BIOCH 755: Biochemistry I Fall 2015

5. Protein Stability and Protein Folding (6.4-5)

Jianhan Chen
Office Hour: M 1:30-2:30PM, Chalmers 034
Email: jianhanc@ksu.edu
Office: 785-2518

6.4 Protein Stability

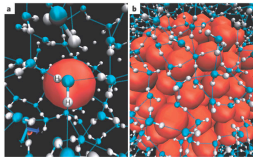
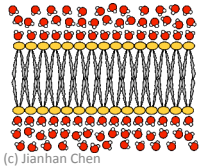
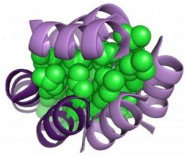
- Key concepts
  - Protein stability depends primarily on hydrophobic effects and secondarily on electrostatic interactions.
  - A protein that has been denatured may undergo renaturation.
  - Protein structures are flexible and may include unfolded regions.

Proteins Are Weakly Stable

- Typical proteins gain ~0.4 KJ/mol stability per residue
  - A typical 100-residue protein is ~40 KJ/mol stable (merely equivalent of a couple hydrogen bonds!)
- The stability depends on a range of factors
  - Intramolecular interactions
    - Bonded: chemical bonds, angles, dihedrals etc
    - Nonbonded: "weak" interactions
      - Charged-charged, van der Waals (dispersion and repulsion)
  - Intermolecular interactions: nonbonded/weak interactions
    - Cellular environment: solvent (water), membrane, salt, pH etc
    - Association with other biomolecules, small molecules, ions, etc
- Protein stability/structure are results of extremely delicate balance among powerful countervailing forces!

Hydrophobic Effect

- The property that nonpolar solutes aggregate in water
- Arise from a combination of elemental physical effects
  - Difference in strengths solute-water and water-water interactions
  - Difference in shapes (sizes) of solutes and water
  - Various entropic contributions
- Considered the main driving force of folding (as well as most self-assembly processes in biology)
  - Biological membrane, micelle formation, protein folding …
  - Complex temperature dependence: cold denaturation of proteins
  - Very difficult to describe theoretically!

Hydropathy

- Quantify "hydrophobic/hydrophilic tendencies
- +: hydrophobic
- -: hydrophilic
- Good indicator of side chain burial

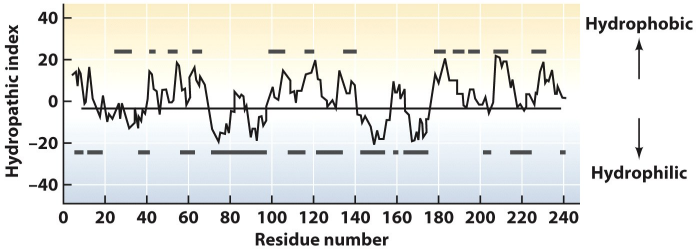


Figure 6-35

Bonvine chymotrypsinogen

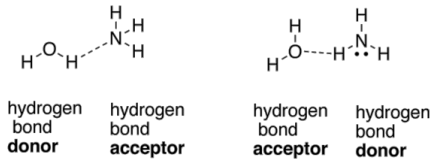
TABLE 6-3 **Hydropathy Scale for Amino Acid Side Chains**

Side Chain	Hydropathy
Ile	4.5
Val	4.2
Leu	3.8
Phe	2.8
Cys	2.5
Met	1.9
Ala	1.8
Gly	−0.4
Thr	−0.7
	−0.8
	−0.9
	−1.3
	−1.6
	−3.2
	−3.5
	−3.5
	−3.5
	−3.5
	−3.9
	−4.5

Source: Kyte, J. and Doolittle, R.F., *J. Mol. Biol.* 157, 110 (1982).

Electrostatic Interactions

- Hydrogen bonds: very important in macromolecule structures
  - Primarily a dipole-dipole interaction, but arguably with some covalent nature (electron sharing in so-called low barrier HBs)



  - The strength of HBs vary greatly and depend on the environments (dielectric screening)
- Salt-bridges: association of opposite charges
  - mostly on protein surface (or at protein-protein interface)
  - Important for specificity but often non-stabilizing or even destabilizing

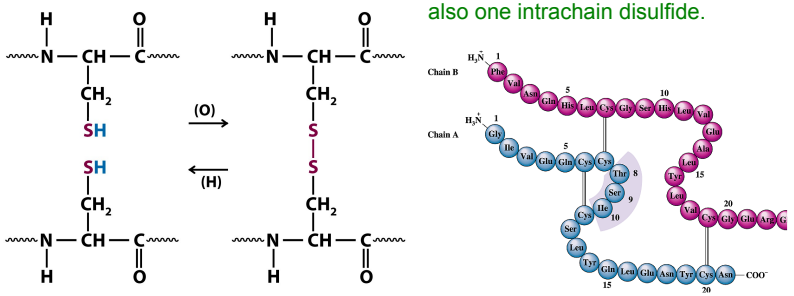
What is a hydrogen bond worth?

