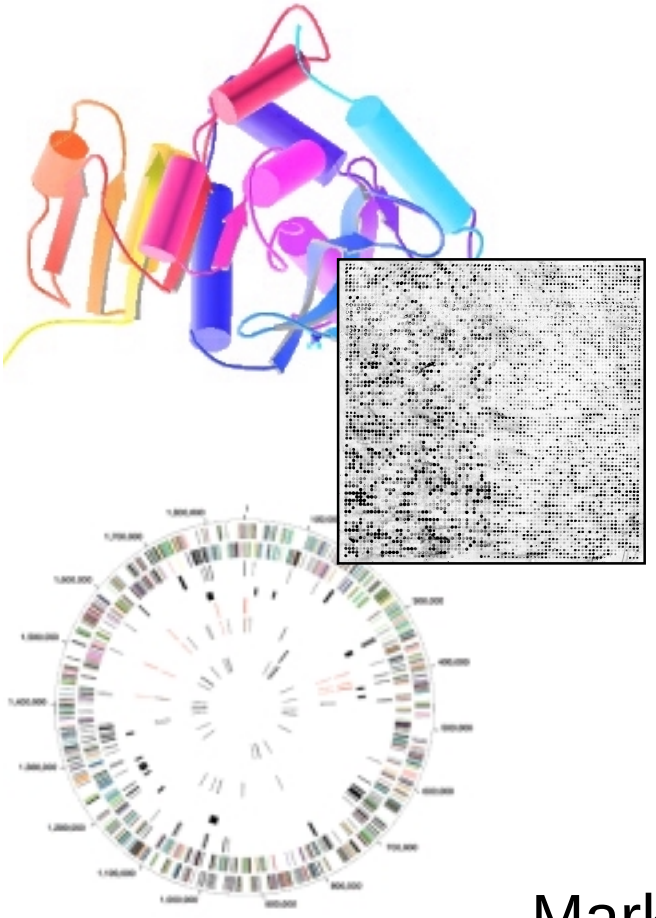
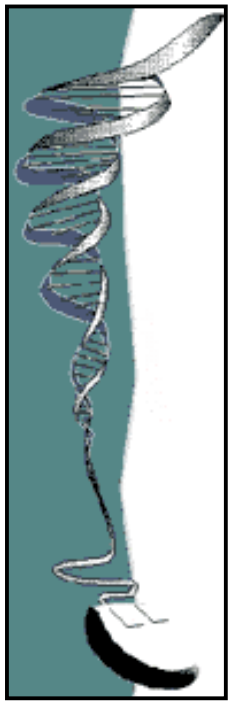
BIOINFORMATICS
Simulation





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Overview:
Electrostatics + Basic Forces

- Electrostatics
  - ◊ Polarization
  - ◊ Multipoles, dipoles
  - ◊ VDW Forces
  - ◊ Electrostatic Interactions
- Basic Forces
  - ◊ Electrical non-bonded interactions
  - ◊ bonded, fundamentally QM but treat as springs
  - ◊ Sum up the energy

- Simple Systems First

Overview:
Methods for the Generation and Analysis of Macromolecular Simulations

1 Simulation Methods
- ◊ Potential Functions
- ◊ Minimization
- ◊ Molecular Dynamics
- ◊ Monte Carlo
- ◊ Simulated Annealing

2 Types of Analysis
- ◊ liquids: RDFs, Diffusion constants
- ◊ proteins: RMS, Volumes, Surfaces

- Established Techniques (chemistry, biology, physics)
- Focus on simple systems first (liquids). Then explain how extended to proteins.

Electric potential, a quick review

- E = electric field = direction that a positive test charge would move
- Force/q = E
- Potential = W/q = work per unit charge = Fx/q = Ex
  - ◊ E = - grad ϕ ; E = ($d\phi/dx$, $d\phi/dy$, $d\phi/dz$)

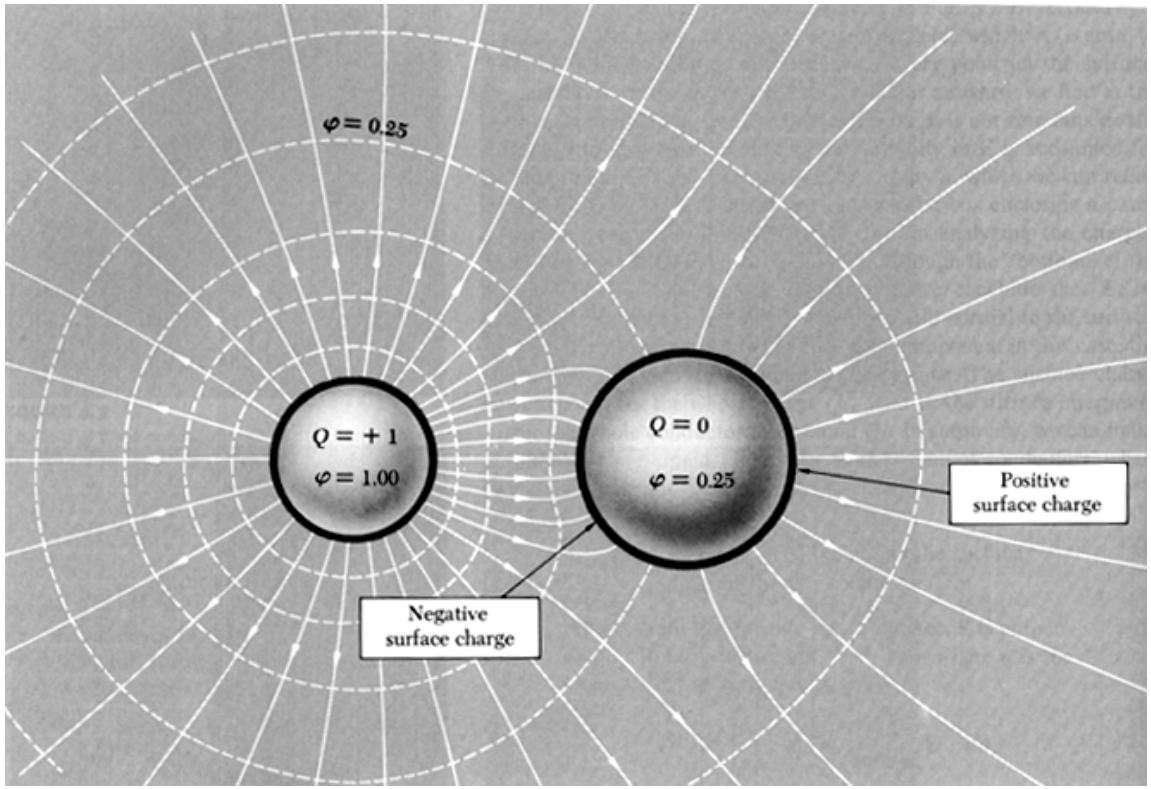


Illustration Credit: Purcell

Maxwell's Equations

- 1st Pair (curl's)
  - ◊ A changing electric field gives rise to magnetic field that circles around it & vice-versa. Electric Current also gives rise to magnetic field. [no discuss here]
- 2nd Pair (div's)
  - ◊ Relationship of a field to sources
  - ◊ no magnetic monopoles and magnetostatics: div B = 0 [no discuss here]
- All of Electrostatics in Gauss's Law!!

$$\text{curl } \mathbf{E} = -\frac{1}{c}\frac{\partial \mathbf{B}}{\partial t}$$

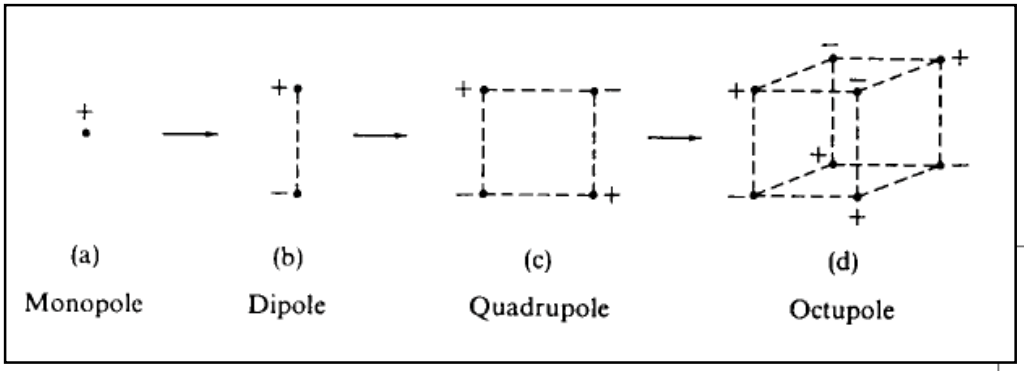
$$\text{curl } \mathbf{B} = \frac{1}{c}\frac{\partial \mathbf{E}}{\partial t} + \frac{4\pi}{c}\mathbf{J}$$

$$\text{div } \mathbf{E} = 4\pi\rho$$

$$\text{div } \mathbf{B} = 0$$

cgs (not mks) units above

Multipole Expansion



- Routinely done when an atom's charge distribution is replaced by a point charge or a point charge and a dipole
  - ◊ Ignore above dipole here
  - ◊ Harmonic expansion of pot.
- Only applicable far from the charge distribution
  - ◊ Helix Dipole not meaningful close-by
- Terms drop off faster with distance

$$\Phi(\mathbf{x}) = \frac{q}{r} + \frac{\mathbf{p} \bullet \mathbf{x}}{r^3} + \frac{1}{2}\sum_{i,j} Q_{ij} \frac{x_i x_j}{r^5} + \cdots$$

$$\Phi(\mathbf{x}) = \frac{K_1 q}{r} + \frac{K_2 q}{r^2} + \frac{K_3 q}{r^3} + \cdots$$

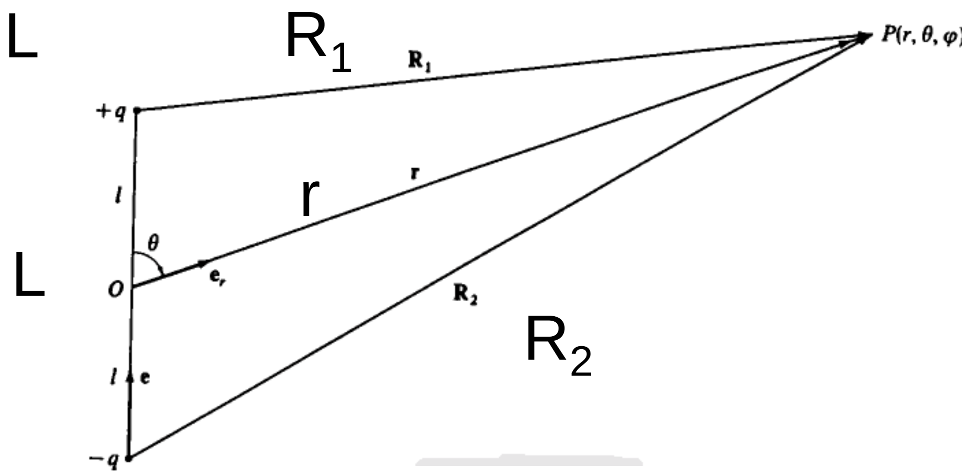
Replace continuous charge distribution with point moments: charge (monopole) + dipole + quadrupole + octupole + ...

Gauss' Law: Electrostatics

- div **E** = $4\pi\rho$
- Coulomb's Law
  - ◊ $\int$ div **E** dV = $\int 4\pi\rho$ dV
  - ◊ $\int$ **E** • d**A** = $\int 4\pi\rho$ dV [Divergence thm.]
  - ◊ Assume spherically symmetrical charge distribution
  - ◊ E ($4\pi r^2$) = 4π Q ==> E = Q/r^2
  - ◊ U = - Q/r [assuming a zero at inf.]
- Equations for the Potential Based on the Charge in a Region plus Boundary Conditions
  - ◊ div grad U = $4\pi\rho$
  - ◊ $\nabla^2 U = 4\pi\rho$ [poisson's equation]
  - ◊ $\nabla^2 U = 0$ [Laplace's equation]

Dipole Derivation

- $\phi(r, \theta) = -q/R_1 + q/R_2$
  - ◊ $\phi(r, \theta) = q(R_1 - R_2)/ R_1R_2$
- If r is very much larger than L
  - ◊ Vectors essentially parallel, like single-slit
  - ◊ $R_1R_2 = r^2$
  - ◊ $R_2 - R_1 = 2L \cos\theta$
  - ◊ $q(R_2-R_1) = 2Lq\cos\theta = p \cos\theta$
    $= \mathbf{p} \bullet \mathbf{r}/|r|$
  - ◊ **p** = dipole moment vector
    = [charge][separation]
    in direction from neg. to positive charge
- $\phi(r, \theta) = p \cos\theta / r^2$
  - ◊ $E = \text{grad}\, \phi(r, \theta) \sim 1/r^3$ with a complex angular dependence
- Monopole is 1/r, which dominates over dipole ($1/r^2$), dipole dominates quadrupole



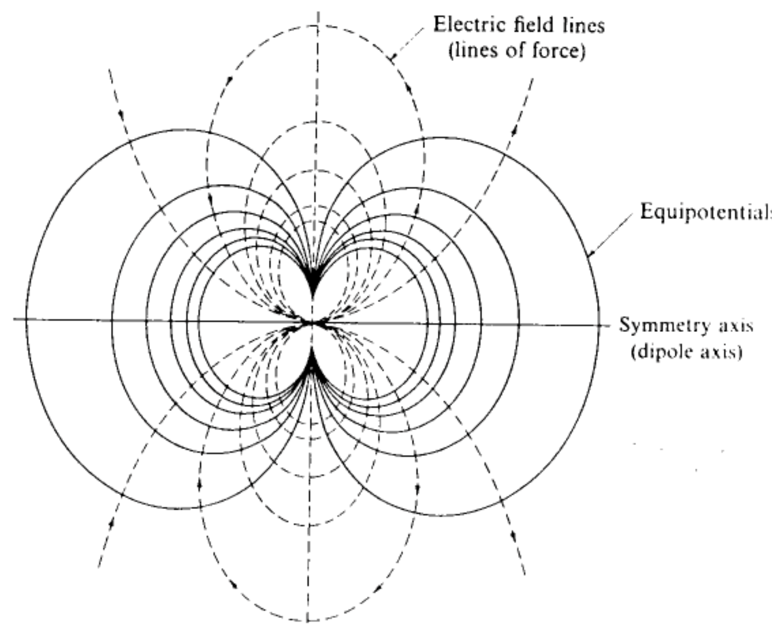
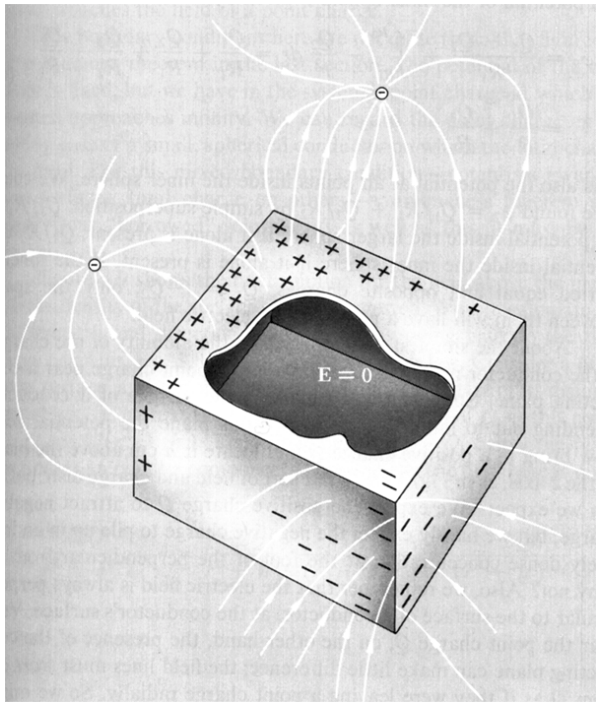
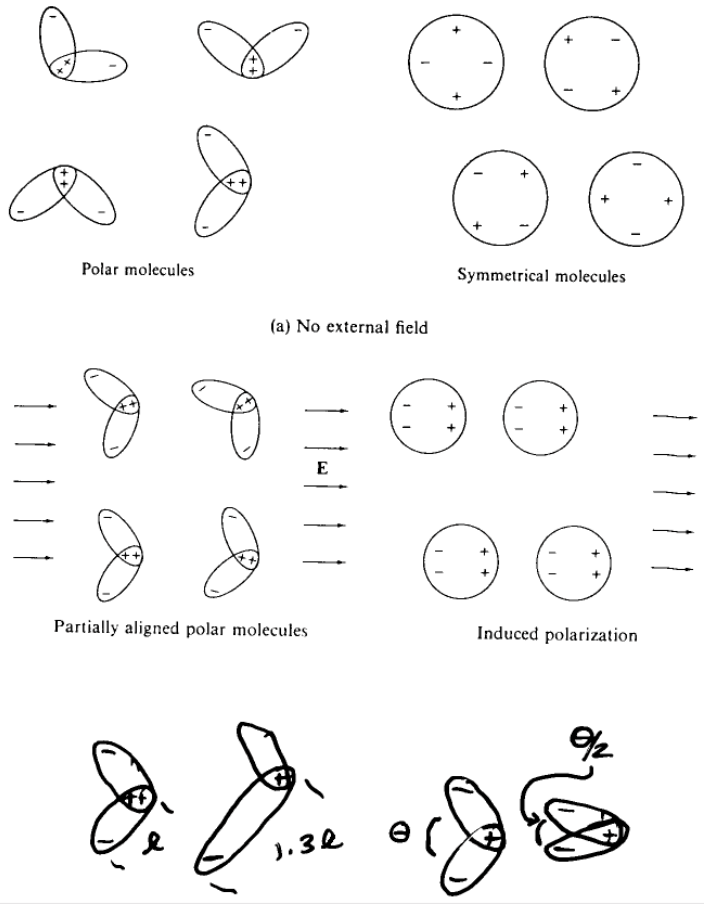


Illustration Credit: Marion & Heald

Polariz-ation





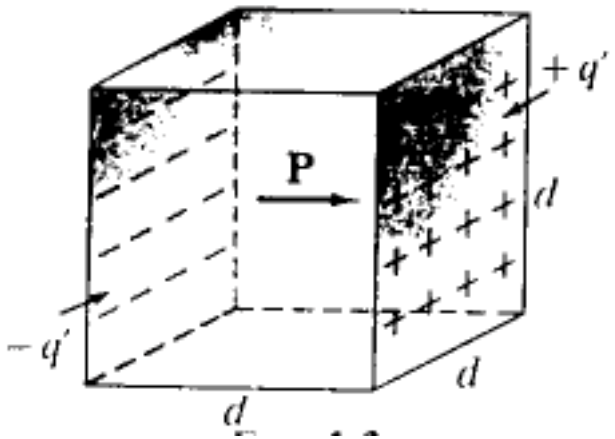
- Charge shifts to resist field
  - ◊ Accomplished perfectly in conductor -- surface charge, no field inside
  - ◊ Insulators partially accommodate via induced dipoles
- Induced dipole
  - ◊ charge/ion movement (slowest)
  - ◊ dipole reorient
  - ◊ molecular distort (bond length and angle)
  - ◊ electronic (fastest)

Illustration Credit: Purcell, Marion & Heald

Dielectric const.

- Macro manifestation of polarization
- Values
  (measured in debye)
  - ◊ Air, 1
  - ◊ Water, 80
  - ◊ Paraffin Wax, 2
  - ◊ Methanol, 33
  - ◊ Non-polar protein, 2
  - ◊ Polar protein, 4
- High-frequency
  - ◊ water re-orient, 1ps
  - ◊ bond, angle stretch
  - ◊ electronic, related to index of refraction

$$q'd = Pd^3$$



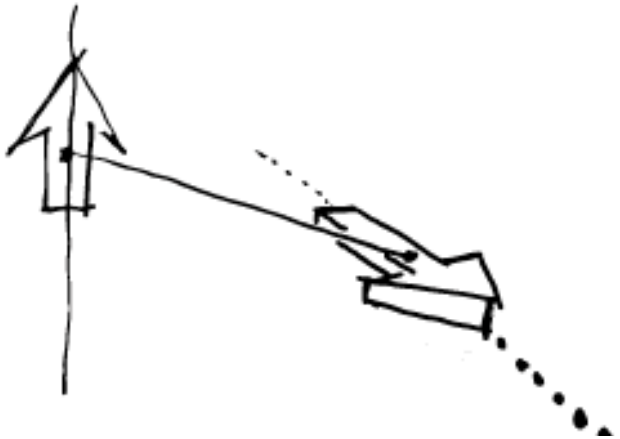
- $P = \alpha E$
  P = dipole moment per unit volume
- $\alpha =$ electric susceptability
- $\alpha = (\varepsilon - 1)/4\pi$
- $\varepsilon =$ dielectric const.
- Effective Field Inside Reduced by Polarization

Polarity vs. Polarizability

From Sharp (1999): “Application of a classical electrostatic view to macromolecular electrostatics involves a number of useful concepts that describe the physical behavior. It should first be recognized that the potential at a particular charged atom *i* includes three physically distinct contributions. **The first is the direct or Coulombic potential of j at i. The second is the potential at i from the polarization (from molecule, water and ionic) induced by j. This is often referred to as the screening potential, since it opposes the direct, Coulombic potential. The third arises from the polarization induced by i itself. This is often referred to as the reaction or self potential,** and if solvent is involved, as the solvation potential. When using models which apply the concept of a dielectric constant (a measure of polarizability) to a macromolecule, **it is important to distinguish between polarity and polarizability.** Briefly, polarity may be thought of as describing the density of charged and dipolar groups in a particular region. Polarizability, by contrast, refers to the *potential* for reorganizing charges, orienting dipoles and inducing dipoles. Thus polarizability depends both on the polarity and the freedom of dipoles to reorganize in response to an applied electric field. When a protein is folding, or undergoing a large conformational rearrangement, the peptide groups may be quite free to reorient. In the folded protein these may become spatially organized so as to stabilize another charge or dipole, creating a region with high polarity, but with low polarizability, since there is much less ability to reorient the dipolar groups in response to a new charge or dipole without significant disruption of the structure. Thus, while there is still some discussion about the value and applicability of a protein dielectric constant, it is generally agreed that the interior of a macromolecule is a low polarizable environment compared to solvent. This difference in polarizability has a significant effect on the potential distribution.”

VDW Forces: Start by Deriving Dipole-Dipole Energy

Simplify. Focus on Formula for Parallel Dipoles



Interaction energy of 2 pair of dipoles is a complex function of two angles (θ, ψ)

$$V(R) = -q p_1 \left(\frac{1}{(R-L)^2} - \frac{1}{(R+L)^2} \right)$$

$$= +q p_1 \left(\frac{4RL}{(R+L)^2 (R-L)^2} \right)$$

IF $R \gg L$

$$V(R) \doteq +2 \frac{p_1 p_2}{R^3}$$

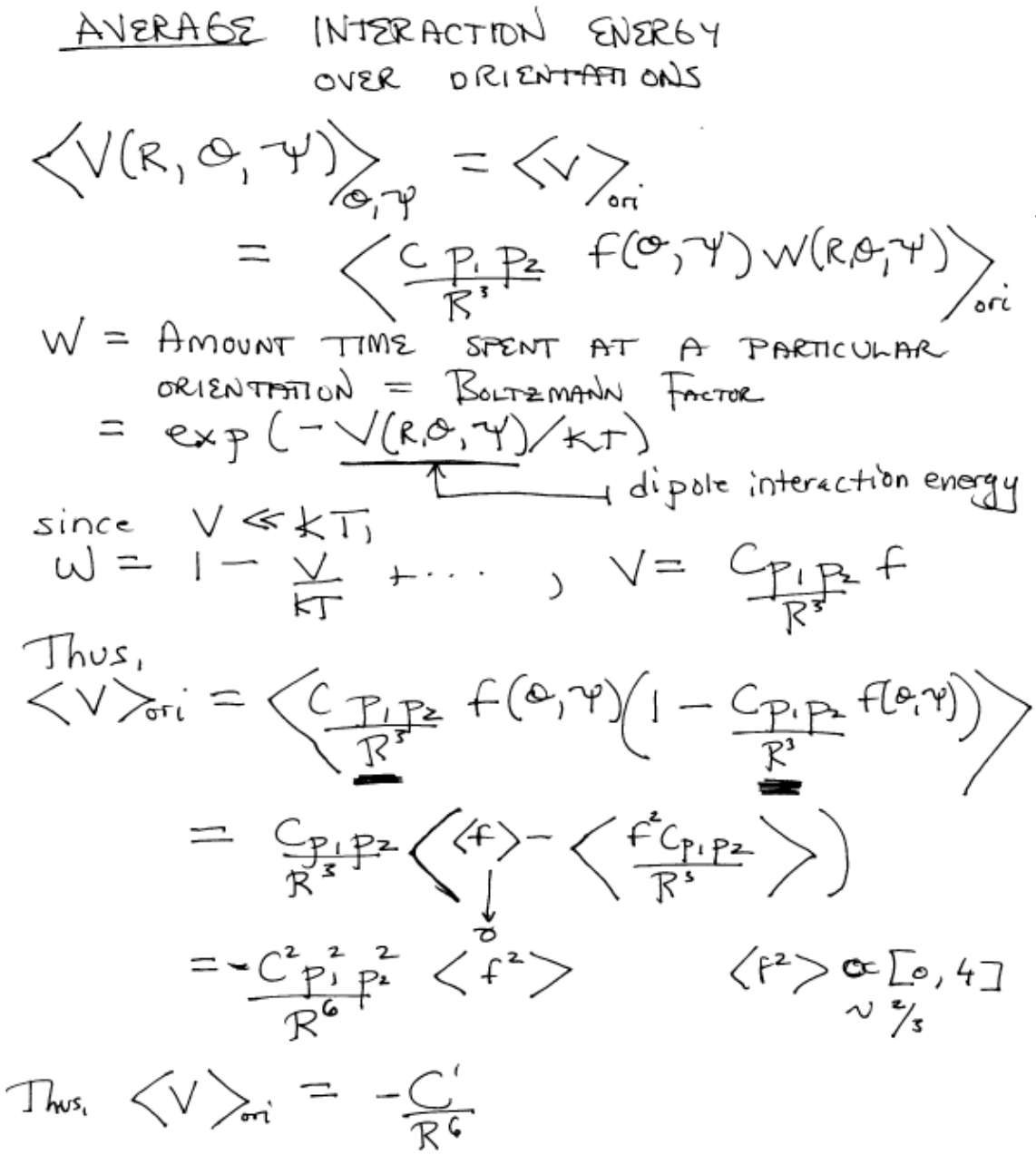
PARALLEL DIPOLES

IN GENERAL, $V = C \frac{p_1 p_2}{R^3} f$

WHERE f IS A FUNCTION OF ORIENTATION ANGLES θ & ψ — $f(\theta, \psi)$

Average Dipole-Dipole Interaction Energy

- Multiplication of dipole-dipole energy ($1/r^3$) and Boltz. Factor (~dipole-dipole energy) gives ($1/r^6$)



Dipole-induced dipole Energy

- Multipl-ication of dipole-dipole energy ($1/r^3$) and amount of induced dipole ($1/r^3$) gives ($1/r^6$)

INDUCED DIPOLE ($\Rightarrow$, p_2^*) is always parallel to permanent dipole ($\Rightarrow$, p_1)

$$\vec{p}_2^{\,*} = \alpha \vec{E}$$

$$\vec{E}_{dipole} = \frac{\nabla p_1 \cdot \hat{r}}{R^2} = -\frac{2p_1}{R^3}$$

Using parallel dipole formula above,

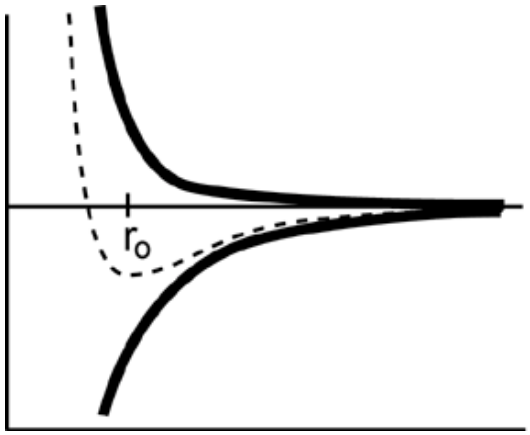
$$V(R) = \frac{2 p_1 p_2^*}{R^3} = -\frac{4 p_1^2 \alpha}{R^6} = -\frac{C}{R^6}$$

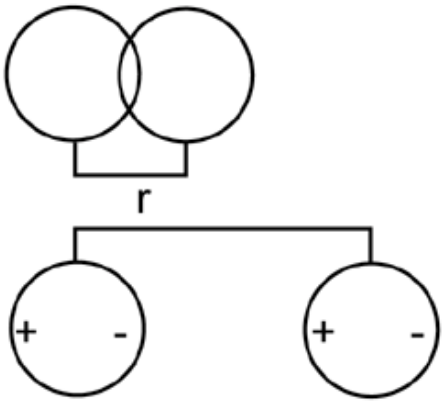
VDW Foces:
Induced dipole-induced dipole

- Too complex to derive induced-dipole-induced dipole formula, but it has essential ingredients of dipole-dipole and dipole-induced dipole calculation, giving an **attractive** $1/r^6$ dependence.
  - ◊ London Forces
- Thus, total dipole cohesive force for molecular system is the sum of three $1/r^6$ terms.
- Repulsive forces result from electron overlap.
  - ◊ Usually modeled as A/r^{12} term. Also one can use exp(-Cr).
- VDW forces: $V(r) = A/r^{12} - B/r^6 = 4\varepsilon((R/r)^{12} - (R/r)^6)$
  - ◊ ε ~ .2 kcal/mole, R ~ 3.5 A, V ~ .1 kcal/mole [favorable]

Packing ~ VDW force

- Longer-range isotropic attractive tail provides general cohesion
- Shorter-ranged repulsion determines detailed geometry of interaction
- Billiard Ball model, WCA Theory





Electron Overlap Replusion $U = \varepsilon \left(\frac{r_0}{r}\right)^{12}$

Dispersion Attraction $U = -4\varepsilon \left(\frac{r_0}{r}\right)^{6}$

Close-packing is Default

- No tight packing when highly directional interactions (such as H-bonds) need to be satisfied
- Packing spheres (.74), hexagonal
- Water (~.35), “Open” tetrahedral, H-bonds

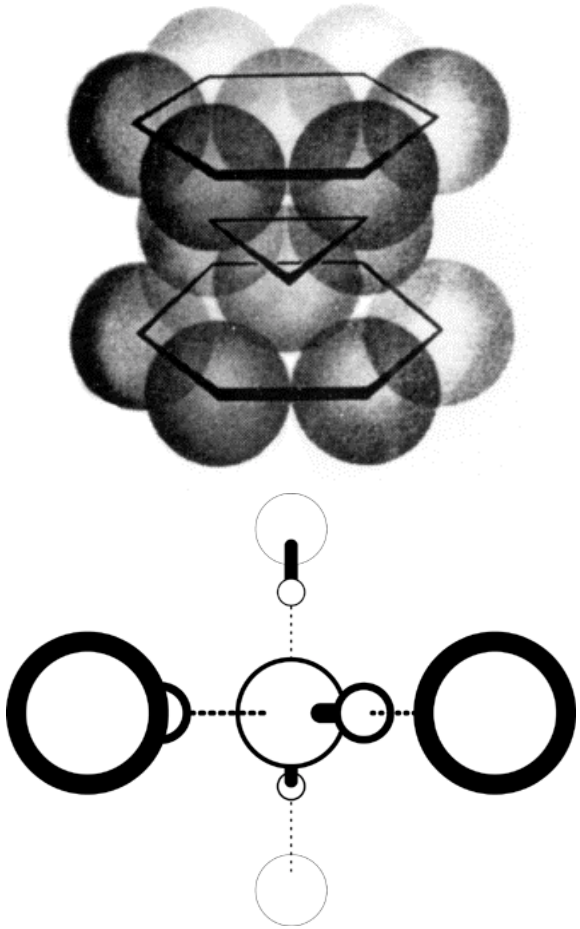
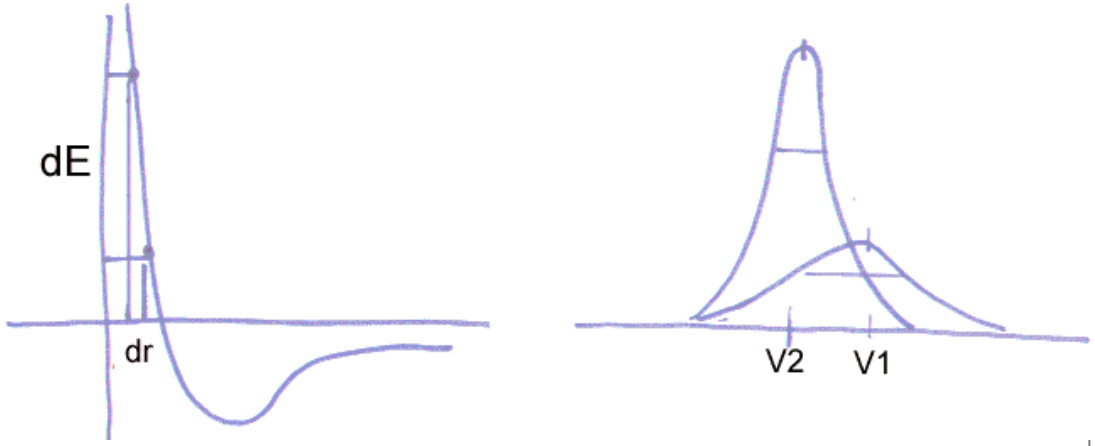


Illustration Credit: Atkins

Small Packing Changes Significant

- Exponential dependence
- Bounded within a range of 0.5 (.8 and .3)
- Many observations in standard volumes gives small error about the mean (SD/sqrt(N))



atom	ε (kJ/ mole)	σ (Å)	charge (electrons)
carbonyl carbon	0.5023	3.7418	0.550
α-carbon (incorporating 1 hydrogen)	0.2034	4.2140	0.100
β-carbon (incorporating 3 hydrogens)	0.7581	3.8576	0.000
amide nitrogen	0.9979	2.8509	-0.350
amide hydrogen	0.2085	1.4254	0.250
carbonyl oxygen	0.6660	2.8509	-0.550
water oxygen in interactions with the helix	0.6660	2.8509	-0.834
water hydrogen in interactions with the helix	0.2085	1.4254	0.417
water O in interactions with other waters	0.6367	3.1506	-0.834
water H in interactions with other waters	0.0000	0.0000	0.417

Different Sets of Radii

Despite sensitivity of VDW radius and r_0 parameter there is considerable disagreement!

