

Physical Chemistry in Action

Arieh Ben-Naim

# Solvent-Induced Interactions and Forces in Protein Folding

Hydrophobic-Hydrophilic Phenomena

 Springer

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*This book is dedicated to my friend and  
colleague,  
Prof. Kesharsingh J. Patil.*



# Preface

In 1980, I published a book titled *Hydrophobic Interactions*, wherein I summarized all that was known at that time about the hydrophobic ( $H\phi O$ ) effects: solvation and hydrophobic interactions. The more general term “solvent-induced interactions” was studied mainly in connection with the effect of a solvent on the interaction between two charged particles.

Toward the end of the 50s, Kauzmann (1959) introduced the idea of “hydrophobic bonds”—later referred to as “hydrophobic interactions”—to explain the stability of the 3D structure of proteins. This idea dominated the field of protein chemistry in its entirety, including the protein folding problem and the self-assembly of proteins. My contributions to the field were: First, a clear-cut *definition* of the concepts of “ $H\phi O$  solvation” and “ $H\phi O$  interaction,” and second, a new and useful *measure* of both the  $H\phi O$  solvation and  $H\phi O$  interactions, Ben-Naim (1971a, b, 1972a, b, 1974).

The application of the new definition and new measure of the  $H\phi O$  effects led to the establishment of the fact that there is a tendency of the two  $H\phi O$  molecules to adhere to each other, and that this tendency is far stronger in aqueous solutions than in any other solvent for which the relevant data was available.

In my book’s preface, Ben-Naim (1980), I wrote:

*...in spite of my research in this field over almost ten years, I cannot confirm that there is at present either theoretical or experimental evidence that unequivocally demonstrates the relative importance of hydrophobic interactions over other types of interaction in aqueous solutions.*

In this quoted paragraph, I did not specify the processes in which the  $H\phi O$  interactions were involved, but it was clear from the context of the preface as a whole that I was referring specifically to the processes of protein folding and protein–protein association.

In the 1980s, it was believed that direct hydrogen bonds (HBs) did not contribute significantly to the stability of proteins. This led to the widespread acceptance of the idea that  $H\phi O$  interactions are the *most important* factors in determining the stability of proteins.

However, the situation changed dramatically in the late 1980s, upon the discovery of some new hydrophilic ( $H\phi I$ ) effects. It was found that some  $H\phi I$  interactions are far stronger than the corresponding  $H\phi O$  interactions. Contrary to common belief, it was also ascertained that direct HBs contribute significantly to the stability of proteins; and that the role of  $H\phi O$  effects in protein was grossly exaggerated.

Unfortunately, there are still numerous scientists who still subscribe to the old and obsolete ideas regarding the role of HBs and  $H\phi O$  effects in protein stability in spite of the overwhelming evidence that there are many more  $H\phi O$  interactions, and that each of these is far stronger than the corresponding  $H\phi O$  effect.

This book is in some sense a sequel to my previous book which was published in 1980. However, its main message is that for quite a long-time people were misled to believe in the idea of the “most important”  $H\phi O$  effects. In a way, this book presents a paradigm shift from  $H\phi O$  to  $H\phi I$ . It will be shown that various  $H\phi I$  effects not only explain the stability of the structure of proteins and the speed of the process of protein folding but also effectively expunge the mystery associated with some biochemical processes such as protein folding and self-assembly of proteins.

The first three chapters deal with some basic definitions of solvent-induced effects and narrate the story of the evolution of the ideas about the factors that are important for protein stability; from early ideas about HBs to  $H\phi O$  effects and finally to the  $H\phi I$  effects. In Chap. 4, we summarized what is known about  $H\phi O$  solvation and interactions. It is shown that these  $H\phi O$  effects are very different in strength when they are applied to *entire* molecules, and on *groups* attached to a protein. Chapter 5 is dedicated to  $H\phi I$  effects. Here we present the evidence regarding their strength based on theoretical, experimental, and simulated calculations.

The last chapter summarizes the implementation of the  $H\phi I$  effects in the explanations of the long-lasting problems such as protein folding and self-assembly of proteins.

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As always, I am very grateful for the graceful help from my wife, Ruby, and for her unwavering involvement in every stage of the writing, typing, editing, re-editing, and polishing of the book.

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# Abbreviations

1D	One Dimensional
CD	Cold Denaturation
GPF	Grand Partition Function
HB	Hydrogen Bond
HBed	Hydrogen Bonded
HBing	Hydrogen Bonding
HBs	Hydrogen Bonds
$H\phi I$	Hydrophilic
$H\phi O$	Hydrophobic
Ig	Ideal Gas
PF	Partition Function
PFP	Protein Folding Problem
PMF	Potential of Mean Force
SIF	Solvent-Induced Forces
SII	Solvent-Induced Interactions
SMI	Shannon's Measure of Information
SPT	Scaled Particle Theory

# Chapter 1

## Introduction, Definitions and Motivations for Studying Solvent-Induced Interactions



In the first part of this chapter we start by defining the *interaction energy* between two or more particles in different media and express these interactions in terms of thermodynamic quantities. In the second part, we discuss the motivation for studying these interactions in the context of the so-called protein folding problem (PFP). We shall survey the main historical landmarks in the history of studying solvent-induced interactions (SII) in connection with the PFP.

