

Lecture 3:

Proteins

Aims:

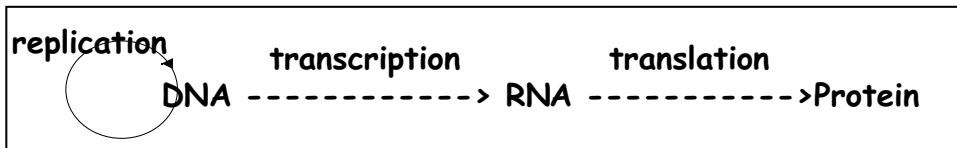
- Significance of proteins
- Protein Structure
- Different kinds of proteins - Function
- Protein purification and kinetics

Alberts *et al*, Chapter 5

Recap :

- Atom, element, molecules, macromolecules -----> cells -> tissues -> organs.....
- Four major families of small organic molecules - building blocks
 - sugar -> polysaccharides
 - fatty acids -> fats, lipids
 - nucleotides -> nucleic acid
 - amino acids -> proteins
- **Energy:** chemical reactions
- Helper molecules: enzymes, activated carriers
- Metabolic pathways are interconnected
 - catabolic/anabolic pathways -> metabolism

Central Dogma:



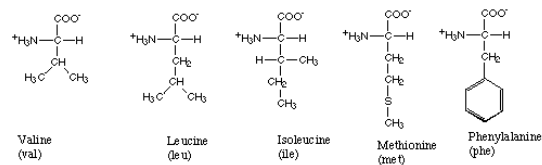
Proteins:

- Building blocks from which cells are built
- Execute nearly all cell functions
 - proteins with different functions
- 3D shape of protein -> functions performed by protein
 - > **Function follows structure**

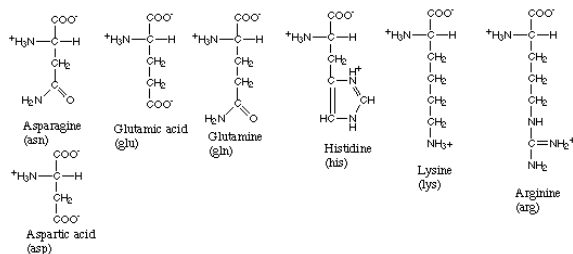
Historical Landmarks in The Understanding of Proteins

- 1838** Name "protein" suggested by Berzelius for the complex organic nitrogen-rich substance found in all plant & animal cells; "Protein": Greek for *proteios* = "primary"
- 1819-1904** Most of the 20 amino acids found in proteins were discovered
- 1864** Hoppe-Seyler crystallized & named hemoglobin
- 1894** Fischer proposed a lock-&-key analog for enzyme-substrate interactions
- 1897** Buchner & Buchner laid the foundation of enzymology by showing that cell-free-extracts of yeast can ferment sucrose to CO_2 and ethanol
- 1926** Sumner crystallized urease - demonstrating that proteins could possess the catalytic activity of enzymes.
Swedberg developed the analytical ultracentrifuge; estimated the correct MW of hemoglobin
- 1933** Tiselius introduced electrophoresis for separating proteins in solution
- 1934** Bernal & Crowfoot presented the first detailed x-ray diffraction patterns of a protein, obtained from crystals of the enzyme pepsin
- 1942** Martin & Syngde developed chromatography for protein separation
- 1951** Pauling & Corey proposed structure of a helical conformation of a chain of L-amino acids - the α helix - and β sheet
- 1955** Sanger completed the analysis of the aa sequence of insulin (first protein sequenced)
- 1956** Ingram produced fist protein fingerprints - a single aa diff in sickle-cell hemoglobin
- 1960** Kendrew described the first detailed structure of a prot. - sperm whale myoglobin - to resolution 0.2 nm; Perutz proposed a lower-resolution structure for hemoglobin
- 1963** Monod, Jacob & Changeux recognized that many enzs are regulated through allosteric changes in their conformation

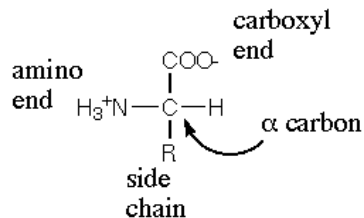
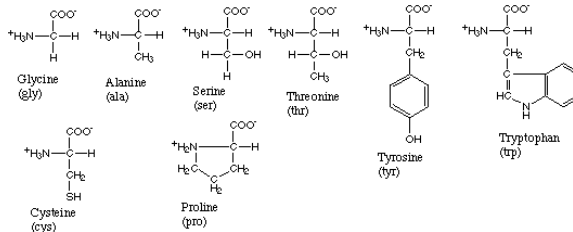
Amino acids with hydrophobic side groups



Amino acids with hydrophilic side groups



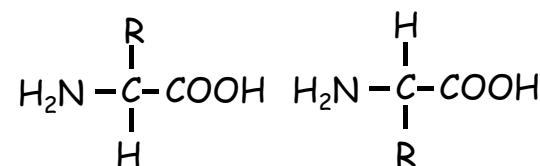
Amino acids that are in between



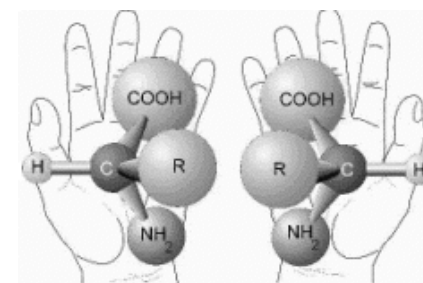
Optical Isomers

When you have a carbon with 4 different groups binding to it (a *chiral center*), you can have optical isomers.

If molecules were flat, these molecules would be the same.



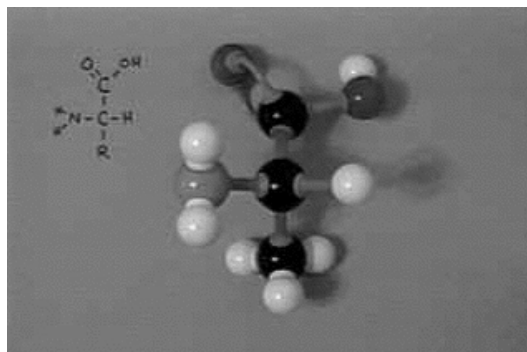
Molecules aren't flat and so molecules with the same formula can be different.



D and L Amino Acids

Most naturally occurring amino acids are L amino acids.

Chemically synthesized amino acids are a mixture of D and L forms.



L amino acids - amino on left side

Nobody knows why naturally occurring amino acids are L instead of D.

Amino Acid	Abbreviation	Side Chain	At neutral ph (7)
Alanine	Ala A	nonpolar	0
Glycine	Gly G	nonpolar	0
Valine	Val V	nonpolar	0
Leucine	Leu L	nonpolar	0
Isoleucine	Ile I	nonpolar	0
Proline	Pro P	nonpolar	0
Phenylalanine	Phe F	nonpolar	0
Methionine	Met M	nonpolar	0
Tryptophan	Trp W	nonpolar	0
Cysteine	Cys C	nonpolar	0
Asparagine	Asn N	uncharged polar	0
Glutamine	Gln Q	uncharged polar	0
Serine	Ser S	uncharged polar	0
Threonine	Thr T	uncharged polar	0
Tyrosine	Tyr Y	uncharged polar	0
Aspartic acid	Asp D	negative	-
Glutamic acid	Glu E	negative	-
Arginine	Arg R	positive	+
Lysine	Lys K	positive	+
Histidine	His H	positive	+

Protein Structure

Protein: subunit is amino acid
amino acid linked by peptide bond

20 amino acids each with different structure
& chemical properties

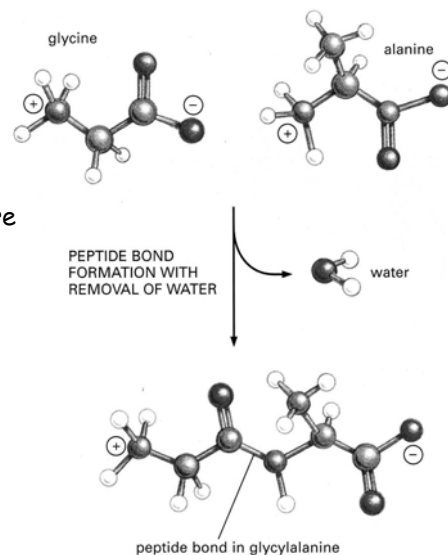
9 essential (we can't make) amino acids:

Thr, Met, Lys, Val, Leu,
Ile, His, Phe, Trp

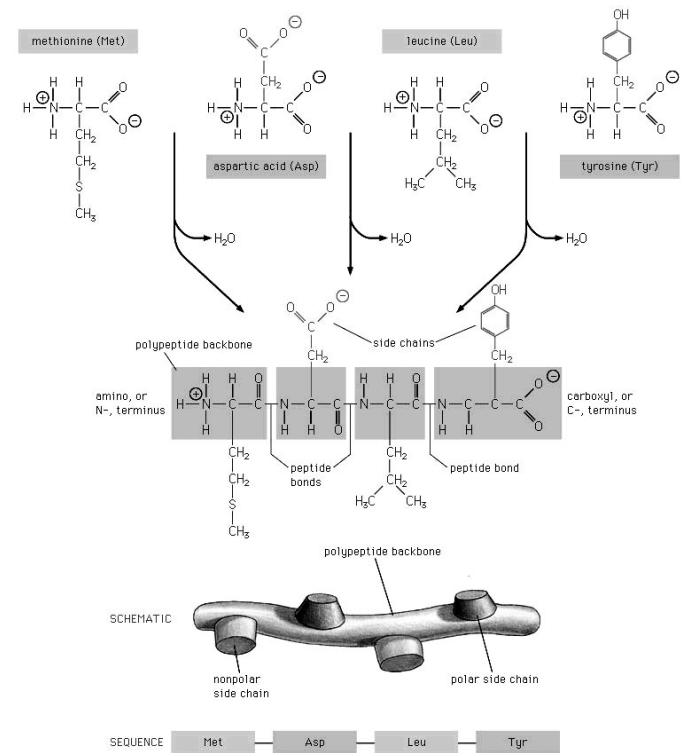
Peptide bond: a covalent bond

C atom from carboxyl group shares electrons with N from amino group
→ condensation reaction with loss of a molecule of water

Side chains: - different, give amino acid its unique property
- not involved in making peptide bond



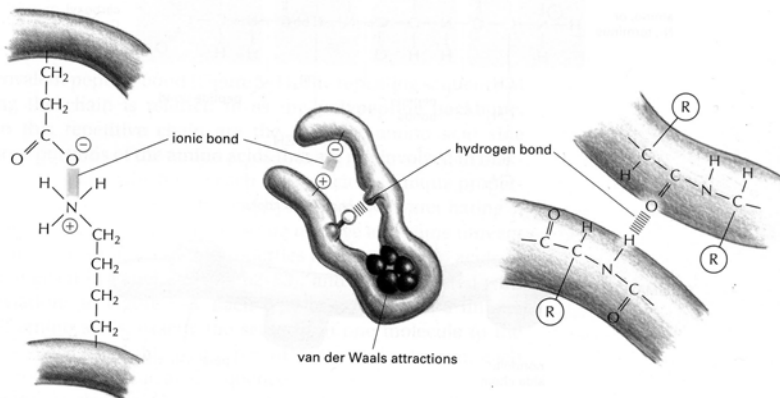
Polypeptide backbone



Protein Folding

- Covalent bonds in amino acid chain allows free rotation of atoms
- folding of polypeptide backbone
- Folding constrained by weak noncovalent bonds
formed by atoms in polypeptide backbone and atoms in amino acid side chains

