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Comparative Study of Electrostatic Models for the Amide-I and -II Modes: Linear and Two-Dimensional Infrared Spectra

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Abstract

We have carried out a comparative study of five *ab initio* electrostatic frequency maps and a semi-empirical model for the amide-I and -II modes. Unrestrained molecular dynamics simulation of a 3_{10} -helical peptide, Z-Aib-L-Leu-(Aib)₂-Gly-OtBu, in CDCl₃ is performed using the AMBER ff99SB force field, and the linear and two-dimensional infrared (2D IR) spectra are simulated based on a vibrational exciton Hamiltonian model. A new electrostatic potential-based amide-I and -II frequency map for *N*-methylacetamide is developed in this study. This map and other maps developed by different research groups are applied to calculate the local mode frequencies of the amide linkages in the hexapeptide. The simulated amide-I lineshape from all models agrees well with the previous experimental results on the same system, except for an overall frequency shift. On the contrary, the simulated amide-II bands are more sensitive to the frequency maps. Essential features obtained in the electrostatic models are captured by the semi-empirical model that takes into account only the intramolecular hydrogen bonding effects and solvent shifts. Detailed comparisons between the models are also drawn through analysis of the local mode frequency shifts. Among all of the maps tested in this study, the new four-site potential map performs quite well in simulating the amide-II bands. It properly predicts the effects of hydrogen bonding on the amide-I and -II frequencies, and reasonably simulates the isotope-dependent amide-I/II cross peaks upon $^{13}\text{C}=^{18}\text{O}/^{15}\text{N}$ substitutions.

I. Introduction

Vibrational modes characteristic to a peptide linkage are referred to as the amide modes, and there are seven amide modes whose atomic motions are well characterized in normal mode analysis of *N*-methylacetamide (NMA), the simplest model compound of peptide linkage.¹ The amide-I mode, mostly consists of the highly localized C=O stretching motion, is known to be sensitive to peptide and protein conformation. Spectral analysis of the amide-I band in linear infrared (IR) and vibrational circular dichroism (VCD) measurements has often been utilized to characterize the basic secondary structures and their content in proteins.^{1–3} Recent intensive application of two-dimensional (2D) IR spectroscopy^{4–6} also focuses on the amide-I modes in different secondary structures, such as α -helix,^{7,8} 3_{10} -helix,^{9–13} antiparallel β -sheet,^{14,15} and β -hairpin,^{16–18} as well as some proteins in aqueous solutions¹⁹ and membranes,²⁰ and aggregated amyloid peptides.^{21,22} It has also been recognized that 2D IR spectroscopy is a promising tool for monitoring dynamical structure change in timescale from picosecond to millisecond.^{19,23} Isotope substitution of the amide-I modes with $^{13}\text{C}=^{16}\text{O}$ and $^{13}\text{C}=^{18}\text{O}$ enables the linear and nonlinear spectroscopic methods to selectively probe structure and dynamics at a specific local position.^{8,12,16–18,20,21,24,25}

Other amide modes have also attracted attentions in spectroscopic studies. For example, the amide-A mode, ascribed to the N–H stretching mode, can be used to investigate intra- and intermolecular hydrogen bonding because hydrogen bonding produces drastic changes in the resonant frequency and absorption cross section of the mode.¹ Mirkin and Krimm found from their DFT calculation of alanine dipeptide (*N*-acetyl-L-alanine-*N'*-methylamide) and tripeptide that the amide-III mode was sensitive to dihedral angles ϕ and ψ .²⁶ The amide-II mode, mainly composed of the C–N stretching mode and the out-of-phase in-plane N–H bending mode, has also been used to study molecular conformation by linear IR and VCD.^{1–3,27} Especially, the significant frequency shift ($\sim 100\text{ cm}^{-1}$) upon deuteration makes the amide-II mode a useful reporter of the H/D exchange process, from

which detailed information on kinetics and structure stability of biological macromolecules has been extracted.²⁸ Recently, 2D IR spectroscopy has also been applied to the amide-II mode. The amide-I/II and -II/II vibrational couplings (or -I'/II' and -II'/II' when the peptide units are nitrogen deuterated) of NMA,²⁹⁻³¹ peptides,^{12,32-34} and proteins^{35,36} have been investigated. Of particular interest are two recent studies^{12,34} which demonstrated that the cross-peaks between the amide-I and -II modes connected through an intramolecular C=O...H-N hydrogen bond are discernable with the aid of ¹³C and ¹⁸O labeling on the amide-I mode and ¹⁵N labeling on the amide-II mode.^{12,34} The demonstrated strategy is a promising method toward detecting the formation of a single helical turn from an extended polypeptide chain. These new studies demonstrated further the potential of 2D IR spectroscopy and demanded a theoretical advancement to properly simulate spectral patterns of the amide-I and -II modes.

Compared with many theoretical studies on the local mode frequency³⁷⁻⁴⁷ and nearest-neighbor coupling^{33,48-51} of the amide-I modes, the amide-II mode has not been extensively studied yet. Only a few groups expanded the calculation protocol to create local mode frequency maps,^{31,33,52} and coupling maps^{33,34,53} for the amide-II modes. These vibrational properties were calculated for peptide linkages in small model peptide compounds, such as NMA and Ac-Gly-NHMe. Therefore, it is necessary to check whether they are applicable to peptides and proteins to properly reproduce the linear and nonlinear responses using, for example, a vibrational exciton model. Several critical tests have been carried out for the amide-I modes based on the 2D IR spectra simulated with the molecular dynamics (MD) simulation in explicit solvent molecules.^{13,47,54} In these comparative studies, different electrostatic and empirical models for the local site-energies were compared by simulating 2D IR spectra of some proteins⁵⁴ and membrane peptide bundles⁴⁷ in aqueous solutions, and a L-(α Me)Val₃₁₀-helical octapeptide in deuterated chloroform.¹³

In order to test the transferability of the different electrostatic frequency maps created for the amide-II mode of NMA, we conduct a comparative study on their performance in simulating the

amide-I/II 2D IR spectra of a 3_{10} -helical hexapeptide, Z-Aib-L-Leu-(Aib)₂-Gly-OtBu (**1**, Z, benzyloxycarbonyl; Aib, C ^{α,α} -dimethylglycine; OtBu, *tert*-butoxy, Figure 1) in CDCl₃. Previous 2D IR measurements revealed that the peptide forms the 3_{10} -helix structure,^{12,34} as expected for Aib-rich oligopeptides in a relatively weak polar solvent like chloroform.^{55,56} Due to methylation of the alpha-carbon, the energetically favorable conformations around the Aib residue are highly restrained to the helical region of the Ramachandran plot ($\phi \sim \pm 60 \pm 20^\circ$, $\psi \sim \pm 30 \pm 20^\circ$).⁵⁷ This propensity leads to a relatively stable 3_{10} - and/or α -helix structure for an Aib-rich peptide in the crystal state and solution phases.^{55,56} If MD simulation is able to predict this structural homogeneity, a short peptide with a robust conformation is a desirable model in the comparative study of 2D IR spectra, because we can rule out the possibility that disagreement between the measured and simulated spectra originates from poor performance of the MD force field. In our previous study on Z-[L-(α Me)Val]₈-OtBu in CDCl₃, the adapted CHARMM22 force field generated a trajectory with a relatively high population of α -helical and nonhelical conformations in an unrestrained NVE MD simulation,¹³ contrary to several experimental results that indicate that the peptide most likely forms a 3_{10} -helix.^{9,10,58,59} The simulated 2D IR spectral patterns based on the unrestrained trajectory disagreed with those observed in measurements, and a restrained trajectory referenced to the 3_{10} -helix crystal structure was required to achieve much better agreement.¹³ In this study, we employ the AMBER force field (ff99SB)⁶⁰⁻⁶² to get a trajectory of the Aib-rich peptide. Several different force fields, such as CHARMM,⁶³ GROMOS,^{64,65} and Cedar,⁶⁶ have been applied to Aib-rich peptides to study the relative stability of α -, 3_{10} - and π -helical conformations in gas and solution phases. The most stable helical conformations obtained from these simulations are quite dependent on the force fields used. It is intriguing to see whether the structure sampled by the AMBER force field can simulate the measured 2D IR spectra, which is very sensitive to peptide helicity.¹³

To model both the amide-I and -II modes, we newly create a four-site electrostatic potential map in this study to compare with the four existing electrostatic maps: three of the six-site electrostatic

potential maps by Bouř et al.⁵² and the four-site electrostatic field/gradient map by Bloem et al.³¹ The comparison provides insights into how the performance depends on the basis set, density functional, the number of parameterized sites, and electrostatic model.

Although the previous study³⁴ reasonably well reproduced the experimental 2D IR spectra of **1** and its $^{13}\text{C}=^{18}\text{O}$ -Leu monolabeled (**1***) and $^{13}\text{C}=^{18}\text{O}$ -Leu/ ^{15}N -Gly bis-labeled (**1****) isotopomers using a model calculation based on the ideal 3_{10} -helix conformation and a semi-empirical model for the amide-I and -II local site energies, some subtle features observed for the amide-II band were still missing. Also, the ^{15}N isotope effects on the amide-II spectral features were less quantitatively reproduced. Thus, another objective in this study is to check whether the combined computation protocol of MD simulation and more sophisticated electrostatic models is able to better reproduce the measured amide-I and -II 2D IR spectra than the semi-empirical model.

This paper is organized as follows. Details on the MD simulation, spectral simulation, and features of the different electrostatic models are given in the next section. Our semi-empirical model and the newly created DFT frequency maps for the amide-I and -II modes are also included. Section III presents the results of MD trajectory analysis and local mode frequency calculation, and the simulated linear IR and 2D IR rephasing (R) and nonrephasing (NR) spectra of the unlabeled hexapeptide **1**. The spectra of the $^{13}\text{C}=^{18}\text{O}$ - and ^{15}N -labeled peptides (**1*** and **1****) are simulated using the frequency maps that best reproduced the amide-I and -II bands of **1**. We discuss the performance of the electrostatic models based on their ability to reasonably predict the frequency shifts and reproduce the linear and 2D IR spectra, especially of the amide-II mode. Concluding remarks are given in the section IV.

II. Methods

A. MD simulation. A nanosecond MD trajectory of **1** in CDCl_3 was obtained with the NAMD simulation program package⁶⁷ and the modified AMBER force field (ff99SB) which has been shown to

achieve a better balance of secondary structure elements than other force fields.⁶⁰⁻⁶² Partial charges of the unnatural amino acid residue, Aib, as well as the capping groups (Z and OtBu) were determined by restrained electrostatic potential (RESP) method, following the same method as in the original determination.^{60,68} These values are presented in Figure 2. During the fitting process for the Aib residue, the partial charges of the C, O, N and H atoms that will participate in the peptide linkages with other residues were fixed to $0.5973e$, $-0.5679e$, $-0.4157e$ and $0.2719e$ (e is the elementary charge), the same values as the other natural amino acid residues in the AMBER force field.⁶⁰ The charges of Aib determined in this study are slightly different from those determined by Ramos and co-workers⁶⁹ perhaps because no charge restraints were applied in their study.