Atom Type & Symbol		Bondi 1968	Lee & Richards 1971	Shrake & Rupley 1973	Richards 1974	Chothia 1975	Rich-mond & Richards 1978	Gelin & Karplus 1979	Dunfield et al. 1979	ENCAD derived 1995	CHARMM derived 1995	Tsai et al. 1998
$-CH_3$	Aliphatic, methyl	2.00	1.80	2.00	2.00	1.87	1.90	1.95	2.13	1.82	1.88	1.88
$-CH_2-$	Aliphatic, methyl	2.00	1.80	2.00	2.00	1.87	1.90	1.90	2.23	1.82	1.88	1.88
>CH-	Aliphatic, CH	-	1.70	2.00	2.00	1.87	1.90	1.85	2.38	1.82	1.88	1.88
=CH	Aromatic, CH	-	1.80	1.85	*	1.76	1.70	1.90	2.10	1.74	1.80	1.76
>C=	Trigonal, aromatic	1.74	1.80	*	1.70	1.76	1.70	1.80	1.85	1.74	1.80	1.61
$-NH_3+$	Amino, protonated	-	1.80	1.50	2.00	1.50	0.70	1.75		1.68	1.40	1.64
$-NH_2$	Amino or amide	1.75	1.80	1.50	-	1.65	1.70	1.70		1.68	1.40	1.64
>NH	Peptide, NH or N	1.65	1.52	1.40	1.70	1.65	1.70	1.65	1.75	1.68	1.40	1.64
=O	Carbonyl Oxygen	1.50	1.80	1.40	1.40	1.40	1.40	1.60	1.56	1.34	1.38	1.42
-OH	Alcoholic hydroxyl	-	1.80	1.40	1.60	1.40	1.40	1.70		1.54	1.53	1.46
-OM	Carboxyl Oxygen	-	1.80	1.89	1.50	1.40	1.40	1.60	1.62	1.34	1.41	1.42
-SH	Sulfhydryl	-	1.80	1.85	-	1.85	1.80	1.90		1.82	1.56	1.77
-S-	Thioether or –S-S-	1.80	-	-	1.80	1.85	1.80	1.90	2.08	1.82	1.56	1.77

Molecular Mechanics: Simple electrostatics

atom	ε (kJ/ mole)	σ (Å)	charge (electrons)
carbonyl carbon	0.5023	3.7418	0.550
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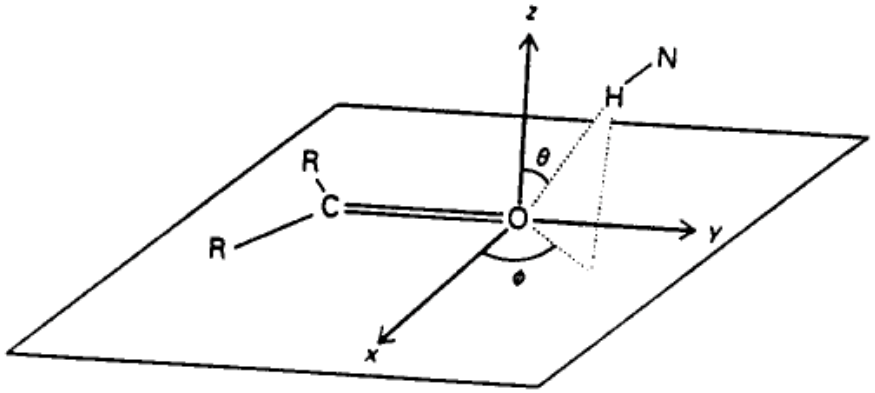
water H in interactions with other waters	0.0000	0.0000	0.417

- U = kqQ/r
- Molecular mechanics uses partial unpaired charges with monopole
  - ◊ usually no dipole
  - ◊ e.g. water has apx. -.8 on O and +.4 on Hs
  - ◊ However, normally only use monopoles for unpaired charges (on charged atoms, asp O)
- Longest-range force
  - ◊ Truncation? Smoothing

H-bonds subsumed by electrostatic interactions

- Naturally arise from partial charges
  - ◊ normally arise from partial charge
- Linear geometry
- Were explicit springs in older models

Illustration Credit: Taylor & Kennard (1984)



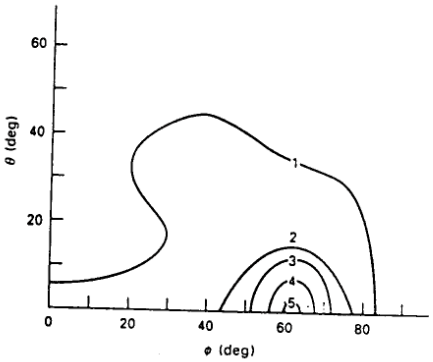


FIGURE 4.4
The geometries of C═O ··· H—N hydrogen bonds observed in crystal structures of small molecules. The definitions of the angles ϕ and θ are illustrated at the *top*, and the relative frequencies of their observed values in intermolecular hydrogen bonds (R. Taylor et al., *J. Amer. Chem. Soc.* 105:5761–5766, 1983) are given by the contours. The angle ϕ measures departures from linearity of the C═O bond and the H atom; the most frequently observed values are in the region of 50°–60°. The angle θ measures the extent to which the H atom lies out of the plane defined by the R, C, and O atoms; the most commonly observed values are in the region of 0°–7°. The lone-pair electrons of the oxygen atom are believed to project at angles of $\phi = 60°$, $\theta = 0°$. The spherical polar coordinate system used here gives a bias toward small values of θ that could be corrected by plotting sin θ.

Table 4.7 *Lengths of* H—N ··· O═C *hydrogen bonds*[a]

Donor	Mean H ··· O Distance for Different Acceptors (Å)		
	Carboxyl[b]	Carboxylate[c]	Amide
>N—H[d]	2.002 ± 0.012	1.928 ± 0.012	1.934 ± 0.005
>N⁺—H[e]	1.983 ± 0.055	1.869 ± 0.028	1.858 ± 0.043
NH_4^+	1.916 ± 0.041	1.886 ± 0.018	1.988 ± 0.075
$R—NH_3^+$	1.936 ± 0.014	1.841 ± 0.008	1.891 ± 0.034
$R_2—NH_2^+$	1.887 ± 0.047	1.796 ± 0.014	1.793 ± 0.070
$R_3—NH^+$		1.722 ± 0.025	1.845 ± 0.014

[a] The N—H distance is generally 1.03 Å; adding this value to the tabulated distances gives the distance between the N and O atoms.

[b] C═O oxygen atom of unionized carboxylic acids and esters.

[c] Oxygen atom of carboxyl anions ($—CO_2^-$).

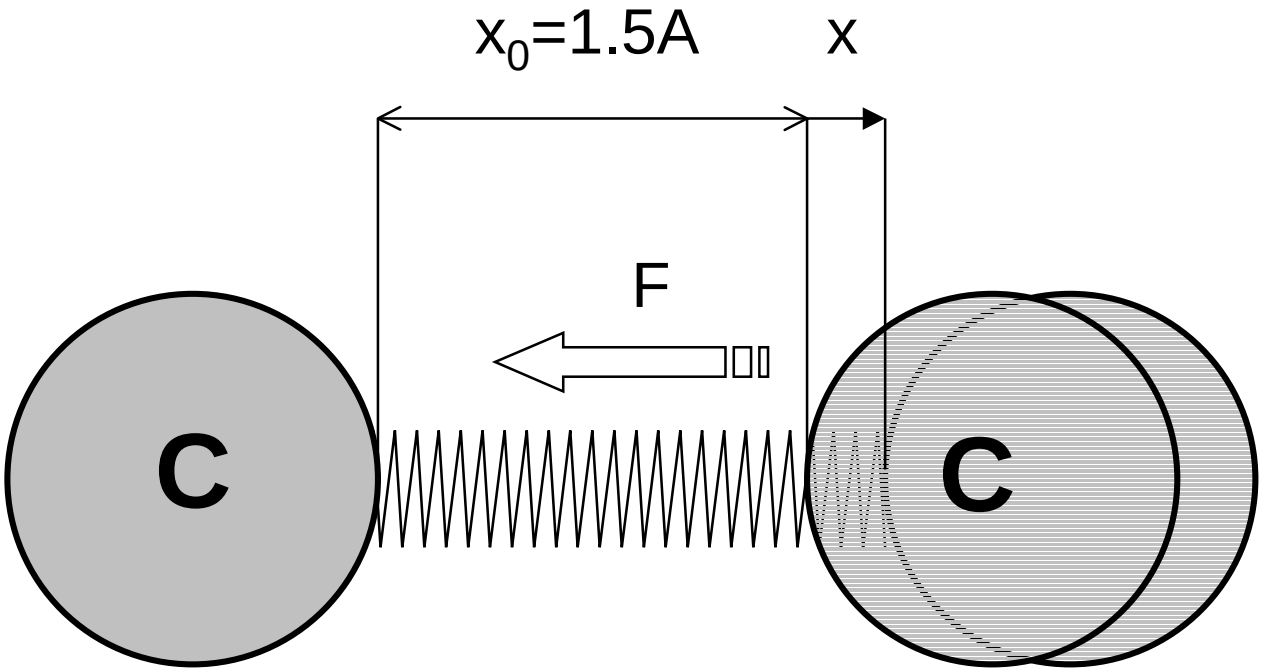
[d] Uncharged donor.

[e] Charged donor with trigonal geometry.

From R. Taylor and O. Kennard, *Acc. Chem. Res.* 17:320–326 (1984).

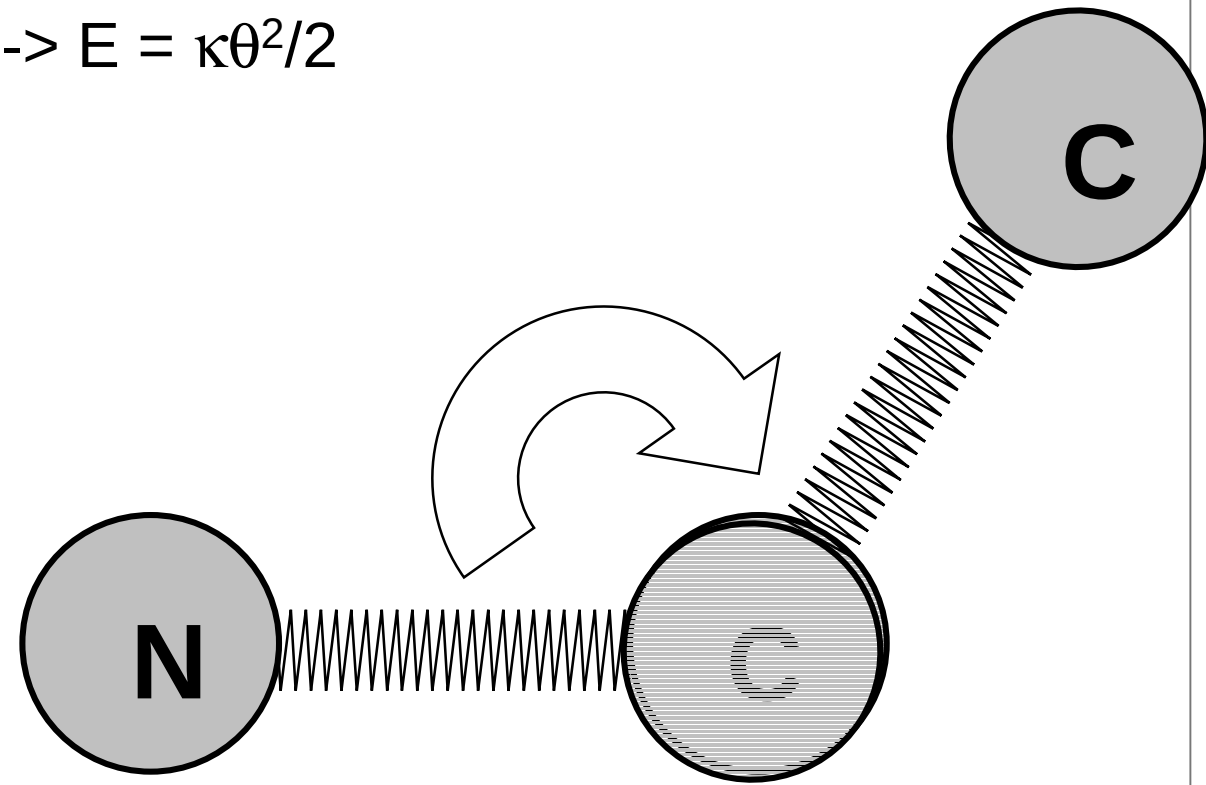
Bond Length Springs

- $F = -kx \rightarrow E = kx^2/2$
- Freq from IR spectroscopy
  - ◊ -> w= sqrt(k/m), m = mass => spring const. k
  - ◊ k ~ 500 kcal/mole*A^2 (stiff!), w corresponds to a period of 10 fs
- Bond length have 2-centers



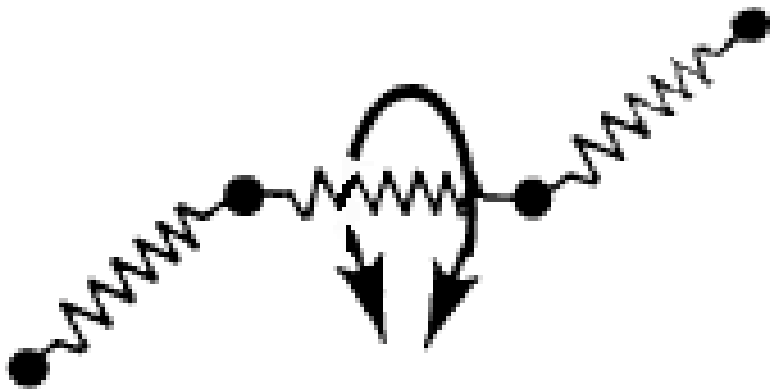
Bond angle, More Springs

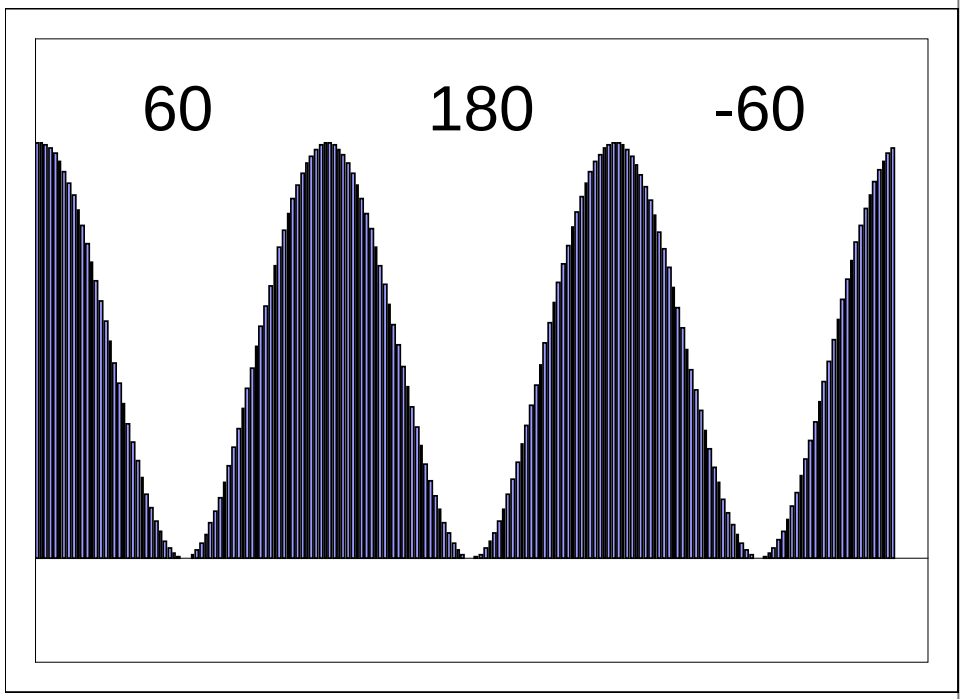
- torque = $\tau = \kappa\theta$ -> $E = \kappa\theta^2/2$
- 3-centers



Torsion angle

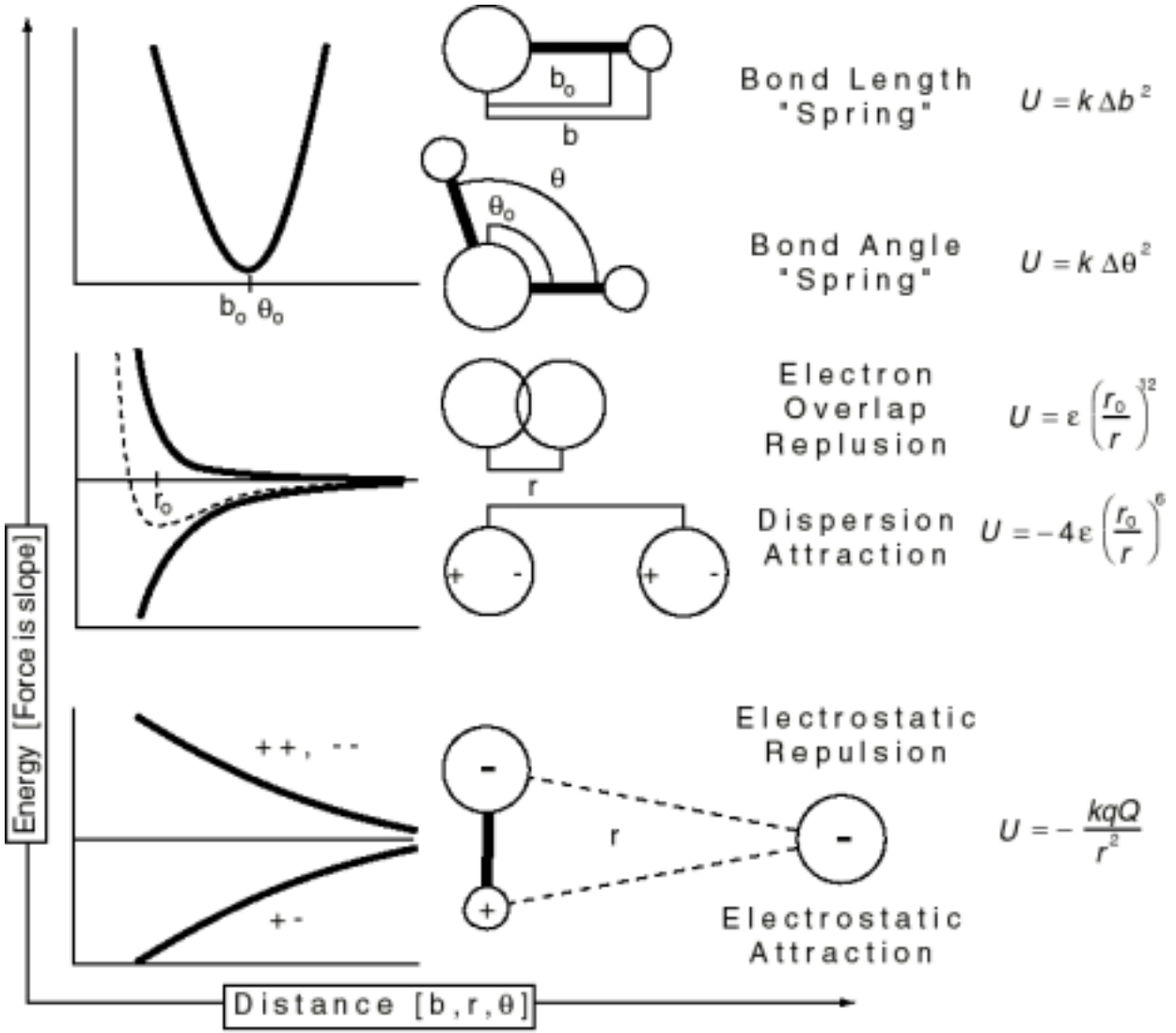
- 4-centers
- U(A)=K(1-cos(nA+d))
  - ◊ cos x = 1 + $x^2/2$ + ... , so minima are quite spring like, but one can hoop between barriers
- K ~ 2 kcal/mole





Potential Functions

- Putting it all together
- Springs + Electrical Forces



Sum up to get total energy

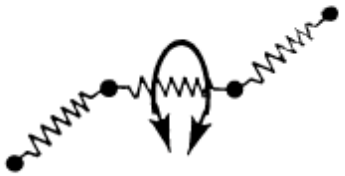
- Each atom is a point mass (m and **x**)
- Sometimes special pseudo-forces: torsions and improper torsions, H-bonds, symmetry.

$$E_{empirical} =$$

$$\sum_{bonds} k_o(b - b_o)^2$$

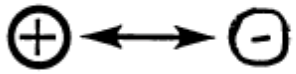
$$+ \sum_{angles} k_\Phi(\Phi - \Phi_o)^2$$

$$+ \sum_{dihedrals} k_\Psi \cos(n\Psi + \delta)$$

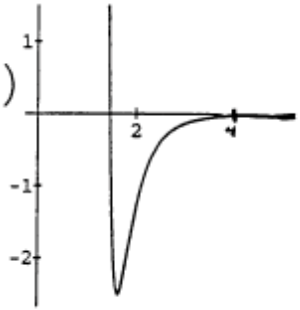


$$+ \sum_{chiral,planar\ centers} k_\omega(\omega - \omega_o)^2$$

$$+ \sum_{non-bonded} (Qr^{-1} + Ar^{-12} - Br^{-6})$$



$$+ \sum_{symmetry\ non-bonded} (Qr^{-1} + Ar^{-12} - Br^{-6})$$



Energy Scale of Interactions

THE SCALE OF INTERACTIONS

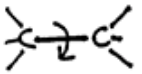
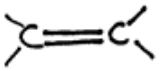
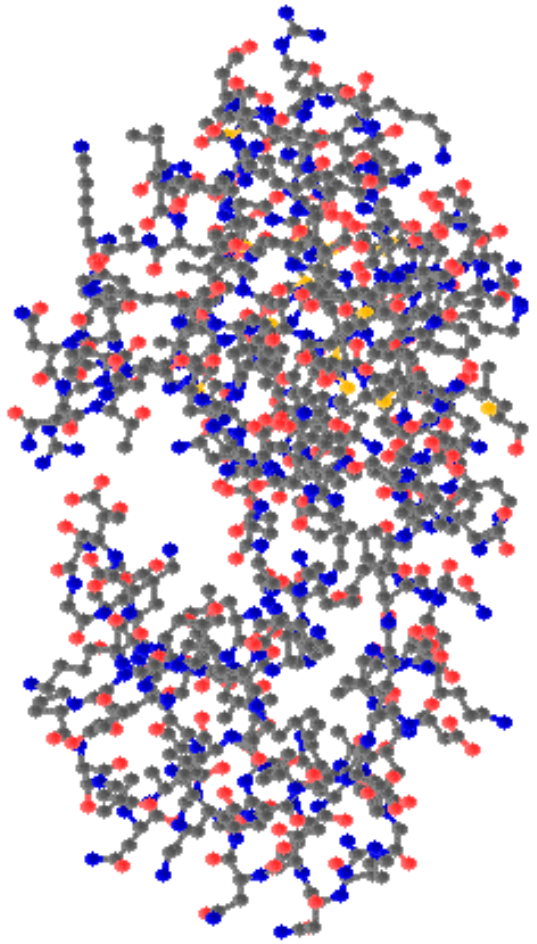
Interaction	Energy (kcal/mole)	
van der Waals in water	−0.1	
van der Waals in vacuo	−0.3	
Hydrogen bond in water	−1.0	
Hydrogen bond in vacuo	−5.0	
Torsion barrier about single bond	+3.0	
Torsion barrier about double bond	+20	
Barrier to breaking a bond	+100	C—C
Energy to change a bond angle by 10°	+2	
Energy to stretch a bond length by 0.1 Å	+2.5	C—C →
Thermal energy at 300°K	0.6	kT

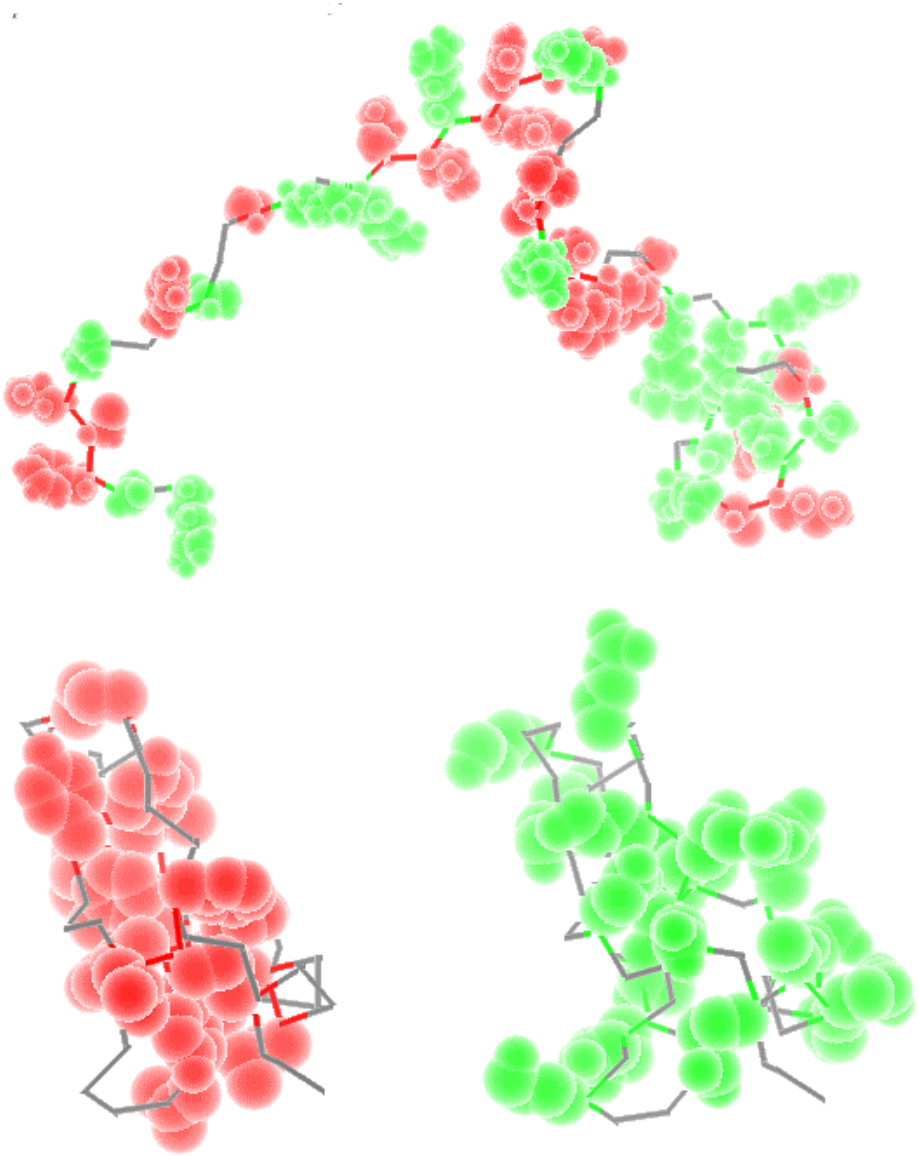
Illustration Credit: M Levitt

Elaboration on the Basic Protein Model

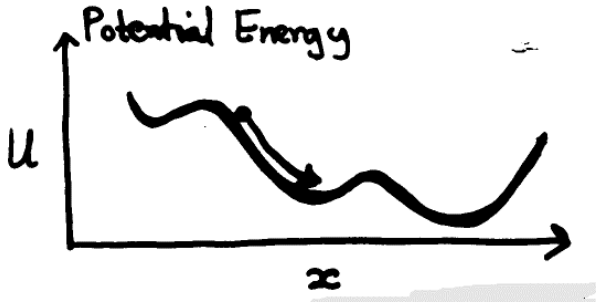
- Geometry
  - ◊ Start with X, Y, Z's (coordinates)
  - ◊ Derive Distance, Surface Area, Volume, Axes, Angle, &c
- Energetics
  - ◊ Add Q's and k's (Charges for electrical forces, Force Constants for springs)
  - ◊ Derive Potential Function U(x)
- Dynamics
  - ◊ Add m's and t (mass and time)
  - ◊ Derive Dynamics (v=dx/dt, F = m dv/dt)



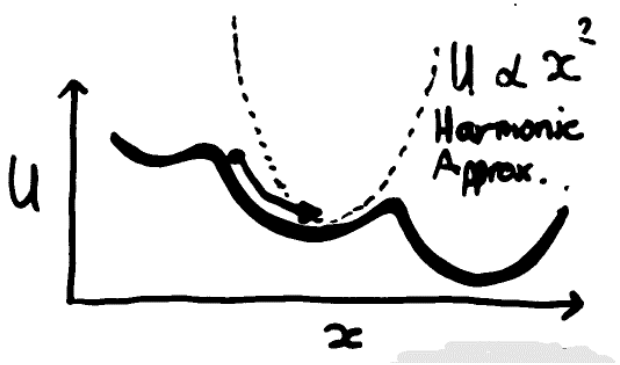
Goal: Model Proteins and Nucleic Acids as Real Physical Molecules



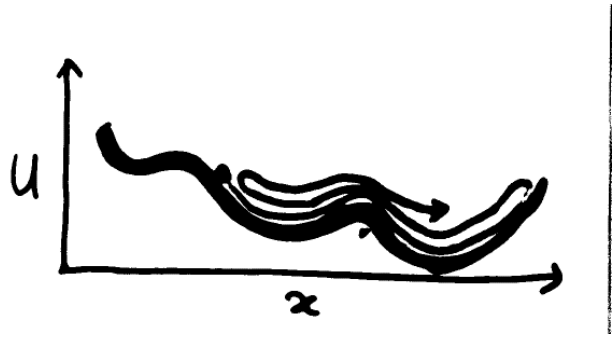
Ways to Move Protein on its Energy Surface



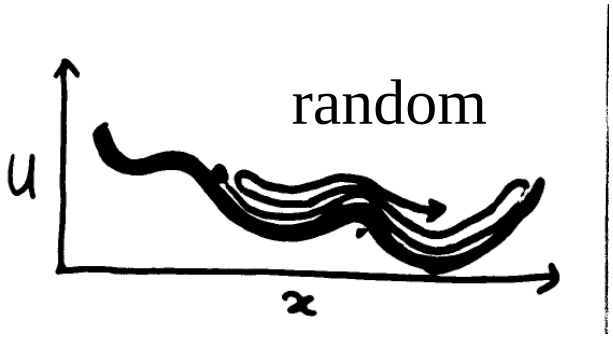
Minimization



Normal Mode Analysis (later?)



