Protein Stability, Folding, and Dynamics

D ⇌ N

(denatured) (native)

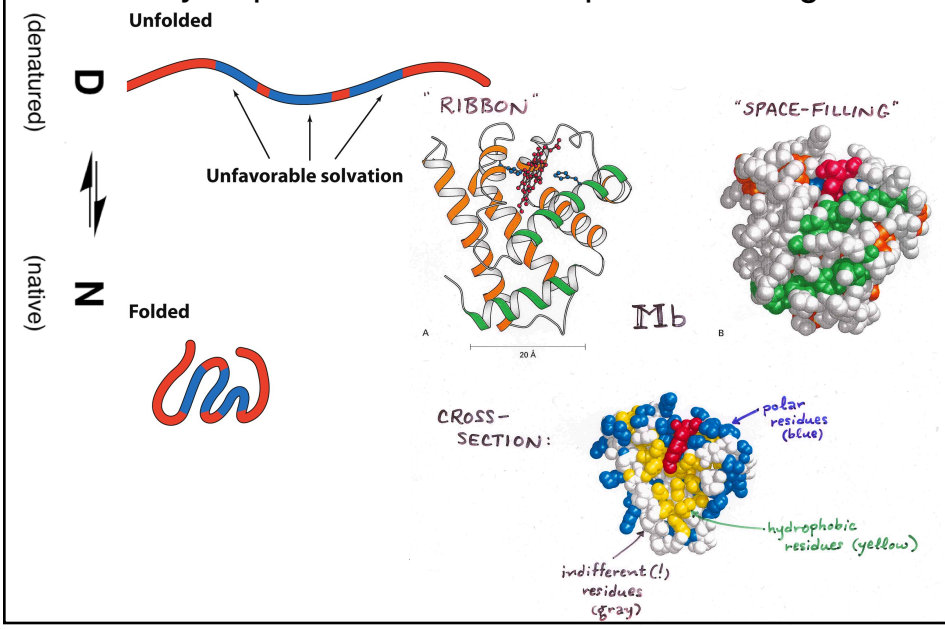
What is the equilibrium?
- Lies to the right
- Therefore, ΔG is negative

What forces operate?
- Non covalent:
  - H-bonds? yes, definitely, but those with water in D-state
  - Ionic (salt-bridges)? yes, but not that many and non specific
  - van der Waals? yes, but not a driving force until there is compaction
  - Hydrophobic? YES, bury hydrophobic residues
- Covalent:
  - Disulfide bonds? yes, but most proteins don't have any

Which force(s) are the most important?
- Hydrophobic!

Protein Stability, Folding, and Dynamics

Hydrophobic effect drives protein folding



Protein Stability, Folding, and Dynamics

Hydrophobic effect drives protein folding

[TABLE 4-3] Hydrophobicity Scales

Residue	Scale A[a]	Scale B[b]
Phe	2.8	3.7
Met	1.9	3.4
Ile	4.5	3.1
Leu	3.8	2.8
Val	4.2	2.6
Cys	2.5	2.0
Trp	−0.9	1.9
Ala	1.8	1.6
Thr	−0.7	1.2
Gly	−0.4	1.0
Ser	−0.8	0.6
Pro	−1.6	−0.2
Tyr	−1.3	−0.7
His	−3.2	−3.0
Gln	−3.5	−4.1
Asn	−3.5	−4.8
Glu	−3.5	−8.2
Lys	−3.9	−8.8
Asp	−3.5	−9.2
Arg	−4.5	−12.3

[a]Scale A is from Kyte, J., and Doolittle, R.F., *J. Mol. Biol.* **157**, 105–132 (1982).

[b]Scale B is from Engelman, D.M., Steitz, T.A., and Goldman, A., *Annu. Rev. Biophys. Chem.* **15**, 321–353 (1986).

