Myths in Modern Science: The Hydrogen Bond and its Surroundings Part 2. The Hydrophobic-Bond-Myth

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Abstract

The fashionable but stereotypic thinking on the concept of the so-called “hydrophobic bond” has been examined in light of criticisms raised by many scientists: Hildebrand, Shinoda, Israelachvili, and so on. The author’s comments are given on the harmful influence of the concept of “hydrophobic bond” in chemistry and biochemistry. In my opinion, this concept can be considered as a myth in modern science.

Key Words: proteins, hydrogen bond, nonpolar side-chain, Gibbs free energy

Area of Interest: In silico drug discovery
1. What is the Hydrophobic Bond?

They were like two drops of oil that have dripped on to the surface of a basin of water. The drops gathered in one place by repelling water; it is more accurate to say that the pressure of water forces them to adhere to each other, making it impossible for either to get away.

The above phrases come from Natsume Soseki’s novel “Mon, The Gate.” In this context we realize that water pushes oil molecules to adhere to each other, but not that oil molecules dislike water. Soseki wrote about a phenomenon similar to the so-called “hydrophobic bonding. We well know that the social atmosphere or environment often rejects foreign elements and peoples (immigrants). Soseki’s comment is quite reasonable in view of our knowledge of physical science.

Hydrophobic force, hydrophobic bond, hydrophobic effect, hydrophobicity, and so on, are commonly used terms. They frequently appear in the literature and textbooks of organic chemistry and biochemistry. Most readers have no sense of strangeness regarding these words; rather, they use them habitually. According to Hildebrand and Shinoda, however, the word “hydrophobic” is incorrect and very misleading.

2. Criticisms on the Concept of Hydrophobic Bond

In 1968, Hildebrand stated the following in his paper, A Criticism of the Term “Hydrophobic Bond” [1].

Sir: The term “hydrophobic bond” that has come into use in the literature of polymers seems to me to be inappropriate for two reasons. One, the alkyl groups of two polymer chains are not forced together by phobia to water.

Furthermore, the energy of evaporating a mole of n-butane from its solution at 1 atm and 25° is 5.46 kcal, greater than it is from its own pure liquid at the boiling point, 4.81 kcal. This represents attraction, not phobia. Ice is wet by octane. The fact that octane is nearly insoluble in water is merely the result of the fact that the attraction is not strong enough to penetrate the high cohesion of water.

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1 Natsume Soseki (1867-1916) is Japan’s greatest and most respected writer, whose works are attracting a number of critical inspections and debates. The title “Gate” had been inspired by Friedrich Nietzsche’s aphorisms “Also sprach Zarathustra” (translated into English by Thomas Common as “Thus spoke Zarathustra”: The name of the gateway (Torweg) is inscribed above ‘This Moment’).
2 The complete works of Natsume Soseki, Iwanami Shoten, Vol. 4, p. 77. Soseki’s granddaughter Yoko Matsuoka-McClain wrote that this novel was Soseki’s favorite of his all novels. (Y. Matsuoka-McClain, 1995, Natsume Soseki, looked at by his granddaughter, Sincho-sha Selected Books, No. 950). See also Damian Flanagan, 2003, Natsume Soseki, who most Japanese do not pertinently appreciate, Sekaishisou-sha. In this book, Flanagan wrote that Soseki is much greater as a novelist than writers of the same period, such as Tolstoy, Dostoyevsky, James, and Proust. Cf. Marvin Marcus, Memory and Melancholy in the Personal Writings of Natsume Soseki, University of Hawai’i Press, 2009.
3 In the Univ. of California at Berkley, there is a house named “Hildebrand Hall” to commemorate his great contributions to chemistry.
4 Kozo Shinoda (1927-2014), Emeritus Professor of Yokohama National Univ. (deceased on 15 April 2014).
5 This part of the quotation has been permitted by the publisher.
The noun, bond, seems likewise inappropriate because the attraction between the alkyl groups of two polymer chains has none of the characteristics that distinguish chemical bonds from van der Waals forces. Any “simple multiple proportions” between such groups result from regularities in the structures of the two chains, not from any valence forces between alkyl groups. The alkyl chains in micelles of soap are not bonded together by phobia for surrounding water; they stick together just as strongly in the absence of water.

