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*Original*

Hydrophobic Hydration Processes: Intensity Entropy and Null Thermal Free Energy and Density Entropy and Motive Free Energy / Fisicaro, Emilia; Compari, Carlotta; Antonio Braibanti, And. - In: ACS OMEGA. - ISSN 2470-1343. - 4:(2019), pp. 19526-19547. [10.1021/acsomega.9b01440]

*Availability:*

This version is available at: 11381/2867202 since: 2019-11-28T11:07:03Z

*Publisher:*

*Published*

DOI:10.1021/acsomega.9b01440

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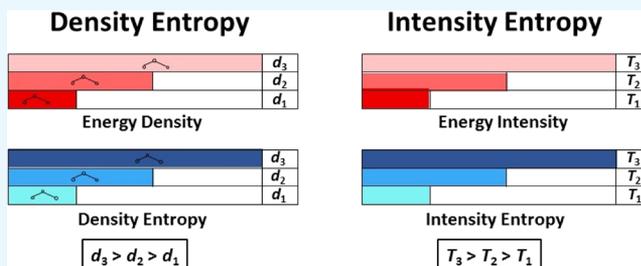
# Hydrophobic Hydration Processes: Intensity Entropy and Null Thermal Free Energy and Density Entropy and Motive Free Energy

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## Supporting Information

**ABSTRACT:** The processes at the molecule level, which are the source of the ergodic properties of thermodynamic systems, are analyzed with special reference to entropy. The entropy change produced by increasing the temperature  $T$  depends on the increase of velocity of the particles with a decrease of the squared mean sojourn time ( $\tau_m^2$ ) and gradual loss of instant energy intensity. The diminution, which is due to dilution, of the number of terms in the summation of cumulative sojourn time ( $\tau_i^2$ )<sub>Σ</sub> produces loss of energy density, thus generating a gradual increase of density entropy,  $dS_{\text{Dens}}$ . The ergodic property of thermodynamic systems consists of the equivalence of density entropy (dependent on dilution) with intensity entropy (dependent on temperature). This equivalence has been experimentally verified in every hydrophobic hydration process as thermal equivalent dilution. An ergodic dual-structure partition function {DS-PF} represents the state probability of every hydrophobic hydration process, corresponding to the biphasic composition of these systems. The dual-structure partition function {DS-PF} ( $K_{\text{mot}} \zeta_{\text{th}}$ ) is the product of a motive partition function {M-PF} ( $K_{\text{mot}}$ ) multiplied by a thermal partition function {T-PF} ( $\zeta_{\text{th}} = 1$ ). {M-PF} gives rise to changes of density entropy, whereas {T-PF} gives rise to changes of intensity entropy. {M-PF} is referred to a reacting mole ensemble (reacting solute) composed of few elements (moles), ruled by binomial distribution, whereas {T-PF} is referred to a nonreacting molecule ensemble (NoremE) (nonreacting solvent), which is composed of a very large population of elements (molecules), ruled by Boltzmann statistics. Statistical thermodynamic methods cannot be applied to {M-PF} that can be calculated by numerical methods from the experimental titration data. By development of the dual-structure partition function {DS-PF}, parabolic convoluted binding functions are obtained. The tangents to the binding functions represent the dual enthalpy,  $-\Delta H_{\text{dual}} = (-\Delta H_{\text{mot}} - \Delta H_{\text{th}})$ , and the dual entropy,  $\Delta S_{\text{dual}} = (\Delta S_{\text{mot}} + \Delta S_{\text{th}})$ . The connections between canonical and grand-canonical partition functions of statistical thermodynamics with thermal and motive partition functions of chemical thermodynamics, respectively, are discussed. Special attention has been devoted to the equality  $\Delta H_{\text{th}}/T + \Delta S_{\text{th}} = 0$ , typical of NoremEs, as an entropy–enthalpy compensation with  $\Delta G_{\text{th}}/T = 0$ . The thermodynamic potential change  $\Delta\mu$ , as proposed by potential distribution theorem (PDT) for iceberg formation from {T-PF} of the solvent, is nonexistent because the excess solvent is at a constant potential ( $\Delta\mu_{\text{solv}} = 0$ ). The information level offered by the ergodic algorithmic model (EAM) is more complete and correct than that offered by the potential distribution theorem (PDT): the stoichiometry of the water reaction in hydrophobic hydration processes is determined by the EAM as the function of the number  $\pm \xi_w$ . Quasi-chemical approximation, renamed the chemical molecule/mole scaling function (Che. m/M. sF), is a fundamental breakthrough in the application of statistical thermodynamics to chemical reactions. Boltzmann statistical molecule distribution of the thermal partition function {T-PF} is scaled with binomial mole distribution of the motive partition function {M-PF}. For computer-assisted drug design, the alternative calculation procedure of Talhout, based on the previous experimental determination of binding functions, is recommended. The ergodic algorithmic model (EAM), applied to the experimental convoluted binding functions, can recover the distinct terms of intensity entropy ( $\Delta H_{\text{mot}}/T$ ) and density entropy ( $\Delta S_{\text{mot}}$ ), together with other essential information elements, lost by computer simulations.



## 1. INTRODUCTION

While studying the thermodynamic properties of hydrophobic hydration processes,<sup>1–6</sup> we have had the opportunity of exploiting the thermal equivalent dilution (TED) principle to get very reliable results valid for a large set of reactions. We have found that the convoluted binding function  $R \ln K_{\text{dual}} = (-\Delta G_{\text{dual}}/T) = \{f(1/T) \times g(T)\}$  in every hydrophobic hydration process presents a curved parabolic shape, parallel

to that of the convoluted binding function  $R \ln K_{\text{dual}} = \{f(1/d_A) \times g(T)\}$  where  $d_A$  is the dilution of the ligand A, expressed in molar fractions. We have verified the equivalence of temperature and dilution, as distinct variables of intensity entropy and

Received: May 17, 2019

Accepted: September 17, 2019

Published: November 11, 2019

density entropy, respectively, as a proof of the ergodic properties of these thermodynamic systems.

Now, we are going to show how the ergodic property of equivalence of intensity entropy with density entropy originated at the molecule level. We are thus setting the basis to find out the connection between chemical thermodynamics and statistical thermodynamics.

This article is the second part of a three-paper series concerning hydrophobic hydration processes:

- I. Dual Structure Partition Function for Biphasic Aqueous Systems.<sup>5</sup>
- II. Intensity Entropy and Null Thermal Free Energy and Density Entropy and Motive Free Energy (Ergodicity)
- III. Ergodic Algorithmic Model (EAM) Validation.<sup>6</sup>

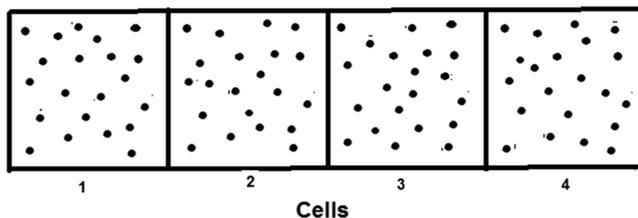
In part I, the hydrophobic aqueous systems have been shown to be biphasic, consisting of the phase “solvent” in excess and phase “diluted solute”. The thermodynamic state of these systems is presented mathematically in the probability space by the exponentials of a dual-structure partition function  $\{\text{DS-PF}\} = \{\text{M-PF}\} \cdot \{\text{T-PF}\}$ , composed of an exponential motive partition function  $\{\text{M-PF}\}$ , referred to the solute, and by an exponential thermal partition function  $\{\text{T-PF}\}$ , referred to the solvent. By taking the exponents of these partition functions, we pass from the probability space to the thermodynamic space where  $\{\text{DS-PF}\}$  gives rise to curved “convoluted” binding functions  $R \ln K_{\text{dual}} = \{f(1/T) \times g(T)\}$  and  $RT \ln K_{\text{dual}} = \{f(T) \times g(\ln T)\}$ , which conform to the binding functions experimentally determined, and constitute an ergodic algorithmic model (EAM).

In part II, the molecular origin of intensity entropy, as a property dependent on temperature and hence on the velocity of the molecules, will be demonstrated as well as the molecular origin of density entropy, as a property dependent on dilution of the solute. Intensity entropy is the function of time through the velocity of the particles, whereas density entropy is the function of space through changes of the solvent volume causing changes in dilution of the solute. The relationships between thermodynamic functions and approximate computer-simulated functions will be discussed. The potential distribution theorem (PDT) will be shown to be not conforming to the biphasic composition of hydrophobic hydration systems.

In part III,<sup>6</sup> the statistical analysis of the whole body of experimental data, determined for approximately 80 different compounds with approximately 600 experimental points, will validate the ergodic algorithmic model (EAM) by statistical inferences.

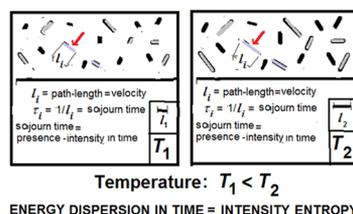
## 2. RESULTS AND DISCUSSION

**2.1. Ergodic Molecular Theory: Intensity Entropy and Density Entropy.** The ergodic theory is based on the principle that, in a system completely disordered such as a gas or a solute, the system has “forgotten” its original distribution, reaching a steady-state distribution, continuously changing but conserving certain average measures. We imagine dividing the whole volume of a solution into many microcells. Then, we imagine taking a set of snapshots, at 1 ns exposure, of a section: we find a set of imaginary cells wherein the disposition of the molecules in each cell is completely different from one another (Figure 1). In each cell, the disposition of molecules, as points, is completely random: the only property conserved is that where the average number of molecules per cell is constant. If we increase the exposure time to 1  $\mu\text{s}$ , we find that each



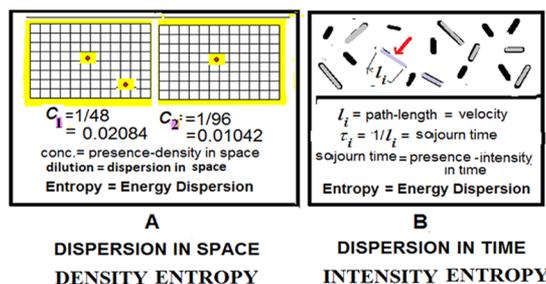
**Figure 1.** Solute molecules in each cell of the solution are completely random. Presence density is measured as a solute-to-solvent ratio.

molecule has left a trace on the photographic plate: the traces are longer, and their color intensity is weaker the higher the temperature of the solution (Figure 2). The molecules moving



**Figure 2.** The traces left by the moving molecules are longer the higher the temperature  $T$ . Left: lower temperature; right: higher temperature.

within the cell are carrying a portion of thermal energy, measured by a molecule thermal factor (mtf)  $\varphi = T^{-(C_p, A/k_B)}$ . If we dilute the solution, we obtain an increased dispersion in space of the thermal energy (Figure 3A). If we increase the



**Figure 3.** Ergodic parameters. A: Density entropy is proportional to dilution (reciprocal concentration): presence density in space  $\rightarrow$  concn  $x$  (molar fraction) ( $\exp(S_{\text{Dens}}/R) = (d_{\text{id}(A)}) \cdot (T^{(C_p, A/R)})$ ). B: Intensity entropy is proportional to velocity (reciprocal sojourn time): presence intensity in time  $\rightarrow$  sojourn time  $\tau_i$  (lengths ( $l_i$ ) are traces of moving molecules impressed on a photographic plate) ( $\exp(S_{\text{Ints}}/R) = (T^{(C_p, A/R)})$ ).

temperature  $T$ , each molecule runs faster, leaving a weaker trace, indicating that the energy intensity  $\varepsilon_{\text{intensity}}$  at each point is lower (Figure 3B). The parameter sojourn time (or persistence time) ( $\tau_i = 1/l_i$ ) of  $i$ th molecule measures the presence intensity in time. The lines represent path lengths ( $l_i$ ) of the molecules. The longer the path length  $l_i$ , the faster the molecule runs ( $v_i = l_i$ ) and the shorter is the sojourn time  $\tau_i$  ( $\tau_i = 1/l_i$ ), that is, the time spent by each molecule to run one single length unit<sup>a</sup>. The parameter controlling the variability in space is concentration  $x_A$  (in molecule fraction) of species A, whereas the parameter controlling the variability in time is squared sojourn time  $\tau_m^2$ . As the time parameter, the squared mean sojourn time  $\tau_m^2$  corresponding to the squared mean

velocity ( $v_m^2 = 1/\tau_m^2$ ) is on the same scale of the kinetic energy ( $v_m^2$ ). By summing up all the squared sojourn times of all the  $N$  molecules within a fixed selected sector of the solution, we obtain the cumulative squared sojourn time  $(\tau_i^2)_\Sigma$ .

$$(\tau_i^2)_\Sigma = \sum_{i=1}^{i=N} \tau_i^2 \quad (1)$$

The cumulative squared sojourn time  $(\tau_i^2)_\Sigma$  is a summation of the energy intensity of the molecules, extended to the whole sector. The slower the molecule is, the longer its energy intensity (persistence or sojourn time) in a site is. For the ergodic theory, the activity of species A is equal to a function of the squared sojourn time, which means that the presence density in space ( $a_A$ ) equals the energy intensity in time ( $f(\tau_i^2)_\Sigma$ ). In general terms, we can express the cumulative sojourn time as the product of two factors

$$a_A = f(\tau_i^2)_\Sigma = [x_A \cdot f(\tau_m^2)] = [x_A \cdot \phi] \quad (2)$$

where  $\tau_m^2$  is the squared mean sojourn time,  $x_A$  is the mole fraction concentration, and  $\phi$  is the molecule thermal energy factor (thef,  $\phi = T^{-(C_{p,A}/k_B)}$ ). If, by increasing temperature, we increase the velocity of the particles (supposing, for simplicity, that we double the temperature  $T$ ), we obtain shorter squared sojourn times. If the squared velocity is doubled, then the squared sojourn time  $\tau_i^2$  of each molecule is halved, and the cumulative squared sojourn time  $(\tau_i^2)_\Sigma$  is halved as well. The activity is halved.

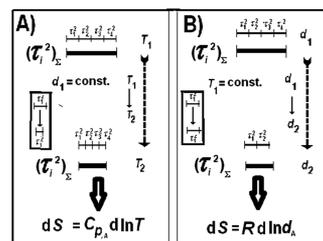
$$\begin{aligned} a_A &= f[(\tau_i^2)_\Sigma] = f\left[\sum_{i=1}^{i=N} (\tau_i^2/2)\right] = [(x_A/2) \cdot f(\tau_m^2)] \\ &= [(x_A/2) \cdot \phi] \end{aligned} \quad (3)$$

In a different sample of the same initial composition, we dilute the solution at constant temperature so that the concentration  $x_A$  of the molecules is halved. Then, we imagine counting again the cumulative squared sojourn time  $(\tau_i^2)_\Sigma$ : we obtain a new value, which is halved because the mean number of terms in the summation is now halved because of doubled dilution.

$$\begin{aligned} a_A &= f[(\tau_i^2)_\Sigma] = \left[f\left(\sum_{i=1}^{i=N/2} \tau_i^2\right)\right] = [(x_A/2) \cdot f(\tau_m^2)] \\ &= [(x_A/2) \cdot \phi] \end{aligned} \quad (4)$$

By doubling dilution, we can obtain the same change in virtual activity as that obtained by doubling temperature  $T$  (Figure 4).

The first factor  $x_A$  in eq 2 depends, by definition, on the concentration of solute A, and the second factor  $\phi = f(\tau_m^2)$  is a function of the reciprocal of the squared mean velocity of the particles. The molecule thermal factor (mtf)  $\phi = f(\tau_m^2) = T^{-(C_{p,A}/k_B)}$  represents the thermal energy associated to each molecule A. We can analyze the two factors in eq 4 separately, starting with  $f(\tau_m^2) = f'(1/v_m^2)$ . If we put  $x_A = 1$  in eq 2, we refer to a homogeneous phase where the concentration is meaningless, for instance, a pure liquid or a solvent in excess (very diluted solutions), and only the function of the squared sojourn time is effective. According to the Maxwell–Boltzmann distribution function, the mean velocity  $v_m$  is connected to the temperature by



**Figure 4.** Ergodic property. A: By increasing  $T$ , we increase molecular velocity thus obtaining shorter cumulative squared sojourn times,  $(\tau_i^2)_\Sigma = \Sigma \tau_i^2$ . B: By increasing  $d$  (e.g., by halving the concn) we obtain shorter cumulative squared sojourn times  $(\tau_i^2)_\Sigma$  due to the halved number of terms of the summation.

$$(\pi/8)(M/R)v_m^2 = T \quad (5)$$

and by assuming that all the molecules as moving at the same mean velocity, we obtain that

$$1/\tau_m^2 = (v_m^2) \quad (6)$$

Then, by passing to the logarithms of eq 5 and differentiation, we obtain

$$d \ln(v_m^2) = d \ln T \quad (7)$$

By changing the temperature  $T$ , we obtain a change of squared mean velocity of the particles so that we can write the entropy differential at the mole thermodynamic level as the function of squared mean velocity at the molecule level.

$$(dS_{\text{Ints}})_{d,id(A)} = C_{p,A} d \ln(v_m^2) \quad (8)$$

Thus, we have shown how any change in the second factor  $\phi = f(\tau_m^2)$  of eq 2 corresponds, through eq 8, to an equivalent change of thermal entropy (intensity entropy) because the velocity of the particles is changed. When the temperature is increased, the velocity increases too, and the thermal change of entropy (intensity entropy) increases as well. By changing the other factor in eq 2, we change the concentration. The reciprocal of concentration is the ideal dilution  $d_{id(A)} = 1/x_A$  of the species A where  $x_A$  is in mole fractions. The ideal calculated dilution (as a solute-to-solvent ratio) is proportional to the number of possible statistical configurations that the solute can occupy. The molecule A reaches its maximum dilution because the associated thermal energy brings the molecules to occupy the maximum accessible volume, thus reaching maximum dilution. Ideal dilution  $d_{id(A)}$  represents the experimental evaluation at the thermodynamic level of the probability state of molecule ensemble (density entropy) of statistical mechanics

$$(dS_{\text{Dens}})_T = R d \ln d_{id(A)} \quad (9)$$

whereas the thermal change of entropy or the intensity entropy change at constant  $d_{id}$  is represented by

$$\begin{aligned} (dS_{\text{Ints}})_{d,id} &= (dH/T)_{d,id} = (d(C_{p,A} dT)/T)_{d,id} \\ &= (C_{p,A} d \ln T)_{d,id} \end{aligned} \quad (10)$$

