

Amides as models to study the hydration of proteins and peptides - spectroscopic and theoretical approach on hydration in various temperatures

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Abstract

Interactions with water are one of the key factors which determine protein stability and activity in aqueous solutions. However, the protein hydration is still insufficiently understood. *N*-methylacetamide (NMA) is regarded as a minimal part of the peptide backbone and the relative simplicity of its structure makes it a good model for studies on protein–water interactions.

In this paper, the influence of NMA and *N,N*-dimethylacetamide (DMA) on surrounding water molecules in a range of temperature (25–75 °C) is studied by means of the FTIR spectroscopy. The results of the difference HDO spectra method are compared with the results of theoretical DFT calculations of NMA and DMA aqueous complexes.

Both NMA and DMA can be regarded as “structure-makers”, yet their hydration spheres are different. These molecules exhibit a mixed and mutually dependent types of hydration: hydrophilic and hydrophobic. In the case of a NMA molecule that has one methyl group less than DMA, the type of

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hydrophobic hydration is more important. The DMA hydration sphere is less stable: the interactions between water molecules around the methyl groups are strained. Moreover, the hydration of NMA is much more temperature dependant than in the case of DMA. The source of the differences may be hidden in the N–H···H₂O interaction.

The delicate nature of water interactions with the peptide block models may be cautiously translated into the much more complicated interactions of proteins with their hydration shells.

Keywords: N-methylacetamide, hydration, FT-IR spectroscopy, DFT calculations

1. Introduction

Proteins play key role in every biological process. Their functionality in living organism is determined by their secondary and tertiary structure, which is formed and preserved by relatively weak interactions called hydrogen bonds. Hydrogen bonds in protein-water systems consist mostly of intra- and intermolecular bonds between protein molecule and water in natural environment.

Interactions of water and proteins is one of the most important topics when it comes to studying behavior of proteins in their native state. Multiple studies have taken on this topic using various methods [1–12], however, it is still poorly understood. The study of proteins hydration experimentally is problematic due to the high level of complexity of these molecules. Additionally, overall hydration of proteins involves two general types of interactions, namely, hydrophilic hydration and hydrophobic hydration of the non-polar



15 fragments. Those contributions are hard to separate in such complex struc-
16 tures.

17 *N*-methylacetamide (NMA) can be regarded as a single peptide bond unit
18 that is used as a model for studying both intra- and intermolecular interac-
19 tions that would occur within proteins backbone in their native states. It has
20 been shown that the NMA's hydration sphere is very similar to the hydration
21 sphere of lysozyme. The lysozyme-affected water spectrum mainly consists of
22 the spectral share of water affected by NMA, which indicates the hydration
23 of the protein backbone.[12] Recent studies have confirmed the resemblance
24 of NMA to peptides as it seemed to form chain like structures similar to
25 protein backbone [13]. Existence of NMA dimers and other oligomers have
26 also been proposed [14–17]. Particularly, it was pointed out that NMA dimer
27 might be even more convenient peptide bond model than NMA itself [15].

28 NMA and its water clusters have been extensively studied both experi-
29 mentally [13–15, 18–28] (mainly by means of vibrational spectroscopy) and
30 theoretically [13, 15, 18, 19, 25, 27–35]. It has been numerously investi-
31 gated using C=O or N–D stretch as a probe of changes in its surrounding
32 [13, 14, 18, 19, 26, 28–30]. Changes of water OD bands in isotopically diluted
33 systems is also a popular probe for studying hydration of NMA [18, 34, 36].
34 Using aforementioned approach it has been stated that at 25°C water in
35 NMAs first hydration shell forms stronger hydrogen bonds than bulk water
36 [36]. Recently, these observations have been supported by Salamatova *et. al*
37 [18], with the results of MD simulations of NMA–water systems. Theoretical
38 studies describing this molecule's hydration have shown that a water molecule
39 forms stronger hydrogen bonds with the C=O group than with the N–H or



40 N–D [29, 37–39]. Also, it has been observed that C=O···H₂O hydrogen
41 bonds are stronger than between water molecules. However, computational
42 studies of deuterated systems carried out by Yadav *et al.* [29] revealed that
43 bonds formed by the C=O group and water are weaker than water–water
44 hydrogen bonds outside NMA first hydration shell.

45 In other works, in which the hydration of this molecule was investigated,
46 it has been pointed out that the dilution of the bulk NMA with water or the
47 increase of temperature leads to the disruption of hydrogen bonded aggre-
48 gates present in the bulk NMA at low temperatures [33]. Its hydration leads
49 to the formation of NMA–water clusters in which at least some of NMA dy-
50 namics seems to “freeze at a measurable time scale. This means that NMA
51 experiences a kind of hydrophobic collapse while interacting with water [18].
52 The *ab initio* analysis of 1·NMA–2·H₂O clusters and their deuterated deriva-
53 tives has allowed to assign normal modes of the aqueous hydrogen bonded
54 NMA [34]. The one water molecule has been hydrogen bonded to C=O group
55 of NMA and another one to the N–H group of those molecule.

56 Yang and Qian [40] conducted MM studies on properties of the isolated
57 *cis*- and *trans*-NMA and NMA–water complexes. Calculation of the interac-
58 tion energies and cooperative effect have revealed that complex of the *trans*-
59 NMA with two water molecules attached to the carbonyl oxygen is energet-
60 ically unfavorable. Buck and Karplus on the basis of MD in a vacuum [39]
61 have found that in the case of such a complex distance between the hydrogen
62 atom of the first water molecule and the carbonyl oxygen atom is 1.71 Å and
63 the second is 1.97 Å. Also, it has been shown that NMA with one hydrogen
64 bonded water molecule on either C=O or N–H prefers a second one interact-



65 ing with the other group [40]. In addition, it has been found that complex
66 with 3 water molecules is stable.

67 Several studies focus on a *cis-trans* equilibrium caused by rotation bar-
68 rier around C–N bond in formamide and acetamide derivatives [23, 41–45].
69 It has been shown that in the case of NMA similarly to proteins *trans* form of
70 NMA is the dominant form in liquid phases. This form also dominates in the
71 gas phase. Theoretical studies on properties of isolated NMA molecule and
72 NMA in aqueous solution revealed also the existence of two other resonance
73 structures in such systems [38, 46–48]. Neutral form of NMA is more pro-
74 nounced in gas phase while zwitterionic structure in water. As a result, the
75 elongation of C=O bond and the shortening of N–C bond passing from gas
76 to water are observed. This effect is caused by the stabilization of zwitteri-
77 onic structure of NMA by hydrogen bonding with water molecules. Also, the
78 rotation of the amide methyl group as an effect of solvation NMA molecule
79 has been observed.

80 The Born–Oppenheimer molecular dynamics and MD/MM have been
81 used to study NMA in the gas phase and in a box of water molecules [48].
82 The resultant frequencies of the amide bond vibrations confirmed other the-
83 oretical and experimental studies [49, 50].

84 In this work we have investigated the water structure around NMA in
85 wide temperature range by means of the FTIR spectroscopy. Vibrational
86 spectroscopy is an excellent method for investigation of solute hydration
87 [51, 52]. Isotopic dilution technique of HDO in water allows us to obtain
88 bands which are more convenient to analyze and highly sensitive to changes
89 in a solution. HDO spectra are narrower and less complex than the OH bands



90 of H₂O. Moreover, the OD vibration is practically free from intramolecular
91 and intermolecular couplings between oscillators. Using difference spectra
92 method [53–55] it is possible to isolate the contribution of HDO affected by
93 the solute from the OD band. Solute-affected HDO spectra can then be
94 compared with HDO spectra of bulk water, thus determining the structural
95 and energetic state of hydrogen bonds of water in the nearest surround-
96 ings of solute. In isotopically diluted solutions of NMA there is significant
97 amount of N-deuterated NMA. N–D bond vibrations happen to manifest in
98 the IR spectrum in the same region as HDO bands. Spectra of water af-
99 fected by *N,N*-dimethylacetamide (DMA) have also been obtained to isolate
100 ND stretching vibration from NMA-affected HDO spectra and to compare
101 those compounds in terms of hydration. To confirm the interpretation of
102 spectral results theoretical calculations were performed.

103 2. Materials and Methods

104 2.1. Chemicals and Solutions

105 *N*-methylacetamide (99+%, Aldrich), *N,N*-dimethylacetamide (Acros Or-
106 ganics, 99.5%) and deuterium oxide (Aldrich, 99.96%) were used to pre-
107 pare solutions without purification. Water used was deionized. For each
108 solute (NMA, DMA) stock solution in deionized water of maximum molal-
109 ity 1 mol · kg⁻¹ was prepared. Less concentrated solutions were prepared by
110 dilution of weighed amounts of stock solutions using deionized water. Each
111 of the solutions was then divided into two parts in order to prepare sample
112 and reference solutions. Sample solution was prepared by adding D₂O to one
113 of the parts in amount of 4% with respect to H₂O (by weight). Reference

114 solution was prepared by adding the same molar amounts of H₂O to the sec-
115 ond part. The amount of used deuterium oxide was confirmed to be enough
116 for reaction $\text{H}_2\text{O} + \text{D}_2\text{O} = 2\text{HDO}$ ($K \approx 4$) to give an almost quantitative
117 amount of HDO. All solutions were prepared by weight. The prepared so-
118 lutions were degassed before density and spectral measurements. Solution
119 densities were measured using the Anton Paar DMA 5000 densitometer at
120 all studied temperatures (25, 35, 45, 55, 65, and 75 °C, with the tolerance of
121 0.001 °C).

