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Simple Theory for Salt Effects on the Solubility of Amide

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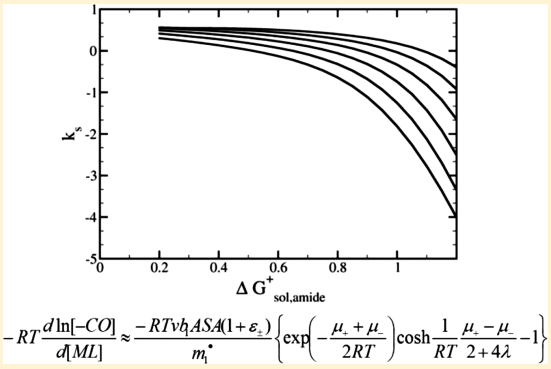
ABSTRACT: Elucidating the interactions of cosolvents and cosolutes, for example, urea and inorganic salts, with proteins plays a very important role in understanding protein structure formation, solubility, and dynamics. In a recent study, we rationalized the experimentally observed salt effects on water/air surface tension and showed the potential importance of cation/anion association/cooperativity. In this paper, we focus on salt effects on the solvation of simple model compounds such as peptides and benzene, hoping to obtain a more general and simple understanding of the Hofmeister series. We show here that preferred cation binding to the carbonyl and anion to the apolar surface of model polypeptides can help explain the experimentally observed salt effects on polypeptide activity coefficient in water. The effects of ions on the solvation of amide group can be both direct and indirect, which together attribute to an effective change of the proton donor/acceptor equilibrium in aqueous solutions. We show that such an argument can be used to understand not only the salt effects on solubility of amides but also why some organic compounds are protein secondary structure denaturants whereas others are protectants.



I. INTRODUCTION

The effects of cosolvents and cosolutes including inorganic salts on various thermodynamic and dynamic properties of water have invoked the interest of many research groups.[1−19] In particular, a lot of efforts have been devoted to understanding how solution conditions affect the solvation, structure formation, dynamics. and function of proteins.[20−34] For example, the Hofmeister series[1] has been known for over a century and still remains one of the most active research area. The molecular mechanisms behind such a series of salts, as well as that of the well documented protein denaturation by urea and guanadinium and the protection by osmolytes such as trimethylamine oxide (TMAO), remain not fully understood.[35−37] Understanding the physical and chemical properties of these aqueous solutions is important for many processes in solution chemistry and protein science, especially those that involve interfaces (e.g., the biomacromolecule/water interface). These interfaces play critical roles in determining protein stability and solvation. Such interfaces are also important in many other physical, chemical, and atmospheric processes. With the over a century of accumulation of experimental data and fast advancements in both experimental and computational tools, we expect a more satisfactory and uniform solution to emerge soon.

For such an old and important problem, not surprisingly, different mechanisms have been proposed, for example, to explain how cosolvents and cosolutes affect protein structures and solubility. Roughly speaking, these mechanisms can be classified into "direct" and "indirect" ones. The direct mechanism involves the direct interaction between the solute of interest such as a protein and the cosolvents/cosolutes and the indirect mechanism is associated with the capability of the latter in affecting the water structure (or dynamics), which in turn affects the physical properties of the protein. Following Hofmeister,[1] cations and anions have often been ordered into the Hofmeister series. Ions "salting-out" proteins are believed by some researchers to make water structures and are called kosmotropes. Ions salting-in proteins are, on the other hand, thought to break water structure and are called chaotropes.[23,33] Similarly, both direct and indirect mechanisms have been proposed for urea denaturation and for protein structure protection by osmolytes. For example, Tanford[24] postulated that urea denatures protein by the indirect change of the hydrophobic interactions, whereas the direct interaction model[25] mainly involves direct interactions of urea with side chains and its hydrogen bonding with backbones of proteins.[25−27,35−37] To test the indirect model, a large number of experiments were performed on water structure and dynamics using different techniques, but controversies remain.[35−40]

Often related to the indirect mechanism, the effects of cosolvents and cosolutes on the water/air surface tension have also been examined carefully. Record and Pelgram[41] analyzed the effects of inorganic salts on the water/air surface tension and calculated the partitioning of ions between bulk and surface. They showed that the surface tension data are

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consistent with chaotropic anions being enriched and kosmotropic ones being depleted near the water/air interface. They also performed similar ion partitioning analyses[42] based on the solubility data of model peptide compounds and concluded that cations, in particular heavily hydrated ones, show strong preferential binding to the amide group. In the previous papers of this series,[43,44] we included both selective binding of anions over cations at interfaces as well as the cation/anion cooperativity to build a simple theoretical model for the salt effects on surface tension. This model provides a qualitative explanation of experimental data and underlines the importance of ion cooperativity in affecting water properties. Here, we investigate the role of preferential ion binding and ion cooperativity in affecting the solvation of solutes. In this theory both cation binding to the amide and cation/anion association based on the "matching water affinity" rule for simple ions are included. Using this theory, we try to establish a relation between the solvation energies of the ions and their effects on the solubility of model compounds, such as benzene and peptides, which provides an explanation for the related experimental observations. More importantly, we propose a simple argument, based on the ion's capability of affecting the hydrogen-bonding proton donor−acceptor equilibrium, to explain the importance of cation binding over anion in salting in the protein backbone and affecting the protein secondary structure. This argument also helps explain why some compounds such as urea are protein secondary structure denaturants whereas others such as TMAO are protectants.

II. SALT EFFECTS ON SOLUBILITY

II.1. Short Summary of Experimental Data. Numerous experimental measurements and excellent reviews exist on how addition of salts to an aqueous solution affects the solubility of solutes ranging from simple molecules such as H_2 and benzene to large biomolecules.[45] It is beyond the scope of this paper to provide an in-depth review on these data and we only focus on a few aspects here. It should be pointed out that the salt effects on solubility are normally both solute and cosolute specific. The salts ranked according to their effects on solubility show an order that is quite different from the one obtained based on their effects on surface tension. These different trends can be seen from Table 1, in which we list, as examples, some experimental data taken from the literature for the salt effects on surface tension, on the solubility of benzene, and on the solubility of AGTEE, a model peptide. For the surface tension, the data shown are $d\gamma/dm$, the increment of surface tension as a function of the molar concentration of the inorganic salts. On the other hand, the effects of salts on solubility at low to medium concentrations are normally written in terms of k_s

$$\ln f = k_s c_s \quad (1)$$

where f is the activity coefficient of the solute, c_s the molar concentration of the salt, and k_s a constant. When k_s is positive, the addition of the cosolute induces an increase of the activity coefficient and thus a reduction of the solubility of the solute. From examining the listed experimental data, several interesting trends will be discussed below and rationalized later.

