Atomistic Modeling of Enantioselective Binding

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ABSTRACT

This Account focuses on computational studies related to chiral recognition. It begins with a description of potential energy surfaces and the computational tools used to explore such surfaces, describes approximations and assumptions made by researchers computing enantioselective binding, and then explains why differential free energies of binding can be computed so accurately. The review focuses on chiral recognition in chromatography, emphasizing binding and enantiodiscriminating forces responsible for chiral recognition. The Account also describes computational studies of chiral recognition in cyclodextrins, proteins, and synthetic receptors.

Introduction

Chirality has been thrust into the scientific forefront in several subdisciplines of the chemical sciences. Reflecting this are new journals dedicated to the topic of chirality, including *Tetrahedron*: *Asymmetry*, *Enantiomer*, *Chiraity*, and *Molecular Asymmetry*, all of which complement existing journals that are themselves replete with papers on this topic. In this Account I focus on computational studies directed toward understanding chiral recognition. Omitted are theoretical studies having analytical solutions because those papers typically use highly stylized representations of "molecules", e.g., cylinders with helical grooves, perfect polygons, continuum models representing surfaces to which antipodes bind, etc. Although these papers provide much of the fundamental groundwork for our understanding of chirality forces, they are generally of little interest to the practicing bench chemist whom I target in this review. Instead, described here are atomistic modeling studies where techniques such as molecular mechanics, quantum mechanics, and molecular dynamics or Monte Carlo simulation methods have been carried out to evaluate enantioselection in proteins, synthetic host−guest complexes, and chromatographic systems.

Kenny Lipkowitz received his B.S. in chemistry from SUNY Geneseo in 1972 and earned his Ph.D. under the supervision of Brad Mundy at Montana State University in 1975. After a postdoctoral at The Ohio State University with Leo Paquette, he initiated his academic career at IUPUI in 1976. Ken was one of the early faculty members at the QCPE Summer Workshops on Practical Applications of Quantum Chemical Methods in the 1980s. He organized numerous workshops on molecular mechanics and was cofounder and first cochairman of the Gordon Research Conference on Computational Chemistry. Ken is currently associate editor of the *Journal of Chemical Information and Computer Science* and is co-editor of *Reviews in Computational Chemistry*.

Potential Energy Surfaces and Computational Tools

Having knowledge of a molecule's potential energy surface (PES) is important because that surface dictates the molecule's shape, its dynamical features, and its reactivity. Unfortunately, it is difficult to know a priori what the PES looks like with great certainty, except for the simplest of examples. Consider a small molecule with two rotatable bonds. A plot of internal (potential) energy as a function of those two degrees of freedom is given in Figure 1. While this PES has some apparent symmetry, it is nonetheless fairly complex; imagine, then, what the PES of a molecule having tens of rotatable bonds would look like! Fortunately, a wide range of computational tools exist that are capable of mapping such surfaces, thus letting us better understand or even predict the shapes and reactivities of molecules.

In this review it is assumed the reader is familiar with, or has at least heard about, these methods. In particular I assume a basic understanding exists concerning quantum mechanics (QM) and molecular mechanics (MM) calculations, and that the reader has a rudimentary knowledge of what molecular dynamics (MD) and Monte Carlo (MC) techniques are used for in simulations. Figure 1 depicts how these methods are used to explore or to map out a PES. The blackened arrow means that a QM or MM geometry optimization (energy minimization) has been done starting from some initial set of torsion angles. These energy minimizations usually seek the nearest minimum on the unknown PES so many such calculations beginning from different starting points are needed to find all the minima. The black dots with lines dropping onto the PES is my representation of what MC calculations involve. Here, randomly selected torsion angles are chosen, and the internal energies at those dihedral angles are computed with either a MM potential energy function or a QM Hamiltonian; millions of configurations are thus sampled for statistical thermodynamic averaging. The key point here is that one can move between minima separated by high potential barriers this way. Finally, the black winding line represents the movement over the PES using MD methods. The most relevant point to be made about MD is that one can sample very effectively *local* regions on the PES, but special methods are needed to make jumps to other regions of the surface. Given the quality of existing hardware along with such computational methodologies, rapid advances in understanding enantioselectivity have been made.