122 2.2. FTIR Measurements

123 FTIR spectra of prepared solutions were recorded on Thermo Electron
124 Co. Nicolet 8700 spectrometer with resolution of 4 cm⁻¹. Each spectrum
125 was an average of 128 independent scans. A liquid cell (model A145, Bruker
126 Optics) with CaF₂ windows separated PTFE spacers was used. The path
127 length was determined interferometrically and was 29.0 μm. The temperature
128 of measurements was kept at 25, 35, 45, 55, 65, and 75 °C with a tolerance
129 of ±0.1 °C and monitored using electronic thermometer with thermocouples
130 placed in the sample. The temperature of 75 °C has been chosen as an upper
131 temperature limit being the highest possible temperature due to technical
132 capabilities of the experimental setup. In the case of DMA solutions, the
133 maximum temperature was limited to 55 °C due to decomposition of solution
134 components to gaseous products at higher temperatures. The spectrometer
135 was purged with dry nitrogen to eliminate the influence of air components
136 on recorded spectra, namely, CO₂ and water vapor.



137 *2.3. Spectral data analysis*

138 Procedures of recording and analyzing spectra were handled using the
139 commercial PC software: OMNIC (Thermo Electron Co.), GRAMS/32 (Galac-
140 tic Industries Corporation, Salem, NH) and RAZOR (Spectrum Square As-
141 sociates, Inc., Ithaca, NY).

142 To obtain the desired information the difference spectra method [56] was
143 applied. This method assumes that water in a solution can be divided into
144 two additive contributions: the “bulk” water—identical to pure water—and
145 “affected” water—under the influence of solute. Therefore information about
146 solute-affected water can be isolated from solutions IR spectrum. The de-
147 tailed procedure of spectral data analysis toward extraction of the solute-
148 affected water spectra has been described in section S1 of Supplementary
149 Data.

150 *2.4. Interpretation of the solute-affected water spectra*

151 To interpret given solute-affected spectra the Badger-Bauer rule is used
152 [57]. This empirical rule states that energy of hydrogen bonds is proportional
153 to the shift of the OD band position, thus it is a convenient method of com-
154 paring energetic state of solute-affected water with bulk water and investigate
155 the changes of this characteristics with temperature [54, 55]. The shape of an
156 OD bands contain information about hydrogen bond energy distribution in
157 water. With the existence of empirical curves that link the oxygen–oxygen
158 intermolecular distance (R_{OO}) with the OD stretching wavenumber (ν_{OD})
159 it is possible to transform the spectral band shapes to the oxygen–oxygen
160 distance distribution function $P(R_{OO})$ [56, 58].



161 *2.5. Theoretical calculations*

162 All calculations involving optimization of NMA or DMA complexes with
163 water molecules were performed with the Gaussian 09 v.D1 software [59]
164 available at the Academic Computer Center in Gdansk (TASK). The analy-
165 sis of resulting wavefunction files involving electron density difference calcu-
166 lations and the reduced density gradient (RDG) method [60] was performed
167 with the Multiwfn software v.3.3.9. [61]. Structures of NMA or DMA com-
168 plexes with water molecules were optimized similarly to the procedure pre-
169 sented in our previous papers [16, 62]. Final structures were optimized at
170 the density functional theory (DFT) level with B3LYP hybrid exchange-
171 correlation functional [63, 64] and 6-311++G(d,p) basis set [65] within the
172 conductor-like polarizable continuum model (CPCM) of water as a solvent
173 [66, 67]. All calculations were performed with the D3 version of Grimme’s
174 empirical dispersion correction including Becke–Johnson damping [68]. Such
175 a selection gives a good consistency of theoretical and experimental results.

176 Differences in electron densities caused by NMA or DMA interactions
177 with water molecules were calculated and visualized according to section
178 4.5.5 of the Multiwfn manual. Shortly, electron densities were calculated
179 for the water complex with NMA or DMA and separately for their isolated
180 fragments: NMA or DMA, and water molecules, and then appropriately
181 subtracted from each other.

182 3. RESULTS AND DISCUSSION

183 3.1. FTIR investigation of water structure

184 3.1.1. Characteristic of solute-affected water spectra and “bulk” water spectra

185 Figure 1 presents the NMA-affected HDO spectra (Figure 1a), DMA-
186 affected HDO spectra (Figure 1b), and the “bulk” water spectra (Figure 1c)
187 as a function of temperature. Band shapes of these spectra were transformed
188 into the oxygen–oxygen distance distribution function $P(R_{OO})$ of the water
189 molecules. The obtained distance probability distributions are shown in Figs.
190 2a and 2b for solute-affected water, $P^a(R_{OO})$, and in Figure 2c for “bulk”
191 water, $P^b(R_{OO})$. The band parameters for affected HDO bands, together
192 with the bulk HDO bands, for measured temperatures are presented in Table
193 1, along with intermolecular oxygen-oxygen distances, R_{OO} .

194 A comparison of the values of the mean oxygen–oxygen distances (R_{OO}^g)
195 for solute-affected water and for “bulk” water (Table 1) points out that water-
196 water hydrogen bonds are shorter in the presence of these solutes. The
197 shift of the values of the gravity center of bands, ν_{OD}^g , (related to the mean
198 energy of water hydrogen bonds) towards lower values with respect to the
199 ones corresponding to pure water at a given temperature (Table 1) suggests
200 that water affected by NMA and DMA forms on average stronger H-bonds
201 than pure water in the whole temperature range. In addition, the hydrogen
202 bonds of water molecules around NMA are stronger and shorter than those in
203 water affected by DMA. The above results indicate that both amides enhance
204 the water structure in their nearest surrounding in the whole temperature
205 range, and can be classified as “structure-making” solutes. This statement is
206 supported by the analysis of radial distribution functions obtained by means

207 of MD simulations at 27°C [18]. Analyzing the effect of temperature variation
208 on the affected HDO spectra, it can be seen that solute-affected water spectra
209 at lower temperature are characterized by stronger hydrogen bonds than at
210 higher temperatures. A similar relationship is shown by the “bulk” water
211 spectrum. This is obvious because at higher temperatures the distances
212 between water molecules increase and hydrogen bonds weaken. Furthermore,
213 the number of moles of affected water molecules (N values from Table 1)
214 around NMA decreases as the temperature increases, and as a result at the
215 highest temperature, the two water molecules are affected by NMA. Water
216 molecules in the hydration sphere of DMA are not susceptible to temperature
217 variations, which is reflected the constant value of number of affected water
218 molecules as a function of temperature.

219 *3.1.2. Differences in oxygen-oxygen distance distributions between “affected*
220 *water” and “bulk” water*

221 To provide greater insight into the difference in intermolecular distances
222 between affected water and “bulk” water the following procedure was used:
223 the distance distribution function for “bulk” water, $P^b(R_{OO})$, (Figure 2c)
224 was subtracted from the distribution function of water affected by solute,
225 $P^a(R_{OO})$, (Figures 2a and 2b) at a given temperature. The results of the
226 subtraction, $\Delta P(R_{OO})$, each analyzed temperature are shown in Figures 3a
227 and 3b for NMA and DMA, respectively. This operation allows to observe
228 subtle changes in the population of hydrogen bonds of water affected by
229 solute relative to “bulk” water at a given temperature.

230 The analysis of distance differences (Figure 3b) clearly shows that the
231 hydration sphere of DMA contains two populations of hydrogen bonds of

232 water for all studied temperatures: the first one corresponds to the weak
233 hydrogen bonds ($R_{OO} \approx 2.9 \text{ \AA}$), while the second one corresponds to the
234 strong water-water hydrogen bonds ($R_{OO} \approx 2.73 \text{ \AA}$). The distances assigned
235 to the second population is close to the distance typical to the water in the ice
236 phase ($R_{OO} = 2.76 \text{ \AA}$) [69]. The creation of the above-mentioned populations
237 takes place mainly at the expense of very weak hydrogen bonds ($R_{OO} \geq 3.0$
238 \AA) and to a lesser extent of hydrogen bonds with mean energy (the population
239 of water-water hydrogen bonds only slightly longer than and equal to the
240 most probable distance in bulk water, value $R_{OO} = 2.826 \text{ \AA}$ at 25°C , see
241 Table 1). A slightly larger population of strong water hydrogen bonds relative
242 to the weak ones causes that in the surrounding of DMA the water structure
243 is strengthened with respect to the “bulk” water. Furthermore, it can be
244 seen that the differences between DMA-affected water and “bulk” water are
245 practically the same at all temperatures. This means that DMA enhances
246 to the same extent the water structure in the whole temperature range, and
247 thus demonstrates the high stability of the DMA hydration shell.