A rectangular box ($60 \text{ \AA} \times 49 \text{ \AA} \times 46 \text{ \AA}$) containing one peptide and 845 CDCl_3 molecules was equilibrated for ~ 3.5 ns before a 1.5-ns production run under three-dimensional periodic boundary conditions. Constant temperature and pressure (293 K and 1 atm) of the system were regulated by the Langevin dynamics method with a damping coefficient of 5 ps^{-1} ⁷⁰ and Langevin piston Nosé-Hoover barostat,^{70,71} respectively. Because there is no structure data available for this peptide, we set its initial conformation to the ideal right-handed 3_{10} -helix with $(\phi, \psi) = (-57^\circ, -30^\circ)$, and the positions of the backbone C^α atoms were harmonically restrained during only the equilibration process. Electrostatic interaction was calculated using the particle-mesh Eward sum method.⁷² All bond lengths involving hydrogen atoms were constrained using the SHAKE algorithm.⁷³ The trajectory was calculated with a 2-fs time step, and 15 000 snapshots were sampled at every 0.1 ps along the 1.5-ns trajectory for the analysis of molecular conformations, hydrogen bonding patterns, as well as the site energies of the amide-I/II modes to compute their linear and 2D IR spectra.

B. Electrostatic and semi-empirical models. Five different electrostatic models and one semi-empirical model are compared below.

Bouř et al. reported an electrostatic potential model with six parameterization sites on C, O, N, H, C(−C) and C(−N) for the amide-I, -II, -III and -A modes.⁵² Their three different sets of parameters were based on DFT calculations of NMA-3H₂O clusters at the three different levels of BPW91/6-31G**, BPW91/6-311++G** and B3LYP/6-311++G**, and correlating the vibrational frequencies with the electrostatic potential created by the water molecules. Ten cluster configurations were generated from MD simulation with the AMBER force field and the potential was modeled by atomic partial charges of water (0.42 e for hydrogen and −0.84 e for oxygen). We applied these maps to the local mode frequency calculation and denote them as the model B1 (BPW91/6-31G**), B2 (BPW91/6-311++G**) and B3 (B3LYP/6-311++G**). It should be noted that our MD simulation was performed using the AMBER force field, and hence the calculated local amide mode frequencies are expected to have less systematic errors that may come from different charges used in the MD simulation and the creation of DFT maps.

The amide-I' and -II' frequencies of NMA-d were correlated with electric fields and their gradients at the amide C, O, N, and D atoms by Knoester and co-workers³¹ based on DFT calculation with the ADF TZ2P basis and the RPBE exchange correlation functional. The map coefficients were fitted to 16 components of electric fields and gradients created in 75 different point charge configurations. The frequencies estimated from their electrostatic map had a correlation factor of 0.97 for the amide-I' mode and 0.90 for the amide-II' mode. It is noted that the map was scaled to give the correct gas phase frequencies, namely, 1717 cm^{−1} for the amide-I' and 1440 cm^{−1} for the amide-II' mode. Although the latter is lower by ~60 cm^{−1} from that of NMA due to the exchanged deuterium, we used their map without additional rescaling, and denote it as the model K.

In the previous study,¹³ the four-site potential model parameterized at the amide C, O, N, and H atoms of NMA by Cho and co-workers⁴¹ reasonably reproduced the observed amide-I R and NR spectral patterns of the 3₁₀-helix octapeptide Z-[L-(α Me)Val]₈-OtBu in CDCl₃. This frequency map was constructed for only the amide-I mode based on ab initio calculation of 96 NMA- n D₂O ($n = 1-5$)

complexes at the RHF/6-311++G** level.⁴¹ However, parameters for the amide-II mode were not reported. The need to model the amide-II mode stimulated us to create a four-site potential map on our own to see whether the spectral features of the amide-II mode can be predicted as well as those of the amide-I mode.

Our potential map was created in a similar way to Cho's except that the electrostatic potential was correlated with the frequencies obtained by DFT calculations at the B3LYP/6-31+G* level, and 13 NMA/D₂O complexes with different intermolecular configurations were considered. DFT calculations were carried out using the Gaussian 03 package.⁷⁴ The optimized structures of NMA-*n*D₂O (*n* = 1–5) are presented in Figure 3. First, we performed normal mode analysis of an isolated NMA in vacuum and obtained a scaling factor by fitting the calculated amide-I and -II mode frequencies to the experimental gas phase values ($\omega_0^{I(II)}$) of 1723 cm⁻¹ and 1499 cm⁻¹,³⁸ respectively. This factor was used to scale the amide-I and -II frequencies of the NMA/D₂O complexes, and the shift ($\delta\omega^{I(II)}$) of the scaled frequency ($\omega^{I(II)}$) from $\omega_0^{I(II)}$ were correlated with electrostatic potentials from the surrounding water molecules:

$$\delta\omega^{I(II)} = \omega^{I(II)} - \omega_0^{I(II)} = 116140.8 \sum_i \sum_k l_i^{I(II)} \frac{q_k}{|r_i - r_k|} \quad (1),$$

where *i* represents the four parameterized sites of NMA, that is, the amide C, O, N, and H atoms, and *k* represents the atoms of water molecules. The partial charges $q_D = 0.412e$ and $q_O = -0.824e$ were used to calculate electrostatic potentials created by the atoms at a distance of $|r_i - r_k|$ (in Å) away from the parameterized site. The correlation coefficients $l_i^{I(II)}$ were listed in Table 1, and the sum of $l_i^{I(II)}$ is zero. As shown in Figure 4, the DFT frequency of the amide-I and -II mode is reproduced to within ± 5 cm⁻¹. It is well known that hydrogen bonding at the oxygen and hydrogen atom decreases (increases) the amide-I (II) mode frequency.³⁷⁻⁴⁰ Also, the hydrogen-bond induced amide-I frequency shift is larger

at the carbonyl site than at the N–H site. This trend is opposite in the case of the amide-II mode: A larger shift is caused by hydrogen-bonding at the N–H group than at the carbonyl group. These characteristics are clearly seen in the one-to-one complexes (Figure 3, 1–3). We denote our four-site potential maps as the model G to compare with the other models.

It is worthwhile to apply the semi-empirical model (Em) that was devised in the previous study of the hexapeptides,³⁴ where the major experimental features were reproduced by simply assuming that all residues have the same average dihedral angles $(\phi, \psi) = (-57^\circ, -30^\circ)$ with Gaussian standard deviations of $\sim 9.5^\circ$. In Em, the intramolecular $\text{C}=\text{O}\cdots\text{H}-\text{N}$ hydrogen bonding is considered as the only source from the peptide that induces frequency shifts of the amide-I and -II modes. Hydrogen bond electrostatic energies are calculated at the amide $\text{C}=\text{O}$ and $\text{N}-\text{H}$ sites separately and they are converted to the frequency shifts by the factors of $\kappa_{\text{CO}}^{\text{I}} = 0.42 \text{ cm}^{-1} \text{ mol kJ}^{-1}$, $\kappa_{\text{NH}}^{\text{I}} = 0.96 \text{ cm}^{-1} \text{ mol kJ}^{-1}$, $\kappa_{\text{CO}}^{\text{II}} = -0.70 \text{ cm}^{-1} \text{ mol kJ}^{-1}$, and $\kappa_{\text{NH}}^{\text{II}} = -2.9 \text{ cm}^{-1} \text{ mol kJ}^{-1}$, where $\kappa_{\text{CO}}^{\text{I(II)}}$ and $\kappa_{\text{NH}}^{\text{I(II)}}$ are conversion factors for the amide-I (II) mode when hydrogen bonding occurs at the $\text{C}=\text{O}$ and $\text{N}-\text{H}$ sites, respectively. Solvent contributions are also empirically included by considering the two amide $\text{C}=\text{O}$ groups that are exposed to CDCl_3 at the C-terminus. The amide-I (II) frequency of the last two linkages was shifted by $-10 (+5) \text{ cm}^{-1}$ with a standard deviation of $7.5 (5) \text{ cm}^{-1}$. Because CDCl_3 molecules do not form hydrogen bonds with $\text{N}-\text{H}$ groups, it is reasonable to ignore the effects on the first $\text{N}-\text{H}$ group at the N-terminus.

All of the electrostatic models described above were built on the basis of NMA or NMA-d. The frequency shifts were correlated with electrostatic potentials from the surrounding water molecules or electrostatic field/gradient from external charges, and the two methyl groups of NMA do not contribute to the calculation of electrostatic properties. When these maps are used to calculate the local mode frequencies of a peptide unit in polypeptides, it is necessary to define a “chromophore”, that is, a group of atoms whose potential, field, and gradient are excluded from the calculation of the local mode

frequency shift. Following the same definition used in the previous simulation studies for the amide-I mode,^{13,54,75} we defined the chromophore as the minimum group of atoms that contains the parameterized atoms and maintains charge neutrality within the group. Table 2 depicts the chromophore and parameterized atoms in each model. In the AMBER force field, the partial charges of amino acid residues have been determined so that their total sum give a net charge of zero.⁶⁰ Thus, the chromophore as defined in the six-site models (B1, B2 and B3) requires excluding two complete residues in the frequency shift calculation. For the four-site models, it is possible to define the chromophore in three different ways. The chromophore of models K and G contains two complete residues. In addition to the parameterized atoms, the model KN chromophore includes the C α atom and the side chain groups on the N-terminal side, whereas the model KC chromophore includes those on C-terminal side.

D. Computation of linear and 2D IR spectra. We calculated the linear and 2D IR spectra by a combination of the vibrational exciton model and a sum-over-states approach. Vibrational exciton Hamiltonian for the amide-I and -II modes was built for each snapshot of the MD trajectory with the local mode frequencies as the diagonal elements and the intermode couplings as the off-diagonal elements. The nearest-neighbor couplings between the amide-I/I, -I/II, -II/I and -II/II modes have been mapped out as a function of ϕ and ψ in the DFT calculation for Ac-Gly-NHMe,³⁴ and the corresponding values were read out for the nearest-neighbored local modes in the peptide. The coupling between the amide-I/II modes in the same peptide linkage was set to -33 cm^{-1} ,³⁴ close in magnitude to the experimentally determined values for NMA (27 cm^{-1} in DMSO²⁹ and 29 cm^{-1} in D₂O³⁰) and a proline dipeptide ($29.6\text{--}32\text{ cm}^{-1}$).⁷⁶ All of the other couplings were approximated by the transition charge coupling.³⁴ The two-exciton Hamiltonian was built from the one-exciton Hamiltonian under the harmonic approximation. The diagonal anharmonicities of the amide-I and -II modes were set to 16 and 11 cm^{-1} , respectively.^{30,77} The urethane and ester C=O stretching modes and the urethane vibration similar to the amide-II mode were also included in the simulation as described previously.³⁴ The amide-I

and -II frequencies of the second peptide unit in **1*** and **1**** were decreased by 69 and 8 cm^{-1} , respectively, to account for the isotope effect of $^{13}\text{C}=^{18}\text{O}$ labeling. The amide-II frequency of the forth peptide unit in **1**** was redshifted by 16 cm^{-1} to account for the ^{15}N isotope shift based on the experimentally observed shift for *i*PrCO–Gly–OtBu.³⁴ The transition dipoles of the amide-I and -II local modes and capping groups have been defined in our previous study.³⁴ Because not all existing models provide correlation maps that can be used to predict vibrational anharmonicity and dipole strength from electrostatic properties, we employed only the correlation maps for the local mode frequency in this comparative study.