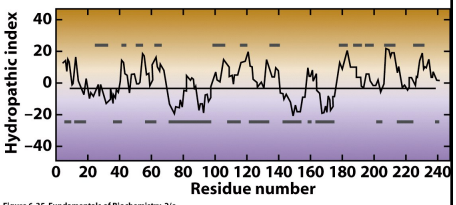


Figure 6-35 Fundamentals of Biochemistry, 2/e
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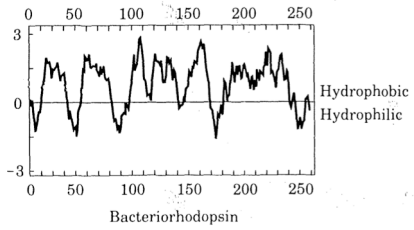
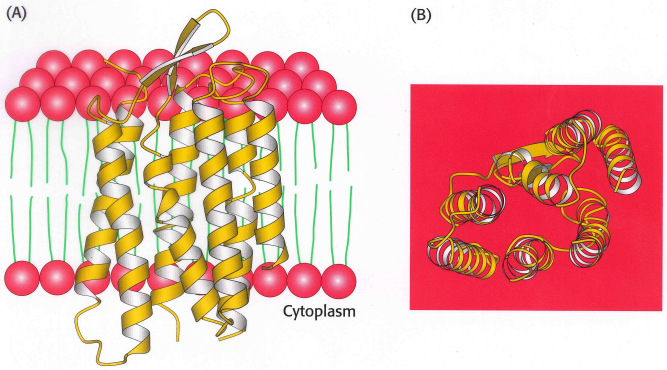
Protein Stability, Folding, and Dynamics

Hydrophobic effect drives protein folding

What about membrane proteins?





ENZYMES: Binding & Catalysis
A. Binding
B. Catalysis
C. Nomenclature
D. Catalysis
1. Transition State Theory
2. Catalytic & Mechanistic strategies
E. Quantifying the Catalytic Power: Kinetics
1. Review of kinetics
2. Enzyme Kinetics (M-M equation; L-B plot; inhibition)
a. M-M equation
b. Lineweaver-Burk; double reciprocal
c. Inhibition
d. Uses of Steady-state kinetics
e. Active-site identification
f. Energetics of Catalysis
F. Enzyme Mechanisms
1. Proteases
2. Serine Proteases
a. Active Site Determination
b. Proposed mechanism
c. Specificity; Chymotrypsin *versus* elastase
3. Other protease mechanisms

Enzyme Regulation
A. Introduction
B. Strategies
1. Gene Regulation
2. Covalent Modification
3. Allosteric Control
C. Covalent Modification
1. Proteolysis
2. Protein modification
a. Phosphorylation
i. Kinases
ii. Phosphatase
i. Example: PKA
D. Allosteric Control
1. Regulation nomenclature
a. Example: ATCase
2. Review; binding curves; why sigmoidal
3. Hill Equation
4. Physical models; 3° and 4° conf. changes
a. T_0 vs. R_6
b. Sequential; KNF
c. Concerted (symmetry); MWC
d. Examples of measuring these parameters

Lecture 20 (10/29/25)

- Reading: Ch4; 128-136
- Homework #20

NEXT
Exam 3

"Enzyme" Regulation: Hemoglobin
A. Roles of Hb
1. Oxygen transport
2. CO_2 binding
3. Blood buffer: Bohr effect
B. Oxygen Binding/role of protein
C. Binding curves
1. oxygen
2. Allosteric effectors (BPG)
3. Bohr effect; (protons)
4. Carbon dioxide
D. Structure-Function; Structural basis for physiology (T & R states)
E. Mechanism of Cooperativity

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
5. Prediction
C. Protein Dynamics

Protein Stability, Folding, and Dynamics

Protein Stability, Folding, and Dynamics

D ⇌ N

(denatured) (native)

What is the magnitude?

About ⁻9-10 kcal/mole = ΔG°'

$$K_{eq} = \frac{[N]}{[D]} = e^{-\Delta G°'/RT}$$

At 25 °C; $= e^{-(-10)/0.59}$

$= 2 \times 10^7$

Protein Stability, Folding, and Dynamics

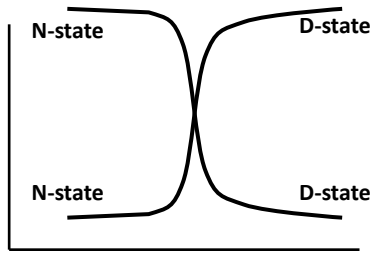
Degradation ← D ⇌ N

Aggregates ← (denatured) (native)

What other fates are there for the D-state?

Degradation (turnover)
Aggregates (precipitation)

What does the change in equilibrium, or transition, look like?



What are some observables?

CD, fluorescence, activity, viscosity, etc.

What are some of these conditions?

Protein Stability, Folding, and Dynamics

What are these conditions?

