Surfaces: Interactions of Proteins with Surfaces

Importance of Protein-Surface Interactions

- Modulate cell adhesion
- Trigger the biological cascade resulting in foreign body response
- Central to diagnostic array/sensor device design & performance
- Initiate other bioadhesion: e.g., marine fouling, bacterial adhesion

Fundamentals on Proteins

- Largest organic component of cells (~18 wt% /H_2O =70%); extra-cellular matrix, and plasma (7wt% /H_2O=90%).

- Many thousands exist—each encoded from a gene in DNA.

- Involved in all work of cells: ex, adhesion, migration, secretion, differentiation, proliferation and apoptosis (death).

- May be soluble or insoluble in body fluids.

  Insoluble proteins—structural & motility functions; can also mediate cell function (ex., via adhesion peptides)

  Soluble proteins—strongly control cell function via binding, adsorption, etc.

- Occur in wide range of molecular weights.

"Peptides" (several amino acids): hormones, pharmacological reagents

e.g., *oxytocin:* stimulates uterine contractions (9 a.a.)
aspartame: NutraSweet (2 a.a.)

"Polypeptides" (~10-100 amino acids): hormones, growth factors

e.g., *insulin:* 2 polypeptide chains (30 & 21 a.a.)
epidermal growth factor (45 a.a.)

"Proteins" 100's-1000's of amino acids

e.g., *serum albumin* (550 a.a.)
apolipoprotein B: cholesterol transport agent (4536 a.a.)

Protein Functions

- *Structural/scaffold*: components of the extracellular matrix (ECM) that physically supports cells

e.g., *collagen*—fibrillar, imparts strength;
elastin—elasticity to ligaments;
adhesion proteins: *fibronectin, laminin, vitronectin*—glycoproteins that mediate cell attachment (bonded to GAGs)

- *Enzymes*: catalyze rxns by lowering E_a thru stabilized transition state, via release of binding energy

e.g., *urease*—catalyzes hydrolysis of urea

Protein Functions (cont.)

- *Transport*: bind and deliver specific molecules to organs or across cell membrane

e.g., *hemoglobin* carries bound O_2 to tissues;
serum albumin transports fatty acids

- *Motile:* provide mechanism for cell motion e.g., via (de)polymerization & contraction

e.g., *actin, myosin* in muscle

Protein Functions (cont.)

- *Defense:* proteins integral to the immune response and coagulation mechanism

e.g., *immunoglobulins (antibodies)*—Y-shaped proteins that bind to antigens (foreign proteins) inducing aggregate formation

fibrinogen & thrombin—induce clots by platelet receptor binding

- *Regulatory:* cytokines—regulate cell activities

e.g., *hormones: insulin* (regulates sugar metabolism); *growth factors*

Protein Structure

- Proteins have multiple structural levels.

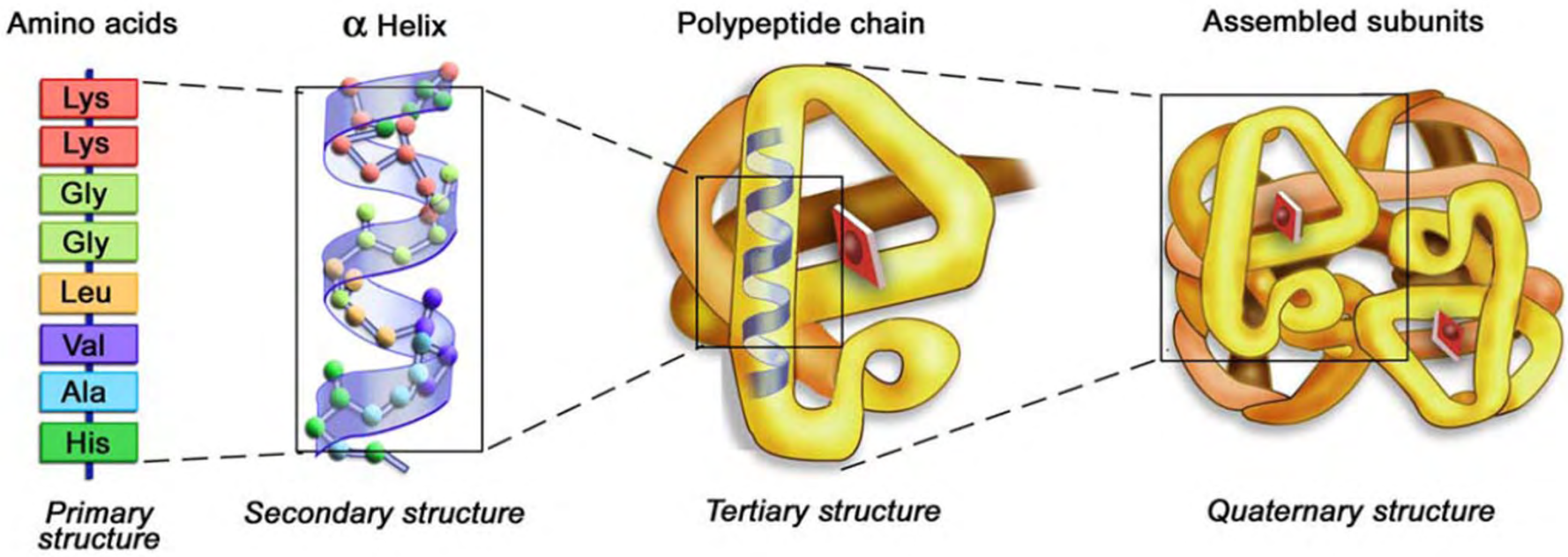
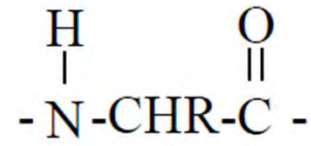


Figure by MIT OCW.

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

Protein Structure

1. Primary Structure

- comprised of amino acid residues: 
- peptide (amide) bond CONH is effectively rigid & planar (partial double-bond character)
- directional character to bonding: amino acids are L stereoisomers

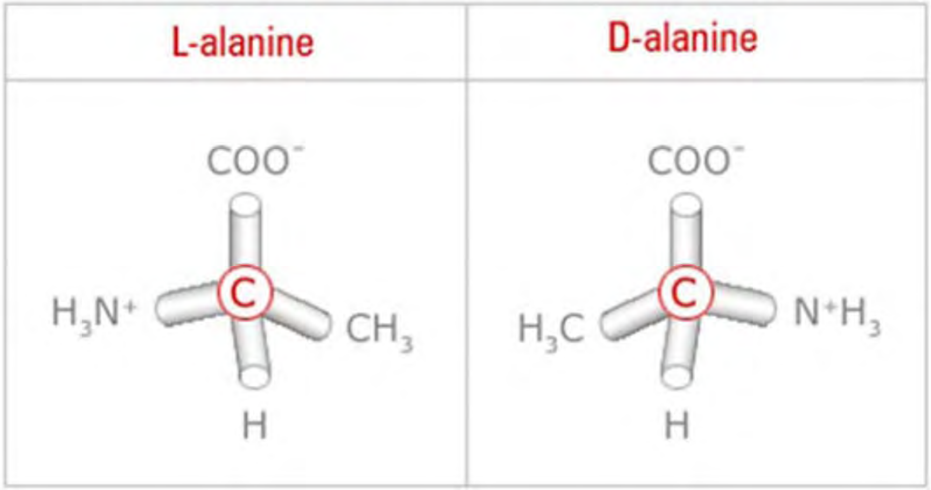


Figure by MIT OCW.

[after A. L. Lehninger, D. L. Nelson and M. M. Cox, Principles of Biochemistry, pg. 115.]

Protein Structure

- AA side groups have variable chemical character

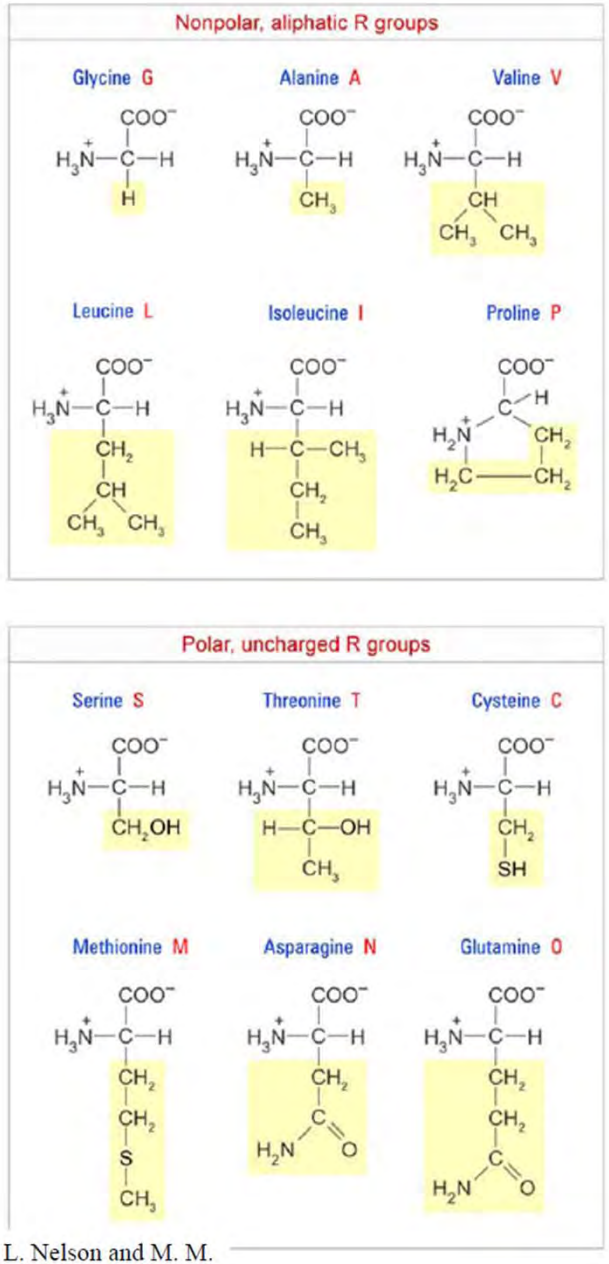


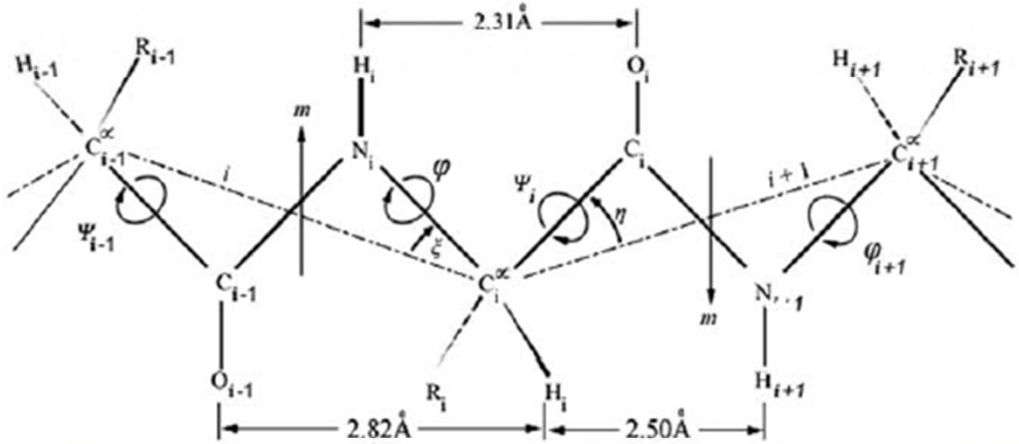
Figure by MIT OCW.

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

Protein Structure

2. Secondary Structure

Spatial configuration determined by the rotation angles φ_i & ψ_i about the single bonds of the α-carbons



[after P. J. Flory. *Statistical Mechanics of Chain Molecules*, pg. 251.]

(φ_i ,ψ_i) are independent of (φ_{i+1},ψ_{i+1})

Figure by MIT OCW.

β–sheets

- backbone has extended "zigzag" structure
- stabilized by intermolecular H-bonding between –NH and C=O of adjacent chains

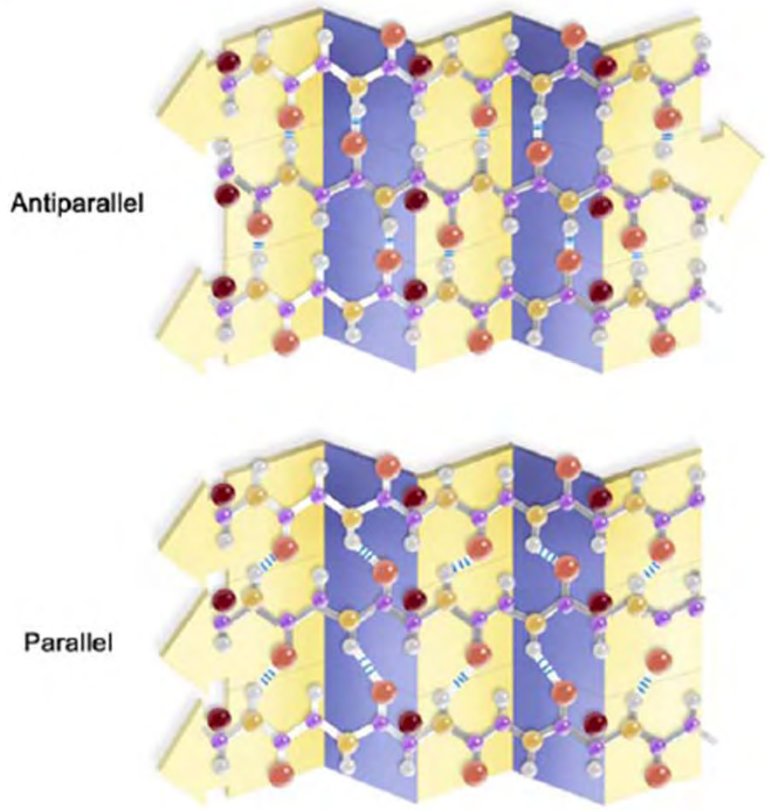


Figure by MIT OCW.

