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Idealized models of protofilaments of human islet amyloid polypeptide

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Abstract

Fibrils formed by assembly of human islet amyloid polypeptide (hIAPP) are found in most patients with type II diabetes. Structurally, these fibrils are composed of multiple protofilaments and are characterized by extended beta sheets, variable helical twists, and different morphologies. We have previously derived models for the hIAPP protofilament using simulations constrained by data from EPR spectroscopy. In the current work, these models were used as a basis for generating idealized hIAPP protofilaments with symmetrical geometrical properties using a new algorithm, MFIBRIL. We show good agreement of the idealized protofilaments with experimental data for amino acid side chain orientations and geometrical features including the inter-beta sheet distance and the protofilament radius. These idealized protofilaments can be used in MFIBRIL to generate fibril models that may be experimentally testable at the molecular level. MFIBRIL can also be used for building structures of any repetitive molecular assembly starting with a single building block obtained from any source.

Introduction

Amyloidogenic peptides and proteins have the intrinsic ability to aggregate and form insoluble fibrils. These aggregates are associated with more than 40 human diseases, including Alzheimer's disease, Parkinson's disease, and prion diseases¹. Human islet amyloid polypeptide (hIAPP) is a 37-residue peptide hormone co-secreted with insulin by pancreatic beta cells in response to an elevated glucose level. Among several physiological roles, soluble hIAPP facilitates the effects of insulin by stimulating neurons to reduce food intake². However, misfolding and aggregation of hIAPP is toxic to beta cells^{3–5} and hIAPP fibrils are found in >90% of patients with type II diabetes⁶. Understanding the structure of these fibrils and earlier aggregates on the fibril pathway may reveal the mechanism of hIAPP aggregation and facilitate the design of therapeutic agents and biomarkers for type II diabetes.

The structure of the hIAPP fibril is difficult to study directly, but structural information has been obtained by indirect methods. Fourier transform infrared spectroscopy and circular

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More information on side chain orientations and energy minimization can be found in the supporting material. This information is available free of charge via the Internet at <http://pubs.acs.org>.

dichroism have shown that the fibril has a significant β -stranded component^{7, 8}. X-ray and electron diffraction data indicate that the β -strands are perpendicular to the fibril axis (i.e. a cross- β -structure) and that the spacing between successive β -strands is about 4.7 Å. A diffuse 9.5-Å reflection is also observed in X-ray diffraction data and is thought to be due to spacing between β -sheets⁹. Electron paramagnetic resonance (EPR) spectroscopy shows that the β -strands are parallel and in-register; i.e., the same residues in neighboring peptides stack on each other^{1, 6}.

Electron microscopy (EM) has shown that hIAPP fibrils are polymorphous, with two major morphologies: a ribbon-like form and a left-handed twisted form¹⁰. The diameter of the fibrils typically varies from 80 to 150 Å^{9, 10} and the pitch of the twisted fibrils varies from about 250 to 500 Å⁹⁻¹¹. Conventional and scanning transmission EM show that a hIAPP fibril consists of 2–4 or more protofilaments¹⁰, where a protofilament is defined as a single peptide stack. EM observations of fibril preparations have also shown some thinner structures of about 50 Å in diameter^{9, 10}.

We recently derived a family of models for protofilaments in twisted hIAPP fibrils using EPR spectroscopy and computational simulated annealing¹². A key element in obtaining these models was computational refinement based on inter-spin label distances obtained using the double electron-electron resonance (DEER) method. The resulting family of protofilament models is characterized by stacked hIAPP monomers that form opposing β -sheets and exhibit a left-handed helical twist. The two β -strands of each monomer adopt out-of-plane positions and are staggered by about 3 peptide layers¹².

The EPR-derived models provide many insights into the structure of the hIAPP protofilament. Our goal is to use these models as a basis for construction of potential hIAPP fibril models that may be validated with further EPR experiments. However, the resolution of the protofilament models is limited, particularly in regions that are less well constrained by experimental data. This leads to variability in the conformation of the constituent peptides and in the local helicity, which restricts our ability to understand the details of the protofilament and to build fibril models. Therefore, we developed an algorithm, MFIBRIL, to extract representative peptides from the models and use these peptides to generate idealized protofilaments with defined geometries. Here, we describe MFIBRIL and discuss its potential for generation of experimentally testable fibril models built from symmetrical and geometrically defined protofilaments.

