

MB&B 300a – Principles of Biochemistry – Fall 2015

Part 1: Proteins – based on lectures by Prof. Matthew Simon

James Diao

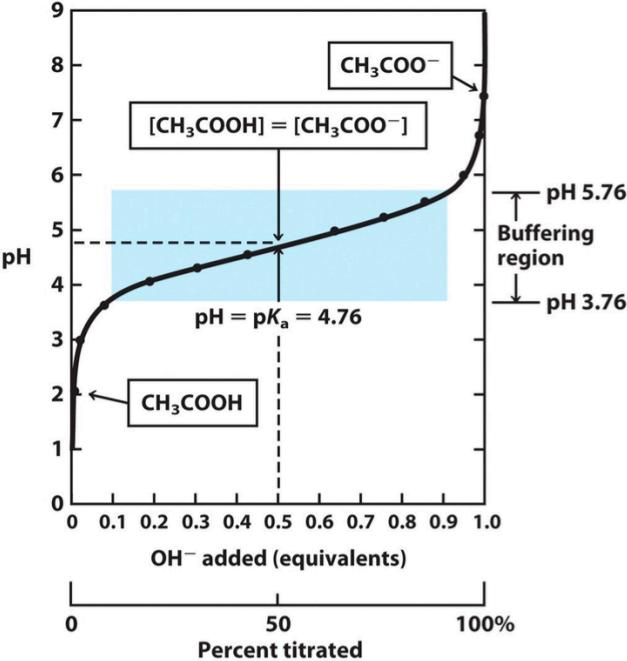
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Lecture 1: Introduction to Biochemistry

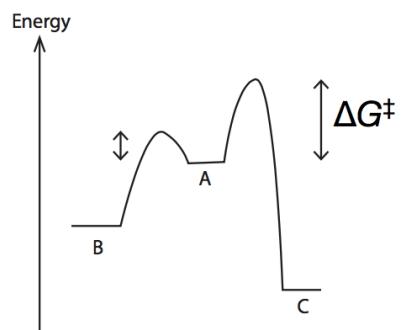
- A. What is biochemistry?
 - a. Study of organic molecules and their chemical reactions
 - b. Descriptive and mechanistic
- B. Representations of molecules: cartoon (functional units), space filling (individual atom volume), and ribbon (path and folding of AA backbone).
- C. Four foundational forces: H-bonds; ionic bonds, van der Waals interactions, hydrophobic effect.
 - a. These are specific, reversible interactions that act en masse.
 - b. Ionic interaction: derived from Coulombic forces: $E = k \frac{q_1 q_2}{\epsilon r}$;
 $k \sim 332 \text{ kcal/mol}$, $\epsilon_{\text{water}} \sim 80$, $\epsilon_{\text{membrane}} \sim 2$. Polar dielectrics have a “shielding” effect, making them easier to separate.
 - c. H-bonds: unequal covalent bonds create H-bonds donors and acceptors
 - i. Directional (donors+ and acceptors-)
 - ii. Vary in strength (0 to ~5 kcal/mol [ideal])
 - iii. C-H bonds ~1 Å, H-bonds ~2 Å;
2 electronegative atoms in H-bonded molecules ~2.5-3.5 Å
 - d. Van der Waals interactions: electrons polarize in a favorable way.
 - i. Weak, but sum together to be significant.
 - ii. At a few angstroms, you get attraction.
Too close- repulsion increases rapidly. Too far- attraction decays.
 - e. Hydrophobic interactions: ordering of water at nonpolar surfaces.
 - i. When you bring together hydrophobic surfaces, you liberate ordered water molecules, which increases disorder/entropy.
 - ii. ~ 25 cal/A² when you cover up exposed surface area.
- D. Thermodynamics: driving forces
 - a. 2nd law: universal entropy (S) increases in every spontaneous process. System + Surroundings.
 - b. Entropy describes disorder; degrees of freedom.
- E. Enthalpy is heat content (H)
 - a. Example: $2 \text{ H}_2 + \text{O}_2 \rightarrow \text{H}_2\text{O} + \text{heat}$
 - i. Entropy of the system decreases (fewer gas molecules).
 - ii. Entropy of surroundings increases because disordered heat energy is released.
 - b. Derivation:
$$\Delta S_{\text{surroundings}} = - \frac{\Delta H_{\text{system}}}{T}$$
$$\Delta S_{\text{total}} = \Delta S_{\text{system}} - \frac{\Delta H_{\text{system}}}{T}$$
$$\Delta S_{\text{total}} = \Delta S_{\text{system}} + \Delta S_{\text{surroundings}}$$
$$-\Delta S_{\text{total}} = \Delta H_{\text{system}} - T\Delta S_{\text{system}}$$
$$\Delta G = \Delta H_{\text{system}} - T\Delta S_{\text{system}}$$
- F. $\Delta S(\text{total}) > 0 // -\Delta S(\text{total}) < 0 // \Delta G(\text{system}) < 0$
- G. The Gibbs free energy is a system parameter that allows us to calculate reaction dynamics without measuring surroundings.

Lecture 2: Chemical Principles of Biochemistry

- A. Gibbs Free Energy: determines spontaneity of reactions
 - a. 2nd Law: reactions are spontaneous iff entropy of the universe increases:
 - b. $\Delta S_{\text{total}} \geq 0$; $\Delta G < 0$
 - c. $\Delta G = \Delta H_{\text{system}} - T\Delta S_{\text{system}} = -T\Delta S_{\text{total}}$ (H_{total} is always 0 by conservation of energy)
 - d. ΔG is a property of the system only, so it can be determined.
- B. Free Energy depends on concentration of reagents and products
 - a. ΔG and K_{eq} express the “driving force” of a reaction
 - b. At equilibrium ($\Delta G=0$, $K=K_{\text{eq}}$), no net change in concentrations
 - c. $\Delta G = \Delta G^{\circ} + RT \ln K$
 - d. For $A + B \rightarrow C + D$ at equilibrium: $\Delta G = 0$; $\Delta G^{\circ} = -RT \ln K_{\text{eq}} = -1.36 \log \frac{[C]_{\text{eq}}[D]_{\text{eq}}}{[A]_{\text{eq}}[B]_{\text{eq}}}$
 - e. Free energies and equilibrium constants are related:
 - i. At 0 free energy, $K_{\text{eq}} = 1$.
 - ii. 1.4 kcal/mol or 5.7 kJ/mol shifts K_{eq} by 10-fold.
- C. Acids and Bases have their own conventions
 - a. $K_{\text{eq}} = K_a = [A^-][H^+]/[HA]$; $pK_a = -\log K_a$
 - b. Titration curves illustrate buffering capacity
 - c. Memorize pK_a values:
 - i. Acetic acid: 4.76
 - ii. Carbonic Acid: 3.66 // Bicarbonate: 10.2
 - iii. Glycine, carboxyl: 2.34 // Glycine, amino: 9.60
 - iv. Phosphoric acid: 2.14 // Dihydrogen phosphate: 6.86 // Monohydrogen phosphate: 12.4
 - d. The environment affects pK_a ; an acid is weakened in acidic environments and strengthened in basic environments (Le Chatelier’s principle).

D. Thermodynamic product: lowest energy state (C)

Kinetic product: lowest energy barrier (B)



Note: assume irreversible reaction

$\Delta\Delta G^\ddagger$ kcal/mol	Amount B
5.46	99.99%
2.73	99%
1.36	90.9%
0	50%

E. Chemical Kinetics

- For $A \rightarrow B$: $\frac{d[B]}{dt} = k[A]$; $k = \frac{k_B T}{h} e^{-\frac{\Delta G}{RT}}$ (Boltzmann, Planck)
- Fast, bimolecular reactions are diffusion limited ($A + B \rightarrow C$); $k_{max} = 10^8 \text{ M}^{-1}\text{s}^{-1}$
- Random collision reactions can be very fast in biological phenomena: aquaporin transports water at 10^9 molecules per second.

F. Overview of Cellular Composition:

- Monomers \rightarrow Macromolecules \rightarrow Supramolecular complexes
- Nucleotides \rightarrow DNA \rightarrow Chromatin
- Amino acids \rightarrow Proteins \rightarrow Plasma membrane
- Sugars \rightarrow Cellulose \rightarrow Cell wall

G. Key elements (making up most biomolecules).

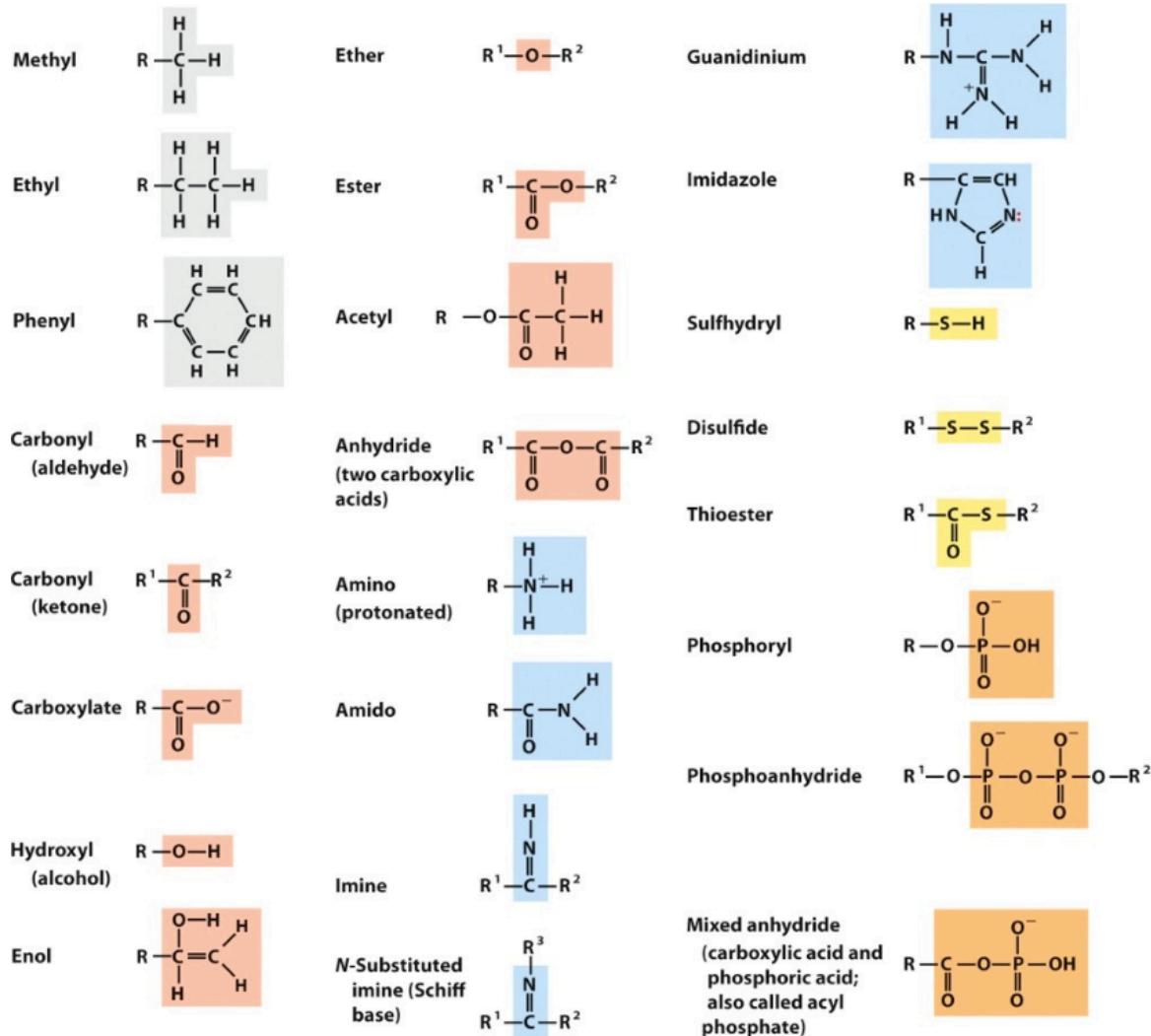
Know bulk elements, abbreviations, and periodic table locations

H. Drawing molecules: draw bond angles correctly, use skeletal structures

- Carbon in biomolecules are usually sp^2 (120°) or sp^3 (109.5°)

I. Oxidation \rightarrow pi-bonds and bonds to electronegative atoms

J. Common chemical groups in biomolecules (know names, be able to identify in molecules)



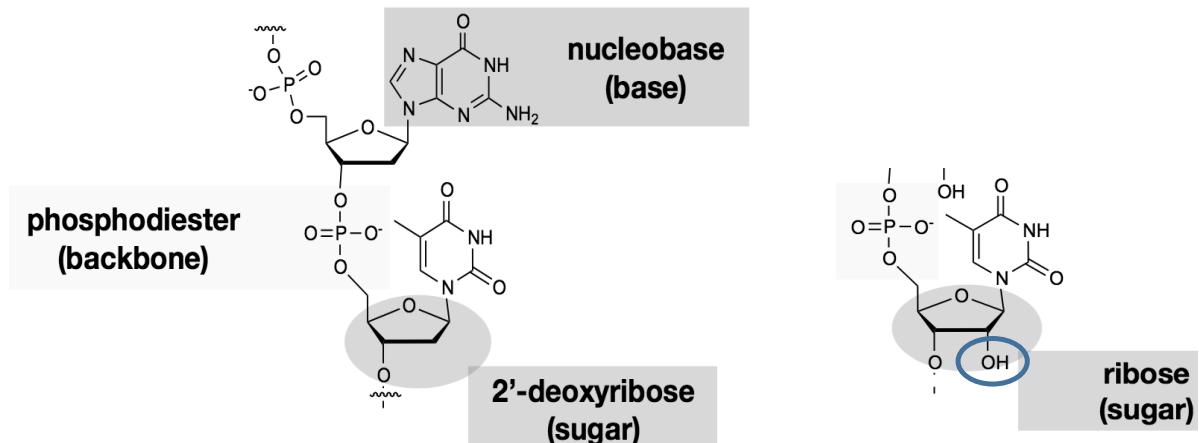
Key Molecular Classes: water, proteins, nucleic acids, polysaccharides, lipids, monomeric subunits and intermediates, inorganic ions.

A. Proteins: polymers of amino acids

B. Nucleic acids: polymers of nucleotides

a. DNA: right-handed double-helix (follows right hand rule)

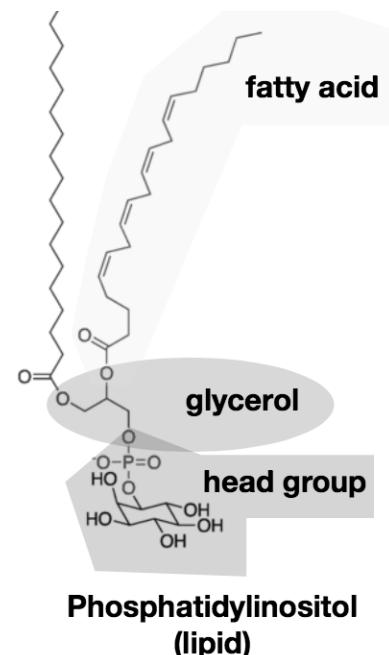
RNA: ribose, uracil



- C. Polysaccharides are made of sugar building blocks
e.g. cellulose from glucose.
- D. Lipids are mostly fatty acid derivatives (fatty acids + head groups)
- E. Inorganic ions
 - a. Enzyme cofactors:
Mg²⁺ coordinates the phosphates of ATP in many enzymes
 - b. Structural roles via chelation in proteins/RNA:
(Chelation is when ions are electrostatically attached)
Zn²⁺ coordinates His and Cys in some transcription factors.
 - c. Signaling molecules:
Ca²⁺ release in response to IP₃

F. Summary

- a. Learn how K_{eq} and standard free energy can determine whether a reaction is thermodynamically favorable
- b. Learn the relationship between equilibrium constant and free energy
- c. Thermodynamic v. kinetic selectivity; ballpark numbers
- d. Major classes of biomolecules and their monomer subunits



Lecture 3: Amino Acids, Peptides, and Secondary Structure

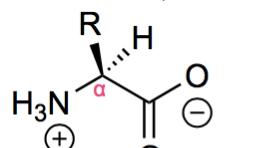
A. Learn figure 3-14.

B. Proteins: molecular machines driving biological function

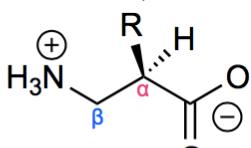
a. Primary sequence: proteins are a linear array of amino acids linked by peptide bonds

b. This linear sequence is generally encoded in DNA. They fold into ordered structures and often self-assemble. Structure drives function.

C. α -amino acids: (α -site is closer to COO^-)



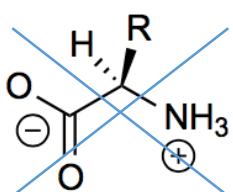
α -amino acid



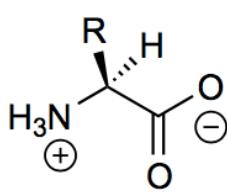
β -amino acid

D. Amino acids in proteins are always L-stereoisomers.

This results in the S conformation for all amino acids except Cys, which is R.

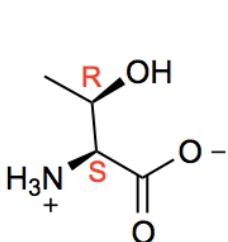


D-amino acid

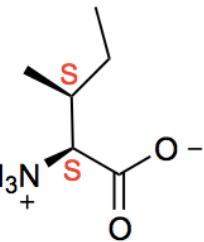


L-amino acid

E. Threonine and Isoleucine have two stereocenters. Don't worry, these are fixed.

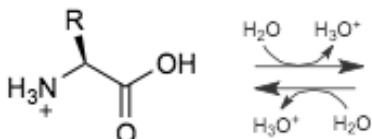


(2S,3R)-Threonine

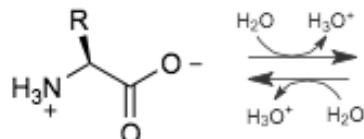


(2S,3S)-Isoleucine

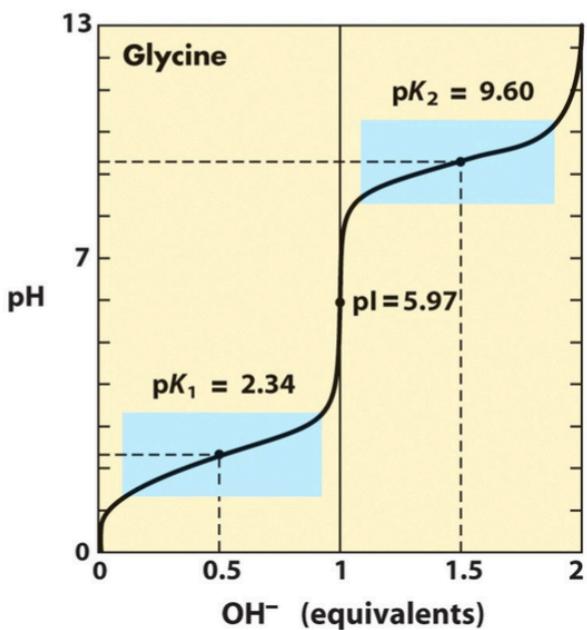
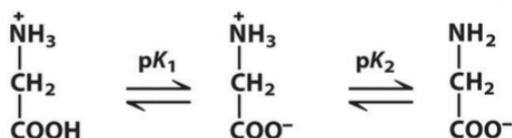
F. Amino acids are zwitterionic (+ and - charges) because COOH will always protonate NH_2



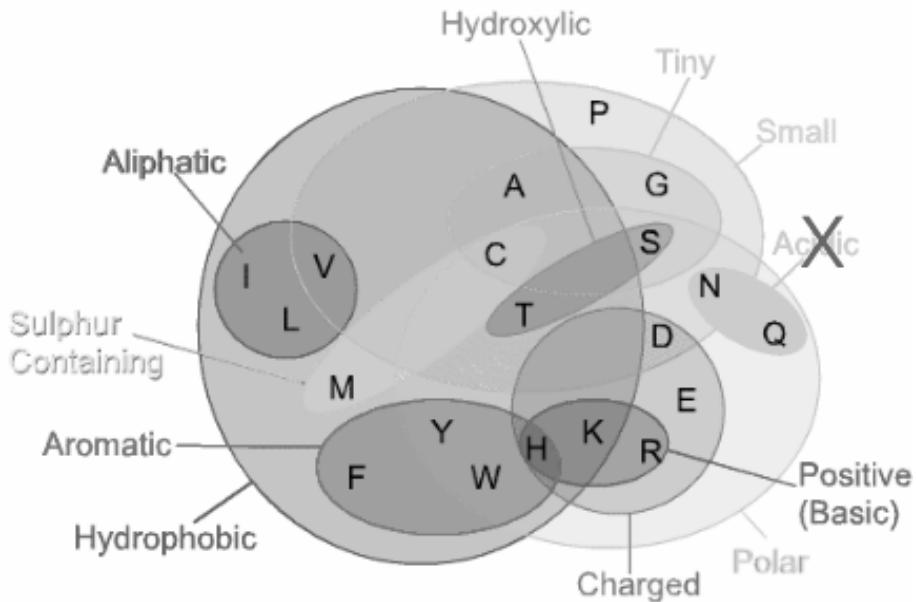
Acidic conditions



Basic conditions

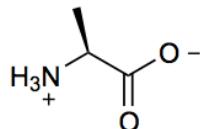


Learn Names and Abbreviations

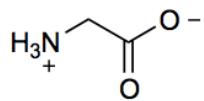


Ala	A	Alanine
Arg	R	Arginine
Asn	N	Asparagine
Asp	D	Aspartic acid
Cys	C	Cysteine
Gln	Q	Glutamine
Glu	E	Glutamic acid
Gly	G	Glycine
His	H	Histidine
Ile	I	Isoleucine
Leu	L	Leucine
Lys	K	Lysine
Met	M	Methionine
Phe	F	Phenylalanine
Pro	P	Proline
Ser	S	Serine
Thr	T	Threonine
Trp	W	Tryptophan
Tyr	Y	Tyrosine
Val	V	Valine

A. Tiny amino acids: Alanine and Glycine



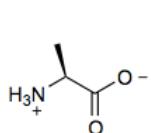
Alanine (Ala, A)



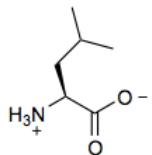
Glycine (Gly, G)

B. Hydrophobic amino acids: Aliphatic + methionine

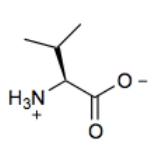
Note the beta-branching.



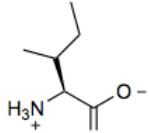
Alanine
(Ala, A)



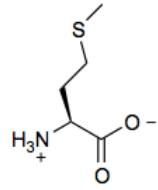
Leucine
(Leu, L)



Valine
(Val, V)

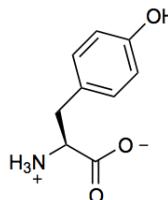


Isoleucine
(Ile, I)

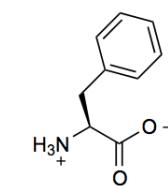


Methionine
(Met, M)

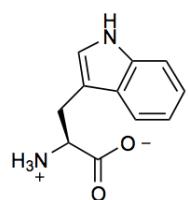
C. Aromatic amino acids: absorb UV light (~280 nm to quantify amount of protein)



Tyrosine
(Tyr, Y)



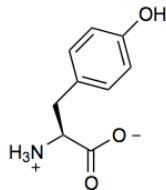
Phenylalanine
(Phe, F)



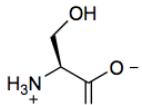
Tryptophan
(Trp, W)

+ Histidine

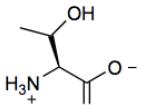
D. Hydroxyl and Sulfhydryl Amino Acids



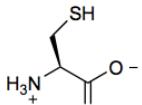
Tyrosine
(Tyr, Y)



Serine
(Ser, S)

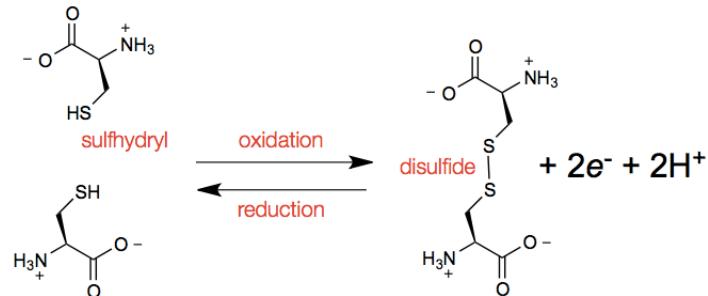


Threonine
(Thr, T)



Cysteine
(Cys, C)

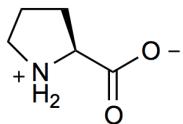
E. Cysteine can be **oxidized** to cystine via disulfide bridge



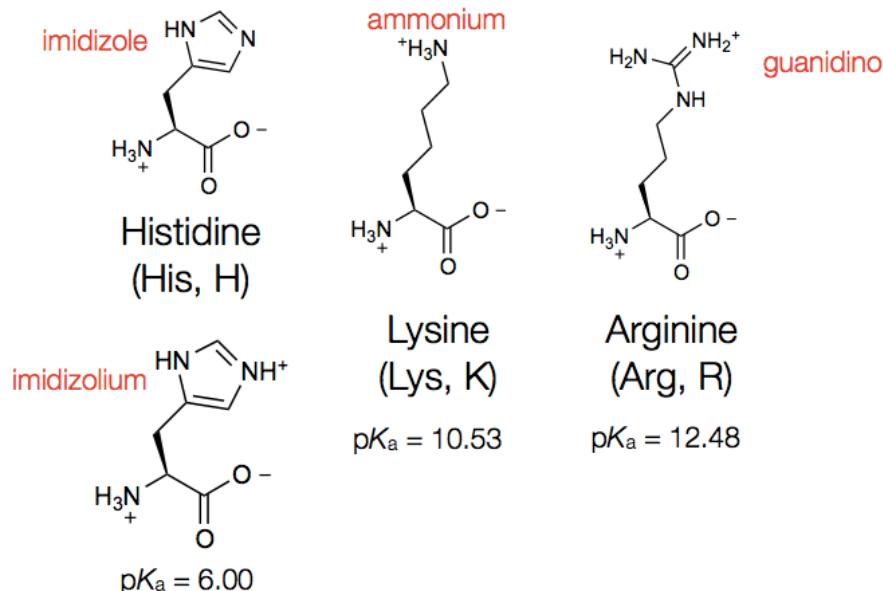
Cysteine
(Cys, C)

Cystine

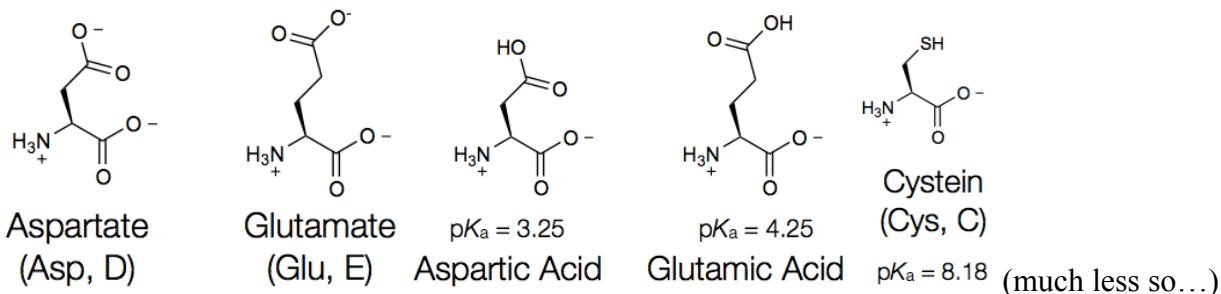
F. Proline: a secondary amine leads to a **constrained** cyclic conformation with limited rotations.



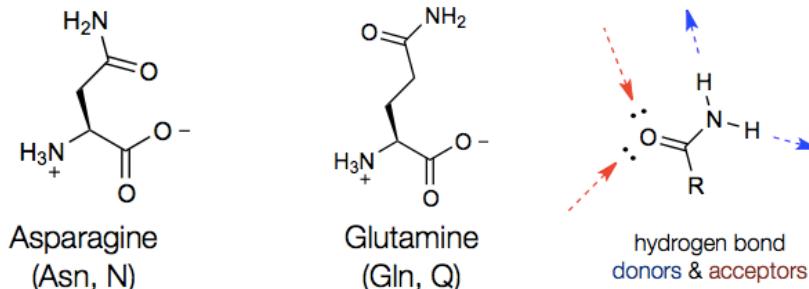
G. Basic Amino Acids



H. Acidic Amino Acids



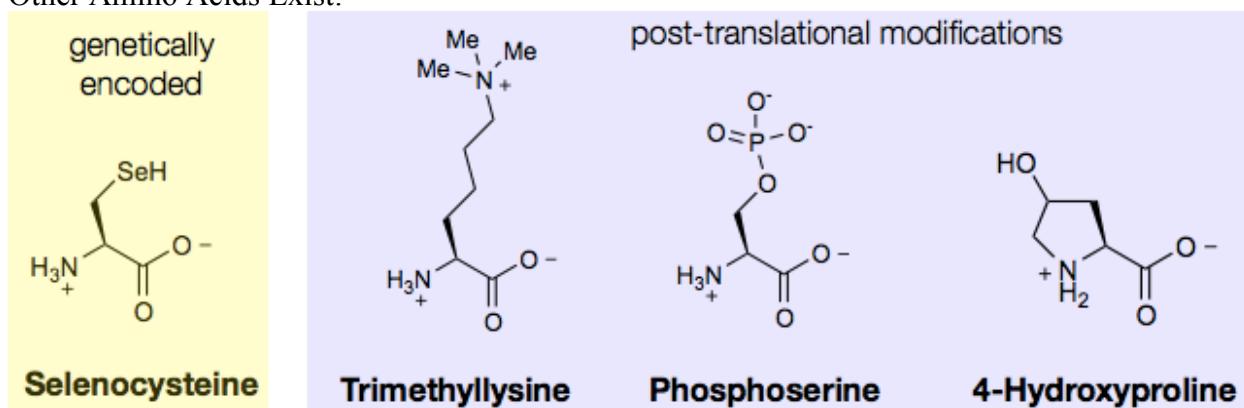
I. Polar Amino Acids



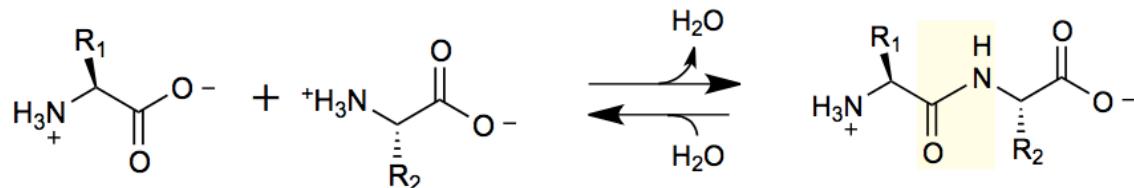
Learn: structures, names, abbreviations, properties, and pKa (for acidic and basic R)

Amino acid	pK_1 (-COOH)	pK_2 (-NH ₃ ⁺)	pK_R (R group)
Nonpolar, aliphatic			
R groups			
Glycine	2.34	9.60	
Alanine	2.34	9.69	
Proline	1.99	10.96	
Valine	2.32	9.62	
Leucine	2.36	9.60	
Isoleucine	2.36	9.68	
Methionine	2.28	9.21	
Aromatic R groups			
Phenylalanine	1.83	9.13	
Tyrosine	2.20	9.11	
Tryptophan	2.38	9.39	
Polar, uncharged			
R groups			
Serine	2.21	9.15	
Threonine	2.11	9.62	
Cysteine	1.96	10.28	
Asparagine	2.02	8.80	
Glutamine	2.17	9.13	
Positively charged			
R groups			
Lysine	2.18	8.95	10.53
Histidine	1.82	9.17	6.00
Arginine	2.17	9.04	12.48
Negatively charged			
R groups			
Aspartate	1.88	9.60	3.65
Glutamate	2.19	9.67	4.25

A. Other Amino Acids Exist:

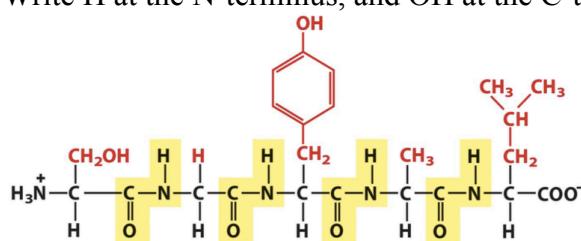


B. Peptide Bond Formation and Hydrolysis

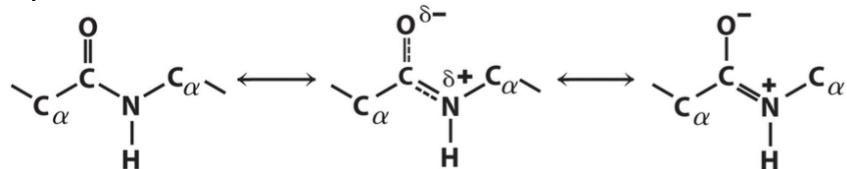


Protein hydrolysis is favorable, but the energy barrier is high and keeps it kinetically stable.

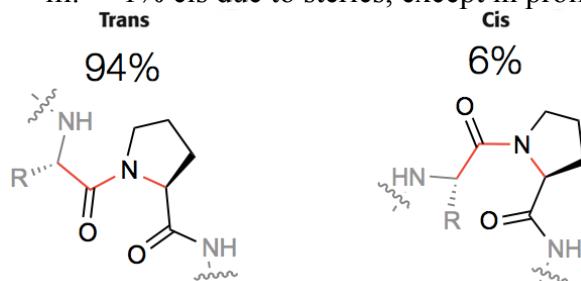
- C. Dipeptide: 2, tripeptide: 3, peptide: several, protein: many (60+)
- D. Most proteins are large; frequency skewed right by a few really big ones.
- E. Proteins and peptides are mostly made via translation of mRNA on ribosomes
 - a. Central dogma: makes the vast majority of proteins and peptides
 - b. Non-ribosomal peptide synthesis: multi-subunit complexes make predefined peptides.
Only a few peptides are made this way, often cyclic.
- F. Peptide nomenclature (shown: peptide bonds, side chains, termini)
Write H at the N-terminus, and OH at the C-terminus



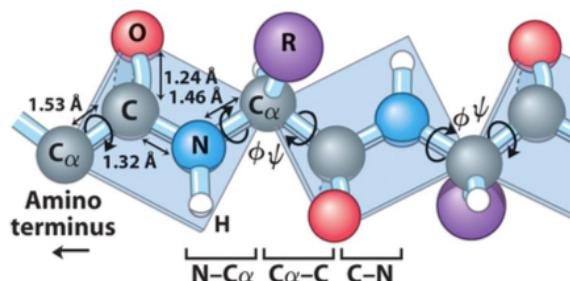
G. Peptide conformation



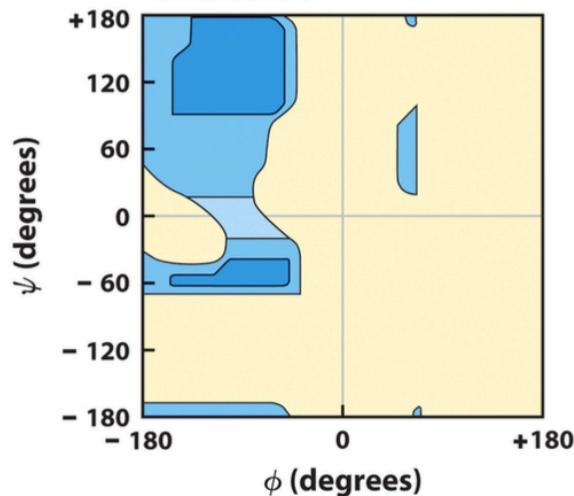
- a. Peptide bonds have double-bond character from resonance, creating 120° angles.
 - i. This makes **ALL SIX** atoms planar; twisting the bond requires ~ 20 kcal/mol
 - ii. The C-N bond is shorter than usual: 1.33 \AA v 1.47 \AA .
 - iii. $<1\%$ cis due to sterics, except in proline, which has 6% cis due to more equal sterics.



