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Computational study of ligand binding to protein receptors

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ABSTRACT

We have determined, for the first time, the enthalpic contributions to the energy change associated with ligand reorganization (LR) upon the binding of the same ligand to multiple sites within human serum albumine (HSA). Quantum mechanics based density functional theory (DFT) has been used for the LR calculations, which provides much better accuracy than previously used molecular mechanics methods (MM). Our findings show that for some ligands these enthalpic contributions can be attributed to specific structural and conformational changes.

1. Introduction

The source of activity of most biologically active compounds (ligands) is their binding to a protein receptor. This binding often proceeds with significant changes in the molecular structure of the ligand itself as well as the receptor. The change in the ligand's molecular structure is accompanied by change in the ligand's total energy which includes electronic, vibrational and possibly rotational contributions. The change in electronic energy can be expected to make the largest contribution because it involves changes to chemical bonding (e.g. changes in bond lengths and angles) which is the strongest intramolecular interaction present. The other two contributions can be considered to be much smaller due to the fact that vibrational and rotational energy levels are much more closely spaced than the electronic energy levels. When the ligand binds to the receptor there are enthalpy and entropy changes associated with the process [1,2]. Enthalpy changes include the conformational changes which are required to enable the ligand molecule to better "fit" its receptor i.e. to form various non-bonded interactions with the receptor. It has long been recognized [2] that neither the global energy minimum in the gas phase nor the single crystal structure of the ligand molecule are good representations of the structure which is realized upon binding to the receptor. One must also take into account possible intra- and intermolecular non-bonded interactions between the ligand and the receptor. This is difficult to do in a quantitative manner because of the large size of most receptors which would require excessive computer resources. Therefore these enthalpic contributions are usually investigated by using molecular mechanics (MM) [3] rather than quantum mechanics (QM). When methods of quantum mechanics have been used [2] they have been used in the form of semiempirical methods (SE) which do not perform well outside their parametrization range. Furthermore, SE methods have been employed for the calculation of only a small subset of the ligands studied, the rest being treated via MM [2]. MM methods are even more restricted by the embedded parametrization and in addition are incapable of describing charged species. Many ligands (see below) bind as ions and cannot be adequately described by MM methods. The magnitude of some (but not all) enthalpic contributions e.g. hydrogen bonding between ligand and receptor, can in principle be determined experimentally. For example, by using solid state NMR one can measure the magnitude of chemical shifts for elements involved in hydrogen bonding which reflects the energy (strength) of such a bond [5]. However, for large ligand–receptor complexes even these experimental methods may fail to give quantitative results due to the large number of overlapping NMR peaks. Besides enthalpic factors, one needs to consider entropic influences [1,4]. Entropic influences arise, for example, from rearrangement of solvent molecules around the ligand which takes place upon binding. The net effect of both contributions must be a stabilizing one: initial deformation of the ligand molecule is an endothermic process but this is compensated for by the establishment of hydrogen bonds and other non-covalent interactions between the ligand and receptor.

It is not feasible to determine experimentally enthalpic and entropic contributions independently so recourse must be made to computational methods. We are interested in the enthalpic contributions stemming from ligand deformation. We also wished to determine which structural changes are associated with particular enthalpic contribution.

2. Materials and methods

We have used a non-empirical, density functional method (DFT) [6] implemented in Gaussian 03 program [7] to study the

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dependence of total electron energies of ligands on their molecular structure. We selected the hybrid B1B95 functional and 6-31 + G* basis set for our calculations because according to a recent report [8] this combination provides the best description of conformational energies. The lowest average error for this functional/basis has been found to be at least 8 kJ/mol, so the energy differences pertaining to LR <10 kJ/mol are not considered sufficiently significant to be included in the discussion.

