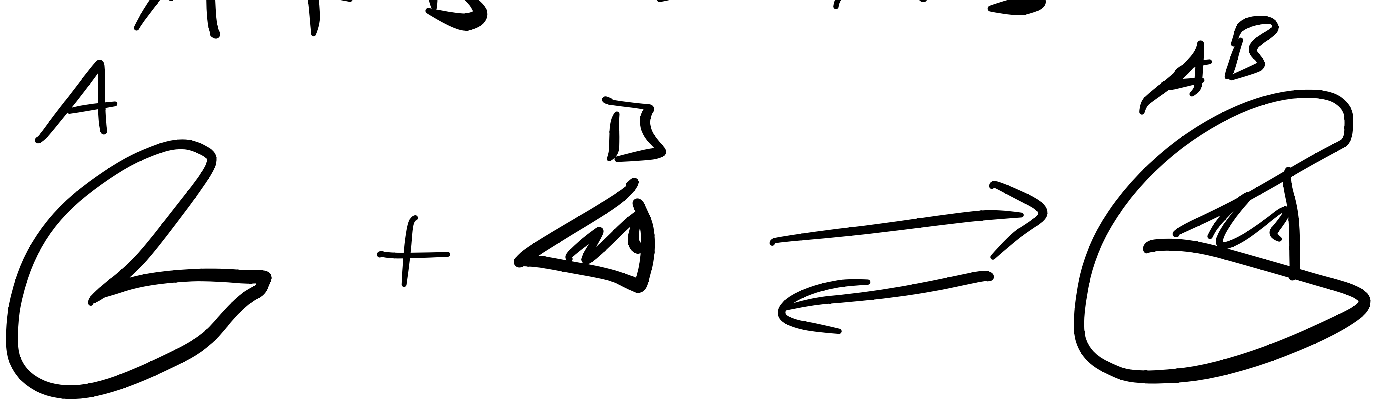
Conformational Equilibrium

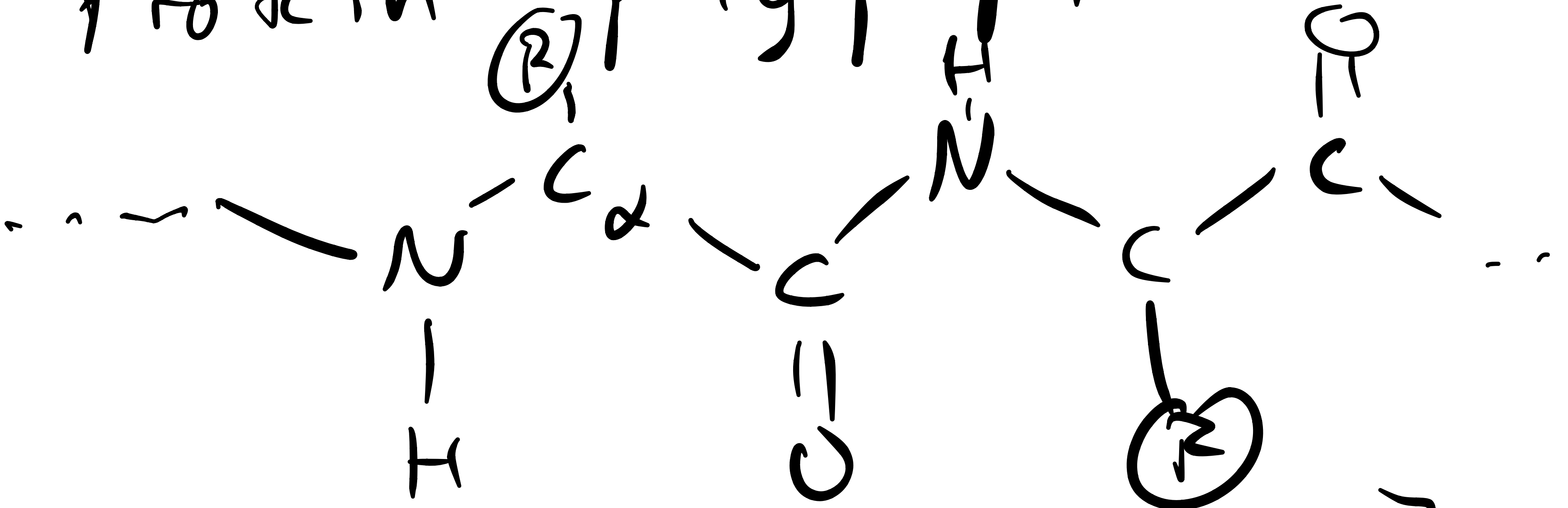
$$A + B \rightleftharpoons G + H$$

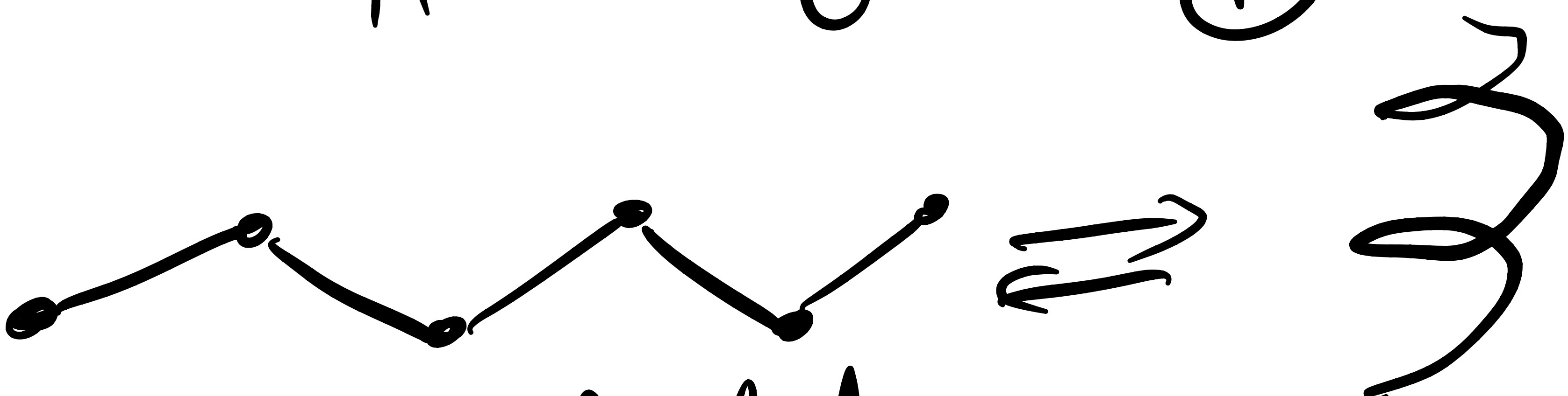
$$A + B \rightarrow AB$$



$A \rightleftharpoons B$

Protein polypeptide





Unfolded Folded

$$\Delta G_{fold} = \Delta H - T\Delta S$$

< 0 want to be folded

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

$\Delta S_{fold} < 0$ "more order"

