Biomaterial Course

Surface and Protein Interactions

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Final Year Bachelor of Chemical Engineering

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Reading

1) B. Ratner, A. Hoffman, F. Schoen, and J. Lemons: *Biomaterials Science,*
$2^{nd}/3^{rd}$ Edition edition (San Diego: Elsevier Academic Press. 2004).

2) Butt, H-J.; Graf, K.; Kappl *Physics and Chemistry of Interfaces, 2nd Edition*
(Wilet-VCH: Weinheim 2006).

Surfaces of Biomaterials

Three points:

1 – Surfaces have unique properties

2 – We can (and do) measure these properties

3 – Because they affect biocompatibility

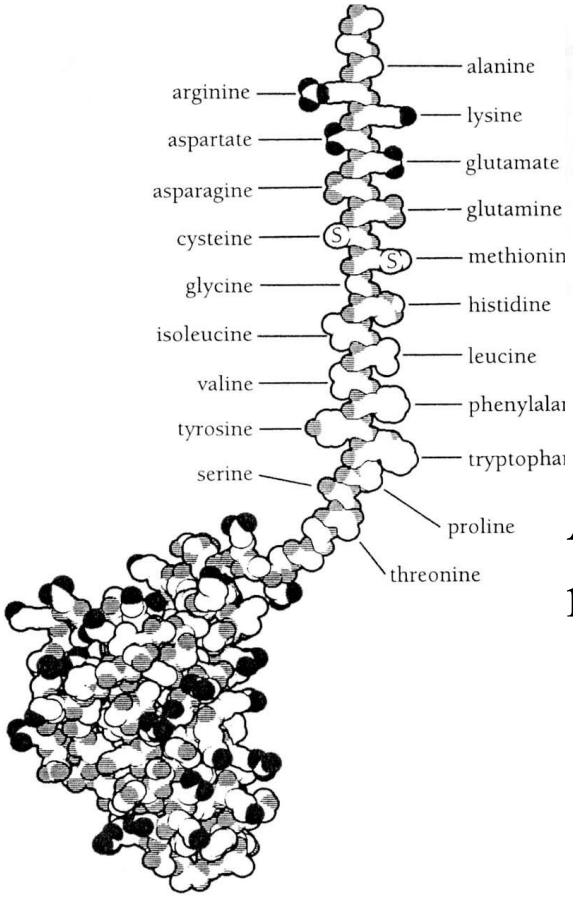
Review

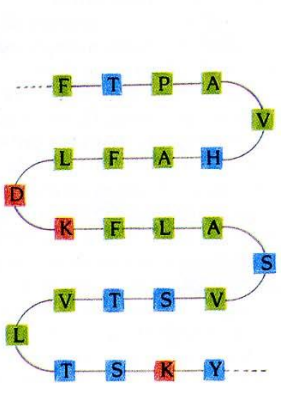
1. Surfaces of materials have unique descriptive properties:
    - Excess surface free energy and Atomic / Molecular composition (vs. Bulk)
    - Chemical composition (reactivity vs. Bulk) and Topography (vs. shape)
2. There are numerous surface specific characterization techniques – the most prominent of these for evaluating biomaterial surfaces are:
    2. Contact Angles and ESCA / SIMS
    3. SPM (AFM, etc)

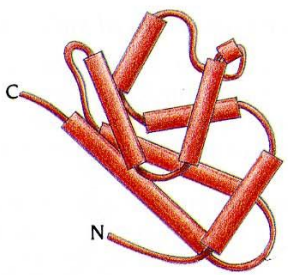
These techniques provide information about surface energetics, atomic and molecular composition, surface chemistry, and topography.

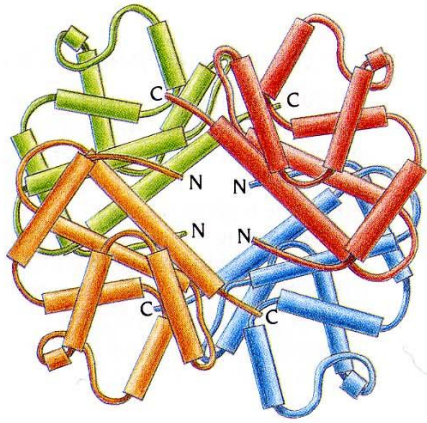
Protein Structure

Proteins are comprised of discrete building blocks (amino acids) assembled into hierarchical structures.









Amino acid side chain heterogeneity manifests in protein surface character:

- charged (acidic / basic) } "hydrophilic"
- non-charged polar } "hydrophobic"
- non-charged, non-polar

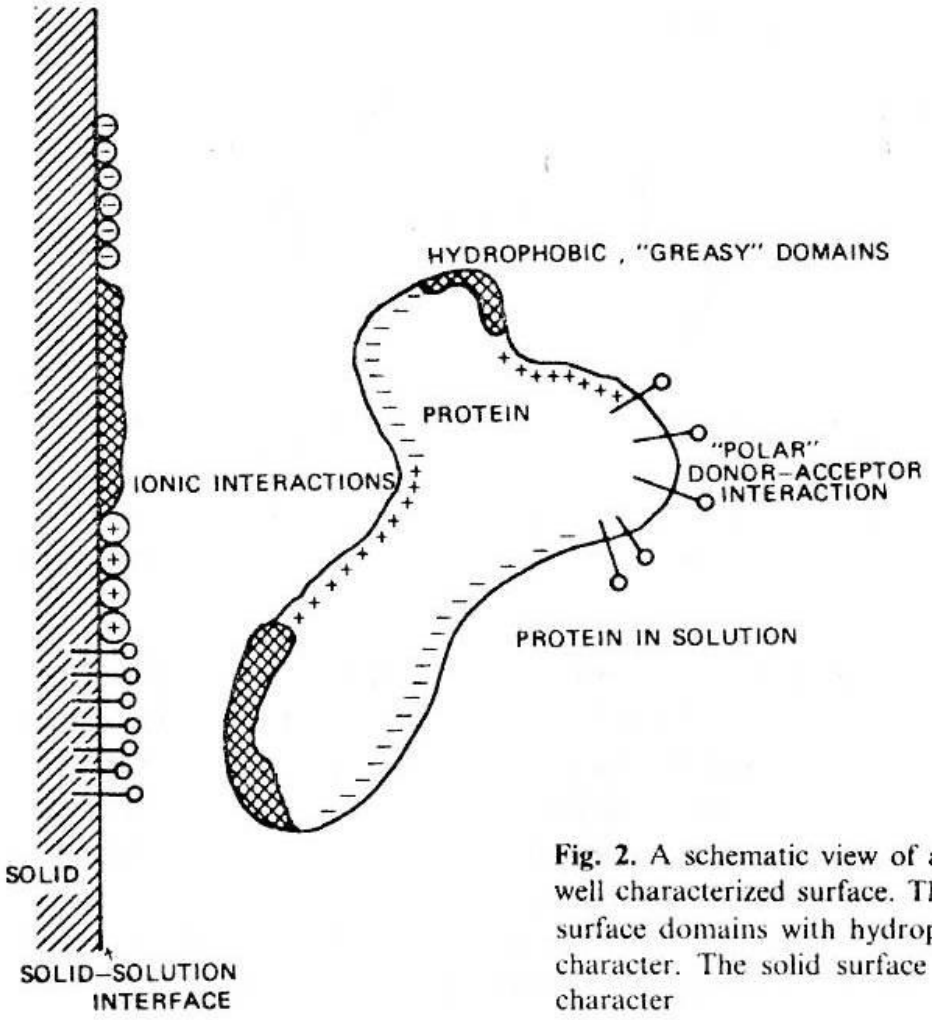
Protein Structure Energetics

A close balance of competing energetics determine protein

Table 1 Interactions that Determine the Structure of a Protein Molecule in an Aqueous Environment

Type of interaction	$\Delta_{\text{compact-unfolded}}G$	Remarks
Coulomb	$\gtrless 0$	Depending on the pH relative to the isoelectric point of the protein/sorbent complex.
Hydrogen bond	≈ 0	Formation of protein–protein and water–water bonds compensated by loss of protein–water bonds.
Dipole	≈ 0	
Dispersion	$\lesssim 0$	Atomic packing densities in compact protein molecules higher than in water.
Hydrophobic dehydration	$<< 0$	Entropy increase in water released from contact with hydrophobic components.
Distortion of bond lengths and angles	> 0	Some bonds are under stress in the folded structure.
Rotational freedom along the polypeptide chain	$>> 0$	Folding reduces the conformational entropy of the polypeptide chain and, possibly, the side groups.

Surface and Protein Domains



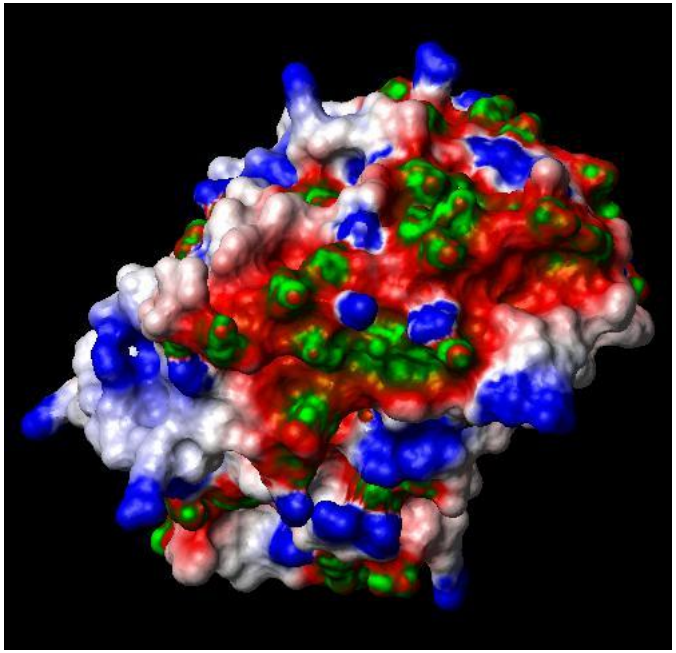


Fig. 2. A schematic view of a protein interacting with a well characterized surface. The protein has a number of surface domains with hydrophobic, charged, and polar character. The solid surface has a similar domain-like character

a**D**sorption, Modes

A*d*sorption is the process of association of solvates (or the solvent) to a material interface

