Lecture 20 (10/28/20)

- Reading: Ch4; 142-151
- Problems: Ch4 (text); 14, 16
  Ch6 (text); 1, 4

NEXT (after exam)

- Reading: Ch8; 310-312, 279-285
  Ch24; 957-961
- Problems: Ch8 (text); 1,2,22
  Ch8 (study-guide:facts); 1,2,4,5,7,8,9

Protein Structure

A. Stability
   1. Two-state model
   2. Energetics
   3. Denaturation
   4. Methods to study

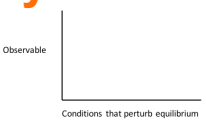
B. Protein Folding
   1. Evidence-Anfinsen
   2. Protein Folding Pathways
   3. Mechanism; *in vitro* vs. *in vivo*
      a. Kinetics
      b. Thermodynamics
   4. Diseases

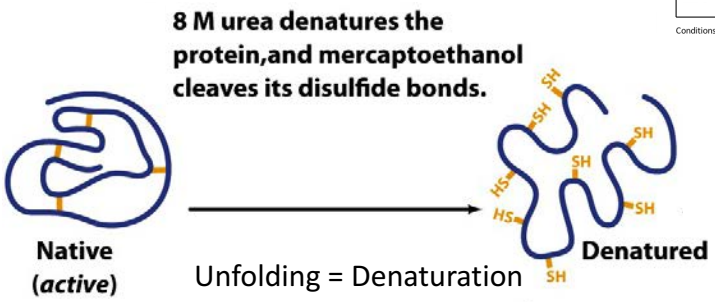
C. Prediction

D. Dynamics

Protein Stability, Folding, and Dynamics

What are these Observables?





Unfolding = Denaturation

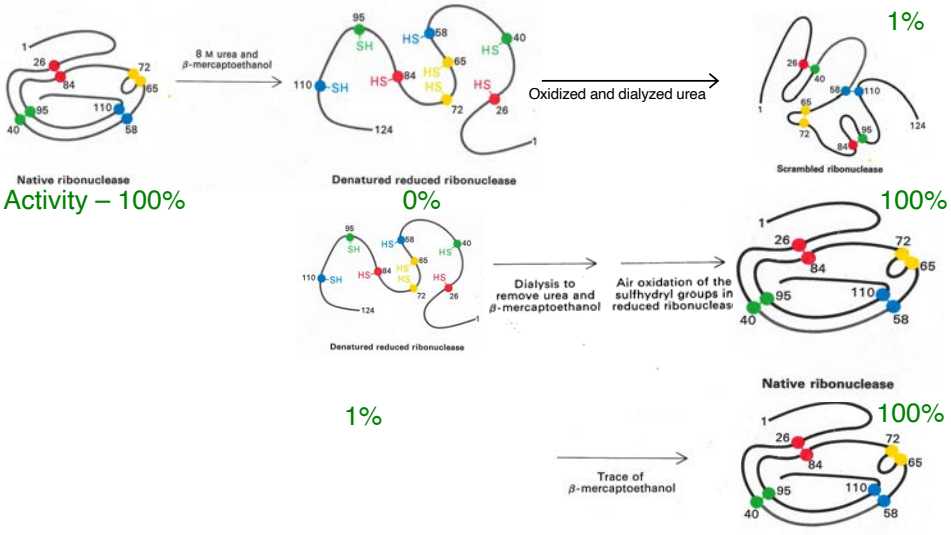
Any techniques that can measure a difference between these two states:

Optical rotation, CD, fluorescence (native (Trp) or ANS), UV absorbance, viscosity, shape

Protein Stability, Folding, and Dynamics

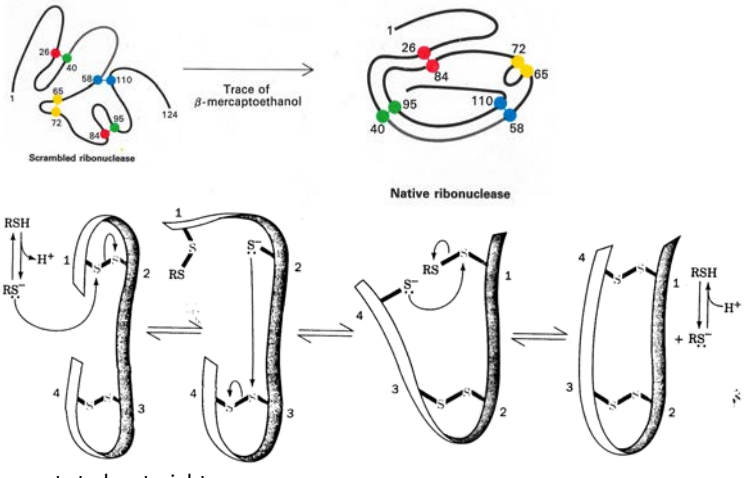
What determines the 3D structure of a given protein?

