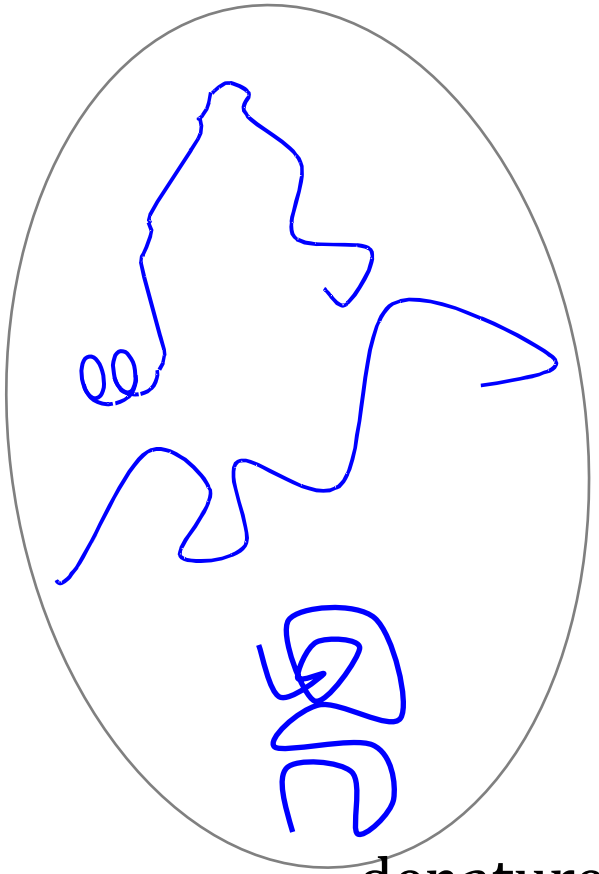
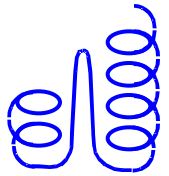
Protein stability

Andrew Torda, Wintersemester 2011 / 2012, GST

- Our model



ΔG



native
(N) folded

denatured (D)
unfolded
non-native

- free energy change on
  - folding
  - denaturing ($-\Delta G$)
- for a stable protein
  - $\Delta G_{N \rightarrow D} > 0$

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)
- some books and papers work with other convention

Empiricism / rules

- more positive ΔG, more stable the protein
- most proteins are marginally stable ($\Delta G \approx 0$)
- proteins can be denatured by
  - pH, concentrated ions, temperature, solvent, surface area
- too hot ?
  - eggs cook, people die (many reasons)
- some bacteria live at 373 K – their proteins are not denatured

Rules

- nature cares about free energy ΔG
  - usually measure free energy
  - $\Delta G = \Delta H - T\Delta S$

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

- chemistry books normally work with ΔG° standard free energy

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

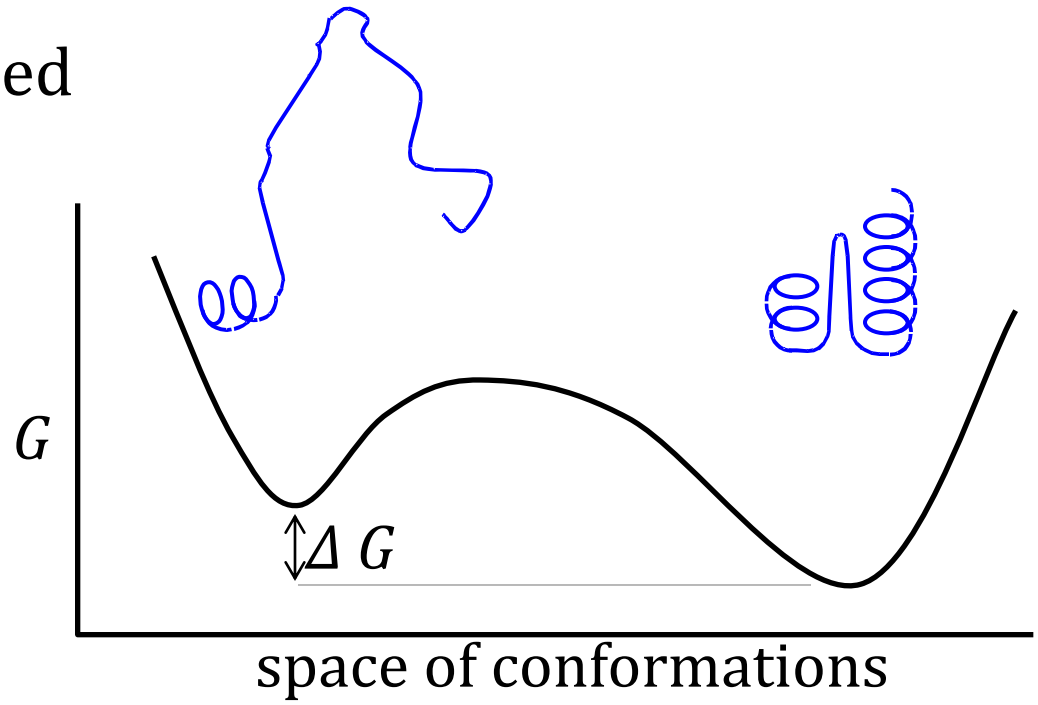
Energies

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

Two state model

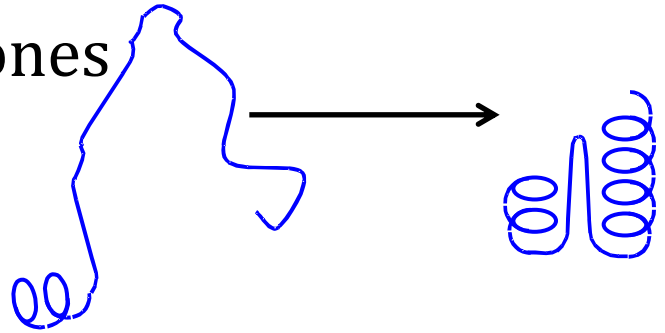
- model requires an energy barrier

- 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



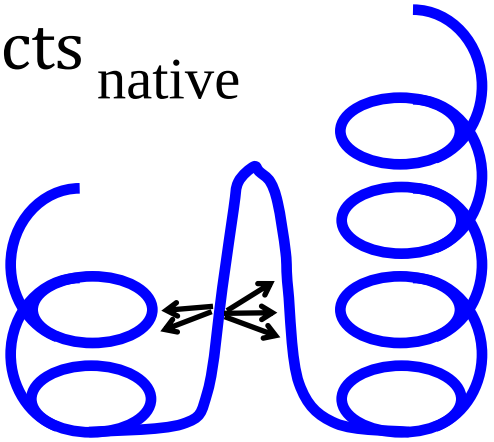
- cannot be so simple
  - atoms would just fall into correct position
  - ΔG would always be very positive
  - missing ?
    - effect of solvent

