Lecture 5:
Protein-Surface Interactions

Importance of Protein-Surface Interactions:

- Modulate cell adhesion
- Trigger the biological cascade resulting in foreign body response
- Central to diagnostic assay/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

- *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

- *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…

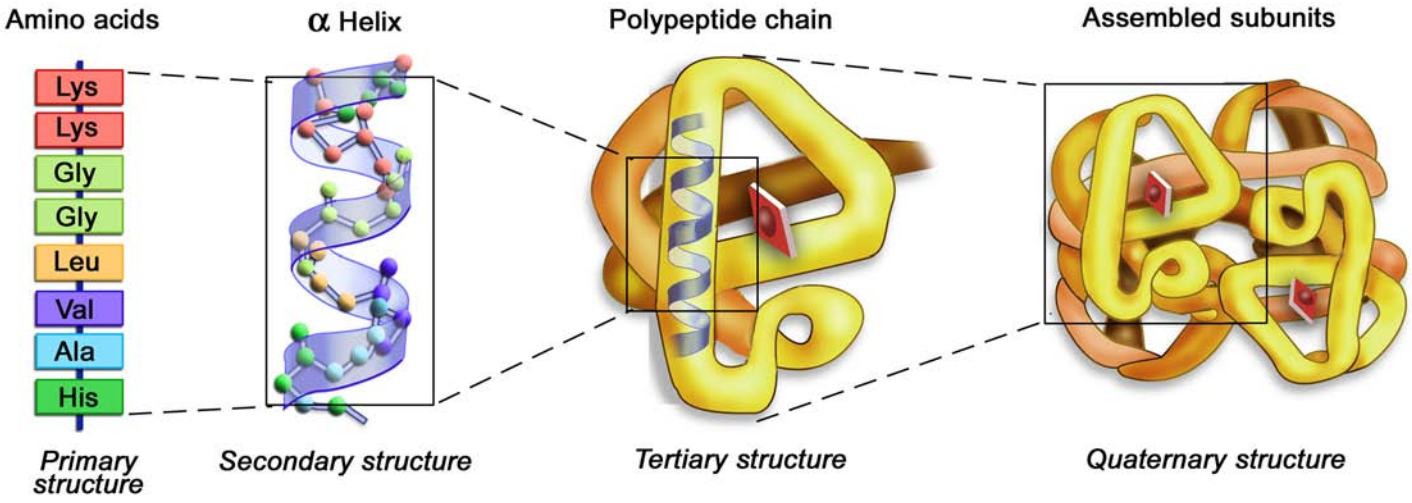


Figure by MIT OCW.

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

1. Primary Structure

- comprised of amino acid residues: - N-CHR-C -

- peptide (amide) bond CONH is effectively rigid & planar (partial double-bond character)

- directional character to bonding: amino acids are L stereoisomers

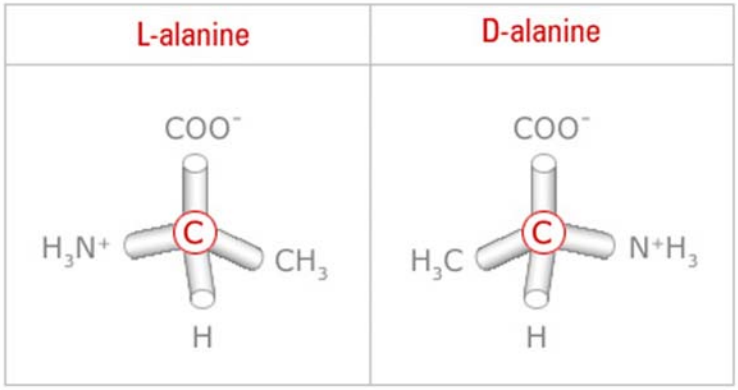


Figure by MIT OCW.

