

Structure of the Alanine Hydration Shell as Probed by NMR Chemical Shifts and Indirect Spin–Spin Coupling

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The structure of the alanine hydration shell was modeled by Carr–Parinello molecular dynamics (CPMD) to explain subtle differences in NMR chemical shifts and indirect spin–spin coupling constants of the neutral (zwitterionic), cationic, and anionic forms of this amino acid. In comparison with classical molecular dynamics (MD), the quantum mechanical CPMD approach revealed a more structured solvent and significant differences in the radial and angular distributions of the water molecules around the solute. In particular, the solvent was predicted to be organized around the uncharged COOH and NH₂ residues to a similar degree as that for the charged ones. This was not the case with MD. For snapshot CPMD configurations, the NMR parameters were computed by density functional theory (DFT) and averaged. Obtained values were significantly closer to experimental parameters known for ¹⁵N and ¹³C isotopically labeled alanine than those calculated by the conventional implicit dielectric solvent model. The NMR results also quantitatively reflect a superiority of the CPMD over the MD explicit solvent treatment. A further improvement of the computed spin–spin coupling constants could be achieved by taking into account vibrational averaging beyond the harmonic approximation. Differently positioned water molecules in the clusters cause an unexpectedly large scattering of the NMR parameters. About 10–15 dynamics snapshots were required for a satisfactory convergence of the shifts and couplings. The NMR chemical shift was found to be much more sensitive to the molecular hydration than the coupling. The results thus indicate a large potential of the NMR spectroscopy and quantum simulations to probe not only the structure of molecules but also their interactions with the environment.

I. Introduction

Structure, reactivity, and physical properties of molecules are, to a large extent, determined by their environment. The characterization of the environment is particularly necessary for a proper understanding of biologically interesting systems, such as proteins, their structure, function, and folding.^{1–4} For example, many peptide molecules may not exist in vacuum at all as their charge must be stabilized by the solvent.^{4–7} Individual solvent components and interactions can further modify the biochemical activity.^{8,9} Although there is a relatively small number of experimental means enabling study of the hydration patterns directly, techniques such as dielectric¹⁰ and terahertz¹¹ spectroscopy, fluorescence labeling,¹² and infrared^{13,14} and other optical¹⁵ spectroscopies revealed a wealth of details about the interaction of peptides and proteins with water in solutions. The nuclear magnetic resonance (NMR) is a particularly sensitive local probe of the chemical environment of atomic nuclei.^{16,17} Although the high-resolution NMR spectroscopy can, in principle, provide a three-dimensional estimate of protein hydration,¹⁸ the data processing is often dependent on computer-extensive simulation and interpretation techniques.

To explore this potential, we concentrate in the present study on a detailed interpretation of the NMR chemical shifts and indirect spin–spin coupling constants (*J*-coupling) of variously charged ¹⁵N and ¹³C isotopically labeled alanine forms. Previous attempts to use conventional dielectric solvent approximations yielded only a limited accuracy in predicting the NMR parameters and their changes upon pH variation.¹⁹ The partially

covalent character of the hydrogen bonds, charge transfer, and many polarization phenomena are not well-represented in the simplified models. Fortunately, the alanine molecule is relatively small and thus accessible for a more accurate modeling of the hydration shell with at least some water molecules explicitly included at the same electronic quantum chemical level of approximation as the amino acid itself.

This is possible with the Carr–Parinello molecular dynamics (CPMD) approach²⁰ that efficiently and simultaneously treats the time development of the electronic wave function and the nuclear motion. CPMD is still based on a classical Newtonian mechanics for the nuclei, but this does not seem to affect the low-frequency molecular motions associated with the vibrations. As shown below, some correction to the NMR parameters for a quantum behavior of the higher-frequency vibrations can be added to the CPMD results as a perturbation. The quantum approach thus ensures a more faithful modeling of the solute–solvent-specific interactions and their time correlation.¹⁵ Some previous simulations of similar highly polar systems indicated significant differences in water structure, as obtained by CPMD and classical MD,²¹ which we confirmed also for alanine. Moreover, the computation based on the CPMD water distribution patterns provided distinctly more accurate values of the chemical shifts and *J*-coupling.

The accurate ab initio computations of NMR parameters in sizable systems were also made possible relatively recently,^{17,22} owing to the establishment of the coupled perturbed methods,^{23,24} overcoming the origin dependence,²⁵ and implementations within the density functional theory (DFT).^{26,27} Although we are not aware of any comprehensive computation involving the vibrational effects and quantum chemical estimation of the hydration

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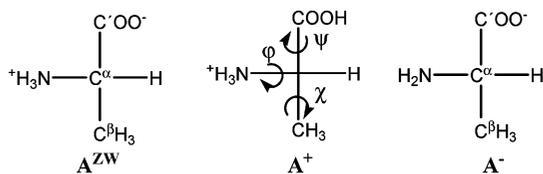


Figure 1. Structure of the three alanine forms and the carbon notation (C^γ , C^α , C^β) used for the indexing of NMR parameters. The main torsional angles are indicated for the cation. Angles φ (NH_3 rotation, $\angle\text{HNC}^\alpha\text{C}^\gamma$) and χ (CH_3 rotation, $\angle\text{HC}^\beta\text{C}^\alpha\text{C}^\gamma$) were defined for the hydrogen atom, providing the value within 0 – 120° ; similarly, the COO^- rotation (angle $\psi = \angle\text{NC}^\alpha\text{C}^\gamma\text{O}$) was defined within -90 – 90° . For the NH_2 group in A^- , $\varphi \in (-180, 180^\circ)$ was defined as an average of the two possible hydrogen dihedral angles φ_1 and φ_2 ($\varphi_2 > \varphi_1$) as $\varphi = (\varphi_1 + \varphi_2)/2$ (for $\varphi_2 - \varphi_1 < 180^\circ$) and $\varphi = (\varphi_1 + \varphi_2 - 360^\circ)/2$ (for $\varphi_2 - \varphi_1 \geq 180^\circ$).

shell geometry, the available experience indicates that in order for the predictions to be accurate, molecular motion and environmental factors have to be taken into account beside the values computed for equilibrium geometries.^{7,17,19,28,29} Therefore, we find the estimation of the hydration effects important also for the methodology and interpretation of the NMR experiment itself. Although simplified dielectric and point charge solvent models can describe with a reasonable accuracy some vibrational solvent effects of water on polar peptide-like molecules,^{30,31} the present results indicate that they may not be appropriate for NMR.

Understanding and precise modeling of the dependence of NMR parameters on the molecular geometry and environment are also of pivotal importance for peptide and protein secondary structure studies,^{32–34} and similarly in the chemistry of nucleic acids^{35,36} and sugars.^{37,38} Empirical or partially rationalized dependencies of the J -coupling on dihedral angles, known as Karplus equations,^{39,40} revolutionized the NMR conformational studies. Currently, computer modeling has become a standard part of such analyses. The structure predictions can thus be made more precise and reliable if the effect of hydration and the molecular environment is included in the calculations as well.