Diagonalization of the one- and two-exciton Hamiltonian gives the transition frequencies and dipole moments between the exciton states. These properties together with the linewidth and orientational factors are fundamental to determine the spectral pattern of linear and 2D IR spectra. Also, the structural inhomogeneity as sampled by MD snapshots causes additional line broadening due to inhomogeneity of the local mode frequencies and vibrational couplings. It was concluded in the previous simulation study that the former was especially dominant for the amide-I spectra.^{13,54} In the simulation below, the fast and slow line broadening effects were assumed to be separable for both of the amide-I and -II modes. We used a Lorentzian function with a half width at half maximum of 6 cm^{-1} to model the homogeneous lineshape, and sampled 15 000 snapshots from the NPT MD trajectory to take into account the inhomogeneous contributions. The formulas to calculate the linear and 2D IR spectra^{10,11} and the orientational factors for the perpendicular polarization¹⁵ have been already reported.

The calculation protocol described above involves static averages over MD snapshots without extracting dynamics directly from the MD simulations. This approach has been applied by a number of groups to simulate amide-I 2D IR spectra.^{6,13,18,54,78} It is adequate for simulating 2D spectra at a very short waiting time T when the effects of dynamical processes, such as spectral diffusion, vibrational energy transfer, and chemical exchange, are not prominent. Recently, several methods in the time

domain have been developed to take into account these dynamics and are more suitable for simulating waiting time dependence of 2D IR spectra.⁷⁹⁻⁸¹

III. Results and Discussion

A. Analysis of MD trajectory. Molecular conformation of the 1.5-ns MD trajectory was analyzed in terms of dihedral angles and the pattern of C=O $\cdots$ H-N intramolecular hydrogen bonding. Figure 5 plots dihedral angle distribution for each pair of ϕ and ψ . The statistical averages and standard deviations are shown in the bottom panels and also tabled in Table 3. The average angles around the first four residues at N-terminus are very close to those of the ideal 3_{10} -helix ($\phi = -57^\circ$, $\psi = -30^\circ$) and their standard deviations ($\sigma_\phi = 7.3\text{--}8.7^\circ$ and $\sigma_\psi = 8.9\text{--}10.2^\circ$) are very close to the value (9.5°) we used in the previous model calculation.³⁴ On the contrary, the last two residues, Gly and Aib, highly fluctuate around the mean structure, which are deviated from the ideal 3_{10} -helix. In particular, the last residue clearly has some population with $(\phi, \psi) = (45^\circ, -120^\circ)$. In the overall 1.5-ns simulation started with the ideal right-handed 3_{10} -helix as the initial structure, we did not detect any occurrence of the left-handed conformation in any of the residues.

Figure 6 shows the time dependent intramolecular hydrogen bonding pattern of the C=O groups in the urethane and the first three peptide linkages at the N-terminus. We used the Kabsch-Sander electrostatic energy criterion of -0.5 kcal/mol to define the existence of a hydrogen bond between the C=O and N-H groups.⁸² In agreement with the results of dihedral angle analysis above, the four C=O groups mainly form 3_{10} -helical intramolecular hydrogen bonds between i and $(i+2)$ linkages with a tiny fraction of $3_{10}/\alpha$ bifurcated pattern. The C=O group in the second linkage, which is labeled with $^{13}\text{C}=^{18}\text{O}$ in **1*** and **1****, is hydrogen-bonded with the N-H group in the forth linkage, which is labeled with ^{15}N in **1****, most of the time ($> 98\%$). The floppier residues at the C-terminus are probably the main reason that the C=O group in the third linkage has a slightly higher percentage of being free

from hydrogen bonding. Note that there is no α -helical hydrogen-bond donor to this C=O group and hence the α -helical or $3_{10}/\alpha$ bifurcated hydrogen bonding patterns are zero at all time.

The results shown in Figures 5 and 6 indicate that the almost ideal 3_{10} -helix is the dominant conformation that the Aib-rich hexapeptide takes in CDCl_3 . This conclusion drawn from the MD simulation study corroborates our structural determination that the peptide **1** forms a 3_{10} -helix in CDCl_3 as concluded from the characteristic 3_{10} -helix doublet cross-peak pattern in the amide-I frequency region.¹² We should emphasize that the simulation was implemented without any restraints imposed on the backbone conformation or side chain groups. In the previous comparative study for $\text{Z}[(\alpha\text{Me})\text{Val}]_8\text{-OtBu}$ in CDCl_3 , the unrestrained MD simulation using CHARMM force field predicted a heterogeneous helical structure ensemble that was too broad to reproduce the experimental 2D IR spectral patterns.¹³ Another example was shown by Kuczera and co-workers that the 3_{10} -helix of $(\text{Aib})_{10}$ peptide was significantly less stable than α - or π -helix in solutions when the CHARMM22 force field was applied.⁶³ However, protected $(\text{Aib})_{10}$ peptides form a regular 3_{10} -helical conformation in the crystal state and in weakly polar solvents like CDCl_3 and DMSO.^{11,83,84} Our result suggests that the AMBER ff99SB force field as adapted here is capable of simulating the helical conformations of Aib peptides better than the CHARMM22 force field with the previous adaptation.^{13,63}

B. Comparisons of local mode frequencies from different electrostatic models. Before drawing detailed comparisons of linear and 2D IR spectra simulated with the different models, it is insightful to check whether there are notable differences in the calculated amide-I and -II local mode frequencies. Figure 7 shows the amide-I and -II local mode frequency shifts obtained from the eight models (B1, B2, B3, K, KN, KC, G, and Em). They are plotted in different scales to clearly reveal the variations in each model. The total frequency shift is separated into the contributions from the solvent and from the peptide atoms outside the chromophore. The mean values and standard deviations are also summarized in Table 2. The calculated frequencies exhibit a significant dependence on models, namely,

the electrostatic properties (potential or field/gradient), the number of parameterization atom, procedure, basis set and functional used in the DFT calculation, and the definition of chromophore.

First, comparisons among the models B1, B2 and B3 are made. For the amide-I mode, the three models show that the solvent shift (open circles) is -6 to -19 cm^{-1} and varies little over the residues. Interestingly, positive peptide shifts (open squares) are obtained for all amide-I modes in the model B1, in contrast to the predictions in B2 and B3. This is counter-intuitive, especially for the two amide-I modes at the second and third peptide linkages, because conformational analysis has shown that these two linkages are involved in intramolecular 3_{10} -helical hydrogen bonding at both the C=O and N-H sites and hydrogen bonding is expected to cause a red shift of the amide-I mode frequency.³⁷⁻⁴⁰ To discuss further this discrepancy, we analyzed the peptide contribution to the frequency shifts by separating it into three components based on their origins: the frequency shifts due to (i) the atoms involved in the intramolecular hydrogen bond (IHB) at the C=O and (ii) N-H sites, and (iii) the other atoms on the side chains and backbone. The average values of these three components and their standard deviations are listed in Table 4. This separation was achieved by turning off contributions from the non-involved atoms. For example, the frequency shift coming from hydrogen bonding at the carbonyl site was evaluated by setting zero charges to all atoms except for the N and H atoms that are involved in forming the 3_{10} -helical hydrogen bond. This analysis shows that the shifts due to IHB at the N-H site is positive in B1, and so is that at the C=O site in B2. Such a behavior contradicts the previous results that the amide-I frequency decreases when forming hydrogen bonds at the carbonyl and amide sites.^{37,39,40} Although red shifts at the C=O and N-H sites are correctly predicted in B3, the magnitude of the former is much smaller than that of the latter, inconsistent with the calculation results.^{37,39,40} Overall, the positive peptide shifts compensate the negative solvent shifts, and the total frequency shifts (solid circles) obtained in the model B1 range from -11 to 1 cm^{-1} with standard deviations of 9 – 11 cm^{-1} . For

the models B2 and B3, the total shifts of the amide-I mode are -8 to -20 cm^{-1} and -15 to -27 cm^{-1} , respectively, and the fluctuations of 5 – 8 cm^{-1} are smaller than those in B1.

Figure 7 shows that the total shifts of the amide-II modes in all models are dominated by the peptide shifts. It should be noted that in B1–B3, the solvent contribution leads to redshift of the amide-II mode frequency, contradictory to the blueshift observed for amide compounds in chloroform.⁸⁵ Comparing to B1, the frequency shifts calculated with the models B2 and B3 exhibit much larger peptide contributions (more than 50 cm^{-1}) and larger deviations (Table 2). The shifts due to the IHB partners and side chain atoms are very large in B2 and B3 (Table 4). The blueshift due to hydrogen bond formation is compensated by the redshift originating from other atoms, and the total peptide shifts become 30 – 99 cm^{-1} in B1–B3. For the amide-II mode, previous calculation results predicted that hydrogen bonding at the N–H site causes a much larger blueshift than at the C=O site.^{39,40} Of the three Bouř maps, only the results from B1 agree with this prediction.

For the model K, the general trend for the variation of the total amide-I and -II frequency shifts across the peptide is similar to that of B3, but the magnitudes of the amide-II frequency shifts are much smaller (Figure 7). The hydrogen bonding effects at the C=O and N–H sites show the correct trend in both modes, in contrast to B3. However, the amide-I shifts of -6 to -9 cm^{-1} at the C=O site and -2 to -6 cm^{-1} at the N–H site are relatively small compared to the calculated values of about -20 cm^{-1} and about -10 cm^{-1} .^{37,39} The amide-II shifts due to the backbone and side chains are larger than the hydrogen bond shifts of 2 – 3 cm^{-1} in this field/gradient model.