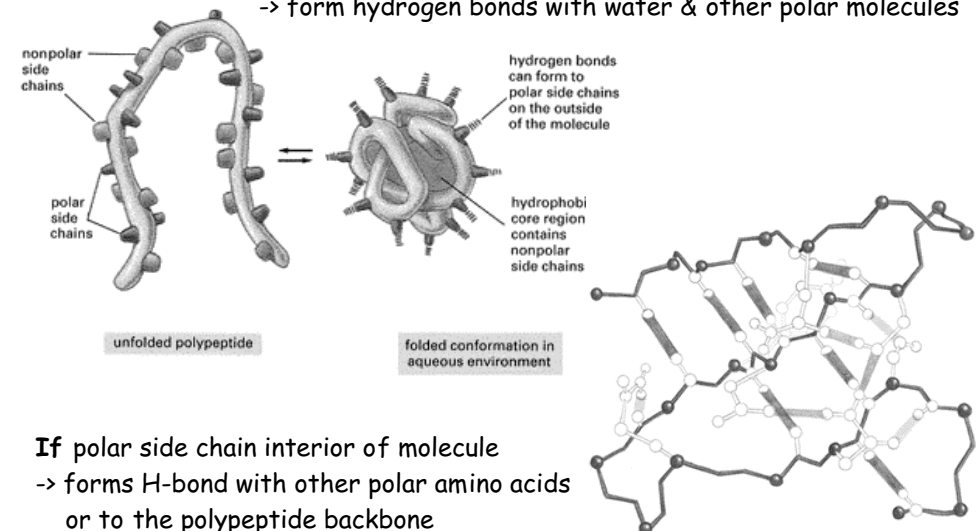
Stability = combination of large number of bonds holding region of polypeptide chain together



...Protein Folding

Side chains: properties polar vs nonpolar govern folding of a protein

- **nonpolar** side chains → **interior** of molecule
 - **polar** side chains **exterior** of molecule
- form hydrogen bonds with water & other polar molecules

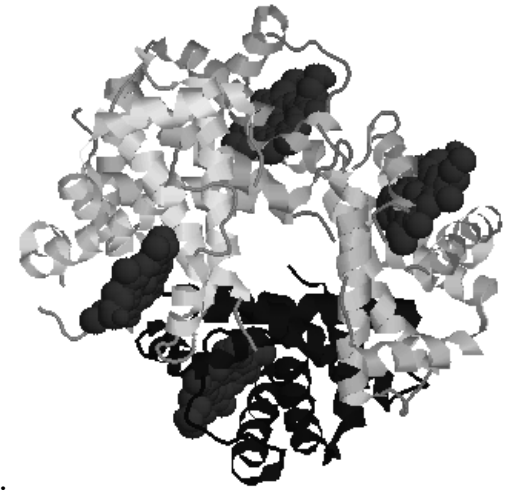
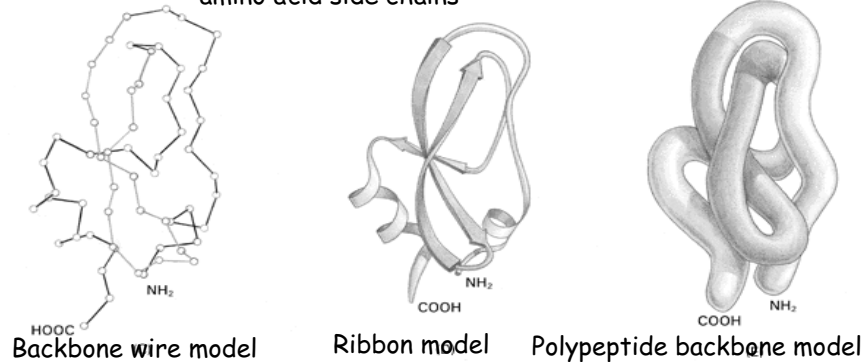
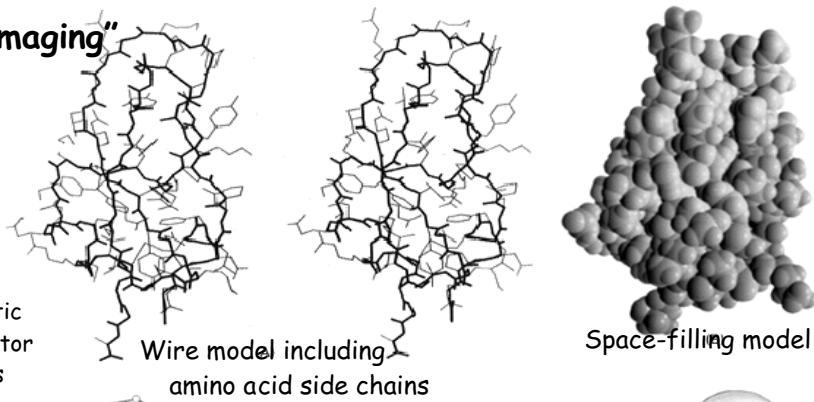


If polar side chain interior of molecule
→ forms H-bond with other polar amino acids
or to the polypeptide backbone

Experimental: x-ray, nuclear magnetic resonance (NMR)

Protein "Imaging"

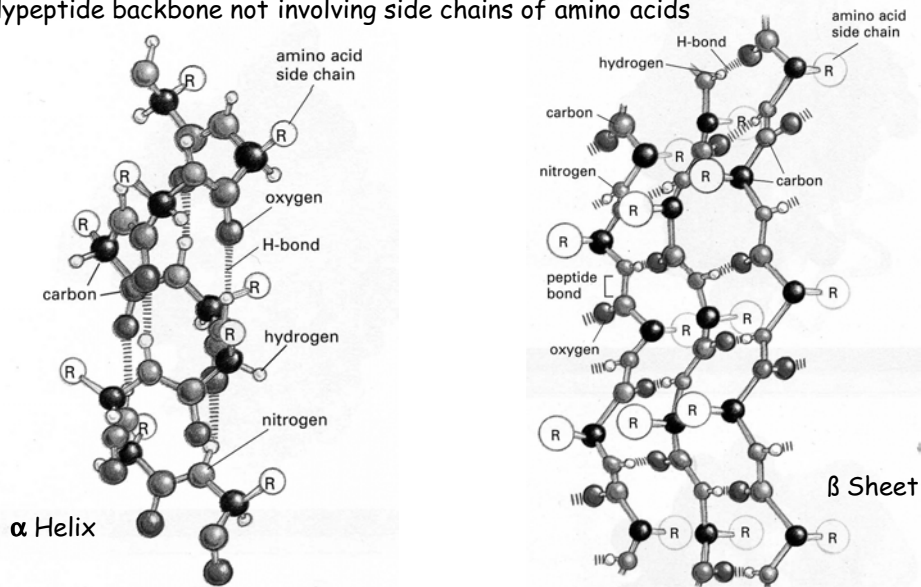
E.g. BPTI
Basic Pancreatic
Trypsin Inhibitor
88 amino acids
..... small !



Hemoglobin:
2 α globin + 2 β globin
4 heme molecules where O_2 binds

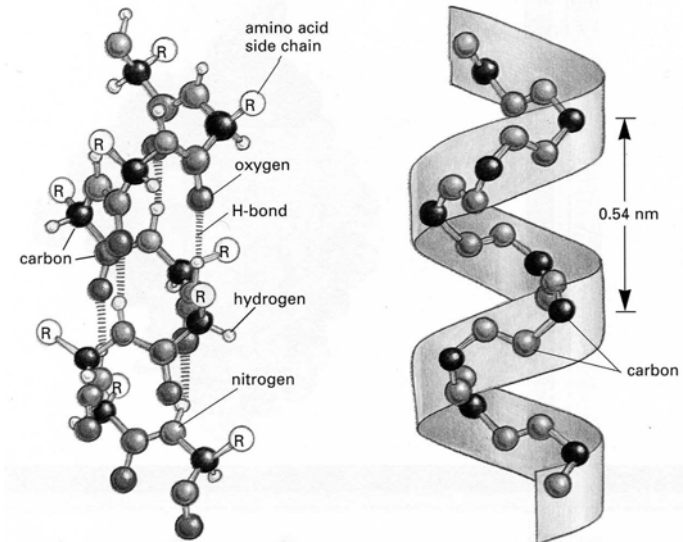
Protein Structural Motifs

- Two regular folding patterns: α Helix and β Sheet
- Stabilized by H bonds between amid (-NH) & carbonyl (-CO) group of polypeptide backbone not involving side chains of amino acids



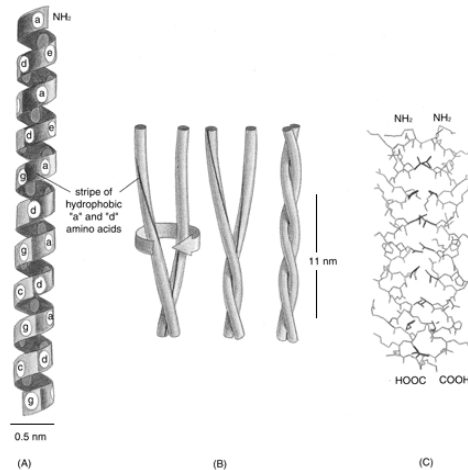
α Helix

- A single polypeptide chain turns around itself to make a rigid cylinder
- H-bond between every 4th peptide bond linking N-H to C=O of another peptide bond
 - Internal H bonding between internal backbone atoms
- > Helix with a complete turn every 3.6 amino acids



.... α Helix

- Found in proteins in cell membrane e.g receptors & transport protein
- Polypeptide backbone
 - hydrophilic,
 - H-bonded to itself
 - shielded from hydrophobic env. by nonpolar side chains



Coiled-coil:

- pair of helices wrapped around each other
- formed when most nonpolar side chains are on one side
 - long rodlike coiled-coils form structural framework for elongated proteins

E.g. α -keratin: intracellular fibers reinforcing outer layer of skin & its appendages
myosin molecules involved in muscle contraction

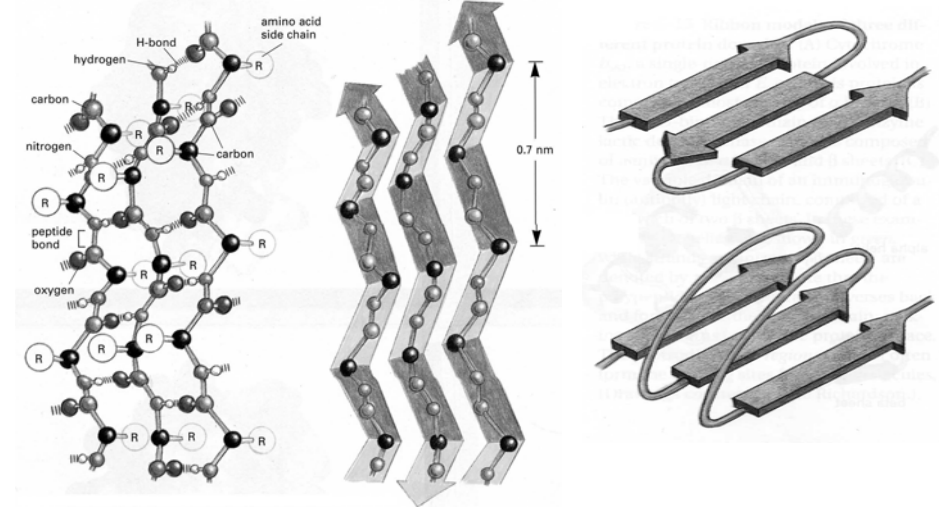
β Sheet

- Core of proteins are β sheets

parallel chains - neighboring polypeptide chains in same orientation

antiparallel chains - polypeptide chains folding back & forth upon itself, running in opposite direction

