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# Two states do not necessarily correspond to a two-state transition: van't Hoff enthalpy in the case of a small entropy difference between the states

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## Abstract

We discuss the van't Hoff enthalpy relation, that is conventionally used to determine the two-stateness of a system, in the presence of a small entropy difference between the states. Specifically, for a two-state system we show in this case that the calorimetric enthalpy does not equal the van't Hoff enthalpy. We also study the van't Hoff enthalpy for an $N$-state system and find that the van't Hoff enthalpy and the calorimetric enthalpy of the $N$-state system can in fact become equal, provided that the entropy differences between the states are finite.
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## 1. Introduction

In order to reveal the two-stateness of a calorimetric transition, comparison between the calorimetric enthalpy ($\Delta H_{cal}$) and the van't Hoff enthalpy ($\Delta H_{vH}$) is widely applied, e.g., in gel–liquid transitions [1], vapor sorption [2], micellar aggregation [3], coil-collapse transition of polymers [4], and protein folding [5]. The general view is that if $\Delta H_{vH} = \Delta H_{cal}$, i.e., the classical calorimetric criterion of a two-state transition, then the transition is regarded as two-state [6,7]. However, the van't Hoff analysis of, e.g., proteins has shown that the interpretation of the results may become ambiguous. The latter may be connected to the determination of the 'baselines' of the heat capacities corresponding to temperatures below and above the heat capacity peak [8]. Moreover, it is demonstrated that the heat capacity corresponding to,

e.g., a three-state system can be indistinguishable from a two-state system over a broad temperature interval for a particular choice of the free energy of the intermediate state [9,10].

In this work, we consider a two-state model for which we will properly explain the concept of the van't Hoff enthalpy. We will only consider the case for vanishing heat capacities at zero and infinite temperatures in order to avoid the above-mentioned problems regarding the heat capacity 'baselines'. We show that $\Delta H_{vH} \neq \Delta H_{cal}$ when the entropy difference between the two states is small. For an $N$-state system we are able to calculate the corresponding entropy difference such that the van't Hoff relation is fulfilled. Our considerations may be relevant to small systems investigated, e.g., by means of single molecule techniques.

## 2. van't Hoff enthalpy

The van't Hoff enthalpy is an effective measure that equals the calorimetric enthalpy for a thermodynamic two-state transition. When discussing the transition,

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the two states are termed the bound state and the unbound state, referring to the enthalpically favorable state (low temperature state) and the entropy-dominated state (high temperature state), respectively. Let

$$\vartheta(T) = \frac{H(T) - H(0)}{\Delta H_{cal}}, \tag{1}$$

be a measure of the progress of the temperature-induced transition, where $H(T)$ is the enthalpy at temperature $T$, $H(0)$ is the enthalpy at zero temperature, and $\Delta H_{cal}$ is the enthalpy difference between the unbound and bound states. In this work, we will only consider models for which the heat capacity vanishes at zero and infinite temperature, thus, $\Delta H_{cal} = H(\infty) - H(0)$ is an unambiguous quantity. $\vartheta$ spans from 0 to 1, and consequently a natural choice of an effective equilibrium constant is [7]

$$K_{eff} = \frac{\vartheta}{1 - \vartheta}. \tag{2}$$

The van't Hoff equation for this transition yields [11]

$$\Delta H_{vH} = k_B T^2 \frac{d \ln K_{eff}}{dT} = \frac{k_B T^2}{\vartheta(1 - \vartheta)} \frac{d\vartheta}{dT}, \tag{3}$$

where $d\vartheta/dT \sim dH(T)/dT = C(T)$, i.e., the heat capacity (see Eq. (1)). We now define $T_d$ as the temperature at which half of the heat is absorbed, i.e., $\vartheta(T_d) = 1/2$ (see Eq. (1)), thus, the van't Hoff enthalpy for a single temperature-induced transition and vanishing heat capacities in the zero and infinite temperature limit becomes

$$\Delta H_{vH} = 4 k_B T_d^2 \frac{C(T_d)}{\Delta H_{cal}}. \tag{4}$$

One usually considers the ratio

$$\kappa \equiv \frac{\Delta H_{cal}}{\Delta H_{vH}}, \tag{5}$$

to quantify the deviation from $\Delta H_{vH}$, and therefore from a thermodynamic two-stateness, for a given transition. Notably, several small globular proteins reveal $\kappa \approx 1$ [6,12,13].