Disrupt forces that hold protein in tertiary structure

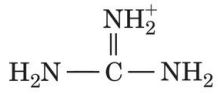
1) Temperature (affects both enthalpy and entropy (-**T**ΔS)
2) pH (changes charges; affects all polar interactions)
3) Detergents (creates micelles; turns proteins inside-out)
4) Chemicals:
   - urea, guanidine HCl (chaotropic agents; complicated)
   - mercaptoethanol, DTT (cleaves disulfide bonds)
   - Salts: Hofmeister Series (stabilizing/destabilizing salts)

Cations: $^{+}NH_4 > {}^{+}Cs > {}^{+}K > {}^{+}Na > {}^{+}Li > {}^{+2}Mg > {}^{+2}Ca > {}^{+2}Ba$

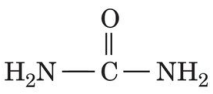
Anions: $^{-2}SO_4 > {}^{-2}PO_4 > CH_3COO^{-} > {}^{-}Cl > {}^{-}Br > {}^{-}I > {}^{-}ClO_4 > {}^{-}SCN$

Stabilizing — Destabilizing

Chaotropic Agents Denature Proteins: Can you see how?



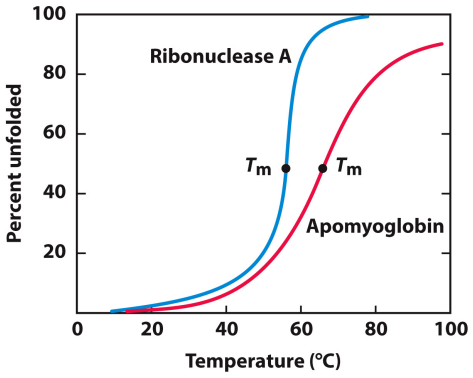
Guanidinium ion

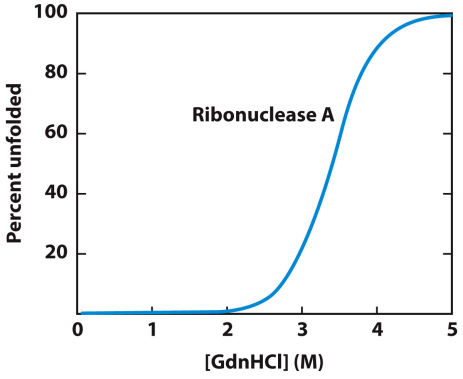


Urea

Protein Stability, Folding, and Dynamics

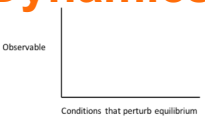
Examples of Protein Denaturation



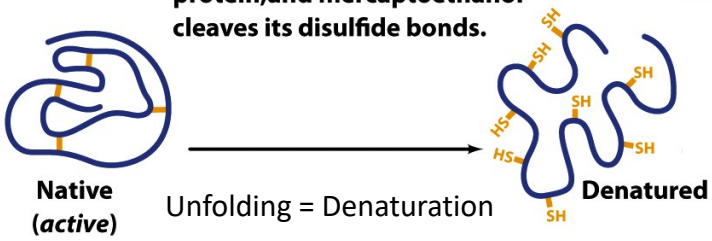


Protein Stability, Folding, and Dynamics

What are these Observables?



8 M urea denatures the protein,and mercaptoethanol cleaves its disulfide bonds.



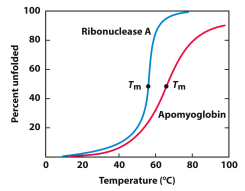
Native (*active*)

Unfolding = Denaturation

Denatured

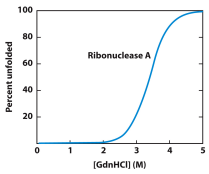
Any techniques that can measure a difference between these two states:

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



fluorescence

CD

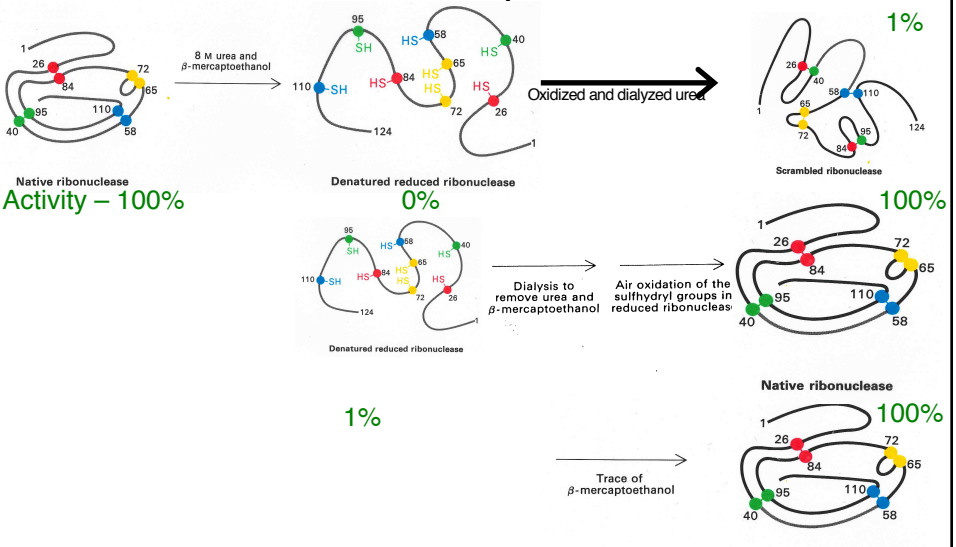


CD

Protein Stability, Folding, and Dynamics

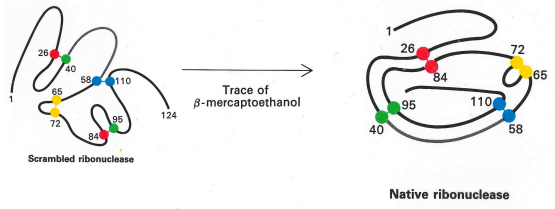
What determines the 3D structure of a given protein?

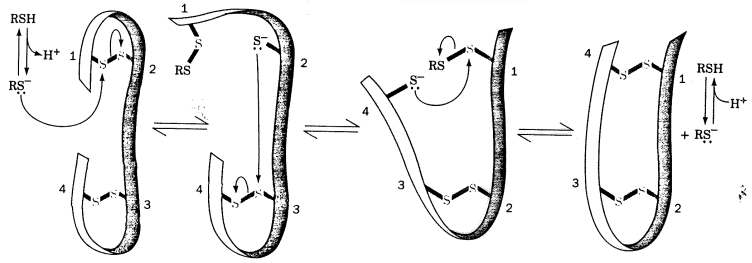
Anfinsen Experiment



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

Primary structure determines Tertiary structure!

Protein Stability, Folding, and Dynamics

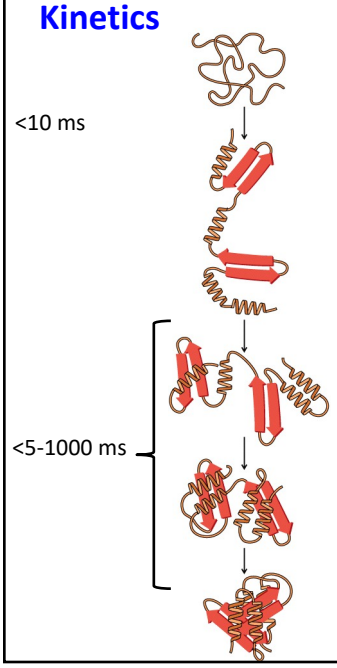
How Can Proteins Fold So Fast?

- Proteins fold to the lowest-energy state in the µsec–sec 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.

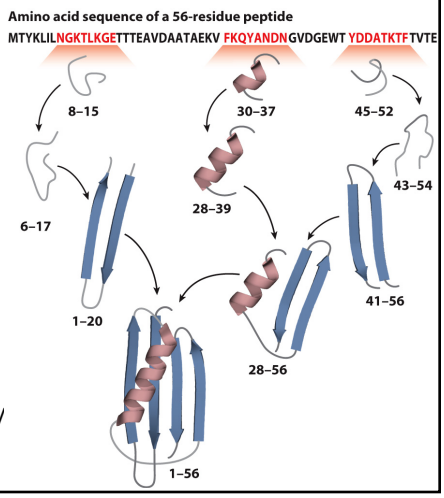
Protein Stability, Folding, and Dynamics

