SUPPORTING INFORMATION

HYDROPHOBIC HYDRATION PROCESSES.
II. INTENSITY ENTROPY AND NULL THERMAL FREE ENERGY,
DENSITY ENTROPY AND MOTIVE FREE ENERGY

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APPENDIX A
Calculation of Thermodynamic Functions for Structure-based Drug Design

The structure-based drug design can follow the procedure of Talhout: we subtract the thermal components $\Delta H_{th}$ and $\Delta S_{th}$ from the observed apparent functions $\Delta H_{dual}$ and $\Delta S_{dual}$, respectively, and we obtain the linear motive function $RT\ln K_{mot}=f(T)$. This procedure, at variance with other procedures usually applied in computer assisted drug design, starts with the experimental determination of an equilibrium constant $\ln K$ at different temperatures.

We have shown how in every hydrophobic process the convoluted Binding Functions $R\ln K_{dual}={f(1/T)*g(T)}$ and $RT\ln K_{dual}={f(T)*g(lnT)}$ necessarily exist. It is not recommendable the application of anyone of the many computer simulations, completely unbound to any experimental evidence: being computer simulations referred to a monocentric partition function, it is impossible to calculate convoluted binding functions. The Ergodic Algorithmic Model (EAM) represents the analytical mathematical properties of the Binding Functions, calculated from a dual structure partition function (DS-PF), conform to the biphasic composition of every hydrophobic hydration system.

We report here the successive steps to be followed for the treatment of the experimental data.

![Figure S1. Class B. Experimental Binding Functions $R\ln K_{dual}={f(1/T)*g(T)}$ and $RT\ln K_{dual}={f(T)*g(lnT)}$.](image-url)

The Bonding Functions are analysed by the Ergodic Algorithmic Model (EAM).

One begins by determining the equilibrium constant at five or six temperatures; then the data are plotted against $1/T$ or against $T$, thus obtaining curved convoluted functions (Figure S1).

The motive Entropy $\Delta S_{mot}$ is contributing to Free Energy as well $\Delta H_{mot}$, whereas the thermal functions $\Delta H_{th}$ and $\Delta S_{th}$ are not contributing to Free Energy; rather the thermal terms are components of the secondary functions $g(T)$ and $g(lnT)$, producing the curvature of the linear motive functions ($f(1/T)$ and ($f(T)$, respectively. The thermal functions can be calculated by the curvature amplitude of the apparent binding function $RT\ln K_{dual}=-\Delta G_{dual} = {f(T)*g(lnT)}$. Then, the thermal components are subtracted from the experimental dual functions $\Delta H_{dual}$ and $T\Delta S_{dual}$, respectively, to get the motive functions. The Binding Function $RT\ln K_{dual}=-\Delta G_{dual} = {f(T)*g(lnT)}$ as convoluted function is expressed as

$$RT\ln K_{dual}=-\Delta G_{dual} = -\Delta H_{dual} + T\Delta S_{dual}$$  \hspace{1cm} (S1)

The Binding Function $RT\ln K_{dual}$ is a parabolic curve, analogous to that in Figure S9 of Supporting Information, APPENDIX F. By including the explicit expressions for thermal enthalpy and entropy, we obtain

$$RT\ln K_{dual}=-\Delta G_{dual} = (-\Delta H_{mot} + \Delta C_{p,hydr} T) + T(\Delta S_{mot} - \Delta C_{v,hydr} \ln T)$$  \hspace{1cm} (S2)

By subtraction of the thermal contributions from the convoluted function, we obtain the motive function

$$RT\ln K_{mot} = -\Delta G_{mot} = -\Delta H_{mot} + T\Delta S_{mot}$$  \hspace{1cm} (S3)
which, reported as the function of $T$, is a straight line, whereas equation (S2), as convoluted function, represents a parabolic curve.

In the following, we indicate how one can calculate the motive functions starting from the experimental data obtained at different temperatures:

1) calculate the polynomials representing:
   (a) experimental Binding Functions $R \ln K_{\text{dual}} = -\Delta G_{\text{dual}} / T = \{f(T) \cdot g(\ln T)\}$
   (b) experimental Binding Function $R T \ln K_{\text{dual}} = -\Delta G_{\text{dual}} = \{f(T) \cdot g(\ln T)\}$
   by interpolating the experimental data ($R \ln K_{\text{dual}}$ or $R T \ln K_{\text{dual}}$), when plotted as the function of $1 / T$ and $T$, respectively.