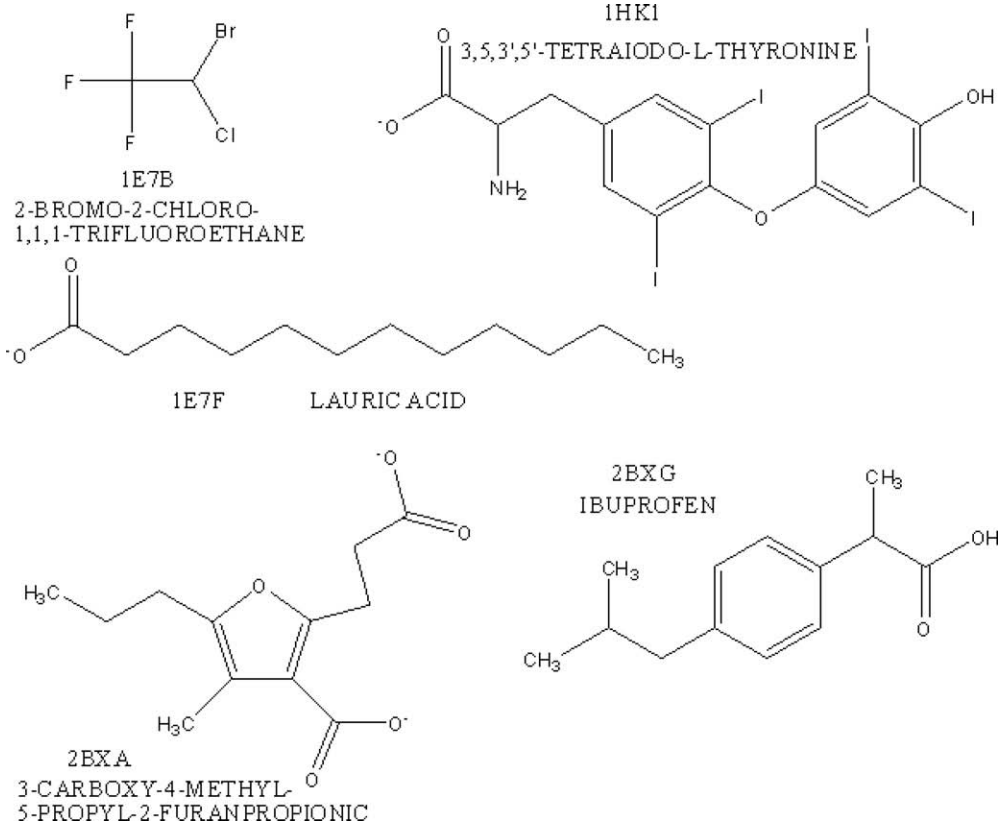
We have selected 12 ligands for this study which bind to multiple sites of the albumin receptor. The molecular structure of each bound ligand (configuration) has been taken from the crystallographic data available in the RSCB Protein Data Bank (PDB) and a single point DFT calculation of total electron energy was performed for that structure. The energies thus obtained (relative LRE) are then "normalized" to the energy of the lowest configuration which is assumed to be zero. This approach, while providing only relative energies of different configurations, allows for compensation of errors in total energy inherent in the used functional (DFT). Furthermore the approach avoids the need for specifying the most stable, reference structure. Even if complete geometry optimization is made and its total energy calculated to obtain global minimum it is not clear whether such reference structure is useful. One may of course calculate the energy difference between global minimum and structure of the bound ligand, but such value does not bear any simple relationship to the binding affinity. This is because the initial state of ligand binding process does not comprise free molecules with identical energies, but consists of the Boltzmann type population of solvated molecules, each with different solvation geometry and different solvation energy. Likewise, the final state of this process may not be well defined. Is the bound ligand molecule to be considered a free molecule, partially solvated molecule or just free molecule existing in the net dielectric medium posed by the receptor cavity? Binding affinity is related to the energy difference between these states. When calculating relative LR energies i.e. energies of the same ligand bound at different sites, such ambiguities are minimized. The population of solvated molecules is not the reference point and cavity effects can be expected to largely cancel out.