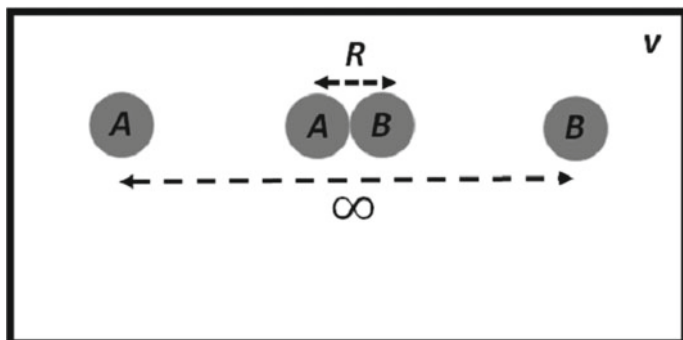
### 1.1 Definitions; Direct and Indirect Interactions

The basic idea of solvent-induced interactions (SII) is quite simple. We can view the SII as an extension of the concept of intermolecular interaction between two atoms or two molecules in a vacuum.

Figure 1.1 shows two spherical particles (A and B which can either be identical or different particles). The intermolecular interaction between these two particles is defined as the work (i.e. the force, times distance) involved in bringing the two particles from infinite separation to the final distance  $R$ . We denote this work by:

$$W^{(v)}(\infty \rightarrow R) = U(R) - U(\infty) = U(R) \quad (1.1)$$

The superscript ( $v$ ) is for “vacuum,” or in an ideal-gas phase. On the left-hand side, we write the work ( $W$ ) associated with the process, denote by  $(\infty \rightarrow R)$ , as the difference between the potential energy of this system at  $R$ , minus the potential energy of the same system at infinite separation ( $\infty$ ). Since the latter is assumed to be zero, we omit  $U(\infty)$  and simply identify the work  $W^{(v)}(\infty \rightarrow R)$  with the potential energy  $U(R)$  which is also referred to as the *direct* interaction energy between the two particles at a distance  $R$  in a vacuum.



**Fig. 1.1** Two simple spherical particles A and B are brought from infinite separation to the final distance  $R$ , in a vacuum or in an *ideal-gas* phase

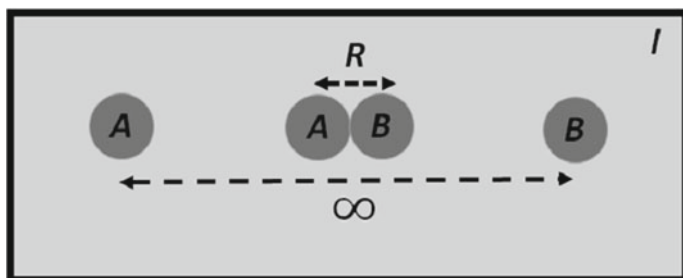
For non-spherical particles, the interaction energy might also be dependent on the orientations of the two particles as well as on the internal configuration of the two molecules, (say, proteins).

We now extend the concept of interaction between the two particles and apply it to the same two particles but in a solvent, or in a liquid phase ( $l$ ), Fig. 1.2. The work associated with the process ( $\infty \rightarrow R$ ) no longer depends only on  $R$  but also on some thermodynamic parameters of the system.

Figure 1.2 shows the same process as in Fig. 1.1 but now the two particles are immersed in some “solvent.” Clearly, the work associated with the process would depend on the density of the solvent molecules, the temperature at which the process is carried out, and on whether the solvent is or is not at equilibrium.

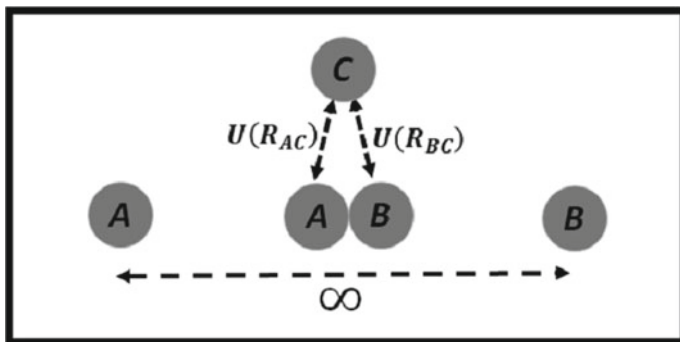
In this book, we always assume that the solvent is at equilibrium. Also, we assume that process ( $\infty \rightarrow R$ ) is carried out very slowly (quasi-statically) in such a way that while the two particles are moved, the state of equilibrium of the solvent is maintained.