Secondary Structure	Stability per H-bond	Model	Reference State
Antiparallel β-sheet	-2.8	$[Ac\text{-}ala\text{-}NHMe]_2$	Infinite separation
Ala-gly Type II turn	-0.6	Ac-ala-gly-NHMe	Extended
Amide H-bond	-0.3	$[formamide]_2$	Infinite separation
1st helical H-bond	-0.2	$Ac\text{-}(ala)_2\text{-}NHMe$	extended
2nd helical H-bond	-0.4/-1.0	$Ac\text{-}(ala)_4\text{-}NHMe$	extended
Ala-gly Type I turn	2.6	Ac-ala-gly-NHMe	extended
Pro-gly Type I turn	2.6	Ac-pro-gly-NHMe	extended

Other Stabilizing Factors

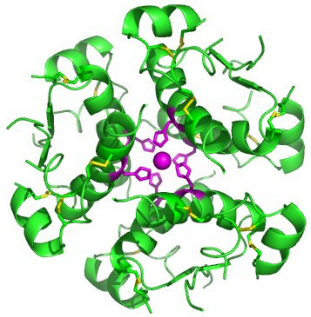
- Disulfide Bonds: frequently involved in stabilizing small proteins and extracellular proteins (oxidative conditions)
  - Not as common for intracellular proteins due to the reducing environment

The small protein insulin has two polypeptide chains connected by two interchain disulfide bonds. There is also one intrachain disulfide.



Other Stabilizing Factors

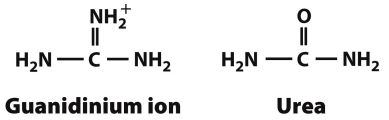
- Disulfide Bonds: frequently involved in stabilizing small proteins and extracellular proteins (oxidative conditions)
  - Not as common for intracellular proteins due to the reducing environment
- Metal ions: many small proteins require binding of ions for maintaining stable folds
  - Common ions: Ca^{2+}, Zn^{2+}
  - associate with Asp/Glu/His/Cys residues)



High-resolution model of six insulin molecules assembled in a hexamer, highlighting the threefold symmetry, the zinc ion holding it together (pink sphere), and the histidine residues (pink sticks) involved in zinc binding. Inactive insulin is stored in the body as a hexamer, while the active form is the monomer (from wikipedia)

Protein Denaturation

- Weakly stable and can be readily denatured by temperature, denaturants, changing pH, etc
  - Offer regulatory mechanisms in cell
- Two commonly used denaturants:



- Many proteins can spontaneous refold once returned to their "native" conditions
- Protein folding and misfolding

Denaturation and Refolding of RNase A

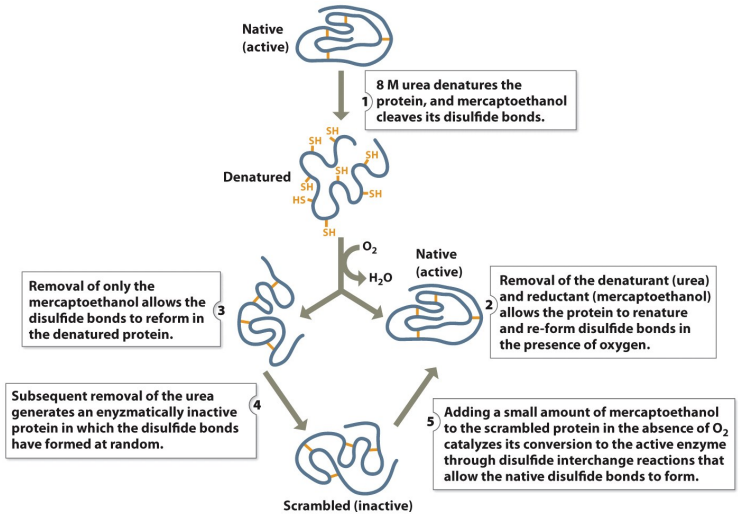


Figure 6-38

Protein Dynamics

- Proteins are living molecules!
- Thermal fluctuations
- Function cycles often involve conformational transitions
- Again, many proteins are "intrinsically disordered"!