The hydrogen atoms for each molecule are added using GaussView software [9] because crystallographic data do not give hydrogen atom positions. Data regarding different structures of the same ligand bound to different albumin sites were labeled in our paper by four alphanumerics which are assigned by the PDB database [10]. The letter which follows refers to the sequence in which these different structures are listed in the PDB file not to the specific receptor site. Ambiguous ligand structures i.e. those for which crystallographic data are incomplete or inconsistent were not used in our study. Ligands whose geometry of the carboxylic group shows equal C–O bond lengths and differs considerably from the corresponding geometry in acetic [11], propanoic [12] or other carboxylic acids were assumed to bind in ionized form i.e. as carboxylate anions.

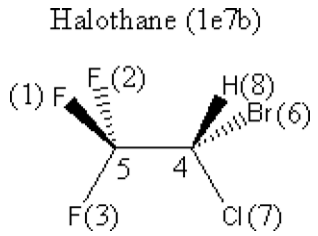
3. Results and discussion

We focused our attention on 12 albumin bound ligands, 5 of which exhibit large (>40 kJ/mol) variation in LR. The five ligands analyzed are shown in Scheme 1 and will be discussed individually.

The binding affinity of drugs for albumin receptor had been studied previously [13] but only a single value was reported; the value does not distinguish between affinities for different sites. Since no experimental data exist on binding affinities to different sites of the same receptor we did not attempt such calculations because we would not be able check their reliability.



Scheme 1.



Scheme 2. Numbering scheme and molecular structure of bound **1e7b**.

3.1. ***1e7b***—2-bromo-2chloro-1,1,1-trifluoroethane (halothane)-1E7B

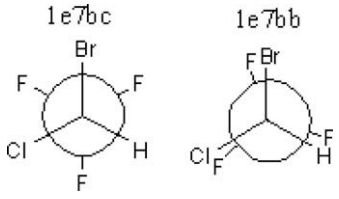
The ligand (Scheme 2) is an important general anesthetic and it can bind to three different sites on albumin as determined by Bhattacharya et al. [14]. The calculated relative energies (LRE) for the three bound ligands (based on the geometry obtained from X-ray data) are given in the Table 1 below. We note that the bound conformation of the ligand in the highest energy configuration is an eclipsed one.

The bulk of differences in LRE for halothane ligands can be accounted for by the conformational energy barrier pertaining to the rotation around the central C–C bond. This conclusion is based on the calculated torsional potential in halothane [15] which gives the value of 21 kJ/mol as the energy barrier for rotation. This value is comparable in magnitude, but smaller than the 39.2 kJ/mol obtained in our calculations for **1e7b**. The discrepancy may be due to the fact that Scharf and Laasonen [15] used different, lower level DFT functional (LDA) and also because their calculations were done for liquid halothane using methods of molecular dynamics.

The Newman projections of the two halothane configurations as bound to different albumin sites are shown in Scheme 3.

Table 1

Ligand	Relative LR (kJ/mol)
1e7ba	8.6
1e7bb	39.2
1e7bc	0



Scheme 3.

Table 2

Ligand				Relative LR (kJ/mol)
1e7flb				61.2
1e7fld				87.7
1e7flf				0.0
Bond	–COOH bond lengths			Lauric acid [16]
	1e7ff	**1e7fb**	**1e7fd**	
O1–C3	1.25	1.25	1.25	1.38
O2–C3	1.25	1.25	1.25	1.17

3.2. ***1e7f***—lauric acid

Lauric acid (Scheme 4) is a fatty acid that binds as a carboxylate anion to six different sites on HSA as determined by Bhattacharya et al. [16]. For three of these sites the X-ray data for ligand structures are incomplete and these three sites have been excluded from our study. The relative LR of the ligand bound to the three remaining sites are given in Table 2. The same Table also shows that this ligand binds in anionic form. This conclusion is supported by the experimentally determined C–O bond lengths of the carboxylic group; both distances are the same. In the free lauric acid the corresponding bond lengths are distinctly different [17].

