The role of the concentration scale in the definition of transfer free energies

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HIGHLIGHTS

- We explain the phenomenon of "concentration-scale dependent" transfer free energies.
- The molarity-scale transfer free energy quantifies solute–solvent interactions.
- Other transfer free energies include unwanted contributions due to volume changes.

GRAPHICAL ABSTRACT

Which transfer free energy relates to solute-solvent interactions?

$\Delta_{tr}G$

x-scale ✗   c-scale ✓   m-scale ✗

J/mol
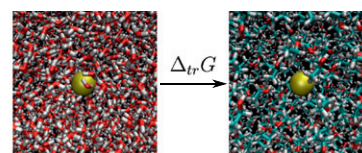
Why? Because it corresponds to a hypothetical transfer between solutions with equal volume.

ABSTRACT

The Gibbs free energy of transferring a solute at infinite dilution between two solvents quantifies differences in solute–solvent interactions — if the transfer takes place at constant molarity of the solute. Yet, many calculation formulae and measuring instructions that are commonly used to quantify solute–solvent interactions correspond to transfer processes in which not the molarity of the solute but its concentration measured in another concentration scale is constant. Here, we demonstrate that in this case, not only the change in solute–solvent interactions is quantified but also the entropic effect of a volume change during the transfer. Consequently, the "phenomenon" which is known as "concentration-scale dependence" of transfer free energies is simply explained by a volume-entropy effect. Our explanations are of high importance for the study of cosolvent effects on protein stability.

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1. Introduction

The knowledge of the preference of a solute for one solvent over another is very important in understanding basic processes in biochemistry, biology, solution chemistry, and related natural sciences. Moreover,

it is an important prerequisite in the design of products and production processes in formulation and engineering. Gibbs free energies of transfer (often abbreviated by "transfer free energies" = TFEs) are quantities that are used to quantify the solvent preferences of solutes. Thus, many explanatory models and design principles are based on measured or calculated TFEs. In biochemistry and biology, TFEs are extensively used in the study of chemical denaturation and renaturation of proteins or other macromolecules. In the framework of the transfer model, for example, TFEs can help to unravel which groups of a protein promote

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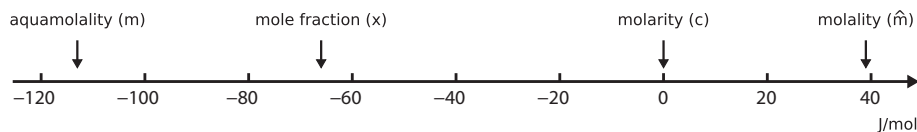*E-mail address:* Beate.Moeser@ur.de (B. Moeser).

Fig. 1. Illustration of the difference between different STFEs by means of the example of transfers between water and a 1 M urea solution. The molarity-scale STFE is arbitrarily set to zero. The different quantities in Table 1 are in the given example: $d_w = 0.99707$ kg/L, $d_{w+co} = 1.01274$ kg/L [23], $m_{co} = 1.0497$ mol/kg [23], $M_{co} = 60.06$ g/mol, $M_w = 18.015$ g/mol. The molarity-scale STFE of glycine between the two solutions is 17.3 J/mol [2], which demonstrates that the illustrated differences between the different STFEs are not negligible compared to the absolute values.

or prevent unfolding in a denaturant or osmolyte [1,2]. Moreover, commonly used hydrophobicity scales are based on TFEs [3].

The underlying idea in TFE analyses is that a solute favors a solvent 'b' over another ('a'), if the transfer of the solute at infinite dilution from solvent 'a' to solvent 'b' is favorable. Whether this is the case can be quantified by the Gibbs free energy of the transfer (TFE). For a transfer at a constant (infinitely small) concentration ξ of the solute 'i', the TFE $\Delta_{tr}G^0_{i,\xi}(a \to b)$ is given by the difference of the solute's standard chemical potentials in the two solvents

$$\Delta_{tr}G^0_{i,\xi}(a \to b) = \mu^0_{i,\xi}(b) - \mu^0_{i,\xi}(a), \tag{1}$$

where ξ can be any common concentration scale (e. g. molarity, molality, or mole fraction). For a long time, it was unclear, whether the change in solute–solvent interactions during a transfer at infinite dilution is best represented by a transfer at constant molarity or at constant mole fraction of the solute. Most researchers favored the mole-fraction scale [4–10] in Eq. (1), whereas others favored the molarity scale [11]. At the latest when one discovered that the sign of the TFE can depend on the used concentration scale [8], it was clear that the choice of concentration scale is highly relevant. In 1978, Ben-Naim was able to resolve the question by means of statistical thermodynamics. In a very in-depth and insightful article [12], he showed that only the Gibbs free energy of a transfer at constant molarity can be interpreted directly in terms of favorable or unfavorable solute–solvent interaction free energy. While Ben-Naim's paper "Standard Thermodynamics of Transfer. Uses and Misuses" [12] by now was cited more than 300 times, the findings reported therein seem to be rather unknown nowadays. This is best illustrated by the fact that there exists a variety of studies, in which TFEs or related quantities are evaluated at constant mole-fraction (e. g. [13,14]) or constant (aqua-)molality (e. g. [15–18]) and nonetheless are interpreted exclusively in terms of solute–solvent interactions. The error due to this can be negligible in some cases, but in others it can be so large that it even affects the classification of the interactions into the categories "favorable" and "unfavorable" as we will show later on in Fig. 1. The fact that the sign of a TFE can depend on the concentration scale for which the standard chemical potentials are defined is sometimes called "concentration-scale dependence" of TFEs and is still described as a source of confusion in the recent literature [19,20].

In the article at hand, we didactically explain why TFEs calculated by Eq. (1) only yield the desired information about solute–solvent interaction free energy if the molarity-scale standard chemical potentials are used. We start out by recapitulating that depending on the choice of concentration scale in Eq. (1), the calculated TFE corresponds to a different *hypothetical* transfer process (insofar as the infinitely small concentration of the transferred substance is kept constant in a different concentration scale). This fact is nowadays often not paid attention to. Subsequently, we show how the TFEs of the different transfer processes can generally be converted into one another and provide a convenient table with explicit conversion terms. A discussion of the conversion equation reveals that even in the limit of infinite dilution of the transferred substance it matters in which concentration scale the concentration is kept constant. Considering this, we address the question which of the different transfer processes at infinite dilution should

be used to quantify the solvent preference of a solute. We show that this is the transfer at constant molarity and we explain comprehensibly how the TFEs corresponding to the other processes can be interpreted. Our results also affect TFE-related quantities as e. g. "chemical potential derivatives".

