9-25-2018

Polymer interfaces and biopharmaceuticals: Chemistry, designs and challenges

David W. Grainger
University of Utah, USA, david.grainger@utah.edu

Polymer interfaces and biopharmaceuticals: chemistry, designs and challenges

David W. Grainger, Ph.D.

Departments of Biomedical Engineering, and Pharmaceutics

University of Utah

Salt Lake City, UT, USA

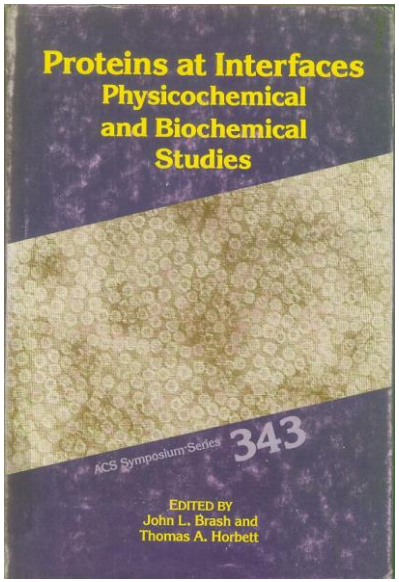
david.grainger@utah.edu

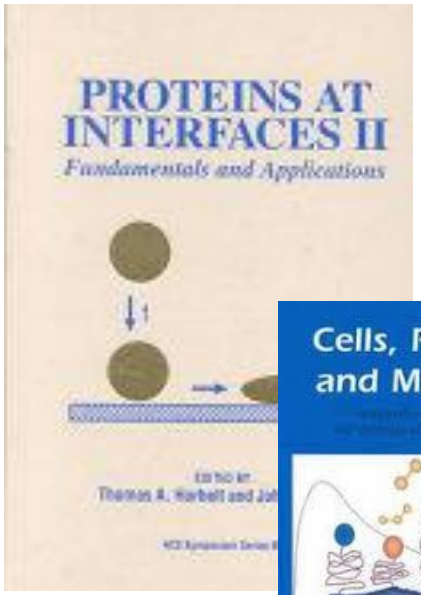
Single Use Technologies III
Snowbird, 2018

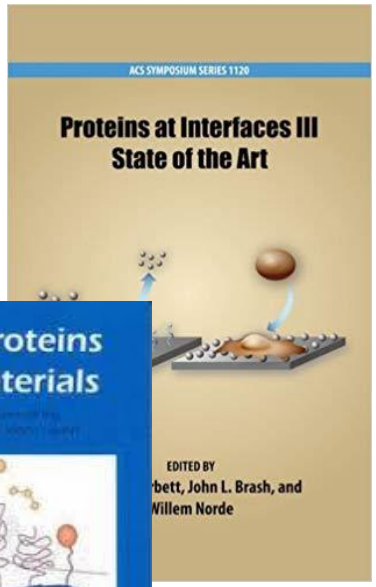
The University of Utah
Department of Pharmaceutics and Pharmaceutical Chemistry

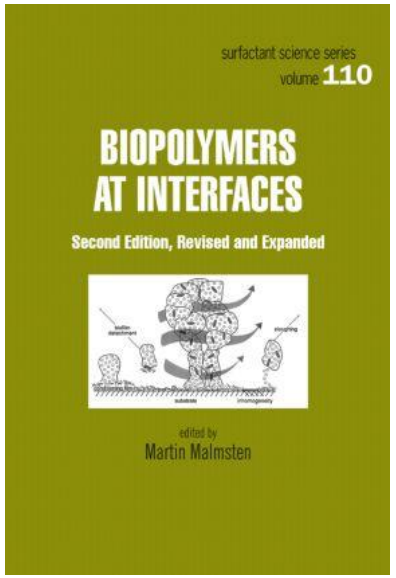
Department of
BIOMEDICAL ENGINEERING
COLLEGE OF ENGINEERING | THE UNIVERSITY OF UTAH

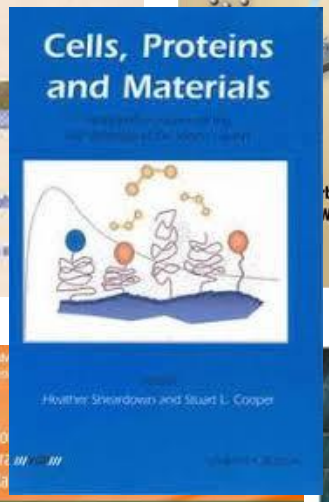
Polymer surface and biological fluids

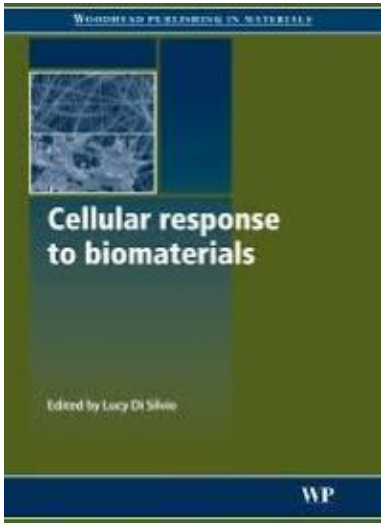


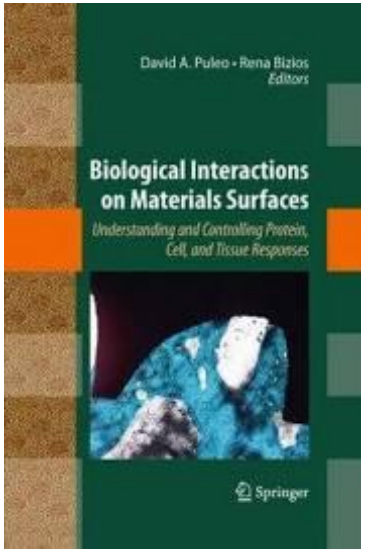


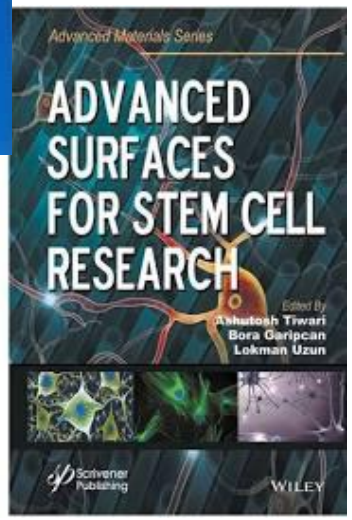


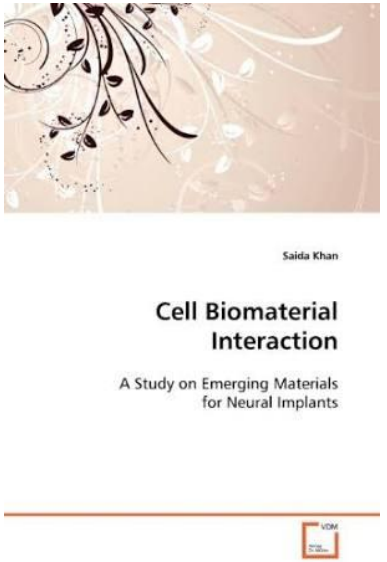




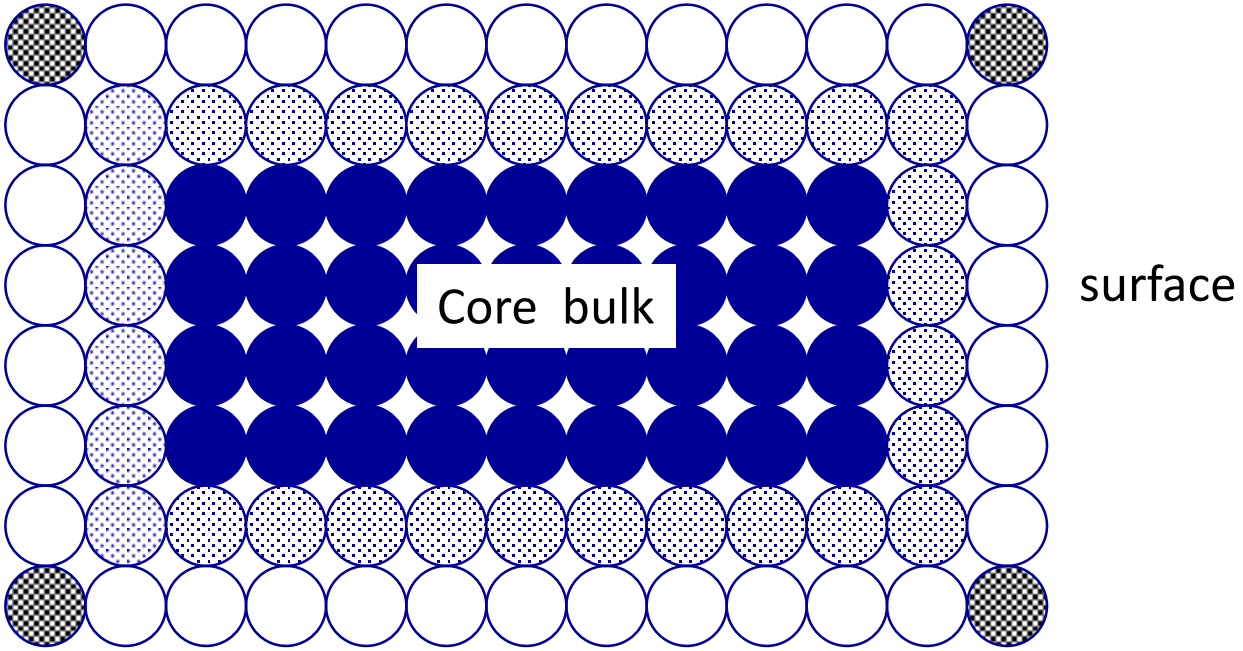








The surface (physics/chemistry) differs from the bulk



- ***Surface energies are different than bulk***
- ***Surface reactivities are different than bulk***
- ***Surface properties are different than bulk***

Defining and Characterizing Interfaces

- general points
  - surfaces are uniquely reactive
  - surface vs. bulk
  - surfaces readily contaminate
  - surface can be mobile
- surface parameters
  - roughness/topography
  - chemical composition
  - surface energy/wettability
  - crystallinity

What's so special about a surface?

Surface phenomena are driven by a reduction in surface (free) energy.

Biomaterials surfaces are sites of:

- *adsorption of a species from the environment*
- *surface segregation of a species from the bulk of biomaterial*
- *surface reconstructions/re-organization*
- *surface reactions*

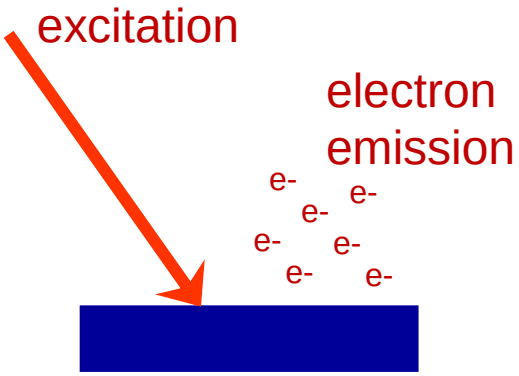
Define "Surface"

What information do you want?

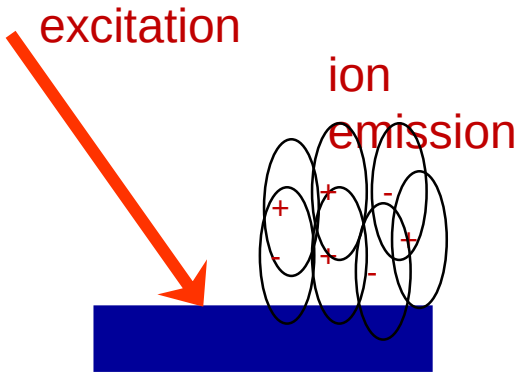
- **In most cases, cells and proteins, micro-organisms respond to outer atomic layers (~3nm) or first few monolayers) of surface**
- **Spatial resolution of method**
- **Topographical information**
- **Gradient between surface and bulk**

Lots of expensive toys and tools for analysis

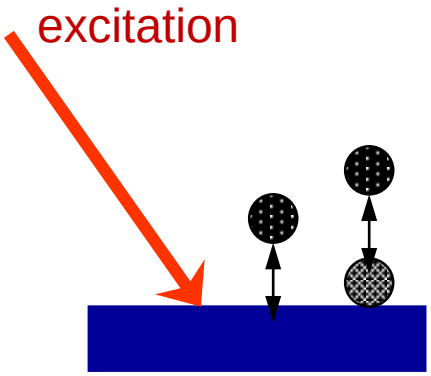
Electron Spectroscopies



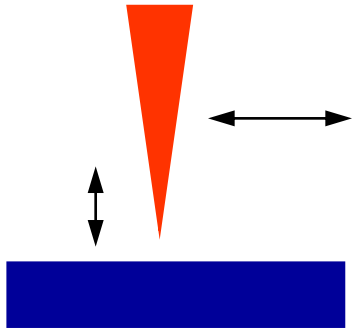
Ion Spectroscopies



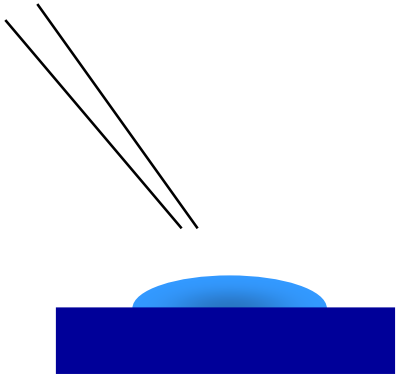
Vibrational Spectroscopies



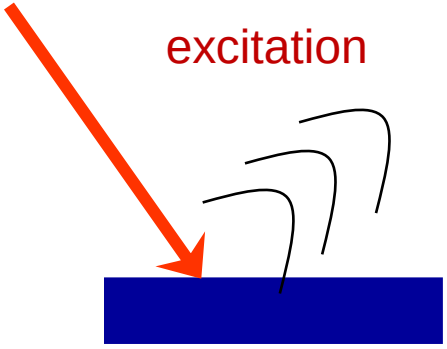
Scanning Probe Microscopies



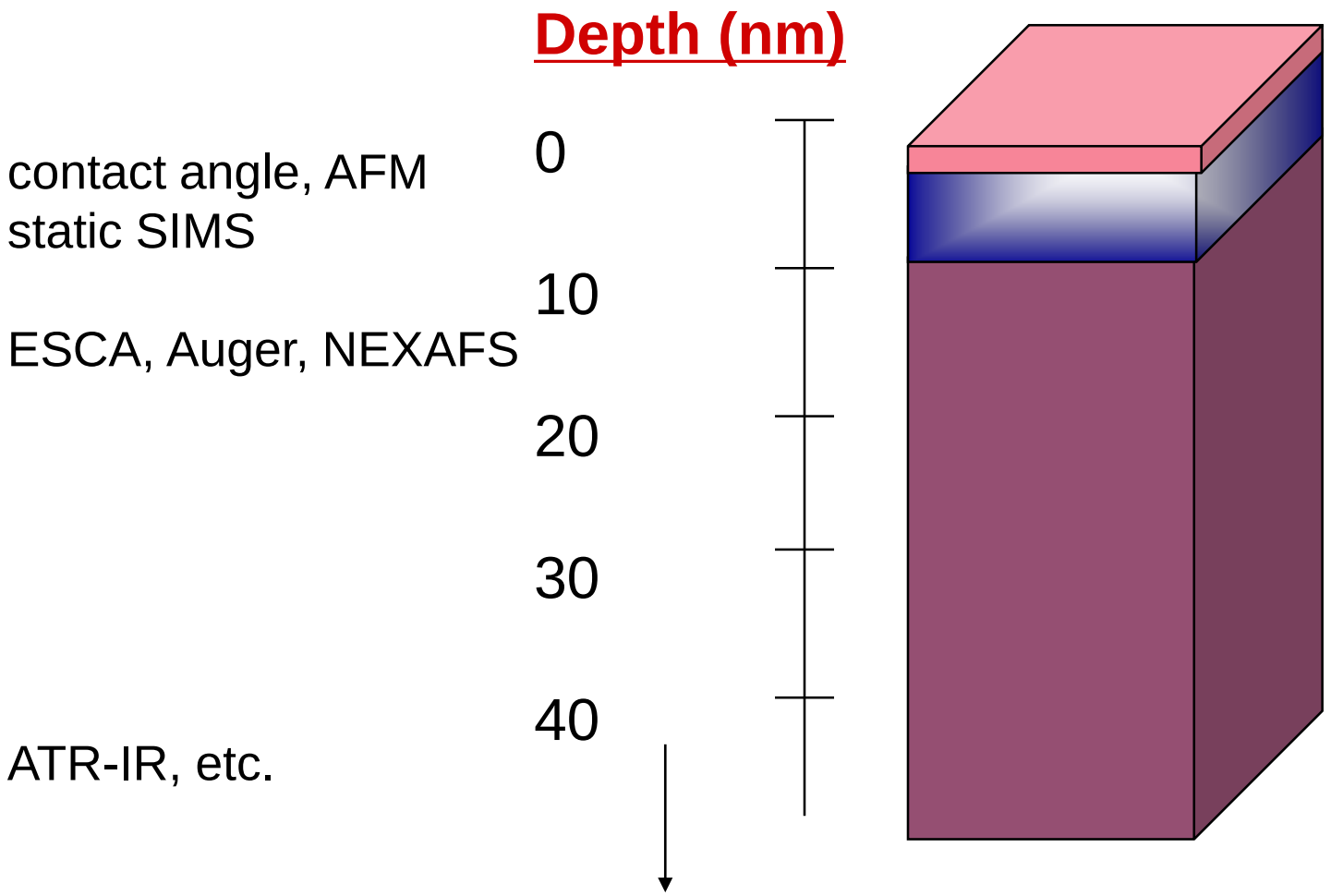
Contact Angle Methods



Diffraction Methods



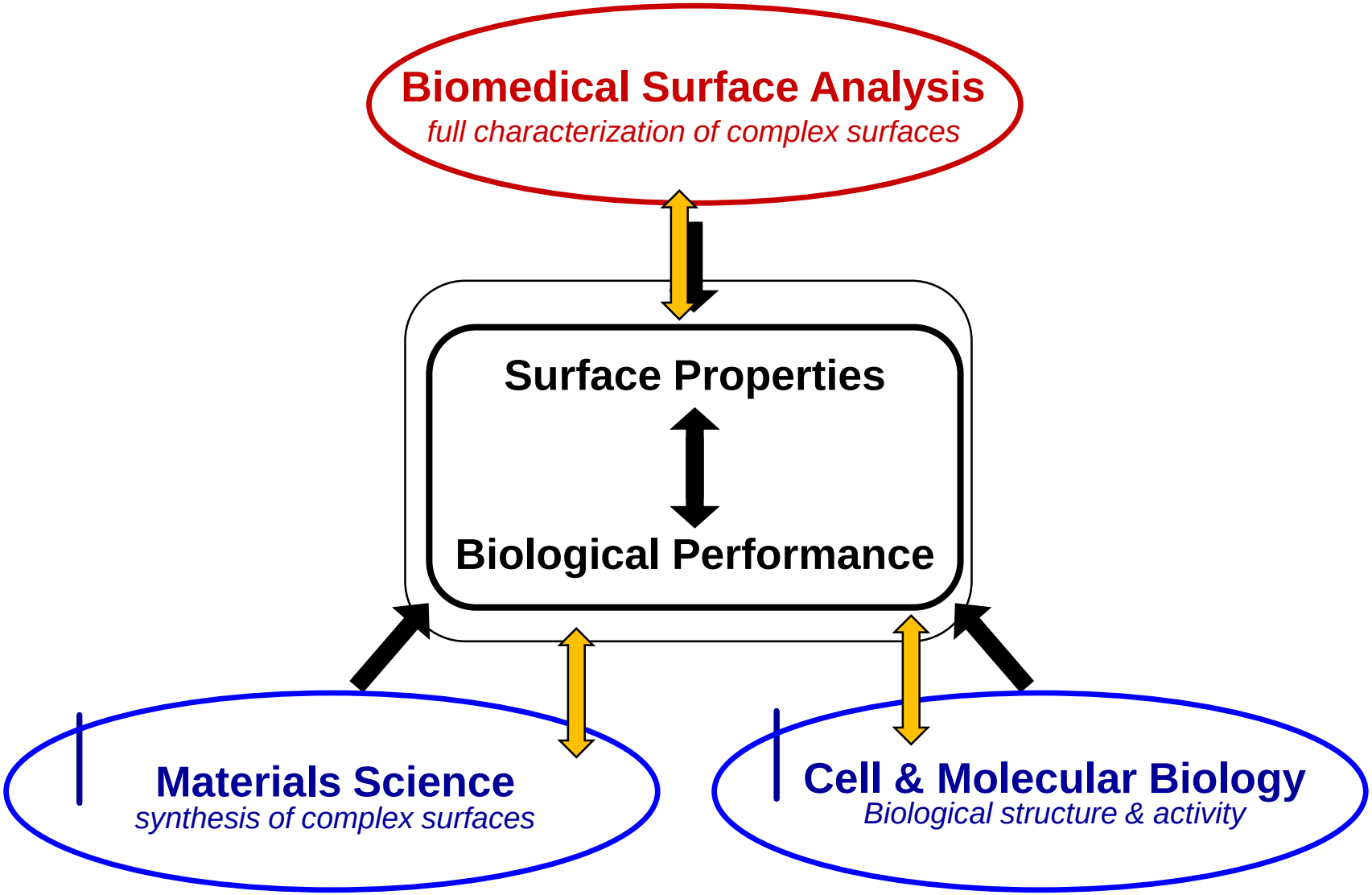
No one technique does it all!!



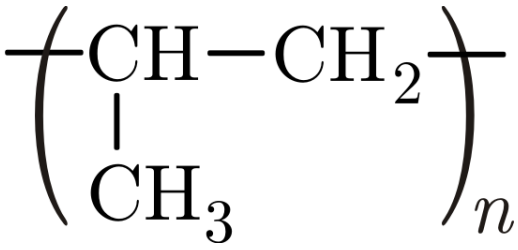
A single technique will provide an answer …..

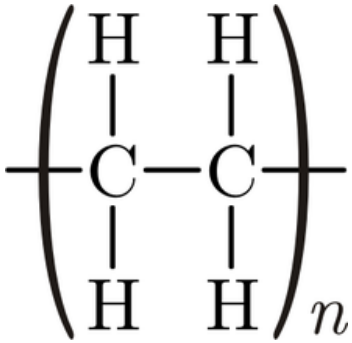
but it might not be the correct answer!

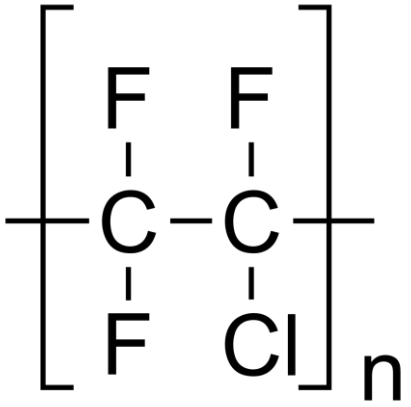
Heuristics and algorithms to provide ideas



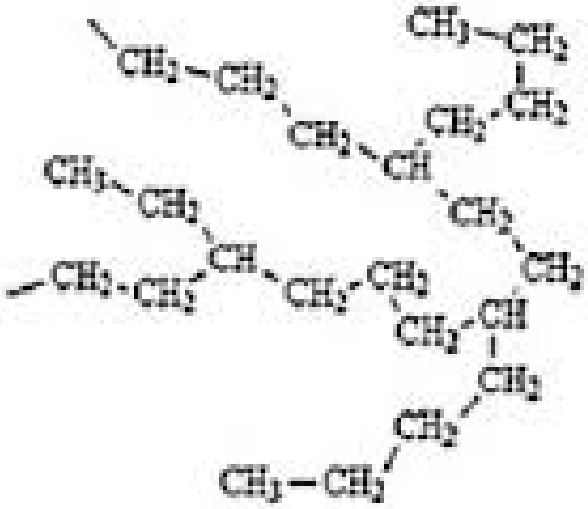
What is your surface?



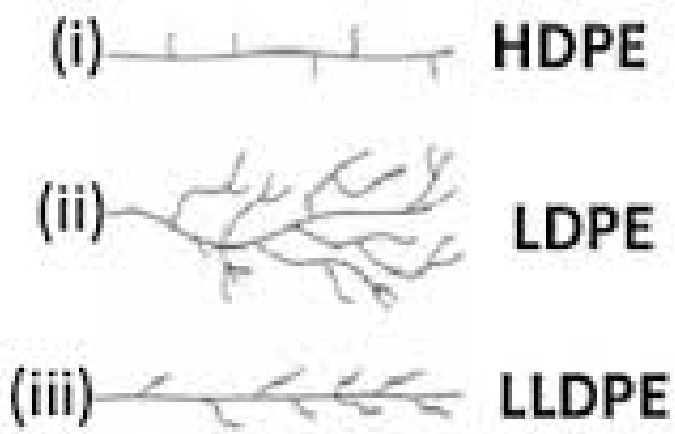




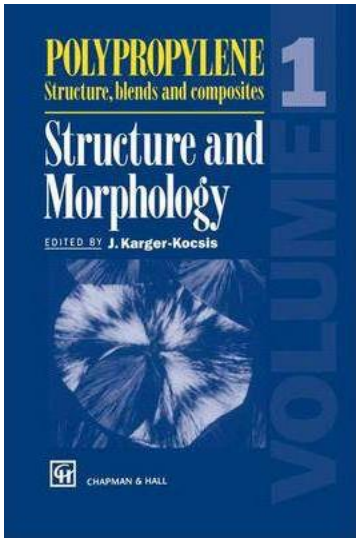
atactic? syndiotactic, isotactic?