Both $\rightarrow$ rigid structure held together by H bonds



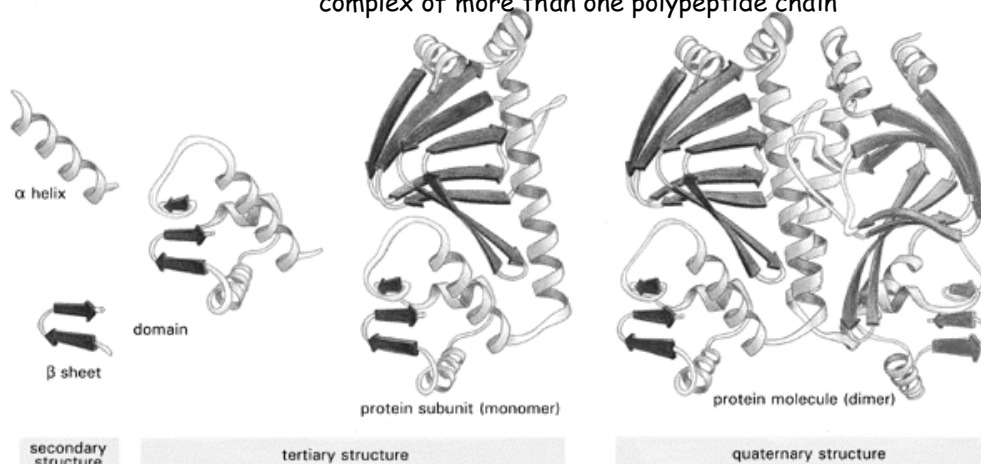
Protein Structure: Levels of organization

Primary structure: amino acid sequence

Secondary structure: α helices and β sheets

Tertiary structure: 3D conformation of polypeptide chain

Quaternary structure: structure of a protein molecule formed as a complex of more than one polypeptide chain



Protein domain:

- Compact and stable folded region of polypeptide
- Individual region (that can fold independently) of a larger structure
- Contains 50-350 amino acids
- The modular unit from which many larger proteins are constructed
- Different domains are often associated with different functions
- Domains usually connected by relatively unstructured lengths of polypeptide



Number of possible proteins:

20 amino acids

Polypeptide chains 4 aa in length

$\rightarrow 20 \times 20 \times 20 \times 20 = 20^4 = 160,000$ possible chains

20^n different possible polypeptide chains "n" aas long

E.g. protein length = 300 aas $\rightarrow 20^{300}$ different polypeptide chains

Unique stable 3D conformation $\rightarrow$ function

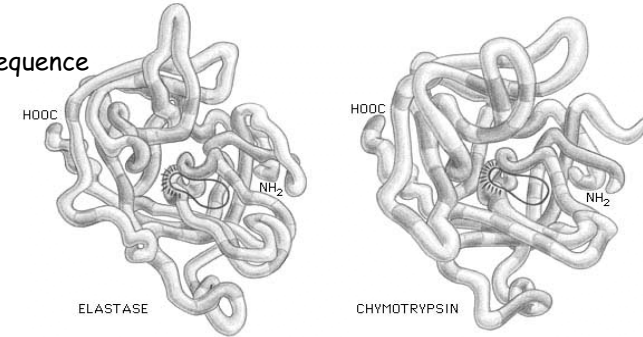
A single change in a few atoms in one aa can disrupt protein structure $\rightarrow$ loss of function

Protein Families

Alteration in structure $\rightarrow$ new function

Group proteins in families

Members have similar aa sequence
& 3D conformation



E.g. Serine proteases: family of proteins cleaving (proteolytic) enzymes

E.g. Digestive enzymes: chymotrypsin, trypsin and elastase

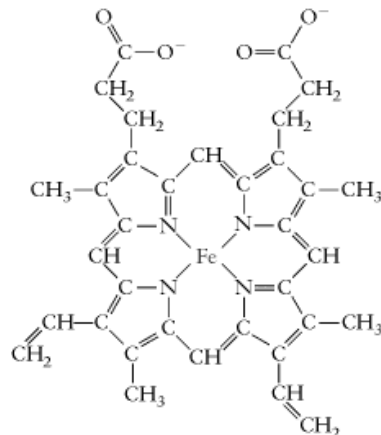
- Comparing these enzs $\rightarrow$ **portions of aa sequences are nearly the same**
 $\rightarrow$ similar 3D conformation
- **BUT various proteases have distinct enzymatic activities**
- cleave different prots or peptide bond between different types of aas
therefore each carries out a distinct function in an organism

Protein subunits

Proteins bind each other via weak noncovalent bonds to produce larger structures

Binding site: region of interaction

Each polypeptide chain $\rightarrow$ a protein subunit
resulting in dimers, tetramers



E.g heme groups of hemoglobin
are the O_2 binding component
Has iron atom encaged

Protein filaments, sheets, tubes, or spheres

Chain of identical protein molecules can form if binding site on a protein is complementary to another region of the surface of the same protein
 $\rightarrow$ produce extended protein filament
e.g. actin filament (helical structure)
important in cytoskeleton

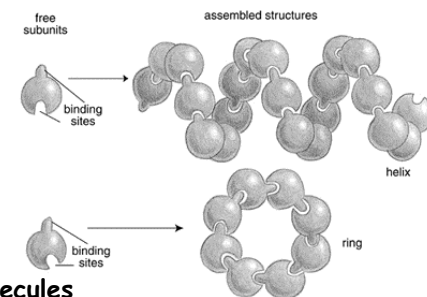
Spherical shells:

protein coats of virus particles

Structures of protein plus RNA or DNA molecules

e.g. viruses, ribosomes

- these structures can be dissociated into their constituent macromolecules



The structure of a cell is self-organizing:
if required proteins are produced, the appropriate structures will form

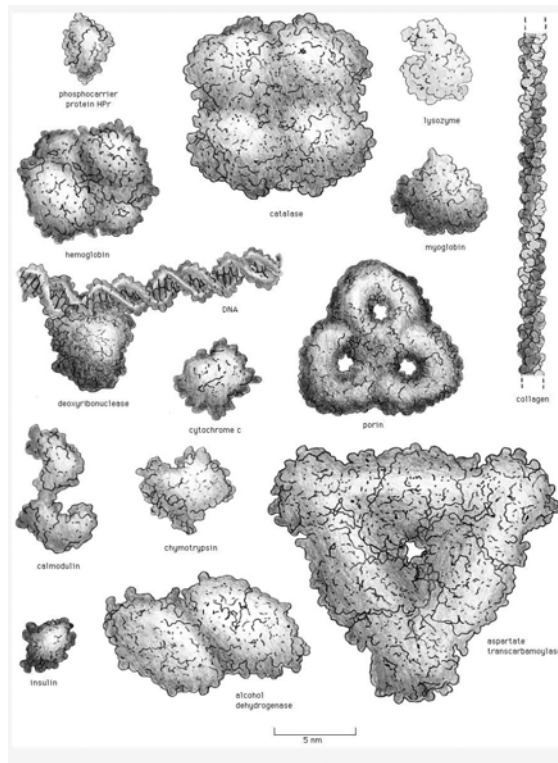
Range of Protein size & shape

Globular proteins:

- polypeptide chains fold up into compact shape like a ball
- irregular surface
- E.g. enzymes

Fibrous proteins:

- simple elongated 3D structure spanning a larger distance
- stable
- long-lived structures
- E.g. keratin: hair, nails, horns



Helix

- Subunits of either amino acids or protein molecules linked to **minimize the free energy of the contact** between them
- Rare for subunits to join up in a straight line → results in a helix
amino acids joined together termed α helix
protein subunits e.g. actin molecules → actin filaments
- **Helix generated by placing many similar subunits next to each other**
- **Twist of helix: right- or left-handed**
Handedness is the same if turn helix upside down
- reversed if helix is reflected in a mirror

Most helices are right handed

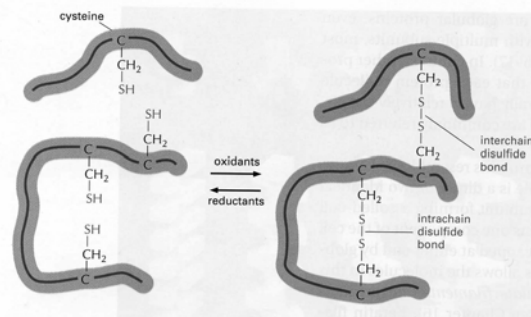


Extracellular proteins: attached to outside of cell's plasma membrane
or secreted as part of the extracellular matrix

- Help maintain cell structure

polypeptide chains may be stabilized by covalent cross-linkages

- between 2 aas in same protein
- or connect different polypeptide chains in a multisubunit protein
- common cross-links: disulphide bonds (S-S bonds)



Fibrous extracellular proteins

- form gel-like extracellular matrix
- helps collections of cells to bind together to form tissues
 - secreted by cells into surroundings, assemble into sheets or long fibrils
- E.g. collagen (3 long polypeptide chains), elastin.

Elastin: → enable skin, arteries, lungs etc to stretch and recoil without tearing

-> **Globular** proteins

-> **Fibrous** proteins

Protein conformation determines function

Proteins: Functions

Enzymes: accelerates a chemical reaction without itself undergoing a change

E.g. catalyses of covalent bond breakage or formation

pepsin degrades dietary proteins in the stomach;

DNA polymerase makes DNA;

proteins kinase adds a phosphate group to a protein

Structural Proteins: provide mechanical support to cells and tissues

E.g. Extracellular matrix: collagen & elastin form fibers in tendons and ligaments

Intracellular: tubulin forms long stiff microtubules & Actin forms actin filaments

-> support the plasma membrane

α -keratin forms fibers that reinforce epithelial cells, hair & horn prot.

Transporter Proteins: carry small molecules or ions

E.g. blood: serum albumin carries lipids; hemoglobin carries O_2

Transmembrane proteins: transport ions, small molecules across membrane

e.g. bacteriorhodopsin: a light-activated proton pump transport H^+ out of cell

Proteins: Functions

Receptor Proteins: detect signals & transmit them to the cell's response machinery

E.g. insulin receptor allows a liver cell to respond to insulin by taking up glucose
adrenergic receptor on heart muscle increases heartbeat rate when bound to adrenalin

Rhodopsin in the retina detects light

acetylcholine receptor in muscle cell membrane receives chemical signals released from a nerve ending

Gene Regulatory Proteins: bind to DNA to switch genes on or off

E.g. lactose repressor in bacteria prevents expression of enzyme proteins that degrade lactose

Special Purpose Proteins: proteins with highly specialized properties

E.g. antifreeze protein of Arctic & Antarctic fish protect blood from freezing

Green fluorescent protein (GFP) in jellyfish emits green light

Monellin found in African plant has sweet taste

Glue proteins secreted by mussels to attach to rocks

Proteins: Functions

Motor Proteins: function to generate movement in cells and tissues

E.g. myosin in skeletal muscle cells provides motive force for animals to move;

kinesin interacts with microtubules to move organelles in cell

dynein enables eucaryotic cilia and flagella to beat

Storage Proteins: store small molecules or ions

E.g. iron is stored in the liver by binding to protein ferritin;

ovalbumin in egg white is a source of amino acids for developing bird embryo;

casein in milk is a source of amino acids for baby animals

Signaling Proteins: carry signals from cell to cell.