2. Different transfer processes at infinite dilution

In the recent literature, the TFE of a solute 'i' between two solutions 'a' and 'b' is often said to be the difference of the solute's standard chemical potentials in the two solutions $\mu^0_i(b) - \mu^0_i(a)$ [19,20]. Even though a standard chemical potential is only defined in connection with a concentration scale (see Appendix B.2), a concentration scale is often not specified. This suggests (incorrectly) that the choice of concentration scale for the standard chemical potential is of no significance. However, here, we show that depending on the concentration scale of the standard chemical potentials, a different TFE is obtained that corresponds to a different transfer process. This was already discussed in the early days of TFE studies [4,11].

We start our reasoning by considering a general transfer of a single solute molecule 'i' from a solution 'a' to a solution 'b'. The Gibbs free energy associated with the removal or the addition of a single solute molecule from respectively to a large solution is by definition the solute's chemical potential μ_i in the considered solution (respectively the negative thereof in case of removals). Hence, the Gibbs free energy of the transfer of a single molecule from a *given* solution 'a' to a *given* solution 'b' is

$$\Delta_{tr}G_i(a \to b) = \mu_i(b) - \mu_i(a). \tag{2}$$

Given that this is a general transfer between two solutions, we realize that a TFE expressed by the difference of standard chemical potentials must correspond to a transfer between special solutions − i. e. solutions for which $\mu_i(b) - \mu_i(a)$ reduces to $\mu^0_i(b) - \mu^0_i(a)$. To learn under which conditions this is the case, it is instructive to express Eq. (2) in an arbitrary concentration scale ξ[1]:

$$\Delta_{tr}G_i(a \to b) = \mu^0_{i,\xi}(b) - \mu^0_{i,\xi}(a) + kTln\left(\frac{\gamma_{i,\xi}(b) \cdot \xi_i(b)}{\gamma_{i,\xi}(a) \cdot \xi_i(a)}\right). \tag{3}$$

The ξ_i describe the concentrations of the solute 'i' in the two solutions 'a' and 'b' and the $\mu^0_{i,\xi}$ and $\gamma_{i,\xi}$ are the standard chemical potentials and activity coefficients of the solute in the two solutions in the concentration scale ξ. From Eq. (3), it is evident that a TFE calculated by the difference of standard chemical potentials corresponds to a transfer process for which the third term on the rhs is zero. This is the case if the solute has the same infinitely small concentration ξ_i in both solutions so that $\gamma_{i,\xi}(b) = \gamma_{i,\xi}(a) = 1$ and $\xi_i(b)/\xi_i(a) = 1$. The condition $\xi_i(b) = \xi_i(a)$ is necessary because the third term on the rhs of Eq. (3) does not vanish if $\xi_i(b) \approx 0$ and $\xi_i(a) \approx 0$ but $\xi_i(b) \neq \xi_i(a)$. Hence,

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[1] ξ may stand for any of the concentration scales listed in Appendix A or more generally for any concentration scale that fulfills the three criteria listed in Appendix B.2.2.

depending on the concentration scale to which the standard chemical potentials in Eq. (1) belong, a *different TFE* is calculated that corresponds to a *different transfer process* because the concentration of the transferred solute is kept constant in a *different concentration scale*. For the commonly used concentration scales listed in Appendix A, this implies concretely: the TFE between a solvent 'a' and a solvent 'b' obtained by Eq. (1) corresponds to the Gibbs free energy of the hypothetical transfer of the solute

- from an infinitely large volume of solvent 'a' to a volume of the same size of solvent 'b' if determined by $\mu_{i,c}^0(b) - \mu_{i,c}^0(a)$ (molarity scale),
- from an infinitely large mass of solvent 'a' to the same mass of solvent 'b' if determined by $\mu_{i,\hat{m}}^0(b) - \mu_{i,\hat{m}}^0(a)$ (molality scale),
- from an infinitely large number of solvent molecules 'a' to the same number of solvent molecules 'b' if determined by $\mu_{i,x}^0(b) - \mu_{i,x}^0(a)$ (mole-fraction scale),
- from an infinitely large mass of water to the same mass of water in a mixed solvent if determined by $\mu_{i,m}^0(b) - \mu_{i,m}^0(a)$ (aquamolality scale).

In the measurement of a TFE, the Gibbs free energy of transfer is not determined by the actual realization of one of the above hypothetical transfer processes. Instead, the difference of the standard chemical potentials is determined from experiments at finite concentrations (as e. g. solubility measurements [19]). Hence, when we discuss the above processes in the following, the discussion is *not about how to transfer* in an experiment, but rather about *which difference of standard chemical potentials to determine* (in any kind of suitable experiment).

Unfortunately, neither consistent nor precise terms are in common use for the description and distinction of different TFEs. In the following, we try to be precise in the choice of words to avoid misunderstandings. We will use the word "TFE" generally for Gibbs free energies of any transfer processes of a solute between different solvents. TFEs that are determined by the difference of "standard" chemical potentials are sometimes called "standard" Gibbs free energy of transfer. Here, we adopt this term and abbreviate them by "STFE". Thus, the different STFEs have in common that they correspond to a transfer process at constant solute concentration in the limit of infinite dilution, but they differ in the concentration scale in which the solute concentration is kept constant. To indicate that the solute concentration is kept constant in a given concentration scale ξ, we use the term "ξ-scale" TFE. It is important to note that in this context, the specification of a concentration scale only defines the underlying transfer process. The concentration units used in experiments are unaffected by this and a ξ-scale TFE can in principle also be determined by using a different concentration scale (plus conversion factors). In symbolic notations in equations, we mark STFEs by the superscript 0 (to indicate that we take the difference

of two infinite-dilution standard state chemical potentials) and ξ-scale TFEs by the subscript ξ.