- in native structure there are "correct contacts"

Balance of energy terms

- in native structure there are correct contacts

native

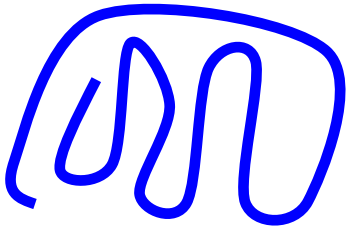


One atom in native structure

- interacts with correct neighbours

In the non-native structures there are also contacts

- atom interacts with “wrong” neighbours



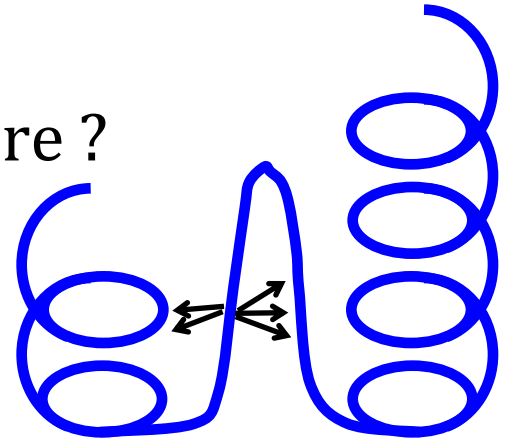
some denatured / non-native

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

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



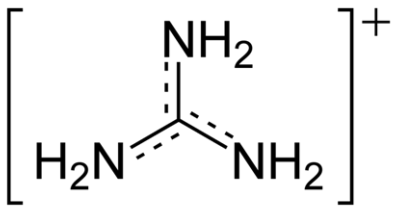
- stability will depend on $H_{\text{native}} - (H_{\text{denatured}} + H_{\text{protein-}H_2O})$

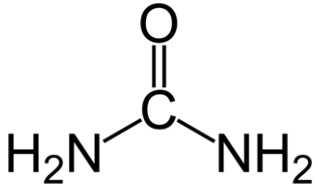
Explain denaturing

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

Entropy version 1

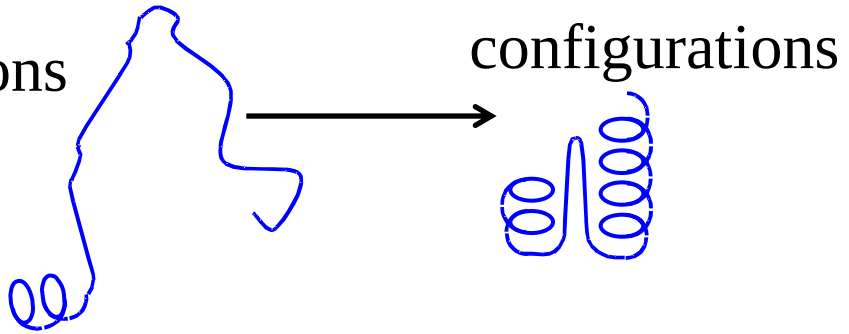
$\Delta G = \Delta H - T\,\Delta S$

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

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

lots of configurations

few configurations

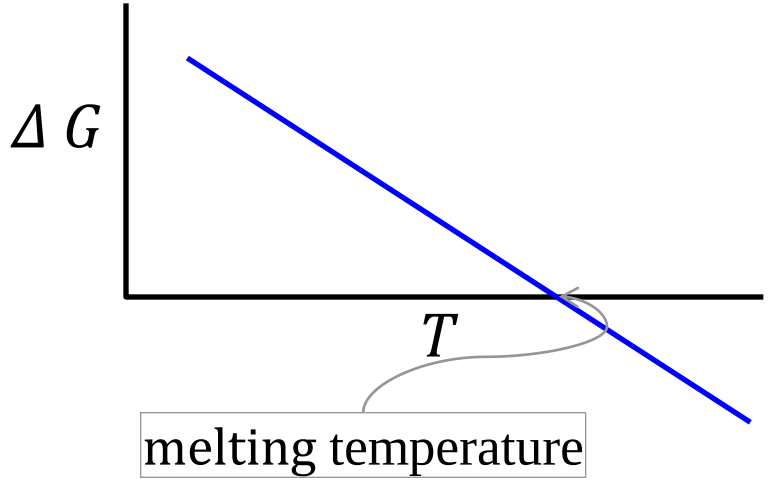


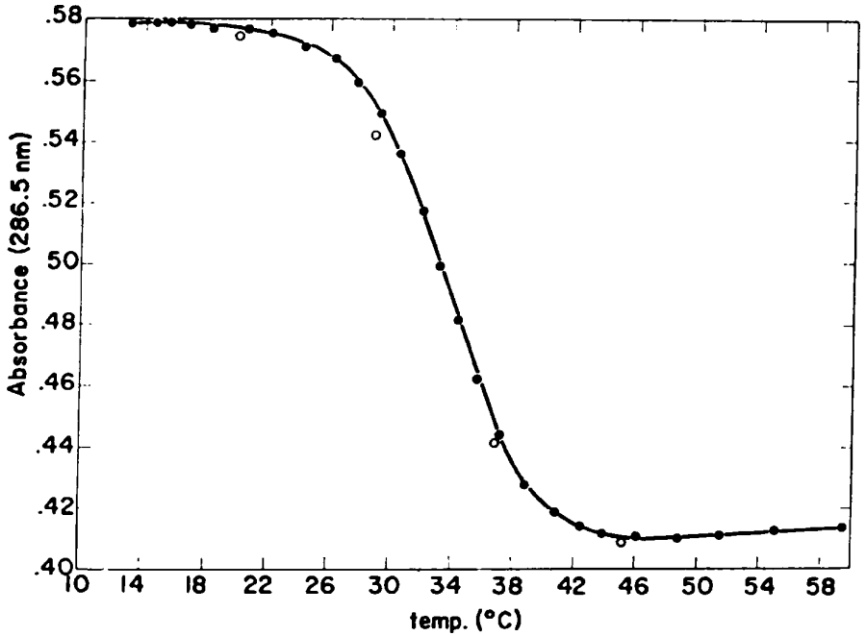
- as a protein unfolds
  - number of conformations ↑
  - entropy goes ↑
- ΔS will favour denaturing