Methods

MFIBRIL

In describing the MFIBRIL algorithm, we refer to models of hIAPP protofilaments obtained from simulated annealing based on EPR constraints¹². These calculations were performed on protofilaments comprising 101 peptides, with every fourth peptide carrying spin labels. Separate calculations were performed with constraints to produce protofilament models with different helical pitches and each calculation gave a family of ten protofilament models that were consistent with the EPR constraints.¹² In all families, the peptide unit adopted a horseshoe conformation, but with one β -strand displaced with respect to the plane of the second β -strand.¹²

Peptides with this general out-of-plane horseshoe conformation were used in MFIBRIL to build an idealized protofilament in three steps, as illustrated in Figure 1: (1) selection of a representative monomeric unit (peptide) from peptide aggregates, for example, a family of protofilament models obtained from simulated annealing; (2) standardization of the orientation of this peptide; (3) rebuilding of an idealized protofilament with a defined pitch,

stagger and axis offset (defined below) using multiple copies of the peptide. Using these three steps, we constructed idealized protofilaments starting with multiple peptides from each of the families of protofilament models. Each step is described in detail in the following sections.

We note that step (1) in this procedure is specifically focused on determination of a representative peptide from a family of protofilament models obtained from simulated annealing calculations for hIAPP. However, MFIBRIL can select representative peptides from any aggregate consisting of identical peptides with similar conformations. Step (1) can also be omitted and MFIBRIL can be used to build a repeating molecular assembly starting from a single unit from any source. The program is not restricted to models obtained from simulation or to molecular assemblies comprising only amyloidogenic peptides. MFIBRIL is available for use at <<http://chemsoft.hsc.usc.edu:8080/MFIBRIL/>>.

Step 1: Selection of representative peptides—Representative peptides are selected from a simulation dataset of protofilament models¹² based on two criteria: the position of the C α of each amino acid and the side chain direction. Each dataset contains 10 models and each model includes 101 peptides¹². First, all the peptides of a dataset are aligned in PyMol¹³ based on a userdefined region, for which we used residues 13–18 and 31–35 (the beta-strand regions) of peptide #50 in the 6th 101-peptide hIAPP protofilament model. Then, for each residue, a boundary cube is defined that covers all the C α atoms from the same residue of different peptides. This cube is divided into 64 smaller sub-cubes of equal volume. The score of each sub-cube is given by the number of C α atoms it contains. The 'C α score' of each residue in a particular peptide is equal to the score of the sub-cube in which the C α atom resides, and the total C α score of the peptide is the sum of the scores of all the residues in that peptide.

Step 2: Standardization of the orientation of the representative peptides—The orientation of each representative peptide is standardized before building a protofilament. To standardize the orientation, a peptide plane is defined perpendicular to the fibril axis. In the hIAPP peptide, this plane is defined by three points (Figure 2A): P1 - the midpoint of the C α atoms of residues 13 and 17, P2 - the midpoint of the C α atoms of residues 13 and 35, and P3 - the midpoint of the C α atoms of residues 31 and 35. The peptide plane is then rotated to $x=0$ and the peptide is shifted to point M, the midpoint of P2 and P4, where P4 is the midpoint of the C α atoms of residues 17 and 31. Alternatively, users can choose the midpoint of P1 and P3 for point M. Finally, the peptide is rotated to project the line M-P2 as the y -axis in the $x=0$ plane. This standardization procedure gives a hIAPP peptide with two β -strands that are almost in the same plane perpendicular to the x -axis, with the open end of the peptide in the positive y direction (Figure 2B). In this configuration, the x -axis is parallel to the growth axis of the protofilament.

Step 3: Building an idealized protofilament—hIAPP protofilaments are left-handed helices that contain peptides staggered by about three peptide layers¹². To reproduce these geometrical properties, peptide stagger and helical pitch (Figure 2C) must be included in the idealized protofilaments. Furthermore, the helical axis of the protofilament may not be positioned centrally. Thus, MFIBRIL permits this axis to be offset to any (x,y) coordinate (Figure 2D).

The stagger of a peptide is defined by the offset of the two β -stands along the x -axis (Figure 2C) and specifically by the difference (in Å) between the x coordinates of the C α atoms of residues 13 and 35. For convenience, we refer to the stagger in terms of peptide spacing, where 1 "peptide spacing" is the distance between neighboring peptides and is 4.7 Å for hIAPP. To obtain a given stagger, the peptide is rotated around the y -axis to give the

appropriate difference between the x coordinates of C α (13) and C α (35), without changing the peptide conformation.

The pitch of the protofilament is defined with the assumption that each peptide rotates by the same angle to achieve the final pitch. That is, given a pitch = p Å, every peptide performs a clockwise rotation with angle $\alpha = 360^\circ / (p/\text{spacing})$. To achieve a left-handed pitch, p must be positive. The axis for helical rotation is parallel to the x -axis (Figure 2C).

The offset is defined as the cross point of the rotational axis and the plane $x=0$, and can be defined by the user. A built-in reference point is the "center" (Figure 2D) of the peptide at point C ($0, y_0, z_0$), where y_0 is the midpoint between the two C α atoms that have the largest and smallest y coordinates (y_1 and y_2), and z_0 is the midpoint of the C α atoms that have the largest and smallest z coordinates (z_1 and z_2). The offset of the rotational axis is defined from the reference point. Other reference points (identified as red dots with blue text in Figure 2D, where $R_y = y_1 - y_0$ and $R_z = z_1 - z_0$) can be defined to build potential fibril models based on peptide-peptide interactions via the loop or β strands.

