Supporting material for:

Human Islet Amyloid Polypeptide Monomers Form Ordered β-hairpins: A Possible Direct Amyloidogenic Precursor

Author Affiliation:

Nicholas F. Dupuis, Chun Wu, Joan-Emma Shea, and Michael T. Bowers*

Department of Chemistry and Biochemistry, University of California Santa Barbara, Santa Barbara, CA 93106

Appendix 1. ATD and simulation structures of IAPP monomers

Figure S1. ATD of the +4 charge state of human IAPP recorded at elevated ion funnel DC and RF voltages which favors conversion of dehydrated solution structures to solvent free conformers. This leads to annealing of the extended feature reducing its intensity relative to normal ion funnel parameters. It also leads to annealing more of the ions into the most compact structural family with a collision cross section of 616 Å² (which matches the cross section of the solvent free simulation family).

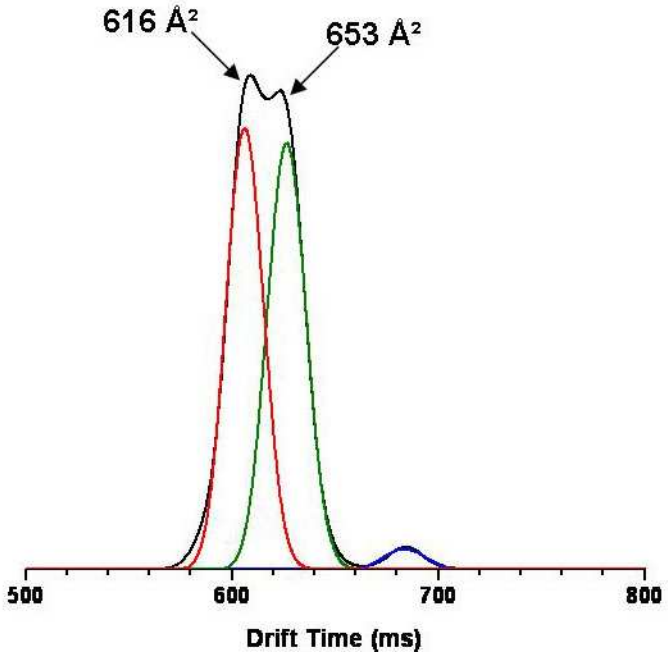


Figure S2. The +4 ATD of human IAPP is shown recorded on a nano-ESI travelling wave ion mobility mass spectrometer. The ATD shows the same two peaks observed in the DC ion mobility results. The mass spectra of the corresponding peaks are included and both show the 0.25 amu spacing indicative of a +4 human IAPP monomers.

