Rational Design of an Orthosteric Regulator of hIAPP Aggregation

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Supporting Information

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Materials and Methods

General. All reagents used in experiments were purchased from Aldrich, Sigma and Acros, and used without further purification if not specified. CH₂Cl₂ were dried prior to use by percolation through anhydrous CaH₂. All reactions were stirred magnetically; moisture-sensitive reactions were performed under nitrogen in flame-dried glassware. Thin-layer chromatography (TLC), usually using either ethyl acetate/hexane as the solvent system, was used to monitor reactions. 9-fluornylmethoxycarbonyl (Fmoc) amino acids and peptide synthesis reagents were purchased from GL Biochem. Hoveyda-Grubbs II catalyst was obtained from Sinocompound. Flash chromatography was performed following the conditions described by Still and coworkers. We removed solvents by rotary evaporation under reduced pressure and then dried the residues using a vacuum pump. Reverse-phase HPLC experiments were conducted with C18 reversing phase columns using a Waters-600-2487 HPLC detected by a UV detector of 215 nm. The typical flow rate for analytical HPLC is 0.8 mL/min and 6 mL/min for preparative HPLC. In all cases, 0.06% aqueous TFA and acetonitrile buffers were used. Proton NMR spectra were obtained on a JEOL-ECA-300 spectrometer (300 M).

Materials. 2-oleoyl-1-pamlitoyl-sn-glyecro-3-glycerol (POPG), 2-oleoyl-1-pamlitoyl-sn-glyecro-3-phosphocholine (POPC), 1,2-dimyristoyl-L-alpha-phosphatidylglycerol (DMPG), 1,2-dimyristoyl-sn-glycero-3-phospho-choline (DMPC) were obtained from Avanti; Dimethyl Sulphoxide (DMSO), thioflavin T (ThT), Calcein and 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) were from Sigma. The hIAPP was purchased from Genscript. The hIAPP₁⁻¹₉ and UC₁ peptides were synthesized using standard FMOC methods. For HBS 1, HBS 2, HBS 3 and HBS 4, the hydrogen bond surrogate (HBS) approach has been used.¹⁻³ All the peptide were dissolved in HFIP to 1mg/ml and incubated overnight at room temperature to obtain homogeneous, aggregate-free preparations. The samples were stored at -20°C. Before usage, HFIP was removed by evaporation under a gentle stream of nitrogen. Stock solution was prepared by dissolving the peptides in DMSO to 1mg/ml, and then added with PBS to final concentration (the final concentration of DMSO is 2%). For the cellular assay, peptide solutions were prepared in cell culture (RPMI-1640 with no fetal calf serum). A small quantity of DMSO was added to promote dissolution and the final concentration of DMSO was less than 1%. For the Dye Leakage experiment, peptide solution was prepared in Tris-HCl buffer (pH=7.4). For Circular Dichroism Spectroscopy, peptide was dissolved in Tris-HCl buffer directly to final concentration.
Synthesis of HBS α-helices The Synthesis of HBS α-helices was synthesized as reported before.

Table S1. The synthesized HBS peptides and control peptide

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<tr>
<th>Compound</th>
<th>Sequencea</th>
<th>Hot spots</th>
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<tr>
<td>UC1</td>
<td>Ac-LAAFEA-NH-Me</td>
<td>L12 F15</td>
</tr>
<tr>
<td>HBS 1</td>
<td>XLA*AFEA-NH-Me</td>
<td>L12 F15</td>
</tr>
<tr>
<td>HBS 2</td>
<td>XAA*AFEA-NH-Me</td>
<td>F15</td>
</tr>
<tr>
<td>HBS 3</td>
<td>XLA*AEEA-NH-Me</td>
<td>L12</td>
</tr>
<tr>
<td>HBS 4</td>
<td>XLA*AFAA-NH-Me</td>
<td>L12 F15</td>
</tr>
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a. X represents a 4-pentenoic acid residue; * = N-allyl residue; Me represents methyl.
Figure S1. Synthesis of the dipeptide building block

Figure S2. Synthesis of the constrained peptide HBS 1
Synthesis of hIAPP<sub>1-19</sub>: Peptides was synthesized as described above. Oxidation of free thiols to disulphides was promoted by a dimethylsulphoxide-mediated oxidation.

Figure S3. Synthesis of the hIAPP<sub>1-19</sub>.
**REMD and MD simulations.** All the REMD and MD simulations were performed using GROMACS-4.5.3 software package. For REMD simulations, the recent implicit solvent model (igb=5), plus the surface term (gbsa=1, 0.005 kcal/Å²/mol) to represent water solvent effects, was used with Amber96 force field. Recent studies have shown that Amber96 force field with implicit solvent can predicts reasonable structures for α, β, and αβ proteins and AMBER ff96 is fairly well-balanced between α-helix and β-sheet secondary structures.

