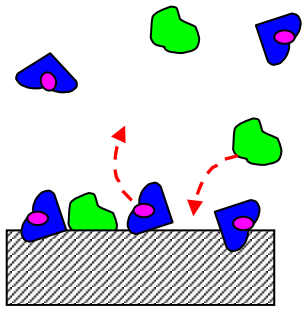
Lecture 6:
Protein-Surface Interactions (Part II)

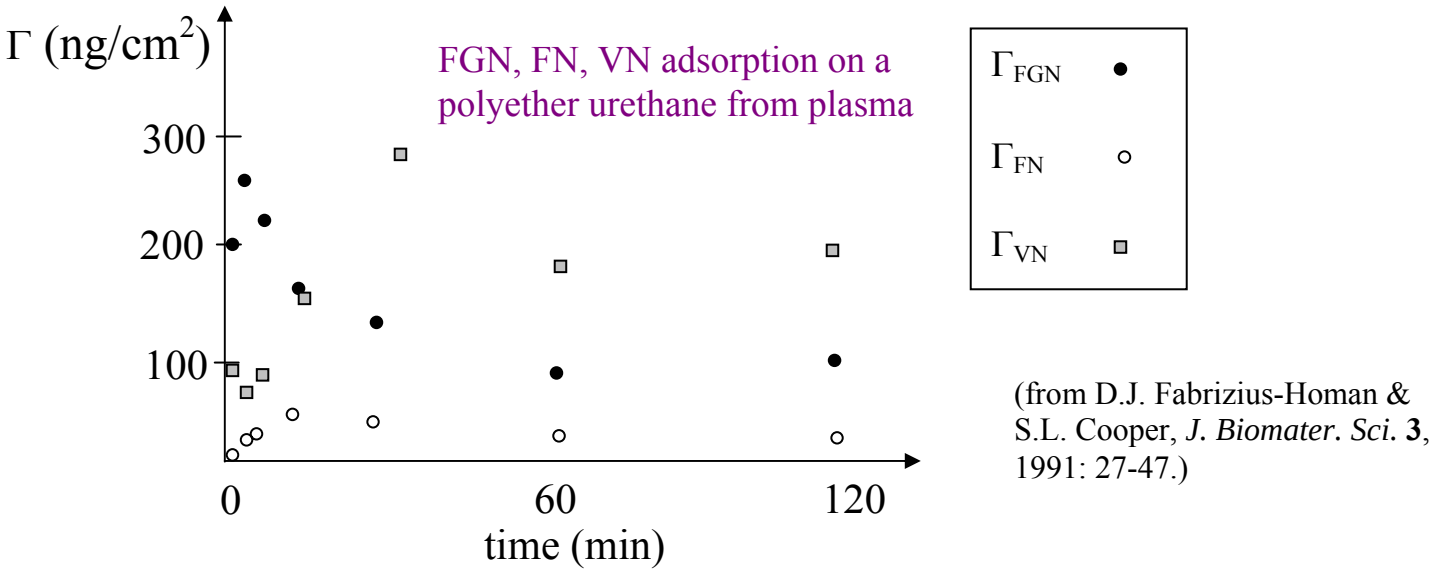
The Langmuir model is applicable to numerous reversible adsorption processes, but fails to capture many aspects of protein adsorption.

1. Competitive Adsorption



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

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

Hypothesis:

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

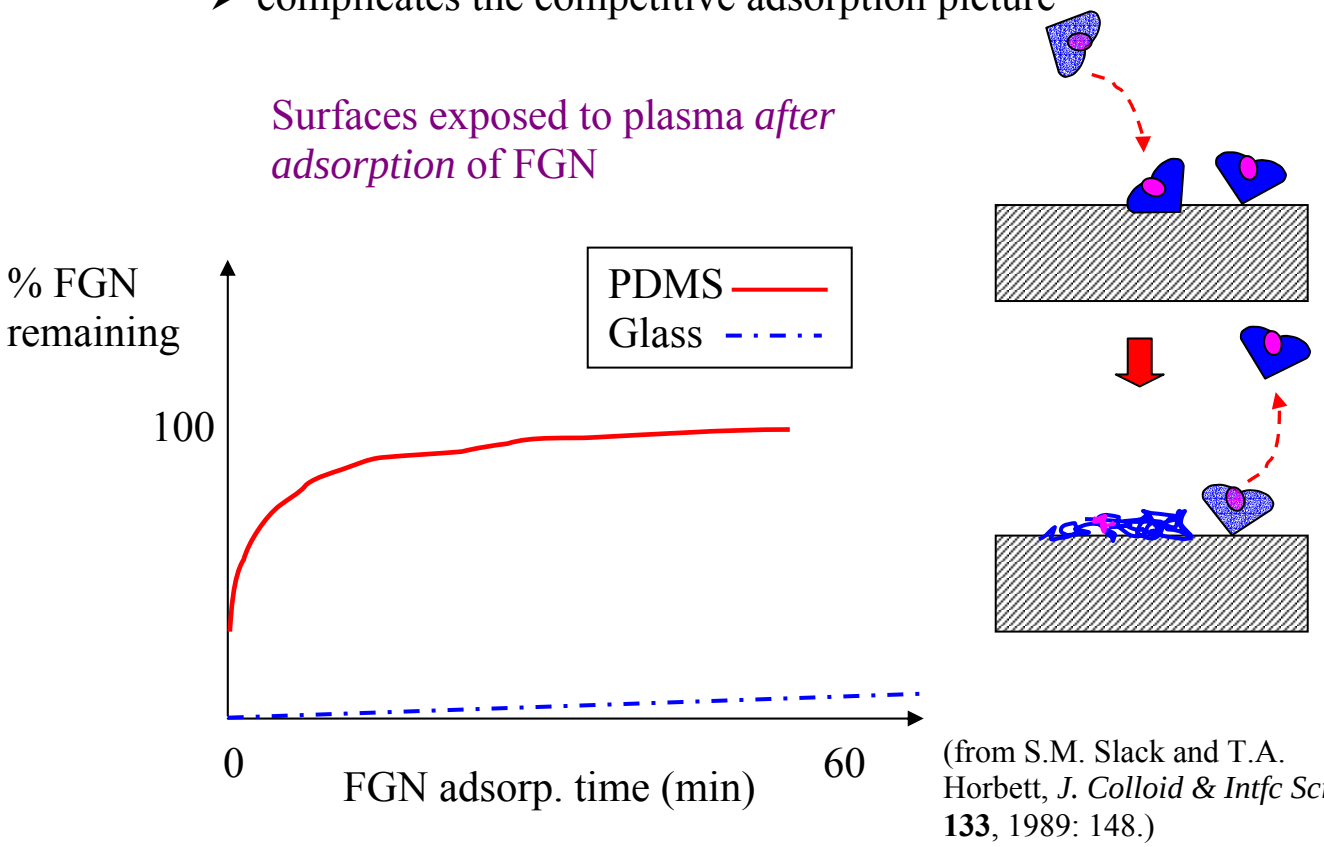
At t>0: local depletion of adsorbed species near surface– exchange with faster diffusing species ensues

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.)

Physiological implications:

a) hydrophobic surfaces cause more denaturing

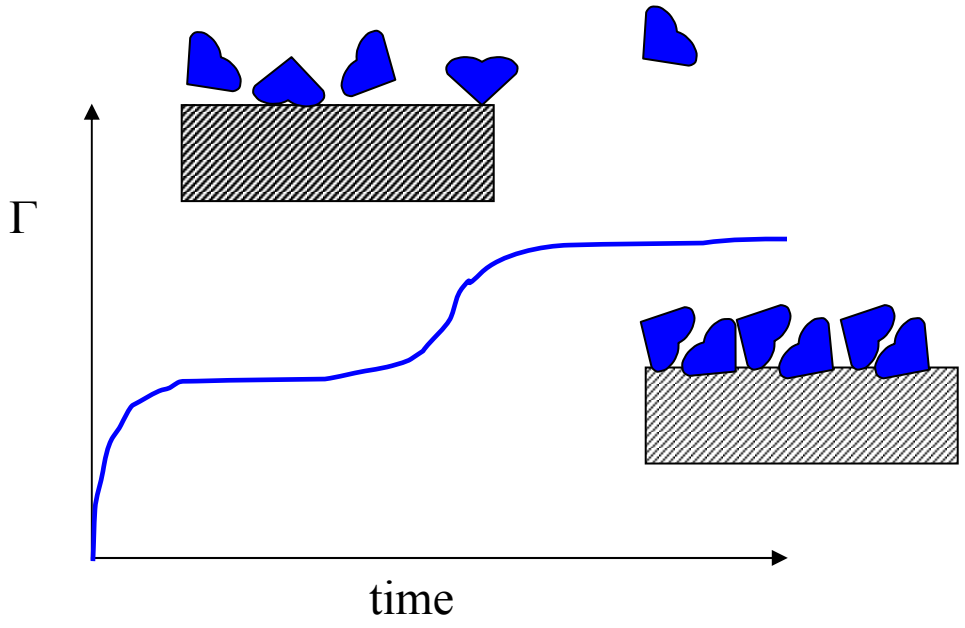
b) denatured proteins may ultimately desorb (by replacement) ⇒ non-native solution behavior