In general all of the data in Table 1 follow the same Hofmeister series for the anions; that is, the higher the charge density, the more efficient they are to increase the water/air surface tension or to salt-out the solute.

The ranking orders for the cations are more complicated and show a strong dependence on the counterions. These data strongly suggest that the effects of salts cannot always be viewed as a simple summation of the effects of anions and cations, which indicates the importance of cation/anion cooperativity in affecting water properties. In fact, we showed in the previous study[43] that ion cooperativity varies with ion types and molecular dynamics simulations showed that ion association of simple ions is consistent with the "matching water affinity" rule. So the inclusion of ion cooperativity roughly following this "matching water affinity" rule provides a possible explanation for the rather complicated salt dependence of water/air surface tension.[44]

It is also easily seen from Table 1 that the ranking of the ions, especially cations, by their capability of affecting solubility is also heavily dependent on the solute itself. Here, we first examine in some detail the salt-dependence of benzene solubility in water. (The results on the other small nonpolar molecules such as H_2 and O_2 are rather similar to that on benzene.[46]) From the available data on NaCl, KCl, CsCl, NaBr, KBr, NaI, and CsI, one observes that the higher the charge density of the ions, the stronger their salting-out effects are. This trend is expected if the portioning of the ions between the solute/water interface and the bulk water is dominated by the hydration of ions. (LiCl appears to be out of order. It will be discussed in more detail later.)

Next, the salts listed in Table 1 affect the solubility of the model peptide ATGEE[47] in a manner distinctly different from that of benzene. It is seen from these data that the salting-in effect roughly increases with the charge density of the cations (in direct contrast to the case of benzene, where the salt-out effect increases with charge density). Earlier molecular dynamics simulations[48] and quantum chemistry calculations[49] have shown that the binding energy between cations and the carbonyl can be significant, although Algaer and van der Vegt[68] showed that the sodium/carbonyl interaction is weak compared to that between water and carbonyl. Experimental evidence are also not clear. For example, infrared spectral[50] and chromatography[51,52] studies suggest that the binding of cations to the carbonyl is weak, although Li NMR data[53] and viscosity[54,55] measurement indicated the opposite. The analysis of the solubility data by Pelgram and Record[42] did show the preferential binding of cations, especially the small and well hydrated ones, to the amide group. However, the thermodynamic data itself does not provide the mode (direct or indirect

Table 1. Salt Effects on Surface Tension and Setschenow Salting-Out Coefficients

	Ca^{2+}	Li^+	Na^+	K^+	Cs^+
	Benzene Setschenow Salting-Out Coefficients[a]				
Cl^-		0.14	0.20	0.17	0.09
Br^-			0.16	0.12	
I^-			0.10		−0.01
	Amide Setschenow Salting-Out Coefficients[b]				
Cl^-	−0.09	0.02	0.05	0.05	0.06
Br^-	−0.36	−0.17	0.00	−0.02	
I^-		−0.28	−0.23	−0.21	
	Surface Tension[c]				
Cl^-		1.65	1.73	1.59	1.57
Br^-		1.31	1.47	1.35	
I^-		0.78	1.14	1.15	

[a]In units of L/mol, from ref 46. [b]In units of L/mol, from ref 47. [c]In units of dyne/(cm^2 mol), from ref 41.

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binding) in which the cations accumulate around the amide backbone.

Two opposing ion binding scenarios may be proposed to explain the rough trend shown in the ATGEE solubility data: from the data given in Table 1, one sees that the salting-in effects are the strongest for iodides, whereas chlorides slightly salt-out the peptide. One straightforward explanation would be that anion binding is the main reason for the peptide to salt-in, and the binding is stronger for larger and less hydrated anions. This argument would, however, have a problem in explaining the larger salting-in effect of salts of strongly hydrated cations than weakly hydrated ones (see the stronger salting-in effect of $CaBr_2$ and LiBr than NaBr and KBr). It would also have trouble explaining the weaker salting-out effect of $CaCl_2$ and LiCl than NaCl, KCl, or CsCl. In fact, $CaCl_2$ was shown to salt-in the peptide, and CsCl is the strongest salt-out agent among the five chlorides studied. These experimental observations, on the other hand, are consistent with the argument that cation binding is responsible for the salting-in effect, and the binding is stronger for cations with higher charge densities. This latter possibility is the focus of the current paper. In section II.2, the "chemical processes" that contribute to the resulted preferred binding of cations and exclusion of anions from the polar amide backbone is discussed and a rational of the observed results of thermodynamics analysis of solubility data is provided.[42] In this derivation the cooperativity between anions and cations is not included. Next, in section II.3, the ion cooperativity is included and the correlation of the solvation (roughly speaking charge density) of the ions with their effects on affecting the solvation of amide is shown qualitatively. In this paper, instead of getting the quantitative results, we show that the simplest model allows us to capture the trends of a salt's capability in affecting the amide activity coefficient by using simply the individual ions' solvation strength.

II.2. Direct versus Indirect Ion Binding. To discuss ion binding in more detail, it is necessary to distinguish between the thermodynamically determined preferential binding and the detailed microscopic molecular mechanism. In fact, as mentioned above, although the solvation data and molecular dynamics simulations are consistent with preferential cation binding, experiments have not been able to unambiguously detect the molecular signature of such a direct binding. Experiments have also shown that single amino acid molecules are well hydrated in water and in fact they do not form hydrogen bonds with each other even at very high concentrations. On the basis of these experimental facts, we postulate that the carbonyl group is a good hydrogen bond acceptor and its solvation dominates (the hydrogen bonding and anion binding to the amino group were shown to be much weaker[48]). The hydration of the amide is then mainly affected by processes that can change the concentration of free proton donors in the solution. We discuss in the following molecular/ion interactions that could affect the binding/solvation of the carbonyl of amides. First, we note that in pure liquid water there exist nonsaturated proton donating and accepting sites, denoted as D and A, respectively,

$$H_2O \overset{K_H}{\leftrightarrow} D + A \tag{2}$$

In the meantime, because the cations M^+ and anions L^- interact with water, preferentially with proton accepting (water oxygen) and donating (proton) sites, respectively, they can generate additional proton donors and acceptors, respectively.

