

Ras-Raf MM_PB(GB)SA Tutorial: AMBER 8

Bethany L. Kormos and David L. Beveridge*

*Department of Chemistry and Molecular Biophysics Program,
Wesleyan University, Middletown, CT 06459*

Introduction

The examples in this MM-PB(GB)SA tutorial are distributed with AMBER 8^{1,2} and may be found in the following directory:

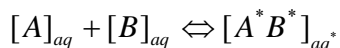
~/amber8/src/mm_pbsa/Examples

This tutorial is based on calculations performed with Amber 8^{1,2} on a PC Cluster running Red Hat Linux. The tutorial content is basically the same for the different versions of Amber, however there are differences in implementation and technical details between the versions, thus they have been divided into separate tutorials.

Contained within the ~/amber8/src/mm_pbsa/Examples directory are six examples for how the MM-PBSA program may typically be used in AMBER. One of the capabilities offered within the MM-PBSA module in AMBER that is not covered in these examples is the alanine scanning technique.³ These examples have been drawn from studies performed by Case and coworkers^{4,5} on a protein-protein complex (H-Ras/C-Raf1).

Summary of Theory

The calculations described herein as MM-PBSA or MM-GBSA may also be more precisely designated as MD-PBSA or MD-GBSA. This genre of theoretical calculations is additionally referred to by several different names in the recent literature, including master equation,⁶ end point⁷ calculations, and component analysis.⁸⁻¹⁰ The overall objective of these methods and this tutorial is to calculate the absolute binding free energy for the non-covalent association of any two molecules, A and B, in solution, i.e.



where $[A]_{aq}$ refers to the dynamical structure of molecule A free in solution, $[B]_{aq}$ refers to the dynamical structure of molecule B free in solution, and $[A^*B^*]_{aq^*}$ represents the complex formed

from molecules A and B , taking into account any structural changes ($[A^*]$ and $[B^*]$) and solvent reorganization (aq^*) that may occur upon complex formation.

In principle, this calculation requires three MD simulations, one each on $[A]_{aq}$, $[B]_{aq}$, and $[A^*B^*]_{aq^*}$. Gohlke and Case⁵ refer to this protocol as “separate trajectories” or “three trajectories” (3T). However, in this tutorial, one makes the (questionable) approximation that structural adaptation is negligible and draws the trajectories for $[A]_{aq}$ and $[B]_{aq}$ from the single MD carried out on $[A^*B^*]_{aq^*}$, simply by separating the complex into its constituent parts, $[A^*]_{aq^*}$ and $[B^*]_{aq^*}$ (1T). The extension to the 3T protocol should be straightforward starting from this tutorial.

The following is a summary of the thermodynamic quantities determined in this tutorial. The binding free energy for the noncovalent association of two molecules may be written as:

$$\Delta G(A + B \rightarrow AB) = G_{AB} - G_A - G_B$$

The free energy of any molecule X may be divided into a contribution from the solute and a contribution from the solvent:

$$G(X) = G^{solute}(X) + G^{solvent}(X)$$

The free energy contribution from the solute may be expressed as:

$$G^{solute}(X) = U(X) - TS(X)$$

where:

$$U(X) = \langle E(X) \rangle$$

$$S(X) = S_{trans} + S_{rot} + S_{vib} + S_{config}$$

Here, $\langle E(X) \rangle$ represents an average of energies obtained from the MD simulation(s),

S_{trans} and S_{rot} are the entropic contributions from translational and rotational motion, respectively, obtained from classical statistical mechanics,

S_{vib} is the entropic contribution from vibrational motion, obtained from a normal mode calculation in this tutorial, and

S_{config} is the configurational entropy contribution from sidechain reorganization. Note that configurational entropy is neglected in this protocol, but may be significant.

The free energy contribution from the solvent may be expressed as:

$$G^{solvent}(X) = G^{es}(X) + G^{nes}(X)$$

where

G^{es} is the electrostatic contribution, which is obtained from a PB or GB method, and

G^{nes} is the nonelectrostatic contribution.

In this tutorial,

$$G^{nes}(X) = \gamma SASA(X) + b$$

where

γ is a surface tension parameter, set to 0.005 for PB and 0.0072 for GB in this tutorial,

$SASA(X)$ is the solvent-accessible surface area of molecule X , determined by LCPO¹¹ or Molsurf¹² in this tutorial, and

b is a parameterized value, set to 0.0 for both PB and GB in this tutorial.

A more complete explanation of the theory may be found in Appendix A. A description of the AMBER output and how it corresponds to the quantities outlined in this summary (and in Appendix A) may be found in Appendix B.

One final consideration that should be addressed before proceeding further is that energy component methods such as the one used in this tutorial involve an assumption of additivity. For a critical perspective on this, see Dill.¹³ Calculations of this genre are susceptible to both errors of omission and commission. Errors of omission involve the neglect of certain contributions, such as configurational entropy or ion effects, while errors of commission refer to uncertainties in the estimates of terms included in a model, i.e. the use of a constant dielectric in calculating the solvation free energy or the use of a harmonic potential in the normal mode calculation.

