Electronic Supplementary Material (ESI) for Chemical Communications

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A foldamer approach to targeting membrane bound helical states of islet amyloid polypeptide

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Experimental Section:

Materials and Methods:

Synthesis of pentapyridyl (**3**) was reported earlier.[1] Compound (**2**) was synthesized using a solid phase submonomer approach.[2] The monomer used in the synthesis was β-alanine tert-butyl ester (Sigma Aldrich, St. Louis, Missouri). Monomer was introduced on bromoacetic acid 2-chlorotrityl resin (1.4 mmol/g) from Novabiochem (Now EMD Millipore Chemicals, USA). Elongation was achieved using bromoacetic anhydride (Acros Organic). The cleavage of O-tert butyl ester and solid phase were achieved simultaneously using TFA cocktail [Trifluoroacetic acid (TFA)/ triethylsilane (TES)/H_2O (95:2.5:2.5, v/v)] (both TFA and TES from Sigma Aldrich, St. Louis, Missouri). The pentapeptoid (**2**) was then purified using reverse phase HPLC (Vydac, C18 resin). Confirmation of (2) was achieved using MALDI-TOF and will be reported in near future.

Synthesis of pentaquinoline (**1**) was achieved using linear solution phase iterative amide coupling as reported earlier for the shorter homologues. The starting material, methyl 8-nitro-(1H)-4-quinolinone-2-carboxylate, was generated using known procedure[3, 4, 5] which is then functionalized by introducing O-tert butyl ester as side chain. Chain elongation was achieved using successive amide coupling and nitro group reduction steps following a solution phase approach. The acid groups, which were protected as tert-butyl esters, were cleaved in the last step using a TFA cocktail [TFA/TES/DCM (dichloromethane) (95:2.5:2.5, v/v)] to give the target compound. The final compound was purified by HPLC and characterized for identity and purity (> 98%) using NMR, HPLC, and MALDI-TOF and will be reported in near future.

ThT was purchased from Acros Organics (Fair Lawn, New Jersey). Both the lipids [dioleoylphosphatidylglycerol (DOPG) and dioleoylphosphatidylcholine (DOPC)] were purchased from Avanti Polar Lipids, Inc. (Alabaster, Alabama). Human IAPP (hIAPP) was synthesized by *t*-Boc methods and purified by the W. M. Keck facility (New Haven, Connecticut). hIAPP stock solutions were prepared using methods adapted from previous work in our lab.[6] Briefly, IAPP (~3 mg) was resolubilized in 7M guanidinium solution. The solution was filtered (0.2 micron filter) transferred to C-18 spin column, washed two times with 10% acetonitrile , 0.1% TFA in water and then two times with milliQ water and then eluted with 50% each of acetonitrile and water (200 µL) and split into aliquots. These aliquots were then lyophilized and stored at -80 ºC as powder and used fresh for every experiment. IAPP concentration was determined by absorbance measurements at 280 nm ($\varepsilon = 1400\ M^{-1}cm^{-1}$).

Unilamellar liposomes were prepared by extrusion through a 100 nm extrusion filter. A 20 mg/mL solution of lipid, 50% DOPG:DOPC was passed through the extrusion film 21 times using a syringe extrusion device. This solution was used to prepare a buffer that would result in a final lipid concentration of 500 µg/mL for each kinetic run. The final concentration of lipid was also confirmed by measurement of total phosphorous[7, 8] which was within 0-2% error limit. All measurements were made in 50 mM sodium phosphate buffer at pH 7.4, 100 mM KCl, and 23°C.

Lipid-Catalyzed Amyloid Formation Kinetics: The liposome stock solution in buffer (500 µg/mL) and 20 µM ThT were added to a Microfluor 1 black 96-well plate (Thermo Electron Corp). This was followed by addition of the small molecule inhibitors dissolved in DMSO (final

DMSO concentration: 0.2%) and gentle mixing. Fiber formation was initiated by addition of hIAPP stock solution to a final concentration of 10 μM. Final volumes were 200 μL. Formation of amyloid was monitored by ThT fluorescence (Ex 450 nm and Em 485 nm) using a FluoDia T70 fluorescence plate reader (PTI) except the experiment where pentaquinoline was added in IAPP at different time frames (Fig. 3). Data for Fig. 3 was performed on QuantaMaster C-61 fluorescence spectrometer (PTI). Kinetic experiments (Fig. 1 and 2) were performed in triplicate, using a fourth well without hIAPP as a control blank. The data were blank subtracted, renormalized to the maximum intensity of a standard hIAPP. Reaction profiles were fit using the built in sigmoidal fit on Origin 5.0 separately for each run. This was used to extract separate t_{50} values from which average and standard errors were calculated. All presented fits were normalized for clarity in the main text, and are shown uncorrected in the supplement.