Major workers in this field are surely well aware of the fact that there is no true bond between alkyl groups of adjacent polymer chains and are, moreover, competent to calculate the thermodynamic quantities involved in the interaction between such chains. Why, then, should a terminology continue in use that misleads some into thinking that the “hydrophobic bond” represents a special concept that must be mastered in order to deal with these systems? Why not speak simply of alkyl interaction free energy, energy, or entropy? I do not find it necessary to invent “fluorophobic bonds” in order to handle the thermodynamics of the limited solubility of heptane in perfluoroheptane.

I thank Nemethy, Scheraga, and Kaufmann for kindly replying in some detail to my request for their views on this matter. However, I do not agree with their statement “... hydrocarbons actually prefer a nonpolar environment to being surrounded by water.” I say rather that molecules of water “prefer” to be hydrogen-bonded together rather than separate to admit alkanes. In order for these to dissolve, a large amount of water must be present per mole of alkanes in order to supply sufficient entropy to offset the unfavorable balance of attractive energies.

3. A response to Hildebrand from three chemists who advocated the concept of the hydrophobic bond

The reply from Nemethy, Scheraga, and Kaufmann to Hildebrand, Comments on the Communication “A Criticism of the Term ‘Hydrophobic Bond’ by Joel H. Hildebrand,” which appeared in the same pages of the journal [2]6, is given below.

Sir: Hildebrand’s discussion of the nature of the forces of interaction governing the solubility of hydrocarbons is incomplete. It is certainly true, as he states, that van der Waals interactions in themselves are on balance favorable between the two substances, and that they therefore would actually favor mixing. As it was noted repeatedly before, this is indicated by a direct calculation of the relevant energies of interaction, just as it is shown by the data cited by Hildebrand. However, the net free energy of solution, which determines solubility, is dominated by a large negative, i.e., unfavorable, excess entropy term. It has been shown that this entropy term arises owing to changes in the state of water and has to be attributed to increased ordering of water molecules, i.e., to an increase in hydrogen bonding. As a result, in spite of the favorable interaction energies, the free energy of solution is positive. This can be expressed by saying that, in over-all terms, i.e., in \( \Delta F_0 \), hydrocarbons actually prefer a nonpolar environment to being surrounded by water. This is implied by the use of the adjective “hydrophobic.”

Because the source of immiscibility is an entropy factor, the water-hydrocarbon system differs qualitatively, and in a unique manner from most systems of low miscibility. Thus the interactions in this system do represent a special concept.

6 This part of the quotation has been permitted by the publisher.
We do not wish to argue about the matter of nomenclature. However, it should be pointed out that the criticism of the use of the term “bond” in the present context, where it does not refer to a chemical bond but to a loose association, has also been recognized repeatedly by various workers in the past. Nevertheless, the term “hydrophobic bond” has proven to be useful as shown by its frequent occurrence in recent physical, chemical, and biochemical nomenclature.

4. Criticism by Shinoda

In the same year (1968), Shinoda wrote a review “Hydrophobic Bonding and Solubility” and stated that the theoretical ground of this effect is erroneous. The abstract of this review [3] is given below. A similar argument is also presented in Shinoda’s monograph “Solution and Solubility” 8.

A molecule dissolved in water, such as a nonpolar component of a water-soluble protein, is hardly miscible, and has a tendency to associate with each other, and separate, from solvent water. This tendency is called the “hydrophobic bonding.” This term, however, is not suitable if one considers the origin of the phenomenon. In the process of dissolving hydrocarbons into water, although the solubility is very small, the heat of dissolution is generally small, the dissolution entropy, which is the difference of dissolution and mixing entropy, becomes a large negative value. This is because water molecules surrounding a hydrocarbon molecule convert into an ice-like state from a solvent state. Therefore, the origin of the hydrophobic bonding has been attributed to 1) it occurs by entropic effect. 2) The entropy of the system decreases when water takes ice-like state, and therefore the solubility decreases further, etc.