248 A common feature of the differences in intermolecular distances distribu-
249 tion for NMA, $\Delta P(R_{OO})$, (Fig. 3a) is the reduction of population of very
250 weak hydrogen bonds of water ($R_{OO} \geq 3.0 \text{ \AA}$) and simultaneous distinct
251 increase the strong ones ($R_{OO} \approx 2.73 \text{ \AA}$), in comparison to “bulk” water.
252 The first population is practically unchanged with increasing temperature.
253 The most significant changes relate to the population of water molecules with
254 mean energy and weak hydrogen bonds of water ($R_{OO} \approx 2.9 \text{ \AA}$). At the lowest
255 temperatures (25°C and 35°C), it may be seen that population of such hy-
256 drogen bonds of water around NMA decreases, relative to the “bulk” water.



257 However, starting from the temperature of 45 °C, the discussed population
258 of hydrogen bonds increases with the temperature increase.

259 3.1.3. Differences between $\Delta P(R_{OO})$ vs. temperature 25 °C

260 Changes in populations of hydrogen bonds in the hydration sphere of
261 NMA, with respect to the temperature of 25 °C as a reference point, are
262 shown in Figure 4. As can be seen, the population of water molecules with
263 mean and weak energy of hydrogen bonds is increased in comparison to
264 $\Delta P(R_{OO})$ at 25 °C. This population seems to be responsible for the weakening
265 of the hydrogen bond network around NMA when the temperature increases,
266 relative to the course of $\Delta P(R_{OO})$ at 25 °C. The NMA hydration sphere at
267 35 °C is additionally characterized by a slightly larger population of strong
268 hydrogen bonds (ca. 2.65 Å) relative to the temperature of 25 °C. Probably,
269 such a population can be caused by the formation of NMA–NMA dimers
270 [14–16] at this temperature, which may break up at higher temperatures.
271 Such an interpretation is however speculative because the presence of dimers
272 in aqueous solutions is difficult to observe.

273 3.2. DFT calculations: sources of differences in NMA and DMA hydration

274 A series of DFT calculations was performed to try to explain the differ-
275 ences in strong and weak populations of hydrogen bonds recognized on the
276 basis of NMA- and DMA-affected water spectra. Undoubtedly, the difference
277 between NMA and DMA hydration lies in the presence of additional methyl
278 group. Thus, we created a closed ring of water molecules encircling those
279 compounds in the closest proximity of the amine proton or methyl group
280 in the case of NMA and DMA, respectively, by a gradual addition of water

281 molecules to optimized NMA and DMA structures (see Figure 5). The water
282 ring is a model of the full hydration shell, which could be too demanding
283 to calculate for the selected set of method and basis set. In each case, the
284 minimal number of water molecules needed to create a closed circle of water
285 molecules was equal to eight. Animations showing optimized structures of
286 these complexes (in two variants: with overlaid differences in electron density
287 or with visualized places of weak interactions determined by RDG method)
288 are included in Supplementary data.

289 3.2.1. Steric effect

290 For both NMA and DMA the ring of water molecules is skewed (Figure
291 5, top view). The skewness seems to be connected with the steric effect
292 that nitrogen-bounded methyl group exerts on water molecules approaching
293 the carbonyl oxygen atom. The starting dihedral angle between C–H and
294 C=O bonds in NMA or DMA isolated molecules is close to 0° . This result is
295 in accordance with ref. [37]. The situation changes when interactions with
296 water molecules occur. When water molecules approach the carbonyl bond of
297 NMA the C–H bond easily adopts the dihedral angle to ca. 30° to fit the water
298 ring encompassing the molecule (Figure 5, front view), hence the skewness
299 of the water ring plane. The rotation of the amide methyl group of NMA,
300 when going from gas phase to solution, has also been found by Mennuci *et.al*
301 [37]. However, the examined clusters have been smaller (they contained up
302 to 3 water molecules) and the value of the dihedral angle was different. The
303 weak steric interaction between carbonyl group and methyl proton (marked
304 with red arrow in Figure 6a) also shifts to one side and makes place for a
305 water molecule to take the optimal location. The position of methyl proton

306 is not as fixed as in the case of DMA, where an additional steric interaction
307 is placed between both amine methyl groups (large green/red patch under
308 carbonyl group in Figure 6a). Moreover, carbonyl group (C=O) and water
309 molecules interacting with it lies in one plane and the water ring is highly
310 symmetrical (Figure 5, front view).

311 Interactions between water molecules or between water molecules and the
312 carbonyl group of NMA or DMA are enhanced with respect to the “bulk”
313 structure ($R_{OO,bulk,av.} = 2.817 \text{ \AA}$, see Fig. S5 in Supplementary Data), thus,
314 an enhanced population is expected to be present in the R_{OO} distance dis-
315 tribution. In fact, both compounds exhibit such an enhancement (Figure 3).
316 The population origins not only in water–carbonyl interactions but can be
317 the effect of the hydrophobic hydration, in which van der Waals interactions
318 have a dominant role (green/olive patches around methyl groups in Fig. 6a).
319 In the case of formamide, i.e. a molecule that does not contain hydrophobic
320 groups in its structure, we observe only an increasing population of weak
321 hydrogen bonds of water, in comparison to the “bulk” water (see Fig. 8a
322 in ref. [36]) This indicates that in the presence of formamide, the hydrogen
323 bonds between water molecules and the oxygen atom of carbonyl group and
324 those between water molecules are weaker.

325 The interaction of carbonyl group of NMA with a single water molecule
326 does not seem to be distinguished, and its energetics is similar to the one
327 between water molecules [16, 29]. However, the closing of the water ring
328 around NMA, involving weak hydrophobic interactions (green/olive patches
329 in Fig. 6a), enhances significantly C=O \cdots H₂O binding. In such a situation,
330 two water molecules interact strongly with carbonyl oxygen and almost to

331 the same degree ($R_{OO} = 2.710 \text{ \AA}$ and 2.728 \AA). Similar length (2.73 \AA)
332 between carbonyl oxygen and water oxygen has been obtained by means of
333 MD simulations for a larger system in ref. [38]. The shape of the electron
334 density differences caused by the interaction of NMA and the water ring
335 confirms high similarity, or symmetry, in both water–carbonyl interactions.

336 Further analysis shows that under the influence of water molecules the
337 electron density increases on the carbonyl oxygen. This is accompanied with
338 the decrease of electron density along the C=O bond. Also, the decrease
339 of electron on the amide hydrogen and its increase along the N–H bond
340 towards the nitrogen atom can be observed. In addition, electrons of N–C
341 bond are pushed towards the carbon atom. This results are in agreement
342 with the analysis of Wannier Centers average positions for NMA molecule in
343 gas phase and in water given in ref. [38]

344 A different picture emerges from the analysis of an analogous DMA data.
345 Such a water ring closure in the DMA system is less favorable. The van der
346 Waals interactions are less organized and differences in C=O \cdots H₂O inter-
347 actions are larger than in the case of NMA. The introduction of additional
348 methyl group simply inhibits the rotation of other two methyl groups in
349 DMA molecule. The nitrogen-bonded methyl group of DMA cannot adopt
350 an optimal angle to fit the water ring as in NMA system. This way, only one
351 water molecule of water can easily interact with the carbonyl group while the
352 second one has to find another—non-optimal with respect to NMA—place
353 at the expense of the interaction energy with the carbonyl group. One water
354 molecule is closer to the carbonyl oxygen atom ($R_{OO} = 2.705 \text{ \AA}$), while the
355 second one interacts poorly ($R_{OO} = 2.886 \text{ \AA}$, higher even than the average



356 “bulk” distance). Such an inequality may contribute to the presence of two
357 distinct water hydrogen bond populations (stronger and weaker relative to
358 the “bulk” water) in the vicinity of DMA molecule, as in experimental results
359 presented in Figure 3. It should be stressed that the population of strong
360 hydrogen bonds includes also interactions between water molecules around
361 methyl groups. The symmetry of water–carbonyl bond–water double inter-
362 action is broken. Such a division is also clearly visible in the electron density
363 difference (Figure 5, top view), where the weakly interacting water molecule
364 is pushed away from the plane of the water ring. The change in electron den-
365 sity for the first water molecule is highly similar to the case of NMA, while
366 the second one is non-symmetrical and exhibits a weaker electron density
367 transfer.