Relative energies of **1e7f** when bound at different receptor sites can be attributed predominantly to conformational changes generated by rotations around CC bonds in the alkyl chain. We approximate the rotation barrier around each CC bond in **1e7f** by the torsional barriers in *n*-butane. The heights of rotational barriers in *n*-butane have been determined computationally [18] and experimentally [19]. Each CC bond in **1e7f** is examined to determine its conformation, the appropriate conformation energy is assigned to it and the energies are then added for the whole molecule. The sums of appropriate rotational barriers [18] around individual CC bonds are given in Table 3 and suggest that the argument regarding dominant contribution of conformational energies to LR is plausible.

The differences in conformation energies account for only a part of relative energies.

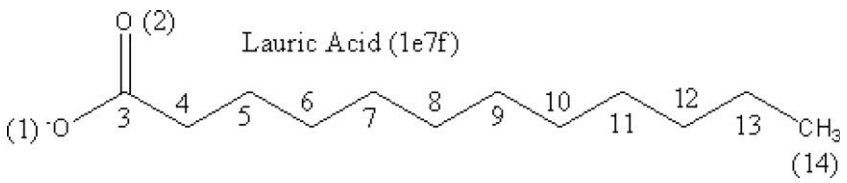
While there are no changes in bond lengths > 0.01 Å in **1e7f** configurations the accumulation of small bond length and bond angle differences may account for the remaining difference in LRE.

3.3. ***1hk1***—thyroxine (3,5,3′,5′-tetraiodo-L-thyronine)

Thyroxine (Scheme 5) is an important hormone necessary for the physiological regulation of growth and metabolism. It binds as an anion to 4 sites on HSA as determined by Petitpas et al. [20]. The relative LRE values determined for the different binding sites of thyroxine are shown in the Table 4 below.

Some of the LRE in **1hk1** can be related to conformational changes induced by the rotation around the C7–C8 bond. **1hk1b** conformer (eclipsed) requires less reorganization energy than **1hk1c** (staggered). This is surprising because eclipsed conformers have higher energies than staggered ones.

A possible explanation for this anomaly could be the presence of steric hindrance between the amino group and the neighboring aromatic ring. Although it may be energy costly for **1hk1b** to reach an eclipsed conformation, this conformation positions the amine



Scheme 4. Numbering scheme and molecular structure of bound **1e7f**.

Table 3

Conformation	1e7ff	1e7fb	1e7fd
Anti	6	6	4
Eclipsed	1	3	5
Gauche	2	0	0
Syn	0	0	0
Relative LR (kJ)	0	61.2	87.7
Energy accounted for in conformational energy (kJ)	20.3	38.4	64

Conformation	Butane conformer energies ΔH/kJmol^{-1}
Anti	0
Eclipsed	12.8
Gauche	3.77
Syn	22.8

group away from the neighboring aromatic ring. On the other hand, in **1hk1c** although the conformation along C7–C8 bond is staggered and thus energetically more favorable, the amine group approaches the aromatic ring more closely as is shown in Scheme 6.

The angle between the two aromatic rings is also different in **1hk1b** and **1hk1c**. C11–C10–O17–C4 dihedral angles for **1hk1b** and **1hk1c** are 76.6° and 95.8°, respectively. This has the consequence of pushing bulky iodine atoms I23 and I21 in **1hk1c** closer to the ring B than in **1hk1b** as shown in Scheme 7. The resulting steric crowding may contribute to higher relative LRE of thyroxine when bound to the site C.

