Supporting Information

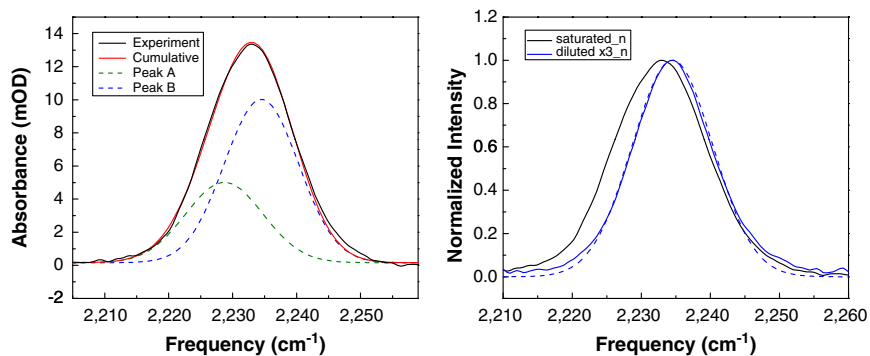## Chung et al. 10.1073/pnas.1100587108

Fig. S1. The FTIR spectrum of the CN stretching mode of a high concentration (~18 mM) HP35-(CN)$_2$ sample. (*Left*) Data (black solid curve) have a small shoulder on the low frequency side of the spectrum. The spectrum is fit with the sum of two Gaussian curves with a peak at 2,229 cm^{-1} (peak A, green dashed curve) in addition to the peak at 2,235 cm^{-1} (peak B, blue dashed curve). The solid red curve is the two Gaussian fit to the experimental spectrum. (*Right*) The same high concentration spectrum is compared to the spectrum of the same sample, diluted to one-third concentration (blue solid curve), both normalized. The diluted spectrum has only one peak at 2,235 cm^{-1}. Its Gaussian fit is shown as the blue dashed curve. These observations demonstrate that peak A develops at high concentration of the peptide and may