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

- side groups R have variable character

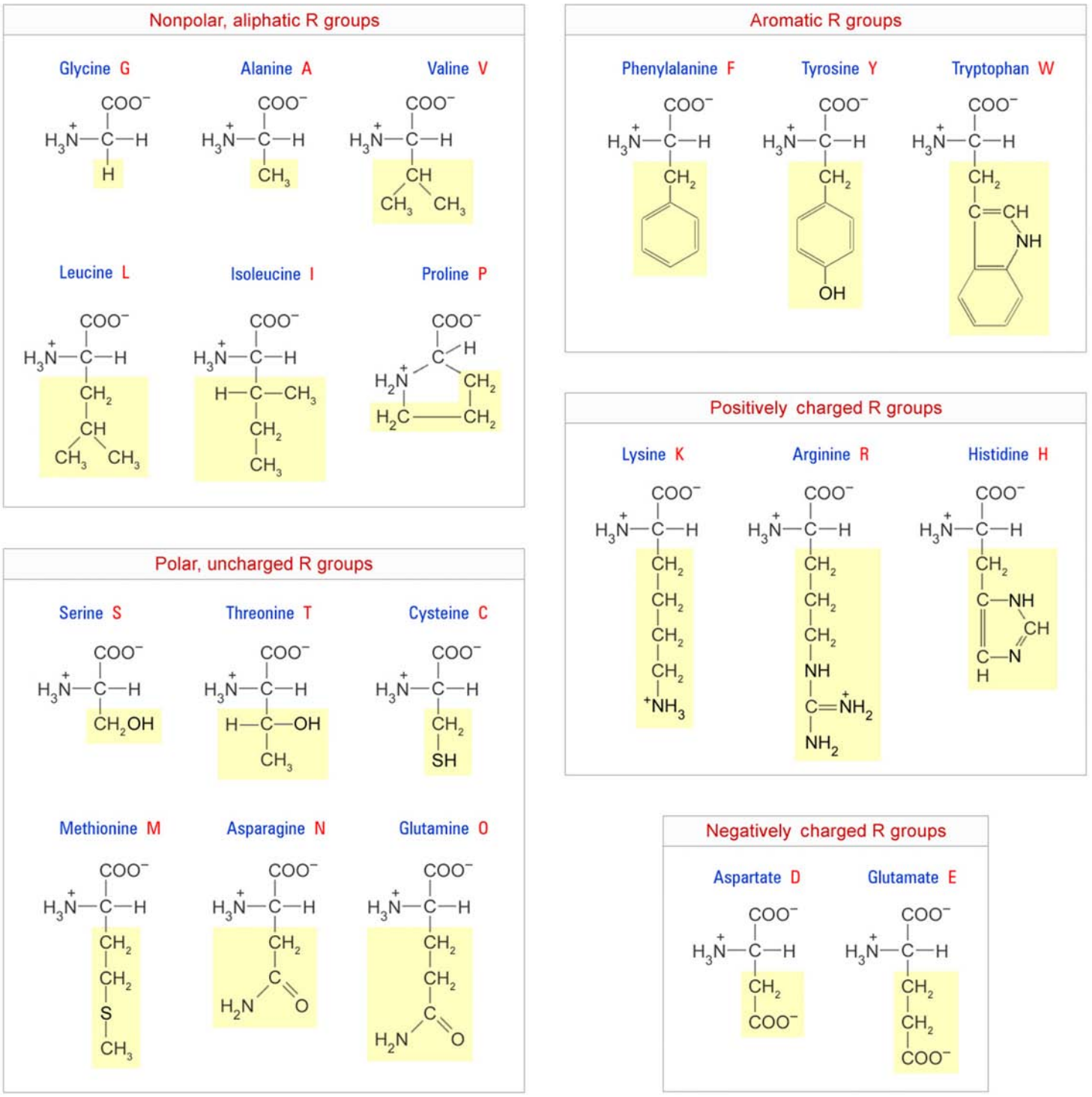
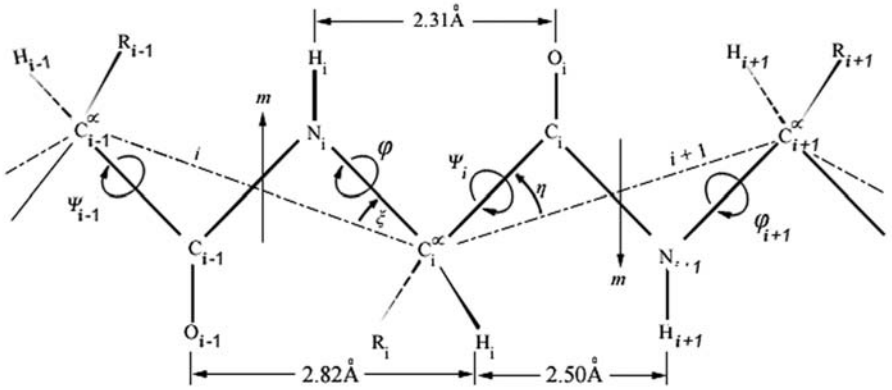


Figure by MIT OCW.