Setup

First, the environmental variables for the AMBER 8 suite of programs must be specified by adding the following lines to your shell initialization file (.cshrc, .bash_profile, etc.):

```
export AMBERHOME=/home/surjit/amber8
```

```
PATH=$PATH:/home/surjit/amber8/exe
```

```
if [ -z "${LD_LIBRARY_PATH}" ]
```

```
then
```

```
LD_LIBRARY_PATH="${IA32ROOT}/lib"; export LD_LIBRARY_PATH
```

else

```
LD_LIBRARY_PATH="${IA32ROOT}/lib:$LD_LIBRARY_PATH"; export
LD_LIBRARY_PATH
fi
```

Next, copy the contents of `~/amber8/src/mm_pbsa/Examples` to your local directory.

Files within the Example directory include:

```
my_amber94_delphi.crg – charge parameter file for DELPHI
my_parse_delphi.siz   – VDW parameter file for DELPHI
raf_wt.prmtop         – prmtop file for ligand
ras_II_wt.prmtop      – prmtop file for receptor
ras_raf_II_wt.prmtop – prmtop file for complex (“prmtop” files contain vital
                        information such as charges and topology for each
                        molecule used in the calculation)
```

Subdirectories within the Example directory include:

```
01_GenerateSnapshots – Snapshots from molecular dynamics trajectory for
                        analysis
02_MMPBSA_Stability – Examples of running a stability calculation, i.e.
                        estimating the free energy of one species
03_MMPBSA_Binding – Examples of running a binding energy calculation, i.e.
                        estimating the  $\Delta G$  for  $A + B \rightarrow AB$ 
04_MMPBSA_Nmode – Example of normal mode analysis, i.e. estimating the
                        entropy change
05_MMPBSA_Decomp_Residue – Example of energy decomposition by residue
06_MMPBSA_Decomp_Pair – Example of pairwise energy decomposition
```

To run an MM-PBSA calculation in AMBER, type the following at the prompt:

```
% mm_pbsa.pl mm_pbsa.in > mm_pbsa.log &
```

where *mm_pbsa.pl* is the executable (a perl script) and *mm_pbsa.in* is the edited input file. The details of the *mm_pbsa.in* file are in the AMBER manual, as well as within the .in file itself, and some of the key points are also outlined throughout the text below.

Within each directory are the appropriate mm_pbsa.in file and the output files that should be generated if the program runs correctly. The correct output files have a *.save extension, so that the *.out files generated can be compared to them to ensure the program has run correctly.

Note

The mm_pbsa.pl executable changed significantly between Amber 7 and Amber 8. In Amber 8, the different subroutines called are found in separate *.pm files in the ~/amber8/src/mm_pbsa directory rather than within the mm_pbsa.pl file. In Amber 7, one could comment out select unlink lines within the mm_pbsa.pl file in order to keep certain output files with which to troubleshoot. In Amber 8, the unlink lines must be commented out in the individual *.pm files.

Note

In the tutorial, which was developed for AMBER 8, the PB method used is the *pbsa* method, which is implemented within the Amber 8 suite of programs. The DelPhi program may also be used, however it is not distributed with AMBER and therefore must be installed locally. As such, the mm_pbsa.in file changes slightly from AMBER 7 to AMBER 8: there is no longer a @DELPHI parameters section, but a @PB parameters section, in which the user may choose the preferred PB method for the calculation of the electrostatic contribution to the solvation free energy. The *pbsa* documentation is found in the AMBER 8 manual in the Miscellaneous chapter, section 11.10, p. 252.

Note

Useful references for this tutorial are:

- The AMBER website, Amber 8 manual, and AMBER mail reflector archive:
<http://amber.scripps.edu/>
- The DelPhi description and manual:
<http://honiglab.cpmc.columbia.edu/>

01 GenerateSnapshots

Purpose: This directory contains a short MD trajectory of the Ras-Raf complex, md_traj_short.mdcrd. In this step, snapshots are generated for the complex, as well as the receptor by itself and the ligand by itself for subsequent MM-PB(GB)SA analysis.

Input file: The AMBER 8 manual, pages 218-227 contains a complete description of the keywords contained in mm_pbsa.in.

The @GENERAL section – General parameters are described in this section. Some of the key points include:

PREFIX		– file name for snapshots that are generated
PATH		– path where the trajectory is contained and where the snapshots will get written
COMPLEX	1	– 1=yes, 0=no. The complex, receptor,
RECEPTOR	1	and ligand must be turned on to
LIGAND	1	generate the respective snapshots
GC	1	– Turns on the option to generate snapshots

The @MAKECRD section – These keywords are dependent on the size of the system, whether periodic boundary conditions were used, the number of structures one desires to extract, and the number of atoms in the ligand, receptor and complex. Descriptions of these keywords are also given under @MAKECRD.

The @TRAJECTORY section – Names and locations of the trajectory files are listed in this section.

The @PROGRAMS section – This section can be ignored for this calculation.

At the prompt, type:

```
% mm_pbsa.pl mm_pbsa.in > mm_pbsa.log &
```

Output files: After you have executed `mm_pbsa.pl` you will see five snapshots of the complex (`ras_raf_II_wt_com.crd.#`), receptor (`ras_raf_II_wt_rec.crd.#`), and ligand (`ras_raf_II_wt_lig.crd.#`).