Anfinsen Experiment



Protein Stability, Folding, and Dynamics

Anfinsen Experiment showed that 1° structure determines 3° structure!

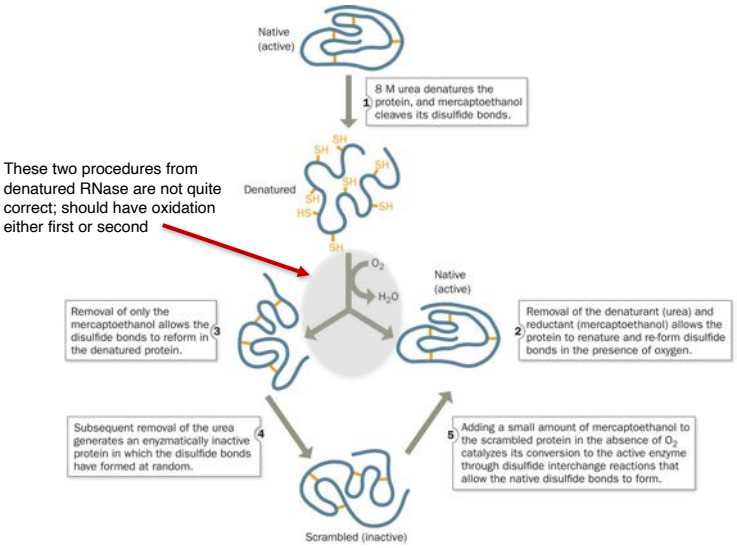


From 1→2, protein wants to be straight;
from 2→3 protein wants to bend

Disulfide Exchange

Protein Stability, Folding, and Dynamics

Process Diagram: Denaturation and Renaturation of RNase A



Protein Stability, Folding, and Dynamics

Primary structure determines Tertiary structure!

Protein Stability, Folding, and Dynamics

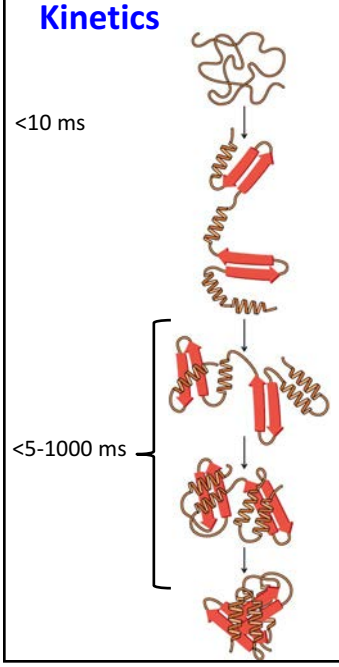
How Can Proteins Fold So Fast?

- Proteins fold to the lowest-energy state in the microsecond to second time scales. How can they find the right fold so fast?
- It is mathematically impossible for protein folding to occur by randomly trying every conformation until the lowest-energy one is found (Levinthal's paradox).
- Search for the minimum is therefore not random; there must be a PATHWAY toward the native structure, which is thermodynamically most favorable.

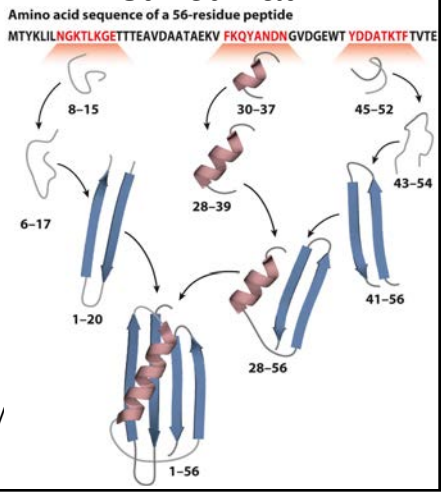
Protein Stability, Folding, and Dynamics

