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The interaction of proteins with solid surfaces

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The interaction of proteins with solid surfaces is a fundamental phenomenon with implications for nanotechnology, biomaterials and biotechnological processes. Kinetic and thermodynamic studies have long indicated that significant conformational changes may occur as a protein encounters a surface; new techniques are measuring and modeling these changes. Combinatorial and directed evolution techniques have created new peptide sequences that bind specifically to solid surfaces, similar to the natural proteins that regulate crystal growth. Modeling efforts capture kinetics and thermodynamics on the colloidal scale, but detailed treatments of atomic structure are still in development and face the usual challenges of protein modeling. Opportunities abound for fundamental discovery, as well as breakthroughs in biomaterials, biotechnology and nanotechnology.

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Abbreviations

MD	molecular dynamics
OWLS	optical waveguide lightmode spectroscopy
SAM	self-assembled monolayer
TIRF	total internal reflectance fluorescence
ToF-SIMS	time-of-flight secondary ion mass spectroscopy

Introduction

The interaction of proteins with solid surfaces is not only a fundamental phenomenon but is also key to several important and novel applications. In the biomaterials field, protein adsorption is the first step in the integration of an implanted device or material with tissue [1,2]. For example, the adsorption of serum proteins, such as fibrinogen, fibronectin or vitronectin, can influence the adhesion of leukocytes, macrophages or platelets, and ultimately lead to fibrous encapsulation [3,4]. In nanotechnology, protein–surface interactions are pivotal for the assembly of interfacial protein constructs, such as sensors, activators and other functional components at the biological/electronic junction. A detailed mechanistic

understanding of the protein–surface interaction would be of value to these fields, and the ability to tailor specific protein–surface interactions would benefit nanoscale materials and bio-nano-assembly technologies [5]. Fundamentally, the interaction of proteins and surfaces involves both protein binding and unfolding; studies may therefore increase our knowledge of protein biophysics in general.

Because of the great relevance of the protein–surface interaction phenomenon, much effort has gone into the development of protein adsorption experiments and models. The ultimate goals of such studies would be to measure, predict and understand the protein conformation, surface coverage, superstructure and kinetic details of the protein–surface interaction. This review will briefly summarize experimental knowledge about protein–surface interactions, survey particular systems with specific interactions, assess modeling approaches from large to small length-scale representations, and finally outline opportunities for future research.

Experimental studies on protein adsorption and surface morphology

Several reviews are available on physicochemical aspects of the adsorption of proteins to solid surfaces [6–9]. Proteins are often thought to denature at both solid–liquid and vapor–liquid interfaces, although they retain more structure on electrostatically neutral hydrophilic surfaces than on hydrophobic or charged surfaces. Kinetic measurements of adsorbed protein as a function of time and equilibrium adsorption isotherms have been measured using a variety of informative techniques, including optical waveguide lightmode spectroscopy (OWLS) [10], ellipsometry [11,12] and total internal reflectance fluorescence (TIRF) [13–15,16*,17,18]. Studies have identified systematic effects of salt concentration, protein charge or dipole moment, and surface charge or hydrophobicity [12,14,19–21]. Kinetic effects are often complex, in that long-time behavior can differ from short-time behavior, and final surface coverage can vary based on the rate at which protein was introduced into the system. Behavior is highly dependent on the individual nature of the protein and the surface involved. These complexities are often interpreted as arising from underlying structural phenomena (i.e. conformational change in the protein). For example, the orientation of fibronectin adsorbed on different polyelectrolytes was inferred from OWLS measurements [10]. Similarly, the orientation and spreading behavior of fibrinogen and lysozyme on hydrophobic and hydrophilic surfaces were inferred from kinetic measurements using TIRF [13–15,16*].