$$M^+ + H_2O \overset{K_+}{\leftrightarrow} M_S + nD$$

$$L^- + H_2O \overset{K_-}{\leftrightarrow} L_S + mA \tag{3}$$

In eq 3, M_S and L_S denote the hydrated cations and anions, respectively (they are thus also prevented from directly interacting with the amide). In the above equation, the hydration of M^+ and L^- each generates n and m proton donors and acceptors, respectively. The proton donors and acceptors interact with the amide groups CO and NH, respectively, through hydrogen bonding

$$-CO + D \overset{K_D}{\leftrightarrow} -COD$$

$$-CO + M^+ \overset{K_M}{\leftrightarrow} -COM$$

$$-NH + A \overset{K_A}{\leftrightarrow} -NHA \tag{4}$$

The "chemical" processes listed above lead to the following simple relations:

$$[D][A] = K_H$$

$$[M_S][D]^n = K_+[M^+]$$

$$[L_S][A]^m = K_-[L^-]$$

$$[-COD] = K_D[-CO][D]$$

$$[-COM] = K_M[-CO][M^+]$$

$$[-NHA] = K_A[-NH][A] \tag{5}$$

Since at low concentrations water concentration is largely a constant, it is included in the equilibrium constant K_H in the first equation given above. For the concentrations of the two ion species, we also have, according to the conservation of matters

$$\begin{aligned}&[M_s] + [M^+] + [-COM]\\ &\quad = [L_s] + [L^-] + [-NHA]\\ &\quad = [ML]\end{aligned} \tag{6}$$

where [ML] is the total concentration of the electrolyte. According to eqs 5 and 6 and when the concentration of the amide is low compared to the electrolyte, we obtain the following equations for the concentrations [A] and [D]

$$\frac{K_H}{[A]} - n\frac{K_+[ML][A]^n}{K_+[A]^n + K_H{}^n} = [A] - m\frac{K_-[ML]}{K_- + [A]^m}$$

$$\frac{K_H}{[D]} - n\frac{K_-[ML][D]^m}{K_-[D]^m + K_H{}^m} = [D] - \frac{K_+[ML]}{K_+ + [D]^n} \tag{7}$$

It is difficult to obtain analytic and general solutions to eq 7, and in what follows we discuss instead several limiting cases to gain insights on how salts with different cation/anion compositions may affect the solvation/hydration of amides. For this purpose, we will first examine how "direct" and "indirect" binding of cations changes the chemical potential of the amide. At low concentrations of salts and when the effect of cations on the hydrogen bond donor is much stronger than the anions, we can consider a "complete" hydration of cations ($K_+ \gg [D]^n$, a weak hydration scenario of cation is also easy to treat and leads to the similar results, but will not be discussed in

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details here) and neglect the effects of anions ($K_- \approx 0$). We then have

$$[\mathrm{D}] = \frac{\sqrt{(n[\mathrm{ML}])^2 + 4K_\mathrm{H}} + n[\mathrm{ML}]}{2} \tag{8}$$

Further, as [ML] is low, we have

$$[\mathrm{D}] = \sqrt{K_\mathrm{H}} + \frac{n[\mathrm{ML}]}{2} + O([\mathrm{ML}]^2) \tag{9}$$

and

$$[\mathrm{M}^+] = \frac{{K_\mathrm{H}}^{n/2}[\mathrm{ML}]}{K_+} + O([\mathrm{ML}]^2) \tag{10}$$

Making use of these equations, we obtain the following expressions for the concentrations [−COD] and [−COM]

$$[-\mathrm{COD}] = \left\{K_\mathrm{D}\sqrt{K_\mathrm{H}} + \frac{nK_\mathrm{D}}{2}[\mathrm{ML}] + O([\mathrm{ML}]^2)\right\}[-\mathrm{CO}]$$

$$[-\mathrm{COM}] = \left\{\frac{K_\mathrm{H}^{n/2}K_\mathrm{M}}{K_+}[\mathrm{ML}] + O([\mathrm{ML}]^2)\right\}[-\mathrm{CO}] \tag{11}$$

As a result, the "free" −CO has a concentration of

$$[-\mathrm{CO}] = \frac{1}{1 + K_\mathrm{D}\sqrt{K_\mathrm{H}} + \left(\frac{K_\mathrm{H}^{n/2}K_\mathrm{M}}{K_+} + \frac{nK_\mathrm{D}}{2}\right)[\mathrm{ML}]} \tag{12}$$

If one writes the above equation in the following form:

$$[-\mathrm{CO}] = \frac{1}{1 + K_\mathrm{D}[\mathrm{D}]_\mathrm{eff}} \tag{13}$$

one can define $[\mathrm{D}]_\mathrm{eff}$ as the effective concentration of proton donors in the presence of ML, and

$$[\mathrm{D}]_\mathrm{eff} = \sqrt{K_\mathrm{H}} + \left(\frac{K_\mathrm{H}^{n/2}K_\mathrm{M}}{K_+K_\mathrm{D}} + \frac{n}{2}\right)[\mathrm{ML}]. \tag{14}$$

According to eq 14, we may treat the enhanced solvation of the solute of interest as a result of the change of the effective concentration of the available proton donors in the solution, a consequence of added salts affecting the proton donor/acceptor balance. Therefore, from the above analysis, it is seen that both direct and indirect ion binding could be important in affecting the solvation of the solute. This analysis also shows that the thermodynamically determined cation preferential binding as calculated from solubility data may not lead to direct experimental observations of cation binding with the solute.[57] Next, if we choose the standard state as the free ("un-solvated") −CO with a concentration of $[-\mathrm{CO}]^\circ$, we write the k_s coefficient of free −CO, following eq 1

$$k_s([-\mathrm{CO}]) = -RT\frac{\mathrm{d}\ln[-\mathrm{CO}]/[-\mathrm{CO}]_0}{\mathrm{d}[\mathrm{ML}]} \approx RT\left(\frac{K_\mathrm{H}^{n/2}K_\mathrm{M}}{K_+K_\mathrm{D}} + \frac{n}{2}\right) \tag{15}$$

In obtaining the above equation, we have used the fact that $[-\mathrm{CO}]_0 = (1/1 + K_\mathrm{D}(K_\mathrm{H})^{1/2})$ and we kept only the linear term in the Taylor expansion for $\ln[-\mathrm{CO}]/[-\mathrm{CO}]_0$.