We can show how the analytical expressions of configuration and thermal changes of entropy follow directly from the conditions at the molecule level foreseen by the ergodic theory. In general, we note (Table S1 in the Supporting Information, Appendix H) that to each ergodic parameter  $x_A$  or  $\tau_m^2$  at the

Table 1. Ergodic Algorithmic Model (EAM)

(A) probability space	
(1) dual-Structure partition function (for biphasic systems)	
$\{DS - PF\} = \{T - PF\} \cdot \{M - PF\}$	(12)
$\exp(-\Delta H_{\text{dual}}/RT) \cdot \exp(\Delta S_{\text{dual}}/R) = \exp(-\Delta G_{\text{dual}}/RT) = K_{\text{dual}} = K_{\text{mot}} \cdot \zeta_{\text{th}}$	(13)
$\exp(-\Delta G_{\text{th}}/RT) = \exp(-\Delta \Gamma_{\text{th}}/k_{\text{B}}T)^{(1/NA_{\text{v}})} = \zeta_{\text{th}} = 1$	(14)
$\exp(S_{\text{dens}}/R) = a_{\text{A}}^{-1} = x_{\text{A}}^{-1} \Phi^{-1} = x_{\text{A}}^{-1} T^{C_{\text{p,A}}/R}$	(15)
$\exp(S_{\text{int}}/C_{\text{p,A}}) = T$	(16)
$\{DS - PF\} = K_{\text{dual}} = K_{\text{mot}} \cdot \zeta_{\text{th}} \rightarrow (\text{Solute} + \text{Solvent})(\zeta_{\text{th}} = 1)$	
$\{M - PF\} = \{f(T) \times f(d_{\text{id(A)}})\} = K_{\text{mot}} \rightarrow \text{solute}$	
$\{T - PF\} = f(T) = \zeta_{\text{th}} = 1 \rightarrow \text{solvent}$	
(2) activity $\rightarrow$ solute	
$a_{\text{A}} = x_{\text{A}} \Phi$ ( $a_{\text{A}}$ = activity of A, $\Phi = T^{-C_{\text{p,A}}/R}$ ; thermal factor)	(17)
(3) ergodicity $\rightarrow$ dilution $\rightarrow$ temperature	
$(1/x_{\text{A}}) = d_{\text{id(A)}} \cdot T^{C_{\text{p,A}}/R}$	(18)
(B) thermodynamic space	
(1) binding function $R \ln K_{\text{dual}} = \{f(1/T) \times g(T)\}$ (convolution, at constant $d_{\text{id(A)}}$ ) curved function (convoluted function)	
$R \ln K_{\text{dual}} = \{(-\Delta H_{\text{dual}}/T) + (\Delta S_{\text{dual}})\}$ $= \{(-\Delta H_{\text{mot}}/T) + (-\Delta H_{\text{th}}/T)\} + \{(\Delta S_{\text{mot}}) + (\Delta S_{\text{th}})\}$	(19)
$\partial(R \ln K_{\text{dual}})/\partial(1/T) = -\Delta H_{\text{dual}} = -\Delta H_{\text{mot}} - \Delta H_{\text{th}}$ $= -\Delta H_{\text{mot}} - \Delta C_{\text{p,hydr}} T$ (J mole <sup>-1</sup> )	(20)
linear function ( $f(1/T)$ ) (primary function)	
$R \ln K_{\text{mot}} = -\Delta H_{\text{mot}}/T + \Delta S_{\text{mot}}$	(21)
$\partial(R \ln K_{\text{mot}})/\partial(1/T) = -\Delta H_{\text{mot}}$ (J mole <sup>-1</sup> )	(22)
(2) binding function $RT \ln K_{\text{dual}} = \{f(T) \times g(\ln T)\}$ (convolution, at constant $d_{\text{id(A)}}$ ) curved function (convoluted function)	
$RT \ln K_{\text{dual}} = \{-\Delta H_{\text{dual}} + T \Delta S_{\text{dual}}\}$ $= \{-\Delta H_{\text{mot}} - \Delta H_{\text{th}}\} + T\{\Delta S_{\text{mot}} + \Delta S_{\text{th}}\}$	(23)
$\partial(RT \ln K_{\text{dual}})/\partial T = \Delta S_{\text{dual}} = \Delta S_{\text{mot}} + \Delta S_{\text{th}} = \Delta S_{\text{mot}} + \Delta C_{\text{p,hydr}} \ln T$ (J K <sup>-1</sup> mole <sup>-1</sup> )	(24)
linear function ( $f(T)$ ) (primary function)	
$RT \ln K_{\text{mot}} = -\Delta H_{\text{mot}} + T \Delta S_{\text{mot}}$	(25)

Table 1. continued

(B) thermodynamic space

$$\partial(RT \ln K_{\text{mot}})/\partial T = \Delta S_{\text{mot}} \quad (26)$$

(3) activity

$$dS_{\text{Dens}} = (-R \, d \ln x_A)_T + (C_{p,A} \, d \ln T)_{x_A} \quad (27)$$

(4) ergodicity

density entropy and intensity entropy

$$(dS_{\text{Dens}})_T = (-R \, d \ln x_A)_T = (R \, d \ln d_{id,A})_T \rightarrow \text{entropy density} \quad (28)$$

$$(dS_{\text{Ints}})_{x_A} = (C_{p,A} \, d \ln T)_{x_A} \rightarrow \text{intensity entropy} \quad (29)$$

thermal equivalent dilution (TED)

$$(dS_{\text{Dens}})_T = (dS_{\text{Ints}})_{x_A} \quad (30)$$

$$n_w(R \, d \ln d_{id,A})_T = n_w(C_{p,A} \, d \ln T)_{x_A} \quad (31)$$

molecule level corresponds a certain change, either a configuration or thermal change of the entropy function at the thermodynamic level, according to the scheme: it is worth noting the equivalence between ideal dilution  $d_{id(A)}$  in eq 9 and temperature  $T$  in eq 10, a property that has been experimentally observed<sup>1–4</sup> as thermal equivalent dilution (TED). The ergodic theory assumes the equivalence between density entropy (dispersion in space, as a function of  $x_A$ ) and intensity entropy (dispersion in time, as a function of  $\tau_m$ )<sup>2</sup> at the molecule level: we should find an analogous equivalence between configuration ( $(dS_{\text{Dens}})_T = (R \, d \ln d_{id(A)})_T$ ) and thermal ( $(dS_{\text{Ints}})_d = (C_{p,A} \, d \ln T)_d$ ) changes of the thermodynamic function entropy at the mole level. This equivalence can be verified by controlling (Table S2 in the Supporting Information, Appendix H) the perfect equivalence of the changes in the properties of the system either by changing ideal dilution  $d_{id(A)}$  or by changing temperature  $T$ .

**2.2. Ergodic Algorithmic Model (EAM): “Convolved” Binding Functions.** We have shown<sup>5</sup> that, starting from the dual-structure partition function {DS-PF}

$$\begin{aligned} & \{(\exp(-\Delta H_{\text{mot}}/RT))x(\exp(\Delta S_{\text{mot}}/R)) \cdot (\exp(-\Delta H_{\text{th}}/RT))\} \\ & \quad x(\exp(\Delta S_{\text{th}}/R))\} \\ & = K_{\text{dual}} \end{aligned} \quad (11)$$

of the probability space, valid for every hydrophobic hydration process, we can pass to the thermodynamic space by taking the logarithms of each factor in eq 11. In such a way, we can develop an ergodic algorithmic model (EAM) consisting of a set of mathematical relationships (eqs 12–31) reported in Table 1.

The binding functions  $R \ln K_{\text{dual}} = \{f(1/T) \times g(T)\}$  and  $RT \ln K_{\text{dual}} = \{f(T) \times g(\ln T)\}$  are “convoluted” functions. Convolution is a mathematical operation on two functions,  $f$  and  $g$ , to produce a third function  $C\{f \times g\}$ , which expresses how the shape of one  $f$  function is modified by the corresponding  $g$  function. The convolution of the two binding functions of the ergodic algorithmic model (EAM) is generated by the dual-structure partition function

$$\{\text{DS} - \text{PF}\} = \{\text{M} - \text{PF}\} \cdot \{\text{T} - \text{PF}\}$$

The primary function  $f(1/T)$  in the convolution  $\{f(1/T) \times g(T)\}$  or the primary function  $f(T)$  in the convolution  $\{f(T) \times g(\ln T)\}$ , representing the modifiable function  $f$ , are generated by {M-PF}, whereas the secondary function  $g(T)$  in the convolution  $\{f(1/T) \times g(T)\}$  or the secondary function  $g(\ln T)$  in the convolution  $\{f(T) \times g(\ln T)\}$ , representing the modifying function  $g$  of the convolution, are generated by {T-PF}. The simple modifiable functions  $f(1/T)$  and  $f(T)$ , prior to modification, are linear functions, whereas the resulting convoluted functions come out to be parabolic curves. The convoluted binding functions of class A processes are convex (see Figure S8 in the Supporting Information, Appendix F) ( $\Delta C_{p,\text{hydr}} > 0$ ), whereas the convoluted binding functions of class B processes are concave (see Figure S9 in the Supporting Information, Appendix F).

An analogous couple (see Figure S9 in the Supporting Information, Appendix F) of concave binding functions is obtained for hydrophobic hydration processes of class B ( $\Delta C_{p,\text{hydr}} < 0$ ). We recall that reaction A  $\{\xi_w W_I (\text{solvent}) \rightarrow \xi_w W_{II} (\text{iceberg})\}$  with phase transition of water from solvent ( $W_I$ ) to iceberg ( $W_{II}$ ) takes place in the hydrophobic hydration processes of class A, whereas reaction B  $\{-\xi_w W_{II} (\text{iceberg}) \rightarrow \xi_w W_I (\text{solvent})\}$  with the opposite phase transition takes place in the hydrophobic hydration processes of class B. The type of curvature has been determined by analyzing the diagrams with the values of  $R \ln K_{\text{dual}}$  (or other equivalent potential parameter) reported as the function of  $1/T$ . The shape of these curves as  $f(1/T)$  repeats exactly the shape of the function representing the variation of  $R \ln K_{\text{dual}}$  as the function of reciprocal dilution (thermal equivalent dilution, TED).

TED represents the first example of experimental determination of an ergodic property: equivalence between density entropy, dependent upon the “space” variable, and intensity entropy, dependent upon the “time” variable.

We have calculated the coefficients of the binding functions of eqs 26 and 27 by interpolation of the experimental data. By calculating the derivative in  $\partial(1/T)$  of eq 26, we have shown how, by taking advantage of the  $\Delta C_{p,\text{hydr}}$  constant, we can calculate separately the thermal functions  $\Delta H_{\text{th}}$  and  $\Delta S_{\text{th}}$  from

the slopes of eqs 28 and 29 (see Figures S10 and S11 in the Supporting Information, Appendix F). Then, we can obtain, by subtraction of the thermal functions from  $\Delta H_{\text{dual}}$  and  $\Delta S_{\text{dual}}$  the intercepts at  $T = 0$  and at  $\ln T = 0$ , respectively. The intercepts are the respective motive functions  $\Delta H_{\text{mot}}$  and  $\Delta S_{\text{mot}}$ . These motive functions, in their turn, are independent from the temperature, and they will be shown to depend on iceberg functions, that is, on the solute enthalpy and entropy changes associated to formation or reduction of the iceberg.

These equalities indicate that the observed total probability factor  $K_{\text{dual}} = (K_{\text{mot}} \cdot \zeta_{\text{th}})$  with  $\zeta_{\text{th}} = 1$ , experimentally determined, is compatible with the product partition function of eq 11. We choose the dual-structure partition function {DS-PF} as the correct function, suited to the interpretation of the experimental data in every hydrophobic hydration process.

The ergodic algorithmic model (EAM) demonstrates that the assignment of distinct partition functions for the solvent and solute in diluted solutions of hydrophobic hydration systems is the correct choice for biphasic systems. The validity of the dual structure of the partition function is shown by the explicit calculations of the network of convoluted curved binding functions, conforming to the EAM and matching the experimental determinations of the respective chosen potential parameter, measured at different temperatures.

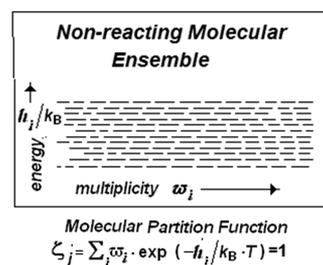
A similar composite structural scheme had been assumed for simulations by Pratt and Rempe.<sup>7</sup> They had, some years ago, a correct insight of the problem of heterogeneity of the hydrophobic systems without stating explicitly of a biphasic character. The distinctions introduced by Pratt and Rempe<sup>7</sup> include (a) a defined proximal volume around the solute, belonging to the solute, that is treated explicitly by the calculations, (b) the solute conformational fluctuations prescribed by statistical thermodynamics and the proximal volume fluctuating if the solute conformation fluctuates, and (c) the interactions of the systems with more distant, extra system solution species treated by approximate physical theories such as dielectric continuum theories. Translated into EAM language, these assumptions mean that (a) solute and the "defined proximal volume" identify with the reacting phase concerning the motive partition function {M-PF}, (b) the volume of the iceberg is variable (i.e., it is fluctuating) with passage of water molecules  $W_{\text{II}}$  (iceberg) to  $W_{\text{I}}$  (solvent) or vice versa (passage of state) concerning {T-PF}, and (c) the variable volume of the excess solvent produces changes in the thermodynamic properties of the solute concerning {M-PF}. These important discoveries by Pratt and Rempe<sup>7</sup> have been nullified by prejudices: (a) a single homogeneous partition function was necessary and enough to describe the whole inhomogeneous system, and (b) the whole system, which is ruled by Boltzmann statistics, is consequently suitable for computer statistical simulations. We recall the point that, according to the EAM, the potential function  $R \ln Z_{\text{M}}$  of the reacting molar phase is ruled by binomial mathematical distribution and can be determined by experiments, whereas the thermal partition function is ruled by statistical distribution.

**2.3. Nonreacting Ensemble {T-PF} and Reacting Ensemble {M-PF}.** The ergodic algorithmic model<sup>5</sup> is founded on the assignment of the dual-structure partition function {DS-PF} of eq 11 to each hydrophobic hydration process. The product partition function

$$K_{\text{dual}} = \exp(-\Delta G_{\text{mot}}/RT) \cdot \exp(-\Delta G_{\text{th}}/RT) \quad (32)$$

is the multiplication of a motive partition function, {M-PF} =  $\exp(-\Delta G_{\text{mot}}/RT) = K_{\text{mot}}$  times a thermal partition function {T-PF} =  $\exp(-\Delta G_{\text{th}}/RT) = \zeta_{\text{th}} = 1$ . The dual-structure partition function {DS-PF} is a product, valid for every hydrophobic hydration process. The motive partition function ({M-PF} =  $\exp(-\Delta G_{\text{mot}}/RT) \neq 1$ ) is referred to the solute, whereas the thermal partition function (i.e., {T-PF} =  $\exp(-\Delta G_{\text{th}}/RT) = 1$ ) is referred to the solvent. {M-PF}, concerning the reacting solute, can give rise to changes of configuration entropy (or density entropy), whereas {T-PF}, concerning the nonreacting solvent, can produce changes of thermal entropy (i.e., intensity entropy) only.

Each partition function, representative of an ensemble in the probability space, gives rise to corresponding functions in the thermodynamic space. The thermal functions enthalpy,  $\Delta H_{\text{th}}$ , and entropy,  $\Delta S_{\text{th}}$ , concerning the solvent, are referred to a nonreacting molecule ensemble (NoremE) of molecules, which is characterized (Figure 5) by enthalpy microlevels



**Figure 5.** The population of a NoremE system is distributed over a unique set of microlevels of enthalpy  $h_i/k_B$ , narrowly spaced. The molecules are moving continuously from one microlevel to another.

( $h_i$ ) very narrowly spaced with interlevel separation of the order of magnitude  $k_B T$  on the molecule scale where  $k_B$  is the Boltzmann constant ( $k_B = 1.3806 \times 10^{-23} \text{ J K}^{-1}$ ). The NoremE is independent from concentration or dilution so that only thermal changes of entropy can be produced ( $(dS)_{x=1} = C_p \ln T$ ). The NoremE ensemble is represented in the probability space by

$$\{T - PF\} \zeta_{\text{th}} = \sum \omega_i \exp(-h_i/k_B T) = 1 \quad (33)$$

which implies that the summation  $\sum \omega_i \exp(-h_i/k_B T)$  can be factorized in entropy and enthalpy probability factors

$$\Omega \cdot (\eta/T)^{-1} = \exp(\Delta S_{\text{th}}/R) \cdot \exp(-\Delta H_{\text{th}}/RT) = 1 \quad (34)$$

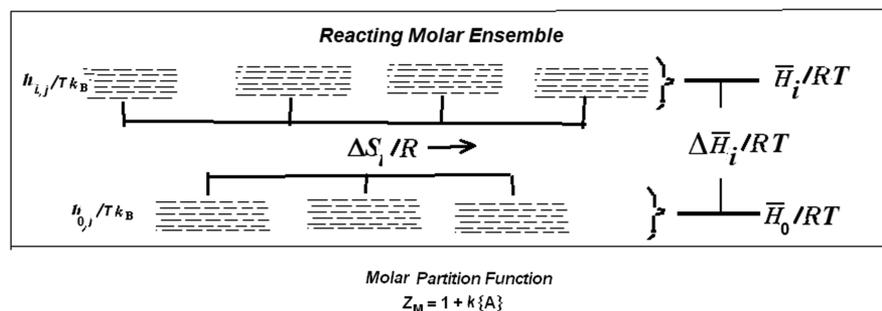
with the ergodic property.  $\Omega$  is the statistical density entropy (in space) and  $\eta/T$  is the statistical intensity entropy (in time). The function  $\Omega$  or the function  $(\eta/T)$  can be calculated by statistical mechanics methods, thus obtaining the Boltzmann entropy for the NoremE by calculation of intensity entropy  $\Omega$  or  $(\eta/T)$ .

$$\Delta S_{\text{th}} \equiv S = k_B \ln \Omega \quad (35)$$

In eq 34, we state the equality of an entropy factor  $\Omega$  with an enthalpy factor  $(\eta/T)$ : this means that NoremE is assumed to be ergodic because, by calculating the multiplicity in space  $\Omega$  (i.e., density entropy), we obtain the intensity entropy  $(\eta/T)$ , that is, dispersion in time.