As mentioned in Methods, we tested two additional definitions of chromophore for this model (KN and KC). The solvent frequency shifts are not affected by the definition of chromophore. On the other hand, the peptide and total shifts in KN and KC are quite different from those in K (Figure 7 and Table 2). For example, some amide-I local modes show positive shifts, and negative shifts are seen for all of the amide-II modes. The acute chromophore dependence indicates that the electric fields and their

gradients created by the atoms neighbored to peptide linkages significantly affect the local mode frequencies. The trend of total frequency shifts observed for KN and KC, implies that the excitonic amide-I and -II bands in CDCl_3 would show blue- and red-shift, respectively, from the peak frequency observed in the gas phase. This is totally opposite to what we observed in the FT IR spectrum. Therefore, we hereafter do not further consider models KN and KC in the calculation of linear and 2D IR spectra and comparisons with the other models.

The pattern of amide-I total frequency shifts calculated using the model G is close to that of K but with a smaller magnitude, about -11 to -18 cm^{-1} . Also, the trend of hydrogen bonding effects is correctly predicted in this model except for the relatively smaller magnitudes than the reported theoretical values, just as in the model K. The analysis for the amide-I mode suggests that the frequency correlation with the electrostatic properties is better parameterized at the four atom sites as in the models K and G, rather than at the six sites in the models B1–B3, in terms of reproducing the proper hydrogen bonding effects. Although the magnitude of the total amide-II frequency shift in model G is similar to that obtained with B1, particulars of the peptide shift are quite different. The solvent shifts of the amide-II mode in this model are positive, contrary to B1–B3 but consistent with the trend found in the experiments.⁸⁵ It is noted that the total amide-II local frequency of the first linkage is always lower than the other linkages in the electrostatic potential-based models (B1, B2, B3 and G). This tendency is not seen in the field/gradient-based model K.

Finally, let us inspect the shifts obtained with our semi-empirical model. In spite of its simplicity, the calculated shifts resemble some of the electrostatic models. The amide-I modes have negative shifts of -20 to -31 cm^{-1} and their pattern is similar to that of B3, K, and G. Comparing to B1 and G, the amide-II total frequency shifts are slightly smaller in magnitude by about 10 cm^{-1} (except for the first residue). The total frequency shifts obtained for the 1.5-ns MD trajectory are very similar to those calculated in the model calculation for the ideal 3_{10} -helix in the previous work,³⁴ most likely

because the molecular conformation of **1** acquired from the MD simulation is quite close to the ideal structure we assumed.

C. Comparisons of linear and 2D IR spectra of the unlabeled peptide. Figure 8 shows linear IR spectra of **1** measured³⁴ (black) and simulated using the six models (red). The unlabeled hexapeptide exhibits the urethane/ester C=O band at 1716 cm⁻¹ and the unlabeled amide-I band at 1666 cm⁻¹. Roughly speaking, the two bands at 1528 cm⁻¹ and 1498 cm⁻¹ can be attributed to the vibrational exciton bands of the intramolecularly hydrogen-bonded amide-II modes and the free amide-II mode, respectively, with the latter being overlapped with the urethane-II mode.^{12,34} For comparison, the frequency origin of the amide-I local mode was shifted from gas phase value by the values reported in each panel of Figure 8 so that the peak of the simulated amide-I exciton band overlaps with the experimental peak. The origin shifts greatly depend on the model: -60.0 cm⁻¹ (B1); -50.5 cm⁻¹ (B2); -43.0 cm⁻¹ (B3); -33.5 cm⁻¹ (K); -49.0 cm⁻¹ (G); and -38.2 cm⁻¹ (Em). Overall, the observed amide-I band was reproduced quite well, especially by B1 and K. The other models predict a slightly narrower (by ~ 6 cm⁻¹) band and hence the relative peak intensity ratio between the amide-I and the capping C=O bands varies. However, considering that B1 predicts a positive amide-I frequency shift upon forming a C=O...H-N intramolecular hydrogen bond and requires the largest shift of the origin, it is not appropriate to advocate that this model can simulate the amide-I spectrum well.

For the free and hydrogen-bonded amide-II bands, none of the models simulated the observed lineshape to the same level as the amide-I mode. The models G and Em generate an intensity ratio close to that of the measurement, but the linewidths are slightly narrower and peaks are not as well resolved. The lower frequency band in the B1 simulated spectrum is quite small. The higher frequency band in B2 and B3 is very broad and is located at 1553 and 1559 cm⁻¹, higher by about 25 and 31 cm⁻¹, respectively, from the observed band at 1528 cm⁻¹. The model K exhibits one asymmetric peak at 1515 cm⁻¹ with quite strong peak intensity.

Bouř and co-workers found from their simulated linear spectra of NMA in water that a bigger basis set is required to obtain reasonable agreement between the experimental and simulated amide-I frequency and linewidth.⁵² They also found that the application of the B3LYP functional resulted in improvement of the amide-II frequency whereas the amide-I frequency shifts away from the experimental value.⁵² These results reflect only the solvent contributions, and hence the basis set and functional dependence may be different when the backbone and side chain atoms are taken into account in the calculation of polypeptides. The linear amide-I band (Figure 8) of **1** simulated with B1, has a similar bandwidth to the experimental values. However, as mentioned above, this model with the lowest basis set (6-31G**) is unable to predict the redshift of the local amide-I mode due to the intramolecular hydrogen bonding and a quite large shift of the frequency origin (-60.0 cm^{-1}) is required. The calculated spectra with B2 and B3 have almost the same linewidth, and a slightly smaller frequency shift is needed for B3 which uses the B3LYP functional. This tendency of peak shift is different from that concluded for the amide-I band of NMA in water.⁵²

A clear dependence on the basis set is noticeable for the simulated amide-II band. Comparing the results between B1 and B2, the higher basis set (B2) leads to a broader high-frequency amide-II band with its frequency overestimated by $\sim 25\text{ cm}^{-1}$ from the experimental value. In B1, the high-frequency amide-II band is narrower than the measurement. The low-frequency peak position is relatively well reproduced to within a few cm^{-1} . On contrary, B2 and B3 use the same basis sets with different functional. They give similar features for the amide-II band. Compared to the models B1-B3, the 6-31+G* basis set used in the model G is relative small and the number of parameterized atoms is fewer. The amide-II band shape obtained from the model G is in reasonable agreement with the experiment. Interestingly, the lower basis set may be capable of reproducing the experimental results better. Keiderling and co-workers reported that a relatively small basis set [6-31G(d)] with the BPW91 functional was able to predict the amide-I and -II frequencies for the isolated and solvated NMA.³⁸ The

dependence which Bouř et al. found in the calculation of NMA spectrum⁵² was not reproduced once the DFT frequency maps were applied to the peptide linkages connected with one another through covalent bonds. These different trends most likely originate from contributions of peptide and backbone atoms.

The model K predicted the amide-I frequency and the bandwidth of linear spectrum better than any other models. However, the high-frequency amide-II band is too narrow and the separation between the high- and low-frequency bands is so small that the overall band shape looks like a single peak with a marginal shoulder. Knoester and coworkers³¹ simulated the linear IR spectrum of NMA-d in D₂O based on their frequency-field/gradient correlation, and found that the calculated solvent shift of the amide-II mode (+21 cm⁻¹) is less than a half of the experimental shift (+53 cm⁻¹). Another correlation based on electric field/gradient and second derivatives by Hayashi and Mukamel⁴⁵ also underestimated the solvent frequency shift of the amide-II band of undeuterated NMA, which was ascribed to insufficient electrostatic sampling. They also reported a similarly narrow amide-II band in the linear IR spectrum computed for a 17 residue helical peptide.⁷⁸ The amide-II mode frequency appears to be less well described by the field and/or gradient model.

In the model G, the lineshape of the amide-I band is almost the same as that simulated in B2 and B3. On the other hand, the amide-II band is not as broad as seen for the spectra of B2 and B3, rather close to that acquired in B1, and the intensity ratio between the high- and low-frequency bands is slightly better.

The semi-empirical model Em could reproduce the measurement relatively well. The amide-I band is very similar to that in B2, B3 and G, and the two amide-II bands have a narrower line shape than the experimental result. In this model, the contributions of backbone and side chain atoms are completely ignored. The intramolecular C=O $\cdots$ H-N hydrogen bond and empirical solvent contributions are the sole sources that affect the site-energy of the amide-I and -II local modes. The total amide-II mode frequency in this model has the trend observed in the potential models, suggesting that the lower

frequency in the first linkage can be attributed to the lack of intramolecular hydrogen bonding at the N–H group. It appears that the local amide-I and -II frequencies can be relatively well modeled by considering only a small number of atoms in the vicinity of peptide linkages. Kubelka and Keiderling³⁸ and Besley⁴⁰ reproduced the amide-I, -II and -III frequencies of NMA quite well by considering three explicit water molecules, two hydrogen-bonded at the carbonyl oxygen atom and one at the amide hydrogen atom, with continuum solvent model. Although the peptide linkage of NMA is fully solvated in water, the situation is different for the linkages connected with others in polypeptide chains to form a certain secondary structure. In the sample we studied, the hydrogen-bonded peptide units are surrounded by the methyl groups attached on the C $^{\alpha}$ carbons, and weakly polar chloroform molecules. These atom groups do not form strong hydrogen bonds with the C=O and N–H groups, and hence the 3_{10} -helical intramolecular hydrogen bonds would play a major role in affecting the local frequencies of the amide modes.

Figure 9 presents the measured³⁴ and simulated absolute magnitude 2D IR R and NR spectra of **1** in the full spectral region. The measured R spectrum exhibits a strong unlabeled amide-I peak, a urethane/ester C=O peak and the amide-II peaks on the diagonal, corresponding to the prominent features in its linear spectrum. The hydrogen-bonded amide-II band at 1530–1550 cm⁻¹ is composed of several coupled peaks. The free amide-II band is a weak shoulder to the red side of the hydrogen-bonded amide-II band and cannot be clearly resolved. There are weak cross-peaks between the amide-I and hydrogen-bonded amide-II modes at $\omega_t = 1525$ and 1548 cm⁻¹ along $\omega_r = -1673$ cm⁻¹, but the cross-peaks between the amide-I and free amide-II modes are not discernable.

On the contrary, the NR spectrum manifests better resolved diagonal peaks and more distinct cross-peaks than the R spectrum. The NR sequence has higher resolving power due to destructive interference effects.³² The clear two cross-peaks between the amide-I and -II modes along $\omega_r = 1662$ cm⁻¹ implicate that the hydrogen-bonded amide-II band has two components, and each of them is

coupled with the unlabeled amide-I modes. The free amide-II mode diagonal peak now clearly appears, and its cross-peaks to the hydrogen-bonded amide-II modes and amide-I modes are also much more clearly resolved.