E.g. hormones & growth factors that contribute to physiological function

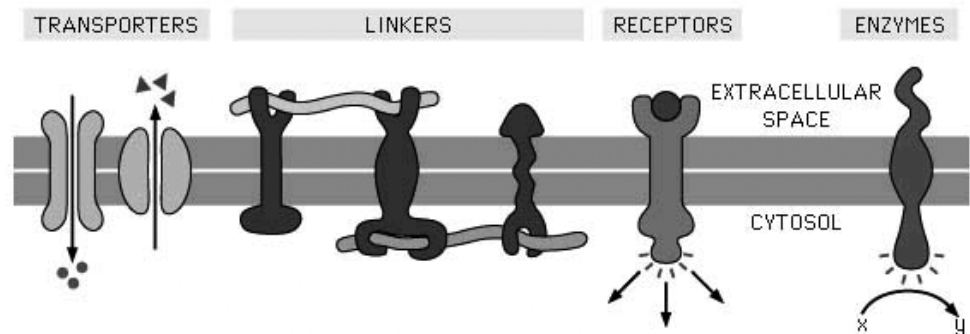
insulin (hormone) controls glucose levels in the blood

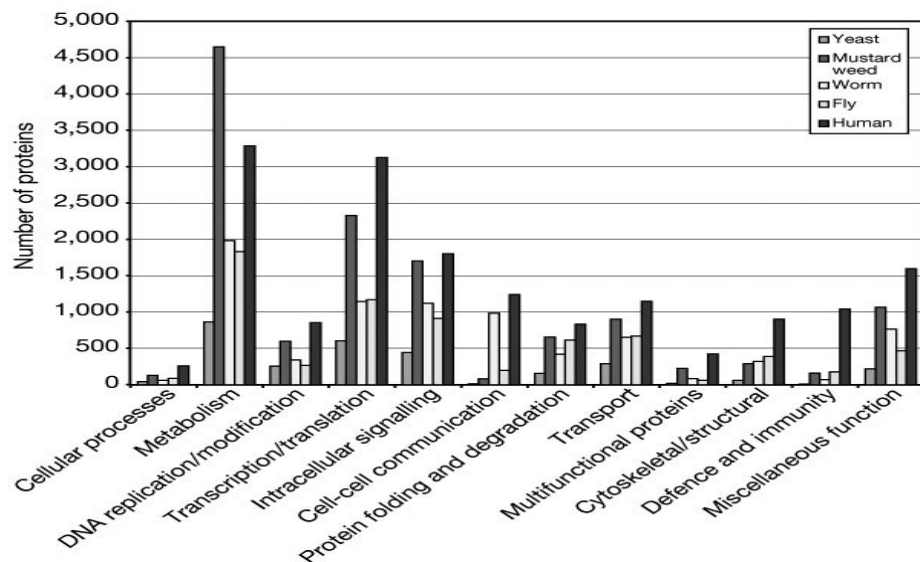
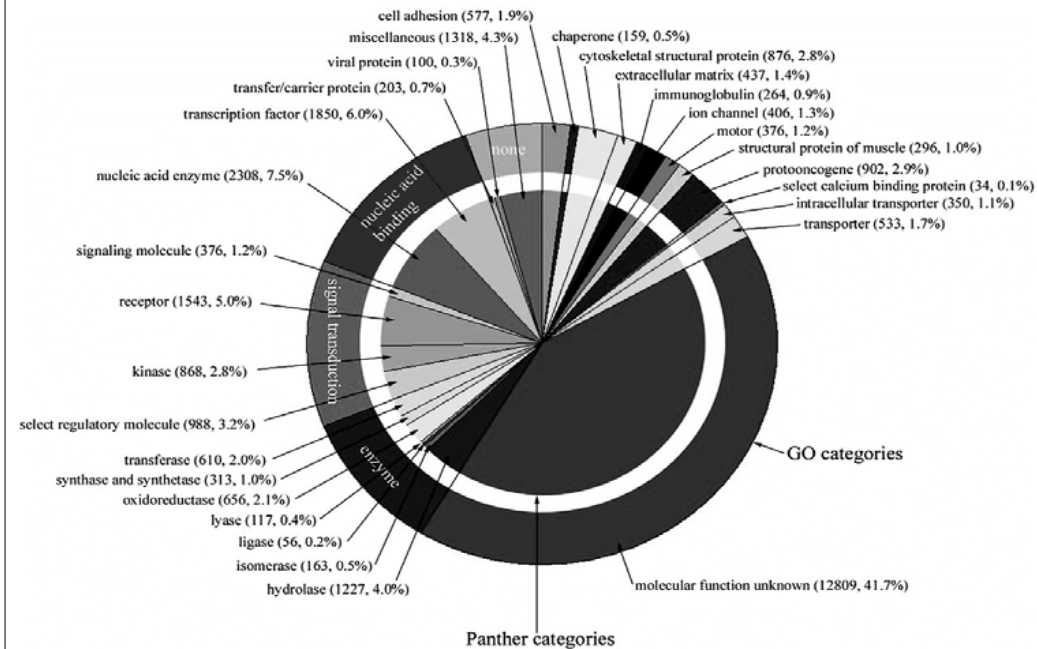
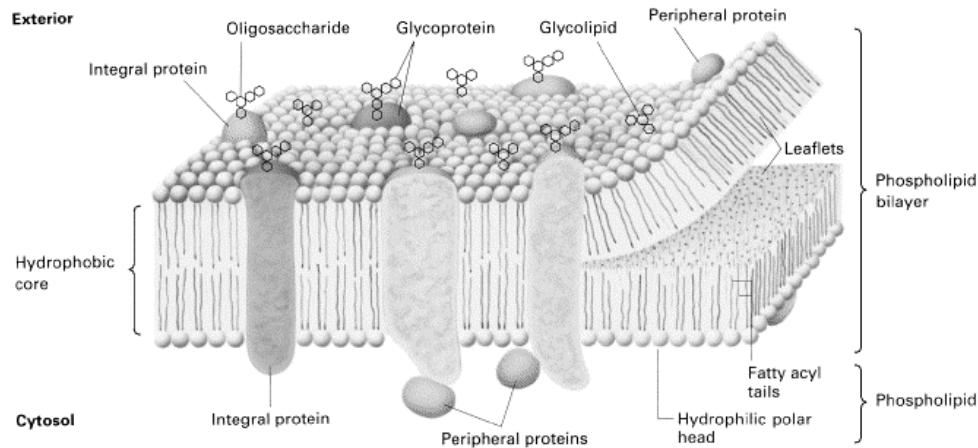
netrin attracts growing nerve cells in a specific direction in developing embryo

nerve growth factor (NGF) stimulates some types of nerve cells to grow axons

epidermal growth factor stimulates growth & division of epithelial cells

Membrane proteins and their functions





Summary re Protein structure.....

- Proteins are polymers of amino acids joined via peptide bonds
→ polypeptides
- The four levels of protein architecture are -
 - Primary structure → amino acid sequence
 - Secondary structure → α helix, β chain
 - Tertiary structure → polypeptide chain
 - Quaternary structure → assembled units
- The amino acid sequence determines the tertiary structure
- Protein folding is driven by the hydrophobic effect
 - nonpolar side chains → interior of complex
- Change the protein environment (from polar: water)
→ change force of protein folding
- Protein folding is mediated by weak noncovalent interactions:
ionic, H bonds, van der Waals, hydrophobic
- Different family of proteins - different functions

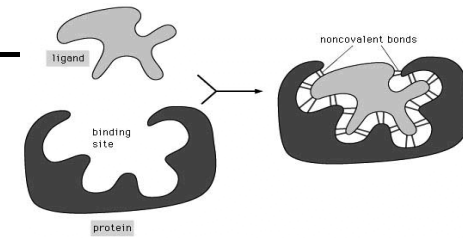
How Proteins Work

- How do proteins accomplish their functions?
 - how proteins bind to selected molecules
 - how their activity depends on such binding
- The ability to bind other molecules allows proteins to act as catalyst, signal receptors, & tiny motors

The binding of proteins to other molecules is specific: each protein molecule can bind just one or a few molecules out of the 1000s of different molecules it encounters.

Protein Ligands

Ligand: a substance bound by the protein
e.g. ion, small molecule, macromolecule
(Latin *ligare*, "to bind")



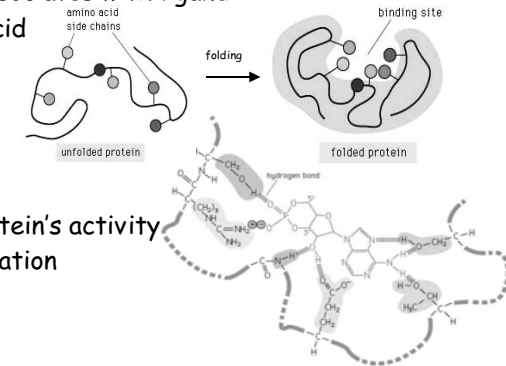
Ability of protein to bind selectively & with high affinity to a ligand is due to:

- the formation of a set of weak bonds thus need many weak bonds

Possible if surface of ligand fits to protein (hand in a glove)

Binding site: region of protein that associates with ligand

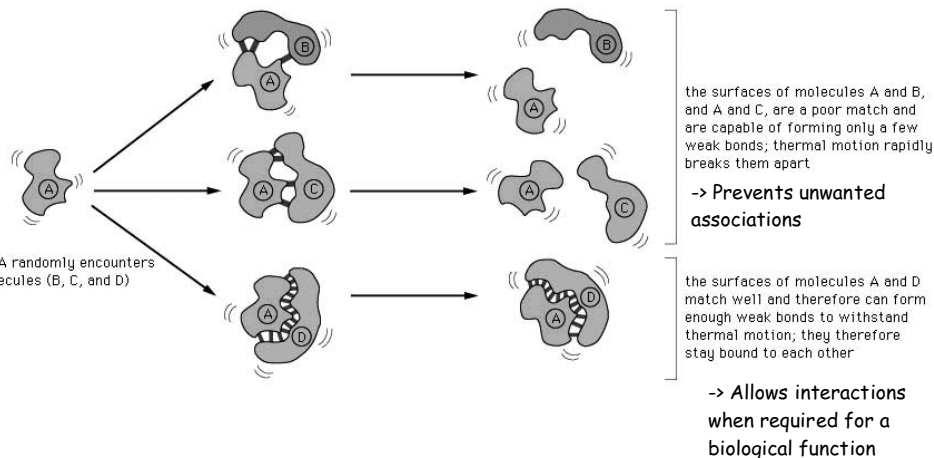
- usually cavity with particular amino acid arrangement formed by folding



- for different ligands that regulate protein's activity
- to place prot in a particular cellular location (a helix of a membrane-spanning prot)

Protein Interactions

- How noncovalent bonds mediate interactions between macromolecules:



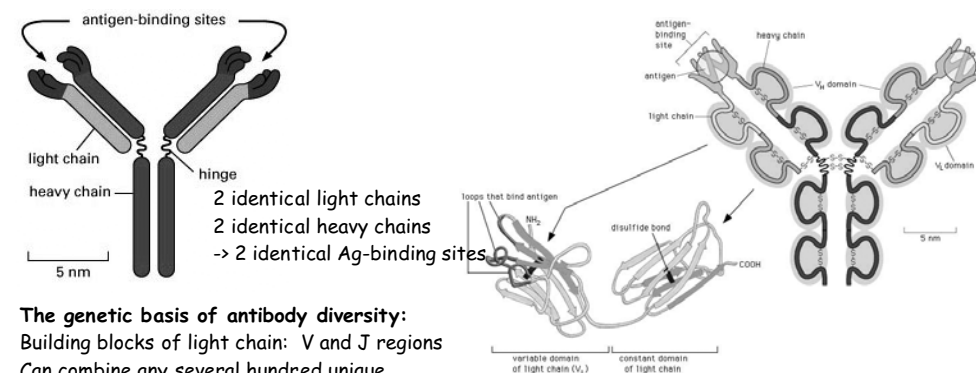
Proteins: Antibodies (Immunoglobulins)

Antibody:

Protein produced by B lymphocytes in response to foreign molecule or invading organism
Binds tightly to the foreign molecule or cell -> inactivating it or marking it for destruction

Antigen:

Molecule that is recognized by and binds specifically to an antibody molecule
It generates/provokes the immune response that produces the antibodies

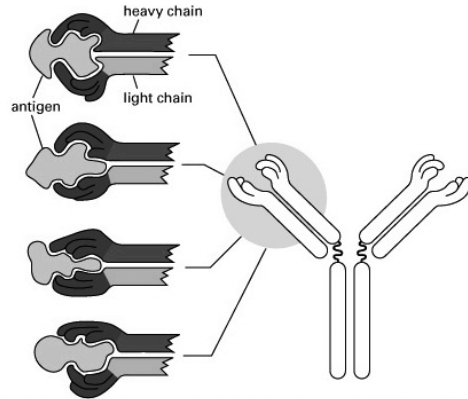


The genetic basis of antibody diversity:

Building blocks of light chain: V and J regions
Can combine any several hundred unique variable (V) segments with any four joiner (J) segments to form unique light chains

Antibody Specificity

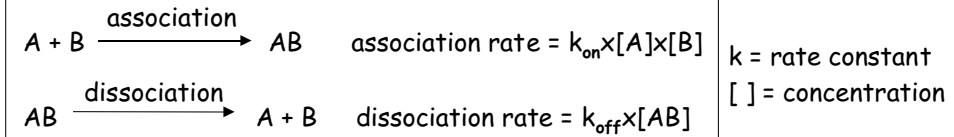
Ab recognizes its Ag with great specificity



Abs bind to their Ags with different strength:
association/dissociation/equilibrium constant

- Binding strength is measured by the **equilibrium constant**

Antibody binds to antigen -----time-----> dissociation



Equilibrium: state when number of binding (**association**) events per second is **equal** to the number of unbinding (**dissociation**) events

$$\text{at equilibrium: } k_{\text{on}} \times [A] \times [B] = k_{\text{off}} \times [AB]$$

Equilibrium constant (K) = the concentration of Ag, Ab, Ab-Ag at equilibrium
=> the ratio of the rate constants for association & dissociation
-> a measure of the strength of binding

$$\text{Equilibrium constant: } K = k_{\text{on}} / k_{\text{off}} = [AB] / [A][B]$$

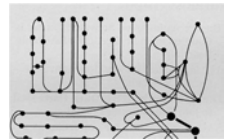
Increase in number of noncovalent bonds between Ab & Ag
-> increase in binding strength between Ab & Ag
-> larger K

-> reflects increase in free-energy difference between the bound and free states

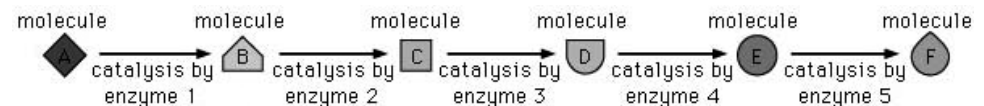
-> an effect of binding strength on the equilibrium state

Protein: Enzymes

Enzymes: proteins that bind to their ligand (**substrate**) and catalyze the breakage or formation of covalent bonds in these molecules
-> **products**



- Catalysts
- Speed
- No change to themselves
- Classes of enzymes: based on function; carry out similar reactions
- Highly specific: catalyzing only a single type of reaction
e.g. thrombin cuts blood protein between arg & gly
- Enzymes work in teams: product of one enzyme becoming substrate for the next
-> metabolic pathways providing cell with energy and generates molecules needed by cell



Common Types of Enzymes:

Enzyme	Reaction catalyzed
Hydrolases	hydrolytic cleavage
Nucleases	break down nucleic acids by hydrolyzing bonds between nucleotides
Proteases	break down proteins by hydrolyzing peptide bonds
Synthases	synthesize molecules by condensing smaller molecules together
Isomerases	rearrangement of bonds within a single molecule
Polymerases	polymerization e.g DNA, RNA synthesis
Kinases	addition of phosphate group to proteins
Phosphatases	hydrolytic removal of phosphate group
Oxido-reductases	reaction in which one molecule is oxidized, and other is reduced enzymes often called oxidases, reductases, dehydrogenases
ATPases	hydrolyze ATP e.g. motor proteins (e.g. myosin) & membrane transport proteins (e.g. Na/K pump)

Common name of enzyme usually indicates substrate and nature of reaction catalyzed
e.g hexokinase adds phosphate group to D-glucose

"-ase" typical ending of enzyme name

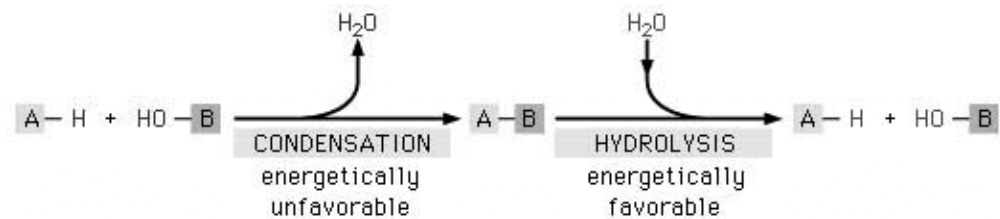
-> some exceptions e.g pepsin, trypsin, thrombin, lysozyme, etc

Note:

For a molecule to undergo any reactions it must overcome an activation energy barrier characteristic for that reaction.

-> May be achieved by putting more energy into the system such as heat.

-> But enzyme reduces the activation energy (input of energy) barrier of only one specific reaction path. Therefore an enzyme allows reactions to proceed at normal temps and directs them into one desired pathway.

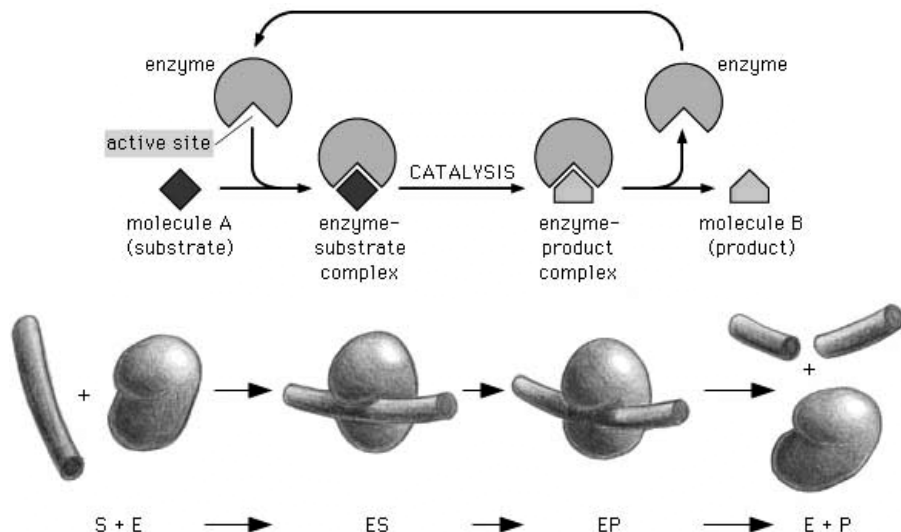


*How can condensation happen in a cell environment that is mostly water?
Nonpolar interior of proteins!*

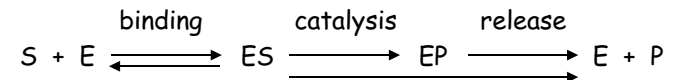
Example of how an enzyme works:

Active site of enzyme: fits the contours of its substrate molecule;
reaction site where the reaction occurs.

Reaction: at least two reactants



Enzymatic Rates:



Increase $[S] \rightarrow$ increase $[ES] \rightarrow$ rate of P in a linear relation

But very high $[S]$ all active sites of E are occupied by S
and rate of P formation depends on how quickly S can be processed

Turnover number = number of S molecules that can be processed per second

$V_{\max}$ = maximum rate of enzymatic reaction

attained after $[S]$ has reached maximum value (saturating levels)

K_M = $[S]$ at which enz. works at half its speed ($0.5 V_{\max}$)

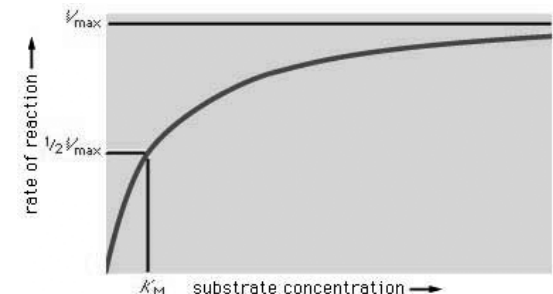
Michaelis-Menten equation:

$$\text{Rate } (V_0) = (V_{\max} [S]) / ([S] + K_M)$$

$$V_{\max} = k_{\text{cat}}[E_t]$$

Low K_M means S & E bind tightly

High K_M means weak binding



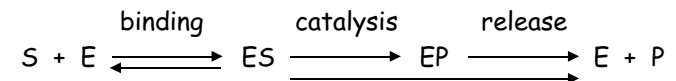
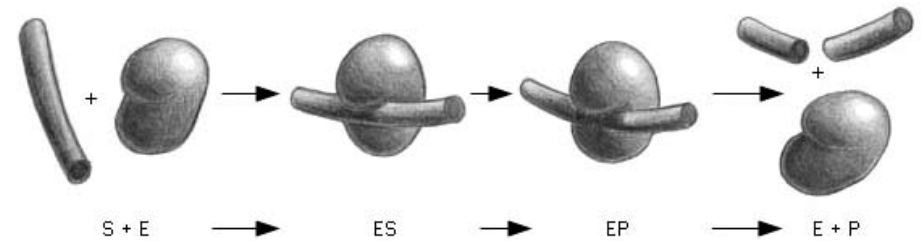
Enzyme rates: K_M values for some enzymes

Enzyme	K_M
Chymotrypsin	$5 \times 10^{-3} \text{ M}$
Lysozyme	$6 \times 10^{-6} \text{ M}$
β -Galactosidase	$4 \times 10^{-3} \text{ M}$
Threonine deaminase	$5 \times 10^{-3} \text{ M}$
Carbonic anhydrase	$8 \times 10^{-3} \text{ M}$
Penicillinase	$5 \times 10^{-5} \text{ M}$
Pyruvate carboxylase	$4 \times 10^{-4} \text{ M}$

Carbonic anhydrase

rate enhancement: 10,000,000

Turnover number = 400,000 kcat/sec



Enzyme regulation

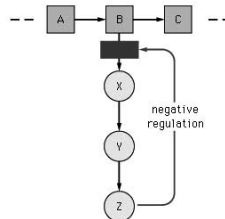
The catalytic activities of enzymes are regulated

- genetic level: expression of gene encoding enzyme
- subcellular compartmentalization of enzyme
- binding of molecule (other than substrate) to regulatory site of enzyme (outside of active site) -> alters rate S converted to P

