BCMB 3100: Partial notes
Chapter 4 (Part 1)

- Diversity of proteins
- 3D structure of proteins
- Fibrous vs globular proteins
- Conformation vs configuration
- $1^\circ$, $2^\circ$, $3^\circ$ and $4^\circ$ structure
- Peptide groups in polypeptide
- $\phi$ vs $\Psi$ angles, Ramachandran plot
- Xray crystallography & NMR
- $\alpha$ helix vs $\beta$-sheet

Diversity of proteins

- ___________ - study of large sets of proteins, such as the entire complement of proteins produced by a cell
- *E. coli* has about _________ different polypeptides (average size 300 amino acids, M, 33,000)
- Fruit fly (*Drosophila melanogaster*) about 16,000, humans/other mammals about ~20,000 - 40,000 different polypeptides

3D STRUCTURE OF PROTEINS

Two classes of proteins:

- ___________: water insoluble, static, "tough", extended, provide mechanical support ($\alpha$-keratin, collagen)
- ___________: compact, "spherical", usually: hydrophobic interior & hydrophilic exterior (enzymes)

*E. coli* proteins on 2D gel electrophoresis

See Fig. 5.11

*E. coli* expresses ~4000 proteins
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The biological activity of a protein depends on its conformation:
- **Conformation**: spatial arrangement of substituent groups that are free to assume different positions in space, without breaking any bonds, because of the freedom of bond rotation.

The number of potential conformations of a protein is **finite**. Under physiological conditions, the protein assumes a single stable shape: **native conformation**
- **Configuration**: a spatial arrangement of atoms that can not be changed without breaking covalent bonds.

### Levels of Protein Structure

- **Primary structure**: the covalent backbone of a polymer (sequence of amino acids)
- **Secondary structure**: the residue-by-residue conformation of the backbone of a polymer (\( \alpha \) helix and \( \beta \)-strands)
- **Tertiary structure**: the 3D conformation of a polymer in its native folded state
- **Quaternary structure**: the 3D structure of a multisubunit, particularly the manner in which the subunits fit together

**Supersecondary structure**: clusters of secondary structure (e.g. \( \beta\alpha\beta \))

**Domain**: a distinct structural unit of a polypeptide; domains have separate functions and may fold as independent, compact units.
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Resonance structure of the peptide bond

(a) Peptide bond shown as a C-N single bond
(b) Peptide bond shown as a double bond
(c) Actual structure is a hybrid of the two resonance forms. Electrons are delocalized over three atoms: O, C, N
(d) The peptide bond is planar with partial double bond characteristics. This prevents rotation of the peptide bond and constrains the conformation of the peptide backbone.

Planar peptide groups in a polypeptide chain

- Rotation around C-N bond is restricted due to the double-bond nature of the resonance hybrid form
- Peptide groups (blue planes) are therefore planar

Nearly all peptide groups in proteins are in the trans conformation

- trans conformation is less favorable than cis due to steric interference of $\alpha$-carbon side chains
- cis conformation is established protein during synthesis
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**Rotation of atoms in a peptide group**

- Peptide group has a repeating $\text{N-C}_\alpha$-$\text{C}$ backbone
- Rotation about both the $\text{N-C}_\alpha$ ($\phi$) and $\text{C}_\alpha$-$\text{C}$ ($\Psi$) bonds is possible
- Rotation of the $\text{N-C}_\alpha$ bond in proline is restricted because of the pyrrolidine ring structure

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See Fig. 4.9 Rotation around the $\text{N-C}_\alpha$ and $\text{C}_\alpha$-$\text{C}$ bonds that link peptide groups

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Steric contour diagram of allowed values of rotation permitted about the $\text{N-C}_\alpha$ ($\phi$, $\phi$) and $\text{C}_\alpha$-$\text{C}$ ($\Psi$, $\Psi$) bonds.

---

developed in 1963 by
G. N. Ramachandran,
C. Ramakrishnan,
and V. Sasisekharan
University of Madras, Madras, India
University of Michigan, Ann Arbor
See Fig. 10 (a) Ramachandran Plot

(a) Solid lines: range of permissible $\phi$ and $\psi$ values
Dashed lines: outer limits for an alanine residue
Blue dots: values for known conformations

(b) Ramachandran Plot

- Observed $\phi$ and $\psi$ values in known structures. Crosses are typical values for a single protein.
- $\alpha$-Helix residues (red), $\beta$-Strand residues (blue), others (green).

