

# Free energy, electrostatics, and the hydrophobic effect

Magnus Andersson

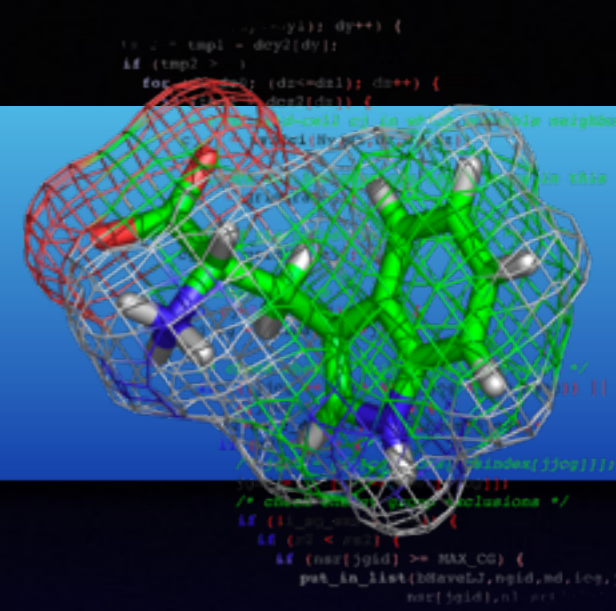
*magnus.andersson@scilifelab.se*

**Theoretical & Computational Biophysics**

SciLifeLab

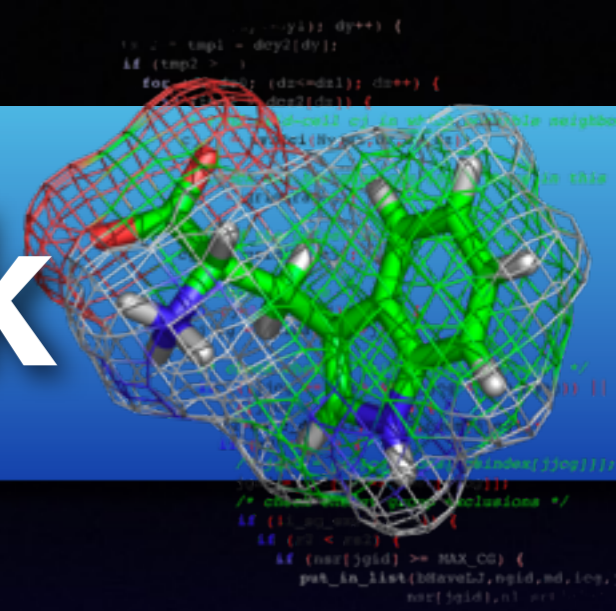


# Recap



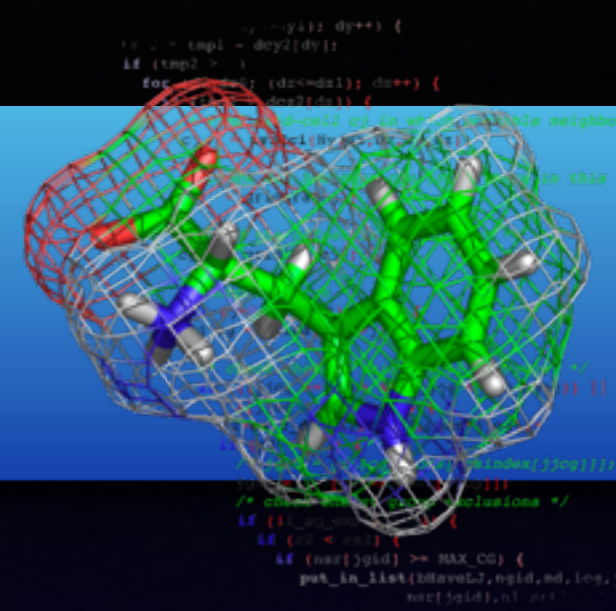
- Protein structure
- Electrostatics & hydrogen bonds
- Van der Waals / Lennard-Jones
- Interaction strengths
- Energy Landscapes
- The Boltzmann Distribution
- Free Energy and entropy

# To sum up last week



- Two critical results:
  - Protein folding is about conformations of long polypeptide chains - how can it find the best structure?
  - Reaction directions are determined by free energy;  $F=E-TS$ . Stable states are  $F$  minima.

# Outline today

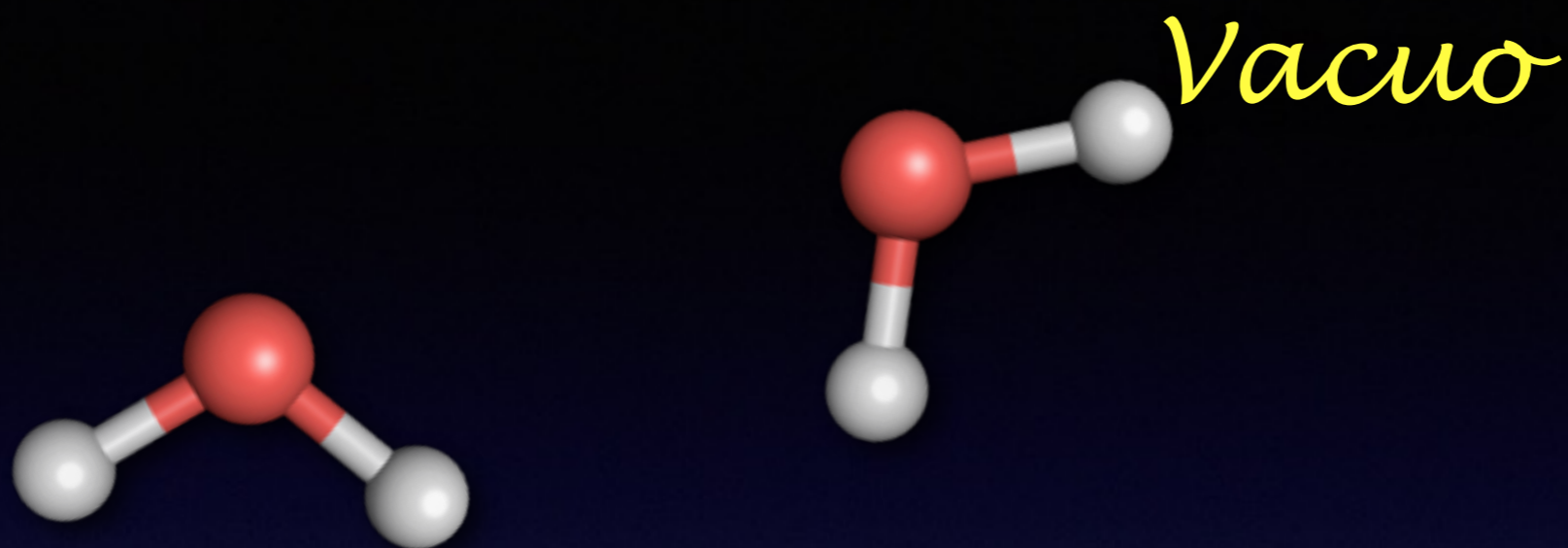


- Hydrophobic effect revisited
- Connection to  $F = E - TS$
- Connection to protein folding
- Strength of electrostatics in proteins
- Titratable amino acid side chains

# Water Phase Transitions

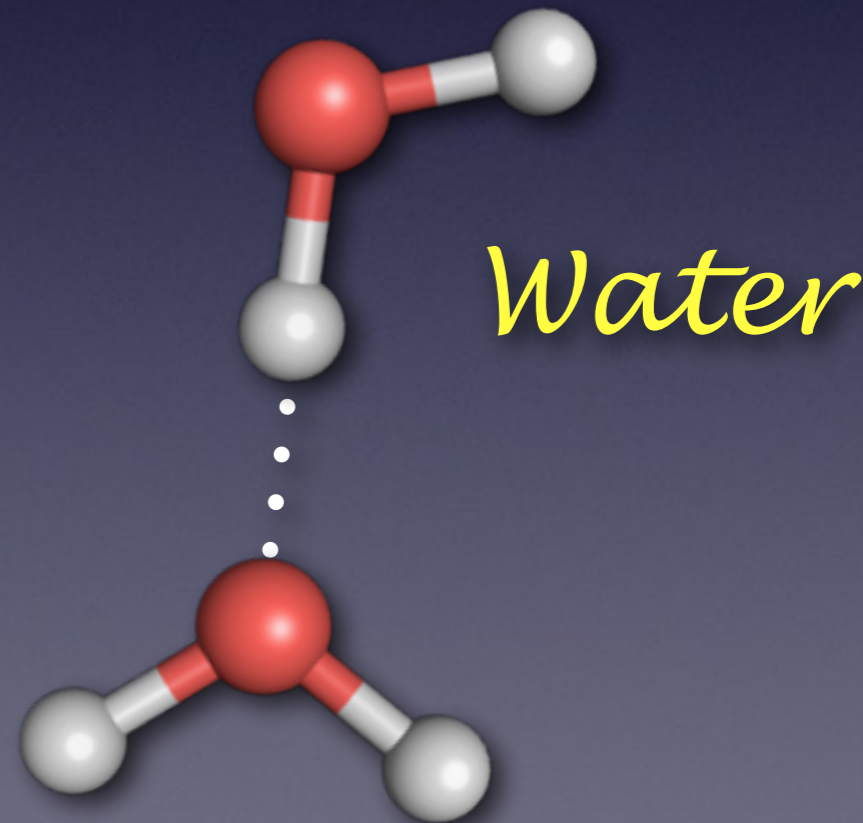


- Systems wants to stay at lowest F
- ICE: Low E, low low S
- Water: Higher E, higher S
- When temperature is low, first term (E) dominates  $F=E-TS$
- When temperature is high, second term (TS) dominates  $F=E-TS$
- Can we use this to understand

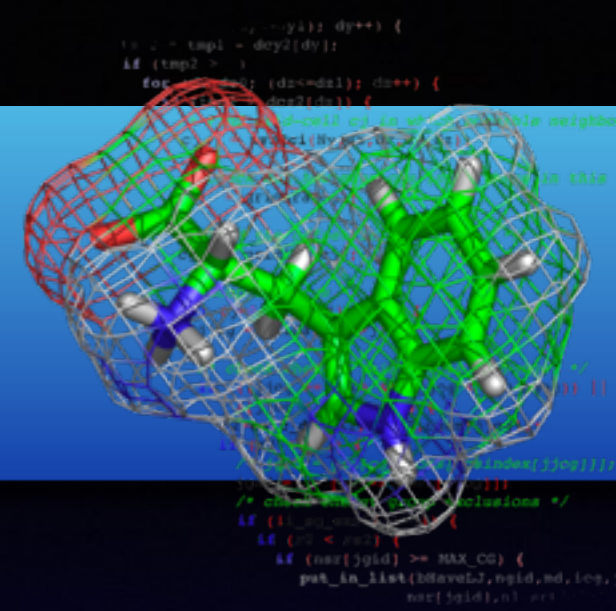


**Gain: Energy of 1 h-bond ( $E_H < 0$ )**

**Loss: Entropy of 1 ( $0.5 * 2$ ) freely rotating water ( $S_H > 0$ )**



# Peer challenge



Which is true for H-bond formation  
at room temperature?

A)  $E_H < TS_H$

B)  $TS_H < E_H$

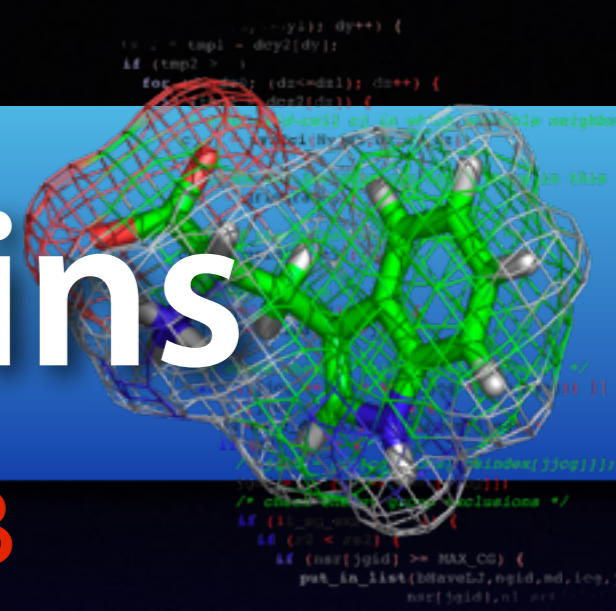
*Don't forget the sign!*



$F_H = E_H - T_S_H$



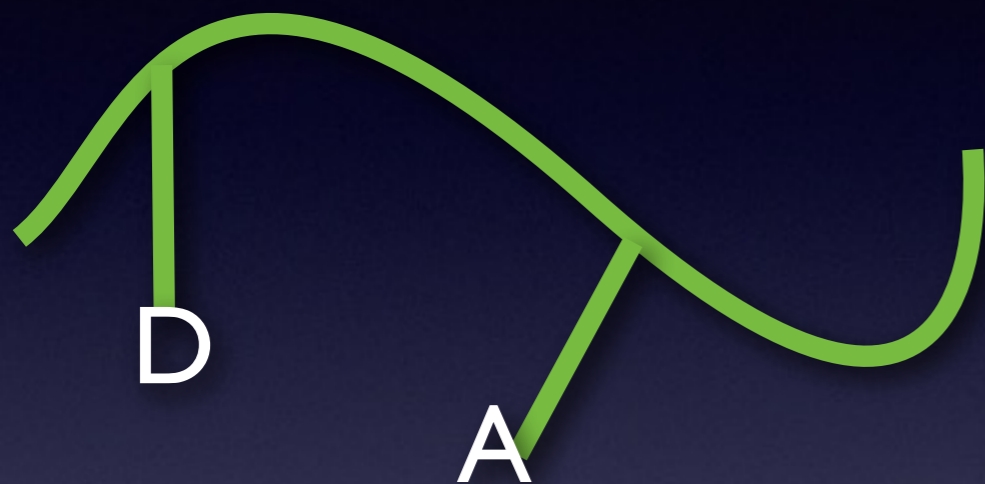
# H-bond $\Delta G$ for proteins



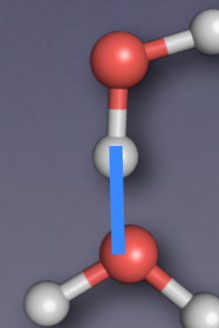
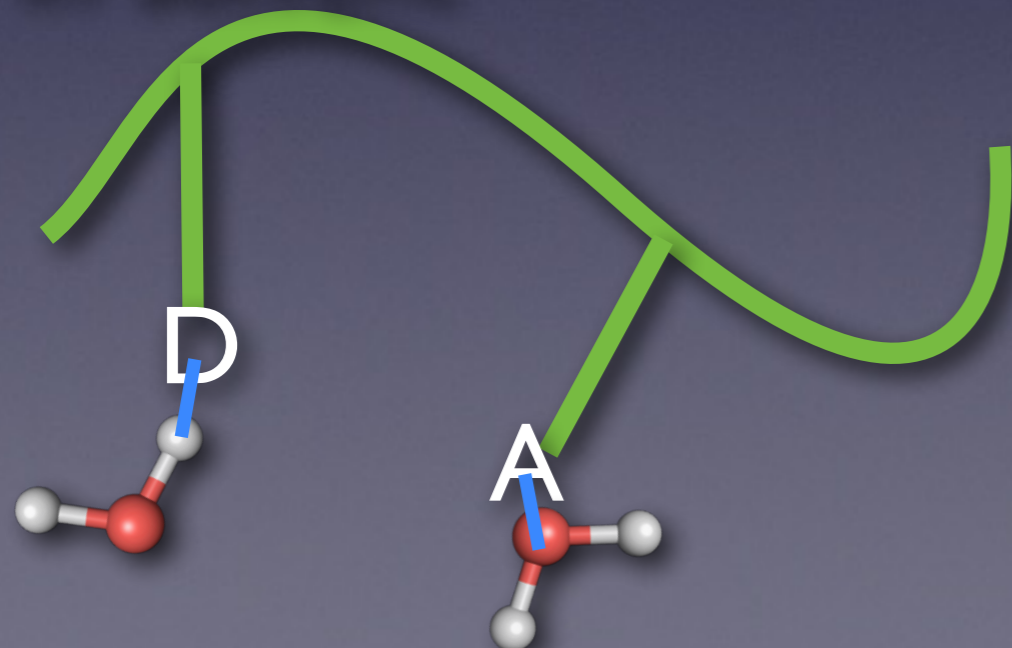
In vacuo

State A

State B



In solvent



1-octanol



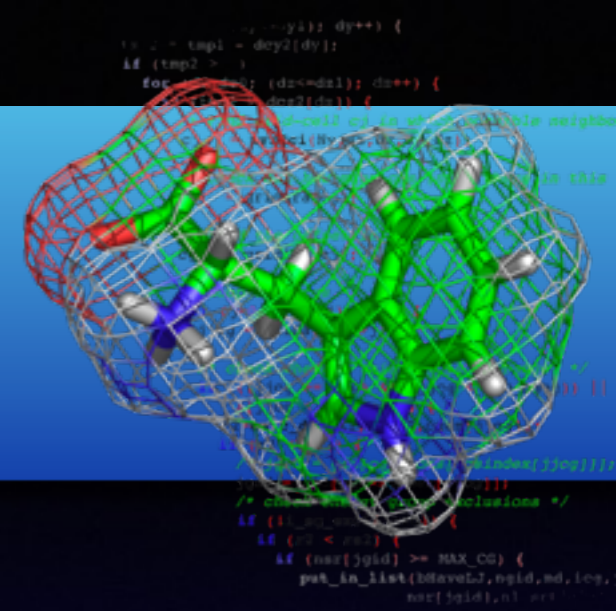
water



Why do some molecules like oil/gas better?

