Supporting Information

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SI Text

SI Materials and Methods. ***Peptide synthesis.*** LKα14 peptides, Ac-LKKLLKLLKKLLKL-OH, were synthesized de novo using standard Fmoc chemistry (1). Natural abundance residues were obtained in protected form from Novabiochem. L-leucine, isopropyl-d_7 (99% atom ^{2}H) was purchased from Cambridge Isotope Laboratories and protected with Fmoc-OSu. Peptides with single-site leucine deuteration are abbreviated as Leu#, where # is the position of the deuterated residue. Wang resins with L-leucine preloaded were used for all samples except L14, for which unloaded Wang resin was linked with deuterated Fmoc-Leu via the MSNT process (2). Peptides were synthesized on a Rainin PS3 instrument using N-methyl-2-pyrrolidone as the primary solvent and capped by reaction with acetic anhydride. After cleavage and lyophilization, purity was confirmed with mass spectrometry and HPLC. The peptides did not require further purification.

Peptide binding and substrate preparation—polystyrene films on CaF_2 prisms Polystyrene films were fabricated by spin-casting a 3% solution (wt/wt in toluene) of polystyrene (Polymer Source Inc.) onto one side of an equilateral CaF_2 prism at 3,000 rpm. The polystyrene film was then brought into contact with a 1× PBS buffer solution and the interface was probed through the backside of the film. The peptide solution was injected into the buffer without exposing the surface to the air/water interface. The concentrations of the peptide solution used in this study were 0.05 mg/mL.

Sum frequency generation vibrational spectroscopy. Details of our sum frequency generation (SFG) setup are published elsewhere (3) and will only be briefly discussed here. Briefly, the visible beam with a wavelength of 532 nm was delivered by an EKSPLA Nd:YAG laser operating at 50 Hz, which was also used to pump an EKSPLA optical parametric generation/amplification and difference frequency unit based on barium borate and $AgGaS_2$ crystals to generate tunable IR laser radiation from 1,000–4,000 cm^{-1}. The bandwidth was 2 cm^{-1} for the visible pump pulses and 4 cm^{-1} for the IR laser. Both beams were focused at the sample with energies of 150 μJ and 200 μJ per pulse for the visible and the IR beam, respectively. The spectra were collected with 200 shots per data point in 2 cm^{-1} increments. The SFG spectra were normalized by the product of the IR and visible pump beam intensities. The input angles of the visible and IR pump beams inside the prism were 47° and 58° versus the surface normal of the polystyrene surface, respectively. Both angles were smaller than the critical angle for total internal reflection for the CaF_2—water interface with a thin polystyrene interlayer (66°).

Peptide binding and substrate preparation—polystyrene beads. 1.0 μm diameter polystyrene beads in aqueous suspension were obtained from Polysciences. An estimated binding area of 80 $Å^2$ per molecule of LKα14 (0.52 mg peptide per 1.0 mL bead suspension) was verified to form monolayer coverage on the polystyrene surface using X-ray photoelectron spectroscopy.

3 mL of bead suspension, 1.55 mg of LK peptide (in aqueous solution at 5.0 mg/mL), and 40 mL of 0.13× PBS buffer (28 mM NaCl, 7 mM KH_2PO_4/K_2HPO_4, pH 7.0) were combined in a 100 mL round bottom flask and stirred gently at room temperature for 4–12 hours, then transferred to a 50 mL centrifuge tube. The suspension was centrifuged for 2 h at 3,000 × g, the supernatant removed, and the pellet resuspended in roughly 1 mL of deuterium-depleted water (Cambridge Isotope Laboratories). This solution was transferred to a 1.5 mL microcentrifuge tube and centrifuged at 10,000 × g for 10 min. The supernatant was discarded and the pellet resuspended and centrifuged again. Following this, the pellet was resuspended a final time, flash-frozen in liquid nitrogen, and lyophilized for a minimum of 3 d, leaving the sample almost entirely dry. Trace residual water can be seen in the form of the central trace water signal (HOD) spike in the NMR line shapes, none of which had a significant integration relative to the line shape area of interest. UV analysis of the supernatants indicated 85–90% peptide binding.

