

LA-UR-97- 2321

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Title: Computer Simulation of Protein Solvation,
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in Radiation Biology

Author(s): Lawrence R. Pratt, Angel E. Garcia and Gerhard Hummer, Theoretical
Division, Los Alamos National Laboratory
Bruce J. Berne, Dept. Chem., Columbia U.
Stephen A. Corcelli, Dept. Chem., Brown U.
Shekhar Garde, Dept. Chem. Eng., U. Delaware
Michael E. Paulaitis, Dept. Chem. Eng., Johns Hopkins U.
Andrew Pohorille, NASA Ames Res. Center and Dept. Pharm.
Chem., U. Cal.-San Francisco
Steven W. Rick and Gregory J. Tawa, Natl. Cancer Inst.,
Frederick Cancer R&D Center

Submitted to: DOE Office of Scientific and Technical Information (OSTI)

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Computer Simulation of Protein Solvation, Hydrophobic Mapping, and the Oxygen Effect in Radiation Biology

Lawrence R. Pratt*, Angel E. Garcia and Gerhard Hummer
Theoretical Division, Los Alamos National Laboratory

Bruce J. Berne
Department of Chemistry, Columbia University

Stephen A. Corcelli
Department of Chemistry, Brown University

Shekhar Garde
Department of Chemical Engineering University of Delaware

Michael E. Paulaitis
Department of Chemical Engineering, Johns Hopkins University

Andrew Pohorille
NASA Ames Research Center and Department of Pharmaceutical Chemistry,
University of California San Francisco

Steven W. Rick and Gregory J. Tawa
National Cancer Institute, Frederick Cancer Research and Development Center

Abstract

This is the final report of a three-year, Laboratory-Directed Research and Development (LDRD) project at the Los Alamos National Laboratory (LANL). Hydrophobic effects are central to the structural stability of biomolecules, particularly proteins, in solution but are not understood at a molecular level. This project developed a new theoretical approach to calculation of hydrophobic effects. This information theory approach can be implemented with experimental, including computer simulation-experimental, information. The new theory is consistent with, builds upon, and subsumes previous integral equation and scaled particle statistical thermodynamic models of hydrophobic effects. The new theory is sufficiently simple to permit application directly to complex biomolecules in solution and to permit further extension to incorporate more subtle effects.

Background and Research Objectives

“No one has yet proposed a quantitative theory of aqueous solutions of nonelectrolytes, and such solutions will probably be the last to be understood fully.”¹

*Principal Investigator, E-mail: lrp@lanl.gov

Hydrophobic and hydrophilic are categories of solvation effects in aqueous liquids. Classical ions such as Na^+ or polar molecules such as NH_3 are easily recognized hydrophilic solutes. In contrast, the interactions of hydrophobic solutes or groups with water molecules do not display classic electrostatic or specific chemical interactions. Primitive hydrophobic solutes are inert gases and simple hydrocarbons that are sparingly soluble in water. However, much of the interest in hydrophobic effects is associated with more complex solutes that contain both hydrophobic and hydrophilic moieties. Surfactant species, for example the decanoate anion, include both hydrophobic and hydrophilic parts and are called amphiphilic.

Solutions containing amphiphiles often show exotic behaviors. The assembly of micelles and bilayer membranes is clearly associated with the bifunctional character of the species that compose them.² These structures attempt to sequester hydrophobic groups away from the aqueous environment while still satisfying the need of hydrophilic groups for contact with water. Amino acids and peptides can also be amphiphilic molecules. Protein molecular structure, function, and aggregation have provided an important motivation for study of hydrophobic effects.³ It is widely believed that hydrophobic interactions drive protein folding by providing a nonspecific, cohesive stabilization of structures that successfully satisfy the contrasting solvation requirements of hydrophilic and hydrophobic molecular parts.⁴

Hydrophobic effects are thus of practical interest. If we accept the goal of a simple, physical, molecularly valid explanation, then hydrophobic effects have also proven to be conceptually subtle. The reason is that hydrophobic phenomena are not tied directly to a simple dominating interaction as is the case for hydrophilic solvation of Na^+ , as an example. Instead hydrophobic effects are built up more collectively. In concert with this indirectness, hydrophobic effects are viewed as entropic interactions and often exhibit counterintuitive temperature dependencies. An example is the cold denaturation of globular proteins. Though it is believed that hydrophobic effects stabilize compact protein structures and proteins denature when heated sufficiently, it now appears common for proteins structures to unfold upon appropriate cooling.⁵ The entropic character of hydrophobic effects makes them more fascinating and more difficult.

The technical problems for molecular theories of hydrophobic effects are of two types. The first technical problem is that water is a singular liquid.⁶ Sensible approximate molecular theories for nonassociated liquids often don't make good sense for aqueous liquids. However, this difficulty is not a special limitation of our ability to perform molecular dynamics or Monte Carlo simulation calculations on water. Most of the modern progress in understanding hydrophobic effects has exploited a divide-and-conquer strategy:

assume that necessary molecular-scale information on water can be obtained by simulation and then build theories of hydrophobic effects from that starting point.

The second category of technical difficulty is more generic. Beyond the interactions among water molecules, the additional interactions involved with a hydrophobic effect are mostly excluded volume interactions. The problems to be solved bear considerable resemblance to the packing problems that underlie the theory of simple liquids.^{7,8} For our problems, however, the solutes must find space in an exotically structured thermal medium, liquid water.

It may be noted here that the theoretical developments below apply also to other solution chemistry problems of current interest. Electronic structure calculations for species in solution often append to the free energy a contribution to represent "cavitation." This is a free energy cost to open space for the solute in the solvent. Theories that solve that problem for water should be carried over to other solvents too.

Importance to LANL's Science and Technology Base and National R&D Needs

Hydrophobic effects are universally involved in aqueous solution chemistry and in the practical problems of environmental remediation and structural biology.