- b. Peptide backbone conformation can be described by two angles: phi and psi
Bonds are on the order of 1.0 \AA — 1.5 \AA



H. Ramachandran plots: accessible conformations of peptides (darker is more allowable)



- I. Primary structure: linear sequence of monomers.
Secondary structure: local conformation of the backbone.
Tertiary structure: interactions of side chains on a single polypeptide to form overall structure.
Quaternary structure: interactions of multiple peptides to form a complex.
- J. Four Foundational Forces in Biochemistry:
Ionic interactions, H-bonds, van der Waals, Hydrophobic effect.
- K. H-bonds can form between NH_3^+ and C=O : in the peptide backbone, forming structural motifs.
Both were predicted by Linus Pauling, who used high-tech modeling to discover the helix.

Lecture 4: Fibrous and Globular Proteins

A. Alpha Helix

- a. Right-handed (allows outward side chains), rigid, filled center.
- b. 1 turn = 5.4 Å and 3.6 residues; 1 amino acid = 1.5 Å
This is a good benchmark for molecular length.
- c. All N-H and C=O groups bonded at optimal length $C_n - \cdots - C_{n+4}$
- d. The α -helix creates a net dipole (negative C=O points toward the C-terminus; positive N-H points toward the N-terminus)

B. Side chain interactions: especially important for side-chains of $C_n - \cdots - C_{n+4}$ residues because they're next to each other.

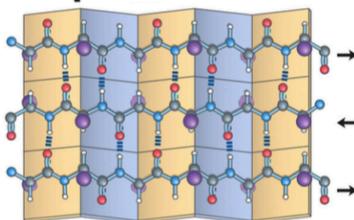
- a. Stabilizing: hydrophobic interactions
- b. Destabilizing: steric hindrance
- c. Either: electrostatic interactions
- d. Proline: produces a kink (because of angles available); thus, usually absent in α -helices.

C. Structure related to function: α -helices can make sequence-specific contacts with DNA.

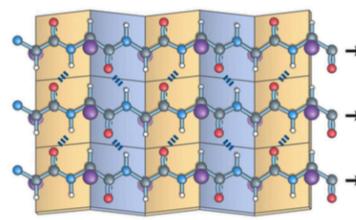
D. Beta-Sheets

- a. Two types exist and are favorable: formation depends on AA sequence.
- b. Antiparallel: peptide chains in opposite directions; parallel: same direction

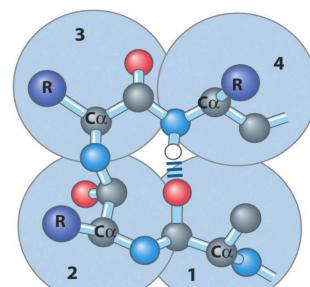
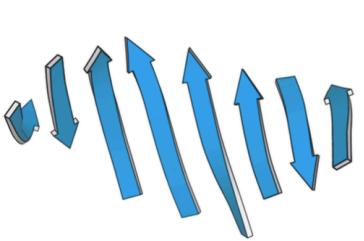
Antiparallel



Parallel

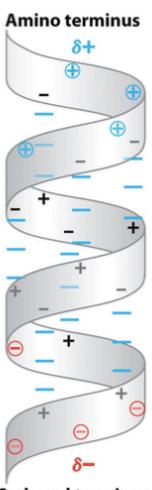


- c. 2-15 extended strands of ~6 residues arranged into β -pleated sheets.
- d. Successive side chains project to opposite sides of the sheet, alternating up and down.
- e. Most N-H and C=O groups are H-bonded.
- f. β -sheets are indicated by numerous directional flat ribbons ($N \rightarrow C$).
These two are the same sheet, shown from 2 different angles.



Type I

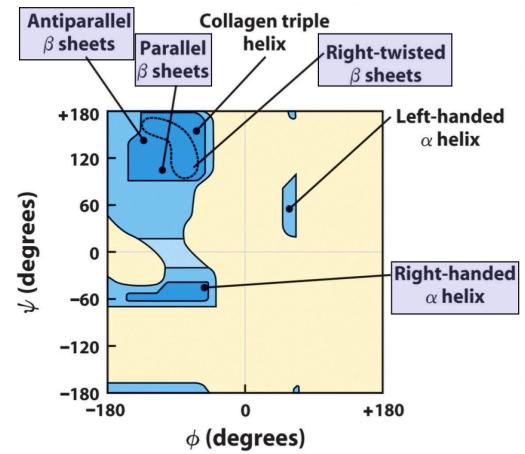
- E. **β -turns** reverse polypeptide chain direction in antiparallel β -sheets:
Tight 180° turns by 4 amino acids, with 1-4 H-bonding.
- F. **Coils** are non-repetitive secondary structures. Coils have ordered geometries, but are not definable by simple structural categories. About half of a protein's structure can be referred to as coil conformation.



The helix creates a net dipole

G. Secondary structures represent certain conformational spaces.

- We plot the amino acids and see where they go.
- Glycine is so small it can adopt a large number of angles, and obscures the overall patterns.



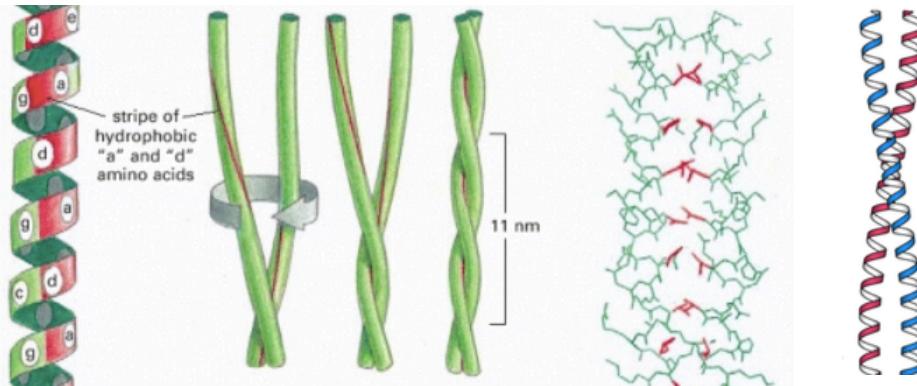
Fibrous Proteins: Long strands/sheets

A. Examples

- Keratin: coiled-coil of α -helices
- Collagen: unique triple helix
- Silk fibroin: smooth sheets

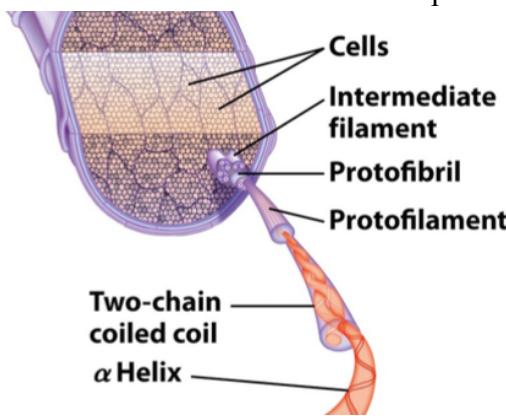
B. Parallel α -helices can dimerize into a coiled-coil (same N→C)

- Hydrophobic side-chains pack at the interface to hold α -helices together in a left-handed twist.
- Heptad (7) pseudo repeat (abcdefg) creating a stripe of hydrophobic residues that hold together at contact areas



C. Keratins are structural proteins: $M_r = 40-67 \text{ kDa}$.

- Formed by coiled-coil α -helices dimers.
- In the intermediate filament (IF) family that assembles into larger complexes e.g. hair, wool.
- Structural levels: IFs are made of protofibrils, made of protofilaments, made of α -helix dimers.

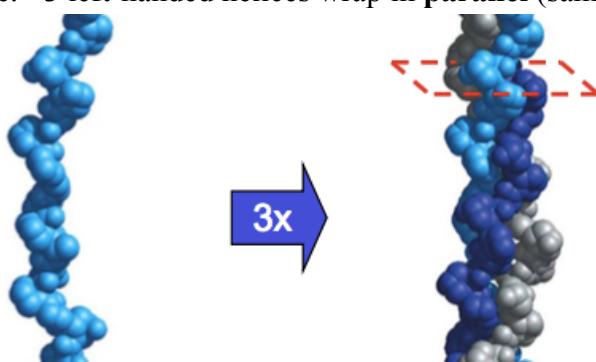


- Application: DNA-binding leucine zippers also allows sequence specific connection. The hydrophobic leucines help “zip up” the molecule, with other interactions helping.

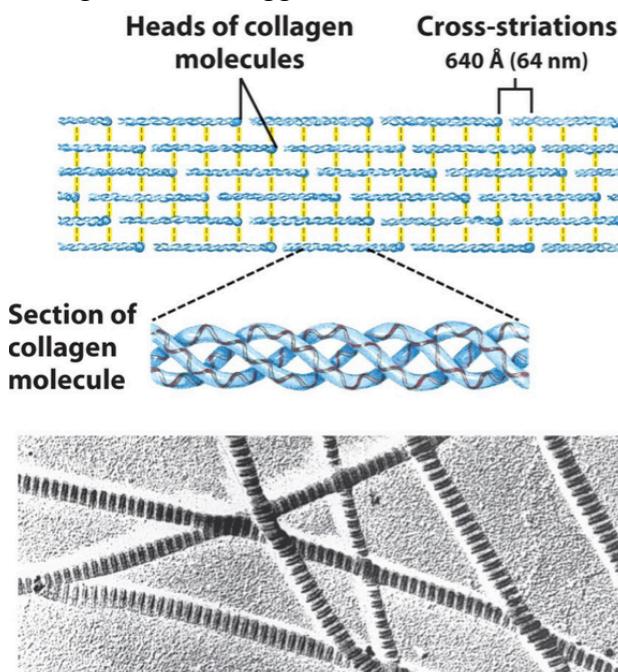
D. Collagen: a triple helix

- Made of ~1050 amino acids and ~120 kDa per chain. 3 residues/turn.
- High tensile strength: structural component of all connective tissues, like cartilage, bone, skin.
- Repetitive motif $(\text{Gly-X-Y})_n$ // X is usually proline, and Y is usually hydroxyproline.

- d. Proline rings on the outside; extensive H-bonding.
- e. 3 left-handed helices wrap in **parallel** (same N→C) to make a right-handed twist.



- f. Collagen molecules: tightly packed helices, intramolecular links
- g. Collagen fibrils: staggered molecules, intermolecular crosslinks.



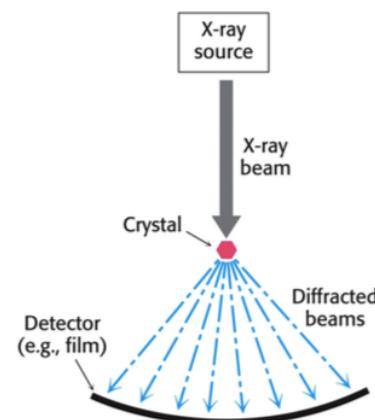
repeat (Gly-Ser-Gly-Ala-Gly-Ala)_n

- E. Silk: Fibroin consists of β -pleated sheets.
- a. Fibroin: 5263 amino acids (391 kDa).
 - b. Tightly packed, stacked antiparallel β -sheets
 - c. Stabilized by extensive H-bonding; no covalent bridges

Structural Determination

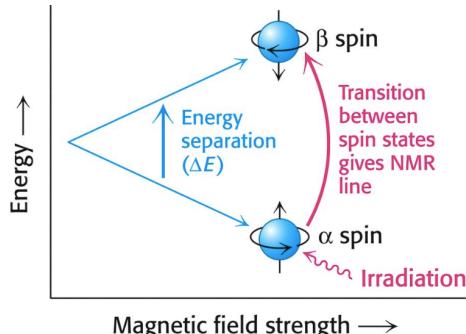
F. X-ray Crystallography

- a. X-rays: 0.7-1.5 Å wavelength
- b. Tightly packed crystals of purified proteins diffract X-rays. Homogeneity is key to tight packing.
- c. Obtaining protein crystals is really hard; frequently the rate limiting step for crystallography.
- d. Diffraction pattern allows calculation of contour map of electron density distribution. The backbone and residues are then fitted in to obtain the structure.



G. NMR Spectroscopy

- e. NMR active nuclei like ^1H , ^{13}C , and ^{15}N have a magnetic dipole that gives them nuclear spin. This gives you information about its electron environment.
- f. Different structures are solutions for NMR data under certain geometric constraints.



H. Cryo-Electron Microscopy (best for atomic structure of proteins)

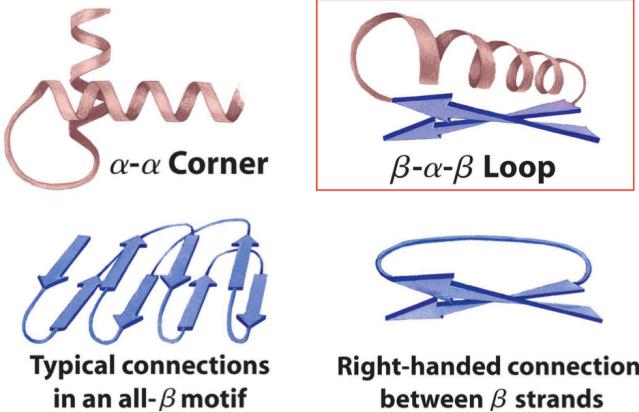
- a. Transmission EM: electrons pass through frozen homogeneous sample and hit a detector screen.
- b. Revolution in usage: much higher resolutions than available in the past.
- c. Sample frozen quickly to prevent ice crystal formation.
 - i. Benefit: observation of the true shape of a hydrated molecule in solution.
 - ii. Cost: No stain, low signal.
- d. Single molecules are aligned and modeled into a 3D picture.

Summary of Methods for Determining Protein Structure

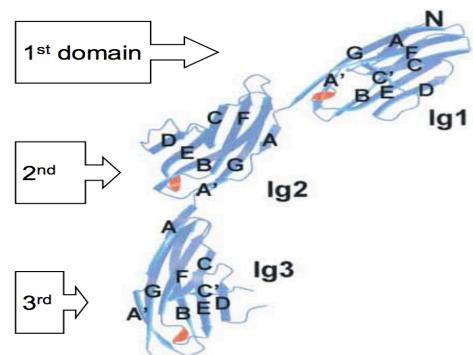
Technique	Advantages	Disadvantages/ Challenges
X-ray Crystallography	<ul style="list-style-type: none"> • Possibility of atomic resolution. 	<ul style="list-style-type: none"> • Requires protein crystals that diffract. • To find conditions, usu. need large quantities of homogenous material. • Possibility of crystallization induced artifacts.
NMR	<ul style="list-style-type: none"> • Can capture protein dynamics. • Structure is determined in solution 	<ul style="list-style-type: none"> • Requires concentrated protein. • Difficult to assign peaks especially for large proteins. • Frequently requires isotopically labeled proteins.
Electron Microscopy	<ul style="list-style-type: none"> • Requires less material. • Especially useful for very large complexes. 	<ul style="list-style-type: none"> • Generally lower resolution. • Better suited for large assemblies that provide high contrast and not small proteins.

Soluble Globular Proteins

- A. Fold into compact structures with polar surfaces and hydrophobic insides
Pockets shaped to accommodate prosthetic groups or binding partners.
- B. These proteins contain varying fractions of secondary structures (helices and sheets).
- C. Combined secondary structures form folds that often compact them heavily (~20x shorter)



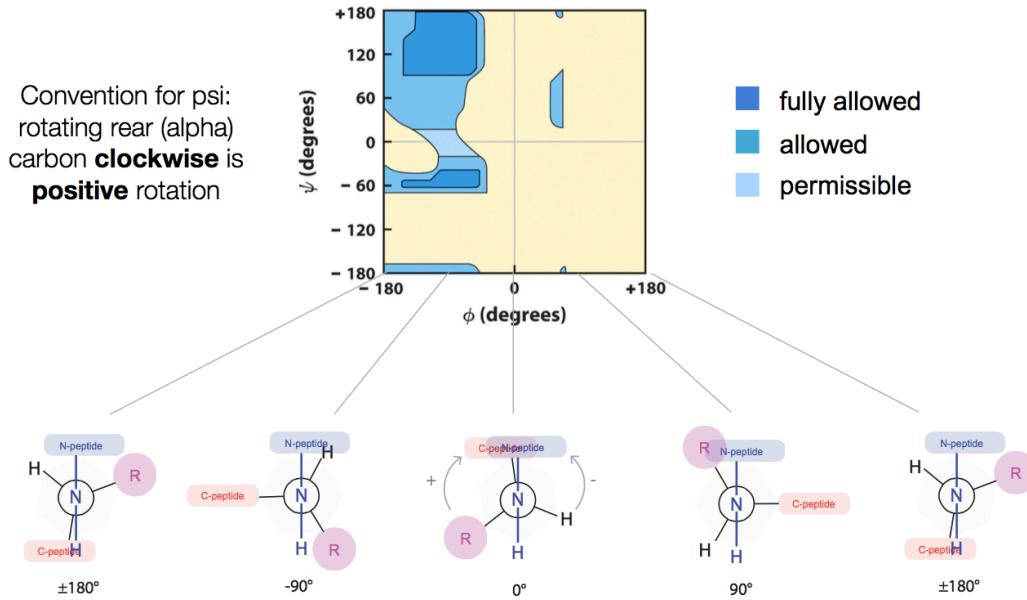
- D. Motif: a repetition of a fold. Stable motifs are classified into four groups.
 - a. Only α, only β, combination α/β, sequential α/β
- E. Polypeptides can fold into multiple domains
 - a. Domains: stable globular units
 - b. Three immunoglobulin domains shown below
- F. Tertiary Structure: Myoglobin as an example:
 - a. Myoglobin has hydrophobic residues, a bound heme group, and 78% α-helix, 0% B-sheet.
 - b. Heme group: protoporphyrin (organic tetrapyrrole ring) with Fe²⁺ in 6 coordination bonds. Oxygen binding site.
 - c. Compact interior stabilized by van der Waals and hydrophobic interactions.
- G. Quaternary Structure: Hemoglobin as an example
 - d. 64-kDa tetramer of 4 subunits: 2 α-globin and 2 β-globin.
 - e. Oligomeric protein; dimer of α-β protomers.
 - f. C₂ rotational symmetry: superimposable by 180° rotations, no reflections.
- H. Summary
 - g. Fibrous proteins:
 - i. Keratin: a coiled coil of alpha helices
 - ii. Collages: a Gly-Pro rich triple helix
 - iii. Fibroin: stacked beta-sheets
 - h. Methods of protein structure determination (x-ray crystallography, NMR, EM)
 - i. Globular Proteins: folds, motifs, domains, and all levels of structure



Lecture 5: Protein Structure, Stability, and Folding

A. Conformational Modeling

- a. Rotating clockwise is positive rotation.
- b. The Ramachandran plot is populated where we expect:



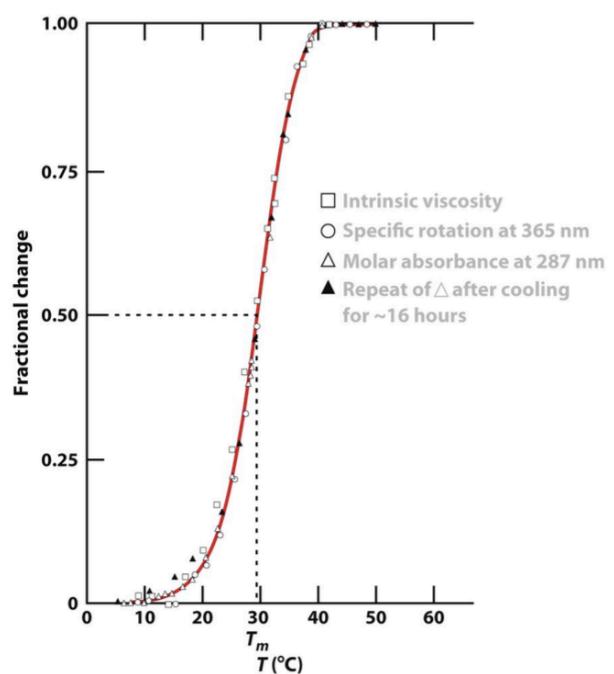
- c. Conventionally, peptides are all trans, fully extended at 180° (first picture, bottom-left corner).

B. Protein Stability

- a. Proteins are only marginally stable: >100 residue proteins generally stable by only ~ 10 kcal/mol
- b. Ionic forces: not main drivers of stability. Well solvated on the surface, rare in the core.
- c. van der Waals forces (including induced dipoles and London dispersion forces): independently weak, but collectively substantial driver of stability.
- d. Hydrophobic effect: very important for stability
- e. H-bonds: generally in the interior, < 2 kcal/mol for $\text{N-H} \cdots \text{O=C}$. Important for stability.

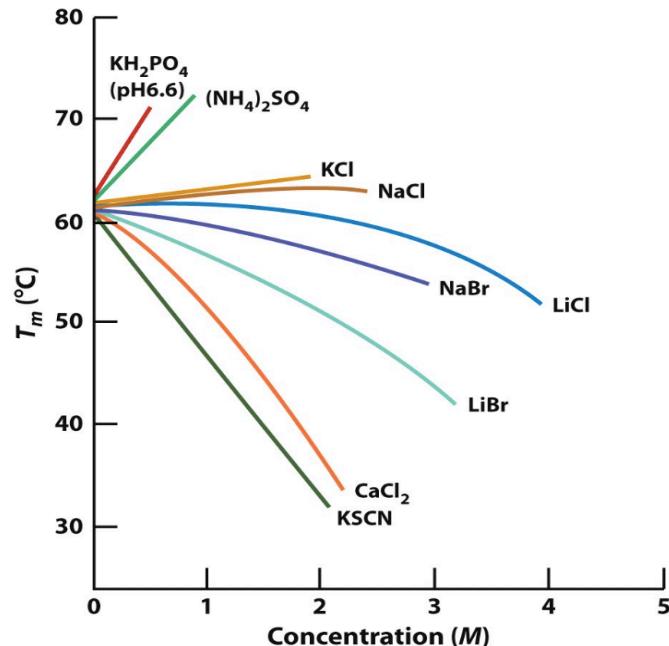
C. Protein Denaturation from Native Conformations

- a. Protein stability is measured by T_m : denaturation midpoint. This is the temperature at which the proportion of folded and unfolded states are both 0.50 – determined by thermal shift assay.
- b. pH (disrupts ionization state)
- c. Detergents (disrupt hydrophobic effects)
e.g. SDS
- d. High concentrations of organic substances and solvents. (disrupt hydrophobic effects).

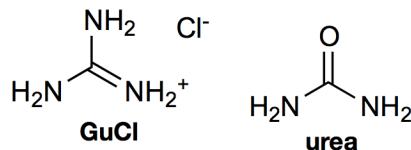


e. Chaotropic salts: salts that cause denaturation

i. Salts can be stabilizing OR destabilizing. Denaturation at high molarity (1-10 M).



ii. Common denaturants are guanidinium chloride and urea.

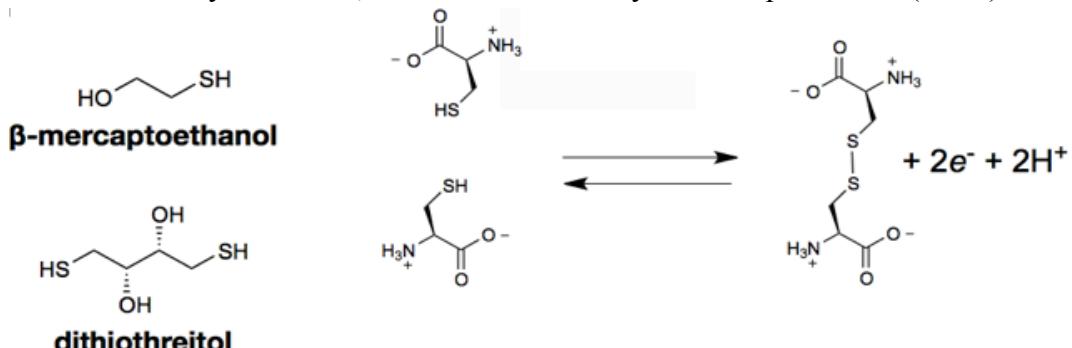


D. Classic experiment to test whether primary sequence directs folding:

Christian B. Anfinsen. "Studies on the Principles that Govern the Folding of Protein Chains."

E. Ribonuclease (RNase) catalyze hydrolysis of RNA

a. Stabilized by disulfides, which are reduced by β -mercaptoethanol (BME) or dithiothreitol (DTT)



b. RNase loses activity reversibly when unfolded with urea + BME.

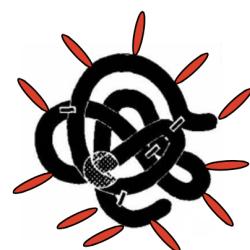
c. Environment affects reversibility i.e. pH. This gives the same S-shaped curve as temperature.

d. Anfinsen's experiment denatured RNase with urea and BME, but it "renatured" afterwards.

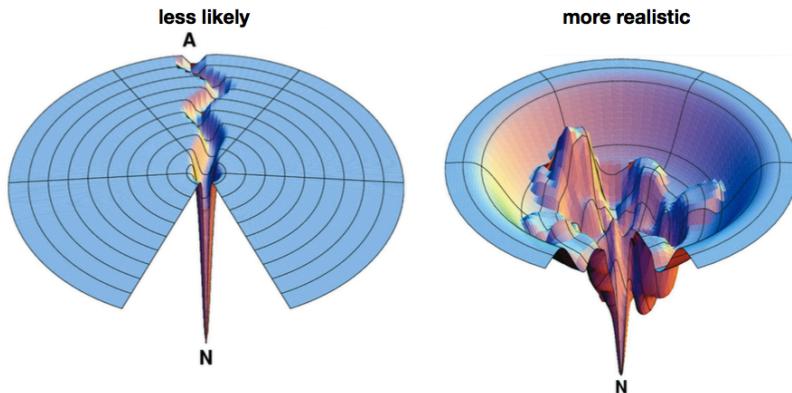
Conclusion: reversibility means complete protein folding information is present in the amino acid sequence.

F. Folding is mainly driven by internal residues

- RNase experiment: derivatize some free surface amino groups with an 8 amino acid peptide (poly-DL-alanine) and check for reversibility.
- Result: no effect on folding. Thus, internal amino acids drive folding.

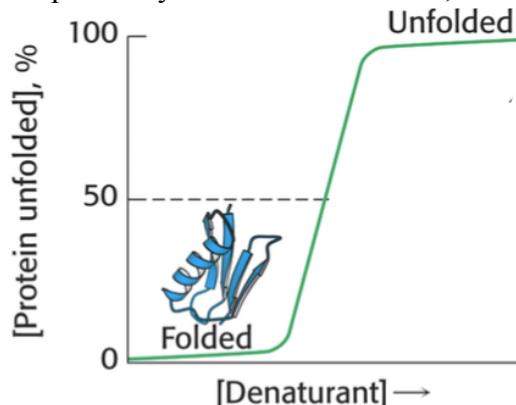


- G. Thermodynamic Hypothesis (for folding): the 3D structure of a native protein in its normal physiological state (solvent, pH, temperature) is at the lowest energy state.
- a. Levinthal-Paradox: If you have a 100 AA polypeptide with 3 main chain rotations (conservative), you get $3^{100} = 5 \times 10^{47}$. Each rotation takes 10^{-13} seconds, so randomly finding the most optimal one would take 1.6×10^{27} years. Thus: folding must follow limited pathways.
 - b. Folding is not a single pathway, but a collection of pathways making a simultaneous local conformational search in parallel – a rugged energy surface with local minima.



H. Denaturation curves and cooperativity

- a. Cooperativity: half-denatured state; mixture of folded and denatured molecules.



I. Folding is a progressive energy-entropy trade

protein	environment	ΔG	ΔH	$-\Delta S$
RNAse	pH 2.5	-1.7	-57.0	55.3
Chymotrypsinogen	pH 3	-7.7	-39.0	31.3
Myoglobin	pH 9	-13.6	-41.8	28.2

J. Chaperones provide microenvironments that assist in folding

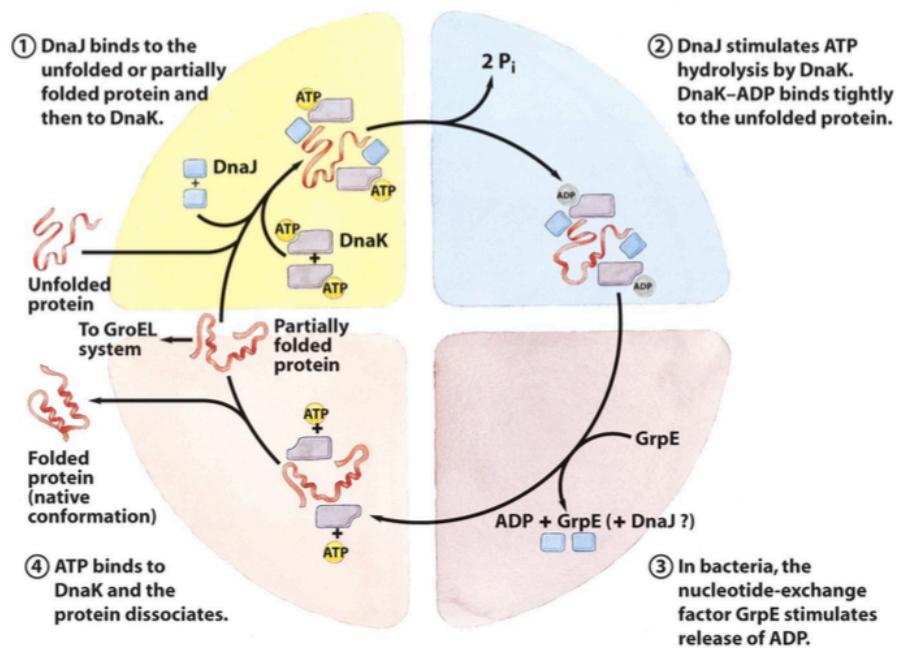
- a. Two major families:
 - i. Heat shock protein 70 (Hsp70): bind to hydrophobic residues in new or partially unfolded proteins (named DnaK in *E. coli*).
 - ii. Chaperonins: give misfolded proteins a second chance (GroEL/ES system in *E. Coli*).
- b. Isomerases assist with configuration
 - i. Protein disulfide isomerase (PDI): forms correct disulfide bridges
 - ii. Peptidyl prolyl cis-trans isomerase: accelerates native cis/trans proline configuration.

c. Mechanism

key: recognition of hydrophobic surfaces

followed by bind/release cycles under ATP hydrolysis

<i>E. coli</i>	<i>human</i>
DnaJ	hsp40
DnaK	hsp70
ATP ADP+Pi	ATP ADP+Pi

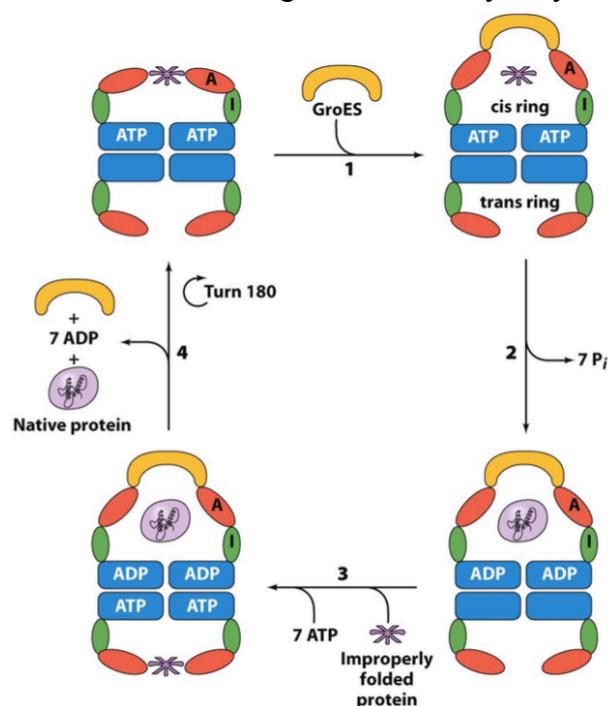


Binding and release of protein helps toggle protein conformation.

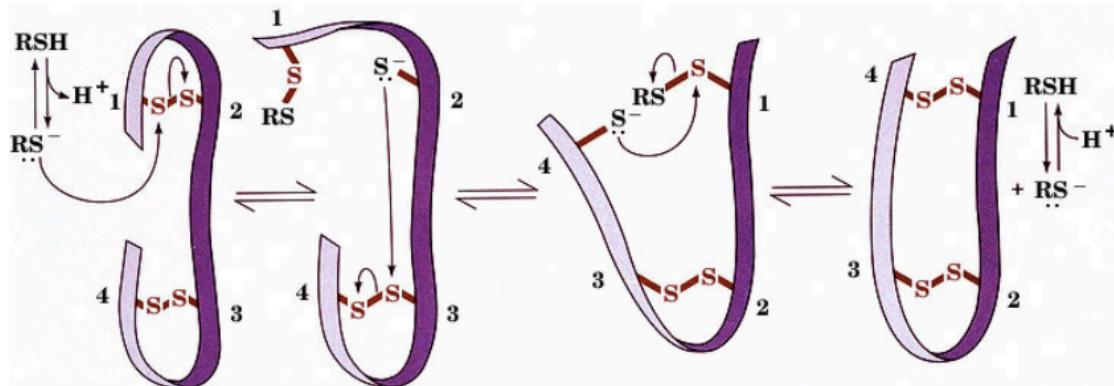
d. GroEL/GroES complex: 2 heptamer rings of ~60 kDa subunits (7-fold rotational symmetry)

EL is the main body, and ES is the cap.