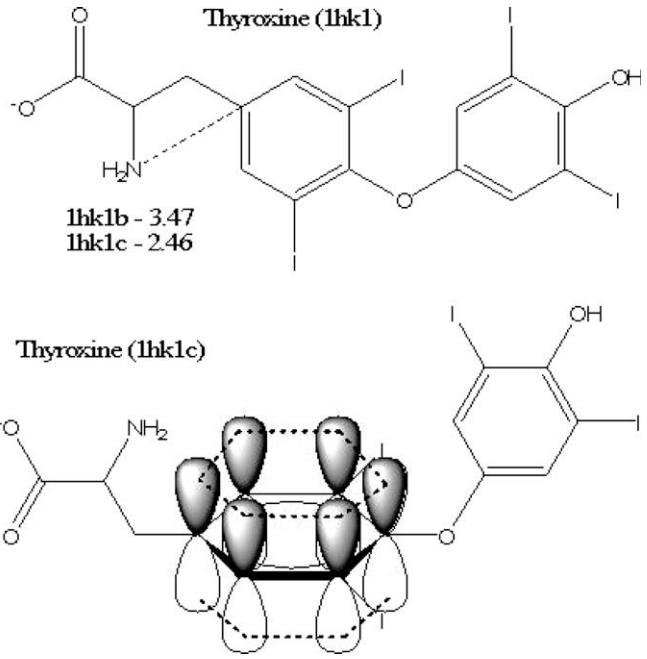
3.4. ***2bxa***—*3-carboxy-4-methyl-5propyl-2-furanpropionic acid*

2bxa (3-carboxy-4-methyl-5-propyl-2-furanpropionic acid; Scheme 8) is a metabolite commonly found in the human body and urine. Furanpropanoic acid is found to bind to HSA as a dicarboxylate anion. Furanpropanoic acid binds to 4 sites on HSA by Ghuman et al. [21]. The relative LRE of furanpropanoic acid in configurations associated with different binding sites at HSA as determined by X-ray diffraction are shown in Table 5.

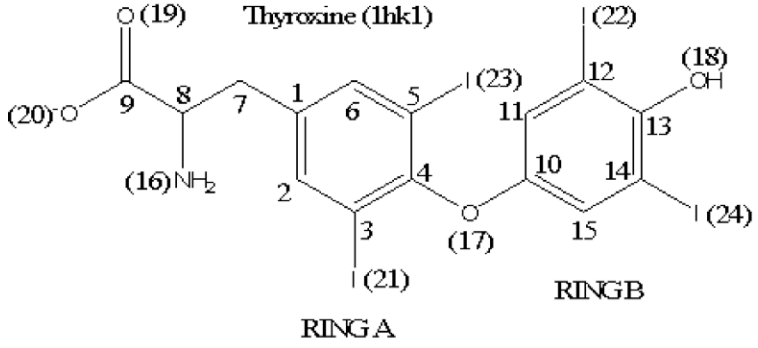
Some of the LRE of furanpropanoic acid are due to conformational rotation around the C9–C11 bond as shown in Table 6 and Scheme 8 There is no significant conformational change around the C8–C4 bond.

