ENZYMES

A. Binding
B. Catalysis
C. Nomenclature
D. Catalysis
E. Quantifying the Catalytic Power: Enzyme Kinetics: Michaelis-Menten Kinetics
   1. Inhibition: Competitive, Uncomp., Noncomp.
F. Active-site identification
G. Energetics of Catalysis
H. Enzyme Mechanisms
   1. Proteases
   2. Serine Proteases
      a. Proposed mechanism
      b. Specificity
   3. Other protease mechanisms
I. Enzyme Regulation
   1. Covalent Modification
      a. Proteolysis
      b. Phosphorylation
   2. Allosteric Control
      a. Regulation nomenclature
      b. Review; binding curves; why sigmoidal
      c. Hill Equation
      d. Physical models; Sequential; KNF, Concerted; MWC
J. Hemoglobin
   1. Roles of Hb
      a. Oxygen transport
      b. CO_2 binding
      c. Blood buffer: Bohr effect
   2. Oxygen Binding/role of protein
   3. Binding
      a. Oxygen; curves
      b. Allosteric effectors
         i. BPG
         ii. Bohr effect; (protons)
         iii. Carbon dioxide

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

NEXT
- EXAM 3
- Reading: Ch7; 229-235

Lecture 20 (10/30/24)

   4. Structure-Function; Structural basis for physiology (T & R states)
   5. Mechanism of Cooperativity
   6. Isoforms during development
   7. Adaptations for high altitude
   8. Molecular disease: sickle-cell anemia

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. Protein Dynamics
D. Protein Prediction
   1. Alpha-fold

Protein Stability, Folding, and Dynamics

Problem

- Which substitution would be more likely to disrupt a protein's structure?
- Val replaced by: Ala or Phe
- Lys replaced by: Asp or Arg
- Gln replaced by: Glu or Asn
- Pro replaced by: His or Gly

Protein Stability, Folding, and Dynamics

Degradation

D ⇌ N

Aggregates

(denatured) (native)

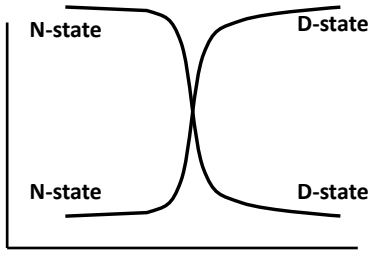
$\Delta G°' = {}^{-}9\text{-}10$ kcal/mole

$K_{eq} = 2 \times 10^{7}$ at 25 °C

What other routes are there for the D-state?

Degradation (turnover)
Aggregates (precipitation)

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



Observable

What are some observables?

CD, fluorescence, activity, viscosity, etc.

Conditions that perturb equilibrium

What are some of these conditions?

Protein Stability, Folding, and Dynamics

What are these conditions?

Disrupt forces that hold protein in tertiary structure

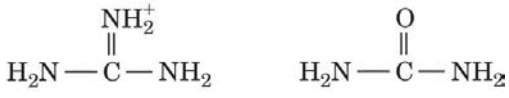
1) Temperature (affects both enthalpy and entropy (-**T**ΔS)
2) pH (changes charges; affects all polar interactions)
3) Detergents (creates micelles; turns proteins inside-out)
4) Chemicals:
   - urea, guanidine HCl (chaotropic agents; complicated)
   - mercaptoethanol, DTT (cleaves disulfide bonds)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

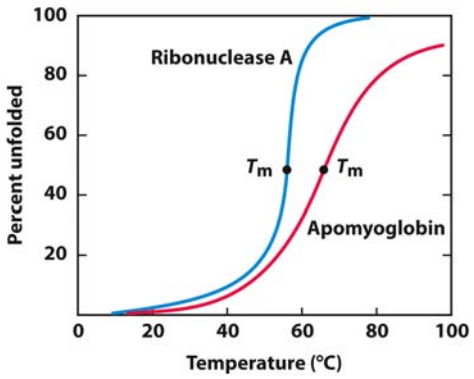
Chaotropic Agents Denature Proteins: Can you see how?

