Designed peptides as nanomolar cross-amyloid inhibitors acting via supramolecular nanofiber co-assembly

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Amyloid self-assembly is linked to numerous devastating cell-degenerative diseases. However, designing inhibitors of this pathogenic process remains a major challenge. Cross-interactions between amyloid-β peptide (Aβ) and islet amyloid polypeptide (IAPP), key polypeptides of Alzheimer’s disease (AD) and type 2 diabetes (T2D), have been suggested to link AD with T2D pathogenesis. Here, we show that constrained peptides designed to mimic the Aβ amyloid core (ACMs) are nanomolar cross-amyloid inhibitors of both IAPP and Aβ42 and effectively suppress reciprocal cross-seeding. Remarkably, ACMs act by co-assembling with IAPP or Aβ42 into amyloid fibril-resembling but non-toxic nanofibers and their highly ordered superstructures. Co-assembled nanofibers exhibit various potentially beneficial features including thermolability, proteolytic degradability, and effective cellular clearance which are reminiscent of labile/reversible functional amyloids. ACMs are thus promising leads for potent anti-amyloid drugs in both T2D and AD while the supramolecular nanofiber co-assemblies should inform the design of novel functional (hetero-) amyloid-based nanomaterials for biomedical/biotechnological applications.

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Fig. 1 | ACM design concept, their effects on IAPP amyloid self-assembly and cytotoxicity, and ACM secondary structures. a Sequences of IAPP and Aβ(40(42)), proposed models of fAβ and fAβ40 folds, and hypothetical IAPP/Aβ40 “hetero-amyloids” β-strands, pink or blue and underlined; “hot segments” of self-/cross-interactions, bold; loop residues, italics)25,30,31. b ACM inhibitor design strategy. Template Aβ(15–40) in a β-strand-loop-β-strand fold proposed for fAβ40 is modified via (a) N-methylations in Aβ(17–20), (b) substitution of Aβ(24-26) by hydrophobic tripeptides, and (c) Met35 substitution by Nle. e Sequences of the six ACMs and negative controls VGS-VF and VGS-LF (Supplementary Table 1). Each sequence corresponds to two different ACMs which contain the same LTS but a different couple of N-methylated residues (dashed boxes). Color code as in a: LTS and tripeptide VGs in red; green or violet for peptide names and corresponding N-methylated residues. d Nle3-VF, L3-VF, and F3-VF block IAPP amyloid self-assembly. Fibrillogenesis of IAPP (16.5 µM) alone or with ACMs or VGS-VF was assessed via ThT binding (IAPP/peptide 1/2) (means ± SD, n = 3 independent assays). e Nle3-VF, L3-VF, and F3-VF suppress the formation of toxic IAPP assemblies. Solutions of f (7-day-aged (VFS-VF 24 h)) added to RIN5fm cells; cell viability determined via MTT reduction (means ± SD, three technical replicates each). g ACMs inhibit fAβ4mediated cross-seeding of IAPP. Fibrillogenesis of IAPP (12 µM) without or with fAβ seeds (10%) and seeded IAPP/ACM mixtures assessed via ThT binding (IAPP/ACM 1/2) (means ± SD, n = 9 (for IAPP alone) or 3 (for all other samples) independent assays). h ACMs inhibit seeding of IAPP by preformed fAβ. Fibrillogenesis of IAPP (12 µM) without or with fAβ seeds (10%) and seeded IAPP/ACM mixtures assessed via ThT binding (IAPP/ACM 1/2) (means ± SD, n = 3 (for all other samples) independent assays). i ACMs inhibit seeding of IAPP by preformed fAβ. Fibrillogenesis of IAPP (12 µM) without or with fAβ seeds (10%) and seeded IAPP/ACM mixtures assessed via ThT binding (IAPP/ACM 1/2) (means ± SD, n = 3 (for all other samples) independent assays).
assemblies and amyloid fibrils mediates pancreatic β-cell degeneration in T2D.

Epidemiological studies suggest that T2D patients have an increased risk of AD and vice versa. In addition, increasing evidence suggests molecular and pathophysiological links between both diseases. Cross-interactions between Aβ and IAPP could be such molecular links. In fact, polymorphic Aβ/IAPP interactions are able to cross-seed or cross-suppress amyloid self-assembly depending on structures and self-assembly states of the interacting polypeptides. To this end, IAPP and Aβ fibrils act as reciprocal cross-seeds of amyloid self-assembly, as shown by both in vitro and experimental in vivo studies. On the other hand, nanomolar affinity interactions between early pre-fibrillar and non-toxic IAPP and Aβ species redirect both polypeptides into initially non-fibrillar and non-toxic co-assemblies, thus delaying amyloid self-assembly. Importantly, Aβ and IAPP were found to colocalize in AD- and T2D-related amyloid deposits both in humans and in mouse models. Aβ/IAPP cross-interactions and putative “hetero-amyloids” could thus be highly relevant to the pathogenesis of both diseases.

Based on the above, molecules targeting amyloid self-assembly and reciprocal cross-seeding effects of IAPP and Aβ could be promising leads for anti-amyloid and anti-bril treatments for T2D. Furthermore, so far, only a few inhibitors of amyloid self-assembly of both polypeptides (termed “cross-amyloid” inhibitors) have been reported and none of them suppressed reciprocal Aβ/IAPP cross-seeding. Moreover, except for a recently approved and controversially discussed anti-Aβ amyloid antibody, no anti-amyloid treatments for AD or T2D have yet reached the clinic.

One reason for the high-affinity IAPP/Aβ(40/42) cross-interactions could be the sequence similarity (50%) and identity (~25%) between both polypeptides. Notably, higher degrees of sequence identity/similarity are observed between their amyloid core segments IAPP(8–28) and Aβ(15–40/42). In addition, the same IAPP- or Aβ(40/42)-“hot segments” within their amyloid core segments were found to mediate both self- and cross-interactions. Strong similarities exist also between their fibril folds and potential cross-seeding interfaces within putative hetero-amyloids were proposed.

Capitalizing on IAPP/Aβ cross-interactions, we have previously designed peptides derived from the IAPP amyloid core IAPP(8–28) as IAPP “interaction surface mimics” (ISMs). ISMs effectively suppressed amyloid self-assembly of Aβ(40/42) and/or IAPP by sequestering them into amorphous, non-toxic aggregates.

Here, we explored the idea of designing peptides derived from the Aβ40 amyloid core Aβ(15–40) as Aβ “amyloid core mimics” (ACMs) and inhibitors of amyloid self-assembly and cross-interactions of IAPP and Aβ. Our inhibitor design concept aimed at distorting the pathogenic fibril fold of Aβ(15–40) and stabilize alternative, amyloid-like but non-amyloidogenic folds. These should yield alternative interaction surfaces with IAPP or Aβ42 and redirect them into non-fibrillar and non-toxic aggregates. A series of conformationally constrained peptides was synthesized and studied. In fact, ACMs were non-amyloidogenic and non-cytotoxic, bound IAPP and Aβ42 with nanomolar affinity, and fully blocked their cytotoxic amyloid self-assembly. Furthermore, ACMs effectively suppressed reciprocal cross-seeding effects. Surprisingly, ACMs exerted their inhibitory function by co-assembling with IAPP or Aβ42 into amyloid fibril-resembling nanofibers and their diverse, highly ordered superstructures. For their characterization, a spectrum of biophysical, biochemical, and advanced microscopy methods, including confocal laser-scanning microscopy (CLSM), stimulated emission depletion (STED) imaging, two-photon microscopy (2PM), and fluorescence lifetime imaging microscopy (FLIM)-based Förster resonance energy transfer (FRET) (FLIM-FRET) was applied. In addition, in vitro and ex vivo cell-based assays were used. In strong contrast to IAPP or Aβ42 fibrils (IAPP or fAβ42), co-assembled nanofibers were “ThT-invisible”, non-cytotoxic, and seeding-incompetent. Moreover, they were thermostable, easily degradable by proteinase K (PK), and became efficiently phagocytosed in vitro by primary macrophages and cultured microglial cells.

Results
Inhibitor design and concept evaluation
For inhibitor design, Aβ(15–40) was used as a template in the context of the fAβ40 fold suggested by Petkova et al., which features a β-strand-loop-β-strand motif with Aβ(12–22) and Aβ(30–40) forming the β-strands and Aβ(23–29) the loop (Fig. 1a, b). Of note, this U-shaped fold has often been applied to model Aβ-IAPP hetero-amyloids. A minimum number of chemical modifications was made aiming at (a) distorting the loop, (b) stabilizing β-sheet structure, and (c) suppressing intrinsic amyloidogenicity of Aβ(15–40) while maintaining its pronounced self-cross-assembly propensity in analogy to the ISM concept (Fig. 1b). The modifications were: (a) substitution of loop tripeptide Aβ(24–26) (Val-Gly-Ser) by β-sheet-propagating tripeptides consisting of identical large hydrophobic residues, which were expected to strengthen β-sheet interaction surfaces while being incompatible with localization in turns/β-arcs and (b) selective amide and N-methylation of two alternate residues within both of the two Aβ β-strand segments, which should suppress intrinsic amyloidogenicity of ACMs and their co-assemblies (Fig. 1b). Positions of N-methylations were based on fAβ40 models and previous SAR studies. Finally, Met35 was replaced by Nle to avoid Met(O)-related side effects.

To evaluate the concept, 13 Aβ(15–40) analogs containing various different “loop tripeptide segments” (LTS), comprising (Nle3)3, (Leu3), (Phe3), (Arg3), (Gly3), or Val-Gly-Ser (control LTS) and one pair of two N-methylated residues were designed, synthesized and studied. First, the effect of unmodified Aβ(15–40) (abbreviated VGS) on IAPP fibrillogenesis was studied by using the amyloid-specific thioflavin T binding assay and was found unable to inhibit (Supplementary Fig. 1a, b). However, non-N-methylated analogs Nle3 and L3 containing LTS (Nle3) or (Leu3) instead of Aβ(24–26) led to some delay in fibrillogenesis (Supplementary Fig. 1a, b). By contrast, analogs R3 and G3 containing LTS (Arg3) and (Gly3), respectively, did not inhibit and far-UV CD spectroscopy indicated less β-sheet structure than in Nle3 and L3 (Supplementary Fig. 1a–c). These findings suggested that Nle3 or L3 might be suitable candidates for further modifications.

Peptide Nle3 was then used as a template to identify best-suited positions for N-methylations. The four Nle3 analogs Nle3-LF, Nle3-VF, Nle3-GI, and Nle3-GG were synthesized, each of them containing two N-methylations placed at specific residues either within the N-terminal region corresponding to Aβ(15–23) (analogs Nle3-VF and Nle3-LF) or within the C-terminal region corresponding to Aβ(27–40) (analogs Nle3-GI & Nle3-GG) (Supplementary Fig. 2a and Supplementary Table 1). Peptides Nle3-VF and Nle3-LF (Fig. 1c) carrying N-methylations at Val8/Phes20 and at Leu17/Phes19, respectively, fully suppressed IAPP fibrillogenesis and cytotoxicity as determined by ThT binding in combination with the 3,4,5-dimethylanilazo-2-yl-L-2,5-diphenyltetrazolium bromide (MTT) reduction assay in cultured rat insulinoma cells (Fig. 1d–g, Supplementary Fig. 2b, c, and Supplementary Fig. 3a, b). By contrast, Nle3-GI and Nle3-GG, carrying N-methylations at Gly29/Ile31 and at Gly29/Ile33, respectively, did not inhibit (Supplementary Fig. 2b, c). Titration of cytotoxic IAPP with Nle3-VF or Nle3-LF revealed nanomolar IC50 values consistent with highly potent inhibitory activities (Table 1 and Supplementary Fig. 4a, b). Of note, the introduction of the N-methylations of Nle3-VF and Nle3-LF into the non-inhibitory peptides VGS, G3 or R3 did not convert them into...
supplementary Fig. 3c were <100 nM and in very good agreement with the determined IC50 results suggested that ACMs bind IAPP monomers/pretripeptide (Nle)3 and one of the two N-methylation patterns within peptides (Leu)3 or (Phe)3 and each of the two identified N-methylation patterns (Table 1 and Supplementary Fig. 4c).