Direct observation of atomic-scale surface morphology is currently impossible, but progress has been made toward determining protein structure on a surface and useful structural information can be obtained. Biophysical methods for determining protein structure in solution (CD, IR spectroscopy, NMR and crystallography) are confronted with the challenge of the small heterogeneous samples present in a monolayer on a surface. Still, attenuated total reflection Fourier transform IR (ATR-FTIR) spectroscopy has been used to track the loss of secondary structure during insulin unfolding on a model lipid–water interface [22], and as albumin or an antibody adsorbs to silica surfaces [23,24]. Giacomelli and Norde [25] have used CD together with probes of thermal stability to measure changes in the secondary structure of BSA before and after thermal denaturation. Long *et al.* [26] used solid-state NMR techniques to determine the structure of the terminal helix of statherin on hydroxyapatite. This first high-resolution structural and dynamic characterization of a hydrated biomineralization protein adsorbed to its substrate is based on accurate measurements of distances between backbone carbonyl carbons and backbone nitrogens. Another study used NMR to isolate secondary structure changes in a model 13-residue peptide before and after adsorption to charged substrates [27]. Biochemical techniques have also enabled researchers to infer much about the morphology of proteins on surfaces. Mutagenesis experiments, radioisotope labeling, antibody or epitope binding [28], surfactant elutability and catalytic activity have all been used to infer knowledge about the (changing) state of the protein on the surface [29,30]. Nanoscale imaging techniques have also been used. Atomic force microscopy (AFM) has been employed to study lysozyme [31], ferritin [32] and insulin at surfaces, including the observation of surface denaturation [22]. Scanning force techniques have been explored to study lysozyme and albumin topology and adhesion force [33], and scanning tunneling microscopy (STM) has also been used with lysozyme [34].

Some methods are able to isolate information about particular regions of the protein. Hydrogen-exchange mass spectroscopy has been used to identify regions of lysozyme and α -lactalbumin that are accessible to solvent (i.e. partially unfolded) during chromatography under a range of solution conditions [35–37]. A particularly promising method is time-of-flight secondary ion mass spectroscopy (ToF-SIMS). In this technique, an adsorbed protein layer is bombarded with ions, breaking off short segments (as much as several peptides) from the upper nanometer of the adsorbed layer. After identifying these segments using mass spectroscopy, the orientation of the protein or its state of unfolding can be inferred [38**], and simultaneous contributions from multiple types of proteins at the surface can be distinguished [39**]. In theory, this technique can be applied in general to most typical proteins at different interfaces to provide structural information.

Structural information about the surface is also available. Tarasevich *et al.* [24] used classic IR techniques to measure the number of various types of hydrogen bonds with the surface and thus infer the loss of secondary structure in the protein [24]. By employing self-assembled monolayers (SAMs), Ostuni *et al.* [40**] were able to introduce varying concentrations of individual hydrophobic end-groups to an otherwise nonadsorbing hydrophilic surface. The authors emphasize the “intrinsic effect of local interactions”, as they can measure the effect of adding single aromatic groups sparsely across an otherwise hydrophilic surface. This study and others [41–43] demonstrate the versatility of SAMs for creating customized surfaces to explore protein–surface interactions.

In summary, protein–surface interactions are highly dependent on the individual properties of the system. Many methods are being developed to observe structure and significant information is already available to begin to decode the phenomenon.

Specific protein–surface binding in natural and engineered systems

Many specific protein–surface interactions have been observed in natural systems or created using new nanoeengineering techniques. There is a dream of emulating biology in the fabrication of materials, assembling them from the bottom-up with a hierarchy of levels of organization [44]. A challenge in chemical engineering and materials science today is the design and construction of self-assembled nanoscale structures. Although microelectronics processing techniques (e.g. lithography) are excellent for creating small features, it is difficult to manipulate nanoscale objects directly. Self-assembly promises to be a sound strategy for creating materials and devices cheaply, using thermal energy, diffusion, recognition, and thermodynamic states and kinetic traps as central themes in the construction. Biological systems, of course, self-assemble routinely: nanoscopic components control and execute the creation of new components and their assembly into larger superstructures. A typical bacterium might contain hundreds to tens of thousands of different proteins at any given time, yet somehow these proteins interact with their proper partners to perform their functions correctly. Clearly, there are engineering principles to discover.