A*b*sorption is when the solvent is taken up by the material

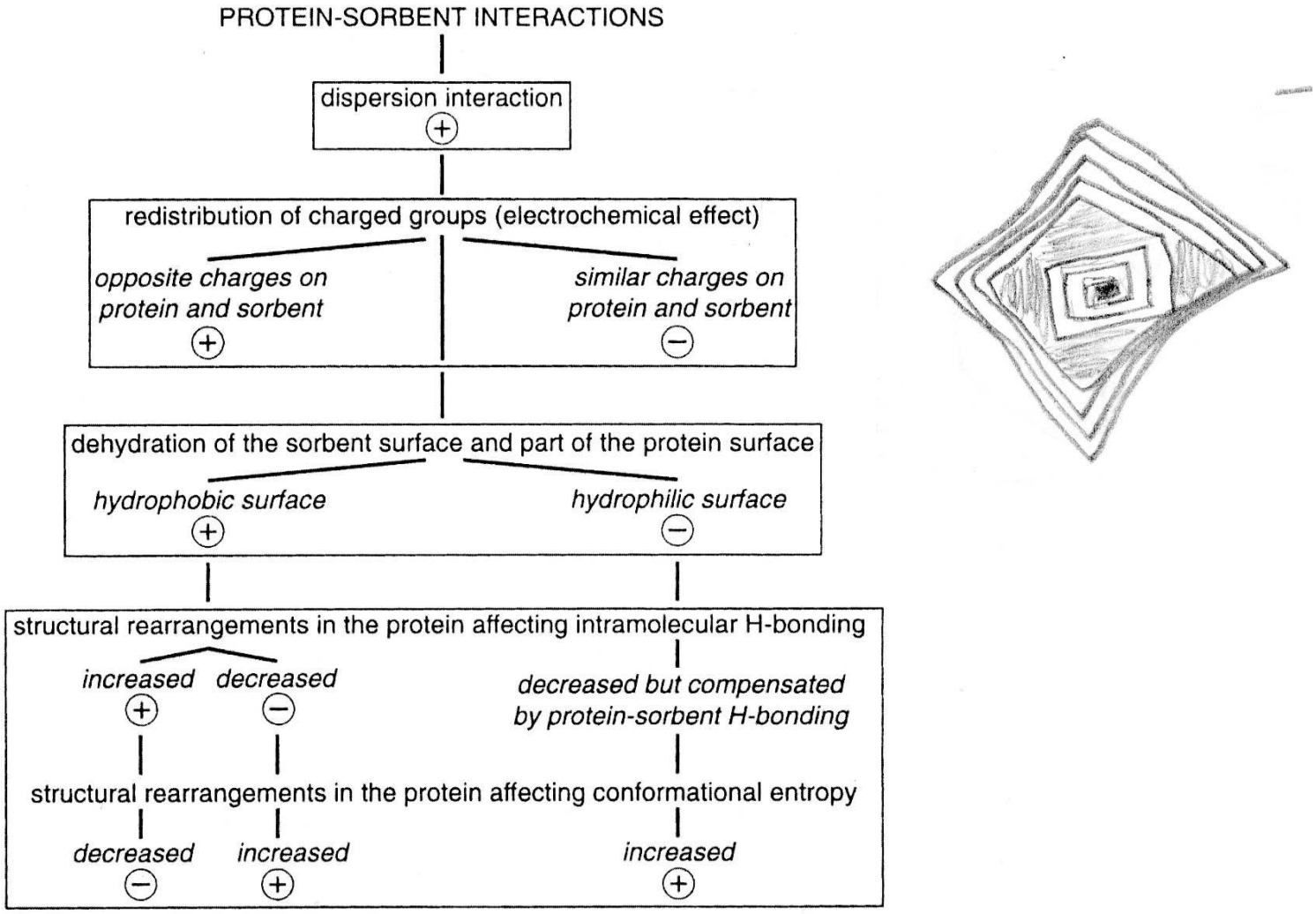
physisorption (physical adsorption): long range and weak van der Waals attraction between adsorbate and substrate ($\Delta H_{physisorption}$ ~ 20 kJ mol^{-1})

- no activation barrier, fast, reversible, surface symmetry insensitive, multilayer formation possible, $T_{surface} < T_{condensation}$

chemisorption: short range and strong bonding between adsorbate and substrate ($\Delta H_{chemisorption}$ ~ 200 kJ mol^{-1})

- activation barrier possible (b), variable uptake kinetics, covalent / ionic / metallic bonding, often irreversible, surface symmetry specific, limited to monolayer, wide range of $T_{surface}$

Overview of Protein Adsorption



Scheme 1 Interdependency of the major subprocesses that are involved in the overall protein adsorption process. Adsorption-promotion is denoted by + and adsorption-opposition by −.

Favorable and Irreversible

Protein adsorption is energetically favorable as the slight increase in enthalpy is more than compensated for by a large decrease in free energy. Increases in the system's entropy contribute to irreversibility

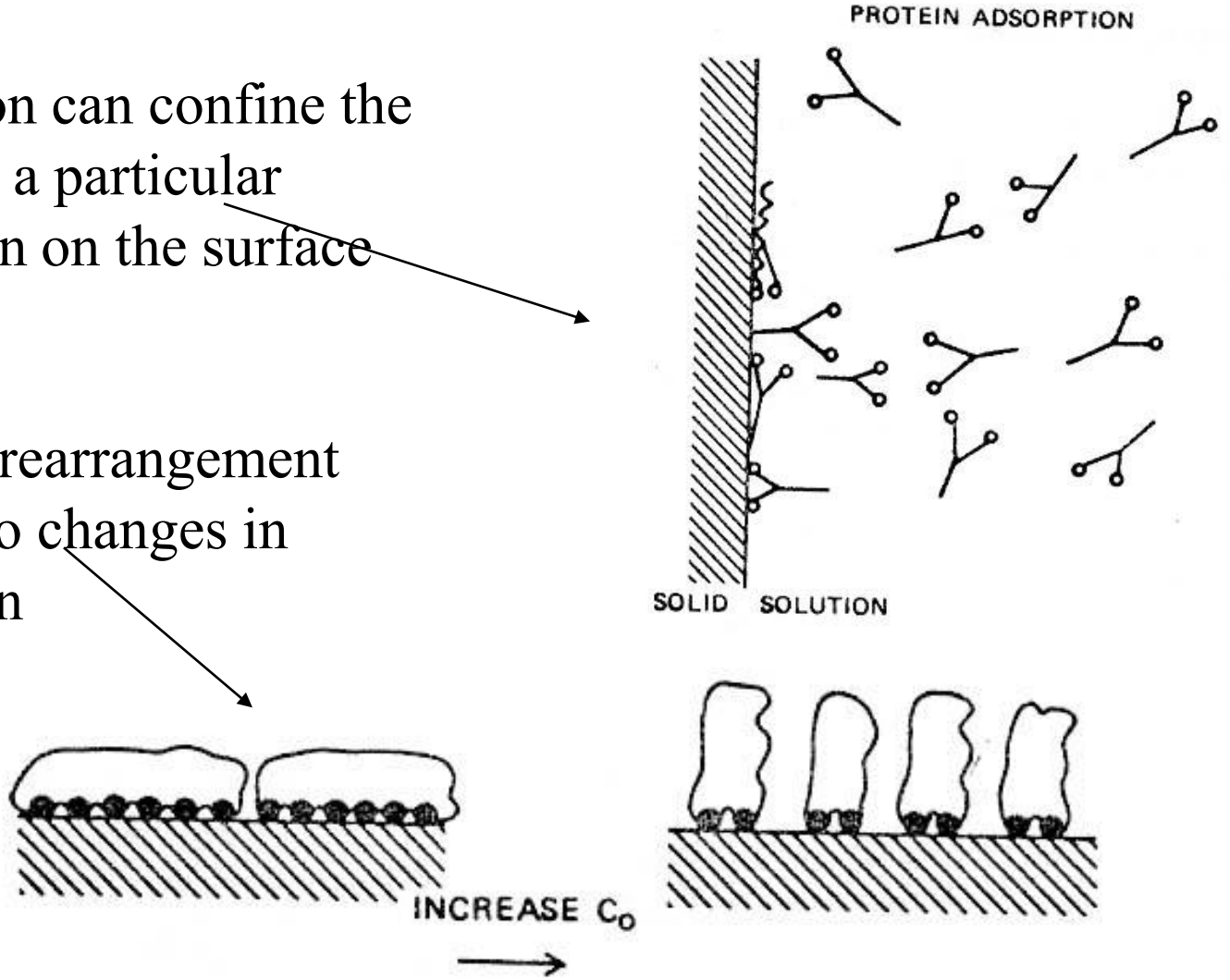
	Lysozyme at pH 10 ($Z_H = +5$)		
	ΔG (kJ/mol)	ΔH	ΔS (kJ/kmol)
Overall protein adsorption process	<< 0	−90	> 0
Dissociation of H+	−20	0	0.07
Overlap of electric fields	−10	−20	−0.03
Change in the chemical medium of the incorporated ions	30	−80	−0.37
Dehydration of the sorbent surface	−220	−40	0.60
Rearrangements in the protein structure	< 0	50	> 0

Plateau adsorption; 0.05 aqueous M KCl; 25°C.

Orientation

Adsorption can confine the protein to a particular orientation on the surface

Dynamic rearrangement can lead to changes in orientation



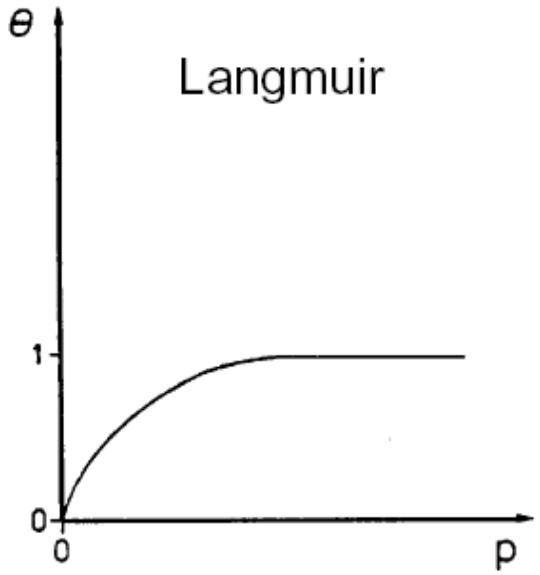
Orientation can affect protein activity!