Importantly, in the presence of ACMs both self- and cross-seeding of IAPP amyloid formation by preformed fIAPP or fAβ40 hetero-oligomers was confirmed by size exclusion chromatography (SEC) (Fig. 2c). In addition, formation of low MW IAPP homo- and IAPP/ACM hetero-oligomers was confirmed by electrospray ionization-mobility spectrometry-mass spectrometry (ESI-MS-MS), a method which had previously been successfully applied to characterize IAPP/Al40 hetero-oligomers (Supplementary Fig. 8).

Far-UV-CD spectroscopy revealed that IAPP/Nle3-VF co-assemblies exhibited a mixture of disordered and β-sheet structures (Fig. 2d). By contrast, the CD spectra of aged IAPP and IAPP/non-inhibitor (VGS-VF) mixtures were typical for β-sheet-rich aggregates (Fig. 2d). Furthermore, anilinonaphthalene 8-sulfonate (ANS) binding studies indicated that IAPP/Nle3-VF co-assembly fully suppressed surface-exposure of hydrophobic clusters, which occurs at early steps of IAPP amyloid self-assembly and is likely related to cytotoxic oligomer formation (Fig. 2e).

To characterize the morphology of the IAPP/ACM co-assemblies, solutions used for ThT binding and MTI reduction assays were examined with transmission electron microscopy (TEM). As expected, fibrillar assemblies were major species in aged IAPP and its mixtures with the non-inhibitor VGS-VF (Fig. 2f). However, we were surprised to see that the aged mixture of IAPP with six ACMs exclusively consisted of fibrillar assemblies as well; these fibrils were indistinguishable from IAPP fibrils by TEM (6–10 nm widths and 100–200 nm lengths) (Fig. 2f and Supplementary Table S2). Notably, in contrast to aged IAPP and IAPP/non-inhibitor mixtures, no turbidity, gelation, or precipitation, was observed in the above IAPP/ACM mixtures. X-ray fiber diffraction then revealed that fibrils in IAPP/Nle3-VF mixtures exhibited the cross-β pattern, which is typical for amyloid fibrils (Fig. 2g).

Because fIAPP strongly binds ThT and ACMs were non-amyloidogenic up to at least 100 μM (Fig. 1d, Supplementary Fig. S5a and b), it seemed reasonable to speculate that the fibrils in the IAPP/ACM mixtures could be: (a) IAPP which escaped detection by the ThT binding assay or (b) fIAPP which was covered with non-specifically bound ACMs; competition of ACMs and ThT for the same fibril binding sites might have blocked ThT binding to fIAPP. However, these possibilities were excluded by a series of experiments (Supplementary Fig. 9). Of note, most of the fibrillar assemblies of IAPP/ACM mixtures did not bind the amyloid dye Congo red as well (Supplementary Fig. 10).

The ThT-invisible and non-cytotoxic fibrils found in the IAPP/ACM incubations (termed “hf-IAPP/ACM”) might thus be heteromeric. To obtain more evidence for this hypothesis, we first applied immunogold-TEM. In fact, aged IAPP/ACM mixtures contained fibrils which bound both the anti-IAPP and the anti-Ab (anti-amyloid) antibody (Fig. 3a and Supplementary Fig. 11a). Additional support was obtained by hetero-complex pull-down assays. Here, ThT-invisible fibrils present in aged mixtures of N-terminal biotin-labeled IAPP (Biotin-IAPP) with Nle3-VF were captured by streptavidin-coated magnetic beads and their components revealed by WB (Fig. 3b).

High-resolution advanced laser-scanning microscopy provided further unequivocal evidence for diverse supramolecular IAPP/ACM nanofiber co-assemblies (Fig. 3c–i, Supplementary Fig. 11b–f, Supplementary Fig. 12, and Supplementary Movies 1, 2, CLSM, STEM, and 2PM visualization of aged IAPP/Nle3-VF mixtures containing N-terminal TAMRA-labeled IAPP (TAMRA-IAPP) and N-terminal Atto647N-labeled Nle3-VF (Atto647N-Nle3-VF) or N-terminal fluorescently-labeled Nle3-VF (Fluos-Nle3-VF) revealed large amounts of μm-long heteromeric nanofiber bundles (Fig. 3c, d, Supplementary Fig. 11b–e, and Supplementary Movies 1, 2). Their widths were 232 ± 76 nm (n = 33), whereas fIAPP homomeric nanofiber assemblies formed under identical conditions were less broad (124 ± 23 nm (n = 19)), as estimated by the more accurate STEM nanoscopy (Fig. 3e and Supplementary Fig. 11f). 3D reconstructions of z-stacks of 2PM pictures suggested that heteromeric nanofiber bundles consist of laterally co-assembled, parallel

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| ACM       | IC50 (±SD) (nM) | app. Kd (±SD) (nM) |
|-----------|----------------|--------------------|
| Nle3-VF   | 65.0 (±5.2)    | 69.5 (±1.4)        |
| Nle3-LF   | 82.1 (±10.2)   | 55.4 (±5.9)        |
| L3-VF     | 112.5 (±21.0)  | 77.3 (±12.9)       |
| L3-LF     | 133.2 (±22.0)  | 143.2 (±25.0)      |
| F3-VF     | 78.5 (±13.6)   | 15.0 (±1.9)        |
| F3-LF     | 41.7 (±5.4)    | 37.6 (±2.9)        |

IC50 values, means (±SD) from three independent titration assays (n = 3 technical replicates each).

Determined by titrations of N-terminal fluorescein-labeled IAPP (5 nM, pH 7.4) with ACMs.

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arranged/in part intertwined stacks of IAPP and ACM molecules (Supplementary Movies 1, 2 and Supplementary Fig. 1Id, e). Additional 2PM studies revealed diverse highly ordered fibrous superstructures, including huge macromolecular loops (>500 µm long) (Fig. 3g, h and Supplementary Fig. 12a) and ribbon- or nanotube-like co-assemblies (widths 5–20 µm, lengths >500 µm) (Fig. 3h, i). Interestingly, parts of the ribbon-like co-assemblies were reminiscent of giant DNA double helices (Fig. 3h, i). Here, IAPP assemblies seemed to “wrap” and “link” two parallel-running heteromeric nanofiber bundles and similar observations were made in the nanotube-like co-assemblies. Twisted heteromeric nanofiber bundles were also observed (Fig. 3h). Notably, IAPP mixtures with other ACMs but not with the non-inhibitor VGS-VF contained similar heteromeric nanofiber superstructures as the IAPP/Nle3-VF mixtures (Supplementary Fig. 12b–d).

At this stage, detailed studies on the interaction of ACMs with IAPP were performed (Supplementary Fig. 13). Dot blots showed that ACMs and the non-inhibitor VGS-VF bind IAPP. However, ACM/IAPP co-assemblies (termed “ACM-coated” IAPP) consisted of IAPP bundles which were randomly covered by amorphous Nle3-VF aggregates and maintained the ThT binding and cytotoxic properties of IAPP.

Fig. 2 | Nanomolar affinity IAPP/ACM interactions yield amyloid fibril‐resembling but ThT‐invisible nanofibers. a, Nanomolar affinity IAPP/ACM interactions as determined by fluorescence spectroscopy. Fluorescence spectra of Fluos-IAPP (5 nM) and its mixtures with Nle3-VF (pH 7.4) at indicated molar ratios (data from one representative binding assay from n = 3 independent assays). Inset, binding curve; data are means ± SD from n = 3 independent assays. b, IAPP/ACM interactions result in hetero‐dimers and medium‐to‐high MW hetero‐assemblies. Characterization of IAPP/Nle3‐VF hetero‐complexes via cross‐linking at different time points and NuPAGE and Western blot using anti‐IAPP (left) or anti‐Aβ (right) antibodies. IAPP/Nle3‐VF mixtures (1/2; IAPP, 30 µM) were cross‐linked with glutaraldehyde; the orange box indicates medium‐to‐high MW hetero‐assemblies (major species); arrows indicate hetero‐di/‐tri/‐tetramers. Representative results from 3 independent assays. c, Characterization of IAPP/Nle3‐VF hetero‐assemblies via size exclusion chromatography (SEC). Chromatograms of IAPP alone (16.5 µM) or its mixtures with Nle3‐VF (1/2) at 0 h and at 96 h (mAU, milli‐absorbance units). The black arrow indicates IAPP monomers and Nle3‐VF dimers; blue arrows indicate IAPP/Nle3‐VF hetero‐dimers and medium‐to‐high MW hetero‐assemblies. Similar results were found in two independent assays. d, IAPP/Nle3‐VF co‐assemblies are more disordered than β‐sheet‐rich IAPP assemblies. Far‐UV CD spectra of 7‐day‐aged IAPP (16.5 µM; pH 7.4) and its mixtures (1/2) with the Nle3‐VF or VGS‐VF (non‐inhibitor) are shown; for comparison, the spectrum of freshly dissolved IAPP (0 h) is also shown. CD spectra are the average of three spectra of the same solution. e, IAPP/Nle3‐VF co‐assembly blocks surface‐exposure of hydrophobic clusters occurring at early steps of IAPP amyloid self‐assembly as determined by anilinonaphthalene 8‐sulfonate (ANS) binding. Fluorescence emission spectra of ANS alone/with IAPP (2 µM) (left) and of ANS alone/with IAPP/ACM (1/2) mixtures (right) (pH 7.4) were measured at various time points of self‐ or co‐assembly as indicated (representative results from two independent assays). fIAPP/ACM interactions result in ThT‐invisible fibrils of indistinguishable appearance to fIAPP by TEM. TEM images of 7‐day‐aged IAPP (16.5 µM) and its mixtures (1/2) with ACMs or VGS‐VF (non‐inhibitor) (solutions from Fig. 1d, f). Scale bars: 100 nm. Representative images from seven independent assays for fIAPP and the IAPP/Nle3‐VF mixture and two to three similar independent assays for the other IAPP/ACM mixtures. g IAPP and fibrils in IAPP/Nle3‐VF mixture exhibit the amyloid cross‐β structure signature. X‐ray fiber diffraction patterns of IAPP and fibrils present in aged IAPP/Nle3‐VF mixture (1/2) showed major meridional and equatorial reflections at ~4.7 and ~10 Å. Data were representative of two independent experiments.
These results further supported the notion that hf-IAPP/ACM were distinct from ACM-coated fIAPP.

To learn more about the molecular architecture of the IAPP/ACM nanofibers, we used FLIM-FRET. Pronounced FLIM-FRET events were observed in TAMRA-IAPP/Fluos-Nle3-VF nano-fiber co-assemblies (Fig. 3j). The faster donor (Fluos-Nle3-VF) fluorescence decay, its strongly reduced lifetime (~0.8 ns) in presence of the acceptor (TAMRA-IAPP), and the appreciable FLIM-FRET efficiency (~55%) were consistent with a very close donor-acceptor proximity i.e., <5.5 nm corresponding to the Förster radius of the TAMRA/Fluos pair (Fig. 3i and Supplementary Fig. 14). The FLIM-FRET data supported the notion that IAPP and the ACM might be part of the same fibril.

Together, these results suggested that the potent inhibitory effect of ACMs is mediated by nanomolar affinity co-assembly with IAPP monomers/prefibrillar species into amyloid fibril-resembling but ThT-invisible and non-cytotoxic nanofibers and their diverse highly ordered superstructures.

