Surfaces in “Biological Environments”

18.11.2020

Ville Jokinen

E5150 Surfaces and Films Lecture 10

Content and Learning outcomes

1. Proteins on surfaces (≈ 60 min) (Quiz will be about this)
2. Antibacterial surfaces (≈ 5-10 min)
3. Blood contacting surfaces (≈ 5-10 min)
4. Implant surfaces (≈ 5-10 min)

The homework will be about surfaces that resist adsorption of blood proteins (related mostly to topic 1 but also 3)

Part 1: Proteins on Surfaces

Why things are a bit different when biology is involved?

Biology is simplified chemistry (applied to a specific field) and chemistry is simplified physics (applied to a specific field).

Biological entities have evolved to fill a purpose. For many purposes, there is an entity…hence to any rule there tends to be an exception.

Some biological entities are alive (e.g. cells, bacteria) and are operating under their own agenda.

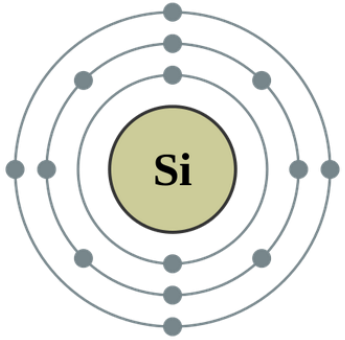
Increasing: amount of different entities, size/complexity of entities, emergent complexity

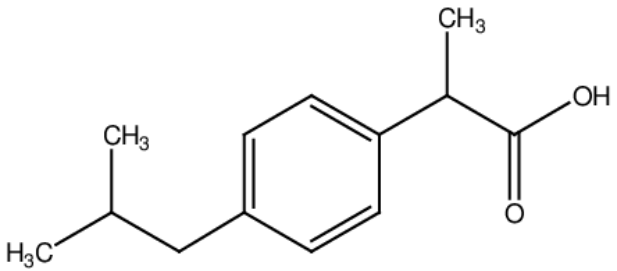
Physics → Chemistry → Biology

Increasing: detail of models, accuracy of models, universality of models

Silicon atom

https://www.quora.com/What-is-the-structure-of-silicon



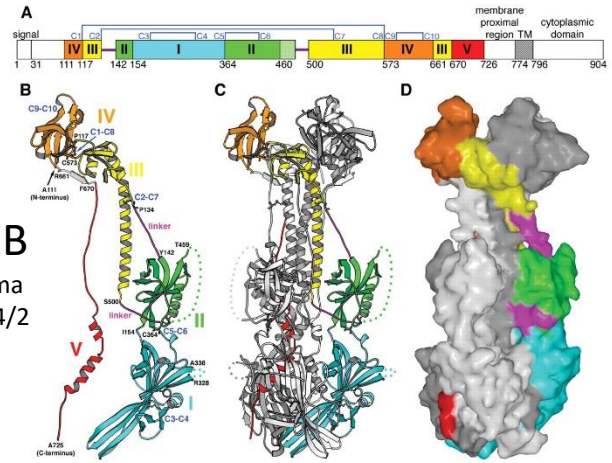


Ibuprofen

International Journal of Pharmacy and Pharmaceutical Sciences
ISSN- 0975-1491 Vol 7, Issue 2, 2015

Herpes Simplex Glycoprotein B

https://science.sciencemag.org/content/313/5784/217/tab-figures-data



Adsorption and adhesion?

Adhesion refers to liquid/solid phases sticking together

Adsorption refers to **individual molecules** sticking to a liquid/solid phase.

What is the same?

The forces/interactions

Free energy considerations

(for adhesion in **energy per area** and for adsorption in **energy per atom/molecule/mole**)

What is different?

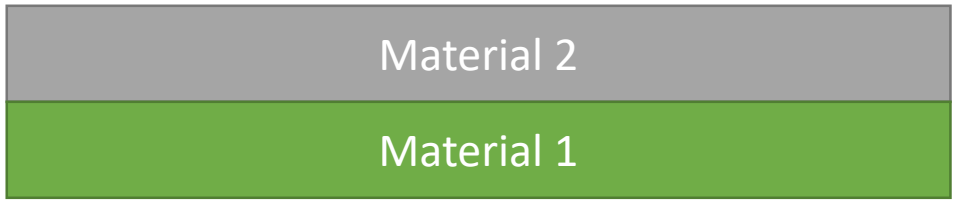
Adsorption is a stochastic equilibrium process whereas adhesion is not.

In this lecture:

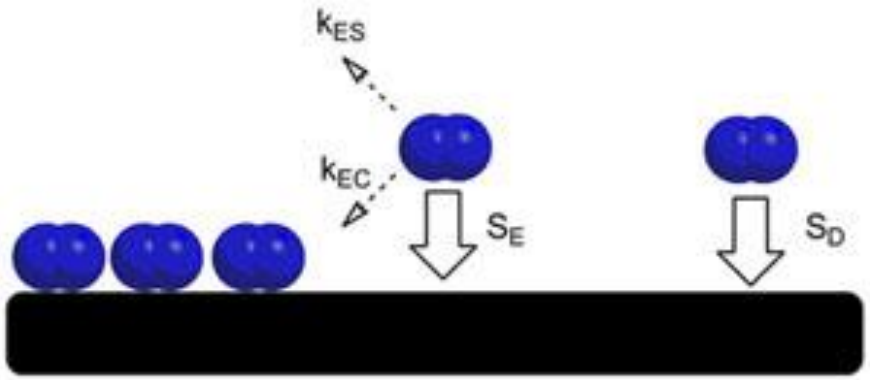
Proteins adsorb

Cells/bacteria adhere

(but this is mostly terminology and not a fruitful starting point for understanding these phenomena)



Two solids adhered together



Adsorption of gas molecules onto a solid surface

Figure: https://en.wikipedia.org/wiki/Adsorption

Protein structure (in one slide!)

Primary structure:

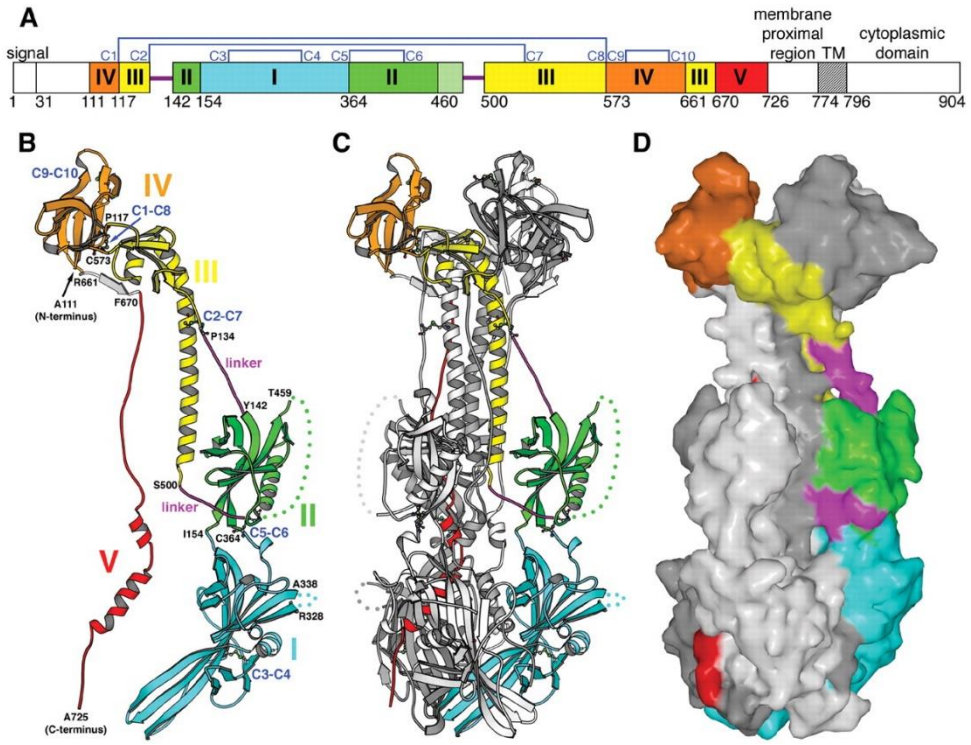
Proteins consist of a chain of amino acids: Some are hydrophilic, some are hydrophobic. Some have positive charge, some have negative charge.

Secondary/tertiary/quaternary structure:

The linear chain makes 3D object that minimizes the surface energy. Main driving force is having **hydrophobic amino acids concentrated on the inside** of the protein and hydrophilic on the outside.

Further structure:

Many proteins are further modified by chemical modification. E.g. adding phosphate groups (phosphorylation) or adding oligosaccharide chains (glycosylation)



Herpes Simplex Glycoprotein B

https://science.sciencemag.org/content/313/5784/217/tab-figures-data

Protein adsorption, basics

Possibly the most important surface-biomolecule interaction: medical diagnostic tests, medical devices, medical implants, biofouling, biochemical processing, cookware etc.

Proteins are the main biological tools. Their specific shape and chemistry is their function.

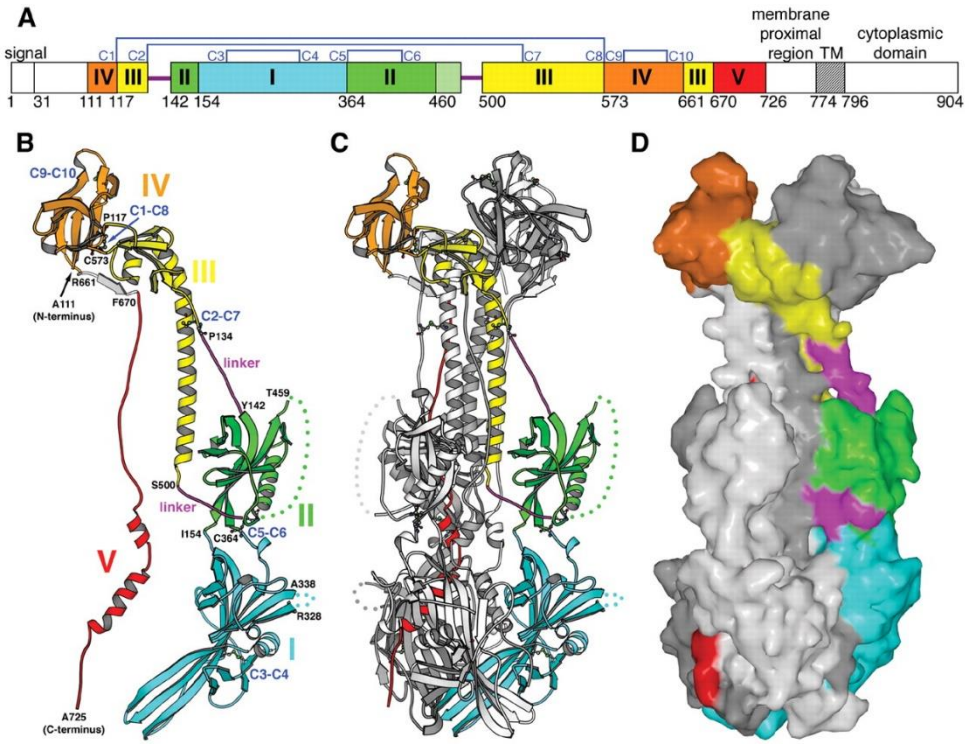
Protein adsorption in most cases happens from **aqueous medium.**

Proteins interact by **both chemical** (amino, carboxylic, sulfide) **and physical bond** formation (hydrogen bonds, hydrophilic and hydrophobic interactions).

Human genome has 20k – 25k genes for proteins.

When proteins adsorb their tertiary structure usually changes (the protein denaturates).

A key driving force for adsorption on many surfaces is **hydrophobic interaction**.



