THE UNIVERSITY of TEXAS

HEALTH SCIENCE CENTER AT HOUSTON

SCHOOL of HEALTH INFORMATION SCIENCES

Continuum Electrostatics

For students of HI 6001-100 “Biomolecular Modeling”

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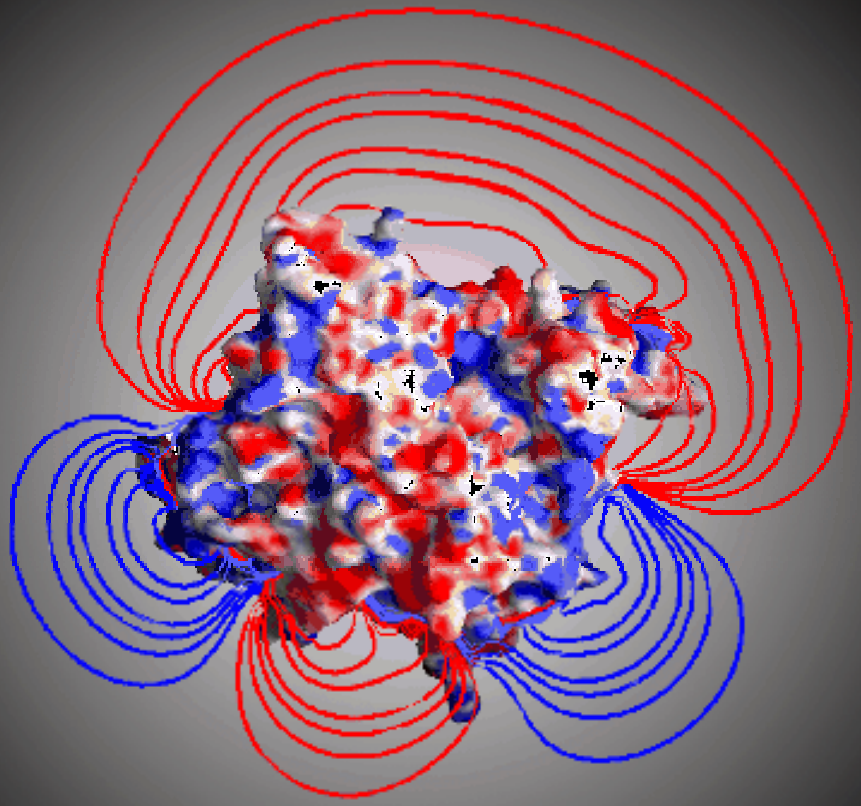
http://biomachina.org/courses/modeling/08.html

Effect of Charges in Biology

- Mg2+ binding to RNA or DNA
- Zn2+ binding in gene regulation
- Ca2+ binding in signal transduction (calmodulin etc.)
- signal transduction through phosphorylation (Tyr, Ser, His)
- ions form organizing centers for protein folding
- steering of protein assembly
- formation of lipid bilayers (membranes)
- etc…

Electrostatic Potential Contours of Mouse Acetylcholinesterase

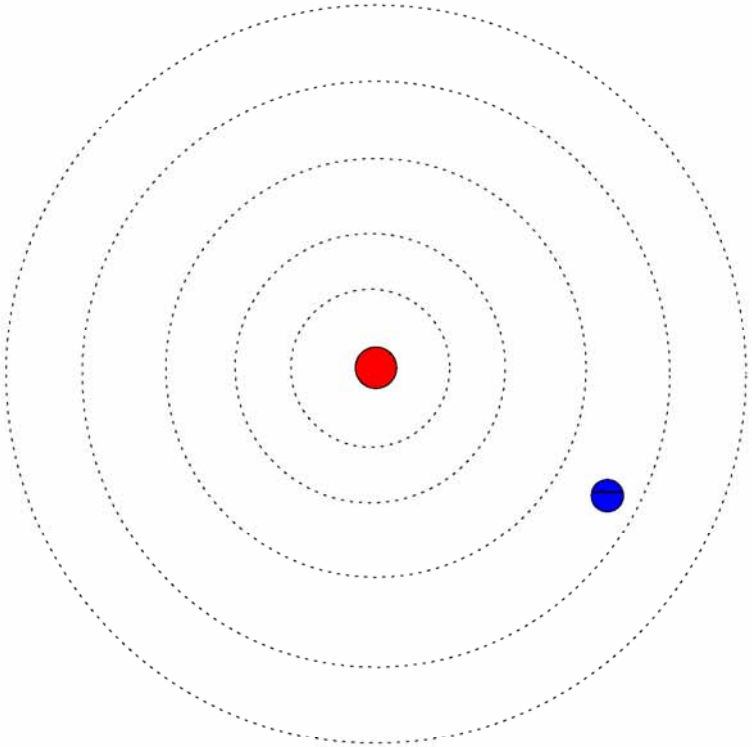


McCammon Group - UCSD

Electrostatic Potentials and Fields

- Electrostatic interactions are very long-ranged (recall the 1/r dependence of the Coulombic term in the MM energy function).
- The electrostatic potential is a scalar quantity, i.e. it has no direction.
- Suppose we place a charged particle into an electric field. The electrostatic potential is the quantity that when multiplied by the charge on the particle tells us the energy required to place the particle in the field.
- The electrostatic field is a vector that tells us the gradient of the electrostatic potential. When multiplied by the charge on the particle it tells us the force acting on the particle.

Coulomb Potential



- Electrostatic Potential:

$$\phi(\mathbf{r}) = \sum_{i=1}^{charges} \frac{q}{\varepsilon \mid \mathbf{r} - \mathbf{r}'_i \mid}$$

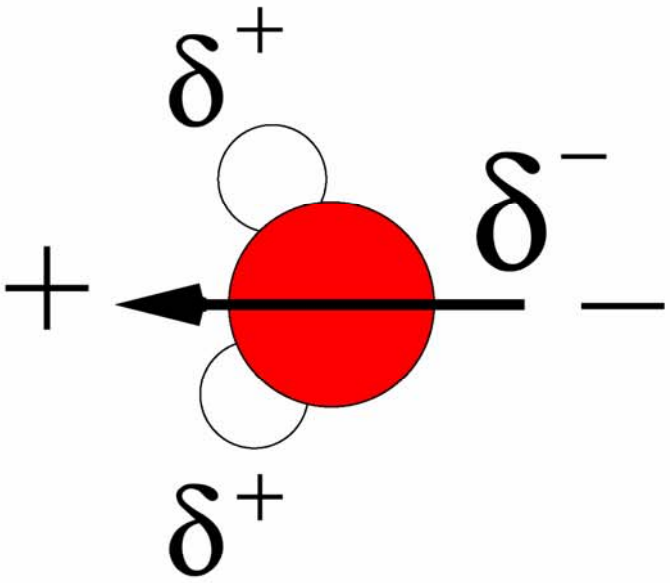
- Electrostatic work required or gained to bring a charge q' to point $\mathbf{r}_{q'}$ in the potential

$$W = q' \, \phi(\mathbf{r}_{q'})$$

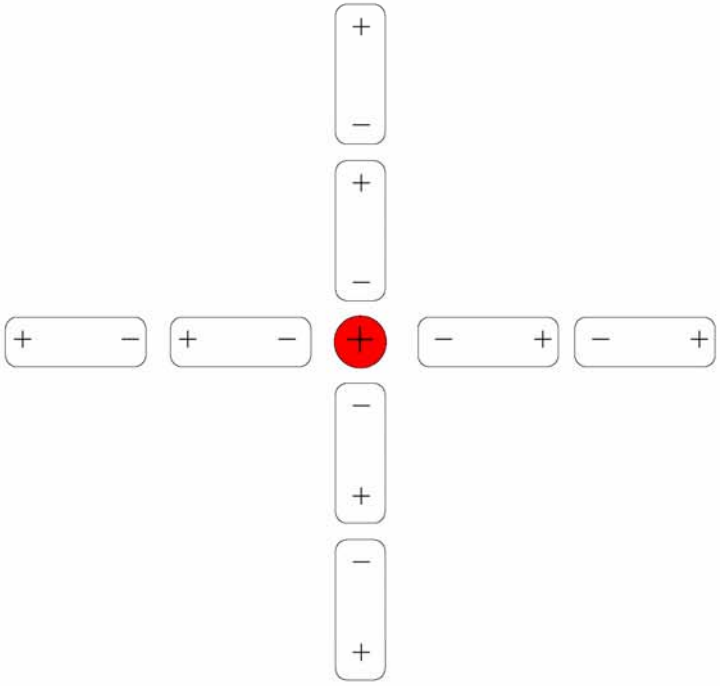
Dieelectric Screening

Permanent Molecular Dipoles

For instance water: 1.9 Debye



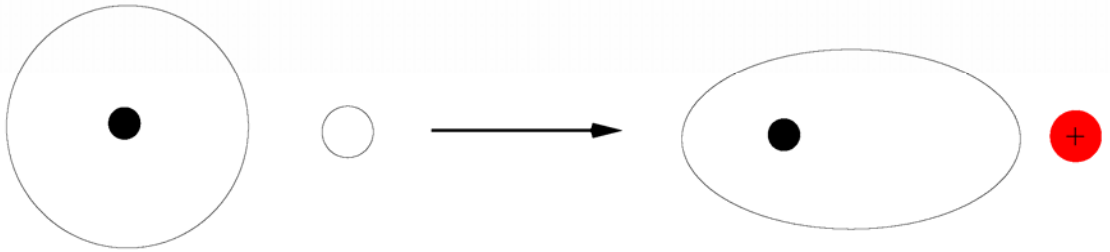
Dipolar molecules orient in an electro-static field