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__________ technique that reveals 3D position of atoms in a protein.

1. Need crystallized protein
2. Source of ________

X-ray strikes protein & some are scattered by electrons in protein.

3. Scattering is detected by X-ray film or solid state electronic detector.

4. Pattern of recombinant waves depends upon atomic structure.

5. Pattern converted to atomic image by computer analysis.
**Methods for Determining Protein Structure**

- X-ray crystallography is used to determine the three-dimensional conformation of proteins.

**Ribonuclease A**

(a) Space-filling model (bound substrate analog black)
(b) Cartoon ribbon model (shows secondary structure)
(c) Substrate-binding site view

As of today, 155,168 Biological Macromolecular Structures (see protein databank, https://www.rcsb.org/)

**NMR determination of protein structure**

A method used to determine structure of small proteins in solution. Nuclear magnetic resonance (NMR) is a physical phenomenon in which nuclei in a magnetic field absorb and re-emit electromagnetic radiation.

Ribonuclease A determined by NMR (polypeptide chain backbone)

Protein structures are deposited in the databases: e.g., Protein Data Bank (PDB)

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Secondary structure in proteins

Linus Pauling & Robert Corey (1950s)
proposed ______ and ______ as types of structures in proteins

right-handed
carbonyl O of aa residue n is H-bonded to α-amino N of residue n+4
rise = 0.15nm, pitch = 0.54, 3.6 residue per turn
Fibrous proteins (e.g. keratin) may be largely α-helix
Globular proteins vary greatly in α-helix content: average α helix content of 26%
All side chains point outward from cylinder of helix
Ala often found in α helix; Pro & Gly usually not present in helix but may be at ends

See Fig. 4.11
The α-helix
• All side chains project outward from helix axis
Helix in horse liver alcohol dehydrogenase

(a) Amino acid sequence, (b) Helical wheel diagram

- Amphoteric α helix (blue ribbon)
- Hydrophobic residues (blue) directed inward, hydrophilic (red) outward

This is an example of an amphoteric α helix

Horse liver alcohol dehydrogenase

has ββββ motif known as Rossman fold

- Amphoteric α helix
- Hydrophobic residues (blue) directed inward, hydrophilic (red) outward

Fig. 4.14 Leucine zipper of yeast

- DNA binding region consists of two amphoteric α helices, one from each of two protein subunits.

This transcription factor has amphoteric helices known as a leucine zipper. Leucine zippers have only a lose coiled structure compared to other coiled-coil amphoteric helices.

β sheet: extended polypeptide strands (β strands) stabilized by H-bonds between carbonyl O and amide H.
Each amino acid accounts for 0.32-0.34 nm of length of polypeptide chain.

β strands may be parallel or antiparallel
side chains point alternatively above and below plane of strand

β sheets contain 2-15 strands with average of 6 aa residues per strand
β strand content of globular proteins is variable:
Average of 19% β structure
Fig 4.15 \( \beta \)-Sheets (a) parallel, (b) antiparallel
(See Figures 4.16 & 4.17)

Interactions of \( \beta \) sheets

- \( \beta \) Sheet side chains project **alternately above and below** the plane of the \( \beta \) strands
- One surface of a \( \beta \) sheet may consist of hydrophobic side chains that can interact with other hydrophobic residues in protein interior
- __________ \( \beta \) sheets have hydrophobic side chains projecting outward that can interact with hydrophobic faces of \( \beta \) sheets or other helices

Stereo view of antiparallel \( \beta \) sheet

- Side chains (front \( \beta \) strand) alternate sides
- \( \beta \)-Strands twist in a right-handed direction

Structure of PHL P2 protein
(grass allergen)

(a) Blue/purple antiparallel \( \beta \)-sheets within a protein
(b) Stereo view of the \( \beta \) sandwich. Polar residues (red), hydrophobic residues (blue)

Example of amphipathic interaction in a \( \beta \) sheet
Loops and Turns (1)

• Loops and turns connect α helices and β strands and allow a peptide chain to fold back on itself to make a compact structure
• ________ - often contain hydrophilic residues and are found on protein surfaces
• ________ - loops containing 5 residues or less
• __________________ - connect different antiparallel β strands (also called hairpin loop)