**ACM/IAPP nanofibers evolve from amorphous co-assemblies and IAPP may act as a template**

We next asked at which stage of the co-assembly process the fibrillar co-assemblies form. The cross-linking and SEC studies indicated that large hetero-assemblies were present already at the beginning of the co-incubation (Fig. 2b, c). TEM only detected amorphous aggregates between 0 and 48 h, whereas fibrils were the most abundant species at
later time points (7 days) (Fig. 4a and Supplementary Fig. 15a). Notably, early amorphous aggregates in the IAPP/ACM mixtures were non-cytotoxic as also found for the fibrils (Supplementary Fig. 15b). Thus, non-toxic hf-IAPP/ACM likely evolve via structural rearrangements of non-toxic amorphous co-aggregates.

Because ACMs were non-amyloidogenic in isolation but co-assembled with IAPP into amyloid fibril-resembling nanofibers, we hypothesized that the amyloidogenic character of IAPP could play a role. In fact, TEM showed that no fibrils formed in mixtures of Nle3-βC with the natively occurring (human) IAPP analog rat IAPP or the earlier designed double N-methylated IAPP analog IAPP-GI, which have high sequence identity to IAPP but are weakly or non-amyloidogenic (Fig. 4b)\(^{31}\). Moreover, “cross-nucleation” studies using 2PM and FLIM-FRET suggested a templating role for IAPP monomers/pre fibrillar species (Fig. 4c, d). Addition of seed amounts (5%) of TAMRA-IAPP monomers to Fluos-Nle3-βC yielded within 48 h nanofiber co-assemblies of similar appearance and FLIM-FRET properties as those present in 6-day-aged TAMRA-IAPP/Fluos-Nle3-βC (1/2) mixtures (Figs. 4c, d, Sf, Supplementary Fig. 16, Supplementary Movie 4). Notably, appreciable FLIM-FRET events were detected when TAMRA-IAPP seeds were used (Supplementary Fig. 17).

Additional properties of hf-IAPP/ACM

We next studied whether non-toxic hf-IAPP/ACM may differ from IAPP regarding other properties as well. First, we asked whether hf-IAPP/ACM can seed IAPP fibrillogenesis\(^{44}\). However, in contrast to IAPP or fibrils present in IAPP/non-inhibitor mixtures, seed amounts of hf-IAPP/ACM were unable to accelerate IAPP fibrillogenesis as assessed by ThT binding (Fig. 4e).

Pathogenic amyloid fibrils are usually characterized by an extraordinarily high stability\(^{44}\). Therefore, we compared the thermo-stabilities of IAPP/ACM nanofibers and IAPP by using ThT binding and TEM. In contrast to IAPP, hf-IAPP/Ne3-βC were fully converted into amorphous aggregates after heating to 95°C for 5 min (Fig. 4f). Furthermore, most pathogenic amyloids are resistant toward proteolytic degradation, including PK degradation\(^{11}\). IAPP and hf-IAPP/ACM were therefore incubated with PK and degradation kinetics followed by dot blot analysis using anti-IAPP and anti-β (anti-ACM) antibodies (Fig. 4g). Remarkably and in contrast to IAPP, which was stable to PK digestion for at least 30 h, hf-IAPP/ACM were fully degraded in <6 h with their ACM component degraded within few minutes. Finally, we studied the cellular uptake efficiency of hf-IAPP/ACM in direct comparison to IAPP. In fact, the uptake of amyloid assemblies by macrophages and microglia is a major mechanism of amyloid clearance in both AD and 2D\(^{-46,48}\). Uptake of IAPP versus hf-IAPP/ACM was studied in primary murine bone marrow-derived macrophages (BMDMs) and the well-established murine microglial cell line BV2 by fluorescence microscopy using TAMRA-IAPP as tracer\(^{17,76}\). We found that three- to ten-fold higher amounts of hf-TAMRA-IAPP/ACM were phagocytosed by both cell types as compared to TAMRA-IAPP (Fig. 4h and Supplementary Fig. 18).

Together, the results revealed several potentially beneficial properties of non-toxic hf-IAPP/ACM, i.e., seeding incompetence, thermostability, high PK sensitivity, and an efficient cellular clearance profile, which clearly distinguished them from pathogenic IAPP.

Proposed hypothetical models of IAPP/ACM nanofiber co-assemble

The structure of Aβ/IAPP hetero-amyloids has not yet been elucidated\(^{31}\). Based on the suggested structures of fAPP and fIAPP\(^{40,42}\) and the polymorphic nature of the self-/cross-amyloid assembly, various different interfaces could be involved in hf-IAPP/ACM formation (Fig. 5). Our data suggested that single IAPP/ACM nanofibers and basic units of their superstructures may form by lateral co-assembly of two or more “protoplan”-like stacks of IAPP and ACM molecules (Fig. 5). Thereby, β-sheet-prone segments of the ACM part corresponding to Aβ(21–40) would become incorporated into the β-sheet H-bond network of the ACM “protoplan” yielding diverse cross-interaction surfaces with both IAPP and ACM stacks (Fig. 5a, b)\(^{31,51,52}\). The proposed arrangement of Aβ(21–40) is also supported by the finding that Aβ(15–40) analogs with N-methylations within Aβ(21–40) did not inhibit (Supplementary Fig. 2a–c). IAPP “protoplan” cross-interactions with the ACM could be mediated via previously suggested IAPP segments and IAPP folds or yet unknown variants thereof\(^{31,51,52}\). The lack of a second IAPP “protoplan” and a non-ideal IAPP/βA(ACM) side chain interdigitalion in the hetero-nanofiber as compared to fAPP and/or intrinsic instability of involved IAPP and or/ACM folds could account for hetero-nanofiber lability\(^{31,51}\). Notably, axial IAPP/ACM “protoplan” co-assembly could also occur (Fig. 5c)\(^{31}\). However, the 2PM and 2PM data, the expected higher instability of such a “protoplan” due to the N-methylations, and the observed lack of inhibitory effects of partial ACM segments (Supplementary Fig. 2f–h) make this scenario less likely.

ACMs inhibit Aβ42 amyloid self-assembly via co-assembly into ThT-invisible and non-toxic nanofibers and their diverse superstructures

In analogy to other Aβ-derived Aβ inhibitors, ACMs would be expected to also interfere with Aβ amyloid self-assembly\(^{31,51,52}\). In fact, ThT...
Fig. 4 | Mechanism of formation and properties of IAPP/ACM nanofiber co-assemblies. a) Evolution of hf-IAPP/ACM from amorphous co-aggregates. TEM images of IAPP (16.5 μM) and IAPP/Nle3-VF mixtures (1/2) between 0 and 7 days of incubation. Scale bars: 100 nm. Data were representative from two independent similar experiments. b) TEM images of 7-day-aged IAPP-Gl/Nle3-VF or rat IAPP/Nle3-VF (1/2) show amorphous aggregates. Images show major aggregate populations present in each sample. c) Consistent results were found by 2PM for IAPP-Gl/Nle3-VF. Scale bars: 100 nm. e) IAPP monomers/pre fibrillar species template nanofiber co-assembly. Representative 2PM images of Fluos-Nle3-VF (33 μM) cross-seeded with freshly made TAMRA-IAPP (5%). Scale bars: 10 μm; inset, 1 μm. Images are from one sample examined at various incubation time points and data were consistent with 2PM data of Fig. 3e–j. d) FLIM-FRET of nanofiber co-assembly of e at 48 h reveals similar features to hf-TAMRA-IAPP/Fluos-Nle3-VF (1/2; 6-day-aged) from Fig. 3j. Left panel, fluorescence decay curves (top) and lifetimes (bottom) of Fluos-Nle3-VF without or with TAMRA-IAPP shows a strong shift of donor lifetime in the presence of acceptor. Middle panel/upper part, FLIM image showing donor lifetime; range as indicated; scale bar, 5 μm. Middle panel/lower part, FLIM-FRET efficiency (%); range as indicated; scale bar, 5 μm. Right panel, distributions donor lifetime (<1 ns) and FLIM-FRET efficiency (75%). Data from one experiment. e) hf-IAPP/ACM are seeding-incompetent. IAPP (12 μM) fibrillogenesis alone or with 10% hf-IAPP/ACM, fIAPP, or IAPP/VGS-VF was followed by ThT binding (means ± SD from n = 4 (IAPP alone and IAPP with 10% fIAPP) or n = 3 (all other samples) independent assays). f) Thermostability of hf-IAPP/ACM versus fIAPP. Left panel, ThT binding of fIAPP and hf-IAPP/Nle3-VF before/after boiling (5 min); means ± SD, three independent assays. Right panel, representative TEM images after boiling; scale bars: 100 nm. Results are representative from two similar independent experiments. g) Degradation of hf-IAPP/ACM versus fIAPP by proteinase K (PK) followed by dot blot. fIAPP or hf-IAPP/Nle3-VF were subjected to PK digestion (37 °C); quantification by anti-fIAPP and anti-β-Aβ antibodies. Representative membranes from three independent assays. h) Phagocytosis of hf-IAPP/ACMs versus fIAPP by primary murine BMDMs and cultured murine BV2 microglia. Left panel, representative microscopic images of cells following incubation (6 h, 37 °C) with TAMRA-IAPP (3.3 μM) or hf-TAMRA-IAPP/Fluos-Nle3-VF (3.3 μM); red dots, TAMRA-IAPP; scale bars, 100 μm. Mid and right panels, amounts of phagocytic cells (% of total). Data means ± SD from 18 or 15 biologically independent samples of TAMRA-IAPP or hf-TAMRA-IAPP/Nle3-VF, respectively. Analyzed in five independent cell assays with each assay well analyzed in three fields of view. **P < 0.0001 for hf-TAMRA-IAPP/Nle3-VF versus TAMRA-fIAPP, i.e., P = 1.2349E-09 in BMDM cells and 1.5781E-06 in BV2 cells (unpaired t-test (two-sided)).
CR, or the non-amyloidogetic ACMs, we assumed that they might be heteromorphic (termed hf-Aβ42/ACM).

2PM examination of aged Aβ42/ACM mixtures containing N-terminal TAMRA-labeled Aβ42 (TAMRA-Aβ42) and Fluos-ACMs revealed diverse heteromorphic fibrous superstructures. These comprised several µm-long heteromorphic nanofiber bundles with widths between 0.5–2 µm and related heterogeneous superstructures, i.e. ribbons, tapes, or nanotube-like ones with widths between 3–14 µm (Fig. 6e and Supplementary Fig. 22). The 2PM images and 3D reconstructions of z-stacks indicated that both axial and lateral co-assembly might underlie their formation, likely enabled by the high degree of sequence identity between ACMs and Aβ42 (Supplementary Movie 5). Pronounced FLIM-FRET events were detected, which were consistent with close distances between the two peptides, i.e., <5.5 nm (Fig. 6f and Supplementary Fig. 23). Notably, a stronger reduction of Fluos-Nle3-VF lifetime in the pre-node region (ROI-1) than in the "cable"-like ones (ROI-2) consistent with their structural heterogeneity (Fig. 6f).

ACM/Aβ42 interactions and hetero-complexes were then studied by fluorescence spectroscopy, SEC, cross-linking in combination with NuPAGE and WB, and far-UV CD spectroscopy (Supplementary Fig. 24). Fluorescence spectroscopic titrations of N-terminal FITC-labeled Aβ42 (FITC-Aβ42, 5 nM) with ACMS yielded nanomolar app. KdS (Table 2 and Supplementary Fig. 24a). SEC and cross-linking studies revealed large MW hetero-assemblies in Aβ42/Ne3-VF mixtures consistent with the TEM and 2PM findings (Supplementary Fig. 24b, c). Cross-linking also identified Aβ42/Nle3-VF hetero-dimers; their formation might underlie hetero-nanofiber co-assembly. Far-UV CD spectroscopy indicated less β-sheet structure in hf-Aβ42/Nle3-VF as compared to fAβ42 (Supplementary Fig. 24d).

