# A Continuum Electrostatic Analysis of Protein Binding: Barnase–Barstar Complex Formation

Lillian T. Chong and Zachary S. Hendsch*
ltchong@mit.edu, hendsch@mit.edu

MIT Department of Chemistry

Understanding the nature of protein–protein recognition is fundamental to the study of biological processes. The complex between barnase, a bacterial RNase, with its intracellular inhibitor, barstar, is a suitable system for the general problem of molecular recognition since the structures of the proteins have been solved, both separately and in the protein complex. This interaction between barnase and barstar is extremely tight with a $K_d$ of $10^{-14}M$ (Schrieber & Fersht, 1993) and is predominantly electrostatic. We are studying the role of electrostatics in the stability and specificity of the barnase–barstar complex using a continuum method which utilizes iterative finite-difference methods offered in the DELPHI (Gilson et al., 1988) software package. Although electrostatic analysis is much less expensive than molecular-dynamics simulation, our computations required about 10 days of CPU time. Quanta, a high-performance interactive color graphics system, has enabled us to visualize the complex in three-dimensions on an SGI workstation by using coordinates extracted from the X-ray crystal structure of the complex.

**Background:** A considerable amount of research has been done on the barnase–barstar complex. The X-ray crystal structures of barnase–barstar (Buckle et al., 1994) and of barnase alone (Mauguen et al., 1982; Bycroft et al., 1991) have been solved to high resolution. The structure of barstar in solution has been solved by NMR (Lubienski et al., 1994). Several mutagenesis studies of barnase–barstar have been conducted (Hartley, 1993; Schrieber & Fersht, 1993; Schrieber et al., 1994). Accurate free energy values from kinetic and thermodynamic measurements are available in a recent single- and double-mutant cycle study of barnase–barstar (Schrieber & Fersht, 1995). The double-mutant cycle study is especially intriguing because, in principle, it isolates the electrostatic interactions between a pair of residues from interactions with the rest of the protein. This study has revealed several important interactions that had not been noted previously from examination of the barnase–barstar crystal structure.

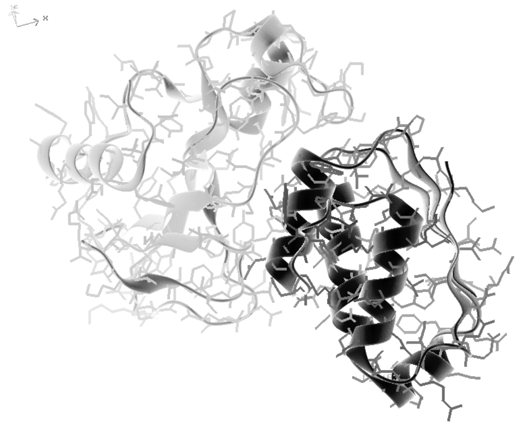

Figure 1: The barnase–barstar complex. The barnase structure is lightly shaded on the left and the barstar structure is heavily shaded on the right.

**Motivation:** Barnase–barstar is an attractive structure for studying the role of electrostatics in the stability and specificity of complex formation. Barstar complements barnase in surface charge, sterically blocking the electropositive base of the barnase active site with its electronegative exposed face in addition to providing 14 intermolecular hydrogen bonds (Schreiber & Fersht, 1995). The presence of extensive electrostatic interactions along the barnase–barstar interface makes the complex ideal for analysis using continuum electrostatic methods.

**Procedure:** We are using a continuum electrostatic method developed by Hendsch & Tidor (1994), which involves using the DELPHI software package on an SGI Indigo 2 Extreme Workstation. Within DELPHI, the coordinates extracted from the X-ray crystal structure of the barnase–barstar complex are transferred onto a 65 x 65 x 65 point lattice. The proteins of the complex are regarded as a set of point charges and hard spheres. The contact surface between the radii of the protein atoms and the radius (1.4 Å) of a water probe molecule defines the

*This work is advised by Prof. Bruce Tidor and supported in part by NIH grant GM-47678 and a Science Partnership from MIT. Address: Room 6-135, 77 Massachusetts Avenue, Cambridge MA 02139.

molecular surface of the proteins. The volume enclosed by this molecular surface is regarded as a low dielectric medium. The solvent water is regarded as a continuum of high dielectric material with a defined ionic strength. The DELPHI software package employs iterative finite-difference methods to solve the Poisson-Boltzmann equation (or, as in our case, its linearized form). The electrostatic free energy contribution of every residue in the complex is thus obtained. That is, the total electrostatic binding free energy is analyzed in terms of a desolvation contribution for every functional group in the complex and an interaction energy between all pairs of functional groups. In this manner, the primary determinants of stability can be analyzed. The number of interactions computed is $O(n^2)$, where $n$ is the number of functional groups. Our barnase–barstar computation has 582 functional groups.