2) calculate the tangents of the Binding Functions as derivatives $\partial (R \ln K_{\text{dual}}) / \partial (1 / T)$ and $\partial (R T \ln K_{\text{dual}}) / \partial (T)$

3) plot $\partial (R \ln K_{\text{dual}}) / \partial (1 / T)$ against $T$ and $\partial (R T \ln K_{\text{dual}}) / \partial (T)$ against $\ln T$, respectively, thus obtaining in both diagrams the same slope $\Delta C_{\text{p,hydr}}$.

4) calculate the motive Free Energy by subtraction of the thermal functions from
   $$\{-\Delta G_{\text{dual}} / T\} = \{-\Delta G_{\text{mol}} / T\} \{-\Delta H_{\text{dual}} / T + \Delta H_{\text{th}} / T + \Delta S_{\text{dual}} + \Delta S_{\text{th}}\} = \{-\Delta H_{\text{mol}} / T\} + \Delta S_{\text{th}}$$  \hspace{1cm} (S4)

5) calculate the iceberg formation function
   $$\{-\Delta G_{\text{for}} / T\} = \{-\langle \Delta h_{\text{for}} \rangle_{A} \cdot \xi_{W} \} / T + \{-\langle \Delta S_{\text{for}} \rangle_{A} \cdot \xi_{W} \}$$  \hspace{1cm} (S5)
   with $\langle \Delta h_{\text{for}} \rangle_{A} = -22.7 \pm 0.7 \text{ J} \cdot \text{mol}^{-1} \cdot \xi_{W}^{-1}$ and $\langle \Delta S_{\text{for}} \rangle_{A} = -445 \pm 3 \text{ J} \cdot \text{K}^{-1} \cdot \text{mol}^{-1} \cdot \xi_{W}^{-1}$

6) subtract the iceberg formation function
   $$\{-\Delta G_{\text{affin}} / T\} = \{-\Delta G_{\text{mol}} / T + \Delta G_{\text{for}} / T\} = \{-\Delta H_{\text{mol}} / T + \Delta H_{\text{for}} / T\} + \{-\Delta S_{\text{app}} - \Delta S_{\text{for}}\}$$  \hspace{1cm} (S6)

7) obtain the affinity of the residual chemical reaction
   $$\{-\Delta G_{\text{affin}} / T\} = \{-\Delta H_{\text{aff}} / T\} + \{-\Delta S_{\text{aff}}\}$$  \hspace{1cm} (S7)

8) compare, whenever possible, the results of computer simulations with the thermodynamic functions experimentally determined.
APPENDIX B

*molecule/Mole* Scaling Function or *Quasi*-Chemical Approximation

A quasi-chemical view of the solvation free energy of hard-core solutes

\[
\beta \mu_c = \ln (\nu \rho_o / \langle \exp(-\beta \Delta U) \rangle \sigma_o) \ z_o \quad (S8)
\]

is presented by Pratt. The activity of an isolated solute \( \nu \rho_o / \sigma_o \) corresponds to the density

\[
\rho_o = \langle \exp(-\beta \Delta U) \rangle \sigma_o \ z_o (q_o / V) \quad (S9)
\]

where

\[
z_o = \exp(\beta \mu_c) \quad (S10)
\]

The Ergodic Algorithmic model has resolved the problem of connection between “molecule” ensembles and “Mole” chemical sets. As shown in equation (44-46), the Quasi-chemical model\(^1\), introduced by the theory of hydrophobic effects, is a possible answer to this question, perfectly conformed to the Ergodic Algorithmic Model (EAM). The need of a Quasi-chemical model arises from the structure of the Dual-Structure Partition Function \( \{DS-PF\} \). The Thermal-Partition Function \( \{T-PF\} \), referred to the solvent, is representative of a Norem\(E \) statistical ensemble constituted by an extremely large population of molecular units (see Figure 5), each molecule being associated to a small energy \( \varepsilon / kT \). This large population is subject to Boltzmann Statistics. The Motive Partition Function \( \{M-PF\} \) referred to the solute, is constituted by a population of few Moles \( \{REME \text{ ensemble}\} \) distributed over macrolevels \( H_i \) distribution not ruled by Boltzmann Statistics (see Figure 6) rather by binomial distribution. Just to give an example, the Stirling approximation is not applicable to a restricted population of Moles and suited to treat problems concerning the solute. The name of “Quasi-Chemical approximation” is not adequate to the real important role played by this relationship. In Norem\(E \) statistical ensembles, the partition function resulting from the calculation is a single number, an extremely large number \( \Omega = z^{N_{rem}} \). An example is Boltzmann equation

\[
S = k_B \ln \Omega \quad (S11)
\]