II. Methods Section

Periodic boxes ($10 \times 10 \times 10 \text{ \AA}$) containing 27 water molecules and alanine in the desired form (zwitterion, A^{ZW} , cation, A^+ , and anion, A^- ; see Figure 1) were created by the HyperChem program.⁴¹ Within HyperChem and the Amber99^{42,43} force field, a classical molecular dynamics (MD) was run for 1 ns with 1 fs integration time steps and at a temperature of 400 K to equilibrate the systems. The TIP3P⁴⁴ force field as a part of Amber99 was used for water. Then, the geometry was optimized and input into the Car–Parrinello CPMD⁴⁵ software package. The same periodic boundary conditions and 4 au (0.09676 fs) time step were maintained for all CPMD calculations performed with the BLYP⁴⁶ functional and Vanderbilt ultrasoft pseudopotentials.⁴⁷ The energy cutoff of 25 Ry was used. The initial configuration was relaxed by six short CPMD runs comprising 200 steps. After each run, the system was quenched to the Born–Oppenheimer surface by reoptimizing the wave function. Longer 10 ps production runs were then performed under a temperature of 300 K maintained with the Nosé–Hoover algorithm,⁴⁸ which also kept the system in the canonical (nVT) ensemble. During the 10 ps time, 20 geometry snapshots were acquired at 0.48 ps intervals.

With our own scripts, the clusters of alanine and water molecules thus obtained were reduced to contain only four to nine water molecules that were hydrogen-bonded and closer than

3.6 \AA to the solute. For some computations, larger clusters were produced when water molecules up to 4.5 \AA were retained, including those around the CH_3 group. As discussed below, however, the cluster extension did not bring about a convincing improvement, and better results were obtained by a constrained solute optimization and an anharmonic vibrational correction to the coupling. As for CPMD, the same procedure of cluster generation was repeated with classical Amber99⁴³ and polarizable Amoeba⁴⁹ force fields in the Tinker MD program.⁵⁰ Standard Amber and Amoeba atomic parameters were used with ad hoc charges for the NH_2 ($q_{\text{N}} = -0.95$, $q_{\text{H}} = -0.42$) and COOH ($q_{=\text{O}} = -0.82$, $q_{-\text{O}^-} = -0.63$, $q_{\text{H}} = 0.44$) groups. The Amoeba geometry distributions were quite close to those obtained by Amber99 and thus were not used for the NMR spectra modeling.

For the MD and CPMD clusters, NMR shielding and J -coupling constants were calculated by the Gaussian suite of programs,⁵¹ the B3LYP^{52,53} methods with the standard Gaussian 6-311++G** basis set by default. For control computations, other methods were tried, as specified below. An aqueous environment extending far from the solute was simulated by placing the clusters into a polarizable continuum using the PCM model.⁵⁴ Default cavity parameters were used, using the Simple united atom topological model for atomic radii with an average tesserae area of 0.2 \AA^2 . For some computations (see below), the alanine molecule geometry was optimized with a fixed configuration of the water molecules, and the NMR parameters were recalculated, both at the same (B3LYP/PCM/6-311++G**) level. NMR shifts were also calculated using the HF, MP2, B3LYP, BPW91, and BP86 approximations. The BPW91 and BP86 GGA functionals gave almost identical results; thus, only the BPW91 numbers are reported.

Isotopically labeled L-alanine (^{13}C , 98%; ^{15}N , 98%) was purchased from Stable Isotopes, Inc., and its NMR spectra were measured with FT NMR spectrometers (Varian UNITY-500 and Bruker AVANCE-500) with ^1H at 500 MHz, ^{13}C at 125.7 MHz, and ^{15}N at 50.7 MHz, in D_2O or in a $\text{H}_2\text{O}/\text{D}_2\text{O}$ mixture (9:1). The solution pH was varied by additions of HCl and NaOH solutions, so that the amino acid was present exclusively in the zwitterionic (A^{ZW} , pH = 7), cationic (A^+ , pH = 2), or anionic (A^- , pH = 12) form. Further details of the measurement can be found elsewhere.¹⁹

III. Results and Discussion

Water Structure. The time of the CPMD simulations was limited due to the computer demands (months of processor time) to a rather short interval of 10 ps. However, because of the fast water relaxation^{21,55} and the previous classical MD equilibration, we suppose that this time is sufficient to yield a meaningful structure of the hydration sphere. Control computations did not indicate significant changes of the results, such as the water distribution, for longer simulations. A similar duration of the CPMD production run was used, for example, for a hydrated semiquinone.⁵⁶ The CPMD water oxygen and hydrogen probability distributions in the A^{ZW} , A^+ , and A^- hydration spheres are plotted in Figure 2 (top). Distributions obtained by the classical molecular dynamics (Tinker, Amber99 force field) are shown in Figure 2 (bottom) for comparison.

We can observe principal differences in the arrangement of the solvent around the three alanine forms. For the zwitterion, the water is very structured around the NH_3^+ and COO^- charged residues, which is apparent as clearly discernible clouds of higher probability. No such structure is formed around the hydrophobic CH_3 group, and such homogeneous distribution is

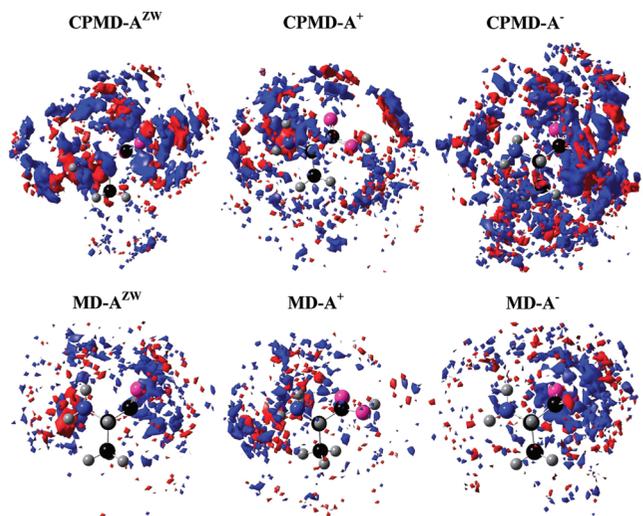


Figure 2. Water oxygen (red) and hydrogen (blue) probability distributions as obtained from the CPMD dynamics (top, BLYP functional) and the classical MD run (bottom, Amber99 force field). The approximate orientation of the alanine is indicated (CH_3 group points down; $\text{C}^\alpha\text{--H}^\alpha$ to the observer; carboxyl top right; amine top left) by plotting a randomly selected geometry; waters farther than 3.6 Å from the amino acid were ignored in the statistics.

represented in the plots by predominantly white areas with occasional fluctuations. As expected, in the A^+ cation simulations, the water is mostly structured in the vicinity of the NH_3^+ group, while for the anion A^- , a similarly organized structure is induced by the COO^- moiety. The negative charge of the carboxyl group in A^{ZW} and A^+ seems to have a slightly more significant impact on the hydration sphere than the positive amine charge in A^{ZW} and A^- , probably because of the greater binding potential (up to four hydrogen bonds can be formed) and polarization properties of the COO^- group.

