MME 4506 Biomaterials

Protein adsorption to biomaterial surfaces

After implantation of a biomaterial in a living system, proteins have been observed on the surface in a short time, less than 1 second later.

A monolayer of protein adsorbs to most surfaces in seconds to minutes

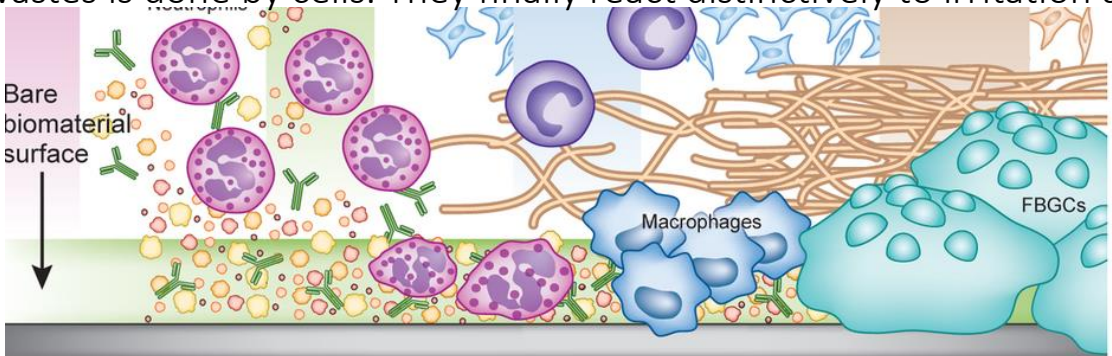
This event occurs well before cells arrive at the surface, therefore they see primarily a protein layer rather than the actual surface of the biomaterial.

Since cell respond specifically to proteins this interfacial protein film may be the event that controls subsequent bioreaction to implants

Cells arrive at an implant surface after proteins adsorb, by diffusive, convective or active locomotion mechanisms

On the surface of the biomaterial cells adhere, differentiate, release active compounds to communicate with other cell types, multiply and organize themselves into tissues comprised of one or more cell types.

Extracellular matrix is secreted by cells to fill the spaces between cells and serve as attachment structure for proteins and cells. Formation of blood vessels that is critical to provide this new tissue with nutrition and to remove wastes is done by cells. They finally react distinctively to irritation and injury.



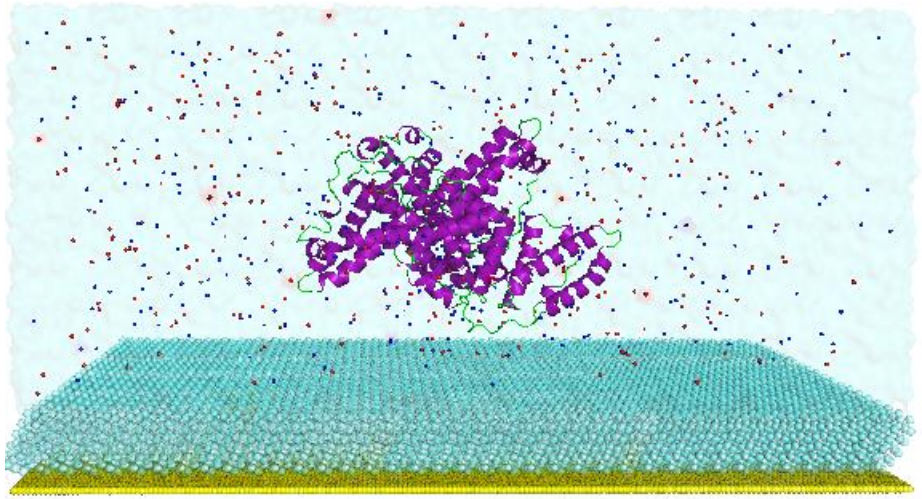
In vivo biocompatibility tests done on 10 common biomaterials shows that they are all found to heal essentially identically after one month implantation in mammals.

gold, polyurethane, silicone rubber, polytetrafluoroethylene (PTFE), polyethylene (PE), poly(methyl methacrylate) (PMMA), poly(2-hydroxyethyl methacrylate) (PHEMA), poly(ethylene terephthalate) (PET), titanium, alumina

These materials are hydrophilic, hydrophobic, hard, soft, polymeric, ceramic and metallic and exhibit wide range of properties

Each material adsorbs different proteins, and shows substantially different cell attachment and cell growth behavior in vitro.

In vivo all materials quickly acquire a layer that contains many proteins (possibly comprised of 200 or more proteins) in many states of orientation and denaturation so that they adsorb a complex, non-specific layer of proteins.

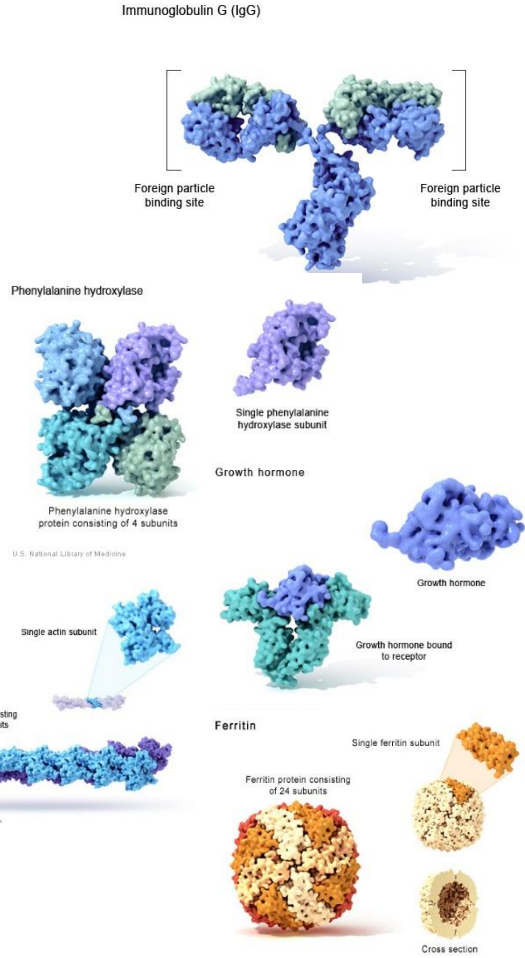


Proteins are large, complex molecules that play many critical roles in the body
They do most of the work in cells and are required for the structure, function, and regulation of the body's tissues and organs

Proteins are made up of hundreds or thousands of smaller units called amino acids which are attached to one another in long chains
There are 20 different types of amino acids that can be combined to make a protein
The sequence of amino acids determines each protein's unique 3D structure and specific function