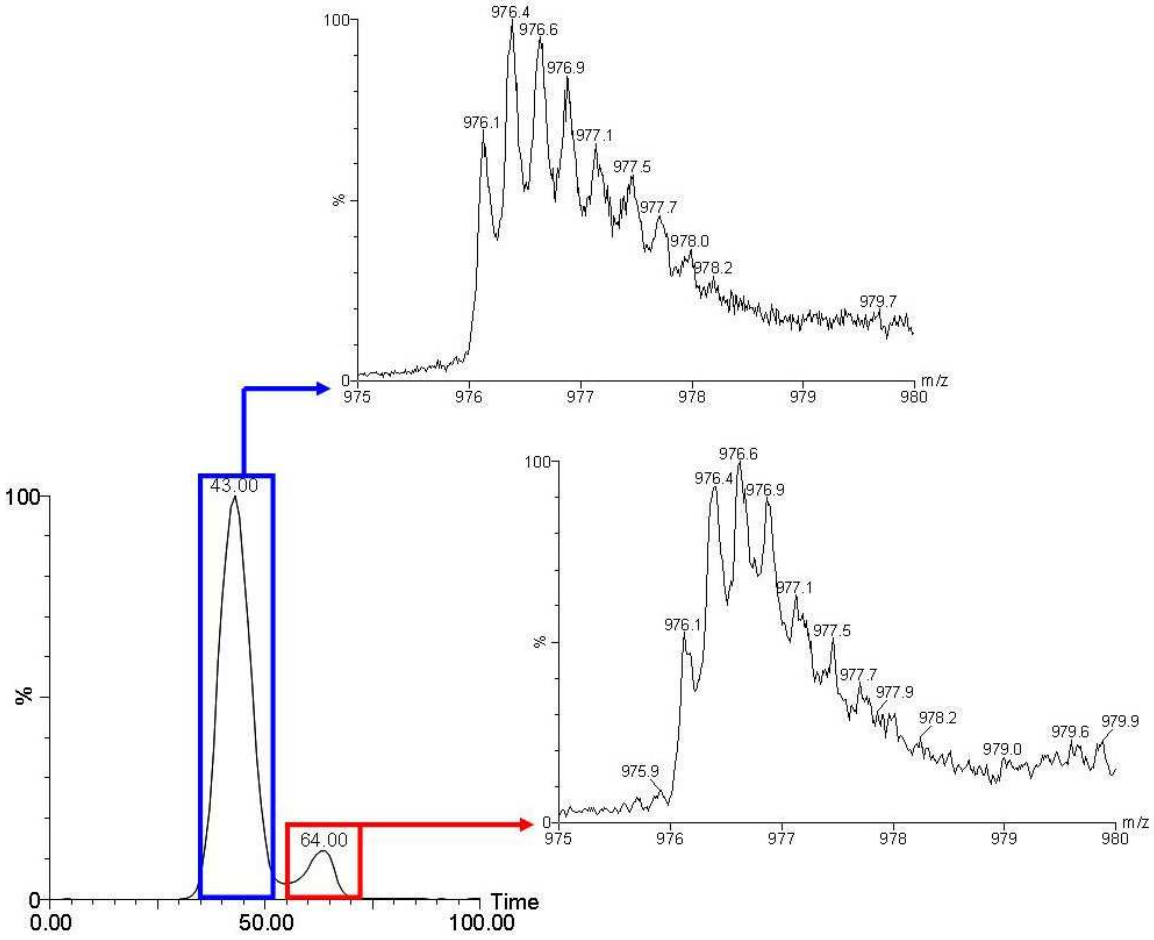
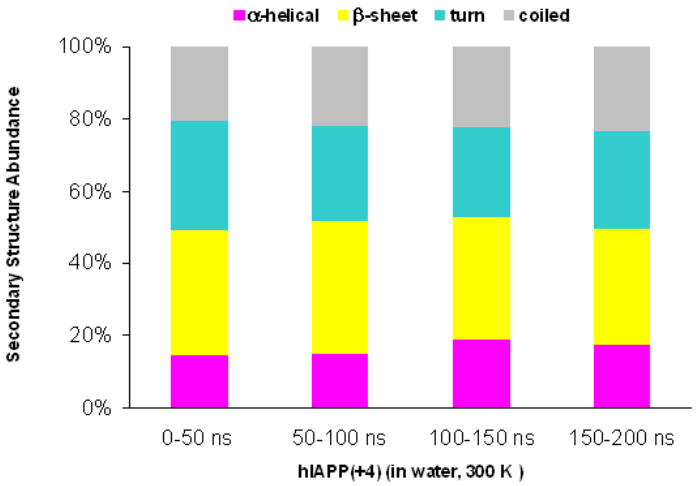
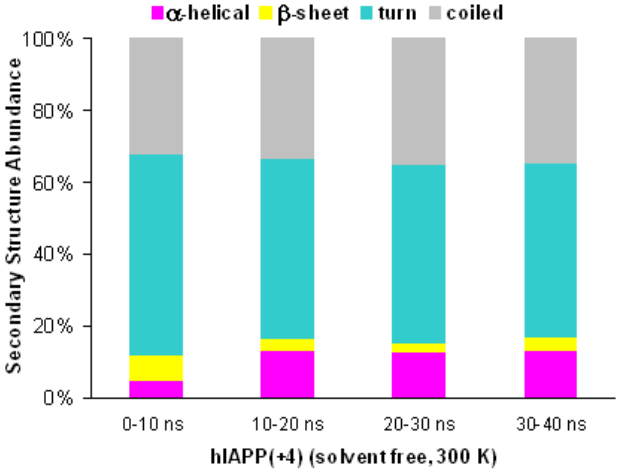