Geometrical analysis of idealized protofilaments

Analysis of the geometric properties of idealized protofilaments can also be performed using MFIBRIL. The sheet-sheet distance (the distance between the two β -sheets in the protofilament) and radius were determined for idealized protofilaments with variation of peptide stagger, helical pitch, and axis offset. These data were compared with those obtained in EPR-constrained simulation models¹² and through direct experiments^{9, 10}. The sheet-sheet distance in the protofilament is calculated as the average of the distance between the C α of residue 12 of peptide i and the closest C α of residue 36 of another peptide (peptide j) and the distance between the C α of residue 13 of peptide i and the C α of residue 35 of peptide j . The radius is defined as the maximum distance among all the C α atoms of a peptide to the rotational axis of the protofilament.

The quality of the constructed protofilaments was examined using Procheck¹⁴ and through energy minimization using AMBER10¹⁵. In this calculation, the side chains of a protofilament were minimized for 1000 steps and then the whole structure was minimized for 5000 steps.

Definition of datasets from simulations¹²

EPR-constrained simulated annealing molecular dynamics calculations¹² gave protofilament models with helical pitches ranging from 250 to 1000 Å and a stagger of about 3 peptides. Four datasets (each with 10 models of 101-peptide protofilaments) were obtained from these calculations. Here, we refer to these datasets as 4_nc, 4_250, 4_500 and 4_1000 based on the helical pitch constraint: no constraint (nc), and 250 Å, 500 Å and 1000 Å, respectively; with 4 referring to placement of a spin label on every 4th peptide. The details of these calculations have been described elsewhere¹².

Results

Construction of protofilament models with varied stagger, pitch and offset

MFIBRIL was used to build idealized protofilament models with variation of stagger, pitch and offset. Fifty peptides with the highest C α score were selected from each of the datasets 4_nc, 4_250, 4_500 and 4_1000. Each combination of stagger, pitch and offset was used to reorient the peptide (Figure 2) and then the side chain direction for each peptide were compared with the consensus directions obtained as indicated in Supplemental Table S1. Peptides were excluded if they did not meet the following criteria: an exact match for side

chains defined as "IN" or "OUT", except for residues 12 and 36; and no more than 2 differences for side chains defined as "in" and "out". We note that the side chain directions of a given peptide can alter with variation of stagger. A peptide was also excluded if the protofilament built from the peptide had a negative sheet-sheet distance (i.e., a clash between β -sheets).

The results of this procedure for protofilament models #1 to #15 are shown in Table 1. For example, for model #1, there were 18, 19, 24 and 19 peptides from datasets 4_nc, 4_250, 4_500 and 4_1000 (out of the original 50 selected from each dataset) that were not excluded by the above criteria. For model #1, this gave a total of 80 peptides (Table 1) that were used to build 80 different idealized protofilaments with stagger = 3 peptides, pitch 250 Å, and offset = center (Figure 2). The sheet-sheet distance and radius were determined for each of these protofilaments and are given as an average $\pm$ SD in Table 1. A similar procedure was performed for a systematic set of protofilaments with varied stagger, pitch and offset. Some examples are shown as models #2 to #15 in Table 1. These results are described in more detail in the following sections.

Models #1 to #15 built from the peptide in Figure 3A were also successfully minimized in AMBER10 (Supplementary Table S2) and the minimized structures had most residues in allowed regions of conformational space based on Procheck analysis. These results indicate that the models constructed in MFIBRIL have reasonable intermolecular distances and hydrogen bonding.

Structures of idealized protofilaments

Geometric analysis of protofilament models #1, #2 and #3 (Table 1) gave sheet-sheet distances of 8.3 ± 1.6 , 10.6 ± 1.6 and 11.7 ± 1.7 Å, respectively. These values were similar to those of 8.8 ± 0.3 , 10.8 ± 0.2 , and 12.6 ± 0.4 Å in the respective simulation datasets 4_250, 4_500, and 4_1000¹². These results show that MFIBRIL can be used to idealize the simulation model without loss of the key geometric features.

Construction of protofilaments corresponding to models #1, #2 and #3 is shown in Figure 3, based on a single peptide selected from dataset 4_500. This peptide (Figure 3A) had the top C α score among the 1010 peptides (10 models $\times$ 101 peptides) in this dataset and had side-chain directions that were largely consistent with the consensus directions (Table 1). A discussion of these side chain directions and a comparison with those in previous structures^{11,16} is given later in the text.

