SUPPLEMENTAL INFORMATION

TABLE S1. Constraints used in SAMD calculations

Geometry Element	Structural Element in Each Peptide	Value [1]	r1 [2]	r2 [2]	r3 [2]	r4 [2]
Inter-label distance	Label pairs in Table 1 for labeled peptides	Exptl. N (Å) [3]	N-R-1 Å [3]	N-R Å [3]	N+R Å [3]	N+R+1 Å [3]
ß-sheet H-bonds	Residues 12 - 18, 31 - 36	2.15 Å	1.3 Å	1.8 Å	2.5 Å	3.0 Å
Peptide bond breaks	Bonds connecting residues 26-27	1.5 Å	1.4 Å	1.5 Å	3.0 Å	5.0 Å
Fibril Linearity [4]	Peptides 1 - 101	475 Å	468 Å	469 Å	480 Å	481 Å
Pitch constraint [5]	Peptides with 1,11 relationship	324º	308.6º	317.6º	328.7º	332.3º
Omega torsion	All peptide bonds	180º	177.0º	178.0º	182.0º	183.0º

[1] The value is an experimental or idealized value for the particular geometry element.
[2] AMBER10 input values for simulated annealing.
[3] N is the experimental DEER distance (Fig. 3B). R is the range shown in column 5 of Table S2.
[4] The distance constraint was applied between the Cα atoms of amino acid (aa) 14 in peptide 1 and aa14 in peptide 101, and between the Cα atoms of aa34 in peptide 1 and aa34 in peptide 101.
[5] Constraints between peptide 1-peptide 11, peptide 2-peptide 12, … peptide 91-peptide 101 in the fibril. The numbers shown are those for a pitch constraint of 500 Å. In general, for a pitch constraint of P Å, the constraint value, r1, r2, r3, and r4 are given by 360-[((10×360)/x)×5)]º, where x = P, P*(1-0.3), P*(1-0.15), P*(1+0.15), and P*(1-0.3), respectively. This gives a constraint value of 324º (as entered in AMBER) or -36º (reflecting a left-handed twist) for a pitch constraint of 500 Å. Each torsional angle constraint was defined using four backbone atoms: for ß-strand 1, Cα of aa13 and aa17 in peptides i and i+10; and for ß-strand 2, Cα of aa31 and aa35 in peptides i and i+10. Input files for all constraints were generated with in-house code and will be made available on request.

TABLE S2. DEER distances determined experimentally, inter-label distances measured in the model, and ranges used in SAMD calculations

Label Pairs	DEER Distance (Å) [1]	Model Distance 1 mean±std (Å) [3]	Model Distance 2 mean±std (Å) [4]	Range (±Å) [2]
12-18	22	21.6±1.3	21.6±1.3	2
13-18	21	20.3±1.2	20.2±1.2	2
13-19	21	20.0±1.2	19.8±1.1	2
13-24	32	32.5±3.8	33.0±3.7	8
13-28	35	32.2±1.5	32.4±1.8	4
13-32	27	22.2±2.5	22.1±2.4	8
13-35	26	26.7±2.1	26.0±2.1	4
13-36	26	24.3±2.2	24.4±2.4	4
14-19	19	19.2±1.3	19.3±1.3	2
14-32	27	26.8±1.3	26.5±1.2	2
15-29	24	20.3±3.1	20.7±3.4	8
16-27	35	30.7±3.6	31.2±4.1	8
17-29	25	18.9±2.1	19.9±3.1	8
17-35	33	29.5±0.9	29.5±1.0	4
19-24	20	18.6±0.9	18.5±0.9	2
24-30	23	21.5±2.3	22.1±2.5	4
24-31	23	22.6±2.0	22.6±2.2	4
27-35	27	26.6±1.3	26.7±1.3	2

[1] Experimental data
[2] Range used in SAMD calculations
[3] Mean inter-label distances in 10 structures obtained by SAMD calculations with no pitch constraint
[4] Mean inter-label distances in 10 structures obtained by SAMD calculations with a 500 Å pitch constraint

