

# TOPICS IN MOLECULAR PHARMACOLOGY VOLUME 2

A. S. V. BURGEN  
AND  
G.C. K. ROBERTS

EDITORS

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## *Preface*

In recent years, there have been considerable advances in our knowledge of the details of small molecule—macromolecule interactions in general, as well as in the characterisation of receptor molecules themselves. These advances have brought us substantially closer to the goal of a detailed understanding of drug, hormone and neurotransmitter action at a truly molecular level.

There is, of course, still a long way to go. Further progress will depend upon the integration of many different kinds of information — from physical chemistry to physiology. We feel that the development of molecular pharmacology would be helped by publication of a series of reviews of relevant advances in these areas.

It is our intention that the series should deal both with the fundamental general principles governing drug-receptor interactions and with analysis of specific receptor systems. Thus in this second volume the articles range from discussions of the basic physical chemistry of intermolecular interactions to descriptions of complex membrane-bound receptor systems. The common feature is a concern to push as far as possible toward the molecular level, to understand drug action in terms of the three-dimensional structure and dynamics of the receptor system.

Arnold S.V. Burgen  
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# *Hydrophobic interactions in biological systems*

A. BEN-NAIM

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## 1. Introduction

The term 'hydrophobic interactions' has been used in the chemical, biochemical and biological literature in connection with several closely related phenomena. As implied by the term, hydrophobic interactions are involved in processes occurring in aqueous solutions, in which solutes (or groups in larger molecules) tend to 'avoid' the aqueous environment. This tendency may be expressed in various ways, some of which are described in Figure 1. In Figure 1a, a partition of a solute between two solvents is depicted. One of the solvents is water, the second may be any organic liquid, say hexane. If the solute distributed between the two phases is, say, methane, we observe that the 'partition coefficient' (i.e., the concentration ratio of methane in the two phases) is in favor of the non-aqueous solvent. This observation may be interpreted in terms of the relative preference of methane for the two environments, or the extent of its *phobia* for the aqueous phase. Equivalently, if we had *one* solute molecule distributed between the two phases we should observe that over a very long period of time, the solute would spend about ten times longer in the organic phase than in the aqueous phase. We shall return to this aspect of hydrophobic effects, which depends upon *solute-solvent* interactions, in section 6.

Figure 1b shows a schematic process of dimerization. Here the two solutes approach each other within the aqueous phase. By forming a dimer they partially avoid the exposure of their surface to the solvent. It is in this respect that we use the term hydrophobic interaction to describe the affinity between the two solutes, or the equilibrium

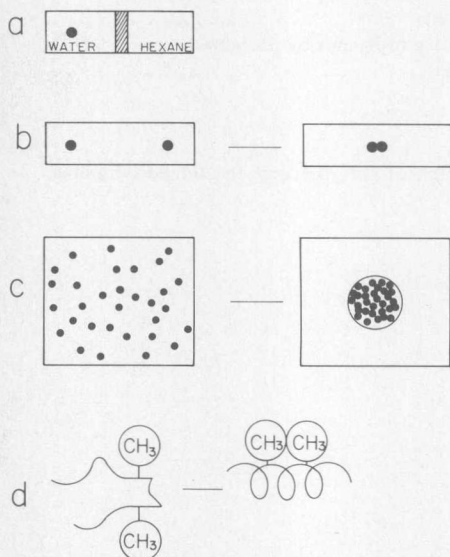


Fig. 1. Schematic examples of processes involving hydrophobic interactions: (a) a non-polar solute distributed between two phases: water and hexane; (b) a process of dimerization; (c) aggregation of a large number of solute particles to form a droplet in water; (d) a conformational change of a polymer. Two methyl groups (encircled) are brought in contact in the conformation on the right hand side.

constant for the dimerization process. This aspect of the subject is dealt with from section 7 through the end of the chapter.