Kinetics

How? Protein folding



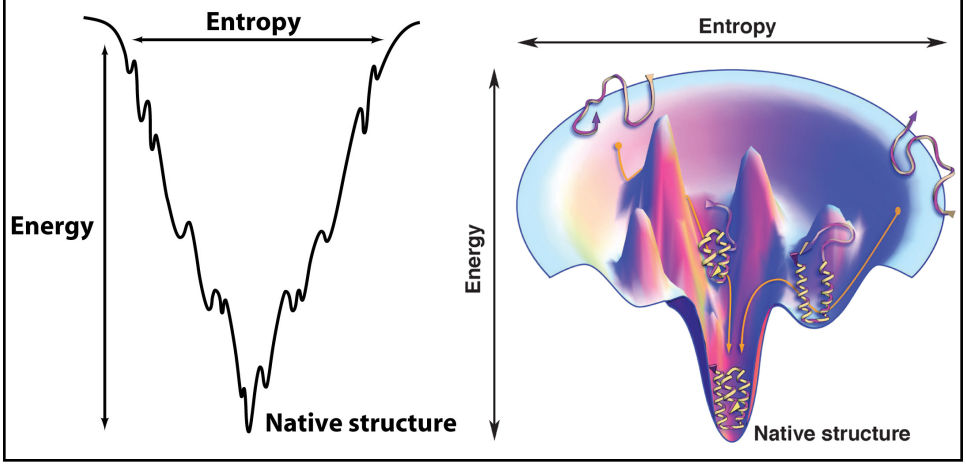
Protein Folding Follows a Distinct Path



Protein Stability, Folding, and Dynamics

Energetics

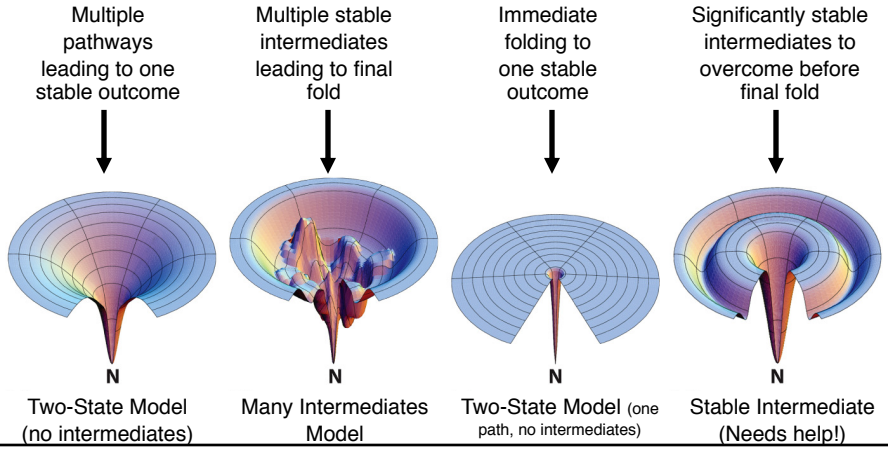
Energy-Entropy Diagram: Protein Folding Funnel



Protein Stability, Folding, and Dynamics

Energy-Entropy Diagram: Protein Folding Funnel

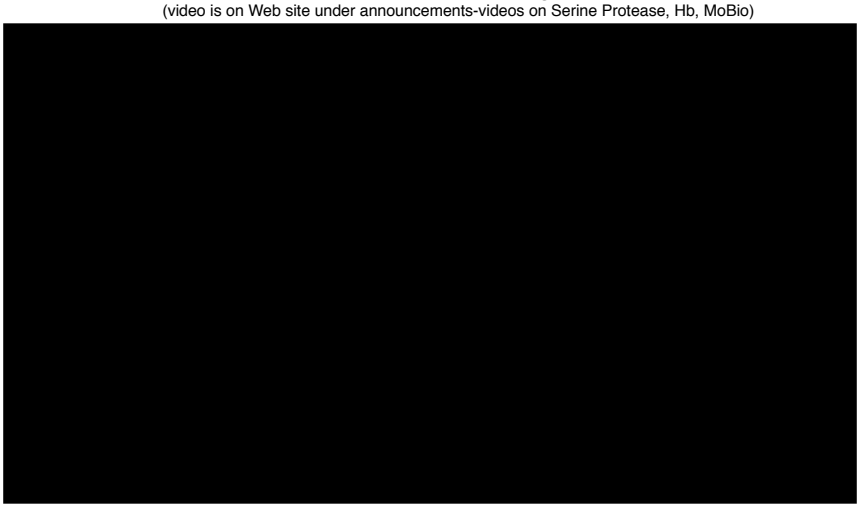
Free-Energy Funnel Model of Protein Folding Is Dependent on the Stability of Motifs Within the Protein