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

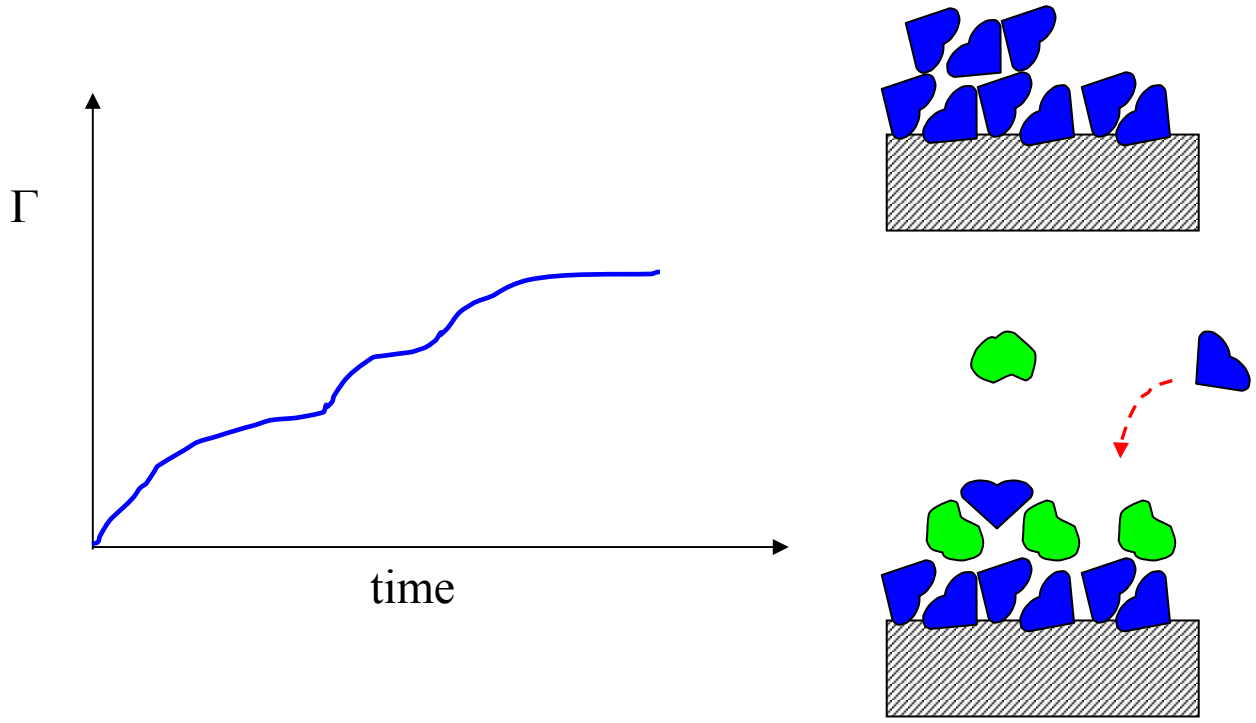
3. Restructuring

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



4. Multilayer Formation

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



Measurement of Adsorbed Proteins

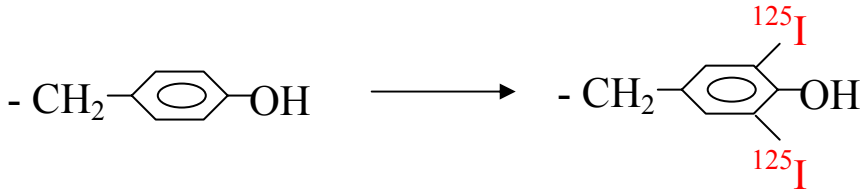
1. Techniques for Quantifying Adsorbed Amount