Several replicas were set up with initial temperatures exponentially spaced from 300.0K to 381.5K for dimer calculation (i.e., 300.0, 315.3, 331.2, 347.7, 365.0, 381.5), 300.0K-514.0K for hIAPP$_{7-29}$ monomer calculation (i.e., 300.0, 321.8, 344.9, 369.3, 395.1, 422.4, 451.2, 481.7, 514.0), 300.0K-455.1K for hIAPP$_{1-29}$ calculation (i.e., 300.0, 312.0, 324.4, 337.2, 350.9, 364.1, 378.6, 391.7, 407.2, 422.6, 438.6, 455.1), and 300.0K-523.8K for HBS 1 monomer calculation (i.e., 300.0, 335.6, 376.9, 421.2, 470.0, 523.8). Initial velocities for each replica system were generated according to the Maxwell−Boltzmann distribution according to the corresponding replica’s initial temperature (300K). The amylin crystal structure (PDB: 3G7V) was used as the initial model and was performed molecule simulation after energy minimization without solvent equilibrium. Exchanges between neighboring replicas were attempted every 2000 MD steps (2.0 ps), and the exchange rate among replicas was ~0.25. SHAKE method was applied to constrain the bonds. Temperature was regulated using Berendsen’s algorithm with a coupling constant of 1.0 ps.

Each hIAPP$_{7-29}$ dimer replica was run for 200.0 ns, giving a cumulative simulation time of 1.2 µs for this system. Each hIAPP$_{7-29}$ monomer replica was run for 400.0 ns, giving a cumulative simulation time of 3.6µs and the box side-length was set as (2.67 nm, 2.12 nm, 2.60 nm) with a total size of 14.7 nm$^3$. Each hIAPP$_{1-29}$ monomer replica was run for 100.0 ns, giving a cumulative simulation time of 1.2µs and the box side-length was set as (4.04 nm, 5.77 nm, 2.61 nm) with a total size of 60.84 nm$^3$. Each HBS 1 monomer replica was run for 200.0 ns, giving a cumulative simulation time of 1.2µs and the box side-length was set as (1.25 nm, 1.21 nm, 1.26 nm) with a total size of 1.91 nm$^3$. For the hIAPP$_{7-29}$–HBS1 system, MD simulations were performed for 500.0ns at 300.0K. The box side-length of this system was set as (3.06 nm, 3.12 nm, 3.60 nm). The RMSD at time t is calculated as flows:

$$RMSD(t) = \sqrt{\frac{1}{M} \sum_{i=1}^{N} m_i \left[ r_i(t) - r^\text{ref} \right]^2}$$

Where $M=\Sigma_i m_i$ and $r_i(t)$ is the position of atom $i$ at time $t$ after least square fitting the structure to the reference structure. For all the simulations, the reference structure is the optimized initial structure. The HBS peptides were simulated through constraining the 1,4-hydrogen bond manually. The free energy was calculated as flows:

$$\Delta G(R) = -k_B T \ln P(R) - \ln P_{\text{max}}$$

where $k_B$ is the Boltzmann constant, $P$ is the probability distribution of the molecular system along some coordinate $R$, and $P_{\text{max}}$ denotes its maximum, which is substracted to ensure $\Delta G = 0$ for the lowest free energy minimum. The FES was generated through a python script.

For MD simulations, the GROMACS force field with simple point charge (SPC) water model has been used. Simulations were carried out by using the GROMACS package with constant number, pressure, and temperature (NPT) and periodic boundary conditions. The linear constraint solver (LINCS) method was used to constrain bond lengths, allowing an integration step of 2 fs. Electrostatic interactions were calculated with the Particle-Mesh Ewald algorithm. A constant pressure of 1 bar was applied with a coupling constant of 1.0 ps. For hIAPP7-29 monomer and hIAPP7-29 dimer, simulations in both 300K and 400K have been performed for 200ns to evaluate the potential energy surface.
Coarse Grain (CG) MD simulations. The conformation has been constrained to helix. Two systems have been prepared. For the first system, 100 hIAPP7-29 monomers have been added to a box (26.47nm, 26.47nm, 26.47nm) randomly with a cut-off of 0.5 nm. For the second system, 100 hIAPP7-29 monomers together with 500 HBS1 monomers have been added to a box (26.47nm, 26.47nm, 26.47nm) randomly with a cut-off of 0.5nm. The CG systems were described with the MARTINI CG force field (version 2.1) together with the EiNeDyn approach. All CG MD simulations were performed using the GROMACS simulation package version 4.5.3. Each CG-MD was run for 0.5μs. The system was weakly coupled independently to an external temperature bath at 300 K. The pressure was weakly coupled to an external bath at 1 bar using a semi-isotropic pressure scheme.

Inhibitor Design. The helical interfaces were evaluated by computational alanine scanning mutagenesis to identify the hot spots using a Robetta Full-Chain Protein Structure Prediction server. The sequence was optimized using Rosetta online server (Sequence Tolerance module). The corresponding models used for docking and docking analysis were prepared by SWISS-MODEL, an Automated Comparative Protein Modelling Server.

Circular Dichroism Spectroscopy. The hIAPP, HBS 1, HBS 2, HBS 3, HBS 4 and UC 1 peptides stock solutions dissolved in HIFP were transferred to EP tubes. HFIP was removed by evaporation under a gentle stream of nitrogen and the peptides were re-solubilized in 10mM Tris-HCl buffer, pH 7.3. After resolution, the peptide solutions were briefly put under vortex movement for 20s and transferred to a 0.1 cm cuvette. Trifluoroethanol (TFE) was also added to the system as controls. Spectra were measured at 1 nm intervals from 190-260 nm at a scanning speed of 50nm/min and a bandwidth of 5 nm. All experiments were conducted at room temperature (~25 °C). All of the CD spectrum has been smoothed consistently.