⟶ additional field: Reaction Field

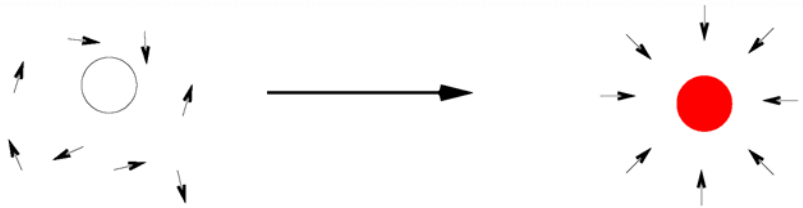
Contributions Inside Molecule

Electronic Polarization



Pure Electronic Polarization: $\varepsilon_{el} \approx 2$

Nuclear Polarization



in a semirigid macromolecule $\varepsilon_{nuc} \approx 2$

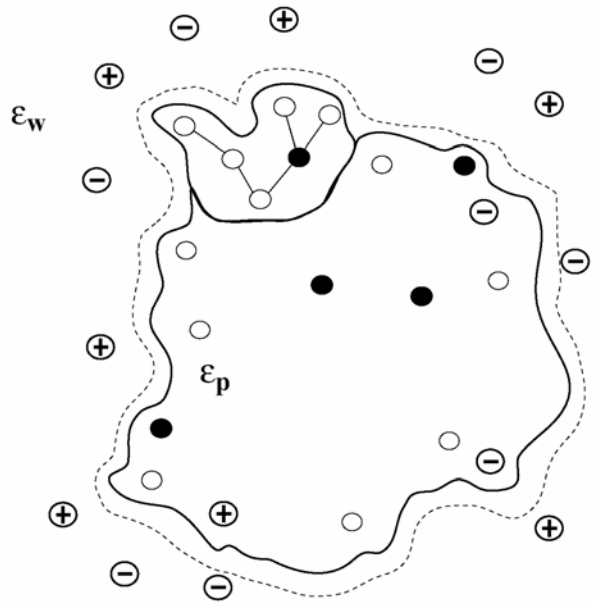
$\longrightarrow$ total polarization: $\varepsilon_{macro} = \varepsilon_{el}\ \varepsilon_{nuc} \approx 4$

A Molecule In Solution

- In MD, screening is sometimes modeled implicitely by distance-dependent dielectric (1/r dependence of ε, $1/r^2$ term in the MM energy function). See earlier notes.

Continuum electrostatics:

- Inside protein ε_p~2-4.
- Outside molecule ε_w~60-80 (solvent)

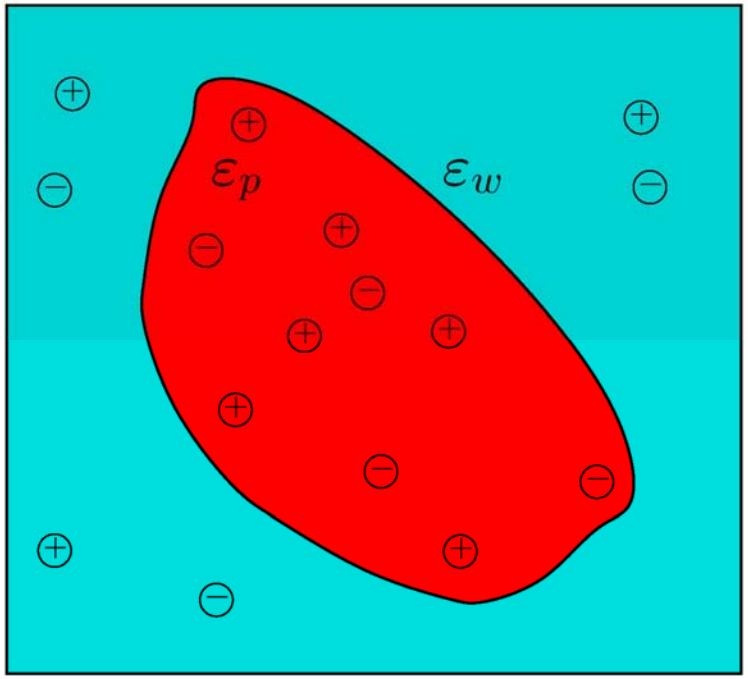


Continuum Electrostatics

Conceptual Model:

Protein: Low dielectric region
With fixed partial charges

Solvent: High dielectric region
with unlocalized (mobile) charges



A continuum electrostatic model describes molecules at atomic detail using a macroscopic description.

Poisson Equation

One of the fundamental equations of classical electrostatics

Electrostatic Field: $\mathbf{E}(\mathbf{r}) = -\nabla\phi(\mathbf{r})$

∇ – differential operator: $\nabla = (\frac{\partial}{\partial x}, \frac{\partial}{\partial y}, \frac{\partial}{\partial z})$

Poisson Equation equation in vacuum (Gauß Theorem):

$$\nabla\mathbf{E}(\mathbf{r}) = 4\pi\rho(\mathbf{r})$$

A dielectric medium screens the field.

$$\nabla\left[\varepsilon(\mathbf{r})\mathbf{E}(\mathbf{r})\right] = 4\pi\rho(\mathbf{r})$$

Poisson Equation:

$$\nabla\left[\varepsilon(\mathbf{r})\nabla\phi(\mathbf{r})\right] = -4\pi\rho(\mathbf{r})$$

$\phi(\mathbf{r})$: electrostatic potential

$\varepsilon(\mathbf{r})$: relative permittity ("dielectric constant")

$\rho(\mathbf{r})$: charge density

Ionic Distribution

Mobile Ions are distributed according to a Boltzmann statistic.

Mean Concentration at $\mathbf{r}$

$$\langle c_i(\mathbf{r}) \rangle = c_i^{\text{bulk}} \exp(-\mathcal{W}_i(\mathbf{r})/RT)$$

Potential of Mean Force

valency

electron charge: 1.602 10^{-19} C

$$\mathcal{W}_i(\mathbf{r}) \approx Z_i e_0 \phi(\mathbf{r})$$

potential in J/mol units

Ion Distribution

$$\rho_{ions}(\mathbf{r}) = \sum_{i=1}^{K} c_i^{\text{bulk}} Z_i e_0 \exp\left(\frac{-Z_i e_0 \phi(\mathbf{r})}{RT}\right)$$

gas constant R = N_A x k_B = 8.315 J/(mol K)

temperature in K

Poisson-Boltzmann Equation

Poisson Equation:

$$\nabla \left[\varepsilon(\mathbf{r})\nabla\phi(\mathbf{r})\right] = -4\pi\rho(\mathbf{r})$$

Charge Distribution

$$\rho(\mathbf{r}) = \rho_{prot}(\mathbf{r}) + \rho_{ions}(\mathbf{r})$$

$$\rho_{ions}(\mathbf{r}) = \sum_{i=1}^{K} c_i^{\text{bulk}} Z_i e_0 \exp\left(\frac{-Z_i e_0 \phi(\mathbf{r})}{RT}\right)$$

Poisson-Boltzmann Equation

$$\nabla \left[\varepsilon(\mathbf{r})\nabla\phi(\mathbf{r})\right] = -4\pi\left(\rho_{prot}(\mathbf{r}) + \sum_{i=1}^{K} c_i^{\text{bulk}} Z_i e_0 \exp\left(\frac{-Z_i e_0 \phi(\mathbf{r})}{RT}\right)\right)$$

Poisson-Boltzmann Equation

Poisson Equation:

$$\nabla \left[\varepsilon(\mathbf{r})\nabla\phi(\mathbf{r})\right] = -4\pi\rho(\mathbf{r})$$

Charge Distribution

$$\rho(\mathbf{r}) = \rho_{prot}(\mathbf{r}) + \rho_{ions}(\mathbf{r})$$

$$\rho_{ions}(\mathbf{r}) = \sum_{i=1}^{K} c_i^{\text{bulk}} Z_i e_0 \exp\left(\frac{-Z_i e_0 \phi(\mathbf{r})}{RT}\right)$$

Poisson-Boltzmann Equation

$$\nabla \left[\varepsilon(\mathbf{r})\nabla\phi(\mathbf{r})\right] = -4\pi\left(\rho_{prot}(\mathbf{r}) + \sum_{i=1}^{K} c_i^{\text{bulk}} Z_i e_0 \exp\left(\frac{-Z_i e_0 \phi(\mathbf{r})}{RT}\right)\right)$$

Linearized Poisson-Boltzmann Equation

Linearization for $(\phi(\mathbf{r})/RT < 1)$:

$$\sum_{i=1}^{K} c_i^{\text{bulk}} Z_i e_0 \exp\left(\frac{-Z_i e_0 \phi(\mathbf{r})}{RT}\right) \approx \sum_{i=1}^{K} c_i^{\text{bulk}} Z_i e_0 - \sum_{i=1}^{K} c_i^{\text{bulk}} Z_i^2 e_0^2 \frac{\phi(\mathbf{r})}{RT}$$

$$\sum_{i=1}^{K} c_i^{\text{bulk}} Z_i e_0 = 0 \qquad \text{Charge Balance}$$

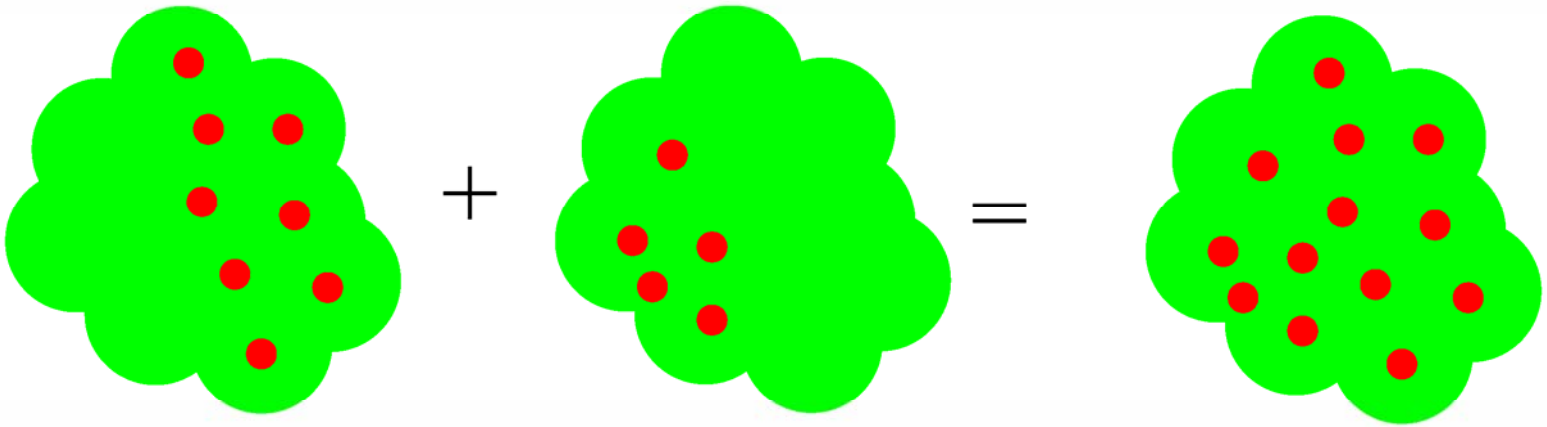
$$\nabla\left[\varepsilon(\mathbf{r})\nabla\phi(\mathbf{r})\right] = -4\pi\left(\rho_{prot}(\mathbf{r}) - \sum_{i=1}^{K} c_i^{\text{bulk}} Z_i^2 e_0^2 \frac{\phi(\mathbf{r})}{RT}\right)$$

$$\text{Define}: \quad I = \frac{1}{2}\sum_{i=1}^{K} c_i^{\text{bulk}} Z_i^2; \qquad \bar{\kappa}^2 = \frac{8\pi N_A e_0^2 I}{k_B T}$$

$$\nabla\left[\varepsilon(\mathbf{r})\nabla\phi(\mathbf{r})\right] = -4\pi\rho_{prot}(\mathbf{r}) + \bar{\kappa}^2(\mathbf{r})\phi(\mathbf{r})$$

