Motions, frequencies and proteins

Andrew Torda, Wintersemester 2017 / 2018 GST

Motions and dynamics in proteins

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

- Examples

Types

- without barriers
- with barriers

Nasty question .. answered later

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

Heat protein to 300 K

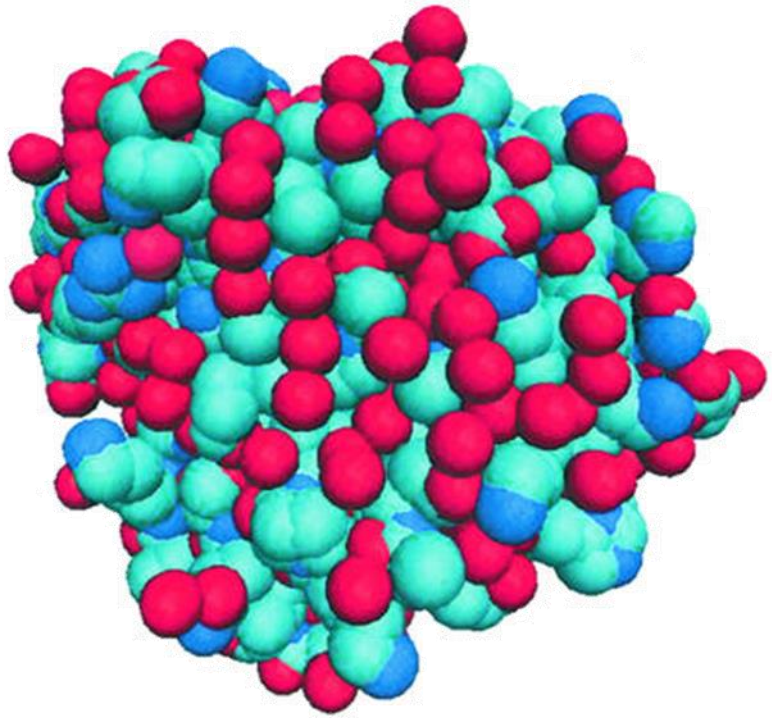
- do the frequencies of motions change ?

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

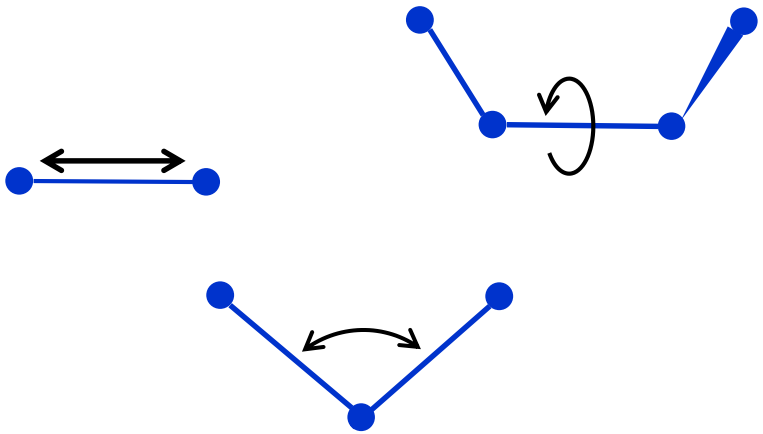


Frauenfelder, McMahon and Fennimore, Proc Natl Acad Sci USA, 100, 8615-8617 (2003)

Protein motion examples

Fundamental arguments

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



More fundamental arguments

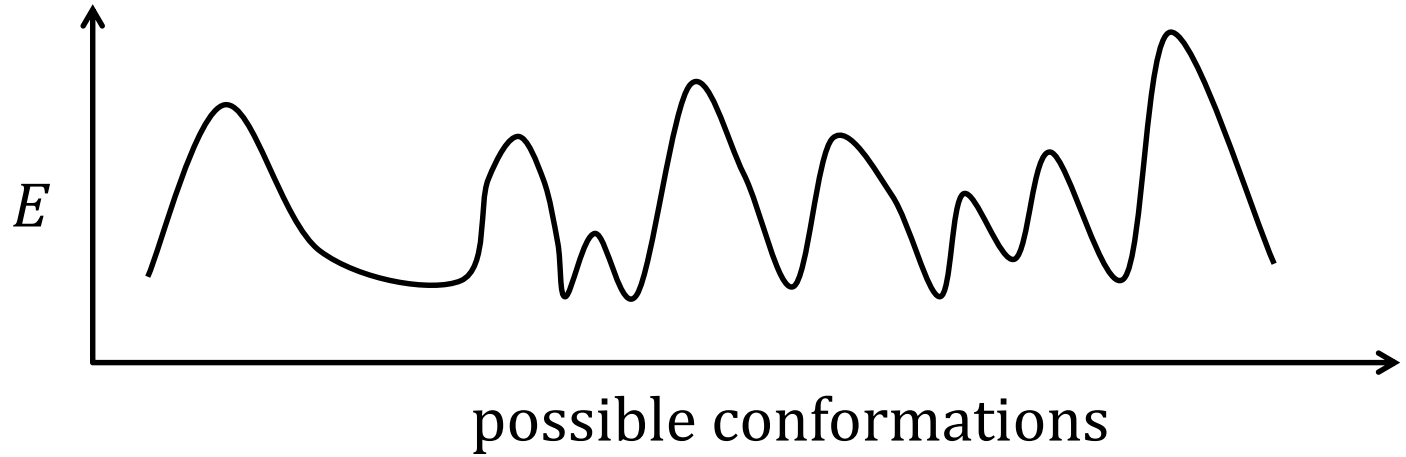
- at $T = 0$ everything dead
- at $T = 300$ (this room) everything has kinetic energy
  - everything is moving
  - meaning of temperature ? T, E_{kin} ?

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

E_{kin}	kinetic energy
T	temperature

Energy surfaces

Run around on an energy surface



Energy surface and energy should determine motions

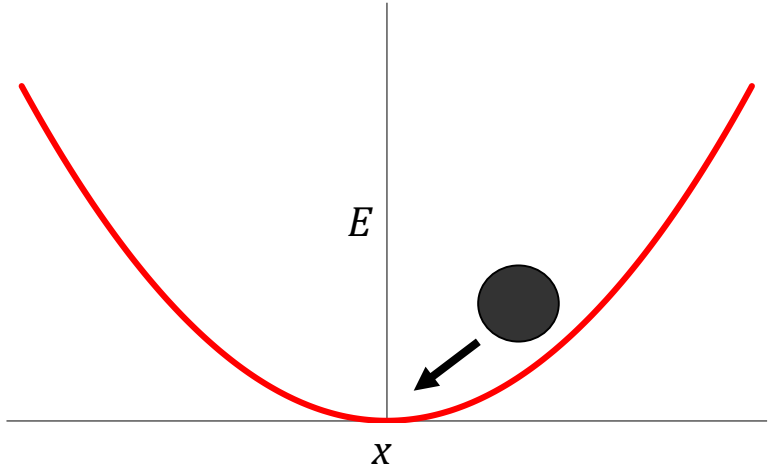
- too complicated
- energy surface not well known

Work with simpler models

Motions with or without barriers

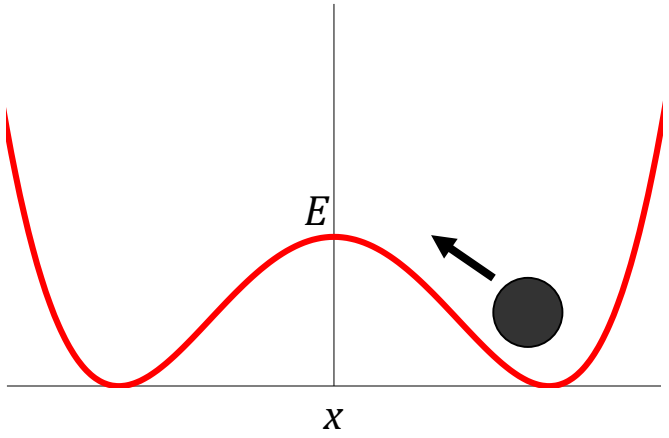
Without barriers

- one state + fluctuations



With a barrier

- two states

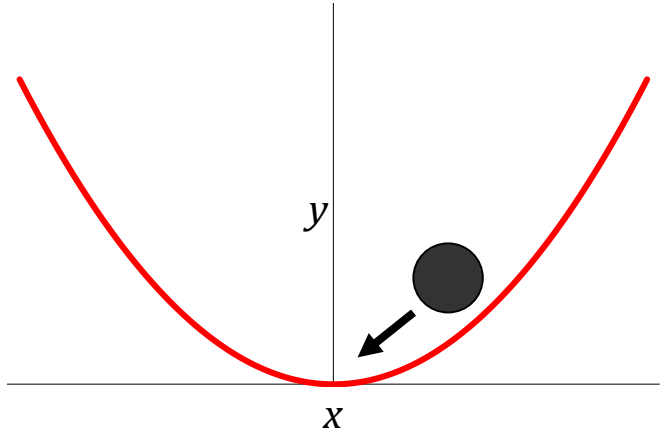


Harmonic oscillators

Find them everywhere..

- potential energy = kx^2

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



A is the amplitude

ω is the frequency

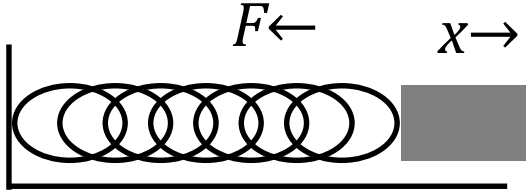
δ is phase

Detour .. why does this make sense ? Is there a physical basis for formula ?

Harmonic oscillator

$F = -kx$

$ma = -kx$



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

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

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

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

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

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

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

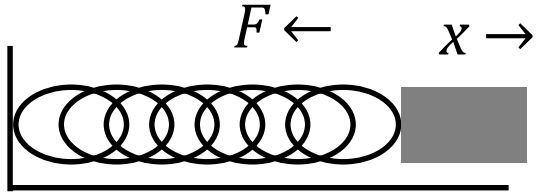
$$= -A\omega^2 \cos(\omega t + \delta)$$

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

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

– periodic form is consequence of Hookes law

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

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

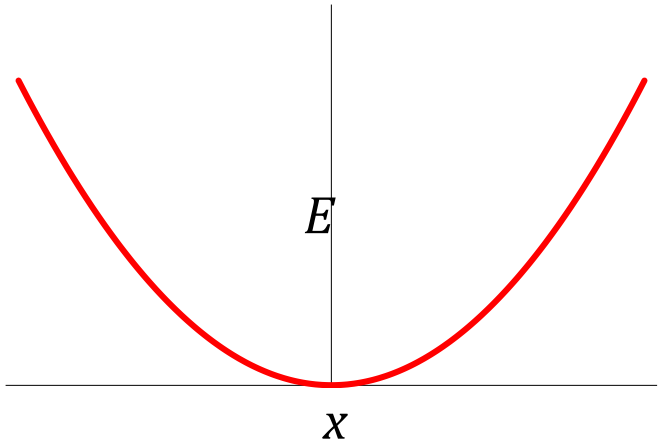
What is meaning of $\frac{d^2x}{dt^2}$?

- 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

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

$$E_{kin} = \frac{1}{2} m v^2$$

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

- amplitude changes

Frequencies and amplitudes

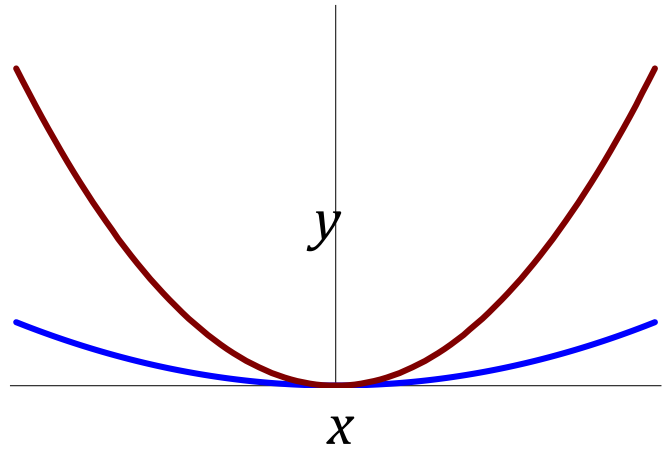
For a given temperature

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

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)

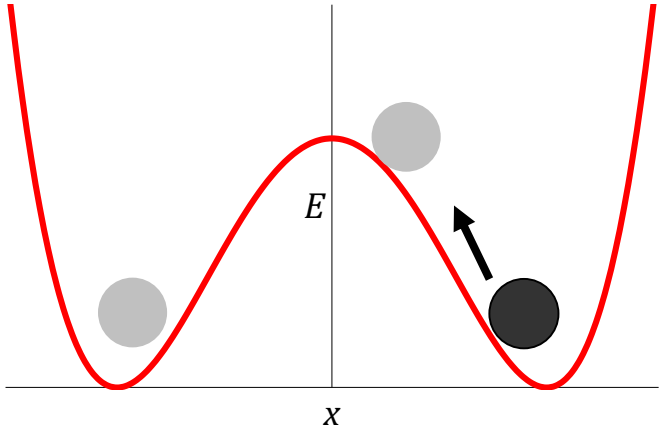
- Maybe appropriate for motion without barriers
- only approximation
- frequencies do not depend on temperature
- we imagine a protein to have
  - many oscillators
  - some fast some slow
- bigger motions have lower frequency

Movement with barriers

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

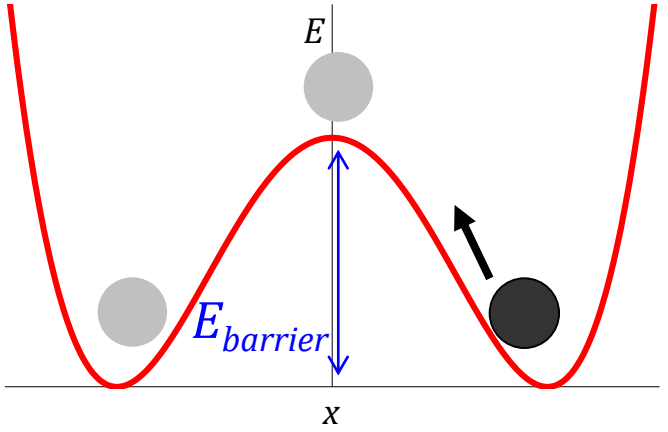
Our model

- assume some random influences
  water and other atoms always hitting you
- velocity from $E_{kin} = \frac{1}{2} mv^2$, $v \propto E_{kin}^{\frac{1}{2}}$
  - better… $E_{kin}^{\frac{1}{2}} \pm$ 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…



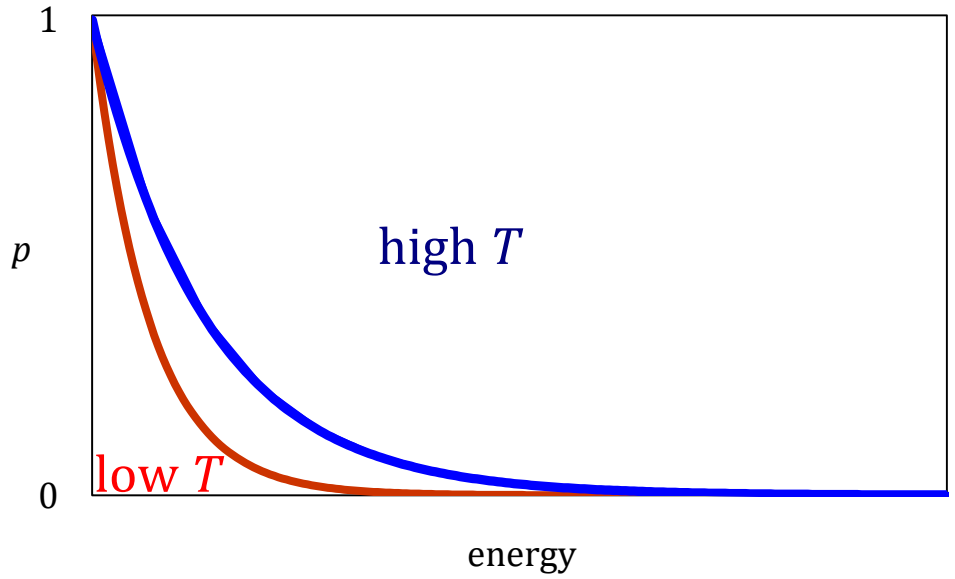
Movement with barriers

Rate directly reflects

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

Boltzmann rule… (more formal next semester)

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



k	Boltzmanns const
T	temperature

Implications

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

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..