Scientific Approach and Accomplishments

A Few Basic Principles. Let's note here some of the basic principles that underlie models, theories, and calculations of hydrophobic effects. The quantity of first interest is the free energy associated with the interaction of the solute with the aqueous environment. This is the chemical potential or partial molar Gibbs free energy of the solute.^{9,10} This quantity provides a driving force for rearranging molecules in thermodynamic systems, and many quantities of interest are fundamentally connected to this free energy. Consider first an atomic solute of type A. For example, perhaps A = Ar. The chemical potential at extreme dilution may be expressed as

$$\mu_A = kT \ln \rho_A \Lambda_A^3 + \Delta\mu_A^{(0)}, \quad (1)$$

where T is the temperature, k is the Boltzmann constant, ρ_A is the number density of A atoms, and Λ_A is the thermal deBroglie wavelength for the A solute, a known function of the temperature. There are several messages from this equation. The first is that the concentration dependence of μ_A is known in the low concentration regime. The second is

that this $\ln \rho_A$ concentration dependence comes with a choice of concentration units so that the argument of the logarithm is dimensionless. The third message we develop further here: $\Delta\mu_A^{(0)}$ is the part of the chemical potential that expresses interactions between the solute and the solvent molecules. This point is captured by the formula¹¹ that identifies how $\Delta\mu_A^{(0)}$ is obtained from the solute-solvent interactions:

$$e^{-\Delta\mu_A^{(0)}/kT} = \left\langle e^{-\Delta U/kT} \right\rangle_0. \quad (2)$$

The brackets indicate a thermal average of the variable enclosed. In this case, it is the average of the Boltzmann factor of the mechanical potential energy of solute-solvent interaction over the thermal motion of the solvent alone. The subscript zero emphasizes that the solute is only a test particle for the purposes of this average.

The equations above bring us from molecular interactions to measurable thermodynamic parameters. We have presented these equations first for atomic hydrophobic solutes because they are easier to describe for that case. But these results have natural elaborations to other circumstances. Often another choice of concentration units will be most natural for the experimental context. A popular but not necessary choice is to use mole fraction concentration units $x_A = \rho_A/\rho$ at low concentration, where ρ is the number density of the solvent.¹² The change of concentration variable in Equation (1) will then induce an additive contribution to the $\Delta\mu_A^{(0)}$. Another elaboration is essential to the treatment of molecular solutes M with internal structure more complicated than atomic solutes. Equations (1) and (2) combined can then be expressed as

$$\mu_M = kT \ln \left[\rho_M \left(V / Q_M^{(0)} \right) \right] - kT \ln \left\langle \left\langle e^{-\Delta U/kT} \right\rangle \right\rangle_0. \quad (3)$$

The double bracket indicates the average over the thermal motion of the solute and solvent under the condition of no interactions between solute and solvent. The quantity to be averaged is the Boltzmann factor of the mechanical potential energy of interaction between solute and solvent. This is a test *molecule* description of the effects of solute-solvent interactions. $Q_M^{(0)}$ is the partition function of the solute M in isolation. For the atomic case, $V/Q_M^{(0)} = \Lambda_A^3$.

These formulae are based upon the classical mechanical limit of statistical mechanics. That limit is the most useful starting point for understanding aqueous solutions. Substantial quantum-mechanical generalization of these results is possible, but we will largely ignore those possibilities. A slight generalization of Equation (3), the final one for this discussion, is associated with conformational free energies and probable solute

structure. We inquire about the number density of solute molecules of type M with a particular conformation, including translational and orientational positioning. Our inquiry might be answered by determining the number density of molecules of type M. In notation established elsewhere,¹³ this density is $\rho_M(I_M)$ with I_M specifying the full positioning of the solute. The potential distribution expression for this density is^{13,14}

$$\rho_M(\mathbf{1}_M) = e^{\mu_M/kT} Q_M^{(0)} s_M^{(0)}(\mathbf{1}_M) \langle e^{-\Delta U/kT} \rangle_{0, \mathbf{1}_M} \quad (4)$$

The normalized probability density for the molecule to adopt conformation I_M in the absence of interactions with the solvent is $s_M^{(0)}(I_M)$. The brackets require the average over the thermal motion of the solvent alone with the solute fixed at the configuration I_M .

Equation (3) can then be obtained from Equation (4) because $\rho_M(I_M)$ is normalized to the total number of molecules of type M. The assumption of classical mechanical motion is more severe for the internal motions of solutes of interest than for the relative motions of solute and solvent. In that case it would be natural to evaluate $s_M^{(0)}(I_M)$ and $Q_M^{(0)}$ with the appropriate quantum mechanical techniques but to obtain the thermal average involving solute-solvent interactions classically.

These formulae clarify how solute-solvent interactions are expressed in the thermodynamic quantities of first interest. The formulae need not be used directly but serve to organize concepts and they may initiate development of computational algorithms. They also emphasize the principle that molecular calculations start from a specification of the mechanical potential energies of interaction between solute and solvent. The formulae do not require that the interaction model be decomposable as interactions between pairs of molecules.

Primitive Hydrophobic Effects. As noted above much of the interest in hydrophobic effects is associated with complicated systems, often biological macromolecules in water. However, hydrophobic effects in those complicated systems can be identified in the simpler circumstances of inert gases dissolved in water. These gases are less soluble in water than in typical hydrocarbon solvents such as cyclohexane. That low solubility is associated with an unfavorable (negative) entropy of solution, extracted through the temperature derivative of the chemical potential, and the heat capacity increment upon solution extracted with the temperature derivative of that solvation entropy is larger than for hydrocarbon solvents. Thus a negative entropy of solution at room temperature decreases in magnitude as the temperature is increased, and it is often said that hydrophobic solubilities are entropy dominated at room temperatures but enthalpy dominated at higher temperatures. These are unusual behaviors that beg a valid molecular explanation. These

are primitive hydrophobic effects. The discussion here will adopt the attitude that if these primitive hydrophobic phenomena are not understood at a molecule level, then hydrophobic effects in the more complicated settings of first interest are also not understood on a molecular basis.

