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On the effect of sodium salts on the coil-to-globule transition of poly(*N*-isopropylacrylamide)

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Summary

It has been shown that sodium salts affect significantly the temperature of the coil-to-globule collapse transition of poly(*N*-isopropylacrylamide) [*J.Am.Chem.Soc.*, 2005, **127**, 14505]. Since this phenomenon resembles the cold renaturation of globular proteins, it can be studied by means of the theoretical approach devised to rationalise the occurrence and the mechanism of cold denaturation [G. Graziano, *Phys.Chem.Chem.Phys.*, 2010, **12**, 14245; *Phys.Chem.Chem.Phys.*, 2014, **16**, 21755]. It emerges that the collapse transition is driven by the decrease in solvent-excluded volume in order to maximise the translational entropy of water molecules and ions. At a given temperature, the aqueous solutions of sodium salts have densities higher than that of water. For this reason, the magnitude of the solvent-excluded volume effect proves to be larger, stabilizing the globular conformations of poly(*N*-isopropylacrylamide). On the other hand, two large ions, iodide and thiocyanate, are poorly hydrated and stabilise the coil conformations of the polymer by a preferential binding mechanism.

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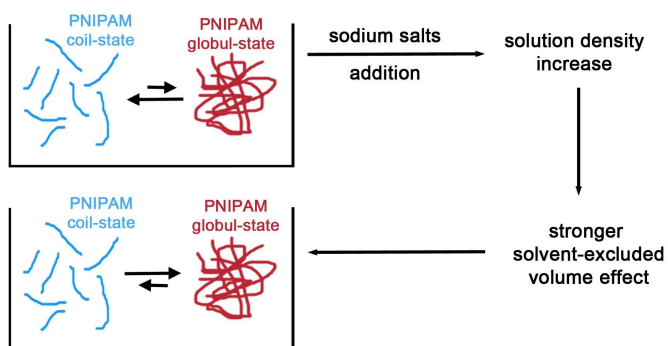
Sodium salts addition to water increases the density, raising the solvent-excluded volume effect magnitude, stabilizing the PNIPAM globule state.

Introduction

  The coil-to-globule collapse transition of poly(*N*-isopropylacrylamide), PNIPAM, has been considered, by a long time, a good model system for the cold denaturation-renaturation of globular proteins.[1-4] On increasing the temperature, PNIPAM chains collapse to globular conformations at T(collapse) $\approx$ 31°C in water.[1-4] The collapse transition proves to be linked to the aggregation of PNIPAM globules (i.e., to the lower critical solution temperature, LCST, of the polymer in water). By taking advantage of the latter feature, Cremer and colleagues have been able to study in detail, by means of a microfluidic device, the effect of several sodium salts on the PNIPAM collapse transition.[5,6] The results indicate that most of the anions are able to decrease the T(collapse) value (i.e., to increase the temperature range where globular conformations are stable; a *salting-out* phenomenon), in line with the famous Hofmeister series.[7] Moreover, the effect depends on salt concentration. Strongly hydrated anions, such as SO_4^{2-} and CO_3^{2-}, cause a significant decrease of T(collapse), whereas poorly hydrated anions, such as I^- and SCN^-, cause a slight increase of T(collapse); the latter would be a *salting-in* phenomenon. For instance, some of the T(collapse) values determined by Cremer and colleagues are the following: T(collapse) = 31°C in water, 32°C in 0.3 M NaSCN, 31.5°C in 0.3 M NaI, 28.5°C in 0.3 M NaCl, 25.5°C in 0.3 M NaF, 18.5°C in Na_2SO_4 and 15°C in 0.3 M Na_2CO_3 (these numbers are obtained from Figure 1c in ref. [5], and for the latter two salts refer to the lower-temperature phase transition of a two-step phase transition).[5,6] It is worth noting that very similar results have been obtained by Cremer and colleagues also for two elastin-like polypeptides.[8] These very interesting findings have been rationalised by means of a combination of the hydration entropy of the anions, the surface tension increase caused by anion addition to water, and the direct binding of poorly hydrated anions to the PNIPAM surface.[5,6,8] However, this heuristic rationalization cannot be considered fully satisfactory.

  One of us has developed a simple and reliable statistical thermodynamic approach to explain the occurrence of cold denaturation in water and its molecular mechanism.[9,10] We would like to show that the same approach can account for the effect of sodium salts on the collapse transition of PNIPAM. It is necessary to recognise that PNIPAM globules do not

exactly correspond to the native state of globular proteins. The *globule* state represents a huge ensemble of collapsed conformations that do not possess the same 3D structural organization; PNIPAM chains have much more conformational freedom in comparison to the polypeptide chains in the native state. The decrease in solvent-excluded volume upon collapse (reliably measured by the decrease in water accessible surface area, WASA[11]) is the actual driving force of the coil-to-globule transition,[9,10,12,13] leading to a marked gain in the translational entropy of water molecules. The main determinant of the anion effect on the T(collapse) value proves to be the basic fact that the experimental density of the aqueous salt solutions is larger than that of water. A liquid density increase leads to an increase in the volume packing density of the solution (i.e., the fraction of the total liquid volume really occupied by molecules and ions). The latter causes an increase in the magnitude of the solvent-excluded volume effect and so an increase in the stability of the PNIPAM globular conformations. In addition, for the two large anions, I^- and SCN^-, causing a slight T(collapse) increase, a preferential binding mechanism has to be taken into account due to their low charge density.

Theoretical approach

The coil-to-globule transition of PNIPAM in water and aqueous salt solutions should mainly be driven by the minimization of the solvent-excluded volume (i.e., WASA minimization) in order to maximise the translational entropy of water molecules and ions.[9,10,12,13] It should be considered an entropy-driven collapse transition.[14,15] The conformations populated by PNIPAM chains can be divided into two large ensembles: (a) the open-swollen conformations whose average properties are represented by the *coil* state, *C*-state; (b) the compact-globular conformations whose average properties are represented by the *globule* state, *G*-state. According to the devised approach,[9,10] the Gibbs energy change associated with the collapse-swelling transition, ΔG_{tr}, is given by (note that the formula is written adopting the globular protein convention: the *G*-state is stable when ΔG_{tr} is positive):

$$\Delta G_{tr} = [\Delta G_c(C) - \Delta G_c(G)] - T \cdot \Delta S_{conf} + [E_a(C) - E_a(G) + \Delta E(intra)] =$$
$$= \Delta\Delta G_c - T \cdot \Delta S_{conf} + \Delta E_a \qquad\qquad (1)$$

