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**Review**

# Hydrophobicity: is $LogP_{o/w}$ more than the sum of its parts?

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**Abstract** – The empirically calculated parameter $LogP_{o/w}$, the $\log_{10}$ of the coefficient for solvent partitioning between 1-octanol and water, has been used to provide the key data for a unique non-covalent interaction force field called HINT (Hydropathic INTeractions). This experimentally-derived force field encodes entropic as well as enthalpic information and also includes some representation of solvation and desolvation energetics in biomolecular associations. The theoretical basis for the HINT model is discussed. This review includes: 1) discussion of calculational representation of the hydrophobic effect, 2) the rationale for describing the experimental $LogP_{o/w}$ based descriptors used in the HINT force field and model as free energy-like, 3) the relationship between hydrophobic fragment constants and partial group electrostatic charge, and 4) the implications of structurally-conserved water molecules on free energy of molecular association. Several recent applications of HINT in structure-based and ligand-based drug discovery are reviewed. Finally, future directions in the HINT model development are proposed. © 2000 Éditions scientifiques et médicales Elsevier SAS

**hydropathy / hydrophobicity / structure-based drug design / ligand-based drug design / free energy / solvation energy**

## 1. Introduction

Our understanding of the biomolecular environment is increasingly relying on a growing appreciation of chemical structure and chemical interactions between and within species. Our comprehension of how biological molecules associate (both intra- or intermolecular), how the omnipresent water molecules affect these associations and how this milieu (somehow) exquisitely works together to produce and sustain life is ultimately based on our understanding of biological structure. These associations and interactions are the underlying cause of nearly all processes at the molecular level, which are in turn the basis of biological action. Flaws of some nature in the structure (and function) of these biological entities, or the presence of some unwanted molecular entity, give rise to disease. Understanding this structural information presents opportunities and approaches for treatments. This is relevant to medicinal chemists and computational chemists engaged in drug discovery because the paradigm of 'structure-based drug design' [1] is based on exploiting the three-dimensional structure of therapeutically important biomacromolecules and complexes.

An even more robust and long-lived technology of medicinal chemistry and drug discovery is the 'ligand-based drug design' paradigm which is also called QSAR (quantitative structure–activity relationships) [2, 3]. In this paradigm the known biological activities of a series of molecules, which are assumed to bind in a similar manner at the same site, are related to their structure and structurally-derived parameters through a mathematical model. If new molecules do not stray far, structure-wise, from the learning set of the model, the activity of the new molecules can often be accurately predicted. In developing QSAR medicinal chemists have learned much about molecular structure and properties that is often not appreciated by scientists in other fields. In particular, medicinal chemists have a very rich understanding of hydrophobicity and hydrophobic interactions, including a large number of computational methods to estimate $LogP_{o/w}$, the partition coefficient for 1-octanol/water solubility, for small, organic, drug-like molecules [4–8].

Interestingly, the hydrophobic interaction is one of the most important, but least understood non-covalent struc-

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tural effects in ligand binding, biomacromolecular associations and related phenomena [9]. The hydrophobic effect is generally regarded as being, at least in part, entropic in origin. Unfortunately the common tools for computational structure creation and evaluation, which are based on molecular mechanics, do not include terms for estimating entropic energy. Molecular mechanics force fields can be used with the thermodynamic cycle-perturbation method to yield free energy estimates [10–12]. This is a computationally-intensive technique that is difficult to implement and interpret, but has yielded excellent results in some laboratories [13, 14]. While the term 'hydrophobic bonding' is clearly a misnomer, there is an associated phenomenon whereby hydrophobic entities tend to congregate and exclude water. This is an ensemble effect that is not derivative of molecular mechanics models or even conventional quantum chemistry, but is clearly related to the unique solution and solvation properties of water.

In this report we will describe how we have built, by combining concepts and fundamentals of molecular biology, structure-based drug design, ligand-based drug design and classical medicinal chemistry, a sophisticated model for biomolecular interaction based on the seemingly simple solvent partitioning data reported as $LogP_{o/w}$. We intend to show that there is substantive thermodynamic free energy data in $LogP_{o/w}$, and despite the relative ease in predicting $LogP_{o/w}$ with a variety of methods (that have been extensively reviewed elsewhere [15, 16]), the value of understanding and exploiting this surprisingly rich thermodynamic data source transcends the method(s) of $LogP_{o/w}$ prediction. An added benefit is that the effects of solvation/desolvation for ligands are incorporated in $LogP_{o/w}$. Like hydrophobic effects, solvation/desolvation is not explicitly treated in commonly used molecular mechanics methods. Our model for these phenomena, based on $LogP_{o/w}$, is called HINT for Hydropathic INTeractions [17–20] and is being used in a growing number of investigations of ligand binding, protein associations and related biomolecular phenomena.

