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Commentary

Recognition forces in ligand–protein complexes: Blending information from different sources

Giuseppe Ermondi *, *Giulia Caron*

Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, Via P. Giuria 9, I 10125 Torino, Italy

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Abbreviations:
α, mean polarizability
aq, aqueous state
COX-1, cyclooxygenase isoenzyme
CSD, Cambridge Structural Database
E, internal energy
E', electric field
$F(r)$, force
G, Gibbs free energy
g, gas state (or vacuum)
H, enthalpy
$\hat{H}$, Hamiltonian operator
hERG, human ether á go-go related gene
MD, molecular dynamics
MIF, molecular interaction field
r, distance between particles
P, pressure
PDB, Protein Data Bank
p, permanent dipole moment
p_{ind}, induced dipole moment
q, charge of a particle
S, entropy
U, potential function
V, volume of the system

ABSTRACT

A variety of ligands interact with proteins in many biological processes; shape complementarity, electrostatic forces and hydrophobicity are the main factors governing these interactions. Although this is accepted by the scientific community, confusion about the significance of certain terms (e.g. hydrophobicity, salt bridge) and the difficulty of discussing the balance of acting forces rather than their single contributions, are two of the main problems encountered by researchers working in the field. These difficulties are sometimes enhanced by the unskilled use of informatics tools, which give great help in understanding the topic (especially from the visual standpoint), but only if used critically. After explaining some general chemical concepts, the commentary discusses the main forces governing ligand–protein interactions, focusing on those generating confusion among scientists with different backgrounds. Three examples of ligand–protein interactions are then discussed to illustrate the advantages and drawbacks of some in silico tools, highlighting the main interactions responsible for complex formation. The same examples are used to point out the limits in separating forces that are mandatory for occurrence of a given interaction and additional forces.

* *Corresponding author*. Tel.: +39 011 670 7282; fax: +39 011 670 7687.
E-mail address: giuseppe.ermondi@unito.it (G. Ermondi).
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1. Introduction

The successful practice of medicinal chemistry crucially depends upon an understanding of the principles of molecular recognition [1]. A molecular recognition event is one in which a host molecule is able to form a complex with a guest molecule [2], and thus such an event is usually categorised as supramolecular chemistry. Any molecular recognition process occurs through a number of interactions between the two molecules, i.e. protein (=receptor) and ligand (i.e. drug).

Eq. (1) describes the non-covalent, reversible binding of one protein (P) and one ligand (L) to form a ligand–protein complex (PL):

$$\mathrm{P} + \mathrm{L} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \mathrm{PL} \tag{1}$$

where k_1 is the rate constant for association complex, k_{-1} is the rate constant for dissociation of the complex. For such a reaction, a binding (association) constant K_B (or its reciprocal dissociation or inhibition constants, K_D or K_i, respectively) can be defined as

$$K_B = \frac{k_1}{k_{-1}} = \frac{[\mathrm{PL}]}{[\mathrm{P}][\mathrm{L}]} \tag{2}$$

it is usually assumed that the biological activity of a ligand is related to its binding constant K_B for the receptor.

Although it is well accepted that the binding of a drug to its receptor is mediated by shape complementarity, electrostatic interactions and hydrophobicity, these concepts are often used either with only limited understanding or with different glossaries.

The main goal of this paper is therefore to alert readers that researchers with different backgrounds (chemists, biologists and pharmacologists) often refer to the same interactions but adopt different viewpoints and vocabularies. To achieve this aim, after explaining some basic concepts, we draw a pragmatic picture of the molecular recognition forces involved in ligand–protein complexes, focusing on those aspects potentially misleading for scientists of different backgrounds.

The second objective the authors want to reach concerns the critical use of in silico tools designed for the inspection of ligand–protein complexes. This is done by the aid of three examples, one concerning the inhibition of the COX-1 isozyme, and two related to the modelling of potassium channels.

2. Basic concepts

In view of the quantum nature of the movement of electrons and nuclei, a consistent theory of intermolecular interactions could be derived only from quantum-mechanical concepts. Quantum mechanics postulates that the wave function of a quantum system completely defines its dynamical state; stated otherwise, all predictions which can be made concerning the dynamical properties of the system at a given instant of time t can be deduced from the knowledge of the wave function $\psi(t, \underline{r})$ at that instant, where $\underline{r}$ is the vector of position coordinates of the particles of the system. The equation of wave propagation is the Schrödinger Eq. (3):

$$i\hbar \frac{\partial \Psi(\underline{r}, t)}{\partial t} = \hat{H}\Psi(\underline{r}, t) \tag{3}$$

where $\hat{H}$ is the Hamiltonian operator that contains information about the force field governing the particles motion and the other symbols have their conventional meaning, not essential for our purpose [3].

It is noteworthy that the force field depends on the particles charges and thus the motion is governed by electrostatic forces. Given the exact wave function one could calculate the electronic density of the system and thus solve mathematically the system independently of its state (vacuum, solvated, solid). In other words, a main concept is that the solution of the problem of determining intermolecular interactions amounts, strictly speaking, to solving Schrödinger equation [4]. Since for complexity reasons this is never the case, different approaches are adopted and generally (and also here) the main intermolecular interactions are described by classical mechanics in vacuum (where in vacuum refers to the gas-phase). In addition, small molecules are used under the assumption that the results are transferable to larger structures (ligands and proteins).

A second concept is that the electrostatic forces are usually considered by classical mechanics as pair-wise interactions and often are represented by a potential function. This latter ($U(r)$) is defined as the work done to bring two atoms from an infinite separation to separation r, and is calculated by Eq. (4) [5]:

$$U(r) = \int_r^\infty F(r)\mathrm{d}r, \quad \text{so that} \quad F(r) = -\frac{\mathrm{d}U}{\mathrm{d}r} \tag{4}$$

where $F(r)$ is the force between the two atoms and by convention is positive when it is repulsive and negative when attractive. Eq. (4) links the forces that govern the interactions (i.e. the subject of this commentary) with the corresponding energy and permits the use of forces and energies as interchangeable terms.

A third concept of utmost importance is that the laws of thermodynamics are the most used tools to transfer the information obtained in vacuum to solvated (aqueous) systems. Briefly, the first law of thermodynamics states that the change in internal energy (E, the relationship with U is shown in Fig. 1A) is equal to the heat added to the system minus the work done by the system. Fundamentally the first law declares that energy is conserved for a closed system, with heat and work being the forms of energy transfer. From this statement it is possible to define some fundamental quantities, such as enthalpy (H) and Gibbs free energy (G), that will be used in Section 4.1. One of the formulation of the second law of thermodynamics states that the entropy of an isolated system not at equilibrium will tend to increase over time, approaching a maximum value. Essentially, the second law of thermodynamics introduces the concept of entropy that plays a crucial role in the definition of the hydrophobic effect (see Section 4.2).