Examples of protein functions

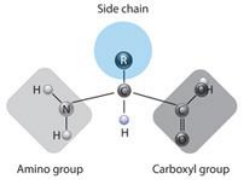
Function	Description	Example
Antibody	Antibodies bind to specific foreign particles, such as viruses and bacteria, to help protect the body	Immunoglobin G
Enzyme	Enzymes carry out almost all of the thousands of chemical reactions that take place in cells	Phenylalanine hydroxylase
Messenger	Messenger proteins transmit signals to coordinate biological processes between different cells, tissues and organs	Growth hormone
Structural component	Provide structure and support for cells and tissues	Actin
Transport/storage	Bind and carry small atoms and molecules within the cells and throughout the body	Ferritin

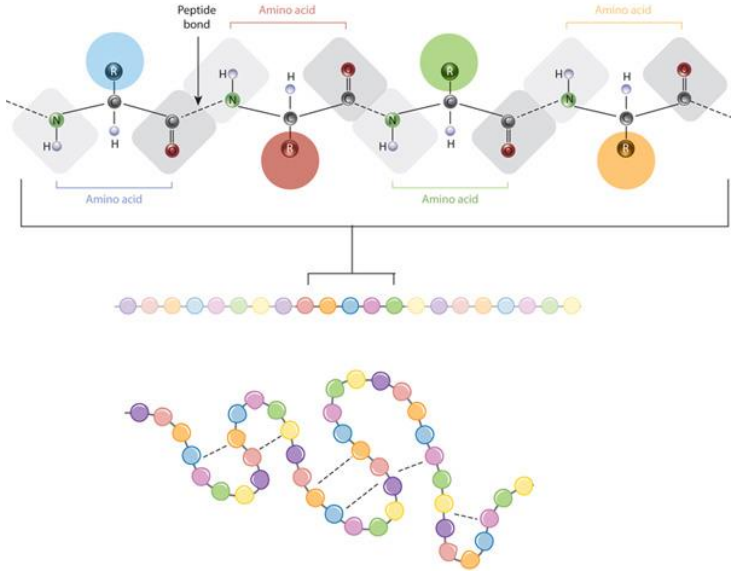


Each gene in cellular DNA contains the code for a unique protein structure
All proteins are assembled with different amino acid sequences that are held together by different bonds and folded into a variety of 3D structures
The folded shape or conformation depends directly on the linear amino aid sequence of the protein

Amino acids are small organic molecules that consist of a central carbon atom linked to an amino group, a carboxyl group, a hydrogen atom and a variable component called a side chain
Peptide bonds link multiple amino acids together in a protein, forming a long chain

Each of 20 amino acids has a unique side chain of different chemistry





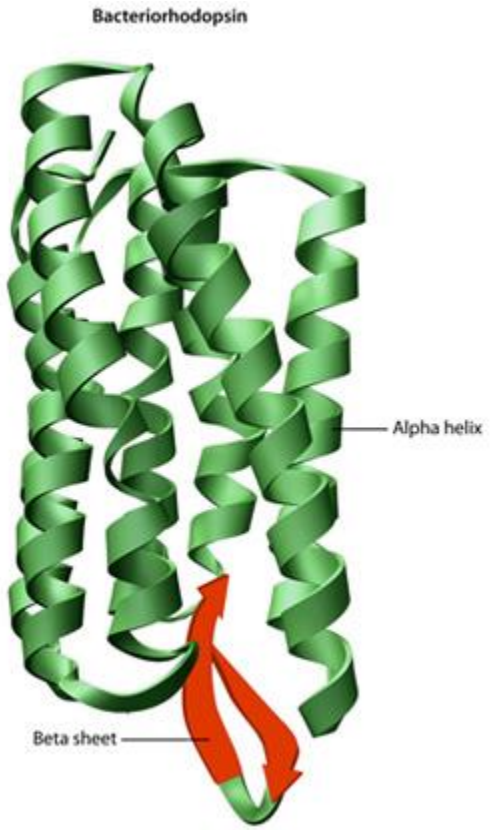
The largest group of amino acids have nonpolar side chains
Some amino acids have side chains with positive or negative charges while others have polar but uncharged side chains
These side chains can bond with one another to hold a length of protein in a certain shape or conformation

Charged amino acid side chains can form ionic bonds
Polar side changes can form hydrogen bonds
Hydrophobic side chains interact with each other via weak van der Waals interactions

The order and location of amino acids in a protein determines where the bends and folds occur in that protein

Certain patterns of folding occur
Alpha helices and beta sheets are present in most proteins
The formations and folds in a single polypeptide are unique

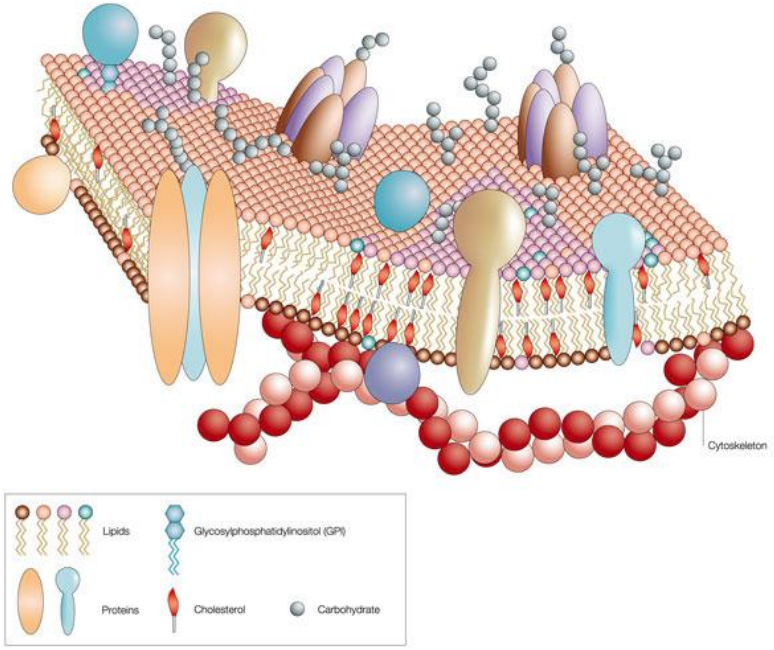
The final shape of the protein is a compact, low energy form
The fully folded protein can still make small shape adjustments



Chemical forces between a protein and its immediate environment contribute to protein shape and stability in addition to thousands of noncovalent bonds between amino acids