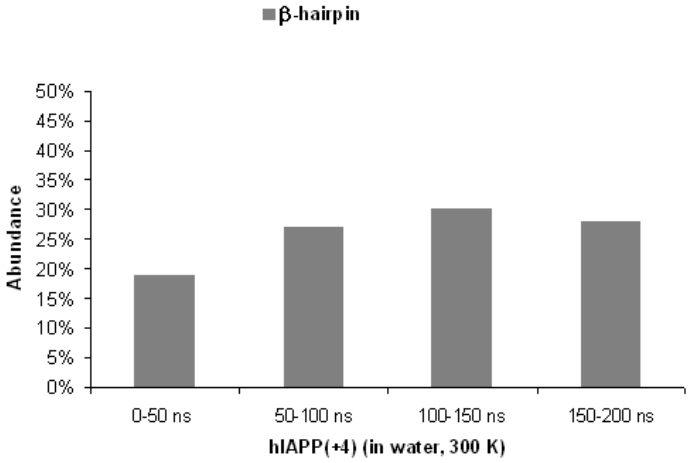
Figure S3 A typical example to show the convergence (within last 100 ns / 20 ns) of the REMD simulations, respectively in water solvent and in solvent-free environment. A total 200 ns / 40 ns sampling of human IAPP(+4) at 300 K are equally divided into four blocks for secondary structure (A-B) and tertiary structure (C-D) analysis. The representative structure of β-hairpin in water solvent is shown in F of Fig. 5, respectively. The representative structure of compact form in solvent free environment is shown in K of Fig. 5.