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

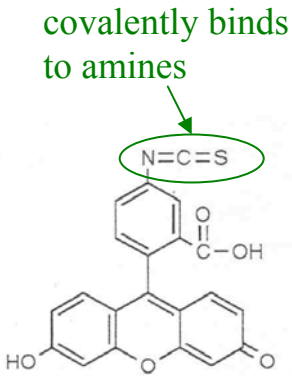
Advantage: high signal-to-noise ⇒ measure small amts (ng)

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

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

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)

2. Techniques for Studying Adsorption Kinetics

a) *In situ Ellipsometry*

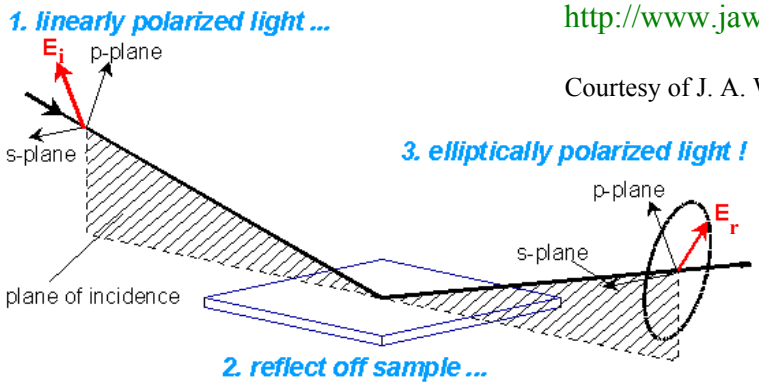


Figure from:
http://www.jawoollam.com/tutorial_1.html

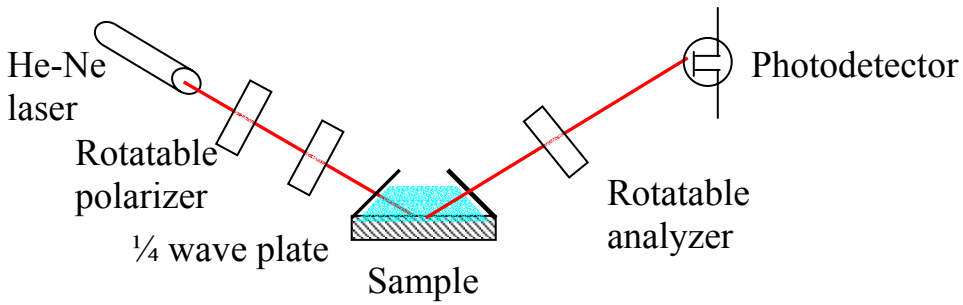
- polarized light reflected from a surface
- phase & amplitude changes to parallel (p) and perpendicular (s) E-field components

E_i , E_r = incident/reflected E-field

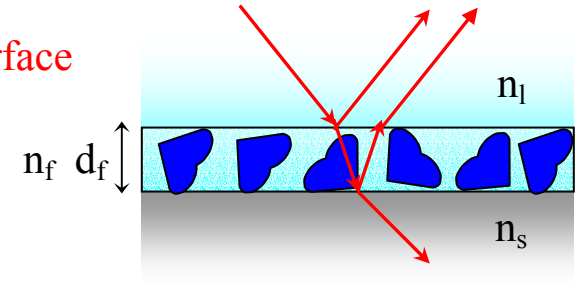
reflection coefficients: $r_p = \frac{E_{rp}}{E_{ip}} = |r_p| \cdot e^{i\delta_p}$ and $r_s = \frac{E_{rs}}{E_{is}} = |r_s| \cdot e^{i\delta_s}$