02 MMPBSA Stability

Purpose: The `mm_pbsa.in` file in this directory computes continuum free energy estimates with both PB and GB methods to verify convergence (stability) of the energies. In this calculation, the energy components for the five snapshots of the COMPLEX that were generated in Part 1 are computed; they could also of course be computed for the ligand and/or the receptor.

Input file:

The @GENERAL section:

The complex is turned on while the ligand and receptor are turned off.

The keywords `COMPT`, `LIGPT`, `RECPT` refer to the path of the `prmtop` files for the various molecular species.

The keywords `MM`, `GB`, `PB`, `MS` are turned on:

MM – Calculation of gas-phase energies using Sander

GB – Calculation of solvation free energies using the GB models in Sander

PB – Calculation of solvation free energies using *pbsa*

MS – Calculation of nonpolar contributions to solvation using Molsurf. If `MS = 0`, nonpolar contributions are instead calculated with the LCPO method in Sander.

Each of the programs used to compute the different energy contributions have different associated parameters to be specified, thus they each have a section in the input file in which this may be done:

The @PB section – these are the keywords supplied to AMBER to calculate the electrostatic and non-electrostatic contributions to the solvation free energy using the Poisson-Boltzmann equation.

`PROC` – the method used, either `DelPhi` (1) or *pbsa* (2).

REFE – the reference state taken for the PB calculation.
INDI – molecule dielectric constant
EXDI – solvent dielectric constant; 80.0=water
SCALE – reciprocal of one grid spacing (grids/angstrom)
LINIT – number of iterations with the linear PB equation.
PRBRAD –solvent probe radius in Angstroms.
RADIOPT – option for atomic cavity radii.
SURFTEN and SURFOFF – parameters used to compute the nonpolar contribution of the solvation free energy.

There are other keywords in the *pbsa* program which may or may not be useful in this section, i.e. PBTEMP (temperature used), ACCEPT (convergence criterion), etc.

The @MM section – These are the keywords supplied to Sander in AMBER 8 for the gas phase MM calculation:

DIELC – dielectric constant for electrostatic interactions.

The @GB section – These are the keywords supplied to Sander in AMBER 8 to run GB:

IGB – the generalized born solvation model used. Choices are outlined in the input file as well as in the AMBER manual. Methods differ between AMBER versions. IGB=3 and 4, available in AMBER 7, are not supported in AMBER 8. See Appendix C for a brief description of the GB methods available in AMBER 8.

GBSA – method for SASA calculation

SALTCON – sets concentration (M) of mobile counterions in solution.

EXTDIEL – solvent dielectric constant (default = 78.5 in Sander).

INTDIEL – solute dielectric constant (default = 1.0 in Sander).

SURFTEN and SURFOFF – parameters used to compute the nonpolar contribution of the solvation free energy. SURFOFF is not a keyword available in Sander, so it will always be set to 0.0 as in the input file. However, SURFTEN = 0.0072 in the input file is slightly different than the default value in Sander (SURFTEN = 0.005). Changing the value of SURFTEN in this test case from 0.0072 to 0.005 changes the resultant energy fairly significantly, with an overall

difference of 25.07 kcal/mol in GBTOT, a value larger than the standard deviations computed. A value of 0.0072 is recommended for calculations in which AMBER charges are used,¹⁴ while the value of 0.005 should be used with PARSE charges.¹⁵

NOTE

The different IGB methods require different atomic radii that are read in from the prmtop files. This is accomplished using different “set default PBradii” commands when preparing the prmtop files in LEaP. Consult the AMBER 8 manual and/or Appendix C of this tutorial for further details.

The @MS section – These are the keywords supplied to the Molsurf program. Molsurf is used to compute the solvent accessible surface area (SASA) of the solute,¹² which is used to determine the hydrophobic contribution to the solvation free energy:

PROBE – radius of the probe used to calculate the SASA

The @PROGRAMS section:

No programs need to be specified for this section.

Again, use the mm_pbsa.in input file provided and type the command:

```
% mm_pbsa.pl mm_pbsa.in > mm_pbsa.log &
```

Output files: The energy components for the five structures are listed in the file ras_raf_II_wt_all.out.save, and the average value for each component averaged over the five snapshots and associated standard deviations are listed in the file ras_raf_II_wt_statistics.out.save. Notice that no entropic terms are included, so this is not a true free energy value.

03 MMPBSA Binding

Purpose: The mm_pbsa.in file in this directory may be used to compute the free energies of binding for the process $A + B \rightarrow AB$, in this case the association of ras with raf to form the complex.

Input file:

The @GENERAL section:

This time, the ligand, receptor, and complex are all turned on, and the terms MM, GB, PB, and MS are computed for EACH species. The remainder of the input file is set up exactly the same as it was in Part 2.

Use the input file provided and type:

```
% mm_pbsa.pl mm_pbsa.in > mm_pbsa.log &
```

Output files: The three output files, ras_raf_II_wt_rec.all.out.save, ras_raf_II_wt_lig.all.out.save, and ras_raf_II_wt_com.all.out.save, contain the energy components for each the five snapshots generated in the first part of the tutorial for the receptor, ligand and complex, respectively. The file, ras_raf_II_wt_statistics.out.save, lists the energy components for complex, receptor, and ligand separately, followed by the difference in energy between the complex and the free species (under the category DELTA). Notice that no entropic terms are included, so this is not a true free energy value.

