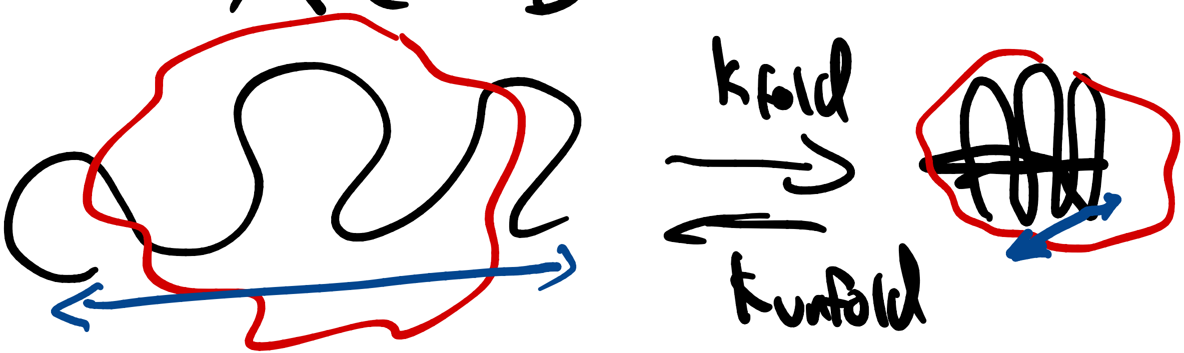
$$A + B \rightleftharpoons C + D$$

chemical eq

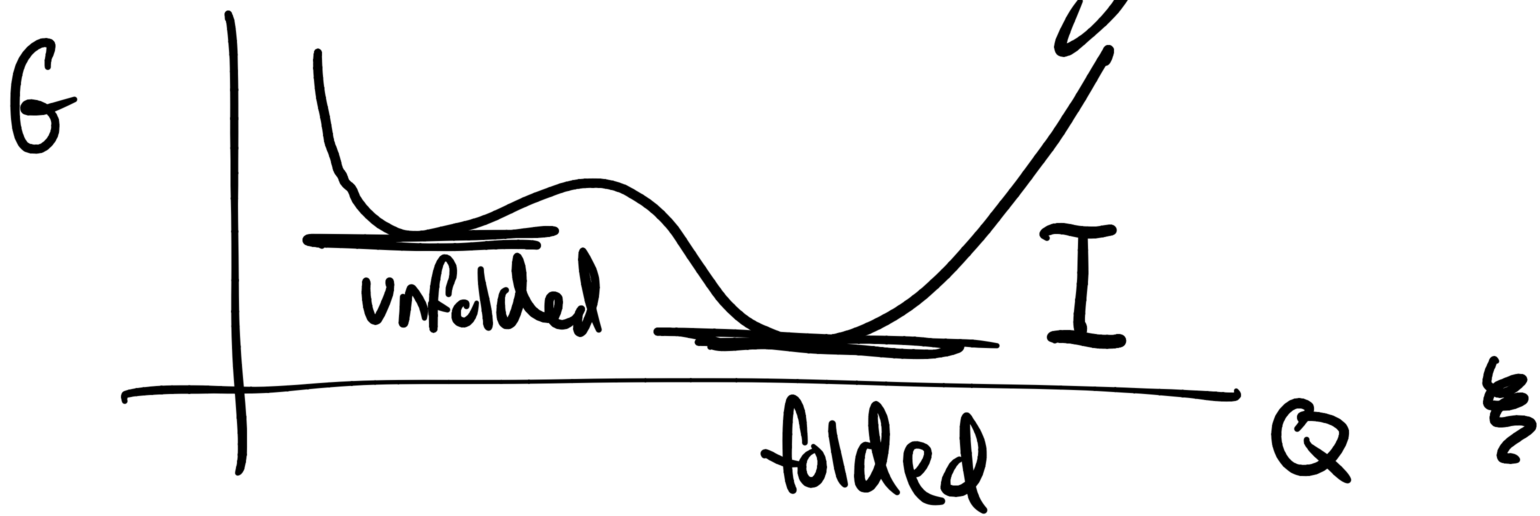
↳

"Biochemical reaction"