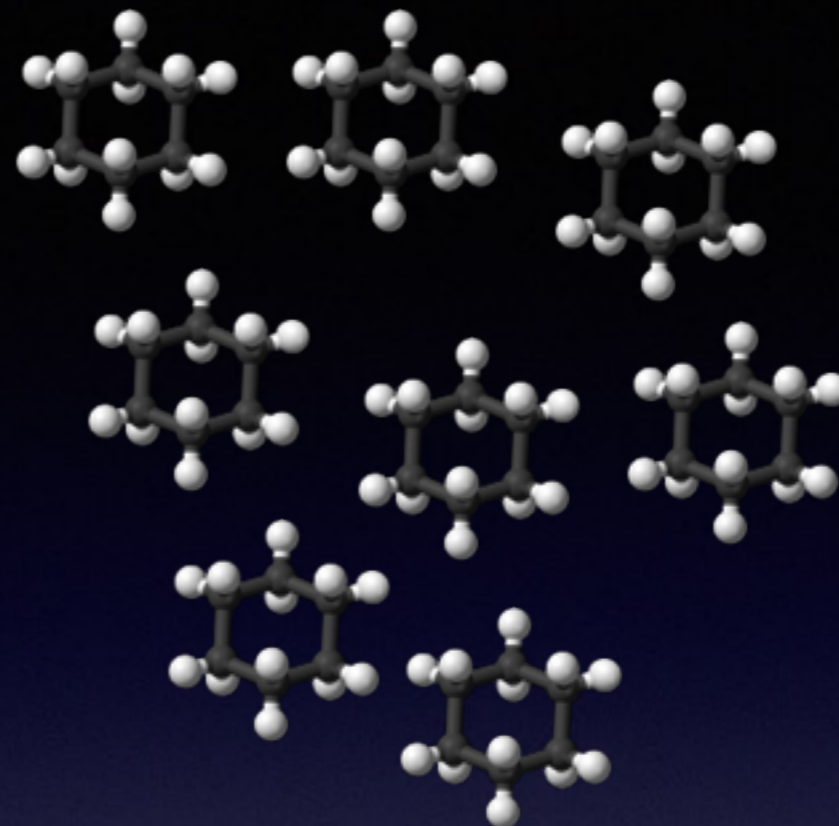
Why do some molecules like water better?

# Partitioning

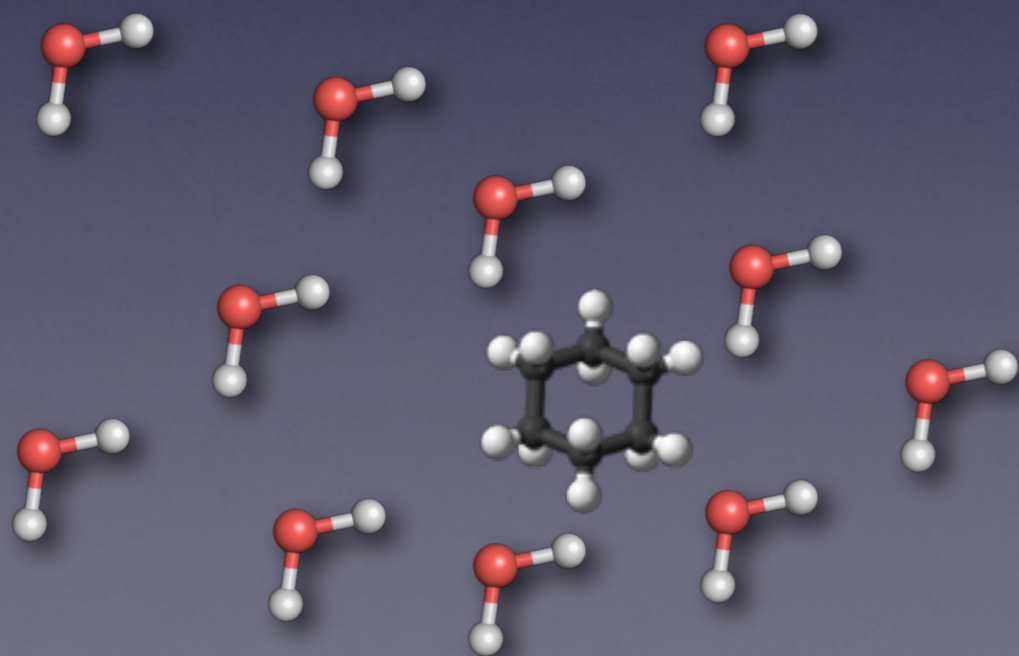


- Consider transfer of hydrocarbon to H<sub>2</sub>O
- Concentrations (X) rather than probability
- Count per mol, so we use R instead of k
- $X \propto \exp\{-G/RT\}$
- $\Delta G_{\text{liq} \rightarrow \text{aq}} = -RT \ln (X_{\text{aq}}/X_{\text{liq}})$

**9.25 mol/l**

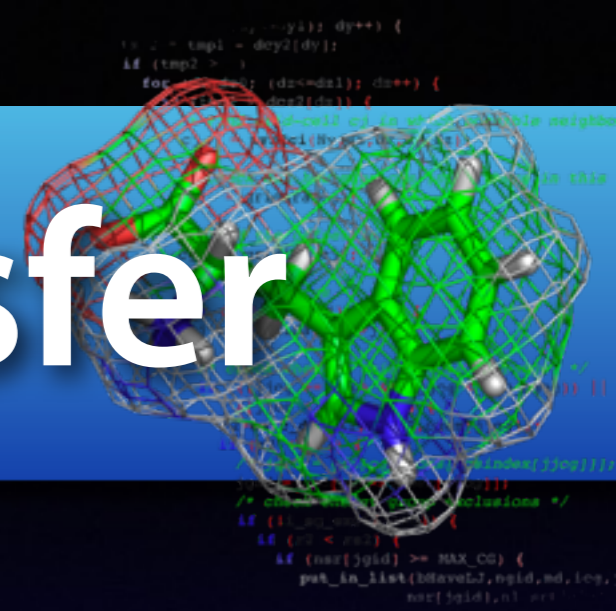


$$\Delta G_{\text{liq} \rightarrow \text{aq}} = +6.7 \text{ kcal/mol}$$



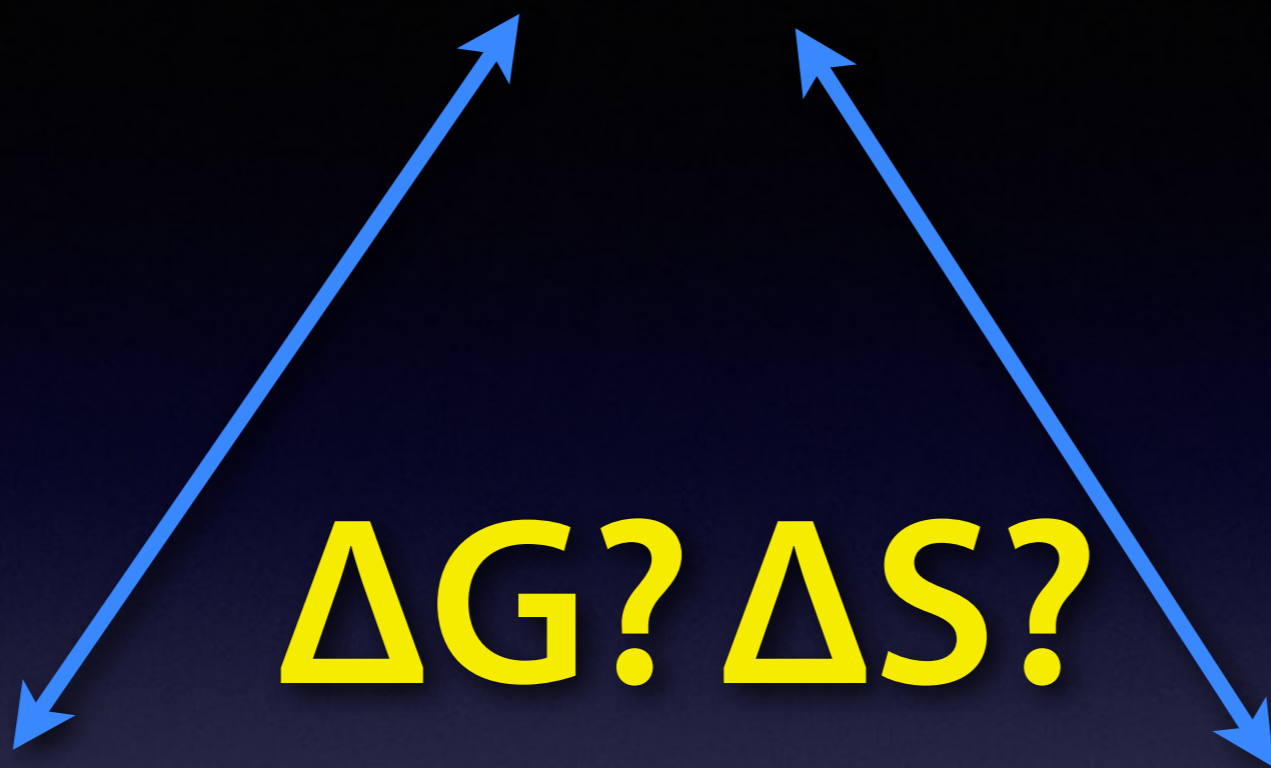
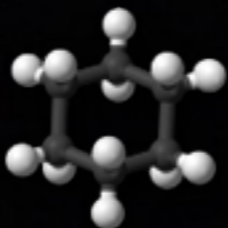
**0.0001 mol/l**

# Hydrocarbon transfer



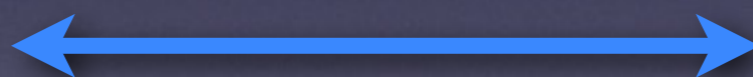
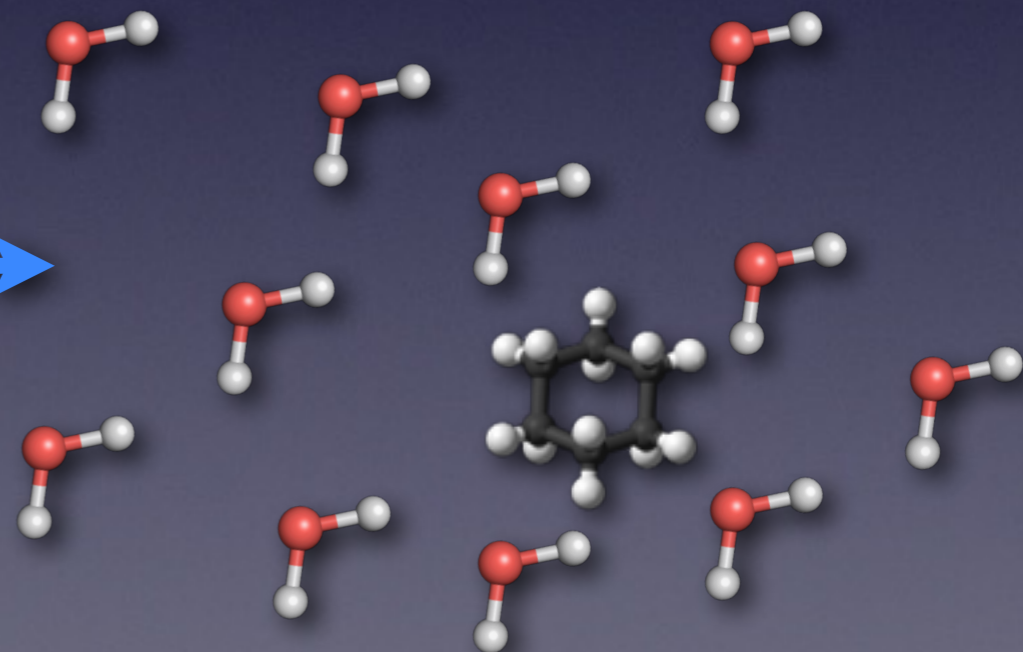
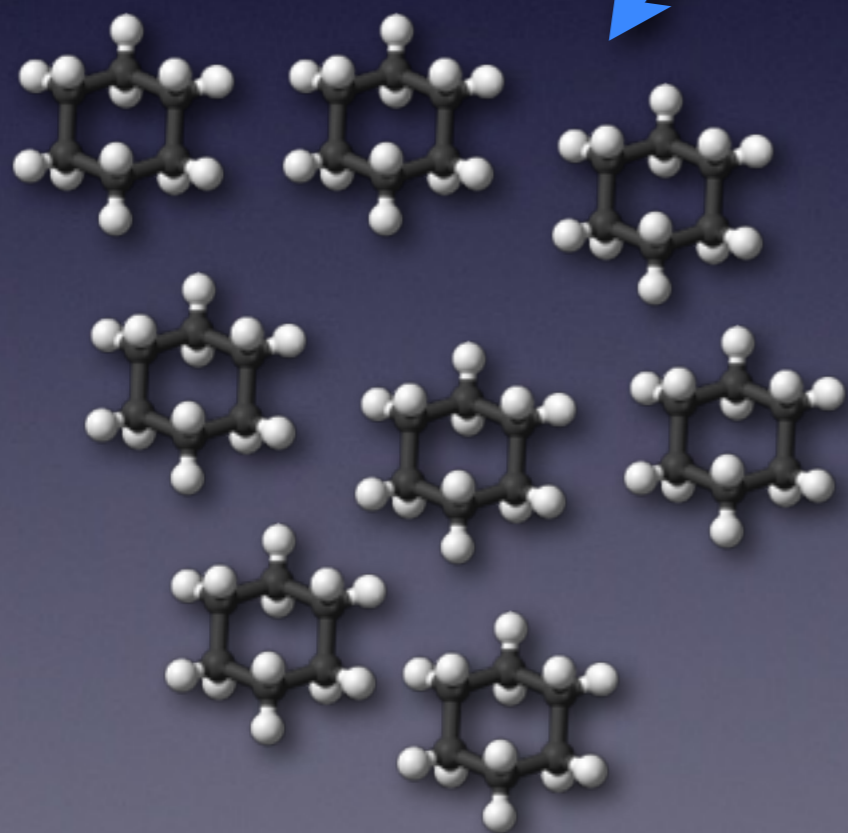
- $\Delta G_{\text{liq} \rightarrow \text{aq}} = +6.7$  kcal/mol at room temp
- Not spontaneous process
- It costs free energy to solvate hexane in  $\text{H}_2\text{O}$
- Why?

$$G = H - TS$$



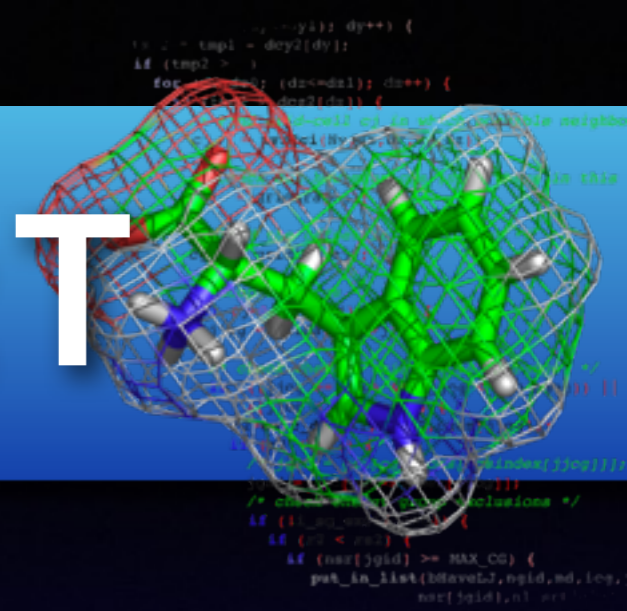
$\Delta G?$   $\Delta S?$

$\Delta H?$



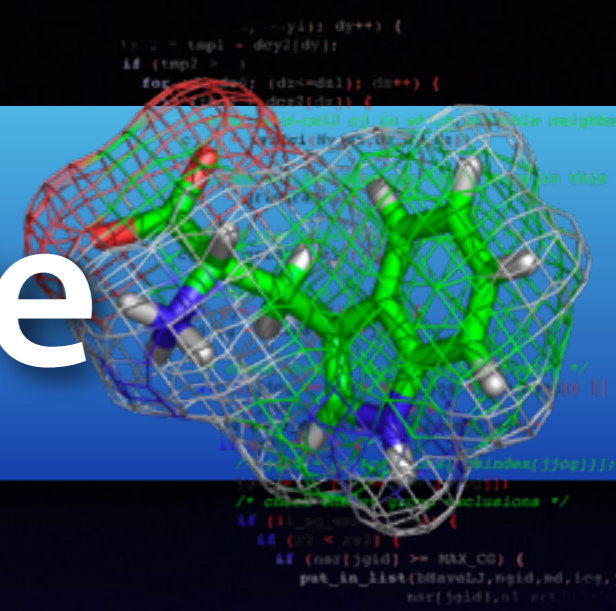
$\Delta G = \Delta H - T\Delta S$

# Thermodynamic T



- Minor perturbations at equilibrium
- $F + dF = 0$        $F + dE - TdS - SdT = 0$
- At equilibrium under constant  $V$  &  $T$ ,  
this leads to:  
 $dF = dE - TdS = 0$
- or:  $T = dE/dS$
- This was the *thermodynamic* definition  
of temperature that we covered last week

# S vs. Temperature



- $dF=d(E-TS)=dE-TdS-SdT$
- at equilibrium,  $dE-TdS=0$  (last slide)
- Thus, at constant volume we get:  
 $S=-dF/dT$
- And at constant pressure it is  $S=-dG/dT$
- Compare  $T = dE/dS$  from last slide
- This solves our problem!
  - Measure  $G$  at multiple  $T$  to get  $S$ !

