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## Adsorption of Protein onto a Solid Surface

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The adsorption of protein onto a solid surface is a far-ranging and complex problem. Issues of electrostatics, conformation and topography, and of course, thermodynamics, all factor significantly into the process. Solution chemistry in all its detail including pH and ionic strength can be used to control the adsorption process, but can also confound it if not properly considered. This article will introduce, briefly, some of these major considerations, and how understanding of protein adsorption can be used for new product development.

### Proteins: a brief introduction

Proteins are **copolymers** of 20 to 23 different **amino acids** linked together to form a **polypeptide** chain [1]. Many proteins consist of a single such polypeptide chain. Others have two or more chains that may or may not be identical to each other. Structural **sidechains** factor heavily into the resulting chemistry of the protein. Within the same protein, some sidechains may be acidic and some basic, making the polypeptide **amphoteric**. The sidechains furthermore vary in their **hydrophobicity** (polarity), rendering the polypeptide **amphiphatic** (a.k.a., **amphiphilic**), which property makes them inherently surface-active [2]. Proteins therefore can display a rich and seemingly daunting chemistry.

Proteins furthermore have a structural hierarchy that may play into their behavior [1]. The **primary structure** is the sequence of amino acids and location of any disulfide bridges in the polypeptide chains. The primary structure determines spatial orientation of the protein in its ambient environment. For single-strand proteins, the spatial orientation is further divided into a **secondary structure** and a **tertiary structure**. The former of these is any regular local structure such as a helix or sheet, and the latter is the overall topology of the polypeptide chain. For multiple-strand proteins, known as **oligomeric proteins**, a **quaternary structure** is defined as the structure formed by monomer-monomer interaction between the strands.

The secondary structure of a protein leads to its **conformation** as determined by the nature of its **folding**. In general, proteins fold themselves into two broad classes, *viz.*, **globular proteins** or **fibrous proteins**. As the names suggest, globular proteins are compactly folded and coiled, and fibrous proteins are more filamentous or elongated [3]. Two

conformations of globular proteins are the  **$\alpha$ -helix**, such as those of myoglobin and hemoglobin, and the  **$\beta$ -sheet** as found with many antibodies. Because different regions within a single protein may assume different conformations, it is possible for example to obtain  **$\alpha/\beta$  proteins**, such as  $\alpha$ -lactalbumin. Filamentous conformations are attained by collagen and silk. Color illustrations of 18 different conformations are given in [4]. A protein that has lost its natural conformation (folding) is said to be **denatured**. Most proteins upon adsorption will lose their native conformation to at least some extent, and a corresponding increase in entropy may be a driver for the adsorption process. Research has been conducted to study protein denaturation in a controlled fashion, using carefully prepared concentrations of **guanidinium chloride**, a salt known for having this property.

Proteins typically carry **electrostatic charge**, often both positive and negative on the same protein. The protein net charge, charge distribution, and decay length of electrostatic interactions with the surface can all affect adsorption with the surface [5]. Some proteins have their adsorption behavior less affected by electrostatics than by other factors such as hydrophobicity. These proteins typically show large conformational changes upon adsorption, and are referred to as “**soft proteins**” [5]. Other proteins, called “**hard proteins,**” are so strongly affected by electrostatics that they begin to behave like simple colloidal systems in some respects.

### **Solution Environment: pH and ionic strength**

The environment of the solution which the protein leaves as it adsorbs to the surface will have an effect on that adsorption. Only two of the many solution conditions will be considered here. For a number of protein adsorption scenarios, adsorption is maximized when solution pH is close to the **isoelectric point** [5], i.e., the pH at which the protein is electrically neutral or minimally charged. This is related to protein-solvent interactions becoming less favorable, and protein-protein interactions becoming less unfavorable, as the protein net charge diminishes. Extreme pH levels will cause any protein to become unstable or to lose its native conformation [1].

