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Supporting Information

Cryo-electron Microscopy Imaging of Alzheimer's Amyloid-beta 42 Oligomer Displayed on a Functionally and Structurally Relevant Scaffold

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Experimental Procedures

Plasmid preparation for the expression of wild type (WT) αHL oligomers, and αHL-displayed Aβ or hIAPP oligomers

The plasmid encoding Aβ42 sequence was obtained from Sara Linse's group in Lund and the pET20b (+) plasmid for WT αHL with D8H6 tag expression in *E.coli* was commercially constructed by TOP Gene Technologies, Inc. The synthetic genes for Aβ42 sequence and the moiety sequence of αHL with overhangs were produced by using Phusion® High-Fidelity DNA Polymerase with the primers as below. PCR was carried out for Aβ42 in the conditions: 30 s at 98°C, 30 cycles of 98°C for 10 s, 72 °C for 5 s. The similar conditions were used for αHL part, except 30 cycles of 98°C for 10 s, 60°C for 10 s, 72°C for 2 min 15 s, as well as a final elongation at 72°C for 5 min.

According to the manufacturing protocol, NEBuilder® HiFi DNA Assembly Master Mix was used to assemble the DNA fragments of αHL and Aβ42/hIAPP with overhangs, followed by the digestion of template DNA with DpnI enzyme. NEB 5-alpha Competent *E. coli* were transformed with 2 µl of the assembled products for over-night incubation at 37°C, yielding colonies on the plate with ampicillin and then for the sequence validation. The other Aβ fragments and αHL hybrid sequences with overhangs were generated by using Phusion® High-Fidelity DNA Polymerase with the following primers. Following the same procedure of Aβ42-αHL, we fully assembled single linear sequence by using NEBuilder® HiFi DNA Assembly Master Mix for the transformation and yielding colonies. The DNA sequence of hIAPP with overhangs was synthesised by Sigma. The synthetic genes were amplified following by the same procedure as the Aβ42.

WT αHL primers

5' TATGT TCAAC CTGAT TTCAA AACAA TTTTAGAGAGCCCA

3' ATTTCTTGGATAGTAATCAGATATTTGAGCTACTTCATTATCAG

Aβ42-αHL primers

5' TACTATCCAAGAAATGACGCTGAATTCCGTCACGACTCTG

3' ATCAGGTTGAACATAAGCGATCACAACGCCACCAACCATCA

hIAPP-αHL primers

5' ATTGATACAAAAAAATGCAACACTGCCACA

3' ATCAG GTTGA ACATAATATGTATTG

The DNA sequence of hIAPP with overhangs

(AAATTCGATTGATACAAAAAAATGCAACACTGCCACATGTGCAACGCAGCGCCTGGCAAATTTTTTAGTTCATTCCAGCAACAAC
TTTGGTGCCATTCTCTCATCTACCAACGTGGGATCCAATACATATTATGT TCAACCTGATTTCAAAACAA)

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Aβ0-αHL
5' TCCAAGAAATTATGTTCAACCTGATTTCAAAACA
3' ATCAG GTTGA ACATA ATTTCTTGGATAGTAATCAGATATTTGAG
Aβ1-17-αHL
5' CCAGAAGCTGTATGTTCAACCTGATTTCAAAACAA
3' ATCAGGTTGAACATACAGCTTCTGGTGGTGAAC
Aβ1-28-αHL
3' TCAGGTTGAACATACTTGTTAGAACCCACGTCTTC
5' GTTCTAACAAGTATGTTCAACCTGATTTCAAAACAA
Aβ11-42-αHL
5' TCCAAGAAATGAAGTTCACCACCAGAAGC
3' CTGGTGGTGAACTTCATTTCTTGGATAGTAATCAGATATTTG

Protein expression and purification

The verified αHL plasmids were transformed into competent *E. coli* BL21(DE3) pLysS cells. The cells were incubated on an ampicillin plate (100 μg/mL) overnight at 37 ℃. Then a single colony was picked for incubation with LB medium (200 mL) at 37 ℃, 180 rpm until the OD600 reached around 0.6. The incubation temperature was set to 18 ℃ for the overnight expression induced by IPTG (final conc. 0.5 mM). The cell pellets were harvested and lysed in lysis buffer (50 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 0.38 mM DDM, 10 mM imidazole) on ice with the addition of a final concentration of 5 mM $MgCl_2$, 1 mg/mL lysozyme and 100 units benzonase (Cat. Number: E1014). The lysis was sonicated three times for each 30 seconds, and centrifuged at 24000g for 1 h to remove cell debris. The immobilized metal-affinity chromatography was used to purify αHL in a high-specificity affinity TALON (Cobalt) column. The supernatant was incubated with the resin for at least 1 h at 4 ℃. To increase the purity of heptameric αHL, Co-NTA chromatography imidazole gradient fractionation (20 mM, 50 mM and 250 mM) was used to elute αHL. SDS-gel (4-20% or 4-12%) electrophoresis was used to determine the elution of monomeric and heptameric αHL. Protein concentration was measured by the Nanodrop Spectrophotometer (ND-2000, Thermos Scientific) at the wavelength of 280 nm. Then the proteins were aliquoted on ice and frozen in liquid N_2 immediately for storage at -80 ℃. For the hybrid Aβ/hIAPP-αHL oligomers, the expression and purification methods were the same with wild type αHL. TCEP was added into the lysis buffer (final conc. 0.5 mM) and elution buffer (final conc. 1 mM) when purifying the hIAPP-αHL oligomer. The procedure was prepared according to the published paper [1].

LC-MS

The purified proteins including wild type αHL and hybrid Aβ/hIAPP-αHL were identified by LC-MS. ESI-TOF MS (LCT Premier Mass Spectrometer, Waters AG, Baden-Dättwil, Switzerland) was combined with the liquid chromatography (LC, Waters 2795). A gradient of ACN/water in the presence of 0.1% formic acid was prepared for the MassPREP Phenyl Guard Column (Waters n°186002785) or the C18 Aeris widepore column (Phenomenex). The obtained MS spectrums for multiply charged protein ions were deconvoluted by using MAxent1 software to obtain the protein mass.

ANS fluorescence assay

The oligomerization of hybrid Aβ/hIAPP-αHL in the solution of 50 mM Tris-HCl, pH 8, 0.5 M NaCl, 0.38 mM DDM, 250 mM imidazole was determined by ANS fluorescence assay on the Synergy™ 4 (BioTek) plate reader. 40 μL solution at the final concentration of 10 μM hybrid Aβ/hIAPP-αHL and 20 μM ANS was prepared on the unsealed OptiPlate™-384 well plate with the material of polystyrene (flat and non-transparent bottom, white, PerkinElmer company, catalogue No.: 6007290). ANS fluorescence was measured with excitation wavelength at 350 nm and emission wavelength at 490 nm. As a control, the buffer was added with 50 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 0.38 mM DDM and 250 mM imidazole. Three independent experiments were conducted**.**

ThT fluorescence assay and transmission electron microscopy (TEM)

Aβ42 and hIAPP powders were purchased from the rPeptide company (catalogue ID: A-1163-1; Purity >97%) and the AnaSpec company (catalogue ID: AS-60804; Purity>95%) respectively. 1 mg Aβ42 or 1 mg hIAPP was dissolved in 1 mL 10 mM NaOH, followed by the sonication in an ice-water bath for 1 min, as previously described [2]. 50 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 250 mM imidazole with or without 0.38 mM DDM was used to dilute the WT Aβ42 and hIAPP peptides. 50 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 250 mM imidazole and 0.38 mM DDM was used to dilute WT αHL and hybrid Aβ/hIAPP-αHL sequences. 40 μL solution was added into a black 384-well, non-treated and flat-bottom microplate with the material of polystyrene (NUNC, Thermo Fisher Scientific, catalogue ID: 24276), which was then sealed with a piece of foil film (Greiner Bio-One GmbH, catalogue No.: 676090). The plate was incubated in a microplate reader (PHERAstar FSX, BMG LABTECH, Germany) at 37 °C without shaking. The final concentration of peptides (WT Aβ42, hIAPP,

αHL and a series of hybrid Aβ/hIAPP-αHL sequences) and ThT was 10 μM and 40 μM respectively. The excitation and emission wavelengths for ThT assay were set up 430 nm and 480 nm respectively. Buffer with 50 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 0.38 mM DDM and 250 mM imidazole was added as a control. Three independent experiments were conducted.

