Thermodynamics of structural transitions, folding & denaturation

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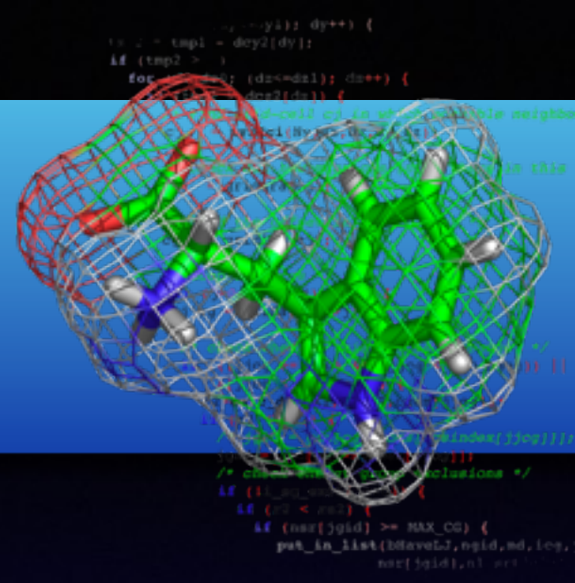
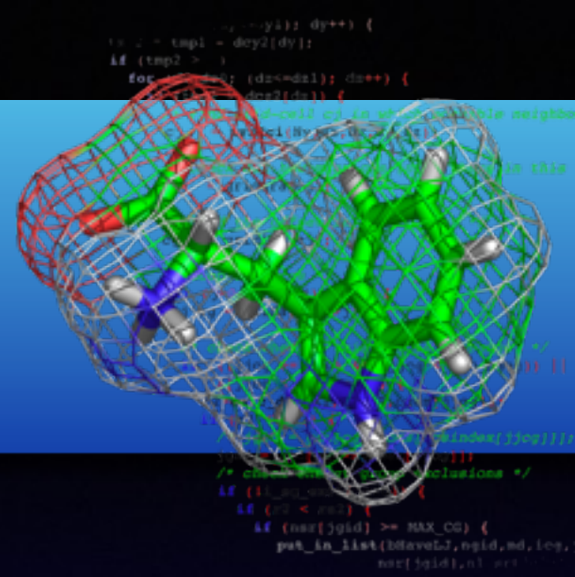
Recap



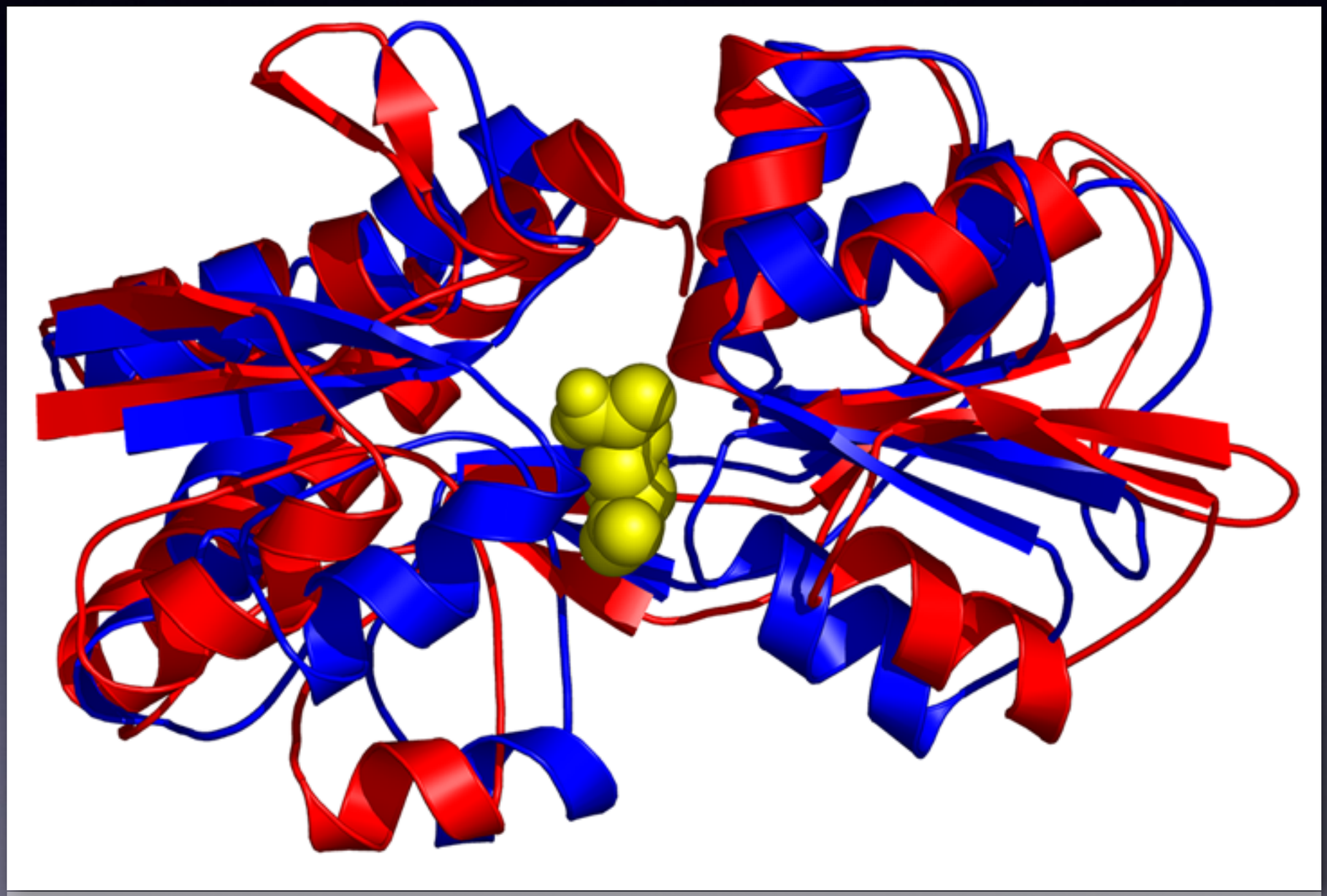
- Structural classification
- Evolution of structure and sequence
- Size of proteins, helices, sheets
- Stabilization of a few kcal/mol
- Common folds are common because they can accommodate lots of sequences
- Most sequences would not form proteins

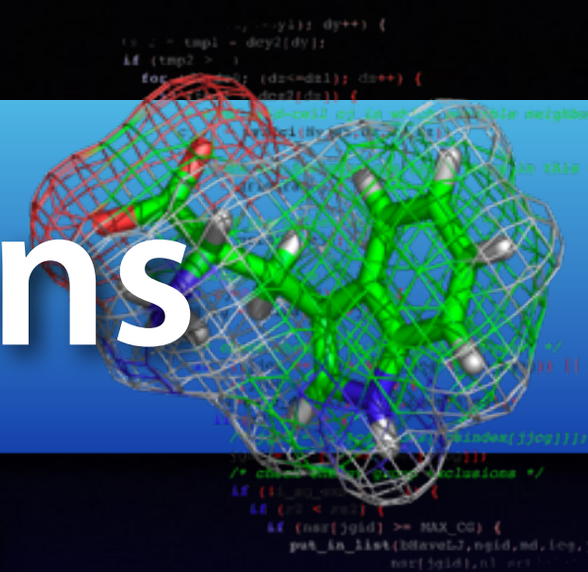
Today



- Properties of structural transitions
- How do proteins fold and unfold?
- What does it mean for stabilization?
- Atomic models/theories of folding
  - Importance of hydrophobic effect
- Molten globule
- Side-chain packing
- Energy gap stabilization