be caused by aggregation or dimerization. The growth of the 2,229 cm^{-1} peak can also occur at lower peptide concentrations 3–4 d after the preparation of the sample. In the experiments presented here, only fresh samples were used, and each sample was checked for the shoulder after the 2D IR experiments were performed.
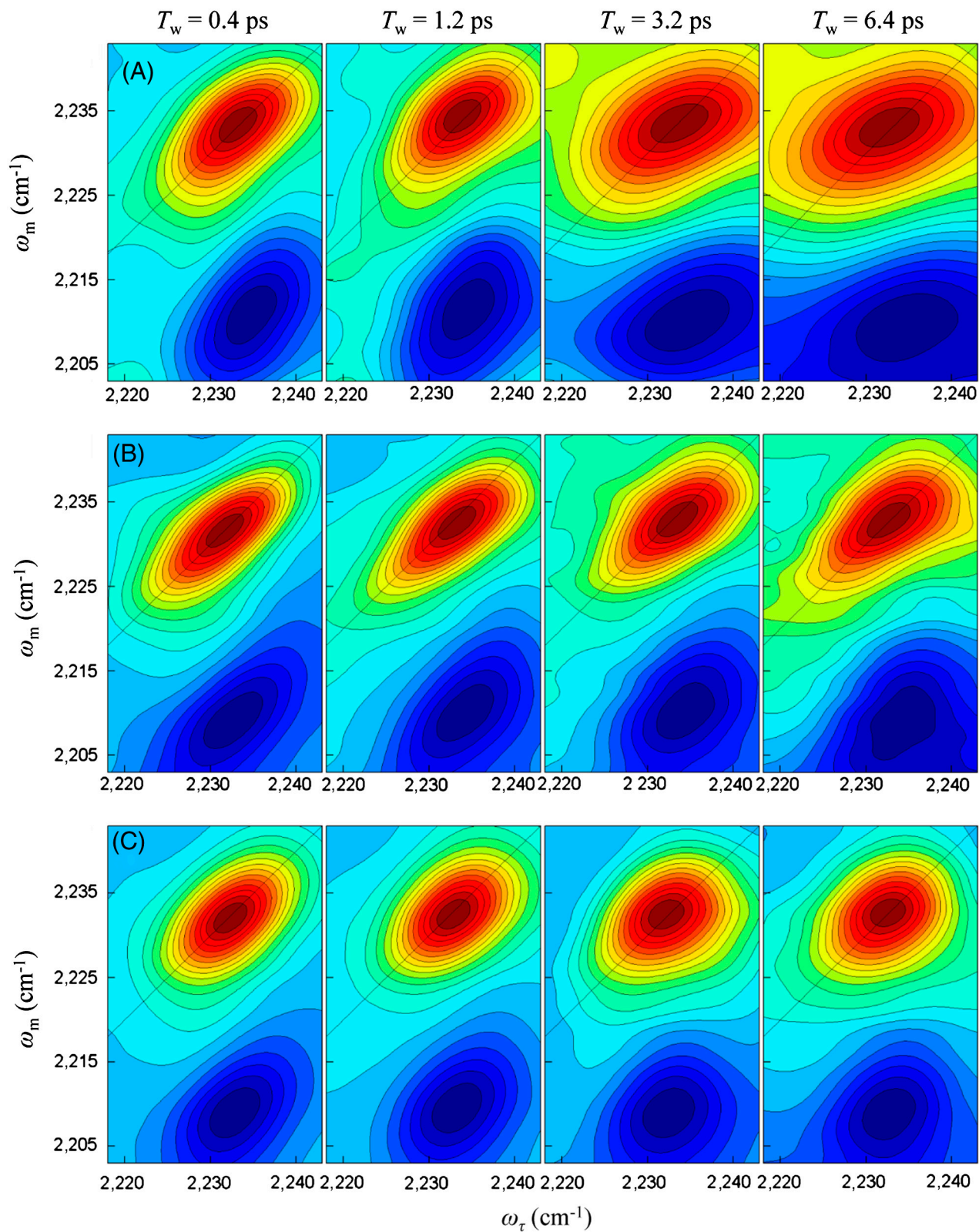
Fig. S2. The time progression of 2D IR spectra of CN stretch of HP35-$(CN)_2$ in various solvents at the waiting times (T_w) of 0.4, 1.2, 3.2, and 6.4 ps. (*A*) HP35-$(CN)_2$ in water; (*B*) HP35-$(CN)_2$ in 0 M GuHCl glycerol/water; (*C*) HP35-$(CN)_2$ in 6 M GuHCl glycerol/water.
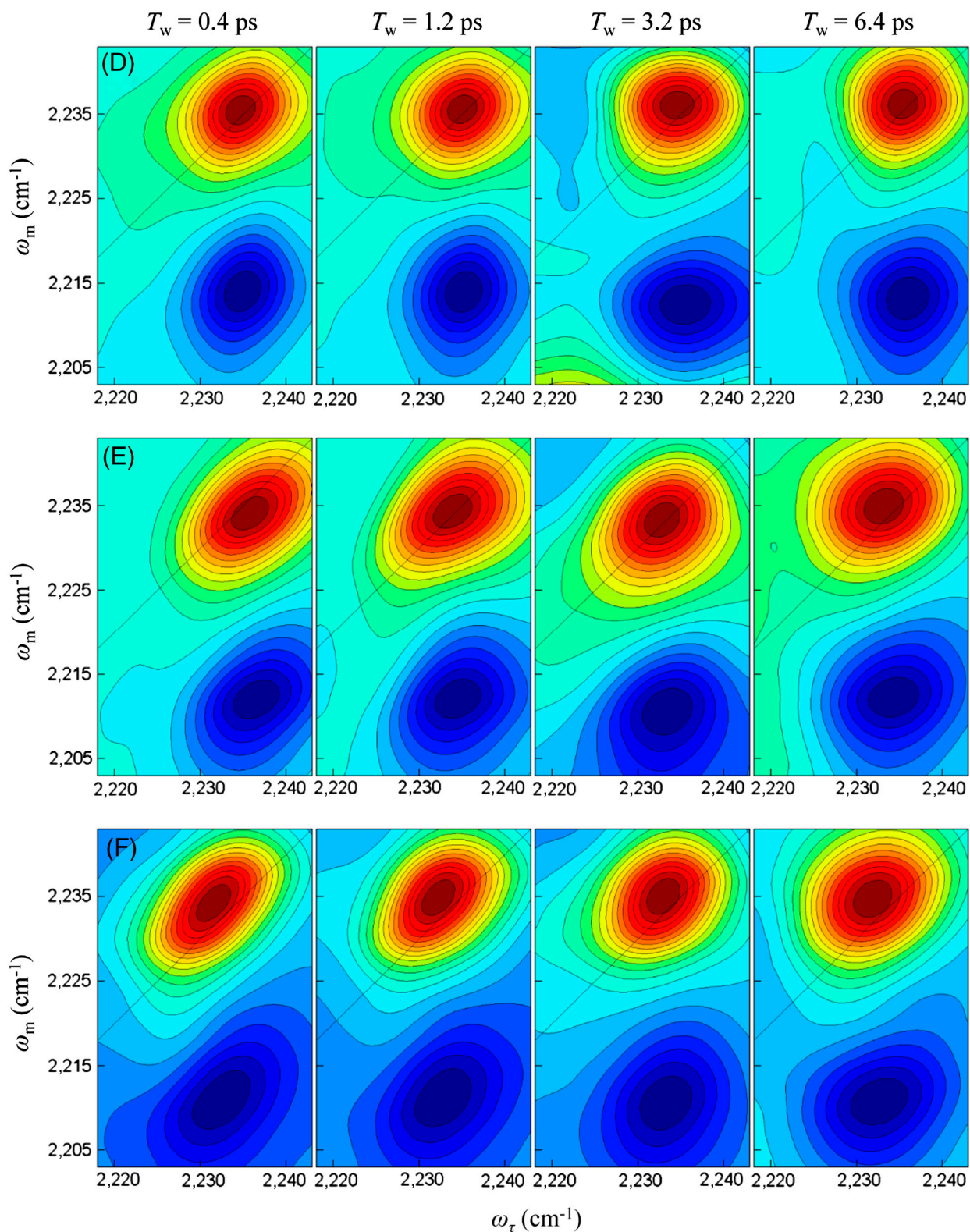
Fig. S3. The time progression of 2D IR spectra of CN stretch of PheCN in various solvents at the waiting times (T_w) of 0.4, 1.2, 3.2, and 6.4 ps. (*D*) PheCN in water; (*E*) PheCN in 0 M GuHCl glycerol/water; (*F*) PheCN in 6 M GuHCl glycerol/water.