Properties of Solutions of Linear PBE

$$If \quad \rho_1 + \rho_2 = \rho \quad then \quad \phi(\mathbf{r}, \rho_1) + \phi(\mathbf{r}, \rho_2) = \phi(\mathbf{r}, \rho)$$

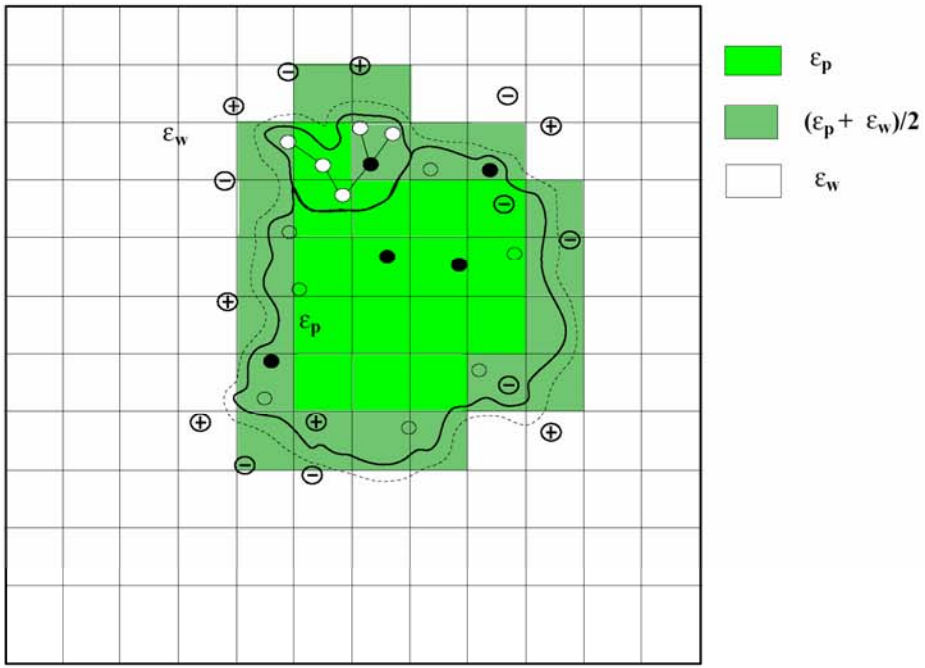


As long as $\varepsilon(r)$ remains unchanged.

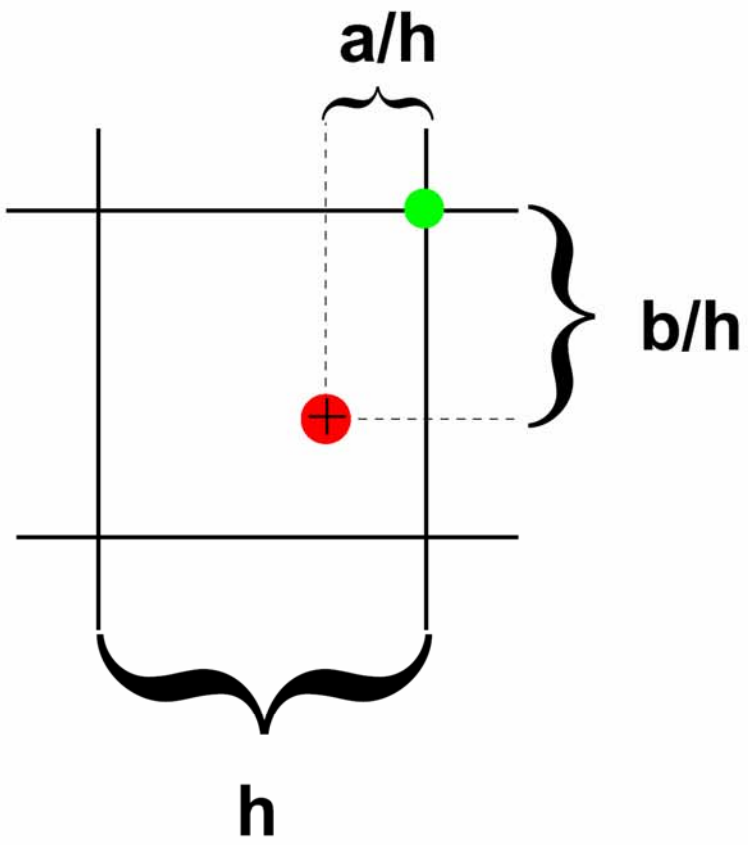
Discrete Model

The boundary molecule/solvent is smoothed.

Charges, dielectic constant, and ionic strength are mapped to the grid.



Assigning Charges to a Grid



Tri-Linear Interpolation

$$q_{grid} = q(1 - \frac{a}{h})(1 - \frac{b}{h})(1 - \frac{c}{h})$$

Problem!! – The splitted charges interact with each other. Grid Artefact!

The Grid Artefact cancels in energy differences!

Numerical Solution (Finite Difference)

$$\nabla\left[\varepsilon(\mathbf{r})\nabla\phi(\mathbf{r})\right] - \bar{\kappa}^2(\mathbf{r})\phi(\mathbf{r}) + 4\pi\rho_{prot}(\mathbf{r}) = 0$$

Integration over grid voxels:

$$\int \nabla\left[\varepsilon(\mathbf{r})\nabla\phi(\mathbf{r})\right]\ \mathrm{d}\mathbf{r} - \int \bar{\kappa}^2(\mathbf{r})\phi(\mathbf{r})\ \mathrm{d}\mathbf{r} + 4\pi\int \rho_{prot}(\mathbf{r})\ \mathrm{d}\mathbf{r} = 0$$

(Gauss theorem) →

$$\int \left[\varepsilon(\mathbf{r})\nabla\phi(\mathbf{r})\right]\ \mathrm{d}\mathbf{A} - h^3\bar{\kappa}_0^2\phi_0 + 4\pi q_0 = 0$$

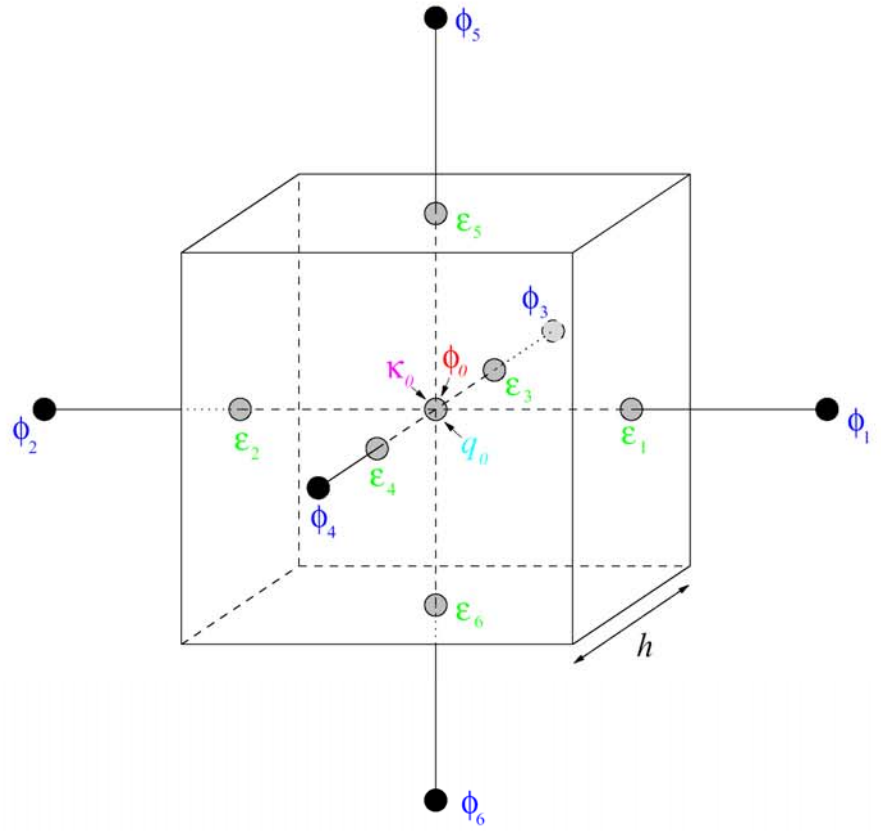
Surface Integral:

$$\int \left[\varepsilon(\mathbf{r})\nabla\phi(\mathbf{r})\right]\ \mathrm{d}\mathbf{A} = \sum_{i=1}^{6} h\varepsilon_i(\phi_i - \phi_0)$$

$$\sum_{i=1}^{6} h\varepsilon_i(\phi_i - \phi_0) - h^3\bar{\kappa}_0^2\phi_0 + 4\pi q_0 = 0$$

$$\phi_0 = \frac{\left(\sum_{i=1}^{6} h\varepsilon_i\phi_i\right) + 4\pi q_0}{\left(\sum_{i=1}^{6} h\varepsilon_i\right) + h^3\bar{\kappa}_0^2}$$