[after A. L. Lehninger, D. L. Nelson and M. M. Cox. *Principles of Biochemistry*, pg. 169.]

α–helices

- stabilized by intramolecular H-bonding between C=O of residue *i* and –NH of residue *i+3* (requires all L or D stereoisomers)

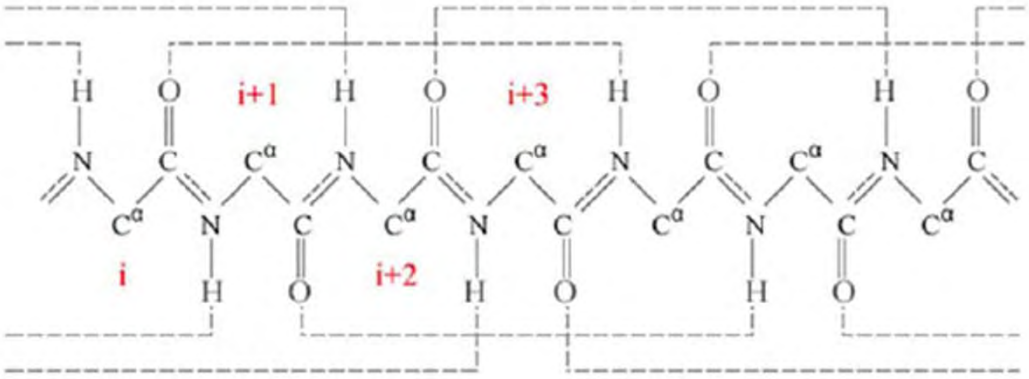
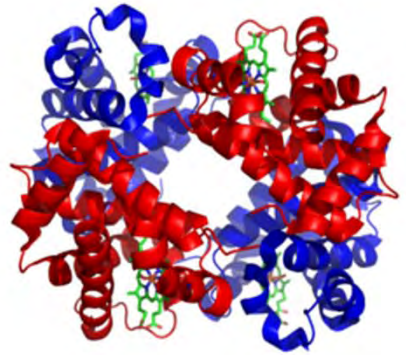


Figure by MIT OCW.

[after P. J. Flory. *Statistical Mechanics of Chain Molecules*, pg. 287]

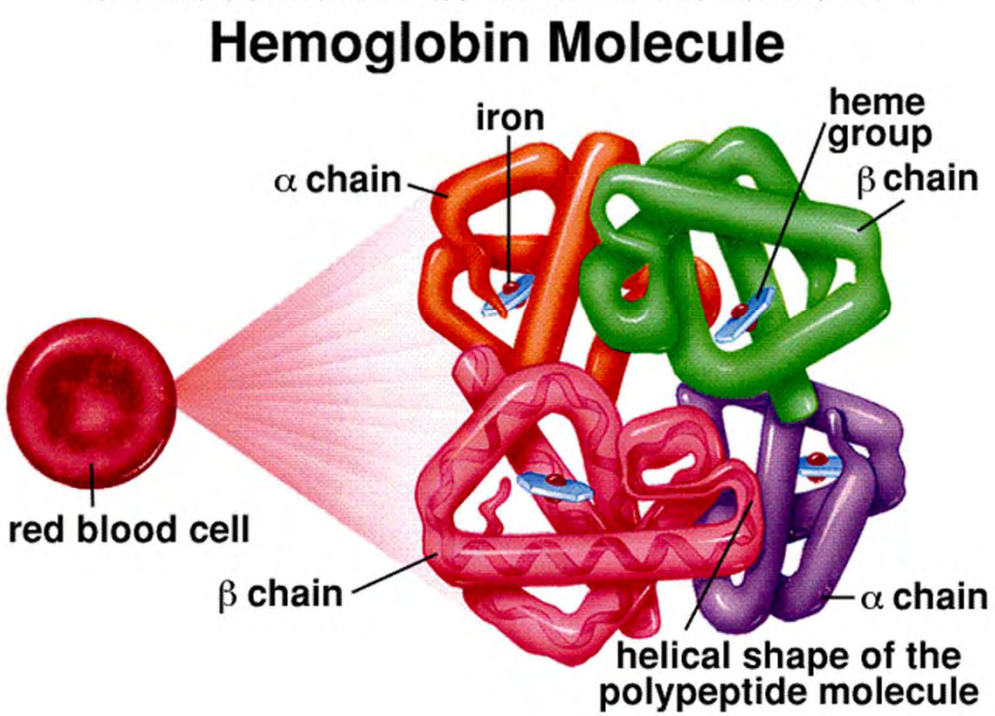
- natural abundance
  - most common secondary structure in proteins
  - in fibrous proteins: α–keratins (hair, skin,…)
  - in globular proteins: avg. ~25% α–helix content

3. Tertiary & Quaternary Structure



- Tertiary: folded arrangements of secondary structure units
- Quaternary: arrangements of tertiary (polypeptide) units

Example: hemoglobin



Synthetic Polymers vs. Proteins

Property	Synthetic Polymers	Polypeptides
Molecular Wt.	$1000\text{-}10^6$ g/mol	$1000\text{-}10^6$ g/mol (typ. <2000 a.a.)
Molecular Wt. Distribution	Always > 1 (M_w/M_n)	Always ≡1
Sequence	i. 1-3 types of repeat units ii. many chemistries	i. many side groups ii. always amides
Solution Structure	Random coils or self-avoiding random coils $R_g \sim N^{0.5}$ (θ solvent) $R_g \sim N^{0.6}$ (good solvent)	Globular –"condensed" chains ($\rho \sim 1.36$ g/cm^3) (hydrophobic R groups sheltered from H_2O) $R_g \sim N_{aa}^{0.33}$
Available Conformations	$\Omega_{ran} \sim z^N$ (z = # n.n.) $\Omega_{SA} \sim z'^N N^{1/6} << \Omega_{ran}$	Ω ~1 (can ↑ if bound or adsorbed!)
Secondary Interactions	van der Waals, H-bonds, electrostatic, "hydrophobic effect"	Same as synthetic, with *" lock-and-key "* topology

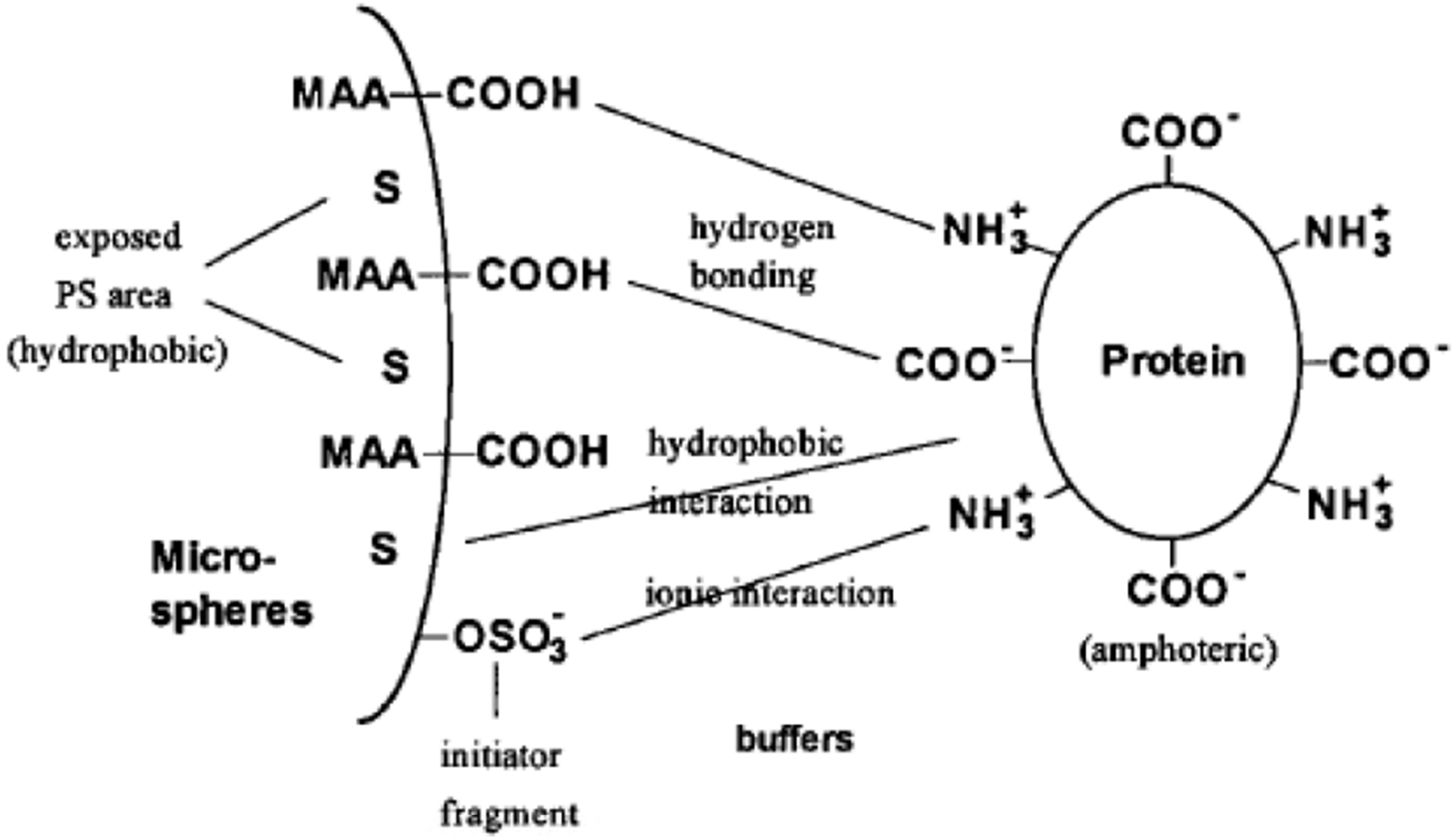
Protein Adsorption on Biomaterial Surfaces

Polypeptides can *transform* to "random coil" conformations, through:

- changes in temperature
- changes in soln. pH or composition (e.g., added salts, urea)
- adsorption to surfaces

⇒ *changes physiological function!*

- In as short a time as can be measured after implantation in a living system (< 1 sec), proteins are already observed on biomaterial surfaces.
- Seconds to minutes: a monolayer of protein absorbs to most surfaces
- Protein adsorption occurs well before arrival of cells – thus cells primarily interact with a protein layer, rather than actual biomaterial surface



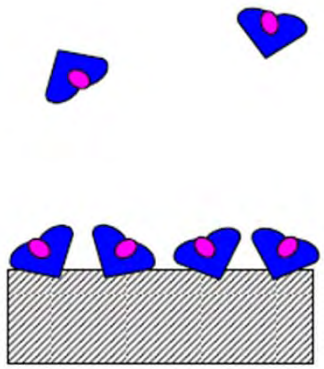
Overall schematic representation of the protein adsorption on carboxylated microspheres. Many types of interactions.

Protein Adsorption on Biomaterial Surfaces

Background

A) Protein activity varies in adsorbed vs. solvated state

Why???

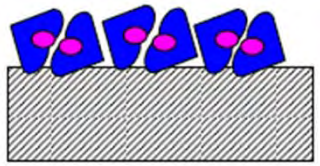


1. higher local concentration— function may be conc. dependent

e.g., cell adhesion increases with adhesion peptide concentration

2. change in reactivity—access to "active" a.a. sequence ↑ or ↓

⇒ enhanced or reduced binding capability

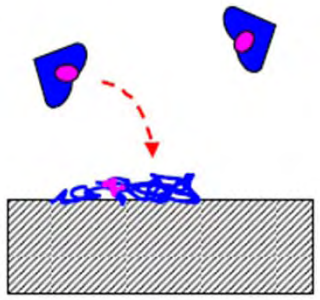


e.g., fibrinogen: platelets adhere when adsorbed, not in soln.