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

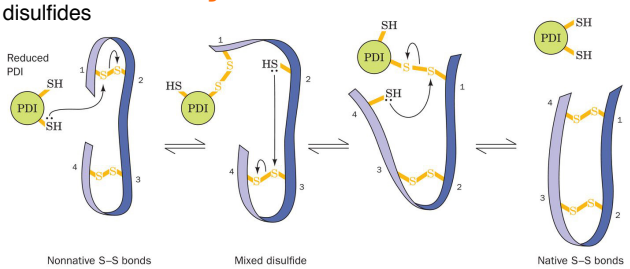
***Thermodynamics**

Protein Stability, Folding, and Dynamics

Kinetic help

Protein Disulfide Isomerase (PDI) Catalyzes Disulfide Interchange

Incorrect disulfides



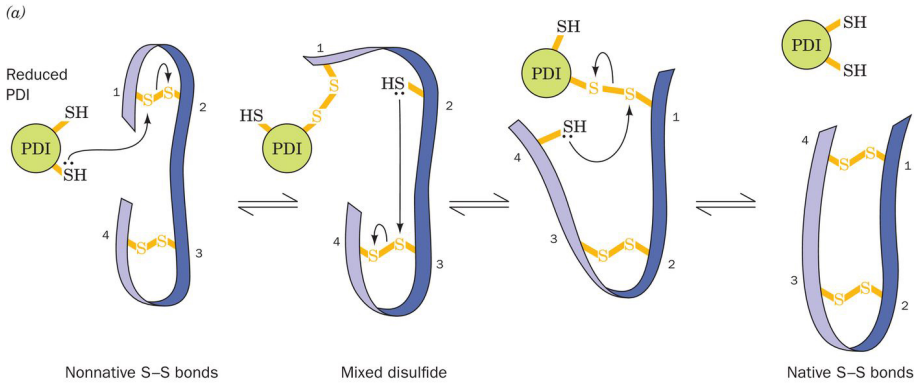
Correct d

Protein Stability, Folding, and Dynamics

Kinetic help

Protein Disulfide Isomerase (PDI) Catalyzes Disulfide Interchange

Incorrect disulfides



Correct disulfides

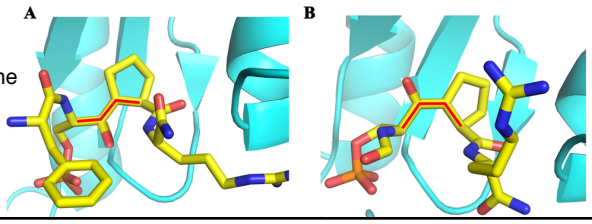
Protein Stability, Folding, and Dynamics

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



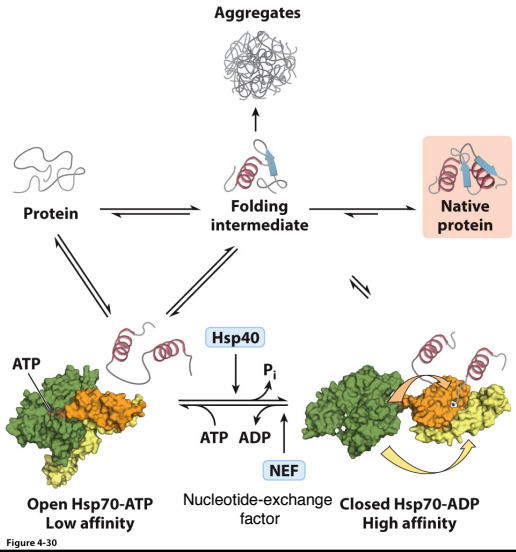
trans-proline

cis-proline

Protein Stability, Folding, and Dynamics

Thermodynamic help

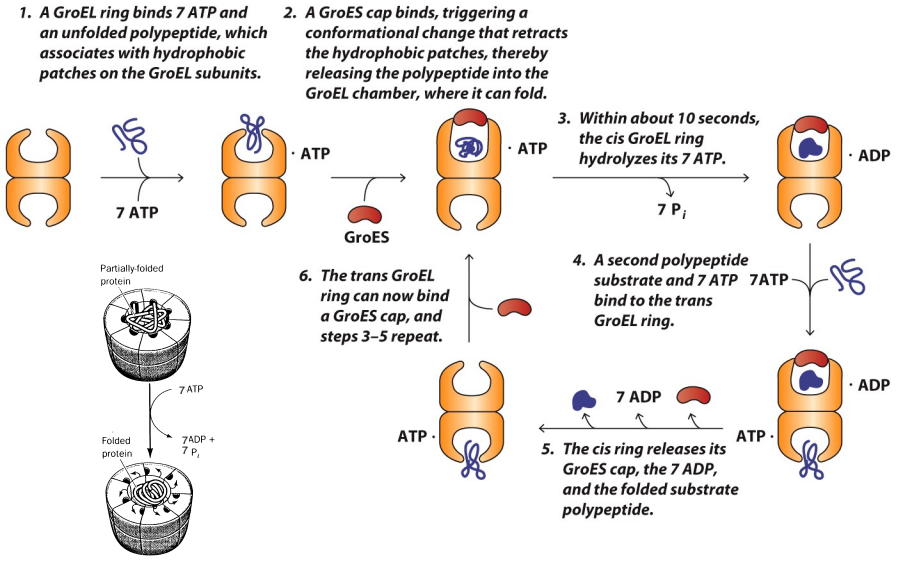
Chaperones Prevent Misfolding and Aggregation



