

# Quantitatively Interpreting Thermal Behavior of Self-Associating Systems

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A general method is presented to extract thermodynamic as well as structural information from calorimetric data on self-associating systems using existing statistical thermodynamic models. The method is illustrated with one simple and one complex ligand binding system taken from the literature. The method is also used to extract the aggregation number using a simple mass balance model for self-assembly of surfactant molecules, and experimental evidence is provided to support this.

## Introduction

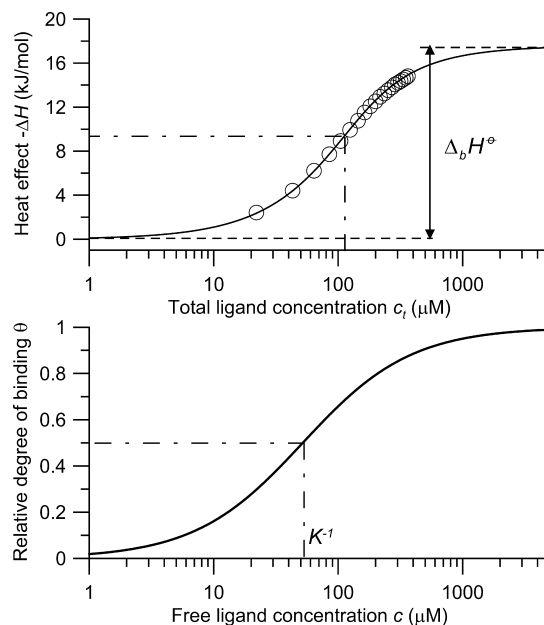
Isothermal titration calorimetry (ITC) is a relatively new technique that is gaining more and more interest because it can provide fast and reliable thermal data on binding equilibria, self-assembly, and other phenomena involving a characteristic enthalpy change.<sup>1–4</sup> The underlying principle is simple: the system at hand, for instance, a macromolecular solution, is titrated by small aliquots of a titrant such as a ligand solution while the temperature of the system is maintained constant. The heat supplied to or removed from the system upon each addition is recorded as being the heat effect associated with the titrant addition. The resulting data are usually depicted as an isotherm of heat effect versus total concentration of titrant; see Figure 1 (top). A model free and often quite sufficient approach to the analysis of ITC data is to take the inflection point to determine the relevant thermodynamic equilibrium constant and the swing of the curve around the inflection point as the value of the associated enthalpy change. A noteworthy method has been developed by Saboury that uses the binding isotherms belonging to two macromolecular solutions of different concentration to extract the isotherm.<sup>5,6</sup>

When potentially more information is available from the ITC data, such as a cooperativity index in the case of ligand binding or an aggregation number in the case of a micellar system, an underlying model is necessary to extract this. While thermodynamic models are available for such systems, these have, to the knowledge of the authors, not yet been used for an adequate interpretation of ITC data.

In this Article, we shall first describe a general method to extract thermal information from ITC data using existing thermodynamic models and we shall illustrate it with a simple example. Subsequently, we shall demonstrate the method by means of two important examples. One is a recent study on the surfactant binding to a polyelectrolyte where cooperativity plays a significant role. The other example is a micellar system of which we shall determine the aggregation number in addition to critical micelle concentration (cmc) and micellization enthalpy. We shall apply the latter model to a collection of isotherms obtained for a variety of surfactant systems.

## Experimental Section

ITC experiments were conducted using a VP-ITC microcalorimeter (MicroCal). The surfactants, *n*-nonyl glucoside (NNON),



**Figure 1.** Heat effect (top) and relative degree of binding (bottom) of calcium ions to human growth hormone (hGH). The circles represent data taken from Saboury,<sup>6</sup> and the drawn lines result from a fit of the simple binding model to the heat effect data.

sodium dodecyl sulfonate (SDS), tetradodecylammonium bromide (TTAB), and sodium deoxycholate (SDC) were obtained from Sigma Aldrich (The Netherlands) and used without further purification. Solutions were made with purified water (Milli Q, Millipore, The Netherlands).

## Theory

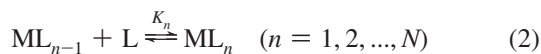
The relation that will be invoked here is generally known as the Gibbs–Helmholtz relation and reads

$$\Delta H = \frac{\partial(\Delta G/T)}{\partial(1/T)} \quad (1)$$

where  $\Delta H$  is the enthalpy change in a process,  $\Delta G$  is the associated Gibbs free energy change, and  $T$  is the absolute temperature. In the following two subsections, it will be

illustrated how this relation is used in different ensembles as occur for ligand binding and self-association.