Structural transitions



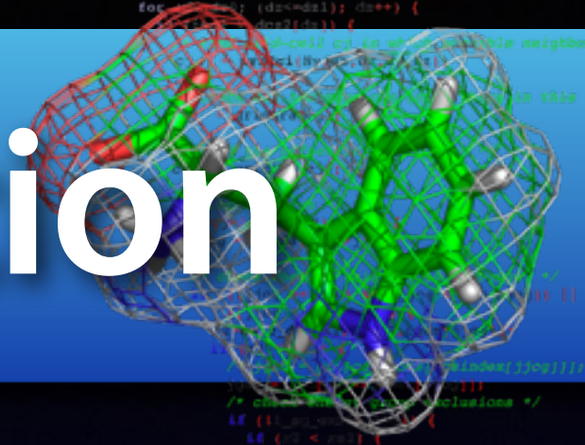


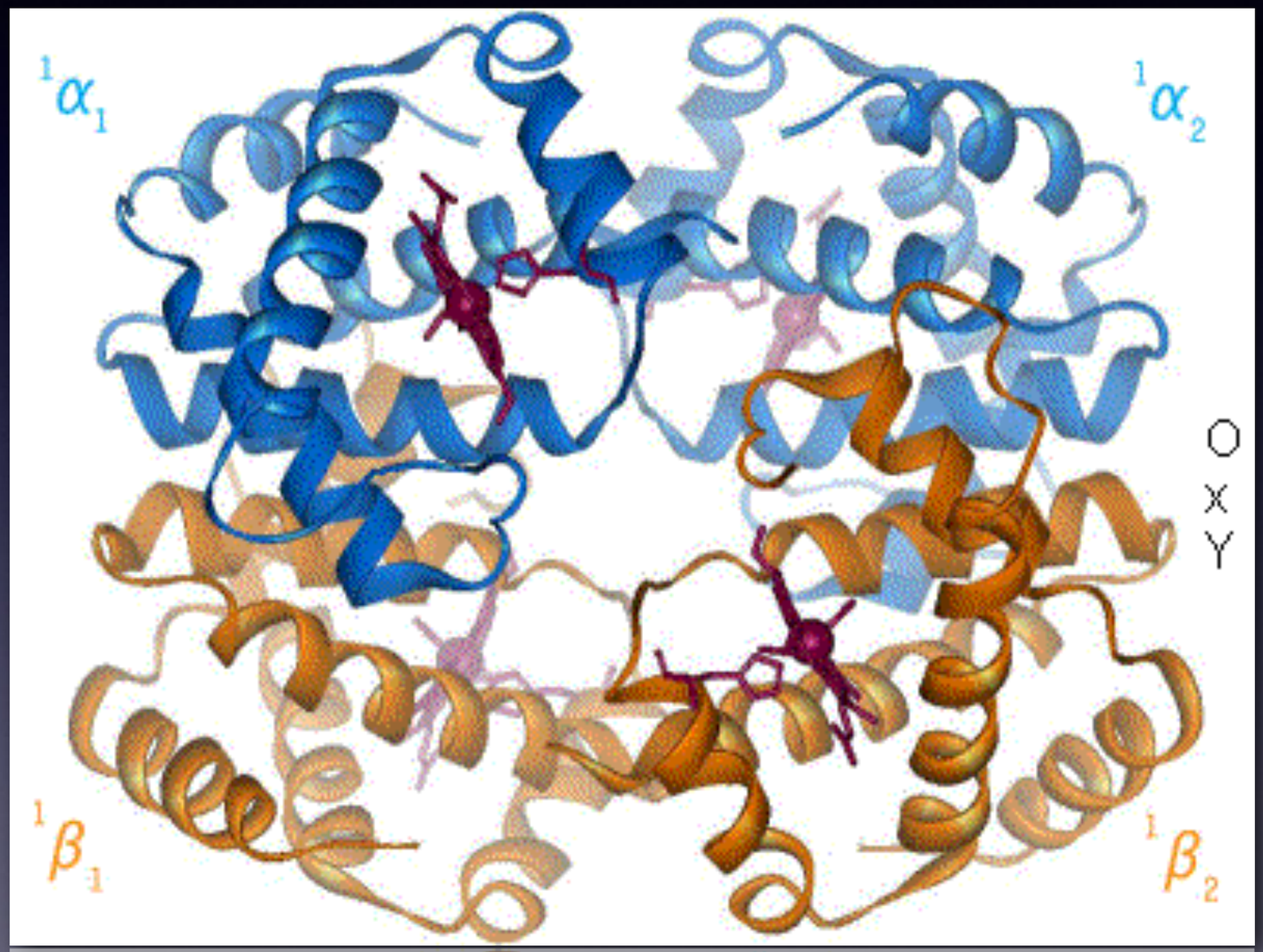
Selected Fit

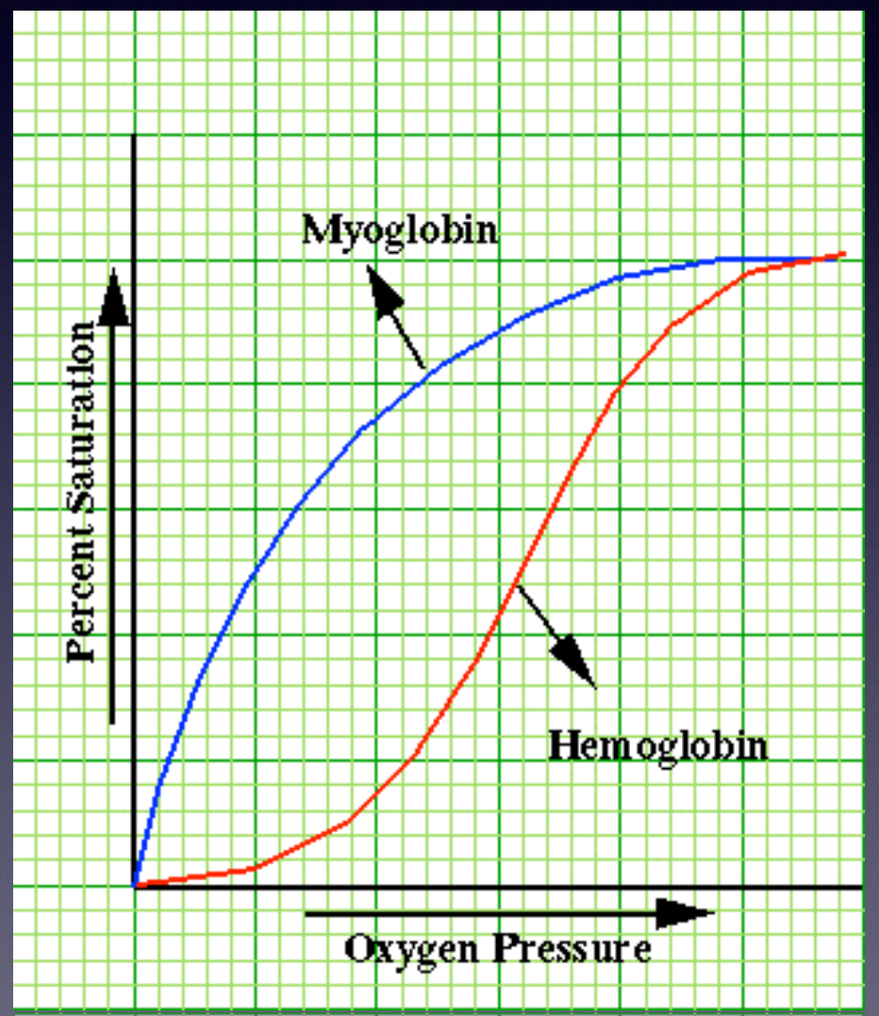
~6Å motion

Fully reversible

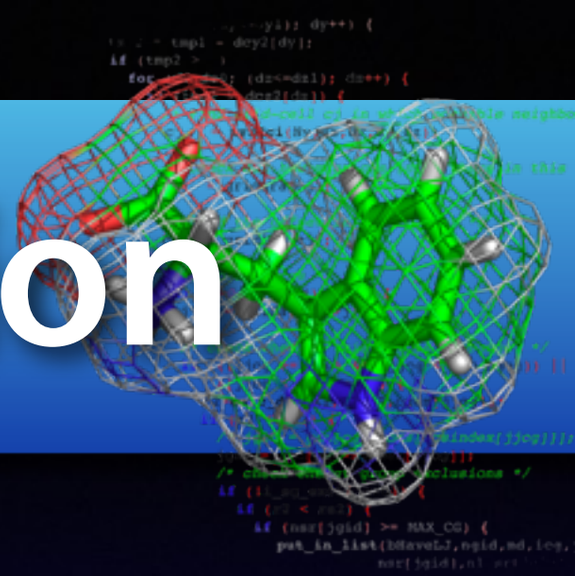
Hemoglobin transition



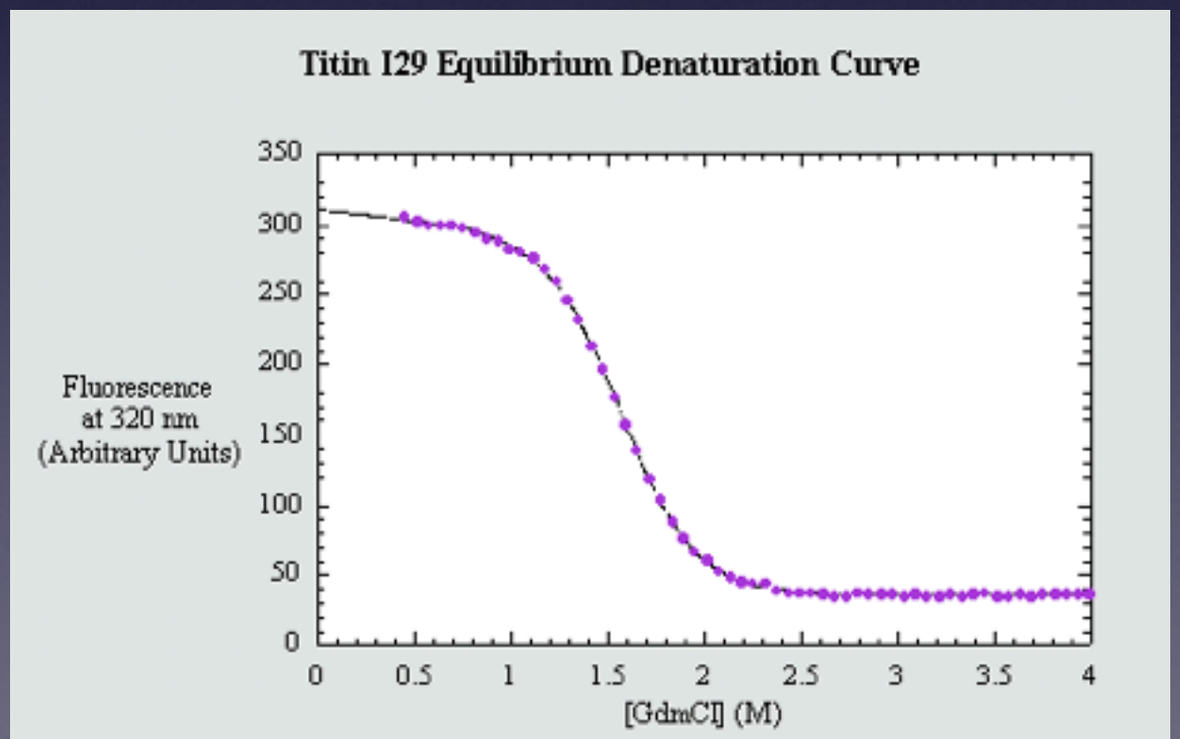




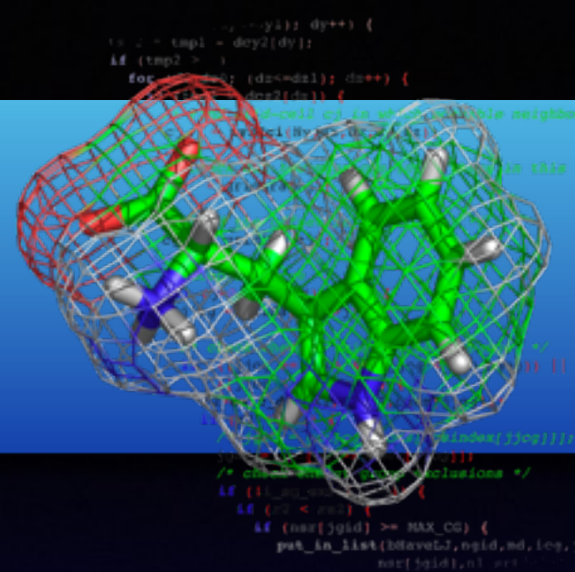
Folding / denaturation



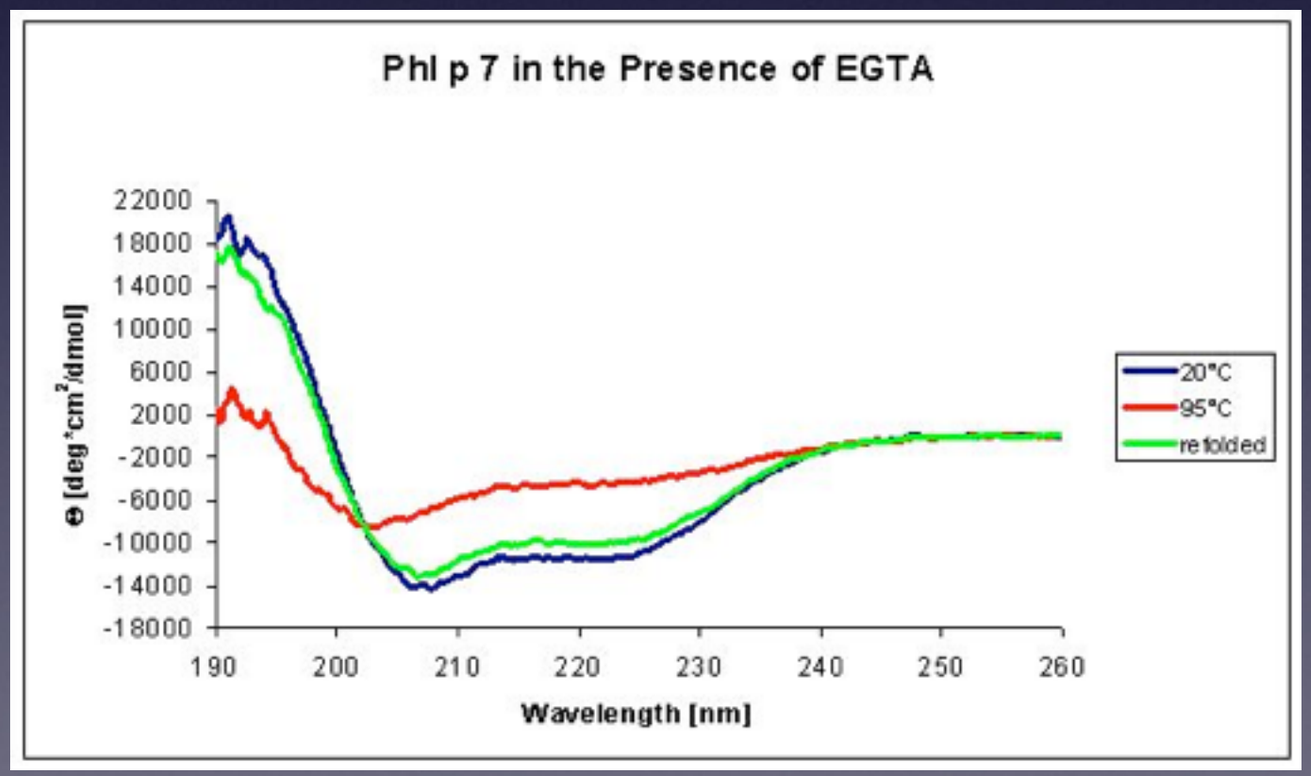
- Thermodynamic stability
- Separate issue: kinetic properties
- S-curves for observables: abrupt change
- Cooperative transition
- Salt concentration
  - Urea, GuHCl
- pH
- Temperature



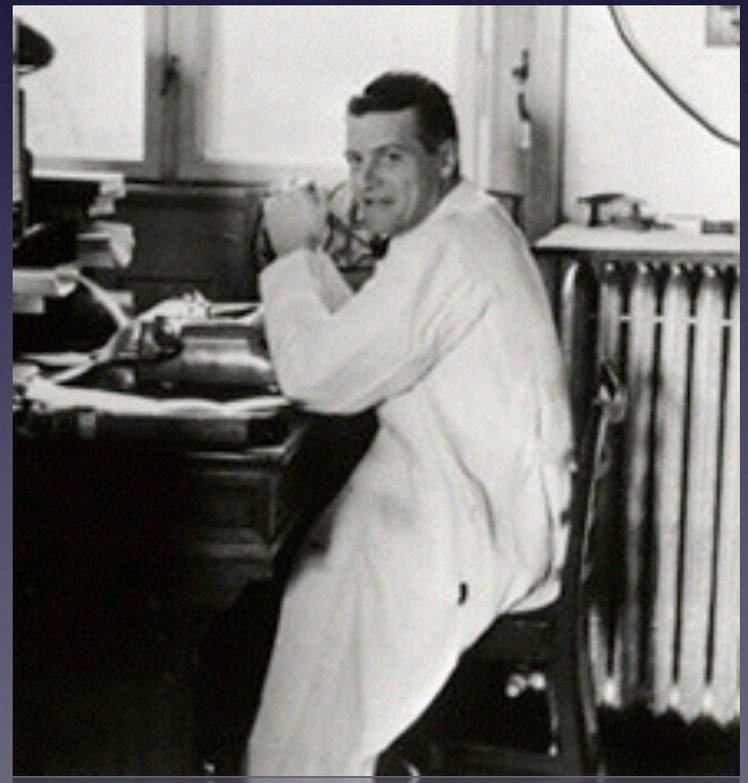
Refolding



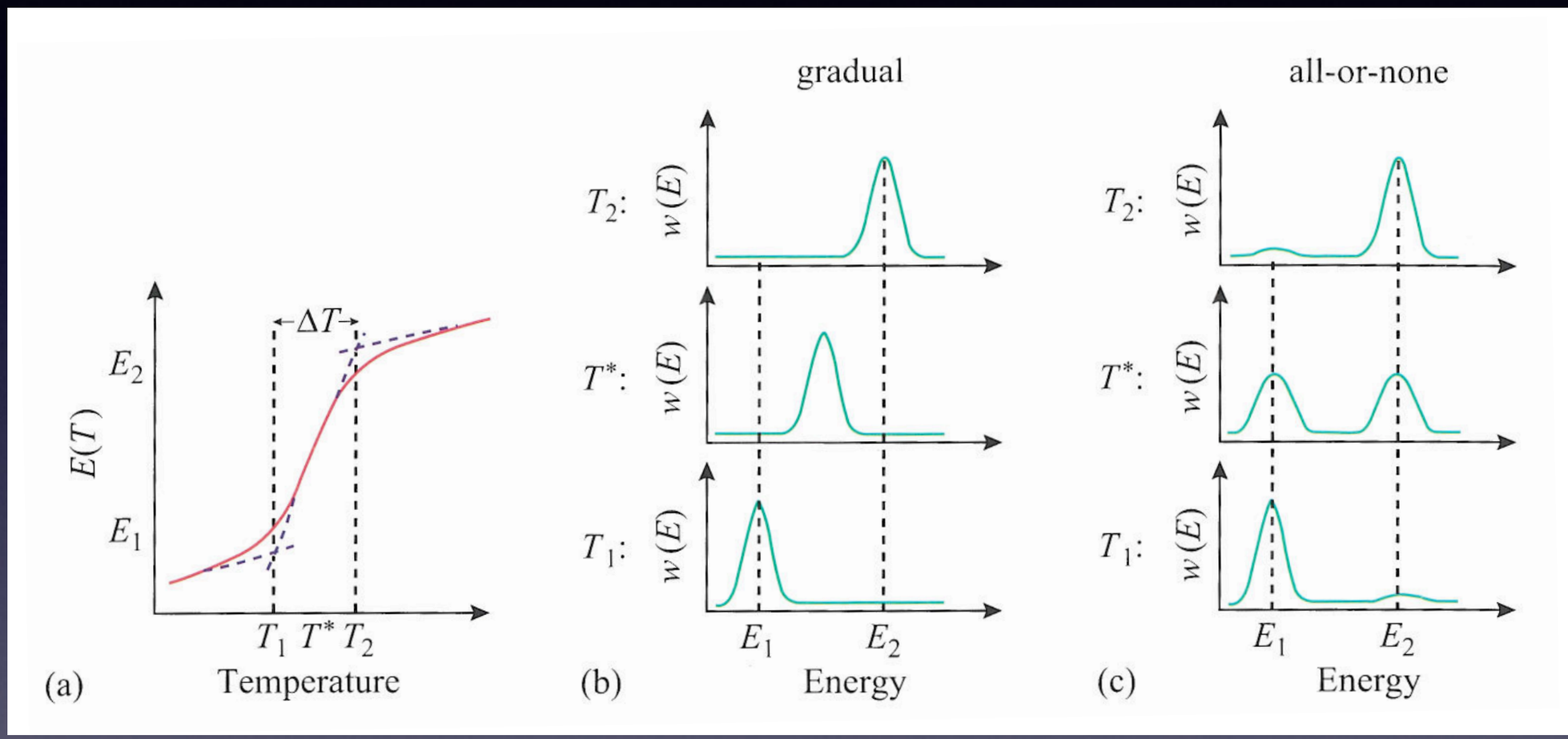
- Christian Anfinsen, 1967
  "Reductive Cleavage of Disulfide Bridges in Ribonuclease"
- Nobel Prize 1969



CD spectroscopy example



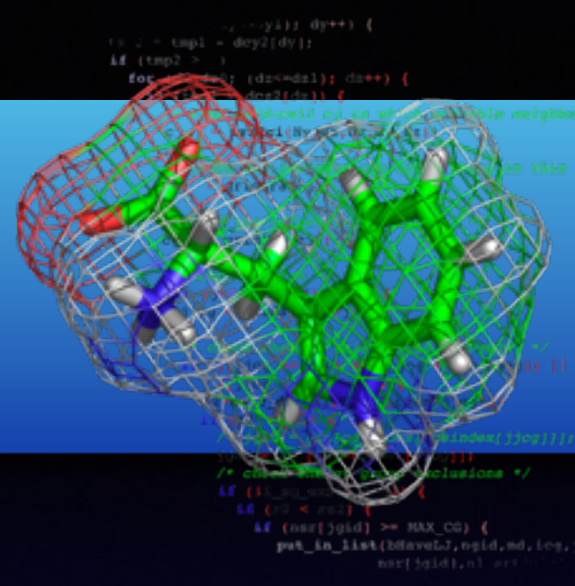
Gradual vs. all-or-none



To what extent do semi-folded states exist?

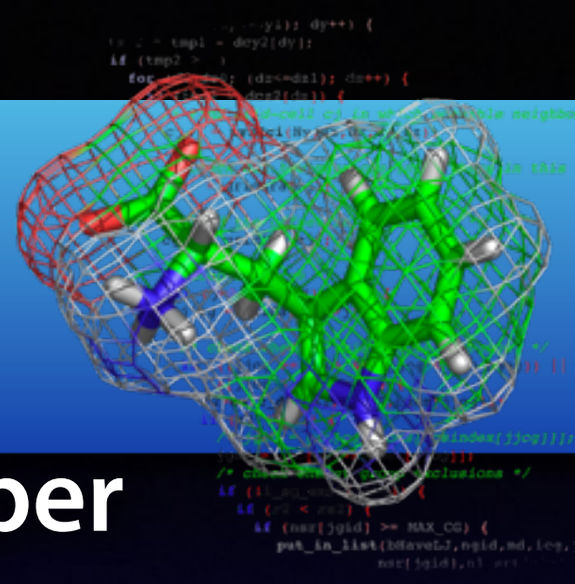
Not obvious from experiment!

Calorimetry



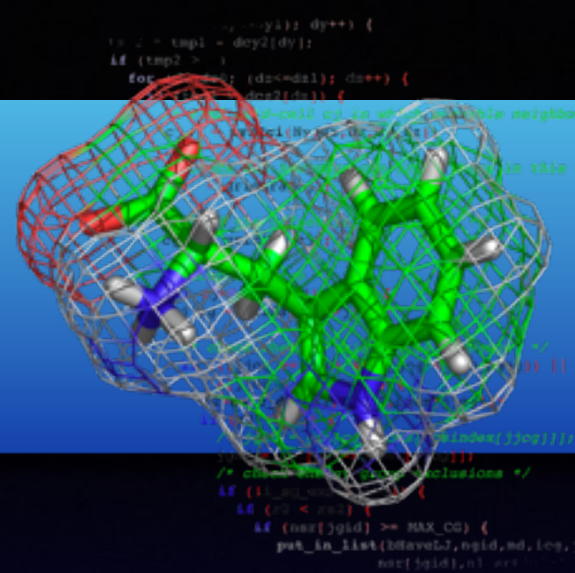
- Measurements of heat capacity per protein
- Does an abrupt change imply cooperativity of the transition?
- No, just that it happens fast!

van't Hoff criterion



- What is the specific heat change "per melting unit" upon denaturation?
- Compare to specific heat per molecule (easy to calculate from concentration)
  - if melting unit = full protein -> all-or-none
  - if melting unit < full protein -> unfolds in smaller parts
  - if melting unit > full protein -> aggregate

Fold fraction

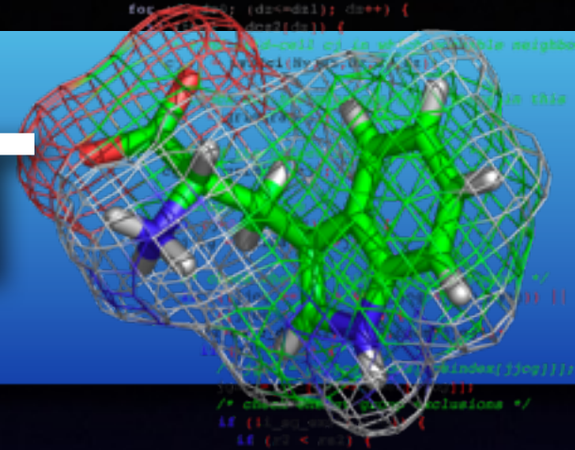


- Native: Energy E, entropy S
- Molten: Energy E′, entropy S′
- Free energy G=E-TS and G′=E′-TS′
- Assume Boltzmann state distribution

$$P_{\text{molten}} = \frac{\exp\left[-(E' - TS')/kT\right]}{\exp\left[-(E - TS)/kT\right] + \exp\left[-(E' - TS')/kT\right]}$$