The various processes of aggregation among many solute particles, shown schematically in Figure 1c, may be considered generalizations of the dimerization process. In the limit of a very large number of particles, the aggregation process leads to the formation of a droplet. Clearly, in this case most of the solute molecules that participate in the aggregate are not exposed to the aqueous environment; only those on the surface of the droplet are in direct contact with the water. Finally, in Figure 1d we show a schematic intramolecular process, where a polymer changes its conformation. If we focus on two (or more) side chains, such as the two methyl groups marked in the figure, we find that their extent of exposure to the solvent is reduced by the folding of the polymer. Historically, it was the puzzling mechanism for the spontaneous folding of a protein into the native (or active) conformation that led the biochemists to suspect that one of the main contributions to the driving force for this process is the hydrophobic interaction.

All of the processes described above have one feature in common, namely the tendency of the non-polar solutes (or groups) to reduce their contact with the water.

From the physicochemical point of view the origin of the hydrophobic interaction, on a molecular level, is not yet understood. This will ultimately be dependent on our understanding of the properties of liquid water on the microscopic level. From the biological point of view one is usually more interested in some practical questions. For example, how to estimate the strength of the interaction between various solutes (or groups in larger molecules), or how to use our knowledge of hydrophobic effects to regulate or control biological processes (such as the adsorption and release of drugs that are bound to proteins)?

In this review we shall survey some of the experimental aspects of hydrophobic interactions. The style is mostly descriptive, except for one appendix where the Langmuir isotherm is derived from statistical mechanical considerations.

## 2. Definitions

We now translate the description we have given of hydrophobic interactions into more precise terms. The simplest case involves two simple solutes, say argon or methane, in water. Let  $R_1$  and  $R_2$  be the position vectors of the centers of these two solutes within the solvent. The process in which we shall be interested is the following: We start with the two solutes at infinite separation from each other, i.e.  $R_{12} = |R_2 - R_1| = \infty$ , and then bring them to some close separation, say  $R_{12} \approx \sigma$ , where  $\sigma$  is of the order of the solute diameter. The process is carried out within the solvent at some fixed temperature  $T$  and pressure  $P$ .

The Gibbs free energy change of the system in this process is written symbolically as

$$\Delta G(\infty \rightarrow \sigma) = G(T, P, \text{Solvent composition}; R_{12} = \sigma) - G(T, P, \text{Solvent composition}; R_{12} = \infty) \quad (1)$$

For reasons that will be discussed later (section 7) it is convenient to split  $\Delta G(\infty \rightarrow \sigma)$  into a direct and an indirect part, namely

$$\Delta G(\infty \rightarrow \sigma) = U(\sigma) + \delta G^{\text{HI}}(\sigma) \quad (2)$$

Here,  $U(\sigma)$  is the direct (or the vacuum) part of the interaction between the two particles. The remaining part of  $\Delta G(\infty \rightarrow \sigma)$ ,  $\delta G^{\text{HI}}$  is the solvent-induced interaction, which, in water, may be referred to as the hydrophobic part of the interaction.

The reason for using the Gibbs free energy function and not any other thermodynamic function in our definition is as follows.  $\Delta G$  of the process is the work required to bring the two solutes from  $R_{12} = \infty$  to  $R_{12} = \sigma$ . It can be shown (for details see Ben-Naim, 1974) that the gradient of  $\Delta G(\infty \rightarrow R)$  with respect to  $R$ , is the force acting between the two solutes at  $R$ , i.e.

$$F_{\text{ss}}(R) = - \frac{\partial \Delta G(\infty \rightarrow R)}{\partial R} = - \frac{\partial U(R)}{\partial R} - \frac{\partial \delta G^{\text{HI}}(R)}{\partial R} \quad (3)$$

This force includes a direct force that originates from the solute particles themselves, and an indirect force that originates from the presence of the solvent.