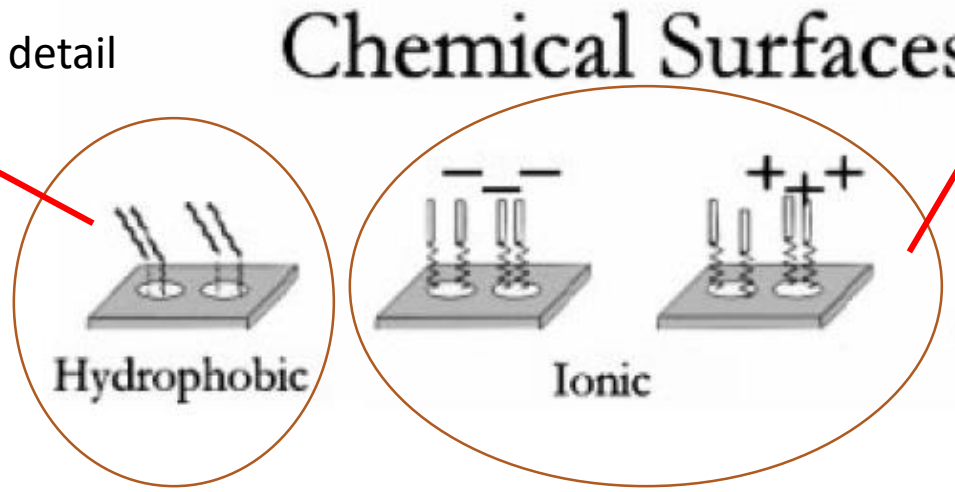
Herpes Simplex Glycoprotein B

https://science.sciencemag.org/content/313/5784/217/tab-figures-data

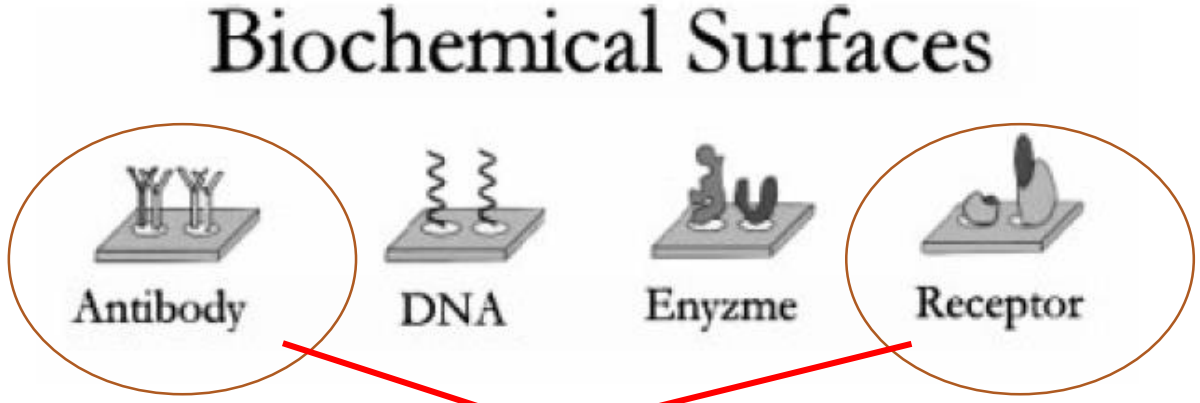
In this lecture:

1. We look in detail

2. Discussed briefly



-<u>Nonspecific</u> binding
-Based on physico-chemical properties of the surfaces



-<u>Specific</u> binding
-Based on shape recognition (shape matching).

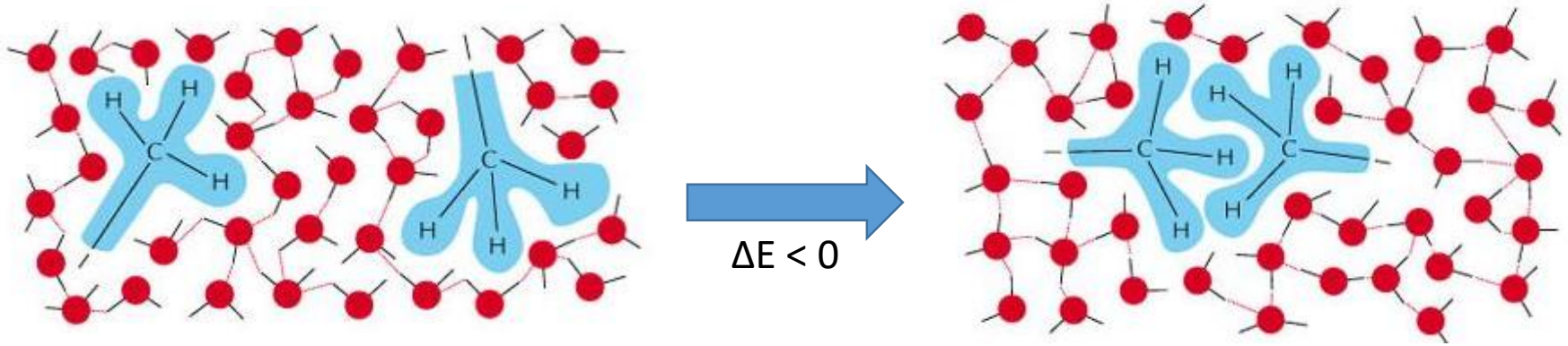
3. Shown in few examples

Hydrophobic interaction

Hydrophobic interaction (also known as hydrophobic effect or hydrophobic force) is a pseudo force that explains why hydrophobic molecules/surfaces adhere/adsorb on each other under water.

What is the physical basis for hydrophobic interaction?

A



B

In figure B compared to figure A we have:

1. More CH_3-CH_3 interaction
2. Less CH_3 – H_20 interaction?
3. More H_20- H_20 interaction?

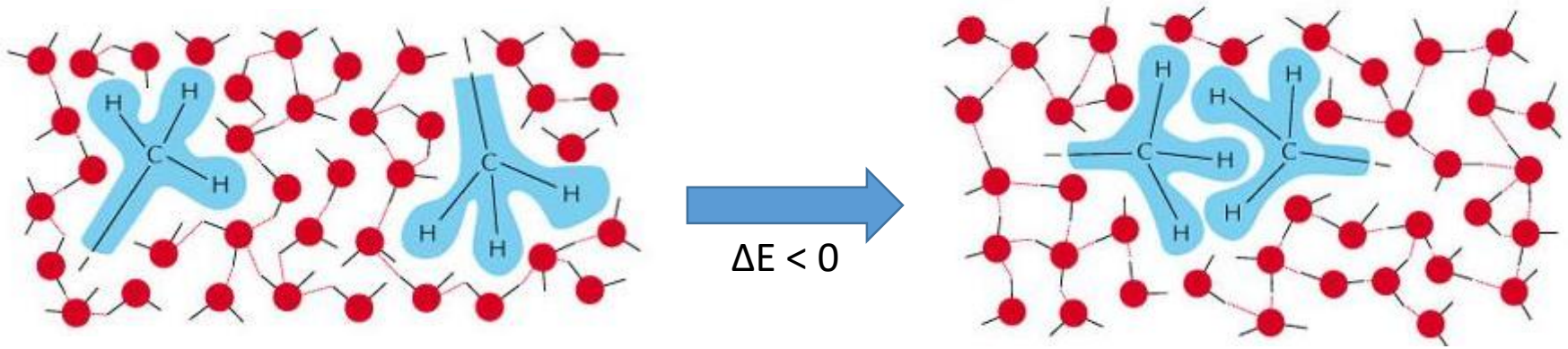
Which of these three do you think is the main driving force? Guess in chat! 1, 2 or 3

Hydrophobic interaction

Hydrophobic interaction (also known as hydrophobic effect or hydrophobic force) is a pseudo force that explains why hydrophobic molecules/surfaces adhere/adsorb on each other under water.

What is the physical basis for hydrophobic interaction?

A



B

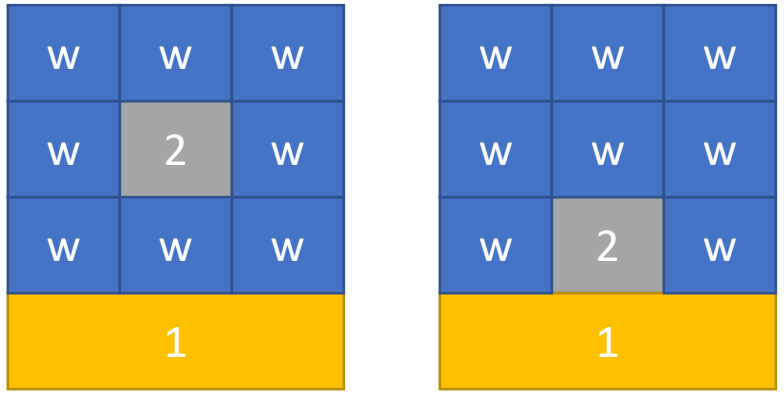
More CH_3-CH_3 interaction? Contributes (Van-der-Waals), but this is not the main reason
Less CH_3 – H_20 interaction? No, weaker but attractive interaction
More H_20- H_20 interaction? YES!! Water-water bonds are strong.

Range of hydrophobic interaction ≈ 2 – 10 nm (mediated by local ordering of water molecules.)

Adsorption, a surface energy point of view.

Let's take a hydrophobic surface (1), a hydrophobic analyte (2) and "blocks" of water (w)

The situation looks like this in adsorbed and nonadsorbed states:



Non-adsorbed Adsorbed

Table 1. Free energy of formation of a liquid–liquid area of contact from surfaces previously exposed to air, in erg/cm² at 25°C

Liquids	Hexane*	Octane†
Hydrocarbon/water	−39.5	−42.0
Hydrocarbon/hydrocarbon	−35.8	−42.4
Water/water	−144.0	−144.0

One erg = 10^{-7} J.

C. Tanford, PNAS 76, p. 4175, 1979

Free energy of formation of liquid-liquid area
≈=
-1 * Surface energy

The interfacial energies that change upon adsorption:
-1 (3 vs 4) water-hydrophobic molecule interaction (≈-40 erg/cm^2)
-1 (2 vs 3) water-hydrophobic surface interaction (≈-40 erg/cm^2)
+1 (1 vs 0) hydrophobic surface – hydrophobic molecule interaction (≈-35 erg/cm^2)
+1 (9 vs 8) water – water interaction (≈-145 erg/cm^2) (the driving force for hydrophobic interaction)

So the total change of energy upon adsorption is – 35 – 145 – (– 40 – 40) = -100 erg/cm^2
(note I am using adhesion data to demonstrate adsorption, but the point I am making about the relative importance of the terms applies to both)

Adhesion under water (or other medium)

Under water: $W_{12} = \gamma_{1,w} + \gamma_{2,w} - \gamma_{12}$

In previous lecture: *Hydrophilic surfaces have less adhesion under water than in air since the surfaces satisfy some of their bonding capability with physical bonds with water (compared to air).*

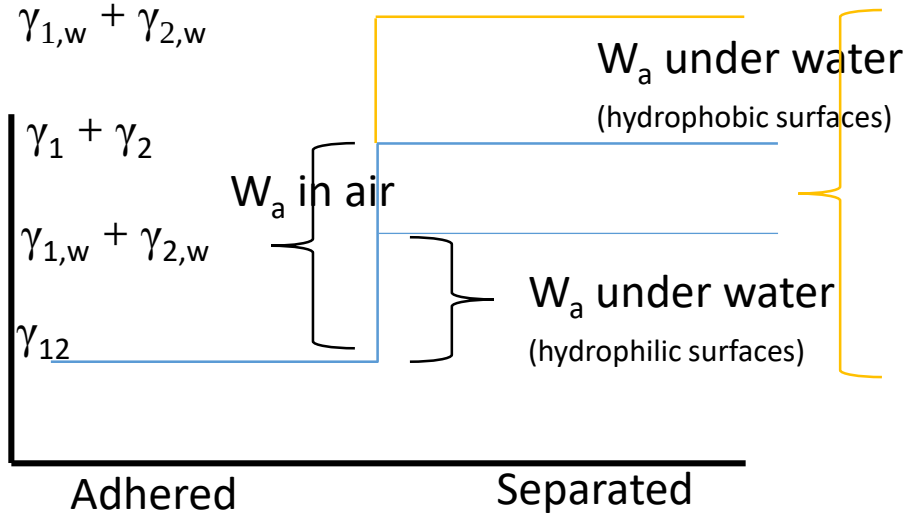
Mystery: Hydrophobic surfaces also interact more with water than air. So shouldn't the same apply but to a lesser extent?

Answer: Air does not interact with itself (to a significant degree) but water does. **So the logic of only thinking of the bonds that form between the surfaces only works in the case of air,** the extension to other media is invalid.