04 MMPBSA Nmode

Purpose: The mm_pbsa.in file in this directory turns on the NM keyword, which is used to calculate the entropy of the ligand using the NMODE program in AMBER. The NMODE program may additionally be used to search for transition states, perform a conjugate gradient minimization, or perform a langevin mode calculation. In this section, first, a Newton-Raphson minimization is performed on each of the five structures of the ligand. Then, the nmode calculation is performed, which computes normal mode frequencies as well as thermodynamic parameters for each minimized structure.

Input file:

The @GENERAL section:

Notice that only the ligand is turned on in the input file, thus entropies are only being calculated for the ligand.

The @NM section - this section contains the parameters for the minimizations and nmode calculations:

DIELC – the distance-dependent dielectric constant for the electrostatic interaction

MAXCYC – the maximum number of minimization cycles

DRMS – the convergence criterion for the energy gradient

*****NOTE*****

The default value for DRMS in Sander is 1.e-5, much smaller than the 0.1 value used in this example. DRMS = 0.1 is only used for demonstration purposes in this tutorial to reduce computation time.

*****NOTE*****

Setting DIELC = 4 (and EEDMETH = 5, which is not a keyword in the mm-pbsa input, but is default in the sander input file that mm-pbsa sets up) corresponds to a distance-dependent dielectric of 4r. See <http://amber.ch.ic.ac.uk/archive/200405/0399.html>.

There are a number of additional NM keywords available in Sander in AMBER that may be useful in other circumstances.

The @PROGRAMS section – this section is not necessary for an NMODE calculation.

Again, use the mm_pbsa.in input file provided and type the command:

```
% mm_pbsa.pl mm_pbsa.in > mm_pbsa.log &
```

Output files: The ras_raf_II_wt_lig.all.out.save file lists the translational, rotational and vibrational entropy components as well as the total entropy for each structure, and the ras_raf_II_wt_statistics.out.save file lists the averages and standard deviations.

05 MMPBSA Decomp Residue

Purpose: The mm_pbsa.in file in this directory turns on the DECOMP keyword, which is used to decompose the free energies into individual contributions from gas phase energies, solvation free energies calculated with GB and nonpolar contributions calculated with the LCPO method. In this example, the energies are decomposed per residue.

Input file:

The @GENERAL section:

Here the DC keyword is turned on so as to perform energy decomposition. Notice that the ligand, receptor and complex are all turned on, and that only the MM and GB energetics are turned on. Energy decomposition is not available for PB in AMBER 8.

The @DECOMP section - this section contains the parameters for the energy decomposition:

The keywords are explained quite well in the input file, so the explanations are not repeated here. However, notice that only a select number of residues are chosen in the ligand and receptor (as well as the complementary residues in the complex) on which to perform the energy decomposition.

The @MM section:

This section is the same as in Sections 2 and 3.

The @GB section:

These parameters are set to the same values seen in Sections 2 and 3.

Again, use the mm_pbsa.in input file provided and type the command:

```
% mm_pbsa.pl mm_pbsa.in > mm_pbsa.log &
```

Output files: The `ras_raf_II_wt_lig.all.out.save`, `ras_raf_II_wt_rec.all.out.save`, and `ras_raf_II_wt_com.all.out.save` files list individual energy contributions for each residue selected of the ligand, receptor and complex.

T – indicates the energy due to the total residue

S – indicates the energy due to the sidechain

B – indicates the energy due to the backbone

The `ras_raf_II_wt_statistics.out.save` file contains the mean energy contributions for each residue in the complex, receptor and ligand, again for the total residue, sidechain and backbone. Additionally, the decomposed energy differences of the residues between the free and bound forms are given in the DELTA section.

06 MMPBSA Decomp Pair

Purpose: The `mm_pbsa.in` file in this directory turns on the DECOMP keyword, which is used to decompose the free energies into individual contributions from gas phase energies, solvation free energies calculated with GB and nonpolar contributions calculated with the LCPO method. In this example, the energies are decomposed pairwise by residue.

Input file: The only difference between this input file and that in Section 5 is in the @DECOMP parameters. `DCTYPE=4` instead of 2, calling for energy decomposition pairwise by residue. Other than this difference, the remainder of the input file is the same.

Again, use the `mm_pbsa.in` input file provided and type the command:

```
% mm_pbsa.pl mm_pbsa.in > mm_pbsa.log &
```

Output files: The `ras_raf_II_wt_lig.all.out.save`, `ras_raf_II_wt_rec.all.out.save`, and `ras_raf_II_wt_com.all.out.save` files list individual energy contributions for each residue with all of the other residues selected for the ligand, receptor and complex.

T – indicates the energy due to the total residue

S – indicates the energy due to the sidechain

B – indicates the energy due to the backbone

The `ras_raf_II_wt_statistics.out.save` output file contains the pairwise energy contributions for each of the species, again for the total residue, sidechain and backbone. Additionally, the decomposed pairwise energy differences of the residues between the free and bound forms are given in the DELTA section.