Clearly, it is very easy to generalise the concept of an interaction between two particles, so that it will include many solute particles. To do this we start with  $n$  simple spherical solutes at fixed positions but at infinite separation from each other. Then we bring them to some compact configuration (say, by forming a spherical droplet). Let us denote the final configuration by  $R_1, R_2, \dots, R_n$  (where  $R_i$  is the position vector of the center of the  $i$ -th solute). The hydrophobic interaction among  $n$  solutes at the configuration  $R_1, R_2, \dots, R_n$  is defined, generalising (Eqn. 2), by

$$\delta G^{\text{HI}}(R_1, R_2, \dots, R_n) = \Delta G(\infty \rightarrow R_1, R_2 \dots R_n) - U(R_1, R_2 \dots R_n) \quad (4)$$

i.e., we extract the direct interaction  $U$ , from the total change in free energy of the process.

In all of the processes mentioned above the concept of the hydrophobic interaction has been used in connection with at least two interacting solute particles. The process of transferring a solute from one solution phase into another involves essentially one solute particle interacting with the solvent and formally does not involve hydrophobic interaction in the sense that was defined above. However, in some limiting cases one can show that information on the free energy of transfer may be used to extract information on hydrophobic interactions. A detailed account of this aspect is given in Ben-Naim (1980). In this review we shall only briefly touch upon the so-called hydrophobic hydration in section 6.

Apart from the process described above, one may use the term hydrophobic interaction for intramolecular processes. We have referred to such an example in Figure 1d where two non-polar groups approach each other on the same molecule. Here the hydrophobic interaction between the two groups is more difficult to measure, since in the



overall process of conformational change of a macromolecule there is no way of separating the hydrophobic interaction between two selected groups from the total free energy change that accompanies the changes in the state of the entire polymer. However, recently a simple model for estimating the intramolecular hydrophobic interaction has been devised (Ben-Naim and Wilf, 1979). In this model one uses a molecule with two alkyl substituents. The transfer of one alkyl group is from one position (where it is far from the second group) to another position (where it is close to the second group). In this process the 'carrier' molecule is assumed to be unaffected, and hence the total free energy change of the process is approximately related only to the intramolecular hydrophobic interaction. This model is further discussed in section 8.

### 3. The fundamental theoretical problem concerning hydrophobic interactions

In this section we present what we believe to be the crux of the theoretical problem of the hydrophobic interaction. The exposition is very sketchy and the reader who is interested in more details on this topic is referred to Ben-Naim (1974, 1980).

The main tool for handling the problem on a molecular level is statistical mechanics. The details of the various theories are quite advanced. However, since the problem is very difficult, all the results obtained so far by theoretical means are quite preliminary.

The statistical mechanical formulation of the hydrophobic interaction between two particles starts from the expression for  $\delta G^{\text{HI}}$  in terms of the intermolecular interactions between all the molecules in the system. In a compact form the expression is (for details see Ben-Naim 1974, 1980)

$$\Delta G(\infty \rightarrow \sigma) = U(\sigma) + \delta G^{\text{HI}}(\sigma) = U(\sigma) - k_{\text{B}} T \ln \left\{ \frac{\langle \exp[-\beta B_{\text{SS}}(R = \sigma)] \rangle_{\text{o}}}{\langle \exp[-\beta B_{\text{SS}}(R = \infty)] \rangle_{\text{o}}} \right\} \quad (5)$$

Here,  $U(\sigma)$  is the direct interaction energy between the two solutes at the distance  $R = \sigma$ ,  $k_{\text{B}}$  is the Boltzmann constant,  $\beta = (k_{\text{B}} T)^{-1}$  and  $B_{\text{SS}}$  is the total interaction energy between the two solutes S and all the solvent molecules at some specific configuration. The initial and final states of the system are indicated by  $R = \infty$  and  $R = \sigma$ , respectively. The notation  $\langle \rangle_{\text{o}}$  stands for an average over all the possible configurations of the solvent molecules (in the  $T, P, N$  ensemble, using the probability density of the pure solvent).

