TERTIARY STRUCTURE (3°)

Global 3-dimensional arrangement of ALL atoms in a protein
- Includes:
  - 2° structural elements (alpha helices and beta sheets)
  - Amino acid side chains
  - Prosthetic groups
    - Small organic molecule or metal ion associated with a protein
- Regions of SECONDARY structure INTERACT to give a protein its TERTIARY structure
  - Major forces stabilizing tertiary structure are hydrophobic interactions among nonpolar side chains in the compact core of the proteins.

QUATERNARY STRUCTURE (4°)
- Arrangement of multiple protein molecules into COMPLEXES
  - The three dimensional structure of a protein made of >1 polypeptide
  - Complexes of 2, 3, 4 etc… protein molecules are called dimers, trimers, tetramers…oligomers
- Oligomers may be:
  - Formed with identical protein monomers = HOMOOLIGOMER
    - Example: Myoglobin
  - Formed with different protein monomers = HETEROOLIGOMERS
    - Example: Hemoglobin:
      - 2 alpha subunits
      - 2 beta subunits
- Protein subunits of oligomers interact through NON-COVALENT interactions

Hemoglobin (4 subunits) Liver Alcohol Dehydrogenase (2 subunits)
Heterotetramer Can be either a homo- or heterodimer
PROTEIN FOLDING

- **Goal:** To achieve the LOWEST energy state

Formation of hydrophobic domains often the primary driving force for tertiary structure

**The hydrophobic effect**

Water and oil: They don’t like each other.

When you drop oil into water, it tends to glob up into little droplets.

Proteins act the same way. All the ‘greasy’ hydrophobic residues tend to go up in the middle of the protein making a ‘hydrophobic core’.

The polar and charged residues tend to line the outside of the protein as they are happy interacting with water.

- **Hydrogen bonding stabilizes interactions between regions of polypeptide chain**
  - Secondary structural elements have H-bonds to stabilize the peptide backbone – 2° structure does not directly involve side chains
Hydrogen bonds

Hydrogen bonds occur when a proton (hydrogen) is shared between a donor group and the unpaired electrons of an acceptor oxygen.

Proteins fold such that all hydrogen bonding groups participate in a hydrogen bond.

- Other forces involving side chains that influence how proteins fold:
  - **Metal ion coordination** to negatively charged amino acid side chains
  - **Hydrophobic interactions** between NON-POLAR side chains
    - Favored on interior – not exposed to water
  - **Ionic/Electrostatic Interactions** between charged side chains
    - Favored on the outside
    - Sometimes on inside if near opposite charge
  - **Hydrogen bonding among side chains** of polar amino acids
  - **Disulfide bridges** between Cys amino acids stabilize tertiary structure COVALENTLY (only covalent interaction – rest are non-covalent)

- Note: Once folded, proteins are not rigid; highly dynamic
FORCES THAT STABILIZE STRUCTURE OF PROTEINS

How do we determine the 3-D structure?

1. **X-ray crystallography** – use crystal of pure protein
2. **NMR (2-D NMR)** – measures magnetic characteristics of each atom
   - Both methods are extremely difficult and require lots of computer power to make sense of data

Protein Folding Interactive Animation:
http://www.wiley.com/legacy/college/boyer/0470003790/animations/animations.htm
CAN WE UNFOLD PROTEINS ONCE THEY ARE FOLDED? YES!

- Proteins can be unfolded = DENATURED
  - Lose most levels of structure
  - Protein adopts a random coil conformation
  - Primary amino acid sequence is maintained
  - Loss of protein function – enzymatic etc…
  - Go from NATIVE (correctly folded, biologically active state) to DENATURED and UNFOLDED (loss of organized structure and function)

- Use denaturing agents: Interfere with the forces that stabilize protein folding

<table>
<thead>
<tr>
<th>DENATUREING AGENT</th>
<th>TARGET</th>
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<tbody>
<tr>
<td>heat</td>
<td>H-bonds, hydrophobic interactions</td>
</tr>
<tr>
<td>agitation</td>
<td>H-bonds, hydrophobic interactions</td>
</tr>
<tr>
<td>pH</td>
<td>salt bridges</td>
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<tr>
<td>mercaptoethanol</td>
<td>disulfide bridges</td>
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<tr>
<td>detergents (SDS)</td>
<td>hydrophobic interactions</td>
</tr>
<tr>
<td>urea, guanidine HCl</td>
<td>H-bonds, hydrophobic interactions</td>
</tr>
</tbody>
</table>

Is this process REVERSIBLE? – i.e. can we restore a protein, once denatured to its original configuration and restore function?

- Yes – Denaturation CAN BE reversible
  - Heat treatment usually is not reversible

- The renaturation of the protein RIBONUCLEASE A (an enzyme that cleaves DNA) won Christian Anfinsen the Nobel Prize in 1972

- Experiment:
  1. Denatured pure Ribonuclease A by treatment with UREA and β-mercaptoethanol to give a completely unfolded, denatured protein
     - β-mercaptoethanol used to reduce disulfide bonds
     - Urea breaks H-bonds and hydrophobic interactions
  2. Then he removed the denaturants and exposed the protein to air
  3. The protein had folded back into its original 3-D shape and activity was restored!!

*This experiment suggested that the unfolded polypeptide refolded by itself in the test tube*

Further experiments determined that it DID refold back to its original state
CONCLUSION: ALL THE NECESSARY INFORMATION AS TO HOW A PROTEIN FOLDS IS ENCODED INTO THE PRIMARY SEQUENCE!