Another primitive hydrophobic effect is the effect of water on the association of simple hydrocarbon molecules in aqueous solution. Presented here are discussion and an example of association of pairs of inert gas atoms; these focus on the function that gives the mean forces between such a pair upon differentiation, i. e., the pair potential of the average forces. These issues broaden the topic from hydration of solitary hydrophobic solutes to the interactions associated with disruption of the water structure by more than one hydrophobic solutes in proximity. A further extension of the study of hydrophobic interactions is the treatment of conformational equilibrium of simple, flexible nonpolar molecules in aqueous solution.

This organization of our subject beginning with primitive hydrophobic effects leaves to an important subsequent stage the integration of valid molecular theories of primitive hydrophobic effects into the more complicated settings of molecular biophysics.

An important part of the puzzle is that the most characteristic hydrophobic effects, the unfavorable entropies and large heat capacity changes, seem to be largely independent of the molecular details of solute-solvent interactions within broad families. This is an awkward point for computational chemistry that naturally invests great effort in getting intermolecular interactions accurately described before entropies are considered. This point emphasizes the utility of studying model problems, primitive hydrophobic effects in the first place and modelistic expressions of those effects. It is helpful first to identify the minimum that must be included in the model in order to get the interesting behavior and only after that to include all features actually present in specific cases.

Theories And Methods. This section discusses theories and calculations that have been used in molecular modeling of primitive hydrophobic effects. There is a basic schism among approaches that have been pursued. One approach is to model hydrophobic effects empirically on the basis of experimental solubilities without direct consideration of solute-water molecular interactions. Hydrophobic effects extracted on the basis of empirical fitting of solubilities are often called *hydrophobicities*. Empirical hydrophobicities have the advantage that they may be widely applied, but the disadvantages are that they refrain from molecular explanations and are of unknown reliability outside the phenomena from which they are derived.

On the other hand, molecular theories must start from a statement of intermolecular interactions. The interactions of hydrophobic species with solvent molecules are generically

of van der Waals type, not classic electrostatic or specific chemical interactions. For such circumstances it is natural to proceed along the conceptual lines of the WCA⁷ organization of the theory of liquids: to develop concepts and theories, we first consider hard core model interactions and expect to build from there. Molecular theories should show also how more realistic interactions can be treated.

We discuss current molecular theories without direct consideration of computation techniques. However, from recent theories conceptual tools have evolved that can be well integrated into standard computational simulation work. It has been explicitly argued that these tools be generally incorporated into simulation packages.¹⁵ In fact, unanticipated results have been obtained through application of these techniques.¹⁶

Insertion theories and models. Direct use of Equation (2) or (4) to study primitive hydrophobic effects started about seven years ago with the realizations that these formulae can be directly feasible for atomic-sized solutes in water at the moderate conditions of greatest interest;^{17,18} and that, when feasible, these results give information of direct conceptual relevance to molecular theories.^{15,19} We anticipate later discussion by asserting here that insights drawn from these direct calculations have recently led to new theories that promise to extend the range of applicability of molecular ideas beyond the primitive hydrophobic effects.

Direct calculations of insertion probabilities. Beginning with hard core interactions between rigid solutes and water molecules, we note that Boltzmann factors of Equation (2) or (4) are either zero or one, respectively, depending on whether any solvent molecules overlap the van der Waals volume of the solute at each solvent configuration sampled. The average collects with weight *one* all configurations leaving the solute-excluded volume empty; this *insertion probability* we will denote as $p_0(I_M)$ so that

$$\Delta\mu_M^{(0)}(\mathbf{1}_M) = -kT \ln p_0(\mathbf{1}_M). \quad (5)$$

This probability is simplest to consider for spherical solutes, and we will discuss that case specifically. In favorable but physically interesting cases, $p_0(I_M)$ can be calculated directly by simulation. Configurations of the bulk water can be sampled with conventional computational methods. Those sampled configurations may then be analyzed by constructing a uniform tessellation of the space I_M for each such point and determining whether the solute could be positioned there without overlap of the van der Waals volume of the solvent. Figure 1 shows the oxygen atoms of a realistic configuration of water molecules drawn from a molecular dynamics simulation; and Figure 2 shows balls of radius 1.5\AA at positions that would permit placement of a hard core model of the Ne atom with exclusion radius of 1.5\AA .

For the case of Figures 1 and 2, where the van der Waals volume of the solvent is modeled by overlapping spheres of one kind only and the solute is a sphere also, a simplification is possible. The calculation can be reduced to the estimation of the distribution, $p_m(\lambda)$ of distances λ from the arbitrarily chosen observation point to the nearest water oxygen center. The desired insertion probability p_0 is the accumulated probability that the nearest neighbor of an arbitrary point is further away than the given distance of closest approach:

$$p_0 = \int_{\lambda=R_A+R_S}^{\infty} p_m(\lambda') d\lambda' \quad (6)$$

We have dropped the argument I_M since we are explicitly discussing spherical solutes. We have also adopted the common special case that the distance of closest approach is the sum of a van der Waals radius of the solute and the solvent atoms, $\lambda = R_A + R_S$.

Distributions derived from p_0 . Equation (6) illustrates that interesting structural information can be derived from p_0 . In the case here, if p_0 is known as function of λ , then the distribution of distances to the nearest neighbor is found as the derivative $p_m(\lambda) = -dp_0/d\lambda$. This distribution helps us to address an interesting hypothesis advanced to explain why the solubility of inert gases is lower in water than in hydrocarbon solvents.²⁰ It was suggested that this relatively low solubility was due to the fact that a water molecule is smaller in size than solvent molecules of typical hydrocarbon liquids such as hexane and that the size of interstitial cavities would therefore be larger in the hydrocarbon liquid than in water. The countervailing view is that typical organic solvents are not composed of spherical molecules much larger than a water molecule. More realistically, they are composed of nearly spherical units like the methyl and methylene groups that are only slightly larger than a water molecule. In addition, organic liquids are typically more dense on a packing fraction basis than is water. Examination^{15,19} of $p_m(\lambda)$ computed for several common solvents showed that the size of the most probable cavities is about the same for the cases examined. The most probable cavity sizes are largely established by the density and radius of the primitive solvent sites not the solvent correlations.