Multiple copies of the peptide in Figure 3A were used to construct a protofilament with a stagger of 3 peptides and a left-handed pitch of 500 Å (Figure 3B). Simplified images of this protofilament and those with pitches of 250 Å and 1000 Å (built from the same peptide) are shown in Figure 3C. In these images, the peptides are colored blue, orange and green to illustrate the 3-peptide stagger.

Variation of stagger and pitch

The general dependence of the sheet-sheet distance on pitch and stagger is shown in Figure 4. A decrease in stagger or an increase in pitch causes an increase in the sheet-sheet distance. At large pitches (>1000 Å) the rate of increase in the sheet-sheet distance is slow and this distance approaches the value for a straight protofilament with the same stagger. The experimental value for the sheet-sheet distance (about 9.5 Å)⁹ is not satisfied for a protofilament with a pitch of 2000 Å and a stagger of 3 peptides (model #4, Table 1) or with a stagger of 2 or 4 peptides and a pitch of 500 Å (models #5 and #6 in Table 1). Thus, the experimental sheet-sheet distance is only obtained in protofilaments with pitches of about 250 Å to 500 Å (Figure 4A) and a stagger of about 3 peptides (14.1 Å) (Figure 4B).

Variation of offset

The results in the previous section assume a helical axis that is positioned centrally with respect to the hIAPP monomers. However, wrapping of multiple protofilaments around each other to give fibrils is likely to require this axis to be offset from the central position. MFIBRIL models built with inclusion of this offset are shown in Figure 5 and the corresponding geometric data are shown in Table 1 and Figure 6. The structures in Figure 6A all have a pitch of 500 Å and a stagger of 3 peptides, with offsets (defined in Figure 2) of center, loop, b1 and b2. These structures are examples of those in models #2, #8, #11 and #14, respectively (Table 1). All were built using the peptide in Figure 3A.

The protofilament radius differs significantly with use of different offsets (Figure 6A). Offsets around the loop region give the largest radius (about 35 Å). This feature can be seen in the structures in Figure 5B for the loopL, loop and loopR offsets. The offset of "center" gives the smallest radius (about 17.5 Å), while the beta strand offsets (b1L, b1, b1R, b2L, b2 and b2R) give similar radii of about 23–28 Å with a stagger of 14.1 Å (3 peptides). The images in Figure 5B also show how different offsets result in different potential interaction interfaces for protofilament-protofilament interactions. These interfaces would occur close to the offset axis, which appears as the "hole" in the models in Figure 5B.

Different offsets resulted in a smaller variation in sheet-sheet distances (Figure 6B and C). Thus, the conclusion that the experimental sheet-sheet distance is obtained for protofilament pitches of 250 Å to 500 Å and a stagger of about 3 peptides (14.1 Å) holds almost regardless of the offset used (Table 1).

Discussion

The MFIBRIL algorithm permits generation of idealized protofilaments based on models from simulation. The idealized models keep the important features of the simulation models, but are more ordered. These idealized protofilaments are suitable for further model building of hIAPP fibrils, which can also be achieved in MFIBRIL. Beyond the specific application to hIAPP, MFIBRIL can be used for building any fibrillar aggregate of identical peptides. Polymorphism is common for amyloid proteins, including amyloid β and α-synuclein, and recent theories suggest that different conformations of amyloid aggregates may play distinct roles in disease¹⁷. Thus, studying the structural details may reveal different aggregation mechanisms that have different roles in disease. Idealized models generated in MFIBRIL will also provide targets for screening for potential biomarkers and therapeutic agents using docking techniques.

The results obtained with variation of stagger and pitch in MFIBRIL indicate that hIAPP protofilaments may be limited to staggers of about 2 to 4 peptides (centered on 3) and pitches between about 250 to 500 Å. These parameters gave a sheet-sheet distance and a protofilament radius consistent with experimental data^{9, 10} and in line with our EPR-constrained simulation models¹². As stagger approaches 4 peptides or the pitch decreases to <250 Å, sheet-sheet clashes prevent formation of the protofilament. Conversely, as the stagger falls below 2 or the pitch increases to 1000 Å and above, the protofilament structure opens up and hydrophobic surfaces within the horseshoe peptide conformation have greater exposure to water. The balance between these extremes may provide the most stable protofilament.