**Progress:** We have completed the continuum electrostatics calculation on the barnase–barstar complex. The overall contribution of electrostatics to the stability of the complex is destabilizing by roughly 14 kcal/mol, suggesting that the hydrophobic effect provides the driving force for association. In light of the theoretical study of salt bridges conducted by Hendsch & Tidor (1994), this destabilization is expected for protein complexes such as barnase–barstar in which there are many buried electrostatic interactions. It is due to incomplete compensation of the large desolvation penalty by favorably electrostatic interactions in the complex. We are presently analyzing the results of our continuum electrostatic calculations on barnase–barstar. Our results complement the double-mutant cycle study of Schreiber & Fersht (1995) in that both methods probe the strength of pairwise interactions in the bound state; our results add new information by also estimating the desolvation penalty for each group. The double-mutant cycle study conducted by Schreiber & Fersht (1995) report the most important interactions in barnase–barstar to be between Asp39b* of barstar and the barnase side chains (Lys27bn, His102bn, Arg83bn, and Arg87bn) that surround it.[1] These interactions (over 6 kcal/mol) are among the strongest interactions yet reported for any protein complex (Schreiber & Fersht, 1995). Yet, including its desolvation penalty, Asp39b* makes little if any net contribution to the overall stability of the complex. Instead, our results indicate Glu76b* as the most stabilizing residue in the complex due to a moderate solvation penalty that is outweighed by favorably electrostatic interactions.

[1] The suffix b* indicates that a residue is in barstar, and bn indicates that it is in barnase.

**Summary:** Barnase is a rich system for exploring the determinants of stability and specificity of complex formation. Thorough experimental examinations of barnase complexes have already been conducted, including studies involving the extremely tight barnase–barstar complex. Electrostatics play a critical, complimentary role in the complex formation of barnase and barstar. A continuum electrostatic analysis of barnase–barstar has revealed that electrostatics destabilize the complex. Further examination of the results from this analysis should provide valuable insight into the role of electrostatics in protein binding. This study may also reveal a rationale that could be used in the design of more effective ligands for drugs.

## References


Buckle, A. M., Schreiber, G. & Fersht, A. R. (1994). Protein–protein recognition: Crystal structural analysis of a barnase–barstar complex at 2.0-Å resolution. *Biochemistry*, 33, 8878–8889.

Gilson, M. K., Sharp K. A. & Honig B. H. (1988). Calculating the electrostatic potential of molecules in solution: Method and error assesment. *J. Comput. Chem.*, 9, 327–335.

Hartley, R. W. (1993). Directed mutagenesis and barnase–barstar recognition. *Biochemistry*, 32, 5978–5984.

Hendsch, Z. S. & Tidor, B. (1994). Do salt bridges stabilize proteins? A continuum electrostatic analysis. *Protein Science*, 3, 211–226.

Lubienski, M. J., Bycroft, M., Freund, S. M. V. & Fersht, A. R. (1994). $^{13}$C assignments and three-dimensional solution structure of barstar using nuclear magnetic resonance spectroscopy. *Biochemistry*, 33, 8877–8877.

Mauguen, Y., Hartley, R. W., Dodson, E. J., Dodson, G. G., Bricogne, G., Chothia, C. & Jack, A. (1982). Molecular structure of a new family of ribonucleases. *Nature (London)*, 29, 162–164.

Schreiber, G. & Fersht, A. R. (1995). Energetics of protein–protein interactions: Analysis of the barnase–barstar interface by single mutations and double mutant cycles. *J. Mol. Biol.*, 248, 476–486.

Schreiber, G., Buckle, A. M. & Fersht, A. R. (1994). Stability versus function: Two competing forces in the evolution of barstar. *Structure*, 2, 945–951.

Schreiber, G. & Fersht, A. R. (1993). The interaction of barnase with its polypeptide inhibitor barstar studied by protein enginnering. *Biochemistry*, 32, 5145–5150.