Loops and Turns (2)

__________: 4 aa, stabilized by H-bonds between α-carbonyl O of residue n and α-NH of residue (n+3); Pro is often 2nd residue

____________: (also called glycine turn, β bend) like Type I Turn but 3rd residue is Gly

Reverse turns

(a) Type I, and (b) Type II

See Fig. 4.20

BCMB 3100 Partial Lecture Notes for Chapter 4 (Part 2)

• Supersecondary Structures (Motifs), Domains, Folds, Quaternary Structure
• Anfinsen’s Experiment denaturation, reduction & refolding
• Protein folding, Chaperones
• Collagen, Myoglobin & Hemoglobin
Common motifs

Motifs - recurring protein structures

- independently folded, compact, distinct structural unit in proteins
- ~25 to ~300 amino acid residues
- connected to each other by loops, bound by weak interactions between side chains
- may have separate functions
- illustrate evolutionary conservation of protein structure

Pyruvate Kinase

- Main polypeptide chain folds into three distinct domains

Last enzyme in glycolysis

DOMAIN: Independently folded, compact, distinct structural unit in proteins

Cytochrome c

- Conservation of cyt c structure

- Tuna (+heme)
- Tuna
- Rice
- Yeast
- Bacteria

See Last slides of Chapter 3 Lecture for Phylogenetic Tree
Structural similarity of (a) lactate dehydrogenase, (b) malate dehydrogenase
(only 23% identical amino acids!!)

CH₃- HOCH-COO⁻ Lactate

COO⁻ CH₃- HOCH-COO⁻ Malate

(a) B. stereothermophilus, (b) E. coli

Four categories of protein domains

1. **All α** - almost entirely α helices and loops
2. **All β** - only β sheets and non-repetitive structures that link the β strands
3. **Mixed α/β** - alternate regions of α helix and β strand (e.g. αβα motif)
4. **α + β** - local clusters of α helices and β sheet in separate, contiguous regions of the polypeptide chain

Folds

- A “fold” is a combination of secondary structures that form the core of a domain
- Some domains have simple folds, others have more complex folds

Common domain folds
Examples of tertiary structure

- Human serum albumin
- E. coli cytochrome b_{562}

Tertiary Protein Structures (cont)

- Jellyfish green fluorescent protein
- Pig retinol-binding protein

Domain Structure and Function

- A ________ may have a particular function
- ________ between 2 domains provide crevices, grooves, and pockets on the surface of a protein for binding or catalytic sites
- In multifunctional enzymes, each catalytic activity can be on one of several domains

Quaternary Structure

- organization of subunits in a protein with multiple subunits (an “oligomer”)
- Subunits may be identical (αα) or different (αβ) and have a defined stoichiometry and arrangement
- Subunits are held together by many weak, noncovalent interactions (hydrophobic, electrostatic)
Quaternary structure of multidomain proteins

Subunits = \( \alpha_2 \)
(each with \( \alpha/\beta \) folds)

Subunits = \( \alpha_2 \)
(each subunit is all \( \beta \) structure)

Subunits = \( \alpha_4 \)

Subunits = \( \alpha_3 \)

Rhodopsin photosynthetic reaction center

Subunits = \( \alpha_2\beta_2 \)

Subunits = \( \alpha_3\beta_\gamma \)

Protein Denaturation and Renaturation

- partial or complete unfolding of native conformation
- causes loss of biological activity
- caused by heat, extreme pH, detergents, chaotropic agents; due to disruption of non-covalent interactions
- some proteins can be refolded or renatured


_"chaos-promoting":_

chemicals that denature proteins (e.g. urea, guanidinium chloride)

chaotropic agents do NOT cleave covalent bonds but disrupt 2°, 3° and 4° structure

---

**Urea and guanidinium chloride**

Two chaotropic agents

Disrupt hydrophobic interactions in interior of protein by disordering water molecular adjacent to the protein.

Chaotropic agents increase entropy of system by interfering with hydrogen bonds, Van der Waals forces, and hydrophobic effects.