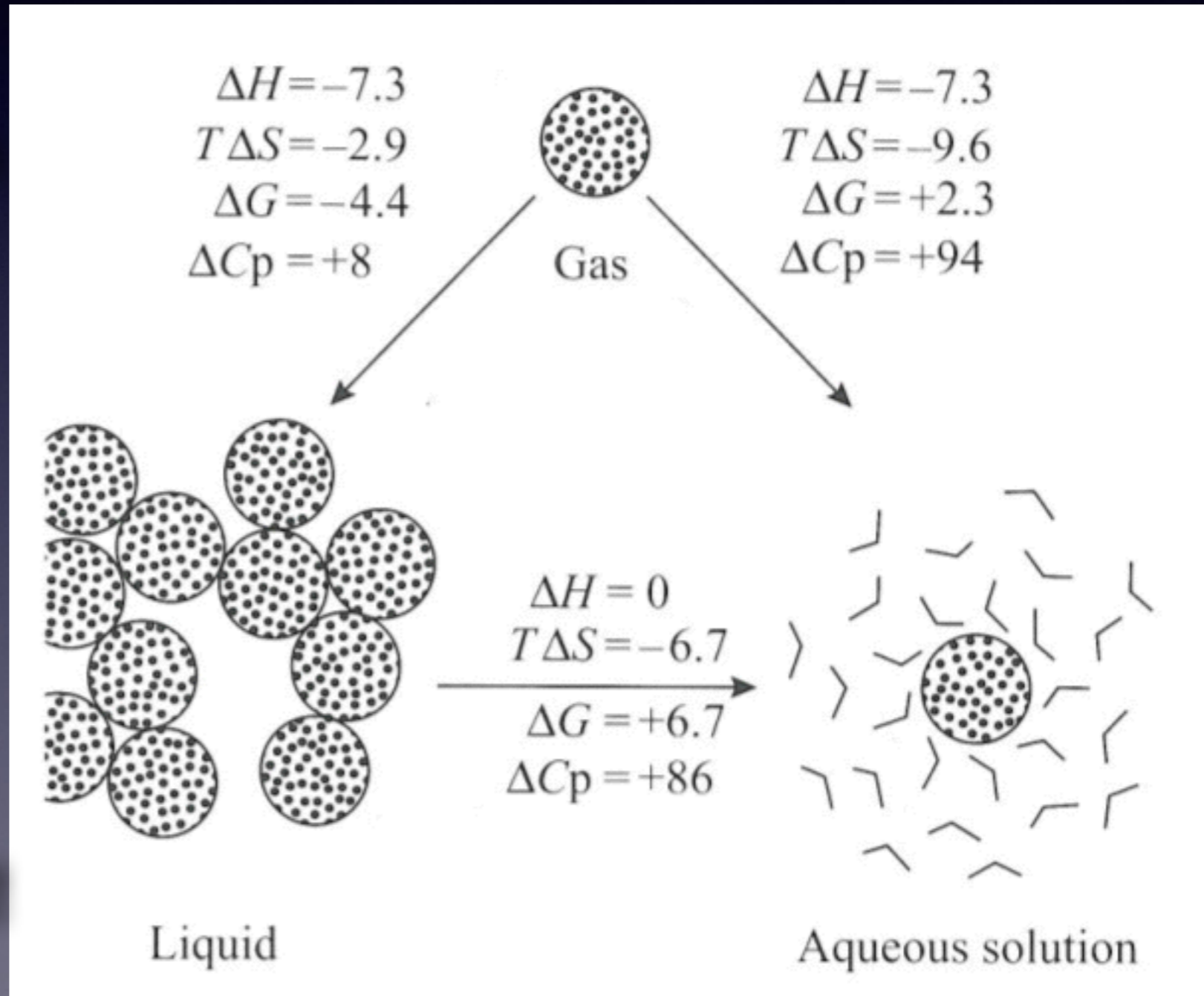


# Hydrophobic solvation

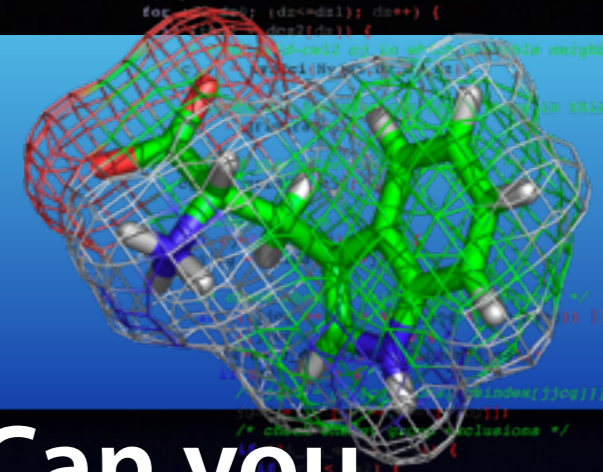


We can compare the gas phase with aqueous or liquid phases the same way!

Knowing  $\Delta G(T)$ , we can calculate the other properties!



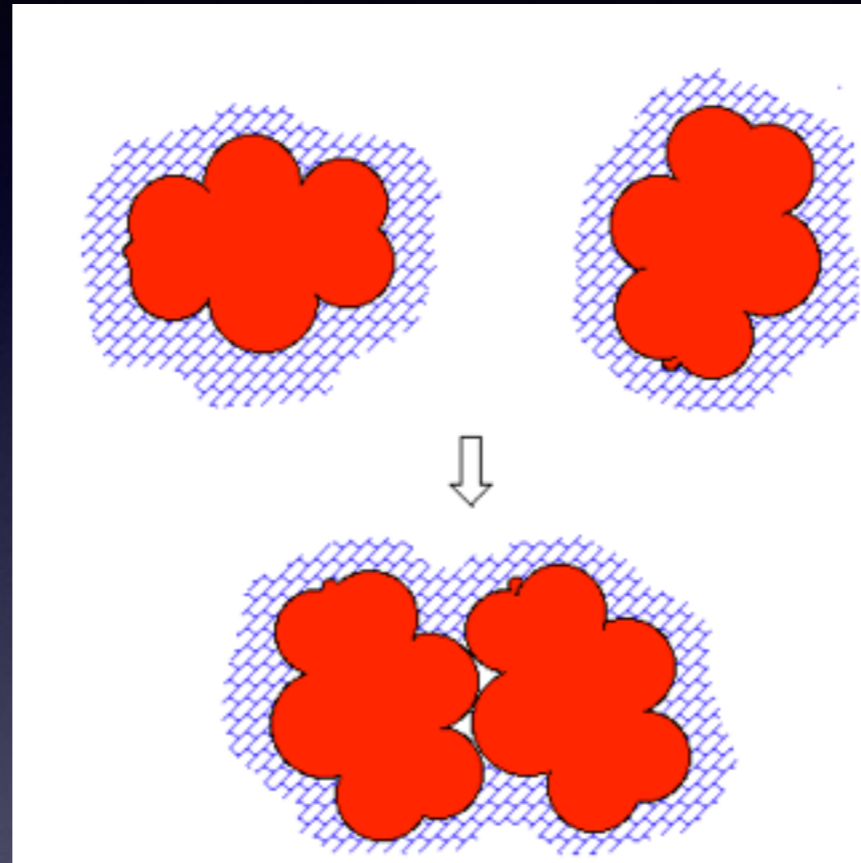
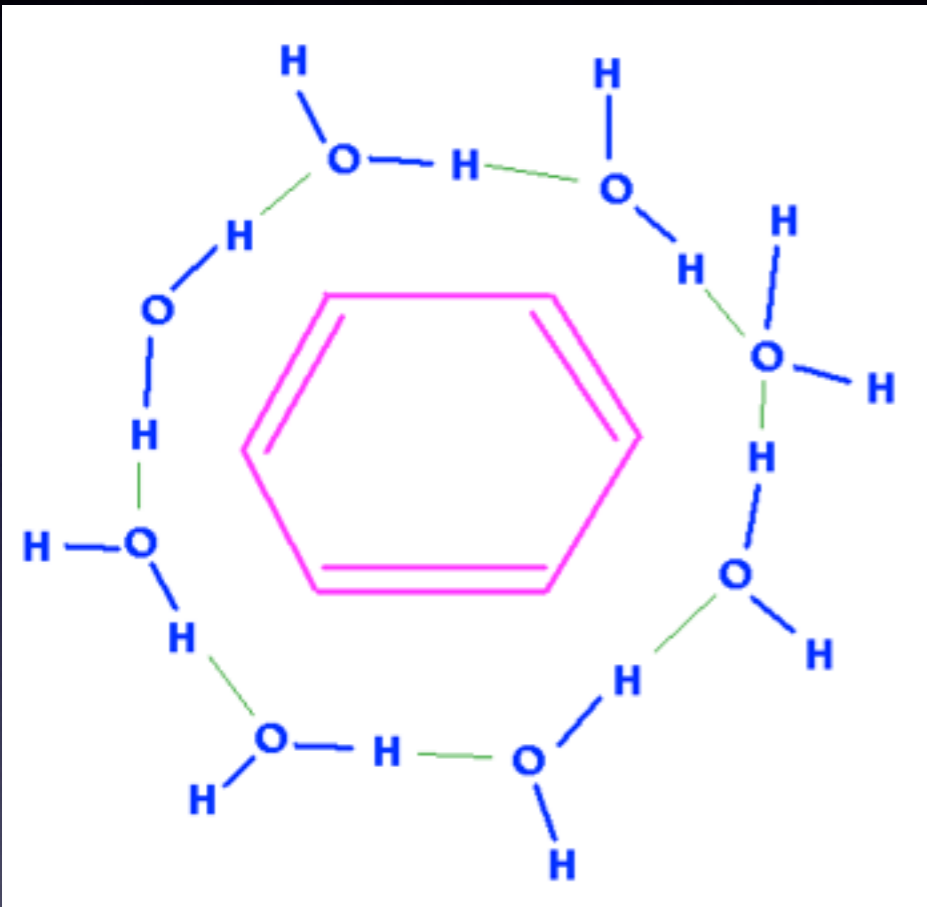
# Hydrophobic effect



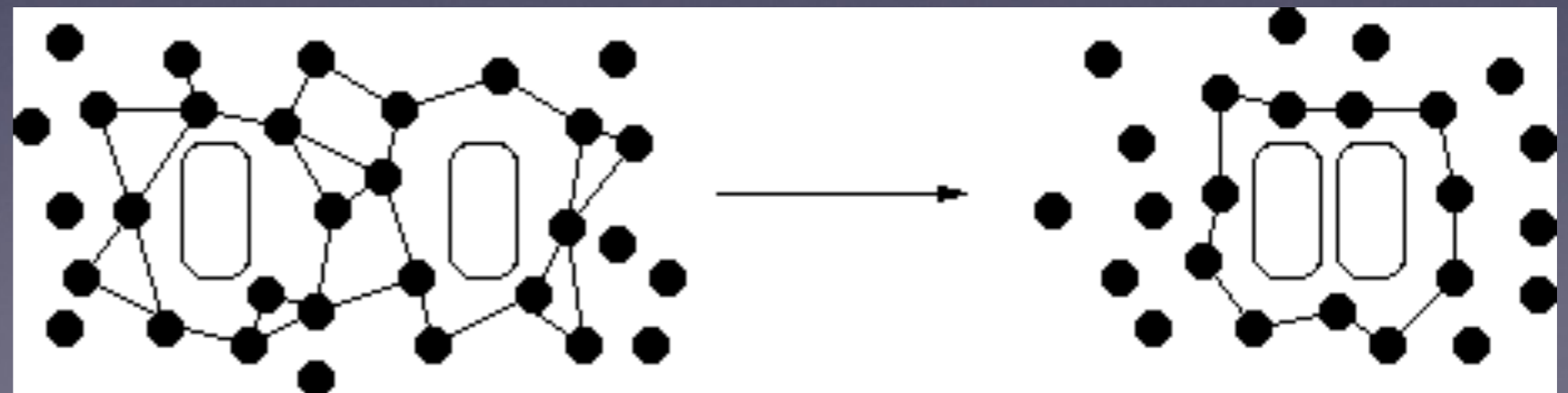
Can you  
account for  
these processes?

$\Delta G?$   $\Delta S?$

$\Delta H?$



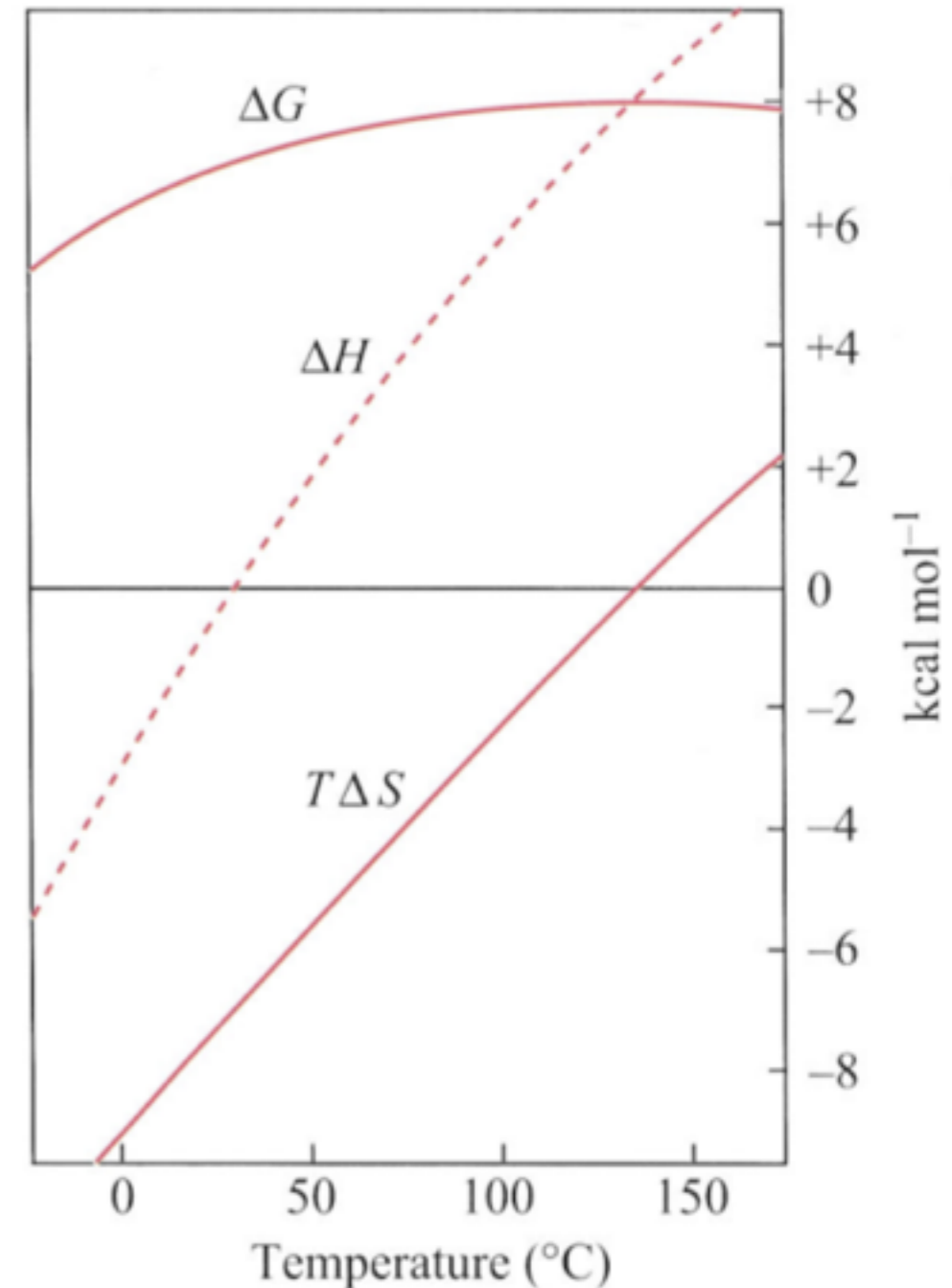
Clathrate  
structures



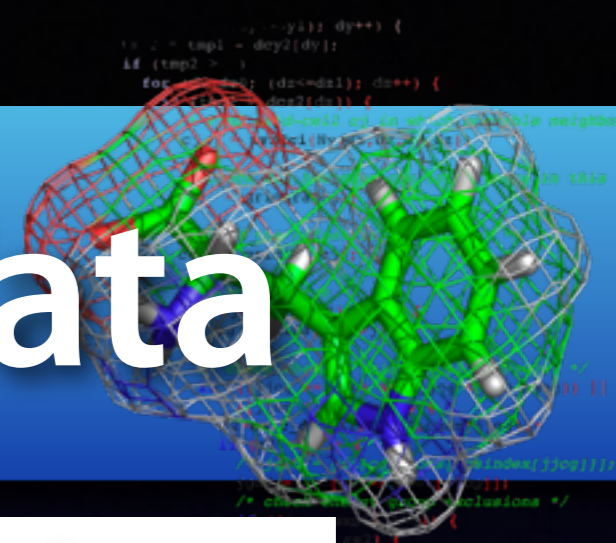
# Temperature dependence



- Strong dependence for H
- Strong dependence for TS
- G is a small difference!