Approximations, Assumptions, and Why Differential Free Energies of Binding Can Be Computed Accurately

There exist many assumptions and approximations concerning the basic computational methods themselves; these issues are beyond the scope of this review, but they

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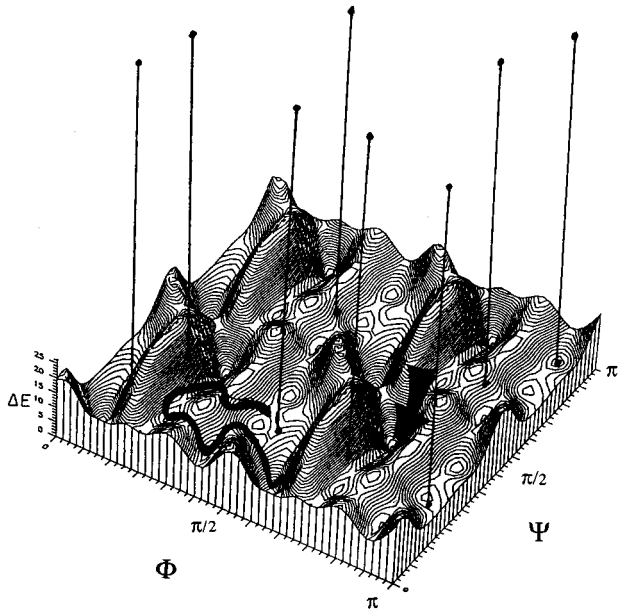


FIGURE 1. Part of a complex potential energy surface illustrating the differences between computational methods used to explore such surfaces.

are important in their own right and should not be slighted. Moreover, as in other molecular modeling studies, many scientists carrying out the calculations will often truncate the size of the receptor or guest or omit buffers, counterions, and even solvent environments to make the system computationally tractable. The repercussions of these omissions and the pitfalls to be aware of when performing such modeling studies have been described.[1] Other approximations related specifically to the calculation of enantioselective binding are also made, and we point them out here.

First, most scientists do not compute absolute free energies but rather determine differential free energies. In all systems where enantioselective binding takes place, there exist two competing equilibria:

$$H^R + G^R \leftrightharpoons H^R{\cdot}G^R \quad (1)$$

$$H^R + G^S \leftrightharpoons H^R{\cdot}G^S \quad (2)$$

H refers to the host molecule serving as a selector, G is the guest molecule that is the selectand, and the superscripted *R* and *S* are stereochemical descriptors. In both equilibria the binary complexes are often weakly bound, diastereomeric complexes held together by van der Waals forces, charge-transfer forces, hydrogen bonding, and so on. Most problematic is that the enantiodiscriminating forces are very small compared to the complexation forces, sometimes by 2−3 orders of magnitude. Hence, the computational chemist is faced with the challenging task of computing very small energy differences, often less than 500 cal/mol!

One could attempt to compute the free energy difference, ΔG, for equilibrium 1, compute the free energy difference for equilibrium 2, and then compare those differences; $\Delta\Delta G$ will thus indicate directly which substrate is more tightly affixed to the host molecule. However, recognize that the left-hand sides of both equilibria are equivalent; one has the same host molecule, and, by virtue of an enantiomeric relationship, (*R*)-guest = (*S*)-guest because they have the same shapes, the same energies, and the same extent of solvation in the unbound state. Thus, all one needs to do is compute the free energies of the bound-state complexes. The differences between the free energies of those complexes can be used to predict preferential guest binding. This approach is a double difference method, and the first successful computational evaluation of enzyme−substrate specificity was carried out this way by DeTar, who used a tetrahedral intermediate to represent a transition state for a tryptophan derivative reacting with chymotrypsin.[2] DeTar assumed that if the competing binding mechanisms were similar enough, influences of polar effects, solvation effects, and entropy differences would cancel, thus making differences in computed energies comparable to differential free energies, $\Delta\Delta G$.

Because differential free energies are being computed, most scientists do not worry much about the quality of the computational method (QM or MM) being implemented to actually compute the energies. For example, it is assumed that if a force field overestimates electrostatics and underestimates hydrogen bonding, a cancellation of force field errors is to be expected because the same problems experienced by the D-isomer exist for the L-isomer. This is a working assumption that is not strictly justifiable because what are actually being computed are energies of diastereomeric complexes rather than of pure enantiomers. Nonetheless, this is an assumption that works very well, as we will see. Most computational papers published to date make use of these error cancellations arising from double difference calculations.

This panacea of "cancellation of errors" fails when one inadequately samples the diastereomeric PESs. Merely taking a guest molecule and docking it somehow to a host molecule followed by a simple energy minimization (for D and then for L-isomers) is a poor way to compute values for comparison with experiment; multiple such calculations are needed. Most published papers concerning calculation of enantioselection take advantage of the double difference approach, but unfortunately they do not adequately sample enough configurations to make their results meaningful for comparison with experiment. The key question is, "How much configurational sampling is needed?"