Kinetics

How? Protein folding



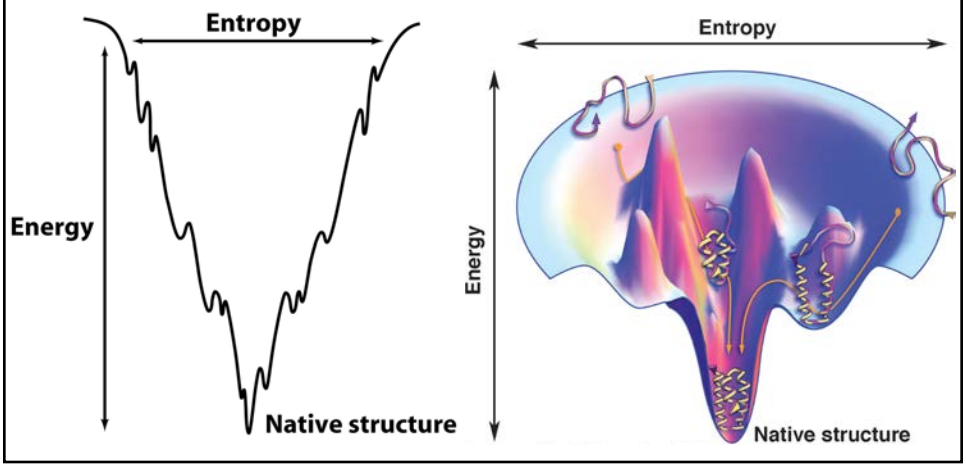
Protein Folding Follows a Distinct Path



Protein Stability, Folding, and Dynamics

Energetics

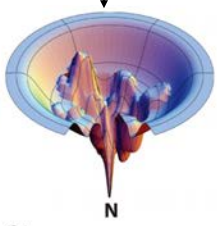
Energy-Entropy Diagram: Protein Folding Funnel



Protein Stability, Folding, and Dynamics

Energy-Entropy Diagram: Protein Folding Funnel

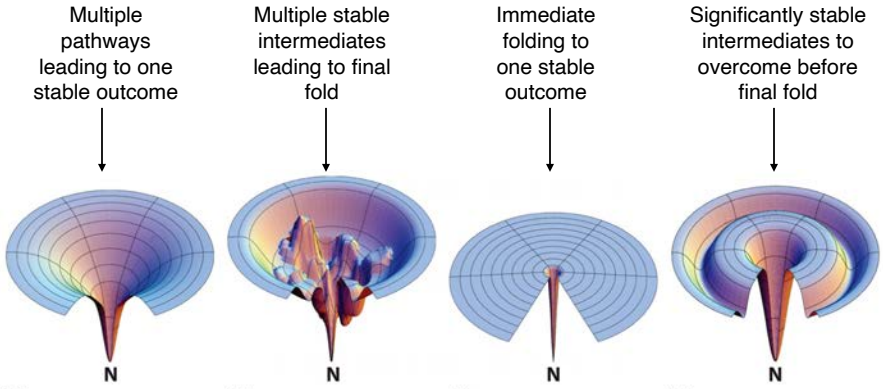
Free-Energy Funnel Model of Protein Folding Is Dependent on the Stability of Motifs Within the Protein

Multiple pathways leading to one stable outcome	Multiple stable intermediates leading to final fold	Immediate folding to one stable outcome	Significantly stable intermediates to overcome before final fold
↓	↓	↓	↓
			
Two-State Model (no intermediates)	Many Intermediates Model	Two-State Model (one path, no intermediates)	Stable Intermediate (Needs help!)

Protein Stability, Folding, and Dynamics

Energy-Entropy Diagram: Protein Folding Funnel

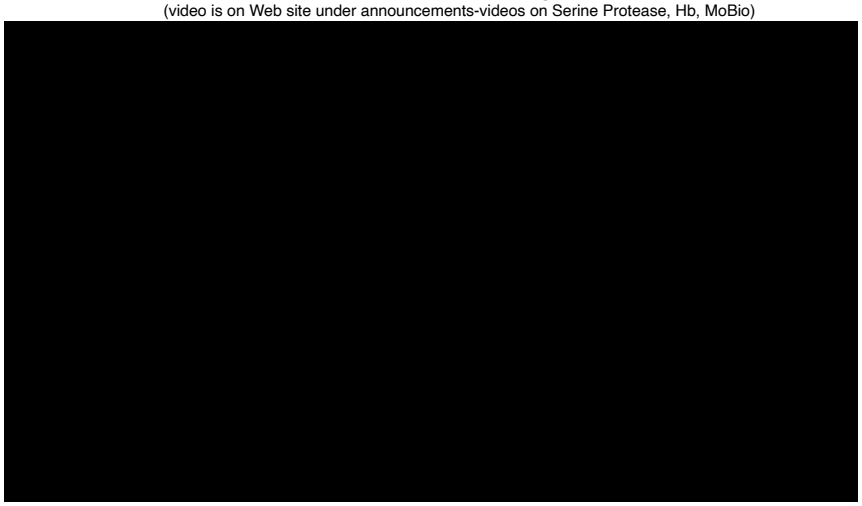
Free-Energy Funnel Model of Protein Folding Is Dependent on the Stability of Motifs Within the Protein