Molecular Dynamics (MD)

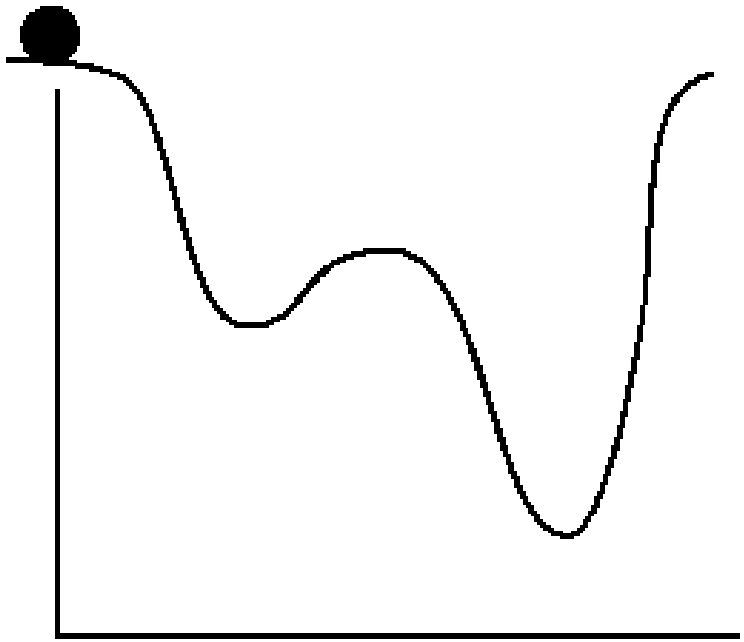


Monte Carlo (MC)

Illustration Credit: M Levitt

Steepest Descent Minimization

- Particles on an “energy landscape.” Search for minimum energy configuration
  - ◊ Get stuck in local minima
- Steepest descent minimization
  - ◊ Follow gradient of energy straight downhill
  - ◊ i.e. Follow the force:
    step ~ **F** = -∇ U
    so
    x(t) = **x**(t-1) + a **F**/|**F**|



Multi-dimensional Minimization

- In many dimensions, minimize along lines one at a time
- Ex: $U = x^2+5y^2$, $F = (2x,10y)$

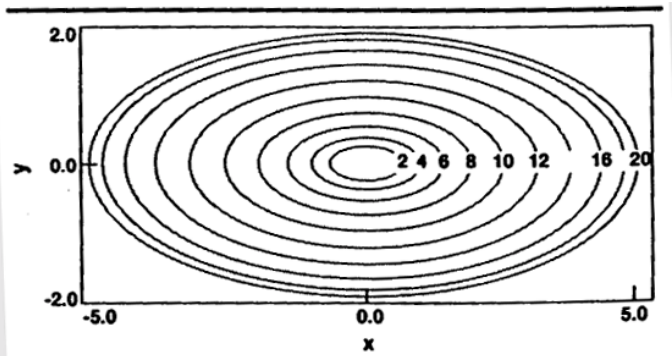


Figure 4–1. Energy Contour Surface of a Simple Function

An energy contour surface for the function $x^2 + 5y^2$. Each contour represents an increase of two arbitrary energy units.

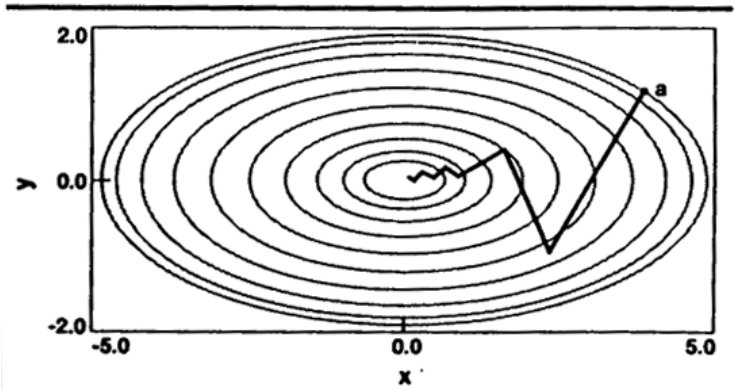


Figure 4–5. Minimization Path following a Steepest-Descents Path without Line Searches

The searching starts from point a and converges on the minimum in about 12 iterations. Although the number of iterations is slightly larger than in Figure 4–4, the total minimization is five times faster since, on average, each iteration used only 1.3 function evaluations. Note that, in most applications in molecular mechanics, the function evaluation is the most time-consuming portion of the calculation.

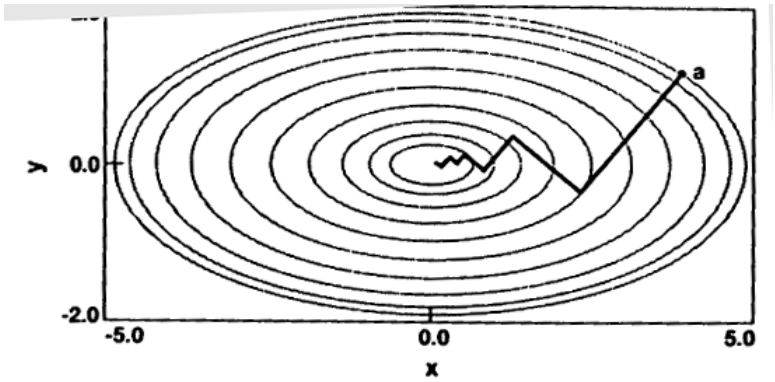


Figure 4–4. Minimization Path following a Steepest-Descents Path

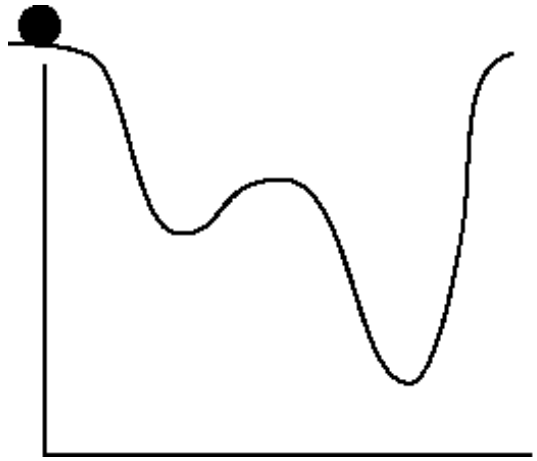
When complete line searches starting from point a are used, the minimum is reached in about 12 iterations. Here, where a rigorous line search is carried out, approximately 8 function evaluations are needed for each line search using a quadratic interpolation scheme. Note how steepest descents consistently overshoots the best path to the minimum, resulting in an inefficient, oscillating trajectory.

Illustration Credit: Biosym, discover manual

Other Minimization Methods

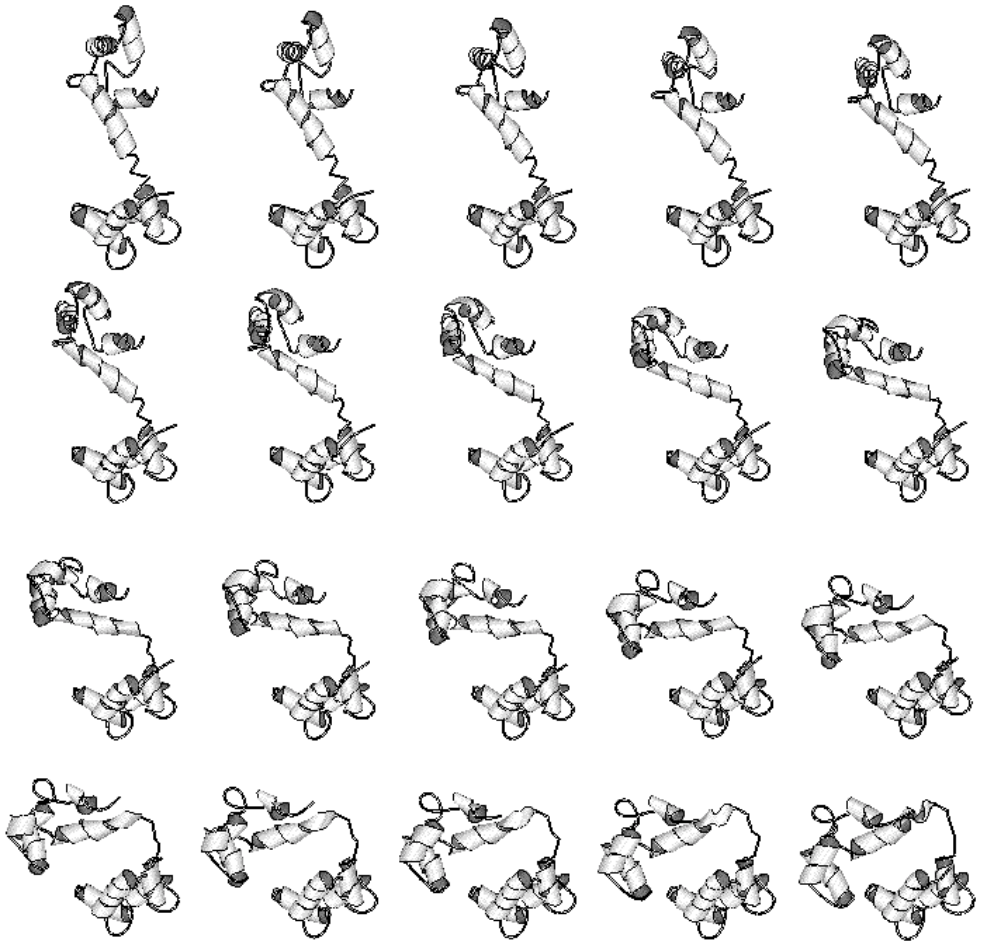
- Simplex, grid search
  - ◊ no derivatives
- Conjugate gradient
  step ~ F(t) **-** b**F**(t-1)
  - ◊ partial 2nd derivative
- Newton-Raphson
  - ◊ using 2nd derivative, find minimum assuming it is parabolic
  - ◊ V = ax2 + bx + c
  - ◊ V' =2ax + b & V'' =2a
  - ◊ V' =0 -> x* = -b/2a

- Problem is that get stuck in local minima
- Steepest descent, least clever but robust, slow at end
- Newton-Raphson faster but 2nd deriv. can be fooled by harmonic assumption
- Recipe: steepest descent 1st, then Newton-raph. (or conj. grad.)



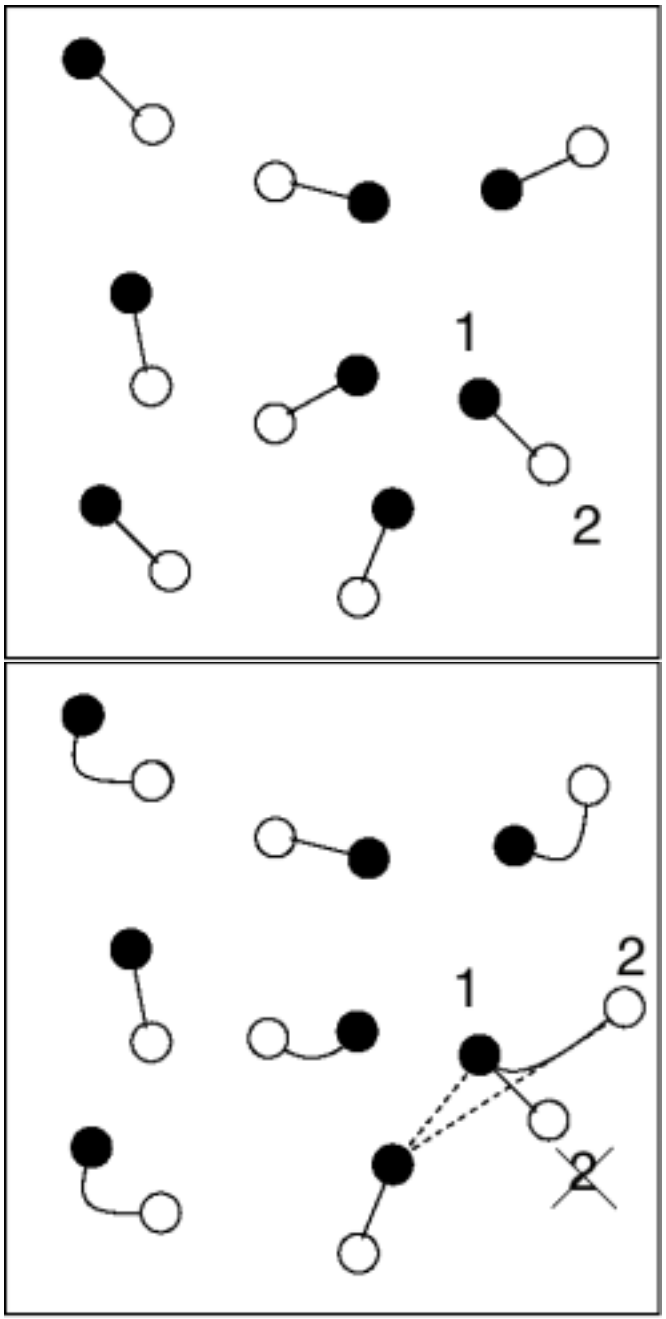
Adiabatic mapping

- Interpolate then minimize
  - ◊ Gives apx. energy (H) landscape through a barrier
  - ◊ can sort of estimate transition rate
    rate = (kT/h) exp (-dG/kT)
  - ◊ Used for ring flips, hinge motions



Molecular Dynamics

- Give each atoms a velocity.
  - ◊ If no forces, new position of atom (at t + dt) would be determined only by velocity
    x(t+dt) = **x**(t) + **v** dt
- Forces change the velocity, complicating things immensely
  - ◊ **F** = d**p**/**dt** = m d**v**/dt



Molecular Dynamics (cont)

- On computer make very small steps so force is nearly constant and velocity change can be calculated (uniform a)

$$\Delta \mathbf{v} = \frac{\mathbf{F}}{m}\Delta t$$

[Avg. **v** over Δt] = (**v** + Δ**v**/2)

- Trivial to update positions:

$$\mathbf{x}(t+\Delta t) = \mathbf{x}(t) + (\mathbf{v} + \frac{\Delta \mathbf{v}}{2})\Delta t$$

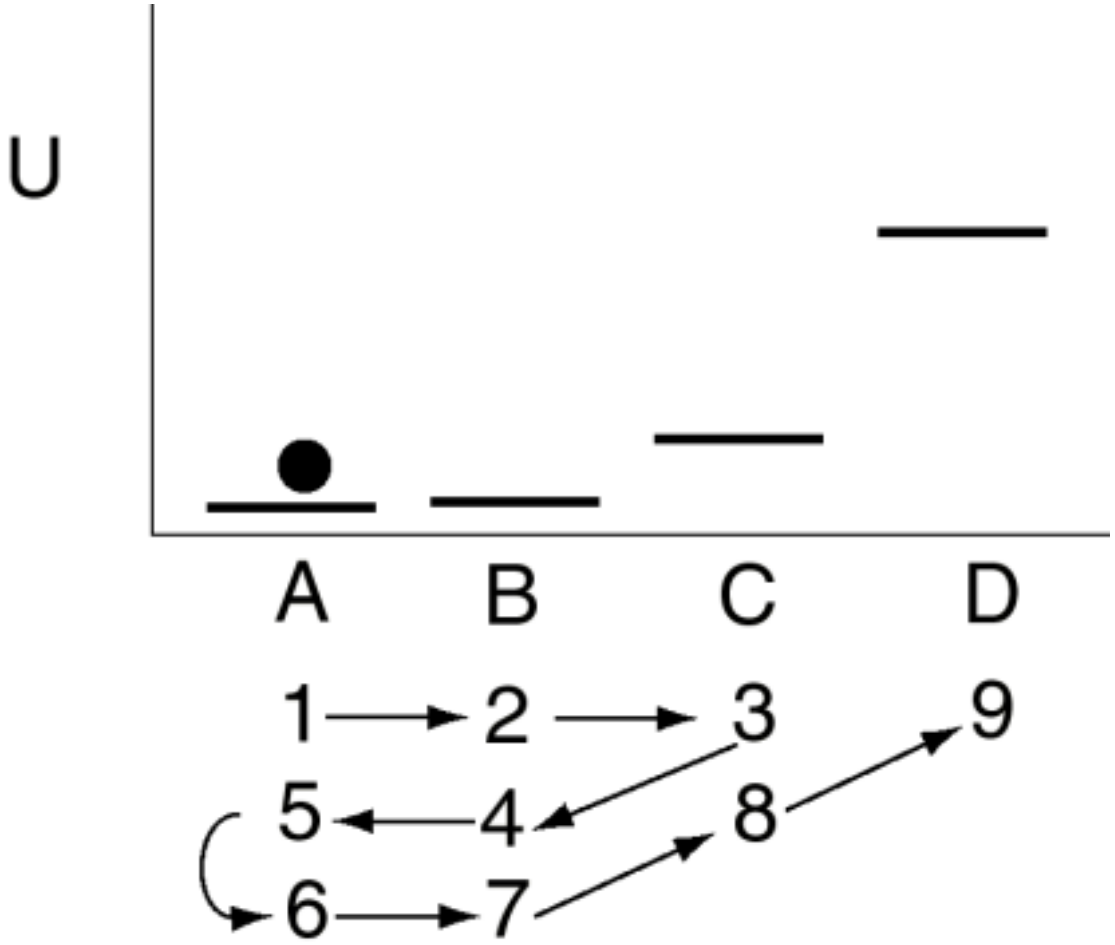
$$= \mathbf{x}(t) + \mathbf{v}\Delta t + \frac{\mathbf{F}}{2m}\Delta t^2$$

- Step must be very small
  - ◊ Δt ~ 1fs (atom moves 1/500 of its diameter)
  - ◊ This is why you need fast computers
- Actual integration schemes slightly more complicated
  - ◊ Verlet (explicit half-step)
  - ◊ Beeman, Gear (higher order terms than acceleration)

Phase Space Walk

- Trajectories of all the particles traverses space of all possible configuration and velocity states (phase space)
- Ergodic Assumption:
  Eventually, trajectory visits every state in phase space
- Boltzmann weighting:
  Throughout, trajectory samples states fairly in terms of system's energy levels
  - ◊ More time in low-U than high-U states
  - ◊ Probability of being in a state ~ exp(-U/kT)
- Consequently, statistics (average properties) over trajectory are thermodynamically correct

Example Phase Space Walk



$$\langle X \rangle = 3X_A + 3X_B + 2X_A + 1X_D$$

$$\langle U \rangle = 6U_{AB} + 2U_A + 1U_D$$

Monte Carlo

- Other ways than MD to sample states fairly and compute correctly weighted averages? Yes, using Monte Carlo calculations.
- Basic Idea:
  Move through states randomly, accepting or rejecting them so one gets a correct "Boltzmann weighting"

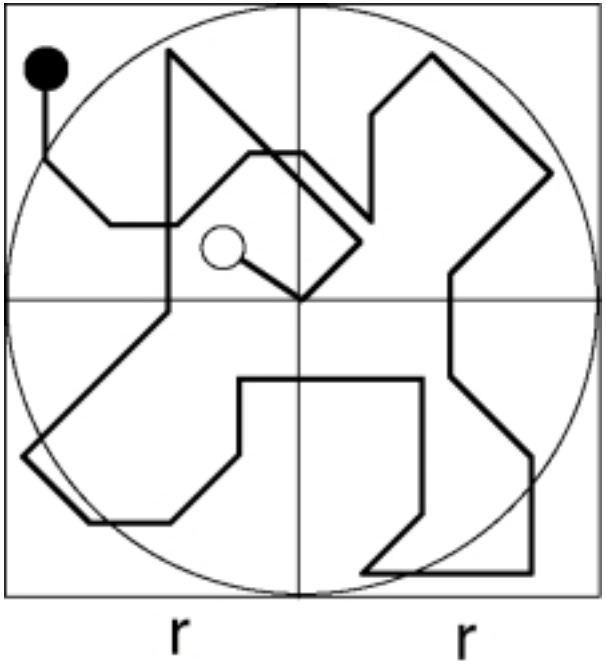
- Formalism:
  - ◊ System described by a probability distribution ρ(n) for it to be in each state n
  - ◊ Random ("Markov") process π operates on the system and changes distribution amongst states to πρ(n)
  - ◊ At equilbrium original distribution and new distribution have to be same as Boltzmann distribution

$$\pi\rho(n) = \rho(n) = \frac{1}{Z}\exp\left(\frac{-U(n)}{kT}\right)$$

Monte Carlo (cont)

- Metropolis Rule
  (for specifying π)
  1. Make a random move to a particle and calculate the energy change dU
  2. dU < 0 –> accept the move
  3. Otherwise, compute a random number R between 0 and 1:
     R < ~ exp(-U/kT) –>
     accept the move
     otherwise –>
     reject the move

- "Fun" example of MC Integration
  - ◊ Particle in empty box of side 2r (energy of all states same)
  - ◊ π = 6 x [Fraction of times particles is within r of center]



MC vs/+ MD

- MD usually used for proteins. Difficult to make moves with complicated chain.
- MC often used for liquids. Can be made into a very efficient sampler.
- Hybrid approaches (Brownian dynamics)
- Simulated Annealing. Heat simulation up to high T then gradually cool and minimize to find global minimum.

Moving Molecules Rigidly

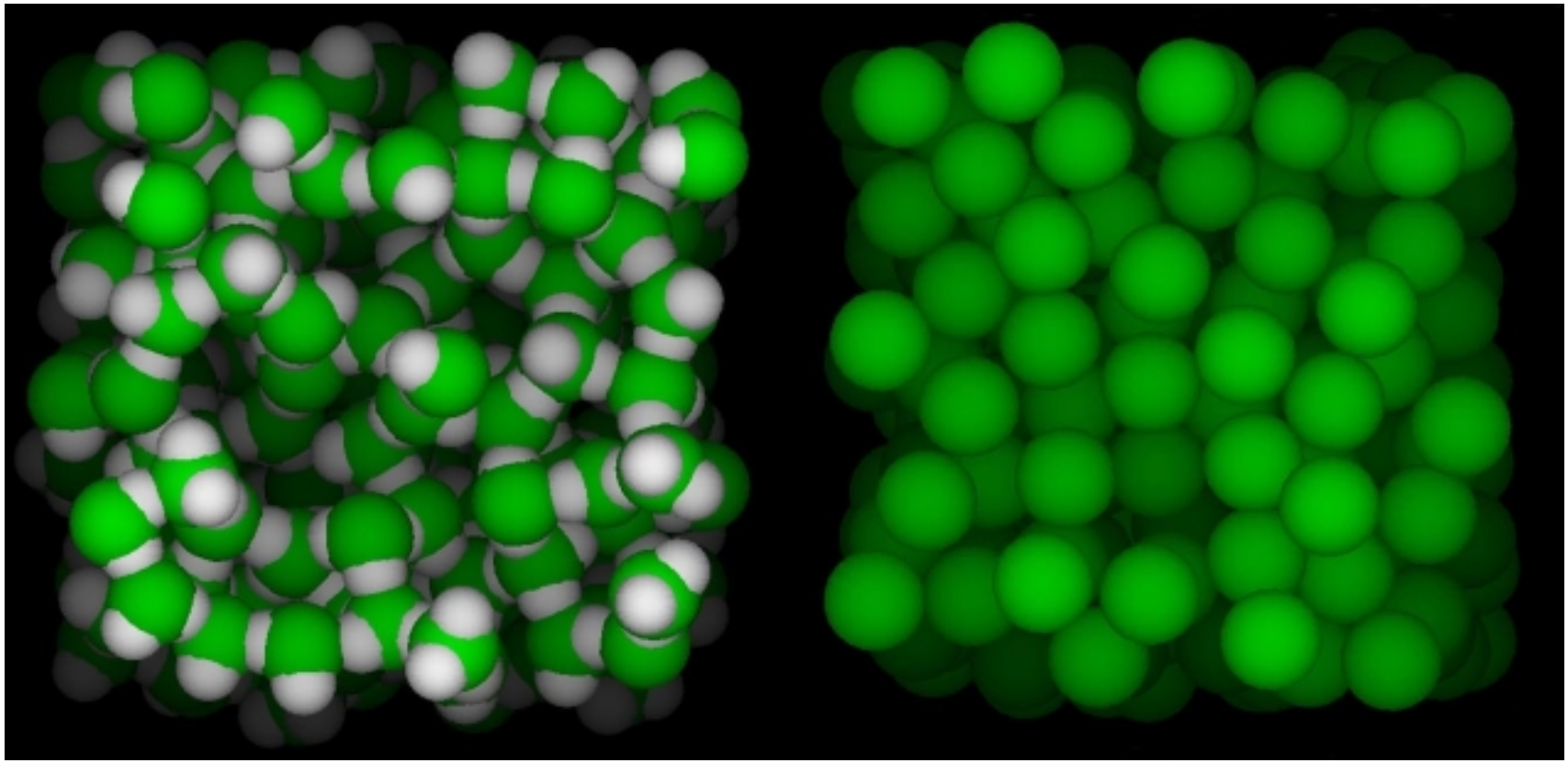
- $\mathbf{X}_i(t+1) = (x_i(t),y_i(t),z_i(t))$ = coordinates of ith atom in the molecule at timestep t
- Rigid-body Translation of all i atoms
  - ◊ For each atom atom i do $\mathbf{x}_i(t+1) = \mathbf{x}_i(t) + \mathbf{v}$

- Rigid-body Rotation of all i atoms
  - ◊ For each atom atom i do $\mathbf{x}_i(t+1) = \mathbf{R}(\phi,\theta,\psi)\ \mathbf{x}_i(t)$
  - ◊ Effectively do a rotation around each axis (x, y, z) by angles ϕ,θ,ψ (see below)
  - ◊ Many conventions for doing this
    - **BELOW IS ONLY FOR MOTIVATION**
    - Consult Allen & Tildesley (1987) or Goldstein for the formulation of the rotation matrix using the usual conventions
  - ◊ How does one do a random rotation? Trickier than it seems

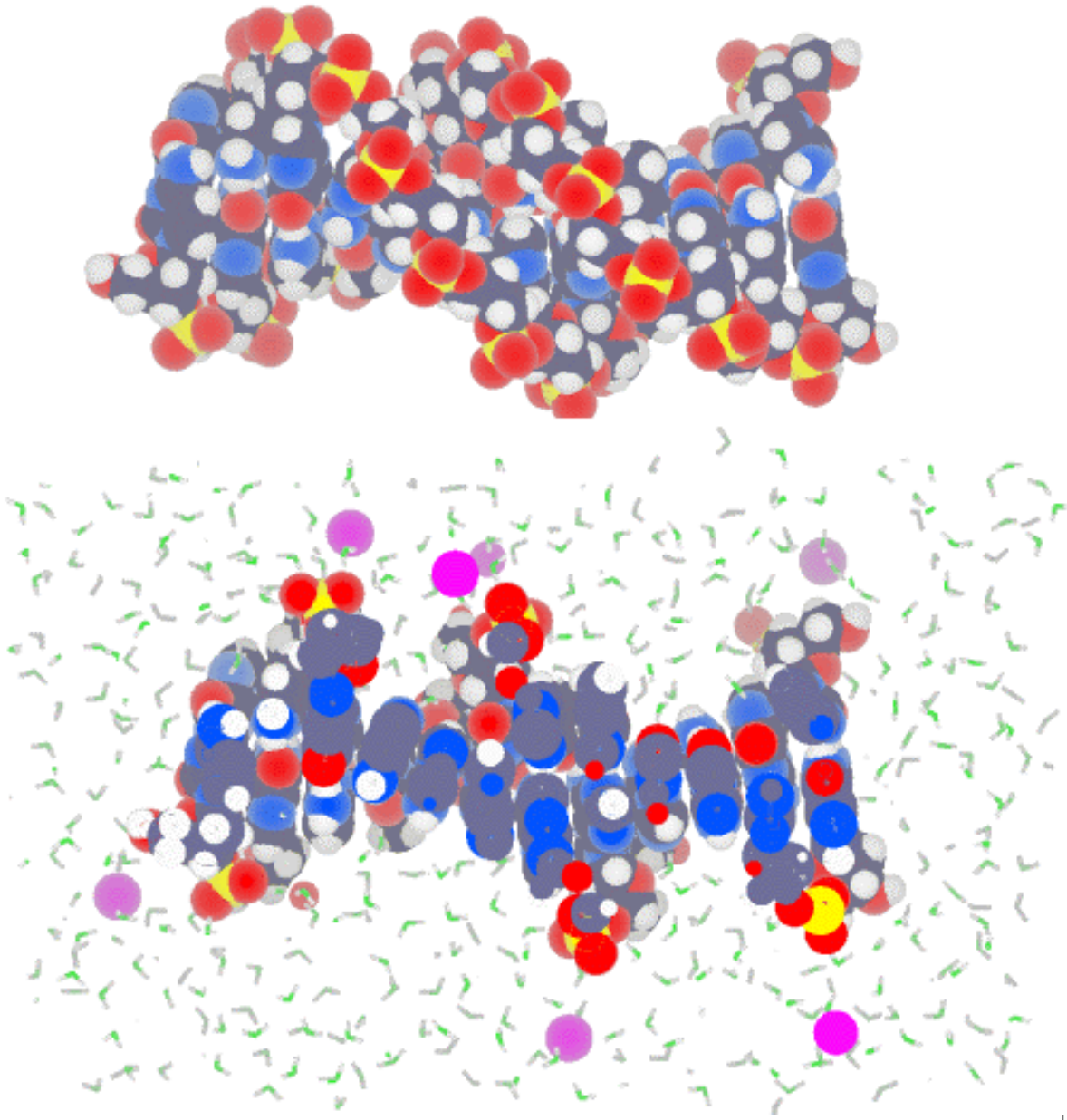
$$\begin{pmatrix} x' \\ y' \end{pmatrix} = \begin{pmatrix} \cos\theta & -\sin\theta \\ \sin\theta & \cos\theta \end{pmatrix} \begin{pmatrix} x \\ y \end{pmatrix}$$

$$\begin{pmatrix} x' \\ y' \\ z' \end{pmatrix} = \underbrace{\begin{pmatrix} \cos\theta & -\sin\theta & 0 \\ \sin\theta & \cos\theta & 0 \\ 0 & 0 & 1 \end{pmatrix}}_{\text{Finally, rotate by } \theta \text{ around z axis}} \underbrace{\begin{pmatrix} \cos\phi & 0 & -\sin\phi \\ 0 & 1 & 0 \\ \sin\phi & 0 & \cos\phi \end{pmatrix}}_{\text{Second, rotate by } \phi \text{ around y axis}} \underbrace{\begin{pmatrix} 1 & 0 & 0 \\ 0 & \cos\psi & -\sin\psi \\ 0 & \sin\psi & \cos\psi \end{pmatrix}}_{\text{First, rotate by } \psi \text{ around x axis}} \begin{pmatrix} x \\ y \\ z \end{pmatrix}$$

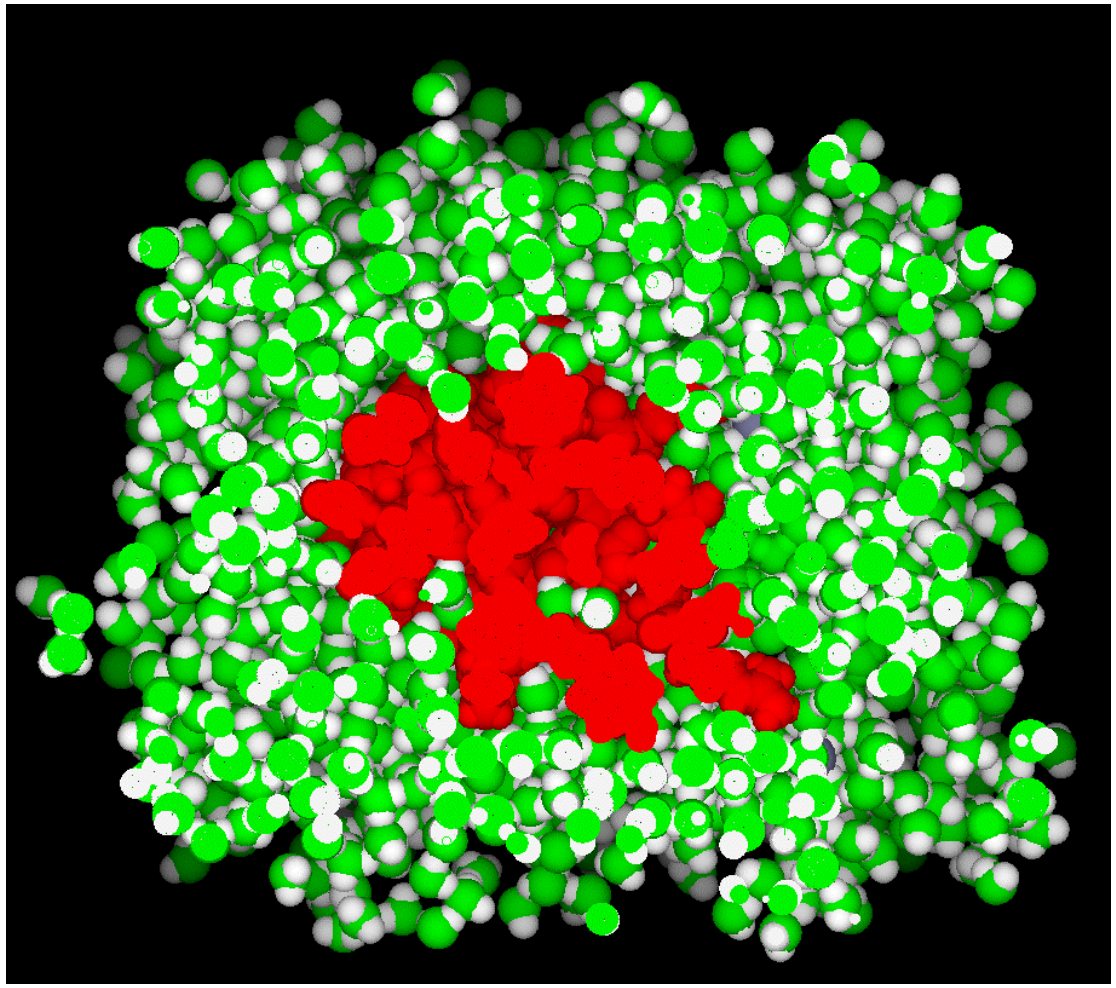
Typical Systems: Water v. Argon



Typical Systems: DNA + Water



Typical Systems: Protein + Water



Practical Aspects: simulation cycle I

- Divide atoms into types (e.g. alpha carbon except for Gly, carbonyl oxygen)
- Initially
  - ◊ Associate each atom with a mass and a point charge
  - ◊ Give each atom an initial velocity
- Calculate Potential
- Calculating non-bonded interactions take up all the time
  - ◊ Electrostatics hardest since longest ranged
  - ◊ Neighbor lists

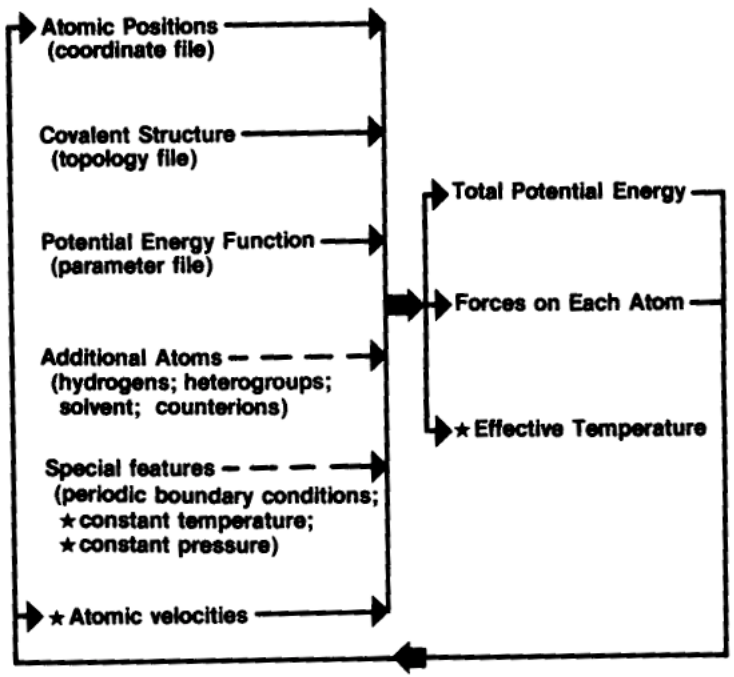


Fig. 4.1. Schematic flow chart of algorithms for energy minimization and molecular dynamics. Features which apply only to molecular dynamics are indicated by asterisks. Dashed lines indicate optional input. Each cycle of energy minimization represents a step in conformation space, while each cycle of molecular dynamics represents a step in time.