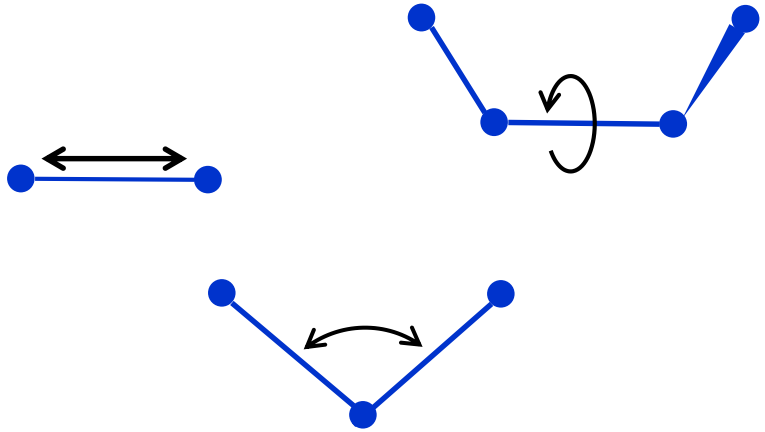
different kinds of motions (smaller)

Bond stretching, angle bending

- nearly harmonic

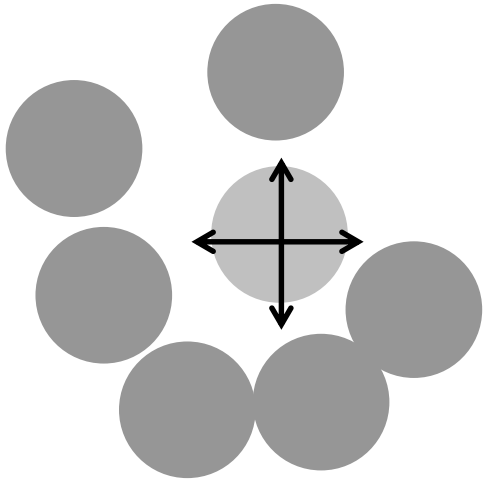
Torsion angles

- separate energy wells



Other motions may be locally like harmonic

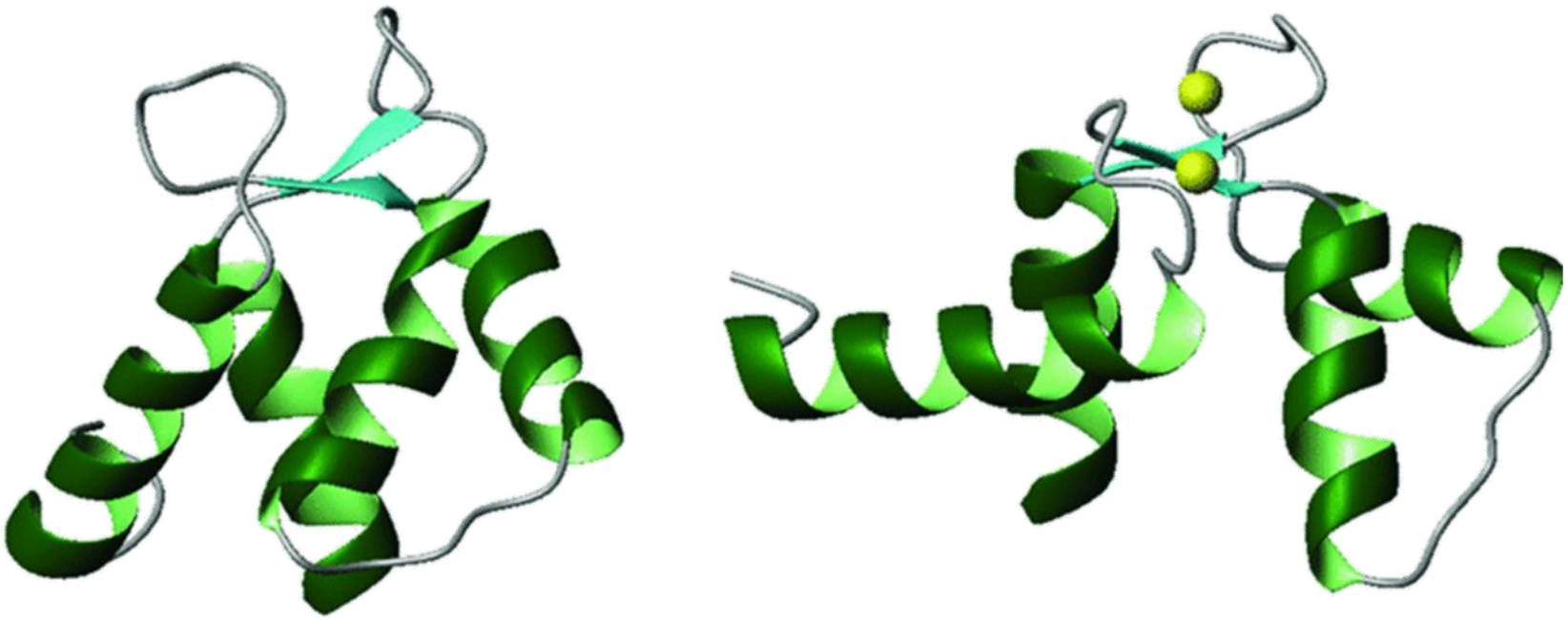
- vibrations of packed atoms



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 of models

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

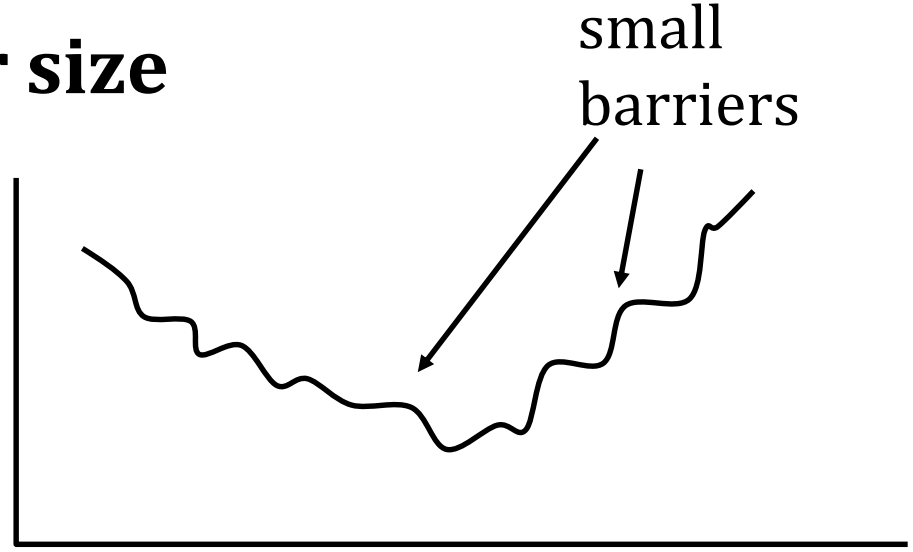
- Are all the different structures present at room temperature ?
- I have two states A and B $\frac{p_a}{p_b} = \exp\left({}^{E_A - E_B}/_{kT}\right)$
  - if $E_A - E_B$ much bigger than kT (some kJ mol^{-1})
    - only one state will be seen

Barrier size

Barriers very small

- all particles have plenty of energy
- effectively not present

- may be the case for some rotations



Types of motions

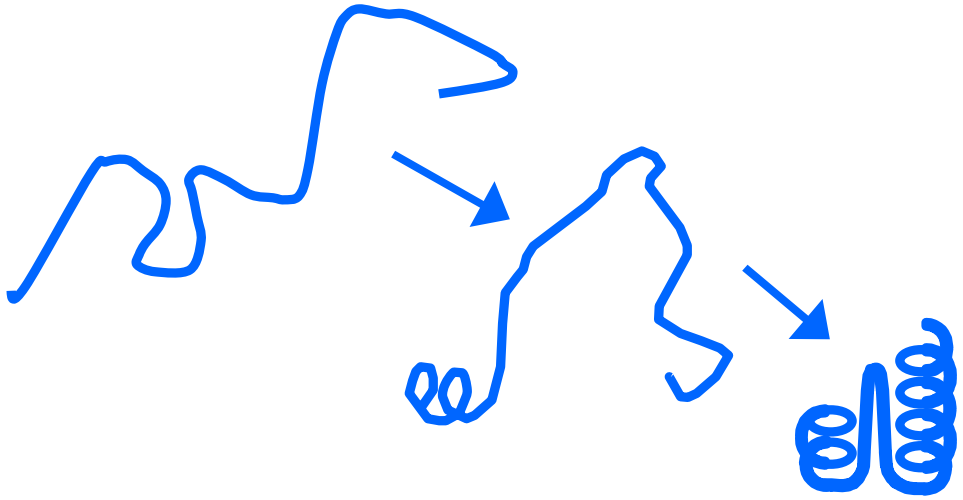
motion	amplitude Å	$\log_{10}$ of time (s)
bond vibration	0.01 – 0.1	-14 to -13
sidechain rotation surface sidechains	5 – 10	-11 to -10
protein hinge bending	1 – 50	-11 to -7
sidechain rotation inside protein	5	-4 to 0
helix / strand breakage	5 – 10	-5 to 1

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

Protein Folding

How does a protein do this ?



If we could understand folding we could

- predict structure
- design proteins that fold better (more stable)
- identify essential residues for folding (not suitable for mutagenesis)

Folding should be easy

From biochemistry Übungen (protein folding – easy)

- take lysozyme / ribonuclease...
- put in 8M urea (unfolds)
- remove urea (refolds)

Conclusion ?

- the protein sequence is all you need to fold a protein
- is this true ? Not always

Alternative (logical reasoning)

- protein folding should be impossible…

Folding should be hard / Levinthal's paradox

Each amino acid has 2 or 3 or n conformations

- for a protein of m residues, it should visit n^m

If it spends 10^{-15} s at each conformation ?

- time to find one conformation for n=3 and 100 residues
  - $3^{100} \times 10^{-15}$ s $\approx 1.6 \times 10^{25}$ years

Consequence

- proteins cannot be exploring space randomly
- historic idea of "folding pathway"

Questions

- Kinetic versus thermodynamic
- What order do events happen in ? (collapse vs secondary struct)
- Is unfolding the same as folding ?
- Is folding in a test tube the same as nature ?

Kinetic versus thermodynamic minima

Anfinsen story..

- proteins can be unfolded and refolded alone
- all the information is in the sequence
- native conformations are the (free) energy minimum
- thermodynamic belief

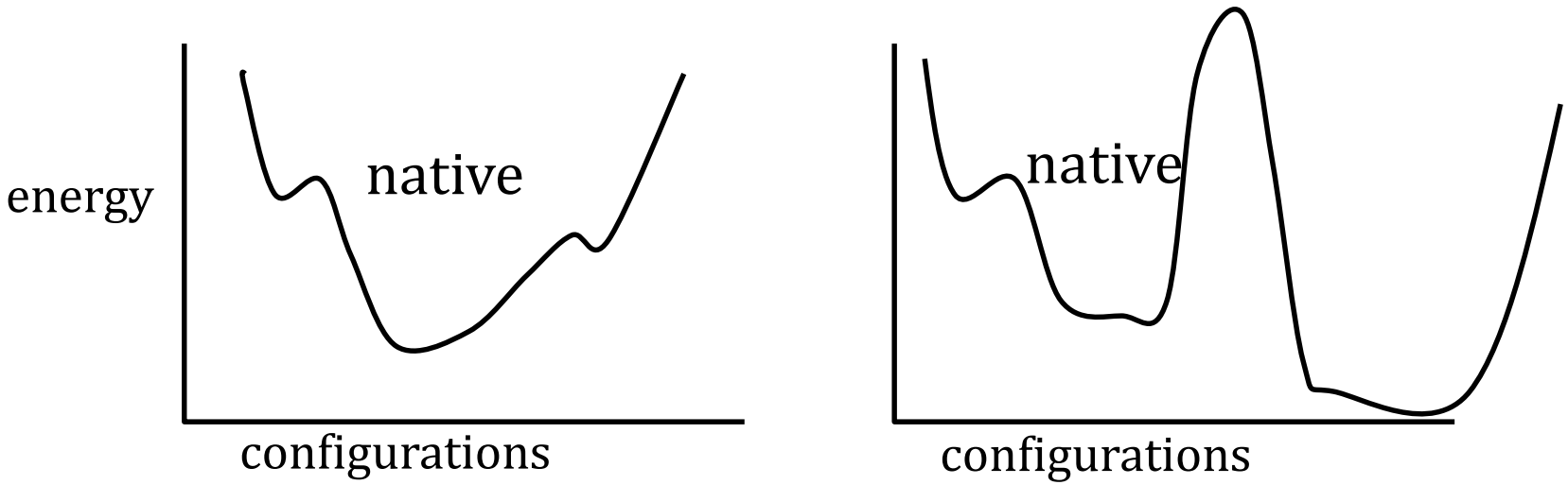
More modern

- many many proteins cannot be refolded in the lab
- consequence .. maybe they need something else
- maybe they are not always in free energy minimum

Kinetically trapped proteins..

Kinetic versus thermodynamic minima

- If proteins fold spontaneously and remain folded, they are thermodynamically determined
- If you leave a protein long enough and it unfolds, it was not in an energy minimum



thermodynamic

kinetically trapped

Kinetic versus thermodynamic minima

Thermodynamic

- protein structure prediction
  - just a matter of modelling the real world

Kinetically trapped

- we cannot predict structure from sequence just by energies

Kinetic versus thermodynamic minima

Can we see which is the case ?