Surface energies take into account the bonds lost/gained in the surrounding medium. Rewritten Young's equation:

$$\gamma_{sl} = \gamma_{sv} - \gamma_{lv}\cos(\theta)$$

$\gamma_{sl} > \gamma_{sv}$ If: $\theta > 90°$



Hydrophilic and hydrophobic surfaces under water and in air

Journal of Adhesion Science and Technology 31(8):1-22

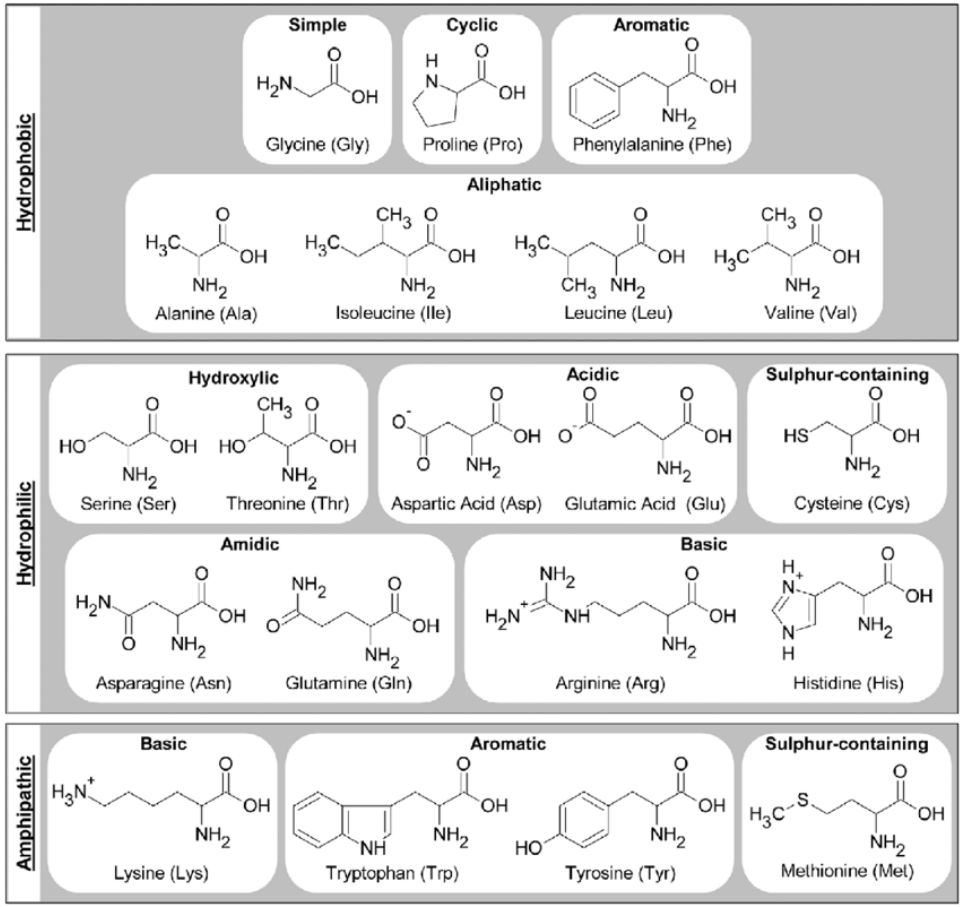
Adsorption by hydrophobic interaction 1

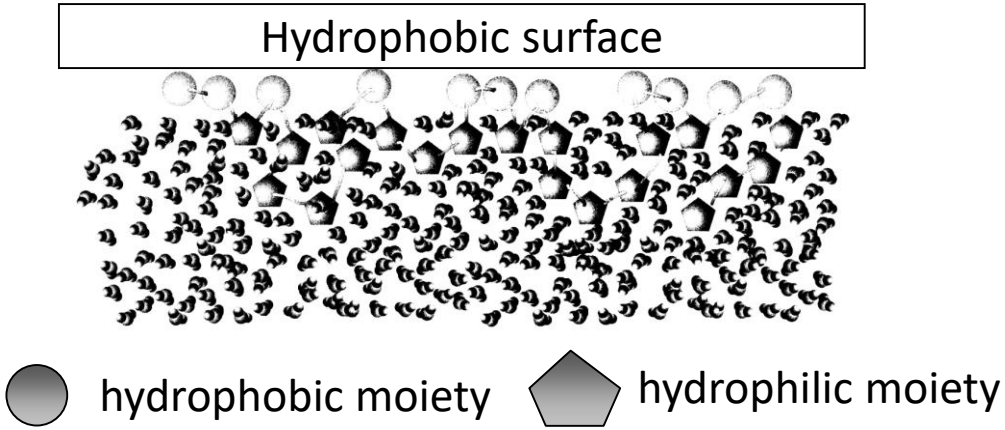
Proteins have hydrophobic and hydrophilic parts. Protein conformation of dissolved proteins is primarily stabilized by having a hydrophobic core and hydrophilic outside.

When a protein comes into contact with a hydrophobic surface, free energy can be lowered by bringing the hydrophobic moieties of the protein in contact with the hydrophobic surface (thus leaving more water molecules to interact with each other).

Proteins denature (change their shape) partially upon adsorbing and also as a function of adsorption density.

In many cases, it is still possible to retain activity (e.g. antigen recognition) even after partial denaturing.



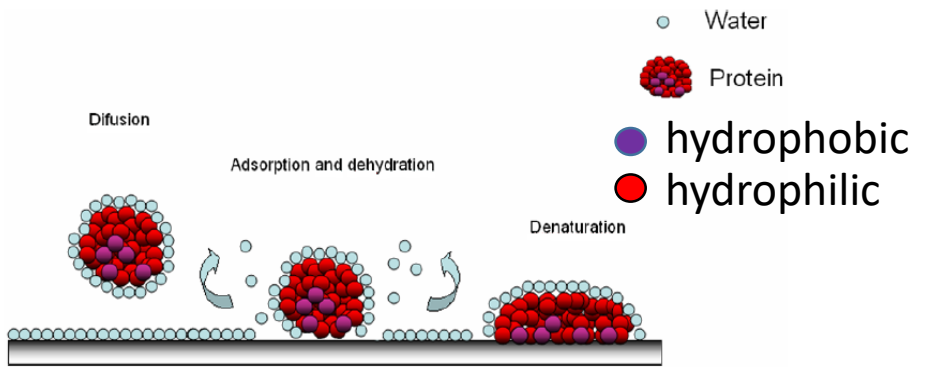


Adsorption by hydrophobic interaction 2

Protein diffuses onto a surface.

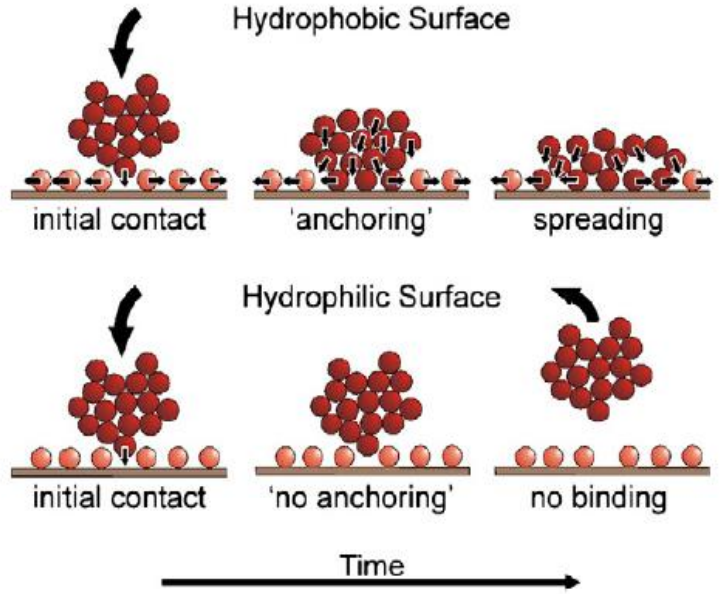
On hydrophobic surface, hydrophobic parts of the protein might come into contact with the hydrophobic surface -> hydrophobic interaction.

The protein denatures to bring more of the hydrophobic parts into contact with the surface.



J. Funct. Biomater. **2012**, *3*, 528-543; doi:10.3390/jfb3030528

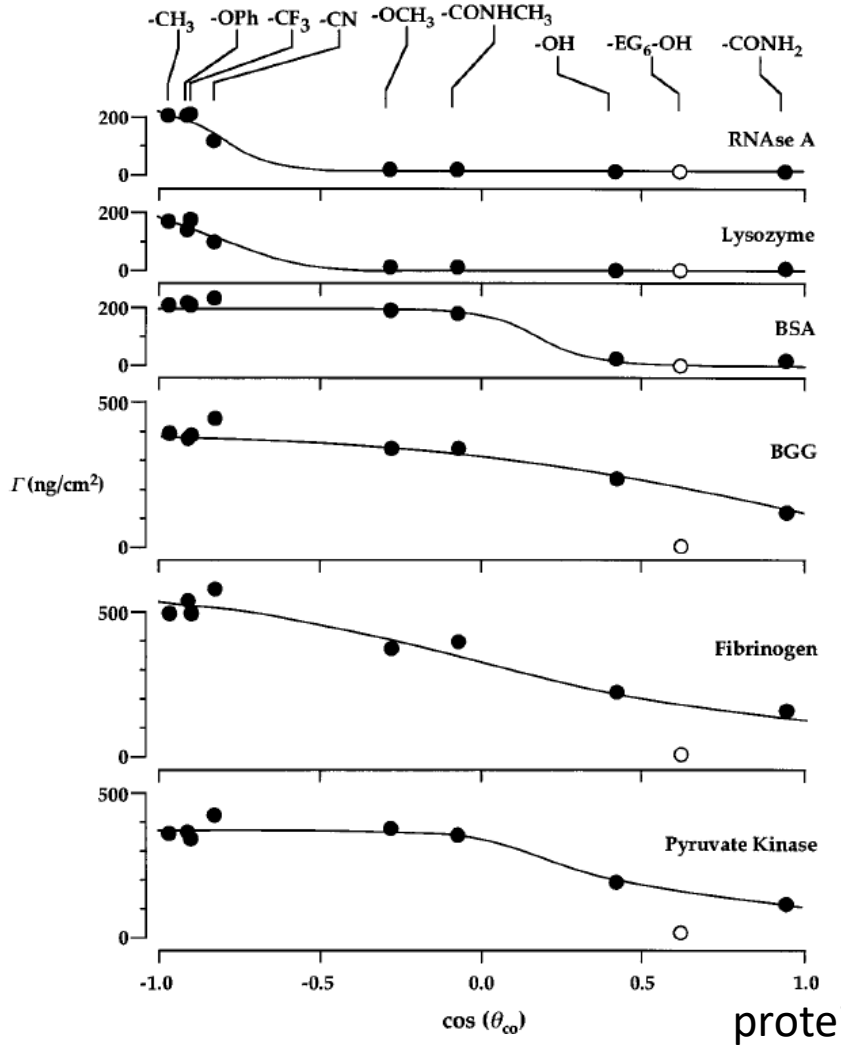
On a hydrophilic surface this does not happen since the free energy change of dewetting the hydrophilic surface is positive.



M. Rabe et al. / Advances in Colloid and Interface Science 162 (2011) 87–106

Adsorption by hydrophobic interaction 3

The vast majority (all?) of proteins adsorb on hydrophobic surfaces by hydrophobic interaction.
Some proteins, especially large ones, seem to adsorb on all surface chemistries, but more strongly on hydrophobic**.**



Small Proteins

Medium

Large

protein size increases

Water contact angles:

$-CH_3$	$\theta \approx 110°$
-OH	$\theta \approx 15°$
$-EG_6$-OH (PEG)	$\theta \approx 40°$

Sigal et al. J. Am. Chem. Soc. 120, p.3464, 1998

Adsorption by electrostatic forces

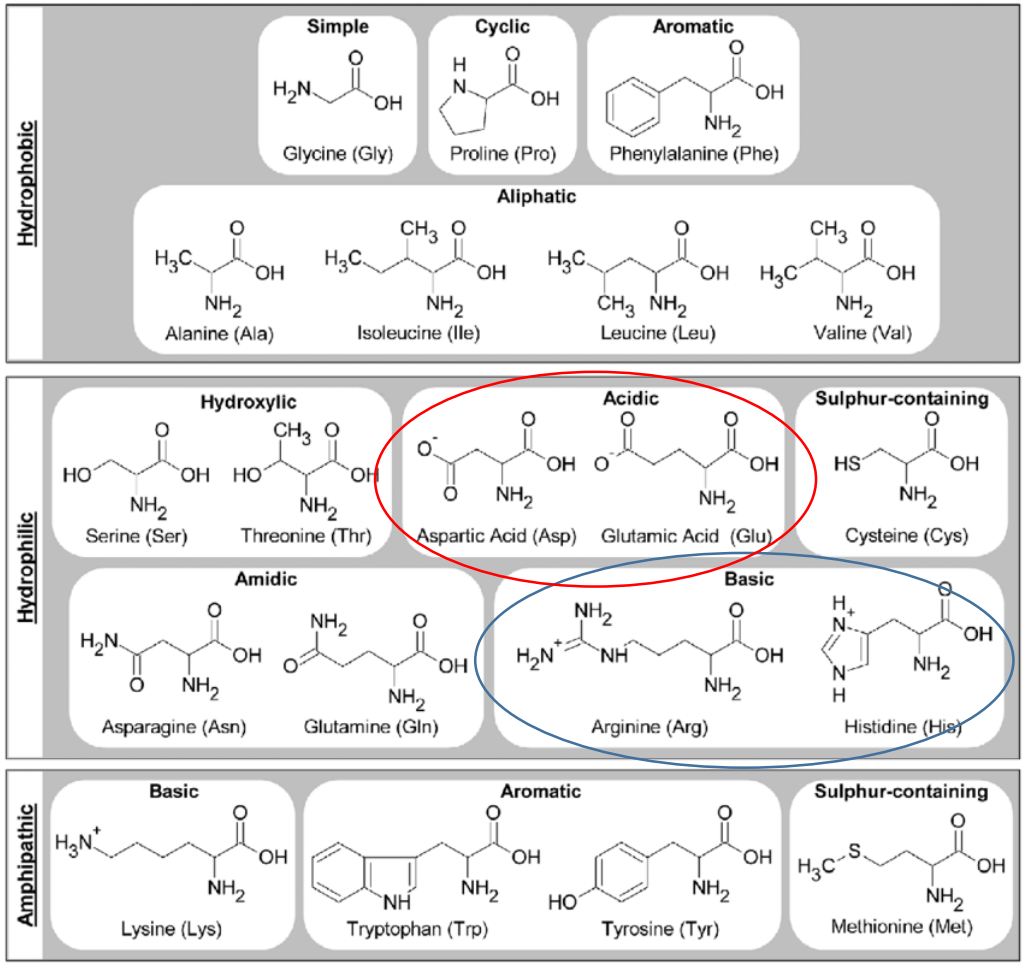
Some amino acids are charged. Both negatively and positively charged amino acids exist.

