Physical Principles in Biology
Biology 3550
Spring 2025

Lecture 33

Protein Folding Thermodynamics

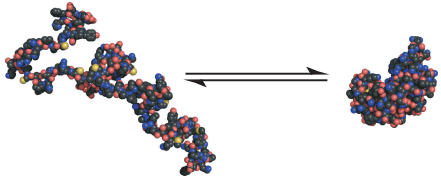
Friday, 4 April 2025

©David P. Goldenberg
University of Utah
goldenberg@biology.utah.edu

Announcements

- Problem Set 5:
  - Due Friday, 11 April at 11:59 PM
  - Submit pdf file on Gradescope
- Quiz 5:
  - Friday, 11 April
  - 25 min, second half of class

Another Thermodynamic Process in Biology: Protein Folding and Unfolding



- Unfolded proteins:
  - Broad ensembles of rapidly interconverting conformations.
- Folded (native) proteins:
  - Compact, well-defined conformations.
  - *Usually* the functional state.
  - Motions are restricted, but can be essential for function.
- Three-dimensional structure forms after (or during) synthesis.
- Many proteins can unfold reversibly, and the process has been extensively studied *in vitro*.

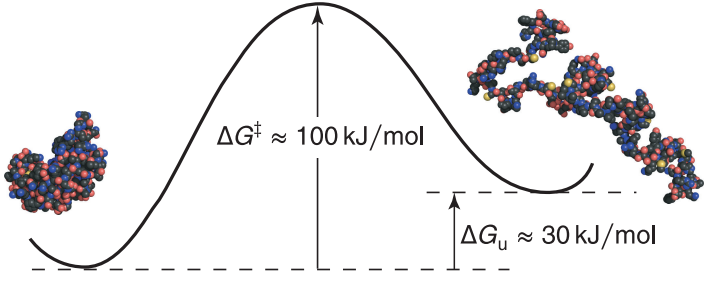
Protein Unfolding: A Simplified Summary

- For small, single-domain proteins ($\approx$ 100 amino acids), unfolding is well described as a “two-state” process:

$$N \rightleftharpoons U$$

  Partially folded molecules are rarely detected at equilibrium.

- Free energy profile for unfolding and refolding:



Equilibrium Constant for Unfolding

- Calculate K_u from ΔG_u° at 298 K

$$\Delta G_u^\circ = -RT \ln K_u$$

$$K_u = e^{-\Delta G_u^\circ/(RT)} = e^{-(30\times10^3\ \mathrm{J/mol})/(8.314\ \mathrm{J/(mol\cdot K)}\times 298\ \mathrm{K})}$$

$$K_u = \frac{[\mathrm{U}]_{eq}}{[\mathrm{N}]_{eq}} \approx 6 \times 10^{-6}$$

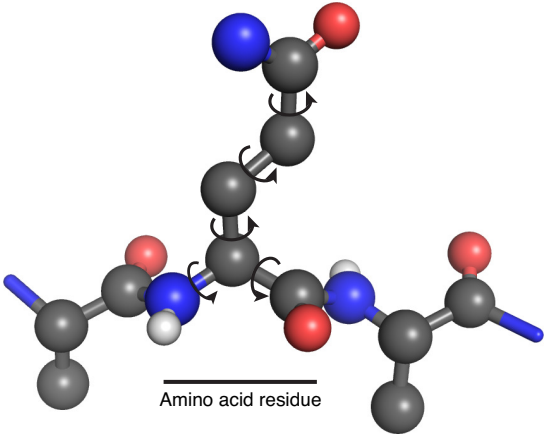
- Only a very small fraction of molecules are unfolded at any instant.
- But, unfolding can be relatively fast, and individual molecules will sample the unfolded state at some point.
- What determines the overall equilibrium between native and unfolded states?
- What determines which three-dimensional structure a particular sequence will form?

Conformational Entropy Change for Protein Unfolding

For now, focus only on the polypeptide chain itself:

- The native protein is a (relatively) unique structure.
- The unfolded state is an ensemble of rapidly interconverting structures.
- From Boltzmann: $S = k \ln \Omega$ (for a single molecule)
- For the native state, assume $\Omega_N = 1$, $S_N = 0$.
  (A questionable assumption, but it turns out to not be so bad.)
- What about the unfolded state?

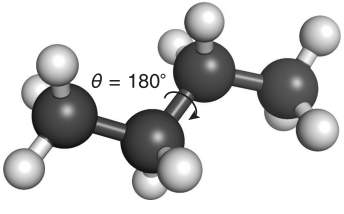
Rotatable Bonds in an Amino Acid Residue

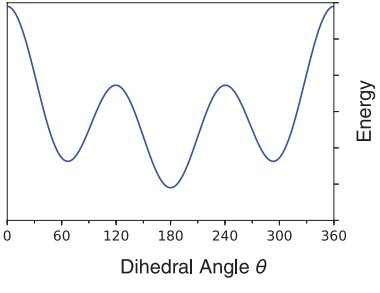


- Amino acid residue: Part of amino acid left in a polypeptide.
- Assume that each residue can take on 10 conformations in the unfolded state.