# Thermodynamic data



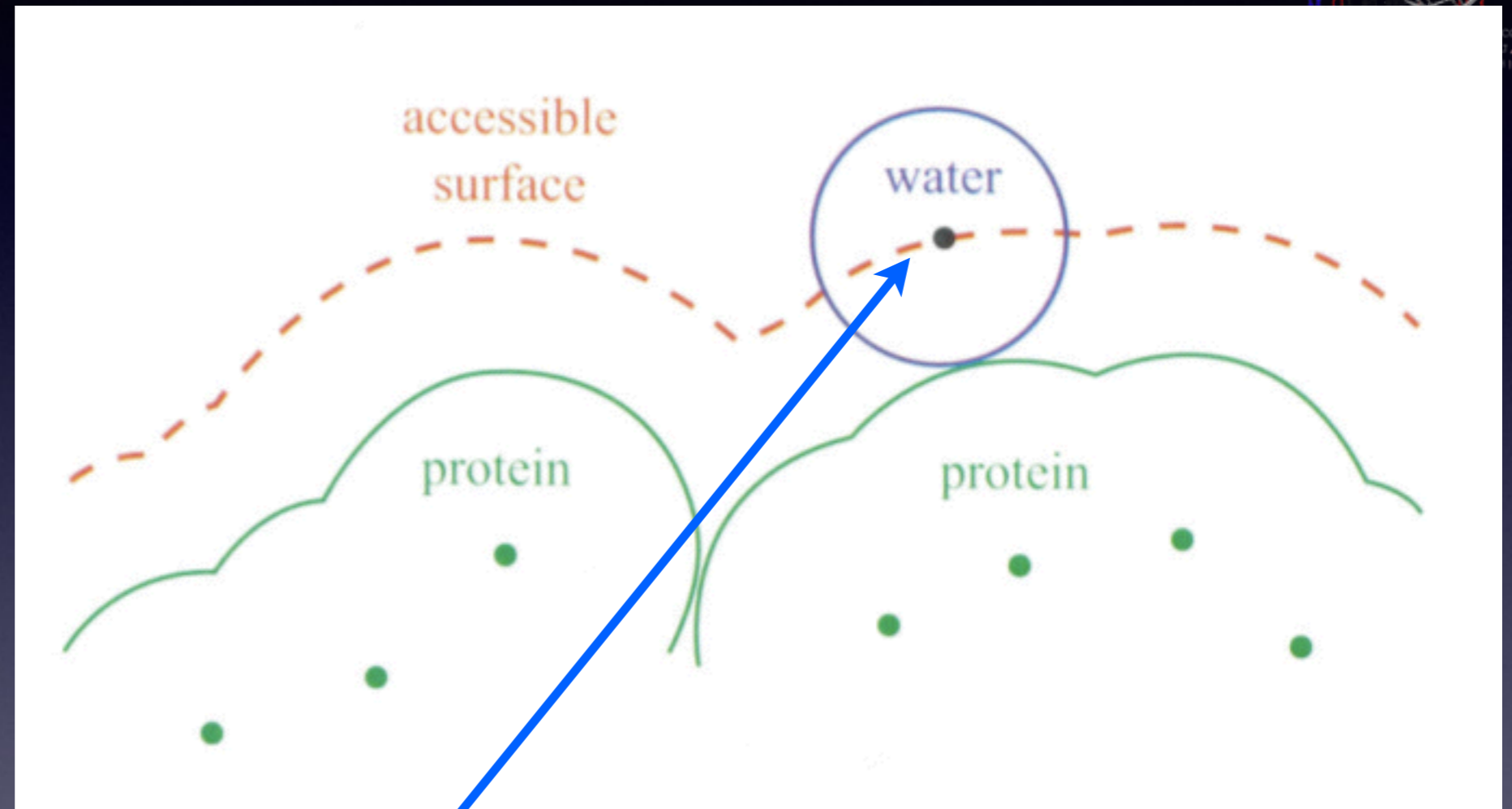
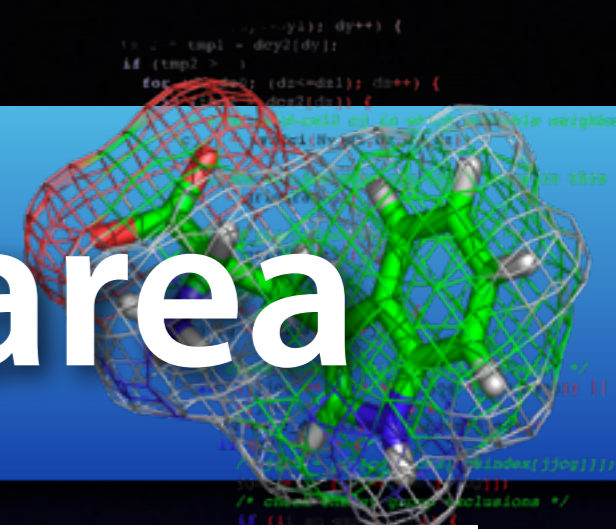
**Table 5.1.** Typical thermodynamic parameters of hydrophobic group transfer from a nonpolar liquid to an aqueous solution at 25°C.

Molecule	Transfer from → to	$\Delta G$ (kcal mol <sup>-1</sup> )	$\Delta H$ (kcal mol <sup>-1</sup> )	$T\Delta S$ (kcal mol <sup>-1</sup> )	$\Delta C_p$ (kcal mol <sup>-1</sup> K <sup>-1</sup> )
Ethane (CH <sub>3</sub> ) <sub>2</sub> (compare with Ala side group: —CH <sub>3</sub> )	benzene→ water	+3.6	-2.2	-5.8	+59
	CCl <sub>4</sub> → water	+3.8	-1.8	-5.4	+59
Benzene C <sub>6</sub> H <sub>6</sub> (compare with Phe side group: —CH <sub>2</sub> —C <sub>6</sub> H <sub>5</sub> )	benzene→ water	+4.6	+0.5	-4.1	+54
Toluene (C <sub>6</sub> H <sub>5</sub> ) <sub>2</sub> CH <sub>3</sub> (compare with Phe side group)	toluene→ water	+5.4	+0.4	-5.8	+63

**$\Delta G$  virtually proportional to area!**

Values taken from Tanford C. *The hydrophobic effect*, 2nd edn, New York: Wiley-Interscience, 1980.

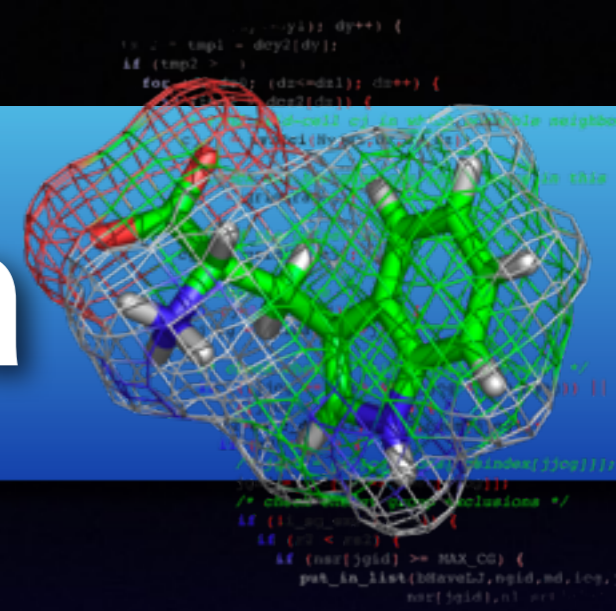
# Accessible surface area



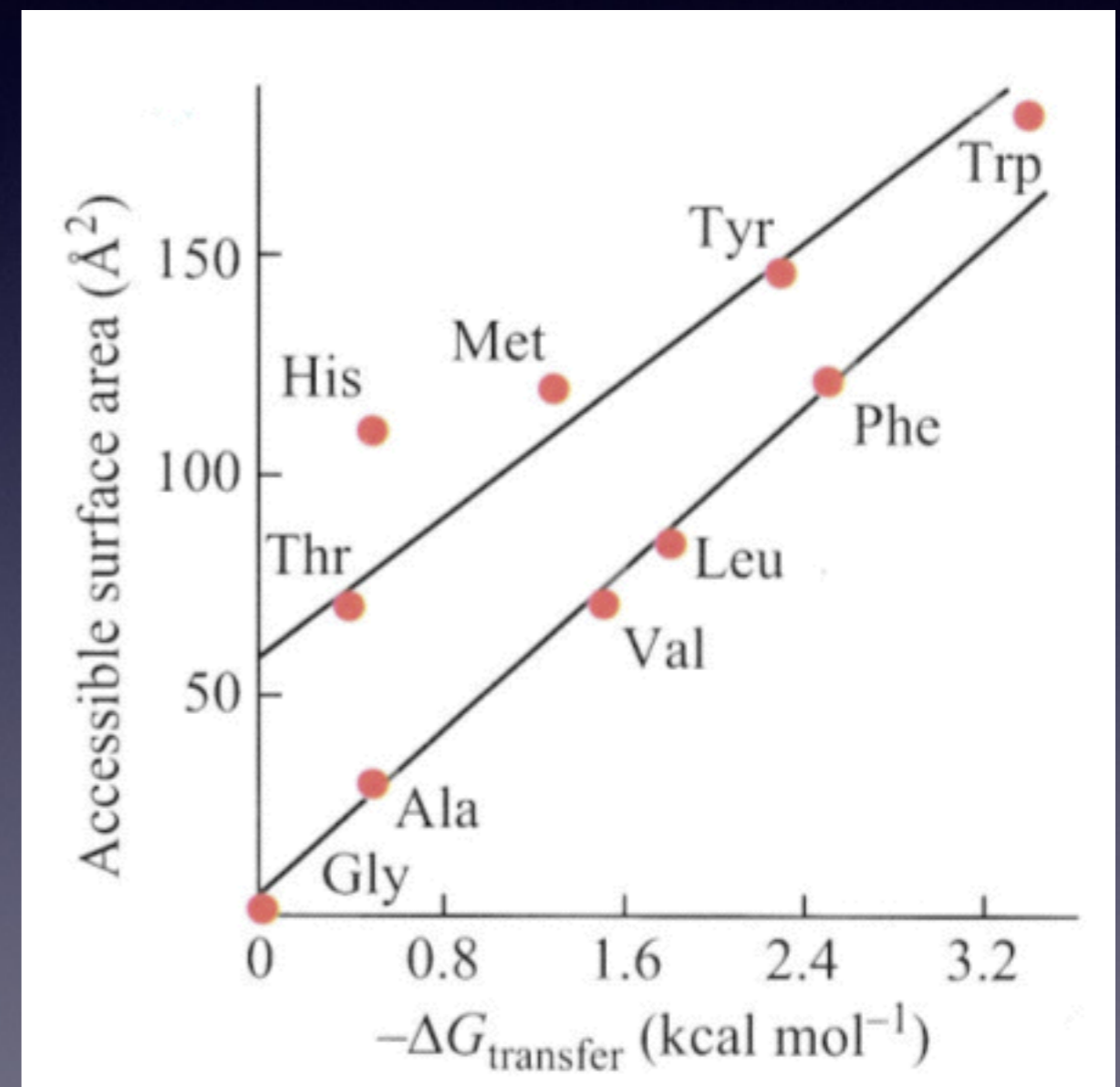
Probe radius 1.4Å

“Solvent accessible surface area”

# Amino acid area



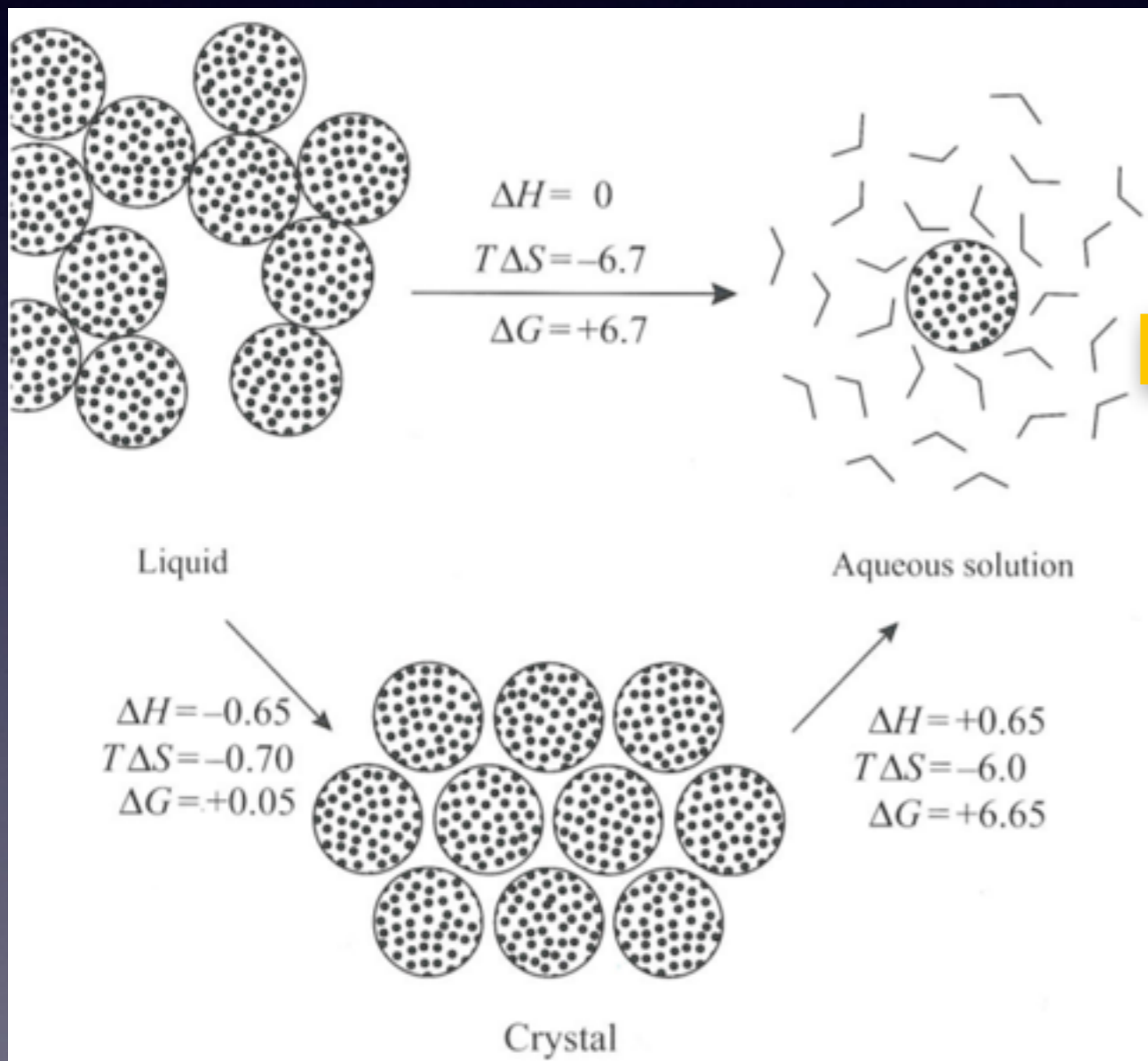
- For amino acids, we get very good agreement if we remove  $\sim 50\text{\AA}^2$  per polar atom!