Proteins that are dissolved in the cell cytoplasm have hydrophilic chemical groups on their surfaces and hydrophobic groups are hidden inside the folded structure

In contrast, the proteins that are inserted into the cell membranes display some hydrophobic chemical groups on their surface where they are exposed to membrane lipids



The driving force for protein folding is the attainment of minimum energy

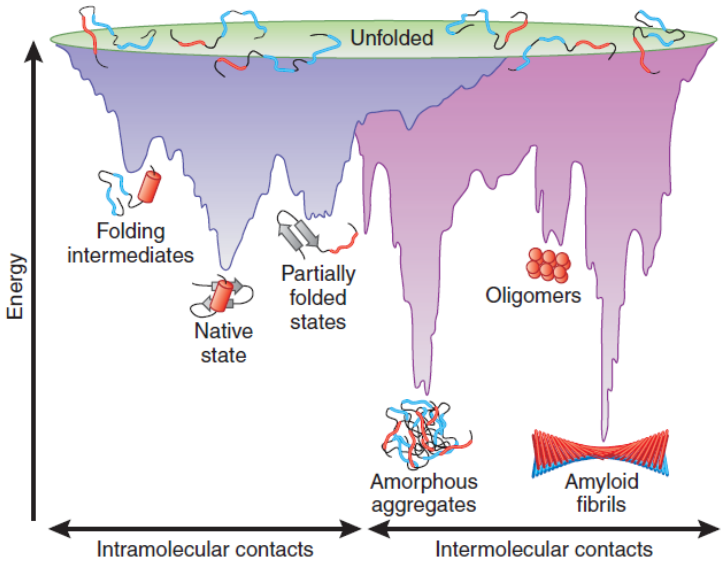
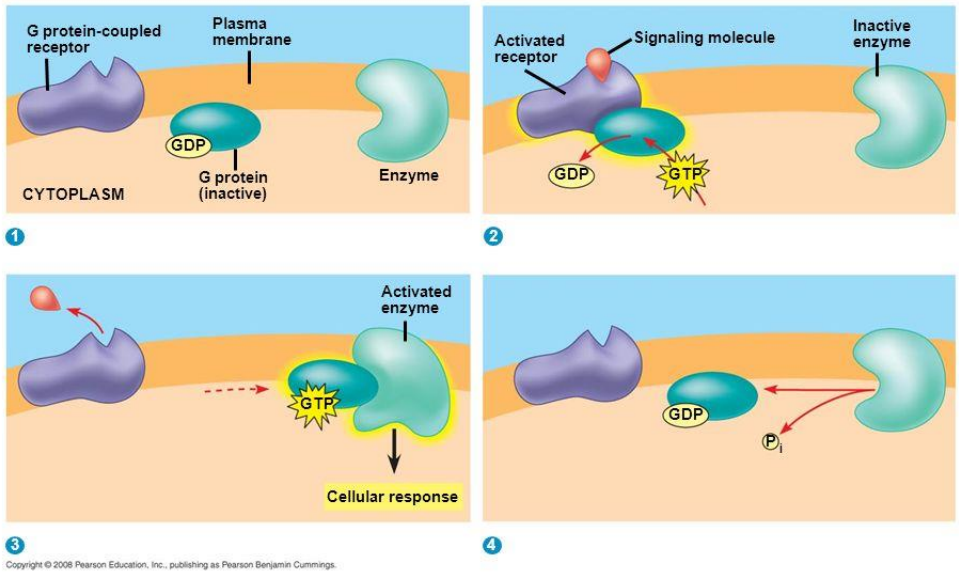


Figure 1 Energy landscape scheme of protein folding and aggregation. The purple surface shows the multitude of conformations 'funneling' to the native state via intramolecular contacts and the pink area shows the conformations moving toward amorphous aggregates or amyloid fibrils via intermolecular contacts. Both parts of the energy surface overlap. Aggregate formation can occur from intermediates populated during *de novo* folding or by destabilization of the native state into partially folded states and is normally prevented by molecular chaperones. Cell-toxic oligomers may occur

Nature's use of proteins as signaling agents comes from one (or a few) specific proteins in fixed conformations and orientations so they optimally deliver signals.



The hypothesis for the slow healing of biomaterials suggests that the body views the non-physiologic proteinaceous foreign body capsule layer as something with which it has no experience and reacts to it as an unrecognized foreign invader that must be walled off

The main strategy for development of healing biomaterials is the synthesis of surfaces of biomaterials that present to the body the same signaling proteins as the surface of a clean, fresh wound

Research to inhibit non-specific protein adsorption is driven by such questions:

How resistant to protein pickup can such surfaces be made?

Why are they resistant to protein adsorption?

How long can they remain resistant to protein fouling?

Can they be functionalized with organic groups permitting the immobilization of active biomolecules on a smooth background?

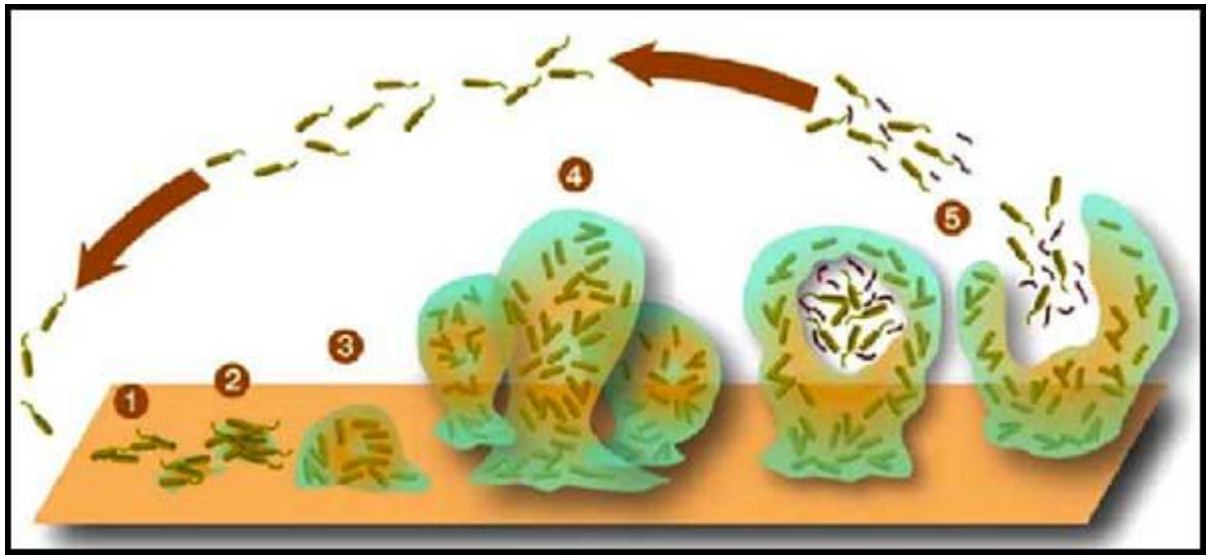
Surfaces that interact with precision with biological systems will be complex:

- multicomponent
- multilayer
- orientated
- patterned

Given the complexity of the molecular structures that make up the individual biomolecules comprising these surfaces, fabrication and characterization of such surfaces requires extreme skill in surface science and engineering.