The end points of ThT assay of WT Aβ42, αHL and hybrid Aβ42-αHL were taken to prepare the TEM samples. Negative-staining method was used to prepare the samples. First, 6 μL each sample was dipped on a freshly glow-charged 400-mesh Formvar-carbon coated copper grid and absorbed for 2 min. The excess solution was then removed by a piece of filter paper, and the grids were washed by 20 μL ddH_2O for 30 s. Next, the grids were stained with 6 μL 2 % uranyl acetate for 60 s and washed by the 20 μL ddH_2O for 15 s twice. Images were taken with a TEM (JEOL 2010) at an voltage of 200 KV.

Limited proteolysis of wild type αHL and hybrid Aβ/hIAPP-αHL oligomers

20 mg/mL, 50 μL Dioleoylphosphatidylcholine (DOPC, Avanti polar lipids), dioleoylphosphatidygly-cerol (DOPG, Avanti polar lipids) mixture at a ratio of 4:1 in pentane was dried by N_2 gas and then put in a desiccator overnight under high vacuum to totally get dried. 1 mL, 10 mM Tris-HCl buffer, pH 8.0 containing 1 mM EDTA was added to suspend the lipid film. To get unilamellar liposomes, the solution was extruded through two 0.1 μm polycarbonate membranes (Whatman™). 1,2-diphytanoyl phosphophatidycholine (DPhPC, Avanti polar lipids) liposomes were prepared using the same method.
10 μL, 1 mg/mL DOPC:DOPG liposomes were incubated with 50 μL, 0.2 mg/mL wild type αHL (50 mM Tris-HCl, pH 8, 0.5 M NaCl, 0.38 mM DDM, 20 mM imidazole) or hybrid Aβ/hIAPP-αHL with the same buffer for 2 h at room temperature. 0.1 mg/mL, 2 μL proteinase K (Sigma Aldrich) in water solution was added to 20 μL of incubating solution at room temperature. After 10 min, 1 μL, 40 mM phenylmethanesulfonyl fluoride (PMSF in ethanol, Thermo Scientific) was added to inactivate the proteinase K. Then the solution was heated for 15 min at 95 ℃ for denaturation. 15 μL solution was added into 5 μL sample loading buffer (5x) and was run the SDS-gel (4%-20%).

Hemolysis assays

MBSA buffer (150 mM NaCl and 10 mM MOPS, pH7.4, containing 0.1% BSA) was prepared to wash rabbit blood cells (rRBC) repeatedly on ice and then centrifuged at 1200 rpm for 2 min until the supernatant becomes clear. 1% rRBC solution was freshly prepared before the experiment. The recombinant wild type αHL and hybrid Aβ42-αHL oligomers in the solution of 50 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 250 mM imidazole and 0.38 mM DDM were used for the hemolysis assay. 2 μL of 1.24 mg/mL wild type αHL or hybrid Aβ42-αHL oligomers were mixed with MBSA (98 μL) in the first well. Then 50 μL was transferred to the second well for the dilution with 50 μL MBSA buffer. Repeat this step to the 12th well. Then, each well had a volume of 50 μL and serially two-fold diluted. To keep the same concentration, 2 μL, 35 μM Aβ42 oligomer was added into the first well instead. Then, 50 μL fresh 1% rRBC was added into each well. The hemolytic activities of wild type αHL oligomers, hybrid Aβ42-αHL oligomers and wild type Aβ42 oligomers were determined in the non-treated, unsealed and flat-bottom 96-well plate (Greiner Bio-One™, catalogue No.: 655101) with the material of polystyrene on the Synergy™ 4 (BioTek) plate reader. The decrease of absorbance in light scattering at 595 nm was recorded for 2 h at room temperature. The concentration of 50 % lysis to show the specific hemolytic activity (HC50) was calculated. Three independent experiments were conducted.

Single-channel electrical recording

Single-channel electrical recordings were performed in an apparatus with two Delrin compartments at room temperature. The compartments consist of two sides, cis- (connected with ground electrode) and trans- (connected with voltage electrode) sides, separated by a 25 μm thick polytetrafluoroethylene (PTFE) film with a 100 μm aperture (Good Fellow Inc., #FP301200). After the painting of the film with 2 μl 2% (v/v) hexadecane in pentane, 10 μL of DOPC:DOPG mixture (4:1) dissolved in pentane at a concentration of 20 mg/mL was added into the cis and trans parts of the home-made chamber which was filled with 500 μL of 10 mM Hepes buffer, pH 7.4 with 1 mM EDTA and 1 M KCl. The black lipid membrane was formed by slowly pipetting the solution. 0.1 mg/mL wild type αHL or hybrid Aβ42-αHL oligomers were diluted 100 times in 50 mM Tris-HCl buffer, pH 8, 0.5 M NaCl, 250 mM imidazole and 0.38 mM DDM detergent. Then 5 μL diluted samples were added into the grounded cis compartment of the chamber for the recordings which were connected by Ag/AgCl electrodes and amplified by the patch-clamp amplifier (Axopatch 200B, Axon instrument). A lowpass-filter at 5 kHz and a sampling frequency at 10 kHz were applied for the data collection at room temperature by a Digidata 1440A digitizer (Axon instrument). Clampfit software was used for the data analysis.

Western blot

To obtain the oligomer-enriched Aβ42, 100 μM Aβ42 was incubated at 37 °C under quiescent conditions for 2 h [3]. 15 μL of 1 mg/mL, wild type αHL or hybrid Aβ/hIAPP-αHL in 50 mM Tris-HCl buffer, pH 8, 0.5 M NaCl, 250 mM imidazole, 0.38 mM DDM were boiled for

5 min at 95 ℃. 5 µL, 4x sample loading buffer was mixed and 7 µL was loaded into the SDS-gel (Bio-Rad 4–20% Mini-PROTEAN® TGX™ Precast Protein Gels). The SDS-PAGE Electrophoresis was conducted at 120 V for 80 min. The whole page was then transferred using trans-blot turbo mini 0.2 µm PVDF transfer pack (Bio-Rad, #1704156) on the transfer apparatus. After blocking the membrane with 3% nonfat milk in TBST (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.5% Tween 20) for 1 h, the membrane was incubated with anti-oligomer A11 antibody (1:500) for 2 h. The membrane was washed 3 times for 10 min by TBST buffer. Then the membrane was incubated with a 1:1500 dilution of goat anti-rabbit HRP-conjugated secondary antibody for 1 h. Blots were washed with TBST three times and developed with the chemiluminescent reagent (LumiGLO, Chemiluminescent Substrate Kit) .

Cell viability assay

Neuroblastoma SH-SY5Y cells were cultured in EMEM medium supplemented with 15 % (v/v) fetal bovine serum, 2 mM Glutamine (final concentration), and 1% (v/v) penicillin/streptomycin. Cells were kept at 37°C, 5 % CO_2 in a petri dish. The maximum passage number is 15 times. 50 µL resuspended cells with 6000 cells/well density were dispensed to a tissue culture treated, flat-bottom 96-well plate (Biofil^R, Item No.: 011096) with the material of polystyrene. The plated cells were incubated for 24 h at 37°C, 5 % CO_2. Aβ42 oligomers were prepared by dissolving the powder in PBS [3] or the buffer with DDM (50 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 250 mM imidazole and 0.38 mM DDM) at a concentration of 1 mg/ml followed by the incubation at 37°C for 2 h. 2 µL of Aβ42 oligomers, wild type αHL, hybrid Aβ42-αHL or Aβ11-42-αHL oligomers at a final concentration of 5 µM was added to each well. PBS and buffer (50 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 250 mM imidazole and 0.38 mM DDM) were used as the control. Afterwards, the wells were incubated with the serum-free medium for 48 h, and then the plate was equilibrated at room temperature for 30 min. A volume of CellTiter-Glo luminescent reagent (Promega, cat. G7571) was equally added to the cell culture medium present in each well. This assay has been applied to study the toxicity of Aβ aggregates on SH-SY5Y cells [4]. To induce cell lysis, the plate was put on an orbital shaker for 2 min and incubated at room temperature for 10 min to stabilize the luminescent signal. The luminescent intensity was measured on the PHERAstar 96-well plate reader and performed three independent experiments. Data are represented as the mean S.E.M (standard error of the mean). Two-tailed student's t-test was applied for statistical significance. **P<0.01 (very significant) and ***P<0.01 (highly significant) were compared to the control.