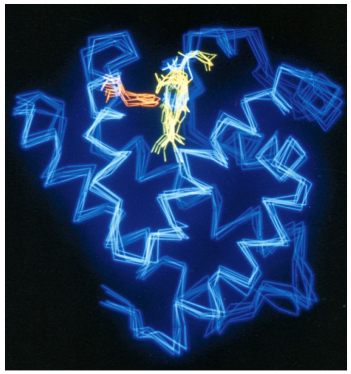


Figure 6-39
Courtesy of Martin Karplus, Harvard University

Molecular Dynamics of Myoglobin: Proteins "Breathing"

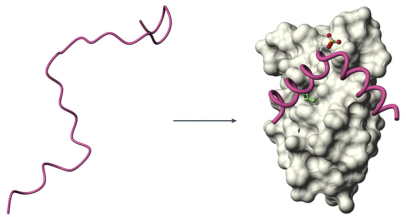


Figure 6-40
Courtesy of Peter Wright, Scripps Research Institute, La Jolla, California

Coupled binding and folding of KID

6.5 Protein Folding

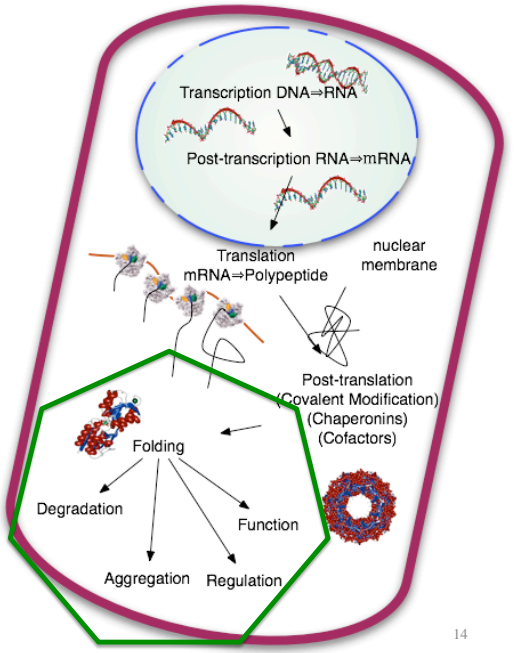
- **Key Concepts 6.5**
  - A folding protein follows multiple pathways from high energy and high entropy to low energy and low entropy.
  - Protein disulfide isomerase catalyzes disulfide bond formation.
  - A variety of molecular chaperones assist protein folding via an ATP-dependent bind-and-release mechanism.
  - Amyloid diseases result from protein misfolding.
  - The misfolded proteins form fibrils containing extensive β structure.

Protein Folding

The newly synthesized peptide chain needs to fold into specific 3D structures before finally becoming functional proteins!

It is a spontaneous process!

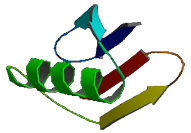
We need to understand how it works!



The Protein Folding Problem

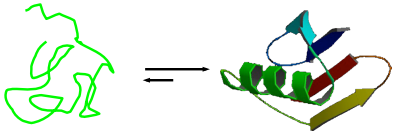
- How does the primary Sequence specify the native fold? (Afinsin, Science, 1973)

MTYKLILNGK TLKGETTTEA
VDAATAEKVF KQYANDNGVD
GEWTYDDATK TFTVTE



- Levinthal's Paradox: how protein folds this fast?

τ_{fold} < 1 second



10 conformations per residue, 10^{-11} (10 ps) per state

10^{60} states for a 60-residue protein, $>10^{40}$ years for random search !

Folding Pathways?

- Hydrophobic collapse
- 2nd structures form
- Tertiary repacking

- Such view is over simplification of the fundamentally diffusive process of protein folding!

- Funneled energy landscape: broad pathways of folding guided by underlying free energy landscapes that are smooth and funneled towards native folds

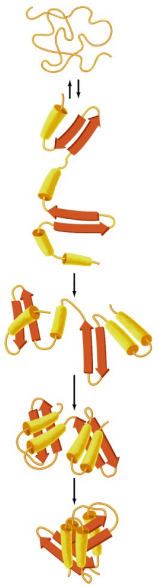
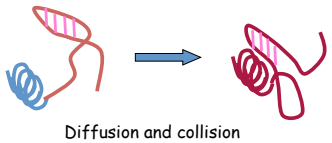


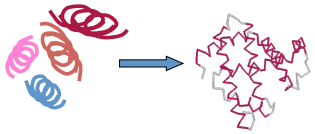
Figure 6-41

How proteins fold?