Illustration Credit: McCammon & Harvey (1987)

Practical Aspects: simulation cycle II

- Update Positions with MD equations, then recalculate potential and continue
- Momentum conservation
- Energy Conserved in NVE ensemble
- Hydrophobic interaction naturally arises from water behavior

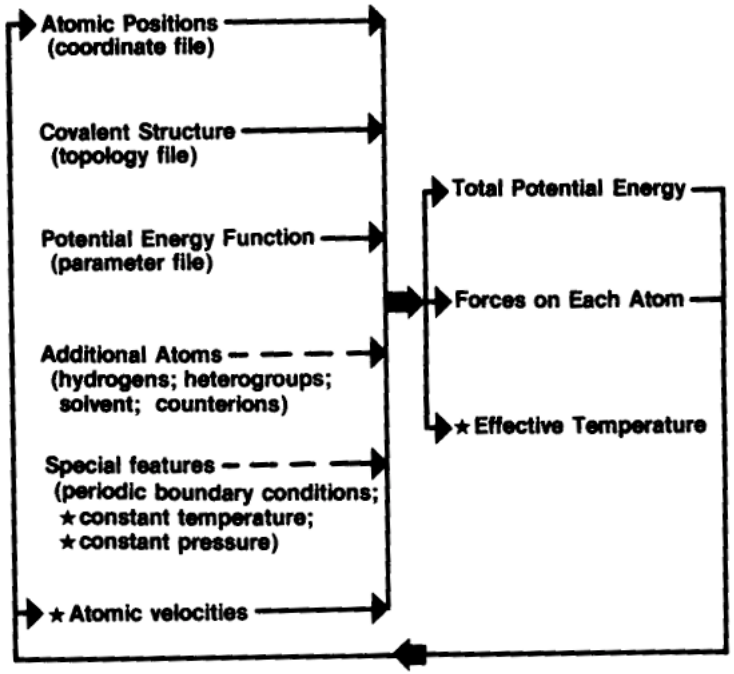


Fig. 4.1. Schematic flow chart of algorithms for energy minimization and molecular dynamics. Features which apply only to molecular dynamics are indicated by asterisks. Dashed lines indicate optional input. Each cycle of energy minimization represents a step in conformation space, while each cycle of molecular dynamics represents a step in time.

Illustration Credit: McCammon & Harvey (1987)

Sample Protein Parameters (toph19.pro)

```
REMARKS TOPH19.PRO ( protein topology )
REMARKS ==============================
REMARKS Charges and atom order modified for neutral GROUPs.
REMARKS Histidine charges set to Del Bene and Cohen sto-3g calculations.
REMARKS Amide charges set to match the experimental dipole moment.
REMARKS Default for HIStidines is the doubly protonated state

set echo=false end
!! for use with PARAM19 parameters ( no special hydrogen bonding potential )
!! donor and acceptor terms just for analysis

AUTOGENERATE  ANGLES=TRUE  END
{*==========================*}

{* protein default masses *}
MASS   H      1.00800! hydrogen which can h-bond to neutral atom
MASS   HC     1.00800!    ="=     ="=     ="=    to charged atom
MASS   HA     1.00800! aliphatic hydrogen
MASS   CT    12.01100! aliphatic carbon
MASS   C     12.01100! carbonyl carbon
MASS   CH1E  13.01900! extended atom carbon with one hydrogen
MASS   CH2E  14.02700!    ="=      ="=     ="=   two hydrogens
MASS   CH3E  15.03500!    ="=      ="=     ="=   three hydrogens
MASS   CR1E  13.01900!    ="=      ="=      in an aromatic ring with one H
MASS   N     14.00670! peptide nitrogen with no hydrogens attached
MASS   NR    14.00670! nitrogen in an aromatic ring with no hydrogens
MASS   NP    14.00670! pyrole nitrogen
MASS   NH1   14.00670! peptide nitrogen bound to one hydrogen
MASS   NH2   14.00670!    ="=       ="=      ="= two hydrogens
MASS   NH3   14.00670! nitrogen bound to three hydrogens
MASS   NC2   14.00670! charged guandinium nitrogen bound to two hydrogens
MASS   O     15.99940! carbonyl oxygen
MASS   OC    15.99940! carboxy oxygen
MASS   OH1   15.99940! hydroxy oxygen
MASS   S     32.06000! sulphur
MASS   SH1E  33.06800! extended atom sulfur with one hydrogen

!some empirical rules for the following topologies:
!
```

Sample Protein Parameters (toph19.pro)

```
. RESIdue ALA
 GROUp
  ATOM N    TYPE=NH1   CHARge=-0.35   END
  ATOM H    TYPE=H     CHARge= 0.25   END
  ATOM CA   TYPE=CH1E  CHARge= 0.10   END
 GROUp
  ATOM CB   TYPE=CH3E  CHARge= 0.00   END
 GROUp
  ATOM C    TYPE=C     CHARge= 0.55   END  !#
  ATOM O    TYPE=O     CHARge=-0.55   END  !#

 BOND N    CA
 BOND CA   C
 BOND C    O
 BOND N    H
 BOND CA   CB

 IMPRoper  CA     N    C  CB  !tetrahedral CA

 DONOr H    N
 ACCEptor O C

 IC   N    C    *CA  CB     0.0000    0.00  120.00    0.00   0.0000

END {ALA}

!---------------------------------------------------------------------

RESIdue ARG
 GROUp
  ATOM N     TYPE=NH1   CHARge=-0.35   END
  ATOM H     TYPE=H     CHARge= 0.25   END
  ATOM CA    TYPE=CH1E  CHARge= 0.10   END
 GROUp
  ATOM CB    TYPE=CH2E  CHARge= 0.00   END
  ATOM CG    TYPE=CH2E  CHARge= 0.00   END
 GROUp
  ATOM CD    TYPE=CH2E  CHARge= 0.10   END  !#
  ATOM NE    TYPE=NH1   CHARge=-0.40   END  !#
```

Sample Protein Parameters (param19.pro)

```
remark - parameter file PARAM19 -

bond C    C      450.0  1.38!  B. R. GELIN THESIS AMIDE AND DIPEPTIDES
bond C    CH1E   405.0  1.52!  EXCEPT WHERE NOTED.  CH1E,CH2E,CH3E, AND CT
bond C    CH2E   405.0  1.52!  ALL TREATED THE SAME. UREY BRADLEY TERMS ADDED
bond C    CH3E   405.0  1.52
bond C    CR1E   450.0  1.38
bond C    CT     405.0  1.53
bond C    N      471.0  1.33
bond C    NC2    400.0  1.33!  BOND LENGTH FROM PARMFIX9 FORCE K APPROXIMATE
bond C    NH1    471.0  1.33
bond C    NH2    471.0  1.33
bond C    NP     471.0  1.33
bond C    NR     471.0  1.33
bond C    O      580.0  1.23
bond C    OC     580.0  1.23!  FORCE DECREASE AND LENGTH INCREASE FROM C O
bond C    OH1    450.0  1.38!  FROM PARMFIX9 (NO VALUE IN GELIN THESIS)
bond C    OS     292.0  1.43!  FROM DEP NORMAL MODE FIT
bond CH1E CH1E   225.0  1.53
bond CH1E CH2E   225.0  1.52
bond CH1E CH3E   225.0  1.52
bond CH1E N      422.0  1.45
bond CH1E NH1    422.0  1.45
bond CH1E NH2    422.0  1.45
bond CH1E NH3    422.0  1.45
bond CH1E OH1    400.0  1.42!  FROM PARMFIX9 (NO VALUE IN GELIN THESIS)
bond CH2E CH2E   225.0  1.52
bond CH2E CH3E   225.0  1.54
bond CH2E CR1E   250.0  1.45!  FROM WARSHEL AND KARPLUS 1972 JACS 96:5612
bond CH2E N      422.0  1.45
bond CH2E NH1    422.0  1.45
bond CH2E NH2    422.0  1.45
bond CH2E NH3    422.0  1.45
bond CH2E OH1    400.0  1.42
bond CH2E S      450.0  1.81!  FROM PARMFIX9
bond CH2E SH1E   450.0  1.81
bond H2E NH1     422.0  1.40
```

Sample Protein Parameters (param19.pro)

```
angle C    C    C       70.0 106.5!  FROM B. R. GELIN THESIS WITH HARMONIC
angle C    C    CH2E    65.0 126.5!  PART OF F TERMS INCORPORATED. ATOMS
angle C    C    CH3E    65.0 126.5!  WITH EXTENDED H COMPENSATED FOR LACK
angle C    C    CR1E    70.0 122.5!  OF H ANGLES.
angle C    C    CT      70.0 126.5
angle C    C    HA      40.0 120.0!  AMIDE PARAMETERS FIT BY LEAST SQUARES
angle C    C    NH1     65.0 109.0!  TO N-METHYL ACETAMIDE VIBRATIONS.
angle C    C    NP      65.0 112.5!  MINIMIZATION OF N-METHYL ACETAMIDE.
angle C    C    NR      65.0 112.5
angle C    C    OH1     65.0 119.0
angle C    C    O       65.0 119.0 ! FOR NETROPSIN
angle CH1E C    N       20.0 117.5
angle CH1E C    NH1     20.0 117.5
angle CH1E C    O       85.0 121.5
angle CH1E C    OC      85.0 117.5
angle CH1E C    OH1     85.0 120.0
angle CH2E C    CR1E    70.0 121.5
angle CH2E C    N       20.0 117.5
angle CH2E C    NH1     20.0 117.5
angle CH2E C    NH2     20.0 117.5
angle CH2E C    NC2     20.0 117.5 ! FOR NETROPSIN
angle CH2E C    NR      60.0 116.0
angle CH2E C    O       85.0 121.6
angle CH2E C    OC      85.0 118.5
angle CH2E C    OH1     85.0 120.0
angle CH3E C    N       20.0 117.5
angle CH3E C    NH1     20.0 117.5
angle CH3E C    O       85.0 121.5
angle CR1E C    CR1E    65.0 120.5
angle CR1E C    NH1     65.0 110.5! USED ONLY IN HIS, NOT IT TRP
angle CR1E C    NP      65.0 122.5
angle CR1E C    NR      65.0 122.5
angle CR1E C    OH1     65.0 119.0
angle CT   C    N       20.0 117.5
angle CT   C    NH1     20.0 117.5
angle CT   C    NH2     20.0 117.5
angle CT   C    O       85.0 121.5
angle CT   C    OC      85.0 118.5
angle CT   C    OH1     85.0 120.0
angle HA   C    NH1     40.0 120.0
angle HA   C    NH2     40.0 120.0
```

Sample Protein Parameters (param19.pro)

```
!angle NR   FE   CM      5.0      180.0
!angle NR   FE   OM      5.0  180.0! JUST A GUESS FROM EXISTING FE CM DATA


dihe CH1E C    N    CH1E 10.0      2     180.0! PRO ISOM. BARRIER 20 KCAL/MOL.
dihe CH2E C    N    CH1E 10.0       2     180.0
dihe CR1E C    C    CR1E 5.0       2     180.0! => TRP OOP. VIB 170CM 1
dihe CR1E C    C    C    2.5       2     180.0! SEE BEHLEN ET AL JCP 75:5685 81
dihe CR1E C    C    NH1  2.5       2     180.0
dihe X    C    CH1E X    0.0       3       0.0! FROM GELIN THESIS AMIDES
dihe X    C    CH2E X    0.0       3       0.0! USING A SINGLE
dihe X    C    CR1E X   10.0       2     180.0! DIHEDRAL PER BOND RATHER
dihe X    C    CT   X    0.0       3       0.0! THAN MULTIPLE TORSIONS
dihe X    C    N    X    8.2       2     180.0! ALKANE TORSION REDUCED TO
dihe X    C    NC2  X    8.2       2     180.0! 1.6 FROM 1.8 TO COINCIDE WITH
dihe X    C    NH1  X    8.2       2     180.0! THE EXPERIMENTAL BARRIER.
dihe X    C    NH2  X    8.2       2     180.0
dihe X    C    OH1  X    1.8       2     180.0
dihe X    C    OS   X    1.8       2     180.0 ! INFERRED FROM C-OH1
dihe X    CH1E CH1E X    1.6       3       0.0
dihe X    CH1E CH2E X    1.6       3       0.0
dihe X    CH1E N    X    0.3       3       0.0! FROM HAGLER ET AL TABULATION OF
dihe X    CH1E NH1  X    0.3       3       0.0! EXP. DATA AND 6 31G CALC.
dihe X    CH1E NH2  X    1.8       3       0.0! PROTONATED SECONDARY AMINE
dihe X    CH1E NH3  X    0.6       3       0.0! 1/PROTON SO 3 FOR THE BOND
dihe X    CH1E OH1  X    0.5       3       0.0! CHANGED TO ROUGHLY MEOH
dihe X    CH2E CH2E X    1.6       3       0.0
dihe X    CH2E N    X    0.3       3       0.0! SEE CH1E COMMENTS
dihe X    CH2E NH1  X    0.3       3       0.0
dihe X    CH2E NH2  X    0.6       3       0.0
dihe X    CH2E NH3  X    0.6       3       0.0
dihe X    CH2E OH1  X    0.5       3       0.0
dihe X    CH2E S    X    1.2       2       0.0
dihe X    CT   CT   X    1.6       3       0.0
dihe X    CT   N    X    0.3       3       0.0! SEE CH1E COMMENTS
dihe X    CT   NC2  X    0.3       3       0.0
dihe X    CT   NH1  X    0.3       3       0.0
dihe X    CT   NH2  X    0.6       3       0.0
dihe X    CT   NH3  X    0.6       3       0.0
dihe X    CT   OH1  X    0.5       3       0.0
dihe X    CT   S    X    1.2       2       0.0
!dihe X    FE   NR   X    0.05      4       0.0
```

Sample Protein Parameters (param19.pro)

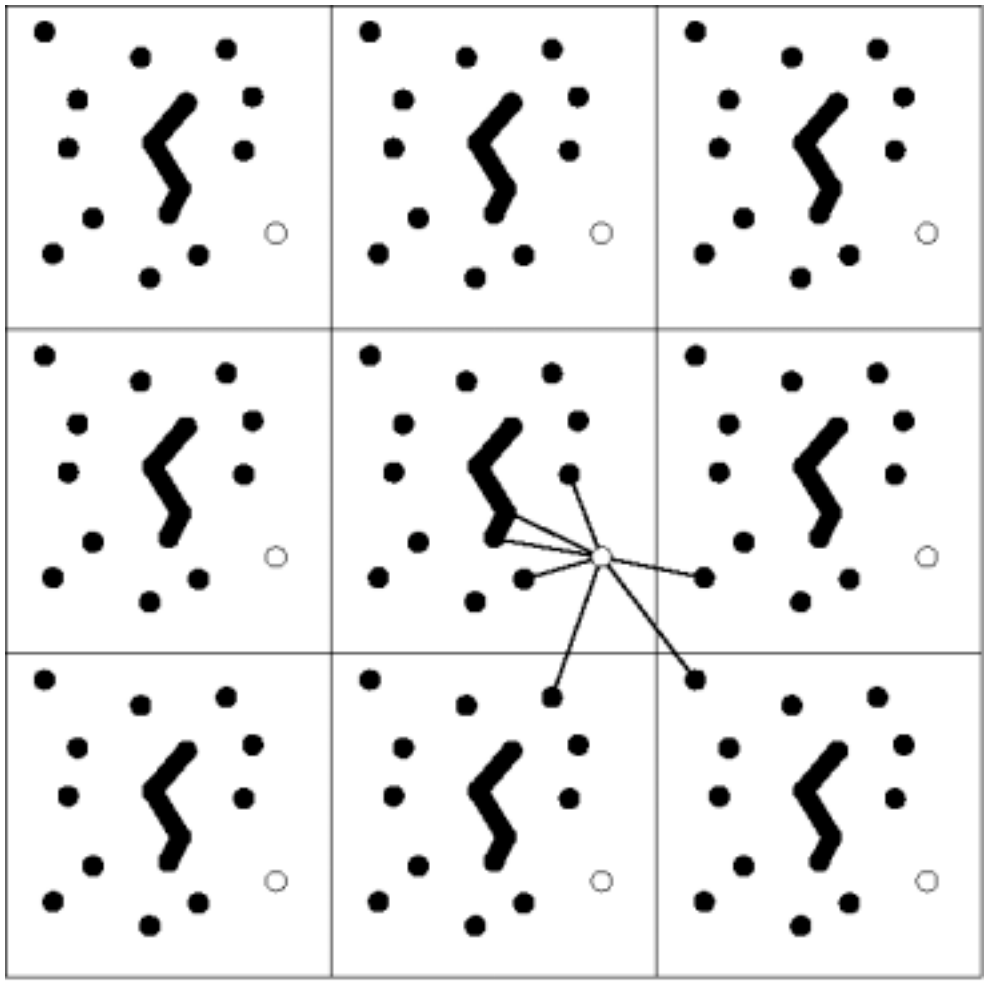
```
{* nonbonding parameter section *}
{* ============================ *}
nbonds
      atom cdie shift eps=1.0  e14fac=0.4   tolerance=0.5
      cutnb=9.0 ctonnb=7.5 ctofnb=8.0
      nbxmod=5 vswitch wmin 1.0
end


 !                eps     sigma       eps(1:4) sigma(1:4)
 !                (kcal/mol) (A)
 !                -------------------------------------
 NONBonded  H       0.0498   1.4254      0.0498   1.4254
 NONBonded  HA      0.0450   2.6157      0.0450   2.6157 !- charged group.
 NONBonded  HC      0.0498   1.0691      0.0498   1.0691 !   Reduced vdw radius
 !
 NONBonded  C       0.1200   3.7418      0.1000   3.3854 ! carbonyl carbon
 NONBonded  CH1E    0.0486   4.2140      0.1000   3.3854 ! \
 NONBonded  CH2E    0.1142   3.9823      0.1000   3.3854 !  extended carbons
 NONBonded  CH3E    0.1811   3.8576      0.1000   3.3854 ! /
!! NONBonded  CM      0.0262   4.4367      0.1000   3.3854
 NONBonded  CR1E    0.1200   3.7418      0.1000   3.3854 !  ring carbons
!! NONBonded  CT      0.0262   4.4367      0.1000   3.3854
 !
 NONBonded  N       0.2384   2.8509      0.2384   2.8509
 NONBonded  NC2     0.2384   2.8509      0.2384   2.8509
 NONBonded  NH1     0.2384   2.8509      0.2384   2.8509
 NONBonded  NH2     0.2384   2.8509      0.2384   2.8509
 NONBonded  NH3     0.2384   2.8509      0.2384   2.8509
 NONBonded  NP      0.2384   2.8509      0.2384   2.8509
 NONBonded  NR      0.2384   2.8509      0.2384   2.8509
 !
 NONBonded  O       0.1591   2.8509      0.1591   2.8509
 NONBonded  OC      0.6469   2.8509      0.6469   2.8509
 NONBonded  OH1     0.1591   2.8509      0.1591   2.8509
!! NONBonded  OM      0.1591   2.8509      0.1591   2.8509
 NONBonded  OS      0.1591   2.8509      0.1591   2.8509
 !
 NONBonded  S       0.0430   3.3676      0.0430   3.3676
 NONBonded  SH1E    0.0430   3.3676      0.0430   3.3676
 !
!! NONBONDED FE        0.0000    1.1582      0.0000 1.1582

set e_bo=true end
```

Periodic Boundary Conditions

- Make simulation system seem larger than it is
- Ewald Summation for electrostatics (Fourier transform)



Average over simulation

- Deceptive Instantaneous Snapshots (almost anything can happen)
- Simple thermodynamic averages
  - ◊ Average potential energy <U>
  - ◊ T ~ < Kinetic Energy > = ½ m < v^2 >
- Some quantities fixed, some fluctuate in different ensembles
  - ◊ NVE protein MD (“microcanonical”)
  - ◊ NVT liquid MC (“canonical”)
  - ◊ NPT more like the real world

Timescales

Motion	length (Å)	time (fs)
bond vibration	0.1	10
water hindered rotation	0.5	1000
surface sidechain rotation	5	10^5
water diffusive motion	4	10^5
buried sidechain libration	0.5	10^5
hinge bending of chain	3	10^6
buried sidechain rotation	5	10^{13}
allosteric transition	3	10^{13}
local denaturation	7	10^{14}

(From McCammon & Harvey, Eisenberg & Kauzmann)

D & RMS

- Diffusion constant
  - ◊ Measures average rate of increase in variance of position of the particles
  - ◊ Suitable for liquids, not really for proteins

$$D = \frac{\langle \Delta r^2 \rangle}{6\Delta t}$$

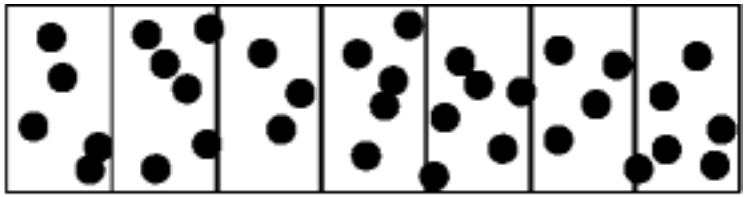
- RMS more suitable to proteins

$$RMS(t) = \sqrt{\frac{\sum_{i=1}^{N} d_i(t)}{N}}$$

$$d_i(t) = \mathbf{R}(\mathbf{x}_i(t) - \mathbf{T}) - \mathbf{x}_i(0)$$

  - ◊ di = Difference in position of protein atom at t from the initial position, after structures have been optimally rotated translated to minimize RMS(t)
  - ◊ Solution of optimal rotation has been solved a number of ways (Kabsch, SVD)

Number Density

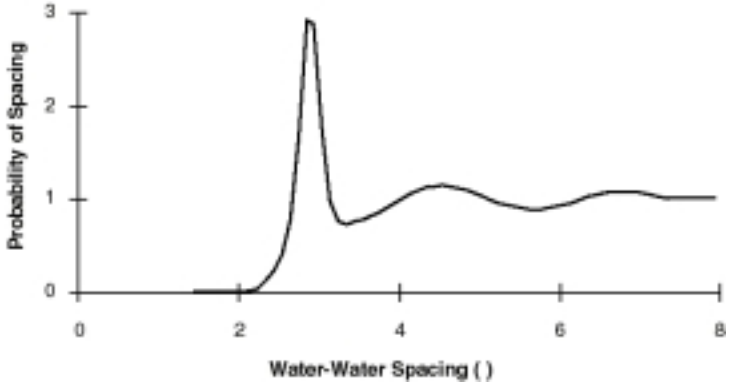


= Number of atoms per unit volume averaged over simulation divided by the number you expect to have in the same volume of an ideal “gas”

Spatially average over all directions gives

1D RDF =

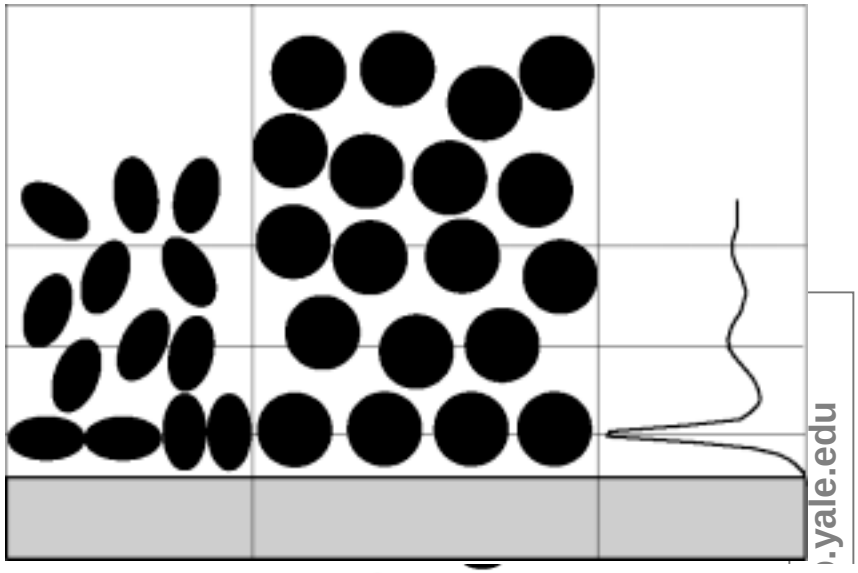
[Avg. Num. Neighbors at r]
[Expected Num. Neighbors at r]

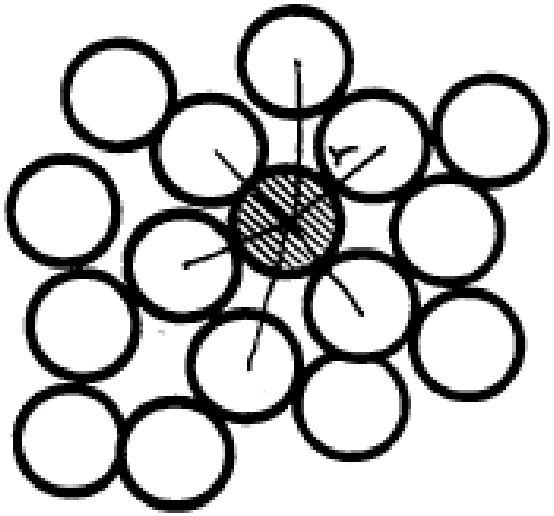


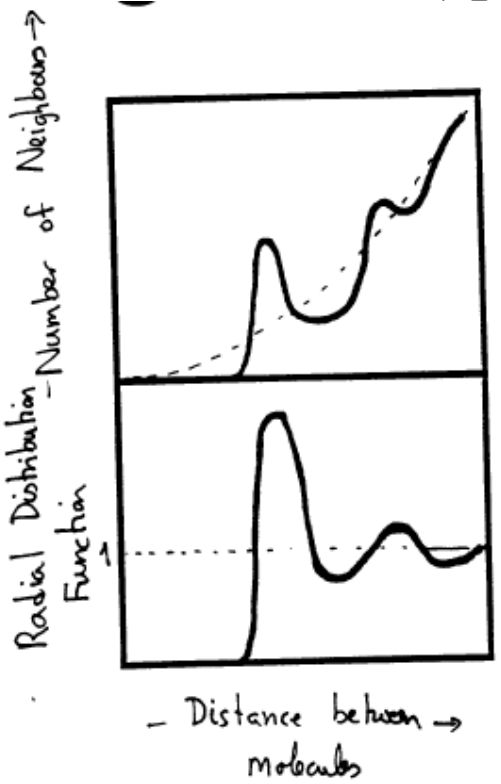
“at r” means contained in a thin shell of thickness dr and radius r.

Number Density (cont)

- Advantages: Intuitive, Relates to scattering expts
- D/A: Not applicable to real proteins
  - ◊ 1D RDF not structural
  - ◊ 2D proj. only useful with "toy" systems
- Number densities measure spatial correlations, not packing
  - ◊ Low value does not imply cavities
  - ◊ Complicated by asymmetric molecules
  - ◊ How things pack and fit is property of instantaneous structure - not average







Major Protein Simulation Packages

- AMBER
  - ◊ http://www.amber.ucsf.edu/amber/amber.html
  - ◊ http://www.amber.ucsf.edu/amber/tutorial/index.html
- CHARMM/XPLOR
  - ◊ http://yuri.harvard.edu/charmm/charmm.html
  - ◊ http://atb.csb.yale.edu/xplor
  - ◊ http://uracil.cmc.uab.edu/Tutorials/default.html
- ENCAD
- GROMOS
  - ◊ http://rugmd0.chem.rug.nl/md.html
  - ◊ “Advanced Crash Course on Electrostatics in Simulations” (!) (http://rugmd0.chem.rug.nl/~berends/course.html)

Molecular Biophysics & Biochemistry 400a/700a (Advanced Biochemistry)

Computational Aspects of:
Simulation (Part II),
Electrostatics (Part II),
Water and Hydrophobicity

Mark Gerstein

Classes on 11/12/98 & 10/17/98

Yale University

The Handouts

- Notes
  - ◊ Coming on Tuesday!!!
  - ◊ Perhaps available on-line at http://bioinfo.mbb.yale.edu/course
- Presentation Paper
  - ◊ Duan, Y. & Kollman, P. A. (1998). Pathways to a protein folding intermediate observed in a 1-microsecond simulation in aqueous solution *Science* **282**, 740-4.
    - http://bioinfo.mbb.yale.edu/course/private-xxx/kollman-science-longsim.pdf
    - http://www.sciencemag.org/cgi/content/abstract/282/5389/740
- Fun
  - ◊ Pollack, A. (1998). Drug Testers Turn to'Virtual Patients' as Guinea Pigs. *New York Times.* Nov. 10
    - http://www.nytimes.com/library/tech/98/11/biztech/articles/10health-virtual.html
    - http://bioinfo.mbb.yale.edu/course/private-xxx/pollack-nytimes-bioinfo.html

The Handouts II

- Review
  - ◊ Sharp, K. (1999). Electrostatic Interactions in Proteins. In *International Tables for Crystallography,* International Union of Crystallography, Chester, UK.
  - ◊ Dill, K. A., Bromberg, S., Yue, K., Fiebig, K. M., Yee, D. P., Thomas, P. D. & Chan, H. S. (1995). Principles of protein folding--a perspective from simple exact models. *Protein Sci* **4**, 561-602.
  - ◊ Gerstein, M. & Levitt, M. (1998). Simulating Water and the Molecules of Life. *Sci. Am.* **279**, 100-105.
    - http://bioinfo.mbb.yale.edu/geometry/sciam
  - ◊ Franks, F. (1983). *Water.* The Royal Society of Chemistry, London. Pages 35-56.