In the form of the Guggenheim equation, the preferential binding of ML can also be written as[42]

$$k_s \approx \frac{-RTvb_1\mathrm{ASA}(1+\varepsilon_\pm)}{m_1^\bullet}\left(\frac{\nu_+K_{\mathrm{P},+} + \nu_-K_{\mathrm{P},-}}{\nu} - 1\right) \tag{16}$$

where R is the gas constant, T is the temperature, $m_1^\bullet$ is the molality of water, and b_1 is the number of water molecules per surface area at the surface. In this equation, $1 + \varepsilon_\pm$ is the factor that converts the concentration derivative to an activity derivative, $\nu = \nu_+ + \nu_-$ is the number of ions per formula unit of the salt, and K_P's are the partition coefficient of the ions. When the effect of the anion is neglected

$$k_s \approx \frac{-RTvb_1\mathrm{ASA}(1+\varepsilon_\pm)}{m_1^\bullet}\frac{K_{\mathrm{P},+} - 1}{2} \tag{17}$$

Comparing eqs 15 and 17, we then see that

$$K_{\mathrm{P},+} \approx \frac{m_1^\bullet}{vb_1\mathrm{ASA}(1+\varepsilon_\pm)}\frac{(2K_\mathrm{H}^{n/2}K_\mathrm{M}/K_+ + nK_\mathrm{D})}{1 + K_\mathrm{D}\sqrt{K_\mathrm{H}}} + 1 \tag{18a}$$

Again, the above equation shows that the thermodynamically defined preferred binding of the ions can be either due to direct (the term containing K_M) or indirect effects (the term containing n).

When the effect of proton accepting interactions of anions are taken into account, similar approximations lead to the following relation:

$$K_{\mathrm{P},-} \approx 1 - \frac{m_1^\bullet}{vb_1\mathrm{ASA}(1+\varepsilon_\pm)}\frac{mK_\mathrm{D}}{1 + K_\mathrm{D}\sqrt{K_\mathrm{H}}} \tag{18b}$$

The fact that $K_{\mathrm{P},-} < 1$, as seen from eq 18b, shows that anions are excluded from the proton accepting carbonyl of amides. Taking eqs 18a and 18b together, it is seen that when −CO hydration is assumed to dominate the solvation of the amide, preferred binding and exclusion of cations and anions would be observed, although preferred binding might not exist in the form of direct binding. This is the most important idea of the present paper, and in the discussion section, I will discuss in more details its broad implication. In the next section, I will use this argument and include ion-cooperativity to show that the experimental results on salt effects can be explained.

II.3. Simple Theory for Salt Effects on Solubility. From the above analysis, it is seen that cations and anions can affect the solubility through its change of the concentration of effective proton donors either by direct binding (mainly cations) or by interacting with water (both cations and anions). Equation thus provides a direct way of calculating the "separate effects" of anions and cations on the solvation (both water and ion binding) of the amide backbone, without considering the cation−anion cooperativity. In the following we formulate a simple theory on salts effects of solubility that also takes into account ion cooperativity. To make use of the Guggenheim equation,[42] the partitioning of ions between the bulk aqueous solution and the surface of solute of interest is needed. Considering ion-cooperativity,[43] we write the Taylor expansion of the grand potential of the solution system as a functional of the local concentrations of the ions, up to the second order

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$$\Omega([M^+], [L^-]) = RT \int \left(\left(\ln \frac{[M^+](\vec{r})}{[ML]} - \frac{\mu_+(\vec{r})}{RT} \right)^2 + \left(\ln \frac{[L^-](\vec{r})}{[ML]} - \frac{\mu_-(\vec{r})}{RT} \right)^2 + \lambda (\ln[M^+](\vec{r}) - \ln[L^-](\vec{r}))^2 \right) d\vec{r} \quad (19)$$

with the constraint

$$\int [M^+](\vec{r})\, d\vec{r} = \int [L^-](\vec{r})\, d\vec{r} = [ML]V \quad (20)$$

The integration of the above equation over the full space with a total volume V can be approximately separated into that over the bulk and interfacial parts. The $[M^+](r)$ and $[L^-](r)$ are the local concentrations of the cations and anions, respectively; $\mu_+(r)$ and $\mu_-(r)$ are the "local external potential" resulted from the differences of ion solvation between the bulk and the surface at the infinite dilute salt concentration. In the absence of cooperativity between cations and anions, $\lambda = 0$, and eq 19 takes a minimum when $\ln([M^+](\vec{r})/[ML]) = (\mu_+\vec{r}/RT)$ and $\ln([L^-](\vec{r})/[ML]) = (\mu_-\vec{r}/RT)$. The last term in the integrand is thus the contribution from ion cooperativity, with the parameter λ describing the strength of ion cooperativity, which vanishes when ion association does not affect the ion distribution. In previous study, we have used a simple Born model to show that λ can be approximately written in the form $\lambda = \exp(-B(\Delta G^+_{sol} - \Delta G^-_{sol})^2)$, where B is a constant and ΔG_{sol} is the Born solvation energy of the ion. Equation 19 thus formally includes preferred ion binding as discussed in the previous section, as well as ion cooperativity, which has been shown important in interfacial distributions of ions.[43,44]

The contribution over the bulk can be neglected (its contribution relative to the interfacial term follows $\propto \int (1/V^2)\, dr = 1/V$, which vanishes as $V \to \infty$). For simplicity, we approximate the interfacial concentrations by average interfacial values (that deviate from their bulk values), and taken the local ion concentration as their average values, $[M^+]_i, [L^-]_i$, the above equation becomes

$$\Omega([M^+]_i, [L^-]_i) \approx RTv \left\{ \left(\ln \frac{[M^+]_i}{[ML]} - \frac{\mu_+}{RT} \right)^2 + \left(\ln \frac{[L^-]_i}{[ML]} - \frac{\mu_-}{RT} \right)^2 + \lambda (\ln[M^+]_i - \ln[L^-]_i)^2 \right\} \quad (21)$$