Single-particle Cryo-EM

3 µl of the protein solution (4.83 mg/ml protein, 50 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 250 mM imidazole and 0.38 mM DDM) was pipetted onto a glow-discharged UltrAuFoil grid (R 1.2/1.3, Au 300). Grids were blotted for 1 s with blot force 2 and plunge-frozen in liquid ethane using a Vitrobot Mark IV (ThermoFisher Scientific) with 100% humidity at 7 ˚C. Data were acquired on a Titan Krios electron microscope at 300 keV (Thermo Fisher), with a GIF Quantum LS Imaging filter (20 eV slit width), a K2 Summit electron counting direct detection camera (Gatan), using a magnification of 48'540x, resulting in a calibrated pixel size of 1.03Å. The defocus varied between –0.9 and –3.0 µm using SerialEM [5]. For helical reconstruction, 4'284 movies were recorded with a total dose of 55 e^-/Å2 per movie (10 sec exposure in total, 0.2 sec per frame, 50 frames in total). The dose rate was ~5.5 e^-/Å2 per second (~1.1 e^-/Å^2 per frame). The Focus software [6] was used to drift-correct and dose-weight using MotionCor2 [7].
The reconstruction was done with RELION 3.0 [8]. We automatically picked 2'438'446 particles from 4'284 micrographs, using RELION 3.0. The particles were extracted using a box size of 200 pixels (~206Å, Table 1). Several rounds of 2D and 3D classification were executed to remove bad particles resulting in 141'366 particles. The particles were used for 3D auto-refine and the beam tilt values for the entire data set and the defocus for each segment was estimated. Afterwards another run of 3D auto-refine was executed followed by Post Process, using a soft-edge mask and an estimated map sharpening B-factor of −124.06 Å^2, was performed resulting in a map with a resolution of 3.6 Å and 3.3 Å (by the FSC 0.5 and 0.143 criterion; Figure 6B).

Structure modelling

Three initial models were built by using Modeller 9.24 [9]. The first model was built using the standard protocols using only the αHL heptameric structure (PDB ID: 7AHL). In order to build the second and third models, the hairpin structure of Aβ42 hairpin NMR (PDB ID: 6RHY) was grafted onto the β-barrel region of 7AHL using two separate methods. In the first method, the symmetry of 7AHL was used in order to graft the structure of 6RHY based on the threading of αHL's β-barrel. For the second method, we performed grafting similar to the first method, but did so without the use of the threading information.
The Aβ42-αHL protein chimera was modeled into the cryo-EM density with homology modeling using Rosetta [10]. First, the chimeric sequence was aligned to the 7AHL sequence with hhalign [11] and the alignment was used to thread the chimeric sequence onto the previously described models. These threaded models were unambiguously docked into the cryo-EM density using UCSF chimera [12] and, using the 7AHL pdb as a reference for symmetry, these models were used as templates for symmetric refinement into the density using RosettaCM [13]. 5,000 trajectories of RosettaCM were run (command line and input files described at 10.5281/zenodo.3967686)

and the model with the lowest energy was the final model. The same methodology was applied to the manual threading model except for substituting the manual alignment in the initial sequence threading step.

In order to generate the shortened beta barrel models, atom pair distance and angle constraints were applied to shift the hydrogen bonding backbone N and O atoms of the adjacent subunits by 2 residues. These models were coarsely minimized using a custom-made set of constraints and the rosetta scripts Rosetta application. The command lines and example constraint files are also described in further detail at: 10.5281/zenodo.3967686. Finally, these coarse models were refined into the cryo-EM density as templates to RosettaCM with the same methodology as described previously. The score of the 5 top scoring models from all 4 conformations are plotted in Figure S8.

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Results and Discussion

Wild-type αHL

ADSD INIKT G TTD I GSNTT V KTGDLVTYDK ENGMHKKVFY SFIDDKNHN K K LLV I RTKG T
IAGQYRVYS E EGANKSGLAW PS AFKVQLQL PDN E VAQ I SD YYPRN SIDT K EYM STLTY G F
NGNVTGDDTG K IGGLIGAN V S IGHT LKYVQ PDF K T I L ESP TDKKVGWKVI FNNMVNQNWG
PYDRD SWNPV YGNQLFMKT R NGSMKAADNF LDPNK A SSL L SSGF S P DFA T V IT MD RK AS K
QQTNI DVIYE RVRDDYQLHW TSTNW KGTNT KDKWT DRSSE RYKID WEKEE MTNDD DDDDD
DHHHHHH

Wild-type Aβ42 DAEFRHDSGY EVHHQKLVFF AEDVGSNKGA I IGLMVGGVV IA

Hybrid Aβ42-αHL

ADSD INIKT G TTD I GSNTT V KTGDLVTYDK ENGMHKKVFY SFIDDKNHN K K LLV I RTKG T
IAGQYRVYS E EGANKSGLAW PS AFKVQLQL PDN E VAQ I SD YYPRN DAEFR HDSG Y EVHHQ
KLVFFAEDVG SNKGAI IGLM VGGVVIAYVQ PDF K T I L ESP TDKKVGWKVI FNNMVNQNWG
PYDRD SWNPV YGNQLFMKT R NGSMKAADNF LDPNK A SSL L SSGF S P DFA T V IT MD RKASK
QQTNI DVIYE RVRDDYQLHW TSTNW KGTNT KDKWT DRSSE RYKID WEKEE MTNDD DDDDD
DHHHHHH

Hybrid Aβ11-42-αHL

ADSD INIKT G TTD I GSNTT V KTGDLVTYDK ENGMHKKVFY SFIDDKNHN K K LLV I RTKG T
IAGQYRVYS E EGANKSGLAW PS AFKVQLQL PDN E VAQ I SD YYPRN EVHHQ K LVF F AEDVG
SNKGAI IGLM VGGVV IAYVQ PDF K T I L ESP TDKKVGWKVI FNNMVNQNWG PYDRD SWNPV
YGNQLFMKT R NGSMKAADNF LDPNK A SSL L SSGF S P DFA T V IT MD RKASK QQTNI DVIYE
RVRDDYQLHW TSTNW KGTNT KDKWT DRSSE RYKID WEKEE MTNDD DDDDD DHHHHHH

Hybrid Aβ1-28-αHL

ADSD INIKT G TTD I GSNTT V KTGDLVTYDK ENGMHKKVFY SFIDDKNHN K K LLV I RTKG T
IAGQYRVYS E EGANKSGLAW PS AFKVQLQL PDN E VAQ I SD YYPRN DAEFR HDSGY EVHHQ
KLVFF AEDVG SNKYV Q PDFK T IL ESP TDKK VGWKV I FNNM VNQNW GPYDR DSWNP VYGNQ
LFMKTRNGSM KAADN FLDPN KA SSL L SSGF S P DFA T V IT M D RKAS KQQTN I DVI Y ERVRD
DYQLH WTSTN WKGTN TKDKW TDRSS ERYKI DWEKE EMTND D DDDDD DHHHHHH