Protein Stability, Folding, and Dynamics

Computer Simulation of Protein Folding: A 40 residue protein

(video is on Web site under announcements-videos on Serine Protease, Hb, MoBio)



Simulating protein folding on the millisecond timescale has been a major challenge for many years. Recently, Folding@home researchers Vincent Voelz, Greg Bowman, Kyle Beauchamp, and Vijay Pande have broken this barrier. This is a movie of one of the trajectories that folded (i.e. started unfolded and ended up in the folded state). See Voelz *et al.* (2010) *J. Am. Chem. Soc.*, **132**:1526 for more details.

Protein Stability, Folding, and Dynamics

Protein folding help

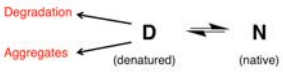
While the primary structure dictates what the fold will be, IN THE CELL, proteins often need help.

Kinetic help

1. Protein disulfide isomerase (PDI)
2. Peptide Prolyl Isomerase (PPI)

***Energetic help** (**prevent aggregation; maintain equilibria**)

1. Molecular chaperones – heat-shock proteins (Hsp)
   - Hsp70, Hsp90, etc.
2. Molecular chaperonin
   - GroEL-GroES



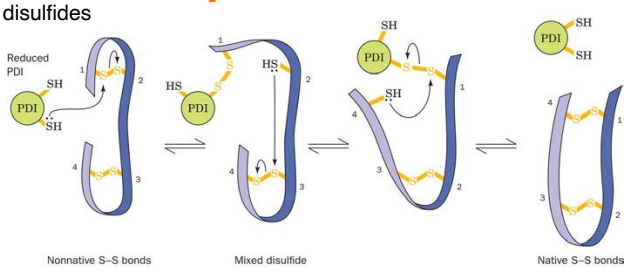
***Thermodynamics**

Protein Stability, Folding, and Dynamics

Kinetic help

Protein Disulfide Isomerase (PDI) Catalyzes Disulfide Interchange

Incorrect disulfides



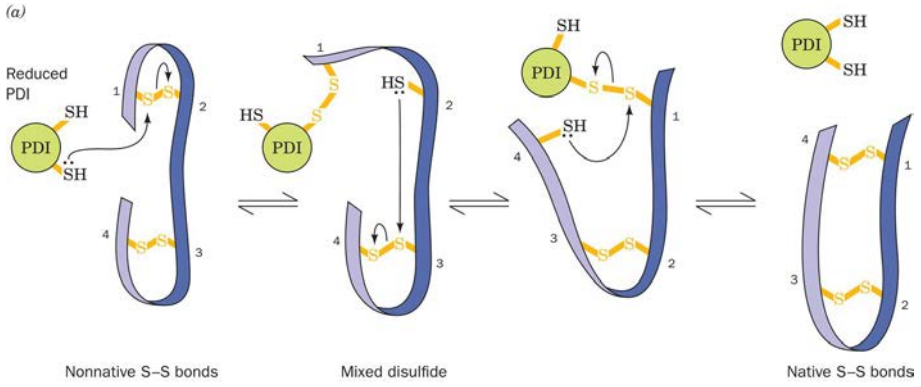
Correct c

Protein Stability, Folding, and Dynamics

Kinetic help

Protein Disulfide Isomerase (PDI) Catalyzes Disulfide Interchange

Incorrect disulfides



Correct disulfides

Protein Stability, Folding, and Dynamics

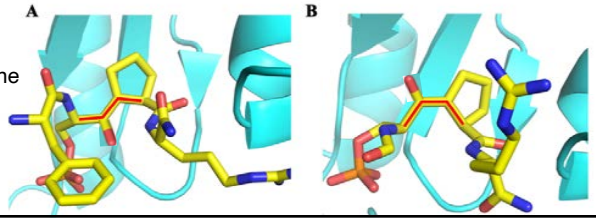
Kinetic help

Peptidyl Prolyl Isomerase (PPI) Catalyzes *trans-to-cis* isomerization

Without catalysis this takes 8-10 min!