### *1.1. The HINT model*

The basis of our investigations is a 'natural' and intuitive free energy force field based on experimentally measured interactions between real molecules and real solvents. Because of this, we believe that the effects of entropy and solvation are inherently included along with hydrogen bonding, Coulombic, acid–base, hydrophobic interactions etc. All of these effects are significant, if not crucial, to understanding and exploiting biological structure. Our model, HINT, uses the experimental data from solvent partitioning experiments between water and 1-octanol ($LogP_{o/w}$) for interaction classification and quantitative scoring. HINT was created to specifically include all non-covalent interactions (*figure 1*). Hydrophobic and polar interactions, which are collectively referred to as hydropathy, between molecules in biologically important systems are empirically quantified. In this scheme, hydropathic attractions between species include hydrogen-bonding, acid–base interactions, Coulombic attractions as well as hydrophobic interactions. All of these are related to solvent partitioning phenomena because the dissolution of a ligand in a mixed solvent system (such as water/1-octanol) involves the same fundamental processes and atom–atom interactions as biomolecular interactions within or between proteins and ligands. In practice, the conceptually simple HINT model scores each atom–atom interaction within or between biological molecules with the following equation:

$$\mathbf{b_{ij}} = \mathbf{a_i}\,\mathbf{S_i}\;\mathbf{a_j}\,\mathbf{S_j}\,\mathbf{T_{ij}}\,\mathbf{R_{ij}} + \mathbf{r_{ij}}, \qquad (1)$$

where $\mathbf{b_{ij}}$ is the interaction score between atoms **i** and **j**, **a** is the hydrophobic atom constant, **S** is the solvent accessible surface area ($H_2O$ probe), $\mathbf{T_{ij}}$ is a logic function described below, and $\mathbf{R_{ij}}$ and $\mathbf{r_{ij}}$ are functions of the distance between atoms **i** and **j** (i.e. **r**). Generally the hydropathic-dependent function, $\mathbf{R_{ij}}$, is the simple exponential $\mathbf{e^{-r}}$ and $\mathbf{r_{ij}}$ is an implementation of the Lennard-Jones potential function [21, 22]. The $\mathbf{r_{ij}}$ term is mostly a penalty function to flag van der Waals violations. The double sum, $\mathbf{\Sigma\Sigma b_{ij}}$, is the total interaction score for the system. The HINT convention is that favourable interactions are scored with $\mathbf{b_{ij}} > 0$ and unfavourable interactions are scored with $\mathbf{b_{ij}} < 0$. The logic function $\mathbf{T_{ij}}$ returns a value of 1 or –1 depending on the character of the interacting polar atoms (i.e. $\mathbf{a} < 0$): there are three possibilities: acid–acid, acid–base, or base–base; only acid–base is scored favourably. $\mathbf{T_{ij}}$ also flags hydrogen bonds which are in the HINT model a special case of acid–base interactions.

The hydrophobic atom constant, **a**, is the key parameter in the HINT model. We calculate **a** by an adaptation of the CLOGP [4] method of Hansch and Leo. As in CLOGP, HINT uses values from a functional group primitive set that is summed and modified by structure-dependent factors that are coded by the connectivity between the group fragments. These factors represent real physical phenomena related to the molecular structure and properties [23, 24]. HINT then calculates the hydrophobic atom constants from the fragment constants after these factors have been applied. This method is, in our model, preferable to LogP estimation methods that de-

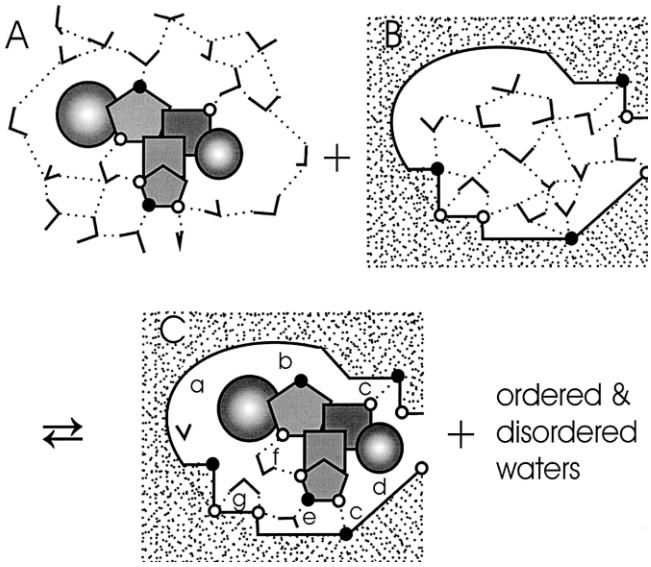


**Figure 1.** Schematic describing the ligand binding process. **A**) A ligand surrounded by water molecules. The 'curved' surfaces of the ligand are hydrophobic, while vertices are polar: open circles are hydrogen bond acceptors and closed circles are hydrogen bond donors. **B**) A macromolecular receptor site occupied by water molecules. **C**) The ligand bound in the site with the following features: a) hydrophobic–hydrophobic interaction surface; b) ligand has a hydrogen bond donor in an inhospitable hydrophobic region of the receptor; c) new hydrogen bond between ligand and receptor; d) hydrophobic groups of ligand in polar region of receptor; e) water-mediated hydrogen bond between ligand and receptor; f) water bound (intramolecular) to ligand and perhaps dragged into pocket with ligand, and g) water associated with receptor and largely unaffected by this binding event.

construct molecules into (only) component atoms [5–8] because the Leo factors provide a layer of second order structural information directly related to molecular shape and solubility. These hydrophobic atom constants along with a small handful of atom-type specific flags encode the thermodynamic information from the experimental measurement of logP. Most importantly, the hydrophobic atom constant is a localized thermodynamic parameter specific to interaction – it reveals the potential type and strength of interaction that the atom may engage in.

