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Supporting Information for "Does Liquid-Liquid Phase Separation Drive Peptide Folding?"

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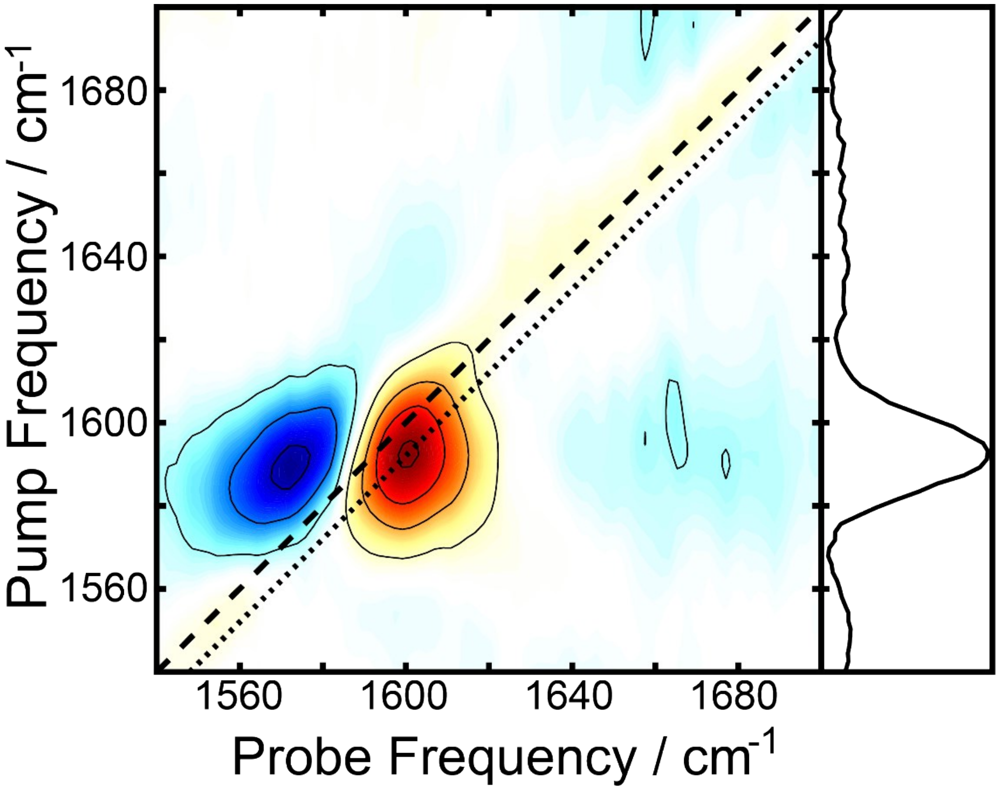

Figure S1. 2DIR spectrum of PEG in D_2O. We attribute the peak that arises at around 1600 cm^{-1} pump frequency in other spectra to PEG.

Methods for Simulating 2DIR Spectra

The polyproline spectra were simulated using a transition dipole coupling, floating oscillator model where we extracted the amide-I transition dipole moment positions and directions from idealized, 40 residue long, PPI and PPII helix structures,[1,2] and calculated the transition dipole coupling energy between all pairs of dipoles, using the dipole coupling parameters of Krimm.[3] The amide-I central frequencies were set to 1650 cm^{-1}. Gaussian diagonal disorder of standard deviation 12 cm^{-1} was added to the exciton Hamiltonian. Diagonalization of the Hamiltonian provided normal mode frequencies and transition dipoles, from which a 2DIR stick spectrum was generated within the harmonic approximation. Stick spectra were averaged over 2000 realizations of diagonal disorder. The final simulated 2DIR spectra were obtained by convolving the 2D stick spectra with a 2D Lorentzian function with 3 cm^{-1} half-width at half max.[4] Random-coil spectra were simulated by first generating structures using a self-avoiding chain model, the details of which can be found in ref 5. We generated 2000 sample structures with 40 residues and used the same spectrum simulation method described above, averaging all spectra together to obtain an ensemble averaged random coil spectrum. The simulated spectrum in Figure 4 is simply a linear combination of the simulated 2DIR spectra for a polyproline I and a polyproline II peptide.