Minimizing $\Omega[M^+]_i[L^-]_i$ with respect to the local concentrations, $[M^+]_i[L^-]_i$, respectively, one obtains

$$[M^+]_i = [ML] \exp\left(-\frac{\mu_+ + \mu_-}{2RT} \right) \exp\left(-\frac{1}{RT} \frac{\mu_+ - \mu_-}{2 + 4\lambda} \right)$$
$$[L^-]_i = [ML] \exp\left(-\frac{\mu_+ + \mu_-}{2RT} \right) \exp\left(\frac{1}{RT} \frac{\mu_+ - \mu_-}{2 + 4\lambda} \right) \quad (22)$$

Substitution of eq 22 ($K_{P,+} = [M^+]_i[ML]$, $K_{P,-} = [L^-]_i[ML]$) into eq 16 yields

$$-RT \frac{d \ln[-CO]}{d [ML]} \approx \frac{-RTvb_1 ASA(1 + \varepsilon_\pm)}{m_1^\bullet} \left\{ \exp\left(-\frac{\mu_+ + \mu_-}{2RT} \right) \cosh \frac{1}{RT} \frac{\mu_+ - \mu_-}{2 + 4\lambda} - 1 \right\} \quad (23a)$$

As discussed above, $\mu_+(r)$ and $\mu_-(r)$ are determined by the difference between the bulk and interface solvation of cations and anions. In the following, we are interested in a qualitative understanding of the salt effects, and we only calculate the quantity k^n_s, defined as

$$k^s_n = \exp\left(-\frac{\mu_+ + \mu_-}{2RT} \right) \cosh \frac{1}{RT} \frac{\mu_+ - \mu_-}{2 + 4\lambda} - 1 \quad (23b)$$

III. RESULTS

In principle μ_+ and μ_- can be determined from eq 18, but a number of equilibrium constant parameters await experimental determination. Therefore, in the following qualitative treatment, we only make use of the argument obtained from the previous section on preferred binding and exclusion of cations and anions, and treat the binding constants in an approximate way. This is made possible by realizing that the binding of M^+ to both water oxygen and carbonyl oxygen is likely of the same electrostatic nature and thus both effects are expected to increase with the charge density of the cation. In the following, we use eq 23 to consider three special cases.

1. Without specific binding between the ions and the solute (and therefore the solute is of the hard-sphere type), we approximate the free energy change for an ion with a radius of $r_\pm$ moved from the bulk to the solute surface by the change of the dielectric constant from the bulk value ε to the interfacial value ε_{int}:[43]

$$\mu_\pm = \frac{q_\pm^2}{8\pi\varepsilon_0 r_\pm} \left(\frac{1}{\varepsilon} - \frac{1}{\varepsilon_{int}} \right) = \Delta G^\pm_{sol} \frac{\varepsilon_{int} - \varepsilon}{\varepsilon_{int}} \quad (24)$$

where again ΔG_{sol} is the Born solvation energy of the ion. Making use of eq 24, k_s as a function of $\Delta G^\pm_{sol}$ are calculated and given in Figure 1. It is seen from this figure that without preferential binding both cations and anions follow the same trend as expected: the higher the charge density, the stronger is the salting-out effect.

2. In the discussion given earlier, we have argued that both direct and indirect preferential binding of cations to amides (which involve cation binding to the carbonyl and water oxygen, respectively) are of the similar electrostatic nature. We therefore assume that this binding is proportional to the charge density and thus to the solvation energy and μ_+. At the same time, anion has the opposite effect and would be expelled from the amide carbonyl (eq 18b, anion binding to the NH group is neglected in this simplified treatment). Under these approximations $\mu_\pm = \Delta G_{sol}(\varepsilon_{int,amide} - \varepsilon/\varepsilon_{int,amide})(1 \mp a) = \Delta G_{sol,amide}(1 \mp a)$, where a describes the preferential binding (exclusion) of the cation (anion) to the carbonyl group. Due to the preferential binding and exclusion of cations and anions, respectively, as discussed in the previous section, a should be

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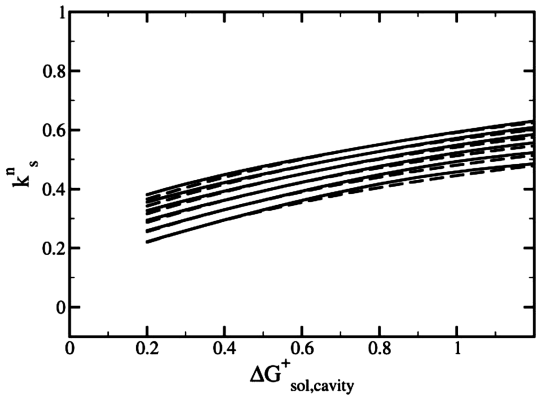

Figure 1. Effects of salts on the activity coefficient of a "cavity"without preferential binding of ions, predicted by eq 24, with ($\lambda = \exp(-4(\Delta G^+_{sol,amide} - \Delta G^-_{sol,amide})^2)$, solid lines, from top to bottom lines, $\Delta G^-_{sol,amide}$ is $0.2RT$, $0.4RT$, $0.6RT$, $0.8RT$, $1.0RT$, and $1.2RT$, respectively) and without ($\lambda = 0$ dashed lines) cooperativity. The calculated results shown are for the quantity k^n_s, which had no units. In the current theory, smaller ΔG_{sol} corresponds to more weakly hydrated ions.

negative. For simplicity, the same a value will be used for cations and anions. As an example, we set $a = -3$ and the calculated results for k_s as a function of ΔG^+_{sol} and ΔG^-_{sol} are given in Figure 2a. From this figure, it is seen that (1) for the same cation, salts formed by larger anions (smaller μ_-) show stronger salting-in effects, (2) for the same anion, salts formed by smaller cations (larger μ_+) generally show stronger salting-in effects or weaker salt-out effect, (3) a minimum salting-in effect might appear for cations with intermediate size, which shows the importance of the cation/anion cooperativity, (4) as a result of salting-in effect of the cations and ion cooperativity, salts can be ranked by the following order according to their capabilities of salting-in a carbonyl: (small cation, large anion) > (small cation, small anion) > (large cation, large anion) > (large cation, small anion). For comparison, the experimental results on ATGEE are shown in Figure 2b, from which a similar trend as the theoretical results are observed, for example, for salting-in effects LiI > CsI (KI) > LiCl > LiI.