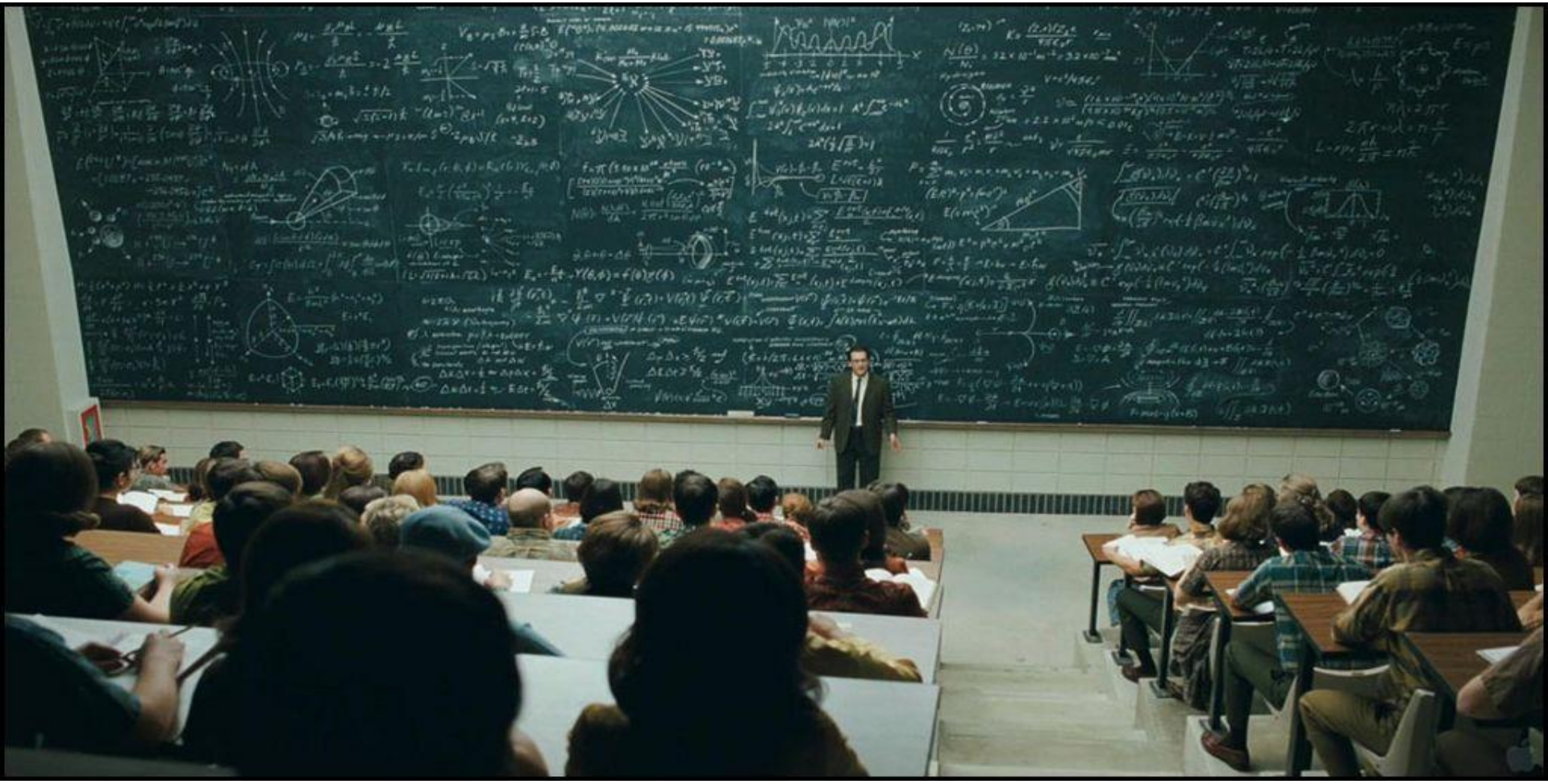
(a)



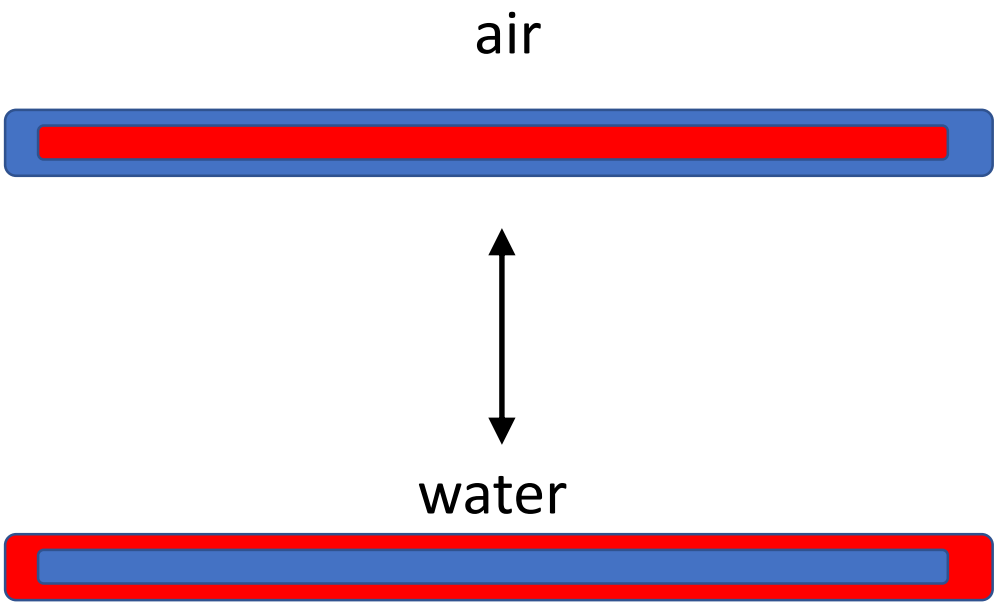
(b)



For biomedical applications, it's complicated……



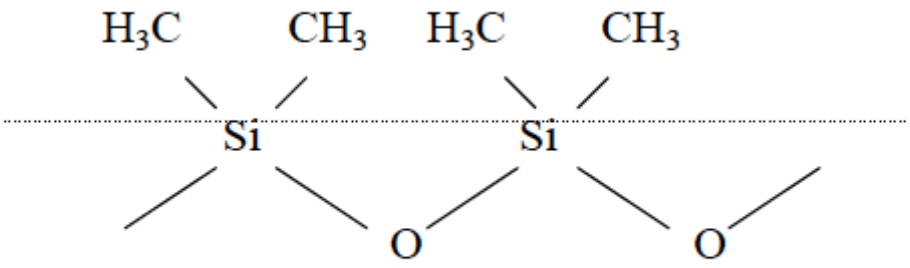
Surface mobility and re-arrangement



Reorientation of polymer chains: water versus air (PDMS)

classical example: silicon rubber, **PDMS**

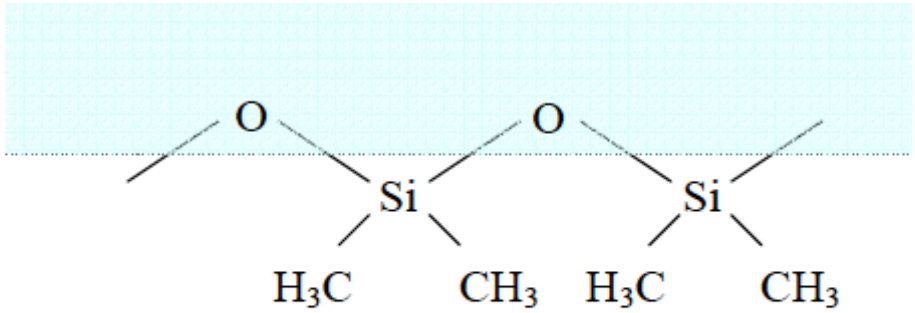
PDMS in air



$\gamma_{PDMS} = 20$ dyn/cm

($\gamma_{CH3} = 23$ dyn/cm)

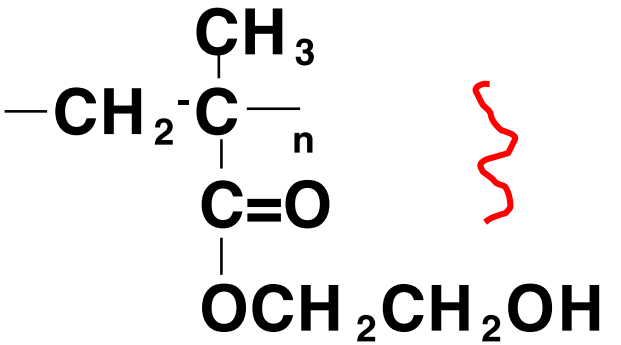
PDMS in H_2O



Demonstrating polymer mobility using XPS

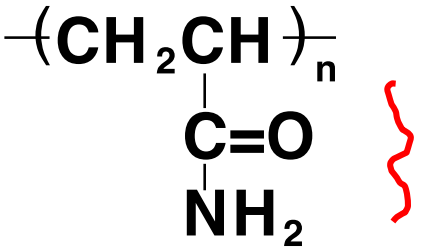
Ratner et. al. J. Appl. Polymer Sci. 22 643 (1978)

Radiation grafted layers of poly(HEMA) and polyacrylamide (>1 μm thick) on silicone rubber core

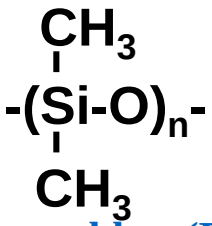


poly(2-hydroxyethyl methacrylate)

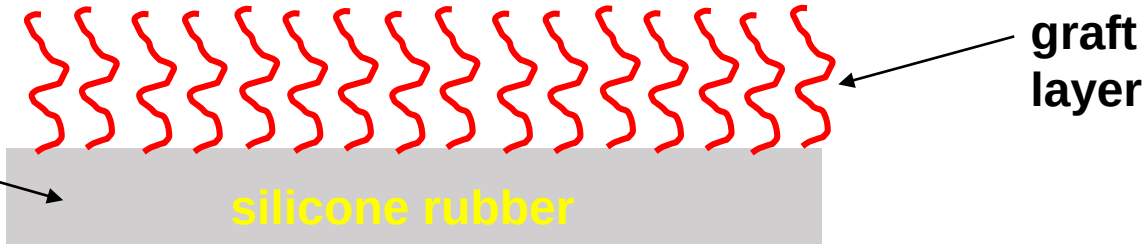
PHEMA



Polyacrylamide



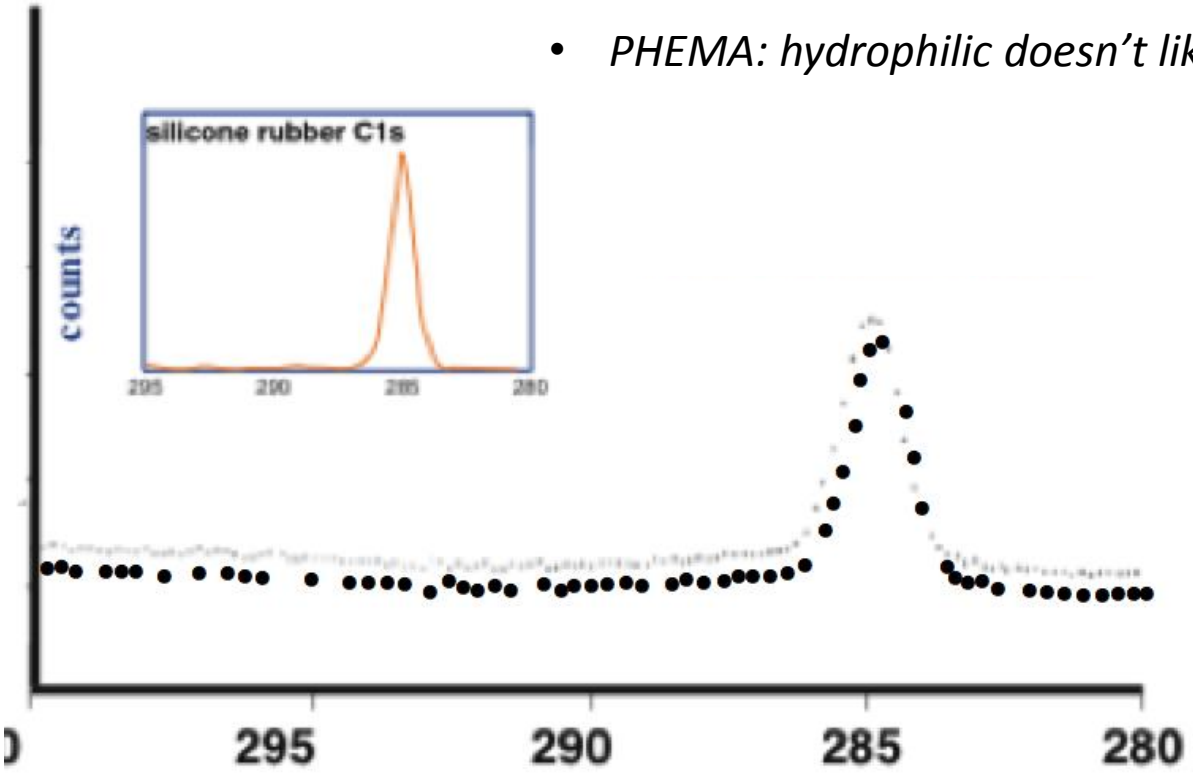
Silicone rubber (PDMS)

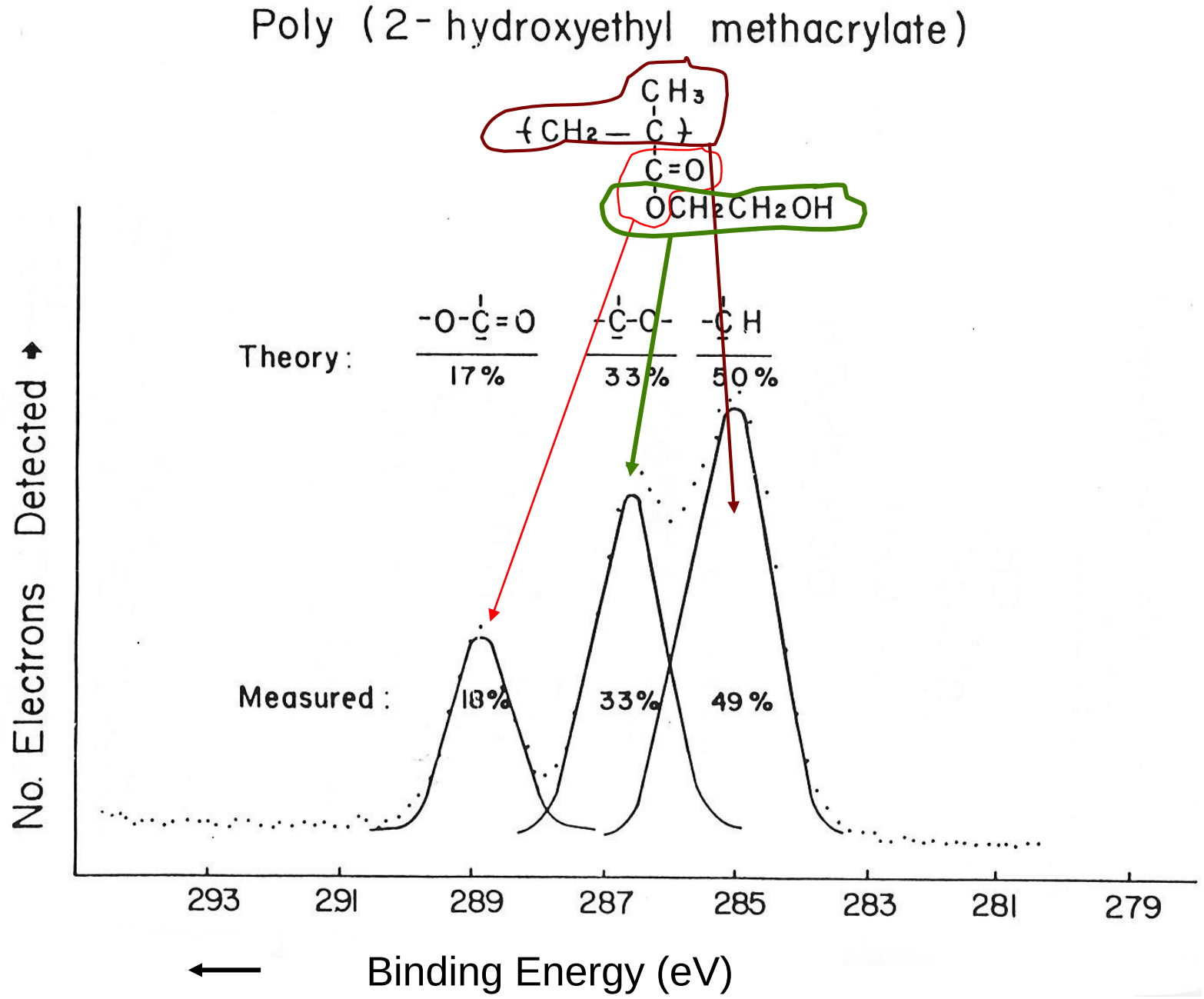


ESCA examination at room temperature showed only PDMS?

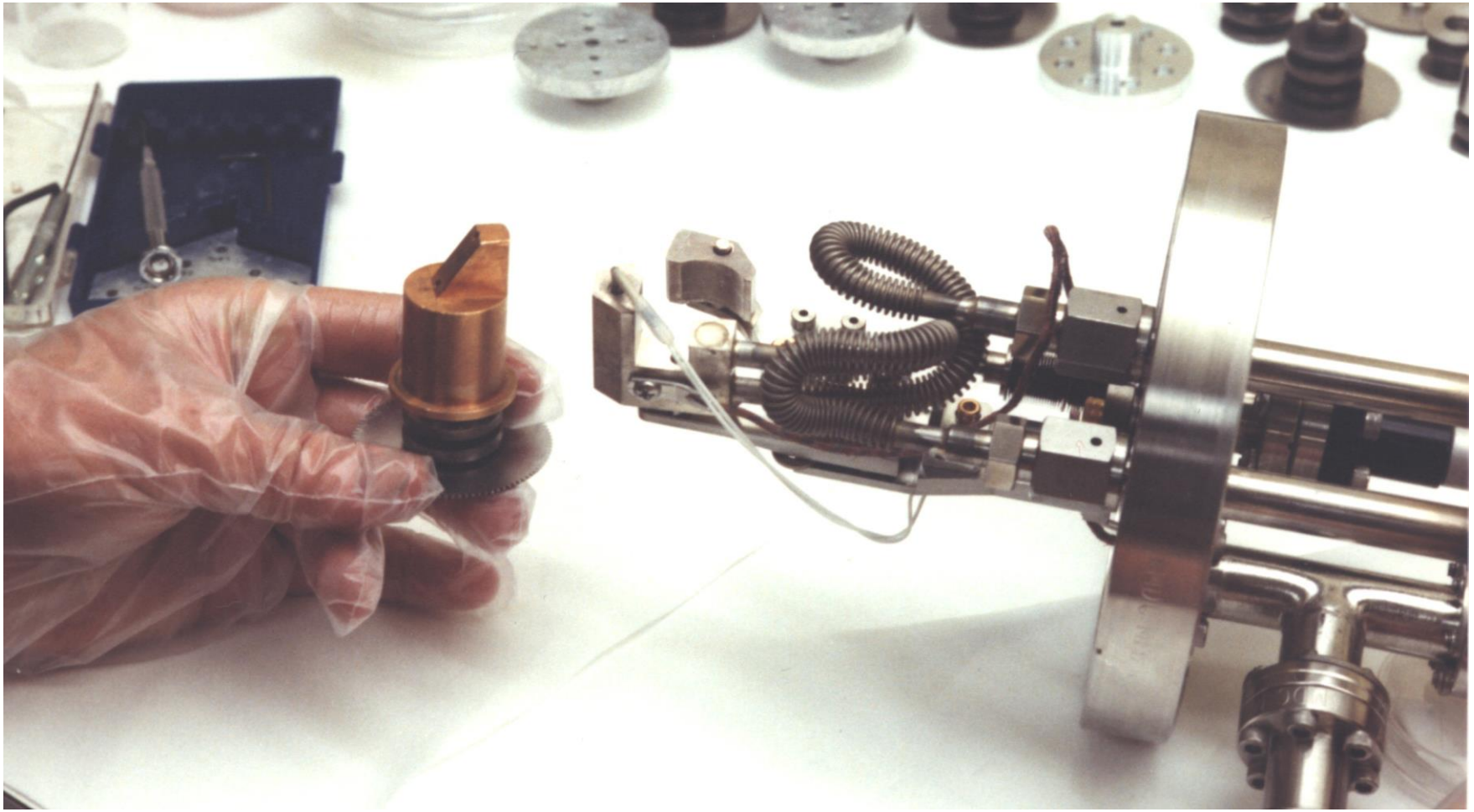
Initial XPS spectra under vacuum looked like pure silicone rubber

- *Silicone rubber: hydrophobic – likes air*
- *PHEMA: hydrophilic doesn't like air or vacuum*




Poly (2-hydroxyethyl methacrylate)
CH3
(CH2 — C)
C=O
OCH2CH2OH
Theory:
-O-C=O
17%
-C-O-
33%
-C H
50%
Measured:
18%
33%
49%
No. Electrons Detected
293
291
289
287
285
283
281
279
Binding Energy (eV)

Install a cold stage on the ESCA/XPS instrument



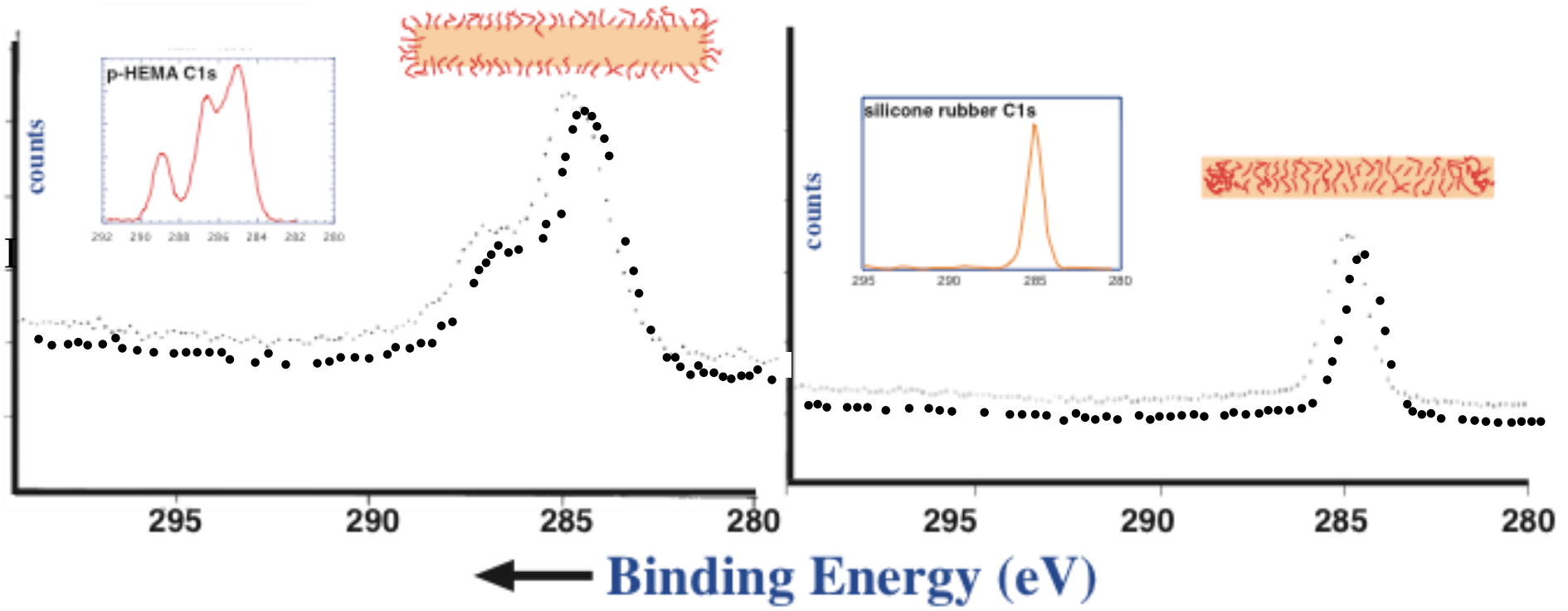
Frozen hydrated samples can be studied

Polymer surface mobility: cold stage XPS/ESCA

Poly(hydroxyethyl methacrylate) grafted to silicone rubber (frozen-hydrated, -120 deg. C.)

⇌

Poly(hydroxyethyl methacrylate) grafted to silicone rubber (dry air - ambient temperature)



same study done with an acrylamide graft (reversal seen); also grafted on polyethylene (no reversal)

Ratner,BD; et al., (1978): Radiation-grafted hydrogels for biomaterial applications as studied by the ESCA technique. *J. Appl. Polym. Sci*. 22, 643-664.