I think this kind of explanation is wholly flawed. In reality, 1) the heat of dissolution of a hydrocarbon chain increases as solubility decreases. However, 2) since the heat is generated by solvent water taking an iceberg state, the heat of dissolution becomes smaller, superficially. 3) Since the decrease of enthalpy in an iceberg formation is larger than the entropy decrease, it is unsuitable to call this phenomenon, hydrophobic bonding. 4) Instead, the solubility of the hydrocarbon is increased by the iceberg formation. 5) This is the reason why rare gasses dissolve rather readily in water.

From the term “hydrophobic effect”, one has the impression that “oil dislikes water.” In actuality, however, “water dislikes oil.” If we examine the reality, however, it should be called a “lipophobic effect.” In addition, do you think it would be appropriate for it to be called a bond? In chemistry, the word “bond” generally means the covalent bond, except for those interactions between molecules such as the hydrogen bond.

5. Hildebrand’s Opinion

Hildebrand’s reply to Tanford, Is there a “hydrophobic effect?” that appeared in 1978 [4], is given below.

In 1968 I published ‘A Criticism of the Term ‘Hydrophobic Bond’ in which I gave evidence that the alkyl groups in polymer chains in water are not forced together by “phobia” for water.

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7 This part of the quotation has been permitted by the publisher.
8 This book is highly regarded as a singularly excellent work in colloid and surface chemistry, in addition to that of Hildebrand et al.: Regular and Related Solutions, Van Nostrand Reinhold, NY, 1970.
In subsequent papers I gave further evidence that throws light upon the forces acting between water and alkanes. These facts have been largely overlooked by biologists, as illustrated by a recent paper on “The Hydrophobic Effect and the Organization of Living Matter,” Alfred North Whitehead 9 has written: “An unflinchible determination to take the whole evidence into account is the only method of preservation against the fluctuating extremes of fashionable opinion.” Indeed, no scientist can afford, in the long run, to overlook any fact that is pertinent to understanding the phenomenon that he is studying. In this paper, I set forth such facts.

The loss of entropy when an alkane gas dissolves in water has been explained by the formation of what has been facetiously called an “iceberg” around the solute molecules. Any such assumption is not tenable; the viscosities of water and carbon tetrachloride at 25°C are nearly the same, 0.880 and 0.895 cp; and the diffusivity of methane in water 105D is 1.72 cm²•sec⁻¹; at 25°C in CCl₄ it is 2.89 cm²•sec⁻¹. If the molecules of methane were encased in “icebergs,” they could not diffuse 0.6 as rapidly in water as in CCl₄.

In conclusion, there is no hydrophobia between water and alkanes; there only is not enough hydrophilia to pry apart the hydrogen bonds of water so that the alkanes can go into solution without assistance from attached polar groups.

In the same year (1978), Tanford wrote a short paper, The Hydrophobic Effect and the Organization of Living Matter [5]10; a part of the abstract is given below.

The hydrophobic effect is a unique organizing force, based on repulsion by the solvent instead of attractive forces at the site of organization. It is responsible for the assembly of membranes of cells and intracellular compartments, and the absence of strong attractive forces makes the membranes fluid and deformable. The spontaneous folding of proteins, however, involves directed polar bonds, leading to more rigid structures. Intercellular organization probably involves polar bonds between cell surface proteins.

In 1997 (ca. 19 years later), Tanford published a review, “How protein chemists learned about the hydrophobic factor [6]11.” Parts of the abstract of this review are given below.12

It is generally accepted today that the hydrophobic force is the dominant energetic factor that leads to the folding of polypeptide chains into compact globular entities. This principle was first explicitly introduced to protein chemists in 1938 by Irving Langmuir, past master in the application of hydrophobicity to other problems, and was enthusiastically endorsed by J. D. Bernal.