One important area of research is on resistance to bacterial biofilms

Bacteria adhere to surfaces via a conditioning film of proteins that adsorb first to the surface

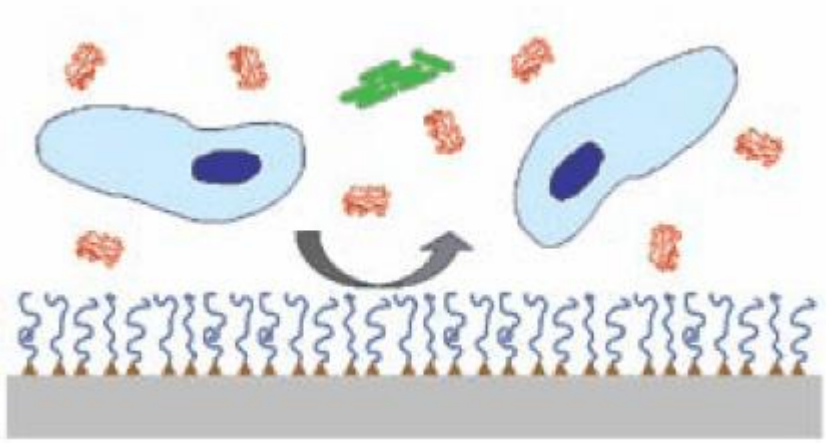


Bacteria stick to this conditioning film and begin to secrete a gelatinous slime layer (the biofilm) that aids in their protection from external agents like antibiotics

Such biofilms cause inflamatory reaction to infected devices such as urinary catheters, endotracheal tubes, vascular grafts, hip joint prostheses, heart valves

If the conditioning film can be inhibited, bacterial adhesion and biofilm formation can be reduced

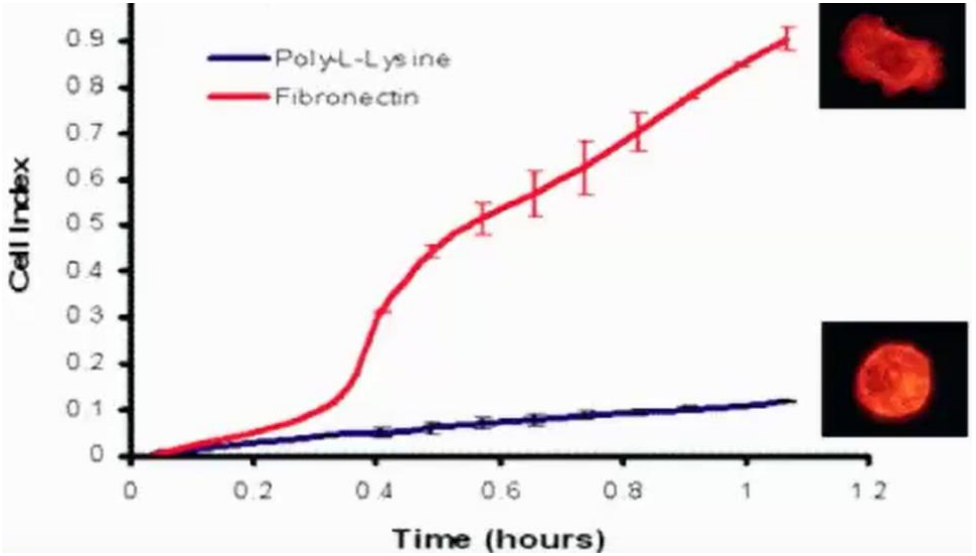
Nonfouling or protein resistant surfaces refer to surfaces that resist the adsorption of proteins and adhesion of cells.



It is generally accepted that:

- Surfaces that resist protein adsorption will also resist cell adhesion
- Hydrophilic surfaces are more likely to resist protein adsorption
- Hydrophobic surfaces usually adsorb a monolayer of tightly adsorbed proteins.

Surfaces with selective protein adsorption in vitro result in different cellular response in vivo



Control over protein adsorption provides control over cell adhesion
Important for a variety of processes:

- Clot formation
- Foreign body response
- Osteoinductivity
- Vascularization
- Tissue growth
- Bacterial colonization

Generally protein adsorption is affected by many factors:

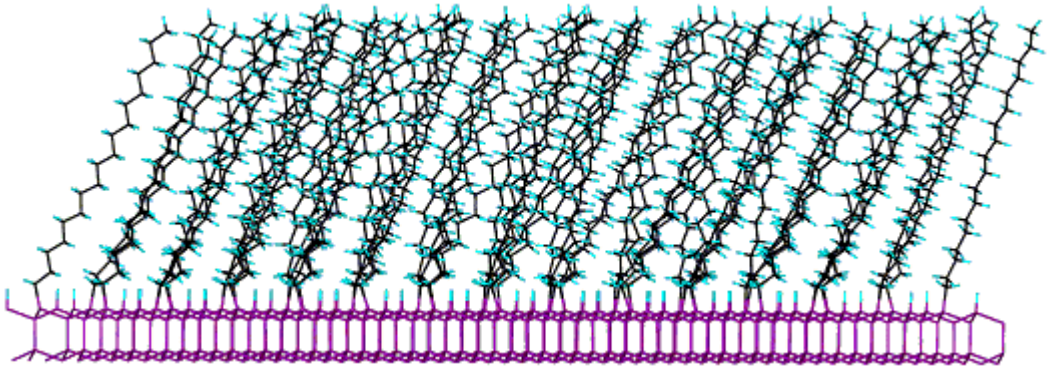
1. Surface characteristics
    1. Hydrophobicity-philicity
    2. Charge (electrostatic effects)
2. Protein characteristics
    1. Charge
    2. Conformation
    3. Stability
    4. Size (small molecules diffuse faster than large molecules)
3. Ambient conditions
    1. Concentration of different proteins in solution
    2. pH
    3. Ionic strength
    4. Temperature

Literature on protein adsorption and cell interaction with nonfouling surfaces reveals many aspects of protein adsorption on biomaterial surfaces:

- Hydrophobic surfaces have a strong tendency to adsorb proteins irreversibly. The driving force for this is the unfolding of the protein on the surface, accompanied by the release of many hydrophobically structured water molecules from the surface. As a result the entropy of the system increases.