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where $\Delta G_c(C)$ and $\Delta G_c(G)$ represent the reversible work to create in water or aqueous salt solutions a cavity suitable to host the *C*-state and the *G*-state, respectively; ΔS_{conf} represents the conformational entropy gain of PNIPAM chains upon swelling; $E_a(C)$ and $E_a(G)$ represent the energetic interactions (i.e., both van der Waals attractions and H-bonds) among the *C*-state or the *G*-state of PNIPAM and surrounding water molecules and ions; $\Delta E(intra)$ is the difference in intra-chain energetic interactions between the *C*-state and the *G*-state. In eqn. (1), terms due to the reorganization of water-water H-bonds are not present because the latter process is characterized by an almost complete enthalpy-entropy compensation.[16,17]

It has already been shown that the $\Delta\Delta G_c$ contribution is always positive, favouring the *G*-state, because the reversible work of cavity creation is proportional to chain WASA, whose value markedly increases upon swelling.[9,10,18-20] In contrast, the $-T \cdot \Delta S_{conf}$ contribution is always negative, favouring the *C*-state. The ΔE_a contribution is expected to be negative,[21,22] because the number of energetic interactions that a PNIPAM chain is able to do intra-molecularly and with water molecules in the *C*-state should be larger than that the same PNIPAM chain is able to do intra-molecularly and with water molecules in the *G*-state. The ΔE_a contribution should be even more negative in aqueous solutions of poorly hydrated ions that are preferentially bound by the PNIPAM surface (a situation similar to that occurring for globular proteins in the presence of denaturants).[18-20] This general reasoning implies that the ΔE_a contribution should favour the *C*-state. Of course, at T(collapse) a perfect balance between the three contributions in eqn. (1) has to hold, so that $\Delta G_{tr} = 0$.

Calculation procedure

To study the PNIPAM collapse-swelling thermodynamics, it is necessary to calculate: (1) the reversible work to create in water and aqueous salt solutions a cavity suitable to host the chain in its *C*-state and *G*-state, respectively, over a large temperature range; (2) the conformational entropy gain of the chain upon swelling; (3) the ΔE_a contribution.

The first task can be accomplished by means of a simple, geometric model.[9,10] On passing from the *G*-state to the *C*-state, the polymeric chain changes conformation but its van der Waals volume is practically not affected: $V_{vdW}(G\text{-state}) \approx V_{vdW}(C\text{-state})$. What

markedly changes is the solvent-excluded volume that can reliably be measured by WASA which strongly increases upon swelling: WASA(*C*-state) >> WASA(*G*-state). It is assumed that the *G*-state is a sphere, whereas the *C*-state is a prolate spherocylinder, having the same V_{vdW} of the sphere representing the *G*-state, as in the case of globular proteins.[9,10] According to the computer simulations of Rodriguez-Ropero and van der Vegt,[23] a collapsed PNIPAM chain made up of 40 monomer units has a radius of gyration equal to 10 Å in the SPC/E water model.[24] These simulations should be reliable because they reproduce the occurrence of collapse at 31°C.[23] As a consequence, a PNIPAM chain of 40 monomer units in the *G*-state is modelled as a sphere of radius a = 10 Å, V_{vdW} = 4189 Å^3 and WASA = 1633 Å^2. The *C*-state is modelled as a prolate spherocylinder possessing radius a = 5 Å, cylindrical length l = 47 Å, so that V_{vdW} = 4189 Å^3 and WASA = 2405 Å^2. It is possible to select different spherocylinders to model the *C*-state of the PNIPAM chain, always with the constraint that V_{vdW} = 4189 Å^3. From the qualitative point of view the outcome of the analysis does not change; see the Appendix A.

The $\Delta\Delta G_c$ contribution is calculated by means of the classic scaled particle theory,[18-20,25-27] SPT, formula for a spherocylindrical cavity of radius a and cylindrical length l in a hard sphere fluid mixture (the pressure-volume term is neglected for its smallness at P = 1 atm):

$$\Delta G_c = RT \cdot \{-\ln(1-\xi_3) + [6\xi_2/(1-\xi_3)]a + [12\xi_1/(1-\xi_3)]a^2 + [18\xi_2^2/(1-\xi_3)^2]a^2 +$$
$$+ [3\xi_2/2(1-\xi_3)]l + [6\xi_1/(1-\xi_3)]a \cdot l + [9\xi_2^2/(1-\xi_3)^2]a \cdot l\} \qquad (2)$$

where $\xi_i = (\pi/6) \cdot \Sigma \rho_j \cdot \sigma_j^i$, and ρ_j is the number density, in molecules per Å^3, of the species j and σ_j is the corresponding hard sphere diameter; $\xi_3 = (\pi/6) \cdot \Sigma \rho_j \cdot \sigma_j^3$ represents the volume packing density of the hard sphere fluid mixture; by setting l = 0, the formula is that for a spherical cavity of radius a; by considering only one component, eqn. (2) is that for a hard sphere fluid.

The experimental values of the density of water, heavy water and all the considered aqueous salt solutions, over the 0-40°C temperature range and 1 atm, have been used to

perform calculations.[28-32] The following effective hard sphere diameters have been selected and considered to be temperature-independent: σ = 2.80 Å for both water and heavy water molecules;[33] 2.02 Å for Na^+ ions;[34] 2.66 Å for F^- ions; 3.62 Å for Cl^- ions; 3.92 Å for Br^- ions; 4.40 Å for I^- ions; 3.58 Å for NO_3^- ions; 4.76 Å for $H_2PO_4^-$ ions; 4.60 Å for SO_4^{2-} ions (all these values come from Table XIII of ref. [35]); 3.94 Å for SCN^- ions, the same hard sphere diameter of carbon dioxide;[36] 4.20 Å for CO_3^{2-} ions.[37]

The second task is accomplished by neglecting the coupling between backbone and side-chain degrees of freedom, so that the $T \cdot \Delta S_{conf}$ term is calculated by considering that each monomer unit gains a temperature-independent conformational entropy upon swelling:[9,10,18-20]

$$T \cdot \Delta S_{conf} = T \cdot N_{res} \cdot \Delta S_{conf}(res) \tag{3}$$

where N_{res} = 40 and $\Delta S_{conf}(res)$ = 4 J K^{-1}molres^{-1}. It has to be stressed that such a value is markedly smaller than that used in the case of globular proteins.[9,10,18-20,38] The *globule* state of PNIPAM does not correspond to a single and unique structure (like the native state of globular proteins), and so the backbone and the side-chains of this polymer have a lot of conformational freedom also in the *globule* state. For this reason a small value has been selected for $\Delta S_{conf}(res)$. It would be possible to assign different values to $\Delta S_{conf}(res)$ without altering the trend of the obtained T(collapse) values as a function of salt type and concentration (see below).