A theoretical paper by H. S. Frank and M. Evans, not itself related to protein folding, probably played a major role in the acceptance of the hydrophobicity concept by protein chemists because it provided a crude but tangible picture of the origin of hydrophobicity per se in terms of water structure.

9     Alfred North Whitehead (1861–1947) was an English mathematician and philosopher. He is best known as the defining figure of the philosophical school known as process philosophy, which today has found application to a wide variety of disciplines, including ecology, theology, education, physics, biology, economics, and psychology, among other areas. (Wikiquote)

10-11 This part of the quotation has been permitted by the publisher.

12    Tanford himself wrote: that ... “It is for this reason, and perhaps especially because of the consequences for biological organization, that a special term to describe hydrocarbon-water antipathy has proved convenient, which does not mean that “hydrophobic” is a good word for it. ...”
It is generally accepted today that the hydrophobic force is the dominant energetic factor in terms of water structure.

This seems to be a self-confident declaration of their triumph. Nevertheless, was the matter in question really settled? I do not think so. Tanford does not seriously respond to Hildebrand’s criticism. Instead, he forcibly insists on his own explanations.

Criticisms by Hildebrand and Shinoda against the concept of a hydrophobic bond were briefly introduced, as above. Since then, few papers have been written in response to the questions cast by these distinguished scientists. Below, we will examine related descriptions from textbooks of physical chemistry.

6. Comments on the concept of hydrophobic bonds, taken from textbooks of physical chemistry

With regard to the hydrophobicity, Israelachvili, in his book, *Intermolecular and Surface Forces* [7], wrote as follows.

> Hydrophobic means “water-fearing”, but it is important to note that the interaction between a hydrophobic molecule and water is actually attractive, due to the dispersion force. However, the interaction of water with itself is much more attractive. Water simply loves itself too much to let some substances get in its way.

Tinoco et al., wrote the following in their textbook *PHYSICAL CHEMISTRY, Principles and Applications in Biological Science*, as follows.

**Hydrophobic Interactions** (from chapter 3 [8])

> One type of interaction that is important in aqueous solutions is the hydrophobic (fear of water) interaction. Water molecules have a strong attraction for each other, primarily as a consequence of hydrogen bond formation.

Now let us consider two such hydrocarbon groups, R. Each separate group when exposed to liquid water will, from the data above, cause an unfavorable free-energy change. If the two groups cluster together, the disruptive effect on the solvent network will be less than the combined effects of the two separate groups. Therefore, the association of the groups will be thermodynamically favored:

\[
R_{(aq)} + R_{(aq)} \rightarrow [R\neg R]_{(aq)}
\]

Separate clustered

*The clustering of the groups is not because they “like” each other, but because they are both “disliked” by water. The clustered arrangement of the hydrocarbons results in a decrease in the*
overall free energy of the system in comparison with the isolated hydrocarbons in water. Such hydrophobic interactions are important in many biological systems. For example, hydrocarbon groups in a water-soluble protein are usually found to cluster in the interior of the protein. Similarly, polar lipid molecules form bilayer sheets or membranes in water, in which the hydrophobic portions are buried inside and the polar or charged portions are on the surface, exposed to the water. Such molecules are called amphiphilic. Hydrophobic interactions are characterized by low enthalpy changes and are entropy driven. We should note that hydrophobic interaction is a term that we use to describe the combined effects of London, van der Waals, and hydrogen bonding-interactions in certain processes in aqueous solutions; it is not a “force” different from the others we have discussed. In particular, there is no such thing as a hydrophobic bond.

Hydrophobic and Hydrophilic Environments (from Chapter 9 [9]15)

The terms hydrophobic (water fearing) and hydrophilic (water loving) are often encountered in the biochemical literature.

Because water is the medium in which biological organisms have developed, the interactions of biomolecules with water is very important. To understand the distinctions between terms like hydrophobic and hydrophilic, we need to recognize that there is always a competition between the interactions of molecules of two substances – for instance, hexane and water – with one another and their interactions with molecules of the same type. It is a mistake to describe such interactions as resulting from “hydrophobic forces” because they occur completely and naturally in the absence of any water whatsoever.

