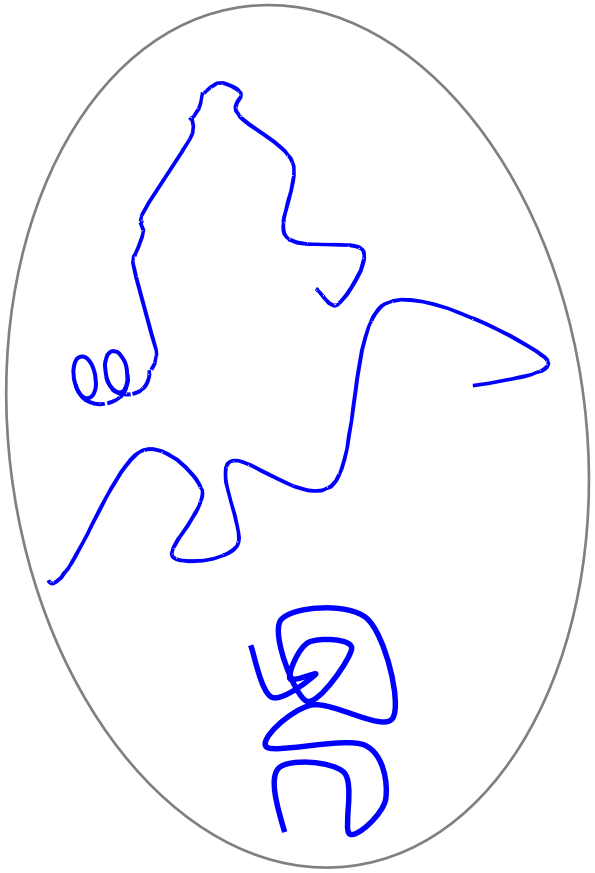
Protein stability

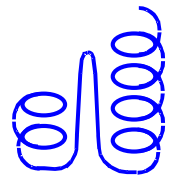
Our model

Andrew Torda, Wintersemester 2016 / 2017 GST



denatured (D)
unfolded
non-native

ΔG



native
(N) folded

Free energy change on

- folding
- denaturing $(-\Delta G)$

For a stable protein

$$\Delta G_{N \rightarrow D} > 0$$

Nomenclature – not for discussion

Make sure the following words are clear

- enthalpy
- free energy
- denatured / unfolded

Protein Stability

- what is known empirically
- definitions
- a stability surprise
- some explanations
- weaknesses of models

ΔG convention

$\Delta G_{folding} = -\Delta G_{unfolding}$

Define $\Delta G > 0$ as stable so reaction is

folded → unfolded (native → denatured)

more positive ΔG, more stable the protein

Empiricism / rules

- most proteins are marginally stable ($\Delta G \approx 0$)
- proteins can be denatured by
  - pH, ions, temperature, solvent, surface area, urea (example later)

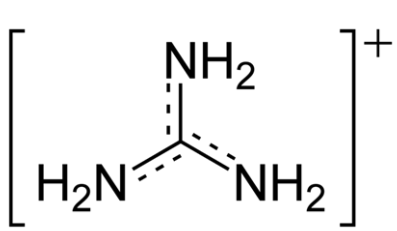
- too hot ?
  - eggs cook, people die
- big variation
  - some bacteria live at 373 K – their proteins are not denatured

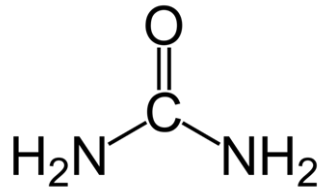
Explain denaturing

Why do these denature a protein ?

pH, concentrated ions, temperature, solvent, surface area

- pH ? change the charge on some groups, remove favourable interactions
- ions ? provide competition for charges, H-bonds





- temperature ? add kinetic energy, push particles out of minima
- solvent ? remove favourable protein-solvent interactions
- surface area ? surface tension / protein – air interactions

Rules

Nature cares about free energy ΔG we measure free energy, not potential energy

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

- G free energy (Gibbs)
- H enthalpy
  potential energy including volume effects $U + PV$
- S entropy

Formula will come back – remember

- two contributions
  1. entropy always favours unfolding
  2. enthalpy / potential energy will favour folding

Measurement

Folded / Unfolded

- how to measure
  - spectroscopy
    - absorbance (optical / UV spectroscopy)
    - rotational (CD / ORD)
    - fluorescence
    - NMR
  - activity
  - …
- usually – two states (native / denatured) that somehow look different

Energies

- calorimetry
- measurements of [native] / [denatured]

Experimental - measure ΔG ?

How do I measure ΔG for a reaction $\mathrm{A} + \mathrm{B} \rightarrow \mathrm{C}$?

- calorimetry ? measure the heat released
  - for a protein, reaction is folded → unfolded
  - Could one prepare protein and watch it fold ? Difficult

Alternative view – for any reaction $\mathrm{A} \rightarrow \mathrm{B}$ at equilibrium

- $\Delta G = RT \ln \frac{[\mathrm{B}]}{[\mathrm{A}]}$ or in our case $\Delta G = RT \ln \frac{[\text{unfolded}]}{[\text{folded}]}$

Problem

- under normal conditions [unfolded] is too small
- if $\frac{[\text{unfolded}]}{[\text{folded}]} = 10^{-4}$ or 10^{-5} could you see it ?

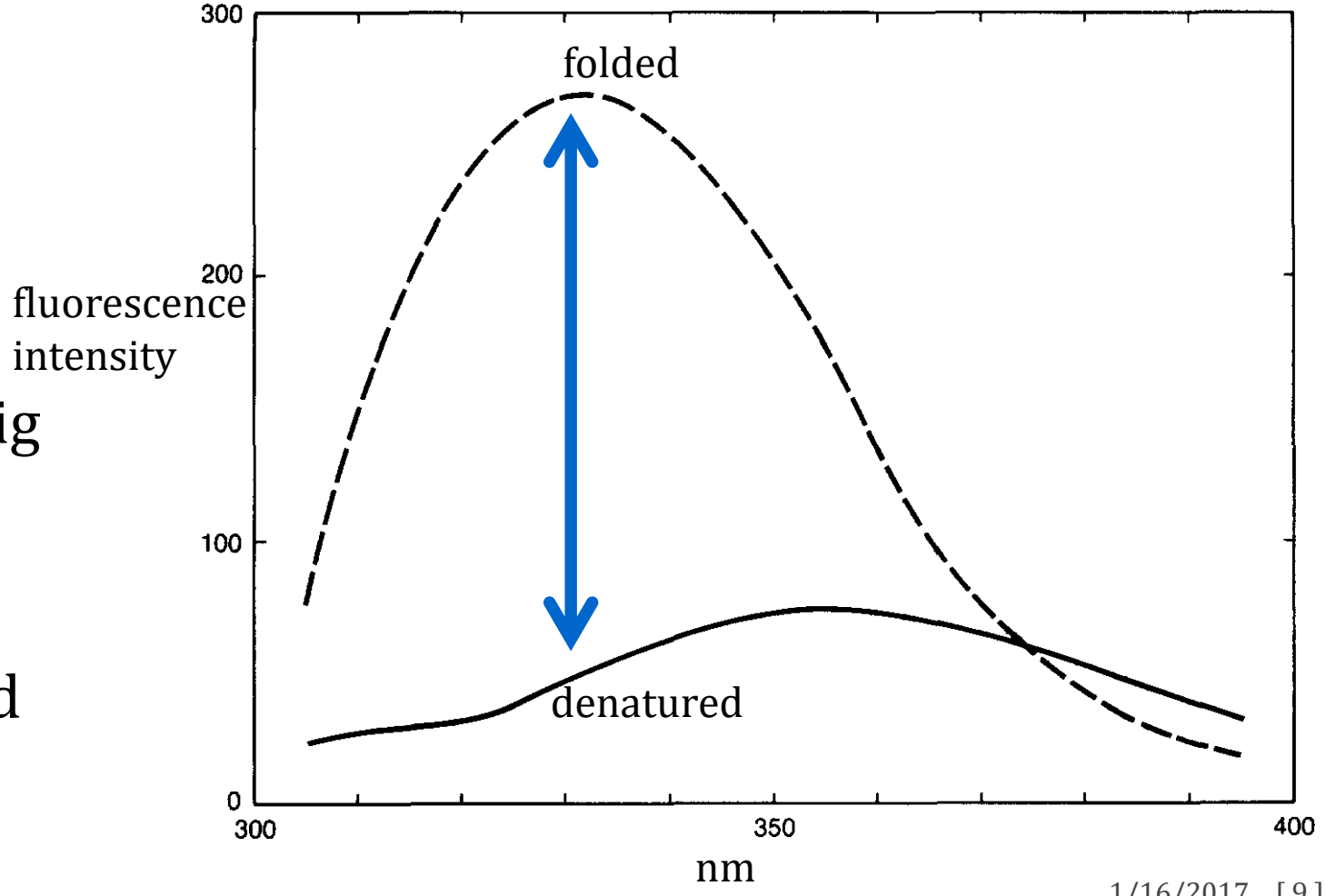
Assume

- you have some kind of spectroscopy (CD, fluorescence, …)
- you can detect a signal for folded and unfolded