Table S1. Solvent conditions used for the experiment

[GuHCl] (M)	% glycerol (vol/vol)	Viscosity (cP)
0	25	2.7 ± 0.1
4	14	2.6 ± 0.1
6	10	2.7 ± 0.1

All measurements were performed at room temperature. The viscosity of each sample was measured using a calibrated Ostwald viscometer.

Table S2. Center frequency and FWHM values of CN stretch of HP35-$(CN)_2$ and PheCN in various solvent conditions

	[GuHCl] (M)	Center (cm^{-1})	FWHM (cm^{-1})
HP35-$(CN)_2$ in water	—	2234.5 ± 0.2	13.5 ± 0.2
HP35-$(CN)_2$ in viscosity-normalized glycerol/water	0	2234.4 ± 0.2	13.6 ± 0.2
	4	2234.0 ± 0.2	13.0 ± 0.2
	6	2233.8 ± 0.2	13.0 ± 0.2
PheCN in water	—	2236.8 ± 0.2	9.3 ± 0.2
PheCN in viscosity-normalized glycerol/water	0	2236.3 ± 0.2	12.2 ± 0.2
	4	2235.2 ± 0.2	12.2 ± 0.2
	6	2234.8 ± 0.2	12.2 ± 0.2

The viscosity of the viscosity-normalized solvents was 2.7 cP.