Early models proposed for hIAPP fibrils contained three β-strands in a hIAPP monomer^{18, 19}, but most recent models have a horseshoe-shaped monomer formed by only two β-strands^{11, 12, 16}. Luca et al.¹¹ determined a model for ribbon-like hIAPP fibrils using scanning transmission EM, solid state NMR (SSNMR) and modeling¹¹, in which each

peptide has a horseshoe shape formed by a β -strand (8 to 16), a connection region (17 to 27), and a second β -strand (28 to 37). Molecular dynamics (MD) simulations based on this model indicated potential formation of fibrils containing two or three protofilaments with different organizations and interaction interfaces^{20, 21}. Further important structural information has been derived by Wiltzius et al.¹⁶ from X-ray structures of several hIAPP fragments, including NFLVHSS (residues 14–20), NNFGAIL (21–27) and SSTNVG (28–33)¹⁶. Hamiltonian-temperature replica exchange MD simulations indicated β -strands for residues 17–26 and 30–35 in a hIAPP dimer²². Another study using NMR, atomic force microscopy and mass spectroscopy revealed that IAPP molecules associate into transient low-order oligomers via interactions between H18 and Y37, and simulations based on this finding rendered a 2-fold (2-protofilament) model²³ similar to that in Luca et al.¹¹ A 3-fold model was proposed from two-dimensional infrared spectroscopy and MD simulations²⁴.

hIAPP fragments of residues 20–29²⁵, 30–37²⁶ and 8–20²⁷ are sufficient to form fibrils in vitro, where 20–29 is the amyloidogenic region, and FLVHS (15–19) and NFLVH (14–18) are the two shortest fragments that have been found self-assemble²⁸. A screening study of hIAPP mutants also revealed the importance of regions 20–29 and 12–17 in fibril formation, with most of the 28 mutations that reduced amyloidogenesis located in these two regions²⁹. Residue 20 is important for fibrilization, since an S20G mutation increased in vitro amyloidogenicity and an S20K mutation greatly delayed fibrilization³⁰. The aromatic residues in hIAPP also play an important role in fibrilization. Substituting one or all the aromatic residues with Leu and Ala decreased the rate of fibril formation^{31, 32}, and fluorescence resonance energy transfer has shown that Y37 interacts with at least one Phe residues during fibrillization³³. It has recently been shown that the effects of the aromatic side chains in fibril formation are likely to be due to their hydrophobicity and planarity³⁴. The nonamyloidogenic rat IAPP differs from hIAPP at only six residues at positions 18, 23, 25, 26, 28 and 29, including three key proline residues (P25, P28, P29) that play a major role in preventing fibril formation³⁵.

Since previous studies have identified residues 20 to 29 as the key amyloidogenic region, it is of interest to evaluate the consistency of side chain orientations in this region among the proposed models. In Table 2, we compare the side chain orientations of the "consensus" peptide derived in this work with those in two other models^{11, 16}. There is excellent agreement between the side chain orientations in this region in our consensus peptide and those in Luca et al.¹¹. Interestingly, we also observe good agreement with the X-ray structure of NNFGAIL (21–27)¹⁶, based on viewing this peptide as two halves separated by a flexible glycine residue. On this basis, the alternating inward and outward patterns observed in Luca et al.¹¹ and in our consensus peptide (with the exception of N22, which we cannot define) are also seen in the NNFGAIL Xray structure¹⁶. The general consistency among the models provides a good basis for further use of these orientations in theoretical studies.

The SSNMR of Luca et al.¹¹ model suggests a structure comprising two protofilaments that interact with each other through C-terminal β -sheet. Consistent with this interaction, the X-ray structure of peptide SSTNVG (28–33)¹⁶ has a steric zipper interface between two peptides, which may also be present in the hIAPP fibril. Recent studies using ion mobility spectrometry combined with mass spectrometry and molecular simulation have also suggested that the interfaces of hIAPP dimers are formed by β -strands³⁶. However, the orientation of the side chains of the beta strands in these models remains uncertain, with several different proposed models^{11, 12, 16}.

Putative models for fibrils based on the structural and mutagenesis information described above can be tested through construction of idealized structures in MFIBRIL. Indeed, our

