Tracking Fiber Formation in Human Islet Amyloid Polypeptide with Automated 2D-IR Spectroscopy

*David B. Strasfeld, Yun L. Ling, Sang-Hee Shim and Martin T. Zanni**

University of Wisconsin—Madison, Department of Chemistry, 1101 University Ave., Madison,WI 53703

zanni@chem.wisc.edu

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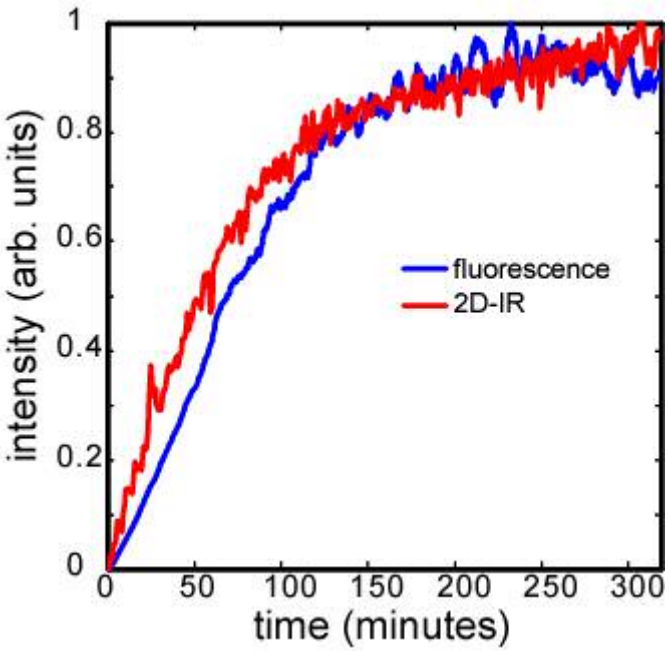
Supporting Information

Sample Preparation. We prepare the samples using a modified procedure by Jayasighe and Langen,[1] to remove amyloid seeds and initiate fiber formation. We denatured commercially obtained hIAPP (BaChem) in deuterated hexafluoro-isopropanol (d-HFIP). An aliquot of the d-HFIP stock solution was dialysized in 0.1 mM DCl and D_2O, respectively, to remove residual TFA. The sample was then lyophilized, re-dissolved in HFIP to denature the peptides, and quickly dried. Aggregation was initiated by adding D_2O (with no salt) at a pH of 6 and a concentration of 1 mM. The aggregating hIAPP/D_2O solution was divided into three samples so that aggregation could be monitored with 2D-IR, FTIR and fluorescence spectroscopies concurrently. These experiments were performed at room temperature (295 K). The earliest 2D IR spectra collected had no β-sheet content. We estimate, given the initial intensity of the 1618 cm^{-1} peak in the 2D IR spectra, that β-sheet seeds cannot comprise more than 1.9% of the sample when aggregation was first initiated, if seeds are present at all.

Automated 2D-IR spectroscopy. The aggregation process, which took place between two CaF_2 windows separated by a 56 μm Teflon spacer, was observed by the rapid scanning of two phase-cycled pulse sequences. We create these 2D-IR pulse sequences using a Ge acousto-optic modulator (AOM) based pulse shaper, as has been reported previously.[3,4] Briefly, femtosecond mid-IR pulses were initially generated by difference frequency mixing (DFM) the two femtosecond near-IR outputs of a BBO-based optical parametric amplifier (OPA). A ruled grating (150 g/mm) was then used to disperse the mid-IR pulses into the frequency domain. The Ge AOM was used to modulate the phase and intensity of the frequency profile so as to create the desired, phase cycled pulse pair. The pump pulse phases were incremented by $\Delta\varphi_1 = \Delta\varphi_2 = \pi/20$ for each τ delay; however, the relative phase between the pump pulses was held constant, $\varphi_1 - \varphi_2 = 0$. Spectra were taken by scanning over 1.92 ps of the first coherence time in 9 fs steps. Rather than implementing a pump-probe experiment in the most traditional sense, so that $\Delta OD = -\ln(\text{transmission}_{\text{pump on}}/\text{transmission}_{\text{pump off}})$, to improve signal to noise, we took the difference in transmission generated from two pump pulse trains with absolute phase shifted by π, so that $\Delta OD = -\ln(\text{transmission}_{\text{pump on, }\varphi=0}/\text{transmission}_{\text{pump on, }\varphi=\pi})$. Thus, it took 426 different pulse shapes to measure a single 2D IR spectrum, which is accomplished in 0.43 seconds at a 1 kHz repetition rate. A running

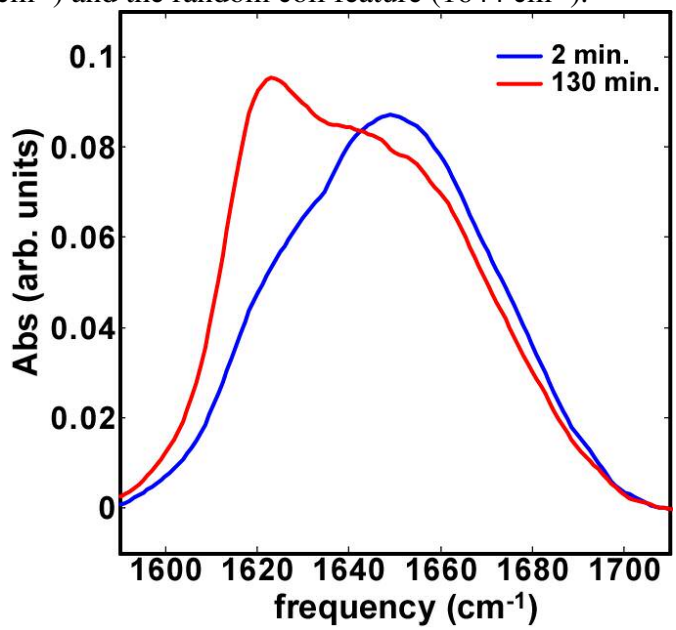
Figure S1. The change in intensity of the fundamental, anti-symmetric β-sheet feature and the change in ThT fluorescence intensity are plotted as a function of aggregation time.