Why This isn't an Absurd Way to Estimate the Entropy Change

- Rotational isomers (rotamers)





- In both native and unfolded states, dihedral angles fluctuate around energy minima.
- In native state, most bonds are restricted to one minima.
- In unfolded state, bonds can sample two or three minima.
- The "rotational isomeric state approximation"

Conformational Entropy Change for Protein Unfolding

- From Boltzmann: $S = k \ln \Omega$
- For the native state, assume $\Omega_N = 1$, $S_N = 0$.
- For the unfolded state assume that each residue can take on 10 possible conformations.
  - For two residues, $\Omega_U = 10^2$
  - For three residues, $\Omega_U = 10^3$
  - For n residues, $\Omega_U = 10^n$
- ΔS_{conf} for unfolding

$$\Delta S_{conf} = S_U - S_N = k \ln \Omega_U - k \ln \Omega_N$$

$$= k \ln \frac{\Omega_U}{\Omega_N} = k \ln 10^n$$

Clicker Question #1

Estimate ΔS_{conf} for a protein 100 amino-acid residues long.

A) 3×10^{-20} J/K

B) 3×10^{-21} J/K

C) 3×10^{-22} J/K

D) 3×10^{-23} J/K

- $k = 1.3806 \times 10^{-23}$ J/K

Conformational Entropy Change for Unfolding

$$\begin{aligned}\Delta S_{\text{conf}} &= k \ln 10^{n} \\ &= 1.3806 \times 10^{-23}\,\text{J/K} \times \ln 10^{100}\end{aligned}$$

$$\ln 10^{100} = 100 \times \ln 10$$

$$\begin{aligned}\Delta S_{\text{conf}} &= 1.3806 \times 10^{-23}\,\text{J/K} \times 100 \times \ln 10 \\ &\approx 3 \times 10^{-21}\,\text{J/K}\end{aligned}$$

Conformational Entropy Change for Protein Unfolding

- From the previous slides:

$$\Delta S_{\text{conf}} = k \ln 10^n$$

n is the number of amino acid residues. Assumes 1 conformation for the native state and 10 conformations for each residue in the unfolded state.

- On a molar basis for $n = 100$

$$\Delta S_{\text{conf}} = R \ln 10^{100} = 8.314\,\text{J}/(\text{mol}\cdot\text{K}) \times \ln 10^{100}$$
$$= 2 \times 10^3\,\text{J}/(\text{mol}\cdot\text{K})$$

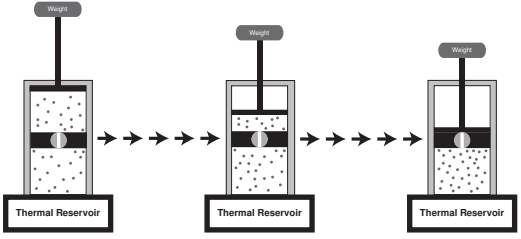
- Corresponding free energy change at 298 K:

$$-T\Delta S_{\text{conf}} = -5.7 \times 10^5\,\text{J/mol} = -570\,\text{kJ/mol}$$

- Compare with the overall free energy change for unfolding, on the order of 30 kJ/mol

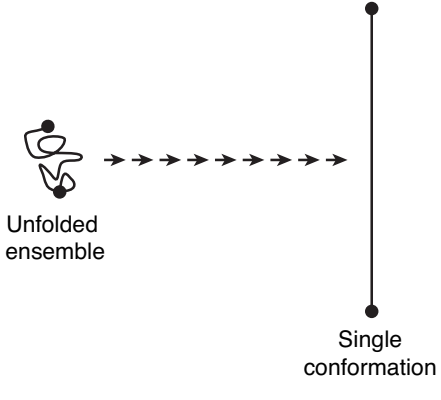
Could We Measure the Conformational Entropy Change for Unfolding a Protein?

- Recall reversible isothermal compression of a gas:



- To measure entropy change:
  - Measure work required for reversible process, w_{rev}.
  - Since $\Delta E = 0$ and $\Delta E = q + w$, $q_{\text{rev}} = -w_{\text{rev}}$
  - $\Delta S_{\text{sys}} = q_{\text{rev}}/T = -w_{\text{rev}}/T$.
- For a protein, measure the work to go from the unfolded ensemble to a single conformation (at constant temperature).