Vesicle Preparation. Lipid sample (POPG: POPC=1:3 for kinetic and dye leakage studies) were first dissolved in chloroform at a concentration of 10 mg/mL. The solvent was removed from the lipid sample by evaporation under a gentle stream of nitrogen. And then the sample was dried under a vacuum overnight. Multilamellar vesicles (MLVs) were prepared by rehydrating the lipid film in the appropriate buffer and then subjecting that to 5 freeze-thaw cycles to equilibrate the vesicles with the buffer. Large unilamellar vesicles were made from MLVs by extrusion through a 100 nm extrusion film. Solution of lipid (2mg/ml) was passed through the extrusion film 25 times using a syringe extrusion device. The stock solution was diluted to the final concentration and used for the kinetic and DSC studies. For the dye leakage experiments, Calcein contained in the lipid (POPG:POPC = 1:3) vesicles was prepared by rehydrating the lipid film in Tris-HCl buffer (10mM) containing 60mM calcein. All the preparations were similar with that in the kinetic studies, except that nonencapsulated calcein was removed from vesicles through size exclusion chromatography using a PD-10 column. Lipid concentrations were quantified by the method of Stewart.

Thioflavin T Fluorescence. Peptides and LUVs were prepared as described above. Thioflavin T stock solution (500 μM) was added to the sample (final concentration 20μM) in the well of 96-well black microplate (Corning Costar Corporation, USA). The fluorescence was measured directly as a function of time using a Synergy 4 Plate Reader. ($\lambda_{\text{ex}} = 440$ nm, slit width = 5nm; $\lambda_{\text{em}} = 482$ nm, slit width = 10nm). The final volume was 200 μL. As controls, TFE or LUV was also added to further research the kinetic mechanism.
Photoinduced cross-linking of unmodified proteins (PICUP) and SDS-PAGE Analysis.\textsuperscript{21} For preparation of crosslinking samples, the peptides solution (18μl, 30μM for hIAPP, 300μM for HBS1) was mixed with ammonium persulfate (20mM, 1μl) and tris (2,2'-bipyridyl) dichlororuthenium(II) hexahydrate (1mM, 1μl). The mixture was then exposed to a filament lamp for 10s. The reaction was stopped by addition of dithiothreitol (1M, 1μl) and stored in ice for the SDS-PAGE Analysis. Peptide samples after PICUP were mixed with SDS-sample buffer and heated to 90°C for 5 min. Then the samples were subjected to Nu-PAGE electrophoresis in 12% Bis-Tris gels with SDS-PAGE running buffer. Silver stain kit (Beyotime Company, China) was used to analyze the protein contents. \textbf{As the limitation of PICUP-SDS-PAGE with silver staining method that peptide of low concentration will be difficult to be observed, we increased the peptide concentration by 3 times.}

\textbf{Dye Leakage Assay}.\textsuperscript{22, 23} The hIAPP, hIAPP\textsubscript{1-19}, and HBS 1 peptides were prepared as described above. For each data point, the baseline fluorescence of the empty and dye-encapsulated lipid vesicles was measured for 15 s. Peptides from DMSO stock solution were added and the fluorescence was recorded every 20 second for 20min. The maximum increase in fluorescence was measured by the addition of Triton X-100 to a final concentration of 0.1%. The dye leakage percentage was calculated according to the follow equation:

\[
\text{percentage of dye leakage} = \frac{I-I_{\text{baseline}}}{I_{\text{detergent}}-I_{\text{baseline}}}
\]

Where \(I\) was the dye leakage fluorescence intensity recorded as a function of time. And the relative dye leakage was calculated as follows:

\[
\text{relative percentage of dye leakage} = \frac{I-I_{\text{baseline}}}{I_{\text{max}}-I_{\text{baseline}}}
\]

Where \(I_{\text{max}}\) was the maximum dye leakage fluorescence intensity among the peptide samples at the same time. All the dye leakage experiments were conducted at room temperature (\(\sim 25 \degree \text{C}\)) in 10 mM Tis-HCl buffer (pH=7.4). Each experiment was repeated four times separately.

\textbf{MTT Assay}. INS-1 cells were cultured in 96-well microplate at 37°C in a humidified 5% CO2 (5% CO2, 95% air) atmosphere for 24 h firstly. The culture medium used here is RPMI-1640 (GIBCO Invitrogen, USA) supplemented with 10% FBS (GIBCO Invitrogen, USA). Different peptide samples were added and the INS-1 cells were cultured in RPMI-1640 medium without FBS for 12 h. Finally, MTT (1 mg/ml, AMRESCO, USA) was added and incubated for another 4 h. The absorbance was measured at 560nm.

\textbf{Transmission Electron Microscopy (TEM)}. Samples (hIAPP, 10μM; HBS1 100μM) were incubated for 1 h or 12 h and placed on 300 mesh formvar-coated copper grids for 1.5 min before removing excess solution. 1% fresh tungstophosphoric acid was then added and incubated for another 1 min. Samples were observed with Hitachi-7650B electron microscope (Hitachi, Japan).