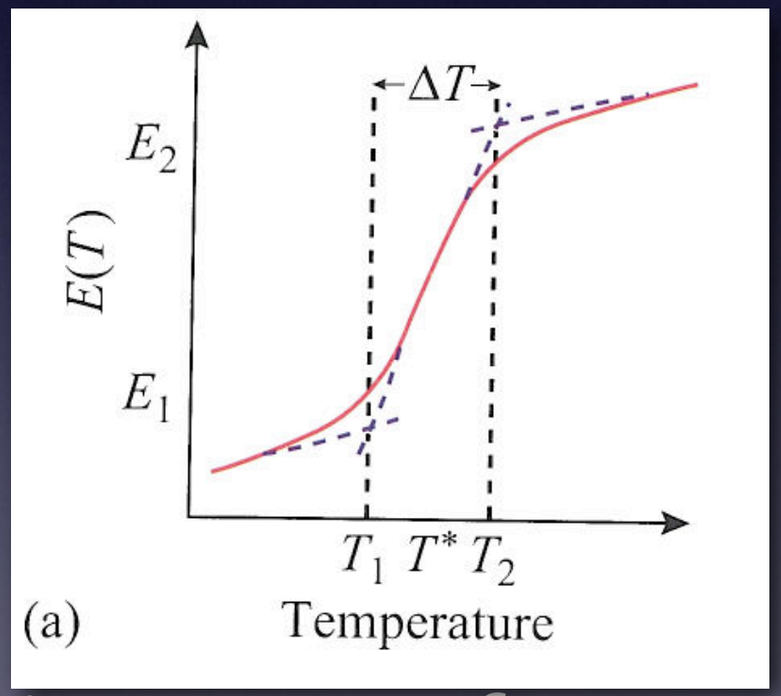
$$= \frac{1}{1 + \exp\left[-(\Delta E - T\Delta S)/kT\right]}$$

Transition width ΔT



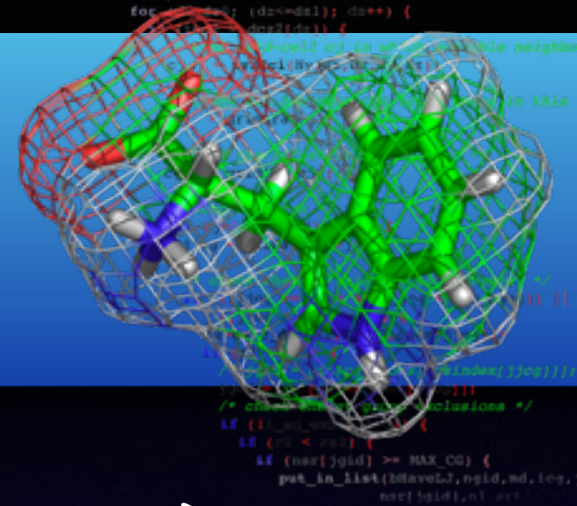
- Fold fraction P and the temperature width ΔT can be obtained from calorimetry

$$\frac{d}{dT}P_{\text{molten}} \approx \frac{\Delta P_{\text{molten}}}{\Delta T} \approx \frac{1}{\Delta T}$$



- Can we calculate the derivative in terms of energy E from the P_{molten} expression?

Specific heat

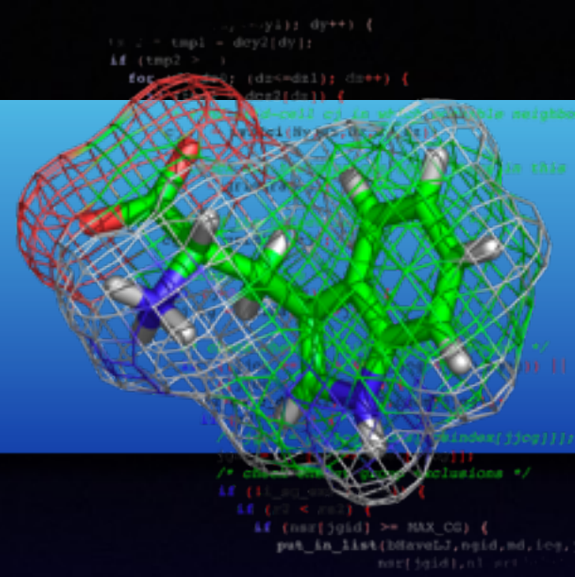


$$\frac{d}{dT}P_{\text{molten}} = \frac{d}{dT}\left\{\frac{1}{1+\exp\left[(\Delta E - T\Delta S)/kT\right]}\right\}$$

$$= -\left\{\frac{1}{1+\exp\left[(\Delta E - T\Delta S)/kT\right]}\right\}^2 \left\{\exp\left[(\Delta E - T\Delta S)/kT\right]\right\} \times \left(-\frac{\Delta E}{kT^2}\right)$$

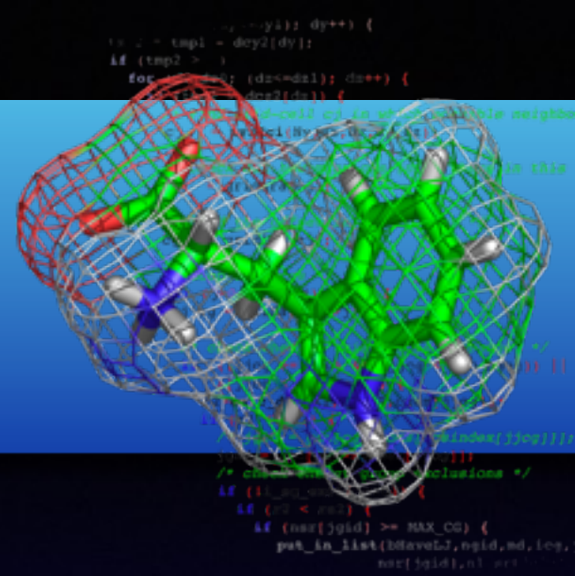
$$= P_{\text{molten}}(1 - P_{\text{molten}})(\Delta E/kT^2)$$

Specific heat



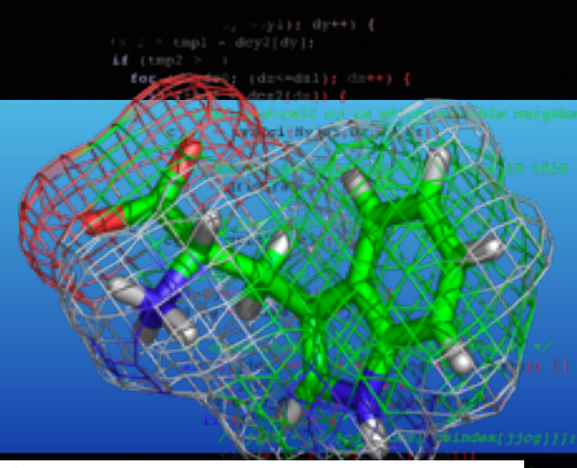
- At transition temperature, $P_{molten}=0.5$
- If $\Delta E/kT >> 1$, this is close to middle point
- $dP/dT = 0.25\ \Delta E/kT_0^2$
- Combine with $dP/dT=1/\Delta T$:
- $\Delta E=4kT_0^2/\Delta T$ for a “melting unit”
- $\Delta E=\Delta H/N$ for entire protein molecules

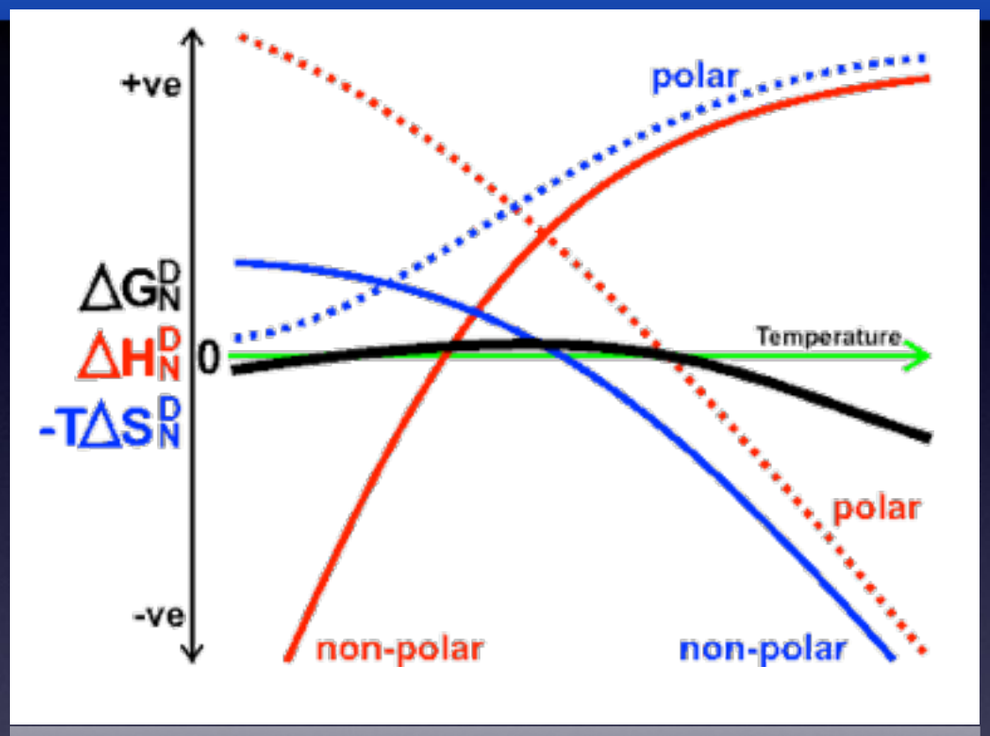
Denaturation

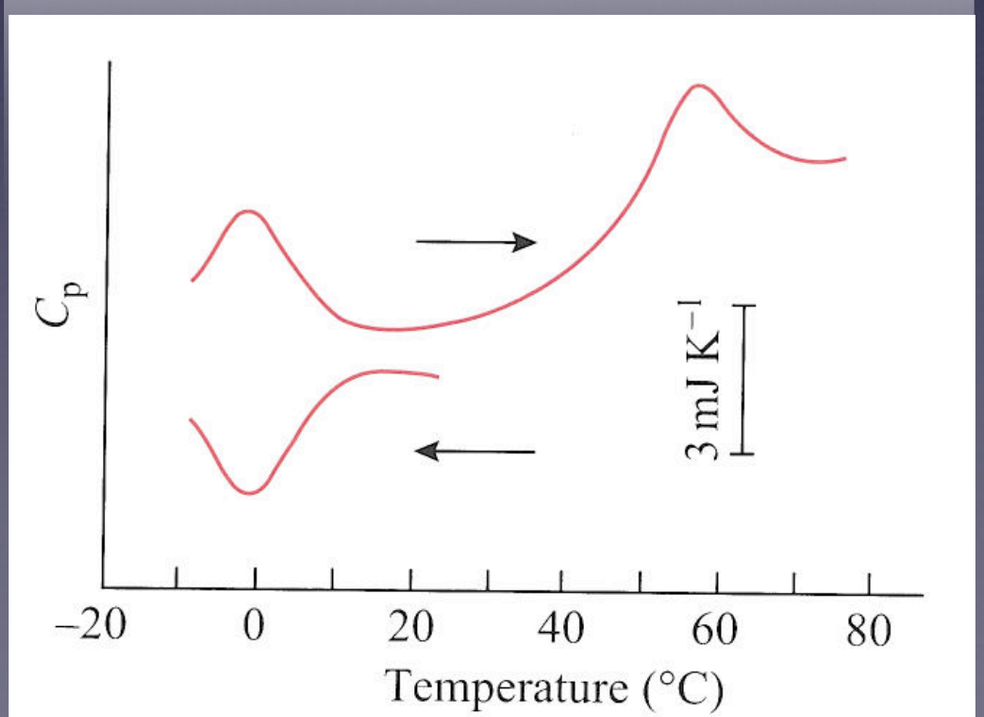


- Why is the protein unfolding?
- $\Delta G = \Delta E - T\Delta S$
- Explained by hydrophobic effect
  - ΔE increases with temperature
  - ΔS positive for unfolding
- But S will drop with temperature (less mobile solvent molecules)

Cold denaturation







Protein Cold Denaturation as Seen From the Solvent

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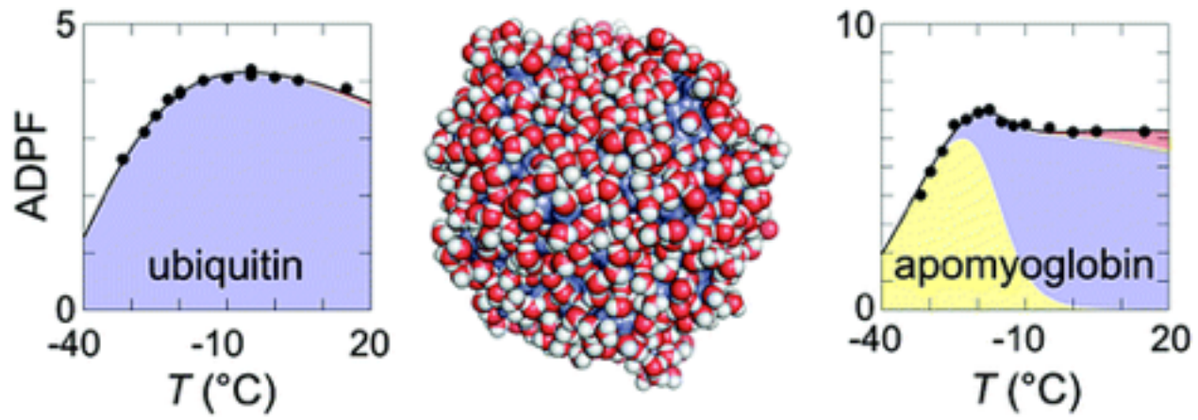
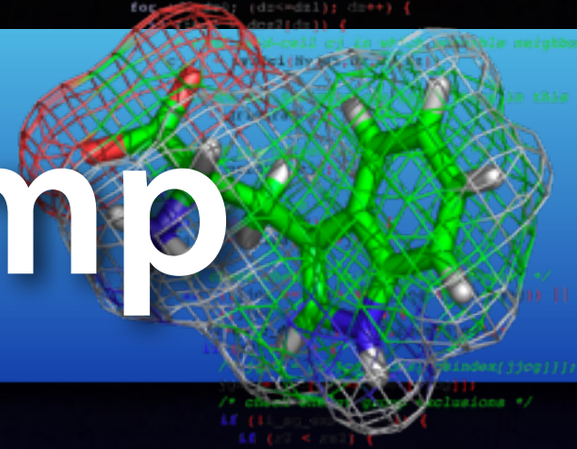
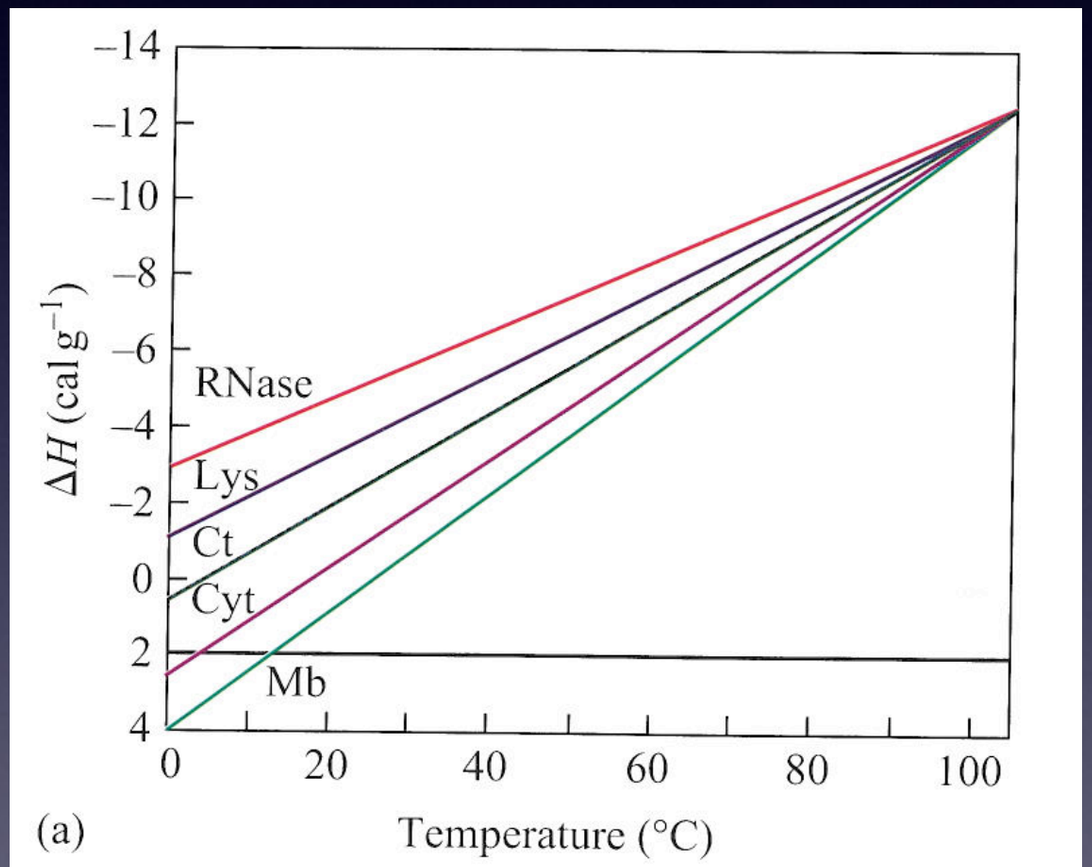
Abstract