- leave a protein for 10 minutes
  - see if it finds another state
- leave it for 10 years ?
- depends on barriers

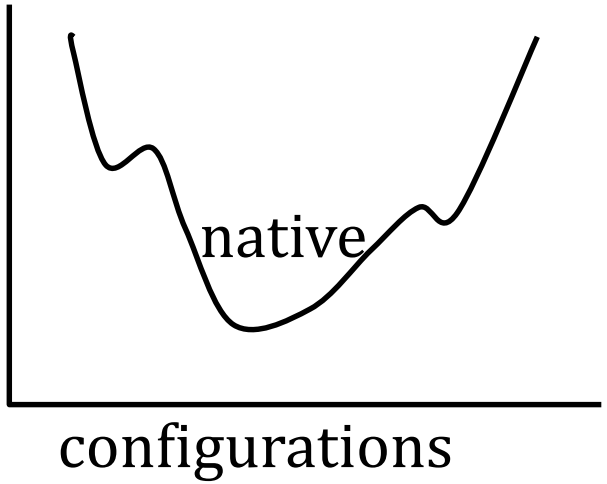
Empirically

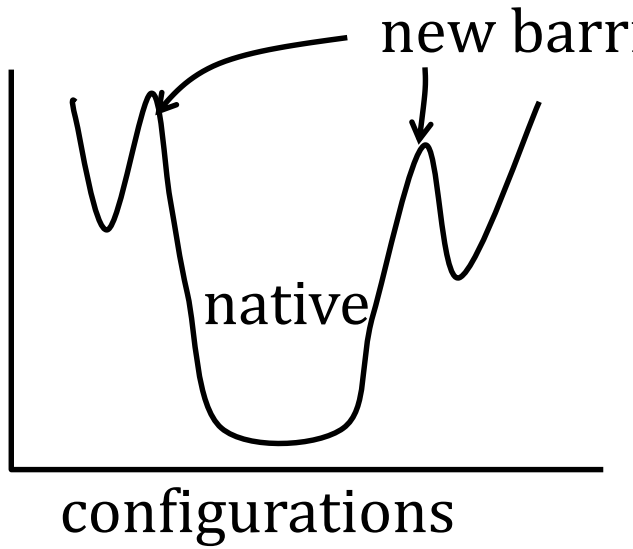
- some evidence of kinetic trapping
- some proteins do have other states
  - β-fibrils, Alzheimers, mad cow disease

Evolution / design consequences

Imagine I can predict structure and stability

- I design a better / more stable protein





- my new protein may be more stable
- it may never fold
- evolutionary implications
  - protein sequences may evolve for folding (+ structure and function)

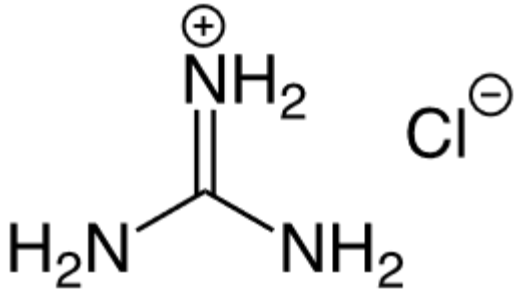
Experiments

Timescales

- maybe 10^{-6} s for folding
- maybe orders of magnitude slower (sometimes faster)

Experimental approaches

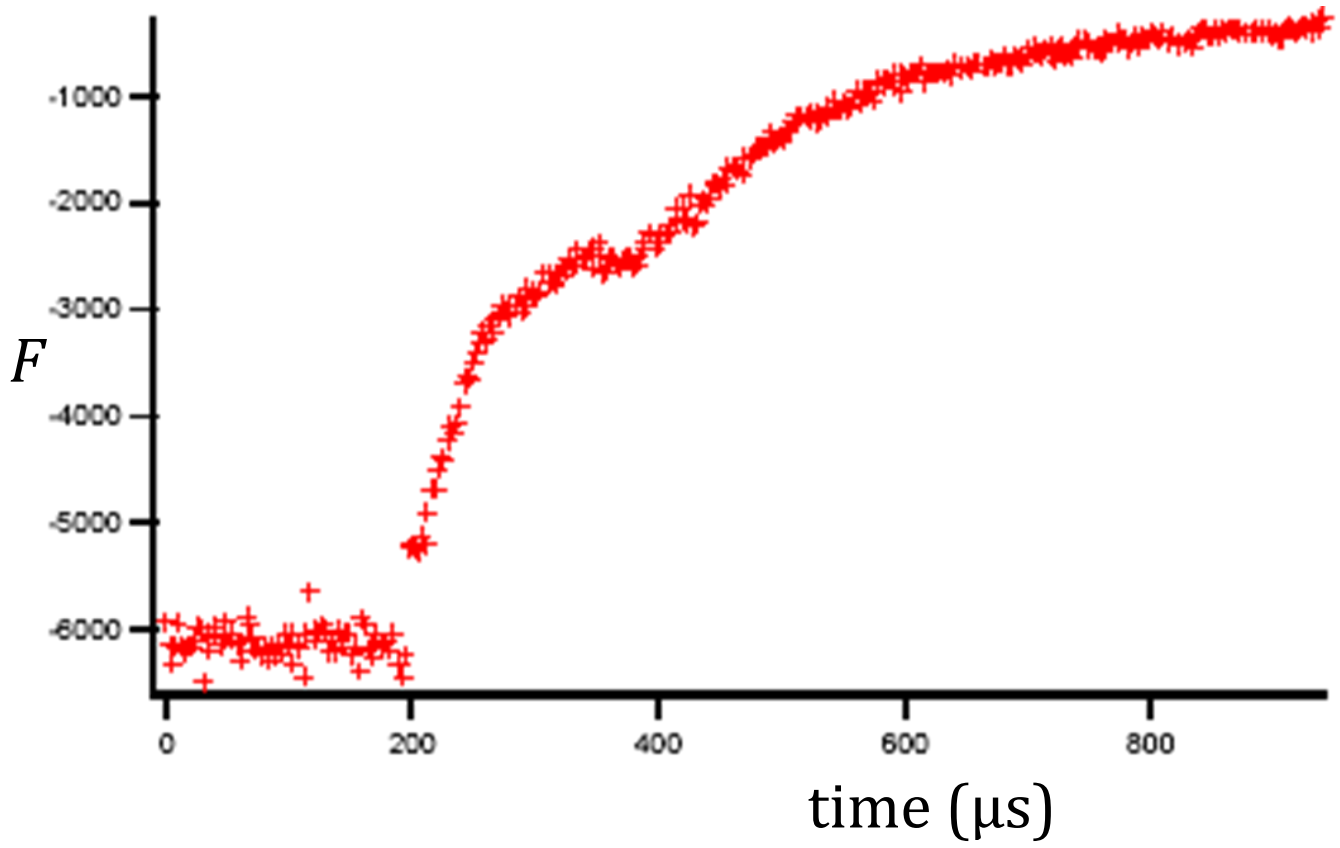
- force protein to unfold
  - chemistry (guanidinium HCL, 8M urea)
  - temperature (heat, cold)
- change conditions and watch
  - try to measure very fast
  - try to change timescale
  - try to measure unfolding



Experiments are difficult

Very difficult to measure on the µs / ms time scale

- temperature jump
- stop - flow
- fluorescence
- NMR
- circular dichroism (CD)



No experiment gives good structural detail on this time scale

Dimitriadis, G, Drysdale, A, Radford, S and Smith, A, "Investigating early events in protein folding by laser induced temperature jump"

How good are experiments ?

Technical difficulties – obvious

How relevant are experiments ?

- Imagine the perfect experiment
  - you unfold a protein (heat, salt, ..)
  - go back to native conditions and watch

  - does this tell you about protein folding ?

Nature versus experimental folding

Nature

- protein is synthesised on ribosome
- 150 mM salt, pH relatively neutral, 300 K

Experiment

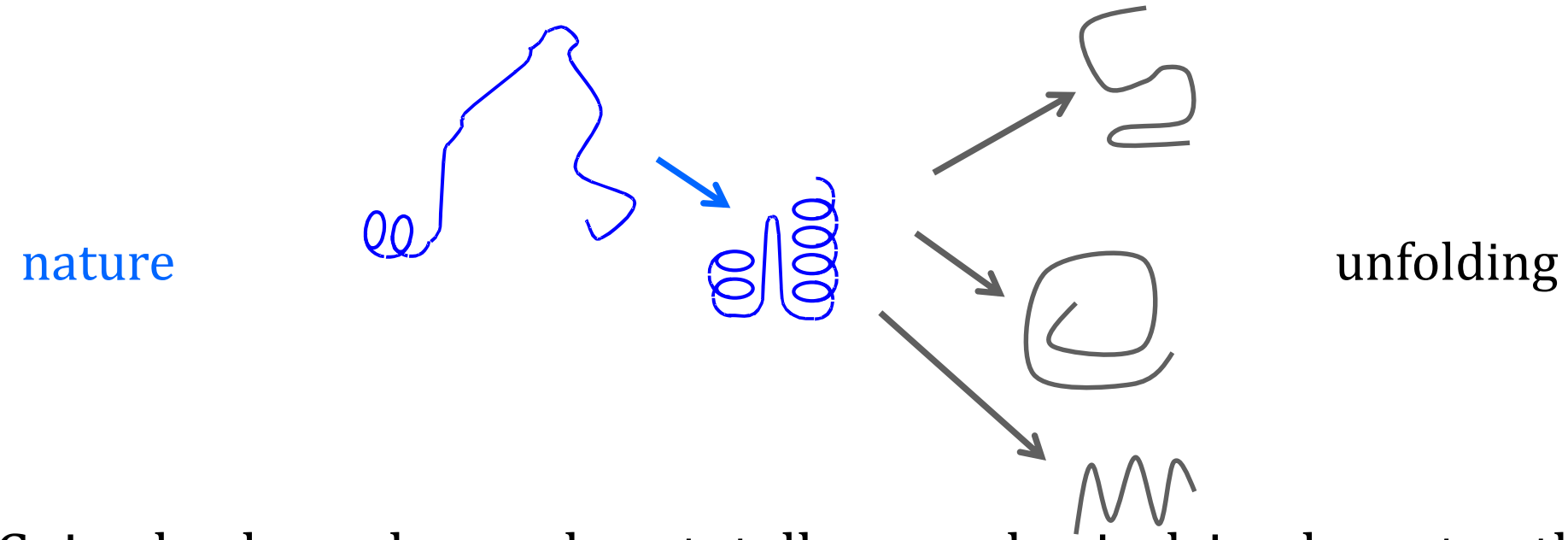
- high salt, temperature, ..

Difference

- under exotic conditions, protein visits unusual conformations

Simulations

- Can one simulate folding ? Not really
- Unfolding ?
  - start with native protein and heat it until it unfolds
  - is this the unfolding pathway ? Is it the opposite of folding ?



- Going backwards may be a totally non-physical, irrelevant pathway

Forgetting atomistic detail

- What are questions we can ask ?
- What can we guess without any calculations ?
- Questions
  - is there such a thing as a folding pathway ?
  - how should we look at folding ?
    - secondary structure forms first and is rearranged
    - hydrophobic residues come together and then secondary structure forms ?
    - a few important contacts are formed, then structure forms

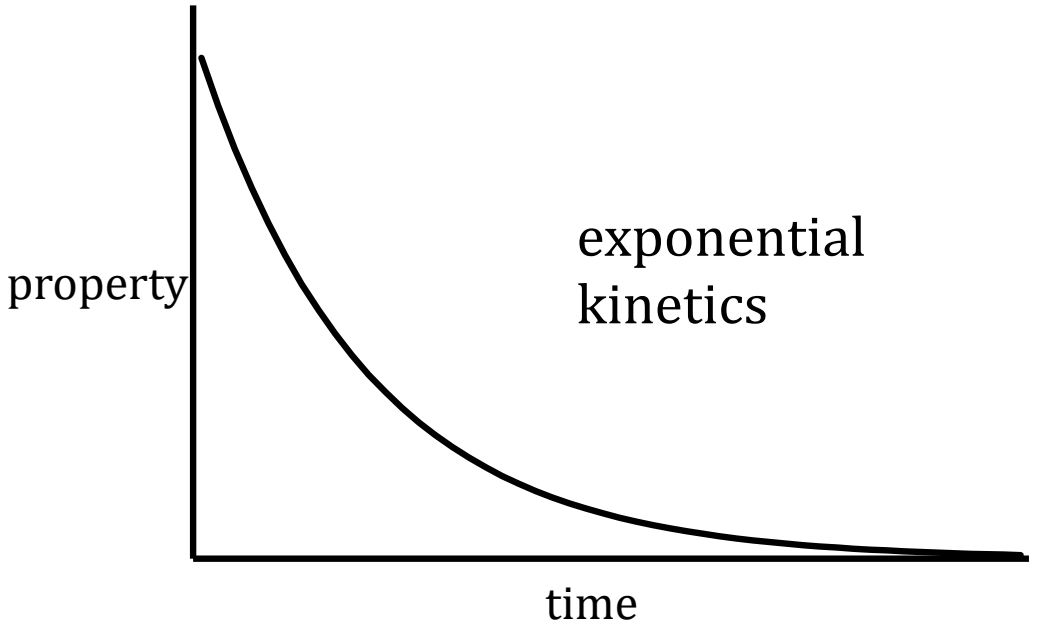
Kinetics in general

What have we seen so far ?

- most properties have something like exponential decay
- property $= ae^{-\alpha t}$
- rate of change proportional to quantity present

A model should explain at least this

- later by ensemble view..
  where are barriers ?



What do we know ?

Possibilities

Backbone:

Proteins form secondary structure first

- helices and sheets then arrange themselves

OR

Sidechains:

Hydrophobic collapse

- hydrophobic residues find each other
- backbone rearranges and secondary structure is fixed

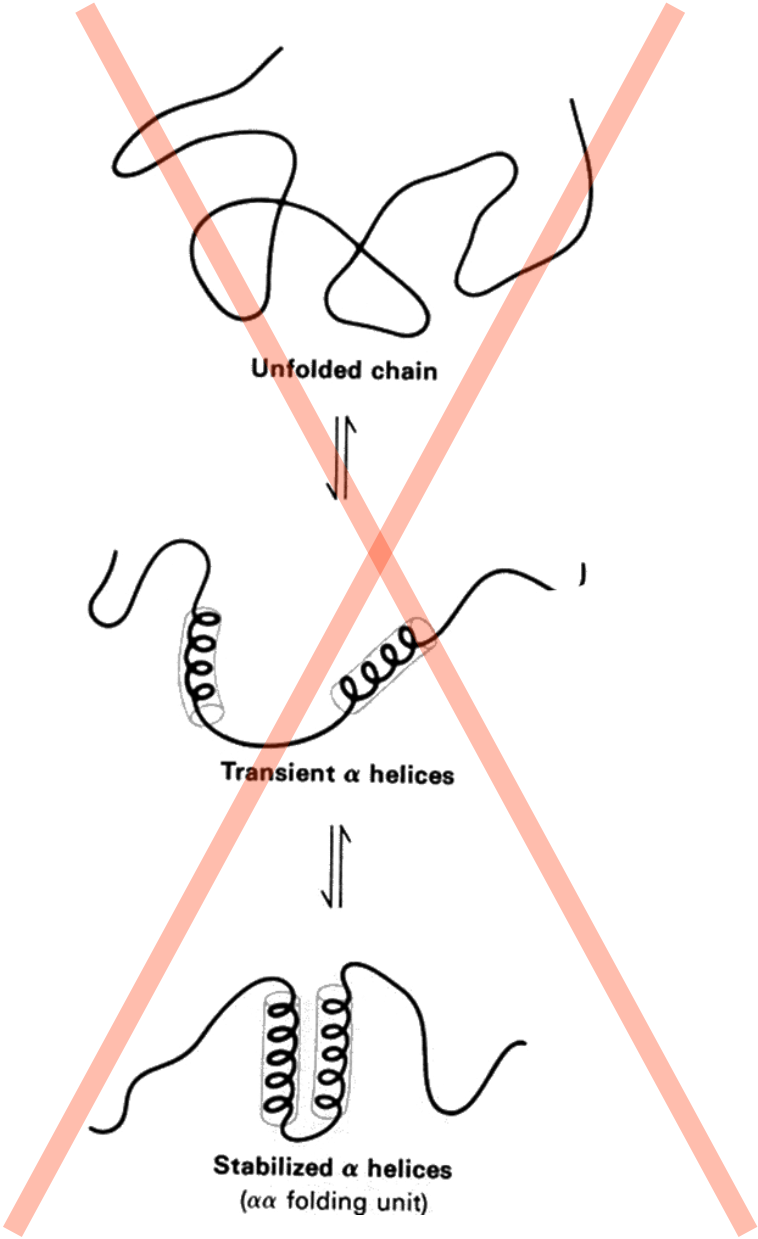
Sidechain or backbone driven

Old textbook

- local secondary structure forms, then reorganises
- secondary structure depends largely on backbone