\textbf{Differential Scanning Calorimetry (DSC)}. The peptide stock solution and lipid (DOPG: DOPC=1:3) in chloroform were codissolved and the solution was removed by evaporation under a gentle stream of nitrogen. The peptide/lipid film sample was then further dried under high vacuum for several hours and dissolved by PBS. Sample was briefly vortexed without freeze thawing and extrusion through film. Phase transition temperature (\(T_m\)) was measured with a TA Q2000 differential scanning calorimeter. DOPG and DOPC were used in view of the \(T_m\) of
POPC and POPG was less than 0 °C. Besides, the hIAPP\textsubscript{1-19} peptide was used to replace the hIAPP peptide as the aggregation of hIAPP will affect the DSC results significantly.

**Dynamic Light Scattering (DLS) Analysis.** The size distribution of aggregates was analyzed at 25 °C by a Malvern ZEN3690 Zetasizer using a monochromatic coherent He-Ne laser as the light source and a detector that detected the scattered light at an angle of 90°.
Supplementary Results

The α-helix-to-β-sheet Transition Mechanism:

Figure S4. Free energy surface for (a) hIAPP\textsubscript{1-29} monomer, (b) hIAPP\textsubscript{7-29} monomer at 300 K as a function of average Psi angles and the mean square deviation (RMSD). The average Psi angles were chosen as one of the reaction coordinate to evaluate the secondary structure conveniently. RMSD was chosen as another reaction coordinate to evaluate the conformation difference directly. All of the average Psi angles mentioned in the paper are all referred to the average of all residues. For hIAPP\textsubscript{7-29} dimer, residues in only one chain has been taken into account to compare with the monomer system directly. The minimum energy basins were determined with a relative binding free energy (ΔG\textsubscript{relative}) less than 0.2. For hIAPP\textsubscript{1-29} monomer, the minimum energy basins centered at (RMSD, Psi) values of (1.267, -8.75), (1.225, 21.25). For hIAPP\textsubscript{7-29} monomer, it centered at values of (1.004, 19.75), (0.996, 18.25), (1.004, 21.25), (1.012, 34.75). For hIAPP\textsubscript{7-29} dimer (Fig. 1a), it centered at values of (0.7575, 56.45), (1.0125, 36.05), (0.8475, 63.25), (0.9225, 44.55), (0.5925, 54.75), (0.6075, 54.75), (0.8625, 64.95), (0.8625, 64.95), (0.9075, 30.95), (0.9225, 44.55), (1.0275, 49.65), (1.0575, 53.05).

It was very important to make clear the hIAPP aggregation mechanism, as identifying the target is a prerequisite for drug design.

Increasing evidence has suggested that some natively unfolded polypeptides populate a helical intermediate.\textsuperscript{24} NMR spectroscopic studies on full-length IAPP peptide revealed a helical conformation of hIAPP and rat IAPP.\textsuperscript{25-27} Recently, the α-helix-to-β-sheet transition of the hIAPP(11–25) dimer has also been investigated by performing extensive REMD.\textsuperscript{28} On the basis of these results, a phase transition mechanism has been proposed.\textsuperscript{29-32} On the other hand, a β-strand rich dimer has been included in the aggregation pathway as it appears to have the greatest likelihood of propagating larger β-structured aggregates and fibrils.\textsuperscript{33, 34} Besides, more and more evidences have suggested that amyloid oligomers are β-sheet–rich structures.\textsuperscript{35, 36} Those results together with the classical aggregation model\textsuperscript{37} indicate an early conformation transition mechanism\textsuperscript{38-40}. Our SDS PAGE experiment suggests that hIAPP dimer was a very important intermediate for hIAPP aggregation (Fig. 2h). CD experiment shows a helix structure at the beginning of the aggregation (Fig. 2b).

Michael T. Bowers proposed an updated assembly mechanism for hIAPP including a β-sheet rich dimer, but how the helix state assembled to β-sheet rich structures kept unknown.\textsuperscript{35, 36}
a hIAPP monomer misfold to β-rich structure firstly and form β-rich oligomers further? Or on the contrary, the helix-rich monomers bind to each other and then convert to β-rich oligomers?

To further study the conformational properties of hIAPP monomer and dimer, we constructed the 2D free energy surface (FES) as a function of average Psi angles and the root-mean-square deviation (RMSD) of hIAPP$_{7-29}$, hIAPP$_{1-29}$ and hIAPP$_{7-29}$ dimer as is shown in Fig. 1a and Figure S4. We were convinced that the conformation was sampled entirely as the replica exchange molecular dynamic is a method to effectively sample high-dimensional rough energy landscapes. And both helix-rich and β-strand rich structures have been observed indicating that the simulation time was enough. The results show us two characters of the hIAPP monomer folding states. First, the conformation of hIAPP monomer is varied. Only two basins (ΔGrelative<0.2) were found for hIAPP1-29 monomer and three for hIAPP$_{7-29}$ monomer. There are a lot of other energy basins which are rather shallow indicating that hIAPP monomer adopt a variety of conformation. Second, the average Psi angle of the deepest basins lies mainly between -8.75 to 34.75. This observation implies that the monomer adopts a conformation with helix and random-coil structures. The barriers between the high value of Psi shadow energy basins (β-rich structures) and the light minimum energy basins (helix and coil rich structures) are high (basins are isolated). It suggests that for the hIAPP monomer, the β-rich structures are difficult to form as it is neither kinetic favorable (high barrier represent a high activation energy and low transition rate) nor thermodynamics favorable (shadow basins represent a low enthalpy of formation).