Hybrid Aβ1-17-αHL

ADSD INIKT G TTD I GSNTT V KTGDLVTYDK ENGMHKKVFY SFIDDKNHN K K LLV I RTKG T
IAGQYRVYS E EGANKSGLAW PS AFKVQLQL PDN E VAQ I SD YYPRN DAEFR HDSGY EVHHQ
KLYVQ PDFKT ILESP TDKKV GWKVIFNNMV NQNWG PYDRD SWNPV YGNQL FMKTR NGSMK
AADNF LDPNK A SSL L SSGFS P DFA T V IT MD RKASK QQTNI DVI YE RVRDD YQLHW TSTNW
KGTNT KDKWT DRSSE RYKID WEKEE MTNDD DDDDD DHHHHHH

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Hybrid Aβ0-αHL

ADSD I NIKT G TTD I GSNTT V KTGDLVTYD K ENGMHKKVFY SFIDDKNHN K K LLV I RTKG T

IAGQY RVYS E EGANKSGLAW PS AFKVQLQ L PDN E VAQ I SD YYPRNYVQPD F KTI L ES P TD

KKVGW KVIFN NMVNQ NWGPY DRDSW NPVYG NQLFM KT RNG SMKAA DNFLD P N KAS SL LSS

GFSPD FATV I TMDRK ASKQ Q TNIDV IYE R V RDDYQ LHWTS TNWKG TNTKD KWTDR S SERY

KIDWE KEEMT NDDDD DDDDH HHHHH

Wild-type hIAPP KCNTA TCATQ (10) RLANF LVHSS (20) NNFGA ILSST (30) NVGSN TY (37)

Hybrid hIAPP-αHL

ADSD INIKT G TTD I GSNTT V KTGDLVTYDK ENGMHKKVFY SFIDDKNHN K K LLV I RTKG T

IAGQYRVYS E EGANKSGLAW PS AFKVQLQL PDN E VAQ I SD YYPRN SIDTK KCNTA T CA T Q

RLANF LVHSS NNFGA ILSST NVGSN TYYVQ PDF K T I L ESP TDKKVGWKVI FNNMVNQNWG

PYDRD SWNPV YGNQLFMKT R NGSMKAADNF LDPNK A SSL L SSGF S P DFA T V IT MD RKASK

QQTNI DVIYE RVRDDYQLHW TSTNW KGTNT KDKWT DRSSE RYKID WEKEE MTNDD DDDDD

DHHHHHH

Figure S1. The full sequences of wild type (WT) αHL, WT Aβ42, a series of hybrid Aβ-αHL peptides, WT hIAPP and hybrid hIAPP- αHL. Aβ amino acids substituted the transmembrane β-hairpin part of αHL (red). The substituted Aβ or hIAPP sequences were shown in blue.

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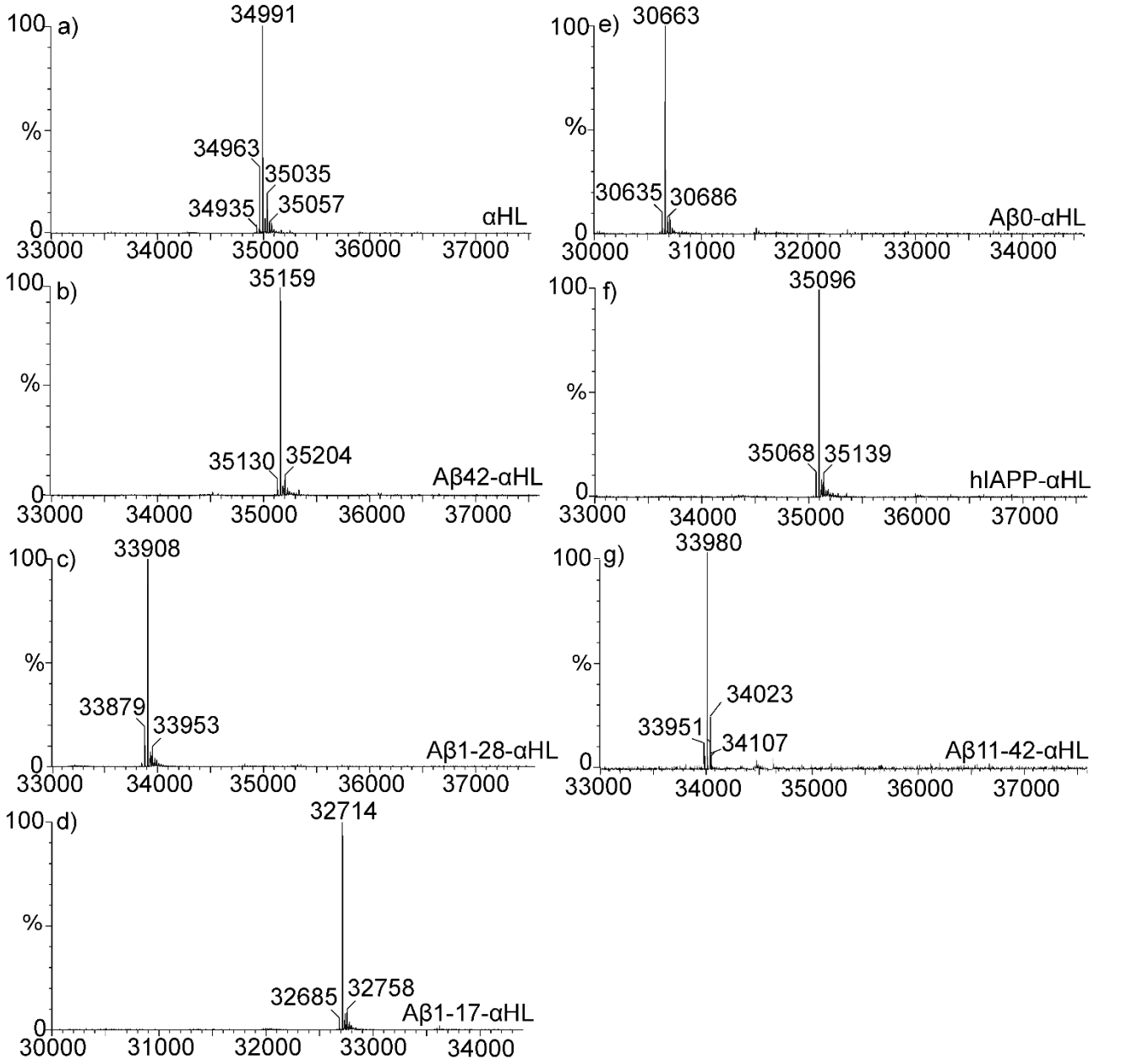

Figure S2. Deconvoluted mass spectrum of the purified WT αHL and a series of hybrid Aβ/hIAPP–αHL peptides. LC-MS experiments were conducted to confirm the molecule weight of a series of hybrid Aβ/hIAPP-αHL proteins including WT αHL. Results are compared to the calculated weight, which have been listed in Table S1.

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Table S1. The theoretical molecule weight of WT αHL and a series of hybrid Aβ/hIAPP-αHL.