where \( S \) is referred to the probability of finding one molecule in an Avogadro population of molecules and \( k_B \) is Boltzmann constant \( (k_B = 1.381 \times 10^{-23} \text{ J·K}^{-1} \cdot \text{molecule}^{-1}) \). It is possible the passage from molecule population scale to Mole population scale by taking the Avogadro \( N_a \)-root of \( \Omega \)

\[
S = (N_{Av} \cdot k_B) \ln \Omega^{1/(N_{Av})} = R \ln x_A \quad (S12)
\]

where \( x_A \) is molar fraction and \( R = N_a \cdot k_B \) \( (R = 8.31451 \text{ J·K}^{-1} \cdot \text{Mole}^{-1}) \). In reacting REME ensembles, the statistical molecular distribution, ruled by Boltzmann statistics, is restricted within the microlevel distribution of each macrolevel \( H_i \). The distribution of Moles among the macrolevels \( H_i \) is ruled by binomial distribution of chemical reactions and is represented by a molar partition function composed by summation of \( (n+1) \) terms. We use \( n=2 \) to simplify the text representation

\[
Z_M = (1+ k \ a_i)^2 = 1 + 2 k a_i + k^2 a_i^2 \quad (S13)
\]

where \( a_i \) is the activity of the ligand \( L \), \( k \) is the site binding constant, and \( 2 \) is the number of binding sites. The Quasi-Chemical approximation guarantees that the resulting Partition Function \( \{DS-PF\} \) has the structure of sum of terms, adequate to the chemical reaction. \( Z_M \) can be rewritten as (see equation (49))

\[
Z_M = (1+ k \ a_i)^n = (1+ [k^* (x_{1,2,3}^0)] \cdot a_i)^n \quad (S14)
\]
where the factor \( \{z_{(N,V,T)}\} \) represents the molecule distributions within each macrolevel, and \([k^*\{z_{(N,V,T)}\}]\) is the computed site constant. The mathematical structure of equation (S14) is conform to PTD because it is focused on a distinguished solute molecule of the species of interest: specifically, the molecule of interest is the central molecule of the hydration complex. The hydrated complex is also suited to application of Quasi-Chemical approximation, which conjoins the Boltzmann statistical distribution function of molecules to the binomial distribution function of Moles.

On the ground of these arguments, we suggest eliminating the word “approximation” and to change the title of this function into that, more appropriate to the relevance of this relationship, of “Chemical molecule/Mole scaling Function” (Che.m/M.sF).

The Molar partition function

\[
Z_M = (1+[k^*\{z_{(N,V,T)}\}] \cdot a_i)^{\xi^*}
\]

\( (S15) \)
can be calculated, with \( \{z_{(N,V,T)}\} \) as molecule statistical distribution around each macrolevel \( H_j \) and \([k^*\{z_{(N,V,T)}\}]\) is a calculated site constant. The number \( \xi^* \) is a reaction coefficient of water \( W_i \) binding to hydrophobic moieties. The coefficient \( \xi^* \) is a number representing the ratio between numbers of molecules whereas \( \xi_w \) is in general non-integer being the ratio between volume of the entering molecule and volume of one cluster \( W_i \). The ratio of volumes is in general non-integer. \( \xi^* \) is smaller than \( \xi_w \) (\( \xi^* < \xi_w \)) if not all the water molecules expelled from the solvent might become iceberg ligands of the central solute molecule.
APPENDIX C

Entropy = Energy Dispersal Parameter

A) Density Entropy:

1) Energy dilution and Mole dilution

![Reacting Ensemble](image)

Figure S2. Reacting Ensemble: Energy concentration ($E_{\text{density}}$) at changing dilution $d$: $E_3 < E_2 < E_1$ with $d_3 > d_2 > d_1$

Red colour intensity is proportional to Energy concentration ($E_{\text{density}}$)

2) Density Entropy, Mole dilution, and energy dilution

![Reacting Ensemble](image)

Figure S3. Reacting Ensemble: Density Entropy at changing dilution $d$: $S_3 > S_2 > S_1$, with $d_3 > d_2 > d_1$

Blue colour intensity is proportional to Density Entropy $S_{\text{Dens}}$

$$S_{\text{Dens}} = 1/E_{\text{density}} = f(d_A) \quad (S16)$$

B) Intensity Entropy:

1) Energy Intensity ($E_{\text{intens}}$) inversely proportional:

to Temperature $T$, to velocity of molecules, and to Energy Dispersal in time ($1/E_{\text{intens}}$)

![Non-Reacting Ensemble](image)