However, there are also a few differences between the CPMD and classical model. Overall, the CPMD water distribution is more discrete than that obtained from MD. For example, for the zwitterion, approximately three-quarters of the CPMD first hydration sphere appears to be structured (at least in the projection shown in Figure 2), while the rest is amorphous with occasional fluctuations only. For the same system, the classical MD indicates a structure only in $\sim 50\%$ of the volume, restricted to the neighborhood of the charged residues. The anion (A^-) appears extreme in this sense as the water “structure” propagates around the whole molecule, even in the vicinity of the hydrophobic methyl group, although the structure is most apparent close to the COO^- part, as expected.

For the anion and cation, we can also clearly see that the largest differences between the classical MD and CPMD originate in the hydration of the noncharged residues (COOH , NH_2). The weaker electrostatic and partially covalent (charge-transfer) forces between the solvent and the solute and mutual water interactions thus seem to be strongly underestimated by the Amber99 force field. Similar water structuring around polar residues was suggested and partially confirmed experimentally in a number of previous protein and peptide hydration studies.^{8,18,55,57–59} The Amoeba MD simulations led to a very similar water distribution as that obtained with the Amber99 force field and thus have not been further analyzed. However, as pointed out previously,²¹ the incorporation of the polarization can occasionally improve the radial distribution parameters if compared with the purely electrostatic models.

In a more quantitative manner, the differences in the hydration of individual alanine forms can be seen in Figure 3 as the radial

distribution functions (RDF). The ambivalent character of the NH_2 group in the anion brings about the most striking difference between the classical and CPMD approach as the group can act both as a hydrogen donor and acceptor. The acceptance mediated by the lone electron pair on the nitrogen, however, is strongly underestimated by MD. In contrast, a much larger peak appears in the radial distribution obtained from CPMD (see the $\text{N}\cdots\text{HOH}$ panel in Figure 3). An average $\text{N}\cdots\text{H}$ hydrogen bond length for this interaction is shorter from CPMD (~ 1.8 Å) than that predicted by MD (2.1 Å). On the contrary, in the $\text{C}'\cdots\text{HOH}$ panel, for example, we can observe an overbinding between the carboxyl group and water hydrogen via the $\text{C}=\text{O}\cdots\text{H}$ bond predicted by MD (dashed red line). For all forms, the CPMD simulation predicts the optimal carboxyl–water oxygen distances (associated with maxima of RDF, $\text{C}'\cdots\text{OH}_2$ panel in Figure 3) about 0.15 Å larger than classical MD. The CPMD amino–water (cf. the $\text{N}\cdots\text{OH}_2$ panel) distances are shorter but by a much smaller margin. A similar tendency was observed for the glycine hydration, where polarization effects could only partially account for the CPMD quantum distributions.²¹ The radial hydration structure seems to be lost at distances of $\sim 4\text{--}5$ Å where RDF becomes constant, which is in agreement with other peptide hydration studies.⁶⁰ More distant water molecules were already outside of the box.

Alanine Flexibility. Judging from the inhomogeneous broadening of Raman and Raman optical activity spectral lines⁶¹ in the alanine molecule, all of the three methyl, amine, and carboxyl residues significantly contribute to the geometry dispersion by a hindered rotation. This is in agreement with the distributions of the dihedral angles obtained by the classical and CPMD runs plotted in Figure 4. Note that the rotation periodicity was used for the statistics of the NH_3 and COO^- rotations, where 120 and 180° changes, respectively, yields the same conformer. The two force field models provide similar torsional distributions for the methyl rotation; for CPMD, the angular range is slightly broader and more centered around the $\chi = 60^\circ$ value (canonical for the sp^3 hybridization).

Even larger differences have been encountered for the movements of the COO^- and COOH groups. In the cation, for example, the Amber99 calculation predicts a wide distribution of the ψ angle, almost reminding one of a free rotation. On the other hand, the CPMD histograms are sharper with well-defined central values ($\psi_{\text{A}^{\text{ZW}}} \sim -45^\circ$; $\psi_{\text{A}^+} \sim -5^\circ$; $\psi_{\text{A}^-} \sim -30^\circ$). For A^{ZW} and A^+ , the Amber99 and CPMD φ angle (NH_3^+ rotation) distributions are very similar and resemble those of the CH_3 group. However, in the A^- anion, the uncharged NH_2 moiety is predicted by CPMD to be rigid with a sharp probability peak at $\varphi \sim -5^\circ$. For the anion, the φ angle is defined as an average of the two possibilities (see the definition in Figure 1); therefore, this result means that the hydrogen atoms point predominantly to the COO^- group as if it were a sphere, that is, without a notable preference for a specific oxygen. The Amber99 force provides a dramatically different result, predicting that the NH_2 group rotates almost freely and the conformations with an amino hydrogen pointing to the carboxyl are preferred by a tiny margin only.

As a minor difference, we noticed that the OH alanine cation group tended to be oriented trans with respect to the carboxyl $\text{C}=\text{O}$ bond in the COOH moiety ($\angle(\text{O}=\text{C}'\text{OH}) \sim 180^\circ$) in the classical MD simulation. On the other hand, the implicit PCM model as well as the CPMD predicted a cis arrangement ($\angle(\text{O}=\text{C}'\text{OH}) \sim 0^\circ$) to be more populated, with a stabilization energy of ~ 5 kcal/mol. The cis geometry additionally provides a better agreement between the experimental and calculated

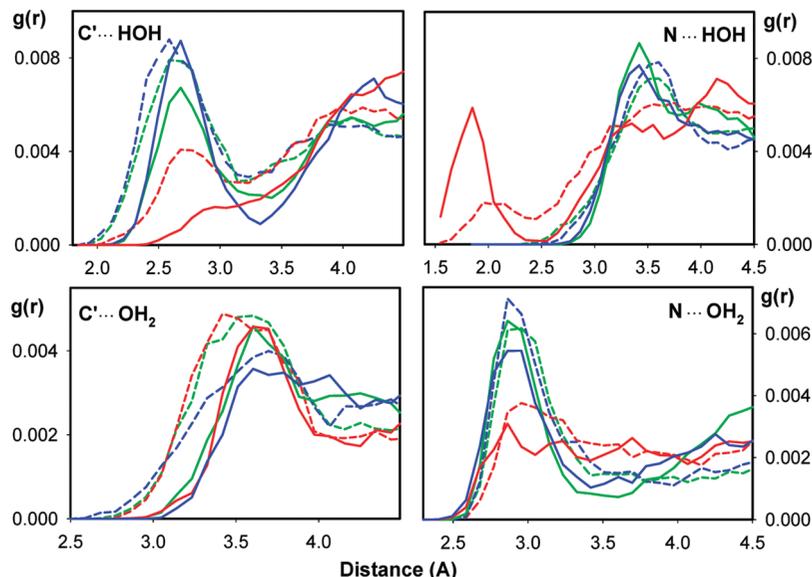


Figure 3. Radial distribution functions for the hydrated alanine zwitterion (A^{ZW} , green), cation (A^+ , red), and anion (A^- , blue) as obtained by the CPMD (solid line) and classical MD (dashed line). Distances from the carboxyl carbon (left) and the amine nitrogen (right) to water hydrogens (top) and oxygens (bottom) are displayed.

