Title: Cryo-EM structure and inhibitor design of human IAPP (amylin) fibrils

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**Supplemental Tables:**

**Supplementary Table 1** Comparison of possible models of hIAPP fibrils

| Model composition | Model 1 | Model 2 | Model 1 (swap) | Model 2 (swap) |
|-------------------|---------|---------|----------------|----------------|
| Nonhydrogen atoms | 1800    | 1600    | 1800           | 1600           |
| Protein residues  | 240     | 220     | 240            | 220            |
| Ligands           | 0       | 0       | 0              | 0              |
| B factors (Å)     | 105.1   | 120.6   | 105.1          | 80.4           |
| R.m.s. deviations |         |         |                |                |
| Bond lengths (Å)  | 0.006   | 0.009   | 0.003          | 0.008          |
| Bond angles (°)   | 1.138   | 1.203   | 0.940          | 1.354          |
| MolProbity score  | 2.69    | 2.58    | 2.67           | 2.84           |
| Clashcore         | 23.8    | 22.4    | 25.9           | 29.6           |
| Poor rotamers (%) | 0       | 0       | 0              | 0              |
| Ramachandran plot |         |         |                |                |
| Favored (%)       | 72.7    | 80      | 77.3           | 65             |
| Allowed (%)        | 27.3    | 20      | 22.7           | 35             |
| Disallowed (%)     | 0       | 0       | 0              | 0              |
| Model vs. Data CC | 0.71    | 0.60    | 0.63           | 0.68           |
| Model resolution (Å) (FSC=0.5) | 3.7 | 4.0 | 4.4 | 3.8 |
| Solvation energy  |         |         |                |                |
| ΔG° per chain (kcal/mol) | -10.8 | -12.2 | -10.6 | -10.3 |
| ΔG° per residue (kcal/mol) | -0.45 | -0.55 | -0.44 | -0.47 |

**Supplementary Table 2** Solvation energy calculation

| Fibril structure | ΔG° per layer (kcal/mol) | ΔG° per residue (kcal/mol) |
|------------------|--------------------------|----------------------------|
| hIAPP (PDB 6VW2) | -21.6                    | -0.45                      |
| TDP-43 SegA-sym (PDB 6N37) | -34.2 | -0.47 |
| Aβ ex vivo (PDB 6SHS) | -33.2 | -0.42 |
| Tau PHF (PDB 5O3L)  | -62.8                    | -0.43                      |
| FUS (PDB 5W3N)     | -12.2                    | -0.20                      |

**Supplementary Table 3** Structure alignments between Aβ and hIAPP

| PBD ID | Residues | Mutation | Methods | r.m.s.d. (Å) | # of atoms | IAPP 24-34 vs. Aβ 19-29 |
|--------|----------|----------|---------|--------------|------------|-------------------------|
| 6OIZ   | 20-34    | isoAsp23 | MicroED | 4.0          | 42         | 2.1                     | 40           |
| 2M4J   | 1-40     | Wild type | NMR     | 3.3          | 55         | 3.5                     | 42           |
| 2MVX   | 1-40     | Osaka (E22Δ) | NMR     | 6.4          | 81         | 1.5                     | 36           |
| 5KK3   | 1-42     | Wild type | NMR     | 4.7          | 62         | 1.5                     | 38           |
| 5OQV   | 1-42     | Wild type | CryoEM  | 4.8          | 62         | 1.9                     | 36           |
| 2NAO   | 1-42     | Wild type | NMR     | 4.8          | 61         | 1.6                     | 40           |
| 2MXU   | 1-42     | Wild type | NMR     | 4.9          | 62         | 1.6                     | 39           |
| 2BEG   | 1-42     | Wild type | NMR     | 3.7          | 55         | 2.0                     | 40           |
| 2LMN   | 1-40     | Wild type | NMR     | 3.5          | 59         | 1.8                     | 42           |
| 2MPZ   | 1-40     | Lowa (D23N) | NMR     | 3.7          | 56         | 2.8                     | 42           |
| 6SHS   | 1-40     | Wild type (ex vivo) | CryoEM | 1.8          | 46         | 2.3                     | 35           |
| Name  | Segment targeted     | Sequence                      | Effect            |
|-------|----------------------|-------------------------------|-------------------|
| N9S-A | Asn21-Ser29          | NNFGAI\{nme-L\}SS            | Delay aggregation |
| N9S-B | Asn21-Ser29          | NN\{nme-F\}GAILSS            | No effect         |
| A9G-A | Ala25-Gly33          | AI\{nme-L\}SSTNVG            | Delay aggregation |
| A9G-B | Ala25-Gly33          | A\{nme-I\}LSSTNVG            | Delay aggregation |
| N4Gm-A| Asn21-Gly24          | N\{nme-N\}FGG\{d-F\}\{d-N\}\{d-N\} | No effect         |
| N4Gm-B| Asn21-Gly24          | NN\{nme-F\}GG\{d-F\}\{d-N\}\{d-N\} | Delay aggregation |