These deep basins in hIAPP$_{7-29}$ dimer FES suggest a thermodynamics favorable formation (Fig. 1a). The barriers among these energy basins are relatively low, indicating that conversions among these conformers can occur easily. The β-sheet-rich conformers were found to coexist with α-helical and random coil structure in the global minimum-energy basin. In other words, the helix-rich dimer will self-promote the conformation transition and promote an “energy gap” formation between the native folding state (mainly helix and random-coil structures) and β-rich structures. The hIAPP can misfold easily (fast transition process and stable conformer) through a helix-rich intermediate.

In general, FES features show that it is difficult for the hIAPP monomer folding to β-rich conformers, and it is the helix dimers that self-promoted the conformation transition. It explains why addition of some helix promoters, such as HFIP, TFE or lipids, will promote hIAPP aggregation distinctly.²⁹, ⁴¹, ⁴² Besides, it may be difficult to block this “energy gap”, as it is not only thermodynamics favorable but also with high kinetic stability. A possible way is to interfere in the formation of helix dimers in the early assembling process.

It can be seen from Fig. 1a that the parallel helix intermediate will be formed firstly and then turned to an antiparallel more stable state. The helix-helix interactions among the N-terminal residues L12, A13, N14, F15 and V16 are prominent before β-sheet formation. The formation of helix dimers were thermodynamic unfavorable (low ΔGrelative with shadow basins) but kinetic favorable (low barriers). They play an important role in promoting the formation of β-sheet-rich conformers. With the unwinding of helix structures, interactions between the N-terminal residues T9, Q10, R11, L12, A13 for one chain, and C-terminal residues F23, G24, A25, I26, L27 for another, were preserved. Meanwhile, an antiparallel β-sheet structure was formed. This correspond with the 2D infrared spectroscopy studies that the hydrophobic sequence FGAIL plays a critical role in the aggregation mechanism and can form a β-sheet during the lag phase.²⁴ Generally, the FES features of hIAPP peptide suggest that aggregation to β-sheet structures can be enhanced by the stabilization of helical intermediates, as the formation of these helical intermediates was thermodynamically unfavorable in the simulations (shadow basins). This finding is in agreement with recent experimental studies which show enhanced aggregation when
the sequence is mutated to increase helicity but preserve hydrophobicity. A reasonable strategy is therefore to design an inhibitor which stabilizes the helical state but prevents the formation of toxic intermediates. Such a strategy has the advantage over directly inhibiting fiber formation, which may be too late to be therapeutically helpful, or directly targeting the disordered structure of the hIAPP monomer, which is difficult.
MD simulations with explicit solvent models:

**Figure S5.** Two-dimensional free energy maps for hIAPP7-29 with explicit solvent model as a function of average Psi angle and the root mean square deviation (nm). (a) hIAPP7-29 monomer in 300 K. (b) hIAPP7-29 monomer in 400 K. (c) hIAPP7-29 dimer in 300 K. (d) hIAPP7-29 dimer in 400 K.

The free energy surface of hIAPP7-29 monomer and dimer with explicit solvent models (Figure S5) suggests that the conventional simulations with explicit solvent models are indeed difficult to overcome the energy barrier in low temperature. As is shown in Figure S5.c, in 300 K, the dimer tend to maintain the initial helix structures ($\Psi$= -40 ~ -20) and we didn’t observe any conformation transition process. In Figure S5.d (400 K), the dimer will fold to thermodynamic favor beta-sheet structures ($\Psi$>60) fast and some detail information are difficult to observed. In Figure S5.b, we also only observed the most stable beta-sheet structures. Some details has been
lost. As a result, it is impossible to observe the process how the helix structures folded to thermodynamic favor beta-sheet structures in 300 K as the energy barrier has not been overcome. It is also difficult to characterize the folding details in 400 K, as the high temperature lead the peptide folding to the most stable structures fast and some folding details will be lost.
**Inhibitor Design:**

The docking results suggest that the HBS 1 peptide will interact with hIAPP by the hydrophobic interaction between residues Leu12 and Phe15 as we have expected.

Figure S6. (a) Plot of RMSD of designed helix peptide versus docking score upon its docking to the helix hIAPP backbone. (b) The best docking poses with the lowest binding free energies.
CD spectrum shows us that the optimized peptide HBS 1 own the most helical content. However, the control peptide HBS 2, HBS 3 and HBS 4 own less helical content. The unconstrained peptide, UC 1, have non helix structures at all. Content of the helix structures was calculated from the ratio $\frac{\Theta_{222}}{\Theta_{\text{max}}}$, where $\Theta_{\text{max}} = (-44 000 + 250T)(1 - k/n)$. The $\Theta_{\text{max}}$ for 6-residue $\alpha$-helices is calculated to be -16714.3 for $k=4.0$ and $T=20^\circ C$.