- if the protein is partially folded
  you can estimate ΔG

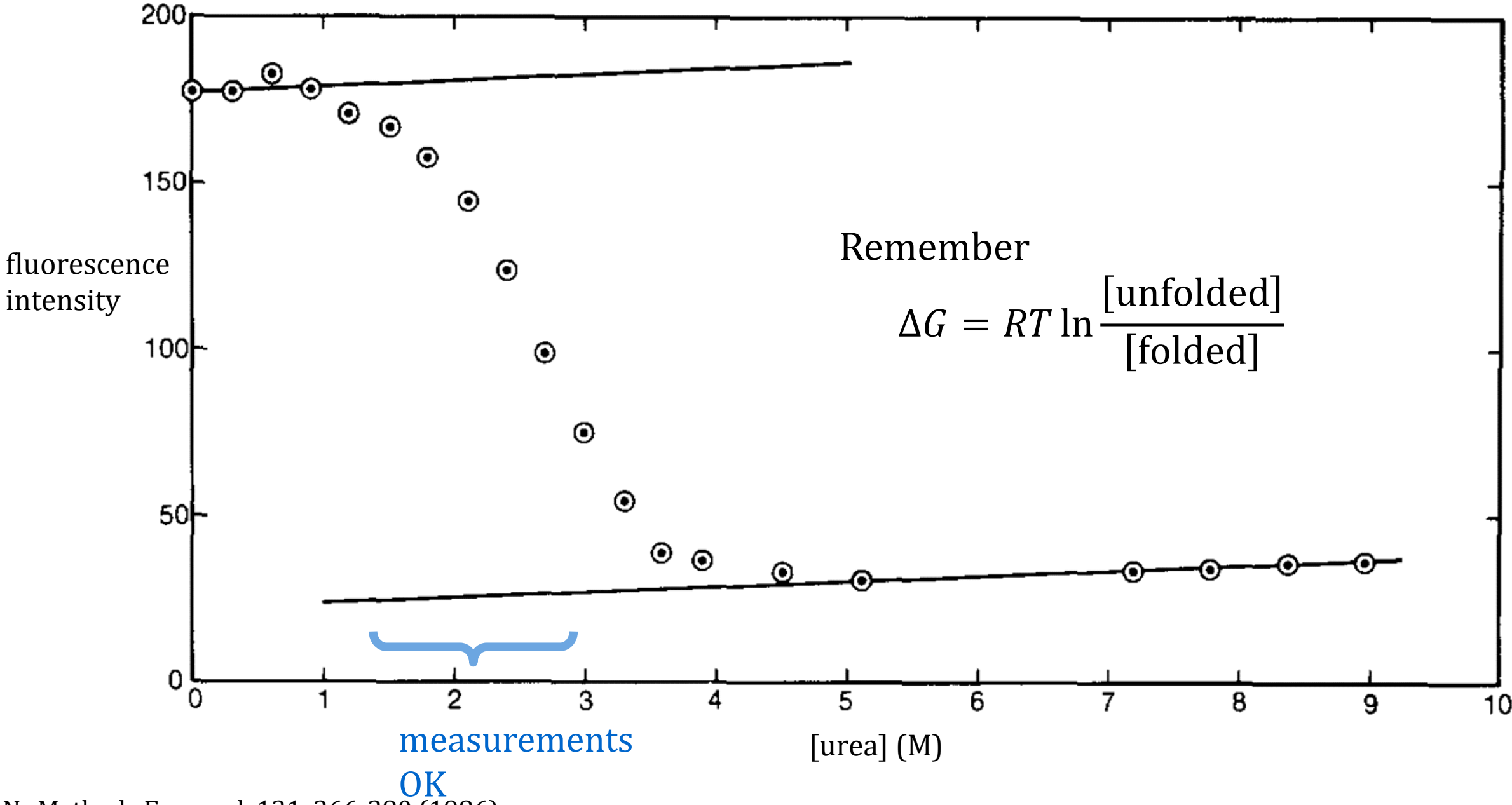
- if $\frac{[\text{unfolded}]}{[\text{folded}]}$ is not too small or big
  I can measure it

- trick is to make it partially unfold



Pace, C.N., Methods Enzymol, 131, 266-280 (1986)

Add denaturant



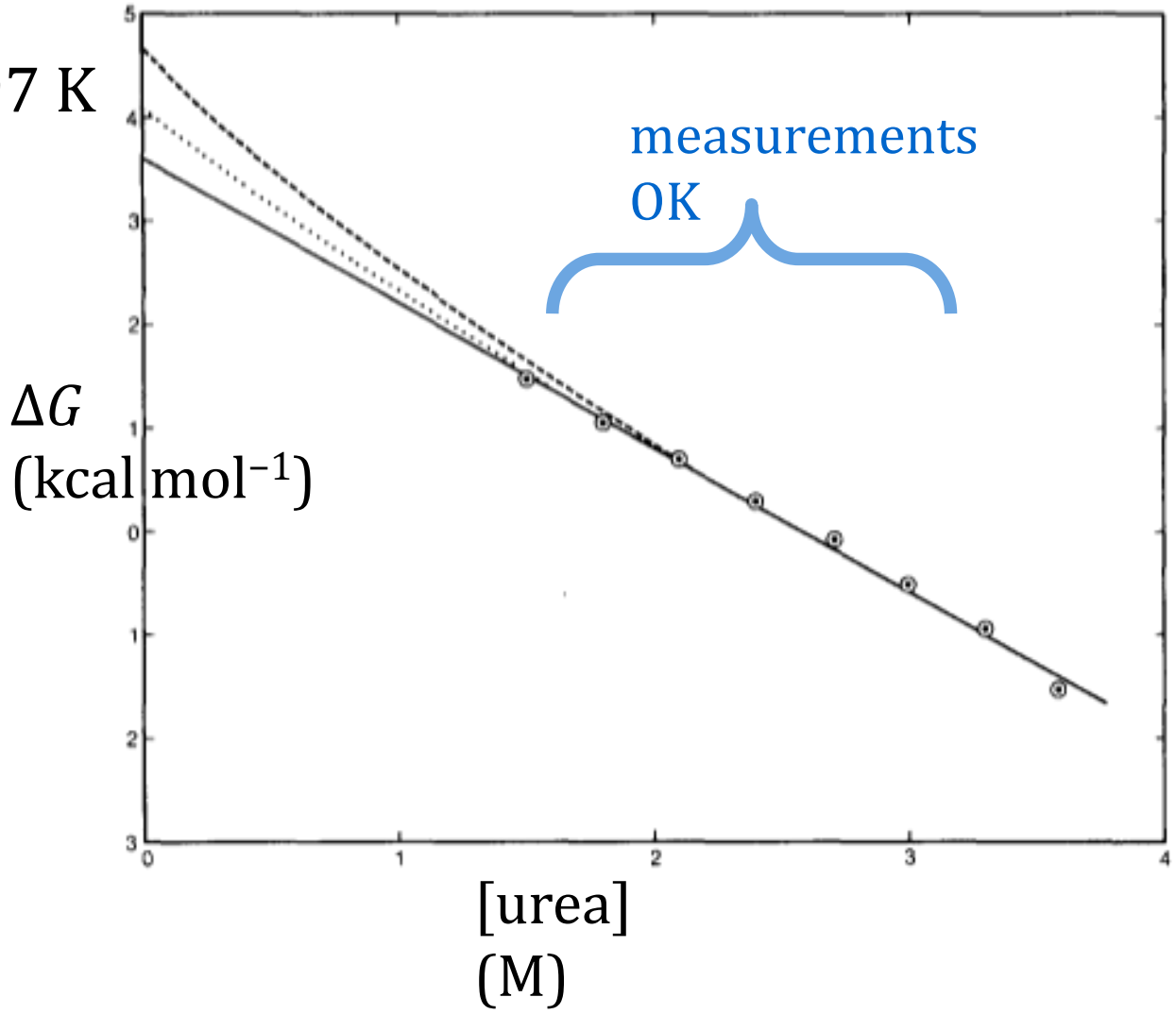
Remember

$$\Delta G = RT \ln \frac{[\text{unfolded}]}{[\text{folded}]}$$

Pace, C.N., Methods Enzymol, 131, 266-280 (1986)

Experimental - measure ΔG ?

- From spectroscopy, estimate ΔG for each temperature / [urea]
- Extrapolate back to zero urea or 297 K
- Far from perfect - do not ask me if the line is really a line



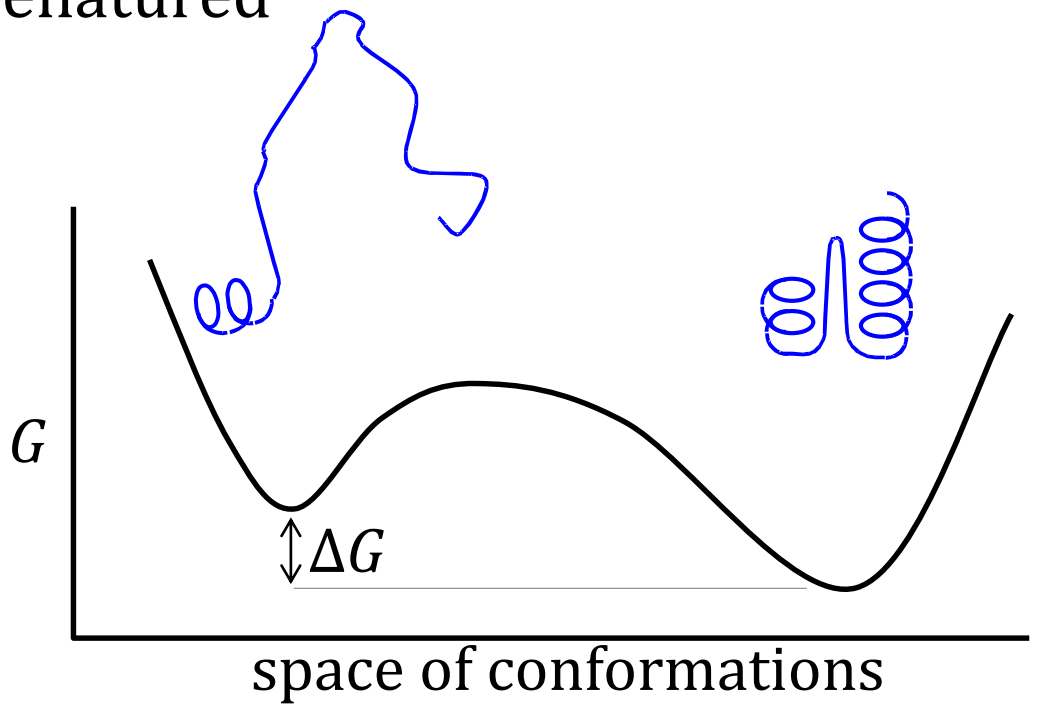
Pace, C.N., Methods Enzymol, 131, 266-280 (1986)

Two state model

Model requires an energy barrier

What if $\Delta G = 0$?