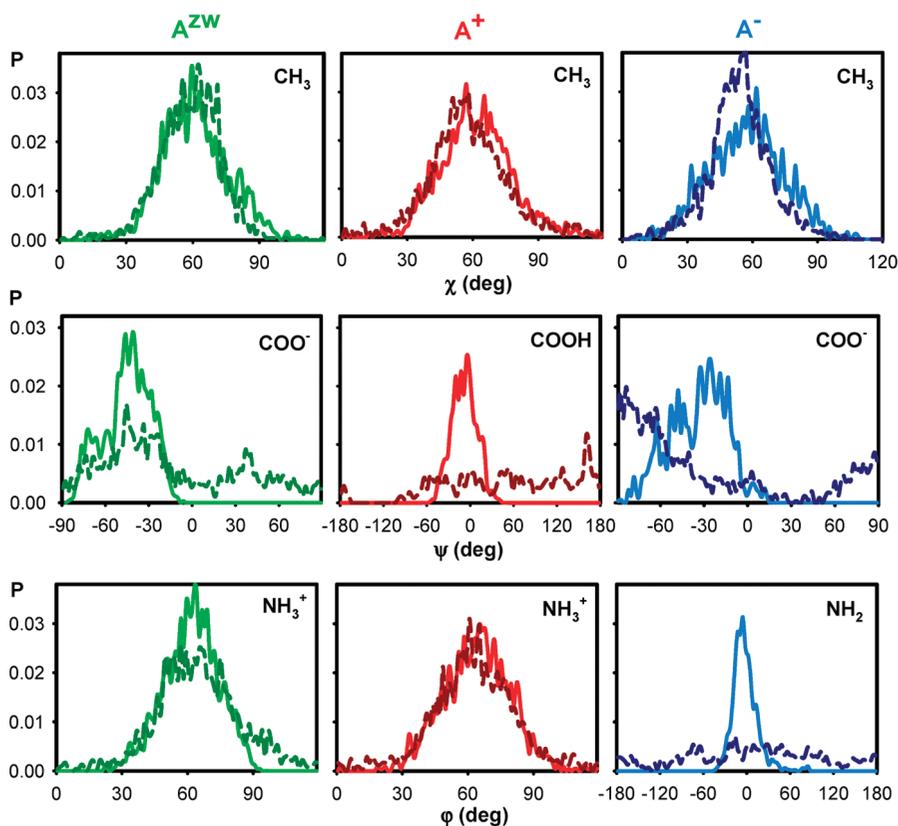


Figure 4. Comparison of the CPMD (solid line) and Amber99 MD (dashed lines) angular probabilities for the rotation of the methyl, carboxyl, and amino group in the three alanine charged forms. The angles are defined in Figure 1.

NMR properties. Therefore, we restricted the computations to the cis conformer. During the CPMD run, the hydroxyl hydrogen minutely (in $\sim <0.05\%$ of the simulation time) dissociated from the COOH cation group, implying it is partially attached to a nearby water molecule. This, however, does not seem to have any experimental implications.

Representative Clusters. It is currently impossible to obtain NMR spectral parameters for all molecular dynamics geometries. Moreover, a higher approximation level is needed for NMR than for the CPMD dynamics. Therefore, the property computations

were limited to a set of 20 clusters constructed from the MD snapshots. Nevertheless, in Figure 5, we can show that the water distribution in the clusters represents the differences between the hydration shell structure predicted by the CPMD and classical MD simulations reasonably well. For example, the average water- $O\cdots N$ distances for A^{ZW} and A^+ in the Amber99 clusters (2.87 and 2.88 Å, magenta numbers in Figure 5) are larger than those in the CPMD clusters (2.80 and 2.80 Å, black numbers), which is in agreement with the radial distributions shown in Figure 3. Similarly, for the $C\cdots H$ distances, the

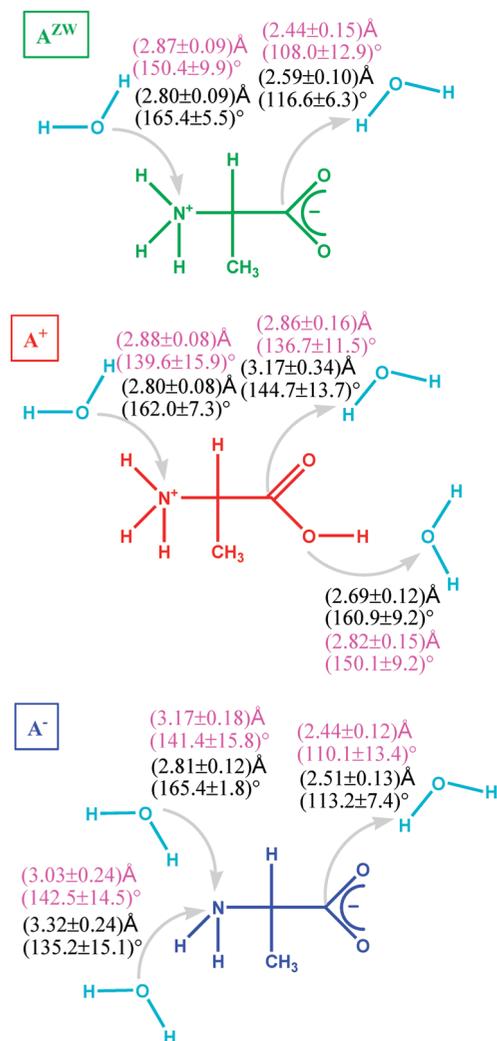


Figure 5. Geometrical parameters in two sets of 20 alanine–water clusters used for the NMR parameter computations; Amber99 (magenta numbers) and CPMD (black) clusters. Average hydrogen bond distances and angles for the hydrogen bonds indicated by the gray arrows are shown with the mean absolute deviations.

Amber99 values are lower, which was already predicted by the RDFs above and so forth. The angular averages (Figure 5) cannot be checked quantitatively, but their mean absolute deviations correspond well to the probability distributions (Figure 2) in that the Amber99 cluster dispersion values are larger. This reflects the lower degree of the hydration shell organization predicted by the classical MD model.

Chemical Shifts. As the chemical shifts are known to be very sensitive to the environment,¹⁷ an improved model of the alanine geometry and hydration sphere is expected to provide better calculated values. This can be documented on the NMR chemical shifts of A^+ and A^- as referenced to the zwitterion in Table 1.

First, the values obtained with the continuum PCM solvent model are compared with the CP cluster results. We can see that the PCM values follow the chemical shift changes observed under the pH variations in alanine with a relatively large error. Accidentally, the supposedly inferior HF approximation performs better than B3LYP for the cation (cf. the average deviations $\Delta\sigma$, 0.52 versus 1.32 ppm); in most cases, the DFT methods are better, although the results obtained with various functionals are quite similar, which is in agreement with latest computations on similar systems.⁶² However, the hydration

model involving explicit water molecules in the CP clusters is clearly superior to PCM. Typically, the mean absolute deviation decreases three times. The mean is somewhat biased because the NMR shifts of heavier atoms are larger; nevertheless, by comparing individual atoms, we see that also the hydrogen shifts are more realistically provided by the cluster model. The approximation levels containing the two-electron exchange (HF, MP2, B3LYP) seem to be slightly better than GGA functionals (BPW91 and B86 (not shown)). The inadequacy of the PCM model is even more apparent for individual atomic shifts; particularly large is the dispersion of the calculated values for C^α . The cluster computations provide correct signs of the shift differences, except for the C' carbon in A^+ , reasonably reproduced by BPW91 only.