- Homework Paper
  - ◊ Honig, B. & Nicholls, A. (1995). Classical electrostatics in biology and chemistry. *Science* **268**, 1144-9.

Outline

- Last Time
  - ◊ Basic Forces
    - Electrostatics
    - Packing as VDW forces
    - Springs
  - ◊ Minimization, Simulation
- Now
  - ◊ Simulation, Part II: Analysis,
    What can be Calculated from Simulation?
  - ◊ Electrostatics Revisited: the Poisson-Boltzmann Equation
  - ◊ Water Simulation and Hydrophobicity
  - ◊ Simplified Simulation

Practical Aspects: simulation cycle I

- Divide atoms into types (e.g. alpha carbon except for Gly, carbonyl oxygen)
- Initially
  - ◊ Associate each atom with a mass and a point charge
  - ◊ Give each atom an initial velocity
- Calculate Potential
- Calculating non-bonded interactions take up all the time
  - ◊ Electrostatics hardest since longest ranged
  - ◊ Neighbor lists

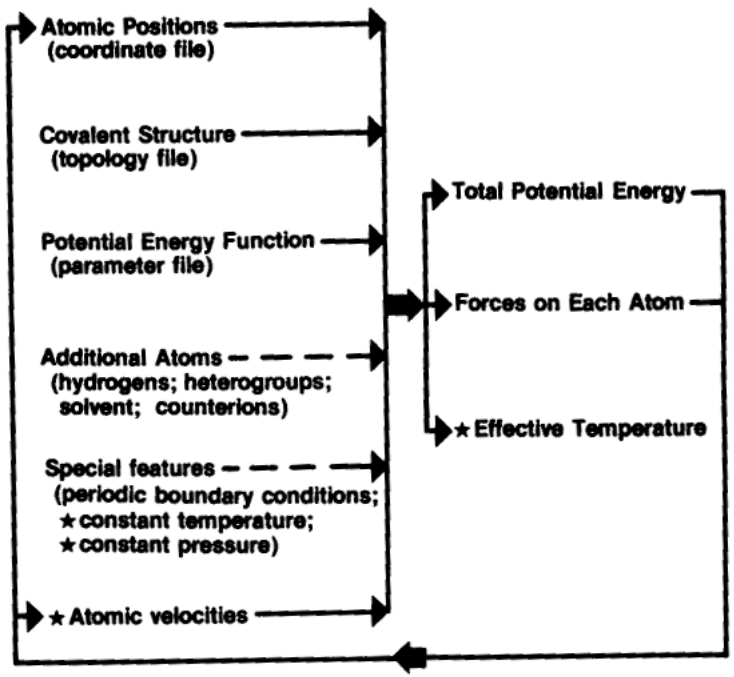


Fig. 4.1. Schematic flow chart of algorithms for energy minimization and molecular dynamics. Features which apply only to molecular dynamics are indicated by asterisks. Dashed lines indicate optional input. Each cycle of energy minimization represents a step in conformation space, while each cycle of molecular dynamics represents a step in time.

Illustration Credit: McCammon & Harvey (1987)

Practical Aspects: simulation cycle II

- Update Positions with MD equations, then recalculate potential and continue
- Momentum conservation
- Energy Conserved in NVE ensemble
- Hydrophobic interaction naturally arises from water behavior

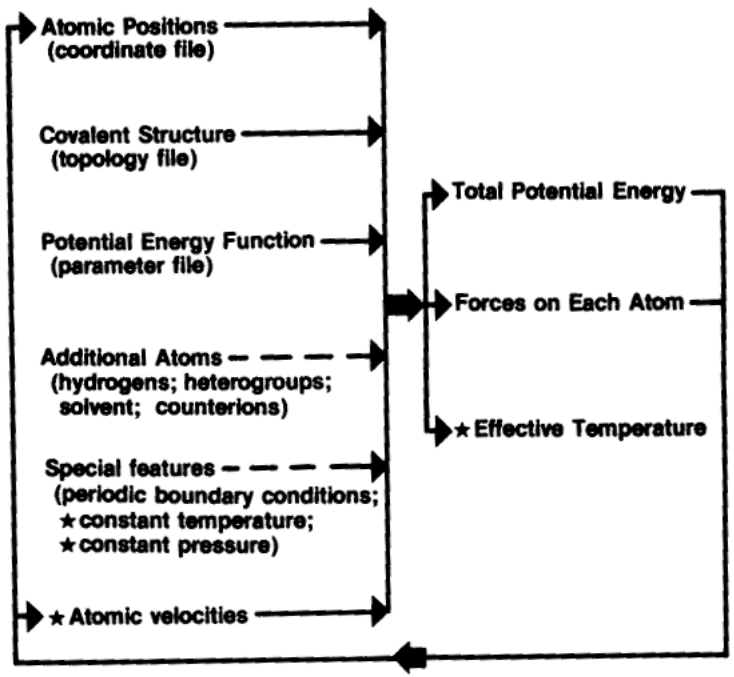


Fig. 4.1. Schematic flow chart of algorithms for energy minimization and molecular dynamics. Features which apply only to molecular dynamics are indicated by asterisks. Dashed lines indicate optional input. Each cycle of energy minimization represents a step in conformation space, while each cycle of molecular dynamics represents a step in time.

Illustration Credit: McCammon & Harvey (1987)

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  - ◊ http://uracil.cmc.uab.edu/Tutorials/default.html
- ENCAD
- GROMOS
  - ◊ http://rugmd0.chem.rug.nl/md.html
  - ◊ “Advanced Crash Course on Electrostatics in Simulations” (!) (http://rugmd0.chem.rug.nl/~berends/course.html)

Moving Molecules Rigidly

- $\mathbf{X}_i(t+1) = (x_i(t),y_i(t),z_i(t))$ = coordinates of ith atom in the molecule at timestep t
- Rigid-body Translation of all i atoms
  - ◊ For each atom atom i do $\mathbf{x}_i(t+1) = \mathbf{x}_i(t) + \mathbf{v}$

- Rigid-body Rotation of all i atoms
  - ◊ For each atom atom i do $\mathbf{x}_i(t+1) = \mathbf{R}(\phi,\theta,\psi)\ \mathbf{x}_i(t)$
  - ◊ Effectively do a rotation around each axis (x, y, z) by angles ϕ,θ,ψ (see below)
  - ◊ Many conventions for doing this
    - **BELOW IS ONLY FOR MOTIVATION**
    - Consult Allen & Tildesley (1987) or Goldstein (1980) for the formulation of the rotation matrix using the usual conventions
  - ◊ How does one do a random rotation? Trickier than it seems

$$\begin{pmatrix} x' \\ y' \end{pmatrix} = \begin{pmatrix} \cos\theta & -\sin\theta \\ \sin\theta & \cos\theta \end{pmatrix} \begin{pmatrix} x \\ y \end{pmatrix}$$

$$\begin{pmatrix} x' \\ y' \\ z' \end{pmatrix} = \underbrace{\begin{pmatrix} \cos\theta & -\sin\theta & 0 \\ \sin\theta & \cos\theta & 0 \\ 0 & 0 & 1 \end{pmatrix}}_{\text{Finally, rotate by } \theta \text{ around z axis}} \underbrace{\begin{pmatrix} \cos\phi & 0 & -\sin\phi \\ 0 & 1 & 0 \\ \sin\phi & 0 & \cos\phi \end{pmatrix}}_{\text{Second, rotate by } \phi \text{ around y axis}} \underbrace{\begin{pmatrix} 1 & 0 & 0 \\ 0 & \cos\psi & -\sin\psi \\ 0 & \sin\psi & \cos\psi \end{pmatrix}}_{\text{First, rotate by } \psi \text{ around x axis}} \begin{pmatrix} x \\ y \\ z \end{pmatrix}$$

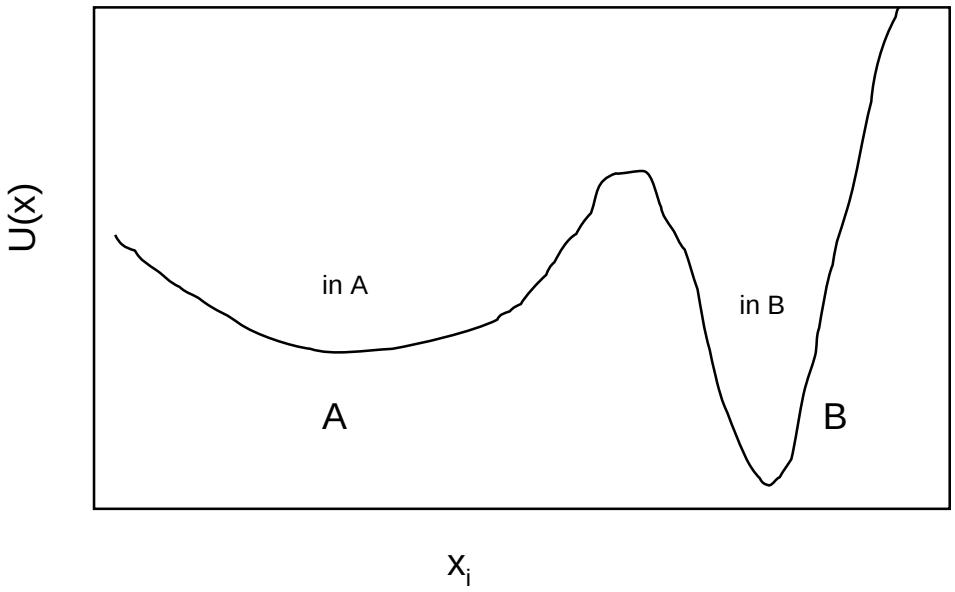
Simulation, Part II: Analysis: What can be Calculated from Simulation?

Average over simulation

- Deceptive Instantaneous Snapshots (almost anything can happen)
- Simple thermodynamic averages
  - ◊ Average potential energy <U>
  - ◊ T ~ < Kinetic Energy > = ½ m < v^2 >
- Some quantities fixed, some fluctuate in different ensembles
  - ◊ NVE protein MD (“microcanonical”)
  - ◊ NVT liquid MC (“canonical”)
  - ◊ NPT more like the real world

Energy and Entropy

- Energy
  - ◊ At each point i (with coordinates x_i) on the pot. energy surface there is a well-defined "energy" $U(x_i)$
- Probability of occurrence
  - ◊ $P_i = \exp(-U_i/kT)/Q$
  - ◊ The boltzmann distribution
  - ◊ Q = Sum over all P_i , to normalize probabilities to 1

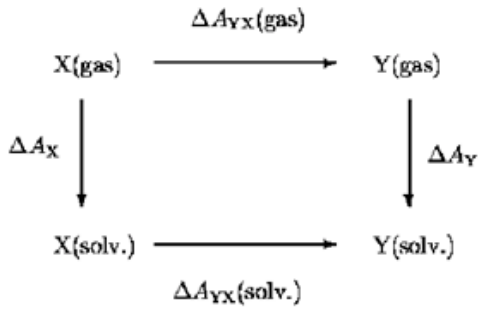


- Entropy
  - ◊ $S(A) = k \sum (P_i \ln P_i)$, where the sum is over points i in A
- Free Energy
  - ◊ G(A) = U(A) - TS(A)
- Entropy and Free Energy are only defined for distinctly diff. "states" -- e.g. A ("unfolded")and B ("folded")
  - ◊ State B has a lower U and its minimum is more probable than State A
  - ◊ However, state A has a broader minimum that can be occupied in more ways
- Relative Prob
  - ◊ $\frac{P(A)}{P(B)} = \frac{\exp(-G(A)/kT)}{\exp(G(B)/kT)}$

Application of Simulation: Thermodynamic Cycles

Molecular mutation

The difference of free energy of solvation $\Delta\Delta\mu_{YX}$ between two solutes X and Y can be calculated by the following thermodynamic cycle:



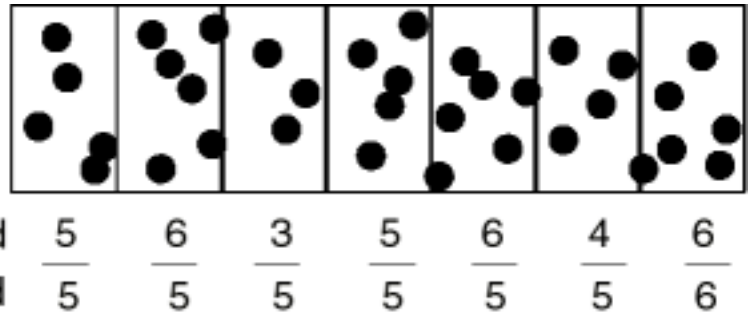
where $\Delta\mu_X$ and $\Delta\mu_Y$ are, respectively, the free energy of solvation of X and Y, and $\Delta\mu_{YX}(\text{gas})$ and $\Delta\mu_{YX}(\text{solv.})$ are the free energies of mutating X in Y in, respectively, in the gas phase and the solution phase. (*Computational alchemy.*)

The differences of free energies of solvation is

$$\Delta\Delta\mu_{YX} = \Delta\mu_Y - \Delta\mu_X = \Delta\mu_{YX}(\text{solv.}) - \Delta\mu_{YX}(\text{gas}) \quad (138)$$

Text block adapted from on-line notes at Rutgers Chemistry

Number Density

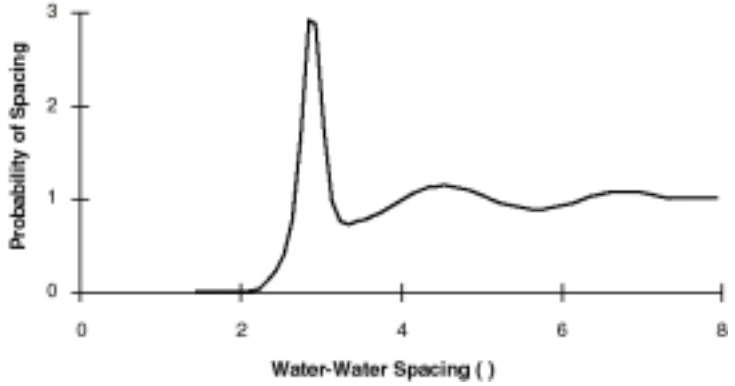


= Number of atoms per unit volume averaged over simulation divided by the number you expect to have in the same volume of an ideal “gas”

Spatially average over all directions gives

1D RDF =

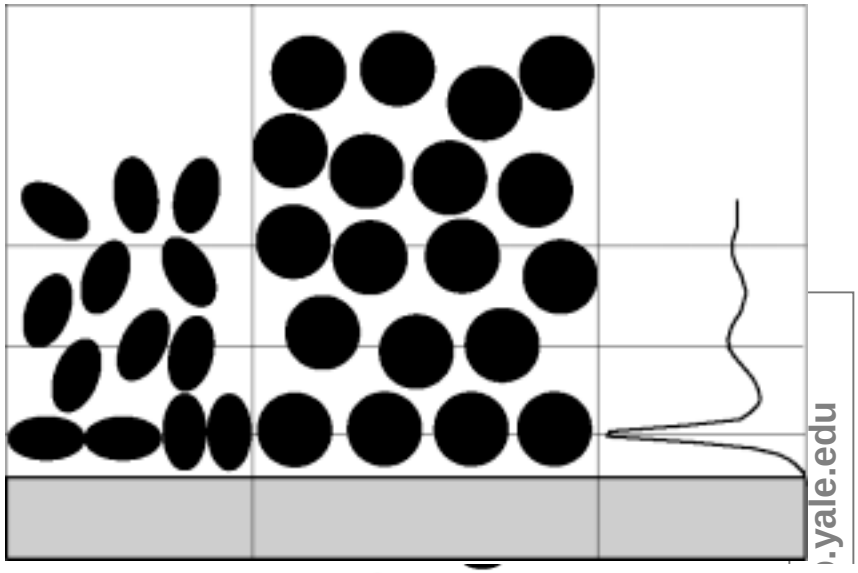
[Avg. Num. Neighbors at r]
[Expected Num. Neighbors at r]

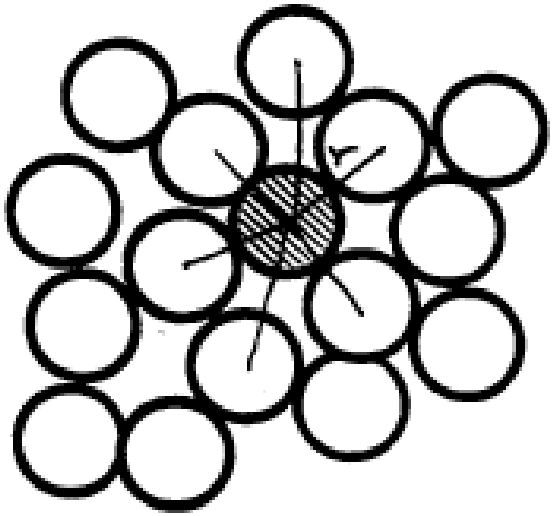


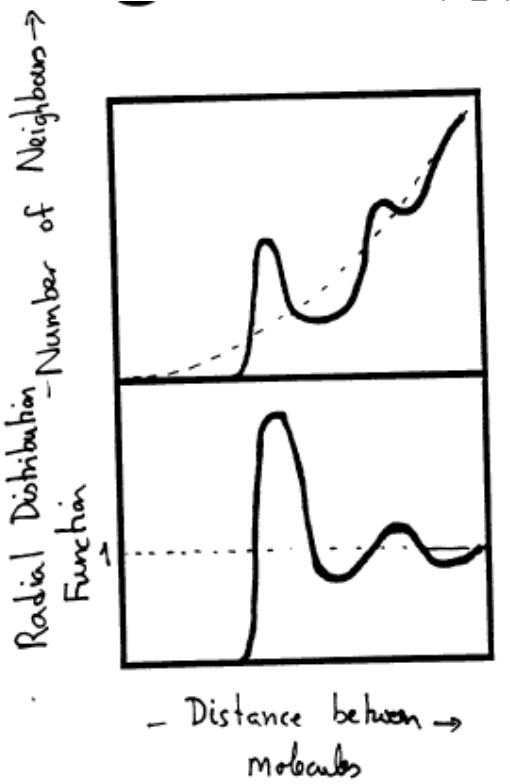
“at r” means contained in a thin shell of thickness dr and radius r.

Number Density (cont)

- Advantages: Intuitive, Relates to scattering expts
- D/A: Not applicable to real proteins
  - ◊ 1D RDF not structural
  - ◊ 2D proj. only useful with "toy" systems
- Number densities measure spatial correlations, not packing
  - ◊ Low value does not imply cavities
  - ◊ Complicated by asymmetric molecules
  - ◊ How things pack and fit is property of instantaneous structure - not average







Measurement of Dynamic Quantities I

- The time-course of a relevant variable is characterized by

(1) Amplitude (or magnitude), usually characterized by an RMS value

$R = \mathrm{sqrt}[< (a(t) - \langle a(t) \rangle)^2 >]$

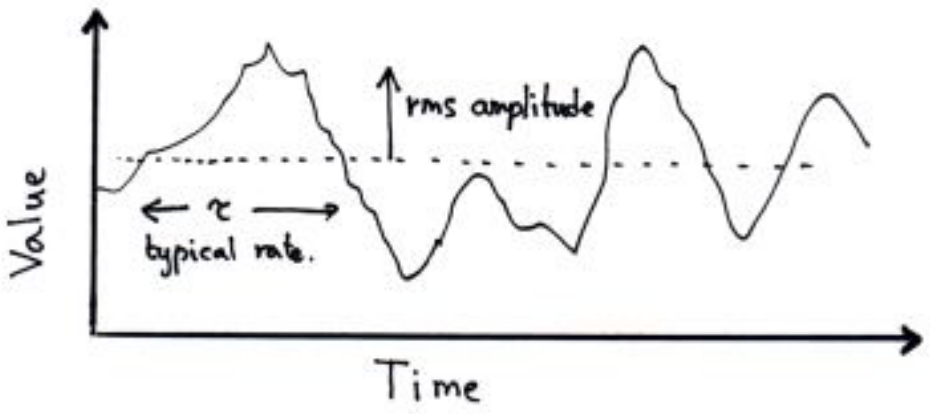
$R = \mathrm{sqrt}[< a(t)^2 - 2a(t)\langle a(t) \rangle + \langle a(t) \rangle^2 >]$

$R = \mathrm{sqrt}[< a(t)^2 > - \langle a(t) \rangle^2]$

  - similar to SD
  - fluctuation

- Relevant variables include bond length, solvent molecule position, H-bond angle, torsion angle

Illustration from M Levitt, Stanford University



Measurement of Dynamic Quantities II

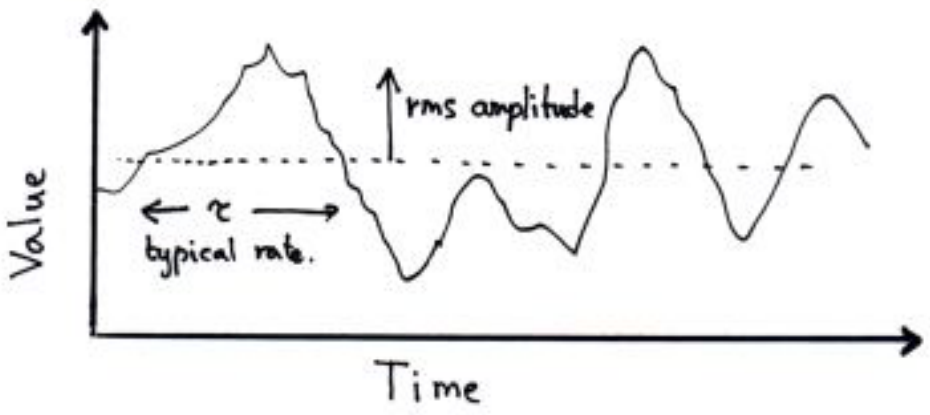
- The time-course of a relevant variable is characterized by

(2) Rate or time-constant

  - ◊ Time Correlation function
  - ◊ $C_A(t) = \langle A(s)A(t+s) \rangle = \langle A(0)A(t) \rangle$ [averaging over all s]
  - ◊ Correlation usually exponentially decays with time t
  - ◊ decay constant is given by the integral of C(t) from t=0 to t=infinity

- Relevant variables include bond length, solvent molecule position, H-bond angle, torsion angle

Illustration from M Levitt, Stanford University



D & RMS

- Diffusion constant
  - ◊ Measures average rate of increase in variance of position of the particles
  - ◊ Suitable for liquids, not really for proteins

$$D = \frac{\langle \Delta r^2 \rangle}{6\Delta t}$$

- RMS more suitable to proteins

$$RMS(t) = \sqrt{\frac{\sum_{i=1}^{N} d_i(t)}{N}}$$

$$d_i(t) = \mathbf{R}(\mathbf{x}_i(t) - \mathbf{T}) - \mathbf{x}_i(0)$$

  - ◊ di = Difference in position of protein atom at t from the initial position, after structures have been optimally rotated translated to minimize RMS(t)
  - ◊ Solution of optimal rotation has been solved a number of ways (Kabsch, SVD)

Observed RMS values

COMPARISON OF OVERALL VALUES

Property	Value in vacuo	Value in soln.	Value expt.
• All-Atom R.M.S. Deviation (Å)	2·60	1·55	1·3 (0·5)
• C^{α} Fluctuation (Å)	0·54	0·43	0·68
• Radius of Gyration (Å)	10·9	11·5	11·5

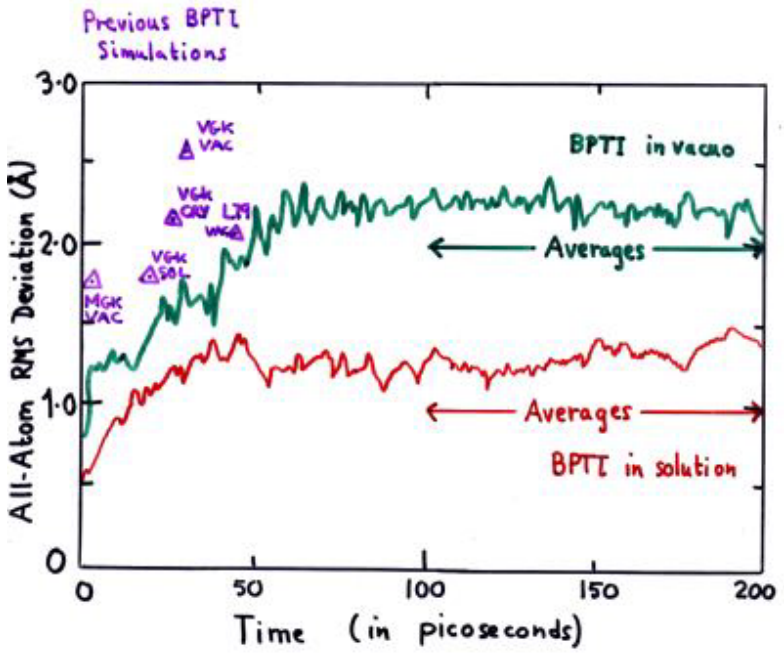


Illustration from M Levitt, Stanford University

Other Things to Calculate

- Fraction of Native Contacts
- Percent Helix
- Radius of Gyration

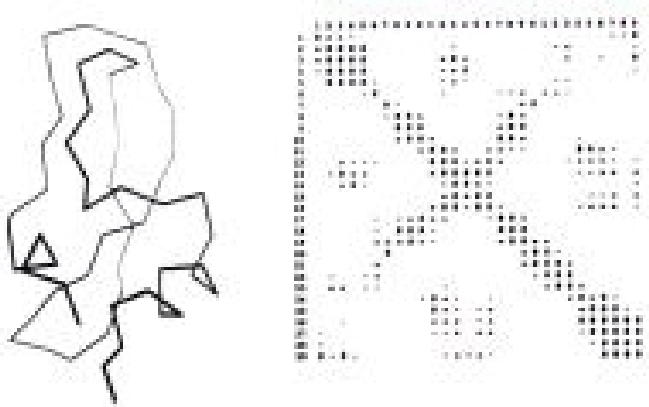
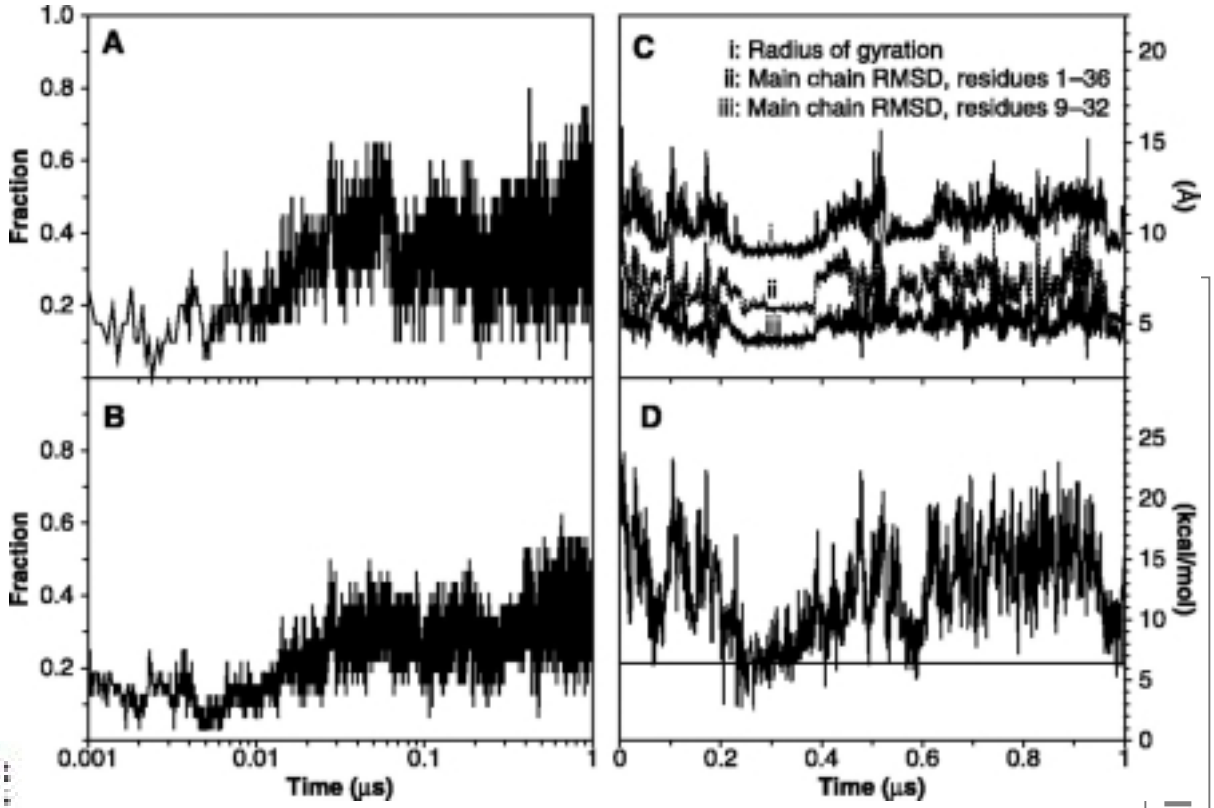


Illustration and Caption from Duan & Kollman (1998)



Caption: Time evolution of (A) fractional native helical content, (B) fractional native contacts, (C) R and the main chain rmsd from the native structure, and (D) SFE of the protein. The helical content and the native contacts are plotted on a logarithmic time scale. The helical content was measured by the main chain - angle
(60° ± 30°, 40° ± 30°). The native contacts were measured as the number of neighboring residues present in 80% of the last 50 ns of the native simulation. Residues are taken to be in contact if any of the atom pairs are closer than 2.8 Å, excluding residues i and i+1, which always have the contacts through main chain atoms. The SFE was calculated as described by Eisenberg and McLachlan (31) using their parameters (0.0163, 0.00637, 0.02114, 0.02376, and 0.05041, in kcal mol Å2, for the surface areas of nonpolar, polar, sulfur, charged oxygen, and charged nitrogen, respectively). The straight line represents the SFE of the native structure.