368 According to the above discussion, the differences in the hydration prop-
369 erties of both molecules are related to the formation of a water cage around
370 them. Both NMA and DMA due to the presence of hydrophilic and hy-
371 drophobic groups in their molecule are characterized by two types of hy-
372 dration, which are mutually dependent. Moreover, hydrogen bonds between
373 water molecules involved in the cage formation and the interaction of water
374 molecules with the carbonyl oxygen atom are cooperative. The formation
375 of a water cage around hydrophobic groups affects the equality/inequality
376 of interactions of water molecules with the carbonyl group. In the NMA
377 molecule, the hydrophobic type of hydration is more important, although
378 it has one methyl group less than DMA. This is due to a worse fit of the
379 “ice-like” water around methyl groups of DMA to the molecular shape of the
380 molecule.



381 *3.2.2. Importance of N-H...H₂O interaction*

382 The interaction of water molecule with hydrogen atom of amino group of
383 NMA ensures the stability of the hydration sphere around NMA. It acts like
384 a "clip" stabilizing the hydration cage around NMA. At lower temperatures,
385 this interaction is incorporated in the hydration cage [36]. When the tem-
386 perature increases, the hydrogen bonds around NMA weaken and eventually
387 break, and as a result, the water molecule interacting with the hydrogen
388 atom of amino group starts to lose its contact with other water molecules.
389 The relatively weak N-H...water interaction (the distance between the ni-
390 trogen atom of NMA and oxygen atom of water is 2.890 Å), freed from other
391 interactions, contributes to the increase of the population of weak hydrogen
392 bonds in the presence of NMA at higher temperatures (see Fig.3a).

393 The hydration shell around DMA, represented with the modeled ring of
394 eight water molecules, is already less stable than in the case of NMA at
395 ambient temperatures (see Figure 6a, the van der Waals interactions are
396 scattered much more than in the case of NMA). The ring of water molecules
397 around DMA is stretched from the very beginning due to the relatively large
398 methyl group in the place of amine proton. In consequence, the already
399 weakened hydration shell may be less prone to the temperature increase.

400 **4. Conclusions**

401 The effect of NMA and DMA on the water structure as a function of tem-
402 perature has been studied by means of FTIR spectroscopy, supported with
403 DFT calculations. The results of theoretical calculations helped to resolve
404 spectral results. The agreement between experimental and computational re-

405 sults was satisfactory and gave a consistent picture of the amides hydration
406 in aqueous solution.

407 The results revealed that hydrogen bonds of water molecules around these
408 solutes are stronger and shorter than those in pure water in the whole tem-
409 perature range. Although both solutes strengthened the water structure,
410 significant differences in the characteristics of their hydration shells could be
411 noticed. The essential difference in the hydration of NMA and DMA is the
412 way they create the water cage around them, which is the result of both
413 hydrophilic and hydrophobic hydration. These two types of hydration are
414 mutually dependent. Thus, the increase in the population of strong hydrogen
415 bonds of water in the surroundings of these solutes is the effect of the inter-
416 actions of water molecules with the oxygen atom of carbonyl group and those
417 between water molecules around hydrophobic groups. The hydration sphere
418 of formamide, a molecule that does not contain any hydrophobic group, is
419 characterized by an increased population of weak hydrogen bonds of water,
420 with respect to the “bulk” water ref. [36], without the strengthening ob-
421 served in the case of NMA or DMA. This suggest that the presence of a
422 hydrophobic element in the molecule is a factor conditioning the enhanced
423 network of hydrogen bonds around the solute.

424 The DMA molecule is additionally surrounded by a population of water
425 molecules that forms weak hydrogen bonds with respect to the “bulk” water
426 (at all given temperatures). The presence of this population results from the
427 poor fit of the water cage to the solute’s geometry. Such a disadvantageous
428 fit turn out to be less susceptible to the temperature change because the
429 hydration cage is already distorted at lower temperatures, in comparison to



430 NMA.

431 On the other hand, the NMA water cage is better organized than in the
432 case of DMA. The hydrophobic type of hydration is more important in the
433 case of NMA molecule, although it has one methyl group less than DMA.
434 The interaction of water molecule with the hydrogen atom of amino group is
435 significant for the stability of the hydration sphere of NMA. Thus, at lower
436 temperatures the hydration sphere of NMA contains only the population of
437 stronger hydrogen bonds, in comparison to the “bulk” water. As the temper-
438 ature increases, the additional population of water molecules, characterized
439 with weak hydrogen bonds, is observed in the water population affected by
440 NMA. It results from the weak N–H···water interaction. At lower tempera-
441 tures, this interaction is incorporated into the hydration cage, while at higher
442 temperatures it starts to be exposed when water molecule interacting with
443 the hydrogen atom of amino group loses its contact with the cage around the
444 molecule.

445 Although both NMA and DMA are simple molecules, their similarity
446 to the basic building block of proteins makes them convenient models of
447 protein–water interactions. The obtained results clearly show that the con-
448 tributions to the hydration sphere of the solute are not additive due to the
449 number of non-polar groups. A stable hydration sphere depends on the pos-
450 sibility of building a cooperatively reinforced network of hydrogen bonding
451 water molecules around the solutes. The changes in the hydration of solutes
452 caused by the temperature increase and their explanation may be cautiously
453 translated into phenomena accompanying the process of protein denatura-
454 tion.

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459 **6. Declaration of interest**

460 The authors declare that they have no conflict of interest.

461 **7. References**

- 462 [1] L. Biedermannova, B. Schneider, Hydration of proteins and nucleic
463 acids: Advances in experiment and theory. A review, *Biochim. Biophys.*
464 *Acta* 1860 (2016) 1821–1835.
- 465 [2] L. Aggarwal, P. Biswas, Hydration water distribution around intrinsi-
466 cally disordered proteins, *J. Phys. Chem. B* 122 (2018) 4206–4218.
- 467 [3] J. N. Dahanayake, K. R. Mitchell-Koch, Entropy connects water struc-
468 ture and dynamics in protein hydration layers, *Phys. Chem. Chem.*
469 *Phys.* 20 (2018) 14765–14777.
- 470 [4] J. T. King, K. J. Kubarych, Site-specific coupling of hydration water and
471 protein flexibility studied in solution with ultrafast 2D-IR spectroscopy,
472 *J. Am. Chem. Soc.* 134 (2012) 18705–18712.
- 473 [5] R. C. Remsing, E. Xi, A. J. Patel, Protein hydration thermodynamics:
474 The influence of flexibility and salt on hydrophobin II hydration, *J.*
475 *Phys. Chem. B.* 122 (2018) 3635–3646.

- 476 [6] V. M. Dadarlat, C. B. Post, Decomposition of protein experimental
477 compressibility into intrinsic and hydration shell contributions, *Biophys.*
478 *J.* 91 (2006) 4544–4554.
- 479 [7] D. Hecht, L. Tadesse, L. Walters, Correlating hydration shell structure
480 with amino acid hydrophobicity, *J. Am. Chem. Soc.* 115 (1993) 3336–
481 3337.
- 482 [8] V. P. Kutysenko, A study of water-protein interactions by high-
483 resolution NMR spectroscopy, *Mol. Biol.* 35 (2001) 90–99.
- 484 [9] D. Russo, J. Olivier, J. Teixeira, Water hydrogen bond analysis on
485 hydrophilic and hydrophobic biomolecule sites, *Phys. Chem. Chem.*
486 *Phys.* 10 (2008) 4968–4974.
- 487 [10] M. Tarek, D. J. Tobias, The dynamics of protein hydration wa-
488 ter: A quantitative comparison of Molecular Dynamics simulations and
489 neutron-scattering experiments, *Biophys. J.* 79 (2000) 3244–3257.
- 490 [11] G. Zaccai, The dynamics of protein hydration water: A quantitative
491 comparison of Molecular Dynamics simulations and neutron-scattering
492 experiments, *Phil. Trans. R. Soc. Lond. B* 359 (2004) 1269–1275.
- 493 [12] A. Panuszko, M. Wojciechowski, P. Bruździak, P. W. Rakowska, J. Stan-
494 gret, Characteristics of hydration water around hen egg lysozyme as the
495 protein model in aqueous solution. FTIR spectroscopy and molecular
496 dynamics simulation, *Physical Chemistry Chemical Physics* 14 (2012)
497 15765.



