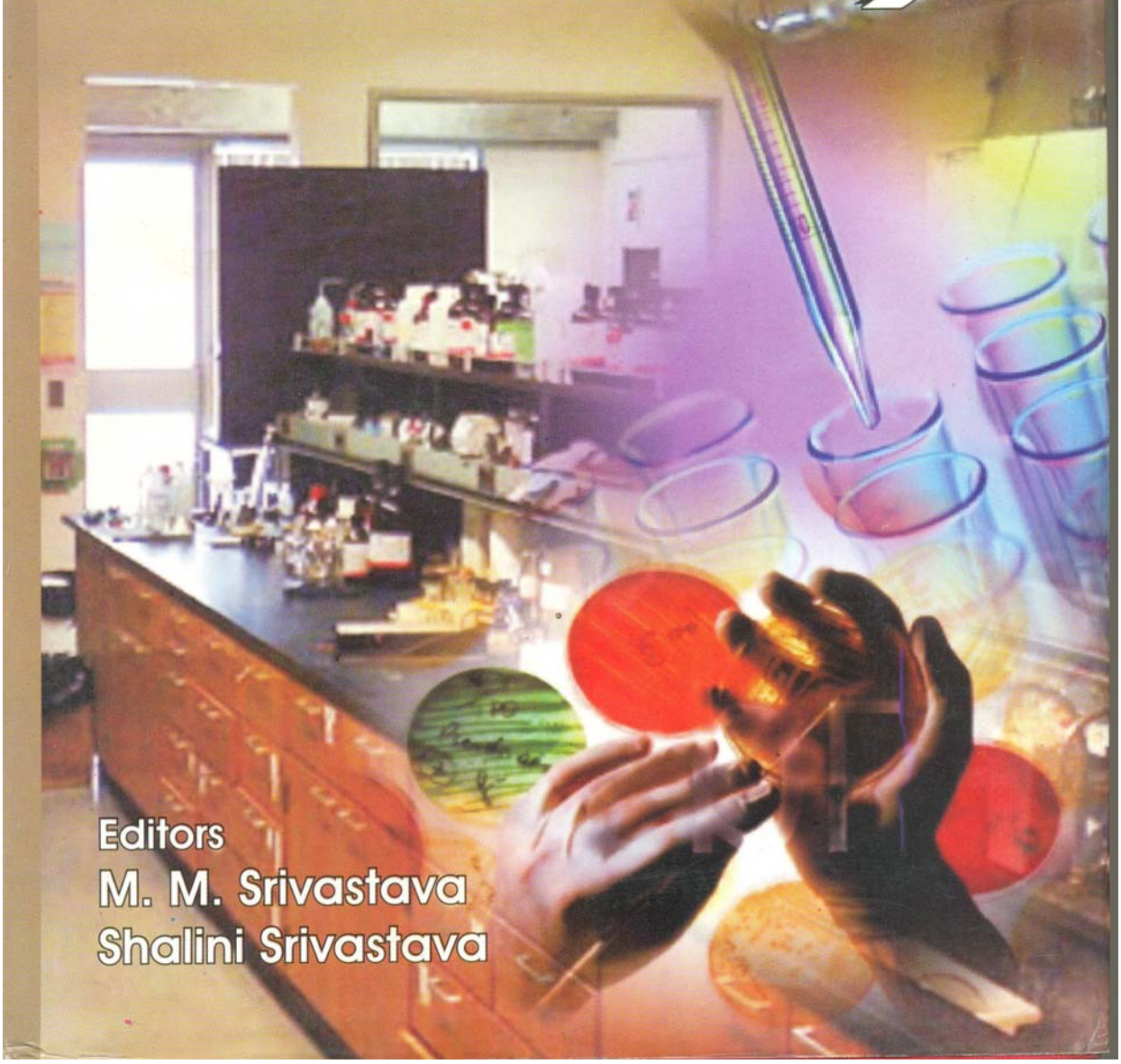
# Recent Trends in Chemistry



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# Computer Aided Drug Designing: Trends in Thermodynamics and Kinetics of Binding Molecular Simulations for De Novo Drug Design