Numerical Solution (Finite Difference)

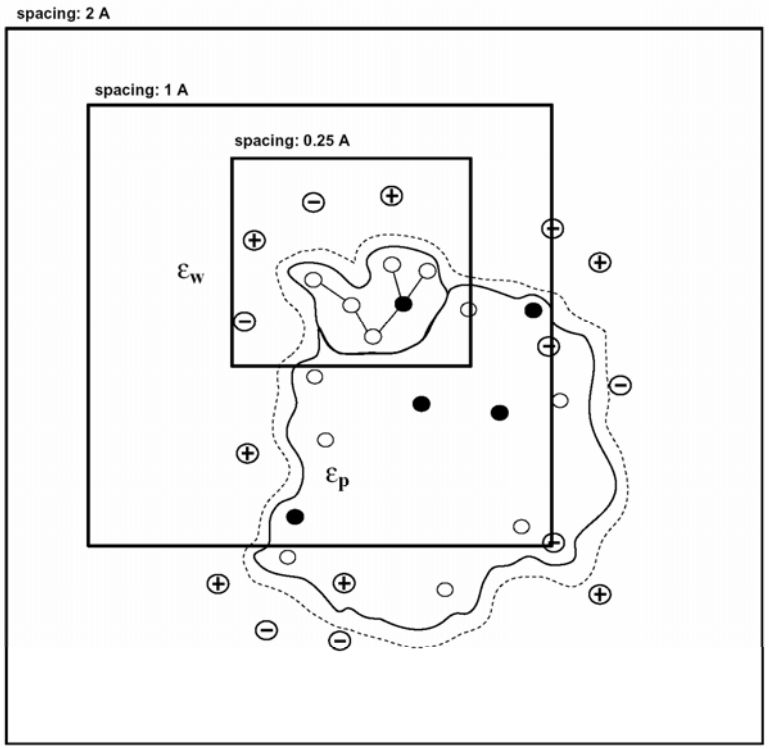


In the nth iteration:

$$\phi_O^n = \frac{\left(\sum_{i=1}^{6} \varepsilon_i \phi_i^{n-1}\right) + \frac{4\pi q_O}{h}}{\left(\sum_{i=1}^{6} \varepsilon_i\right) + \varepsilon_i \kappa_O{}^2 h^2}$$

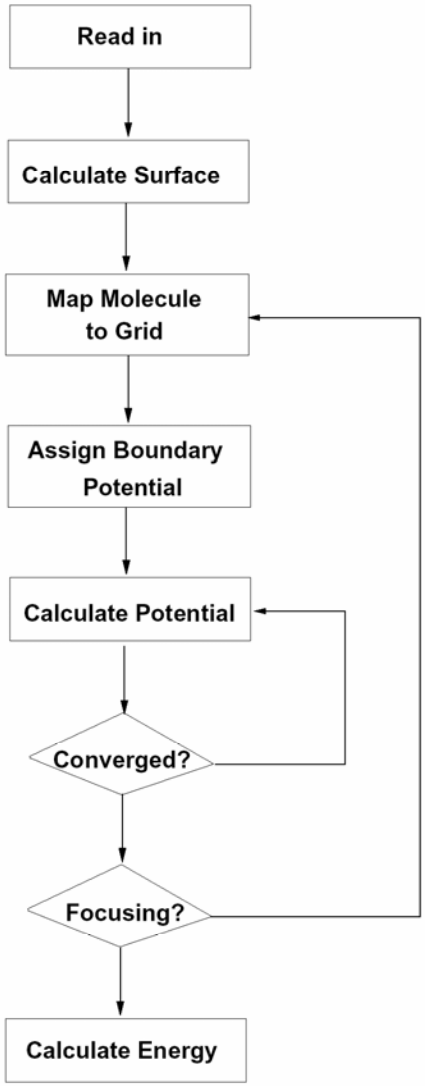
Iteration until selfconsistency.

Focusing/Boundary Conditions



- outer grid – boundary condition from an analytical solution (Debye-Hückel theory, Kirkwood - spherical molecules with charges, Born model)
- inner grids – initialized from grid one level up