3. denaturation—conformation varies from soln. conformation

⇒ different a.a. sequences exposed

*enhance or deactivate normal function
*elicit unintended function



e.g., natural polymers used as biomaterials are more immunogenic than synthetic polymers

Protein Adsorption on Biomaterial Surfaces

Background

B. Entropic forces

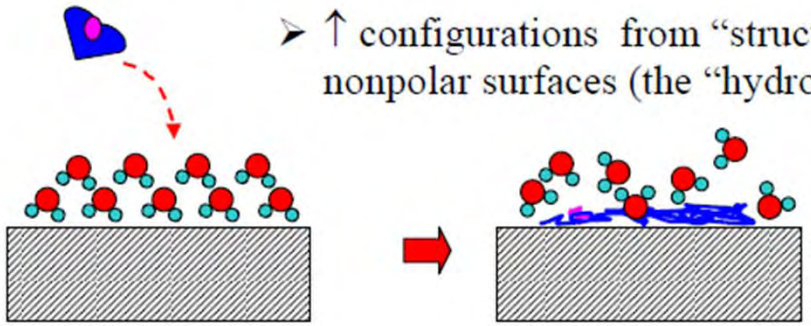
1. secondary bond formation

electrostatic > H-bonding > dispersive

BOND ENERGY

Depends on material's surface chemistry

2. entropic forces



- ↑ configurations from "structured" H_2O adjacent to nonpolar surfaces (the "hydrophobic effect")

- less translational entropy loss (ΔS_{mix}) for adsorbed proteins (macromolecules) vs. H_2O

$$\frac{\Delta S_{mix}}{k} = n_p \ln \phi_p + n_{H_2O} \ln \phi_{H_2O}$$

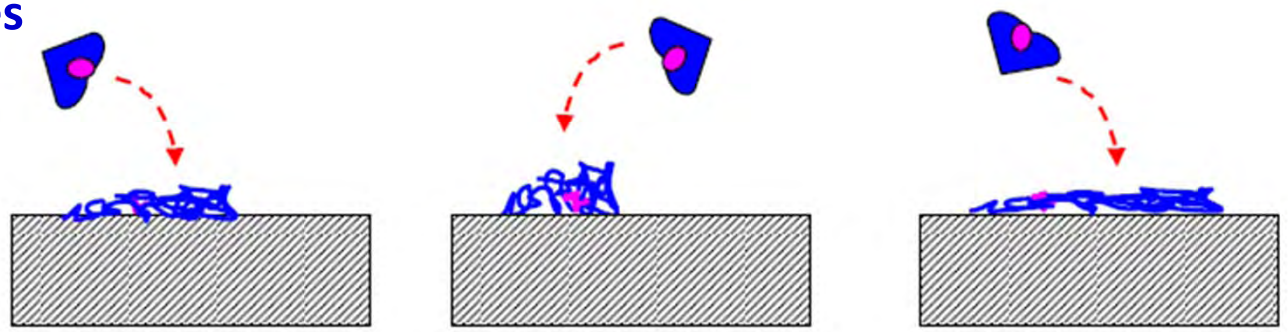
For a given ϕ_p, n_p decreases as protein MW ↑ ⇒ ↓ ΔS_{mix}

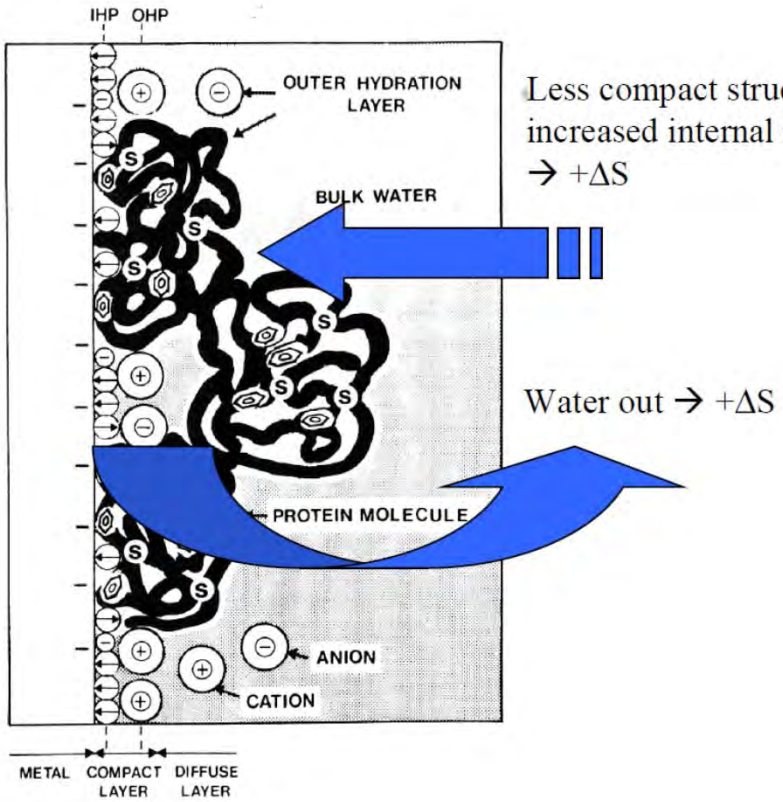
Protein Adsorption on Biomaterial Surfaces

Background

- ↑ configurations for denatured vs. solvated proteins

B. Entropic forces





Less compact structure, i.e. increased internal mobility → +ΔS

Water out → +ΔS

Gain in entropy probably is the main driving force during spontaneous protein adsorption!!

Protein Adsorption on Biomaterial Surfaces

Background

C. Adsorbed proteins initiate physiological responses to biomaterials

c) Adsorbed proteins initiate physiological responses to biomaterials

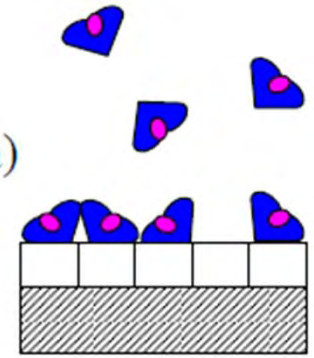
- coagulation mechanism
- alternative pathway of complement system (vs. antigen-antibody)
- *in vitro* protein adsorption experiments → 1st test of "biocompatibility"

Models for Protein Adsorption: Langmuir Model

The simplest picture: *Langmuir model* for reversible adsorption

Makes analogy to chemical reaction kinetics:

[P] = protein concentration in solution (e.g., #/vol)
[S] = density of unoccupied surface sites (e.g., #/area)
[PS] = density of surface sites occupied by protein



$P + S \leftrightarrow PS$

Assumes: 1 protein binds 1 surface "site"—
can involve multiple secondary bonds

Assuming the "reaction" follows 1st order kinetics:

adsorption rate = $k_a[P][S]$
desorption rate = $k_d[PS]$

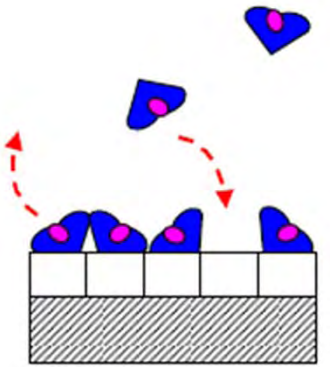
Assumes: dilute [P] (in plasma: 90% H_2O)

Models for Protein Adsorption: Langmuir Model

At equilibrium: adsorption rate = desorption rate

$$k_a[P][S] = k_d[PS]$$

Assumes: reversibility



Can define an "affinity" const, K (or K_a): $K = k_a/k_d = \dfrac{[PS]}{[P][S]}$

(a.k.a. "binding" or "association" const; typical units = L/mol)

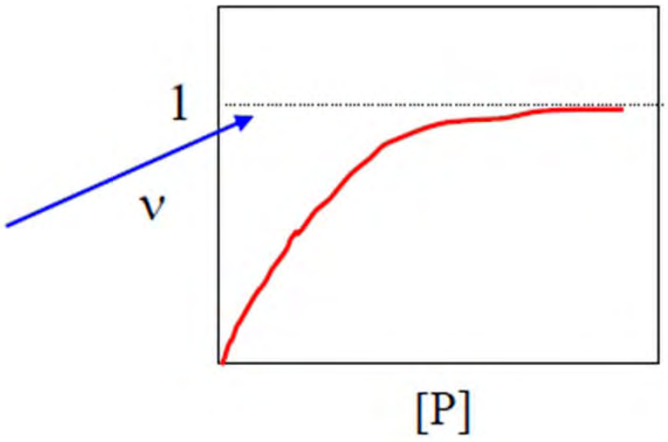
Models for Protein Adsorption: Langmuir Model

K obtained experimentally by measure of fraction occupied sites:

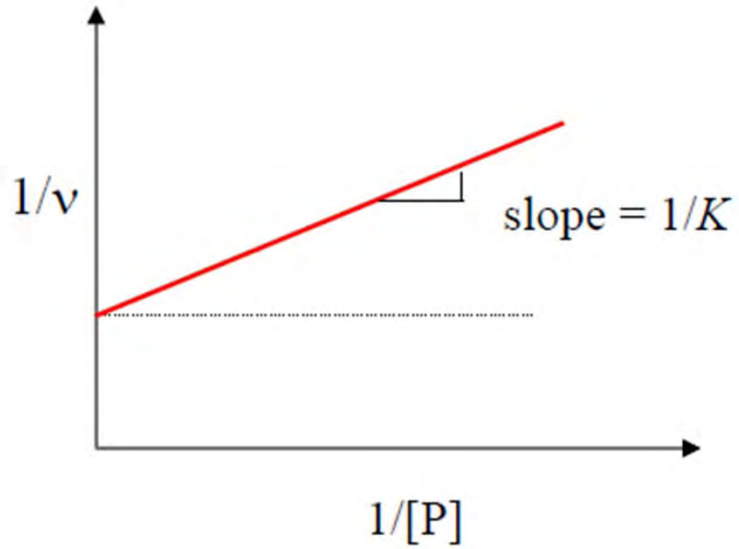
ν = # filled sites/total # surface sites

$$\nu = \frac{[PS]}{([S]+[PS])} = \frac{K[P]}{(1+K[P])}$$

Binding plateaus at ν=1, monolayer coverage.



To obtain *K*:



Models for Protein Adsorption: Langmuir Model

K_a is an indicator of the favorableness of adsorption. Note that K_a is the inverse of the dissociation constant, K_d, which has units of concentration, e.g., mol/L.

- For [P] < K_d, few occupied surface sites.
- For [P] = K_d, half of the surface sites will be occupied.

Models for Protein Adsorption: Scatchard Plot

A second approach used to extract K is known as a Scatchard plot.

Rearranging: $$K[S] = \frac{[PS]}{[P]}$$

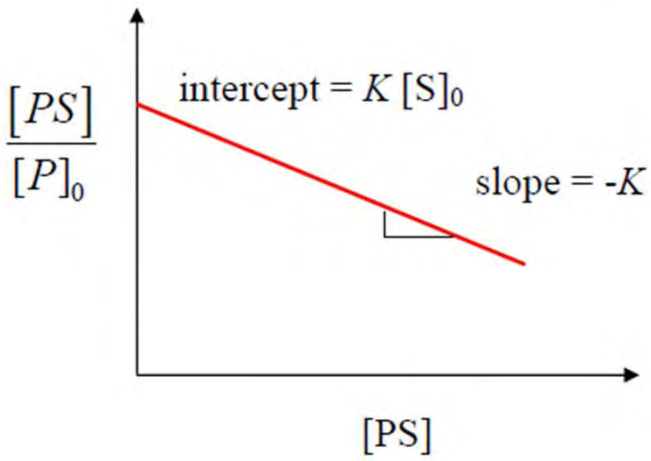
Defining the total number of surface sites: $[S]_0 = [S] + [PS]$,

And substituting for [S]: $$K([S]_0 - [PS]) = \frac{[PS]}{[P]}$$

If the protein solution concentration is not significantly depleted upon adsorption, then [P] ≈ $[P]_0$ (the initial protein concentration):

$$\frac{[PS]}{[P]_0} = -K[PS] + K[S]_0$$

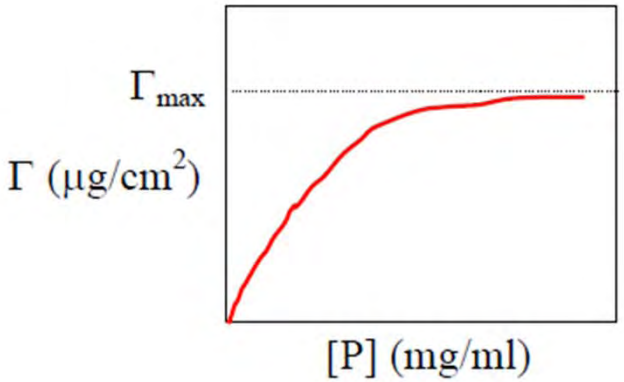
Scatchard Plot



Provides a measure of $[S]_0$

Models for Protein Adsorption: Scatchard Plot

In adsorption experiments, the value usually measured is a surface concentration, e.g., ng/cm^2 or µg/cm^2 – often denoted as Γ or θ



Surface Sensitive Measurements Necessary

1. Ellipsometry
2. Surface Plasmon Spectroscopy
3. Quartz Crystal Microbalance

If we assume a *monolayer* coverage at Γ_{max}, we can calculate the *effective area per protein molecule* on the surface:

$$A_{eff} = \frac{M_{protein}}{N_{Av}\Gamma_{max}}$$

➡ Related to protein conformation on surface!