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Introduction A basic premise of current day structure based drug design endeavours is that the interactions between the drug molecule and the target receptor are optimal. Initial drug discovery processes were based solely on synthetic approaches but over the years many computer based techniques coined as computer aided drug design (CADD), computer aided molecular design (CAMD) have been used to discover new compounds with specific and desired properties. Typically, the structure of the active site of the receptor or a pharmacophore model is built, molecular fragments are picked up from the databases and fitted into the active site. If a high scoring function suitably defined, is achieved the fragment is accepted and built further. These approaches more often than not involve system specific protocols and some times unavoidable but beneficial subjectivity. The obvious advantage is the rapidity with which lead compounds could be generated for testing. Any theoretical connection to the observed kinetic and thermodynamic data on binding however, is tenuous. While such methods aid in expediting the drug design process

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tremendously, there can be no two opinions on the need for the development of a universal methodology that is system independent and based on the laws of physics. Molecular simulations provide the necessary platform to construct theoretically rigorous methodologies to strive for this goal.

According to Beveridge & DiCapua [1989], molecular simulations can be defined as "the numerical determination of energetics (thermodynamics), structural and dynamic properties of a mathematical model of a molecular assembly on a computer". Molecular simulations with a complete atomic level description of the macromolecular systems but with the electronic coordinates averaged out (i.e. representing the systems at the Born-Oppenheimer level), considering the surrounding solvent and salt conditions are feasible in principle and form the current state of the art [Jayaram, 1998a], but these are in the domain of extensive supercomputing. With the increasing popularity of the adaptations of simulation algorithms to massively parallel environments (>100 processors), many of the chemical and biochemical problems are becoming computationally tractable. This has far reaching ramifications in the area of drug design. The challenge is to configure simulations to elicit verifiable properties of interest in realistic time frames and thus aid in drug design.

The search for a comprehensive solution to drug design involves three key questions: (a) Where does a drug bind? (b) How fast does it bind? (c) How well does it bind? These issues in conventional physical chemistry when reframed in the context of molecular simulations provide objective leads to the path for *de novo* drug design (Figure 1). The first question is addressed by docking studies which locate the binding site on the receptor and place the ligand there. Most docking algorithms use system specific biases in some form. Brownian dynamics simulations serving as an unbiased search technique for docking are ideally suited for letting the ligand (candidate drug molecule) diffuse to the active site based on the laws of physics. As a microscopic alternative to analytical theories based on simple models [Berg & von Hippel, 1985], Brownian dynamics simulations are proving essential to understand the influence of various steering and orientational effects on the kinetics of macromolecular association in complex systems where a variety of interactions are occurring simultaneously [McCammon & Harvey, 1987; Sharp et al., 1987; Brune & Kim, 1994; Madura et al., 1994, 1995; Jayaram et al., 1996; Northrup, 1996; Das & Jayaram, 1998; Wade et al., 1998]. Theory for extracting the rate constant from Brownian dynamics simulations for diffusion-controlled association, which is often the rate limiting step, is also in place thus addressing the second question. Some novel methodologies for *a priori* binding free energy estimates based on analyses of