Peptide	Calculated weight (MW)/Da	Observed $[M+H]^+$/Da
Wild type αHL	34991	34991
Hybrid Aβ42-αHL	35158	35159
Hybrid Aβ11-42-αHL	33980	33980
Hybrid Aβ1-28-αHL	33907	33908
Hybrid Aβ1-17-αHL	32712	32714
Hybrid Aβ0-αHL	30662	30663
Hybrid hIAPP-αHL	35095	35096

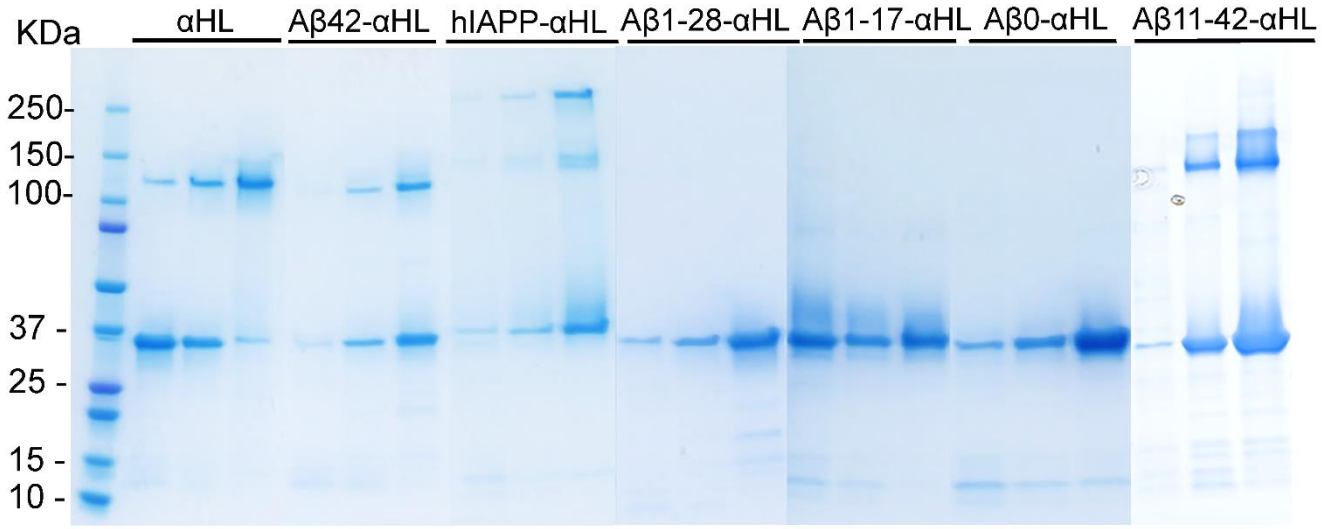

Figure S3. Purification of WT αHL and a series of hybrid Aβ/hIAPP–αHL proteins. All the proteins were expressed in *E. coli* and purified on the Co-NTA affinity column by using the His-tag at the C-terminus. SDS-PAGE Electrophoresis (Bio-Rad, 4-20 %) was conducted at 200 mV to monitor the protein quality. There was an imidazole concentration gradient (20 mM, 50 mM and 250 mM respectively) in the elution buffer (50 mM Tris-HCl, pH 8.0, 500 mM NaCl and 0.38 mM DDM) to separate the monomer and oligomer. The SDS-PAGE Electrophoresis was conducted at 200 V for 25 min.

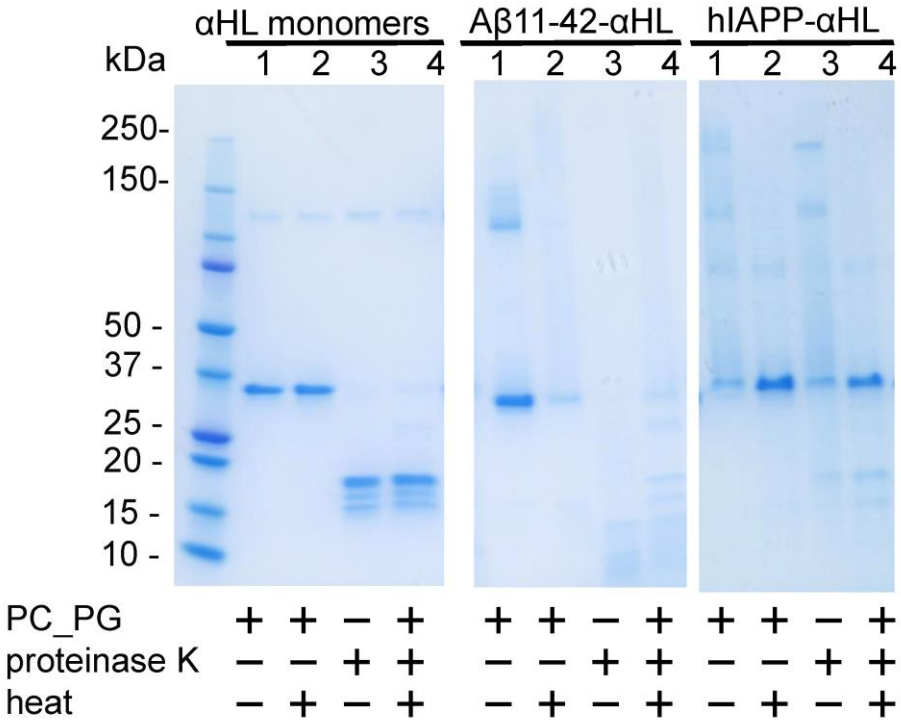

Figure S4. Limited proteolysis with proteinase K of the WT αHL monomers, hybrid Aβ11-42-αHL and hybrid hIAPP-αHL oligomers in the presence of DOPC:DOPG (4:1) liposomes (1 mg/ mL). For WT αHL monomers, Lane 1: αHL monomers were treated with DOPC:DOPG liposomes; lane 2: αHL monomers were treated with DOPC:DOPG liposomes and then got heat denatured at 95 °C for 15 min; lane 3: αHL monomers were digested with proteinase K; lane 4: αHL monomers treated with DOPC:DOPG liposomes were further digested by proteinase K and then heat-denatured at 95 °C. There were the same conditions for hybrid Aβ11-42-αHL and hybrid hIAPP-αHL oligomers. Monomeric WT αHL in the presence of proteinase K is taken as a control and shows more prone to breakdown, as reported previously [14]. Hybrid Aβ11-42-αHL and hybrid hIAPP-αHL oligomers exhibit the obvious proteolysis and heat denaturation.