A study was made by Höök et al. of the adsorption of the globular protein hemoglobin (Hb) onto a hydrophobic methyl-terminated thiol monolayer that had been deposited onto a gold surface [6]. This made use of a quartz crystal microbalance (QCM) technique that monitors frequency change in real time upon mass loading. At low ionic strength (low salt concentration) near the isoelectric point (about 6.9), a two-phase adsorption was observed. The first layer was thought to be more rigidly coupled to the surface, due to dissociation of subunits in the Hb molecule and thus partial denaturation. The second layer, which showed little or no denaturation, was more loosely bound. The molecules in this layer retained some functionality, as evidenced by an ability to be chemically reduced and to bind CO. At high salt concentration (200 mM KCl), also near the isoelectric point, stability against denaturation is higher than it is at low ionic strength. Also, electrostatic interactions between protein molecules are weaker at higher vs. lower ionic strength. As a result, only one monolayer is adsorbed at high ionic strength. At low ionic strength, maximum adsorption (of the bilayer) occurs at or very near the isoelectric point. This has been reported to occur even on charged surfaces. At pH values much different (higher or lower)

than the isoelectric point, formation of the bilayer is likely to be discouraged due to electrostatic repulsion resulting from an increasing amount of surface charge on the protein. At high ionic strength, adsorption shows relatively little dependence on pH in the range 6.5-7.5.

In general, as the solution environment becomes less hospitable to a protein, there is a greater tendency for the protein to adsorb to a surface, thereby leaving the solution. Altering the solution environment after protein adsorption may affect properties of the adsorbed layer, such as conformation, adsorption strength, or adsorption extent (see below).

### **Surface Environment: hydrophobicity and topography**

A general rule of thumb is that *proteins prefer to adsorb to a hydrophobic surface* rather than to a hydrophilic surface; there are exceptions to this. Proteins tend to conserve more of their native structure on hydrophilic rather than hydrophobic surfaces, because the former have little or no electrostatic charge [7]. The preference for hydrophobic surfaces may be related to differences in conformation of the adsorbed protein on the two types of surfaces [5].

Many studies that have examined the effect of surface hydrophobicity on protein adsorption have used polymer microspheres as the substrate. Microspheres of polystyrene/poly (methylmethacrylate) (PS/PMMA) were used by Yoon et al. in their study of adsorption of bovine serum albumin (BSA) [8]. By varying the ratio of the two monomers used during preparation of the microspheres, the level of surface carboxylation, expressed as number of surface carboxyl groups/nm<sup>2</sup>,  $N_C$ , could be varied (carboxyl groups come from the methacrylic acid). They report that protein adsorption is due primarily to hydrophobic interactions and hydrogen bonding, with ionic interactions and van der Waals interactions considered to be much less significant. At low  $N_C$  (<1), adsorption is due primarily to hydrophobic interactions, and is sensitive to pH. The adsorption is maximized near the isoelectric point of BSA, which is 4.7. At higher  $N_C$  (>2), adsorption is due more to hydrogen bonding, and is relatively insensitive to pH.

Ayhan reports another study using PMMA microspheres for the adsorption of BSA [9]. Microspheres of neat PMMA, and microspheres of methylmethacrylate co-polymerized with 2-hydroxyethyl methacrylate (HEMA), were each sterically stabilized with poly(vinylpyrrolidone) (PVP), or with poly(vinyl alcohol) (PVA). In addition, some P(MMA/HEMA) microspheres were left unstabilized (five types of microspheres total). It was found that microspheres of the two types of stabilized PMMA and of the unstabilized copolymer showed increasing BSA adsorption with increasing hydrophobicity. In both the Yoon and Ayhan studies, other factors such as ionic strength may have had an effect.

A study by Bruinsma et al. with immediate product application examined the hydrophobicity (and other characteristics) of the surface of contact lenses (CL) for the adsorption of protein and the bacterium *Pseudomonas aeruginosa*, as a function of time that the lenses were worn [10]. The lenses were a hydrogel containing 2-hydroxyethyl methacrylate, 2% methacrylic acid, and water. After CL were worn for 10 days, and more so after 50 days (“overwear”),

there was a significant increase in both surface hydrophobicity as indicated by water contact angle measurements, and in the amount of adsorbed proteinaceous material, including the natural tear component lysozyme. Other factors may well have played into the increase in adsorbed protein, such as a measured increase in surface roughness (topography).