Figure S4. Non-reacting Ensemble: Energy Intensity ($E_{\text{intens}}$) at changing Temperature $T$: $E_3 < E_2 < E_1$, with $T_3 > T_2 > T_1$

Red colour intensity is proportional to Energy Intensity ($E_{\text{intens}}$)
2) **Intensity Entropy** \(S_{\text{Ints}}\) proportional to Energy Dispersal in time \(1/\varepsilon_{\text{Inten}}\)

As shown in Section 2, the parameter characterising the energy dispersal in time \(1/\varepsilon_{\text{Inten}}\) is the sojourn time \(\tau\), which is the time spent by each molecule to run the length unit. The mean sojourn time \(\tau_m\) is inversely proportional to mean velocity \(v_m\). We use the squared mean sojourn time \(\tau_m^2\) because it is inversely proportional to the squared mean velocity \(v_m^2\). According to the kinetic theory, the squared mean velocity is directly proportional to the absolute temperature \(\text{dln}(v_m^2) = \text{dln}T\), thus connecting the energy dispersal in time (Intensity Entropy) with the reciprocal of squared mean sojourn time \(1/\tau_m^2\) and, consequently, with the absolute temperature \(T\), which is experimentally detectable.

\[
S_{\text{Ints}} = 1/\varepsilon_{\text{time}} = f(T) \quad (S17)
\]

Ergodicity guarantees that, at null concentration (e.g. a solvent, Figure 17), a change of Intensity Entropy is obtained by changing the temperature \(T\).

In a solution, ergodicity guarantees that, by changing the temperature \(T\), a change of Intensity Entropy can be obtained like as the change of Density Entropy obtained by changing dilution, by Thermal Equivalent Dilution (TED),

\[
-R\text{dln}(a_i)^n = n \ C_{p,L}\text{ dln}T \quad (S18)
\]

where activity \(a_i\) is

\[
a_i = x_i \cdot \Phi \quad (S19)
\]

with Lambert Thermal Energy Factor \(\Phi\)

\[
\Phi = f(1/\tau_m^2) = T^{-((C_{p,L}/R))} \quad (S20)
\]

The Lambert thermal Energy Factor \(\Phi\) in equation (S20) is the source of Density Entropy. The relationship implicit in \(\Phi\) between reciprocal squared sojourn time \(1/\tau_m^2\) and temperature \(T\) is the same as that between reciprocal squared sojourn time \(1/\tau_m^2\) and temperature \(T\) in Intensity Entropy. This means that Density Entropy and Intensity Entropy are intrinsically the same physical process, modulated by the velocity of the molecules, whereby energy dispersal in time is produced: this explains why they can be summed to each other. The only difference (quantitative but not qualitative) between them derives from solution (i.e. \(x_i\)) and thermal (i.e. \(C_{p,L}\)) properties of the molecules which they are referred to, respectively. The concentration factor \(x_i\) connected to Density Entropy explains why Density Entropy is contributing to free energy, which depends on concentration, whereas Intensity Entropy is not contributing to free energy, being independent from concentration.
APPENDIX D

Exponential Probability Space. Logarithmic Thermodynamic Space

The probability space can be represented by the exponential expression referred to the specific system

$$\exp(-\Delta G/RT) = \exp(-\Delta H/RT) \exp(\Delta S/R) \quad (S21)$$

If we plot this expression in an orthogonal diagram with abscissa $x(\exp(-\Delta H/RT))$ and ordinate $y(\exp(-\Delta S/R))$, (Figure 18) we obtain an equilateral hyperbola. A set of expressions $\exp(-\Delta G/RT) = f(x,y)$ with increasing values $(-\Delta G/RT)$ is represented by a set of homologous equilateral 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 ordinate

$$y(\exp(-\Delta H/RT))$$

or the value of abscissa $x(\exp(\Delta S/R))$ of point A, we choose a point on that hyperbola, thus reaching all the information elements available. This point cannot be chosen by computer simulation. This is the essential information element that we lose by computer simulation: we cannot fix a point referred to the specific system on the chosen hyperbola. On the auxiliary coplanar axis $z$, we read the scale of values $\exp(-\Delta G/RT) = f(x,y)$. By computer simulation, we might perhaps choose the correct hyperbola, but we cannot fix any point A for that specific reaction, losing essential information elements.