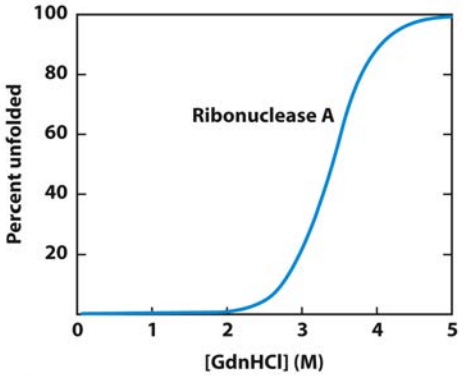


Guanidinium ion Urea

Protein Stability, Folding, and Dynamics

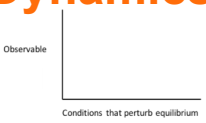
Examples of Protein Denaturation

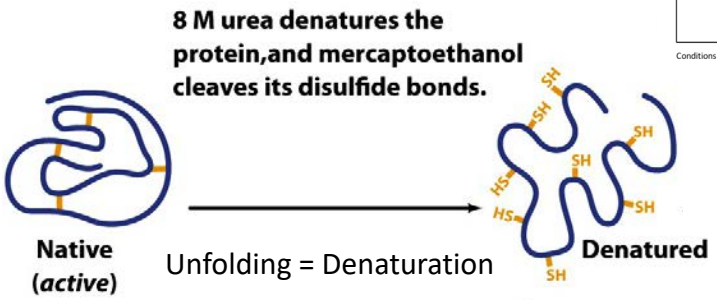




Protein Stability, Folding, and Dynamics

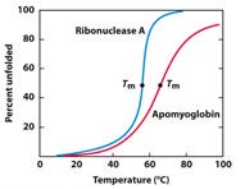
What are these Observables?





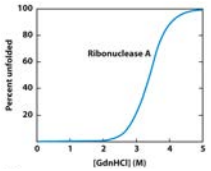
Any techniques that can measure a difference between these two states:

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



fluorescence

CD

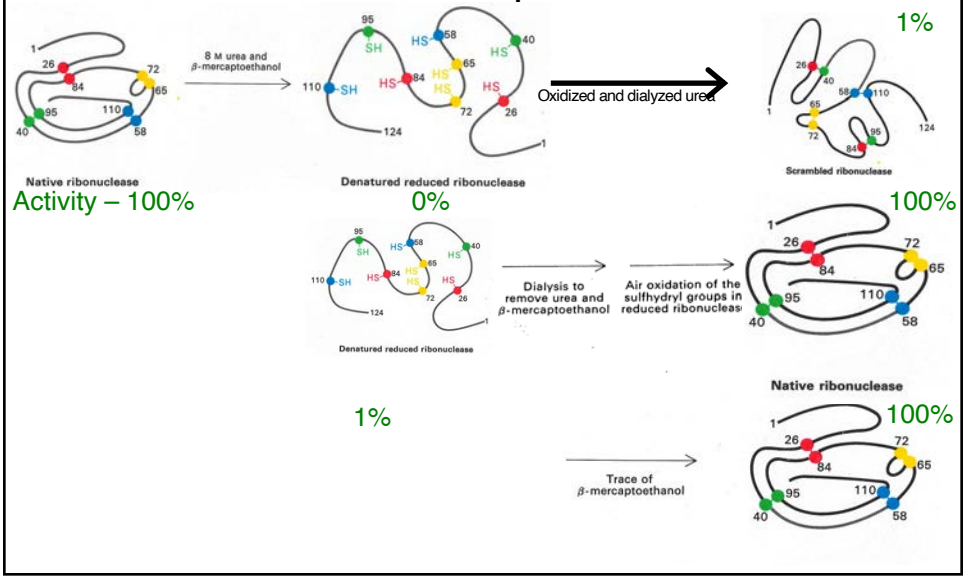


CD

Protein Stability, Folding, and Dynamics

What determines the 3D structure of a given protein?

Anfinsen Experiment



Activity – 100%

0%

1%

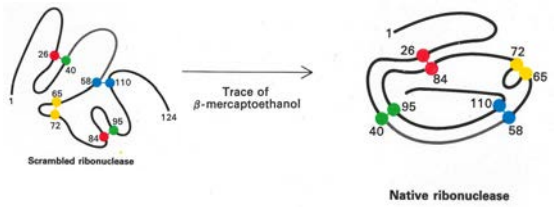
100%

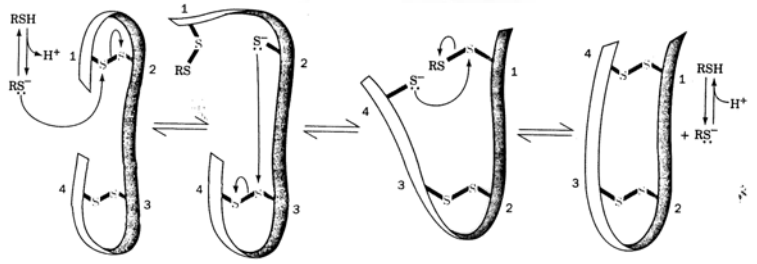
1%

100%

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.