A second type of structural information may be obtained from the derivative $d \ln p_0 / d\lambda$. Because of the connection with the thermodynamic Equation (2), this derivative can be interpreted as a compressive force. Comparison of water and typical organic solvents¹⁹ leads to the conclusion that water exerts a relatively higher compressive force on the surface of an inert solute. The helpful physical view²¹ that water squeezes out small

hydrophobic solutes was thus confirmed by the molecular scale calculations studying this compressive force.

Information Theory Model (ITM) of p_0 . We now come to the question of whether simulation calculations will always be required to obtain information on p_0 . We discuss a recently proposed^{22,23} information theory that predicts p_0 approximately. The idea is to consider p_0 but the $k=0$ member of a distribution p_k of the numbers of solvent centers in the observation volume v , i.e., within the surface outlining the solute excluded volume. (If several different types of solvent centers are present, we will study the distribution giving probabilities of finding a specified number of centers of each type in the volume excluded to each.) The plan is to collect the available information on this distribution and to construct a heuristic model by maximizing the information entropy

$$\eta = - \sum_{k \geq 0} p_k \ln \left[\frac{p_k}{w_k} \right] \quad (7)$$

subject to the constraints of the known information. Here w_k is an initial guess at the distribution and is called the default model. The predicted distribution will equal the default model if no further information is supplied. Moments such as the power moments $m_k = \langle n^k \rangle$ are the typically considered information. Surprisingly, it has been found that the simplest possibilities for these models work well for primitive hydrophobic effects. In particular, the flat default model,²² together with the experimentally available moments $m_0=1$, $m_1=\rho v$, and

$$m_2 = \rho v + \rho^2 \int_v d^3 r \int_v d^3 r' g_{OO}(|\mathbf{r} - \mathbf{r}'|), \quad (8)$$

describes primitive hydrophobic effects accurately. Here $g_{OO}(r)$ is the oxygen-oxygen radial distribution function for water. This simplest model takes the functional form

$$\ln p_n = \zeta_0 + n\zeta_1 + n^2\zeta_2, \quad (9)$$

fitted to the information with the parameters ζ_k . Then $\Delta\mu_A = \zeta_0$. Considerations of scaled particle models (below) suggest that this good agreement will not hold up for arbitrarily large hard-core solutes. But the flexibility provided through the default model suggests that this information theory modeling should be extendible to macromolecules and their aggregates in aqueous solution. The discovery of informative default models is an important line of development of further improvements of this approach.

An advantage of this approach is that numerical application is only limited by the evaluation of the moment integrals. Such moments can be obtained directly from stored simulation data. Thus, no important computational obstacle prevents application of this information theory to nonspherical solutes or odd geometric configurations of solutes.

Entropy convergence. The ITM provides an explanation of a puzzling hydrophobic phenomenon known as entropy convergence. We discuss this phenomenon and the explanation here.²⁴ The following facts constitute the entropy convergence phenomena: free energies of solvation of gases in water along the saturation curve show a maximum and this maximum occurs at about the same temperature for different gases.²⁵ Similarly, if hydrophobic contributions to protein folding are extracted from calorimetry, they also appear to show a maximum in the same range (130°C –140°C).²⁶ At the maximum, the temperature derivative of the solvation free energy is zero, and this temperature derivative contributes most of the solvation entropy. Thus, the solvation entropy is nearly zero at the convergence temperature. This is approximately independent of the detailed nature of the hydrophobic solute so that hydrophobic entropies for many solutes intersect at a common temperature. Why is this?

The two-moment ITM applied to hard sphere solutes predicts entropy convergence for those cases.²⁴ Additionally, test particle simulation methods used to study more realistic, Lennard-Jones models of inert gas atoms in water also provide a reliable description of the temperature dependence of the solvation free energy.²⁷ This theoretical success permits a simpler understanding of entropy convergence. We argue that a continuous Gaussian distribution reliably approximates the two-moment information model,^{22,24,28,29} exhibits the entropy convergence, and produces an explicit result for the excess chemical potential:

$$\Delta\mu_A^{(0)} \approx kT\rho_{sat}(T)^2 v^2 / 2 \langle \delta n^2 \rangle + kT \ln(2\pi \langle \delta n^2 \rangle) \quad (10)$$

where $\rho_{sat}(T)$ is the density of liquid along the vapor saturation curve. $\rho_{sat}(T)$ decreases with increasing temperature along the saturation curve, and $T\rho_{sat}(T)^2$ shows a maximum (Figure 3). But $\langle \delta n^2 \rangle$ has been found to be relatively independent of temperature along the saturation curve outside the critical region. Thus, the maximum in the solvation free energy derives from the maximum in $T\rho_{sat}(T)^2$. Analysis²⁴ of the temperature derivative of Equation (10) with these understandings then shows why a reasonable entropy convergence is obtained but with a convergence entropy that is slightly *negative*.

The ITM is expected to be accurate for sufficiently small solutes because the moments available could saturate the distribution. The ITM and its Gaussian limit are expected to be accurate for the center of the distribution p_k for large volumes v but not accurate for the wings of that distribution when v is large. The isothermal compressibility β_T is the thermodynamic parameter that reflects σ^2 in the macroscopic limit. Even though

the ITM as not expected to be accurate in the macroscopic limit, it is β_T that reflects the difference in the information used with the ITM is applied to water and organic solvents. It is remarkable that water appears less compressible than organic solvents and displays a minimum in the isothermal compressibility within the temperature range of interest here (Figure 4).³⁰

Scaled particle models. The scaled particle model (SPM) was the first essentially molecular theory of hydrophobicities. It derived from an earlier scaled particle theory, a successful theoretical calculation of the thermodynamic properties of the hard sphere liquid. Pierotti³¹ then adapted the scaled particle theory to produce a solubility model for realistic liquids by a natural replacement of the hard sphere pressure with the measured pressure of the real solvent of interest. With attractive solute-solvent interactions treated perturbatively, this scaled particle model was remarkably successful. The SPM is the molecular theory of hydrophobicities most widely considered among biomolecular modelers. However, its success is somewhat fortuitous.^{32,33} For example, though the SPM predicts a reasonable value for the surface tension for the water liquid-vapor interface at room temperature, the predicted temperature dependence is wrong. Since entropies and temperature dependencies are special goals of theories of hydrophobic effects, this incorrect temperature dependence is important.