- 498 [13] Y. Zhou, G. Deng, Y.-Z. Zheng, J. Xu, H. Ashraf, Z.-W. Yu, Evidences
499 for cooperative resonance-assisted hydrogen bonds in protein secondary
500 structure analogs, *Sci. Rep.* 6 (2016) 36932.
- 501 [14] M. Albrecht, C. A. Rice, M. A. Suhm, Elementary peptide motifs
502 in the gas phase: Ftir aggregation study of formamide, acetamide,
503 N-methylformamide, and N-methylacetamide, *J. Phys. Chem. A* 112
504 (2008) 7530–7542.
- 505 [15] T. Forsting, H. C. Gottshalk, B. Hartwig, M. Mons, M. A. Suhm, Cor-
506 recting the record: the dimers and trimers of trans-N-methylacetamide,
507 *Phys. Chem. Chem. Phys.* 19 (2017) 10727–10737.
- 508 [16] K. A. Biernacki, E. Kaczkowska, P. Bruździak, Aqueous solutions of
509 NMA, Na_2HPO_4 , and NaH_2PO_4 as models for interaction studies in
510 phosphate–protein systems, *Journal of Molecular Liquids* 265 (2018)
511 361–371.
- 512 [17] W. A. Herrebout, K. Clou, H. O. Desseyn, Vibrational spectroscopy of
513 N-methylacetamide revisited, *J. Phys. Chem. A* 105 (2001) 4865–4881.
- 514 [18] E. Salamatova, A. V. Cunha, R. Bloem, S. J. Roeters, S. Woutersen,
515 T. L. C. Jansen, M. S. Pshenichnikov, Hydrophobic collapse in N-
516 methylacetamide-water mixtures, *J. Phys. Chem. A* 122 (2018) 2468–
517 247.
- 518 [19] L. C. Mayne, B. Hudson, Resonance Raman spectroscopy of N-
519 methylacetamide: Overtones and combinations of the C-N stretch

- 520 (Amide II) and effect of solvation on the C=O stretch (Amide I) in-
521 tensity, *J. Phys. Chem.* 95 (1991) 2962–2967.
- 522 [20] J. M. Dudik, C. R. Johnson, S. A. Asher, UV resonance Raman studies
523 of acetone, acetamide, and N-methylacetamide: Models for the peptide
524 bond, *J. Phys. Chem.* 89 (1985) 3805–3814.
- 525 [21] X. G. Chen, R. Schweitzer-Stenner, N. G. Mirkin, S. A. Asher, N-
526 methylacetamide and its hydrogen-bonded water molecules are vibra-
527 tionally coupled, *J. Am. Chem. Soc.* 116 (1994) 11141–11142.
- 528 [22] X. G. Chen, R. Schweitzer-Stenner, S. A. Asher, N. G. Mirkin,
529 S. Krimm, Vibrational assignments of trans-N-methylacetamide and
530 some of its deuterated isotopomers from band decomposition of IR, visi-
531 ble, and resonance Raman spectra, *J. Phys. Chem.* 99 (1995) 3074–3083.
- 532 [23] S. Song, S. A. Asher, S. Krimm, K. D. Shaw, Ultraviolet resonance
533 Raman studies of trans and cis peptides: Photochemical consequences
534 of the twisted π^* excited state, *J. Am. Chem. Soc.* 113 (1991) 1155–
535 1163.
- 536 [24] V. Vasylyeva, S. K. Nayak, G. Terraneo, G. Cavallo, P. Metrangolo,
537 G. Resnati, Orthogonal halogen and hydrogen bonds involving a peptide
538 bond model, *CrystEngComm* 16 (2014) 8102–8105.
- 539 [25] S. Woutersen, Y. Mu, G. Stock, P. Hamm, Hydrogen-bond lifetime
540 measured by time-resolved 2D-IR spectroscopy: N-methylacetamide in
541 methanol, *Chemical Physics* 266 (2001) 137–147.



- 542 [26] M. F. DeCamp, L. DeFlores, J. M. McCracken, A. Tokmakoff, Amide I
543 vibrational dynamics of N-methylacetamide in polar solvents: The role
544 of electrostatic interactions, *J. Phys. Chem. B* 109 (2005) 11016–11026.
- 545 [27] R. Schweitzer-Stenner, G. Sieler, Intermolecular coupling in liquid and
546 crystalline states of trans-N-methylacetamide investigated by polarized
547 Raman and FT-IR spectroscopies, *J. Phys. Chem. A* 102 (1998) 118–
548 127.
- 549 [28] R. Zhang, H. Li, Y. Lei, S. Han, All-atom Molecular Dynamic simula-
550 tions and relative NMR spectra study of weak c-h o contacts in amide-
551 water systems, *J. Phys. Chem. B* 109 (2005) 7482–7487.
- 552 [29] V. K. Yadav, A. Chandra, First-principles simulation study of vibra-
553 tional spectral diffusion and hydrogen bond fluctuations in aqueous so-
554 lution of Nmethylacetamide, *J. Phys. Chem. B* 119 (2015) 9858–9867.
- 555 [30] M. H. Farag, M. F. Ruiz-Lopez, A. Bastida, G. Monard, F. Ingrosso,
556 Hydration effect on amide I infrared bands in water: An interpretation
557 based on an interaction energy decomposition scheme, *J. Phys. Chem.*
558 *B* 119 (2015) 9056–9067.
- 559 [31] J. Heyda, J. C. Vincent, D. J. Tobias, J. Dzubiella, P. Jungwirth, Ion
560 specificity at the peptide bond: Molecular Dynamics simulations of N-
561 methylacetamide in aqueous salt solutions, *J. Phys. Chem. B* 114 (2010)
562 1213–1220.
- 563 [32] W.-G. Han, S. Suhai, Density functional studies on N-methylacetamide-
564 water complexes, *J. Phys. Chem. B* 100 (1996) 3942–3949.



- 565 [33] N. T. Hunt, K. Wynne, The effect of temperature and solvation on the
566 ultrafast dynamics of N-methylacetamide, *Chem. Phys. Lett.* 431 (2006)
567 155–159.
- 568 [34] N. G. Mirkin, S. Krimm, Ab initio vibrational analysis of isotopic deriva-
569 tives of aqueous hydrogen-bonded trans-N-methylacetamide, *J. Mol.*
570 *Struct.* 377 (1996) 219–234.
- 571 [35] J. Gao, M. Freindorf, Hybrid ab initio QM/MM simulation of N-
572 methylacetamide in aqueous solution, *J. Phys. Chem. A* 101 (1997)
573 3182–3188.
- 574 [36] A. Panuszko, E. Gojlo, J. Zielkiewicz, M. Smiechowski, J. Krakowiak,
575 J. Stangret, Hydration of simple amides. FTIR spectra of HDO and
576 theoretical studies, *J. Phys. Chem. B* 112 (2008) 2483–2493.
- 577 [37] B. Mennucci, J. M. Martínez, How to model solvation of peptides?
578 Insights from a quantum-mechanical and molecular dynamics study of
579 N-methylacetamide. 1. Geometries, infrared, and ultraviolet spectra in
580 water, *Journal of Physical Chemistry B* 109 (2005) 9818–9829.
- 581 [38] M. P. Gaigeot, R. Vuilleumier, M. Sprik, D. Borgis, Infrared spec-
582 troscopy of N-methylacetamide revisited by ab initio molecular dynam-
583 ics simulations, *Journal of Chemical Theory and Computation* 1 (2005)
584 772–789.
- 585 [39] M. Buck, M. Karplus, Hydrogen bond energetics: A simulation and
586 statistical analysis of N-methyl acetamide (NMA), water, and human
587 lysozyme, *Journal of Physical Chemistry B* 105 (2001) 11000–11015.

- 588 [40] Z.-Z. Yang, P. Qian, A study of N-methylacetamide in water clus-
589 ters: Based on atom-bond electronegativity equalization method fused
590 into molecular mechanics, *The Journal of Chemical Physics* 125 (2006)
591 064311.
- 592 [41] W. L. Jorgensen, J. Gao, Cis-Trans energy difference for the peptide
593 bond in the gas phase and in aqueous solution, *J. Am. Chem. Soc.* 110
594 (1988) 4212–4216.
- 595 [42] G. Nandini, D. N. Sathyanarayana, Ab initio studies on geometry and
596 vibrational spectra of N-methylformamide and N-methylacetamide, *J.*
597 *Mol. Struct.(TEOCHEM)* 579 (2002) 1–9.
- 598 [43] S. Shin, A. Kurawaki, Y. Hamada, K. Shinya, K. Ohno, A. Tohara,
599 M. Sato, Conformational behavior of N-methylformamide in the gas,
600 matrix, and solution states as revealed by IR and NMR spectroscopic
601 measurements and by theoretical calculations, *J. Mol. Struct.* 791 (2006)
602 30–40.
- 603 [44] A. C. Fantoni, W. Caminati, Rotational spectrum and ab initio calcu-
604 lations of N-methylformamide, *J. Chem. Soc., Faraday Trans.* 92 (1996)
605 343–346.
- 606 [45] Y. K. Kang, H. S. Park, Internal rotation about the CN bond of amides,
607 *J. Mol. Struct.(TEOCHEM)* 676 (2004) 171–176.
- 608 [46] H. Torii, T. Tatsumi, M. Tasumi, Effects of hydration on the structure,
609 vibrational wavenumbers, vibrational force field and resonance raman