Together, these results suggested that the potent inhibitory effect of ACMs on Aβ42 amyloid self-assembly is mediated by nanomolar affinity interactions of ACMs with Aβ42 monomers/prefibrillar species, which redirect them into long ThT-invisible and non-toxic hetero-nanofibers and their diverse µm-scaled superstructures. Further studies suggested that the binding of ACMs to preformed fAβ42 does not result in this kind of co-assemblies (Supplementary Fig. 25).

**Additional properties of Aβ42/ACM co-assemblies**

Hippocampal synaptic plasticity is regarded as a key mediator of learning and memory processes; its damage by toxic Aβ42 aggregates is a major responsible factor in AD pathogenesis44,45. Our ex vivo electrophysiological studies in mouse brains revealed that in the presence of various different ACMs, Aβ42-mediated inhibition of hippocampal long-term potentiation (LTP) was fully ameliorated (Fig. 7a). Of note, ACMs exerted their inhibitory effects when added to both Aβ42 monomers and pre-oligomerized Aβ42 (Fig. 7a and Supplementary Fig. 26). As inhibition of LTP by Aβ42 is linked to loss of memory and cognitive functions in AD, this data supported the potential physiological relevance of the in vitro determined inhibitory effects44,45.

We then investigated whether hf-Aβ42/ACM might differ from fAβ42 with respect to their seeding competence. In fact, ThT binding showed that, in contrast to fAβ42, hf-Aβ42/Nle3-VF, and hf-Aβ42/L3-VF were seeding-incompetent (Fig. 7b). Furthermore, we asked whether hf-Aβ42/ACM might exhibit similar proteolytic degradation, thermolability, and cellular clearance features as hf-IAPP/ACM. Kinetics of PK-mediated degradation of fAβ42 versus hf-Aβ42/Nle3-VF were studied by dot blot analysis and the 6E10 antibody, which specifically recognizes Aβ42 (Aβ1-17) but not the ACMs. hf-Aβ42/Nle3-VF were
degraded within ~30 min, whereas degradation of fAβ42 took ~2 h, i.e., was ~4 times slower (Fig. 7c). Thermostability studies using TEM then showed that hf-Aβ42/Nle3-VF were fully converted into amorphous aggregates after 5 min at 95 °C; by contrast, fAβ42 were stable at 95 °C for at least 15 min (Fig. 7d). Finally, phagocytosis of TAMRA-fAβ42 and TAMRA-Aβ42/ACM by cultured BV2 microglia cells was quantified by fluorescence microscopy46,48. Significantly higher amounts of hf-TAMRA-Aβ42/ACM became phagocytosed as compared to TAMRA-fAβ42 (Fig. 7e). Of note, ACM addition to preformed cytotoxic TAMRA-Aβ42 oligomers (TAMRA-oAβ42) resulted in a delay of fibrillogenesis, less cytotoxicity, and a significantly increased cellular uptake (Supplementary Fig. 27).

Together, these findings revealed that ThT-invisible and non-toxic hf-Aβ42/ACM were seeding-incompetent and less thermostable than...
Fig. 6 | Inhibition of Aβ42 amyloid self-assembly via ThT-invisible and non-toxic Aβ42/ACM nano-fibril/co-assembly. a ACMs inhibit Aβ42 amyloid self-assembly. Fibrillogenesis of Aβ42 (5 μM) and Aβ42/ACM (1/1) followed by ThT binding (means ± SD, three independent assays). b ACMs suppress Aβ42 cytotoxicity. Aged Aβ42 (5 μM) or Aβ42/ACM (1/1) (6 days) (without ThT) were added to PC12 cells; cell damage determined via MTT reduction (means ± SD, three independent assays, n = 3 technical replicates each). c ACMs suppressed seeding of Aβ42 by fIAPP. Fibrillogenesis of Aβ42 (5 μM) without or with fIAPP seeds (10%) and of fIAPP-seeded Aβ42/ACM (1/1) followed by ThT binding (means ± SD, three independent assays). d Aged Aβ42/ACM consists of ThT-invisible fibrils (fIAPP/ACM). Representative TEM images of Aβ42 (fIAPP) and Aβ42/ACM mixtures (1/1) (from b: 6-day-aged) are shown; scale bars, 100 nm. Images represent results from eight (Aβ42), three (Aβ42/Nle3-VF), two (Aβ42/L3-VF, Aβ42/F3-VF, Aβ42/L3-F3), or one (Aβ42/Nle3-LF, Aβ42/L3-LF, L3-LF) independent experiments(s). Bottom right, bar diagram showing fibril lengths; data from n = 22 fibrils in Aβ42 and n = 20, 15, 20, 15, 20, 23, and 22 fibrils in Aβ42 mixtures with Nle3-VF, Nle3-LF, L3-VF, L3-LF, F3-VF, and F3-LF, respectively. ***p < 0.001 or ****p < 0.0001 for lengths of hf-Aβ42/ACMs versus fAβ42 as indicated (one-way ANOVA & Bonferroni). P-values: 3.36E 10, 7.41E-17, 3.40E-04, 9.25E-05, and 7.52E-05 for Aβ42/Nle3-VF, Nle3-LF, L3-VF, L3-LF, F3-VF, and F3-LF, respectively.

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Inhibition of fIAPP-mediated cross-seeding of Aβ42 amyloid self-assembly by ACMs

Cross-seeding of Aβ42 amyloid self-assembly by fIAPP accelerates Aβ42 amyloid self-assembly and could link the onset and pathogenesis of T2D with AD.25,26 In a simplified mechanistic scenario, fIAPP seeds will template the formation of IAPP/Aβ hetero-amyloids, which will then be degraded by PK and PK-resistant products.25 Importantly, cross-seeding by fIAPP was performed in the presence of ACMs. Therefore, polymorphic cross-interactions between amyloid core regions may play an important role in the formation of fibrils and various amyloidic species (e.g., rods, ribbons, and nanotube-like structures).27-29

Because ACMs contains the Aβ amyloid core, bind with high affinity to both IAPP and Aβ, incl. fIAPP and Aβ42, and inhibit their amyloid self-assembly, we assumed that they might also interfere with the cross-seeding of Aβ42 by fIAPP. In fact, ACMs effectively suppressed fIAPP-mediated cross-seeding of Aβ42 fibrillogenesis and cytotoxicity (Fig. 7j). Involved supramolecular (co-)assemblies were then studied by 2PM (Fig. 7g-j). First, preformed TAMRA-fIAPP seeds were added to Aβ42-containing terminal HiLyte647-labeled Aβ42 (HiLyte647-Aβ42). 2PM at the fibrillogenic plateau revealed large Aβ42 clusters, consisting of apparently amorphous aggregates or fibrils, bound to/mixing with/from fIAPP surfaces (Fig. 7g, h and Supplementary Movies 6, 7). This data was consistent with secondary (cross)-nucleation.30 However, a completely different picture was obtained when TAMRA-fIAPP seeds were added to a mixture of Aβ42 with Nle3-VF containing HiLyte647-Aβ42 and Fluos-Nle3-VF (Fig. 7j). Major species were: (a) large fibrous Aβ42/Nle3-VF/IAPP co-assemblies (many µm long; widths ~1.5 µm) and (b) diverse roundish/elliptical Aβ42/Nle3-VF/IAPP or Aβ42/Nle3-VF co-assemblies (up to ~10 µm) (Fig. 7h-j). Analysis of 2PM images and 3D-reconstructions suggested that fibrous co-assemblies consisted of Aβ42, Nle3-VF, and Aβ42/Nle3-VF bound to fIAPP bundles (Fig. 7j) and Supplementary Movies 8, 9). Thus, suppression of Aβ42 cross-seeding likely occurs via a dual mechanism (Fig. 5): (1) sequestration of Aβ42 into non-toxic ACM/Aβ42 co-assemblies (both fibrillar and amorphous ones) and (2) binding of non-toxic ACM and Aβ42/ACM co-assemblies to fIAPP yielding cross-seeding-incompetent and non-toxic ternary nano-fibril co-assemblies.

Discussion

Here we exploited Aβ/IAPP cross-interactions to design Aβ amyloid core mimics (ACMs) as inhibitors of amyloid self-assembly of both IAPP and Aβ42. Collectively, we identified six 26-residue peptides as effective amyloid inhibitors of both IAPP and Aβ42. All six ACMs bound IAPP with nanomolar affinity and blocked its cytotoxic amyloid self-assembly with nanomolar IC50 values. In addition, all six ACMs bound Aβ42 with nanomolar affinity and blocked its cytotoxic self-assembly, three of them with nanomolar IC50 values. Moreover, ex vivo electrophysiology in murine brains showed a full amelioration of Aβ42-mediated damage of synaptic plasticity by ACMs. Importantly, ACMs also inhibited reciprocal cross-seeding of IAPP and Aβ42 amyloid self-assembly29. ACMs thus belong to the most effective inhibitors of in vitro amyloid self-assembly of IAPP, Aβ42, or both polypeptides.29

Our most remarkable finding was that ACMs, which were non-amyloidogenic in isolation, exerted their potent amyloid inhibitor function via an unexpected mechanism, i.e. by co-assembling with IAPP or Aβ42 into amyloid fibril-resembling but ThT-invisible and non-toxic nano-fibrils and their diverse highly ordered fibril superstructures. The latter ones comprised large heteromeric nano-fibrils and several µm-sized loops, ribbons, and nanotube-like superstructures. Furthermore, non-toxic ternary fibrous co-assemblies consisting of IAPP, Aβ42, and ACM formed when Aβ42 cross-seeding by fIAPP was performed in the presence of ACMs. Although unexpected, IAPP/Aβ42/ACM nano-fibril co-assembly was in line with the ACM design concept. In fact, ACMs contained all three Aβ hot segments required for high-affinity interactions with IAPP, Aβ42, and themselves, and combined inbuilt β-sheet extension blocking (N-methylations) with β-sheet stabilization/extension enabling (LTS and Aβ(21–40)) elements26,29,30,31,32,33.
The identified IAPP/ACM nanofibers were indistinguishable from fIAPP by TEM and had the cross-β amyloid core signature by XRD, but were less ordered than fIAPP according to CD spectroscopy. Our TEM, STED, 2PM, and FLIM-FRET studies suggested that nanofibers and basic parts of their fibrous superstructures consisted of laterally co-assembled, parallel arranged or intertwined/twisted, “protofibril-like” IAPP and ACM stacks. In addition, our studies showed that hf-IAPP/ACM evolve from large amorphous co-assemblies and suggested that IAPP monomers/prefibrillar species might template this process likely via hetero-dimers. Although the mechanistic steps are yet unclear, IAPP/ACM nanofiber co-assembly could proceed in analogy to proposed mechanisms of self- and co-assembly of IAPP and Aβ8,12,13,24,29,56.

The identified Aβ42/ACM nanofibers had similar widths but two to four times greater lengths than fAβ42. Our imaging results were consistent with both axial and lateral co-assembly, the former likely underlying hetero-nanofiber elongation. Notably, Aβ42/ACM fiber co-assembly was in line with earlier findings by Mihara and coworkers57.

