Supporting Information For
Kinetic Profile of Amyloid Formation in the Presence of an Aromatic Inhibitor by Nuclear Magnetic Resonance

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MATERIALS AND METHODS

Preparation of stock solutions of IAPP and curcumin

IAPP was purchased from NeoBioSci (Cambridge, MA). Aggregate-free IAPP in buffer was prepared using a protocol similar to that reported by O'Nuallain and coworkers.[1] Preexisting aggregates were removed by dissolving IAPP in 100% hexafluoroisopropanol (HFIP) (Sigma). After 30 minutes of incubation, HFIP was evaporated under a gentle stream of N_2 gas. To ensure that all HFIP is removed, the sample was lyophilized overnight. IAPP was then dissolved in ice-cold buffer containing 50 mM KH_2PO_4 and 100 mM KCl (pH 5.4). To remove undissolved peptide and large aggregates, the samples were centrifuged at 16000 × *g* for 30 s, followed by careful transfer of the supernatant into clean tubes. The concentration of IAPP in the final supernatant was determined by UV absorbance at 214 nm as described elsewhere.[2]

The fragment IAPP(11-25) containing the S20G mutation (Acetyl-RLANFLVHSGNNFGA-NH_2) was synthesized in house by solid-phase peptide synthesis using 9-fluorenylmethoxycarbonyl chemistry, purified by reversed-phase HPLC, and then characterized by mass spectrometry as described in our previous work.[2] NMR samples of IAPP(11-25)S20G were prepared in the same way as described previously.[2] Briefly, the lyophilized peptide was dissolved in ice-cold 10 mM NaH_2PO_4 buffer (pH 4.3) followed by centrifugation at 16000 × *g* for 30 s, and then careful transfer of supernatant to another tube. This sequence of steps was done three times. The peptide concentration was then determined by UV absorbance at 214 nm.[2]

Curcumin was purchased from Acros Organics (Geel, Belgium). Because it is insoluble in water, we prepared stock solutions of the polyphenol in 100% ethanol. Curcumin concentrations were determined by absorbance at 429 nm. The molar extinction coefficient of curcumin at this wavelength is 55000 M^{-1} cm^{-1}.[3]

Mass Spectrometry

Mass spectra of phosphate-buffered acidic solutions containing curcumin were obtained using a Finnigan LTQ linear ion trap mass spectrometer (Thermo Fisher Scientific, MA) housed in the core mass spectrometry facility of the University of Massachusetts Medical School.

CD spectroscopy

All dichroic spectra were recorded at 4 °C using a JASCO J-815 spectrometer. Stoppered quartz cuvettes with a path length of 1mm were used for all samples. Spectra were acquired by taking readings every 1 nm with averaging time and bandwidth set at 4 s and 1 nm, respectively. All samples were incubated in the cuvettes and kept at 4 °C in between acquisition of spectra.

NMR spectroscopy

All NMR spectra were recorded at 4 °C using a 600 MHz Varian INOVA spectrometer. Water suppression in one-dimensional ^{1}H NMR spectra was accomplished by presaturation. Parameters used include 1400 transients, relaxation delay of 2 s, and a saturation delay of 3 s. All spectra were processed using a line broadening of 0.3 Hz. Chemical shifts are reported relative to the methyl resonance of 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) at 0 ppm. The peak area of the methyl peak of DSS which does not change with time was arbitrarily assigned a value of 1. The peak areas in the backbone amide (7.9-8.9 ppm), sidechain amide (6.8-7.2 ppm and 7.5-7.8 ppm), and methyl (0.8-1.0 ppm) regions were then measured and the average of the three values determined. The y-values for the kinetic plot shown in Figures 3B and 3C were then determined as follows:

$$\frac{average\ at\ day\ x}{average\ at\ day\ 0} \times 100$$

Total correlation spectroscopy (TOCSY) spectra of IAPP(11-25)S20G in the absence or presence of curcumin in ethanol were obtained with a mixing time of 80 ms using the MLEV-17 spin-lock sequence for coherence transfer.[4] All cross peaks were identified according to our assignments published elsewhere.[2] Volumes of the NH → H^{α} and NH → H^{β} cross peaks were measured relative to that of the methyl resonance of

DSS which was arbitrarily set to 1. The average of the cross peak volumes from all residues on day *x* was then determined. This was divided by the corresponding average at day 0, multiplied by 100, and then plotted against incubation time. All NMR samples were stored at 4 °C in between acquisition of spectra.

Transmission electron microscopy of IAPP assemblies

Aliquots taken from the NMR samples (~ 7 μL) were spotted on carbon-coated copper grids, incubated for two minutes and then stained with 1% uranyl acetate. TEM images were recorded at the Core Electron Microscopy Facility of the University of Massachusetts Medical School.