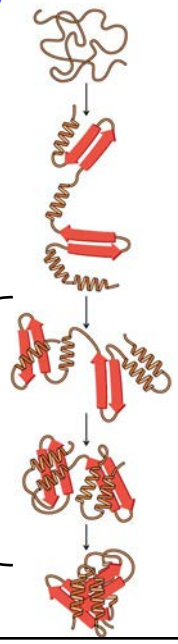
Protein Stability, Folding, and Dynamics

Kinetics

How? Protein folding

<10 ms

<5-1000 ms



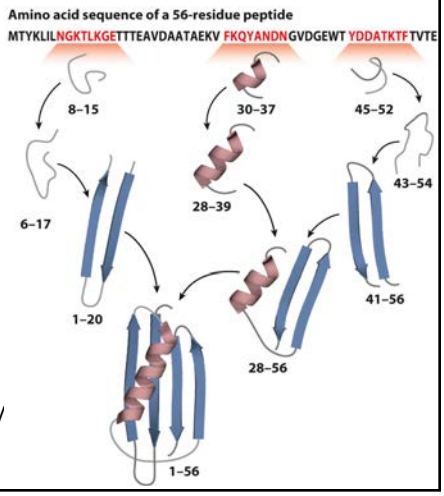
2° Structures form

3° Structural Domains form

Molten Globule

3° Structure forms/ structure forms

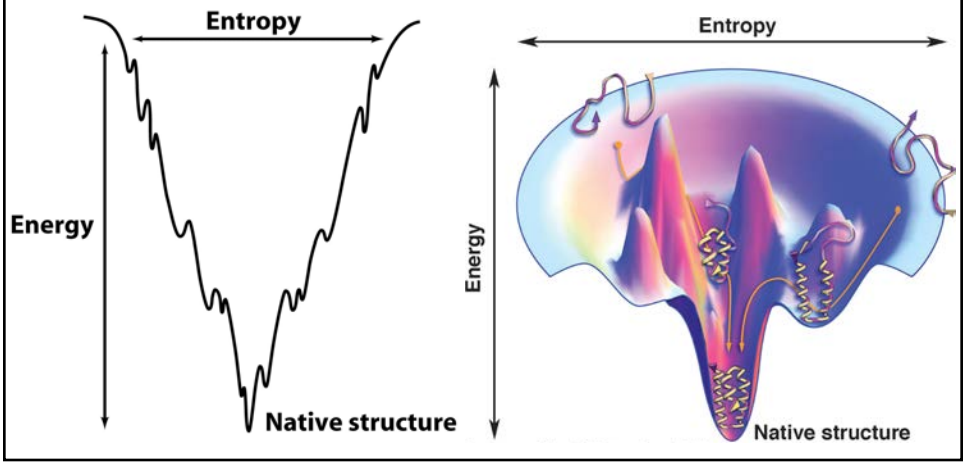
Protein Folding Follows a Distinct Path



Protein Stability, Folding, and Dynamics

Energetics

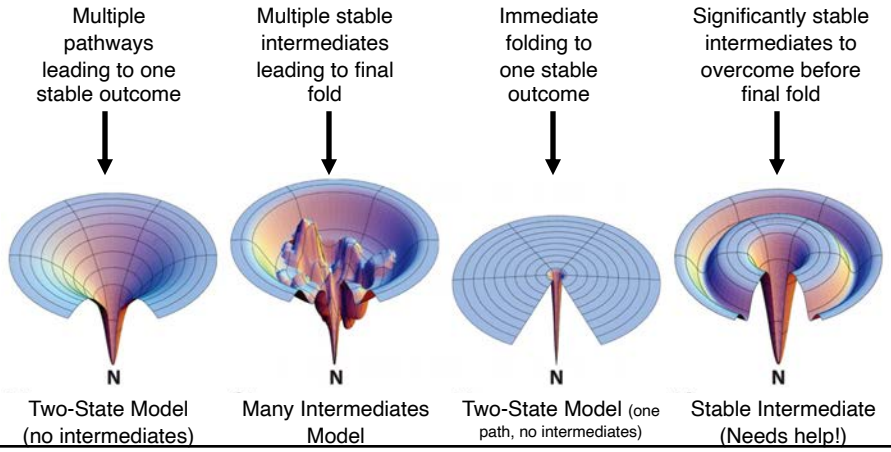
Energy-Entropy Diagram: Protein Folding Funnel