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

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

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

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

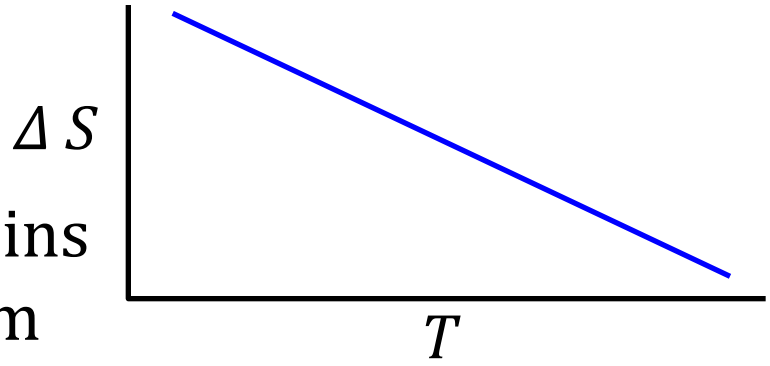
$$= k\left(\ln \Omega_{folded} - \ln \Omega_{unfolded}\right)$$

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

- Ω_{folded} definitely goes up with temperature

Entropy change on unfolding

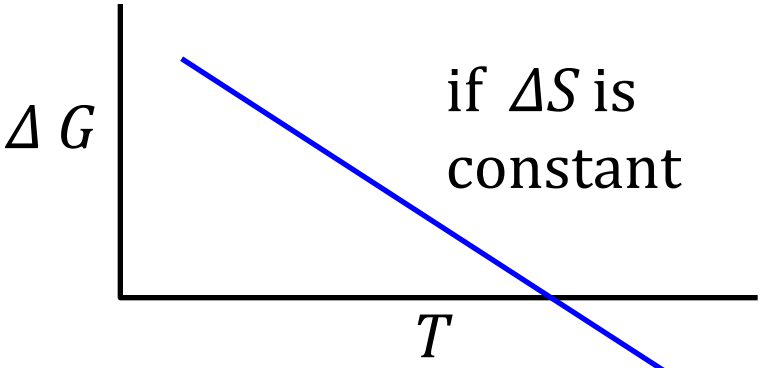
- depending on heat capacity one has a curve like

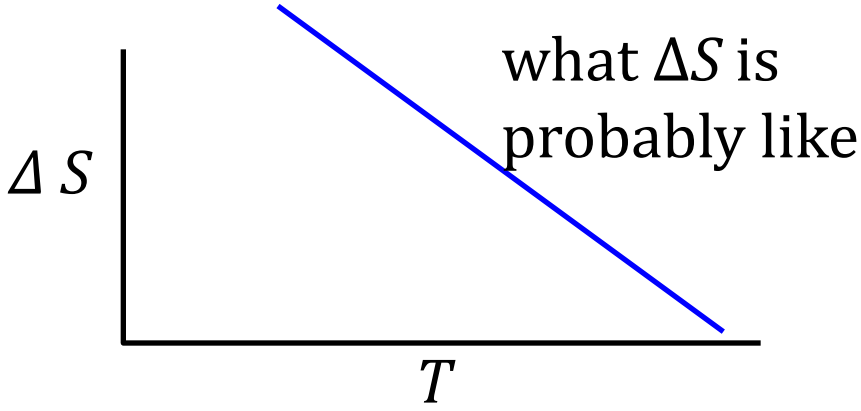


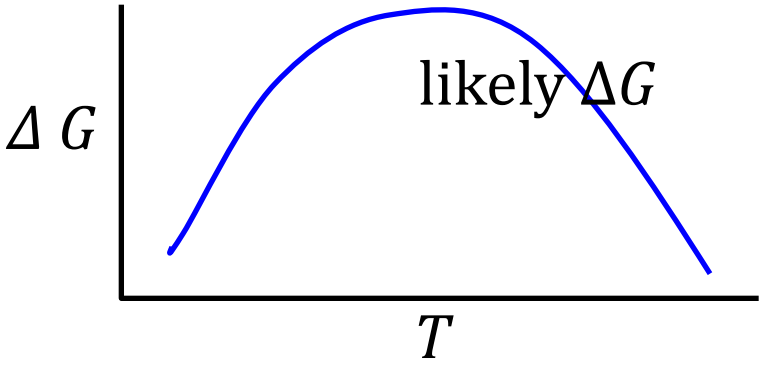
- radical consequence
  - entropy might make proteins less stable as you cool them
  - can one cook an egg by cooling it ?
- combining these properties

protein stability

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







- what is seen / claimed ?