- 610 intensities of N-methylacetamide, *Journal of Raman Spectroscopy* 29
611 (1998) 537–546.
- 612 [47] M. P. Gaigeot, Theoretical spectroscopy of floppy peptides at room
613 temperature. A DFTMD perspective: Gas and aqueous phase, *Physical
614 Chemistry Chemical Physics* 12 (2010) 3336–3359.
- 615 [48] F. Ingrosso, G. Monard, M. Hamdi Farag, A. Bastida, M. F. Ruiz-Lopez,
616 Importance of Polarization and Charge Transfer Effects to Model the
617 Infrared Spectra of Peptides in Solution, *Journal of Chemical Theory
618 and Computation* 7 (2011) 1840–1849.
- 619 [49] K. Kwac, M. Cho, Molecular dynamics simulation study of *N*-
620 -methylacetamide in water. I. Amide I mode frequency fluctuation, *The
621 Journal of Chemical Physics* 119 (2003) 2247–2255.
- 622 [50] S. Ham, J.-H. Kim, H. Lee, M. Cho, Correlation between electronic and
623 molecular structure distortions and vibrational properties. II. Amide I
624 modes of NMA-D₂O complexes, *The Journal of Chemical Physics* 118
625 (2003) 3491–3498.
- 626 [51] M. Falk, T. A. Ford, Infrared spectrum and structure of liquid water,
627 *Can. J. Chem.* 44 (1966) 1699–1707.
- 628 [52] D. F. Hornig, On the spectrum and structure of water and ionic solu-
629 tions, *J. Chem. Phys.* 40 (1964) 3119.
- 630 [53] J. Stangret, Solute-affected vibrational spectra of water in Ca(ClO₄)₂
631 aqueous solution, *Spect. Lett.* 21 (1988) 369–381.



- 632 [54] J. Stangret, T. Gampe, Hydration sphere of tetrabutylammonium
633 cation. FTIR of HDO spectra, *J. Phys. Chem. B* 103 (1988) 3778–3783.
- 634 [55] J. Stangret, T. Gampe, Ionic hydration behavior derived from infrared
635 spectra in HDO, *J. Phys. Chem. A* 106 (2002) 5393–5402.
- 636 [56] M. Smiechowski, J. Stangret, Vibrational spectroscopy of semiheavy
637 water (HDO) as a probe of solute hydration, *Pure Appl. Chem.* 82
638 (2010) 1869–1887.
- 639 [57] R. M. Badger, B. S. H. Bauer, Spectroscopic studies of the hydrogen
640 bond II the shift of the OH vibrational frequency in the formation of
641 the hydrogen bond, *J. Chem. Phys.* 5 (1937) 839–851.
- 642 [58] S. Bratos, J.-C. Leicknam, S. Pommeret, Relation between the OH
643 stretching frequency and the OO distance in time-resolved infrared spec-
644 troscopy of hydrogen bonding, *Chem. Phys.* 359 (2009) 53–57.
- 645 [59] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb,
646 J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Pe-
647 tersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Iz-
648 maylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara,
649 K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda,
650 O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery Jr., J. E. Peralta,
651 F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N.
652 Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell,
653 J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam,
654 M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo,

- 655 R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi,
656 C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Za-
657 krzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D.
658 Daniels, Ö. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, D. J. Fox,
659 Gaussian 09, Revision D.01, 2009.
- 660 [60] E. R. Johnson, S. Keinan, P. Mori-Sanchez, J. Contreras-Garcia, A. J.
661 Cohen, W. Yang, Revealing noncovalent interactions, *Journal of the*
662 *American Chemical Society* 132 (2010) 6498–6506.
- 663 [61] T. Lu, F. Chen, Multiwfn: A multifunctional wavefunction analyzer,
664 *Journal of Computational Chemistry* 33 (2012) 580–592.
- 665 [62] Taurine as a water structure breaker and protein stabilizer, *Amino Acids*
666 50 (2018) 125–140.
- 667 [63] C. Lee, W. Yang, R. G. Parr, Development of the colle-salvetti
668 correlation-energy formula into a functional of the electron density,
669 *Physical Review B* 37 (1988) 785–789.
- 670 [64] A. D. Becke, Density-functional thermochemistry. III. the role of exact
671 exchange, *The Journal of Chemical Physics* 98 (1993) 5648–5652.
- 672 [65] R. Ditchfield, W. J. Hehre, J. A. Pople, Self-consistent molecular-orbital
673 methods. IX. an extended gaussian-type basis for molecular-orbital stud-
674 ies of organic molecules, *The Journal of Chemical Physics* 54 (1971)
675 724–728.
- 676 [66] M. Cossi, N. Rega, G. Scalmani, V. Barone, Energies, structures, and

677 electronic properties of molecules in solution with the C-PCM solvation
678 model, *Journal of Computational Chemistry* 24 (2003) 669–681.

679 [67] V. Barone, M. Cossi, Quantum calculation of molecular energies and
680 energy gradients in solution by a conductor solvent model, *Journal of*
681 *Physical Chemistry A* 102 (1998) 1995–2001.

682 [68] S. Grimme, S. Ehrlich, L. Goerigk, Effect of the damping function in
683 dispersion corrected density functional theory, *Journal of Computational*
684 *Chemistry* 32 (2011) 1456–1465.

685 [69] D. Eisenberg, W. Kauzmann, *The Structure and Properties of Water.*,
686 Oxford University Press, 1969.

Table 1: The parameters of HDO bands of water affected by NMA (Figure 1a), water affected by DMA (Figure 1b), “bulk” water (Figure 1c), and the respective intermolecular oxygen–oxygen distances. R_{OO} errors have been estimated on the basis of the HDO bands position errors.

T^a	N^b	$\nu_{OD}^o{}^c$	$\nu_{OD}^g{}^d$	$fwhh^e$	I^f	$R_{OO}^o{}^g$	$R_{OO}^g{}^h$
NMA-affected water spectrum							
25	3.0±0.5	2505±2	2486±2	177±4	13120	2.821±0.003	2.821±0.003
35	2.8±0.5	2517±2	2492±2	178±4	11836	2.821±0.003	2.828±0.003
45	2.6±0.5	2518±2	2495±2	167±4	10640	2.836±0.003	2.833±0.003
55	2.5±0.5	2526±2	2501±2	175±4	10059	2.836±0.003	2.838±0.003
65	2.4±0.5	2526±2	2505±2	175±4	9400	2.841±0.003	2.844±0.003
75	2.2±0.5	2534±2	2511±2	169±4	8447	2.851±0.003	2.849±0.003
DMA-affected water spectrum							
25	2.5±0.5	2517±2	2497±2	165±4	16169	2.836±0.003	2.836±0.003
35	2.5±0.5	2524±2	2503±2	167±4	14666	2.836±0.003	2.836±0.003
45	2.6±0.5	2528±2	2505±2	167±4	13467	2.841±0.003	2.841±0.003
55	2.5±0.5	2534±2	2511±2	166±4	12066	2.851±0.003	2.849±0.003
“bulk” water spectrum							
25	-	2509±2	2505±2	162±4	10434	2.826±0.003	2.844±0.003
35	-	2513±2	2509±2	164±4	10022	2.836±0.003	2.849±0.003
45	-	2519±2	2513±2	164±4	9670	2.836±0.003	2.854±0.003
55	-	2522±2	2517±2	167±4	9281	2.838±0.003	2.859±0.003
65	-	2528±2	2521±2	170±4	8965	2.844±0.003	2.861±0.003
75	-	2532±2	2524±2	169±4	8612	2.849±0.003	2.867±0.003

^a Temperature (°C). ^b Affected number, equal to the number of moles of water affected by one mole of solute. ^c Band position at maximum (cm⁻¹). ^d Band position at gravity center (cm⁻¹). ^e Full width at half-height (cm⁻¹). ^f Integrated intensity (dm³ · mol⁻¹ · cm⁻¹). ^g The most probable O···O distance (Å). ^h Mean O···O distance (Å).