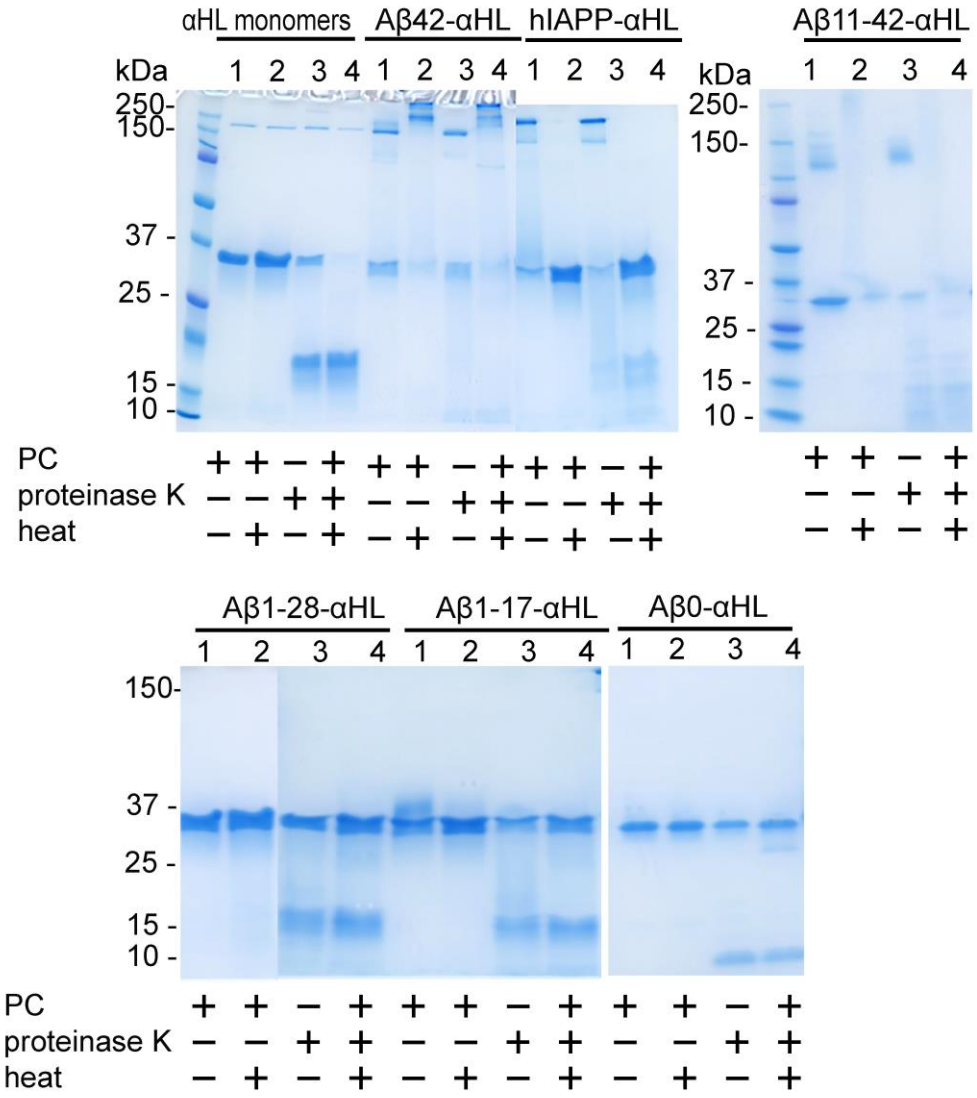

Figure S5. Limited proteolysis with proteinase K of WT αHL monomers and hybrid Aβ/hIAPP-αHL oligomers in the presence of DPhPC liposomes (1 mg/ mL). For αHL, Lane 1: αHL monomers were treated with DPhPC liposomes; lane 2: αHL monomers were treated with DPhPC liposomes and then got heat denatured at 95 °C for 15 min; lane 3: αHL monomers were digested with proteinase K; lane 4: αHL monomers treated with DPhPC liposomes were further digested by proteinase K and then heat-denatured at 95 °C. There were the same conditions for hybrid Aβ/hIAPP-αHL proteins.

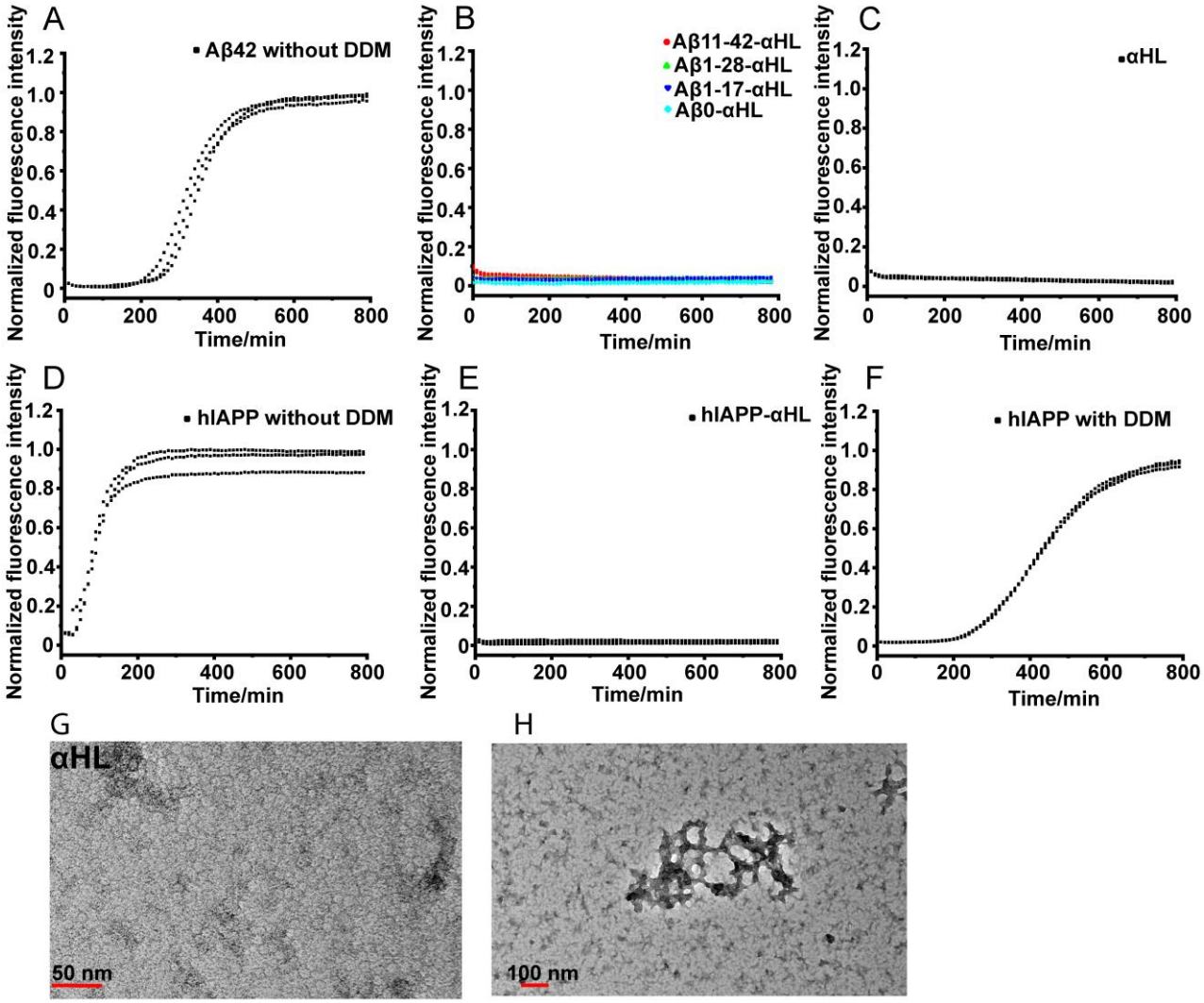

Figure S6. ThT fibrillation kinetics of the WT amyloid peptides, αHL displayed Aβ, or WT αHL proteins (A-F) and TEM images of WT αHL oligomers (G) as well as the amorphous aggregates of hybrid Aβ42-αHL (H). Higher ThT fluorescence intensity indicates more fibril formation. (A) WT Aβ42 in the buffer of 50 mM Tris-HCl, pH 8.0, 0.5 M NaCl and 250 mM imidazole. (B) A series of hybrid Aβ-αHL oligomers including Aβ11-42-αHL, Aβ1-28-αHL, Aβ1-17-αHL or Aβ0-αHL, prepared in the same condition as Aβ42-αHL in Figure 3B (50 mM Tris-HCl, pH 8.0, 500 mM NaCl, 250 mM imidazole and 0.38 mM DDM). (C) WT αHL in the buffer of 50 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 250 mM imidazole and 0.38 mM DDM. (D) WT hIAPP amyloid aggregation assay in the buffer of 50 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 250 mM imidazole. (E) Hybrid hIAPP-αHL or (F) WT hIAPP was used for the kinetic assay in the buffer of 50 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 250 mM imidazole and 0.38 mM DDM. The ratio of ThT and proteins was 4:1 with a ThT final concentration at 40 μM. The excitation and emission filters were 430 and 480 nm, respectively. (G-H) The samples were obtained from the end of ThT kinetics for TEM imaging. Scale bars of WT αHL oligomers (G) and the amorphous aggregates of hybrid Aβ42-αHL (H) are 50 nm and 100 nm respectively.

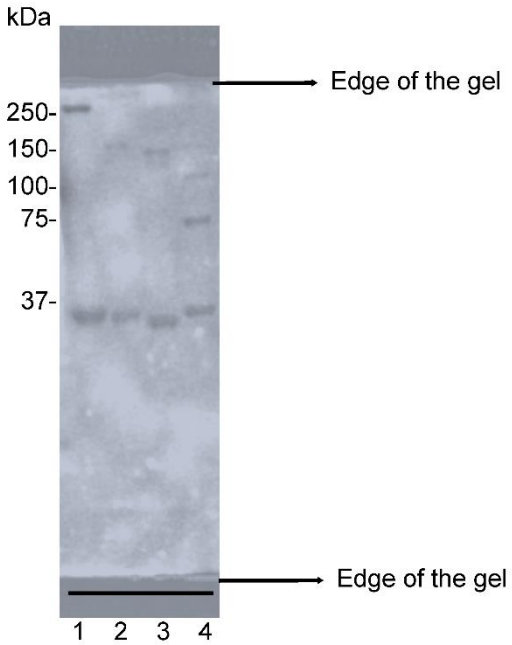

Figure S7. Immunogenic similarity of WT αHL, hybrid Aβ42/11-42-αHL and hybrid hIAPP- αHL by western blot. The anti-Aβ42 oligomer conformation-dependent antibody A11 recognized αHL oligomers (lane 1); hybrid Aβ42-αHL oligomers (lane 2); hybrid Aβ11-42-αHL oligomers (lane 3); hybrid hIAPP-αHL oligomers (lane 4). The SDS-PAGE electrophoresis prior to blotting, was conducted at 120 V for 80 min. No bands with molecule weight lower than the monomer bands (around 30-35 kDa) were found on the gel.