Another notable finding of our study relates to the potential beneficial properties of IAPP/ACM and Aβ42/ACM nanofibers. Their properties clearly distinguished from pathogenic fIAPP and fAβ42 and, in addition to their non-toxic nature and seeding incompetence, comprised thermolability, proteolytic degradability, and more efficient phagocytosis than fIAPP and fAβ42. Such features are reminiscent of labile/reversible functional amyloids, in which they may serve...
to control their formation/storage/disassembly related to their diverse biological functions. Examples are amyloids from certain secreted peptide hormones or from proteins forming reversible subcellular condensates. By contrast, most “pathological amyloids” are linked to cell damage and characterized by high stability and resistance to proteolysis. Furthermore, increasing evidence suggests that amyloid fold polymorphism underlies amyloid pathogenicity and functional diversity. Our results suggest that, in addition to pathogenic IAPP/β hetero-amylogens generated by fibril-mediated cross-seeding, potentially beneficial IAPP/β hetero-amylogens, such as those mimicked by IAPP/ACM nanofibers, might also exist and could be also the case for other cross-interacting amyloids. The structural characterization of IAPP/ACM nanofibers should help in identifying molecular factors that may redirect cytotoxic amyloid self-assembly into non-toxic and labile hetero-amylogens and enable the exploitation of amyloid fold versatility to design effective anti-amyloid molecules.

In conclusion, our work offers a series of designed peptides as highly potent inhibitors of amyloid self-assembly and reciprocal cross-seeding of IAPP and Aβ42 and as promising leads for effective anti-amyloid drugs in both T2D and AD. In addition, the identified nanofiber co-assemblies should guide the design of future functional (hetero-) amyloid-based supramolecular nanomaterials for biomedical and biotechnological applications.

**Methods**

**Peptides and peptide synthesis**

IAPP, IAPP-GI, rat IAPP, and their N-terminal fluorescein- or biotin-labeled analogs were synthesized by FMoc-based solid phase synthesis (SPPS), subjected to air-oxidation, and purified by RP-HPLC as previously described in refs. 25, 26, 32, 64. Their stock solutions were prepared in 1,1,3,3,3-hexafluoro-2-isopropanol (HFIP) (4 °C), filtered over 0.2 µm filters (Millipore), and concentrations were determined by UV spectroscopy. TAMRA-IAPP was synthesized by overnight coupling of 5,6-carboxytetramethylrhodamine (TAMRA) (Novabiochem/Merck) to RINK-resin-bound IAPP using a threefold molar excess of 2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium-hexafluorophosphate (HBTU) and a 4.5 molar excess of N,N-diisopropylethylamine (DIPEA) in N,N-dimethylformamide (DMF). TAMRA-IAPP cleavage from the resin and RP-HPLC purification were performed as for the other labeled IAPP analogs; stocks were made in HFIP (4 °C). Aβ42 was synthesized on Tentagel R PHB resin (0.18 mmol/g; Rapp Polymer) by Fmoc-SPPS using previously reported protocols.

Seed-free aqueous Aβ42 stock solutions (10–20 µM) were obtained by SEC fractionation according to published protocols. Briefly, HPLC-purified Aβ42 (purification by Peptide Specialty Laboratories) was dissolved (1 mg/ml) in a solution of 5 M GdnHCl in 10 mM TRIS/HCl pH 6.0 and loaded onto a Superdex 75 10/300 GL column (eluent: 50 mM ammonium acetate pH 8.5, 0.5 ml/min). The monomeric Aβ42 elution peak was collected on ice, stored at 4 °C, and used within 1 week; peptide concentration was determined by UV spectroscopy. Fluorescein-isothiocyanate-β-Ala-labeled Aβ42 (FITC-Aβ42) and TAMRA-labeled Aβ42 (TAMRA-Aβ42) were from Bachem and HiLyte647-Aβ42 from AnaSpec; their stocks were prepared in HFIP (4 °C).

Aβ(15–40) analogs comprising ACMS, non-inhibitors, and partial segments thereof (Supplementary Tables 1, 4) were synthesized using previously described standard Fmoc-SPPS protocols and, in most cases, WANG-resin (0.3–0.5 mmol/g; Iris Biotech); Tentagel R PHB resin was used for Ne3, R3, and G3-VF (0.16 mmol/g; Rapp Polymer). Briefly, double couplings were usually performed using threefold molar excess protected amino acid and HBTU and 4.5-fold molar excess of DIEX in DMF. For difficult couplings, we applied either 2-(7-aza-1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium-hexafluorophosphate (HATU) or 4-(N,N-diisopropylamino)pyridine (DIPEA) in N,N-dimethylformamide (9:1) as coupling reagents. N-terminal fluorescein-labeled Aβ(15–40) analogs were synthesized by coupling peptide-resins with 5,6-carboxyfluorescein (Sigma-Aldrich) using threefold molar excess protected amino acid and HATU and 4.5-fold molar excess of DIEX (double couplings). N-terminal Atto647N-labeled Ne3-VF was synthesized by coupling peptide-resin with Atto647N (carboxy-derivative) (ATTO-TEC) using HATU. Peptide cleavage from the resin was performed with 95% TFA/H2O. All peptides were purified by RP-HPLC on Nucleosil 100 C18 (Grace) or Reprosil Gold 200 C18 columns (Dr. Maisch) according to previously described protocols.

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solutions were made in HFIP (4 °C); peptide concentrations were determined by peptide weight or by UV spectroscopy (fluorescently-labeled analogs)25,32. All synthetic peptides were characterized by matrix-assisted laser desorption ionization (MALDI-MS) or electrospray ionization (ESI-MS) mass spectrometry (Supplementary Table 4).

Thioflavin T (ThT) binding assays

IAPP fibrillogenesis-related studies. Effects of the different peptides on IAPP fibrillogenesis, including self- and cross-seeded fibrillogenesis, were studied in combination with TEM and MTT reduction assays according to previously established ThT binding assay systems12,20,21,32. At the indicated time points, aliquots of peptide incubations (made as described below) were gently mixed with the ThT solution (20 μM ThT in 0.05 M glycine/NaOH, pH 8.5, if not stated otherwise) in a 96-well black MTP (FluoroNunc/Thermo Fisher Scientific). ThT binding was determined immediately by measuring fluorescence emission at 486 nm following excitation at 450 nm using a 2030 Multilabel Reader VictorX3 instrument (PerkinElmer Life Sciences) (software: PerkinElmer 2030 Manager (V4.0))20. ThT binding of seeds/buffer were determined by peptide weight or by UV spectroscopy (fluorescently-labeled analogs)25,32. Non-toxic ACMs and ACM/A42 co-assemblies (both fibrillar and amorph) bind to IAPP yielding non-toxic and cross-seeding-incompetent fibrous co-assemblies.

Fig. 8 | Schematic overview of identified co- assemblies and proposed mechanisms of ACM-mediated suppression of Aβ42 amyloid self assembly (a) and its cross-seeding by fIAPP (b, c). a Lower row, Aβ42 self-assembles into toxic oligomers and fAβ42. Upper row, non-toxic ACMs bind with low nanomolar affinity Aβ42 and redirect it into heteromeric nanofiber co-assemblies (hf-Aβ42/ACM), which are non-toxic, seeding-incompetent, and thermolabile and become easier degraded and more effectively phagocytosed than fAβ42. hf-Aβ42/ACM, which may form by lateral or axial co-assembly are shown. b Cross-seeding of Aβ42 by fIAPP yields via secondary nucleation Aβ42/fIAPP co-assemblies, Aβ42, and toxic Aβ42 oligomers. c ACM-mediated inhibition of cross-seeding of Aβ42 by fIAPP. Non-toxic ACMs and ACM/A42 co-assemblies (both fibrillar and amorph) bind to fIAPP yielding non-toxic and cross-seeding-incompetent fibrous co-assemblies.
that similar amounts of fIAPP and hf-IAPP/ACM were used for seeding or other assays. To determine the effects of ACMs on fAβ42-mediated cross-seeding of IAPP fibrillogenesis, seed amounts (10%) of fAβ42 (made by incubating Aβ42 (88 μM) in ThT buffer containing 1% HFIP for 19 days at 37 °C; fibril formation confirmed by ThT binding and TEM) were added to freshly made IAPP (12 μM in ThT buffer) or IAPP/peptide mixtures (1/2); incubations were performed for 48 h.

ACM fibrillogenesis-related studies. To study the fibrillogenic potential of ACMs, peptides, and Aβ40 (positive control) (100 μM) were incubated in 10 mM aqueous sodium phosphate buffer, pH 7.4 (1% HFIP) for 4 days. ThT fluorescence was measured at 0 h and 4 days by mixing an aliquot with a ThT-containing solution (121 μM ThT, 0.05 M glycine/NaOH, pH 8.5); buffer values were subtracted from the data shown in Supplementary Fig. 5b. The absence of fibrils and cytotoxic aggregates from these solutions was confirmed by TEM (Supplementary Fig. 5a) and MTT reduction assays (Supplementary Fig. Sc).

Aβ42 fibrillogenesis-related studies. To study the effects of the different peptides on Aβ42 fibrillogenesis, synthetic Aβ42 isolated from SEC (see “Peptides and peptide synthesis”) was used. Peptide incubations were performed in the presence of IAPP in 1/1 well black MTPs (FluoroNunc, Thermo Fisher Scientific) based on previously developed protocols. Incubation conditions for all assays were (if not stated otherwise): Aβ42 (5 μM) alone or its mixture with the peptide (at the indicated molar ratios) in 45 mM ammonium acetate, pH 8.5, containing 10 μM ThT (37 °C); MTPs were shaken (500 rpm; orbital shaker (CAT S20)) for the first 5 h of the fibrillogenesis. ThT fluorescence was measured with a 2030 Multilabel Reader VictorX3 instrument at the indicated time points as under ACM-related assays. Values of seeds or buffer alone were subtracted from the data in self-/cross-seeding assays; all other data shown are raw data except for data in Supplementary Figs. 25b, 27a, which were normalized.

For studying the effects of ACMs on fAβ42-mediated seeding of Aβ42, preformed fAβ42 (made by incubating Aβ42 (5 μM) as above but without ThT for 6 days (TEM, Fig. 6d)) were added to freshly made Aβ42 (5 μM) or Aβ42/ACM mixtures (5 μM each; made on ice) just before the addition of ThT (10 μM). fAβ42 seed concentration was 0.5 μM (10%) and incubations (37 °C) were performed as above. Note, fAβ42 were quantified/verified by ThT binding, dot blots, and TEM. For studying whether hf-Aβ42/ACM might seed Aβ42 fibrillogenesis, Aβ42 (5 μM) and Aβ42/ACM mixtures (5 μM each) were first incubated for 6 days without ThT as described in the top section to obtain fAβ42 and hf-Aβ42/ACM; fibrils were quantified/verified by ThT binding (fAβ42), TEM (fAβ42 and hf-Aβ42/ACM), and dot blots. Seed amounts of fAβ42 or hf-Aβ42/ACM (10%, 0.5 μM) were then mixed (on ice) with freshly made Aβ42 (5 μM) in 45 mM ammonium acetate, pH 8.5, and following the addition of ThT (10 μM) incubations (37 °C) were performed as described above. To study the effects of ACMs added at post-nucleation time points of Aβ42 fibrillogenesis (including effects on preformed Aβ42 oligomers (2 h-aged Aβ42)), Aβ42 (5 μM) was incubated in the presence of ThT as described in the top section and mixed with the ACM (1/1) at the indicated time points of fibrillogenesis. Effects of ACMs on fAβ42-mediated cross-seeding of Aβ42 were studied as follows: Aβ42 (10 μM) alone and Aβ42/ACM mixtures (1/2) were prepared as above on ice. Preformed fAβ42 (2 μM) (made by incubating IAPP (128 μM) in ThT buffer for 9–12 days) was added to the above solutions just prior to the addition of ThT (10 μM). The final composition of the assay buffer was: 45 mM ammonium acetate, pH 8.5, containing 10 μM ThT and <2% of ThT buffer resulting from the fIAPP seed or the buffer alone solution (in the Aβ42 without seed control solution). Incubations (37 °C) and determination of ThT fluorescence were performed as for all other Aβ42-related studies.
Fig. 27d) was performed by one-way ANOVA and Bonferroni (software: OriginPro 2021).