TABLE S3. Structural data from SAMD calculations with no pitch constraint

Cycle	Pitch (Å)	Radius (Å)	Sheet-Sheet Å)
1	240.0	22.8	10.6
2	239.8	25.9	9.9
3	249.0	23.3	8.8
4	245.6	25.0	9.2
5	233.5	23.4	9.0
6	226.2	28.4	8.9
7	251.2	24.8	9.3
8	221.1	26.4	8.8
9	251.8	25.0	9.9
10	212.4	25.9	9.1
Average	237.0	25.1	9.4
SD	13.5	1.7	0.6

TABLE S4. Structural data from SAMD calculations with a 250 Å pitch constraint

Cycle	Pitch (Å)	Radius (Å)	Sheet-Sheet Å)
1	253.0	29.2	8.8
2	242.4	25.0	8.6
3	241.4	26.1	8.9
4	241.8	25.1	9.2
5	236.8	26.9	8.7
6	239.3	22.2	8.8
7	239.0	23.5	8.2
8	244.1	23.1	8.8
9	244.3	25.7	8.6
10	245.1	22.9	8.9
Average	242.7	24.9	8.8
SD	4.4	2.1	0.3

TABLE S5. Structural data from SAMD calculations with a 500 Å pitch constraint

Cycle	Pitch (Å)	Radius (Å)	Sheet-Sheet Å)
1	436.9	28.0	10.8
2	436.4	23.2	11.0
3	439.1	26.9	10.8
4	440.4	23.0	10.7
5	444.7	33.4	10.7
6	459.6	25.3	11.0
7	454.2	31.0	10.5
8	455.4	22.7	10.7
9	467.6	33.1	11.1
10	448.3	24.9	11.1
Average	448.3	27.1	10.8
SD	10.6	4.1	0.2

TABLE S6. Structural data from SAMD calculations with a 1000 Å pitch constraint

Cycle	Pitch (Å)	Radius (Å)	Sheet-Sheet (Å)
1	817.0	23.1	11.7
2	810.2	19.4	12.6
3	897.8	25.0	12.2
4	829.7	22.4	13.0
5	799.5	24.6	12.5
6	820.0	26.7	12.3
7	862.5	31.3	12.9
8	884.3	19.1	13.0
9	756.1	21.3	12.9
10	853.1	24.1	12.5
Average	833.0	23.7	12.6
SD	42.3	3.6	0.4

TABLE S7. Mean geometrical data from SAMD calculations with various pitch constraints[1]

Input Pitch[2]	Pitch (Å)	Radius (Å)	Sheet-Sheet (Å)
250	242.7±4.4	24.9±2.1	8.8±0.3
500	448.3±10.6	27.1±4.1	10.8±0.2
1000	833.0±42.3	23.7±3.6	12.6±0.4
1500	1227.5±124.7	20.4±2.5	18.1±1.2
2000	1409.4±131.2	19.0±1.5	15.6±1.5
2500	2115.7±128.5	22.8±2.8	20.8±1.3
3000	2517.0±154.9	21.0±2.5	20.8±1.1
4000	3286.1±152.3	19.2±3.8	20.0±2.2

[1] All means are calculated for 10 structures.
[2] The formal pitch defined by constraints in the calculation (see footnote 5 in Table S1). The results of the calculation do not necessarily match this pitch exactly.