The NoremE is characterized in the thermodynamic space by the property

$$\Delta H_{\text{th}}/T + \Delta S_{\text{th}} = \Delta G_{\text{th}}/T = 0 \quad (36)$$



**Figure 6.** The mole population of the REME is distributed over a set of macrolevels  $\langle H_i/RT \rangle$ . Each macrolevel, representing the enthalpy macrolevel of each chemical species, distributed over  $\Delta S_i/R$  multiple cells consists of a set of molecule microlevels  $\langle h_{i,j}/T k_B \rangle$ , and each of which, at the mole level, give a mean value of  $\langle H_i/RT \rangle$ . The interlevel separation  $\Delta H_i/RT$  between macrolevels is much larger than the separation  $\Delta h_{i,j}/k_B T$  between microlevels. Density entropy is represented as energy dispersion over multiple cells on the scale of  $R \ln(1/x_i)$ .

which means that this ensemble is constitutionally and experimentally “ergodic”. This relationship implies that we can calculate the integrals in thermodynamic space

$$(1/T_{\text{up}}) \int_{T=0}^{T_{\text{up}}} \Delta C_{p,\text{hydr}} dT = \Delta H_{\text{th}}/T_{\text{up}} \quad (37)$$

$$\int_{T=1}^{T_{\text{up}}} \Delta C_{p,\text{hydr}} d \ln T = \Delta S_{\text{th}} \quad (38)$$

for which there holds the relation

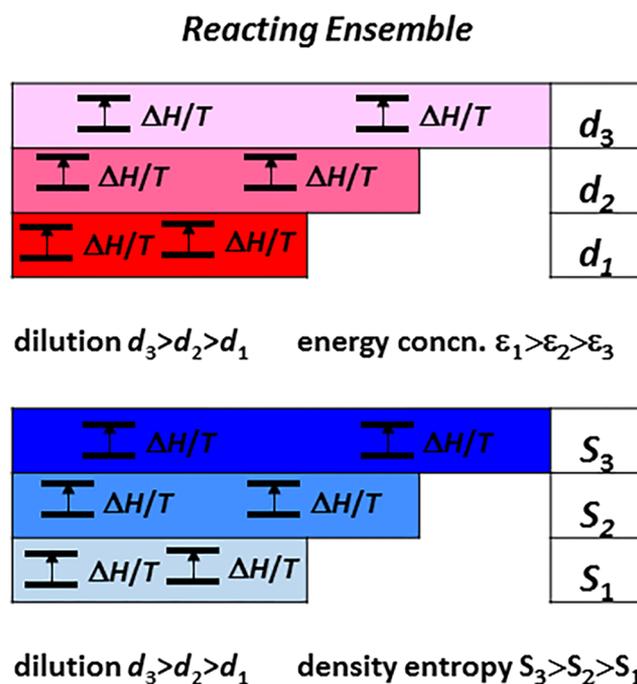
$$(1/T_{\text{up}}) \int_{T=0}^{T_{\text{up}}} \Delta C_{p,\text{hydr}} dT \cong \int_{T=1}^{T_{\text{up}}} \Delta C_{p,\text{hydr}} d \ln T \quad (39)$$

in accordance with eq 36. The symbol “ $\cong$ ”, that is, almost equal, in eq 39 is indicative of the small ineffectual difference in inferior integration limits ( $T = 0$  and  $T = 1$ ) under the condition that  $T_{\text{up}} > 273 \gg 1$ .

The motive thermodynamic function enthalpy,  $\Delta H_{\text{mot}}$  and entropy,  $\Delta S_{\text{mot}}$  are referred to the reacting solute. The solute moles constitute a reacting system represented by a reacting mole ensemble (REME) (Figure 6) where the difference  $\Delta H_i$  between macrolevels is, on the mole scale, a multiple of  $RT$ . This ensemble is at variable entropic multiplicity  $\Delta S_i$  on the mole scale of the order of multiples of  $R \ln x_A$ . The REME is constituted by few elements (moles). Approximations typical of Boltzmann statistics (e.g., Stirling approximation) cannot be applied to this small size set, and the REMEs cannot be calculated by computer statistical simulations but only by mathematical distributions.

Dispersion over the cells by dilution (Figure 6) yields an increase of density entropy. On the other hand, we recall the point that distribution of molecules (not moles!) over the microlevels ( $h_{i,j}$ ) of each  $H_i$  macrolevel follows the thermal statistical distribution with the molecule thermal factor (mtf):  $\varphi = f(\tau_m^2) = T^{-(C_p, A/k_B)}$ , corresponding, in the mole thermodynamic space, to the Lambert thermal energy factor (THEF):  $\Phi = T^{-(C_p, A/R)}$ . The Lambert THEF transforms each solute molecule into a carrier of energy, so that dilution of solute means dilution of energy also. Being density entropy directly proportional to energy dilution, by measuring solute dilution we are measuring density entropy as well (Figure 7).

**2.4. From Chemical Thermodynamics to Statistical Thermodynamics.** The REME, representing the reacting components, is composed of few elements, and it is not ruled by Boltzmann statistics. Because of the limited number of elements, a mole partition function  $Z_M$  cannot be calculated by statistical mechanics methods, rather by numerical methods of



**Figure 7.** Moles of the REME ensemble are dispersed (dilution) over solvent cells. (Upper diagram) decrease of energy concn ( $\epsilon_1 > \epsilon_2 > \epsilon_3$ ); red color intensity proportional to energy concn. (Lower diagram) increase of density entropy; blue color intensity proportional to density entropy.

type HYPERQUAD<sup>8</sup> by interpolation of titration data or by quasi-chemical approximation,<sup>9</sup> now renamed the chemical molecule/mole scaling function (Che. m/M. sF).

This point deserves a special comment because it concerns the relationships between chemical and statistical thermodynamics.

In conclusion, we can state that computer simulations are inadequate to reproduce the motive partition function  $\{M\text{-PF}\}$ , which is dependent on three distinct quantum level functions  $\exp(-\Delta G_{\text{mot}}/RT)$ ,  $\exp(-\Delta H_{\text{mot}}/RT)$ , and  $\exp(\Delta S_{\text{mot}}/R)$ . The essential information produced by the difference between  $\exp(-\Delta H_{\text{mot}}/RT)$  and  $\exp(\Delta S_{\text{mot}}/R)$ , which is specific for each compound, has been ignored and arbitrarily cancelled in the simulation.

The statement that computer simulations are not appropriate deserves a comment. With reference to the equation

$$\exp(-\Delta G_{\text{mot}}/RT) = \exp(-\Delta H_{\text{mot}}/RT) \cdot \exp(\Delta S_{\text{mot}}/R) \quad (40)$$

we observe that, if we plot the expression of eq 40 in an orthogonal diagram with abscissa  $x(\exp(-\Delta H/RT))$  and ordinate  $y(\exp(\Delta S/R))$ , we obtain an equilateral hyperbola (see the Supporting Information, Appendix D). A set of expressions  $\exp(-\Delta G/RT) = f(x,y)$  with increasing values  $(-\Delta G/RT)$  are represented by a set of homologous hyperbolas. On the diagonal coplanar axis  $z$ , we read the numerical scale of  $\exp(-\Delta G/RT)$ . If we determine or computer-simulate the numerical value of  $(-\Delta G/RT)$ , we are choosing one precise hyperbola on the scale. Then, if we determine separately the value of abscissa  $x(\exp(\Delta S/R))$  and the value of ordinate  $y(\exp(-\Delta H/RT))$ , we choose a point on that hyperbola, thus reaching all the information on elements disposable. If we do not introduce specific values of the two factors, the intensity entropy factor  $(-\Delta H_{\text{mot}}/T)$  and density entropy factor  $(\Delta S_{\text{mot}})$ , specific for each compound, we cannot define exactly the point inherent in that process. This essential information element is lost by computer simulations.

The most direct way to recover the fundamental information necessary to get the correct expression for free energy  $[(-\Delta G_{\text{mot}}/RT) = (-\Delta H_{\text{mot}}/RT) + (\Delta S_{\text{mot}}/R)]$  is the experimental determination of the equilibrium constant  $R \ln K_{\text{dual}}$  at five or six different temperatures to obtain, by processing the data by the ergodic algorithmic model (EAM), the curved convoluted binding functions  $R \ln K_{\text{dual}} = \{f(1/T) \times g(T)\}$  and  $RT \ln K_{\text{dual}} = \{f(T) \times g(\ln T)\}$ . These convoluted binding functions contain in them the information necessary to solve the problem.

In eq 48, we state the fundamental connection of statistical thermodynamics to the experimental data of chemical thermodynamics for hydrophobic hydration processes.

An analogous mathematical procedure that transforms the set of statistical partition functions  $Z(N, V, T)$ , each referred to one macrolevel  $H_i$ , composed of a population of  $h_{i,j}$  microlevels, into a polynomial at the mole level is the quasi-chemical theory.<sup>9,11</sup> The quasi-chemical approximation (actually, it is not an “approximation”; rather, it is a correct fitting procedure) should be more appropriately named as the chemical molecule/mole scaling function, (Che. m/M. sF), representing a fundamental breakthrough in the connection of statistical thermodynamics with chemical thermodynamics. The Che. m/M. sF scaling function does not assume that it is possible to extract information from the thermal partition function {T-PF} of the solvent to calculate free energy and employs the stoichiometry  $\pm \xi_w$  of a chemical reaction as “additional information” element to build up the motive partition function {M-PF} of the solute. In addition, the ergodic algorithmic model (EAM) guarantees that the stoichiometric information  $\pm \xi_w$ , calculated by  $\Delta C_{p,\text{hydr}}$  is pertinent to that specific reaction because it has been extracted from the experimental data through a thermal equivalent dilution (TED) analysis.

**2.5. Dual-Structure Partition Function.** The correct interpretation of the thermodynamic behavior of hydrophobic hydration processes is based on an ergodic algorithmic model (EAM). This model consists of the dual-structure partition function {DS-PF}, which is a product function, constituted by a thermal partition function {T-PF} multiplied by a motive partition function {M-PF}:

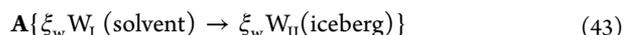
$$\{\text{DS} - \text{PF}\} = \{\text{T} - \text{PF}\} \cdot \{\text{M} - \text{PF}\} \quad (41)$$

The dual-structure partition function {DS-PF} in an explicit mathematical format results in being

$$\begin{aligned} \exp(-\Delta G_{\text{dual}}/RT) &= K_{\text{dual}} = (K_{\text{mot}} \cdot \zeta_{\text{th}}) \\ &= \{(\exp(-\Delta H_{\text{mot}}/RT))(\exp(\Delta S_{\text{mot}}/R))\} \\ &\cdot \{\exp(-\Delta H_{\text{th}}/RT)\exp(\Delta S_{\text{th}}/R)\} \end{aligned} \quad (42)$$

By mathematical development of the exponential partition function {DS-PF}, we get a very essential mathematical representation of the properties of each hydrophobic hydration process.

The types of hydrophobic hydration processes examined by means of the ergodic algorithmic model are very different from one another in molecular size, ranging from small gaseous molecules to macromolecular proteins and from monocarboxylic acids to micelles: for every class A process, the reaction



with iceberg formation has been observed, and for every class B process, the reaction



with iceberg reduction (i.e., water phase transition iceberg-to-solvent = reduction of iceberg) has been observed.  $\xi_w$  is an absolute number, indicating the pseudo-stoichiometric number of clusters of  $W_{\text{I}}$  or (i) melted in class A from the solvent to form the iceberg clusters of  $W_{\text{II}}$  or (ii) condensed in class B into the solvent  $W_{\text{I}}$  to reduce the iceberg. The size of the iceberg is proportional to the size of the incoming molecule for small solute molecules or to the size of the branching moiety in denaturing macromolecules. The iceberg can be considered as a soft niche in the structure of the solvent where a solute can enter in. The number  $\xi_w$  does not correspond, in general, to an integer number and is described pseudo-stoichiometric because it is not indicative of an integer number of molecular or atomic units; rather, it indicates the ratio between volume  $V_{W_{\text{II}}}(\text{iceberg})$  entering the unit and volume  $V_{W_{\text{I}}}$  of one cluster ( $W_{\text{I}}$ ). The ratio between volumes is not, in general, an integer number. The only reaction taking place at constant potential in the solvent for class A is melting of  $\xi_w W_{\text{I}}$  clusters, forming  $\xi_w W_{\text{II}}$  iceberg clusters. As a state transition, each melting reaction yields an entropy change

$$\Delta C_{p,\text{hydr}} = \xi_w C_{p,w} = \Delta H_{\text{hydr}}/T \quad (45)$$

that, in analogy with Trouton's rule, is a constant. For Trouton's rule, the ratio between evaporation heat and boiling temperature  $\Delta H_{\text{eb}}/T_{\text{eb}}$  is a constant entropy change for evaporation in many liquids,  $\Delta H_{\text{eb}}/T_{\text{eb}} = \Delta S_{\text{evap}} = +86.9 \pm 1.4 \text{ J K}^{-1} \text{ mol}^{-1}$ . In class B,  $-\xi_w W_{\text{I}}$  water clusters condense, thus producing iceberg reduction with transformations exactly opposite of those in class A. The value at evaporation for liquids of  $\Delta S_{\text{evap}} = +86.9 \pm 1.4 \text{ J K}^{-1} \text{ mol}^{-1}$  is coincident by changing the sign with the extrapolated value at a null iceberg,  $\Delta S_0^{(\xi_w=0)} = -86.4 \text{ J K}^{-1} \text{ mol}^{-1}$ , common to many gases. This coincidence, involving so many elements, cannot be by chance. It means that the intensity entropy change at the trapping of gas in water is equal, but with an opposite sign, to the intensity entropy change  $\Delta S_{\text{evap}}$  of the passage of a liquid to the vapor phase. Alternatively, it is equal to the entropy change  $\Delta S_{\text{cond}}$  of a vapor condensing to the liquid phase. We observe that both entropy changes  $\Delta S_{\text{evap}}$  and  $\Delta S_{\text{cond}}$  are intensity entropy changes with opposite signs ( $\Delta S_{\text{evap}} = \Delta S_{\text{Int}} > 0$  and  $\Delta S_{\text{cond}} =$

$\Delta S_{\text{int}} < 0$ ) due to velocity gain or velocity loss by the molecules, respectively.

We call the attention of the reader on the peculiar property of the hydrophobic isobaric heat capacity  $\Delta C_{p,\text{hydr}}$  which is different from the usual heat capacity  $C_p$ . The heat capacity  $C_p$  is usually attributed to the distribution of energy over translational, vibrational, and rotational motions of the molecules. The different energy levels corresponding to each kind of motion can be reached successively by increasing the temperature: in such a way,  $C_p$  is very often variable with temperature  $T$ . In contrast,  $\Delta C_{p,\text{hydr}}$  as the thermal intensity entropy change can be attributed only to changes of translational motions of water molecules ( $W_{\text{II}}$ ), produced by the passage of state of clusters of  $W_{\text{I}}$  of the solvent to icebergs of  $W_{\text{II}}$  of the solute. The water molecules of  $W_{\text{I}}$  are moving from the resting state of the liquid solvent to the moving state of the solute in the solution as  $W_{\text{II}}$  by acquiring thermal intensity entropy (we remind that intensity entropy changes cannot contribute to reaction free energy).

It is worth noting also that, in class A, the melting units of  $\xi_w W_{\text{I}}$  from the solvent are transformed into icebergs at the very moment of receiving the incoming molecule. The process of iceberg formation from the solvent at constant potential generates a change in the thermodynamic potential of the solute because the transformation of  $\xi_w W_{\text{I}}$  clusters of the solvent into icebergs of  $\xi_w W_{\text{II}}$  of the solute means reduction of the solvent volume (the solvent identifies with  $W_{\text{I}}$ ) with a consequent concentration of the solute. The process of iceberg formation is characterized by a large negative unitary density entropy change, indicating an increase of solute concentration (or diminution of dilution and hence diminution of density entropy) and the reduction of solvent volume<sup>6</sup>

$$\langle \Delta s_{\text{for}} \rangle_{\text{A}} = -445 \pm 3 \text{ J K}^{-1} \text{ mol}^{-1} \xi_w^{-1} \text{ with } \sigma = \pm 0.7\% \quad (46)$$

In class B,  $-\xi_w W_{\text{I}}$  water clusters, condensing into the solvent at constant potential, produce an increase of solvent volume with dilution of the solute, thus increasing the density entropy of the solute<sup>6</sup>

$$\langle \Delta s_{\text{red}} \rangle_{\text{B}} = +432 \pm 4 \text{ J K}^{-1} \text{ mol}^{-1} \xi_w^{-1} \text{ with } \sigma = \pm 0.9\% \quad (47)$$

The calculations, concerning motive functions, to get the unitary values  $\langle \Delta s_{\text{for}} \rangle_{\text{A}}$  and  $\langle \Delta s_{\text{red}} \rangle_{\text{B}}$  have been applied<sup>6</sup> to approximately 80 hydrophobic processes of very different molecular sizes and very different condensation states with approximately 600 data points. The mean unitary values (unitary means referred to  $\xi_w = \pm 1$  water cluster  $W_{\text{I}}$ ) reported above show variability within the limits of experimental errors, presenting in such a way a very good statistical validation of the ergodic algorithmic model over a statistically significant population of experimental data, as shown in detail in part III<sup>6</sup> of this series. The agreement between mean values  $\langle \Delta s_{\text{for}} \rangle_{\text{A}}$  and  $\langle \Delta s_{\text{red}} \rangle_{\text{B}}$  reported above with significant very low variability is reinforced by the numerical agreement between the condensation Trouton constant  $\Delta S_{\text{conds}} = -86.9 \pm 1.4 \text{ J K}^{-1} \text{ mol}^{-1}$  and the extrapolated value at the null iceberg for a set of inert gases,  $\Delta S_0^{(\xi_w=0)} = -86.4 \text{ J K}^{-1} \text{ mol}^{-1}$ .