3. Conversion between standard TFEs

One might think that during a transfer at infinite dilution, only changes in solute–solvent interactions can contribute to the TFE and that at the most the size of this contribution differs between the different infinite-dilution processes. In this case, the sign of all STFEs could be used as an indicator for the solvent preference of the solute. However, this notion is not correct as we clearly show in the following by a discussion of the conversion terms between the different STFEs that all correspond to different infinite-dilution transfer processes. The conversion terms can be derived from the definition of the standard chemical potential for different concentration scales. This is done in Appendix B and here we focus on a discussion of the result:

Two STFEs, $\Delta_{tr}G_{i,\xi}^0(a \to b)$ and $\Delta_{tr}G_{i,\theta}^0(a \to b)$, that correspond to a transfer of a solute 'i' at constant concentration ξ respectively θ in the limit of infinite dilution from a solvent 'a' to a solvent 'b' are converted by

$$\Delta_{tr}G_{i,\xi}^0(a \to b) = \Delta_{tr}G_{i,\theta}^0(a \to b) - kT\ln\left(\frac{\lim\limits_{\theta_i(b)\to 0}\left(\frac{\xi_i(b)}{\theta_i(b)}\right)}{\lim\limits_{\theta_i(a)\to 0}\left(\frac{\xi_i(a)}{\theta_i(a)}\right)}\right). \tag{4}$$

$\xi_i(s)$ and $\theta_i(s)$ express the concentration of the solute 'i' in a solution with solvent 's' in the two different concentration scales ξ and θ.

In Table 1, we list explicit expressions for the conversion term evaluated for the complete set of commonly used concentration scales. It is important to note that the conversion term is an additive term and not a factor. This implies that if the STFE is zero for one of the transfer processes it differs from zero for the others. This is illustrated in Fig. 1 by means of the example of transfers between water and a 1 M urea solution. Hence, we see: It is not possible that the sign of all STFEs (given by Eq. (1)) provides information about the solvent preference of the solute. Thus, the question arises: Does any one of the STFEs at all quantify the solute–solvent preference? If yes, which one? And how are then the other STFEs interpreted?

To our knowledge, Ben-Naim [12] was the first to identify the molarity-scale STFE as the TFE that indeed provides the desired information about the solvent preferences of solutes, which can also be quantified in the framework of the *solvation thermodynamics* introduced by him [21,22]. In the following, we explain why the molarity-scale STFE has this outstanding interpretation and explain the physical meaning of the conversion terms.
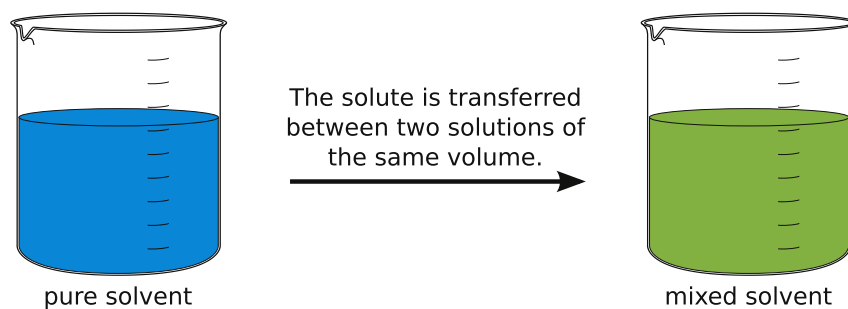
4. Interpretation of standard TFEs

The outstanding interpretation of the molarity-scale STFE can be qualitatively discussed by means of Fig. 2. The figure schematically illustrates the difference of transfer processes between a pure solvent and a mixed solvent at constant molarity and at constant aquamolality of the transferred solute: while the transfer at constant molarity only involves a change in solvent, the transfer at constant aquamolality involves in addition to this a change in accessible volume to the solute. In fact, all possible transfer processes except for those at constant molarity involve a change in accessible volume. This can be entropically favorable or unfavorable — depending on whether the accessible volume increases or decreases. Yet, TFE studies usually do not aim at such entropic volume contributions but only at changes in the solute–solvent interaction free energy between different solvents. Hence, we see that the molarity-scale STFE is outstanding because it corresponds to a hypothetical transfer process that does not involve a change in volume and thus exclusively probes changes in interactions with the solvents. While a negative molarity-scale STFE implies that the change of solvent is favorable, a negative non-molarity-scale STFE implies that the change of solvent in

Table 1
Conversion between different STFEs. The argument of the logarithm in Eq. (4) is given for pairs of the concentration scales defined in Appendix A. In the second column, it is given for general transfers between two solvents 'a' and 'b'. d_a and d_b are the mass densities and M_a and M_b the molar masses of the solvents. In the third column, the argument of the logarithm is given for the special case of transfers between water w and a mixed solvent of water and cosolvent $w + co$. d_w and d_{w+co} are the mass densities of water and the mixed water–cosolvent solution, M_w and M_{co} are the molar masses of water and the cosolvent, and m_{co} is the aquamolality of the cosolvent in the mixed water–cosolvent solution.

θ, ξ	$a \to b$	$w \to w + co$
x, c	$\dfrac{d_b \cdot M_a}{d_a \cdot M_b}$	$\dfrac{d_{w+co}}{d_w} \cdot \dfrac{1 + m_{co}M_w}{1 + m_{co}M_{co}}$
$\hat{m}, c$	$\dfrac{d_b}{d_a}$	$\dfrac{d_{w+co}}{d_w}$
m, c	–	$\dfrac{d_{w+co}}{d_w} \cdot \dfrac{1}{1 + m_{co}M_{co}}$
$\hat{m}, x$	$\dfrac{M_b}{M_a}$	$\dfrac{1 + m_{co}M_{co}}{1 + m_{co}M_w}$
m, x	–	$\dfrac{1}{1 + m_{co}M_w}$
$m, \hat{m}$	–	$\dfrac{1}{1 + m_{co}M_{co}}$

A) Transfer at constant **molarity** of the solute

The solute is transferred between two solutions of the same volume.

pure solvent   mixed solvent

B) Transfer at constant **aquamolality** of the solute

Cosolvent is added until the mixed solvent has the right composition.