Conclusions

This tutorial has provided an introduction to the methods provided within the AMBER 8 MM-PB(GB)SA module, including solvation free energy computation, normal mode analysis and energy decomposition. With a basic familiarity of these concepts, the user should be able to reliably compute the binding free energy of two macromolecules with an understanding of the advantages of these methods, as well as assumptions made in the process.

References

1. Case, D. A.; Darden, T. A.; Cheatham III, T. E.; Simmerling, C. L.; Wang, J.; Duke, R. E.; Luo, R.; Merz, K. M.; Wang, B.; Pearlman, D. A.; Crowley, M.; Brozell, S.; Tsui, V.; Gohlke, H.; Mongan, J.; Hornak, V.; Cui, G.; Beroza, P.; Schafmeister, C.; Caldwell, J. W.; Ross, W. S.; Kollman, P. (2004) AMBER 8. University of California, San Francisco.
2. Pearlman, D. A.; Case, D. A.; Caldwell, J. W.; Ross, W. S.; Cheatham III, T. E.; DeBolt, S.; Ferguson, D.; Seibel, G.; Kollman, P. (1995) "AMBER, a package of computer programs for applying molecular mechanics, normal mode analysis, molecular dynamics and free energy calculations to simulate the structural and energetic properties of molecules." *Comp. Phys. Commun.* **91**, 1-41.
3. Huo, S.; Massova, I.; Kollman, P. A. (2002) "Computational Alanine Scanning of the 1:1 Human Growth Hormone-Receptor Complex." *J. Comput. Chem.* **23**, 15-27.
4. Gohlke, H.; Kiel, C.; Case, D. (2003) "Insights into Protein-Protein Binding Free Energy Calculation and Free Energy Decomposition for the Ras-Raf and Ras-RalGDS Complexes." *J. Mol. Biol.* **330**, 891-913.
5. Gohlke, H.; Case, D. A. (2004) "Converging Free Energy Estimates: MM-PB(GB)SA Studies on the Protein-Protein Complex Ras-Raf." *J. Comput. Chem.* **25**, 238-250.

6. Ajay, A.; Murcko, M. A. (1995) "Computational Methods to Predict Binding Free Energies in Ligand- Receptor Complexes." *J. Med. Chem.* **38**, 4953-4967.
7. Swanson, J. M.; Henchman, R. H.; McCammon, J. A. (2004) "Revisiting free energy calculations: a theoretical connection to MM/PBSA and direct calculation of the association free energy." *Biophys. J.* **86**, 67-74.
8. Jayaram, B.; McConnell, K.; Dixit, S. B.; Beveridge, D. L. (1999) "Free Energy Analysis of Protein-DNA Binding: The EcoRI Endonuclease - DNA Complex." *J. Comput. Phys.* **151**, 333-357.
9. Kombo, D. C.; B., J.; J., M. K.; Beveridge, D. L. (2002) "Calculation of the Affinity of the lambda Repressor-Operator Complex Based on free Energy Component Analysis." *Molecular Simulation* **28**, 187-211.
10. Jayaram, B.; McConnell, K.; Dixit, S. B.; Das, A.; Beveridge, D. L. (2002) "Free-energy component analysis of 40 protein-DNA complexes: A consensus view on the thermodynamics of binding at the molecular level." *J. Comput. Chem.* **23**, 1-14.
11. Weiser, J.; Shenkin, P. S.; Still, W. C. (1999) "Approximate Atomic Surfaces from Linear Combinations of Pairwise Overlaps (LCPO)." *J. Comput. Chem.* **20**, 217-230.
12. Connolly, M. L. (1983) "Analytical Molecular Surface Calculation." *J. Appl. Cryst.* **16**, 548-558.
13. Dill, K. A. (1997) "Additivity principles in biochemistry." *J. Biol. Chem.* **272**, 701-704.
14. Still, W. C.; Tempczyk, A.; Hawley, R. C.; Hendrickson, T. (1990) "Semianalytical Treatment of Solvation for Molecular Mechanics and Dynamics." *J. Am. Chem. Soc.* **112**, 6127-6129.
15. Sitkoff, D.; Sharp, K. A.; Honig, B. (1994) "Accurate Calculation of Hydration Free Energies Using Macroscopic Solvent Models." *J. Phys. Chem.* **98**, 1978-1988.
16. Schlitter, J. (1993) "Estimation of Absolute and Relative Entropies of Macromolecules Using the Covariance Matrix." *Chem. Phys. Lett.* **215**, 617-621.
17. Dixit, S. B.; Andrews, D. Q.; Beveridge, D. L. (2005) "Induced Fit and the Entropy of Structural Adaptation in the Complexation of CAP and lambda Repressor with Cognate DNA Sequences." *Biophys. J.* **88**, 3147-3157.
18. Hawkins, G. D.; Cramer, C. J.; Truhlar, D. G. (1995) "Pairwise Solute Descreening of Solute Charges from a Dielectric Medium." *Chem. Phys. Lett.* **246**, 122-129.