Polymer surface mobility by ESCA

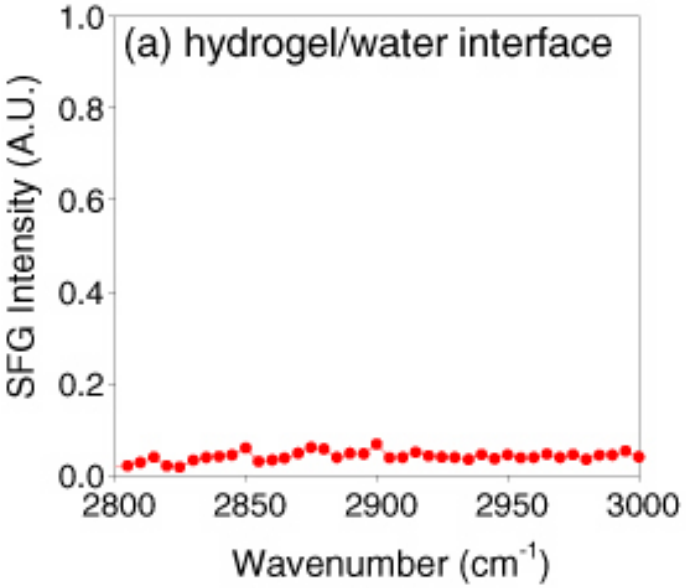
Ratner et al., J. Appl. Polym. Sci. 22, 643-664

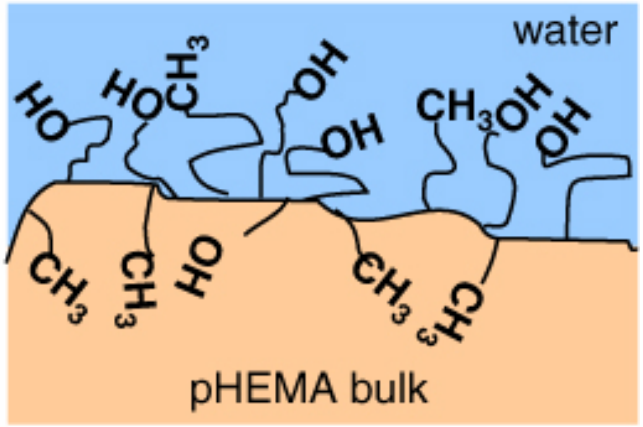
Considerations for this study

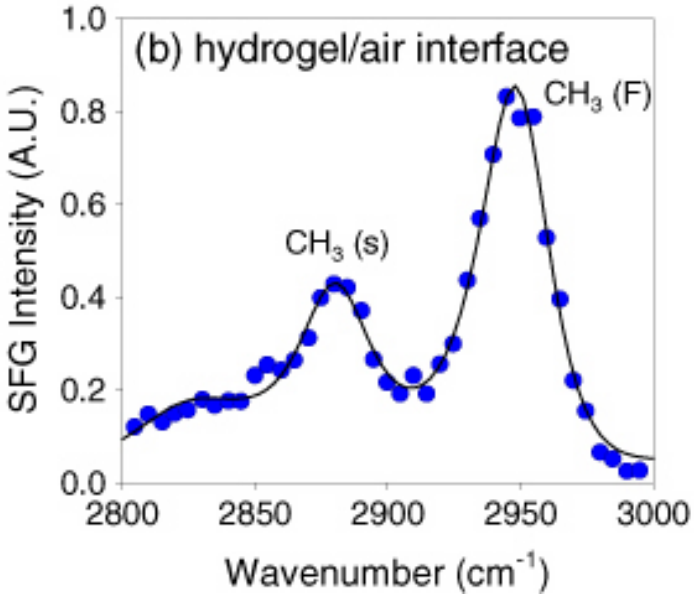
1. Vacuum pumping on frozen samples (particularly with ion pumps)
2. Molecular mobility and glass transition temperature of polymers (also, did the grafted layer migrate into water or did the silicone migrate into air?)
3. Penetration depth and graft layer thickness

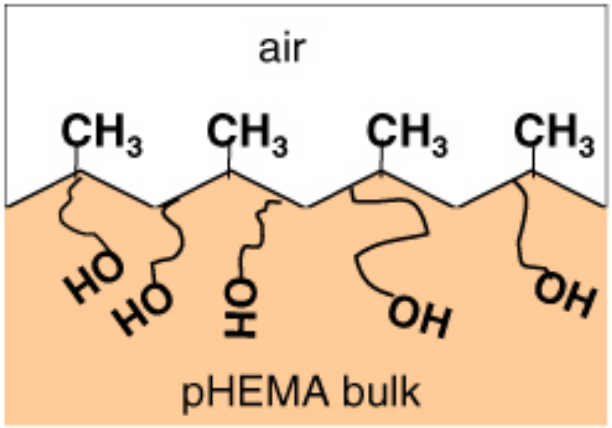
More polymer mobility: hydrogels

Sum Frequency Generation (SFG) technique



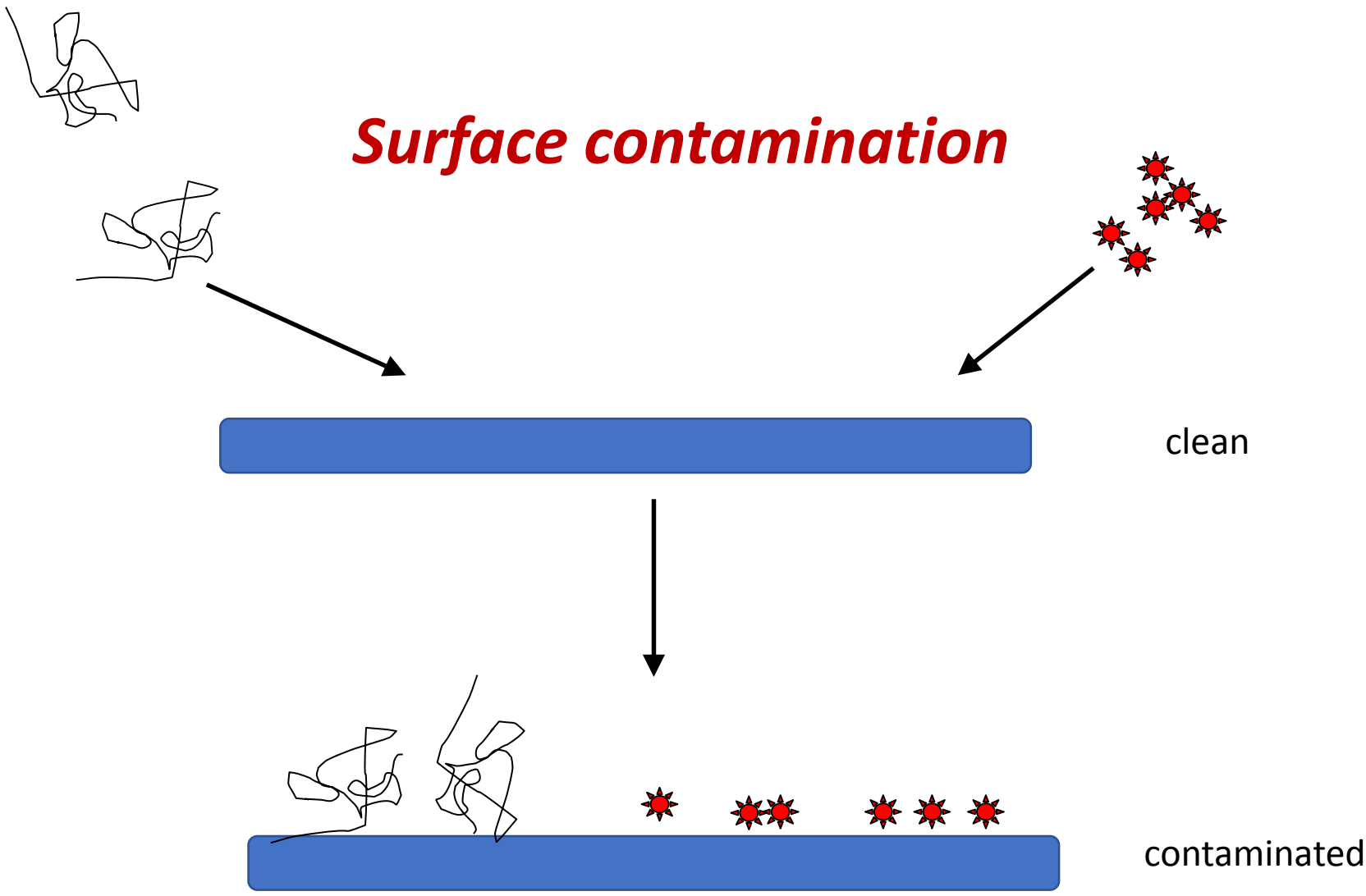






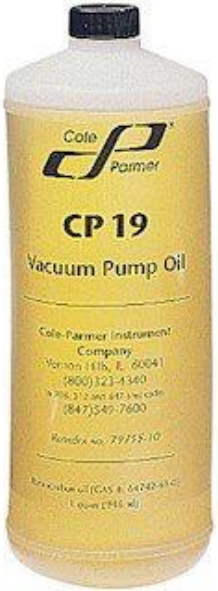
A. Opdahl et al., J. Phys. Condensed Matt., 16(21) (2004).

Surface contamination

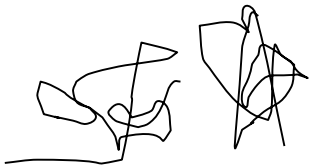


Common lab surface-active agents = ubiquitous surface contaminants

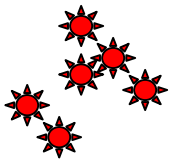
Glassware, pump oil



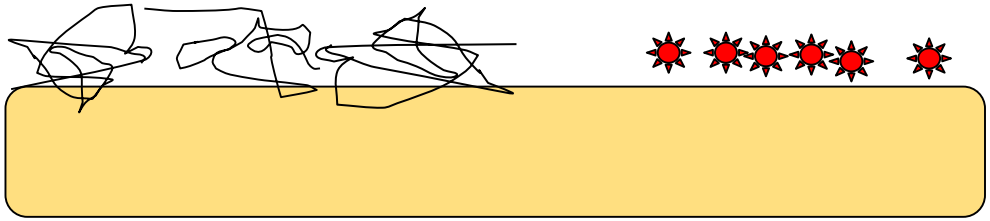
silicones



phthalates



adsorbed contaminant overlayers

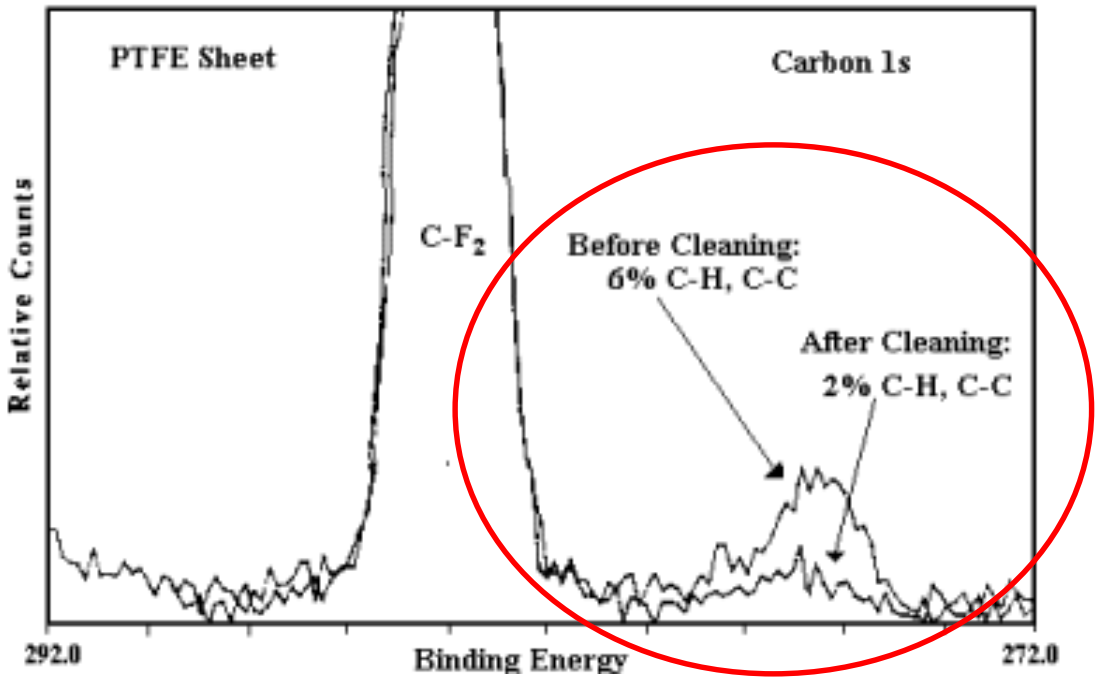


t= seconds-minutes

Table 1. Principal outgassed species from a post-cured silicone rubber compound.

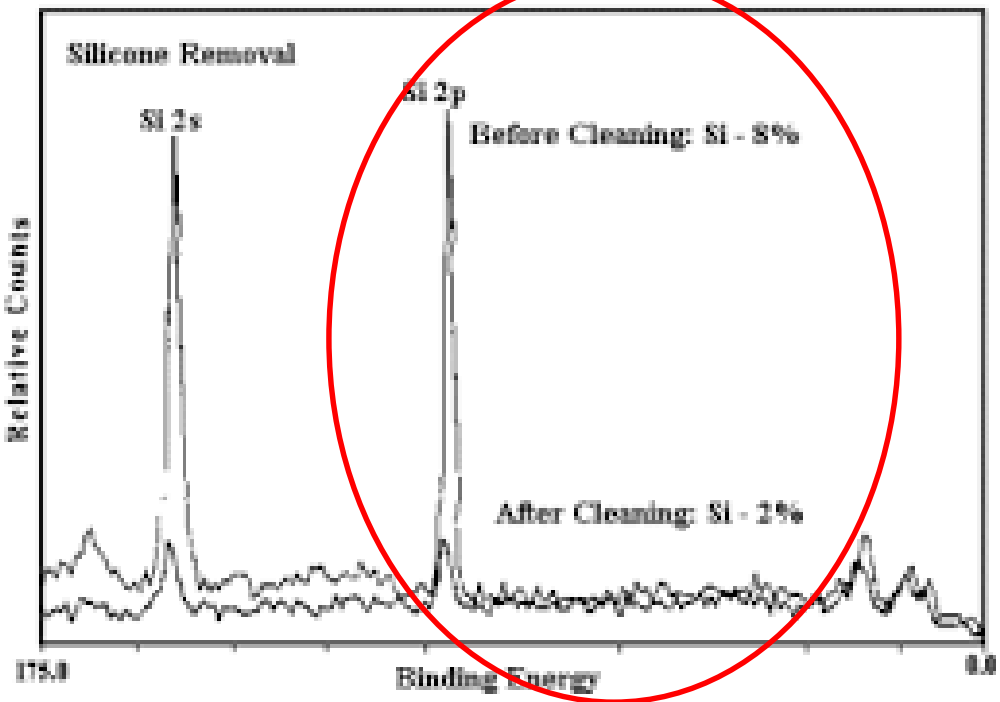
Component	Probable Source
cyclohexasiloxane	LMW silicone fluid
phenyl benzoate	catalyst byproduct
linear hexasiloxane	LMW silicone fluid
linear pentasiloxane	LMW silicone fluid
propanoic acid ester	catalyst byproduct
diethylphthalate	pigment wetting agent
Cx hydrocarbons	Silane coupling agent
cyclopentasiloxane	LMW silicone fluid
biphenyl hydrocarbon	catalyst byproduct
linear heptasiloxane	LMW silicone fluid
Cx aldehydes	catalyst decomposition product
cyclotrisiloxane	LMW silicone fluid

https://imageserv5.team-logic.com/mediaLibrary/99/Outgassing_20of_20Silicone_20Elastomers.pdf



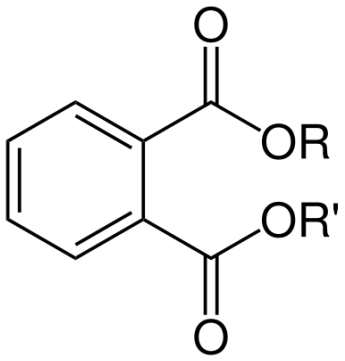
We can see silicon on almost every surface under XPS, even when it's not supposed to be there!

Ambient phthalates in air are used to calibrate mass spec instruments!



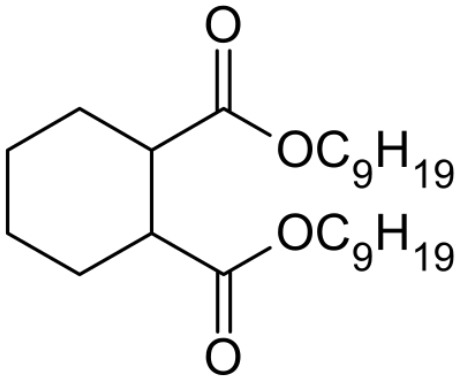
Common plasticizing agents

Phthalate esters



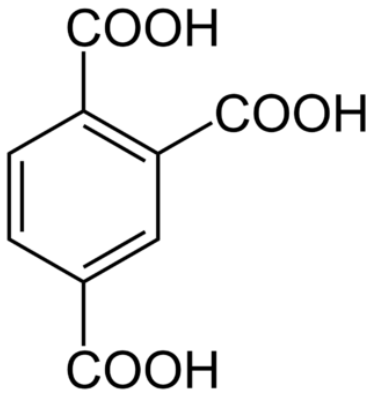
1,2-cyclohexane dicarboxylic acid diisononyl ester

Hexamoll DINCH

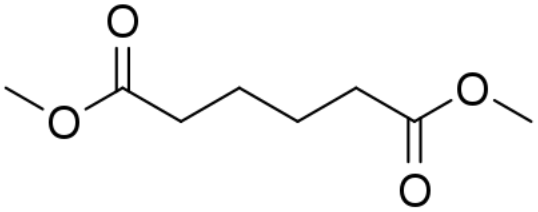


inert organic materials with high boiling points and low vapor pressures.

Trimellitic acids/esters



Adipate salts and esters



Mechanism of plasticization

External:

Small molecules that "get between' polymer chains in amorphous thermoplastics to disrupt polymer-polymer interactions, lower Tg, act as lubricants to allow chain motions

Internal:

changing polymerization chemistry (copolymerization) to introduce polymer chain structures that disrupt chain-chain interactions and lower Tg. More porous, less cohesive structure, more flexible. Formable.

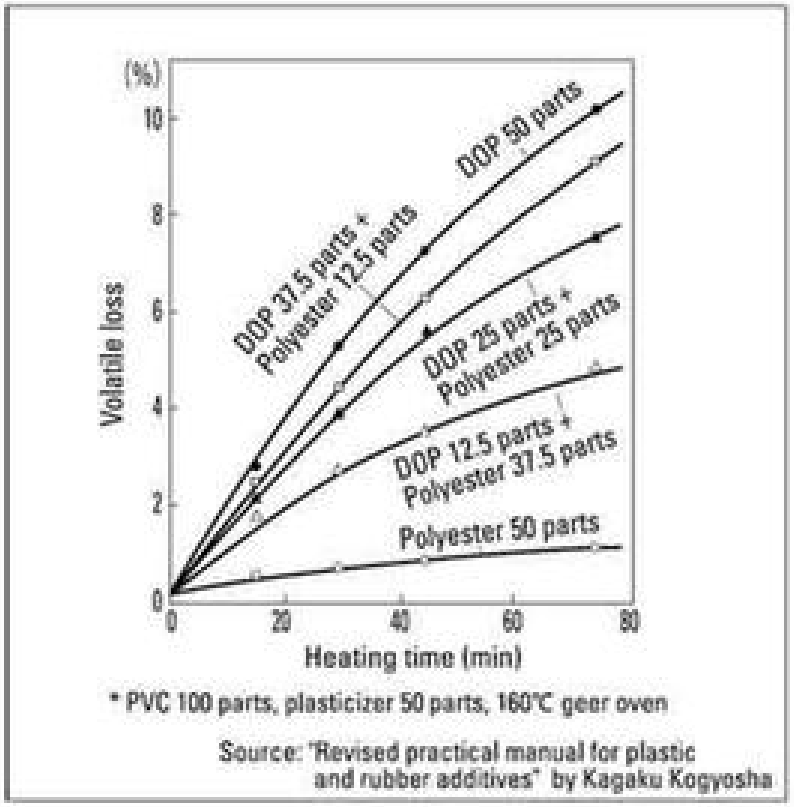
Desired Result: eliminate brittle, stiff character, deform at lower bending or tensile forces, imparting flexibility,

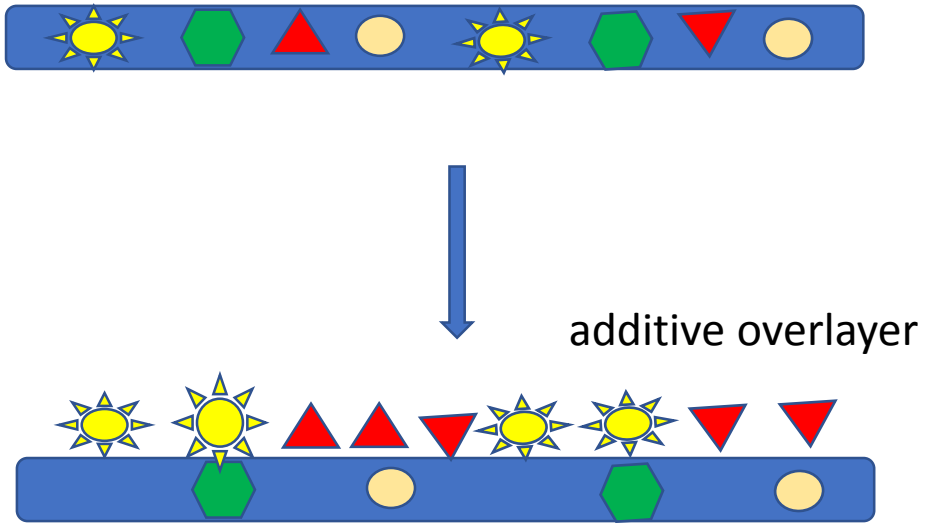
Additive bleed to surfaces:

Small molecule and oligomer additives bleed to polymer film surfaces

- ***Dyes, antioxidants, plasticizers, fillers, oligomers etc.***

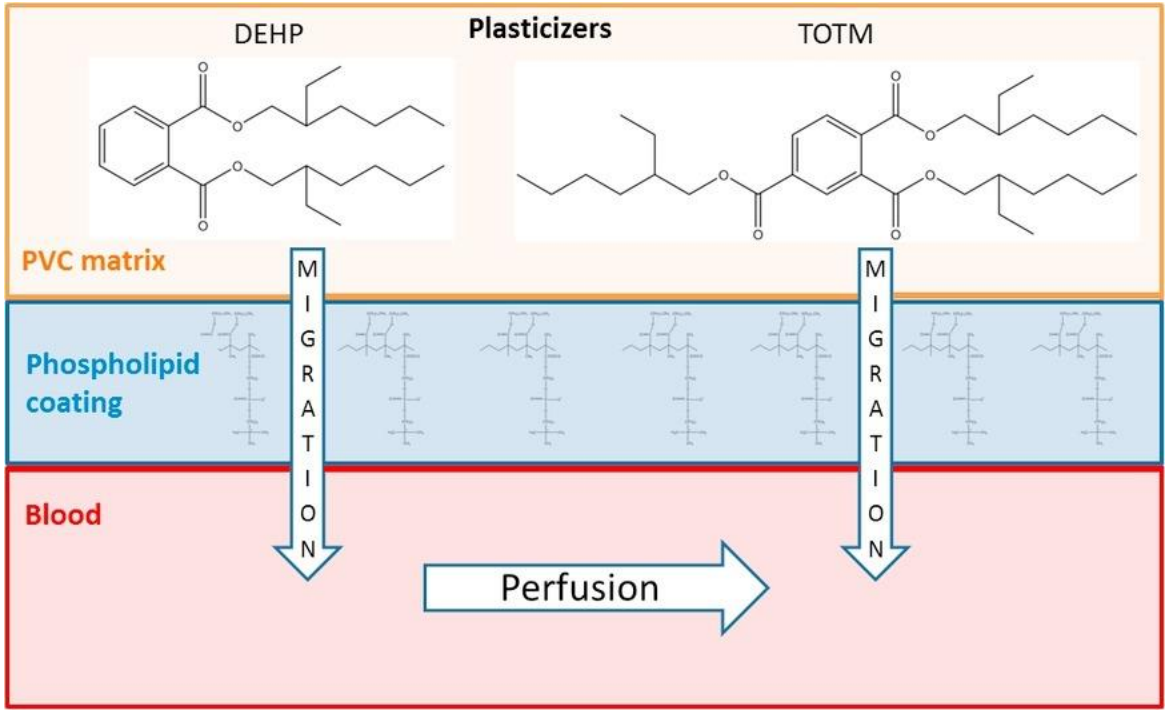
Your polymer <u>may not</u> be presenting the surface chemistry you think due to bleed.





http://www.pvc.org/en/p/property-modification-of-pvc-products

Münch et al., *Chemosphere* 202 (2018) 742e749



"Migration behavior of both DEHP and TOTM was slightly, even though not significantly, increased by the anti-coagulation coating"

PDMS elastomer bleeds oligo-PDMS continuously to its surface, unless extracted in solvent or oxidized using plasma.