Folding is a competition between entropy & enthalpy

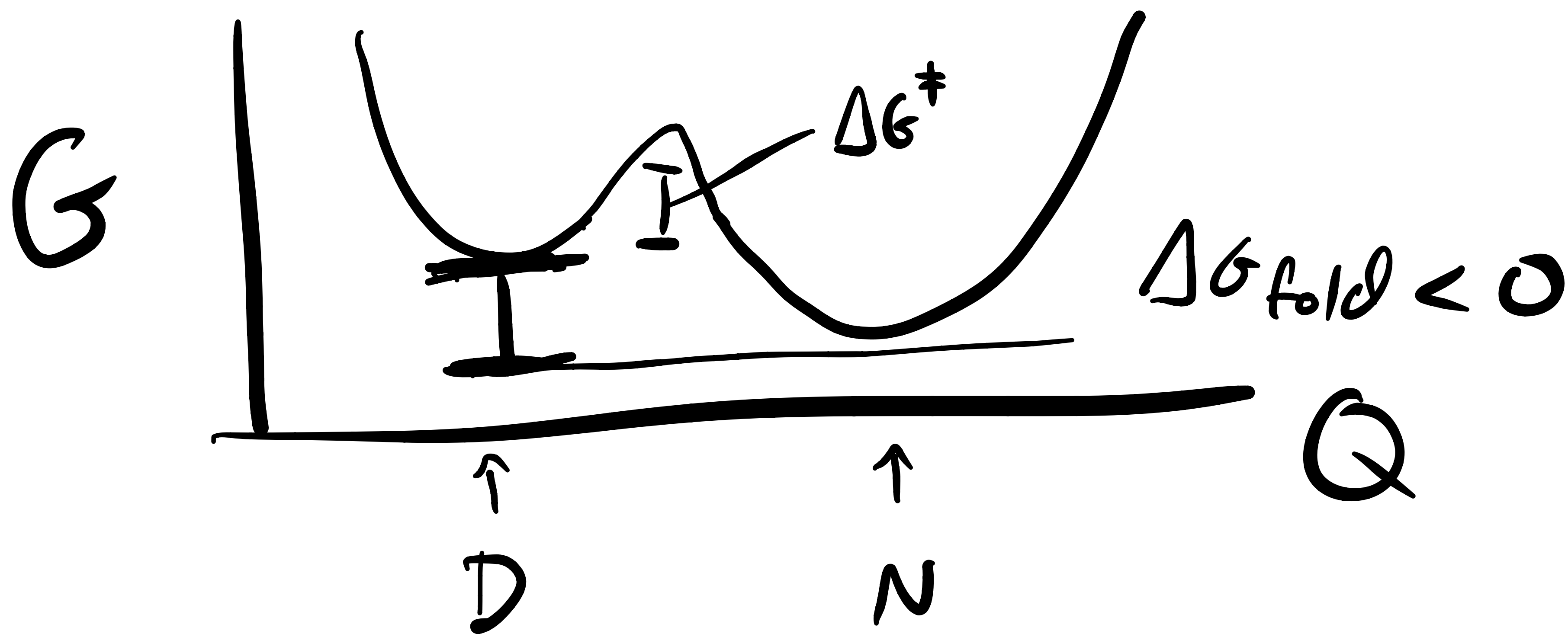
$\Delta G_{folding} \sim -5 \to -10$ kcal/mol

