Physical Principles in Biology
Biology 3550
Spring 2024

Lecture 33

Protein Folding Thermodynamics

Friday, 5 April 2024

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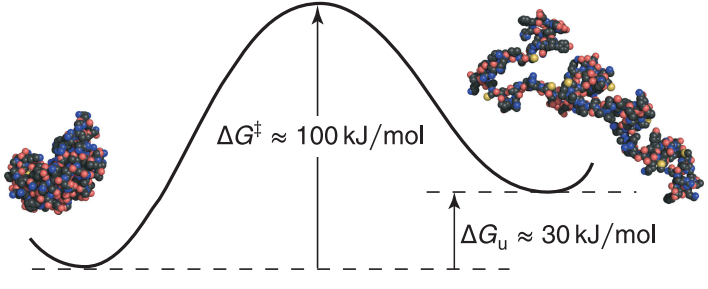
Announcements

- Class WILL be held on Monday, 8 April!
- Problem Set 5:
  - Due Monday, 15 April at 11:59 PM
  - Submit pdf file on Gradescope
- Quiz 5:
  - Friday, 12 April
  - 25 min, second half of class
  - Will cover thermodynamics
  - 50 min
- Review Session:
  - 5:15 PM, Thursday, 11 April
  - HEB 2002
  - Come with questions!

Protein Unfolding/Refolding: A Simplified Summary

- Free energy profile for unfolding and refolding:



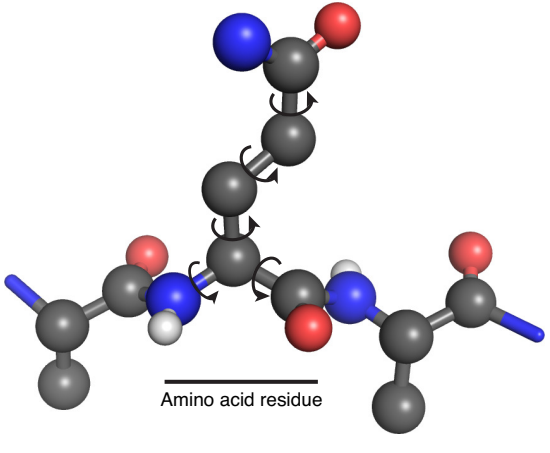
- What determines the overall equilibrium between native and unfolded states?
- What determines which three-dimensional structure a particular sequence will form?

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?

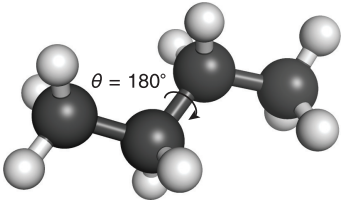
Rotatable Bonds in an Amino Acid Residue

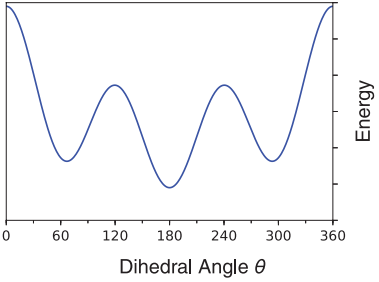


- Amino acid residue: Part of amino acid left in a polypeptide.
- Assume that each residue can take on 10 conformations in the unfolded state.

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 $n = 100$

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

$$= 2 \times 10^3\,\text{J}/(\text{mol}\cdot\text{K})$$

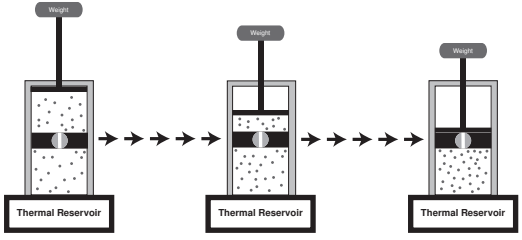
- Corresponding free energy change at 298 K:

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

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

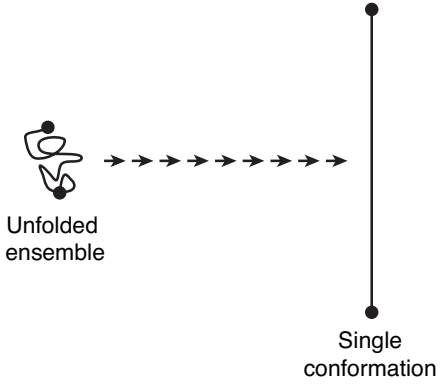
Could We Measure the Conformational Entropy Change for Unfolding a Protein?