The Probability Space of Figure S6, representing the exponential functions, generates the Thermodynamic Space of Figure S7, where the exponents are represented in an orthogonal axis system. In this system, the abscissa represents Density Entropy and the ordinate represents Intensity Entropy. The hyperbolas are transformed into a set of parallel lines, orthogonal to the diagonal auxiliary axis $(-\Delta f/T) = (-\Delta G/RT) \cdot \cos 45^\circ$. The coordinates of point A can be determined by experimental determination of

![Figure S6. Probability space represented by exponential probability functions.](image)
Figure S7. Vector representation of free energy in Thermodynamic Space

\[ (-\Delta G/RT) = R \ln K \], measured at different temperatures \( T \), and analysed following the Ergodic Algorithmic Model (EAM). Computer simulations might even guess (Figure S7) the correct blue vector \((-\Delta \Gamma/T)\) but ignore the red vectors \((\Delta S)\) and \((-\Delta H/RT)\).

Computer simulations calculate the function

\[ R \ln K = (-\Delta G^*/RT) = \left( -\Delta \Gamma/T \right) / \cos 45^\circ \]  \hspace{1cm} (S22)

arbitrarily ignoring any difference between the two terms \((\Delta S)\) and \((-\Delta H/T)\) and, consequently, cancelling all the information elements characterising each specific reaction. By applying the Ergodic Algorithmic Model (EAM) to a set of potential parameters (e.g. \( R \ln K \)), referred to one specific hydrophobic hydration process, experimentally determined at different temperatures, one can calculate the function

\[ R \ln K_{mot} = (-\Delta H_{mot}/T) + (\Delta S_{mot}) \]  \hspace{1cm} (S23)

The two terms of this motive function are specific of each compound and suited to recover the information elements of each hydrophobic hydration process, cancelled and lost by computer simulations, together with other additional important information elements, like as \( \pm \xi_w \), pseudo-stoichiometric number of water molecules \( W_i \).
APPENDIX E

Approximations of Computer Simulations

The usual procedures employed in computer simulations can be distinguished, roughly speaking, in Molecular Dynamics Methods and Monte Carlo Methods. The former methods calculate Free Energy (or thermodynamic potential $\mu$) as the function of a time variable (temperature and molecule velocity) whereas the latter methods calculate Free Energy (or thermodynamic potential $\mu$ as the function of a space variable (density, dilution, Boltzmann distribution). Free energy calculated by Molecular Dynamics Methods is obtained as total mean Intensity Entropy $\langle -\Delta G_{\text{cal}}/T \rangle = -\langle -\Delta H/T \rangle$ whereas Free Energy calculated by Monte Carlo Methods is obtained as total mean Density Entropy $\langle -\Delta G_{\text{cal}}/T \rangle = -\langle \Delta S \rangle$. In computer simulation processes, the two procedures are assumed to be equivalent: the assumption is defined as ergodic property of the system. In this context, the definition of ergodic property is misleading because “ergodicity” means that Intensity Entropy and Density Entropy, which as physical processes are intrinsically different, as “ergodic” they might be in part equivalent as far as entropic effects of energy dispersal: dispersal of energy in time, as Intensity Entropy, equivalent to energy dispersal in space, as Density Entropy. Altogether, the two types of Entropy keep the point that the respective amounts of the two components terms of free energy $\langle -\Delta G^{\text{mot}}/T = -\Delta H_{\text{mot}}/T + \Delta S_{\text{mot}} \rangle$, experimentally determined by Ergodic Algorithmic Model (EAM), and Density Entropy $\langle -\Delta H_{\text{mot}}/T \rangle$ and Density Entropy $\langle \Delta S_{\text{mot}} \rangle$, are necessarily different from each other $\langle -\Delta H_{\text{mot}}/T \neq \Delta S_{\text{mot}} \rangle$, as characteristic property of each chemical process. We recall that the two separate terms can be experimentally determined by Ergodic Algorithmic Model (EAM). If we assume that we can calculate Free Energy indifferently by both methods, MD and MC, we assume, erroneously, that they are intrinsically identical physical processes with equal, indistinguishable, intensity $\langle -\Delta H/T \rangle$ and density $\langle \Delta S \rangle$ entropic functions. In such a way, the information elements of the reaction, provided by the different specific values of Intensity Entropy $\langle -\Delta H_{\text{mot}}/T \rangle$ and Density Entropy $\langle \Delta S_{\text{mot}} \rangle$, experimentally determinable by application of the Ergodic Algorithmic Model (EAM), are completely ignored and lost by computer simulations. All computer simulations are arbitrarily approximated, with inherent uncertainty and loss of information.