Monitor Stability of Specific Hydrogen Bonds

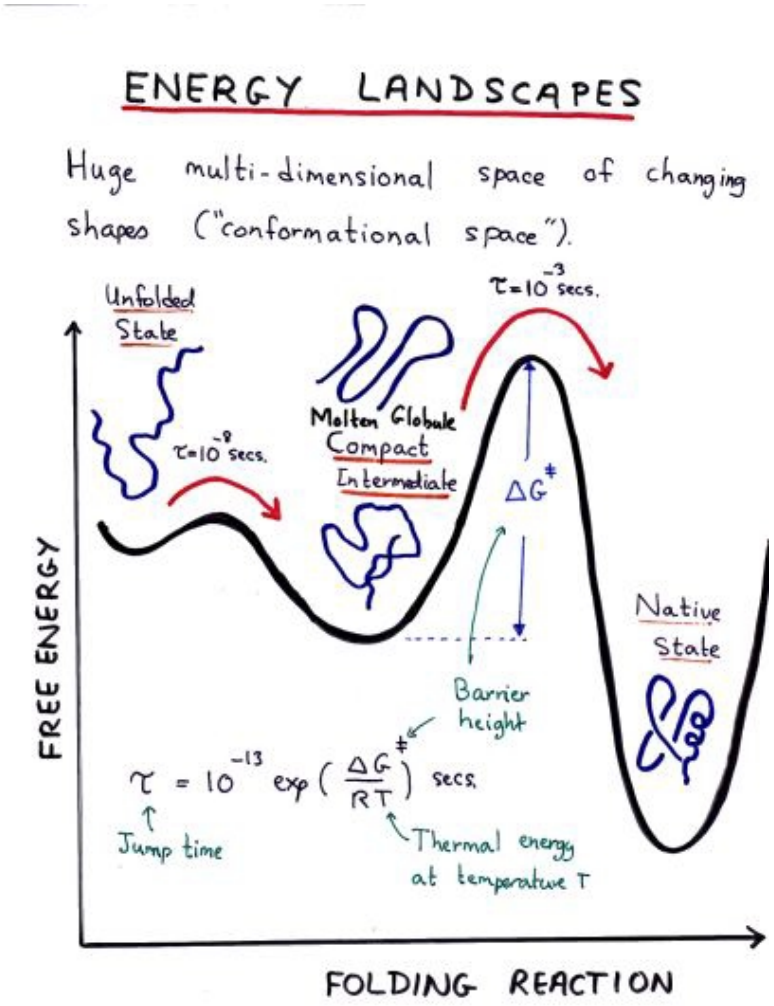
HYDROGEN BONDS

Secondary Structure	O.. H Pair	Stability (%) in vacuo	Stability (%) in soln.
β hairpin	35 .. 18	12	57
	18 .. 35	85	63
	33 .. 20	91	76
	20 .. 33	80	86
	31 .. 22	53	93
	22 .. 31	82	87
	29 .. 24	72	67
	24 .. 29	37	34
	45 .. 21	63	86
	21 .. 45	14	42
α-helix	47 .. 51	76	66
	48 .. 52	93	90
	49 .. 53	90	98
	50 .. 54	78	90
	51 .. 55	73	93
	52 .. 56	-	42

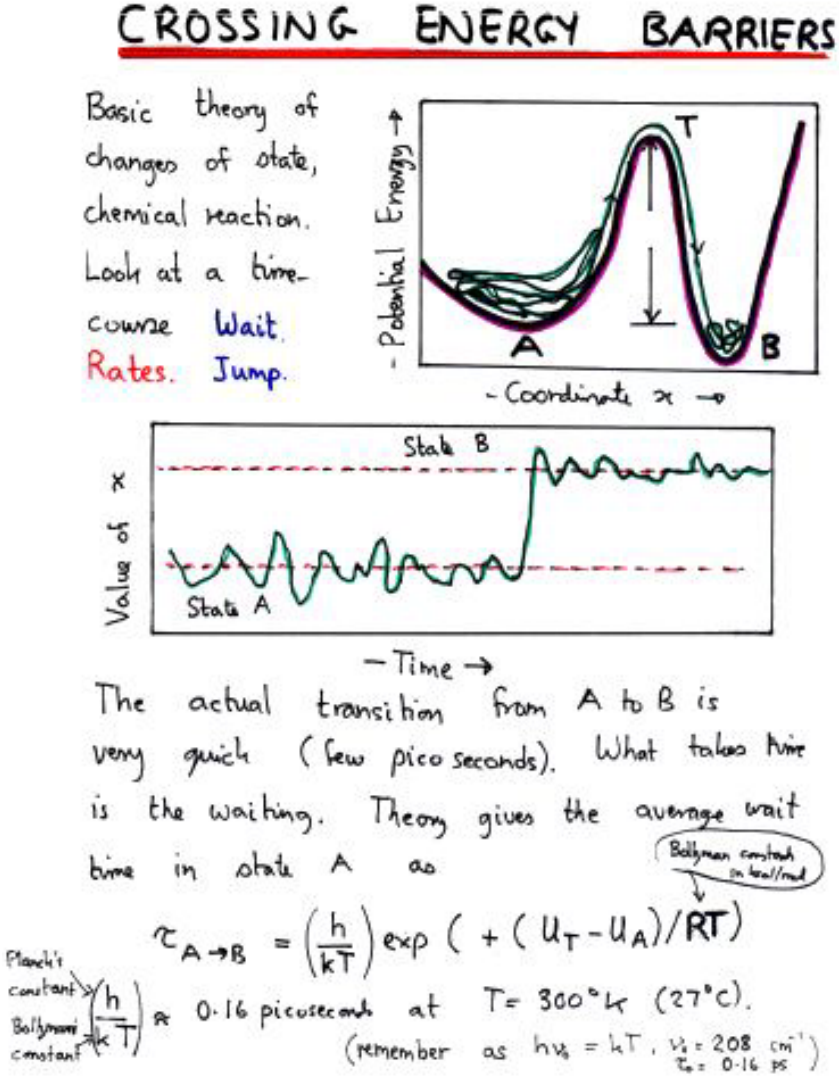
- Hydrogen bonds in solution are as strong as in vacuo
- Relative strength on position in secondary structure

Illustration from M Levitt, Stanford University

Energy Landscapes and Barriers Traversed in a Simulation



Illustrations from M Levitt, Stanford University



Timescales

Motion	length (Å)	time (fs)
bond vibration	0.1	10
water hindered rotation	0.5	1000
surface sidechain rotation	5	10^5
water diffusive motion	4	10^5
buried sidechain libration	0.5	10^5
hinge bending of chain	3	10^6
buried sidechain rotation	5	10^{13}
allosteric transition	3	10^{13}
local denaturation	7	10^{14}

Values from McCammon & Harvey (1987) and Eisenberg & Kauzmann

Electrostatics Revisited: the Poisson-Boltzmann Equation

Poisson-Boltzmann equation

- Macroscopic dielectric
  - ◊ As opposed to microscopic one as for realistic waters
- Linearized: sinh $\phi = \phi$
  - ◊ counter-ion condense

- The model
  - ◊ Protein is point charges embedded in a low dielectric.
  - ◊ Boundary at accesible surface
  - ◊ Discontinuous change to a new dielectric
    (no dipoles, no smoothly varying dielectric)

PBE Eq. Ugh!

- $$\vec{\nabla} \cdot [\varepsilon(\vec{r}) \vec{\nabla} \varphi(\vec{r})] - \varepsilon(\vec{r}) \bar{\kappa}(\vec{r}) \sinh[\varphi(\vec{r})] - \frac{4\pi}{kT} \rho^f(\vec{r}) = 0$$

dielectric const — IN

potential — OUT

ionic strength — IN

fixed charges — IN

Simplifications of the Poisson-Boltzmann equation

- Laplace eq.
  - ◊ div grad V = ρ
  - ◊ grad V = **E** field
  - ◊ Only have divergence when have charge source

PBE Eq. Ugh!

- $\vec{\nabla} \cdot [\varepsilon(\vec{r}) \vec{\nabla} \varphi(\vec{r})] - \varepsilon(\vec{r}) \bar{K}(\vec{r}) \sinh[\varphi(\vec{r})] - \frac{4\pi}{kT} \rho^f(\vec{r}) = 0$

dielectric const — IN

potential — OUT

ionic strength — IN

fixed charges — IN

- No moving ions, Constant Dielectric → Poisson's Eq.

$$\vec{\nabla}^2 \varphi(\vec{r}) = \frac{4\pi}{kT\varepsilon} \rho(\vec{r})$$

- Finite Difference Soln. to PDE

  (PDE has deriv. WRT to 2 var.
  ODE like Newton's Eq. has deriv. WRT to 1 var.)

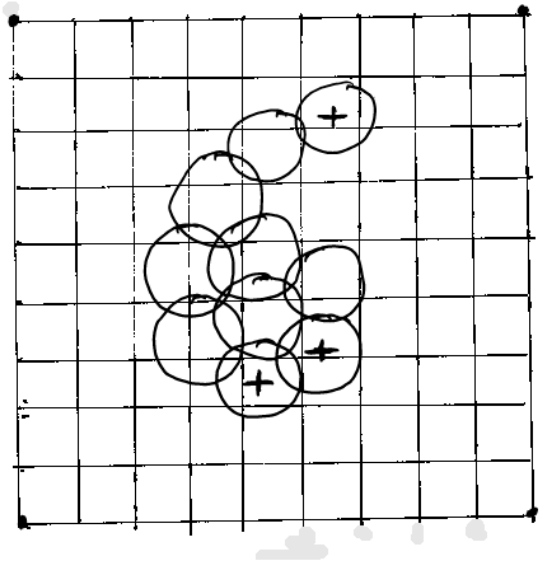
- $\vec{\nabla}^2 \varphi(\vec{r}) = \frac{4\pi}{KT\varepsilon} \rho(\vec{r})$

- in 2D $\frac{\partial^2 \varphi}{\partial x^2} + \frac{\partial^2 \varphi}{\partial y^2} = C\rho(\vec{r})$ (const.)

- $\frac{\partial^2 \varphi}{\partial x^2} = \frac{\partial}{\partial x}\left(\frac{\partial \varphi}{\partial x}\right) = \frac{\partial}{\partial x}\left(\frac{V_{j+1} - V_j}{\Delta}\right) = \frac{(V_{j+1} - V_j) - (V_j - V_{j-1})}{\Delta^2}$

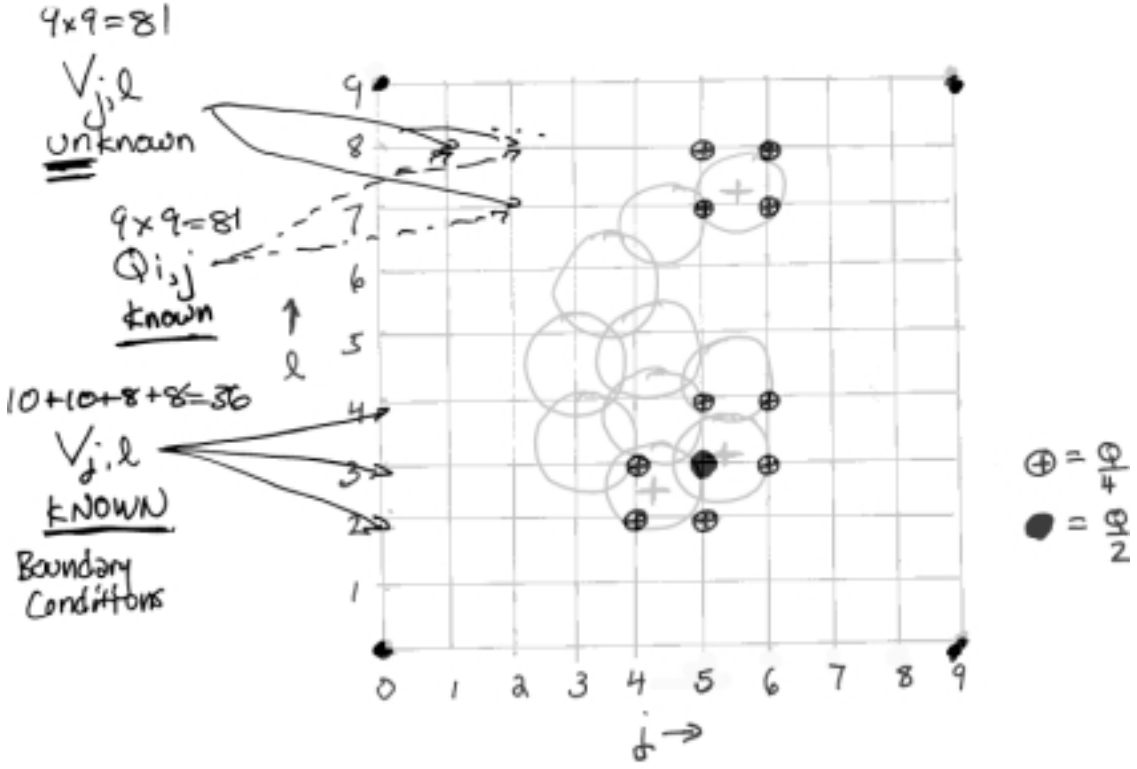
  $(\partial x = \Delta \;,\; \partial \varphi = V_{j+1} - V_j)$

- $V_{j+1,l} + V_{j-1,l} + V_{j,l+1} + V_{j,l-1} - 4V_{j,l} = \Delta^2 C Q_{j,l}$



Protein on a Grid

For intuition ONLY -- Don't need to know in detail!!



Demand Consistency on the Grid

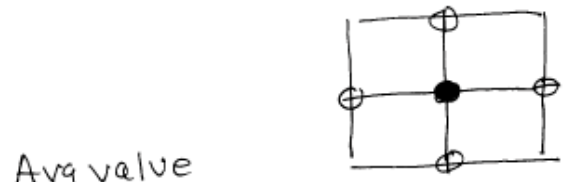
- $V_{j+1,l} + V_{j-1,l} + V_{j,l+1} + V_{j,l-1} - 4V_{j,l} = \Delta^2 C Q_{j,l}$
- System of Equations → solve for unknown $V_{j,l}$
- Matrix Inversion in Finite Diff. method

Relaxation: Deviation from consistency should vanish at $t \to \infty$

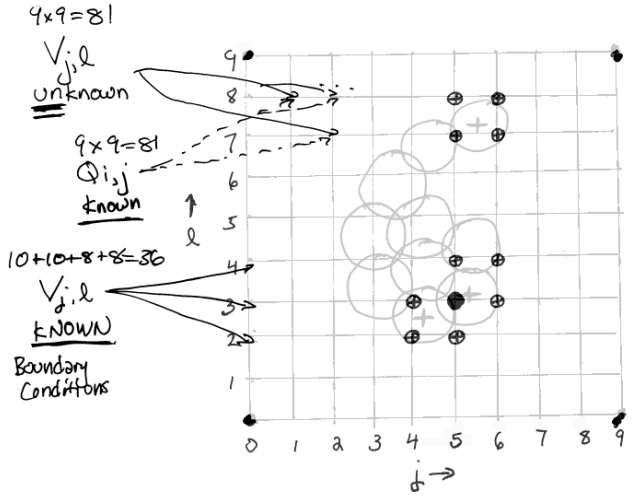
$$\nabla^2 V - 4\pi\rho = \left(\frac{\partial V}{\partial t}\right) \to 0 \text{ at } t=\infty$$

$$V_{j,l}^{t+1} \leftarrow V_{j,l}^{t} + \Delta t \left(\frac{V_{j+1,l}^{t} + V_{j-1,l}^{t} + V_{j,l+1}^{t} + V_{j,l-1}^{t} - 4V_{j,l}^{t}}{\Delta^2} - Q_{j,l} \right)$$

For intuition ONLY -- Don't need to know in detail!!

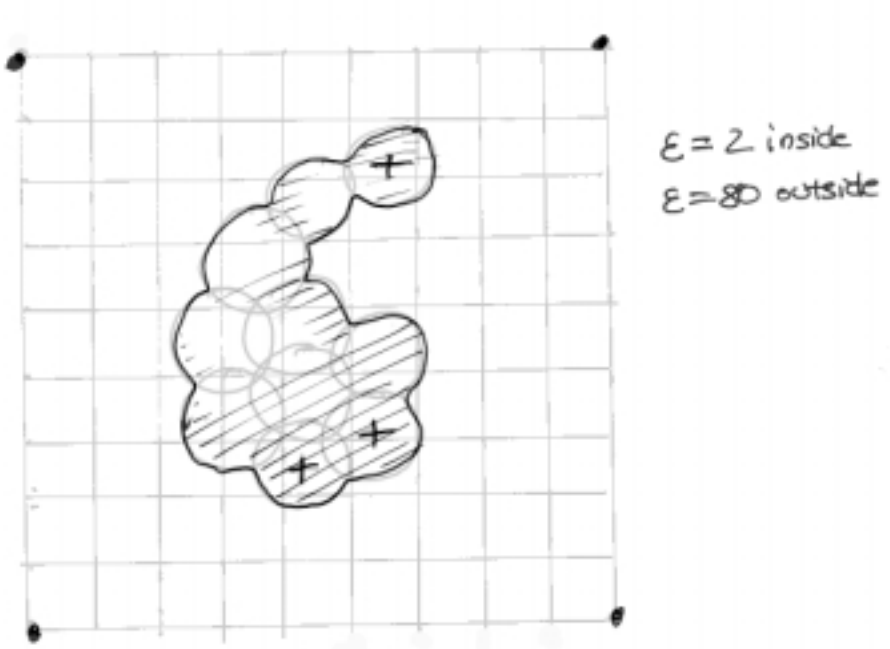


Avg value at center (●) is avg. value at 4 outside nodes (⊕) plus charge at center



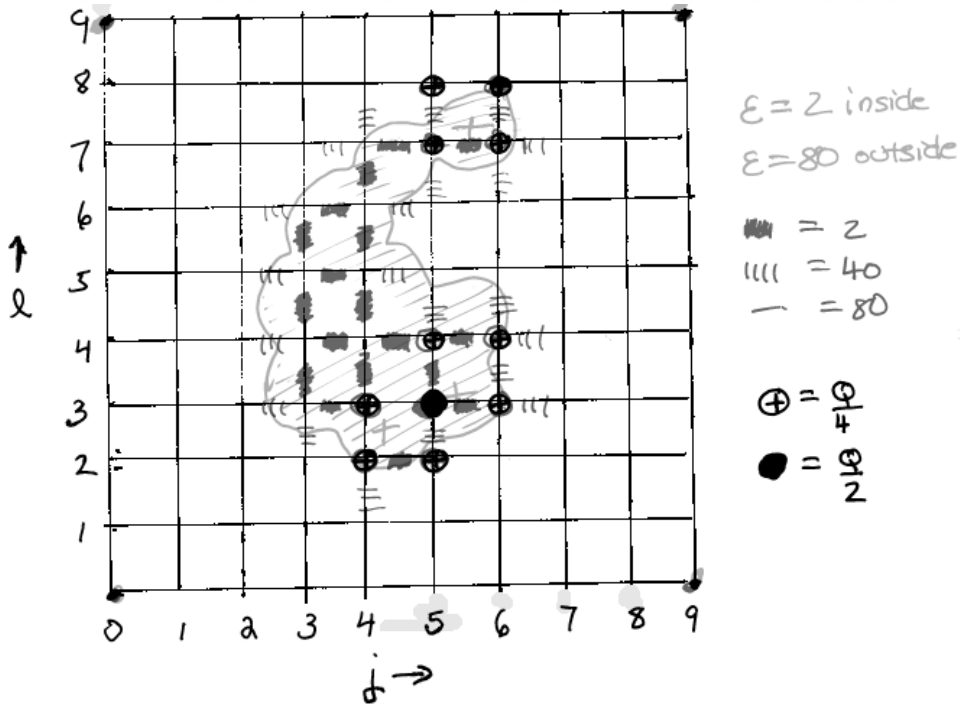
(c) Mark Gerstein, 1999, Yale, bioinfo.mbb.yale.edu

Adding a Dielectric Boundary into the Model

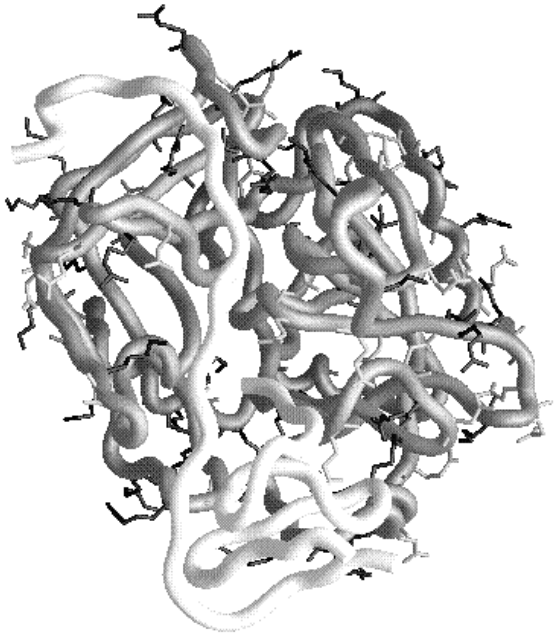


$$\nabla \cdot (\epsilon(r) \nabla \varphi) \Rightarrow$$

$$\frac{1}{\Delta^2}\left(\epsilon(j \to j+1)(V_{j+1} - V_j) - \epsilon(j-1 \to j)(V_j - V_{j-1})\right)$$

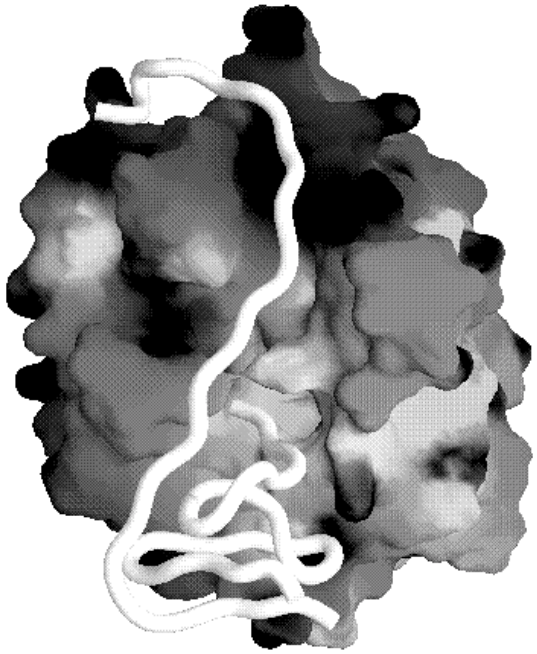


Electrostatic Potential of Thrombin



The proteolytic enzyme Thrombin (dark backbone worm) complexed with an inhibitor, hirudin (light backbone worm). The negatively charged (Light gray) and positively charged (dark gray) sidechains of thrombin are shown in bond representation.

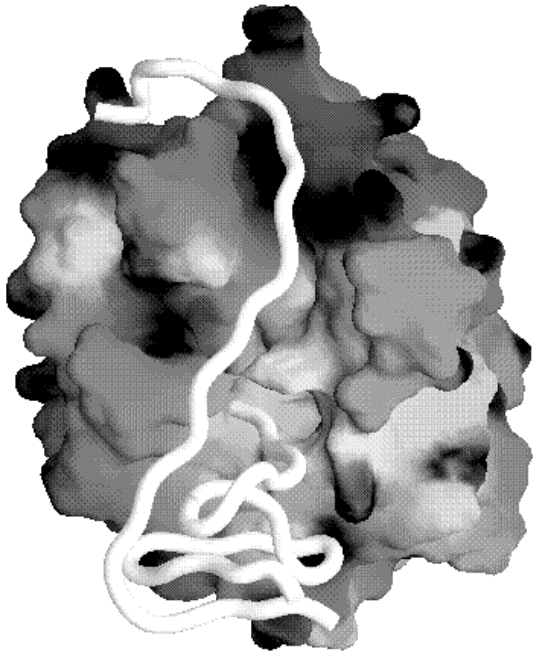
Graphical analysis of electrostatic potential distributions often reveals features about the structure that complement analysis of the atomic coordinates. For example, LEFT shows the distribution of charged residues in the binding site of the proteolytic enzyme thrombin. RIGHT shows the resulting electrostatic potential distribution on the protein surface. The basic (positive) region in the fibrinogen binding, while it could be inferred from close inspection of the distribution of charged residues in TOP, is more apparent in the potential distribution.



Solvent accessible surface of thrombin coded by electrostatic potential (dark: positive, light: negative). Hirudin is shown as a light backbone worm. Potential is calculated at zero ionic strength.

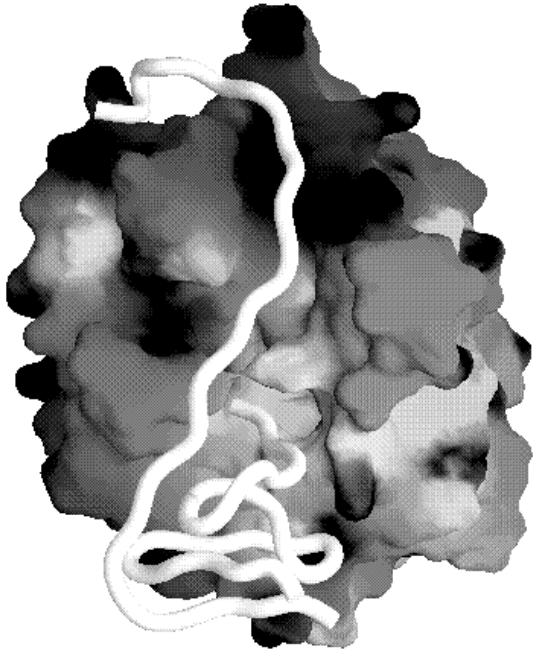
Illustration Credit: Sharp (1999)
Text captions also from Sharp (1999)

Increasing Ionic Strength



Solvent accessible surface of thrombin coded by electrostatic potential (dark: positive, light: negative). Hirudin is shown as a light backbone worm. Potential is calculated at physiological ionic strength (0.145M)

TOP shows the effect of increasing ionic strength on the potential distribution, shrinking the regions of strong potential in comparison to BOTTOM.

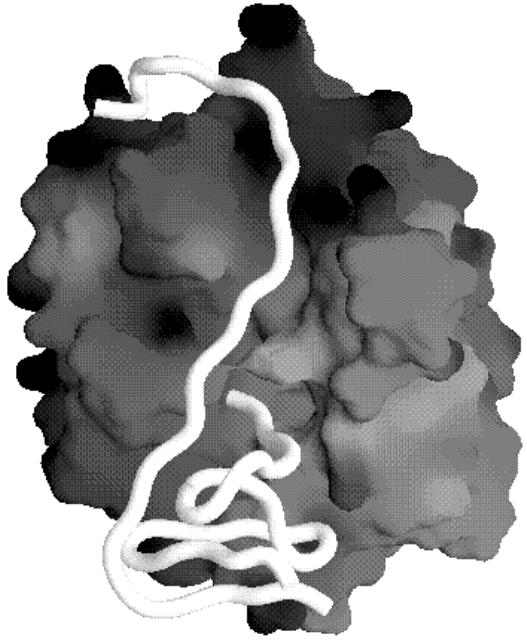


Solvent accessible surface of thrombin coded by electrostatic potential (dark: positive, light: negative). Hirudin is shown as a light backbone worm. Potential is calculated at zero ionic strength.

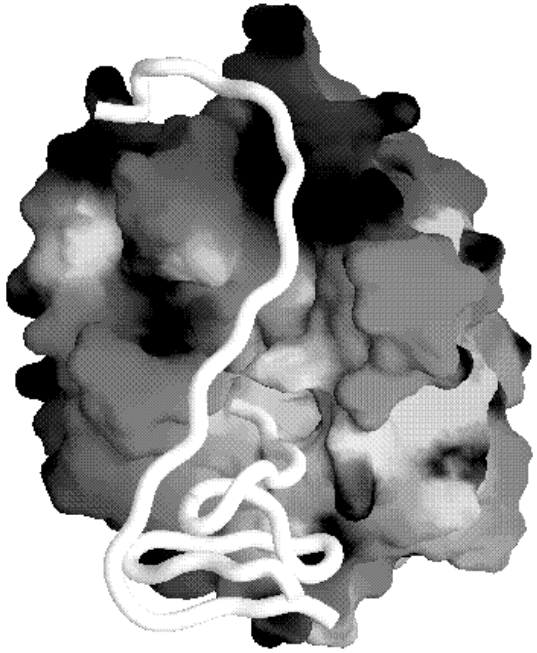
Illustration Credit: Sharp (1999)
Text captions also from Sharp (1999)

Increasing Dielectric

Solvent accessible surface of thrombin coded by electrostatic potential (dark: positive, light: negative). Hirudin is shown as a light backbone worm. Potential is calculated using the same polarizability for protein and solvent.



TOP is calculated assuming the same dielectric for the solvent and protein. The more uniform potential distribution compared to BOTTOM shows the focusing effect that the low dielectric interior has on the field emanating from charges in active sites and other cleft regions.



Solvent accessible surface of thrombin coded by electrostatic potential (dark: positive, light: negative). Hirudin is shown as a light backbone worm. Potential is calculated at zero ionic strength.

Illustration Credit: Sharp (1999)
Text captions also from Sharp (1999)

pKa shifts

Charge transfer processes are important in protein catalysis, binding, conformational changes and many other functions. The primary examples are acid-base equilibria, electron transfer and ion binding, in which the transferred species is a proton, an electron or a salt ion respectively. The theory of the dependence of these three equilibria within the classical electrostatic framework can be treated in an identical manner, and will be illustrated with acid-base equilibria. A titratable group will have an intrinsic ionization equilibrium, expressed in terms of a known intrinsic $pK^{o}a$. Where $pK^{o}a = -\log_{10}(K^{o}a)$, $K^{o}a$ is the dissociation constant for the reaction $H^{+}A = H^{+}+A$ and A can be an acid or a base. The $pK^{o}a$ is determined by all the quantum chemical, electrostatic and environmental effects operating on that group in some reference state. For example a reference state for the aspartic acid side-chain ionization might be the isolated amino acid in water, for which $pK^{o}a = 3.85$. In the environment of the protein the pKa will be altered by three electrostatic effects. The first occurs because the group is positioned in a protein environment with a different polarizability, the second is due to interaction with permanent dipoles in the protein, the third is due to charged, perhaps titratable, groups. The effective pKa is given by (where the factor of 1/2.303kT converts units of energy to units of pKa):

$$pKa = pK^{o}a + (\Delta\Delta G^{rf}+\Delta\Delta G^{perm}+\Delta\Delta G^{tit})/2.303kT$$

Text block from Sharp (1999)

1. Desolvation, Rx Field

2. Permanent Dipoles

3. Other Charges

pKa continued I

Text block from Sharp (1999)

1. Desolvation, Rx Field

The first contribution, $\Delta\Delta G^{rf}$, arises because the completely solvated group induces a strong favorable reaction field (See section 22.3.2.3) in the high dielectric water, which stabilizes the charged form of the group (The neutral form is also stabilized by the solvent reaction field induced by any dipolar groups, but to a lesser extent). Desolvating the group to any degree by moving it into a less polarizable environment will preferentially destabilize the charged form of that group, shifting the pKa by an amount

$$\Delta\Delta G^{rf} = \frac{1}{2}\sum_i \left(q_i^d \Delta\phi_i^{rf,d} - q_i^p \Delta\phi_i^{rf,p} \right) \qquad (12)$$

where q_i^p and q_i^d are the charge distributions on the group, $\Delta\phi_i^{rf,p}$ and $\Delta\phi_i^{rf,d}$ are the changes in the group's reaction potential upon moving it from its reference state into the protein, in the protonated (superscript p) and deprotonated (superscript p) forms respectively, and the sum is over the group's charges.