3. Molecular dynamics simulations have shown that anions, especially large ones with low charge density and high polarizibility tend to accumulate near the apolar surfaces including the water/air interface. In this special case, we take into account anion binding empirically by introducing an attractive (and thus negative) term ν, so that

$$\mu_+ = \Delta G_{sol}{}^+ \frac{\varepsilon_{int,apolar} - \varepsilon}{\varepsilon_{int,apolar}} = \Delta G^+_{sol,apolar}$$

$$\mu_+ = \Delta G_{sol}{}^- \frac{\varepsilon_{int,apolar} - \varepsilon}{\varepsilon_{int,apolar}} = \Delta G^-_{sol,apolar} - \Gamma \quad (25)$$

IV. DISCUSSION

The current study strives to provide a simple, molecular, although at this stage, qualitative (or semiquantitative) mechanism for the different behaviors of salts (as well as other cosolutes and cosolvents) in affecting the hydration/solvation of the amides and protein backbone. Especially, a physical picture was proposed to understand the thermodynamics analyses of solubility data (e.g., the use of the Guggenheim equation or the Kirkwood-Buff integration),

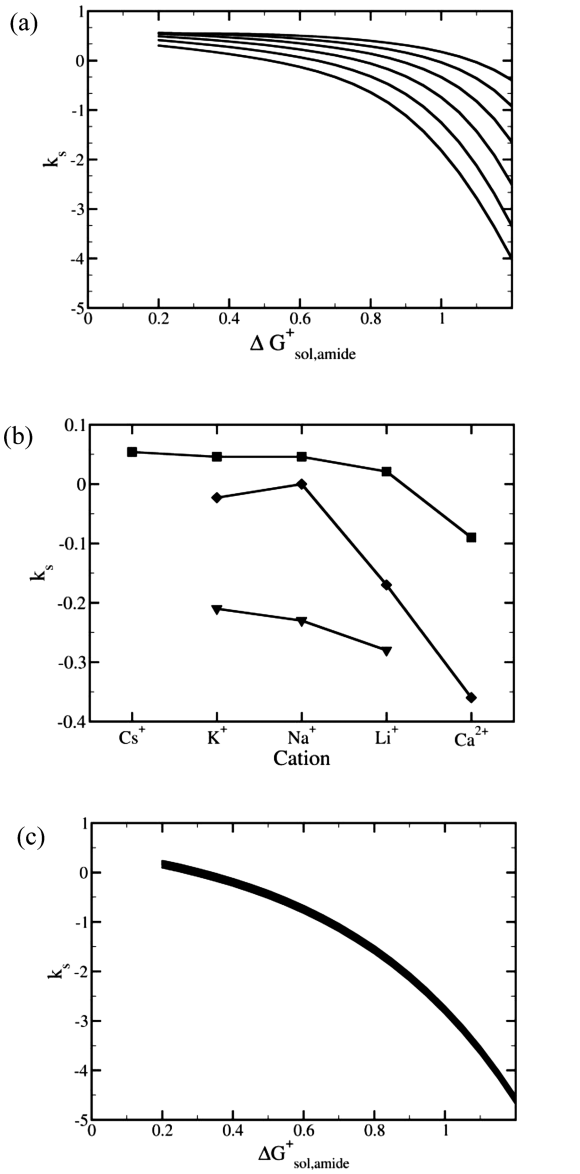

Figure 2. Theoretical (a) and experimental (b) results on the effects of salts (with different ion compositions) on the activity coefficient of molecules containing amides. The calculated results shown are for the quantity k^n_s, using eqs 24 and 25, $\Gamma = 1RT$ and $\lambda = \exp(-4(\Delta G^+_{sol,amide} - \Delta G^-_{sol,amide})^2)$. The experimental results (in units of L/mol) are for ATGEE and taken from ref 47. The theoretical results without ion cooperativity, $\lambda = 0$, are given in panel c. In panel a, from top to bottom lines, $\Delta G^-_{sol,amide}$ is $0.2RT$, $0.4RT$, $0.6RT$, $0.8RT$, $1.0RT$, and $1.2RT$, respectively.

which starts from experimental data to obtain ion partitioning coefficients. Such an analysis does not yield molecular information either on the mode of ion partitions or on the physical reason of preferred binding or exclusion from the protein surface. We presented here a simple explanation for the different ranking orders for salt effects observed for the solubility of nonpolar molecules (e.g., benzene) and amides. Consistent with earlier thermodynamics analyses,[42] the current analysis shows that cation binding plays an important role in salting-in the peptides. Although direct binding of cations to the carbonyl oxygen has been observed in a number of molecular dynamics simulations, as discussed earlier, direct experimental evidence has been elusive. Similarly, thermodynamics analysis

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does not allow one to distinguish between direct and indirect effects. These two effects represent two possible binding modes: cations of high charge density can either preferentially "bind" a good proton acceptor such as the carbonyl oxygen of amide through a direct interaction or by increasing the concentration and/or activity of water proton donors and thus enhancing the hydrogen bonding and hydration of the carbonyl. In this study, we show that the direct and indirect effects can be combined together to represent an effective change of the "proton donor concentration" in the aqueous solution.

This simple argument can be used to understand the observed ranking order of salts in affecting the activity of model peptides in water (which is also consistent with other experimental measurements such as protein conformation changes[45]). For example, it provides a straightforward understanding of the observed salting in effect of strongly hydrated cations such as Li^+ and Ca^{2+}. It predicts that when the cation charge density and thus its interacting strength with both water and carbonyl decrease, the salting-in effect of the corresponding salt also decreases, consistent with experimental observations. For example, the salting-out capabilities of chlorides increase in the order $CaCl_2$ < LiCl < NaCl = KCl < CsCl. (Cation/anion cooperativity also affects the ranking order, see below.) The other important conclusion of the present model is that the thermodynamic preferential binding of cations to the protein surface does not necessarily reflect in the direct binding of the cation.[57]