Reducing the Amount of Sampling on Potential Surfaces

Most enantioselective binding studies involve binary complexes between one chiral host molecule and one chiral guest molecule (ternary structures are less common, except for studies of cyclodextrins, where two cyclodextrins can encapsulate one guest molecule or where a chiral molecule is entrapped in a lattice). Hence, the first objective of a molecular modeler is to decide where to place the guest in or around the host (and in what relative

orientation) before further searching on the PES is done. Some methods used to accomplish this include the following:

1. Select unambiguous examples to study. In other words, pick a system in which there can be only one docking site and one orientation, or for which only a limited number configurations can exist. Indeed, this was one of several desiderata listed by DeTar in his selection of α-chymotrypsin as an enzyme system for computing stereospecific hydrolysis rates.

2. Limit the sampling by using experimental data. Intermolecular NOEs can provide a lot of information about where a guest binds to a host, and, in some cases, it can provide orientational information as well. Alternatively, using crystallographic data of, e.g., a protein with a (related) bound substrate can provide a good starting place for docking the pair of molecules.

3. Use "motif-based" docking strategies.[3] Here one takes advantage of the known interactions that can take place between two molecules. For example if one molecule has a π-acidic ring and the other a π-basic ring, the modeler would lay one ring over the other to account for the charge-transfer complex that will probably occur. Simultaneously, one would associate a hydrogen bond donor on one molecule with a hydrogen bond acceptor on the other and likewise attempt to match steric and electrostatic interactions between molecules. Published examples have shown that this approach works quite well for focusing on the important part of an otherwise complex PES.

4. Search all of the potential energy surface without making any a priori assumptions; in other words, let the computer do the search for you without any user intervention or bias.

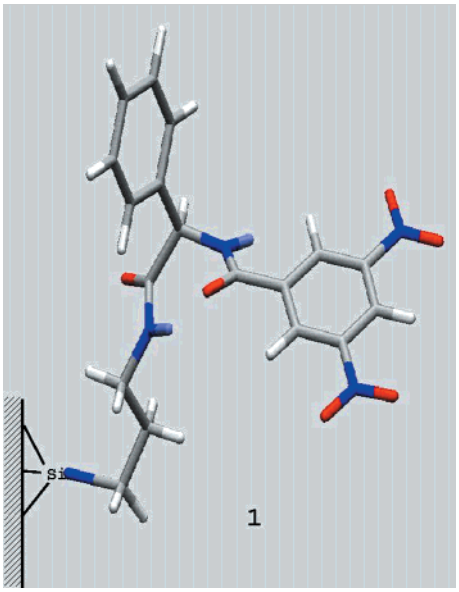
Using one or more of these strategies allows the molecular modeler to generate the initial binary complexes. From this starting point, local or even global searches of the PES can be made. The examples given below illustrate how this has been accomplished in recent years.

Enantioselective Binding in Chromatography

The idea of using chiral stationary phases (CSPs) for chromatographic resolutions of enantiomeric mixtures has been around for many years, but advances have been made in this field of technology only in the past decade. This work has had an enormous impact on the way bench chemists now go about separating enantiomeric mixtures. Despite this success, the question I have heard repeatedly from both the developers and users of those CSPs was, "How do they work?"

To answer this question as well as to assist in the design of enhanced stationary phases, our group began computing the enantioselectivities of these materials. The type of CSP we focused our attention on initially is referred to as type I, or brushlike CSPs that are relatively small pendant organic molecules linked to silica gel like **1**.

The approach Lipkowitz and Darden developed[4] for



sampling the microstates needed for statistical averaging is illustrated in Figure 2. Here the CSP molecule's center of mass (or any atom) is placed at the origin of a coordinate system. An origin is likewise selected on the analyte, and the position of the analyte relative to the CSP is given in polar coordinates.

At each latitude, Θ, and longitude, Φ, a large number of orientations of the analyte with respect to the CSP is generated. The key to this sampling strategy was to always ensure that the van der Waals surfaces of the two molecules just touched one another for each unique orientation generated. Thus, during the sampling, the value of *r*, the intermolecular separation between selector and selectand, was allowed to vary so that a bulky part of one molecule would not overlap a bulky part of the other molecule. A large number of orientations were sampled at given values of Θ and Φ. All values of Θ and Φ were then evaluated this way by moving the analyte around the CSP molecule in very small increments so that all minima on the intermolecular potential energy surface were located. In essence, what was done was to roll the analyte molecule over the van der Waals surface of the CSP, sampling configurations for the statistical averaging.