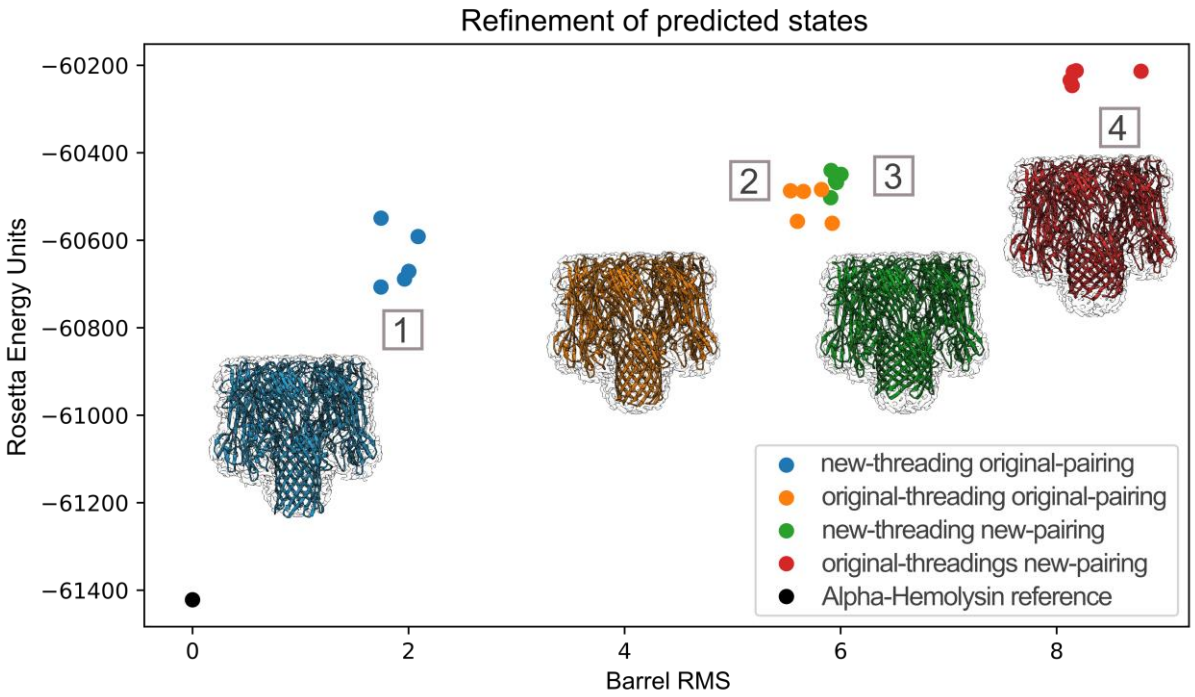

Figure S8. Total score comparison between the four assessed states of the Aβ-αHL chimera as evaluated by the Rosetta full atom score function (lower score is better/more stable). The x-axis is the RMS (Root Mean Square) deviation of our models to the αHL (PDB: 7AHL) scaffold, and each of the clusters of points represent a particular modeled conformation of our Aβ42-αHL. The y-axis represents the energy landscape of the possible conformations of Aβ42 region on the αHL scaffold, given the low-resolution electron density. We found the models to be ranked as follows: 1. original β-barrel strand pairing with custom alignment (blue), 2. original β-barrel strand pairing with hhpred alignment (orange), 3. shifted β-barrel strand pairing with custom alignment (green), 4. shifted β-barrel strand pairing with hhpred alignment (red).

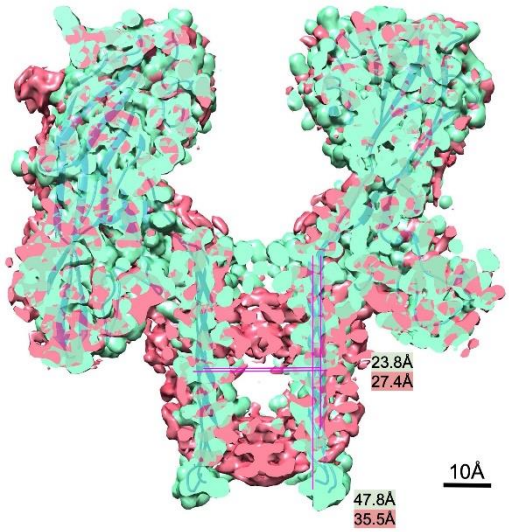

Figure S9. Map superposition of WT αHL (green) and the hybrid Aβ42-αHL (red). The hybrid Aβ42-αHL β-barrel pore has a shorter length (35.5 Å) and a similar inner diameter (27.4 Å) of the largest circular cross-section, compared to the WT αHL barrel (47.8 Å high and 23.8 Å wide).

Table S2. Structure refinement by Phenix software

Model		
Composition (#)		
Chains	7	
Atoms	32434 (Hydrogens: 15921)	
Residues	Protein: 2051 Nucleotide: 0	
Water	0	
Ligands	0	
Bonds (RMSD)		
Length (Å) (# > 4σ)	0.007 (0)	
Angles (°) (# > 4σ)	0.560 (0)	
MolProbity score	1.35	
Clash score	4.28	
Ramachandran plot (%)		
Outliers	0	
Allowed	2.75	
Favored	97.25	
Rama-Z (Ramachandran plot Z-score, RMSD)		
Whole (N = 2037)	0.21 (0.18)	
Helix (N = 42)	4.43 (0.08)	
Sheet (N = 1057)	0.32 (0.16)	
Loop (N = 938)	0.35 (0.20)	
Rotamer outliers (%)	0.39	
Cβ outliers (%)	0	
Peptide plane (%)		
Cis proline/general	11.1/0.0	
Twisted proline/general	0.0/0.0	
CaBLAM outliers (%)	0.69	
ADP (B-factors)		
Iso/Aniso (#)	16513/0	
min/max/mean		
Protein	28.63/173.52/59.28	
Nucleotide	---	
Ligand	---	
Water	---	
Occupancy		
Mean	1	
occ = 1 (%)	100	
0 < occ < 1 (%)	0	
occ > 1 (%)	0	
Data		
Box		
Lengths (Å)	112.27, 112.27, 111.24	
Angles (°)	90.00, 90.00, 90.00	
Supplied Resolution (Å)	3.3	
Resolution Estimates (Å)	Masked	Unmasked
d FSC (half maps; 0.143)	---	---
d 99 (full/half1/half2)	3.4/---/---	3.4/---/---
d model	3.3	3.3
d FSC model (0/0.143/0.5)	2.7/3.1/3.3	2.7/3.1/3.3
Map min/max/mean	-0.08/0.14/0.00	
Model vs. Data		
CC (mask)	0.8	
CC (box)	0.73	
CC (peaks)	0.74	
CC (volume)	0.79	