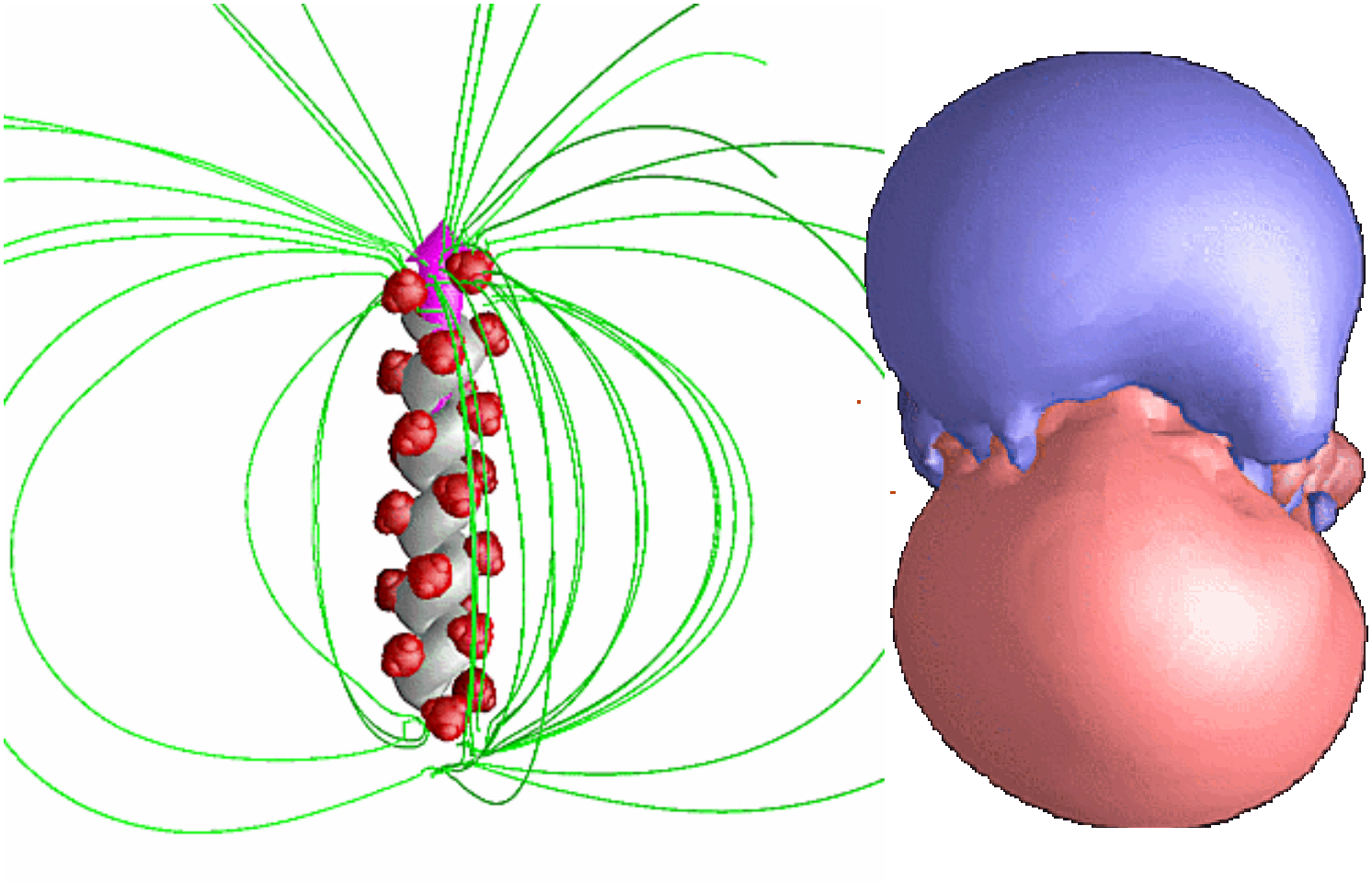
PBE FlowChart

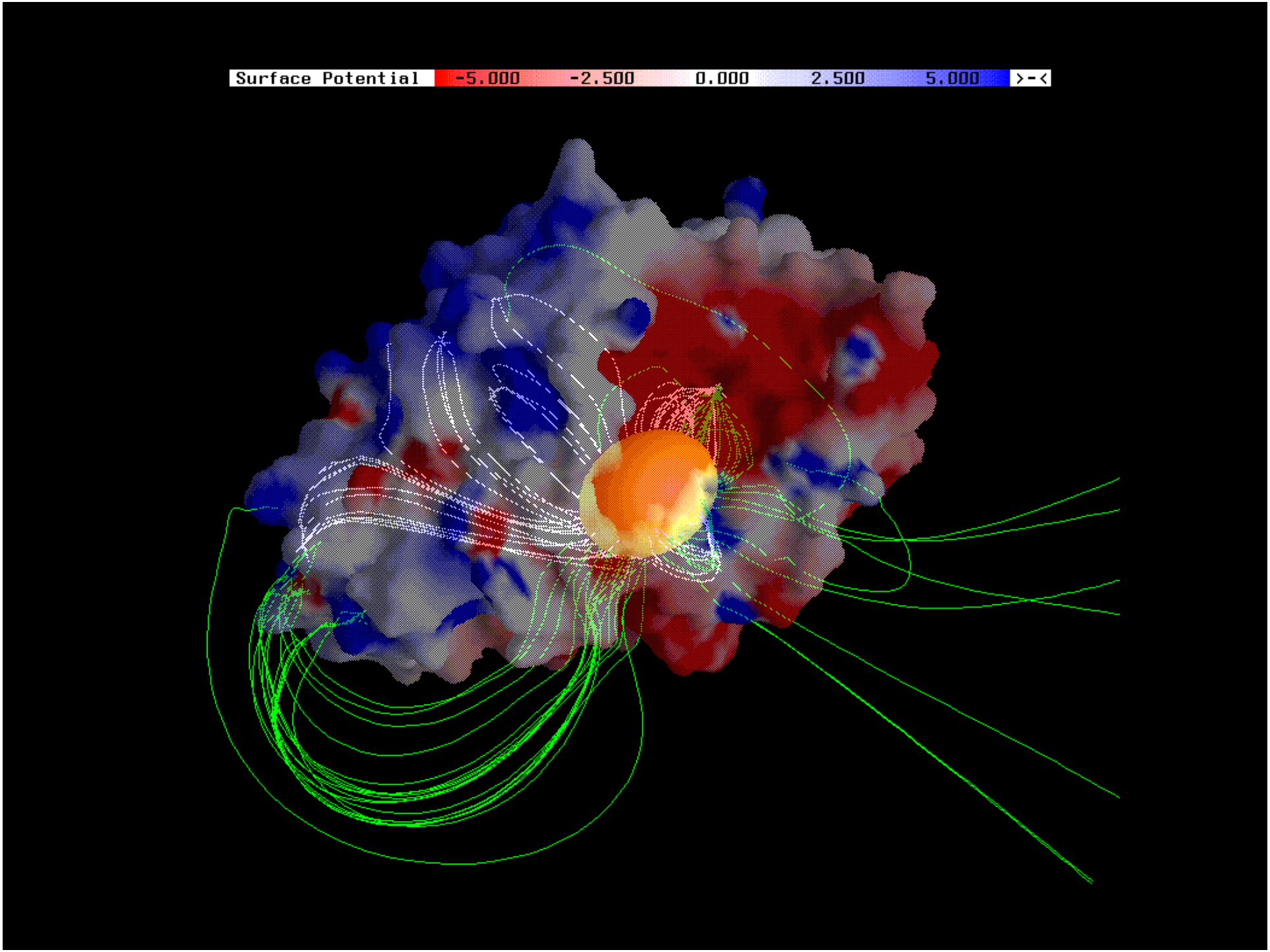


- an analytical solution is needed for the potential at the outer boundary
- alternatively, periodic boundary conditions could be used
- different numerical procedures exist (for instance boundary element method)
- different programs exist: Delphi, MEAD, UHBD, Charmm Module, GRASP

GRASP

- http://trantor.bioc.columbia.edu/grasp/
- free for academic use
- currently runs on SGI only




Surface Potential
-5.000
-2.500
0.000
2.500
5.000
>-<

DelPhi

- Developed, along with Grasp, by Barry Honig's group, now at Columbia
- Difference between DelPhi and GRASP:
  - Grasp was intended to be an interactive molecular graphics program with a very rough PDB solver. Uses a 32x32x32 grid size.
  - DelPhi is intended for quantitative analysis, and therefore is more robust. Uses a 65x65x65 grid size.
- For the most accurate figures, use DelPhi to solve the PB-equation then use a visualization program to create images.

DelPhi Input File

```
gsize=165
scale=2.5
in(pdb,file="bb_cmplx_h.pdb")
in(siz,file="charm22.siz")
in(crg,file="charm22.crg")
acenter(28.114,40.477,9.909)
indi=2.0
exdi=80.0
prbrad=1.4
salt=0.10
ionrad=2.0
bndcon=4
maxc=0.0001
linit=400
!nonit=800
energy(s,c,g)
```

Radius File

```
!my siz based on PARSE
!(value for P taken from Pauling,
! for Mg from Biophys J 2001, 80, 1151)
atom__res_radius_
O         1.4
H         1.0
C         1.7
N         1.5
S         1.85
P         1.90
Mg        0.99
```

DelPhi Charge File

```
!
!    Delphi charge file generated from CHARMM
!      top22.pro
!        (c) 1995 Andreas Windemuth
atom__resnumbc_charge_
N      ALA       -0.470
HN     ALA        0.310
CA     ALA        0.070
HA     ALA        0.090
CB     ALA       -0.270
```

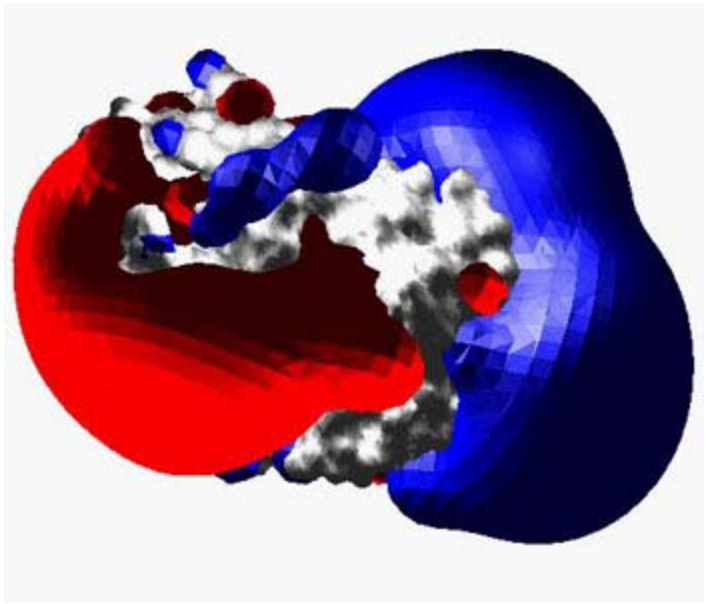
Example DelPhi Output File

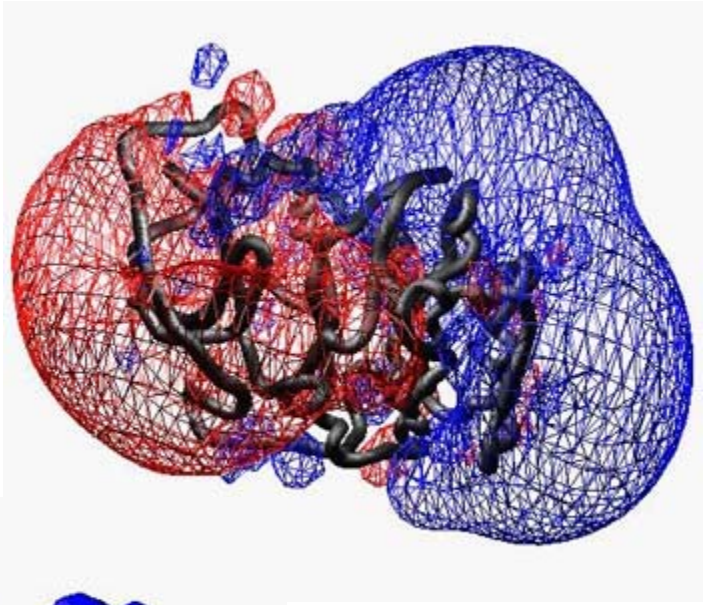
```
DELPHI SITE POTENTIAL FILE
grid size,percent fill:          59   80.00000
inner,outer dielectric:   2.000000       80.00000
ionic strength (M):  0.0000000E+00
ion excl., probe radius:  0.0000000E+00   1.400000
linear, nolinear iterations:         121           0
boundary condition:           2
Data Output: COORDINATES CHARGE POTENTIALS REACTION COULOMBIC
title: qdiffxas: qdiffxs4 with an improved surfacing routine
```

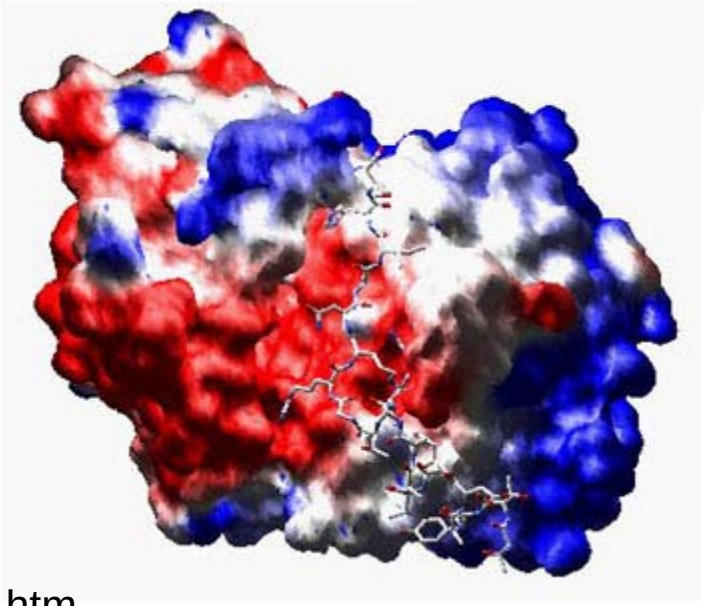
ATOM COORDINATES (X,Y,Z)			CHARGE	GRID PT.	REAC. PT.	COUL. POT
17.9100	39.4200	41.1000	0.0000	-0.3997	16.7603	-17.1328
17.3200	40.2300	41.0900	0.0000	-0.3630	15.6865	-16.0201
18.1100	39.1200	40.1600	0.0000	-0.3454	17.2304	-17.5536
17.4500	38.6700	41.5700	0.0000	-0.4081	16.4920	-16.8791
19.0900	39.8400	41.8700	0.0000	-0.5228	17.9606	-18.4438
19.5400	38.5900	42.6200	0.0000	-0.6829	19.6806	-20.3204
20.6700	38.9100	43.6000	0.0000	-0.8435	21.7513	-22.5361
21.7900	39.5500	43.2700	0.0000	-0.9294	22.9364	-23.7841
20.6200	38.8000	44.9200	0.0000	-0.8785	22.3664	-23.1802
22.5500	39.6200	44.3600	0.0000	-1.0767	25.3352	-26.3132
21.8200	39.1500	45.3700	0.0000	-1.0302	24.9292	-25.8796
22.0200	39.0700	46.3500	0.0000	-1.0996	26.0130	-27.0211
20.0800	40.4500	40.8800	0.0000	-0.4794	17.8343	-18.2561
20.3800	39.8600	39.8500	0.0000	-0.4536	18.5558	-18.9569
20.3600	41.7200	41.1700	0.0000	-0.4297	16.3155	-16.6724
19.8800	42.2500	41.8700	0.0000	-0.4658	16.0082	-16.4187
21.2800	42.5400	40.3600	0.0000	-0.2490	13.9603	-14.1032
20.6200	43.9200	40.4300	0.0000	-0.2036	11.5971	-11.7285
22.7700	42.4800	40.7200	0.0000	-0.3196	14.3997	-14.5307
23.5700	43.0700	39.9900	0.0000	0.0466	10.0525	-9.7317
23.1200	41.5000	41.5500	0.0000	-0.6889	18.8949	-19.3878
22.6000	40.6400	41.5500	0.0000	-0.7699	20.4193	-21.0508
24.5000	41.3900	42.0400	0.0000	-0.9091	21.8624	-22.5334
25.6800	41.3000	41.0700	0.0000	-0.7988	21.0879	-21.5502
25.6900	40.5000	40.1300	0.0000	-0.8230	21.5642	-22.0923
26.6700	42.1400	41.3400	0.0000	-0.7973	20.2470	-20.5840
26.7700	42.6600	42.1900	0.0000	-1.0643	22.3860	-23.0180
27.7900	42.2600	40.4000	0.0000	-0.3994	16.9163	-16.7950
28.4300	43.6500	40.3300	0.0000	0.0680	7.8928	-6.8582
27.4400	44.7400	39.8900	0.0000	1.5067	-7.7255	11.4788
27.9000	46.2000	39.8700	0.0000	5.9398	-19.7567	28.0624
28.8400	46.2500	38.7500	0.0000	20.0697	-37.0564	62.6851
29.7600	46.0900	39.1200	0.0000	7.3198	-25.0434	34.7500
28.6500	46.7600	37.5200	0.0000	81.6300	-64.7456	157.5255