- Cationic proteins bind to anionic surfaces and anionic proteins bind to carionic surfaces. The driving force is a combination of enthalpy decrease due to ion-ion bondings and entropy increase due to the accompanying release of counterions with their waters of hydration.

- It has been commonly observed that proteins adsorb in monolayers (they do not adsorb nonspecifically onto their own monolayers).



Proteins and polymers have similarities in their adsorption behaviors

Polymers in solution have flexible, coily, high entropic structures
Their conformational entropy is lower when adsorbed on a surface

Fig. 1. Flexible, unordered polymer molecule adsorbed at a surface

A polyampholyte (a polymer that carries both positive and negative charges) is similar to proteins in structure
In its isoelectric state (when the numbers of positive and negative charges are equal and evenly distributed over the molecule), intramolecular electrostatic interaction is attractive and the molecules shrink. At a pH away from the isoelectric point, they spread

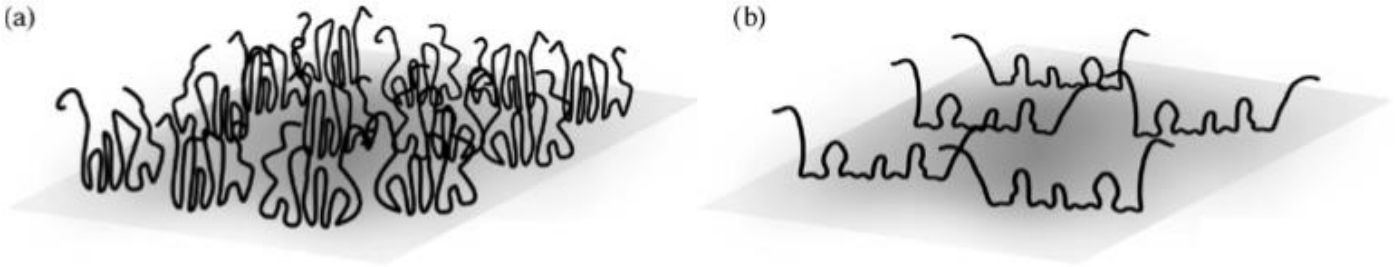


Fig. 2. Adsorbed polyampholyte molecules. (a) In the isoelectric state intramolecular electrostatic interaction renders the molecules relatively compact and because of the overall electroneutrality they may pack in a dense layer. (b) At pH values away from the isoelectric point, when the molecules possess a net amount of charge, intra- and inter-molecular electrostatic repulsion results in more expanded molecules that adsorb in a less dense layer.

Proteins have more stability and order in the solution and change their structure on the surface depending on their stability

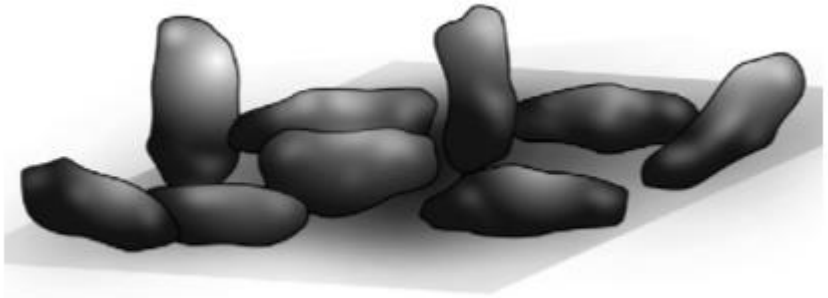
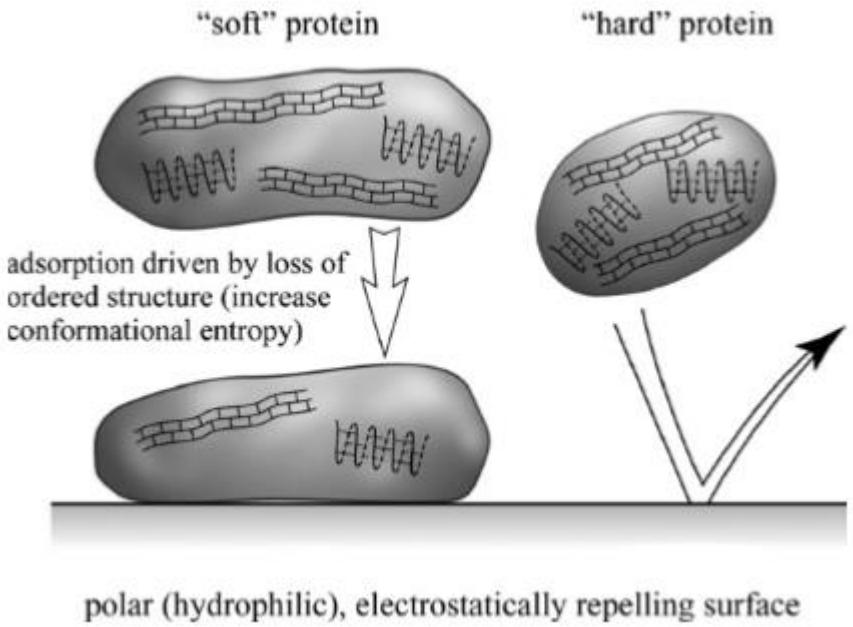
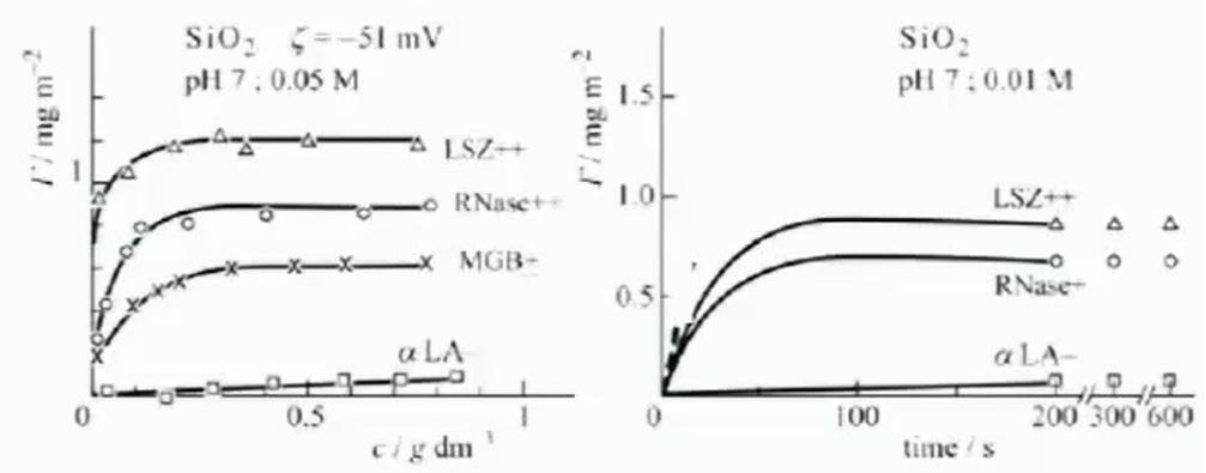


Fig. 3. Impression of a monolayer of adsorbed globular protein molecules.