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

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 $(\varphi_{i+1}, \psi_{i+1})$

Figure by MIT OCW.

Ramachandran plots: designate permitted ranges of φ & ψ for a.a. residues

[from A.L. Lehninger, D.L. Nelson & M.M. Cox, pg. 171.]

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β–sheets

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

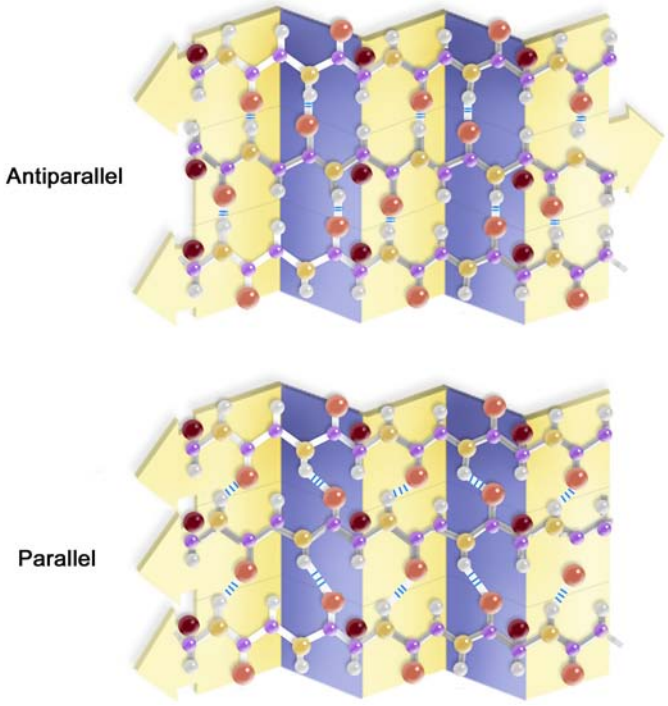

Figure by MIT OCW.

[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)

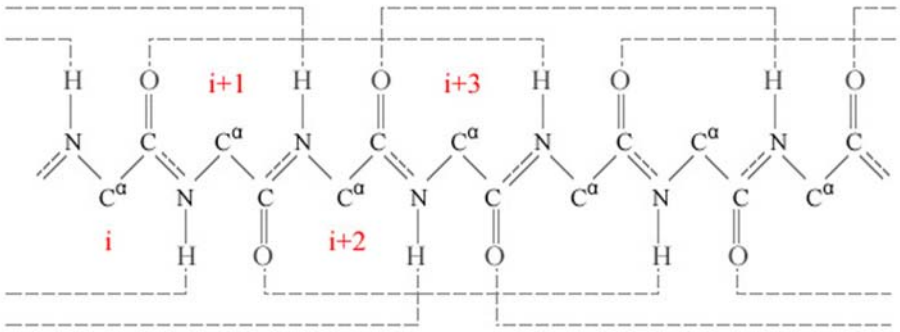

Figure by MIT OCW.

[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

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[from A.L. Lehninger, D.L. Nelson & M.M. Cox, pg. 187.]

Synthetic Polymers vs. Proteins

Property	Synthetic Polymers	Polypeptides
Molecular Wt.	1000-10^6 g/mol	1000-10^6 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 (ρ~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

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

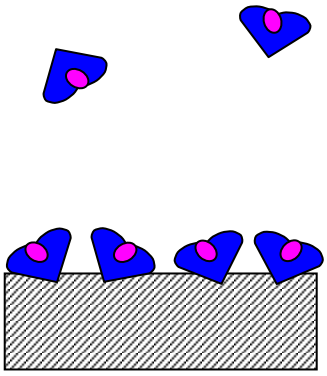
⇒ *changes physiological function!*

Protein Adsorption on Biomaterial Surfaces

Background

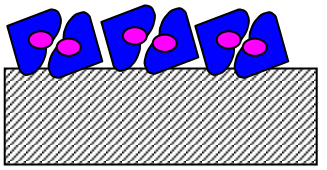
a) Protein activity varies in adsorbed vs. solvated state

Why?