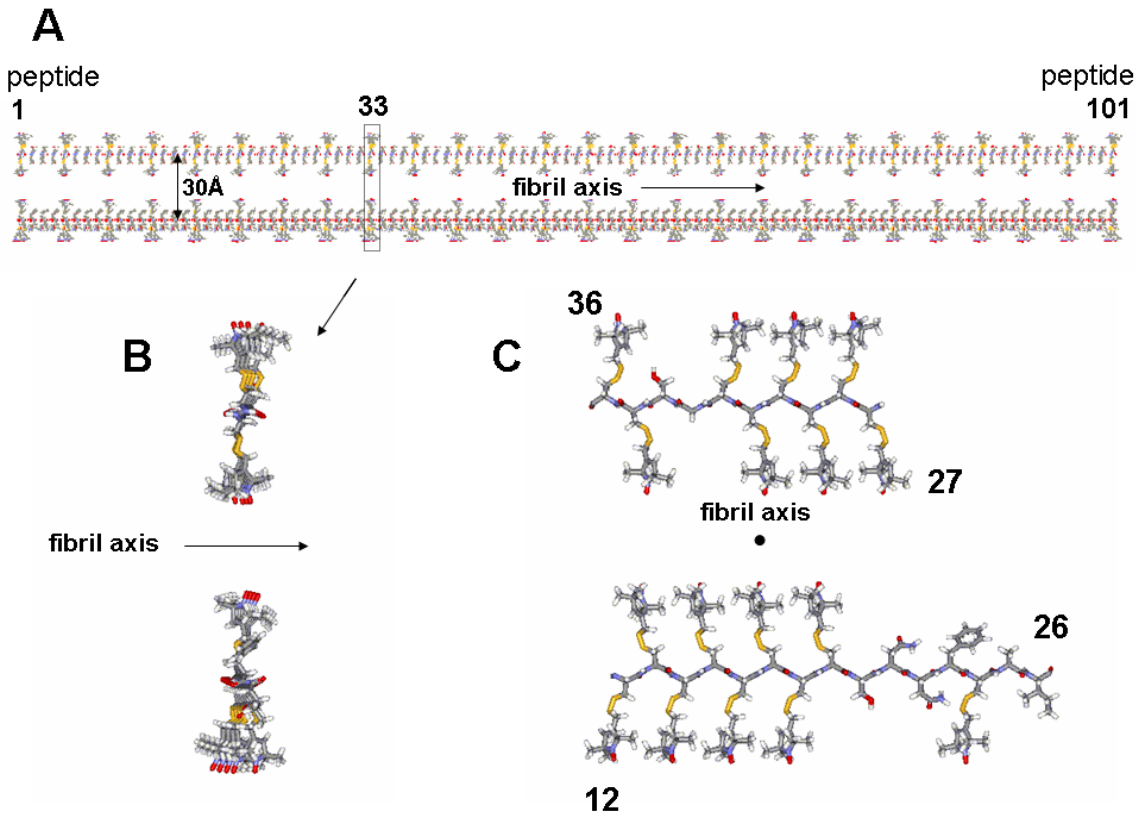

FIGURE S1. The starting structure for refinement by SAMD. (A) A model with 101 peptides constructed as two fragments (residues 12-26 and 27-36 of hIAPP) in beta strand conformations and orthogonal to the fibril axis. Every fourth peptide includes spin labels, starting from peptide 1. (B) A view of peptide 33 (a spin-labeled peptide) in the same orientation to that shown in A. (C) The same peptide following rotation through 90°. The fibril axis is now going into the page. The 17 individual spin labels are at positions 12, 13, 14, 15, 16, 17, 18, 19 and 24 (fragment 1) and 27, 28, 29, 30, 31, 32, 35 and 36 (fragment 2).