**Ligand Binding Equilibria.** Consider the binding of ligands L to substrates M having  $N$  binding sites according to



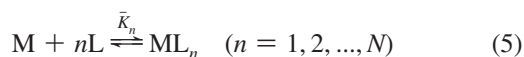
with  $K_n$  being the successive equilibrium constants. In the dilute limit, a full thermodynamic description of this binding process is given by the semi-grand-canonical partition function or *binding polynomial*<sup>7</sup>

$$\Xi(T, N, a_L) = 1 + \bar{K}_1 a_L + \bar{K}_2 a_L^2 + \dots + \bar{K}_N a_L^N \quad (3)$$

in which  $a_L$  denotes the ligand activity that is related to the ligand chemical potential by

$$\mu_L = \mu_L^\circ + RT \ln a_L \quad (4)$$

with  $R$  being the molar gas constant and  $\mu_L^\circ$  being the standard chemical potential of the ligands. The formation constants  $\bar{K}_n$  are defined by the equilibria



and hence the equilibrium constants and the formation constants are related by  $K_n = \bar{K}_n / \bar{K}_{n-1}$ .

One important method to obtain the semi-grand-canonical partition function for linear macromolecules, as will be dealt with here, is the transfer matrix method.<sup>8</sup> A transfer matrix describes how the semi-grand-canonical partition function for a macromolecule is changed when one site is added. Given the transfer matrix, the partition function is found by

$$\Xi(T, N, a_L) \simeq \lambda^N \quad (6)$$

with  $\lambda$  being the largest eigenvalue of the transfer matrix. It is our experience that already for quite small values of  $N$ , such as 12, the asymptotic behavior is reached. For a linear macromolecule with pairwise interacting groups, the transfer matrix is given by

$$T_1 = \begin{pmatrix} 1 & z \\ 1 & zu \end{pmatrix} \quad (7)$$

where  $z = Ka_L$  with  $K$  being the binding constant of a single group and with  $u$  being the cooperativity parameter. For  $u = 1$ , one finds the independent site binding model, for  $u > 1$  one has cooperativity, and for  $u < 1$  anticooperativity. The model goes under various names: it is related to the so-called Ising model for magnetism and was used by many including Katchalsky, Rice and Harris and Marcus to describe protonation of weak polyelectrolytes.<sup>8</sup> Another name is the Satake–Yang model as has been used for the treatment of surfactant–polyelectrolyte binding equilibria.<sup>9</sup> A more involved model has the transfer matrix

$$T_2 = \begin{pmatrix} 1 & z & z & z^2 u \\ 1 & zu & zu & z^2 u^2 vw \\ 1 & z & zu & z^2 uv \\ 1 & zu & zuvw & z^2 u^2 v^2 w^2 \end{pmatrix} \quad (8)$$

In this model, two more interaction parameters are included:  $v$  for next-nearest neighbor interactions and  $w$  for triplet interactions.<sup>8</sup>

In order to extract information from the semi-grand-canonical partition function, derivatives are calculated. For what follows, the two important quantities are the relative degree of binding

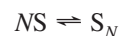
$$\theta = \frac{1}{N} \frac{\partial \ln \Xi}{\partial \ln a_L} \quad (9)$$

and the heat of binding that follows from the appropriate Gibbs–Helmholtz relation as

$$\Delta H = -R \frac{\partial \ln \Xi}{\partial (1/T)} \quad (10)$$

with  $R$  being the molar gas constant. In the latter case, one has to keep in mind that any binding constant is related to the standard enthalpy of binding through a van't Hoff relation. Therefore, the differentiation in the above eq 10 actually proceeds in two steps: one first differentiates versus the acting binding constants which are subsequently differentiated versus inverse temperature so as to extract the relevant enthalpy. In the following section, this will be illustrated by the two examples.