References

(1) O'Nuallain, B.; Williams, A. D.; Westermark, P.; Wetzel, R. Seeding specificity in amyloid growth induced by heterologous fibrils. *J. Biol. Chem.* **2004**, *279*, 17490-17499.
(2) Liu, G.; Prabhakar, A.; Aucoin, D.; Simon, M.; Sparks, S.; Robbins, K. J.; Sheen, A.; Petty, S. A.; Lazo, N. D. Mechanistic studies of peptide self-assembly: transient α-helices to stable β-sheets. *J. Am. Chem. Soc*. **2010**, *132*, 18223-18232.
(3) Zsila, F.; Bikadi, Z.; Simonyi, M. Induced circular dichroism spectra reveal binding of the antiinflammatory curcumin to human α_1-acid glycoprotein. *Bioorg. Med. Chem.* **2004**, *12*, 3239-3245.
(4) Bax, A.; Davis, D. G. MLEV-17-based two-dimensional homonuclear magnetization transfer spectroscopy. *J. Magn. Reson.* **1985**, *65*, 355-360.

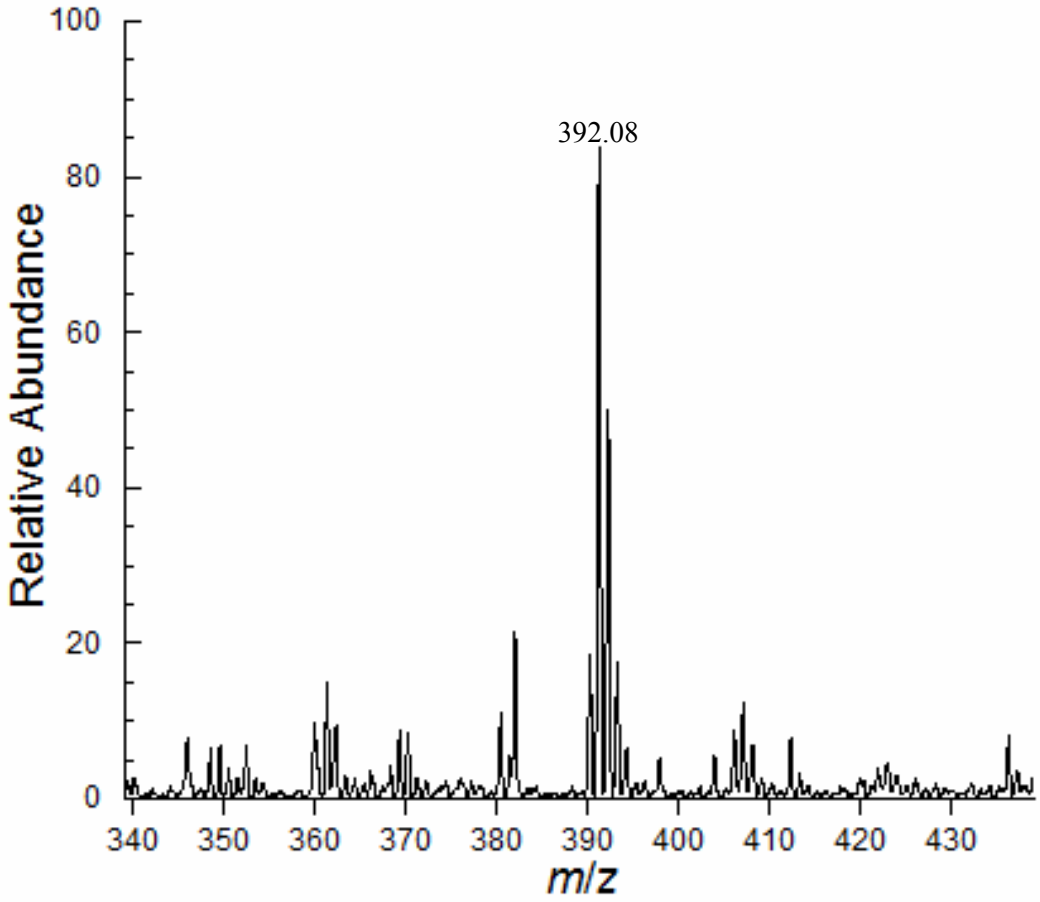

Figure S1. Portion of the electrospray ionization mass spectrum of a sample containing an aliquot of curcumin dissolved in ethanol incubated in the presence of 10 mM NaH_2PO_4 buffer (pH 4.3) for 2 weeks at 4°C. The peak labeled at 392.08 *m*/*z* is due to the ion [curcumin (368 Da) + Na^+]. The high relative abundance of the ion indicates that curcumin does not degrade under the conditions used during the incubation.