After so many promising results confirming the reliability of the ergodic algorithmic model (EAM), we have raised the problem of comparing the data obtained by us with the results of other experimental determinations of thermodynamic properties of other systems and the many computational

works now frequently appearing in the physical chemistry journals.

**2.6. Thermal Partition Function {T-PF}. 2.6.1. Enthalpy–Entropy Compensation.** The first problem considered by us, concerning general molecular properties of hydrophobic hydration processes, has been the discussion of enthalpy–entropy compensation, largely debated in the literature, as shown by the many papers that have been appearing over the past 50 years. No author is completely convinced that such compensation corresponds to definite thermodynamic changes of the structure of the solute. The scientists are skeptical on this point. The skepticism is revealed by a report in Wikipedia under the heading “enthalpy–entropy compensation”: “The existence of any real compensation effect has been widely ‘derided’ in recent years and attributed to the analysis of interdependent factors and chance.” According to Gallicchio, Kubo, and Levy,<sup>13</sup> in the phenomenon of entropy–enthalpy compensation, the transformations, when carried out on a computer by free-energy perturbation simulations, must be dubbed as “computational alchemy”. Sometimes, the title itself of the paper reveals uncertainty as shown in “Entropy–Enthalpy compensation: Fact or Artifact?” by Sharp.<sup>14</sup>

None of the many articles that have been appearing in the literature over the past 50 years has mentioned a possible essential contribution to entropy–enthalpy compensation by both thermal enthalpy and thermal entropy. For the ergodic algorithmic model (EAM), the following equality holds

$$\Delta H_{\text{th}}/T \approx \Delta S_{\text{th}} \quad (48)$$

which is itself an entropy–enthalpy ergodic compensation as

$$\Delta H_{\text{th}}/T - \Delta S_{\text{th}} = 0 \quad (49)$$

The relevance of the high contribution of  $\Delta H_{\text{th}}$  and  $T\Delta S_{\text{th}}$  from eq 73 to compensation can be appreciated if we recall that the two thermal functions are calculated as

$$\Delta H_{\text{th}} = \Delta C_{p,\text{hydr}} \cdot T = \pm \xi_w C_{p,w} \cdot T \quad (50)$$

and

$$\Delta S_{\text{th}} = \Delta C_{p,\text{hydr}} \cdot \ln T = \pm \xi_w C_{p,w} \cdot \ln T \quad (51)$$

where the heat capacity of water is  $C_{p,w} = 75.36 \text{ J K}^{-1} \text{ mole}^{-1}$  and the absolute number  $\xi_w$  can be as high as 120 in some proteins. The ratio between the two functions gives

$$(\xi_w C_{p,w} \cdot dT) / (\xi_w C_{p,w} \cdot d \ln T) = dT / d \ln T = dT / (dT/T) = T \quad (52)$$

This assessment of entropy–enthalpy compensation based on the special structure of the thermal partition function {T-PF} concerning the solvent represents a further validation of the ergodic algorithmic model and a condemnation of all the theories and speculations on the molecular origin of entropy–enthalpy compensation, other than those implicit in eq 48.

**2.6.2. Null Thermal Free Energy.** Lee and Graziano<sup>15</sup> expressed the opinion that, in biochemical processes, there are some side reactions where enthalpy and entropy compensate for each other and do not influence the free energy. The same hypothesis had been launched by Benzinger.<sup>16</sup> Thermal enthalpy,  $\Delta H_{\text{th}}$ , and thermal entropy,  $\Delta S_{\text{th}}$ , satisfy the conditions foreseen by these authors.

The analysis of the function free energy, enthalpy, and entropy in hydrophobic hydration processes as development of the dual-structure partition function {DS-PF} with distinction into motive and thermal functions has made possible a

comparison of the properties of the thermal functions of {T-PF} with functions reported in the literature for the thermal properties of hydrophobic hydration processes. We have verified that the thermal free energy is erroneously considered different from zero in some textbooks<sup>17</sup> and articles. No mention of the motive functions is seen in these texts, missing another essential feature of these reactions. The formulas reported in the literature concerning the functions  $\Delta H(T)$ ,  $\Delta S(T)$ , and  $\Delta G(T)$  for protein unfolding, and for micelle formation are reported in the Supporting Information, Appendix F. The formulas<sup>17</sup> reported in the literature to calculate thermal free energy  $\Delta G(T)$  as different from zero are erroneous, as shown in the Supporting Information, Appendix G.

Even Kronberg,<sup>18</sup> discussing the origin of the hydrophobic effect (HE), considers the compensation between enthalpy and entropy as significant, although the HE does not exclude the existence of a certain portion of residual active thermal free energy. In principle, any active thermal free energy is absolutely excluded (see eq 69) by the ergodic algorithmic model (EAM).

We would like to underline that this identification of the solvent as a nonreacting separate phase has been possible because of the introduction of distinct partition functions for the solvent with the intensity entropy thermal probability factor and solute with the density entropy motive probability factor. The null free energy is a constitutional invariable property of every nonreacting molecule ensemble (NoremE) (see Figure 5).

We will show (see Section 2.9, Figure 11, below) how the distinction into thermal and motive functions can help us solve one of the most debated problems of biochemical thermodynamics: the interpretation of cold denaturation of proteins. Having established that motive functions only contribute to free energy, the problem of determining the cold denaturation consists of determining the conditions of temperature at which the motive free energy  $\Delta G_{\text{mot}}$  becomes nil; above that temperature with  $\Delta G_{\text{mot}} < 0$ , the folded protein is the stable form, whereas below that temperature with  $\Delta G_{\text{mot}} > 0$ , the protein denatures.

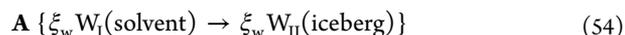
**2.7. Iceberg Formation/Reduction.** **2.7.1. {T-PF}: Solvent and Niche for the Iceberg.** The topics concerning “iceberg formation” or “iceberg reduction” are particularly suited to show the differences between the dual-structure partition function {DS-PF} and statistical mechanics calculations. Pohorille and Pratt<sup>22</sup> have analyzed the problem of the cavities in molecular liquids in connection with hydrophobic compound solubility. Other researchers have put the question how the icebergs proposed in our model were comparable with the cavities found by statistical calculations. The cavities considered by Pohorille and Pratt<sup>22</sup> and by Pratt and Chandler<sup>27</sup> theory, such as cavities obtained by statistical calculations, concern NoremEs; the thermodynamic properties of these cavities are assumed to be calculated from the partition function {T-PF} of the solvent, and the thermal partition function {T-PF} represents the thermodynamic properties of the solvent in hydrophobic hydration processes. Thermal enthalpy and thermal entropy concerning {T-PF} do not contribute to free energy because thermal free energy is invariably zero, being  $-\Delta G_{\text{th}}/T = -\Delta H_{\text{th}}/T + \Delta S_{\text{th}} = 0$  with the solvent at constant potential. In conclusion, {T-PF} cannot generate any potential function  $\Delta\mu$  because  $-\Delta G_{\text{th}}/T = 0$  invariably. The thermodynamic functions for iceberg for-

mation/reduction must be searched for and found as development of the motive partition function {M-PF} without any reference to the thermal partition function of the solvent. We remind once again that thermal entropy  $\Delta S_{\text{th}}$  cannot contribute to free energy and cannot be employed to calculate thermodynamic potentials (see the Supporting Information, Appendix G). On the other hand, we have shown how, according to the ergodic algorithmic model (EAM), the experimental dual functions  $\Delta H_{\text{dual}}$  and  $\Delta S_{\text{dual}}$  can be subdivided into motive and thermal components, reflecting the biphasic structure of these solutions. The motive entropy  $\Delta S_{\text{mot}}$  contributes to free energy as well as  $\Delta H_{\text{mot}}$  whereas the functions  $\Delta S_{\text{th}}$  and  $\Delta H_{\text{th}}$  do not contribute to free energy. The motive entropy  $\Delta S_{\text{mot}}$  can be obtained as the slope from the equation

$$-\Delta G_{\text{mot}} = -\Delta H_{\text{mot}} + T\Delta S_{\text{mot}} \quad (53)$$

if motive free energy  $-\Delta G_{\text{mot}} = RT \ln K_{\text{mot}}$  (which is a simple primary function and not a “convolution”) is reported as a function of  $T$ . Otherwise, the motive entropy  $\Delta S_{\text{mot}}$  can be calculated by extrapolation of  $\Delta S_{\text{dual}}$  to  $\ln T = 0$ .

Further essential information elements can be achieved by analyzing the motive functions.<sup>6</sup> The unitary density entropy in processes of class A,  $\langle \Delta s_{\text{for}} \rangle_{\text{A}} = -445 \pm 3 \text{ J K}^{-1} \text{ mol}^{-1} \xi_{\text{w}}^{-1}$ , is calculated for every compound from the slope of the motive function, concerning the solute,  $\Delta S_{\text{mot}} = f(\xi_{\text{w}})$ , reported as the function of the pseudo-stoichiometric number  $\pm \xi_{\text{w}}$ . The negative unitary entropy change  $\langle \Delta s_{\text{for}} \rangle_{\text{A}}$  for class A reported above indicates a density entropy loss by the solute, not by the solvent. Iceberg formation represents for the solvent a change of phase at constant potential with a decrease of solvent volume for the reaction



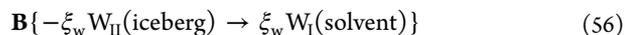
with change in intensity entropy

$$dS_{\text{th}} = \Delta C_p d \ln T = (\Delta C_p dT)/T = dH_i/T \quad (55)$$

$dS_{\text{th}} \equiv dS_{\text{Ints}}$  as the Intensity Entropy change does not contribute to free energy; the density entropy change  $\langle \Delta s_{\text{for}} \rangle_{\text{A}}$  cannot be of concern of the solvent but rather of the thermodynamic properties of the solute.

**2.7.2. {M-PF}: Solute and Iceberg Formation/Reduction.** We call again the attention of the reader on the molecular and thermodynamic process taking place at iceberg formation. Iceberg formation generates a change of density entropy of the solute: iceberg formation corresponds to a decrease in volume of the solvent ( $\Delta V_{\text{solvent}} < 0$ ) thus generating a higher concentration of the solute and a density entropy loss by the solute (not by the solvent, which is at constant potential).

Analogously, the process of iceberg reduction, by the reaction



implying condensation of cluster  $W_{\text{I}}$ , leads to an expansion of solvent volume ( $\Delta V_{\text{solvent}} > 0$ ) with dilution of the solute and a corresponding increase of density entropy by the solute. The unitary entropy function for iceberg reduction<sup>6</sup>  $\langle \Delta s_{\text{red}} \rangle_{\text{B}} = +432 \pm 4 \text{ J K}^{-1} \text{ mol}^{-1} \xi_{\text{w}}^{-1}$  indicates a positive gain of density entropy by the solute (not by the solvent, which is kept at constant potential).

The changes of density entropy, either positive or negative, belong to changes of the motive function {M-PF} concerning

REME (reacting ensembles), composed of few elements, that cannot be treated by statistical mechanics calculations. Pratt et al.<sup>9,11</sup> have introduced for the purpose of overcoming the gap between NoremEs and REMEs the quasi-chemical approximation, now renamed chemical molecule/mole scaling function (Che. m/M. sF) with dismissal of the word “approximation” (see the Supporting Information, Appendix B).

Some examples of entropy-driven reactions of the solute where the density entropy contribution is produced by  $\langle \Delta s_{\text{red}} \rangle_B$  are the dissociation of carboxylic acids and the formation of hydrophobic bonds (see Figure 12 below). As far as hydrophobic bonds are concerned, on the grounds of the arguments exposed above, the widely convoluted accepted interpretation that the hydrophobic bond should be stabilized because it is entropy-driven by the increase of thermal intensity entropy of the solvent is aberrant. A thermal entropy increase would mean increase of intensity entropy, and as such, it could not contribute to free energy. Intensity entropy cannot in principle stabilize any bond whatsoever, hydrophobic or not hydrophobic. The driving reaction is necessarily associated to a density entropy gain by the reacting solute and not by the solvent, which is at constant potential  $\mu$ .

**2.8. Ergodic Algorithmic Model (EAM) and Free Energy Simulations.** **2.8.1. Free Energy from the Motive Partition Function {M-PF}.** In recent decades, the calculation of free energy by computer simulations has become popular in physical and biophysical chemistry.<sup>10</sup> The purpose of these calculations is the determination of free energy changes using numerical simulations based on the fundamental principles of statistical mechanics. Developments of computational power and of mathematical probability theories have contributed to the advancement of free energy calculations. The result of any computer simulation should be a representation of the thermodynamic potential  $\Delta\mu_A$  and in general a simulation of the motive binding functions  $R \ln K_{\text{mot}} = -\Delta G_{\text{mot}}/T = f(1/T)$  and  $RT \ln K_{\text{mot}} = -\Delta G_{\text{mot}} = f(T)$ , derived from the monocentric partition function {M-PF}. The functions employed in statistical thermodynamics are continuous and not quantum functions as the exponentials of {DS-PF}. The computer simulations do not reproduce the motive binding functions  $R \ln K_{\text{mot}} = -\Delta G_{\text{mot}}/T = f(1/T)$  and  $RT \ln K_{\text{mot}} = -\Delta G_{\text{mot}} = f(T)$  as distinct components of the curved convoluted binding functions  $R \ln K_{\text{dual}} = -\Delta G_{\text{dual}}/T = \{f(1/T) \times g(T)\}$  and  $RT \ln K_{\text{dual}} = -\Delta G_{\text{dual}} = \{f(T) \times g(\ln T)\}$ , found by interpolation of the experimental data determined at different temperatures. The convoluted functions are studied and explained by the ergodic algorithmic model (EAM), as derived from the dual-structure partition function {DS-PF}. The ergodic algorithmic model (EAM) is complementary to computer simulations and very often substitutive of computer simulations because the free energy functions are calculated by the EAM directly from the experimental data and found to be conforming to the curved convoluted functions offered by the EAM. The results of the ergodic algorithmic model (EAM) are highly reliable because they are obtained by mathematical procedures directly from specific experimental data and not guessed as the simulated ones.

Regarding the application of computer simulations to the representation of the thermodynamic properties of hydrophobic hydration processes, we observe that many computer simulations present two decisive drawbacks, which exclude the possibility of using these methods, at least in the formats used

so far, to treat the hydrophobic hydration processes. It is nonsense, in connection to the dual-structure partition function {DS-PF} of hydrophobic hydration processes, to calculate a thermodynamic potential  $\Delta\mu_A = \Delta G_A/n$ , which is a unitary free energy, from thermal entropy function  $\Delta S_{\text{th}}$  belonging to the thermal partition function {T-PF}. We remind that the thermal partition function gives rise to intensity entropy changes, which do not contribute to free energy. The theory of Pratt and Chandler<sup>27</sup> based on the calculation of an oxygen-pair correlation function of water around a cavity is clearly referred to the thermal partition function {T-PF} of the solvent, not contributing to free energy. The distinct behaviors of thermal and motive partition functions depend on the biphasic structure of every hydrophobic hydration solutions with each phase being referred to different statistical ensembles: NoremE for the solvent and REME for the solute. The dual structure of the partition function {DS-PF} gives rise in thermodynamic space to “convoluted” functions with complex mathematical structures. The computer simulations are applicable to Boltzmann ensembles whereby large-number approximations can be safely applied. The number of elements (molecules) of the NoremE, representing the solvent, is very, very large and of the order of some Avogadro numbers ( $N_{\text{Av}} = 6.22 \times 10^{23}$ ), and this makes possible the application of approximations for large numbers to these ensembles. The same approximations cannot be applied to the REME representing the reacting solute: an REME is composed of very few elements (moles) for which mathematical binomial distribution holds instead of Boltzmann statistical distribution. The chemical molecule/mole scaling function (Che. m/M. sF), that is, quasi-chemical approximation,<sup>9,11</sup> must be applied, or alternatively, the ergodic algorithmic model (EAM) can be used. The properties of the motive partition function {M-PF}, referred to a mole population, are suited to rule the progress of the chemical reaction. The concept itself of the chemical reaction means transformation of moles (or fractions of a mole) of a substance, not of molecules. We can experimentally detect a reaction whenever we observe at least a minimum detectable change of fractions of moles. This means that in these cases, we find  $-\Delta G_{\text{mot}} \neq 0$ .

The functions  $\Delta H_{\text{th}}$  and  $\Delta S_{\text{th}}$  as secondary functions  $g(T)$  and  $g(\ln T)$  of the convolutions modify the linear motive binding functions  $R \ln K_{\text{mot}} = f(1/T)$  and  $RT \ln K_{\text{mot}} = f(T)$ , thus determining the curvature amplitudes of the convoluted binding functions at constant  $d_{\text{id}(A)}$ ,  $R \ln K_{\text{dual}} = -\Delta G_{\text{dual}}/T = \{f(1/T) \times g(T)\}$  and  $RT \ln K_{\text{dual}} = -\Delta G_{\text{dual}} = \{f(T) \times g(\ln T)\}$ . The curvature amplitude, referred to constant  $\Delta C_{p,\text{hydr}}$  contains information ( $\pm \xi_w$ ) concerning iceberg formation in class A or iceberg reduction in class B. Losing this information means losing the possibility of distinguishing between the unitary density entropy changes  $\Delta s_{\text{for}}$  or  $\Delta s_{\text{red}}$ , referred to the solute, and the thermal intensity entropy changes, referred to the solvent. Another essential information lost by computer simulations<sup>19</sup> is the distinct values of intensity entropy ( $\Delta H_{\text{mot}}/T$ ) and density entropy ( $\Delta S_{\text{mot}}$ ). The formal ergodic equivalence, assumed in computer simulations, between molecular dynamics methods and Monte Carlo methods is a decisive drawback of computer simulations, leading to the loss of essential information elements (see the Supporting Information, Appendix D).

Even the potential distribution theorem (PDT),<sup>20</sup> because it is not based on a dual-structure partition function {DS-PF} of

a biphasic system, lacks much information on hydrophobic hydration processes and cannot be employed as such to calculate free energy. Both motive functions with the reaction of iceberg formation/reduction in hydrophobic hydration processes must be considered to calculate free energy by the chemical molecule/mole scaling Function (Che. m/M. sF), that is, by quasi-chemical approximation.<sup>9</sup> These quasi-chemical calculations cannot be defined an “approximate”; rather, they are the correct acceptable development of PDT, referred to constant  $\Delta C_{p,\text{hydr}}$  and known  $\pm\xi_w$ . The reaction stoichiometry  $\pm\xi_w$  introduced by quasi-chemical calculations is an additional information element obtained from the thermal partition function {T-PF}, necessary to make the calculations conformed to the biphasic structure of every hydrophobic hydration systems.