---

Detecting denatured Proteins

- Heat denaturation of ribonuclease A
- Unfolding monitored by changes in ultraviolet (blue), viscosity (red), optical rotation (green)

T_m (melting temperature) = temperature when 50% of particular protein is denatured

---

oxidation

R-SH + HS-R $\rightarrow$ R-S-S-R

---

reduction

---

: the loss of electrons from a compound

: the gain of electrons by a compound or ion

Common reducing agents: β mercaptoethanol; dithiothreitol
Disulfide bridges in bovine ribonuclease A

Ribonuclease:
- 124 aa,
- mainly β sheet,
- 4 disulfide bonds

(a) Location of disulfide bridges
(b) Stereo view of Cys-26 and Cys-84

(1950s) showed that the information necessary to specify 3D structure of ribonuclease came from the amino acid sequence

The amino acid sequence specifies 3D structure!!

The native form of a protein (e.g., ribonuclease) appears to be the thermodynamically most stable structure

Denaturation and renaturation of ribonuclease A

see Fig. 4.33

Protein Folding and stabilization (1)

Cooperativity of folding: formation of one part of structure (e.g., initial aa interactions) leads to formation of remaining structure.

is MAJOR driving force in protein folding
Protein Folding and stabilization (2)

Protein folding:
* nonpolar side chains inside
* most polar chains outside
* those polar chains inside H-bond with each other

→ 2° structure

H-bonds and van der Waals forces stabilize globular protein folding

Covalent cross links (R-S-S-R) and ionic interactions may stabilize some globular proteins

CURRENT THEORY OF PROTEIN FOLDING

1. not random
2. cooperative and sequential
3. dependent on 1° structure
4. for some proteins 1° structure alone determines folding
5. some proteins require help to fold (e.g. enzymes or chaperones)
6. extremely rapid, native conformation is generally reached < 1 second
7. NOTE: most protein have single native 3D shape; but some exceptions (e.g. IUP and metamorphic proteins)

• __________: proteins that bind newly synthesized polypeptides & assist in proper folding
• __________ increase rate of correct folding and prevent the formation of incorrectly folded intermediates
• __________ bind unassembled protein subunits, prevent incorrect aggregation before assembly into multisubunit protein
• Most chaperones are heat shock proteins

E. coli chaperonin

(a) (b) Core consists of 2 identical rings (7 GroE subunits in each ring)
(c) Protein folding takes place inside the central cavity
Chaperonin-assisted protein folding
• Hydrolysis of several ATP molecules is required

Collagen triple helix (1)
- Most abundant vertebrate protein (25-35% total protein); fibrous; found in bone, tendons, skin, blood vessels, cartilage; gelatin, glue
- Collagen is an aggregate of 3 left-handed helices coiled into a right-handed super helix!
  [3 aa per turn, pitch = 0.94 nm, rise = 0.31 nm]
- Has high percentage of Pro, Gly, Hyp (4-hydroxyproline), also contains Hyl (hydroxylysine), also a glycoprotein
- Structure elucidated by G. N. Ramachandran

Collagen triple helix (2)
- Hyp & Hyl are formed posttranslationally
- Hyp allows more H-bonding & stabilization of collagen triple helix
- Pro & Hyp prevent formation of α-helices & make collagen fibers rigid
- Strengthened by inter- and intra-covalent crosslinks between allysine-Lys (allysine)
- Vitamin C (ascorbate) deficiency → decrease in Hyp & Hyl formation → __________ (weakness in blood vessels & skin)
Ascorbate (vitamin C) is a reducing agent that keeps prolyl hydroxylase in an active form. Primates and guinea pigs have lost ability to synthesize ascorbic acid, so they must obtain it from their diet.

Collagen triple helix (3)

- Multiple repeats of -Gly-X-Y-: X often proline, Y often 4-hydroxyproline [Gly-Pro-Hyp]

- Glycine residues located along central axis of a triple helix (other residues cannot fit)

- For each -Gly-X-Y- triplet, one interchain H bond forms between amide H of Gly in one chain and -C=O of residue X in an adjacent chain

- No intrachain H bonds exist in the collagen helix

Interchain H bonding in collagen

- Amide H of Gly in one chain is H-bonded to C=O in another chain
Some proteins require **cofactors** or **prosthetic groups** at their active sites for activity