**Figure S7.** Circular Dichroism Spectra of the HBS and control peptides. (a) Helical Percentage of HBS peptides and control peptides. (b) CD Spectroscopy studies on HBS1 (20 μM) and unconstrained control peptide UC1 (20 μM). 30% TFE was also added to the system as control. (c) CD Spectroscopy studies on HBS 2 (20μM), HBS 3 (20μM) and HBS4 (20 μM) in PBS. (d) CD Spectroscopy studies on HBS2 (20 μM), HBS3 (20μM) and HBS4 (20 μM) in 30% TFE and PBS.
We performed REMD simulation for HBS 1 peptide. It is difficult to optimize the force field constant. We simulated HBS peptide by constraining the 1,4-hydrogen bond manually. We found that the distance between N and H atom was basically unchanged. It suggests that this method can simulate the HBS peptide successfully. From Figure 5.a, we found that HBS 1 peptide can fold to helix conformation with a high probability.

Figure S8. REMD simulation of HBS 1 peptide. (a) FES for HBS1 at 300 K as a function of average Psi angles and RMSD. (b) Sketch map for the simulation of HBS1 peptide. We constrained the 1,4- hydrogen bond manually to simulate the hydrogen bond surrogate. (c) Length of the 1,4-hydrogen bond as a function of the simulation time.
Kinetic Studies:

Figure S9. Kinetic studies of hIAPP (10 μM) incubated with stirring at 25°C. (a) Samples were treated with DMSO after the HFIP treatment step. (b) Samples were treated with dilute acid after the HFIP treatment step before readjusting the pH to 7.3. Results are the average of three experiments.
Figure S10. Kinetic studies of hIAPP (1 μM) incubated with stirring at 25°C. (a) Samples were treated with DMSO after the HFIP treatment step. (b) Samples were treated with dilute acid after the HFIP treatment step before readjusting the pH to 7.3. Results are the average of three experiments.
It can be found that the UC 1 peptide can increase the content of fibril simply. There are no intermediates formed any more. We supposed that the UC1 peptide will co-aggregate with hIAPP through a simple β-sheet stacking mechanism.

Kinetic studies of HBS 1 show us that it cannot aggregate any more. Besides, DLS measurement of HBS 1 shows a low count rate (less than 10 kcps) which indicates that it will not assemble any more. (Date not shown here)
Figure S13. The TEM images of hIAPP fibrils. (a) The hIAPP peptides incubated for 12h; (b) The hIAPP peptides incubated with HBS 1 for 12h.
Conformation transition analysis:

CD spectrum suggests that hIAPP will fold to slight helix structure at the beginning of the aggregation process as the MD simulation has predicted and switch to sheet rich structures quickly. For hIAPP only system, the last conformation also contain some random coil structures except the sheet structures. After addition of HBS 1 or TFE, the helix structures were promoted to formation and it will change to sheet-rich structures quickly. That is to say, similar with TFE, addition of HBS 1 will promote formation of helix intermediates and enhance the conformation transition to sheet rich structures.

What’s more, Figures S12c suggests that during the process of hIAPP aggregation in the presence of HBS 1, HBS 1 will maintain helix conformation. That is to say, the high ThT fluorescence intensity was caused by hIAPP only and not lead by HBS 1 as the ThT will tend to bind to sheet-rich structures. Figure S10 also shows us that HBS 1 can’t bind with ThT.

![Figure S14](image)

Figure S14. (a) Conformation change of hIAPP (10μM) only during the aggregation process. (b) Conformation change of hIAPP (10μM) only during the aggregation process in the presence of 10% TFE. (c) Conformation change in the presence of HBS1 (100μM) during the hIAPP assembling process. Absorb induced by HBS 1 was not deducted. (d) Conformation change in the presence of HBS1 (100μM) during the hIAPP assembling process. Absorb induced by HBS 1 has been deducted.
CG-MD simulations.

Actually speaking, it is rather difficult to simulate protein folding and assembling at the same time, as the calculation cost is very huge. Usually, people often research on the folding of protein monomer or small oligomers with an all atom force field. However, for protein assembling simulation, a coarse-grained force field have to be used to save calculation cost. As a result, some folding details have to be given up.

In this paper, martini coarse-grained force field has been used. The conformation has been constrained to helix. Two systems have been prepared. For the first system, 100 hIAPP7-29 monomers have been added to a box (26.47nm, 26.47nm, 26.47nm) randomly with a cut-off of 0.5 nm. For the second system, 100 hIAPP7-29 monomers together with 500 HBS1 monomers have been added to a box (26.47nm, 26.47nm, 26.47nm) randomly with a cut-off of 0.5 nm.

To evaluate the assemble rate conveniently, we defined the aggregation ratio function p:

\[
p = \left(1 - \frac{N_t}{N_0}\right) \times 100%
\]

Where, \(N_t\) is the total number of clusters (cut-off is 0.4 nm) at time \(t\). \(N_0\) is the number of clusters at the beginning of the aggregation simulation process.

![Figure S15](image)

**Figure S15.** Kinetic study of 100 hIAPP7-29 monomers alone (blue line) and in the presence of 500 HBS1 peptides (red line). Aggregation ratio function \(p\) has been employed to evaluate the aggregation level.