**2.8.2. Ergodic Algorithmic Model (EAM) and Theory of the Hydrophobic Effect.** The ergodic algorithmic model (EAM) founded on a dual-structure partition function {DS-PF} as the product of a motive partition function {M-PF} for the solute phase times a thermal partition {T-PF} for the solvent phase

$$\{\text{DS} - \text{PF}\} = \{\text{M} - \text{PF}\} \cdot \{\text{T} - \text{PF}\} \quad (57)$$

offers a sound theoretical basis to the theory of the hydrophobic effect.

The ergodic algorithmic model (EAM) offers a scientific basis for the interpretation of the hydrophobic effects. We observe that the expression  $(-\ln p_n \approx \zeta_0 + \zeta_1 n + \zeta_2 n^2)$  reported by Pratt<sup>23</sup> in the review “Molecular Theory of Hydrophobic Effects” is the equation of a concave parabola, which repeats exactly the shape of the experimental binding function  $RT \ln K_{\text{dual}} = -\Delta G_{\text{dual}} = \{f(T) \times g(\ln T)\}$  for a reaction of class B. The curved shape of the apparent binding function is caused by the thermal component  $\Delta S_{\text{th}}$  as the function of heat capacity  $\Delta C_{p,\text{hydr}}$  (curvature amplitude  $C_{\text{ampl}} = 0.7071/\Delta C_{p,\text{hydr}}$ ). The identification of the potential curve with the binding function  $RT \ln K_{\text{dual}} = -\Delta G_{\text{dual}} = \{f(T) \times g(\ln T)\}$  comprehending thermal functions is confirmed by Pratt<sup>23</sup> who states that the maximum of the potential curve coincides with the point of entropy convergence as determined by Baldwin<sup>25</sup> and Lee<sup>26</sup> for thermal entropy (see eq 18).

$$\Delta S^\circ(T_2) = \Delta S^\circ(T_1) + \Delta C_{p,\text{hydr}} \ln(T_2/T_1) \quad (58)$$

Pratt<sup>23</sup> has not recognized the double dependence on both  $T$  and  $\ln T$  of eq 58 as a property of a convoluted function. We remind once again that thermal entropy  $\Delta S_{\text{th}}$  cannot contribute to free energy and cannot be employed to calculate thermodynamic potentials. The ergodic algorithmic model (EAM) offers a different interpretation of the maximum of the parabolic function. According to the EAM, the concave function is typical of the processes of class B with iceberg reduction (see Figures S2 and S9 in the Supporting Information, Appendices A and F, respectively). In the processes of class B, the maximum corresponds to the temperature  $T$  at which  $\Delta S_{\text{dual}} = \Delta S_{\text{mot}} + \Delta S_{\text{th}} = 0$ , and there is exact compensation between motive entropy,  $\Delta S_{\text{mot}}$ , and thermal entropy,  $\Delta S_{\text{th}}$ ; this means that the point of entropy convergence is at  $\Delta S^\circ(T_1) = -\Delta S_{\text{mot}}$ .

The molecular theory of hydrophobic effects and some parts of the potential distribution theorem (PDT)<sup>20</sup> need revision to become applicable to hydrophobic hydration processes. The distinct behaviors of thermal and motive components are not considered by potential distribution theorem (PDT); this

theorem assumes that the hydrophobic systems are monophasic, and a lot of information is lost. Another fundamental limitation of PDT when applied to hydrophobic hydration systems is that of not considering the possible alternative of a concave or convex curve (alternative class A or class B) as proposed by the ergodic algorithmic model (EAM). The concave/convex alternative or class A/class B alternative, bound to the alternative of either iceberg formation or iceberg reduction with phase transition of water at constant potential, is completely ignored by PDT. The ergodic algorithmic model (EAM) keeps for sure that every hydrophobic hydration system is biphasic. A dual-structure partition function {DS-PF} = {M-PF} · {T-PF} represents the biphasic system. Iceberg formation is a phase transition of  $\xi_w W_I$  from solvent ( $W_I$ ) to solute ( $W_{II}$  (iceberg)), that is, from an element of {T-PF} to an element of {M-PF} with constant potential of  $W_I$ . The iceberg function is not the probability of forming a cavity in the solvent, as assumed by PDT; rather, the iceberg derives from the transformation of portions of the solvent,  $\xi_w W_I$ , into a niche of  $\xi_w W_{II}$ , thus generating a phase transition. This phase transition modifies the solvent volume, which generates changes of the thermodynamic motive potential function {M-PF} of the solute (see Section 2.9 below) but keeps the solvent at constant potential. In other words, if we want to look for the origin of the potential  $\Delta\mu$ , we must search, as a driving force of any hydrophobic hydration processes, among the factors of the motive function  $RT \ln K_{\text{mot}} = f(T)$  (Table 1). We can establish the stoichiometry ( $\pm\xi_w$ ) of the chemical reaction of iceberg formation/reduction from the thermal partition function {T-PF} of the solvent, but the free energy change of the reaction is produced by the motive partition function {M-PF} of the solute. Specifically, in the reactions of class A, the formation of the iceberg reduces the solvent volume and reduces the dilution  $1/x_A$  of the solute, thus modifying the partition function {M-PF} of the solute. By recognizing that the thermal functions  $\Delta H_{\text{th}}$  and  $T\Delta S_{\text{th}}$  cannot contribute directly to free energy, the ergodic algorithmic model (EAM) excludes the possibility that the driving reaction might be found in the solvent. The opposite reaction of iceberg reduction takes place in reactions of class B where the volume of the solvent is expanded, thus increasing the dilution  $1/x_A$  of the solute. On the ground of these transformations of the partition function {M-PF} of the solute, we can state that the choices of Pratt<sup>23</sup> and Pratt and Chandler<sup>27</sup> of calculating the potential  $\Delta\mu$  from the partition function {T-PF} of the solvent or from the oxygen–oxygen correlation function of solvent water are unacceptable. In consequence, we must search for a different explanation, coherent with the ergodic algorithmic model (EAM), of the origin of the changes of potential  $\mu$  within the motive binding functions  $R \ln K_{\text{mot}} = f(1/T)$  and  $RT \ln K_{\text{mot}} = f(T)$  concerning the motive partition function {M-PF} of the solute.

The statement that the potential distribution theorem is unreliable is referred only to the application of the theory to evaluate the potential  $\Delta\mu$  of the solvent in hydrophobic hydration processes, but it is not referred to the whole theory in general. The criticism on the specific point of hydrophobic hydration processes is well founded. The foundations of the potential distribution theorem have been searched for by Pratt<sup>11</sup> et al. in the analogies with the information theory. Some reflections on this choice are in order. The information theory has been invoked by Pratt et al.<sup>11</sup> with reference to the connection between “information-theoretic entropy” and

“thermodynamic entropy” where the thermodynamic entropy is considered a parameter of disorder in chemical systems. This kind of information-entropy disorder can be identified with “energy dispersion in space”, that is, with density entropy. In contrast with this, we have shown that every hydrophobic hydration system is represented by a dual-structure partition function  $\{\text{DS-PF}\} = \{\text{M-PF}\} \cdot \{\text{T-PF}\}$  where the thermal partition function  $\{\text{T-PF}\}$  is representative of the solvent. This thermal partition function of the solvent can give rise to changes of dispersion of energy in time (i.e., to intensity entropy change) at constant potential. It is impossible to extract any potential function change  $\Delta\mu$  from thermal partition function  $\{\text{T-PF}\}$  because changes of  $\Delta\mu$  as a temperature-dependent property of the thermal partition function of the solvent are nonexistent.

The inadequacy of PDT to explain properties of the solvent is confirmed by realizing that so many pretended results obtained by PDT in relation to hydrophobic hydration processes are inconsistent with numerical and geometrical properties of the functions obtained by application of the ergodic algorithmic model (EAM). We remind that the ergodic algorithmic model (EAM) is presenting convoluted binding functions  $R \ln K_{\text{dual}} = -\Delta G_{\text{dual}}/T = \{f(1/T) \times g(T)\}$  and  $RT \ln K_{\text{dual}} = -\Delta G_{\text{dual}} = \{f(T) \times g(\ln T)\}$ , which are free energy functions experimentally determined. As obtained from experimental data, these functions do not need any simulation with inherent uncertainties and loss of information.

Molecular dynamics and Monte Carlo calculations need corrections with respect to the format employed so far. The maximum in the binding function  $RT \ln K_{\text{dual}} = -\Delta G_{\text{dual}} = \{f(T) \times g(\ln T)\}$ , which is a curved concave function, is explained by the EAM by considering that the maximum corresponds to the temperature at which  $\Delta S_{\text{dual}} = \Delta S_{\text{mot}} + \Delta S_{\text{th}} = 0$ . The potential distribution theorem (PDT) with missing essential information elements does not even mention that, for the same reaction of class B, another parallel concave binding function  $R \ln K_{\text{dual}} = -\Delta G_{\text{dual}}/T = \{f(1/T) \times g(T)\}$  exists whose tangent is the dual enthalpy ( $-\Delta H_{\text{dual}} = -\Delta H_{\text{mot}} - \Delta H_{\text{th}}$ ) with a minimum for  $\Delta H_{\text{dual}} = 0$ . This concave binding function  $R \ln K_{\text{dual}} = \{f(1/T) \times g(T)\}$  presents in each compound the same curvature amplitude as the other concave binding function  $RT \ln K_{\text{dual}} = -\Delta G_{\text{dual}} = \{f(T) \times g(\ln T)\}$  and an equal (negative) value of hydrophobic heat capacity  $\Delta C_{p,\text{hydr}} < 0$ . By passing to more general considerations concerning both class A and class B and not concerning one single reaction of class B only, the potential distribution theorem (PDT) ignores the fact that we can find hydrophobic hydration processes of class A also (e.g., the solubility in water of inert gases) presenting for each compound couples of “convex” binding functions  $R \ln K_{\text{dual}} = -\Delta G_{\text{dual}}/T = \{f(1/T) \times g(T)\}$  and  $RT \ln K_{\text{dual}} = -\Delta G_{\text{dual}} = \{f(T) \times g(\ln T)\}$  (see Figure S8 in the Supporting Information, Appendix F). Both these convex free energy functions have in each compound the same curvature amplitude and the same positive hydrophobic heat capacity,  $\Delta C_{p,\text{hydr}} > 0$ .

**2.8.3. Molecule/Mole Scaling Function or Quasi-Chemical Approximation.** The failure of PDT to explain the formation of the cavity from the partition function of the solvent has surprised us and suggested us to analyze the foundations of the theory.<sup>23</sup> The potential distribution theorem originated from the work of Widom<sup>28</sup> on the theory of fluids, and it seems that the theory concerned the solvent. The PDT has been applied also in connection to computer simulations of chemical

reactions and free energy functions. Pratt et al.<sup>22</sup> represent the potential as

$$\beta\mu = \ln[x_j p \Lambda_a^3 / q_a^{\text{int}}] \quad (59)$$

where  $x_j$  is the mole fraction of species  $j$  for this ideal case,  $x_j p$  is the partial pressure of species  $j$ , and  $p$  is the total pressure. The factor  $\beta = 1/k_B T$  indicates that it is referred to a population of molecules. This expression and the parallelism of PDT with computer simulations indicate that these relationships are referred to a statistical population, ruled by Boltzmann statistics, whereas the mole population of the solute in hydrophobic hydration systems is a reacting ensemble (REME), composed of few elements and ruled by binomial distribution. The direct application of PDT to the study of chemical reactions is inappropriate if no distinction is done between binomial and Boltzmann statistical distributions. Pratt et al.<sup>24</sup> have correctly adapted the potential distribution theorem to handle REMEs by considering quasi-chemical approximation. They have introduced partition functions analogous to the motive partition function  $\{\text{M-PF}\}$ , generating the motive binding functions  $R \ln K_{\text{mot}} = f(1/T)$  and  $RT \ln K_{\text{mot}} = f(T)$  (see the Supporting Information, Appendix B), thus obtaining polynomial molar partition functions. They consider that the components of the solute originate clusters. They consider two levels of clustering of the solute: (a) inner level clusters (icebergs) and (b) outer level clusters (icebergs). The equilibrium hydration constants of eqs 63 and 64 are very likely referred to outer level water clusters with stoichiometry  $\pm \xi_w$  and suitable for quasi-chemical approximation.

quasi-chemical approximation proposed by Pratt et al.,<sup>24</sup> now renamed the chemical molecule/mole scaling function (Che. m/M. sF) is coherent with the ergodic algorithmic model (EAM) founded on appropriate reference to chemical potential  $\mu$  of the solute on the mole scale  $1/RT$  as a factor of the motive partition function  $\{\text{M-PF}\}$ . In fact, we have shown in part I,<sup>5</sup> Table 1, that the chemical potential  $\mu$  is a dilution-equivalent density entropy function in REMEs

$$dS = d(-\mu/T) = R \ln d_A \quad (60)$$

where active dilution,  $d_A = (1/a_A)$ , is the reciprocal of activity  $a_A$ . The basic setting of the thermodynamic potential ( $-\mu/T$ ) is the definition of activity  $a_A$ . We propose to change the expression for the activity of a species  $\sigma$

$$z_\sigma = \exp(\beta\mu_\sigma) = [V\rho_\sigma / (\lll e^{-\beta\Delta U} \ggg q_\sigma)] \quad (61)$$

as assumed by PDT<sup>22</sup> into the expression (cf. eq 2)

$$a_\sigma = \exp(-\beta\mu_\sigma) = (T^{-(\Delta C_{p,A}/R)} \cdot \lll \exp(-U/(kBT)) \ggg) \quad (62)$$

The latter expression is considered at the molecule level with both components of activity, thermal (as the thermal factor  $\Phi$  from thermal equivalent dilution) and motive. The activity  $a_\sigma$  treated by the chemical molecule/mole scaling function (Che. m/M. sF) or quasi-chemical approximation generates at the mole level the motive partition function  $K_{\text{mot}}$ . The molecule distribution of each chemical species A over the microlevels of  $h_{ij}$  generates at the mole level the Lambert thermal activity factor  $\Phi = T^{-(\Delta C_{p,A}/R)}$ , which is the source of the ergodic property of chemical solutions. Equation 61 is based on the principle that entropy  $S$  can depend on both density entropy and intensity entropy. We keep this same structure for activity  $a_A$  when we apply the quasi-chemical approximation (Pratt et

Table 2. Terms Composing the Free Energy Function  $-\Delta G_{\text{app}}$  in Some Hydrophobic Hydration Processes

$-\Delta G_{\text{dual}} = -\Delta H_{\text{dual}} + T\Delta S_{\text{dual}}$	$-\Delta G_{\text{mot}} - \Delta G_{\text{th}} = (-\Delta H_{\text{mot}} - \Delta G_{\text{th}}) + T(\Delta S_{\text{dual}} + \Delta S_{\text{dual}})$	$RT \ln K_{\text{dual}} = f(T)$	curved
$-\Delta G_{\text{mot}}$	$-\Delta G_{\text{mot}} = -\Delta H_{\text{mot}} + T\Delta S_{\text{mot}}$	$RT \ln K_{\text{mot}} = f(T)$	straight
$-\Delta G_{\text{th}} = -\Delta H_{\text{th}} + T\Delta S_{\text{th}} = 0$	$\Delta H_{\text{th}} = \Delta C_{p,\text{hydr}}T; \Delta S_{\text{th}} = \Delta C_{p,\text{hydr}} \ln T$	$\rightarrow$ solvent	
$-\Delta G_{\text{app}} = -\Delta G_0 - 0 - \Delta G_{\text{cav}}$	$-\Delta G_0: (\text{gas} \rightarrow \text{solute}), (\text{liq} \rightarrow \text{solute}), (\text{undiss} \rightarrow \text{diss.})$	$\rightarrow$ solution	
$-\Delta G_{\text{cav}} = -\Delta H_{\text{cav}} + T\Delta S_{\text{cav}}$	$\Delta H_{\text{cav}} = \xi_w \cdot \langle \Delta h_{\text{for}} \rangle_A; \Delta S_{\text{cav}} = \xi_w \cdot \langle \Delta s_{\text{for}} \rangle_A$	$\rightarrow$ solution	
$RT \ln K_{\text{dual}} = -\Delta H_{\text{dual}} + T\Delta S_{\text{dual}}$	$-\Delta G_0 - \Delta G_1 - \Delta G_2 - \Delta G_{\text{dual}} = -\Delta G_0 - \Delta G_{\text{th}} - \Delta G_{\text{mot}}$	$\rightarrow$ solvent + solution	

al.<sup>22</sup>) to the hydration equilibria involved in water-clustering reactions of iceberg formation and iceberg reduction, concerning the solute. The Lambert thermal factor  $\Phi = T^{-(\Delta C_p, \text{A}/R)}$ , applied to the solvent molecules, is the source of intensity entropy, whereas when applied to solute molecules is the source of density entropy.

We have shown (see Section 2.9 below) that the reactions of iceberg formation/reduction are of concern with the water species  $W_I$  and  $W_{II}$ , generating changes of solvent volume. The change of solvent volume modifies the properties of the solute of partition function  $\{M\text{-PF}\}$  as distinct from the bulk solvent. The bulk solvent corresponds to thermal partition function  $\{T\text{-PF}\}$ . The solvent/solute change of phase of water, direct or inverse, can take place by two opposite reactions. The reaction of iceberg formation, typical of class A with  $\xi_w > 0$  is a hydration clustering reaction with constant  $\xi_w$  (or  $\xi_w^*$  with  $\xi_w > \xi_w^*$  if not all the water molecules expelled from  $W_I$  to form a niche with icebergs become ligands of the solute)

$$K_{\text{diss}} = (a_A) \cdot (a_B)^{-1} \cdot (a_{WII})^{-\xi_w \text{ or } -\xi_w^*} \quad (63)$$

whereas the reaction of iceberg reduction, eq 64, typical of class B with  $-\xi_w$  is a dehydration reaction with a constant of

$$K_{\text{assoc}} = (a_A) \cdot (a_B)^{-1} \cdot (a_{WII})^{-\xi_w \text{ or } -\xi_w^*} \quad (64)$$

( $-\xi_w$  or  $-\xi_w^*$  with  $\xi_w > \xi_w^*$  because not all the water molecules reducing the iceberg niche might become ligands of the solute)

Following Pratt,<sup>22</sup> we transform the expression of activity in eq 62 from the molecule scale to the mole scale by means of the chemical molecule/mole scaling function (Che. m/M. sF) or quasi-chemical approximation. The scaling ratio from molecule to mole yields

$$(A) (T^{(\Delta C_{p,A}/k_B)})^{(1/N_{Av})} = T^{(\Delta C_{p,A}/R)} \quad (65)$$

$$(B) (\ll \exp(-U/(k_B T)) \gg)^{(1/N_{Av})} = x_A \quad (66)$$

for the two factors of eq 66. We insist that the name of quasi-chemical approximation should be changed into that more appropriate, chemical molecule/mole scaling function (Che. m/M. sF) (see the Supporting Information, Appendix B).