Stillinger³³ analyzed the SPM and proposed a revised scale particle model (RSPM) that incorporates the measured surface tension in addition to the measured pressure, in this way correcting the most direct error of the SPM. The RSPM deserves note because it is more accurate for primitive hydrophobic effects^{15,17,19} than the SPM but not more difficult to use.

Stillinger also discussed physical expectations for the structure of the solvent outside a hard sphere solute and clarified the following issue: when the hard sphere radius is large, the water is expected to pull away from the hard sphere surface. This dewetting behavior is associated with the lack of attractive solute-solvent forces and with the fact that thermodynamic conditions of greatest interest are on the saturation curve at low vapor pressures. The material immediately surrounding such a solute would be recognized as water vapor. The ITM above does not produce this behavior; that is the most severe failing of that simple model. A default model w_n that describes a generic liquid with incipient vapor separation should build such a feature into the ITM approach.

Whether this dewetting phenomenon is important to biophysical systems with solute-solvent attractive forces hasn't been studied conclusively. However, related dewetting has

recently been discovered when stacked hard surfaces are positioned closer than two water molecular layers from one another.³⁴

Integral equation approximation. Equilibrium theories of classical fluids³⁵ are often formulated as integral equations for the distribution functions, e. g., $g_{AA}(r)$ giving the probability distribution of AA pairs at a specified separation in water. The PC theory³⁶ was the first theory of hydrophobic effects largely built on that conventional basis. A less conventional feature was that the PC theory avoided calculating separately the structure of water in the absence of hydrophobic solutes, much in the spirit of the scaled particle models. Instead, the measured $g_{OO}(r)$ was used as input to the theoretical calculation of $g_{AA}(r)$. The idea was to use what is known about pure water to make predictions about hydrophobic effects. The ITM makes this heuristic point of view explicit.^{21,22} Moreover, the success of the simplest two moment ITM provides important support for the earlier PC theory: the PC theory can be derived on the assumption that fluctuations of density fields are given by a Gaussian probability functional.²⁹ The two moment ITM is not precisely the same as the PC theory because the ITM preserves also the fact that the spatial integrals of density fields are nonnegative integers. However, that distinction is secondary except for small solutes. A more important distinction that the ITM suggests constructive extensions of current theories.

The most noteworthy prediction of the PC theory was the potential of mean force for model methane pairs in water (Figure 5). This was the first molecularly realistic prediction of a potential of mean force between hydrophobic spheres in water. It showed structural oscillations that are natural features of these functions in simpler liquids: a solvent-separated minimum free energy configuration, a thermally substantial barrier to desolvation, and a free energy minimum at contact of the two methane molecules. These qualitative features of potential of mean force between spherical hydrophobic solutes have subsequently been observed in simulation many times.²³ The result shown in Figure 5 was produced with the two-moment ITM, is substantially the same as the prediction of PC, and is in good agreement with the available *computer simulation data*.

Conclusions. Hydrophobic effects are linked with entropic contributions associated with the structure of water abutting space-filling solution impurities without classic electrostatic or chemical interactions with the solvent. Theories that have broken new ground in recent years have focused on finding space for hydrophobic solutes in water, transplanting statistical and geometric concepts associated with scaled particle theories of the hard sphere fluid to more general solution environments.^{8,37} Similar concepts have found use in studies of polymeric glasses. The theoretical tools are readily

incorporated into computational simulation efforts¹⁵ and can give fresh insight into the structure and function of solvents.^{16,24}