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

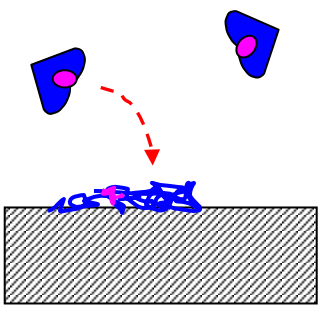
e.g., cell adhesion increases with adhesion peptide concentration



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

b) Driving forces for protein adsorption

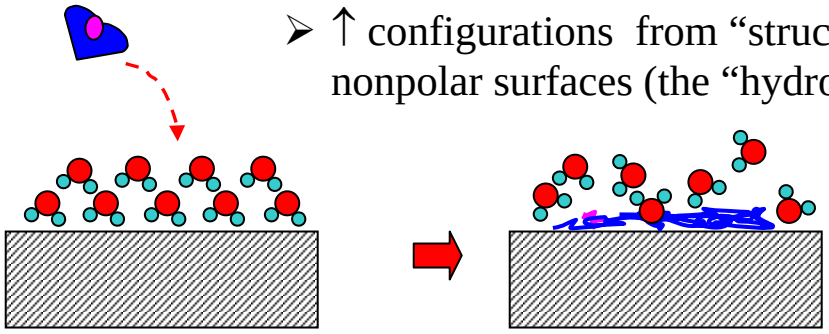
1. secondary bond formation

electrostatic > H-bonding > dispersive

BOND ENERGY

Depends on material's surface chemistry

2. entropic forces



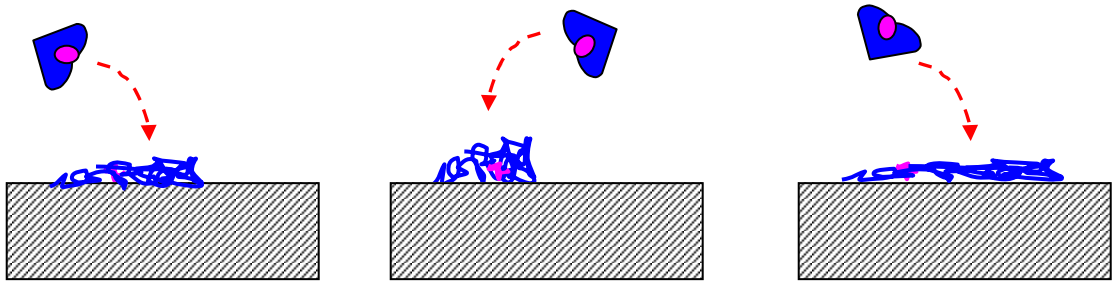
- ↑ configurations from "structured" H_2O adjacent to nonpolar surfaces (the "hydrophobic effect")

- 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 ↑ $\Rightarrow \downarrow \Delta S_{mix}$

- ↑ configurations for denatured vs. solvated proteins



c) Adsorbed proteins initiate physiological responses to biomaterials

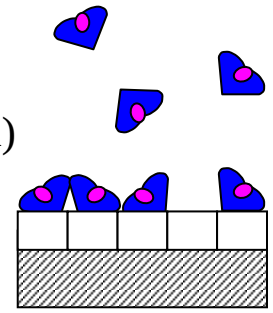
- coagulation mechanism
- alternative pathway of complement system (vs. antigen-antibody)
- *in vitro* protein adsorption experiments → 1st test of "biocompatibility"

Models for Protein Adsorption

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

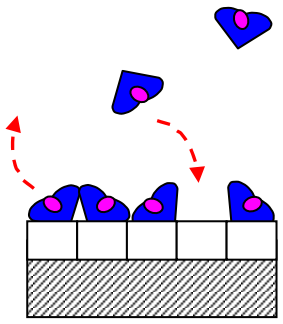
Assuming the "reaction" follows 1st order kinetics:

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

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

At equilibrium: adsorption rate = desorption rate

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



Assumes: reversibility

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

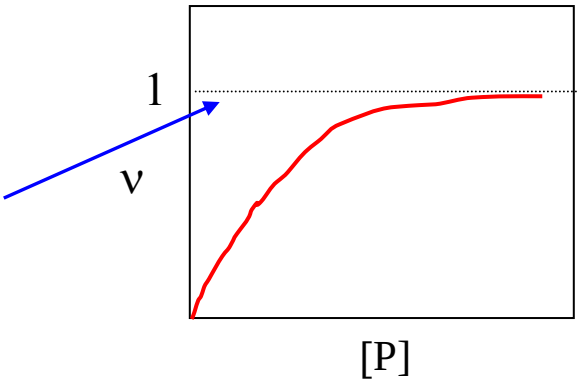
(a.k.a. "binding" or "association" const; typical units = L/mol)

K obtained experimentally by measure of fraction occupied sites:

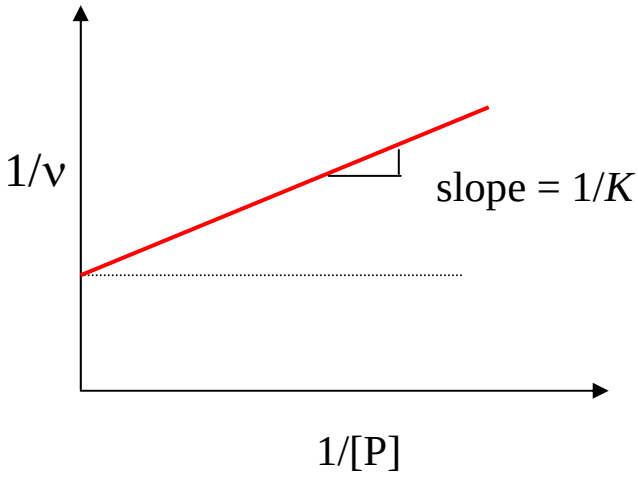
ν = # filled sites/total # surface sites

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

Binding plateaus at ν=1, monolayer coverage.



To obtain K:



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.

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: $[S]_0 = [S] + [PS]$,

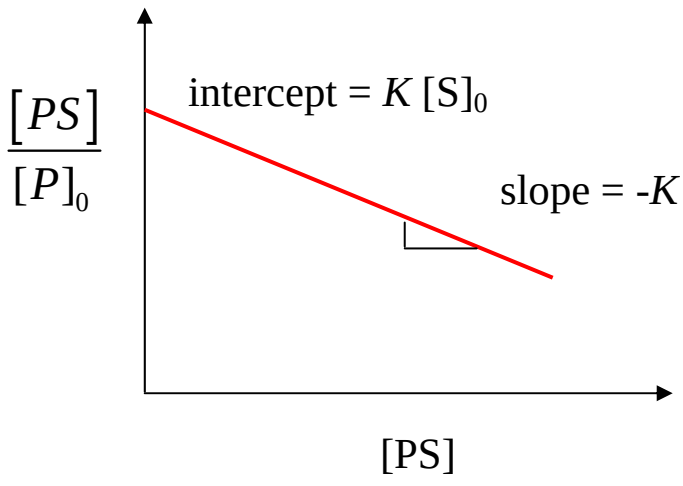
And substituting for [S]: $$K([S]_0 - [PS]) = \frac{[PS]}{[P]}$$

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

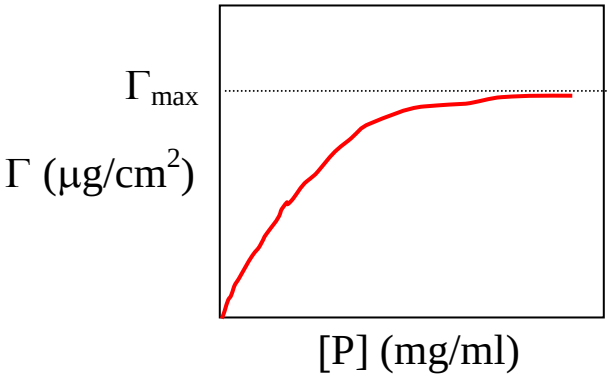
$$\frac{[PS]}{[P]_0} = -K[PS] + K[S]_0$$

Scatchard Plot

Provides a measure of $[S]_0$



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



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:

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