Conformational Equilibrium

Last time, discussed

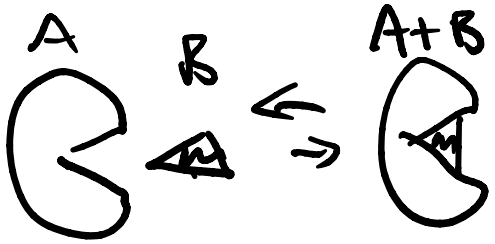
$A + B \rightleftarrows G + H$ reactions,

exchanging # of moles of atoms between chemical species

But the same kind of analysis can be useful for biological systems

Here we have non covalent interactions taking use between different states of a molecule

This includes classic

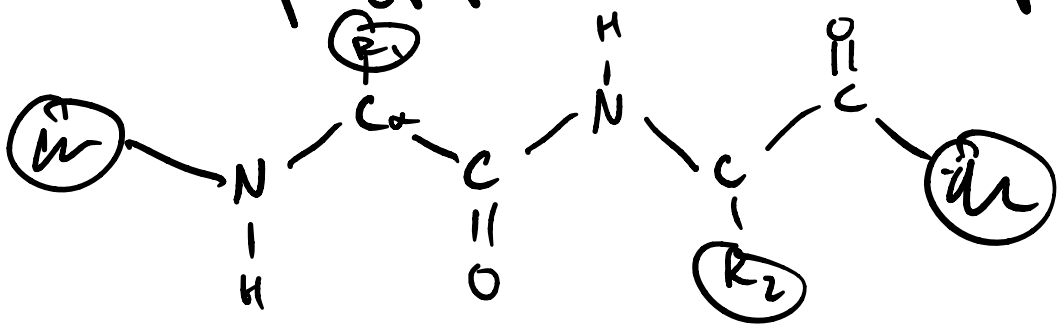


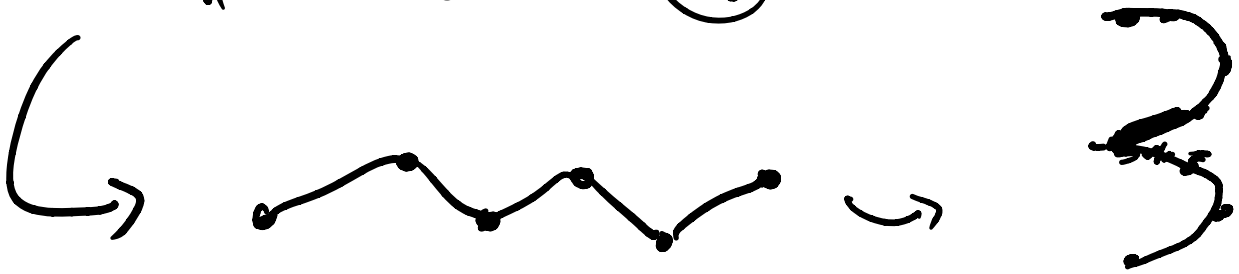
reaction

There is one kind of reaction scheme even simpler, which is

$$A \rightleftharpoons B$$

A very important example of this kind of reaction is the "folding" of polypeptide chains (proteins)





Folding controlled by $\Delta G = \Delta H - T\Delta S$

in general $\Delta H_{fold} < 0$ ← more favorable interactions

hbond, coulomb

consider H_2O also

$\Delta S_{fold} < 0$ ← "more order"

smaller number of microstates

This means protein folding is a competition between entropy and enthalpy. Many proteins have $\Delta G \sim -10$ kcal/mol because of cancelation of large ΔH and $-T\Delta S$

This chapter is about how to measure and extract these quantities from experimental data

Start with 2-state model

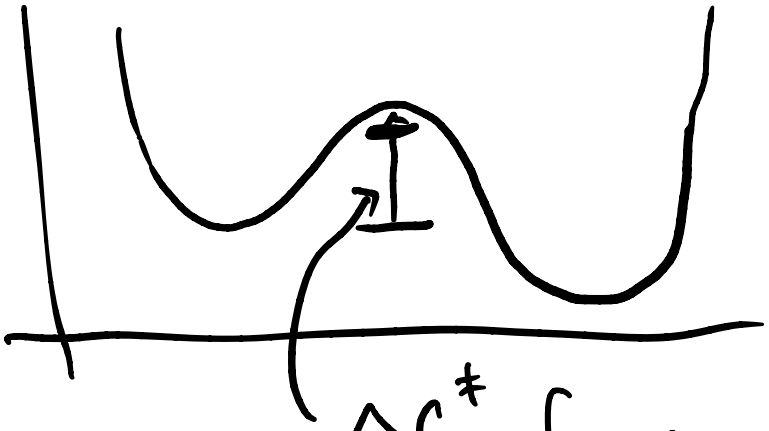
So reaction is $D \rightleftharpoons N$

↑ denatured ↑ native

with $K_{eq} = [N]/[D]$ and

$\Delta G^{o}_{fold} = -RT \ln K_{eq}$

Aside:



$\Delta G^{\ddagger}$ free energy barrier controls rate of folding

Can be too high for large proteins so folding may need to be catalyzed by a "chaperone"

But how can we measure $[N]/[D]$?

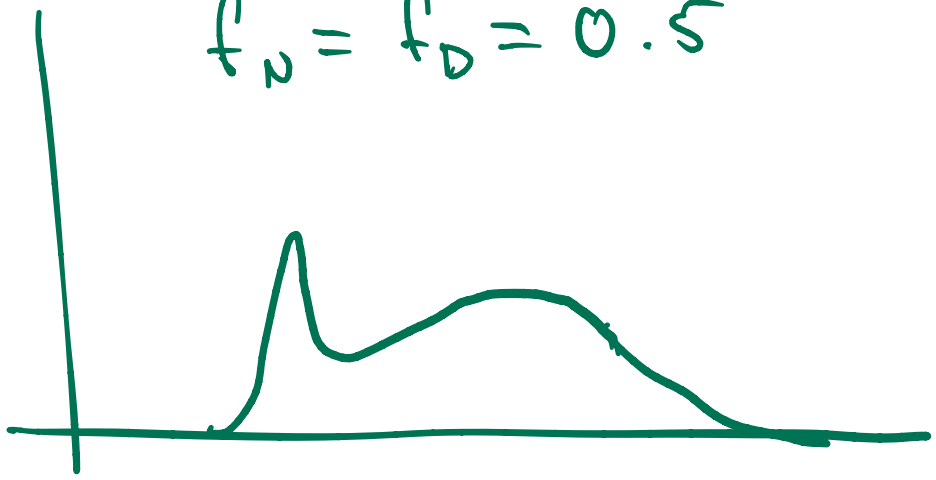
Need some spectroscopic technique that can distinguish between N & D

Lets first imagine what this could look like in general

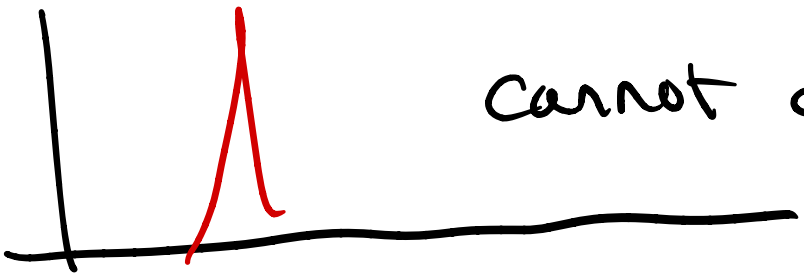
What if we have a mixture?

$$y^{obs}(x) = f_N\, y_N(x) + f_D\, y_D(x)$$

Eg $f_N = f_D = 0.5$



But now consider $f_N = 1.0$, vs 0.99 vs 0.98



cannot distinguish!

Hence realistically we can only evaluate $k = [N]/[D]$

when $f_N = \frac{[N]}{[D]+[N]}$ is intermediate

This applies to other measurements

- FRET – can be used to infer distances like above
- Circular Dichroism (example in book) absorption ~ 220 nm shows secondary structure vs unfolded
- NMR – different pattern of peaks when in native conformation

So when can we use this combination idea to measure K?