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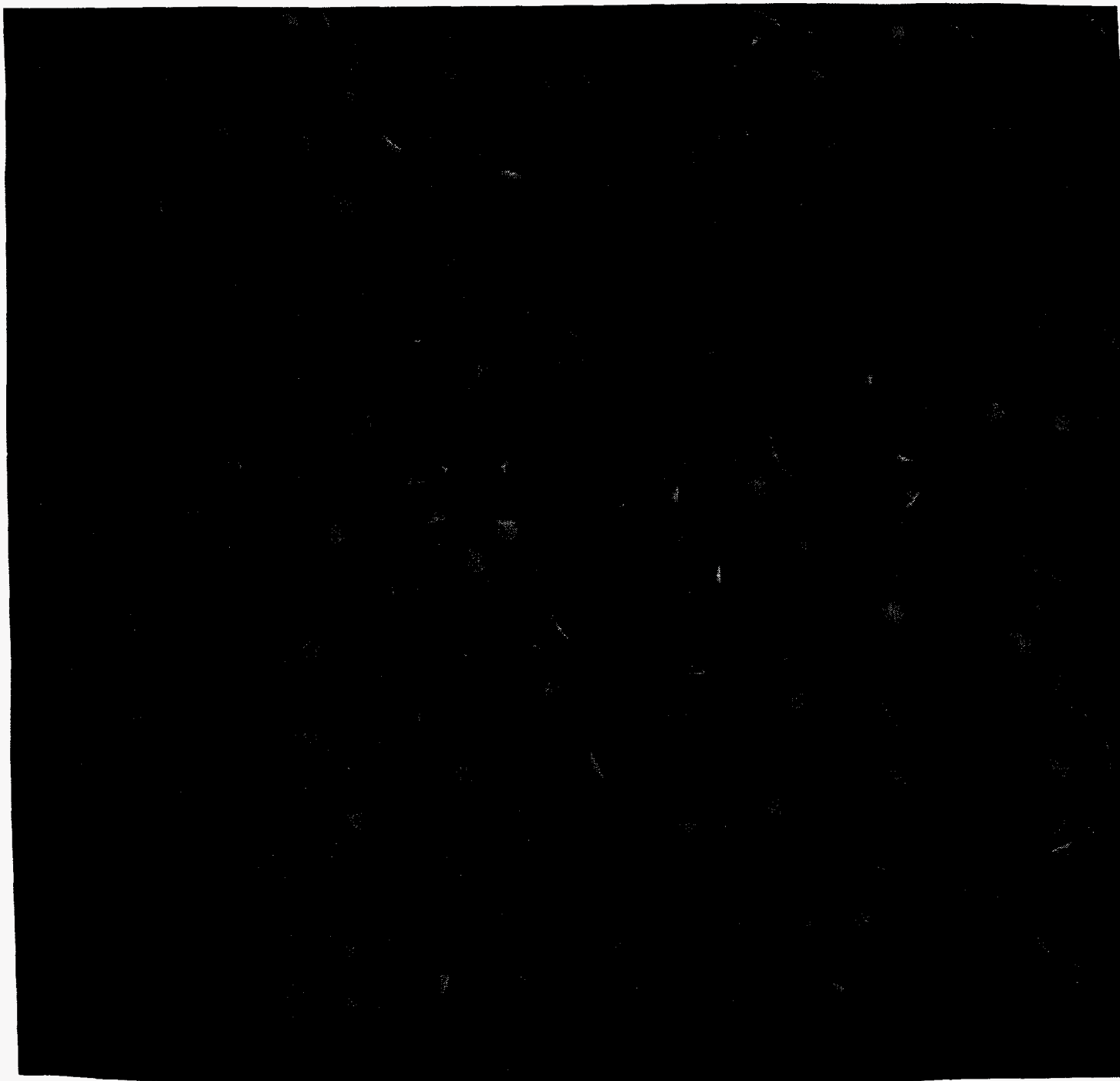


Figure 1. Configuration of 343 water molecules (periodic boundary conditions) drawn from a canonical ensemble with $T = 300K$ and $\rho = 1.0g/cm^3$ —only the oxygen atoms are shown.

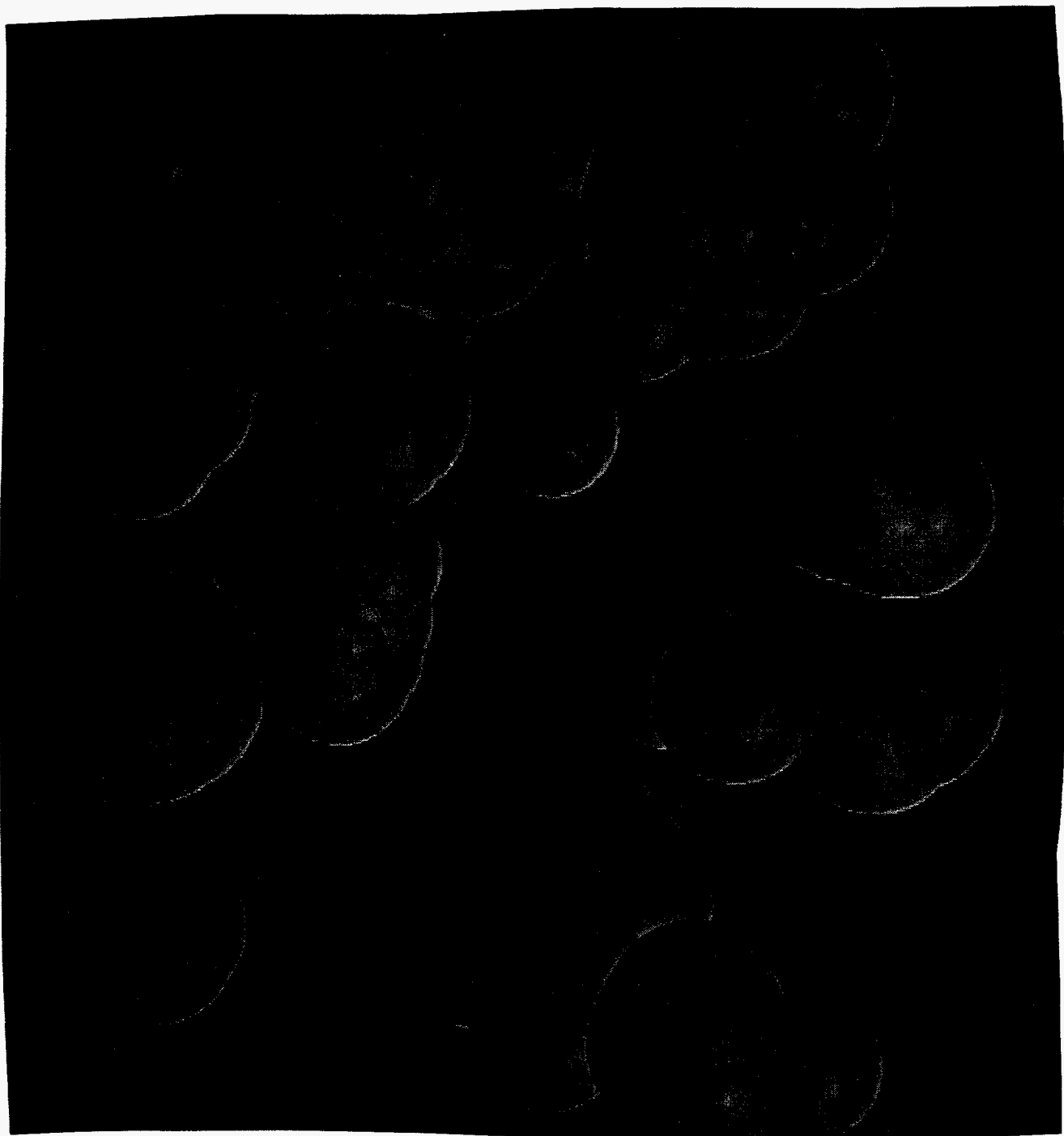


Figure 2 Successful insertions of spheres of radius 1.5\AA in a water configuration such as that shown in Figure 1.