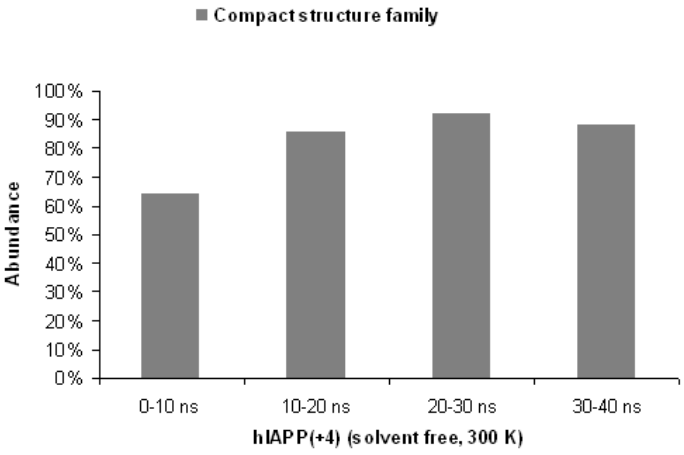
A



B

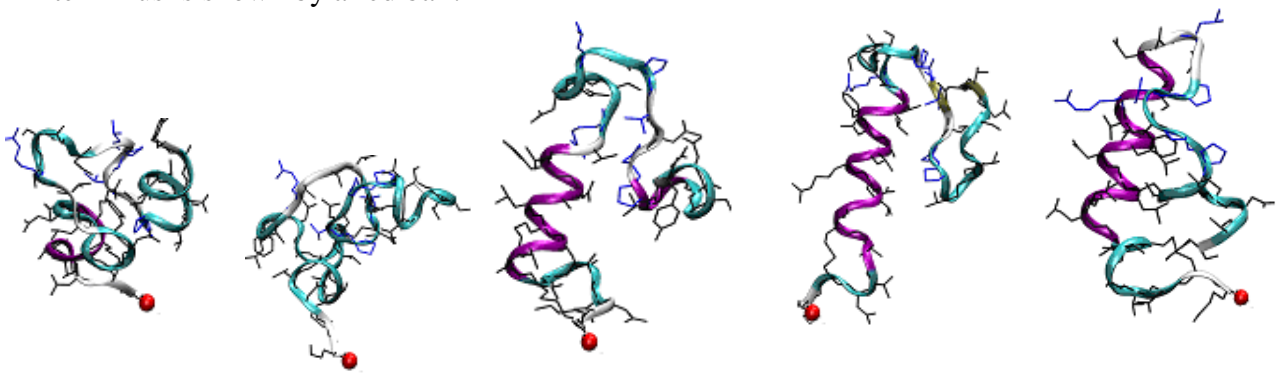


C



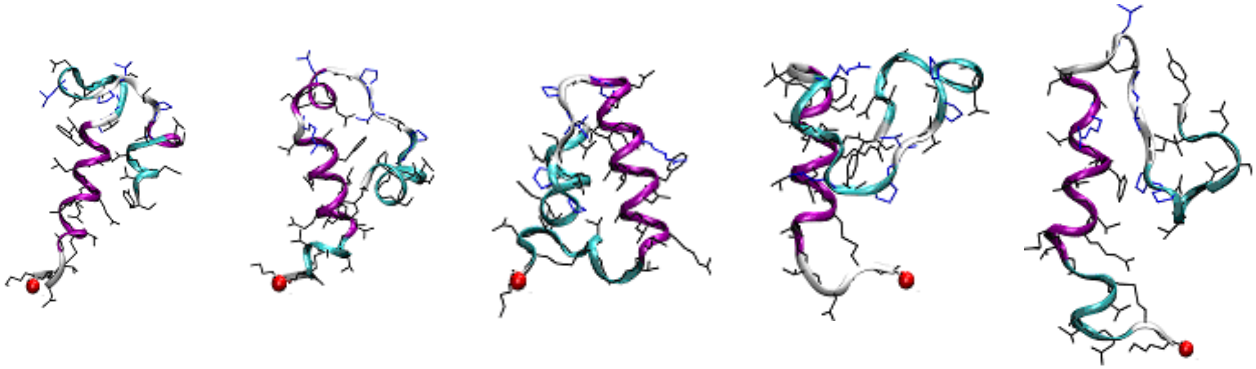
D

Figure S4 Representative solution structures of the top 10 structural families for each peptide from the clustering analysis with a cutoff Cα rmsd of 3.0 Å. A1-B8: rat IAPP(+3) D1-E5: human IAPP (+3); F1-H4: human IAPP(+4). The side chains of the protonated histine of human IAPP(+4) and the 'mutated' residues (R18, L23, P25, V26, P28, P29) of rat IAPP(+4) are in blue. The abundance and the calculated cross section ($Å^2$) are shown in parenthesis. The backbone is in cartoon; α-helical, β-sheet, β-bridged, turn and coiled conformations are colored in purple, yellow, tan, cyan and white respectively. The N-terminus is shown by a red ball.

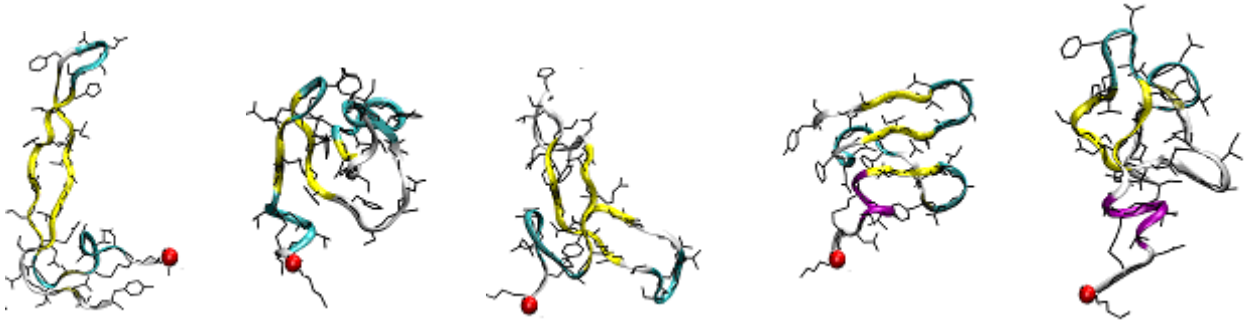


rIAPP(+4)

A1(14%,644) A2(11%,665) B1(10%,657) B2(9%,661) B3(8%,648)



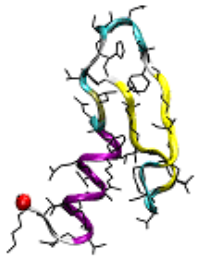
B4(5%, 674) B5(4%,683) B6(4%, 638) B7(3%,673) B8(3%,730)



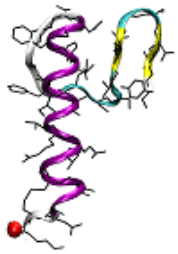
hIAPP(+3)

C1(11%,700) D1(12%,637) D2(7%,620) D3(6%,632) D4(5%,657)

hIAPP(+3)