Conformational changes from ATP hydrolysis influence the microenvironment to help folding.



- e. Protein disulfide isomerase (PDI) assists with disulfide exchange in the endoplasmic reticulum.



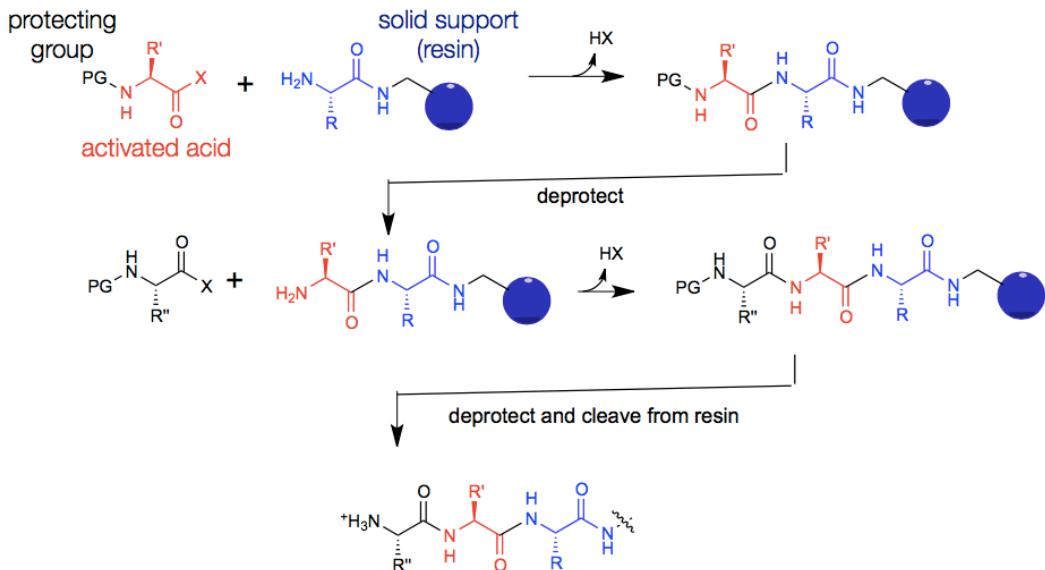
- f. Disulfide bridges are mostly found in secreted extracellular proteins, or in the secretory pathway: the endoplasmic environments are oxidizing, and the cytosol environment is reducing.

- K. Proteins are generally only as stable as they need to be for proper function
- Enzyme-substrate-protein interactions depend on conformational flexibility
 - Proteins need to be degraded to allow turnover.
 - Super-stable proteins would inhibit these two processes.

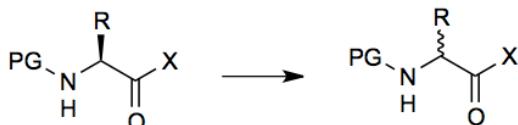
Lecture 6: Purification and Analysis of Proteins

A. Peptide Synthesis

- Difficulties: selectivity and dimerization (1-2 v. 1-1, 2-2, 2-1).
- Carboxylate is not a great electrophile, and ammonium is not a great nucleophile.



- Solid-state chemistry v. resin support is great because you can wash away unreacted reagents.
- You can activate the acid by converting to an acid chloride, and prevent dimerization by adding a protecting group.
- Racemization via the enolate may be problematic (deprotonation and backside addition of H).



- f. Yield is a limitation because you take yield^{^(cycles)}, and that decays very quickly.
- g. Peptide synthesis: standard for up to ~30 amino acids; can incorporate non-standard residues. Generally makes ~10 mg of material at a time (but can be scaled up).

B. Studying mechanisms by isolating components

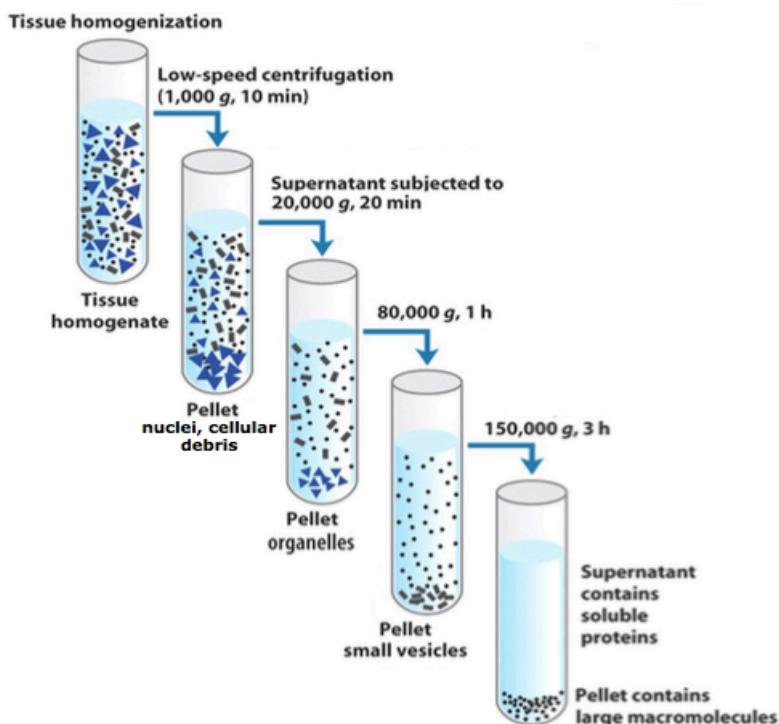
- Determining which proteins/enzymes are responsible for certain phenomena is difficult
- Human cells have tens of thousands of proteins expressed at different levels.
The range of [protein] spans $> 10^6$. Many important proteins exist at $< 100/\text{cell}$.

C. Cell lysis yields crude extract

- Osmotic lysis using hypotonic solution
- Detergents: blocked by a cell wall, which may be removed by lysozyme.
Caveat: this may denature proteins.
- Mechanical homogenization: dounce homogenizer
- French press: high pressure extrusion through a small hole.
- Sonication: sound waves

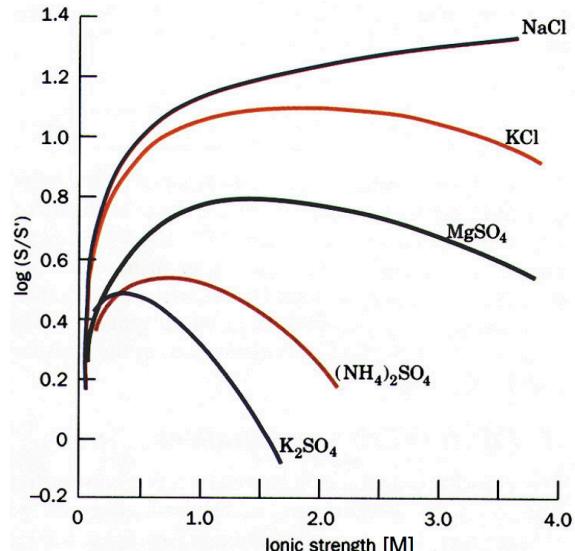
D. Centrifugation is used for subcellular fractionation of crude extract

- Relative centrifugal force (RGF) in g's = 1.12 (radius in cm) $(\text{RPM}/1000)^2$



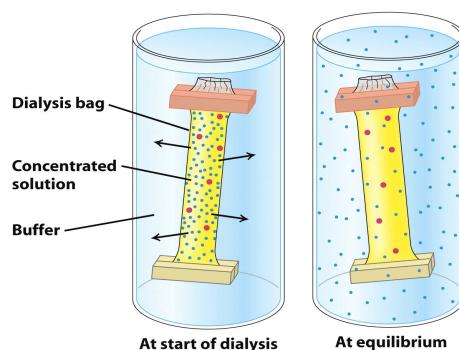
E. Summary of strategies for protein purification

Characteristic	Procedure
Solubility	1. Salting in 2. Salting out
Ionic Charge	1. Ion exchange chromatography 2. Electrophoresis 3. Isoelectric focusing
Polarity	1. Adsorption chromatography 2. Paper chromatography 3. Reverse-phase chromatography 4. Hydrophobic interaction chromatography
Molecular Size	1. Dialysis and ultrafiltration 2. Gel electrophoresis 3. Gel filtration chromatography 4. Ultracentrifugation
Binding Specificity	1. Affinity chromatography



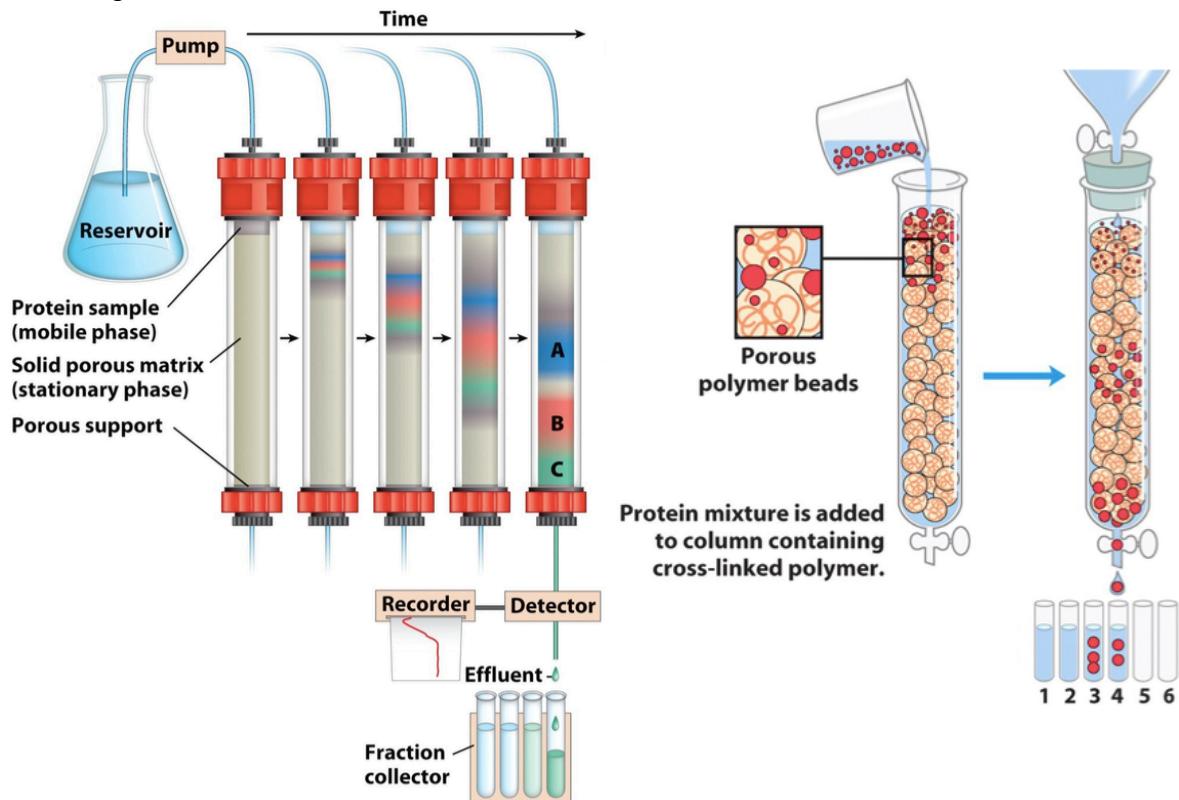
F. Salting proteins out of solution with ammonium sulfate:

- At higher concentration, $(\text{NH}_4)_2\text{SO}_4$ outcompetes proteins for solvation.
- Proteins sometimes precipitate at low ionic strength (needs charge neutralization).
- Dialysis: a semi-permeable membrane with a high MW/M_r cutoff (e.g. 5 kDa) and excess new buffer. Used to exchange buffers after salting or other purification steps.



G. Column Chromatography

a. General picture:

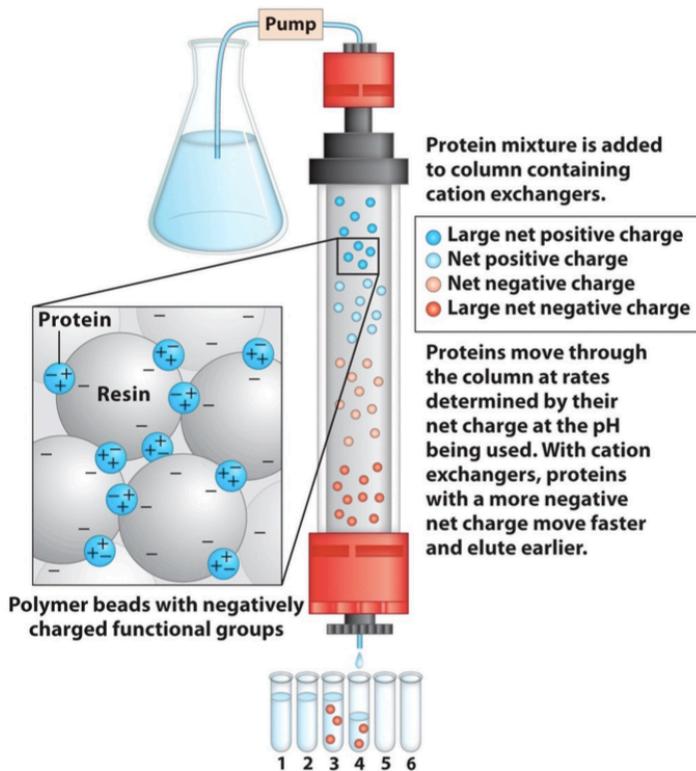


b. Gel filtration chromatography (size exclusion chromatography)

- Stationary phase: matrix of porous polymer; V_x (bead volume), typically 65%.
Mobile phase: protein solution; V_0 (void volume), typically 35%.
 V_t (bed volume) = $V_x + V_0$
- Small molecules enter the bead pores and are impeded, longer path.
- Large molecules do not enter the bead pores and quickly migrate to the bottom.
- Another way to swap buffers. Can desalt samples.

c. Ion exchange chromatography

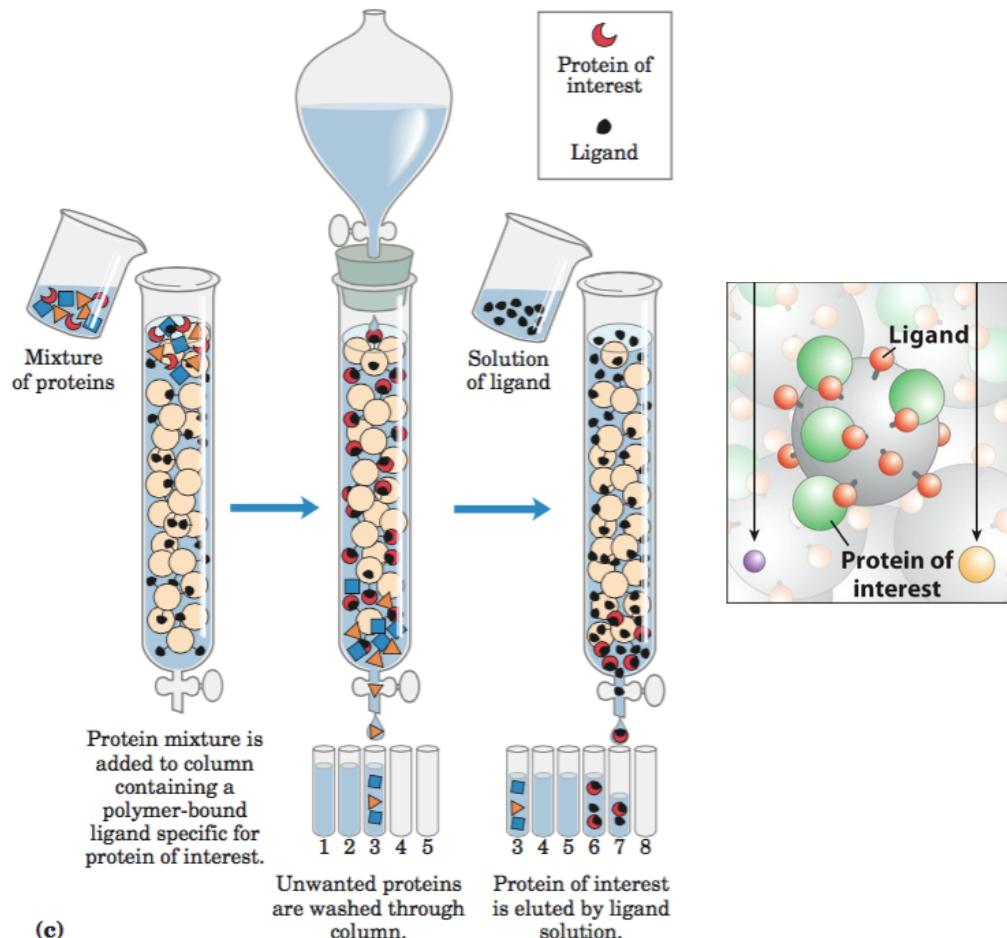
- i. Matrix of derivatized charged beads bind to and impede ionic proteins.
- ii. Cation exchange resin uses negative charges to bind cations. Anions come out earlier.
Anion exchange resin uses positive charges to bind anions. Cations come out earlier.



- iii. Basic proteins (+) typically bind to cation exchange resins (-). However, an acidic (-) protein may still bind to cation-exchange resin (-) if it has a local basic (+) patch.
pI: pH at which a charged molecule is neutral.
- iv. Something important about salts and interaction with proteins coming down.
- v. The range of ion exchange resins means that you can run your sample over and over until you get a very specific output.
- vi. Resin use limited by: cost, resin stability, resolution/specifity, flow rate, reproducibility.

d. Affinity Chromatography

- Immobilize a ligand that specifically binds your protein. Rinse, separate, and elute.
- Separation may involve: (a) disrupting the protein-ligand interaction e.g. low pH, or (b) wash with ligand solution to rebind protein and elute (competitive elution).
- Requires: (a) knowledge of protein binding partners, and (b) a way to immobilize the ligand without disrupting protein-ligand interaction.



e. Specific enrichment of an enzymatic activity

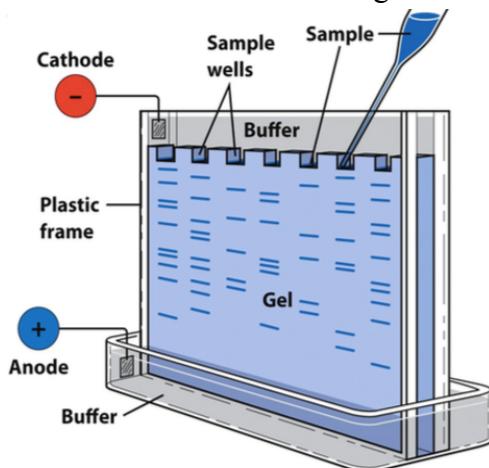
- Activity: total units of active protein in preparation
- Specific activity: units of activity/mg TOTAL protein
- Yield: amount of recovered activity

TABLE 3-5 A Purification Table for a Hypothetical Enzyme

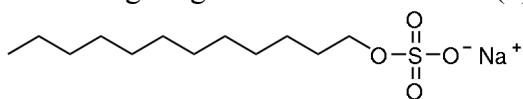
Procedure or step	Fraction volume (ml)	Total protein (mg)	Activity (units)	Specific activity (units/mg)
1. Crude cellular extract	1,400	10,000	100,000	10
2. Precipitation with ammonium sulfate	280	3,000	96,000	32
3. Ion-exchange chromatography	90	400	80,000	200
4. Size-exclusion chromatography	80	100	60,000	600
5. Affinity chromatography	6	3	45,000	15,000

H. SDS-PAGE: Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis: separates proteins

a. The cathode is the negative end. This is different from most conventions in physics.



- b. Polyacrylamide gel: variable density (4-20%) separates variable protein sizes.
Acrylamide polymerizes readily. N,N'-methylenebisacrylamide can also help form cross-links.
 - c. Process: reduce S-S bonds with BME or DTT, then add SDS to denature and give (-) charge.
SDS: sodium dodecyl sulfate: binds proteins (~1 SDS per 2 AAs at saturation).
Even though big molecules have more (-) charge, size dominates and they travel more slowly.

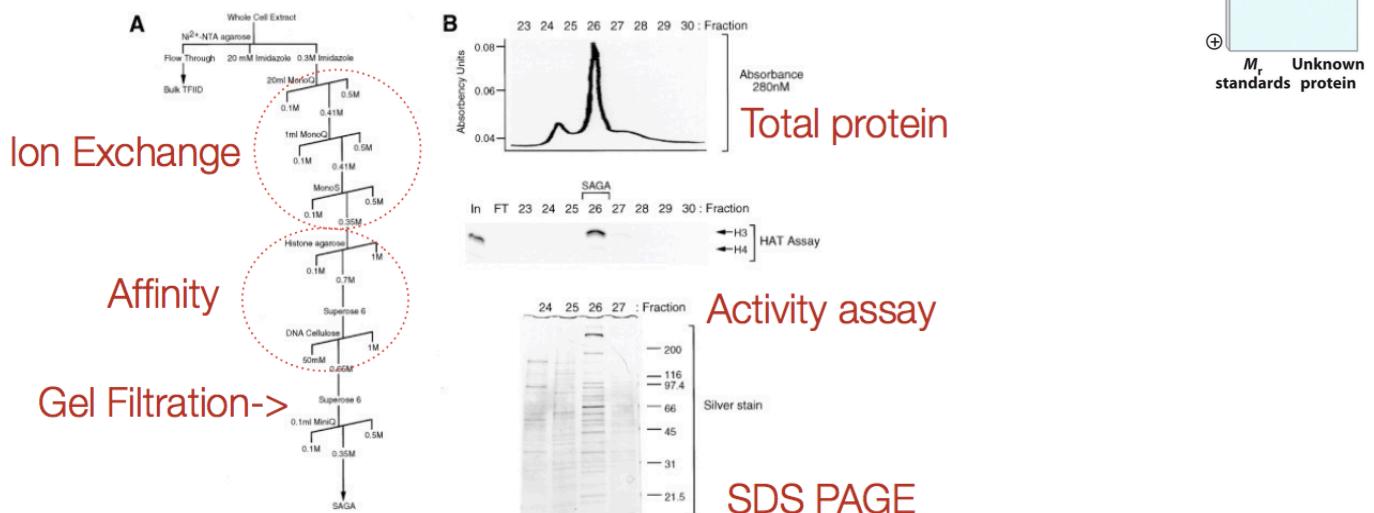


- d. Non-specific stains:

- i. Coomassie brilliant blue (>50 ng)
 - ii. Silver stain (>1 ng)
 - iii. SYPRO fluorescent dyes (>1 ng)

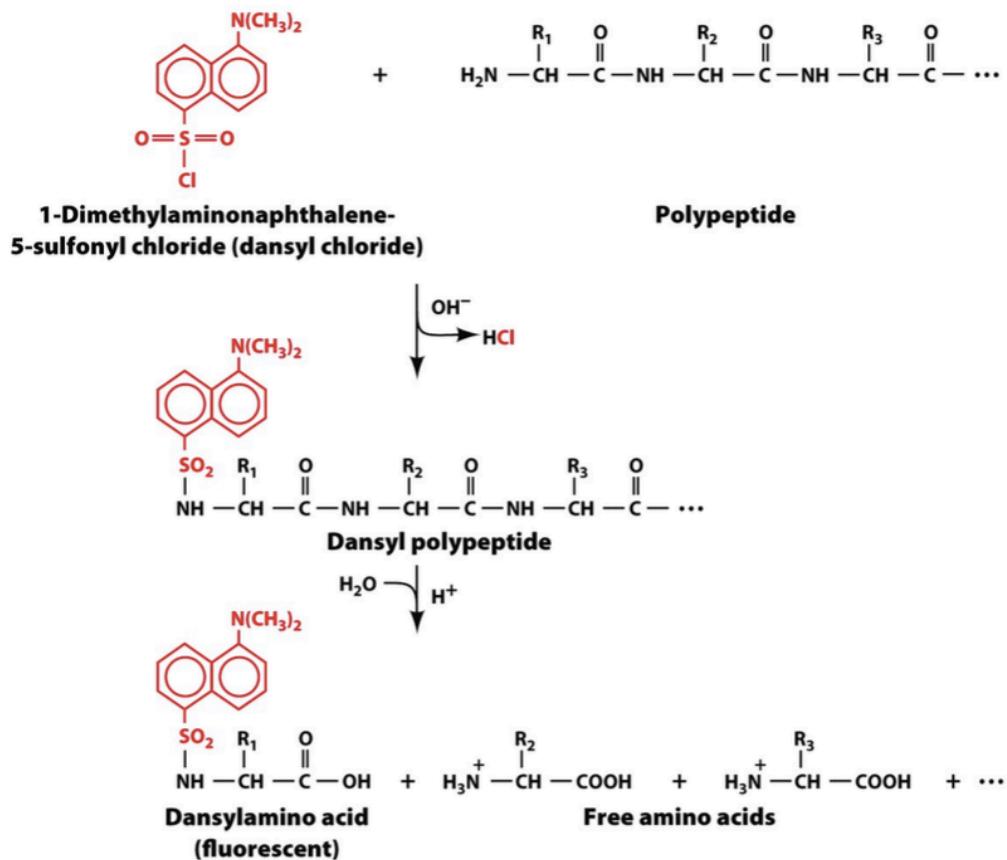
e. Western blotting allows specific detection of proteins.

I. Protein Purification Example: SAGA HAT complex

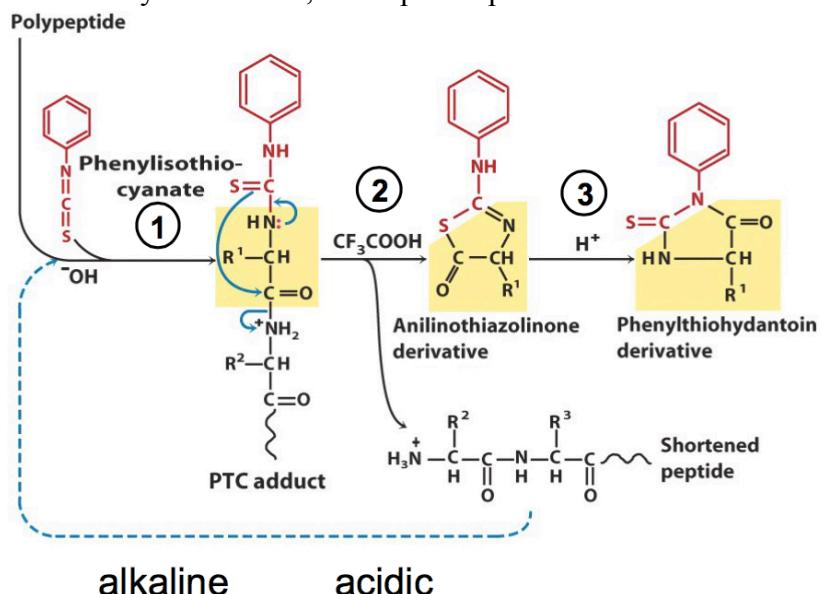


J. Protein Sequencing by Edman Degradation

1. N-terminus labeling (with fluorophore)



2. Treat with acid to promote cyclization and cleavage.
3. Rearrangement of thiazolinone to thiohydantoin.
4. Identify amino acid, and repeat- up to about 40 amino acids.

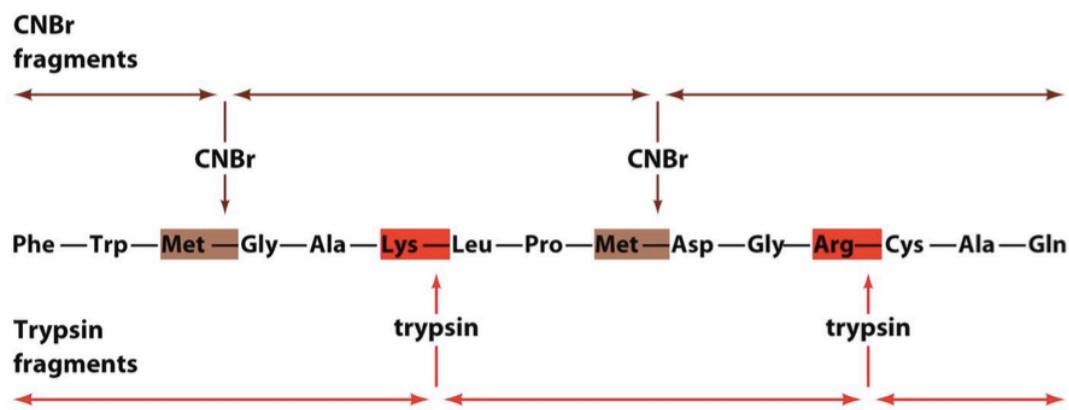


K. Protein cleavage into peptides

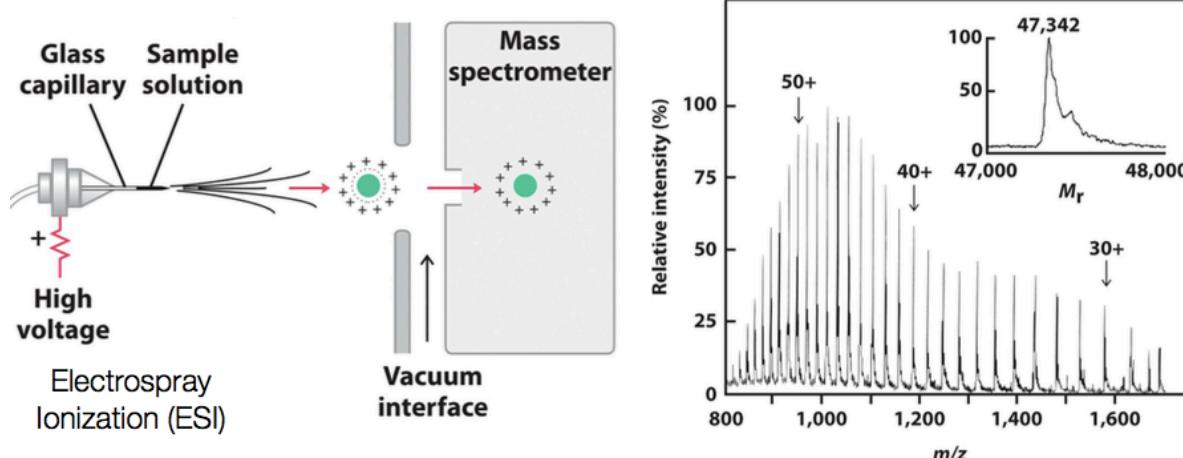
- Acid hydrolysis: random; gives a heterogeneous mixture.
- Chemical cleavage: cyanogen bromide cleaves at (M)
- Other proteases: all cleave to the C-terminal side of the residue
 - Trypsin (R, K)
 - Chymotrypsin (F, W, Y)
- Reduce Cys to SH and treat with iodoacetate to prevent disulfide bridging.



L. Sequencing large proteins: cleave with multiple proteases, sequence overlapping smaller peptides and compare (used to sequence bovine insulin).



M. Mass spectrometry: determines mass to charge (m/z) ratios of small proteins.



N. ESI sprays the solution through a high-voltage glass capillary to give it a charge, and then through a vacuum to evaporate the solvent. The charged peptides remain and go into the analyzer.

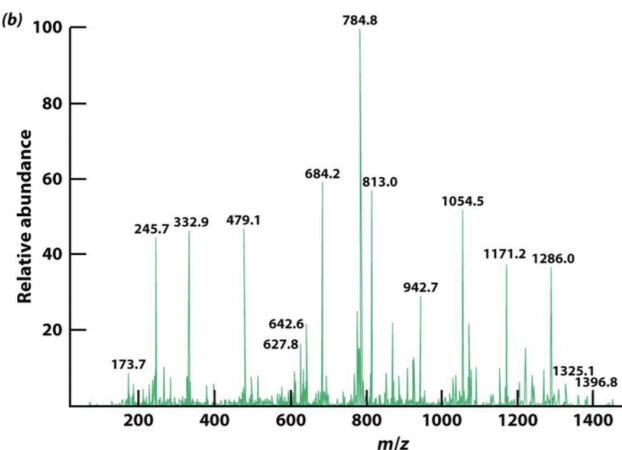
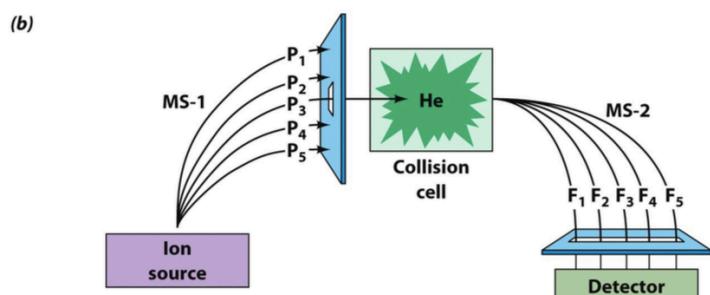
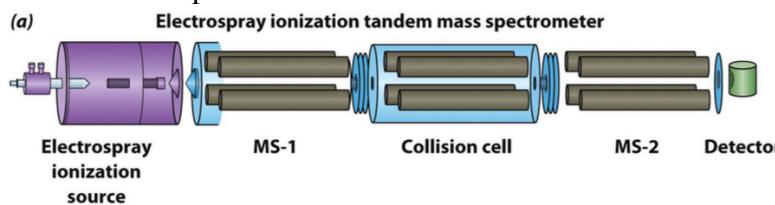
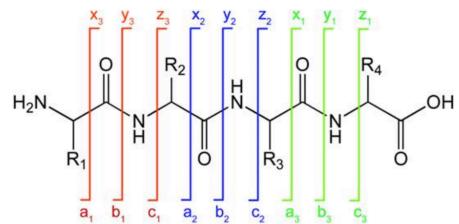
O. Common proteomics workflow based on mass spectrometry

- Separate protein of interest by SDS-PAGE, cut out the stained band, and digest with protease.
- Separate smaller peptides by reverse phase chromatography (hydrophobic stationary phase binds nonpolar; polar elutes first) – column outputs directly into mass spectrometer.
- Analyze parent peptide masses
- Fragment and sequence abundant peptides in the mass spectrometer.
- Use spectra to identify proteins in sample.

P. MS-MS (tandem mass spectrometer) used to sequence peptides

- a. Peptides fragment in the chamber in different ways.

Analyze the y-ion and b-ion series (fragments between the carbonyl and the amino group) and measure between the peaks to calculate the mass of each amino acid.