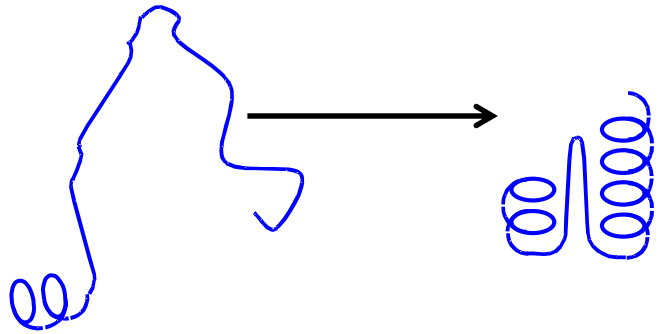
- ½ molecules folded
- ½ molecules denatured



First picture of stability

What holds a protein together ?

- forces between atoms
  - bonds, electrostatic, Lennard-Jones
- atoms also repel



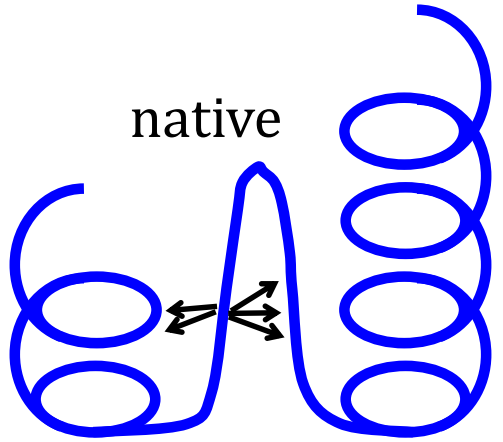
Cannot be so simple

- atoms would just fall into correct position
- ΔG would always be very positive
- missing ?
  - effect of solvent

- in native structure there are "correct contacts"

Balance of energy terms

Refer to contacts in native structure as correct



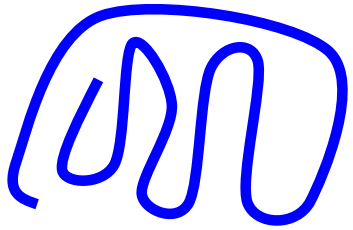
One atom in native structure

- interacts with correct neighbours

In the non-native structures there are also contacts

- atom interacts with “wrong” neighbours

- balance of forces
- stability has to do with
  - energy / enthalpy $H_{\text{native}} - H_{\text{denatured}}$

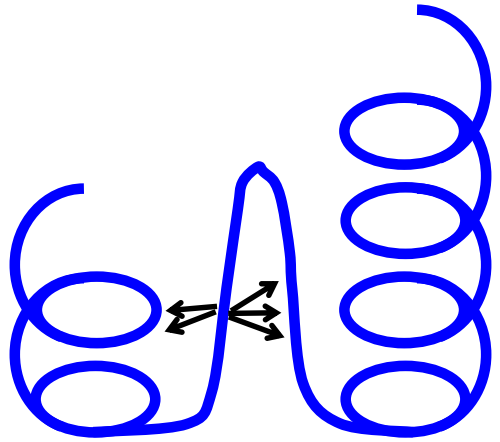


some denatured / non-native

Balance of energy terms

More forces

- how many polar / charged groups are there ?
  - NH + CO backbone every residue
  - polar / charged sidechains
- all can interact with water



- stability will also depend on

$$H_{native\_water} - H_{unfolded\_water}$$

Balance of energies (Klausur Frage)

- contacts in native structure
- contacts in unfolded structure
- contacts of native protein with water
- contacts of unfolded protein with water

Entropy version 1

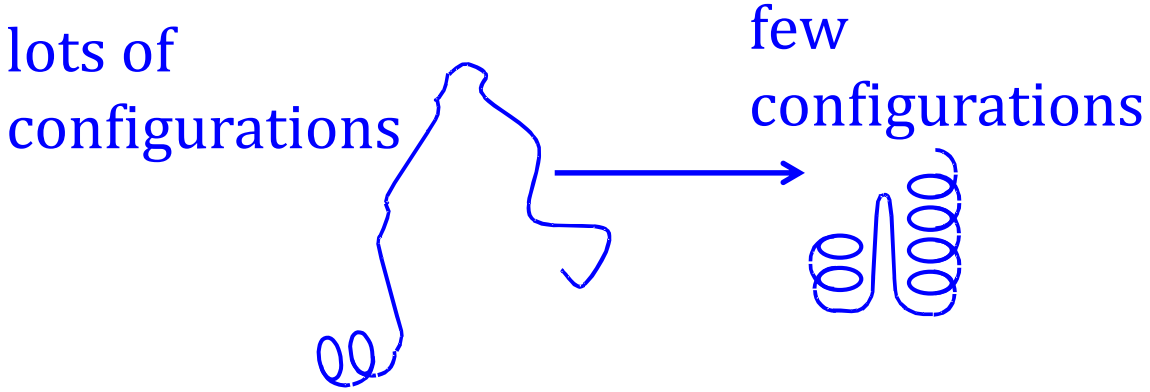
$\Delta G = \Delta H - T\,\Delta S$

- considered ΔH terms, what is $\Delta S_{folded\ unfolded}$?
  - entropy depends on the number of conformations ($k \ln \Omega$) or better

$S = -k \sum_{i=1}^{N_{states}} p_i \ln p_i$

As a protein unfolds

- number of conformations ↑
- entropy goes ↑



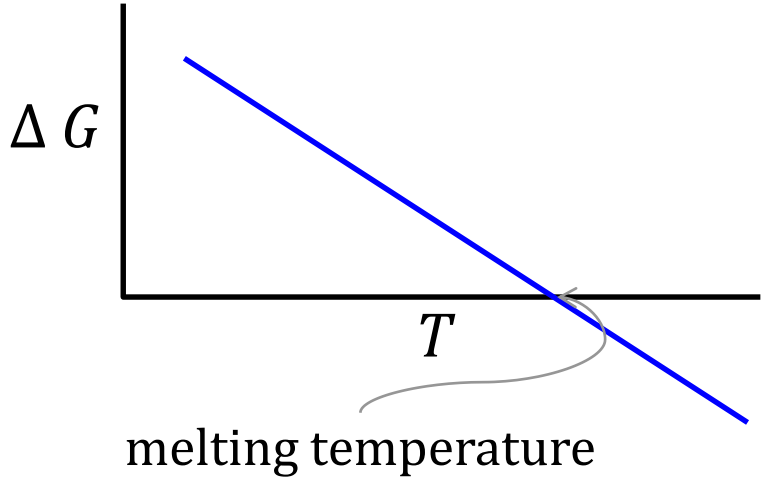
- entropy / ΔS will favour denaturing

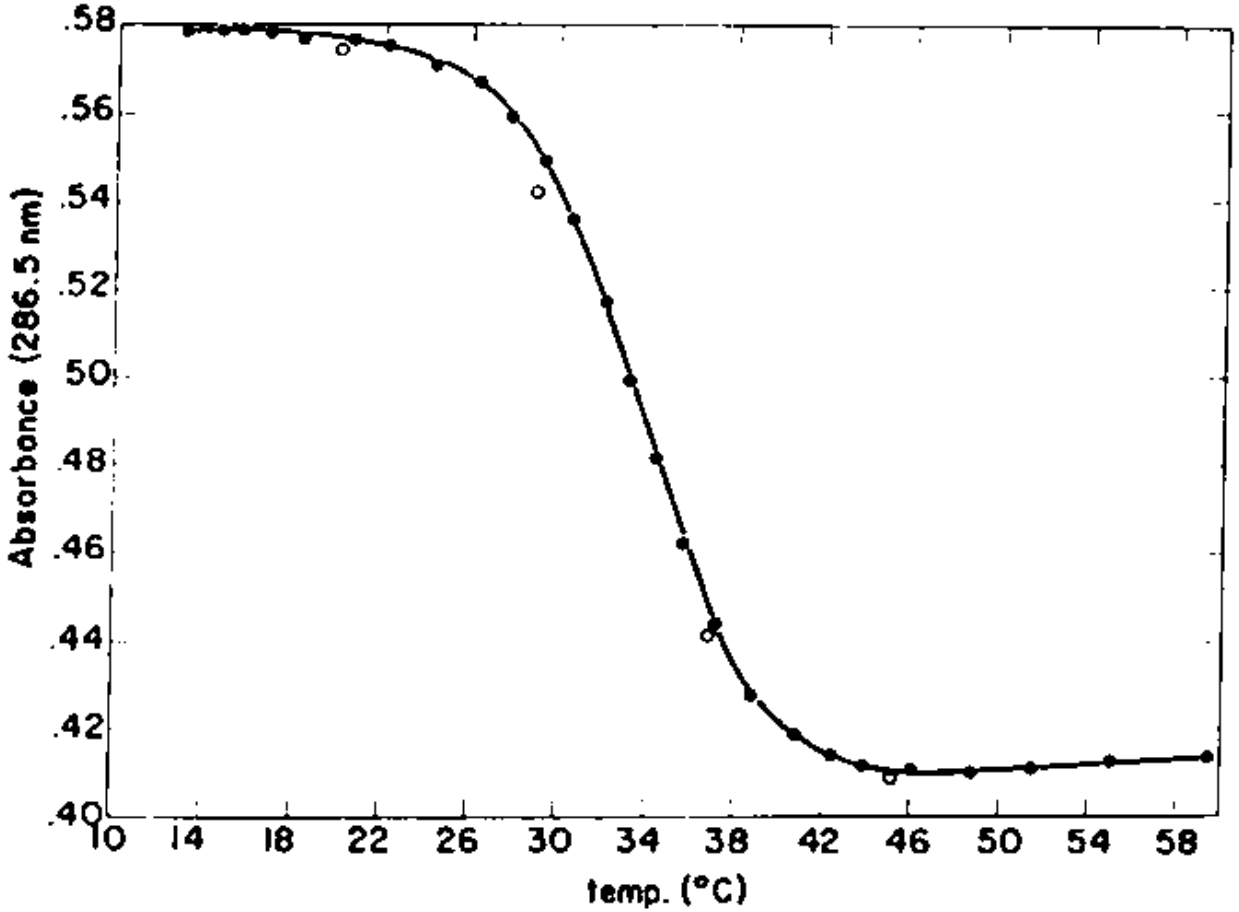
Ω_x number of states in x
k Boltzman constant