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

Protein folding help

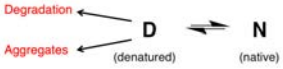
While the primary structure dictates what the fold will be, IN THE CELL, proteins often need help.

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



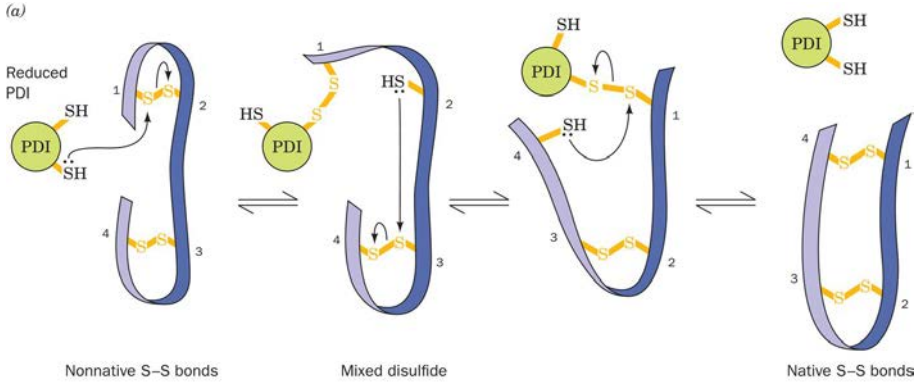
*Thermodynamics

Protein Stability, Folding, and Dynamics

Kinetic help

Protein Disulfide Isomerase (PDI) Catalyzes Disulfide Interchange

Incorrect disulfides



Correct disulfides

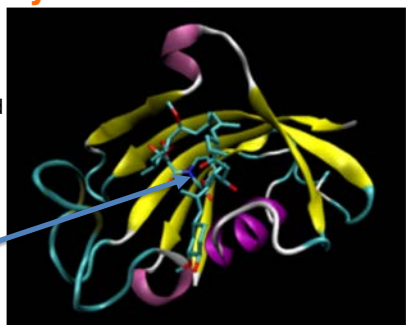
Protein Stability, Folding, and Dynamics

Kinetic help

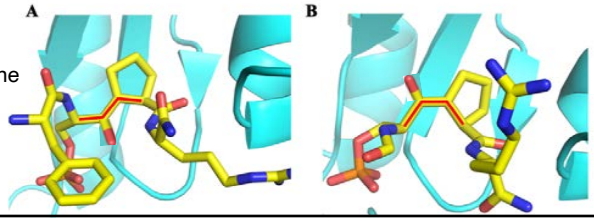
Peptidyl Prolyl Isomerase (PPI) Catalyzes *trans-to-cis* isomerization

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)