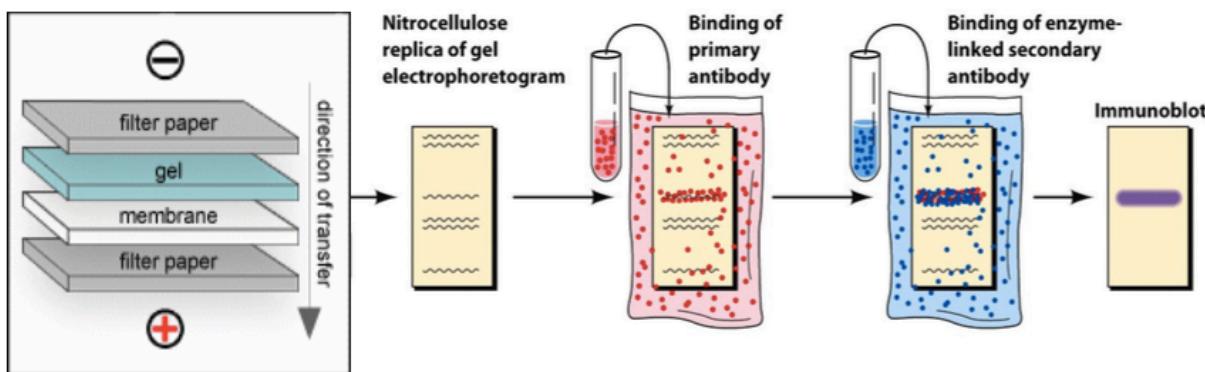
- b. See textbook 3-31 in Lehninger 6e.

Q. Antibodies as Tools to Study Proteins

- a. Immunoglobulin domains are highly specific to certain epitopes on antigens.
- b. Polyclonal antibodies: (1) inject an animal (rabbit, goat, mouse, donkey) with an antigen, (2) many B cells generate different antibodies for different epitopes on the same antigen, and (3) antibodies are extracted from serum.
- c. Monoclonal antibodies: (1) inject an animal with antigen, (2) isolate individual B cells and immortalize by fusion with hybridoma line, (3) collect antibodies from the B cell lines that recognize a *single* antigen epitope. Nobel Prize 1984 (Köhler and Milstein).

R. Western blotting detects specific proteins

1. Perform gel electrophoresis on a sample containing the protein of interest
2. Block the unoccupied binding sites on the nitrocellulose with casein
3. Incubate with rabbit antibody to the protein of interest
4. Wash and incubate with an enzyme-linked goat anti-rabbit antibody
5. Assay the linked enzyme with a colorimetric reaction



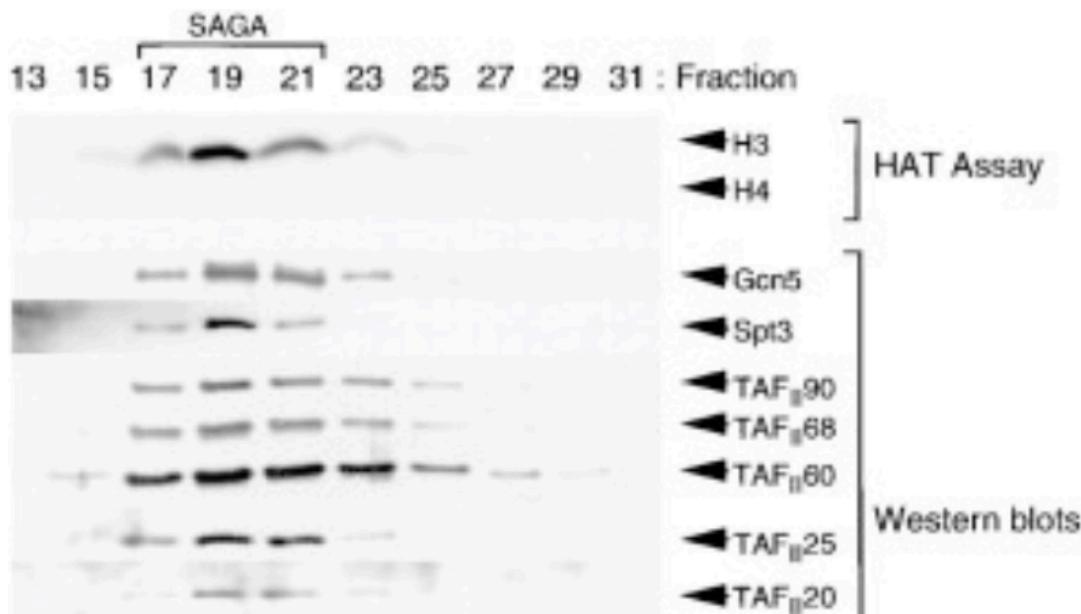
transfer to nitrocellulose
(or PVDF) membrane

Can use a horse radish peroxidase (HRP) conjugated 2° antibody
or

Can use a fluorescently labeled 2° antibody

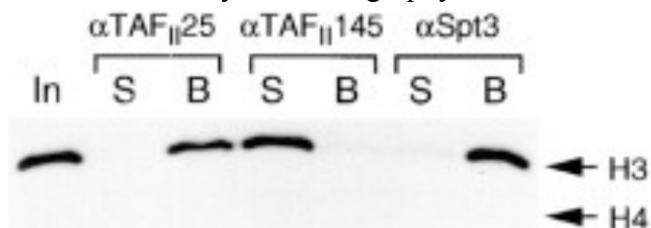
S. Using antibodies to confirm complex associations

- a. Co-fractionation: do the factors behave as if they are in one complex?



- b. Co-immunoprecipitation: does affinity enrichment via antibody enrich the expected activity and other complex members?

Similar to affinity chromatography: use beads coated with antibody that react with your protein.



(S: Supernatant – unbound // B: Beads – bound)

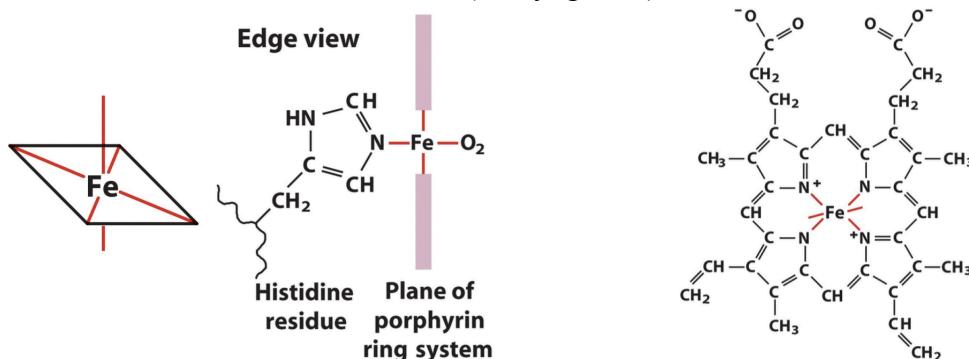
T. Summary

- a. Learn the steps of protein purification (cell lysis, sub-cellular fractionation, purification).
- b. Learn the principles behind different approaches to column chromatography.
- c. Learn how SDS-PAGE works.
- d. Learn how antibodies are made and how they are used to detect proteins.
- e. Learn how proteins can be sequenced chemically or by using mass spectrometry.

Lecture 7: Allostery, Myoglobin, and Hemoglobin

A. Binding of Oxygen to Myoglobin

- a. Myoglobin (153 AA, 17 kDa): structure solved by Kohn Kendrew 1957.
- b. Monomeric member of the globin family; transports oxygen within muscles.
- c. Contains heme-ring based on porphyrin with 4 coordination bonds to Fe^{2+} .
The 2+ state is important. 3+ is oxidized (rust) and unable to coordinate with O_2 .
- d. The other 2 bonds coordinate 1 His (in myoglobin) and 1 O_2 molecule.



- B. Carbon monoxide (CO) is toxic because it binds heme 200x better than O_2 .
- C. Dissociation constant (K_d) measures the ability of bound groups to separate.
- D. Lower K_d = tighter bond = less ligand needed to occupy ½ of the binding sites.

Protein	Ligand	K_d (M)*
Avidin (egg white) [†]	Biotin	1×10^{-15}
Insulin receptor (human)	Insulin	1×10^{-10}
Nickel-binding protein (<i>E. coli</i>)	Ni^{2+}	1×10^{-7}
Calmodulin (rat) [§]	Ca^{2+}	3×10^{-6} 2×10^{-5}

*Avidin-biotin is one of the strongest interactions known: femtomolar [ligand] binds ½ sites.

- E. Ligand binding is a function of its concentration and the dissociation constant. $\text{P} + \text{L} \rightleftharpoons \text{PL}$
 θ = fraction of occupied binding sites.

$$K_d = \frac{C_P C_L}{C_{PL}}$$

$$C_{PL} = \frac{C_P C_L}{K_d}$$

$$\theta = \frac{\frac{C_P C_L}{K_d}}{\frac{C_P C_L}{K_d} + C_P} = \frac{\frac{C_P}{K_d} C_L}{\frac{C_P}{K_d} C_L + C_P} = \frac{\frac{C_P}{K_d} C_L}{\frac{C_P}{K_d} (C_L + K_d)}$$

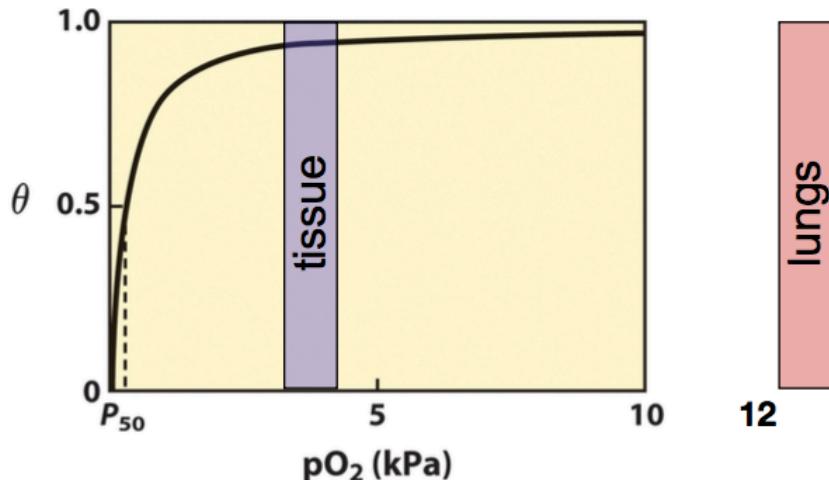
$$\theta = \frac{C_{PL}}{C_{PL} + C_P}$$

$$\theta = \frac{C_L}{C_L + K_d}$$

- a. When ½ sites are occupied, then $C_{PL} = C_P$ and $K_d = C_L$.
- b. Protein: myoglobin, ligand: oxygen
 P_{50} is partial pressure at half-saturation, i.e. when ½ of sites are occupied.

$$\theta = \frac{CO_2}{CO_2 + K_d}$$

θ = fraction of occupied oxygen binding sites



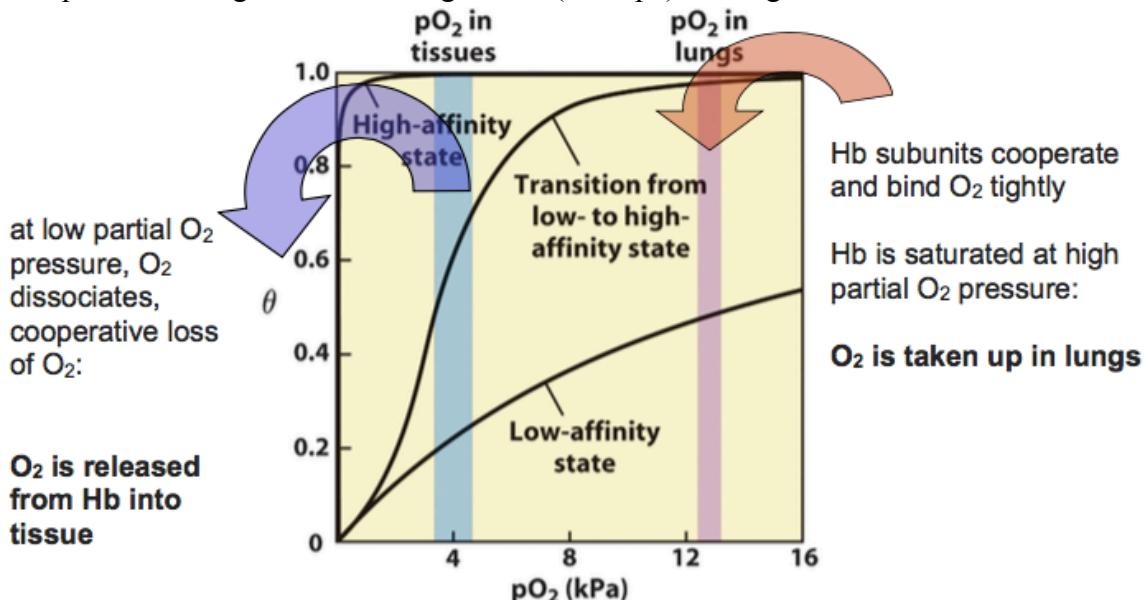
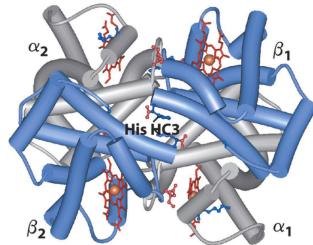
- c. Myoglobin: good for transport within muscles, not useful for transporting from lungs to tissues
BECAUSE of its uniformly tight ligand binding.

F. Binding of Oxygen to Hemoglobin

- a. Globin family tetramer
- b. Each heme-containing subunit is similar to myoglobin

G. Oxygen-binding curve of hemoglobin

- a. More variable affinity based on surrounding $[O_2]$ makes it ideal for transport from lungs to tissues – sigmoidal (S-shape) binding curve.



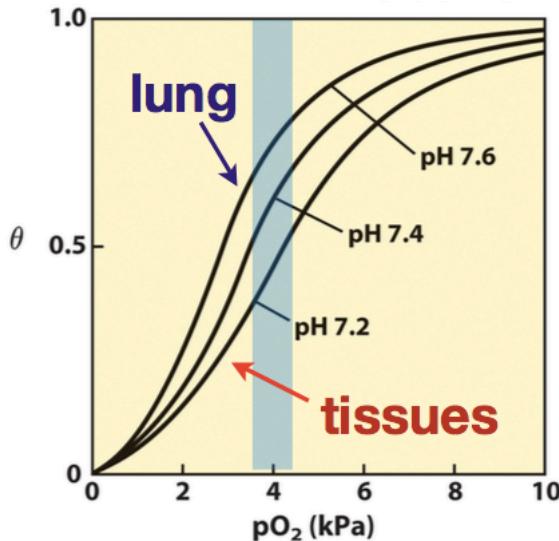
- b. Oxygen binding to a heme group creates a conformational change in the subunit. The oxygen-free form has its B-subunit His engaged in an ion pair, which is broken by the change. The new conformation increases oxygen binding by other subunits.

H. Allostery: “Allos stereos” – “other shape”

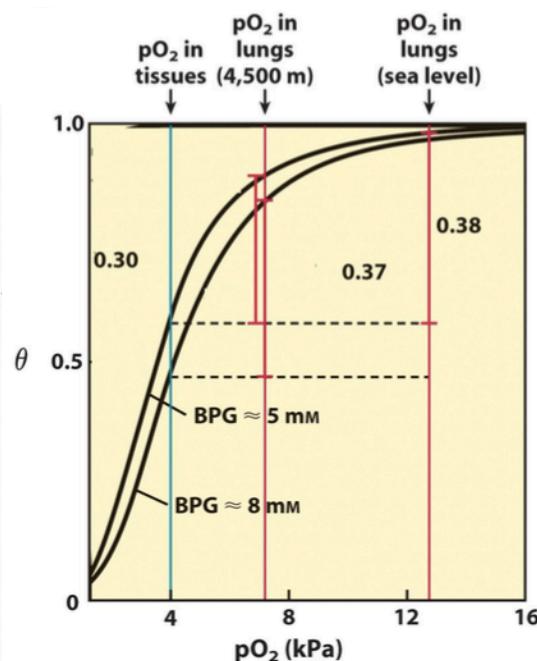
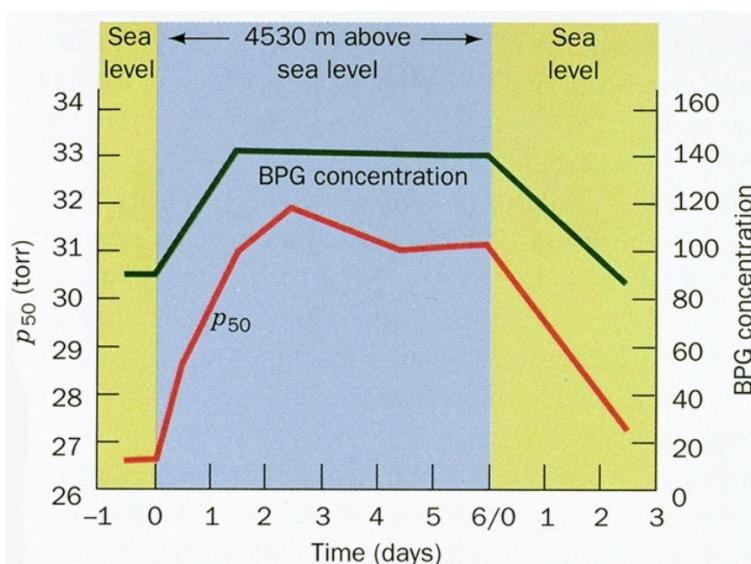
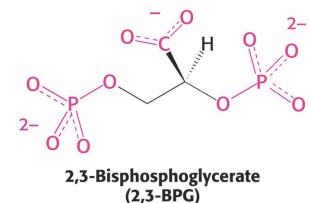
- a. Typically in multi-subunit proteins.
- b. Oligomerization allows cooperativity between multiple binding sites. Modulators bind elsewhere and induce a conformational change. The substrate can be a modulator (as in hemoglobin).
- c. Examples: hemoglobin, ATCase, acetylcholine receptor (target of sarin).
- d. General model: (T) tense → (R) relaxed = less binding → more binding = sigmoid curve.

I. The Bohr effect (1904)

- a. Metabolism and respiration produce $\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}^+$ and HCO_3^-
- b. In tissues, high CO_2 and high H^+ acidity promote O_2 release from Hb.

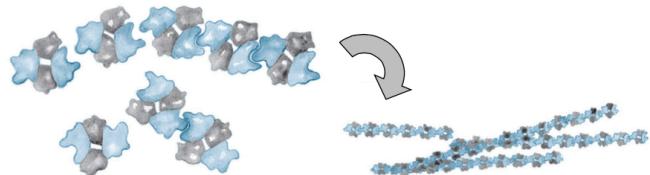
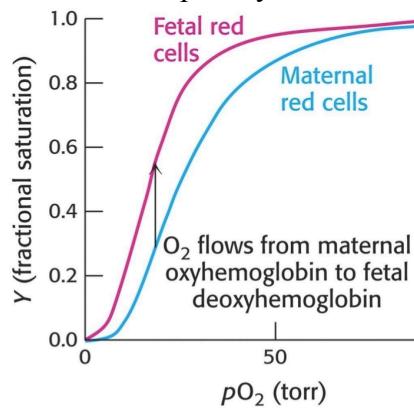


- c. Both H^+ and CO_2 are allosteric regulators bound by Hb.
 - i. H^+ binds at multiple sites in Hb (including His HC3)
 - ii. CO_2 reacts reversibly with N-terminal amino groups in Hb subunits:
 $\text{Hb-NH}_3^+ + \text{CO}_2 \rightarrow \text{Hb-NHCOO}^- + 2\text{H}^+$
 - iii. This stabilizes the T-state, lowers local O_2 affinity, and promotes O_2 release.
- d. In the lungs, high $[\text{O}_2]$ favors the R-state, which releases CO_2
- J. Regulation of Hb- O_2 -binding by 2,3-BPG (biphosphoglycerate)
 - a. Hb in blood contains high amounts of BPG.
 - b. 1 BPG binds to the center of a Hb tetramer.
 - This stabilizes the T-state, greatly reducing O_2 affinity.
 - c. Altitude adaptation: BPG rises at high altitude/low- O_2 and increase Hb's O_2 release capacity.



K. Fetal Hemoglobin

- a. Different subunits: 2α and 2γ, to shift affinity and transfer oxygen from maternal → fetal blood.
- b. β-subunits replace γ-subunits a few days after birth.



L. Sickle Cell Anemia

- a. Abnormally formed erythrocytes: easily rupture, clog capillaries
- b. Caused by point mutation on the surface triggering aggregation into strands.

M. Treatment of multiple ligand binding – A. Hill (1910)

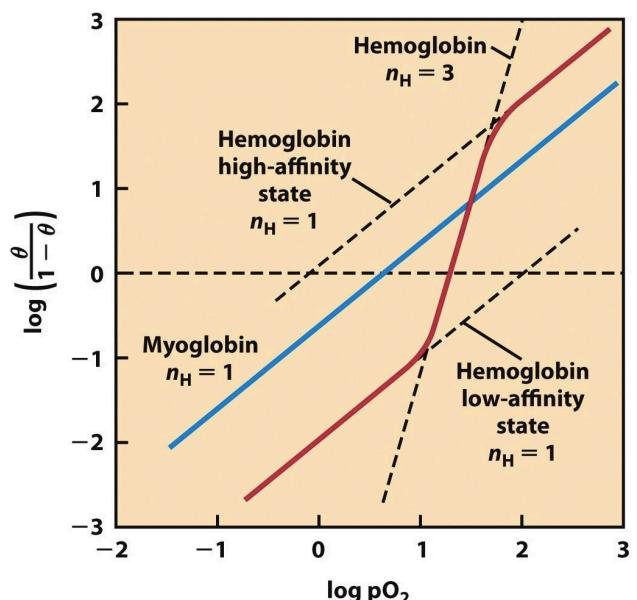
- a. ADD MORE INFO FROM SLIDES
- b. θ = fraction of occupied binding sites.
- c. n_H : Hill coefficient: slope, measure of cooperativity ($n > 1$ = positive cooperation)

$$\frac{1}{\theta} = \frac{C_L^n + K_d}{C_L^n} = 1 + \frac{K_d}{C_L^n}$$

$$\frac{1}{\theta} - 1 = \frac{K_d}{C_L^n} = \frac{1 - \theta}{\theta}$$

$$\frac{\theta}{1 - \theta} = \frac{C_L^n}{K_d}$$

$$\log \left(\frac{\theta}{1 - \theta} \right) = n \log C_L - \log K_d$$



N. Summary

- a. Myoglobin and hemoglobin
- b. Basic equations for ligand binding
- c. Allostery and regulation
- d. Hill equation and cooperativity.

Lecture 8: Enzymes I: Basic Concepts and Kinetics

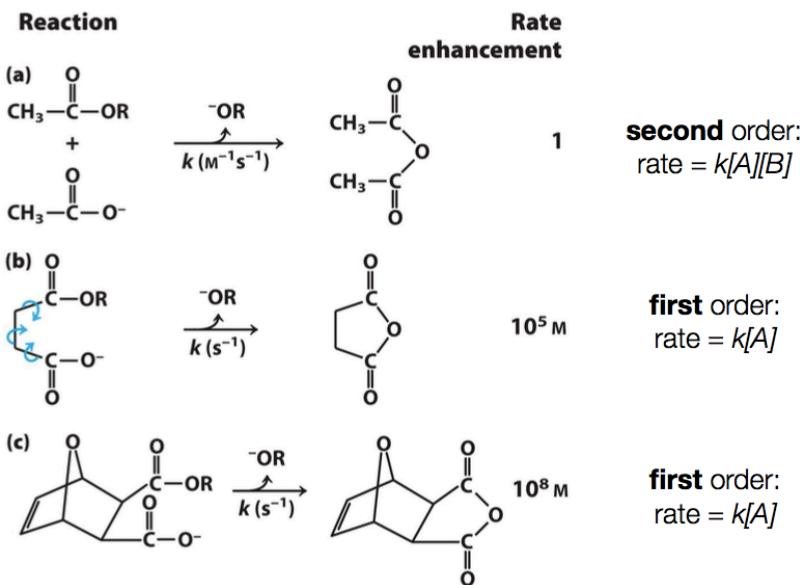
- A. Function: control reaction rate and specificity.
 B. E.C. numbers denote class, type of group transferred, group donor, and its acceptor.

No.	Class	Type of reaction catalyzed
1	Oxidoreductases	Transfer of electrons (hydride ions or H atoms)
2	Transferases	Group transfer reactions
3	Hydrolases	Hydrolysis reactions (transfer of functional groups to water)
4	Lyases	Addition of groups to double bonds, or formation of double bonds by removal of groups
5	Isomerases	Transfer of groups within molecules to yield isomeric forms
6	Ligases	Formation of C—C, C—S, C—O, and C—N bonds by condensation reactions coupled to ATP cleavage

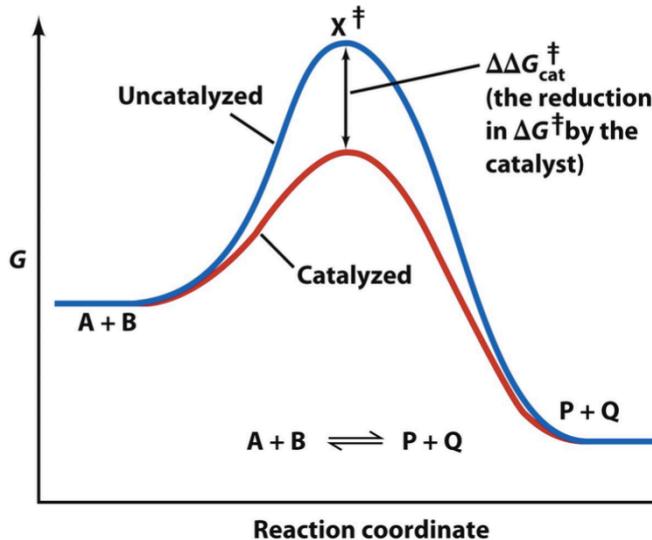
- C. Rate acceleration is dramatic, protease example
- Chemical hydrolysis of peptide bonds requires 6 M HCl at 110°C for 24 hours.
 - Enzymes do this rapidly at pH 7, 25°C.

Enzyme	Nonenzymatic half-life of substrate	Rate enhancement (k_{cat}/k_{un})
OMP decarboxylase	78,000,000 years	1.4×10^{17}
Staphylococcal nuclease	130,000 years	5.6×10^{14}
AMP nucleosidase	69,000 years	6.0×10^{12}
Carboxypeptidase A	7.3 years	1.9×10^{11}
Triose phosphate isomerase	1.9 days	1.0×10^9
Carbonic anhydrase	5 seconds	7.7×10^6

- D. Entropic reduction can increase reaction rates
- Second order limited by diffusion/collision rate.
 - Intramolecular first order limited by energy barrier of adopting the s-cis-conformation.
 - Intramolecular first order, locked into s-cis conformation.



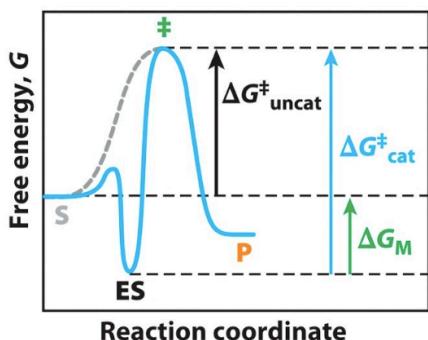
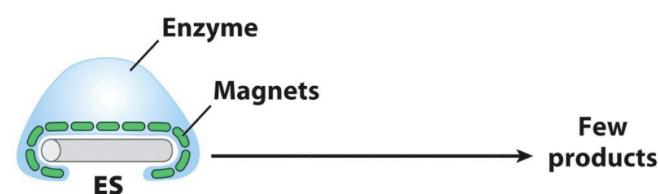
E. Enzymes lower the activation energy of a reaction



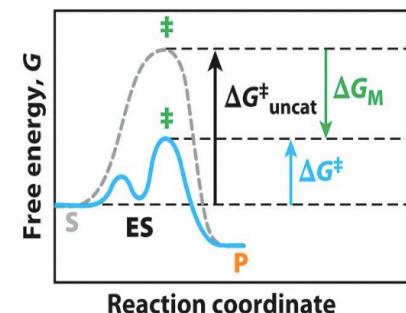
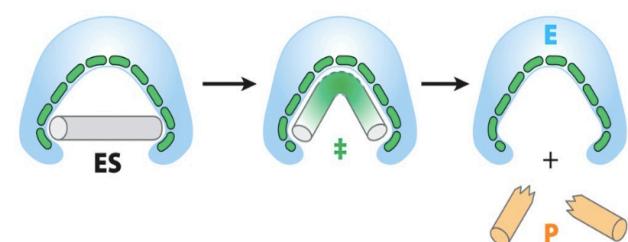
F. Enzymes bring substrates together, provide a cleft for the substrate, properly orient it, and bind and stabilize the transition state.

G. Binding to substrate can slow down a reaction ("magnets" = weak interactions)
Instead, enzymes complement and stabilize the transition state

Enzyme complementary to substrate



Enzyme complementary to transition state



H. Review of chemical kinetics

$$\text{a. For } A \rightarrow B: \frac{d[B]}{dt} = k[A] \quad k = \frac{k_B T}{h} e^{\frac{-\Delta G^\ddagger}{RT}}$$

- b. Transition state theory: attaining the transition state allows for the reaction.
TS theory is an idealized but useful model.

I. Properties of an enzymatic active site

- a. Usually a cleft for desolvation
- b. Only a few amino acids are directly involved
- c. Multiple interactions: ensure specificity
- d. Weak interactions: ensure reversibility
- e. H-bonds often used to orient substrates

J. Other factors relevant to catalysis

a. Cofactors: inorganic ions

- i. Mg^{2+} Hexokinase, glucose 6-phosphatase, pyruvate kinase
- ii. Fe^{2+}/Fe^{3+} Cytochrome oxidase, catalase, peroxidase
- iii. Ni^{2+} Urease
- iv. Zn^{2+} Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B

b. Coenzymes: complex organic molecules; connection with nutrition.

Coenzyme	Examples of chemical groups transferred	Dietary precursor in mammals
Coenzyme A	Acyl groups	Pantothenic acid and other compounds
5'-Deoxyadenosylcobalamin (coenzyme B_{12})	H atoms and alkyl groups	Vitamin B_{12}
Flavin adenine dinucleotide	Electrons	Riboflavin (vitamin B_2)
Lipoate	Electrons and acyl groups	Not required in diet
Nicotinamide adenine dinucleotide	Hydride ion ($:H^-$)	Nicotinic acid (niacin)
Pyridoxal phosphate	Amino groups	Pyridoxine (vitamin B_6)

c. Prosthetic groups (tightly bound coenzyme)

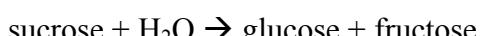
- d. Holoenzyme: the catalytically active enzyme with cofactors and/or coenzymes.
e. Apoprotein: the protein component of a holoenzyme.

K. Enzyme kinetics:

a. Saturation: when an enzyme-catalyzed reaction reaches V_{max} , independent of [S].

At low [S], 1st order dependence. At higher [S], rate approaches limit.

b. Leonor Michaelis and Maud Menten analyzed invertase kinetics (1913):



c. Supposed mechanism:



d. Assumption 1: Pre-equilibrium

$$i. v = \frac{d[P]}{dt} = k_2[ES]$$

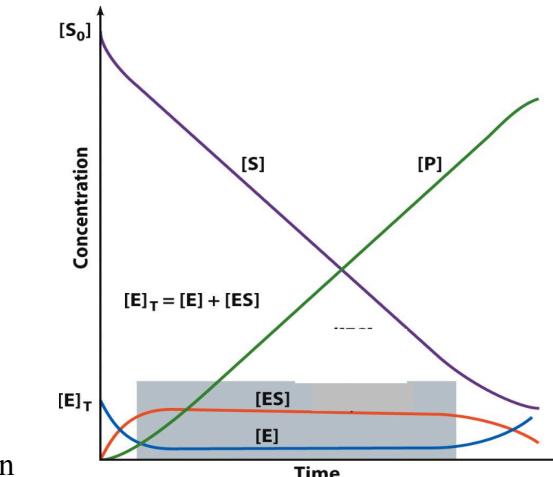
$$ii. K_S = \frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]}$$

e. Assumption 2: Steady state; flat [ES] curve:

$$\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES] = 0$$

f. Derivation of the Michaelis-Menten equation

$$i. \text{ Begin with } \frac{d[ES]}{dt} = 0$$



$$[ES](k_{-1} + k_2) = k_1[E][S]$$

$$[ES](k_{-1} + k_2 + k_1[S]) = k_1[E]_T[S]$$

$$[ES] = \frac{k_1[E]_T[S]}{(k_{-1} + k_2 + k_1[S])} = \frac{[E]_T[S]}{(k_{-1} + k_2)/k_1 + [S]}$$

$$[ES] = \frac{[E]_T[S]}{K_M + [S]}$$

$$k_2[ES] = \frac{k_2[E]_T[S]}{K_M + [S]} = \frac{d[P]}{dt} = v_0$$

$$v_0 = \frac{V_{max}[S]}{K_M + [S]}$$

$$ii. \text{ Substitute } [E] = [E]_T - [ES]$$

$$iii. \text{ Solve for } [ES]$$

$$iv. \text{ Substitute } K_M = (k_{-1} + k_2) / k_1$$

$$v. \text{ Multiply by } k_2$$

$$vi. \text{ Substitute } V_{max} = k_2[E]_T$$

g. Interpretations of the MM equation

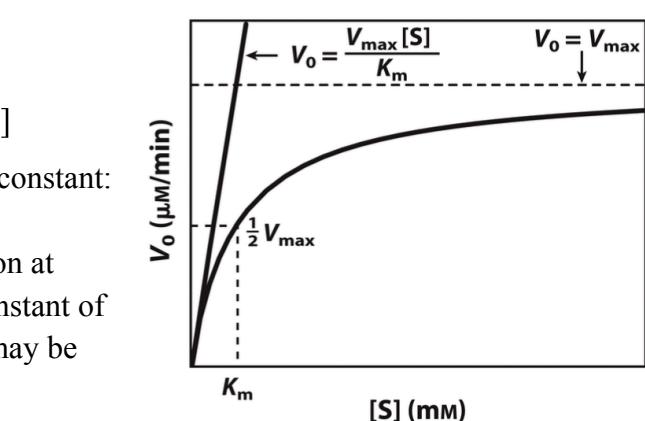
- At $v_0 = \frac{1}{2} V_{\max}$, $K_M = [S]$
- At low $[S]$, $v_0 = \frac{V_{\max}[S]}{K_M} \approx \left(\frac{k_{\text{cat}}}{K_M}\right) [E]_T [S]$
- k_{cat}/K_M is the “apparent” 2nd order rate constant: a measure of catalytic efficiency.
- k_{cat} describes the limiting rate of reaction at saturation. This is generally the rate constant of the limiting step (in this case, k_2), but may be more complex.
- $\frac{k_2}{K_M} = \frac{k_1 k_2}{k_{-1} + k_2}$ When $k_2 \gg k_{-1}$, $\left(\frac{k_{\text{cat}}}{K_M}\right) \approx k_1$

Since k_1 describes the rate of association, this means that the reaction has attained “catalytic perfection” and is diffusion limited.