These studies originally implemented rigid body molecules, but that constraint was lifted once the computing horsepower became available. Most notable in this regard is the work from Gasparrini et al.,[5] who have developed a robust program for such enantioselective binding calculations called Glob-MolInE, complete with a graphical front end. For us and for Gasparrini, it was clear that using only the lowest energy conformations (obtained from a conformational analysis) of each molecule would be inappropriate. Just as in the pharmaceutical sciences where it is recognized that the "bioactive conformation" of a drug molecule need not be the global minimum, it was recognized that the most effective binding shape of the CSP (and/or the analyte) need not be the lowest energy structures either. Indeed, this was found to be the case in several systems we eventually studied. Hence, it

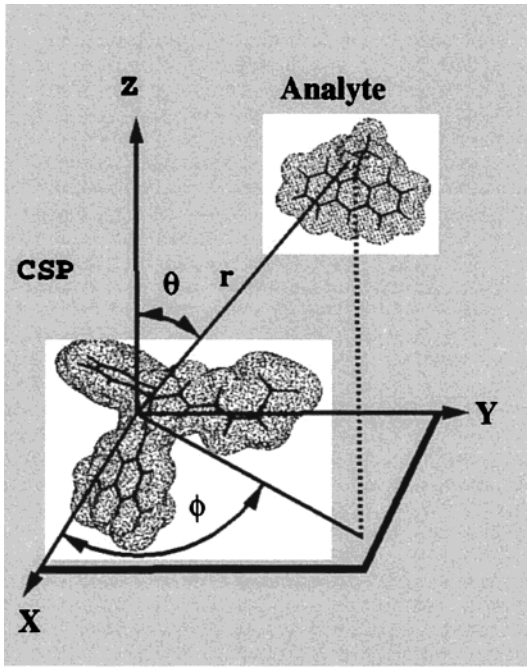


FIGURE 2. Relative position and orientation of analyte with respect to the CSP.

was necessary to account for all reasonable shapes of the CSP as well as of the analyte.

Because all microstates are being sampled this way, their energies and probabilities are known, so one can also determine entropies as well as enthalpies of binding. Hence, the final energies computed are free energy differences which can be compared to experimental values directly.

This method works reliably; for all analyte mixtures we considered, we were able to predict the correct retention order (i.e., the sign of $\Delta\Delta G$ was always correct), and when our differential free energies were converted to separation factors, α, plots of computed versus observed α values were linear and usually had high correlation coefficients,[6−9] as was the case with the results from the Italian group and others. Results of interest to the general scientific community derived from such calculations involve the following issues.[4,6−9]

Binding Site. In contrast to immobilized enzymes that are used as CSPs and which are known to sometimes have different binding sites for the optical isomers, it was not clear to most chromatographers if both antipodes bind to the same or to different regions on the CSP. From the intermolecular potential energy surfaces of the binary complexes, we find the lowest energy structures to have both isomers binding around the same values of latitude, Θ, and longitude, Φ, indicating that the enantioselection does not arise from differences in binding site, but instead from *how* the analytes bind.

Intermolecular Forces Responsible for Binding. Most analytes separated on type I chromatographic phases are neutral molecules; the binding free energies and association constants are thus small. The intermolecular forces responsible for analyte binding include charge-transfer complexation between π-basic aryl rings of the analyte with the π-acidic dinitrobenzoyl moiety of **1**, dipolar association of analyte functionality with the amide linkage of **1**, hydrogen bonding, and dispersion forces. The type and amount of forces responsible for complexation depend on the composition of the CSP and the analyte, however.

Enantiodifferentiation. Energy partitioning schemes have been developed by several authors to evaluate the fragments of the CSP doing most of the work holding the complexes together. While this is useful in its own right (for design of new CSPs), the more significant finding involves differences those fragments feel between mirror image probes. Fragments sensing little or no difference between enantiomers are nondiscriminating, while fragments experiencing large differences are deemed to be enantiodiscriminating. A generalization derived from these simulations is that the molecular fragments most responsible for analyte binding are also the most stereodiscriminating. There is a less well-defined summary of enantiodiscriminating forces. In some cases the short-range dispersion forces are most discriminating, while in other cases the long-range electrostatic forces are most responsible for enantioselectivity; enantiodiscriminating forces are thus case dependent.