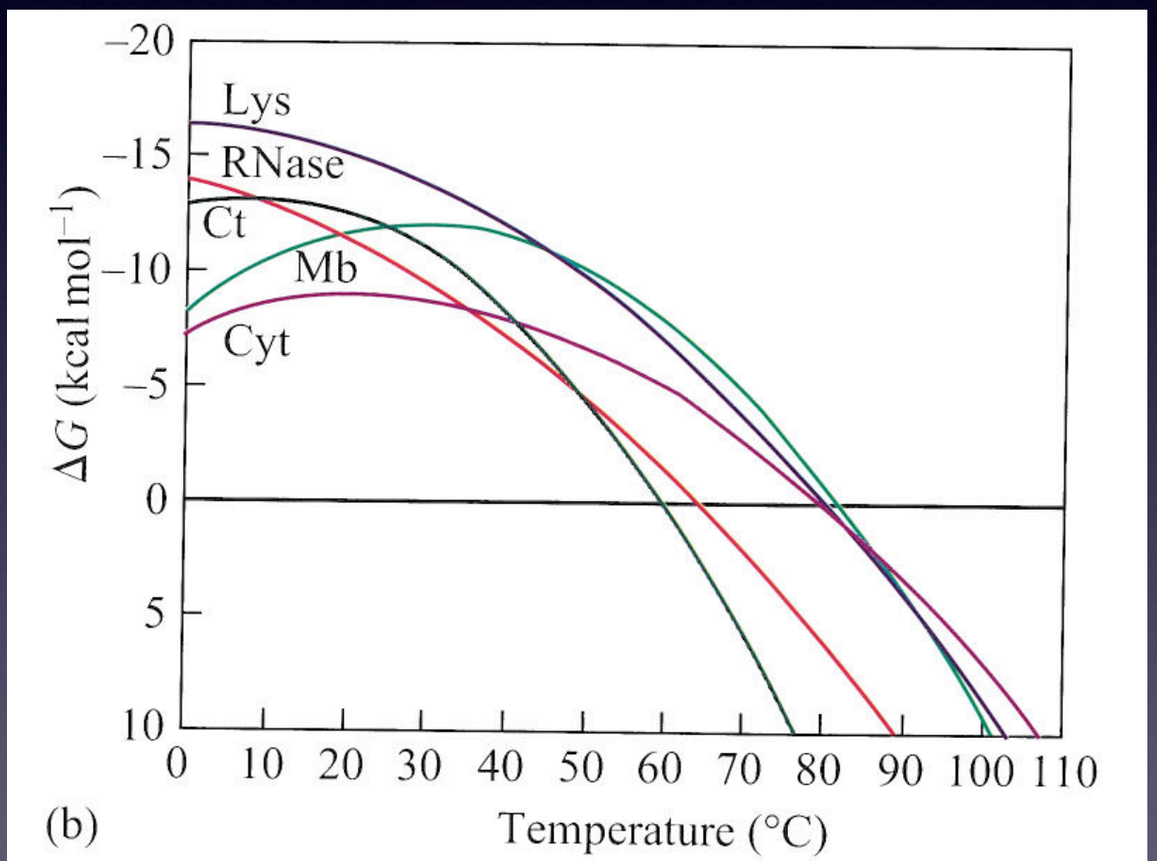


Unlike most ordered molecular systems, globular proteins exhibit a temperature of maximum stability, implying that the structure can be disrupted by cooling. This cold denaturation phenomenon is usually linked to the temperature-dependent hydrophobic driving force for protein folding. Yet, despite the key role played by protein–water interactions, hydration changes during cold denaturation have not been investigated

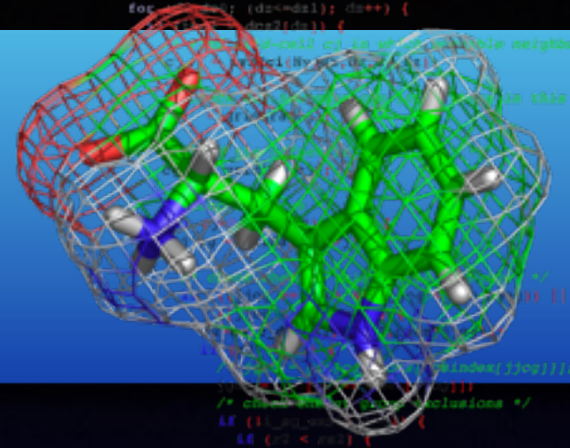
Specific heat vs. temp

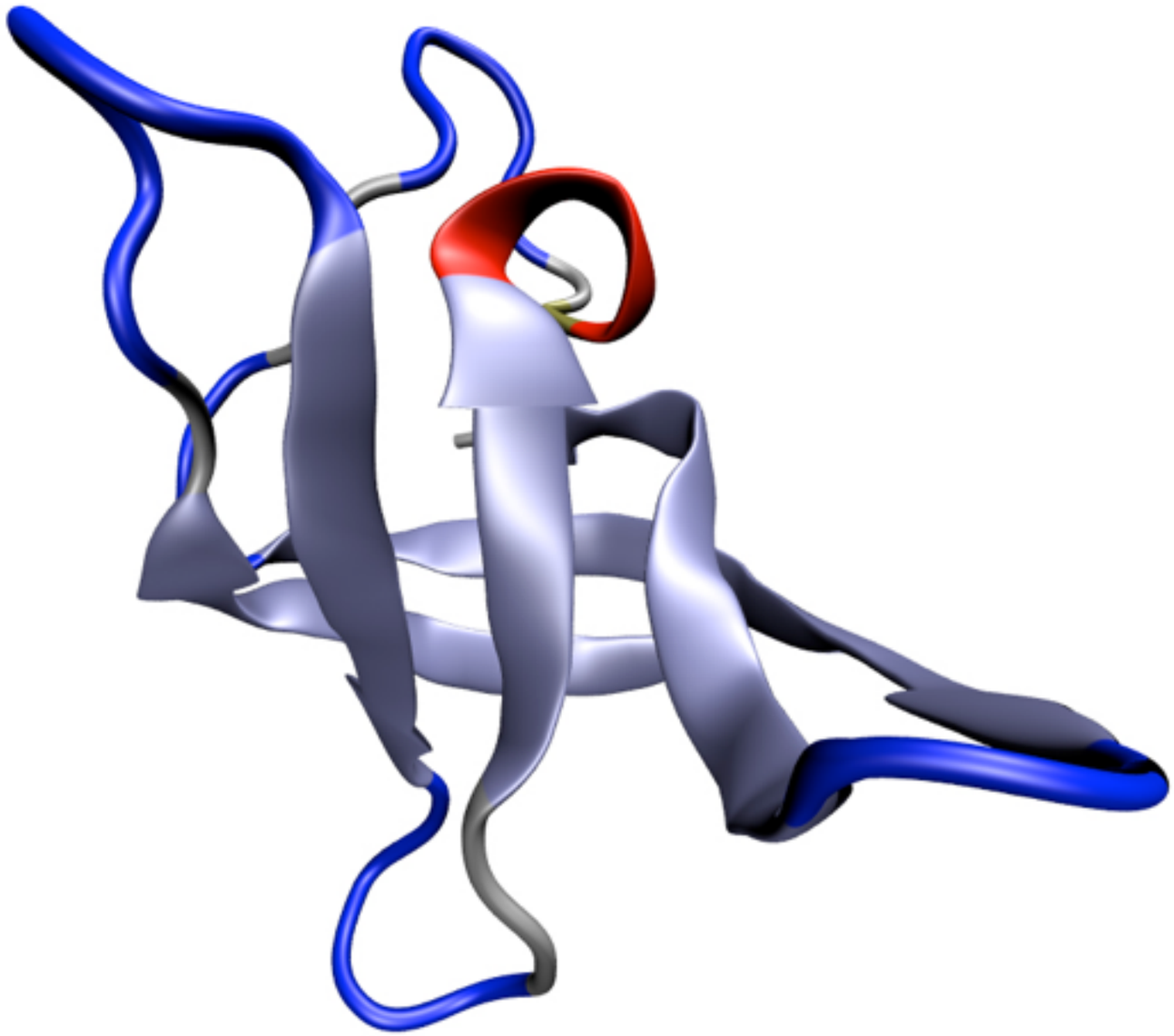






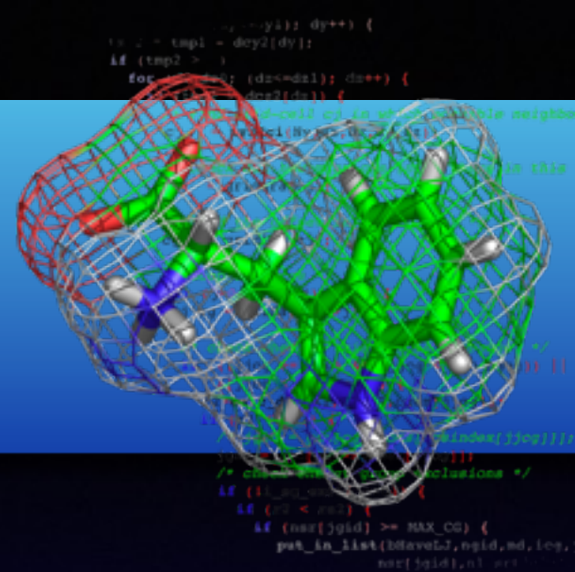
Cold shock proteins!





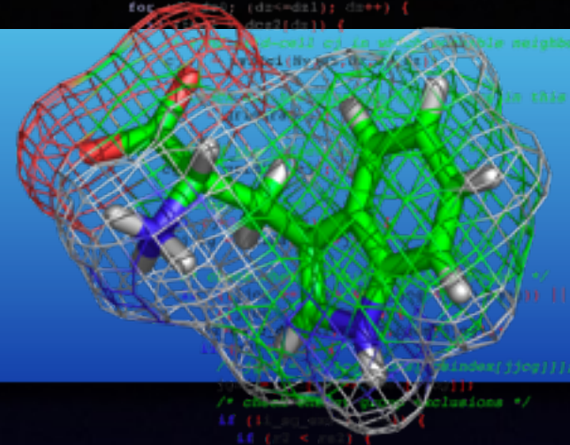
Structures evolved to survive low temperatures

Denatured state

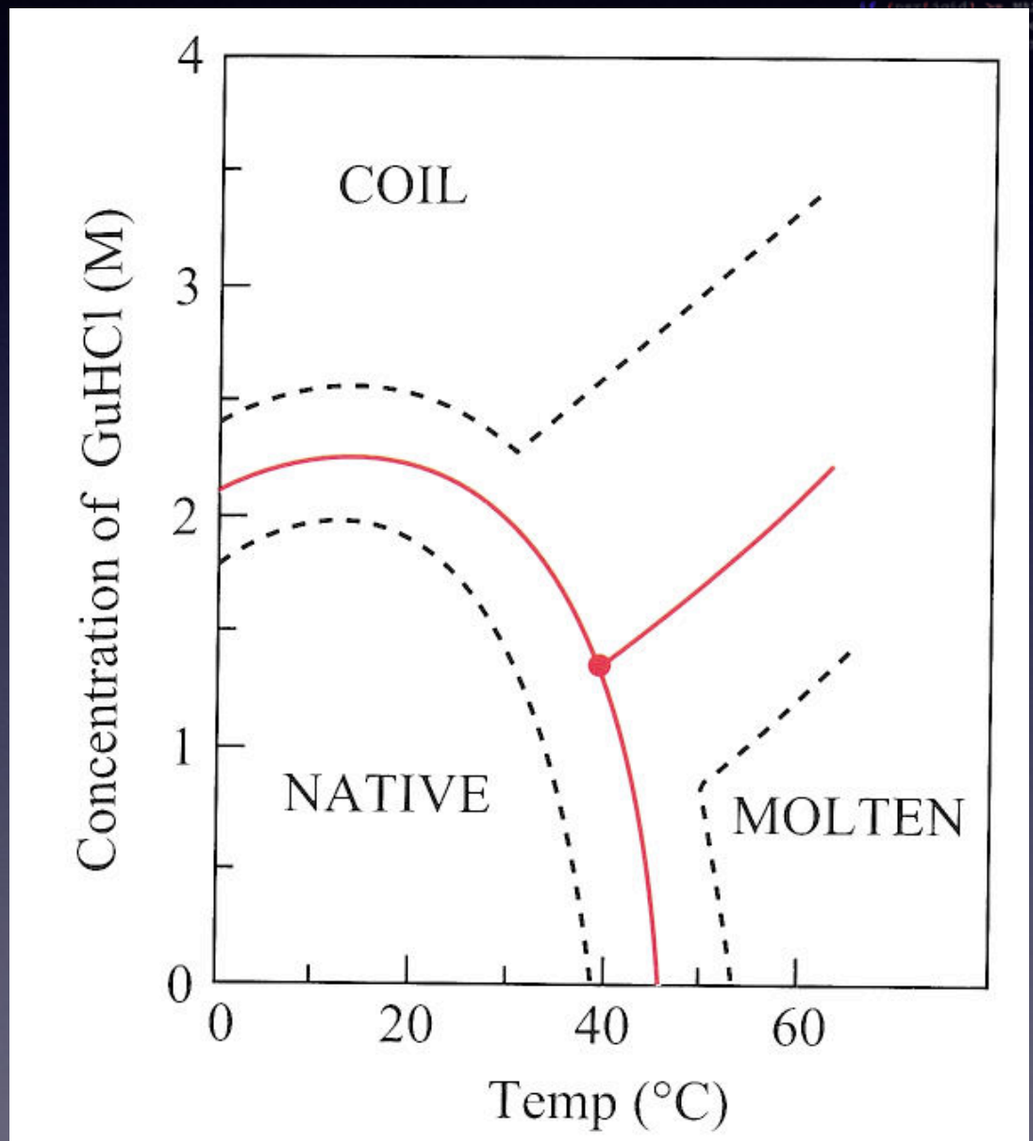


- What does the denatured state look like?
- Extended coil?
- Surprisingly, experiments indicate they can be quite compact - almost like a native protein
- Secondary structure still present
- Complete unfolding requires strong denaturants like GuHCl in high conc.

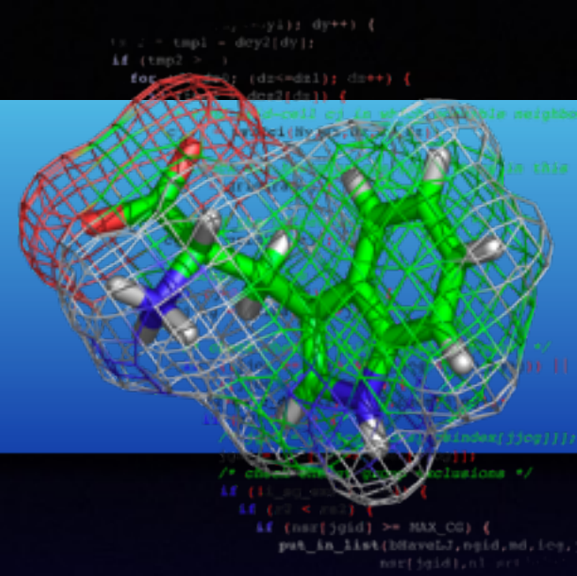
Multiple states



Proteins seem to have both a non-native but still compact “molten globule” state, as well as completely unfolded coils!