Note that $[S]_0$ (in #/area) is the inverse of the area per molecule:

$$A_{eff} = \frac{1}{[S]_0}$$

Summary: Protein Adsorption

Pro-adsorption factors

- Dehydration of the interface
- Increase of the protein interior mobility
- Columbic interactions
- Van der Wall interactions

Anti-adsorption factors

- Strong water binding to interface
- Protein rigidity
- Surface mobility
- Low net charge

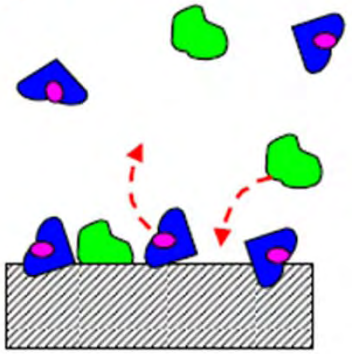
Surfaces: Interactions of Proteins with Surfaces: Part 2

Protein-Surface Interactions: Part 2

The Langmuir model is applicable to numerous reversible adsorption processes, but fails to capture many aspects of protein adsorption.

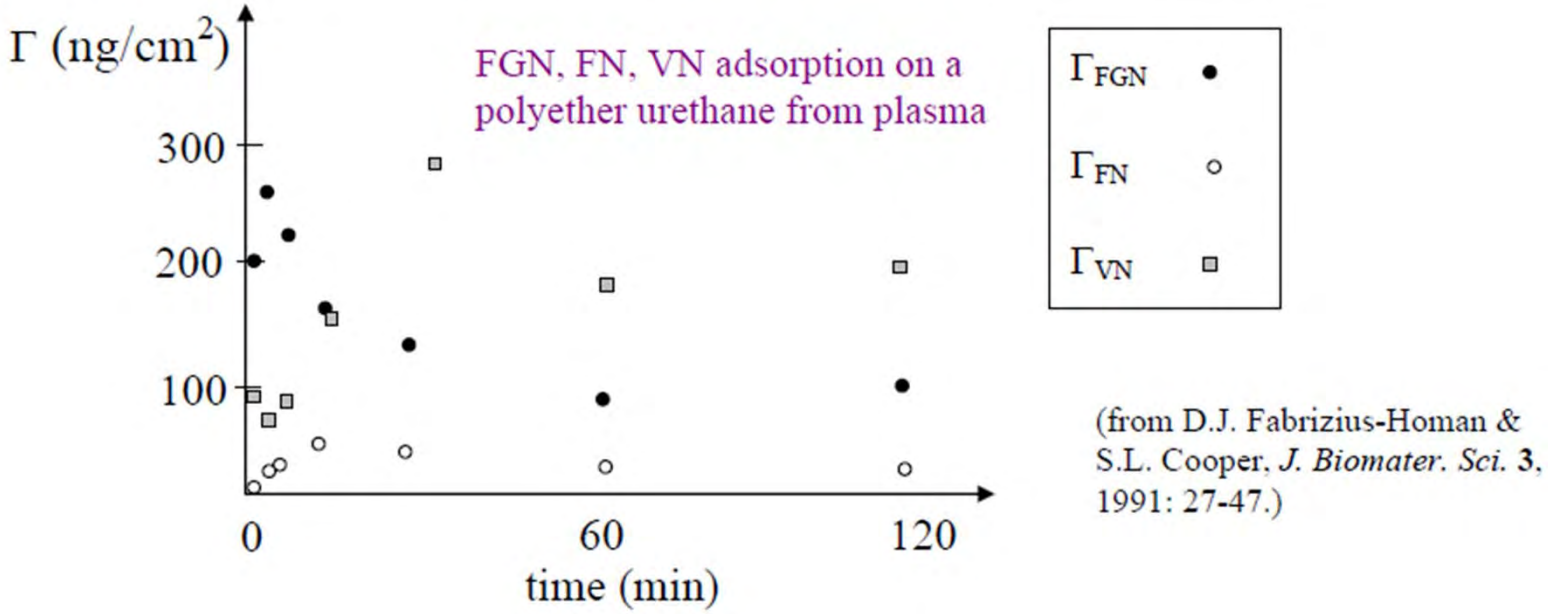
1. Competitive Adsorption

- many different globular proteins *in vivo*
- surface distribution depends on $[P_i]$'s & *time*



Vroman Effect

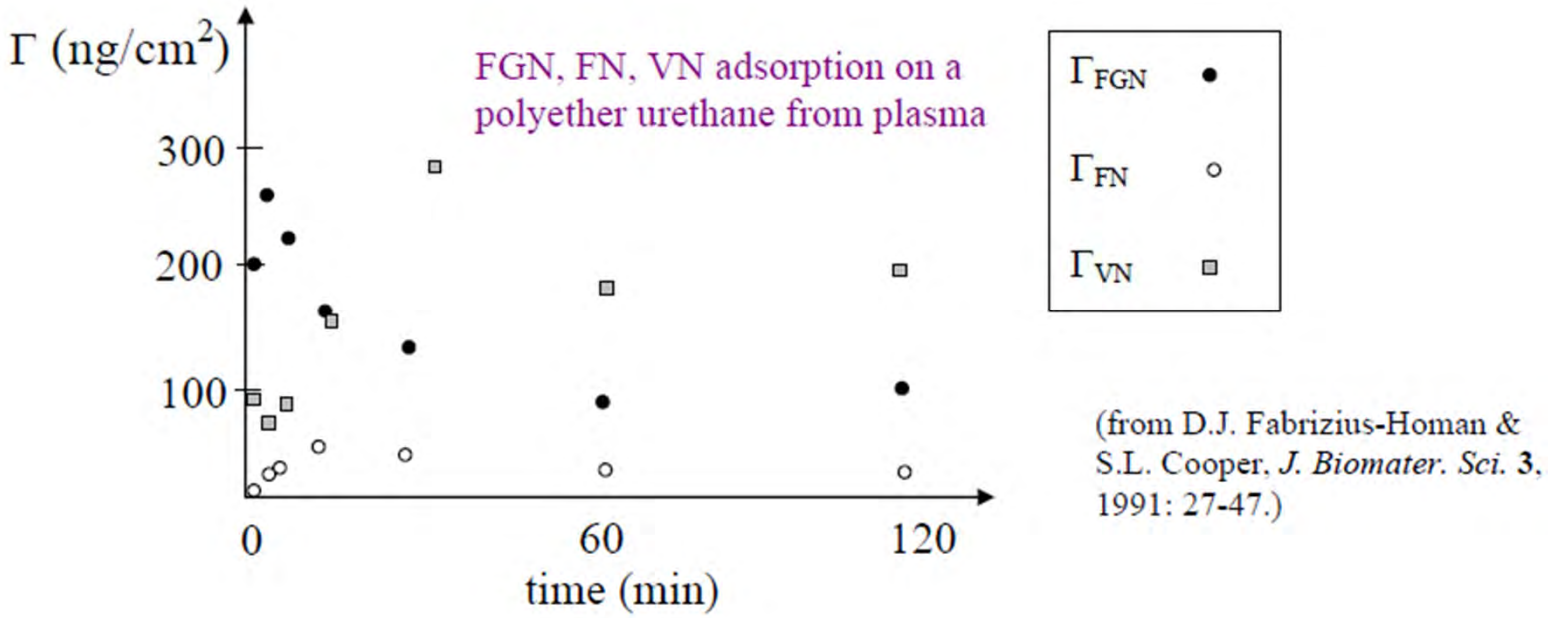
The Vroman effect: Displacement (over time) of initially adsorbed protein by a second protein.



(from D.J. Fabrizius-Homan & S.L. Cooper, *J. Biomater. Sci.* **3**, 1991: 27-47.)

Protein	Plasma conc. (mg/ml)	MW (Daltons)
Human serum albumin	42	68,500
Immunoglobulins	28	145,000 (IgG)
Fibrinogen	3.0	340,000
Fibronectin	0.3	240,000
Vitronectin	0.2	60,000

Plasma – fluid component of blood with anticoagulant added
Serum – fluid component of blood with coagulants removed



(from D.J. Fabrizius-Homan & S.L. Cooper, *J. Biomater. Sci.* **3**, 1991: 27-47.)

Observations

At t~0: uniform $[P_i]$'s everywhere ⇒ protein with highest concentration dominates initial adsorption

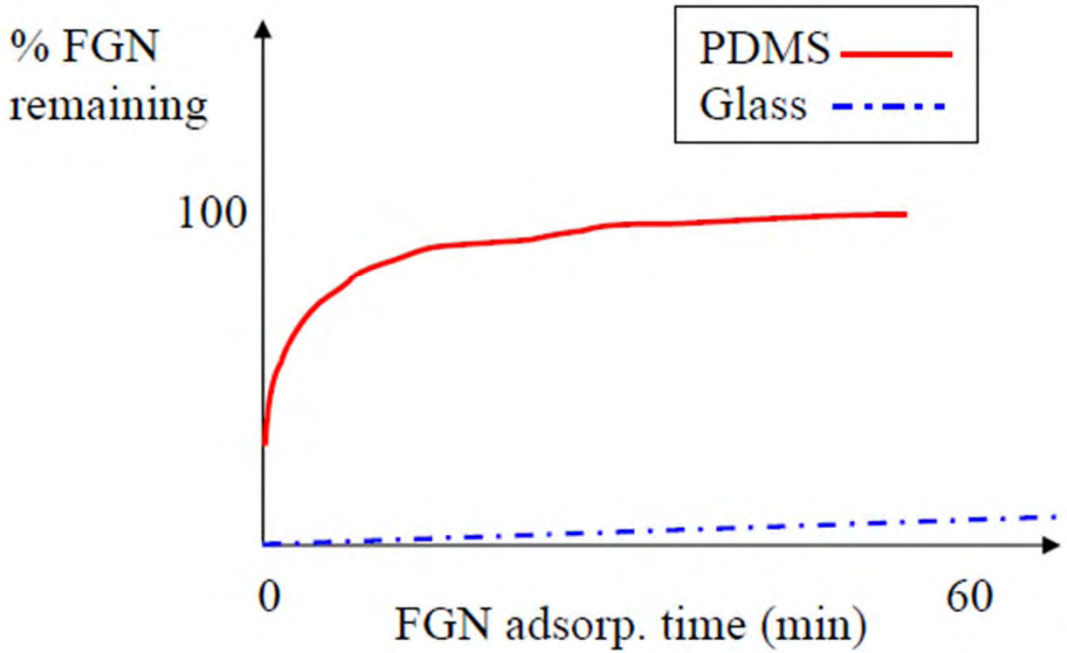
At t>0: local depletion of adsorbed species near surface– exchange with faster diffusing species ensues

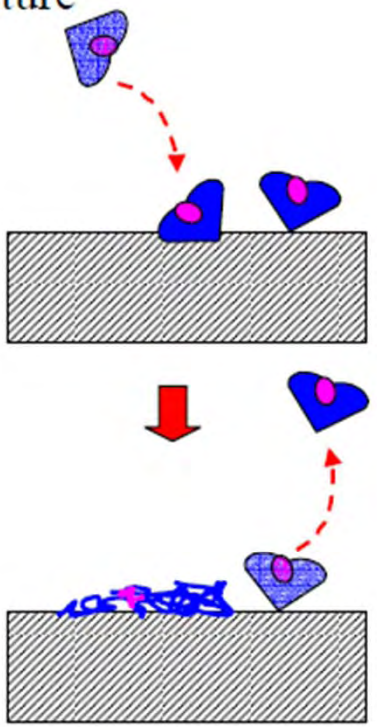
At t>>0: gradual exchange with higher affinity species

2. Irreversible Adsorption

- occurs *in vivo* & *in vitro:* proteins often do not desorb after prolonged exposure to protein solutions
- complicates the competitive adsorption picture

Surfaces exposed to plasma *after adsorption* of FGN





(from S.M. Slack and T.A. Horbett, *J. Colloid & Intfc Sci.* **133**, 1989: 148.)

