

Delphi Workshop

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Delphi is a powerful computational tool developed by Barry Honig and his research group at Columbia University. Delphi is a solver of the Poisson-Boltzmann (PB) Equation. [1-3] The PB equation is used in implicit solvation calculations. Implicit solvation techniques are used to account for the effects of a solvent (e.g. water) without having actual solvent (water) molecules included in the model.

$$\nabla \cdot [\epsilon(r) \cdot \nabla \phi(r)] - \kappa_0^2 \phi(r) + 4\pi\rho_{\text{int}}(r) = 0 \quad \text{The linearized PB equation.}$$

∇ = vector differential operator $[(\delta/\delta x) + (\delta/\delta y) + (\delta/\delta z)]$

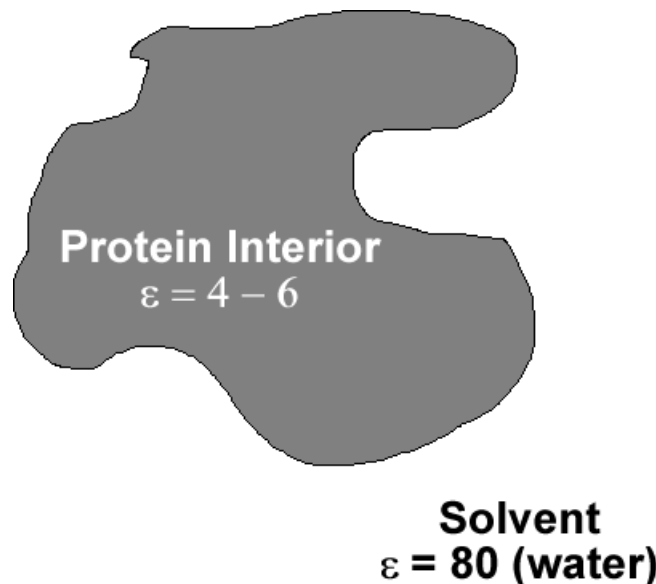
$\epsilon(r)$ = dielectric function

$\phi(r)$ = electrostatic potential

κ = Debye-Hückel inverse length.

$\rho_{\text{int}}(r)$ = interior charge density

A three dimensional grid of defined density surrounds the system in a cubic box. Each grid point represents a dielectric constant (either in solvent (water) or in solute (protein interior)). Either the molecular surface or solvent accessible surface is used to determine the boundary between the solute and the solvent. Typically, the solvent accessible surface is used.



NOTE: Delphi reports energy in units of kT. (1 kT = 0.592 kcal/mol for T = 298 K and k = 0.001986577 kcal/mol•K)

Make a separate project directory (e.g. del_tutor). You will study the binding in the barstar/barnase complex. We will use PDB entry 1BRS for this study. [4] This complex has been investigated extensively using Delphi and is a good instructional example. [5, 6]

Download **1BRS.PDB** from the Protein Data Bank (<http://rutgers.rcsb.org/pdb/>).

This structure has missing side chains and residues. We used the “**profix**” program in the JACKAL molecular modeling package[7] to replace missing residues and missing sidechains. JACKAL is not yet available on UMDNJ servers/workstations other than linux boxes; however, it is freely available for unix/linux platforms at <http://trantor.bioc.columbia.edu/programs/jackal/> . The fixed pdb file (bb_cmplx_fix.pdb) is available from the course webpage. This file was built from the A/D chains of 1BRS.PDB and the SEQRES information block for those chains (there are 3 complexes in the PDB file; we only need one.).

Prepare the PDB File: We must add hydrogen atoms to our pdb file. We will begin with the complex.

Begin an insightII session. Type “insightII” then <enter>.

Load the Biopolymer Module – Click on the Accelrys Logo in the upper left hand corner and select Biopolymer from the menu.

Molecule > Get: Get File Type: PDB
 Archive Frames: Frame
 Mol File Name: bb_cmplx_fix.pdb

Click on Execute then Cancel

<i>Mouse Review:</i>	Rotate – Right Mouse Button Translate – Middle Mouse Button Select – Left Mouse Button Zoom – Middle & Right Mouse Buttons
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Color the atoms by atom type.

Molecule > Color: Molecule Spec: BB_CMPLX_FIX
Color Method: By_Atom

Click on Execute then Cancel

Add Hydrogen atoms to the structure.

Modify > Hydrogens Molecule Spec: BB_CMPLX_FIX
 Set_pH > pH Value 7.4
 Capping Mode: Off

Click on Execute then Cancel

Protein > Cap Molecule Name: BB_CMPLX_FIX
 C_Terminus: checked
 C_Cap Groups: COO- checked

N_Terminus: checked
N Cap Groups: NH3 checked

Click on Execute then Cancel

Energy minimization: Open the Discover Module from the Accelrys logo.