Cancelation of a large $\overset{\text{neg}}{\Delta H}$ and $-T\Delta S$

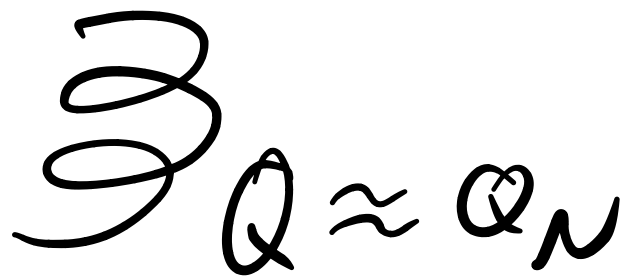
2 state model $D \rightleftharpoons N$

denatured native

$$D \rightleftharpoons N$$



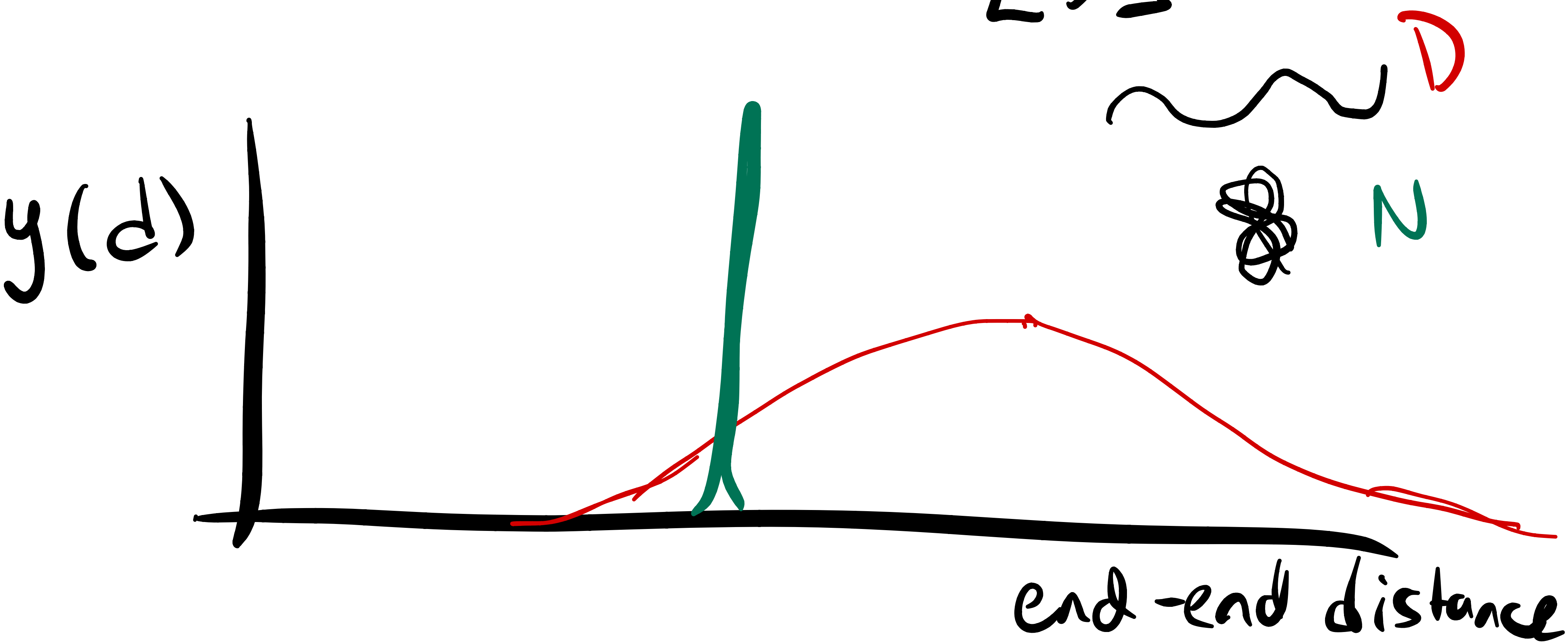
Q = # of native contacts



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