Irreversible Adsorption

Physiological implications:

a) hydrophobic surfaces cause more denaturing

b) denatured proteins may ultimately desorb (by replacement) $\Rightarrow$ non-native solution behavior

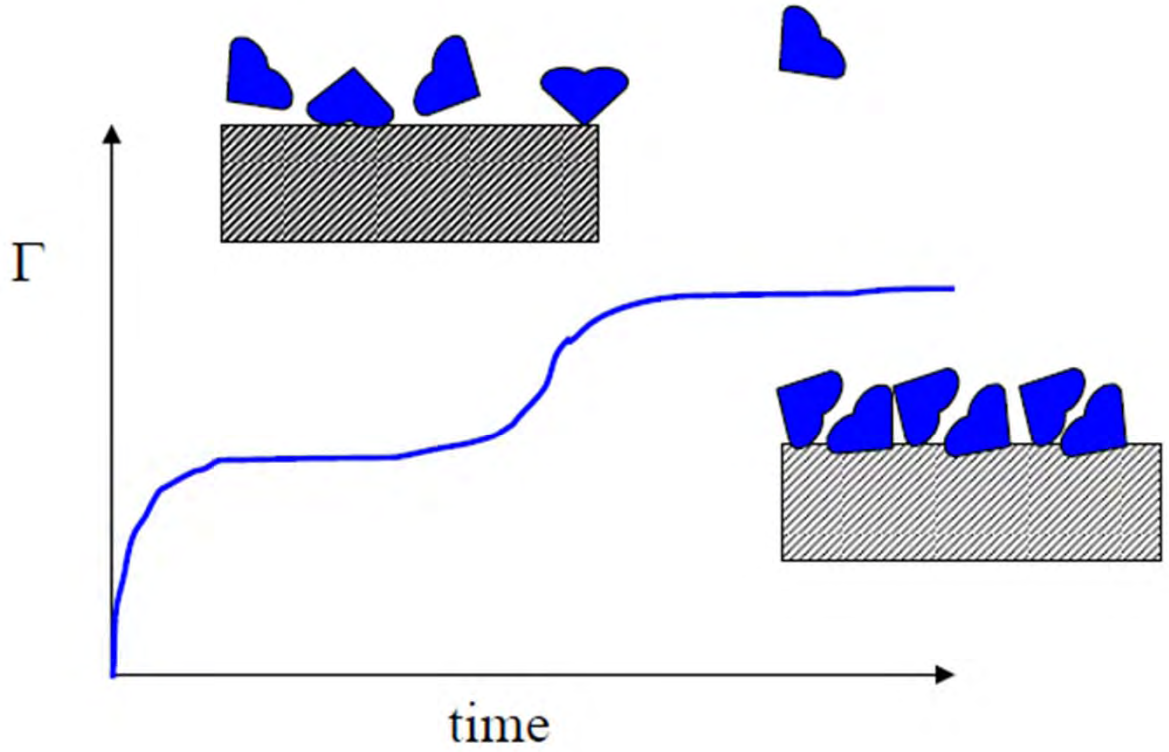
Models that attempt to account for 1 & 2:

S.M. Slack and T.A. Horbett, *J. Colloid & Intfc Sci*. 133, 1989 p. 148
I. Lundstroem and H. Elwing, *J. Colloid & Intfc Sci*. 136, 1990 p. 68
C.F. Lu, A. Nadarajah, and K.K. Chittur, *J. Coll. & Intfc Sci*. 168, 1994 p. 152

Restructuring

3. Restructuring

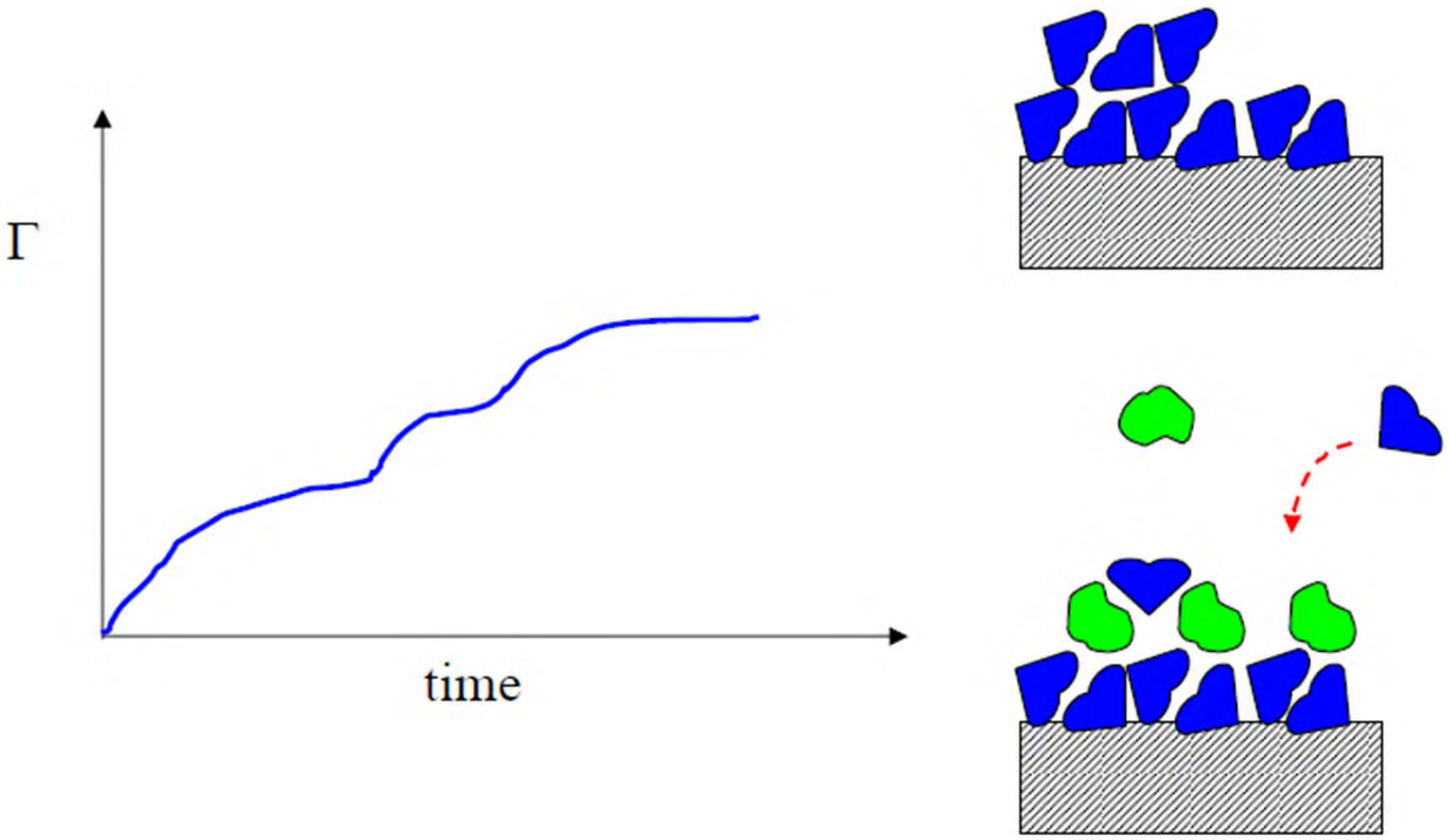
- Protein layers reaching monolayer saturation can reorganize (e.g., crystallize) on surface, creating a stepped isotherm



Multilayer Formation

4. Multilayer Formation

- Proteins can adsorb atop protein monolayers or sublayers, creating complicated adsorption profiles



Measurement of Adsorbed Protein

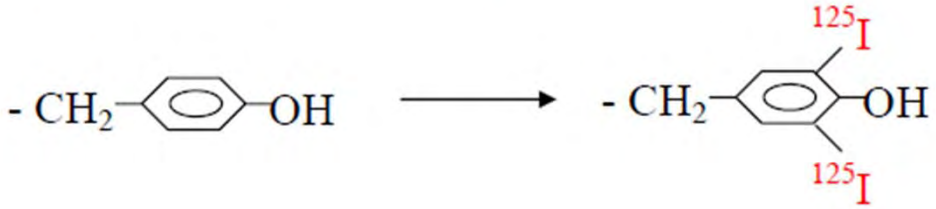
1) Techniques for quantifying adsorbed amounts

a) *Labeling Methods*: tag protein for quantification, use known standards for calibration

i) *Radioisotopic labeling*

- proteins labeled with radioactive isotopes that react with specific a.a. residues

e.g., tyrosine labeling with ^{125}I; ^{131}I; ^{32}P



- Small % radioactive proteins added to unlabelled protein
- γ counts measured and calibrated to give cpm/μg

Advantage: high signal-to-noise ⇒ measure small amts (ng)

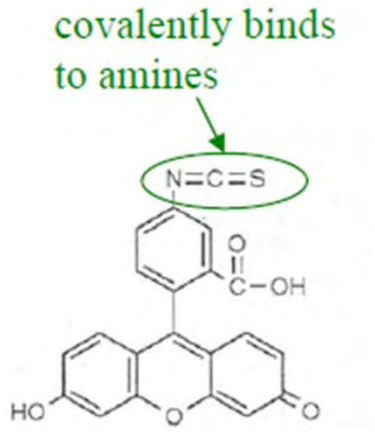
Disads: dangerous γ emissions, waste disposal, requires protein isolation

Measurement of Adsorbed Protein

ii) *Fluorescent labels*

- measure fluorescence from optical excitation of tag

e.g., fluorescein isothiocyanate (FITC)



Advantage: safe chemistry

Disads: tag may interfere with adsorption, requires protein isolation, low signal

Measurement of Adsorbed Protein

iii) *Staining*

- molecular label is adsorbed to proteins *post facto*

e.g., organic dyes; antibodies (e.g, FITC-labeled)

Advantages: safe chemistry, no protein isolation/modification

Disads: nonspecific adsorption of staining agents (high noise)

b) *Other Quantification Methods*

i) HPLC on supernatants (w/ UV detection)

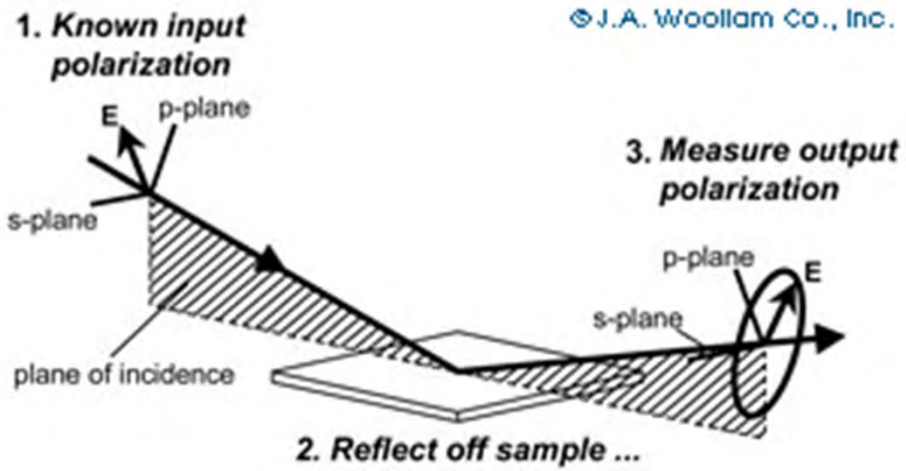
ii) XPS signal intensity, e.g., N^{1s} (relative to controls)

iii) Ellipsometry—adsorbed layer thickness (dry)

Measurement of Adsorbed Protein

2) Techniques for quantifying adsorbed amounts

- **In-Situ Ellipsometry**



- polarized light reflected from a surface
- phase & amplitude changes to parallel (p) and perpendicular (s) E-field components

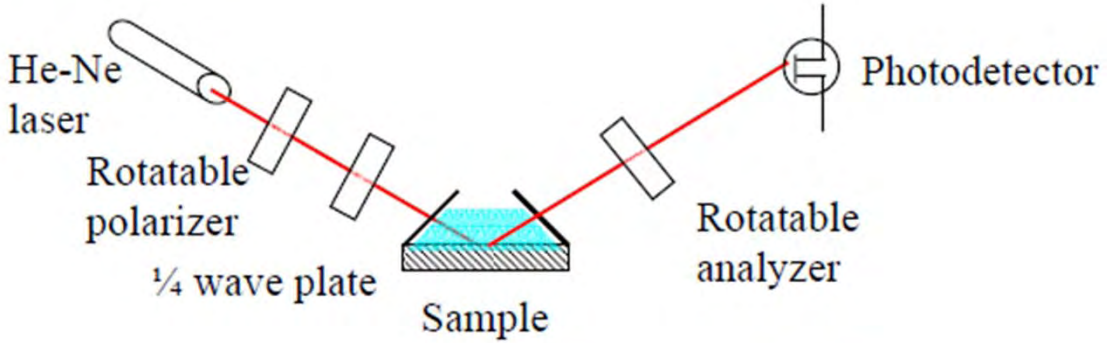
E_i , E_r = incident/reflected E-field

reflection coefficients: $r_p = \frac{E_{rp}}{E_{ip}} = |r_p| \cdot e^{i\delta_p}$ and $r_s = \frac{E_{rs}}{E_{is}} = |r_s| \cdot e^{i\delta_s}$

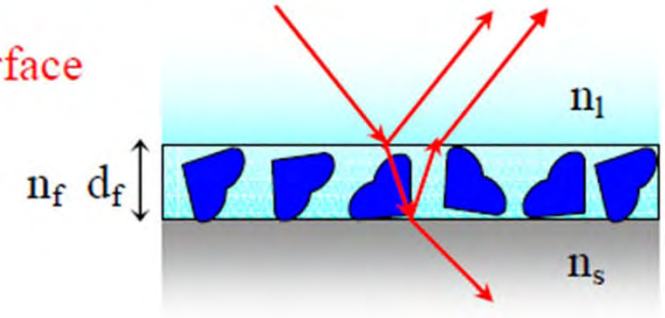
ratio of amplitudes: $\tan\Psi = \frac{|r_p|}{|r_s|}$ phase difference: $\Delta = \delta_p - \delta_s$

Ellipsometry

- Experimental set-up



Proteins adsorbed to a surface



Adsorbed protein layer changes the refractive index adjacent to the substrate.