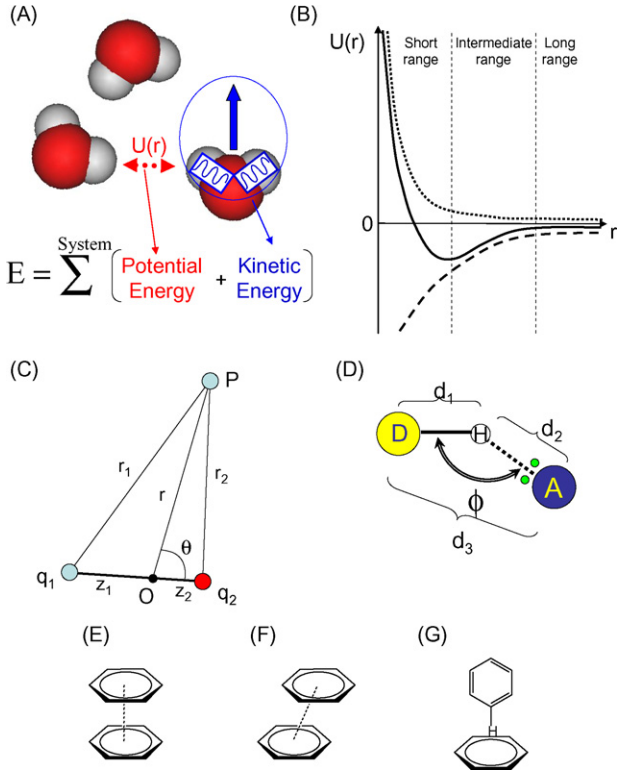


Fig. 1 – The Van der Waals curve, the difference between *E* (=internal energy) and *U* (=interaction potential function), and schematic representation of some intermolecular forces listed in Table 1. The symbols are reported in the correspondent equations in the text and in Appendix A. (A) Schematic representation of the relationship between internal energy (*E*) of the system and energy of the molecules, including intermolecular potential function *U*(*r*) and translational, vibrational and rotational kinetic energy. (B) The Van der Waals curve: the interaction potential funtion (*U*(*r*)) as a function of the intermolecular distance (*r*). The curve (—) represents the potential resulting from the combination of the attractive (- - -) and repulsive (· · ·) contributions. (C) Ion–permanent dipole (fundamental). (D) Hydrogen bond (combined). (E) Interaction involving the π-system: face-to-face (combined). (F) Interaction involving the π-system: parallel-displaced (combined). (G) Interaction involving the π-system: T-shaped (combined).

Finally, it must be kept in mind that the growing impact of crystallography in drug–receptor studies is due to the observation that crystals can be seen as supramolecular entities in that they are built from molecules held together by non-covalent forces [6], the same forces that govern other molecular recognition events as ligand–protein binding [7,8].

3. Vacuum state: the simplified kingdom

Despite the vacuum state (also called the vacuum) having no biological correspondence, interaction theories were developed mainly for this state since the absence of solvent interactions permits a simpler treatment.

Intermolecular interactions are of electrostatic nature, i.e. depend on the presence of electrical charges and/or dipoles. Whereas the definition of atomic charge is evident, that of dipole moment, polarisability and induced dipole moment is briefly recalled below. The dipole moment $\underline{p}$ (Eq. (5)) (vector entities are underscored) between two charges equal but of opposite sign separated by a distance $\underline{r}$ is defined as the product of the charge q with the distance r. The direction of the dipole moment is from negative to positive sign.

$$\underline{p} = q \cdot \underline{r} \tag{5}$$

the polarizability α is the ease of distortion of the electron cloud of a molecular entity by an electrical field (Eq. (6)). It is experimentally measured as the ratio of induced dipole moment (p_{ind}) to the electric field E' which induces it:

$$\alpha = \frac{p_{ind}}{E'} \tag{6}$$

in ordinary usage the term refers to the 'mean polarizability', i.e. the average over three rectilinear axes of the molecule [9].

In general, the work of Van der Waals has suggested, and later developments have confirmed, that the form of the potential function governing the electrostatic interactions between two particles is the sum of an attractive and a repulsive contribution, as shown in Fig. 1B [4]. The expression of the potential function basically depends on the nature of the interacting charges and some outlines are given below to explain the physical origin of the interactions.

Electrostatic forces can be grouped in two main categories: simple forces that arise from the charged entities of which the molecules are made up, and combined forces that are a combination of simple ones (Table 1).

3.1. *Simple electrostatic forces*

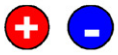
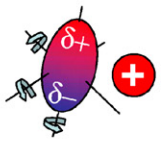
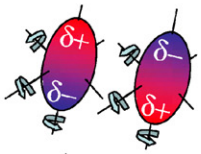
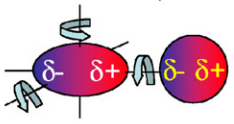
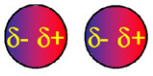
Simple electrostatic forces are schematically represented in the upper part of Table 1 with the equations of the corresponding potential ((T1)–(T4)) and an approximate value of the energy involved in the interaction. In general (T1)–(T4) are the product of two factors: one depends on the particles properties (e.g. the charge) whereas the second depends on a geometrical factor involving the particles position.

3.1.1. *Ion–ion and ion–permanent dipole interactions*

The basic equation of all electrostatic interactions is Coulomb's law (Eq. (T1)) for which the potential energy falls off as $1/r$. Interactions that vanish with certain powers of $1/r$ are named long-range interactions and usually have an attractive nature. Contribution of ion–ion interactions can be positive or negative based on the sign of the involved charges. Ion–permanent dipole interactions (Fig. 1C) can be used to illustrate how all electrostatic interactions descend from the basic Coulomb's law. By developing the Coulombic equation (the mathematical treatment is reported in Appendix A) one finds that the distance potential dependence becomes $1/r^4$ (Eq. (T2)).

Table 1 – Electrostatic forces

Simple

	Category		Schematic representation[a]	Potential equation[b] and number		Approximate energy
	Ion - Ion			$\frac{q_1 q_2}{4\pi\varepsilon_0}\cdot\frac{1}{r}$	(T1)	25 kJ mol^{-1}
	Ion - Permanent dipole			$-\frac{q^2 p^2}{(4\pi\varepsilon_0)}\cdot\frac{1}{kTr^4}$	(T2)	50-200 kJ mol^{-1}
Van der Waals forces	Permanent dipole - Permanent dipole (Keesom Forces)			$-\frac{2}{3}\frac{p_1^2 p_2^2}{(4\pi\varepsilon_0)^2}\cdot\frac{1}{kTr^6}$	(T3)	50-500 kJ mol^{-1}
	Permanent dipole - Induced dipole			$-\frac{p^2\alpha_p}{(4\pi\varepsilon_0)^2}\cdot\frac{1}{r^6}$	(T4)	<5 kJ mol^{-1}
	Induced dipole - Induced dipole (Dispersion or London forces)			Always present Are attractive and depends on $1/r^6$ Quantum mechanical origin		< 5 kJ mol^{-1}
	Repulsive forces			Always present Quantum mechanical origin Repulsion derived from overlapping electron clouds		

Combined

Category	Classification and approximate energy of interaction	
Hydrogen bond	Between H and atoms with low electronegativity Between H and atoms with high electronegativity (Between the partially positive H and electrons in double/triple bond)	4-60 kJ mol^{-1}
Involving π systems	Face-to-face Parallel-displaced T-shaped Cation-π	5-80 kJ mol^{-1} and 0-50 kJ mol^{-1}

(a) One-color circles refer to ions; two-color ovals to permanent dipoles; two-color circles refer to induced dipoles; the arrows are used to indicate the random motion of the object responsible of the averaging of the interaction. (b) q_i is the charge of the ion i; p_i the dipole moment of the dipole i; α_p the polarizability; r the distance between objects; k the Boltzmann constant; ε_0 the permittivity of the free space; T is the temperature; the subscript i is omitted where unnecessary.

3.1.2. *Van der Waals interactions*

Van der Waals interactions include permanent dipole–permanent dipole, permanent dipole–induced dipole and induced dipole–induced dipole forces (Table 1).

The interaction between two permanent dipoles, $\underline{p}_1$ and $\underline{p}_2$ (also called Keesom forces), can be treated following a procedure similar to the one outlined in Appendix A to obtain the final Eq. (T3). This interaction depends on the sixth power of $1/r$ and thus is a long-range interaction.

The method outlined in Appendix A can also be applied to the permanent dipole–induced dipole interaction to obtain the Eq. (T4). This potential is not temperature-dependent, unlike the interaction between permanent dipoles, since the induced dipole always follows the instantaneous direction of the

electrical field generated by the inducing dipole. It is an attractive long-range contribution.