- 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).

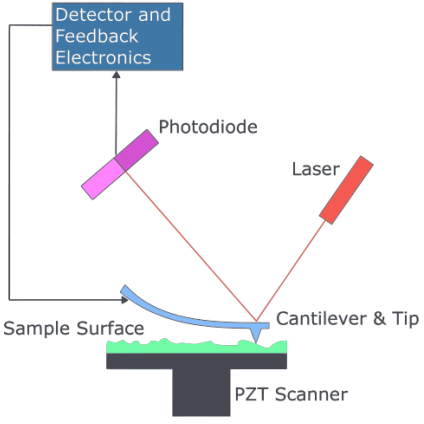
Stretching an Unfolded Protein



- 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.

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

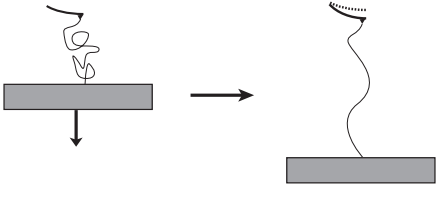
An Atomic Force Microscope (AFM)



- 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 Folding

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

- Measured experimentally for unfolding:

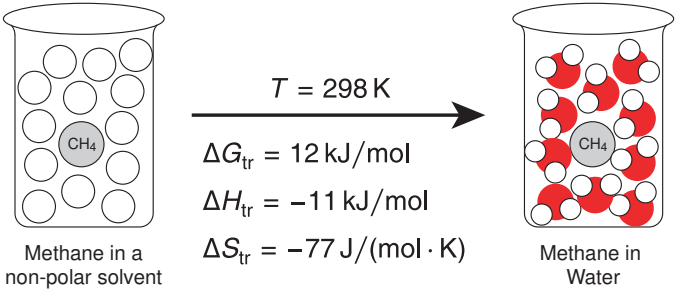
$$\Delta G_{\mathrm{u}} = 30\,\mathrm{kJ/mol}$$
$$\Delta H_{\mathrm{u}} = 100\,\mathrm{kJ/mol}$$
$$\Delta S_{\mathrm{u}} = 230\,\mathrm{J/(mol \cdot K)}$$

- 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



- $\Delta G_{\text{tr}} = \Delta H_{\text{tr}} - T\Delta S_{\text{tr}}$
- 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.

Transfer Free Energy versus Accessible Surface Area

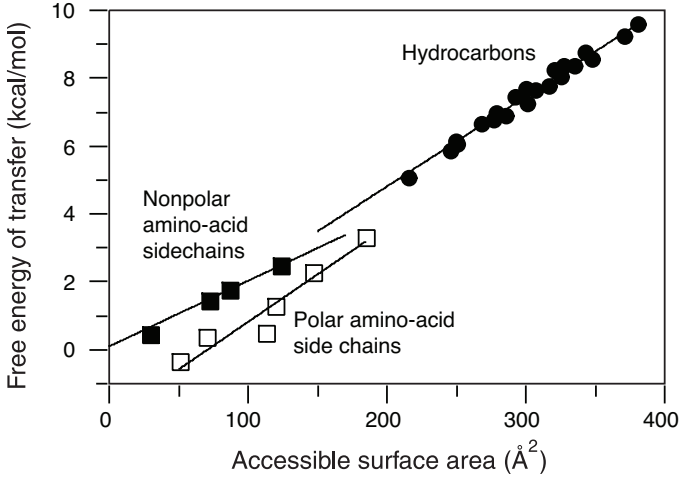


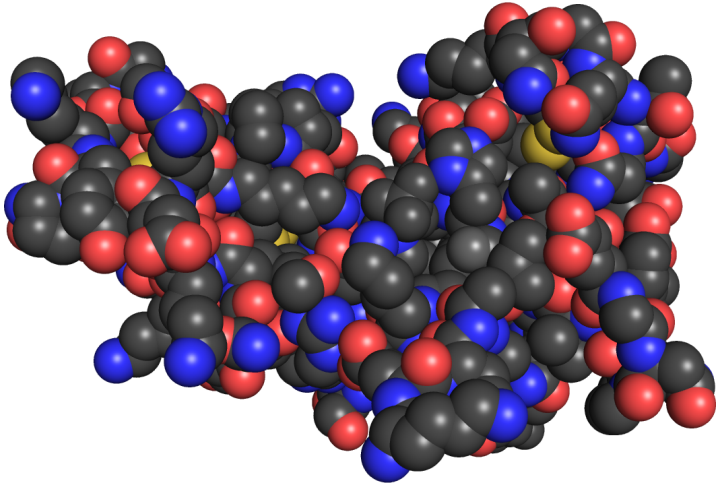
Figure adapted from: F. M. Richards. Areas, volumes, packing and protein structure. *Annu. Rev. Biophys. Bioeng.*, 6:151–176, 1977. http://dx.doi.org/10.1146/annurev.bb.06.060177.001055