In the second part of Table 1, four cluster models are compared for the most common B3LYP functional. The classical MD clusters produced with the Amber99 force field provide very unrealistic NMR shifts. Note that the clusters are submerged in the PCM dielectrics as well to mimic the distant aqueous environment. For the cation, the mean deviation increases to 4.64 ppm, and the carboxyl carbon shift becomes -23.88 ppm, which is $7.6\times$ more than that observed experimentally (-3.11 ppm). For the anion, the MD cluster results are equally unrealistic as for most atoms, the signs of the calculated shifts do not agree with the experiment.

Unlike the Amber99 MD results, the shifts calculated for the CPMD clusters are mostly superior to the PCM model. The computations with larger clusters yield practically the same numbers as those obtained with the hydrogen-bonded waters only. Only the shifts of the β -hydrogens, presumably most sensitive to this variation, appear to be reproduced more realistically with the explicit methyl hydration (cf. the A^+ and A^- experimental shifts of 0.08 and -0.26 ppm, calculated as 0.15 and -0.12 ppm, respectively) than those without it (0.00 and -0.40 ppm). However, this difference is comparable with the statistical error. The results for larger clusters can also be affected by the limited size of the simulation box. We therefore consider the restriction to the hydrogen-bonded waters as being adequate. The average error of 1.38 ppm obtained for the cation with the smaller clusters is similar to that for the PCM statistics ($\Delta\sigma = 1.32$ ppm). The cluster method corrected the rather unrealistic PCM anion C' shift (-0.42 ppm) to -4.91 ppm, which better corresponds to the experimental number -3.11 ppm; similar improvements can be seen for other atoms. Understandably, the inclusion of the explicit solvent seems to be most important for the polar molecular residues. For the anion, the average deviation is even reduced to $\sim 50\%$ ($1.93 \rightarrow 0.96$ ppm).

The unoptimized CPMD cluster average deviations (1.38 and 0.96 ppm for the cation and anion, respectively) are further reduced by a restricted optimization (to 0.29 and 0.62 ppm) up to $\sim 25\text{--}30\%$ of the original PCM error. This can be understood as the geometries obtained from the CPMD method are hampered by the limited electronic basis and the necessary restriction to the general gradient approximation (GGA) functional (BLYP). Upon further improving the snapshot structures based on the simplified BLYP model by a constrained optimization of the amino acid geometry with fixed water positions at the B3LYP/PCM(H_2O)/6-311++G** level, the resultant chemical shift errors thus become smaller. For the Amber99 MD clusters, the alanine optimization reduces the error too (results not shown), but the results are still much worse than those for CPMD. Therefore, we can attribute a large part of the improve-

TABLE 1: Calculated and Experimental Chemical Shifts (σ , in ppm) in the Charged A^+ and A^- Forms with Respect to A^{ZW}

(a) Method Comparison										
atom	single molecule, PCM				CP-opt clusters					
	HF	MP2	BPW91	B3LYP	HF	MP2	BPW91	B3LYP	exptl. ^a	
N	-2.93	-5.21	-5.66	-4.94	$\sigma_{A^+} - \sigma_{A^{ZW}}$ -0.96	-2.28	-2.98	-2.47	-2.20	
C'	-3.44	-2.05	-0.23	-0.42	-2.45	-3.11	-1.56	-2.14	-1.77	
C $^\alpha$	-2.20	-1.77	0.69	-1.21	-4.62	-3.22	-1.53	-2.47	-3.11	
C $^\beta$	-1.89	-2.28	-2.13	-2.19	-0.92	-1.06	-0.87	-0.95	-0.83	
H $^\alpha$	0.71	0.78	0.78	0.76	0.62	0.70	0.58	0.58	0.37	
H $^\beta$	0.28	0.28	0.26	0.27	0.24	0.24	0.22	0.23	0.08	
$\Delta\sigma^b$	0.52	1.12	1.78	1.32	0.66	0.38	0.49	0.29	0.00	
N	-10.50	-13.07	-12.96	-11.71	$\sigma_{A^-} - \sigma_{A^{ZW}}$ -5.54	-8.55	-7.93	-6.91	-6.60	
C'	12.38	0.99	1.36	14.31	-1.67	-0.50	0.23	-0.24	0.93	
C $^\alpha$	-1.05	13.56	14.27	0.87	8.59	9.72	9.78	9.80	8.94	
C $^\beta$	4.21	4.51	4.55	4.72	2.67	3.50	3.13	3.22	4.25	
H $^\alpha$	-1.13	-0.86	-0.82	-0.90	-0.91	-0.66	-0.73	-0.77	-0.48	
H $^\beta$	-0.46	-0.39	-0.39	-0.41	-0.42	-0.41	-0.33	-0.35	-0.26	
$\Delta\sigma^c$	1.70	1.99	2.15	1.93	1.03	0.87	0.72	0.62	0.00	
(b) Cluster Type Comparison (for B3LYP, standard errors of the mean are shown)										
atom	MD	CP ^c	CP	CP-opt	exptl. ^a					
N	-0.61 ± 1.96	0.88 ± 2.18	$\sigma_{A^+} - \sigma_{A^{ZW}}$ 0.62 ± 2.16	-2.47 ± 0.29	-2.20					
C'	-23.88 ± 2.31	-5.94 ± 1.99	-4.91 ± 1.95	-2.47 ± 0.34	-3.11					
C $^\alpha$	-4.99 ± 1.55	-1.15 ± 2.16	-1.16 ± 2.10	-2.14 ± 0.40	-1.77					
C $^\beta$	-1.38 ± 1.35	-3.66 ± 1.30	-3.50 ± 1.36	-0.95 ± 0.29	-0.83					
H $^\alpha$	-0.90 ± 0.14	0.52 ± 0.30	0.66 ± 0.30	0.58 ± 0.12	0.37					
H $^\beta$	-0.34 ± 0.12	0.15 ± 0.15	0.00 ± 0.13	0.23 ± 0.02	0.08					
$\Delta\sigma^b$	4.64	1.60	1.38	0.29	0.00					
N	7.96 ± 1.56	-4.34 ± 2.37	$\sigma_{A^-} - \sigma_{A^{ZW}}$ -5.44 ± 2.48	-6.91 ± 0.85	-6.60					
C'	-7.44 ± 1.88	7.98 ± 1.70	8.05 ± 1.63	9.80 ± 0.42	8.94					
C $^\alpha$	-5.63 ± 1.45	-0.81 ± 1.71	-0.71 ± 1.66	-0.24 ± 0.50	0.93					
C $^\beta$	-3.71 ± 1.01	3.75 ± 1.36	2.55 ± 1.46	3.22 ± 0.21	4.25					
H $^\alpha$	0.55 ± 0.21	-0.94 ± 0.22	-0.67 ± 0.20	-0.77 ± 0.09	-0.48					
H $^\beta$	0.55 ± 0.15	-0.12 ± 0.19	-0.40 ± 0.19	-0.35 ± 0.03	-0.26					
$\Delta\sigma^b$	7.89	1.10	0.96	0.62	0.00					

^a Reference 19. ^b Mean absolute deviation from the experiment. ^c Large clusters, where the CH₃ group hydration was included.

ment of the NMR shifts obtained by CPMD to a more realistic water structure.