# Hardening of structure

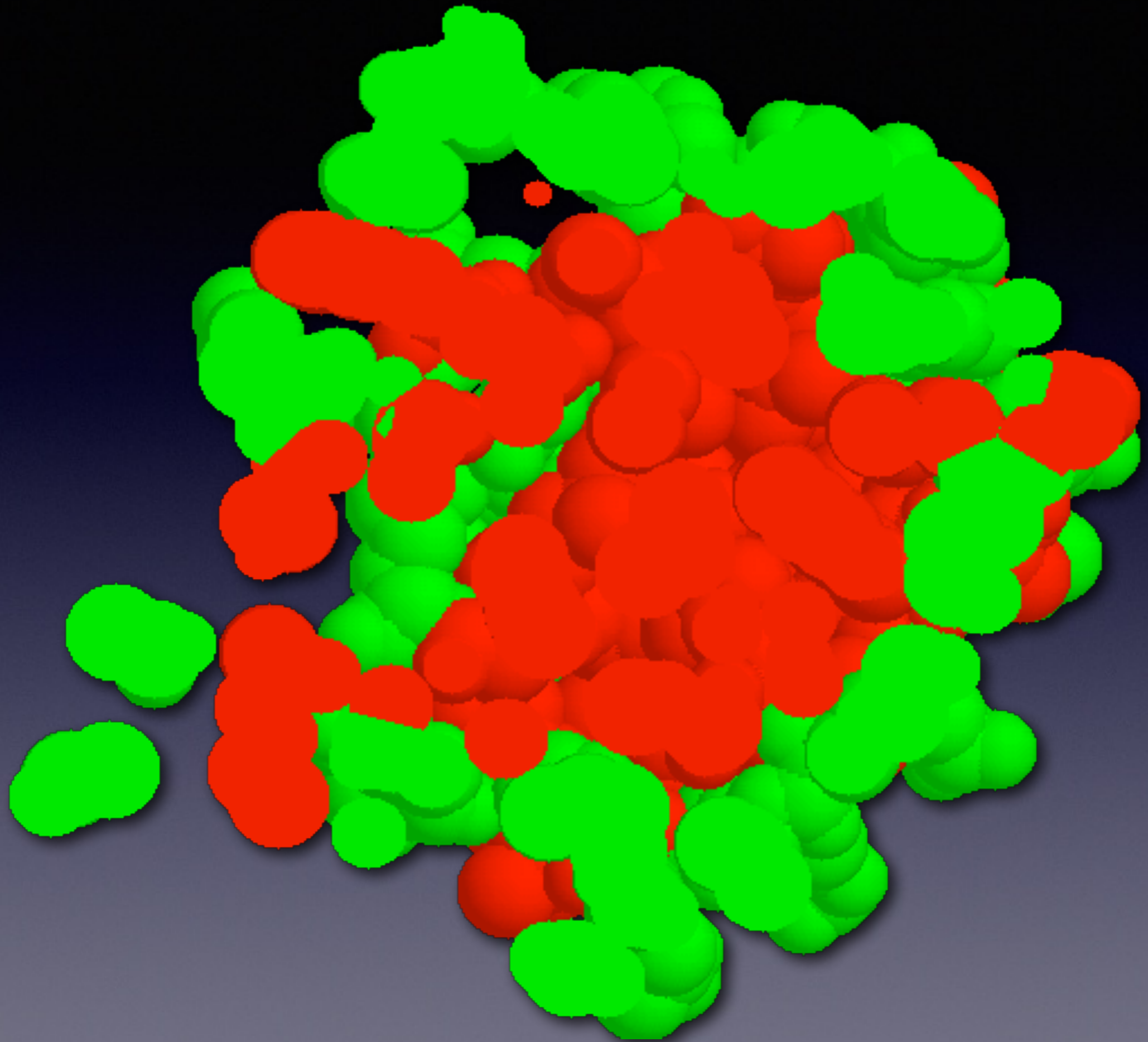


- What happens after hydrophobic collapse?

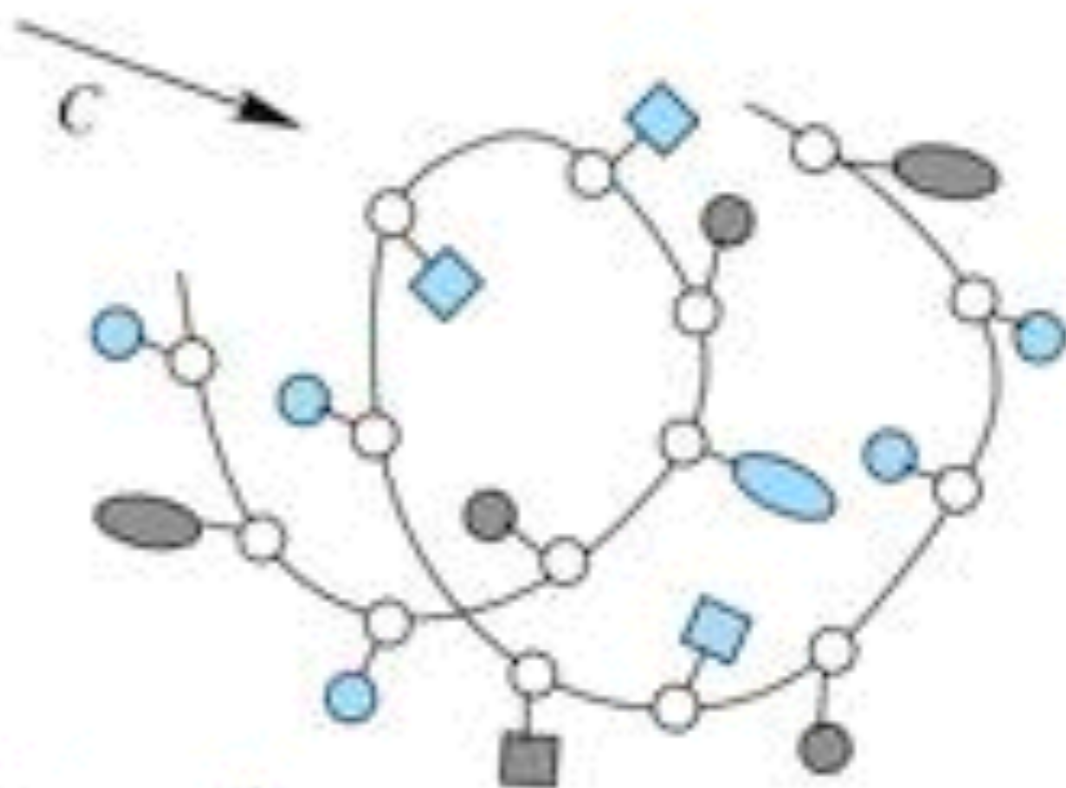
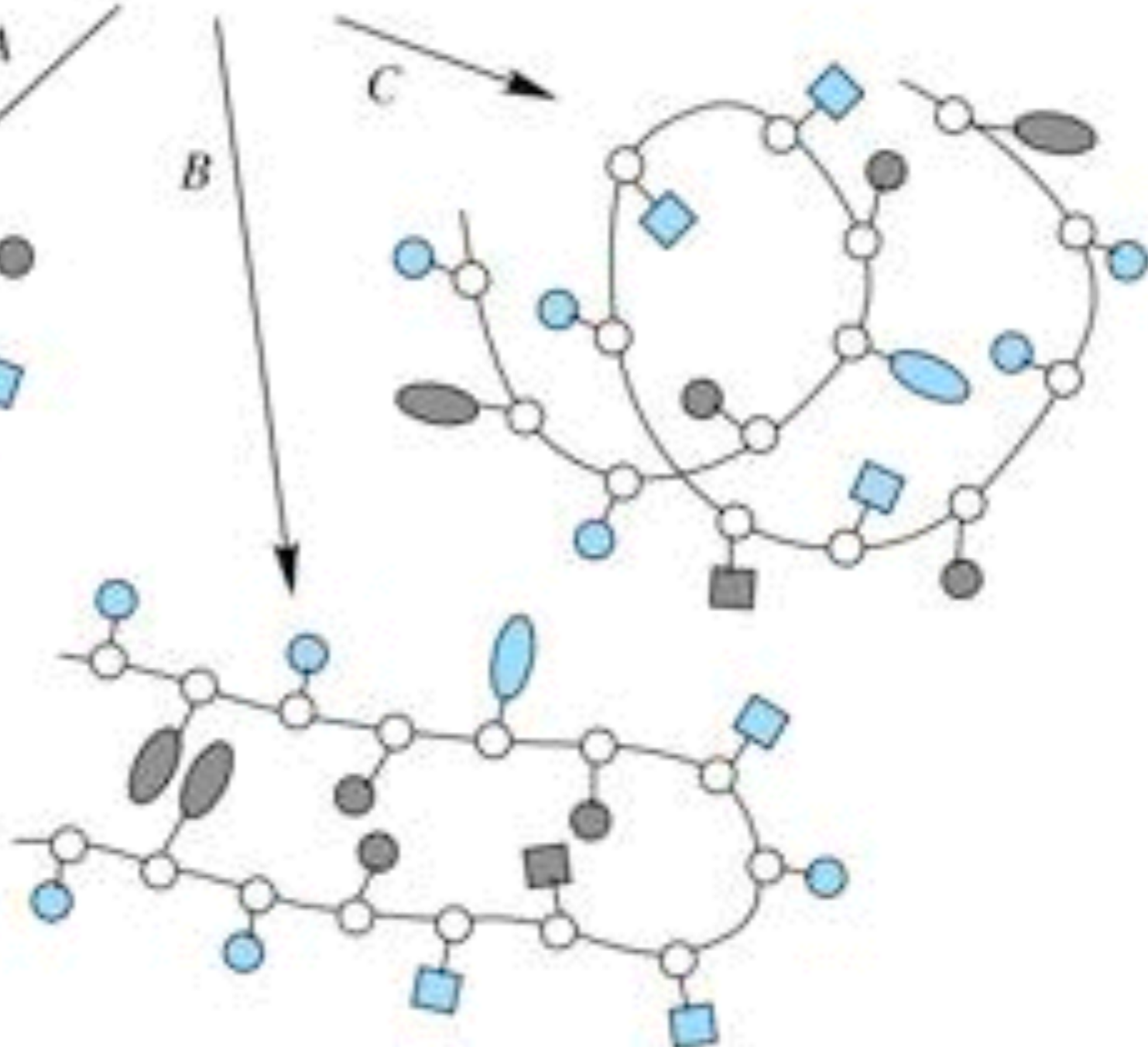
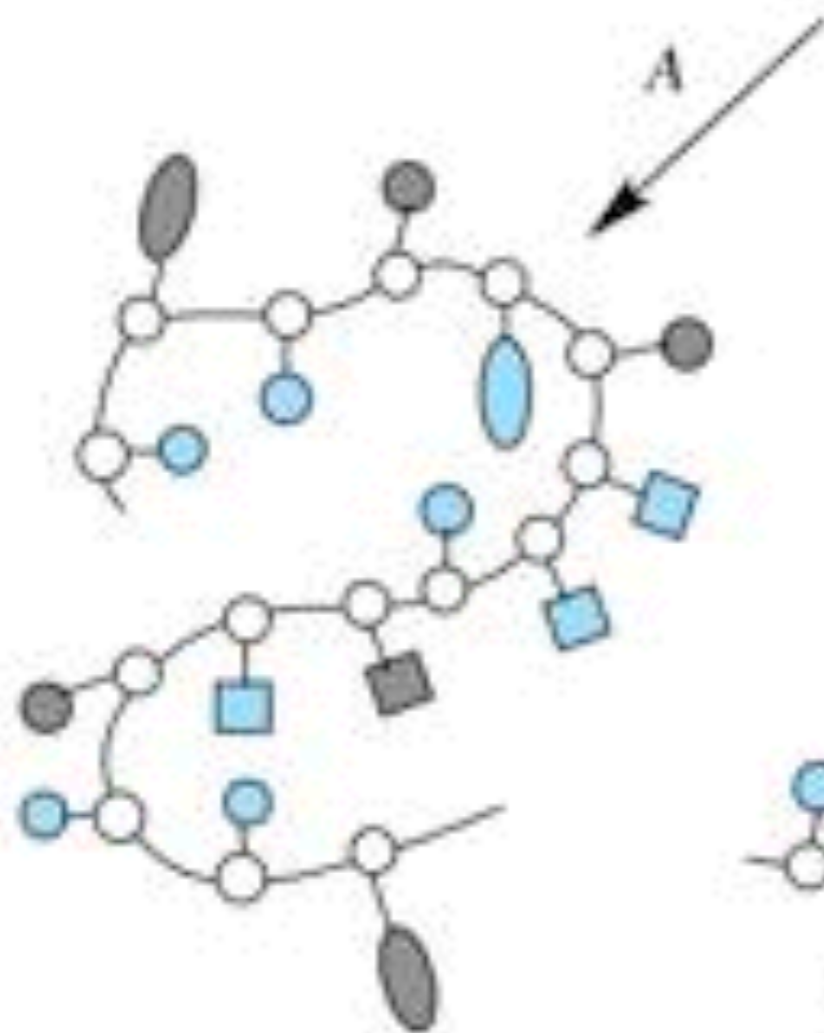
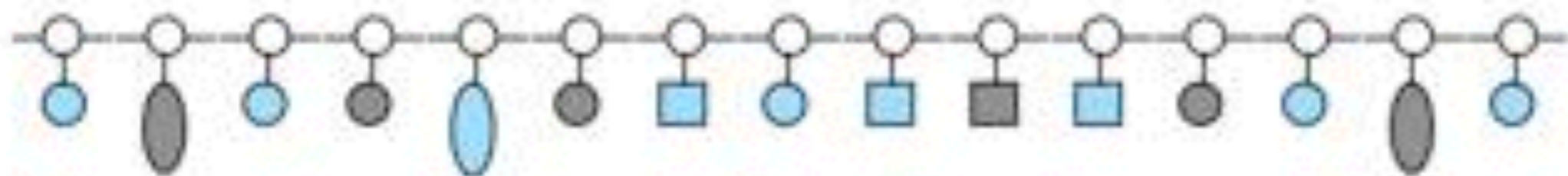


Once we have a separate hydrophobic phase, the cost is very low to “harden” it, or even form a crystal

What does this mean for proteins?