Galli and co-workers studied the effects of topography for adsorption of the protein F-actin onto modified surfaces of evaporated titanium (Ti) [11]. F-actin is a fibrous protein having filaments of width 6.5-8.2 nm and an isoelectric point between 5.06 and 5.27. The titanium film had a thickness of 10 nm and was deposited onto Si <111> at a rate of 50 Å/sec. It had an RMS roughness of 0.2 nm. Using the technique of Local Anodic Oxidation [12] and an atomic force microscope (AFM), two sets of titania structures were produced, one with a height of 3-4 nm, the other with a height of 1-2 nm (both had a full-width-half-maximum (FWHM) of about 40 nm). These structural changes reportedly occurred with no accompanying chemical change. The workers found that more F-actin adsorbed on the structures with less height than on the structures of greater height. Also, F-actin adsorbed onto the structures of less height seemed to display a preferred orientation, whereas the protein adsorbed onto the structures of greater height did not. The explanation is that the protein adsorbs according to a “lock and key” mechanism that prefers surface features of a size very similar to feature sizes of the protein.

### **Adsorption: energetics and structure**

Two ways of characterizing a layer of adsorbed protein are the **adsorption strength** and the **adsorption extent** (i.e., amount of adsorbed material). In many cases, one does not necessarily imply anything about the other, although both are related to the structural stability of the protein. For homopolymers and copolymers, the extent of adsorption often increases with increasing molecular weight; this does not apply in a straightforward way to all proteins. How closely the adsorbed protein molecules are “crowded together” on the surface will affect their orientation, deformation, and denaturation upon adsorption. Previous workers have shown for several protein systems that conformational change decreases with increasing amount of adsorbed protein, simply because the molecules have less room to spread out on the surface [5]. Malmsten goes on to describe a “**random sequential adsorption**” model presented by Adamczyk and co-workers for adsorption of colloidal particles. This model predicts that the maximum packing density on the surface, called a “jamming limit,” will be about 50% of the surface area, perhaps as high as about 60% in some special cases. Extending this to adsorbed proteins is difficult, although “hard” proteins may begin to behave like colloidal particles (see above). Some adsorbed proteins will change their orientation to accommodate more molecules being adsorbed, from a “side-on” to an “end-on” contact with the surface. While this does not change the percent coverage of the surface *per se*, it does increase the extent of adsorption as well as change the chemically-active sites the protein presents to the solution.

During adsorption, a protein may rapidly unfold to “conform” to the new environment of the surface. After adsorption, the protein layer may relax, achieving a new conformation with lower energy. The adsorption event itself occurs within microseconds to

milliseconds, but relaxation occurs over a much longer timeframe, of the order of hours or even days [7]. As a general rule, the thickness of many adsorbed protein layers on many surfaces is of the order of a few tens of nanometers [5]. As with many adsorbates, there may be significant energetic and mechanistic differences between adsorption of a protein and its desorption [2].

A particularly useful approach for studying protein adsorption is formation of **self-assembled monolayers** (SAMs) on a rigid substrate, such as a glass slide [2]. Very often, these alkanethiol films may be further chemically modified to tailor their surface properties. Adsorption may be studied using gradient surfaces, such as positive-negative charge gradients or surface density gradients of chemically-active sites.

## **Applications**

Because proteins are so ubiquitous, their adsorption factors into very many everyday products and applications. Among these are medical and industrial uses, plus some more novel applications that are beginning to emerge.

### Medical applications include:

- biocompatibility of medical and dental implants
- lysine-laden surfaces that can adsorb proteins to dissolve blood clots
- diagnostic devices and biosensors
- drug delivery schemes for pharmaceuticals
- biomedical research and genetic engineering
- sterile surfaces in hospitals, clinics, and biomedical labs

### Industrial applications include:

- food and beverage processing equipment
- production equipment for protein purification and separation
- optical switching via protein adsorbed to an optical surface

### Emerging applications include:

- biomimetics, e.g., self-healing surfaces mimic protein adsorption such as natural “anti-freeze proteins” [7]
- materials engineering, e.g., antibodies binding to specific crystal surfaces to control crystal growth [7]

## **Summary**

Protein adsorption and biotechnology can be used to help improve products during the product development cycle. Modifying solution chemistry and surface environment are ways to improve performance of adsorbed protein layers. Other improvements can be tailored to meet your needs. Contact Bjorksten | bit 7 for more information about enhancing your product performance.

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## **Key Words**

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