Short Announcements

1st Quiz today: 15 minutes

Homework 3: Due next Wednesday.

Next Lecture, on Visualizing Molecular Dynamics (VMD) by Klaus Schulten

Today’s Lecture: Protein Folding, Misfolding, Aggregation
Quiz

You have 15 minutes to answer the following questions

1. Starting with double-stranded DNA, explain step-by-step, the process of making RNA and a protein. Tell about what are (thermodynamically) stable molecules and how to make them chemically reactive, including small molecules or other molecules/proteins which need to interact with them. [You may use drawing in your explanation.]

\[
\begin{array}{c}
\text{dsDNA} \\
5' \ldots 3' \\
3' \ldots 5'
\end{array}
\]
The Protein Energy Landscape

Largely from Martin Gruebele, Chemistry, Physics UIUC
Also from Maria Spies, Biochemistry, UIUC
Protein Folding Summary

• Proteins 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.

• $\Delta G$ 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.
How does a Protein go from unfolded to folded
a) at all; b) in 1 msec; c) with no chaperones?

(Helping proteins)

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 – 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? → 3^{100}

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

How long to fold?
→ longer than the age of the universe!!!
Proteins: A short, hard life.

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

\[
A_{\text{unfolded}} \xleftrightarrow[k_f]{k_{uf}} A_{\text{folded}}
\]

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

This means a protein is unfolded for how much time/day?

24 sec/day once/hr! 20-60% are natively unfolded—bind to negatively charged substrate and then folds.

Hydrophobic regions become exposed, becomes ubiquitinated. Reused aa in proteasomes.

Not nearly enough chaperones to help re-fold. Tend to do this by itself.

\( k_f/k_{uf} = 1 \text{ hr (if } K_{eq}=3600) \)
Simple Calculation of $\Delta G$ from $K_{eq}$.

$$K_{eq} = \exp(-\Delta G/kT) = 1000$$

Therefore $\Delta G = 7k_BT$

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

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 (Lots of bonds form but loss of a lot of entropy)
Protein folding: the energy landscape theory

Native state

Intermediate states

Unfolded state

Molten Globule State

\( I_A \)

\( I_B \)

Energy

Native state
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
Energy Funnel and Free Energy Surface

\[ \Delta G = \Delta H - T \Delta S \]

Work of: Wolynes, Bryngelson, Onuchic, Luthey-Schulten, Dill, Thirumalai
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 (=1000J = \(1/3 \ kT\)) and P-P (=250J) bonds favorable
- Single 90° rotation per time step allowed.

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.
Conformation Analysis

[Add up E, $S = k_b \ln W$]

Energy = 0 kJ
$W=14$
$S=\textbf{R} \ln(14) \approx 22 \text{JK}^{-1}\text{mol}^{-1}$

Energy = -0.25 kJ; **-0.5 kJ**
$W=7$
$S=\textbf{R} \ln(7) \approx 16 \text{JK}^{-1}\text{mol}^{-1}$

Energy = -1 kJ
$W=2$
$S=\textbf{R} \ln(2) \approx 5.8 \text{JK}^{-1}\text{mol}^{-1}$

Energy = -1.5 kJ
$W=1$
$S=\textbf{R} \ln(1)=0$

**Kinetic trap**
(Have to break two bonds)

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

Entropy : horizontal scale

$k \ln 1 = 0$

$k \ln 14$
The folded state (-1.5 kJ) has the lowest entropy, and the unfolded states have the highest entropy.
Entropy vs. Reaction Coordinate

![Graph showing the relationship between entropy and reaction coordinate. The graph depicts a decreasing trend as the reaction coordinate increases.]
Free Energy Analysis (200K)

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.
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.