

Chapter 10

General Conclusions

This thesis embodies a rigorous analytical and computational treatment of important biophysical problems using methods from physics and mathematics. All biological and chemical systems obey the laws of physics; however, the complexity of these systems makes their mathematical description and computational modeling extremely difficult, especially in situations of practical interest. Application of physical principles to the modeling of biophysical systems is usually only possible through intelligent approximations which reduce the systems' complexity with acceptable losses in accuracy. One such tool, the continuum electrostatic approximation as expressed through the linearized Poisson–Boltzmann equation, has been shown to be generally applicable and accurate in calculation of electrostatic free energies, especially differences in electrostatic free energies, and is the basis of all work in this thesis.

A particularly nice feature of the continuum electrostatic approximation, and any linear-response electrostatic model in general, is that the electrostatic portion of a molecule's free energy is also a quadratic function of the molecule's electrostatic charge distribution. This feature implies that the electrostatic component of the free energy of association of two rigid molecules is a quadratic function of both molecule's charge distributions. Using properties of the continuum electrostatic model, we have shown that this quadratic electrostatic binding free energy can be minimized, yielding optimal electrostatic charge distributions for one of the binding partners with respect to the other. We have been able to obtain these optimal charge distributions analytically in spherical, spheroidal and planar geometries with ionless solvent, as well as in spherical geometries where the solvent has mobile ions. We have also been able to obtain these charge distributions numerically in arbitrarily shaped molecular geometries with and without mobile ions in the solvent. We have seen the electrostatic portion of the optimized binding free energy to be favorable (negative) in almost every case, contrary to the observation that the electrostatic contribution is unfavorable for most natural molecular complexes. In fact, we have been able to prove that this must be the case in many situations of physical interest. There is generally a large gap between the electrostatic binding free energy of a natural complex and the electrostatic binding free energy that it could have if optimal. Even though it may not be possible to find a physical perturbation of an existing binding partner that would

yield optimal binding, there seems to be a significant range for improvement in that direction, often tens of kcal/mol.

Application of electrostatic charge optimization methods of the transition state analog of *B. subtilis* chorismate mutase has explicitly shown that optimal charge distributions can be used as templates, indicating regions where natural molecules are non-optimal, as well as which of these regions are energetically more important. Using the electrostatic charge optima for this system, we have been able to predict several different physical variations of the original analog which could be better enzyme inhibitors by as much as 3.8 kcal/mol (6.4 kT at 25°C). This is the first time that charge optimization has led to the prediction of novel compounds for improved functionality.

As an extension to the optimization of binding free energy, we have developed a general formalism for specificity optimization — designing molecules to bind tightly to one or more particular target molecules while explicitly avoiding binding to one or more particular undesirable (decoy) molecules. We have even been able to formulate a theory of general specificity — the avoidance of all decoy molecules similar to the target. We have shown through simple example how these specificity optimization theories may be successfully applied to molecular design. Application to the chorismate mutase family of enzymes has indicated that natural enzymes seem to be much more evolved for general specificity than affinity for their transition states. This may be important as catalytic efficiency often depends not only on stabilizing the transition state, but also on not being product inhibited, not stabilizing the substrate and not being inhibited by other molecules present in the system.¹³⁶ Comparison of the natural enzymes from *B. subtilis* and *E. coli* to the catalytic antibody 1F7 indicates that the antibody is much less specificity optimized, and perhaps more affinity optimized. This could be one factor resulting in the general deficiency of catalytic antibodies compared to their natural counterparts.

A final application of the general specificity optimization and affinity optimization theories involves the development of improved haptens for catalytic antibody design. Under the assumption that catalytic antibodies are affinity optimized for their haptens, we provide a formalism allowing one to design haptens which induce the creation of generally specific antibodies. I.e., a good antibody for one of these haptens would have been indirectly general specificity optimized for the desired transition state. Such antibodies may be more catalytically efficient than those created using standard transition state analogs as haptens.

As a whole, this thesis lays down a firm and general theoretical framework for electrostatic affinity and specificity optimization in the context of molecular association. Examples provided herein, both simple toy problems and application to important biomolecular systems, develop the necessary computational tools for implementation of these optimization procedures and demonstrate the real world applicability and immediate usefulness of the concepts involved. It is believed that the work presented herein shall provide a basis for much productive research and development in fields such as pharmaceutical design, molecular recognition, enzymology, and protein folding.