Alternative

- sidechains are very important



Stryer, L. Biochemistry, 1981, WH Freeman, "Biochemistry", page 36

Sidechains might be important

- backbone view does not predict collapse of protein
  why would helices and sheets find each other ?
- α-helix and β-strand propensity is weak
  - isolated peptides are not stable
  - β-strands often depend on long-range H bonds
- helix / strand formation depends on environment
  - similar sequences form α-helix in this protein, β-strand in another
- fold is largely predictable / characterised by pattern of HP (sidechains)
- proteins are most sensitive to mutations in core
  (they are important for stability)

Types of pathway

From classical chemistry we would like a path

unfolded → A→ B→C→ folded (native)

Basic idea - molecules may get side-tracked, but

- every molecule sees A, B, C...
- where does it come from ?
  - Levinthal's answer to paradox - there must be a preferred pathway
- old view / microstate pathway approach

Consequence of simple pathway

Two state kinetics

- unfolded → A , A → B or B → C might have a barrier

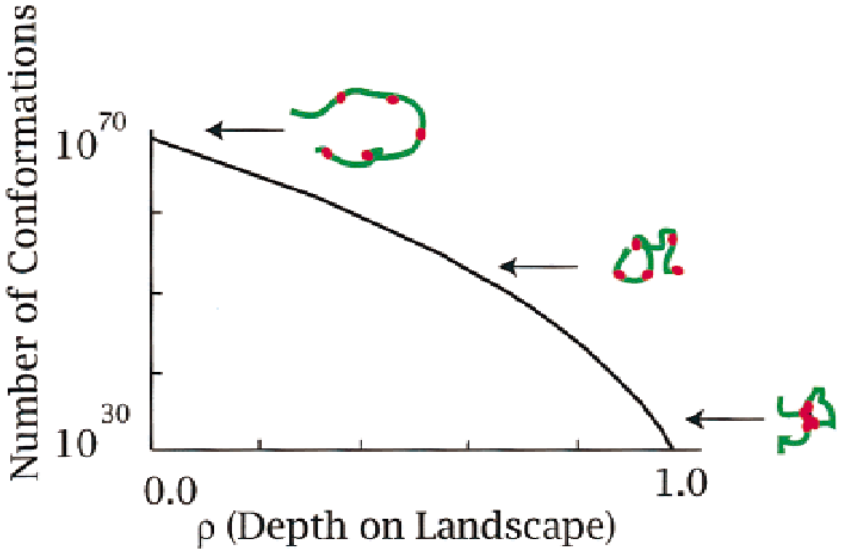
Does it sound intuitively reasonable ?

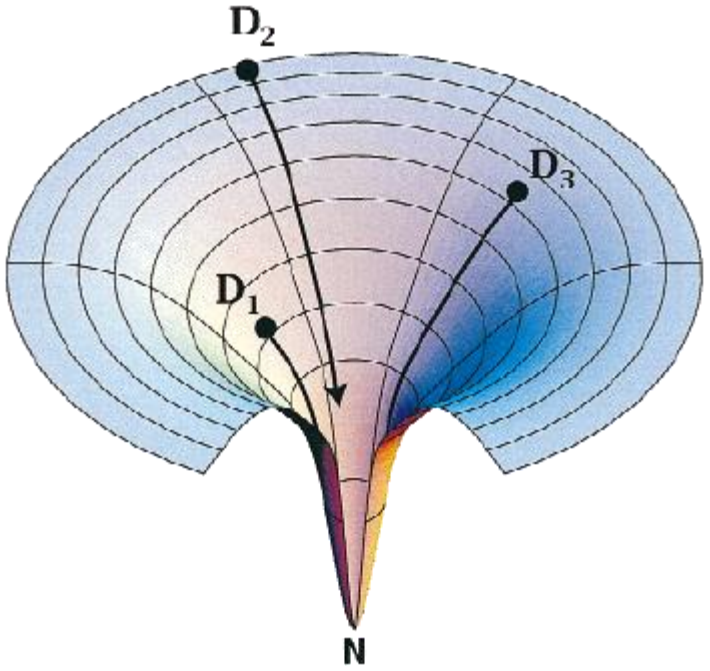
- what if a mutation perturbs A → B or B → C ?
  - whole pathway might break
  - some mutants do not fold

Do you need conventional pathway to explain barriers ? Needs long explanation

Ensemble view

- conformation space is huge
- will a protein be able to find a neat path through it ?
- should we even look for paths ?
- consider a multitude of paths...
- is this merely a cute picture ?
- first implication...
  - entropy vs energy





Dill, K.A., Protein Sci., 8, 1166-1180, 1999
Polymer principles and protein folding

Ensemble view

As a protein folds

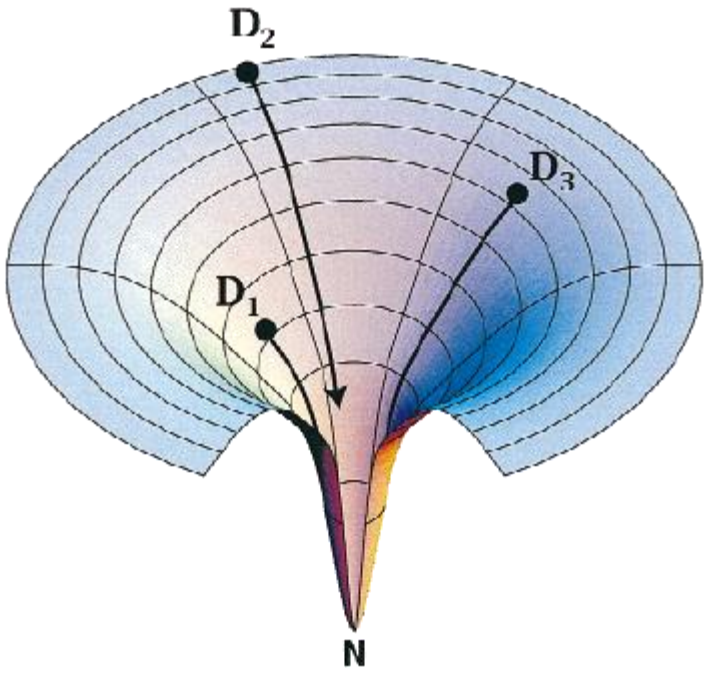
- potential energy goes down (happy)
- number of possible states goes down
- entropy goes down (unhappy)

Interpretation in chemical terms

- do we have a off-pathway intermediate ?

Do we have a reaction coordinate ?

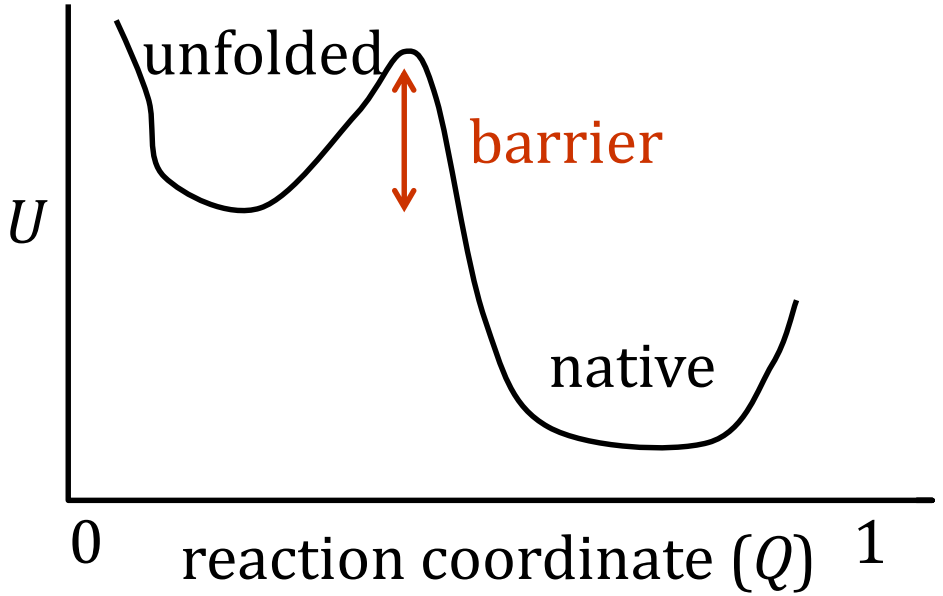
- not a classic one
- can we invent one ? yes
  - if two atoms are in contact in the final structure
    - native contact
  - Q = fraction of contacts which are correct



Reaction path

Is this like a chemical reaction ?

- no
- many molecules have same Q, but different conformations



We want at least two state kinetics

- where does barrier come from ?

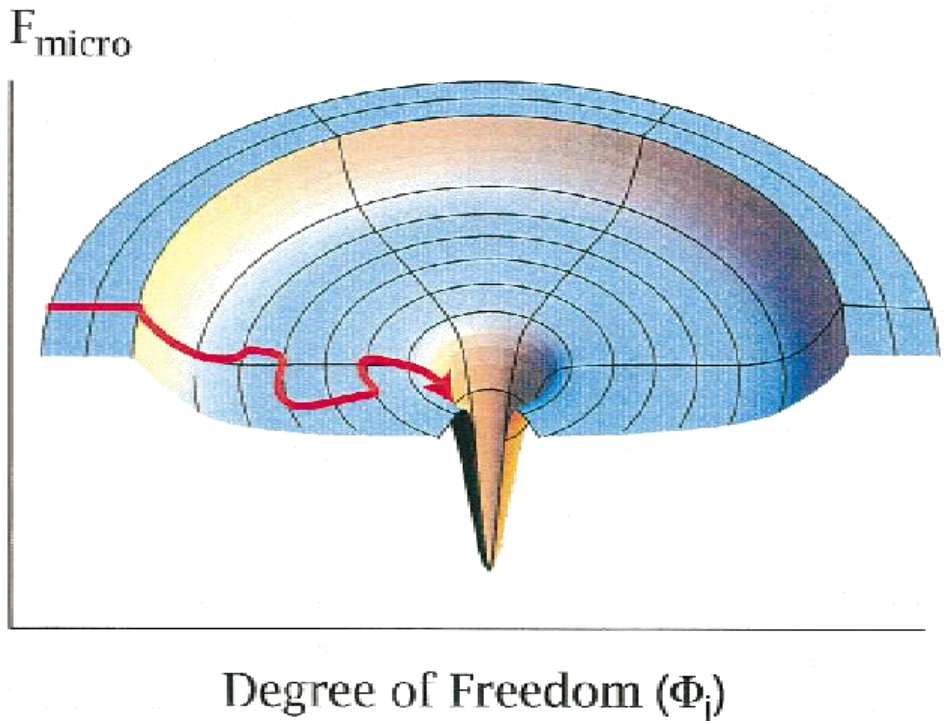
Entropic barriers

Nature cares about free energies

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

If a molecule walks around

- it takes a long time
- looks the same as an energy barrier



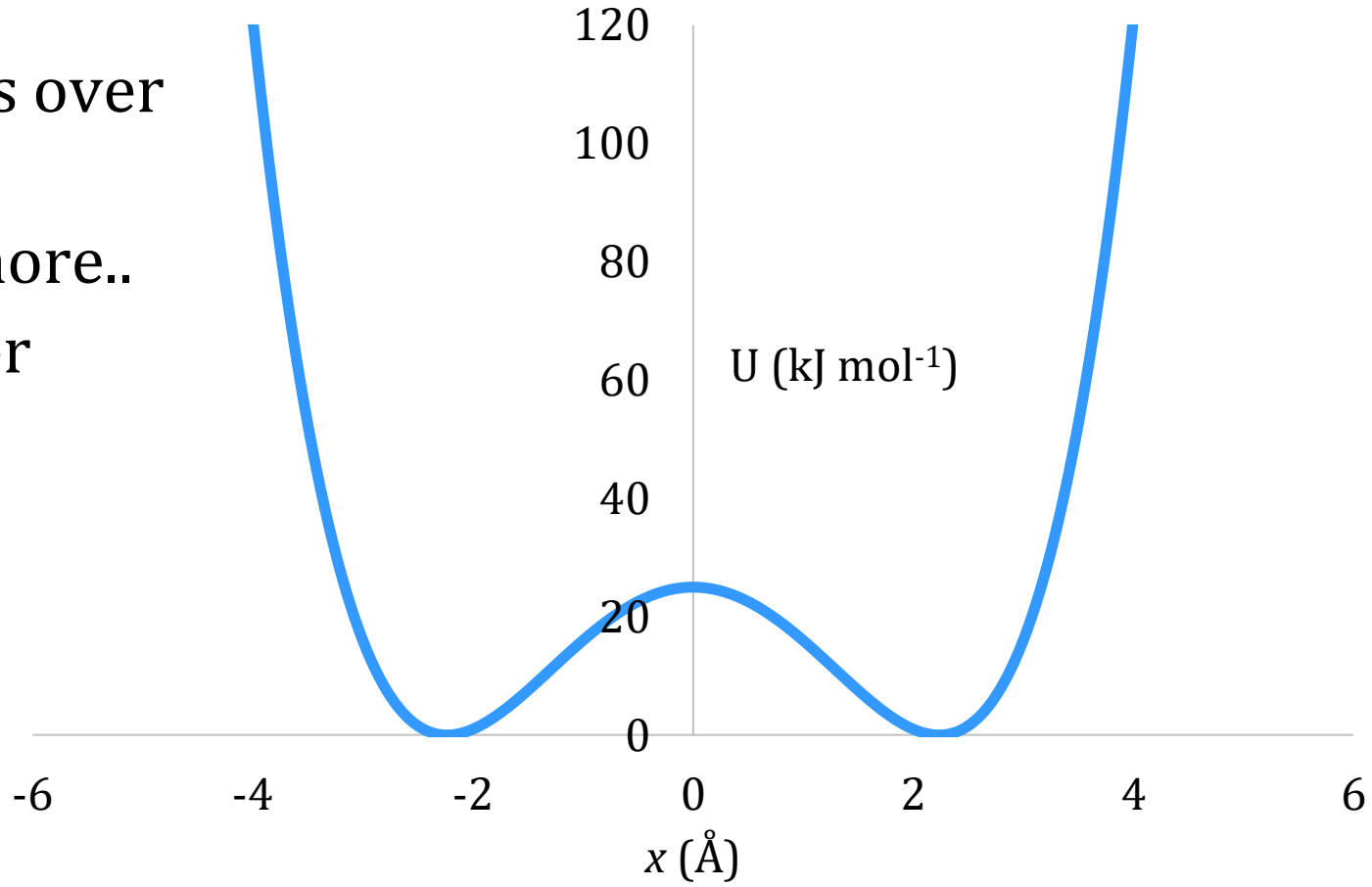
- Are these pictures useful ?
- Do they agree with calculation ?

- have we seen an entropic barrier before ?
  - an entropic valley ?

Dill, K.A., Protein Sci., 8, 1166-1180, 1999, Polymer principles and protein folding

entropy – story from the Übung

- we have an energy surface
- system prefers low energy regions
  - no surprise
- heat the system and it jumps over barrier
- heat the system more and more..
  - it did not cross the barrier more often
  - why ?