Entropic and Solvation Effects. At this time we cannot say if there exist compensating enthalpy−entropy effects on chiral chromatography on the basis of the results of computational studies because we have not studied enough systems to derive a sound conclusion and because relatively few other investigators have computed entropic influences adequately in their modeling studies (see later, however). While entropy of mixing does influence the differential free energies of binding, $\Delta\Delta S$ is not the major contribution to discrimination in chiral chromatography. Differential solvation of the competing binary diastereomeric complexes has been studied and found to be of minor importance in chiral discrimination,[10] though it is recognized that in some instances loss of resolving power can be induced by solvents that competitively bind to the CSP, especially by solvents or additives capable of hydrogen bonding.

Other research groups were also carrying out similar work on type I CSPs using a variety of docking strategies and sampling protocols. The idea of carrying out full geometry relaxation of selective initial diastereomeric complexes was also promulgated by Still and Rogers.[11,12] Their docking strategy was based on NMR data, but they also used motif-based strategies to generate initial complexes that were then energy minimized to account for induced-fit structural changes. In that first paper, only the global minimum located for each diastereomeric complex was used for prediction purposes, leading to poor results. In their following papers they carried out a statistical treatment where $\Delta\Delta H$ and $\Delta\Delta S$ were computed following Lipkowitz et al. Good results were obtained this way, and conclusions comparable to those of Lipkowitz were derived, albeit for different CSPs.

Another significant paper in this area is that by Däppen, Karfunkel, and Leusen.[3] Their approach to the sampling

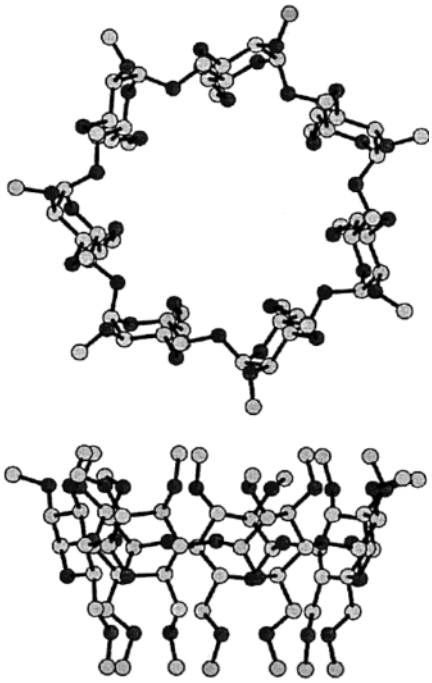


FIGURE 3. Top and side view of a permethylated cyclodextrin molecule. The gray spheres are carbons, while the dark spheres represent oxygen atoms. Hydrogen atoms are omitted for clarity.

issue was based on the binding motif methodology, which was compared to grid-based methods in their paper. The focus of their work was on designing a new CSP that would have enhanced selectivity. Another group that was very active in this area at that time was Topiol's.[13] Their research was directed more toward understanding chirality forces and focused less on how to compute the differential binding energies, however. Several other studies where multiple conformers of CSP and analyte were docked using rigid body orientations but then geometry optimized were published by Norinder and Sundholm[14] and also by Edge et al.,[15] who were the first to consider the influence of the solid matrix to which the CSP is attached. Finally we note that other types of modeling studies with predictive power have been carried out on this type of stationary phase. In particular, we point out two studies where quantitative structure−enantioselective retention relationships (QSERRs) and comparative molecular field analyses (CoMFAs) have been performed at an atomic level.[16,17]

Enantioselective Binding in Cyclodextrins

Cyclodextrins are cyclic oligomers of α-glucose that are capable of host−guest complexation. Cyclodextrins are chiral and, accordingly, serve as chiral microenvironments for asymmetric induction, but they are also used as mobile-phase additives for capillary electrophoresis as well as for stationary phases in various types of chromatography. Figure 3 shows the structure of a permethylated cyclodextrin molecule. A large number of computational publications directed toward understanding host−guest binding of native and derivatized cyclodextrins have appeared in the literature,[18] but most of those papers were not concerned about chiral discrimination, nor were simulations carried out (simple docking and energy minimization is more typical in that literature).

