Lecture 5  
Tuesday, January 15, 2008

1. Protein Structure

- Helices, β-strands
- Protein tertiary, quaternary structure
- Protein folding

2. Exploring Proteins

- Purification
- Sequencing
- Identifying proteins in vivo.
  - Proteomics
  - Laser Scanning Confocal Microscopy
  - Fluorescent proteins

Chapter Problems:

Chapter 2: 1, 2, 5, 6, 7, 8, 9, 11, 13. For problem 9, assuming the membrane is 25 Å thick, how many amino acid residues, forming a perfect α-helix, are required to span that distance?

Chapter 3: 1, 4, 10, 11, 14, 15, 17, 18.

Chapter 4: 1, 3 (assume B-form helix), 5, 7, 10 (why would U be used in RNA but T in DNA, given this chemistry?), 11, 12, 13, 20, 22, 23 (why might the protein sequence be more conserved than the DNA sequence?)

Note that many of the problems in Chapters 3 and 4 are “thought” problems, meaning you will probably have no idea how to approach them at first. Still, you should think about them a bit and then check the answer in the back of the book. I am not interested so much in that you get them right without checking, but that you understand the thinking behind the answer.
3. Peptide structures

- Peptide backbone: forms α-helix and β-sheet.

- Structure: Ψ, φ angles.

- Ramachandran Plot
  
  - Plots energy of a peptide as a function of all possible Ψ, φ angles.
  
  - Regions of low energy (≈ minimum stability).

4. α-helix

- Barrel-like, cylinder-like

- Stabilized by N-H...O=C H bonds

- R groups on outside → helical wheel representation

- Different chemical properties on different sides of helix

- Non-polar

- Amphipathic helices
2. β-strands
   - the other major 2° structure
   - R, O angles make peptide side be extended.
   - look like ribbons.

Properties:
   a. R groups alternate above & below peptide plane

- non-polar
- polar

- Form sheets of several strands H-bonded together

β-sheets - made of multiple β-strands.

c. 2 orientations of adjacent strands.

\[ N \rightarrow \_\_\_\_ \_\_\_ C \]

\[ C \_\_\_\_ \_\_\_ \_\_\_ \_\_\_ N \]

anti-parallel

\[ N \rightarrow \_\_\_\_ \_\_\_ C \]

\[ N \_\_\_\_ \_\_\_ C \]

parallel
-can be mixed

\[ \begin{align*}
    N & \rightarrow C \triangleright \text{ap} \\
    C & \leftarrow N \triangleright \text{par} \\
    C & \leftarrow N \triangleright \text{par} \\
    N & \rightarrow C \triangleright \text{ap}
\end{align*} \]

C. turn.

180° reversal of peptide chain direction; in ~4 residues.
- Turns connect anti-parallel β-strands.

\[ \begin{align*}
\text{β-sheet.}
\end{align*} \]

Blaber.
4. Protein Structure:
levels of structural organization:

- \(1^\circ\) (primary) struct. \(\equiv\) sequence.
- \(2^\circ\) (secondary): local interactions that give rise to spec. \(\chi\) of
- \(3^\circ\) (tertiary): how \(2^\circ\) struct. pack together.
  - folded, native protein.
- \(4^\circ\) (quaternary): \(3^\circ\) struct. packing

![Diagram](image)

\(\beta\)-strand.
interested in 3° because structure related to function.

structure = function

[ - all proteins adopt 3° structures. ]

- not quite true.
  - family of unstructured proteins.
  - not quite true
    - plaques. → alzheimers.

- families of 3° structures
  - some all α helix
    - myoglobin, hemoglobin
  - some all β
    - anti bodies
    - immuno globulins
  - coiled coil
- Mixed of
  - largest family
  - different sub families

- all these proteins have some common features.

- polar residues are on outside
- non-polar resin are on inside.

Ser, Thr, E, Q, R, K
N, D,

Ala, V, L, I, M, C

- F, Y, N, H.

- folded proteins interact with other
  folded proteins → 40
- pair up via electrostatic interact.
  via hydrophobic.
5. Protein folding.
- Random search for every \( \Phi, \Psi \)
- Time to find native structure: billions of years.
- Understand how they fold, \( \Phi \) why they fold to that \( 3^\circ \) structure.

Known facts.
1. Proteins are marginally stable

\[ \text{unfolded} \rightleftharpoons \text{folded} \]

\[ \Delta G \approx 30 \text{ kJ/mol} \]

Several factors that contribute to protein folding:

- Electrostatics
- H-bonds
- van der Waals
- Hydrophobic
electrostatic enthalpy $\Delta H$ favors Native.

H-bonding enthalpy $\Delta H$ favors N

van der Waals $\Delta H$ favors N

hydrophobic effect. $\Delta S$ favors N

* chain entropy $\Delta S$ favors Unf.
  - although $\Delta G$ is small, $\Delta H$ & $\Delta S$ huge.

2. protein folding & unfolding is cooperative.

[Diagram showing a graph with temperature on the x-axis and protein concentration on the y-axis, with a sharp drop around the middle temperature (Tm).]

all-or-none

- doesn't mean no intermediate
- intermediates don't accumulate.
Cooperativity means proteins resist denaturation (i.e., loss of function) up to some point and then feels change over small range in temperature.

- means they maintain function as long as possible.

3. Stability is fairly robust to changes in a.a. sequence.
   - Mutations show us that many sites are not critical for stability or function.
   - Are a few critical residues.
     - "Hot spots"

4. Protein folding & unfolding is fast.
   - Proteins begin to fold on ribosome.
   - No random search.
   - Transition from U → N has relatively few "pathways"
Most proteins fold in <1 sec.
- α helices very fast. < μsec. 10^-6 s
- β strands/β-sheets take longer
  μsec → sec.

- Slow parts:
  things get stuck
  - cis/trans isomerization involving
    X-Pro peptide bonds.
    - minutes.
    - mis folding; aggregate.

- Forming disulfide bonds.

\[
\text{thiol.} \quad \rightarrow \quad \text{disulfide}
\]
- Responses by your cells:
  - degrade
  - enzymes that help fix these problems.
  - catalyze cis \rightarrow trans
  - catalyze s-s exchange.

- chaperones: chaperonin.

5. Some amino acids prefer helix

- formers
  - A, L, K, M, Q, E

- strand
  - V, I, Y, Cys, N, F \text{ bulky, large atoms/grps at } \text{CB}

- not exclusive:
  - can only predict structure a \text{2x better than chance.}
- because 3D structure can bias 2D structures.

6. general model for protein.
Figure 2.29 Structure of the $\alpha$ helix. (A) A ribbon depiction with the $\alpha$-carbon atoms and side chains (green) shown. (B) A side view of a ball-and-stick version depicts the hydrogen bonds (dashed lines) between NH and CO groups. (C) An end view shows the coiled backbone as the inside of the helix and the side chains (green) projecting outward. (D) A space-filling view of part C shows the tightly packed interior core of the helix.
Figure 2.35 Structure of a β strand. The side chains (green) are alternately above and below the plane of the strand.
For each amino acid, the NH group is hydrogen bonded to the CO group of one amino acid on the adjacent strand, whereas the CO group is hydrogen bonded to the NH group on the amino acid two residues farther along the chain (Figure 2.37). Many strands, typically 4 or 5 but as many as 10 or more, can come together in β sheets. Such β sheets can be purely antiparallel, purely parallel, or mixed (Figure 2.38).
Figure 2.41 Structure of a reverse turn. The CO group of residue $i$ of the polypeptide chain is hydrogen bonded to the NH group of residue $i + 3$ to stabilize the turn.