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Ultrafast Chemical Exchange Dynamics of Hydrogen Bonds Observed via Isonitrile Infrared Sensors: Implications for Biomolecular Studies

Joachim Kübel,[†] Giseong Lee,[‡] Saik Ann Ooi,[†] Sebastian Westenhoff,[†] Hogyu Han,[‡] Minhaeng Cho,[‡,§] and Michał Maj*[,†]

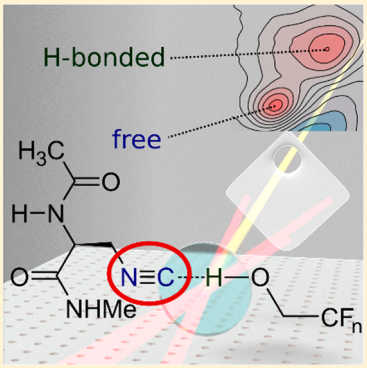
[†]Department of Chemistry and Molecular Biology, University of Gothenburg, 40530 Gothenburg, Sweden

[‡]Department of Chemistry, Korea University, Seoul 02841, South Korea

[§]Center for Molecular Spectroscopy and Dynamics, Institute for Basic Science, Seoul 02841, South Korea

Supporting Information

ABSTRACT: Local probes are indispensable to study protein structure and dynamics with site-specificity. The isonitrile functional group is a highly sensitive and H-bonding interaction-specific probe. Isonitriles exhibit large spectral shifts and transition dipole moment changes upon H-bonding while being weakly affected by solvent polarity. These unique properties allow a clear separation of distinct subpopulations of interacting species and an elucidation of their ultrafast dynamics with two-dimensional infrared (2D-IR) spectroscopy. Here, we apply 2D-IR to quantify the picosecond chemical exchange dynamics of solute−solvent complexes forming between isonitrile-derivatized alanine and fluorinated ethanol, where the degree of fluorination controls their H-bond-donating ability. We show that the molecules undergo faster exchange in the presence of more acidic H-bond donors, indicating that the exchange process is primarily dependent on the nature of solvent−solvent interactions. We foresee isonitrile as a highly promising probe for studying of H-bonds dynamics in the active site of enzymes.



The development of new infrared techniques has gained significant momentum due to recent advances in laser technology, yet application of vibrational spectroscopy to studying structure and dynamics of proteins is facing great difficulties.[1] Site-specific structural information is often inaccessible as many vibrational modes are coupled with each other and strongly overlap in frequency. At the same time, many localized vibrational modes are characterized by low oscillator strengths, imposing the requirement for highly concentrated samples, which in turn limits the application of infrared (IR) spectroscopy to smaller protein systems.

These limitations have been partially overcome by introducing non-natural IR-active probes into protein side-chains.[2−4] Many IR-active functional groups have been successfully incorporated into proteins. Stretch vibrations of azides, nitriles, and thiocyanates have often been selected for studies due to their well-pronounced absorption peaks in a spectral window above 2000 cm^{-1}, which are well separated from typical protein absorption bands.

A challenge connected with these probes is that their response to intermolecular interactions is complicated in nature. Besides Coulomb electrostatics, other interactions such as exchange-repulsion, polarization, and dispersion have been shown to play a role.[5,6] Although it is relatively straightforward to predict how these non-Coulombic terms contribute to the frequency shift in bulk solvents, quantitative determination of their magnitudes in a complex protein environment proves too difficult for most IR sensors.

Recently, we have introduced isonitrile functional group as a highly specific probe of H-bonding interactions.[7] Such unique specificity toward H-bonds stems from the fact that vibrational frequency shifts of the isonitrile (N≡C) stretch mode are dominated by blue-shifting exchange-repulsion and Coulombic interactions, which are particularly strong in magnitude upon H-bonding. Furthermore, these specific interaction contributions are significantly large in magnitude compared to the bulk solvent effects like polarity.[8] Such a rather simple solvatochromic response of isonitrile makes the interpretation of experimental data considerably easier and characterizes isonitrile as a highly promising IR sensor of structure and dynamics of H-bonds that can be studied with two-dimensional infrared (2D-IR) spectroscopy and other laser spectroscopic techniques.

