Today, Lec 9, Protein Folding

Homework #5: due at beginning of next class (as usual); Homework #6 assigned Thursday

Mid-term: Tuesday Oct 20\textsuperscript{th}, in class

Go over reading, HW, Class notes.

Concepts—\sim \frac{1}{2}; write about them

Calculations— about \sim \frac{1}{2}

Today: Very difficult for detailed calculation

Martin Gruebele-experimentalist;
Klaus Schulten – computation (next class!)

Tons of computational work for physicist-types

9/29/15
Protein Folding: primary, secondary, tertiary, quaternary

https://artofbiochemistry.wordpress.com/2013/02/20/1226
Figure 4-4 Three types of noncovalent bonds help proteins fold. Although a single one of any of these bonds is quite weak, many of them can form together to create a strong bonding arrangement that stabilizes a particular three-dimensional structure, as in the small polypeptide shown here (center). R is a general designation for a side chain. Protein folding is also aided by hydrophobic forces, as shown in Figure 4-5.
Secondary structure

Tertiary structure

Quaternary structure

α helix

β pleated sheet

β strand

Hydrogen bond

Hydrogen bond

Transthyretin polypeptide

Transthyretin protein

Boiling an egg
What happens? Why?

Answer in molecular terms. Energy, entropy
The white part, or albumin, is initially clear. You heat up the egg and then cool it down and the egg white is completely scattering because the protein has become disordered. The reason is that there is enough energy at the higher temperature, $T \sim 398^\circ K$, instead of the normal room temperature of $298^\circ K$, to break the regular non-covalent bonds in the protein which keeps it’s shape. The process of taking an unfolded protein, and folding it up, is not a reversible reaction. The hydrogen bonds, van der Waal bonds, and the ionic bonds are a few KT, but water comes in and bashes into then and breaks those bonds. In the white part, there is a ton of albumin, and so rather than just refolding after it’s heated up, the bonds will more likely reform with other potential partners.

See the web site above.
The Protein Energy Landscape

Largely from Martin Gruebele, Chemistry, Physics UIUC
Also from Maria Spies, Biochemistry, UIUC
Protein folding – the process that results in acquisition of the native structure from a completely or partially unfolded state

Protein folding cannot proceed by purely random search among ALL possible conformations:

Imagine:
100 aa protein (M.W. 10kDaltons – very small)
Let’s say 3 configurations for each step

How Many possible configurations? \( \rightarrow 3^{100} \)

It takes at minimum \( 10^{-15} \) sec for each step:
(time scale required for bond rotation)

How long to fold?
\( \rightarrow \) longer than the age of the universe!!!
How does a Protein go from unfolded to folded
a) at all; b) in 1 msec; c) with no (help), chaperones?

(Helping proteins)

Unfolded $\rightarrow$ Folded

Inactive $\rightarrow$ Active

Main driving forces:
1) Shield hydrophobic (black spheres) residues/a.a. from water;
2) Formation of intramolecular hydrogen bonds.

Active areas: 4 centuries on it
Predicting tertiary structures from primary sequence still not solved!
Difficulty relating to experimental observations.
Protein Folding Summary

• Proteins are made as a string of amino acids, supposedly unstructured, and then fold up into its shape.
• Can fold and do say fairly fast (< second).

• In most cases, don’t need help. In complicated cases (big proteins, very crowded conditions such as in a cell) proteins get help: proteins called chaperones.

• ΔG is almost always small: (5-10 kT—few H-bonds). E goes down; S goes down. They compensate.

• Kinetics – fast cause not huge barriers. (Detailed calculations necessary.)

• Protein Funnel is a good model.
Proteins: A short, hard life.

A typical protein folding equilibrium constant $K_{eq} \approx 3600$.

$$A_{\text{unfolded}} \leftrightarrow A_{\text{folded}}^{k_f \ k_{uf}}$$

$$K_{eq} = [A_{\text{folded}}]/[A_{\text{unfolded}}] = k_f / k_{uf}$$

This means a protein is unfolded for how much time/day? 24 times/day = once/hr!

Not nearly enough chaperones to help re-fold. Tend to do this by itself. 20-60% are natively unfolded–bind to negatively charged substrate and then folds.

50-100 aa
Let’s say you have protein $K_{eq} = 1000$

So what fraction of states are folded? (Recall: $\Delta G = \Delta E - T\Delta S$)

So what’s $\Delta G$? $K_{eq} = \exp(-\Delta G/kT)$

$\Delta G = 7 \, k_B T$

How many hydrogen bonds is this?