**Association Equilibria.** Consider the micellization equilibrium where monomers S assemble into aggregates  $S_N$ .



It constitutes a simple albeit useful model to describe the association of surfactant molecules into micelles.<sup>10</sup> The chemical potential of the monomers is written as  $\mu_1 = \mu_1^\circ + RT \ln x_1$  with  $x_1$  being their mole fraction and  $\mu_1^\circ$  being the standard chemical potential. Likewise, the chemical potential per monomer in the aggregates is written as  $\mu_N = \mu_N^\circ + RT/N \ln(x_N/N)$ . In equilibrium, these chemical potentials are identical from which follows

$$x_N = N(K_a x_1)^N \quad (11)$$

with the equilibrium association constant given by  $K_a = \exp\{-(\mu_N^\circ - N\mu_1^\circ)/(RT)\}$ . The total amount of surfactant in the system is given by  $x_t = x_1 + x_N$ . This mass balance and the law of mass action (11) can be solved numerically. The model predicts the well-known result of a sharp increase of the aggregate mole fraction with overall mole fraction beyond a certain critical value, the critical micelle concentration (cmc). The Gibbs free energy of this system is

$$\Delta_a G = x_t \mu_1 \quad (12)$$

and, using again the Gibbs–Helmholtz relation, the molar enthalpy change is given as

$$\Delta_a H = \frac{\partial(\mu_1/T)}{\partial(1/T)} = h\Delta_a H^\circ \quad \text{with} \quad h \equiv -\frac{\partial \ln x_1}{\partial \ln K} \quad (13)$$

The scaled enthalpy  $h(x_i)$  varies monotonically with total monomer mole fraction  $x_i$  and has a sharp transition around the “critical micelle concentration” for which we conveniently take the monomer mole fraction  $x_h$  where  $h = 1/2$ . At this point, the slope of the scaled enthalpy function is given by

$$\left(\frac{dh}{dx_t}\right)_{x_h} = \frac{1}{8x_h} \left(N - \frac{1}{N}\right) \quad (14)$$

The above analysis implies that it is possible to extract the aggregation number from ITC data on micellar systems as has been shown before by Marcelis et al.<sup>11</sup> using an enthalpy balance. The purpose of bringing it forward here is to present this finding for a wider audience and to demonstrate its validity.

## Results and Discussion

**Simple Ligand Binding.** Figure 1 (top) gives the molar heat of binding calcium ions to human growth hormone (hGH) taken from Saboury.<sup>6</sup> As this binding is considered to be noncooperative, a simple binding polynomial as

$$\Xi = 1 + Ka_L \quad (15)$$

should suffice. The relative degree of binding follows using eq 9 as

$$\theta = \frac{Ka_L}{1 + Ka_L} \quad (16)$$

which is indeed equivalent to a Langmuir isotherm describing binding to independent groups. Likewise, the heat of binding follows using eq 10 as

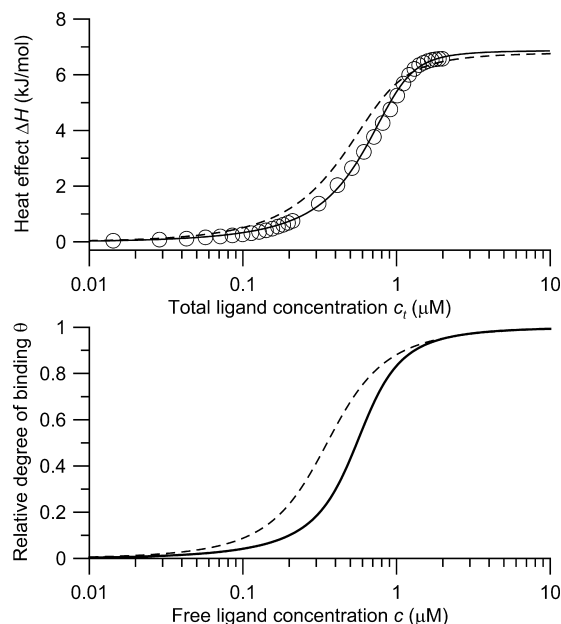
$$\Delta H = \theta \Delta_b H^\circ \quad (17)$$

where we have used the van't Hoff relation between the binding constant  $K$  and the standard enthalpy of binding, that is