The approximation produced by the omission by computer simulations of the essential parameter of Intensity Entropy factor $\langle -\Delta H_{\text{mot}}/T \rangle$ and Density Entropy factor $\langle \Delta S_{\text{mot}} \rangle$, specific for each compound, represents a serious problem for the widely diffused method of analysis by computer simulations of biochemical processes and of structure-based drug design. It is an ideological misconception by computer simulations, the application of mathematical functions derived for molecule ensembles, composed by myriads of particles, which are ruled by Boltzmann statistics, to reacting Mole ensembles composed by few elements, ruled by mathematical binary distributions.

We presume that from now on, it will be impossible to undertake any study of biochemical processes in water or any research of drug design without a previous collection of sets of experimental determinations of convoluted binding functions

$$ R\ln K_{\text{dual}} = -\Delta G_{\text{dual}}/T = f(1/T) \star g(T) \quad \text{and} \quad R\ln K_{\text{dual}} = (f(T) \star g(\ln T)), $$

(S24)

to be analysed by Ergodic Algorithmic Model (EAM). It is useless to employ larger and larger capacities of computer memory or prolong the reaction time, if we employ inadequate algorithms. It will be essential finding out the mathematical rational connections between binomial distributions of few reacting Moles and statistical distributions of molecule ensembles, composed by myriads of elements, occupying the microlevels $h_{ij}$ of each single macrolevel $H_j$ of a quantised model.
APPENDIX F

Experimental Convoluted Binding Functions

Figure S8. Class A. Convex ($\Delta C_{p,hydr}>0$) Convoluted Binding Functions $R\ln K_{dual}=[f(1/T)*g(T)]$ (A) and $RT\ln K_{dual}=[f(T)*g(ln T)]$ (B), (at constant $d_{dual}$). $T_H$ (for Enthalpy, $H$) and $T_S$ (for Entropy, $S$) are the minima of the curves.

Figure S9. Class B. Concave Convoluted Binding Functions.

Convoluted functions $R\ln K_{dual}=[f(1/T)*g(T)]$ (A) and $RT\ln K_{dual}=[f(T)*g(ln T)]$ (B), (Class B, $\Delta C_{p,hydr}<0$) at constant $d_{dual}$. $T_H$ (for Enthalpy, $H$) and $T_S$ (for Entropy, $S$) are the maxima of the curves.

Figure S10. Class A: Tangents to the convoluted binding functions

A) $\partial (R\ln K_{app})/\partial (1/T) = - \Delta H_{app} = - \Delta H_{mot} - \Delta C_{p,hydr} T$ (J Mole$^{-1}$)

B) $\partial (RT\ln K_{app})/\partial T = \Delta S_{app} = \Delta S_{mot} + \Delta C_{p,hydr} \ln T$ (JK$^{-1}$ Mole$^{-1}$).

Slope $\Delta C_{p,hydr}$: equal in the two diagrams, due to equal curvature amplitudes.
Figure S11. Class B. Tangents to the binding functions.

A) \( \frac{\partial (R \ln K_{\text{dual}})}{\partial (1/T)} = -\Delta H_{\text{dual}} = -\Delta H_{\text{mot}} - \Delta C_{p,\text{hydr}} T \) (J\,Mole\(^{-1}\))

B) \( \frac{\partial (RT \ln K_{\text{dual}})}{\partial T} = \Delta S_{\text{dual}} = \Delta S_{\text{mot}} + \Delta C_{p,\text{hydr}} \ln T \) (JK\,Mole\(^{-1}\)).

Slope \( \Delta C_{p,\text{hydr}} < 0 \). equal in the two diagrams, for equal curvature amplitudes.
Null Thermal Free Energy

The formulas reported in the literature concerning the thermal functions $\Delta H(T)$, $\Delta S(T)$ and $\Delta G(T)$ for protein unfolding, and for micelle formation are the following

\[
\Delta H(T) = \Delta H(T_o) + \int_{T_o}^{T} \Delta C_p,\text{hydr} \, dT = \Delta H(T_o) + \Delta C_p,\text{hydr} \,(T- T_o)
\]  

(S25)

\[
\Delta S(T) = \{\Delta H(T_o)/T\} + \int_{T_o}^{T} \Delta C_p,\text{hydr} \, d\ln T = \{\Delta H(T_o)/T\} + \Delta C_p,\text{hydr} \, \ln(T/T_o)
\]  

(S26)

\[
\Delta G(T) = 0 - (\Delta C_p,\text{hydr}/T) \,(T_o - T) + T \ln(T/T_o)
\]  

(S27)

where $T_o$ is the temperature at which half of the molecules in the system are unfolded.