Equation 66 considers at the molecule level both components of activity, thermal (as for thermal equivalent dilution) and motive (as for dilution). The activity  $a_\sigma$  treated by the chemical molecule/mole scaling function (Che. m/M. sF) or quasi-chemical approximation generates at the mole level the motive partition function  $K_{\text{mot}}$ . The factor  $T^{(\Delta C_{p,A}/k_B)}$  generates at the mole level the Lambert thermal factor  $\Phi = T^{-(\Delta C_{p,A}/R)}$  of thermal equivalent dilution (TED).  $\Delta C_p$  had been previously calculated as  $\Delta C_{p,\text{hydr}}$  from the curvature amplitude of the experimental binding functions  $R \ln K_{\text{dual}} = -\Delta G_{\text{dual}}/T = \{f(1/T) \times g(T)\}$  and  $RT \ln K_{\text{dual}} = -\Delta G_{\text{dual}} = \{f(T) \times g(\ln T)\}$  of each compound. The activity  $a_\sigma$  of eq 66 repeats at the molecular level the dual structure of the second principle of thermodynamics (see part I<sup>5</sup>, Appendix C). The activity is transformed from the molecule scale to the mole

scale of probability space; then, it is transformed into the logarithmic form of thermodynamic space.

Equation 66, associated to the thermal functions  $-\Delta H_{\text{th}}$  and  $T\Delta S_{\text{th}}$ , gives rise to calculated curved convoluted binding function  $RT \ln K_{\text{calc}} = \{f(T) \times g(\ln T)\}^b$ , corresponding to the experimental dual function  $RT \ln K_{\text{dual}} = \{f(T) \times g(\ln T)\}$ . The same relationship can be found between the calculated binding function  $R \ln K_{\text{calc}} = \{f(1/T) \times g(T)\}$  and the experimental function  $R \ln K_{\text{dual}} = \{f(1/T) \times g(T)\}$ .

Following the ergodic algorithmic model (EAM), we can find the driving reactions among the reactions contributing to the motive function, concerning the solute. We search for the factor components of the motive binding functions  $RT \ln K_{\text{mot}} = f(T)$  and  $R \ln K_{\text{mot}} = f(1/T)$  after separation from the thermal functions. The dual experimental binding functions are

$$RT \ln K_{\text{dual}} = \{f(T) \times g(\ln T)\} = \{-\Delta H_{\text{mot}} - \Delta H_{\text{th}}\} + T(-S_{\text{mot}} + -S_{\text{th}}) = -\Delta G_{\text{dual}} \quad (67)$$

and

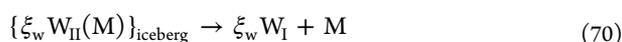
$$R \ln K_{\text{dual}} = \{f(1/T) \times g(T)\} = \{(-\Delta H_{\text{mot}}/T) + (-\Delta H_{\text{th}}/T)\} + \{(\Delta S_{\text{mot}}) + (\Delta S_{\text{th}})\} = \Delta G_{\text{dual}}/T \quad (68)$$

The first step is the separation of the thermal functions from the motive functions. This means that we must transform the parabolic binding functions  $RT \ln K_{\text{dual}} = \{f(T) \times g(\ln T)\}$  and  $R \ln K_{\text{dual}} = \{f(1/T) \times g(T)\}$  into the rectilinear binding functions  $RT \ln K_{\text{mot}} = f(T)$  and  $R \ln K_{\text{mot}} = f(1/T)$ , respectively. We note that, in the partition function  $\{DS\text{-PF}\}$ , the multiplication ( $K_{\text{mot}} \cdot \zeta_{\text{th}}$ ) by  $\zeta_{\text{th}} = 1$  yields such a significant change in the geometrical diagram. The next step is the analysis of the structure (see part III,<sup>6</sup> Section 5, Table 2), constituted by three terms, of the binding functions  $RT \ln K_{\text{dual}} = \{f(T) \times g(\ln T)\}$  (see Table 2) corresponding to free energy  $-\Delta G_{\text{dual}} = -\Delta G_0 - \Delta G_{\text{th}} - \Delta G_{\text{iceberg}}$ . (A similar analysis could be performed on  $R \ln K_{\text{dual}} = \{f(1/T) \times g(T)\}$ ). One term, the thermal free energy  $-\Delta G_{\text{th}}$  is equal to zero. The term  $-\Delta G_0$  is referred to the initial transformation undergone by the solute at zero iceberg (see part III,<sup>6</sup> Table 2). This first step is, for instance, (i) the passage of a gas (phase transformation in a biphasic system) from the kinetically active gaseous state to the resting trapped state into the solvent, or (ii) the passage of a liquid from the free pure state to the trapped state in the solution, or (iii) the passage of a carboxylic acid from the nondissociated state to the dissociated state in the solution. The first reaction step for a series of gases corresponds to intensity entropy change at zero iceberg ( $\xi_w = 0$ ),  $\Delta S_0^{(\xi_w=0)} = -86.4 \text{ J K}^{-1} \text{ mol}^{-1}$ , (intensity entropy change means loss of velocity of the molecules) calculated in nonpolar gases (see ref 3, paragraph 6.1, page 124) with the entropy change  $\Delta S_{\text{condens}} = -86.9 \pm 1.4 \text{ J K}^{-1} \text{ mol}^{-1}$  (thermal entropy change). This change in kinetic energy or intensity entropy change is equal to that given by the Trouton constant, referring to the passage

from vapor to liquid. This interpretation of this state passage as “lost kinetic energy” is indirectly confirmed by analyzing the first-step entropy at zero iceberg formation ( $\xi_w = 0$ ) of a set of liquids dissolving in water. In these liquids, the entropy change at zero iceberg is  $\Delta S_0^{(\xi_w=0)} = -0.54 \text{ J K}^{-1} \text{ mol}^{-1}$ . The small value of entropy changes of  $\Delta S_0^{(\xi_w=0)}$  at zero iceberg of the liquids depends on their condition. The liquids are already condensed prior to dissolution in water, and there is no loss of velocity of the molecules (cf. part III,<sup>6</sup> Table 5) at condensation. The third term of free energy  $-\Delta G_{\text{iceberg}}$  concerns the formation or reduction of the iceberg. The term can be either  $-\Delta G_{\text{for}}$  (iceberg formation) in processes of class A or  $-\Delta G_{\text{red}}$  (iceberg reduction) in processes of class B. We remind the reader that thermodynamic functions for iceberg formation or reduction are changes of the thermodynamic potential functions of the solute, not of the solvent, and correspond to water iceberg reactions. This is because the only changes in the solvent at constant potential are changes of volume ( $\pm\Delta V_{\text{solvent}}$ ), which produce changes (concentration or dilution) in the thermodynamic state of the solute. The unitary entropy for iceberg formation (“unitary” means referred to unity ( $\pm\xi_w = \pm 1$ ) of water cluster  $W_I$ ) is  $\langle\Delta s_{\text{for}}\rangle_A = -445 \pm 3 \text{ J K}^{-1} \text{ mol}^{-1} \xi_w^{-1}$  ( $\sigma = \pm 0.7\%$ ) with a very low standard deviation.

On the grounds of the assignment of the main reaction in each hydrophobic hydration processes to the motive partition function  $\{M\text{-PF}\}$  of the solute, we have examined which reaction of the solute was more suitable to be treated by quasi-chemical approximation of Pratt et al.<sup>11</sup> These authors define the clustering of solvent molecules around a distinguished central molecule or single atom. In our contest, the hydration reactions of eqs 63 and 64 can be considered. Clustering means that there is a central unit, belonging to the solute, attracting a cluster of  $\xi_w$  water molecules of  $W_{II}$ , also belonging to the solute. The number  $\xi_w$  measured by us might be and almost always a non-integer. This non-integer number cannot be considered a stoichiometric number between a central unit  $M$  and  $n_w$  clustered molecules. The chemical reaction more credited to be accepted as a factor of quasi-chemical approximation is the reaction for iceberg formation in class A and the reaction for iceberg reduction in class B, both with stoichiometry  $\pm\xi_w$ . Formation or reduction of the iceberg produces entropic effects bound to a change of volume rather than an enthalpic effect bound to some energy difference.

**2.8.4. Free Energy for Iceberg Formation/Reduction from  $\{M\text{-PF}\}$ .** The knowledge of the parameter  $\pm\xi_w$  allows us to calculate the free energy change  $-\Delta G_{\text{red}}$  for iceberg reduction or the free energy change  $-\Delta G_{\text{for}}$  for iceberg formation. For every process of class A, the reaction with iceberg formation has been observed, and for every process of class B, the reaction with iceberg reduction was observed. By processing the thermodynamic data for approximately 80 different hydrophobic hydration systems, we have calculated<sup>6</sup> unitary values (unitary are referred to  $\xi_w = 1$  water cluster  $W_I$ ) for processes of iceberg formation and opposite iceberg reduction, respectively (Table 3).



The processes of iceberg formation/reduction are related to the motive partition function  $\{M\text{-PF}\}$  of the solute. Specifically, the process of iceberg formation generates

**Table 3. Unitary Values ( $\pm\xi_w = \pm 1$ ) of Thermodynamic Functions for Every Hydrophobic Hydration Process**

class A: iceberg formation	unit	$\sigma$ values
$\langle\Delta h_{\text{for}}\rangle_A = -22.7 \pm 0.7$	$\text{kJ mol}^{-1} \xi_w^{-1}$	$\sigma = \pm 3.1\%$
$\langle\Delta s_{\text{for}}\rangle_A = -445 \pm 3$	$\text{J K}^{-1} \text{ mol}^{-1} \xi_w^{-1}$	$\sigma = \pm 0.7\%$
class B: iceberg reduction	unit	$\sigma$ values
$\langle\Delta h_{\text{red}}\rangle_B = +23.7 \pm 0.6$	$\text{kJ mol}^{-1} \xi_w^{-1}$	$\sigma = \pm 2.51\%$
$\langle\Delta s_{\text{red}}\rangle_B = +432 \pm 4$	$\text{J K}^{-1} \text{ mol}^{-1} \xi_w^{-1}$	$\sigma = \pm 0.9\%$

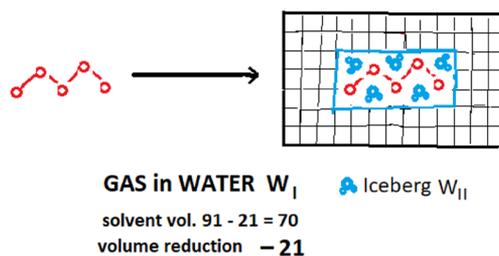
reduction of solvent volume and consequent concentration of the solute. This is a process producing a negative change of density entropy. As an example of a process of a class A reaction, we consider the reaction of a gas molecule or liquid molecule with water  $W_I$ . By considering the unitary functions

$$\langle\Delta h_{\text{for}}\rangle_A = -22.7 \pm 0.7 \text{ and } \langle\Delta s_{\text{for}}\rangle_A = -445 \pm 3$$

we obtain the unitary free energy for iceberg formation

$$\begin{aligned} \Delta g_{\text{for}} &= \Delta h_{\text{for}} - T\Delta s_{\text{for}} = -22,700 + 298 \times 445 \\ &= +109,910 \text{ J mol}^{-1} = +109.91 \text{ kJ mol}^{-1} \end{aligned} \quad (71)$$

The process of iceberg formation (Figure 8) results in being thermodynamically disfavored. How can this counteraction be

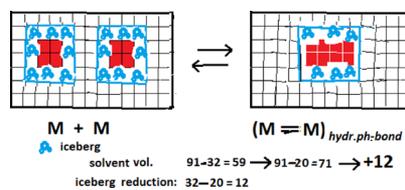


**Figure 8.** Iceberg formation at dissolution of a gas in water  $W_I$ .

overcome? We must consider that this process takes place under pressure: this pressure is forcing the gas to enter the solvent. If the pressure were absent, the gas would be spontaneously leaving the solvent with disruption of the iceberg. Other molecular processes, which possibly can favor iceberg formation, are

- entropy contribution by dilution,  $RT \ln(1/x_A)$ , of gas molecules in the aqueous phase and
- hydration affinity between  $\xi_w'$  water molecules of  $W_{II}$  and the hydrophilic solute unit, as by the reaction of eq 54 for iceberg formation.

For the processes of class B, hydrophobic bonding (Figure 9) can be taken as a representative. The molecule  $M$  in its iceberg is binding to another molecule  $M$  in its own iceberg by hydrophobic bonding: the icebergs coalesce to a common iceberg whose volume is smaller than the sum of component



**Figure 9.** Iceberg reduction at hydrophobic bonding (iceberg contour in azure color).

icebergs. By considering the unitary values for iceberg reduction  $\langle \Delta h_{\text{red}} \rangle_{\text{B}} = +23.7 \pm 0.6 \text{ kJ mol}^{-1} \xi_{\text{w}}^{-1}$  and  $\langle \Delta s_{\text{red}} \rangle_{\text{B}} = +432 \pm 4 \text{ J K}^{-1} \text{ mol}^{-1} \xi_{\text{w}}^{-1}$ , the unitary free energy for iceberg reduction can be calculated

$$\begin{aligned} \Delta g_{\text{red}} &= \Delta h_{\text{red}} - T \Delta s_{\text{red}} = +23,700 - 128,736 \\ &= -105,036 \text{ J mol}^{-1} = -105.04 \text{ kJ mol}^{-1} \end{aligned} \quad (72)$$

A negative  $\Delta g_{\text{red}}$  indicates that the process of iceberg reduction at the formation of a hydrophobic bond is thermodynamically favored, driven by the positive entropic contribution by the solute for iceberg reduction. This result is contradicting the widely accepted interpretation that hydrophobic bonding is entropically favored because of the degrees of freedom acquired by water molecules, set free at the very moment of formation of the hydrophobic bond. These people ignore that the thermal partition function of the solvent (NoremE) is unsuitable for producing free energy changes because it can give rise to changes of intensity entropy (solvent/iceberg phase change) only but not to changes of density entropy.

At any rate, the reaction steps contributing to free energy are those composing the equilibrium constant  $K_{\text{mot}}$ . In the processes with iceberg formation (class A), the favorable contributions are (i) the formation enthalpy  $\Delta H_{\text{for}} = (-21.6 \cdot \xi_{\text{w}}) \text{ kJ mol}^{-1}$  that indicates the affinity of the aliphatic chain for a water sheath of molecules of  $W_{\text{II}}$  and (ii) the enthalpy at zero iceberg  $\Delta H_0^{(\xi_{\text{w}}=0)} = -31.7 \text{ kJ mol}^{-1}$ . This enthalpy change at zero iceberg in gaseous or liquid solutes indicates the affinity between solutes and the solvent sheath. Through this bonding, the continuous source of water clusters by the passage of states with entropy change at constant potential of water  $W_{\text{I}}$ ,  $\Delta H_{\text{hydr}}/T = \Delta C_{p,\text{hydr}}$  is maintained. The unfavorable negative entropic contributions contrast the favorable enthalpic contributions. The balance of these opposite effects leads to significant chemical properties:

- (i) very low solubility of gas molecules as measured by the thermodynamic solubility parameter;
- (ii) free energy change at zero iceberg  $\Delta G_0^{(\xi_{\text{w}}=0)}$  in the dissociation of carboxylic acids, corresponding to the dissociation process of the acid. This property has been mathematically calculated for several acids by subtracting the free energy  $\Delta G_{\text{for}}$  for iceberg formation; the residual free energy calculated as  $\ln K_x = f(1/T)$  for a set of acids comes out to be linearly dependent on the respective Hammett coefficient.<sup>4</sup>

Moreover, the ergodic algorithmic model offers a clear explanation<sup>5</sup> of the processes of

- (i) protein folding, ruled by the favorable entropic contribution of iceberg reduction,
- (ii) cold denaturation ruled exclusively by positive or negative motive free energy,
- (iii) thermal and chemical denaturation by acting on water clustering equilibrium
- (iv) formation of hydrophobic bonds, entropy-driven by solute dilution by formation of volume of a solvent of  $W_{\text{I}}$ .

The ergodic algorithmic model (EAM) indicates the correct procedure to calculate the motive free energy functions or motive binding functions  $RT \ln K_{\text{mot}}$  (enthalpy units) and  $R \ln K_{\text{mot}}$  (entropy units) and to analyze the free energy for iceberg formation or iceberg reduction. We report below, to show the

potency of the procedure, the calculation of free energy for iceberg formation in a series of carboxylic acids for which the constant value of  $+\xi_{\text{w}} = +2.1$  was found.

**2.8.5. From the Thermal Partition Function {T-PF} to the Motive Partition Function {M-PF}.** In conformity with the ergodic algorithmic model (EAM), the quasi-chemical approximation, now renamed the “chemical molecule/mole scaling function” (Che. m/M. sF), adds new information beyond the thermal partition function {T-PF} (i.e., solvent) just as the stoichiometry  $\pm \xi_{\text{w}}$  of the chemical reaction concerning the motive partition function {M-PF} (i.e., solute). In such a way, the quasi-chemical approximation recognizes that the passage from the thermal partition function {T-PF} to the motive partition function {M-PF} has taken place and, by indicating the stoichiometry of the reaction, shows us that the calculations therefrom will be ruled by binomial mole distribution and no longer by Boltzmann statistical molecule distribution.