- "Classical" understandings
  - Diffusion-collision
  - Hierarchical folding
  - Assembly of foldons

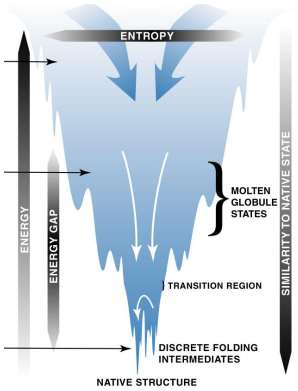


Diffusion and collision
Karplus and Weaver, *Biopolymers.*, 18, 1421 ('77).



Folding via modular assembly
Ptitsyn and Rashin, *Biophys. Chem.*, 3, 1 ('75).

The Energy Landscape Theory
(arguably the prevailing theory)



Wolynes et al., *Science* (1995).

Protein Disulfide Isomerase (PDI) Catalyzes Disulfide Interchange

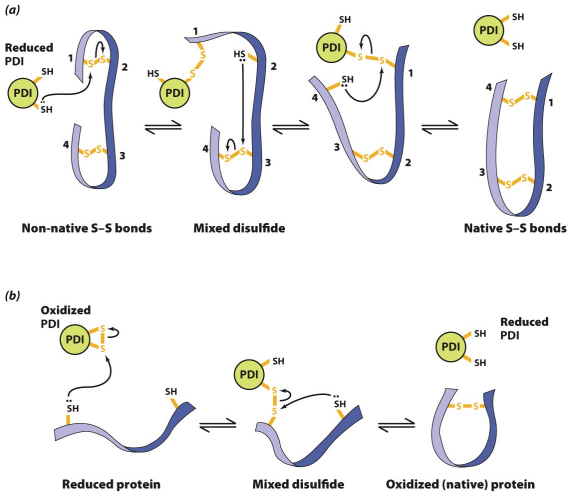


Figure 6-43

Molecular Chaperones

- Assist protein folding: bind to unfolded/misfolded proteins to prevent aggregation and allow re-folding
- Heat shock proteins (Hsp): elevated level under heat shock (many proteins can misfold under heat shock and thus an increase need for chaperones)
- Most chaperones require ATP:

GroEL/ES

- Central chamber
- Large-scale conformational transitions involved function

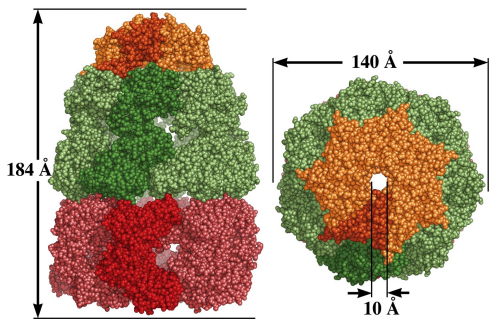


Figure 6-44ab

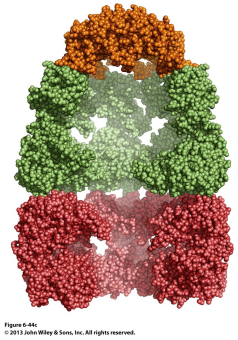


Figure 6-44c

Process Diagram: GroEL/ES Chaperonin

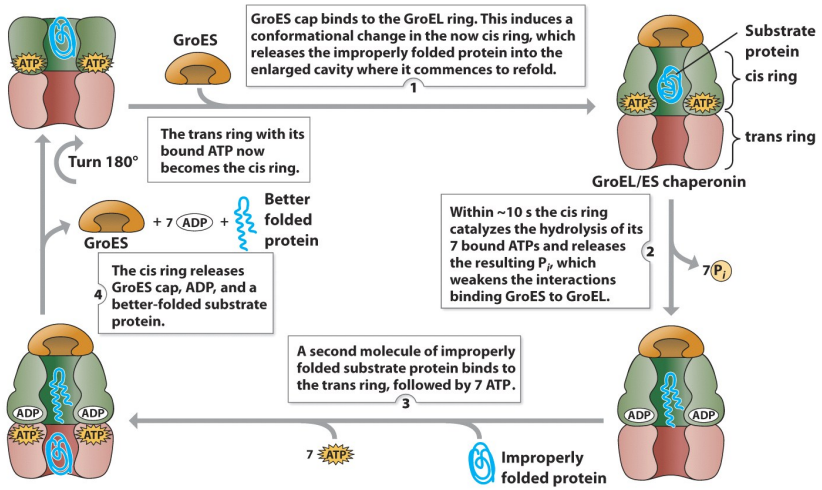
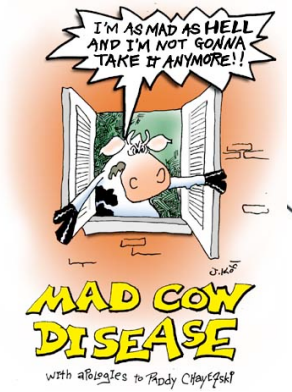
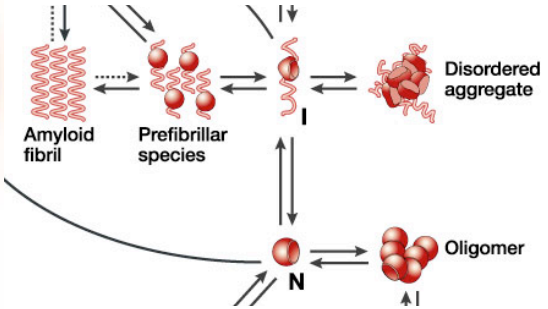