An attentive consideration of the expression of equation (S25) for enthalpy, $\Delta H(T)$ leads to the hypothesis that the expression is dealing exactly and only with thermal enthalpy, $\Delta H_{\text{th}}$. The integration of $\Delta C_p \, dT$ between the limits (inferior) $T_o$ and (superior) $T$ would be only a small interval with respect to the integral of equation (35) in Part I which is integrated between the limits $T=0$ (i.e. $\zeta_w$ molecules $W_i$ at rest) and $T$ (i.e. $\zeta_w$ molecules $W_i$ brought in thermal agitation at the temperature $T$). We could assume that the remaining part of our integral between $T=0$ and $T=T_o$ is contained in the constant term $\Delta H(T_o)$ of equation (S25). This hypothesis is confirmed if we analyse equation (S26) concerning entropy, where the integration constant $\Delta S(T_o)$ is calculated as $S(T_o) = \{\Delta H(T_o)/T\}$. This equality can be assigned only to thermal enthalpy and thermal entropy. Alternatively, we can assume the hypothesis that $\Delta H_{\text{mot}}$ is implicitly included in $\Delta H(T_o)$: were this the case, however, then it should be written $\Delta S(T_o) \neq \{\Delta H(T_o)/T\}$ and equation (66) would be not correct. In any case, the existence of separated specific motive functions is completely ignored by these Authors. The true catastrophe, however, is in equation (S27) where the thermal free energy is considered different from zero whereas, according to the Ergodic Algorithmic Model (EAM) it would be invariably zero, as shown in equation (4) (conform to equation (44) in Part I). On the other hand, the motive free energy of these processes, according to the Ergodic Algorithmic Model (EAM), can be calculated only by considering the process, ruled by $\{\text{M-PF}\}$, of iceberg formation (Ref. 5, Section 5), together with other processes of protonation or complex formation, all of them being reactions of the solute and not of the solvent.

It is worth mentioning that equation (S27) represents a heresy for general thermodynamic theory because thermal energy, as Intensity Entropy, cannot produce any chemical work, in a non-reacting system (NoremEn ensemble), such as the solvent, wherein no concentration change and hence no potential change $\Delta \mu$ is possible. Intensity Entropy change only can be produced by the solvent, with null thermal Free Energy ($\Delta G_{\text{th}} = 0$). It is disappointing that too many textbooks and articles, diffuse this wrong information. Mechanical work is possible in these non-reacting systems (e.g. Carnot cycle). On the other hand, the expression of equation (S27) is mathematically incorrect. If correctly developed, it results to be equal to zero, as required by thermal partition functions. In fact, equation (S25) and equation (S26) represent equal functions, because we should rewrite equation (S27) as
\[ \Delta G_{th}/T = 0 - (\Delta C_{p,hyd}/T) \left\{ [T_d - T] + T \ln(T/T_d) \right\} \]

\[
= 0 - \left\{ \left( \frac{1}{T_d} \int_{T=0}^{T_d} \Delta C_{p,hyd} dT - \int_{T=0}^{T=1} \Delta C_{p,hyd} d\ln T \right) \right\}
\]

\[
= 0 - \left\{ (\Delta H_{th}/T_d) - (\Delta S_{th}) \right\} = 0 - 0 \quad \text{(S28)}
\]

Please, note that the two integrals are practically equal (=), because the integration low limits are \( T=0 \) and \( \ln T=1 \) (1=0), respectively, and the two integrals are practically equal under the normal condition that \( T_d>273K>>1K \). We can write, at variance with the erroneous equation (S27),

\[ \Delta G(T)/T = \Delta H(T)/T - \Delta S(T) = 0 \quad \text{(S29)} \]

(see equation (43) in Part I)\(^4\) what is conform to the invariable property of null thermal free energy \( (\Delta G_{th}/T = 0) \) of thermal functions.
**APPENDIX H**

**Thermal Equivalent Dilution (TED) and the 2\textsuperscript{nd} Law**

We recall that the two types of differential variation of Entropy are expressed by different equations. Configuration change of Entropy or *Density Entropy* change at constant $T$ is represented by

$$ (dS_{Dens})_T = -R \ln n_x A = R \ln d_{id(A)} $$

whereas thermal change of Entropy or *Intensity Entropy* change at constant $d_{id}$ is represented by

$$ (dS_{Ints})_{d, id} = (d(H/T))_{d, id} = (d(C_p, A dT))_{d, id} = (C_p, A d\ln T)_{d, id} $$