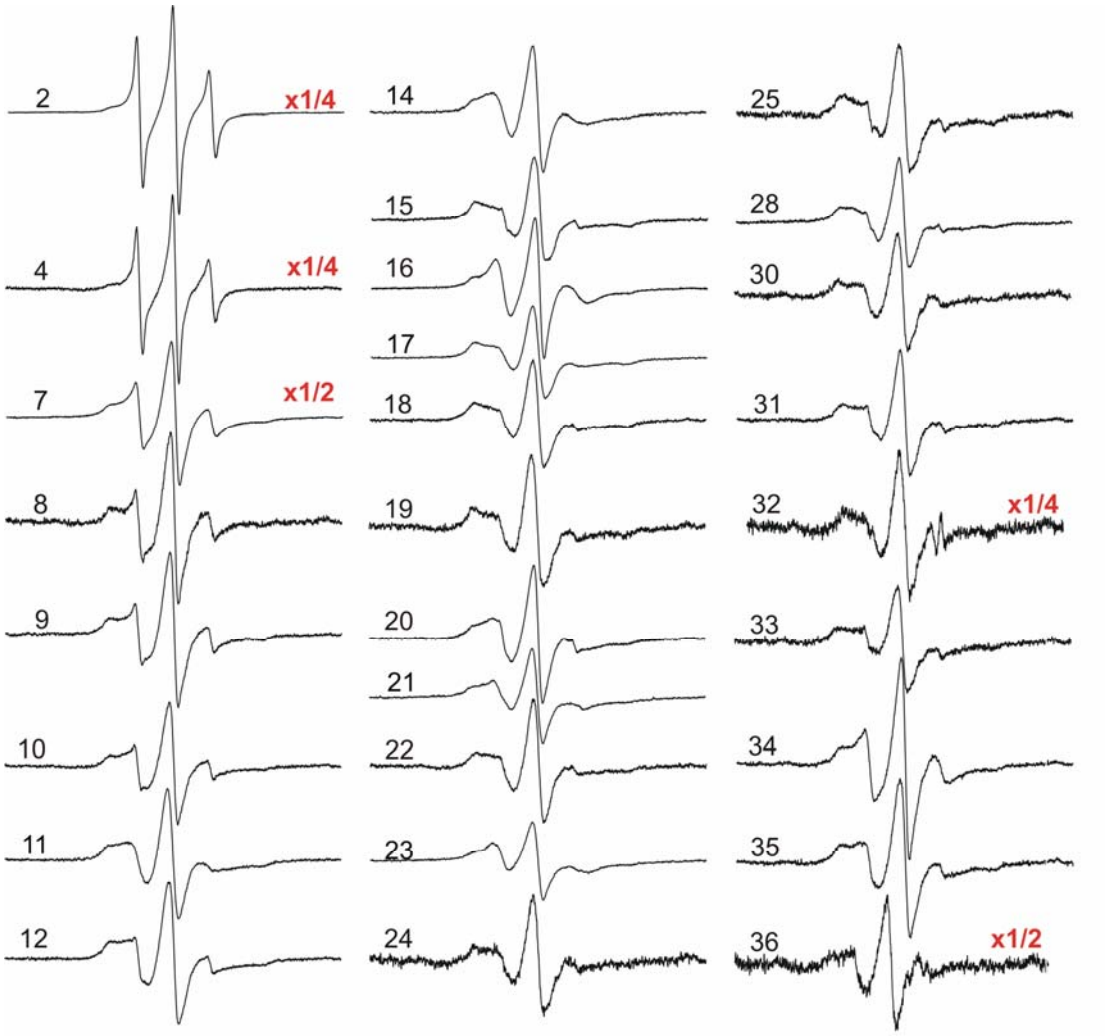

FIGURE S2. EPR spectra of hIAPP fibrils containing single labels at positions 2-36. The fibrils were formed from a mixture of 25% R1 and 75% wild-type peptide. To reduce spectral effects caused by spin-spin interactions and make the EPR spectral lines, we used the commonly employed spin-dilution approach, in which fibrils were grown from a mixture of 25% labeled and 75% wildtype hIAPP. EPR spectra from N-terminal sites (especially positions 2 and 4) have sharper lines than other regions of the molecule, indicating that the N-terminus is more dynamic and outside of the core region. All spectra were obtained using a scan width of 150 Gauss and were normalized to the same number of spins.