Figures and Tables:

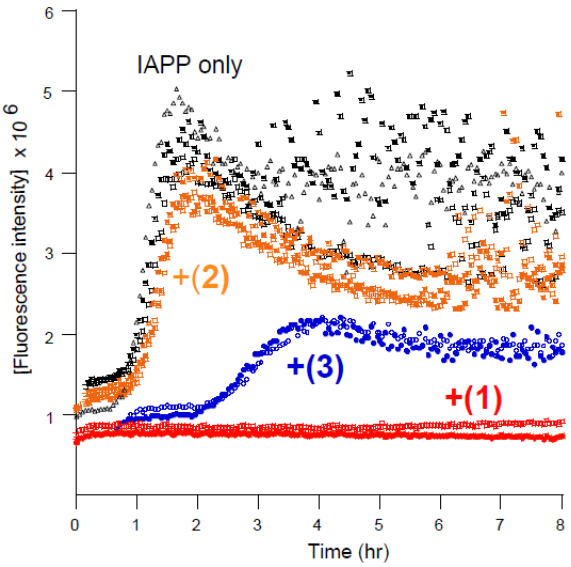


Fig. S1. Representative plots for raw fluorescence from ThT for inhibition of fiber of IAPP in the presence of the indicated ligands. Triplicate experiments are shown for each compound. Data was collected as described in legend to Fig. 1.

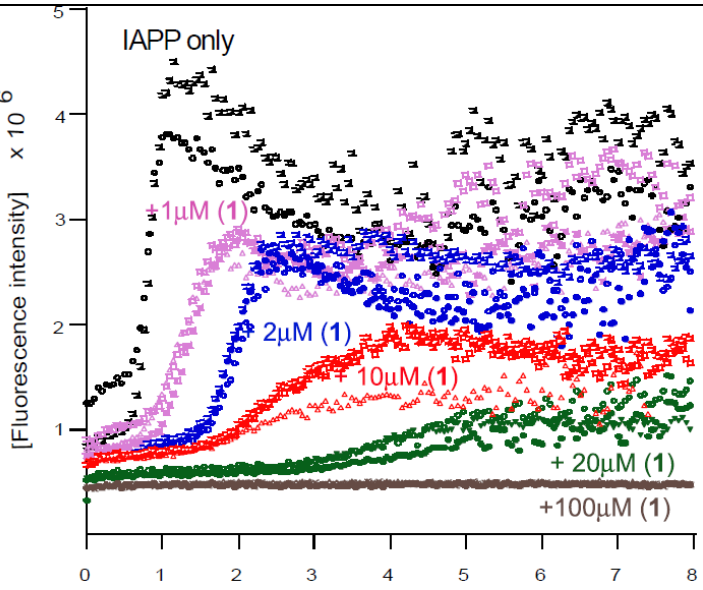
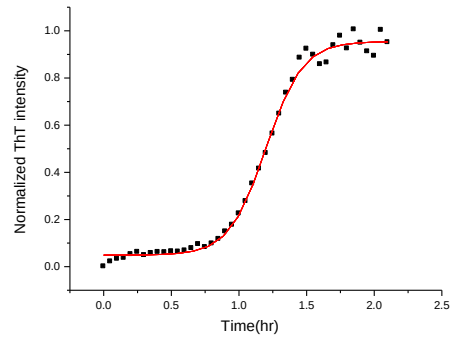
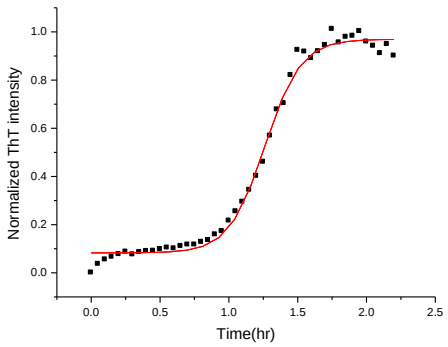


Fig. S2. Representative plots for raw fluorescence from ThT assay for inhibition of fiber of IAPP in the presence of the indicated concentrations of pentaquinoline (**3**). Triplicate experiments are shown for each compound. Data was collected as described in legend to Fig. 2.