## 3. Results and discussion

### 3.1. System of two states

The result $\Delta H_{vH} = \Delta H_{cal}$, i.e., $\kappa = 1$ (see Eq. (5)), is only valid for a system of two states when the entropy difference between the states are infinite. To see this we define a two-state partition function

$$Z = e^{-\varepsilon/T} + \Omega, \tag{6}$$

where $\varepsilon < 0$ is the energy of the bound state and $\Omega$ measures the degrees of freedom of the unbound state (i.e., giving rise to an entropy-difference $\Delta S = T \ln \Omega$ between the two states). In Eq. (6) the Boltzmann constant is set

to unity such that $T$ is in units of $\varepsilon$. To calculate $\kappa$ in Eq. (5) we need to find $T_d$ that is the temperature for which half of the enthalpy of the transition is released. The enthalpy is given by

$$H(T) = T^2 \frac{d \ln Z}{dT} = \frac{\varepsilon e^{-\varepsilon/T}}{e^{-\varepsilon/T} + \Omega} \tag{7}$$

and thus, $H(T_d) = 1/2 \, [H(0) + H(\infty)]$ yields

$$H(T_d) = \varepsilon \frac{2 + \Omega}{2(1 + \Omega)}, \tag{8}$$

as $H(0) = \varepsilon$ and $H(\infty) = \varepsilon(1 + \Omega)^{-1}$. In the limit $\Omega \gg 1$ we obtain the simple result $T_d = -\varepsilon/\ln \Omega$ [14]. To evaluate $\kappa$ in Eq. (5) we also need to calculate the heat capacity, resulting in

$$C(T) = \frac{dH(T)}{dT} = \frac{\varepsilon^2}{T^2} \frac{\Omega e^{-\varepsilon/T}}{(e^{-\varepsilon/T} + \Omega)^2}. \tag{9}$$

Using $\Delta H_{cal} = H(\infty) - H(0) = -\varepsilon \Omega (1 + \Omega)^{-1}$ and inserting $H(T_d)$ from Eq. (8) into Eq. (9) we obtain from Eq. (5)

$$\kappa = \frac{\Omega}{2 + \Omega}, \tag{10}$$

valid for the partition function in Eq. (6). Notably, this result is independent of the binding energy $\varepsilon$. The ratio $\kappa$ becomes 1 for $\Omega \to \infty$ and $\kappa < 1$ for a finite $\Omega$. Thus, a system that consists of two states does not necessarily satisfy the traditionally defined calorimetric criterion of a two-state transition. This effect is illustrated in Fig. 1, where we plot the heat capacities for the partition function in Eq. (6) for $\Omega = 10$ and 100. Both heat capacity curves are approaching zero when $T \to \infty$, however, the fraction in the bound state approaches the value $(1 + \Omega)^{-1}$ for $T \to \infty$, and is therefore appreciably different from zero for smaller $\Omega$. Thus, the high-temperature state for a finite $\Omega$ is a mixture of bound and unbound states. The consequence is that two states in general do not correspond to a two-state transition in the van't Hoff sense.

### 3.2. System of N states

To further investigate the consequence for the van't Hoff enthalpy of a finite entropy difference between the states, we generalize the partition function in Eq. (6) to $N$ states. Assuming that all of the states are equidistant with respect to the free energy we obtain the partition function [15]

$$Z_N = e^{(-N-1)\varepsilon/T} + \Omega e^{(-N-2)\varepsilon/T} + \cdots + \Omega^{N-1}$$
$$= \frac{e^{-N\varepsilon/T} - \Omega^N}{e^{-\varepsilon/T} - \Omega}, \tag{11}$$

where $Z_N$ is a geometrical sum valid for any integer $N \geqslant 2$. Note that $\varepsilon$ and $\Omega$ correspond to the enthalpical and entropical differences between neighboring states in
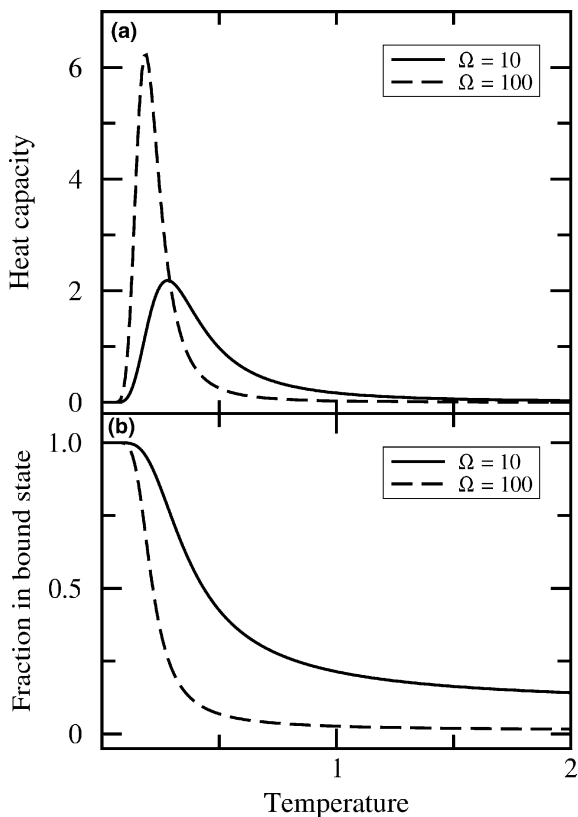
Fig. 1. (a) Heat capacity vs. temperature (see Eq. (9)). (b) Fraction in the bound state calculated from Eq. (6). Temperature in units of $\varepsilon$ (that is set to unity).