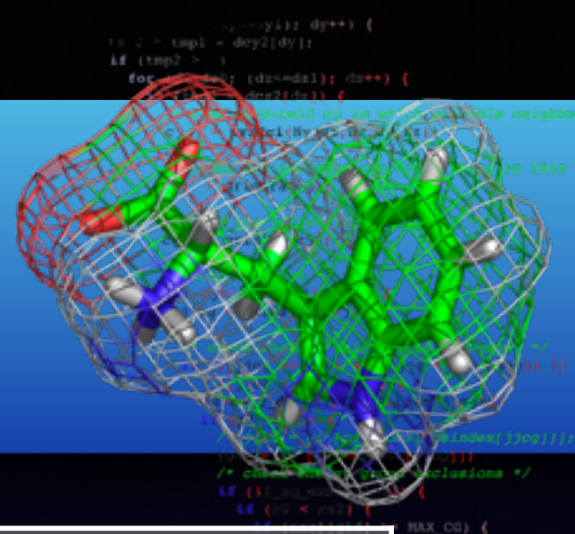


Molten globule



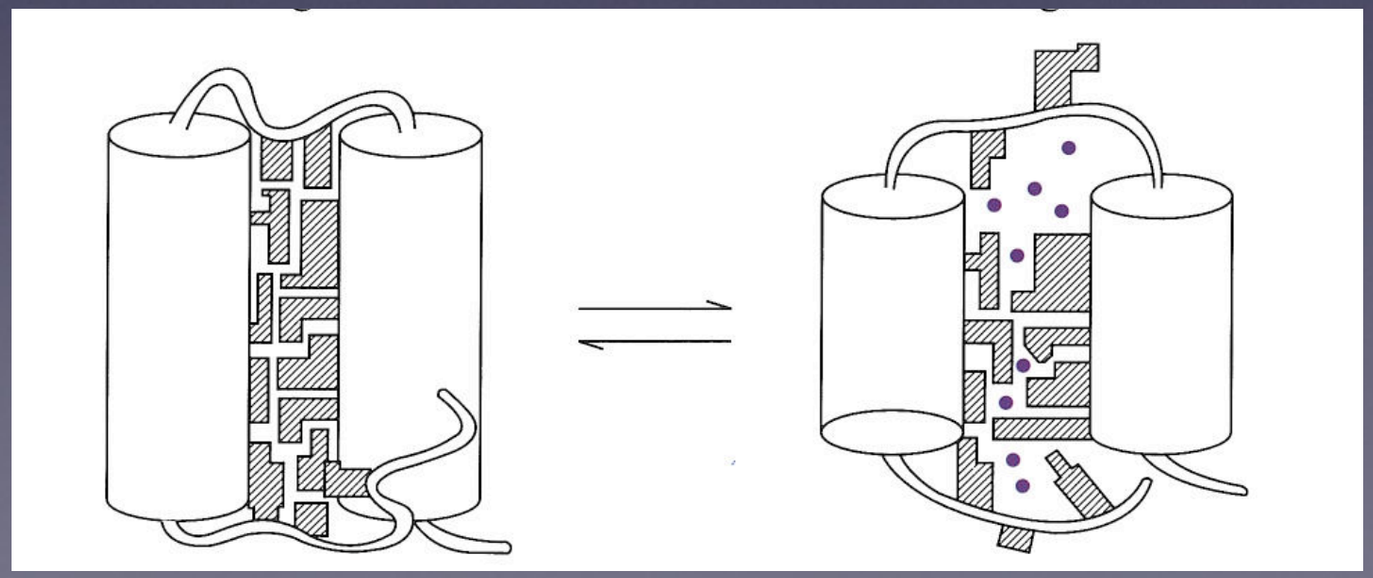
- What is the molten globule?
- Main chain ordering (trace & structure)
- Hydrophobic core size & density
- Volume of the protein molecule (radius)
- Transition native-globule is well defined, yet the structures are quite similar
- Transition globule-coil less sharp

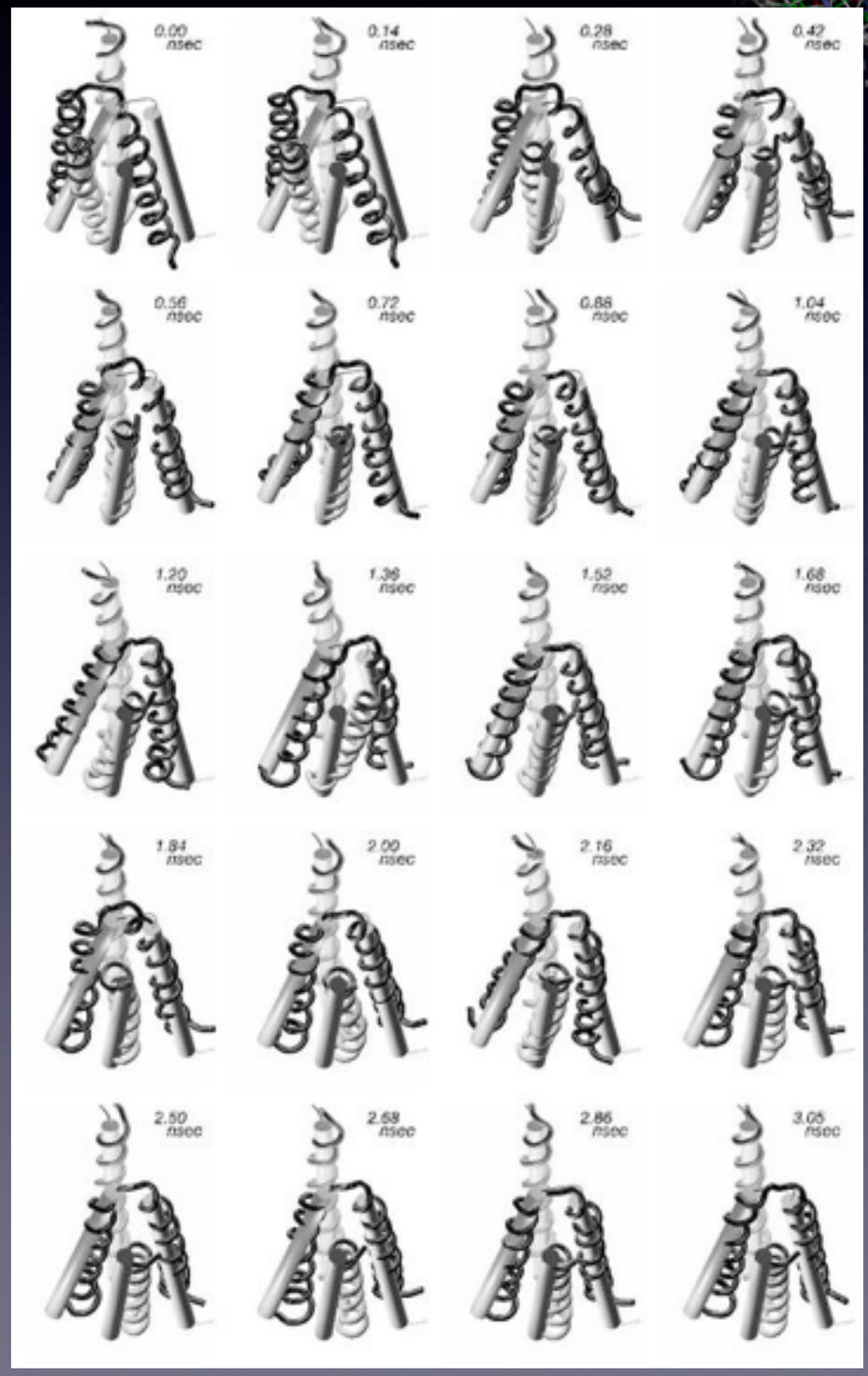
The molten globule



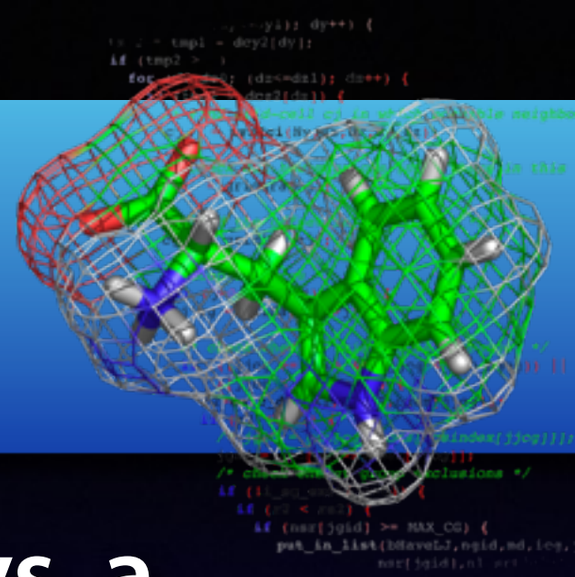
Like native	Like unfolded
Compact, hydrophobic core	Not rigid structure
Secondary structure	No second melting transition to coil
Partial sidechain order (TRP buried)	No unique sidechain packing
Partial S-S bond formation	Not functional

Molten globule

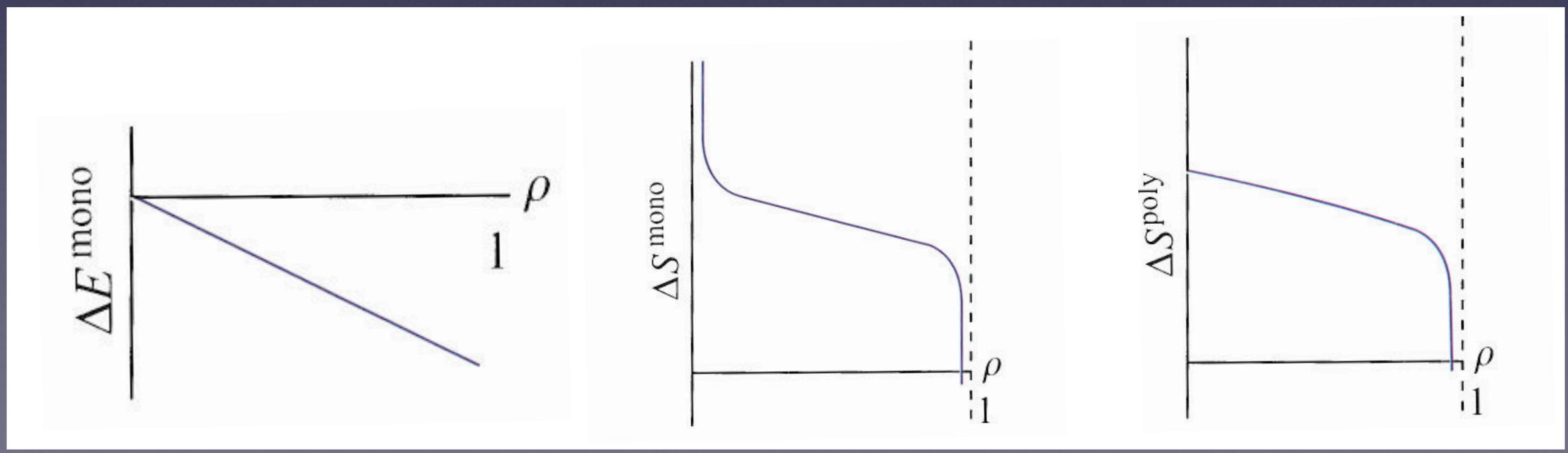




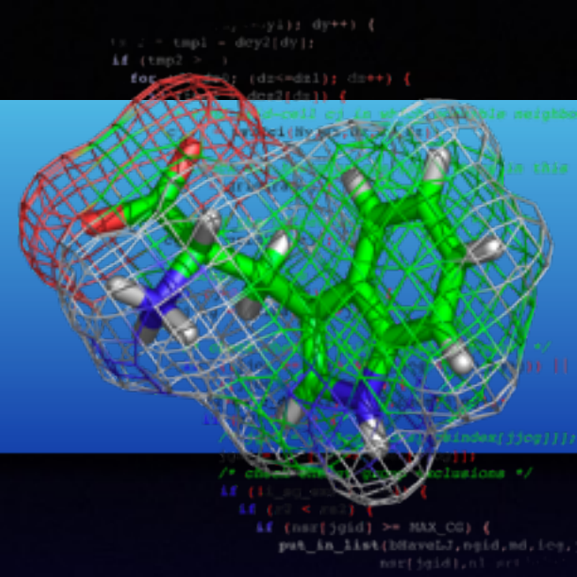
Homopolymers



- Consider disconnected monomers vs. a simple homopolymer chain
- Energy is roughly the same
- Disconnected particles achieve very high entropy when completely separated

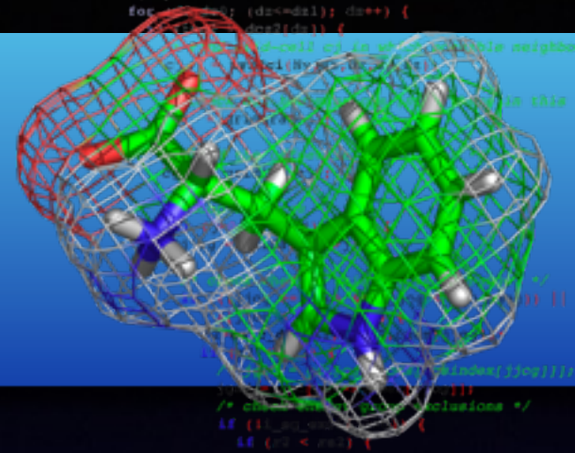


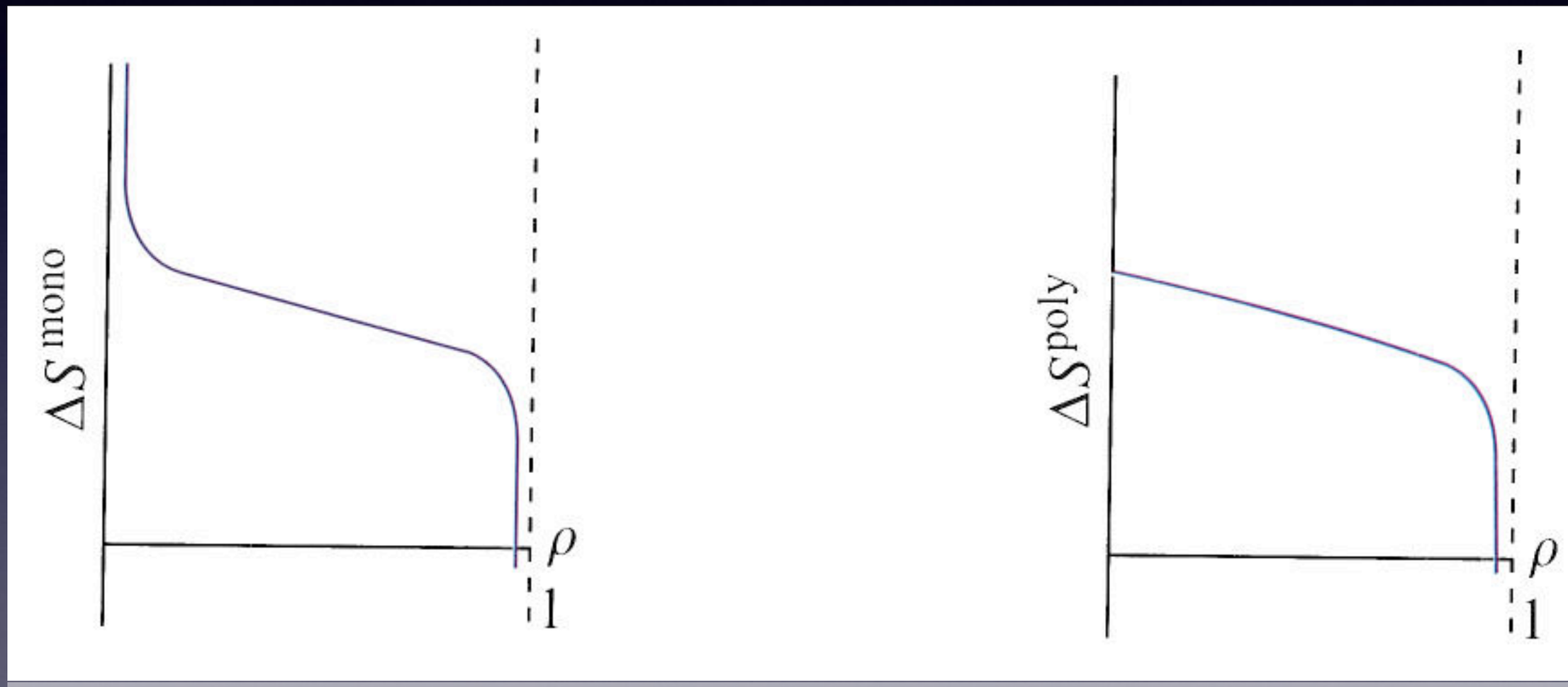
Entropy effects



- The energy per monomer is roughly the same in a cloud and chain
- How does the entropy change?
  - Accessible volume per monomer
- Cloud: $V' = (V - N\omega)/N = V/N(1-\rho) = \omega/\rho(1-\rho)$
- Chain: $V' = \Omega(1 - \rho)$
- Entropy $S = k \ln V'$