main goal in developing the program is to build experimentally testable models for amyloid fibrils with defined staggers, pitches and offsets. The initial definition of the layer will be based on the proposed peptide-peptide interactions in a particular fibril, with two or more interacting protofilaments. It is likely that only specific combinations of stagger, pitch and offset will be viable for a particular assembly, based on steric considerations only, and this will limit the number of models. For models that are sterically viable, we can then determine pairwise inter-spin label distances for multiple pairs of sites using another algorithm in our laboratory, PRONOX³⁷. With this algorithm, we can generate data in a form consistent with that obtained from EPR DEER experiments, which may allow the predicted data to be validated experimentally.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Margittai M, Langen R. Fibrils with parallel in-register structure constitute a major class of amyloid fibrils: molecular insights from electron paramagnetic resonance spectroscopy. *Q. Rev. Biophys.* 2008; 41:265–297. [PubMed: 19079806]
2. Woods SC, Lutz TA, Geary N, Langhans W. Pancreatic signals controlling food intake: insulin, glucagon and amylin. *Philos. Trans. R Soc. Lond. B Biol. Sci.* 2006; 361:1219–1235. [PubMed: 16815800]
3. Meier JJ, Kayed R, Lin CY, Gurlo T, Haataja L, Jayasinghe S, Langen R, Glabe CG, Butler PC. Inhibition of human IAPP fibril formation does not prevent beta-cell death: evidence for distinct actions of oligomers and fibrils of human IAPP. *Am. J Physiol. Endocrinol. Metab.* 2006; 291:E1317–E1324. [PubMed: 16849627]
4. Zraika S, Hull RL, Verchere CB, Clark A, Potter KJ, Fraser PE, Raleigh DP, Kahn SE. Toxic oligomers and islet beta cell death: guilty by association or convicted by circumstantial evidence? *Diabetologia.* 2010; 53:1046–1056. [PubMed: 20182863]
5. Chiti F, Dobson CM. Protein misfolding, functional amyloid, and human disease. *Annu. Rev. Biochem.* 2006; 75:333–366. [PubMed: 16756495]
6. Jayasinghe SA, Langen R. Identifying structural features of fibrillar islet amyloid polypeptide using site-directed spin labeling. *J. Biol. Chem.* 2004; 279:48420–48425. [PubMed: 15358791]
7. Higham CE, Jaikaran ET, Fraser PE, Gross M, Clark A. Preparation of synthetic human islet amyloid polypeptide (IAPP) in a stable conformation to enable study of conversion to amyloid-like fibrils. *FEBS Lett.* 2000; 470:55–60. [PubMed: 10722845]
8. Kaye 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. [PubMed: 10191146]
9. Sumner Makin O, Serpell LC. Structural characterisation of islet amyloid polypeptide fibrils. *J. Mol. Biol.* 2004; 335:1279–1288. [PubMed: 14729343]
10. Goldsbury CS, Cooper GJ, Goldie KN, Muller SA, Saafi EL, Gruijters WT, Misur MP, Engel A, Aebi U, Kistler J. Polymorphic fibrillar assembly of human amylin. *J. Struct. Biol.* 1997; 119:17–27. [PubMed: 9216085]
11. Luca S, Yau WM, Leapman R, Tycko R. Peptide conformation and supramolecular organization in amylin fibrils: constraints from solid-state NMR. *Biochemistry.* 2007; 46:13505–13522. [PubMed: 17979302]

12. Bedrood S, Li Y, Isas JM, Hegde BG, Baxa U, Haworth IS, Langen R. Fibril structure of human islet amyloid polypeptide. *J. Biol. Chem.* 2012; 287:5235–5241. [PubMed: 22187437]
13. The PyMOL Molecular Graphics System, version 1.3r1. Schrödinger, LLC; 2010.
14. Laskowski RA, MacArthur MW, Moss DS, Thornton JM. PROCHECK: a program to check the stereochemical quality of protein structures. *J. App. Cryst.* 1993; 26:283–291.
15. Case DA, Cheatham TE 3rd, Darden T, Gohlke H, Luo R, Merz KM Jr, Onufriev A, Simmerling C, Wang B, Woods RJ. The AMBER biomolecular simulation programs. *J. Comput. Chem.* 2005; 26:1668–1688. [PubMed: 16200636]
16. Wiltzius JJ, Sievers SA, Sawaya MR, Cascio D, Popov D, Riek C, Eisenberg D. Atomic structure of the cross-beta spine of islet amyloid polypeptide (amylin). *Protein Sci.* 2008; 17:1467–1474. [PubMed: 18556473]
17. Reinke AA, Gestwicki JE. Insight into amyloid structure using chemical probes. *Chem. Biol. Drug Des.* 2011; 77:399–411. [PubMed: 21457473]
18. Jaikaran ET, Clark A. Islet amyloid and type 2 diabetes: from molecular misfolding to islet pathophysiology. *Biochim. Biophys. Acta.* 2001; 1537:179–203. [PubMed: 11731221]
19. Kajava AV, Aeby U, Steven AC. The parallel superpleated beta-structure as a model for amyloid fibrils of human amylin. *J. Mol. Biol.* 2005; 348:247–252. [PubMed: 15811365]
20. Zhao J, Yu X, Liang G, Zheng J. Structural polymorphism of human islet amyloid polypeptide (hIAPP) oligomers highlights the importance of interfacial residue interactions. *Biomacromolecules.* 2011; 12:210–220. [PubMed: 21158384]
21. Zhao J, Yu X, Liang G, Zheng J. Heterogeneous triangular structures of human islet amyloid polypeptide (amylin) with internal hydrophobic cavity and external wrapping morphology reveal the polymorphic nature of amyloid fibrils. *Biomacromolecules.* 2011; 12:1781–1794. [PubMed: 21428404]
22. Laghaei R, Mousseau N, Wei G. Structure and thermodynamics of amylin dimer studied by Hamiltonian-temperature replica exchange molecular dynamics simulations. *J. Phys. Chem. B.* 2011; 115:3146–3154. [PubMed: 21384830]
23. Wei L, Jiang P, Xu W, Li H, Zhang H, Yan L, Chan-Park MB, Liu XW, Tang K, Mu Y, Pervushin K. The molecular basis of distinct aggregation pathways of islet amyloid polypeptide. *J. Biol. Chem.* 2011; 286:6291–6300. [PubMed: 21148563]
24. Wang L, Middleton CT, Singh S, Reddy AS, Woys AM, Strasfeld DB, Marek P, Raleigh DP, de Pablo JJ, Zanni MT, Skinner JL. 2DIR spectroscopy of human amylin fibrils reflects stable beta-sheet structure. *J. Am. Chem. Soc.* 2011; 133:16062–16071. [PubMed: 21916515]
25. Westermark P, Engstrom U, Johnson KH, Westermark GT, Betsholtz C. Islet amyloid polypeptide: pinpointing amino acid residues linked to amyloid fibril formation. *Proc. Natl. Acad. Sci. U.S.A.* 1990; 87:5036–5040. [PubMed: 2195544]
26. Nilsson MR, Raleigh DP. Analysis of amylin cleavage products provides new insights into the amyloidogenic region of human amylin. *J. Mol. Biol.* 1999; 294:1375–1385. [PubMed: 10600392]
27. Jaikaran ET, Higham CE, Serpell LC, Zurdo J, Gross M, Clark A, Fraser PE. Identification of a novel human islet amyloid polypeptide beta-sheet domain and factors influencing fibrillogenesis. *J. Mol. Biol.* 2001; 308:515–525. [PubMed: 11327784]
28. Mazor Y, Gilead S, Benhar I, Gazit E. Identification and characterization of a novel molecular-recognition and self-assembly domain within the islet amyloid polypeptide. *J. Mol. Biol.* 2002; 322:1013–1024. [PubMed: 12367525]
29. Fox A, Snollaerts T, Errecart Casanova C, Calciano A, Nogaj LA, Moffet DA. Selection for nonamyloidogenic mutants of islet amyloid polypeptide (IAPP) identifies an extended region for amyloidogenicity. *Biochemistry.* 2010; 49:7783–7789. [PubMed: 20698575]
30. Cao P, Tu LH, Abedini A, Levsh O, Akter R, Patsalo V, Schmidt AM, Raleigh DP. Sensitivity of amyloid formation by human islet amyloid polypeptide to mutations at residue 20. *J. Mol. Biol.* 2012; 421:282–295. [PubMed: 22206987]
31. Marek P, Abedini A, Song B, Kanungo M, Johnson ME, Gupta R, Zaman W, Wong SS, Raleigh DP. 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.* 2007; 46:3255–3261. [PubMed: 17311418]