$$A \rightleftharpoons B$$



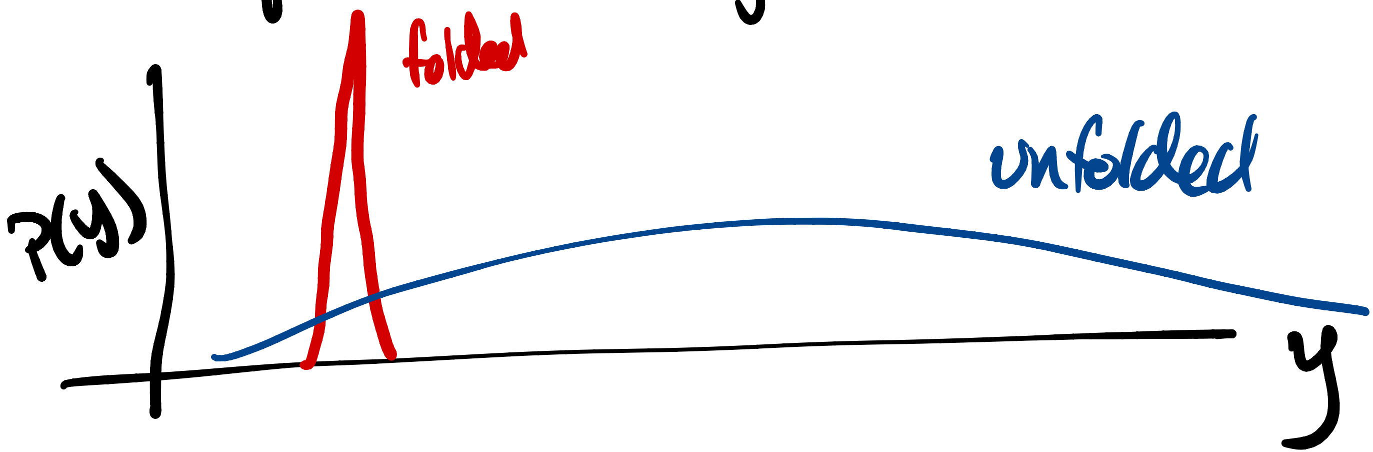
Controlled by $\Delta G_{folding}$




$$\Delta \bar{G}^{\circ} = -RT \ln K_{eq}$$

Measurement (Spectroscopy)

Properties very different




Eg: protein, end-end distance

radius of gyration

mixture:

50% $f_{fold}$ 50% $f_{unf}$

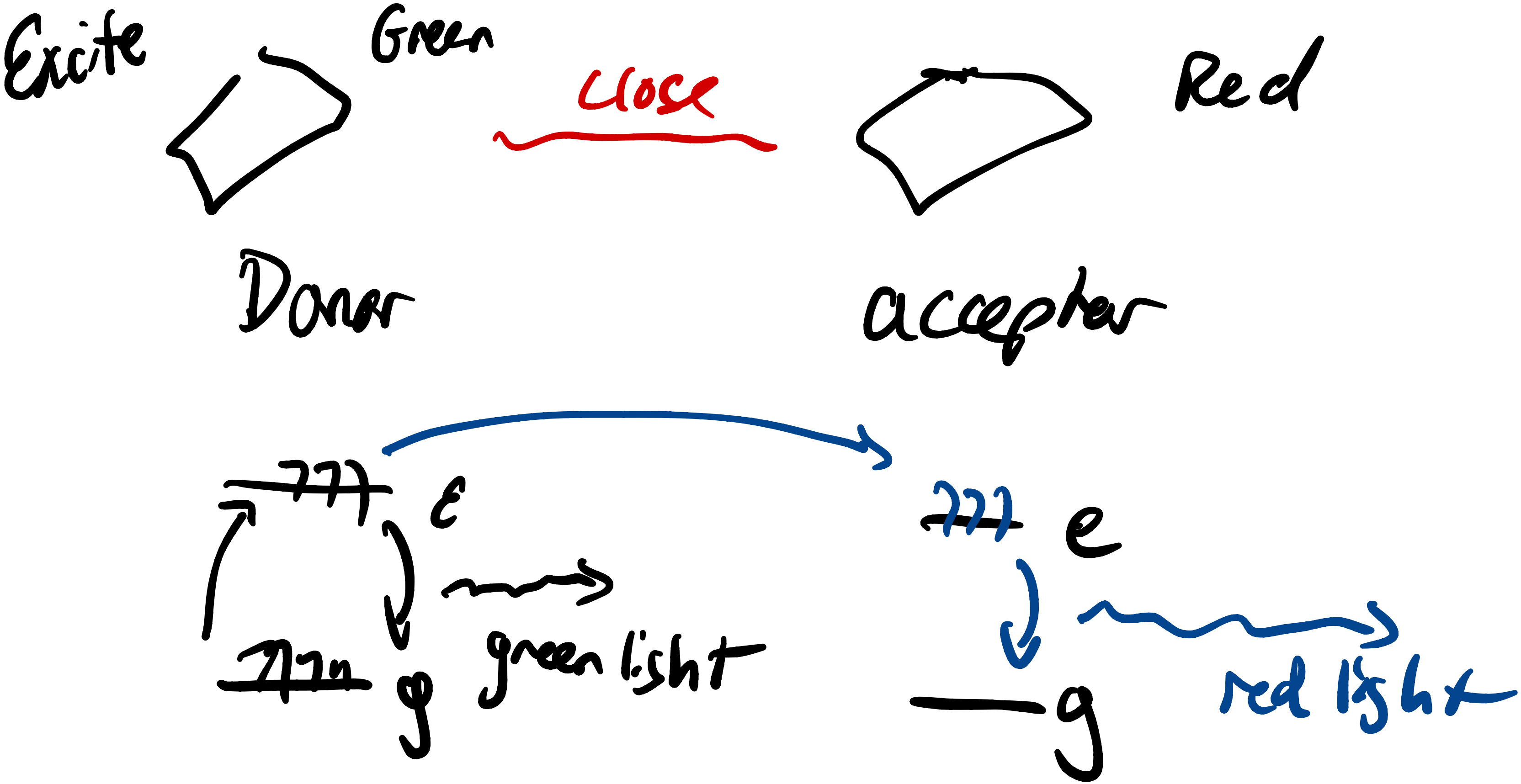

imagine here 99.999% folded

Goal: $K_{fold} = \frac{[\text{Folded}]}{[\text{Unfolded}]}$

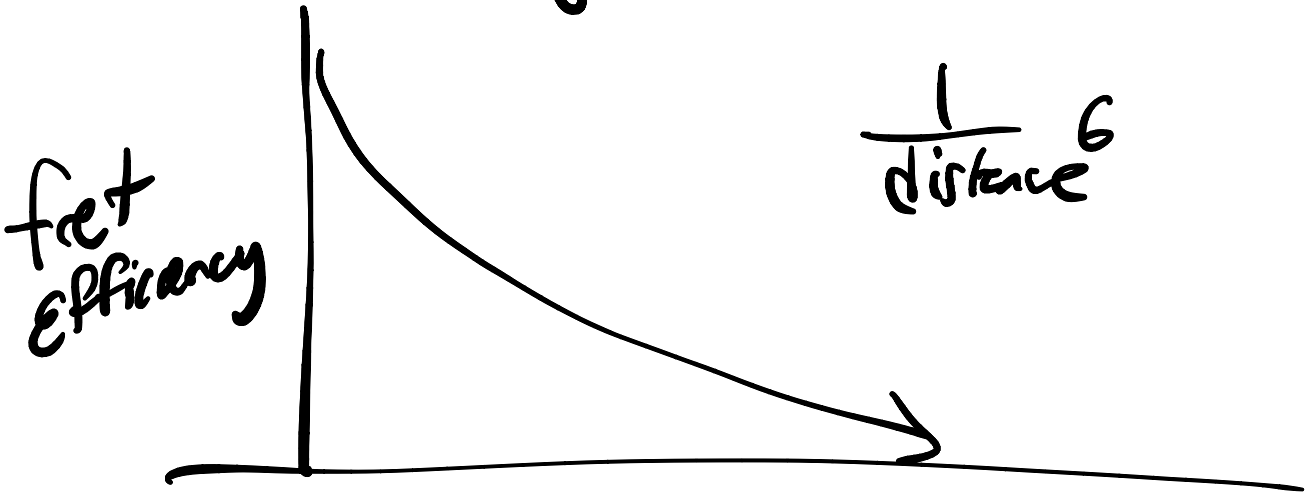
$f_{folded}$ is intermediate ~ 0.5

Eg measurements

FRET: Förster / Fluorescence Resonance Energy transfer

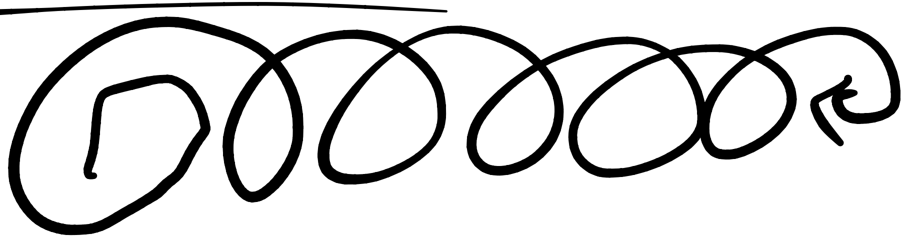

Excite
Green
Close
Red
Donor
acceptor
e
g