Proteins with less stability in solution tend to rearrange its structure upon adsorption
Some proteins are hard (resilient against structural alterations) which influences the adsorption behavior



The adsorption of proteins onto a biomaterial surface from the surrounding fluid phase is rapid, with a fast diffusion controlled initial adsorption accompanied by a heat uptake from the surrounding



The adsorbed proteins are more packed and less relaxed when the adsorption kinetics is fast

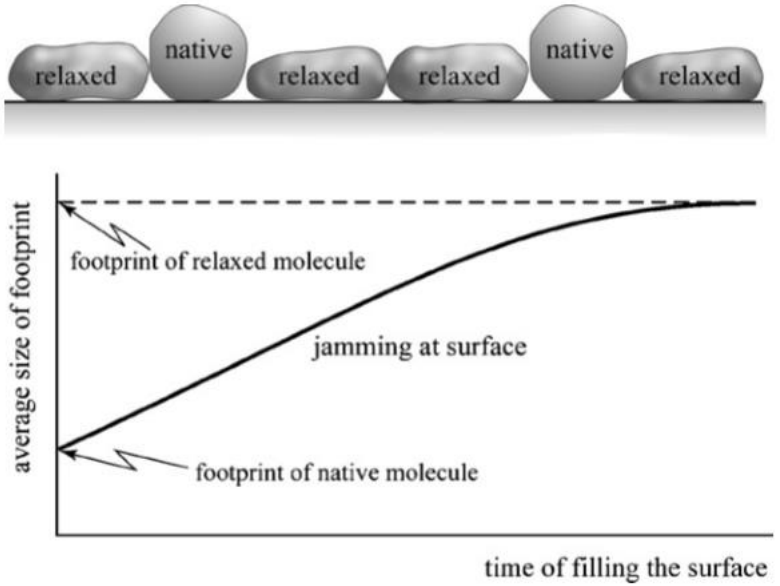


Fig. 8. Degree of relaxation of adsorbed protein molecules depends on the rate at which the surface becomes covered.

Both the surface and protein influence the adsorption
The charge on the protein molecule adapts when it senses the electrostatic field of the surface

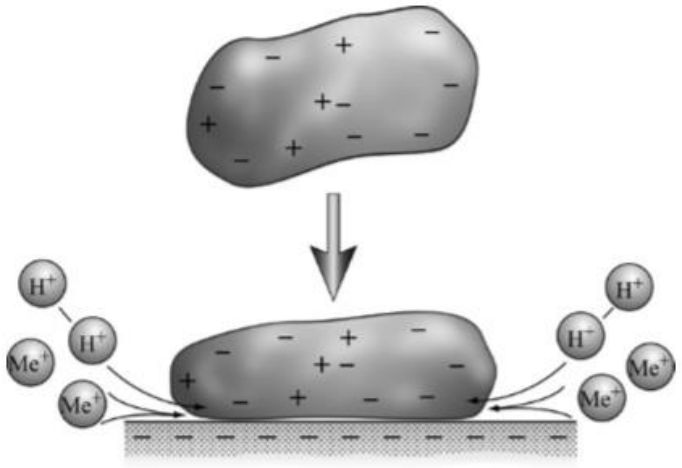


Fig. 4. Charge regulation in protein adsorption. In this figure positive ions (protons and other cations) are incorporated to prevent charge accumulation in the

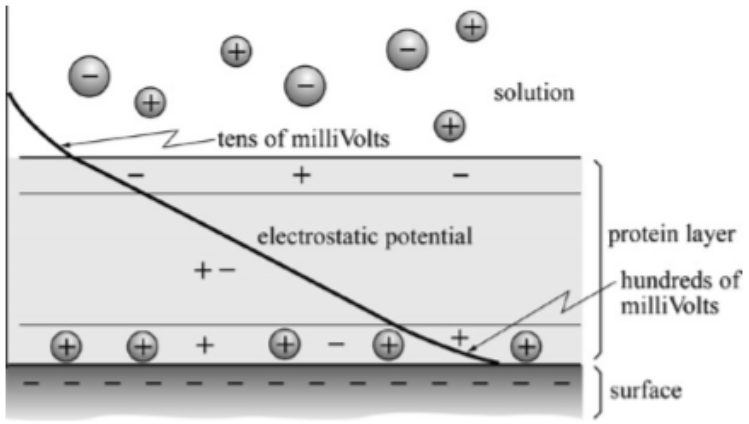
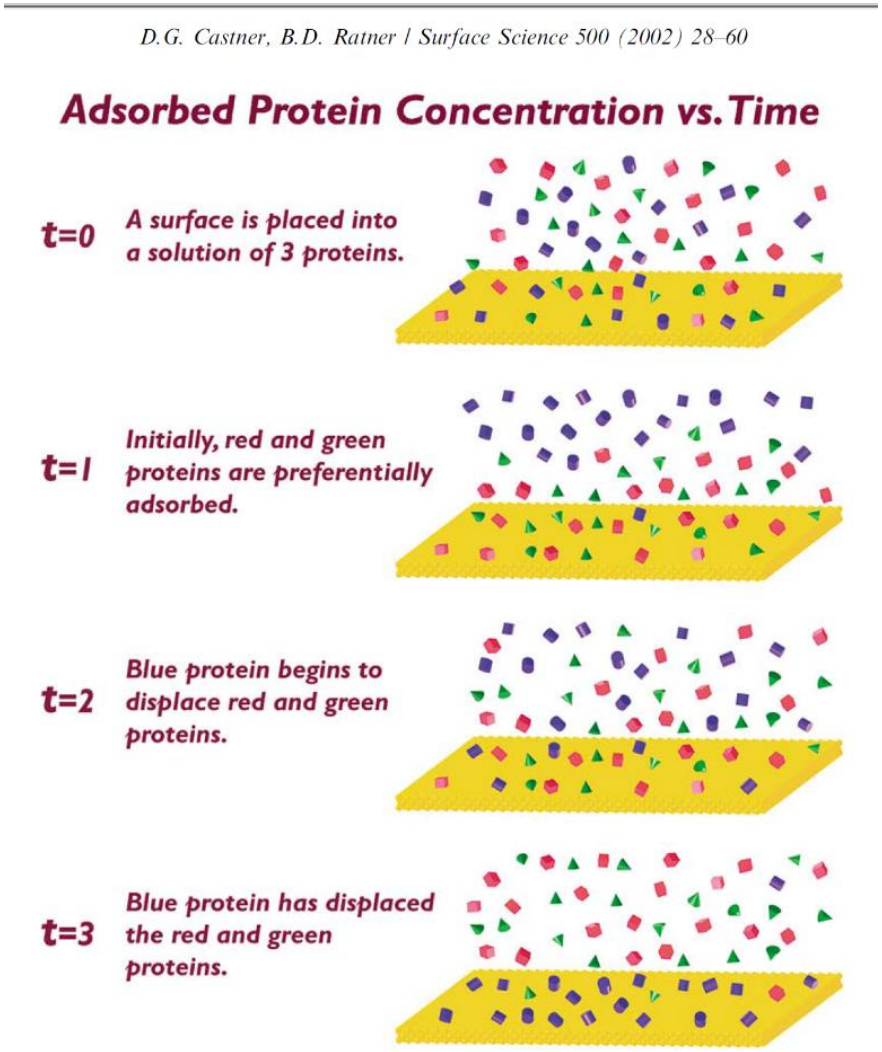