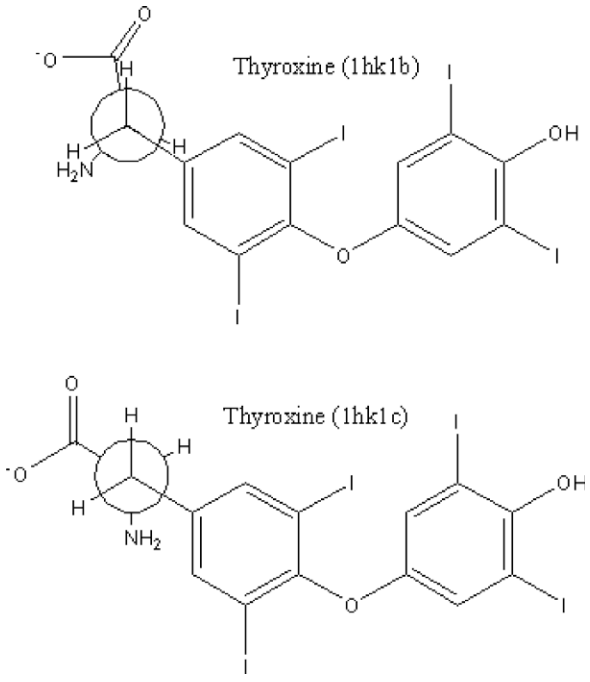
Table 4

Ligand	Relative LR (kJ/mol)
1hkla	71.5
1hklb	0
1hklc	111
1hkd	58.6

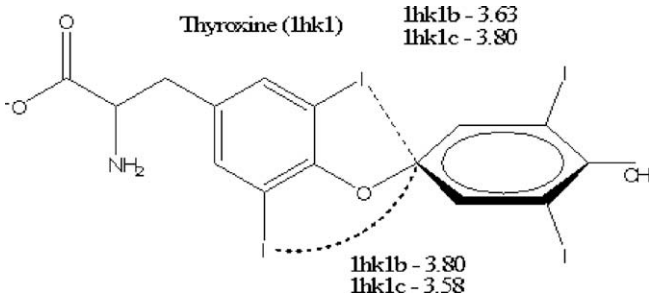


Scheme 6. Molecular structures of **1hk1** with reference to the relative positions of NH_2 vs. the aromatic ring.





Scheme 5. Numbering scheme and molecular structures of bound **1hk1**.



Scheme 7. Positioning of two rings in thyroxine.

Table 5

Ligand	Relative LR (kJ/mol)
2bxala	0
2bxalb	44.6
2bxalc	20.5
2bxald	31.2

Bond	Bond lengths (Å)		Propanoic acid [12]	Acetic acid [11]
	2bxas	**2bxac**		
O13–C14	1.22	1.21	1.23	1.24
O15–C14	1.23	1.22	1.32	1.29
O16–C12	1.23	1.22		
O17–C12	1.23	1.24		

Table 6

Bond	**2bxaa**	**2bxab**
C8–C4	Anti	Anti
C9–C11	Gauche	Eclipsed
Relative LR (kJ/mol)	0	44.6

In butane, the two torsional energy barriers around the central CC bond are 9.03 and 19.03 kJmol^{-1} [18] which are well below the energy difference between **2bxaa** and **2bxab** configurations. The analogous torsional energy barrier around the C9–C11 bond in **2bxaa** is likely to be higher due to the bulky nature of the furane ring. The bulk of the LR energy variation may occur due to bond deformation occurring within the heteroaromatic ring, particularly the stretching of the C3–O6 bond by 0.02 Å.