Our interest in chiral discrimination by cyclodextrins began when we learned that amino acids such as tryptophan could be resolved by cyclodextrins. We first carried out NMR studies using the same solvent medium as was used for the chromatography experiments, and based on NOE intensities of key protons of guest with those of host, we had some a priori knowledge about the geometry of the complexes that we needed for our simulations.[19] Cyclodextrins are not symmetric structures. Moreover, they are very flexible and prone to induced-fit structural changes. Hence molecular dynamics calculations, which account well for this flexibility, were carried out using the same explicit solvents as in the chromatographic and NMR studies. The short simulation times (100 ps) were adequate for reproducing all experimental data. Subsequently, we were able to provide an explanation of how these materials discriminate between enantiomers based on that simulation. Of particular significance is the clockwise versus counterclockwise orientation of the secondary hydroxyl groups on the upper rim of the cyclodextrin that influences strongly the ability of the host molecule to form hydrogen bonds with the guest molecules. For the less tightly bound enantiomer, only single intermolecular hydrogen bonds form between host and guest, but for the more tightly bound isomer, multiple-contact (simultaneous) intermolecular hydrogen bonds form. Moreover, the ratio of multiple-contact hydrogen bonding (leading to preferential enantiomer stabilization) to single hydrogen bond formation is about 2:1. That work was followed by an assessment of mandelic acid binding to other cyclodextrins,[12] but a more challenging problem emerged involving cyclodextrins used for gas chromatography.

Most liquid chromatography and NMR studies of cyclodextrins are done in an aqueous or polar medium where hydrophobic forces push guests into the interior of the macrocyclic cavity. This force is absent in gas chromatographic systems, however, and the key issue we addressed centered on knowing *where* the analyte molecules prefer to bind (the exteriors of these CSPs are as chiral as the interiors, so external binding could also lead to chiral discrimination). The computational protocol we used takes advantage of the good local sampling ability of molecular dynamics together with global structural moves, so that the analyte was forced to visit all regions of the PES.[21] To ensure multiple collisions would occur between host and guest, we placed a spherical reflective wall around the cyclodextrin so that when the analyte detaches itself from the cyclodextrin it hits that wall and is gently shoved back to re-encounter the cyclodextrin molecule. In all examples, the correct retention orders and energetics of chiral discrimination were reproduced, thus validating the method. Energies were used as a convergence criterion, but so were plots such as that in Figure 4. In this figure we show the location of the center of mass of 2-methylbutanoic acid relative to permethyl-β-cyclodextrin over a 50-ns simulation time period. It is clear that

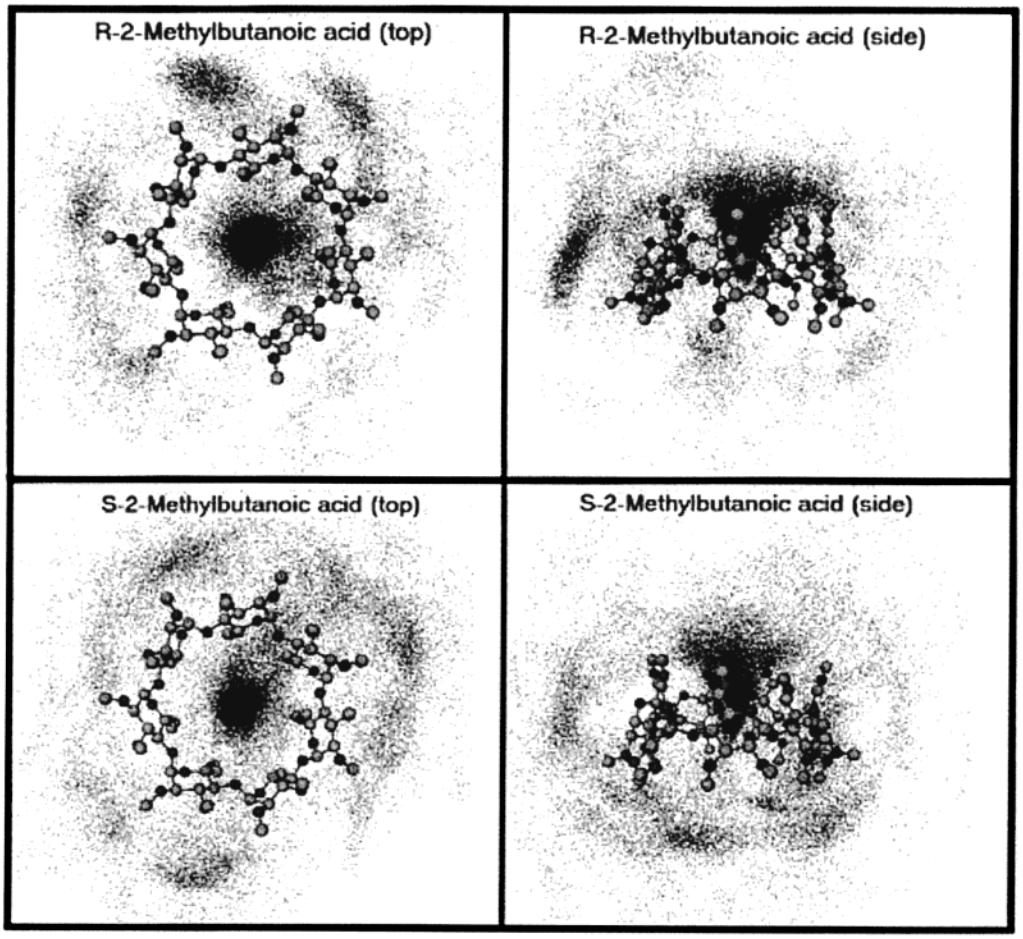


FIGURE 4. Distribution of an analyte molecule's center of mass around a cyclodextrin.