Visualization of DelPhi Electrostatic Potentials

SPDV (Expasy):



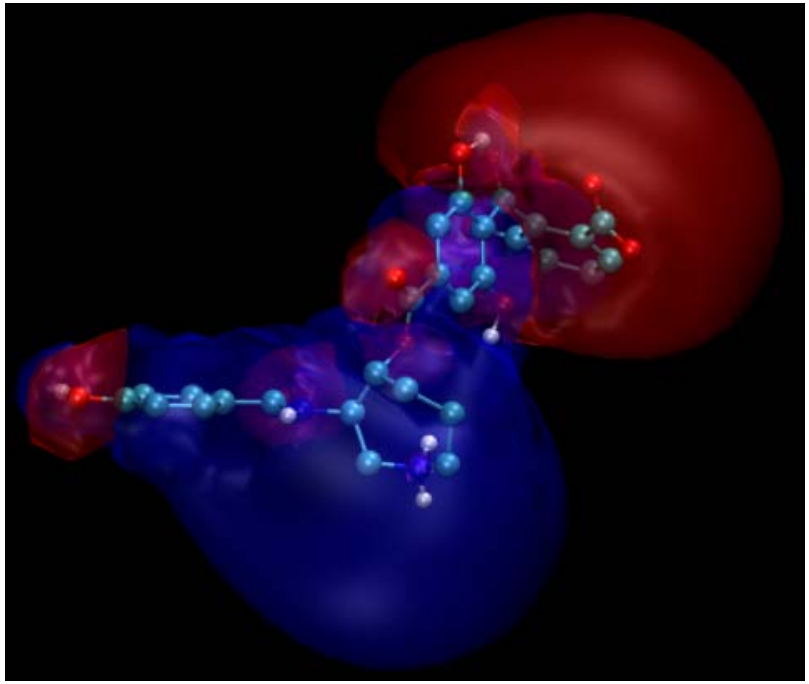


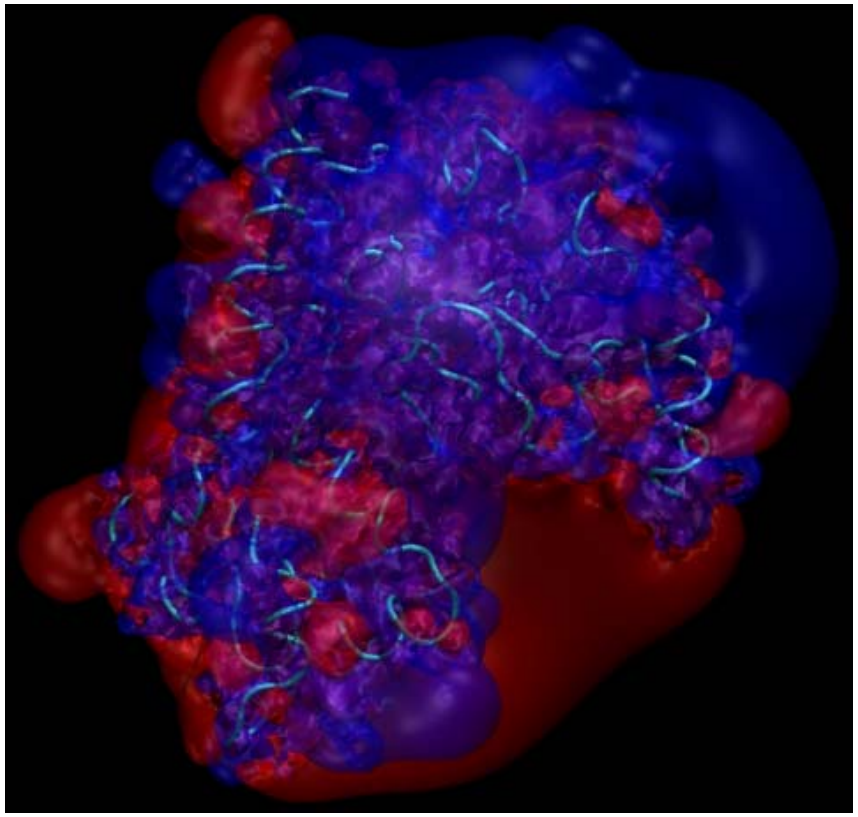


http://au.expasy.org/spdbv/text/epot.htm

Visualization of DelPhi Electrostatic Potentials

VMD

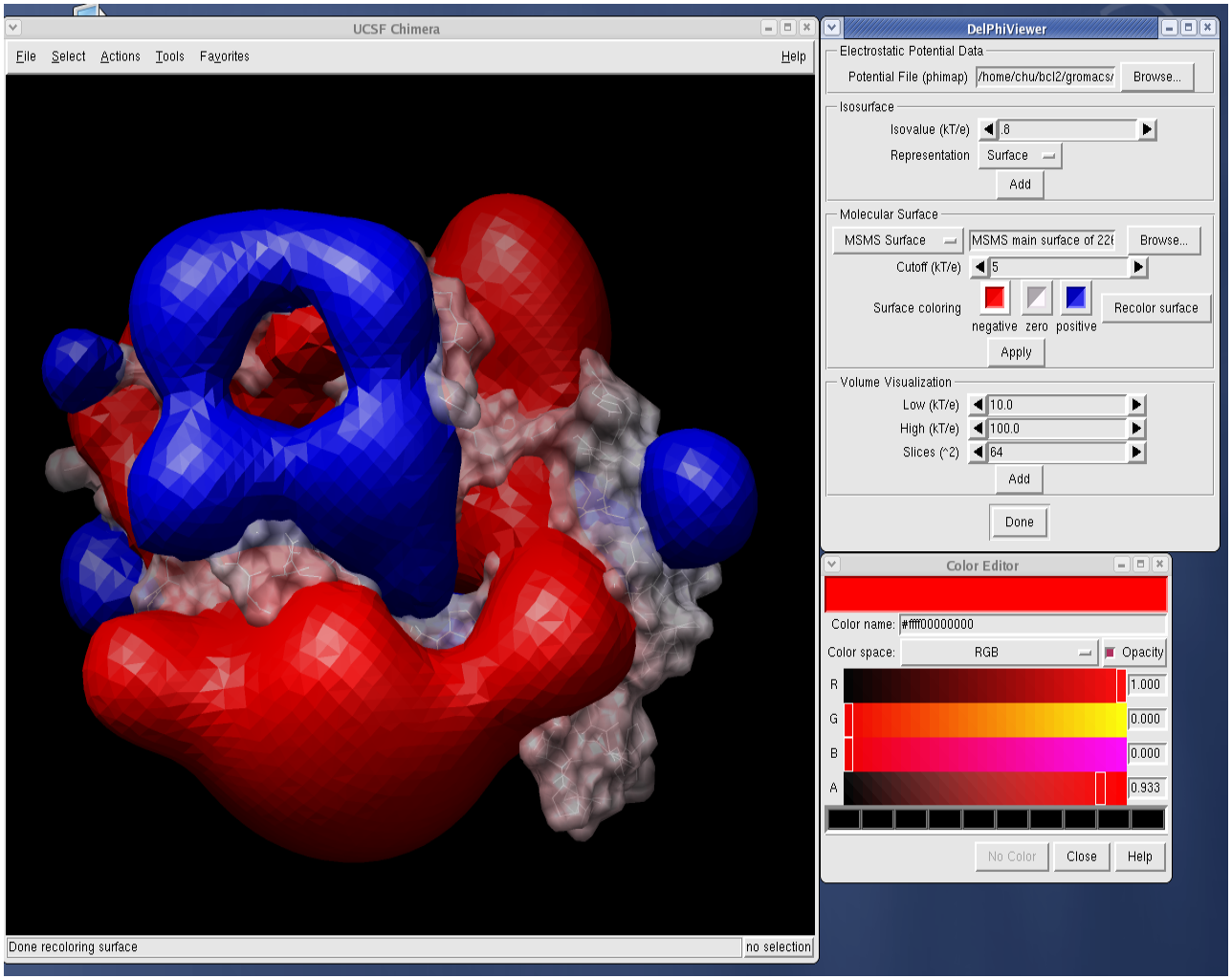




http://agave.wustl.edu/apbs/doc/html/tutorial/x265.html

Visualization of DelPhi Electrostatic Potentials (cont.)

UCSF Chimera



http://www.cgl.ucsf.edu/chimera/

Questions (PBE)?