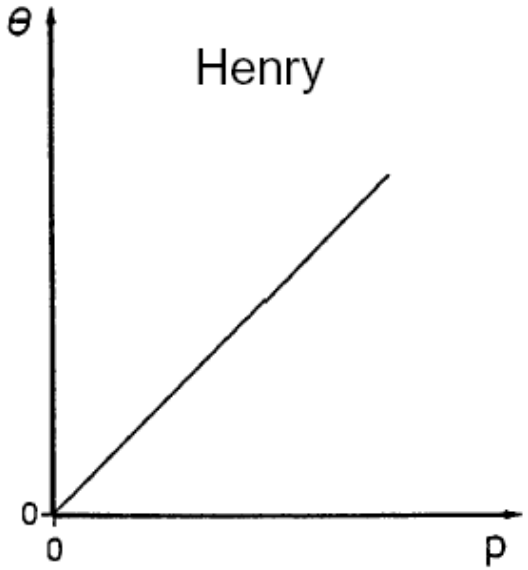
Thermodynamic Models

Henry isotherm: surface coverage θ depends linearly on pressure p (special case of Langmuir for $\theta \rightarrow 0$)

Langmuir isotherm: assumption of a) maximum monolayer coverage (θ = 1); b) no interaction between adsorbate atoms / molecules; c) coverage-independent binding energy; e) thermodynamic equilibrium of adsorption (k_a) and desorption (k_d) rate, (dθ / dt) being equal

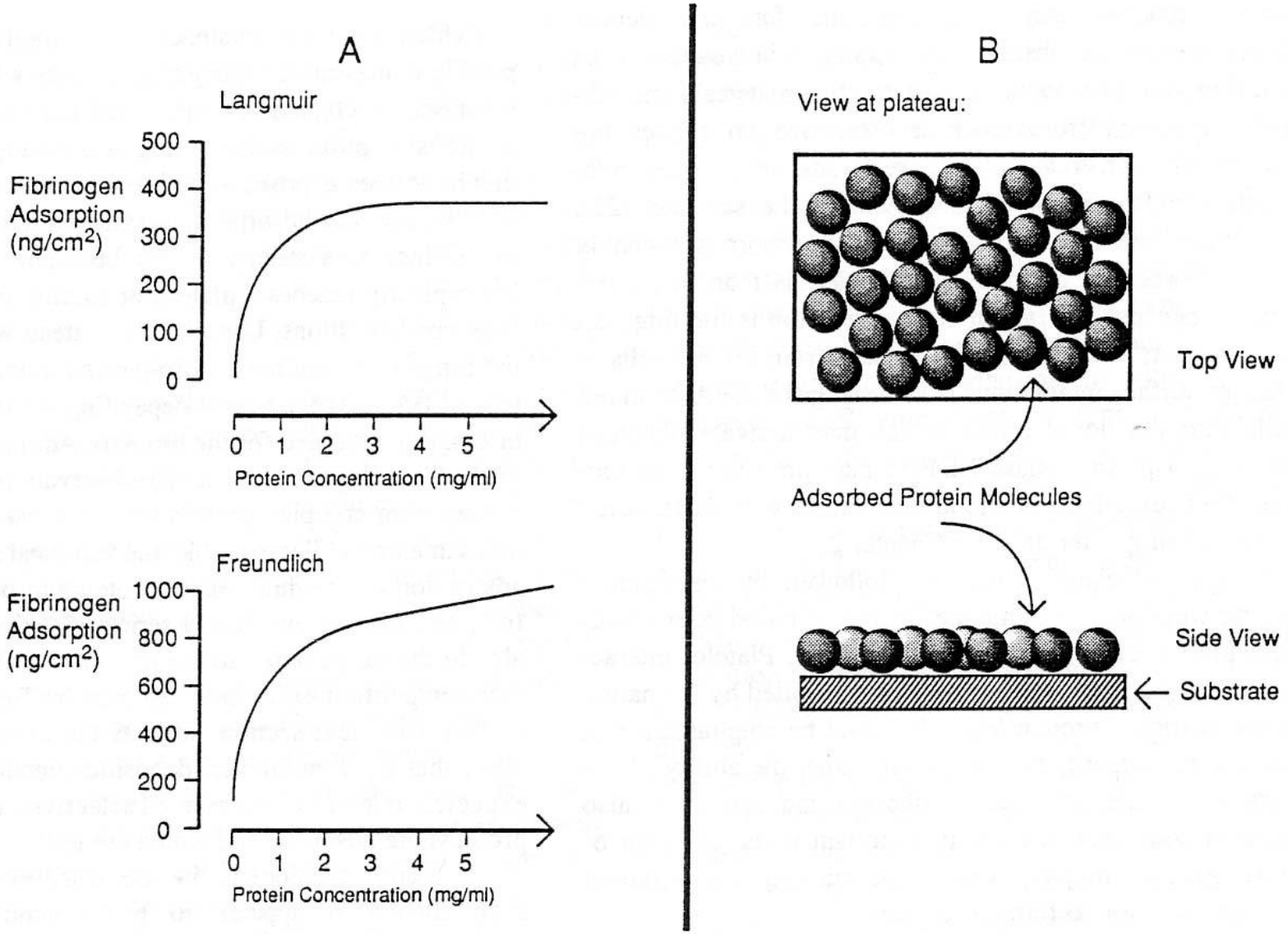
adsorption: $\frac{d\Theta}{dt} = k_a p N (1-\Theta)$ desorption: $\frac{d\Theta}{dt} = k_d N \Theta \longrightarrow \Theta = \frac{K p}{(1+K p)}$ $K = k_a / k_d$





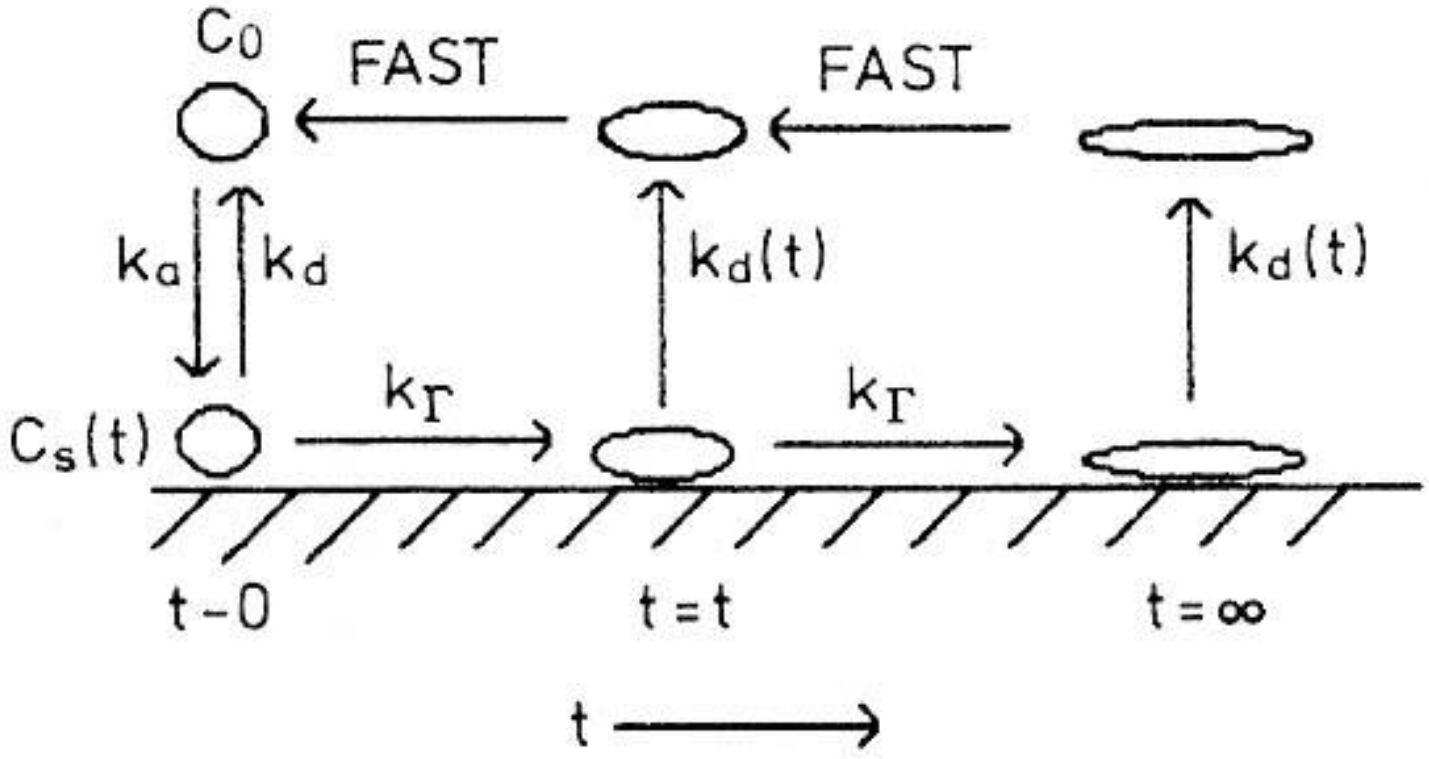
Monolayer?

The Langmuir model assumes a monolayer:



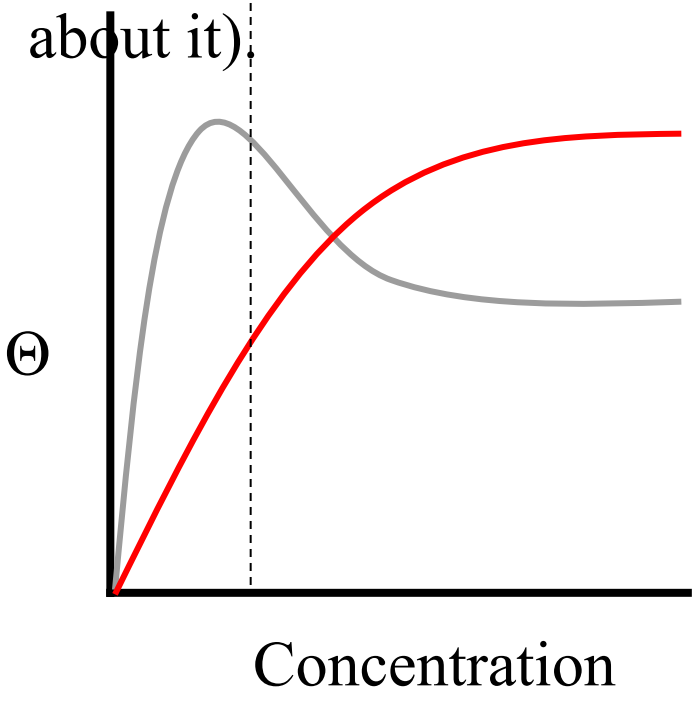
Kinetic Models of Adsorption

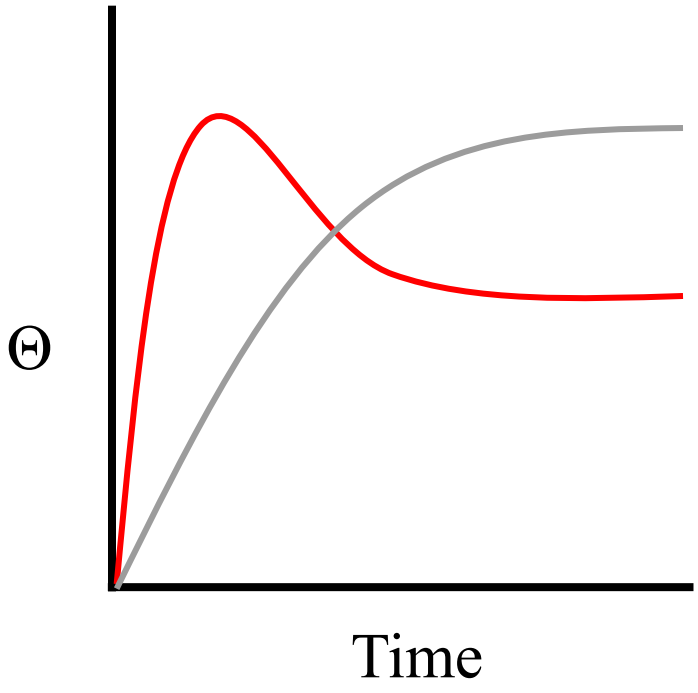
A general model includes adsorption, desorption, conformational changes, rearrangements, etc.