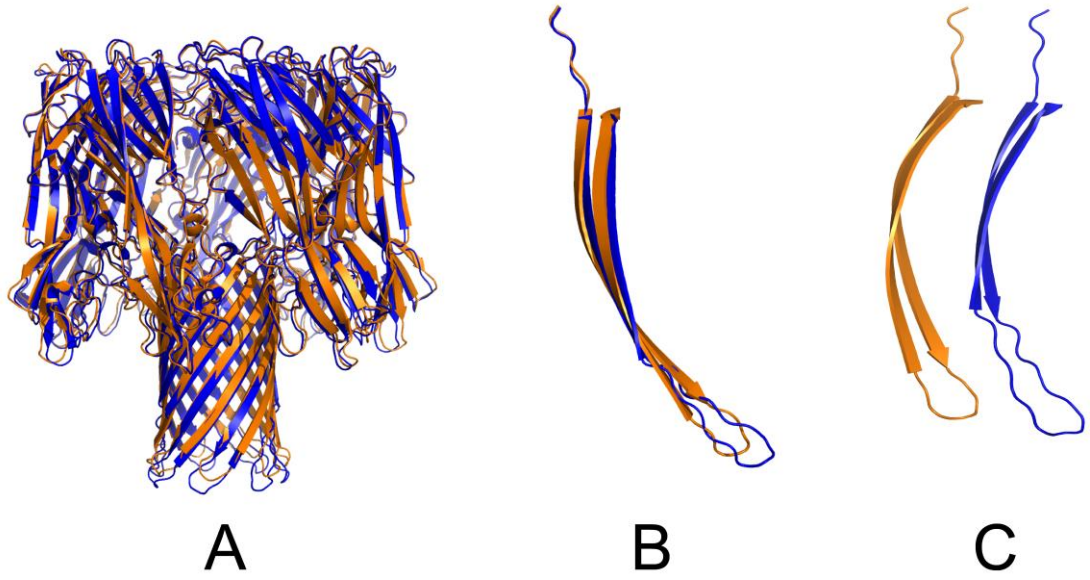

Figure S10. A**:** Superposition of heptameric Aβ42 displayed on the αHL scaffold (in orange) and WT αHL (in blue). B-C: Comparison of the displayed Aβ42 sequence (in orange) and αHL pre-stem domain (residues 106-147 in blue).

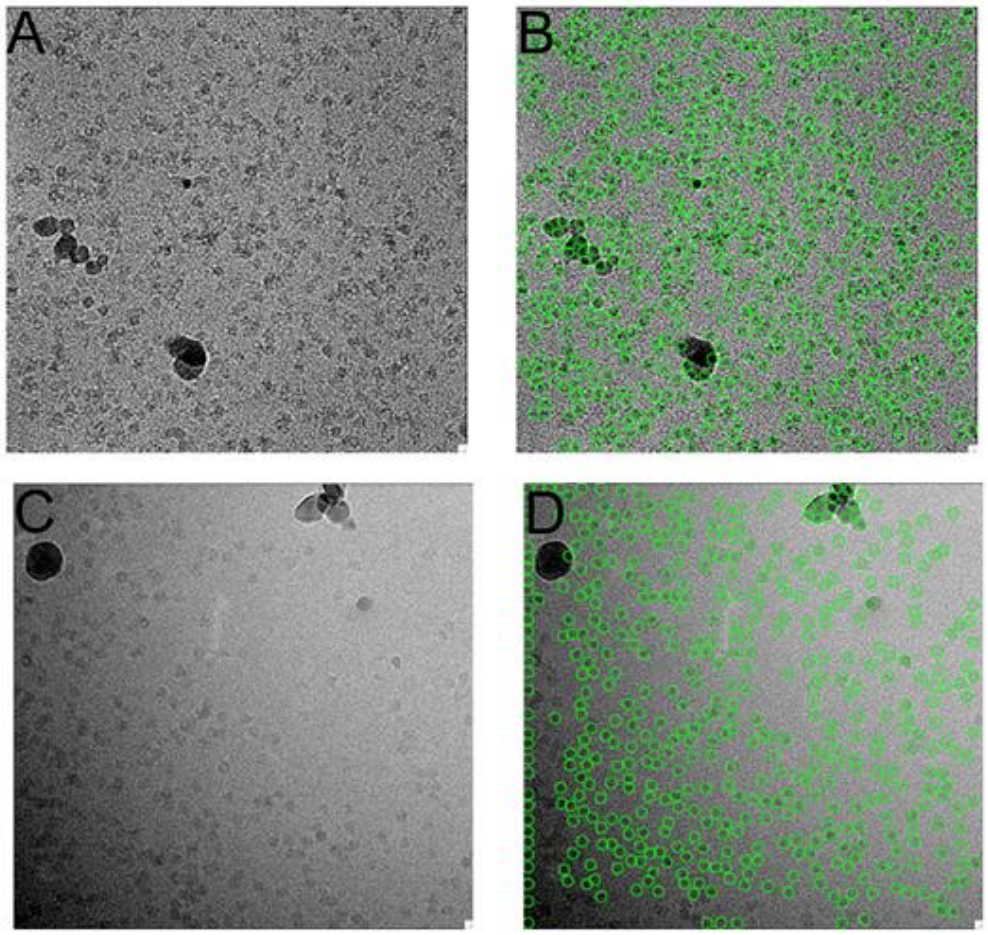

Figure S11. The visualization of hybrid Aβ42-αHL particles in two regions by cryo-EM. 2'438'446 particles (green box) from 4'284 micrographs were automatically picked by RELION 3.0.

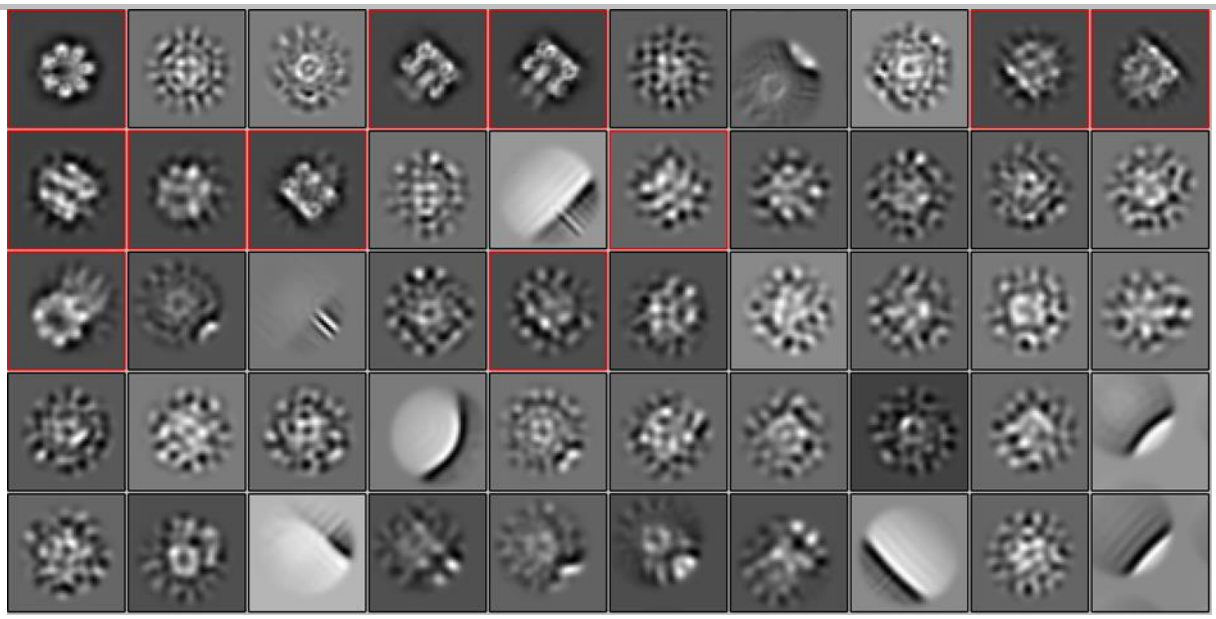

Figure S12. The primary 2D classification of hybrid Aβ42-αHL particles. Some classes that are not framed in red boxes contained noise or contamination and thus were dropped before proceeding on the density map. The particles being framed in the red box were used for 3D auto-refine and the beam tilt values for the entire data set.

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Jinming Wu: Investigation, validation, project administration. Thorsten B. Blum: Investigation, validation. Daniel P Farrell and Frank DiMaio: Investigation, validation. Jan Pieter Abrahams: data analysis, validation. Jinghui Luo: Conceptualization, validation, the project administration and funding acquisition.