- k_{cat} and K_M value ranges

Table 14-1 Values of K_M , k_{cat} , and k_{cat}/K_M for Some Enzymes and Substrates

Enzyme	Substrate	K_M (M)	k_{cat} (s ⁻¹)	k_{cat}/K_M (M ⁻¹ · s ⁻¹)
Acetylcholinesterase	Acetylcholine	9.5×10^{-5}	1.4×10^4	1.5×10^8
Carbonic anhydrase	CO_2	1.2×10^{-2}	1.0×10^6	8.3×10^7
	HCO_3^-	2.6×10^{-2}	4.0×10^5	1.5×10^7
Catalase	H_2O_2	2.5×10^{-2}	1.0×10^7	4.0×10^8
Chymotrypsin	<i>N</i> -Acetylglycine ethyl ester	4.4×10^{-1}	5.1×10^{-2}	1.2×10^{-1}
	<i>N</i> -Acetylvaline ethyl ester	8.8×10^{-2}	1.7×10^{-1}	1.9
	<i>N</i> -Acetyltyrosine ethyl ester	6.6×10^{-4}	1.9×10^2	2.9×10^5
Fumarase	Fumarate	5.0×10^{-6}	8.0×10^2	1.6×10^8
	Malate	2.5×10^{-5}	9.0×10^2	3.6×10^7
Superoxide dismutase	Superoxide ion (O_2^-)	3.6×10^{-4}	1.0×10^6	2.8×10^9
Urease	Urea	2.5×10^{-2}	1.0×10^4	4.0×10^5



L. Lineweaver-Burk (Double-Reciprocal) Plot

- Take the reciprocal and simplify:

$$\frac{1}{v_0} = \frac{K_M + [S]}{V_{\max}[S]} ; \quad \frac{1}{v_0} = \left(\frac{K_M}{V_{\max}}\right) \frac{1}{[S]} + \frac{1}{V_{\max}}$$

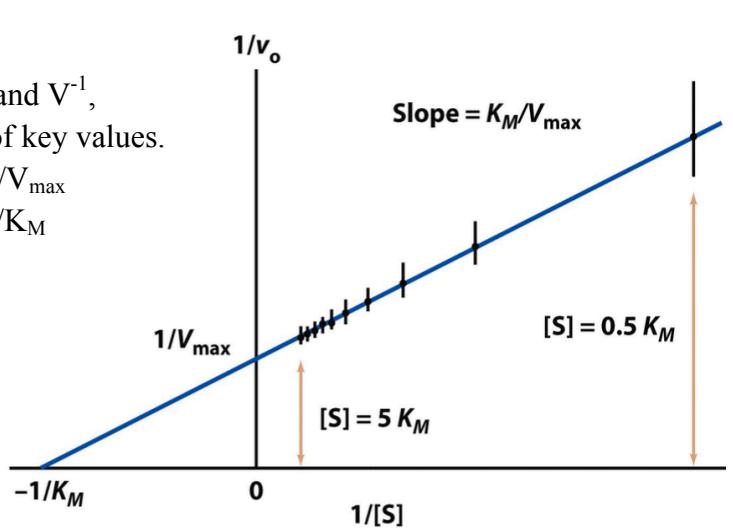
- This creates a linear plot relating $[S]^{-1}$ and V^{-1} , allowing more accurate measurement of key values.

- y-intercept: infinite substrate: $1/V_0 = 1/V_{\max}$

$$x\text{-intercept: infinite velocity: } 1/[S] = -1/K_M$$

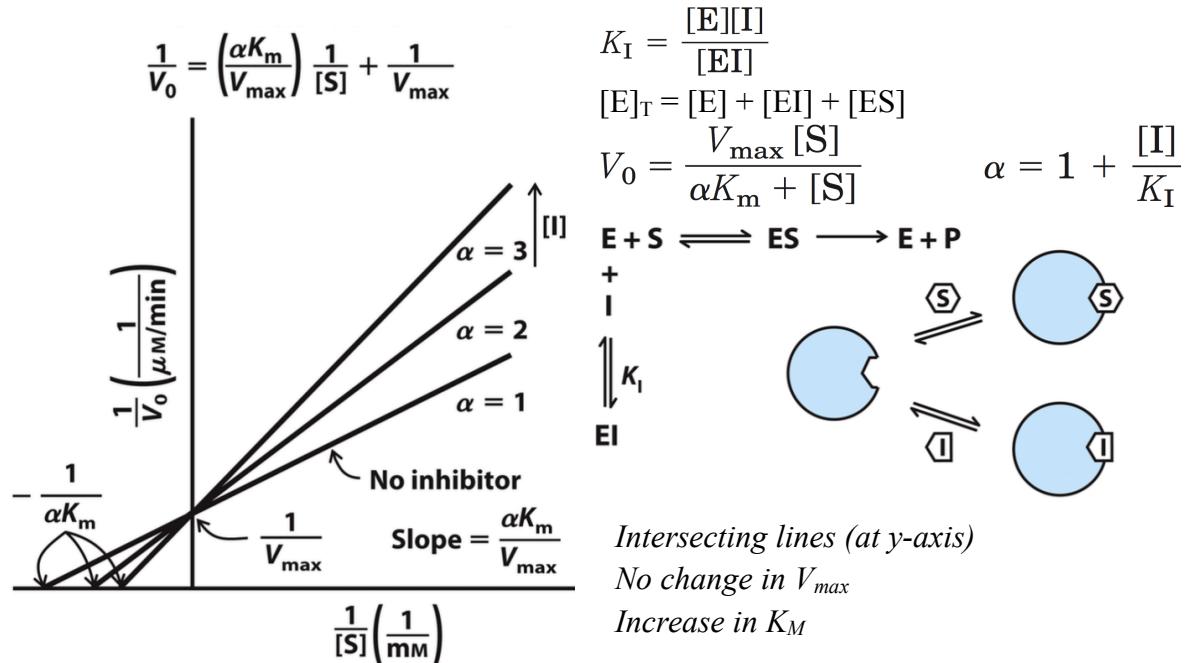
$$\text{Slope: } K_M/V_{\max}$$

Lower is more efficient

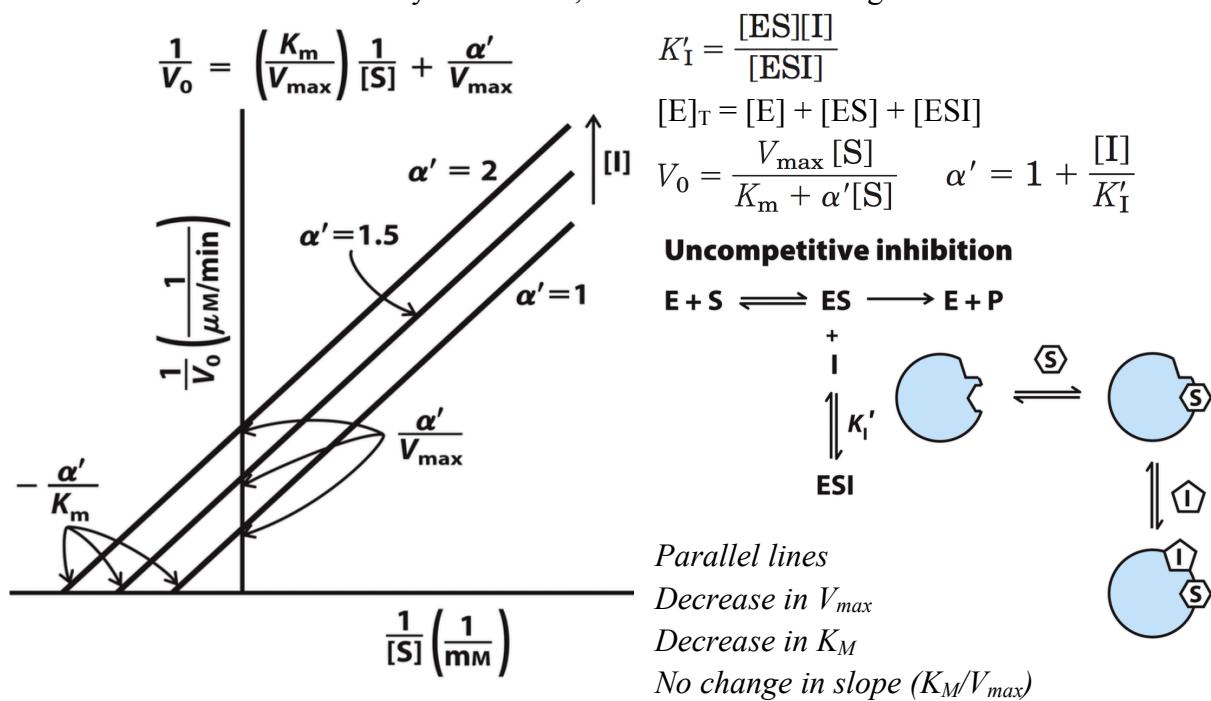


M. Modes of inhibition

- Substrates and products can be inhibitors!
- Irreversible inhibition: dissociates very slowly or not at all
- Reversible inhibition:
 - Inhibitor competes for active site (competitive)
 - Inhibitor binds only to ES complexes (uncompetitive)
 - Inhibitor binds to both free E and ES complexes (mixed)
- Competitive inhibitors: take the place of substrate in the active site

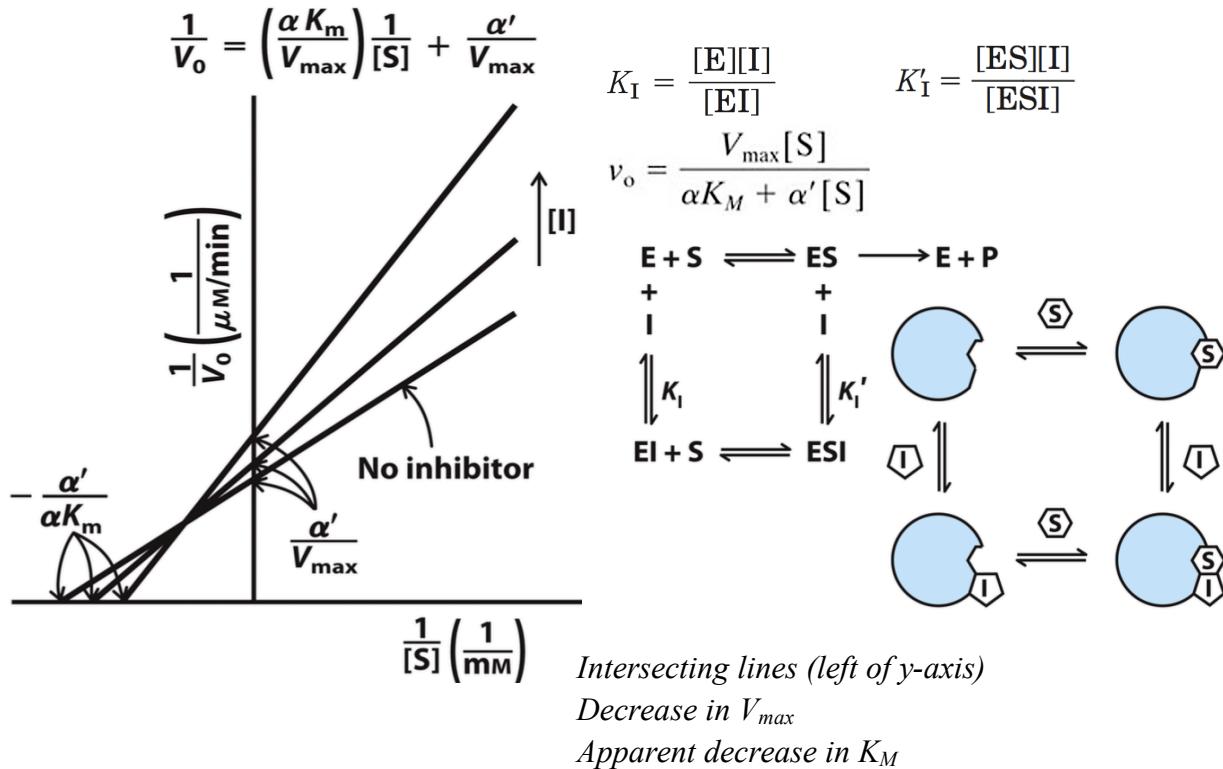


- Uncompetitive inhibition:
 - Allosteric binding to a site distinct from the active site
 - Affects catalytic function, NOT substrate binding.



f. Mixed inhibition

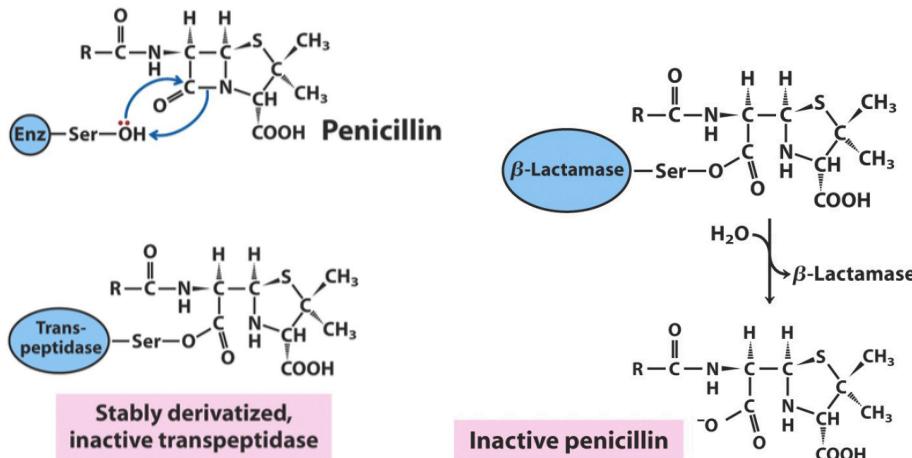
- Inhibitor binds enzyme (at regulatory site) with OR without substrate
- Inhibits BOTH substrate binding AND catalysis
- Noncompetitive inhibitors are mixed inhibitors such $K_I = K_I'$ and $\alpha = \alpha'$.
 K_M does not change, so all lines intersect at the x-axis.



Lecture 9: Enzymes II: Inhibition and Catalysis

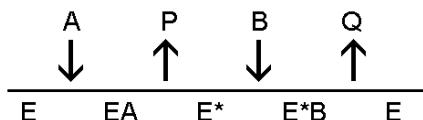
A. Irreversible Inhibitors: β -lactam Penicillin

- Inhibits bacterial transpeptidase involved in peptidoglycan synthesis (blocks cell wall formation)
- Suicide inhibitor: similar to the substrate; binds to the active site of the enzyme, which converts it into a reactive intermediate that permanently binds and inhibits it (activation).
- Penicillin resistance: β -lactamases can hydrolyze the lactam ring.

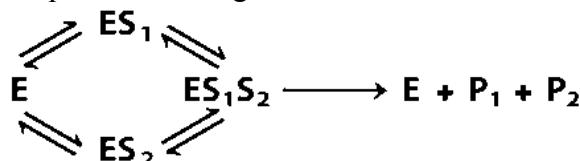


B. Reaction mechanisms with 1 or 2 substrates

- 1 substrate: relatively rare (e.g. isomerases)
- Most common: 2 substrates \rightarrow 2 products, especially group-transfer reaction
- Possible mechanisms:
 - Ping-Pong mechanism: first product released before second substrate bound.



- Sequential binding until both substrates are in the enzyme-substrate complex

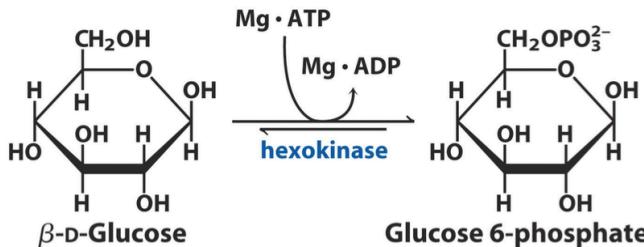


C. Typical catalytic mechanisms

- The active site makes the transition state more favorable
- Conformational constraints: distorts substrate to transition state;
Ensures proximity and orientation (with two substrates)
- Covalent catalysis: transient bond with substrate
- General acid-base catalysis
- Metal ions: stabilize charged transition state or involvement in oxidoreductase reactions

D. Hexokinase: catalyzed reaction

- Multiple isozymes; widely distributed in eukaryotes ($M_r = 107.9$ kDa in yeast)
- Catalyzes initial step of glycolysis: phosphorylation of hexose prevents cellular export.



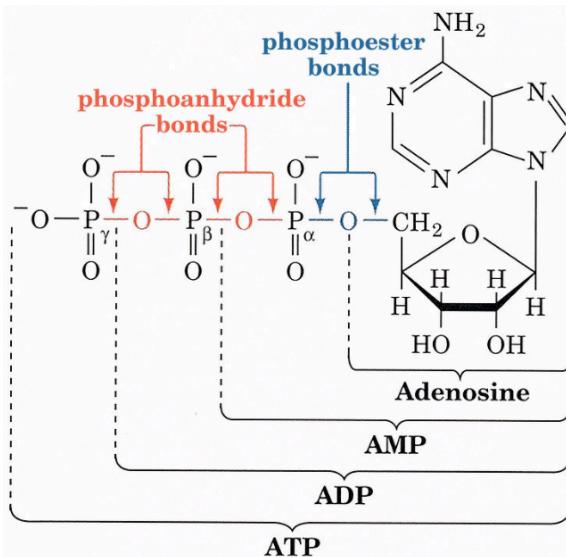
- c. Glucose-binding induces closed conformation, enhances ATP binding, and catalyzes C6 phosphorylation.

E. Group-transfer potentials

- Transfer of activated groups: energy-rich bonds
- Hydrolysis of these bonds yields very negative $\Delta G < -30 \text{ kJ/mol}$

F. Nucleoside triphosphates: energy currency of cells

- ATP in cells is found as a complex with Mg^{2+} – partially neutralizing the negative charge.
- Allows for specific, weak interactions; defines ATP conformation.



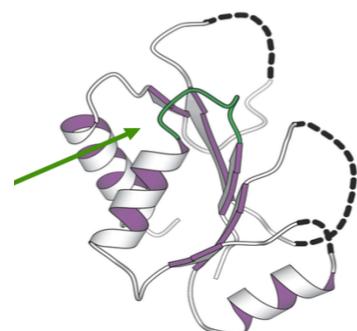
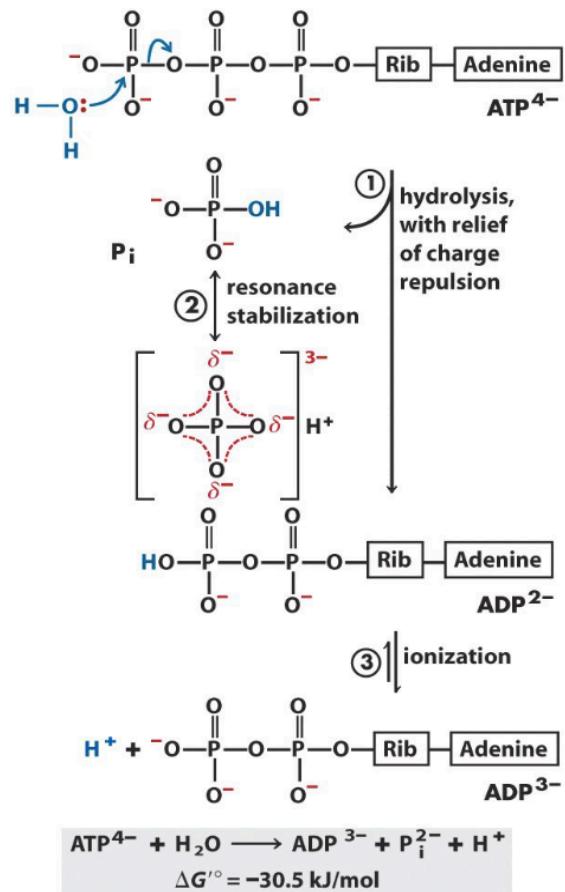
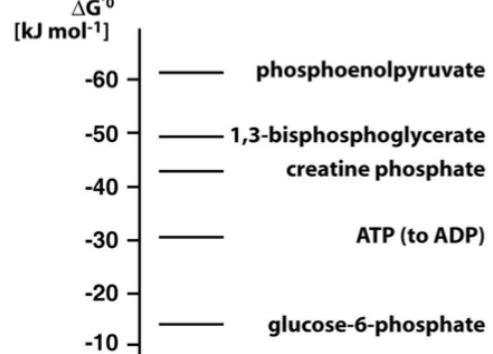
G. ADP and P_i are highly stabilized

- $\Delta G = -30.5 \text{ kJ/mol} = -7.3 \text{ kcal/mol}$
- Charge separation relieves electrostatic repulsion
- P_i (inorganic phosphate) is resonance-stabilized; each bond has double-bond character
- ADP can ionize (by releasing H^+)
- Products better solvated than starting material.

H. Kinases

- Use NTPs to add P_i groups for energy capture, energy transfer, energy use, and cell signaling.
- NTP binding domains share structural features:
 - Central β-sheet with α-helices on both sides.
 - P loop domains interact with NTPs
 - Typical GxxxGK sequence
 - Common motif in adenylate kinase (nucleotide phosphorylation), transducing (vision), ATP synthase (ATP synthesis), and others.

Figure 1: Phosphyl-group transfer potentials

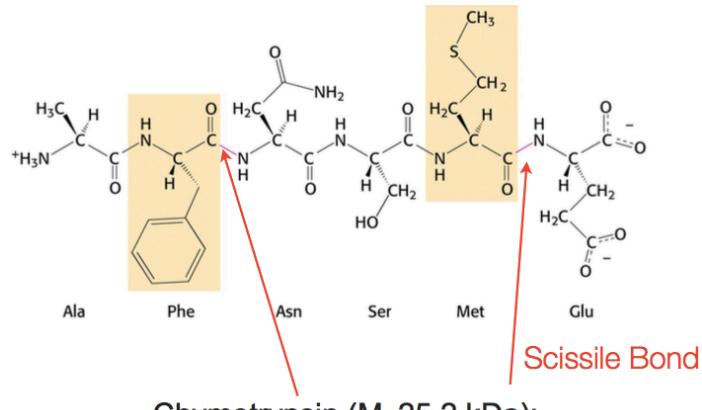


I. Proteases are classified by catalytic mechanism

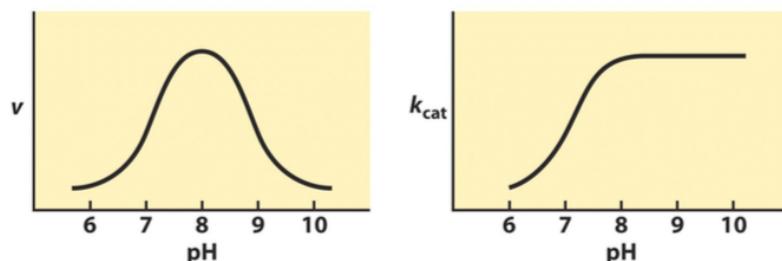
- Serine/Cysteine protease: nucleophile is Ser/Cys
- Aspartyl protease: 2 Asp activate H₂O
- Metalloprotease: Zn²⁺ activates H₂O

J. Chymotrypsin (pancreatic serine protease)

- Specific for peptide bonds C-terminal of aromatic/bulky AAs (F, W, Y, M).
- Scissile bond: covalent bond that can be broken by an enzyme.



- Enzyme reaction velocity and k_{cat} ($=V_{max}/[E]$) display strong pH dependence. Sharp transition at pH 8 – His protonation plays a key role during catalysis.



- In the active site, His⁵⁷ is stabilized and oriented by charged Asp¹⁰², allowing it to accept a proton from Ser¹⁹⁵. This stabilizes Ser¹⁹⁵ as a nucleophilic alkoxide ion.



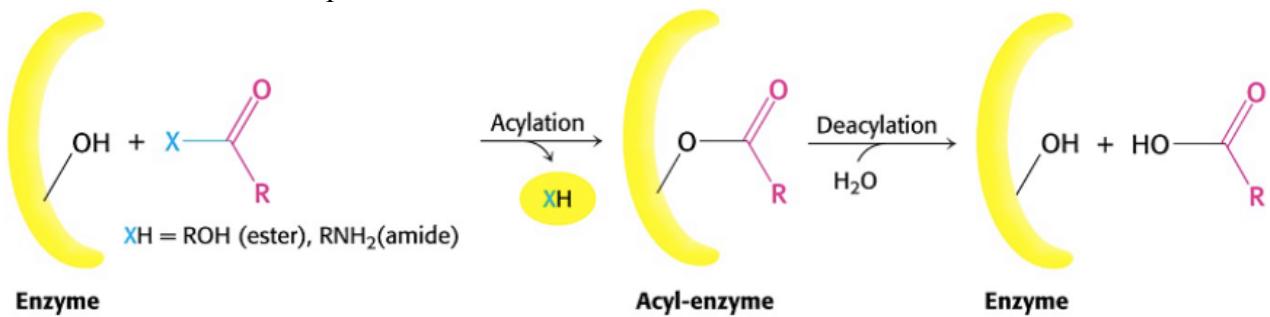
- Ser¹⁹⁵ attacks the substrate carbonyl and initiates the first phase of hydrolytic cleavage.

f. Overall mechanism: 2 steps

i. *Acylation*: Nucleophilic attack of Ser¹⁹⁵ → acyl-enzyme intermediate.

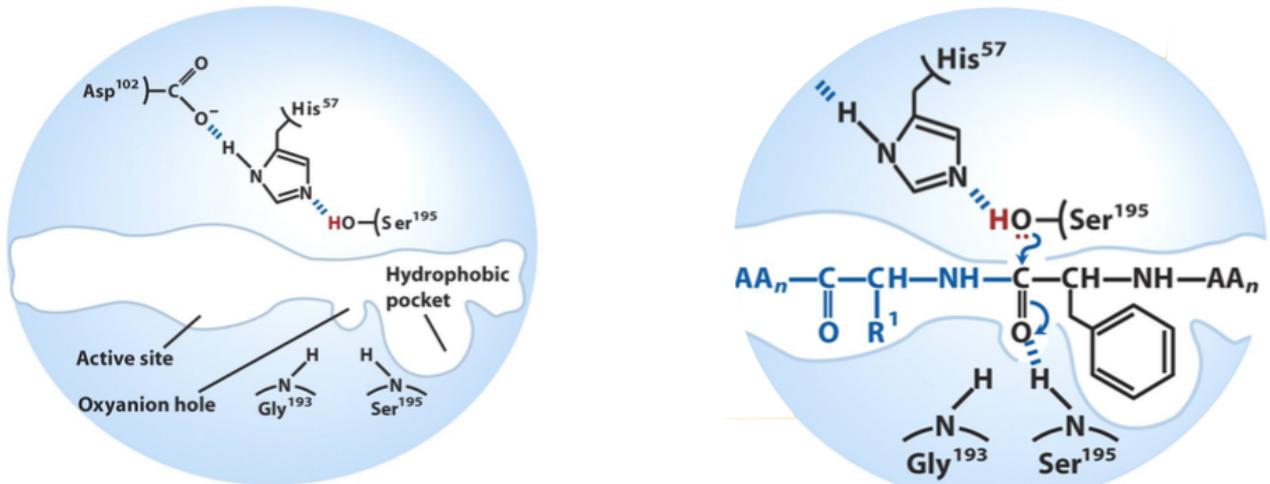
The peptide bond is cleaved, an ester linkage is formed, and product 1 (XH) is released.

ii. *Deacylation*: Slow hydrolysis of ester linkage, regeneration of nonacylated enzyme, and release of product 2.

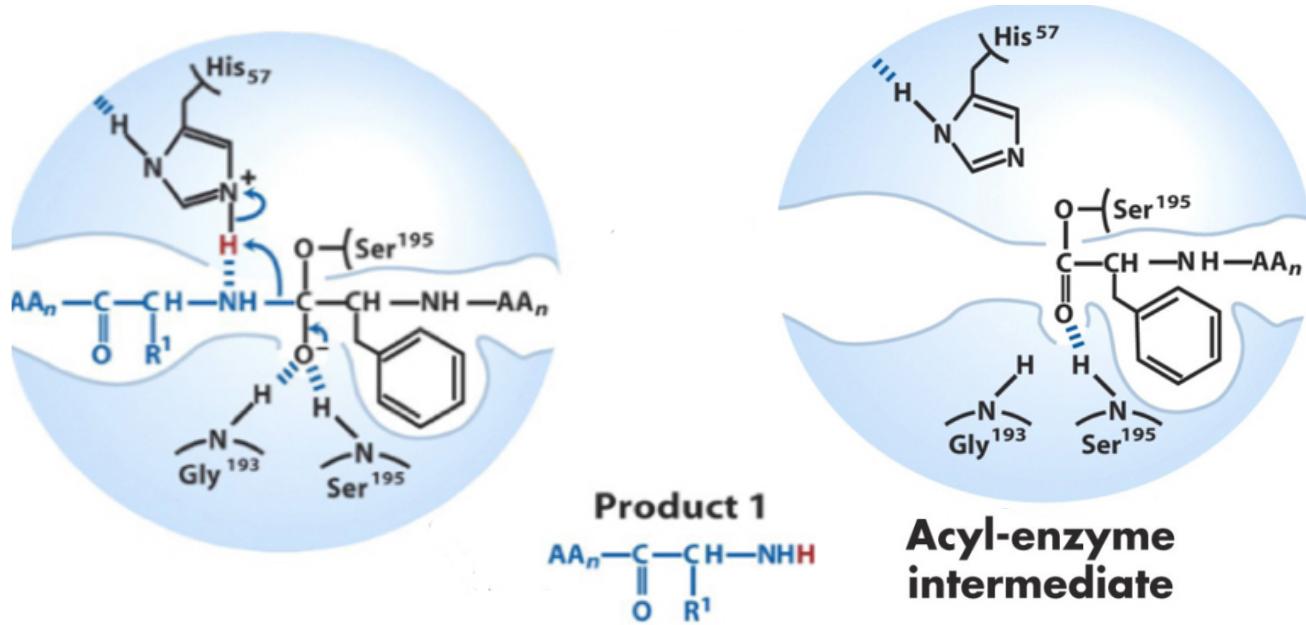


g. Detailed mechanism (p.g. 216-217)

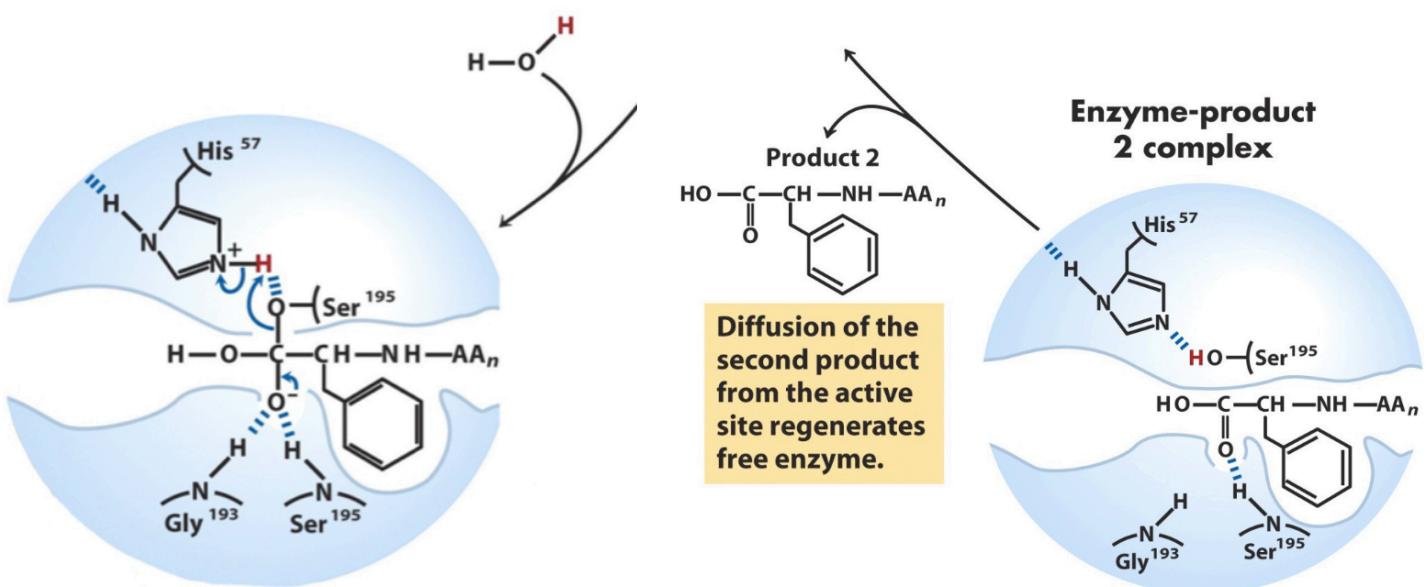
Attachment: Side chain N-terminal of substrate peptide bond stabilized in hydrophobic binding pocket.
Acylation: Ser nucleophilic attack forms tetrahedral intermediate. Charge on substrate carbonyl stabilized in *oxyanion hole*.



The intermediate collapses, releasing product 1 and forming the acyl-enzyme.

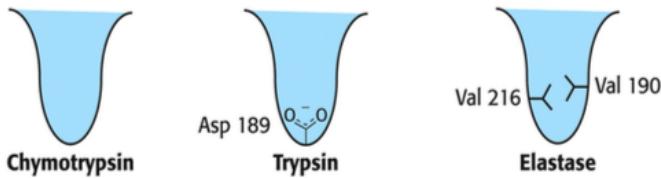


Deacylation: H_2O attacks the carbonyl and protonates the imidazole.
This intermediate collapses; this regenerates the enzyme and releases product 2



A. Serine proteases achieve unique substrate specificity

Pockets for hydrophobic residues, positively charged residues, small residues



F, Y, W, M

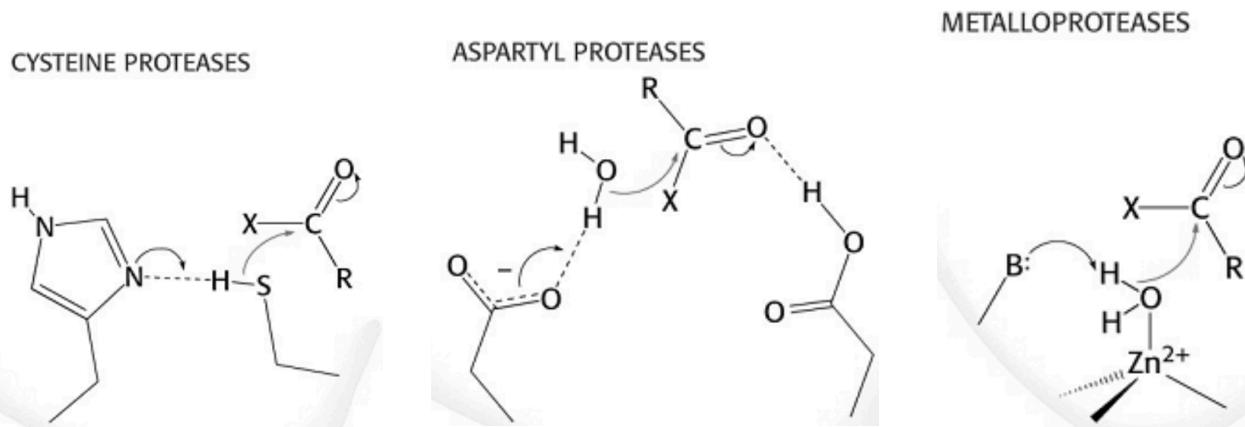
K, R

A, S

B. Cysteine proteases: Cys nucleophile, covalent catalysis, tetrahedral intermediate

C. Aspartyl proteases: 2 Asp form catalytic diad; acid-base catalysis, non-covalent intermediate, proton transfer from activated H_2O .