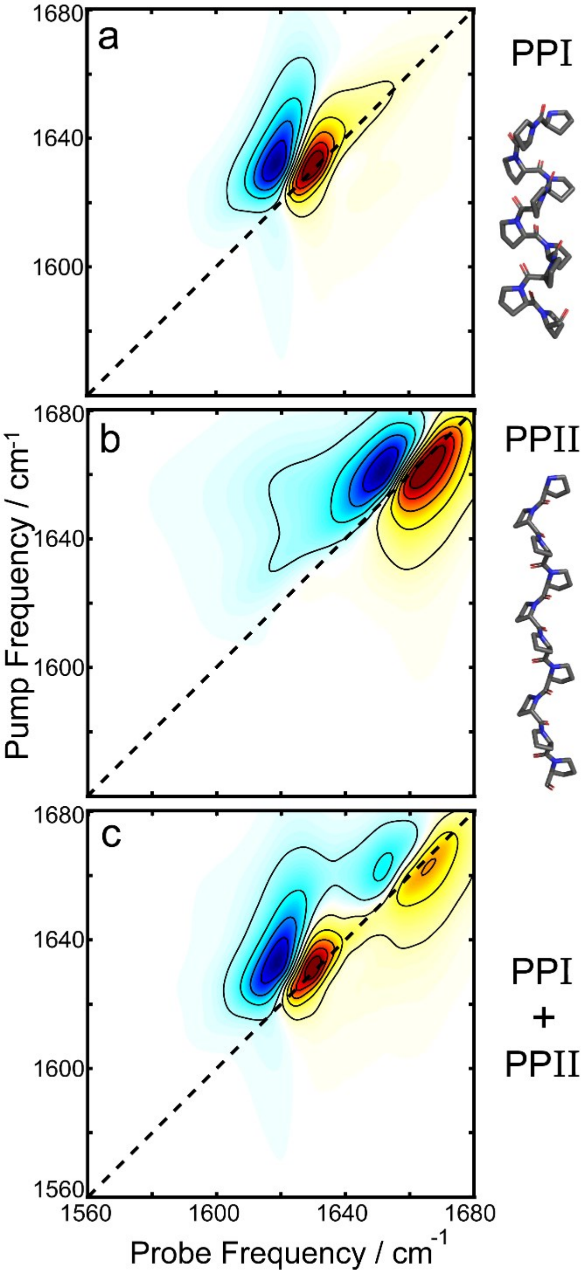

Figure S2. a) Simulated 2DIR spectrum of PPI structure. b) Simulated 2DIR spectrum of PPII structure. c) Summed spectrum of both polyproline structures. A segment of ten residues of the associated structure is shown on the right for each spectrum.

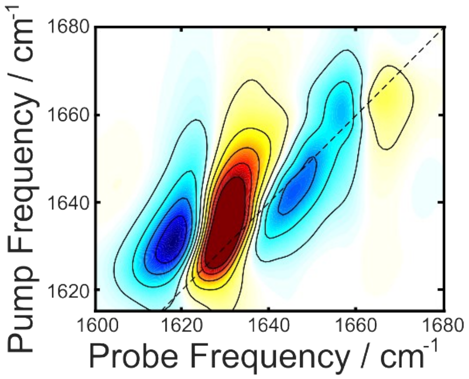

Figure S3. Simulated difference spectrum obtained by subtracting a random coil 2DIR spectrum from one containing 80% random coil, 10% PPI, and 10%. Polyproline peaks at 1630 and 1660 cm^{-1} stand out in the difference spectrum.