Homopolymers

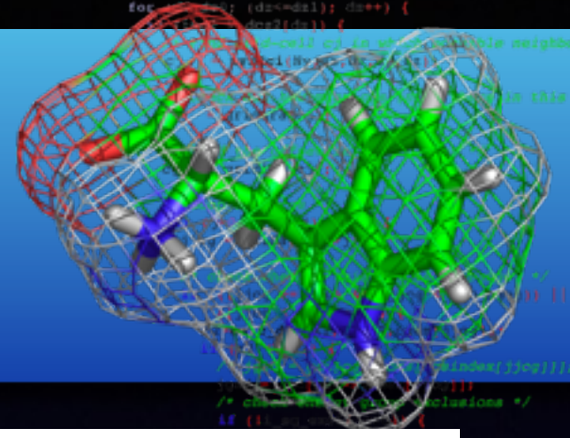


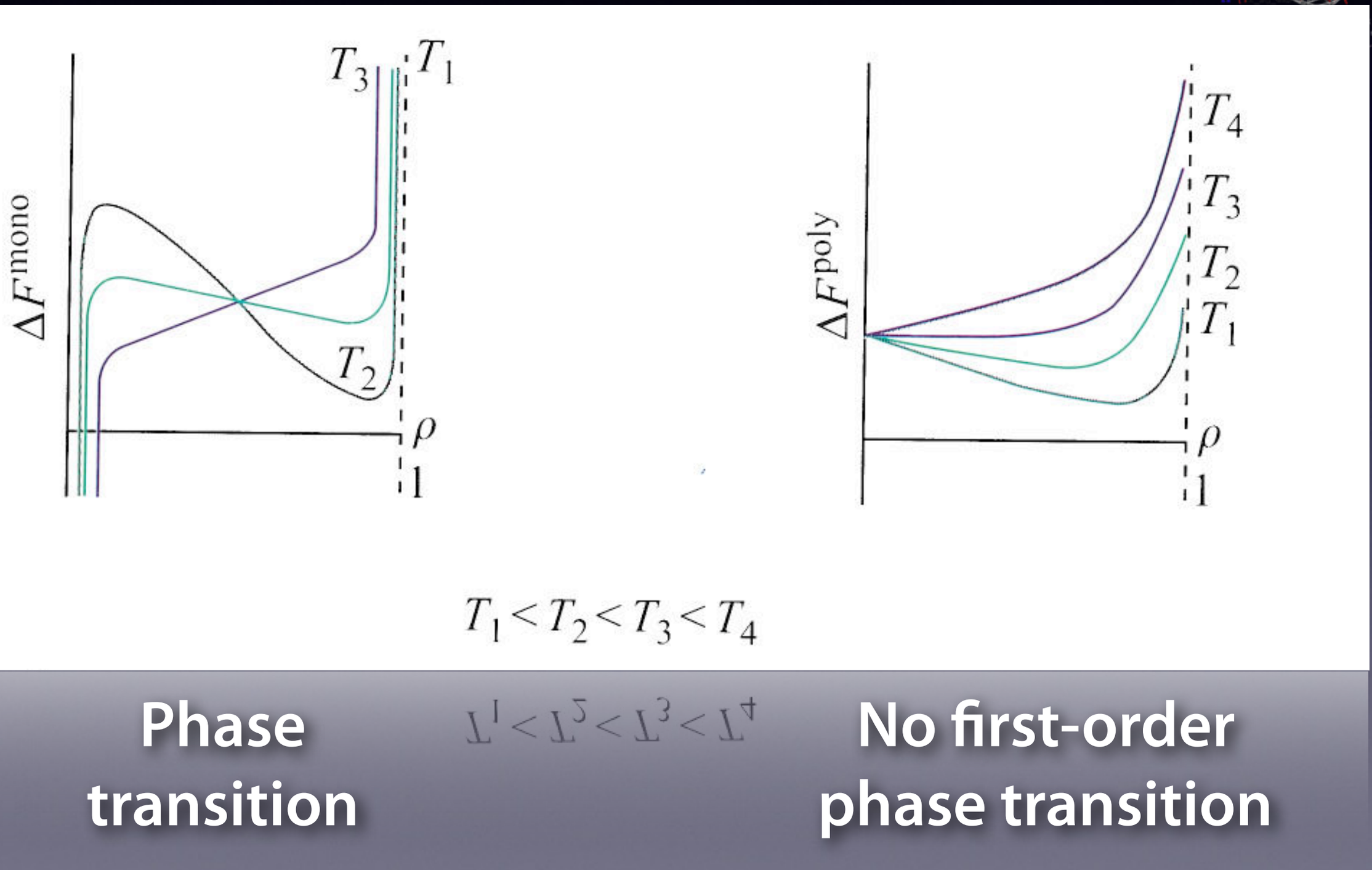


$S=k \ln \omega/\rho(1-\rho)$

$S=k \ln \Omega(1-\rho)$

Homopolymers



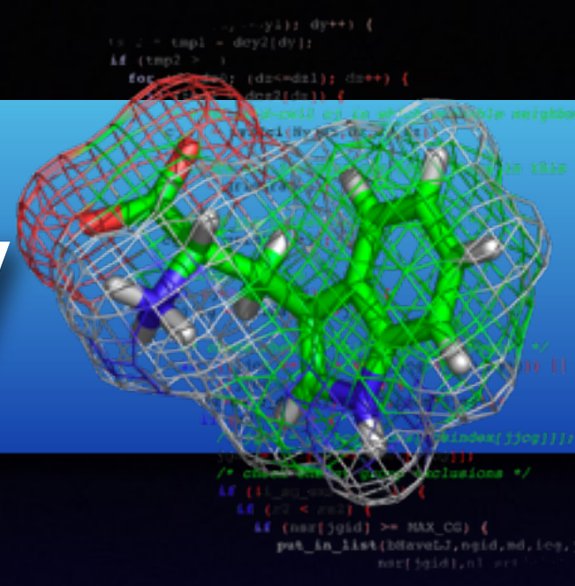


$$T_1 < T_2 < T_3 < T_4$$

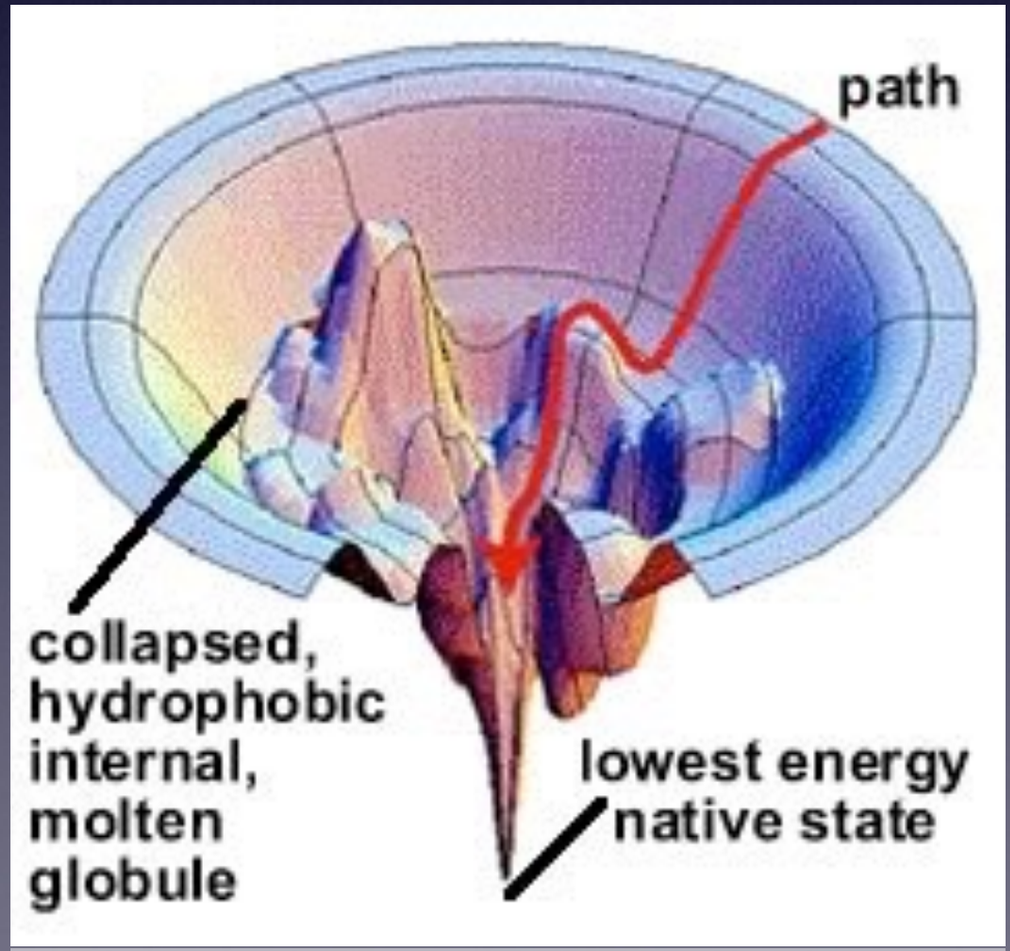
Phase transition

No first-order phase transition

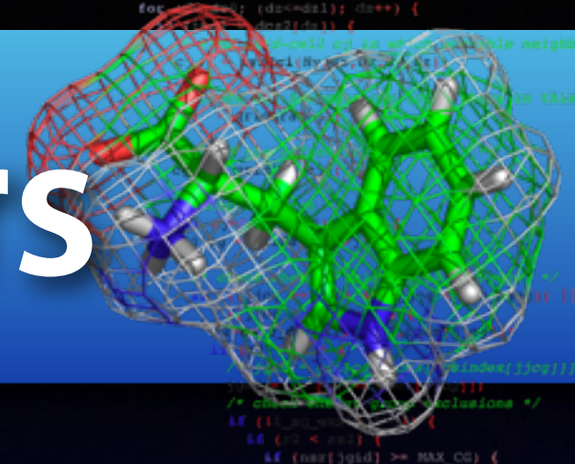
Protein free energy

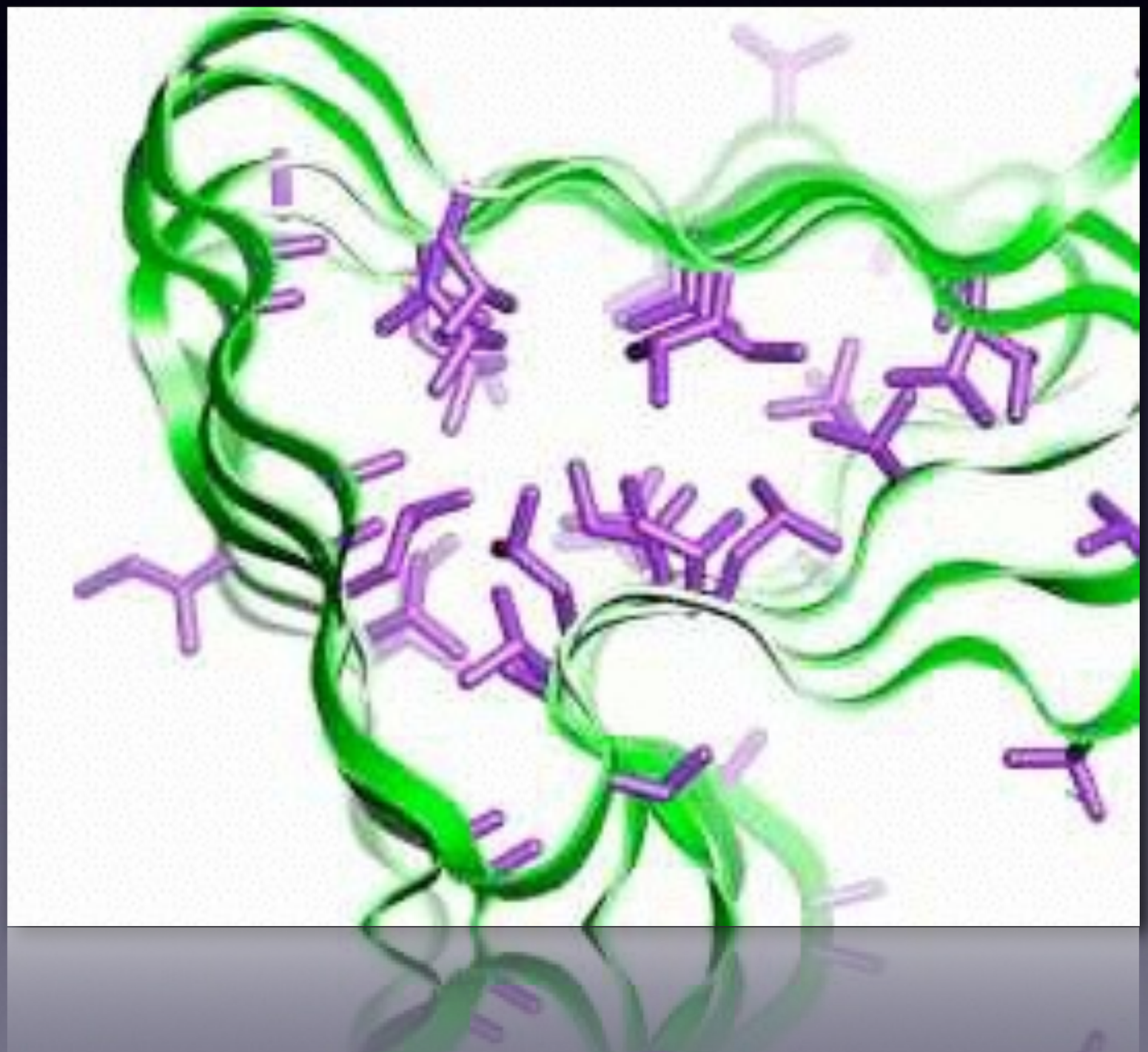


- Free energy barriers is a basic requirement of an all-or-none / first-order phase transition
- Where does it come from in proteins?



Free energy barriers

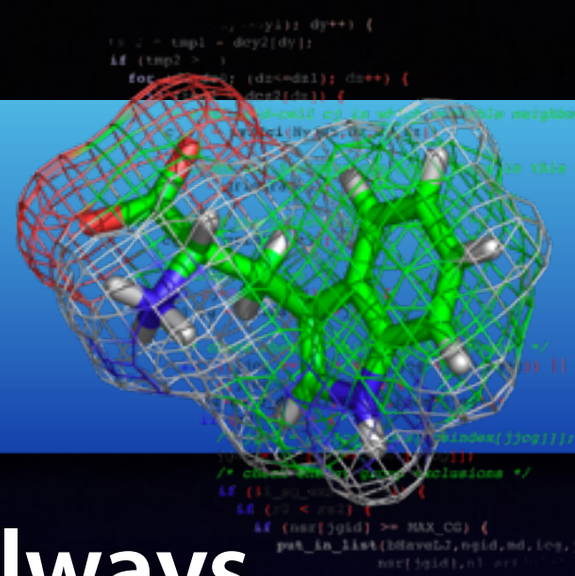




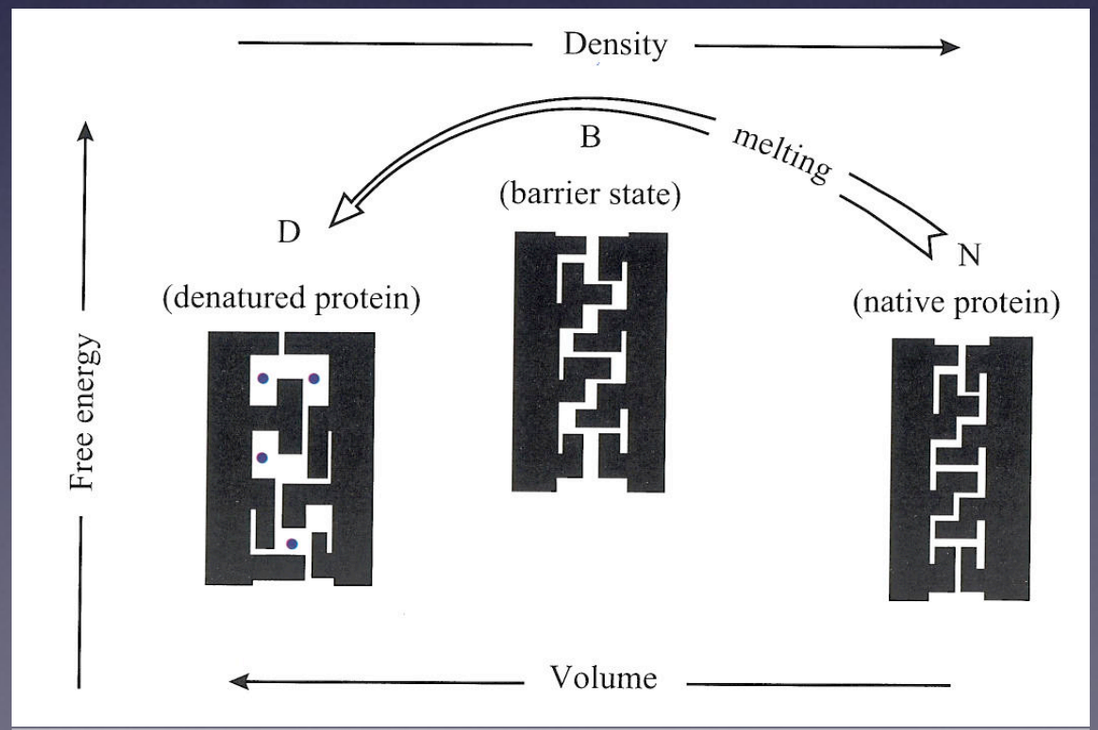
Sidechains are very well packed in native state

More like a solid than a liquid - 80% of the interior volume filled

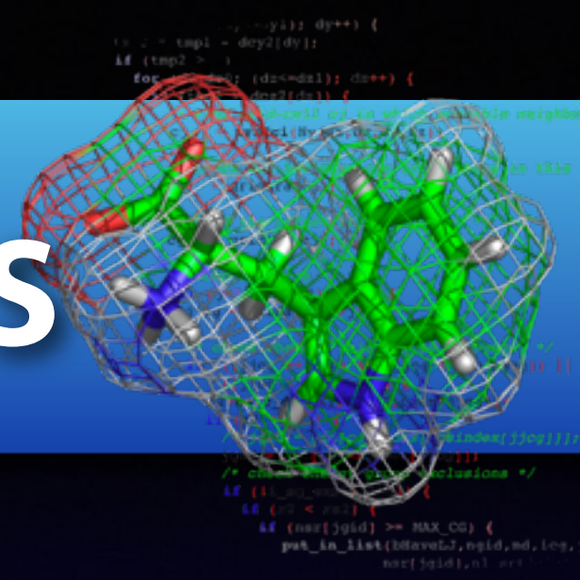
Melting dynamics



- The initial denaturation process is always unfavorable, since the protein expands and ruins sidechain packing
- Water cannot yet enter (too packed)
- Eventually water gets in, barrier is crossed