D. Metalloproteases: Zn^{2+} (bound by His, Glu) activates H_2O , general base catalysis.

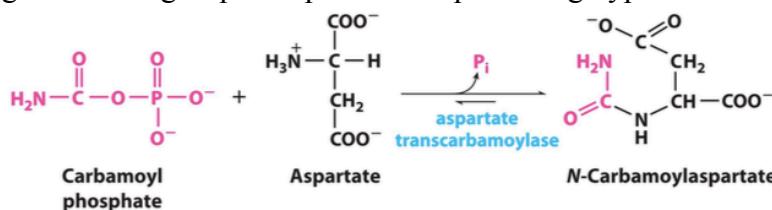


E. Summary

- Types of inhibition and signatures
- General enzyme, kinase, protease mechanism (especially serine protease chymotrypsin)

Lecture 10: Regulation at the Protein Level

- A. Mechanisms for controlling enzymatic activity under fluctuating resources and needs
 - a. Regulation of enzyme amount by synthesis/degradation is important but *slow*.
 - b. Other regulatory processes: allostery, covalent modification, local inhibitors, compartmentalization of enzymatic activity
 - c. Enzymes frequently contain regulatory domains, which control protein localization, substrate specificity, enzymatic activity, and binding partners. Often, a single catalytic site is expressed with a diversity of regulatory elements.
 - d. Allostery: typically in multi-sub-unit proteins. Includes cooperativity between binding sites, and modulators for T \rightarrow R transitions. May have sigmoid curves for binding and reaction velocity.
- B. Ex: Aspartate Transcarbamoylase (ATCase) catalyzes early biosynthesis of pyrimidines (TC) by carbamylating the amino group of aspartate and producing byproduct P_i.

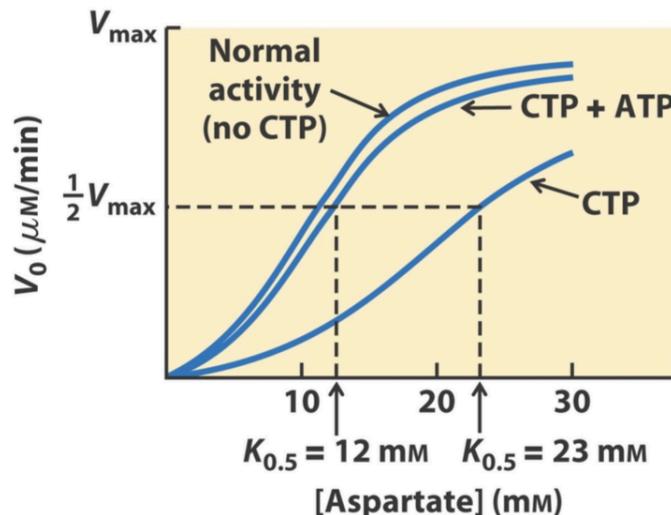


N-carbamoyl aspartate is committed to go on to cytidine 5'-triphosphate (CTP):



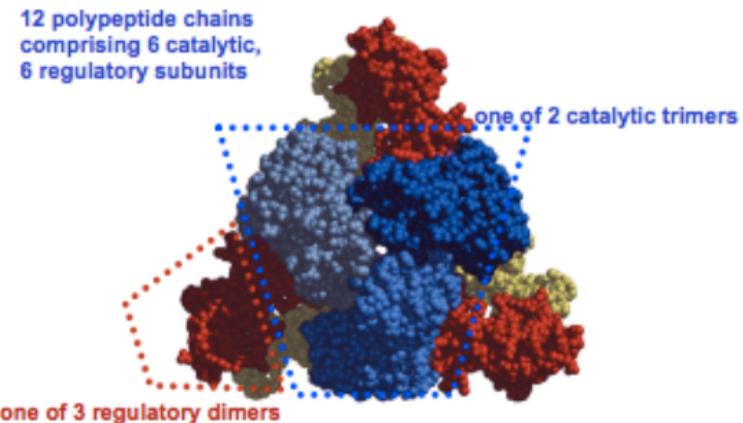
The final product, CTP, inhibits the first reaction catalyzed by ATCase.

- C. ATCase: kinetics of feedback inhibition
 - a. Sigmoidal V₀ behavior: not Michael-Menten, but still demonstrates saturation.

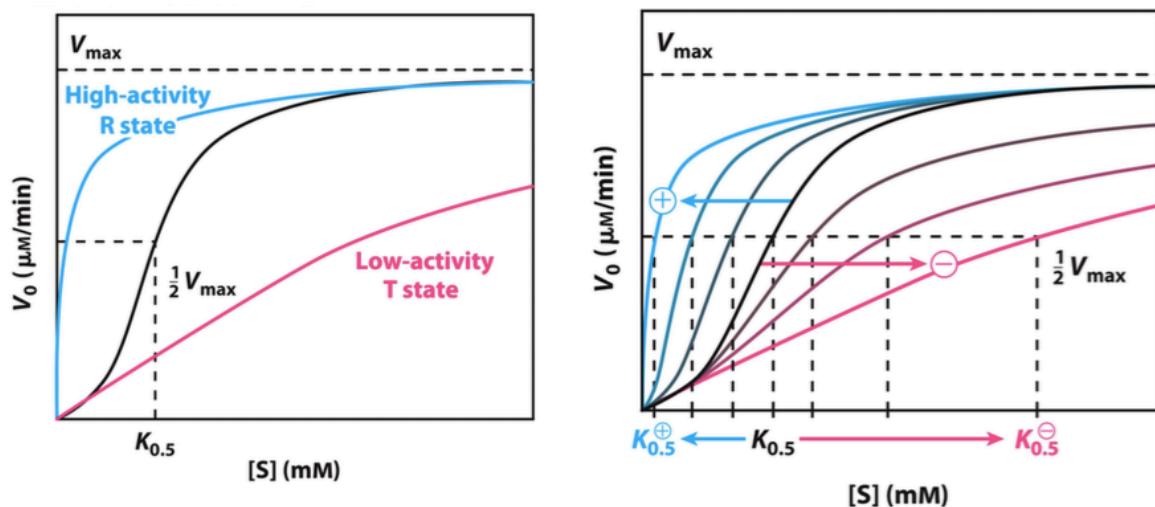


- b. The final product (CTP) inhibits ATCase, while the final product of the purine pathway (ATP) stimulates ATCase activity.
 - c. K_{0.5} is the equivalent of K_m, which denotes the concentration that gives 1/2 V_{max}.

d. ATCase structure: 12 polypeptide chains: 2 catalytic trimers, 3 regulatory dimers.



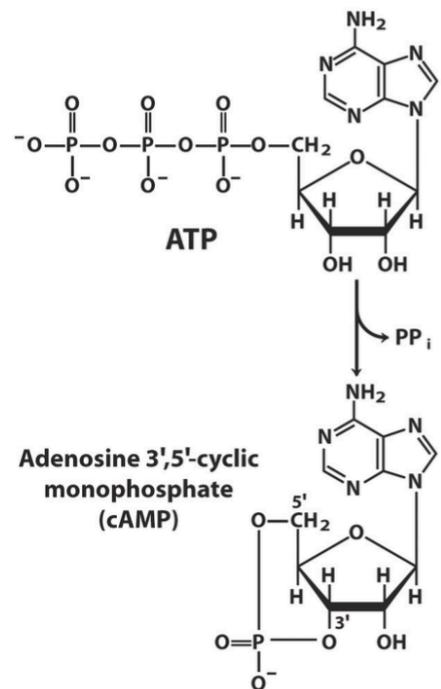
e. Allosterically regulated enzyme behavior and T/R states deviate from MM kinetics



D. Ex: Protein Kinase A (PKA)

- PKA phosphorylates Ser/Thr side chains, and is activated by signaling (adrenaline \rightarrow cAMP \rightarrow PKA).
- Consensus sequence defines amino acids as phosphorylation substrate for active PKA.

Consensus sequence	
Protein kinase A	-X-R-(R/K)-X-(S/T)-B-
B: any hydrophobic residue	



- c. PKA is normally an inactive complex: 2 catalytic subunits (C) and 2 regulatory imposter subunits (R)
- d. Here, the regulatory subunit inserts into the groove. NOT recognized consensus sequence; A is not S/T
- e. cAMP binds the regulatory R subunits, leading to a conformational change: R subunits dissociate off.
- f. The active sites of the catalytic C subunits are freed.

E. Phosphorylation

- a. New H-bonds and ionic interactions with amino acids can (in)activate proteins by changing conformations
- b. Signal amplification: phosphorylation of multiple target proteins by one kinase
- c. High energy → favorable catalysis

F. Eukaryotes have 2 sites of phosphorylation (-OH)

- a. Ser/Thr hydroxyl group
- b. Tyr phenol group

G. Specificity of protein kinases:

- a. Most kinases recognize consensus motifs in multiple substrates; some are substrate-specific.
- b. Specificity controlled by non-catalytic domains, as well as temporal and spacial controls
- c. Signaled by cNTPs, Ca^{2+} and calmodulin, DAG, and phosphoproteins.

Enzyme/protein	Sequence phosphorylated	Pathway/process regulated
Glycogen synthase	RASCTSSS	Glycogen synthesis
Phosphorylase b kinase		
α subunit	VEFRRRLSI	Glycogen breakdown
β subunit	RTKRSGSV	
Pyruvate kinase (rat liver)	GVLRRASVAZL	Glycolysis
Hormone-sensitive lipase	PMRRRSV	Triacylglycerol mobilization and fatty acid oxidation
Phosphofructokinase-2/fructose 2,6-bisphosphatase	LQRRRGSSIPQ	Glycolysis/gluconeogenesis
Tyrosine hydroxylase	FIGRRQSL	Synthesis of L-DOPA, dopamine, norepinephrine, and epinephrine
PKA consensus sequence [†]	XR(R/K)X(S/T)B	

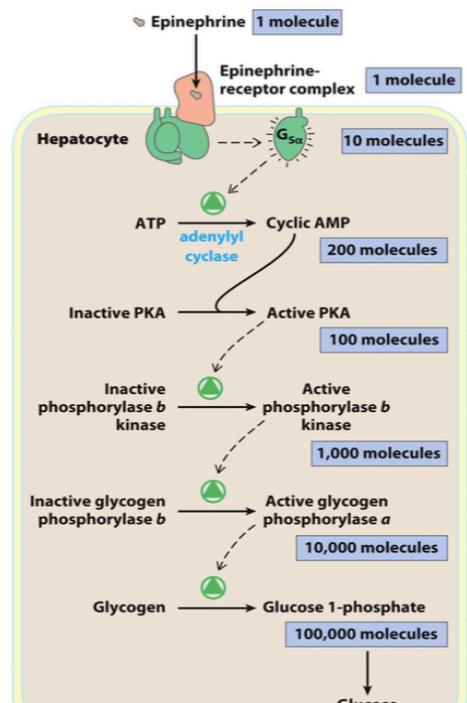
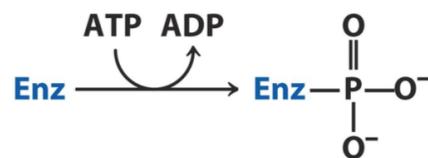
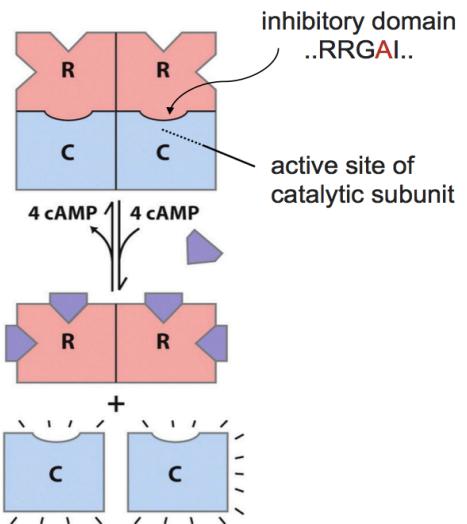
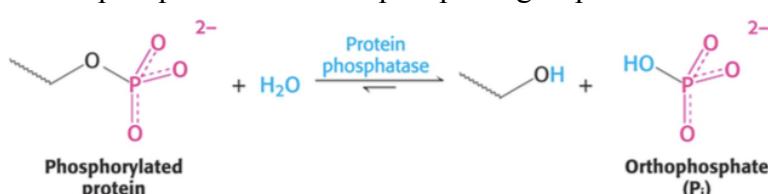
- d. Red: phosphorylated, X: anything, B: hydrophobic
- e. Regulates carbohydrate/lipid metabolism and hormone production itself (feedback loop)

H. PKA in the signal transduction pathway

- a. PKA → phosphorylase kinase → glycogen phosphorylase → glycogen metabolism
- b. Glycogen phosphorylase expressed in liver and skeletal muscle. Phosphorylates and removes glucose from glycogen, one by one.

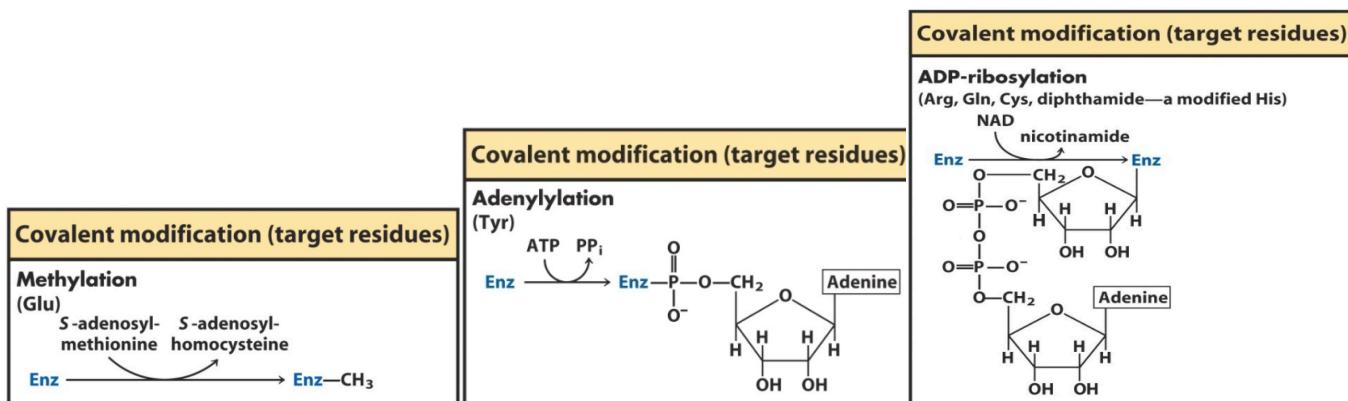
NOT a kinase: phosphate from P_i , not NTP.

I. Protein phosphatases remove phosphate groups. Reversibility allows finely-controlled responses.



J. Other covalent modifications:

- Methylation (methyl-transferase) of bacterial chemotaxis receptors regulates flagellar movement.
- Adenylylation (adenylyl transferase) modifies bacterial AMP to limit glutamine synthesis.
- ADP-ribosylation (cholera toxin) inhibits intracellular signaling pathway.

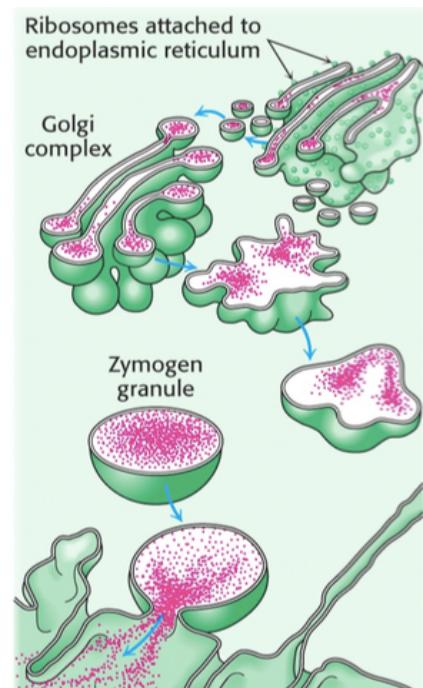
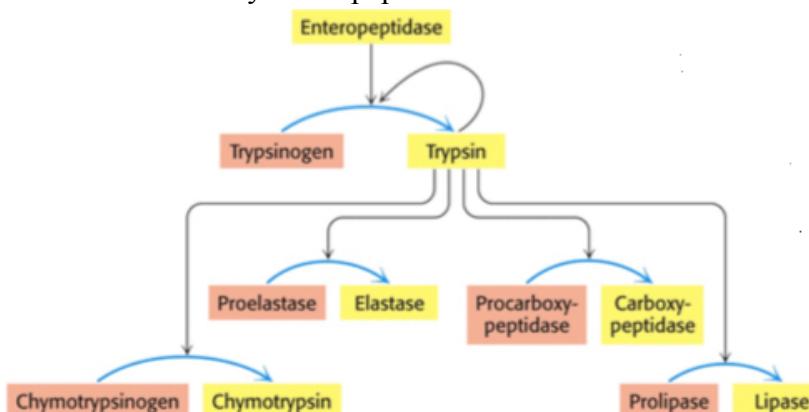


K. Proteolytic cleavage

- Intracellular and extracellular
- Irreversible; activates or inactivates proteins
- Target proteins (proteins): enzymes, hormone precursors, proteins for apoptosis, or structural proteins.

L. Zymogens (proenzymes) are enzyme precursors activated by proteolytic cleavage:

- Released from pancreatic cells via zymogen granules
- Activated by enteropeptidase in the duodenum.

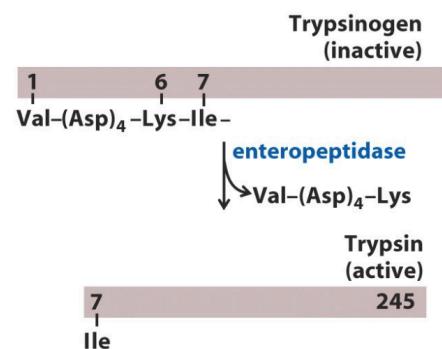


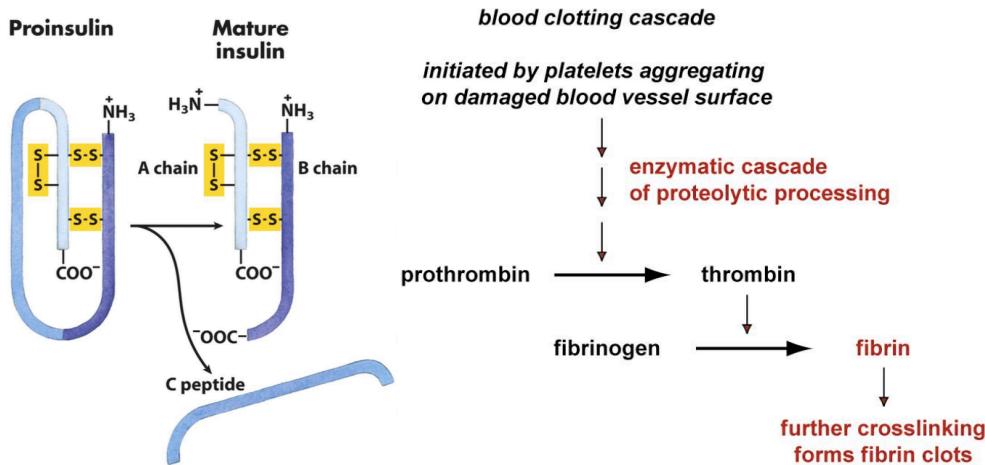
M. Trypsin and trypsinogen

- Pancreatic trypsin inhibitor: binds trypsin tightly to guard against trypsin activity. This inserts a loop resembling a trypsin substrate into the catalytic cleft.
- Zymogen granules in trypsinogen-secreting cells contain PTI, which soaks up inadvertently formed trypsins.

N. Peptide hormones begin as prohormones

- Insulin: packaged/stored in secretory granules, processed by proteolysis before release.
- Blood clotting: series of proteolytic steps (e.g. thrombin cleaves fibrinogen → fibrin).

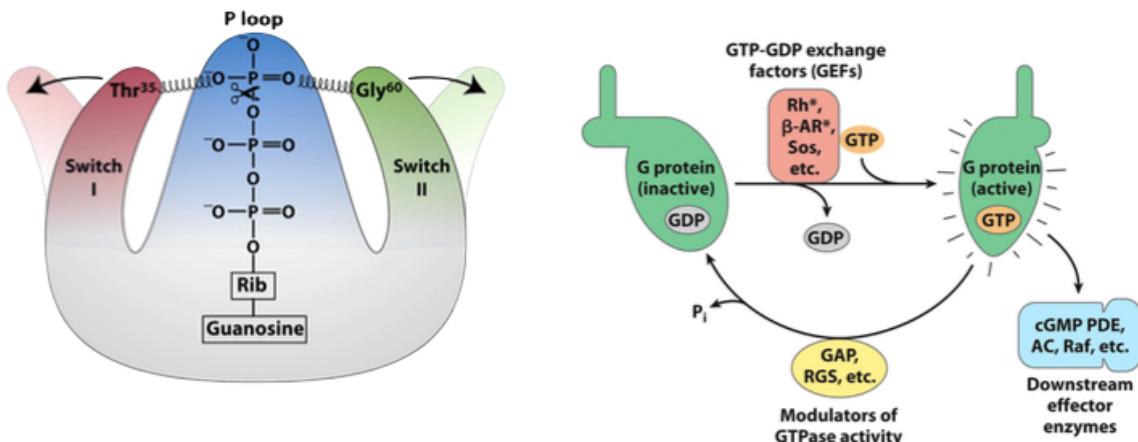




- O. Special and temporal control: compartmentalization influences enzyme access to substrates
- Lysosome: low pH environment dedicated to proteolysis
 - Nucleus: temporal localization of proteins during cell cycle.
Only proteins expressed at that time can be modified.

P. G-proteins

- Switchable signaling proteins: bind and hydrolyze GTP to change conformational states.
- GTPase activator proteins (GAPs) stimulate GTP hydrolysis to GDP.
- GTP-GDP exchange factors (GEFs) restore the GTP-bound state.



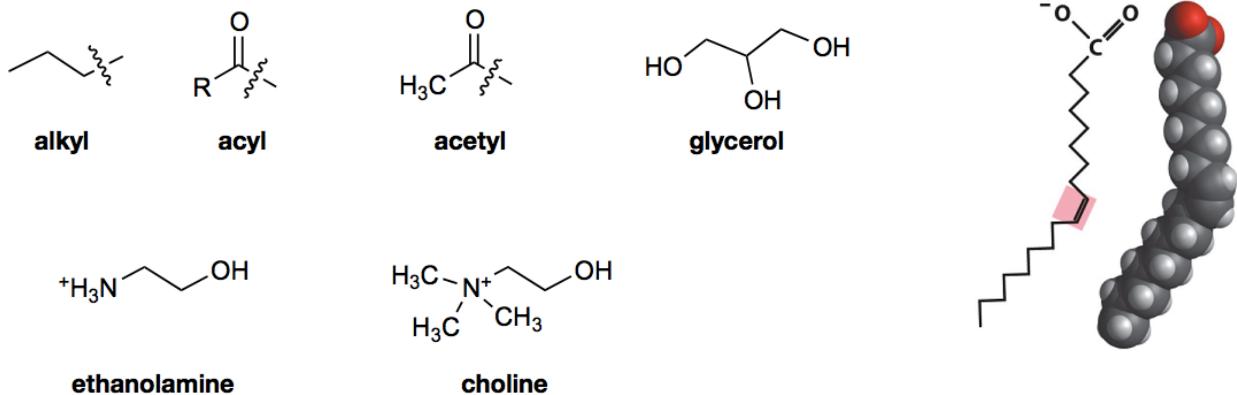
Q. Summary

- Allotropy in enzymes
- Regulatory influence of covalent modification, proteolytic cleavage, and phosphorylation
- Basics of GTPases and how they function as switches

Lecture 11: Membranes I: Bilayers and Compartments

A. Overview

a. Nomenclature



- b. Structure: fluid and flexible lipid bilayers embedded with membrane proteins
- c. Function: define cell boundaries and intracellular compartments, selectively permeable, maintain electric and chemical potentials, actively transport molecules and ions

B. Fatty acids: unbranched carboxylic acids attached to hydrocarbon chains; 4-36 (even) carbons long.

- a. Amphipathic/amphiphilic: polar head group and nonpolar moiety
- b. Most double bonds are cis and unconjugated.
- c. Unsaturation improves fluidity and decreases melting point.
- d. Length increases melting point.
- e. 18:1(Δ^9) means 18 carbons, 1 double bond at position 19 from the COOH.

C. Triacylglycerols: 3 fatty acids in ester linkage with glycerol

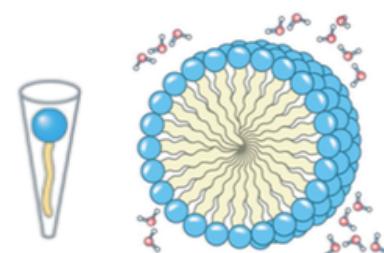
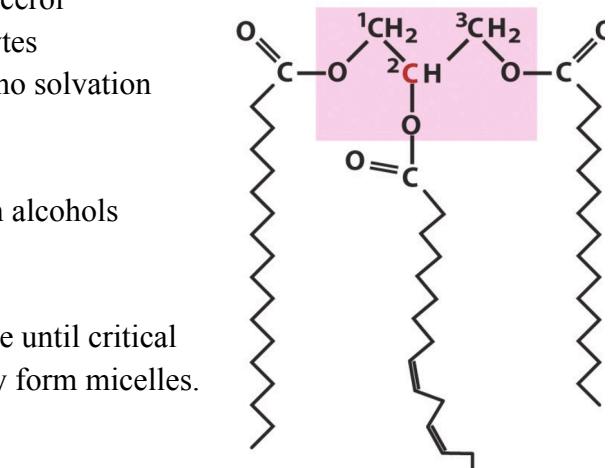
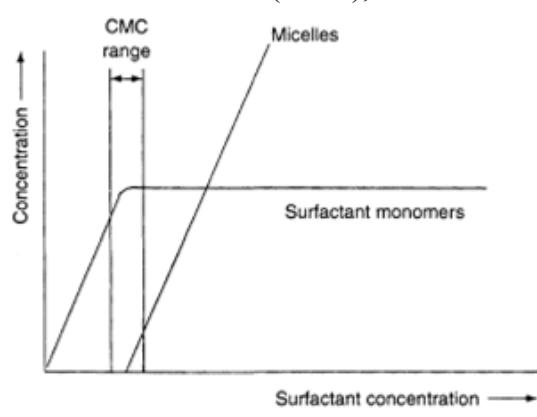
- a. Function: storage lipids; fat droplets in adipocytes
- b. Benefits: high energy per weight, unhydrated (no solvation water weight), thermal insulation

D. Biological waxes

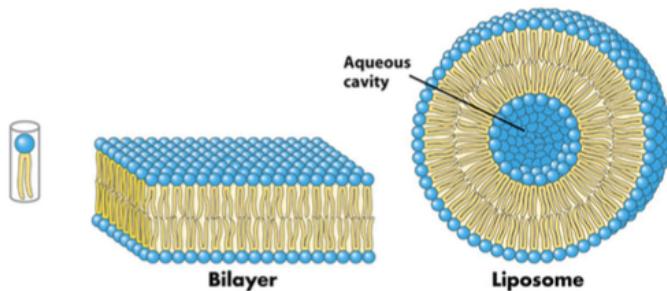
- a. Esters of long-chain fatty acids with long-chain alcohols
- b. Function: water repellent, protection

E. Micelles and bilayers

- a. Wedge-shaped single-chain amphiphiles solvate until critical micellar concentration (CMC), after which they form micelles.

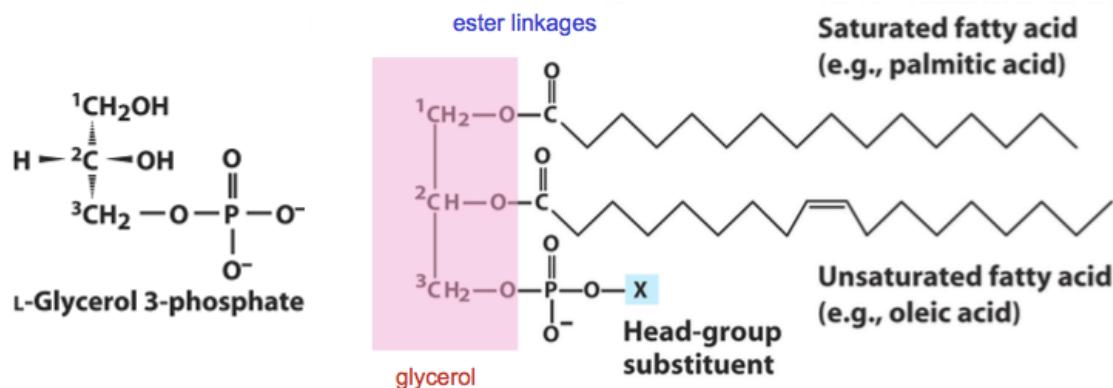


- b. Cylindrical double chain amphiphiles assemble into bilayers.



F. Phosphatidate: lipid backbone 1

- Diacylglycerol phosphorylated at C₃ → diacylglycerol 3-phosphate.
- Phosphatidate: when head group X = H.

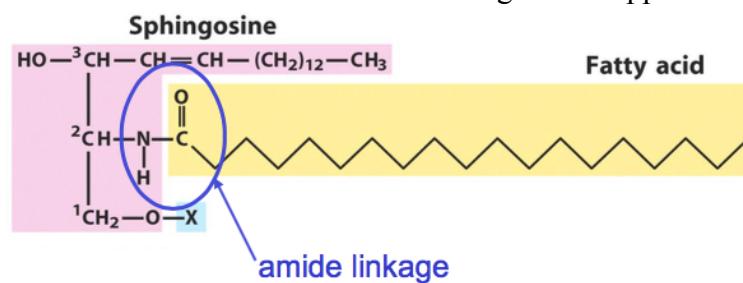


- c. Common polar head groups

Phosphatidylethanolamine	Ethanolamine	$-\text{CH}_2\text{CH}_2\text{NH}_3^+$	0
Phosphatidylcholine	Choline	$-\text{CH}_2\text{CH}_2\text{N}(\text{CH}_3)_3^+$	0
Phosphatidylserine	Serine	$-\text{CH}_2\text{CH}(\text{NH}_3^+)\text{COO}^-$	-1
Phosphatidylglycerol	Glycerol	$-\text{CH}_2\text{CH}(\text{OH})\text{CH}_2\text{OH}$	-1
Phosphatidylinositol 4,5-bisphosphate	myo-Inositol 4,5-bisphosphate		-4

G. Acylated sphingosine: lipid backbone 2

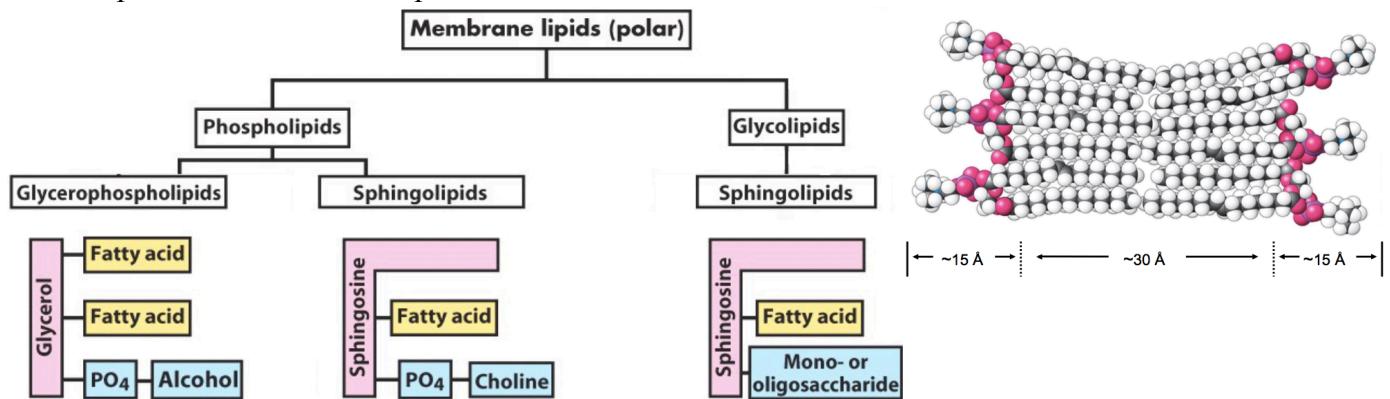
- Sphingosine (long chain amino alcohol) + acyl group → amide linkage
The backbone is unbroken- the long chain supplies the third chain.



- b. Ceramide: when head group X = H.
- c. Glycolipids: when head group X = saccharide(s)

This often lines the outside of the plasma membrane and determines human blood groups:

H. Comparison of membrane lipid classes



- a. Phospholipids: alcohol head group linked through a phosphodiester bond.
- b. Glycolipids: sugar head group linked through glycosidic bonds
- c. Sterols: steroid nucleus with a polar hydroxyl group

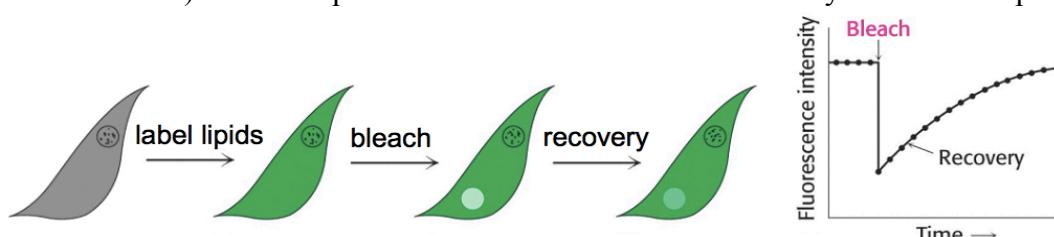
I. Phospholipids are asymmetrically distributed across the inner and outer monolayers

J. Lipid bilayer composition assist signaling: group transfer (especially P_i) on lipid components and head groups allow/prevent recognition by other proteins.