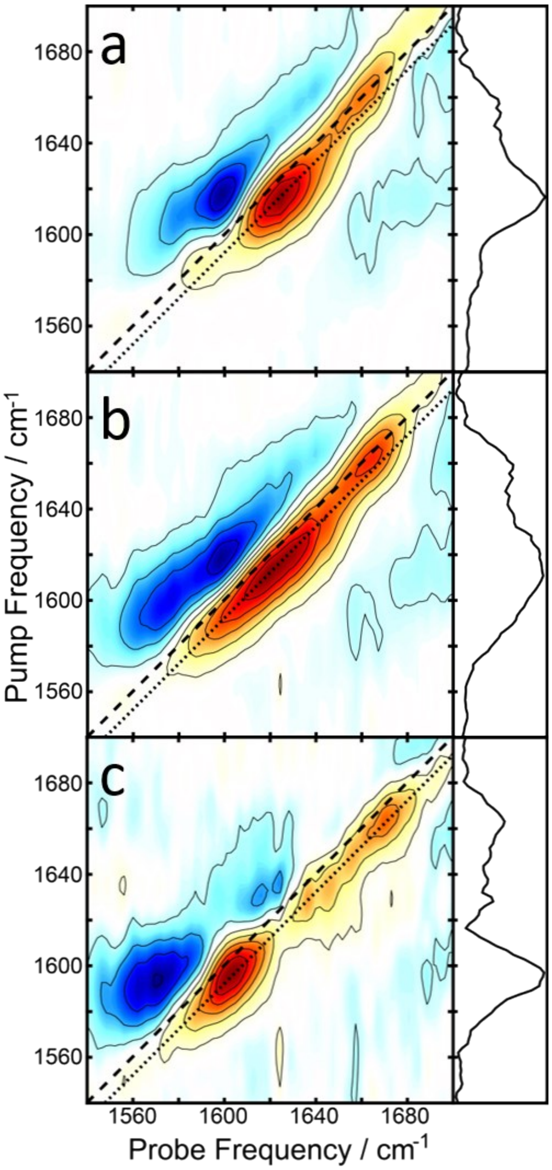

Figure S4. Reproduction of the 2DIR spectra shown in Figure 3 of the main text. 2D IR spectra of PR20 (a) without PEG and (b) with PEG to induce phase separation. c) Difference spectrum. The peak intensities differ from the main text due to the pump spectrum having drifted in intervening months, however the peak positions remain the same when compared to Figure 3 of the main text.

Reweighing of the Experimental Difference Spectra

To determine if the peaks in the difference spectrum come from new peaks in the spectrum after phase separation rather than artifacts from subtraction, we compared the subtraction over a wide range of subtraction ratios to show that the peaks hidden by the main random coil peak remain visible in the difference spectrum. This suggests that our identification of the peaks can tolerate an experimental error in amplitude of at least 25%. By measuring PR_{20} in D_2O in replicate, 4 hours apart, with freshly prepared sample, we found an "error" spectrum (Figure S6) that has an amplitude of about 19%. In addition, the error spectrum has a similar lineshape to PR_{20} in D_2O, whereas the difference spectra in Figure S5 clearly has a different peak pattern and lineshape from PR_{20} in D_2O.

