

Chapter 1

General Introduction

Many fields of molecular science consider a detailed understanding of the fundamental mechanisms by which molecules interact with each other to be essential if progress is to be made. In pharmaceutical or molecular sensor design, for example, a primary goal is the development of molecules that bind tightly and specifically to certain target molecules. In protein folding, one seeks to understand an intramolecular free energy landscape in order to predict low-energy folded structures. In enzyme design, one approach is to create molecules which bind tightly to a transition state structure, while avoiding overly tight binding to the substrate or product molecules. These examples are all founded upon the same conceptual framework — application of knowledge of the forces and energetics of atomic and molecular interactions *in vivo*.

Much of the interest of molecular science is focused on the association of molecules or molecular fragments. Along with this binding process comes a binding free energy — the change in the total free energy of the solution due to the binding event. Together with the concentrations of the associating molecules, this quantity determines the equilibrium fraction of bound molecular complexes. In many situations of interest, the overall binding free energy can be separated into independent electrostatic and non-electrostatic contributions;^{1,2} the electrostatic contribution arising solely from the electrostatic charges of the reactants. The apparent driving force in natural molecular association reactions is the non-electrostatic contribution, which includes the so-called hydrophobic effect. One explanation for the favorability of the hydrophobic effect to binding is that water molecules can make much more favorable interactions with each other than with most solute molecules. Therefore, because association reactions reduce the net solvent–solute surface area, fewer solvent molecules are required to surround the solutes and thus the free energy of the solution goes down, resulting in a propensity for binding. However, by the same standard, the hydrophobic effect provides no mechanism for specificity – i.e., selectively binding to one molecule but not another similarly shaped molecule. Some specificity can be attained by making the surfaces of the molecules shape-complementary, so that they fit together well, making good van der Waals interactions. Most recent calculations indicate that electrostatic interactions actually disfavor binding^{3–9} — the electrostatic contribution to the binding free energy is almost always positive, making binding harder than it

would be if the molecules did not have charge. In natural complexes, electrostatics is commonly used to create specificity^{9–13} — similarly shaped reactants can have different distributions of electrostatic charge resulting in distinctly different binding free energies. A theoretical discussion of the binding free energy and a derivation of the electrostatic contribution is given in Chapter 2.

The electrostatic contribution to binding is particularly non-intuitive because the relevant binding often occurs in aqueous solvent, in which the favorable electrostatic interactions of the unbound reactants with water are sacrificed for (usually less) favorable interactions between the reactants in the bound state. One question that has arisen in the study of molecular binding is whether the electrostatic contribution needs to be unfavorable — is there some underlying physical principle which dictates that electrostatics cannot help to lower the binding free energy *in vivo*, or is nature simply not taking full advantage of these interactions? If there are no physical restrictions, is it possible to predict feasible chemical modifications to existing molecules that may enhance binding electrostatically? Is there a “best” electrostatic contribution to binding for a given pair of molecules, and if so, can it be calculated?

The work of Lee-Peng Lee began to answer these questions. She showed that in an approximation where the molecules in question are spherical in shape (one reactant having a spherical hole for the other to fit into) there is an optimal electrostatic binding free energy which can be analytically calculated.¹⁴ Later, Lillian T. Chong and others showed that by approximating the molecule barnase as a sphere with a spherical binding cavity, a small set of charges of physical magnitudes separated by physical bond lengths could be found inside the spherical ligand which would yield a favorable electrostatic contribution to the binding free energy, where the natural ligand, barstar, would not.¹⁵ It seemed that the electrostatic charge optimization procedure might be useful in chemical and biological situations.

Chapters 3–6 generalize the work of Lee-Peng Lee, providing a theoretical foundation for charge optimization in molecules of any shape, and an analytic solution for slabs (planar, membrane-like molecules), spheroids, and spheres in ionic solution. There it is shown that the optimized electrostatic binding free energy contribution will be favorable (negative) in many situations of physical interest. This result is particularly important because this contribution in natural complexes is usually positive and therefore there is usually considerable room for improvement. It is also shown that the optimal electrostatic charge distributions exist and are not unique — there are many different possible molecular charge distributions yielding the best possible electrostatic binding free energy contribution for a given molecular association reaction. This is important as it may facilitate the design of real molecules with these properties. Furthermore, these optimal charges depend little on the ionic concentration of solvent. Chapter 7 applies these theories to the optimization of a physical molecule, the transition state analog of *Bacillus subtilis* chorismate mutase. Optimization of the analog’s partial charges shows that while it is already well optimized by evolution for binding to the enzyme, certain simple modifications may yield improved inhibitors. Affinity optimization theory has also been applied by Sara Dempster in the study of HIV protease inhibition.¹⁶ She has shown

that the protonation state of the target molecule matters little in the charge optimization process — the ligand can induce the targeted protonation state, although different targeted protonation states may yield different optimal binding free energies. Lee-Peng Lee has also applied charge optimization methods to the optimization of barstar, the natural inhibitor of the enzyme barnase.¹⁷ It was found that the natural complex was well optimized by nature and that optimization could find no mutations to other natural amino acid residues that would improve binding.

The success of these forays into the application of electrostatic charge optimization have led to even more questions than they have answered. Because electrostatics is used by nature for specificity, perhaps charge optimization methods can be generalized to provide improved specificity as well as affinity. Perhaps there is a specificity optimum as well as an affinity optimum? Can one obtain specificity without knowledge of the undesirable binding partners (decoys)? Many of these questions are addressed in Chapter 8, where a general formalism for affinity and specificity optimization is developed. Therein cases of one or more known decoys, unknown decoys, and one or more target molecules are treated. Simple examples show the potential applicability of this theory. In Chapter 9, the theory for general specificity, where specificity is optimized against many types of unknown decoys, is applied to study the mechanism chosen by evolution for the creation of the chorismate mutase enzymes in *B. subtilis* and *E. coli*, as well as the catalytic antibody 1F7. It is found that natural enzymes seem to be optimized more for specificity to the transition state than the catalytic antibody is; this may have some bearing on why catalytic antibodies rarely perform as well as their natural counterparts. We have also developed a methodology for the design of improved haptens which may yield more efficient catalytic antibodies through increased specificity for their transition states. Chapter 10 presents the general conclusions of this thesis.