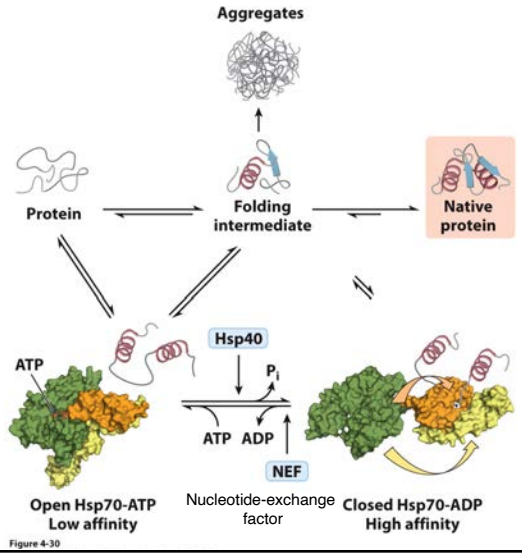
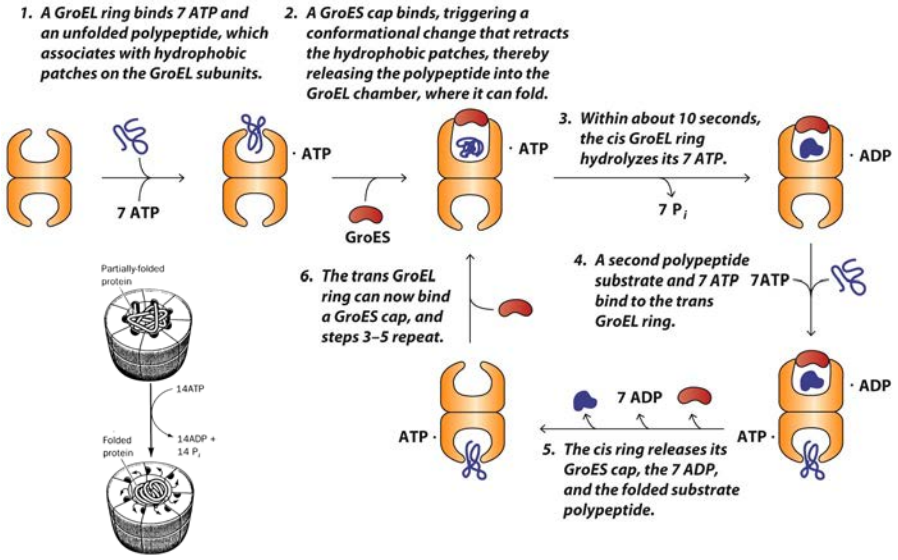