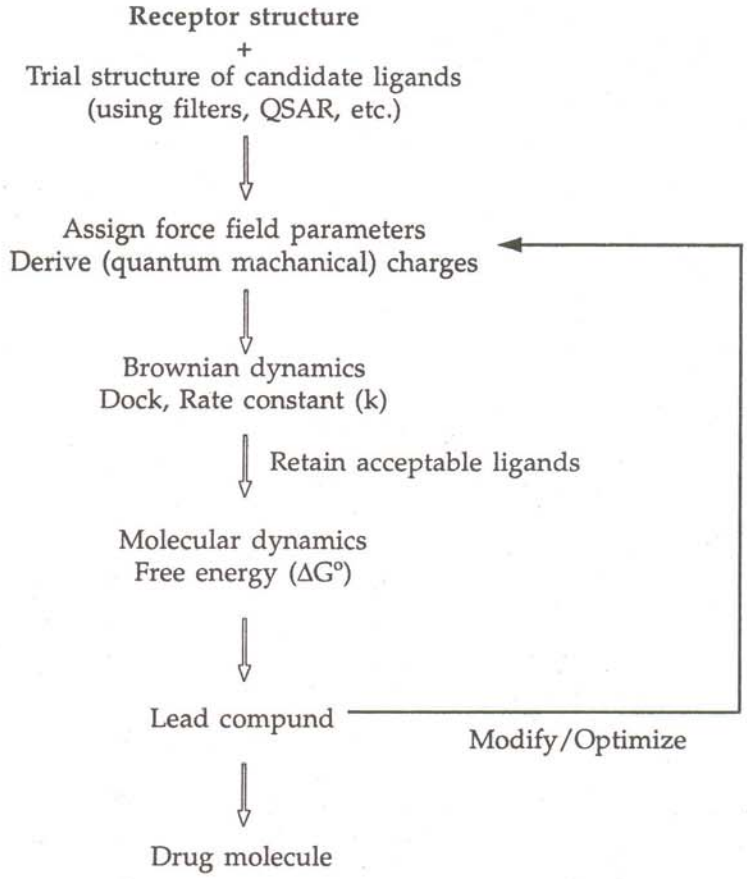


*Figure 1. A molecular simulation based protocol for de novo drug design.*

molecular dynamics trajectories of bound and unbound species have been introduced in the last three years providing indicators to how well a drug binds to the receptor. We present here a brief overview of the theory and practice of molecular simulations to determine the thermodynamic and kinetic parameters of binding and illustrate the methodologies with a DNA-drug and a protein-drug complex.

## Theory and Methodology

To describe the interactions between the drug and its receptor, a force field is required which given the positions of the atoms of the molecules, quantifies the forces on each atom as well as the interaction energy. Several accurate force fields are now available [Cornell et al., 1995; MacKerell et al., 1998; van Gunsteren et al., 1997; Maple et al., 1994] for representing proteins and nucleic acids, but force field

compatible charges have to be derived for the drug/ligand moiety. This can be done by ab initio quantum calculations [Bayly et al., 1993; Kalra et al., 2001a].

Brownian dynamics describes the diffusive motion of solute molecules in a continuum solvent by combining the stochastic Brownian motion with direct forces between the molecules and hydrodynamical interactions. The motion is described by the Langevin equations, which for a system of N Brownian particles are

$$m_i \ddot{r}_i \; -\sum_j \zeta_{ij} \dot{r}_i + F_i + \sum_j \alpha_{ij} f_j \tag{1}$$

where mi is the mass of the $i$ th particle, $r_i$ its position, $\dot{r}_i$ its velocity and its $\zeta_{ij}$ acceleration, is the configuration dependent friction tensor, and $F_i$ the force on the particle due to the other particles (j) and any external force. $\alpha_{ij} f_i$ is a randomly fluctuating force on the particle due to the solvent, where $f_j$ obeys a Gaussian distribution. Trajectories are developed by displacements over successive time intervals Δt, obtained by integrating the Langevin equations of motion [Ermak & McCammon, 1978].

$$r(t+\Delta t) = r(t) + \frac{1}{kT}\sum_{i,j} D_{ij}(t) F_{ij}(t)\Delta t + R(D_{ij}, \Delta t) \tag{2}$$

$D_{ij}$ is the configuration dependent diffusion tensor that can be modeled through the modified Oseen Tensor [Garcia de la Torre & Bloomfield, 1981]:

$$D_{ij} = \frac{kT}{8\pi\eta r_{ij}}\left[\hat{\mathbf{I}} + \frac{\mathbf{r}_{ij}\mathbf{r}_{ij}}{r_{ij}^2} + \frac{\sigma_i^2 + \sigma_j^2}{r_{ij}^2}\left(\frac{\hat{\mathbf{I}}}{3} - \frac{\mathbf{r}_{ij}\mathbf{r}_{ij}}{r_{ij}^2}\right)\right] \tag{3}$$

where I is the unit tensor, η the coefficient of viscosity, σ the hydrodynamic radius and $kT$ the thermal energy. In the case of no hydrodynamic interactions, the tensor is replaced by a diffusion constant representing the motion of the particle

$$D = \frac{kT}{6\,\pi\eta\sigma} \tag{4}$$

R is a random displacement that obeys a Gaussian distribution that can be calculated by generating normal random deviates

$$\left[\ \{x_i\} : < x_i = 0 >, < x_i x_j = 2D_{ij}(t)\Delta t >\ \right] \tag{5}$$