- Ellipsometric angles Ψ and Δ can be converted to adsorbed layer thickness (d_f) & refractive index (n_f) assuming 3-layer model & Fresnel optics

Ellipsometry

- adsorbed amount: $\Gamma = d_f \frac{n_f - n_l}{dn/dc}$

R.I. increment of protein solution vs. protein conc. (~0.2 ml/g)

Advantages: no protein isolation; fast; easy; *in situ*; sensitive

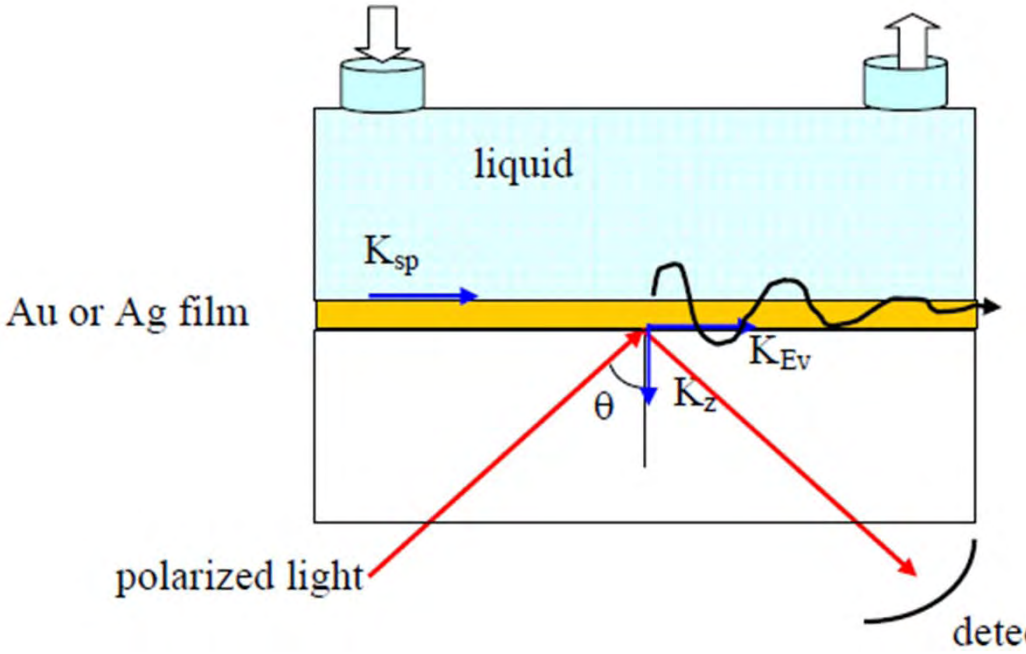
Disads: quantitation requires a model, optically flat & reflective substrates required; can't distinguish different proteins

References:
P. Tengvall, I. Lundstrom, B. Liedburg, *Biomaterials* **19**, 1998: 407-422.
H.G. Tompkins, A User's Guide to Ellipsometry, Academic Press: San Diego, 1993.

Surface Plasmon Resonance Spectroscopy (SPR)

b) *Surface Plasmon Resonance*

- Experimental set-up: polarized light reflects at interface between glass with deposited metal film and liquid flow cell



Total internal reflection

For $\theta > \theta_{critical}$, transmitted intensity decays exponentially into liquid (evanescent wave).

Analogous to QM tunneling—wave at a boundary

Surface Plasmon Resonance Spectroscopy (SPR)

- Theoretical basis:
  - light traveling through high *n* medium (glass) will reflect back into that medium at an interface with material of lower *n* (air/water)
  - total internal reflection for $\theta > \theta_{critical}$

$$\theta_{critical} = \sin^{-1}\left(\frac{n_{low}}{n_{high}}\right)$$

  - surface plasmons—charge density waves (free oscillating electrons) that propagate along interface between metal and dielectric (protein soln)
  - coupling of evanescent wave to plasmons in metal film occurs for $\theta = \theta_{spr}$ ($> \theta_{critcal}$) corresponding to the condition:

$$K_{sp} = K_{Ev}$$

Surface Plasmon Resonance Spectroscopy (SPR)

c/ω_0 = incident light λ

ε_{metal} = metal dielectric const.

K_{sp}, K_{Ev} = wavevector of surface plasmon/evanescent field

$$K_{Ev} = n_{glass}\frac{\omega_0}{c}\sin\theta$$

$$K_{sp} = \frac{\omega_0}{c}\sqrt{\frac{\varepsilon_{metal} n_{surface}^2}{\varepsilon_{metal} + n_{surface}^2}}$$

- Energy transfer to metal film reduces reflected light intensity
- change of $n_{surface}$ due to adsorption of protein at interface will shift θ_{spr} where $K_{sp} = K_{Ev}$

Surface Plasmon Resonance Spectroscopy

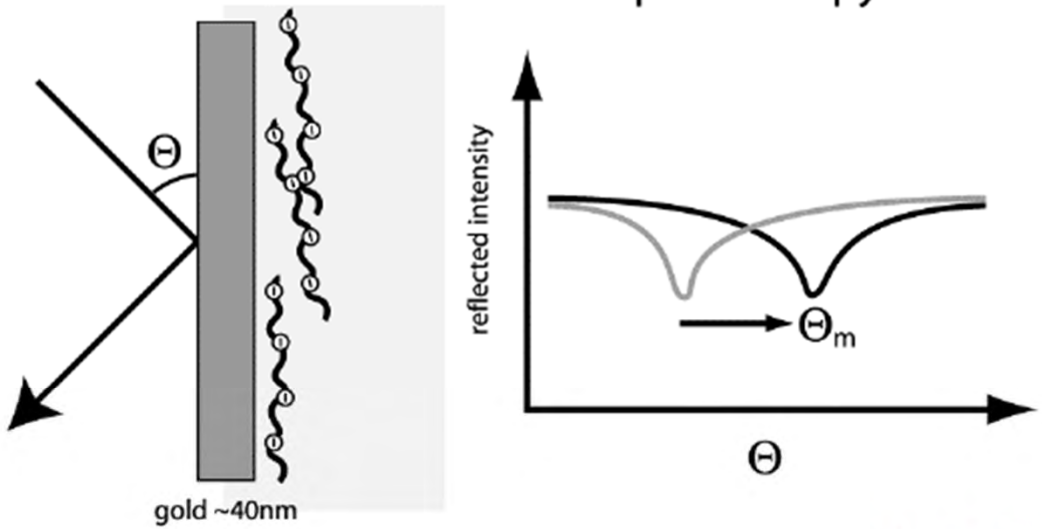
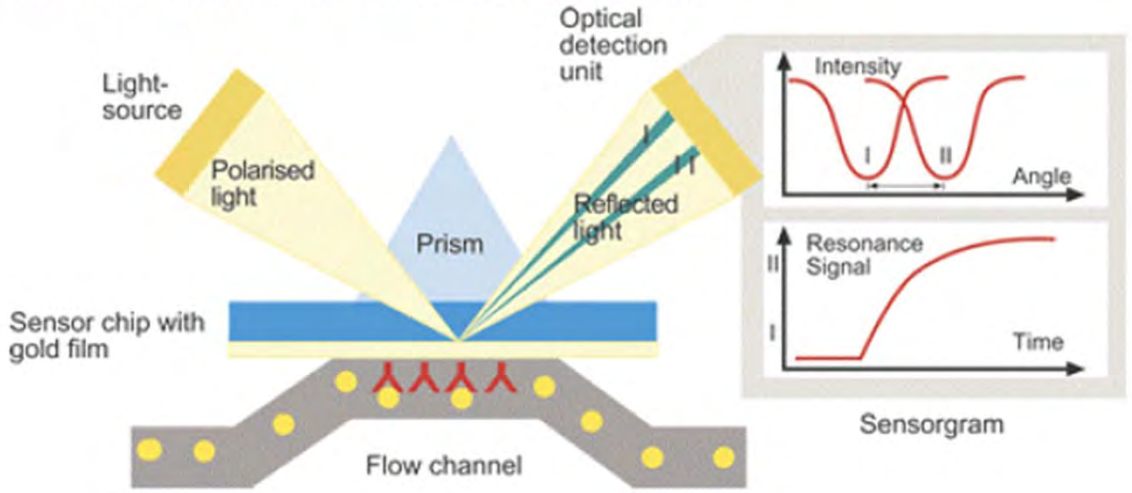