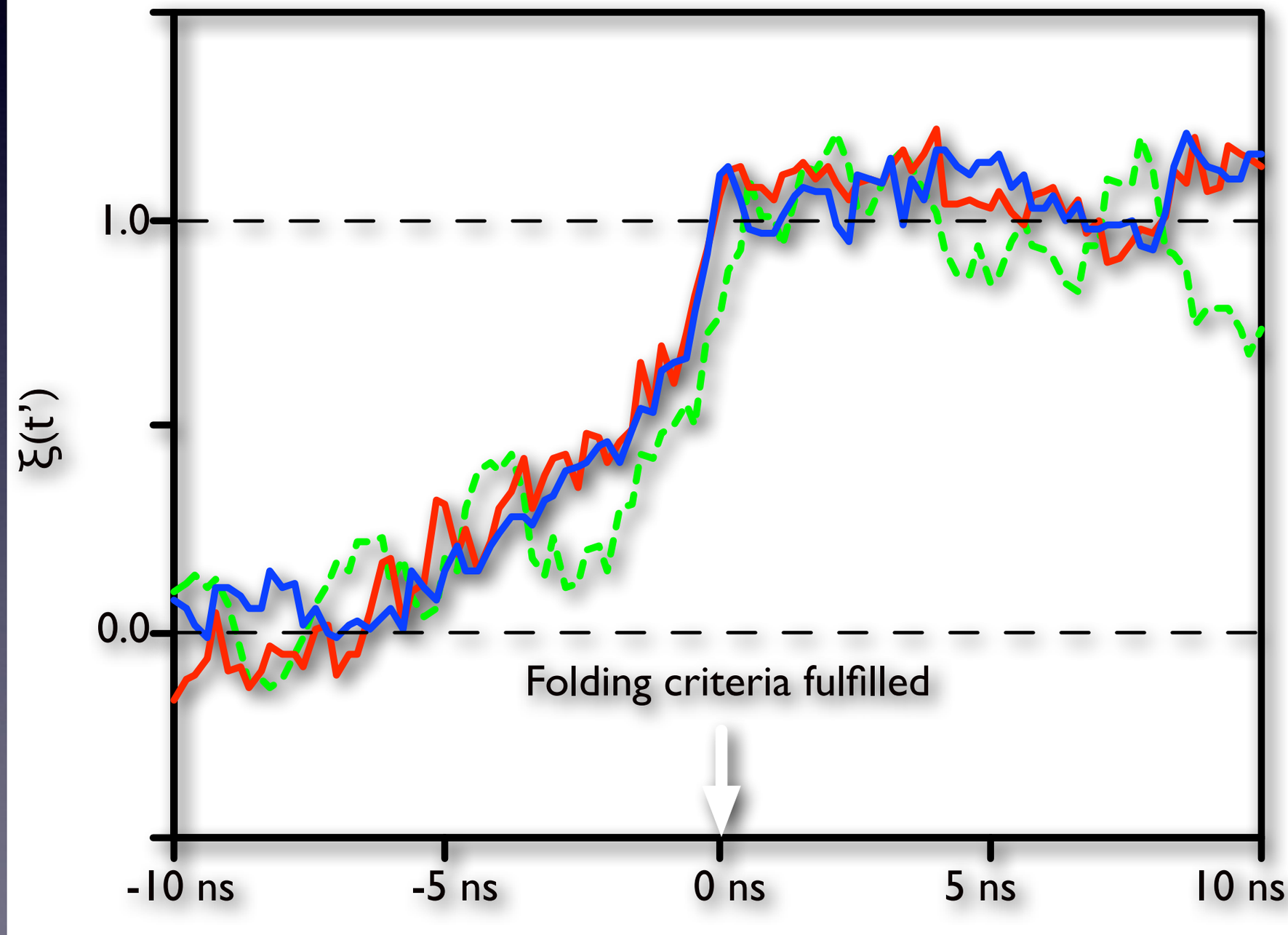
Folding simulations



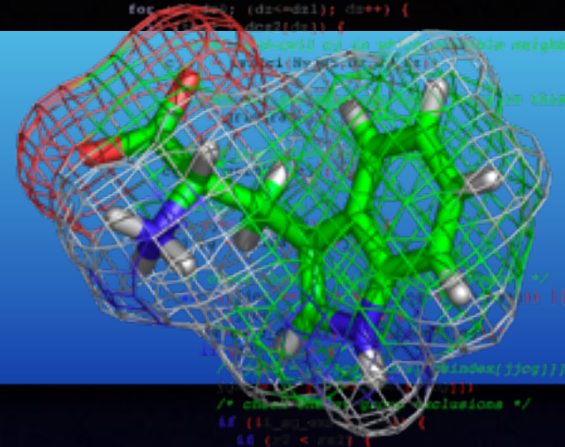
RMSD

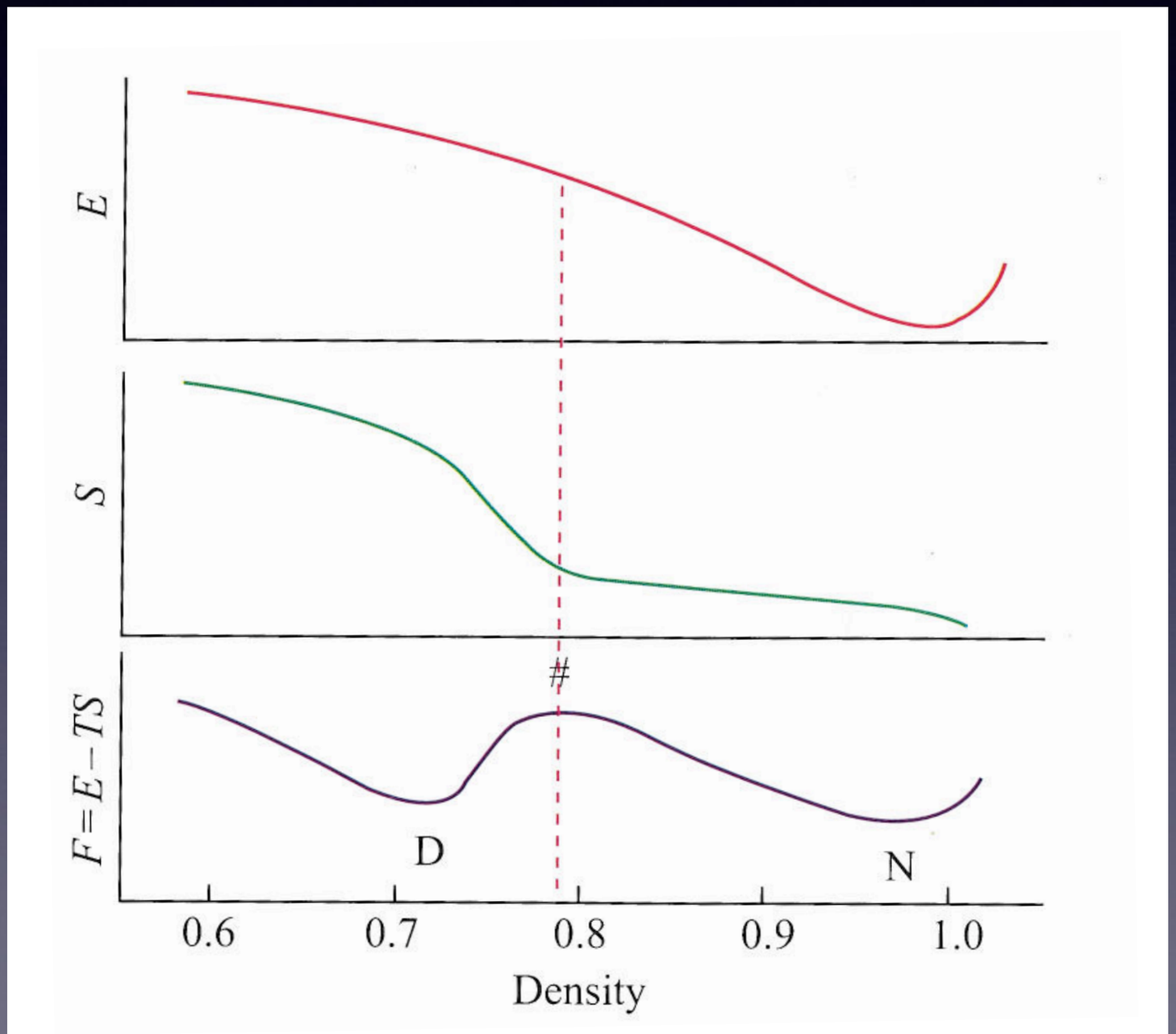
Rgyr of core

Solvent density in core



Energy vs. entropy



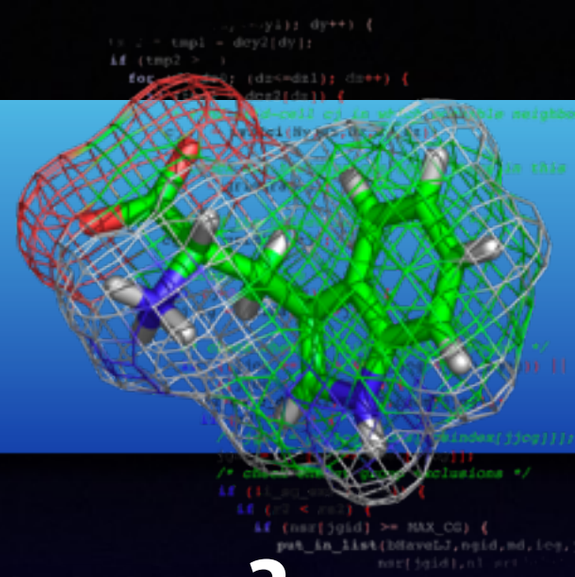


Energy changes gradually

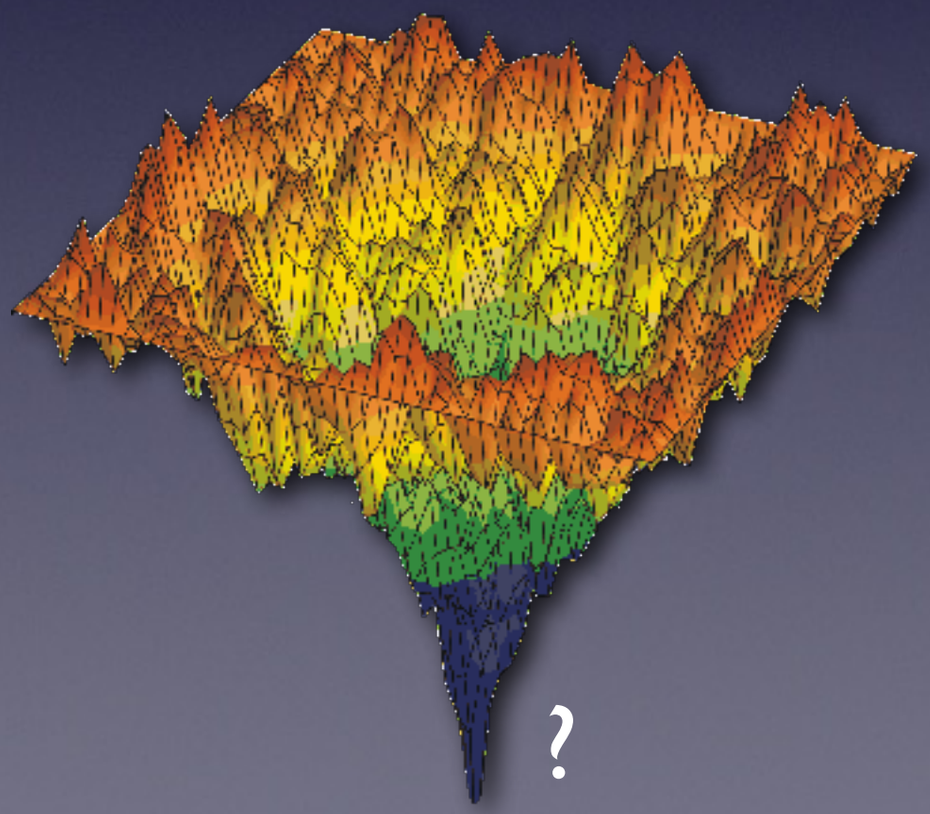
Entropy shows a jump when volume has increased enough for sidechains to move

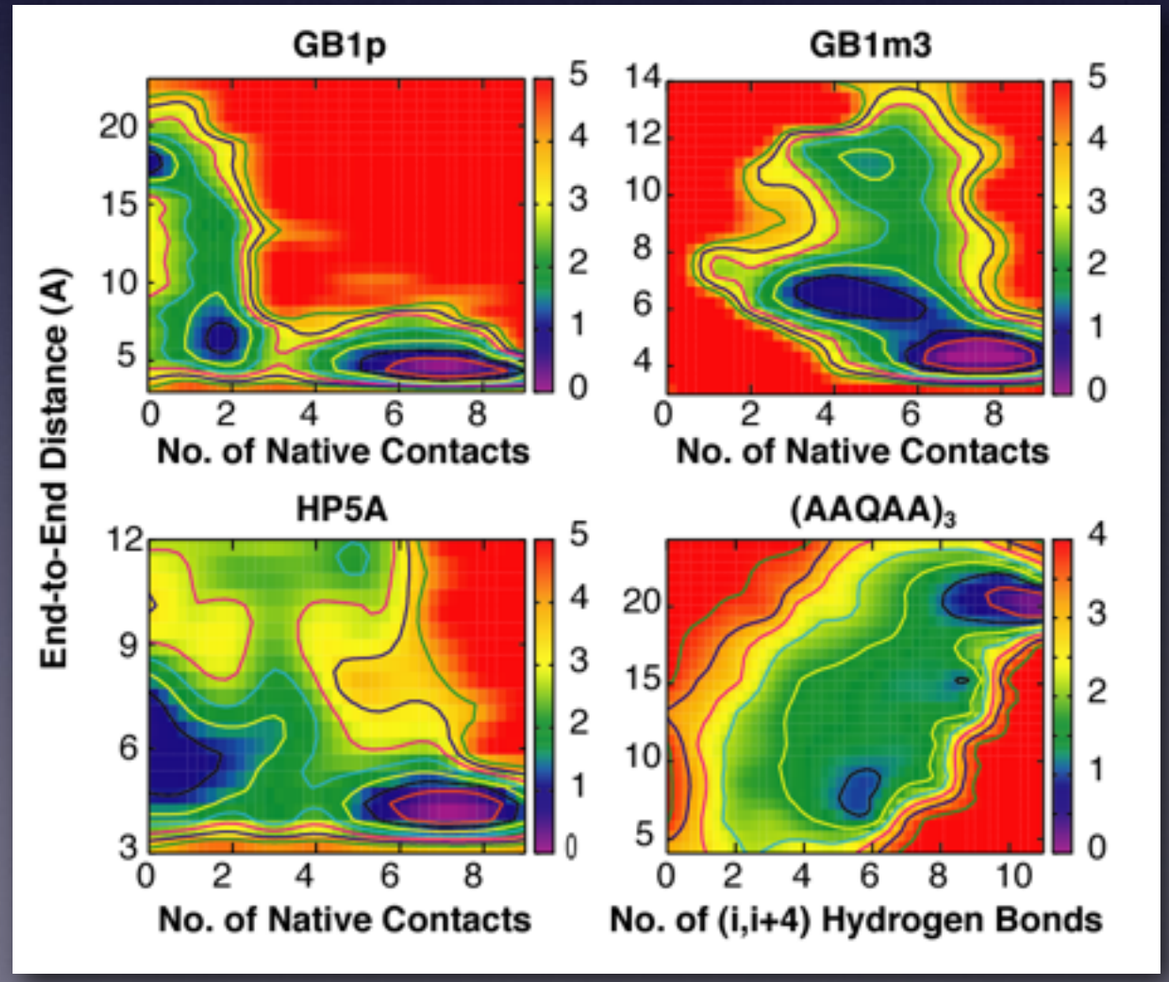
Gives a free energy barrier and all-or-none transition