Finally, electrons in a molecule with no permanent dipole moment are in continuous motion so that the electron density in a molecule oscillates continuously in time and space. Thus at any instant any molecule possesses an instantaneous electrical dipole which fluctuates as the electron density fluctuates. This instantaneous dipole in one molecule induces an instantaneous dipole in a second molecule. The induced dipole in the second molecule and the inducing dipole in the first interact to produce an attractive energy, called the dispersion energy. The latter is attractive and inversely proportional to the sixth power of the intermolecular separation (Table 1). The dispersion energy cannot be analysed by classical mechanics since its origin is purely quantum mechanical.

3.1.3. *Repulsive forces*

As the atoms get too close, at some point there is a strong repulsion from overlapping electron clouds and Pauli's exclusion principle whereby filled electron shells of an atom cannot accommodate any more electrons. The repulsive interaction between electron densities are named short-range interactions and simply define the molecular volume.

All chemists are familiar with the idea that atoms of a given element may be regarded as hard spheres whose radius, the Van der Waals radius [10], is characteristic of that element. The hard-sphere atomic model is an approximation because atoms are not completely hard and their effective size depends on their environment and hybridization state [11].

3.2. *Combined intermolecular forces*

These forces are a combination of the forces discussed above. Because of the distances involved and their mixed classical and quantum mechanical treatment they are considered intermediate forces (Fig. 1B).

3.2.1. *Hydrogen bond (HB) interactions*

The hydrogen bond is an interaction which, according to the IUPAC, occurs between an electronegative atom (conventionally named here A, acceptor) and a hydrogen atom (H) attached to a second, relatively electronegative atom (D, donor) (Fig. 1D). It is an electrostatic interaction, augmented by the small size of the hydrogen, which permits the proximity of the interacting dipoles or charges [12]. Because of its definition HB can occur both in inter- and intramolecular contexts.

The Cambridge Structural Database (CSD) has been the principal source of knowledge about HB for many years. Crystal structures have been used extensively in studies of HBs in small molecules (see below), particularly those having N or O as donors and acceptors [13–16], to quantify the metrical and directional properties of these bonds [14].

HBs are characterised by four geometrical descriptors (Fig. 1D): the distances D–H (d_1), H–A (d_2) and D–A (d_3) and the angle D–H$\cdots$A (ϕ). Typical values of d_3 covering a range of 2.5–3.2 Å [16] and ϕ angles of 130–180° are found. Whereas no or only a slight dependence of the hydrogen bond strength with angular changes are observed in the range of 130–180°, shorter distances down to 2.3 Å result in a more covalent bond character and a larger binding energy, although, the latter aspect does not hold in general [17].

The analysis of crystallographic data shows that the tendency for H-bonds to form along lone-pair directions varies greatly according to the nature of the acceptor atom. Strong lone-pair directionality is found for carboxylates, and many types of aromatic nitrogens, e.g in pyridine. At the other extreme, some types of H-bonds show little or no lone-pair directionality, for example the carbonyl oxygen shows only a limited preference direction along its lone-pairs [18].

Among the plethora of HB classifications described in the literature, we report here the one by Kollman and Allen [19] which groups HBs in two (three) main classes: (a) HB between the hydrogen atom and atoms of high electronegativity (C, N, O, F, P, S, Cl, Se, Br and I), and (b) HB between the hydrogen atom and atoms of low electronegativity (such as HB bonds present in the boranes [19]). Additionally, a third class of HBs (named π/HB) and involving an interaction between the partially positive hydrogen and the electrons in a double and triple bond can be considered. The strength of HB ranges from 4 to 60 kJ mol^{-1} [20] (Table 1).

Most strong HBs are formed by groups in which there is either a charge in the donor group or in the acceptor group. Because of the presence of charged substructures, this category of HBs are often called reinforced/ionic HBs.

Finally, salt bridges and salt bonds are colloquial protein chemistry terms (a salt bridge, in chemistry, is a laboratory device used to connect the oxidation and reduction half-cells of a galvanic cell (electrochemical cell)) for reinforced/ionic HBs involving negative acceptor charged groups derived from Asp, Glu, Tyr, Cys and C-terminal carboxylate groups and positive donor charged group derived from His, Lys, Arg and N-terminal amino groups [21]. Apparently salt bridges are ion–ion interactions governed by Coulomb's law and in vacuum are probably predominant. In solvated systems, as it will be discussed in Section 4.3, the effect of the interactions with water has to be considered.

3.2.2. *Interactions involving π-systems*

Attractive interactions involving π-systems have been known for over half a century [22–24]. The characteristics of the interaction energies of these π-complexes depend on both the nature of the interacting molecules and the π-system.

Non-covalent interactions involving aromatic rings are pivotal to protein–ligand recognition. Indeed, the vast majority of X-ray crystal structures of protein complexes with small molecules reveals bonding interactions involving aromatic residue side chains of the receptor and/or aromatic and heteroaromatic rings of the ligand [7]. The X-ray data and computational works suggest a competition between T-shaped and parallel-displaced geometries, and face-to-face geometry is not found [7] (Fig. 1E–G). London dispersion interactions (see Section 3.1.2) are the major source of stabilization energy between two aromatic molecules. The introduction of heteroatoms into aromatic rings, for example in Trp, His and nucleobases, has significant influence on π–π interaction. Electrostatic attraction between atoms with positive or negative partial charges

and the alignment of molecular dipoles become important in determining how two or more heterocyclic π-systems interact, in addition to the dispersion component.

Another important interaction involving π-systems is the cation–π interaction that, for example, involves cation side chains (Arg, Lys, or protonated His) with aromatic residues (Phe, Tyr and Trp) [23]. Of particular interest is the interaction of the cationic Arg residue with aromatic side chains. Two limiting geometries are possible: a perpendicular arrangement in which the NH's of the Arg point into the face of the aromatic ring, and a parallel or stacked arrangement of the planar guanidinium or Arg and the aromatic moiety. The presence of amino groups in various drugs suggests an important role of this interaction as confirmed by various examples [25].

4. Solvated systems: water is essential for life

In biological systems a central role is played by water because molecular recognition takes place in an aqueous environment. For this reason in this paper we refer to aqueous systems when solvated systems are mentioned. As the result of the mediating effect of water, the intermolecular forces described in vacuum are not valid in solvated systems without adaptation.

4.1. *The thermodynamics of binding*

To this point, the focus has mainly been on the interaction forces, which change the internal energy of the system (ΔE) in vacuum [26]. On the other hand the "driving force" of the binding between molecules is expressed by the Gibbs free energy (ΔG) which is linked to the experimental constant of binding (or association) K_B. The relation between the internal energy and the Gibbs free energy requires the introduction of some thermodynamic definitions.

The Gibbs free energy change for the binding reaction in Eq. (1) (ΔG_B) is related to the standard free energy change (ΔG_B°) by Eq. (7):

$$\Delta G_B = \Delta G_B^\circ + RT \ln \frac{[PL]}{[P][L]} \quad (7)$$

where $R = 8.31\ J\ mol^{-1}\ K^{-1}$ and T is the absolute temperature. The standard state is usually taken to be 298 K, 1 atm pressure and with all components at unit activity [26], under equilibrium conditions $\Delta G_B = 0$, so that Eq. (7) becomes

$$\Delta G_B^\circ = -RT \ln \frac{[PL]}{[P][L]} = -RT \ln K_B \quad (8)$$

where K_B is the binding constant (Eq. (2)) and is the measurable parameter. From Eq. (8) we can see that K_B increases when ΔG_B° becomes more negative.

The Gibbs free energy change is the sum of two contributions according to

$$\Delta G = \Delta H - T\Delta S \quad (9)$$

where ΔH and ΔS are respectively enthalpy and entropy change of the system.

Finally, the enthalpy is related to the internal energy by

$$\Delta H = \Delta E - (\Delta PV) \quad (10)$$

where P and V are the pressure and volume of the system.