The third task is more difficult and direct computer simulations would be required to obtain a reliable ΔE_a value. The latter is estimated by combining two pieces of information. The computer simulations of Rodriguez-Ropero and van der Vegt have shown that the collapse of the 40-monomer unit PNIPAM chain, in the SPC/E water model, causes the loss of 9 H-bonds between the side-chain amide groups and water molecules that are not regained intra-molecularly.[23] This means that ΔE_a has to be a negative and significant quantity. In addition, since ΔG_{tr}[T(collapse)] = 0 and T(collapse) = 31°C in water, and $\Delta \Delta G_c$(water) =

$\Delta G_c(C) - \Delta G_c(G) = 215.2$ kJ mol^{-1} at T(collapse) = 31°C, it is possible to take advantage of this constraint via eqn. (1) and of the T·ΔS_{conf} estimate reported above, fixing:

$$\Delta E_a = T \cdot \Delta S_{conf} - \Delta\Delta G_c = 48.7 - 215.2 = -166.5 \text{ kJ mol}^{-1} \tag{4}$$

This ΔE_a value is reliable by considering the loss of 9 H-bonds upon collapse. It is considered to be temperature-independent over the 0-40°C temperature range and to be right for all the considered aqueous salt solutions, at least as a first approximation (see also the Appendix B).

Results

The experimental values of the density of water and the 0.3 M aqueous salt solutions, over the 0-40°C temperature range are shown in Figure 1A: it is evident that all the considered sodium salts have a density higher than that of water. The corresponding estimates of the volume packing density, ξ_3, calculated using the hard sphere diameter values reported above, are shown in Figure 1B as a function of temperature. The calculated $\Delta\Delta G_c = \Delta G_c(C) - \Delta G_c(G)$ functions for water and all the 0.3 M sodium salt solutions are reported in Figure 2 over the 0-40°C temperature range (their values at 25°C are listed in the fifth column of Table 1). All the $\Delta\Delta G_c$ functions are large positive and increase with temperature. The latter feature is due to (a) the very small density decrease of water and aqueous salt solutions on increasing the temperature, and (b) the rise of the RT factor in eqn. (2), that represents the average thermal energy of the liquid molecules bombarding the cavity surface, and occurs also in the general statistical mechanical formula of ΔG_c.[26,27] The rank order magnitude of the $\Delta\Delta G_c$ functions calculated for 0.3 M sodium salt solutions is: water < NaSCN < NaNO$_3$ < NaI < NaBr < NaCl < NaF < NaH$_2$PO$_4$ < Na$_2$SO$_4$ < Na$_2$CO$_3$ (see Table 1). This rank order corresponds to that found by Cremer and colleagues[5,6] for the sodium salts that stabilise the *globule* state of PNIPAM [i.e., those causing a T(collapse) decrease], but cannot account for the ability of NaSCN and NaI to stabilise the *coil* state of PNIPAM [i.e., those causing a slight T(collapse) increase].

By superimposing the $T \cdot \Delta S_{conf} - \Delta E_a$ straight line (calculated as explained in the Calculation Procedure section) over all the $\Delta \Delta G_c$ functions, the intersection points should correspond to the T(collapse) estimates. The following values have been obtained: T(collapse) = 31°C in water, 28.5°C in 0.3 M NaSCN, 27°C in 0.3 M $NaNO_3$, 23°C in 0.3 M NaI, 23°C in 0.3 M NaCl, 19.5°C in 0.3 M NaH_2PO_4, 11.5°C in 0.3 M Na_2SO_4, 10.0°C in 0.3 M Na_2CO_3 (see the last column of Table 1 for a complete list). These values are in line with those experimentally determined by Cremer and colleagues for the sodium salts stabilizing the *globule* state of PNIPAM.[5,6] In addition, a small vertical shift of the $T \cdot \Delta S_{conf} - \Delta E_a$ straight line, by selecting a ΔE_a value slightly larger in magnitude (i.e., -170 versus -166.5 kJ mol^{-1}), would produce T(collapse) numbers for NaI and NaSCN larger than that of water, in line with the experimental results by Cremer and colleagues (see Figure 3). This should not be considered an *ad hoc* modification because it is well established that large ions, with a low charge density, interact weakly with water molecules and prefer to have dispersion-type interactions with the nonpolar-polar surface of globular proteins or polymers.[39-42]

By repeating the same calculations at a larger salt concentration, 0.5 M for instance, the scenario does not change from the qualitative point of view (see Figure 4). The magnitude of the calculated $\Delta \Delta G_c$ functions increases with salt concentration because the volume packing density rises (due to the density increase), whereas the $T \cdot \Delta S_{conf} - \Delta E_a$ straight line is considered to be not affected by salt concentration. As a consequence, the T(collapse) values decrease on increasing the salt concentration, in line with experimental results for the sodium salts stabilizing the *globule* state of PNIPAM (see Figure 1c in ref. [5]). As before, for NaI and NaSCN, which slightly stabilise the *coil* state of PNIPAM, it is necessary to increase the ΔE_a magnitude in order to have T(collapse) values slightly larger than that of water.

Discussion

The present analysis suggests that the effect of sodium salts on the T(collapse) value of PNIPAM can largely be captured by looking at the magnitude of the $\Delta \Delta G_c$ contribution, which is always a large positive number simply because WASA(*C*-state) >> WASA(*G*-state) and so the solvent-excluded volume effect caused by the *C*-state is larger than that caused by

the *G*-state. According to classic SPT formulae, the latter magnitude depends both on the volume packing density ξ_3 of the solution and on the average hard sphere diameter $<\sigma>$ of the solution particles.[34] By keeping $<\sigma>$ fixed, the ΔG_c magnitude rises on increasing ξ_3 because the void space in solution decreases and the probability of finding molecular-sized cavities decreases. By keeping ξ_3 fixed, the ΔG_c magnitude rises on decreasing $<\sigma>$ because the void space in solution is partitioned in smaller pieces (i.e., the particle size is a characteristic length-scale of the liquid), and the probability of finding molecular-sized cavities decreases. The effective hard sphere diameter of water molecules is 2.8 Å;[39,43] the $<\sigma>$ value of all the considered sodium salt solutions is close to 2.8 Å because water is, by far, the most abundant component. Therefore, the main role is played by the increase in volume packing density ξ_3 caused by the addition of sodium salts to water. In any case, a small $<\sigma>$ effect is evident by looking at the sodium halides (see the sixth column of Table 1): the largest $\Delta\Delta G_c$ value is produced by the salt having the smallest anion, NaF (of course, the differences are very small). It is worth noting that the density changes are not large and so also the changes in the $\Delta\Delta G_c$ contribution are small, but they produce significant effects on T(collapse) because a delicate balance is operative in eqn. (1).