7. Comments from Recent Papers and Reviews

Papers commenting on the concept of “the hydrophobic bond” appeared continuously. In 1990, Dill wrote a review, Dominant Forces in Protein Folding [10], and discussed it with Privalov, Gill, and Murphy [11] [12].

In 1993, Blokzijl and Engberts wrote a review, “Hydrophobic Effects: Opinions and Facts [13]16”, and cast doubt on the possibility of the hydrophobic bond. A part of the abstract is given below.

The term hydrophobic interaction denotes the tendency of relatively apolar molecules to stick together in aqueous solution. These interactions are of importance in many chemical disciplines, including the chemistry of in vivo processes. Enzyme-substrate interactions, the assembly of lipids in biomembranes, surfactant aggregation, and kinetic solvent effects in water-rich solutions are all predominantly governed by hydrophobic interactions. ... Several traditional views have been found to be deeply unsatisfactory, and courageous attempts have been made to conceptualize the driving force behind pairwise and bulk hydrophobic interactions. The review presents an admittedly personal selection of the recent experimental and theoretical developments, and when necessary, reference is made to relevant studies of earlier date.

And in the text they wrote:
Traditionally, the reluctance of apolar compounds to dissolve in water has been attributed to the hydrophobicity of these compounds, in other words, their fear of water. In fact, the term hydrophobicity is misleading.

Further, in epilogue they wrote:

Traditionally, chemists have attributed a pivotal role to the structure of water in the vicinity of apolar solutes or apolar moieties in aqueous solutions as expressed in the concept of hydrophobic hydration and hydrophobic interaction. ... In particular, the idea of clathrate-like hydration layer led to the exaggerated picture of iceberg formation at the surface of apolar solute particles in water. Recent computational studies have yielded little evidence for a significant increase of either the number or the strength of hydrogen bonds in aqueous solutions of apolar compounds when compared to pure water.

In 2001, Lazaridis published a review, “Solvent Size vs. Cohesive Energy as the Origin of Hydrophobicity” [14], casting doubt about the use of the term “hydrophobic.” Thus, in the text, he wrote:

These ideas are lucidly presented in a review by Dill and are adopted in most physical chemistry and biochemical textbooks ... Obviously, in this view the word “hydrophobic” is a misnomer; it would be more accurate to refer to water being “lipophobic”.

In 2002, Southall, Dill, and Haymet wrote a review, “A View of the Hydrophobic Effect,” and surveyed the experimental and theoretical studies of nonpolar solute partitioning into water. There, they noted that the hydrophobic effect is not just due to “water ordering” and not merely due to small size effects of water [15]. A part of the abstract of this review is reproduced below.

We note that the hydrophobic effect is not just due to “water ordering” and nor merely due to small size effect of water. The properties vary substantially with temperature and solute shape. Also, we discuss the limitations of using oil/water partitioning as the basis of thermodynamic models in chemistry and biology.

The following is a part of a review by Meyer, Rosenberg, and Israelachvili, “Recent progress in understanding hydrophobic interactions,” which appeared in 2006 [16]. There they noted that several researchers had begun to take issue with the description by Kauzmann of the hydrophobic interaction.

in 1959, the term “hydrophobic bond” was coined by Kauzmann to describe a tendency toward adhesion between the nonpolar groups of proteins in aqueous solution. Kauzmann suggested that this bond was probably among the most important factors in the stabilization of certain folded configurations in native proteins.

Although the term hydrophobic bond is still used today, as early as 1968, several researchers began to take issue with this description of the hydrophobic interaction.

Computational methods combined with the application of protein engineering to directly study the hydrophobic amino acid residues in protein folding continue to produce evidence that is contradictory to the traditional interpretation of the hydrophobic effect.

17-19  This part of the quotation has been permitted by the publisher.
No one really understands water. It's embarrassing to admit, but the stuff that covers two-thirds of our planet is still a mystery. Worse, the more we look, the more the problems accumulate: new techniques probing deeper into the molecular architecture of liquid water are throwing up more puzzles.