In the case of ligand–protein complexes in aqueous systems, ΔPV can be usually omitted (there is no changes in pressure and volume), whereas for gas-phase association $\Delta PV \cong -RT$, which is about $-2.5\ kJ\ mol^{-1}$ [27] at room temperature. Thus this negative term, when added to ΔE, favours association.

In vacuum, the enthalpy change of the binding reaction, $\Delta H_B(g)$ (where 'g' denotes the gas or the vacuum state), is generally negative if the reactants approach each other in an appropriate orientation, whereas the entropy change, $\Delta S_B(g)$, is generally positive since the formation of the ligand–protein complex produces the reduction of freedom of the two molecules involved in the complex. The three rotational and translational degrees of freedom of the free ligand are replaced by six vibrational degrees of freedom in the complex (the extent of the change ranges, at 310 K, from 12 kJ mol^{-1} for a loose interaction to 60 kJ mol^{-1} for tightly bound complexes [28]).

In summary, since the vacuum entropic contribution of the binding is invariably unfavourable, a negative enthalpic contribution is always required in vacuum to promote binding. Thus in vacuum the electrostatic interactions are the unique force responsible for the formation of the complex. However, since ligand–protein complexes are generally formed in aqueous solution, the main differences affecting the change of the Gibbs free energy of the binding process in the aqueous ($\Delta G_B(aq)$) and gas state ($\Delta G_B(g)$) should be investigated. The laws of thermodynamics (see Section 2) are the most used and simple tools to transfer the information content obtained from vacuum to solvated systems.

The thermodynamic cycle (Fig. 2) furnishes the link between the two states and thus the desired information. In particular the cycle illustrated in Fig. 2 points out that $\Delta G_B(aq)$ differs from $\Delta G_B(g)$ by a contribution due to solvation as shown in Eq. (11):

$$\Delta G_B(aq) = \Delta G_B(g) - \Delta G_P^{Solv} - \Delta G_L^{Solv} + \Delta G_{PL}^{Solv} \quad (11)$$

where ΔG_P^{Solv}, ΔG_L^{Solv}, and ΔG_{PL}^{Solv} are the change of the Gibbs free energy of solvation of the protein, ligand and complex,

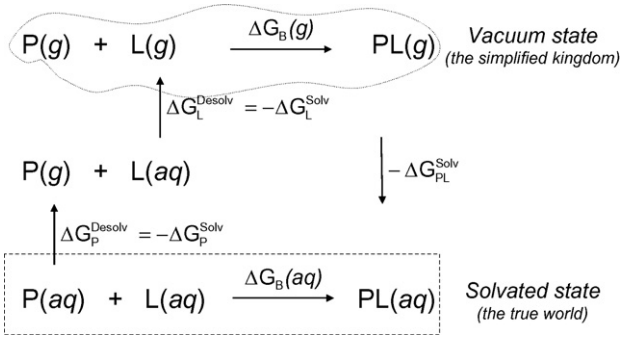


Fig. 2 – A schematic representation of the thermodynamic cycle for ligand–protein binding in the gas-phase and in solution.

respectively. These three solvation parameters take into account both hydrophobic interactions (see Section 4.2) and the modifications induced by water on electrostatic forces (see Section 4.3).

4.2. *Hydrophobic interactions*

The concept of hydrophobic interactions (HI) was introduced by Kauzmann [29] in the field of protein chemistry since it plays an important role in stabilizing the conformation of proteins (see below) [30]. But hydrophobic interactions also explain the low solubility of hydrocarbons in water. The IUPAC defines hydrophobic interactions ''as the tendency of hydrocarbons (or of lipophilic hydrocarbon-like groups in solutes) to form intermolecular aggregates in an aqueous medium. The name arises from the apparent repulsion between water and hydrocarbons. However, the phenomenon ought to be attributed to the effect of the hydrocarbon-like groups on the water–water interaction'' [12].

It is generally assumed that a hydrophobic interaction is entropically rather than enthalpically driven even if controversies are reported in the literature [31]. Briefly, because the molecules of a hydrocarbon are not solvated in water owing to their inability to form HB with water molecules, the latter become more ordered around the hydrocarbon molecule. The resulting increase in solvent structure leads to a higher degree of order in the system than in bulk water, and thus a loss of entropy. When the hydrocarbon structures (whether two protein side chains or alkane molecules) come together they will squeeze out the ordered water molecules that lie between them. Since the displaced water is no longer a boundary domain, it reverts to a less ordered structure, which results in an entropy gain. Once the hydrocarbon chains are in sufficient proximity, dispersion forces (see Section 3.1.2) become operative between them [32].

HI are therefore very different from all other non-covalent interactions in the liquid-phase in the sense that they do not primarily depend on direct attractive intermolecular interactions between the species that interact. Instead they are driven by the tendency of water molecules to retain their own water–water hydrogen bond interactions as much as possible, leading to a tendency to arrange non-polar entities such that the contact surface area between these and water is minimised [33].

The hydrophobic effect is considered to be the major driving force for the folding of globular proteins. It results in the burial of the hydrophobic residues in the core of the protein. As in the folding of protein structures, hydrophobicity is also one of the major forces in molecular recognition. It is noteworthy that in aqueous systems, the presence of HI is necessary for the association of apolar molecular moieties, whereas in vacuum dispersion forces are sufficiently strong to guarantee this association. In water electrostatic interactions decrease in strength because of the presence of the solvent (see Section 4.3). HI can thus be perceived as an emergent property [34] of the aqueous system which permits the maintenance of such relevant interactions, such as dispersion, which could be lost because of other molecular properties of water.

4.3. *The presence of water modulates electrostatic forces*

The repulsive interactions mainly define the molecular volume and surface in solvated systems too as in the vacuum, but in the presence of water the surface of the molecule is not free but fully coated by solvent molecules. Thus, the interaction between ligand and protein is mediated by water molecules. In Section 4.2 the entropic effect of this ''mediation'' has been discussed but water influences electrostatic interactions, too.

First, it is well known that in a medium Coulomb interactions are reduced because of the presence of solvent molecules. The effect of the medium on Coulomb interactions can be considered substituting the permittivity of free space in Eqs. (T1)–(T4) with the dielectric constant of the medium. The dielectric constant of water is about 80, whereas it is 1 in vacuum. Thus a dielectric constant of 1–20 (mostly 2–8) is assumed in proteins interior and of about 80 at the protein periphery next to the surrounding water.

All electrostatic interactions are affected by the modification of the dielectric constant but the effect is particular evident in stronger interactions, i.e. ion–ion (see Section 3.1.1). In addition HB strength (see Section 3.2.1), that in a first approximation comprises an important ion–ion contribute, is particularly sensitive to this effect. In fact buried hydrogen bonds are more important for ligand–protein interactions than those formed in water-exposed regions (where the polar environment correspond to higher ε values). The contribution of HBs to binding affinity have been estimated to be about -1 to -7 kJ mol^{-1} [1,17].

Van der Waals interactions (see Section 3.1.2) between ligand and protein compete with analogous interactions with water, and thus their contribution is weak, even if abundant. The same considerations are valid for the interactions involving π-systems (see Section 3.2.2).

Hydrophobic interactions are often associated with conformational changes of the receptor. Conformational change in a receptor upon ligand binding is usually termed induced fit. In theory such changes could be the result of polar or hydrophobic interactions of the receptor with the ligand. However, examples in the literature are overwhelmingly the result of hydrophobic interactions. In these cases they could equally well be thought of as hydrophobic collapse of a receptor around a ligand [1].

5. Solid state: X-ray data as a source of information

Whereas the vacuum is the ideal state in which intermolecular forces are defined and the solvated system represents the true biological environment, solid state is the operative system from which most research information is extracted, and thus it is the system you want to reproduce in molecular-modelling simulations.