A



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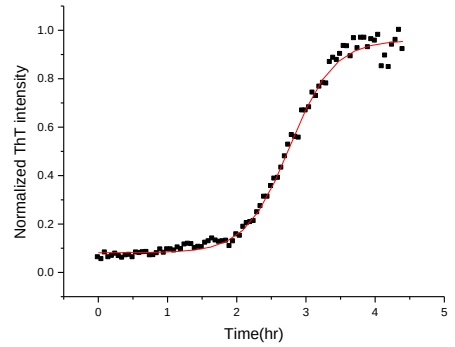
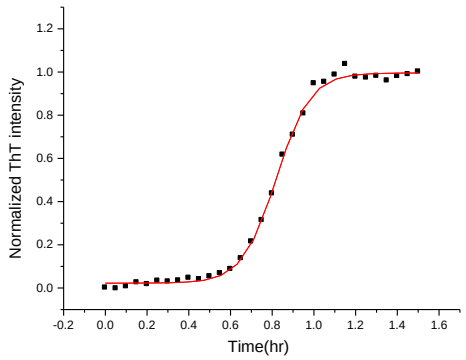


C

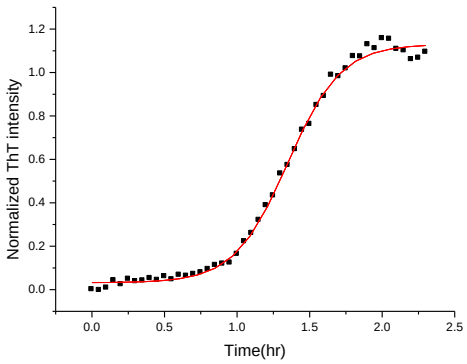
Fig. S3. Graphical presentation of representative sigmoidal curve fits used to calculate t_{50}. Shown are IAPP alone (A), in presence of (**2**) (B), and (**3**) (C). A plot for pentaquinoline (**1**) is not shown because there was no fiber formation observed.

Compound	t_{50} (hr)
IAPP only	1.1±0.1
Pentapeptoid (**2**)	1.2±0.1
Pentapyridyl (**3**)	2.9±0.3
Pentaquinoline (**1**)	NA

Table S1. Statistics of t_{50} values for the inhibition of the fiber formation of IAPP in the presence of various ligands. [IAPP] = 10 µM. [small molecule] = 100 µM.



A



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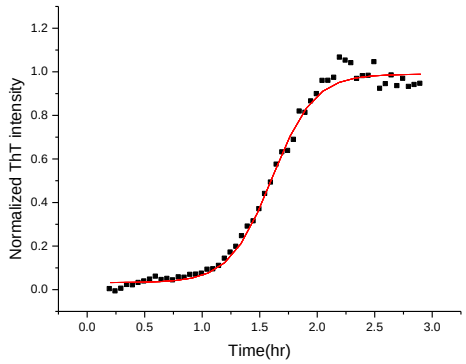

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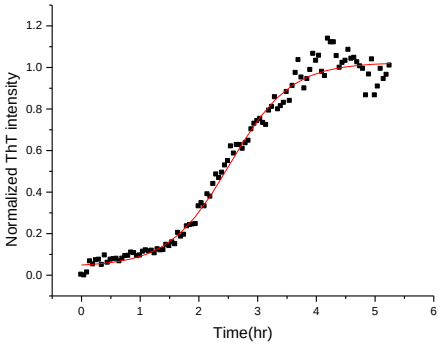

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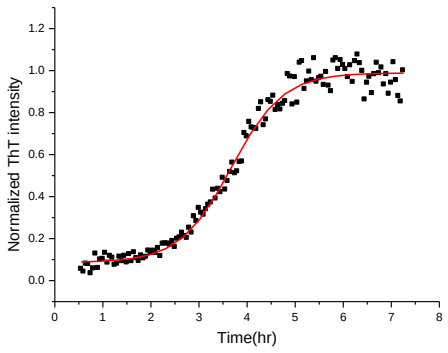

E

Fig. S4. Graphical presentation for the sigmoidal curve fits using Origin 5.0 to determine t_{50} values for representative fiber formation kinetics of IAPP in the presence of (**1**) at (A) 0 μM, (B) 1 μM, (C) 2 μM, (D) 10 μM, and (E) 20 μM. The plot for pentaquinoline (**1**) at 100 μM was not shown because there was no fiber formation observed during the course of the reaction time.

Compound	Concentration (μM)	t_{50} (hr)
IAPP only	NA	0.8±0.1
Pentaquinoline (**1**)	1	1.4±0.1
Pentaquinoline (**1**)	2	1.7±0.2
Pentaquinoline (**1**)	10	2.7±0.2
Pentaquinoline (**1**)	20	4.1±0.3
Pentaquinoline (**1**)	100	NA

Table S2. Calculated t_{50} values for the inhibition of the fiber formation of IAPP in the presence of various concentrations of pentaquinoline (**1**). [IAPP] = 10 μM.

REFERENCES

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