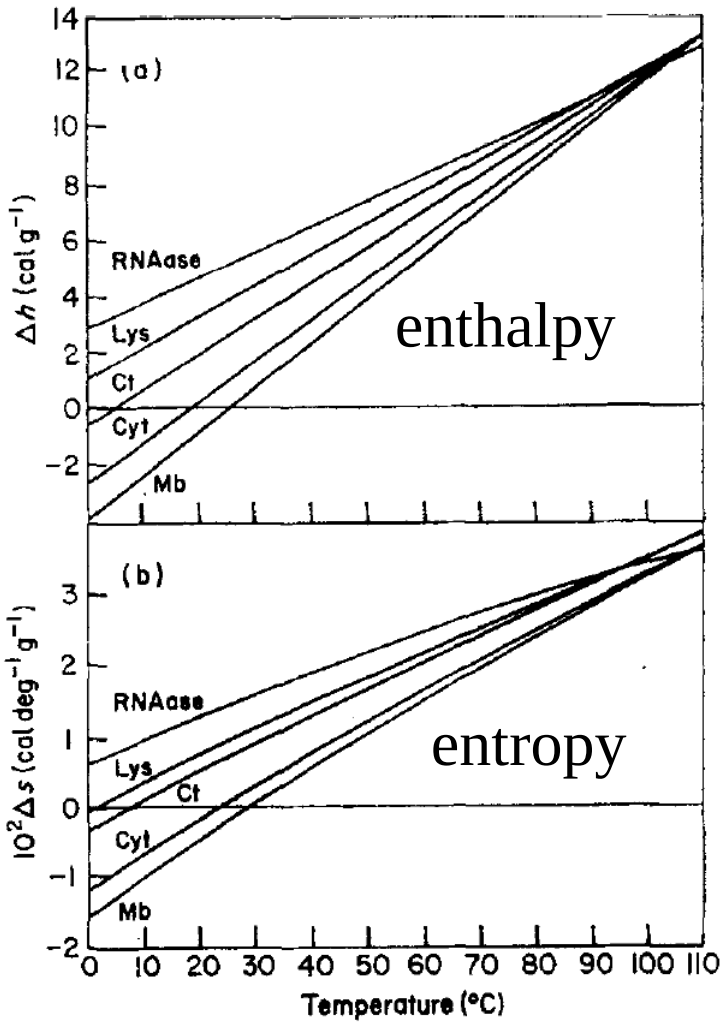
Old measurements

- 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

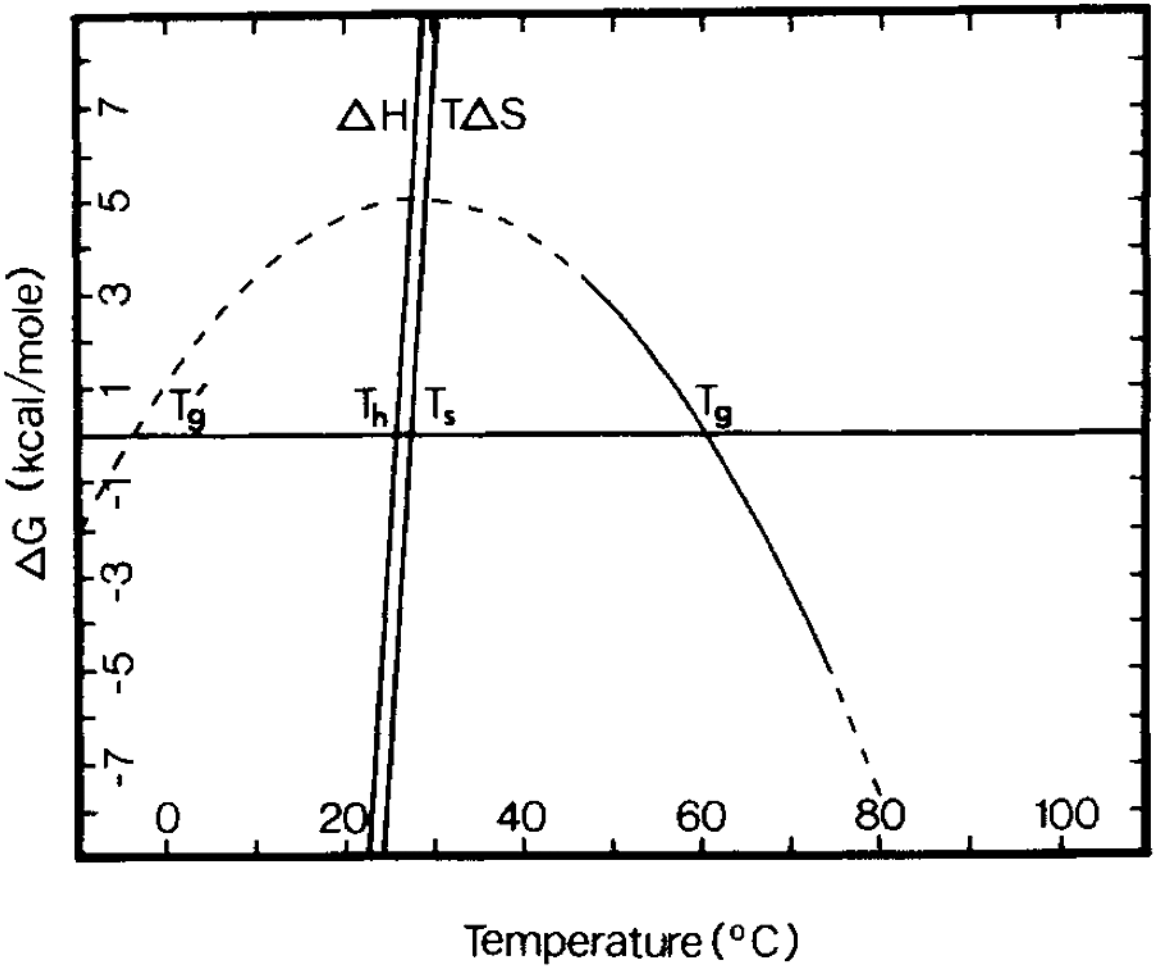


Privalov, P.L., Khechinashvili, N.N, J. Mol. Biol. 86, 665-684, 1974

final version

- claim.. for typical protein
- bold line –measured
- dashed – extrapolate

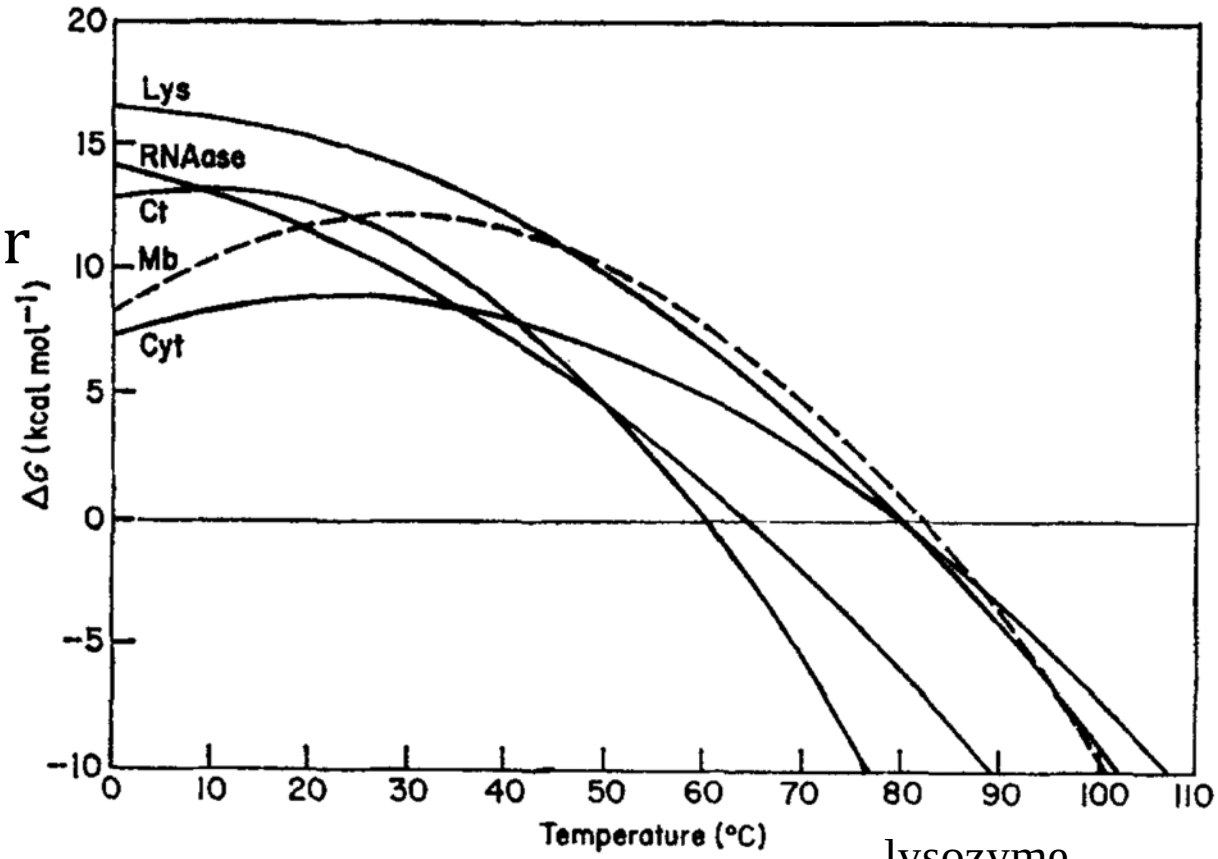
- implies
  - you can denature protein by cooling (cook egg by freezing ?)
- what is measured ?