The ergodic algorithmic model (EAM) suggests that one basic reaction step of the passage from {T-PF} to {M-PF} is the reaction of iceberg formation or, for the opposite passage, from {M-PF} to {T-PF}, the reaction of iceberg reduction where the number  $\xi_{\text{w}}$ , experimentally determined by the curvature amplitude of the binding functions, is proportional to the size of the iceberg formed (if positive,  $+\xi_{\text{w}}$ ) in class A or of the iceberg reduced (if negative,  $-\xi_{\text{w}}$ ) in class B. By employing the unitary values reported in Table 3, it is possible to calculate the free energy for iceberg formation or for iceberg reduction in each hydrophobic reaction if we have previously determined the number  $\pm \xi_{\text{w}}$  by treatment of the experimental data.

As an example of the type of information that can be extracted from the experimental data by means of the ergodic algorithmic model (EAM), we can consider the set of protonation reactions of carboxylic acids (Table 4). We can

**Table 4. Residual Equilibrium Constant in Carboxylic Acids with  $\xi_{\text{w}} = 2.1$**

acid	$\Delta H_x$	$\Delta S_x$	$\Delta G_x$	$\sigma_{\text{Ham}}$
Cl-ethane	9.21	-5.7	1707.81	0.37
ethane	-1.34	14.59	-4349.16	-0.17
CN-ethane	0.15	-13.81	4123.53	0.63
methane	-0.91	0.75	-224.41	0.15
propane	-0.99	17.8	-5305.39	-0.34

show how the equilibrium constant determined by the EAM is composed by two factors, at least, and one of them is always the constant for iceberg formation or reduction. The reaction of iceberg formation or reduction is necessarily present in every hydrophobic hydration process. We wonder how in the computer simulations of free energy calculations<sup>25,29</sup> the reaction of iceberg formation or that of iceberg reduction is systematically ignored.

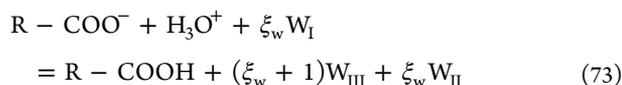
Even Chipot, Kollman, and Pearlman<sup>31</sup> investigated the convergence behavior of potential of mean force (PMF) calculations using free energy perturbation (FEP),<sup>17</sup> thermodynamic integration (TI), and “slow growth” (SG) techniques. “Iceberg formation” or “iceberg reduction” with distinction of reactions of class A or class B is not even mentioned by these authors.

This survey could be extended to many other articles in the literature, but we realize that the conclusion will be the same: free energy changes, as produced in the partition function of

the solute by the processes of “iceberg formation” or “iceberg reduction”, are never mentioned. We recall the point that, according to the ergodic algorithmic model (EAM), the iceberg reaction is a process of phase transition, in class A from water  $W_I$  (solvent) to water  $W_{II}$  (iceberg, solute) with reduction of solvent volume with the solvent at constant potential (thermal free energy  $\Delta G_{th} = 0$ ). In class B, the opposite reaction is taking place with the opposite phase transition from solute to solvent. These phase transitions in the solvent induce variations in the solvent volume and, in consequence, variations in the thermodynamic functions of the solute, concerning  $\{M\text{-PF}\}$  ( $\Delta G_{mot} \neq 0$ ), because they change the dilution state of the solute.

The confirmatory statistical inference of the validation process of the ergodic algorithmic model (EAM) (see part III<sup>6</sup>) demonstrates that the reaction of iceberg formation/reduction is ubiquitous in every hydrophobic hydration process. The statistical inference stating the rule that, in every hydrophobic hydration process, we must find an iceberg reaction as a factor of  $\{DS\text{-PF}\}$  holds not only for the processes and compounds examined in the present articles but must be extended with a high probability of success to whatever hydrophobic hydration process thermodynamically analyzed in the past and in the future. By applying the unitary thermodynamic functions reported in Table 3 together with the number  $\pm\xi_w$  experimentally determined for each compound, we can calculate the equilibrium constants for iceberg  $\ln K_{icbg}$  for any new hydrophobic process eventually examined. Then, we can disaggregate the equilibrium constant  $\ln K_{mot}$  experimentally determined, by subtracting the calculated iceberg equilibrium constant  $\ln K_{icbg}$  and obtain a residual reliable equilibrium constant  $\ln K_x$  relative to the ancillary reactions associated to the reaction of iceberg formation or reduction.

As an example of the type of information that can be extracted from the experimental data by means of the ergodic algorithmic model (EAM), we can consider the set of protonation reactions of carboxylic acids (Table 4). We can show how the equilibrium constant determined by the ergodic algorithmic model (EAM) is composed of two factors at least, and one of them is the constant for iceberg formation or iceberg reduction. In the protonation of carboxylate anions,<sup>3</sup> the total reaction can be written

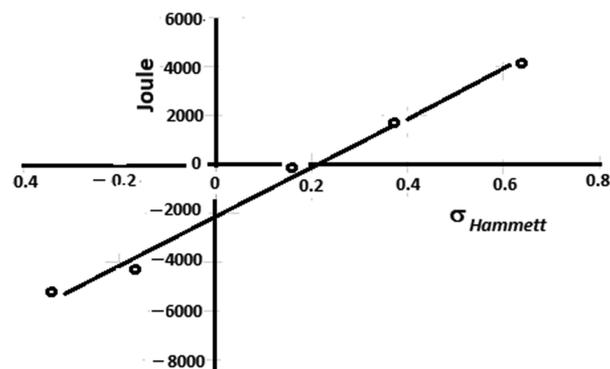


which includes reaction A. The analysis of the residual protonation constant  $\ln K_x$

$$\ln K_x = \ln K_{mot} - \ln K_{for} \quad (74)$$

for five carboxylic acids with  $\xi_w = 2.1$  has given the values of residual thermodynamic functions in Table 4.

The values of free energy  $\Delta G_x$  correlate well as the function of the Hammett constant (Figure 10). This correlation is a proof of validity of the disaggregation of  $\ln K_{mot}$  into two components: (i) iceberg formation constants and (ii) proton affinity constants,  $\ln K_x$ . Probably, if we consider the remaining carboxylic acids of the list examined by us with more complex molecule structures, it could be possible to show how the values of the numbers  $\xi_w > 2.1$  ( $\xi_w^* = \xi_w - 2.1$ ) could be attributed to the formation of icebergs between water  $W_{II}$  and



**Figure 10.** Free energy  $\Delta G_x$  as the function of Hammett constant  $\sigma_{Hammett}$ .

aliphatic and aromatic chains, belonging to more complex carboxylic acids.

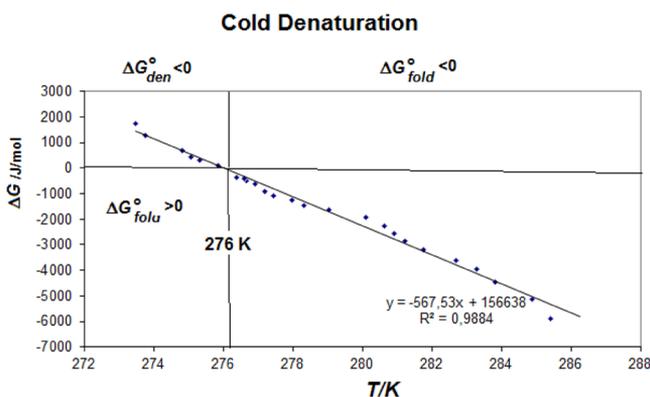
**2.8.6. Hydrophobic Hydration and Structure-Based Drug Design.** Thermodynamics is a fundamental interpretative tool for structure-based drug design. Experimental and theoretical thermodynamic principles are applied to design compounds, structured in such a way to be binding to macromolecular receptors for therapeutic activity.<sup>30</sup> The purpose of these studies is calculating reaction free energy  $\Delta G$  as a parameter of potential energy of the chemical reaction.<sup>32–34</sup> Free energy can be calculated by using molecular dynamics (MD) in two ways: thermodynamic integration (TI) and free energy perturbation<sup>21</sup> (FEP). Both methods are used to determine the free energy difference between two molecules or between two molecular conformations. These usual procedures employed in drug design must be changed to make them conform to an ergodic algorithmic model (EAM). The methodology followed by Talhout et al.<sup>32</sup> for the study of the binding of a series of hydrophobically modified benzamidine chloride inhibitors to trypsin, must be applied. This procedure consists of the preliminary experimental determination of the thermodynamic properties of the system by ITC (isothermal titration calorimetry). We note that whatever experimental method might be used for measurements of a potential function or an equilibrium constant at various temperatures. Enthalpy of binding, entropy of binding, and Gibbs energy are the information acquired. The corresponding binding functions  $R \ln K_{dual} = -\Delta G_{dual}/T = \{f(1/T) \times g(T)\}$  and  $RT \ln K_{dual} = -\Delta G_{dual} = \{f(T) \times g(\ln T)\}$  of each compound are concave functions with equal curvature amplitude, connected by thermal equivalent dilution (TED) to the potential parameter (see Appendix A). Then, the curved functions  $R \ln K_{dual}$  and  $RT \ln K_{dual}$ , by taking advantage of the stoichiometry  $\pm\xi_w$  of the water reaction calculated from the curvature, are transformed into the linear functions  $R \ln K_{mot} = f(1/T)$  and  $RT \ln K_{mot} = f(T)$ , respectively, which are the real potential functions generating free energy  $-\Delta G_{mot}$ . MD computer simulations have provided the values of statistical partition functions  $Z(N,V,T)$ , each referred to one macrolevel  $H_j$ . These molecular values  $Z(N,V,T)$  must be transformed into the molar values  $\{z_{(N,V,T)}\}$  and then linked, by reference to the experimentally measured thermodynamic properties  $R \ln K_{mot}$  and  $RT \ln K_{mot}$  to the mole structure of the system. In such a way, information for potential function  $R \ln K_{mot}$  and  $RT \ln K_{mot}$  and for stoichiometry  $\xi_w$  of the water reaction can be achieved from the experimental molar binding functions and connected to the molecular computer simulations by

appropriate scaling functions. Anyway, one point must be kept in mind: the mathematical algorithm employed to calculate the simulated function must necessarily contain in principle the basic constriction that  $\Delta C_{p,\text{hydr}}$  is constant in each compound ( $\Delta C_{p,\text{hydr}} = -\xi_w C_{p,w}$  class B), and even the coefficient  $\pm\xi_w$  should be explicitly expressed in the new simulation algorithm.

The same procedure of associating the information obtained from the curved experimental convoluted binding functions  $R \ln K_{\text{dual}} = -\Delta G_{\text{dual}}/T = \{f(1/T) \times g(T)\}$  and  $RT \ln K_{\text{dual}} = -\Delta G_{\text{dual}} = \{f(T) \times g(\ln T)\}$  with the equilibrium calculations could be employed by Di Cera et al.<sup>33</sup> for the energetic dissection of serine proteases.

**2.9. Motive Free Energy and Stability of Chemical Compounds.** The need of calculating motive functions, separately from thermal functions, is clearly apparent if we consider the suitability of motive free energy to measure the stability of chemical compounds or chemical conformations.

Typical characteristics of the hydrophobic interactions are the temperature dependences exemplified by cold denaturation.<sup>6</sup> We can show how the ergodic algorithmic model (EAM) can offer a convincing explanation of this phenomenon (Figure 11), an explanation based on the analysis of the properties of R



**Figure 11.** Application of the ergodic algorithmic model (EAM): motive function  $\Delta G_{\text{fold}} = \Delta H_{\text{fold}} - T\Delta S_{\text{fold}}$ , calculated from the denaturation quotient at different  $T$  of a protein. Cold denaturation or folding stabilization depends on the sign of the motive function (critical  $T_{\text{fold}}: 156,638/567.53 = 276$  K).

$\ln K_{\text{mot}} = -\Delta G_{\text{mot}}/T = f(1/T)$  or  $RT \ln K_{\text{mot}} = -\Delta G_{\text{mot}} = f(T)$ . We consider that only the motive functions contribute to free energy, and in consequence, the motive functions only are suited to determine if a reaction is thermodynamically favored or not. We subtract the thermal components  $\Delta H_{\text{th}}$  and  $\Delta S_{\text{th}}$  from the observed apparent functions  $\Delta H_{\text{dual}}$  and  $\Delta S_{\text{dual}}$ , respectively, and we obtain the linear motive function  $RT \ln K_{\text{mot}} = f(T)$ . For the specific case (Figure 11), we label the resulting motive function with the subscript “fold” (folding) instead of “mot” and obtain

$$\Delta G_{\text{fold}} = \Delta H_{\text{fold}} - T\Delta S_{\text{fold}} \quad (75)$$

If  $\Delta H_{\text{fold}} > 0$  ( $\Delta H_{\text{fold}} = +156.638$  kJ mol<sup>-1</sup>) and  $\Delta S_{\text{fold}} > 0$  ( $\Delta S_{\text{fold}} = +567.53$  J K<sup>-1</sup> mol<sup>-1</sup>), at the folding temperature  $T_{\text{fold}}$ , we have  $\Delta H_{\text{fold}} - T\Delta S_{\text{fold}} = 0$ . Above  $T_{\text{fold}}$ , the folded state is the stable one ( $\Delta G_{\text{fold}} < 0$ ), whereas below  $T_{\text{fold}}$ , the stable state is the denatured protein ( $\Delta G_{\text{fold}} > 0$ ;  $\Delta G_{\text{den}} < 0$ ). From this discussion, we can draw the conclusion that the potential  $\Delta\mu$  ( $\Delta G_{\text{fold}}/n = \Delta\mu$ ) we must refer to is that calculated from the linear motive function  $RT \ln K_{\text{mot}} = f(T)$ ,

obtained by exclusion of the thermal components from  $RT \ln K_{\text{dual}} = \{f(T) \times g(\ln T)\}$ .

Another example (Figure 12) where the motive function of the ergodic algorithmic model  $RT \ln K_{\text{mot}} = f(T)$  is working concerns the new view of the hydrophobic bonding, as shown above.<sup>4</sup> The entropy gain stabilizing the hydrophobic bond is the increase of density entropy (i.e., configuration entropy) by the solute and no longer the increase of intensity entropy (i.e., thermal entropy) by the solvent, as erroneously repeated in the literature. In the ergodic algorithmic model, the entropy-producing process of “iceberg reduction” means extension of the solvent volume with equivalent increasing of solute dilution and hence increasing of the density entropy of the solute.

### 3. CONCLUSIONS

The ergodic theory assumes the equivalence between changes of a thermodynamic function at variable temperature  $T$  with changes of the same function on dependence of a space parameter. We have found how this condition can be verified for the function entropy  $S$ . We have shown, in fact, how it is possible to have a picture at the molecular level of the changes of the path length of the molecules as the function of temperature  $T$ . The increased velocity of the particles obtained by increasing the temperature  $T$  reduces the sojourn time of each molecule, thus producing a decrease of instant energy intensity. A decrease of energy intensity means an increase of intensity entropy at constant density, being entropy inversely proportional to thermal energy.

$$dS_{(\text{Ints})} = (d \ln T^{(C_{p,A}/R)})_d \quad (76)$$

Alternatively, by reducing the concentration of the molecules, we reduce the cumulative residence time  $\{(\tau_i^2)_\Sigma\}$  by decreasing the number of molecules contributing to the summation  $\Sigma$ . In such a way, we obtain a decrease of energy density, that is, an increase of energy dilution and increase of density entropy. The increase of density entropy is exactly parallel to the decrease of energy intensity ( $\epsilon_{\text{intens}}$ ) obtained by increasing the temperature  $T$ . A decrease of energy density ( $\epsilon_{(\text{density})}$ ) means, again, an increase of density entropy.

$$dS_{(\text{Dens})} = (d \ln d_A)_T \quad (77)$$

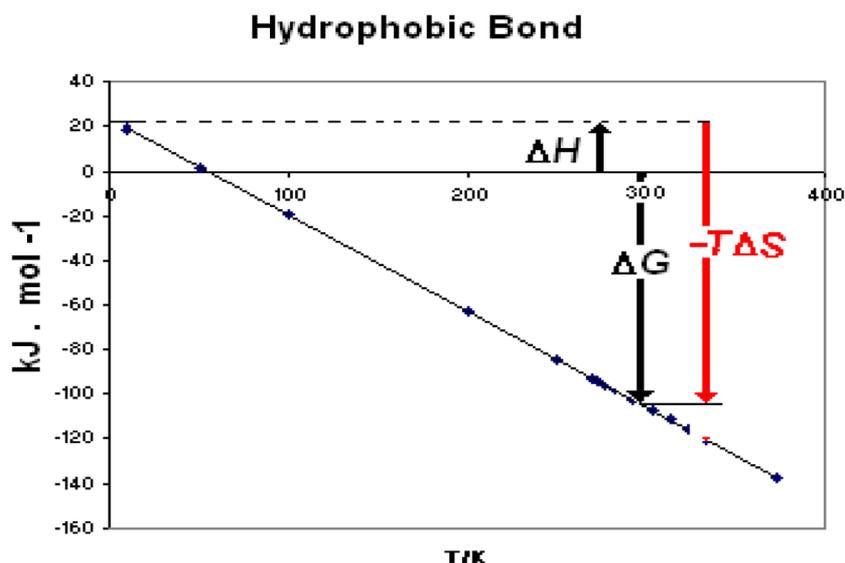
The parallelism of the changes of intensity entropy as the function of temperature  $T$  with the changes of density entropy as the function of dilution  $d_A$  is representative of the ergodic property of the thermodynamic systems.