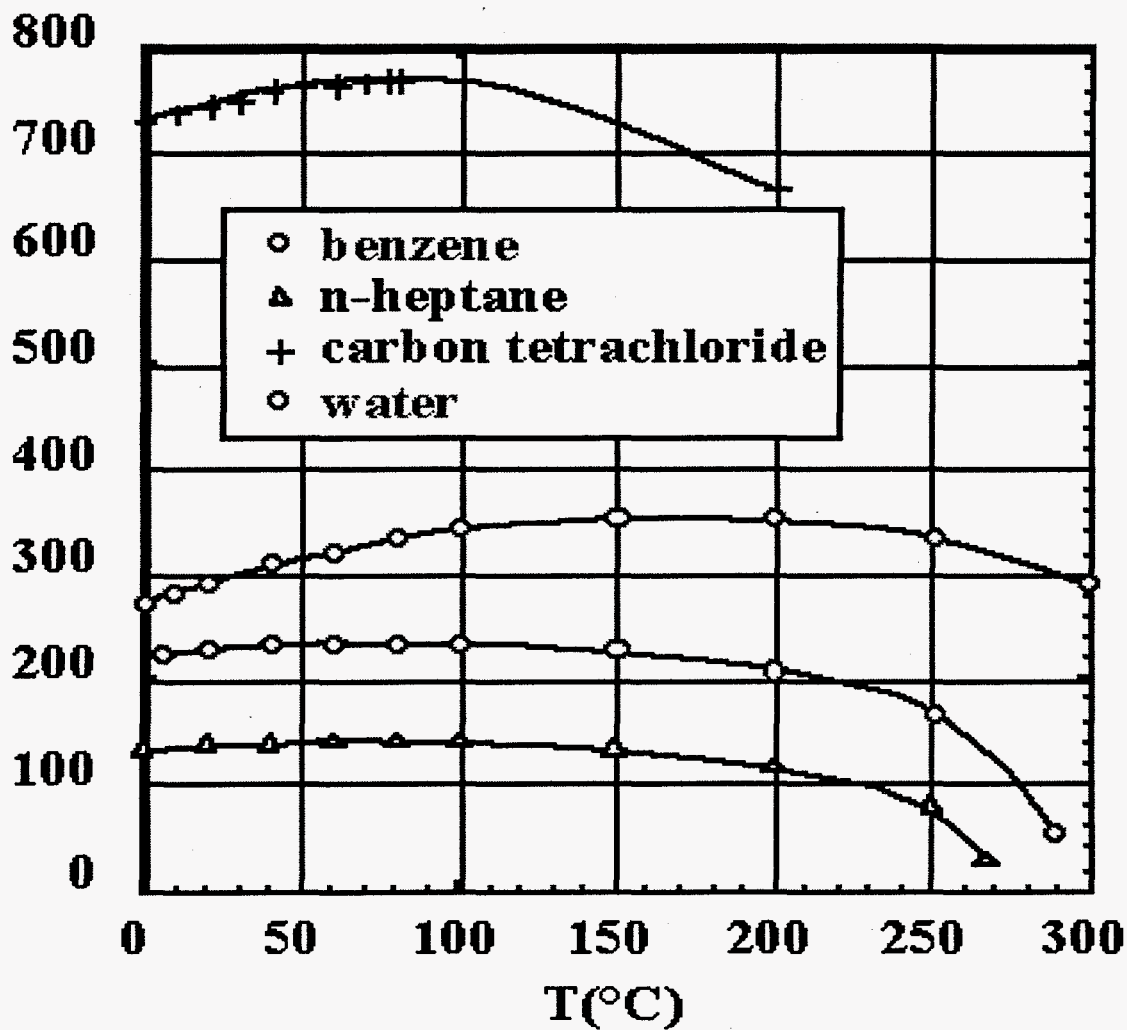


Figure 3. $T\rho_{sat}(T)^2$ (Kg^2/cm^6) for several solvents along their liquid-gas coexistence curve.¹