That’s equivalent to just a couple of Hydrogen bonds. $\Delta G$ is (almost flat).

How can this be? What about $\Delta E$, $\Delta S$? (Recall: $\Delta G = \Delta E - T\Delta S$)

If $\Delta S$ is large and $\Delta E$ is large, then $\Delta G$ can be small.

This is what happens: $\Delta E, T\Delta S \approx -100$’s kJ/mole $\sim 33 \, k_B T$/molecule

(Lots of bonds form but loss of a lot of entropy, $\rightarrow \Delta G$ can be small)
Protein folding: the energy landscape theory

Is this “free energy”, or “energy = enthalpy”?

Ans: Energy (Enthalpy)
Protein folding: the energy landscape theory


2. Highly cooperative – intermediates are rarely observed.

3. Heterogeneity of the starting points – each unfolded molecule has different conformation and different path downhill the folding funnel.

4. In many cases is coupled to translation.
Example: the lattice model

A simplified model of protein folding:
Only 2-D motion allowed; only 90° motion.
(Real proteins are 3D; are not restricted to 90° rotation.)

- 6-mer peptide (2 hydrophobic and 4 hydrophilic amino acids)
- Each amino acid is represented as a bead
  - Black bead: hydrophobic (H)
  - White bead: hydrophilic (P)
- Bonds represented by straight lines
- H-H (=1 KJ/mole = 1/3 k_B T/molecule) and P-P (=250J) bonds favorable
- Single 90° rotation per time step allowed.

What about length-dependence:
do peptides (short proteins) fold-up?

Note: Proteins fold; Peptides don’t fold
Peptides have too few H-H and P-P to fold stably.

Based on work from Ken A. Dill, 1989, and Peter Wolynes, 1987
Chirality in Amino acids

Although most amino acids can exist in both left and right handed forms, Life on Earth is made of left handed amino acids, almost exclusively. Why?

Not really known. Meteorites have left-handed aa.


Alpha helix is a right-handed coil, with left-handed amino acids. (There is steric hinderance for a left-handed helix from left-handed amino acids.)

Similar for β-sheets.

• In 2D: To avoid issues with chirality, all molecules are made so that the first two amino acids go upwards.

• Also, the first kink always goes to the right.
Rotation rules under Lattice Model

- 2-D model - no rotations allowed. [Don’t allow over-counting: horizontal = vertical configuration]

- Molecules are only allowed to change in a single “kink” in 90° increments per time.

![Diagram showing allowed and not allowed moves in a lattice model diagram.](image-url)
Note: these two states would be equivalent by an out-of-plane rotation, but this is not allowed. Consequently only one is allowed.

A student pointed out, This appears to be 180°! Is something wrong?
Conformation Analysis

[Add up E, S = k_b lnW]

Energy = 0 kJ
W=14
S=Rln(14)≈22JK^{-1}mol^{-1}

Energy = -0.25 kJ; -0.5 kJ
W=7
S=Rln(7)≈16JK^{-1}mol^{-1}

Energy = -1 kJ
W=2
S=Rln(2)≈5.8 JK^{-1}mol^{-1}

Energy = -1.5 kJ
W=1
S=Rln(1)=0

Reaction Coordinate

x
0
0.33
0.66
1

Note: Only nearest neighbors that count
Molecular Dynamics has actually taken over to make it more realistic
The Protein Folding funnel

Entropy : horizontal scale

$E$

$\ln 1 = 0$

$k \ln 14$

$\ln 1 = 0$
Entropy vs. Energy
(correlated monotonic function)

The folded state (-1.5kJ) has the lowest entropy, and the unfolded states have the highest entropy.
Entropy vs. Reaction Coordinate
Free Energy Analysis (200°K)

Downhill folding (but in reality, at 200K, nothing moves)

At low temperatures, the lowest free energy state is the most ordered state, in this case the native state.
At room temperature, the folded state (-1500J) has the lowest free energy, and thus is the most energetically favorable conformation to be formed.
At very high temperatures, the fully denatured state has the lowest free energy.

**Downhill unfold**
Free Energy Analysis (360 °K)

This is likely the equilibrium of 50:50 where they are interconverting and equally stable.

Two state folder

Unfolded state—has some structure
Summary of Protein Folding

Proteins can fold.

Don’t need chaperones.

ΔG is always about zero.

Kinetics – fast cause not huge barriers
Class evaluation

1. What was the most interesting thing you learned in class today?

2. What are you confused about?

3. Related to today’s subject, what would you like to know more about?

4. Any helpful comments.

Answer, and turn in at the end of class.