Fig. 5. Model for the distribution of charge in a protein layer at a charged surface and the resulting potential profile.

The composition of the adsorbed protein layer (i.e., the type and concentration of the proteins present in the adsorbed film) can differ from the fluid phase composition and can change with time adsorbed (Vroman effect)



In addition, the surface properties of the biomaterial determines the type, amount, and conformation of the adsorbed proteins.

Different proteins have different surface binding affinities
For example heparin inhibits adsorption of fibronectin

Proteins are selectively adsorbed from mixtures of proteins
Fraction of proteins on the surface are higher than the fraction in mixture

Protein activity in adsorbed state is different than that in solvated state

- Higher local concentration
- Steric hinderance (access to active site is inhibited)
- Change in protein conformation

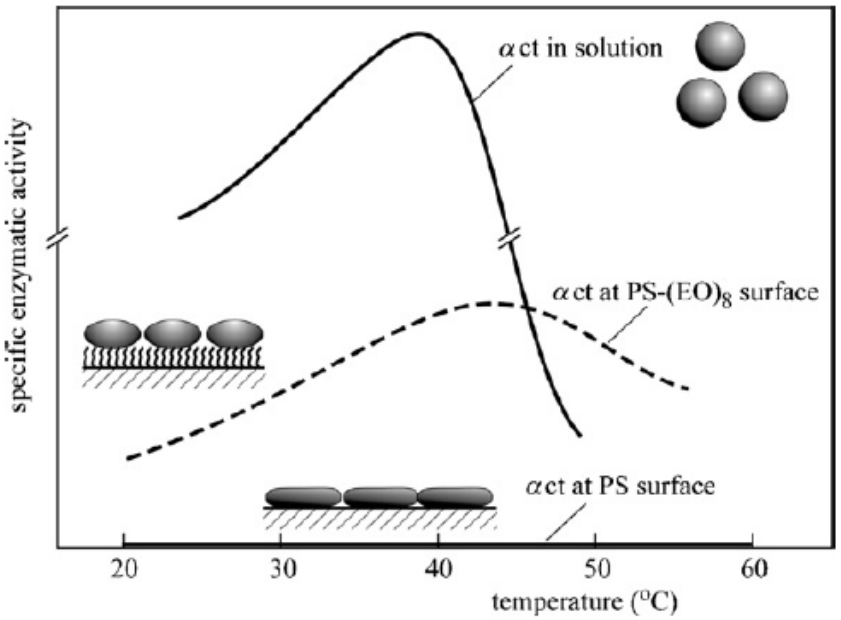


Fig. 12. Enzymatic activity of α-chymotrypsin in solution and at surfaces. Surface-attached oligomers weaken protein–surface interactions resulting in less adsorption-induced structural changes and, hence, less loss of biological activity of the protein.

Properties of the surface strongly influence the composition and recognizability of the adsorbed protein layer, which in turn affects the subsequent cellular interactions.

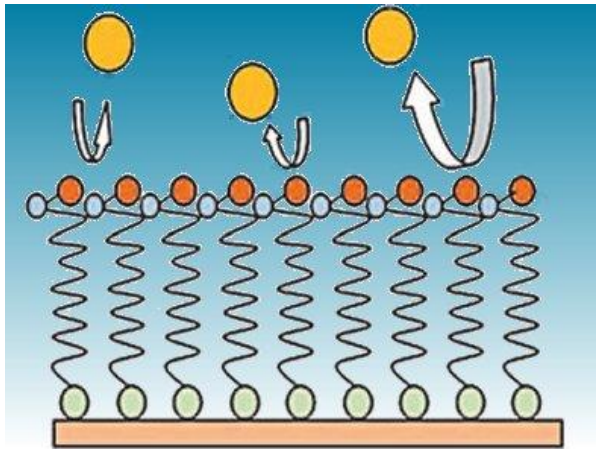
- At least 50% of the surface should be covered before resistance to protein adsorption is observed

- Adsorbed protein films are nonfouling surfaces for other proteins but not to cells

- Surfaces coated with physically or chemically immobilized high molecular weight PEG are highly resistant to protein adsorption. This is due to a combination of 2 factors:

i. The resistance of the polymer coil to compression due to its desire to retain the volume of a random coil (entropic repulsion)

ii. The resistance of the PEG molecule to release bound and free water from the hydrated coıl. (osmotic repulsion)



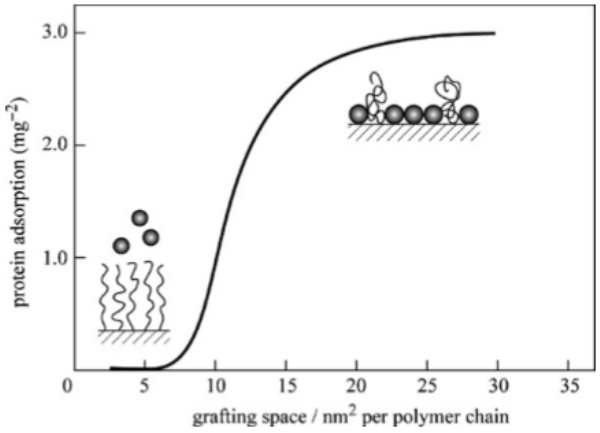


Fig. 11. Adsorption of blood plasma proteins. High-density polymer brushes suppress deposition of proteins at surfaces.