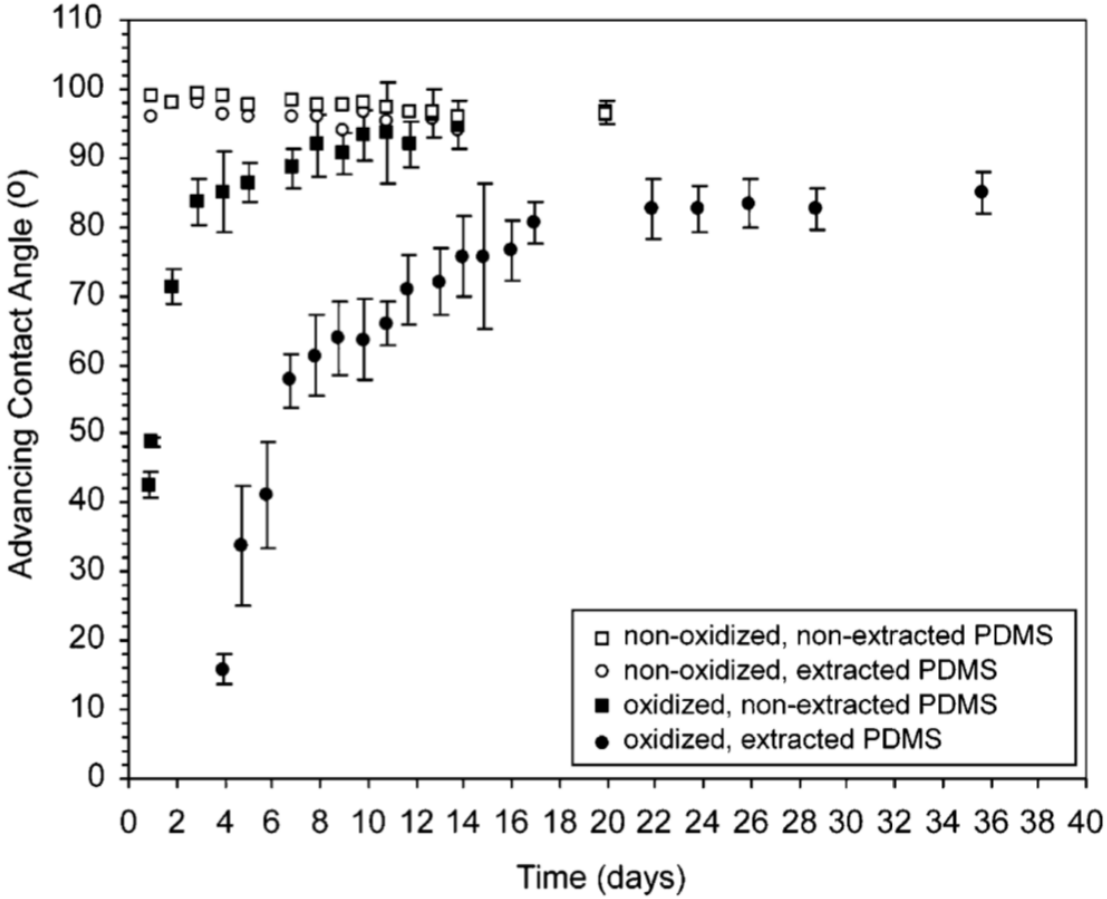
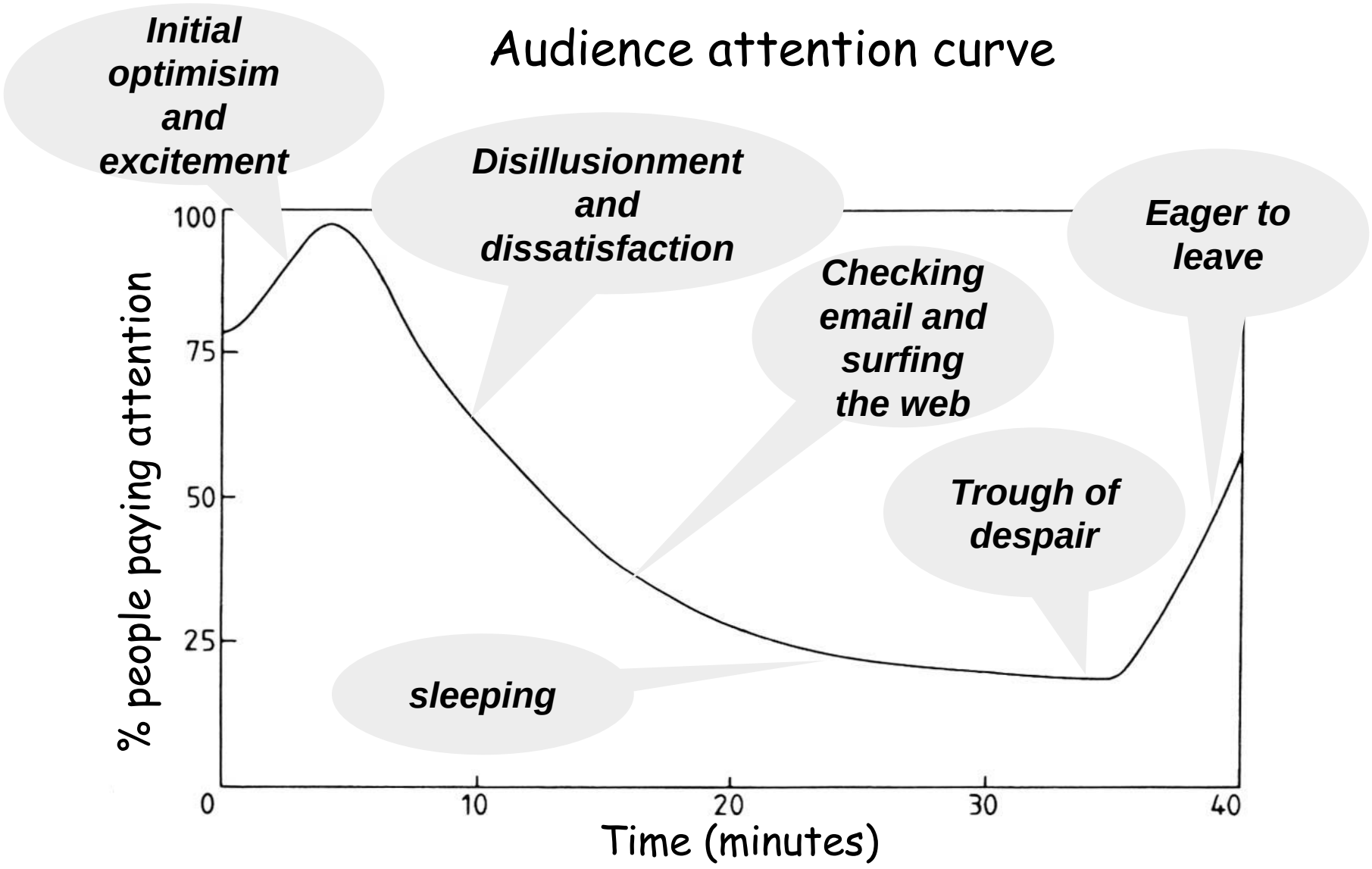


Figure 2. Advancing contact angle measurements of water on PDMS surfaces that were extracted or nonextracted and oxidized or nonoxidized. Surfaces that were extracted and oxidized remained hydrophilic in air for days; surfaces that were not extracted and oxidized regenerated the hydrophobic surface within hours. Error bars for the oxidized surfaces are shown and give an error of ±1 standard deviation (sample size $N = 30$). Surfaces that were not oxidized (either extracted or not extracted) remained hydrophobic. An average error of ±2° (1 standard deviation) was measured for these surfaces; error bars were omitted on the graph for clarity.

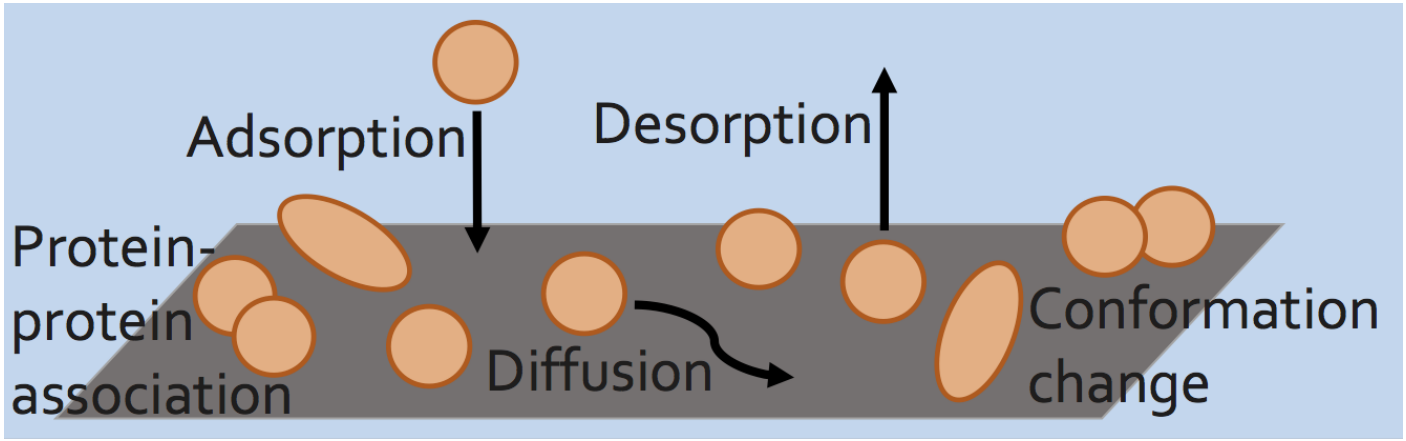
Anal Chem 75, 2003 6548

Audiences have limited attention spans



Surface

Biological milieu



Let's look at protein interfacial behavior:

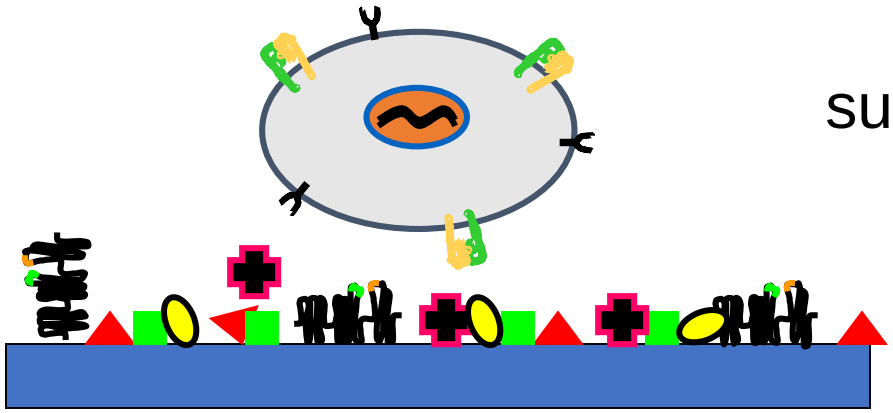
- ***non-specific adsorption (all proteins, all surfaces)***

- ***specific immobilization (desired proteins on certain surface locations)***

Protein surface adsorption is a long story with a long history of study but few technical solutions for serious problems:

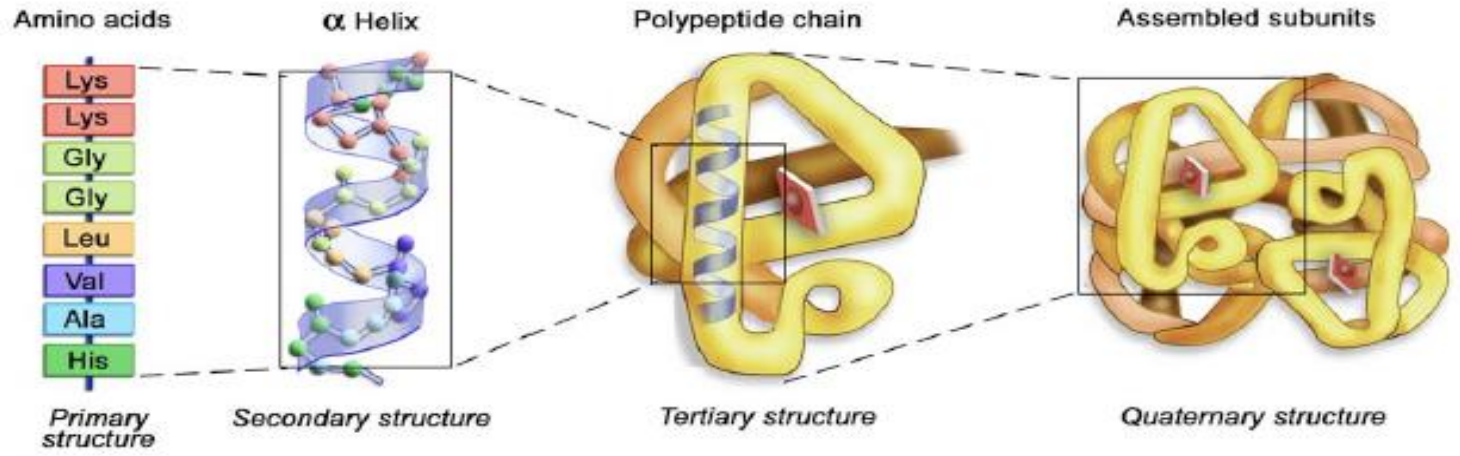
Protein Adsorption

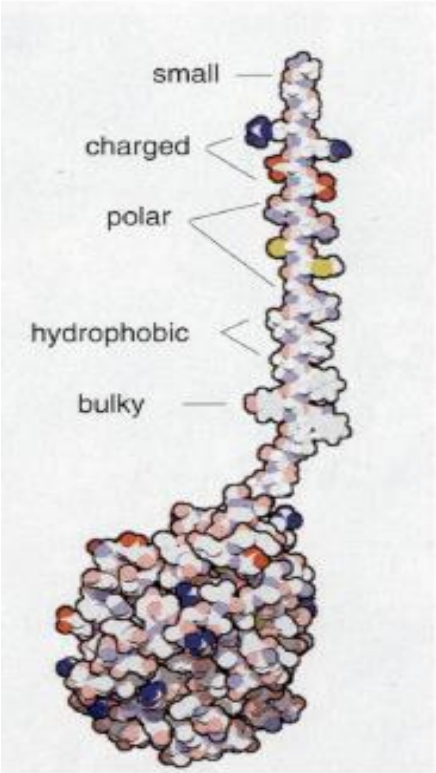
- first “observable” event when a surface interacts with biology
- energy-driven dynamic process, dependent on proteins & surface
- mediates subsequent cascades/responses
  - clotting
  - cell adhesion
  - inflammation & wound healing
- adsorbed proteins as signal transduction elements



surface → **proteins** → cells

Proteins comprise discrete building blocks (amino acids) assembled into hierarchical structures.



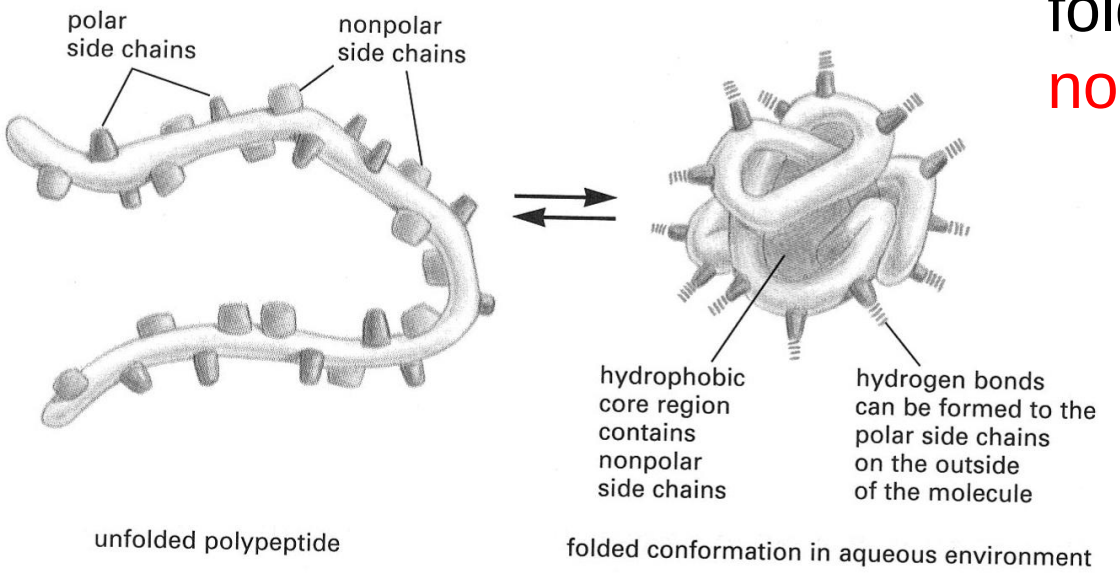


[after A. L. Lehninger, D. L. Nelson and M. M. Cox. *Principles of Biochemistry*, pg. 171.]

Contrary to polymers that have many statistically determined conformations in solution, proteins have one unique conformation that is determined by amino acid sequence and achieved by unique folding pattern.

Protein structure also produces interfacial reactivity

- high MW polymers of 20 different amino acids
- 1º structure controls protein 3D structure
- conformation – 3D folded structure
- structure = function



folding controlled by
non-covalent interactions

- **electrostatic**
- **hydrogen bonding**
- **van der Waals**
- **hydrophobic**

non-covalent interactions also control protein-surface interactions

Protein Structure Energetics: Stability

A close balance of competing energetics determine protein structure.

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

5-20% of protein amino acids contact the surface

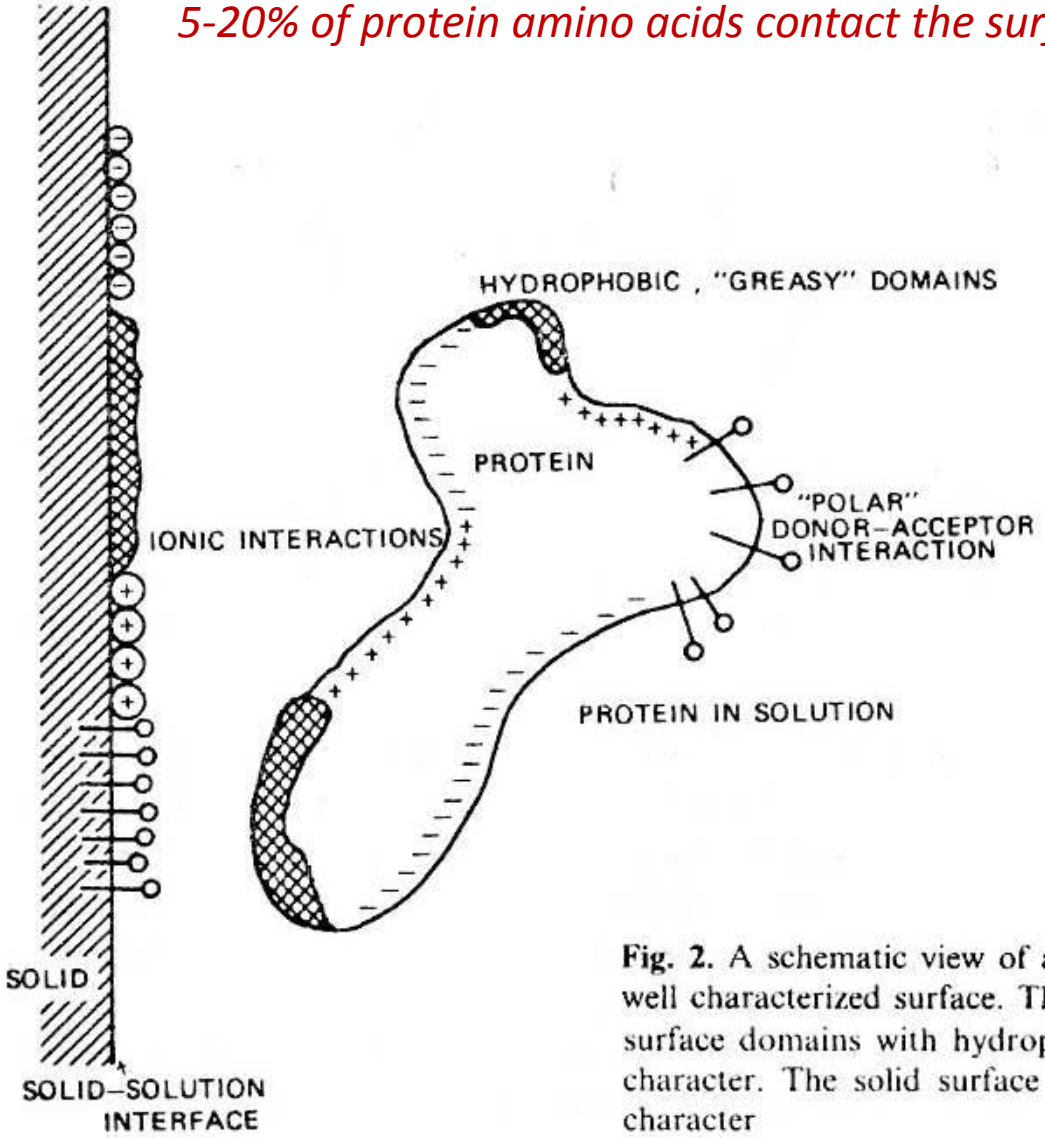
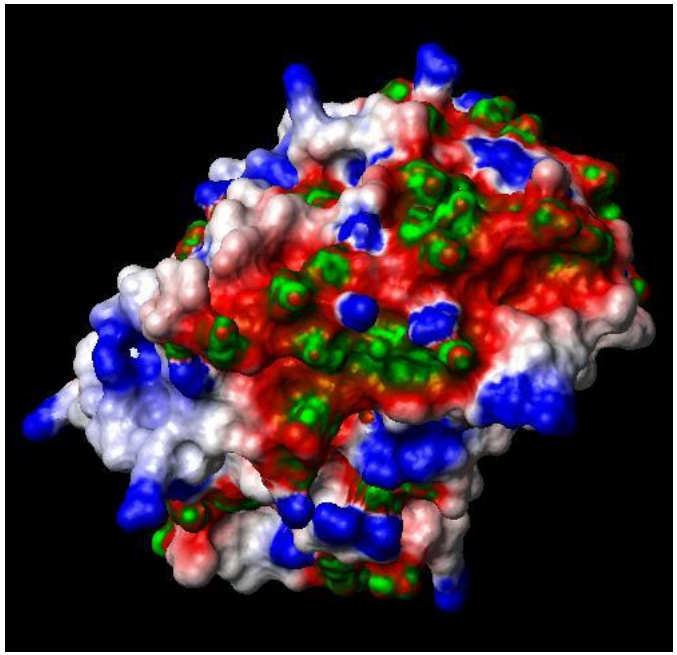
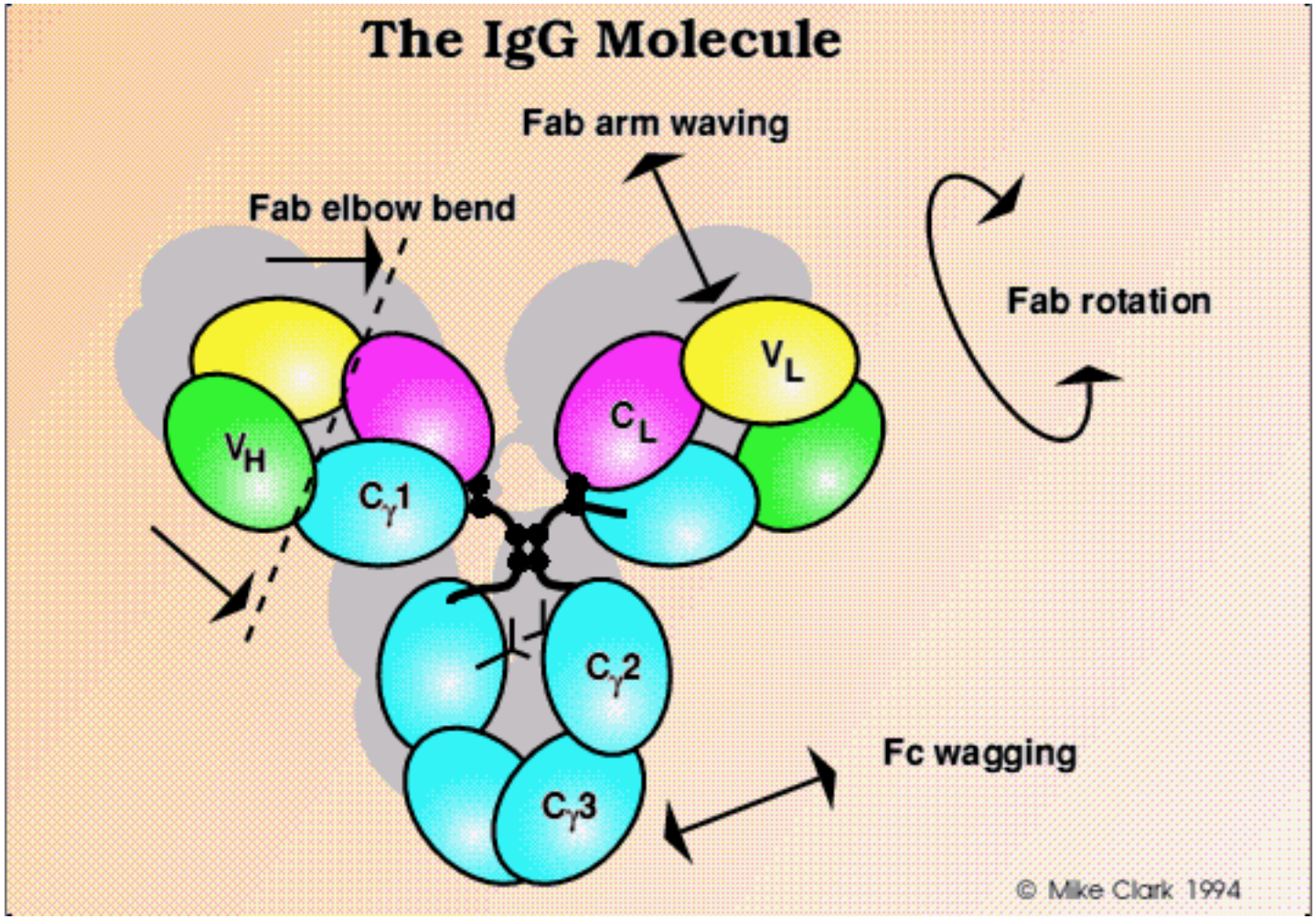