Feedback inhibition: late product (P) inhibits enzyme early in reaction pathway

-> P controls its own level in the cell

----> **negative regulation:** prevents enzyme from acting



Positive regulation: enzyme's activity is stimulated

by a regulatory molecule

-> P in one pathway stimulates E in another pathway

Enzymes have two different binding sites:
for substrate & regulatory molecule

-> **Allosteric enzymes:** 2 or more conformations -> 2 binding sites

(Greek allo, "other" & stere, "solid" or "shape")

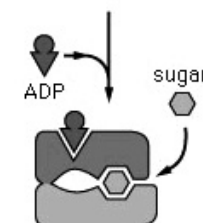
- communication/interaction via conformational change in enzyme

Other allosteric proteins: receptors, structural proteins, motor proteins

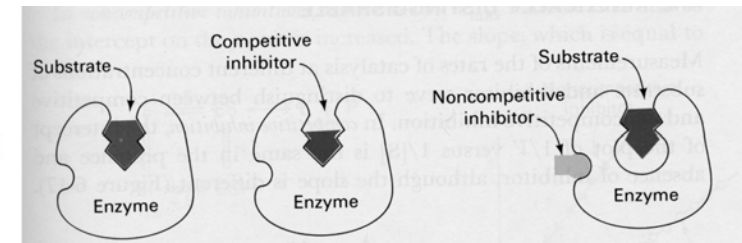
INACTIVE



E.g. oxidation of sugar molecule



ACTIVE



Induced Fit



Noncompetitive Inhibition

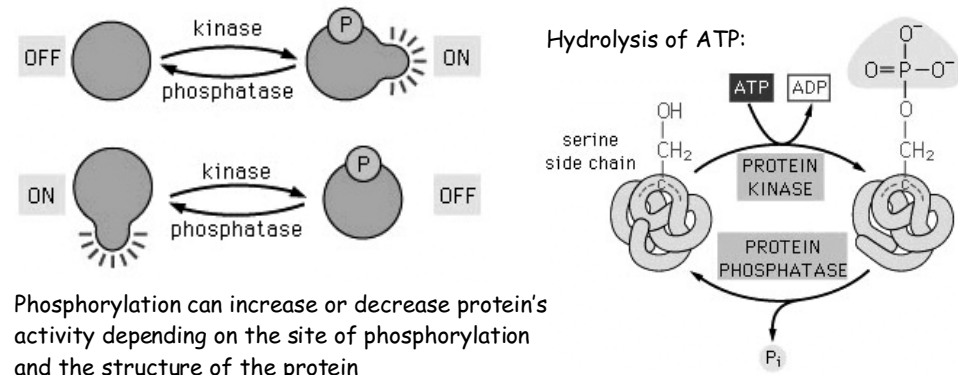


Competitive Inhibition



Protein Phosphorylation:

- Can drive a conformational change
 - because phosphate group carries two negative charges
 - reversible
 - involves ATP: addition of P group from ATP to -OH group on ser, thr or tyr
- **Catalyzed** by a protein **kinase**
- **Dephosphorylation** catalyzed by a protein **phosphatase**
- Different kinases & phosphatases for different sets of proteins



Phosphorylation can increase or decrease protein's activity depending on the site of phosphorylation and the structure of the protein

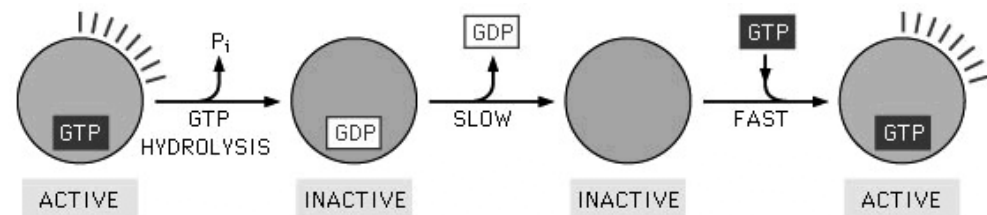
Example of phosphorylation:

glycogen phosphorylase:

- catalyzes breakdown of glycogen
 - a storage carbohydrate
 - releases glucose monomers
- has two forms distinguished by phosphorylation
 - phosphorylase a - is more active (+P)
 - phosphorylase b - is less active (-P)
- different hormones trigger phosphorylation (Pn) of enzyme
 - vigorous muscle activity → epinephrine → Pn in muscle
 - blood glucose too low → glucagon → Pn in liver

GTP binding proteins undergo conformational change

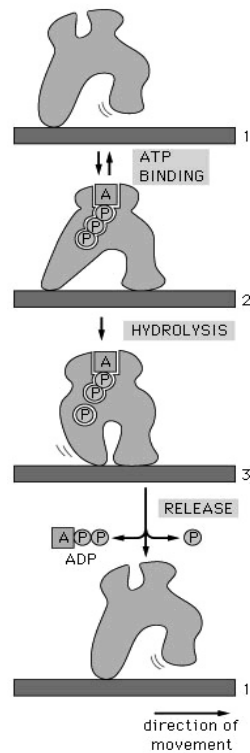
- binding a guanine nucleotide either guanosine triphosphate (GTP) or GDP
- also control enzymatic activity or involved in intracellular signaling



Motor Proteins

Motor proteins produce large movements in cells
ATP motor, Actin-myosin, Flagellum

Protein machine...



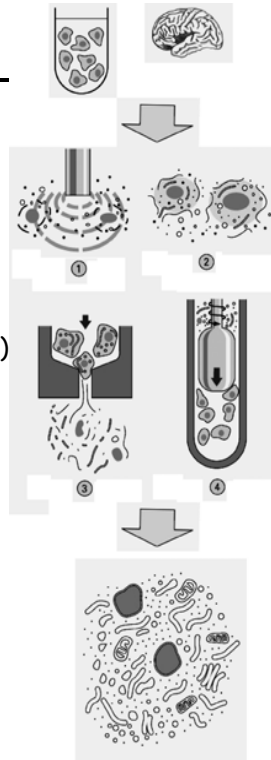
How do we purify proteins so as to study them.....

Protein Purification:

Cell Breakage & Fractionation

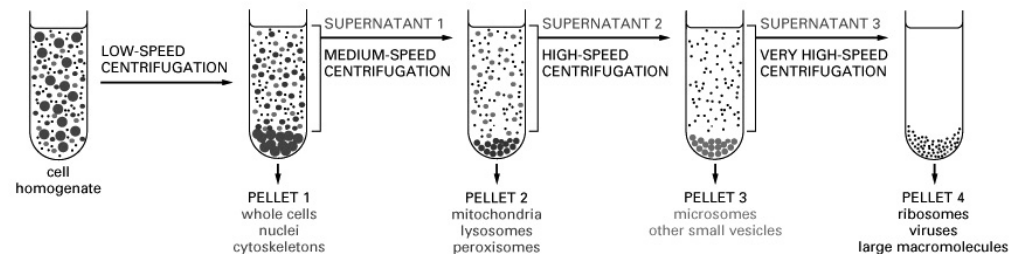
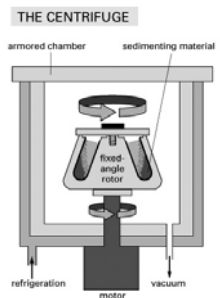
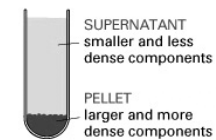
1. Breaking cells and tissues:

- high frequency sound (sonication)
 - mild detergent -> holes in plasma membrane
 - pressure driven: injection through a small hole (syringe)
 - shear cells via homogenization
- > extract or homogenate



2. Centrifugation:

- separation of components based on size and density
- > larger, greatest centrifugal force, move rapidly -> pellet

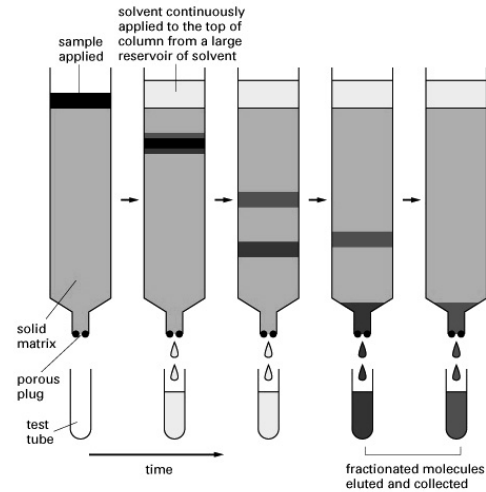


Protein Separation

- Proteins are diverse: size, shape, charge, hydrophobicity, affinity
- Properties used in protein separation

Column chromatography:

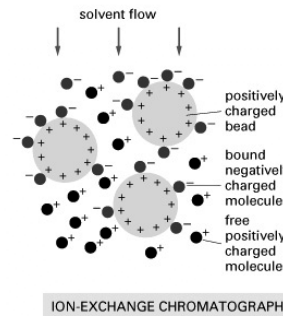
- column: permeable solid matrix (beads) immersed in solvent



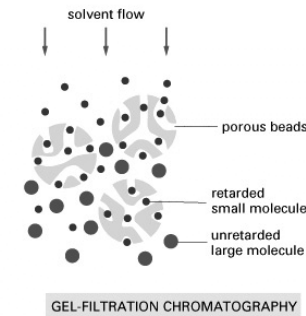
Protein Separation: Chromatography

Column chromatography: 3 types

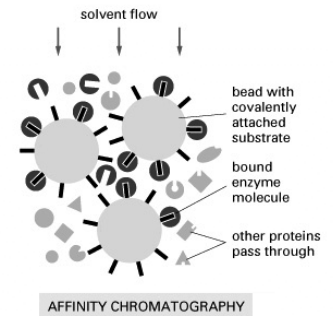
- Ion-Exchange
- Gel-Filtration chromatography
- Affinity chromatography



Charged beads.
Association depends on pH
& ionic strength of solution



Separation according to size.
Porous beads.
Small proteins enter beads
& are delayed



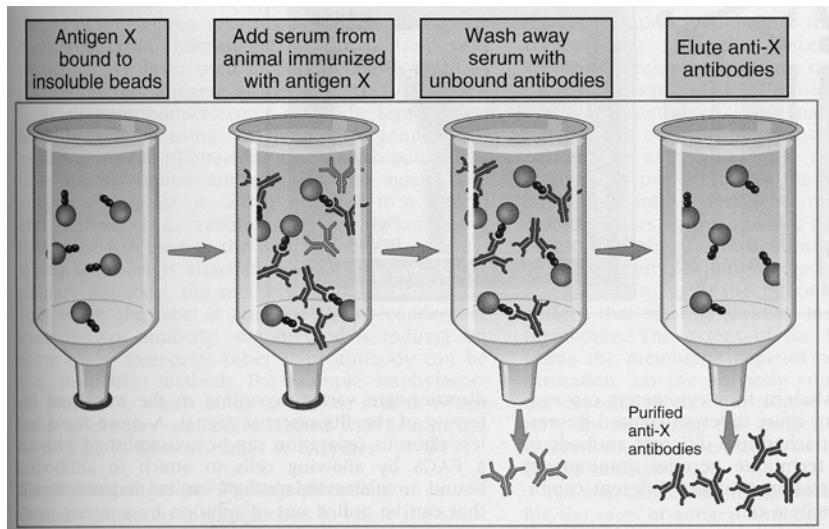
Matrix covalently coupled to
specific molecule (Ab, enzyme)
Proteins bound to column are
released by alter pH,
concentrated salt solutions

-> Purity can be assessed by gel electrophoresis.....