## 2. Understanding the HINT model

One of the more consequential aspects of the HINT model is that it is a unified description of all non-covalent interactions in the biological environment including hydrogen bonding, electrostatic and hydrophobic. HINT uses the same interaction protocol for all interaction types: the differences in score arise (only) from the hydropathic properties of the interacting atoms and not from customized functions tailored for hydrogen bonding, electrostatics, etc. This mirrors nature, where ligand binding or any non-covalent association event, is concerted and not a neat sum of terms where each represents an ingredient of the overall process. In this way the score components of a HINT calculation are on a common basis and valid for internal comparison. Dill [25] has observed that the assumption of additivity in $\Delta G$, implicit in molecular mechanics and related approaches where terms from different types of interactions (e.g., electrostatic, hydrophobic, solvation, etc.) are summed, may be invalid. The HINT model is fundamentally different: each atom–atom interaction, $\mathbf{b_{ij}}$, is related to $\mathbf{\delta g}$, a partial $\mathbf{\Delta G_{interaction}}$, such that $\mathbf{\Delta G_{interaction} = \Sigma \delta g}$.

In the Results and discussion section we will briefly review some of the modelling studies undertaken using the HINT paradigm. Because of the empirical derivation and nature of HINT, the published results themselves must ultimately underpin the validity of the model: the HINT model cannot be 'proven' from quantum mechanics or Newtonian physics. However, some issues concerning the specifics and details of the model warrant the discussion in this section. Indeed, much of the following paragraphs has been inspired by questions about the HINT model that we have received over the last several years. Key, however, in all of this discussion, is that while we can show that most, if not all, important energy contributions are implicitly included in the HINT 'force field' model, there is no rational way to separate them into specific terms associated with enthalpy, entropy, solvation, etc.

### *2.1. What is the hydrophobic effect and how can it be represented?*

There is general agreement that the 'hydrophobic effect' is a consequence (or side effect) of the tendency of hydrogen bond-forming species to congregate in such a manner to maximize formation of hydrogen bonds. This would include such observations as proteins organizing themselves such that the polar side-chains are solvent-exposed, the tendency of water molecules to form networked structures, and even the simple observation that oil and water don't mix. The consequence of this is that lipophilic species or groups are often excluded from the hydrogen-bonding environment, and themselves appear to congregate. Whether there is actually a hydrophobic force is a matter of debate [26–29]. Certainly a fraction of hydrophobic attractions are London forces [30]. In prac-

tice, most conventional molecular mechanics codes attribute the hydrophobic effect (only) to the van der Waals (London force) term. This is partly an implementation issue. Newtonian physics, the backbone of molecular mechanics, can not predict a complex phenomenological occurrence like water congregation and organization and the concomitant exclusion of hydrophobic entities.

However, for our purposes it is pragmatically and conceptually useful to assume that there is a hydrophobic force, and to consider hydrophobic–hydrophobic interactions as components in ligand binding or related phenomena. When the key HINT parameter, $\mathbf{a_i}$ (the hydrophobic atom constant) is greater than zero the atom will favourably interact with another atom whose $\mathbf{a_j}$ is greater than zero – a hydrophobic–hydrophobic interaction. How strong is the interaction? Clearly, it depends on the distance between the atoms. Compared to electrostatic or even London force attractions, there is little experimental data from which to derive a hydrophobic distance relationship law. The HINT modelling program is actually written to allow the user to choose the distance functional relationship. In our studies we have consistently chosen the simple exponential:

$$\mathbf{b_{ij} = a_i S_i \; a_j S_j \, e^{-r}}, \quad (2)$$

i.e. $\mathbf{R_{ij} = e^{-r}}$. This relationship can be grossly derived from the Leo Polar Proximity Factors [4] if they are plotted on an actual distance scale (rather than topological distance). More convincing is the report by Israelachvili and Pashley that the hydrophobic interaction is long range, decaying exponentially with distance [31].

### *2.2. How are entropy and solvation/desolvation implicit in LogP data?*

This is a very crucial question at the heart of understanding the hydrophobic effect. In a 1995 text [32] Hansch and Leo state "it is not possible to state whether hydrophobicity (is) either primarily an enthalpic or entropic phenomenon – it is probably a mixture of both." Classic theoretical treatments by Tanford [9], Nemethy and Scheraga [33, 34] and Ben-Naim [35] have failed to unequivocally resolve this issue. The pragmatic approach used in the development of HINT is, that since LogP is derived almost directly from the results of a real experiment, performed in a way in which no component terms of intermolecular or intramolecular attraction have been isolated or discarded, measured $\mathrm{LogP_{o/w}}$, and by inference the component hydrophobic fragment constants and hydrophobic atom constants, are free-energy-like thermodynamic parameters. As $\mathbf{P_{o/w}}$ is an equilibrium constant for solute transfer between the two solvents:

$$\mathbf{\log P_{o/w} = -\Delta G / 2.303\, RT}, \quad (3)$$

where **R** and (generally) **T** are constants. Thus:

$$\mathbf{Log\ P_{o/w} = k\ \Delta G}, \quad (4)$$

where, for example, $\mathbf{k} \approx -0.733$ kcal.mol$^{-1}$ at 298 K. Since:

$$\mathbf{\Sigma a_i = Log\ P_{o/w}}, \quad (5)$$

then $\mathbf{a_i}$ (the hydrophobic atom constants including Leo factors [4]) are also directly related to $\mathbf{\Delta G}$, i.e. having both enthalpic and entropic components.