The proton donor/acceptor balance can also help us understand denaturation or protection of protein by other solutes/solvents. For example, since urea and Gdm^+ are both rich in proton donors for hydrogen bonding, they tend to increase the hydrogen bonding between the protein backbone and the rest of the solution, and as a result denature protein secondary structures. The increase of amide solvation by the denaturants again can be a result of both direct and indirect effects.[58,59] In contrast, because alcohols[60,61] are rich in proton donors (the hydroxyl group of alcohols contains two proton acceptors and one donor), their addition to water tilts the proton donor/acceptor balance toward lower proton donor concentrations. Their effects thus reflect in weakening the solvation of the carbonyl group and enhancing the secondary structure. The strength of this effect increases with the number of hydroxyl groups and polyhydrolic alcohols are expected to be more efficient in strengthening the secondary structures of proteins. On the other hand, the fluorine substitution in trifluorine ethanol (TFE) increases the acidity of its hydroxyl group and thus its proton donating ability. Therefore, TFE has a weaker effect in promoting the secondary structure formation compared to the alcohols without the fluorine substitution. These predictions are consistent with the experimental observations as well as computer simulation studies.[60,61]

A widely used protein structure renaturant trimethylamine N-oxide (or as another example, glycine betaine, GB[62]) contains only proton acceptors and not donors. Its addition to water is expected to reduce the concentration of free proton donors and thus weakens the hydrogen bonds between water and the amide carbonyl oxygen (which is an indirect effect). Consistently, our earlier simulation study showed that upon the addition of TMAO to the model peptide or protein aqueous solutions, the hydrogen bonds between the amide carbonyl oxygen and water become elongated.[63] Additionally, according to this argument, most amino acids, because of their proton-accepting carboxyl groups, should also act as protein secondary structure protectants, whereas residues rich in proton donors, such as lysine and arginine, are expected to have stronger denaturing effects. Certainly, other properties, such as dielectric constant, polarity and packing density of the protein solution will also affect the protein hydration and solvation, but the consistency between the abovementioned cosolvents' proton donating capability and their effects on protein secondary structures indicates that the proton donor balance plays a major role for amide (at least carbonyl) hydration.

The other deduction from the theory based on the proton donor/acceptor balance is that at low concentrations, since water largely determines the chemical equilibrium between proton donors and acceptors, solutes rich in proton donors and those rich in acceptors have opposite effects and compensate each other when mixed together. Anions, due to their interactions with proton donors, would reduce the salt-in capability of their counterions. As a result, a salt with a strongly solvated cation and a weakly solvated anion (e.g., MgI_2 or even $MgCl_2$) has a strong salting-in effect, whereas with an opposite composition, a salt with a weakly solvated cation and a strongly solvated anion (such as K_2SO_4) would have a strong salting-out effect on the solvation of the protein backbone. Such expectations are consistent with experimental observations.[45] Similarly, proton-acceptor rich cosolvents such as TMAO[63] counteract the denaturing effects of proton donor rich molecules such as urea, which is again a well-known experimental observation. The currently analysis allowed the separation of the effects of anions and cations as well as their cooperativity in affecting the solvation of the protein backbone, and thus provides a "molecular" explanation of the thermodynamics solubility measurement. Therefore, it leads to conclusions that can be tested directly by spectroscopic measurement: for example, the current theory predicts that the addition of strongly solvated anions (or renaturants TMAO) will weaken the hydrogen bond between the CO and water, thus will generate a blue shift in the CO vibration frequency. In addition, earlier simulations papers dealing with ion pairing showed that differences in the H-bonding donor ability of hydrated cations can provide an explanation for Hofmeister series of osmotic/salt activity coefficients of carboxylate and phosphate salts. It was found that the hydrated Li^+ ion is a better H-bonding donor than a hydrated Na^+ ion.[64,65]

Besides the cation/anion compensating and cooperative effects (through proton donor/acceptor equilibrium) on proton donor availability, their association may also play an important role in rendering the corresponding salt its effect on solubility. The cation/anion cooperativity, which is the most obvious for bromides, can be seen from the solubility data of ATGEE. Without cooperativity, one would expect that as the charge density of the counterion decreases, the salting-in effect of the bromide would also gradually decrease. In contrast, their salting-in effect shows a minimum (with a vanishing k_s) for NaBr, which corresponds well to the strong cation/anion association for this salt, indicated by both solubility and water/air surface tension data.[42] In fact, when ion cooperativity is not included in eq 23, the calculated results deviate significantly from experimental data (Figures 2c and 3c).

The above discussion centers at the salt effects on the solvation of the polar carbonyl group. In a real system, one should also take into account the salt effects on the apolar component. A very simple model taking into account the

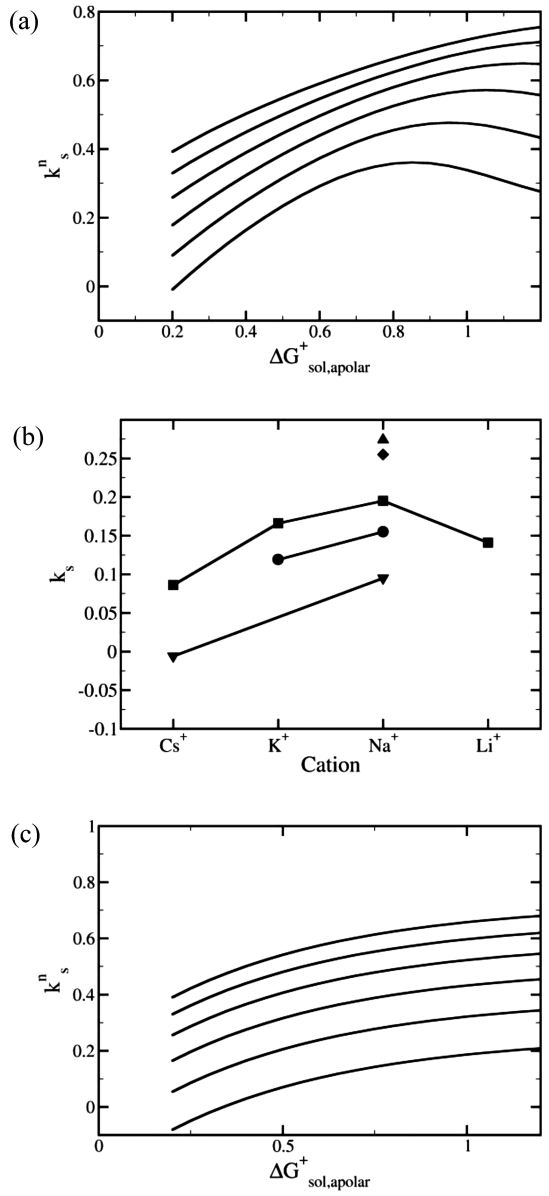

Figure 3. Calculated (a) and experimental (b) effects of salts on the activity coefficient of apolar molecules. The calculated results shown are for the quantity k_s^n, using eq 24 and $\mu_{\pm} = \Delta G_{sol}(\varepsilon_{int,amide} - \varepsilon/\varepsilon_{int,amide})(1 \mp 3) = \Delta G_{sol,amide}(1 \mp 3)$ and $\lambda = \exp(-4(\Delta G^+_{sol,amide} - \Delta G^-_{sol,amide})^2)$. The experimental results (in units of L/mol) are for benzene and are taken from ref 46. The theoretical results without ion cooperativity are given in (c). In panels (a) and (c), from top to bottom lines, $\Delta G^-_{sol,amide}$ is 0.2*RT*, 0.4*RT*, 0.6*RT*, 0.8*RT*, 1.0*RT*, and 1.2*RT*, respectively.