first, lets connect f_N, f_D to k_{fold}

$$f_N = \frac{[N]}{[N]+[D]} \quad \text{and} \quad k_{fold} = \frac{[N]}{[D]}$$

$$\equiv \frac{[D]k}{[D]k+[D]} = \frac{k_{fold}}{k_{fold}+1}$$

$$f_D = \frac{1}{k+1} \quad \text{s.t.} \quad f_N + f_D = 1$$

If $f_N \simeq 0.1 \Rightarrow .1k + .1 = k$

$$\Rightarrow k = 1/9 \simeq .111$$

$f_N = 0.9 \Rightarrow .9k + .9 = k$

$$\Rightarrow k = 9$$

So $\frac{1}{9} < k < 9 \Rightarrow -\ln 9 < \ln k < \ln 9$

$-2.2 < \ln k < 2.2$ $\quad \Delta G^\circ = -RT \ln k$

$\uparrow \approx .6 \frac{kcal}{mol}$

$\Rightarrow 1.3 \geq \Delta G^\circ \geq -1.3 \frac{kcal}{mol}$

Whereas for a stable protein expect $\Delta G^\circ \sim -10 \frac{kcal}{mol}$

$$K_{eq} = e^{-\Delta G^\circ / RT}$$

Rule of thumb: $e^{2.3} \approx 10$

$RT = 0.6$ so $\Delta G = -2.3RT$ or

$\Delta G^\circ \approx -1.4$ is a factor of 10

So states less stable than

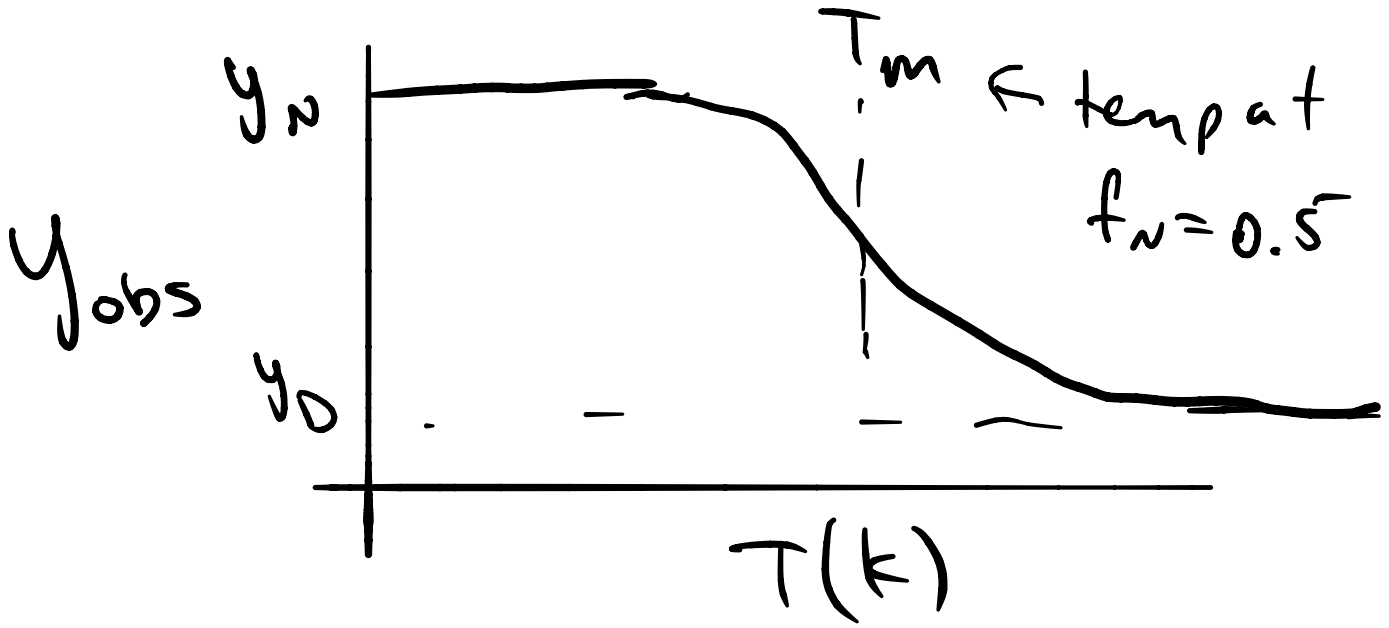
-2.4 kcal/mol will have

a lot of unfolded protein

Typical protein $k_{fold} > 10^3$

* at room temp!