32. Tracz SM, Abedini A, Driscoll M, Raleigh DP. Role of aromatic interactions in amyloid formation by peptides derived from human Amylin. *Biochemistry*. 2004; 43:15901–15908. [PubMed: 15595845]
33. Padrick SB, Miranker AD. Islet amyloid polypeptide: identification of long-range contacts and local order on the fibrillogenesis pathway. *J. Mol. Biol.* 2001; 308:783–794. [PubMed: 11350174]
34. Doran TM, Kamens AJ, Byrnes NK, Nilsson BL. Role of amino acid hydrophobicity, aromaticity, and molecular volume on IAPP(20–29) amyloid selfassembly. *Proteins*. 2012; 80:1053–1065. [PubMed: 22253015]
35. Green J, Goldsbury C, Mini T, Sunderji S, Frey P, Kistler J, Cooper G, Aepli U. Full-length rat amylin forms fibrils following substitution of single residues from human amylin. *J. Mol. Biol.* 2003; 326:1147–1156. [PubMed: 12589759]
36. Dupuis NF, Wu C, Shea JE, Bowers MT. The amyloid formation mechanism in human IAPP: Dimers have beta-strand monomer-monomer interfaces. *J. Am. Chem. Soc.* 2011; 133:7240–7243. [PubMed: 21517093]
37. Hatmal MM, Li Y, Hegde BG, Hegde PB, Jao CC, Langen R, Haworth IS. Computer modeling of nitroxide spin labels on proteins. *Biopolymers*. 2012; 97:35–44. [PubMed: 21792846]

Figure 1.
A flowchart showing the three steps in the MFIBRIL program.

Figure 2. Definitions in the MFIBRIL program. A. Orientation of the peptide in the yz plane at $x=0$ and perpendicular to the fibril (x) axis. B. A different view of the oriented peptide, with the open end of the hairpin in the positive y direction. C. Introduction of stagger into the peptide. D. Different offsets of rotational axes (red dots). The "center" point is the default.

Figure 3.