The $\mathbf{a_i}$ are dimensionless parameters directly related to the free energy of atom transfer (as a part of a specific solute molecule) between two solvents, water and 1-octanol. The suppositions inherent in the HINT model are: 1) these solvents are model environments for polar and hydrophobic regions, respectively, in biomacromolecules, e.g., proteins, enzymes or nucleotides; and 2) the free energy of atom 'transfer' between polar (hydrogen bonding) and hydrophobic regions of biomolecules is the same as that between water and 1-octanol. In other words, each atom's $\mathbf{a_i}$ encodes how it will interact with other atoms (polar, acidic, basic, hydrophobic, etc.), in (we assume) the same way the atom would interact with solvent molecules/atoms. The hydrophobic atom constants are used to characterize atoms from both the ligand and biomacromolecule in the case of small molecule binding, while the HINT algorithm (eq. 1) identifies and scores favourable and unfavourable matches of $\mathbf{a_i}$ vs. $\mathbf{a_j}$ for atoms **i** and **j**. For example, if $\mathbf{a_i}$ and $\mathbf{a_j}$ are both positive, implying that they are both hydrophobic, this is scored favourably as a hydrophobic interaction not unlike a lipophilic solute/1-octanol interaction in the shake flask. Similarly, if $\mathbf{a_i}$ and $\mathbf{a_j}$ are both negative, and one is a Lewis acid while the other is a Lewis base, this **i–j** interaction would also be scored favourably like a hydrogen bond donor or acceptor group on a ligand establishing interactions and solubility in water (*table I*). These scores have been shown ([20, 36–37] and unpublished data) to convincingly correlate with free energy. In effect, the free energy of association between two species in the biological environment is directly related to the sum of these scored matches.

Similarly, energetic effects related to solvation/desolvation must be implicit in $\mathrm{LogP_{o/w}}$ data. The free energy term that comprises $\mathrm{LogP_{o/w}}$ includes experimentally observed effects of water solvation such as water molecules being 'dragged' [38] into the 1-octanol layer by the solute and the entropy associated with the network of water molecules gaining and/or losing 'structure'. These effects are implicit in the hydrophobic fragment and atom constants, and cannot be numerically extracted from $\mathrm{LogP_{o/w}}$ data.

**Table I.** Matrix of atom–atom interaction types characterized and scored by the HINT algorithm.

| | **Hydrophobic** | **Polar:** Lewis acid (H-bond donor) | **Polar:** Lewis base (H-bond acceptor) |
|---|---|---|---|
| **Hydrophobic** | hydrophobic 'Interaction' | hydrophobic–polar (desolvation energy) | hydrophobic-polar (desolvation energy) |
| **Polar:** Lewis acid (H-bond donor) | hydrophobic–polar (desolvation energy) | Coulombic repulsion | acid–base (hydrogen bond) |
| **Polar:** Lewis base (H-bond acceptor) | hydrophobic–polar (desolvation energy) | acid–base (hydrogen bond) | Coulombic repulsion |

However, a portion of the energy of desolvation in an association event between two molecules can be sequestered. It is related to the HINT score arising from hydrophobic–polar interactions. This class of interaction occurs when polar atoms, either acidic or basic, are proximal to atoms with primarily hydrophobic character. While an unavoidable background of hydrophobic–polar interaction must exist in biomolecular systems that (always) have both polar and hydrophobic atoms, some hydrophobic–polar interactions are the result of polar atoms/groups forced into hydrophobic pockets or vice versa. HINT scores these interactions unfavourably (negatively), which in effect represents the energy cost to desolvate those polar groups and place them in an inhospitable (hydrophobic) environment. It is not known at this point what fraction of the HINT hydrophobic–polar interaction scores results from desolvation or other effects, but hydrophobic–polar interaction scores are generally the largest source of unfavourable interactions in HINT studies. In a sense these parts of the HINT model account for water that can be considered 'bulk' and its general effect on thermodynamics, dielectric, etc. A subsection below will describe treatment of structurally conserved water molecules.

### *2.3. Can fragment (and atom) hydrophobicity data represent electrostatics and hydrogen bonding?*

As noted above, one of the powerful attributes of the HINT model is that all interaction types are implicitly encoded in the model. The hydrophobic fragment constants, and likewise the atom constants, are very versatile thermodynamic parameters. *Figure 2* shows the result of plotting Leo hydrophobic fragment constants [4] against the absolute value of the fragment's charge (as calculated by the Gasteiger-Hückel algorithm of Sybyl) [39]. While the correlation is certainly improved by the small handful of highly polar fragments such as $COO^-$ and $NH_3^+$ (upper left), the trend is as expected – more polar groups are likely to be more charged. To add the sign of the (implicit) charge, HINT maintains a database of descriptors (generally –1, 0, +1) representing formal charge, Brønsted acid/base character, Lewis acid/base character, and hydrogen bonding donor/acceptor character indexed by atom potential types. These data are applied in a simple functional algorithm ($\mathbf{T_{ij}}$, eq. 1) whenever the sign of a charge is needed to describe an interaction. Thus, we believe that significant and quantitatively reasonable partial charge information is encoded in hydrophobic fragment constants, and that this information continues to be reliable in the hydrophobic atom constants, $\mathbf{a_i}$. Obviously, however, this charge information is not as robust or detailed as may be obtained by quantum chemical methods, specialized electrostatic potential codes, or even simple charge approximation methods.