The large development of crystallographic databases, such as the Protein Data Bank (PDB) for proteins [35] and the Cambridge Structural Database (CSD) for small organic and organometallic molecules [13], permits to individuate and characterise structural elements and intermolecular

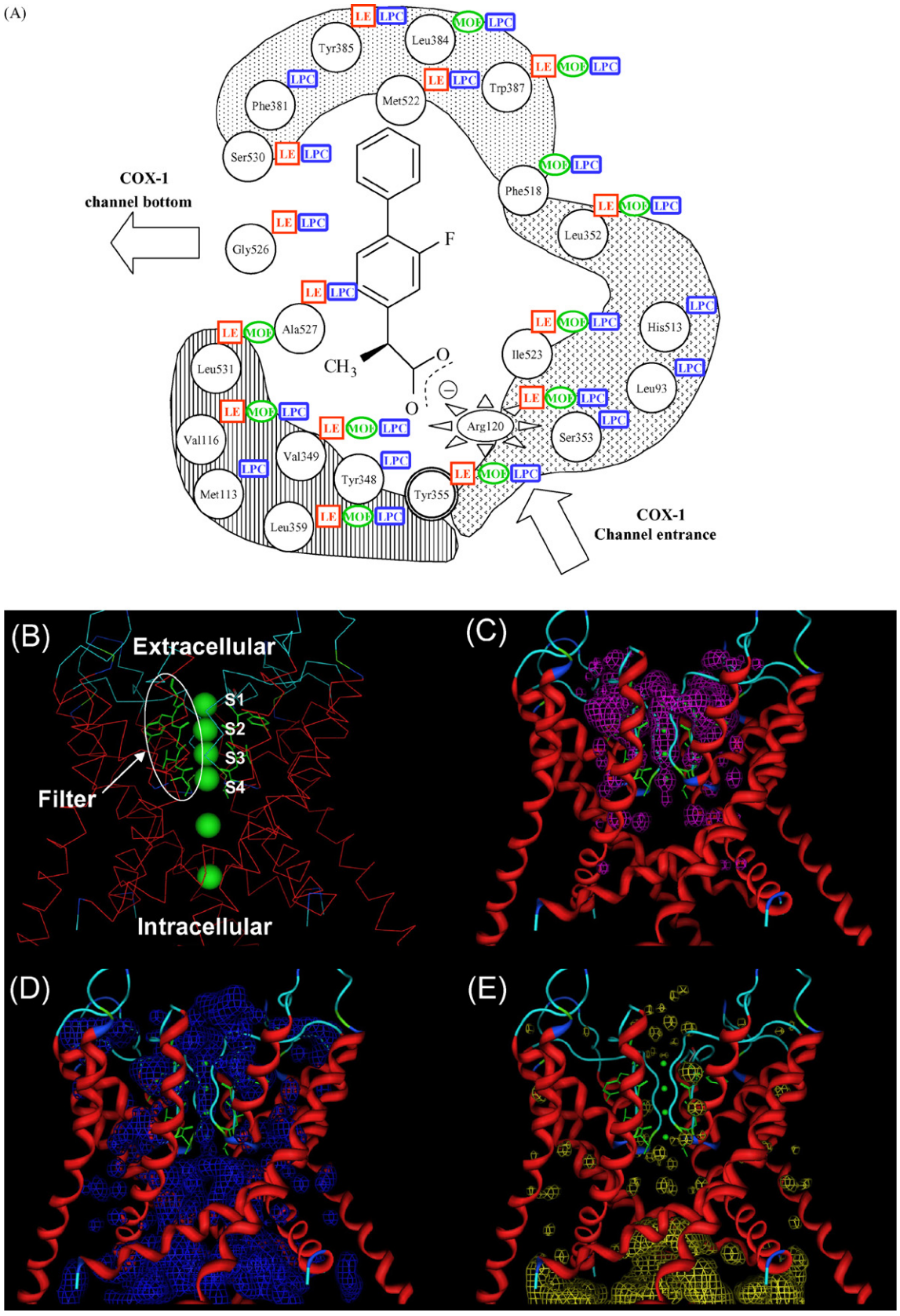


Fig. 3 – (A) Schematic representation of the ligand/COX-1 contacts (PDB code: 1EQH) as obtained using three different software: Ligand Explorer (LE), the MOE Protein Contacts tool (MOE), and Weizman Ligand–Protein Contacts analyser (LPC). The main interactions highlighted by the three tools are shown as follows: red squares for LE, green ovals for MOE and blue squares for LPC. (B) Crystallographic structure of KvAP channel (PDB code: 1ORQ): two of the four subunits comprising the

interactions. This has been achieved by the development of new database search tools, e.g. Relibase [36].

X-ray structure is normally considered as a more accurate representation of a protein structure than NMR spectroscopy because of the lack of a clear quality assessment of NMR structures [37]. It has been shown that protein structures determined by both NMR and X-rays have, in general, very similar backbone folds, but differ in details, such as, packing surface loops and side chains [37]. A recent study [37] shows as the structures obtained with the two techniques differ in the contacts and HB networks formed by the protein residues.

Nevertheless some remarks are necessary whenever intermolecular interactions are discussed on the basis of protein crystal structures [17].

First, experimental limits of protein crystallography must be taken into consideration when large molecules (e.g. receptors and enzymes) are discussed. Resolution below 1.5 Å (coinciding with the mean length of a covalent bond) are rarely obtained for protein crystal (value between 2 and 3 Å are more usual), whereas atomic resolution is achieved below 1.2 Å. As a consequence X-ray diffraction hardly differentiates between isotopes and elements of similar atomic number because of their comparable diffraction power. Except for protein structures at very high resolution, the position of terminal N and O atoms, for example in asparagine and glutamine [17,38], can only be assigned on the basis of a self-consistent HB network. In addition the position of hydrogen atoms remains undetermined and thus HBs are not usually identified. Finally, the estimated standard deviation value in atomic coordinates is inherently related to resolution (a resolution of 2.5 Å correspond to a standard deviation in atomic coordinate of about 0.4 Å).

A second comment is that the nature of the solid state governs experimental results and thus crystallography averages in time and space the individual molecules forming the crystal. Crystal contacts between neighbouring molecules can result in intermolecular interactions which may affect part of a structure (influence of the solid state force field).

In addition, besides positional disorder, which results in distinct occupancies of alternative atomic positions, dynamic disorder also results from thermal motion of the atom about their equilibrium position. Because of time and space averaging during data collection, only spatial restricted atoms contribute constructively to diffraction and thus only their position can be located (for example water molecules can make up to 70% of the number of atoms in a protein crystal but only the water molecules of the first hydration shell surrounding protein or ligand are generally well-ordered thus localizable). Moreover, multiple binding modes of the ligands can occur as a result of spatial averaging. In such situations the same ligand can occupy several energetically equivalent orientations in the binding pocket.

Finally, deviating binding modes can occur in different polymorphic forms of the crystalline state [17]. The influence of crystallization conditions (which may not be the same as those employed in the biological assay) is often unknown or not considered. For example, normally the pH during protein crystallization has no effect upon the formation of various crystal forms, but in the literature various exceptions are reported [38]. A complex of trypsin shows a different ligand conformation, active-site conformation, and crystal morphology due to the change in the pH of the crystallization conditions [38].

6. In silico applications to shed light on the balance of forces governing ligand–protein interaction

In previous sections, forces responsible for ligand–protein interactions have been listed and discussed. Most of them act simultaneously during the formation of a complex, but analysis of their balance is not straightforward, as demonstrated below by three examples: the first concerns the inhibition of cyclooxygenase-1 (COX-1) isoform, an enzyme involved in the conversion of arachidonic acid to prostaglandins [39]; the second the interaction of potassium ions with KvAP Aeropyrum pernix potassium channel (the first reported crystal structure of a voltage-dependent potassium channel in its open state sharing several structural features with eukaryotic Kv channels [40] and the third the *hERG* potassium channels blocking action of sertindole [41–43].