Activation energy reduced 20 kcal/mole by PPI → 10^{15}-fold rate increase

Pro



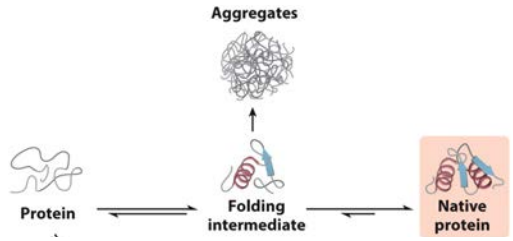
trans-proline

cis-proline

Protein Stability, Folding, and Dynamics

Thermodynamic help

Chaperones Prevent Misfolding and Aggregation

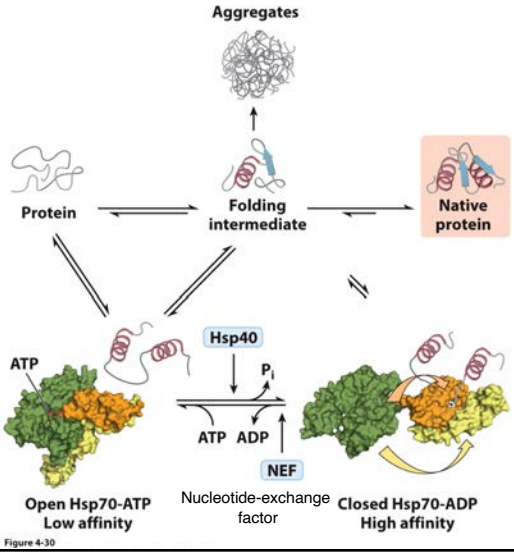


Heat-shock proteins (HSP)

Protein Stability, Folding, and Dynamics

Thermodynamic help

Chaperones Prevent Misfolding and Aggregation



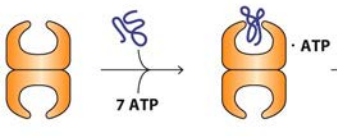
Heat-shock proteins (HSP)

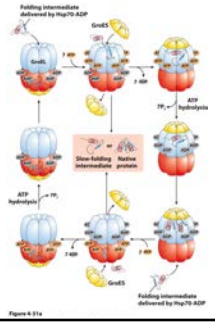
Protein Stability, Folding, and Dynamics

Thermodynamic help

Facilitated folding: Chaperonin

1. A GroEL ring binds 7 ATP and an unfolded polypeptide, which associates with hydrophobic patches on the GroEL subunits.





Protein Stability, Folding, and Dynamics

Thermodynamic help

Facilitated folding: Chaperonin

1. A GroEL ring binds 7 ATP and an unfolded polypeptide, which associates with hydrophobic patches on the GroEL subunits.

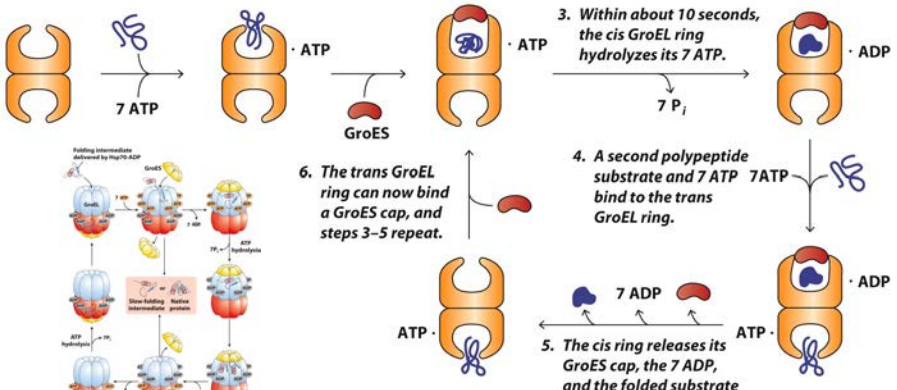
2. A GroES cap binds, triggering a conformational change that retracts the hydrophobic patches, thereby releasing the polypeptide into the GroEL chamber, where it can fold.