From Figure S15, we found that in the presence HBS1, hIAPP7-29 tend to co-assemble to larger helix oligomers quickly at the very early stage of the simulation and aggregate faster than hIAPP alone notably. From Figure S16, we found that in the absence of HBS1, hIAPP tend to aggregate to small oligomers (dimer and trimer). However, in the presence of HBS1, hIAPP will co-assemble with HBS1 to large oligomers. This results correspond with the SDS-PAGE experiment.
Figure S16. Weighted cluster size distribution for hIAPP7-29 of helix state during the assembling process. (a) 100 hIAPP alone. (b) 100 hIAPP in the presence of 500 HBS1. The ordinate represent the number of peptides in each cluster (oligomer). Fraction represent the number of each kind of clusters.
Figure S17. Enlargement of TEM image of large oligomers. The aggregate unit in the lower right corner was got from CG-MD simulation studies.
Dye leakage assay:

**Figure S18.** Mean (3) kinetic traces (scatter diagram) of membrane disruption by (a) hIAPP (1μM) and (b) hIAPP/HBSi (1μM:100μM) mixtures. The exponential fit to the initial (0–23 min) phase of membrane disruption is shown for comparison (red line).
Figure 19. Comparison of the kinetic profile of (a) dye leakage with the kinetic profile of (b) amyloid formation (hIAPP: 1μM; HBS1 10μM). The initial phase (0–23 min) of membrane disruption was fit to an exponential curve. Vesicles were stable over the time course of the experiment in the absence of IAPP.

As the lag time of hIAPP is measured to be about 23min (DMSO), we extended the dye leakage determination to about 1h to determine if the HBS1 inhibitor blocks fiber mediated or oligomer mediated membrane damage. hIAPP (1μM ) and hIAPP/HBS1 mixtures (1μM/100μM) has been used to get the kinetic curve of the dye leakage. 100mM NaCl has been added to the system to balance the osmotic pressure and the Vesicles were proved to be stable over the time course of the experiment in the absence of IAPP (Figure S19.a). The initial phase (0–23 min) of membrane disruption in the absence or presence of HBS1 was fitted to an exponential curve (Figure S18). As has been shown in Figure S18, the R-square of the fit curve for hIAPP alone is 99.758% and
99.352% for hIAPP and HBS1 mixtures. The early stage of membrane disruption by IAPP or hIAPP and HBS1 mixtures (0~23 min) indeed displays the exponential kinetics with the initial rise in fluorescence followed by the gradual decrease in the rate-of-efflux from the vesicle. However, a second phase is also apparent after ~23 min in which the fluorescence begins to rise slowly once again, as shown in Fig.S19. This second phase has been linked to damage to the membrane through the growth of amyloid fibers on the membrane, because the kinetic profile of the second phase of dye leakage is consistent with the kinetic profile of amyloid formation as is shown in the ThT experiment. In the presence of HBS1, although it can prevent the dye leakage notably in the early stage of membrane disruption, the second phase is also apparent and the kinetic profile of the second phase of hIAPP alone is similar with that in the presence of HBS1. As a result, we may concluded that the HBS1 can only block oligomer mediated membrane damage.
Figure S20. (a) Dye leakage assay induced by hIAPP (1μM, blue line), HBS 1 (1μM, red line) and hIAPP (1μM) + HBS 1 (1μM) (green line). (b) Dye leakage assay induced by hIAPP (1μM, blue line), HBS 1 (100 μM, red line) and hIAPP (1μM) + HBS 1 (100μM) (green line). Both of the dye leakage assay were performed with POPC and POPG (3:1) vesicles (15.68 μM).

Figure S21. Dye leakage assay induced by hIAPP (1μM) and hIAPP (1μM) + UC 1 (10μM).
Cell Viability Assay:

Figure S22. Cell morphology in the presence of hIAPP or HBS 1. Different concentration of hIAPP or HBS 1 have been added to the system. (a) 1μM hIAPP; (b) 1μM hIAPP + 10 μM HBS 1; (c) 5 μM hIAPP; (d) 5μM hIAPP + 10 μM HBS 1; (e) 10 μM hIAPP; (f) 10μM hIAPP + 10 μM HBS 1;
DOPG and DOPC (3:1) were used to replace POPC and POPG in view of the $T_m$ of POPC and POPG was less than 0 °C. Besides, the hIAPP$_{1-19}$ peptide was used to replace the hIAPP full length peptide as the aggregation of hIAPP will affect the DSC results markedly.

It has been reported that helical structures of hIAPP can drive early stages of self-assembly of amyloid formation in membranes. To further ascertain that HBS 1 decrease dye leakage fluorescence by effecting hIAPP assembling in solution and not in membrane environment, we performed kinetic studies in a high concentration lipid vesicles environment. It shows us that in this particular environment, HBS 1 will affect the aggregation of hIAPP slightly (Fig. 3c). Differential Scanning Calorimetry (DSC) shows us that addition of HBS 1 or hIAPP1-19 both decrease the phase transition temperature ($T_m$) notably which suggest both insert into the membrane at least if they are codissolved with the lipid before formation of the vesicle$^{43}$.