Balance of forces version 1

$\Delta G = \Delta H - T\,\Delta S$

- proteins should melt / cook / fall apart if you heat them





ribonuclease unfolding

Tsong, T.Y, Baldwin, R.L, Elson, E.L., Proc Natl Acad Sci, 68, 2712-2715, 1971

Entropy version 2

$\Delta G = \Delta H - T\,\Delta S$

- is ΔS a constant ? Is it T dependent ?
- meaning of $\Delta S_{folded\text{-}unfolded}$
- roughly how does the number of states change ?

$$\Delta S = S_{folded} - S_{unfolded}$$

$$= k\left(\ln \Omega_{folded} - \ln \Omega_{unfolded}\right)$$

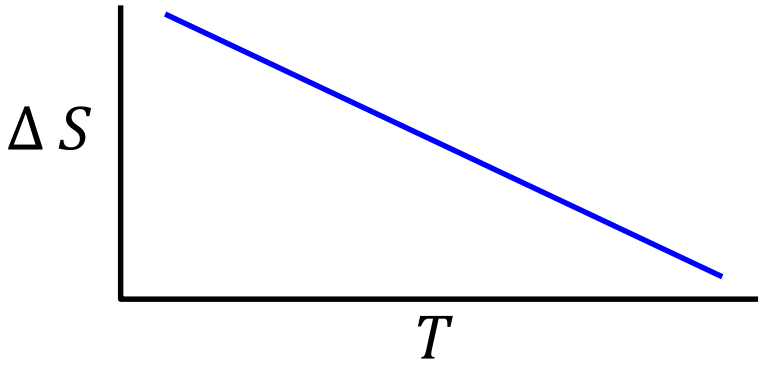
$$= k \ln \frac{\Omega_{folded}}{\Omega_{unfolded}}$$

- Ω_{folded} definitely goes up with temperature

Ω_x number of states in x

Entropy change on unfolding

One has a curve like



Radical consequence

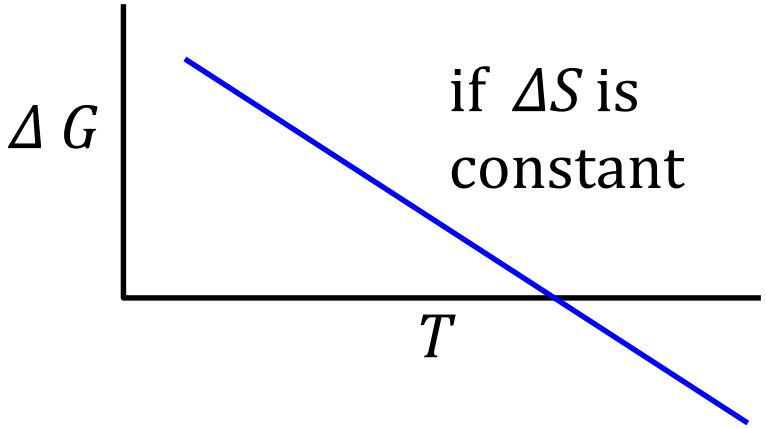
- entropy might make proteins less stable as you cool them

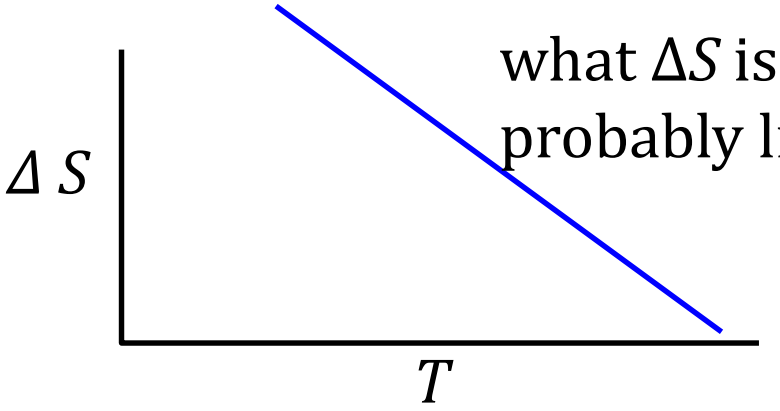
Can one cook an egg by cooling it ?

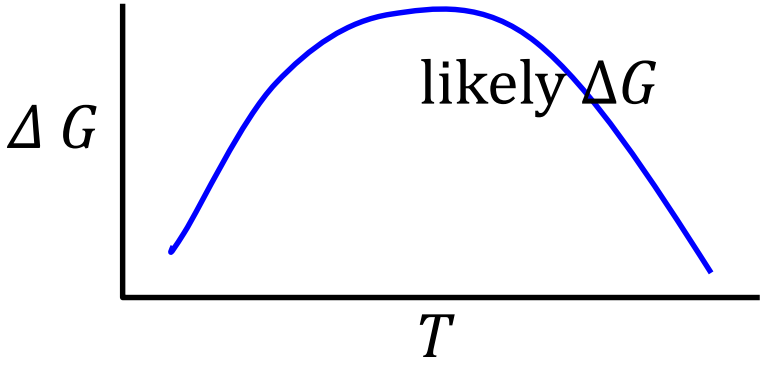
Combining these properties

protein stability

$\Delta G = \Delta H - T\,\Delta S$







What is seen / claimed ?

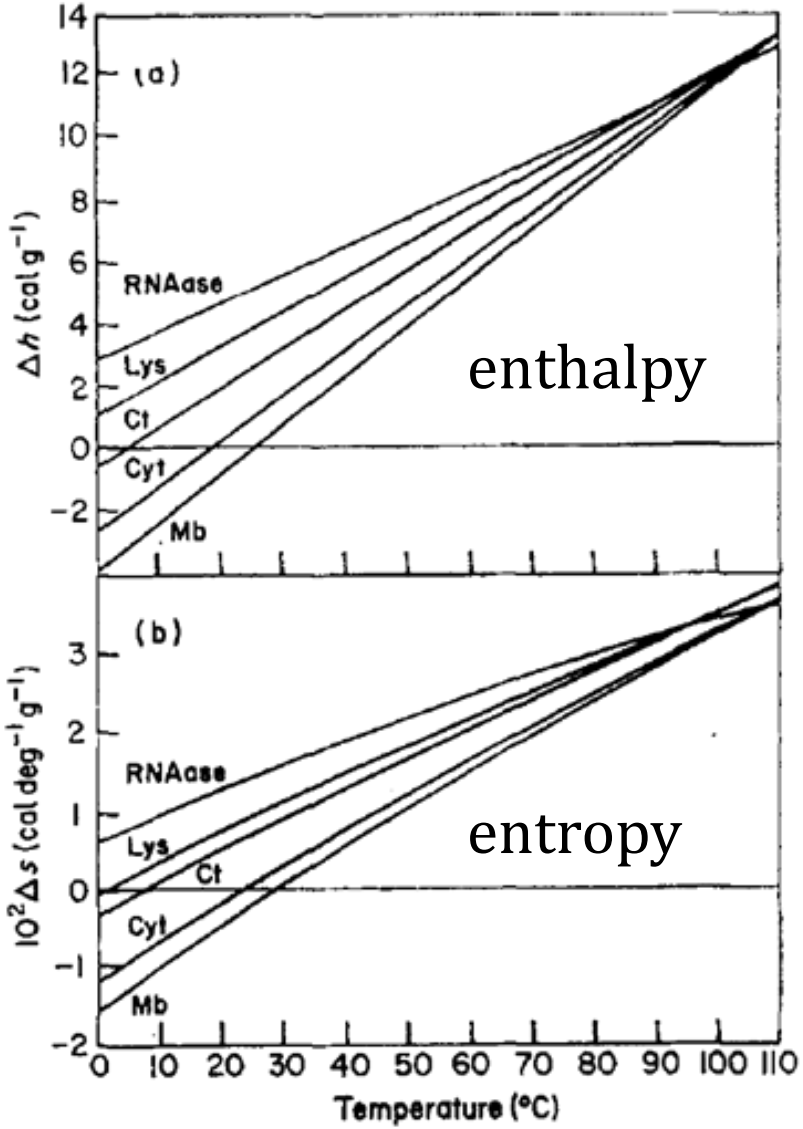
Old measurements

- protein stability (ΔG) is a balance of energy and entropy
- note sign convention

$$\Delta G = \Delta H - T\,\Delta S$$

- difference of two large numbers comes to nearly zero
- what might you expect ?