Figure 4-30

Protein Stability, Folding, and Dynamics

Thermodynamic help

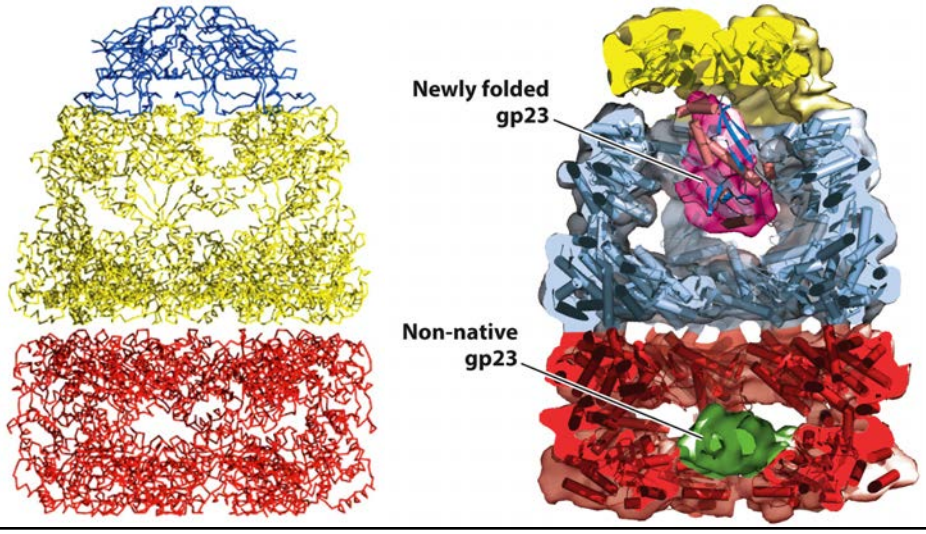
Facilitated folding: Chaperonin



Protein Stability, Folding, and Dynamics

Thermodynamic help

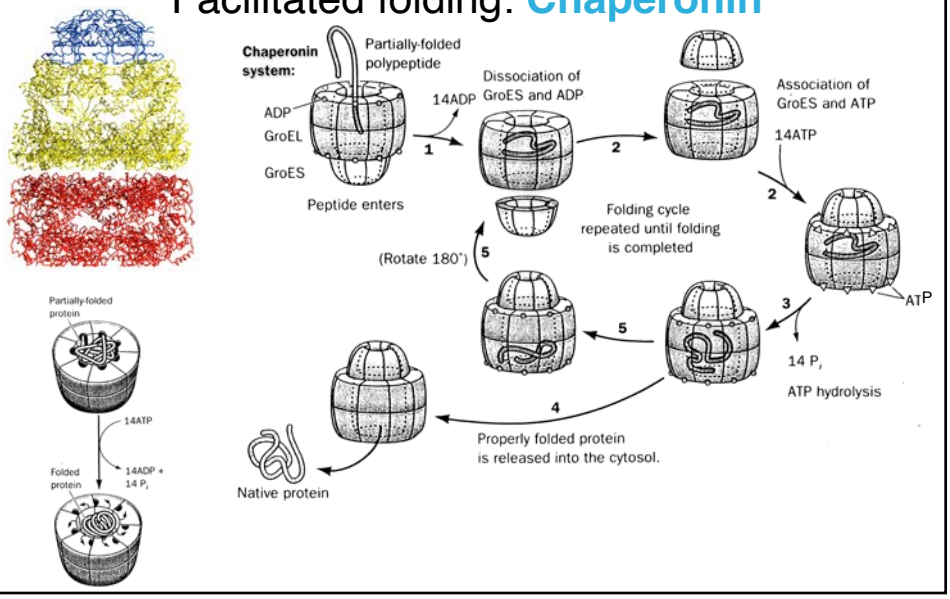
Facilitated folding: Chaperonin



Protein Stability, Folding, and Dynamics

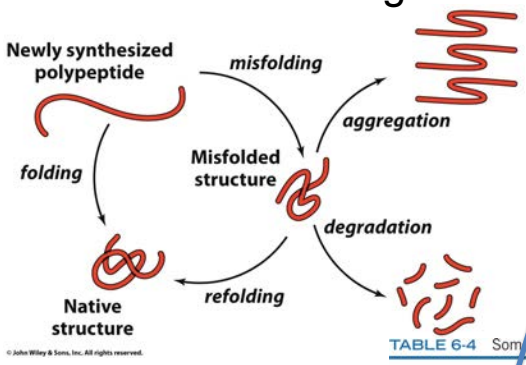
Thermodynamic help

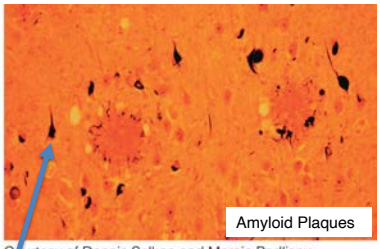
Facilitated folding: Chaperonin



Protein Stability, Folding, and Disease

Misfolding and Disease



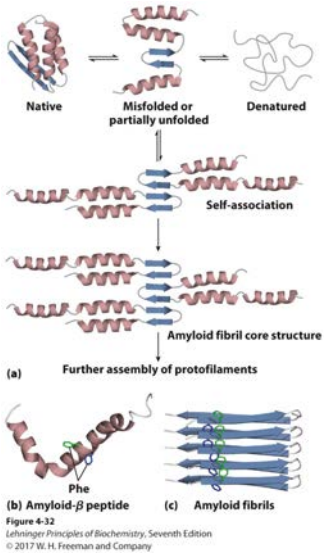


Courtesy of Dennis Selkoe and Marcia Podlisny, Harvard University Medical School

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

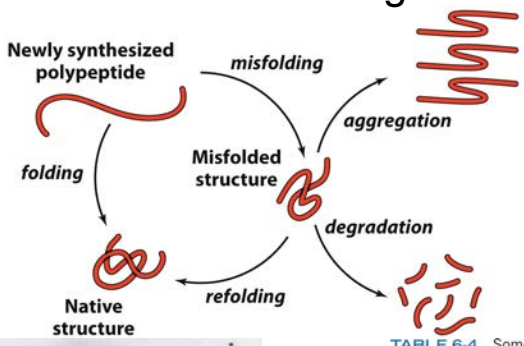
Protein Misfolding Is the Basis of Numerous Human Diseases

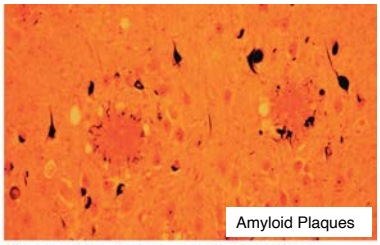


- Native (correctly folded) β amyloid is a soluble globular protein,
- Misfolded β amyloid promotes aggregation at newly exposed protein-protein interface.
- Correctly folded helices are lost and peptides form β strands, β helices, and β sheets.