We can show how the analytical expressions of configuration and thermal changes of Entropy, respectively, follow directly from the conditions at molecule level foreseen by the ergodic theory. In general, we note (Table S1) that to each ergodic parameter $x_A$ or $\tau_m^2$ at molecule level corresponds a certain change, either configuration or thermal change of the entropy function at thermodynamic level, according to the scheme: it is worth noting the equivalence between ideal dilution $d_{id(A)}$ in equation (9) and temperature $T$ in equation (10), a property that has been experimentally observed\textsuperscript{5-8} as *Thermal Equivalent Dilution (TED)*. The ergodic theory assumes the equivalence between *Density Entropy* (dispersion in space, as function of $x_A$) and *Intensity Entropy* (dispersion in time, as function of $\tau_m^2$) at molecule level: we should find analogous equivalence between configuration $(dS_{Dens})_T = R \ln d_{id(A)}$ and thermal $(dS_{Ints})_{d, id} = (C_p, A d\ln T)$ changes of the thermodynamic function entropy, at Mole level. This equivalence can be verified by controlling (Table S2) the perfect equivalence of the changes in the properties of the system either by changing ideal dilution $d_{id(A)}$ either by changing temperature $T$.
Table S2. Configuration Entropy and Thermal Entropy

| ENTROPY (Energy Dispersion) |
|----------------------------|
| Configuration Entropy      | Thermal Entropy |
| Energy Dispersion in Space | Energy Dispersion in Time |

| ENTROPY DENSITY | ENTROPY INTENSITY |
|-----------------|-------------------|
| Variable\(^{(i)}\): \(d_{id}(A)\) \((d_1 < d_2)/(*)\) | \(T\) \((T_1 < T_2)\) |
| \(^{(i)}\) Factor in bold type is constant | \(^{(i)}\) Factor in bold type is constant |
| Molecule-Concentr. Low | Sojourn-Time Low |
| Molecule-Dilution High | Molecule-Velocity\(^{*}\) High |
| Presence-Density Low | Presence-Intensity Low |
| Th. Ener. Density \([(x_A) \cdot (T - (C_{p,A}/R))]\) Low | Th. Ener. Intensity \([(x_A) \cdot (T - (C_{p,A}/R))]\) Low |
| Th. Ener. Dispers. \([(1/x_A) \cdot (T(5/2R))]\) High | Th. Ener. Dispers \([(1/x_A) \cdot (T(5/2R))]\) High |
| ConFigure Entropy \(\exp(S/R) = (d_{id}(A) \cdot (T(5/2R))]\) High | Therm. Entropy \(\exp(S/R) = (d_{id}(A) \cdot (T(5/2R))]\) High |
| Density Entropy \(\exp(S_{Dens}/R) = (d_{id}(A) \cdot (T(5/2R))]\) High | Intensity Entropy \(\exp(S_{Ints}/R) = (d_{id}(A) \cdot (T(5/2R))]\) High |
| \(^{*}\) Ideal Dilution \(d_{id}(A) = 1/x_A\) | \(^{*}\) \(\ln(T) \rightarrow \ln(v^2)\) |

This equivalence will be exploited to find out, by treating the experimental data, the pseudo-stoichiometric coefficient \(\pm \xi_w\), by measuring the equilibrium constant \(\ln K_{dual}\), or other appropriate potential function, at different temperatures.

The TED principle can be assimilated to an equivalence between Entropy fluxes (Figure 24). Each flux can be represented by one cylinder with base \(R\) or \(C_p\) \((C_p = (5/2)R)\) and height \(\ln d_{id}(A)\) or \(\ln T\), respectively.

Each flux represents a change of energy dispersal, i.e. a change of Entropy. If the volumes of the two cylinders are equal, they represent equal fluxes and equivalent Entropy changes. Equation (9) and equation (10) can be joined into a unique differential equation

\[
dS = (\delta Q/T_d) + (R \ln d_{id}(A))/T \\
(532)
\]
Figure S12. TED principle. Equivalence of Entropy fluxes:

*Density Entropy* (configurational) and *Intensity Entropy* (thermal)

where the first term indicates an *Intensity Entropy* change, and the second term a *Density Entropy* change, respectively. For both terms, the 2nd Law of Thermodynamics is valid. The introduction of both terms requires an extension of this law. The usual expression ($dS_{Int} = C_p d\ln T = \delta Q/T \geq 0$) concerning the only *Intensity Entropy* must be completed by $dS_{Dens} = R d\ln A \geq 0$. Hence, the definition of the 2nd Law by R. Clausius (see Part I, APPENDIX A)\textsuperscript{4} can be implemented and now reads:

-(i) Heat can never pass, at constant dilution, from colder to warmer body without any other change, connected therewith, occurring at the same time-

“(ii) molecules can never pass, at constant temperature, from more diluted to more concentrated solutions without spending chemical or electrochemical work”
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