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

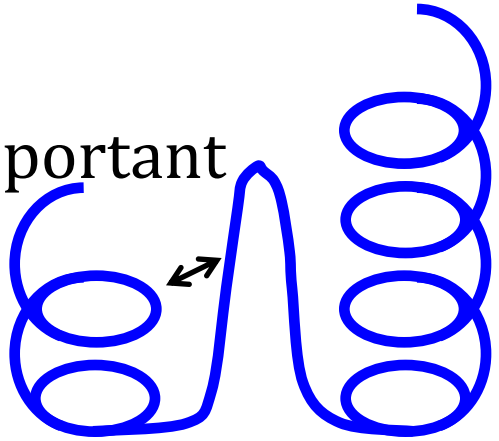
Debatable

- how much does entropy change with temperature ?
- depends on heat capacity of protein

- 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

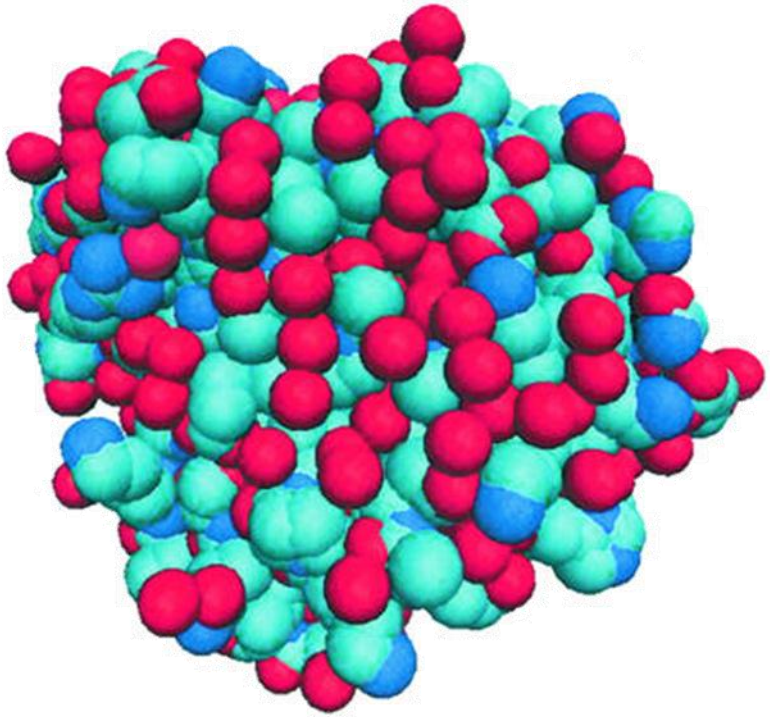
Nasty question .. answered later

- 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

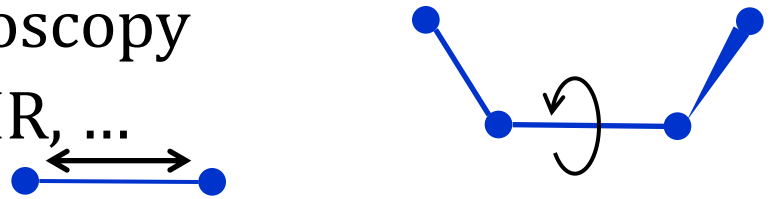
- many similar stories
- activity of protein cannot be explained by simple structure

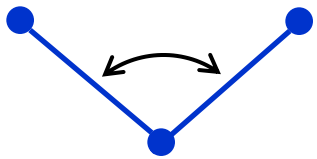


Frauenfelder, McMahon and Fennimore, Proc Natl Acad Sci USA, 100, 8615-8617 (2003)

Protein motion examples

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



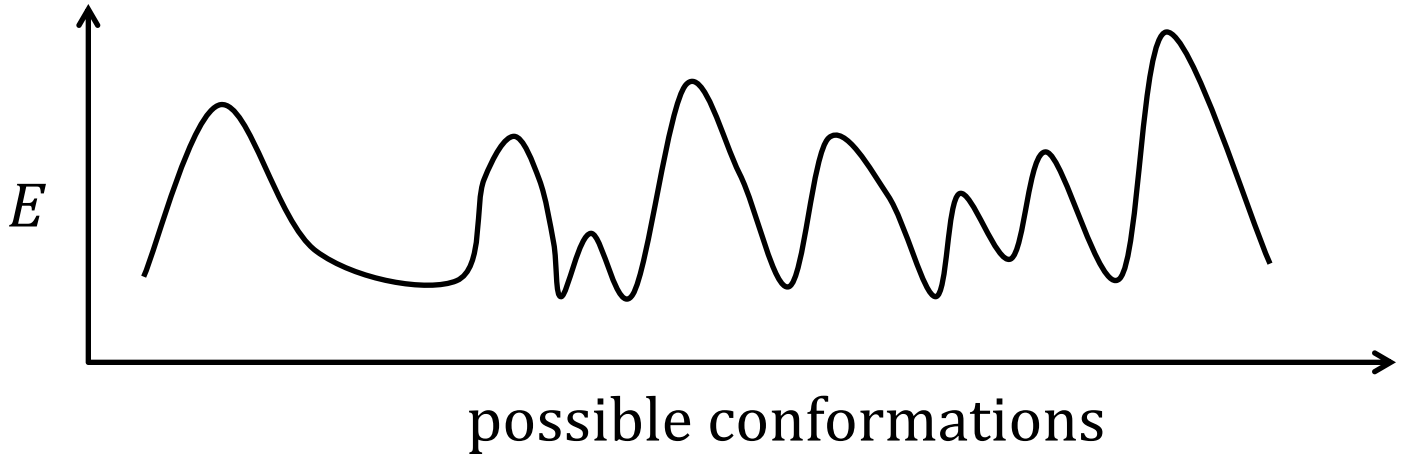


- More fundamental arguments
  - at $T = 0$ everything dead
  - at $T = 300$ (this room) everything has kinetic energy
    - everything is moving
    - meaning of temperature ? T, E_{kin} ?
      - $\frac{1}{2}mv^2$