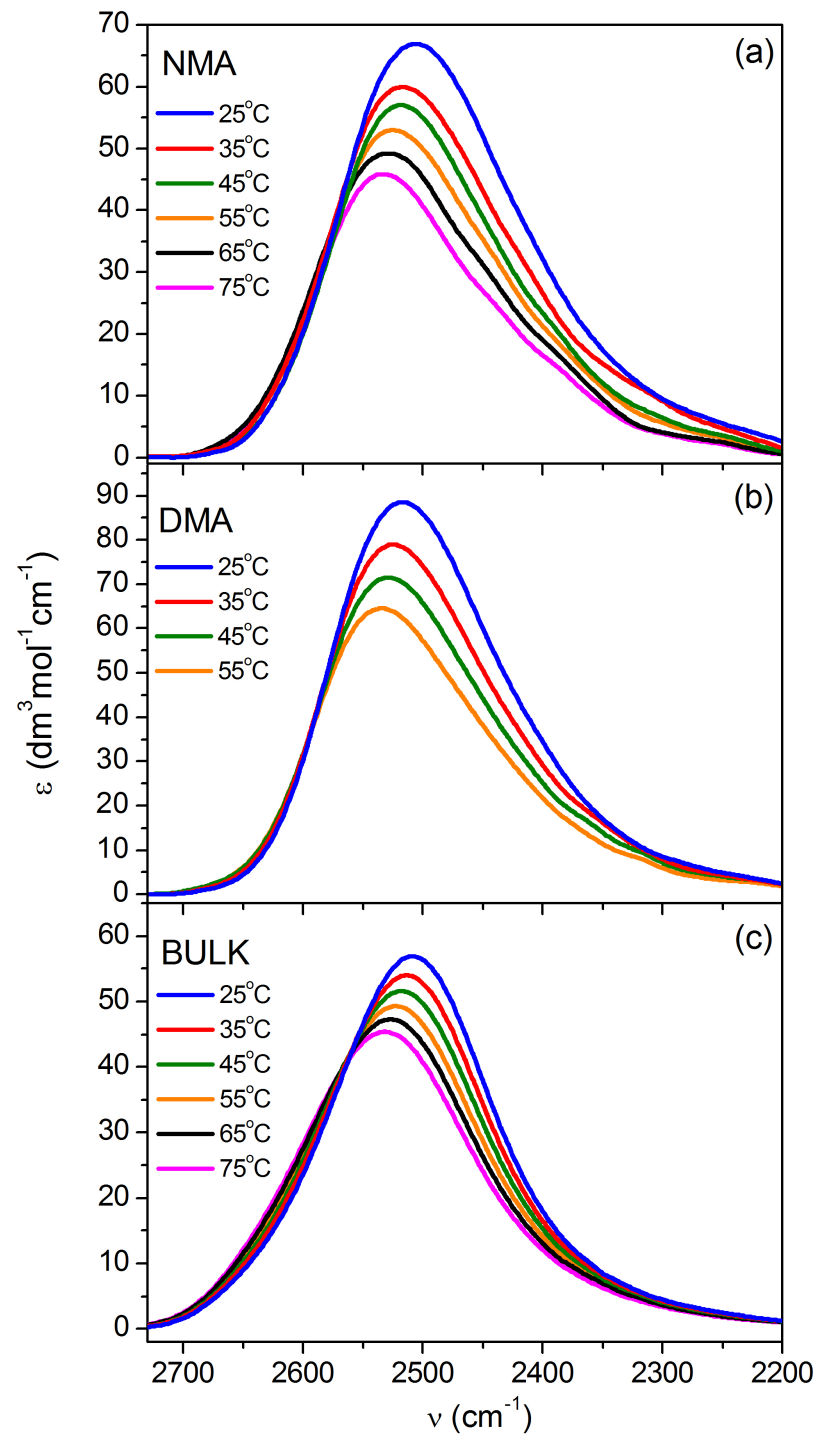


Figure 1: Solute-affected spectra in the OD stretching region for (a) NMA, (b) DMA, and (c) “bulk” HDO spectra as a function of temperature.

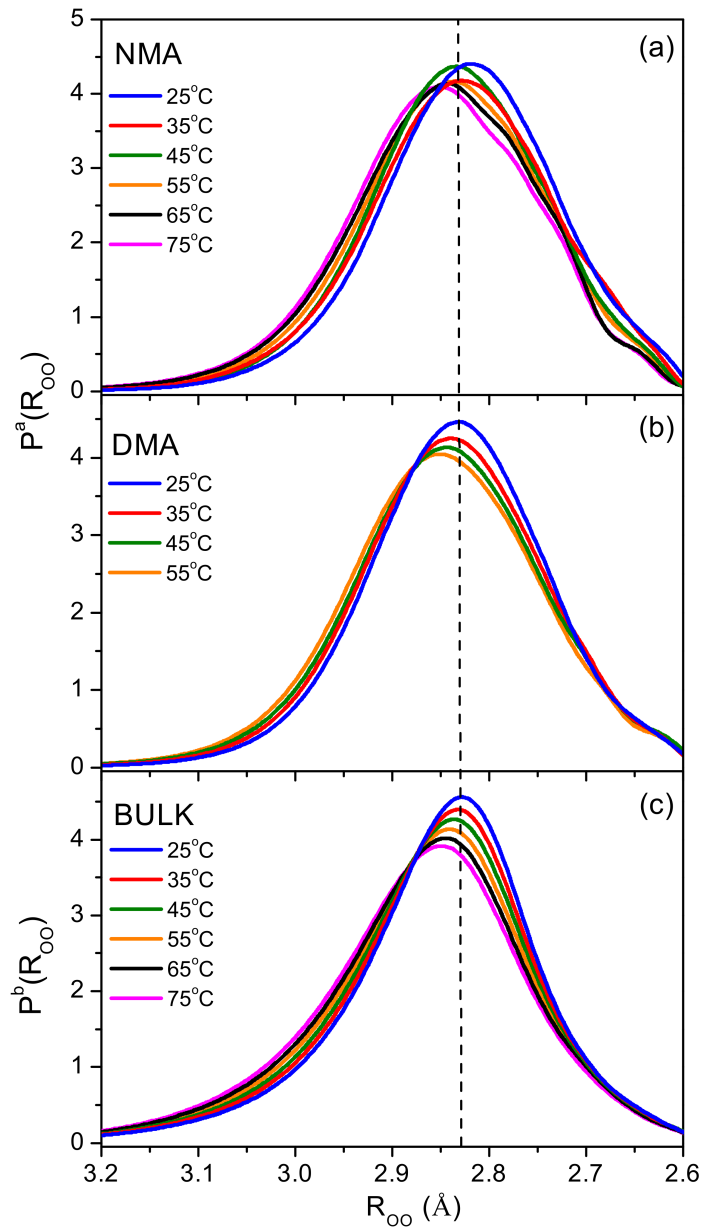


Figure 2: Interatomic oxygen-oxygen distance distributions function derived from the HDO spectra affected by (a) NMA, (b) DMA, and (c) “bulk” water spectra (Figure 1) for all studied temperatures. The vertical dashed line corresponds to the value of the most probable oxygen-oxygen distance in bulk water at 25 °C (2.826 Å, see Table 1).

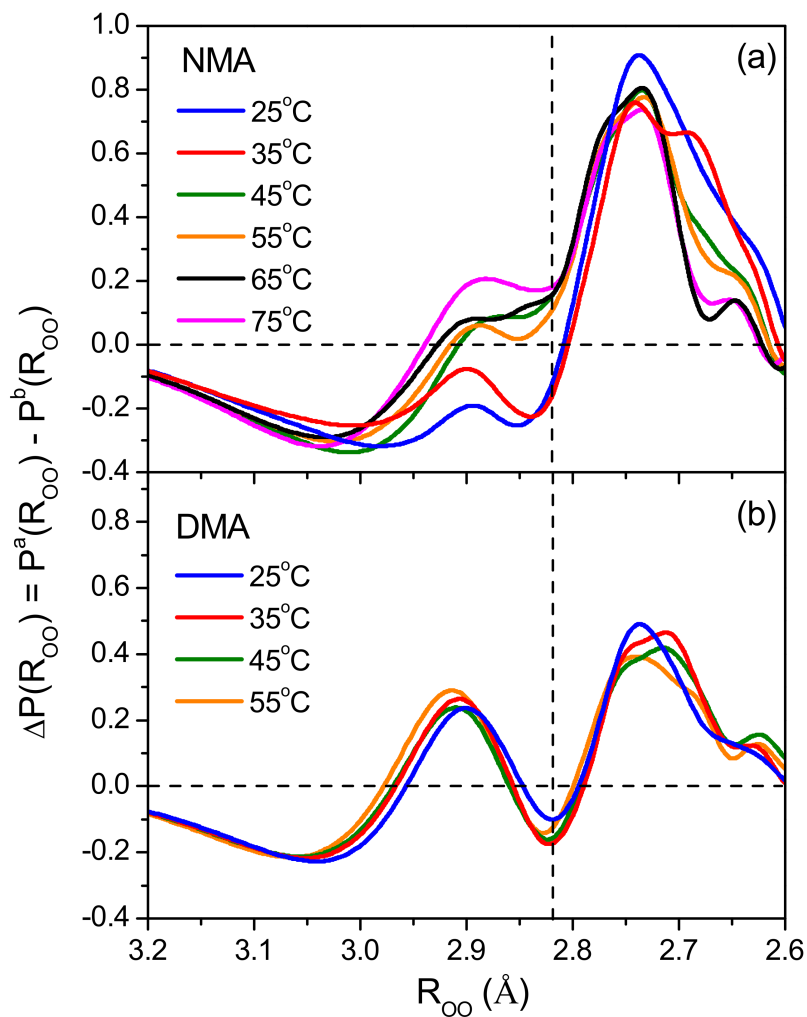


Figure 3: Differences between interatomic oxygen–oxygen distance distribution function of solute-affected water, $P^a(R_{OO})$, and the “bulk” water, $P^b(R_{OO})$, (Figure 2) at given temperature for (a) NMA and (b) DMA. The vertical dashed line corresponds to the value of the most probable oxygen-oxygen distance in bulk water at 25 °C (2.826 Å, see Table 1).