Protein Stability, Folding, and Dynamics

Thermodynamic help

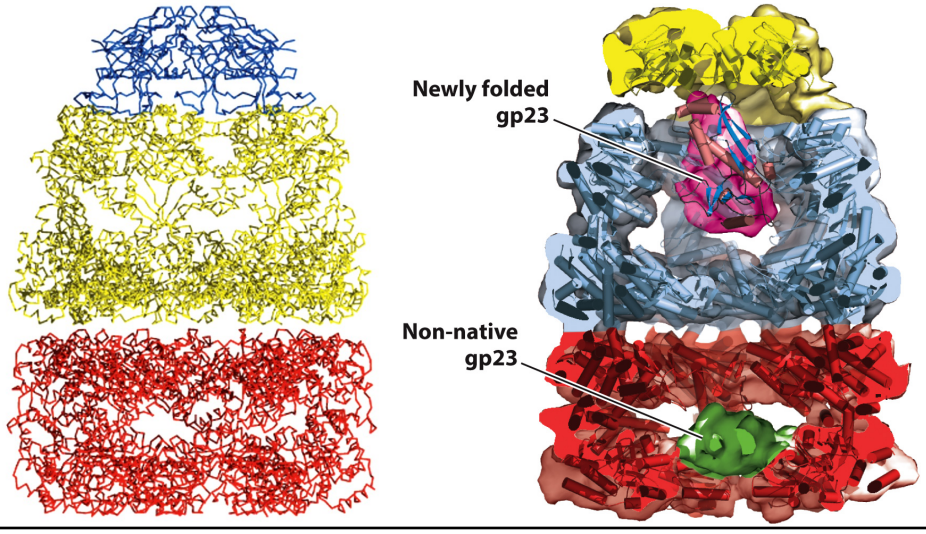
Facilitated folding: Chaperonin



Protein Stability, Folding, and Dynamics

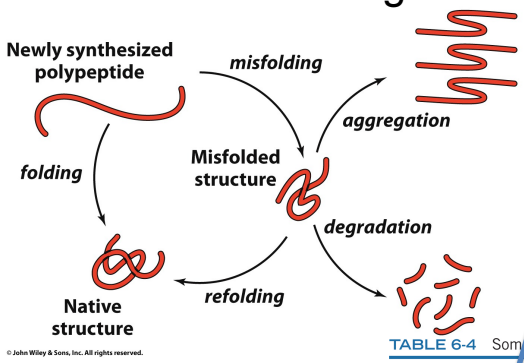
Thermodynamic help

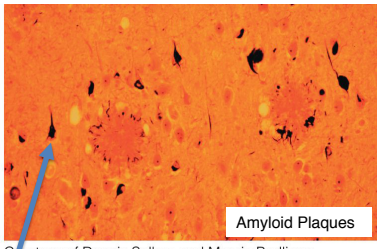
Facilitated folding: Chaperonin



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

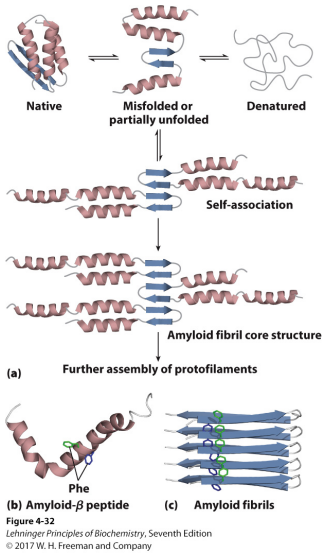
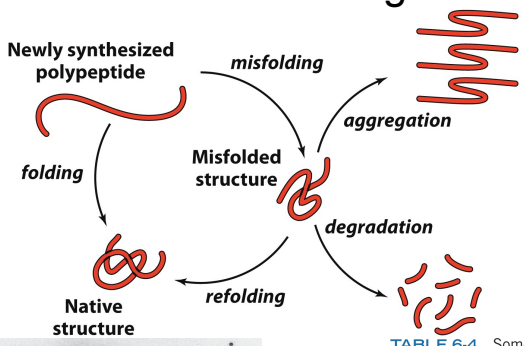


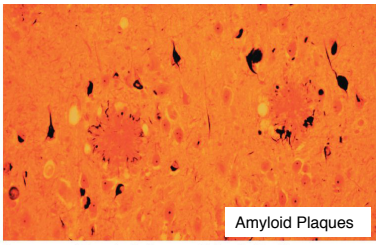
Figure 4-32
Lehninger Principles of Biochemistry, Seventh Edition
© 2017 W. H. Freeman and Company

- 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.