19. Hawkins, G. D.; Cramer, C. J.; Truhlar, D. G. (1996) "Parametrized models of aqueous free energies of solvation based on pairwise descreening of solute atomic charges from a dielectric medium." *J. Phys. Chem.* **100**, 19824-19839.
20. Tsui, V.; Case, D. A. (2001) "Theory and Applications of the Generalized Born Solvation Model in Macromolecular Simulations." *Biopolymers (Nucleic Acid Sciences)*. **56**, 257-291.
21. Onufriev, A.; Bashford, D.; Case, D. A. (2000) "Modification of the Generalized Born Model Suitable for Macromolecules." *J. Phys. Chem. B.* **104**, 3712-3720.
22. Feig, M.; Onufriev, A.; Lee, M. S.; Im, W.; Case, D. A.; III, C. L. B. (2004) "Performance Comparison of Generalized Born and Poisson Methods in the Calculation of Electrostatic Solvation Energies for Protein Structures." *J. Comput. Chem.* **25**, 265-284.

Appendix A

Included in this appendix is a description of the thermodynamic quantities determined in this tutorial.

In MM-PB(GB)SA or MD-PB(GB)SA,

$$\Delta G(A + B \rightarrow AB) = \Delta G^{solute}(A + B \rightarrow AB) + G^{solvent}(A + B \rightarrow AB)$$

Solute Contributions

For ΔG^{solute} :

$$\Delta G^{solute} = G^{solute}(AB) - G^{solute}(A) - G^{solute}(B)$$

For any species on the r.h.s.:

$$G(X) = H(X) - TS(X)$$

$$G(X) = U(X) + PV(X) - TS(X)$$

In this tutorial, any changes in volume upon $A + B \rightarrow AB$ are neglected, hence:

$$G(X) = U(X) - TS(X)$$

For $U^{solute}(X)$:

$$U(X) = \langle E(X) \rangle$$

In the AMBER model,

$$E(X) = E_{val} + E_{ele} + E_{VDW}$$

where

E_{val} is the internal energy contribution from bonds, angles and torsions,

E_{ele} is the electronic energy contribution calculated from the Coulomb potential, $\frac{1}{4\pi\epsilon_0} \frac{q_i q_j}{R_{ij}}$, and

E_{VDW} is the van der Waals distance-dependent interaction energy calculated from the Lennard-

Jones potential, $4\epsilon \left[\left(\frac{\sigma}{r} \right)^{12} - \left(\frac{\sigma}{r} \right)^6 \right]$

The quantity $\langle E(X) \rangle$ is calculated by performing an MD simulation on $[X]$, extracting structures from the simulation, 1, 2, 3, ..., calculating $E(X_1)$, $E(X_2)$, $E(X_3)$, ..., and averaging over the energies,

$$\langle E(X) \rangle = \frac{1}{N} \sum_{i=1, N} E(X_i)$$

For $S^{solute}(X)$, motional modes make the contributions to the free energy:

$$S(X) = S_{trans} + S_{rot} + S_{vib} + S_{config}$$

S_{trans} and S_{rot} are the entropic contributions from translational and rotational motion, respectively. In this tutorial, these quantities are calculated from their ideal gas partition functions, Q , using classical statistical mechanics:

$$S_0 = k \ln Q - \left(\frac{\partial \ln Q}{\partial k_B T} \right)_v$$

where

$$Q_{trans} = \frac{V}{h(k_B T / 2\pi m)^{3/2}}$$

$$Q_{rot} = \left(\frac{\pi^{1/2}}{\sigma} \right) \left(\frac{1}{hck_B T} \right)^{3/2} \left(\frac{1}{I_A I_B I_C} \right)^{1/2}$$

and V is the volume, h is Planck's constant, k_B is the Boltzmann constant, T is temperature, m is the molecular mass, σ is the symmetry number, c is the speed of light, and I_x are the three inertial rotational constants. These contributions are hard-wired into the program.

Complex formation results in a loss of translational and rotational degrees of freedom. As such, $\Delta S_{trans}(A + B \rightarrow AB)$ and $\Delta S_{rot}(A + B \rightarrow AB)$ are both negative and $-T\Delta S$ terms are positive. Therefore, these terms contribute a net positive contribution to the binding affinity.

S_{vib} is the entropic contribution from vibrational motion. There are two approaches to obtain this contribution: normal mode analysis (NMA) or using a quasiharmonic (QH) approach. This tutorial uses only the NMA technique to obtain S_{vib} .

In NMA, one first minimizes X , then performs a normal mode calculation on the minimized structure. The normal mode frequencies ν_i obtained from this calculation are then used to obtain S_{vib} :

$$S_{vib} = \sum_{i=1}^{3N-6} \left[\frac{h\nu_i}{k_B T (e^{h\nu_i/k_B T} - 1)} - \ln(1 - e^{-h\nu_i/k_B T}) \right]$$

S_{config} is the configurational entropy contribution from sidechain reorganization effects, which is typically ignored when calculating S_{vib} by NMA and is ignored in this tutorial.

The other approach for the calculation of entropic terms from MD trajectories involves computing the QH entropy, in which S_{vib} and S_{config} are computed together.^{16,17} This approach is not covered here as the tutorial does not cover this technique.