The examples are of increasing computational complexity, due both to the availability or otherwise of experimental crystallographic data and to the theoretical level of calculations.

6.1. *Analysis of the contacts to describe flurbiprofen/COX-1 interaction*

As previously discussed, X-ray crystallography represents the most widely available tool to extract experimental information about ligand–protein interaction. We thus downloaded the X-ray structures of the COX-1 in complex with flurbirprofen from PDB [35] (PDB code: 1EQH). This compound (2-(3-fluoro-4-phenyl-phenyl)propanoic acid) is a NSAID inhibitor able to block both COX isoforms [44].

Flurbiprofen binds in the COX-1 active-site, which lies at the apex of a long narrow hydrophobic channel extending from the membrane-binding surface to the centre of the protein [45] (Fig. 3A). In the paper reporting the crystallographic structure, the authors claim that the inhibitor's carboxylate moiety takes part in a network of polar interactions which includes two HBs between the inhibitor and Arg120 and a third HB with the phenolic hydroxyl of Tyr355. The atoms at the extreme distal end make Van der Waals contacts with protein residues, whereas the atoms in the

tetrameric channel are shown, the filter residues (Thr-Val-Gly-Tyr-Gly) and the potassium ions (S1–S4) are in green. (C) Crystallographic structure in 3B and MIF for the probe K+ at −20 kcal mol^{-1}: the energy iso-surface is represented in pink mesh. (D) Crystallographic structure in 3B and MIF for the probe OH2 at −5 kcal mol^{-1}: the energy iso-surface is represented in blue mesh. (E) Crystallographic structure in 3B and MIF for the probe DRY at −0.5 kcal mol^{-1}: the energy iso-surface is represented in yellow mesh.

middle part of the inhibitors do not contact any protein atom [46] (see Fig. 3A for a more detailed analysis).

For a better understanding of the balance of forces involved in flurbiprofen/COX-1 interaction, we submitted the complex (as obtained from crystallographic database) to analysis using software in common use designed for rapid inspection of biological structures in PDB format. Briefly, these in silico tools automatically highlight the main contacts present in the complex, and may be seen as the evolution of contact maps, which represent the distances between every pair of residues of a three-dimensional protein structure in a two-dimensional matrix. In particular we used Ligand Explorer (LE) [47], the Protein Contacts tool of the commercial software MOE [48] (herein simply called MOE) and a second free on-line tool, the Weizman Ligand–Protein Contacts (LPC) analyser [49]. All software was used with default parameters.

LE and MOE mainly base contact analysis on the distances between ligand and protein atoms: when the distance is shorter than a given threshold a contact is highlighted. LE distinguishes four classes of contacts: contacts between pairs of carbon atoms are called *hydrophobic interactions*; contacts between potential HB donors and HB acceptors are called *hydrophilic interactions*; contacts between ligand atoms and water are called *ligand–H_2O–protein interactions* and all remaining contacts fall into a generic class and are indicated as *other interactions* [47]. In MOE contacts are considered *hydrophobic* when they involve hydrophobic residues (Val, Ile, Phe, Trp and optionally Met), *ionic* when they involve basic nitrogen atoms and acidic oxygen and, finally, are classified as *HB* when they are determined according to the criteria proposed by Stickle et al. [50]. Finally, LPC software first defines atom classes using a more composite classification (for example nitrogen and oxygen atoms belong to different classes depending on their HB donor or acceptor or donor/acceptor capacity) and second calculates contacts on the basis of the distances between atoms, as done by the LPC and MOE [49].

Fig. 3A illustrates in detail the results given by the three in silico tools. Basically their analysis indicates that HB interactions are always clearly identified, whereas the Van der Waals pattern differs with the software (agreement is slightly improved when default parameters are modified). This finding is in line with the uncertainty affecting the criterion to discriminate Van der Waals interactions (a Van der Waals contact is assumed to be present if the distance is within a certain value). The situation is further complicated because the hydrophobic effect is neglected.

However, the clear identification of a HB network combined with the vague description of Van der Waals forces does not mean that HB is the driving force for the formation of the flurbiprofen/COX-1 complex: we cannot exclude that the sum of the Van der Waals contributions is more important than HB, since the information arising from X-ray analysis only identifies with certainty the polar component of the interaction. Indeed to check whether the polar interaction is mandatory or not for the binding, other evidence is required. In the case of COX-1 inhibition, the doubt has been unravelled by mutational analyses [51] which revealed that only seven residues are critical to allow COX-1 mediated catalysis.

6.2. *Molecular Interactions Fields (MIFs) to investigate the forces of the potassium ions/KvAP potassium channel complex*

GRID [52,53] is a well-known software used to calculate the molecular interaction fields (MIF). A MIF is a collection of energy values calculated from the sum of the attractive and repulsive forces between a molecule (the target) and an interacting partner (the probe), positioned in a lattice of points (or nodes) surrounding the target [52]. Nodes with negative energy values correspond to favourable interactions between molecule and probe, and vice versa. Since the use of different probes evidences the predisposition of the target towards various interaction types, the GRID approach can be used to analyse the nature of the interactions present in crystallographic complexes.

Many traditionally used drugs have had the ability to block a particular cardiac ion channel known as hERG and ultimately to cause serious cardiac effects. For this reason intense effort is now being made to develop in silico tools to predict drug-induced block of *hERG* channels [41]. Since there is no *hERG* potassium channel crystallographic structure, more insights into the hERG structure can be obtained either from bacterial X-ray data or from homology model (see Section 6.3).

As an example of GRID application, the X-ray structure of the voltage-dependent potassium channel KvAP from the bacteria Aeropyrum pernix (Fig. 3B) was downloaded from PDB (PDB code: 1ORQ). Starting from the monomer coordinates, the symmetry operators present in the PDB file were used to obtain the entire 4-fold symmetric structure of the pore with MOE. Finally, the pore was submitted to GRID runs varying three probes (K+, OH2 and DRY) and thus obtaining the corresponding MIFs (Fig. 3C–E). Fig. 3C shows in pink the iso-surfaces joining all points having a very favourable value of interaction energy with the K+ probe (-20.0 kcal mol^{-1} was selected as threshold value). Interestingly, these calculated regions are largely superposable with the four potassium sites located in the X-ray structure in the channel pore in proximity to the potassium selectivity filter (shown in green in Fig. 3B and characterized by the well-known sequence motif Thr-Val-Gly-Tyr-Gly). Fig. 3D shows in blue the iso-surfaces obtained with the probe OH2 at -5 kcal mol^{-1}. It is noteworthy that favourable interactions with water are also predicted to take place in the region of location of potassium ions. This result is in agreement with experimental data that suggest that water molecules replace potassium ions during the potassium ion permeation process [54]. Fig. 3E shows in yellow the iso-surfaces obtained with the probe DRY at -0.05 kcal mol^{-1}. This probe is designed to describe hydrophobic interactions occurring between the ligand and the target. In Fig. 3E yellow regions as obtained by GRID are located mainly in the mouth of the intracellular region of the channel, whereas no hydrophobic region is present in the pore or in the extracellular mouth where potassium ions S1–S4 are located. These findings confirm that the interaction of potassium ions with the bacterial channel is, as expected, largely dominated by electrostatic contributions. Moreover, these results can be used to obtain preliminary but clear information about the binding of drugs with *hERG* potassium channel, since the latter shares several structural features

with eukaryotic Kv channels. In particular, drugs withdrawn from the market due to QT prolongation concerns show lipophilic moieties which, in the binding process, are expected to be located in the hydrophobic regions of the target. In hERG the hydrophobic content is increased compared to the bacterial channel (but analogously located), because of the presence of Tyr and Phe, which respectively replace Leu and Ile in positions 652 and 656. Taken together these observations undoubtedly strengthen the role played by hydrophobic interactions in the binding of drugs with the *hERG* potassium channel.