Interestingly, the values of isotropic shielding obtained for optimized geometries (not shown) are always shifted upfield, and the nuclei are thus more shielded than those for the raw clusters, which may correspond to a geometry compacting upon relaxation to the equilibrium. Although we find that the cluster model best reproduces the experimental numbers, currently, we cannot explain the discrepancy regarding the C $^\alpha$ anion shift, which was found to be 0.93 ppm experimentally but reproduced as negative in most computations. The large difference for this atom between the HF and B3LYP values suggests that the electron correlation inadequately represented in these approximation levels might be responsible for this inconsistency.

The geometry optimization also significantly reduced the dispersion of the chemical shifts and, consequently, standard errors of the mean values (cf. Table 1). As illustrated in Figure 6, where the calculated isotropic shielding values are plotted for individual clusters, the magnetic shielding alterations caused by the water positions are huge within the entire alanine molecule. As expected, the scattering is very large for the N and C' atoms in the polar amino and carboxyl groups. Note, for example, the enormous interval of ~40 ppm for the nitrogen shielding in the anion; the dispersion of ~3–5 ppm calculated

for the hydrogen atoms is impressive as well. However, the considerable dispersion of the shifts comprising atoms in the hydrophobic part of the molecule is equally surprising and, to the best of our knowledge, has not been reported yet. As the averaged values reproduce the experimental data so well, we conclude that the measured shifts must result from a fairly complex interplay of the alanine motion and the structure and dynamics of the hydration sphere.

The large dispersion inevitably results in a relatively slow convergence of the average shift values with the number of clusters (Figure 7). This makes the required computer time longer, which reminds one of a situation when excited electronic states of polar systems have to be modeled with a huge number (~100) of averaged clusters.^{15,63} Fortunately, in the case of NMR spectroscopy, the averages converge much sooner (Figure 7), and 10–15 clusters seem already sufficient to provide reasonable estimates of the shifts.

Coupling Constants. Similar but somewhat finer effects of the hydration and geometry averaging can be found for the *J*-coupling. Here, we omit the HF computation because this approximation is known to be inadequate for the coupling.^{64,65} The agreement of the calculated DFT coupling values with the experiment is documented in Table 2 for the alanine zwitterion. The DFT values obtained with the PCM solvent correction

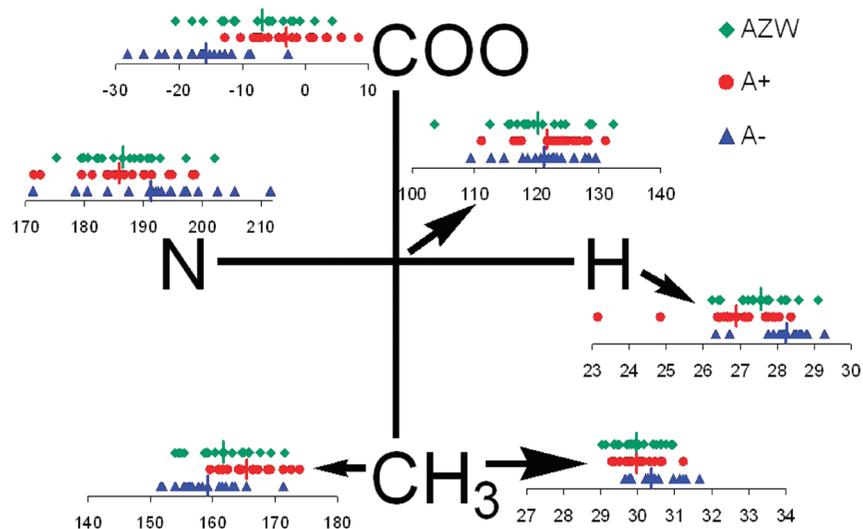


Figure 6. Individual isotropic NMR shieldings (ppm) for the three alanine charged forms as calculated in the 20 CPMD clusters. Average values are indicated by the vertical lines.

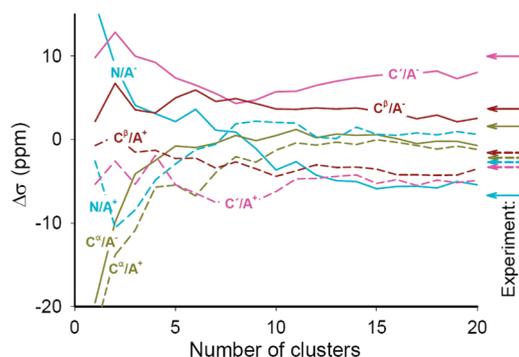


Figure 7. Convergence of selected calculated chemical shifts in the alanine charged forms ($\sigma_{A^-} - \sigma_{AZW}$, full line, and $\sigma_{A^+} - \sigma_{AZW}$, dashed line) with the number of the CPMD clusters taken into account. Experimental values are indicated to the right (arrows).

follow the experimental results reasonably well, with an average deviation of 1.5 Hz. The deviation can be further reduced to 1.2–1.3 Hz when the explicit water effect is included with the CPMD clusters. Similarly as for the shifts, the structures based on classical MD with the Amber99 force field do not seem to be appropriate for improvement of the NMR parameters as the average error rises (relative to PCM) to 2.2 Hz.

Unlike for the shifts, however, the reoptimization of the CPMD geometries brings about significant changes of neither the coupling constants nor the average error. We explain the relative insensitivity of the couplings to the hydration model by the rather different nature of the two magnetic phenomena. Since the NMR shielding is caused by both localized and delocalized electric currents, it senses tiny changes in molecular structure, including a wide solvent shell. On the other hand, the indirect spin–spin coupling is mediated chiefly by electronic clouds in the vicinity of the interacting atoms^{17,66} and is thus less sensitive to the hydration. Indeed, the dispersion of the J -constants caused primarily by the water arrangement reflected in the mean error is much smaller (typically 1–10%; cf. Table 2) than that for the shifts (up to several hundred %; Table 1).

The results also suggest that the shifts are more sensitive to soft low-frequency vibrational modes associated with the solvent and some alanine torsional motions, whereas the coupling is dependent on more localized higher-frequency vibrations, such as bond bending and stretching. As the quantum vibrational phenomena might not be sufficiently characterized by the CPMD

dynamics that still uses Newtonian mechanics for the nuclei, we add to the J -couplings calculated for the clusters the anharmonic vibrational corrections previously determined for the alanine molecule.⁶⁷ This perturbational treatment further improves the cluster results and reduces the average error (0.7 Hz; the cluster-CP^c column in Table 2) to less than 50% compared to that of the original PCM model. Alternatively, one could think about using directly the CPMD geometries to take into account the anharmonic effects as the leading-order anharmonic zero-point contribution to a molecular property is equivalent to evaluating the property at a vibrationally averaged geometry.⁶⁸ This, however, would make the number of required configurations too high and the results susceptible to the inaccuracies of the simplified DFT method used for CPMD.