3. Within about 10 seconds, the cis GroEL ring hydrolyzes its 7 ATP.

4. A second polypeptide substrate and 7 ATP bind to the trans GroEL ring.

5. The cis ring releases its GroES cap, the 7 ADP, and the folded substrate polypeptide.

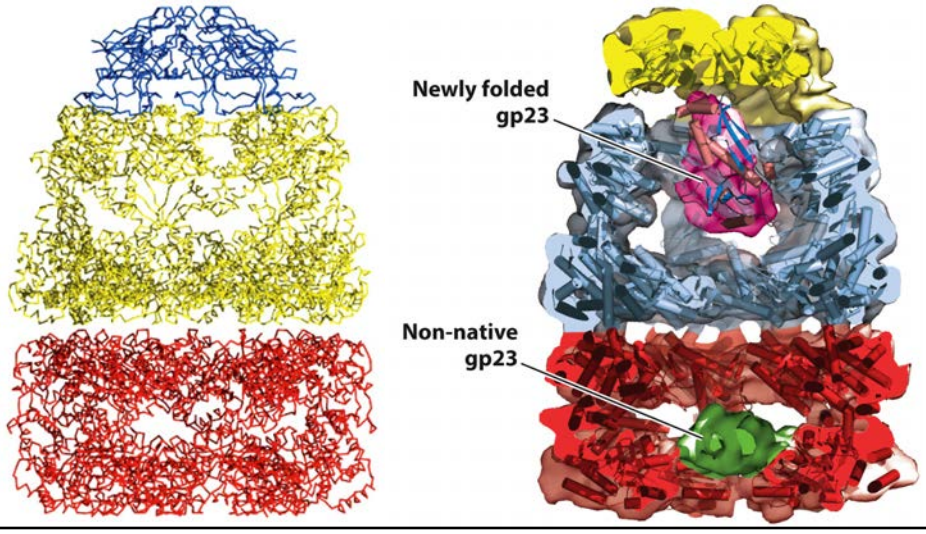
6. The trans GroEL ring can now bind a GroES cap, and steps 3–5 repeat.