For the simulated R and NR spectra, all models produced a diagonal amide-I band that resembles the experimental results, and thus it is difficult to use this band as the criterion to compare different models. This behavior is consistent with the results from our previous study of a 3_{10} -helical octapeptide: The linear spectra and parallel polarization 2D spectra are much less sensitive to the different electrostatic models than the amide-I 2D cross-peak patterns obtained by removing the diagonal peaks with the double-crossed polarization configuration.¹³ Some cross-peaks between the amide-I and -II modes clearly show up in the simulated spectra although those originate from the shoulder of the amide-II band, which shows up in the off-diagonal region $(\omega_i, \omega_f) = (1661, 1549) \text{ cm}^{-1}$ of the measured NR spectrum, are not reproduced.

To investigate the amide-II band more clearly, the measured^{12,34} and simulated spectra with the IR pulses centered at 1550 cm^{-1} are plotted in Figure 10. The spectra are normalized with the maximum intensity of the strongest diagonal amide-II band. Although the higher frequency shoulder is missing in all of the model simulations, qualitative reproduction of the experimental results is achieved by B1, G, and Em. As deduced from the simulated linear spectra, the diagonal amide-II band in B2 and B3 is highly broadened along the diagonal direction, indicating that the inhomogeneity of the local amide-II mode is quite large in these models. The model K generates a narrower diagonal band with a clear cross-peak between the lower and higher frequency band. Comparing to the amide-I mode, simulation of the amide-II mode exhibits larger spectral variations between different models.

D. Simulated linear and 2D IR spectra of 1^* and 1^{} .** For further simulation of the isotope labeled peptides 1^* and 1^{**} , we have chosen the model K and G, which relatively well reproduced the amide-I and -II profiles of **1**, respectively. Figure 11 shows the measured^{12,34} and simulated linear IR

spectra after normalization with the band intensity of the urethane/ester C=O band at $\sim 1716\text{ cm}^{-1}$, a common internal reference band for the spectra of **1**, **1*** and **1****, as well as the difference spectra of **1*** – **1**, **1**** – **1**, and **1**** – **1***. A new band at 1598 cm^{-1} is due to the $^{13}\text{C}=^{18}\text{O}$ labeled amide-I band in the second linkage. Again, the simulated labeled and unlabeled amide-I band is slightly narrower than the measured one. The effects of $^{13}\text{C}=^{18}\text{O}$ and ^{15}N on the amide-II mode are clearly observed in the difference spectra. The complicated difference profiles were only partially replicated. In the difference spectrum between **1*** and **1**, the positive and negative couplet are more separated in frequency. The increased absorbance at $\sim 1518\text{ cm}^{-1}$ by the ^{15}N label was reproduced in the simulated difference spectra **1**** – **1**, and **1**** – **1***. The increase at 1495 cm^{-1} is overestimated and the decreased absorbance between 1530 and 1550 cm^{-1} is different from the experimental results. These discrepancies require further refinement.

Figures 12 and 13 show the measured³⁴ and simulated absolute magnitude 2D IR R and NR spectra, respectively, with the IR pulses centered at 1600 cm^{-1} . The spectra at 1550 cm^{-1} including the $^{13}\text{C}=^{18}\text{O}$ labeled amide-I and amide-II bands are plotted in Figures 14 and 15. The top panels in Figure 15 show the sliced NR spectra at $\omega_r = 1598\text{ cm}^{-1}$. In the previous studies,^{12,34} it has been demonstrated that a combination of the $^{13}\text{C}=^{18}\text{O}$ label on the amide-I mode and the ^{15}N label on the amide-II mode is able to select out a specific amide-I/II cross-peak from among the others, as featured the cross-peak of **1**** at $\omega_t \sim 1520\text{ cm}^{-1}$ that is red-shifted by about 9 cm^{-1} from the cross-peak of **1*** (Figure 15, left-top panel). The simulation in this study resulted in a smaller red shift (5 cm^{-1}). The cross-peak intensity between the $^{13}\text{C}=^{18}\text{O}$ amide-I and hydrogen-bonded amide-II mode for the mono- and bis-labeled peptides is not as strong as the measured one.

The disagreement with the experimental results suggests that improvements on the simulation of amide 2D IR spectra are needed. For example, it is still an open question how the vibrational coupling strength between the amide-I and -II modes is affected by the intramolecular C=O $\cdots$ H–N hydrogen

bond connecting them in a helical structure, and it might be quite different from the transition charge coupling assumed in our simulation. The amide-I/II nearest neighbor coupling estimated by us³⁴ and other groups^{33,53} need to be experimentally verified for various peptide conformations. The spatial extent and conformational dependence of the amide-II/II couplings also require further experimental determination.^{33,34,36} The electrostatic model calculations in this study chose the chromophore definitions that maintain charge neutrality. A recent study employed another definition that removes the atoms which are away by three covalent bonds from the parameterized sites.⁴⁷ Further systematic study is required to clarify the effects of chromophore definition.

IV. Concluding Remarks

Our comparative study of different electrostatic and semi-empirical models has revealed several intriguing points. The simulated amide-I bands obtained from all models are very similar in their linewidths and closely resemble the experimental data, except that the frequency red shifts required to overlap with the experiment are quite different between the models. However, the six-site models may be less suitable for modeling the amide-I and -II local mode frequencies in peptides and proteins because it cannot properly describe the hydrogen bonding effects on the frequencies. In contrast to the model insensitivity observed in the amide-I mode, the six models led to drastically different amide-II bands in the linear and 2D IR spectra. On the basis of the five electrostatic models studied here, the average and fluctuations of the amide-II local site-energy appear to be better computed when it is correlated with the electrostatic potential, rather than the electric field and gradient, created by the atoms surrounding the peptide linkages. Also, a larger basis set does not necessarily generate a more accurate DFT frequency map, at least, for the amide-II mode. The model G based on a relatively small basis set performs reasonably well. Despite the simplicity of our semi-empirical model, which takes into account only the intramolecular hydrogen bonding effects and empirical solvent shifts, it is able to reproduce many of the

amide-I/II spectral features. This indicates that hydrogen bonding effects in the vicinity of the peptide unit need to be carefully modeled in order to accurately estimate the site-energy of the amide-I and amide-II modes. Further refinements of the modeling are needed to obtain more quantitative agreement and better estimation of the isotope effects on the amide-II spectral profiles. Given that vibrational couplings and correlations among different amide modes contain a wealth of information on peptide structure and dynamics, improvements in theoretical modeling and comprehensive comparisons between experimental and simulated amide 2D IR spectra will be essential to successful extraction of such information.

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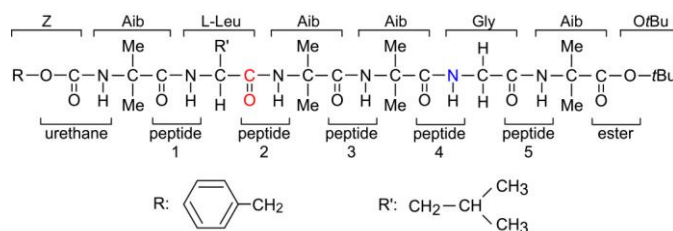


Figure 1. Structural formula of Z-Aib-L-Leu-(Aib)₂-Gly-Aib-OBu (**1**). The carbonyl carbon and oxygen atoms shown in red in the second peptide unit are labeled with ¹³C=¹⁸O in the mono-labeled peptide (**1***). The nitrogen atom shown in blue in the fourth peptide unit is labeled with ¹⁵N in the bis-labeled peptide (**1****) in addition to the ¹³C=¹⁸O label in the second unit.

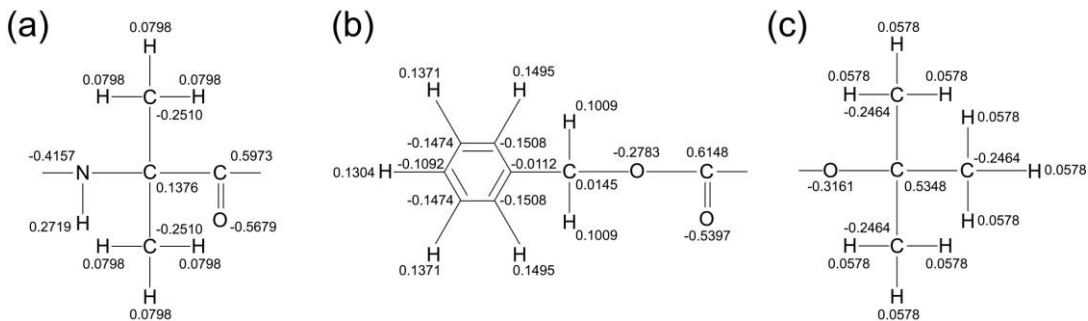


Figure 2. Partial charges of the unnatural amino acid residue (a) Aib ($C^{\alpha,\alpha}$ -dimethylglycine) and protecting groups (b) Z (benzyloxycarbonyl) and (c) OtBu (*tert*-butoxy) determined by RESP method. Bond lengths in these schematic pictures are not in scale.

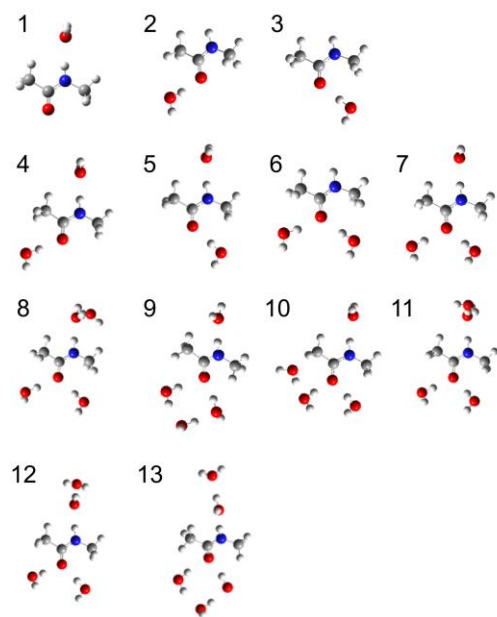


Figure 3. Optimized structures of *N*-methylacetamide/*n*-D₂O complexes (*n* = 1–5) obtained from DFT calculations at the B3LYP/6-31+G* level.

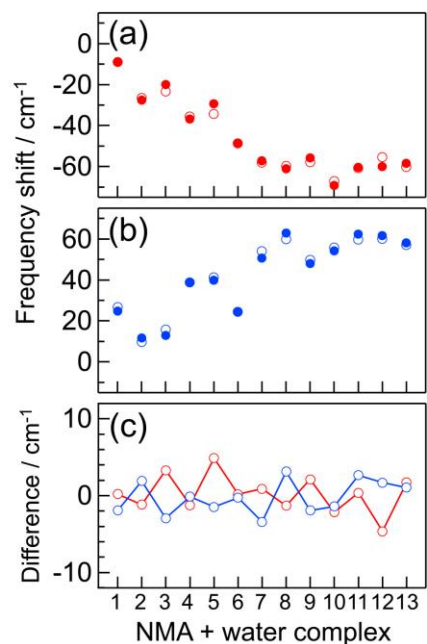


Figure 4. The amide-I (a) and amide-II (b) mode frequency shift of *N*-methylacetamide solvated with 1–5 D₂O molecules: (filled circles) DFT calculation results at the B3LYP/6-31+G(d) level; (open circles) frequency shifts calculated based on eqn (1) of the four-site electrostatic potential model G. The differences between the DFT and the four-site potential model calculation of the amide-I (red) and amide-II (blue) mode are present in (c). The numbering of the NMA/D₂O complexes corresponds to the optimized configurations shown in Figure 3.