cosolvent

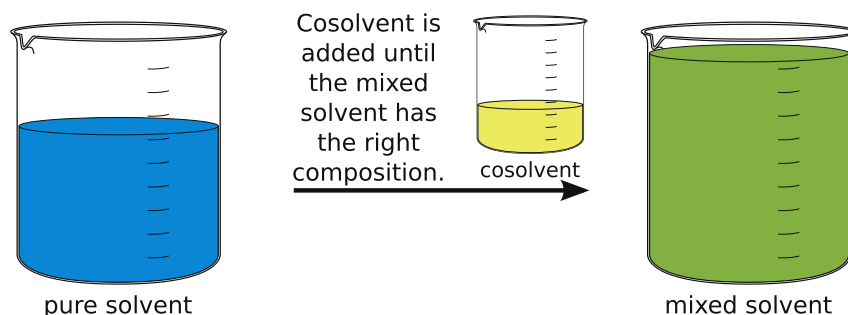pure solvent   mixed solvent

Fig. 2. Illustration of two different transfer processes between a pure and a mixed solvent. Panel A shows a transfer whose change in Gibbs free energy is described by the molarity-scale TFE (i. e. with equal volumes), and panel B shows a transfer whose change in Gibbs free energy is described by the aquamolality-scale TFE (i. e. with equal masses of the principal solvent water). The schematic drawing depicts that the two transfer processes are different because the transfer at constant aquamolality involves a change in accessible volume. This is entropically favorable so that the aquamolality-scale TFE is more negative than the molarity-scale TFE for all solutes transferred between the two depicted solvents. It is important to note that the figure does not describe how molarity-scale and aquamolality-scale TFEs are measured. The figure illustrates the essential qualitative difference between the two different hypothetical transfer processes whose changes in Gibbs free energy are quantified by molarity-scale respectively aquamolality-scale TFEs. See Section 2 for more explanations.

combination with the change in volume is favorable. Thus, it is fully explainable that the sign of the different STFEs can differ for transfers of a given solute between two given solvents.

The validity of this qualitative argument can be proven by means of statistical thermodynamics and simple mathematics: From the expression of the standard chemical potential in terms of statistical thermodynamics as derived by Ben-Naim [12] and given in the appendix (Eqs. (17) and (20)), we get for the molarity-scale STFE[2]

$$\Delta_{tr}G^0_{i,c}(a \to b) = W\left(i|b^0\right) - W\left(i|a^0\right). \tag{5}$$

$W(i|b^0)$ and $W(i|a^0)$ are the coupling works of the solute 'i' to the pure solvents 'a' and 'b'. Thus, Eq. (5) proves that the STFE of a transfer at constant molarity directly reflects differences in the solute–solvent interaction free energy — expressed here as differences in the coupling works $W(i|b^0)$ and $W(i|a^0)$. According to the conversion equation (Eq. (4)), the STFEs of transfers in which the solute concentration is kept constant in scale ξ are given by

$$\Delta_{tr}G^0_{i,\xi}(a \to b) = W\left(i|b^0\right) - W\left(i|a^0\right) - kTln\left(\frac{\lim\limits_{c_i(b) \to 0}\left(\frac{\xi_i(b)}{c_i(b)}\right)}{\lim\limits_{c_i(a) \to 0}\left(\frac{\xi_i(a)}{c_i(a)}\right)}\right). \tag{6}$$

The additional term on the rhs does not depend on the type of solute 'i' but only on the concentration-scale conversions in the two solutions 'a' and 'b' between which the transfer takes place. This fact already excludes that the term is related to solute–solvent interactions (which moreover are already covered by the first two summands on the rhs). Instead, the term directly corresponds to the aforementioned contribution due to a change in volume. In Appendix C, we prove that

$$-kTln\left(\frac{\lim\limits_{c_i(b) \to 0}\left(\frac{\xi_i(b)}{c_i(b)}\right)}{\lim\limits_{c_i(a) \to 0}\left(\frac{\xi_i(a)}{c_i(a)}\right)}\right) = -kTln\left(\left.\frac{V(b)}{V(a)}\right|_{\xi_i}\right), \tag{7}$$

where $\left.\frac{V(b)}{V(a)}\right|_{\xi_i}$ is the relative increase in volume during a transfer at constant ξ_i. This shows that differences in the various STFEs can be completely traced back to differences in changes in the accessible volume. It is important to note that the change in volume in Eq. (7) is due to different sizes of the two solutions 'a' and 'b' and thus is independent of whether the transfer is conducted at constant pressure or at constant volume (see Appendix E for further explanations).

With the knowledge of Eq. (7), we can easily identify the conversion terms in Table 1 with differences in relative increases in volume between different transfer processes. This is best illustrated by an example: During a transfer at constant molarity the volume does not change, but during a transfer at constant molality it changes because the volume of a solution 'b' is a factor of d_a/d_b larger than that of a solution 'a' with the same mass of another solvent. Hence, we have $\Delta_{tr}G^0_{\tilde{m}} = \Delta_{tr}G^0_c - kTln(d_a/d_b)$ which agrees with Table 1.

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[2] using that the thermal de Broglie wavelength Λ_i does not change upon transfer at constant temperature and assuming that the internal partition function q_i does not change either (see Appendix B.1 for details).

The most relevant implication of the above proof is that non-molarity-scale STFEs cannot be interpreted solely in terms of solute–solvent interaction free energy. This does not only apply to STFEs as discussed here but also to related quantities. In protein science, TFEs for transfers between water and mixed water–cosolvent solutions are for example often defined by the following equation (e. g. [17,18])

$$\Delta\mu_{tr,2} = \int_0^{m_3} \left(\frac{\partial\mu_2}{\partial m_3}\right)_{T,P,m_2} dm_3, \tag{8}$$

where the index 2 stands for the solute and 3 for the cosolvent. m is the concentration in the aquamolality scale. Evaluation of the integral yields

$$\Delta\mu_{tr,2} = \mu_2(m_2, m_3) - \mu_2(m_2, 0). \tag{9}$$

Hence, Eq. (8) corresponds to the Gibbs free energy of a transfer of a solute molecule from an aqueous solution to a water–cosolvent solution that both contain the same aquamolality m_2 of the solute. In contrast to the cases discussed before, m_2 does not need to be infinitely small. As motivated by Fig. 2 and proven in Appendix D, also TFEs at constant finite solute concentrations have a contribution due to volume changes if the transfer is not performed at constant molarity. Thus, the sign of $\Delta\mu_{tr,2}$ as defined in Eq. (8) cannot be interpreted solely in terms of interactions. Similar arguments apply to the "preferential interaction parameter" [18] which is also called "chemical potential derivative" [15,16,24,25]

$$\mu_{23} = \left(\frac{\partial\mu_2}{\partial m_3}\right)_{m_2}. \tag{10}$$

This is the integrand of the integral in Eq. (8). Under the assumption that the integrand is constant in the considered interval (cosolvent aquamolality between 0 and m_3), the "μ_{23} value" is often determined and interpreted instead of the TFE. If defined at constant aquamolality as in Eq. (10), it also contains a volume contribution so that its sign does not directly provide information about whether or not the interactions between the solute and the cosolvent are favorable. Consequently, if an aquamolality-scale "μ_{23} value" of a molecule is dissected into contributions of its different surface types (as done in the solute-partitioning model [15,16,24,25]), the entropic volume term is distributed among all surface types present in the molecule proportionally to the respective areas. Thus, it affects the thereby determined "interaction potentials" of the surface types, which are meant to quantify interactions between the surface types and the cosolvent.