Solid-state deuterium NMR spectroscopy. For each experiment, approximately 40 mg of the bead/peptide powder was placed in a Kel-F sample holder, sealed with Teflon tape, and placed in a home-built static solid-state NMR probe. Spectra were collected with a 17.6 Tesla magnet and a Bruker Avance II NMR spectrometer, operating at a ^{2}H frequency of 115.135 MHz. A two-pulse quadrupole echo pulse sequence with 2.65 μs $\pi/2$ pulses, a 40 μs delay between pulses, and a recycle delay of 0.35 s were used throughout. Each experiment consisted of 360,000–400,000 acquisitions. Line shapes were obtained with minimal processing consisting of 1 kHz line broadening and phase adjustment. For comparison, a spectrum of the unbound peptide labeled at Leu8 was also taken. Any difference between the unbound Leu8 line shape and the other line shapes is presumably due to the effects of binding. None of the spectra resemble the line shape of monomeric leucine-d_7, which is a classic Pake doublet with a quadrupolar coupling constant (QCC) of ∼50 kHz.

Theory and Analysis. ***Quantitative SFG analysis.*** In the SFG data modeling we assume the azimuthal angle ϕ has an isotropic distribution over macroscopic regions of the samples. We further assume here that both methyl groups of the terminal isopropyl groups rotate rapidly and that their internal angle of rotation does not affect the transformation of the respective hyperpolarizabilities ($\beta_{A,B,C}$) into the molecular frame ($\chi^{(2)}_{A,B,C}$). This assumption is generally accepted and also verified by our ssNMR experiments (above). The nonvanishing components of $\beta_{A,B,C}$ for the resulting quasi-$C_{2\nu}$ symmetry of the different resonance modes are the following:

$$\text{a1: } \beta_{AAC},\beta_{BBC},\beta_{CCC} \qquad \text{b1: } \beta_{ACA}=\beta_{CAA} \qquad \text{b2: } \beta_{BCB}=\beta_{CBB} \quad \textbf{[S1]}$$

"a" and "b" denote symmetric and asymmetric vibrations, respectively, "1" and "2" stand for in-plane and out-of-plane modes. Using the transformation from the individual methyl coordinates (a,b,c) to the unified atom frame (A,B,C) given in ref (4) we can relate the susceptibilities in molecular coordinates to the laboratory coordinate frame using established procedures and transformation tables in (5) to obtain the relation between the macroscopic tensor elements with the Euler angles θ and η:

For the a modes:

$$\chi_{yyz} = \chi_{xxz} = \frac{1}{2}(\beta_{aac} - \beta_{ccc})[\{(\cos\alpha - \cos^3\alpha)\times (5 + 3\cos 2\eta) - 2\cos\alpha\}(\cos\theta - \cos^3\theta) - 2(\cos\alpha - \cos^3\alpha)\cos\theta] + \beta_{aac}\cos\alpha\cos\theta$$

$$\chi_{yzy} = \chi_{xzx} = \chi_{zxx} = \frac{1}{2}(\beta_{aac} - \beta_{ccc})\{(\cos\alpha - \cos^3\alpha)\times (5 + 3\cos 2\eta)(\cos\theta - \cos^3\theta) + 2\cos\alpha\cos\theta(\cos^2\alpha + \cos^2\theta - 2)\}$$

$$\chi_{zzz} = (\beta_{aac} - \beta_{ccc})[(\cos\alpha - \cos^3\alpha)\times \{2\cos^3\theta - 3(1 + \cos 2\eta)(\cos\theta - \cos^3\theta)\} - 2\cos\alpha\cos^3\theta] + (\beta_{aac}\cos\alpha\cos\theta) \quad \textbf{[S2]}$$

For the b modes:

$$\chi_{yyz} = \chi_{xxz} = \beta_{caa}[(\cos\alpha - \cos^3\alpha)\{-2\cos\theta + 3(\cos\theta - \cos^3\theta)(1 + \cos 2\eta)\} - 2\cos^3\alpha(\cos\theta - \cos^3\theta)]$$

$$\chi_{yzy} = \chi_{zxx} = \chi_{xzx} = \beta_{caa}[3(\cos\alpha - \cos^3\alpha)\times (\cos\theta - \cos^3\theta)(1 + \cos 2\eta) + 2\cos^3\alpha\cos^3\theta]$$

$$\chi_{zzz} = 2\beta_{caa}[(\cos\alpha - \cos^3\alpha)\{2\cos\theta - 3(\cos\theta - \cos^3\theta)\times (1 + \cos 2\eta)\} + 2\cos^3\alpha(\cos\theta - \cos^3\theta)] \quad \textbf{[S3]}$$

Note, that we omitted N/ε_0 in these equations since the term cancels out in the analysis and α is held constant at 55°. Since the values for the molecular hyperpolarizabilities are unknown, ratios of Eq. **S2**, Eq. **S3** must be employed in combination with the ratios of resonance strengths A for different polarization combinations to uniquely determine θ and η. For the ratio β_{aac}/β_{ccc}, which can be estimated from Raman depolarization data and theoretical calculations, we assumed a value of 2.1. This value has been used for leucine isopropyl groups before (6) and is also close to the values for acetone and DMSO. For sake of simplicity we assumed a δ-function distribution for θ and η. The relatively high signal to noise ratio observed for individual deuterated isopropyl units is consistent with this assumption.