Hydrogen bonds are the essential polar–polar interaction. The water solubility inherent in $LogP_{o/w}$ is largely due to the fact that water is both a superior hydrogen bond donor and hydrogen bond acceptor. Hydrogen

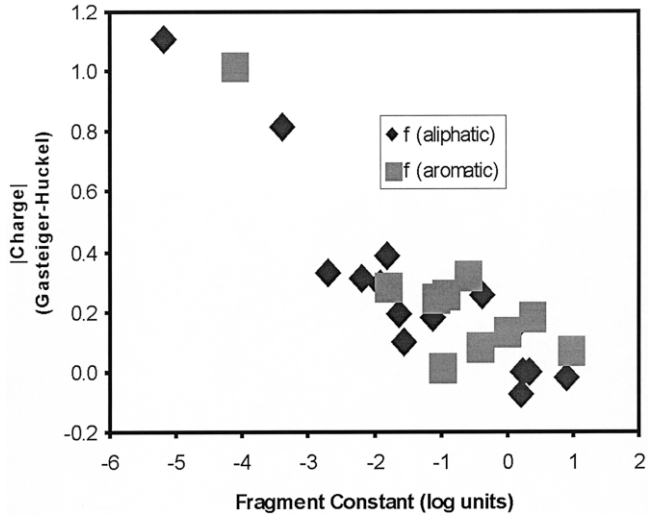


**Figure 2.** Correlation of absolute value of Gasteiger-Hückel charge with hydrophobic fragment constants from Hansch and Leo [4].

bonding does not have to be added to the model; it is a primary outcome of the experiment. In analytical terms, hydrogen bonds are just a special case of favourable (acid–base) Coulombic interactions. The best hydrogen bond donor and acceptor fragments are those that are most polar; i.e. having the most negative hydrophobic fragment constants. In the HINT model hydrogen bonding is promoted by assigning the hydrogen atoms differential hydrophobic atom constants scaled by their parent's intramolecular hydrogen bonding ability, as represented by Leo (CLOGP method) correction factors (0.0 for C; 0.6 for N,P; 1.0 for O,S), originally created to compensate for the effect of intramolecular hydrogen bonding on $LogP_{o/w}$ [4].

### *2.4. How do structurally-conserved waters contribute to free energy of association?*

One of the most intriguing and difficult issues of modelling in the biological environment is accounting for the effects of water. Water is crucial; not only because of its role as a potent hydrogen bond mediator, but also because significant entropy arises from displacement and/or movement of water in and out of the system. However, the modelling of water is difficult. Where should water be considered as bulk, which is conventionally dismissed by using a relatively large dielectric, and where should individual water molecules be explicitly modelled? HINT has some advantages over purely Newtonian simulation methods because of its derivation from experimental solvation data (vide supra). This advantage applies only to bulk water. Explicitly modelled water molecules that bridge between or within biological molecules continue to be necessary to fully define and characterize a biomolecular interaction with HINT ([37, 40] and unpublished data). It is not at all obvious which water molecules, even when they are located crystallographically, should be explicitly considered [41]. It should be pointed out that the GRID program of Peter Goodford [42] has excellent algorithms to locate energetically favourable loci for water molecules in biological systems. Despite the fact that water is one of the two solvents in the $LogP_{o/w}$ experiment, it has a measurable $LogP_{o/w}$ (–1.38) and corresponding hydrophobic atom constants. Thus, water molecules can be treated by the HINT model the same as any other molecule in terms of scoring interactions. In three component systems, e.g., receptor, ligand and water(s), the total score for interaction is as follows:

$$\mathbf{B_{TOTAL}} = \mathbf{B_{R-L}} + \mathbf{B_{RL-W}}, \quad (6)$$

where $\mathbf{B_{R-L}}$ is the total interaction between receptor and ligand and $\mathbf{B_{RL-W}}$ is the total interaction between the receptor–ligand complex and the water molecule(s). The inclusion of the water term is crucial for correctly modelling the free energy of association ([37] and unpublished data).

## 3. Results and discussion

We and others have undertaken a number of studies using HINT on a wide variety of systems as we have developed the model. The feedback we have received from users as we have designed, built and validated the HINT software has been crucial to its continuing emergence as a tool for understanding biomolecular structure. In this section we wish to review some of the published studies utilizing HINT. We want to highlight the work of some of our collaborators and colleagues as well as some of our own results. As we described in the Introduction, there are two limiting case paradigms for computational drug discovery: ligand-based drug design and structure-based drug design. Because we designed HINT to be a bridge between, and useful in, both of these approaches, the applications we report here seem to be best divided in this way.