Purification of antibody by affinity chromatography

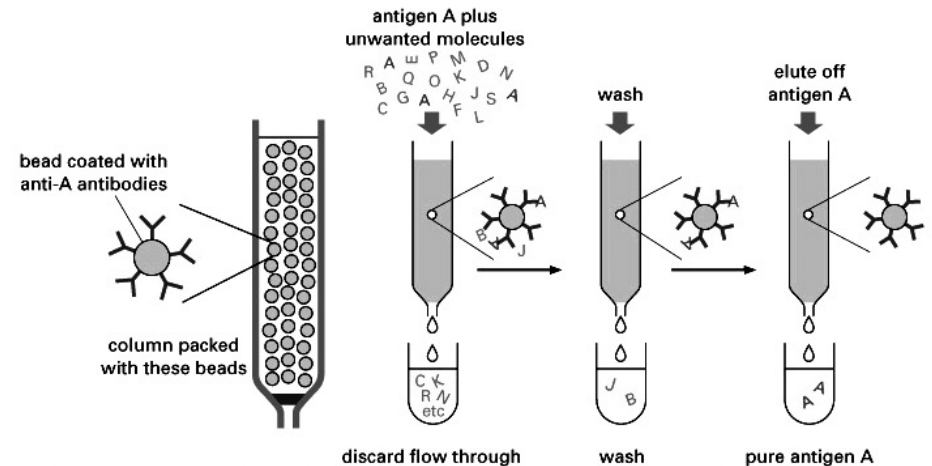
Antigen attached to matrix of insoluble beads

Elute (recover) bound antibodies by changing pH or ionic strength of solution so affinity of antigen binding is lowered



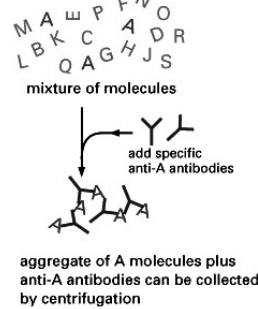
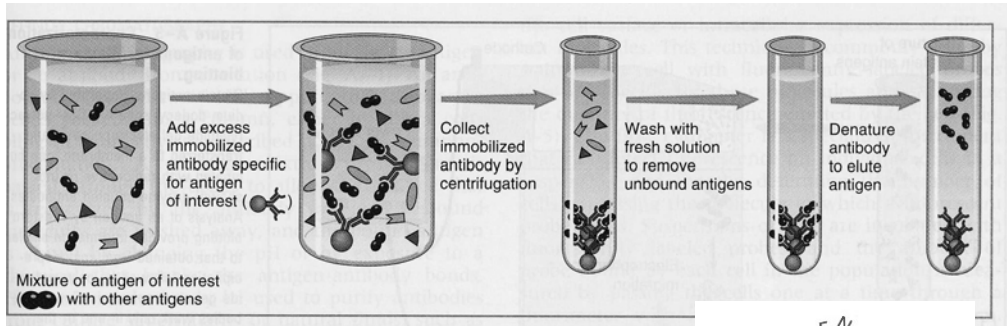
Antibodies used to purify molecules.....

Immunoaffinity column chromatography:



Immunoprecipitation can be used as a means of.....

.....Purification, quantification or identification of an antigen

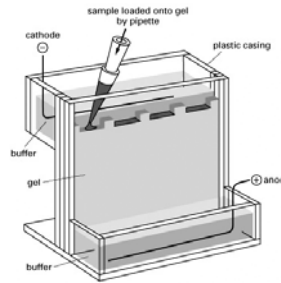


Antigens may be further analyzed by SDS-polyacrylamide gel electrophoresis

Protein Separation: Electrophoresis

Electrophoresis: application of electric field to solution of proteins

-> proteins migrate according to size and net charge



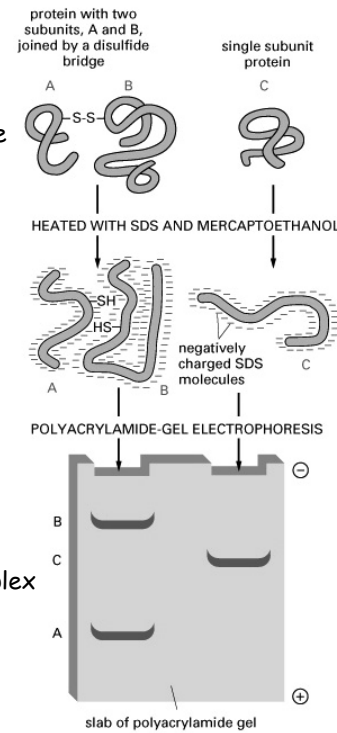
Sodium dodecyl sulfate (SDS): negatively charged

- Complexes with polypeptide chain

-> migration as negatively charged SDS-protein complex

Mercaptoethanol: reducing agent to break -S-S- linkages

-> protein migrates at rate reflecting its MW



Protein Separation: Isoelectric Focusing & 2D

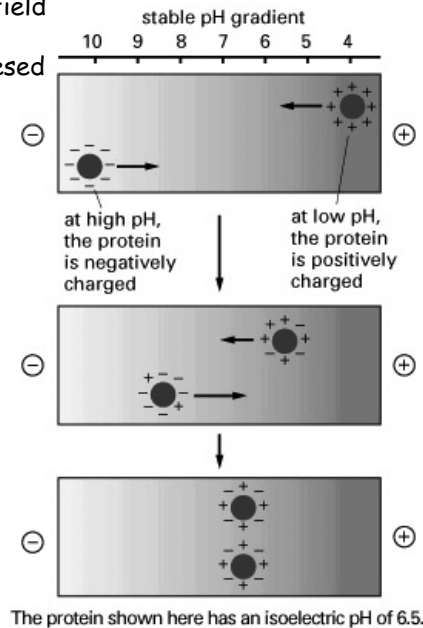
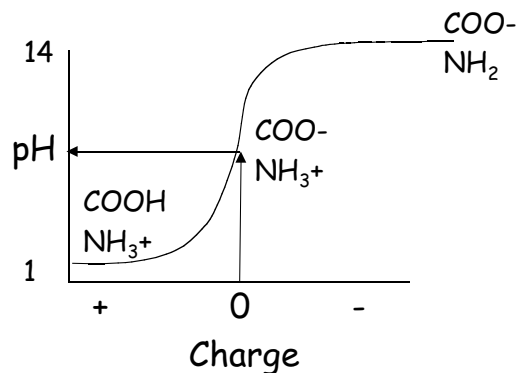
Isoelectric point: pH at which protein has no net charge

-> will not move in an electric field

Isoelectric Focusing: proteins are electrophoresed

in a polyacrylamide gel with an established pH gradient (by a mixture of special buffers)

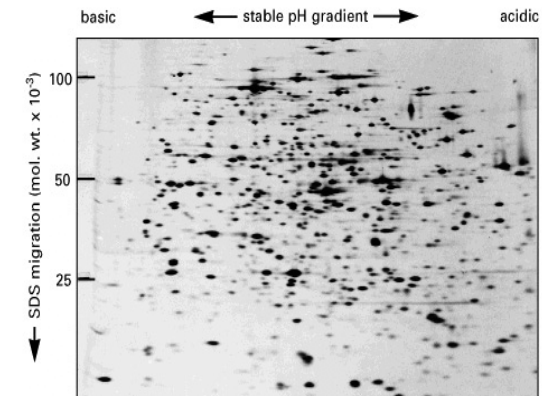
- Each protein moves to a point in gradient corresponding to its isoelectric point



2D gel electrophoresis: separation of protein mixture based on

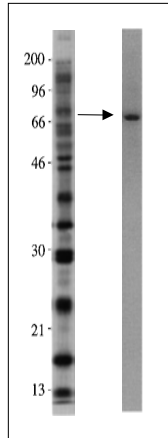
1) Charge, then 2) Molecular weight

Each spot is a protein



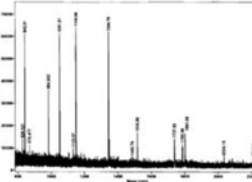
The Study of Proteins (Proteomics) :

Cell Lysis Fractionation Separation media Identification/Characterization



Coomassie blue stained 2D-gel of a total cellular protein extract of colon carcinoma

Protein spot (arrowed) removed from gel and digested with trypsin



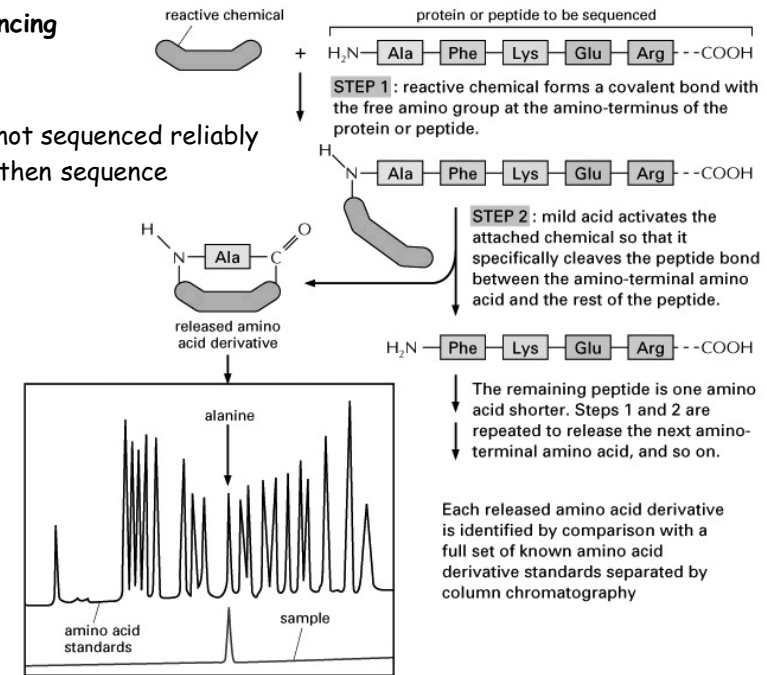
Mass spectrum of peptide fragments obtained by MALDI-TOF mass spectrometry

Database matching of peptide mass information identifies spot as nucleoside diphosphate kinase A (NM23)