____________: small, usually nonprotein molecule required for enzyme activity

____________: a metal ion or other non-amino acid molecule tightly bound to a protein and essential for its activity

____________: active protein with all its cofactors

____________: protein without its cofactors

---

**Myoglobin (Mb)** [See Chapter 9 !]

Binds $O_2$
Stores & transports $O_2$ in muscle

**Prosthetic group:** heme = Fe-protoporphyrin IX

Single polypeptide (153 aa) + heme

Globular protein with 8 $\alpha$-helices

Oxygenated myoglobin = oxymyoglobin

All polar residues (except 2 His's) are located on protein surface

---

**Hemoglobin (Hb)**

Binds $O_2$

Transports $O_2$ in vertebrate blood

**Prosthetic group:** heme = Fe-protoporphyrin IX

Tetramer: $\alpha_2\beta_2$; $\alpha$ has 141 aa; $\beta$ has 146 aa; acts as dimer of $\alpha\beta$

$3^o$ structure of each subunit similar to myoglobin

---

**Heme Fe(II)-protoporphyrin IX**

• Porphyrin ring provides four of the six ligands surrounding iron atom

The ferrous iron is held in place by binding of nitrogens of the 4 pyrrole rings

See Pg. 163
The structure of globins has been conserved in many species.

Amino acid changes (substitutions) may or may not affect protein conformation:

- ____________ do NOT significantly affect conformation (i.e. Val → Ile)
- ____________ DO effect conformation (Glu → Val)

---

**Sperm whale oxymyoglobin**

- Oxygen (red)
- His-93 and His-64 (green)

---

**Hemoglobin tetramer**

See Fig. 9.6

(a) Human oxyhemoglobin  (b) Tetramer schematic

---

**Tertiary structure of myoglobin, α-globin and β-globin**

- α-Globin (blue)
- β-Globin (purple)
- Myoglobin (green)
Oxygen-binding site of whale oxymyoglobin

- Octahedral geometry of coordination complex (six ligands around iron)
- His-93 (proximal histidine) liganded to Fe
- His-64 (distal histidine)

See Fig. 9.4

Oxygen-binding curves

(a) Comparison of O₂-binding to Mb and Hb

Hyperbolic curve (single equilibrium constant)

\[
\text{Mb} + \text{O}_2 \rightleftharpoons \text{MbO}_2
\]

Fractional Saturation = \( Y = \frac{[\text{MbO}_2]}{[\text{Mb}]} \)

\( p_{50} \) = partial pressure at half saturation

See Figures 9.1 & 9.2

(b) Oxygen-binding curves for observed and for high and low affinity forms of hemoglobin

High affinity form of hemoglobin

Low affinity form of hemoglobin

What is the molecular basis for the cooperativity of O₂ binding by oxygen?

Conformational changes in a hemoglobin chain induced by oxygenation

- Oxygen binding to Fe pulls the proximal His toward ring plane
- Helix with His shifts position, disrupting some ion pairs between subunits (blue to red position)

See Fig. 9.7
Enhanced activity resulting from cooperation between subunits of an allosteric protein

A protein having multiple active sites as well as distinct regulatory sites that control the flux of biochemicals through a metabolic pathway.

Hemoglobin is an allosteric protein:

- Regulatory protein whose activity is modulated by noncovalent binding of a specific metabolite at a site other than the active site
- Small molecules that bind to allosteric proteins and regulate their activity
- Allosteric regulation is caused by small changes in native conformation of a protein

**Allostery**

active shape = R (relaxed)
inactive shape = T (taut)

allosteric inhibitor
R  \[\rightarrow\] T

allosteric activator

2,3-bisphospho-D-glycerate (2,3BPG) is an allosteric effector of hemoglobin. It lowers the affinity of deoxyhemoglobin for O₂ (raises P50). [Know physiological significance for Exam]

2,3-Bisphospho-D-glycerate (2,3BPG)

See Fig. 9.10
Binding of 2,3BPG to deoxyhemoglobin

See Fig. 9.10

- (-) Charges on 2,3BPG pair with (+) charges lining the central cavity, stabilizing the DeoxyHb form
- α-Subunits pink, β-subunits blue, heme groups red

(3) *Bohr effect: the increase in p50 of hemoglobin caused by a lowered pH due to an increase in CO₂