Figure S23. (a) Thioflavin T fluorescence of hIAPP only (red line, 10μM) and in the presence of HBS 1 (blue line, 100μM) in a high concentration (500μM) lipid vesicles environment. (b) Effect of hIAPP$_{1-19}$ and HBS 1 on lipid vesicles' phase transition temperature ($T_m$) and enthalpy.
Analytical HPLC traces of peptides:

HBS 1:

![Chemical structure of HBS 1](image)

**Figure S24.** ESIMS of HBS 1. ESIMS m/z for C$_{36}$H$_{53}$N$_7$O$_9$ [M-H]$^-$. Calcd. 727.39, found 727.3

**Figure S25.** Analytical HPLC plot of HBS 1. HPLC conditions: C$_8$ reversed-phase column. 20% B to 60% B in 35 min, A: 0.06% aqueous TFA, B: 80% acetonitrile; flow rate: 0.8 mL/min; monitored at 215 nm. The secondary peak lies on the right of the first peak is the isomer.
**HBS 2:**

![Chemical Structure](image)

*Figure S26.* ESIMS of HBS 2. ESIMS m/z for C$_{33}$H$_{47}$N$_{7}$O$_{9}$ [M-H]. Calcd. 684.78, found 684.7

*Figure S27.* Analytical HPLC plot of HBS 2. HPLC conditions: C$_{18}$ reversed-phase column. 20% B to 60% B in 35 min, A: 0.06% aqueous TFA, B: 80% acetonitrile; flow rate: 0.8 mL/min; monitored at 215 nm. **The secondary peak lies on the right of the first peak is the isomer.**
HBS 3

Figure S28. ESIMS of HBS 3. ESIMS m/z for C30H49N7O9 [M-H]⁻ Calcd. 650.76, found 651.0

Figure S29. Analytical HPLC plot of HBS 3. HPLC conditions: C18 reversed-phase column. 20% B to 60% B in 35 min, A: 0.06% aqueous TFA, B: 80% acetonitrile; flow rate: 0.8 mL/min; monitored at 215 nm. The secondary peak lies on the right of the first peak is the isomer.
Figure S30. ESI-MS of HBS 4. ESIMS m/z for C$_{34}$H$_{51}$N$_{7}$O$_{7}$ [M+H]$^+$ Calcd. 670.82, found 670.6.

Figure S31. Analytical HPLC plot of HBS4. HPLC conditions: C18 reversed-phase column. 20% B to 60% B in 35 min, A: 0.06% aqueous TFA, B: 80% acetonitrile; flow rate: 0.8 mL/min; monitored at 215 nm. **The first peak is the isomer.**
**Figure S32.** ESIMS of UC 1. ESIMS m/z for C_{32}H_{49}N_{7}O_{9} [M-H]. Calcd. 674.36, found 674.8

**Figure S33.** Analytical HPLC plot of HBS 4. HPLC conditions: C18 reversed-phase column. 20% B to 60% B in 35 min. A: 0.06% aqueous TFA, B: 80% acetonitrile; flow rate: 0.8 mL/min; monitored at 215 nm.
**hIAPP1-19:**

**Figure S34.** MALDI-TOF of hIAPP1-19 m/z for C87H143N29O26S2 [M+H]^+ Calcd. 2075.4, found 2074.9143

**Figure S35.** MALDI-TOF isotopic peak of hIAPP1-19
Figure S36. Analytical HPLC plot of hIAPP1-19. HPLC conditions: C18 reversed-phase column. 20% B to 60% B in 35 min, A: 0.06% aqueous TFA, B: 80% acetonitrile; flow rate: 0.8 mL/min; monitored at 215 nm.
NMR data of the synthesized peptide and building block

1. HBS1 peptide.

Figure S37. TOCSY spectrum (600 MHz, 298 K) of HBS1 in D$_2$O/H$_2$O (10/90).
Figure S38. Homonuclear decoupled $^1$H-NMR spectrum for trans HBS1.

Table 2. $^1$H NMR assignments and chemical shifts (δ, ppm) for HBS1 (293 K) in D$_2$O/H$_2$O (10/90).

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<th>$C_\beta H$</th>
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2. Fmoc-Ala-N(ally)-Ala-OH
Figure S39. Structure of Fmoc-Ala-N(ally)-Ala-OH

Figure S40. COSY spectrum (300 MHz, 298 K) of Fmoc-Ala-N(ally)-Ala-OH in CDCl₃.
Figure S41. 1H NMR (300 MHz, 298K) of Fmoc-Ala-N(ally)-Ala-OH in CDCl3.

1H NMR (300 MHz, CDCl3) δ7.75 (d, J=7.47Hz, 2H, Fmoc CζH), 7.61 (t, J=7.4Hz, 2H, Fmoc CβH), 7.39 (t, J=7.73Hz, 2H, Fmoc CεH), 7.30 (m, J=7.4Hz, 1.0 Hz, 2H, Fmoc CζH), 6.13 (d, J =8.27Hz, 1H, A1NH), 5.91 (m, 1H, ally CγH), 5.28 (m, 1H, ally CβH), 4.79 (q, J =7.93Hz, 1H, A2 CαH), 4.67 (m, 1H, A1 CαH), 4.33 (d, 2H), 4.21 (m, 1H, Fmoc CαH), 4.01 (m, 2H), 1.46 (d, J =7.15Hz, 3H, A2 CβH), 1.38 (d, J =6.8Hz, 3H, A1 CβH)
Supplementary Reference:

41 J. D. Knight, J. A. Hebda and A. D. Miranker, Biochemistry, 2006, 45, 9496-9508.