Fig. 2. A schematic view of a protein interacting with a well characterized surface. The protein has a number of surface domains with hydrophobic, charged, and polar character. The solid surface has a similar domain-like character

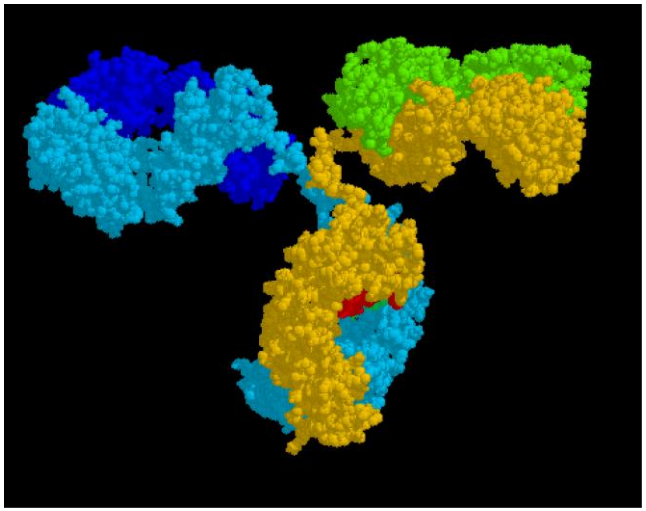


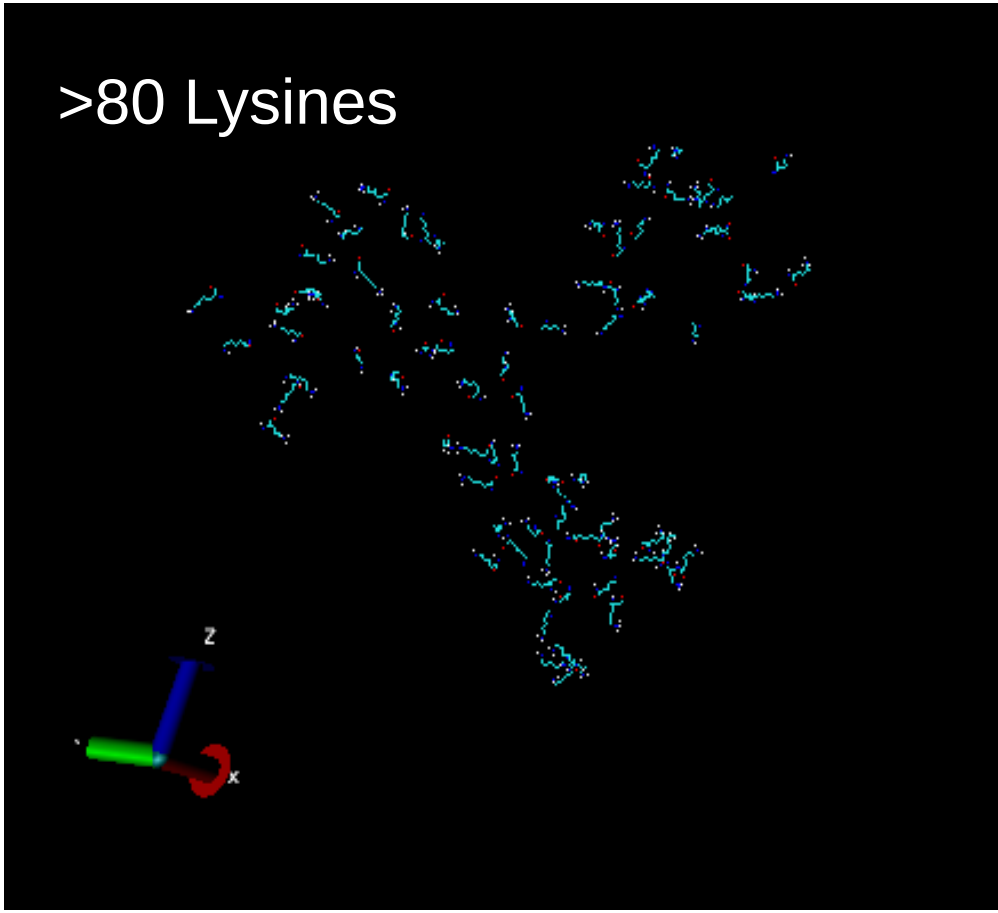
The globular protein model: dynamic, flexible, hydrated and meta-stable

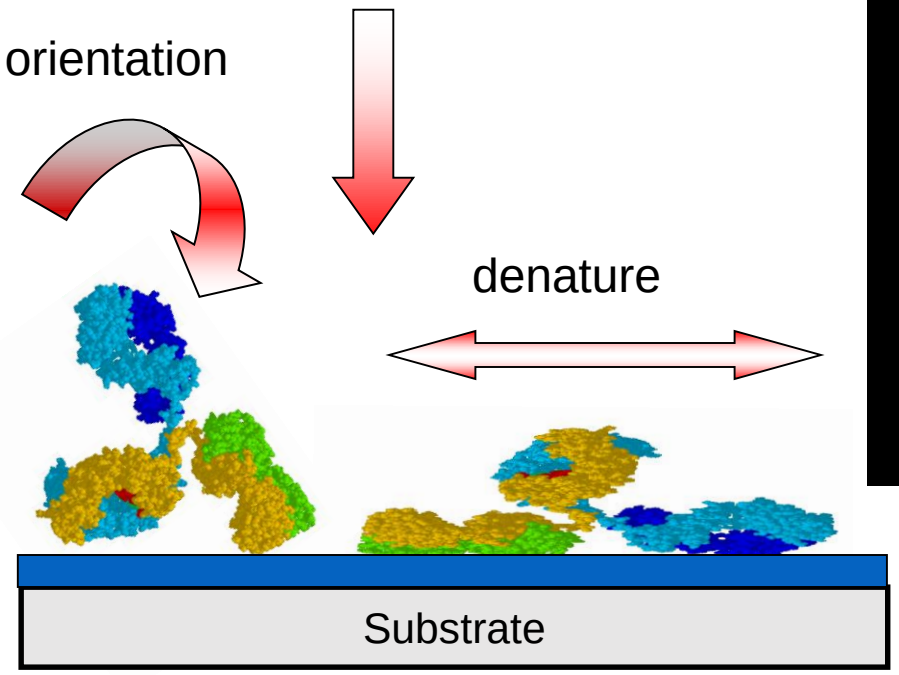


Proteins are dynamic, moving and elastic structures: respond to local environments

Antibody Immobilization: (lysine-surface reaction)







Which Lys residue finds the surface?

a**D**sorption, Modes

Adsorption is the process of association of solutes (or the solvent) ONTO a material interface

Absorption is when the solvent is taken up by the material (inside)

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})

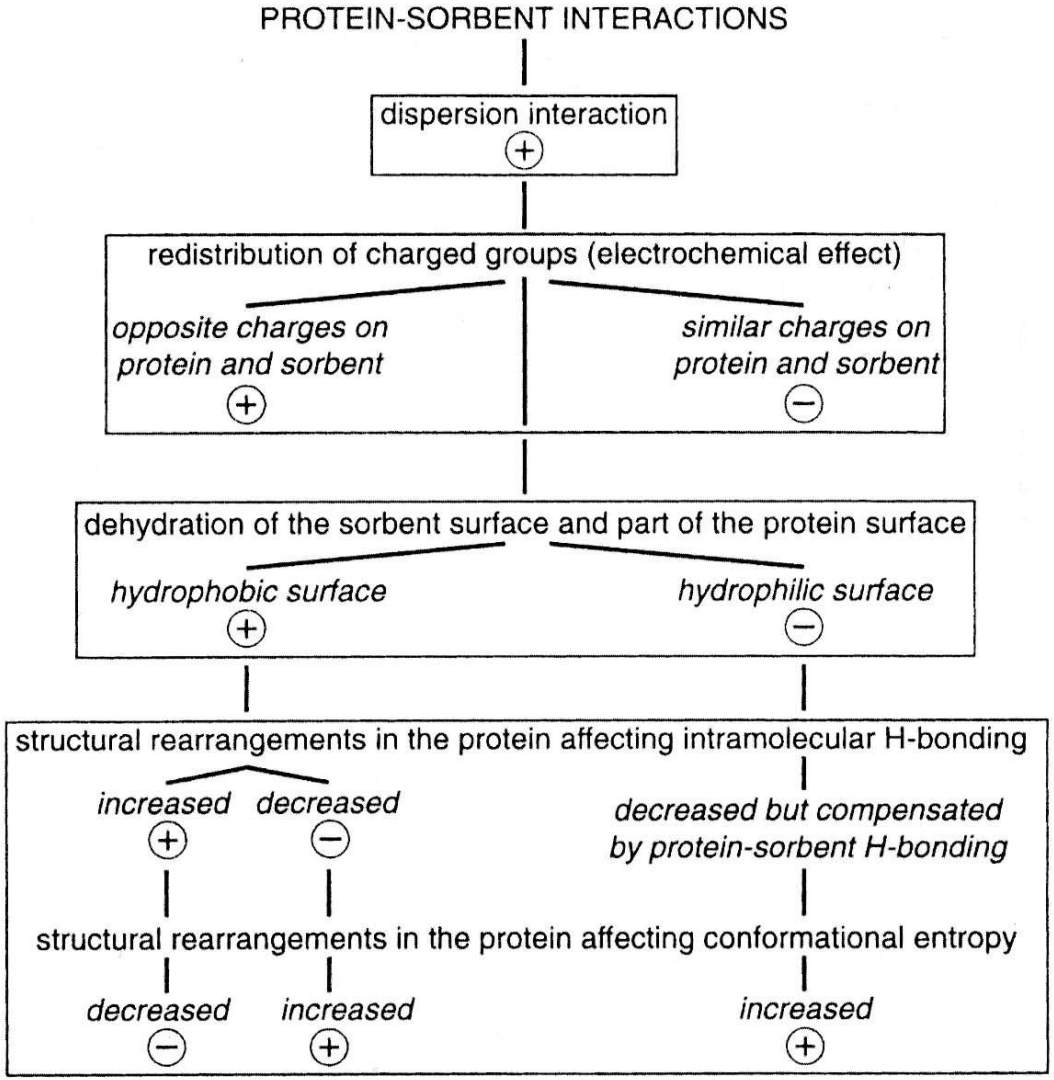
- activation barrier possible (b), variable uptake kinetics, covalent / ionic / metallic bonding, often irreversible, surface symmetry specific, limited to monolayer, wide range of $T_{surface}$

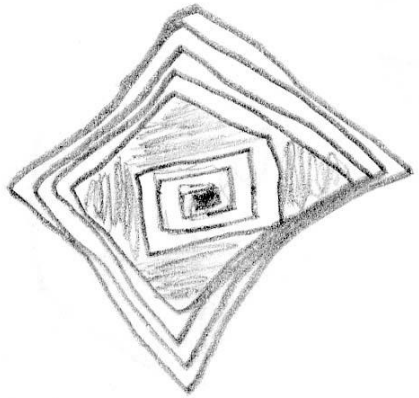
Properties of typical soluble proteins

- Proteins > 8kDa begin to fold and exhibit higher order structure: domains
- Peptides (small chains) vs. proteins (folded larger chains)
- Proteins bury hydrophobic amino acids away into interior of domains - avoid water
- Proteins expose hydrophilic amino acids in their hydration shells facing solutions
- Both energy demands compel proteins to fold and find a **local** energy minimum
- Membrane spanning proteins (cell channels, receptors) are largely insoluble and highly hydrophobic: only active in membranes
- Domains are held together by weak forces (H-bonds, acid-base, van der Waals)
  - small energy input can disrupt domain structures (shaking, heating, ultrasonic, electrochemical, surfaces): denaturation = loss of protein bioactivity
  - Domains can 'breathe' - reversible excursions due to flexible conformations
- Glycosylation (attachment of sugars) renders proteins "sticky" to surfaces
- Balance of unfolding tendency vs. exterior hydration stability plays off on surfaces

- **All proteins have some interfacial activity, stability and affinity on surfaces**

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: 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 adsorption irreversibility.

Table 3 Thermodynamic Analysis of the Adsorption of Lysozyme on Negatively Charged Polystyrene Surfaces

	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.

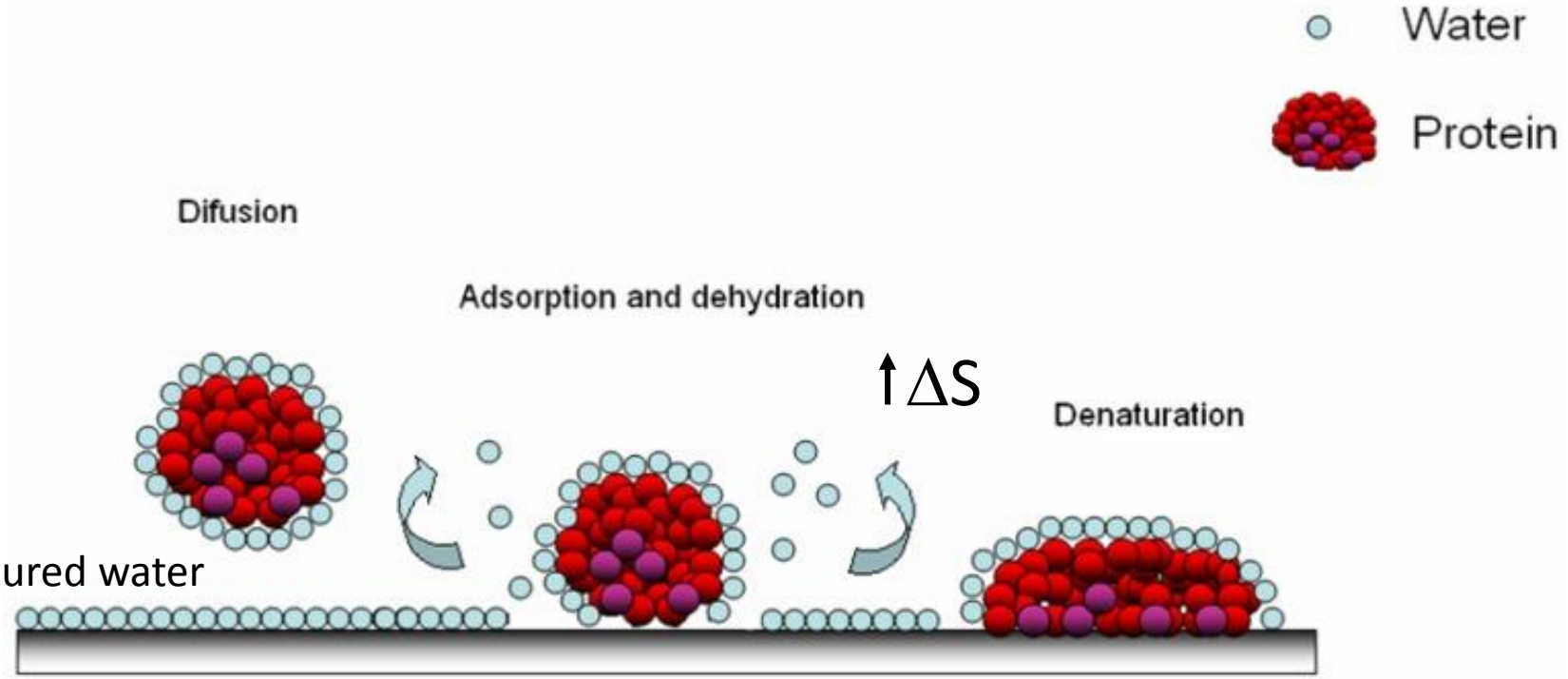
$$\Delta_{ads}G = \Delta_{ads}H - T\Delta_{ads}S$$

largest contribution: dehydration of polystyrene (which means that *protein binding is driven by surface desire to shed its neighboring water molecules*)

negative enthalpy favors adsorption

these numbers look small but they have to be multiplied by 300 K (*T*); negative entropy opposes adsorption

Entropy of adsorption:
protein and surface dehydration drive adsorption

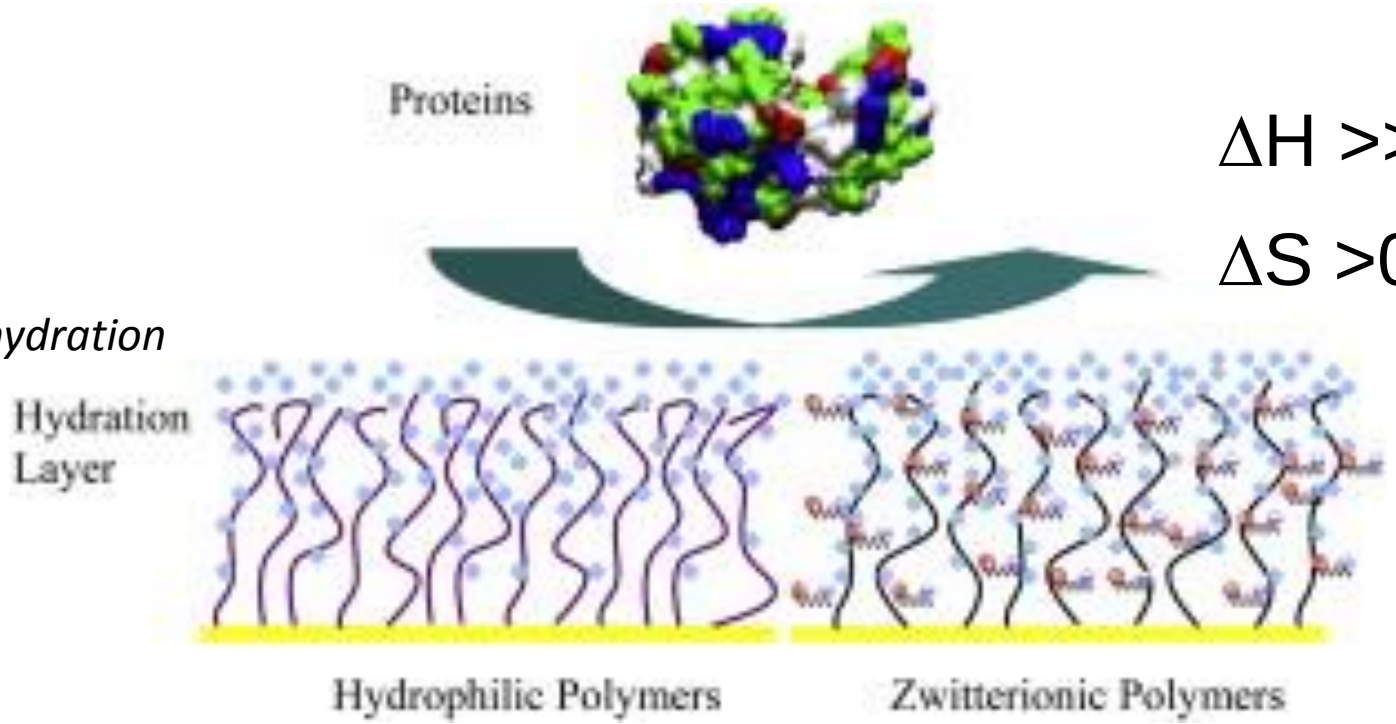


$\downarrow \Delta G_{ads}$ (spontaneous)

Energy of interaction:

stable protein and surface hydration hinder adsorption

$\uparrow \Delta G_{ads}$ (not spontaneous)



$\Delta H >> 0$

$\Delta S > 0$

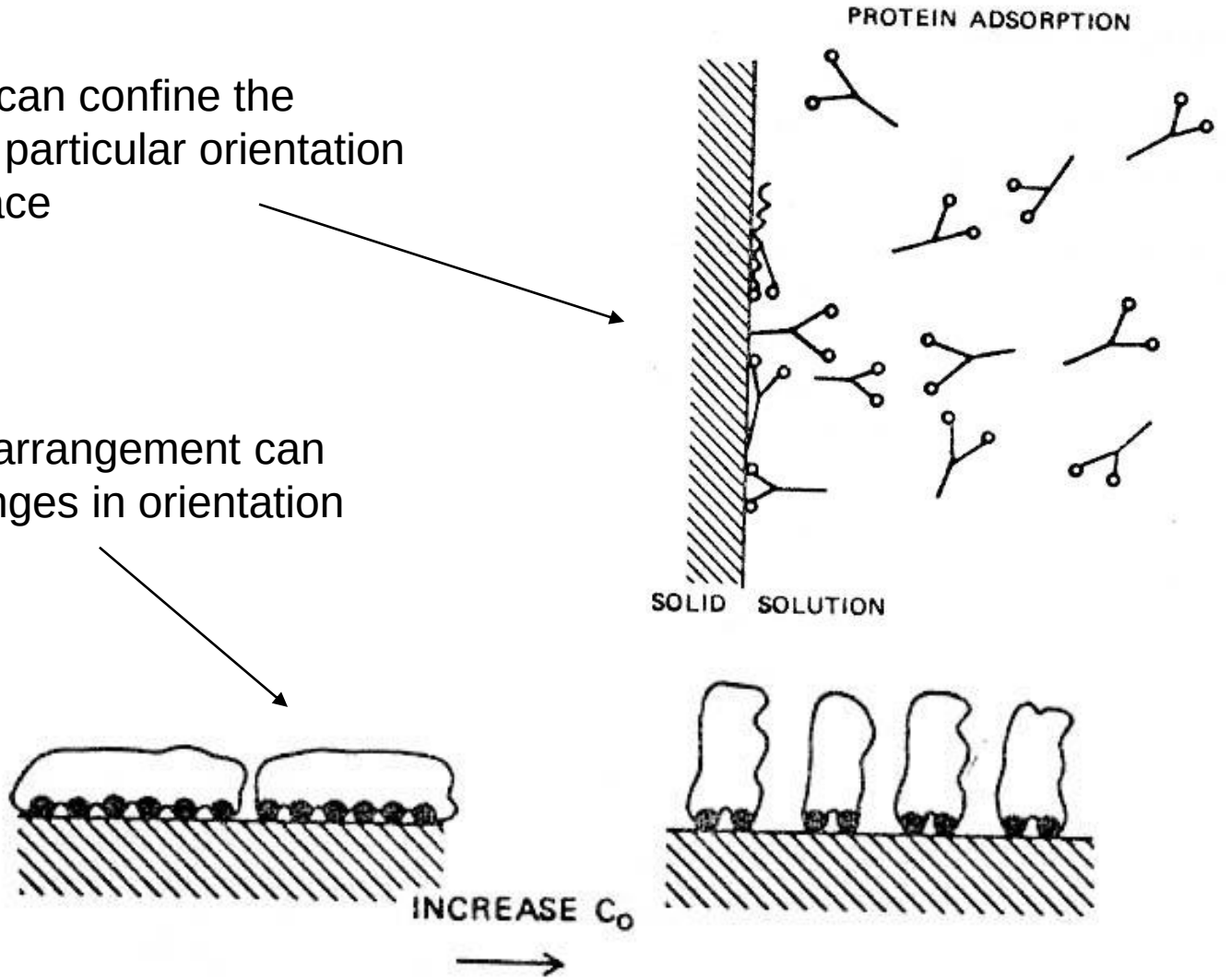
Stable hydration

https://doi.org/10.1016/j.polymer.2010.08.022

Protein Surface Orientation

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

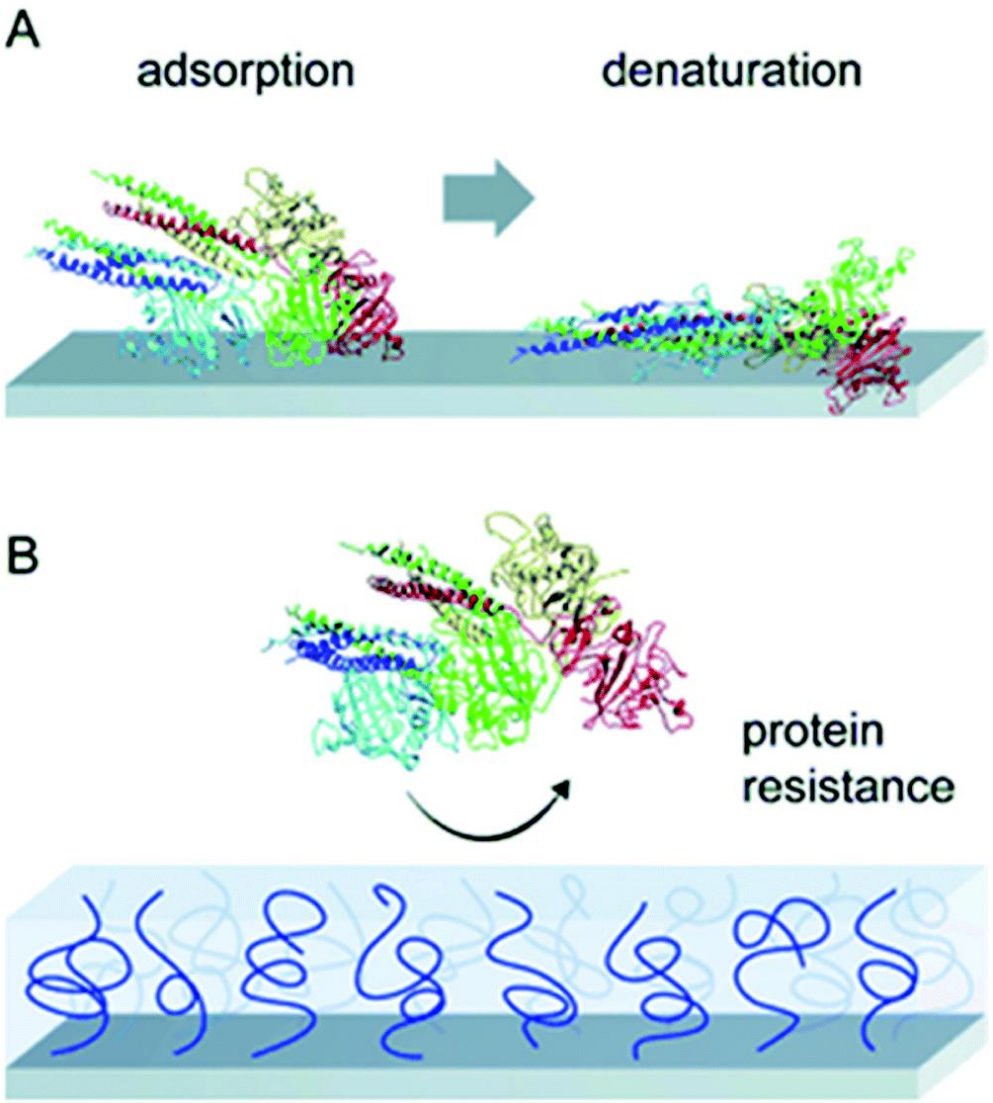
Dynamic rearrangement can lead to changes in orientation



Orientation can affect protein activity!