Bohr effect

- Lowering the pH decreases the affinity of Hb for oxygen

Fetal hemoglobin has lower binding affinity for 2,3-BPG than maternal hemoglobin. Thus, oxygen affinity of fetal red blood cells is higher than maternal red blood cells. [Fetal hemoglobin has 2 α and 2 γ subunits.]
Antibodies Bind Specific Antigens

- Vertebrate immune systems synthesize protein antibodies (immunoglobulins) to eliminate bacteria, viruses, other foreign substances
- Antibodies specifically recognize and bind antigens
- Antibodies are synthesized by lymphocytes (white blood cells)

(a) Human antibody structure
Immunoglobulin G class; IgG) (see Fig. 5.16)

Light Chain contains 2 domains; Heavy Chain contains 4 domains
Each domain: 110 aa in common motif known as immunoglobulin fold

Figure 5.16
Antibody (IgG) Structure
and their use in characterizing and purifying proteins

(see Fig. 5.16)

- Heavy chains (blue) and light chains (red)
- Disulfide bonds (yellow)
- Variable domains colored darker
Both Polyclonal and Monoclonal antibodies can be used to characterize proteins.

Both can be used for Western Blotting and ELISAs and “immunoprecipitation” (in the broadest sense).

Purification of proteins by “immunoprecipitation”

Antibody is attached to insoluble and/or magnetic bead

Enzyme-linked immunosorbent assay (ELISA)

Indirect and Direct ELISAs

Western Blotting
The following are additional notes that will help you in your studying.

**Review of Globular Protein 3D Structure**

Most globular proteins have compact globular shape due to many reversible turns in direction combined with the $\alpha$ helix and/or $\beta$ structure. Usually, hydrophobic aa residues are in the interior and hydrophilic aa residues on the exterior of the protein.

The loops & turns contain nonrepetitive regions of 2° structure.

- Loops: range from ~ 2-16 residues, many hydrophilic residues found at surface of protein (can H-bond with water)
- Turn: loops having only a few residues (<6)

**Average structure of Globular Protein**

- $\alpha$ helix: 26%
- $\beta$ structure: 19%
- Turns: 15%
- Simple loops: 21%
- Complex loops: 10%

**Four categories of protein domains**

1. **All $\alpha$ - domains almost entirely $\alpha$ helices and loops**
2. **All $\beta$ - domains contain only $\beta$ sheets and non-repetitive structures that link the $\beta$ strands**
3. **Mixed $\alpha/\beta$ - supersecondary structures where regions of $\alpha$ helix and $\beta$ strand alternate (e.g. $\alpha\beta\alpha$ motif)**
4. **$\alpha + \beta$ - local clusters of $\alpha$ helices and $\beta$ sheet in separate, contiguous regions of the polypeptide chain**

**Protein Denaturation and Renaturation**

- **Denaturation** - disruption of native conformation of a protein, with loss of biological activity
- Most denatured proteins adopt a random-coil conformation
- Proteins denatured by heating or chemicals
- Some proteins can be refolded or renatured
Protein Folding and stabilization (1)

Cooperativity of folding: formation of one part of structure (e.g. initial aa interactions) leads to formation of remaining structure.

Hydrophobic effect is MAJOR driving force in protein folding

Folded proteins occupy a low-energy well that makes the native structure most stable

Many proteins can fold spontaneously to this low-energy conformation

Proteins are thought to fold “cooperatively” … the first few interactions assist subsequent alignment and folding

Protein folding (more detail)

- extremely rapid, native conformation is generally reached < 1 second
- During folding the polypeptide collapses in upon itself due to the hydrophobic effect
- An intermediate “molten globule” forms with elements of secondary structure
- The backbone is rearranged to achieve a stable native conformation

• Chaperones: proteins that bind newly synthesized polypeptides & assist in proper folding
• Chaperones increase rate of correct folding and prevent the formation of incorrectly folded intermediates
• Chaperones bind to unassembled protein subunits to prevent incorrect aggregation before they are assembled into a multisubunit protein
• Most chaperones are heat shock proteins (synthesized as temperature increases)

Two conformations of hemoglobin: T and R

• Active (R state) and inactive (T state) forms are in rapid equilibrium in allosteric proteins
• Binding of substrates and allosteric activators stabilize the R state and shift the equilibrium in the R direction
• Allosteric inhibitors stabilize the T state and shift the equilibrium in the T direction