all regions inside and around the macrocycle have been sampled and that the interior of the cavity has a higher probability of binding than the exterior. Indeed, for all analytes examined we found that interior binding is preferred because dispersion interactions are maximized there. We then generated a method for determining which region inside or around the host is most discriminating for a given analyte.[22] It was discovered that the greatest amount of chiral discrimination also exists in the interior of the cavity; the region of maximum chiral recognition of the macrocycle is thus spatially coincident with the analytes' preferred binding sites. Hence, nature places the analyte in the most enantiodiscriminating region of the macrocycle to maximize its ability to discriminate, and this is one reason for the success and popularity of cyclodextrins as host molecules for uses in enantioselection.[23] Other findings of general interest are that the forces holding the complexes together are dominated by van der Waals forces, and that enantiodifferentiation is dominated by differences in van der Waals energies. We conclude, then, that chiral recognition in these systems arises from short-range dispersion forces rather than long-range Coulomb forces.

Enantioselective Binding in Proteins

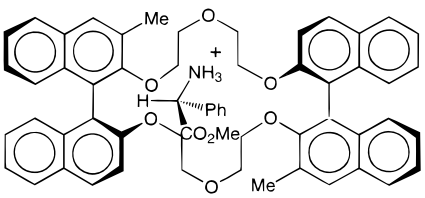
Computational studies of stereodifferentiation in proteins have been carried out in part because of the inherent significance of these systems but also because they have well-defined binding sites that limit both the number of orientations and conformations of substrate. The two most extensively studied enzymes are chymotrypsin and lipase. A large number of studies have focused on chymotrypsin beginning with the early work of DeTar[2] and Wipff et al.[24] Because crystallographic data were available for this hydrolytic enzyme and because motif-based docking of tetrahedral intermediates (used to mimic transition states) provides adequate starting orientations, simple energy minimizations with molecular mechanics gave very good numerical agreement with observed enantioselection, and compelling arguments could be made about how the protein stabilized the preferred transition states. In particular, for the stereoselective hydrolysis of L- and D-*N*-acetyltryptophanamide, it was found that the Michaelis complex for the faster reacting L-isomer is close to the geometry of the tetrahedral intermediate while the D-isomer requires more extensive conformational changes to adopt the geometry of the tetrahedral complex. Most of the L–D stereoselectivity, though, is due to the poorer interaction of the amide group of the D-isomer with the enzyme in the tetrahedral intermediate, and from the favorable association of both the *N*-acetyl and the aryl ring with the enzyme. The major conclusion, confirming

speculation from experimentalists, is that the enantioselectivity displayed upon hydrolysis of peptides arises from the differential transition-state energies leading to the tetrahedral intermediate rather than from the initial Michaelis complex.

Mammalian lipases start the digestion of fats, and a significant number of computational studies directed toward elucidating structure-mechanism relationships in this class of enzymes have appeared. In general, these publications focused more on computational procedures that could be used to predict stereoselectivity, as exemplified by studies from Haeffner[25] and from Orrenius.[26] Nonetheless, there are results derived from published works of general interest to the scientific community explaining unanticipated reversals in stereopreference. In particular is the work from Holmquist et al.,[27] who provided a structural basis for enantioselective inhibition of a lipase by long-chain aliphatic alcohols. For the esterification of 2-methyldecanoic acid, the enantioselective step is the deacylation of the lipase, which in turn was studied with transition-state models that were docked using a crystal structure of a complex containing a cocrystallized transition-state analogue molecule. The faster reacting *S*-isomer occupies a well-established acyl-binding tunnel in the active site of the enzyme and does so in an extended, low-energy conformation that is not possible for its antipode. An alternative mode of binding into the active site for both isomers that allows for the formation of all catalytically crucial hydrogen bonds to the transition state was found, however. In this binding mode, the lipid adopts a "hairpin" conformation, and the computed enantiopreference is opposite to that of the standard model. This in turn explains the opposite stereoselectivity derived from empirical rules for several substrates containing bulky substituents proximal to the stereogenic center. Leading references of additional modeling studies directed toward explaining the molecular basis for enantioselectivity of lipase can be found in a recent paper by Kazlauskas.[28]