Solvent Contributions

For $\Delta G^{solvent}$:

$$\Delta G^{solvent} = G^{solvent}(AB) - G^{solvent}(A) - G^{solvent}(B)$$

The free energy contribution from the solvent for any of these species X may be expressed as:

$$G^{solvent}(X) = G_{es}^{solvent}(X) + G_{nes}^{solvent}(X)$$

G_{es} is the electrostatic contribution, which is obtained from a Poisson-Boltzmann (PB) or Generalized Born (GB) method.

In the PB method, the electrostatic potential produced by a molecular charge distribution is determined by solving the Poisson-Boltzmann equation (given here in its linearized form both for simplicity and because no salt effects are taken into account in this tutorial):

$$\nabla \epsilon(r) \nabla \phi(r) = -4\pi \rho_m(r) + \kappa^2 \epsilon(r) \phi(r)$$

where

$\epsilon(r)$ is the position-dependent dielectric constant,

$\phi(r)$ is the electrostatic potential,

$\rho_m(r)$ is the molecular charge distribution, and

κ is the Debye-Huckel screening parameter to take into account the electrostatic screening effects of (monovalent) salt.

Once the electrostatic potential is computed, the electrostatic contribution to the solvation free energy is given by $\sum_i q_i [\phi(r_i) - \phi(r_i)_{vac}]$, where q_i are the partial atomic charges at positions r_i

making up the molecular charge density [$\rho_m(r) = \sum_i \delta(r - r_i)$] and $\phi(r_i)_{vac}$ is the electrostatic potential computed for the same charge distribution in vacuum.

In this tutorial, the PB method implemented in AMBER 8 is used to compute the electrostatic contribution to the solvation free energy.

The Generalized Born (GB) method is an analytic approximation to the PB method for solving the electrostatic contribution to the solvation free energy:

$$\Delta G_{es}^{solvent} \approx \Delta G_{GB} = -\frac{1}{2} \sum_{ij} \frac{q_i q_j}{f_{GB}(r_{ij}, \alpha_i, \alpha_j)} \left(1 - \frac{e^{-\kappa f_{GBij}}}{\epsilon_w} \right)$$

where r_{ij} is the distance between atoms i and j , α_x are the effective Born radii of the atoms, and f_{GB} is a smooth function, of which a common form is:

$$f_{GB} = \left[r_{ij}^2 + \alpha_i \alpha_j \exp\left(\frac{-r_{ij}^2}{4\alpha_i \alpha_j}\right) \right]^{1/2}$$

There are several GB methods from which to choose in the AMBER program. Further descriptions of these different methods may be found in the AMBER manual and references therein, as well as in Appendix C. The GB method used in the Gohlke and Case Ras-Raf paper (and the AMBER 7 tutorial) is the MGB method; it is not supported in AMBER 8. The GB method used in this AMBER 8 tutorial is the modified Onufriev, Bashford and Case (OBC) method, IGB = 2.

G_{nes} is the nonelectrostatic contribution to the solvation free energy.

In this tutorial,

$$G_{nes}^{solvent}(X) = \gamma SASA(X) + b$$

where

γ is a surface tension parameter, set to 0.005 for PB and 0.0072 for GB in this tutorial,

$SASA(X)$ is the solvent-accessible surface area of molecule X , determined by LCPO or Molsurf in this tutorial, and b is a parameterized value, set to 0.0 for PB and GB in this tutorial.

Appendix B

Included in this appendix is a description of the AMBER output from a PB(GB) calculation and how it corresponds to the thermodynamic quantities outlined in the theory section and Appendix A.

The output in Examples 2 and 3 are energetic quantities and correspond as follows:

<u>AMBER output</u>	<u>Corresponds to</u>	
	<u>In Gohlke and Case,⁵</u> <u>Table 1</u>	<u>In this tutorial</u>
ELE	H_{elec}	E_{ele}
VDW	H_{vdw}	E_{VDW}
INT	H_{int}	E_{val}
GAS	H_{gas}	$E_{\text{ele}}+E_{\text{VDW}}+E_{\text{val}}$ (or ELE+VDW+INT)
PBSUR	N/A	G_{nes}
PBCAL	N/A	G_{es}
PBSOL	N/A	$G_{\text{nes}}+G_{\text{es}}$ (or PBSUR+PBCAL)
PBELE	N/A	$G_{\text{es}}+E_{\text{ele}}$ (or PBCAL+ELE)
PBTOT	N/A	$G_{\text{nes}}+G_{\text{es}}+E_{\text{ele}}+E_{\text{VDW}}+E_{\text{val}}$ (or PBSOL+GAS)
GBSUR	$G_{\text{np,MGB}}$	G_{nes}
GB	G_{MGB}	G_{es}
GBSOL	$G_{\text{solv,MGB}}$	$G_{\text{nes}}+G_{\text{es}}$ (or GBSUR+GB)
GBELE		$G_{\text{es}}+E_{\text{ele}}$ (or GB+ELE)
GBTOT	$G_{\text{gas+solv,MGB}}$	$G_{\text{nes}}+G_{\text{es}}+E_{\text{ele}}+E_{\text{VDW}}+E_{\text{val}}$ (or GBSOL+GAS)

Appendix C

Included in this appendix are descriptions of the Generalized Born (or IGB) methods implemented for use within AMBER 8.