Femtosecond (fs) 2D-IR spectroscopy has been tremendously successful in solving chemical problems that occur on time scales as short as a picosecond.[9−12] Observing events on such fast time scales is not possible with conventional IR and nuclear magnetic resonance (NMR) techniques. Thus, the technique has found a large variety of distinctive applications

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in the fields of chemistry,[13−15] nanomaterials,[16−18] and medicinal research.[19−21]

Most notable applications, which demonstrate the unique strength of 2D-IR over linear IR techniques, are chemical exchange experiments.[22] In a chemical exchange experiment, one can directly observe picosecond structural changes that happen under equilibrium conditions by following the appearance of cross-peaks between two populations of species. The processes that have been studied include single bond isomerization,[23] formation and dissociation of solute−solvent complexes,[24−26] fast conformational changes of protein residues,[27,28] and so on. Despite their unique capabilities, chemical exchange experiments in the IR have never entered the mainstream of analytical methods. The primary reason is that most molecular probes are unable to resolve individual subpopulations of interacting species clearly. Isonitriles, on the other hand, produce separated peaks even in the presence of weak H-bonds, such as those formed by chloroform.[7] This opens up the possibility of applying chemical exchange 2D-IR to more complex molecular systems, such as proteins and RNAs.

To demonstrate the case, we conduct a series of chemical exchange experiments on solute−solvent complexation dynamics between β-isocyanoalanine and protic solvents of varying H-bonding ability. The termini of β-isocyanoalanine are capped as amides (see Figure 1) to eliminate charged

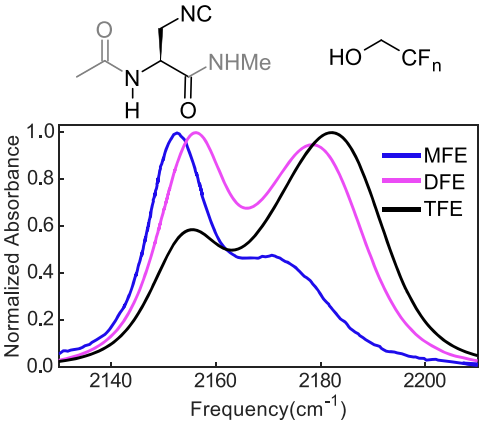

Figure 1. Molecular structures of the capped β-isocyanoalanine (capping groups shown in gray color) as well as the fluorinated alcohols under study (n = 1−3). Normalized Fourier transform infrared spectroscopy (FTIR) data of β-isocyanoalanine in 2-fluoroethanol, 2,2-difluoroethanol, and 2,2,2- trifluoroethanol (in short MFE, DFE, and TFE). Two peaks below and above ca. 2165 cm^{-1} are observed in each solvent, corresponding to the isonitrile resonance in "free" and H-bonded condition, respectively.

groups and to avoid additional H-bonding interactions other than those associated with the isonitrile group. We select MFE (2-fluoroethanol), DFE (2,2-difluoroethanol), and TFE (2,2,2-trifluoroethanol) as solvents. Fluorine atoms, due to their electron-withdrawing properties, increase the acidity of the hydroxyl group and, consequently, the availability of the hydrogen atom for H-bonding interactions in a solute−solvent complex. This allows us to control the nature of the solute−solvent interactions and elucidate vibrational dynamics as a function of H-bond strength.

Before discussing the 2D-IR experiments, we first analyze the FTIR spectra of β-isocyanoalanine, which are presented in Figure 1. Each spectrum is well-described by a sum of two Voigt profiles, which enables the extraction of vibrational frequencies and line widths (Table 1 and Supporting Information Figure S3). Two populations of species can be distinguished in all solvents, with low-frequency peaks corresponding to the N≡C stretch of "free" molecules, whereas the high-frequency peaks to that of H-bonded solute−solvent complexes. The high-frequency peaks are blue-shifted by 20, 23, and 26 cm^{-1} with respect to the low-frequency peak, following the increase in the degree of fluorination.

Table 1. Spectroscopic Parameters of the Isonitrile Resonance of β-Isocyanoalanine in Three Fluorinated Alcohols[a]

	MFE		DFE		TFE	
ν (cm^{-1})	2172	(2152)	2179	(2156)	2182	(2156)
FWHM (cm^{-1})	23	(13)	23	(19)	23	(19)
μ^2(b)/μ^2(f)	0.46		0.61		0.86	
p(f)/p(b)	0.5		0.45		0.45	

[a]Frequency values are given for the H-bonded (free) isonitrile: center frequency (ν), full-width at half-maximum (FWHM), and ratios of transition dipole moments (μ^2) and populations (p).