Most proteins contain both types of amino acids and thus can interact with both positively and negatively charged surfaces. Some proteins have a strong bias in their amino acid composition toward either positive or negative charge.

Many hydrophilic surfaces also contain some amount of (pH dependent) charge. For example: Silanol groups on silicon (Si-O-H) have pKa = 5, so they are slightly negatively charged a neutral pH

What does this mean for adsorption of proteins on surfaces?

It is complicated…



Journal of Adhesion Science and Technology 31(8):1-22

Adsorption by electrostatic forces 2

First approximation: proteins with overall negative charge tend to adsorb on positively charged surfaces and vice versa.

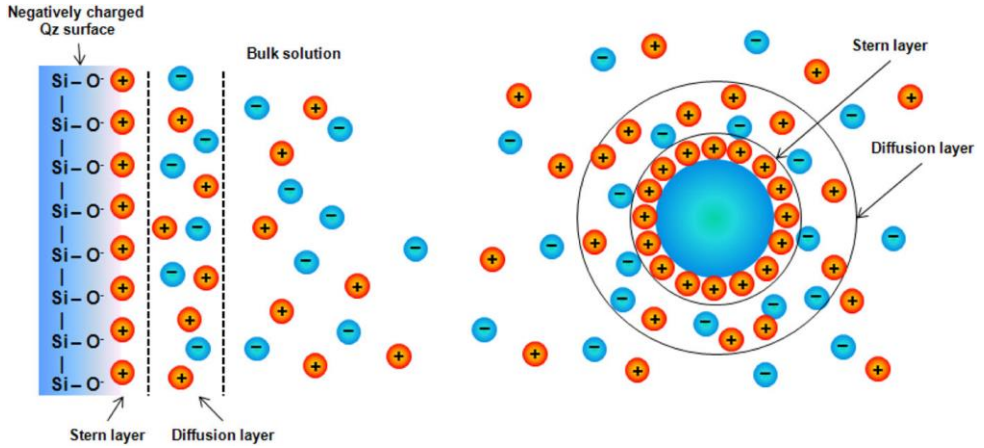
However:

1. Adsorption does not happen over the whole protein but at some localized area. So what matters more is **an area of high charge density orienting itself against the surface.**

2. Water molecules and especially **salts screen electrostatic interaction** so they are not as strong as in a vacuum.

3. Proteins that adsorb through electrostatic interaction start to interact with each other as well when the surface density increases.

4. And many more…for example negatively charged molecules can adsorb on negatively charged surfaces if there are divalent positive ions in the solution to bridge them.



Stern model of electric double layer: immobilized counterions + loosely bound diffuse layer

Adsorption by electrostatic forces 3

Example: β-lactoglobulin adsorption on a negatively charged surface

The protein surface has areas where there is more positive charge density and negative charge density.

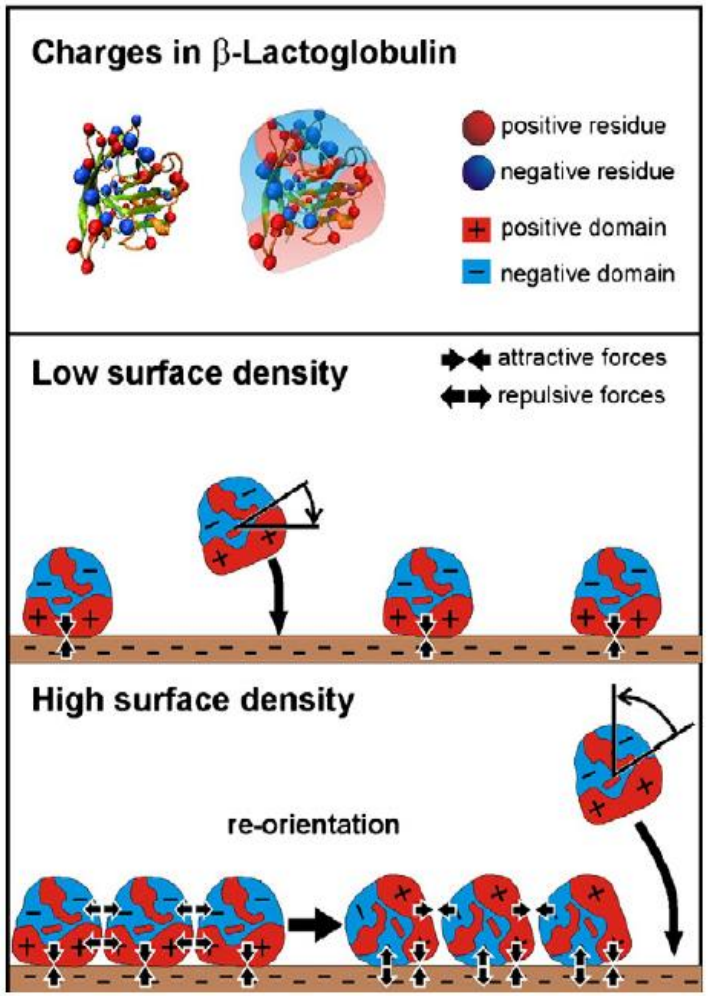
Protein diffuses on the negatively charged surfaces. It can orient itself so that the positively charged areas come into contact. (water and ions help screen the repulsive forces)

As more proteins adsorb, the negatively charged areas start to interact (repel).

In response, the proteins re-align to relieve this…but at the same time the original attractive forces are lessened.

An equilibrium coverage is reached.

Note: **β-lactoglobulin has an overall negative charge** at neutral pH.



M. Rabe et al. / Advances in Colloid and Interface Science 162 (2011) 87–106

Measuring protein adsorption

Parameters of interest:

Total adsorbed amount (typically in ng/m^2 or $\mu g/m^2$)

Thickness of the adsorbed layer (in nm)

- Thickness of the layer gives information on the orientation of adsorption as well

All of the above as a function of time, temperature, pH, other molecules in the suspension etc.

For example:

Immunoglobulin G adsorbed on a surface as a 20 nm layer and the amount of protein adsorbed was 1 mg/m^2. Size of IgG is 4.5 x 4.5 x 23.5 nm^3. Density of pure protein is ≈ 1.35 g /cm^3

We can conclude: the orientation of the adsorption is likely “end on”

We can calculate that the overall density of the layer would be ≈ 0.1 g /cm^3. Comparing this to the theoretical maximum density we conclude that the adsorbed film is quite sparse.

Measurement by QCM

Quartz Crystal Microbalance (QCM) + Dissipation (QCM-D)

QCM is based on a thin piezoelectric disc with driving electrodes on both sides.

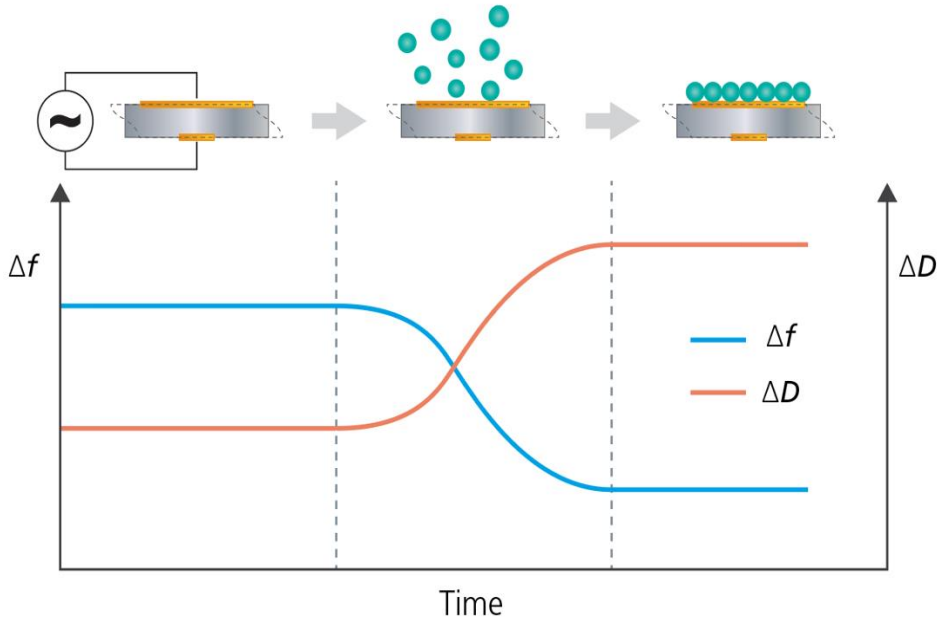
The disk has a characteristic resonant frequency.

As material adsorbs (or desorbs) from the disc surface, the resonant frequency Δf changes as a function of adsorbed mass.

The dissipation component ΔD gives information on the density and elastic vs viscoelastic behavior of the adsorbed layer

By QCM you can study:
-The total amount adsorbed in equilibrium
-The kinetics of protein adsorption
-Thickness of the protein layer can be estimated from ΔD

A downside: coating the sensor with the film/surface chemistry that you are interested in is not always simple.



QCM-D principle

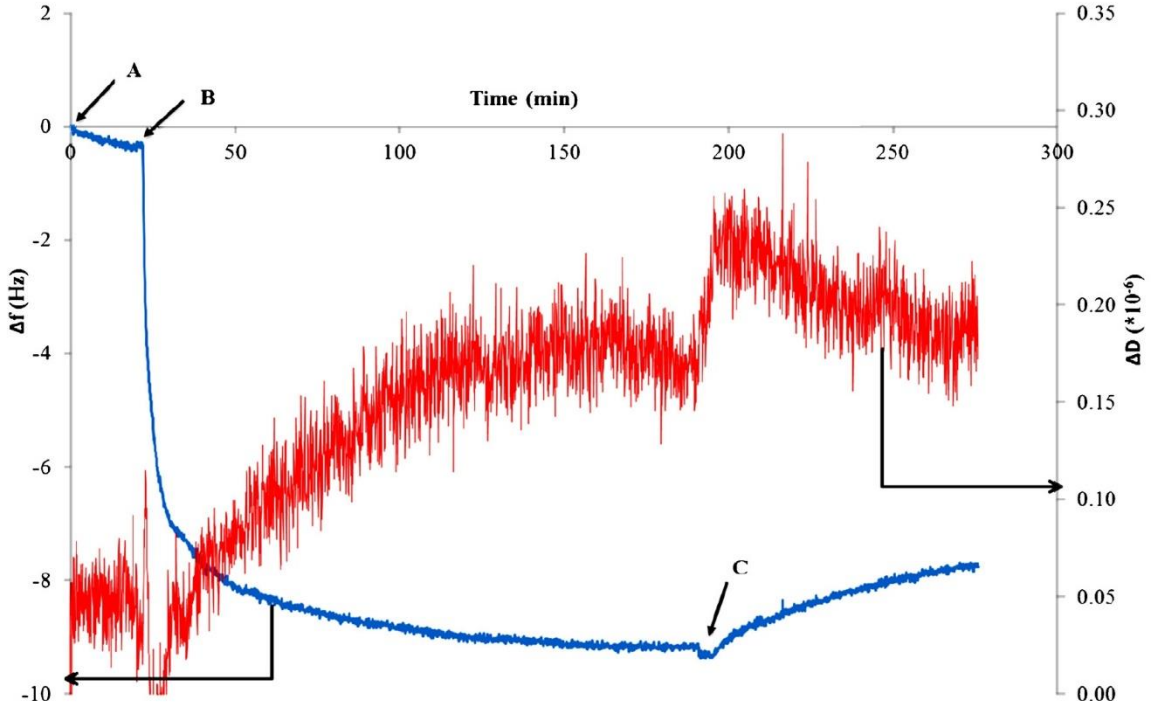
Figure: https://www.biolinscientific.com/measurements/qcm-d