Protein Stability, Folding, and Dynamics

Thermodynamic help

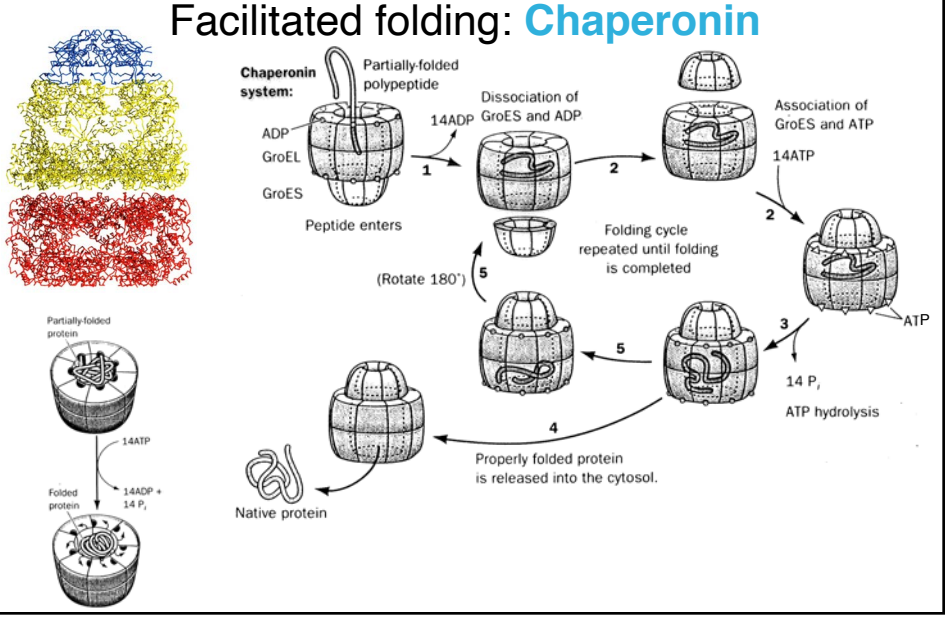
Facilitated folding: Chaperonin



Protein Stability, Folding, and Dynamics

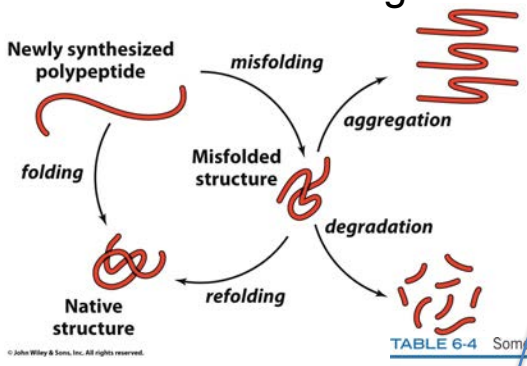
Thermodynamic help

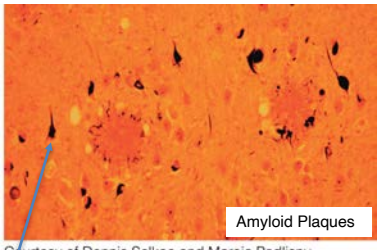
Facilitated folding: Chaperonin



Protein Stability, Folding, and Disease

Misfolding and Disease





Courtesy of Dennis Selkoe and Marcia Podlisny, Harvard University Medical School

TABLE 6-4 Some Protein Misfolding Diseases

Disease	Defective Protein
Alzheimer's disease	Amyloid-β protein
Amyotrophic lateral sclerosis	Superoxide dismutase
Fibrinogen amyloidosis	Fibrinogen α chain
Huntington's disease	Huntingtin with polyglutamate expansion
Light chain amyloidosis	Immunoglobulin light chain
Lysozyme amyloidosis	Lysozyme
Parkinson's disease	α-Synuclein
Transmissible spongiform encephalopathies (TSEs)	Prion protein

Protein Misfolding Is the Basis of Numerous Human Diseases

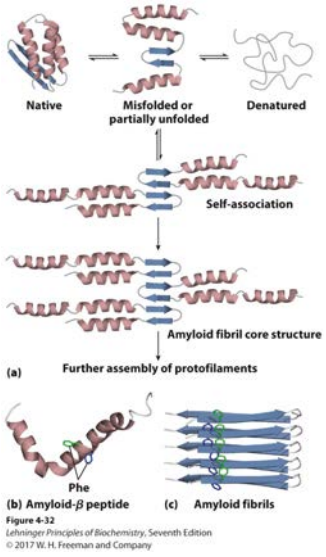
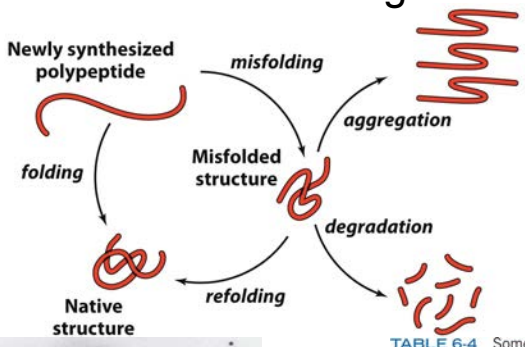


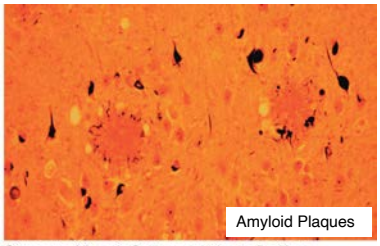
Figure 4-32
Lehninger Principles of Biochemistry, Seventh Edition
© 2017 W. H. Freeman and Company

- Native (correctly folded) β amyloid is a soluble globular protein,
- Misfolded β amyloid promotes aggregation at newly exposed protein-protein interface.
- Correctly folded helices are lost and peptides form β strands, β helices, and β sheets.

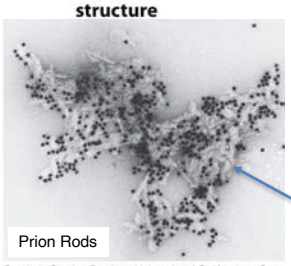
Protein Stability, Folding, and Disease

Misfolding and Disease





Courtesy of Dennis Selkoe and Marcia Podlisny, Harvard University Medical School



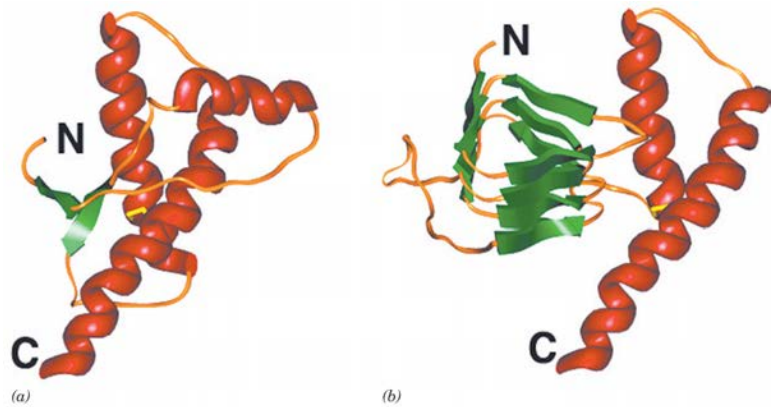
Courtesy Stanley Prusiner, University of California at San Francisco Medical Center