$$D \rightleftarrows N$$

$$K_{fold} = [N]/[D]$$



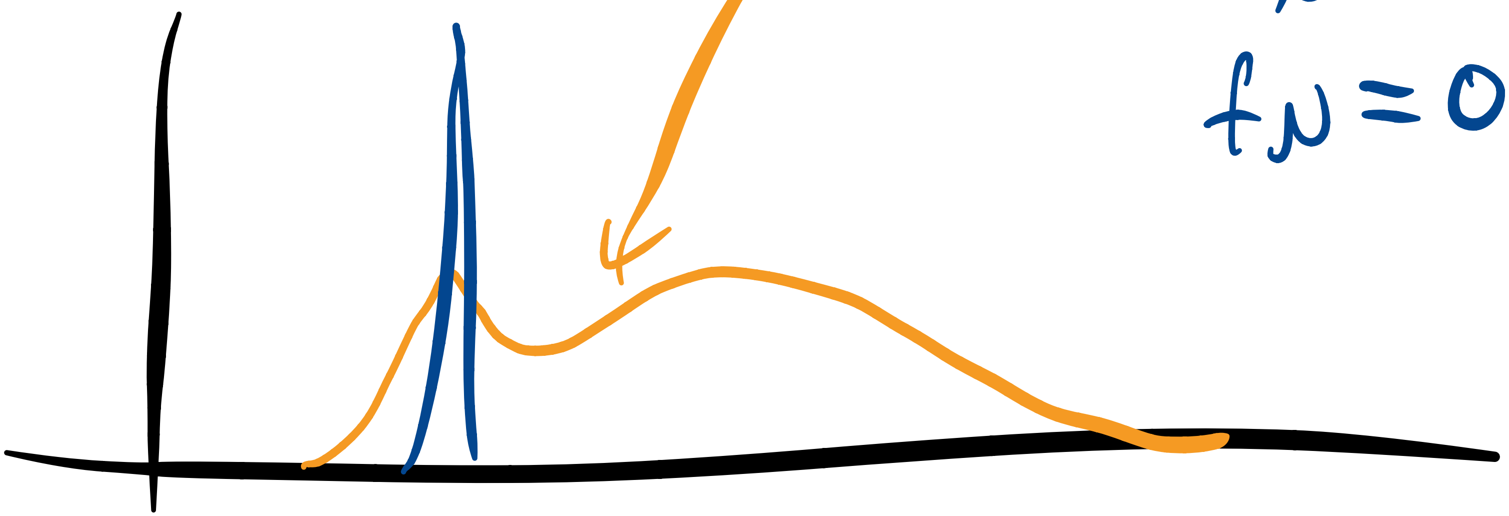
Do measurement

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

$$f_N = f_D = 0.5$$

$$f_N = 1.0$$

$$f_N = 0.995$$



Examples

- FRET – infer distances
- Circular Dichroism (CD)
  absorption around 220nm
  shows secondary structure
- NMR