1° SEQUENCE DICTATES 2° AND 3° STRUCTURE!

ANFINSEN:

AMINO ACID SEQUENCE DETERMINES PROTEIN SHAPE

Primary Sequence = Structure

-Leu - Arg - Asp - Asp - Ser - Leu - Ala - Asp - Glu - Leu - Tyr - Phe - Glu -

Proteins can self-assemble!

All the information needed to make a working 3-D machine is encoded in the amino acid sequence!

Unfortunately, we haven’t figured out the code yet. We can’t effectively predict 3-D structure of a protein from looking at the primary amino acid sequence.
Diseases Associated with Defects in Primary Structure:

1. **Cystic Fibrosis (CF)**
   a. Inherited disease that affects breathing, digestion, reproduction and other functions
   b. 1000 cases/year in the US
   c. Symptoms:
      i. Chronic cough, wheezing and breathing problems
      ii. Frequent sinus and respiratory infections
      iii. Excessive mucous production
      iv. Recurrent pneumonia
      v. Salty skin
      vi. Sterility in males
   d. CF attacks endocrine (outwardly secreting) glands, preventing them from functioning normally
   e. In CF, exocrine glands produce thick, sticky mucous secretions that plug up the body’s ducts and passages
   f. When mucous clogs the respiratory system, bacteria and microorganisms can grow and impair body’s defenses
   g. Sweat glands affected: Abnormal amount of chloride in sweat
      i. Use “sweat test” to identify CF patients
   h. In CF patients, CF ions don’t move properly resulting in reduced or eliminated chloride transport.
   i. Salt stays in sweat and doesn’t escape into epithelium. Cells don’t secrete normal mucous
   j. Also causes deficiency in WATER transport
      Not enough water to wash away mucous from surface and consequently is abnormally sticky.
   k. Leads to obstruction and inflammation in glands/ducts and ultimately tissue damage and death
   l. Disease caused by mutations in CFTR gene – both alleles must be mutated otherwise “carriers”
CFTR = cystic fibrosis transmembrane regulator
   i. Protein expressed at the plasma membrane of epithelial cells
   ii. Acts as a chloride channel
   iii. Way the salt component enters and leaves cells
      1. Deficiency in chloride transport is basis for the symptoms

Most severe mutation is deletion of amino acid 508 – Phenylalanine
   - Mutation causes the protein to get stuck in the endoplasmic reticulum on its way to
     the plasma membrane
   - Other mutations (over hundreds identified) have varying effects and affect
     severity of the disease.

Treatments:
  - Pancreatic enzymes to aid in digestion – pancreatic ducts get clogged
  - Aerosols to help breathing
  - Antibiotics to help respiratory infections
  - Exercise
  - Chest physical therapy
  - Proper nutrition and vitamins
  - Gene therapy – introduce “good” copy of the gene into the genome

Sickled and Normal Red Blood Cells

2. Sickle Cell Anemia
   a. Inherited blood disorder
   b. Chronic anemia and periodic episodes of pain
   c. Defective hemoglobin in red blood cells – has consequences in oxygen transport in blood
   d. After hemoglobin is deoxygenated, hemoglobin clusters together forming rod-like structures
   e. Cause red blood cells to become stiff and assume a sickle shape
   f. Get trapped in capillaries and block circulation to organs, producing pain along with
      many other problems.
   g. Sickle cells are more fragile because their membranes are stretched – break and lyse easily
   h. Red blood cells only live 10-20 days versus 120 days (normal)
STRUCTURE OF HEMOGLOBIN:
- Tetramer (4 subunits)
  - 2 alpha (α) subunits
  - 2 beta (β) subunits
  - Mutation occurs in the beta subunit (Glu → Val; position 6)
  - Sickle cell has 2 abnormal β-chains and 2 normal α-chains

See:
CHIME Models of Hemoglobin and Sickle Hemoglobin
http://www.umass.edu/microbio/chime/hemoglob/index.htm

Electron microscopy picture of Fibrils:
Mutations:
- Most common
  - Single amino acid change from Glu → Val at position 6
  - Places hydrophobic side chain on surface of the protein
  - When deoxygenated, having this hydrophobic group on the surface causes a decrease in protein solubility and rod-like structure production
- Heterozygotes
  - Carriers without symptoms
  - Selective advantage
    - Survive malarial outbreaks
  - Homozygotes – have the disease

<table>
<thead>
<tr>
<th>Mutant Hemoglobin</th>
<th>Position Number</th>
<th>Normal Residue</th>
<th>Substitution</th>
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<tbody>
<tr>
<td>α Chain</td>
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<tr>
<td>G Honolulu</td>
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<tr>
<td>Zurich</td>
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<td>His</td>
<td>Arg</td>
</tr>
</tbody>
</table>

- The hemoglobins are often named for the cities where they were first discovered.
- The numbering for an amino acid position begins at the N-terminus.

A sickled red cell filled with sickle hemoglobin fibers.
Model of Polymerized Hemoglobin

- **Therapies:**
  - Pain Killers
  - Prevent cell dehydration
    - Use of *clotrimazole* – drug that prevents loss of water
  - Gene therapy with fetal hemoglobin or induce fetal hemoglobin expression
    - Fetal hemoglobin seems to prevent sickling of red cells and cells containing fetal hemoglobin tend to survive longer in the bloodstream
      - Hydroxyurea stimulates production of fetal hemoglobin
  - Blood transfusions
  - Antibiotics