E_{kin}	kinetic energy
T	temperature

Energy surfaces

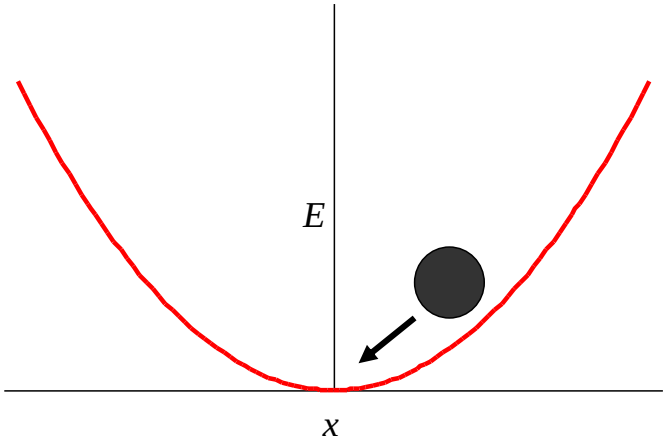
- run around on an energy surface



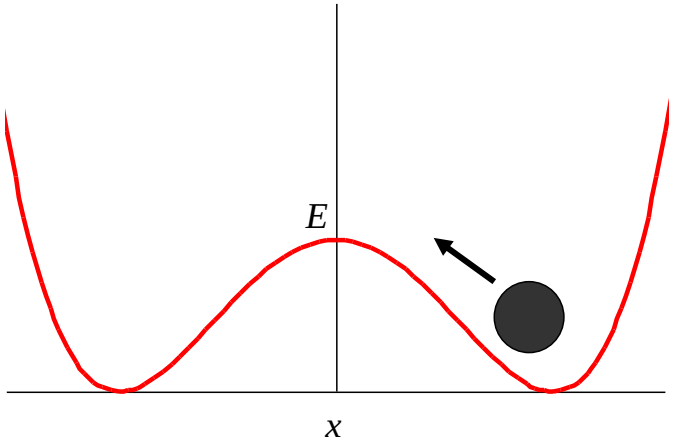
- energy surface and energy should determine motions
  - too complicated
  - energy surface not well known
- work with simpler models

Motions with or without barriers

- Without barriers
  - one state + fluctuations

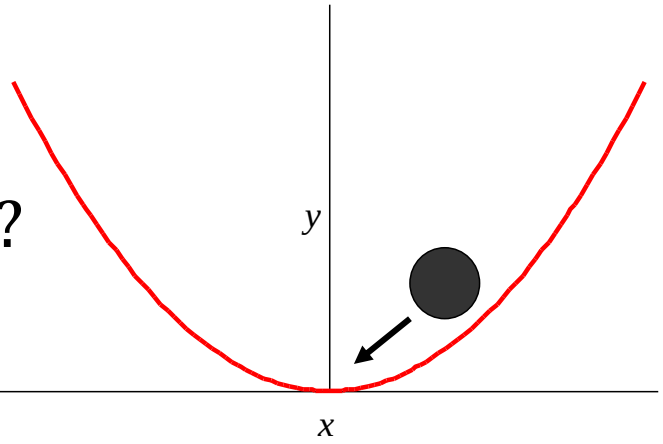


- With a barrier
  - two states



Harmonic oscillators

- find them everywhere..
  - potential energy = kx^2
  - what is the frequency of motion ω ?



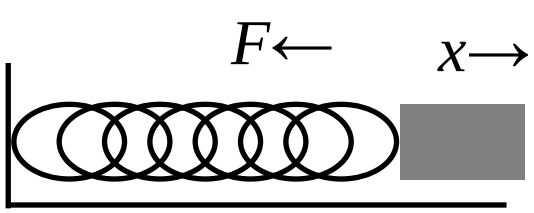
$$x(t) = A\cos(\omega t + \delta)$$

- A is the amplitude
- ω is the frequency
- δ is phase

- Detour .. why does this make sense ?

Harmonic oscillator

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



$$F = -kx$$

$$ma = -kx$$

$$m\ddot{x} = -kx$$

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

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

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

- has a solution.. $x(t) = A\cos(\omega t + \delta)$
  - can I convince you ?

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

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

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

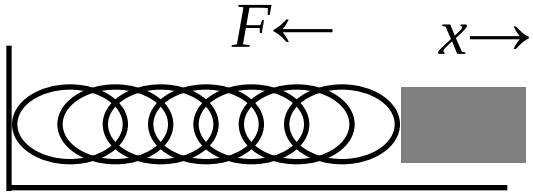
$$\frac{d^2x}{dt^2} = -A\omega \cos(\omega t + \delta)\omega$$

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

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

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

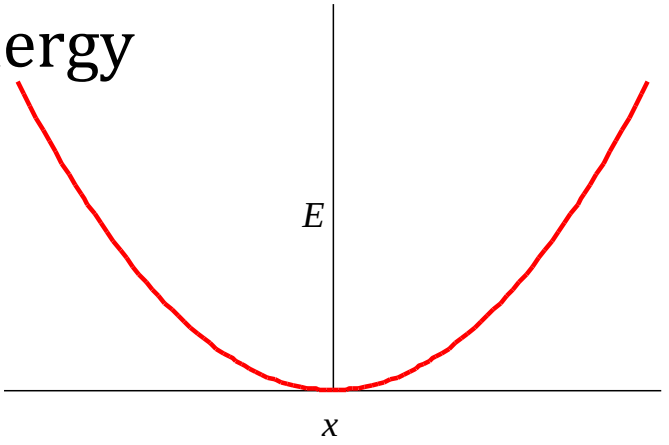
Is the solution valid ? $x(t) = A\cos(\omega t + \delta)$