### *3.1. HINT applications in ligand-based design*

One of our earliest applications of HINT was in creating a HINT hydropathic field to be incorporated in the exciting new (at the time) 3-D QSAR program called CoMFA [43] that was just becoming popular. Basic CoMFA had two fields, steric and electrostatic, and it was clear to us as medicinal chemists that a hydrophobic/hydropathic field was needed for CoMFA to fully describe drug binding and design new drugs. In our 1991 paper [18] we re-analysed the steroid data set that was reported in the original Cramer et al. CoMFA report [43] using a three-field (steric, electrostatic and hydropathic) methodology. This nascent study actually predicted much of the future work with HINT fields in CoMFA. We found little statistical advantage to the addition of a hydropathic field, probably for a variety of reasons. First, some quantity of the information in the HINT field is already present in the steric (i.e. van der Waals/London force) and electrostatic fields. We have discussed this above. Of course, we don't know exactly how much overlap there is. Second, the statistical PLS models severely penalize the addition of more variables to the analysis. By adding approximately 50% more independent variables, we are fighting an uphill battle to improve statistics. On the positive side, however, it turns out that the HINT hydropathic field and the resulting PLS coefficient fields from the analysis are quite easy to interpret, in chemical terms.

This is especially true for cases where there apparently is significant hydrophobic-driven binding, and can be especially useful when the goal of the QSAR study is to design new compounds. Finally, useful information can often be extracted from the HINT fields even if the statistical metrics of the three-field 3-D QSAR models are poorer than the steric/electrostatic models.

There have been numerous CoMFA reports [44–69] published using the HINT field since our first paper [18]. As an aside, that paper has been referenced over 100 times to date, for the most part because it was one of the earlier papers describing the use of CoMFA. Debnath [44, 45], in a detailed 3-D QSAR study of cyclic urea derivatives as potential HIV-1 inhibitors, created several QSAR models utilizing the standard steric and electrostatic CoMFA and the HINT field. While it did not have the best $r^2$, the QSAR model with the best $q^2$ (cross-validated $r^2$), was one derived from only the HINT field. This model also had the lowest number of components. Three-field models had little statistical advantage over the two-field models and were not pursued further despite the HINT field contribution of greater than 20%. It appears in this study that the bulk of HINT field contribution is coming at the expense of the steric field, as the electrostatic field maintains its contribution at 43–49% to the resulting model. This is not always the case, however, as the HINT field does in some data sets extract from the CoMFA electrostatic field.

Pajeva and Wiese have used the HINT hydropathic field in several CoMFA studies [46–49]. Recently they have reported 3-D QSAR studies on multidrug resistance modifiers based on phenothiazines and related compounds [46]. Cross-validated statistics ($q^2$) as high as 0.93 were reported when the HINT field was combined with the steric and electrostatic fields. More importantly, however, is the observation that hydrophobicity is a molecular property that significantly influences multidrug resistance reversals. Furthermore, describing hydrophobicity as a space-directed molecular property, i.e. a field, is preferable to the use of scalar ($LogP_{o/w}$) representations of hydrophobicity. This particular activity involves penetration/crossing of membranes, which is usually regarded as a function of hydrophobicity.

Our approach to 3-D field-based QSAR is to use a ‘palette’ of fields [50], from which the scientist can choose depending on the type of activity associated with the data set. Certainly such a field set should contain a hydrophobic field, although it will certainly not always be part of the best final model. It is, in fact, an oversimplification of the true nature of binding events to ascribe discrete interaction types, e.g., steric, electrostatic, hydrogen bonding, hydrophobic, etc. to the process. However, characterizing binding with these types of terms is a useful mathematical construct for our purposes. In our opinion, the most important aspect of QSAR models, particularly three-dimensional models, is interpretability. That is, finding the model useful for understanding the biological action of a series of drugs, and being able to use this information for further design and development. In that sense, the choice of fields for a 3-D QSAR model should be driven by practical, chemical concerns, not just statistical metrics.

### *3.2. HINT applications in structure-based design*

The very first application of the HINT model, and in fact the purpose behind writing the program, was in examining and understanding X-ray crystallography and biological activity data for compounds binding in the central water cavity of haemoglobin [17]. We had four qualitatively similar classes of compounds that bind in a similar manner to haemoglobin, but have significant variance in their biological activity as measured by the right shift parameter $P_{50}{}^{d}/P_{50}{}^{c}$ (where $P_{50}$ is the partial pressure of oxygen that results in 50% oxygenation of haemoglobin, d = drug, c = control). The most striking effect is that two of these classes differ only in the order of a linker $–CH_2NHCO–$ vs. $–CH_2CONH–$ between the two aromatic rings of the compounds but have an average right shift difference of more than one. Careful analysis of the ligand–protein interactions guided by the HINT model revealed and quantified the specific protein–drug interactions and showed that because the linker order change also caused the carbonyls to be directed antiparallel, the loss of a hydrogen bonding interaction with Lys 99α is probably responsible for the observed right shift difference. However, even more subtle effects were detected. For example, a longer chain molecule that had an inserted methylene in the linker, had poorer hydrophobic interactions between one of the phenyl rings and Phe 36α. In addition, the HINT model recognized as a favourable interaction the weak hydrogen bond force between donors such as $–NH_2$ and the π cloud of aromatic rings [22, 70]. In particular there is an interaction of this nature between Asn 108β and the ligand(s) [17]. The continuing development of these molecules has led to a new class of haemoglobin allosteric effector drug that is currently proceeding through clinical evaluation. Even in the beginning with HINT, now about ten years ago, we recognized the power of the information inherent in $LogP_{o/w}$, but also the critical importance of high quality structural data. We also found in these studies the first correlations between HINT score and binding affinity data [19].