average was then performed until adequate signal to noise was achieved. The spectra shown in Fig. 1 of the text have been averaged over a 5 minute window while the 2D IR spectra used for the kinetic traces are averaged over a 1 min. window. There is a ~2 min. dead-time between initiation of the folding and the collection of the first 2D IR spectrum.

FTIR Measurements. Concurrent FTIR data was taken during aggregation using identical sample cells composed of CaF_2 windows separated by a 56 μm Teflon spacer. We do not report the concurrent FTIR spectra here because the signal-to-noise was insufficient for quantitative analysis. Fiber formation causes background scatter in FTIR spectra, but scatter cannot be suppressed with phase cycling like it is

Fig. S2. The amide I region of FTIR spectra taken at t=2 and t=130 min. after initiation of hIAPP aggregation is shown. Considerable overlap occurs between the β-sheet anti-symmetric feature (1618 cm^{-1}) and the random coil feature (1644 cm^{-1}).

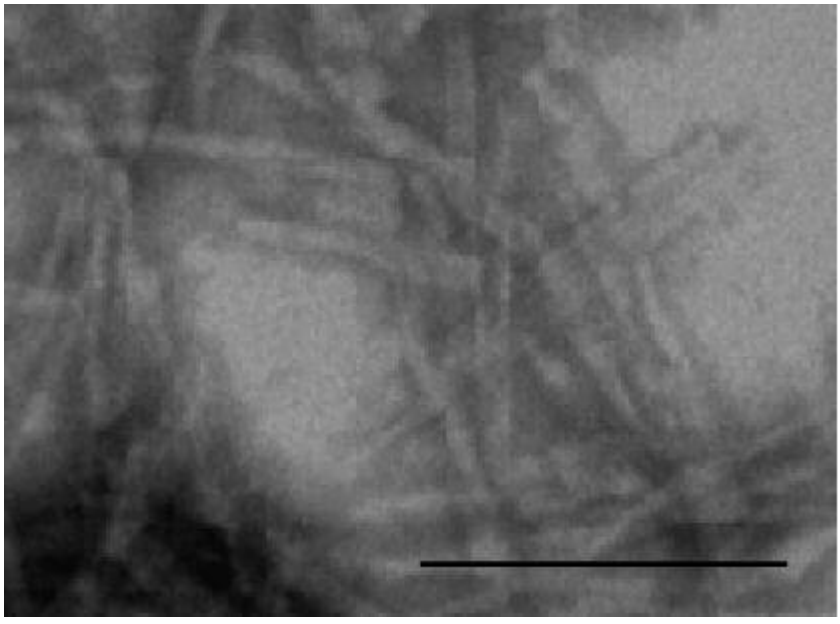


in our automated 2D IR spectrometer. Nonetheless, we show in Fig. S2 the FTIR spectra collected at t=2 and t=130 min. after folding. This data set has better signal-to-noise, but was collected from a different kinetics scan and so is not directly comparable to the kinetics reported with 2D IR spectroscopy. However, it does illustrate a few advantages of 2D IR over FTIR spectroscopy. The random coil and β-sheet features are strongly overlapped in the FTIR, which makes it difficult to quantitatively assess their linewidths, frequency and intensity changes. Even the kinetics are harder to measure, since the random coil intensity only decreases 9.5% during folding. The difference in relative intensity changes is a consequence of 2D IR spectroscopy being a third-order technique, which means that it scales as $|\mu|^4$, where μ is the transition dipole strength. FTIR scales as $|\mu|^2$. Thus, transition dipole changes, which are a strong indication of structure change, are better highlighted in the 2D IR spectra.

Fluorescence Measurements. Concurrent fluorescence data was taken during aggregation using a Hitachi F-4500 fluorescence spectrometer. The fluorescence shift due to binding of Thioflavin T to *β*-sheets was measured with excitation wavelength at 450 nm and emission at 482 nm. The fluorescence data and growth of the β-sheet peak are compared in Fig. S1, where it is seen that the β-sheet peak in the 2D IR spectrum rises more quickly than does the fluorescence.

TEM Measurements. Transmission electron microscopy images were taken of the 5 μl aliquot used to generate the 2D-IR spectra after aggregation was run to completion. The aliquot was loaded onto a polyvinyl butyral coated copper grid and stained with methylamine tungstate (Nanoprobes, Inc.). Excess stain was blotted off and the sample was allowed to air dry. Images were acquired using a Philips CM120 microscope with an accelerating voltage of 80 kV (Fig. S3). A mean diameter of 10.7 ± 1.6 nm was observed, which agrees well with previously reported values.[5]

Figure S3. A representative TEM image of the hIAPP fibers formed during aggregation. The scale bar corresponds to 100 nm.



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