preferential binding of anions at apolar interfaces was also discussed. This preferential binding when combined with the ion-cooperativity effect provides an explanation of the salt effects on the solubility of benzene in water: Although normally the increase of the ionic size decreases its salting-out effect, strong association between Na^+ and Cl^- reduces the preferential binding of anions at the water/benzene interface and makes it an even stronger salting-out agent than LiCl. In this case, as mentioned earlier, Li^+ being an ion with a tight solvation shell could also contribute to the low salting-out effect of LiCl. For comparison, in Figure 4, we also show a simple case where polar (amide) and apolar solvation are both present (each contribute 50%).

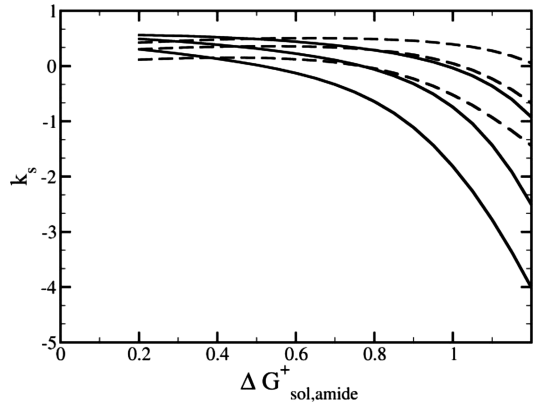

Figure 4. Theoretical results of salts effect (dashed lines) on a model system containing both polar and apolar terms (each contribute 50% in the calculation using eq 24), compared to the results of the "polar solute" reported in Figure 3 (solid lines). From top to bottom lines, $\Delta G^-_{sol,amide}$ is 0.2*RT*, 0.6*RT*, and 1.2*RT*, respectively.

Further, the existence of both apolar side chains and polar backbone is expected to make ion-cooperativity more important in affecting the solubility of a polypeptide or protein. For example, under such a condition, iodides are much stronger salting-in agents than corresponding chlorides: the Li^+ of both LiCl and LiI are by itself has a strong salting-in effect, through improved solvation of the amide carbonyl group. However, the I^- but not Cl^- binds preferentially the apolar surface, and the latter is expelled to a larger extent from the solute of interest. Due to the strong ion cooperativity/association between Li^+ and Cl^-, the cation preferential binding and solvation of the former to the solute is weakened. Consistently, in our recent MD simulation study,[66] it was found that NaI but not NaF or Na_2SO_3 denatures BBA5. The latter two enhances polypeptide secondary structure formations. The cation in the former but not the latter two shows significant preferential binding to the carbonyl oxygen. In NaF or Na_2SO_3 the cations are pulled away by the anions, which are expelled from the polypeptide surface.

Similar effects were observed for GdmCl and GdmSCN. Our simulation study[67] for the denaturation of the B domain of protein A by these two electrolytes showed that they both change the proton donor/acceptor balance of water, so that there are more non-hydrogen bonded water molecules with free proton donors than those with free unsaturated proton acceptors, consistent with them being protein denaturants. Furthermore, it was also found that GdmSCN has a stronger effect in changing this water proton donor/acceptor balance than GdmCl, rendering it a higher denaturing capability. It is known from experiments that GdmSCN is a stronger denaturant than GdmCl. $(Gdm)_2SO_4$, on the other hand, slightly promotes protein structure formation. The difference between these salts again suggests the importance of the compensating effects between cations and anions. Using MD simulations with both polarizable and nonpolarizable force fields, Heyda et al. have shown[48] that Na^+ and K^+, especially the former, interacts with the carbonyl oxygen of N-methylacetamide (NMA). The halides, on the other hand, do not show any significant attraction to the NH group. In a different simulation using a nonpolarizable force field,[68] not only cation binding to the carbonyl but also anion binding to the NH was observed for

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NMA, with the cation binding being more strongly. Anion binding was much weaker to the PNiPAM NH group, showing that at least with this force field the ion direct binding is sensitive to the local environment. Further, the ion partitioning study of Leontidis et al. showed[69] that Na^+ binds with the lipid head groups whereas anion partitions within the lipid monolayers.

Finally, we have to emphasize here again that to obtain simple physical pictures, only limiting cases are discussed in the current study. And for protein solvation, we only focus on the amide backbone. For a real system where protein domains are of different polarities, solvation of backbone and side chains are expected to affect each other and results in more complicated behavior. For example, at high concentrations of salts, the salting-out effect on apolar side chains can lead to hydrophobic collapse, which will change the local polarity of the main chains, and affect the hydration of the latter. We should also note that the current study of the salt effects on amide (polypeptide and protein backbone) focuses on the low concentrations and thus mainly the salting-in effect due to the increased solvation of individual amides. At high concentrations, the direct binding of ions could interlink amides and may result in a salting-out effect instead.

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Notes

The authors declare no competing financial interest.

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(57) Effective binding constant of M+ to −CO: from Eq. 11, we can also estimate the binding constant of M+ to the −CO group.

$$[-\mathrm{COM}]\% = \frac{\frac{K_H^{n/2}K_M}{K_+}[\mathrm{ML}]}{1 + K_D\sqrt{K_H} + \frac{nK_D}{2}[\mathrm{ML}] + \frac{K_H^{n/2}K_M}{K_+}[\mathrm{ML}]}100$$

From the above equation it is easily seen that one can get a wrong estimation of the binding constant of M+ based on a measurement of [−COM] as a function of [ML], especially it can be severely underestimated if

$$\frac{nK_D}{2} >> \frac{K_H^{n/2}K_M}{K_+}$$

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