The contribution of the permanent dipoles is given by

$$\Delta\Delta G^{tit} = \sum_i \left(q_i^d - q_i^p \right) \phi_i^{perm} \qquad (13)$$

2. Permanent Dipoles

where ϕ_i^{perm} is the interaction potential at the *i*'th charge due to all the permanent dipoles in the protein, including the effect of screening. It is observed that intrinsic pKa's of groups in proteins are rarely shifted by more than 1 pKa unit indicating that the effects of desolvation are often compensated to a large degree by the $\Delta\Delta G^{perm}$ term.

pKa continued II

3. Other Charges

The final term accounts for the contribution of all the other charge groups:

$$\Delta\Delta G^{titr} = \sum_i \left(q_i^d < \phi_i >^d_{pH,c,\Delta V} - q_i^p < \phi_i >^p_{pH,c,\Delta V} \right) \quad (14)$$

where $< \phi_i >$ is the mean potential at group charge *i* from all the other titratable groups. The charge state of the other groups in the protein depend in turn on their intrinsic "pKa's", on the external pH if they are acid-base groups, the external redox potential ΔV if they are redox groups, and the concentration of ions, c, if they are ion binding sites, as indicated by the subscript on $<\phi_i>$. Moreover, the charge state of the group itself will affect the equilibrium at the other sites. Because of this linkage, exact determination of the complete charged state of a protein is a complex procedure. If there are N such groups, the rigorous approach is to compute the titration state partition function by evaluating the relative electrostatic free energies of all 2^N ionization states for a given set of pH, c, ΔV. From this one may calculate the mean ionization state of any group as a function of pH, ΔV etc. For large N this becomes impractical, but various approximate schemes work well, including a Monte-Carlo procedure

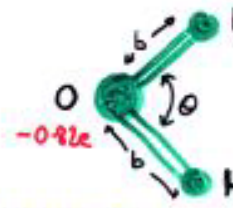
Text block from Sharp (1999)

Water Simulation and Hydrophobicity

Simulating Liquid Water

SIMULATING LIQUID WATER

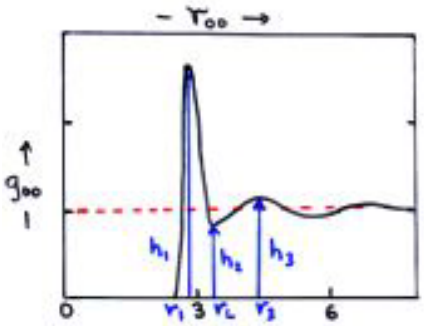
- Very simple model
  - 3 interaction centers
  - Completely flexible
  - Smooth cutoff at 6 Å (list to 8Å)



Electrostatics, Van der Waals } long range forces

- Good fit to experiment

Property		(25 °C) Experiment	Simulation
Potential energy (kcal/mol)		−9.2	−9.5
Pressure (atmospheres)		1	−61
Classical Specific Heat (cal/°K)		27	26
Diffusion Constant (Å²/ps)		0.23	0.22
Rotational Relaxation Time (ps)		2	1.6
Radial Distribution Function	r_1	2.8	2.7
	h_1	~~2.5~~ 3.0*	3.2
	r_2	3.3	3.3
	h_2	0.8	0.8
	r_3	4.6	4.3
	h_3	1.11	1.09

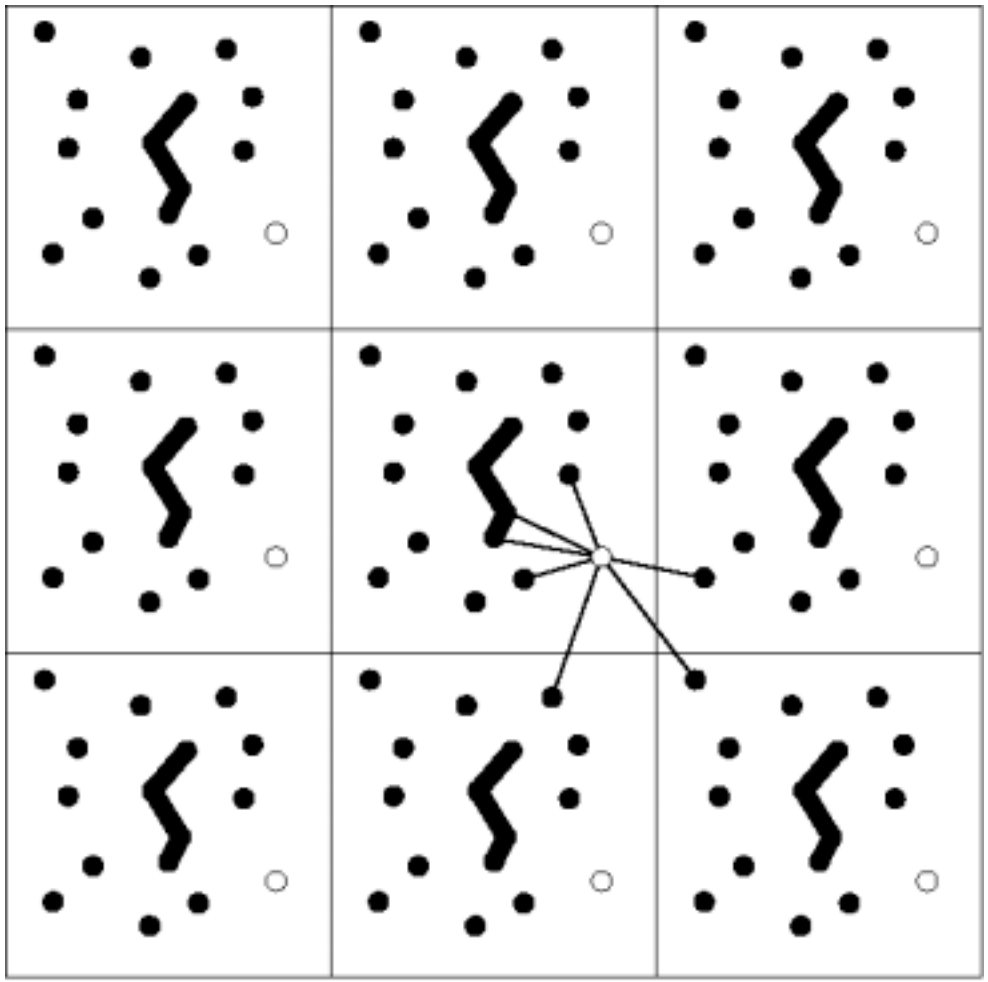


* Calibration error fixed after 15 years of experiment

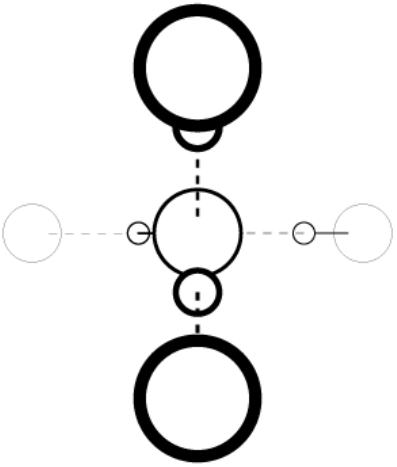
Illustrations from M Levitt, Stanford University

Periodic Boundary Conditions

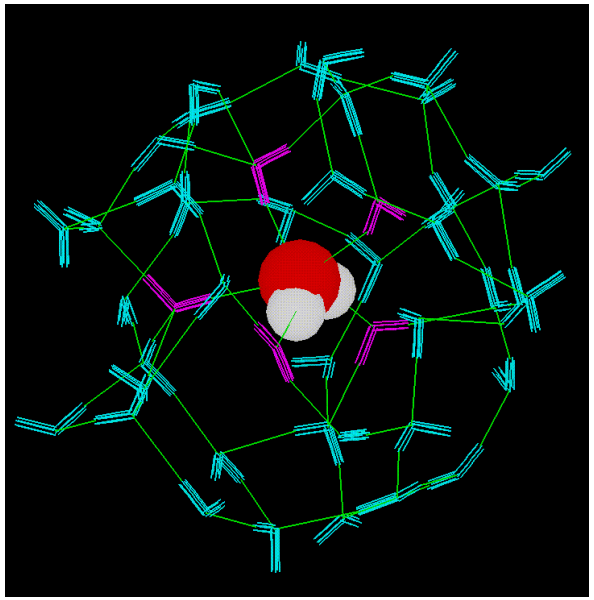
- Make simulation system seem larger than it is
- Ewald Summation for electrostatics (Fourier transform)



Tetrahedral Geometry of Water



HYDROGEN BONDS give water its unique properties. The hydrogen bond is a consequence of the electrical attraction between the positively charged hydrogen on one water molecule *(H1)* and the negatively charged oxygen on another water molecule *(O')*. The electrostatic repulsion between this oxygen and the oxygen that the hydrogen is covalently bonded to *(O)* gives the hydrogen bond a nearly linear geometry. Each water molecule can act as a donor of two hydrogen bonds to neighboring water oxygens. Each water can also accept two hydrogen bonds. This double-donor, double-acceptor situation naturally tends to favor a tetrahedral geometry with four waters around each water oxygen, as shown. Ice has this perfect tetrahedral geometry. However, in water, the tetrahedral geometry is distorted, and it is possible for a water molecule to accept or donate more than two hydrogen bonds (which are consequently highly distorted). Such a distortions of tetrahedral geometry are shown, which is taken from a frame in a simulation. Note that the central water molecule accepts three hydrogen bonds.



Hydrophobicity Arises Naturally in Simulation

- Add no hydrophobic Effect
  - ◊ This arises naturally from entropic effects during the simulation

Mixing is a spontaneous process: a substance will naturally dissolve in water unless there are manifestly unfavorable interactions between it and water. Scientists usually discuss the favorableness of particular interactions in terms of the energy associated with the intermolecular forces. Almost always there are at least some energetically favorable dispersion interactions between the solute and the water. However, the more salient issue is how the interaction between a solute and a water molecule *compares* in strength to the interaction between two water molecules or between two solute molecules. For instance, a polar molecule such as glucose is able to make comparable hydrogen bonds to water as water molecules can make with each other. Thus, there are no unfavorable interactions preventing it from dissolving and it is very soluble.

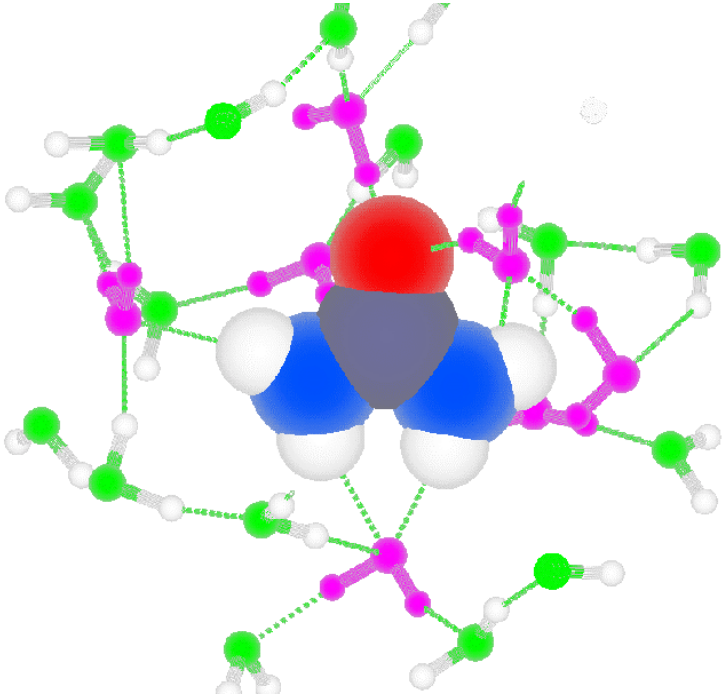
In contrast, water molecules are not able to hydrogen bond to methane, an insoluble, non-polar solute. They would rather interact with each other. The methane molecules, moreover, can favorably interact with each other through attractive dispersion forces. One can see how this situation leads to methane molecules trying to minimize their *relatively* unfavorable interactions with water molecules. An obvious way they can do this is by clumping together, aggregating, and coming out solution. Such aggregation of non-polar solutes in water is often called the *hydrophobic effect* and, as we shall, it is very important in macromolecular structure.

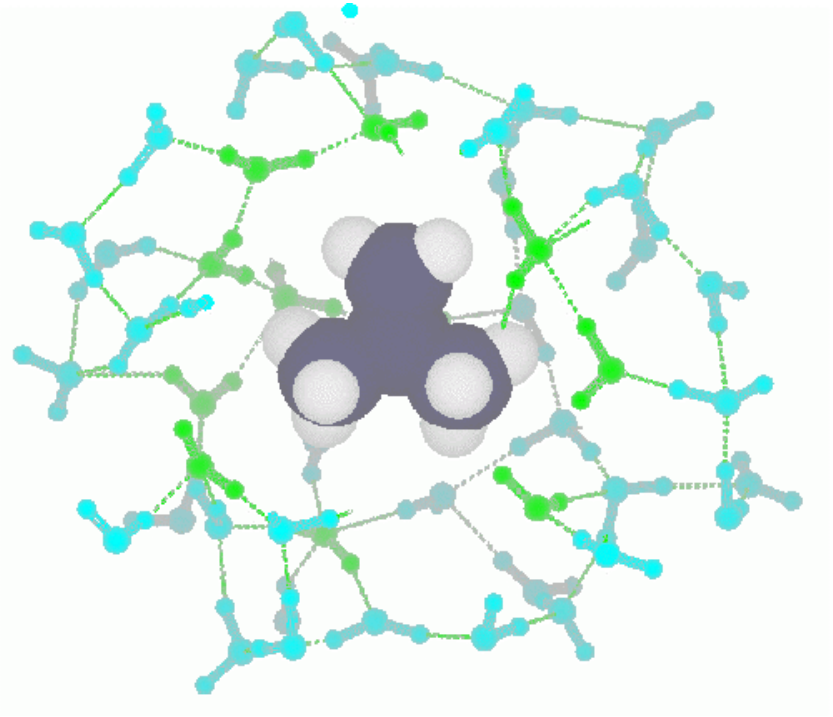
In terms of water structure at room temperature, the relatively unfavorable interaction between water and methane induces each water molecule next to methane to "turn away" from it and hydrogen bond to neighboring water molecules. If one of these turned water molecules manages to keep itself correctly oriented over time, it will have will not have to sacrifice any of its usual four to five hydrogen bonds. This brings up an interesting paradox: From the standpoint of favorable interactions, or energy in more formal terminology, water has not paid any price in solvating the methane. Consequently, there appears to be no energetic reason for methane to be insoluble in water.

This paradox is resolved by entropy. According to one way of thinking, entropy reflects the number of possible states a molecule can exist in. Thus, the more states a water molecule can exist in, the better its situation is entropically, and if a solute "pins down" a water molecule or restricts its freedom of motion, it is entropically unfavorable. All solutes restrict the freedom of motion of water molecules to some degree, but this is particularly true for a non-polar solute, such as methane. Thus, since turning away from methane "pins down" each water molecule slightly, the price of hydrating this non-polar solute is paid indirectly in terms of entropy and not directly in terms of energy.

The hydrophobic effect is currently receiving intense scrutiny from simulation and experiment. The picture that is emerging is somewhat more complicated than the simplified account presented here since at high temperatures, hydrophobic hydration is still unfavorable but for energetic and not entropic reasons. Nevertheless, irrespective of whether the price is paid in terms of energy or entropy, the hydrophobic effect is fundamentally caused by the *relatively* unfavorable interactions between water and hydrophobic solutes.

Different Behavior of Water around Hydrophobic and Hydrophilic Solutes



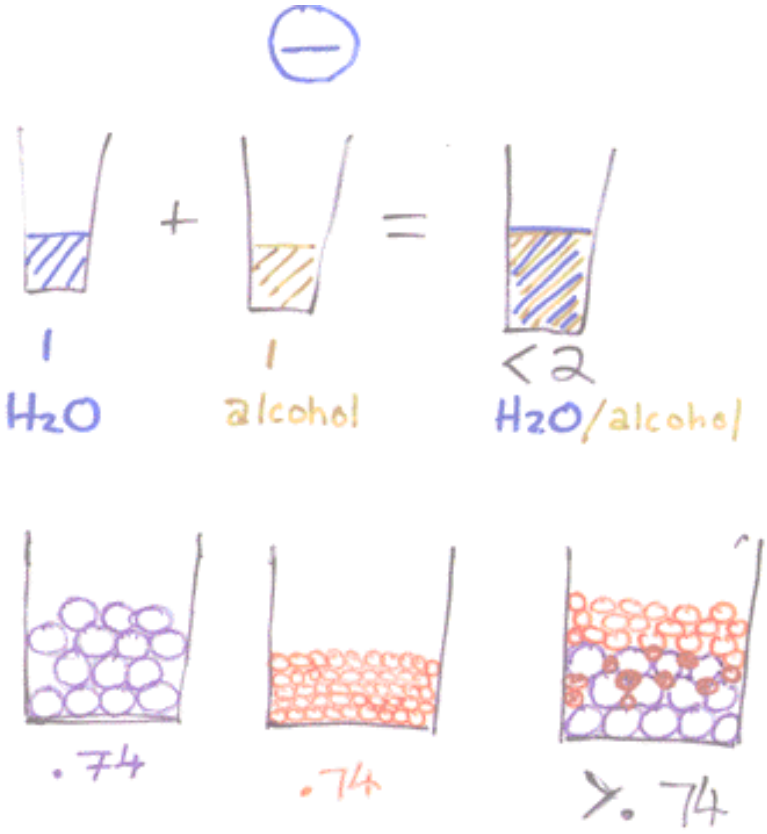


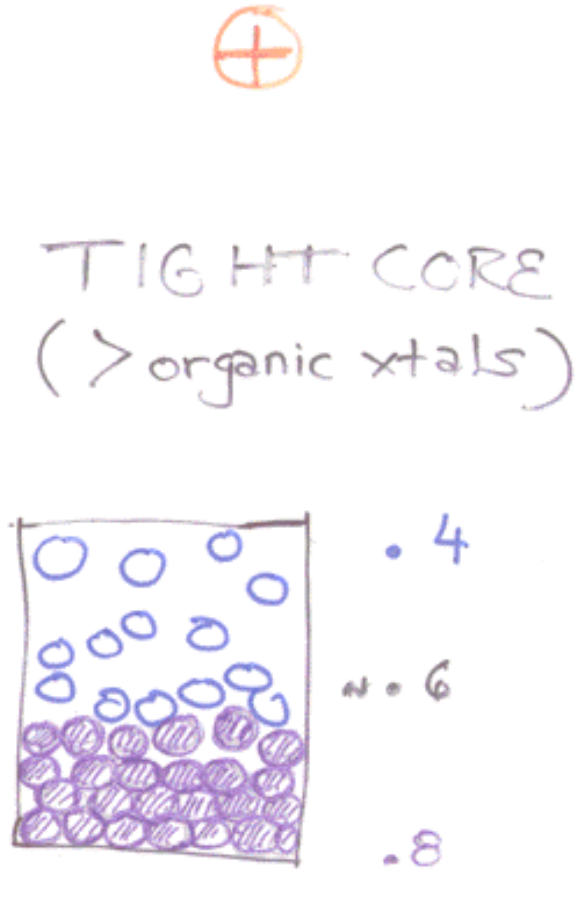
POLAR AND NON-POLAR SOLUTES have very different effects on water structure. We show two solutes that have the same Y-shaped geometry but different partial charges. The polar solute, urea *(left)*, has partial charges on its atoms. Consequently, it is able hydrogen-bond to water molecules and to fit right into the water hydrogen-bond network. In contrast, the non-polar solute, isobutene *(right)*, does not have (substantial) partial charges on any of its atoms. It, thus, can not hydrogen-bond to water. Rather, the water molecules around it "turn away" and interact strongly only with other water molecules, forming a sort of hydrogen-bond "cage" around the isobutene.

Consequences of Hydrophobic Hydration and "Clathrate" Formation

- Hydrophobic hydration is unfavorable (G) but the reason is different at different T
  - ◊ entropically (S) unfavorable at low temperatures because of ordering
  - ◊ enthalpically (H) unfavorable at high temperatures because of unsatisified H-bonds
- Volume of mixing is negative
- Compressibility
- High heat capacity of hydrophobic solvation
  - ◊ Signature of hydrophobic hydration
  - ◊ Hydration creates new temperature "labile" structures

Ways of Rationalizing Packing





Compare standard volumes with amino acids CRYSTAL volumes

Example residue volume: Leu (Å^3)

- Residue in the protein core	165
- VDW envelope	128
- Absolute packing efficiency	78 %
- Sidechain in the protein core	101
- Sidechain in a.a. crystal	110
- Sidechain in solution	107

Example atomic volume: -CH_2- (Å^3)

Protein core	23.5
In solution	26.5
In organic solvent	29.0

Overall comparison to crystal volumes

- 4% less on avg.
- Exceptionally tight core packing

Compare Standard Core Volumes with Amino Acid Solution Volumes

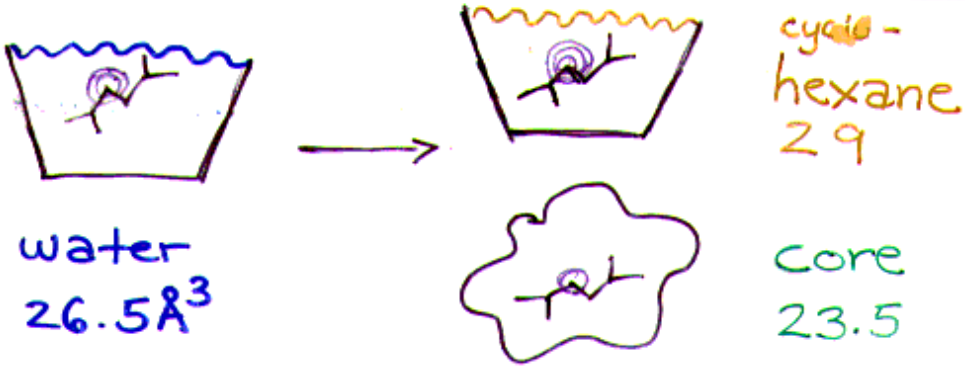
$V^{SIDECHAIN}_{SOLUTION} = V^{SIDECHAIN}_{CORE}$ (Cohn et al. '34, Rao et al. '84)

$= +4$ ALIPHATICS (AYLIP)

$= \sim 0$ POLARS, AROMATICS (MCFY WST)

$= -7$ CHARGED, AMIDE (HNDQ ERK)

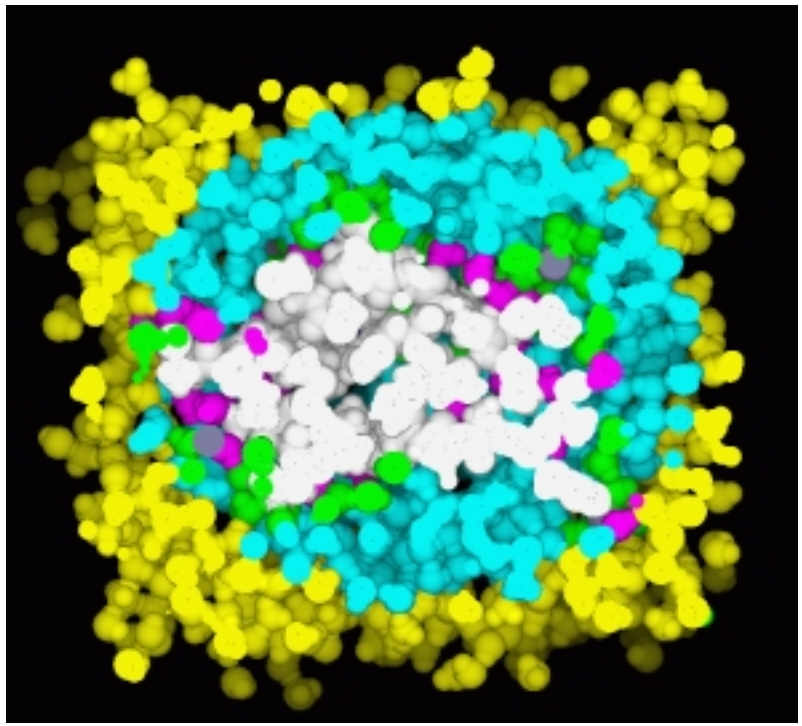
Solution-Transfer Models Predict Opposite Result for Aliphatics

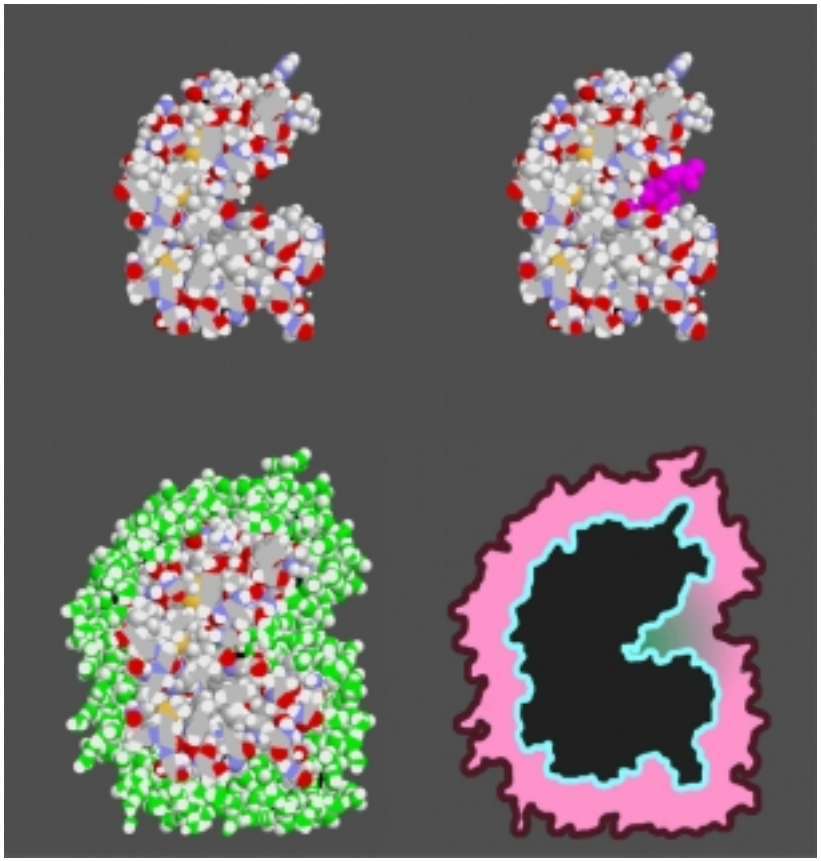


Water around Hydrophobic Groups on protein surface is more Compressible

- Fluctuations in polyhedra volume over simulation related to compressibility
  - ◊ Same way amplitude of a spring is related to spring constant
  - ◊ Rigorous for NPT only, approximately true for part of NVE
- Simulation Results (avg. fluctuations as %SD and compressibility)
  - ◊ Protein core 9.7 % .14
  - ◊ Protein surface 11.7 % .29
  - ◊ Water near protein 13.2 % .50
  - ◊ Bulk water 11.9 % .41
  - ◊ Consistent with more variable packing at protein surface
- Results verified by doing high-pressure simulation (5000 atm, 10000 atm)
  - ◊ Allows calculation of compressibility from definition

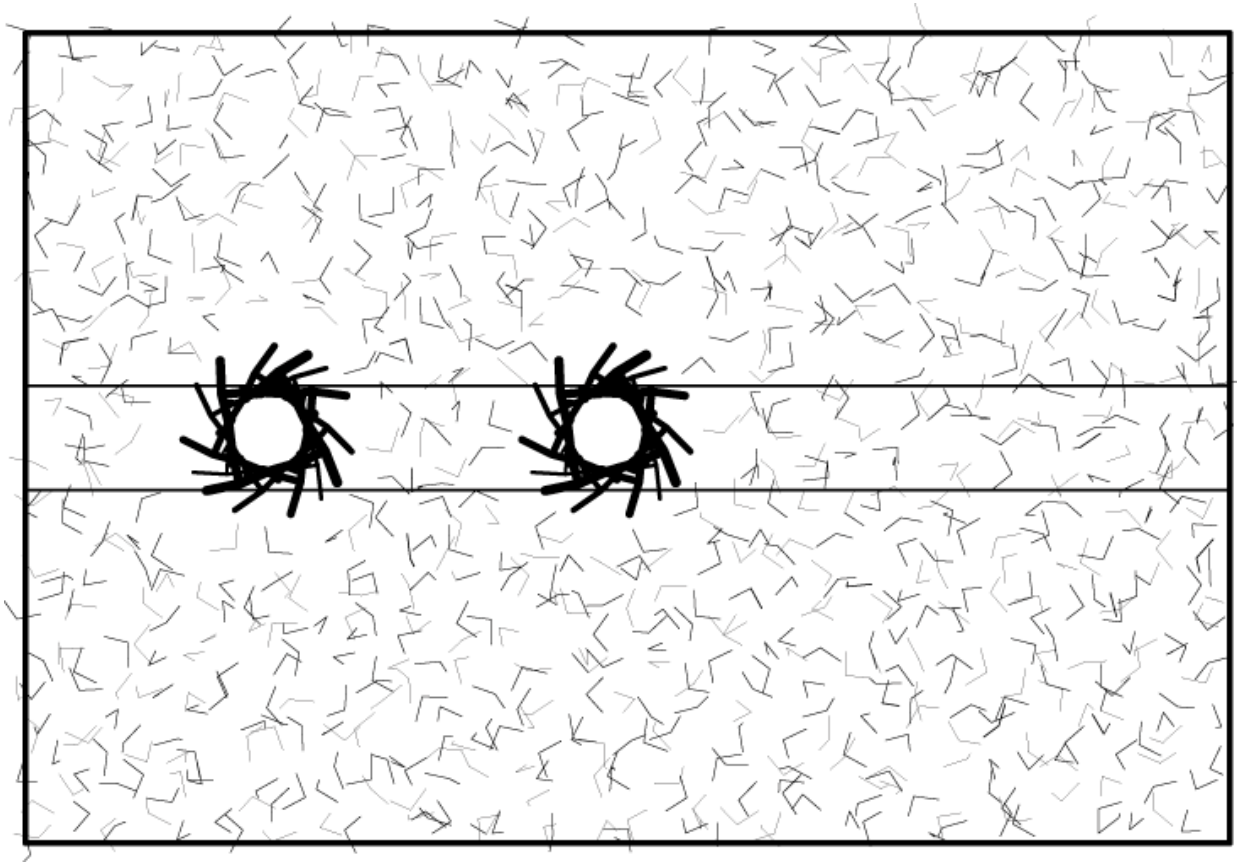
Interaction Between Water and the Protein Surface





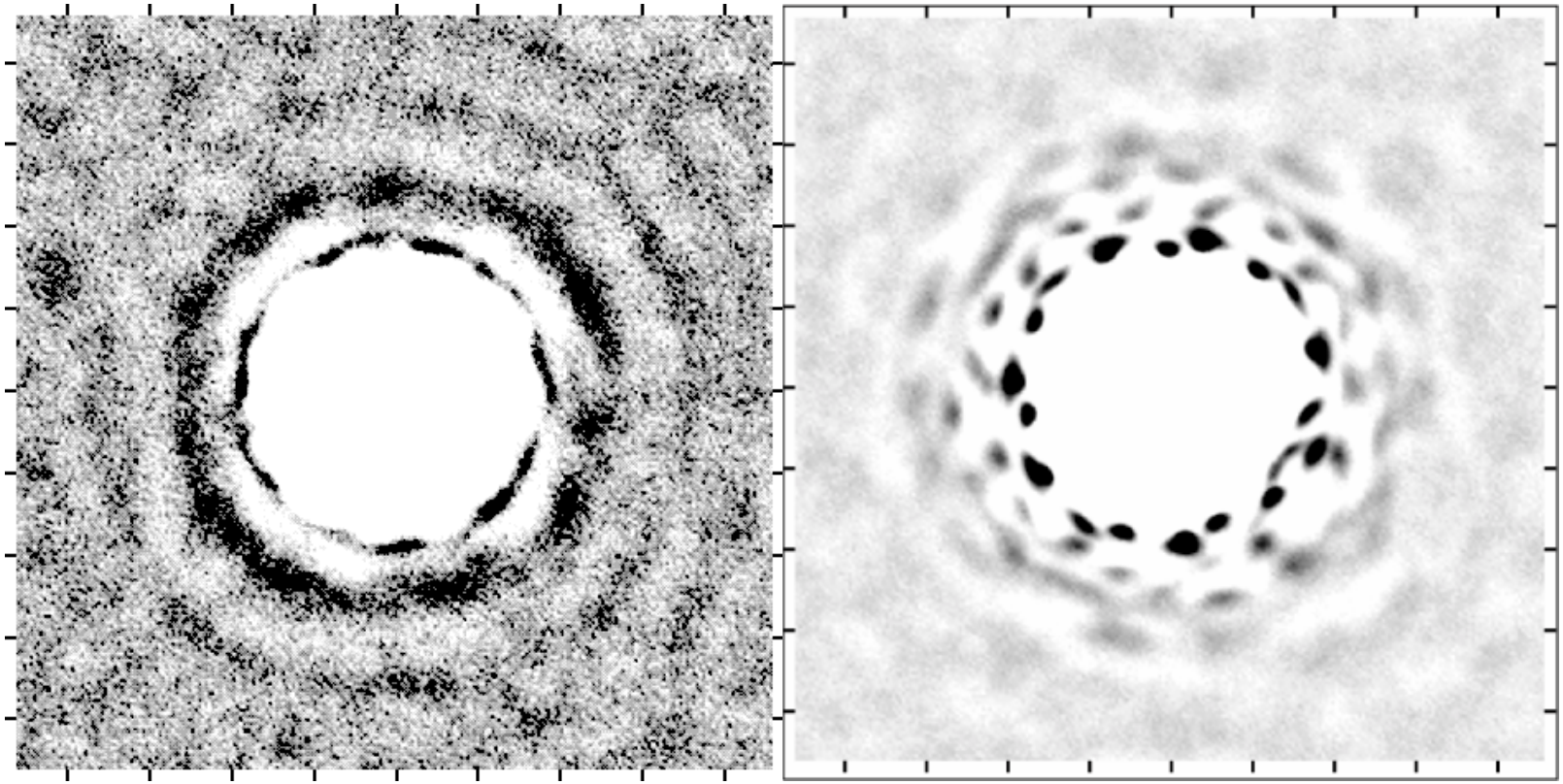
THE PROTEIN SURFACE presents a very interesting interface from the point of view of water structure since it has a very irregular shape and has polar and non-polar atoms juxtaposed in close proximity. A slice through one frame of a simulation of water around a protein is shown. The protein is shown with white atoms in the center. Water molecules strongly interacting with polar and non-polar atoms on the protein surface are shown in magenta and green, respectively. Water molecules weakly interacting with protein are shown in blue. The “region of influence” of the protein extends to roughly the second layer of water molecules. After that the water molecules are not strongly perturbed by the protein. These unperturbed, “bulk” water molecules are shown in yellow. Also, at the center of the protein one can see two buried waters *(magenta)*.