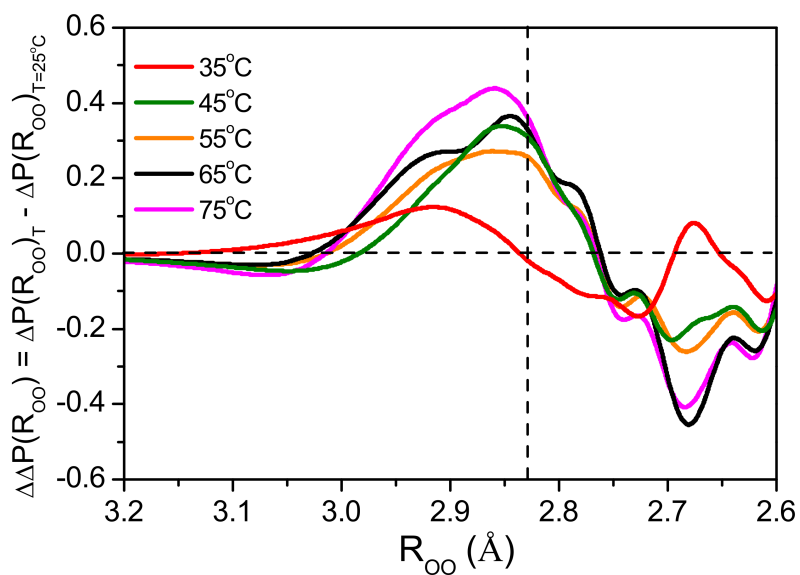


Figure 4: The result of subtraction of the oxygen-oxygen distance function at 25 °C, $\Delta P(R_{OO})_{25\text{ }^{\circ}\text{C}}$, (blue line in Figure 3a) from the distance function at different temperatures, $\Delta P(R_{OO})_T$, obtained for NMA. The vertical dashed line shown to the position of the most probable oxygen-oxygen distance in bulk water at 25 °C (2.826 Å, see Table 1).

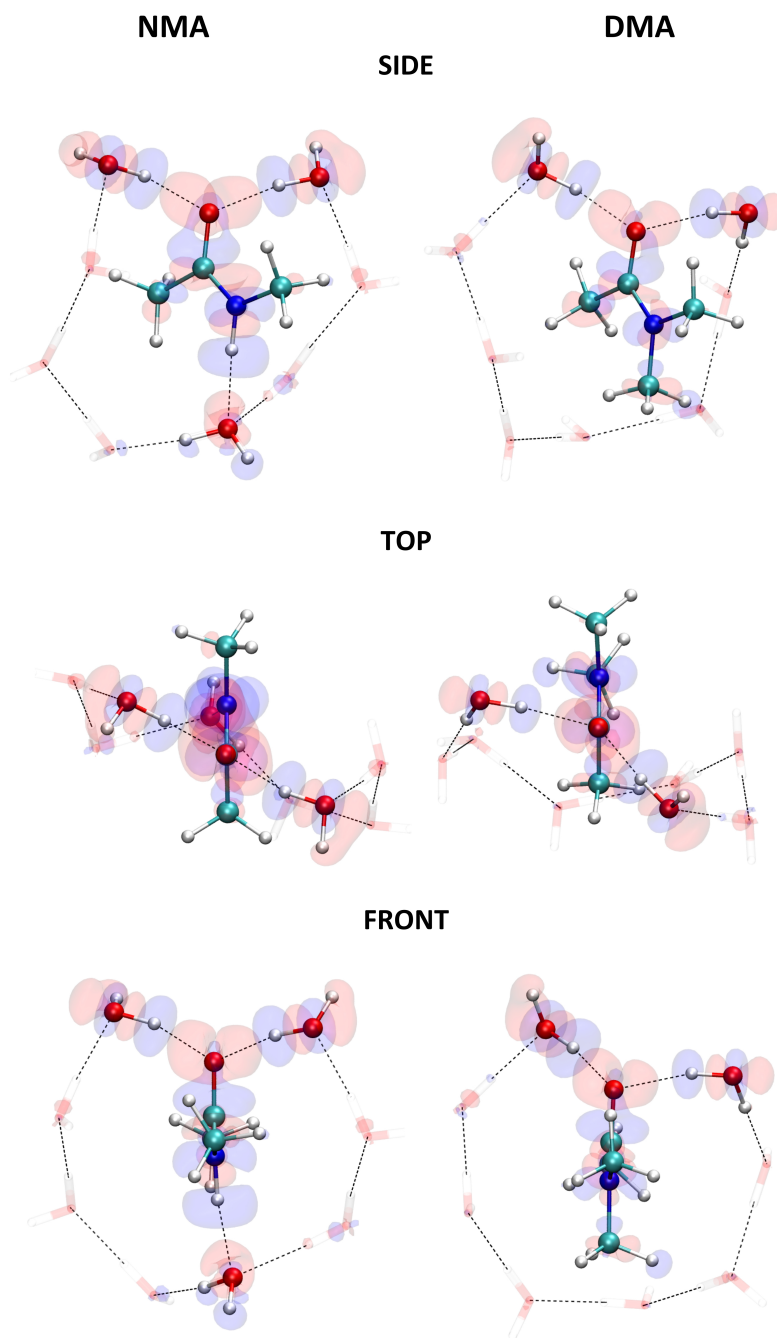
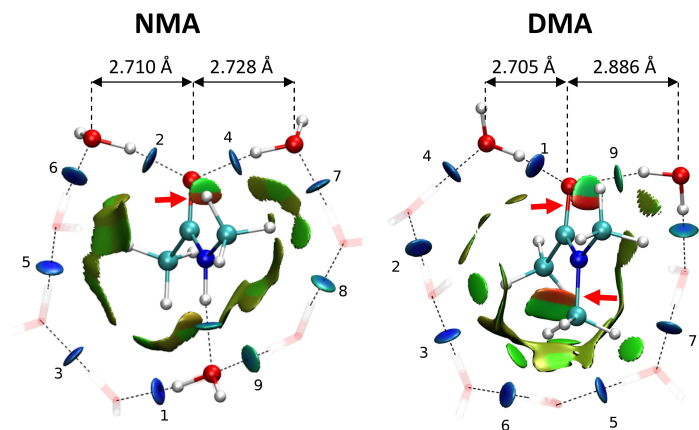


Figure 5: Differences in electron density of NMA and DMA complexes with water molecules caused by interactions of a central molecule with the water ring. Red regions correspond to increase of the electron density, blue regions mark its decrease. Additional movie files with the same structures are available in Supplementary Data.



(a)



(b)

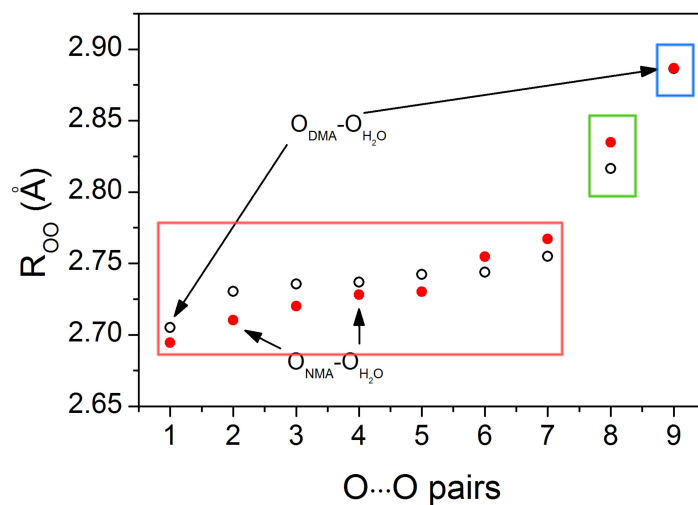


Figure 6: (a) Visualization of weak interactions in NMA and DMA complexes with water molecules. Blue disks correspond to hydrogen bonds, green/olive patches correspond to weak van der Waals interactions, red (marked with red arrows) correspond to steric repulsion. Selected water molecules are made transparent for clarity. Hydrogen bonds are marked with dashed black lines. Numbers indicate $O \cdots O$ pairs sorted according to their length (as in Figure b). Additional movie files with the same structures are available in Supplementary Data. (b) Distribution of oxygen \cdots oxygen distances in NMA (red) and DMA (white) complexes with water. Only distances shorter than 3 Å are shown, and pairs corresponding to interaction of water molecules with the carbonyl oxygen are marked with arrows. The $O \cdots O$ pairs in red, green, or blue frames correspond to strengthened, “bulk-like”, and weakened hydrogen bonds, respectively.