Ex: PI3K phosphorylates PIP2 → PIP3 to activate PKB. This is turned off by phosphatases.

K. Lateral diffusion of lipids

- a. Common and rapid (~1 μm/s; around the cell in seconds)
- b. Probing lipids with fluorescent labels (e.g. TNBS reacts with amines in lipids via EAS)
- c. FRAP (fluorescence recovery after photobleaching): live cells
 - 1) Bleach labeled lipids in plasma membrane with laser
 - 2) Follow lipid diffusion and fluorescence recovery in bleached spot



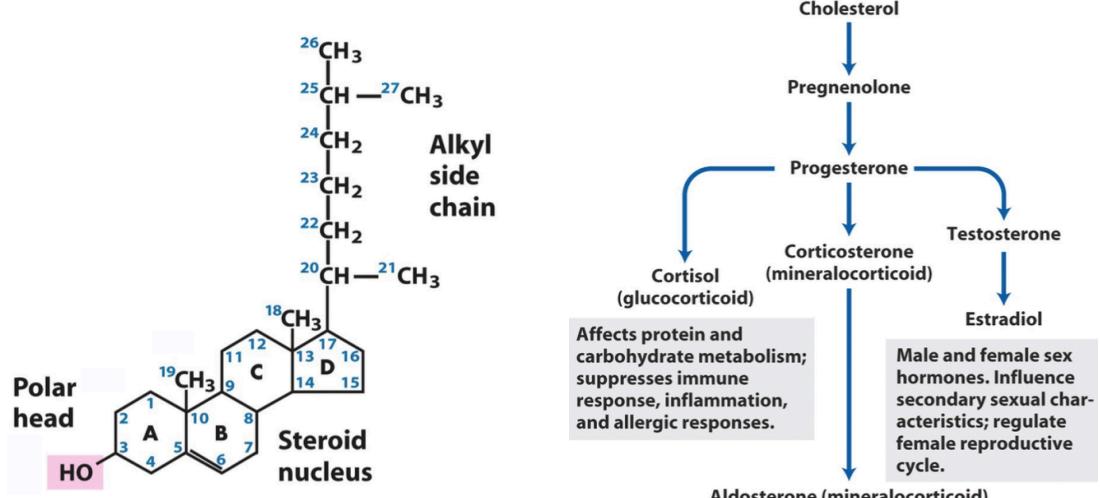
- d. Spontaneous flip-flop of lipids are rare (~t_{1/2} in days)

L. Phase transitions

- a. Fluid <→ paracrystalline (gel)
- b. Heat increases fluidity, which is necessary for proper membrane function.

M. Cholesterol: C₃ hydroxyl constitutes a small polar head group

- a. When cold: maintains fluidity by breaking up close packing of alkyl chains.
- b. When normal temperature: sterically hinders lipid diffusion.
- c. Precursor for all steroid hormones
 - 1) Formed by side-chain cleavage and oxidation of sterol rings.
 - 2) Synthesis of hormones usually in adrenal glands and gonads.

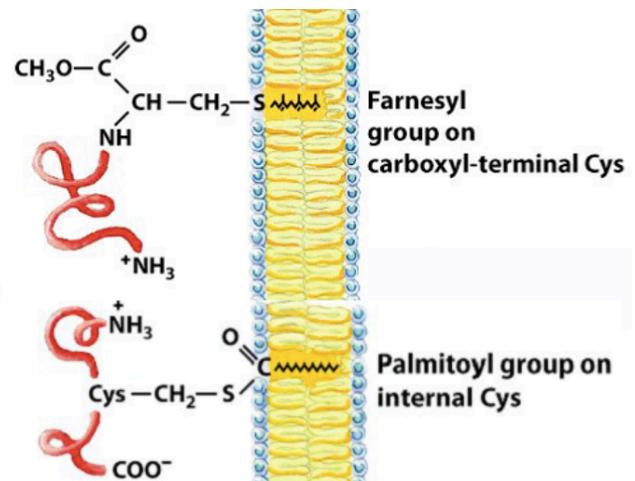
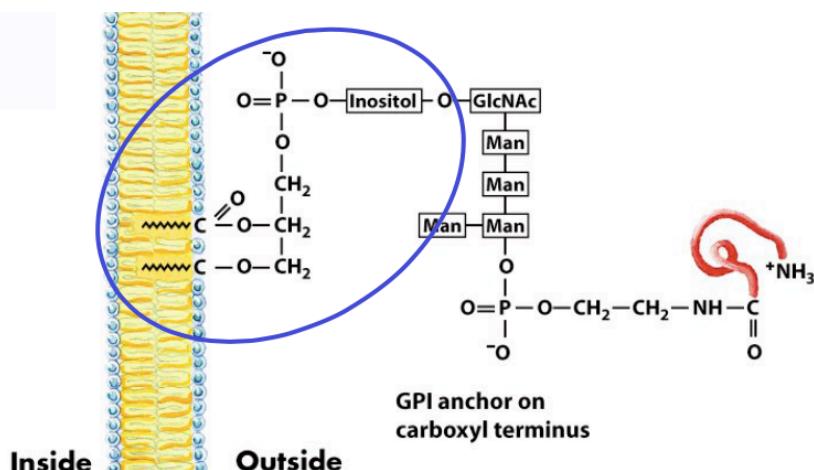


N. Proteins and lipids form membranes together

- Specific composition of lipids and proteins vary
- Interaction of proteins with membranes
 - Hydrophobic effect across bilayer (many)
 - Hydrophobic effect at bilayer surface (few)
 - Covalent lipid anchors
 - Interaction with transmembrane proteins
 - Ionic interaction at lipid surface

- Lipids as protein anchors: glycosylphosphatidylinositol (GPI)
(Anchors to C-terminus)

link via phosphatidylinositol:



- Other lipid anchors exist only on the inner leaflet (left)
 - Isoprenylation of C-terminal Cys
 - Fatty acylation of an internal Cys

O. Ras is a farnesyl anchored GTPase central to growth: implicated in cancers.

Ras-GDP to Ras-GTP triggers downstream signaling.

P. Summary

- Lipids are classified by their fatty acids, backbone structure, and head groups
- Membrane lipids are fluid; lateral diffusion, rare flipping

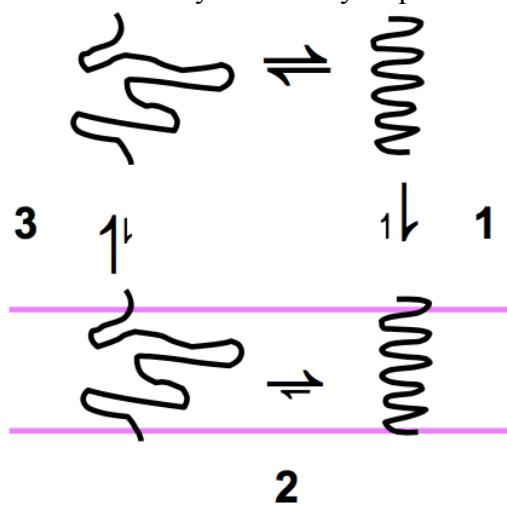
Lecture 12: Membranes II: Proteins, Ion Channels, and Pores

A. Transfer free energy: amino acids out of bilayers

- a. Hydropathy scale: combines hydrophilicity and hydrophobicity of R groups to find an amino acid side chain's tendency to seek a polar (-) or nonpolar (+) environment. Measures energy required to transfer the R group from within a membrane into aqueous surroundings.
- b. Phe 3.7, Ala 1.6, Gln -4.1, Arg -12.3, Backbone peptide bonds: -1 (kcal/mol)
- c. Ex: 18-residue Poly-A peptide into water: $(18)(1.6) + (17)(-1) = 11.8 \text{ kcal/mol}$.
- d. Ion transfer into bilayers is really bad; amino acids are neutralized to COOH and NH₂ first.
- e. H-bonds between amino acids is strongly favored in a lipid environment.
This is because “free” donors and acceptors lose their H-bonds when they are desolvated, but “paired” donors and acceptors do not (6 kcal:1 kcal advantage)

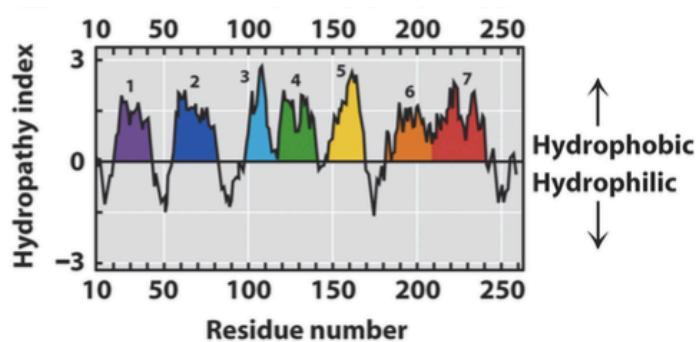
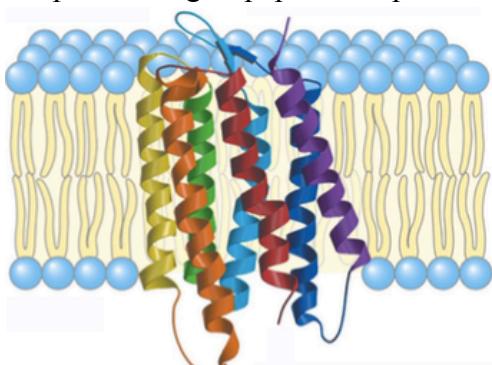
B. Hydrophobic helices are common transmembrane proteins

- a. Review of α -helices
 - i. 5.4 Å pitch/turn, 3.6 residues/turn, 1.5 Å pitch/residue
 - ii. Backbone diameter: 5 Å, backbone+side chains: 10 Å
 - iii. The backbone is completely H-bonded; most favorable 2° structures
 - iv. Why else are hydrophobic helices the most common theme for transmembrane regions



1. Hydrophobic side chains partition out of water.
2. Forming H-bonds is favored in bilayers and strongly drives helical structure.
3. Moving unfolded peptide into bilayer is resisted by polar backbone H-bonds removed from water

- b. Prediction of transmembrane helices: amino acids are assigned hydropathy index based on partitioning of peptides in polar/nonpolar solvents.

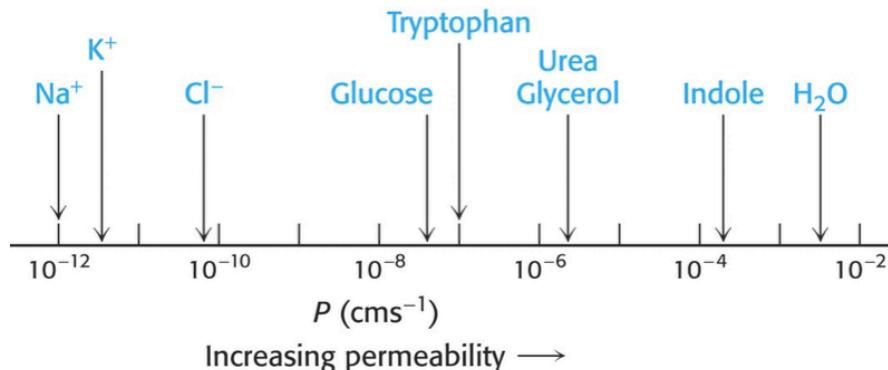


- c. Sequence hydropathy is scanned over a defined length. A hydrophobic sequence over 20 amino acids long a potential α -helix transmembrane region.

- d. Membrane-spanning protein folding is different from soluble protein folding
 - i. Loop folding in polar environment, and transmembrane folding in nonpolar environment
 - ii. Dielectric constant in lipid bilayers is different from aqueous phase: electrostatic interactions become very strong (undiluted by polar bonds)
- e. Hydrophobic helices continued
 - i. Distance between helices (center to center) is 5-12 Å
 - ii. ~20 hydrophobic residues span a bilayer.
 - iii. Individual helices are self-contained – no minimum number to build a membrane protein.
 - iv. Knob-into-hole model of side chains is when side chains from 1 helix intercalate between side chain positions in a 2nd helix. This gives sterically acceptable tight packing (may fit better than helix-lipid packing).
 - v. Packed helices are oriented relative to each other to define structures.

C. Membrane permeability

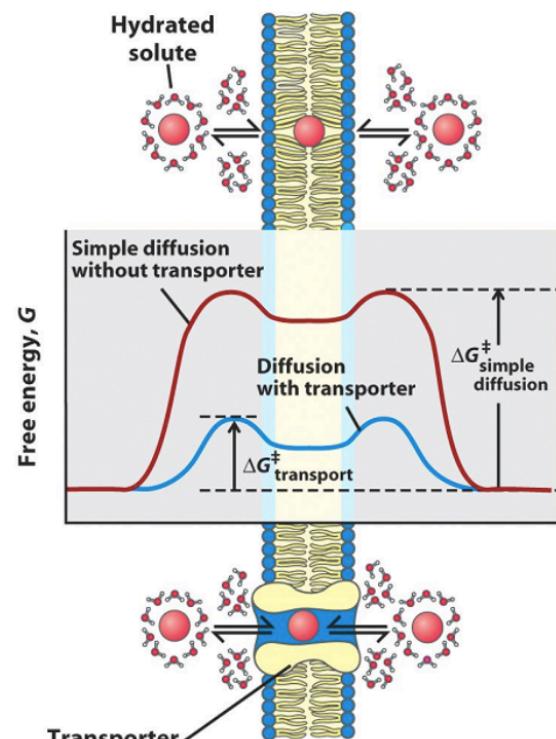
- a. Permeable to most hydrophobic molecules and some small polar molecules (water)
- b. NOT permeable to ions or large polar molecules (glucose, sodium, calcium).



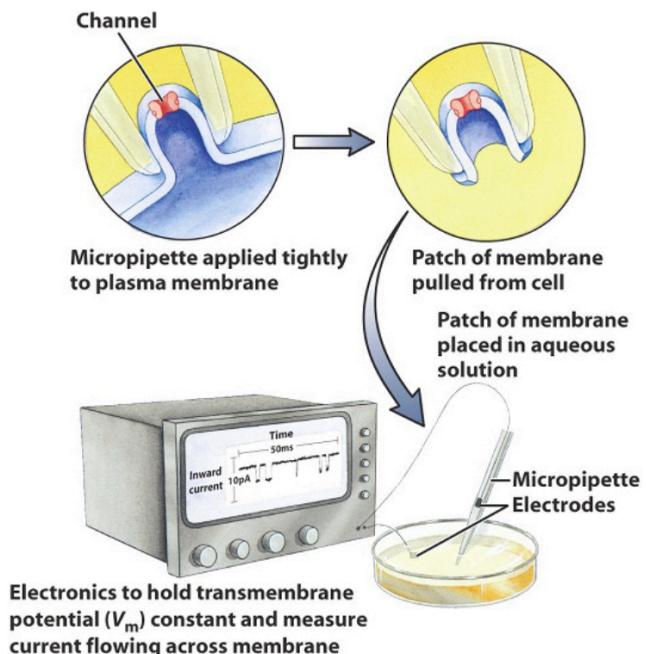
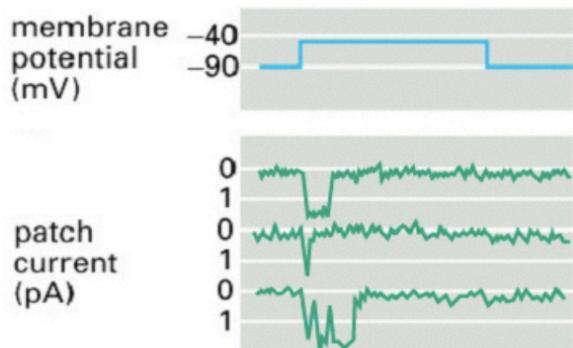
- c. Normal diffusion
 - i. Step 1: removal of hydration shell
 - ii. Step 2: dissolution in nonpolar lipid (high activation cost)
 - iii. Step 3: reformation of hydration shell
- d. Facilitated diffusion: polar environments in membrane proteins lower activation energy.
- e. Ion channels are highly selective.

Relative permeabilities:

	Na^+ channel	K^+ channel	Acetylcholine receptor	Chloride channel
Li^+	0.93	< 0.01	0.87	< 0.01
Na^+	1.00	< 0.01	1.00	< 0.01
K^+	0.09	1.00	1.11	< 0.01
Rb^+	< 0.01	0.91		
Cs^+	< 0.01	< 0.08	1.42	
NH_4^+	0.16	0.13	1.79	
H_3NOH^+	0.94	< 0.03	1.92	
H_2NNH_3^+	0.59	< 0.03		
H_3CNH_3^+	< 0.01	< 0.02		
Cl^-	< 0.01	< 0.01	< 0.01	1.00

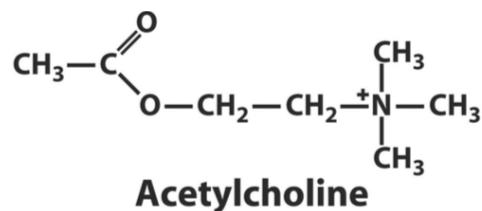
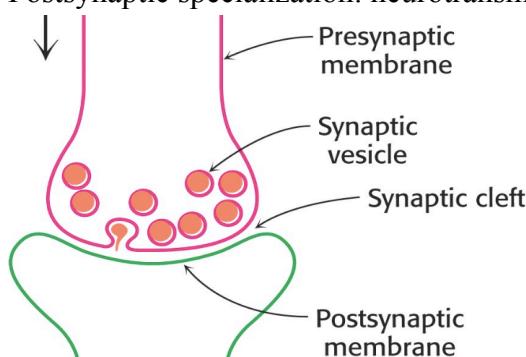


- f. Porins are beta-barrels that form specific transport channels
 - i. Nonpolar exterior and polar interior
 - ii. Polypeptide loops can protrude into lumen
 - iii. Only found in outer membrane of mitochondria/chloroplasts and gram-negative bacteria.
- g. Gated ion channels have controlled opening and spontaneous closing
 - i. Ligand-gated (AChR)
 - ii. Voltage-gated (Na^+ , K^+)
- h. Patch clamping
 - i. Records opening and closing via current flow, with single channel resolution.
 - ii. Patch pipette: gigaohms-tight seal.
 - iii. High resolution on ms time scales and pA current scales.

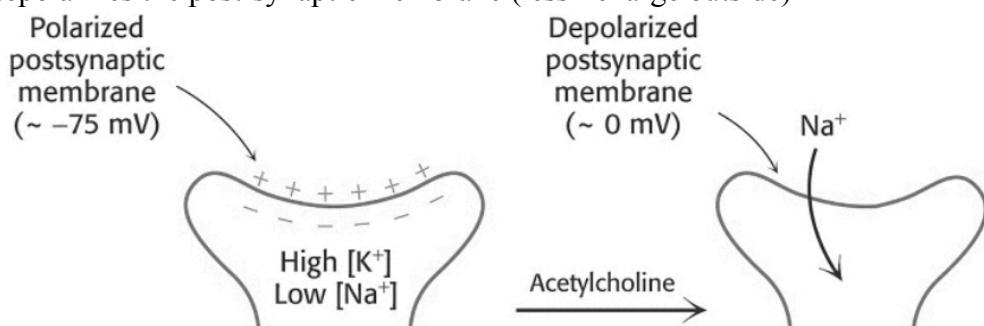


D. Part 1- Acetylcholine and initiation

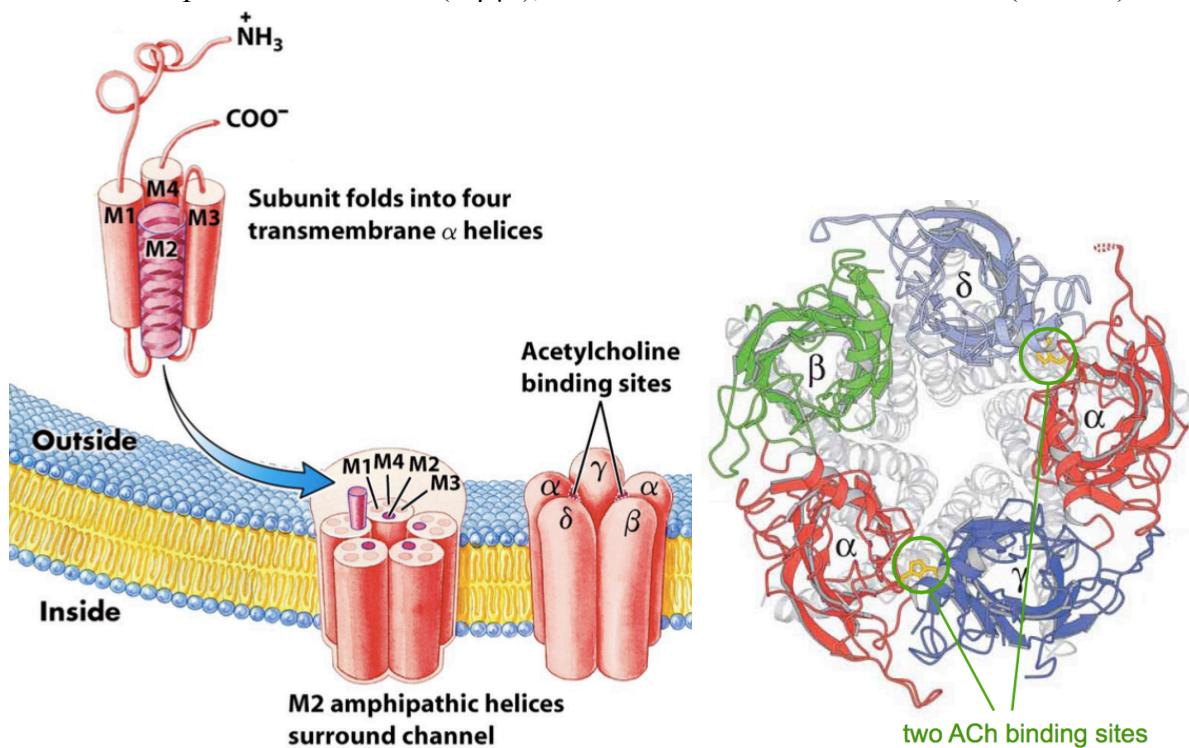
- a. Presynaptic terminal: impulse triggers release of neurotransmitter from synaptic vesicles
- b. Postsynaptic specialization: neurotransmitter receptors bind and open ion channels (in)



- c. Acetylcholine-binding opens channel for ~1 ms; extracellular Na^+ flows in through the AChR and depolarizes the post-synaptic membrane (less +charge outside)

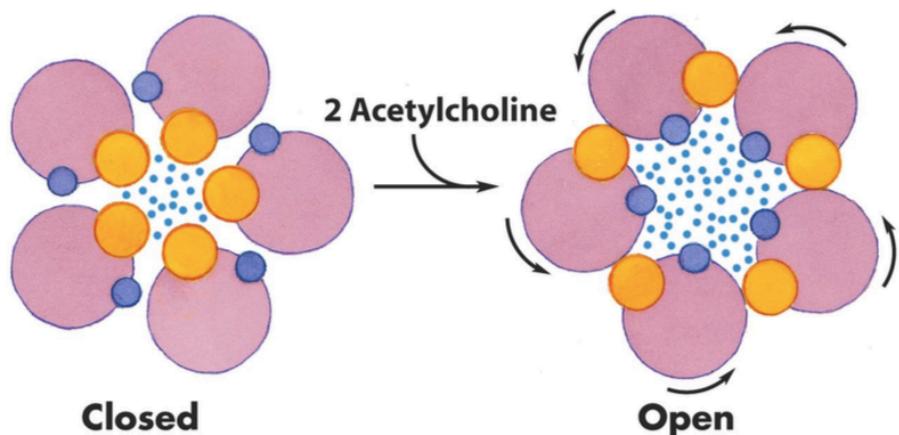


d. AChR is composed of 5 subunits ($\alpha_2\beta\gamma\delta$), each with 4 transmembrane helices (M1-M4)



e. Ligand-gated opening of the AChR

Bulky hydrophobic Leu side chains of M2 helices close the channel.	Binding of two acetylcholine molecules causes twisting of the M2 helices.	M2 helices now have smaller, polar residues lining the channel.
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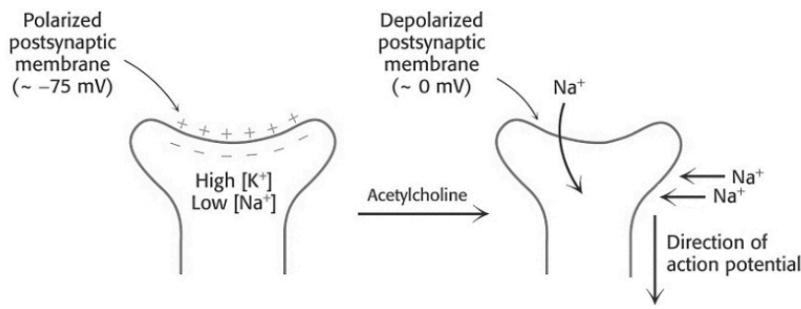
f. Source of AChRs for research

- i. The pacific electric ray has an organ full of presynaptic terminals.
- ii. ACh is released onto AChRs on modified muscle fibers to activate a huge electric shock.

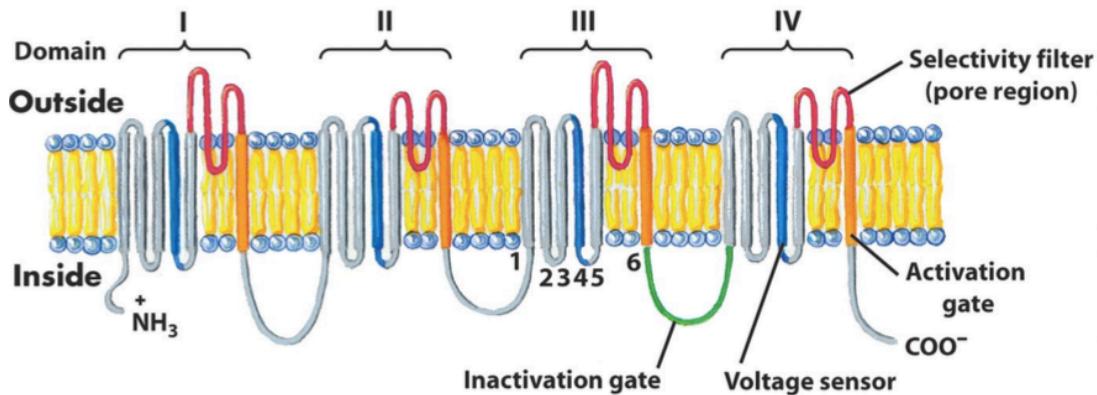
E. Part 2- Na^+ channels and propagation

a. Action potentials: spreading depolarization

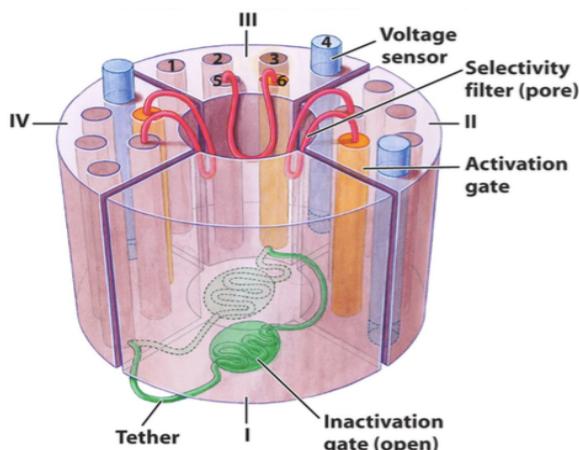
After AChR opening depolarizes the postsynaptic membrane, voltage-gated Na^+ channels at the postsynaptic membrane (and beyond) open to propagate depolarization.



- b. Japanese puffer fish: tetrodotoxin blocks Na^+ channels
- c. Voltage-gated sodium channels



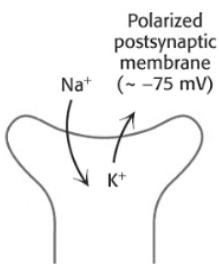
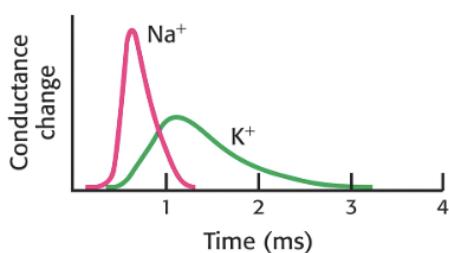
- i. 4 internal repeats of 6 transmembrane regions each.
- ii. Charged TM voltage sensor: during depolarization, there's less +charge outside, and the sensor moves closer to the extracellular part of the bilayer – conformational change.



- d. Voltage-gated channels open in concert to allow Na^+ influx spread membrane depolarization

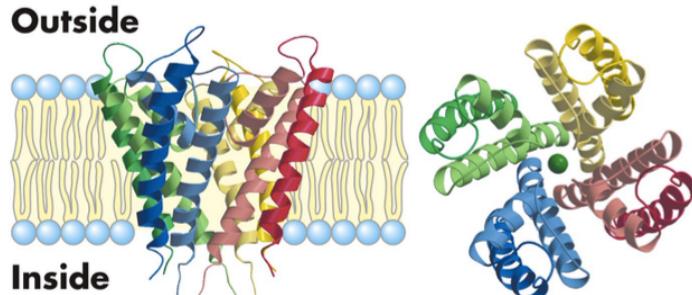
F. Part 3- K^+ channels and repolarization

- a. After voltage-gated Na^+ channels opened and propagated depolarization, voltage-gated K^+ channels open (with short delay) to reverse depolarization

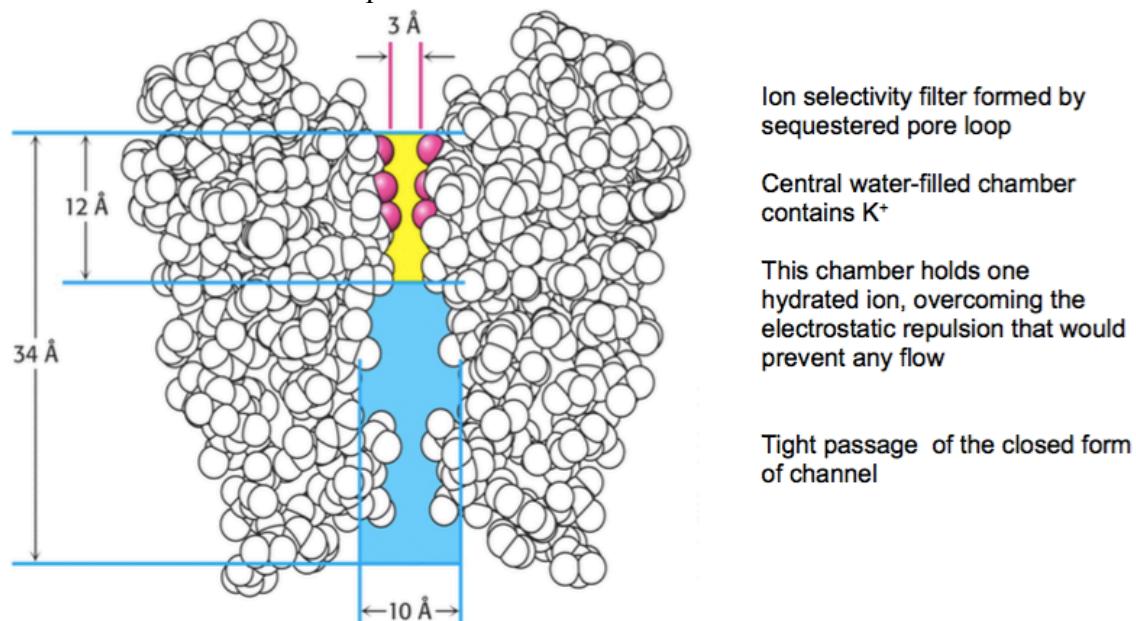


G. Voltage-dependent potassium channels

- Extreme specificity: K^+ over Na^+ > 1000-fold, despite similar atomic radii (1.33: 0.95 Å)
- Structure-function analysis most detailed for *Streptomyces lividans* K^+ channels; prototype
- Four identical subunits, 2 α -helical TM regions each. The channel is the tetramer center.

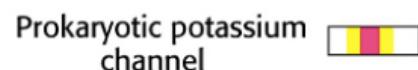
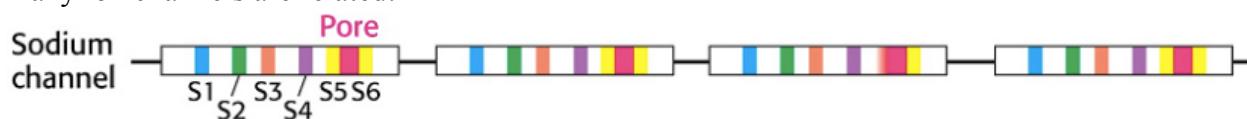


i. Inside view of a potassium channel



- Selectivity is reinforced by 4 layers of carbonyl oxygen atoms in a selectivity filter:
 - K^+ ions stabilized by backbone carbonyls (substituting for hydration)
 - These interactions are NOT like the solvating shell that would stabilize Na^+
- Conformational change during voltage-gated opening
 - Choke point by the intracellular membrane:
 - Closed conformation narrows the pore to ~3.5 Å
 - Open conformation: opens the pore to 12 Å.
 - The central chamber is confluent with cytosol, and the inner α -helix is bent.

H. Many ion channels are related:



- Potassium channels are homologues of sodium channels
- The prokaryotic potassium channel is a shortened homologue of the eukaryotic one.

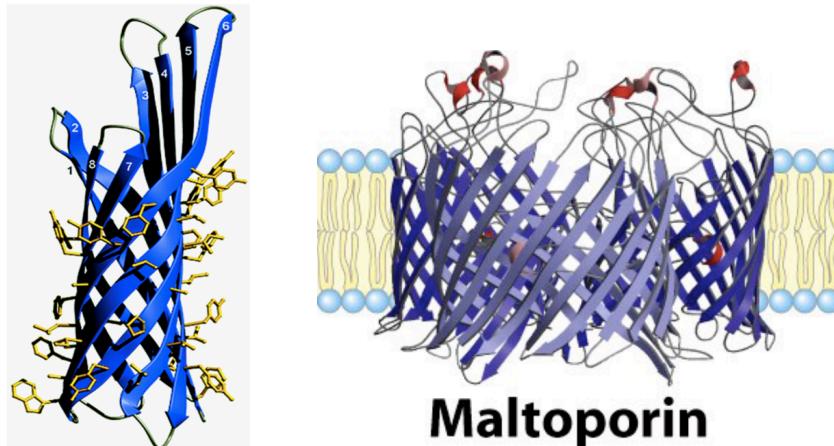
I. Transport across bacterial outer membranes

a. E. coli has two membranes:

- i. Inner membrane: only α -helical proteins
- ii. Outer membrane: α -helical and also β -barrel proteins.