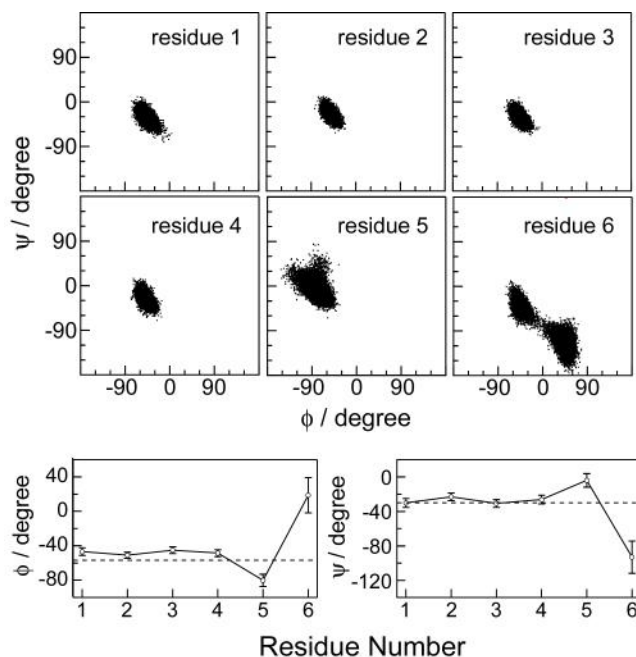


Figure 5. Dihedral angles (ϕ , ψ) of **1** in CDCl_3 extracted from the 1.5-ns MD trajectory. The bottom panels show the average and standard deviation of ϕ (left) and ψ (right) at each residue. The angles of the ideal 3_{10} -helix structure, $\phi = -57^\circ$ and $\psi = -30^\circ$, are indicated by the dashed lines.

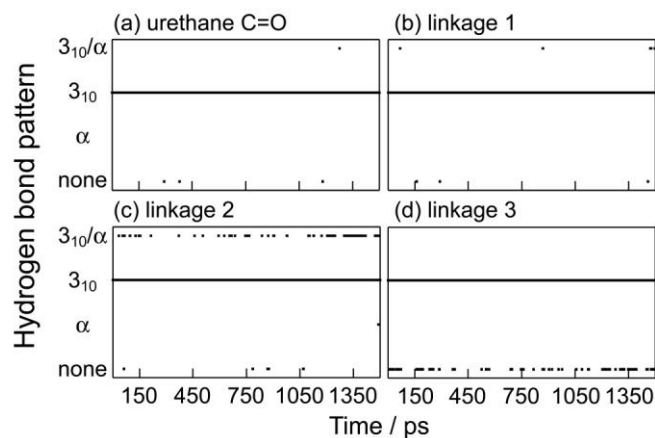


Figure 6. Analysis of the intramolecular C=O $\cdots$ H–N hydrogen bonding pattern along the 1.5-ns MD trajectory of **1** in CDCl₃: (a) the urethane C=O; (b) the C=O in the first peptide linkage; (c) in the second linkage; (d) in the third linkage. The hydrogen bonding pattern is classified into 3₁₀ (*i* and *i*+3 residues), α (*i* and *i*+4 residues) 3₁₀/ α (bifurcated) and no bonding based on the Kabsch-Sander hydrogen bond energy criterion of –0.5 kcal/mol.⁸²

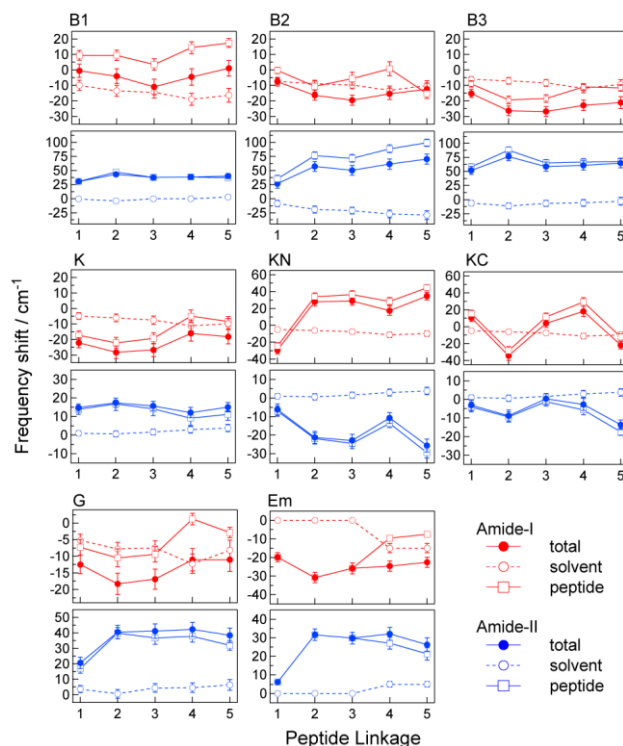


Figure 7. The average amide-I (red) and amide-II (blue) local mode frequency shifts calculated with the different models for the 1.5-ns MD trajectory of **1** in CDCl₃: frequency shift contribution from CDCl₃ molecules (open circles); frequency shift contribution from the peptide backbone and side chain atoms outside the chromophore (squares); the total frequency shifts (filled circles). The standard deviations of each component are indicated by the vertical bars. See Table 2 and text for the details of each model.

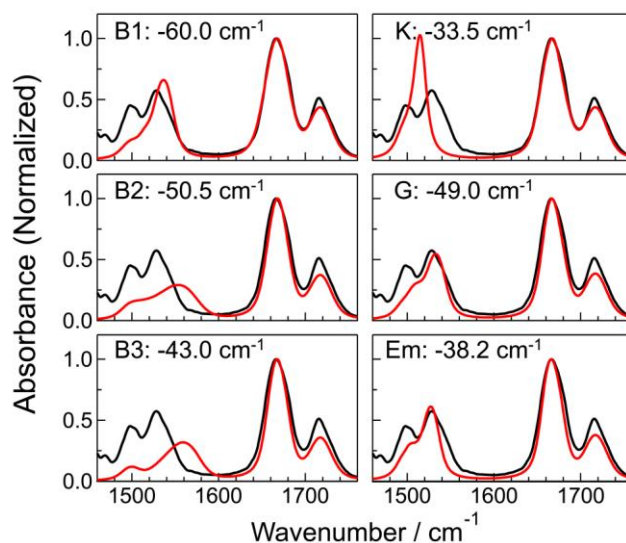


Figure 8. Experimental³⁴ (black) and simulated (red) linear IR spectra of the unlabeled peptide **1** in CDCl₃. Each spectrum is normalized by the peak intensity of the amide-I band at $\sim 1660\text{ cm}^{-1}$. The local amide-I frequency was shifted from the gas phase value by the value reported in each panel to make the peak position of the amide-I exciton band coincide with that of the experimental spectrum.

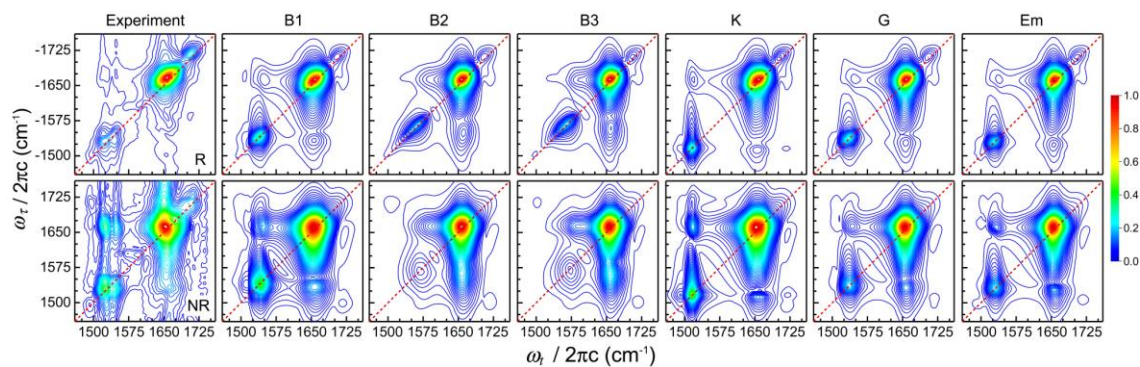


Figure 9. Experimental³⁴ and simulated absolute magnitude 2D IR spectra of **1** obtained under the perpendicular polarization configuration: R (top); NR (bottom). Each spectrum is normalized by the amide-I peak intensity. The experimental and simulated spectra are plotted with 40 and 80 equally spaced contour lines, respectively.

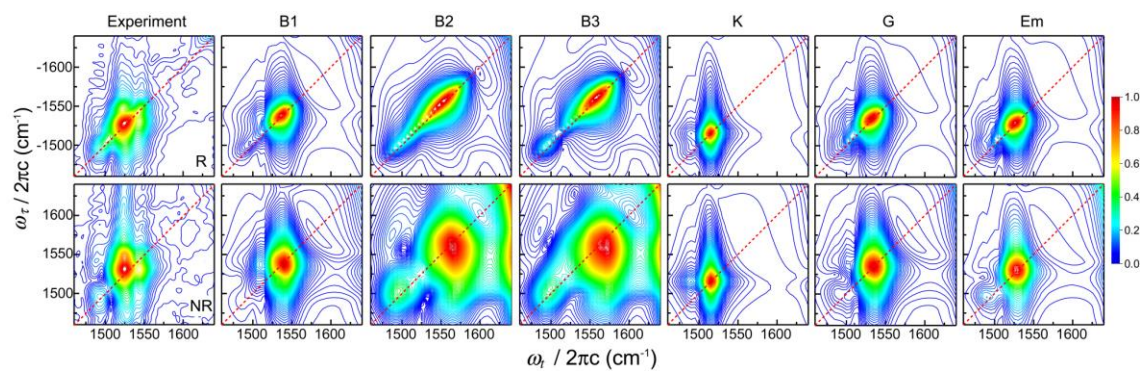


Figure 10. Experimental^{12,34} and simulated absolute magnitude 2D IR spectra of **1** in the amide-II frequency region: R (top); NR (bottom). Each spectrum is normalized by the peak intensity of the diagonal amide-II band and plotted with 40 equally spaced contour lines.