The ultimate aim of a molecular theory should be to evaluate the two average quantities in Eqn. 5. However, this goal is certainly not going to be attained in the foreseeable future. Therefore various approximations have been devised to estimate these averages. It is also important to recognize the fact that the mere knowledge of the form of the expression (Eqn. 5) for  $\delta G^{\text{HI}}$  is of some importance, even if we cannot compute its value. For example, the very fact that the *direct* solute-solute potential does not contribute to  $\delta G^{\text{HI}}$  has been exploited to derive a measure of the hydrophobic interaction. This measure will be described shortly. The important point which we would like to emphasize at this stage is that sometimes the very form of the expression itself may lead to some partial answers to our problem, which do not require a full evaluation (exact or approximate) of the average quantities in Eqn. 5.

There is another interpretation of the quantity  $\Delta G(\infty \rightarrow R)$  which is worth noting. We have defined this quantity as the free energy change for a certain process. This process, though well defined, is not one that may be carried out in an actual experiment. However, the same quantity may be related to the probability of finding the two solute particles at a distance  $R$  from each other. More precisely, if we have a system consisting of a solvent at some temperature  $T$  and pressure  $P$ , and two solute particles, then the probability density of finding the two solutes at a distance  $R$  is related to the solute-solute pair correlation function  $g_{SS}(R)$  by

$$P(R) = \rho_S^2 g_{SS}(R) \quad (6)$$

where  $\rho_S$  is the solute density (which in this case is  $2/V$ , where  $V$  is the volume of the system). The relation between  $g_{SS}(R)$  and the free energy change  $\Delta G(\infty \rightarrow R)$  is simply

$$g_{SS}(R) = \exp [-\Delta G(\infty \rightarrow R)/k_B T] \quad (7)$$

This relation is valid for any concentration of the solute. For more details on the derivation of this relation the reader is referred to Ben-Naim (1974). Note that the quantities  $g_{SS}(R)$  and  $\Delta G(\infty \rightarrow R)$  pertain to two different systems. In one case the two solutes  $S$  are free to wander in the volume  $V$  and we look at the distribution of the intermolecular distances between them. In the second system, we start with the two solutes at *fixed* positions but at infinite separation from each other and bring them to *fixed* positions at a distance  $R$  from each other. The equivalence between the two quantities is expressed in Eqn. 7.

Knowledge of the form of the function  $g_{SS}(R)$  for two solutes  $S$  in water is perhaps the most important goal of any theory of hydrophobic interactions. At present we have gained only partial and very approximate information on  $g_{SS}(R)$ . However, once this function is known, we can estimate the hydrophobic 'forces' operating between two solutes in water. A comparison between such functions in different solvents could, in principle, tell us to what extent water is indeed a unique liquid.

Of course, there are other problems related to hydrophobic effects which are of interest, especially if we aim at understanding molecular processes in biological systems. One important problem, about which we know almost nothing, is to what extent are hydrophobic interactions among many solute particles pairwise additive? Alternatively, can we learn anything from studies of pairwise interactions about the cooperative interaction among many solutes, or non-polar groups attached to a biopolymer? Such questions should be of importance in the future. At present we should focus on the simplest aspect of the theoretical problem, which involves the function  $g_{SS}(R)$ .

#### 4. Some recent progress on the theoretical front

We shall now turn to a brief outline of the recent progress in the theory of hydrophobic interactions. As we have just noted, a full knowledge of the function  $g_{SS}(R)$  in water

(and in some other solvents, for comparison) contains all the information we need. (Of course, this knowledge is restricted to one specific set of thermodynamic variables; for a broader knowledge one needs to know the dependence of  $g_{ss}(R)$  on temperature, pressure and solvent composition.) Most of the theoretical effort in this field is thus focused on methods of computing, or estimating, this function. So far there have been essentially two routes along which the function  $g_{ss}(R)$  has been studied. One is based on a solution of a system of integral equations for the various pair correlation functions in the system. The second depends on one of the computer simulation techniques. Both methods use drastic approximations and therefore the results obtained so far should be regarded as being tentative only.