lysozyme
RNAase
chymotrypsin
myoglobin
cytochrome C



Privalov, P.L., Khechinashvili, N.N, J. Mol. Biol. 86, 665-684, 1974

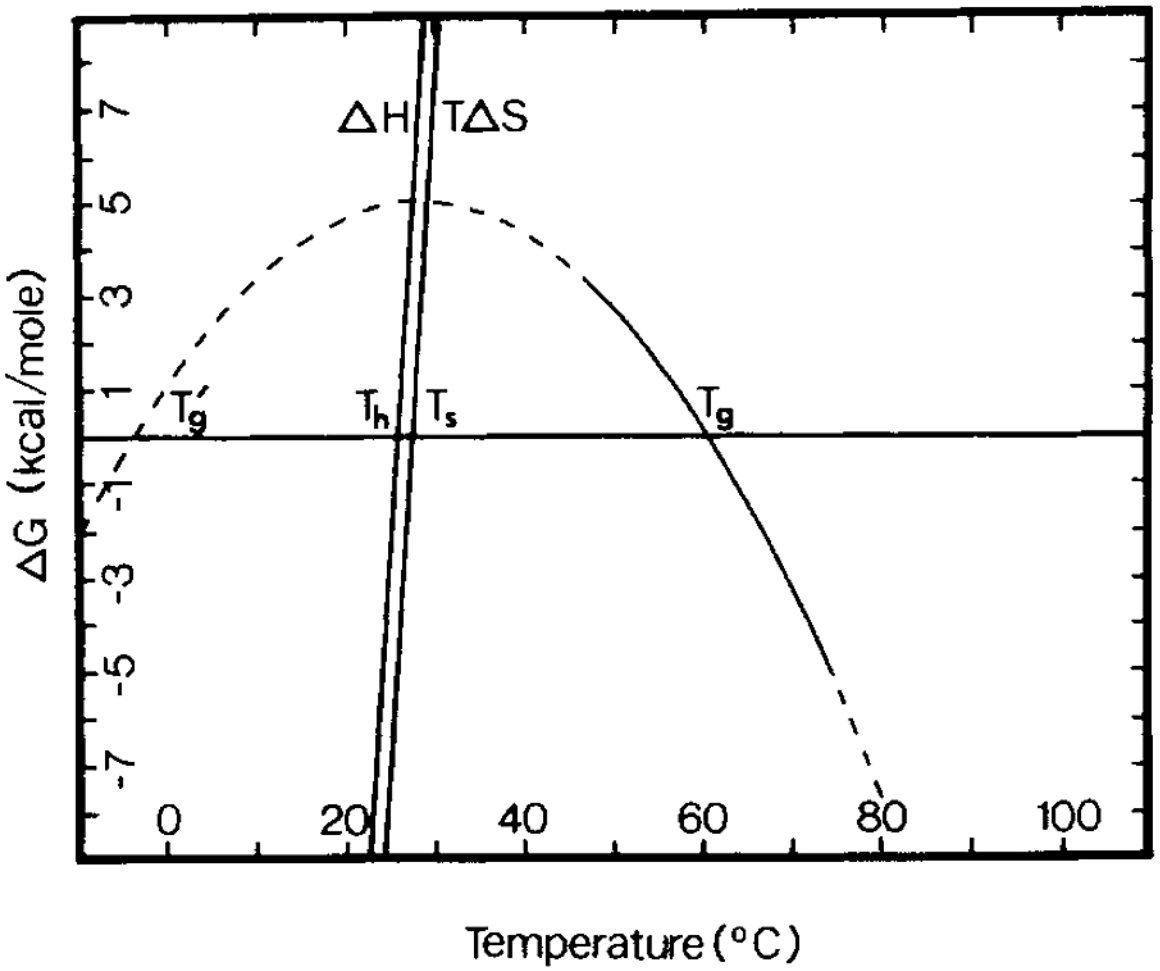
final version

Claim.. for typical protein

- bold line –measured
- dashed – extrapolated

Implies

- you can denature a protein by cooling (cook egg by freezing ?)
- what is measured ?



Becktel, W.J., Schellman, J.A., Biopolymers, 26, 1859-1877, 1987

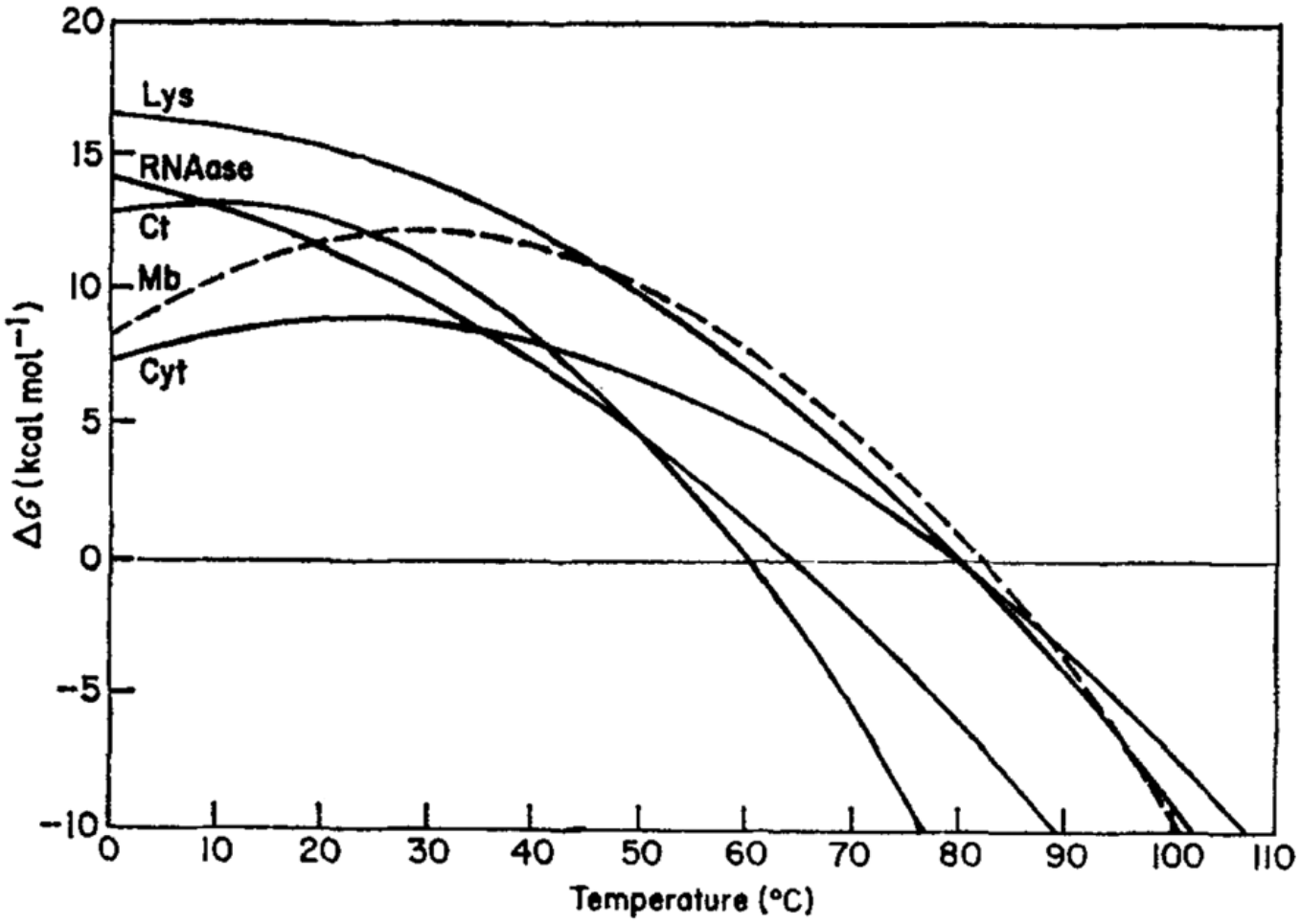
free energy experimental

Looks like there will be cold denaturing

- below zero

Curve is different for all proteins

Does this prove "cold denaturing" ?



lysozyme
RNAase
chymotrypsin
myoglobin
cytochrome C

Privalov, P.L., Khechinashvili, N.N, J. Mol. Biol. 86, 665-684, 1974

Cold denaturing

Controversial

Often predicted to happen below 0°

- hard to measure

Entropy and Enthalpy Summary / Consequences

Enthalpy H / potential energy

- will always favour folding

Entropy

- will always favour unfolding

Debatable

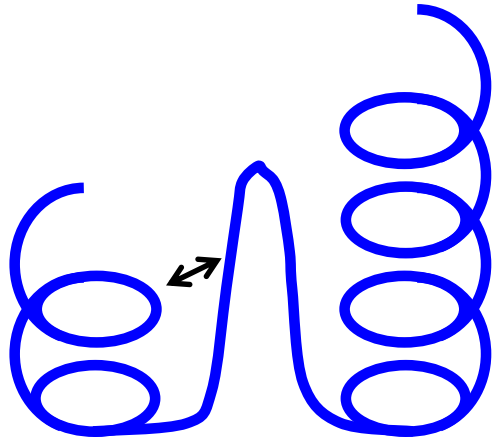
- how much does entropy change with temperature ?