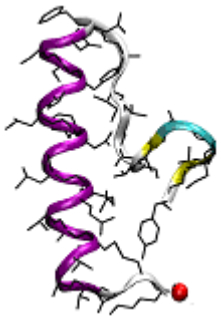


E1(21%,668)

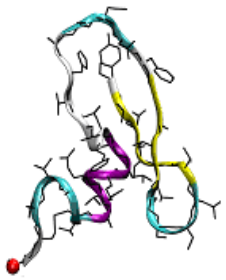


E2(4%,701)

E3(4%,704)

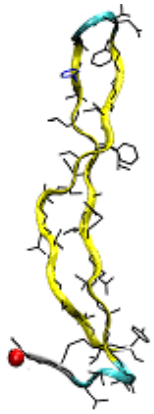


E4(3%,686)

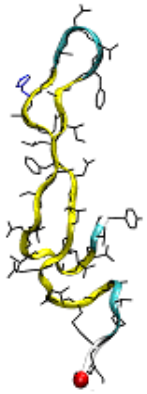


E5(3%,670)

hIAPP(+4)

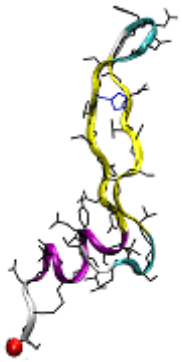


F1(15%,753)

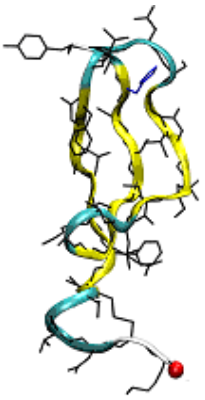


F2(8%,733)

F3(8%,758)

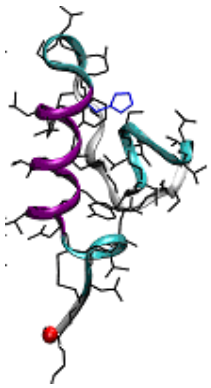


F4(2%,763)



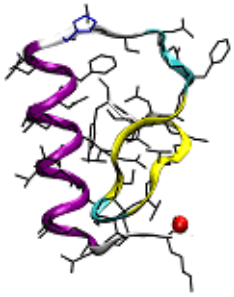
G1(8%,691)

G2(4%,669)

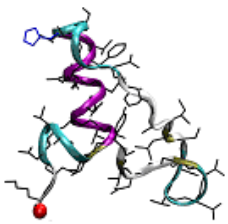


H1(11%,640)

H2(9%,688)