Protein Stability, Folding, and Disease

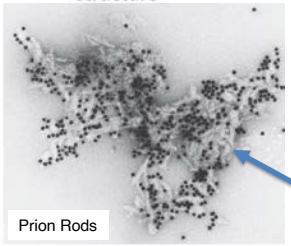
Misfolding and Disease





Amyloid Plaques

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



Prion Rods

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
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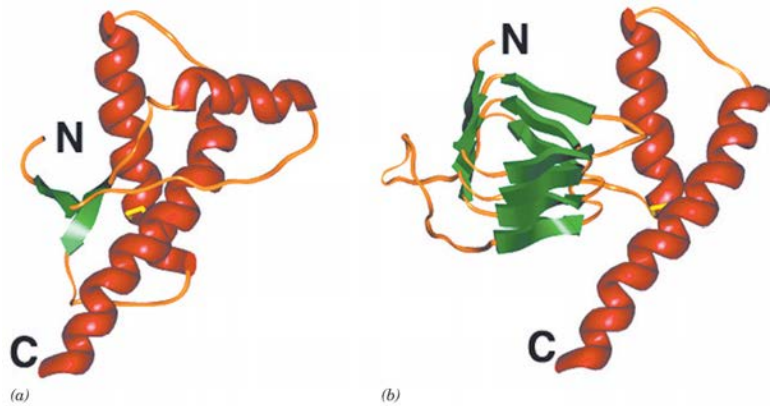
Fibrinogen amyloidosis	Fibrinogen α chain
Huntington's disease	Huntingtin with polyglutamate expansion
Light chain amyloidosis	Immunoglobulin light chain
Lysozyme amyloidosis	Lysozyme
Parkinson's disease	α-Synuclein
Transmissible spongiform encephalopathies (TSEs)	Prion protein

Protein Stability, Folding, and Dynamics

Prion Protein Conformations



(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 Dynamics: Mb

Rotomer conformations:
psec → msec

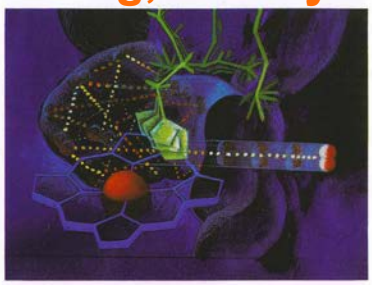
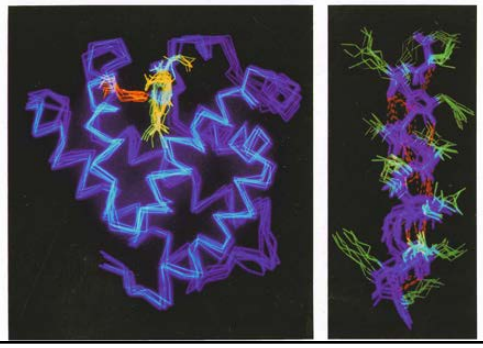


Figure 8-9. "Breathing" motions in myoglobin that permit the escape of its bound O_2.



Domain conformations:
μsec → msec

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

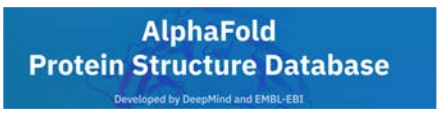
Protein Stability & Protein Folding and Dynamics

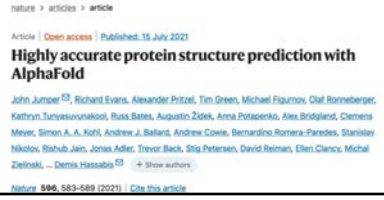
Protein Prediction

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 were developed to predict to about 90% certainty where these 2° structures will be in a given 1° sequence. And, the overall-fold prediction was pretty good (>80%). See Alpha-Fold:





Protein Stability & Protein Folding and Dynamics

Protein Prediction

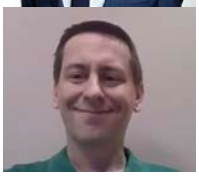
- Alpha-Fold**2**, with the incorporation of AI, can now predict the structure to >95% accuracy.

"Since their breakthrough, AlphaFold2 has been used by more than two million people from 190 countries.
Life could not exist without proteins. That we can now predict protein structures and design our own proteins confers the greatest benefit to humankind."

The Nobel Prize in Chemistry 2024

David Baker (University of Washington) for computational protein design

Demis Hassabis
Google DeepMind, London, UK



John Jumper
Google DeepMind, London, UK
"for protein structure prediction"

Detailed account of how **AlphaFold2** works will be online.

Rosetta is computerized methods for achieving what many people believed was impossible: creating proteins that did not previously exist and which, in many cases, have entirely new functions.