In the natural world, there are several examples of specific protein–surface interactions that can result in useful functions or materials. Antifreeze proteins function by binding to ice crystal nuclei, presumably preventing the spread of crystallization. The crystal structure of an insect antifreeze protein shows protein sidechains positioned in a regular fashion such that threonine groups can make hydrogen bonds with oxygen molecules in the ice lattice [45]. A second well-studied example is the proteins involved in the deposition of calcium carbonate in abalone shells [46]. Proteins associated with the aragonite

crystals both control mineralization and impart material strength several orders of magnitude higher than the inorganic crystal alone [47,48]. Ossification is likely to be similar; ameloblastin and amelogenin are known to control the assembly of hydroxyapatite into tooth enamel, and probably contribute to the mechanical properties [49–51].

Biological examples have inspired the development of engineered systems that can take advantage of the functional properties of proteins to operate on the nanoscale. Using directed evolution techniques, Whaley *et al.* [52] found 12-mer peptides that could bind specifically and with high affinity to the semiconductor surfaces of GaAs, Si and InP. This is significant as proof of concept that tailoring new protein–surface interactions is possible. The paper inspired much follow-up work, including a simulation attempt to create proteins from repeats of similarly designed gold-binding peptides [53]. Sarikaya *et al.* [54•] have recently reviewed such molecular biomimetics, including a summary of 28 short peptide sequences that have been found to bind to solid surfaces ranging from platinum to zeolites to gallium arsenide. For materials synthesis, antibodies have been raised to bind to specific crystal surfaces to help control crystal growth [28,55], and the growth of gold crystals has been controlled by peptide binding [56]. One group has focused on the integration of nanoparticles with proteins by systematically searching for surface treatments to adsorb various proteins on their surfaces [19,20,57]. Brash and co-workers have explored adsorption onto liposomes [58], and have created lysine-laden surfaces that can adsorb proteins that might dissolve clots [59•]. Related ideas are explored in a recent review of the design of nanostructured biological materials through self-assembly [60]. In another study, biotinylated peptide linkers were attached to a surface via streptavidin to bind fibronectin in an oriented manner [61]. Such a material is then hoped to solicit a desired biological response (i.e. tissue integration). Perhaps the most exciting application is optical switching and modulation behavior based on proteins affixed to a substrate [62•]. Clearly, there is great promise for careful control of the protein–surface interface to accomplish much, perhaps someday integrating electronics and nanotechnology with cell biology at the molecular level.

Modeling of proteins on surfaces

Many protein adsorption modeling approaches have been tried and several have been refined to be considerably successful [6–8,63]. Colloidal-scale models represent the protein as a particle and can accurately predict adsorption kinetics and isotherms. These colloidal-scale models include explicit Brownian dynamics type models [64,65], random sequential adsorption models [66–69], scaled particle theory [70,71], slab models [72] and molecular theoretic approaches [73–75]. Most of these approaches treat the electrostatics and van der Waals interactions between the colloidal ‘particle’ and the surface, and thus can capture

dependencies on surface charge, protein dipole moment, protein size or solution ionic strength.