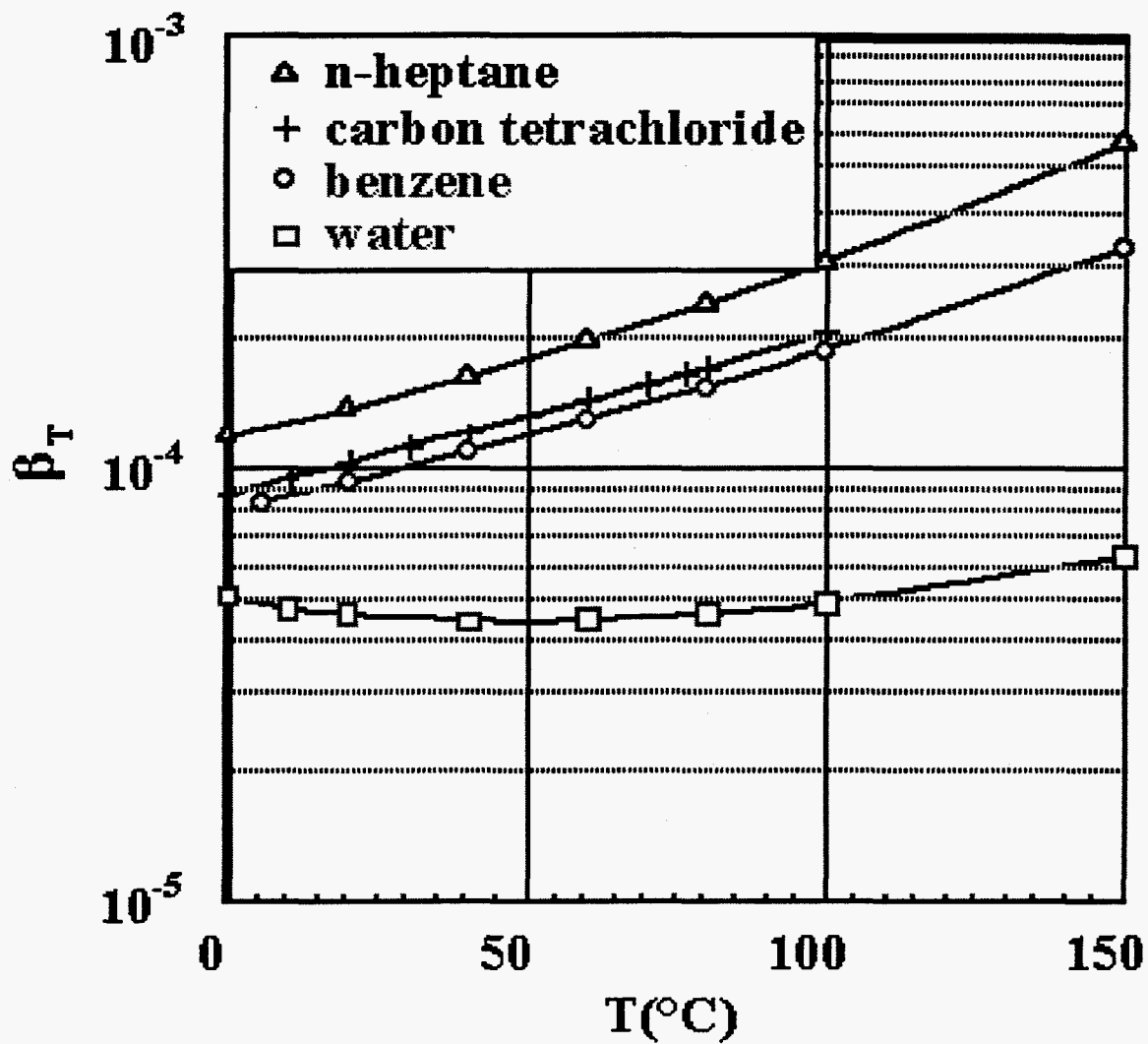


Figure 4. Isothermal compressibilities, β_T (bar^{-1}) of several solvents along their liquid-gas coexistence curve.¹

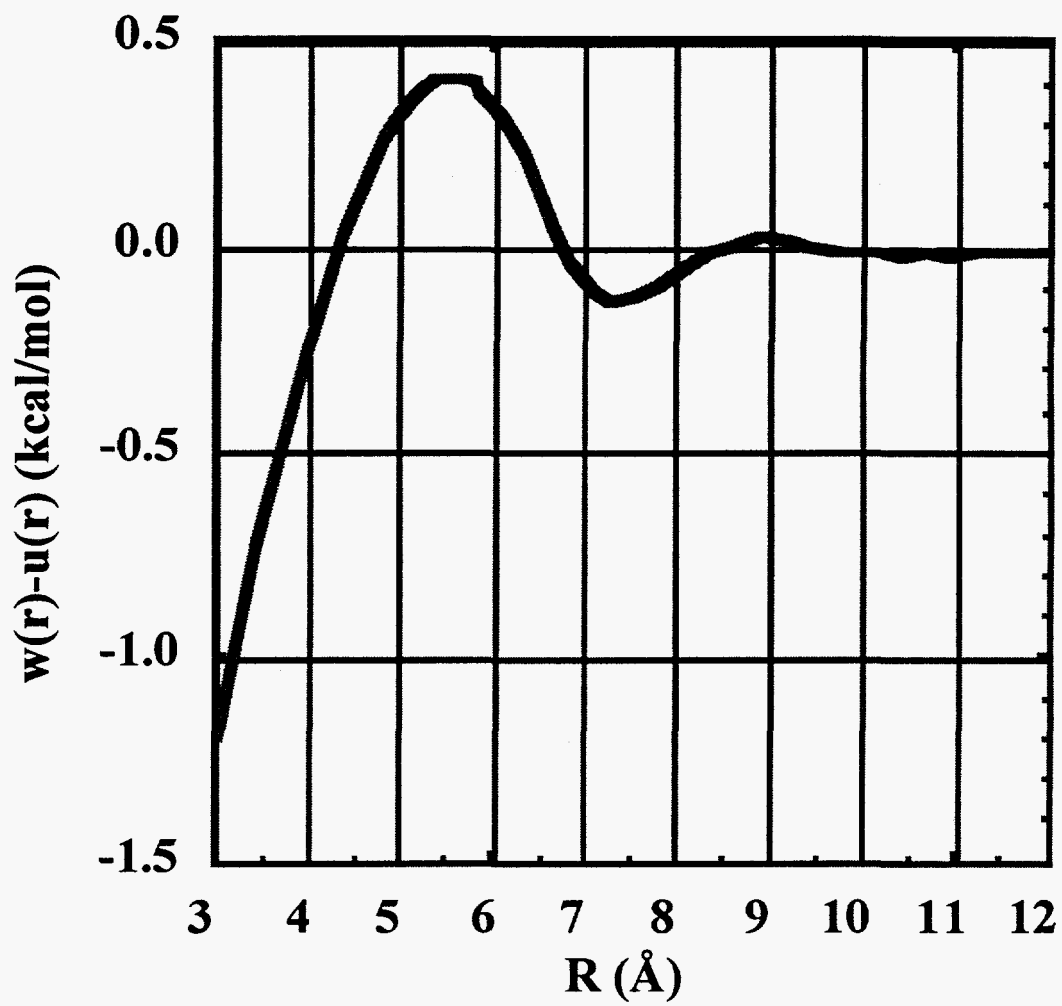


Figure 5. Cavity potential of mean force $kT \ln[e^{u(r)/kT} g_{AA}(r)]$ for two hard sphere model methane molecules in water.²²