The dispersion of selected J -coupling constants is documented in Figure 8. Given the experimental accuracy (~ 0.1 Hz), the differences are considerably large, up to ~ 40 Hz. The wide interval found for the coupling of the C $^{\alpha}$ –H $^{\alpha}$ hydrophobic system is particularly surprising, and the explanation is not straightforward. We did not observe, for example, any correlation between this constant and the C $^{\alpha}$ –H $^{\alpha}$ bond length. Probably, the local electronic structure is significantly perturbed by both the alanine geometry changes and the interactions with the solvent. On the other hand, the variation in the couplings is small compared to the shifts. Consequently, the number of clusters required for results to converge (~ 5 – 10) is also somewhat smaller, as estimated from the convergence plots of selected constants in Figure 9.

The convergence of the calculated NMR constants with the number of the clusters taken into the averaging (Figure 9) and the associated average deviations (Table 2) is in agreement with the latest results obtained for MD/DFT simulations for similar systems.^{69,70} The deviations decrease relatively slowly with the numbers of the clusters; for example, to achieve a precision of 0.05 ppm on the water hydrogen shift, about 1000 clusters were required.⁶⁹ On the other hand, much smaller numbers of configurations give reasonable average values. Also, as can be seen in Table 2, the constrained optimization significantly reduces the dispersion caused by different water positioning around the solute.

J -Coupling pH Dependence. Taking into account the hydration and cluster averaging is even more important for modeling the pH-induced changes of the coupling constants. In Table 3,

TABLE 2: Calculated (B3LYP/PCM/6-311++G) and Experimental J -Coupling Constants (Hz) in the Alanine Zwitterion**

coupled atoms	PCM	clusters-MD ^a	clusters-CP ^a	clusters-CP ^{a,b}	clusters-CP ^c	exptl. ^d
N-C ^α	-3.0	3.9 ± 0.3	-3.5 ± 0.5	-3.8 ± 0.2	-3.6	-5.7
C ^α -C ^β	34.4	31.9 ± 0.8	33.4 ± 1.1	33.5 ± 0.1	34.7	34.9
C ^α -C'	50.4	54.5 ± 1.0	55.2 ± 0.9	53.9 ± 0.1	53.8	54.0
C ^α -H ^α	146.5	139.3 ± 1.6	146.9 ± 2.2	142.7 ± 0.4	148.1	145.1
C ^β -H ^β	123.3	125.7 ± 0.7	125.7 ± 0.8	123.0 ± 0.1	129.8	129.7
N-H ^α	-1.4	0.4 ± 0.1	-1.1 ± 0.2	-1.1 ± 0.1	-1.1	0.0
N-C ^β	-0.5	0.2 ± 0.1	-0.3 ± 0.1	-0.2 ± 0.0	-0.1	0.0
N-C'	-0.1	0.1 ± 0.0	-0.2 ± 0.1	-0.1 ± 0.0	0.0	0.0
C ^α -H ^β	-3.1	-3.2 ± 0.3	-2.8 ± 0.2	-3.0 ± 0.0	-3.7	-4.4
C ^β -H ^α	-3.0	-4.1 ± 0.4	-3.1 ± 0.4	-2.8 ± 0.1	-3.5	-4.6
C'-C ^β	-1.1	-0.9 ± 0.1	-1.3 ± 0.1	-1.1 ± 0.0	-1.3	-1.2
C'-H ^α	-3.7	-5.6 ± 0.4	-4.0 ± 0.5	-3.7 ± 0.1	-4.4	-5.0
N-H ^β	-3.5	2.0 ± 0.1	-3.1 ± 0.1	-2.9 ± 0.1	-3.4	-3.1
C'-H ^β	3.6	4.0 ± 0.1	3.9 ± 0.1	4.0 ± 0.1	4.4	4.2
H ^α -H ^β	6.5	6.0 ± 0.2	6.4 ± 0.3	6.3 ± 0.0	7.3	7.3
ΔJ^e	1.5	2.2	1.2	1.3	0.7	0.0

^a The standard error of the mean is indicated. ^b Reoptimized (B3LYP/PCM/6-311++G**) alanine geometry with fixed water positions. ^c Anharmonic vibrational correction included, according to ref 67. ^d Reference 19. ^e Mean absolute deviation from the experiment.

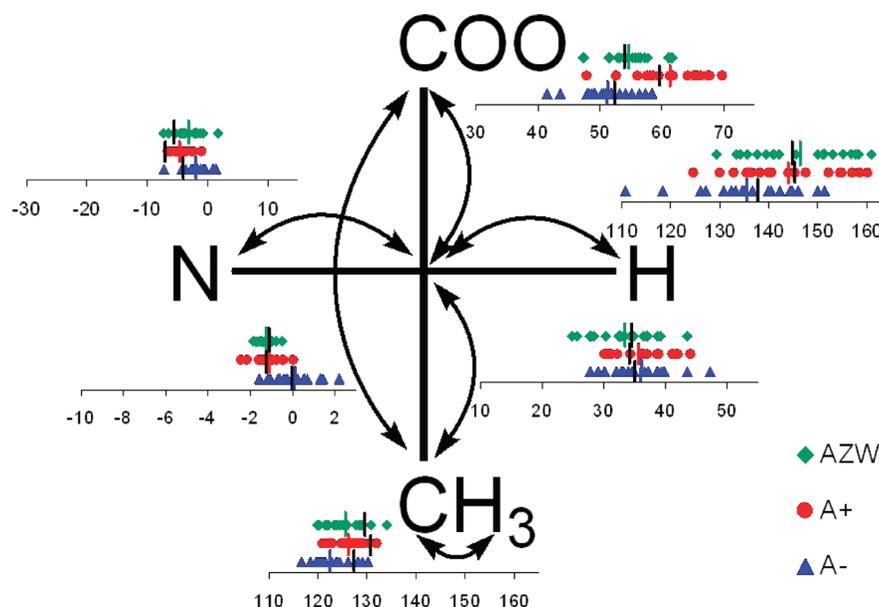


Figure 8. Selected J -coupling constants (in Hz) as calculated for 20 selected CPMD clusters of the hydrated alanine forms and the average values (vertical color lines). The experimental values¹⁹ are indicated by vertical black lines.

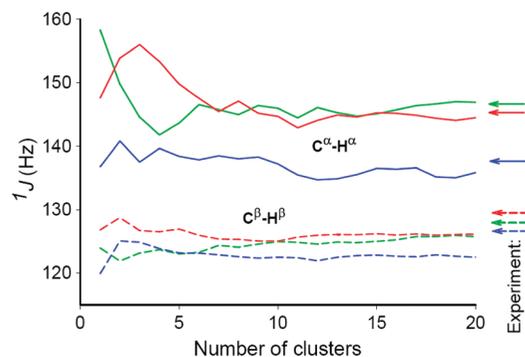


Figure 9. Convergence of selected calculated coupling constants ($^1J(C^{\alpha},H^{\alpha})$, solid lines; $^1J(C^{\beta},H^{\beta})$, dashed lines) in A^{ZW} (green lines), A⁺ (red), and A⁻ (blue) with the number of averaged CPMD alanine-water clusters. Experimental values are indicated to the right (arrows).

the calculated coupling variations in A⁺ and A⁻ with respect to the zwitterion are compared to the experiment. The pH-induced changes are relatively modest. Neither MD nor the CPMD averages convincingly improve the PCM results with

raw cluster geometries. However, the CPMD cluster calculations followed by the alanine reoptimization (column clusters-CP^{a,b} in Table 3) do bring a significant improvement. The average deviations drop from 1.0 to 0.7 and from 1.1 to 0.8 Hz for the cation and anion, respectively.