**Supplementary Note 1**

**Construct design**

SUMO-hIAPP constructs were designed with a (His)$_6$-tag for Ni-column purification, SUMO protein as a solubility tag and full-length hIAPP. hIAPP cDNA was inserted into a pET28a vector containing an amino terminus conjugation of SUMO protein. We initially designed one glycine residue as a linker between SUMO and hIAPP, and we found the SUMO tag cannot be cleaved from this construct. We then extended the linker to three glycine residues to generate a SUMO-tag removable construct. The sequences of both SUMO tag unremovable version (one-glycine linker, 1xG) and removable version (three-glycine linker, 3xG) are shown as follows:

**SUMO-hIAPP (1xG)**

MGSSHHHHHHHGSGLVPRGSASMSDSEVNQEAKEVKPEVKEVKEPTHEHNLKVSDGSEIFFKIKKTTPLRLM
EAFAKRQGKEMDRLRFYDGIQADQTPEDLDMDNDIIEAHRERQIGKCNATCATQRNLAFHLHSSNN
FGAILSSSTNVGSNTY

**SUMO-hIAPP (3xG)**

MGSSHHHHHHHGSGLVPRGSASMSDSEVNQEAKEVKPEVKEVKEPTHEHNLKVSDGSEIFFKIKKTTPLRLM
EAFAKRQGKEMDRLRFYDGIQADQTPEDLDMDNDIIEAHRERQIGGKCNATCATQRNLAFHLHVS
SNNFGAILSSSTNVGSNTY

**Protein purification and SUMO-tag cleavage assays**

Both 1xG and 3xG SUMO-hIAPP were expressed in the Escherichia coli BL21 (DE3) strain, which grows in LB media with 50 μg/ml kanamycin. Protein expression was induced by adding 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) to the cell culture when an OD600 of 0.6-0.8 was reached. The bacterial cells were further cultured at 25 °C for 3 hours and then were harvested and resuspended in 20 mM Tris-HCl, pH 8.0, 500 mM NaCl, 20 mM imidazole and 10% (v/v) glycerol, supplemented with 1% (v/v) halt protease inhibitor single-
use cocktail (Thermo Scientific), and sonicated (3 s on/3 s of cycle, 10 min) and centrifuged (24,000g for 20 min) to obtain the cell lysate. We added our homemade NucA nuclease (5000 U per liter of cell culture) to the cell lysate, filtered the mixed solution and then loaded it onto a HisTrap HP column (GE healthcare). The column was pre-equilibrated with 20 mM Tris-HCl, pH 8.0, 500 mM NaCl and 20 mM imidazole before loading the sample, and after the sample was loaded, the column was washed with 20 mM Tris-HCl, pH 8.0, 500 mM NaCl and 200 mM imidazole and eluted with 20 mM Tris-HCl, pH 8.0, 500 mM NaCl and 500 mM imidazole. Purified protein was concentrated using Amicon Ultra-15 centrifugal filters (Millipore) and stored at −80 °C for future use.

To remove the SUMO-tag, ULP1 protease cleavage assays were performed. Both 1xG and 3xG SUMO-hIAPP protein were mixed with 100:1 (weight basis) with homemade ULP1 protease and samples were analyzed via SDS-PAGE (Extended Data Fig. 3b). At 0 h, samples mixed with ULP1 showed bands of intact SUMO-hIAPP; at 1 h of cleavage, only 3xG SUMO-hIAPP showed band of free SUMO and hIAPP, whereas 1xG still showed intact SUMO-hIAPP, indicating 3xG is SUMO removable but 1xG is not; after more than one month (> 1 m) of incubation at fibril growth condition, SUMO-hIAPP form fibrils and still showed intact SUMO-hIAPP band but not free SUMO and hIAPP bands, indicating 1xG SUMO-hIAPP fibrils contain SUMO tags.

**Synthetic peptide preparation**

Full-length hIAPP wild type and S20G were synthesized by InnoPep with amination at the carboxyl terminal and an intramolecular disulfide bridge between Cys2 and Cys7 with a purity higher than 95%. The inhibitors were synthesized by GenScript at a purity of 95% or higher. The sequences of all inhibitors designed are summarized in Supplementary Table 4, and the sequences of wild type and S20G hIAPP peptides are as follows:

**hIAPP wild type:**

KCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTY-NH₂

**hIAPP S20G:**

KCNTATCATQRLANFLVHSGNNFGAILSSTNVGSNTY-NH₂

The synthetic hIAPP peptide was first dissolved in 100% HFIP at a concentration of 1 mM, sonicated at 4 °C for 1 min, and incubated at room temperature for 5 hours. The HFIP was then removed with a CentriVap Concentrator (Labconco) and treated peptides were stored at -20 °C. Before use, the peptides were freshly dissolved at 1 mM or 5 mM in 100% DMSO, and further diluted 100-fold in PBS and filtered using 0.1 μm Ultrafree-MC-VV centrifugal filters (Millipore) to form 10 μM and 50 μM hIAPP solutions. The peptide inhibitors were dissolved in 100% DMSO at a concentration of 30 mM and stored at -20 °C. Before use, the
DMSO inhibitor stocks were diluted to 10 mM or 3 mM with 100% DMSO, and then diluted 100-fold into hIAPP PBS solution to form 300 μm, 100 μm and 30 μm inhibitor mixtures.