Competitive Adsorption

Competitive adsorption in multi-component mixtures can lead to changes in their relative surface concentration as a function of mass action (concentration) and over time. Transient competition is known as the "Vroman effect" – named for the early researcher into blood-material interaction that first wrote about it).

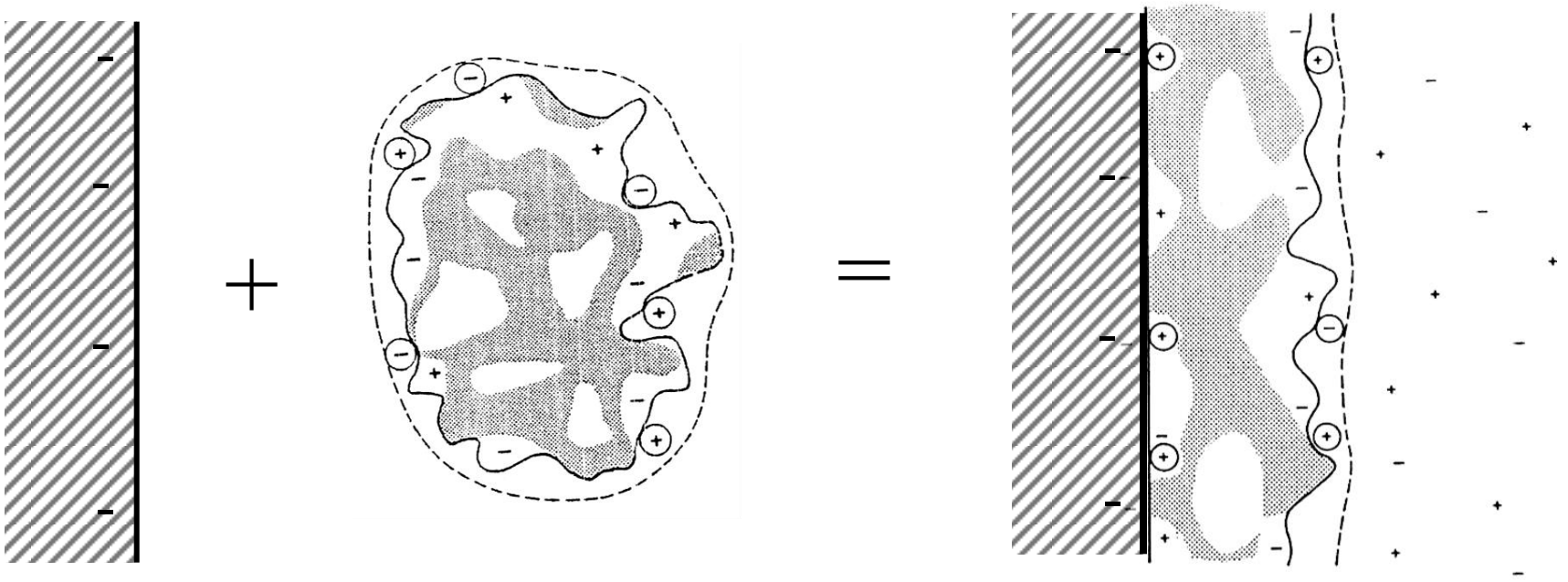




One is **Fast, Weak** and one is **Slow, Strong**, which is which

Protein Coating

Adsorption of proteins to a surface creates a new surface



Surface Protein Solution New Surface

Incremental, Dynamic Process

Protein adsorption to surfaces is followed by higher order interactions

Table 1 Important Processes in the Formation of an Adsorbed Layer of Cells or Proteins

Approach	The transport of cells, proteins, and other biomolecules to the surface. The surface is only briefly clean and is quickly "conditioned" beginning with a layer of small, abundant proteins or nonprotein macromolecules.
Initial attachment	Biofluid components adsorb/adhere to a clean surface by a reversibly bound contact point.
Arrangement	Bound components increase their strength and/or number of surface bonds, while decreasing the reversibility of their attachment. Conformational, positional, and orientational changes occur. Denaturation allows normally hidden hydrophobic groups to seek out nonpolar regions on the surface.
Interactions	Competition, cooperation, displacement, and exchange lead to a steady-state surface composition markedly different from that in the biological fluid source.

A Short History

It has long been noted that blood coagulated more rapidly on negatively charged glass than on hydrophobically modified glass or on polymers.

This affect was first attributed to a simple relationship of charge up until ~1960. The idea was that negatively charged surfaces decreased coagulation times in a way that is analogous to the proposed action of negatively charged heparin, an anticoagulant.

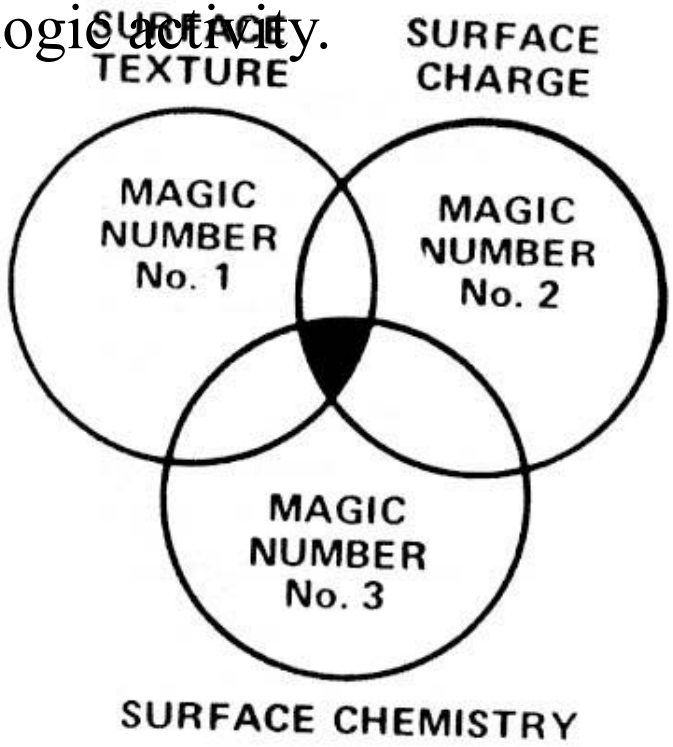
Proteins largely have an overall negative charge and were thought to avoid negatively charged surfaces

The discovery of the surface coagulation activation properties of the negatively-charged protein "Hageman Factor" left some doubt about this theory. It turns out that Hageman Factor was activated on negatively charged surfaces, leading to coagulation.

(Hence begins the study in earnest of proteins on biomaterial surfaces...)

The Search for Heuristics

Using the method of "critical surface energy" developed by Zisman, researchers were able to measure a specific surface property and correlate it to biologic activity.



THE ROLE OF SURFACE ENERGY IN THROMBOGENESIS*

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Buffalo, N.Y.

THE present situation with respect to the evaluation of biomedical materials might be likened to that of a large number of individuals all busily exploring mechanisms for exit from their own independent circular mazes. After final breakthrough of the walls of any independent circular maze we discover only that we have all along been within a much larger, more intricate maze—the whole complex maze of biomedical problems—and that there are many other investigators still within isolated circular loops like the one from which we have recently emerged.

Figure 1 illustrates an overview of three such confining rings, one labeled surface texture; one, surface charge; and the other, surface chemistry. These rings represent three of the primary surface characteristics that are now being carefully examined in the hope of discovering "magic numbers" which might apply in determining the ultimate thrombogenicity or thromboresistance of a candidate biomaterial. The figure takes the optimistic option of showing regions where these three circles may overlap. Stated in the simplest possible terms, the general goal of many of the investigators contributing to this program is to find that specific area where their own special interest, their own "magic number" if you will, overlaps any or all of the others. Our common goal is eventually to provide the biomedical engineer and the general medical community with a specific set of parameters that can guide them in the formulation of new and better blood-contacting materials. Still, it is recognizable that the surface qualities of the materials are not the only factors to be considered. They can be contrasted with all of those

*Presented as part of a *Symposium on Problems in Evaluating the Blood Compatibility of Biomaterials* held by the Section on Basic Medical Sciences and the Section on Biomedical Engineering of the New York Academy of Medicine February 18 and 19, 1971.

This study was based upon research internally supported by Cornell Aeronautical Laboratory, Inc.

Low Critical Surface Energy Hypothesis

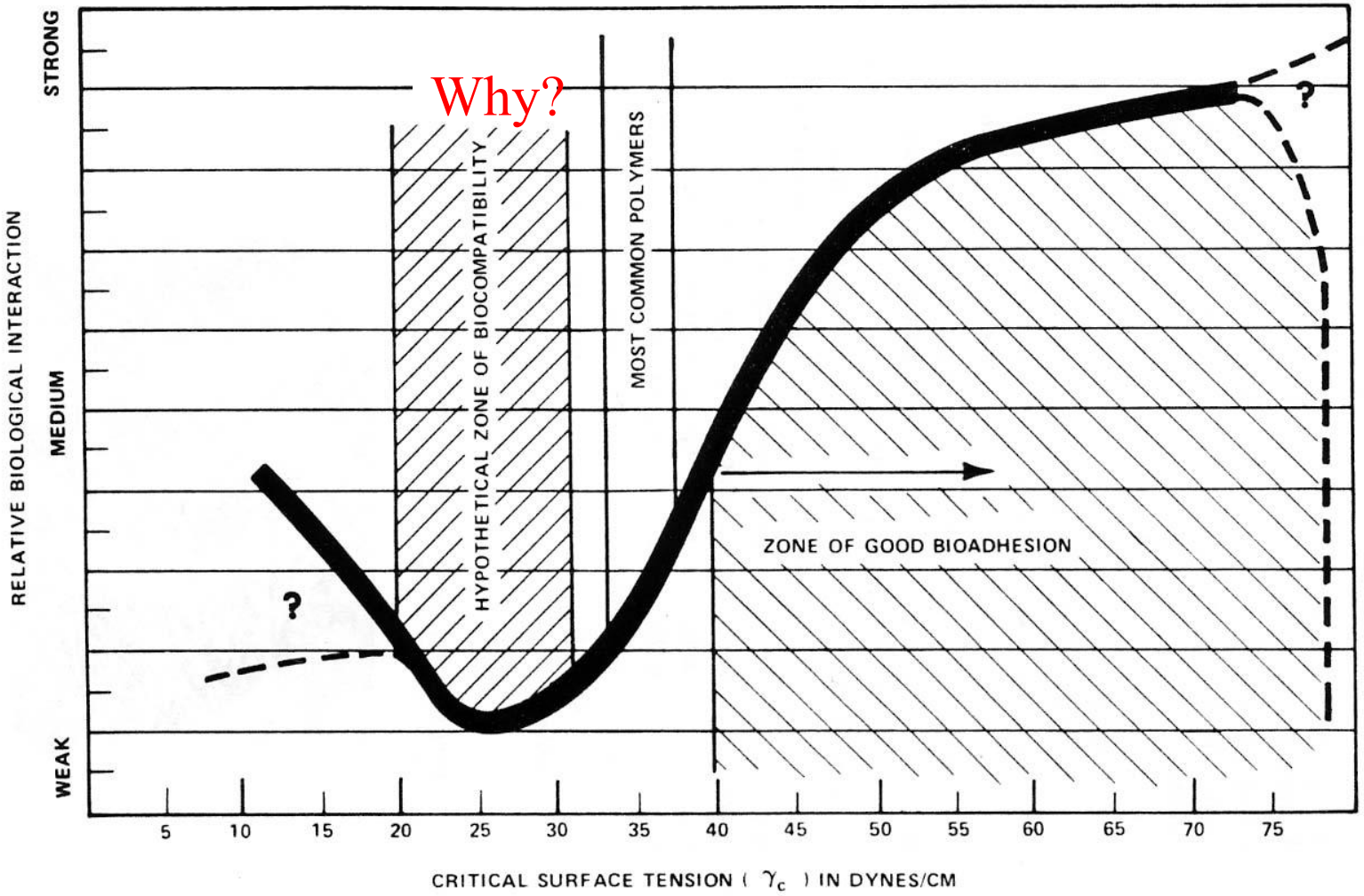


Fig. 4. Tentative correlation of relative surface energies of osolids with their biological interactions. Reproduced by permission from: Baier, R. E. and DePalma, V. A.: *Management of Occlusive Arterial Disease*, Dale, W. A., editor. Chicago, Year Book Med. Publ., 1971.