TABLE 6-4 Some Protein Misfolding Diseases

Disease	Defective Protein
Alzheimer's disease	Amyloid-β protein
Amyotrophic lateral sclerosis	Superoxide dismutase
Fibrinogen amyloidosis	Fibrinogen α chain
Huntington's disease	Huntingtin with polyglutamate expansion
Light chain amyloidosis	Immunoglobulin light chain
Lysozyme amyloidosis	Lysozyme
Parkinson's disease	α-Synuclein
Transmissible spongiform encephalopathies (TSEs)	Prion protein

Protein Stability, Folding, and Dynamics

Prion Protein Conformations



Courtesy of Fred Cohen, University of California at San Francisco. Part a based on an NMR structure by Kurt Wüthrich, Eidgenössische Technische Hochschule, Zurich, Switzerland. PDBid 1QLX.]

Human prion protein
PDBid 1QLX

Protein Stability, Folding, and Dynamics

Protein Prediction

If the 1° structure is known, but only the 3° structure of a *related* homologous protein is known, a prediction of your protein can be done by "homology modeling" (threading).

BUT, WHAT IF NO STRUCTURE?

- Given all the known 3D structures, predictions of propensities to find residues and/or sequences of residues in certain structures have been effective.
  - e.g., already discussed propensities of residues to be in α-helices, β-sheets, and β-turns.
- Computer programs can now predict to about 80% certainty where these 2° structures will be in a given 1° sequence.
- But, the overall-fold prediction is not as good.
- As computers are getting better, the *ab initio* calculation of the lowest energy conformations are getting more reliable. e.g., **C**ritical **A**ssessment of protein **S**tructure **P**rediction (CASP) competition in 2018 gave about 31% correct predictions.

Computational protein design http://www.predictioncenter.org/

Protein Stability, Folding, and Dynamics

Protein Dynamics: Mb

Rotomer conformations:
psec → msec

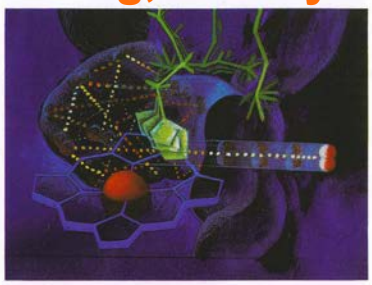
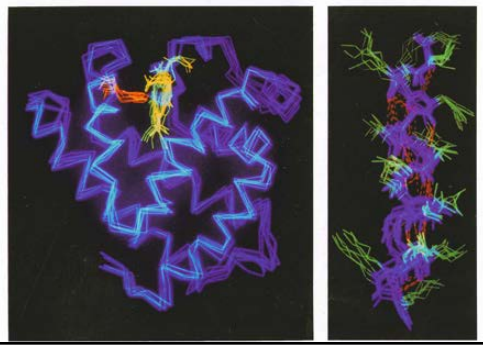


Figure 8-9. "Breathing" motions in myoglobin that permit the escape of its bound O_2.



Domain conformations:
μsec → msec

Molecular dynamics calculations of Mb (Cα-backbone) and the Heme prosthetic group

Protein Stability

Key Concepts

- Protein stability depends primarily on hydrophobic effects and secondarily on electrostatic interactions.
- A protein that has been denatured may undergo renaturation.
- Protein structures are flexible and dynamic; may include unfolded regions.

Protein Folding

Key Concepts

- A folding protein follows a pathway from high energy and high entropy to low energy and low entropy.
- Protein disulfide isomerase catalyzes disulfide bond formation.
- A variety of molecular chaperones assist protein folding via an ATP-dependent bind-and-release mechanism.
- Amyloid diseases result from protein misfolding, with many misfolded proteins forming fibrils withextensive β structure.

Protein Stability

Checkpoint

- Describe the hydropathic index plot for a fibrous protein such as collagen or keratin.
- Describe the forces that stabilize proteins, and rank their relative importance.

Protein Folding

Checkpoint

- Summarize the results of Anfinsen's experiment with RNase A.
- Describe the energy and entropy changes that occur during protein folding.
- Explain why it is important for protein disulfide isomerase to catalyze both the breaking and formation of disulfide bonds.
- How does protein renaturation in vitro differ from protein folding in vivo?
- Explain why a protein such as RNase A can be easily denatured and renatured in vitro, whereas most proteins that are denatured do not refold property in vitro.
- Explain the role of ATP in the action of Hsp70 and GroEL/ES.
- Why would cells need more than one type of chaperone?
- Why do proteins vary in their need for chaperones?
- What are amyloid fibrils, what is their origin, and why are they harmful?