Similar displacement equations can be set up to describe the rotational diffusive motion of the molecules. The diffusion controlled bimolecular rate constant of

association $k$ can be estimated from the encounter probabilities [Northrup et al. 1984]. If β is the probability that a ligand initially at $b$ reaches the target rather than escapes beyond a truncation radius $q$ then $k$ is given by

$$k = \frac{k_D(b)\beta}{1-(1-\beta)\frac{k_D(b)}{k_D(q)}} \tag{6}$$

$$k_D(b) = \left[\int_b^\infty \frac{e^{V(r)/kT}}{4\pi r^2 D(r)} dr\right]^{-1} \tag{7}$$

A number of theoretical methods have been proposed to describe the thermodynamics of drug-receptor binding, ranging from the free energy perturbation [Straatsma et al., 1992; Reddy et al., 1991; Ferguson et al., 1991; Cieplak & Kollman, 1993; Rao & Murcko, 1996] and thermodynamic integration [Warde & McCammon, 1992; Ota et al., 1999] methods at one end to the analysis of energy minimized structures at the other [Holloway et al., 1995; Kalra et al., 2000]. Even though the free energy perturbation and thermodynamic integration yield rigorous and accurate free energy differences on model potential surfaces of interest, they are extremely compute intensive and sufficient statistical sampling is required [Kollman, 1993, Beveridge & Dicapua, 1989]. At the other extreme is the simplest method that calculates only the enzyme inhibitor interaction at the minimum energy configuration and does not include the solvent molecules or Boltzmann averaging. This method is very fast, but could be oversimplified if the desolvation energy and entropic contributions are important. The Molecular Mechanics-Generalized Born Solvent Accessiblity, MMGBSA [Still et al., 1990; Hawkins et al., 1995, 1996; Jayaram et al., 1998b, 1998c; Reddy et al., 1998] and the Molecular Mechanics-Poisson Boltzmann Surface Area, MMPBSA [Wang et al., 2001; Srinivasan et al., 1998] methodologies which are based on the master equation approach proposed by Ajay and Murcko [Murcko, 1995] offer a middle ground between the two extremes and allow for the development of computationally rapid methods.

These methods, based on a divide and conquer philosophy, constitute the current state of the art in terms of theoretical rigor for developing reliable estimates of binding free energies. A phenomenological enumeration of the energy components [Jayaram et al., 1998d, 1999, 2001] contributing to binding, each of which is individually amenable to computation is undertaken, with the implicit assumption of additivity [Dill, 1997]. The net binding process is taken through a thermodynamic cycle (Figure 2) comprising six steps. Step I is the process of converting the uncomplexed protein denoted "P", to the form "P*" in which the protein has adapted its structure to that of the drug bound form. The free energy of this step is