To study the effects of ACMs on fIAPP-mediating cross-seeding of formation of cell-damaging Aβ42 assemblies, incubations were made for the corresponding ThT binding assays but without ThT. Solutions were aged for 1.5 h (37 °C, shaking at 500 rpm). Incubation with the cells and the MTT reduction assay were performed as described above.

Effects of ACMs on PC12 cell viability were studied using the 4 day-aged solutions applied in the ThT binding assays, which were performed to determine their amyloidogenic potential (see under "ThT binding assays"). Following incubation with the cells (at 20 µM) for 20 h, cell damage was assessed by MTT reduction; data were corrected for buffer effects. For comparison, effects of aged Aβ40 were also studied and cytotoxicity was as expected.

Transmission electron microscopy (TEM)
Aliquots of solutions used for ThT binding, MTT reduction, or other assays were applied on formvar/carbon-coated grids at the indicated incubation time points. Grids were washed with ddH2O and stained using aqueous 2% (w/v) uranyl acetate as described in ref. 44. Examination of the grids was done with a JEOL 1400 Plus electron microscope (120 kV) (data acquisition software: TEM Center v. 1.7.19.2439; data analysis with Image J (1.50i)). For Aβ42-related studies, solutions made as for the ThT binding assay but without containing ThT were used for TEM and the MTT reduction assays. Kinetics of evolution of IAPP homo- and IAPP/Nle3-VF hetero-fibrils from amorphous aggregates was followed by TEM in solutions made in 10 mM sodium phosphate buffer, pH 7.4 (Fig. 4a) and also in solutions made in ThT buffer and very similar results were found.

Immunogold-TEM
Immunogold-TEM was performed based on previously described protocols. Briefly, peptide solutions made as described for the corresponding ThT binding assays were applied onto the grids at the indicated incubation time points. Grids were blocked with 0.1% BSA in 1xPBS. fIAPP was detected with a fibril-specific mouse anti-fIAPP antibody (Syntactic Systems; Cl. 91E7)). Nle3-VF was revealed by a rabbit anti-Aβ40 polyclonal antibody (Sigma-Aldrich) exhibiting 10–20% NSB to IAPP. The two antibodies (in 0.1% BSA in 1xPBS; dilution 1/10) were deposited simultaneously onto the grid and incubated for 20 min. Following washing with 1xPBS, grids were incubated (20 min) with secondary antibodies goat anti-rabbit gold-conjugate (10 nm) and goat anti-mouse gold-conjugate (5 nm) (Sigma-Aldrich) (in 0.1% BSA in 1xPBS; dilution 1/10) as above. Following 1xPBS and ddH2O washings, uranyl acetate staining and grid examination were performed as described under "TEM". To quantify IAPP and Nle3-VF contents of fibrils, 5 and 10 nm gold particles were counted (software: Image J (1.50i)). "Antibody reactivity" is expressed as % of a total number of gold particles bound.

Far-UV CD spectroscopy
CD spectra were recorded using a Jasco 715 spectropolarimeter (software: Spectra Manager V1.35.00 (Build 2)). CD spectra (average of three spectra) were measured between 195–250 nm, at 0.1 nm intervals, with a response time of 1 s, and at RT. The spectrum of the buffer was always subtracted from the spectra of the peptide solutions. Peptide incubations related to ACM alone or ACM/IAPP interactions were performed according to previously established protocols 12,20,32. Briefly, to study peptide conformations and oligomerization propensities, CD spectra of freshly made solutions in 10 mM sodium phosphate buffer, pH 7.4, containing 1% HFIP were measured at 5 µM or at the indicated concentrations in concentration-dependence studies. For studying hf-IAPP/ACM, IAPP (16.5 µM) was incubated with Nle3-VF or VGS-VF (33 µM) in ThT buffer for the ThT binding assay for 7 days and spectra were measured at the indicated time points. For comparison, spectra of IAPP, Nle3-VF, and VGS-VF alone were also measured. For studying the structure of hf-Aβ42/Nle3-VF, incubations were performed for the ThT binding assays but in the absence of ThT. Briefly, Aβ42 alone (5 µM), Nle3-VF alone (5 µM), and their mixtures (5 µM each) in 45 mM ammonium acetate (pH 8.5) were incubated for 6 days at 37 °C and CD spectra were measured.

Fluorescence spectroscopic titration assays
Fluorescence spectroscopic studies were performed with a Jasco FP-6500 fluorescence spectrophotometer (software: Spectra Manager V1.54.03 (Build 1)) using previously established protocols. Briefly, excitation was at 492 nm and spectra were measured between 500 and 600 nm. All titrations were performed in freshly made solutions of synthetic N-terminal fluorescently-labeled peptide (5 µM) and various amounts of unlabeled peptide in 10 mM sodium phosphate buffer, pH 7.4 (% HFIP) within 2–5 min following solution preparation as described in ref. 20, 25, 26. Under these experimental conditions, freshly made solutions of Fluoro-IAPP and FITC-Aβ42 (5 µM) consist mostly of monomers, and the same was found for Fluoro-ACMs (5 µM) (e.g., Supplementary Fig. 5e). Apparent binding affinities (app. Kd) were estimated by using 1/1 binding models (software: OriginPro 2016G, Origin 2021, and GraFit v. 5.3) as described in refs. 20, 25, 26, 32. However, due to the high self-assembly propensities of involved peptides, more complex models might also apply. Determined app. Kd means (µSD) from three binding curves derived from three independent titration assays.

Cross-linking, NuPAGE, and Western blot (WB)
Hetero-complex cross-linking studies in combination with NuPAGE and WB were performed with a previously developed assay system used for the characterization of Aβ and IAPP homo- and hetero-assemblies. Briefly, for characterizing IAPP homo-/hetero-assemblies, IAPP (30 µM), IAPP/ACM mixtures (1/2), and ACMs alone (60 µM) were incubated in 10 mM sodium phosphate buffer (pH 7.4) for up to 7 days (20 °C). In the case of Aβ42 homo-/hetero-assemblies, Aβ42 (30 µM) and Aβ42/ACM mixtures (1/2) were incubated in 10 mM sodium phosphate buffer (pH 7.4) for up to 6 days. At the indicated time points (0 h, 24 h, and 7 days (IAPP studies) or 0 h, 3 h, 24 h, and 6 days (Aβ42 studies)), aliquots were cross-linked (2 min) with 25% aqueous glutaraldehyde (Sigma-Aldrich) and treated with a 2 M NaBH₄ solution (in 0.1 M NaOH, 20 min). Following precipitation with trichloroacetic acid (10%) (4°C) and centrifugation (10 min, 12000 g), pellets were dissolved in reducing NuPAGE sample buffer, boiled (5 min, 95°C) and subjected to NuPAGE gel electrophoresis as described using 4–12% Bis-Tris gels and MES running buffer (Thermo Fisher Scientific). Equal amounts of IAPP or Aβ42 were loaded in all lanes. Peptides were transferred onto nitrocellulose membranes (XCell II Blot Module, Thermo Fisher Scientific). Membranes were blocked overnight (10°C) with 5% milk in TBS-T (20 mM Tris/HCl, 150 mM NaCl, and 0.5% Tween-20). To reveal homo-/hetero-assemblies, membranes were incubated (2 h) with one of the following primary antibodies (5% milk in TBS-T): rabbit polyclonal anti-IAPP (Peninsula; 1:1000) for IAPP-containing assemblies, rabbit polyclonal anti-Aβ40 (Sigma-Aldrich; 1:2000) for ACM-containing assemblies, or mouse monoclonal anti-Aβ(1–17) (6610, BIOZOL; 1:2000) for Aβ42-containing assemblies (no cross-reactivity with ACMs). Primary antibodies were combined with suitable peroxidase (POD)-coupled secondary antibodies (donkey anti-rabbit-POD (1:5000) or goat anti-mouse-POD (1:1000)) and homo-/hetero-assemblies were revealed with SuperSignal West Dura Extended Duration Substrate (Thermo Fisher Scientific) (visualization by LAS-4000 mini (Fujifilm); software: Image Reader LAS-4000 mini (V2.0)). Membranes were stripped by incubating in stripping buffer (2% SDS, 100 mM β-mercaptoethanol, 50 mM
mostly of binding. For the CR spectral shift assay, 7-day-aged IAPP or IAPP/ACM solutions were mixed with CR (70 µM) or IAPP/Nle3-VF (33 µM) and spectra were recorded at the indicated time points.

Solutions of IAPP (16.5 µM) or IAPP/ACM (1/2) mixtures were freshly made in 10 mM sodium phosphate buffer, pH 7.4, containing 1% HFIP, and spectra were measured at 214 nm. For IAPP-related studies, elution buffer was 50 mM ammonium acetate, pH 8.5. Aβ42 (5 µM) or Aβ42/ACM (1/1) mixtures were incubated under ThT binding assay conditions (without ThT) and loaded onto the column at the indicated time points. The column was calibrated with proteins/peptides of known molecular weights.

ESI-IMS-MS
A Synapt XS HDMS mass spectrometer (Waters) equipped with an Acquity Premier nano-ESI interface (Waters) was used (software: MassLynx (v4.2)). MS spectra were obtained using the following parameters: Positive mode nano-ESI; capillary voltage of 1.7 kV; nitrogen nebulizing gas pressure 0.8 psi; cone voltage 40 V; source temperature 60 °C; backing pressure 1.6 mBar; Trap Collision Energy Control, ramping 10–100 V; ramping traveling wave height 7–20 V; IMS nitrogen gas flow 20 ml/min, traveling wave speed 300 m/s and IMS cell pressure 0.35 mBar. The m/z scale was calibrated with NaCl cluster ions and Glufbic (m/z 785.8427) as lock mass. For analysis, freshly made solutions of IAPP (30 µM), Nle3-VF (60 µM), and IAPP/Nle3-VF (1/2) in 10 mM sodium phosphate buffer (pH 7.4) were used. The samples were vortexed and directly applied to nano-ESI, operating the nano-HPLC system at a constant flow rate of 0.2 ml/min in bypass mode for direct sample injection. MS analysis was carried out -2 min after sample preparation.

ANS binding fluorescence spectroscopy
ANS binding studies were performed with a Jasco FP-6500 fluorescence spectrophotometer (software: Spectra Manager V1.54.03 (Build 1)) as previously described in refs. 20, 44. Briefly, excitation was at 355 nm and fluorescence emission spectra were recorded between 355 and 650 nm. Solutions of ANS alone (5 µM) and its mixtures with IAPP (2 µM) or IAPP/Nle3-VF (1/2) mixtures were freshly made in 10 mM sodium phosphate buffer, pH 7.4, containing 1% HFIP, and spectra were recorded at the indicated time points.

Congo red (CR) binding
Assays were performed based on previously described protocols. Solutions of IAPP (16.5 µM), Aβ42 (5 µM), and their mixtures with ACMs (IAPP/ACM, 1/2; Aβ42/ACM, 1/1) were prepared as described for the ThT binding assay (Aβ42 without ThT). After 7-day- IAPP-related samples or 6-day-aging (Aβ42-related samples), solutions consisted mostly of fibrillar assemblies (Figs. 1, 2) and were used for studying CR binding. For the CR spectral shift assay, 7-day-aged IAPP or IAPP/ACM or buffer alone solutions were mixed with CR (7 µM) and CR absorption spectra between 400–700 nm were measured using a Clariostar Plus MTP reader (BMG Labtech) (software: Clariostar Software (v.5.70 R3)). Spectra are presented after subtraction of a spectrum taken in the absence of CR, to account for light scattering effects. For CR staining studies, solutions were spotted on a glass slide, air-dried, stained with 200 µM CR in 80% EOH, and examined between a cross-polarizer/ analyzer with an Olympus CKX41 light microscope.