Brownian Dynamics

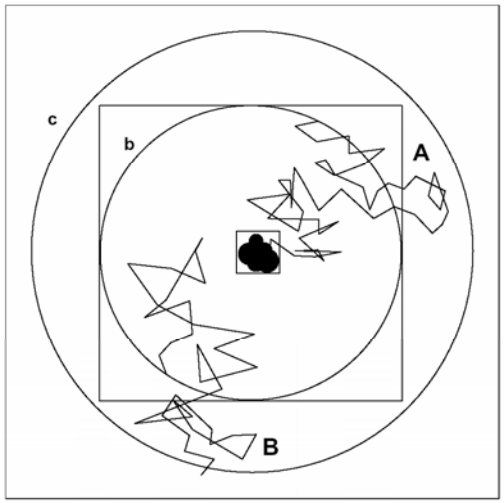
Brownian Dynamics = Newtonian Dynamics + Random Terms

$$\frac{d^2\vec{r}_i(t)}{dt^2} = m_i^{-1}\vec{F}_i + m_i^{-1}\vec{R}_i - \beta_i\frac{d\vec{r}(t)}{dt}$$

In Biomolecular Simulations:

- Diffusion of Macromolecules
- Simulation of Association Processes

Molecules are treated as rigid or only semirigid macroscopic objects.



BD Simulation

• Brownian dynamics (BD) simulations can be used to simulate the diffusion and association of molecules in solution.

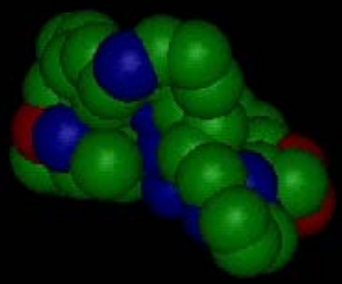
• Brownian motion is the random movement of solute molecules in dilute solution that results from repeated collisions with solvent molecules.

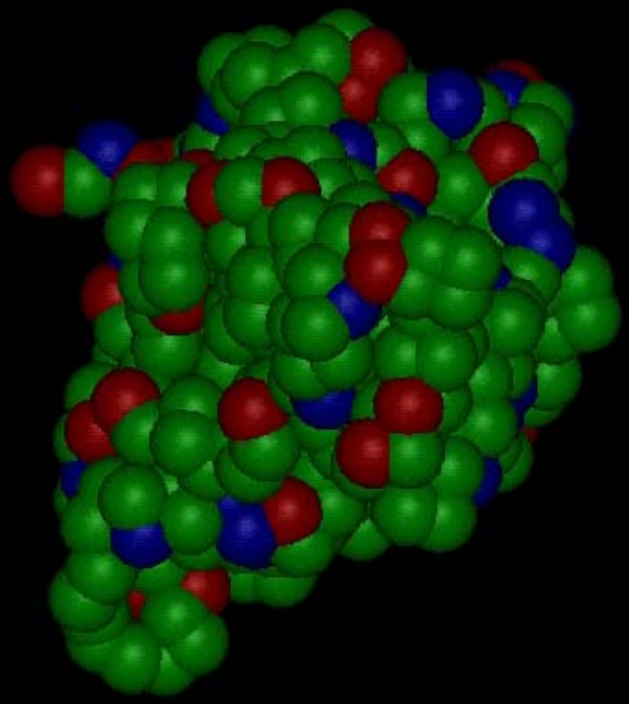
• The basic principle involved in BD simulations is similar to that involved in molecular dynamics simulations, but introduces a few new approximations that allow us to perform simulations on the microsecond timescale (remember that MD of proteins is limited to around 10 nanoseconds).

• The technique has been used to calculate the association rates of enzymes with their substrates (e.g. acetylcholinesterase with its substrate acetylcholine). For diffusion-limited enzymes, this association of the enzyme and substrate is the rate-limiting step of the reaction.

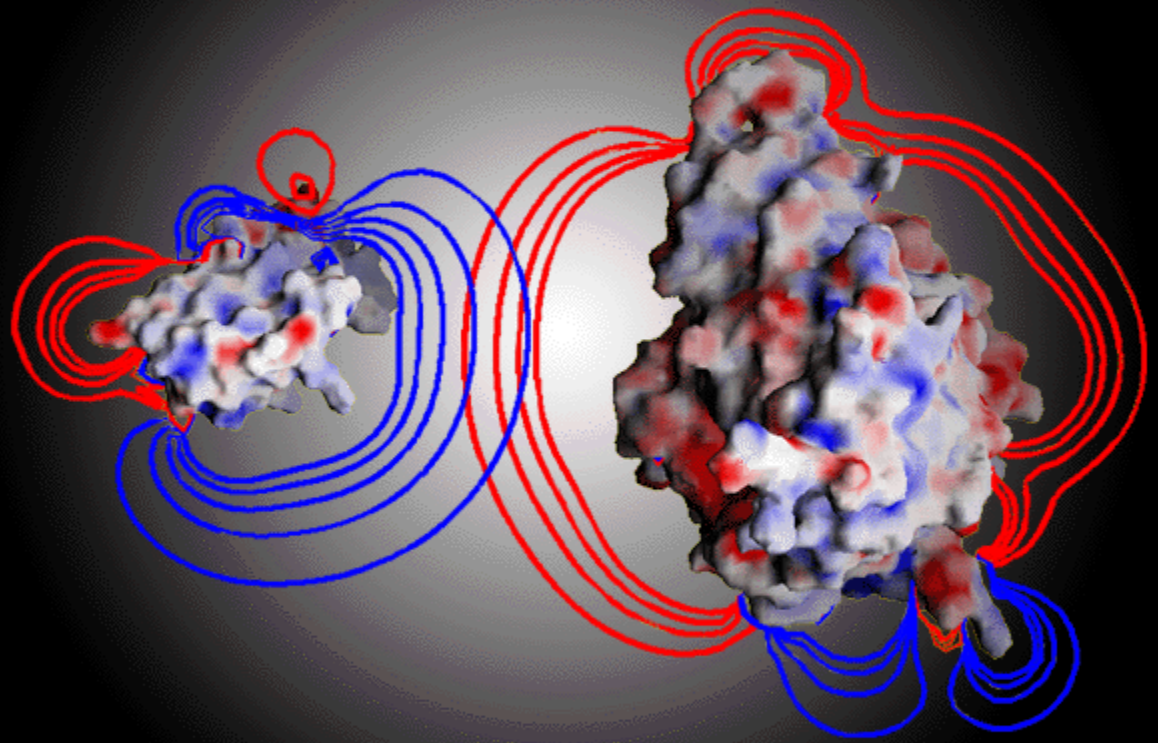
• The simulations allow us to understand how association rates are affected by mutations in the protein, and by the presence of dissolved ions such as Na+ and Cl- in the solution.

Example: Fasciculin - AchE





Electrostatically Accelerated Protein-Protein Association



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Overview of BD Method

• When we replace explicit solvent by an implicit representation, we must make sure that we don't neglect any important properties of the solvent.

• We have already discussed the effects of water on the electrostatic properties of molecules (i.e. its screening behaviour). We have seen how these effects can be approximated in a simplified solvent model by setting the dielectric constant appropriately (see the Electrostatics pages).

• Now, we must also take account of water's effects on the dynamic behaviour of solute molecules in solution. Water has two main effects:

• It is a viscous solvent: it exerts a frictional force on a diffusing solute, slowing it down.

• Collisions with water molecules add a random component to a solute's motion.

• By incorporating both of these effects, BD techniques allow realistic simulation of the diffusion of molecules in solution without the need to include any explicit solvent molecules.

Theory (I)

- The basic algorithm used in BD is similar to that in MD: we use the positions of our particles at time *t,* together with the forces acting on them, to estimate their positions at some later time $t + \Delta t$. However, in BD we typically use much larger time-steps (>1ps) since we don't have to worry about bond stretching etc.

- The algorithm that we use in BD is due to Ermak & McCammon. The translational behaviour of a particle is dictated by:

$$r(t+\Delta t) = r(t) + DF\Delta t/kT + R$$

where D is the translational diffusion constant of the particle, F is the force acting on the particle, and R is a random displacement added in to mimic the effects of collisions with solvent molecules.

Theory (II)

- The translational diffusion constant of a particle is a measure of the speed with which it diffuses through solution: the higher the diffusion constant, the faster it diffuses.

- Translation diffusion constants can be estimated using the Stokes-Einstein relationship:

$$D = kT/6\pi\eta a$$

where η is the solvent viscosity and a is the radius of the particle, i.e. bigger particles diffuse slower. A similar expression can be used to estimate the rotational diffusion constant.

Theory (III)

• R, the random displacement, is dependent on D. R is obtained using a random number generator, and is required to have the following statistical properties:

$$<R> = 0$$

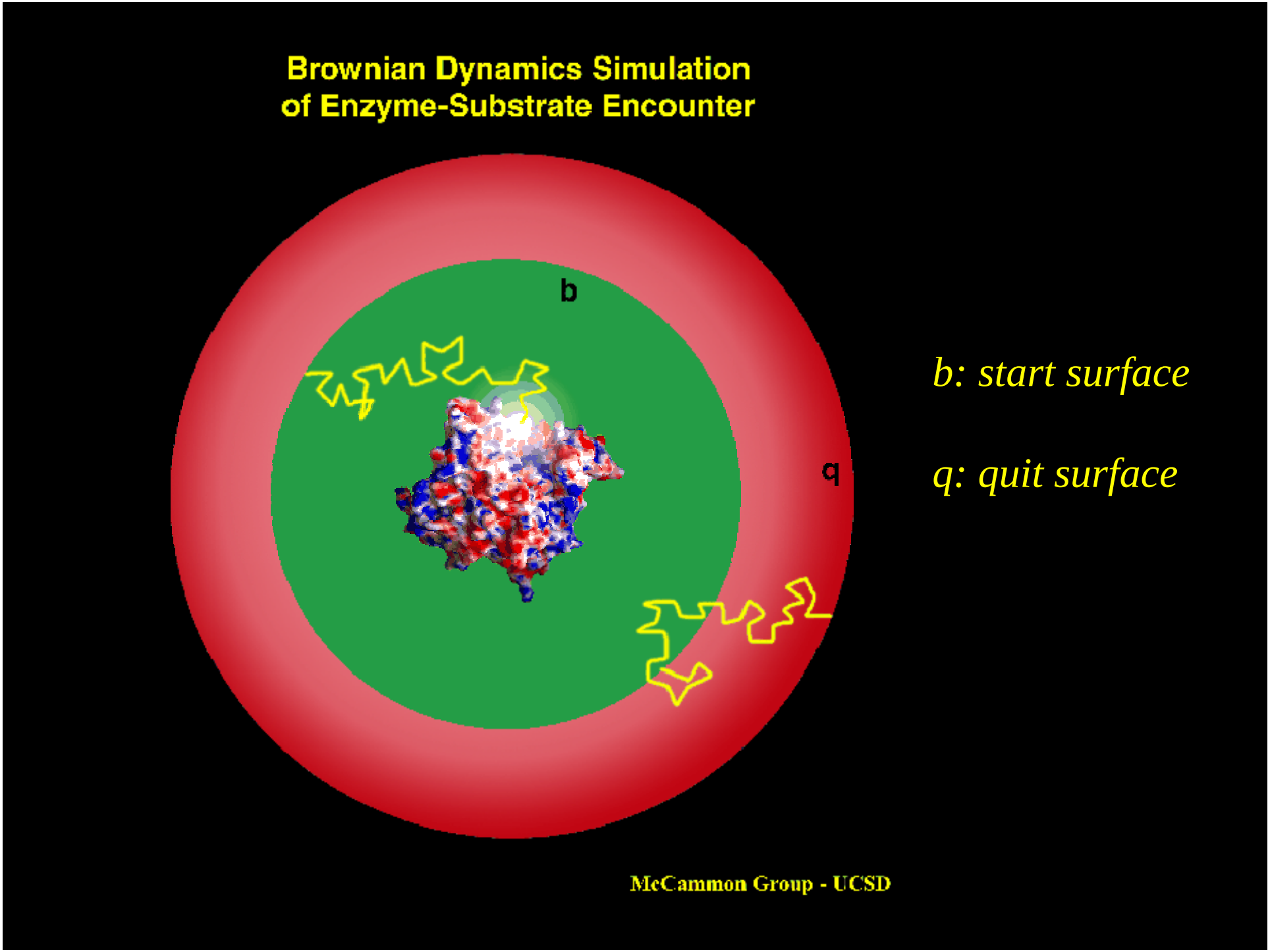
$$<R*R> = 6D\Delta t$$

The first expression says that the average value of the random displacement is zero. This has to be true, otherwise, even with no other forces acting on the particle, it would gradually drift in one direction, which would make no sense. The second expression ensures that the diffusive behavior of the particle is correctly reproduced (Einstein diffusion equation).

Theory (IV)

•In BD simulations, F, the force acting on the particles, is generally assumed to be purely electrostatic and is computed from solving the PBE numerically.

•We reject any step that causes overlap of the particles, i.e. we ask the program to pick another random number that doesn't cause overlap.


Brownian Dynamics Simulation
of Enzyme-Substrate Encounter
b
q
b: start surface
q: quit surface
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Calculating Association Rate Constants

We can use BD to calculate the association rate constant for an enzyme binding its substrate using the following relation:

$$k = k(b) * \beta$$

k is the association rate constant, i.e. the quantity we wish to compute and $k(b)$ is the steady state rate at which a diffusing substrate molecule first comes within distance b of the enzyme. β is the probability that having come within this distance b, the substrate proceeds to associate with the enzyme.

The Smoluchowski Equation

The rate at which two particles come within a given separation *b* can be calculated analytically using the result obtained by Smoluchowski:

$$k(b)=4\pi Db$$

where D is the relative diffusion constant of the two particles. This is simply the sum of the diffusion constants of the enzyme and substrate - note that because the diffusion constant of the substrate is much larger than that of the enzyme, it dominates D.

Calculating $k(b)$ is therefore easy.

Obtaining the Association Probability β

•To calculate β, we perform many separate BD trajectories.

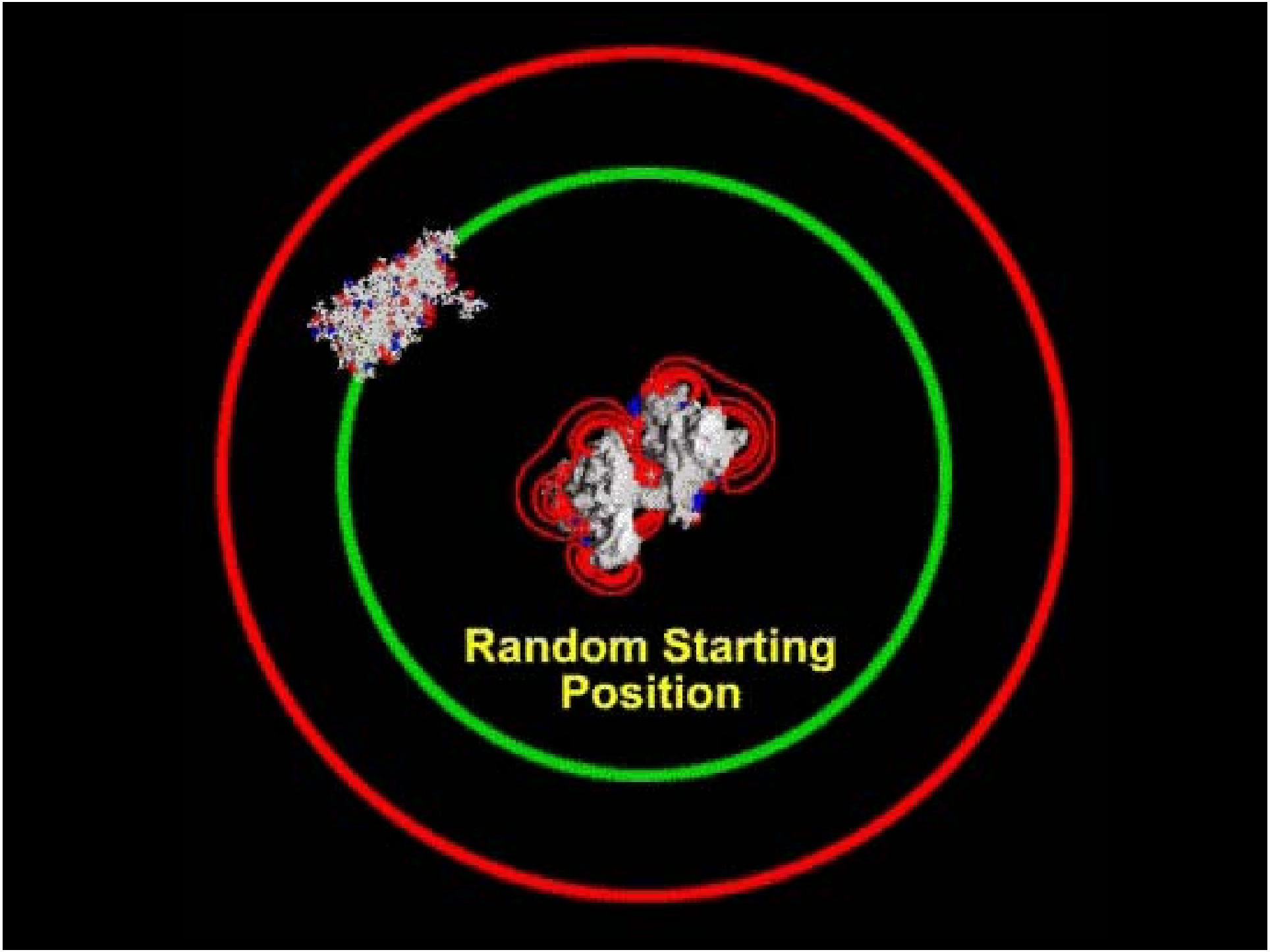
•Each simulation starts with the substrate at a distance b from the enzyme. The electrostatic potential should be approximately constant over the b-surface.

•In principle, all we have to do now is simulate the motion of the substrate until it either binds or escapes (quit or q-surface).

•Note that some substrate molecules that pass through the q-surface might return and bind to the enzyme if we continued the simulation, i.e. they may not actually go on to fully escape. To account for this possibility, we have to correct our calculated value of β (see references at end of class notes).

•We define binding using a set of reaction criteria. We monitor the distance between an atom of the substrate and a point on the enzyme that defines the entrance to the active site.

•In order to obtain statistically meaningful estimates of β, we may have to carry out thousands of trajectories. β is simply the fraction of successful trajectories.


Random Starting
Position

UHBD

- UHBD is a free, well-documented program developed by J. Andrew McCammon's group (originally at UH, now at UCSD) for carrying out Brownian dynamics simulations of protein-ligand association events.

- Local development at UH continued by Prof. Jim Briggs.

- Available at http://adrik.bchs.uh.edu/uhbd.html

Summary

- Electrostatic forces are the most important forces in chemistry and biology.
- The electrostatics of a macromolecule can be approximated by continuum electrostatics (Poisson-Boltzmann Equation).
- The Poisson-Boltzmann Equation can be solved numerically for arbitrarily shaped molecules.
- Brownian Dynamics simulations mimick protein-ligand association and allow calculation of binding rate constants.

Pros/Cons: Continuum Electrostatics

Pros:

- simple model that describes electrostatics aspects of biomolecules very well
- computationally fast
- suitable for binding energy calculations (see following class)

Cons:

- limited conformational flexibility (requires modification of model)
- model may break down when ions from solvent become localized

Resources and Further Reading

WWW:
http://mccammon.ucsd.edu/~chem215
http://trantor.bioc.columbia.edu/programs.html (GRASP, DelPhi)
http://adrik.bchs.uh.edu/uhbd.html

Textbooks:
Bourne & Weissig, Chapter 21

Papers:
Davis and McCammon, *Chem. Rev.* 1990. 90:509-521.
Warshel and Papazyan, *Current Opinion Struct. Biol.* 1998. 8:211-217.
Davis et al. *Comput. Phys. Commun.* 1991. 62:187-197.

Acknowledgement:
G. Matthias Ullmann, Uni Heidelberg