$$\Delta G^0_I = \Delta G_I^{adpt,P} \tag{8}$$

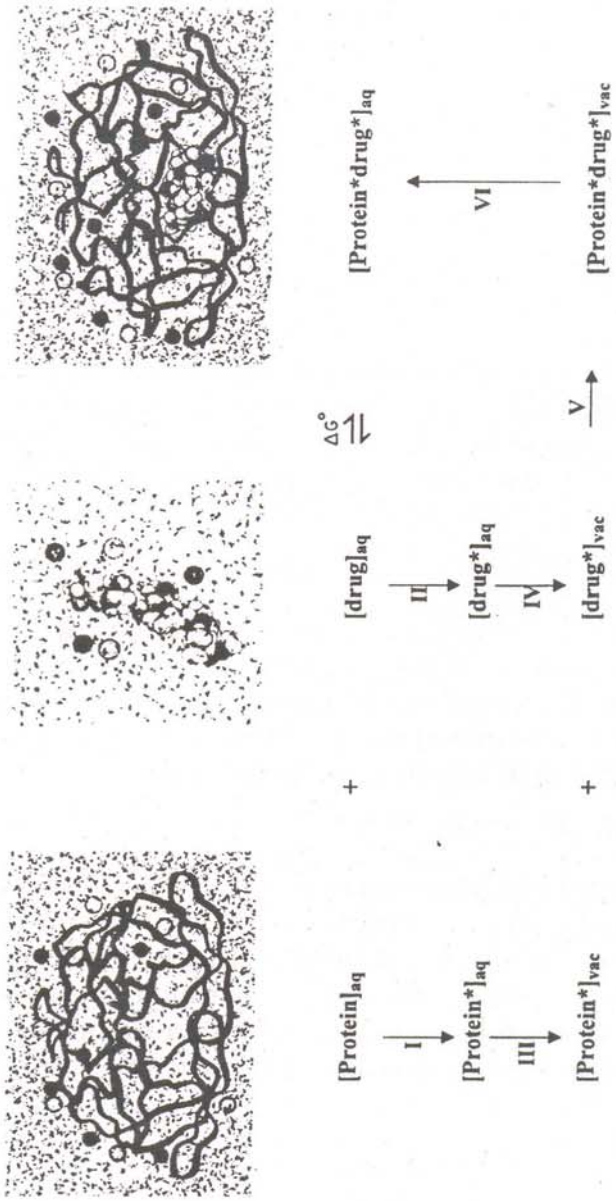


*Figure 2. The thermodynamic cycle used to determine the free energy components in protein-drug binding.*

Step II is the corresponding adaptation required of the drug molecule, converting the uncomplexed form "d" to the complexed form "d*" in solution. The free energy is given as

$$\Delta G^0_{II} = \Delta G_{II}^{adpt,d} \tag{9}$$

The next two steps (III and IV) involve desolvation of P* and d* from aqueous medium to vacuum. The free energy of each of these steps is written as a sum of four components.

$$\Delta G^0_{III} = \Delta G_3^{el,P} + \Delta G_4^{vdw,P} + \Delta G_5^{cav,P} + \Delta G_6^{DH,P} \tag{10}$$

$$\Delta G^0_{IV} = \Delta G_7^{el,d} + \Delta G_8^{vdw,d} + \Delta G_9^{cav,d} + \Delta G_{10}^{DH,d} \tag{11}$$

with contributions from electrostatic effects of desolvating the macromolecule, the van der Waals interactions with the solvent, elimination of the solvent cavity in which the molecule is accomodated and the change in added salt effects. In step V, the protein and the drug associate as a non-covalently bound complex. The thermodynamics of this step can be described as

$$\Delta G^0_V = \Delta H_{11}^{el,C} + \Delta H_{12}^{vdw,C} - T\Delta S_{13}^{tr+rot} - T\Delta S_{14}^{vib+conf} \tag{12}$$

Complexation involves introducing the electrostatic and van der Waals interactions between the protein and the drug in vacuo. A change in external entropy due to loss of translational and rotational degrees of freedom also enters this step. In step VI, the complex is transferred from vacuum back to aqueous solution and the free energy change is due to solvation of the complex.

$$\Delta G^0_{VI} = \Delta G_{15}^{el,C} + \Delta G_{16}^{vdw,C} + \Delta G_{17}^{cav,C} + \Delta G_{18}^{DH,C} \tag{13}$$

Here again an electrostatic component, a van der Waals component, a cavity formation component and added salt effects are involved. In summary, the binding process in solution consists of six well-defined thermodynamic steps each of which can be decomposed into physically meaningful thermodynamic components. The theoretical estimates of values for the various contributions proceed as follows. The standard free energy of a given macromolecular structure (chemical potential) in solution $G^0$as

$$G^0 = G^0_{int} + g^0_{solv} \tag{14}$$

where $G^0$ is the free energy intrinsic to the molecule or complex and $g^0_{solv}$ is the standard free energy of solvation.

$$G^0_{int} = H^0_{int} - T\,S^0_{int} \tag{15}$$

$$H^0_{int} = \langle E^0_{int} \rangle + PV \tag{16}$$

$< E^0_{int} >$ is a Boltzmann average of $E^0_{int}$ and PV is the pressure-volume work.