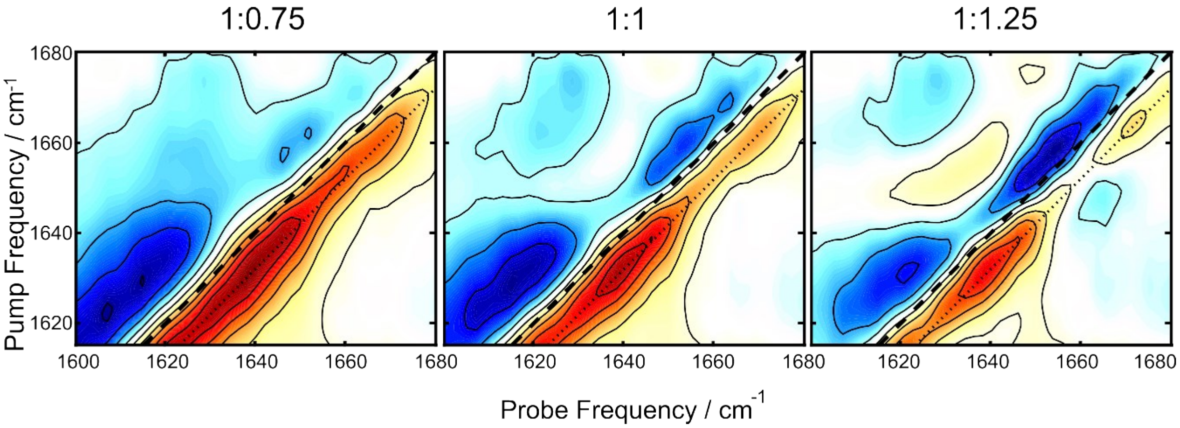

Figure S5. Difference spectra between the 2DIR spectra in **Figures 3a** and **3b**.

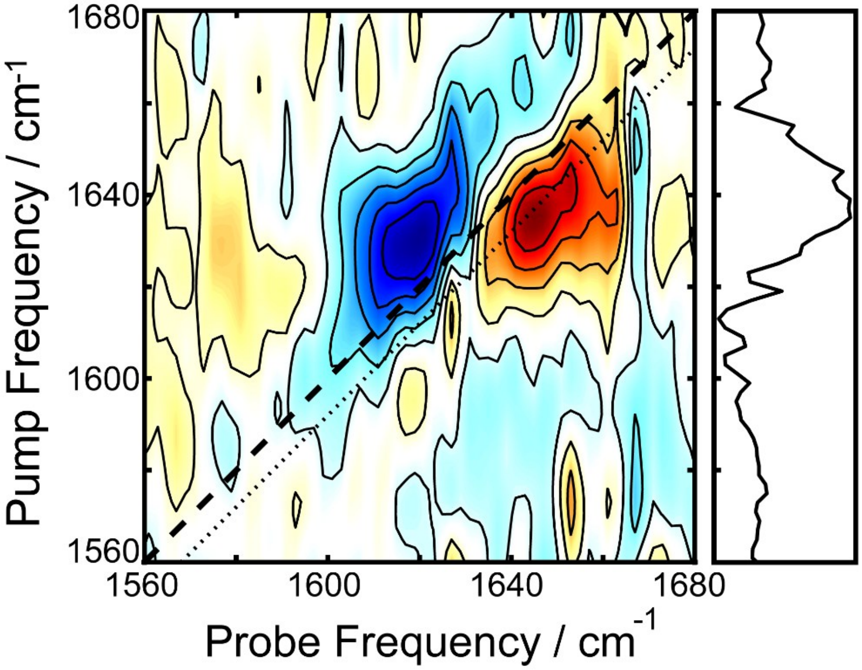

Figure S6. "Error" spectrum produced from two separate 2DIR experiments of PR$_{20}$ in D$_2$O taken about 4 hours apart. The amplitude of the error spectrum 1630 cm^{-1} was measured to be 19% of the amplitude of the original.

Measuring Laser Stability as a Source of Noise

In order to rule out changes in laser intensity contributing to formation of new peaks in the difference spectrum, we measured the laser intensity over a period of 24 hours to test if the new peaks arise due to changes in laser intensity. Fluctuations in the laser intensity over a typical collection period were too small to explain the changes in the spectra.