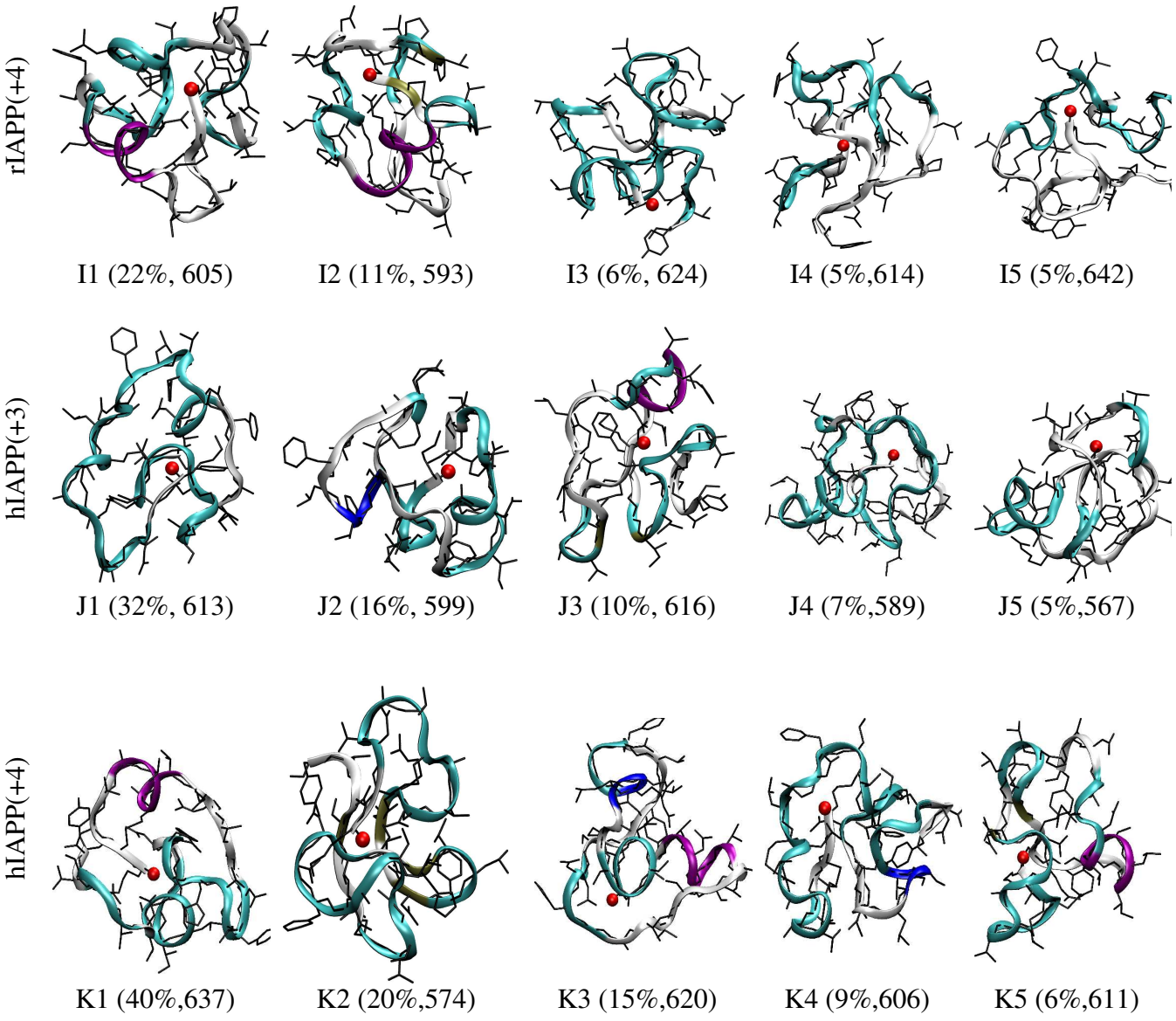


H3(4%,668)



H4(3%,666)

Figure S5 Representative solvent free structures of the top 5 structural families from the clustering analysis with a cutoff Cα rmsd of 3.0 Å. I1-I5: human IAPP (+3) J1-J5: human IAPP(+4) K1-K5: rat IAPP(+4) The abundance and the calculated cross section ($Å^2$) are shown in parenthesis. α-helical, 3-10-helical, β-sheet, β-bridged, turn and coiled conformations are colored in purple, blue, yellow, tan, cyan and white, respectively. The N-terminus is shown by a red ball. The abundance and the calculated cross section ($Å^2$) are shown in parenthesis.



Appendix 2. AFM of human IAPP Fibrils

To confirm that the specific solution conditions used for the mass spectrometry and ion mobility studies were appropriate for amyloid fibril formation, the presence of fibrils investigated using atomic force microscopy (AFM). Briefly, the samples had ~20 μM peptide concentrations in 50 mM Ammonium Acetate buffer at pH 7.4. A 5 μL aliquot of the sample solution was dropped onto a freshly cleaved mica surface and dried under vacuum. The samples were imaged on an Asylum Research MFP-3D-SA atomic force microscope (Asylum Research, Santa Barbara, CA). The AFM was operated in air, at room temperature, in tapping mode with NSCIS/AIBS silicon tips (MikroMasch USA, San Jose, CA). The cantilevers had force constants of ~40 N/m and resonance frequencies of ~300 kHz. The results are given in Figures S6 and S7.