$$S^0_{int} = S^0_{tr+rot} + S^0_{vib+conf} \tag{17}$$

The underlying energetics intrinsic to macromolecules and complexes thereof is written in the conventional form of an empirical energy function

$$E_{int} = E_{bonds} + E_{angles} + E_{dihedrals} + E_{nb} \tag{18}$$

where $E_{bonds}$, $E_{angles}$, $E_{dihedrals}$ describe bond stretching, angle bending and dihedral displacements. The nonbonded interaction term $E_{nb}$ is written as a sum of electrostatics (el) and van der Waals (vdw) terms.

$$E_{nb} = E_{el} + E_{vdw} \tag{19}$$

The rotational and translational entropies are calculated from ideal gas statistical mechanics. These are introduced into the thermocycle at the step of complexation of the protein and the inhibitor in vacuum which obviates concerns regarding the validity of ideal gas statistical mechanics under solution conditions. The vibrational and configurational entropy can be estimated from a quasiharmonic analysis of molecular dynamics trajectories [Karplus & Kushick, 1981; Schafer et al., 2000] or via some approximate methods based on residue contacts at the receptor-ligand interface [Finkelstein & Janin, 1989].

The solvation energy is given as

$$g^0_{solv} = g^0_{el} + g^0_{salt} + g^0_{nel} \tag{20}$$

Here, the electrostatic contribution to the solvation energy, $g^0_{el}$ is estimated via the generalized Born (GB) model:

$$g^0_{el} = -166\ (1 - 1/\epsilon) \sum_{i=1}^{n} \sum_{j=1}^{n} q_i q_j / f_{m2GB} \tag{21}$$

where $f_{m2GB}$ is an effective atomic size/distance parameter derived from the Born radii $\alpha_i$ and pairwise distance $r_{ij}$. With suitable values for i, the solvation energy of a given molecule in a specified conformation can be computed. Added salt effects can be incorporated into GB theory via the Debye-Huckel theory, resulting in the expression

$$g^0_{salt} = -\ (166/\epsilon) \sum_{i=1}^{n} \sum_{j=1}^{n} q_i q_j / f_{m2GBDH} \tag{22}$$

$$f_{m2GBDH} = (K^{-1} + r_{ij})\ (f_{m2GBDH}/r_{ij}) \quad \text{for } i \neq k \tag{23}$$

$$f_{m2GBDH} = (K^{-1} + r_{vdw})\ (\alpha_i/r_{ij}) \quad \text{for } i \neq k \tag{23b}$$

where $f_{m2GBDH}$ is the effective Born radius parameter, including the Debye-Huckel modification. With this addition, the solvent model becomes a combination of GB and Debye-Huckel theory. The non-electrostatic (nel) contributions to the standard free energy are due to van der Waals interactions between the solute and solvent and the work required to alter the cavitation in water in going from initial to final conditions. The total non-electrostatic free energy is written as a function of the solvent accessible surface area (A)

$$\gamma^0_{nel} = \gamma_{nel} A \tag{24}$$

with an empirical coefficient $\gamma_{nel}$ defining the proportionality. Still and coworkers [1990] found that a value of $\gamma_{nel}$ = 7.2 cal/mol/Å$^2$ gave reasonable results for a large number of cases. The quantity nel can be considered as the sum of van der Waals and cavitation term

$$\gamma_{nel} = \gamma_{vdw} + \gamma_{cav} \tag{25}$$

with a value of 7.2 cal/mol/Å$^2$ considered as a resultant of +47cal/mol/Å$^2$ from the cavity term [Sharp et al., 1991] and -39.8 cal/mol/Å$^2$ from van der Waals interactions of the solute and the solvent. An important aspect in a conventional free energy analysis study is the net contribution of electrostatics, shape complementarity, hydrophobic effects, structural adaptation etc. to the binding. The answers to these types of questions can be obtained from a combination of the values associated with the primary terms mentioned above.