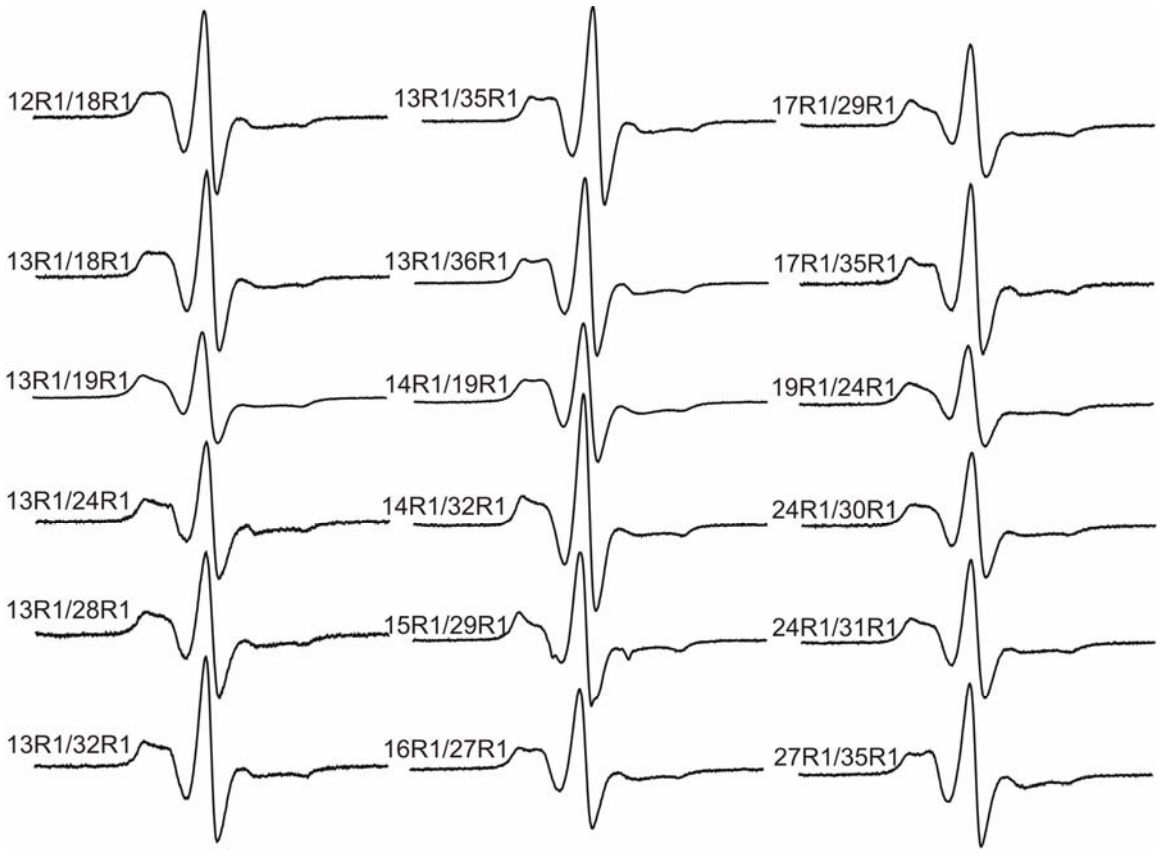

FIGURE S3. Continuous-wave EPR spectra of doubly-labeled hIAPP fibrils. The fibrils were formed from a mixture of 3% R1 and 97% wild-type peptide. All spectra were obtained using a scan width of 150 Gauss and were normalized to the same number of spins.

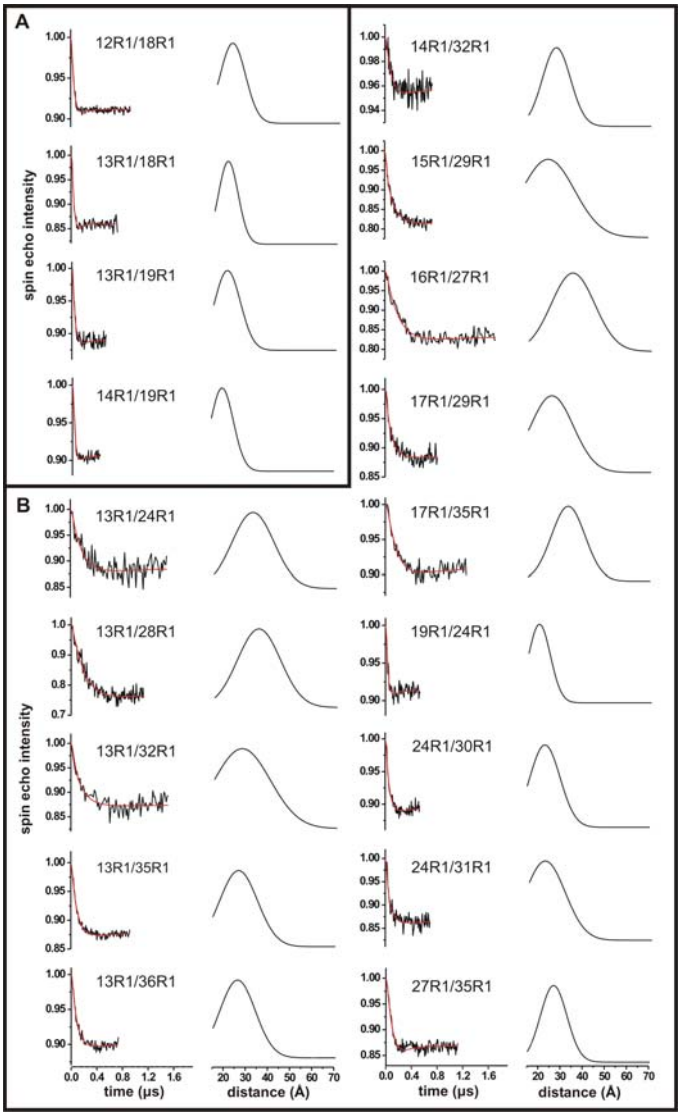

FIGURE S4. Intramolecular distances from four-pulse DEER experiments. The *left* panels show the dipolar evolution time for each of the indicated doubly labeled derivatives of hIAPP fibrils. The data shown in A) are for intra-strand distances. The black traces are background-corrected experimental data and the red lines represent the results of the fits using single Gaussian distribution. The background subtractions were confirmed by using longer dipolar evolution times, but due to better signal-to-noise the shorter scans were used for fitting. The dipolar evolution times from these data are still long enough to satisfy previously published criteria for obtaining reliable distances (ref. 24). The *right* panel shows resulting distance distributions, whose peaks are tabulated in Fig. 3.