Surface ↔ Free Energy ↔ Interfacial

Lyman argued that the surface free energies (vs critical surface energy) drives protein adsorption and therefore biological activation (as in the case of Hageman Factor). Thus highly charged surfaces are less biocompatible. Examples are glass and blood activation.

(New Method: Fowkes)

Andrade argued that the free energy of a polymer-water interface is what governs protein adsorption – so as the solid looks more and more like water there is an increase in biocompatibility. Examples are hydrogels and PEO-modified surfaces have reduced coagulation effects.

(New Materials: Hydrogels)

(New Method: SFG)

What we want to know...

What properties of a biomaterial surface mediate biological response?

To what extent?

Example: Surface Coagulation

Hageman Factor (Factor XII) is surface activated!

So control adsorption to control coagulation.. how?

Surface energetics?

What else?

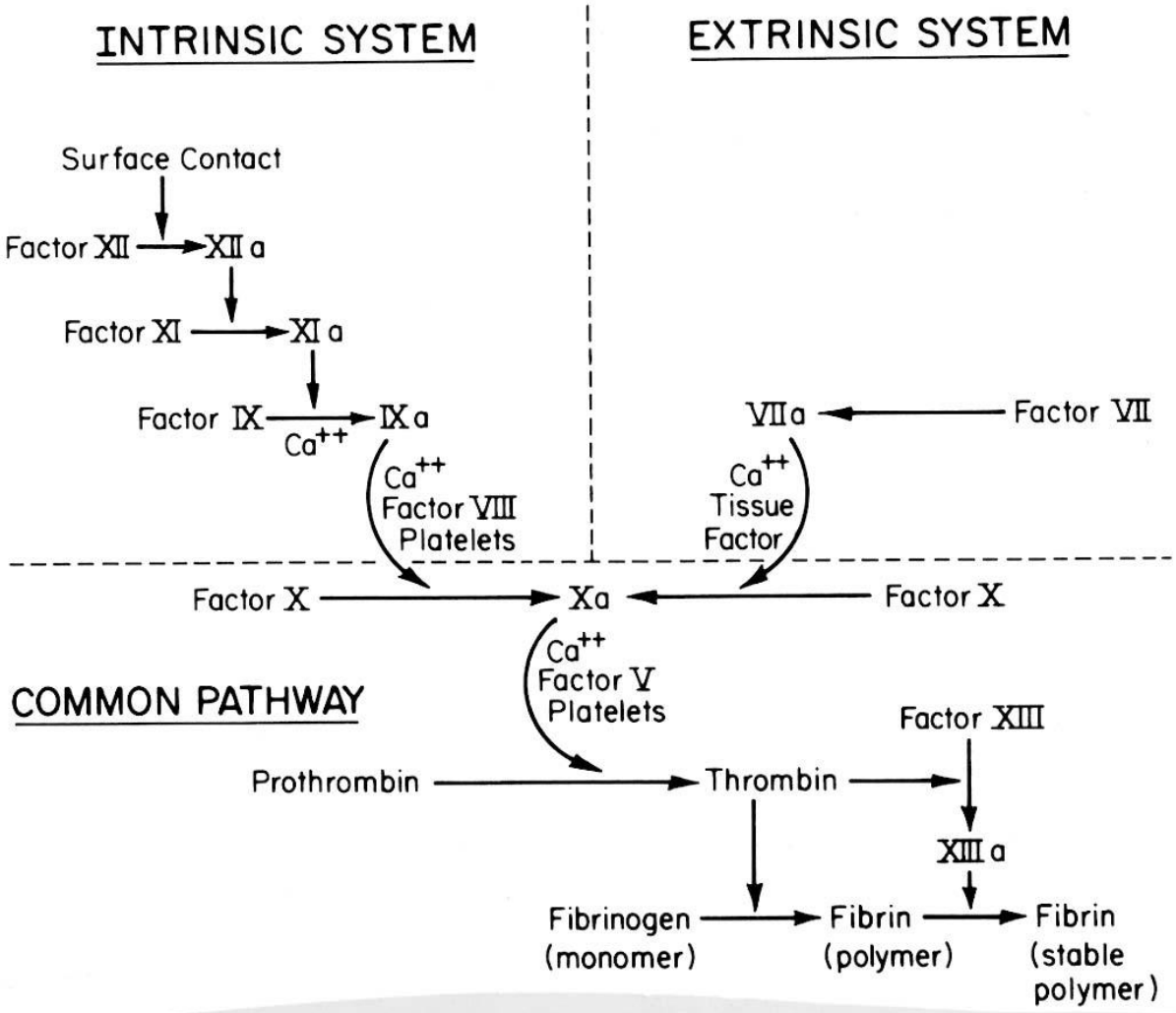


FIG. 3. Mechanisms of clotting factor interactions. Clotting is initiated by either an intrinsic or extrinsic pathway with subsequent factor interactions which converge upon a final, common path.

Example: Bacterial Adhesion

Bacteria take advantage of surface effects to gain a foothold – then they rework the surface!

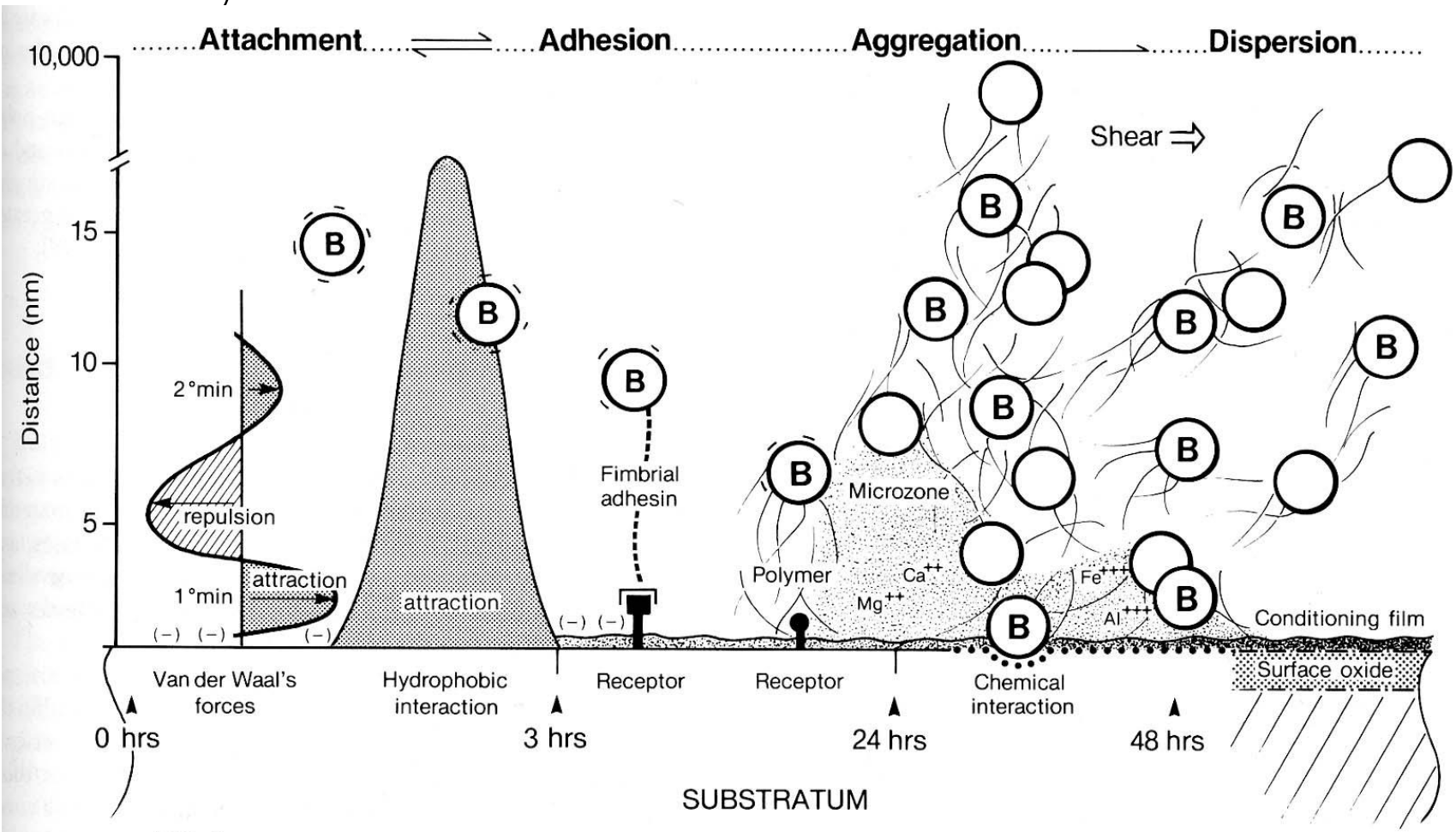


FIG. 4. Molecular sequences in bacterial (B) attachment, adhesion, and aggregation to substratum. (Reprinted with permission from *Science* 237: 1588–1595, 1987.)

Example: Foreign Body Response

Surface properties have been shown to mediate the FBR to a certain degree – however...