Measurement by QCM 2

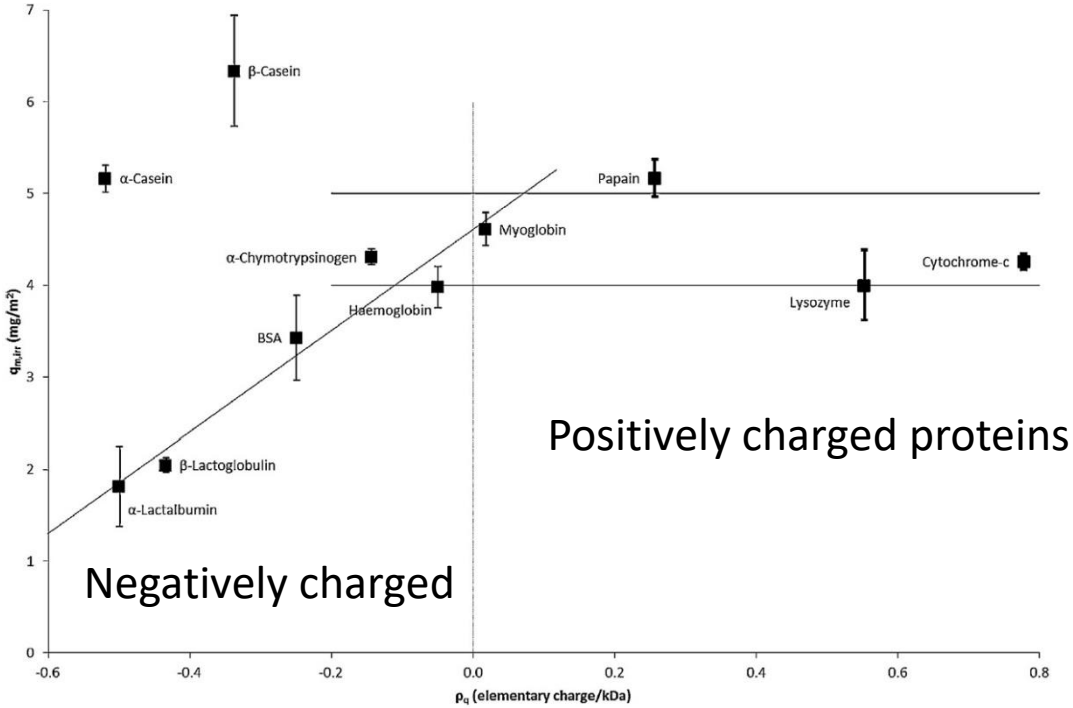
QCM-D, **stainless steel coated sensors** (pI ≈3.5). Experiments done in pH 7.5 buffer so the steel is **negatively charged**.
A: buffer introduced, **B:** protein introduced, **C:** switching back to pure buffer

Their explanations of the data were as follows:

1. Negative charge of protein lead to electrostatic repulsion and thus less total adsorbed protein.
2. Neutral or positive charge has no effect since the driving force is not electrostatic attraction.
3. Caseins were the exception because: "*Caseins have a high number of phospho-substituted serine … with high affinity to iron oxides*"



β-lactoglobulin adsorption



Total protein adsorbed vs protein charge

Measurement by ellipsometry

Ellipsometry measures the thickness and the index of refraction of thin films.

Some assumptions are needed: 1. proteins adsorb as a uniform thin film. 2. An assumption about how the index of refraction of the layer is formed

Ellipsometry gives:

The index of refraction of the adsorbed protein layer (not directly useful usually)

The thickness of the adsorbed layer (useful…but the thickness given is open to interpretation)

The density of the adsorbed layer (calculated from the index of refraction)

The amount of protein adsorbed per unit area (calculated from the density and the thickness of the film. This parameter is very useful and possibly the most accurate as well.)

All of the above can be obtained as function of time so kinetics can also be studied.

Drawbacks:

Multiple assumptions need to be made which can lead to data interpretation errors

The surface needs to be flat and its optical properties need to be known.

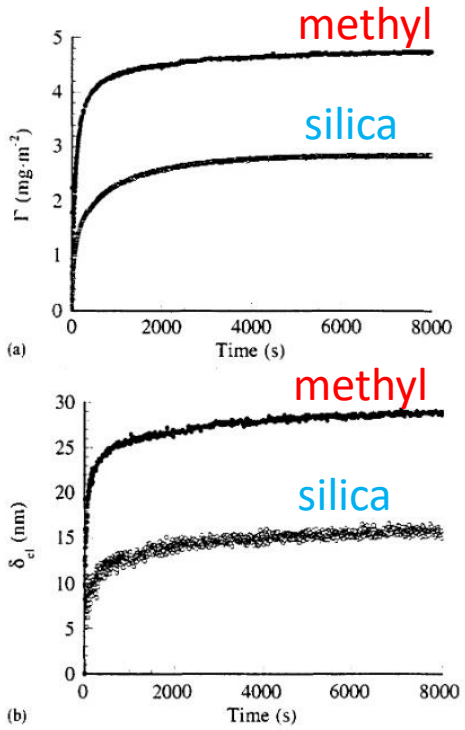
Measurement by ellipsometry 2

Two surfaces: hydrophilic silica (thermally oxidized silicon wafer, weakly negatively charge) and hydrophobic methylated surface (the same silica surface functionalized by trichloroethylene)

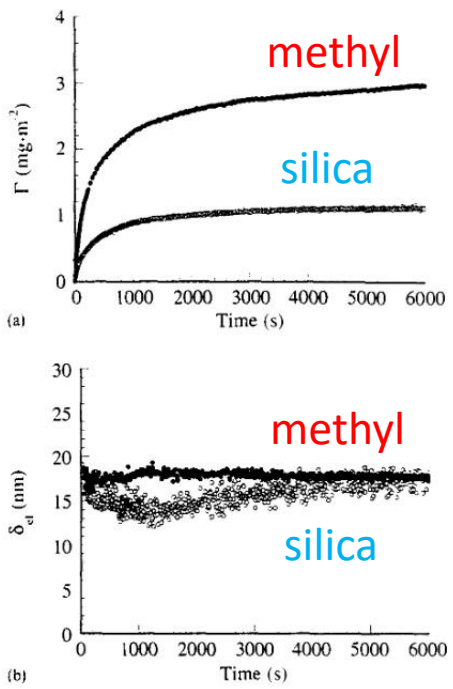
Fibrinogen (a blood clotting protein): 6 x 6 x 45 nm^3 , pI ≈ 6
IgG (an antibody): 4.5 x 4.5 x 23.5 nm^3, pI ≈ 7
Lysozyme (an antibacterial enzyme): 3 x 3 x 4.5 nm^3, pI ≈ 10.7 strong positive charge

Layer mass (ng/m^2)

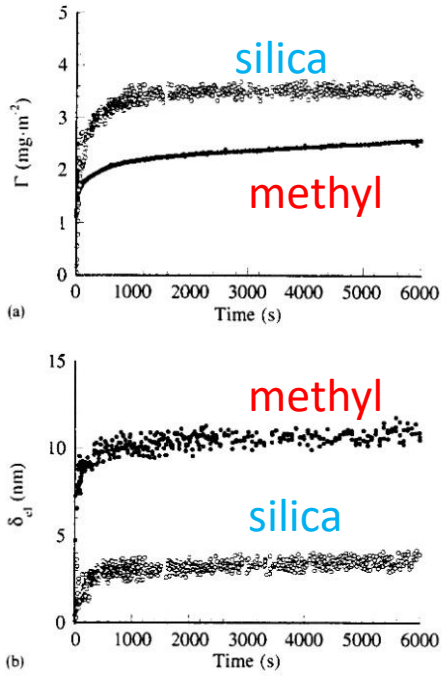
Layer thickness (nm)



Fibrinogen



IgG

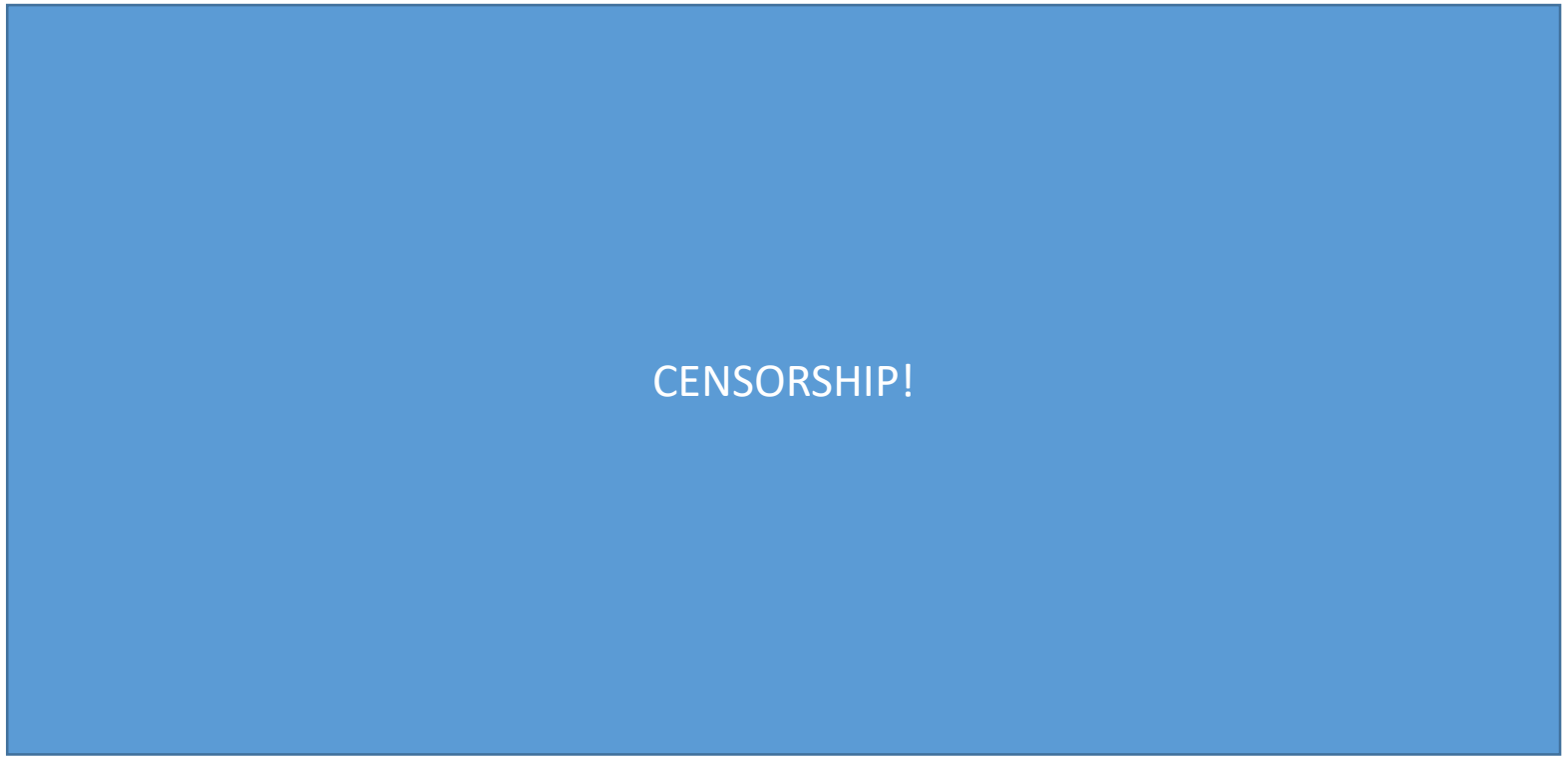


Lysozyme

M. Malmsten/Colloids Surfaces B: Biointerfaces 3 (1995) 297-308

What else could be used to measure protein adsorption?

Any thoughts from studentry?



What else could be used to measure protein adsorption?

Any thoughts from studentry?

Fluorescent staining + microscopy
Radioactive labeling
Contact angle measurements
Desorption ionization mass spectrometry
Reflectometry
AFM
STM
+ many others

You just saw many examples of all kinds of proteins adsorbing on all kinds of surfaces through hydrophobic interaction and through electrostatic effects.

How to design a protein adsorption resistant surface?

The problem is that proteins are a varied bunch.

Some have positive charge. Some have negative charge. Some have no net charge.

Some have more hydrophobic batches on the outside while some have less.

So it would seem like whatever your surface is, how can it not adsorb some class of proteins by either hydrophobic interaction or electrostatic forces?

Any thoughts?

Non-adsorbing surfaces

A non-adsorbing surface important for many applications. For example: 1. when working with small concentrations (depletion) or 2. on any surface e.g. a sensor whose function is adversely affected by adsorption.

On aqueous solvents, the least adsorbing surfaces tend to be **hydrophilic** and **electrically neutral**, in order to minimize **hydrophobic** and **electrostatic** interactions, respectively.