As the pK_a of the hydroxyl group decreases, the blue-shifted peak becomes more intense and significant line broadening is also observed. In MFE, the low-frequency peak is 1.5 times stronger than the high-frequency peak. The peaks are nearly equal in DFE, whereas the high-frequency peak becomes the dominant feature in TFE. Interestingly, the line width of the peak originating from H-bonded species is always relatively broader than that of the "free" species but exhibits very small solvent dependence. The low-frequency peak, on the other hand, shows significant broadening when going from MFE (13 cm^{-1}) to TFE (19 cm^{-1}) solvents. This broadening indicates a significant change in the distribution of local solvent configurations around the probe, but the corresponding frequency shifts remain relatively insignificant due to the absence of specific interactions, to which isonitriles are particularly sensitive.

The physical origins of these spectral features are further investigated with 2D-IR spectroscopy. 2D-IR spectra presented in this study were collected using heterodyne four-wave mixing.[29,30] The details of the experimental setup are given in the Supporting Information. In a 2D-IR experiment, two fs IR pump pulses are used to selectively excite vibrations and transfer the resulting coherence to a population state. The 2D-IR signal field is then generated by a third pulse (from the probe beam) arriving at the sample a certain waiting time T_w after the excitation. A 2D map is created, which allows one to correlate the initial excitation frequencies with the frequencies detected at any desired waiting times after the excitation. Many dynamic events occur during the waiting time, which include chemical exchange processes between vibrationally excited molecules. Because the population state, which is created by the interaction with the pump pulses, can be that of either the ground or the excited state, interaction with the probe pulse induces both $\Delta\nu$ = 0−1 and $\Delta\nu$ = 1−2 coherences, which are manifested in 2D-IR spectra as diagonal (positive) and anharmonically shifted (negative) peaks, respectively.

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The 2D-IR spectra of β-isocyanoalanine at early (0.2 ps) and late (12 ps) waiting times are plotted in Figure 2. Vibrational

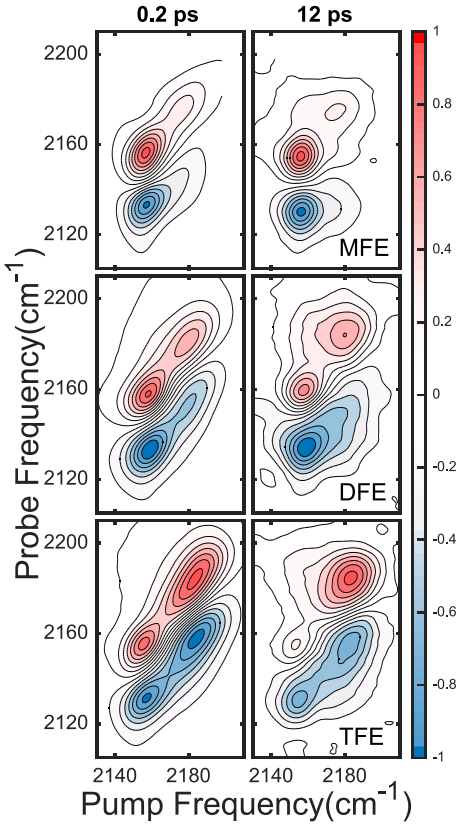

Figure 2. Normalized 2D-IR spectra of β-isocyanoalanine in 2-fluoroethanol, 2,2-difluoroethanol, and 2,2,2-trifluoroethanol (in short MFE, DFE, and TFE) at waiting times of 0.2 and 12 ps.

signals corresponding to the N≡C stretch of free and H-bonded molecules are clearly discernible. There are no cross-peaks present at early waiting times, which indicates the absence of intermolecular coupling between vibrations. As T_w increases, intense cross-peak features emerge due to the formation and breaking of H-bonds in chemical equilibrium.