IGB=1

The Hawkins, Cramer, Truhlar pairwise generalized Born model^{18,19} (GB^{HCT}) is used, with parameters described by Tsui and Case.²⁰

prmtop file: This model uses the default radii set up by LEaP.

Implementation:

$$\Delta G_{pol}^{GB} = -\frac{1}{2} \left(1 - \frac{e^{-\kappa f_{GB}}}{\epsilon} \right) \sum_{ij} \frac{q_i q_j}{f_{GB}}$$

q_i and q_j are partial atomic charges of atoms i and j ,

ϵ is the solvent dielectric constant,

κ is the Debye-Huckel screening parameter, which throughout this tutorial is 0, since there is no salt concentration. Thus in this tutorial, the expression inside the parentheses reduces to $\left(1 - \frac{1}{\epsilon} \right)$.

f_{GB} is defined as

$$f_{GB} = \left[r_{ij}^2 + \alpha_i \alpha_j \exp\left(\frac{-r_{ij}^2}{4\alpha_i \alpha_j} \right) \right]^{1/2}$$

r_{ij} is the distance between atoms i and j ,

α_i is the effective Born radius of atom i

$$\alpha_i^{-1} = \rho_i^{-1} - \frac{1}{2} \sum_{j \neq i} g(\mathbf{r}, \rho)$$

ρ_i is the intrinsic radius of atom i ,

$g(\mathbf{r}, \rho)$ is defined as

$$g = \frac{1}{L_{ij}} - \frac{1}{U_{ij}} + \frac{r_{ij}}{4} \left(\frac{1}{U_{ij}^2} - \frac{1}{L_{ij}^2} \right) + \frac{1}{2r_{ij}} \ln \frac{L_{ij}}{U_{ij}} + \frac{\rho_j^2}{4r_{ij}} \left(\frac{1}{L_{ij}^2} - \frac{1}{U_{ij}^2} \right)$$

$$\begin{aligned}
L_{ij} &= 1 && \text{if } r_{ij} + \rho_j \leq \rho_i, \\
L_{ij} &= \rho_i && \text{if } r_{ij} - \rho_j \leq \rho_i < r_{ij} + \rho_j, \\
L_{ij} &= r_{ij} - \rho_j && \text{if } \rho_i \leq r_{ij} - \rho_j
\end{aligned}$$

$$\begin{aligned}
U_{ij} &= 1 && \text{if } r_{ij} + \rho_j \leq \rho_i, \\
U_{ij} &= r_{ij} - \rho_j && \text{if } \rho_i < r_{ij} + \rho_j
\end{aligned}$$

and ρ_i is related to the atomic radius R_i by

$$\rho_i = S_i(R_i + b_{\text{offset}})$$

where the offset is used to adjust the magnitudes of the solvation energies to those from PB calculations.

IGB = 2

A modified GB model developed by A. Onufriev, D. Bashford and D.A. Case²¹ (GB^{OBC}) is used. This method was still under development in its AMBER 7 implementation, but published for the AMBER 8 implementation.

prmtop file: This model requires the LEaP command “set default PBradii mbondi2” or “set default PBradii bondi” when setting up the prmtop file.

Implementation:

$\Delta G_{\text{pol}}^{\text{GB}}$ is defined as in IGB = 1,

f_{GB} is defined as in IGB = 1,

the effective Born radii, α_i (R_i in the paper) are defined as

$$\alpha_i^{-1} = \tilde{\rho}_i^{-1} - \rho_i^{-1} \tanh(\alpha\Psi - \beta\Psi^2 + \gamma\Psi^3)$$

$\tilde{\rho}_i^{-1}$ is defined as $\rho_i - 0.09 \text{ \AA}$

Ψ is defined as $I\tilde{\rho}_i$

I is defined as

$$I = \frac{1}{4\pi} \int_{\text{VDW}} \theta(|\mathbf{r}| - \tilde{\rho}_i) \frac{1}{r^4} d^3\mathbf{r}$$

and α , β and γ are adjustable, dimensionless parameters optimized to the following for this IGB model: $\alpha = 0.8$, $\beta = 0$ and $\gamma = 2.91$.

IGB = 3

Not an option in AMBER 8.

IGB = 4

Not an option in AMBER 8.

IGB = 5

Same as IGB = 2, with α , β and γ parameters optimized to: $\alpha = 1.0$, $\beta = 0.8$ and $\gamma = 4.85$. IGB = 5 was shown to agree better with the Poisson-Boltzmann treatment in the calculation of the electrostatic component of the solvation free energy.²²

Observations/Recommendations:

Gohlke and Case⁵ found that IGB = 4 predicts the best binding energy compared to experiment when used in conjunction with the normal mode analysis to obtain entropic terms for their protein-protein system, Ras-Raf.

In our studies of the protein-RNA system, U1A-RNA, we have observed that IGB = 2 seems to yield the most reasonable overall binding energies in conjunction with normal mode analysis. The correct trends for the binding energies of mutants is observed by all of the GB methods, however IGB = 2 performs best, even compared to PB. However, in our studies of DNA in a GB solvent, IGB = 5 performed best, giving results most similar to an all-atom simulation.