The validity of the above arguments is confirmed by looking at the small T(collapse) increase, 0.6°C, found by Cremer and colleagues on dissolving PNIPAM in D_2O instead of H_2O.[6] The molecules of heavy water and water have the same effective hard sphere diameter, but the former liquid has a slightly larger molar volume, and so a slightly smaller ξ_3 value (see Table 1 and Figures 1a and 1b): 0.3817 versus 0.3831 at 25°C and 1 atm.[33] As a consequence, the magnitude of the solvent-excluded volume effect is smaller in heavy water and, in fact, nonpolar species are slightly more soluble in D_2O than H_2O.[33] Thus, turning to the PNIPAM collapse, the magnitude of the $\Delta\Delta G_c$ function in D_2O is slightly smaller than that in H_2O and, by keeping the $T \cdot \Delta S_{conf} - \Delta E_a$ straight line fixed, the T(collapse) value proves to increase by a small quantity (see Figures 2 and 4).

All the considered sodium salts, in view of the strength of the electrostatic interactions between ions and water molecules, cause a density increase, which leads to a ξ_3 increase, and so to an increase in the $\Delta\Delta G_c$ magnitude. This reasoning underscores that the stabilizing

action toward the *globule* state of PNIPAM of most sodium salts largely reflects the density increase caused by their addition to water. Also the two salts, NaI and NaSCN, causing a small increase in the T(collapse) value, are able to increase the density and the $\Delta\Delta G_c$ magnitude with respect to the pure water case. However, both the iodide and thiocyanate anions are poorly hydrated because they are characterised by a low charge density,[44,45] and have preferential attractive interactions with the polymer (or protein) surface.[39-42] Therefore, the *salting-in* ability of NaI and NaSCN should be caused by the anion preferential binding on the PNIPAM surface (i.e., the *coil* state is favoured because it has a WASA larger than that of the *globule* state).

The anions causing a T(collapse) decrease are strongly hydrated because they have a high charge density, and exert their stabilizing action by increasing the magnitude of the solvent-excluded volume effect (i.e., increase of the solution density). The anions causing a T(collapse) increase are poorly hydrated because have a low charge density, and exert their destabilizing action of the *globule* state by means of direct interactions on the exposed PNIPAM surface. The anions of the first type are usually called kosmotropes, the anions of the second type are usually called chaotropes, making reference to an imagined ability to "increase" or "decrease" the structure of water.[46,47] Actually, direct structural data have shown that strongly hydrated ions cause a breakage of the tetrahedral network and cannot be classified as kosmotropes.[48,49] The fundamental point is not the structure of the aqueous salt solutions, which is obviously different from that of water. This can readily be appreciated by looking at the experimental partial molar volumes, PMV, at infinite dilution in water, at 25 °C and 1 atm, determined by Millero,[50] of the various ions; see the values listed in the third column of Table 2. All these values are markedly different from the molar volume of water. In addition, the comparison of Millero's PMV with the ion van der Waals volumes[37] emphasises that the ions having the stronger electrostrictive effect (i.e., negative ΔV values) are those causing the larger T(collapse) decrease for PNIPAM. Note that: (a) the ΔV values are correlated with the ionic B coefficients determined by means of NMR relaxation measurements at 25°C[47]; negative ΔV numbers correspond to positive B numbers; (b) the

electrostrictive effect, determined by the strength of the electric field of the ion, is the ultimate reason of the density increase associated with the addition of sodium salts to water.

In conclusion, according to the devised approach, the coil-to-globule transition of PNIPAM is governed by the balance of three contributions: (a) the gain in translational entropy of water molecules and ions associated with the decrease in solvent-excluded volume (i.e., WASA decrease) upon polymer collapse; (b) the loss in conformational entropy upon polymer collapse; (c) the loss of energetic interactions associated with collapse. The addition of sodium salts to water affects this balance in a definite way and the calculated T(collapse) values are in line with the experimental ones. The two main factors are: (a) the density of the aqueous salt solutions, affecting the magnitude of the solvent-excluded volume effect (i.e., the $\Delta\Delta G_c$ magnitude;[9,10,18-20] and (b) the preferential interactions of poorly hydrated anions with the polymer exposed surface.[18-20,39-41] The ability of the devised theoretical approach to provide a rationalization of the conformational stability of globular proteins as a function of temperature (with the straightforward occurrence of cold denaturation), and of the effect of sodium salts on the coil-to-globule transition of PNIPAM merits attention.

Appendix A. Effect of the geometric model of the C-state

It is important to show that the devised approach works well also on changing the geometric model of the *C*-state. Two further prolate spherocylinders have been considered, always with the constraint that their V_{vdW} is equal to that of the sphere modelling the *G*-state (that is not modified). The first one has radius $a = 4.5$ Å, cylindrical length $l = 59.8$ Å, and WASA = 2654 Å^2; the second one has radius $a = 5.5$ Å, cylindrical length $l = 36.7$ Å, and WASA = 2189 Å^2. By performing the same classic SPT calculations for the aqueous 0.3 M sodium salt solutions, one obtains the $\Delta\Delta G_c$ functions reported in Figure A1 (panel A and panel B, respectively), whose qualitative trend is closely similar to that of Figure 2. Moreover, by using, in any case, $\Delta S_{conf}(res) = 4.0$ J K^{-1}molres^{-1}, and by fixing $\Delta E_a = -237.0$ kJ mol^{-1} in the case of the first spherocylinder and -111.5 kJ mol^{-1} in the case of the second spherocylinder (the ΔE_a magnitude should be proportional to ΔWASA), one obtains two straight lines that are reported in Figure A1, panel A and B, respectively. The intersection points between the latter straight lines and the $\Delta\Delta G_c$ functions produce T(collapse) values that are in line with those obtained in Figure 2. Therefore, it should be concluded that the devised approach is robust to changes in the geometric model of the *C*-state.