This example clearly shows how the use of MIFs can shed light on the analysis of the balance of forces governing ligand–protein interactions. In fact, even if quantitative analysis of the single contributions to the interaction is not possible because of the method's dependence on the threshold values selected, a general indication of the occurring factors can easily be obtained even by non-experts in the molecular-modelling field.

6.3. *Docking and molecular dynamics to study drug/hERG interactions*

In the absence of the crystal structure for *hERG* potassium channels, insight into hERG-blocker binding interactions can also be obtained, as mentioned above, from analysis of the homology model (see above). This method has been included in two different in silico approaches, described by Farid et al. [42] and by Österberg and Åqvist [43]. In both studies a homology model of the homo-tetrameric pore domain of hERG was first created using the bacterial potassium channel KvAP as template. Then, to study the binding of some blockers to *hERG* channels, Farid et al. [42] applied a docking procedure, whereas Österberg and Åqvist [43] used molecular dynamics simulations. Since both studies considered sertindole (a blockbuster drug withdrawn from the market due to reports of sudden cardiac death related to its binding to *hERG* potassium channels [41]), a comparative analysis of the two studies was performed by evaluating results for this ligand.

In the first example, the ligand was docked in the putative binding site of the hERG homology model using GLIDE 3.0 [55]. A post-docking procedure was also applied, to take into account the flexibility of the protein. Finally, the predicted docked pose was analysed and the main interactions were deduced probably by simple visual inspection of the complex, in synthesis simultaneous interactions are predicted between sertindole and four aromatic side chains including three Tyr652 residues and one Phe656 residue.

The MD approach used by Österberg and Åqvist [43] approximately confirms the docking results described above, but the results obtained also include two numerical values corresponding to the van der Waals and electrostatic contributions (see Section 3.1 for the classification of forces) governing the interaction with the potassium channel. These values underline the main role played by electrostatic forces in the interaction between sertindole and *hERG* potassium channel.

In the ligand–protein field, docking and molecular dynamics are computer simulations whose main goal is to predict the ligand binding free energy in the aqueous state, $\Delta G_B(aq)$ in Fig. 2. The two methods differ considerably in how they reach the prediction: docking procedures focus exclusively on the binding equilibrium in water, whereas molecular dynamics (or Monte Carlo) free energy simulations consider the whole thermodynamics cycle. Docking procedures, which require an empirical energy function (called scoring function) dependent on the position of the ligand in the binding site and correlated to experimental $\Delta G_B(aq)$, are more intuitive and do not require a lot of computer time or effort, whereas the reverse is true for molecular dynamics simulations. On the other hand, docking suffers from the limitations of all empirical methods: the results depend to a considerable extent on the database used to calibrate the empirical energy function (other drawbacks are beyond the scope of this discussion). In addition, the accuracy of docking when used to investigate the balance of forces governing the interaction closely depends on the chosen scoring function and is thus a questionable strategy.

Molecular dynamics free energy simulations take solvation effects into account and thus hydrophobic effects are included in the evaluation of the binding free energy, but the complexity of the theory and the need for long CPU effort make it necessary to introduce various approximations. In addition, evaluation of the results is far from obvious and moreover, as seen in the example above, the procedures cannot furnish straightforward indications about the balance of the forces involved in the binding.

7. Conclusions

Since any molecular recognition process occurs through a number of interactions between molecules, this paper first highlights the relevance of fundamentals aspects of ligand–protein interactions: their electrostatic nature, the fundamental role played by the hydrophobic effect which is the most evident result of the role played by water, and the use of crystallographic databases as sources of experimental data.

The second part of the commentary focused on some in silico tools that are very useful in the analysis of ligand–protein interactions. These tools include the very simple analysis of the contacts (something more than the results obtained by a simple visual inspection) but also docking and molecular dynamics strategies, these latter listed in order of increasing theoretical level. Shown applications indicate that a precise analysis of the balance of forces governing any ligand–receptor interaction is to date rather difficult to achieve in a straightforward and general manner. This limitation arises from two main reasons: the neglection of the hydrophobic effect and the uncertainty in the definition of Van der Waals contribution. The missing information about the balance of intermolecular forces represents therefore a great limitation for the design of drug candidates.

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Appendix A. Mathematical treatment of ion–permanent dipole interaction

The potential energy between a unit positive charge, which is chosen for simplicity, and two charges is given by

$$U(r_1, r_2) = \frac{1}{4\pi\varepsilon_0}\left[\frac{q_1}{r_1} + \frac{q_2}{r_2}\right] \tag{A.1}$$

in the simple configuration of Fig. 1C, the distances r_1 and r_2 can be expressed in term of z_1, z_2, the distance r between the point O (which is arbitrarily chosen on the line that joins the two charges) and the unit charge, located in the point P, Eq. (A.1) becomes

$$U(r,\theta) = \frac{1}{4\pi\varepsilon_0} \times \left[\frac{q_1}{\left(r^2 + z_1^2 + 2z_1 r\cos\theta\right)^{1/2}} + \frac{q_2}{\left(r^2 + z_2^2 + 2z_2 r\cos\theta\right)^{1/2}}\right] \tag{A.2}$$

the potential is now expressed in term of the ''distance'' of the ion by the dipole r, the orientation of the dipole trough θ, the characteristics of the dipole (charges and z_1 and z_2 values, that are not variable when the dipole is defined). Then the situation in which $r \gg z_1$, z_2 is considered, if $r \sim z_1$, z_2 it should be impossible to distinguish a dipole! When $r \gg z_1, z_2$ it is possible to rearrange Eq. (A.2) in term of variables z_1/r and z_2/r, then Eq. (A.2) can be expand in series of $z_{1,2}/r$ to obtain:

$$U(r,\theta) = \frac{1}{4\pi\varepsilon_0}\left[\frac{q_1 + q_2}{r} + \frac{(q_1 z_1 - q_2 z_2)\cos\theta}{r^2} + \cdots\right] \tag{A.3}$$

some of the terms of the series have a physical meaning but the discussion is beyond the scopes of theses chapter.

In the case of a dipole $q_1 = -q_2 = q$ so the first term of the Eq. (A.3) is null and the second becomes

$$U(r,\theta) = \frac{1}{4\pi\varepsilon_0}\frac{q(z_1 + z_2)\cos\theta}{r^2} = \frac{1}{4\pi\varepsilon_0}\frac{p\cos\theta}{r^2} \tag{A.4}$$

the potential U depends on $1/r^2$ and contains an angular part representing the dependence of the orientation of the dipole respect to the charged ion. If the two particles are free to move, the interaction is averaged by thermal motion, the angular factor is lost and the distance dependence becomes $1/r^4$ (Eq. (T2)).