When can we measure K

$$K_{fold} = [N]/[D] \Rightarrow [N] = [D]K$$

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

$$= \frac{[D]K}{[D]K+[D]} = \frac{K_{fold}}{K_{fold}+1}$$

$$f_N + f_D = 1 \Rightarrow F_D = \frac{1}{K_{fold}+1}$$

$$f_N = 0.1 \sim 0.9$$

$$f_N = 0.1 = \frac{K_{fold}}{K_{fold}+1}$$

$$0.1K + 0.1 = K \Rightarrow 0.1 = 0.9K$$

$$K_{min} = 1/9$$

$$f_N = 0.9 \Rightarrow 0.9K + 0.9 = K$$

$$\Rightarrow K_{max} = 9$$

$$\frac{1}{9} < K_{fold} < 9 \qquad \Delta G^\circ = -RT\ln K$$

$\downarrow \ln()$

$$-\ln 9 < \ln K_{fold} < \ln 9$$

$$-2.2 < \ln K < 2.2 \qquad \times -RT$$

$\approx 0.6 \frac{kcal}{mol}$

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

stability range where K_{eq} can be measured

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

$RT \approx 0.6 \frac{\text{kcal}}{\text{mol}}$

$e^{2.3} \approx 10$

$\Delta G = -2.3 RT \approx -1.4$ kcal/mol

~ factor of 10

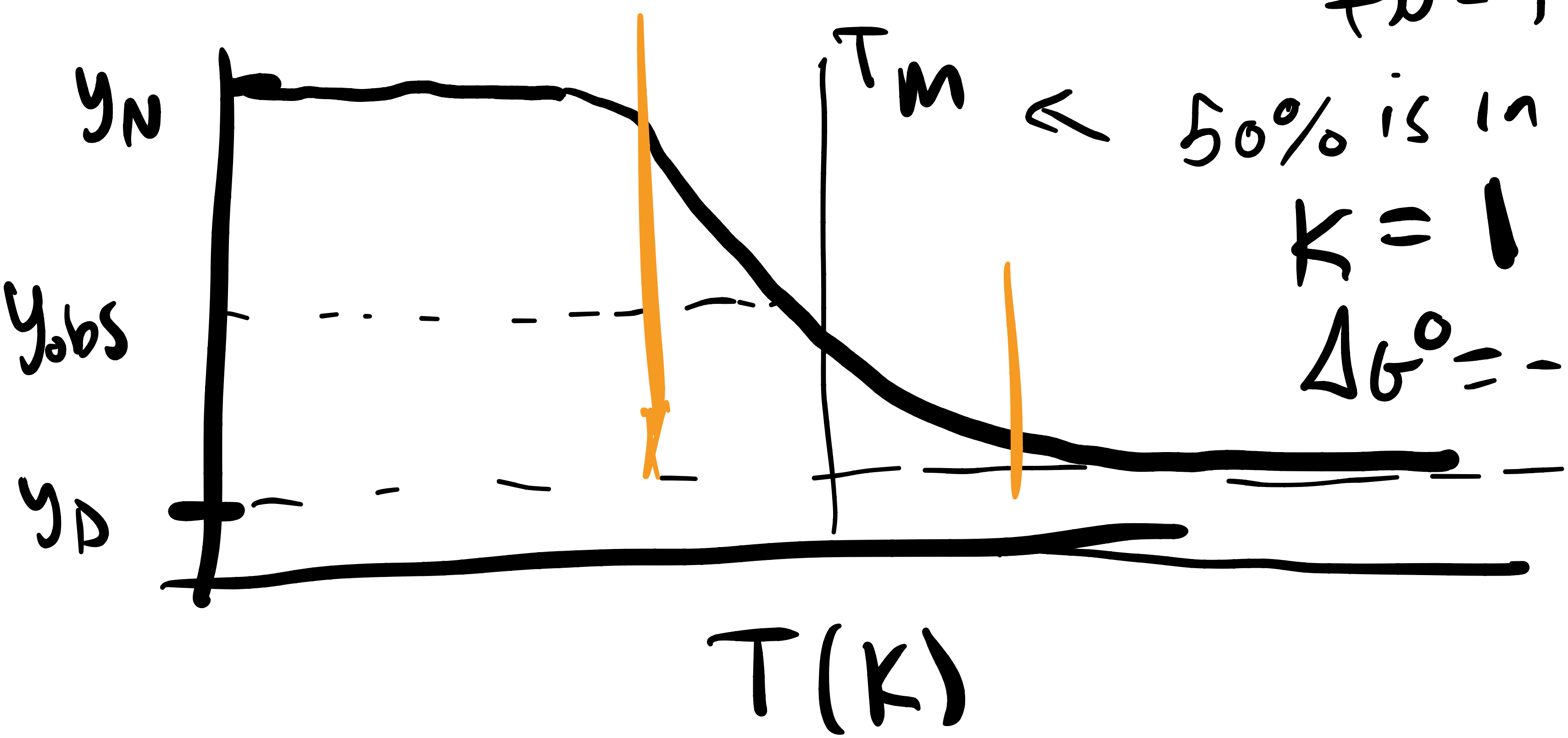
$K_{fold} > 10^3$

at room temperature

Do a denaturing experiment

increasing temperature

"melt" protein



$f_N = f_D = 0.5$