Appendix B. On the slope of the $T \cdot \Delta S_{conf}$ - ΔE_a straight line

It is worth noting that a change in the sign of the slope of the $T \cdot \Delta S_{conf}$ - ΔE_a straight line does not modify the conclusion of the analysis: the gain in translational entropy of water molecules and ions is the driving force of the PNIPAM collapse. In Figure B1, largely corresponding to Figure 2 of the main text, three different straight lines [all producing T(collapse) = 31°C in the case of water] are reported: (1) the original one with positive slope; (2) a second one with zero slope; (3) a third one with negative slope. It is not useful to provide numbers, but to clarify the physical grounds of the possible different slopes. The negative ΔE_a quantity has been considered to be temperature-independent over the 0-40°C temperature range. However, it should depend on temperature and the corresponding heat capacity change should be a positive number. This implies a trade-off between the positive slope of the $T \cdot \Delta S_{conf}$ term and the negative slope of the $-\Delta E_a$ term, and so the overall straight line slope could be positive, negative or zero. In any case, by looking at the intersection points of the three straight lines with the $\Delta \Delta G_c$ functions in Figure B1, it is evident that the slope change does not modify the scenario from the qualitative point of view [i.e., the trend of the T(collapse) values is not affected, and corresponds to that reported in the last column of Table 1]. We still think that the slope should be a slightly positive number.

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Table 1. Values of the experimental density, water molar concentration and volume packing density, at 25°C and 1 atm, for heavy water, water and the 0.3 M aqueous solutions of all the considered sodium salts; effective hard sphere diameters of the anions, considered to be temperature-independent. The classic SPT estimates of the $\Delta\Delta G_c$ quantity at 25°C and 1 atm and the calculated T(collapse) numbers of the PNIPAM model are listed in the last two columns. The experimental values of T(collapse), obtained from Figure 1c in ref. [5], are reported in parentheses. See text for further details.

	d	[H_2O]	σ	ξ_3	$\Delta\Delta G_c$	T(collapse)
	g ml^{-1}	M	Å		kJ mol^{-1}	°C
D_2O	1.1045	55.15	- -	0.3817	209.8	35.5 (31.6)
H_2O	0.9970	55.34	- -	0.3831	211.5	31.0 (31.0)
NaSCN	1.0095	55.29	3.94	0.3851	212.8	28.5 (32.0)
$NaNO_3$	1.0140	55.47	3.58	0.3849	213.3	27.0 (30.0)
NaI	1.0314	55.35	4.40	0.3874	214.8	23.0 (31.5)
NaBr	1.0207	55.54	3.92	0.3868	215.0	23.0 (30.0)
NaCl	1.0095	55.66	3.62	0.3864	215.1	23.0 (28.5)
NaF	1.0099	55.96	2.66	0.3858	215.4	22.0 (25.5)
NaH_2PO_4	1.0221	55.34	4.76	0.3899	216.7	19.5 (24.5)
Na_2SO_4	1.0345	55.96	4.60	0.3919	220.3	11.5 (18.5)
Na_2CO_3	1.0289	56.22	4.20	0.3916	221.0	10.0 (15.0)

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Table 2. Estimates of the van der Waals volume of the ions,[37] their partial molar volumes at infinite dilution in water at 25°C,[50] and the difference between the latter two numbers. Values of the ionic B coefficients, from NMR relaxation measurements at 25°C,[47] are listed in the last column. See text for further details.

| | V_{vdW} | PMV | ΔV | B_{NMR} |
	cm^3mol^{-1}	cm^3mol^{-1}	cm^3mol^{-1}	
Na^+	2.2	-1.2	-3.4	0.06
SCN^-	19.7	35.7	16.0	-0.07
NO_3^-	23.4	29.0	5.6	-0.05
I^-	25.4	36.2	10.8	-0.08
Br^-	18.7	24.7	6.0	-0.04
Cl^-	14.9	17.8	2.9	-0.01
F^-	6.3	-1.2	-5.1	0.14
$H_2PO_4^-$	36.1	29.1	-7.0	- -
SO_4^{2-}	31.5	14.0	-17.5	0.12
CO_3^{2-}	24.5	-4.3	-28.8	0.25

Captions to the Figures

Figure 1. Experimental density of water, heavy water and the 0.3 M aqueous solutions of all the considered sodium salts over the 0-40°C temperature range (panel A). Values of the volume packing density, ξ_3, for water, heavy water and the 0.3 M aqueous solutions of all the considered sodium salts over the 0-40°C temperature range (panel B).

Figure 2. Temperature dependence of the $\Delta\Delta G_c$ functions for water, heavy water and the 0.3 M aqueous solutions of all the considered sodium salts, and of the single $T \cdot \Delta S_{conf} - \Delta E_a$ straight line. The T(collapse) values correspond to the intersection points between this straight line and the $\Delta\Delta G_c$ functions. See text for further details.