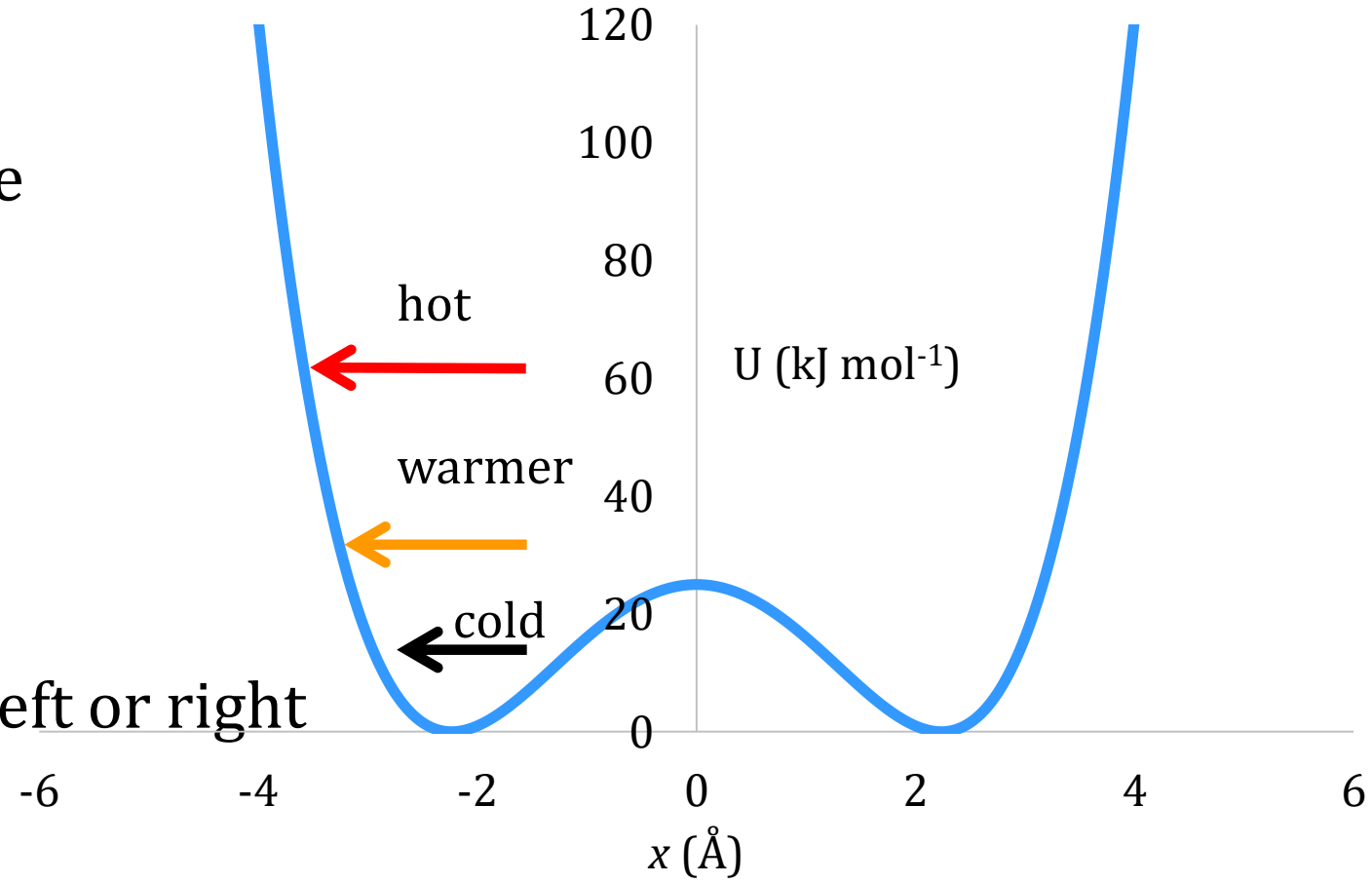


entropy – story from the Übung

- from potential energy
  - system does not want move far from centre

- as we heat the system
  - there is more space to the far left or far right

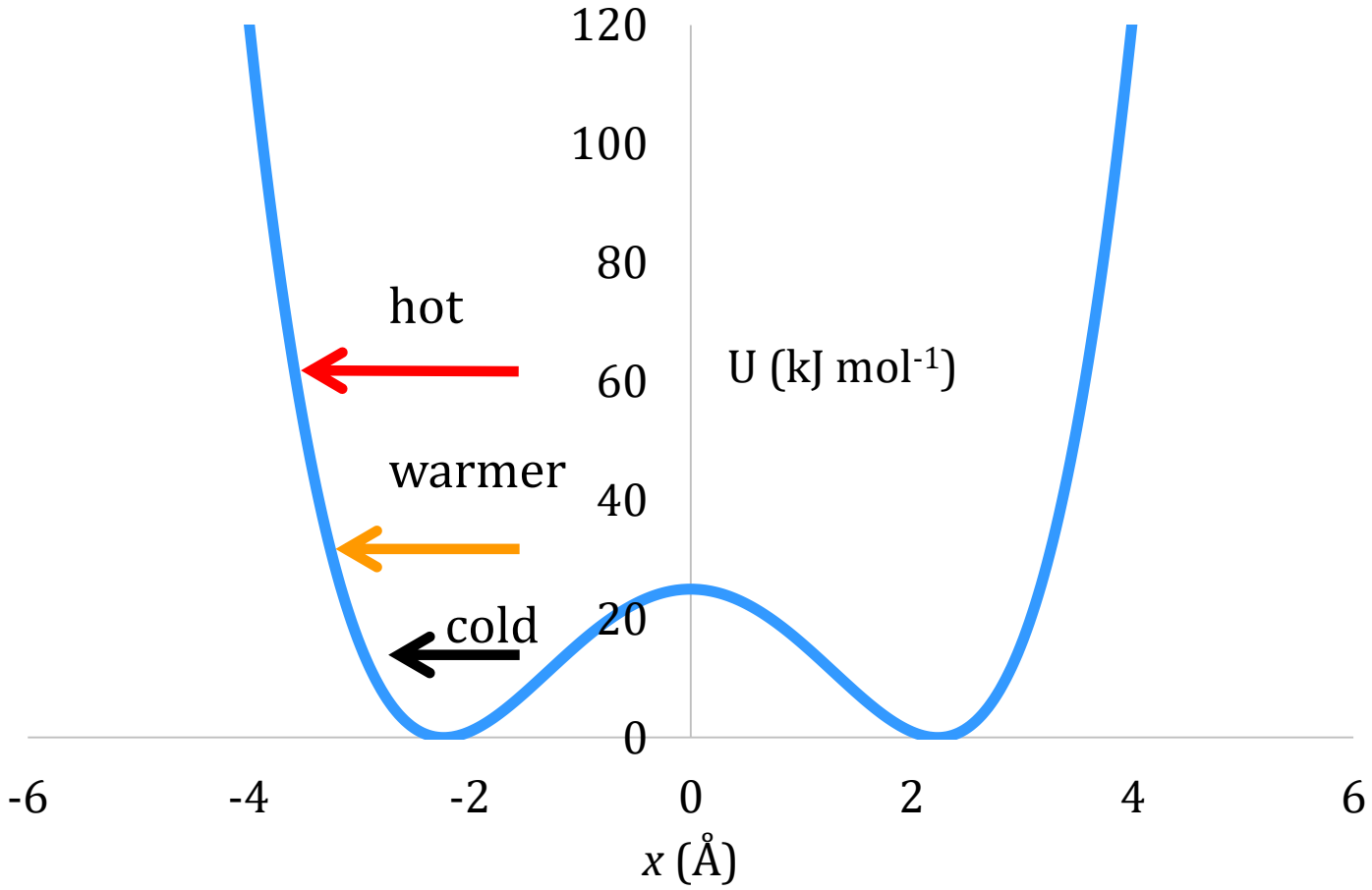
  - how much space ?
    - potentially infinite
- when it is hot
  - the system can be far to left or right



entropy – story from the Übung

regions far left or far right seem attractive

- because they are potentially large (at high temperature)

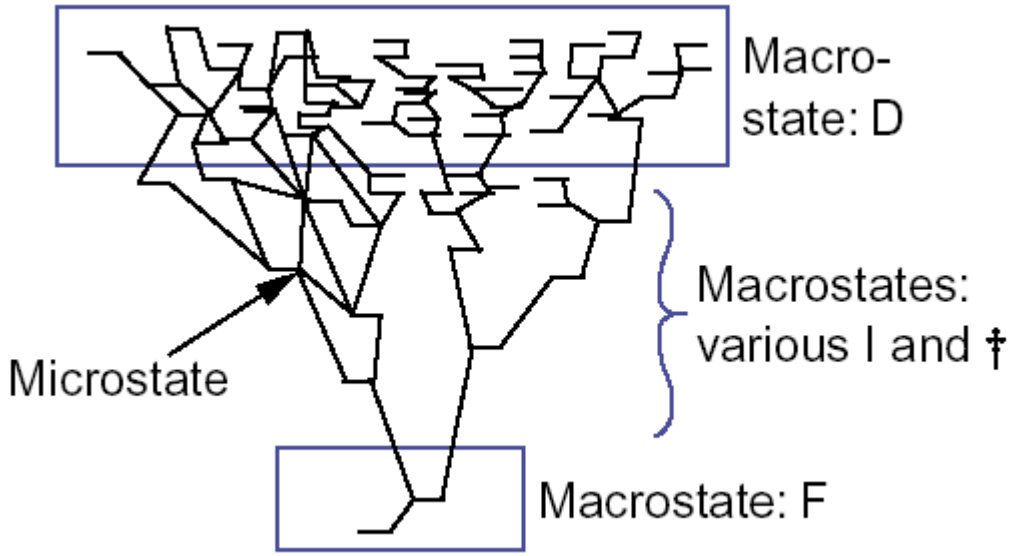


Interpretation of landscape

Does this disagree with conventional pathway ?

$A \rightarrow B \rightarrow C$?

- mostly at early stages
- there is a multiplicity of "A"
- when near native, there are relatively few conformations, so there may be something more like a pathway

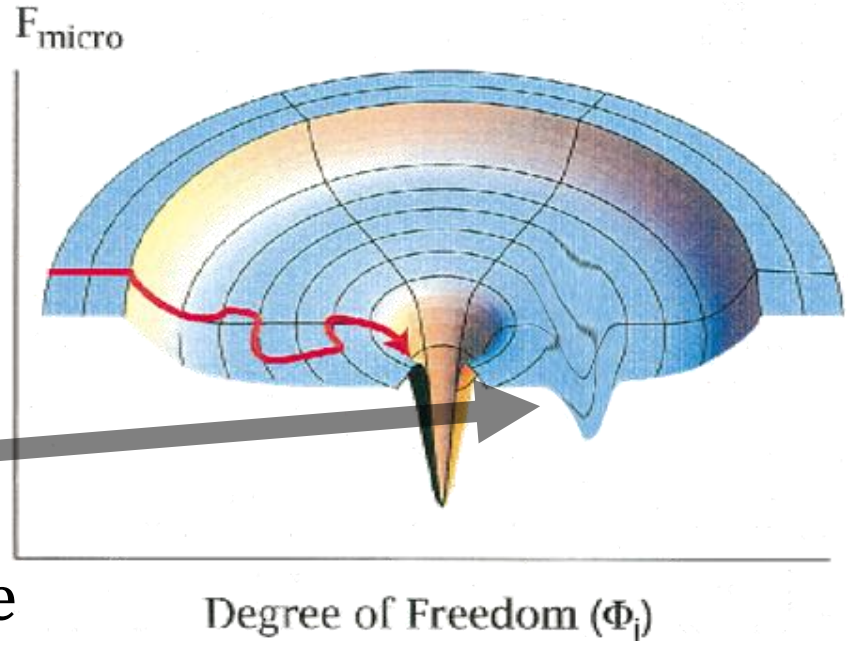


Gruebele, M, Curr. Opin. Struct. Biol. 12, 161-168, 2002, Protein folding: the free energy surface

Plausible ? Agreement with experiment ?

Difficult ..

- Experiments measure an average over all molecules
  - these are the same in different models
  - should we expect well defined intermediates ?
    - not really
    - what if one sees them ?
      - say they are valleys on the energy landscape
- Hard to find testable predictions

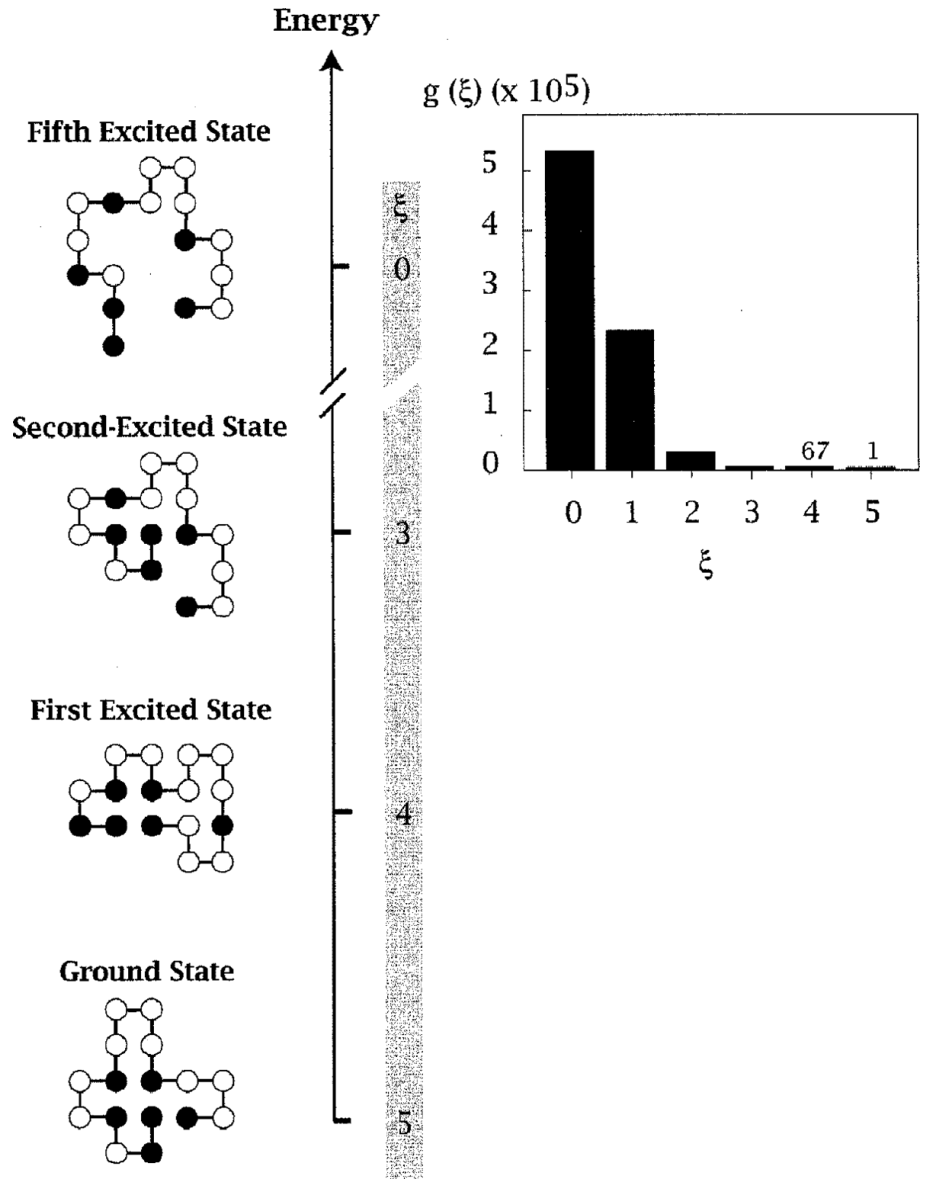


Simulations ?