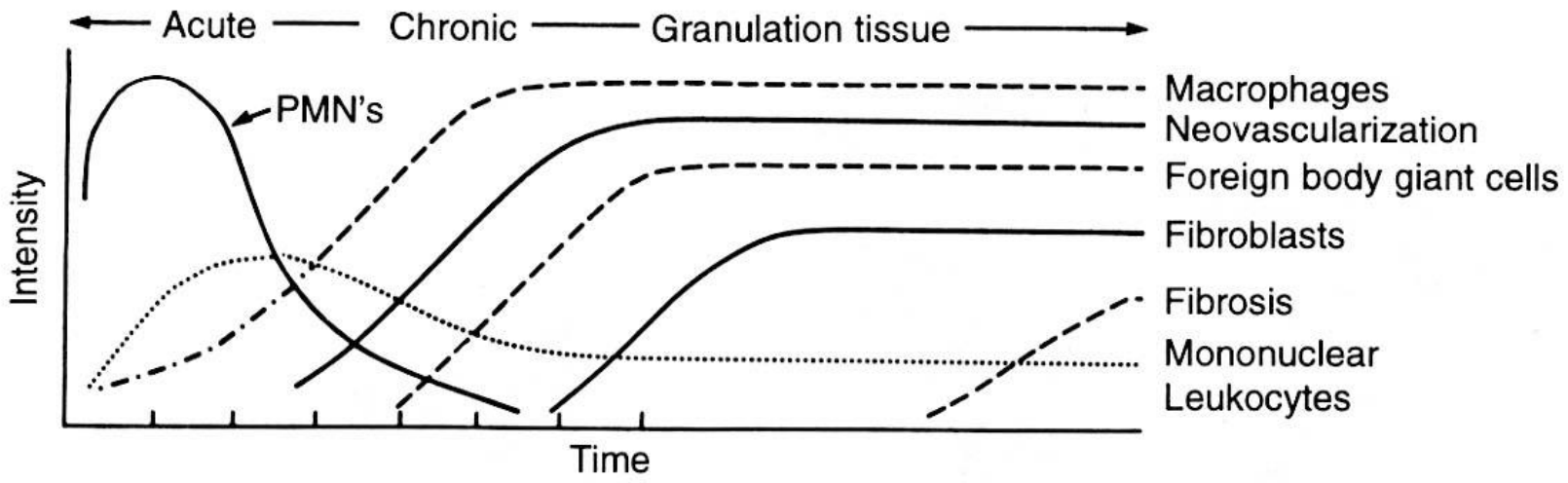


FIG. 1. The temporal variation in the acute inflammatory response, chronic inflammatory response, granulation tissue development, and foreign body reaction to implanted biomaterials. The intensity and time variables are dependent upon the extent of injury created by implantation and the size, shape, topography, and chemical and physical properties of the biomaterial.

Bioreaction – Short and Long Term

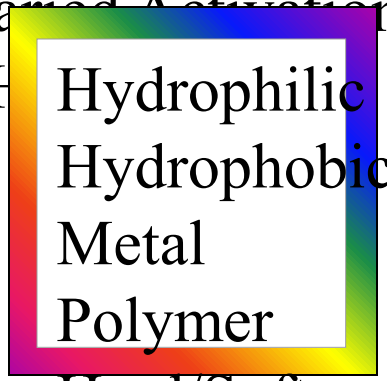
Implant into soft tissue:

9 Different Materials:

- Polyethylene
- Hydroxyapatite
- Polyurethane
- Silicone
- pHEMA
- PTFE (Gore-tex)
- Pyrolytic carbon
- Gold

Short Term Reaction:

- Differential Protein Adsorption
- Varied Activation of H



→ The SAME RESULT!

Long Term Reaction:

- Fibrous Encapsulation

Protein Adsorption to Surfaces

Does it even matter? Not in a great deal of cases!

Nonetheless, it plays a significant role in:

- Complement activation (IgG, IgM)
- Coagulation activation (Hageman Factor)
- Fouling of contact lenses (Albumin, lysozyme)
- Interesting scientific pursuits
- Initial response to implants
- Where transport is important (drug delivery)
- etc.

The goal has shifted from understanding the adsorption properties of unmodified materials to intelligent design of materials to mediate the adsorption process. (Or highjack it entirely.)

Surface Design

⇒ **inorganic** layer formation by:

– thermal conversion: $Si + O_2 \rightarrow SiO_2$ (~1000°C)

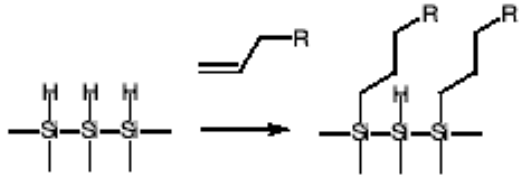
$Si + N_2 \rightarrow Si_3N_4$ (~900°C)

– chemical vapor conversion

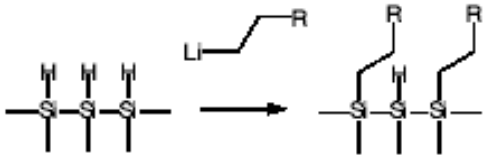
– electrolytic deposition (electro– and electroless plating)

⇒ **organic** layer formation by:

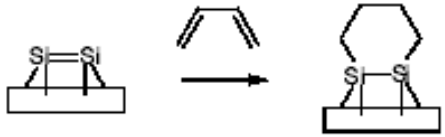
– hydrosilylation (radical, thermal, photochem., catalytic):



– alkyl / aryl carbanions (Grignard, alkyl lithium):



– [2+2] and [4+2] reactions (UHV):



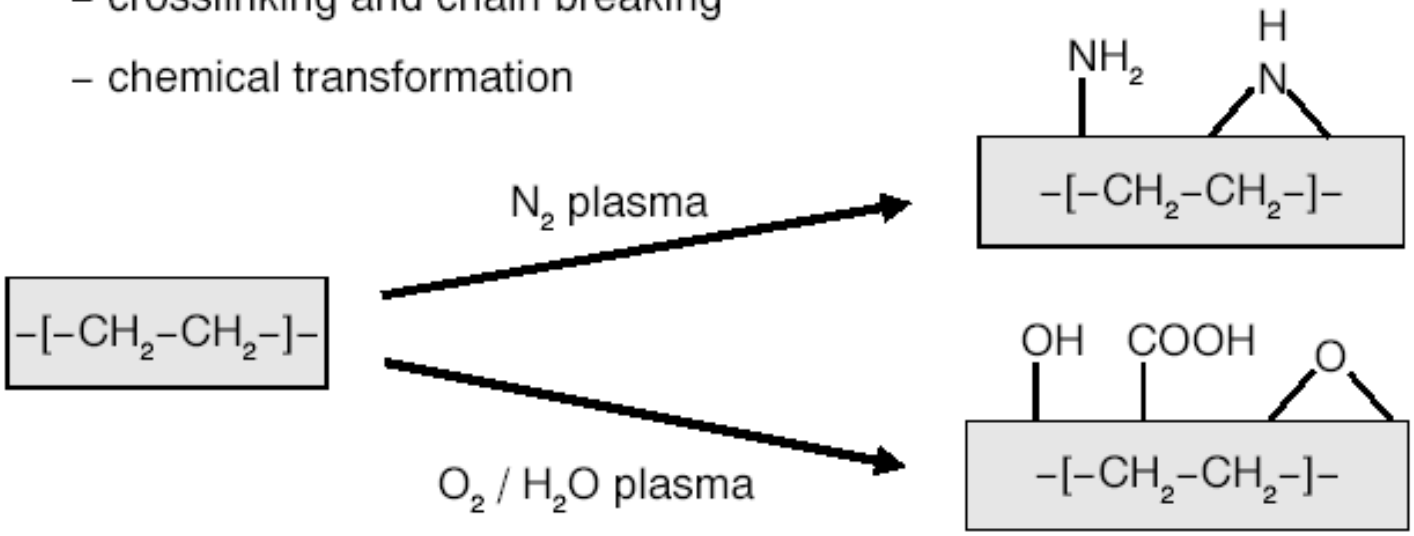
Surface Design

Chemical modification of **polymer** surfaces with **plasma**:

⇒ **inorganic** and **organic** layer formation by plasma treatment of polymer surface:

low pressure ~ 1 torr, high frequency (≥ 1 MHz) **discharge** generates **electrons** (bond breaking), **reactive atoms** and molecular **fragments** (radicals, ions)

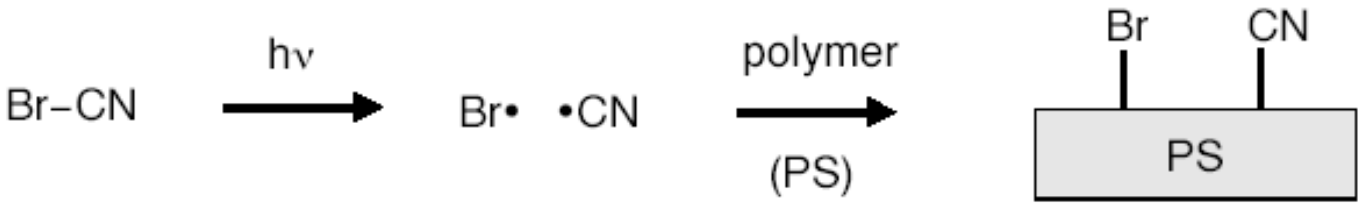
- cleaning (remove organic contaminants)
- ablation / etching (remove substrate material)
- crosslinking and chain breaking
- chemical transformation



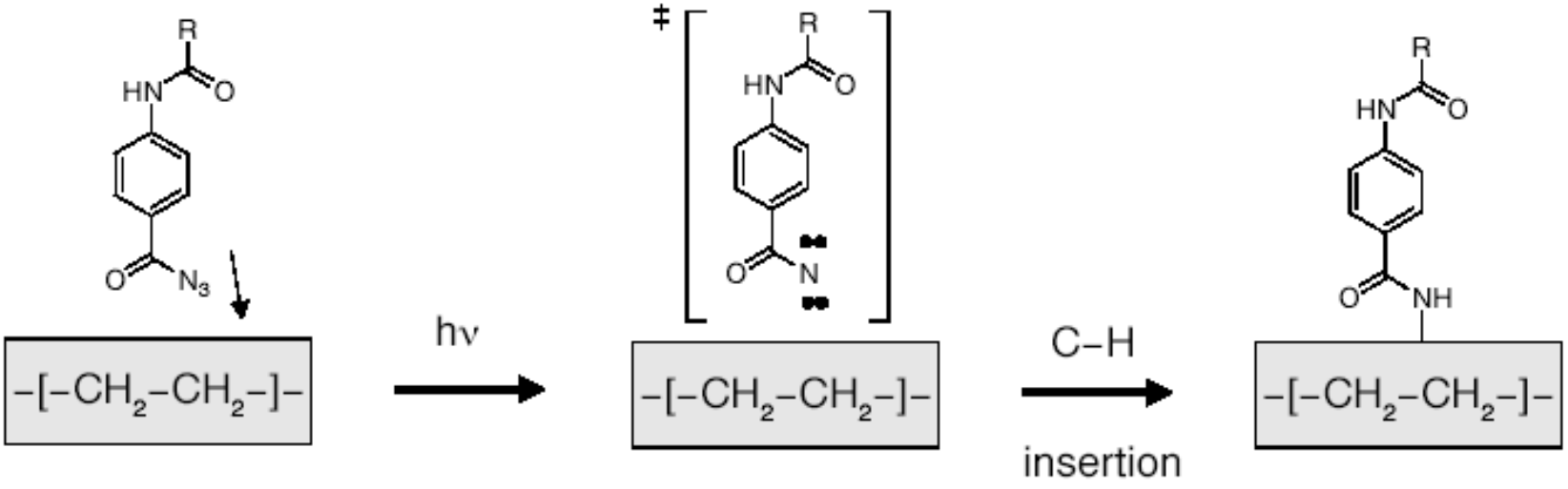
Surface Design

- **Chemical modification** of **polymer** surfaces with **photochemically**:

  – **activation** of reactive molecules by **light** in the **gas** phase:

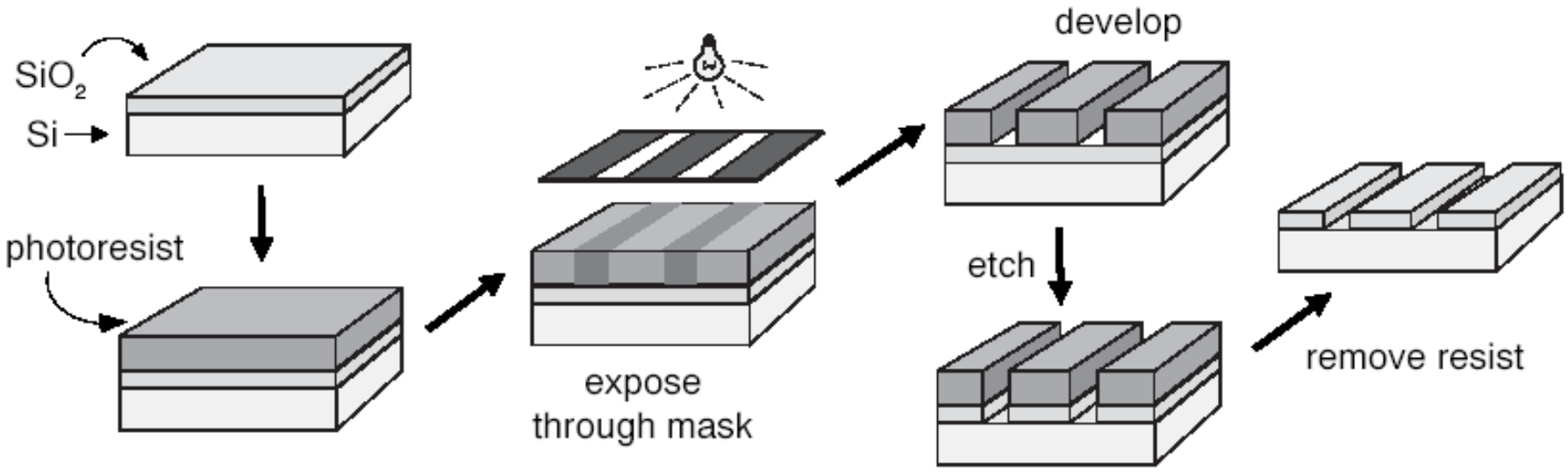


  – photochemical **activation** of molecules **adsorbed** onto the **polymer surface**:

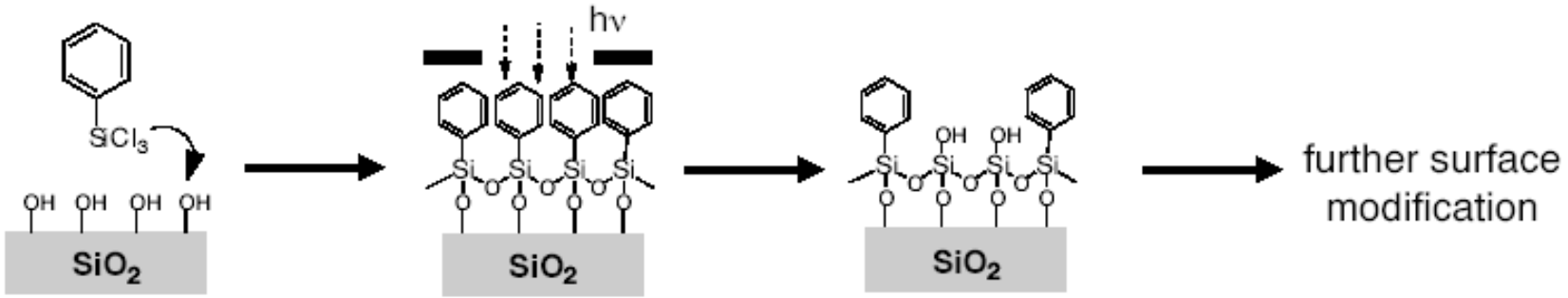


Surface Design

- **Spin coating** of a substrate with a **photosensitive polymer** layer as **mask** material for deposition or etching process, **exposure**, and **development**:



Direct patterning of **organic monolayer** by **deep UV** (DUV) irradiation through mask:



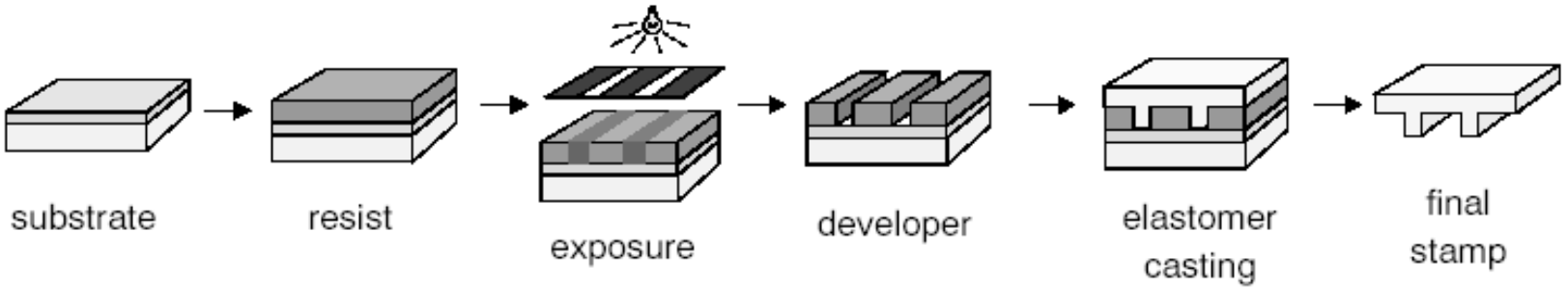
Lit.: Calvert et al., *Science* **1991**, *252*, 551–554

Surface Design

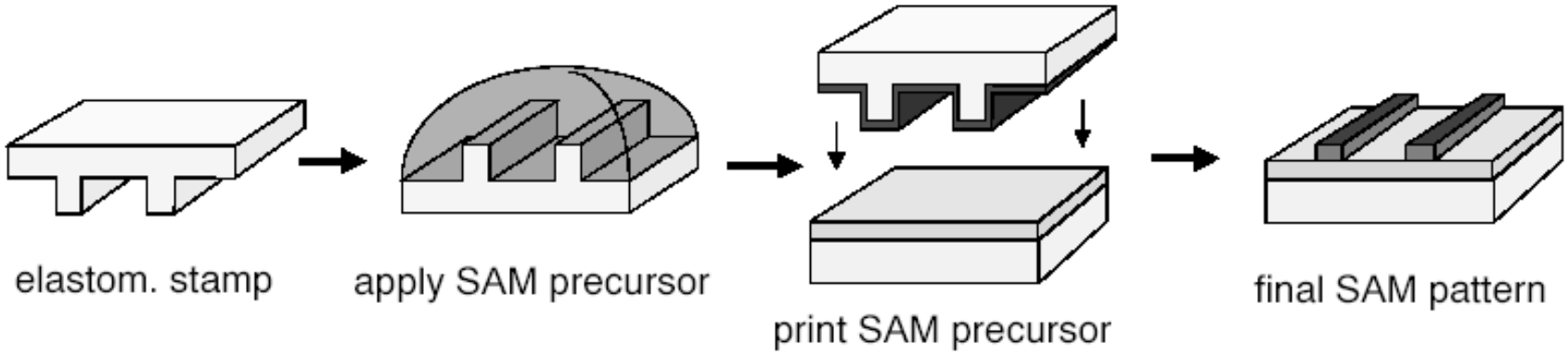
- **Transfer** of **SAM precursor** with elastomeric **stamp** onto substrate:

  ⇒ **master** generation by **photolithography** and similar techniques:

  – stamp is obtained by **casting** of **elastomer** (PDMS, e.g.) over **master**

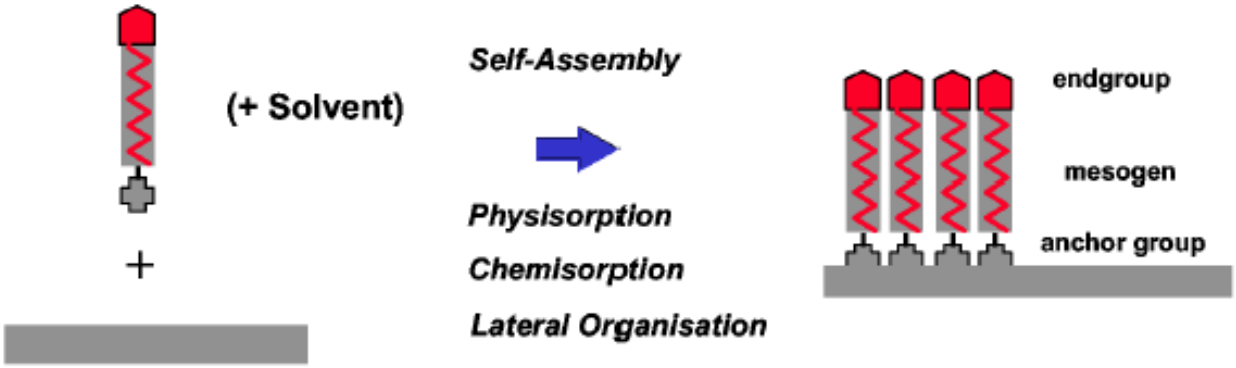


⇒ **pattern** generation by **stamping** of SAM precursor onto substrate:

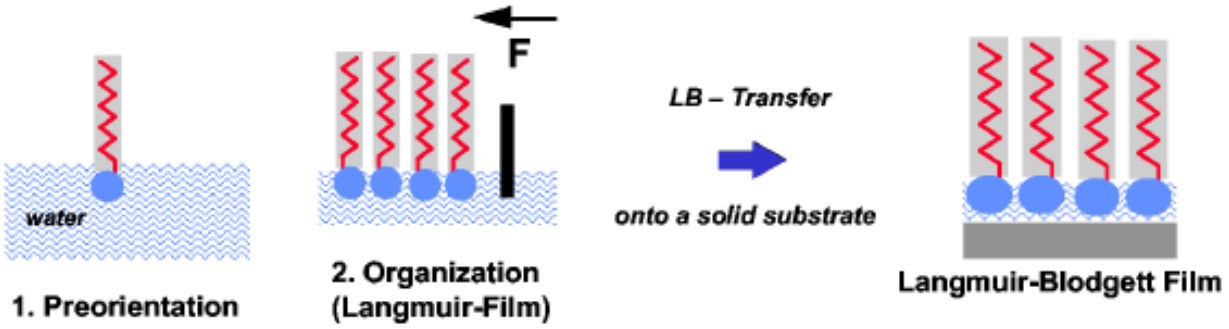


Surface Design

SAM (self-assembled monolayers): adsorption of molecules from solution onto solid substrates to form ordered molecular monolayers (e.g. alkylthiols on gold)



LB (Langmuir-Blodgett mono- and multilayers): transfer of molecules from the air-water interface onto solid substrates to form ordered molecular mono- and multilayers (e.g. phospholipids)



Surface Design

polymer brushes: polymer chains terminally tethered to a solid surface at high anchoring density → controls surface properties like tribological behavior, corrosion resistance or biocompatibility, e.g.