green light
e
g
red light

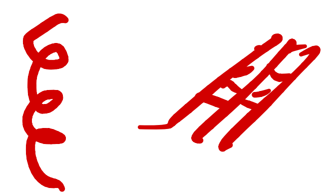
# Fret tells you about distance

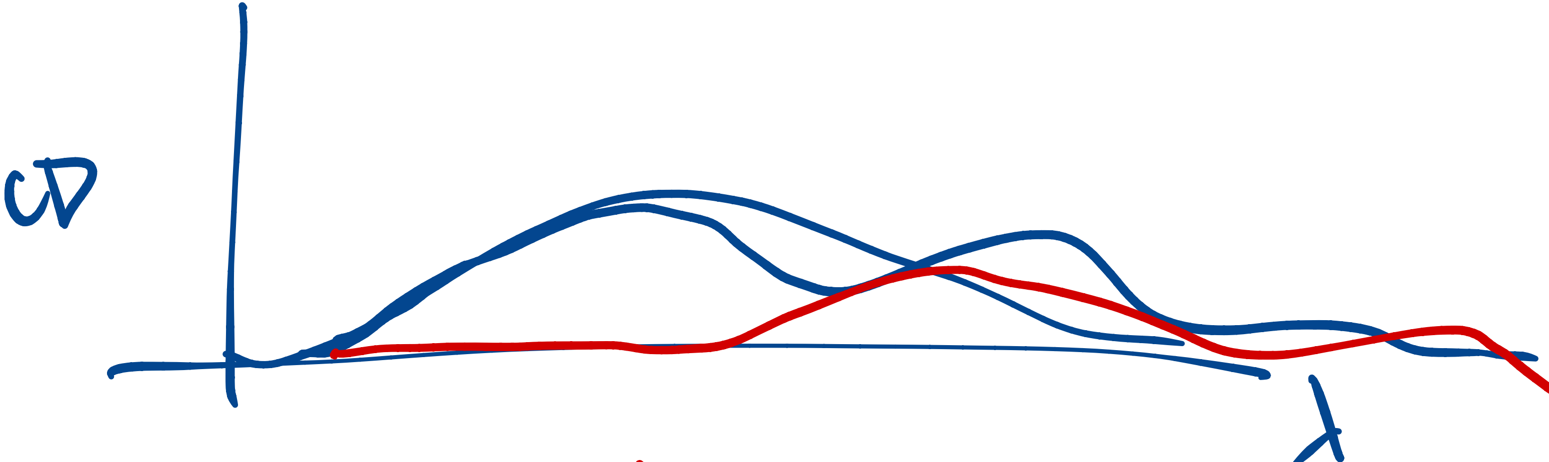




## Circular Dichroism

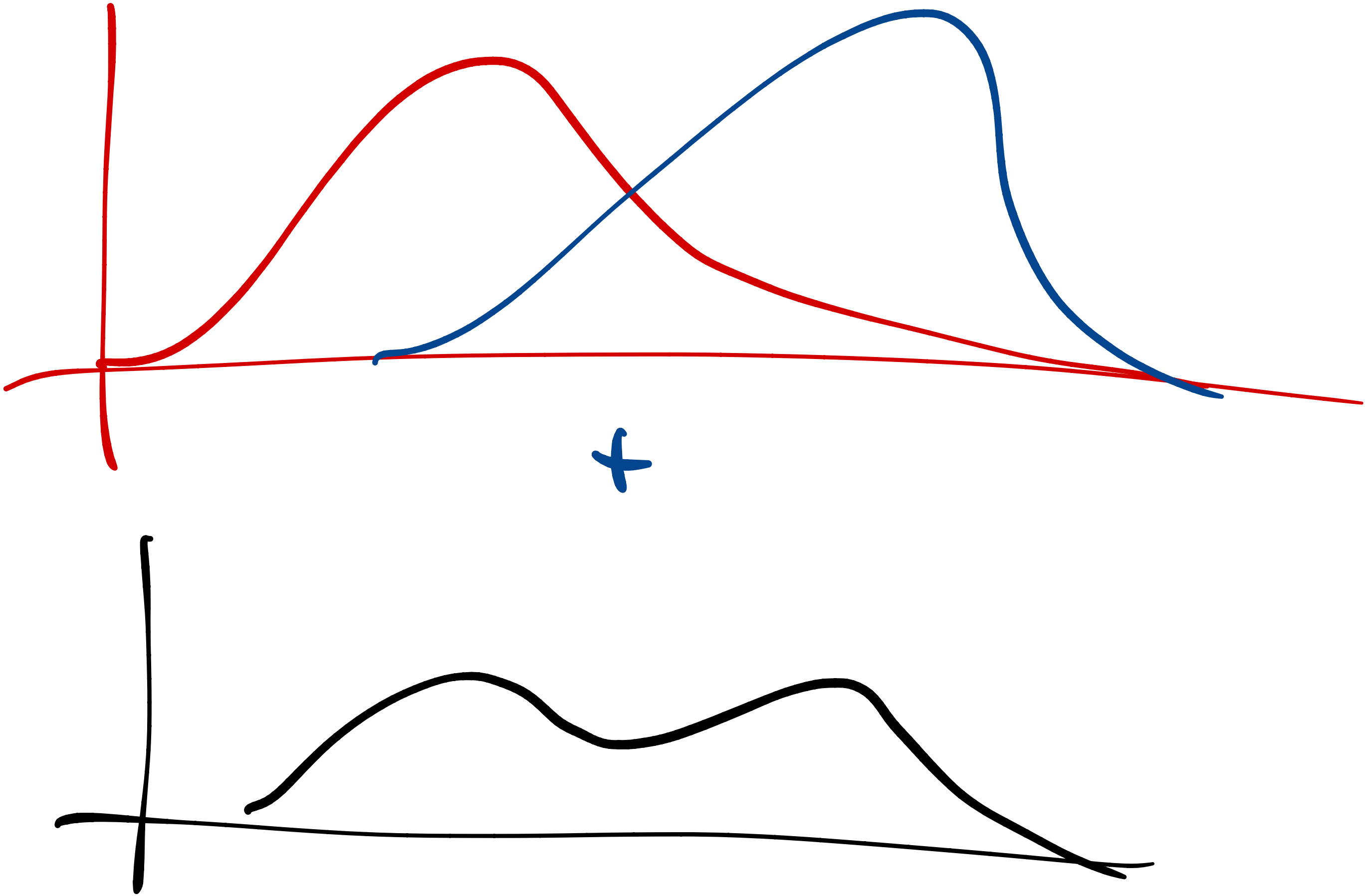







220 nm
particular secondary structure

NMR –



$$f_N = \frac{\#\text{ folded}}{\text{total }\#\text{ molecules}}$$

$$= \frac{\#N\ /V}{\#N + \#D\ /V}$$

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

Native

Denatured

$$D \rightleftharpoons N$$

$$K_{eq} = \frac{[N]}{[D]}$$

$$f_D = 1 - f_N = \frac{[D]}{[N]+[D]}$$

$$0 \le f \le 1$$

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

$$K = \frac{[N]}{[D]}$$

$$[N] = [D]K$$

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

$$\Rightarrow \quad f_N = \frac{K_{fold}}{1+K_{fold}} = \frac{1}{1+{}^{1}/_{K_{fold}}}$$