- Cannot simulate a protein in detail
- Can simulate a discretised, simple protein
  - one point per amino acid
  - coordinates site on grid /lattice / gitter points

Question

- lowest possible energy E_{min}
- how many conformations have
  - $E = E_{mi}$? $E = E_{mi}$ + a bit ? …

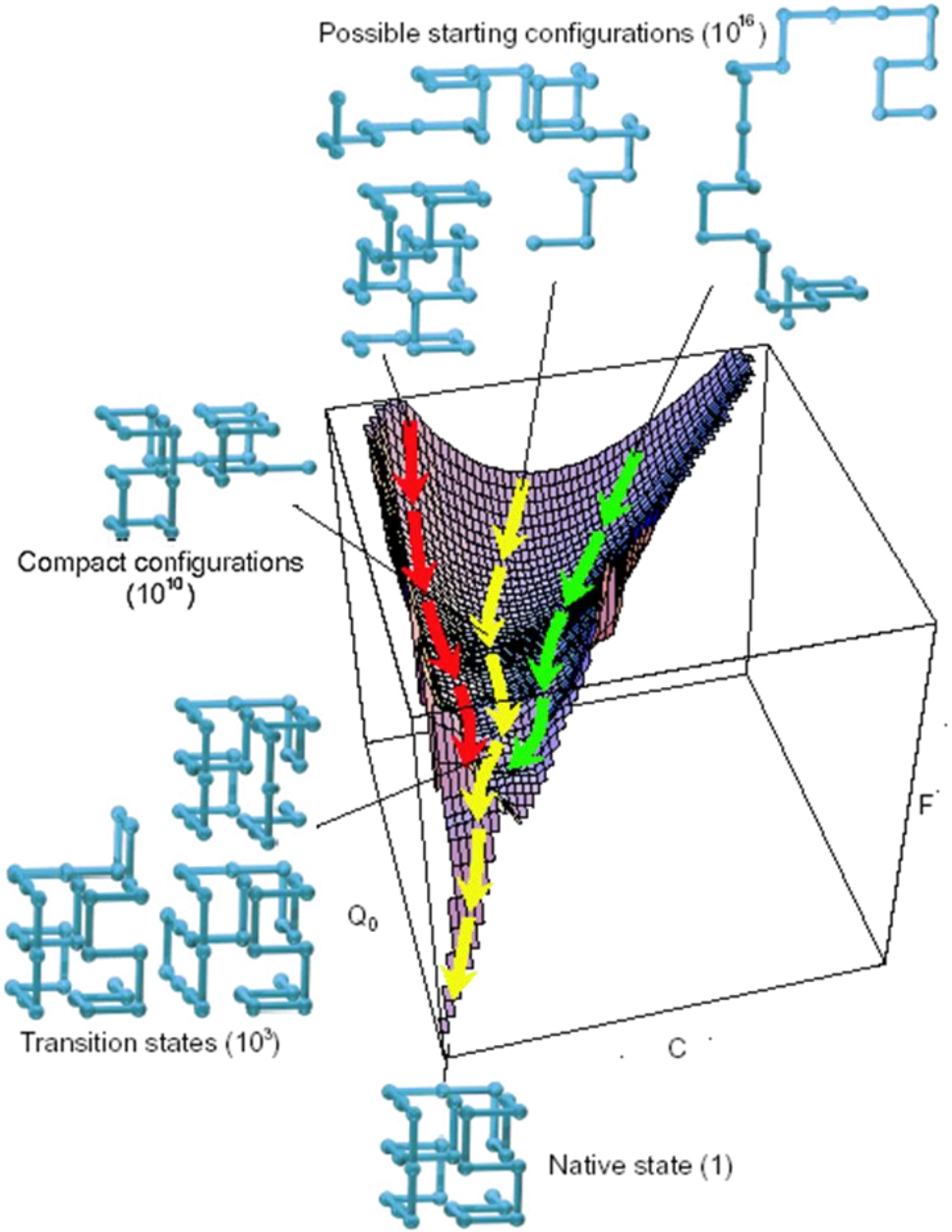


from Dill, K.A., Protein Sci., 8, 1166-1180, 1999, Polymer principles and protein folding

A larger calculation

27 residue

- simple lattice model
- estimations by sampling
  - not exhaustive
  - Q_0 correct contacts
  - C total contacts
  - F free energy



Summary

Experiment vs. theory

- experiment usually gives us averages
- most calculations look at details

Very different views on folding may be hard to distinguish

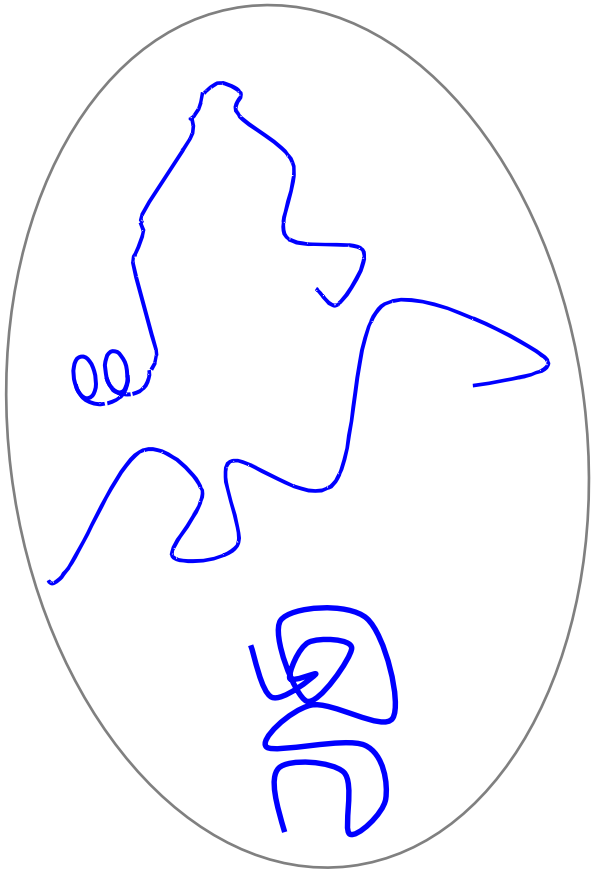
Folding may be guided by sidechains (not hierarchical)

Early folding may be best modelled by very crude models (so space can be sampled)

Any useful model should predict exponential kinetics (or more complicated)

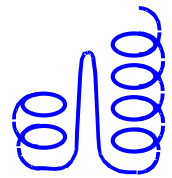
Protein stability

Our model



denatured (D)
unfolded
non-native

ΔG



native
(N) folded

Free energy change on

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

For a stable protein

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

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)

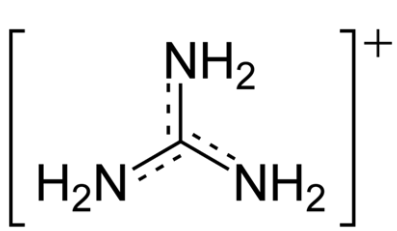
- too hot ?
  - eggs cook, people die
- big variation
  - some bacteria live at 373 K – their proteins are not denatured

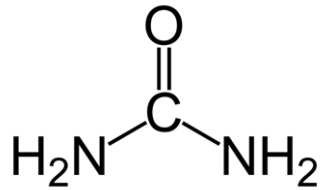
Explain denaturing

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 ?

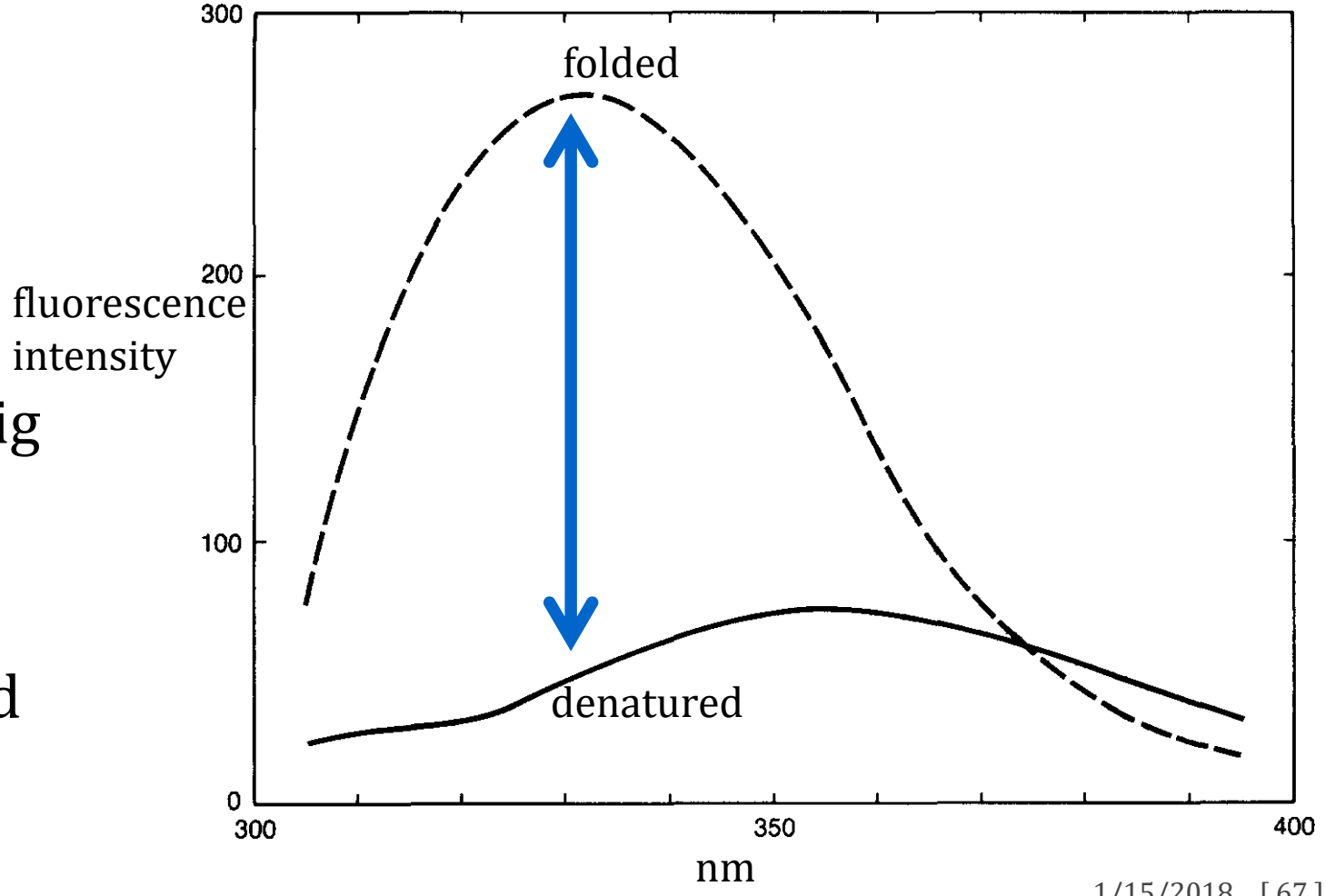
Assume

- 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

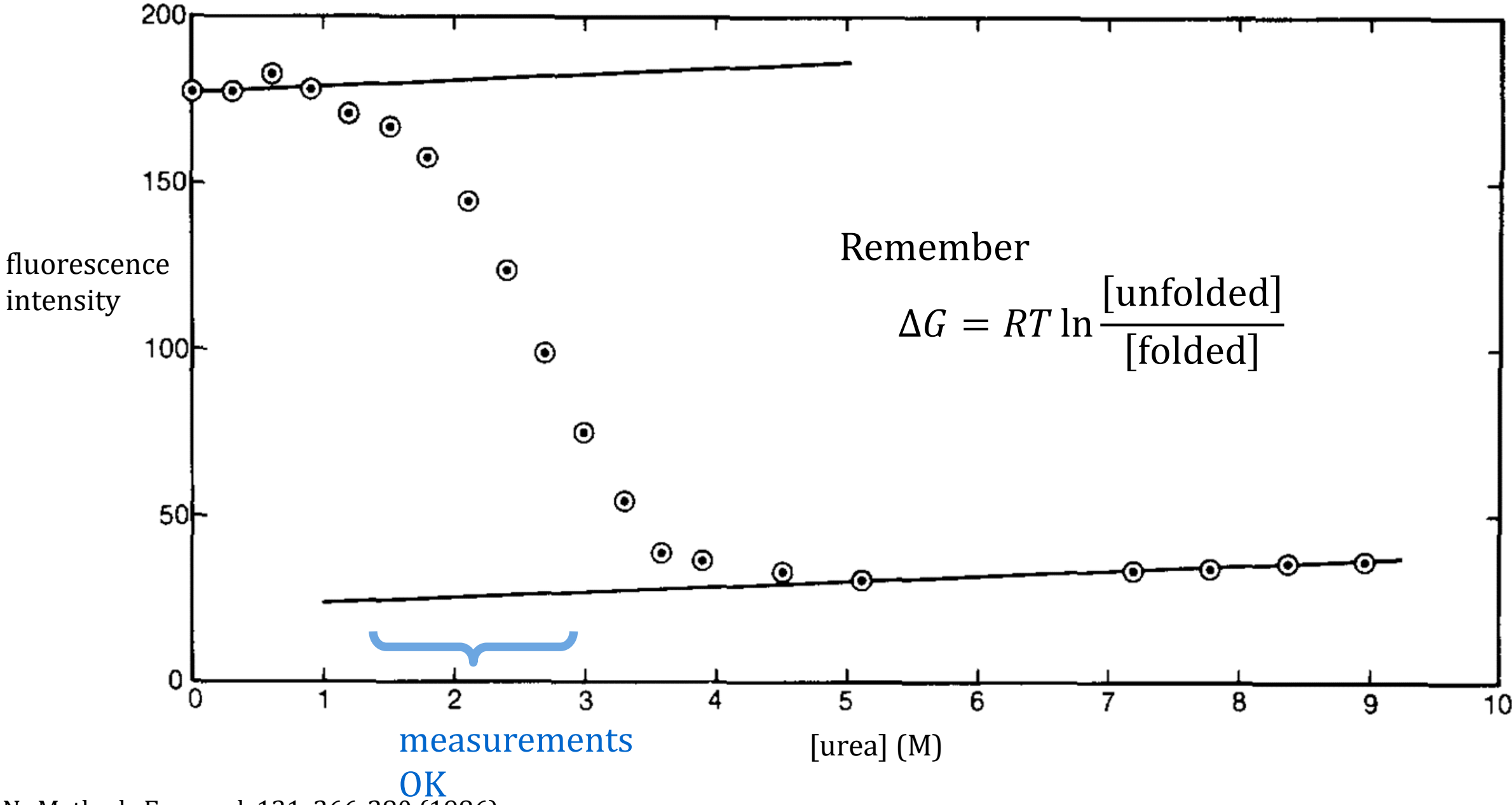
- if $\frac{[\text{unfolded}]}{[\text{folded}]}$ is not too small or big
  I can measure it