5. Differences in TFEs of different solutes

The change in volume during a transfer at infinite dilution but constant concentration of the solute in a given concentration scale is independent of the type of solute that is transferred. It depends only on the two solutions between which the transfer takes place, which is also reflected by the fact that the conversion terms in Table 1 are independent of the solute. Thus, differences in STFEs of different solutes 'i' and 'j' between the same solvents 'a' and 'b' are always independent of the concentration scale in which the solute concentration is kept constant (provided that it is the same concentration scale for both solutes). Such differences directly correspond to the differences in solute–solvent interaction free energy between the two solutes.

Hence, whenever a study *exclusively* aims at a comparison of solvent preferences of different solutes, also non-molarity-scale STFEs can be used (as long as the comparison is accomplished in terms of differences and not factors). This is for example the case in many studies that deal with cosolvent effects on protein stability: The effect of a cosolvent on the folding equilibrium of a protein can be described by the difference of the TFEs of the native and the denatured protein structure from

water to a cosolvent solution [1]. If $TFE_D - TFE_N < 0$, the transfer of the denatured state to the cosolvent solution is more favorable than the transfer of the native state and the protein is denatured by the cosolvent. Analogously, $TFE_D - TFE_N > 0$ implies stabilization of the protein by the cosolvent. The quantity $TFE_D - TFE_N$, which for transfers to a 1 M cosolvent solution corresponds to an m value [26], is a difference of TFEs and thus independent of the underlying type of transfer process.

Also the STFEs of amino acid side chains that are used in the transfer model for the prediction of cosolvent effects on protein stabilities [4,19] are independent of the chosen standard chemical potential difference. This is because they are defined as the difference of the TFE of the amino acid with the given side chain and the TFE of glycine, which does not have a side chain.

6. Advantageous concentration scales in experiments

The specification of a concentration in the molarity scale depends on the temperature, the pressure, and the density of the solution, whereas mole fractions and (aqua-)molalities are independent of these influences. Therefore, the preparation of solutions in the latter concentration scales is much easier than in molarity. Hence, it can be advantageous to use an experimental setup that determines a non-molarity-scale STFE and to convert the result to the molarity-scale STFE afterwards by means of the conversion terms in Table 1.

Either way — using the molarity scale right from the start or converting to molarity scale in the end — there is no way around using densities which depend on temperature and pressure. However, during a measurement of a well-defined and meaningful quantity, the temperature and the pressure must be fixed anyways — also if the concentrations are specified in non-molarity units that are independent of temperature and pressure.

7. Summary and discussion

We summarize our main messages:

- The TFE that is determined by the difference of standard chemical potentials of a concentration scale ξ is the Gibbs free energy associated with the hypothetical transfer of a solute at (i) infinite dilution and (ii) constant concentration ξ from one solvent to another.
- The sign of the molarity-scale STFE directly provides information about whether or not the transferred solute prefers one solvent over another. The sign of non-molarity-scale STFEs cannot as readily be interpreted because these TFEs involve a contribution due to changes in accessible volume, which is not related to the solute–solvent interaction free energy.
- Also TFE-related quantities like "chemical potential derivatives" involve contributions due to volume changes if the derivative is not taken at constant molarity.
- The conversion terms between different STFEs account for differences in the increase in volume during the different underlying hypothetical transfer processes.
- Differences in STFEs of different solutes (transferred between the same two solvents) are independent of the underlying transfer process (as long as the same process is chosen for both of them).
- It does not matter which concentration scale is used during experiments. However, if the experiment aims at studying solute–solvent interactions, the measured TFE has to be converted to a molarity-scale TFE for interpretation.

It is obvious that the above insights are of high practical relevance for the design and analysis of TFE studies for all purposes.

The statistical–thermodynamical ansatz used by Ben-Naim and taken up by us here is a very straightforward and effective way to find out which of the different STFEs provides the desired information about solute–solvent interactions. In 2004, a comprehensive study [20] based on a large variety of experiments was published that among

other things also aimed at identifying the STFE that reflects solute–solvent interactions. In contrast to our reasoning, in that study the molality-scale TFE is presented as the one that most likely describes solute–solvent interactions and it is termed "intrinsic" TFE. Yet, in the paper it is stressed that — based on the experimental evidence — it cannot be excluded that the molarity-scale TFE is the sought one. Therefore, the authors of the study concluded: "To rigorously test the question of preference regarding molal- and molar-based transfer free energies or whether neither is adequate in all cases, experiments performed in solvents of widely differing densities will be required." [20] As explained in the paper at hand, statistical thermodynamics answers this question without the need for further experiments: the molarity-scale STFE is the TFE that quantifies the preference of a solute for one solvent over another.

Appendix A. Notation

The definition and notation for the different concentration scales used in this article are:

- molarity c ($\hat{=}$ number density ρ): molecules per volume of the solution.
- mole fraction x: molecules per total number of molecules.
- molality $\hat{m}$: molecules per mass of solvent (being the mixture of the principal solvent and the cosolvent in a ternary mixtures).
- (aqua-)molality m: molecules per mass of water.

When referring to concentration scales in general, we employ the characters ξ and θ. Whenever a quantity is only defined in the context of a given concentration scale (e. g. a standard chemical potential or an activity coefficient), it has an index referring to the concentration scale.

When different solutions are discussed at the same time, we indicate in brackets to which solution a given concentration refers.

Appendix B. Standard chemical potentials for different concentration scales

B.1. Representation of the chemical potential in terms of statistical thermodynamics

We consider a solution of N_i solute particles and N_j solvent particles. The chemical potential μ_i of the solute in the solution at constant pressure and temperature is defined by

$$\mu_i = \left(\frac{\partial G}{\partial N_i}\right)_{p,T,N_j}. \tag{11}$$

For sufficiently large solutions, it corresponds to the change in Gibbs free energy upon the addition of a single solute molecule to the solution. In [12], Ben-Naim derives an expression for μ_i by means of statistical thermodynamics that reads

$$\mu_i = -kTln\left(\left\langle exp\left(-\frac{\Delta U_i(r_0)}{kT}\right)\right\rangle_0\right) + kTln\left(\frac{\rho_i \Lambda_i^3}{q_i}\right). \tag{12}$$

ρ_i is the number density (i. e. molecules per volume) of the solute 'i' in the solution, and Λ_i and q_i are the thermal de Broglie wavelength and the internal partition function of a molecule of type 'i'. $\Delta U_i(r_0)$ is the

change in system energy upon the addition of a solute molecule 'i' at any fixed position r_0 to the solution at some specific configuration:

$$\Delta U_i(r_0) = U\left(N_i + 1, N_j\right) - U\left(N_i, N_j\right). \tag{13}$$

Here, (N_i, N_j) stands for a specific configuration of the system and the average $\langle\rangle_0$ in Eq. (12) is over all configurations of the molecules in the system except the one that was added.