Non-adsorbing surfaces are quite often hydrated polymer brushes, leading to a steric component of the adsorption resistance

The most commonly used coating is poly-ethylene glycol (PEG). Other candidates: Poly(vinylalcohol) (PVA), Poly(acrylamide) (PAA), Zwitterionic polymers

A study by Whitesides et al.*:
-PEG surfaces resist protein adsorption and bacterial adhesion, but equally good other alternatives were found utilizing a varied set of self-assembled monolayers.
-The key characteristics of non-adsorbing surface were found to be: **i)** hydrophilic **(ii)** hydrogen bond acceptors but **(iii)** *not* hydrogen bond donors.

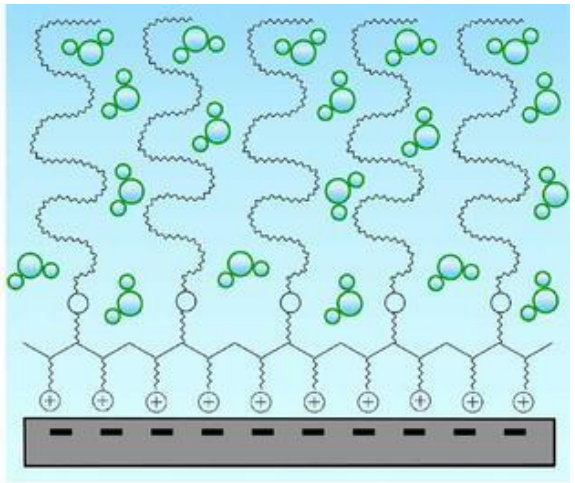
* *Langmuir* **2001,** *17,* 1225-1233

PEG

Poly(ethylene glycol) PEG, alternatively called Poly(ethylene oxide) PEO is a widely used protein adsorption resistant coating.

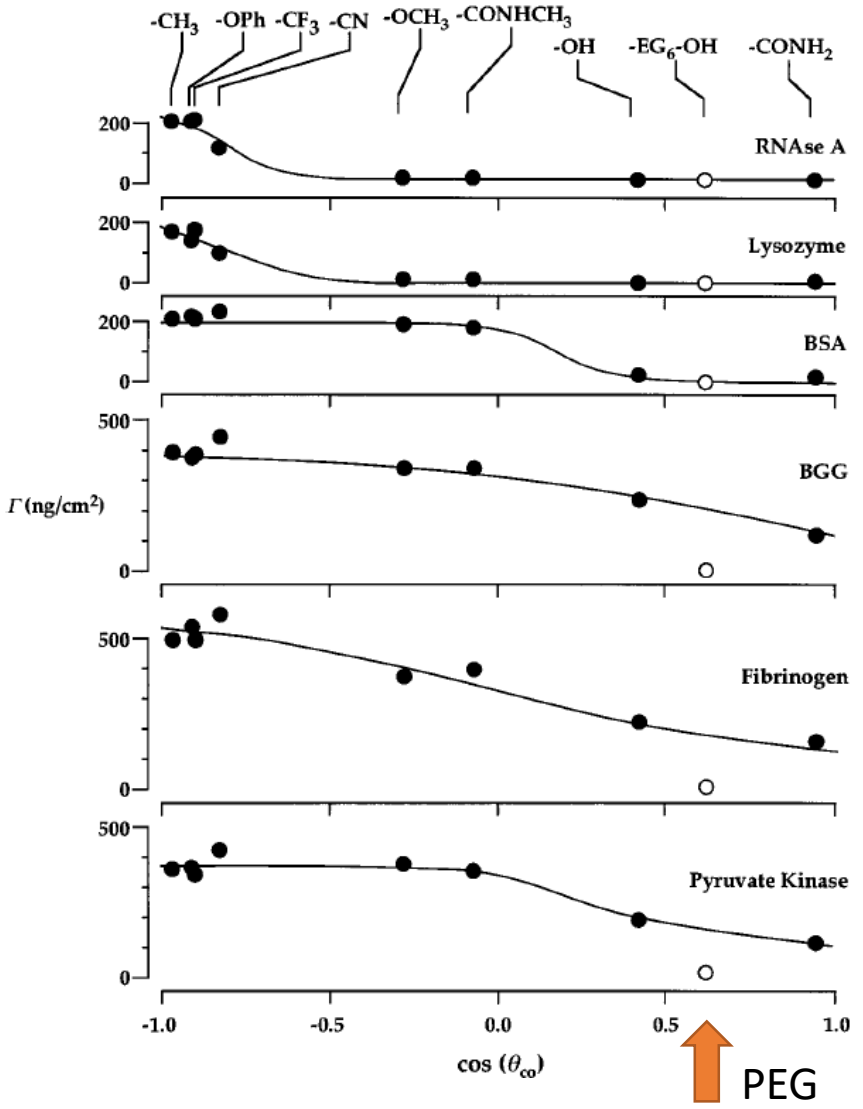
Generally resistant to protein adsorption. It also is resistant toward cell and bacterial adhesion. However, exceptions do exist.

In addition to the properties from the previous slide, PEG forms a loosely bound brush structure filled with water. Proteins therefore cannot approach the “real” surface due to steric hinderance.



PEG brush structure

PEG chemical formula



PEG is a rare surface on which most proteins do not adsorb

Blocking

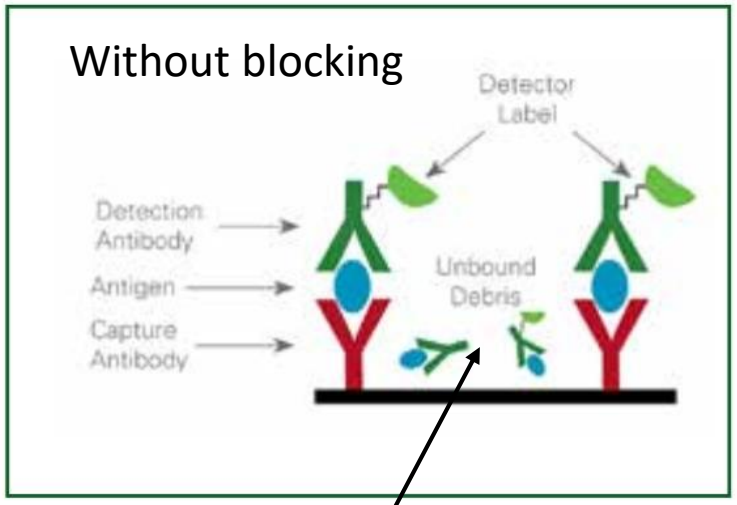
Blocking can be utilized when it is not a problem if there is an adsorbed layer of proteins on the surface as well as it is known and controlled.

Commonly used in immunoassays. You detect e.g. a specific protein marker by it binding to an **antibody selective to that pathogen.** But if all kinds of proteins adsorb on the surface by nonspecific forces, you lose the specificity.

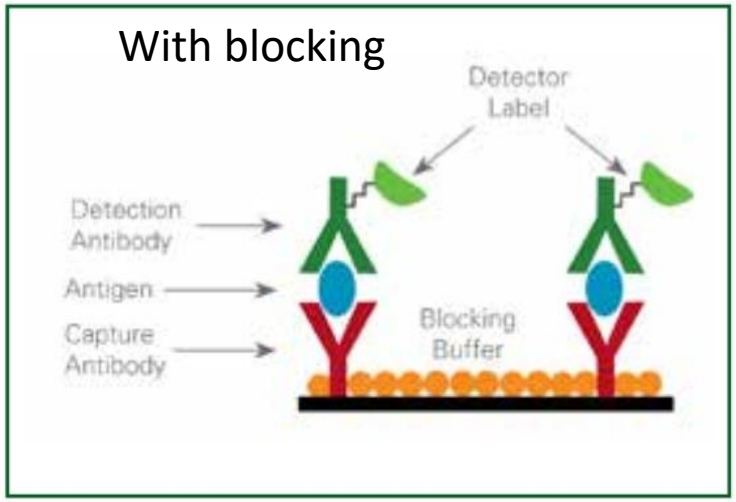
Most common blocking protein is bovine serum albumin BSA

Why does this work? Why do proteins typically not adsorb on other proteins?

You can suggest mechanistic (how something is) or teleological (why something is) reasons, in chat!



Nonspecific binding, false signals



Blocking prevents nonspecific binding, leading to high specificity of immunoassays

https://meridianlifescience.com/documents/Blocker%20insert/MLS%20Blocker%20Practical%20Guide%20English.pdf

Application example

A microfluidic immunoassay which utilizes two things we have learned in this lecture:

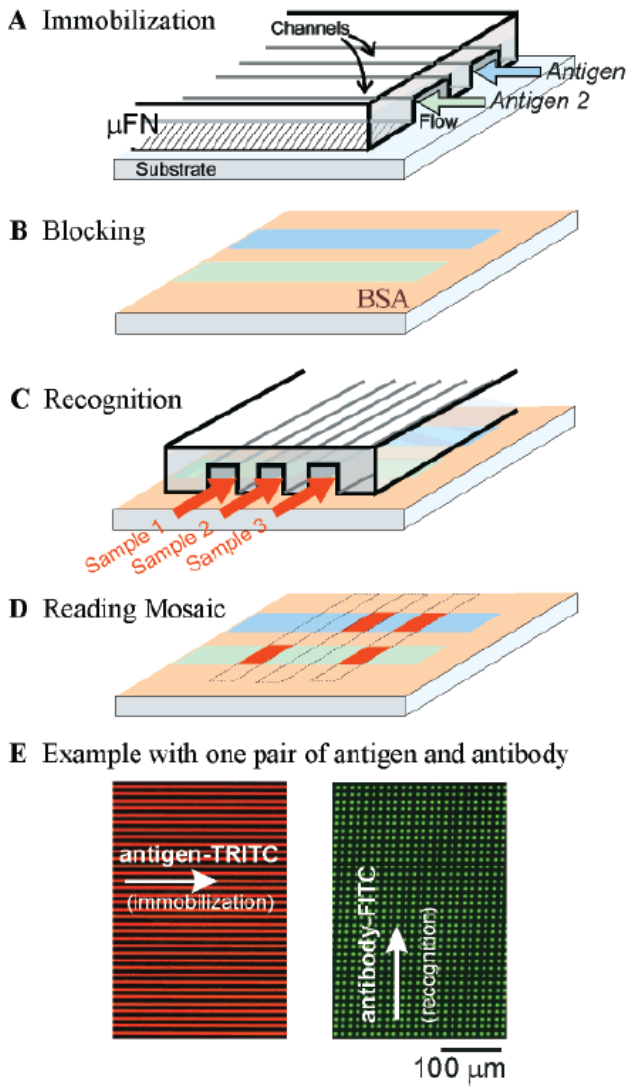
Nonspecific binding through hydrophobic interaction (step A).

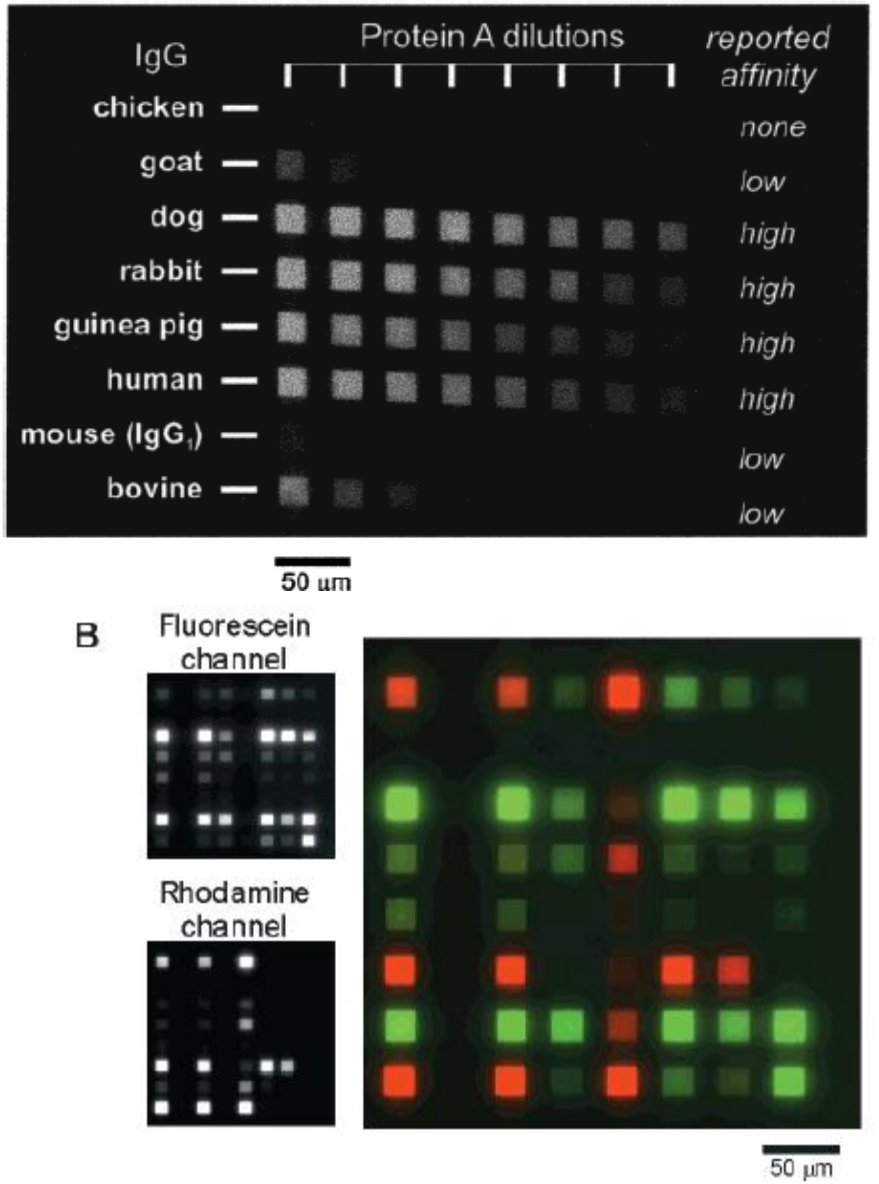
Blocking (step B)