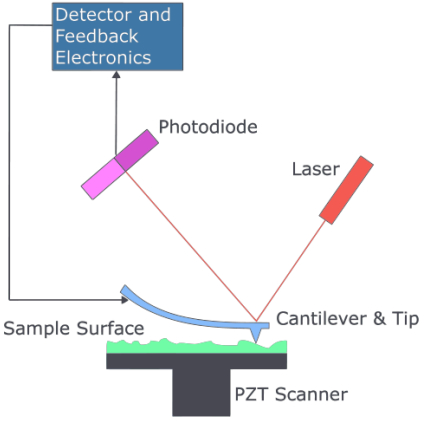
Stretching an Unfolded Protein



- Entropically, the single stretched-out conformation is approximately equivalent to the single folded conformation.
- This transition (probably) doesn't involve net change in hydrogen bonds, the hydrophobic effect or or other interactions.
- Have to stretch very slowly, to ensure reversibility.
- Have to measure very small forces as a function of distance.

$$w_{\text{rev}} = -\int F dx$$

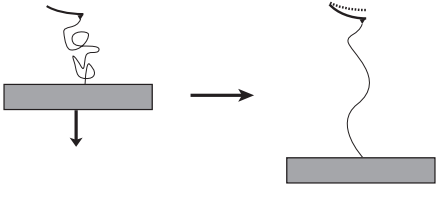
An Atomic Force Microscope (AFM)



- Usual purpose is to make images of surfaces.
- Very fine tip (a few nm in radius) held on flexible cantilever.
- Sample is scanned below probe.
- Movement of cantilever is monitored optically.
- Image of surface is constructed from data.
- Cantilever can be calibrated to measure force as a function of displacement.
  (spring constant)

https://en.wikipedia.org/wiki/Atomic-force_microscopy

Stretching an Unfolded Protein with AFM



- Stage is moved downwards very slowly, as deflection of cantilever is monitored.
- Deflection represents force as a function of distance.
- Force integrated over distance gives w_{rev}.
- $\Delta S_{\text{conf}} = -w_{\text{rev}}/T$
- This experiment is “anti-trivial!”
- Results are consistent with calculation based on rotational isomers!

Thompson, J. B., Hansma, H. G., Hansma, P. K. & Plaxco, K. W. (2002). *J. Mol. Biol.*, 322, 645–652.
http://dx.doi.org/10.1016/S0022-2836(02)00801-X

Observed Thermodynamics for Protein Folding

For a “typical” single-domain protein of 100 amino-acid residues at room temperature (300 K):

- ΔG_u: 5 kJ/mol to 50 kJ/mol
- ΔH_u: 0 kJ/mol to 200 kJ/mol
- ΔS_u:

$$\Delta G_\mathrm{u} = \Delta H_\mathrm{u} - T\Delta S_\mathrm{u}$$

$$\Delta S_\mathrm{u} = \frac{\Delta H_\mathrm{u} - \Delta G_\mathrm{u}}{T} = \frac{100\,\mathrm{kJ/mol} - 30\,\mathrm{kJ/mol}}{300\,\mathrm{K}}$$

$$\Delta S_\mathrm{u} = 230\,\mathrm{J/(mol \cdot K)}$$

Observed Thermodynamics for Protein Unfolding

For our (hypothetical) example at room temperature (300 K):

- Measured experimentally for unfolding:

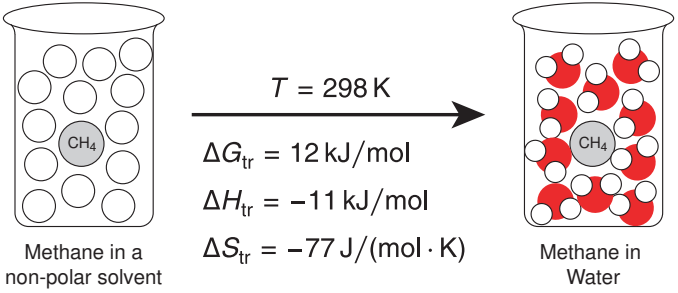
$$\Delta G_{\mathrm{u}} = 30\,\mathrm{kJ/mol}$$
$$\Delta H_{\mathrm{u}} = 100\,\mathrm{kJ/mol}$$
$$\Delta S_{\mathrm{u}} = 230\,\mathrm{J/(mol \cdot K)}$$

- Estimated change in conformational entropy:

$$\Delta S_{\mathrm{conf}} = 2 \times 10^3\,\mathrm{J/(mol \cdot K)}$$
$$-T\Delta S_{\mathrm{conf}} = -570\,\mathrm{kJ/mol}$$

- What we need to explain:
  - Why is $\Delta S_{\mathrm{u}} \ll \Delta S_{\mathrm{conf}}$?
  - Why is $\Delta G_{\mathrm{u}} \gg -T\Delta S_{\mathrm{conf}}$?

Thermodynamics of Transfer of a Non-polar Molecule to Water



- $\Delta G_{tr} = \Delta H_{tr} - T\Delta S_{tr}$
- ΔG_{tr} is positive because ΔS_{tr} is negative! (an “entropically driven” process).
- Water molecules become more ordered when a non-polar molecule is introduced.
- Non-polar groups buried in the interior of folded proteins become exposed to water on unfolding.