Simple Two Helix System



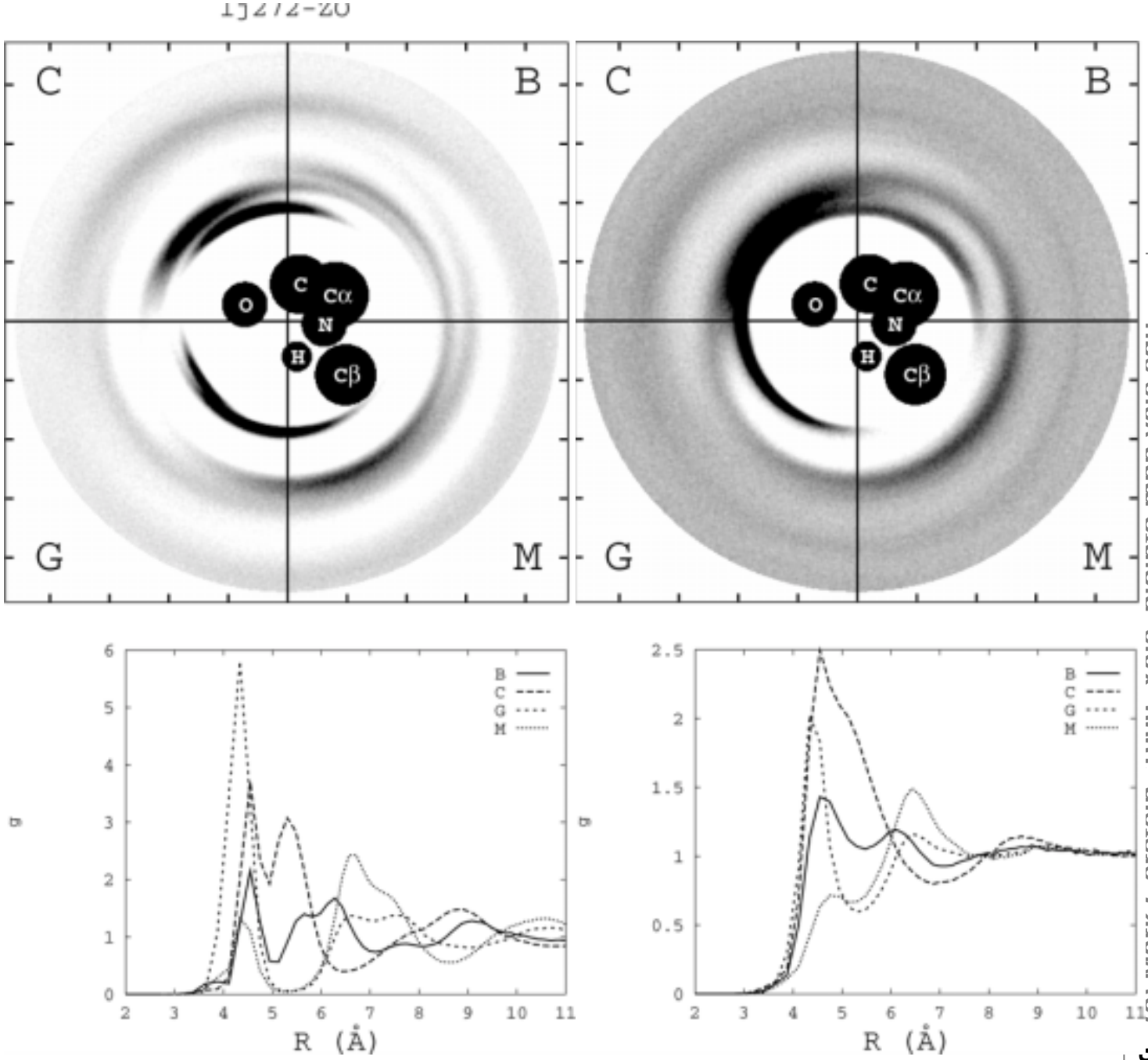
- Number density
  - ◊ g = Normal water,straight & helical projections
  - ◊ For usual RDF "volume elements" are concentric spherical shells
  - ◊ Here, they are tiny vertical columns and helices perpendicular to page
  - ◊ More intuition about groove expansion
- Compare water packing with that of simple liquid ("re-scaled Ar")

Second Solvent Shell: Water v LJ Liquid



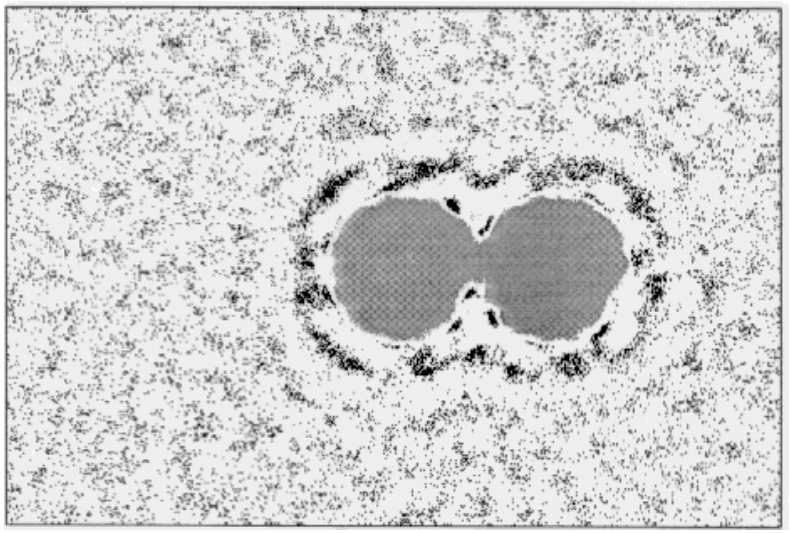
Water
vs.
Ar

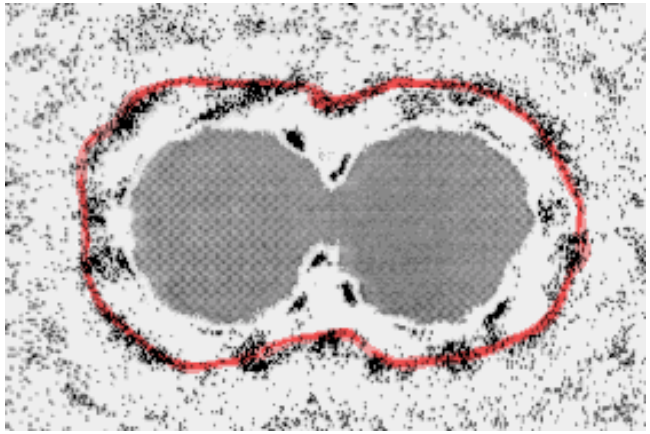
(Helical
Project-
ions)



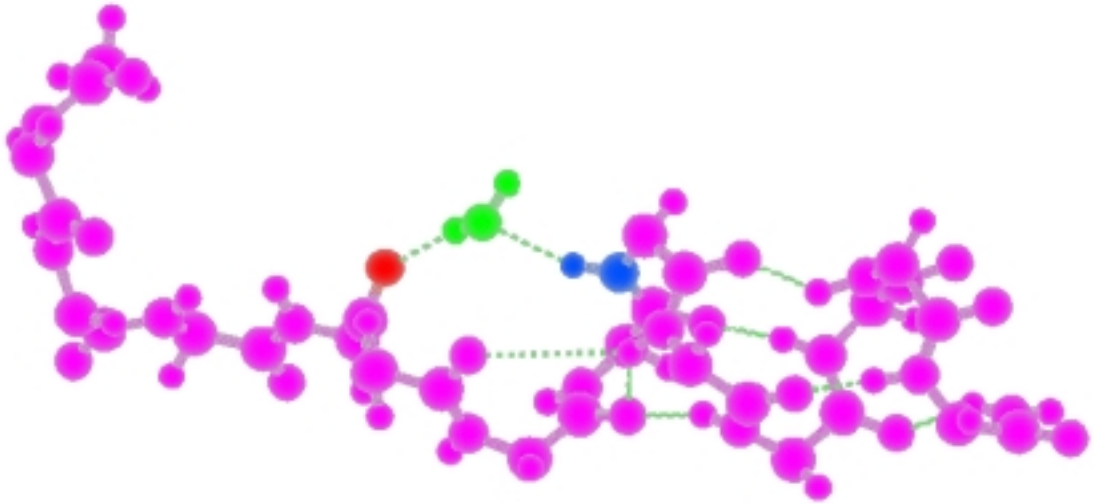
Hydration Surface

- Bring together two helices
  - ◊ Unusually low water density in grooves and crevices — especially, as compared to uncharged water
  - ◊ Fit line through second shell





Water Participates in Protein Unfolding



A PROTEIN HELIX CAN UNFOLD more easily in solution (than in vacuum) because water molecules can replace its helical hydrogen bonds. An unfolding helix is shown. The bottom half the helix is intact and has its helical hydrogen bonds while the top half is unfolded. In the middle a water molecule *(green)* is shown bridging between two atoms that would be hydrogen-bonded in a folded helix: the carbonyl oxygen *(red)* and the amide nitrogen *(blue)*.

Simplified Simulation

Simplification

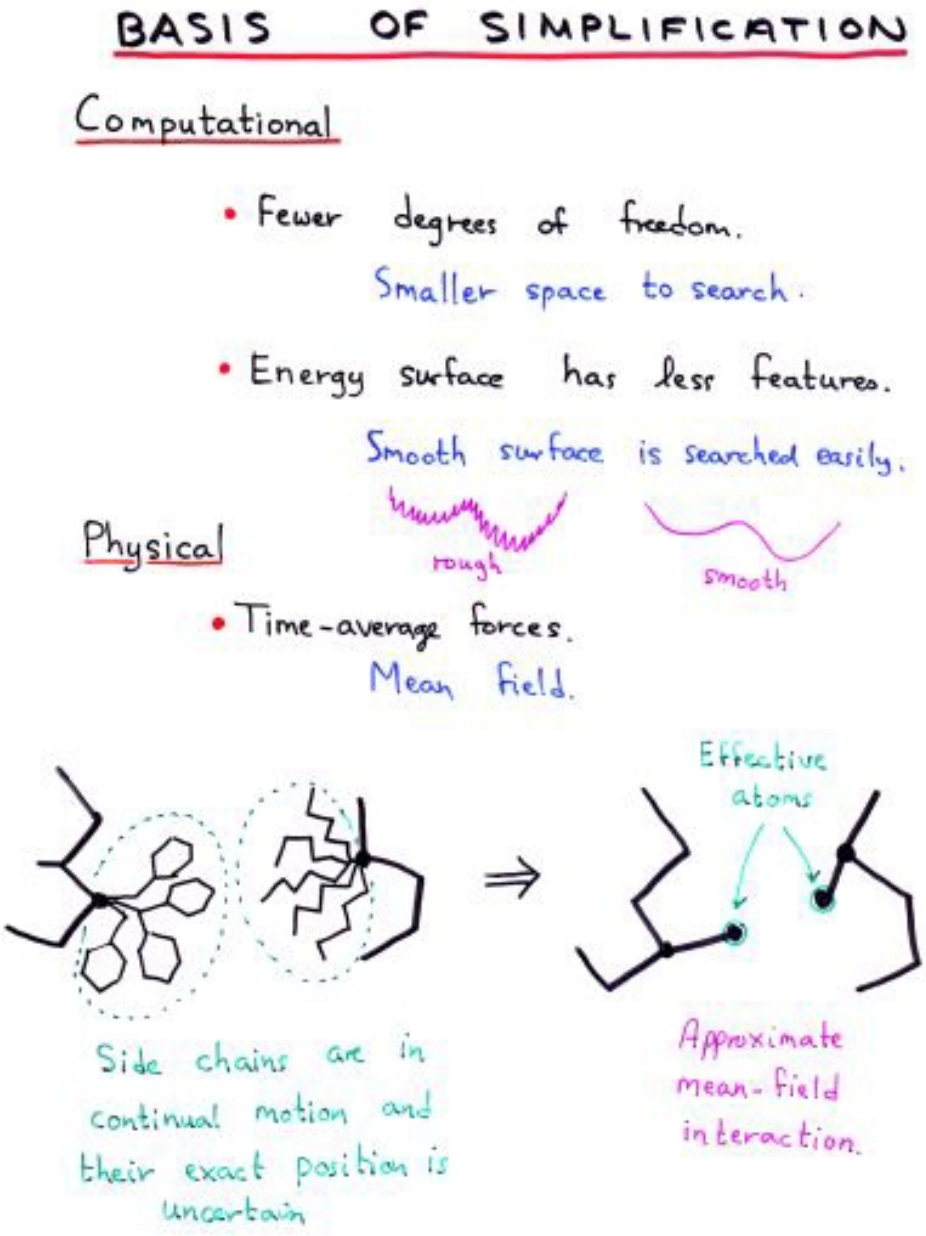


Illustration from M Levitt, Stanford University

Simplified Protein: Lattice Models

- Cubic Lattice
- Tetra-hedral Lattice

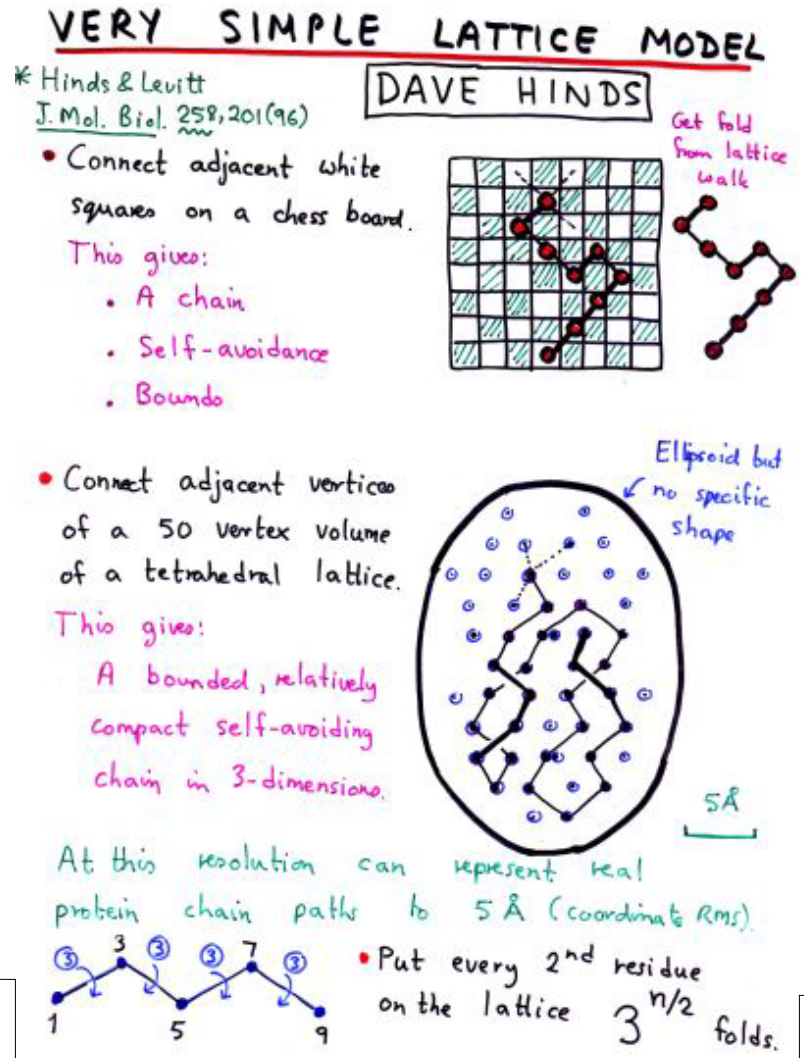


Illustration from M Levitt, Stanford University

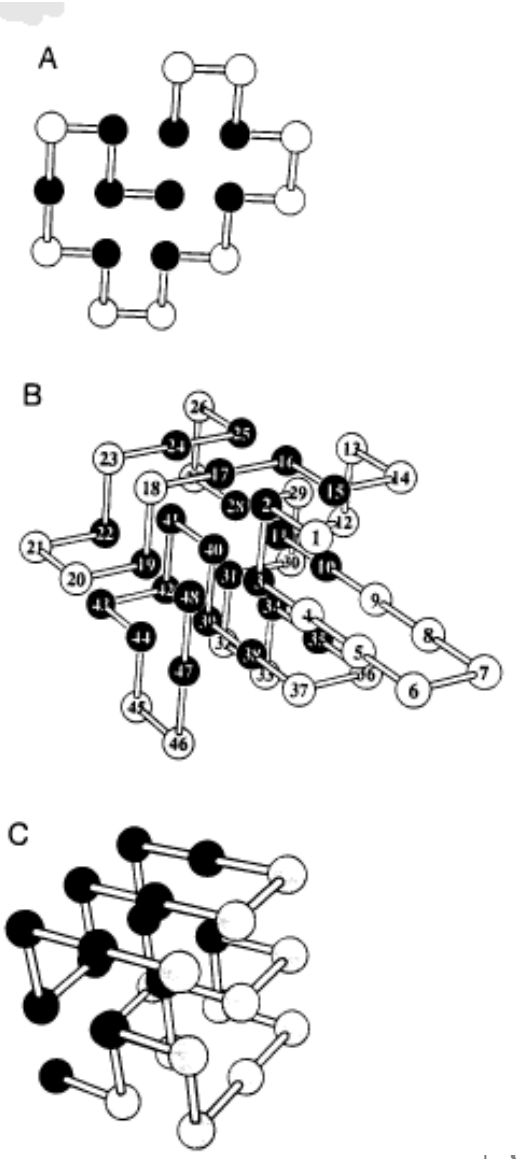
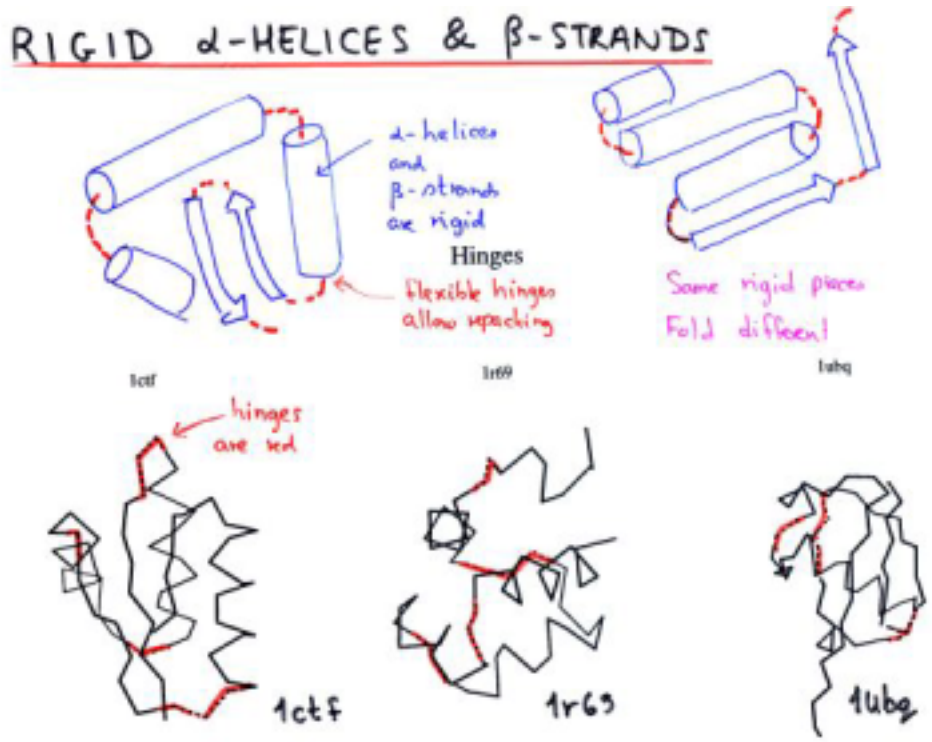


Illustration from Dill et al. (1990)

Off-lattice Discrete State Models



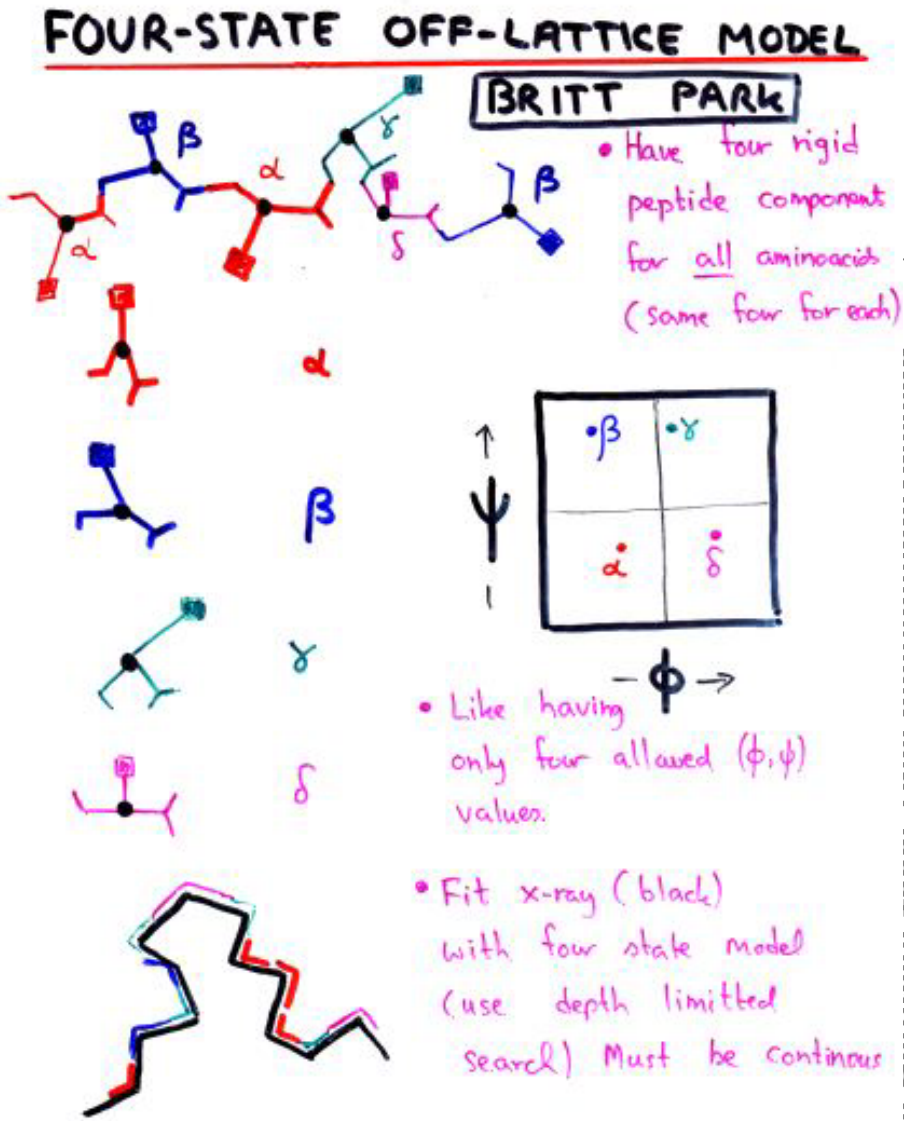


Illustration from M Levitt, Stanford University

How Well Do Lattice Structures Match Real Protein Structure?

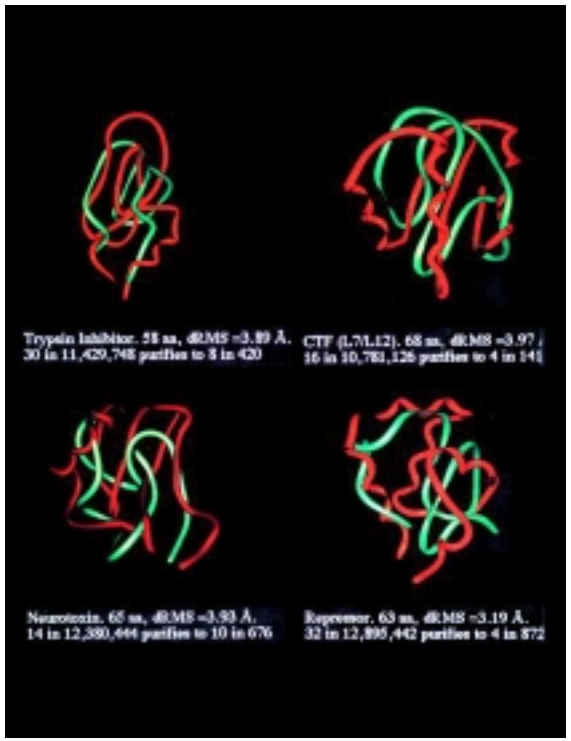
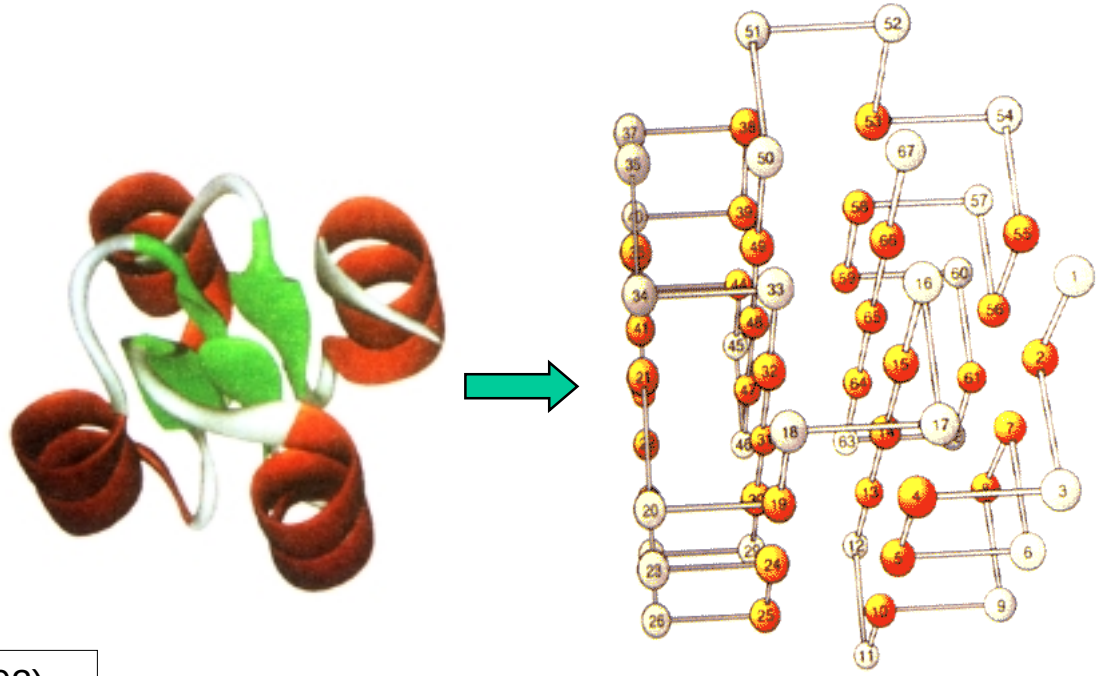
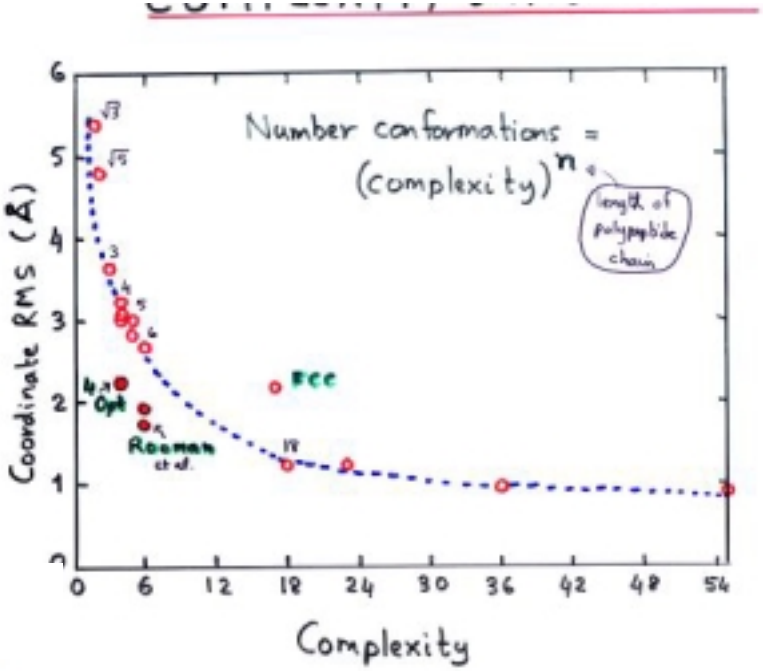


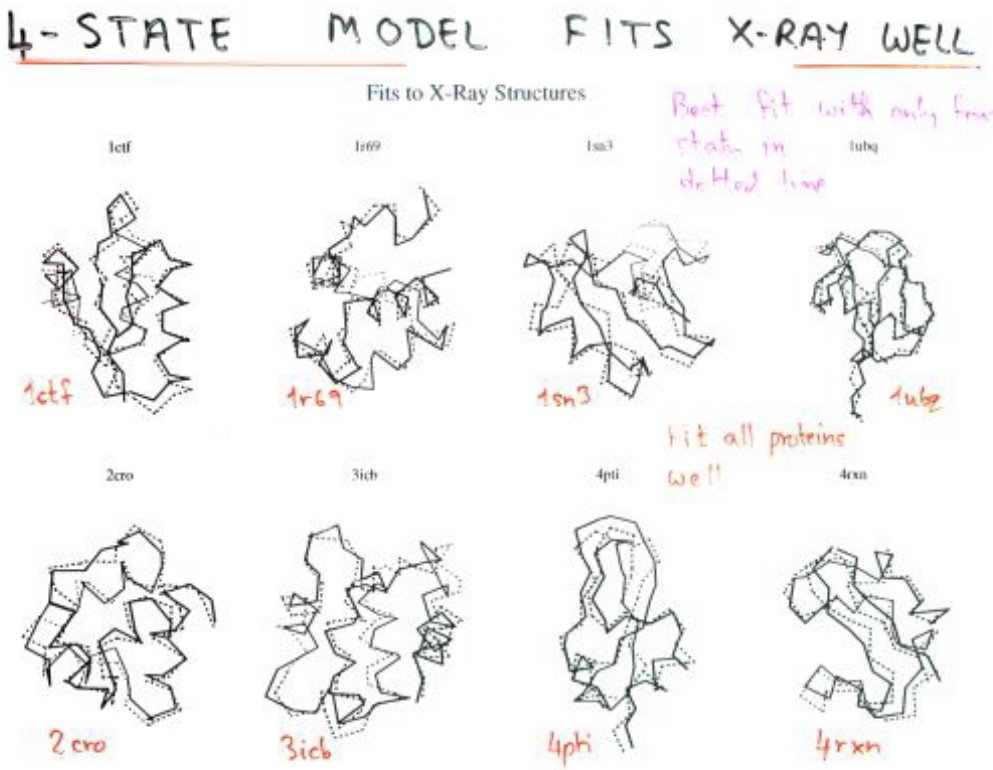
Illustration Credit: Hinds & Levitt (1992)

Illustration Credit: Dill et al. (1995)



How well does the off-lattice model fit?



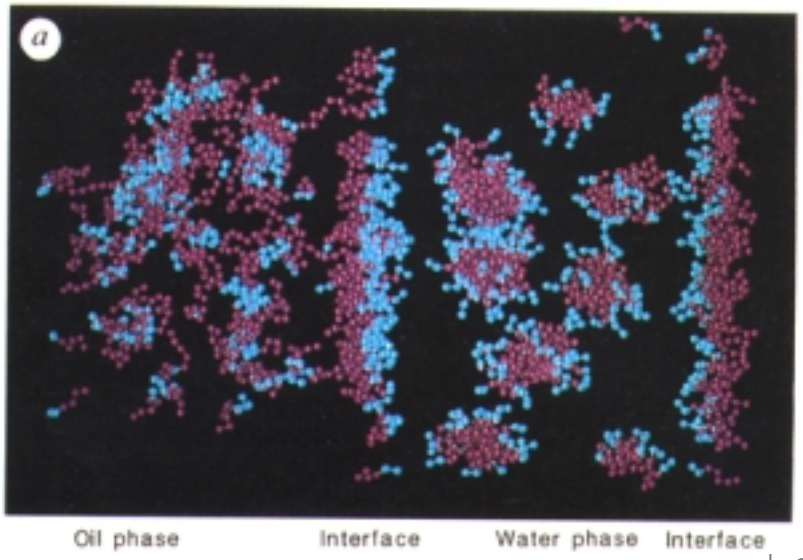


Model Complexity vs Fit to Reality

Illustration from M Levitt, Stanford University

Simplified Solvent

- Smit et al. (1990) Surfactant simulation
- Three types of particles, o, w and s
  - ◊ s consists of w-w-o-o-o-o
  - ◊ s has additional springs
- all particles interact through L-J potential
  - ◊ o-w interaction truncated so purely repulsive
- Above sufficient to give rise to the formation of micelles, membranes, &c



Figures from Smit et al. (1990)

Review -- Basic Forces

- Basic Forces
  - ◊ Springs --> Bonds
  - ◊ Electrical
    - dipoles and induced dipoles --> VDW force --> Packing
    - unpaired charges --> Electrostatics --> charge-charge
- Electrostatics
  - ◊ All described the PBE
  - ◊ kqQ/r -- the simplest case for point charges
    - Multipoles for more complex dist.
    - Validity of monopole or dipole Apx. (helix dipole?)
  - ◊ Polarization (epsilon)
    - Qualitative understanding of what it does
    - 80 vs 3

Review -- Simulation

- Moving on an Energy Landscape
  - ◊ Minimization -- steepest descent
  - ◊ Monte Carlo
  - ◊ Molecular Dynamics
    - Know how an atom will move
  - ◊ The problems
    - Too complex --> Simplified Models
    - Potential Problems
- Analysis
  - ◊ Number density --> RDF, structural quantities
  - ◊ Dynamic quantities, correlation functions, diffusion
    - time course of variables
  - ◊ Hydrophobicity arises naturally in water simulation
    - clathrate formation
    - high heat capacity, volume effects, &c.

Demos

- Minimization Demo
  - ◊ http://www.javasoft.com/applets/jdk/1.0/demo/GraphLayout/example2.html
- Adiabatic Mapping Demo
  - ◊ Molecular Motions Database
  - ◊ http://bioinfo.mbb.yale.edu/MolMovDB
- Rotation Matrices, Rigid Body Motion Demo
  - ◊ 1swm, 2hbs, rasmol

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