And some others from earlier lectures:

Capillary forces based on surface tension (steps A and C)

Adhesion of soft enough polymer onto a smooth enough surface (steps A and C)



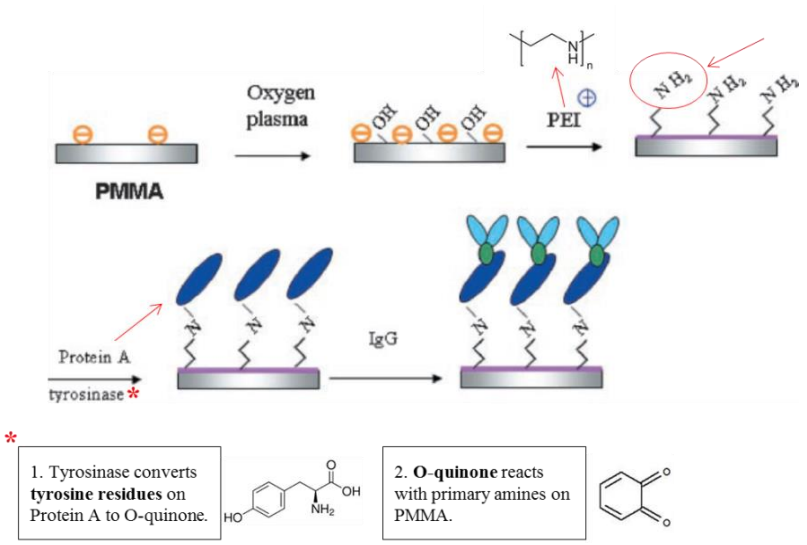


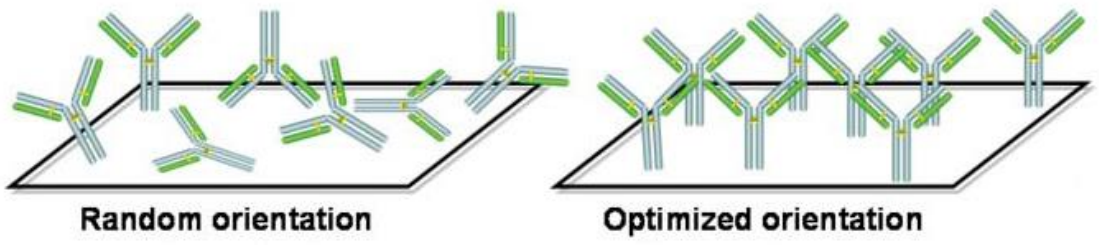
Bernard et al. Anal. Chem. 2001

Covalent/oriented attachment of proteins

Antibodies (and other proteins) can retain part of their activity even when they adsorb by physical forces.

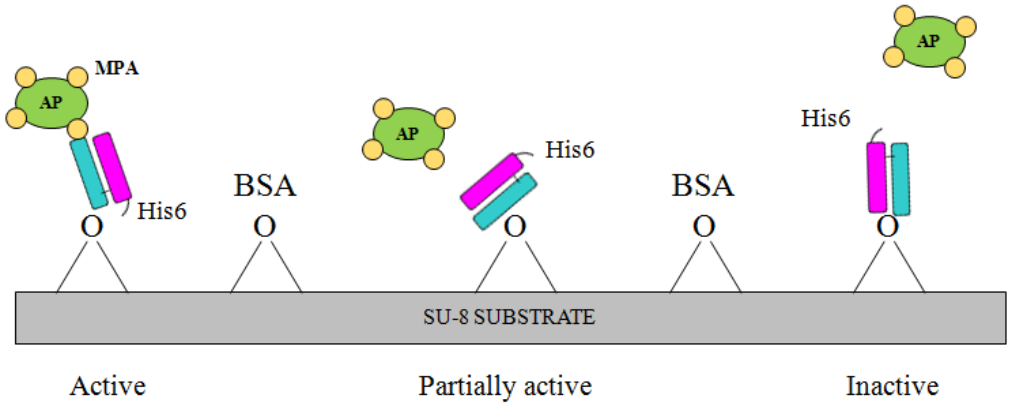
But oriented grafting can lead to better activity.





Some activity

More activity



Example 1:

- PMMA surface is activated by oxygen plasma
- Surface is coated with polyethylenimine (PEI) to have amino groups
- Protein A + tyrosinase mixture is incubated. Action of tyrosinase creates O-quinone from tyrosinase that react with the amino groups on the surface.

Example 2:

-Amino groups from histidine can react with epoxy groups on an epoxy polymer SU-8 surface.

-High activity since the only available histidine is located on the other side of protein to the active site.

Figures: M. Leinikka M.Sc. Thesis. Recombinant antibody coated SU-8 microfluidic devices

Quiz time!

3 questions, 5 minutes.

Part 2: Antibacterial surfaces

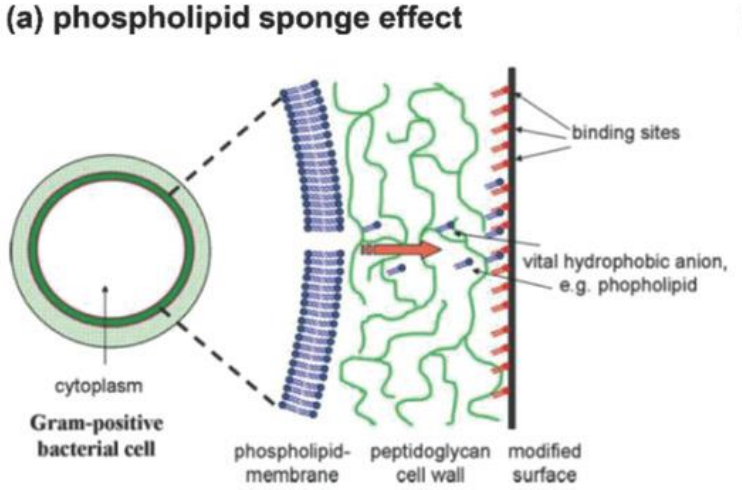
Antibacterial surface chemistries

In most places, bacteria are undesired. Surface options:

Positive charge:

-Quaternary ammonium groups

-Polycations



Strong positive charge of the surface sponges negatively charged phospholipids and thus causes bacterial cell content to leak out. https://doi.org/10.1002/adfm.201802140

Biological surfaces:

-Antibacterial enzymes and peptides

Metal ions:

-Silver

-Copper

Metal ions can potentially damage bacterial proteins/enzymes, while being relatively safe for humans.

Effect usually enhanced by having the silver in a form with high surface area -> nanoparticles or potentially nanostructures?

How about antiviral surfaces?

Very challenging, viruses are very small and they are not even alive.

Not that much is known but it has been shown that viruses remain infective significantly shorter times on Cu surfaces than most others.

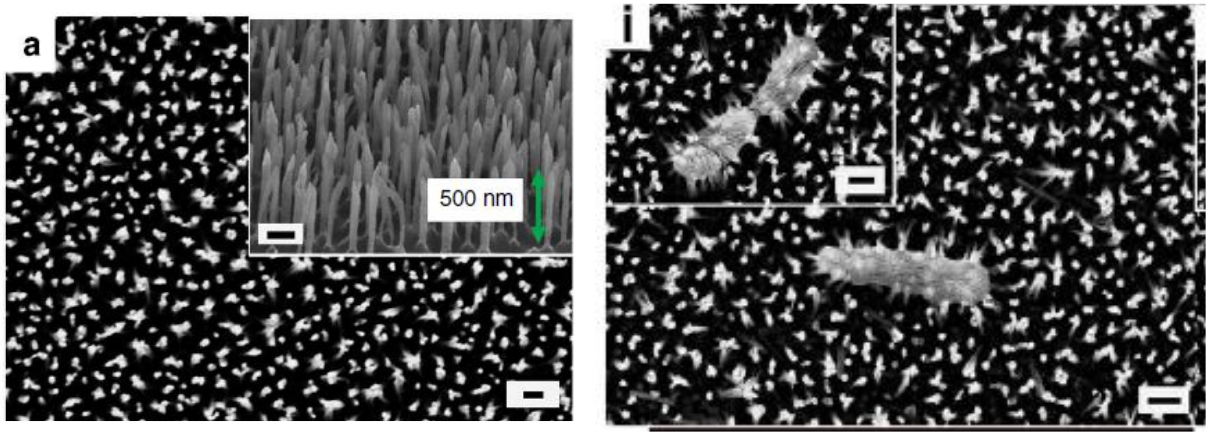
Bactericidal activity of black silicon

Nanospikes are able to kill bacteria

Mechanism is based on mechanical lysis: The bacteria try to make contact with the surface but if the nanospikes have small enough radius of curvature the bacteria impale themselves.

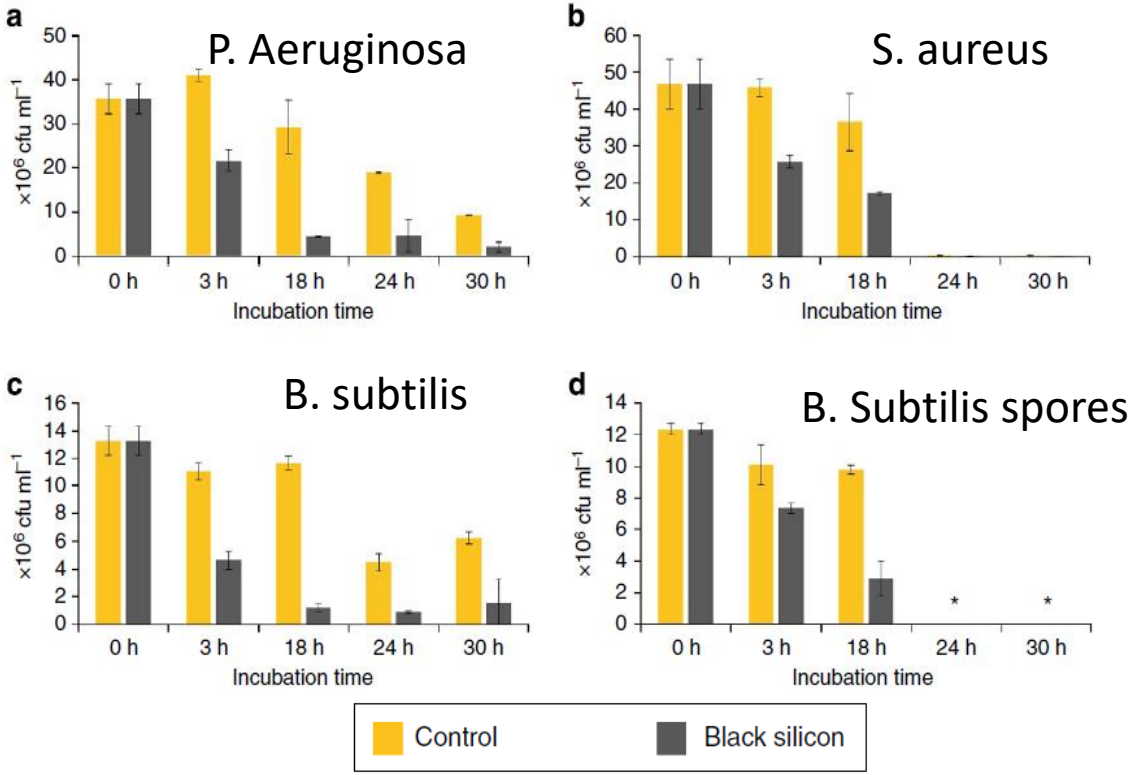
Pros: Chemistry independent, works on many types of bacteria.

Cons: Fragility, unproven in applications



bSi structure

P. Aeruginosa on bSi



NATURE COMMUNICATIONS | 4:2838 | DOI: 10.1038/ncomms3838

Part 3: Blood contacting surfaces

Blood coagulation and surfaces

One function of blood is to coagulate when it senses damage to limit bleeding. This can be triggered both by contact with foreign/damaged surfaces or air.