FF > Select (We will use the default forcefield)

Forcefield Type: Discover
Files: cvff.frc

FF > Potentials > Accept the defaults and click on Execute

Parameters > Minimize

Conjugate
Iterations: 100
Derivative: 0.01
Check Charges
Check Morse

Run > Run

Discover Hosts: Local
Computation Mode: Interactive
Check Run Minimization and Reduce_Output

Click Execute

Save the protein as a new PDB file.

Molecule > Put Put File Type: PDB
 Assembly/Molecule
 Mol File Name: **bb_cmplx_h.pdb**
 Insight_Style: Checked

Click on Execute then Cancel

Use **Session > Quit** when you are done.

Use the **insght_fix.perl** script to fix the residue names in your pdb files. Download the insgth_fix.perl file from the course website into your working directory and use as follows; for example...

```
./insght_fix.perl bb_cmplx_h.pdb
```

****/ Use nedit to Split the resulting bb_cmplx_h.pdb file into two more files each named as bnase_h.pdb(chain A) and bstar_h.pdb (chain D). /****

The Delphi run input file (parameter file: param)

```
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)
```

What the parameters mean:

gsize – GRID SIZE: must be an odd number. A larger grid size will give more accurate potentials; however, will require more cpu time. (NOTE: min = 5; max = 571)

scale – Reciprocal of one grid spacing (grids/angstrom).

in – Used to designate an input file. Three input files are required: a PDB file (the coordinates); an atomic radius file (the siz file); and a partial atomic charge (crg) file.

indi – The molecule's interior dielectric constant.

indi = 1 – molecule with no polarizability

indi = 2 – molecule with only electronic polarizability (good for small molecules)

indi = 4 to 6 – Good for semi rigid large molecules where you have some small reorganization of dipoles. A value from 4 to 6 is best for globular proteins.

acenter – Takes 3 coordinates (in Å) and uses those coordinates for positioning of the molecule center. Use the coordinates of the most central atom in the complex being studied. For example, view the molecule in MOE, select the central atom with the mouse and use the “atoms” dialog to read the x, y, and z coordinates. You may use any viewer for this purpose.

exdi – The exterior dielectric constant (i.e. the dielectric of the solvent; Use $\text{exdi} = 80$ for water).

prbrad – Probe radius. Used for the solvent accessible surface calculation. ($\text{prbrad} = 1.4$ for water.)

salt – The concentration of the first kind of salt (in moles/liter).

salt2 – Used to handle multiple valence salts (Use in conjunction with $\text{val}+2$ and $\text{val}-2$ to designate the valence of the positive and negative ions. Likewise, use $\text{val}+1$ and $\text{val}-1$ for **salt**) See Delphi manual for more information.

ionrad = The ion exclusion layer around the molecule (in Å). Use $\text{ionrad} = 2.0$ for sodium chloride.

bndcon – An integer flag used to specify the type of boundary condition.

$\text{bndcon} = 1$ – potential is zero

$\text{bndcon} = 2$ – dipolar, boundary potentials are approximated by the Debye-Hückel potential.

$\text{bndcon} = 3$ – focusing, (requires a potential map from a prior calculation) See Delphi manual for more details.

$\text{bndcon} = 4$ – Coulombic, Approximate from the sum of the Debye-Hückel potentials of all charges q_i .

gc – The grid convergence threshold. (Another convergence option)

maxc – The maximum convergence threshold based on change in potential.

linit – An integer number (> 3) used to designate the number of iterations with the linear PB equation.

nonit - An integer number (> 0) used to designate the number of iterations with the nonlinear PB equation.

energy(s,c,g) – Tells Delphi what energies to include in output.

S or SOL = Solvation energy or Reaction field energy

C or COULOMBIC = Coulombic energy

G or GRID = Grid energy

ION or IONIC = Use for the direct ionic contribution

OTHER Options:

out – Used to designate output files (surface, phi maps)

For Example Use

```
out(phi, file="bnase.phi", format=grasp)
```

to output a phi map in grasp format.

write(eps) – Writes the electrostatic potential surface to fort.17

The Input Files – These files are text files and can be edited.

The PDB File – Contains your molecular coordinates.