Aside from the cross-peaks, the diagonal peaks themselves carry a considerable amount of information. From the inspection of the FTIR spectra, one may conclude that the population of H-bonded species is much higher in the most acidic solvent. It must be noted, however, that IR intensities, besides concentration, are also dependent on the transition dipole moment (TDM) of the vibrational mode of interest. Since the TDM is a molecular property, it is often assumed to be weakly dependent on the local solvation structure. Such assumption is indeed very convenient to make, as the determination of TDMs requires using a complementary spectroscopic technique. 2D-IR spectroscopy makes it possible to factor out the relative populations and dipole ratios with no extra effort. This is due to the fact that 2D-IR intensities scale with the fourth power of the TDM, as opposed to the quadratic dependence in linear IR experiments. As will become evident, the changes in dipole strengths are not insignificant once strong H-bonding interactions take place. It has been shown before that the dipole strength of the backbone Amide I band is sensitive to the protein secondary structure.[31] To the best of our knowledge, such detailed studies have not been carried out for any of the commonly used, non-natural side-chain probes of protein structure.

The extracted dipole strength and population ratios are presented in Table 1, along with other spectroscopic data. Surprisingly, the population of H-bonded species is always higher than that of the free molecules, even in the case of MFE where the high-frequency peak is considerably weaker A_1/A_2 = 0.45. The low-intensity of the high-frequency peak in MFE is attributed to a relatively large dipole strength of the low-frequency peak, where $(\mu_2/\mu_1)^2$ = 0.46. In DFE, the dipole strength ratio increases to 0.61. At the same time, the population of H-bonded species does not change considerably compared to MFE. In TFE, the relative dipole strength is even higher and equals to 0.86, but the population ratio remains unchanged. Our results indicate that the strength of a H-bond correlates positively with the magnitude of the transition dipole moment.

Besides the dipole strengths, the diagonal peaks can also provide critical information on the ultrafast solvation dynamics. By analyzing the time-dependent line shape evolution, one can separate homogeneous and inhomogeneous line broadening contributions and elucidate the time scales of the solvent reorganization around the vibrationally excited molecule. Here, the crucial quantity is the frequency-frequency correlation function (FFCF), which describes intra- and intermolecular structural fluctuations of a molecular system. The FFCF is treated as a sum of two exponential functions, whose parameters can be directly obtained from 2D-IR spectra. This approximated formula is then used to simulate linear and nonlinear IR spectra. Several different methods of extracting FFCF parameters from 2D-IR spectra have been proposed and published in detail before.[32] The diagonal peaks at early delay times are elongated along the diagonal, which reflects that the homogeneous line width is smaller than the frequency distribution due to an inhomogeneous ensemble of oscillators in the excited sample volume. At longer waiting times, excited molecules have had time to reorient and relax, giving rise to a round peak shape. Already in the 2D-IR spectrum collected at a waiting time of 12 ps (Figure 2), the peaks are essentially round, such that the diagonal elongation is almost gone. The time scales on which the diagonal elongation is reduced, i.e., the parameters of the FFCF, are given in Table 2. The line shape dynamics are different for H-bonded and free molecules in solution, while there is no obvious dependence on the

Table 2. Parameters of the Frequency−Frequency Correlation Function (FFCF), i.e., the time Constants τ_1 (τ_2 is treated as 50 ps, expect where marked c, where it is 13.8 ps), the Respective Spectral Diffusion Constants Δ_1, Δ_2, and the Pure Dephasing Time T_2* for H-Bonded (b) and Free (f) β-Isocyanoalanine in the Three Fluorinated Alcohols as Well as Chemical Exchange Time Constants Extracted from a Global Fit with Error Intervals

	MFE		DFE		TFE	
	b	f	b	f	b	f
τ_1 (ps)	0.9[c]	3.0	5.3	2.9	3.5	6.7
Δ_1 (rad/ps)	1.4	0.7	1.4	1	1.4	1.1
Δ_2 (rad/ps)	0.7	0.4	0.4	0.3	0.5	0.4
T_2* (ps)	3.3	1.9	3.8	2.0	8.7	6.1
$\tau_{f\to b}$ (ps)	81 ± 40		46 ± 20		26 ± 9	
$\tau_{b\to f}$ (ps)	161 ± 80		102 ± 44		59 ± 20	

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solvent polarity of these parameters. It may be noted that the pure dephasing time T_2* is larger for the H-bonding complexes as compared to the free isonitrile species and is furthermore larger with increasing H-bond donor strength, which may suggest more rigid H-bond pairs with stronger donors.

In the present study, we apply the extracted parameters to globally fit the experimental data with the simulated 2D-IR lineshapes based on a full response function theory treatment. This approach allows us to determine the rate of the ultrafast chemical exchange process. In principle, one could derive the rate constant by integrating the volume of the cross-peaks at different T_w however, due to a substantial overlap between the peaks, global fitting provides more accurate estimates.

