 nm. Future directions of research with these tools involve the study of the time dependence of phenomena at interfaces. Another quickly evolving technique, complementary to neutron and X-ray tools, is *Brewster Angle Microscopy*, which allows the visualization of lateral features on surfaces in the micron regime. Finally, *nanofluidics* is the study of the behaviour, manipulation, and control of fluids that are confined to structures of nanometre (typically 1–100 nm) characteristic dimensions. These confined fluids exhibit physical behaviours not observed in larger structures because the characteristic physical scaling lengths of the fluid (*e.g.*, Debye length, hydrodynamic radius) very closely coincide with the dimensions of the nanostructure itself. The new properties not observed in bulk, as for example the vastly increased viscosity near the pore wall, may effect changes in thermodynamic properties and may also alter the chemical reactivity of species at the fluid-solid interface. Nanofluidics has had a significant impact in biotechnology, medicine and clinical diagnostics [10]. Rapid development of new applications is expected in the coming years.

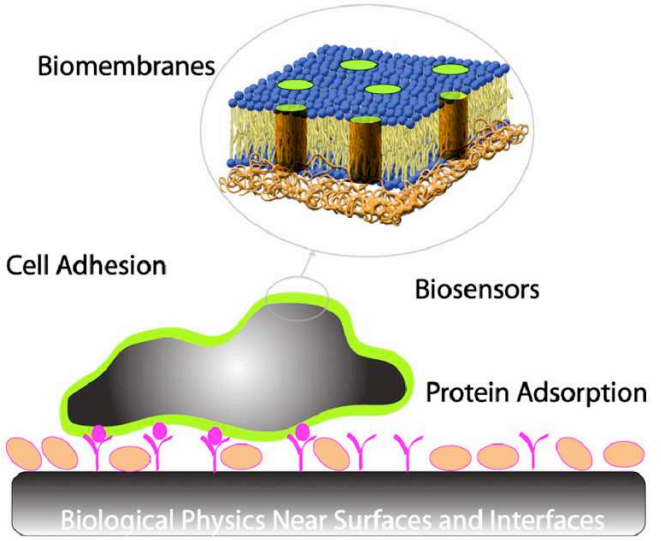

Fig. 1. Cartoon representing the cross-over of the areas of research in biological physics near surfaces and interfaces represented in this special issue.

Bio-interfaces: a liaison between Physics and Biology

The design of bio-functionalized systems on solids, such as semiconductors or electro-optical devices, is a challenging area of research with potential application in areas like [1]: the design of smart biosensors on miniaturized semiconductor chips or electro-optical devices; the design of tissue surface mimetics for the controlled growth of cells or tissue and the maintenance of their functional state; the stress-free immobilization of cells on semiconductor devices as monitors and alarm systems for the sensitive detection of cell-damaging substances.

One major advantage of mimicking these processes on planar supports is the possibility to apply a plethora of surface-sensitive techniques for studying the structure, dynamics and function of complex soft interfaces. In "real" space, probes like atomic force and electron microscopies provide localized images of structures on surfaces. Scattering techniques employing neutrons or X-rays have proven invaluable for looking at large areas and can contribute to the elucidation of the molecular structure as well as to the understanding of molecular and supra-molecular dynamics of complex systems at interfaces [7].

This special issue has provided examples from the great variety of systems and techniques that are nowadays focus of research in biological physics at interfaces. They span from studies of cell adhesion, to protein adsorption, to structural characterization of lipid bilayers and the design of biosensors. Figure 1 depicts schematically the cross-over of these areas that is such that any progress in one field will prove important for the progress of the others.

Cell adhesion and motility are processes involved in fundamental biological phenomena. They imply multi-molecular scaffolds as anchorage points and actin cytoskeleton filaments to build internal stress and eventually crawl onto the substrate. The understanding of the behaviour of cells at surfaces is a fundamental issue both with regard to the cultivation of cell cultures *in vitro* as well as for tissue engineering and other health-related research (implants, cancer therapy, ...). The role of the surface chemistry and topography for the evolution and function of cells has been demonstrated in many experiments but it is not yet fully understood. The recent rapid development in the area of so-called stem cells is particularly interesting. Stem cells are cells that have not reached a high degree of specialization but, depending on the local environment, they can differentiate into different functions and can even move from a specialized to a less specialized state. One key research area for the future will be how tailored surfaces should be designed to act as stimuli to guide cell differentiation in tissue engineering, bioelectronics and cell-based sensors.

Protein adsorption is a widespread event occurring at biological interfaces [11]. Proteins are molecules composed of both hydrophilic and hydrophobic moieties. If proteins are present in aqueous solution, part of them will migrate at the interface and expose the hydrophobic areas to air. When a protein-containing solution comes in contact with a solid surface, it results in the spontaneous accumulation of protein molecules at the interface. Protein adsorption, be it specific or not, is important to numerous biotechnology and biomedical applications. Examples can be found in biomedical engineering, biosensors, immobilized delivery systems, pharmaceuticals, wastewater and soil treatments, etc. Protein adsorption is of academic interest too. It induces a modification of the properties of the surface and, in most cases, of the protein molecules as they can undergo structural rearrangements. Studying the interac-

tion between proteins and interfaces may therefore contribute to the understanding of the mechanism that determines the 3-D structure of protein molecules. Knowledge of the adsorption behavior of proteins has largely progressed in recent years but a unified predictive theory is still lacking. The role of protein adsorption is often negative: clogging of ultra-filtration membranes for water purification or hemodialysis, triggering foreign-body response to implanted biomedical devices, immune response to protein drugs or blood clotting on blood-contacting surfaces. These observations have motivated a strenuous and ongoing search for surface treatment capable of repressing protein adsorption.