$$f_D = \frac{1}{1+K_{fold}}$$

$$f_N = \frac{K}{1+K}$$

$f_N = 0.1$ up to $0.9$

10% $0.1(1+K) = K$

$.1 = 0.9K, \quad K = 1/9 = 0.111\ldots$

90% $0.9(1+K) = K$

$0.9 = 0.1K \Rightarrow K = 9$

$$\frac{1}{9} < K < 9 \quad \text{range}$$

$$\ln(1/9) < \ln K < \ln(9)$$

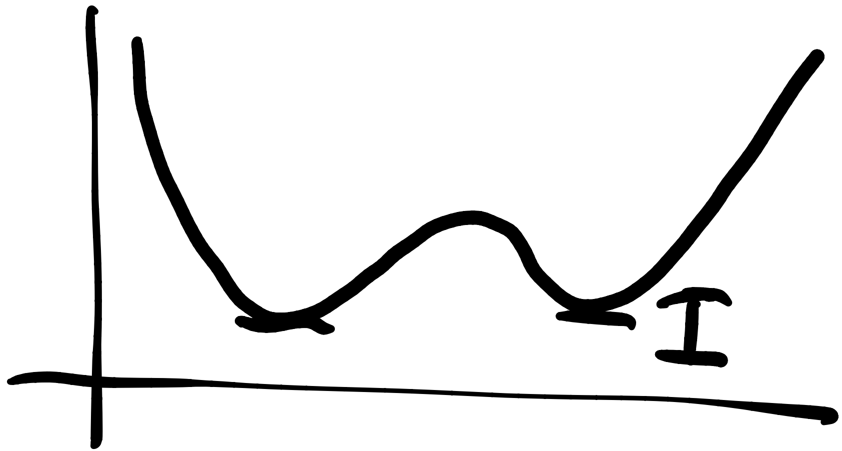
$$-2.2 < \ln K < 2.2$$

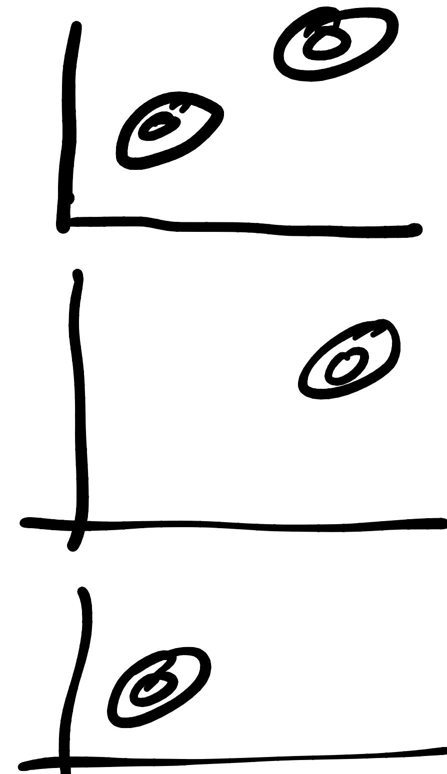
$$\Delta \bar{G}^0 = -RT \ln K$$

$RT \approx .6$ kcal/mol

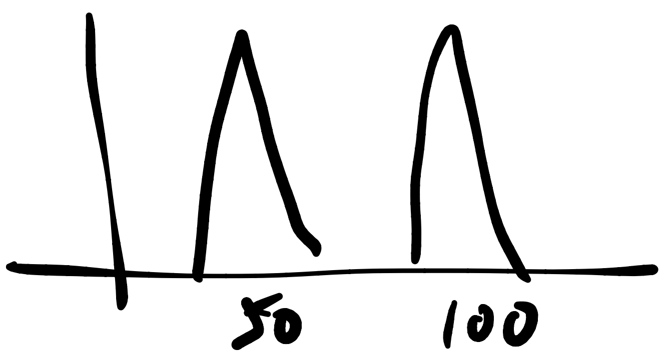
$$2.2 > \Delta G^0/RT > -2.2$$

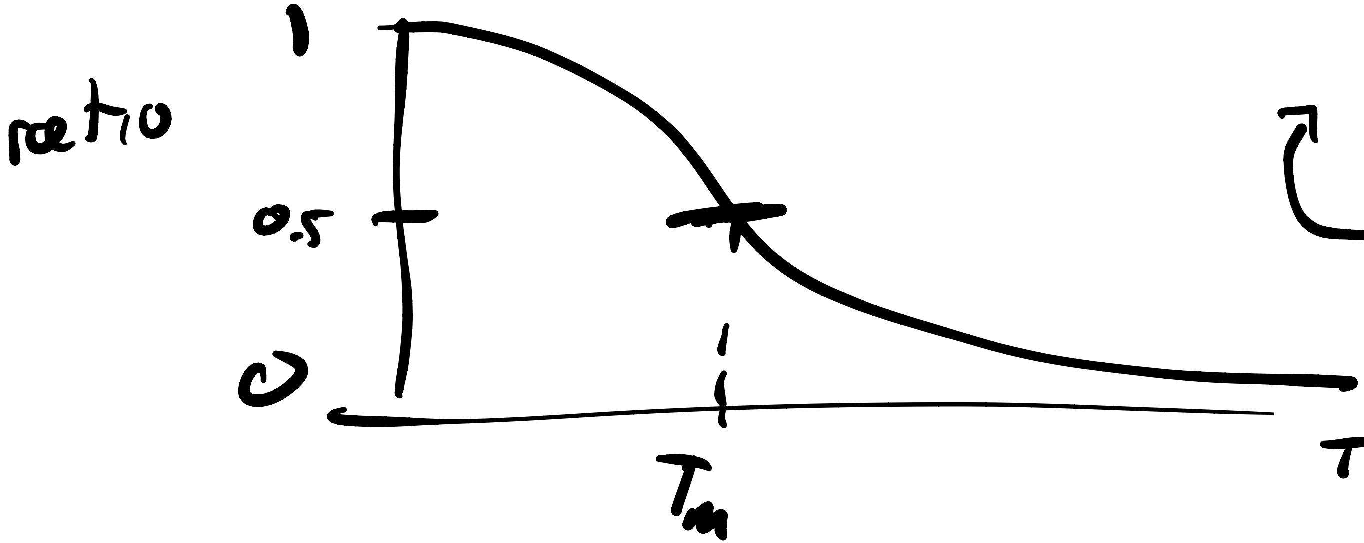
$$-1.3\,\frac{\text{kcal}}{\text{mol}} < \Delta G^{\circ} < 1.3\,\frac{\text{kcal}}{\text{mol}}$$




$$\frac{y - y_N}{y_N - y_D}$$






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

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

$$f = \frac{K}{1+K}$$

$$= \frac{e^{-\Delta G/RT}}{1+e^{-\Delta G/RT}}$$

$$\frac{1}{1+e^{\Delta G/RT}}$$

$$f = \frac{1}{1 + e^{\Delta G/RT}}$$