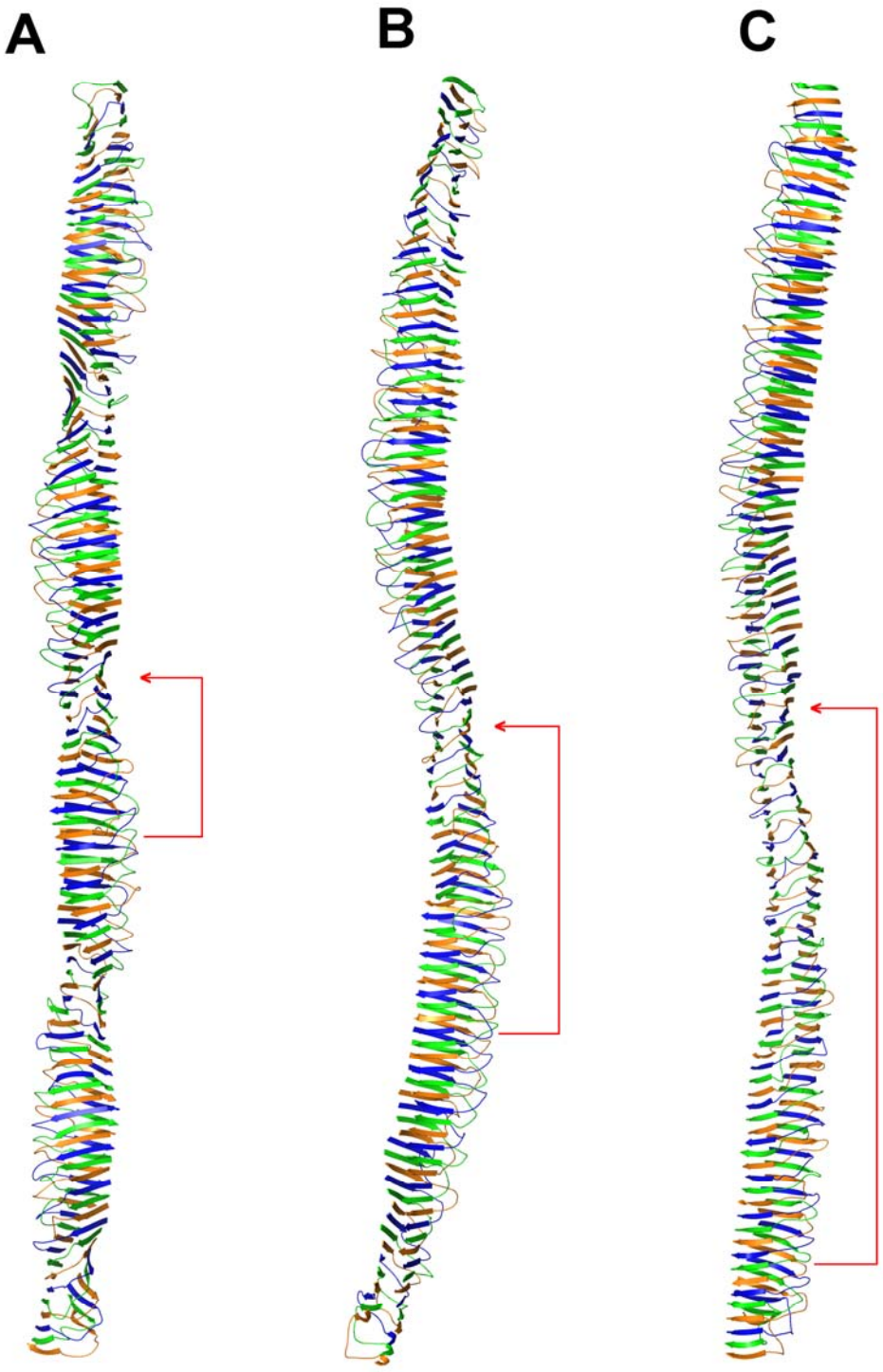

FIGURE S5. Models of hIAPP protofilaments with pitches of (A) 242 Å, (B) 440 Å, and (C) 830 Å. The red arrow indicates a left-handed turn of ~90° in each model. The structures shown in the figure were obtained from cycle 4 of SAMD calculations performed with constraints to give nominal pitches of 250 Å (Table S4), 500 Å (Table S5), and 1000 Å (Table S6), respectively.

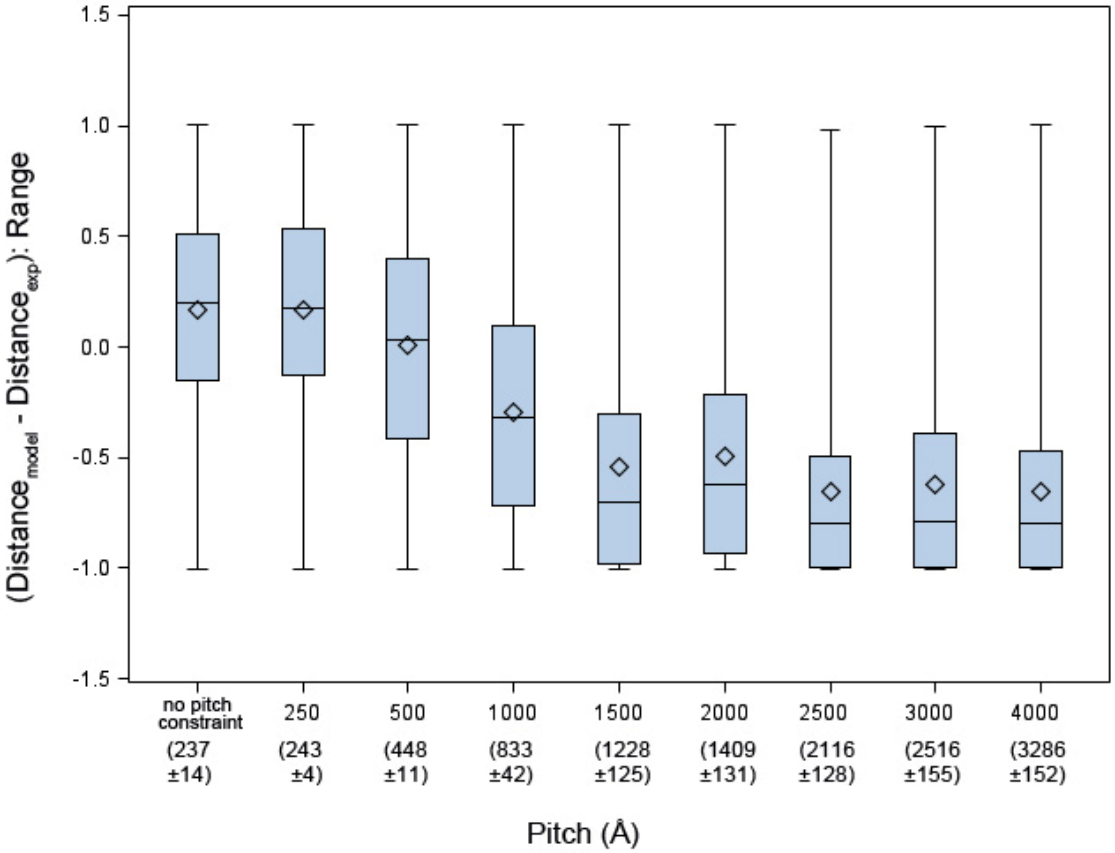

FIGURE S6. A box plot showing the variation of the 13-35 distance in SAMD calculations with various pitch constraints. The Y axis shows the distance differences between the model and the DEER data, normalized by the "range" used in SAMD calculations. The mean values of the distance differences are also shown as diamonds in the plot. The bar is the median value and the top and bottom of the box are the 25th and 75th percentiles, respectively. The X axis shows the formal pitch constraint (upper number) applied in different SAMD runs and the pitches (mean ± standard deviation) obtained in the calculations.