3.5. *2bxg—ibuprofen*

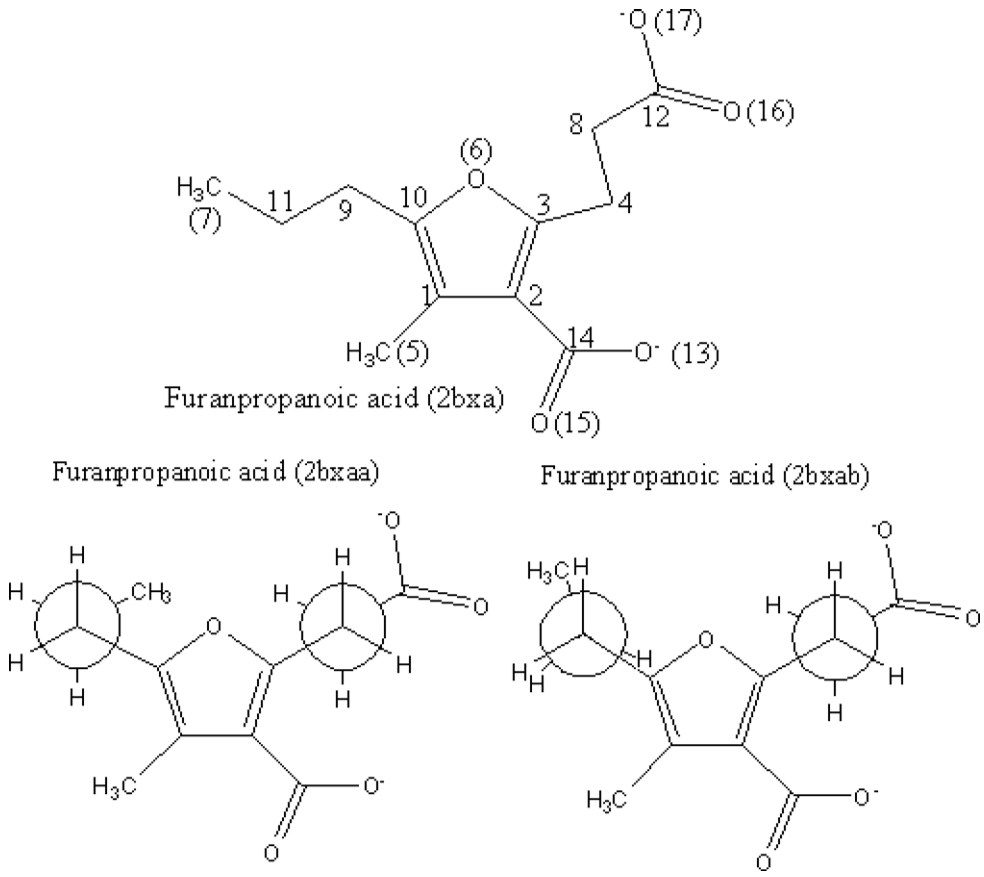
Ibuprofen (Scheme 9) is a commonly used non-steroidal anti-inflammatory drug (NSAID). The Ibuprofen molecule is found to bind in protonated, nonionized form to 4 sites on HSA by Ghuman et al. [21]. The non-ionised form is inferred from the C1–O15 and C1–O14 bond lengths as shown in Table 7. The relative LRE of ibuprofen configurations at different sites of HSA are shown in Table 7.

How can we account for relative LRE difference between **2bxga** and **2bxgb** configurations? Data in Table 8 indicate that the geometry of the aromatic ring differs considerably for the two configurations. The ring of the bound ligand is also considerably larger than in the free ibuprofen molecule [22].

In order to quantify these changes we have attempted to estimate the energy change associated with the stretching of several bonds for which X-ray data indicate largest changes.

The procedure is outlined below and data summarized in Table 9. In Table 9 the energy change associated with the change in particular bond length occurring when ligand binds to a receptor was determined by the following formula (where $r_2 > r_1$)

$$E = \frac{1}{2}k(r_2 - r_e)^2 - \frac{1}{2}k(r_1 - r_e)^2 \quad (1)$$



Scheme 8. Numbering scheme and molecular structures of bound **2bxa**.

Table 7

Ligand	Relative LR (kJ/mol)
2bxga	0
2bxgb	87.3
2bxgc	59.2
2bxgd	58.8

Bond	Bond lengths		Propanoic acid [12]	Acetic acid	Ibuprofen molecule B [21]	Ibuprofen molecule A
	2bxga	**2bxgb**				
C1–O15	1.25	1.23	1.24	1.24	1.22	1.23
Cl–O14	1.31	1.30	1.32	1.29	1.30	1.30

In deriving this equation we assumed that the harmonic potential satisfactorily describes bond length changes. In Eq. (1) E is the potential energy change pertaining to change in the bond length of a specific bond X–Y. XY bond length of a free ligand molecule is r_e; upon binding to the first receptor site this bond length changes to the value r_1, and upon binding to the second site the XY bond length assumes the value r_2. The experimental bond length obtained for a small molecule with the same bond type was used to approximate r_e. Eq. (1) was then used to estimate contribution to LRE from bond length changes in XY. Adding the contributions from individual bonds gives total estimated LRE variation on going from **2bxga** to **2bxgb**. Force constants for particular bonds were taken from Fadini et al. [23] and reference bond lengths (r_e) from Harmony et al. [24].