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

➢ 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

- 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

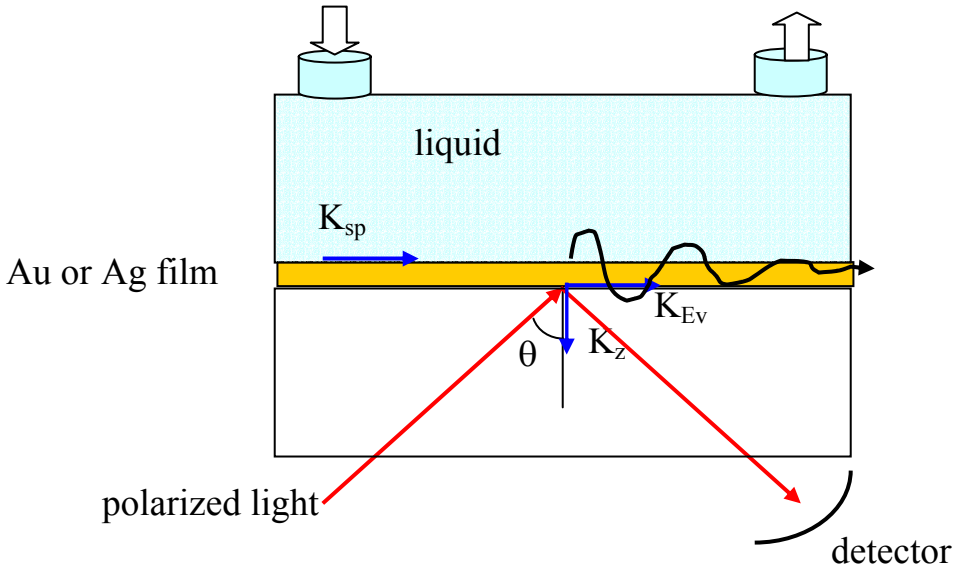
Disads: quantitation requires a model, optically flat & reflective substrates required; can't distinguish different proteins

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.

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

➢ 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:

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

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

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

c/ω_0 = incident light λ

ε_{metal} = metal dielectric const.

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

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

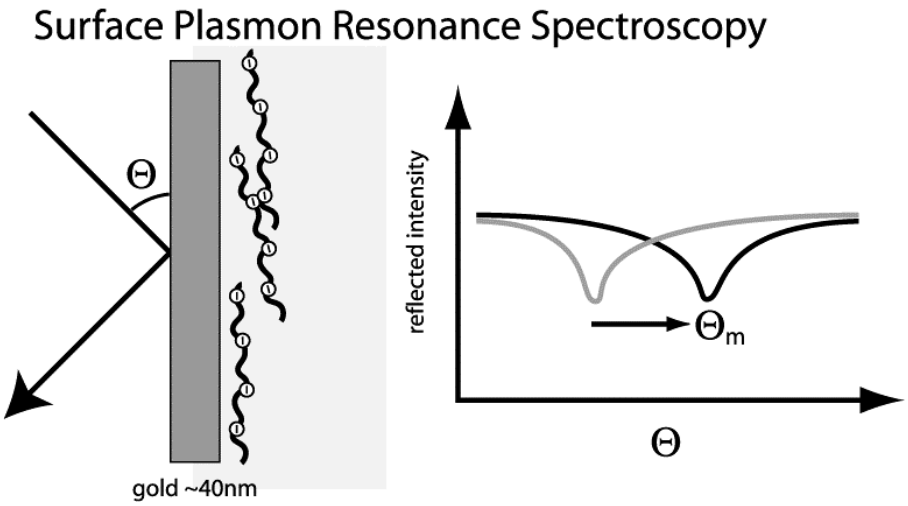
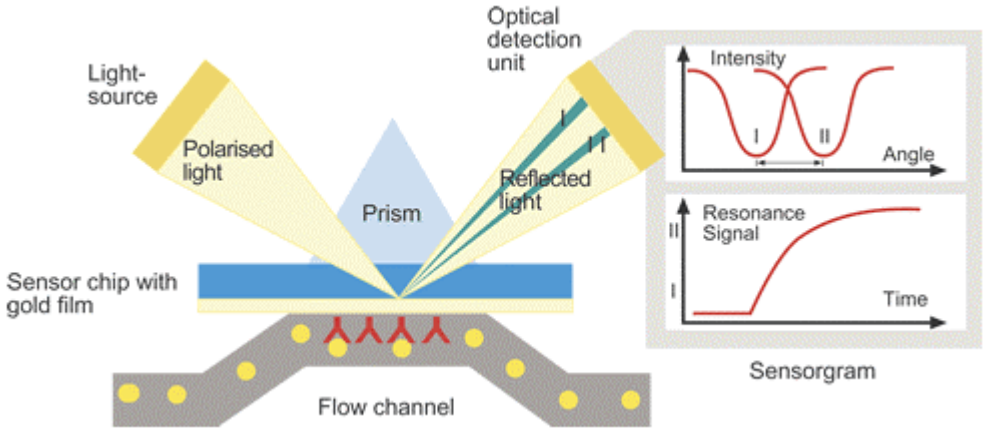
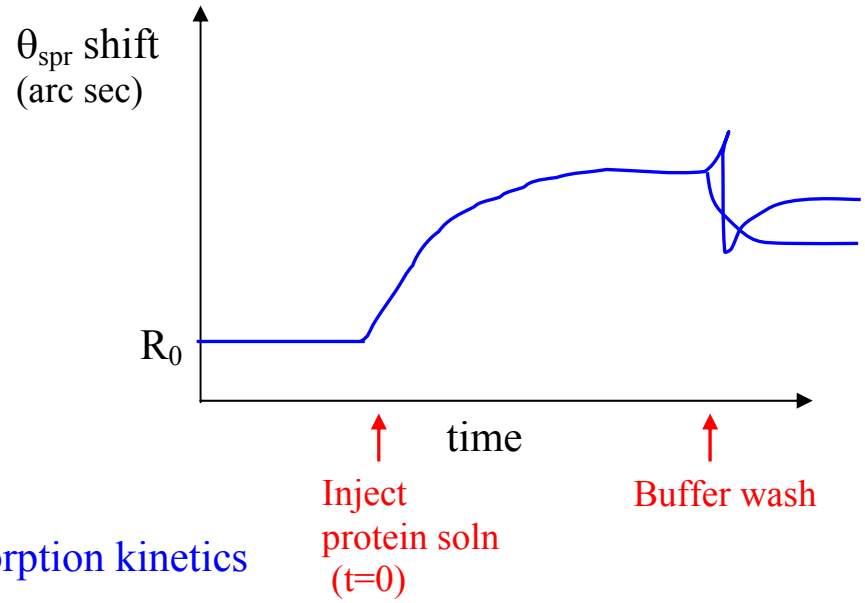