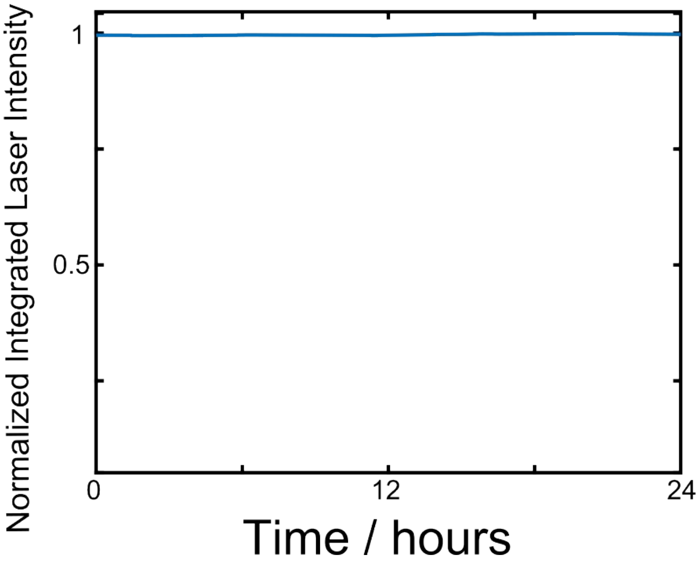

Figure S7. Laser intensity normalized to the peak laser intensity over a 24 hour period. The laser intensity was measured by integrating the spectral intensity of a reference beam collected on the camera and taking the moving average over a four hour period, comparable to the collection time for the 2D spectra. The standard deviation of the intensity is 0.14% of the mean.

Analysis of the Effect of PEG and Peptide Concentration on Spectral Features

The PR_{20} experiments were repeated using varied concentrations of both peptide and PEG to determine whether the new peaks in the spectra were related to phase separation of the peptide. When using a concentration of 5% PEG, which is below conditions need for phase separation (Figure S8a), no significant change in the spectrum was found. In Figure S8b, we repeat the phase separation experiment in 30% PEG, using a higher concentration of PR_{20} of 1000 µM. The FTIR spectrum with higher concentration PEG has similar features to the spectrum in Figure 2, meaning the new peaks appear to scale with the concentration of PR_{20}, implying that increased concentration does not induce any secondary effects such as amyloid aggregation.

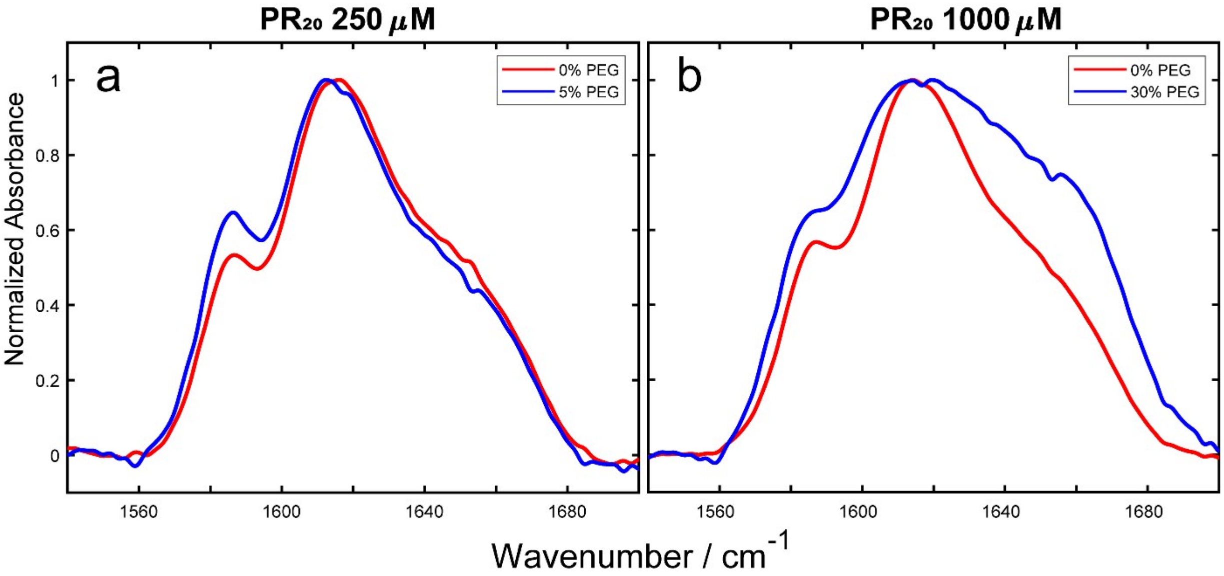

Figure S8. FTIR Spectra of PR_{20} with varying concentrations of PEG and peptide. Spectra were collected from peptide in D_2O with potassium phosphate buffer as described in the main text. The spectra were processed by using pure D_2O and buffer as a reference spectrum, subtracting the baseline, and normalizing the spectrum to the peak absorbance in the amide I region.