The native state

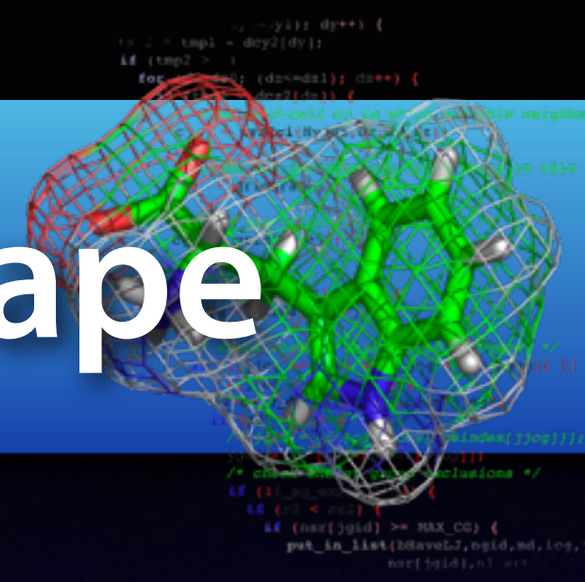


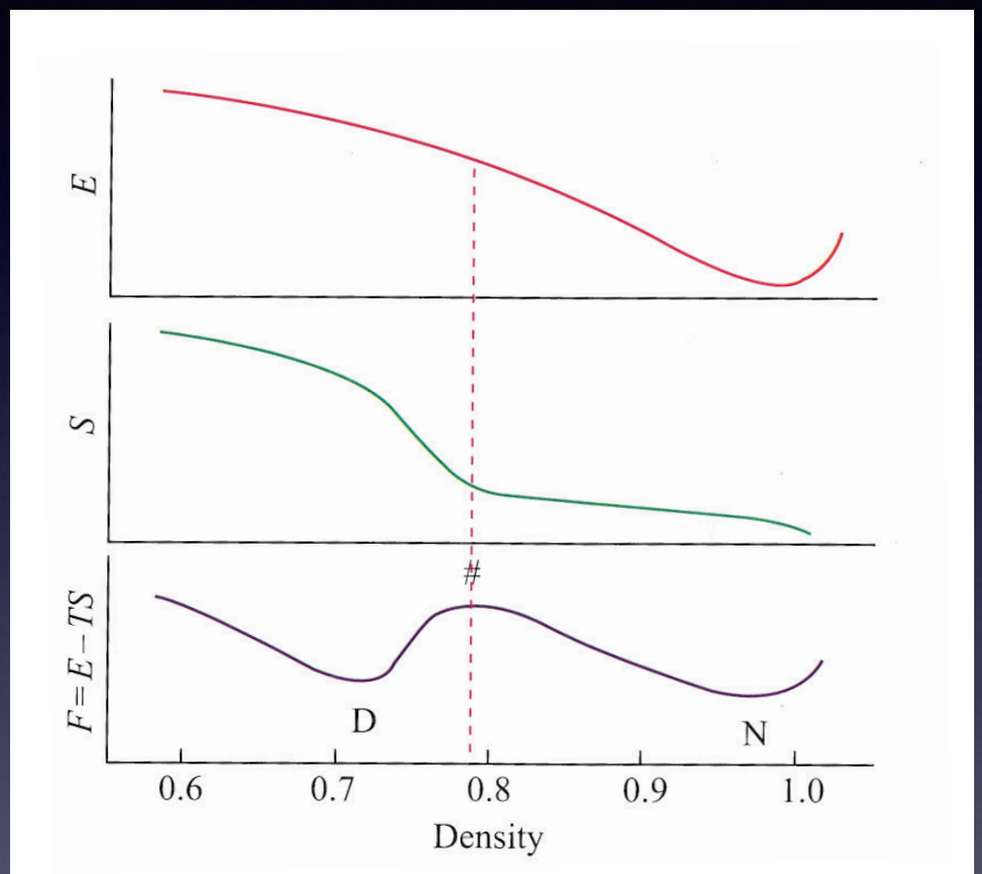
- What defines the native state? Uniqueness?
- Close packing
- Low energy





Entropy in the landscape

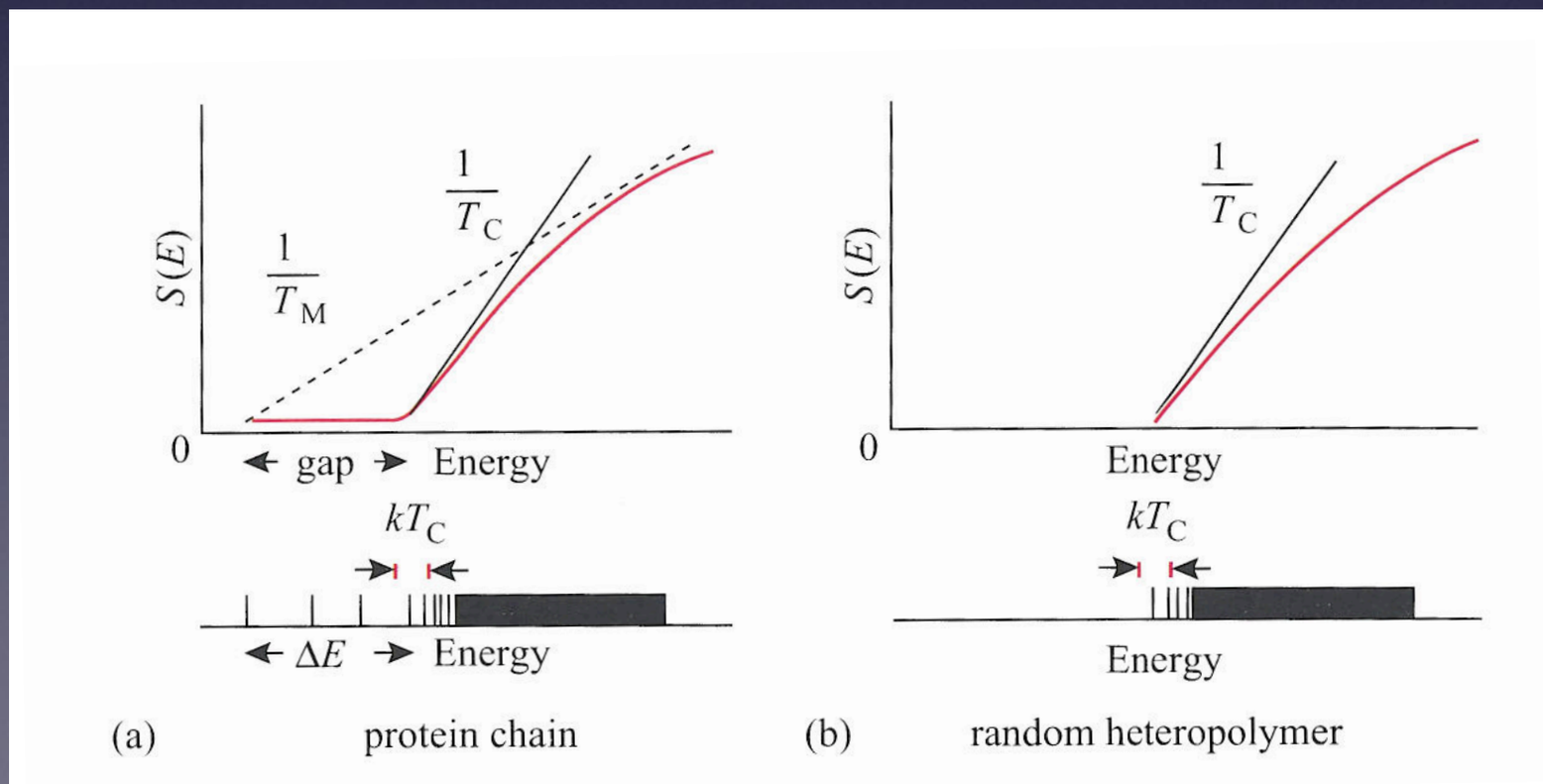




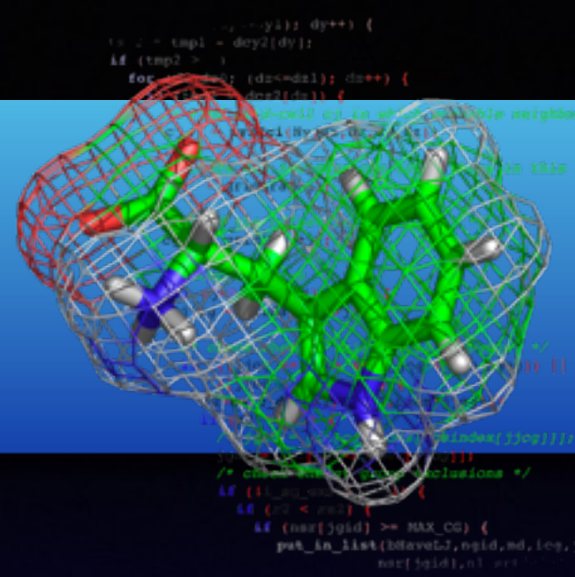
E, S, and F vs. density

$$\rho(r) \propto \exp -\Delta E / kT$$

Plot S vs E:

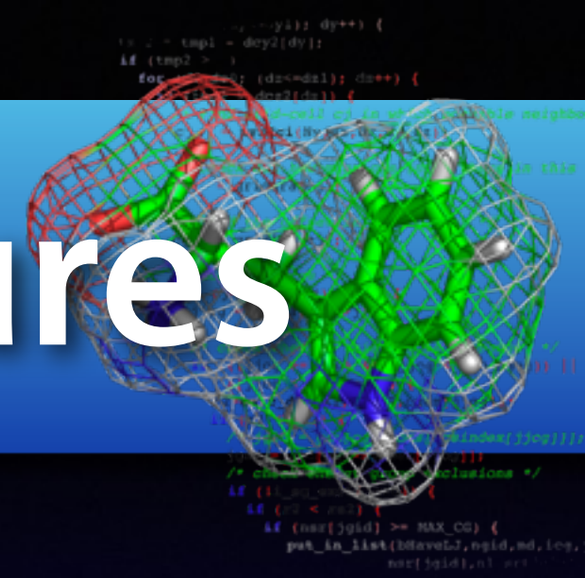


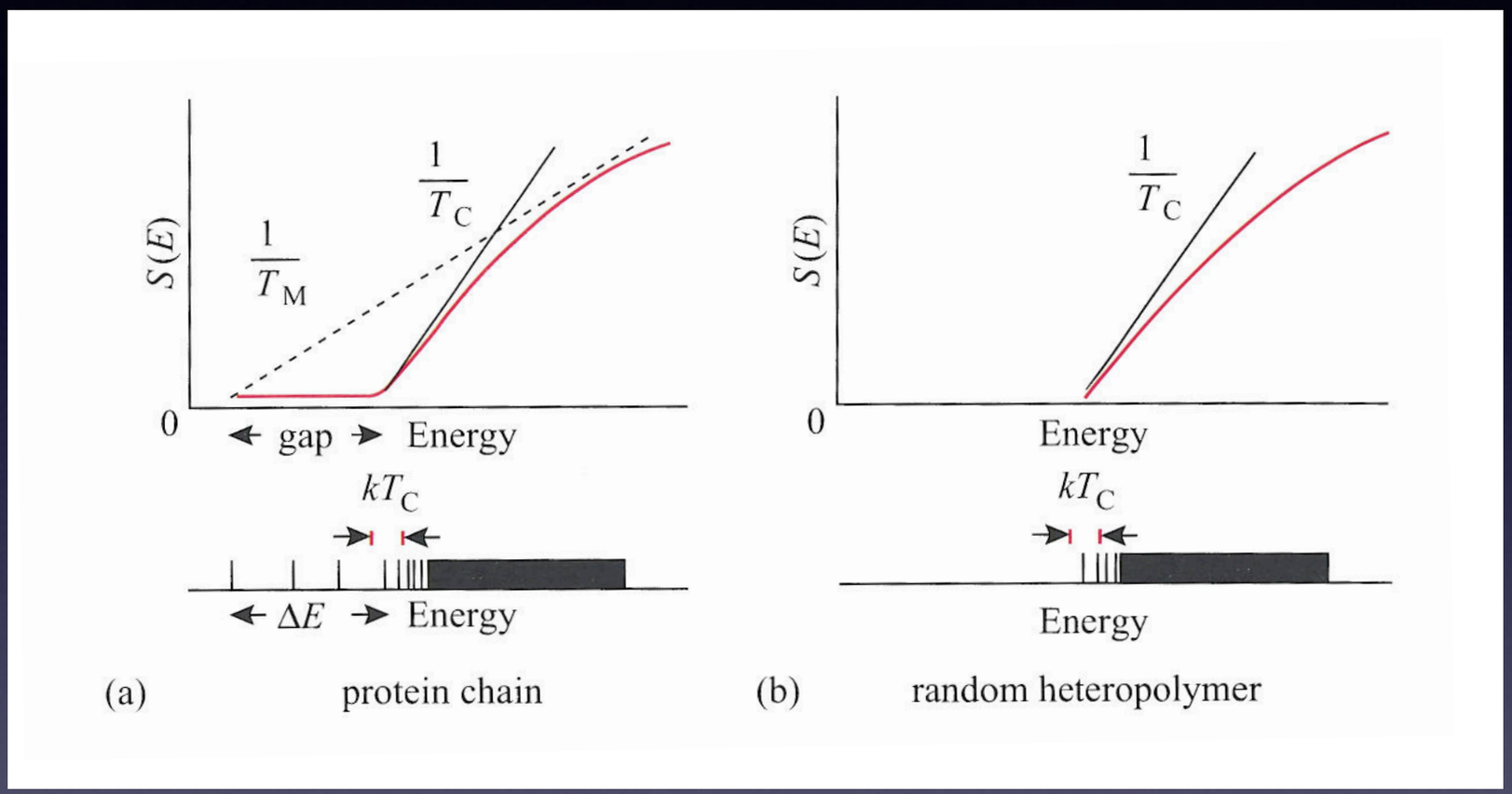
Energy gaps



- Very few structures have low energy
- The lowest-energy structures seem to be separated from the rest by an energy gap
- If gap is large compared to kT, there will be a free energy barrier
- Specific for proteins because of packing
- *Not* valid for general polymers
- *Not* valid for random polypeptides!

Transition temperatures



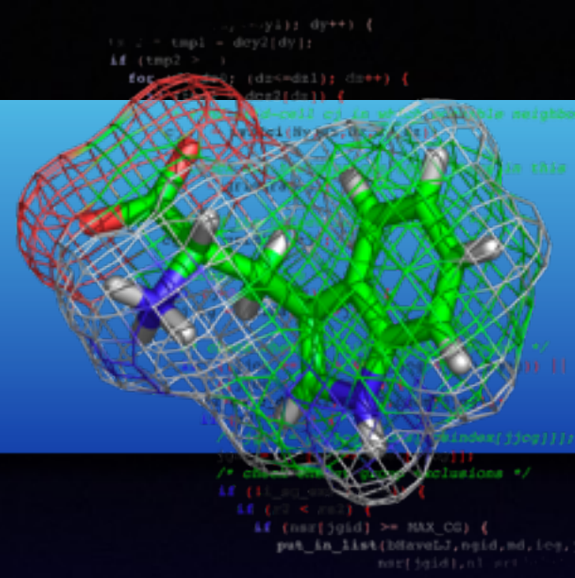


What determines if the barrier to the native state can be surmounted and if it is stable?

Folding/melting: transition between native and other states
Vitrification: chain gets stuck in glass-like low-energy state

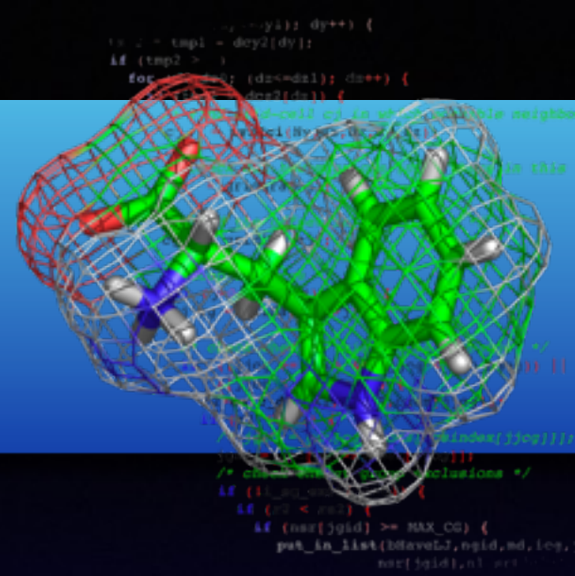
What does $T_{melting} > T_{vitrification}$ mean for proteins?

Fold uniqueness



- Sequences that fold into stable proteins do so because their native structure is separated by an energy gap from the rest
  - Natural selection
- How common is this?
- Use the distribution from last lecture:
- $P_{fold} \simeq \exp(-\Delta E/kT_{vitrification})$
- With $\Delta E >> kT_{vitrification}$ (say, 20x) we get $P_{fold} \simeq 10^{-8}$

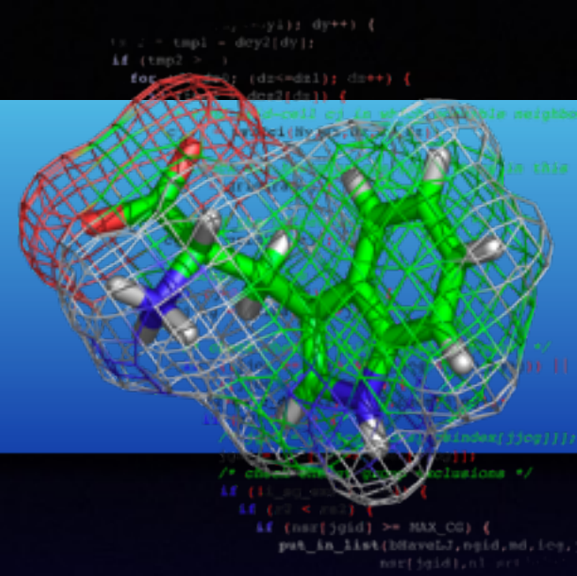
Fold uniqueness



- But why is the native state unique?
- What about two stable native states?

- $P_{fold}^2 \simeq 10^{-16}$ $P_{fold}^3 \simeq 10^{-24}$
- Very rare, but it does happen!
  - Amyloid peptides
  - Remember: Prions!
  - Mad cow disease (BSE)

Summary



- Folding, denaturation, refolding
- Cooperative *and* all-or-none transition
- Cold & hot denaturation
- Molten globule and coil conformations
- Free energy barriers & energy gaps
- Proteins are different from random chains!

Book: chapters 17 & 18