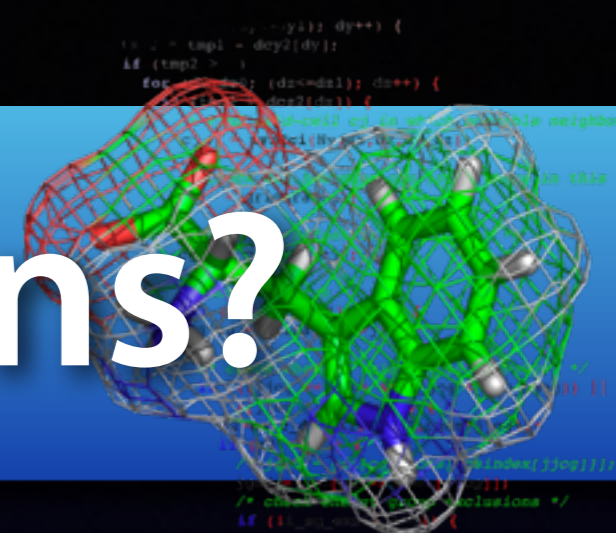




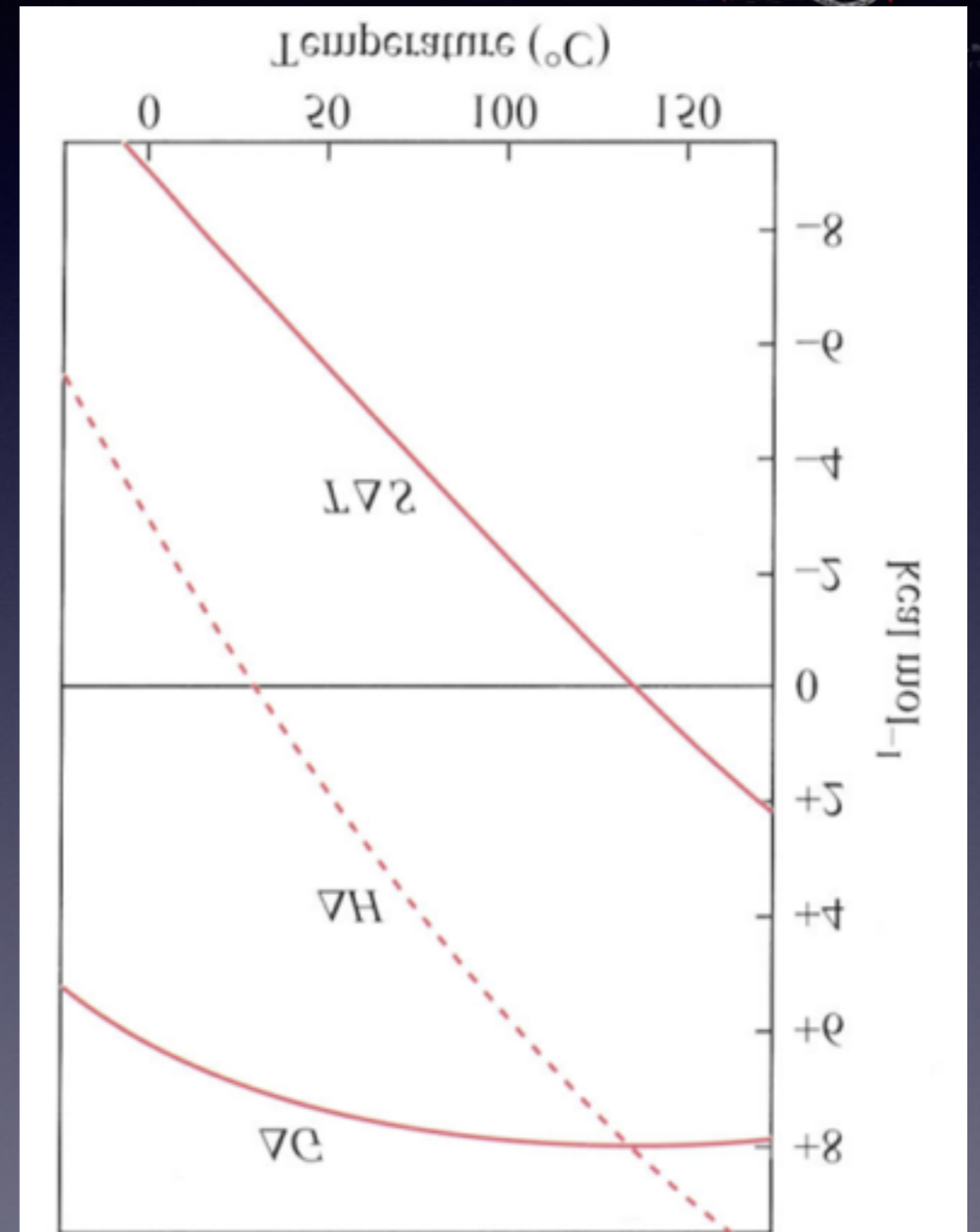
Hydrophobic ●

Hydrophilic ●

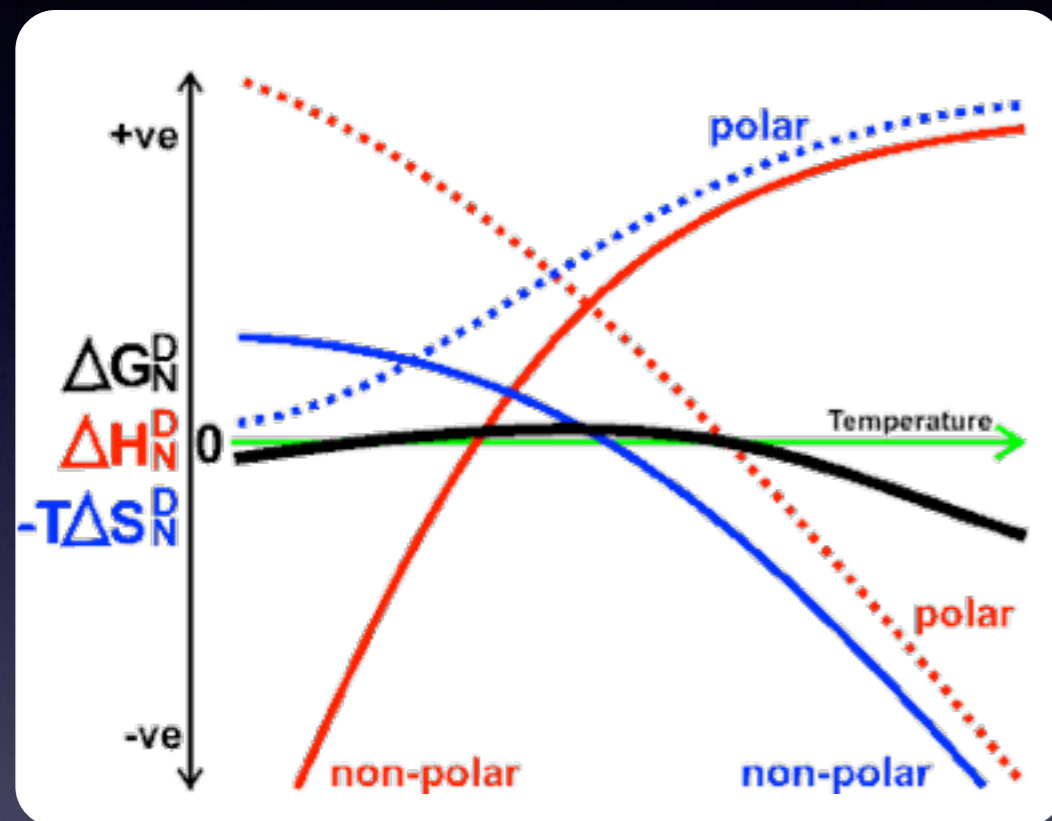
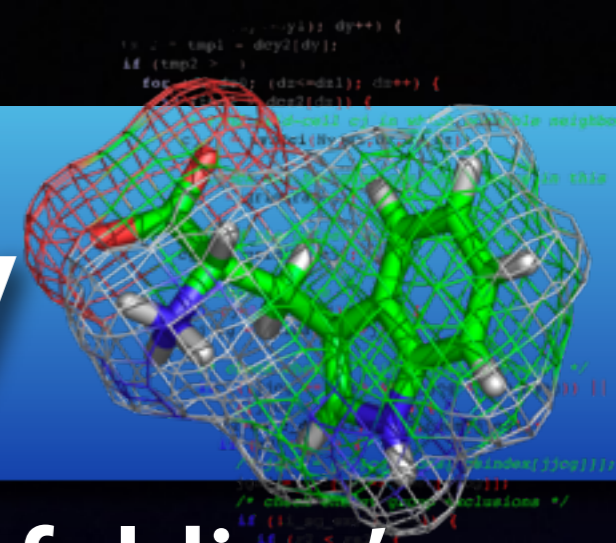
# What about proteins?



- Folding moves hydrophobic residues from water to liquid/interior phase
- Opposite process to solvation, so we use the opposite sign
- Or....
- Flip the plots!



# Protein stability

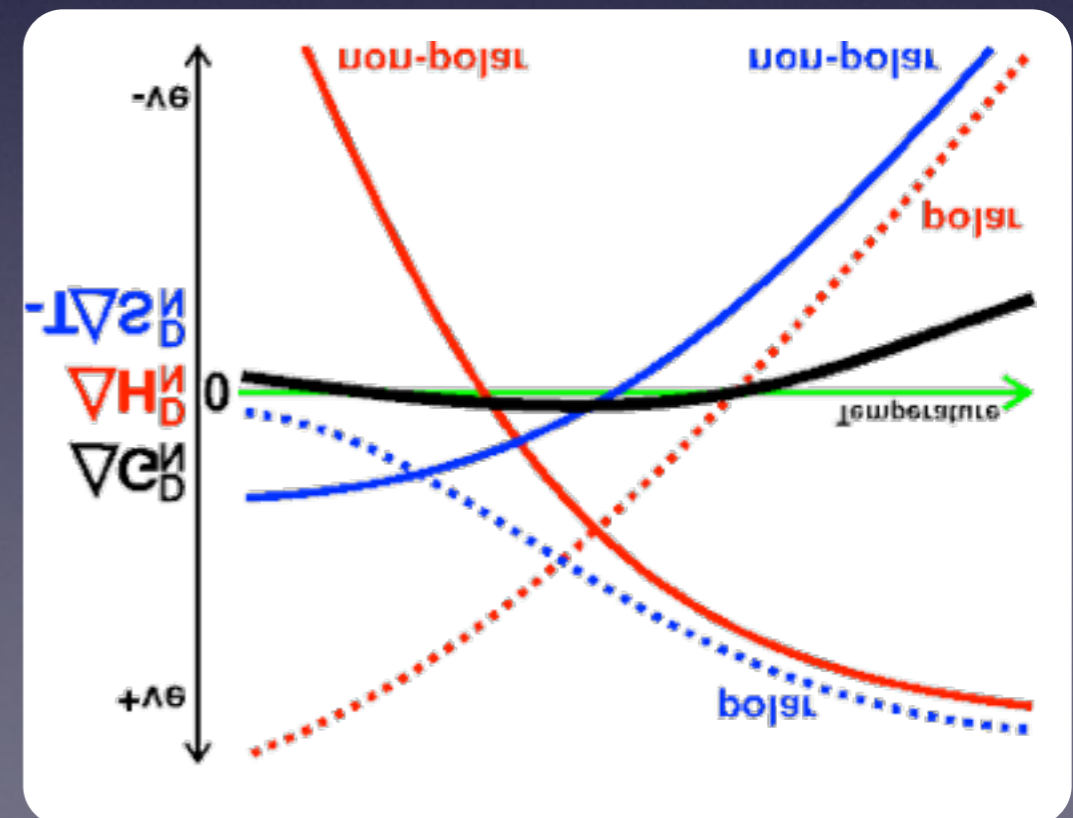


Free energy of 'unfolding'

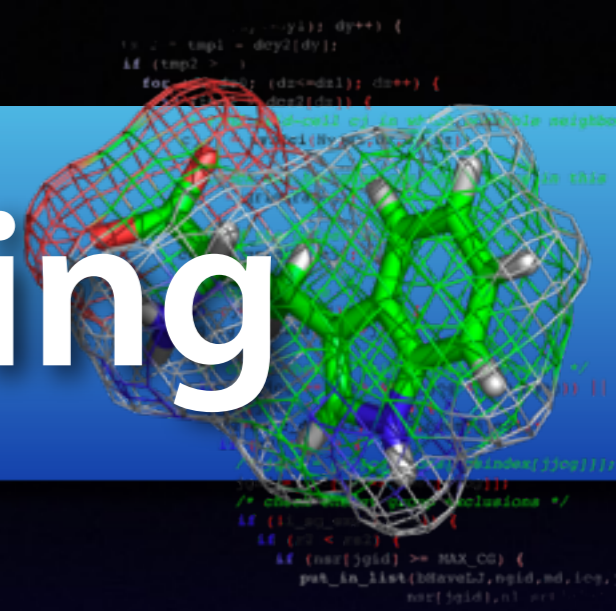
Solvate hydrocarbon in water,  
like we did earlier

Free energy of 'folding'  
(flipped y axis)

Going from water to hydrocarbon,  
which is the opposite process



# $\Delta G$ of Protein Folding

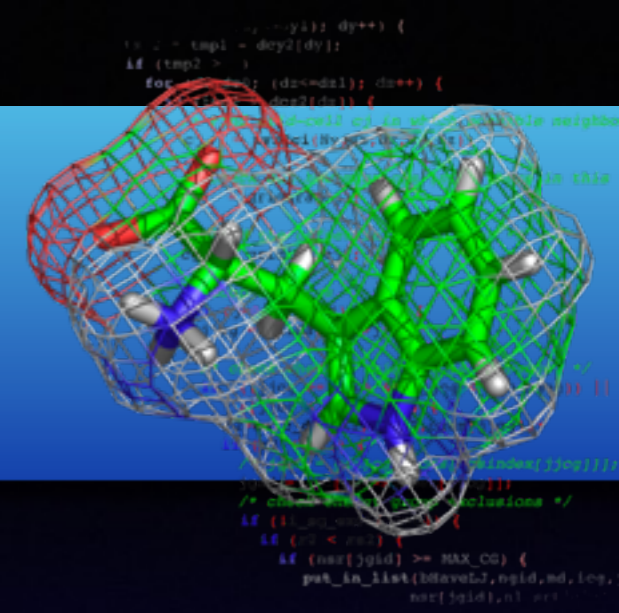


**90% Hydrophobic effect**

**10% “Polishing”**

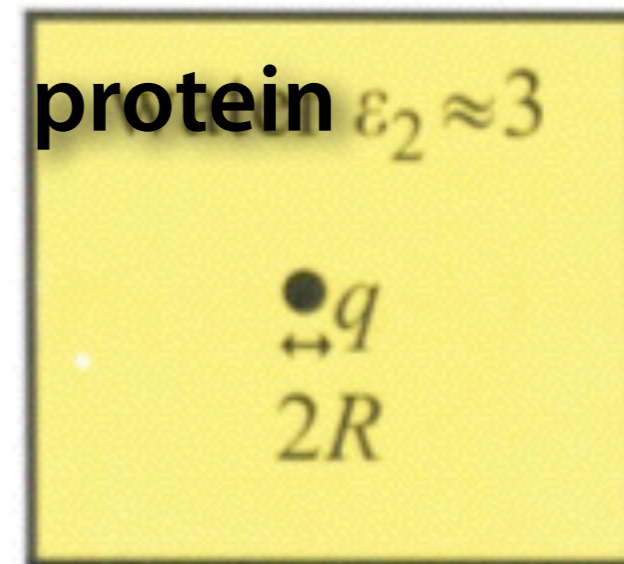
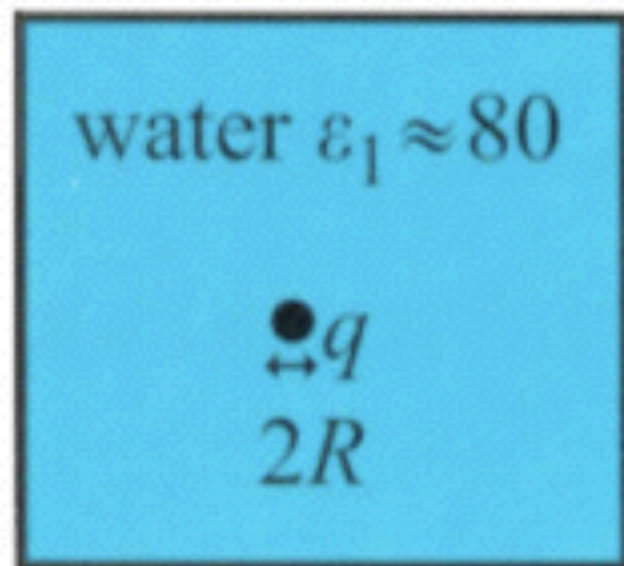
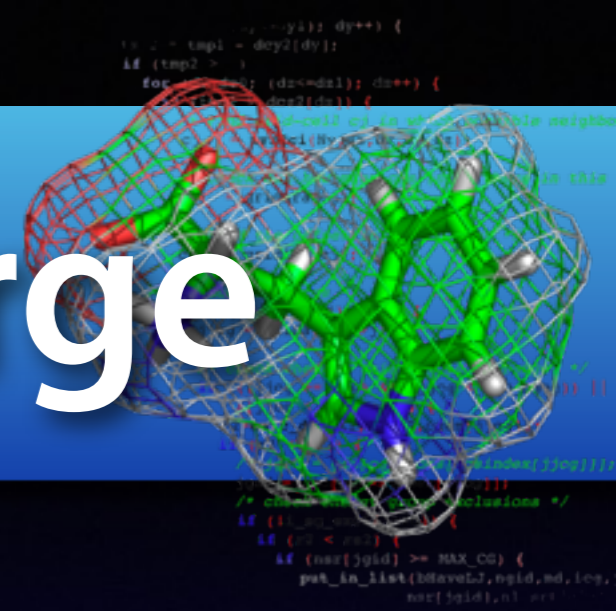
**(Van der Waals packing)**

# Electrostatics



- So, hydrogen bonds are important
- Governed by electrostatics
- $V = q_1 q_2 / \epsilon r$
- What is  $\epsilon$  for us?