J. β -barrel membrane proteins

- a. A closed barrel is created from antiparallel β -strands, forming a nonpolar ribbon.
- b. The surface contacting the lipid bilayer consists of aliphatic, nonpolar side-chains.
- c. Polar and nonpolar amino acids alternate (recall alternation of side-chains in sheets).



- d. Porins: β -barrel proteins that form selective hydrophilic transport channels.
 - i. Maltoporin: trimeric maltose transporter in bacterial outer membranes, based on 16-stranded antiparallel β -sheets forming a barrel.
 - ii. ONLY found in outer membrane of gram (-) bacteria, mitochondria, and chloroplasts.
 - iii. Polypeptide loops can protrude into the lumen.

K. Summary of Topics

- a. Amino acids and proteins partitioning and folding into membranes
- b. Membrane permeability and protein assistance in ion transport
- c. Depolarization and spread of membrane depolarization.
- d. Basis of ion selectivity
- e. Porin structure and function.

Lecture 13: Membranes III: Active Transport

- A. Active transport uses energy to transport against gradients.
- Ex: P-type ATPases, ABC transporters, bacteriorhodopsin.
 - Powered by ATP/chemical gradients/voltage/light/redox
- B. Thermodynamics of transport
- Permeable membranes: gradients are driven to equilibrium by chemical potential due to Δconc
 - Uncharged diffusion is like a chemical equilibrium ($A_{\text{out}} \rightleftharpoons A_{\text{in}}$)
 - Solute free energy varies with concentration
 - $G_A = G_A^0 + RT \ln[A]$ where G_A is the chemical potential (partial molar free energy) of A, and G_A^0 is its standard state.
 - Chemical potential: $\Delta G_A = G_A(\text{in}) - G_A(\text{out}) = RT \ln [A]_{\text{in}}/[A]_{\text{out}}$
 - For charged solutes, electrical potential provides an additional driving force.
 - Electrostatic potential: $\Delta\Psi = \Psi_{\text{in}} - \Psi_{\text{out}} = z_A F \Delta\Psi$
 - Electrochemical potential: $\Delta G_A = RT \ln [A]_{\text{in}}/[A]_{\text{out}} + z_A F \Delta\Psi$
 z_A is the charge on A, and F is Faraday's constant (charge of 1 mole e-, 96,500 C/mol)

$$\Delta\Psi = \frac{RT}{F} \ln \frac{\sum P_c[C(\text{out})] + \sum P_a[A(\text{in})]}{\sum P_c[C(\text{in})] + \sum P_a[A(\text{out})]}$$

Ion	Cell (mM)	Blood (mM)	Permeability Coef (cm s ⁻¹)
K ⁺	139	4	5×10^{-7}
Na ⁺	12	145	5×10^{-9}
Cl ⁻	4	116	1×10^{-8}
X ^{-a}	138	9	0

^aMacromolecules that are negatively charged under physiological conditions.

- d. Expected membrane potential at 25°C = -83 mV

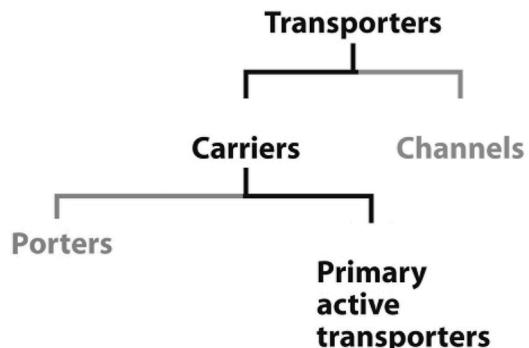
C. Classification of Transporters:

Carriers

- Saturable, bind substrate
- Series of conformational changes during transport
- Slow transport
- Often monomeric

Channels

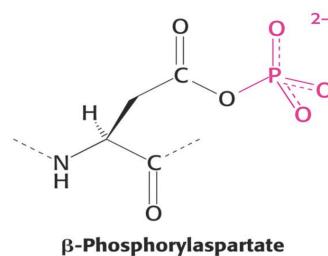
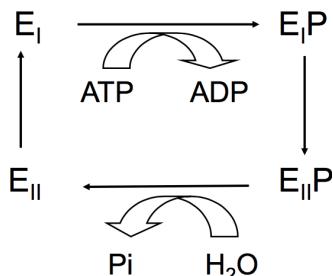
- Typically not saturable at biological concentrations
- Aqueous pore that opens and closes
- Very fast transport
- Often oligomeric



D. Carriers may be primary active transporters: drive transport by direct energy supply

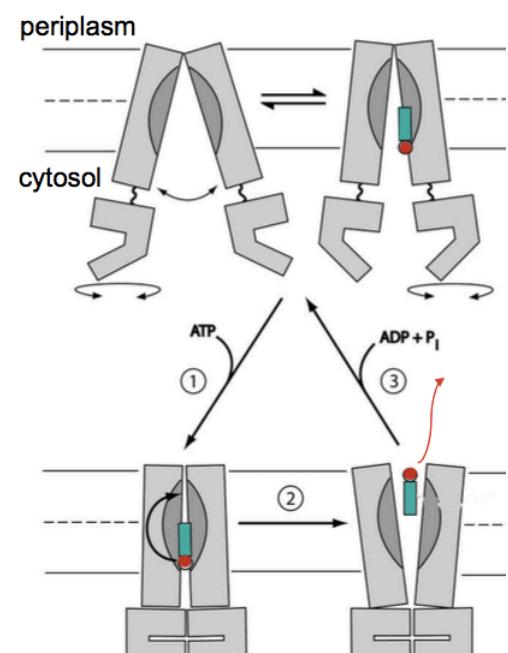
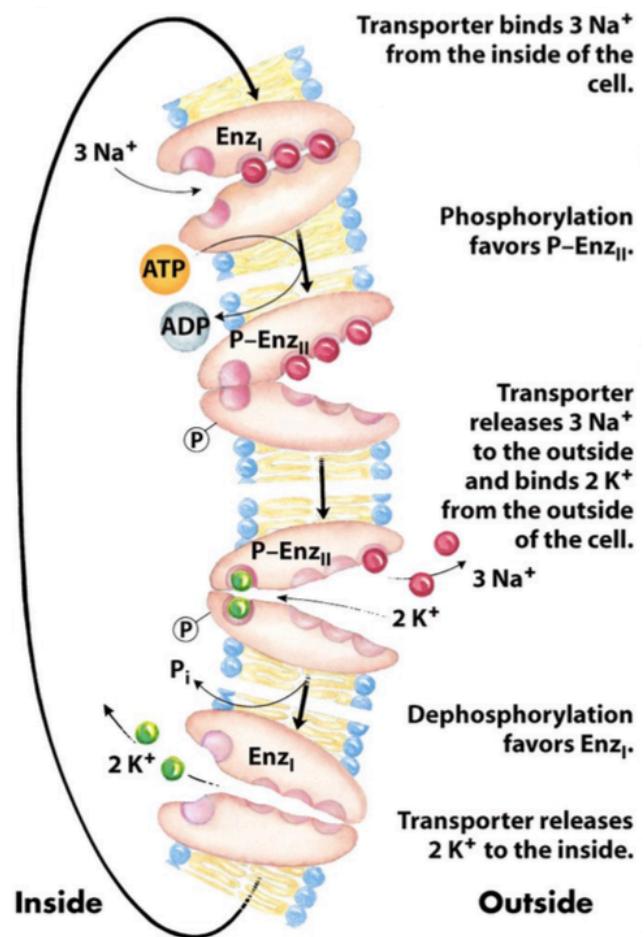
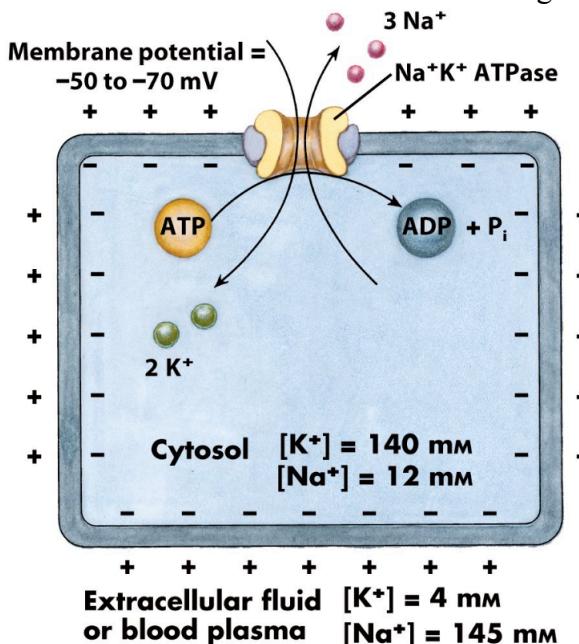
E. P-type ATPases: widely expressed primary active transporters

- Cycle between the E_I and E_{II} states via a phosphoenzyme intermediate
- P-type ATPases are phosphorylated at Asp in the conserved sequence DKTGT



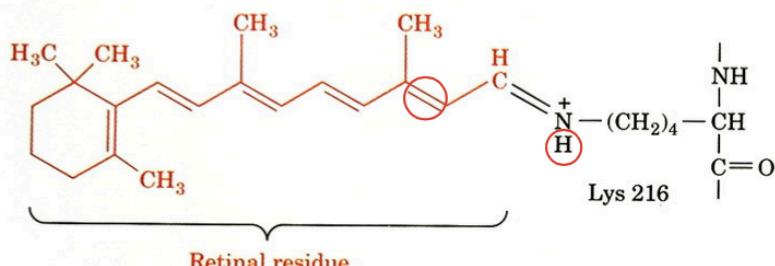
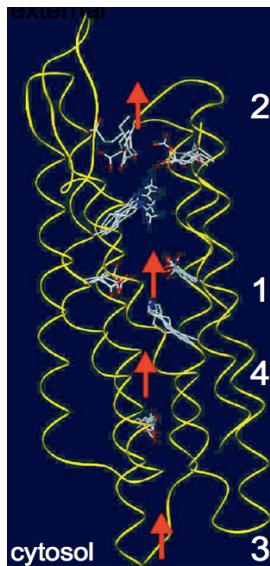
F. Sodium-potassium ATPase

- a. Electrogenic pump: 2 K⁺ in, 3 Na⁺ out; generates membrane potential.
 - b. Present in animals, plants, and bacteria; supports many membrane-bound processes.
 - c. Consumes ~1/3 of the ATP in a resting animal.



- f. Bacteriorhodopsin: light-driven proton pump (cytosol → extracellular space)
- One of the simplest active ion transporters; found in halophilic bacteria.
 - The H^+ concentration gradient fuels ATP synthesis and drives the flagellar motor.
 - Made of 7 TM helices connected by small loops.
 - Retinal (prosthetic group) is bound about halfway through membrane, as a Schiff base (imine) to lysine. Conjugated structure captures visible light photons to pump protons.
 - Amino group of lysine can attach an aldehyde to replace the O and form an imine.

all-trans-retinal



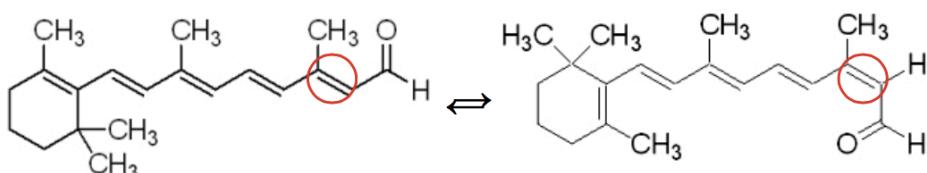
Mechanism:

- All-trans-retinal is photoisomerized to 13-cis-retinal (within 1 ms). This lowers the pKa of the Lys in the Schiff base, causing:
 - Proton transfer to an Asp
 - Opening the H^+ path
- The protonated Asp releases a proton to the outside.
- A proton is taken up from the cytosol
- Re-protonation of the Schiff base and isomerization back to all-trans-retinal.

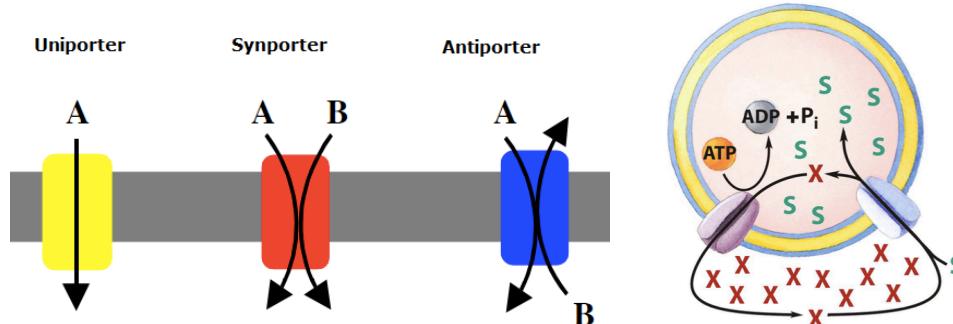
Note that other residues and H-bonds in the path allow proton hopping.

all-trans-retinal

13-cis-retinal



G. Porters

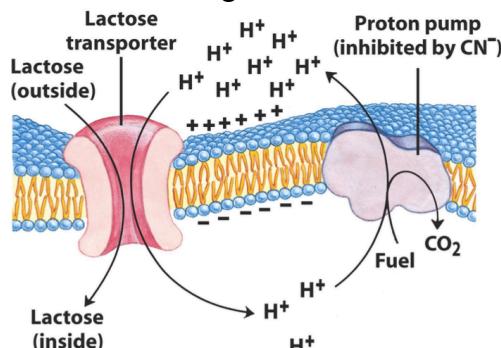


- Uniporters: one substrate, unidirectional, facilitated diffusion
- Cotransporters: symporters (same direction) and antiporters (different direction): Secondary active transport: couples favorable and unfavorable transport.

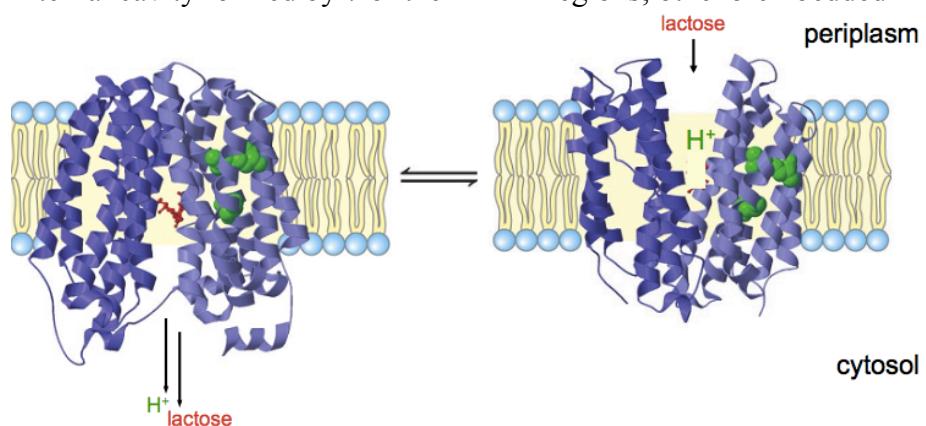
Organism/tissue/cell type	Transported solute (moving against its gradient)	Cotransported solute (moving down its gradient)	Type of transport
<i>E. coli</i>	Lactose	H ⁺	Symport
	Proline	H ⁺	Symport
	Dicarboxylic acids	H ⁺	Symport
Intestine, kidney (vertebrates)	Glucose	Na ⁺	Symport
	Amino acids	Na ⁺	Symport
Vertebrate cells (many types)	Ca ²⁺	Na ⁺	Antiport
Higher plants	K ⁺	H ⁺	Antiport
Fungi (<i>Neurospora</i>)	K ⁺	H ⁺	Antiport

c. Lactose permease

- i. Symporter inner membrane protein in *E. coli*; single polypeptide
- ii. Transports 1:1 proton + lactose into the cell. This converts the energy from an electrochemical gradient of H⁺ to a concentration gradient of lactose.



- iii. Add together the ΔGs for both [H⁺] and [lactose] to determine favorability.
- iv. Substrate binding triggers turnover, so no sugar = no H⁺ transport.
- v. Structure: 12 TM helices; N- and C-terminal 6 helices form 2 distinct bundles. Internal cavity formed by 7 of the 12 TM regions; other 5 embedded in the bilayer.



- d. Right: the ground-state permease is protonated (H⁺ shared between His and Glu), and this creates a high affinity for ligand (lactose) binding from the outside.
- e. Left: substrate binding induces a conformational change that transfers the H⁺ to Glu and reorients the substrate-binding site to the inner surface. This decreases affinity, releasing substrate and H⁺.

H. Summary

- a. The Nernst equation can describe and predict energy associated with movement across electrochemical gradients
- b. Properties, similarities and differences between types of transporters, carriers, and porters.
- c. Detailed knowledge on the Na-K pump and bacteriorhodopsin: how they create gradients.

Lecture 14: Carbohydrates

A. Carbohydrates/sugars: $(\text{CH}_2\text{O})_n$ or $\text{C}_n(\text{H}_2\text{O})_n$, where $n \geq 3$.

B. Roles

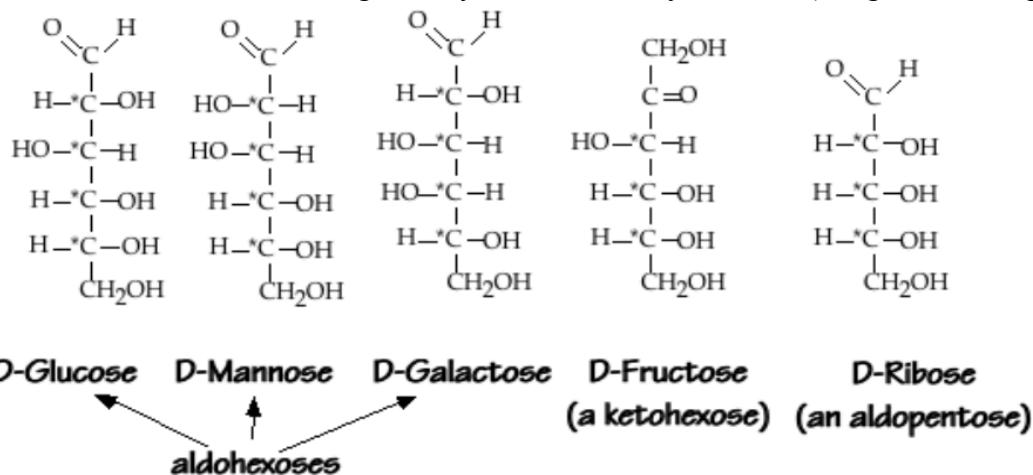
- *Oxidized for energy
- *Stored as food
- Components of bigger molecules
- Components of structures (e.g. wood)
- Attachments to proteins and lipids

C. Origins

- Plants “fix” CO_2 in the atmosphere to make sugars: $6 \text{ CO}_2 + 6 \text{ H}_2\text{O} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6 \text{ O}_2$
- Heterotrophs eat sugars or make them from smaller organic molecules
- Humans and animals have limited ability to make carbohydrates.

D. Types

- Sugars need a carbonyl and ≥ 2 OH groups (simplest is a triose).
Ketoses have a ketone group and aldoses have an aldehyde group.
- Monosaccharides: single monomeric unit.
- Oligosaccharides: a few linked monosaccharides; often linked to proteins (glycoproteins) or lipids (glycolipids)
- Polysaccharides: large linear polymers for structure (cellulose), storage (starch), etc.
- A few are abundant – this is generally an evolutionary accident (but glucose is especially stable)

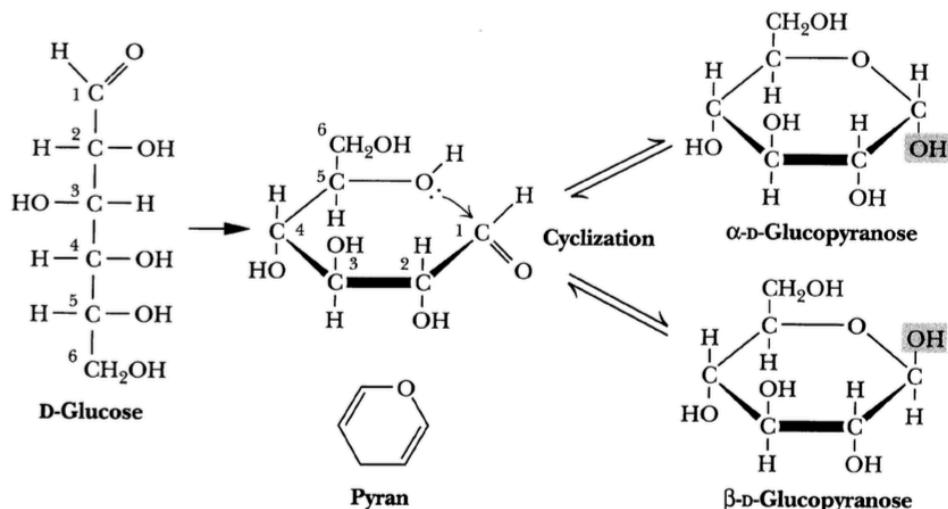


E. Stereochemistry

- Use Fischer projections: horizontal is toward you, vertical is away from you.
- N asymmetric carbons means 2^N stereoisomers
- L/D sugars: D-sugars have the same configuration at the chiral carbon farthest from the carbonyl.
This is generally R. D is the more common one.
- Carbon #X is numbered from the side closest to the carbonyl.

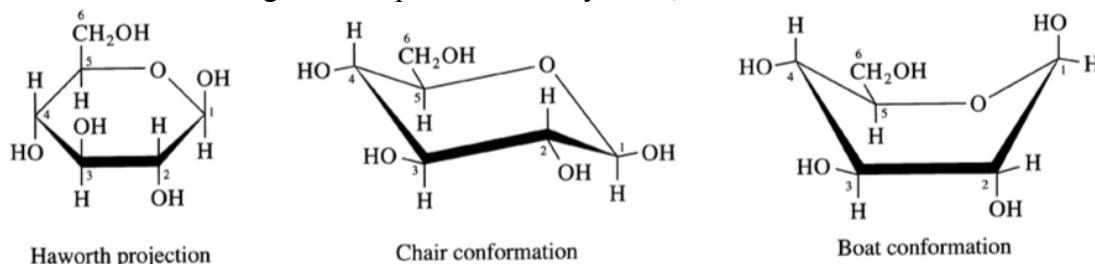
F. Monosaccharide cyclization

- Monosaccharides spontaneously cyclize and linearize.
- Anomeric carbon: the carbonyl carbon that gains chirality upon cyclization
- Stereoisomers differing at the anomeric carbons are the α - and β - anomers.
 - α -configuration is when the anomeric site has the $-OH$ goes DOWN.
 - β - configuration is when the anomeric site has the $-OH$ goes UP.
- Mutarotation: when anomers interconvert in solution.



G. Ring Conformations

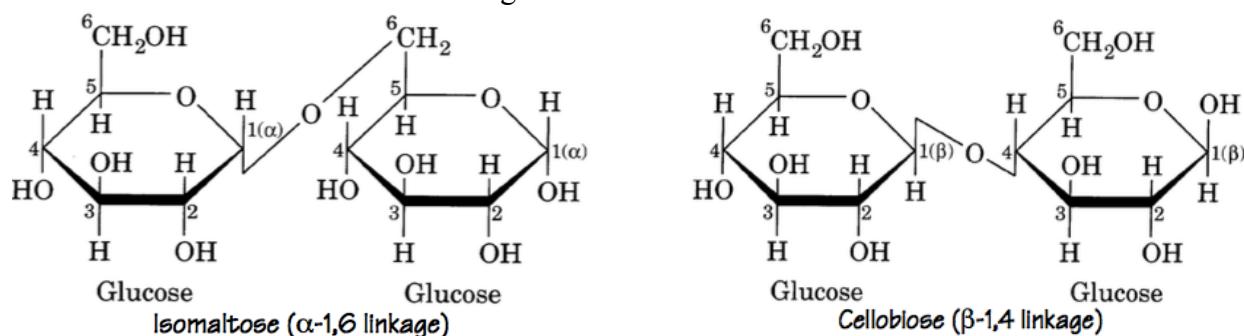
- Different $-OH$ can attack the carbonyl.
- 4- and 7- membered rings are unstable and are not formed.
- In equilibrium solution, open linear chains of 5-6 carbon sugars are disfavored ($<<1\%$).
- Monosaccharide rings are not planar – usually chair, but sometimes boat.



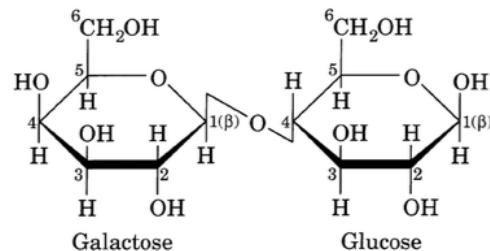
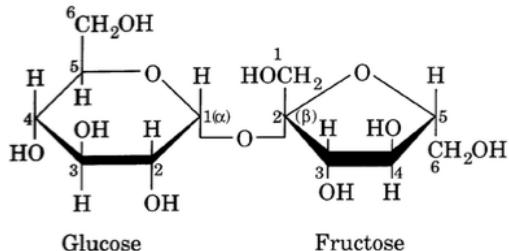
- Modified monosaccharides exist, e.g. in “complex” oligosaccharides on glycoproteins. This may involve amide groups or longer carbohydrate chains attached.

H. Oligosaccharide and Polysaccharide Chemical Structure

- Glycosidic bonds: the anomeric OH can condense with another OH to form $-OR$. Denoted by the numbers of the carbons and the configuration of the anomeric carbon. Prevents mutarotation.



- b. Glycosidic linkages are stable and can “trap” α - or β - isomers.
 - c. Oligo- and polysaccharides have polarity.
 - i. Reducing end: has a free reactive anomeric carbon (can be reduced by a chemical dye)
 - ii. Nonreducing end: lacks an anomeric carbon.
 - d. Disaccharides: a few abundant in biology: sucrose and lactose.



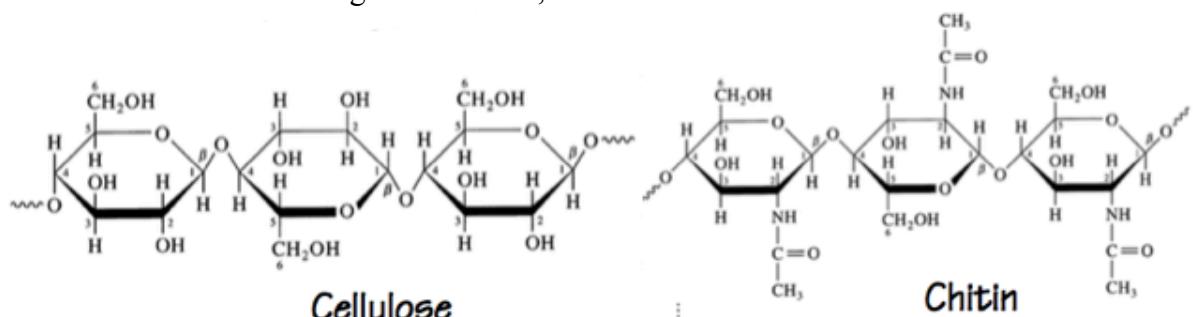
Sucrose

Lactose

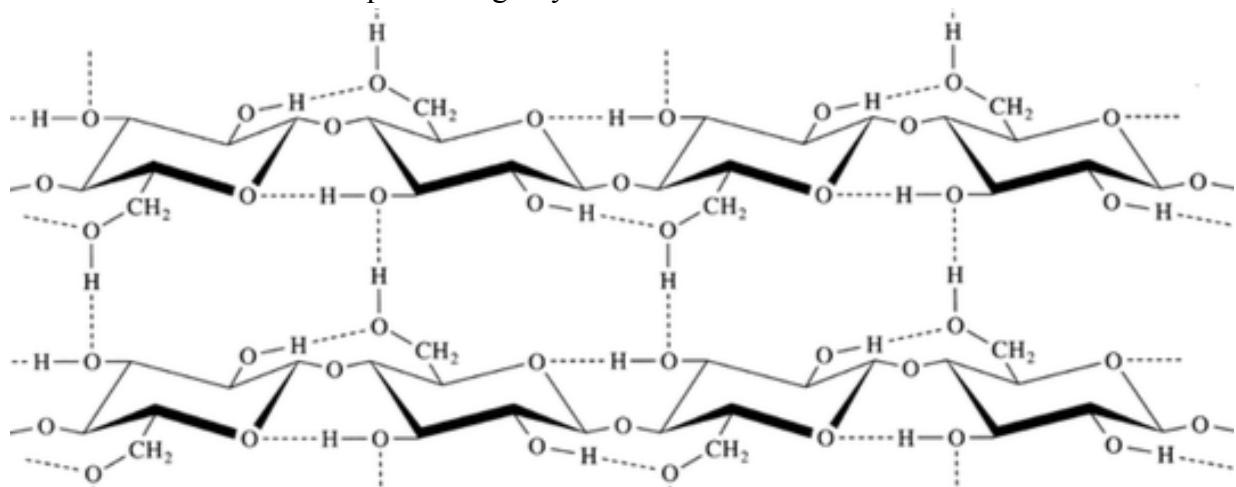
- i. Sucrose cannot be reduced because neither anomeric carbons is free (no reducing end)
 - e. Glycosidases hydrolyze glycosidic bonds: specific for particular linkages.
 - f. Lactose intolerance: most adults lack lactase.

I. Structural Polysaccharides

- a. Cellulose is made of β -glucose chains, and chitin is made of β -GluNAc chains.

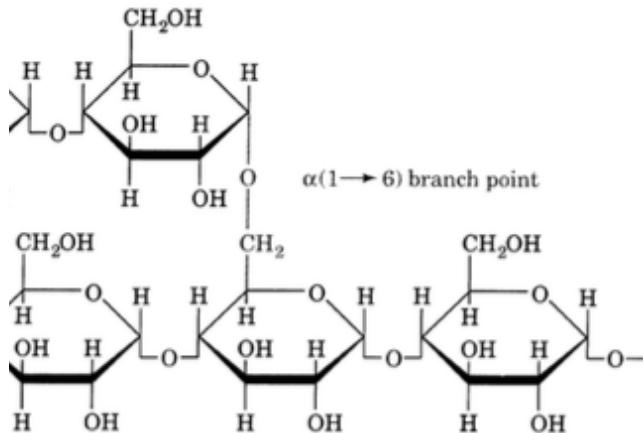


- b. Structural: H-bonds provide rigidity

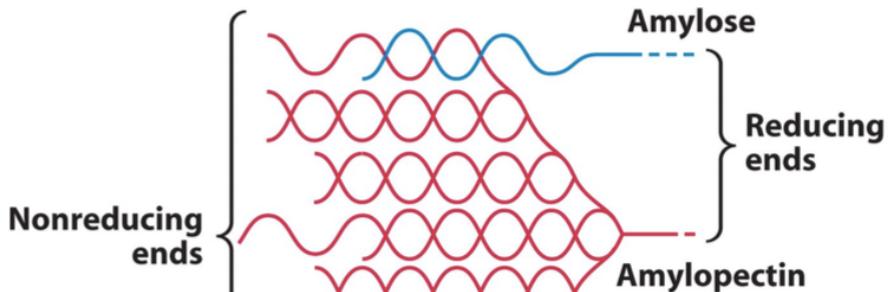


J. Storage Polysaccharides

- a. Plants use starch
 - i. amylose (α -1,4 linkages, floppy left-handed helix)
 - ii. amylopectin (amylose that branches at α -1,6 linkages every 30 units)
- b. Animals use glycogen: α -D-glucopyranose (branches every 10 units)



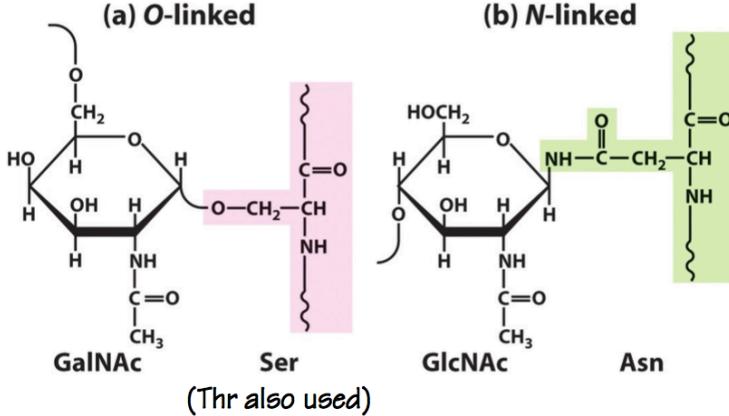
- c. Branching creates many nonreducing ends that can be used to quickly mobilize glucose.



- d. Storage as polymers rather than monosaccharides solves the osmotic problem
(osmotic pressure from high concentration of simple sugars)

K. Glycoconjugates

- a. Glycoproteins: sugars attached to peptide side chains by N-glycosidic or O-glycosidic bonds



- b. Found in eukaryotic cells; large proportion of extracellular proteins (not bacteria)
- c. Glycoprotein sugar structures are information-rich. 8 different mono-units + different linkages.
- d. Making glycoproteins: the polypeptide is synthesized, “sugar transferases” add sugars in the ER/Golgi. For N-linked sugars, a common “core” is added first, then added to.
- e. Glycolipids: sugars make glycosidic bonds to lipid OH groups on the outer plasma membrane.

- L. Blood group antigens are O-linked oligosaccharide glycoproteins.
 - a. Types A and B carry antigens that may stimulate an immune response.
 - b. Type O lacks either group, and is thus the universal donor.
 - c. Glycoproteins are cell markers that help things recognize and adhere to particular cells.
This includes viruses, bacteria, and leukocytes.
- M. Examples of cell-surface carbohydrates used for recognition
 - a. Fertilization (sperm-egg recognition and union)
 - b. Oligosaccharide-coated paths show neurons where to send/grow axons
 - c. Injured endothelial cells express p-selectin to attract leukocytes
^Oligosaccharides may be anti-inflammatory drugs by saturating p-selectin binding sites.
 - d. Influenza virus attaches by binding the saccharide sialic acid on the cell.
Viral neuraminidase removes sialic acid from the infected cell surface so that new virions can escape. Tamiflu (oseltamivir) competitively inhibits neuraminidase, but the virus mutates.