A. Two views of a consensus IAPP peptide (residues 12–36) obtained from MFIBRIL analysis of simulation models. B. Stacking of the same peptide in a protofilament built in MFIBRIL. The stagger of 3 peptides is illustrated by the coloring of orange, blue and green. It is apparent that one beta strand of, for example, an orange peptide is in the same plane as the other beta strand of the next orange peptide in the structure. C. Protofilament models (101 peptides) built from the consensus peptide by MFIBRIL, with staggers of 3 peptides and pitches of 250 Å, 500 Å, and 1000 Å. These are models #1, #2 and #3, respectively, in Table 1.

Figure 4.

A. Dependence of the sheet-sheet distance on pitch in a protofilament built in MFIBRIL with a stagger of 3 peptides. B. Dependence of the sheet-sheet distance on peptide stagger (shown as the distance with the number of peptides in parentheses) in a protofilament built in MFIBRIL as a left-handed helix with a pitch of 500 Å.

Figure 5.

A. Protofilament models (101 peptides) built in MFIBRIL with a stagger of 3 peptides and a left-handed helical pitch of 500 Å, using axis offset points indicated in blue (refer to Figure 2D). Successive peptides are colored green, orange and blue. B. Protofilament models (3-peptide stagger, 500 Å pitch) viewed along the helical axis, with construction based on the axis offset points shown in Figure 2D.

Figure 6.

A. Dependence of the protofilament radius on stagger (shown in A: 1 peptide stagger = 4.7 Å) in protofilaments built in MFIBRIL with a left-handed helical pitch of 500 Å and using the indicated axis offset points. B, C. Dependence of the sheet-sheet distance on (B) pitch (with stagger fixed at 3 peptides) and (C) stagger (with pitch fixed at 500 Å) in protofilaments built using the axis offset points indicated in A.

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Table 1
Geometrical analysis of hIAPP protofilament models built in MFIBRIL

MFIBRIL model #	Stagger (peptides)	Pitch (Å)	Offset ^a	4_ nc	4_ 250	4_ 500	4_ 1000	Total ^b	Sheet- sheet (Å)	Radius (Å)
1	3	250	center	18	19	24	19	80	8.3±1.6	17.5±0.9
2	3	500	center	18	19	24	19	80	10.6±1.6	17.5±0.9
3	3	1000	center	18	19	24	19	80	11.7±1.7	17.5±0.9
4	3	2000	center	18	19	24	19	80	12.3±1.7	17.5±0.9
5	2	500	center	23	23	25	24	95	14.9±1.3	18.2±0.8
6	4	500	center	16	13	11	3	43	5.2±1.6	16.6±0.9
7	3	250	loop	16	16	11	1	44	3.8±0.9	33.0±1.7
8	3	500	loop	18	19	24	18	79	7.8±1.6	33.6±1.7
9	3	1000	loop	18	19	24	19	80	10.3±1.6	33.6±1.7
10	3	250	b1	18	19	24	18	79	10.0±1.4	23.8±1.2
11	3	500	b1	18	19	24	19	80	11.2±1.6	23.8±1.2
12	3	1000	b1	18	19	24	19	80	12.0±1.6	23.8±1.2
13	3	250	b2	18	19	24	19	80	7.9±1.8	22.9±1.2
14	3	500	b2	18	19	24	19	80	10.3±1.7	22.9±1.2
15	3	1000	b2	18	19	24	19	80	11.6±1.7	22.9±1.2

^aFor definitions, see Figure 2D.

^bThe sum of the previous four columns. Each of these columns indicates the sample size of each dataset (i.e. the number of protofilaments modeled from different representative peptides obtained from each dataset).

Table 2

Side-chain directions in the loop region of hIAPP in protofilaments.

Amino Acid	Consensus ^a	Representative peptide ^b	Luca et al. ^{11c}	Wiltzius et al. ^{16d}
S20	out	OUT	O	
N21	i/o	OUT	O	→
N22	i/o	OUT	I	←
F23	out	OUT	O	→
G24	out	OUT	-	
A25	in	IN	I	→
I26	out	OUT	O	←
L27	i/o	IN	I	→
S28	out	OUT	O	
S29	in	IN	I	
T30	OUT	OUT	O	

^aConsensus side chain directions from simulations constrained by EPR data¹². “OUT” and “out” indicate >80% and >60% of side chains pointing to the outside of the protofilament, respectively; “IN” (see Supplemental Table S1) and “in” indicate >80% and >60% of side chains are pointing inward, respectively; “i/o” indicates none of these criteria were met. Data are taken from simulations described in reference¹².

^bA representative peptide (Figure 3A) determined in this work (also see Supplemental Table S1).

^cTaken from Figure 11 of reference¹¹. O and I indicate side chains pointing to the outside and inside of the peptide hairpin, respectively. “-” indicates that the direction could not be determined from the figure.

^dData from the X-ray structure¹⁶ for peptide NNFGAIL (21–27) (PDB ID 3DGJ), with relative side chain directions shown for two halves of the peptide.