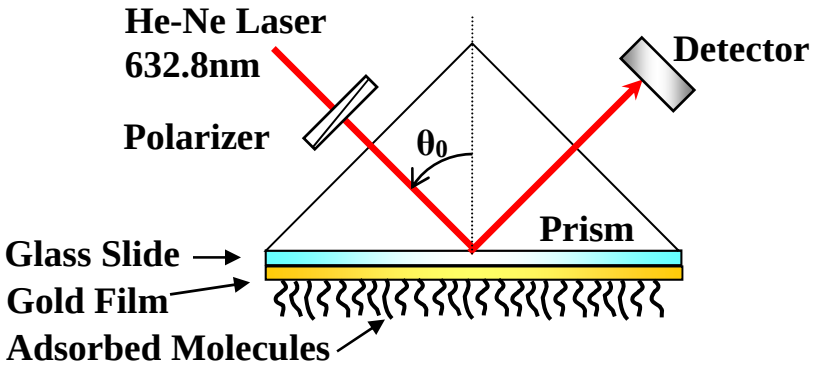
Figure by MIT OCW

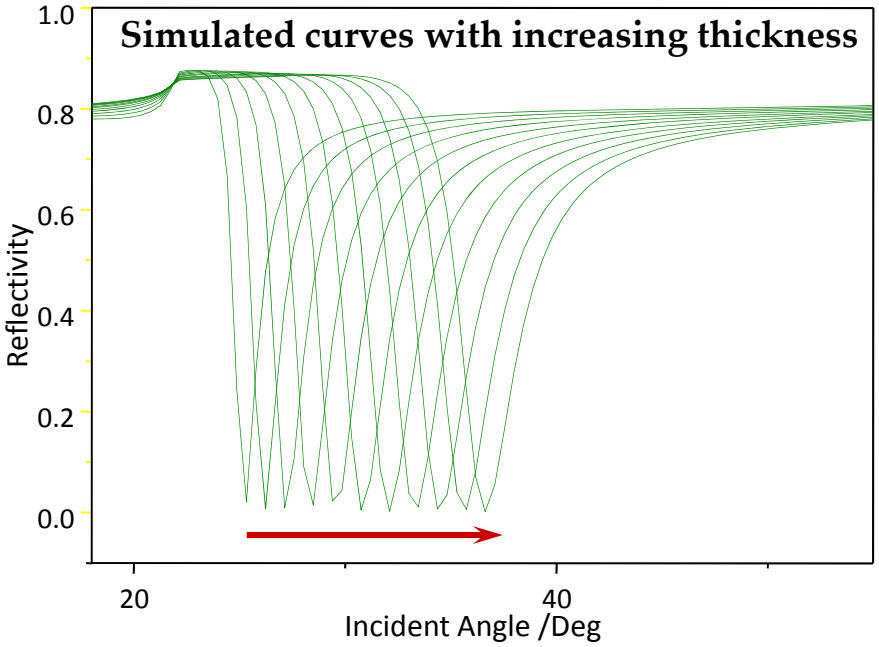
Biacore Commercial SPR Instrument

from *Biacore* website: www.biacore.com/lifesciences/index.html

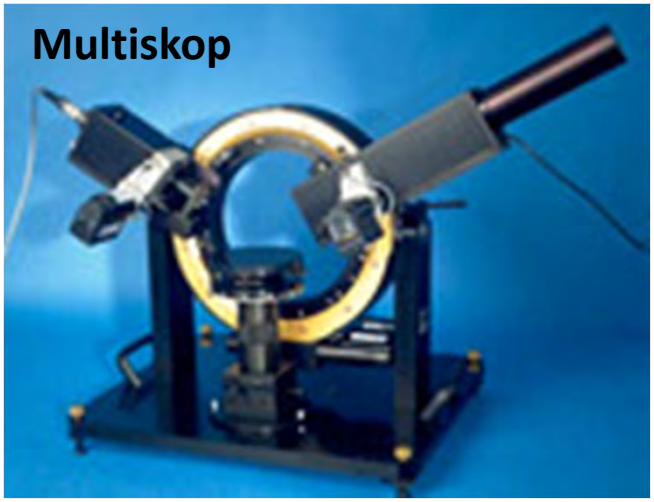


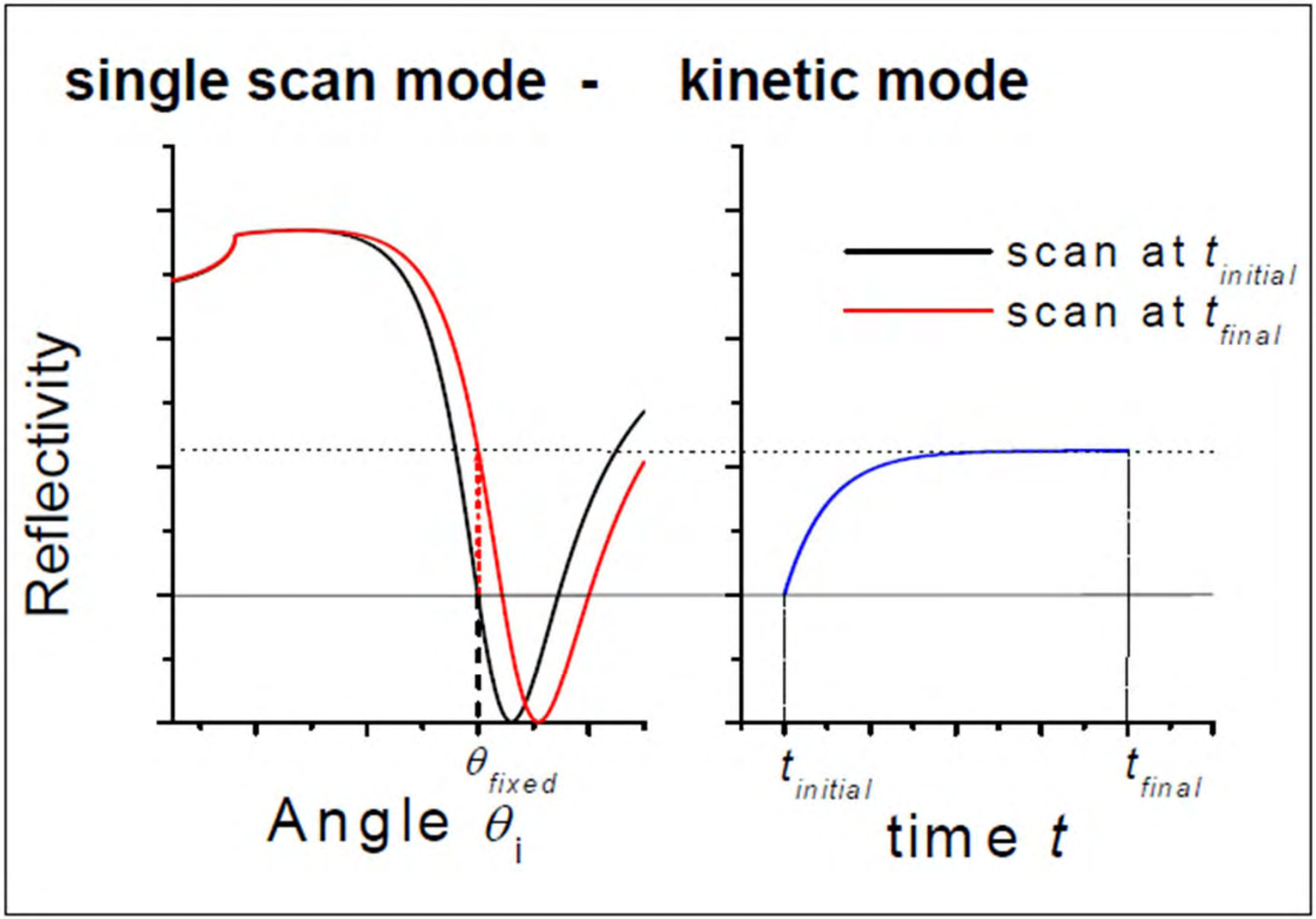
Courtesy of Biacore. Used with permission.





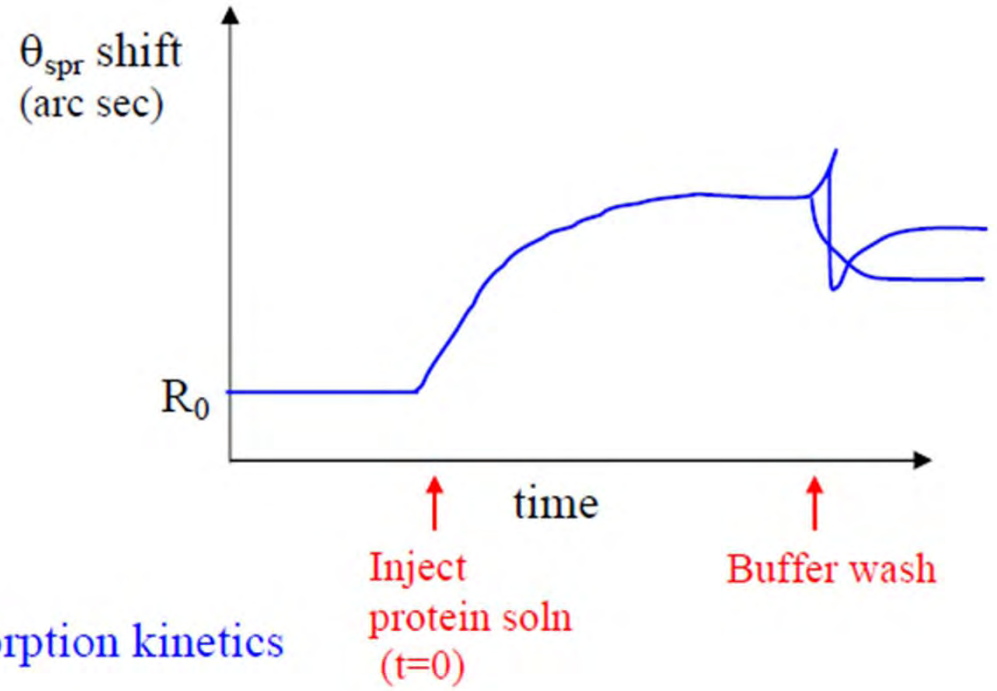
- Evanescent wave optical technique sensitive to changes in thickness and optical properties of thin and ultrathin films – **Angstrom sensitivity**
- Non-destructive to samples
- Attenuated total reflection (ATR) setup in a Kretschmann configuration, optics are away from the sample and subphase
- Surface plasmon excitation observed in reflectivity-angular scan
- Relatively insensitive to environmental changes – temperature, viscosity, etc.





The reflectivity – time curve can then be used for kinetic analysis

Surface Plasmon Resonance Spectroscopy (SPR)



Determining adsorption kinetics

Resonance shift fitted to:

$$R(t) = (R_\infty - R_0)\left[1 - \exp(-k_{obs}t)\right] + R_0 \;\rightarrow\; \text{obtain } k_{obs}$$

linear fit of:

$$k_{obs} = k_d + k_a\left[P\right] \;\rightarrow\; \text{obtain } k_d,\ k_a$$

Surface Plasmon Resonance Spectroscopy (SPR)

- more complex fitting expressions for $R(t)$ often required
- k_d alternatively obtained from dissociation data: $R(t) = R_0 \exp(-k_d t)$

Advantages: no protein labeling, controlled kinetic studies, sensitive

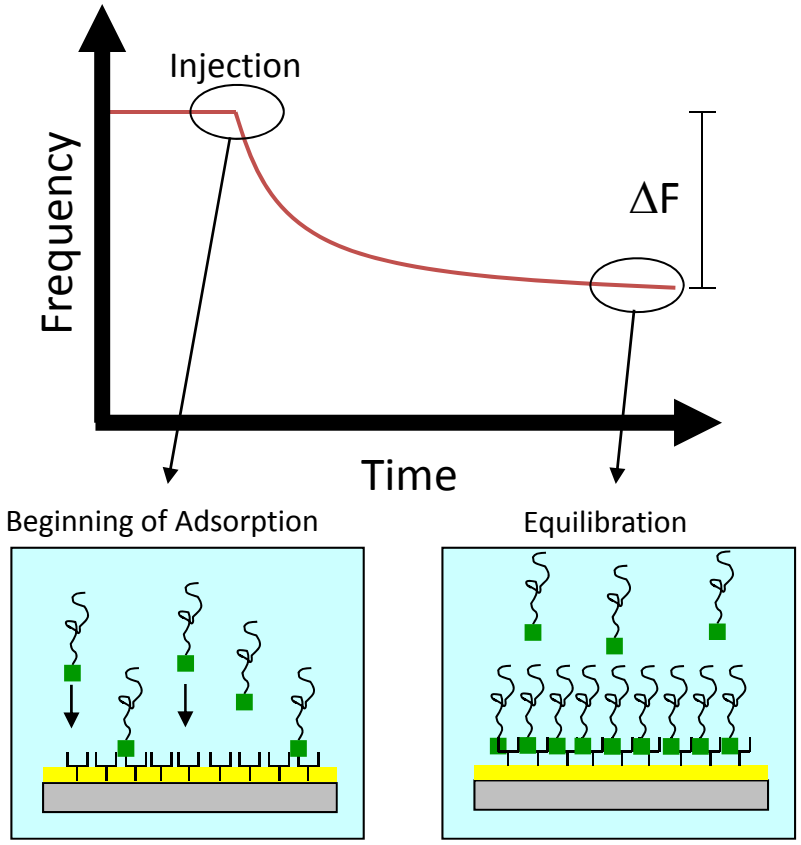
Disads: requires "model" surface preparation—limited applicability

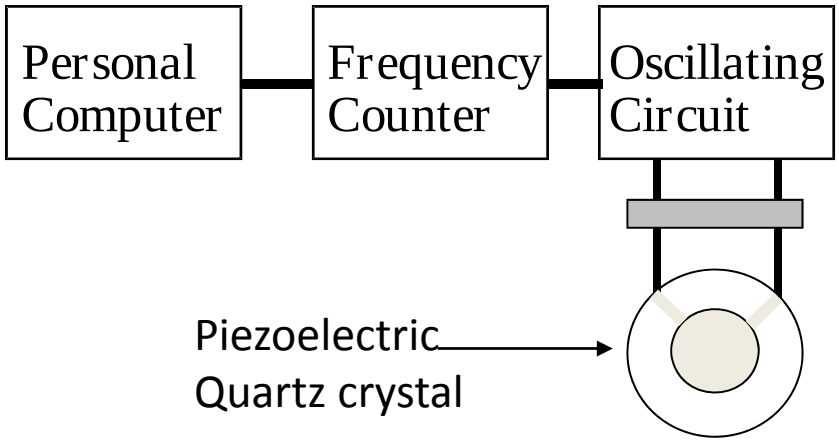
References:

R.J. Green, et al., *Biomaterials* **21**, 2000: 1823-1835.
P.R. Edwards et al., *J. Molec. Recog.* **10**, 1997: 128-134.

Quartz Crystal Microbalance (QCM)

How to measure binding constants?





Sauerbrey Equation

$$\Delta F = \Delta m \left(-2F_q^2 \Big/ A\sqrt{\rho_q \mu_q} \right)$$

Scatchard Equation

$$\Delta F / F_o c = K_b N - K_b \Delta F / F_o$$

Sauerbrey, G., *Z. Phys.* **1959,** *155*, 206.
Sauerbrey, G., *Ann. N.Y. Acad. Sci.* **1949,** *51*, 660-672.

QCM-D (Dissipation)

- Simultaneous monitoring of Δ frequency (ΔF) and Δ dissipation (ΔD) at multiple harmonics of the quartz resonator
- Dissipation reveals qualitative information about the viscoelastic properties of the adsorbed layer
- Dissipation of a viscoelastic polymer layer on a quartz resonator is heavily influence by its structure. Rigid films show small ΔD values while soft or flexible films show larger ΔD values
- Modeling the viscoelastic properties using a Voight model can yield quantitative information

Dissipation

$$\Delta D = \frac{E_{dissipated}}{2\pi \cdot E_{stored}}$$

Larger $E_{dissipated}$ yields more viscoelastic response

http://www.q-sense.com/$2/qcm-d.swf

3. Extent of Denaturing

Ellipsometry

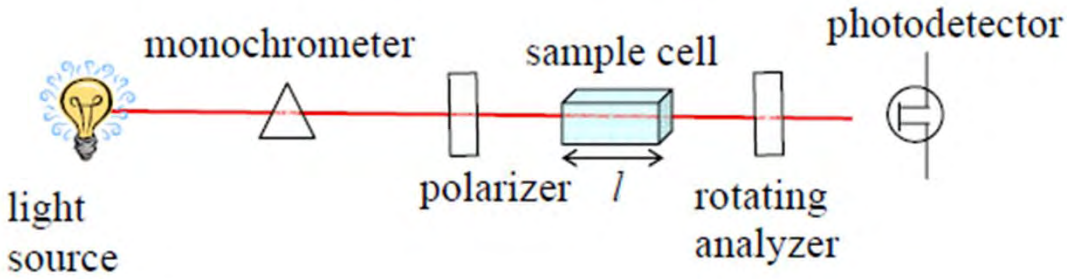
- Variations in thickness (d_f) & refractive index (n_f) of adsorbed layer over time gives indication of denaturation (inconclusive)

Must use a technique sensitive to the structure of the protein

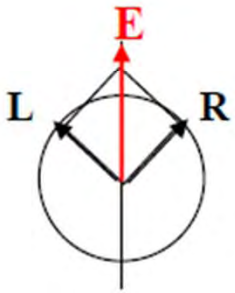
Circular Dichroism

Circular Dichroism

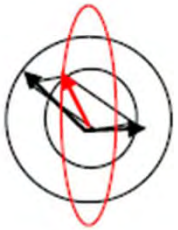
- Experimental set-up: monochromatic, plane-polarized light is passed through a sample solution and detected



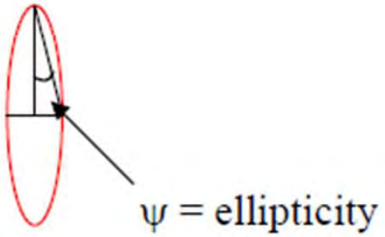
- Theoretical basis: unequal absorption of R- and L-components of polarized light by *chiral molecules* (e.g., proteins!)