The first term on the rhs of Eq. (12) is the coupling work of a molecule of type 'i' to the solution consisting of N_i solute and N_j solvent molecules. It accounts for the interactions between the added particle and the rest of the solution. From now on we will abbreviate it by $W(i|s)$ [12] denoting that we couple a molecule of type 'i' to a solution 's', which is a mixture of molecules of the types 'i' and 'j' with a given composition. If the solute 'i' is infinitely dilute in the solution, we write s^0 so that $W(i|s^0)$ is the coupling work of the solute to the pure solvent 'j'. The second term on the rhs of Eq. (12) corresponds to the chemical potential that the solute 'i' had if the solution were an ideal gas. This term accounts for changes in the Gibbs free energy G due to internal and translational degrees of freedom of the added particle and entropic contributions from the indistinguishability of molecules of type 'i'.

Using the new notation for the coupling work, we can rewrite Eq. (12)

$$\mu_i = W(i|s) + kTln\left(\frac{\Lambda_i^3}{q_i}\right) + kTln(\rho_i), \tag{14}$$

and see that the chemical potential μ_i of the solute in the solution depends in two ways on its concentration in the number–density scale: explicitly through the logarithmic term $kTln(\rho_i)$ and implicitly through the dependence of the coupling work $W(i|s)$ and the internal partition function q_i on the solution composition. For simplicity, we assume throughout the paper, that the internal partition function of a solute is independent of the surrounding solution. It is possible to account for solvent effects on the internal partition function [21] and thus to present our results in a more general form. However, the results themselves do not change if these effects are taken into account.

B.2. Representation of the chemical potential in terms of a standard chemical potential and an activity coefficient

B.2.1. Number–density concentration scale

In the following, we will map the expression in Eq. (14) on a representation of the chemical potential in terms of a standard chemical potential and an activity coefficient. This representation is usually used in thermodynamics and here it is needed to derive the conversion terms between different STFEs. Instead of Eq. (14), we want to use the following functional form to express μ_i

$$\mu_i = \mu_{i,\rho}^0 + kTln(\rho_i) + kTln\left(\gamma_{i,\rho}\right) \quad with \quad \lim_{\rho_i \to 0}\left(\gamma_{i,\rho}\right) = 1. \tag{15}$$

Here, $\mu_{i,\rho}^0$ is the standard chemical potential defined by

$$\mu_{i,\rho}^0 = \lim_{\rho_i \to 0}(\mu_i - kTln(\rho_i)). \tag{16}$$

The subscript ρ denotes that it is defined in the number–density scale. Evaluation of Eq. (16) with help of Eq. (14) shows that $\mu_{i,\rho}^0$ accounts for the coupling work $W(i|s^0)$ at infinite dilution of the solute as well as for its internal partition function and parts of the translational partition function $q_{trans} = V/\Lambda_i^3$

$$\mu_{i,\rho}^0 = W\left(i|s^0\right) + kTln\left(\frac{\Lambda_i^3}{q_i}\right). \tag{17}$$

$\gamma_{i,\rho}$ is the activity coefficient of the number–density scale. It is a function of the solute concentration ρ_i. Insertion of Eq. (17) in Eq. (15) and comparison with Eq. (14) reveals that the term $kT\ln(\gamma_{i,\rho})$ accounts for the dependence of the coupling work on the solute concentration, i. e.

$$kT\ln\left(\gamma_{i,\rho}\right) = W(i|s) - W\left(i|s^0\right). \tag{18}$$

Hence, we have shown that with Eqs. (17) and (18), it is possible to express the chemical potential μ_i in terms of a standard chemical potential and an activity coefficient.

B.2.2. Other concentration scales

The molarity scale (moles per volume) is essentially the same concentration scale as the number–density scale (molecules per volume). It differs from the latter only by a concentration-independent factor N_A, the Avogadro constant. Therefore, we do not discriminate between the two in the main text of the article and call both of them molarity. We get for the molarity scale in analogy to Eq. (15)

$$\mu_{i,c} = \mu_{i,c}^0 + kT\ln\left(\gamma_{i,c} \cdot c_i\right) \quad \text{with} \quad \lim_{c_i \to 0}\left(\gamma_{i,c}\right) = 1. \tag{19}$$

$$\text{with} \quad \mu_{i,c}^0 = \mu_{i,\rho}^0 + kT\ln(N_A) \tag{20}$$

$$\text{and} \quad \gamma_{i,c} = \gamma_{i,\rho}. \tag{21}$$

Most other concentration scales differ from the molarity and the number–density scale by a factor that depends on the concentration of the solute. Nevertheless, μ_i can be expressed in the same functional form as in Eqs. (15) and (19) for all concentration scales ξ that fulfill the following three criteria:

1. The zero point is the same as in the molarity scale: $\xi(c = 0) = 0$.
2. $\xi(c)$ is strictly monotonic.
3. $\xi(c)$ is continuous.

If these criteria hold, the zeroth-order term of the Taylor expansion of $\xi(c)$ in the point $c = 0$ is zero and the first-order term exists:

$$\xi(c) = \left.\frac{\partial \xi}{\partial c}\right|_{c=0} \cdot c + \mathcal{O}\left(c^2\right). \tag{22}$$

All concentration scales listed in Appendix A fulfill these criteria and thus ξ may represent any of them. To express μ_i in the concentration scale ξ by

$$\mu_i = \mu_{i,\xi}^0 + kT\ln\left(\gamma_{i,\xi} \cdot \xi_i\right) \quad \text{with} \quad \lim_{\xi_i \to 0}\left(\gamma_{i,\xi}\right) = 1, \tag{23}$$

we have to find expressions for $\mu_{i,\xi}^0$ and $\gamma_{i,\xi}$ that guarantee that the right-hand sides of Eqs. (23) and (19) are identical for all concentrations. From this requirement follows

$$\mu_{i,c}^0 - \mu_{i,\xi}^0 = kT\ln\left(\frac{\xi_i}{c_i}\right) + kT\ln\left(\frac{\gamma_{i,\xi}}{\gamma_{i,c}}\right). \tag{24}$$