Figure by MIT OCW

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



Courtesy of Biacore. Used with permission.



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:

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

- 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.

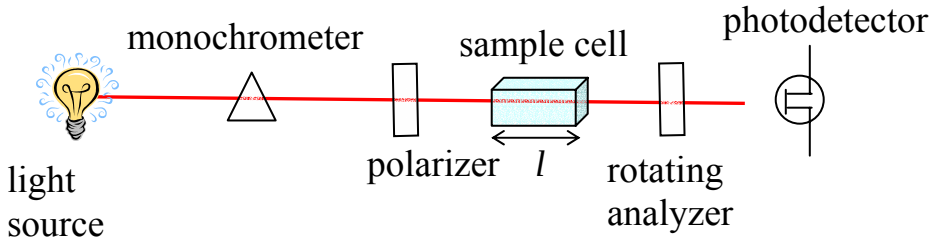
3. Extent of Denaturing

Ellipsometry

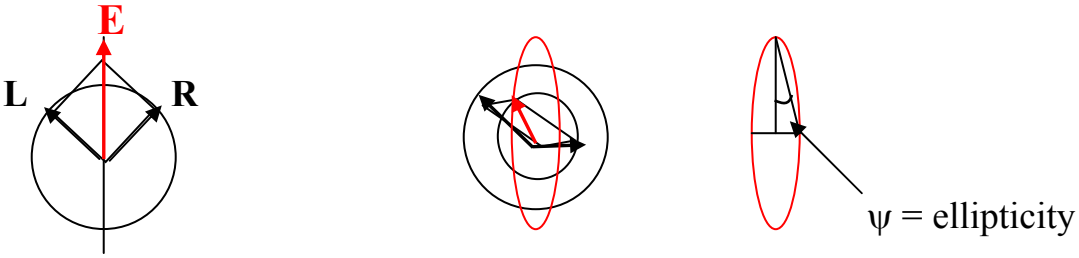
- Variations in thickness (d_f) & refractive index (n_f) of adsorbed layer over time gives indication of denaturation (inconclusive)

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:

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

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

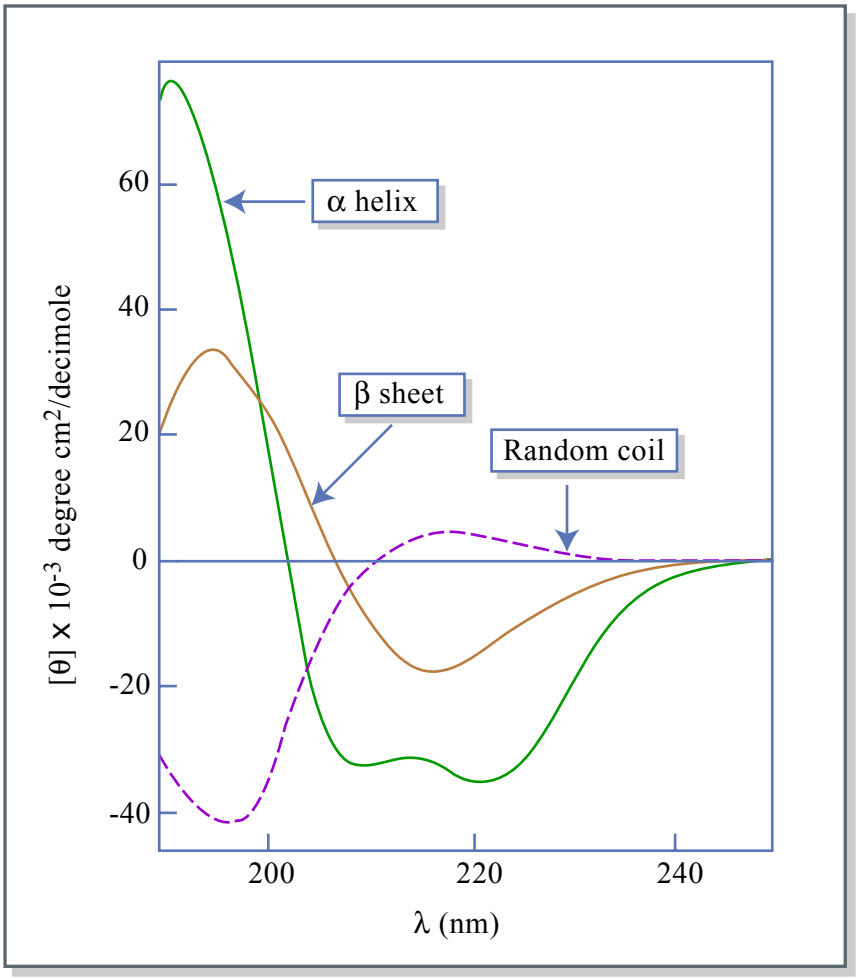
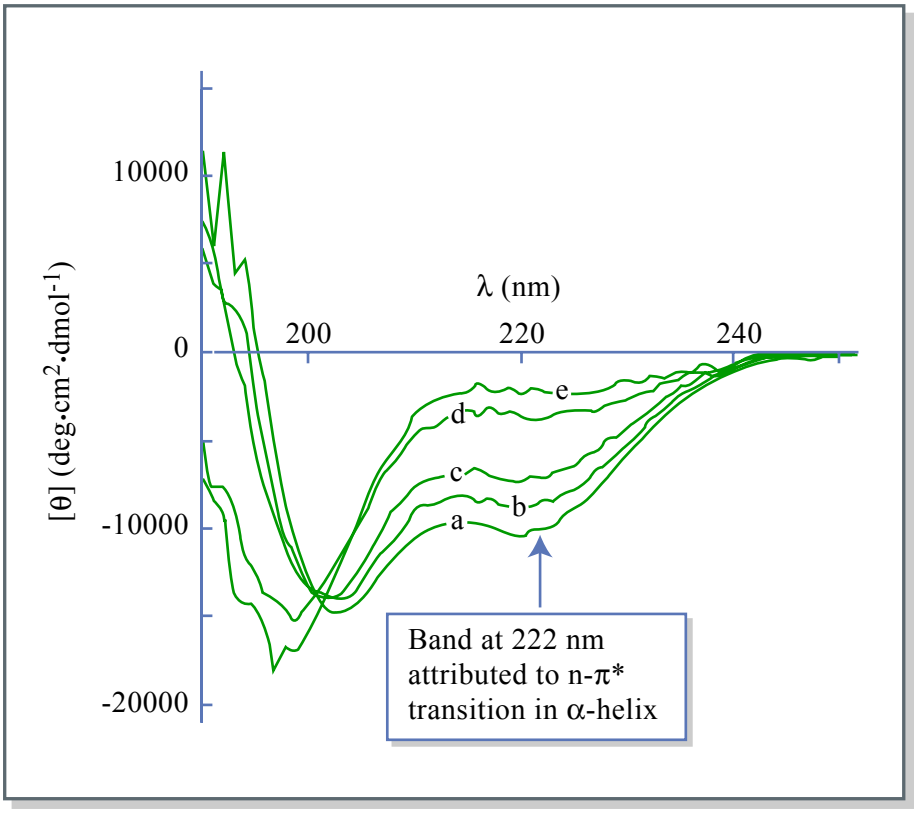