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

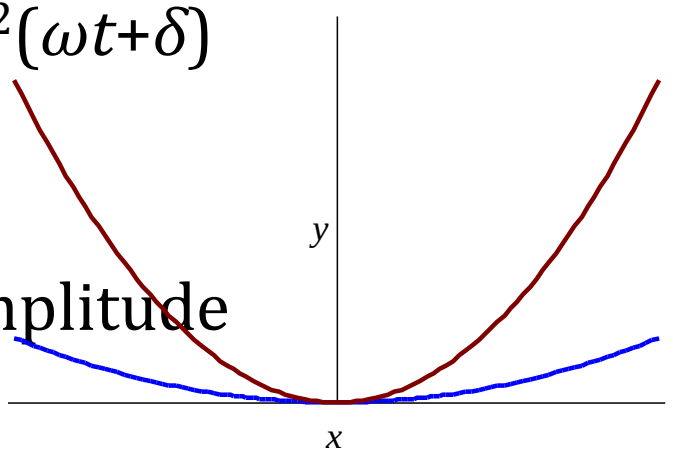
Frequencies and amplitudes

- For a given temperature
  - energy distributes amongst modes/ degrees of freedom
  - E_{kin} the same for different modes
  - $E_{kin} = ½\ mv^2 = ½\ mA^2\omega^2\sin^2(\omega t+\delta)$

- I have two modes in one protein
  - a slow motion / low frequency (bending of a hinge)
  - a fast / high frequency (movement of a sidechain)

$$E_{kin} = ½\ mv^2 = ½\ mA^2\omega^2\sin^2(\omega t+\delta)$$

  - if ω is low A is high

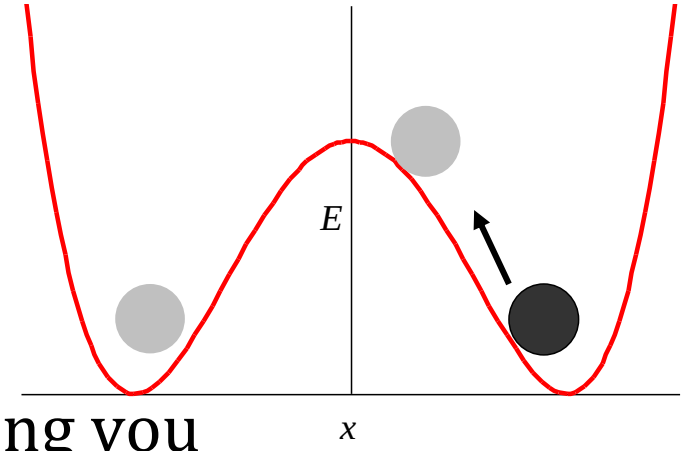


  - low frequency motions are large amplitude
  - big motions are slow

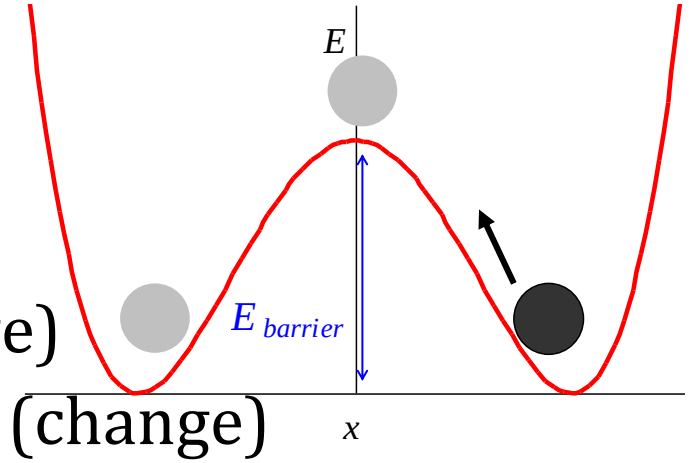
Summary so far (harmonic oscillator)

- 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 $E_{kin} = ½\ mv^2$, $v \propto E_{kin}^{½}$
    - better… $E_{kin}^{½} \pm$ random



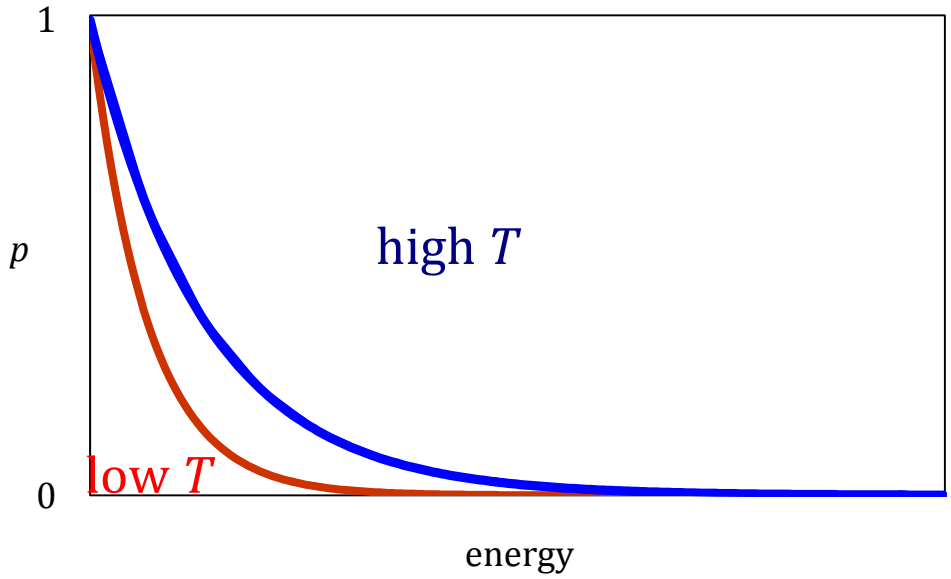
  - if we reach the top
    - we may be moving slowly
      - may move to right (no change)
      - may fall into left energy well (change)
  - consequence

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 early question

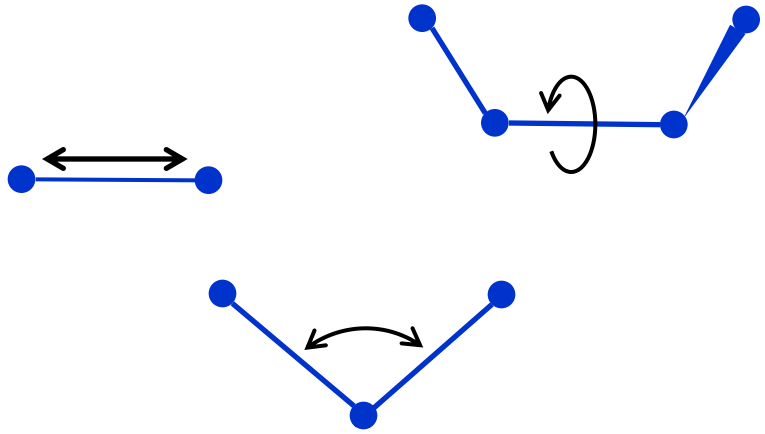
- do frequencies of motions change ?
  - if we have motion in a well (harmonic oscillator) .. No
  - if we move between energy minima … Yes

Which model is better ?