This guilty secret has myriad ramifications. Water defines the terrestrial environment. It is central to Earth and atmospheric sciences, to biology and to many technologies. The common assumption that water is well characterized has led to explanatory edifices built on shaky ground. The situation is unsatisfactory intellectually and hazardous in practice.

Kauzmann argued that water becomes more ‘highly structured’ around hydrophobic solutes, and that the release of some of this water into the relative chaos of the bulk liquid when two such solutes stick together produces a favourable increase in entropy.

This is almost certainly wrong. For one thing, the hydrophobic interaction may operate in different ways for small solutes such as methane, and for the kind of extended nanoscale surfaces that proteins have. Such surfaces may stick together via a sudden, coordinated expulsion of many water molecules, although whether and how much this happens in real protein folding and associations remains unclear. The point is that all such mechanisms depend fundamentally on the structure of bulk water and how that alters near a hydrophobic entity.

In 2008, Ball also wrote a review, Water as an active constituent in cell biology [18]. A part of the conclusion is given below. On page 80, the possible intervention of π-interactions [19]21 has been commented on, as below.

Another model small hydrophobe is benzene, since aromatic groups are common in biological molecules. But while phenyl groups are generally regarded as hydrophobic, it has been known for some time that the π-electron system can act as a weak hydrogen-bond acceptor, creating strong orientational preferences in the bound water molecules.

For other type of π-interactions and consequences, see references [20] - [22]22.

In 2011, Ball published a short review, More than a bystander [23]23, saying that ‘Although still routinely used, this picture receives no real support from experiments, etc. Thus:

Proteins typically bury their hydrophobic amino–acid residues in their interior as they fold; and hydrophobic groups on ligands are generally juxtaposed to similar surfaces in an enzyme’s binding site. Proteins themselves associate into larger aggregates - as functional assemblies, for example, or as fibrillar misfolded structures in neurodegenerative diseases - by marrying up their hydrophobic surfaces. Yet there is still no consensus on how these hydrophobic interactions to operate.

The traditional picture, now decades old, invokes an enhanced ordering of water molecules in the bulk. In this view, the coming together of hydrophobes expels the intervening 'ordered'

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20-21 This part of the quotation has been permitted by the publisher.
22 See also the author’s website http://tim.hi-ho.ne.jp@dionisio.
23 This part of the quotation has been permitted by the publisher.
water into the bulk phase, an entropically favourable process. Although still routinely used, this picture receives no real support from experiments probing hydration structures.

8. Conclusions

To summarize, arguments between advocators of the concept of “hydrophobic bonding” and Hildebrand (and Shinoda) have been introduced. Next, descriptions in textbooks of physical chemistry by Israelachvili and Tinoco et al. were examined. Finally, criticisms against the concept of the hydrophobic bond by several scientists were presented.

In view of this, I think that the concept of the so-called “hydrophobic bonding” is a myth in modern science. And it is a very malignant one. The “hydrophobic-bond-myth” has adverse effects on investigations of chemistry and biochemistry, as well as on protein science, including studies on the mechanisms of protein folding and rational drug design.

The conclusion of this paper is that the concept of the hydrophobic bond is having harmful influences on science. Today, leading scientists do not trust the concept of a hydrophobic force; nevertheless, it remains widespread in the scientific community. The improper usage of this term will surely lead to incorrect conclusions. One should use a more correct and appropriate term, such as lipophilic, nonpolar, or apolar, etc., instead. Without an agreement on an appropriate definition, a lucid conclusion will never be obtained. If, in the future, a common understanding is reached by IUPAC, a more appropriate description will be agreed upon. This state-of-affairs should be set right, as soon as possible. Lectures in universities and descriptions in textbooks should be corrected.

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This paper is dedicated to the late Joel H. Hildebrand (1881-1983) and the late Kozo Shinoda (1927-2014).

GASSHO

25 合掌: Declaration for the confidence to Buddha.