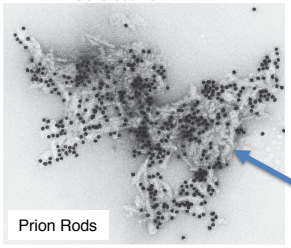
Protein Stability, Folding, and Disease

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

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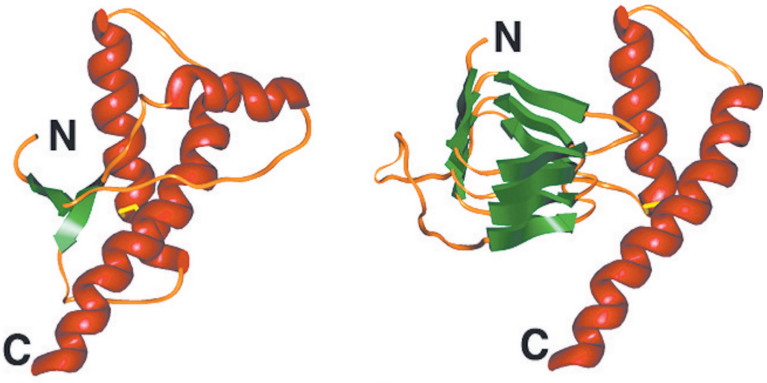
Light chain amyloidosis	Immunoglobulin light chain
Lysozyme amyloidosis	Lysozyme
Parkinson's disease	α-Synuclein
Transmissible spongiform encephalopathies (TSEs)	Prion protein

Protein Stability, Folding, and Dynamics

Prion Protein Conformations



(a) (b)

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).

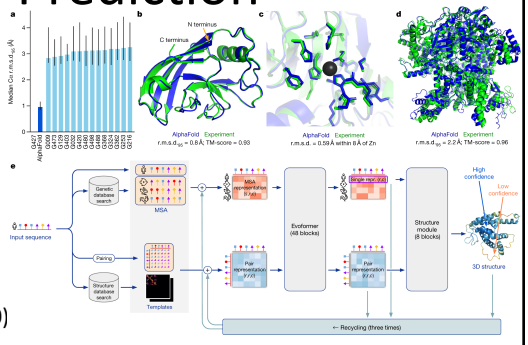
BUT, WHAT IF NO STRUCTURE?

- 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.
- Then came Alpha-fold……

Protein Stability, Folding, and Dynamics

Protein Prediction

AlphaFold: Deep Learning for Protein Structure Prediction Revolutionizing Protein Structure Prediction

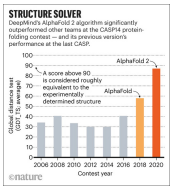


Input: Amino acid sequence → **Model:** Deep learning network → **Output:** 3D protein fold

Overview:
Developed by DeepMind, Lead by Demis Hassabis and John Jumper. They a**chieved** **n**ear-experimental accuracy (CASP14, 2020)

How It Works:
Uses deep neural networks with **attention** and **transformer** architecture; essentially AI. Trained on **Protein Data Bank (PDB)** structures and **evolutionary MSAs**.

There is now an AlphaFold DB with over **200 million** publicly available structures. Extended to **AlphaFold3** (handles complexes, RNA, ligands).



Demis Hassabis John Jumper David Baker
Nobel Prize in Chemistry 2024

Protein Stability, Folding, and Dynamics

Protein Dynamics: Mb

Rotomer conformations:
psec → msec

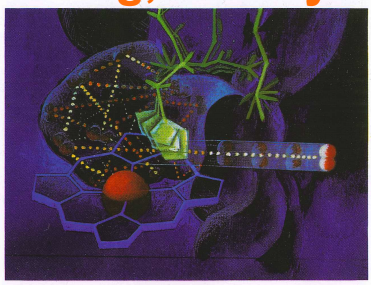
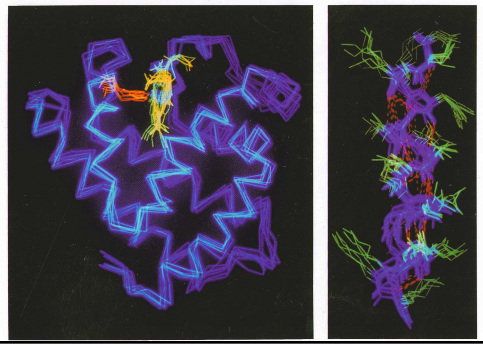


Figure 8-9. "Breathing" motions in myoglobin that permit the escape of its bound O_2.



Domain conformations:
μsec → msec

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