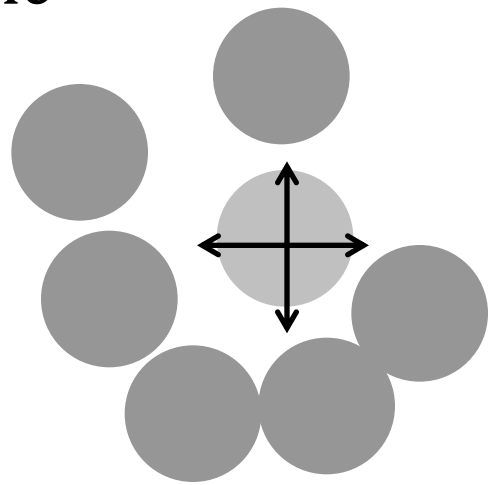
- empirical
  - raise temperature and see if it changes
- physical model
  - what you believe in..

different kinds of motions (smaller)

- bond stretching, angle bending
  - nearly harmonic
- torsion angles
  - separate energy wells

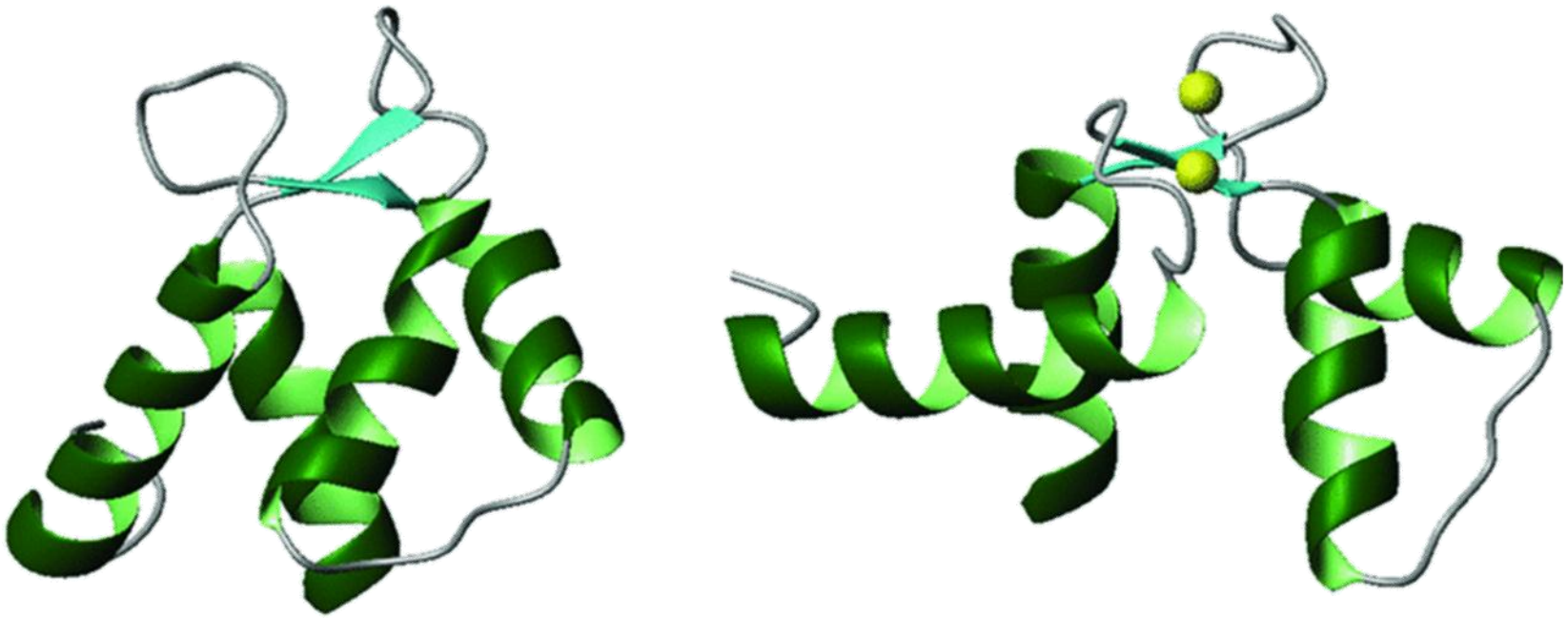


- other motions may be locally like harmonic
  - vibrations of packed atoms



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

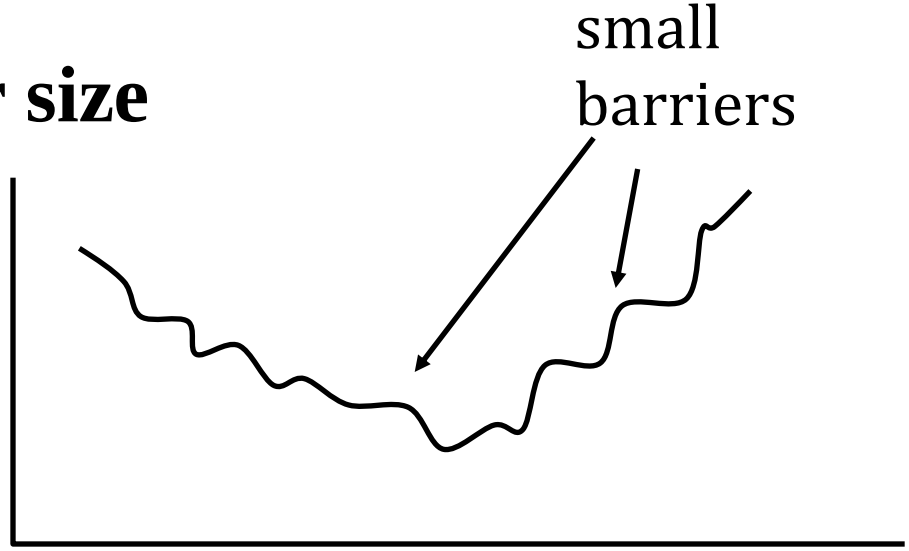
- models are too simple
  - most energy terms are not kx^2
    - locally not a bad approximation
  - we do not have simple energy barriers
  - many more than two states
    - spectrum of motions
- many motions are a mixture (concerted)

Detection of states

- Are all the different structures present at room temperature ?
- I have two states A and B $\frac{p_a}{p_b} = \exp\left(\frac{E_A - E_B}{kT}\right)$
  - if $E_A - E_B$ 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 – 5	-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