The origin of the difference in the amount of work associated with the processes in Figs. 1.1 and 1.2 is easy to understand, qualitatively. Let us start with two simple



**Fig. 1.2** The same process as in Fig. 1.1, but the two spherical particles A and B are in a liquid phase





**Fig. 1.3** The same process as in Fig. 1.1, but the “solvent” consists of a single particle C

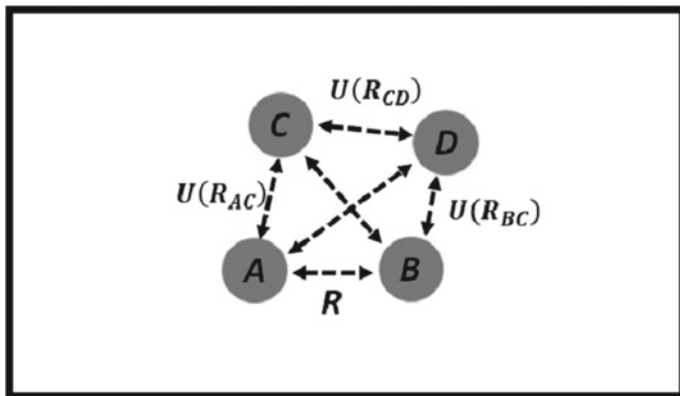
cases of “solvents.” Suppose for simplicity that there is only one solvent particle in the system, Fig. 1.3.

In this case, in addition to the *direct* interaction  $U(R)$  between the two particles, A and B (which is presumed to be the same as in Fig. 1.1), we also have *indirect* interactions mediated through the “solvent.” In the simplest case of Fig. 1.3, we have only one solvent molecule, denoted by C. Here, we have in addition to the interaction  $U(R_{AB})$ , also the two additional interactions  $U(R_{AC})$  and  $U(R_{BC})$ . These two interactions can either cause attraction or repulsion between the two particles A and B, depending on the type of interactions  $U(R_{AC})$  and  $U(R_{BC})$ , and on the location of the solvent particle. For instance, if A attracts C, and C attracts B, then these two interactions cause an *indirect attraction* between A and B. It should be noted that in a liquid of hard-sphere particles, all the *direct* pairwise interactions are repulsive, yet these repulsive interactions produce *indirect attractive* interactions. For details see Refs. [1–3].

Remember that the process we carried out is the same as in Fig. 1.1. The “solvent” particle is free to move around while the two particles A and B approach each other. Therefore, in studying the work  $W(\infty \rightarrow R)$  we need to account for the *average* indirect interactions over all possible configurations of the solvent molecule.

Another simple example is shown in Fig. 1.4. Here we have a “solvent” consisting of two, denoted by C and D. In this case, we have in addition to the *direct* interaction  $U(R_{AB})$ , five additional interactions  $U(R_{AC})$ ,  $U(R_{AD})$ ,  $U(R_{BC})$ ,  $U(R_{BD})$  and  $U(R_{CD})$ . These five interactions can either cause attraction or repulsion between the two particles A and B. Again, recall that the process we carried out is the same as in Fig. 1.1. The “solvent” particles are free to move around while the two particles A and B approach each other. Therefore, in studying the work  $W(\infty \rightarrow R)$  we need to account for the *average* indirect interactions over all possible configurations of the solvent molecules.

Clearly, when the solvent consists of many molecules, the difference between the *direct* interaction  $U(R)$ , and the *work function*  $W(\infty \rightarrow R)$ , becomes more and more complicated. This is *a fortiori* true when the solvent is a complicated liquid like water. It is here that statistical mechanics is most useful in studying the SII between two (or more) particles (or groups of proteins) in different solvents.



**Fig. 1.4** Two particles A and B at fixed positions and at a distance  $R$ , in a “solvent” consists of two particles C and D. Here, in addition to the *direct* interaction between A and B, there are five more interactions that contribute to the *indirect* interaction between A and B

In most of this book, we will assume that the solvent is maintained at equilibrium and at a constant volume and temperature. Also, the total number of solvent molecules will always be assumed to be constant. In such a case, the work  $W(\infty \rightarrow R)$  is related to the change in the Helmholtz energy change in a system consisting of two particles at fixed positions and at a distance  $R$ , and a solvent of  $N$  molecules in a volume  $V$ , and at temperature  $T$ . Thus, we write:

$$W^{(l)}(\infty \rightarrow R) = A(T, V, N; \infty) - A(T, V, N; R) \quad (1.2)$$

The superscript  $(l)$  is for the “liquid phase.” In most of the experiments, in which SII has been studied the solvent was maintained at constant temperature and pressure (rather than volume). In this case, the work  $W(\infty \rightarrow R)$  is related to the Gibbs energy change in the process, i.e.

$$W^{(l)}(\infty \rightarrow R) = G(T, P, N; \infty) - G(T, P, N; R) \quad (1.3)$$

Fortunately, for the processes we will be discussing in this book, there is not much difference in the numerical values of the Helmholtz and Gibbs energies, see Ben-Naim [2], for some numerical examples.

Figure 1.5 shows the same process  $(\infty \rightarrow R)$ , as discussed above carried out in both an ideal gas (or in a vacuum) and in a liquid phase  $(l)$ . We now define the solvent-induced interactions (SII) by difference:

$$\begin{aligned} \delta W(R) &= W^{(l)}(\infty \rightarrow R) - W^{(v)}(\infty \rightarrow R) \\ &= \Delta A(R) - U(R) \\ &= \Delta G(R) - U(R) \end{aligned} \quad (1.4)$$