- Two state model ? native → denatured
  - what does $\Delta G = 0$ mean ?
  - definitely too simple – proteins partially unfold

proteins and systems

Energy

- energy of native state is important but
- energy of denatured state is equally important

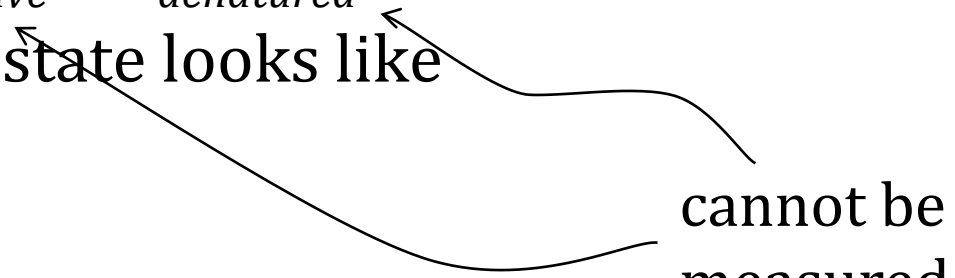


Consequence

- naïve optimisation may not work
- you propose to make a protein more stable by putting in residues with opposite charge
  - lowers energy of native structure
  - also lowers energy of non-native structure

proteins and systems

- you are always looking at $\Delta G = G_{native} - G_{denatured}$
- nobody knows what the denatured state looks like



Not just for temperature

- chemical denaturant ?
  - maybe simply binds to unfolded protein
    - lower energy – more stable

Water is important (water entropy)

- unfolding a protein changes water order

- correct way to look at system is

$$\Delta G = G_{native\_protein+water} - G_{denatured\_protein+water}$$

- next … motions in proteins

Motions, frequencies and proteins

Motions and dynamics in proteins

- how big are they ?
- how fast are they ?

- Examples

Types

- without barriers
- with barriers

Energies

- equal probability
- not equal probability

Nasty question .. answered later

I have motions in a protein at T=273 (cold)

Heat protein to 300 K

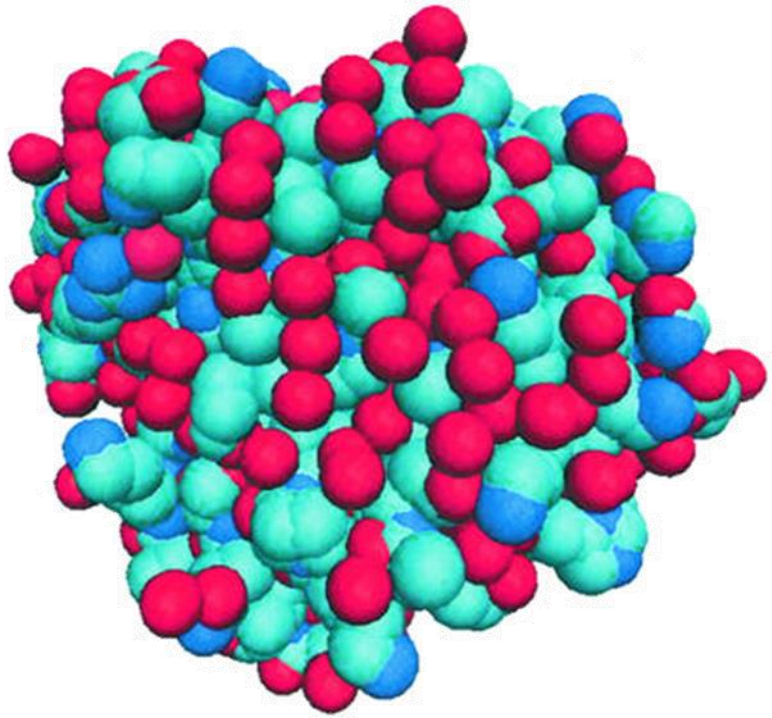
- do the frequencies of motions change ?

Protein motion examples

Early evidence that motions are important

- myoglobin structure (1962)
  - carries O_2, maybe first protein structure solved
- no channel could be found for O_2 to reach haem group
- could only be explained if parts of protein move and open up

- many similar stories
- activity of protein cannot be explained by simple structure

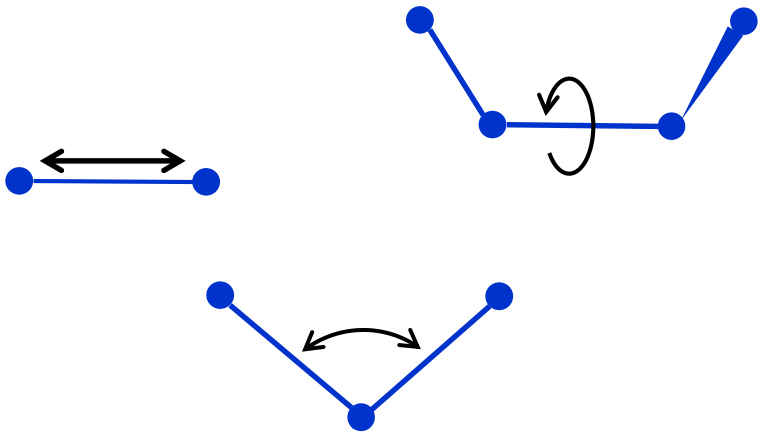


Frauenfelder, McMahon and Fennimore, Proc Natl Acad Sci USA, 100, 8615-8617 (2003)

Protein motion examples

Fundamental arguments

- bonds, angles vibrate, rotate
- basis of many kinds of spectroscopy
  - infra red, fluorescence, NMR, …



More fundamental arguments

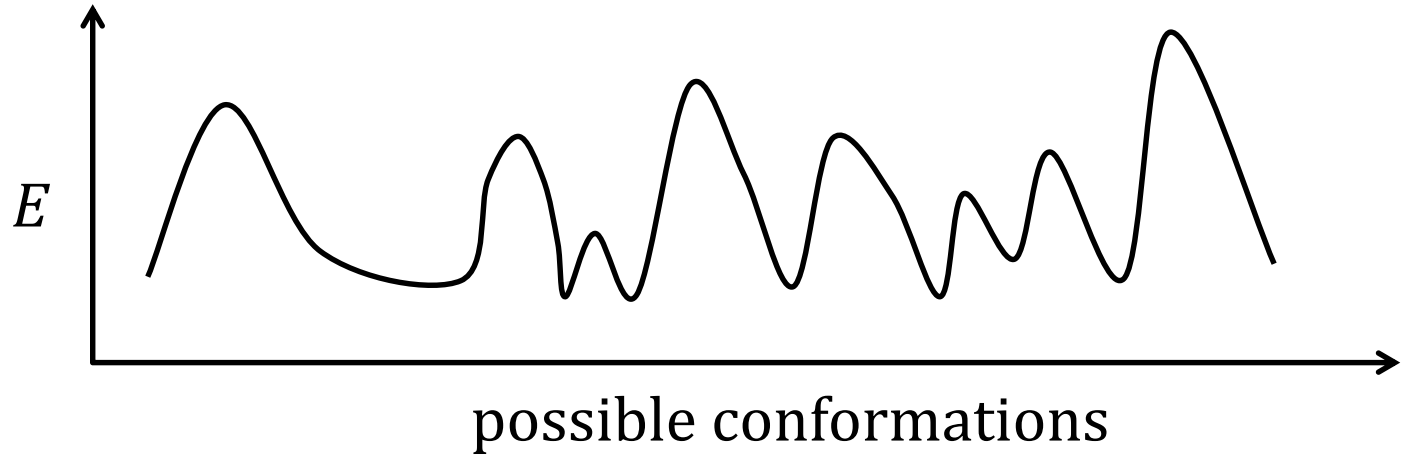
- at $T = 0$ everything dead
- at $T = 300$ (this room) everything has kinetic energy
  - everything is moving
  - meaning of temperature ? T, E_{kin} ?

$$\frac{1}{2}mv^2$$

E_{kin}	kinetic energy
T	temperature

Energy surfaces

Run around on an energy surface



Energy surface and energy should determine motions

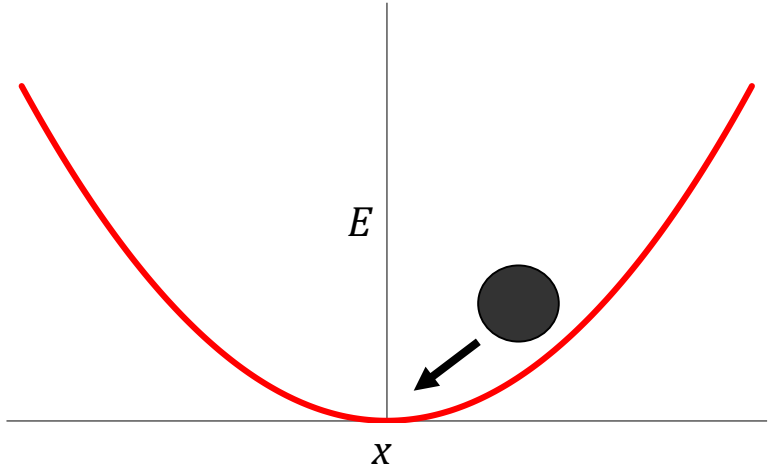
- too complicated
- energy surface not well known

Work with simpler models

Motions with or without barriers

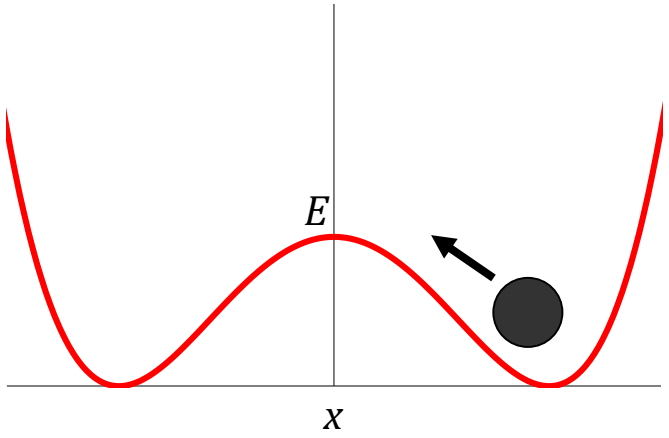
Without barriers

- one state + fluctuations



With a barrier

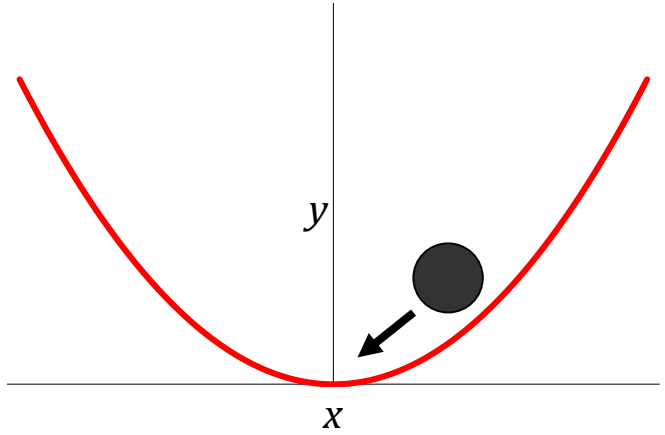
- two states



Harmonic oscillators

Find them everywhere..