The physical dimensions of the fibrils were characterized using the analysis software provided with the AFM. A cross section of the height image is shown in Figure S7. The cross section trace shows that on average the fibrils are ~60 nm wide and have a height of 6 nm. In the bottom panel of Figure S7 a cross section is shown that is taken down the length of the fibrils. The image shows an axial periodicity of 24-40 nm. The three parameters, height, width and periodicity are all consistent with the values previously reported in the literature.[1-4]

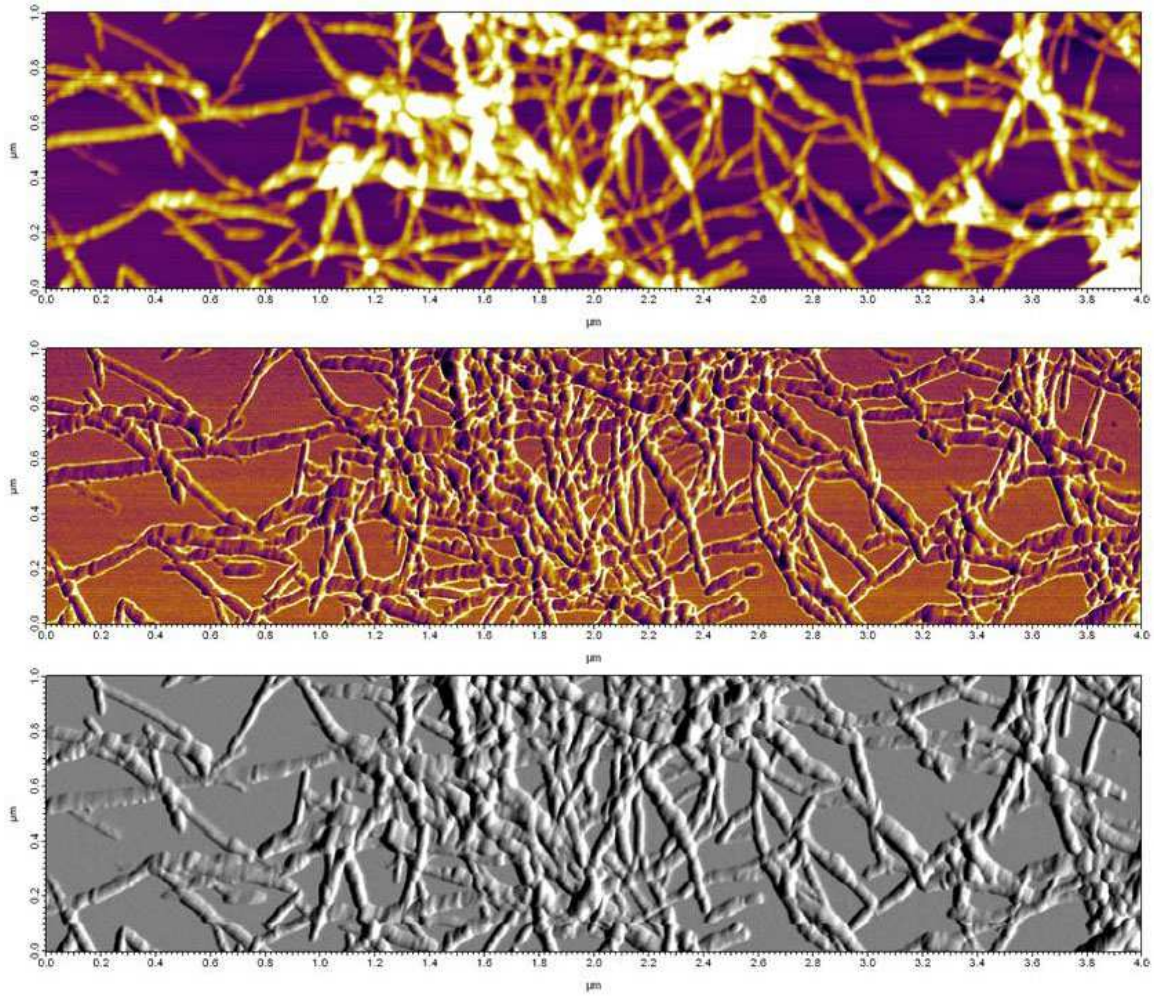


Figure S6. The AFM images of human IAPP fibrils on freshly cleaved mica. The top frame is height image. The middle frame is the phase image. And the bottom frame is the amplitude image.