Protein adsorption principles: take-home summary

All proteins adsorb: some irreversibly (denature)



All surfaces adsorb proteins: some more than others

Faraday Discuss., 2016,191, 435-464

Biomaterials-relevance: proteins at surfaces

- > 500 serum proteins, but only a few crystal structures known, more protein sequences known, and more identified simply as ‘present’ without info on function
- Relatively few studied in competitive adsorption experiments on surfaces
  - more is known about single protein adsorption in buffer: relevance to in vivo?
  - empirical correlations between surface chemistries, amounts of proteins and in vivo responses
- Adhesion vs. non-adhesion protein ratios important for cell attachment to surfaces
- High albumin adsorption correlated with low platelet and low macrophage activation
- Hydrophobic surfaces generally adsorb more protein because of favorable gain from both enthalpy (<0) and entropy (>0 for both protein and surface) [$\Delta G = \Delta H - T\Delta S < 0$]
- Hydrophilic surfaces adsorb less proteins because of opposing energy cost for dehydrating both surfaces to impart adsorption contact -> stable hydration, low protein
- Many hypotheses correlating short-term cause-effect for protein adsorption and response in vivo, but few long-term correlations are observed --> always inflammation

- **To date, no surface chemistry can control types and amounts of proteins**

Properties of some major plasma proteins

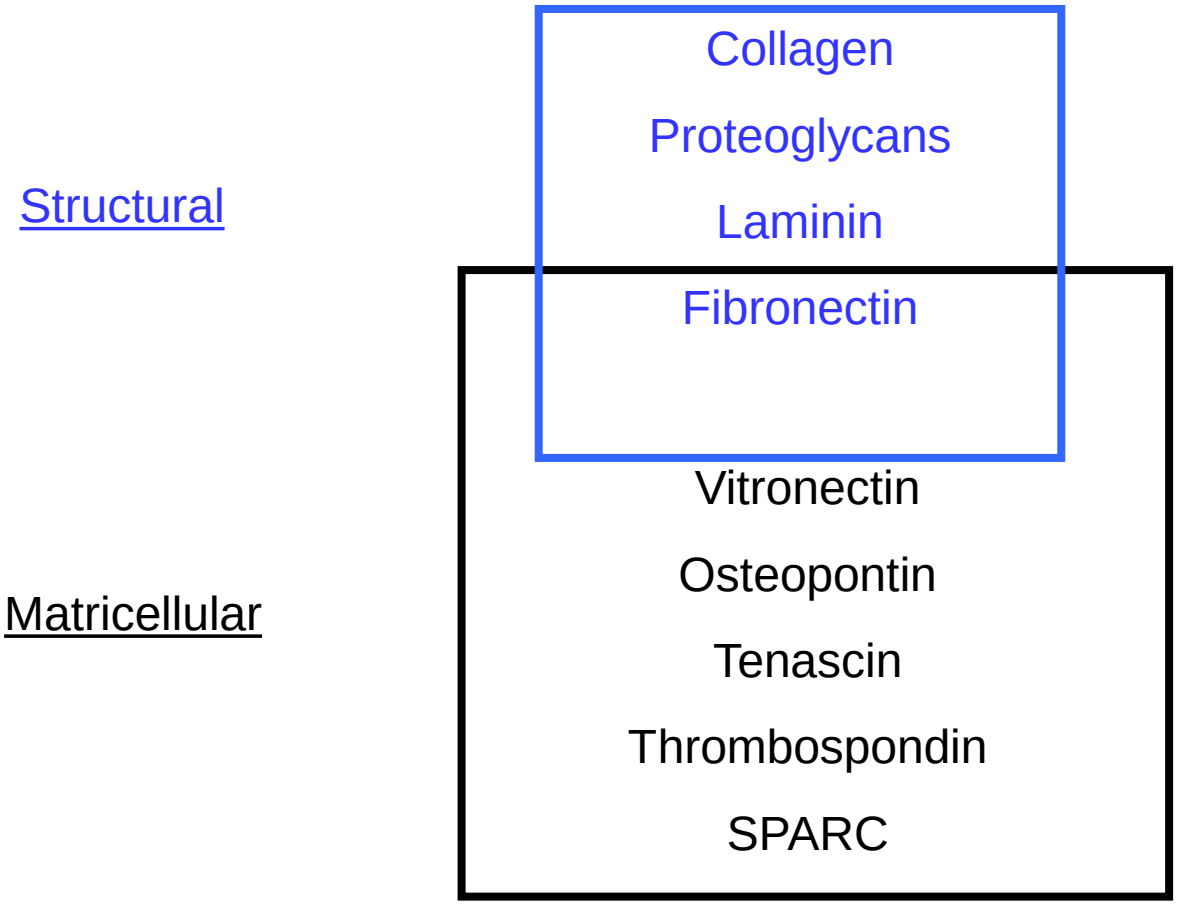
> 500 soluble serum proteins → all compete for the surface!

Protein type	Plasma concentration (mg/ml)	Monomer Molecular weight (daltons)
Prealbumin	10 - 40	54,900
Albumin	35 - 45 ← highest abundance	66,500 Low M.W.
IgG	6 - 17	150,000
Fibrinogen*	2.0 - 4.0	340,000
Fibronectin*	0.26-0.38	250,000 (but dimeric)

- **Mass transfer flux favors high albumin loading on surfaces**
- **Adhesion proteins have integrin binding sites; **albumin non-adhesive****

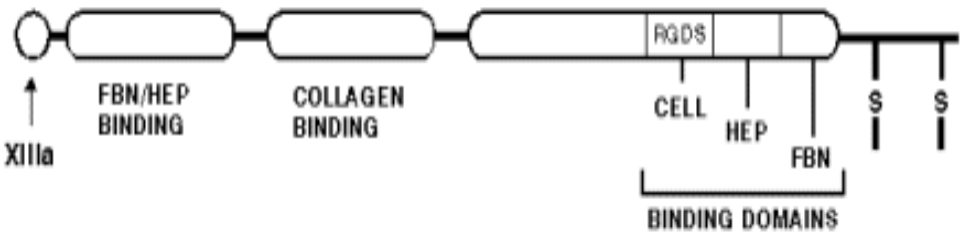
Trace Cell-Adhesive Extracellular Matrix (ECM) Proteins

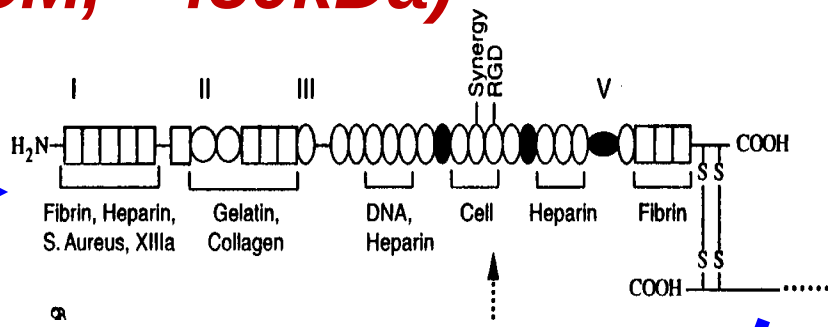
Soluble → Adsorbed/Processed → Recognized by cells



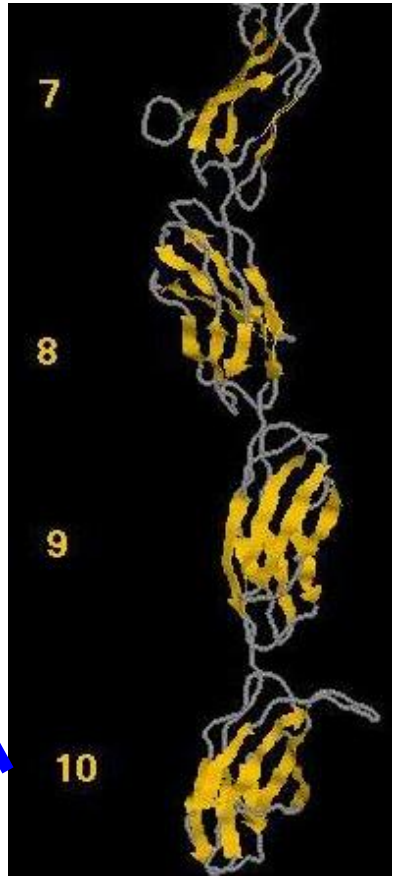
Fibronectin (trace ECM, ~450kDa)

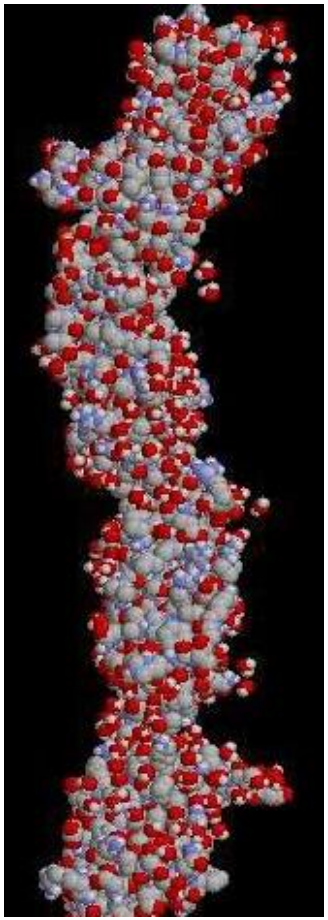
Heterodimer



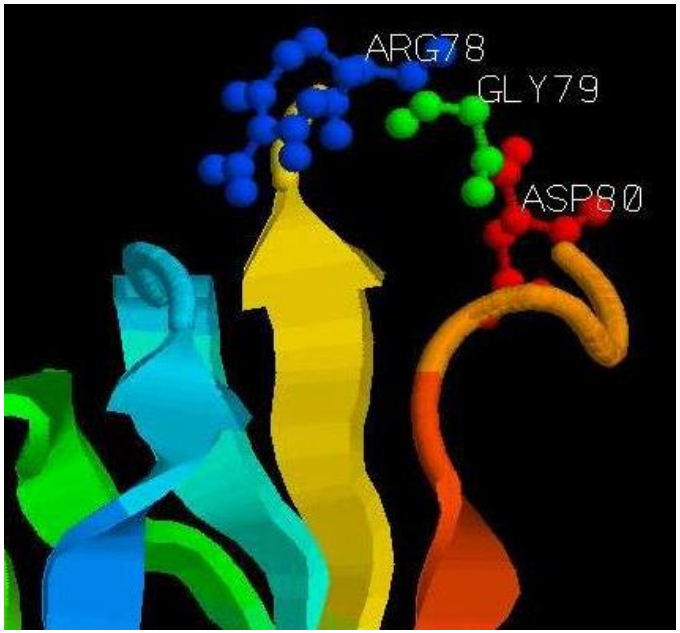


Type III Repeats





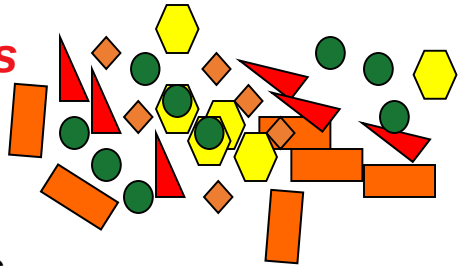
RGD Cell Binding Domain



Recognized by cell Integrin receptor

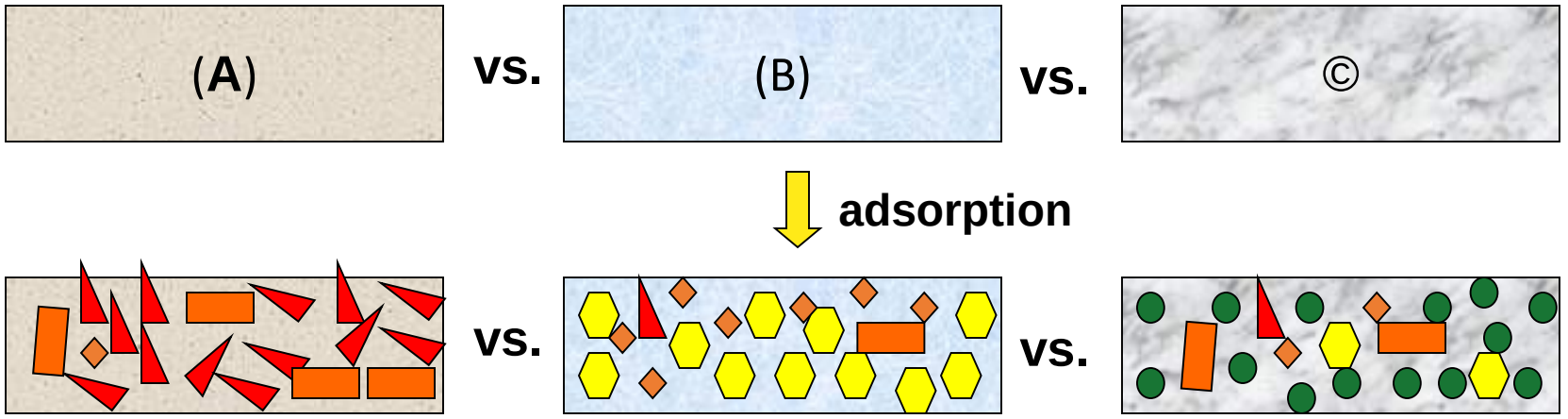
Surface 'Selection' of Proteins from Complex Milieu

- Many clinical and non-clinical samples contain *hundreds* of soluble proteins: serum, cells, tissue, ocean water



- All of them will bind to surfaces, some more than others

- Surfaces can select certain proteins more than others:

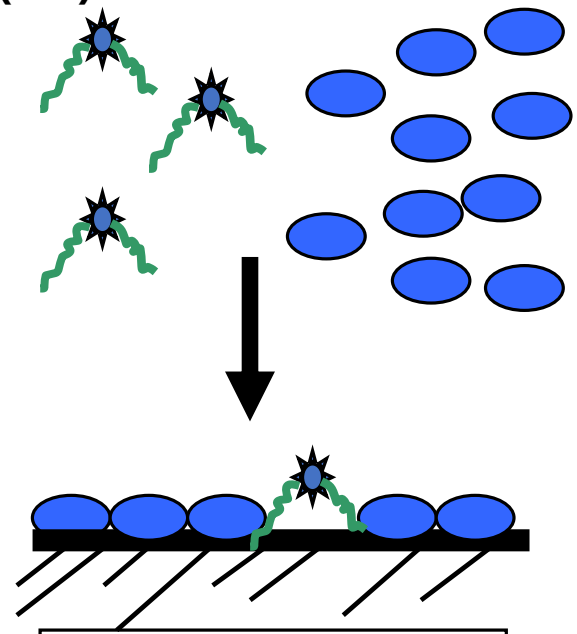


- Hydrophobic surfaces often select albumin from serum
- Albumin has no recognition features - used as a 'blocking agent' on surfaces
- Challenge to create selective adsorption surfaces

Serum Proteins Surfaces Determine Cell Engagement

Density/Concentration

Fibronectin **(Fn)** vs Albumin **(Alb)**

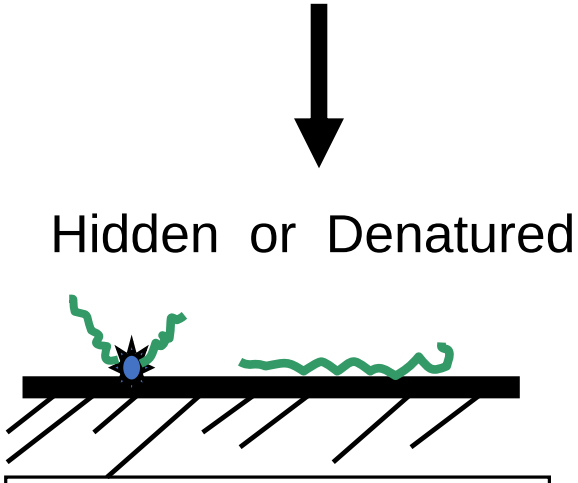


Alb Flux > Fn Flux

Conformation

RGD = Cell binding site

Fibronectin (Fn)

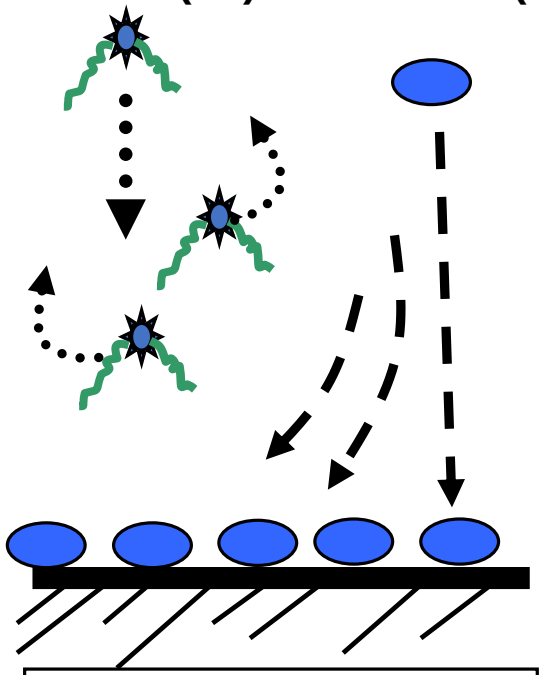


Hidden or Denatured

Fn = globular protein

Competition

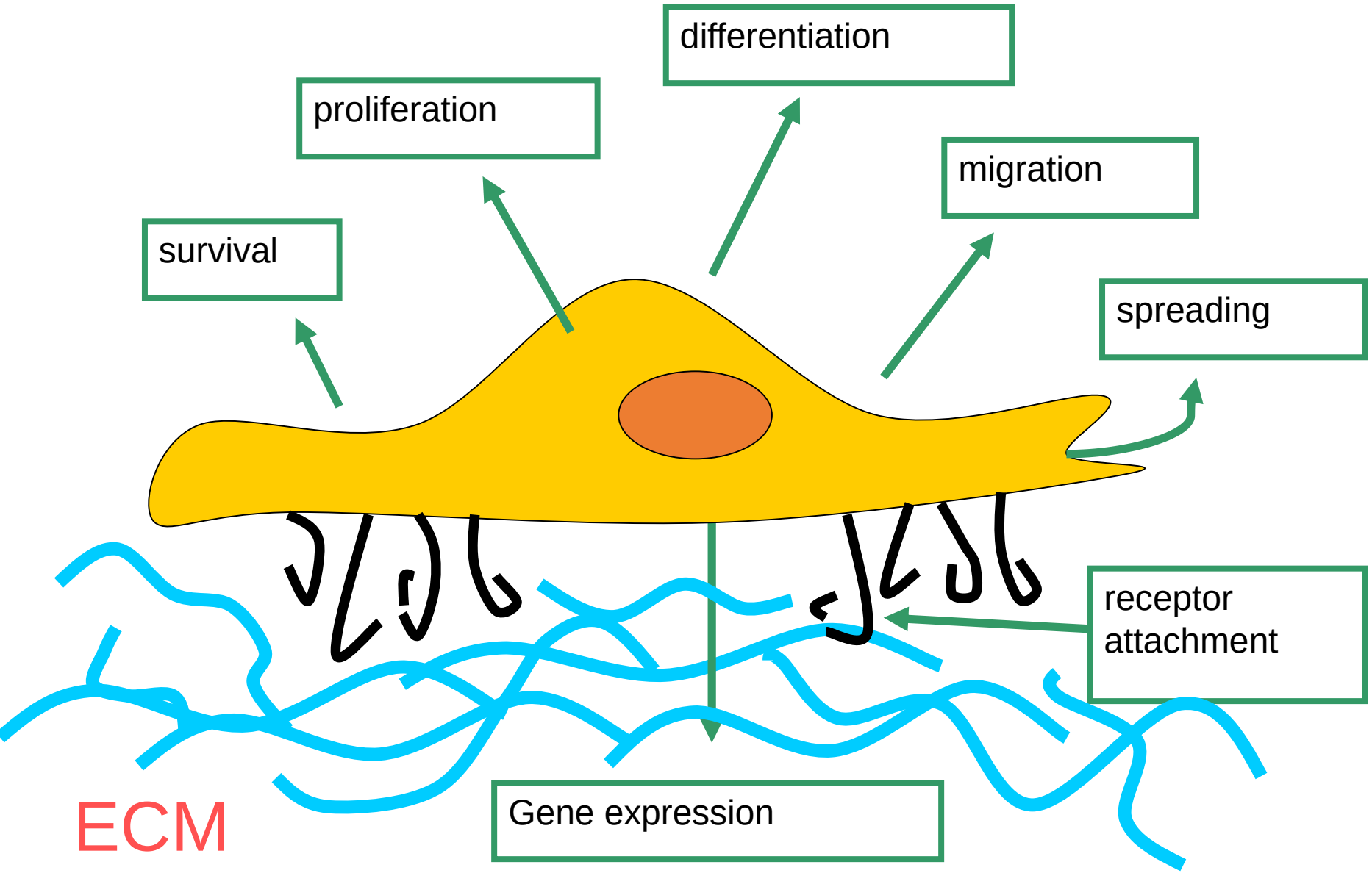
Fibronectin**(Fn)** vs Albumin**(Alb)**



Affinity of Alb vs. Fn

- **These events determine cell adhesion to surfaces**
- **Surface chemistry-dependent protein carpet**

Many Cell are Attachment Dependent



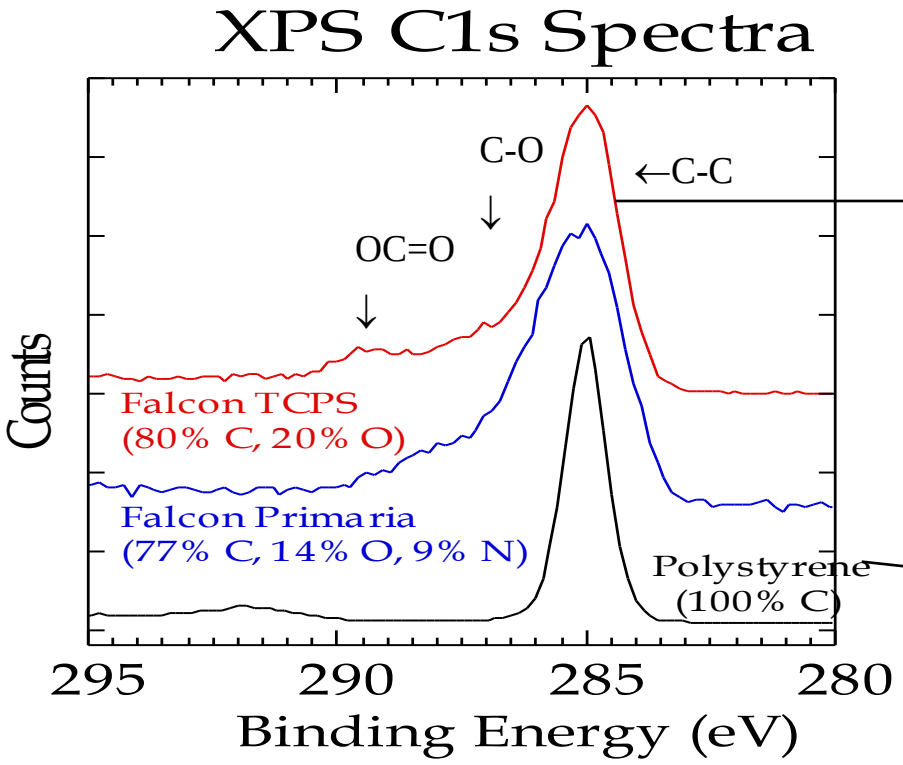
Cell Substrate Adhesive Interactions

For attachment-dependent cells, essential for:

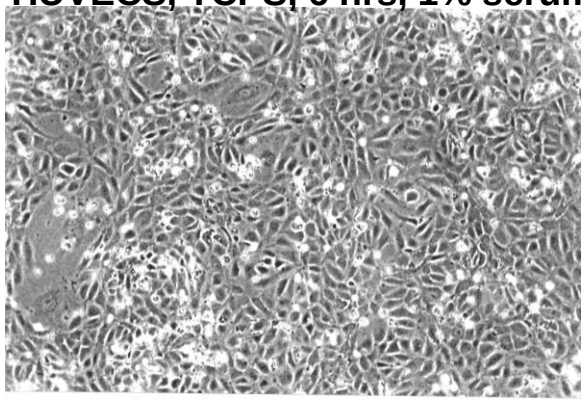
- Cell Adhesion
- Cell Migration
- Cell Shape
- Cell Differentiation
- Cell Proliferation
- Cell Survival
- Cell Matrix Assembly
- Gene Expression
- Mechanosensors

- **Cells never “see” a bare surface**
- **Cells always encounter a protein carpet**

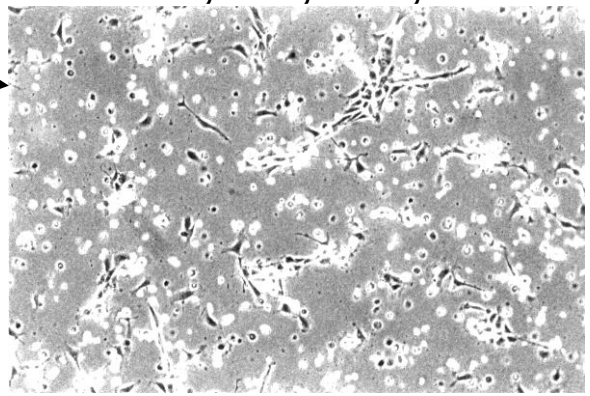
XPS surface analysis of TCPS and BPS substrates vs. cell culture

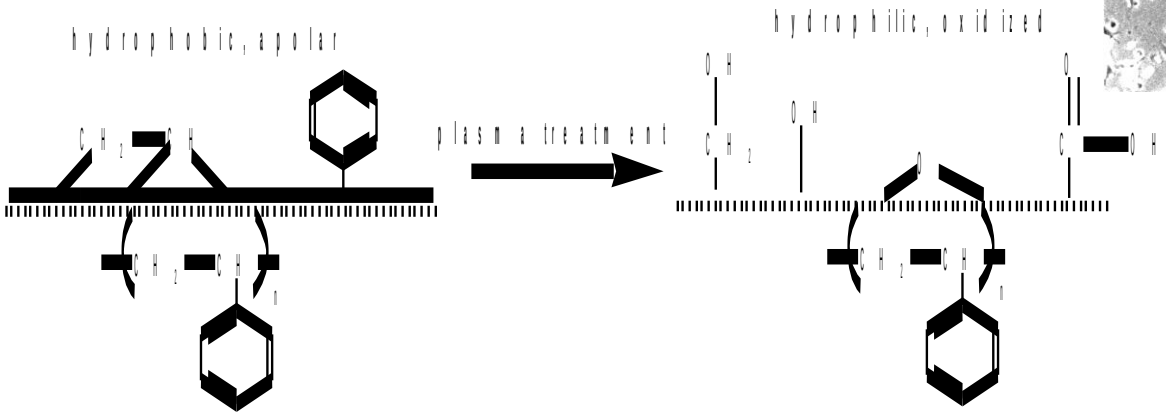


HUVECS, TCPS, 6 hrs, 1% serum

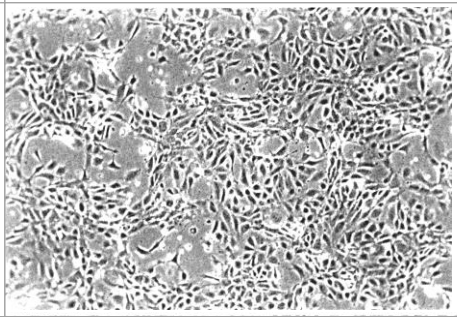
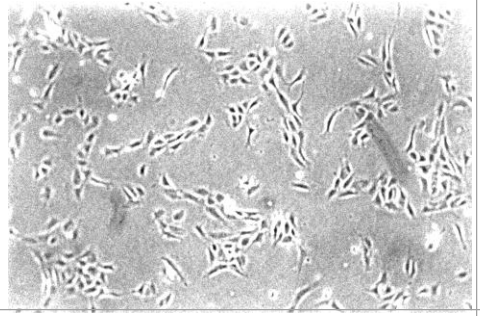
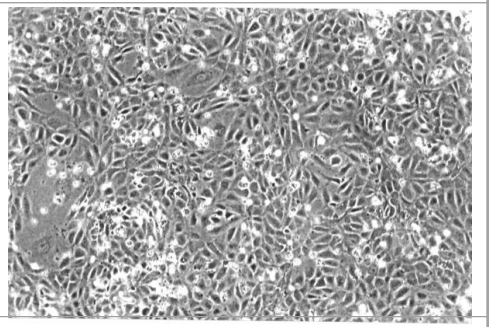
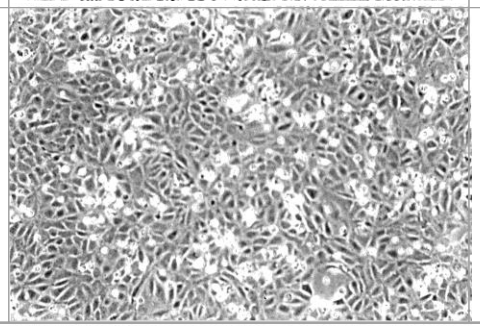
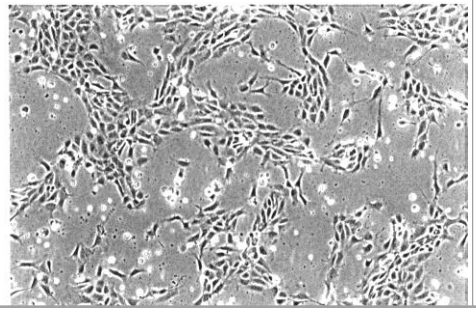
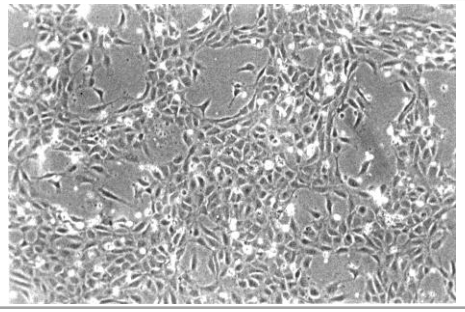


HUVECS, BPS, 6 hrs, 1% serum



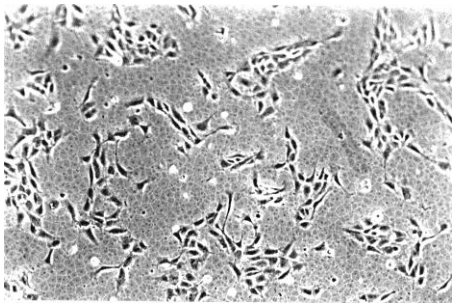
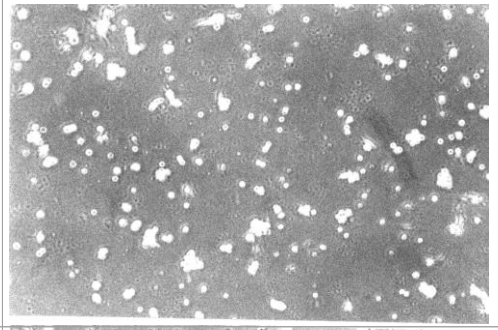
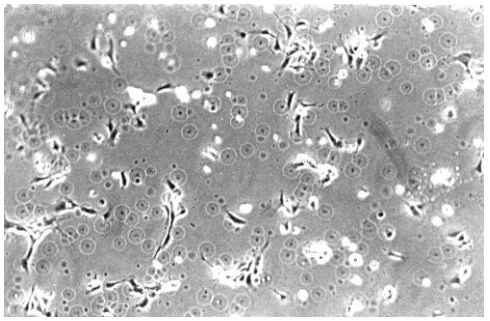
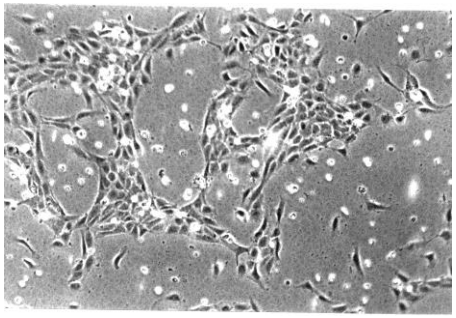
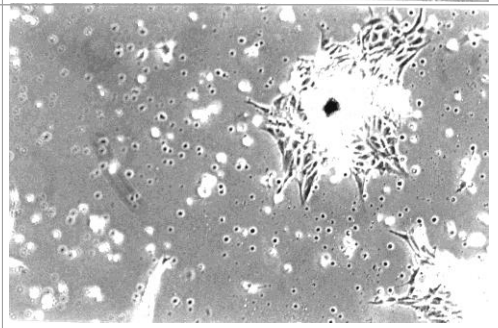
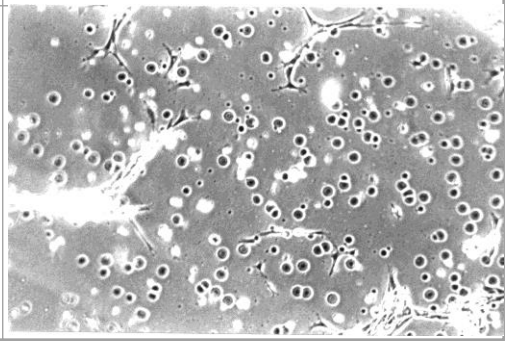


HUVEC culture on (oxidized) TCPS after various protein pre-adsorption conditions

	Fn (3μg/ml)	Fn:BSA (3μg/ml:400μg/ml)	1% serum
a) after 6 hours			
b) after 24 hours			

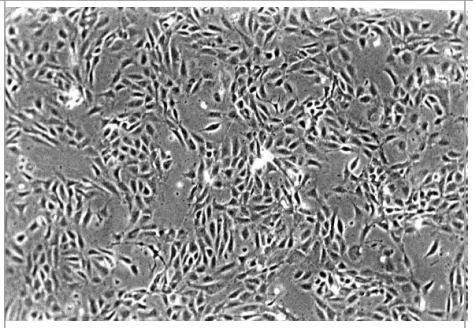
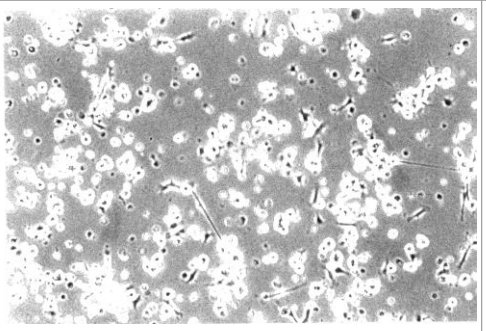
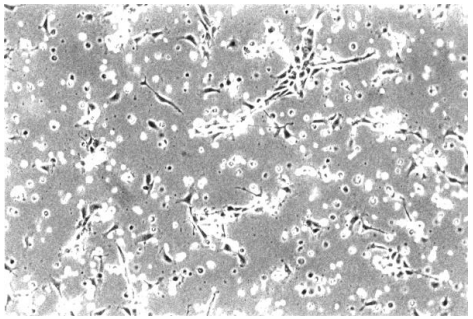
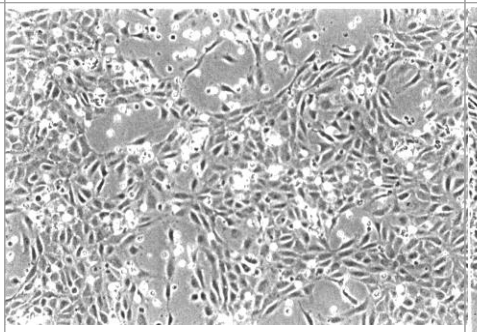
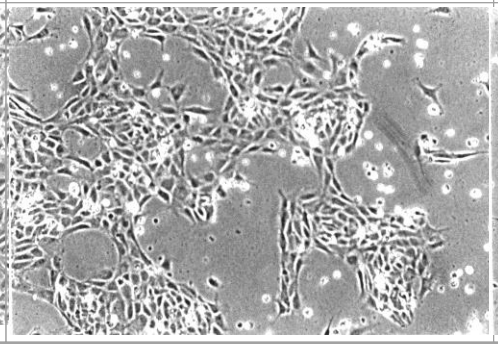
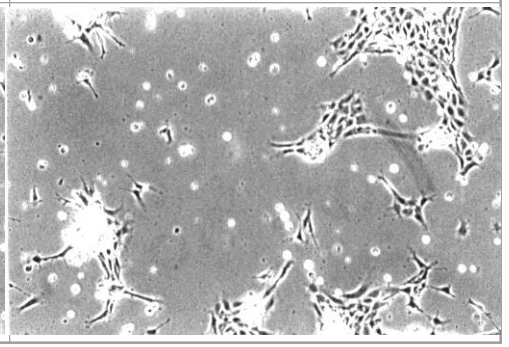
- TCPS promotes cell attachment and spreading with various proteins
- “Gold standard” material for cell-surface interactions

HUVEC culture on hydrophobic PLLA after various protein pre-adsorption conditions

	Fn (3μg/ml)	Fn:BSA (3μg/ml:400μg/ml)	1% serum
after 6 hours			
after 24 hours			

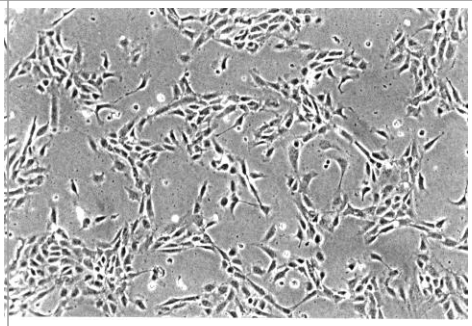
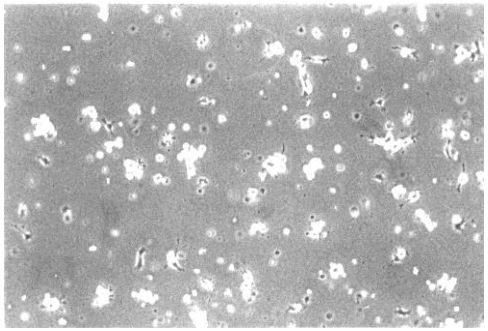
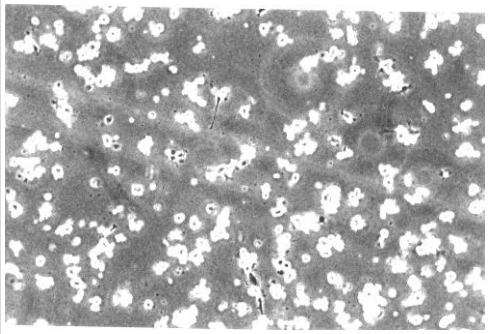
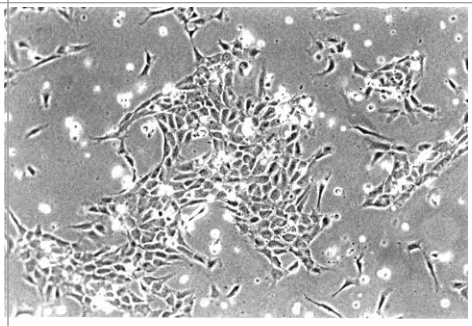
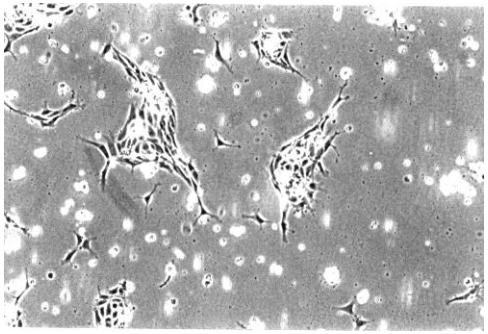
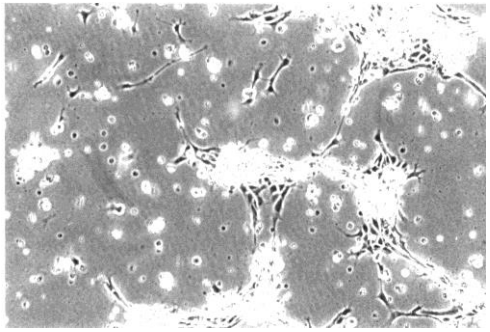
- Cells fail to attach and spread in presence of competing proteins
- Fibronectin alone restores attachment and spreading

HUVEC culture on hydrophobic BPS after various protein pre-adsorption conditions

	Fn (3μg/ml)	Fn:BSA (3μg/ml:400μg/ml)	1% serum
after 6 hours			
after 24 hours			

- **hydrophobic polystyrene fails to promote cell attachment in competitive protein conditions**

HUVEC culture on hydrophobic TeflonAF® following various protein pre-adsorption conditions

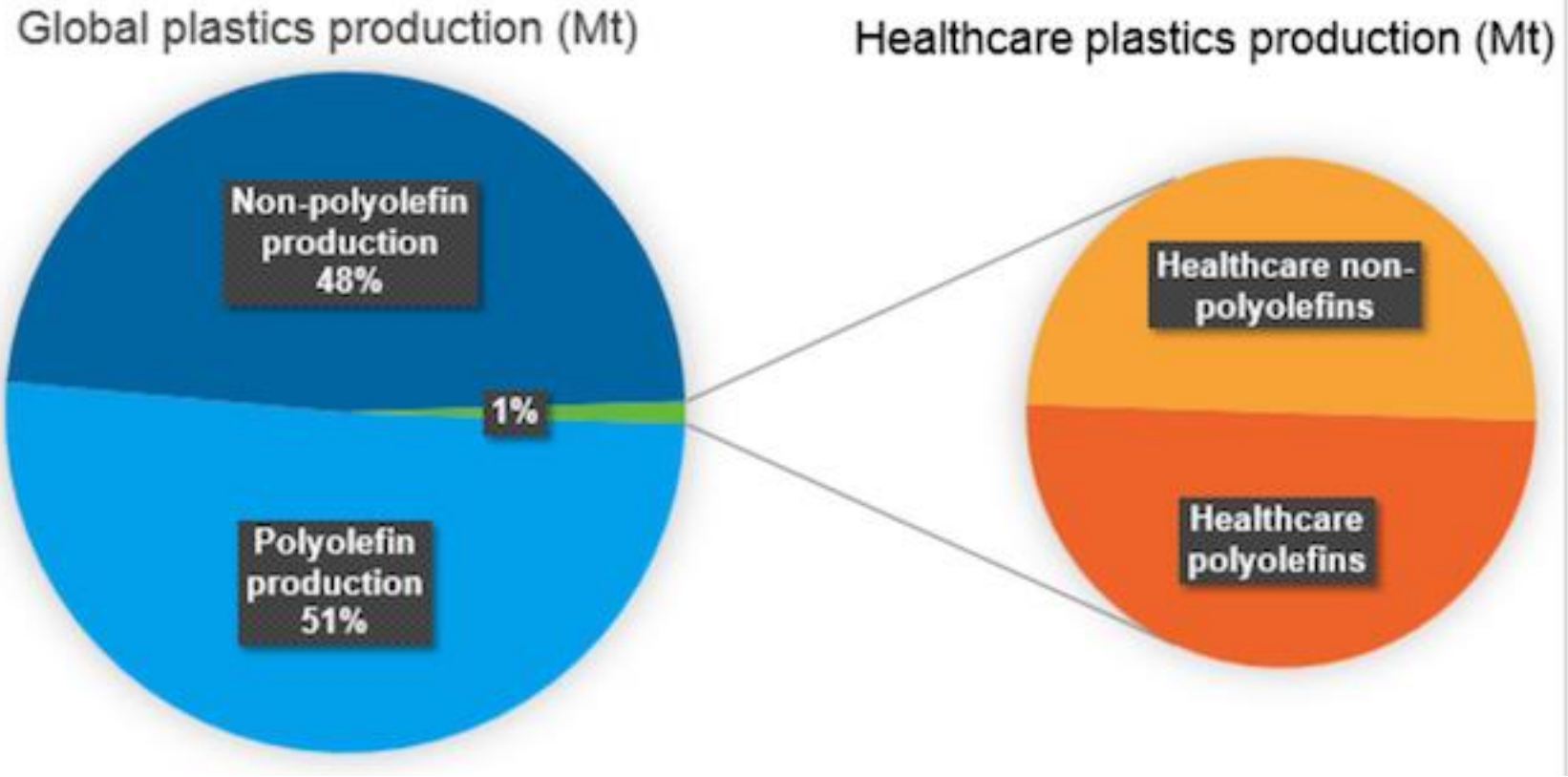
	Fn (3μg/ml)	Fn:BSA (3μg/ml:400μg/ml)	1% serum
6 hrs after plating			
24 hrs after plating			

- Cells fail to attach and spread in presence of competing proteins
- Fibronectin alone restores cell attachment and spreading

THE UNIVERSITY OF UTAH

Department of BIOENGINEERING
UNIVERSITY OF UTAH

The University of Utah
Department of Pharmaceutics and Pharmaceutical Chemistry



Only about 1% of the 311 million tons of plastics, or about 3.1 million tons, go into healthcare," said Petzold. And yet, medical plastics get an awful lot of attention. They punch above their weight because they are subject to "the highest quality requirements and most stringent regulations. Yes, it's a tiny market," said Petzold, "but it has been a large focus for Borealis."

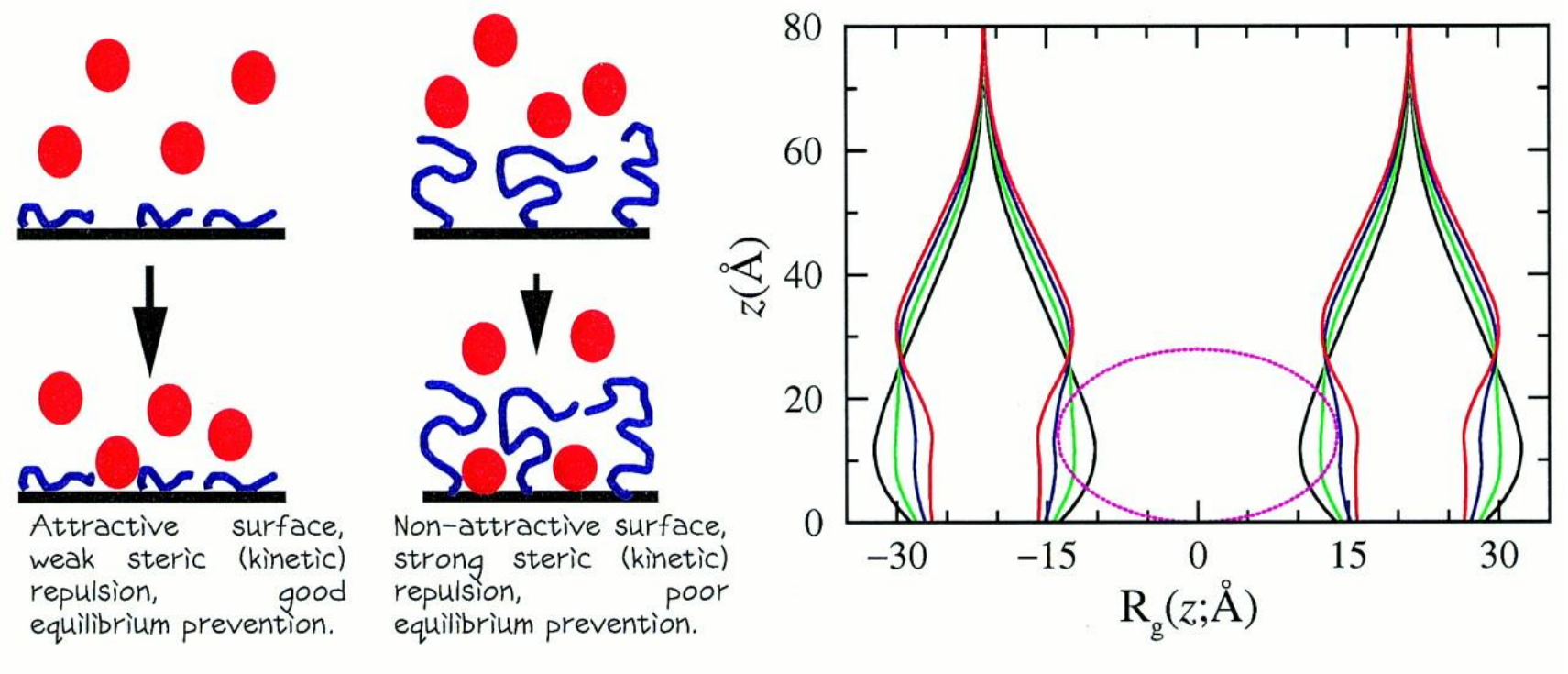
Bormed SB815MO was developed for blow-fill-seal applications, such as IV bottles and ampoules in the medical space. “The material of choice has been LDPE,” said Petzold, which has all the requisite properties for this application—softness, transparency and processability—save one: “The material must be sterilized at low temperatures and, thus, requires longer sterilization cycles.” Random PP co-polymer has also been used, and while it withstands high sterilization temperatures, it also exhibits high stiffness. “So you will have the problem of not being able to empty all of the IV liquid from the bottle or ampoule,” said Petzold. The solution developed by Borealis combines the properties of both materials: Bormed SB815MO is as soft as LDPE and can be sterilized at 121° C, thus allowing short sterilization cycles, and its transparency matches random PP co-polymers.

