Supplementary Information

Structural polymorphs suggest competing pathways for the formation of amyloid fibrils that diverge from a common intermediate species

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Supplemental Methods

Fitting of Diagonal Slices. Diagonal slices were fit to a sum of pseudo-Voigt functions.[1] The unlabeled region of the spectrum was fit with two functions: one centered around 1620 cm^{-1} with a FWHM of 10-15 cm^{-1}, which corresponds to the amyloid β-sheet mode, and one centered around 1630-1640 cm^{-1} with a FWHM of 30-60 cm^{-1}, which accounts for disordered structures and scatter. These were added together to create the green trace shown in Figures 1, 3, and S2. The isotope-labeled peaks were each fit with their own functions, which are shown in either red or blue. The frequencies of the isotope-labeled peaks vary between 1570 and 1594 cm^{-1}, depending on the extent of coupling at that position. Frequency and FWHM parameters for the isotope-labeled peaks are given in the Table S1. For quantitative comparison of the polymorph populations in Figure 3, the FWHM of the fits for the isotope-labeled modes were held constant across all scans. A FWHM of 7.91 cm^{-1} was used for the lower frequency mode (blue trace) and a FWHM of 10.14 cm^{-1} was used for the higher frequency mode (red trace). The peak area was determined for both modes and used to calculate the low-frequency to high-frequency peak area ratio discussed in the main text.

Table S1. Fitting parameters for the various isotope-labeled positions.

Label position	**Frequency (cm^{-1})**	**FWHM (cm^{-1})**
Val17	1583	19.76
Phe23	1593	13.95
Ala25	1570 / 1585	7.91 / 10.14
Ala25 – 2.5% HFIP	1586	14.98
Ala25 – 1:3 isotope dilution	1593	21.91
Gly24	1586	13.55
Leu27	1580 / 1594 (weak)	21.91 / 8.80
Gly33	1583 / 1593 (weak)	11.43 / 8.04

Supplemental Data

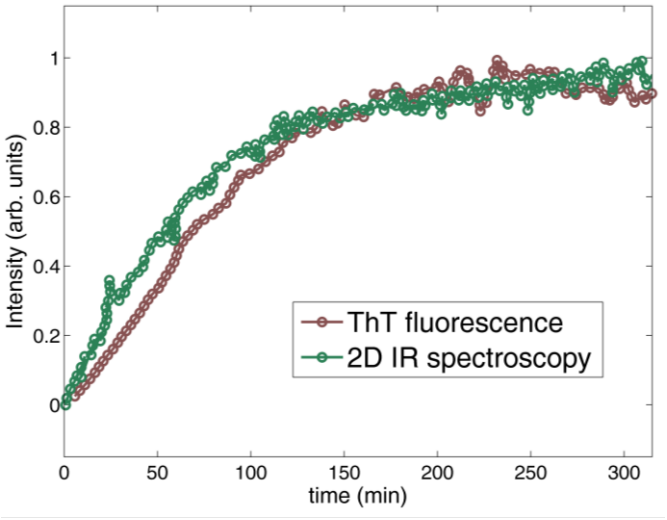

Figure S1. Intensity changes of the unlabeled β-sheet feature extracted from 2D IR spectra and ThT fluorescence plotted as a function of aggregation time.

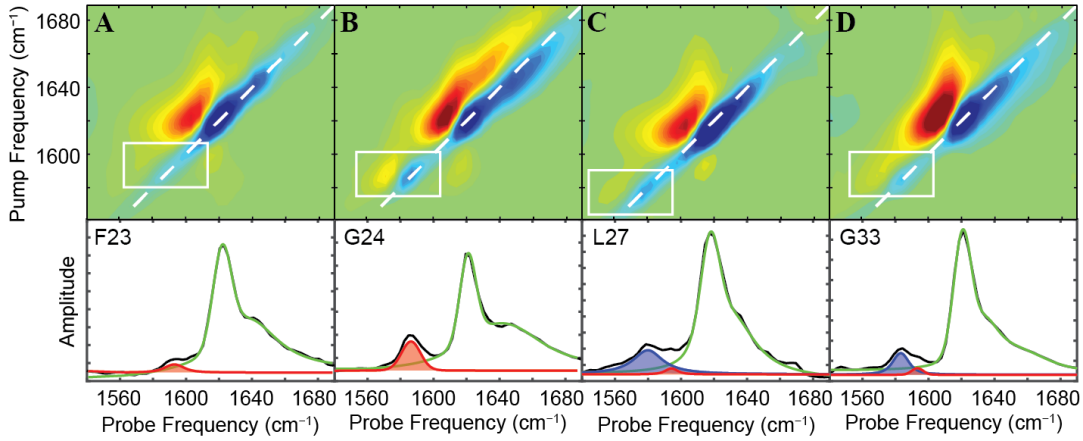

Figure S2. 2D IR spectra measured for four labels not discussed in the main text: Phe23, Gly24, Leu27 and Gly33. All spectra show a single isotope peak, contrary to Ala25. Spectra were measured in the absence of macrocycles. This data was previously reported in Buchanan, L. E., *et al*. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 19285, but additional analysis has been performed for this study.

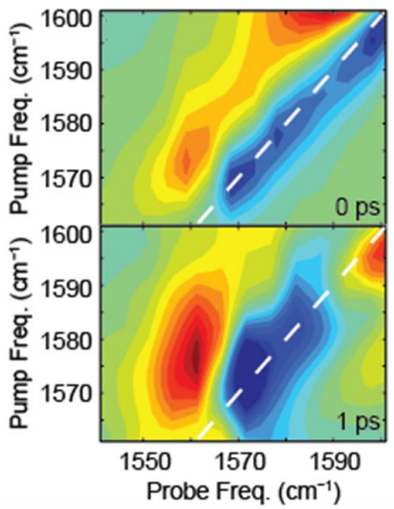

Figure S3. 2D IR spectra of Ala25 isotope modes at 0 ps (top) and 1 ps (bottom) waiting time.

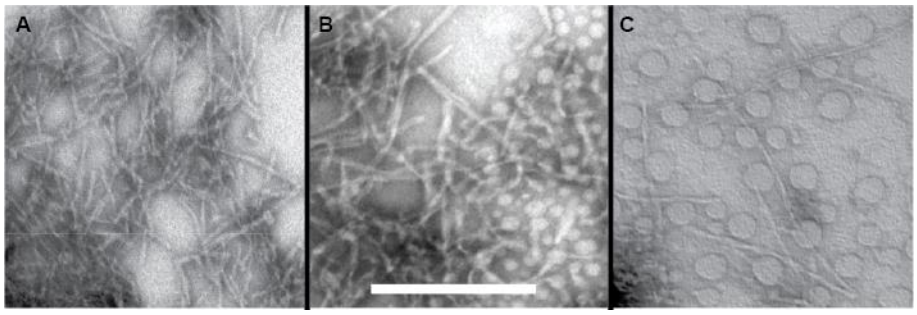

Figure S4. TEM images of hIAPP fibrils**.** TEM images of equilibrated fibrils of (A) pure hIAPP and hIAPP fibrils formed in the presence of (B) seeding macrocycle $Mac_{26\text{-}32}$ and (C) inhibiting macrocycle $Mac_{21\text{-}27}$. The large spheres are likely aggregated macrocycles (2). The scale bar is the same for each image and represents 200 nm. This data was previously reported in Buchanan, L. E., *et al*. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 19285.

Reference

(1) Ida, T., Ando, M., and Toraya, H. (2000) Extended pseudo-Voigt function for approximating the Voigt profile. *J. Appl. Crystallogr. 33*, 1311–1316.