$T_m \Leftarrow$ 50% is in N & D

$K = 1$

$\Delta G^o = -RT\ln K$

$= 0$

fit curve to a model to get ΔG°, ΔH° & ΔS°

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

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

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

$$y_{obs} = \frac{K}{1+K}\, y_N + \frac{1}{1+K}\, y_D$$

$$= \frac{y_D + K y_N}{1+K}$$

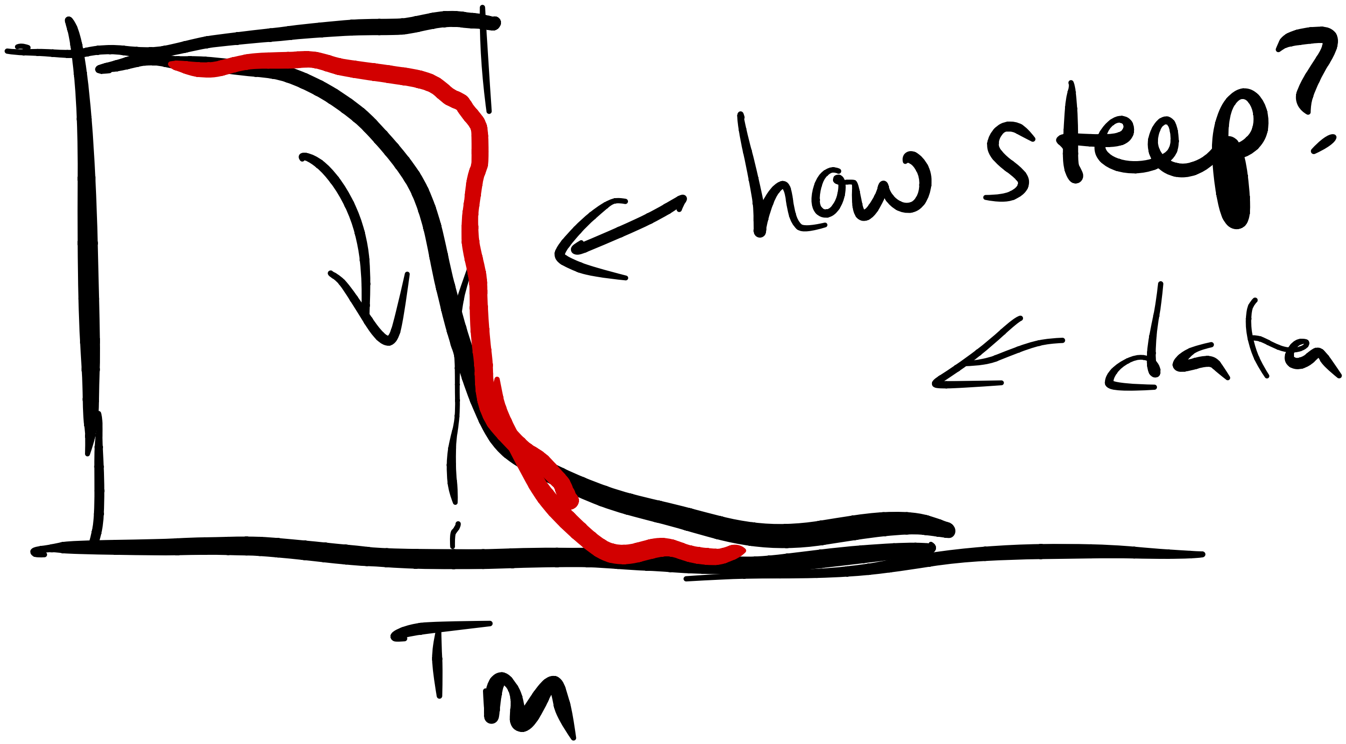
$$= y_D - (y_D - y_N)\frac{K}{1+K}$$

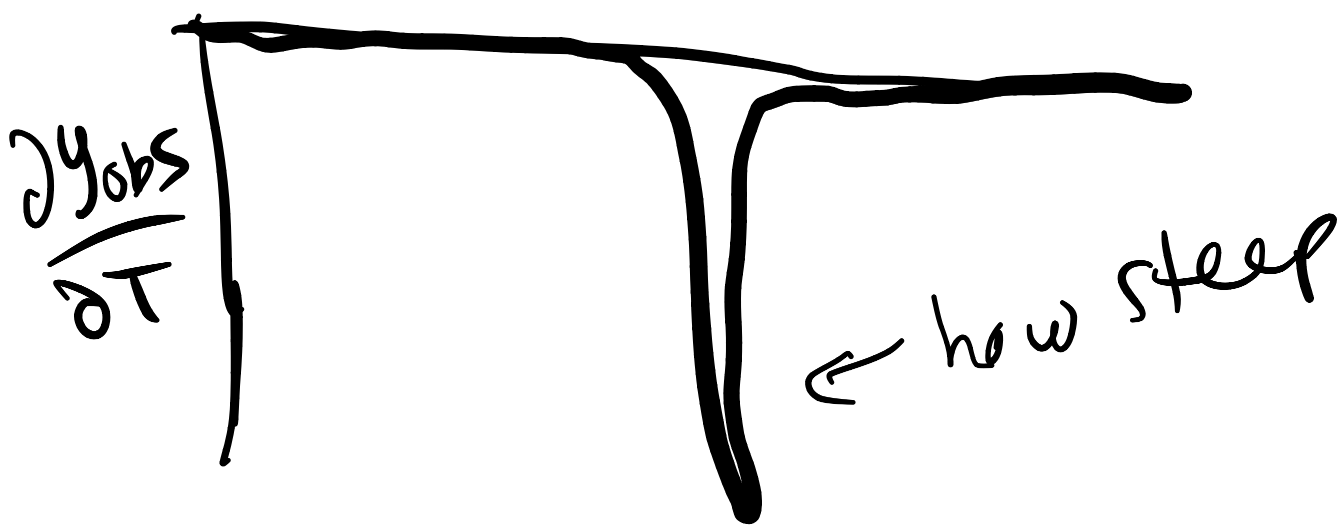
$$y_{obs} = \frac{y_D + K y_N}{1+K} = \frac{y_D + y_N e^{-\Delta\bar{G}^\circ/RT}}{1 + e^{-\Delta\bar{G}^\circ/RT}}$$

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

$$\Delta\bar{G}^\circ = 0 \Rightarrow \Delta\bar{H}^\circ - T_m \Delta\bar{S}^\circ = 0$$

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


how steep?
data
Tm


$\frac{\partial y_{obs}}{\partial T}$
how steep

Simple model ΔH^0, ΔS^0 are constentant

$$\frac{\partial y}{\partial T} = (y_N - y_D)\frac{K}{(1+K)^2}\frac{\Delta \bar{H}^0}{RT^2}$$

> 0

$\Delta H^0 < 0$

$$T_m = \Delta \bar{H}^0 / \Delta \bar{S}^0$$