The study of the adsorption of proteins to nanomaterials has received much attention in recent years due to their ever-increasing use in cancer therapy or medical diagnostics. From a therapy-optimization or health regulation standpoint it is important to understand interactions on a molecular level.

After the pioneering studies of the '70s, the last fifteen years have witnessed an increased interest in the role of *lipids in biological membranes*. This is due mainly to the availability of a certain number (although still low) of membrane protein structures, that have elucidated some of the mechanisms happening at cell surfaces, and to the advent of new or improvement of existing structural techniques enabling studies of single bilayers with a fraction of nanometer resolution. In the recent *Nature* Insight [12] a snapshot of current research in membrane protein biophysics and the emerging role of lipids in shaping membrane protein function are presented. Cells and organisms have developed sophisticated mechanisms for controlling the lipid composition by regulating free cholesterol levels as well as other properties like the degree of saturation of fatty acids. Many diseases are related to the failure of these homeostatic regulatory mechanisms. For example, in both the early and late stages of atherosclerosis there is evidence that changes in membrane bilayer properties influence disease progression. The role of changes in bilayer properties in other diseases such as Alzheimer's or type-II diabetes/metabolic syndrome is less clear, but this may be an area for significant new discoveries of disease mechanisms and treatments. The role of lipids and sterol derivatives as signaling molecules and second messengers is well established even though new discoveries are continuously made. A review on the subtler role of lipids and cholesterol in regulating the biophysical properties of membranes and how this affects cell physiology can be found in [13]. One of the recent advances in the field is the discovery of the existence of coexisting micro-domains within a single membrane. Many important properties of these domains remain poorly characterized. They are important for regulating some signaling pathways, and there is some understanding of how this may work in a few cases. Much more work is needed to better characterize the biophysical properties of cell membranes and the effects that these properties have on membrane proteins. It will be a challenge to the vast amount of work existing on pure lipids to assimilate the perturbations induced by proteins.

Biosensors are portable analytical devices needing to have great selectivity for the target analyte since complex samples, like for example the blood, present a great variety of components. To achieve this specificity, biosensors borrow mechanisms from nature using the recognition molecules of living systems like enzymes, antibodies, peptides, DNA. A biosensor then comprises a bio-recognition molecule integrated with a single transducer to give a reagentless analytical device. The signal transducer converts the bio-recognition event into an electronic signal. Interfacial design of bio-sensing material has been an active field of research [2]. The demanding criteria that bio-sensing interfaces must fulfil are similar to those of biological systems. In the latter case the selective adsorption of a species to an interface or the transport of a species across an interface must be achieved despite the presence of many other species in the surrounding environment. Moreover, it is at the cell surface that many important interactions in biology occur and many bio-recognition molecules function effectively only when inserted in a lipid membrane. Therefore using bio-mimetic membranes to fabricate biosensors is a key factor for success.

Future directions and multi-disciplinarity

Exciting progress in the understanding of bio-molecules at surfaces is on its way that will lead eventually to a detailed understanding of cell-surface interactions. These basic research endeavors will be driven both by curiosity and by the large number of applications of this knowledge. This will happen in parallel with dramatic improvements of existing experimental methods, and development of new ones, to prepare and characterize surfaces on the atomic and mesoscopic scale, including dynamics and kinetics determinations. These methods will be adopted for real-time measurements at solid-liquid interfaces. Theoretical methods and simulations will contribute in an even stronger way to the development of surface science [14]. Whether in bulk solution or at interfaces research in biophysics is characterized by a higher and higher degree of complexity. New techniques have been developed and are being used successfully to deal with complexity. With a better and better structural characterization of complex systems at interfaces, future developments and directions in science will include the study of faster dynamics (in the μs regime) and further development of single-molecule techniques as well as the study of higher spatial resolution (larger areas on the surface). It will also be important to assess the degree to which artificial model membranes can be used to study the functioning of real ones and eventually move to the use of real systems. Investigators will need to deal with the natural lipid asymmetry of real membranes as well as energetics. There is a clear need for the development of new computational models and algorithms that will allow simulations of large assemblies of molecules found for example in domains. In terms of structural characterization, important to assess function, one of the problems to face is the development of non-destructive techniques able to

probe *buried* systems with fraction of nanometer resolution in the three dimensions of space and insensitive to the bulk sides of the interface as well as to disordered components of higher-scale size (*i.e.* receptor-ligand interactions, bacteriophage-cell wall interactions, etc.).

The study of the structural and functional properties of biological systems, whether they are at interfaces or not, requires the concerted application of an arsenal of complementary techniques. Often, in current research laboratories, scientists content themselves as designers of new instrumentation and methodological approaches are developed to characterise by a given technique as many biological molecules as possible. The future perspectives in the field are to tackle a biological problem with a multidisciplinary approach and seek at defining as well as possible the functional, structural and dynamic properties of the molecules implicated in the physiological process together with their interactions [1].

Physicists are moving towards a more active involvement in the search for physical principles governing the assembly and function of biomaterials. The cell produces electrical signals from the diffusion of ions through ion-channel proteins in the lipid bilayer. This electrochemical signaling should allow for efficient communication between cells and microelectronics. Even though research in microelectronics and molecular biology are separately well advanced, the understanding of the interface between a cell and a silicon chip for the production of reliable devices to restore or sense cell function represents still a challenge [2]. The critical issue of the complexity of biomaterials needs to be understood and scientists need to become familiar with the central questions of biology and whenever possible avoid oversimplification of the systems [3].

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