# Cost of forming charge



$$\Delta U_{1 \rightarrow 2} = \frac{q^2}{2\epsilon_2 R} - \frac{q^2}{2\epsilon_1 R}$$

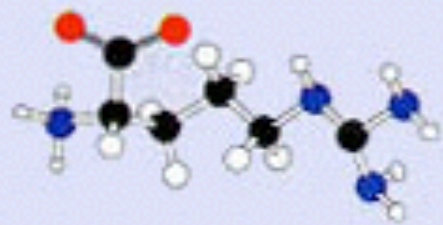
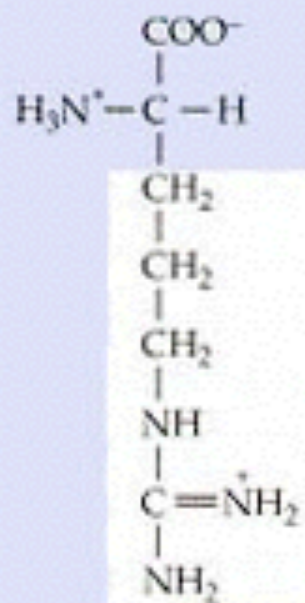
# Charged amino acids



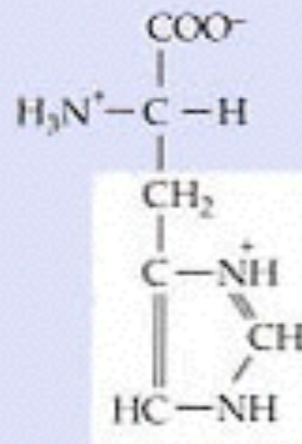
## A. Amino acids with electrically charged side chains

### Positive

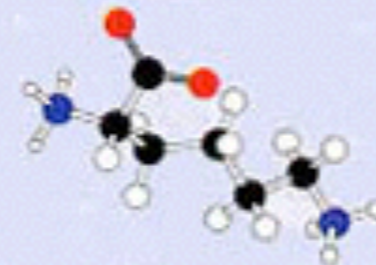
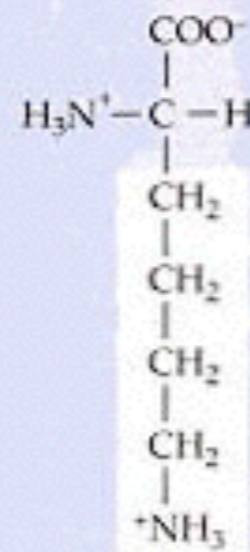
Arginine  
(Arg)



Histidine  
(His)

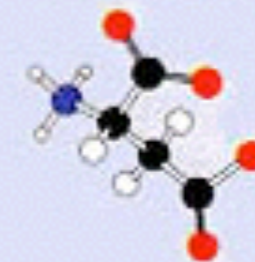
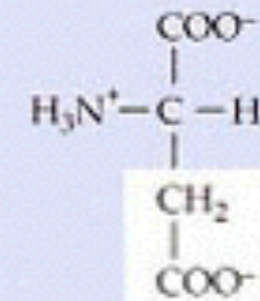


Lysine  
(Lys)

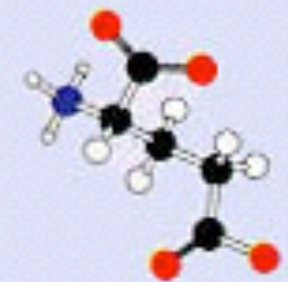
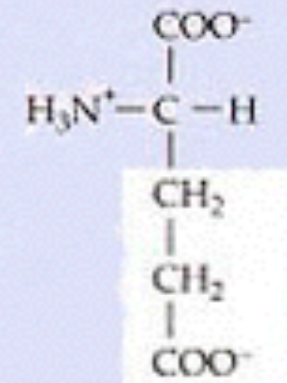


### Negative

Aspartic acid  
(Asp)

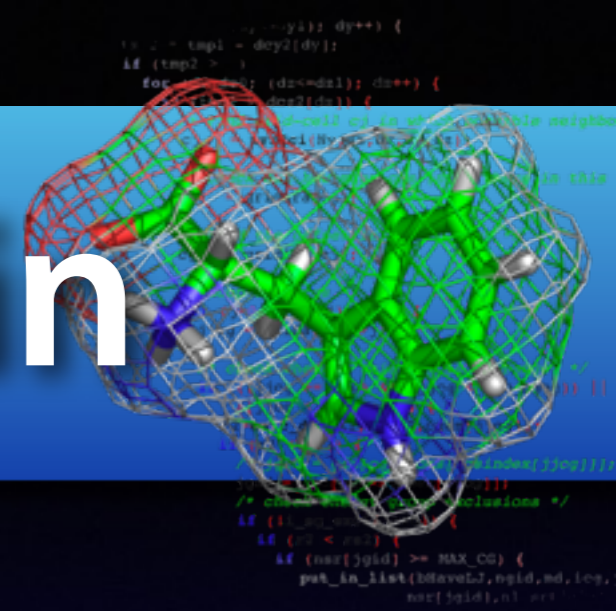


Glutamic acid  
(Glu)



'Titratable'

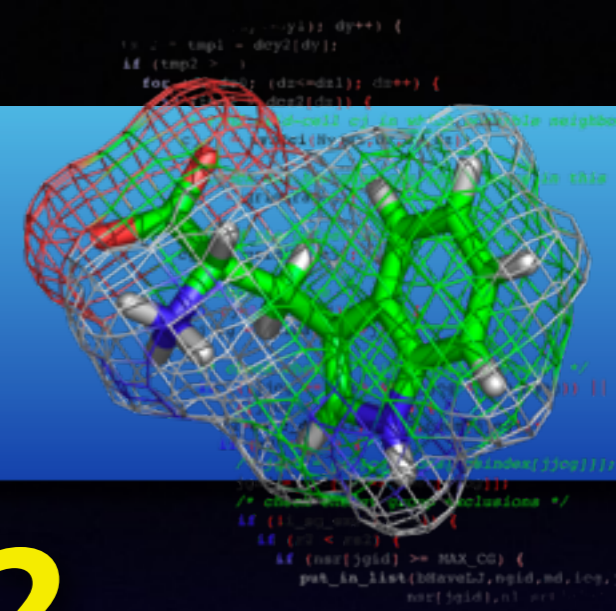
# Charges in protein



- It costs roughly 40kcal to introduce a unit charge in a protein ( $\epsilon=3$ )!
- Compare to  $\sim 1.5$ kcal in water ( $\epsilon=80$ )
- In practice, charges are rare inside proteins
- Titratable amino acids typically uncharged instead.



# Compare



Hydrogen bonds?

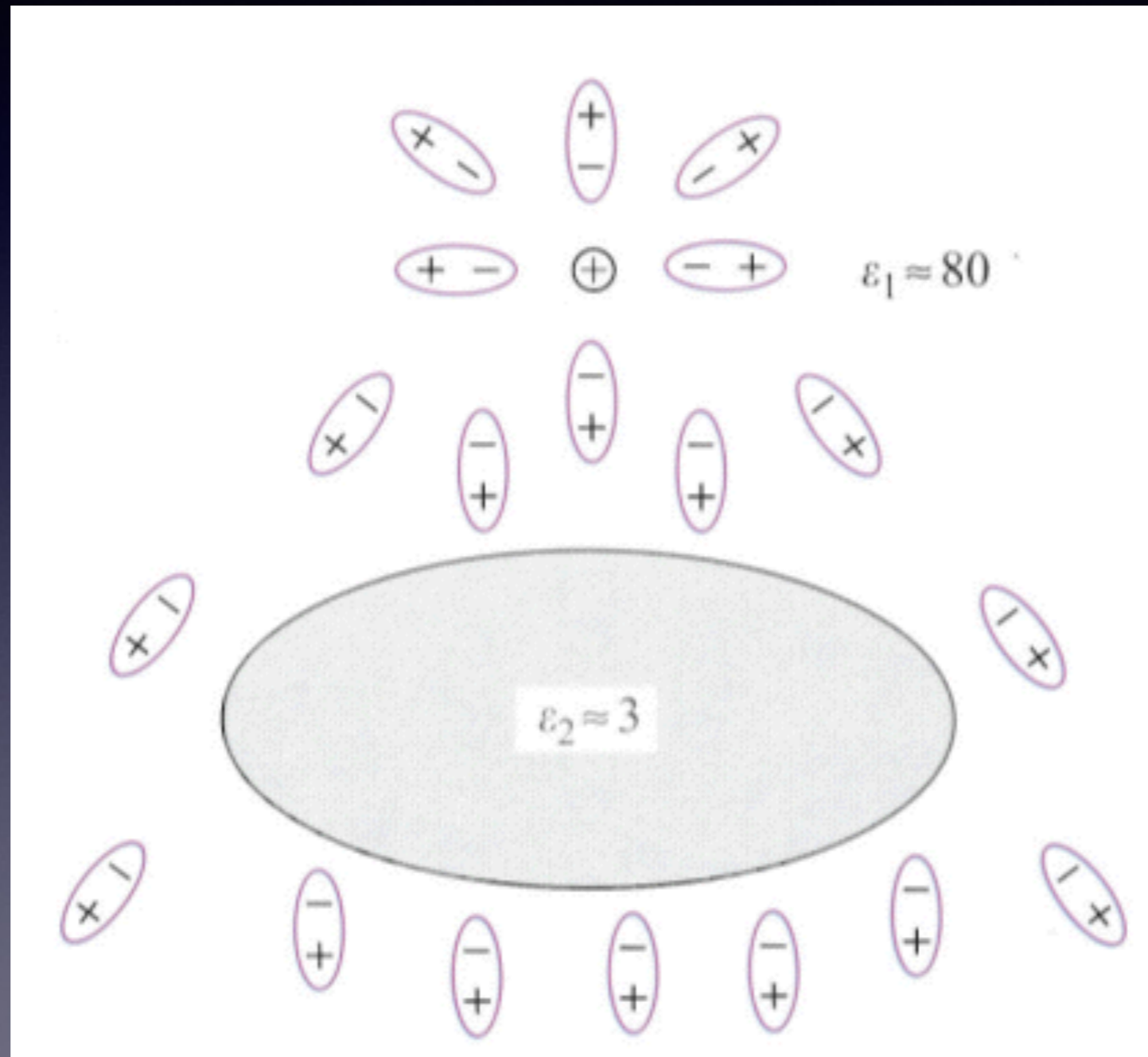
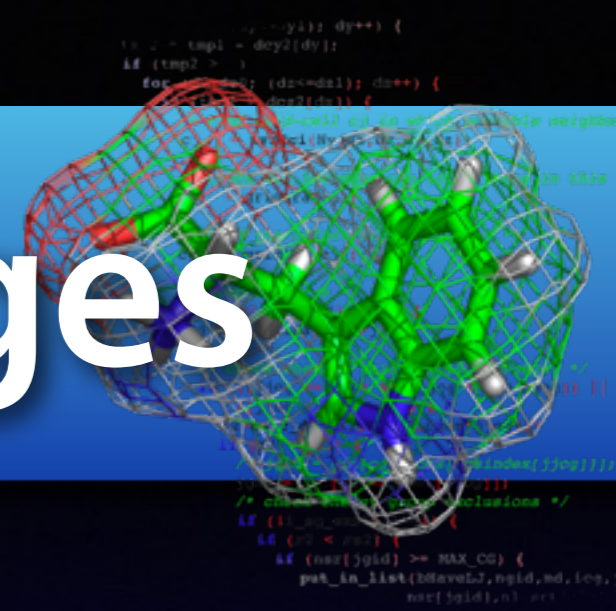
$kT$ ?

(thermal energy)

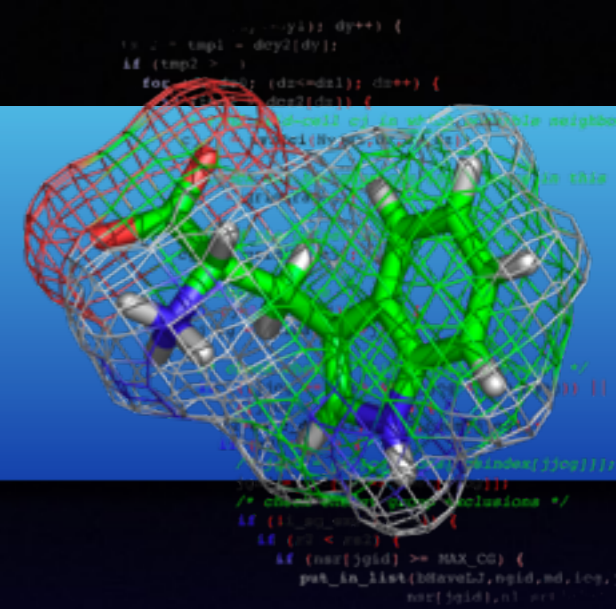
Stability of a protein?