Synthetic Receptors

Many computational studies related to host−guest complexes of non-natural receptors exist, but few have focused on chiral discrimination until recently. Several pertinent papers in this regard include the prediction of enantioselectivity of protonated phenylglycine methyl ester in the Cram crown ether, **2**, by Gehin, Kollman, and Wipff,[29] who



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used configurational sampling prior to energy minimization, and by Raj, Morley, and Jackson,[30] who developed a dynamic Monte Carlo (DMC) method. It is found that

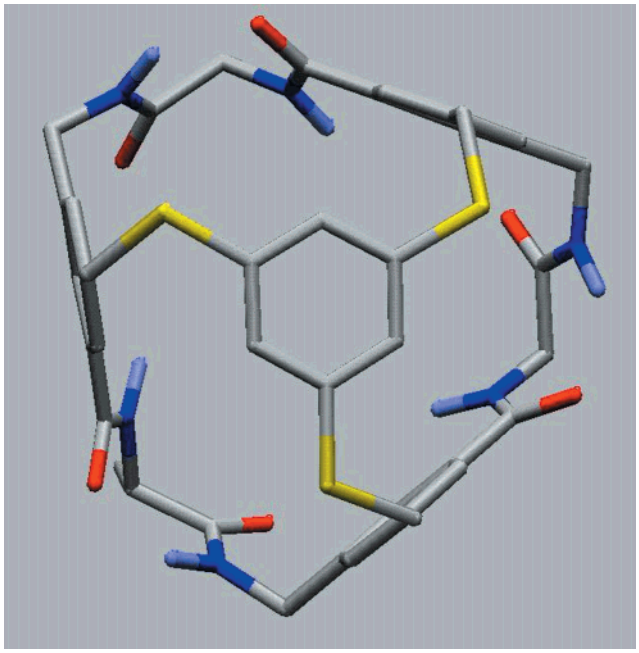

FIGURE 5. Bottom view of Still's C_3-symmetric receptor (provided by F. Gasparrini).

while the electrostatic components of the binding energies are comparable, the van der Waals energies are not; stereoselection thus arises from steric factors.

Most of the published work concerning stereodifferentiation focuses more on techniques that can provide the right answers rather than a full assessment of where and how stereodifferentiation takes place. The most innovative and well-documented work in this area comes from Still and his collaborators, who investigated the enantioselectivity of podands[31−34] and a C_3-symmetric receptor shown in Figure 5.[35] While some of their calculations involved either MC searches[32] or stochastic dynamics simulations alone,[31] these authors also developed a mixed-mode (MC/MD) sampling procedure to generate the ensemble averages for their free energy perturbation (FEP) calculations.[33] This work was followed by papers presenting enhanced sampling strategies that again use the good local sampling of MD methods with the more global sampling on the PES afforded by Monte Carlo moves. Their Monte Carlo jump-between-wells strategy, MC(JBW), as well as their use of stochastic dynamics, called MC(JBW)SD, wastes little time sampling unimportant (high-energy) regions of the diastereomeric PESs, thus making this an especially fast and effective search strategy.[34] The results derived from these simulations are well converged, and small free energy differences can be computed reliably for medium-sized, flexible molecules.

A new method that can evaluate configuration integrals in all degrees of freedom for free energy calculations has been developed by Kolossváry.[36] His Monte Carlo integration scheme is called mode integration (MINTA). It performs well compared to JBW for conformational analysis, and an evaluation of its performance for enantiomeric free energy calculations on Still's receptors shows that it can pick up entropy effects very well, and it is especially

fast. A noteworthy finding from Kolossváry's study is that the preferential binding of an L-guest embedded in the host arises from a dominating entropic stabilization that outweighs the enthalpic stabilization which tends to stabilize the D-isomer. The genesis of this entropy effect arises from the completely different hydrogen-bonding patterns between the host and the two enantiomeric guests: one isomer is locked into a rigid binding mode, while the other is free to rotate.

Summary

With a modern force field one can compute differential free energies of enantiomer binding with high precision because double differences are being calculated. These calculations are becoming more feasible presently because of the advances in computing hardware, and we anticipate new chiral receptors will be designed de novo soon using these computational tools. Although differential binding can be studied in this way, computing the enantioinduction of chemical reactions, where bond-making/breaking takes place, will require developments in reactive potentials or the use of quantum chemistry directly (quantum MD) or coupled with a force field (QM/MM). Such studies are under way.

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