Bormed SC876CF was developed for complexly structured primary and secondary IV packaging, where each layer has its own functionality. Petzold illustrated the level of complexity in a three-layer film:

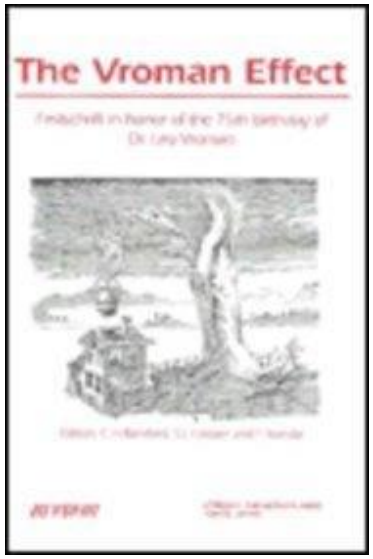
•The 20-micron outer layer, made of homo or random PP, must be heat resistant;
•the 130- to 160-micron core layer of soft or random PP must be soft *and* tough; and
•the 30- to 50-micron sealing layer, random PP or terpolymer, must be transparent and sealable.

All of the layers must withstand sterilization and retain transparency. All of them also may contain impact modifiers to a lesser or greater extent, which pouch producers often require to deliver toughness and softness, especially in the core layer. Impact modifiers are pricey, and “one way that pouch producers can reduce cost is by reducing the quantity of impact modifiers,” explained Petzold.

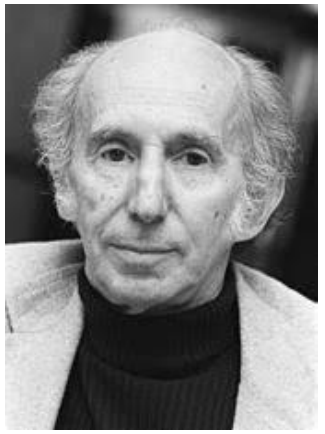
By using Bormed SC876CF for this application, the outer layers do not change, but the amount of impact modifier used in the core layer can be reduced significantly

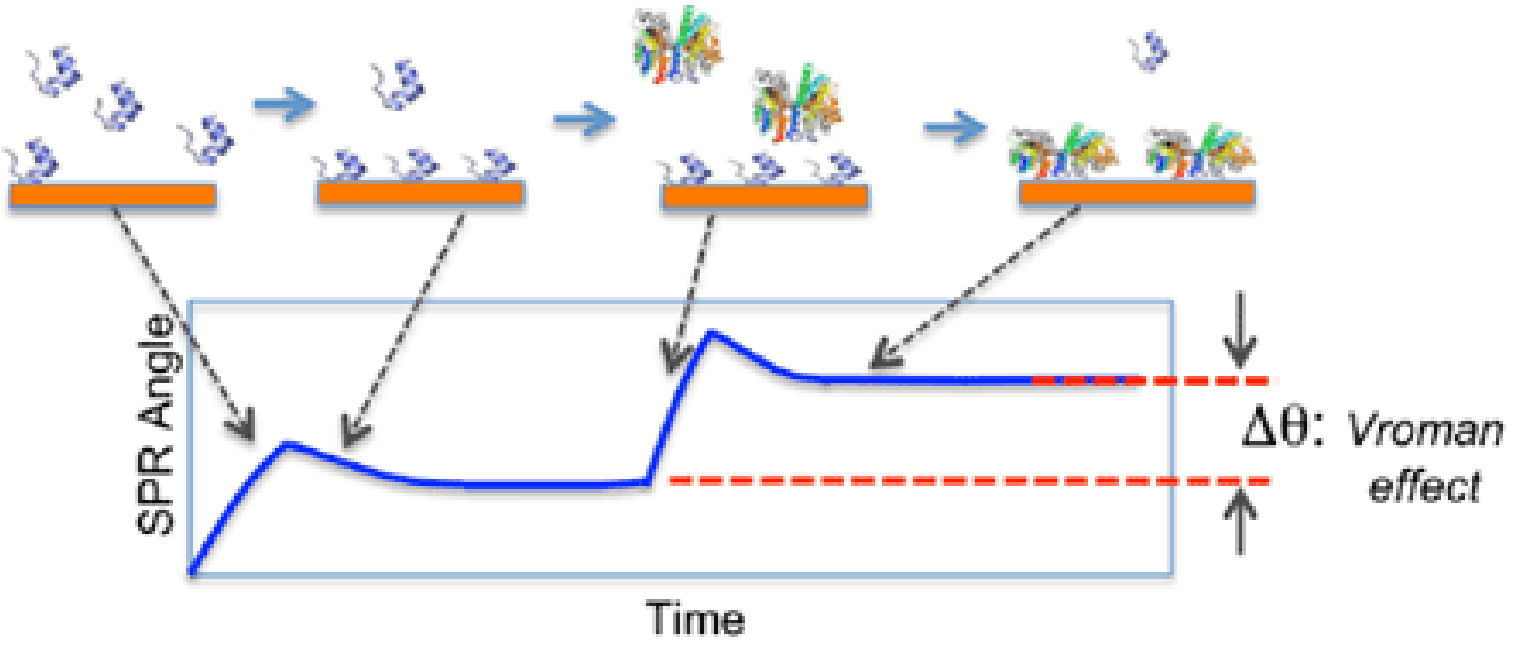


J. Satulovsky, M. A. Carignano, I. Szleifer
PNAS 2000 97 (16) 9037-9041; https://doi.org/10.1073/pnas.150236197



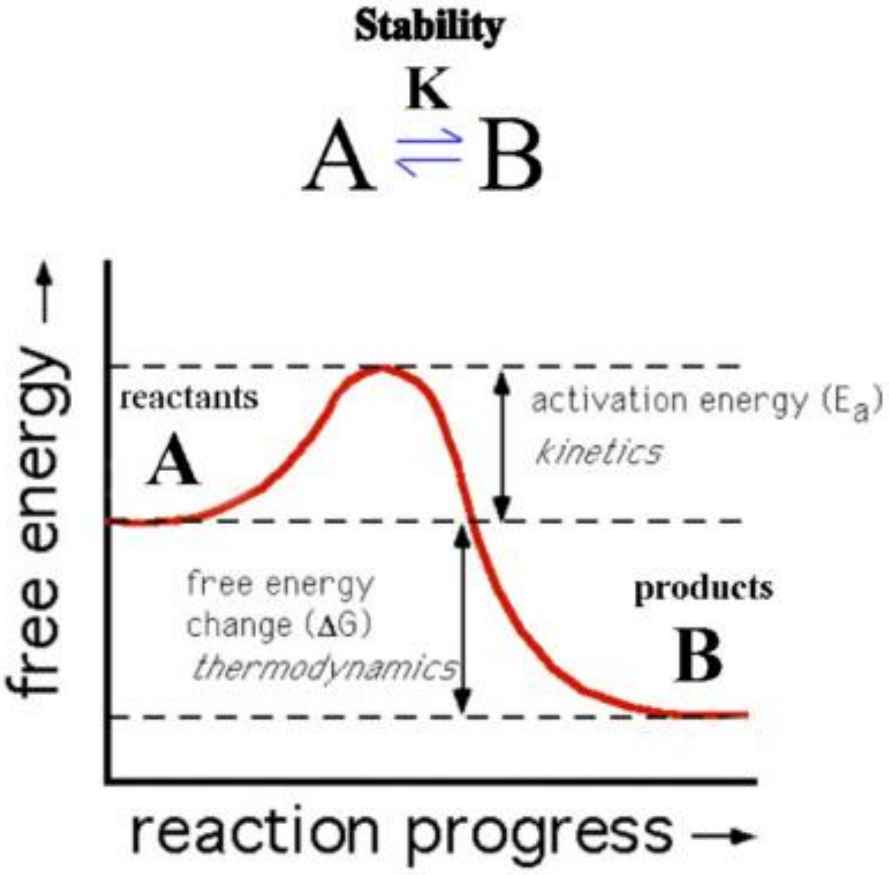
Vroman effect





Kinetics vs Thermodynamics

Rate vs Stability, Spontaneity



Biomaterials-relevance: proteins at surfaces

Serum proteins studied most at interfaces:

- **The Big Three: Albumin, Immunoglobulin G, Fibrinogen**
  - **(Andrade, Hlady)**

- **The Big Ten: add trace serum proteins with certain physiological relevance or abundance:**
  - **α-macroglobulin, fibronectin, apolipoproteins A and E, von Willebrandt Factor, complement C3b, collagen**

- **Still, very limited set studied on limited set of materials surfaces**

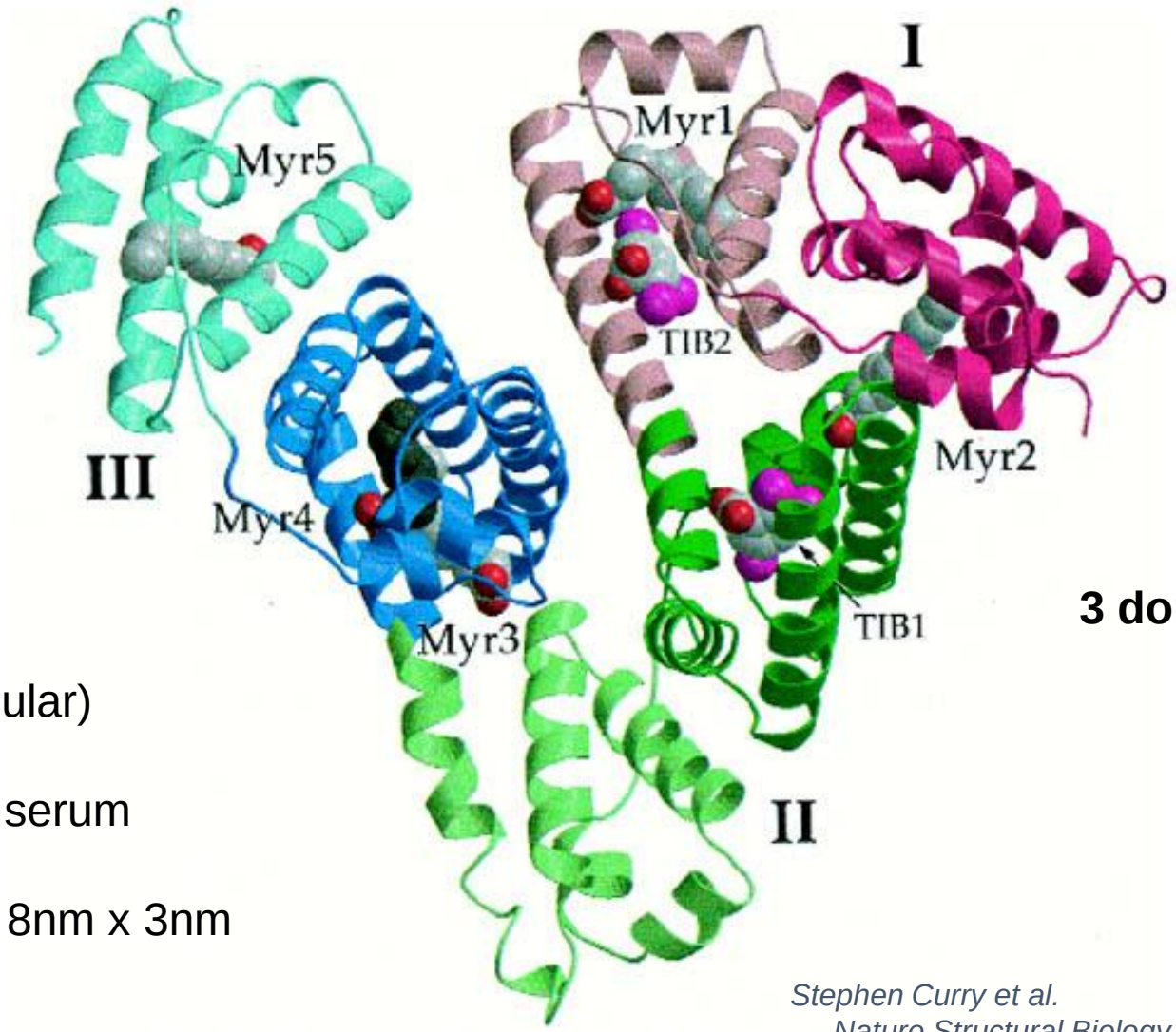
- **Let's look at the Big Three**

Biomaterials-relevance: proteins at surfaces

- Protein adsorption from single-component solutions is different from multi-component solutions
    - Isotherms show surface loading behavior for one protein as a function of conc.
- Equilibrium can be attained in minutes --> hours --> days, depending…..
- A typical protein monolayer is disorganized, denatured and about 350 ng/cm^2
- Multi-layers can form on top of an initial denatured layer (conc/species dependent)
- In competition, high affinity proteins win equilibrium (high k_{on}, low k_{off})
    - faster (high diffusivity) proteins find surface first (rapid diffusion)
    - are displaced later by proteins with higher “sticking coefficients” (affinity)
    - this is also concentration and protein dependent (*Vroman effect*)
- Competition between adsorbed proteins that cells and platelets recognize (adhesion proteins) and non-adhesion proteins (most) determines tissue/cell response
- Adsorbed proteins ‘signals’ combine with soluble cytokine ‘signals’ in vivo to produce a ubiquitous acute inflammatory response (might resolve)

- **All surfaces adsorb some protein (detection limit ~ 1 ng/cm^2 or about 0.3%)**

Serum's most abundant: Human serum albumin



3 domains

66kDa (globular)

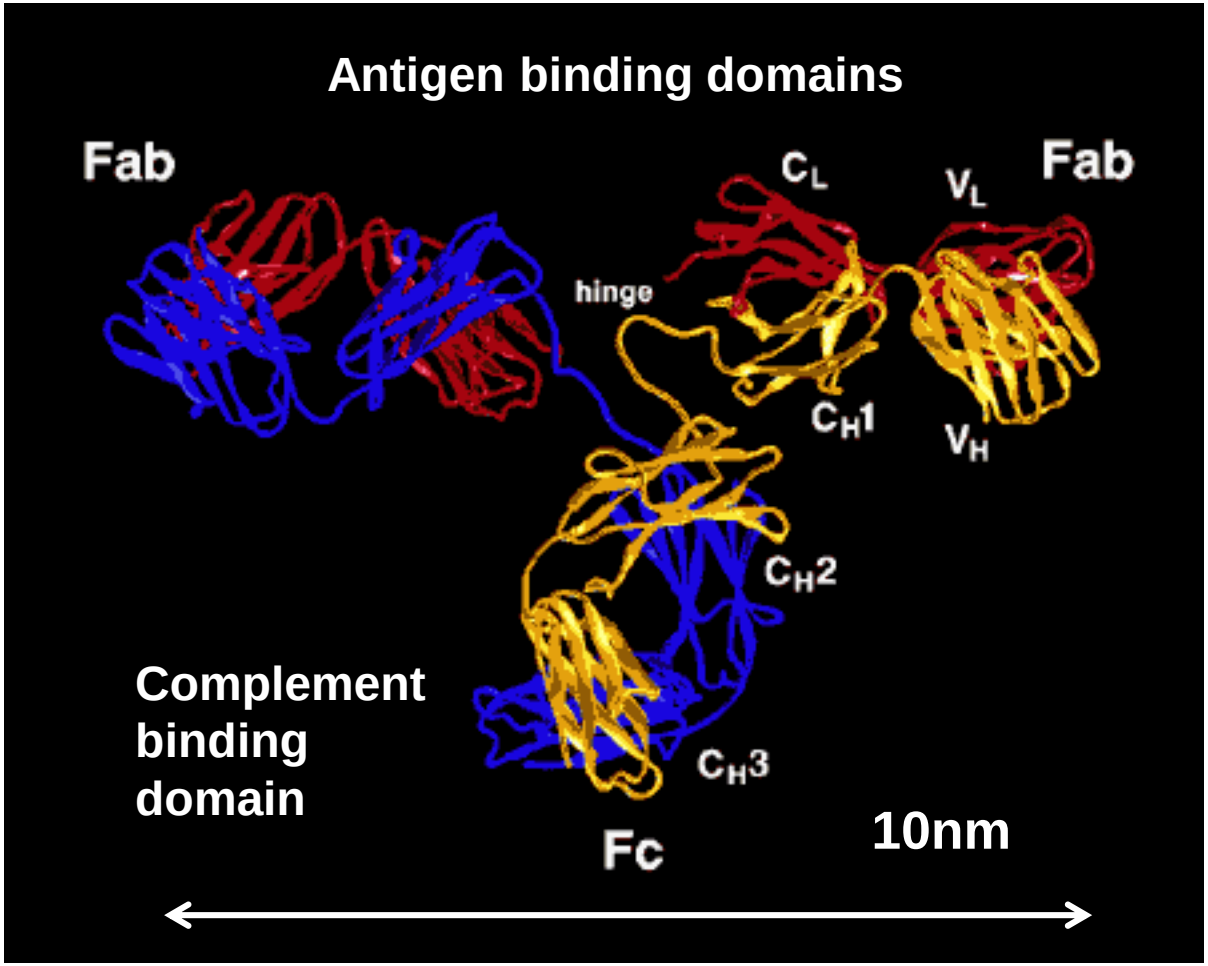
40 mg/ml in serum

Size: 3nm x 8nm x 3nm

Stephen Curry et al.
Nature Structural Biology 5, 827 - 835 (1998)

The Antibody: Immunoglobulin G (IgG)

Second-most abundant protein in serum, 160kDa, glycosylated, 10mg/ml in serum

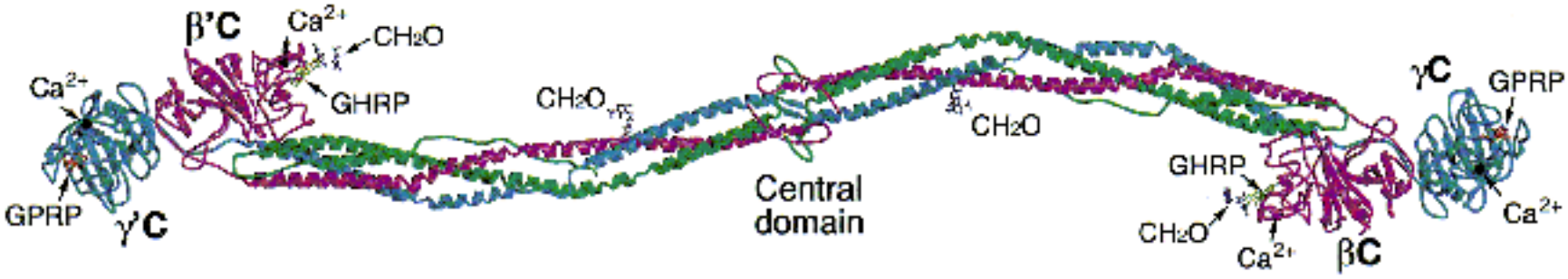


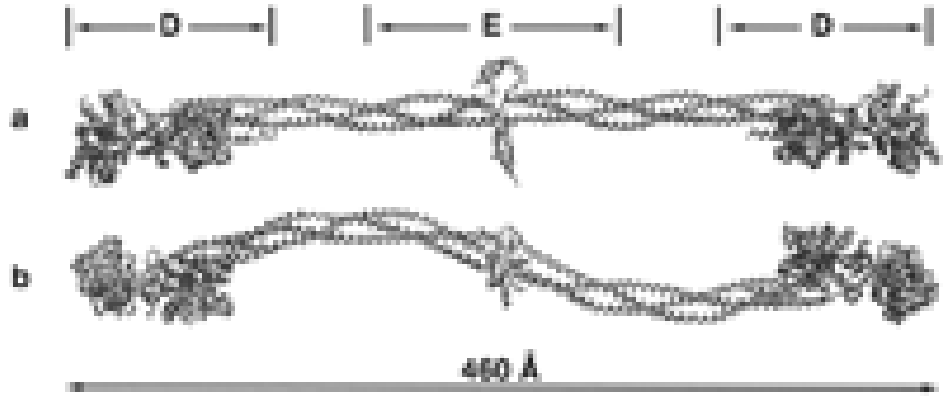
Multi-domain

8 nm 'thick'

IgG binds on one end to targets, interacts on the other with complement

Fibrinogen: #3 in serum

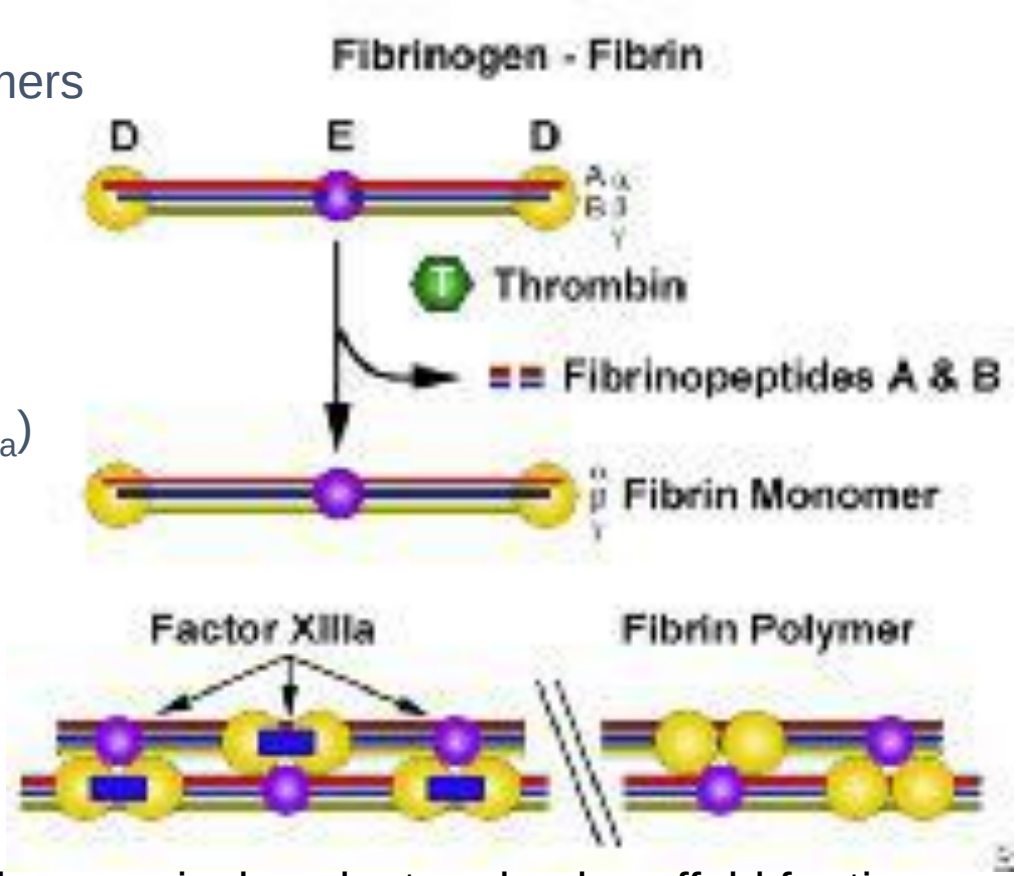




Globular multi-domain glycoprotein, 440kDa, 2 mg/ml in serum

Fibrinogen: responsible for blood clotting

- fibrinogen cleaves to fibrin monomers
- fibrin is crosslinked by FXIIIa:
  --> insoluble gel clot
- fibrin gel entraps platelets and activates platelets' integrin (gII_bIII_a) receptor
- cycle enhanced by platelet de-granulation



- Fibrin gel is FDA-approved, used as surgical sealant and gel scaffold for tissue engineering (Baxter, UVA)
- Fibrinogen deposition on biomaterials is linked to undesired blood coagulation and macrophage activation (inflammatory response)


5950B ESCA SPECTROMETER