Since then, we ([20, 36–37, 40, 71–78] and unpublished data) and others [63, 79–85], have published several studies that utilize HINT to analyse biomolecular complex structures. Wang et al. [79] have recently examined the binding of indolactam-V to protein kinase C with docking and scoring simulations. HINT was used to quantitate the location and magnitude of hydrophobic interactions between the indolactam-V ligand and protein kinase C. They did not, however, characterize the relative contribution of hydrophobic and other interactions in this system. Mozzarelli and co-workers have examined several ligand/protein systems with HINT (unpublished data), and have found fairly good linear correlations between total HINT score, $\mathbf{\Sigma b_{ij}}$ (eq. 1), and binding affinities for these systems.

Gussio, Zaharevitz and Pattabiraman have developed elegant technology for data base searching, virtual screening and lead evolution using the total HINT score as a fitness function or as the key parameters of a QSAR. They have found that multilinear regressions built from partial HINT score sums differentiated by interaction type, i.e. hydrogen bond, hydrophobic, acid–base, etc., can form the basis of quite robust and predictive QSARs. First with HIV-1 reverse transcriptase inhibitors [71–73], and more recently with cyclin-dependent kinase inhibitors [74], well-designed searches of the NCI database, followed by a battery of carefully executed in silico screens (both of which utilize HINT technology), have yielded new lead compounds for further studies. In this latter work, several derivatives of a new class of compounds, called the paullones, were thoroughly modelled with hydropathic (HINT), molecular mechanics, and quantum mechanics to identify a highly active compound, 9-cyanopaullone, which upon synthesis and testing was shown to have an $IC_{50}$ of 24 nM in the p34cdc2/histone H1 kinase assay [74]. Utilizing this unique combination of computational techniques was key to the success of this study.

We have been interested in the binding of doxorubicin and analogues to DNA oligomers. This was investigated by constructing and structure optimizing ligand-bound (doxorubicin) models for the 64 pyrimidine-(3′5′)-purine tetrameric sequence variants [75]. Each of these were then analysed with the HINT model scoring function. The CAAT quartet sequence was shown to have the highest binding score of the 64 combinations, and in general CAX and TAX triplets have the highest scores. Interestingly, although interactions of the chromophore with the DNA base pairs on either side of the intercalation site [I – 1; I + 1], and the neighbouring [I + 2] base pair, are predominant, the results obtained with HINT indicated that the base pair [I + 3] contributes significantly to the sequence selectivity of doxorubicin by providing an additional hydrogen bonding opportunity for the N3′ ammonium of the doxorubicin (daunosamine) sugar moiety in about a quarter of the sequences [75]. This observation, that interactions involving a base pair [I + 3] distal to the intercalation site play a significant role in stabilizing/destabilizing the intercalation of doxorubicin into the various DNA sequences, had not been previously reported experimentally [86] or theoretically [87]. We have also reported a study of 24 doxorubicin analogues [36], some of which have clinical utility, in a smaller set of DNA quartets (CAAT, CAAG, CGAT and CGAG). We are beginning to develop a composite pharmacophore model relating specific chemical features of the drugs to their effects on specific base pairs. This may lead to the design of DNA intercalating agents that can selectively target certain DNA triplet or quartet sequences. It is important to note that the available experimental data for sequence specificity doxorubicin and analogues are inconclusive due to difficulties in procedure and uncertainties arising from preferential binding to the oligonucleotide ends [86, 88–90]. Because of these issues, our computational results may actually be more representative of DNA intercalator sequence structure and specificity than can be obtained by experiment.

### *3.3. Is $LogP_{o/w}$ more than the sum of its parts?*

As mentioned above, $LogP_{o/w}$ for small molecules is easily estimated with a large variety of computational algorithms. These can generally be separated into two major classes: those based on the summation of fragment values (with sets of structural factors), or those based on the summation of atomic values. Both types of methods can yield good estimates of $LogP_{o/w}$, and for series of closely related compounds, e.g., with common chemical templates, all methods yield excellent relative $LogP_{o/w}$ estimates. We believe that working with just the numerical value of $LogP_{o/w}$ is under-utilizing the potential of this data source – its thermodynamic information content is significantly more important and useful. Much of our work in validating the HINT model has been performed in detailed studies of native and mutant haemoglobins, for which high quality X-ray structural [91–93] and thermochemical data [94–96] are available. This is, of course, an extremely well-characterized system, but one with fascinating properties such as allostery, and significant historical information relating site mutations to disease states. In this biomolecular system we have developed a simple HINT-based computational methodology that gives persuasive predictions of free energy changes due to single-site mutations. We calculate inter-

dimer HINT scores and using a simple conversion factor, ca. –515 HINT score units per kcal.mol$^{-1}$, estimate the free energy of dimer–tetramer assembly, $\Delta\Delta G_{D \rightarrow T}$. Key in obtaining accurate estimates has been the careful consideration of structurally relevant water molecules at the interfaces [37].