Figure 3. Temperature dependence of the $\Delta\Delta G_c$ functions for water and the 0.3 M aqueous solutions of NaI and NaSCN, and of two $T \cdot \Delta S_{conf} - \Delta E_a$ straight lines, one with $\Delta E_a = -166.5$ kJ/mol (line 1, solid) and the other with $\Delta E_a = -170$ kJ/mol (line 2, dashed). The dashed line is slightly translated in the vertical direction with respect to the original solid one. In this way, the T(collapse) values of the two salts prove to be higher than that of water. See text for further details.
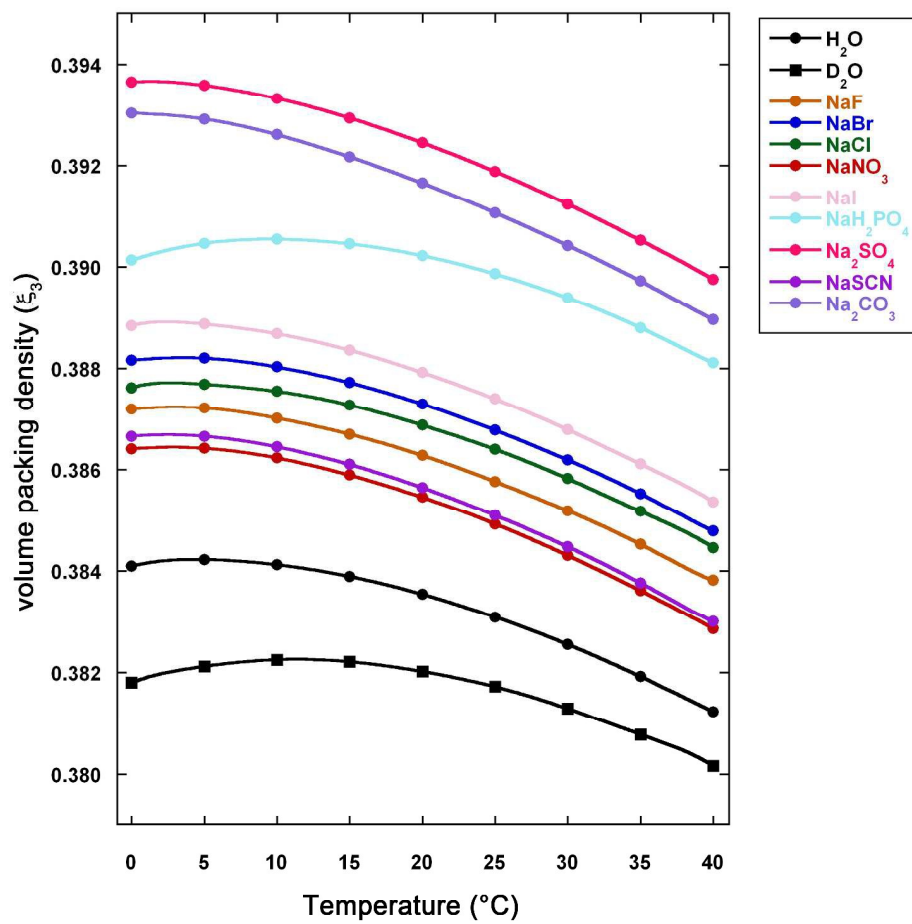
Figure 4. Temperature dependence of the $\Delta\Delta G_c$ functions for water, heavy water and the 0.5 M aqueous solutions of all the considered sodium salts, and of the single $T \cdot \Delta S_{conf} - \Delta E_a$ straight line. The T(collapse) values correspond to the intersection points between this straight line and the $\Delta\Delta G_c$ functions. See text for further details.
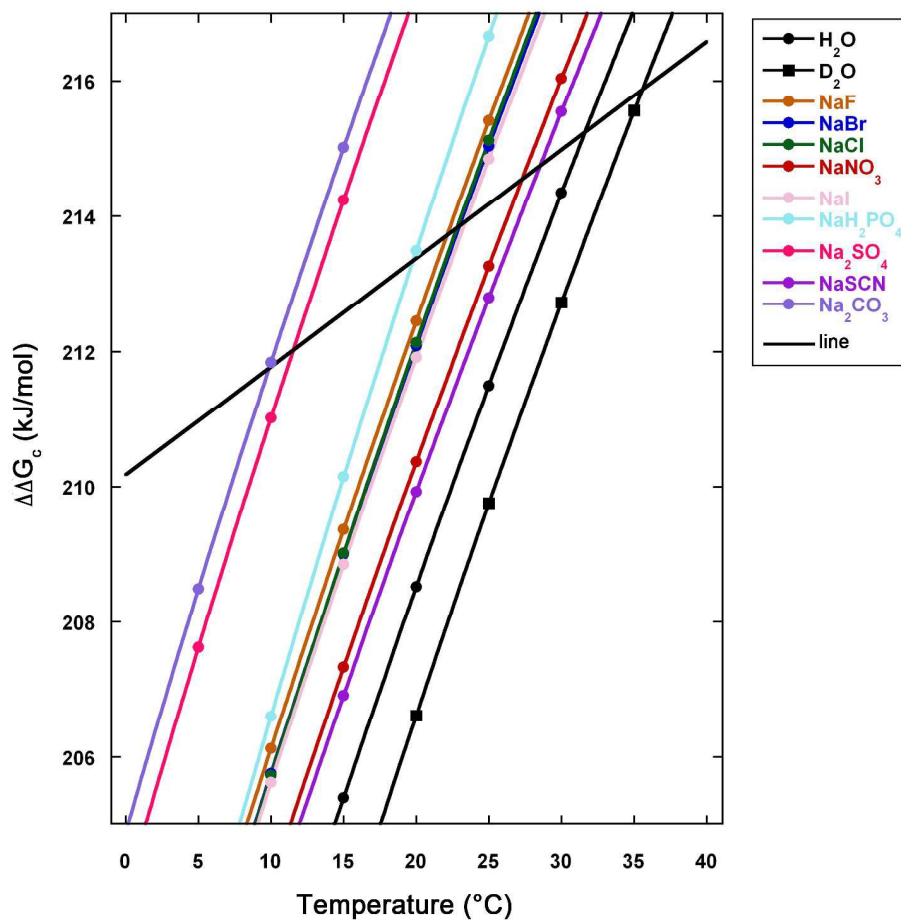
Figure 1. Experimental density of water, heavy water and the 0.3 M aqueous solutions of all the considered sodium salts over the 0-40°C temperature range (panel A). Values of the volume packing density, ξ_3, for water, heavy water and the 0.3 M aqueous solutions of all the considered sodium salts over the 0-40°C temperature range (panel B).
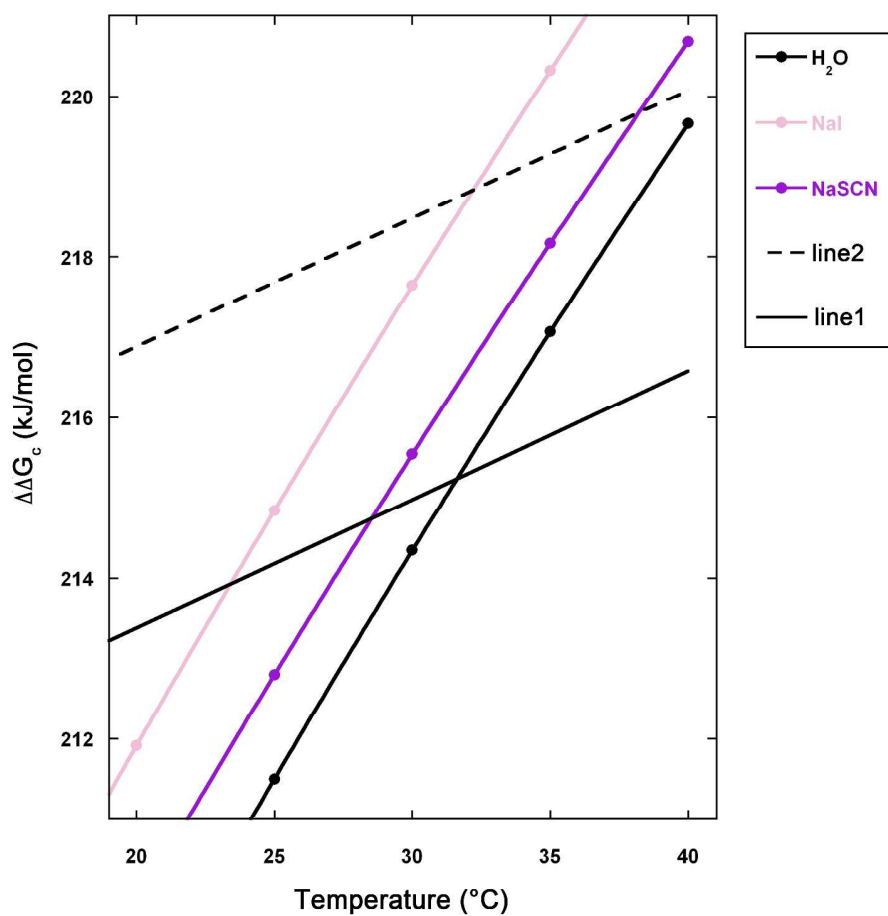
A