The Radius or SIZE file. This file contains the atomic radii for the atoms in your PDB file. The PARSE[8] atom radii file is illustrated below. The ! is used for comments and the atom__res_radius_ line tells Delphi that the information to follow designates the atom, residue name (if given) followed by the radius in angstroms. The file below assigns radii for all atoms in the file and does not specify by residue. You may use the residue designation to specify specific atomic radii for specific residues.

```
!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
```

The partial atomic charge file (crg). A portion of the charm22.crg file is illustrated below. Notice that the atom name is given followed by the residue name (if no residue number is given, then all residues of that name will be given the same charge assignment), followed by the partial atomic charge. While atom names for heavy atoms in proteins and DNA from the Protein Data Bank are very well standardized, atom names for hydrogen atoms are not! Be very careful when using PDB files from various modeling software packages. The charge files (amber94.crg[9] and charm22.crg[10, 11]) used in this exercise have been modified to be compatible with insightII style PDB files.

```
!
!   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
```

HB1	ALA	0.090
HB2	ALA	0.090
HB3	ALA	0.090
C	ALA	0.510
O	ALA	-0.510

Use the following command to run your job.

delphi param > yourjobname.out

or use the following if you want to run the computation in background (not necessary).

nohup delphi param > yourjobname.out &

Perform the same run with salt = 0.10 for bnase_h.pdb and bstar_h.pdb. Then repeat the runs for all three files with salt = 0.00 (use nosalt in the file names).

It's easier to setup all of your run parameter input files (param1, param2, etc.) first, then use a perl script to submit the jobs in sequence. Use delauto.perl from the website. Copy delauto.perl to your working directory then run it using **./delauto.perl** command. Before you do this, examine the script using "nedit" to make sure that the file names make sense or reflect what each calculation is doing.

Analyzing the Output

Portion of the output file (bb_cmplx.out) from the completion of iterations.

```

7.9958527E-06 3.0517578E-04 at          390 iterations
7.0826345E-06 2.7465820E-04 at          400 iterations
finished qdiffx linear iterations
at          : 10:31:48
total time elapsed so far: 251.1740
# loops    : 400
mean,max change (kT/e)  : 7.0826345E-06 2.7465820E-04

total grid energy      : 133166.0      kt
self-reaction field energy : -49041.92    kt
total s.charge,no epsin carrying : 1.9499
corrected reaction field energy: -1368.311    kt
total reaction field energy : -50410.23    kt

coulombic energy      : -44734.99    kt
All energy terms but grid and self_react.: -46103.30    kt
energy calculations done at 259.4767

total cpu time was (sec) 259.4767

DelPhi exited at 10:31:57

```

To obtain the total grid energies for each run use the following command.

grep 'total grid' *.out

Our data is as follows:

(1) bb_cmplx.out;	total grid energy = 133,166.0 kT
(2) bnase.out;	total grid energy = 73,712.15 kT
(3) bstar.out;	total grid energy = 59,427.63 kT
(4) bbcmplx_nosalt.out;	total grid energy = 133,168.90 kT
(5) bnase_nosalt.out;	total grid energy = 73,714.27 kT
(6) bstar_nosalt.out;	total grid energy = 59,433.08 kT

We also need the coulombic energies at zero salt.

grep 'coulombic energy' *nosalt.out

(7) bb_cmplx_nosalt.out;	total grid energy = -44,734.99 kT
(8) bnase_nosalt.out;	total grid energy = -24,760.95 kT
(9) bstar_nosalt.out;	total grid energy = -19,401.52 kT

grep 'corrected reaction field' *nosalt.out

The corrected reaction field energies at zero salt:

(10) bbcmplx_nosalt.out;	total grid energy = -1,367.96 kT
(11) bnase_nosalt.out;	total grid energy = -912.43 kT
(12) bstar_nosalt.out;	total grid energy = -1,038.36 kT

A quick and dirty method to compute ΔG_{elec} (electrostatic enhancement to) is as follows:

$$\Delta G_{\text{elec}} = \Delta G_{\text{grid-cmplx}} - \Delta G_{\text{grid-bnase}} - \Delta G_{\text{grid-bstar}}$$

$$\Delta G_{\text{elec}} = (1) - (2) - (3) = +26.2 \text{ kT} = +15.5 \text{ kcal/mol}$$

Compare to Tidor's value of +14.3 kcal/mol[6] and Honig's value of +3.5 kcal/mol.[12] Honig used CHARMM22 charges and radii, and fixed the structure as we did; however, Honig used the CHARMM forcefield to energy minimize his structure. The positive # indicates that the electrostatic contribution to the free energy is destabilizing for this model.