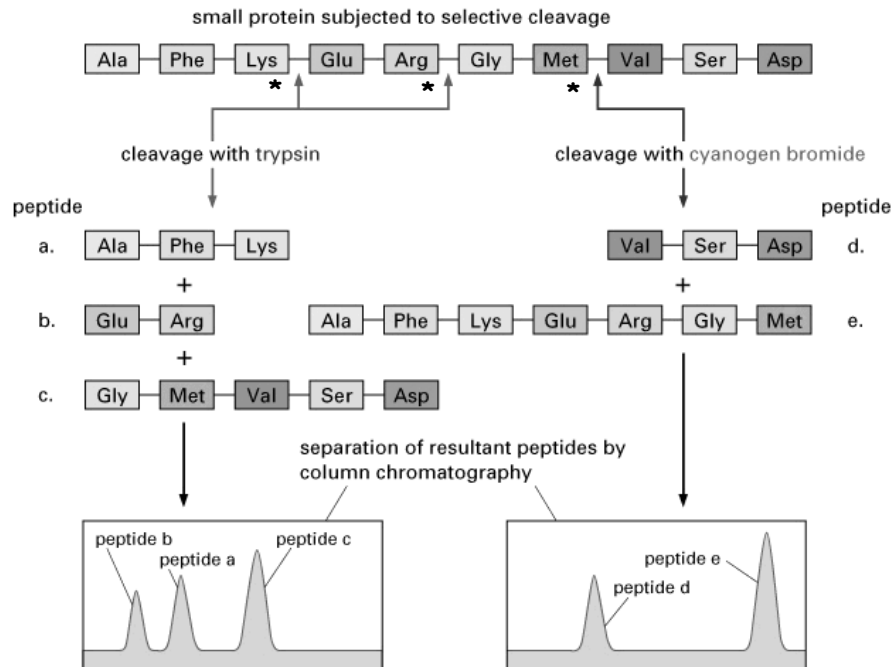
Protein Structure Determination

Amino Acid sequencing

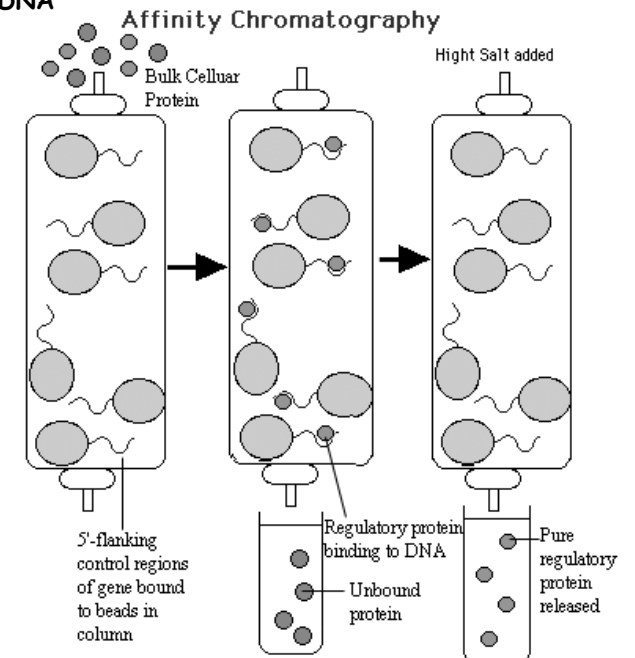
BUT Prots > 50 aa not sequenced reliably
→ cleave protein then sequence



Selective Protein Cleavage

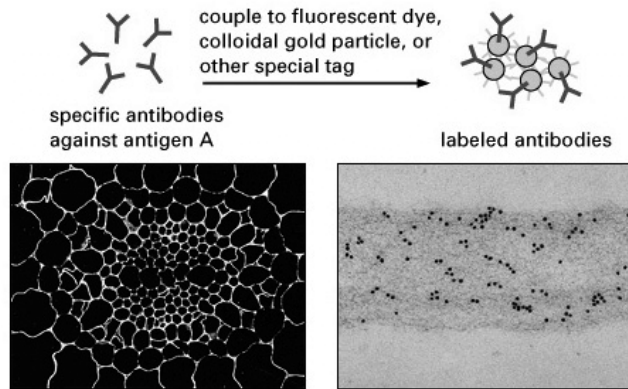


....or purification of a DNA Binding Protein



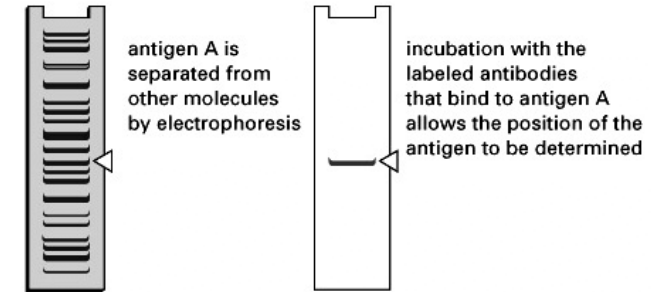
Antibodies used as molecular tags:

Microscopic detection



Antibodies used as molecular tags:

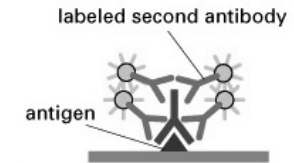
Biochemical detection



Sensitivity is increased by using multiple layers of Abs

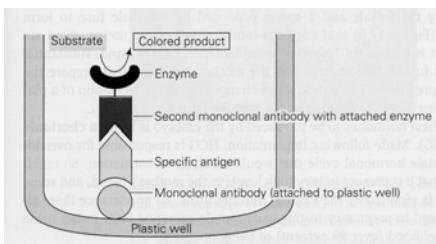
-> sandwich

Detection of smaller numbers of Ag molecules

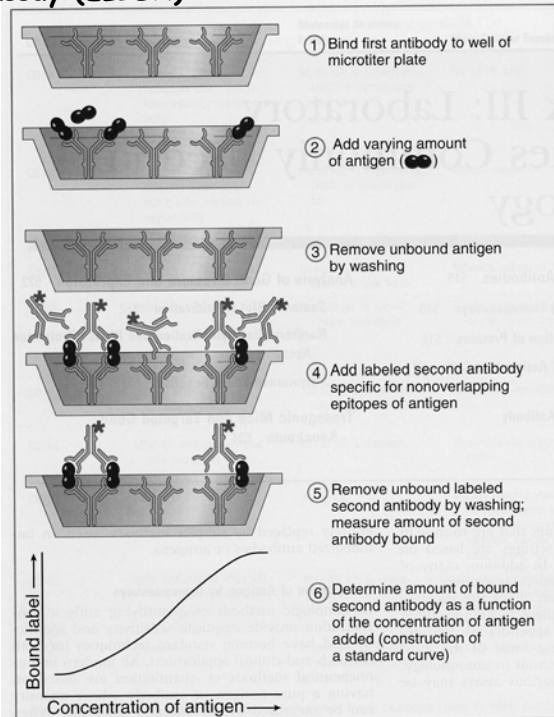


Enzyme linked immunosorbent assay (ELISA)

Or radioimmunoassay

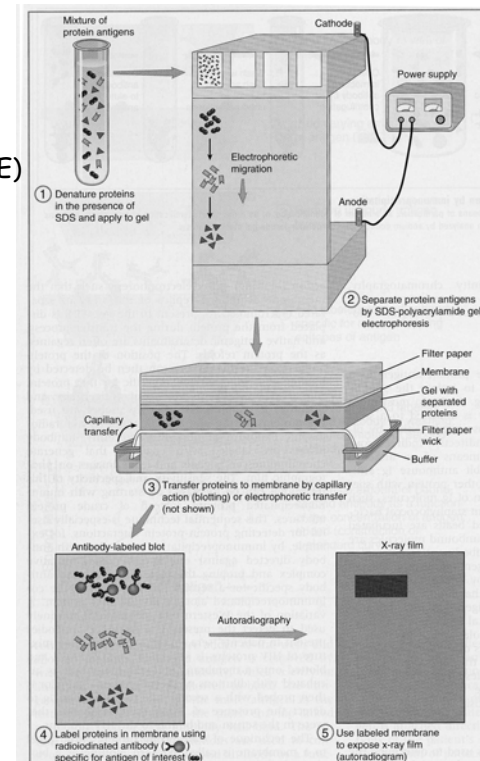


Conformation!



Western Blotting

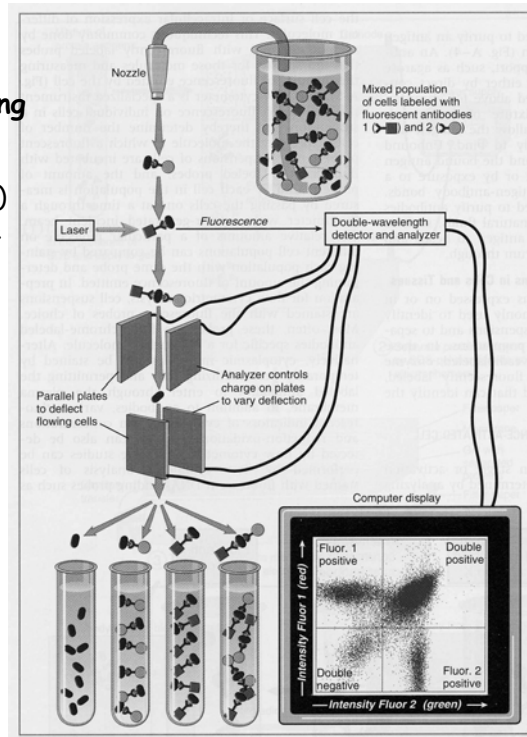
- Protein antigens separated by polyacrylamide gel electrophoresis (PAGE)
- Proteins are transferred to membrane
- Proteins can be probed with radioactive or enzyme coupled antibodies



Flow cytometry and Fluorescence-activated cell sorting

E.g of separation based on two antigenic markers ("two-color sorting")
Able to routinely analyze and separate cell populations based on
> 2 colored probes

Notes: Can detect fluorescence on individual cells in a suspension and thereby determine the number of cells expressing the molecule to which a fluorescent probe binds. Fluorescently labeled cell is measured by passing cells one at a time through a fluorimeter with a laser-generated incident beam.



Proteins

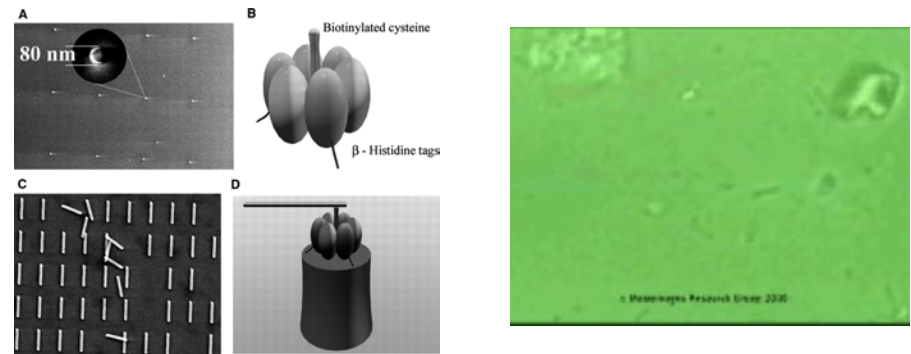
- Proteins are the working components of the cell:
 - proteins execute work in the cell, not genes
 - thousands of proteins → thousands of specific functions
- Noncovalent forces that mediate proteins structure and function:
 - ionic, H bonds, van der Waals, hydrophobic
- Protein function is characterized by reversible processes:
 - chemical interactions - ligand → any molecule bound reversibly
 - binding site → area where ligand binds
 - covalent modifications - phosphates → phosphorylation
 - methyls → methylation
 - adenyl - adenylation
- Reversible interactions and modifications often cause changes in protein shape

Proteins summary

How proteins work

- Proteins bind to other molecules
- The binding sites of antibodies are specific
- Binding strength is measured by the equilibrium constant
- Enzymes are powerful and highly specific catalysts
- V_{max} and K_m measure enzyme performance
- Tightly bound small molecules add extra functions to proteins
- The catalytic activities of enzymes are regulated
- Allosteric enzymes have two binding sites that interact
- A conformation change can be driven by protein phosphorylation
- GTP-binding proteins can undergo dramatic conformational changes
- Motor proteins produce large movements in cells
- Proteins often form large complexes that function as protein machines

Protein machine



Next Lecture

DNA

- The process of DNA replication
- Possible mutations; Repair of mutations
- The Central Dogma of Molecular Biology:
 - Replication of DNA
 - The process of Transcription (DNA -> RNA)
 - The process of Translation (RNA -> Protein)
-
- Alberts, Chapter 6 & 7
- In Class Homework #3
- (answers will be provided afterwards)
- Midterm in 2 weeks.