The lhs of Eq. (24) is per definition independent of the solute concentration. Thus, we can determine $\mu_{i,c}^0 - \mu_{i,\xi}^0$ by evaluating the rhs in the limit of infinite dilution

$$\mu_{i,c}^0 - \mu_{i,\xi}^0 = kT\ln\left(\lim_{c_i \to 0}\left(\frac{\xi_i}{c_i}\right)\right) + kT\ln\left(\lim_{c_i \to 0}\left(\frac{\gamma_{i,\xi}}{\gamma_{i,c}}\right)\right). \tag{25}$$

Due to the first criterion above, $\gamma_{i,\xi}$ and $\gamma_{i,c}$ are both unity in the considered limit so that the second term on the rhs of Eq. (25) vanishes.

According to Eq. (22), the limit $\lim_{c_i \to 0}\left(\frac{\xi_i}{c_i}\right)$ exists and equals $\left.\frac{\partial \xi_i}{\partial c_i}\right|_{c_i=0}$. Hence, we can identify $\mu_{i,\xi}^0$ as

$$\mu_{i,\xi}^0 = \mu_{i,c}^0 - kT\ln\left(\lim_{c_i \to 0}\left(\frac{\xi_i}{c_i}\right)\right) = \mu_{i,c}^0 - kT\ln\left(\left.\frac{\partial \xi_i}{\partial c_i}\right|_{c_i=0}\right). \tag{26}$$

Inserting this back into Eq. (24), we get an expression for $\gamma_{i,\xi}$:

$$\gamma_{i,\xi} = \lim_{c_i \to 0}\left(\frac{\xi_i}{c_i}\right) \cdot \frac{c_i}{\xi_i} \cdot \gamma_{i,c}, \tag{27}$$

$$= \left.\frac{\partial \xi_i}{\partial c_i}\right|_{c_i=0} \cdot \frac{c_i}{\xi_i} \cdot \gamma_{i,c}. \tag{28}$$

Thus, with Eqs. (26)–(28), it is possible to express the chemical potential in any concentration scale ξ that fulfills the above criteria in terms of a standard chemical potential and an activity coefficient.

B.2.3. General conversions between standard chemical potentials

Eqs. (26)–(28) can easily be generalized to relations between the chemical potentials and activity coefficients of any two concentration scales that fulfill the above listed criteria. This is because if the criteria hold for two scales ξ and θ, then they also hold between them. Thus, having shown that $\mu_{i,\xi}^0$ and $\gamma_{i,\xi}$ exist for the concentration scale ξ, we can repeat the derivations (Eqs. (24)–(28)) with the concentration scales θ and ξ instead of ξ and c, and obtain the general conversion equations:

Standard chemical potentials of any two concentration scales ξ and θ that fulfill the above listed criteria are converted by

$$\mu_{i,\xi}^0 = \mu_{i,\theta}^0 - kT\ln\left(\lim_{\theta_i \to 0}\left(\frac{\xi_i}{\theta_i}\right)\right) = \mu_{i,\theta}^0 - kT\ln\left(\left.\frac{\partial \xi_i}{\partial \theta_i}\right|_{\theta_i=0}\right). \tag{29}$$

The corresponding activity coefficients are converted by

$$\gamma_{i,\xi} = \lim_{\theta_i \to 0}\left(\frac{\xi_i}{\theta_i}\right) \cdot \frac{\theta_i}{\xi_i} \cdot \gamma_{i,\theta}. \tag{30}$$

B.3. Conversion between standard TFEs

A STFE $\Delta_{tr}G_{i,\xi}^0(a \to b)$ corresponding to a transfer process at constant ξ is given by the difference of the ξ-scale standard chemical potentials of the solute 'i' in the two solutions 'a' and 'b' between which it is transferred

$$\Delta_{tr}G_{i,\xi}^0(a \to b) = \mu_{i,\xi}^0(b) - \mu_{i,\xi}^0(a). \tag{31}$$

Thus, the difference between a ξ-scale STFE $\Delta_{tr}G_{i,\xi}^0(a \to b)$ and a θ-scale STFE $\Delta_{tr}G_{i,\theta}^0(a \to b)$ follows directly from Eq. (29) and can be expressed by Eq. (4).

Appendix C. Proof that the conversion term corresponds to the relative increase in accessible volume

Here, we prove the validity of Eq. (7). We start out by recasting the argument of the logarithm in the conversion term on the lhs of Eq. (7)

in a different form. In the course of this, we employ several times that the three conditions for $\xi(c)$ listed in Appendix B.2.2 hold:

$$\frac{\lim\limits_{c_i(b)\to 0}\left(\frac{\xi_i(b)}{c_i(b)}\right)}{\lim\limits_{c_i(a)\to 0}\left(\frac{\xi_i(a)}{c_i(a)}\right)} = \frac{\lim\limits_{c_i(a)\to 0}\left(\frac{c_i(a)}{\xi_i(a)}\right)}{\lim\limits_{c_i(b)\to 0}\left(\frac{c_i(b)}{\xi_i(b)}\right)} \tag{32}$$

$$= \frac{\left.\dfrac{\partial\left(\frac{N_i(a)}{V(a)}\right)}{\partial\xi_i(a)}\right|_{c_i(a)=\xi_i(a)=0}}{\left.\dfrac{\partial\left(\frac{N_i(b)}{V(b)}\right)}{\partial\xi_i(b)}\right|_{c_i(b)=\xi_i(b)=0}} \tag{33}$$

$$= \frac{V(b)}{V(a)} \cdot \frac{\left(\frac{\partial N_i(a)}{\partial\xi_i(a)} - \frac{N_i(a)}{V(a)}\cdot\frac{\partial V(a)}{\partial\xi_i(a)}\right)_{c_i(a)=\xi_i(a)=0}}{\left(\frac{\partial N_i(b)}{\partial\xi_i(b)} - \frac{N_i(b)}{V(b)}\cdot\frac{\partial V(b)}{\partial\xi_i(b)}\right)_{c_i(a)=\xi_i(a)=0}} \tag{34}$$

$$= \frac{V(b)}{V(a)} \cdot \frac{\left.\dfrac{\partial N_i(a)}{\partial\xi_i(a)}\right|_{c_i(a)=\xi_i(a)=0}}{\left.\dfrac{\partial N_i(b)}{\partial\xi_i(b)}\right|_{c_i(b)=\xi_i(b)=0}}. \tag{35}$$