The equivalence of intensity entropy with density entropy has been experimentally verified in the study of thermodynamic properties of hydrophobic hydration processes as thermal equivalent dilution (TED). TED consists of the parallelism of the binding function  $R \ln K_{\text{dual}} = \{f(1/T) \times g(T)\}$  with  $R \ln K_{\text{dual}} = \{f(1/d_A) \times g(T)\}$  and also of the parallelism between  $RT \ln K_{\text{dual}} = \{f(T) \times g(\ln T)\}$  and  $RT \ln K_{\text{dual}} = \{f(d_A) \times g(\ln T)\}$ . The parallelism of the two functions gets

$$-R d \ln(a_L)^n = nC_{p,L} d \ln T \quad (78)$$

that represents the mathematical formulation of TED in hydrophobic hydration processes.<sup>c</sup>

The dual-structure partition function {DS-PF} represents the state probability of every hydrophobic hydration process:



**Figure 12.** Hydrophobic bonding: density entropy term is the prominent contributor to the negative free energy (at 298 K) of the entropy-driven hydrophobic bond as an element of the motive partition function {M-PF} of the solute.<sup>4</sup>

$$\begin{aligned}
 K_{\text{dual}} &= K_{\text{mot}} \cdot \zeta_{\text{th}} = \exp(-\Delta G_{\text{mot}}/RT) \exp(-\Delta G_{\text{th}}/RT) \\
 &= \{\exp(-\Delta H_{\text{mot}}/RT) \exp(\Delta S_{\text{mot}}/R)\} \{\exp(\Delta H_{\text{th}}/RT) \\
 &\quad \exp(\Delta S_{\text{th}}/R)\} \quad (79)
 \end{aligned}$$

The dual-structure partition function {DS-PF} is the product of a thermal partition function {T-PF} multiplied by a motive partition function {M-PF}. The thermal partition function {T-PF}, referred to the solvent, can give rise to changes of intensity entropy that do not have any influence upon free energy, whereas {M-PF}, referred to the reacting solute, gives rise to changes of density entropy, representing the essential and only contributions to free energy. A NoremE is representative of the solvent (see Figure 5). It is composed of an extremely large set of elements (molecules), a large enough set to be ruled by Boltzmann statistics. Calculation of the thermal partition function {T-PF} can be performed by applying the approximations of statistical thermodynamics (e.g., Stirling approximation). In contrast, a REME (see Figure 6) is representative of the solute and is composed of few elements (moles), distributed over few macrolevels of  $H_i$ . The ergodic algorithmic model (EAM) can offer for hydrophobic hydration processes correct information by permitting the calculation of the binding functions (i.e., free energy convoluted functions)  $R \ln K_{\text{dual}} = -\Delta G_{\text{dual}}/T = \{f(1/T) \times g(T)\}$  and  $RT \ln K_{\text{dual}} = -\Delta G_{\text{dual}} = \{f(T) \times g(\ln T)\}$ . These convoluted free energy functions are obtained directly by treating the experimental data without any support of computer simulations. By analyzing the convoluted binding functions and then the component motive functions  $\Delta G_{\text{mot}}$ ,  $\Delta H_{\text{mot}}$ , and  $\Delta S_{\text{mot}}$ , essential information elements for hydrophobic hydration processes can be achieved.

We examine further the relationships between the ergodic algorithmic model and statistical thermodynamics by recalling the fundamental achievement of the ergodic algorithmic model (EAM), which combines the peculiar statistical “molecule” distribution of the thermal partition functions {T-PF}, concerning both the solvent and microlevels of  $h_{ij}$  of each macrolevel  $H_i$  with the binomial “mole” distribution of the

solute over the macrolevels of  $H_i$  of the motive partition function {M-PF}. The property of the NoremE (see Figure 5)

$$\Delta H_{\text{th}} - T\Delta S_{\text{th}} = 0 \quad (80)$$

represents a general case of entropy–enthalpy compensation. Strangely enough, any one of the many articles in the literature published for the last 50 years does not even mention the possibility that large parts of entropy and enthalpy giving origin to compensation come from changes (phase transition) taking place within the solvent and concerning the thermal partition function {T-PF} of the solvent. On the other hand, the solvent in hydrophobic hydration processes behaves in a way conforming to the molecular frame approach (MF) whereby, according to Henschman et al.,<sup>12</sup> the explicit nature of the solvent is completely ignored using vacuum in the ideal gas as a reference model of an implicit “continuum solvent”. Clusters of  $W_I$  constitute the solvent water. In contrast, clusters of  $W_{II}$  belong to the solute. The thermal partition function {T-PF} corresponding to the NoremE represents the solvent. It is composed of very many elements (molecules), but it cannot give origin to any changes of free energy and consequently to any change of chemical potential  $\mu$ . The potential change  $\Delta\mu$ , assumed for the solvent by the potential distribution theorem (PDT), is nonexistent. The solvent in diluted solutions is at constant potential because it is at constant concentration.

A motive partition function {M-PF} represents the solute and corresponds to a REME, composed of few elements (moles). By developing the dual-structure partition function {DS-PF}, the binding function  $RT \ln K_{\text{dual}} = -\Delta G_{\text{dual}} = \{f(T) \times g(\ln T)\}$

$$\begin{aligned}
 RT \ln K_{\text{dual}} &= -\Delta G_{\text{dual}} \\
 &= -(\Delta H_{\text{mot}} + \Delta C_{p,\text{hydr}} T) \\
 &\quad + T(\Delta S_{\text{mot}} + \Delta C_{p,\text{hydr}} \ln T) \quad (81)
 \end{aligned}$$

can be calculated. This function can be offered as a sound basis for the theory of hydrophobic effects. As for computer simulations, the distinction between thermal and motive components is ignored unfortunately, thus losing essential information elements. Alternatively, the “chemical molecule/

mole scaling function" (Che. m/M. sF), that is, quasi-chemical Approximation, is suited to calculate potentials by computer simulations: the binomial distribution of motive free energy of the solute is combined with Boltzmann statistical distribution of thermal partition functions of solvent and of single separated  $H_j$  macrolevels.

At the end of the analysis, at both molecule and mole levels, of the algorithmic model (EAM) for hydrophobic hydration reactions, we can conclude that the statistical computations known so far must be substantially changed to make them conform to the biphasic structure of hydrophobic hydration systems. Computer simulations of monophasic systems are inconsistent with the biphasic composition of these systems. Monte Carlo computations and molecular dynamics simulations present drawbacks deriving from the unsuitability of the thermal partition function {T-PF} of the solvent to derive factors of the motive partition function {M-PF} of the solute. The results of Monte Carlo computations and molecular dynamics simulations must be corrected for the stoichiometric information supported by the thermal components produced by the solvent. The corrected results should be compared with the curved binding function  $RT \ln K_{\text{dual}} = \{f(T) \times g(\ln T)\}$  or  $RT \ln K_{\text{dual}} = \{f(1/T) \times g(T)\}$  obtained from the experimental data. Alternatively, the chemical molecule/mole scaling function (Che. m/M. sF) or quasi-chemical approximation can be applied either through the water cluster dissociation constant

$$K_{\text{diss}} = (a_A) \cdot (a_B)^{-1} \cdot (a_{\text{WII}})^{\xi_w} \quad (82)$$

or through the water cluster association constant

$$K_{\text{assoc}} = (a_A) \cdot (a_B)^{-1} \cdot (a_{\text{WII}})^{-\xi_w} \quad (83)$$

and each of which was chosen by reference to the sign of  $\pm \xi_w$ , experimentally determined. The ergodic activity  $a_A$  is calculated as a product of the Lambert thermal activity factor  $\Phi$  times molar fraction  $x_A$ .

$$a_A = \Phi \cdot x_A \quad (84)$$

where  $\Phi = T^{-(\Delta C_{p,A}/R)}$ , preserving the ergodic property of the solution, and so forth for components B and W.

Alternatively, calculations of iceberg volumes compatible with the volumes of molecules or moieties of the molecule involved in each hydrophobic hydration process could substitute the computer simulations of statistical distributions. By assuming that the volume of one cluster of  $W_1$  is  $V_{(W1)} = 19.9 \text{ cm}^3 \text{ mol}^{-1} \xi_w^{-1}$  (see part III,<sup>6</sup> Section 4) with reference to isobaric heat capacity

$$\Delta C_{p,\text{hydr}} = \pm \xi_w C_{p,w} \quad (85)$$

the number  $(\xi_w)_{\text{calc}}$  will be calculated from the ratio between the molecular volume  $V_{\text{mol}}$  and volume  $V_{(W1)}$  of water cluster  $W_1$  involved in the reaction

$$(\xi_w)_{\text{calc}} = V_{\text{mol}}/V_{(W1)} \quad (86)$$

thus offering a possibility of comparison between calculated  $(\xi_w)_{\text{calc}}$  and observed  $\xi_w$ .

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.9b01440.

Calculation of thermodynamic functions for structure-based drug design, molecule/mole scaling function or quasi-chemical approximation, appendix C: entropy = energy dispersal parameter, appendix D: exponential probability space and logarithmic thermodynamic space, appendix E: approximations of computer simulations, appendix F: experimental convoluted binding functions, appendix G: null thermal free energy, and appendix H: thermal equivalent dilution (TED) and the second law (PDF)

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### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

This work is dedicated to the memory of Professor Gian Paolo Chiusoli, Emeritus, University of Parma, who had highly appreciated the ergodic algorithmic model (EAM) as a landmark in the study of hydrophobic hydration processes and to the memory of Professor Frank L. Lambert,<sup>35</sup> Emeritus, Occidental College, Los Angeles, (USA) for his advocacy of changing the definition of thermodynamic entropy as "disorder" to its replacement by viewing entropy as a measure of energy dispersal with energy generated by the Lambert thermal factor  $\Phi = T^{-C_{p,A}/R}$ .

## ■ LIST OF SYMBOLS

- $x_A$  = concentration of A, in molar fractions
- $(\tau_i^2)$  = squared sojourn time of the  $i$ th molecule
- $(\tau_i^2)_\Sigma$  = cumulative squared sojourn time
- $a_A$  = activity of A
- $a_A = f(\tau_i^2)_\Sigma = [c_A \cdot f(\tau_m^2)]$
- $a_L$  = activity of ligand L
- $\tau_m^2$  = squared mean sojourn time
- $\Phi = f(\tau_m^2) = T^{-(C_{p,R})}$  = thermal energy factor (THEF) for moles as the function of  $\tau_m^2$
- $1/\Phi = f'(v_m^2) = f'(T) = T^{(C_{p,R})}$  = reciprocal THEF, to be associated to ideal dilution
- $\phi = f(\tau_m^2) = T^{-(C_{p,kB})}$  = molecule thermal factor (mtf) for molecules as the function of  $\tau_m^2$
- $d_{id(A)} = (1/x_A)$  = ideal calculated dilution of A in the reciprocal molar fraction
- $d_A = 1/a_A = (1/x_A) \cdot (1/\phi)$  = active dilution of A
- $k_B$  = Boltzmann constant =  $1.381 \times 10^{-23} \text{ J K}^{-1}$
- $K_{\text{dual}}$  = experimental equilibrium constant
- $K_{\text{dual}} = \zeta_{\text{th}} \cdot K_{\text{mot}}$  = product partition function with  $\zeta_{\text{th}} = 1$
- $K_{\text{mot}}$  = motive equilibrium constant (partition function of the REME, concerning the solute)
- $\zeta_{\text{th}} = 1 \cdot \Omega \cdot (\eta/T)^{-1}$  = partition function of the NoremE, concerning the solvent
- {DS-PF} = dual-structure partition function (product partition function)
- {T-PF} = thermal partition function (for NoremE)
- {M-PF} = motive partition function (for REME)
- $C_{p,w} = 75.36 \text{ J K}^{-1} \text{ mole}^{-1}$  = molar heat capacity for liquid water
- $\Delta s_{p,w} = C_{p,w} = 75.36 \text{ J K}^{-1} \text{ mole}^{-1}$  = entropy change for phase transition in pure water  $W_I \rightarrow W_{II}$

$\Delta C_{p,\text{hydr}}$  = heat capacity in hydrophobic hydration processes  
 $(\Delta C_{p,\text{hydr}} = \pm \xi_w C_{p,w})$   
 $\Delta S_{p,\text{hydr}} = \Delta C_{p,\text{hydr}}$  = entropy change for water phase transition: **A** (solvent  $\rightarrow$  solute) or **B** (solute  $\rightarrow$  solvent)  
 class **A** = hydrophobic hydration process with phase transition: **A**{ $\xi_w W_{\text{I}}$  (solvent)  $\rightarrow$   $\xi_w W_{\text{II}}$  (iceberg)}  
 class **B** = hydrophobic hydration process with phase transition: **B**{ $-\xi_w W_{\text{II}}$  (iceberg)  $\rightarrow$   $\xi_w W_{\text{I}}$  (solvent)}  
 $W_{\text{I}}$  (cluster),  $W_{\text{II}}$  (cluster) = types of water  
 $n_w$  = number of water molecules of  $W_{\text{I}}$  changing phase in a hydrophobic hydration process ( $\xi_w = |n_w|$ )  
 $\pm \xi_w^*$  = ( $\xi_w = |n_w|$ ) =  $\pm$  pseudo-stoichiometric number of water molecules of  $W_{\text{I}}$   
 $\pm \xi^*$  = coefficient of water iceberg  $W_{\text{II}}$  binding to the solute (+) or dissociating (-) from the solute  
 TED = thermal equivalent dilution principle ( $-R \ln(a_i)^n = n C_{p,L} \ln T$ )  
 $\Delta H_{\text{dual}}$  = experimental enthalpy ( $\Delta H_{\text{dual}} = \Delta H_{\text{mot}} + \Delta H_{\text{th}} = \Delta H_0^{(\xi_w=0)} + \Delta H_{\text{for}} \pm \xi_w C_{p,w} T$ )  
 $\Delta S_{\text{dual}}$  = experimental entropy ( $\Delta S_{\text{dual}} = \Delta S_{\text{mot}} + \Delta S_{\text{th}} = \Delta S_0^{(\xi_w=0)} + \Delta S_{\text{for}} \pm \xi_w C_{p,w} \ln T$ )  
 $\Delta H_{\text{th}}$  = thermal enthalpy ( $\Delta H_{\text{th}} = \Delta C_{p,\text{hydr}} \cdot T$ )  
 $dS_{\text{th}}$  = differential change of thermal entropy; differential of intensity entropy  
 $dS_{\text{Ints}}$  = differential of intensity entropy  
 $S_{\text{th}}$  = thermal entropy ( $\Delta S_{\text{th}} = \Delta C_{p,\text{hydr}} \cdot \ln T$ ); intensity entropy  
 $\Delta G_{\text{th}}$  = thermal free energy (null) ( $-\Delta G_{\text{th}}/T = -\Delta H_{\text{th}}/T + \Delta S_{\text{th}} = 0$ )  
 $dS_{\text{conf}}$  = differential change of configuration entropy; differential change of density entropy  
 $dS_{\text{Dens}}$  = differential of density entropy  
 $\Delta S_{\text{conf}}$  = configuration entropy or density entropy ( $\Delta S_{\text{conf}} = R \ln d_{\text{id}}$ )  
 $\Delta S_{\text{dual}}$  = dual (experimental) entropy = intensity entropy + density entropy (ergodic property)  
 $\Delta H_{\text{mot}} \equiv \Delta H_0$  = motive enthalpy, from  $\Delta H_{\text{dual}}$  extrapolated to  $T = 0$   
 $\Delta S_{\text{mot}} \equiv \Delta S_0$  = motive entropy, from  $\Delta S_{\text{dual}}$  extrapolated to  $\ln T = 0$   
 $\varepsilon_{\text{density}}$  = energy concentration  
 $1/\varepsilon_{\text{density}}$  = energy dispersal in space (proportional to density entropy)  
 $\varepsilon_{\text{intens}}$  = energy intensity  
 $1/\varepsilon_{\text{intens}}$  = energy dispersal in time (proportional to intensity entropy)  
 $\{f(1/T) \times g(T)\}$  = convoluted function  
 $\{f(T) \times g(\ln T)\}$  = convoluted function  
 $f(1/T)$  or  $f(T)$  = linear primary function of the convolution ("modified" to a curve by  $g(T)$ ) or  $g(\ln T)$ , respectively)  
 $g(T)$  or  $g(\ln T)$  = "modifying" secondary function of the convolution  
 $\Delta G_{\text{mot}}$  = motive free energy ( $-\Delta G_{\text{mot}} = -\Delta H_{\text{mot}} + T \Delta S_{\text{mot}} \equiv -\Delta H_0 + T \Delta S_0$ );  $-\Delta G^\circ/RT = \ln K$   
 $R \ln K_{\text{mot}} = f(1/T)$  = linear motive binding function in entropy units  
 $RT \ln K_{\text{mot}} = f(T)$  = linear motive binding function in enthalpy units  
 $R \ln K_{\text{dual}} = \{f(1/T) \times g(T)\}$  = curved convoluted experimental binding function  
 $RT \ln K_{\text{dual}} = \{f(T) \times g(\ln T)\}$  = curved convoluted experimental binding function  
 $R = 8.31451 \text{ J K}^{-1} \text{ mole}^{-1}$  = gas constant

$k_B = 1.381 \times 10^{-23} \text{ J K}^{-1} \text{ molecule}^{-1}$  = Boltzmann constant  
 $\xi_w^{-1}$  = unitary, for  $\xi_w = 1$   
 $T_d$  = denaturation temperature  
 $T_H$  = temperature at which  $\Delta H_{\text{dual}} = 0$  (at minimum in class **A** or at maximum in class **B**)  
 $T_S$  = temperature at which  $\Delta S_{\text{dual}} = 0$  (at minimum in class **A** or at maximum in class **B**)  
 $N_{\text{Av}}$  = Avogadro's number  
 $S = k_B \ln \Omega$  = Boltzmann statistical entropy,  $k_B = 1.38064852(79) \times 10^{-23} \text{ J K}^{-1}$   
 $k_B N_{\text{Av}} \ln \Omega^{(1/N_{\text{Av}})} = R \ln Z_M$  = molar partition function  
 $\Omega$  = statistical energy density (multiplicity)  
 $\eta/T$  = statistical energy intensity  
 $C_{p,A}$  = molar heat capacity of A  
 $\tau_t$  = sojourn time (or persistence time), time spent by molecule  $i$  to run one length unit  
 $\tau_i^2$  = squared sojourn time of each molecule  $i$   
 $(\tau_i^2)_\Sigma$  = cumulative squared sojourn time  
 NoremE = nonreacting molecule ensemble  
 REME = reacting mole ensemble  
 Che. m/M. sF = chemical molecule/mole scaling function

## ■ ADDITIONAL NOTES

<sup>a</sup>Sojourn time (or persistence time) is calculated as  $\tau_i = 1/l_i$  where  $l_i$  is the path length of molecule  $i$ .

<sup>b</sup>For the different roles of the  $f$  function and  $g$  function in the convoluted function, see Appendix D in part I.<sup>5</sup>

<sup>c</sup>As far as we know, these are the first examples of experimental determinations of ergodic property of TED (thermal equivalent dilution). Up to now, ergodicity had been an assumed property of simulation calculations with supposed equivalence of Monte Carlo and molecular dynamics calculations. TED is an intrinsic invariable potential molecular property of every hydrophobic hydration system, but it becomes active in some cases only.

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