Table 1
Corresponding values of $N$ and $\Omega$ that yield $\kappa = 1$ (see Eq. (5))

| $N$ | $\Omega$ |
|-----|----------|
| 2 | $\infty$ |
| 3 | 2.85 |
| 4 | 1.70 |
| 5 | 1.36 |
| 6 | 1.21 |
| 7 | 1.13 |
| 8 | 1.08 |
| 9 | 1.05 |
| 10 | 1.03 |

The calculations are based upon the $N$-state model in Eq. (11).

the $N$-state model, respectively. The corresponding enthalpy becomes

$$H_N(T) = \frac{\mathrm{d}\ln Z_N}{\mathrm{d}(1/T)}$$
$$= \varepsilon \frac{(N-1)\mathrm{e}^{-(N+1)\varepsilon/T} - N\Omega \mathrm{e}^{-N\varepsilon/T} + \Omega^N \mathrm{e}^{-\varepsilon/T}}{(\mathrm{e}^{-N\varepsilon/T} - \Omega^N)(\mathrm{e}^{-\varepsilon/T} - \Omega)},$$
(12)

The heat capacity is calculated as $C_N(T) = \mathrm{d}H_N(T)/\mathrm{d}T$ and is not shown here due to its rather complex structure.

Traditionally, the calorimetric criterion $\Delta H_{\mathrm{vH}} = \Delta H_{\mathrm{cal}}$ has been the hallmark of a two-state transition [13]. To be more specific, by the $\Delta H_{\mathrm{cal}}$ for the $N$-state model we mean the enthalpy difference between the high temperature and the low-temperature states, i.e., $\Delta H_{\mathrm{cal}}(N\text{-state}) = H_N(\infty) - H_N(0)$, as the heat capacities below and above the (single) transition vanish. It is well known that $\Delta H_{\mathrm{vH}} \neq \Delta H_{\mathrm{cal}}$ for the $N$-state model provided that $\Omega \gg 1$ and $N > 2$ (see Eq. (13) below). However, one can now ask on the basis of the $N$-state model whether the calorimetric criterion that was derived for a two-state transition, may be fulfilled for finite $\Omega$ of a system with $N$ states. One should note here that the calorimetric criterion (for two-states) requires that below the transition-temper-

ature only the bound state exists and that above the transition-temperature only the unbound state exists. Thus, the entropy difference between the bound state and the unbound state is infinite, i.e., the two states are thermodynamically well separated, in order to fulfill the calorimetric criterion. As we show in Table 1 for $N > 2$ in the $N$-state model defined in Eq. (11), the corresponding $\Omega$ that fulfills $\Delta H_{\mathrm{vH}} = \Delta H_{\mathrm{cal}}$ actually exists and is finite. However, in contrast to the pure two-state case, this requires a finite entropy difference between the different states (corresponding to a finite $\Omega$). We also note that the values for $\Omega$ listed in Table 1 are comparable to real systems such as in small denaturation-bubbles in double stranded DNA [17].

In the limit $\Omega \gg 1$ for the $N$-state model we obtain $T_{\mathrm{d}} = 1$, $\Delta H_{\mathrm{cal}} = (N-1)\varepsilon$, and $C_N(T_{\mathrm{d}}) = T^{-2}\varepsilon^2(N^2 - 1)/12$, thus, we obtain the simple expression for the ratio in Eq. (5)

$$\kappa(\Omega \gg 1) = \frac{3(N-1)}{N+1},$$
(13)

such that $\kappa \leqslant 3$ for $N \geqslant 2$. In particular, $N = 2$ yields $\kappa = 1$, i.e., the calorimetric criterion for a two-state transition is fulfilled, as discussed above. Furthermore, $\kappa \rightarrow 3$ when $N \rightarrow \infty$ [14,16].

## 4. Summary and conclusion

We study thermally induced transitions when the entropy difference between the different states is small. The motivation for doing this is that traditionally van't Hoff enthalpy measurements have been performed for systems where the bound and unbound states are well separated. This means that well below the transition temperature a single state exists (bound state) and well above the transition temperature another state exists (unbound state), i.e., there is no mixture of states at low and high temperatures, respectively.

Conversely, there exist systems, where the high-temperature state may be a mixture of two or more states. Examples of such systems may be small atomic or

molecular systems, or small units in larger molecules, such as in small denaturation-bubbles in double-stranded DNA under external constraints like in optical tweezers setups [17]. Motivated by this fact we calculate for a system, where the high-temperature state of a two-state system is a mixture of the two states, the deviation of the calorimetric enthalpy ($\Delta H_{cal}$) from the van't Hoff enthalpy ($\Delta H_{vH}$). We show that $\Delta H_{vH} > \Delta H_{cal}$ when the entropy difference between the two states is finite. In contrast, the traditional hallmark property that $\Delta H_{vH} = \Delta H_{cal}$ of a pure two-state system is only valid when the states are entropically well separated. The consequence of this is that two states not necessarily imply a two-state transition in the traditional sense. If we expand the system to $N$ states we show that the calorimetric criterion can be fulfilled, i.e., $\Delta H_{vH} = \Delta H_{cal}$, for small entropy differences between the states. The latter challenges the traditional assumption according to which a transition that involves an intermediate state implies $\Delta H_{vH} \neq \Delta H_{cal}$. We believe that these considerations are useful in the study of thermodynamical transitions in small physical systems.

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