- potential energy = kx^2
- what is the frequency of motion ω ?



$$x(t) = A\cos(\omega t + \delta)$$

A is the amplitude

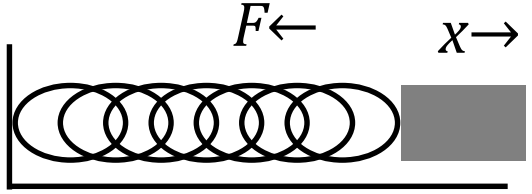
ω is the frequency

δ is phase

Detour .. why does this make sense ?

Harmonic oscillator

$$m\frac{d^2x}{dt^2} + kx = 0$$



$$F = -kx$$

$$ma = -kx$$

$$m\frac{d^2x}{dt^2} = -kx$$

say $\omega^2 = \frac{k}{m}$ so $\omega = \left(\frac{k}{m}\right)^{1/2}$

$$\frac{d^2x}{dt^2} + \omega^2 x = 0$$

has a solution.. $x(t) = A\cos(\omega t + \delta)$

- can I convince you ?

Is the solution valid ? $\boldsymbol{x(t) = A\cos(\omega t + \delta)}$

$$\frac{dx}{dt} = A(-\sin(\omega t + \delta))\omega$$

$$= -A\omega \sin(\omega t + \delta)$$

$$\frac{d^2x}{dt^2} = -A\omega \cos(\omega t + \delta)\omega$$

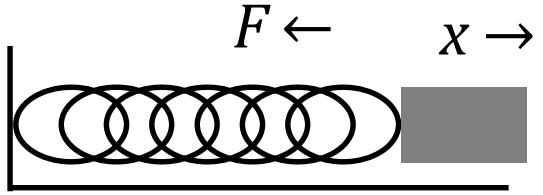
$$= -A\omega^2 \cos(\omega t + \delta)$$

$$= -\omega^2(A\cos(\omega t + \delta))$$

which can be re-written as $\frac{d^2x}{dt^2} = -\omega^2 x$

Is the solution valid ? $x(t) = A\cos(\omega t + \delta)$

From first arguments $\frac{d^2x}{dt^2} + \omega^2 x = 0$



$-\omega^2 x + \omega^2 x = 0$

so $x(t) = A\cos(\omega t + \delta)$ is a solution

and back to $\frac{d^2x}{dt^2} = -\omega^2 x$

Frequency and energy models $\frac{d^2x}{dt^2} = -\omega^2 x$

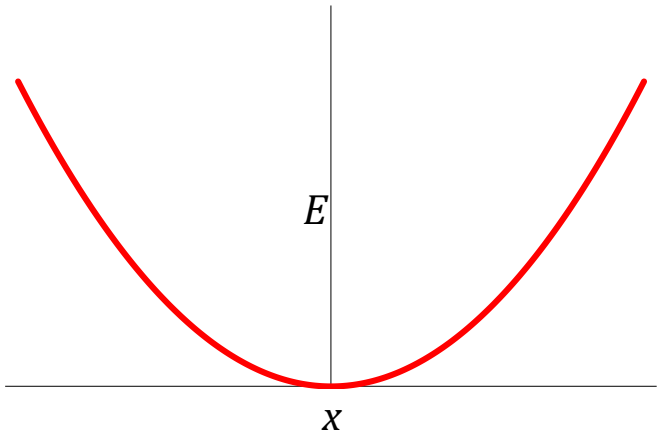
What is meaning of $\frac{d^2x}{dt^2}$?

- second derivative / curvature

What happens if you change temperature ?

- angular frequency ω depends on energy surface
- temperature does not appear here

- what does change ?



Changing temperature (oscillator)

Change temperature

- E_{kin} changes
- $E_{kin} = \frac{1}{2} m v^2$
- $E_{kin} = \frac{1}{2} m A^2 \omega^2 \sin^2(\omega t + \delta)$
- amplitude changes

$$\frac{dx}{dt} = \dot{x} = v = -A\omega \sin(\omega t + \delta)$$

Frequencies and amplitudes

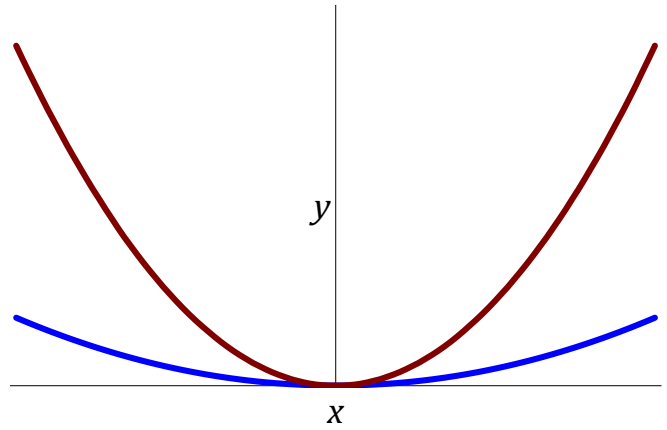
For a given temperature

- energy distributes amongst modes/ degrees of freedom
- E_{kin} the same for different modes
- $E_{kin} = \frac{1}{2} mv^2 = \frac{1}{2} mA^2\omega^2\sin^2(\omega t + \delta)$

I have two modes in one protein

- a slow motion / low frequency (bending of a hinge)
- a fast / high frequency (movement of a sidechain)

$E_{kin} = \frac{1}{2} mv^2 = \frac{1}{2} mA^2\omega^2\sin^2(\omega t + \delta)$



- if ω is low, A is high
- low frequency motions are large amplitude
- big motions are slow

Summary so far (harmonic oscillator)

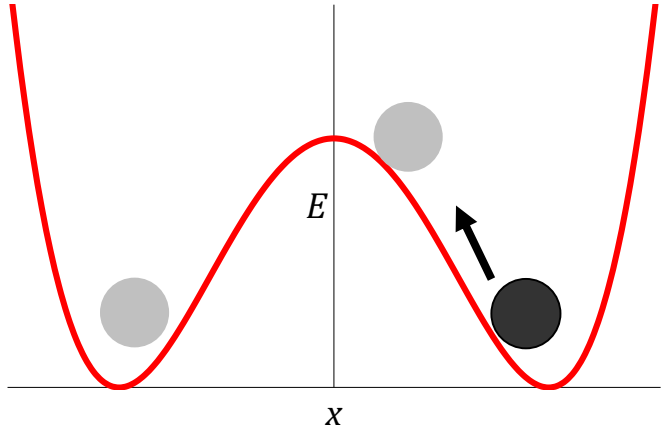
- Maybe appropriate for motion without barriers
- only approximation
- frequencies do not depend on temperature
- we imagine a protein to have
  - many oscillators
  - some fast some slow
- bigger motions have lower frequency

Movement with barriers

How often do you move right to left (and left to right) ?

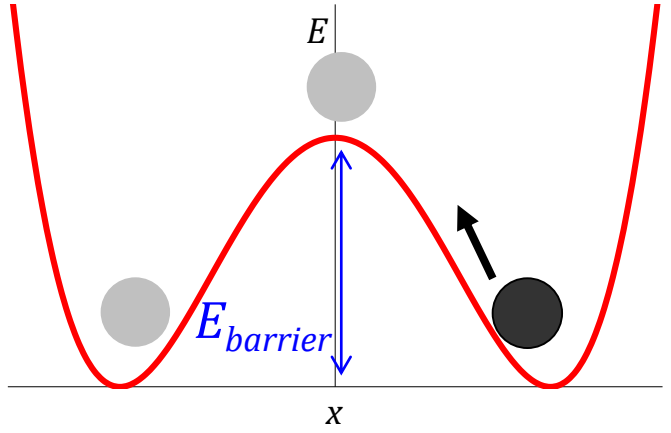
Our model

- assume some random influences
  water and other atoms always hitting you
- velocity from $E_{kin} = ½\, mv^2$, $v \propto Ekin^{½}$
  - better… $E_{kin}{}^{½} \pm$ random



If we reach the top

- we may be moving slowly
  - may move to right (no change)
  - may fall into left energy well (change)
- consequence



Movement with barriers

Rate directly reflects

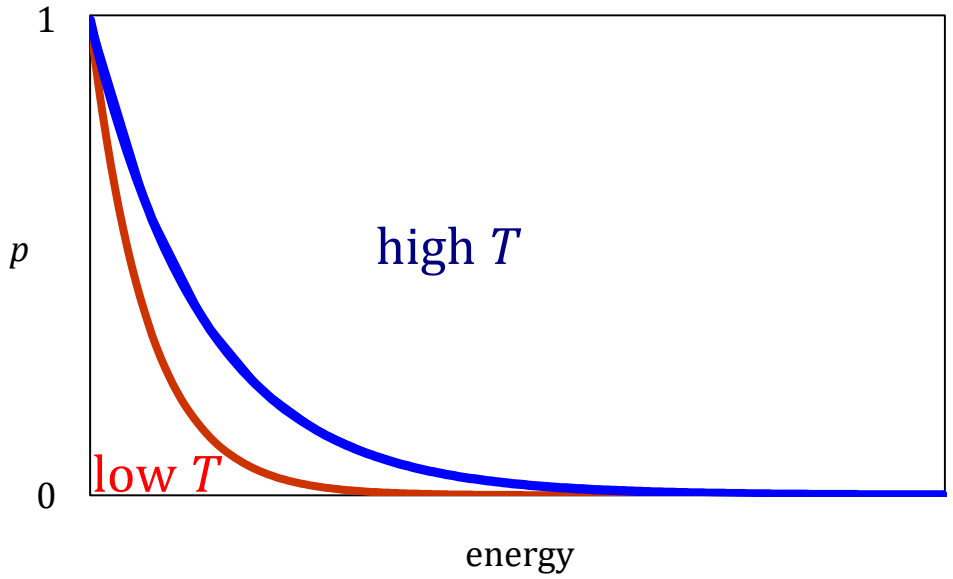
- how often does a particle have enough energy to reach top of barrier ?