## Illustrative Cases

### *(a) Kinetics of DAPI-DNA Complexation*

Diffusion of DAPI (4′,6-diamidino-2-phenylindole) to its DNA binding site d(AATT) is simulated by Brownian dynamics. The simulation was performed using the AMBER force field and a distant-dependent dielectric function [Ramstein & Lavery, 1988; Jayaram et al., 1996; Arora & Jayaram, 1997, 1998]. The drug was initially placed 65 Å away from the DNA and $10^4$ trajectories generated. A trajectory is said to be successful if the drug encounters the minor groove of the DNA with a root mean square deviation of within 3 Å from its crystal structure and unsuccessful if it diffuses to more than 200 Å away from the helical axis of the DNA. A successful trajectory is depicted in Figure 3. The steering and orientational effects of electrostatic forces are evident from the trajectory. Initially the motion of the drug is primarily diffusive and random and as it gets nearer the DNA electrostatic forces guide it. The drug encounters the DNA via a three-dimensional diffusion to a nonspecific site on the DNA and then slides to the right site.

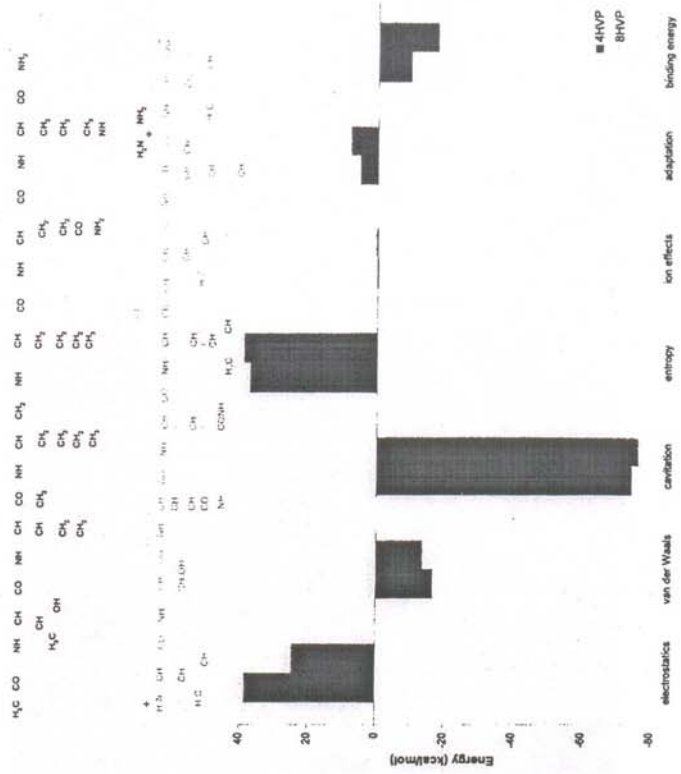


*Figure 3. A trajectory of DAPI, an anti-tumour antibiotic, diffusing to its DNA binding site as simulated by Brownian dynamics. [Typical CPU time for a trajectory is ~100 hours on an R10000 based SGI workstation.]*

The association rate constant without hydrodynamic forces was $9.5 \times 10^6$ $M^{-1}s^{-1}$ without intermolecular forces and $102.9 \times 10^6$ $M^{-1}s^{-1}$ with intermolecular forces. With hydrodynamic interactions included, these were $2.2 \times 10^6$ $M^{-1}s^{-1}$ and $68.4 \times 10^6$ $M^{-1}s^{-1}$ respectively. The experimental value is $63.3 \times 10^6$ $M^{-1}s^{-1}$. This brings out the importance of intermolecular forces in steering the drug to the right site while hydrodynamic steering effects are minor. The role of hydrodynamic forces is basically to slow down the reaction rate.

From the standpoint of drug design, two points are heartening to note. One is that the simulations succeed in bringing the drug molecule to the crystallographically found site on the receptor and second is the magnitude of the calculated rate constants which are in conformity with experiment.

### *(b) Free Energy Analysis of inhibitors of HIV-1 protease*

Molecular dynamics based free energy analyses were carried out on two complexes of HIV-1 protease (PDB codes: 4HVP and 8HVP). The binding free energy values obtained (Figure 4) were in close accord with experiment (-8.4 kcal/mol for 4HVP and -12.3 kcal/mol for 8HVP). Contributions from net van der Waals interactions and hydrophobic effects were found to be favorable to binding while those from net electrostatics, entropy and adaptation expense were found to be unfavorable to binding (Figure 4) [Kalra et al., 2001b]