B

Figure 2. Temperature dependence of the $\Delta\Delta G_c$ functions for water, heavy water and the 0.3 M aqueous solutions of all the considered sodium salts, and of the single $T \cdot \Delta S_{conf} - \Delta E_a$ straight line. The T(collapse) values correspond to the intersection points between this straight line and the $\Delta\Delta G_c$ functions. See text for further details.

Figure 3. Temperature dependence of the $\Delta\Delta G_c$ functions for water and the 0.3 M aqueous solutions of NaI and NaSCN, and of two $T\cdot\Delta S_{conf} - \Delta E_a$ straight lines, one with $\Delta E_a = -166.5$ kJ/mol (line1, solid) and the other with $\Delta E_a = -170$ kJ/mol (line2, dashed). The dashed line is slightly translated in the vertical direction with respect to the original solid one. In this way, the T(collapse) values of the two salts prove to be higher than that of water. See text for further details.

Figure 4. Temperature dependence of the $\Delta\Delta G_c$ functions for water, heavy water and the 0.5 M aqueous solutions of all the considered sodium salts, and of the single $T\cdot\Delta S_{conf}$ - ΔE_a straight line. The T(collapse) values correspond to the intersection points between this straight line and the $\Delta\Delta G_c$ functions. See text for further details.
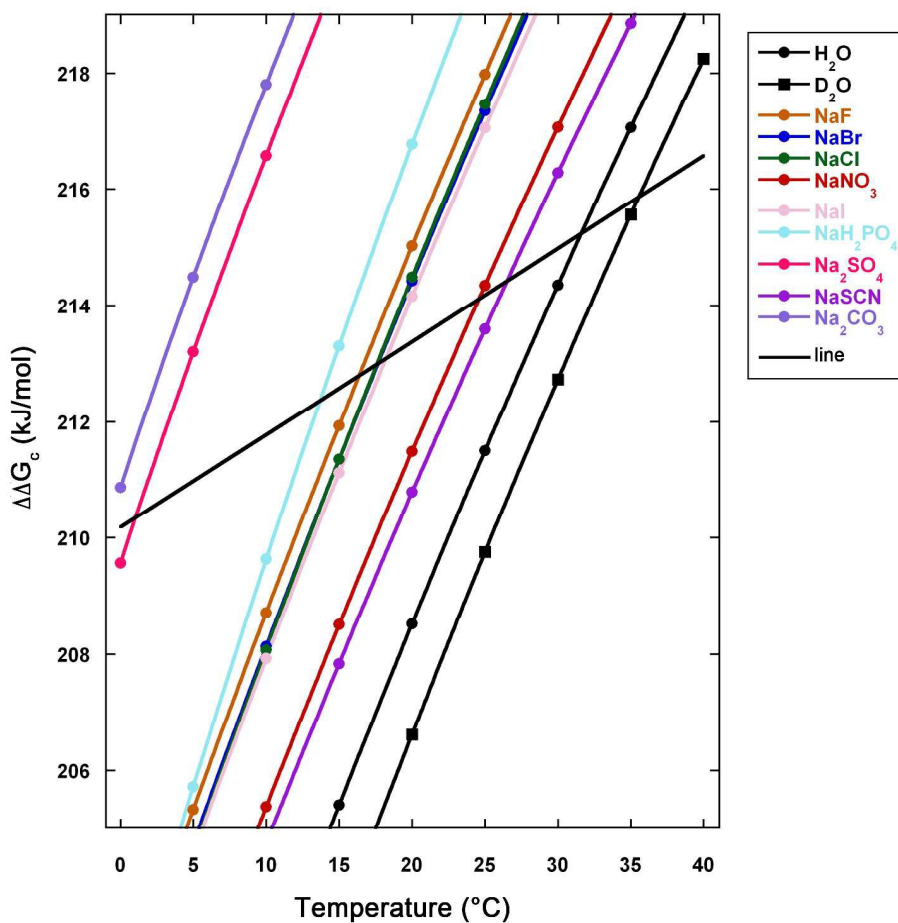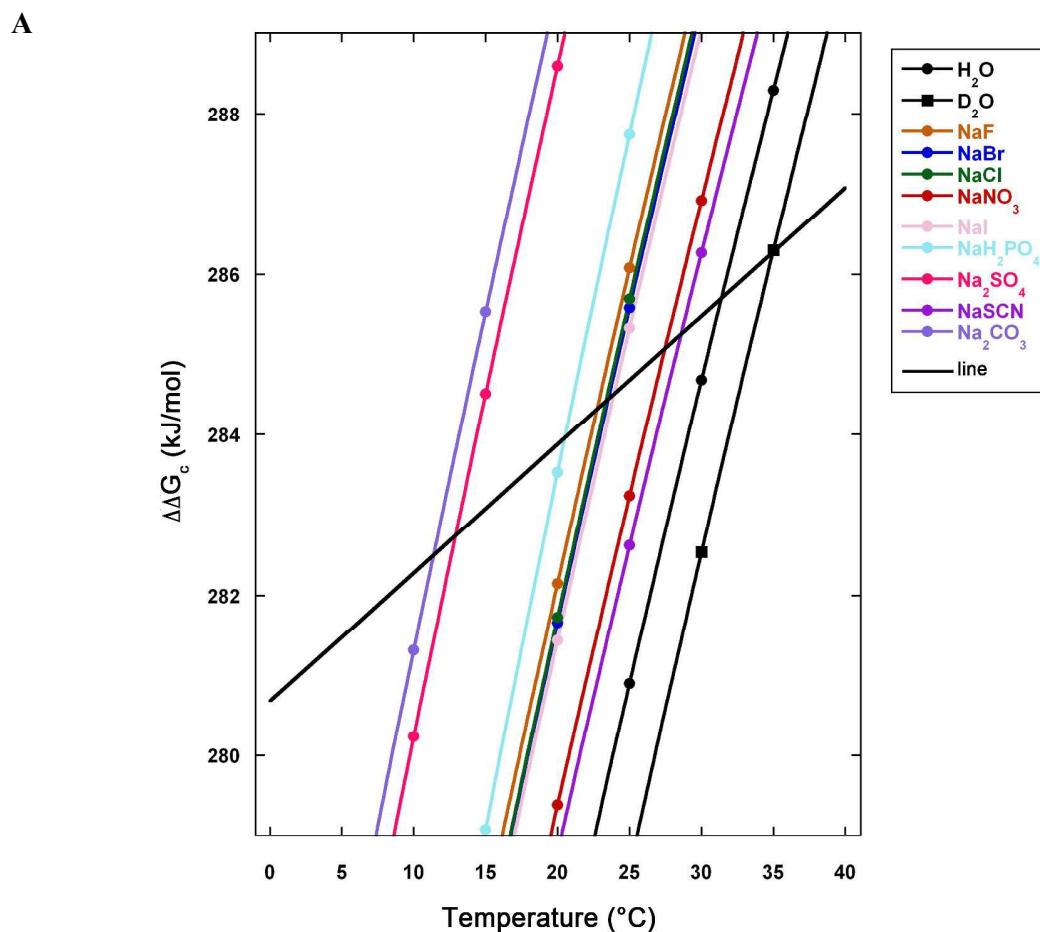
Figure A1. Temperature dependence of the $\Delta\Delta G_c$ functions for water, heavy water and the 0.3 M aqueous solutions of all the considered sodium salts by considering the first (panel A) and the second spherocylinder (panel B) as models of the *C*-state. The intersection points between the $T \cdot \Delta S_{conf} - \Delta E_a$ straight line (calculated as explained in the text of Appendix A), and the $\Delta\Delta G_c$ functions correspond to the T(collapse) values.
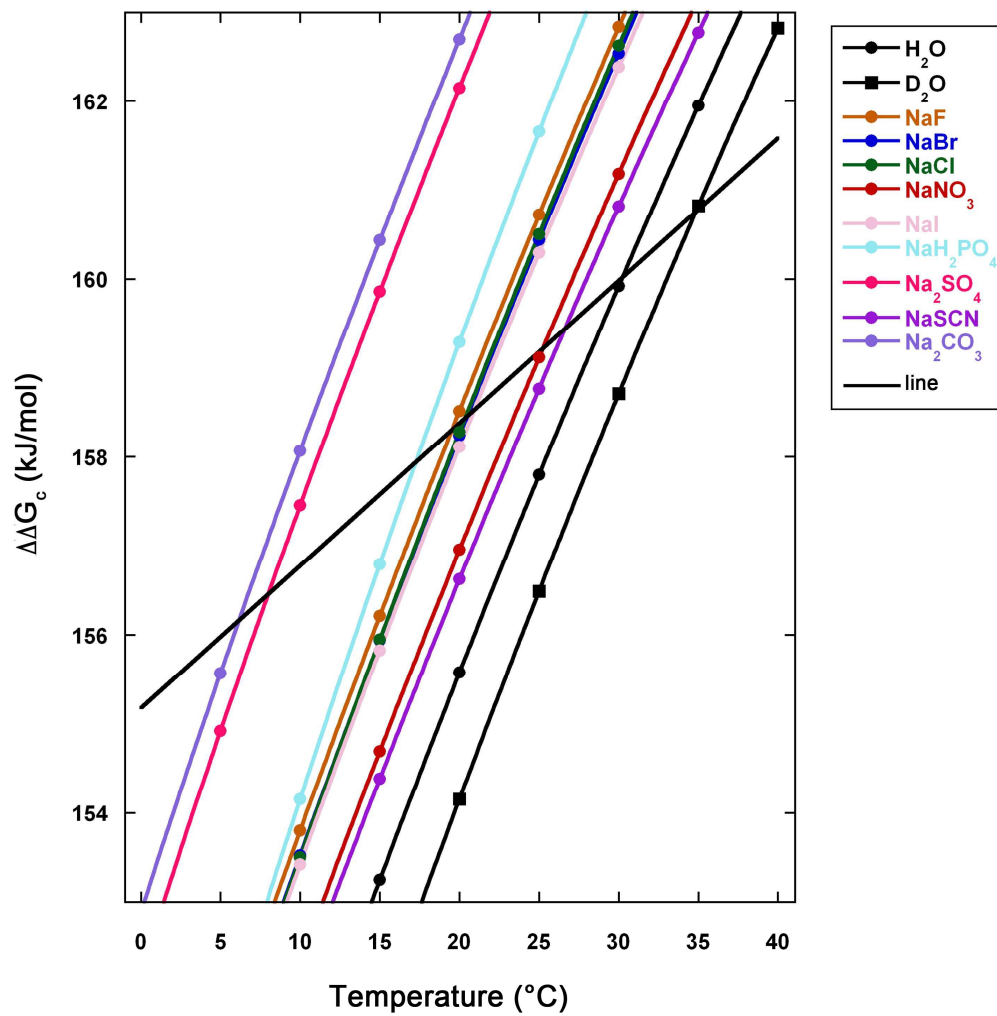
B

Figure B1. Temperature dependence of the $\Delta\Delta G_c$ functions for water, heavy water and the 0.3 M aqueous solutions of all the considered sodium salts (as in Figure 2), and of three $T\cdot\Delta S_{conf} - \Delta E_a$ straight lines, having positive slope (line 1, the original one), zero slope (line 2), negative slope (line 3). See the text of Appendix B for further details.

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