Boltzmann rule… (more formal next semester)

$$p_i \propto e^{\frac{-E_i}{kT}}$$

Implications

- small barriers (small E)
  - easy / fast to cross
- as temperature ↑ rate ↑



k	Boltzmanns const
T	temperature

Return to original question

Do frequencies of motions change ?

- if we have motion in a well (harmonic oscillator) .. No
- if we move between energy minima … Yes

Which model is better ?

Empirical

- raise temperature and see if it changes

Physical model

- what you believe in..

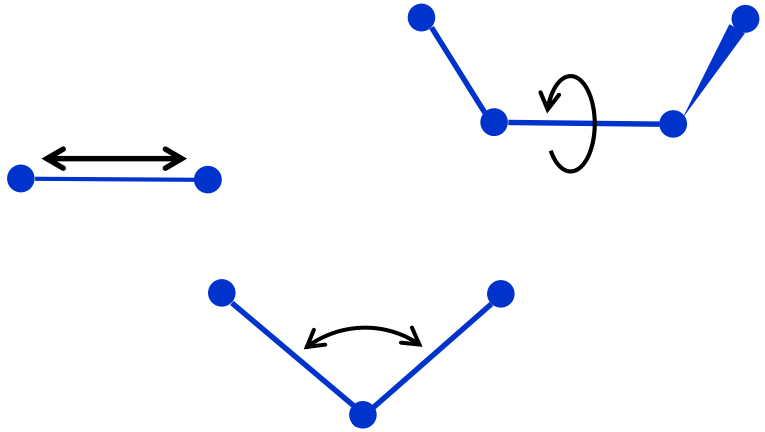
different kinds of motions (smaller)

Bond stretching, angle bending

- nearly harmonic

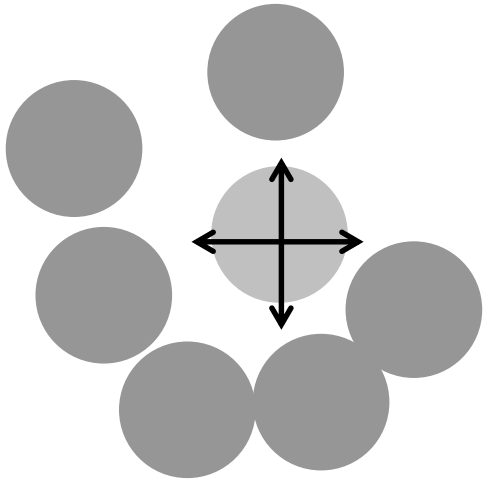
Torsion angles

- separate energy wells



Other motions may be locally like harmonic

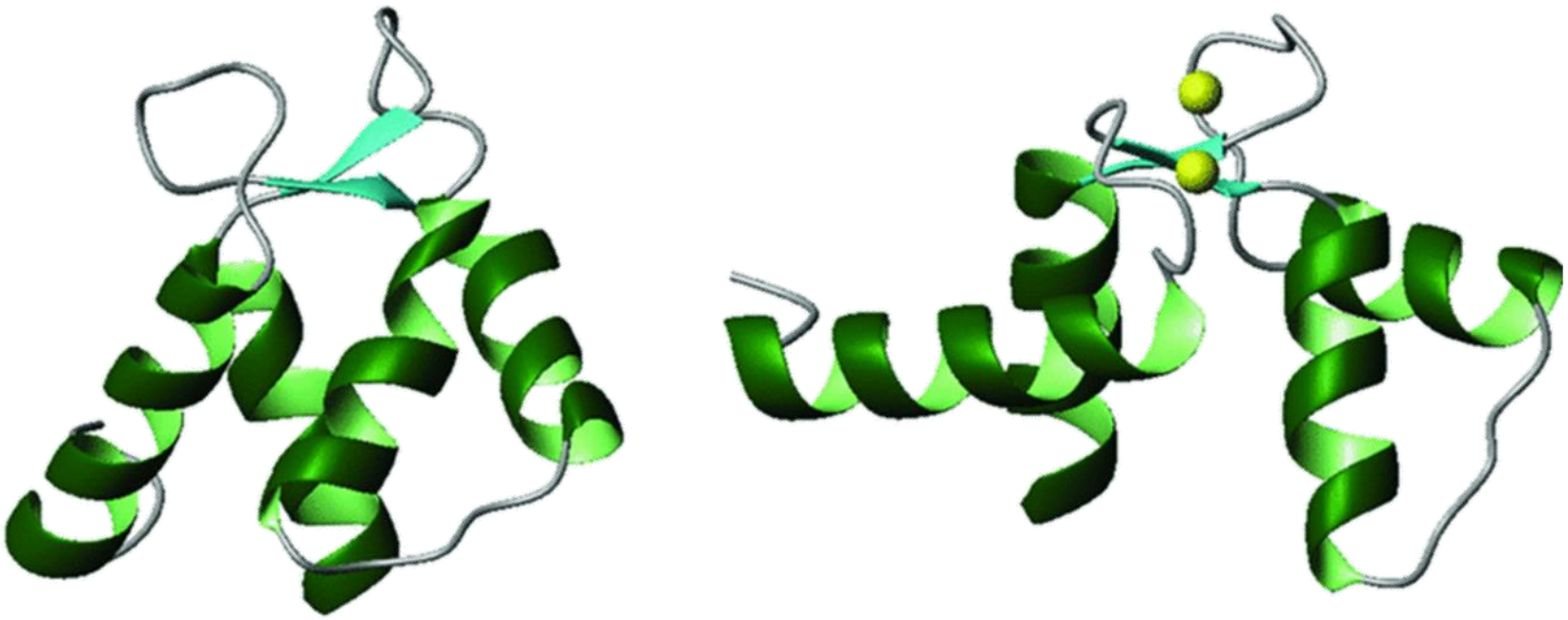
- vibrations of packed atoms



Bigger motions

Calmodulin example

- many Å and probably rather slow
- too move from one conformation to the next – many barriers



Gifford, Walsh, Vogel, Biochem J. 405, 199-221(2007), Structures and … EF-hand motifs

Summary

Models are too simple

- most energy terms are not kx^2
  - locally not a bad approximation
- we do not have simple energy barriers
- many more than two states
  - spectrum of motions
- many motions are a mixture (concerted)

Detection of states

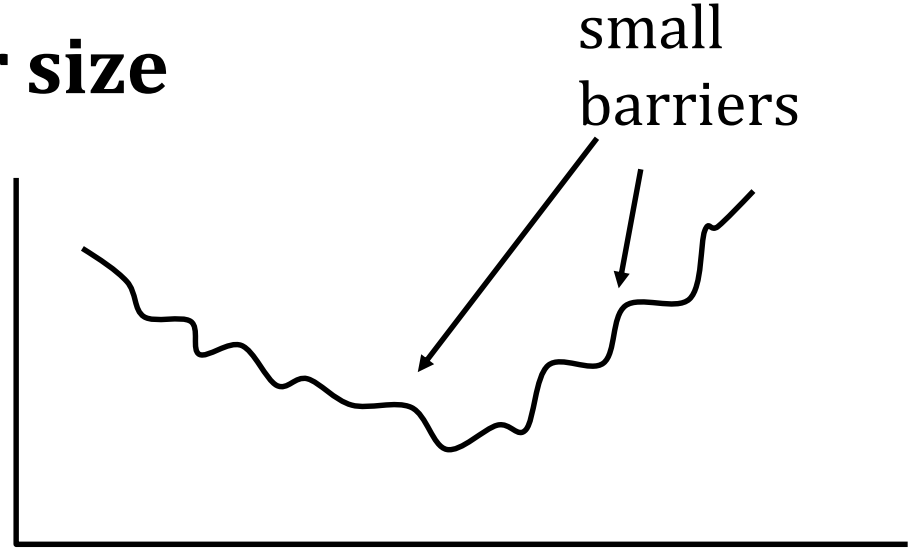
- Are all the different structures present at room temperature ?
- I have two states A and B $\frac{p_a}{p_b} = \exp\left({}^{E_A - E_B}/_{kT}\right)$
  - if $E_A - EB$ much bigger than kT (some kJ mol^{-1})
    - only one state will be seen

Barrier size

Barriers very small

- all particles have plenty of energy
- effectively not present

- may be the case for some rotations



Types of motions

motion	amplitude Å	$\log_{10}$ of time (s)
bond vibration	0.01 – 0.1	-14 to -13
sidechain rotation surface sidechains	5 – 10	-11 to -10
protein hinge bending	1 – 50	-11 to -7
sidechain rotation inside protein	5	-4 to 0
helix / strand breakage	5 – 10	-5 to 1

Summary

- Motions are necessary to explain chemistry
- NMR and X-ray structures are time averages
- usually
  - small motions fast
  - big movements slow
- temperature dependence
  - different for different kinds of movement
  - can be used to estimate energy barriers