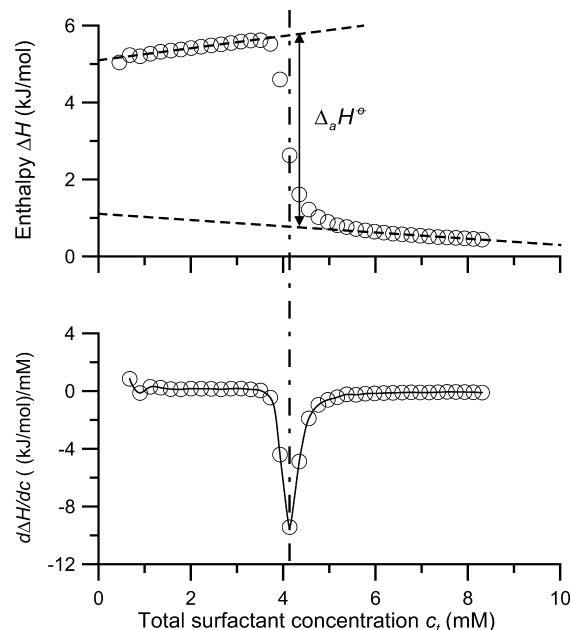
$$\Delta_b H^\circ = -R \frac{\partial \ln K}{\partial(1/T)} \quad (18)$$

The above result states that the heat effect per mole of substrate is proportional to the relative degree of binding. This proportionality is in essence the feature that is used in most analyses of ITC data. The experimental data are fit with the above eq 17, and the resulting isotherm is plotted as a continuous line; the resulting values for the binding constant and the standard binding enthalpy are  $K = 52 \mu\text{M}^{-1}$  and  $\Delta_b H^\circ = -17.4 \text{ kJ/mol}$ . For comparison, Figure 1 (bottom) gives the relative degree of binding versus free ligand concentration. Saboury obtained exactly the same values using an enthalpy balance<sup>6</sup> which demonstrates the equivalence of the two methods for this simple ligand binding system.

**Surfactant Binding to Polyelectrolyte.** The simple relation between the relative degree of binding and the heat effect (see



**Figure 2.** Heat effect (top) and relative degree of binding (bottom) of sodium perfluorooctanoate (FC<sub>7</sub>) to cationically modified hydroxyethyl cellulose. The circles represent the experimental data,<sup>12</sup> the dashed lines result from a fit of the cooperative binding model to the heat effect data by Lapitsky et al.,<sup>12</sup> and the drawn lines result from a fit by the authors where also triplet interactions are taken into account.



**Figure 3.** Top graph gives the evolution of the association heat for the surfactant TTAB around the cmc. Dashed lines give the extrapolated dilution lines away from the cmc. Bottom graph shows the numerical derivative of the top graph. Dash-dotted line indicates the cmc which is taken to be the concentration where the derivative is largest in magnitude.

Figure 1) is lost for more complex binding processes such as when cooperativity plays a role. For a linear macromolecule with a large number,  $N \gg 1$ , of pairwise interacting binding sites, the binding polynomial is given by eq 6 as

$$\Xi = \lambda^N \quad \text{with} \quad \lambda = \frac{1 + Kux + \sqrt{(1 - Kux)^2 + 4Kx}}{2} \quad (19)$$

**TABLE 1: Measured and Literature Values, Taken from the Cited References, of Association Enthalpy ( $\Delta_a H^\circ$ ), Critical Micellar Concentration (cmc), and Aggregation Number ( $N$ ) for Four Different Surfactant Systems**

	$\Delta_a H^\circ$ (kJ/mol)		cmc (mM)		$N$	
	exp	lit	exp	lit	exp	lit
NNON <sup>17</sup>	5.7 $\pm$ 1.2	5.6	8.3 $\pm$ 0.4	7.9	44 $\pm$ 10	58 $\pm$ 6
SDS <sup>15</sup>	-2.7 $\pm$ 0.2	-2.5	8.7 $\pm$ 0.4	8.6	34 $\pm$ 4	36–42
TTAB <sup>14,18,19</sup>	-4.7 $\pm$ 0.7	-5	4.1 $\pm$ 0.2	3.6	63 $\pm$ 7	64
SDC <sup>16,20</sup>	-7 $\pm$ 1.0	-7.4	6.9 $\pm$ 0.7	6.2	10 $\pm$ 3	3–12