Figure by MIT OCW.
After T.E. Creighton, ed., *Proteins: Structures and Molecular Principles*, W.H. Freeman & Co: NY; 1983, p. 181.

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

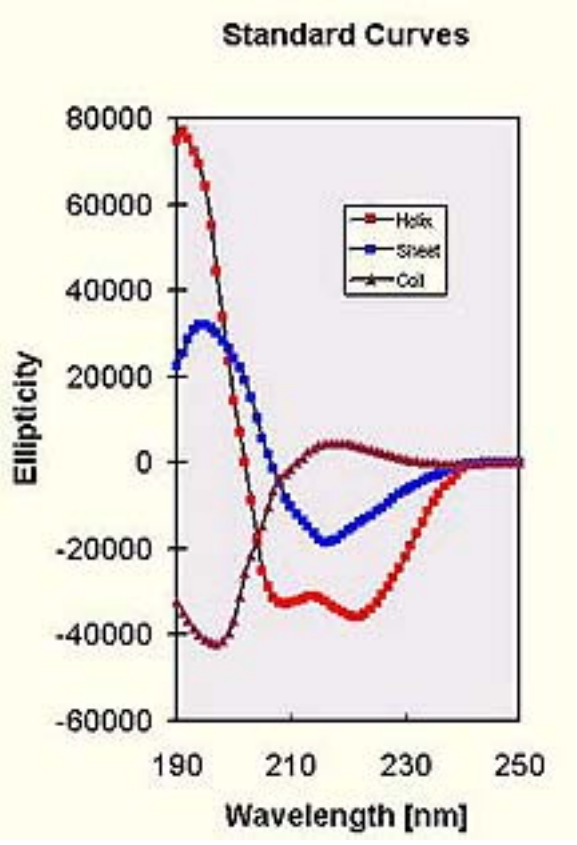
(b-e) adsorbed to colloidal silica: b) pH 6.8; c) pH 7.9; d) pH 9.2; e) pH 11.3 After

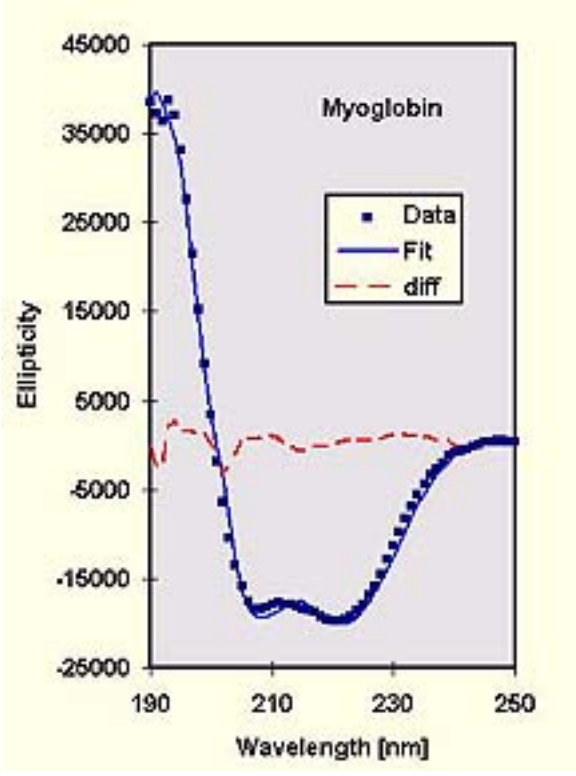
Figure by MIT OCW.
[After S.L. Burkett and M.J. Read, Langmuir 17, 5059 (2001).]

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.