Figure 6-45

Protein can misfold too!

- Our body has many built-in mechanisms to prevent misfolding, such as simply by removing misfolded protiens
- If out of control, the consequences can be severe (protein misfolding diseases)





Protein Folding & Disease

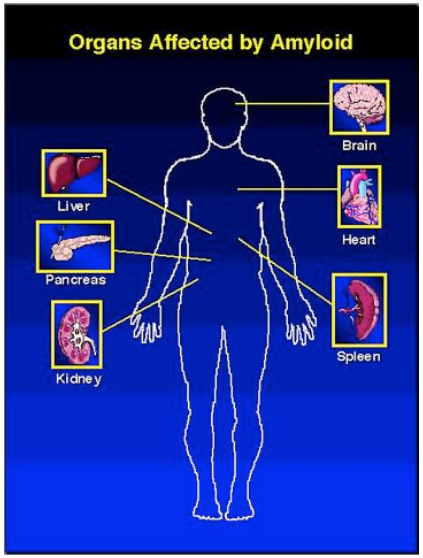
TABLE 6-4 Some Protein Misfolding Diseases

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

Table 6-4

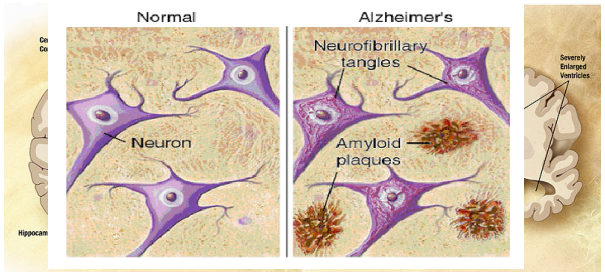
Amyloidosis

- Abnormal accumulation of insoluble fibrous protein aggregates in various organs
- Implicated in various neurodegenerative diseases such as Alzheimer's diseases, type II diabetes and over a dozen others



Alzheimer's Disease (AD)

- Incurable neurodegenerative disease
- Generally diagnosed for people >65 years old
  - >20 million people affected world wide
  - 1 out 14 of age 65-70 and ¼ among 85+
  - Predicted to affect 1 in 85 people by 2050
- Aggregation of Abeta (Aβ) peptide
  - 39-43 residues long with unknown function



Prion Diseases

- First discovery that proteins (or protein conformations) themselves can be infectious agent

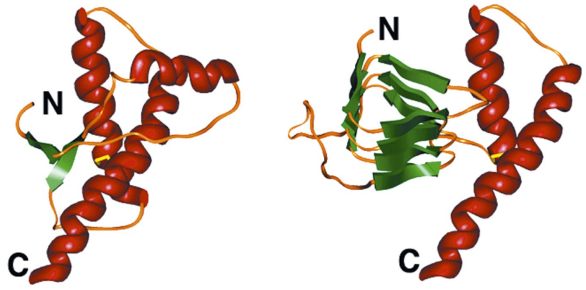


Figure 6-48
Courtesy of Fred Cohen, University of California at San Francisco. Part a based on an NMR structure by Kurt Wüthrich, Eidgenössische Technische Hochschule, Zurich, Switzerland.

Summary

- Proteins are weakly stable: major contributions of stability
  - Hypropathy scales
  - Denaturation
  - Dynamics: IDPs
- Protein folding
  - Protein can fold by themselves
  - Protein folding problem
  - Protein misfolding and related diseases
  - Fibril: beta-sheet structures
- Coming up: molecular mechanics and molecular dynamics

What do a boiled egg and curdled milk have in common?

A. Both smell revolting.
B. Both are usually found in a kitchen.
C. Both would be found in my kitchen if I actually ever cooked.
D. Both illustrate the denaturation of proteins.