Inverse Participation Ratio Analysis of Polyproline Peak Intensities

A method by which we can compare the exciton delocalization of a structure is by calculating the inverse participation ratio (IPR), which is approximately the number of subunits over which a transition is delocalized.[6] For a single replica in our spectral simulations we can use the eigenvectors of the Hamiltonian, V, to calculate the IPR for the k^{th} excitonic mode, P_k:

$$P_k = \left(\sum_i V_{i,k}{}^4 \right)^{-1} \tag{1}$$

We then calculate the IPR(ω) spectrum by averaging the P_k for each exciton mode within a given frequency window, normalized by the density of states:

$$IPR(\omega) = \frac{\left\langle \sum_k P_k \cdot \delta(\omega - \omega_k) \right\rangle}{\left\langle \sum_k \delta(\omega - \omega_k) \right\rangle} \tag{2}$$

Here, ω_k is the frequency of the k^{th} mode. We calculated the stick IPR spectra on a grid with 1 cm^{-1} spacing, and smoothed them with a 4 cm^{-1} moving average. The IPR spectra in Figure S9 shows that all the modes for random coil structures are mostly localized whereas PPI and PPII have peaks in the spectra corresponding to delocalization of specific exciton modes over 2-3 subunits.

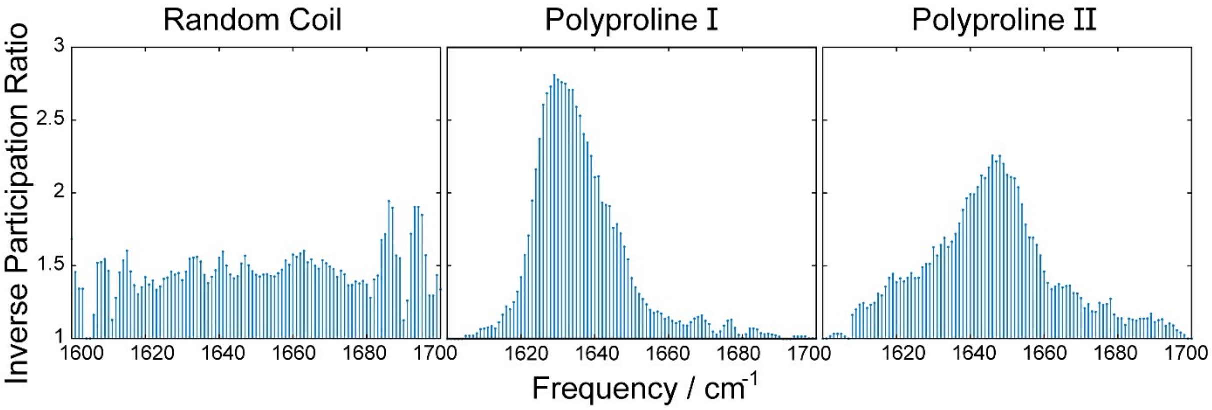

Figure S9. Inverse participation ratio spectra calculated for simulated random coils, PPI, and PPII structures. The peaks in the PPI and PPII indicate delocalization of exciton modes.

References

1 W. TRAUB and U. SHMUELI, *Nature*, 1963, **198**, 1165–1166.
2 V. Sasisekharan, *Acta Crystallogr.*, 1959, **12**, 897–903.
3 W. H. Moore and S. Krimm, *Proc. Natl. Acad. Sci.*, 1975, **72**, 4933–4935.
4 Z. Ganim and A. Tokmakoff, *Biophys. J.*, 2006, **91**, 2636–2646.
5 A. L. Serrano, J. P. Lomont, L.-H. Tu, D. P. Raleigh and M. T. Zanni, *J. Am. Chem. Soc.*, 2017, **139**, 16748–16758.
6 S. Hahn, S. Ham and M. Cho, *J. Phys. Chem. B*, 2005, **109**, 11789–11801.