Another method is the energy partitioning method to compute ΔG_{elec} . This method computes ΔG_{elec} as follows ...

$$\Delta G_{\text{elec}} = \Delta G_{\text{coul}} + \Delta G_{\text{rxnfld}} + \Delta G_{\text{ions}} = +15.0 = +8.9 \text{ kcal/mol}$$

$$\Delta G_{\text{coul}} = (7) - (8) - (9) = -572.52 \text{ kT}$$

$$\Delta G_{\text{rxnfld}} = (10) - (11) - (12) = +582.8 \text{ kT}$$

$$\Delta G_{\text{ions}} = ((1) - (4)) - ((2) - (5)) - ((3) - (6)) = +4.7 \text{ kT}$$

Generate a PHI map for bnase_h.pdb. Add the “out” option for phi maps in your Delphi param file for barnase (bnase_h.pdb). For example ...

```
gsize=165
scale=2.5
in (pdb, file="bnase_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)
out (phi, file="bnase.phi", format=grasp)
```

To view your phi map, use the Grasp[13] program. To start Grasp just type “grasp” in the unix shell.

Right-click in the middle of the graphics window using your mouse (A menu pops up).

Read > PDB File > Show List > bnase_h.pdb

Miscellaneous > toggle cross hairs on and off

Read > DelPhi Potential Map > Show List > bnase.phi

Select Panels (a new window appears)

Click on Electrostatics (an Electrostatics window appears)

Click on Contours (a Contours window appears)

Click on Build Contour

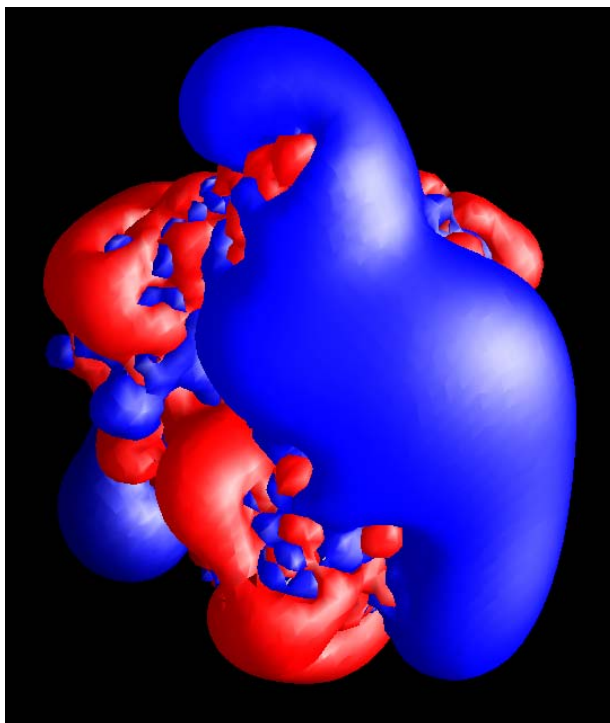
Contour Color: 2 (red)
Contour Value: -0.50 (in kt)

Click on Build

Contour Color: 4 (blue)
Contour Value: +0.50 (in kt)

Click on Build
Quit the Builder; Quit Contours

Write > RGB File



Potential contour looking in at the binding site of barnase (-0.5 kT (red) to +0.5 kT (blue)). Note the large region of positive potential in the binding site of barnase.

A few additional exercises:

1. Compute a phi map for barstar from your bstar_h.pdb file. Does the predominant region of negative potential match up with the region of positive potential in bnase?
2. A recent study by Dong, et al. reveals that one must be careful in the selection of interior dielectric constant for PB computations. [5] This study finds that using an $\text{indi}=20$ gives results that would indicate a net stabilization for ΔG_{elec} . Repeat the computation performed in this tutorial changing the indi value from 2 to 20. Do you observe a negative value for ΔG_{elec} ? This study also reported good agreement with experiment when using $\text{ionrad}=0$ (vdW surface), and $\text{prbrad}=0.0$ with $\text{indi}=4$ and $\text{salt}=0.025$. Try these settings for an additional comparison study.
3. Mutate ASP39 to ALA in barstar (chain D). Perform the mutation in the complex (start from bb_cmplx_fix.pdb), minimize the complex then save the new PDB for the mutant. Split out the bnase and bstar portions as separate files and perform the same series of Delphi calculations. What are your values for ΔG_{elec} with the mutant? Is there a gain or loss in stabilization? What is the impact on the PHI map?

How to mutate the residue in InsightII. Use the Biopolymer module.

Load the PDB file and Color by Atom as you did before.

Residue > Replace

Residue to Replace: BB_CMPLX_FIX:D39

Parameters > Residue Type: ALA

Chirality: L

Click Execute (however, should replace as soon as you pick ALA in Parameters) then Cancel

References:

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2. Rocchia, W., S. Sridharan, A. Nicholls, E. Alexov, A. Chiabrera, and B. Honig, *Rapid grid-based construction of the molecular surface and the use of induced surface charge to calculate reaction field energies: applications to the molecular systems and geometric objects*. J. Comput. Chem., 2002. **23**(1): p. 128-37.
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