the largest eigenvalue of the transfer matrix in eq 7; because of the low ligand levels, the ligand activity is replaced by the mole fraction  $x$ . For the binding polynomial, there are two enthalpy contributions,  $\Delta_K H^\circ$  due to the site binding constant  $K$  and  $\Delta_u H^\circ$  for the cooperativity parameter  $u$ . These contributions are weighted differently by the binding activity and hence result in a dependence that differs from the relative degree of binding. To illustrate this effect, we have applied the cooperative binding model to ITC data taken by Lapitsky et al.<sup>12</sup> on sodium perfluorooctanoate (FC<sub>7</sub>) adsorption to cationically modified hydroxyethyl cellulose, a high molecular mass polyelectrolyte. In Figure 2, these data are plotted together with a best fit result using the parameters,  $K = 0.6 \text{ mM}^{-1}$ ,  $u = 4.7$ ,  $\Delta_K H^\circ = 8.4 \text{ kJ/mol}$ , and  $\Delta_u H^\circ = -1.6 \text{ kJ/mol}$  as provided by the authors.<sup>12</sup> These authors obtained their results by approximating the binding enthalpy by a weighted average of cooperative and noncooperative enthalpy contributions.

The steepness of the ITC graph calls for a more involved model, and we applied one with, in addition to nearest neighbor pair interactions, triplet interactions (see eq 8). The associated parameters are denoted by  $w$  and  $\Delta_w H^\circ$ . We did not include next nearest neighbor interactions, the parameter  $v$ , as these are not expected to be effective. A least-squares fit yields parameter values  $K = 0.36 \text{ mM}^{-1}$ ,  $u = 3.2$ ,  $w = 1.7$ ,  $\Delta_K H^\circ = 9.2 \text{ kJ/mol}$ ,  $\Delta_u H^\circ = 0.11 \text{ kJ/mol}$ , and  $\Delta_w H^\circ = -2.5 \text{ kJ/mol}$ . The result is also shown in Figure 2 and, given the reliability of the data, satisfactorily describes the data. In addition, the adsorption isotherm is shown; the binding isotherm and the heat effect do not have the same functional dependence on the ligand concentration. The resulting value that we obtain for the triplet interaction strength demonstrates that its significance is greater than the nearest neighbor interactions. In the case of proton binding to polyelectrolytes, strong triplet interactions imply that the polyelectrolyte chain is stretching upon increased ligand loading (in other words, the persistence length is increasing);<sup>13</sup> we expect the effect to be similar here.

This example demonstrates that using the Gibbs–Helmholtz relation in conjunction with existing models on ligand binding allows for more detailed and more reliable information to be obtained from calorimetric data.

**Micellization Equilibrium.** We conducted a series of ITC experiments on some well-known surfactant systems labeled NNON, SDS, TTAB, and SDC. As an example, the measured heat effect as a function of total TTAB concentration is given in Figure 3 (top). The numerical derivative of the graph is presented at the bottom of Figure 3. The concentration at which this derivative is maximum is taken as the cmc value. The measured enthalpy values for low concentrations and high concentrations follow straight lines due to dilution effects. The difference between the extrapolated values at the cmc is taken as the value for the standard enthalpy of association. The maximum value of the derivative, the cmc value, and the value for the standard enthalpy of association

are used in eq 14 to calculate the aggregation number. The values determined with this procedure are, together with literature values, presented in Table 1. The literature values for the aggregation numbers have been obtained using static light scattering<sup>14</sup> and spin probes,<sup>15</sup> and by performing a nonlinear least-squares fitting procedure to the obtained isotherms.<sup>16,17</sup> The latter method is hampered by a large number of unknowns and is only expected to produce sufficiently accurate results for low aggregation numbers.

Despite the simplicity of the method, the correspondence between the values obtained with this procedure and those from the literature is striking. Only the SDS value is deviating, which is most likely due to the fact that the micellar size for this system is known to vary roughly as  $x_i^{1/4}$  with surfactant concentration.<sup>15</sup> The literature value given in the table has been obtained by extrapolating this law to the cmc value where we determined the aggregation number.

The above analysis demonstrates that the three important parameters of a micellization process, being the cmc, enthalpy change, and aggregation number, can be extracted from a single calorimetric experiment. The variety of systems chosen warrants a general applicability of this analysis method.

## Conclusion

In summary, we have shown that the very long known Gibbs–Helmholtz relation can be used to turn existing thermodynamic models into predictive tools for enthalpy determinations. Although we have illustrated this for a simple and a complex ligand binding system and for a collection of self-associating surfactant systems, there are many more potential applications such as conformational changes in protein solutions and osmotic stress effects.

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