- trick is to make it partially unfold



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

Add denaturant



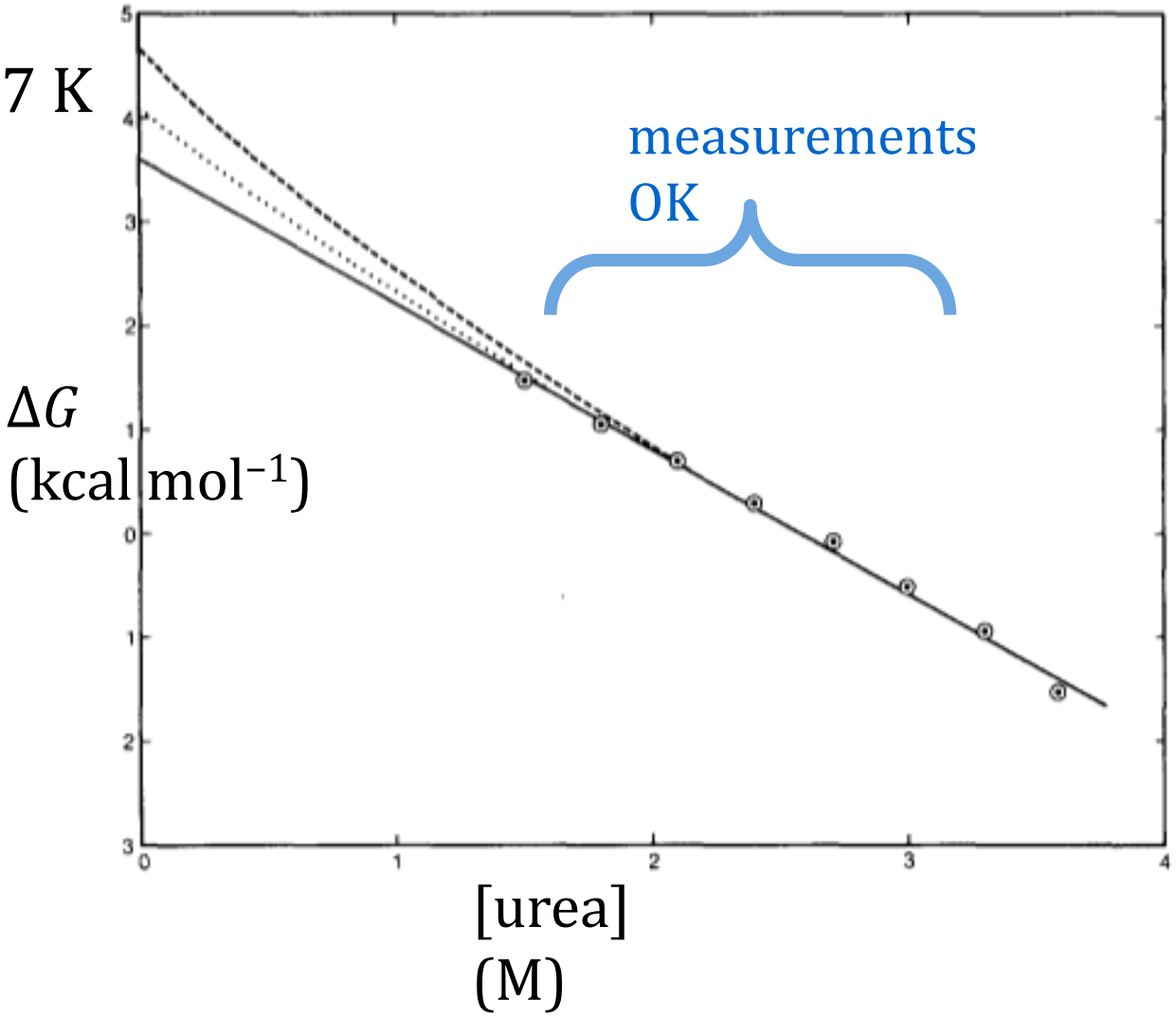
Remember

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

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



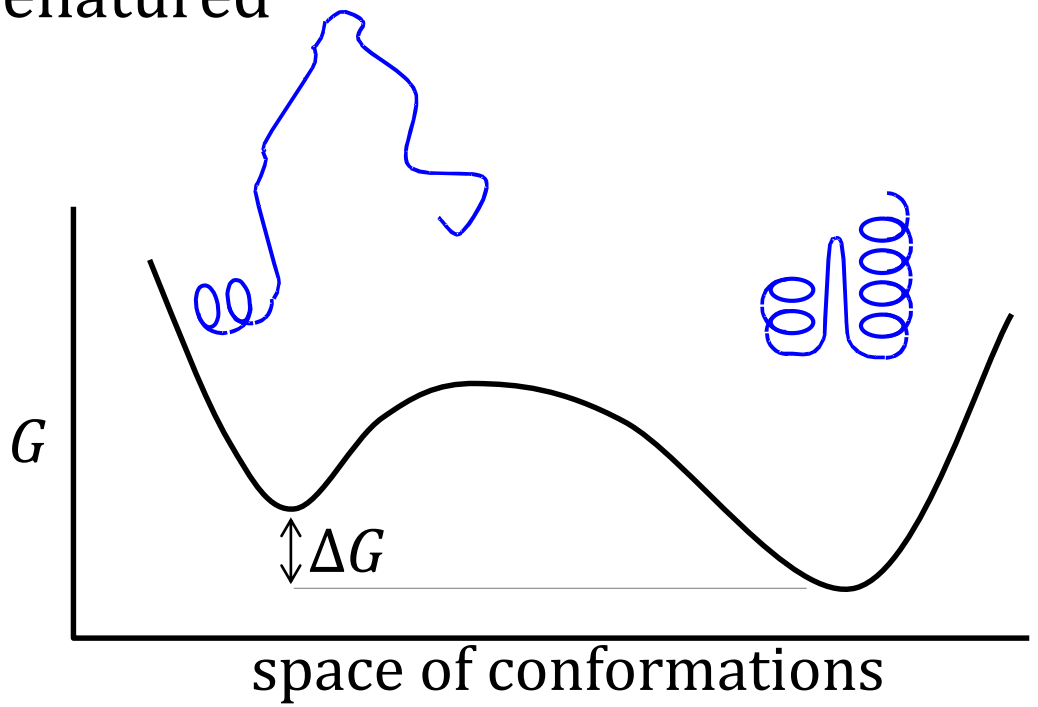
Pace, C.N., Methods Enzymol, 131, 266-280 (1986)

Two state model

Model requires an energy barrier

What if $\Delta G = 0$?

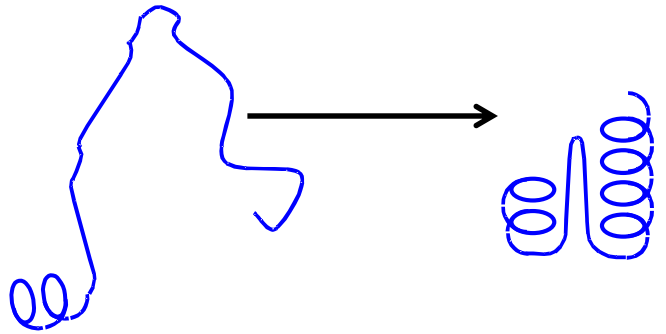
- ½ molecules folded
- ½ molecules denatured



First picture of stability

What holds a protein together ?

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



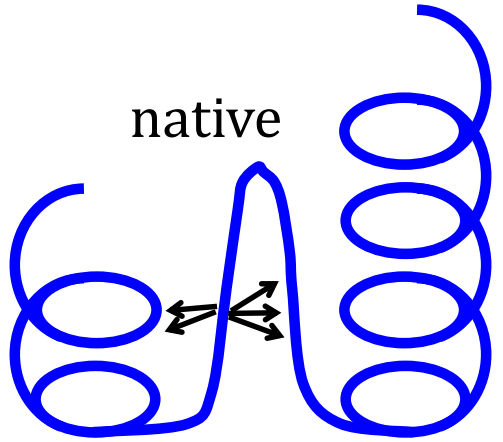
Cannot be so simple

- 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



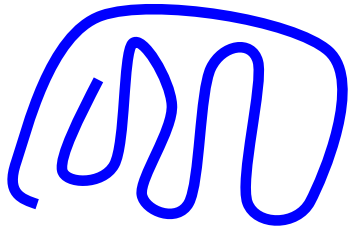
One atom in native structure

- 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 $H_{\text{native}} - H_{\text{denatured}}$

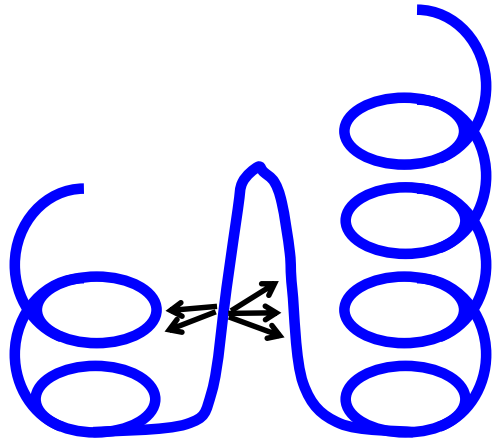


some denatured / non-native

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

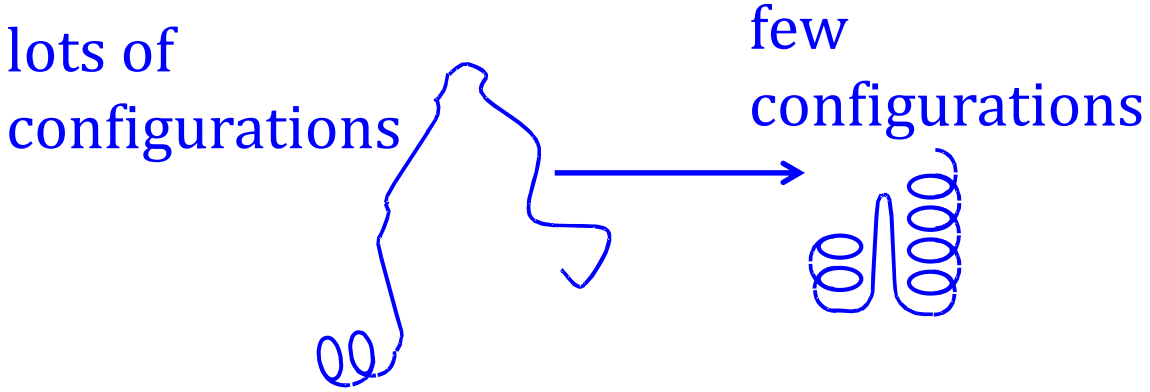
$\Delta G = \Delta H - T\,\Delta S$

- 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 ↑



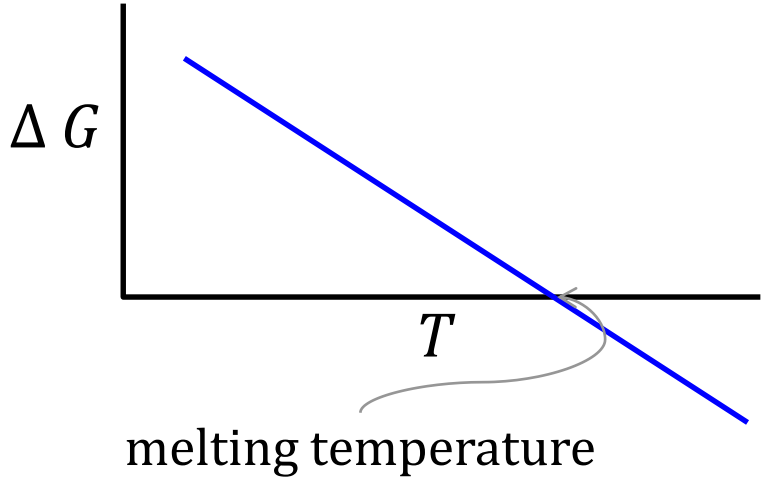
- entropy / ΔS will favour denaturing

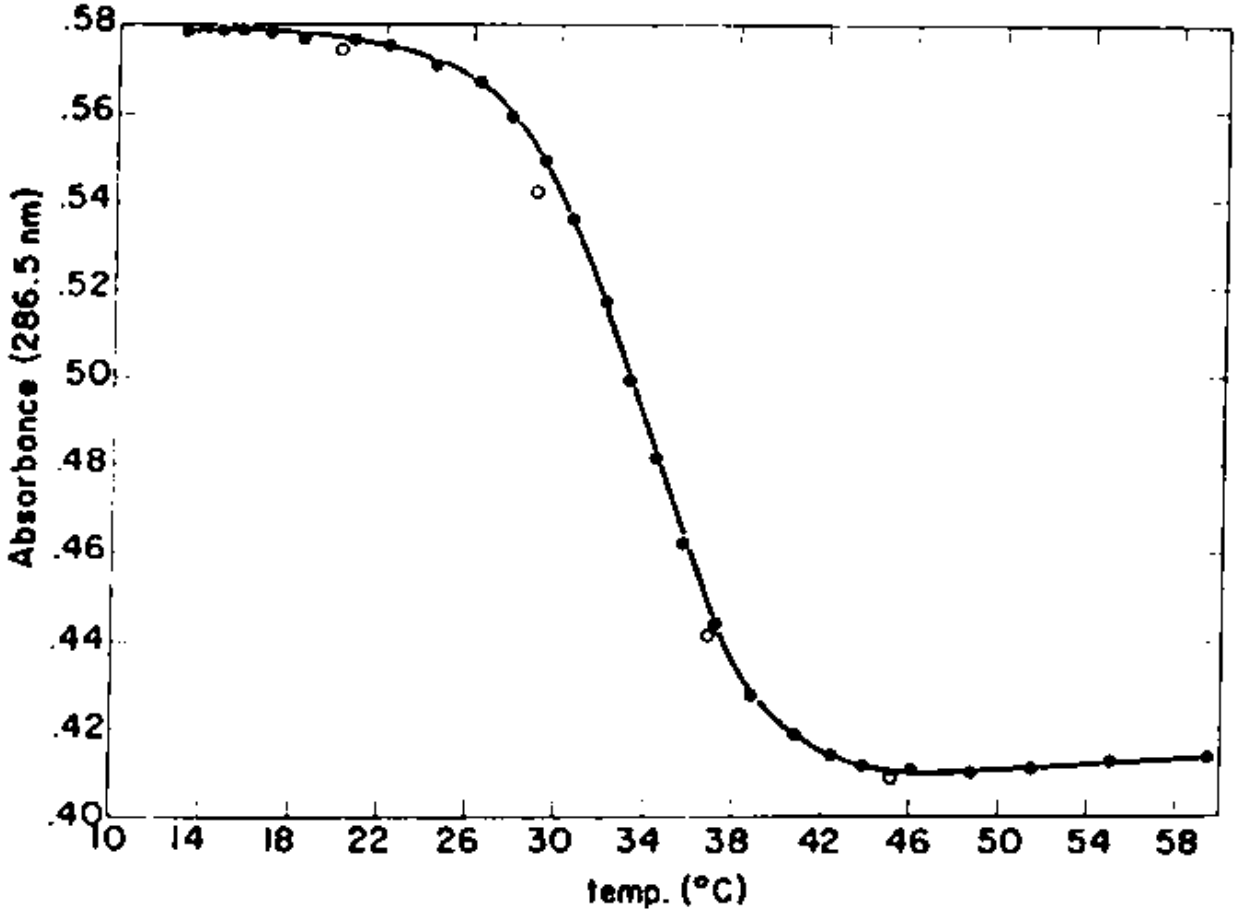
Ω_x number of states in x
k Boltzman constant

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 ?