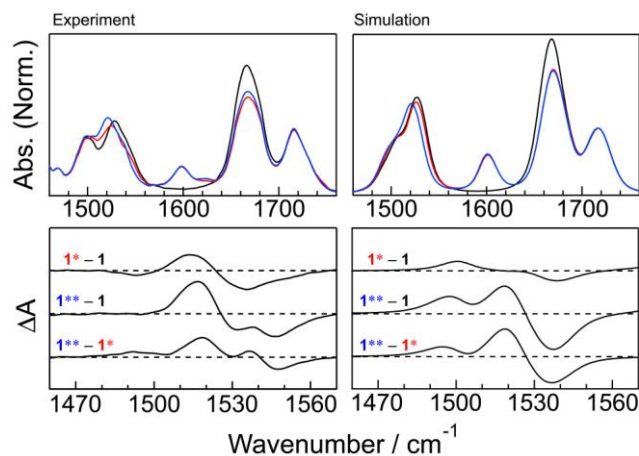


Figure 11. Experimental³⁴ (top left) and simulated (top right) linear IR spectra of **1** (black), **1*** (red) and **1**** (blue) in CDCl₃. Each spectra is normalized by the peak intensity of the urethane/ester C=O band at ~1716 cm⁻¹ after subtracting the background spectrum of neat CDCl₃. Experimental^{12,34} (bottom left) and simulated (bottom right) difference spectra between **1*** and **1**, **1**** and **1**, and **1**** and **1***.

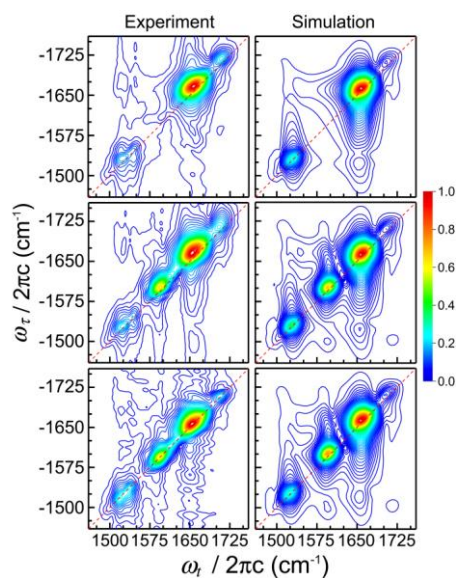


Figure 12. Experimental³⁴ (left) and simulated (right) absolute magnitude 2D IR R spectra obtained under the perpendicular polarization configuration: **1** (top); **1*** (middle); **1**** (bottom). The simulation was carried out using the models K and G for the amide-I and II mode, respectively. Each spectrum is normalized by the maximum intensity of the unlabeled amide-I peak. The experimental and simulated spectra are plotted with 40 and 80 equally spaced contour lines, respectively.

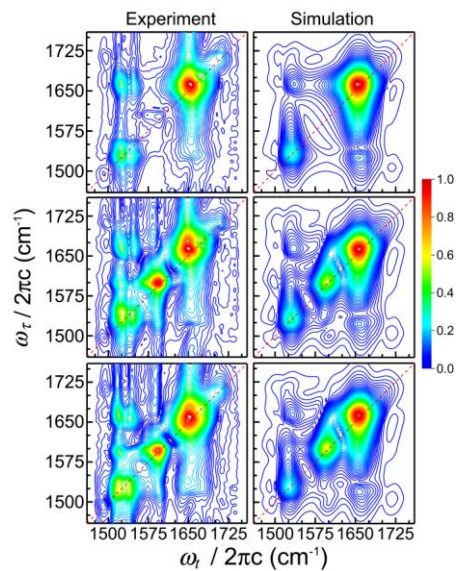


Figure 13. Experimental³⁴ (left) and simulated (right) absolute magnitude 2D IR NR spectra (right): **1** (top); **1*** (middle); **1**** (bottom). See the caption of Figure 12 for other details.

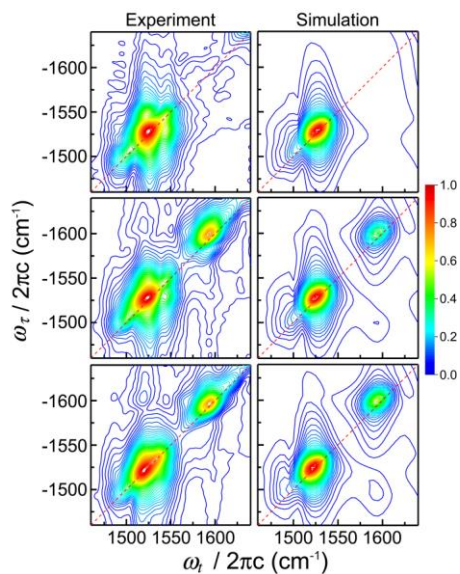


Figure 14. Experimental^{12,34} (left) and simulated (right) absolute magnitude 2D IR R spectra in the amide-II frequency region: **1** (top); **1*** (middle); **1**** (bottom). Each spectrum is normalized by the maximum intensity of the diagonal amide-II band and plotted with 40 equally spaced contour lines.

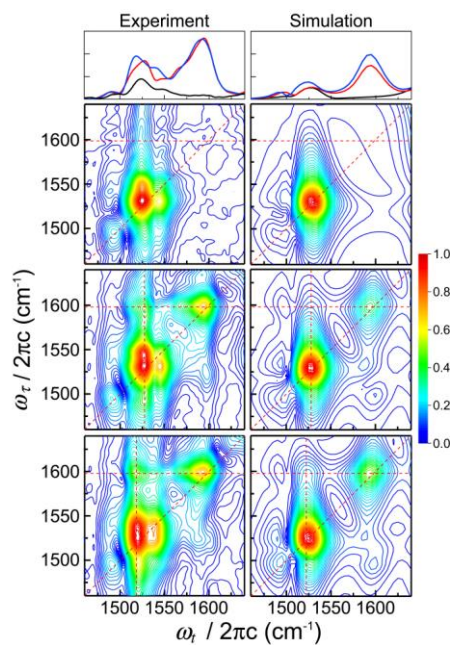
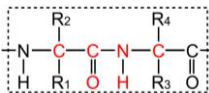
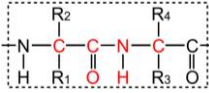
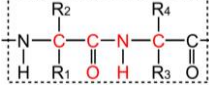
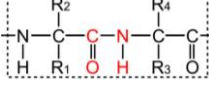
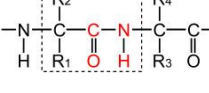
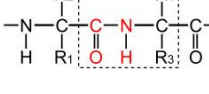
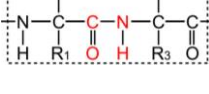


Figure 15. Experimental^{12,34} (left) and simulated (right) absolute magnitude 2D IR NR spectra in the amide-II frequency region: **1** (top); **1*** (middle); **1**** (bottom). Each spectrum is normalized by the maximum intensity of the diagonal amide-II band and plotted with 40 equally spaced contour lines. Slices of the 2D spectra at $\omega_\tau = 1598 \text{ cm}^{-1}$ for **1** (black), **1*** (red), and **1**** (blue) are plotted in the top panels.

TABLE 1: Correlation Parameters $l_i^{I(II)}$ (as a fraction of e) between the Electrostatic Potential at the Four Amide Atom Sites (C, O, N, and H) and the Amide-I and Amide-II Frequency Shifts, as Determined in the Model G

parameterized site	Amide-I	Amide-II
C	0.01179	0.01270
O	−0.00756	−0.00246
N	−0.00549	−0.00876
H	0.00126	−0.00148

TABLE 2: Description of the Models, Definition of Chromophore and Parameterized Atoms, and Solvent, Peptide and Total Frequency Shift of the Amide-I and -II Local Modes