Plane-polarized light resolved into circular components **R** & **L**



More absorption of **R** causes **E** to follow elliptical path



The ellipticity ψ is related to the difference in **L** and **R** absorption by:

$$\psi = \frac{2.303}{4}(A_L - A_R)\frac{180}{\pi} \text{ (degrees)}$$

where $A = -\log T = -\log\frac{I}{I_0} = \varepsilon c_p l$ (Beer's Law)

Molar ellipticity: $[\theta] = \frac{\psi \cdot M_p}{c_p l}$

c_p = protein conc. (g/cm^3)
ε = molar extinction coeff. (cm^2/g)
l = path length (cm)
M_p = protein mol. weight (g/mol)
T = transmittance

- Ellipticity can be + or –; depends on electronic transition (π–π* vs. n-π*)
- Proteins exhibit different values of [θ] for α helix, β sheet, and random coil conformations in the far UV.

Conformation	Wavelength (nm)	Transition
α helix	222 (–)	n-π* peptide
α helix	208 (–)	π–π* peptide
α helix	192 (+)	π–π* peptide
β sheet	216 (–)	n-π* peptide
β sheet	195 (+)	π–π* peptide
β sheet	175 (–)	π–π* peptide

Circular Dichroism

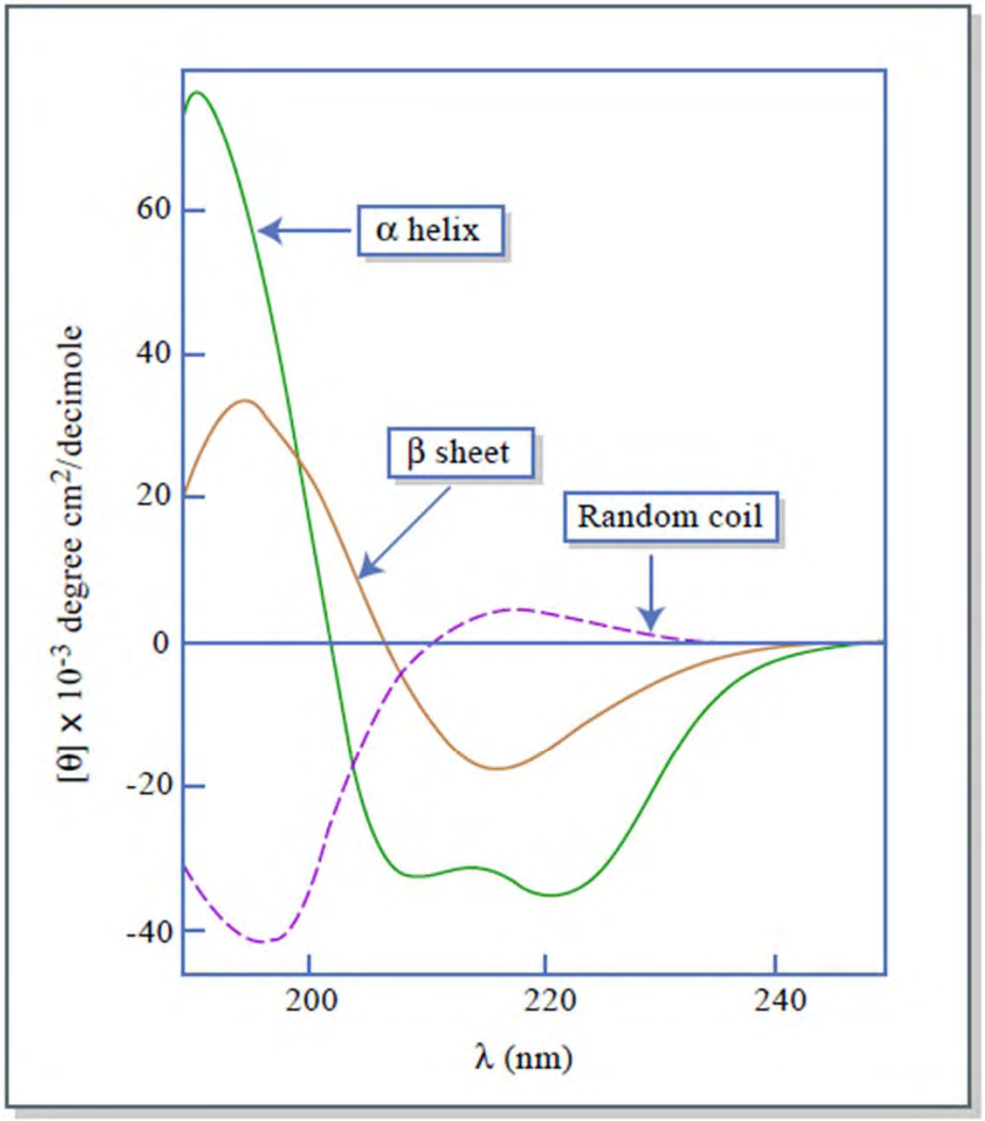
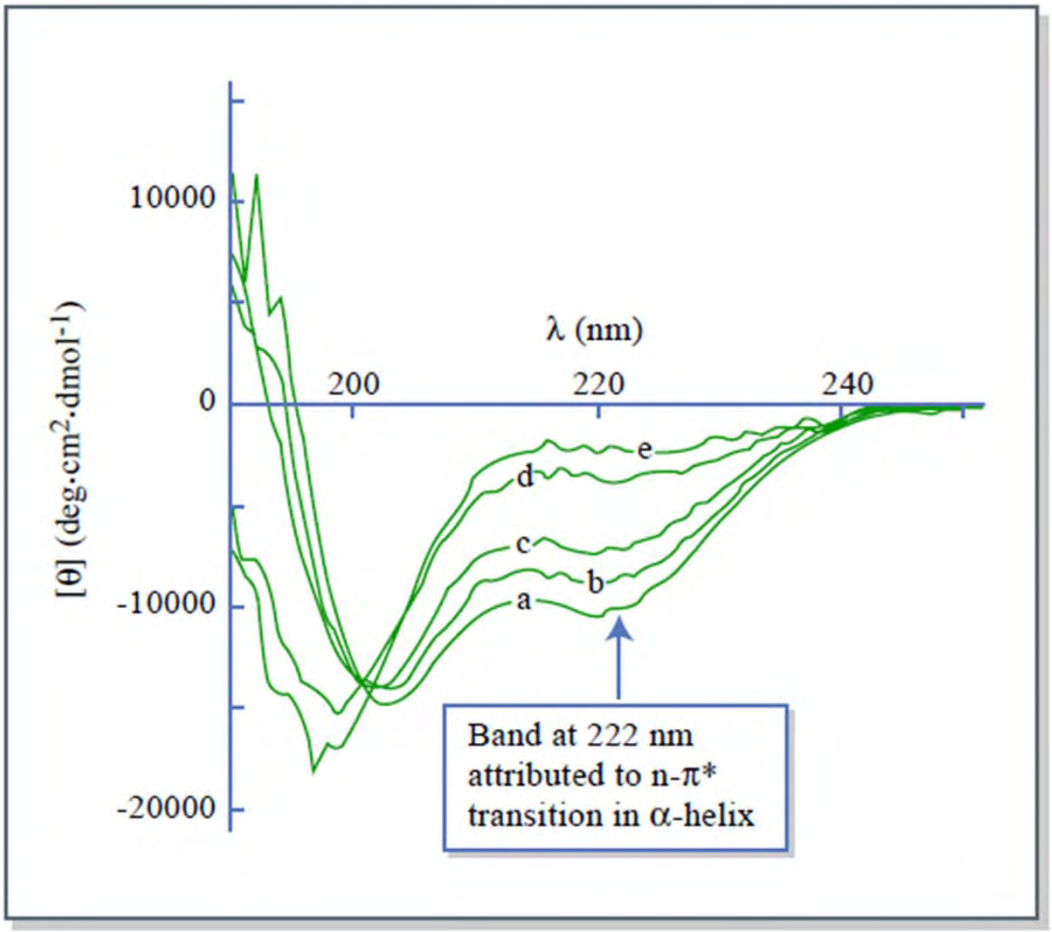


Figure by MIT OCW.

After T.E. Creighton, ed., *Proteins: Structures and Molecular Principles*, W.H. Freeman & Co: NY; 1983, p. 181.

Changes to CD spectra give a measure of *denaturation*, e.g., due to adsorption at a surface



CD spectra for the synthetic peptide:
Ac-DDDDDAAAARRRRR-Am

(a) in pH 7 solution

(b-e) adsorbed to colloidal silica: b) pH 6.8; c) pH 7.9; d) pH 9.2; e) pH 11.3

Figure by MIT OCW.

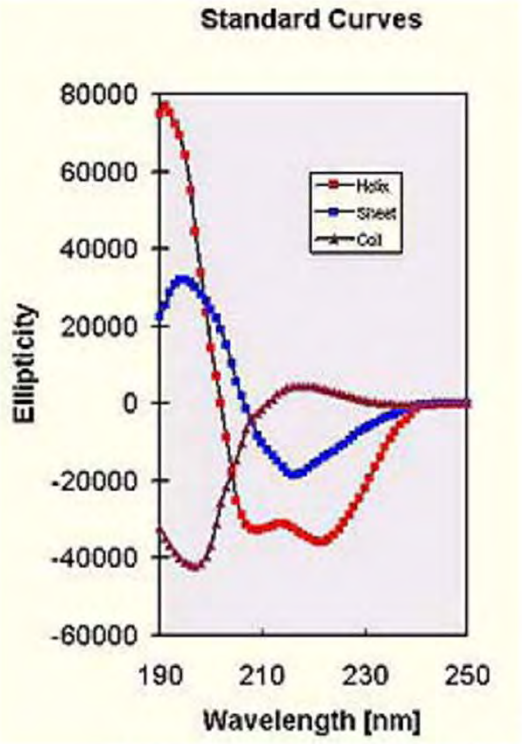
[After S.L. Burkett and M.J. Read, Langmuir 17, 5059 (2001).]

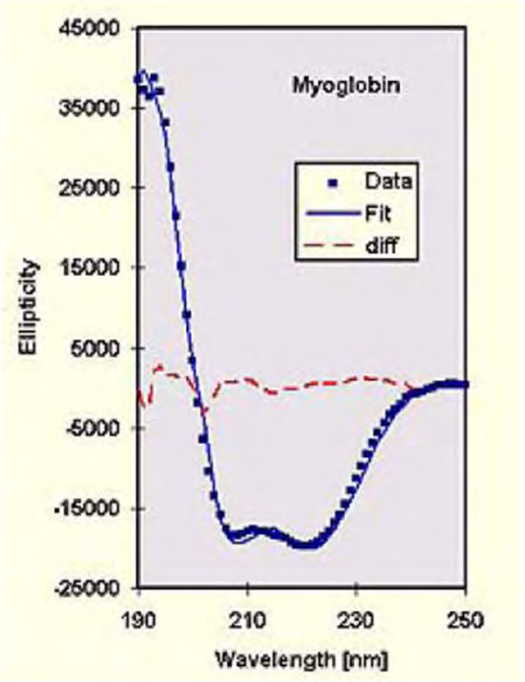
For quantitative comparisons, molar ellipticity per residue is computed, by dividing [θ] by the number of residues in the protein (n_r).

$$[\theta]_{mrd} = \frac{\psi \cdot M_p}{10 n_r c_p l} = \frac{\psi \cdot \overline{M_r}}{10 c_p l}$$

units: deg cm^2 dmol^{-1}

% of α helix, β sheet, and random coil conformations obtained by linear deconvolution using "standard curves" from homopolypeptides such as poly(L-lysine) in 100% α helix, β sheet, and random coil conformations.





"Circular Dichroism Spectroscopy" by Bernhard Rupp.
http://web.archive.org/web/20050208092958/http://www-structure.llnl.gov/cd/cdtutorial.htm

For a rough estimate of α-helix content, the following expressions have been employed:

$$\alpha - helix\% = \frac{[\theta]_{208} - 4000}{33,000 - 4000}$$ from $[\theta]_{mrd}$ data at 208 nm

$$\alpha - helix\% = \frac{[\theta]_{222}}{40,000}$$ from $[\theta]_{mrd}$ data at 222 nm

Advantages: no labeling required; simple set-up

Disads: need experimental geometry with high surface area, e.g., colloidal particles (high signal)

References:

N. Berova, K. Nakanishi and R.W. Woody, eds., Circular Dichroism: Principles and Applications, 2nd ed.,Wiley-VCH: NY; 2000.

N. Greenfield and G.D. Fasman, *Biochemistry* **8** (1969) 4108-4116.