Along the first route, Pratt and Chandler (1977), have applied a sort of semi-fundamental theory for the computation of  $g_{ss}(R)$ . It is not fundamental in the traditional sense where the ingredients of a fundamental molecular theory are the parameters of a single molecule and the intermolecular potentials operating between two (or a small number) of molecules. In contrast, Pratt and Chandler have used the statistical mechanical formalism into which they injected experimental information on the solvent-solvent pair correlation function. This function would have normally been an output rather than an input of the theory. However, by using this information in a set of integral equations (the four Ornstein Zernicke equations) they obtained a short-cut to the computation of the solute-solute pair correlation function. (A previous attempt to compute the hole-hole distribution function for liquid argon and for water has been reported by Hill (1958) and by Ben-Naim (1969).)

As an illustration we present in Figure 2a a result obtained by Pratt and Chandler (1977). Here the function  $\Delta G(\infty \rightarrow R)$  at 30°C and 1 atm pressure is plotted for two hard spheres of diameter  $\sigma_A = 5.0$  Å (a) in a hard sphere solvent with a diameter of 2.7 Å and the same density as liquid water (dashed line), and (b) in water (solid line). From this result alone it is impossible to reach any conclusion regarding the outstanding nature of water as a solvent. In fact, the two curves are very similar in their gross features. They are

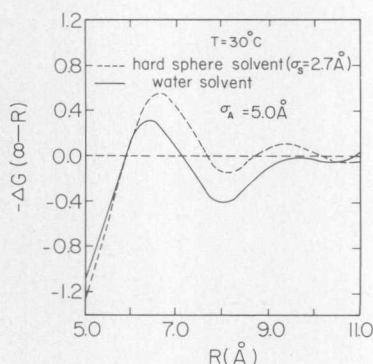


Fig. 2. The free energy change  $\Delta G(\infty \rightarrow R)$  (or the potential of average force) in units of  $k_B T$  between two hard spheres of diameter  $\sigma_A = 5.0$  Å in water (—) and in a hard-sphere solvent with diameter 2.7 Å, and the same density as liquid water at 30°C and 1 atm pressure (---). Redrawn with changes from Pratt and Chandler (1977).

almost identical at contact distance between the two solutes ( $R \approx 5.0 \text{ \AA}$ ) and the general appearance at larger separations are much the same in the two cases. This result is quite surprising to those who still believe that the difference between the two curves should be more pronounced and remarkable.

Regarding the temperature dependence of the hydrophobic interaction (as measured by  $\delta G^{\text{HI}}$ ; for more details see section 5), Pratt and Chandler (1977) found that at some distance, say around  $R \sim 6 \text{ \AA}$ , the values of  $|\delta G^{\text{HI}}|$  increase with temperature. However, at some other distances they obtained either the opposite effect or almost no effect of temperature. As we shall discuss in section 5, it is very important to specify which is the quantity we choose to define the hydrophobic interaction. For example, taking either  $g_{\text{SS}}(R)$  or  $\delta G^{\text{HI}}(\infty \rightarrow R)$  at some fixed value of  $R$  (say  $R \sim \sigma$ ) as our measure, we expect that these two quantities should have different temperature dependences. This is obvious from the relation (Eqn. 7) which connects the two quantities.

From all the results obtained by Pratt and Chandler (1977), it seems that water as a solvent does not manifest an outstanding behaviour with respect to the properties that were examined. It is not clear whether this result is relevant to real water or is an artifact of the approximate treatment. The authors were well aware that the most questionable part of their theory is the neglect of the role of the angular correlation between water molecules (i.e., the use of  $g_{\text{ww}}(R)$  rather than the full pair correlation function for water molecules).

As an example of work on the alternative route we cite here the paper by Pangali et al. (1979). These authors applied the Monte Carlo technique to simulate the behaviour of a system consisting of 214 water-like particles (which are characterized by a model pair potential denoted ST2) and two Lennard-Jones particles serving as solutes. (Previous work along similar lines was reported by Dashevesky and Srakiso, 1974; Geiger et al., 1979; and by Swaminathan and Beveridge, 1979.)