$$\Delta \bar{S}^0 = \Delta \hat{H}^0 / T_m$$

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

$$K = e^{-\Delta \bar{G}^o/RT} = e^{\left(-\Delta \bar{H}^o/RT + \Delta S^o/R\right)}$$

$$\Delta G^o = \Delta \bar{H}^o - T\Delta \bar{S}^o = \Delta \bar{H}^o - T\frac{\Delta \bar{H}^o}{T_m}$$

$$= \Delta H^o (T_m - T)/T_m$$

$$K \approx e^{\Delta H^\circ (T - T_m)/(R T T_m)}$$

can fit $y_{obs}(T)$ to

ΔH°, T_m,

A better model

Constant heat capacity

C_p for Denatured & Native

Native:

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

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

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

$$-\left(\bar{H}_D = \bar{H}_D^{ref} + \bar{C}_p^D (T - T_{ref}) \right)$$

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

$$T_m$$

Can do same for dS

$$d\bar{S} = \bar{C_p}/T \, dT$$

$$S^{N,D} = S^{ref}_{N,D} + \bar{C_p}^{N,D} \ln(T/T_{ref})$$

$$\Delta\bar{S} = \Delta\bar{S}_{ref} + \Delta\bar{C_p} \ln(T/T_{ref})$$

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

$$K = e^{-\Delta G/RT} \rightarrow \gamma_N$$