The agreement between the calculation and the experiment varies according to the coupling type, and some of the calculated results still exhibit a significant error. Most of it can probably be attributed to a limited precision of the density functional approximation,^{17,67,71} apart from restrictions from the basis set, solvent modeling, and the dynamical factors. Typically, with the PCM model, the coupling constants are reproduced with correct signs but a large error, which is only somewhat reduced after the averaging over the CPMD geometry snapshots. For example, the $\Delta J(A^+, N-C^{\alpha}) = -2.5$ Hz in PCM changes to -2.1 Hz within the CPMD approach, which is still rather far from the experiment (-0.9 Hz). The vibrational anharmonic correction did not improve the J -coupling pH dependence (data not shown).

The reoptimized alanine geometries thus resulted in an improved modeling of the J -coupling pH dependence, predomi-

TABLE 3: Calculated and Experimental J -Coupling Differences in Alanine Charged Forms (A^+ , A^-) with Respect to the Zwitterion

coupled atoms	PCM	clusters-MD ^a	clusters-CP ^a	clusters-CP ^{a,b}	exptl.
$J(A^+) - J(A^{ZW})$ [Hz]					
N-C ^α	-2.5	-0.2 ± 0.4	-1.0 ± 0.6	-2.1 ± 0.2	-0.9
C ^α -C ^β	-0.9	0.6 ± 1.1	2.1 ± 1.7	-0.4 ± 0.2	-0.8
C ^α -C'	11.0	9.2 ± 1.8	6.4 ± 1.7	7.2 ± 0.2	5.6
C ^α -H ^α	-2.3	8.4 ± 3.7	-2.4 ± 3.4	-0.7 ± 0.6	1.5
C ^β -H ^β	2.6	1.3 ± 0.8	0.4 ± 1.2	2.1 ± 0.1	1.3
C ^α -H ^β	-0.3	0.2 ± 0.3	-0.8 ± 0.2	-0.4 ± 0.0	-0.2
C ^β -H ^α	-1.0	-1.2 ± 0.6	-0.7 ± 0.5	-1.4 ± 0.1	-0.4
C'-C ^β	-0.2	-1.4 ± 0.3	0.1 ± 0.1	-0.1 ± 0.1	-0.1
C'-H ^α	-2.0	-0.4 ± 1.1	-2.5 ± 0.7	-1.8 ± 0.1	-1.0
N-H ^β	0.0	0.2 ± 0.1	-0.2 ± 0.2	0.0 ± 0.1	0.1
C'-H ^β	0.8	1.2 ± 0.2	0.7 ± 0.2	0.4 ± 0.1	0.4
H ^α -H ^β	0.2	0.2 ± 0.2	0.7 ± 0.4	0.4 ± 0.0	0.0
ΔΔJ ^c	1.1	1.4	1.0	0.7	0.0
$J(A^-) - J(A^{ZW})$ [Hz]					
N-C ^α	0.5	0.9 ± 0.5	1.8 ± 0.6	0.7 ± 0.2	1.4
C ^α -C ^β	1.3	1.9 ± 1.0	2.7 ± 1.5	1.7 ± 0.2	0.3
C ^α -C'	-0.3	1.5 ± 1.3	-4.1 ± 1.3	-3.5 ± 0.2	-1.3
C ^α -H ^α	-14.1	-6.2 ± 2.4	-11.1 ± 3.2	-10.2 ± 0.4	-6.7
C ^β -H ^β	-3.2	-3.9 ± 0.9	-3.2 ± 1.2	-2.1 ± 0.1	-2.1
C ^α -H ^β	-2.1	-0.5 ± 0.2	-1.8 ± 0.5	-1.6 ± 0.2	-2.2
C ^β -H ^α	-1.2	0.8 ± 0.5	-1.6 ± 0.7	-1.1 ± 0.1	-0.2
C'-C ^β	1.3	1.1 ± 0.3	1.3 ± 0.2	1.2 ± 0.1	1.2
C'-H ^α	0.4	2.3 ± 0.6	1.7 ± 0.6	0.7 ± 0.1	0.7
N-H ^β	-0.1	0.4 ± 0.1	-0.1 ± 0.1	-0.4 ± 0.1	0.1
C'-H ^β	0.0	-0.2 ± 0.2	0.1 ± 0.2	-0.2 ± 0.1	0.1
H ^α -H ^β	-0.1	0.0 ± 0.2	-0.1 ± 0.3	0.1 ± 0.0	-0.2
ΔΔJ ^c	1.0	1.1	1.1	0.8	0.0

^a Standard error of the mean is indicated. ^b Reoptimized (B3LYP/PCM/6-311++G**) alanine geometry. ^c Mean absolute deviation from the experiment.

nantly by accounting for the structured hydration shell reproduced by the CPMD. In our opinion, the dynamic and anharmonic geometry variations may potentially play a more important role in the future, too, if better functionals or faster wave function methods enabling a greater precision become available. In any case, the molecular hydration is an important factor influencing the values of the spin-spin coupling constants and should be taken into account in precise computations.

IV. Conclusions

We have performed Carr-Parinello molecular dynamics modeling of hydrated and variously charged alanine forms, thus obtaining significantly different structures of the first hydration spheres than those with the classical force fields. Unlike for the empirical force fields, the CPMD run predicted a large-scale ordering of the water molecules also around the neutral COOH and NH₂ groups. The NH₂ moiety acted both as a hydrogen donor and acceptor within the CPMD model of the alanine anion. The force field differences were also reflected in the geometry of the solute as the CPMD model provided a more rigid amino acid structure. The water structure could not be verified directly, but when we employed the CPMD geometries in computations of the chemical shifts and indirect spin-spin coupling constants, we were able to reproduce the experimental NMR parameters and their pH dependencies observed in the ¹⁵N and ¹³C isotopically labeled amino acid much more faithfully. Both the shifts and couplings exhibited an unexpectedly large dispersion originating not only in the alanine geometry dispersion but also in the interactions with differently positioned solvent molecules. After the averaging with 10–20 snapshot geometries, we obtained shifts and couplings that yielded a better agreement with the experiment

than those calculated with the dielectric continuum solvent correction or even with similar explicit clusters based on the classical MD simulations with empirical force fields. The shifts were found to be much more sensitive to the hydration structure and required a more extensive averaging than the couplings. NMR spectroscopy thus probes both the local chemical environment and the molecular interactions with the solvent. The more faithful CPMD model including the quantum electronic effects may make the structural and dynamical studies more reliable, especially for the polar biologically interesting compounds.

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