The function  $\delta G^{\text{HI}}(\infty \rightarrow R)$  calculated in this way is reproduced in Figure 3. Note that

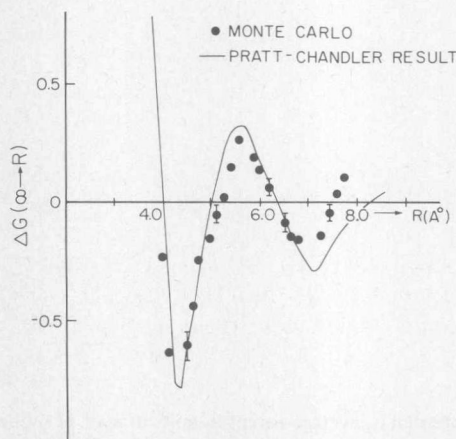


Fig. 3. The free energy change  $\Delta G(\infty \rightarrow R)$  in units of  $k_B T$  between two Lennard-Jones particles dissolved in 214 water molecules (ST2 model) as computed by Monte Carlo method ( $\cdots$ ), and by Pratt and Chandler (—). Redrawn with changes from Pangali et al. (1979).



the first minimum ( $R = \sigma_s = 4.12 \text{ \AA}$ ) corresponds to the distance of contact between the two solutes. The second minimum occurs at  $R = 7.13 \text{ \AA}$ , which roughly corresponds to the distance between the two solutes separated by a single water molecule of a diameter of about  $3.0 \text{ \AA}$ . This pattern of minima and maxima in  $g_{ss}(R)$  in dilute solutions of a solute in a solvent is typical of a mixture of simple fluids (see for more details Ben-Naim, 1974, chapter 4) and does not indicate any peculiarities due to the structure of water.

One way of interpreting the computed results is to define an 'equilibrium constant' corresponding to two kinds of 'dimers'. A dimer at contact, denoted by AA, is defined as any pair of solutes, the separation of which is from zero to  $r_1$  ( $r_1 = 5.73 \text{ \AA}$  is the position of the first maximum in  $\delta G^{\text{HI}}(\infty \rightarrow R)$ ). The second species is a dimer separated by a water molecule, denoted by AWA, and is defined by any pair of solutes, the separation of which is between  $r_1$  and  $r_2$  ( $r_2$  being the position of the second maximum in  $\delta G^{\text{HI}}$ ).

The equilibrium constant is thus

$$K = \frac{X_{\text{AWA}}}{X_{\text{AA}}} = \frac{\int_{r_1}^{r_2} g_{ss}(R) R^2 dR}{\int_0^{r_1} g_{ss}(R) R^2 dR} \quad (8)$$

Calculations by the authors led to a value of  $K = 1.8$  which was interpreted as indicating that the AWA dimer is more stable than the AA dimer. One remarkable point is the nice agreement between the Monte Carlo and the Pratt and Chandler results as demonstrated in Figure 3.

We shall close this brief section with a general comment on the theoretical effort in this field. It is clear that a knowledge of the function  $g_{ss}(R)$  [or equivalently  $\delta G^{\text{HI}}(\infty \rightarrow R)$ ] should be the aim of any theoretical effort. It is also clear that this information is not going to be gained in the near future. Therefore one must try to gain partial information on this function by whatever theoretical or experimental means available. We shall later discuss two such approaches. One involves integrals over  $g_{ss}(R)$ , the other involves the estimation of  $\delta G^{\text{HI}}$  at some specific separations.

## 5. Temperature and pressure dependence

Throughout this chapter we shall encounter many examples of the temperature, and sometimes the pressure, dependence of various quantities that are associated with hydrophobic interactions. These are manifested through the entropy, enthalpy and the volume changes associated with the various processes under discussion. The literature is full of tables of  $\Delta S^\circ$ ,  $\Delta H^\circ$  and, less often, of  $\Delta V^\circ$  that are claimed to represent the temperature and pressure coefficients of the hydrophobic interaction. However, there is a large amount of confusion involved in both the definition and the interpretation of these quantities. We shall not elaborate on this aspect here. A critical account of one example has been presented recently (Ben-Naim, 1978). Instead, we shall stress only one general aspect of