In the last conversion we used that $N_i/V = c_i \cdot N_A = 0$ in the considered limit. The numerator and the denominator of the second factor in Eq. (35) are equal, which is proven in the following by taking into account that the particle numbers $N_i(a)$ and $N_i(b)$ are equal because all solute particles that are in 'a' are transferred to 'b'. At infinite dilution, this is a single molecule. Moreover, the concentrations $\xi_i(a)$ and $\xi_i(b)$ are equal because of the construction of the transfer process, in which ξ_i is the very property kept constant. Therefore, the ratios of N and ξ are equal as well:

$$\frac{N_i(a)}{\xi_i(a)} = \frac{N_i(b)}{\xi_i(b)}. \tag{36}$$

Inserting the Taylor expansion of $N(\xi)$ in the dilute limit $\xi \to 0$

$$N(\xi) = \left.\frac{\partial N}{\partial\xi}\right|_{\xi=0} \cdot \xi + \mathcal{O}\left(\xi^2\right) \tag{37}$$

yields the desired relation

$$\left.\frac{\partial N_i(b)}{\partial\xi_i(b)}\right|_{\xi_i(b)=0} = \left.\frac{\partial N_i(a)}{\partial\xi_i(a)}\right|_{\xi_i(a)=0}, \tag{38}$$

which was to be proven. Inserting this back into Eq. (35), finally yields

$$\frac{\lim\limits_{c_i(b)\to 0}\left(\frac{\xi_i(b)}{c_i(b)}\right)}{\lim\limits_{c_i(a)\to 0}\left(\frac{\xi_i(a)}{c_i(a)}\right)} = \frac{V(b)}{V(a)}. \tag{39}$$

$V(b)/V(a)$ is the relative increase in volume during the considered transfer process at constant ξ.

Evaluation of $\partial N_i/\partial\xi_i|_{\xi_i=0}$ in concrete terms reveals that it is the quantity that needs to be the same in both solutions 'a' and 'b' so that the transfer takes place at constant ξ: it is the volume for $\xi = c$, the solvent mass for $\xi = \hat{m}$, the mass of the principal solvent (water) for $\xi = m$, and the number of molecules for $\xi = x$.

Appendix D. TFEs at constant finite concentrations

According to Eq. (3), the Gibbs free energy $\Delta_{tr}G_{i,\xi}^f$ of the transfer of a solute molecule between two solutions with the same solute concentration ξ that is not infinitely small but finite is given by

$$\Delta_{tr}G_{i,\xi}^f(a\to b) = \mu_{i,\xi}^0(b) - \mu_{i,\xi}^0(a) - kT\ln\left(\frac{\gamma_{i,\xi}(b)}{\gamma_{i,\xi}(a)}\right). \tag{40}$$

With Eqs. (6), (27), (21), and (18) this reduces to

$$\Delta_{tr}G_{i,\xi}^f(a\to b) = W(i|b) - W(i|a) - kT\ln\left(\frac{\frac{\xi_i(b)}{c_i(b)}}{\frac{\xi_i(a)}{c_i(a)}}\right). \tag{41}$$

Eq. (41) corresponds to a generalization of Eq. (6) to transfers at finite concentrations. Also here, the argument of the logarithm can be identified with the relative increase in volume during a transfer at constant ξ_i and constant[3] particle number N_i. Hence, we have

$$\Delta_{tr}G_{i,\xi}^f(a\to b) = W(i|b) - W(i|a) - kT\ln\left(\left.\frac{V(b)}{V(a)}\right|_{\xi_i}\right), \tag{42}$$

and see that the TFE at finite concentration also consists of an interaction term and a term due to an increase in volume (which is only zero for transfers at constant molarity). The interaction term describes the difference in coupling work of a solute molecule 'i' to the two given solutions with different solvents and a finite solute concentration ξ_i. A range of transfers that all start from the same solution 'a' and keep the solute concentration constant (in different concentration scales) in general results in different solution compositions of the second solution 'b'. Thus, the interaction term in Eq. (42) also depends on the concentration scale in which the solute concentration is kept constant. This is different from the case of STFEs (Eq. (6)) where the solutions to which we couple are always pure solvents so that the interaction term is the same for all STFEs (of a solute transferred between the same two solvents).

Appendix E. The meaning of constant pressure and constant volume

Finally, we elucidate the meaning of "constant pressure" and "constant volume" conditions during a transfer process. The (hypothetical) process discussed in this paper is the transfer of a single solute molecule 'i' from a solution 'a' to a solution 'b', which comprises two steps: the removal of the solute from system 'a' and its insertion into system 'b'. Each of the two steps can be performed either at constant volumes $V(a)$ and $V(b)$, or at constant pressure, in which case the two volumes are NPT *ensemble averages*. As nicely discussed by Ben-Naim [21], the chemical potential is identical in both ensembles for macroscopically large systems. The crucial difference is that at constant pressure the chemical potential relates to a change in Gibbs free energy, at constant volume it relates to a change in Helmholtz free energy. Hence, the Helmholtz free energy $\Delta_{tr}F_{i,\xi}^0$ of a transfer at constant volume and the Gibbs free energy $\Delta_{tr}G_{i,\xi}^0$ of a transfer at constant pressure between the same two systems are identical. Both are given as $\mu_{i,\xi}^0(b) - \mu_{i,\xi}^0(a)$ (compare Eq. (1)).

Finally, we stress that the transfer under NVT conditions must not be confused with a hypothetical transfer process, in which the volumes $V(a)$ and $V(b)$ are equal. A process with $V(a) = V(b)$ can either be performed at constant pressure or at constant volume. The condition

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[3] Usually, also at finite concentration, transfer processes with a constant number of solute particles are considered. If however, a transfer process is considered in which a solute is transferred between two solutions with $\xi_i(a) = \xi_i(b)$ but $N_i(a) \neq N_i(b)$, the argument of the logarithm in Eq. (41) does not reflect the relative increase in volume during the considered process. Instead, it reflects the relative increase in volume during the corresponding constant-particle-number transfer between the two considered solutions with composition $\xi_i(a) = \xi_i(b)$.

$V(a) = V(b)$ only fixes the scale for which this hypothetical process is characteristic (the molarity scale) and is no contradiction to the fact that Gibbs free energies are measured at constant pressure and not at constant volume.

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