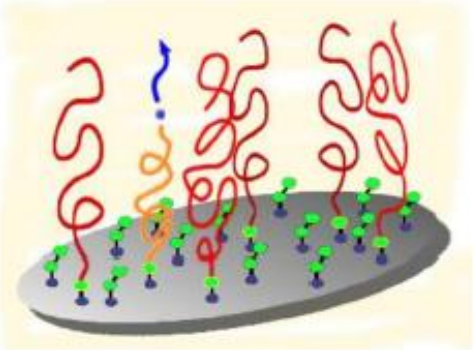
methods to generate polymers brushes:

a) "grafting from": the polymer is **grown from** initiator sites attached to the surface

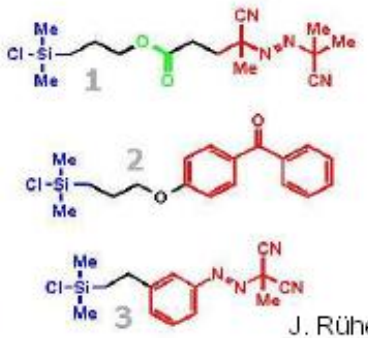
b) "grafting onto": the polymer chains are **attached to** reactive groups on the surface

depending on the solvent the modified surface is exposed to the polymer brushes swell (good solvent) or collapse into their coil state (bad solvent), limit between good and bad solvent is theta-solvent (interaction between solvent molecules,solvent / polymer, and polymer / polymer is balanced for a given temperature)

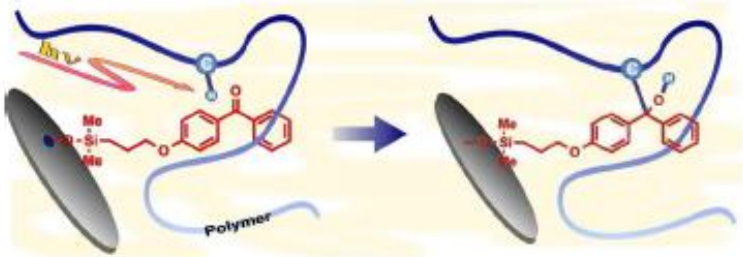
"grafting from"



silane initiators / anchors



"grafting onto"



J. Rühe; http://www.imtek.uni-freiburg.de/cpi/science/brushes/Brushes.htm

Activated Surfaces

Use the preceding techniques to add functional groups to the surface.

Examples are:

- Avidination / Biotinylation
- Epitopes (e.g. RGD for promoting cell adhesion)
- Plasma treatment (promotes protein adhesion)
- Adsorption of whole bioactive molecules (patterns)

Protein Resistant Surfaces

PolyEthylene Oxide (PEO) is a highly mobile, hydrophilic polymer that can be grafted onto a surface (or protein) to render resistance to adsorption. This is a very effective way to

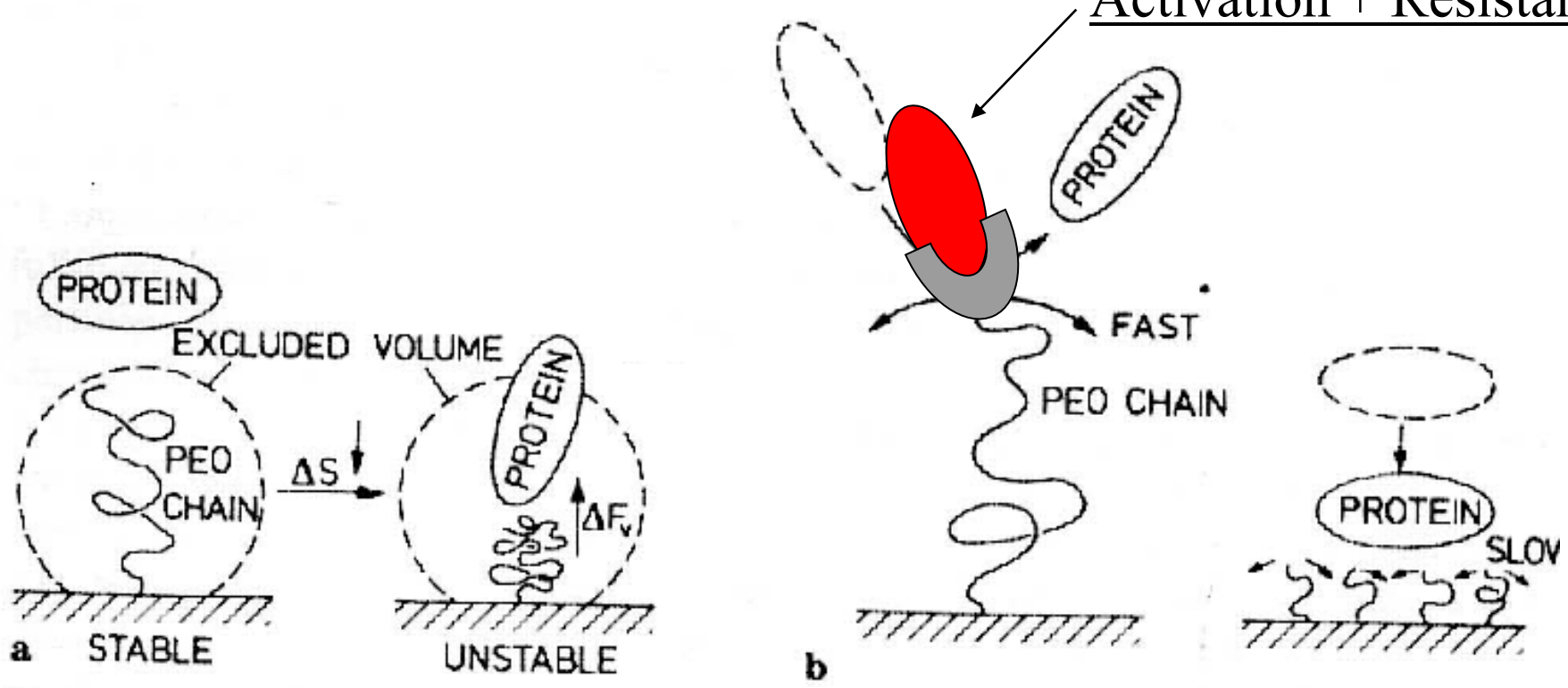


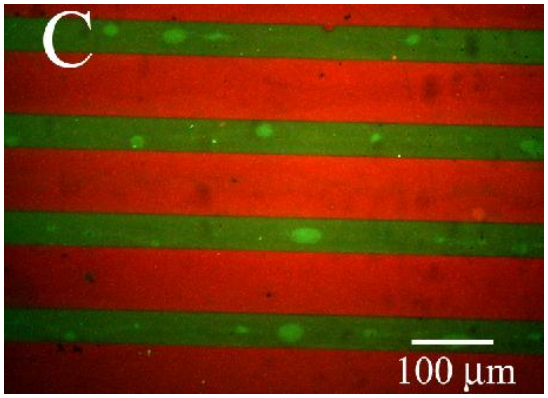
Fig. 25a and b. A protein resistant surface based on the steric repulsion argument commonly used in the colloid stability field [120]. The interaction between a polyethylene oxide grafted surface and a protein solution is shown. a. suggests an excluded volume or steric repulsion mechanism; b. the surface

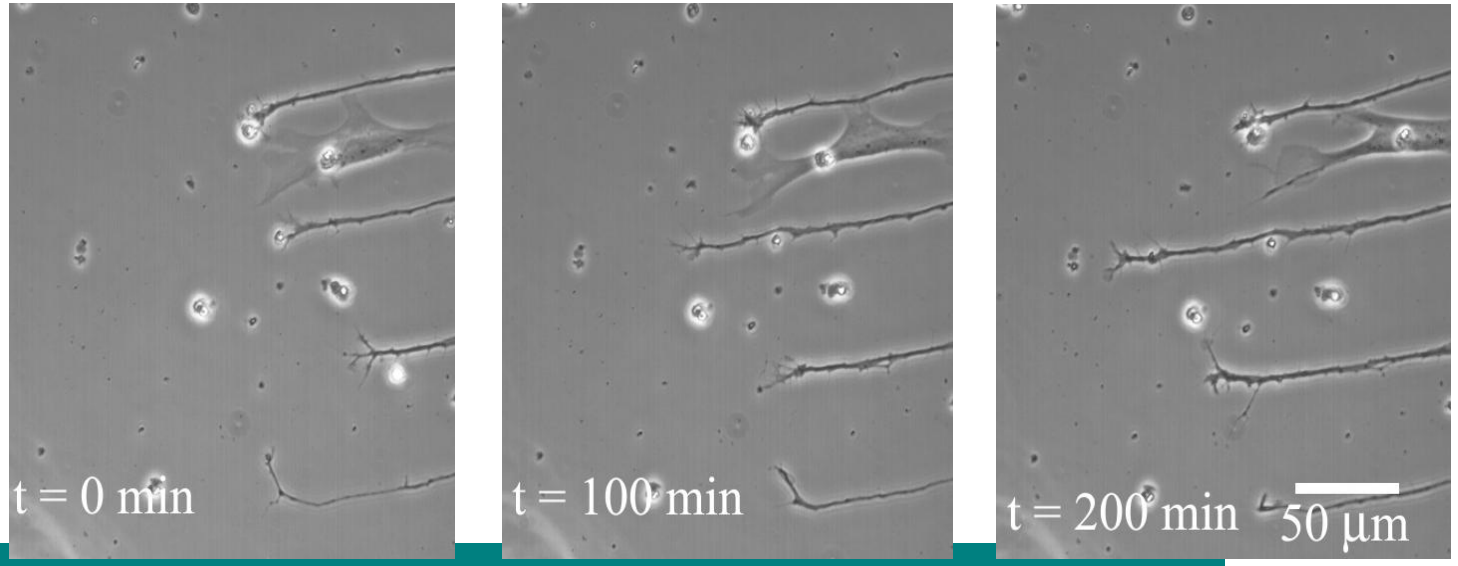
Tissue Engineering

Many tissue engineering design strategies rely on seeding a biomaterial construct with cells. Different strategies are then employed to get the cells to migrate, differentiate, and ultimately to develop into functional tissue.

Surface modification strategies employed include:

- Topographic modification (cell alignment)
- Spatial patterning of cell adhesive zones
- Integration of adhesion epitopes
- Switchable





Conclusions

Three points:

1 – Surfaces have unique properties

2 – We can (and do) measure these properties

3 – Because they affect biocompatibility

Thank You