model	chromophore and parameterized atoms ^a	ref	amide-I frequency shift ^b					amide-II frequency shift ^b					
			linkage	1	2	3	4	5	1	2	3	4	5
B1 ^c potential 6-site		52	solvent	-9.9 (6.4)	-13.5 (7.1)	-14.4 (7.1)	-18.9 (7.9)	-16.3 (9.0)	-0.4 (4.6)	-4.0 (5.6)	-0.2 (5.6)	-0.1 (6.6)	3.1 (7.2)
			peptide	9.4 (6.3)	9.4 (6.8)	3.5 (7.5)	14.4 (7.8)	17.4 (5.8)	30.7 (5.6)	47.2 (6.2)	37.9 (6.6)	38.6 (5.7)	37.1 (6.2)
			total	-0.5 (8.6)	-4.0 (9.6)	-10.9 (10.0)	-4.5 (10.6)	1.1 (10.0)	30.3 (6.8)	43.2 (7.5)	37.7 (8.1)	38.5 (8.3)	40.2 (9.0)
			solvent	-7.3 (4.2)	-8.9 (4.6)	-9.8 (4.7)	-13.2 (5.6)	-10.1 (6.2)	-8.6 (10.8)	-19.2 (13.0)	-21.2 (12.3)	-27.1 (13.5)	-29.1 (15.1)
B2 ^d potential 6-site		52	peptide	-0.2 (4.1)	-10.6 (7.4)	-5.6 (8.2)	0.7 (9.0)	-15.3 (6.2)	35.3 (12.3)	76.6 (12.9)	71.6 (13.5)	88.5 (13.7)	99.4 (12.8)
			total	-7.6 (5.6)	-16.3 (6.5)	-19.5 (6.6)	-15.4 (7.3)	-12.5 (7.7)	26.7 (15.9)	57.4 (17.2)	50.4 (17.7)	61.5 (18.1)	70.2 (18.0)
			solvent	-6.1 (3.7)	-7.0 (4.2)	-8.3 (4.3)	-11.9 (5.5)	-9.4 (6.0)	-6.2 (9.0)	-11.2 (10.5)	-6.6 (10.8)	-5.9 (12.0)	-2.9 (13.8)
			peptide	-9.0 (4.1)	-19.3 (4.9)	-18.5 (5.0)	-11.0 (4.3)	-11.7 (4.2)	58.1 (11.3)	87.8 (11.8)	65.1 (12.6)	66.7 (12.5)	67.7 (11.6)
B3 ^e potential 6-site		52	total	-15.1 (5.2)	-26.3 (6.4)	-26.8 (6.4)	-22.8 (6.9)	-21.0 (7.8)	51.9 (14.0)	76.5 (15.1)	58.6 (16.1)	60.8 (16.5)	64.9 (16.4)
			solvent	-4.9 (4.2)	-6.0 (4.9)	-7.5 (5.3)	-11.1 (6.6)	-9.8 (6.6)	1.0 (2.8)	0.6 (3.3)	1.6 (3.1)	2.9 (3.8)	3.8 (3.8)
			peptide	-17.2 (5.3)	-22.2 (7.1)	-19.3 (7.3)	-4.9 (7.8)	-8.4 (6.4)	13.8 (3.1)	16.7 (4.1)	14.1 (4.4)	9.1 (4.3)	11.2 (3.7)
			total	-22.1 (6.4)	-28.3 (8.3)	-26.8 (8.6)	-16.1 (10.0)	-18.2 (9.2)	14.7 (4.0)	17.4 (5.0)	15.7 (5.2)	12.1 (5.5)	15.0 (5.1)
K ^f field/ gradient 4-site		31	solvent	-4.9 (4.2)	-6.0 (4.9)	-7.5 (5.3)	-11.1 (6.6)	-9.8 (6.6)	1.0 (2.8)	0.6 (3.3)	1.6 (3.1)	2.9 (3.8)	3.8 (3.8)
			peptide	-17.2 (5.3)	-22.2 (7.1)	-19.3 (7.3)	-4.9 (7.8)	-8.4 (6.4)	13.8 (3.1)	16.7 (4.1)	14.1 (4.4)	9.1 (4.3)	11.2 (3.7)
			total	-22.1 (6.4)	-28.3 (8.3)	-26.8 (8.6)	-16.1 (10.0)	-18.2 (9.2)	14.7 (4.0)	17.4 (5.0)	15.7 (5.2)	12.1 (5.5)	15.0 (5.1)
			solvent	-4.9 (4.2)	-6.0 (4.9)	-7.5 (5.3)	-11.1 (6.6)	-9.8 (6.6)	1.0 (2.8)	0.6 (3.3)	1.6 (3.1)	2.9 (3.8)	3.8 (3.8)
KN field/ gradient 4-site		31	peptide	-24.6 (8.1)	33.9 (8.7)	36.3 (9.1)	28.5 (8.9)	44.4 (7.2)	-7.1 (5.6)	-21.9 (6.0)	-24.4 (5.8)	-13.7 (4.5)	-29.4 (5.9)
			total	-29.5 (8.9)	27.9 (9.6)	28.8 (10.0)	17.4 (10.8)	34.7 (9.1)	-6.1 (6.2)	-21.2 (6.5)	-22.8 (6.5)	-10.7 (5.8)	-25.6 (6.9)
			solvent	-4.9 (4.2)	-6.0 (4.9)	-7.5 (5.3)	-11.1 (6.6)	-9.8 (6.6)	1.0 (2.8)	0.6 (3.3)	1.6 (3.1)	2.9 (3.8)	3.8 (3.8)
			peptide	15.5 (7.9)	-28.4 (9.6)	11.5 (9.2)	29.2 (10.4)	-12.2 (7.0)	-4.1 (5.3)	-9.4 (5.4)	-1.3 (4.6)	-5.6 (5.3)	-17.5 (3.7)
KC field/ gradient 4-site		31	total	10.6 (8.7)	-34.4 (10.5)	4.0 (10.2)	18.1 (12.0)	-21.9 (8.9)	-3.1 (6.0)	-8.8 (6.2)	0.3 (5.6)	-2.7 (6.4)	-13.7 (5.2)
			solvent	-5.5 (3.9)	-8.2 (4.2)	-7.9 (4.6)	-12.9 (6.1)	-8.7 (6.5)	2.5 (4.3)	-1.0 (4.9)	2.5 (5.0)	1.2 (5.3)	3.7 (6.1)
			peptide	-8.2 (4.8)	-11.5 (5.6)	-10.3 (4.9)	1.0 (3.6)	-3.3 (3.2)	11.5 (5.5)	34.8 (7.2)	33.1 (7.8)	37.5 (7.5)	30.0 (6.1)
			total	-13.6 (5.8)	-19.7 (6.9)	-18.2 (6.4)	-11.9 (7.1)	-12.0 (7.5)	14.0 (6.7)	33.8 (8.4)	35.5 (8.9)	38.7 (8.9)	33.6 (8.5)
G ^g potential 4-site		this study	solvent	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	-10.0 (7.5)	-10.0 (7.5)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	5.0 (3.0)	5.0 (3.0)
			peptide	-19.8 (4.8)	-30.8 (5.6)	-25.8 (4.9)	1.0 (3.6)	-3.3 (3.2)	11.5 (5.5)	34.8 (7.2)	33.1 (7.8)	37.5 (7.5)	30.0 (6.1)
			total	-13.6 (5.8)	-19.7 (6.9)	-18.2 (6.4)	-11.9 (7.1)	-12.0 (7.5)	14.0 (6.7)	33.8 (8.4)	35.5 (8.9)	38.7 (8.9)	33.6 (8.5)
			solvent	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	-10.0 (7.5)	-10.0 (7.5)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	5.0 (3.0)	5.0 (3.0)
Em semi- empirical		34	peptide	-19.8 (4.9)	-30.8 (5.6)	-25.8 (6.0)	-9.6 (2.2)	-7.6 (2.4)	6.2 (1.5)	31.6 (6.2)	29.8 (6.3)	27.1 (6.3)	21.3 (6.8)
			total	-19.8 (4.9)	-30.8 (5.6)	-25.8 (6.0)	-24.6 (5.5)	-22.6 (5.5)	6.2 (1.5)	31.6 (6.2)	29.8 (6.3)	32.1 (7.0)	26.3 (7.4)
			solvent	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	-10.0 (7.5)	-10.0 (7.5)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	5.0 (3.0)	5.0 (3.0)
			peptide	-19.8 (4.9)	-30.8 (5.6)	-25.8 (6.0)	-9.6 (2.2)	-7.6 (2.4)	6.2 (1.5)	31.6 (6.2)	29.8 (6.3)	27.1 (6.3)	21.3 (6.8)

^a The parameterized atoms are in shown red.

^b The frequency shift (in cm⁻¹) is averaged over 15000 snapshots along the 1.5-ns MD trajectory. The numbers in parenthesis represent the standard deviations of the frequency shifts.

^c DFT calculation at the level of BPW91/6-31G**.

^d DFT calculation at the level of BPW91/6-311++G**.

^e DFT calculation at the level of B3LYP/6-311++G**.

^f DFT calculation with TZ2P basis and the RPBE exchange correlation function.

^g DFT calculation at the level of B3LYP/6-31+G*

TABLE 3: Average (ϕ_{ave} , ψ_{ave}) and Standard Deviation (σ_{ϕ} , σ_{ψ}) of Dihedral Angles Obtained from the 1.5-ns MD Trajectory Analysis of **1 in CDCl_3 (in degree)**

residue	ϕ_{ave}	σ_{ϕ}	ψ_{ave}	σ_{ψ}
1	−46.9	8.7	−30.1	10.3
2	−50.9	7.3	−23.2	8.9
3	−45.1	7.7	−30.9	9.2
4	−48.6	7.7	−26.4	10.3
5	−80.2	14.8	−4.1	15.8
6	−43.6	10.5	−39.9	15.2
	43.6	10.8	−114.4	17.0

TABLE 4: Amide-I and -II Local Mode Frequency Shifts due to the Intramolecular Hydrogen Bonding (IHB) Partners at the C=O and N–H sites, and the Other Atoms in the Backbone and Side Chains (BS)

model	linkage	amide-I frequency shift ^a					amide-II frequency shift ^a				
		1	2	3	4	5	1	2	3	4	5
B1	IHB (C=O)	−18.3 (1.9)	−17.6 (3.1)	−20.9 (5.8)	—	—	26.9 (1.3)	26.2 (1.8)	27.5 (3.5)	—	—
	IHB (N–H)	—	11.4 (5.3)	3.0 (5.2)	5.8 (5.9)	7.2 (5.3)	—	33.9 (10.8)	51.4 (12.0)	55.0 (11.9)	43.1 (13.1)
	BS	27.7 (6.9)	15.7 (9.2)	21.4 (11.5)	8.6 (9.7)	10.2 (7.8)	3.8 (5.7)	−12.8 (12.5)	−41.0 (14.0)	−16.5 (12.6)	−5.9 (14.0)
B2	IHB (C=O)	2.9 (4.0)	1.7 (5.1)	7.5 (7.7)	—	—	146.9 (13.8)	138.5 (12.8)	138.2 (12.3)	—	—
	IHB (N–H)	—	−32.9 (5.7)	−31.9 (6.2)	−37.2 (6.9)	−33.0 (6.5)	—	92.1 (22.0)	169.6 (24.5)	184.6 (26.3)	160.1 (34.6)
	BS	−3.2 (3.9)	20.6 (11.0)	18.8 (14.3)	38.0 (11.0)	17.7 (9.3)	−111.6 (18.3)	−154.1 (27.2)	−236.2 (28.2)	−96.0 (28.3)	−60.8 (34.1)
B3	IHB (C=O)	−14.3 (6.8)	−16.4 (7.9)	−8.7 (9.6)	—	—	223.3 (19.5)	211.4 (18.0)	211.2 (17.9)	—	—
	IHB (N–H)	—	−44.4 (7.9)	−50.5 (9.2)	−56.2 (9.3)	−47.3 (10.2)	—	128.2 (32.6)	242.5 (35.2)	262.3 (37.6)	227.6 (50.3)
	BS	5.3 (4.8)	41.5 (8.2)	40.7 (10.3)	45.3 (8.0)	35.7 (9.0)	−165.1 (22.4)	−251.8 (38.5)	−388.6 (40.1)	−195.6 (39.0)	−159.9 (50.0)
K	IHB (C=O)	−7.7 (2.3)	−8.5 (2.6)	−5.9 (2.9)	—	—	3.1 (1.2)	3.2 (1.4)	1.9 (1.4)	—	—
	IHB (N–H)	—	−4.5 (4.0)	−5.6 (4.4)	−1.7 (6.3)	−3.4 (4.1)	—	2.8 (1.8)	3.2 (2.0)	1.7 (2.8)	3.0 (2.1)
	BS	−9.6 (4.0)	−9.2 (4.1)	−7.8 (4.3)	−3.2 (4.6)	−5.0 (4.7)	10.7 (2.5)	10.8 (2.7)	9.0 (3.0)	7.5 (2.9)	8.2 (2.5)
G	IHB (C=O)	−5.9 (2.6)	−7.0 (2.9)	−5.2 (3.0)	—	—	0.9 (1.0)	0.6 (1.3)	−0.1 (1.8)	—	—
	IHB (N–H)	—	−1.3 (1.9)	−2.0 (2.0)	−1.3 (2.3)	−0.4 (1.9)	—	24.1 (4.6)	28.0 (5.3)	31.9 (5.4)	26.8 (5.7)
	BS	−2.2 (3.1)	−3.2 (3.5)	−3.1 (2.8)	2.3 (3.1)	−3.0 (3.0)	10.6 (5.4)	10.1 (5.2)	5.2 (5.3)	5.6 (5.3)	3.2 (5.0)

^a The frequency shift (in cm^{−1}) is averaged over 15000 snapshots along the 1.5-ns MD trajectory. The numbers in parenthesis represent the standard deviations of the frequency shifts.