Hetero-complex pull-down assays
Pull-down assays were performed using streptavidin-coupled magnetic beads (Dynabeads M-280 Streptavidin, Dynal) as described in refs. 12, 32. Briefly, solutions of Biotin-IAPP (16.5 µM), Biotin-IAPP/Nle3-VF mixtures (1/2), and Nle3-VF (33 µM; control for non-specific binding) in 10 mM sodium phosphate buffer, pH 7.4 were aged for 7 days (20 °C) and subsequently incubated with the beads for 4 h at RT. Bound complexes were isolated by magnetic affinity. Following washing, beads were boiled with reducing NuPAGE sample buffer (5 min, 95 °C) and supernatants subjected to NuPAGE electrophoresis and WB as described under “Cross-linking, NuPAGE and WB”. Equal amounts were loaded in all lanes; lane “Nle3-VF (control)”, freshly dissolved Nle3-VF without incubation with the beads. Scans of uncropped blots are provided in the Source Data file.

CLSM and STED imaging
IAPP or IAPP/ACM mixtures (1/2) containing 10% N-terminal fluorescently-labeled analogs TAMRA-IAPP and Atto647N-ACM (IAPP(total), 16.5 µM; ACM(total), 33 µM) were incubated in 10 mM sodium phosphate buffer, pH 7.4 for 7 days (20 °C). Aliquots (30–40 µl) were pipetted onto SuperFrost Plus adhesion slides (Thermo Fisher Scientific), air-dried, covered with a high precision coverslip (ILC; Ibidi), and embedded using Prolong Diamond Antifade Mountant (Thermo Fisher Scientific). CLSM and STED were performed using a Leica SP8 SPS 3X microscope (HC PL APO 93x/1.30 GLYC CORR STED objective) with a tunable white light laser source to excite fluorophores. Depletion power (660 nm (TAMRA), 775 nm (Atto647N)), and time-gated detection of excited light were chosen to minimize sample damage while optimizing x/y-resolutions. Images were collected in a sequential scanning mode (hybrid-diopte detectors) to maximize signal collection while minimizing channel cross-talk (TAMRA: excitation 552 nm/emission 557–645 nm; Atto647N: excitation 646 nm/emission 651–700 nm). 3D reconstructions/lifibril measurements were performed using Leica’s LAS-X software package (v1.2). Datasets were deconvoluted using Leica’s LIGHTNING application.

2PM and FLIM-FRET studies
Solutions analyzed by 2PM or FLIM-FRET consisted of either N-terminal fluorescently-labeled peptides (100%) or mixtures of labeled with non-labeled peptides (when indicated) and were prepared as follows: For most IAPP-related studies, hIAPP/ACM were prepared by incubating TAMRA-IAPP (16.5 µM) with synthetic N-terminal fluorescent- or Atto647N-labeled ACMs (33 µM) (as indicated in the figure/figure legend) in 10 mM sodium phosphate buffer, pH 7.4 (abbreviated “1xb”) for 6–7 days (20 °C). For comparison, aged TAMRA-IAPP (16.5 µM) was also examined and consisted mostly of fibrillar assemblies (TAMRA-IAPP; Supplementary Fig. 12d). In some cases (i.e. samples examined by both STED and 2PM), IAPP/ACM mixtures (6–7 days aged, 1xb (20 °C)) consisting of 16.5 µM IAPP (total) and 33 µM ACM (total) with each of them containing 10% of labeled peptide were used as indicated. For 2PM studies related to ACM-coated IAPP (Supplementary Fig. 13e), first, IAPP were made by incubating TAMRA-IAPP (16.5 µM) in 1xb for 48 h. IAPP were then co-incubated with a mixture of Nle3-VF/Atto647-Nle3-VF (9/1; 33 µM Nle3-VF (total)) in 1xb for 1 day (20 °C) to yield ACM-coated IAPP. For 2PM and FLIM-FRET studies regarding the role of monomeric/pre fibrillar IAPP on the formation of IAPP/Nle3-VF nanofiber co-assemblies (Fig. 4c, d and Supplementary Fig. 16), aliquots from a freshly made mixture of Fluos-Nle3-VF (33 µM) with TAMRA-IAPP (1.65 µM) in 1xb (20 °C) were examined at indicated incubation times. For the corresponding studies on the role of IAPP (Supplementary Fig. 17), preformed TAMRA-IAPP seeds (made by incubating TAMRA-IAPP (16.5 µM) in 1xb for 5 days (20 °C)) were added to Fluos-Nle3-VF (33 µM); the final concentration of TAMRA-IAPP was 3.3 µM. For FLIM-FRET studies of the different TAMRA-IAPP/Fluos-Nle3-VF co-assemblies (Figs. 3j, 4d and 4f).
Supplementary Fig. 17a–c), the donor Fluos-Nle3-VF alone (data shown in Supplementary Figs. 14, 16, 17d, e) was incubated under the same experimental conditions as the corresponding co-assemblies.

For all Aβ42-related studies, solutions consisted of 1/1 mixtures of unlabeled/labeled peptides (as indicated) and were prepared as for the ThT binding assays (without ThT) (45 mM ammonium acetate, pH 8.5, 37 °C; shaking between 0–5 h aging as indicated) as follows: For the studies on Fig. 4A, Fluos-Nle3-VF/Fluos-ACMs, Aβ42 solutions (Aβ42(total) 5 μM) consisted of 50% TAMRA-Aβ42 and 50% Aβ42 (6-day-aging); Aβ42/ACM mixtures (1/2) contained, in addition to Aβ42/TAMRA-Aβ42 (1/4; Aβ42(total), 5 μM), ACM/Fluos-ACM (1/2; ACM(total), 10 μM) (4–6 day-aging). For FLIM-FRET analysis of hF-TAMRA-Aβ42/Fluos-Nle3-VF (Fig. 6f and Supplementary Fig. 23), Fluos-Nle3-VF alone (mixture of Fluos-Nle3-VF/Nle3-VF) (1/1), Nle3-VF (total) 10 μM was incubated under the same experimental conditions (see above: 4 day-aging) as the TAMRA-Aβ42/Fluos-Nle3-VF (1/1) mixture (consisting of TAMRA-Aβ42/Aβ42, (Aβ42(total) 5 μM) and Fluos-Nle3-VF/Nle3-VF (1/1) (Nle3-VF(total), 10 μM). Of note, no time-dependent pH changes were observed in buffer solutions incubated under the experimental conditions applied for peptide incubations used for FLIM-FRET studies. For studies on IAPP-mediated cross-seeding of Aβ42, the Aβ42 alone solution (Aβ42(total), 10 μM) contained HiLyte647-Aβ42 (50%); the Aβ42/ACM (1/2) mixture (consisting of Aβ42, HiLyte647-Aβ42 (1/2) (Aβ42(total), 10 μM) and Nle3-VF/Fluos-Nle3-VF (1/1) (Nle3-VF(total), 20 μM); solutions were aged for 1.5 h. TAMRA-IAPP seeds for cross-seeding were prepared by incubating TAMRA-IAPP (128 μM) in ThT buffer for 6 days (20 °C); their concentration in the cross-seeded solution was 2 μM.

Aliquots from the above mentioned samples were applied onto SuperFrost Plus adhesion slides, air-dried, washed (Aβ42-related studies), and embedded with Prolong Diamond Antifade Mountant as for CLSM and STEM with one exception: hF-Aβ42/Nle3-VF co-assembly-related samples (incl. the sample of donor alone) (Fig. 6e, f and Supplementary Fig. 23), were centrifuged (20,000× g, 20 min) and pellets resuspended in 1xP before application onto the slide to minimize interference of the sample preparation buffer. Samples were imaged with a twomulti(Photon) Leica TCS SP8 DIVE multispectral two (multi-)photon microscope with 4TUNE NDD detection module, LIGHTNING adaptive deconvolution, and fast lifetime contrast (FLAMON) modality, equipped with extended IR spectrum tunable laser (680–1300 nm) (New InSight X3™, Spectra-Physics) and fixed IR laser (1045 nm), advanced Vario Beam Expander (VBE), ultra-high-speed resonance scanner (8 kHz), HC PL, IAPo25x/1.0 WATER objective, and FLIM-FRET modality. Images were collected in sequential scanning mode (hybrid-diode detectors; TAMRA: excitation 1100 nm/emission 560–630 nm; fluorescence (Fluos): excitation 920 nm/emission 480–550 nm; HiLyte647: excitation 1280 nm/emission 635–715 nm) and handled using Leica’s LAS-X software package. Deconversions were performed using Huygens Professional or Leica’s LIGHTNING application.

For fluorescence lifetime imaging (FLIM), up to 1000 photons/pixel were captured (time-correlated single-photon counting (TCSPC) mode). Samples were prepared as described above. Fluorescence decays were fit using Leica’s FALCON software applying multi-exponential models. The quality of fits was assessed by randomly distributed residuals/low Chi-square values. The number of components (n) used for fittings was manually fixed to values (n = 2–4) that minimized Chi-square statistic. In control experiments, the fluorescence lifetime of the donor (Fluos-Nle3-VF) (prepared as the corresponding mixtures) in absence of the acceptor was acquired similarly (multiexponential model was applied). Since both donor alone and its mixtures with the acceptor showed multiexponential decays, “amplitude-weighted average lifetime” (τAmp) was used to calculate FLIM-FRET efficiency. This lifetime value was calculated by the Leica FALCON software after fitting and specified according to the following formula: τAmp = ∑Aiτi(Ai/ ∑Ai) (with τAmp: amplitude-weighted average lifetime, Ai: amplitude, and τ: lifetime). By comparing the amplitude-weighted average lifetimes of the quenched donor with the donor undergoing FRET, the software extracted the FLIM-FRET efficiency according to the following formula: FRET EFF(E) = 1 − τAmp/τD, (with τAmp: amplitude-weighted average lifetime of the quenched donor (undergoing FRET); τD, amplitude-weighted average lifetime of the unquenched donor).

**Dot blot assays to assess binding of ACMs to IAPP and Aβ42 fibrils and monomers**

IAPP (128 μM) and Aβ42 (11 μM) incubations were made as for ThT binding assays (Aβ42 without ThT) and deposited onto nitrocellulose membranes (IAPP: 40 μg, Aβ42: 10 μg) either directly following their preparation (for monomers) or after 2 days of aging (for fibrils; confirmed by ThT binding and TEM). After blocking (5% milk in TBS-T, 2 h, RT) and several washing steps (with TBS-T and ThT buffer), membranes were incubated with N-terminal fluorescein-labeled ACMs (Fluos-ACMs) at 0.2 μM for IAPP-related membranes or 2 μM for Aβ42-related membranes; incubation was overnight (10 °C) in ThT buffer (containing 1% HEPES). To control for fibril autofluorescence, similar membranes were incubated in parallel with buffer only. Bound peptides were visualized using a LAS-400mini instrument (Fujifilm) (software: Image Reader LAS-4000 mini (V2.0)) equipped with a suitable fluorescence filter. Scans of uncropped blots are provided in the Source Data file.