For clarity, we limit the presentation of the spectral simulation and 2D-IR cross-peak analysis to DFE solvent only, but the same approach was applied to data measured in other solvents (for the full data set, see the Supporting Information). The experimental and simulated 2D-IR spectra at selected waiting times are presented in Figure 3. The 2D-IR

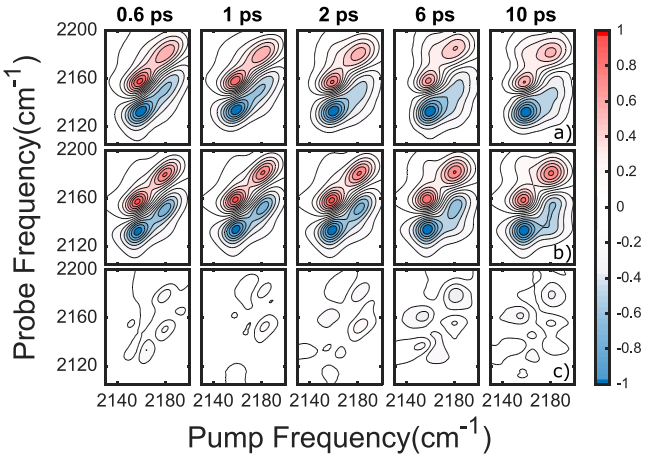

Figure 3. Normalized 2D-IR spectra of β-isocyanoalanine in 2,2-difluoroethanol: Experimental data (top), simulated data (middle), and difference thereof, i.e., residuals (bottom) at selected waiting times.

spectra are normalized for each waiting time to emphasize the relative peak ratios and shapes as well as their corresponding dynamics. The overall agreement between experimental and simulated data is excellent, and minor deviations regarding the relative intensity of the high-frequency peak are probably caused by nonideal FFCF parameters; as experimental, simulated, and residuals data are all plotted with the same color map and contour levels, already small deviations in peak shape will cause distortions of the observed contour plots. To extract the time constant for chemical exchange, both the relative change in diagonal peak intensity with T_w and the grow-in of cross-peaks should be considered, which are both included in our simulations. The time constants for H-bond complex formation and dissociation are included in Table 2 and strongly depend on the choice of solvent: with an increasing number of fluorine atoms, and the associated higher acidity of the alcohols, the time constants for both H-bond complex formation and dissociation decrease considerably.

This observation may be unexpected when thinking of H-bond strength being controlled by the acidity of the H-bond donor and that a more stable H-bonding complex correlates with a longer time constant for dissociation. We suggest that as chemical exchange occurs in chemical equilibrium, the accelerated complex formation, due to a higher driving force of the solute to form H-bonds, also causes the complex dissociation to speed up.

We had shown that the vibrational shift of isonitrile correlates with the Kamlet–Taft parameter α, which is an empirical descriptor of solvent acidity.[7] Here, to provide more accurate estimates of the magnitudes of the interaction energy in the molecular H-bonding complexes under investigation, we performed ab initio calculations at the MP2/aug-cc-pVTZ level of theory (see the Supporting Information). The molecular model consisted of β-isocyanoalanine forming a H-bond with a single alcohol molecule. The complexation energies were found to be −8.9. −9.3, and −8.9 kcal/mol in MFE, DFE, and TFE, respectively. At the same time, the H-bond distance in TFE was found to be nearly 0.03 Å shorter than that in MFE. This indicates that although the attractive H-bonding interactions become stronger, the simultaneous increase of repulsive forces, due to the increasing number of fluorine atoms, results in approximately equal complexation energies. Interestingly, this suggests that the modulation of the transition dipole moment of isonitrile is specific to H-bond length, not the overall interaction energy, unlike the equilibrium constant. Since the relative energy differences between the free and bound isonitriles show negligible solvent dependence, the rate of chemical exchange has to be controlled by the energetic barrier for H-bond formation. This energy barrier must be solvent-dependent, with the barrier being highest for MFE and lowest for TFE (see Figure 4).