Key is to "denature"
the protein with temperature
or chemicals in order to
"melt" the protein, and extract
ΔH & ΔS from this analysis



We will see later, like a phase
transition, but not sharp

How do we get k_{fold}

$$y_{obs} = f_N y_N + (1 - f_N) y_D$$

$$= y_D + f_N (y_N - y_D)$$

$$\Rightarrow f_N = \frac{y_{obs} - y_D}{y_N - y_D}$$

partial drop

total drop

Geometric, can get from plot

$$= \frac{k_{fold}}{1 + k_{fold}} y_N + \frac{1}{1 + k_f} y_D$$

$$= \frac{y_D + y_N k}{1 + k} = y_D - (y_D - y_N) \cdot \frac{k}{1 + k}$$

taller

$$y_{obs} = \frac{y_D + y_N e^{-\Delta G^\circ/RT}}{1 + e^{-\Delta G^\circ/RT}}$$

Can sub in $\Delta \bar{G}^\circ = \Delta \bar{H}^\circ - T\Delta \bar{S}^\circ$

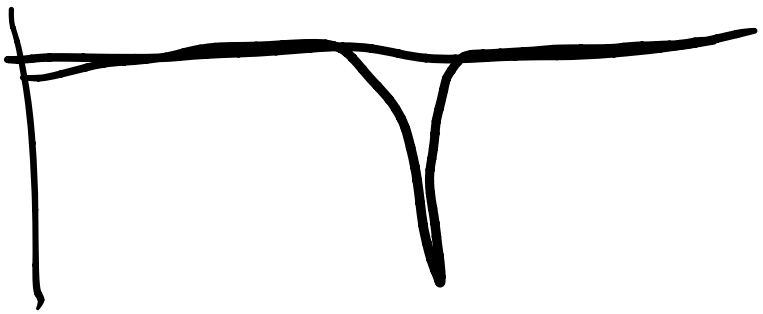
to fit these terms

also $\Delta G^\circ = 0$ @ $f_w = 0.5$

so $T_m = \Delta \bar{H}^\circ / \Delta \bar{S}^\circ$

How steep is the transition? Get derivative

dy/dT



Simple model, ΔH° & ΔS° don't depend on T

Skip, for completeness

$$y = \frac{y_N e^{\Delta S/R} e^{-\Delta H/RT} + y_D}{1 + e^{\Delta S/R} e^{-\Delta H/RT}}$$

$$\frac{dy}{dT} = \frac{\left(1 + e^{\Delta S/R} e^{-\Delta H/RT}\right)\left(+\frac{\Delta H}{RT^2} y_N e^{\Delta S/R} e^{-\Delta H/RT}\right)}{\left(1 + e^{\Delta S/R} e^{-\Delta H/RT}\right)^2}$$

$$- \frac{\left(y_N e^{\Delta S/R} e^{-\Delta H/RT} + y_D\right) \times \left(e^{\Delta S/R} e^{-\Delta H/RT} + \frac{\Delta H}{RT^2}\right)}{\left(1 + e^{\Delta S/R} e^{-\Delta H/RT}\right)^2}$$

$$= \frac{(1+K)(K) y_N - y_N K^2 - y_D K}{(1+K)^2} \frac{\Delta H^\circ}{RT^2}$$

$$= (y_N - y_D) \frac{K}{(1+K)^2} \frac{\Delta H^\circ}{RT^2}$$

and $\frac{k^2}{1+k} = \frac{k}{1+k} \cdot \frac{1}{1+k}$

So $\frac{\partial y}{\partial T} = (y_N - y_D) f_N f_D \frac{\Delta H^\circ}{RT^2}$

Enthalpy or entropy control steepness of the transition

Going back to $y_{obs} = y_D + (y_N - y_D)\frac{K}{1+K}$

$= y_{D+}$

$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ = \Delta H^\circ - T\Delta H^\circ/T_m$

$= \frac{\Delta H^\circ (T_m - T)}{T_m}$

$k = e^{\frac{\Delta H^\circ (T - T_m)}{RTT_m}}$

Can fit to get T_m, $\Delta H^\circ \Rightarrow \Delta S^\circ$ & ΔG°

In reality, better model is constant heat capacity for each stable state

$$d\bar{H}_N = \bar{C}_p \, dT$$

$$\bar{H}_N = H_N^{ref} + \bar{C}_p (T - T_{ref})$$

also for Denatured state

$$\Delta \bar{H} = \Delta \bar{H}_{ref} + \Delta \bar{C}_p (T - T_{ref})$$

eg ref temp at T_m

Can do same for $d\bar{S} = \bar{C}_{p/T} \, dT$

$$\Delta \bar{S} = \Delta S_{ref} + \Delta \bar{C}_p \ln(T/T_r)$$

and use this $\Delta \bar{G}^\circ = \Delta H^\circ - T\Delta S^\circ$ to fit