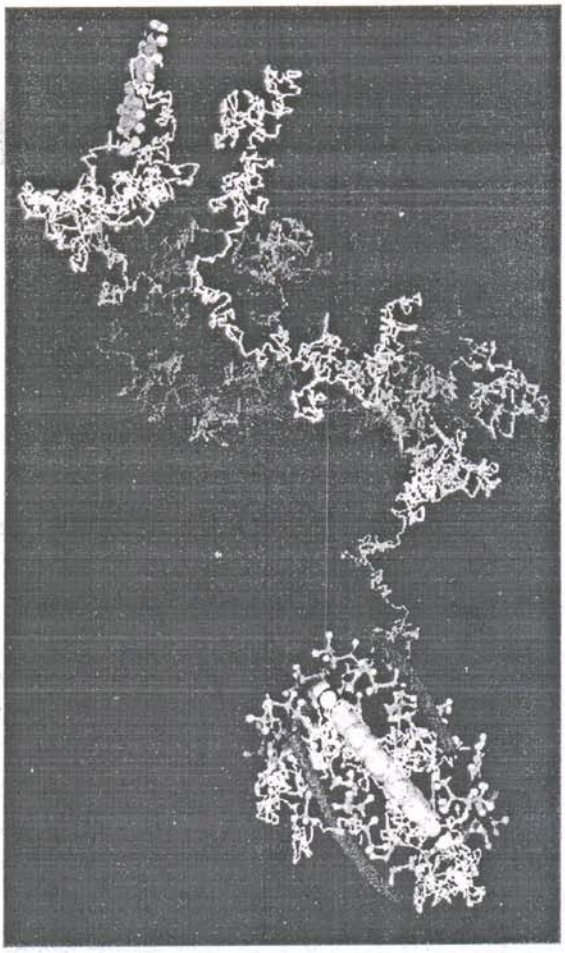

*Figure 4. Calculated standard free energies of binding of HIV-1 protease with two inhibitors (PDB codes: 4HVP and 8HVP) shown as a sum of individual energy components [CPU time required to generate the above histogram is ~540 hours on a 4 processor (R10000) SGI Origin 200.]*

A free energy component analysis as sketched above gives an indication of the type of modifications that could be carried out in the drug molecule so as to optimize binding. For example van der Waals forces can be increased by taking such side chains on the drug molecule that completely fill the subsites in the enzyme. Also unfavorable entropy contributions can be reduced by taking a rigid inhibitor structure. Electrostatic effects display a fine balance between direct interactions and desolvation effects and are difficult to guess from structure alone, thus requiring inputs from theoretical studies as above for deciding the substituents on the drug.

Overall the MMGBSA/MMPBSA methods facilitate an affinity ordering of ligands, targetted to a given receptor.

## Perspectives and Challenges

Accurate determinations of the binding free energies and rate constants of drugs/inhibitors targeted against receptor proteins or specified DNA base sequences are of immense interest in structure based drug design efforts to identify lead compounds (Figure 1). The above discussion is an abridged exposition of some emerging techniques. The applicability of the above methodologies is currently restricted to non-covalent macromolecular associations. Further extensions/ refinements involve inclusion of bond making and breaking steps to encompass covalently bonded drug molecules. One practical approach to a cognition of these events is the combined quantum mechanical / molecular mechanical method (QM/ MM) [Warshel & Levitt, 1976; Singh & Kollman, 1986; Bash et al., 1987; Field et al., 1990; Warshel, 1991; Aqvist et al., 1993]. The reacting parts (such as the active site region) of the system are treated quantum mechanically with the remainder being modeled with the faster molecular mechanical force field calculations. The promise of the QM/MM methods is that they allow for simulations of bond breakage and formation at the active site, while still allowing for the role of the extended system to be modeled in an efficient and computationally expedient manner.

The quest to develop individualized medicine-an aspiration of drug designers in the 21st century-has already triggered research for reliable algorithmic solutions to genome analysis, identification of defective stretches of DNA/genes for a particular disease, determination of three dimensional structure of the target DNA/ RNA, design of base sequence specific drugs, protein structure prediction, active site identification and site directed molecular modeling. All these factors in combination with experiment, informatics and molecular simulations are expected to contribute towards accelerating the drug discovery process if adequate supercomputing resources are available.

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