**What is  $\epsilon$  in a  
protein?**

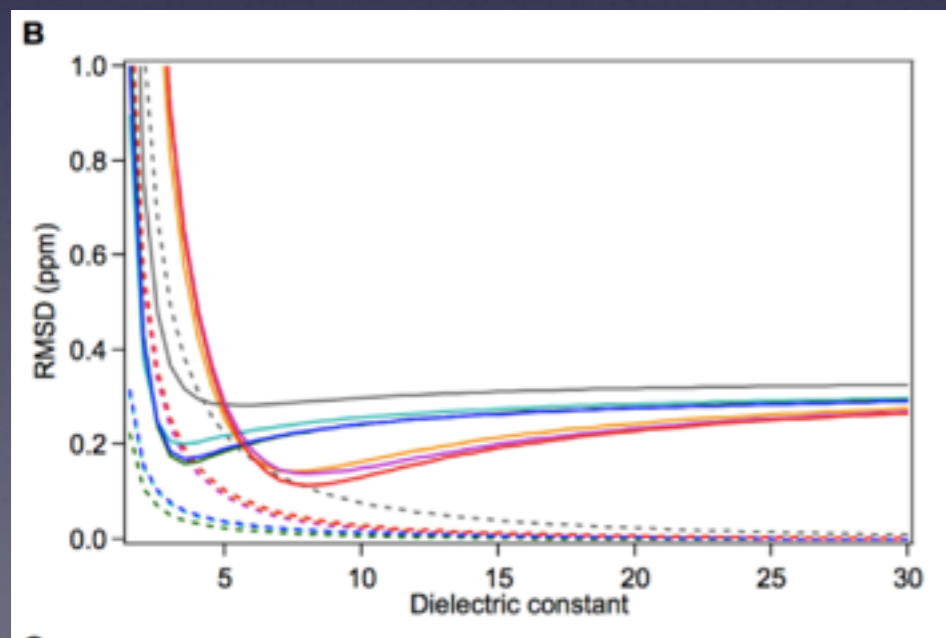
# Screening of charges



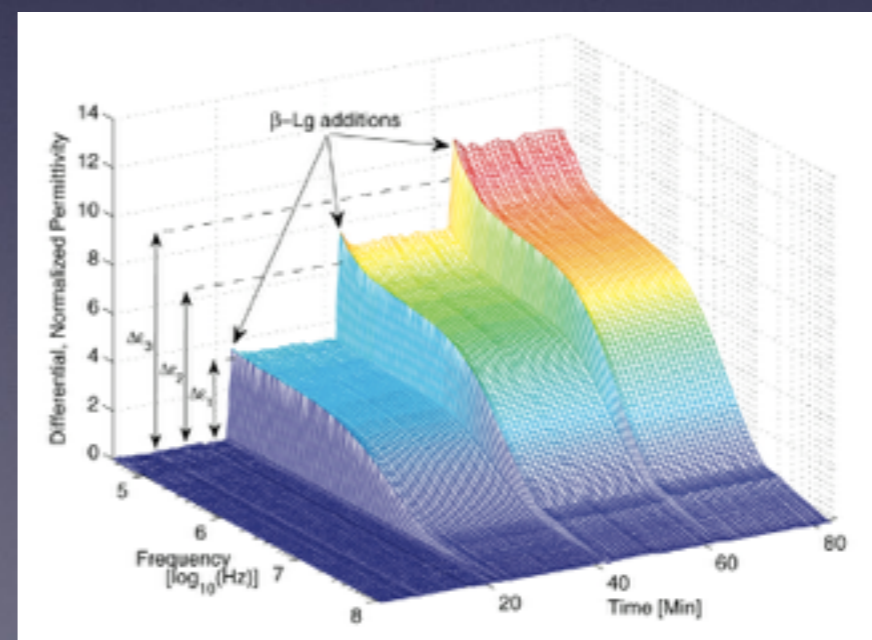
# Electrostatics



# Permittivity, $\epsilon$ (farads/m)

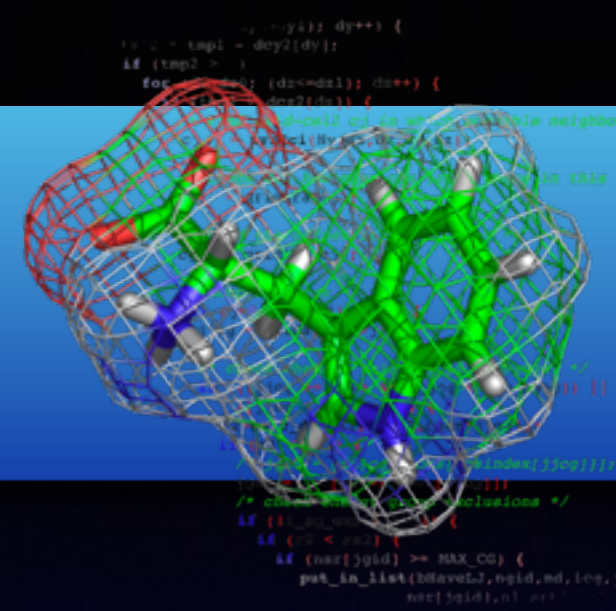


Jens Erik Nielsen, JACS, 2013



Brian Mazzeo, JPCB, 2011

# Electrostatics on the atomic level



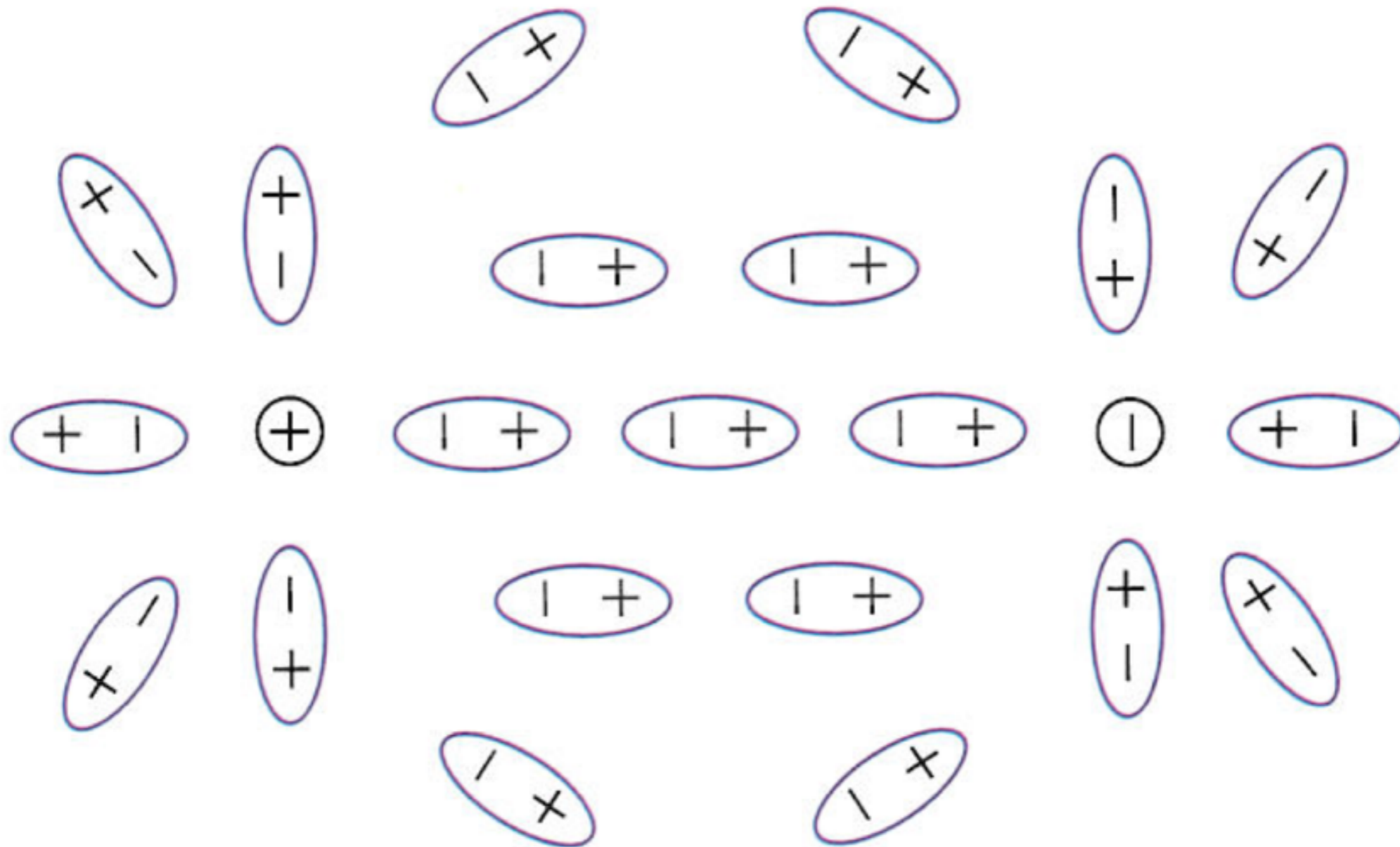
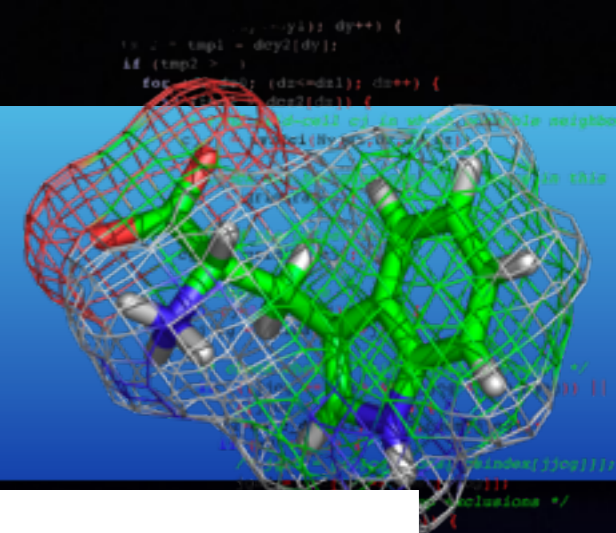
## Vacuo



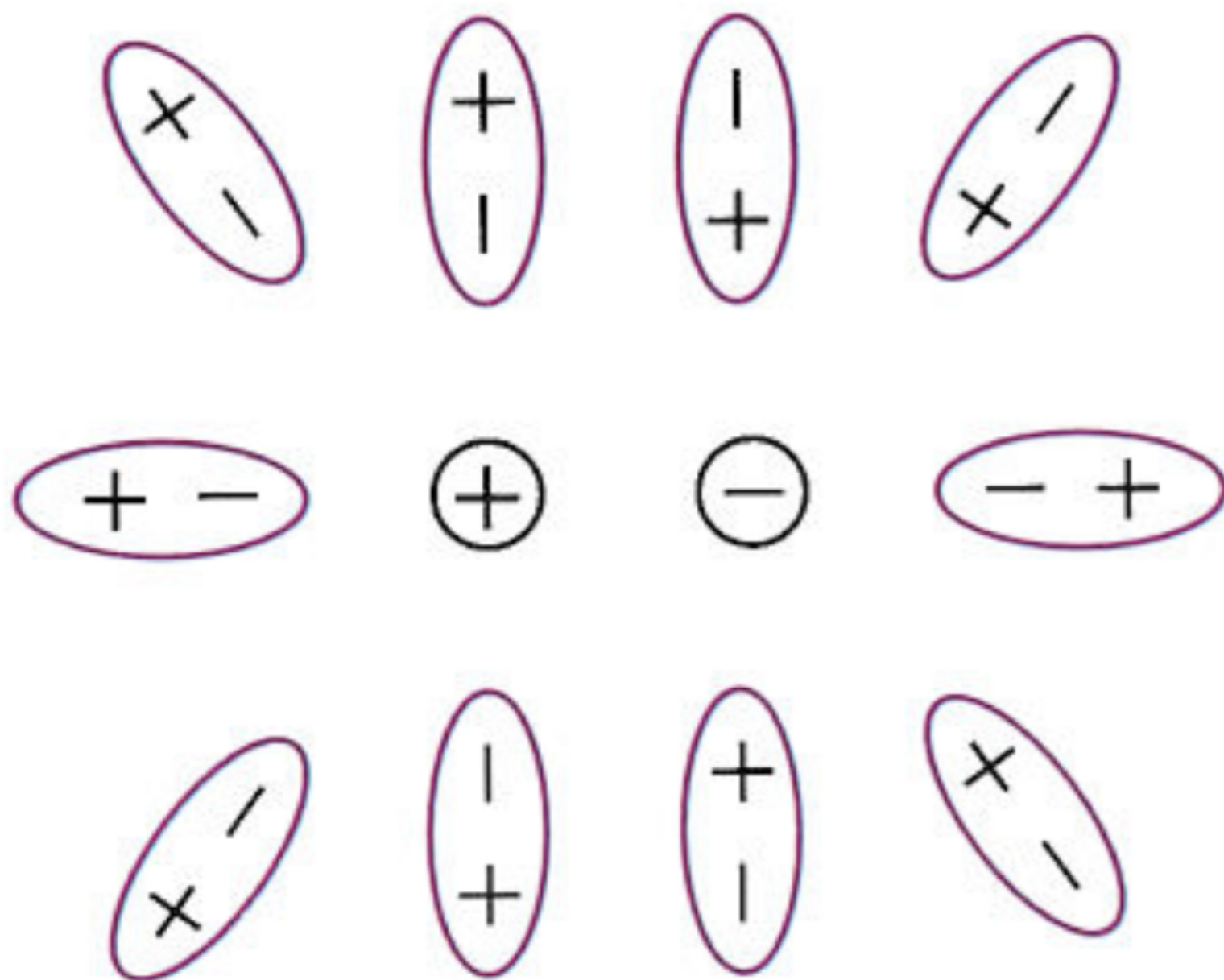
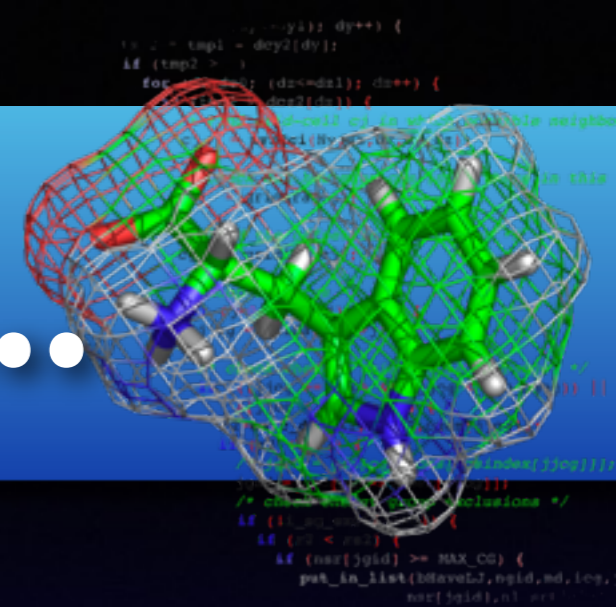
$$V = q_1 q_2 / \epsilon_0 r$$



# In a medium

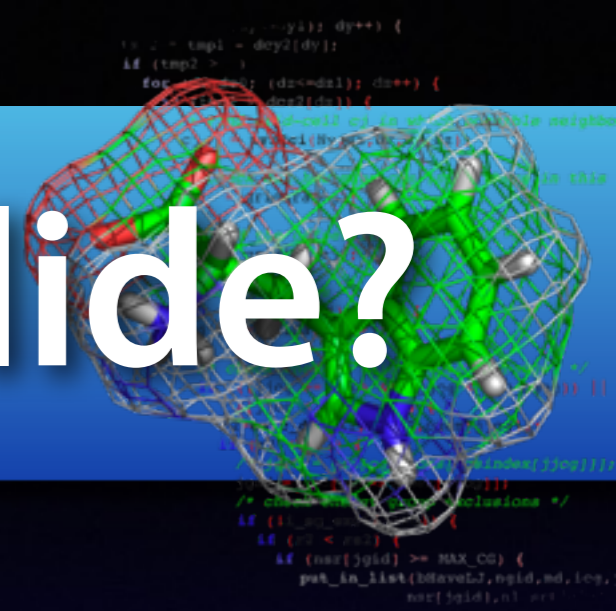


# And even closer...



# ε?

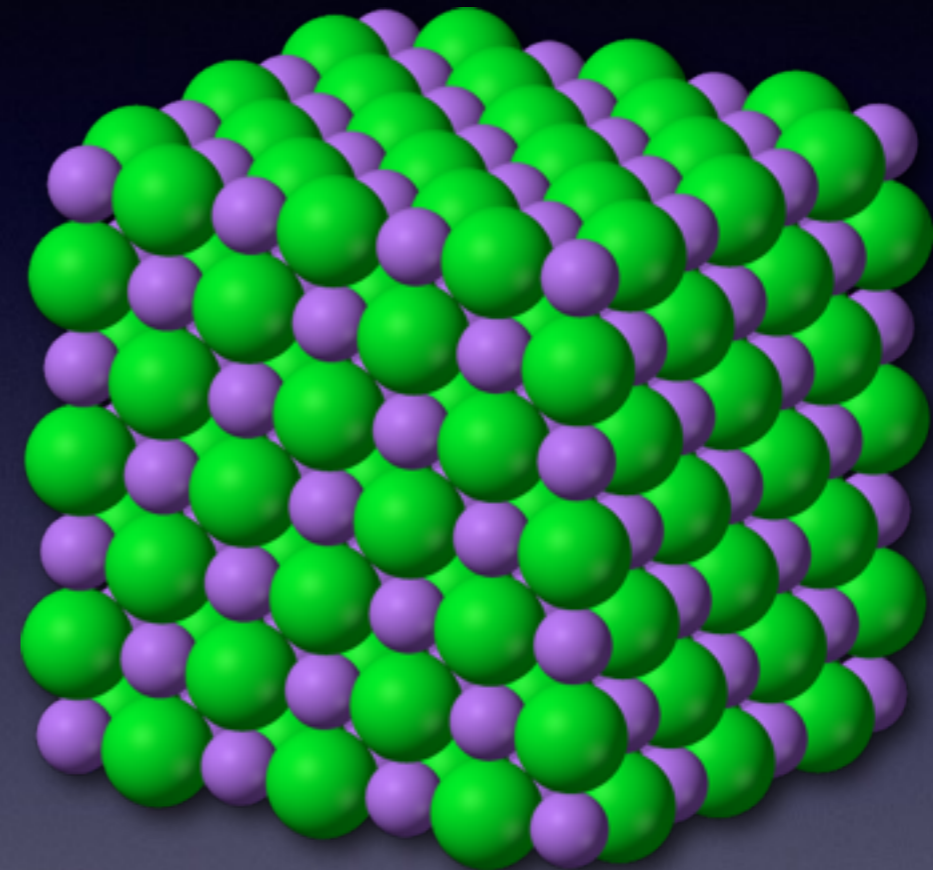
# What is $\epsilon$ in the last slide?



- A)  $\epsilon \cong 1$       100 kcal/mol
- B)  $\epsilon \cong 3-4$       30 kcal/mol
- C)  $\epsilon \cong 20$       6 kcal/mol
- D)  $\epsilon \cong 40-80$       1.5 kcal/mol



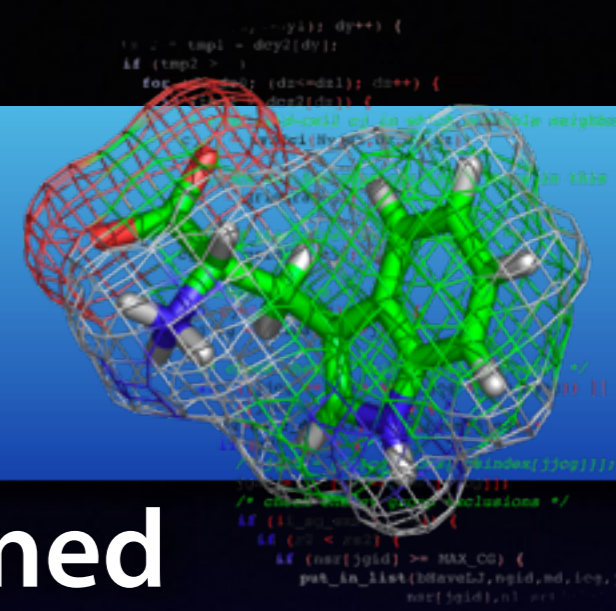
# Salt solubility in water



Energy between two charges  
at 3Å with  $\epsilon=80$ : 1.5kcal/mol

Compare with hydrogen bonds!

# Summary



- Protein folding is largely determined by hydrophobicity
- Hydrophobic effect
- Applications of enthalpy, entropy
- Free Energy of processes
- Protein folding, “molten globule”
- Electrostatics in water is mostly entropy!
- Chapters 5 & 6 in the Protein Physics book