Thermodynamics of Non-polar Surface Transfer to Water

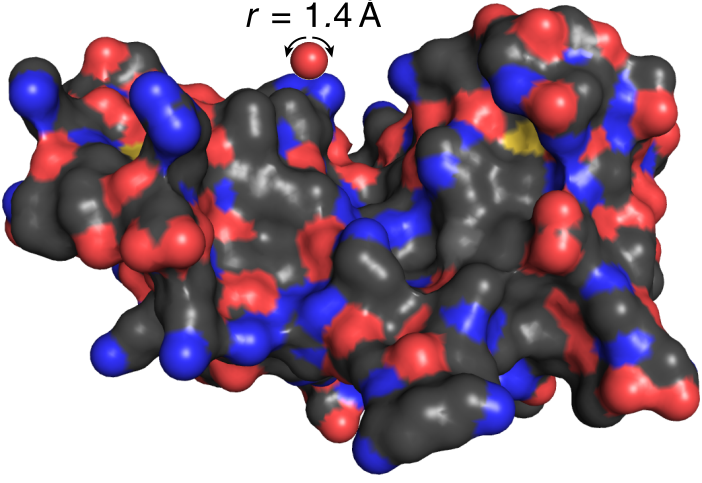
- At 300 K
  - $\Delta G_{\mathrm{tr}} = A_{\mathrm{np}} \times 97\,\mathrm{J/mol/\AA^2}$
  - $\Delta H_{\mathrm{tr}} = A_{\mathrm{np}} \times 7\,\mathrm{J/mol/\AA^2}$
  - $\Delta S_{\mathrm{tr}} = -A_{\mathrm{np}} \times 0.3\,\mathrm{J/(mol \cdot K)/\AA^2}$
  - $-T\Delta S_{\mathrm{tr}} = A_{\mathrm{np}} \times 90\,\mathrm{J/mol/\AA^2}$
- A_{np}: Non-polar surface area ($\mathrm{\AA^2}$) transferred from non-polar environment to water.
- How does the surface area exposed to water change when a protein unfolds?

Estimates are from transfer measurements summarized in:
Baldwin, R. L. (1986). *Proc. Natl. Acad. Sci., USA*, 83, 8069–8072. http://dx.doi.org/10.1073/pnas.83.21.8069
and
Spolar, R. S., Livingstone, J. R. & Record, T. M. (1992). *Biochemistry*, 31, 3947–3955.
http://dx.doi.org/10.1021/bi00131a009

Folded Structure of a Small Protein: Ribonuclease A

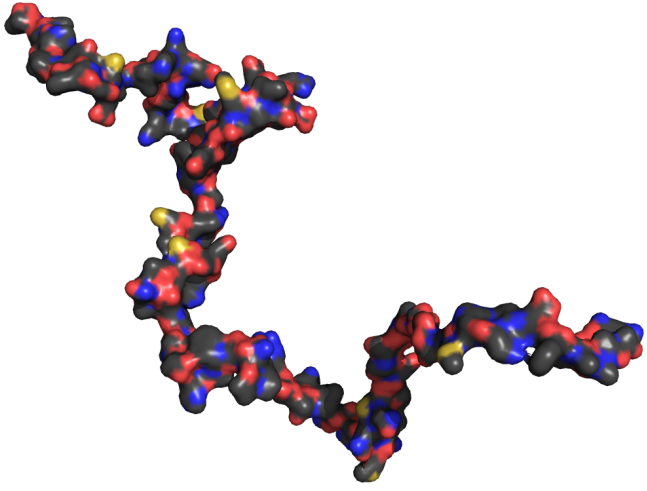


Solvent-accessible Surface of Folded Ribonuclease A



Solvent-accessible Surface of Unfolded Ribonuclease A

(one representative conformation)



Change in Accessible Surface Area for Unfolding for a Protein of About 100 Residues

	Folded ($Å^2$)	Unfolded ($Å^2$)	Difference ($Å^2$)
Total	7,000	14,700	7,700
Non-polar	3,800	8,800	5,000
Polar	3,200	5,900	2,700