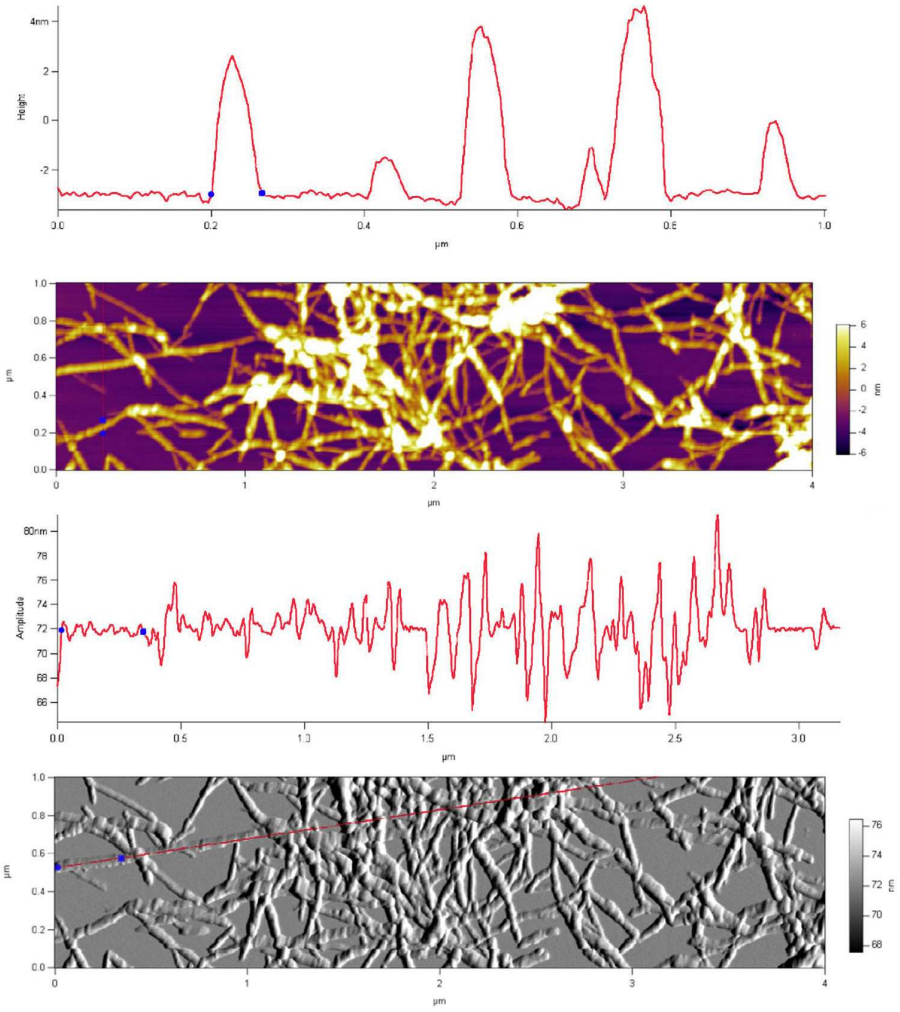


Figure S7. Characterization of the human IAPP fibrils. (Top) The cross section of the height image gives information about the height and width of the fibrils. (Bottom) The tapping amplitude image gives a cross section showing the axial periodicity of the fibrils.

References

(1) Goldsbury, C.; Kistler, J.; Aebi, U.; Arvinte, T.; Cooper, G. J. S.: *Watching amyloid fibrils grow by time-lapse atomic force microscopy*. J Mol Biol **1999,** *285,* 33-39.

(2) Green, J. D.; Goldsbury, C.; Kistler, J.; Cooper, G. J. S.; Aebi, U.: *Human amylin oligomer growth and fibril elongation define two distinct phases in amyloid formation*. J Biol Chem **2004,** *279,* 12206-12212.

(3) Kayed, R.; Bernhagen, J.; Greenfield, N.; Sweimeh, K.; Brunner, H.; Voelter, W.; Kapurniotu, A.: *Conformational transitions of islet amyloid polypeptide (IAPP) in amyloid formation in vitro*. J Mol Biol **1999,** *287,* 781-796.

(4) Marek, P.; Abedini, A.; Song, B. B.; Kanungo, M.; Johnson, M. E.; Gupta, R.; Zaman, W.; Wong, S. S.; Raleigh, D. P.: *Aromatic interactions are not required for amyloid fibril formation by islet amyloid polypeptide but do influence the rate of fibril formation and fibril morphology*. Biochemistry-Us **2007,** *46,* 3255-3261.