Colloidal models often capture complex behavior through some inclusion of structural information, that is, a representation of conformational change. Molecular theoretic, scaled particle theory and random sequential adsorption techniques account for conformational change using multistate representations (e.g. adsorbed folded and adsorbed denatured) [76]. Asthagiri and Lenhoff [77] represented the protein colloid by accounting for patches of positive and negative electrostatic potential on the protein surface, yielding better predictions of interactions between like-charged proteins and surfaces. Recent work has captured the unfolding of a model lattice protein, observing fast unfolding on the surface and slower refolding to a new, low-energy conformation [78•]. Jiang and co-workers represented the protein domains of immunoglobulins (IgGs) as connected spheres; this treatment successfully predicted the orientation of experimental IgG adsorption on surfaces of varying charge [42,79]. Latour and co-workers [80,81,82••] have used detailed molecular calculations, including enthalpic and entropic (solvation) contributions, to determine averaged interaction parameters between residues and surfaces as a function of distance. These parameterizations promise to be useful in building residue-scale models of proteins interacting with surfaces.

Finally, several researchers are exploring detailed atomic representations of proteins. The earliest studies to use protein crystal structures to simulate the adsorption process assumed a completely rigid protein and calculated screened coulomb and Lennard–Jones interactions over all protein rotations and distances [83,84]. Ravichandran *et al.* [85] have similarly explored rigid atomistic models with electrostatic treatments, and found that a net positively charged protein (lysozyme) could adsorb on a positively charged surface, due to the nonuniformity of the charge distribution on the protein. Recently, Zhou *et al.* [86•] predicted the orientations of an adsorbed antibody on a surface using a united residue model, whereby each amino acid is represented by a group with averaged electrostatic and van der Waals interactions. Finally, molecular dynamics (MD) was used to simulate 5 ns of multipeptides interacting with gold [53]. Also, MD-based simulations were used to find minimal energy orientations and unfolding trajectories of albumin subunits on graphite [87].

Research opportunities

Despite extensive study and superb descriptions on the colloidal scale, basic questions concerning the structural details of a protein in its adsorbed state are still difficult to answer. Some of these questions include: does the protein denature; is the protein still active; if the protein has not unfolded, what is its orientation; what conformation does the protein assume; what is the effect of crowding on the surface by other proteins; what are good strategies for

controlling protein adsorption to create a biocompatible surface? High-resolution experimental measurements and detailed structural models are needed to address these issues.

In particular, techniques that could measure atomic structures of proteins on surfaces would be immensely revealing. Time-resolved structural data, even of low resolution, are rare. Many experiments already infer gross knowledge of structure from isotherms and kinetic coverage data; however, the tighter integration of these measurements with detailed models is needed, although the data may not be sufficiently informative to distinguish correct underlying physical principles.

Most current modeling is limited to either large (colloidal) length scales or very small length and time scales (MD). Because initial protein adsorption events occur quickly (microseconds to milliseconds) and unfolding relaxations can occur over much longer times (hours or even days), mesoscopic and multiscale models are needed that can sample details over these relevant scales.

The design of specific interactions for nanotechnological applications is currently dominated by experimental, evolutionary techniques. However, these techniques often do not reveal underlying principles for extrapolation. Design can be limited by technical issues, such as library size and the availability of an appropriate screen. No rational design methodology exists, especially one that takes into account individual protein and surface structures. Quantitative methods to pursue design objectives could greatly supplement existing experimental techniques. For example, the application of quantitative methods to evolutionarily derived proteins could identify cold- and hot-spot residues for modulating binding affinity.

Recent techniques in protein biophysics should be extended to the study of proteins on surfaces; denaturation on a surface is, in some ways, the reverse of the protein folding problem. The application of recent protein modeling methods to this engineering challenge will provide a new means to test potential functions and methods of searching protein conformations. In fact, the interaction of β -amyloid with Teflon particles has recently been used to explore the nature of the formation and conversion of secondary structure in this medically important protein [88].

Conclusions

Although protein adsorption is well studied, we are only beginning to understand the phenomenon on the molecular level. As molecular details ultimately determine the answer to many relevant questions (especially those concerning structure, activity and function), emphasis should be placed on structural biology and biophysics. There is now great opportunity to use the latest biophysical and

structural techniques to reveal fundamental folding and binding principles, to design or control new structures and materials, and to develop applications in biomaterials, biotechnology and nanotechnology.

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