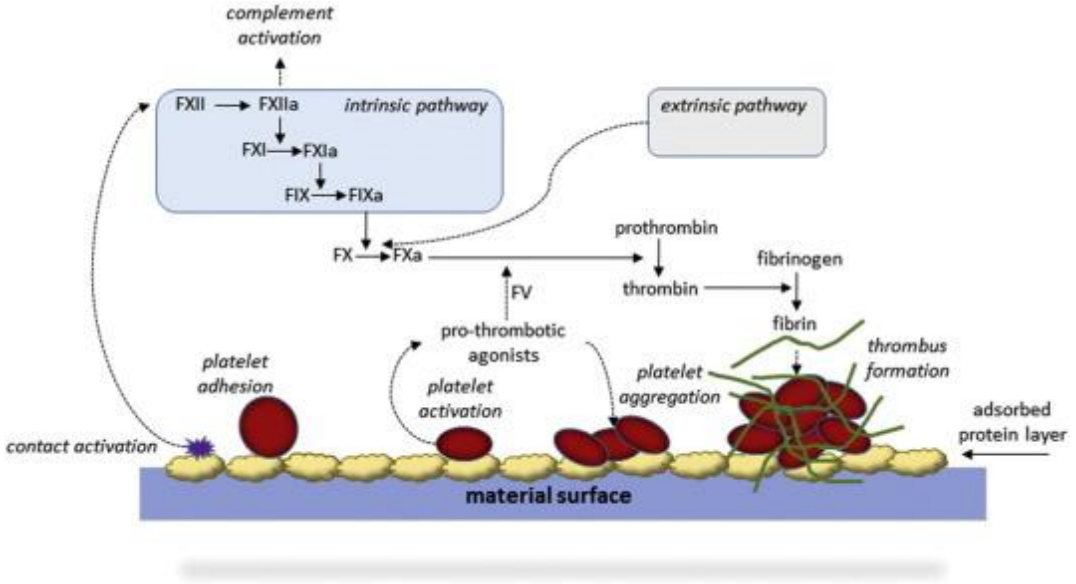
Usually great … but not for example in dialysis or diagnostic tests that use blood (or implants).

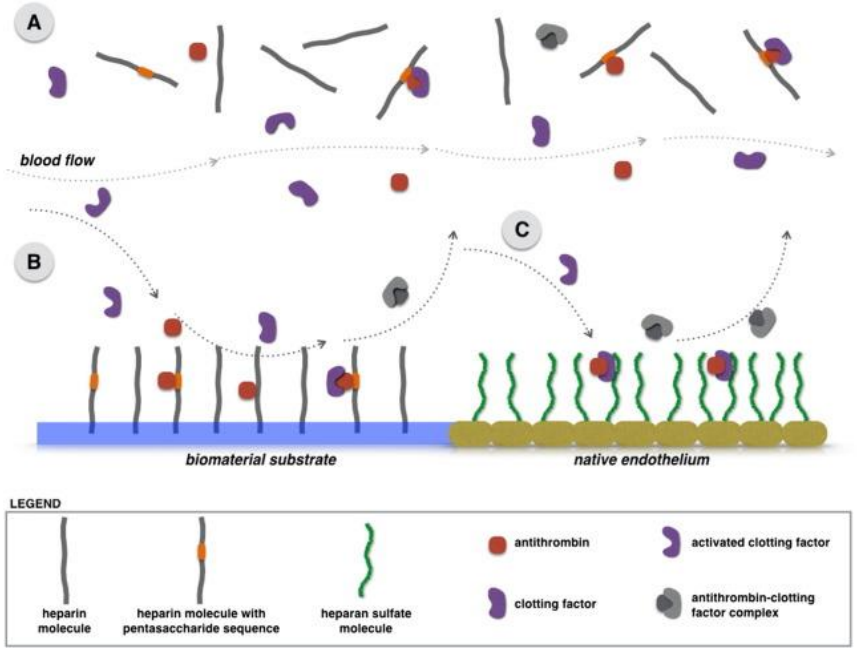
Mechanism is complex, it start with protein adsorption, which leads to coagulation factors activating which leads to platelets to aggregate on the surface. The platelets are small (≈ 2 µm) cells without nuclei.

Standard solution: Heparin coating. Heparin is a strongly negatively charged polysaccharide that is similar to a naturally occurring (in cells) heparan sulfate.

Problem: Heparin coating wears off over time.

Other options: Some smooth polymers, both hydrophilic and hydrophobic, have been indicated to have anticoagulation properties.



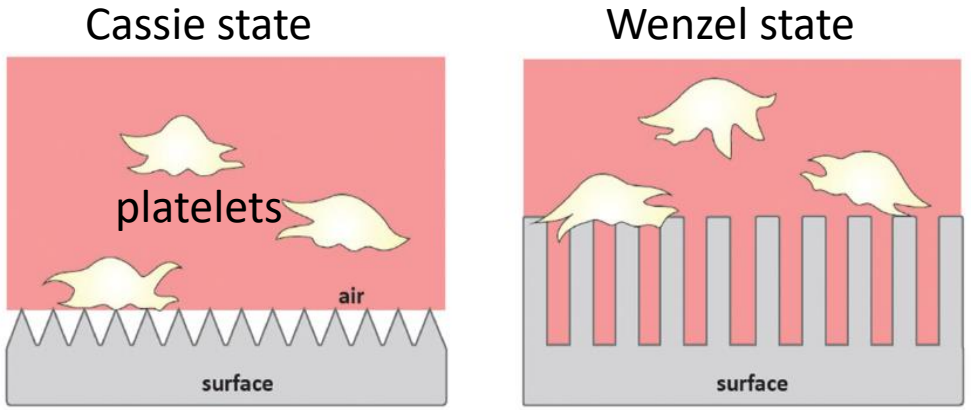


https://doi.org/10.1016/j.addr.2016.12.002

Anticoagulation by structured surfaces?

Platelets are ≈ 2 µm. Coagulation requires platelets to adhere on the surface.

What if the surface has features that are smaller than 2 µm? This limits the contact area of platelets and thus the adhesion.

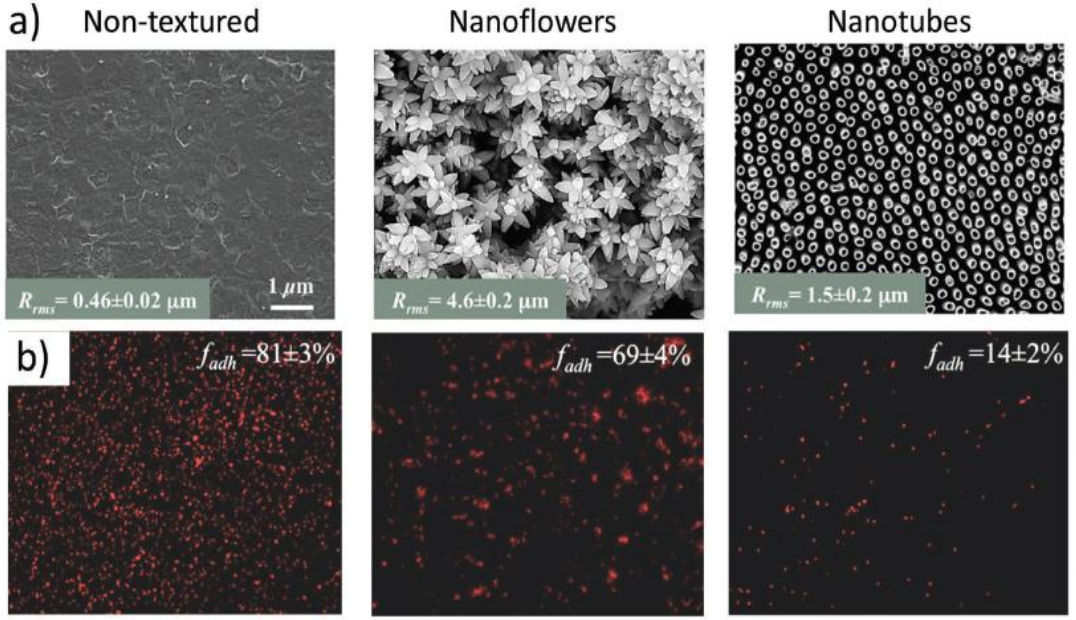


Jokinen et al. 2018 DOI: 10.1002/adma.201705104

Smooth titania vs two types of titania nanostructures

Smooth titania had on average 81% coverage of adhered platelets.

The smaller scale nanostructure (nanotubes) reduced the platelet coverage to 14%



S. Movafaghi, V. Leszczak, W. Wang, J. A. Sorkin, L. P. Dasi, K. C. Popat, A. K. Kota, *Adv. Healthcare Mater.* **2017**, *6*, 1600717.

Part 4: Implant surfaces

Implants and biocompatibility

Permanent (or long term) implants into the human body places a lot of requirements for the surface. There are many materials and applications.

-All should be nontoxic

-Proteins will adsorb on all of them

-In some application cellular adhesion is desired (e.g. orthopedic implants)

-In some applications cellular adhesion is not desired (e.g. stents)

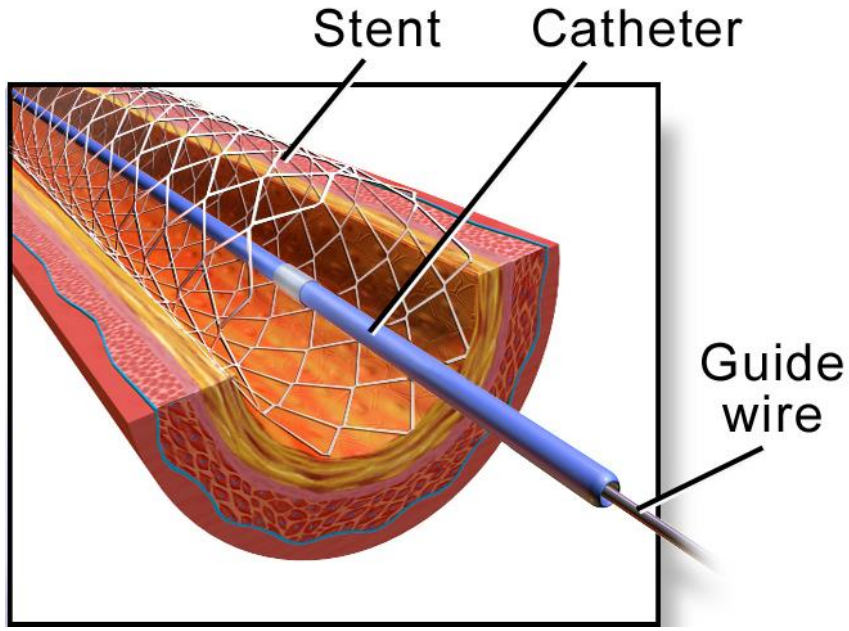
Surprisingly, the list of materials used is large and contains many common materials:

Metals: Stainless steel, Titanium, Cobal-chromium alloys

Ceramics: Alumina, Zirconia, Bioative glasss

Polymers: PE, PP, PDMS, +many others

Biocompatibility: "*the ability of a material to perform with an appropriate host response in a specific application*".



Stent in a blood vessel.
Commonly made of stainless steel.

Figure: https://en.wikipedia.org/wiki/Stent

Homework

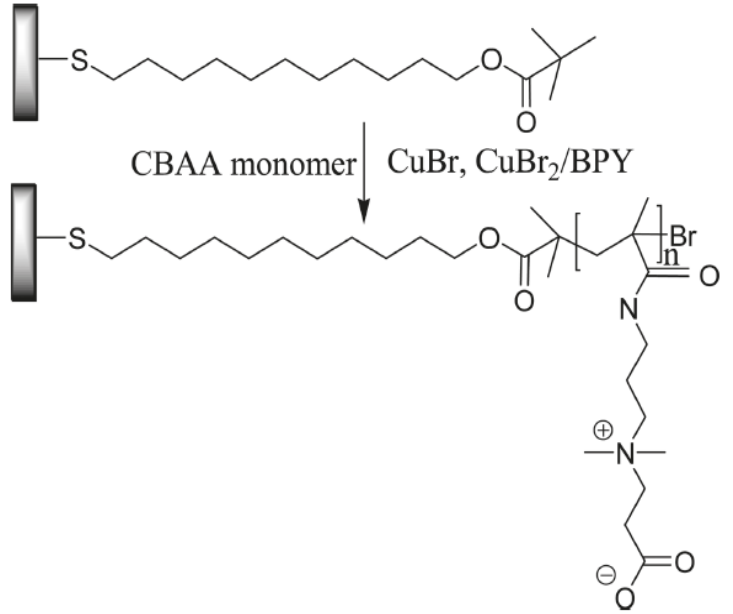
Zwitterionic anti protein adsorption coating.

How does it work?

How well does it work?

What results are shown?

Article utilizes SPR to measure the protein adsorbance



Zwitterionic coating
(same molecule has + and – charges)