Our analysis demonstrates that approximately one third of the LRE difference between **2bxga** and **2bxgb** comes from bond length changes. Force constants for angle deformation are smaller, nonetheless their inclusion may recover even more of the LRE difference.

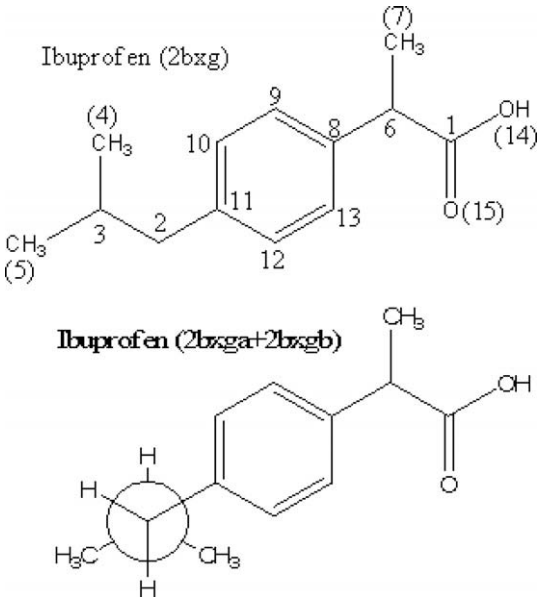
We have also considered possible conformations of the C2–C3 bond (Scheme 9). Conformations in both **2bxaa** and **2bxab** configurations are staggered so the conformational energies cannot contribute significantly to relative LRE.

Table 8

Bond	Bond lengths		Ibuprofen molecule A [21]	Ibuprofen molecule B [21]
	2bxga	**2bxgb**		
C12–C13	1.41	1.46	1.38	1.38
C10–C11	1.46	1.45	1.38	1.39
C9–C10	1.43	1.45	1.39	1.38
C8–C13	1.44	1.46	1.3S	1.38
C8–C9	1.43	1.44	1.38	1.38
C8–C9	1.43	1.44	1.38	1.34

Table 9

Bond	Bond lengths (Å)		Standard bond length (Å)	Bond force constant (N/m)	Energy difference (kJ/mol}	
	2bxga	**2bxgb**				
C(12)–C(13)	1.41	1.46	1.40	760		8.35
C(ll)–C(12)	1.41	1.45	1.40	760		6.50
C(9)–C(10)	1.43	1.45	1.40	760		3.55
C(8)–C{13)	1.44	1.46	1.40	760		4.70
C(6)–C(S)	1.53	1.59	1.53	440		6.25
C(6)–C(7)	1.47	1.50	1.53	441		−3.26
C(3)–C(4)	1.51	1.53	1.53	440		−0.52
C(2)–C(ll)	1.51	1.56	1.53	440		−1.55
C(2)–C(3)	1.52	1.55	1.53	440		1.01
					Total energy (kJ/mol)	25.07



Scheme 9. Numbering scheme and molecular structure of bound **2bxg**.

4. Conclusion

We have used a quantum mechanical method to investigate relative energies of ligands bound to different active sites of the same receptor. The quantum mechanical method is generally more accurate than the previously used molecular mechanics methods and it can account for charged species (ions) while MM methods cannot. This is important in view of the observation that many ligands bind to receptors in ionized form e.g. as carboxylate anions. We have succeeded in attributing some differences in LRE to specific geometrical changes which occur when the ligand molecule binds to different sites at the same receptor. For some ligands, however we could not identify the single most important geometry change but have shown that accumulation of small changes in the multitude of geometrical parameters adds up to significant LRE differences between the ligand configurations.

To the best of our knowledge this work is the first attempt to separate enthalpic from total energy changes pertaining to the ligand binding. This is also the first report which attributes given enthalpic change to specific structural changes in the ligand.

Acknowledgment

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