The size of the adsorbing protein and its stability in the folded state are important factors determining the extent of adsorption on any surface

The thermodynamic principles governing the adsorption of proteins onto surfaces involve various enthalpic and entropic terms favoring or resisting adsorption:

Favoring adsorption		Opposing adsorption	
(-) $\Delta H_{adsorption}$	(+) $\Delta S_{adsorption}$	(+) $\Delta H_{adsorption}$	(-) $\Delta S_{adsorption}$
Short range VdW bonds	Desorption of structured water	Dehydration of the interface between surface and protein	Adsorption of proteins
Long range ion-ion bonds	Unfolding of proteins	Unfolding of protein	PEG chain compression
		PEG chain compression	PEG osmotic repulsion

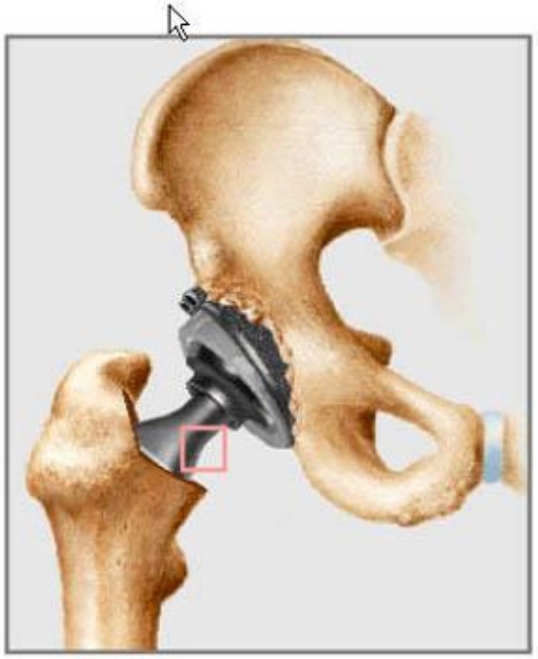
The overall conclusion is that resistance to protein adsorption at biomaterial interfaces is directly related to resistance of interfacial groups to the release of their bound waters
Water may be bound to surface groups by both hydrophobic and hydrophilic interactions.

Scheme to predict whether ("yes") or not ("no") proteins adsorb at surfaces

Protein	Surface			
	Hydrophobic		Hydrophilic	
	+	−	+	−
Stable structure				
+	Yes	Yes	No	Yes
−	Yes	Yes	Yes	No
Labile structure				
+	Yes	Yes	Yes	Yes
−	Yes	Yes	Yes	Yes

The "+" and "−" signs refer to the net electric charge of the protein and the surface.

Adsorbed proteins change surface properties of the material



Hydrophilic surfaces have more resistance to the release of their bound waters

So the most common approach to reducing protein and cell binding to biomaterial surfaces have been to make them more hydrophilic

The approaches to make surfaces more hydrophilic are:

Chemical immobilization of a hydrophilic polymer such as PEG, on the biomaterial surface by

a. Using UV or ionizing radiation to graft copolymerize a hydrophilic monomer onto surface groups
b. Depositing such a polymer from the vapor of a precursor monomer in a gas discharge process

Other approaches are physical adsorption of surfactants, chemical derivatization of surface groups polar groups like hydroxyls or negatively charged groups like carboxylic acids since most proteins and cells are negatively charged.

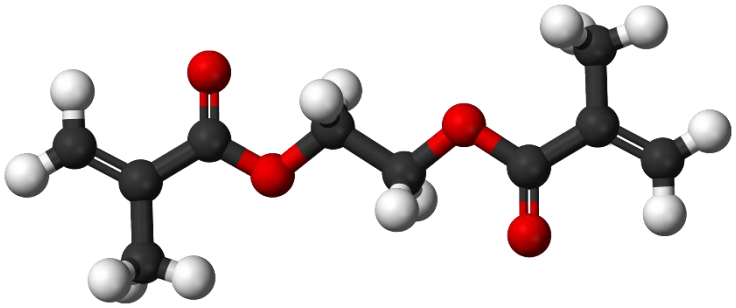
In addition coating with hydrogels and natural biomolecules like albumin, casein, hyaluronic acid exhibit resistance to nonspecific adsorption of proteins.

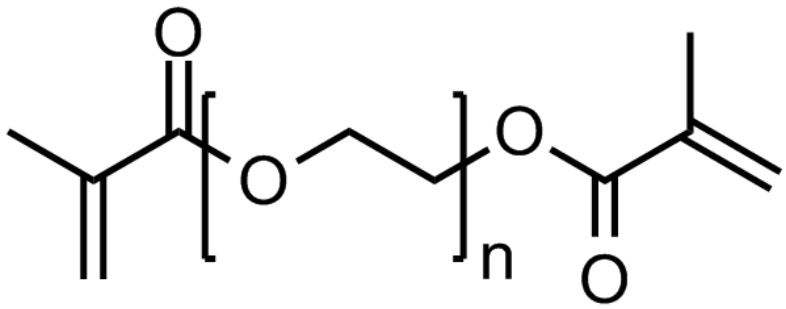
Nonfouling surfaces have medical and nonmedical applications such as

Blood compatible materials,
Implanted devices,
Urinary catheters,
Diagnostic assays,
Biosensors,
Intravenous syringes and tubing,
Heat exchangers,
Ship bottoms

In addition to their medical and economic importance, research on nonfouling surfaces provide important experimental and theoretical insights into the phenomenon of protein adsorption

Surfaces containing poly ethylene glycol (PEG) make up the majority of materials researched as nonfouling surfaces.





Research on bacteria shows that the nonfouling surfaces provide weak resistance to bacteria and biofilm buildup, only the best nonfouling surfaces slowing down the adhesion and colonization.

PEG groups on nonfouling surfaces are susceptible to oxidative damage and this reduces their effectiveness in bacteria resistance.

Defects on nonfouling surfaces such as pits, uncoated areas also provide footholds for bacteria and cells to begin colonization.

Resistance to bacterial adhesion remains an unsolved problem in the surface science.