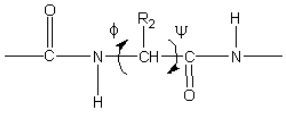
Protein Structure (and Determination of)

Primary structure - determined by amino acid (aa) sequence. Includes disulfide bonds between cysteines.

- Determine the composition (not sequence) of protein.
  * add 6N HCl to protein; incubate at 110°C for 24 hrs. Cleaves peptide bonds.
  * Run through ion exchange chromatography. Collect fractions.
  * It is known which fraction each aa would be in if present. Add ninhydrin to each fraction; if color change to blue occurs, aa is present.
- Edman degradation determines the sequence of a protein.
  * Label the first (amino end) residue of a protein with phenyl-isothiocyanate.
  * Cleave that residue and identify it with chromatography.
  * Repeat again and again for every residue in protein.
  * See lecture handout for chemical steps, v. important!

Secondary structure - local structure.

- α-helix - structure based on rigid transpeptide bond
  * very dense helix with right-handed twist
  * all NH and CO groups are H-bonded together
  * R groups point directly out of α-helix.
  * 3.6 residues per turn, 100° between.
  * Rise = distance between adjacent C_α's = 1.5Å.
  * Pitch = (rise / residues per turn) = 5.4Å per turn.
- β-sheet - again, structure based on rigid transpeptide bond
  * composed of β-strands
  * parallel (strands run same direction) - H-bonds with the aa directly across from it
  * antiparallel (strands run opposite direction) - H-bonds with the two amino acids on either side of it on the other chain
- reverse turns (also known as β-turns) - 6 types
  * within a few aa, the protein chain has made a 180° turn
  * This cannot occur with just any R groups, only certain ones, that must be small: Proline, Glycine and Asparagine most common
- Dihedral angles
  * ϕ (phi) - angle between NH and C_α
  * ψ (psi) - angle between C_α and C=O



  * Can change one of the angles by fixing one and rotating the other
  * Not every combination of ϕ and ψ are possible, because the R group may collide into the NH or C=O. Can plot the allowed combinations on a Ramachandran Plot (see Fig 3.31, p. 57, 5th edition of text)
  * Alpha Helix: ϕ = -57°, ψ = -47°

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    * Beta Sheet: ϕ = -130°, ψ = +125°

Tertiary structure - folding of the entire chain.

- proteins found in an aqueous environment:
    * interior of protein formed of aa with hydrophobic side chains
    * exterior of protein formed of aa with hydrophilic side chains
- proteins found in membranes are reversed from above
- common conserved structural classes, even between proteins totally unrelated in sequence
    * 4-helix bundle - side chains stabilize tertiary structure
    * β-sheets can be twisted into barrels and saddles

Quaternary structure - arrangement of subunits.

- Classic example: 4 subunits of hemoglobin
- Subunits usually held together by noncovalent bonds
- Can be simple, like 2 identical subunits, or complex, like dozens of different subunits

Determination of structure - X-ray crystallization

- Shine X-rays on crystal
- Defracted X-rays imaged on film
- Due to destructive & constructive interference, see spots on film that tell you about the amplitude and phase of the X-rays at that spot. Use Fourier Transform to put together the waves to give picture of the electron density of the crystal.
- If there was such a thing as a lens that could focus X-rays, we could attain a direct image of the crystal; however, no such lens currently exists
- Amplitude of wave is relatively easy to determine; related to intensity of spot
- Phase of wave is harder to determine
    * Make heavy atom derivatives (Hg, Au, U) to act as markers so that we can reference the phase of the waves
- Use info about amplitude and phase to reverse Fourier Transform the scatter, which gives you an image of that which scattered the rays in the first place - called electron density map
- Resolution determines how much detail of the structure you can figure out
    * Higher resolution allows you to use info on the outside of the diffraction pattern
    * This is important because Fourier Transforms produce something in an inverse space - close scatter is a long distance, far scatter is close space, so resolution is dependent upon how far out you have scattered points
    * 6Å resolution - can see polypeptide chain
    * 3Å resolution - can see α-helices and β-sheets
    * 1.5Å resolution - can see atoms (but not hydrogen)