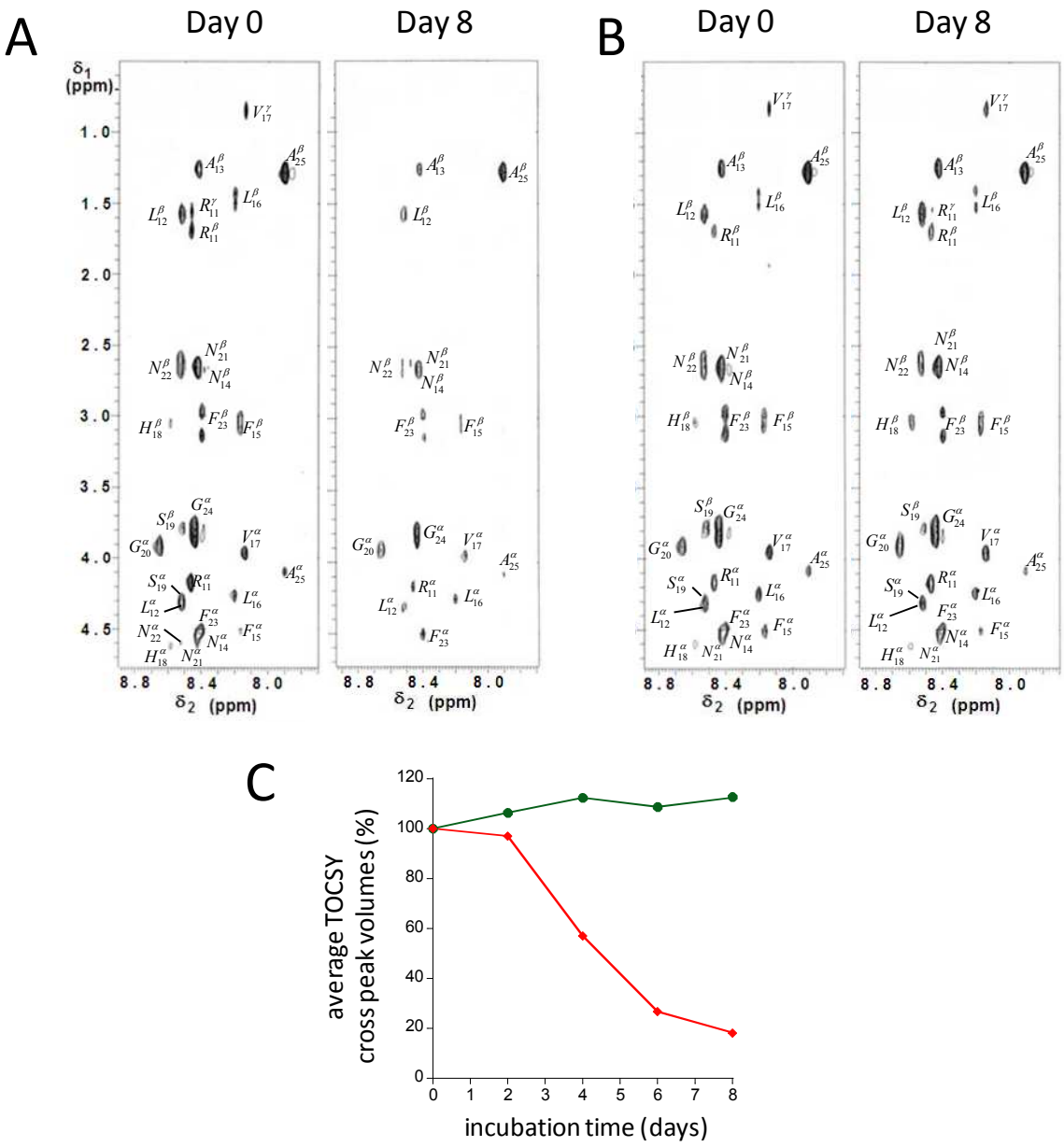

Figure S2. Curcumin inhibits the self-assembly of IAPP(11-25) containing the S20G mutation. (A) The volumes of TOCSY cross peaks of IAPP(11-25)S20G decrease significantly after 8 days of incubation in the absence of curcumin. (B) When the peptide was incubated in the presence of curcumin (1:1, mole:mole), TOCSY spectra taken on day 0 (left) and day 8 (right) are essentially identical. (C) The average TOCSY peak volumes were calculated and plotted against incubation time. The cross peak volumes of the peptide in the absence of curcumin (red) decreased slightly during the first two days, followed by a rapid decrease between day 2 and day 6, consistent with peptide self-assembly. On the contrary, the cross peak volumes of the peptide in the presence of curcumin (green) remained essentially unchanged during the same period of time.

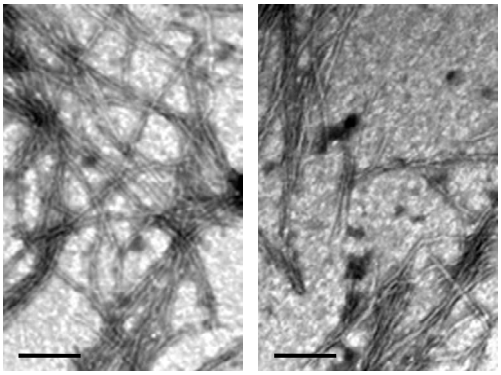

Figure S3. Curcumin did not inhibit fibril formation of IAPP. Transmission electron microscopy (TEM) images show that assemblies are formed after 44 days of incubation in the absence (left image) and presence (right image) of curcumin. IAPP in the absence of curcumin formed mature fibrils of indeterminate lengths. In the presence of curcumin, a mixture of mature and short fibrils (~160 nm long) was formed. Bar corresponds to 100 nm.