In a recent study we have examined mutants that have not been crystallographically characterized. *Figure 3* sets out a correlation between measured and estimated $\Delta\Delta G_{D \rightarrow T}$ for 23 deoxy (T-state) and 23 oxy (R-state) mutants. The few outliers, notably from oxy mutants, may be the result of unknown structural perturbations. There is precedence for this, as haemoglobin Ypslanti (Asp99β→Tyr) has been observed in a different crystal form, called R2, in the oxy state [97]. The correlation of *figure 3* is a remarkable result: mutant protein structures, built from the native crystallographic coordinates with molecular mechanics, are analysed with hydropathic parameters derived from solubility measurements of small organic molecules. Thus are derived free energy estimates that compare favourably with solution phase thermochemical measurements of dimer–tetramer assembly! One would have to agree that there certainly is significant thermodynamic information in $LogP_{o/w}$, and that it is more rich and complex than previously presumed. $LogP_{o/w}$ does apply to the relatively simple questions of solubility and membrane transport, but also provides a unique insight into understanding the general free energy of biomolecular associations. While the controversy over discovering the best statistical method for estimating $LogP_{o/w}$ continues, broad issues such as the real meaning of $LogP_{o/w}$ and hydropathy need further exploration.

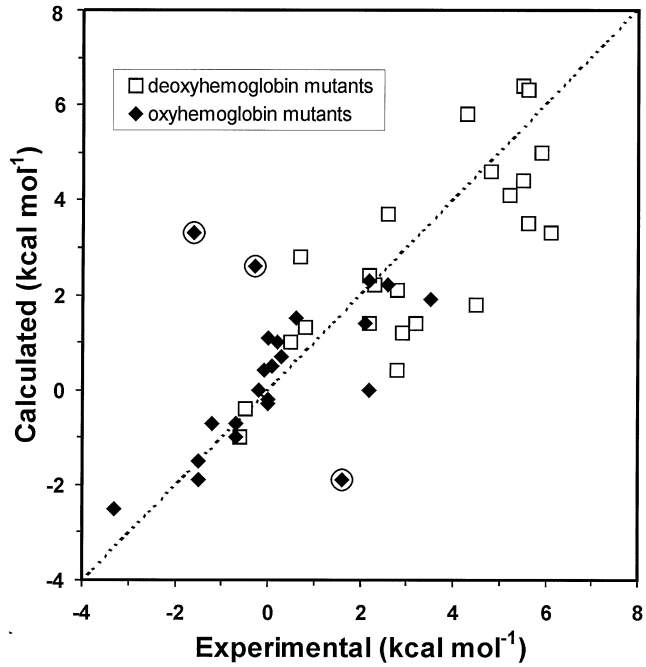


**Figure 3.** Correlation between calculated (from HINT structural analysis) and experimental [94] ΔΔG for dimer–dimer association. Data from deoxyhaemoglobin mutants (squares) have a correlation coefficient (r) of 0.79. Data from (all) oxyhaemoglobin mutants (diamonds) have a correlation coefficient of 0.48; however, removal of outliers (circled) yields a correlation of r = 0.86. The outliers can be rationally discarded with structural arguments. The dashed line indicates perfect correlation, where the slope would be 1.

## 4. Summary and future directions

We are interested in understanding and exploiting biological structure as related to developing new disease treatments. Ligand binding, protein–protein associations, protein–DNA associations etc. are crucial components of structure-based and ligand-based drug design strategies. We are developing a simple computational model based on an experimental free energy measurement: $LogP_{o/w}$, the partition coefficient for octanol/water solubility. $LogP_{o/w}$, as a key parameter in QSAR, has been shown to often correlate with ligand binding. $LogP_{o/w}$ and its component fragment and atom terms reveal the type of interactions that the molecule/fragment/atom are able to make with another species. Values less than zero suggest a polar (hydrophilic) species that would best interact with a polar environment containing hydrogen bond donors and/or acceptors. Positive values represent a hydrophobic moiety. The magnitude of these constants is indicative of the strength of potential interaction. In addition, since the thermodynamic data encoded in $LogP_{o/w}$ is free energy, there is useful, although not necessarily independently extractable, information about entropy and solvation in the hydrophobic atom constants and fragment constants. Our developments of the HINT model have focused on this expansive information content from $LogP_{o/w}$. In this paper we have described how many of the fundamental biomolecular non-covalent interactions associated with drug binding, protein–protein associations, etc. are represented and modelled by the HINT paradigm. Numerous investigations in a score of labs have demonstrated the utility of the methods included in the HINT model.

However, a limitation of HINT actually provides an opportunity for exploration, and will be the direction of our future developments of the model. The molecular mechanics force field methodology required to create and optimize molecular models is often at odds with the intuitive logic of HINT. Two significant classes of inter-

actions can be, according to the HINT model, incorrectly calculated by these force fields. First, hydrophobic–hydrophobic interactions are calculated as favourable by molecular mechanics only insofar as the van der Waals term contributes. The Coulombic electrostatic term is actually in this case repulsive, as hydrophobic groups most often have the same sign of partial charge. Second, many interactions that HINT classifies as unfavourable hydrophobic–polar are according to molecular mechanics electrostatically attractive. For example, carbonyl or carboxylate oxygens have negative partial charges and are thus electrostatically attracted to hydrophobic groups which usually have a small positive partial charge. These apparent systematic errors of molecular mechanics force field methods are fairly small but significant in cases where subtle effects drive the structure formation, such as protein folding. More of a long term concern, however, is that the evolution in crystallographic structure solution and refinement software has increasingly incorporated molecular mechanics force fields that are now introducing this kind of bias into 'experimental' macromolecular structures. We plan to develop a new generation of HINT model that includes structure optimization protocols [98]. Our first step toward that goal is a water (solvent) optimization algorithm, in the current versions of the program that utilizes the HINT score as the 'energy' term in a pseudo-force field.

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