Determination of Protein Structure:

a) x-ray crystallography
b) NMR
c) sequence data

$^1$H chemical shift (ppm)
Figure 4-26

Formation of the native conformation
(information derived from denaturation)

net $\Delta G = -40 \text{ kJ/mol}$

Stabilizing Forces
- hydrophobic effect
- van der Waals
- hydrogen bonds
- ion-ion
- disulfide

Denaturation is ______________
Suggests ______________
"m_______ t____________

Figure 4-28

Primary sequence

determines

tertiary structure

Denaturation - ____________________________
can be reversible (renaturation)

Ribonuclease can be denatured by urea and β-mercaptoethanol

1) remove urea and β-ME by dialysis – native protein within hours
2) remove only β-ME and expose to air – inactive enzyme
   if warm and dialyze in the presence of β-ME
   remove urea – native protein
3) rapidly remove urea and β-ME – inactive enzyme
   add protein disulfide isomerase – native protein

Figure 4-27

Native state; catalytically active.

addition of urea and mercapto-ethanol

Unfolded state; inactive. Disulfide cross-links reduced to yield Cys residues.

Unfolded state; inactive. Disulfide cross-links reduced to yield Cys residues.

removal of urea and mercapto-ethanol

Native, catalytically active state. Disulfide cross-links correctly re-formed.
Folding is not a random sampling of possible conformations

1) Hierarchical
2) Molten Globule

Thermodynamics of Folding
1) A loss of entropy
2) A lower free energy

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Within the protein chain are required to achieve the ___________________

Protein folding in the cell occurs __________________

Protein folding in the cell occurs with __________________

Misfolded proteins as a result of a mutation can result in disease
- cystic fibrosis
- mad cow disease

Some protein folding requires assistance

While primary sequence is the predominant factor in proper protein folding there is also some facilitation

1) Peptidyl-prolyl cis-trans isomerase
2) Protein disulfide isomerase
3) Molecular chaperones
   a) heat shock proteins – bind to unassembled protein regions and prevent aggregation
   damage, synthesis, membrane transport, multiprotein complexes
   b) Chaperonins – elaborate protein complex that assists in protein folding
Protein Folding Assisted by HSP

1. DnaJ binds to the unfolded or partially folded protein and then to DnaK.
2. DnaK stimulates ATP hydrolysis by GroEL. DnaK-ADP binds tightly to the unfolded protein.
3. ATP binds to GroEL and the protein dissociates.
4. In bacteria, the nucleotide-exchange factor GroEI stimulates release of ADP.
5. GroEL binds to DnaK, the GroES-Po1 trimer, and ATP.
7. GroEL and GroES bind to DnaK, with a further step.
8. ATP binds to each subunit of the GroEL heptamer.
9. ATP binds to each subunit of the GroEL heptamer.
10. Protein is fully folded or is a partially folded state that is committed to adopt the native conformation.

Thematic:
1) ___________ sequence determines ___________ structure
2) Function of a protein is dictated by its ___________ 
3) Each protein has a ___________ structure
4) The stabilizing forces in the native conformation are ___________
5) There are common structural patterns

Folded vs Denatured Protein
- is folding thermodynamically favored?
  - enthalpy: bonds with water vs bonds with self
  - entropy: loss of entropy for protein, gain of entropy for water

Can changes be made in the primary structure?
  - invariant
  - variant
  - conserved

Globular and Fibrous Proteins