signal $\quad y_N f_N + y_D (1 - f_N)$

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

@ $T_m$, $\Delta \bar{G}^\circ = 0 = \Delta \bar{H} - T\Delta \bar{S}$

and $\Delta \bar{H} / T_m = \Delta \bar{S}$

$RT = 8.314 \, J/K\,mol \cdot 300$

$\approx 2400 = 2.4 \, kJ/mol$

$1 \, kcal = 4.184 \, kJ \Rightarrow .6 \, kcal/mol$

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

$$K_{eq}(T_2) / K_{eq}(T_1) = e^{-\Delta G \left( \frac{1}{RT_2} - \frac{1}{RT_1} \right)}$$

$$e^x = 10 \qquad \ln(10) = x$$

$$x \approx 2.3$$

$RT = 0.6$

10 factor $\quad 2.3 RT$

$\sim 1.4$ kcal/mol

$e^{\text{what}/RT} = 10 \qquad e^{2.3} = e^{1.4/RT}$

$$e^{x+y} = \underbrace{e^{x}}_{K_{eq}} e^{y} \leftarrow 1.4\ \text{kcal/mol}/\beta\Gamma$$

$$K_{eq} \cdot 10$$

$$-1.4\ \text{kcal/mol} \sim 1/10$$

$\Delta G$ folded protein $\sim 5-10$ kcal/mol if $7$ kcal/mol $= 5 \cdot 1.4$ kcal/mol

$10^5$ fold change