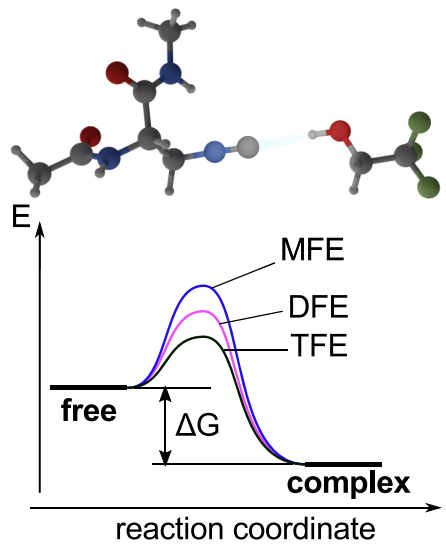

Figure 4. Molecular structure of the H-bonding complex between β-isocyanoalanine and trifluoroethanol and energy scheme showing the relative energetics of H-bonding interactions in the fluorinated ethanol molecules. While the free energy is essentially unchanged in the different solvents, the activation energy, i.e., the barrier height for H-bonding complex formation, is solvent-dependent.

A possible explanation for the accelerated exchange process relates to the *angular jump mechanism* proposed by Laage and Hynes for the reorientation of water molecules.[33] This mechanism relies on large angular jumps to form a H-bond with another binding partner, as opposed to a diffusive reorientation. Angular jumps as large as 49° have been reported before in the case of water and perchlorate

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acceptors.[34] During H-bond reforming, a transition state with a bifurcated H-bond relays the H-bond between two acceptor molecules, making the process of H-bond formation fast and highly directed.

In the case of the fluorinated alcohols under investigation, the individual H-bond network formed by either type of solvent molecules is likely influenced by the presence of the fluorine atoms in the molecules. On the one hand, as noted above, the number of fluorine atoms increases the acidity of the alcoholic hydroxyl group, which can directly influence the transition dipole moment of the isonitrile resonance when H-bonded. Second, the number of fluorine atoms in the solvent molecules changes the energy landscape of intermolecular interactions between solvent molecules; quantum chemical calculations of individual solvents pairs (see the Supporting Information) suggest that indeed dispersive interactions of non-H-bonded solvents pairs of two fluorinated alcohol molecules are strongest for MFE and weakest for TFE. This may hint to the higher relevance of intermolecular H-bonding between solvent molecules for TFE than for MFE, giving rise to a more ordered H-bonding network for TFE as compared to other solvents. With the H-bonding network being more ordered, the formation of bifurcated H-bonds between the solute in the respective solvent environments will be more likely for the solvent with a higher number of fluorine atoms. This could be a reason for the different activation energies and, consequently, the chemical exchange time constants extracted from our global fits. Further computational efforts in terms of classical and QM/EFP molecular dynamics simulations addressing the H-bonding structure in halogenated solvents are currently underway. A piece of experimental evidence for the angular jump mechanism may potentially be obtained by polarization-resolved multidimensional IR spectroscopy.[35]

This report demonstrates the potential of β-isocyanoalanine as a highly sensitive probe for H-bonding in particular when probed using 2D-IR spectroscopy. FTIR and 2D-IR data of the solute interacting with three different solvents contain a vast amount of spectroscopic information: the degree of fluorination of the solvent tunes the acidity of the alcohols and with that specific changes in resonance frequency and transition dipole strength of the solute can be determined. A comparison of experimental with simulated 2D-IR data allows quantifying the time scales for chemical exchange between solute and the respective solvents. For the isonitrile probe discussed here, the slowest exchange interaction that can be observed should be at least two times slower than the values reported in our study, assuming a vibrational lifetime of ca. 6 ps, which should allow one to record 2D-IR data at waiting times of up to 20 ps. We find that the direct vicinity of the H-bonding partner markedly influences the speed of chemical exchange here, suggesting that the isonitrile probe is highly sensitive not just to its H-bonding partner but also to its nearest environment. Especially the latter aspect is a fascinating property that is of particular interest when studying protein active sites with modifications with only minor steric demand. We expect that isonitrile functional groups will make an impact as H-bond reporters in biomolecular research.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jpclett.9b03144.

Detailed description of the experimental 2D-IR setup, solvent-dependent FTIR spectra of β-isocyanoalanine, complete data set of chemical exchange 2D-IR spectra, volume fitting analysis of 2D-IR spectra, and ab initio calculations for the solute−solvent complex and solvent−solvent pairs (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: michal.maj@gu.se.

ORCID

Joachim Kübel: 0000-0002-1613-0315
Minhaeng Cho: 0000-0003-1618-1056
Michał Maj: 0000-0003-1567-9514

Notes

The authors declare no competing financial interest.

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