Solid-state NMR deuterium line shapes. The theory of the dynamically modulated solid-state ^{2}H NMR line shape is well documented (7) and will be described only briefly here as it relates to the major motion of the leucyl side chains in LK peptides. A line shape is measured using a quadrupolar echo experiment, where two 90° pulses with a relative phase shift of $\pi/2$ and separated by a time duration τ_1 are applied to the deuterium spin system. As a result of this pulsed irradiation, a quadrupolar echo is produced at a time τ_2 after the second pulse. The general expression for the dynamically modulated quadrupolar echoes is given by (8):

$$F(t;\tau_1,\tau_2) = \vec{W} \bullet e^{A_+\tau_1} e^{A_-(t-\tau_2)} \bullet \vec{I}, \quad \textbf{[S4]}$$

where $\vec{W}$ is a row vector whose elements are the a priori site occupation probabilities, $\vec{I}$ is a column vector with all elements unity, $A_+ = i\overleftrightarrow{\omega} + \overleftrightarrow{\pi}$, and $A_- = -i\overleftrightarrow{\omega} + \overleftrightarrow{\pi}$. Geometric information resides in the ω matrix which has the diagonal form:

$$i\overleftrightarrow{\omega} = \begin{pmatrix} i\omega_+(\Omega_1) & 0 \\ 0 & -i\omega_+(\Omega_2) \end{pmatrix}, \quad \textbf{[S5]}$$

where

$$\omega_+(\Omega_{1,2}) = \frac{3}{4}\frac{e^2qQ}{\hbar}\sum_{a=-2}^{+2} D^{(2)}_{0,a}(\Omega_{1,2})D^{(2)}_{a,0}(\Omega_{CL}) \quad \textbf{[S6]}$$

which is the resonance frequency associated with the $m = 1$ to $m = 0$ transition of the spin 1 deuterium nucleus. An analogous expression is associated with the $m = 0$ to $m = -1$ transition (9, 10). In Eq. **S3** the solid angles Ω_1 and Ω_2 orient the C–D bond axis at sites 1 and 2 in a molecule-fixed frame, and the solid angle Ω_{CL} relates the molecule-fixed frame to the direction of the external magnetic field, which is considered the z axis of the lab-fixed frame.

Information on the site transition rates per unit time reside in the π matrix which has the form:

$$\pi = \begin{pmatrix} -k_{12} & k_{21} \\ k_{12} & -k_{21} \end{pmatrix}.$$

The kinetic constants are related to the a priori site populations by $p_1 = \frac{k_{21}}{k_{12}+k_{21}}$ and $p_2 = 1 - p_1 = \frac{k_{12}}{k_{12}+k_{21}}$. Because CD_3 group rotation occurs on a time scale much faster than that of configurational changes of the leucine side chain, the effect of methyl rotation can be accounted for by scaling the quadrupolar coupling constant $\frac{e^2qQ}{\hbar}$ in Eq. **S6**. Additional motions at time scales comparable to or slower than the configurational change can be incorporated as described in Greenfield et al. (11) and calculated numerically using MXET1.

Deuterium NMR line shape simulations. Simulations could not be fit automatically to the data due to the difficulty of obtaining proper line shapes in the "shoulder" region at the base of the primary spectral peak, which is disproportionately affected by intermediate geometries with very low (≪1%) populations (12). Although the two-conformer model used here does not consider those subconformers, their populations are far smaller than the experimental error on any dataset, and can be neglected. Similarly, the effect of the carbon-deuterium quadrupolar line shape has been determined to be insignificant under these conditions (13) and was therefore not simulated. Line shapes were instead fit by inspection, and parameters varied by the smallest amounts that resulted in visible changes to the simulated spectra, as follows:

Population ratio	2%
γ-deuteron fraction	1%
Cone angle	2°
QCC_{eff}	1 kHz

Additionally, the effect of the single C_γ deuteron was modeled with a scaling factor, as its longer relaxation time and wider line shape meant that it was generally observed at less than 1/7 of the overall spectral integral.

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