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

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

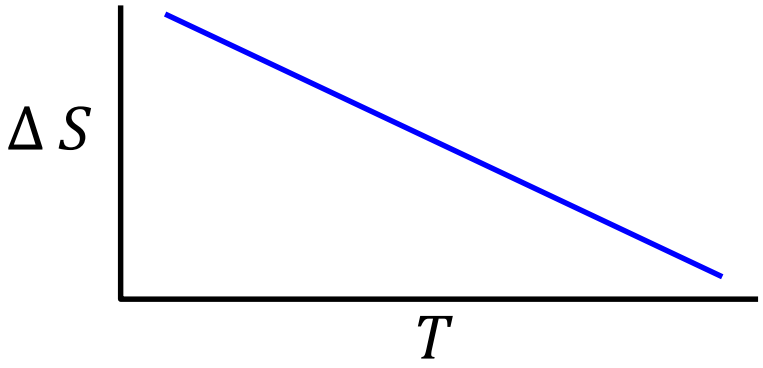
$$= k \ln \frac{\Omega_{folded}}{\Omega_{unfolded}}$$

- 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

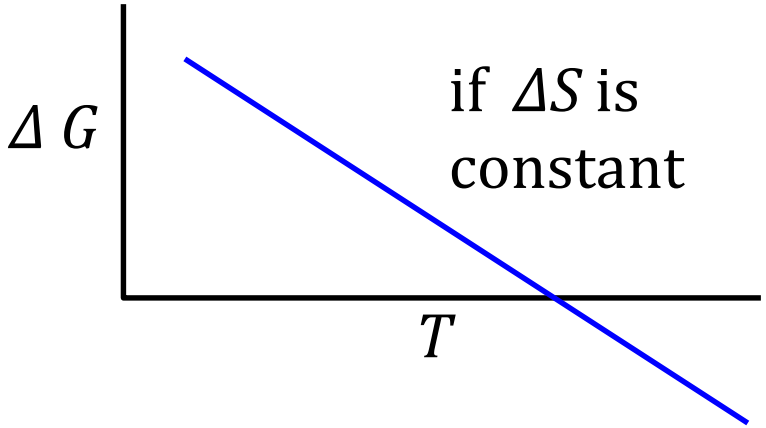
- entropy might make proteins less stable as you cool them

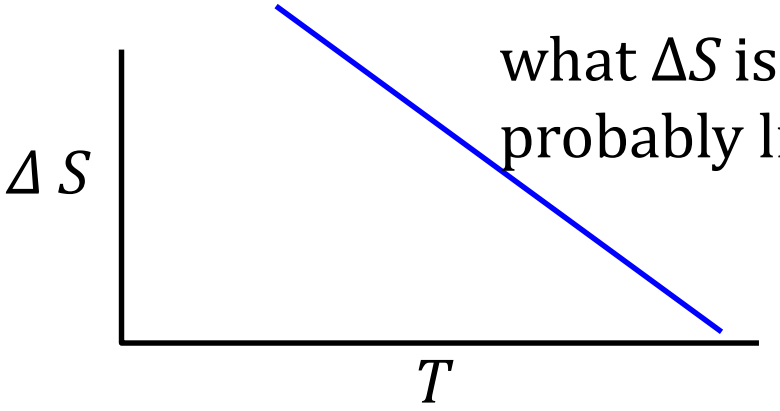
Can one cook an egg by cooling it ?

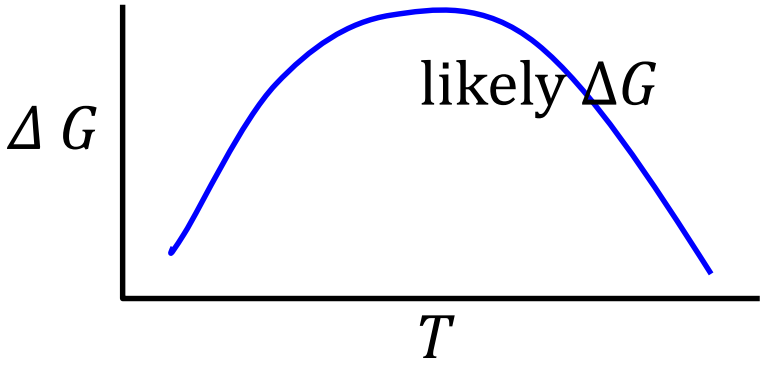
Combining these properties

protein stability

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







What is seen / claimed ?

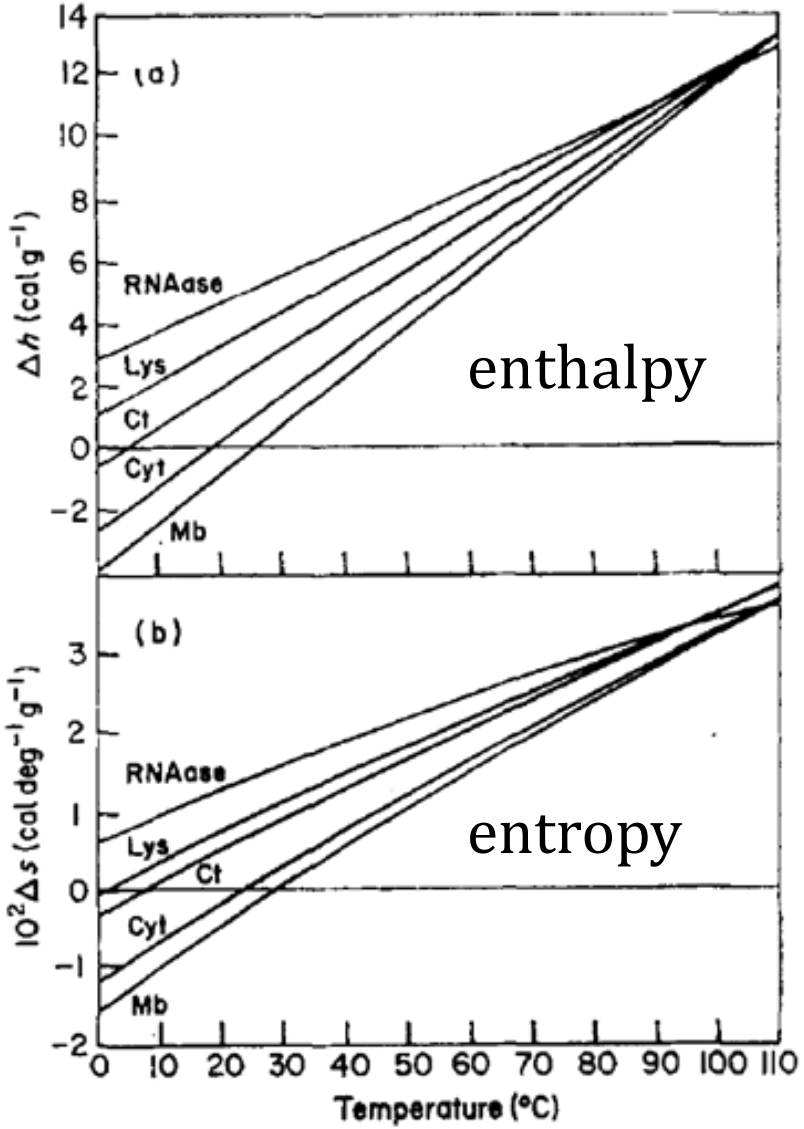
Old measurements

- 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

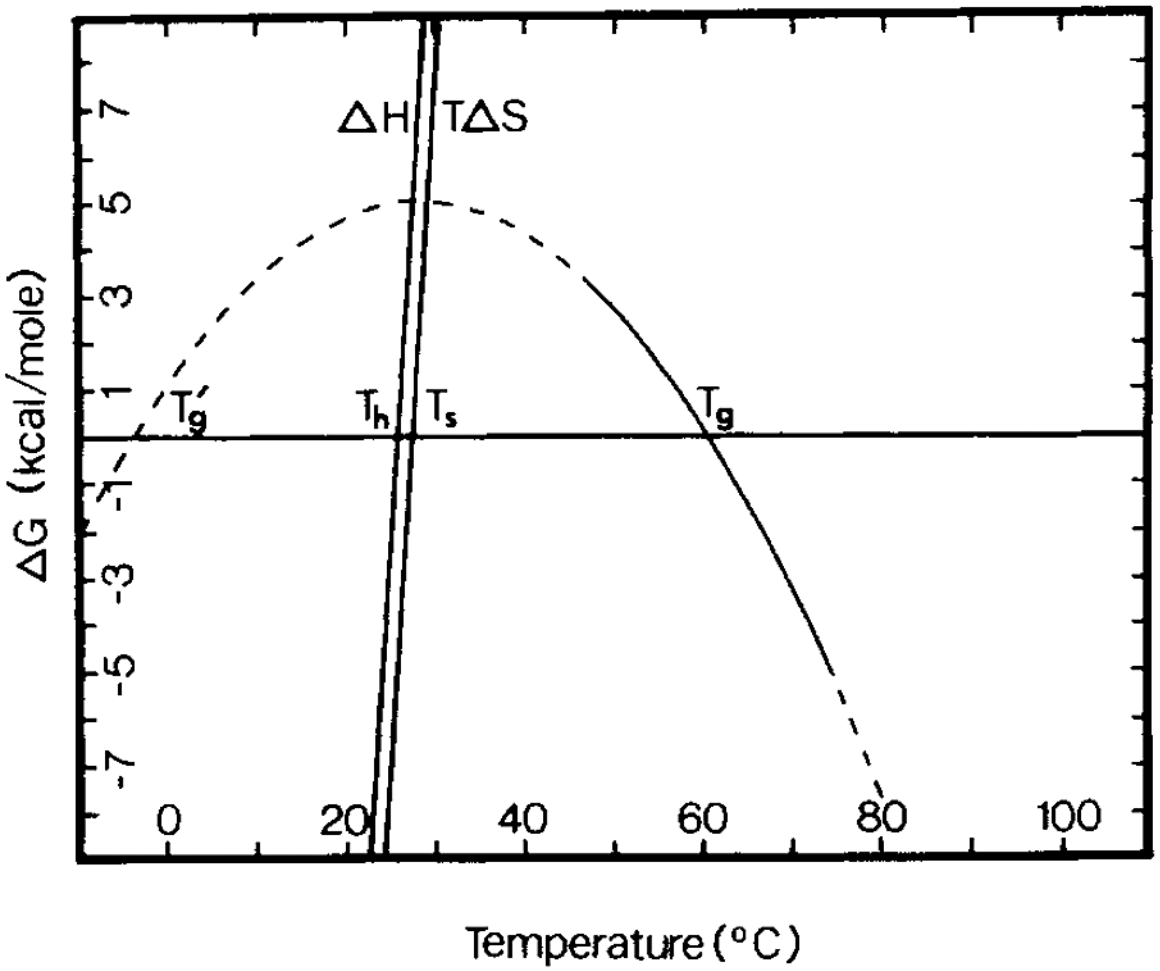
final version

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

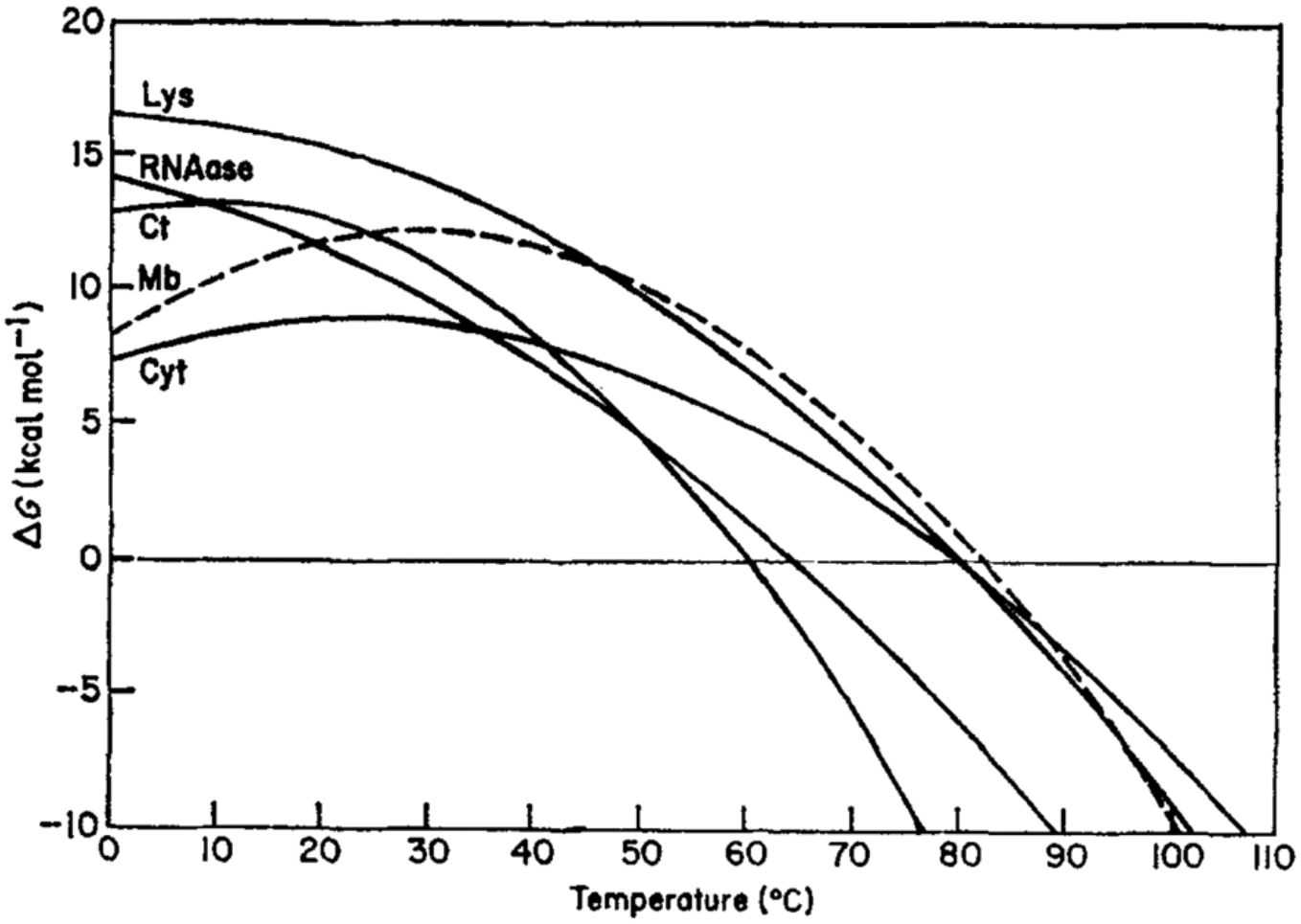
free energy experimental

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

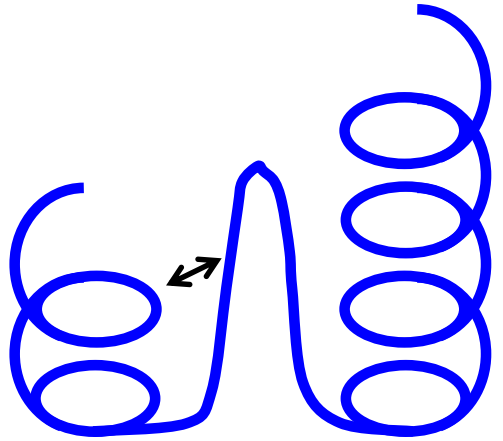
- how much does entropy change with temperature ?

- 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

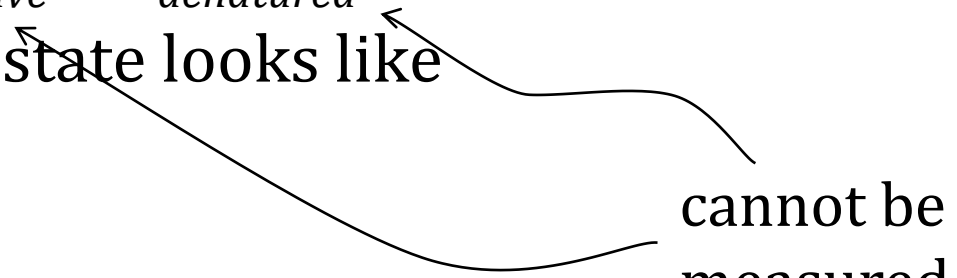


Consequence

- 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

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