REFERENCE

[1] Davis AM, Teague SJ. Hydrogen bonding, hydrophobic interactions, and failure of the rigid receptor hypothesis. Angew Chem Int Ed 1999;38:737–49.
[2] Wikipedia. http://www.wikipedia.org/; 2006.
[3] Messiah A. Quantum mechanics North-Holland; 1970.
[4] Kaplan IG. Modern state of intermolecular interaction theory. Int J Quantum Chem 2004;16:445–65.
[5] Maitland GC, Rigby M, Smith EB, Wakeham WA. Intermolecular forces. In: Breslow R, Goodenough JB, Halpern J, Rowlinson JS, editors. International series of monographs on chemistry, 2nd ed. New York: Oxford University Press; 1987.
[6] Desiraju GR. Chemistry beyond the molecule. Nature 2001;412:397–400.
[7] Meyer EA, Castellano RK, Diederich F. Interactions with aromatic rings in chemical and biological recognition. Angew Chem Int Ed 2003;42:1210–50.
[8] Dunitz JD, Gavezzotti A. Attractions and repulsions in molecular crystals: what can be learned from the crystal structures of condensed ring aromatic hydrocarbons? Acc Chem Res 1999;32:677–84.
[9] Müller P. Glossary of terms used in physical organic chemistry. http://www.chem.qmul.ac.uk/iupac/gtpoc/PQ.html; 1994.
[10] Bondi A, Van der Waals. Volumes and radii. J Phys Chem 1964;68:441–51.
[11] Rowland RS, Taylor R. Intermolecular nonbonded contact distances in organic crystal structures: comparison with distances expected from Van der Waals radii. J Phys Chem 1996;100:7384–91.
[12] McNaught AD, Wilkinson A. IUPAC compendium of chemical terminology Oxford, UK: Blackwell Science; 1997.
[13] Allen FH, Taylor R. Research applications of the Cambridge Structural Database (CSD). Chem Soc Rev 2004;33:463–75.
[14] Allen FH, Motherwell WDS. Applications of the Cambridge Structural Database in organic chemistry and crystal chemistry. Acta Crystallogr Sect B—Struct Sci 2002;58:407–22.
[15] Taylor R. Life-science applications of the Cambridge Structural Database. Acta Cryst Sect D Biol Crystallogr 2002;58:879–88.
[16] Gilli G, Gilli P. Towards an unified hydrogen-bond theory. J Mol Struct 2000;552:1–15.
[17] Gohlke H, Klebe G. Approaches to the description and prediction of the binding affinity of small-molecule ligands to macromolecular receptors. Angew Chem Int Ed 2002;41:2645–76.
[18] Bruno IJ, Cole JC, Lommerse JPM, Rowland RS, Taylor R, Verdonk ML. IsoStar: a library of information about nonbonded interactions. J Computer-aided Mol Des 1997;11:525–37.
[19] Kollman PA, Allen LC. The theory of the hydrogen bond. Chem Rev 1972;72:283–303.
[20] Steed JW, Atwood JL. Supramolecular. Chemistry 2000.
[21] Bosshard HR, Marti DN, Jelesarov I. Protein stabilization by salt bridges: concepts, experimental approaches and clarification of some misunderstandings. J Mol Recognit 2004;17:1–16.
[22] Hunter CA, Sanders JKM. The nature of pi–pi interactions. J Am Chem Soc 1990;112:5525–34.
[23] Ma JC, Dougherty DA. The cation–π interaction. Chem Rev 1997;97:1303–423.
[24] Kim KS, Tarakeshwar P, Lee JY. Molecular clusters of pi-systems: theoretical studies of structures, spectra, and origin of interaction energies. Chem Rev 2000;100:4145–85.
[25] Testa B, Carrupt PA, Gaillard P, Billois F, Weber P. Lipophilicity in molecular modeling. Pharm Res 1996;13:335–43.
[26] Raffa RB. (Extra)thermodynamics of the drug-receptor interaction. Life Sci 1999;65:967–80.
[27] Kollman PA. Drug-target binding forces. In: Wolff ME, editor. Principles and practise, vol. 1. John Wiley & Sons Inc.; 1995. p. 399–412.

[28] Kubinyi H. 3D QSAR in drug design: theory, methods and applications Leiden: ESCOM; 1993.
[29] Kauzmann W. Some factors in the interpretation of protein denaturation. Adv Protein Chem 1959;14:1–63.
[30] Betts MJ, Russel RB. Amino acid properties and consequences of substitutions. In: Barnes MR, Gray IC, editors. Bioinformatics for geneticists. Chichester, West Sussex, England: J. WIley & Sons Ltd.; 2003.
[31] ten Wolde PR. Hydrophobic interactions: an overview. J Phys—Condensed Matter 2002;14:9445–60.
[32] Nogrady T. Medicinal chemistry: a biochimical approach New York: Oxford University Press; 1988.
[33] Sijbren O, Engberts JBFN. Hydrophobic interactions and chemical reactivity. Org Biomol Chem 2003;1:2820.
[34] Testa B, Kier LB, Carrupt PA. A systems approach to molecular structure, intermolecular recognition, and emergence-dissolvence in medicinal research. Med Res Rev 1997;17:303–26.
[35] Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, et al. The Protein Data Bank. Nucl Acids Res 2000;28:235.
[36] Hendlich M, Bergner A, Gunther J, Klebe G. Relibase: design and development of a database for comprehensive analysis of protein–ligand interactions. J Mol Biol 2003;326:607–20.
[37] Garbuzynskiy SO, Melnik BS, Lobanov MY, Finkelstein AV, Galzitskaya OV. Comparison of X-ray and NMR structures: is there a systematic difference in residue contacts between X-ray and NMR-resolved protein structures? Proteins—Struct Funct Bioinf 2005;60:139–47.
[38] Davis AM, Teague SJ, Kleywegt GJ. Application and limitations of X-ray crystallographic data in structure-based ligand and drug design. Angew Chem Int Ed 2003;42:2718–36.
[39] Marnett LJ, Kalgutkar AS. Cyclooxygenase 2 inhibitors: discovery, selectivity and the future. Trends Pharmacol Sci 1999;20:465–9.
[40] Jiang YX, Lee A, Chen JY, Ruta V, Cadene M, Chait BT, et al. X-ray structure of a voltage-dependent K+ channel. Nature 2003;423:33–41.
[41] Aronov MM. Predictive in silico modeling for hERG channelblockers. Drug Discovery Today 2005;10:149–55.
[42] Farid R, Day T, Friesner RA, Pearlstein RA. New insights about HERG blockade obtained from protein modeling, potential energy mapping, and docking studies. Bioorg Med Chem 2006;14:3160–73.
[43] Österberg F, Åqvist J. Exploring blocker binding to a homology model of the open hERG K^+ channel using docking and molecular dynamics methods. FEBS Lett 2005;579:2939–44.
[44] Kurumbail RG, Stevens AM, Gierse JK, McDonald JJ, Stegeman RA, Pak JY, et al. Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. Nature 1996;384:644–8 [published erratum appears in Nature 1997;385(6616):555].
[45] Picot D, Loll PJ, Garavito RM. The X-ray crystal structure of the membrane protein prostaglandin H2 synthase-1. Nature 1994;367:243–9.
[46] Selinsky BS, Gupta K, Sharkey CT, Loll PJ. Structural analysis of NSAID binding by prostaglandin H-2 synthase: time-dependent and time-independent inhibitors elicit identical enzyme conformations. Biochemistry 2001;40:5172–80.
[47] Moreland J, Gramada A, Buzko O, Zhang Q, Bourne P. The molecular biology toolkit (MBT): a modular platform for developing molecular visualization applications. BMC Bioinf 2005;6:21.
[48] MOE. [2005.06]. Montreal, Quebec, Canada: Chemical Computing Group Inc.; 2005.
[49] Sobolev V, Sorokine A, Prilusky J, Abola EE, Edelman M. Automated analysis of interatomic contacts in proteins. Bioinformatics 1999;15:327–32.
[50] Stickle DF, Presta LG, Dill KA, Rose GD. Hydrogen-bonding in globular-proteins. J Mol Biol 1992;226:1143–59.
[51] Thuresson ED, Malkowski MG, Lakkides KM, Rieke CJ, Mulichak AM, Ginell SL, et al. Mutational and X-ray crystallographic analysis of the interaction of dihomo-gamma-linolenic acid with prostaglandin endoperoxide H syntheses. J Biol Chem 2001;276:10358–65.
[52] Goodford PJ. A computational procedure for determining energetically favorable binding sites on biologically important macromolecules. J Med Chem 1985;28:849–57.
[53] GRID. [2.2]. Pinner, Middlesex, UK: Molecular Discovery Ltd.; 2005.
[54] Noskov SY, Berneche S, Roux B. Control of ion selectivity in potassium channels by electrostatic and dynamic properties of carbonyl ligands. Nature 2004;431:830–4.
[55] GLIDE. [3.0]. Portland, OR: Schrödinger, LLC; 2006.