**Dot blot analysis for quantification of fibrils**

Dot blot analysis was used to verify the presence of equal amounts of homo- and heteromeric fibrillar assemblies in the aliquots of solutions examined by the various different assays, e.g., the ThT binding assay, the MTT reduction assay, or the PK digestion assay. For example, in the case of the solutions used for ThT binding and MTT reduction assays, 7-day-aged IAPP (16.5 μM) or IAPP/ACM (1/2) mixtures were prepared as described under “ThT binding assays”; TEM showed that fibrils were major species in both kinds of solutions (Fig. 2f). Aliquots (1.3 μg IAPP) were spotted onto 0.2 μm-nitrocellulose membranes. For comparison, freshly made solutions (containing no IAPP or hF-IAPP/ACM according to ThT binding (IAPP) and TEM) were made as above and spotted immediately. IAPP present in 0-h-aged solutions of IAPP alone and solutions containing non-fibrillar IAPP/ACM co-assemblies (TEM data Fig. 4a) was revealed using a rabbit polyclonal anti-IAPP antibody (Peninsula; 1:1000), whereas for fIAPP present in 7-day-aged solutions the fIAPP-specific mouse anti-IAPP antibody23 (Synaptic Systems; Cl. 91E7, 1:500) was used. Incubations with antibodies and membrane development were done under "PK digestion assays". Scans of uncropped blots are provided in the Source Data file.

**Dot blot analysis to detect (TAMRA)-Aβ42 oligomers**

The presence of oligomers at various incubation time points of (TAMRA)-Aβ42 (5 μM) solutions (prepared as for the ThT binding assay (Supplementary Fig. 27a) but without ThT or as for the MTT reduction assay (Supplementary Fig. 27b)) was studied by dot blot analysis (Supplementary Fig. 27c). At the indicated time points, Aβ42 (4.5 μg) or TAMRA-Aβ42 (4.9 μg) was spotted onto a nitrocellulose membrane. After blocking (5% BSA in ddH2O, 2 h, RT) and several washing steps with TBS-T, membranes were incubated overnight (10 °C) with (anti-) oligomer A11 polyclonal antibody (Thermo Fisher Scientific) (1:1000 in 5% BSA in ddH2O). The primary antibody was combined with donkey anti-rabbit-P (1:5000); detection was under “Cross-linking, NuPAGE, and Western blot”.

**Assessment of fibril thermostability by TEM and ThT binding**

Solutions consisting mainly of IAPP, hF-IAPP/Nle3-VF, fAβ42, or hF-Aβ42/Nle3-VF were prepared as described under "ThT binding assays".
(fIAPP 16.5 μM, 7-day-aged; fAP42/NIe3-VF, IAPP 16.5 μM, Nle3-VF 33 μM, 7-day-aged; fAP42 5 μM, 6-day-aged; hfA42/Nle3-VF, 5 μM each, 6-day-aged; Aβ42 incubations without ThT) and boiled (95 °C) for 5 min except for fAP42 which was boiled for 15 min. TEM grids were loaded, stained, and analyzed under "TEM." ThT binding of fAP42 and hf-IAPP/Nle3-VF solutions was assessed by mixing aliquots before or after boiling with a ThT solution as described under "ThT binding assays." Buffer values were subtracted from the data.

**Proteinase K (PK) fibril digestion assay in combination with dot blot**

The PK digestion assay was performed based on protocols by refs. **71, 72.** Briefly, PK stocks (100 μg/ml) were prepared in 50 mM TRIS/HCl, pH 8.0 containing 10 mM CaCl₂, the final PK concentration in the assay was 0.5 μg/ml. fIAPP (16.5 μM) or fAP42 as PK stock. Solutions made as above but without PK were used as controls for 100% undigested membrane development (2 h, in 5% milk in TBS-T (RT)): mouse anti-

A related assays, solutions were made by mixing 200 (20 min, 20,000×

β42 (5 μM) and hf-Aβ42/ACM (1/1) were made as described under "ThT binding assays" (without ThT; 7-day-aging). For IAPP-related assays, solutions were made by mixing 60 μl of the IAPP or hf-IAPP/ACM solutions with 0.3 μl of the PK stock solution. For Aβ42-related assays, solutions were made by mixing 200 μl of fAP42 or hf-Aβ42/ACM solutions with 1 μl PK stock. Solutions made as above but without PK were used as controls for 100% undigested fibrils. Solutions were incubated at 37 °C and at indicated time points, aliquots were chased from ATCC (EOC2 (CRL-2467)) and were maintained in culture medium (RPMI1640, 10% FBS, 1% penicillin/streptomycin) for 7 days. Following centrifugation (7 days, 20 °C); the mixture of IAPP (1024 μM) and hf-Aβ42/ACM mixtures (1/10) or ACMs alone (500 nM) were incubated with the cells for 6 h at a final homo-/hetero-fibril concentration of 1 μM. Of note, dot blot analysis and BCA showed that the main peptide fraction was present in the pellet. To address the question of whether the addition of Nle3-VF or L3-VF to preformed Aβ42 oligomers may affect their phagocytosis, preformed TAMRA-Aβ42 oligomers (TAMRA-oAβ42) were prepared as for MTT reduction assays incubating TAMRA-Aβ42 (5 μM) for 2 h under ThT binding assay conditions. For studying the effect of the ACMs, TAMRA-oAβ42 was mixed with Nle3-VF or L3-VF (1/1) and, following co-incubation for 2 h (ThT binding assay conditions), mixtures were diluted with cell medium (TAMRA-Aβ42, 1 μM) and incubated with the BV2 cells for 6 h as described above. TAMRA-oAβ42 alone was incubated for 2 h as well and treated thereafter as its mixtures with the ACMs.

Following peptide incubation with the cells, supernatants were removed and cells on the coverslips were washed five times with ice-cold 1xPBS, fixed with 4% paraformaldehyde, washed with 1xPBS, permeabilized with 0.2% Triton-X 100, and rinsed three times with cold 1xPBS. Coverslips were mounted with Vectashield Antifade mounting medium containing DAPI (Vector Laboratories). Images were acquired using a Leica DMi8 Fluorescence microscope. The percentage of cells that had taken up peptides was calculated by dividing the number of BV2 cells or BMDMs that phagocytosed TAMRA-labeled peptide by the total cell count, multiplied by 100. Significance was analyzed (GraphPad 5 and 9) by unpaired student’s t-test (2-sided) or one-way ANOVA and Bonferroni as indicated.

**Hippocampal LTP measurements**

LTP measurements were performed as previously described in refs. **20, 21, 73, 74.** Briefly, sagittal hippocampal slices (350 μm) were obtained from C57BL/6N mice (6–8 weeks of age, male (Charles River Laboratories)) in ice-cold Ringer solution bubbled with a mixture of 95% O₂ and 5% CO₂ as described in ref. 20 and according to protocols approved by the ethical committee on animal care and use of the government of Bavaria Germany. Ethics oversight by ethical committee on animal care and use of the government of Bavaria Germany. Extracellular recordings were performed using artificial cerebrospinal fluid (ACSF)-filled glass microelectrodes (2–3 MΩ) at RT. ACSF consisted of 125 mM NaCl, 2.5 mM KCl, 25 mM NaHCO₃, 2 mM CaCl₂, 1 mM MgCl₂, 25 mM d-glucose, and 1.25 mM NaH₂PO₄ (pH 7.3) and was bubbled with 95% O₂ and 5% CO₂. Field excitatory post-synaptic potentials (fEPSPs) were evoked in the hippocampal CA1 dendritic region via two independent inputs by stimulating the Schaffer collateral commissural pathway (Sccp). For LTP induction, high-frequency stimulation (HFS; 100 Hz/100 pulses) conditioning pulses were delivered to the same Sccp inputs. Both stimulating electrodes were used to utilize the input specificity of LTP, thus allowing for the measurement of internal control within the same slice. Aβ42 (50 nM), Aβ42/ACM mixtures (1/10) or ACMs alone (500 nM) were freshly dissolved in ACSF and applied 60–90 min before HFS. Responses were measured for 60 min after HFS pre-conditioning in ACSF or in ACSF supplemented with pre-oligomerized fAP42 (50 nM) or Aβ42 alone (500 nM) and were analyzed using articial cerebrospinal fluid (ACSF)-filled glass microelectrodes (2–3 MΩ) at RT. ACSF consisted of 125 mM NaCl, 2.5 mM KCl, 25 mM NaHCO₃, 2 mM CaCl₂, 1 mM MgCl₂, 25 mM d-glucose, and 1.25 mM NaH₂PO₄ (pH 7.3) and was bubbled with 95% O₂ and 5% CO₂. Field excitatory post-synaptic potentials (fEPSPs) were evoked in the hippocampal CA1 dendritic region via two independent inputs by stimulating the Schaffer collateral commissural pathway (Sccp). For LTP induction, high-frequency stimulation (HFS; 100 Hz/100 pulses) conditioning pulses were delivered to the same Sccp inputs. Both stimulating electrodes were used to utilize the input specificity of LTP, thus allowing for the measurement of the internal control within the same slice. Aβ42 (50 nM), Aβ42/ACM mixtures (1/10) or ACMs alone (500 nM) were freshly dissolved in ACSF and applied 60–90 min before HFS. Responses were measured for 60 min after HFS. To study the effects of ACMs on the LTP impairment mediated by pre-oligomerized Aβ42, Aβ42 (50 nM) was pre-incubated in ACSF for 24 h (30 °C) and then mixed with L3-VF or F3-LF (1/10) (in ACSF); pre-oligomerized Aβ42 alone and Aβ42/ACM mixtures were applied to the slices 90 min before HFS. fEPSP slope measurements (20–80% of peak amplitude) are presented as % fEPSP slope of baseline (the 20 min control period before tetanic stimulation was set to 100%). Data analysis by one-way ANOVA and Bonferroni’s multiple comparison test or by Kruskal–Wallis test with Dunn’s multiple comparisons test (GraphPad 6) as indicated.

**X-ray fiber diffraction**

fAP42 and hf-IAPP/Nle3-VF were made by aging IAPP (1024 μM) or a mixture of IAPP (1024 μM) and Nle3-VF (2048 μM) in ddH₂O for 3 days. A droplet of each solution was placed between glass rods supported by plasticine balls and allowed to dry (humidified atmosphere, 2–4 days,
X-ray diffraction data were collected at the facility Single-Crystal X-Ray Diffactometry of the TUM Catalysis Research Center (CRC) using a Bruker D8 Venture diffractometer equipped with a CPAD detector (Bruker Photon II), an IMS micro source with CuKα radiation (λ = 1.54178 Å) and a Helios optic using the APEX3 software package (Version 2019-1.0, Bruker AXS Inc., Madison, Wisconsin, USA, 2019).

**Reporting summary**

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

The data supporting the findings of this study are available within the paper and its Supplementary Information files. Source data are provided with this paper. A.K., J.B., and K.T. are co-inventors of the European patent application 22 158 021.0 (applicant Technical University of Munich) (status: pending) related to ACMs, their hetero-assemblies, and potential biomedical applications. Source data are provided with this paper.

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Author contributions
A.K. conceived the project. A.K., K.T., J.B., R.T.A.M., G.R., and M.H. designed experiments with contributions from B.D.V., C.L., O.E.B., Y.T., X.P.-B., M.B., and S.H. K.T., B.D.V., C.L., O.E.B., K.H., Y.T., X.P.B., M.B., S.H., M.O., S.P., L.M., and R.T.A.M. conducted experiments. A.K., K.T., B.D.V., C.L., O.E.B., K.H., Y.T., X.P.B., M.B., S.H., L.M., M.H., G.R., M.O., S.P., C.W., R.T.A.M., and J.B. contributed to data analysis or interpretation. A.K. and K.T. wrote and edited the manuscript with contributions from all authors.

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Competing interests
A.K., J.B., and K.T. are co-inventors of the European patent application 22158021.0 (applicant Technical University of Munich) (status: pending) related to ACMs, their hetero-assemblies, and potential biomedical applications. The remaining authors declare no competing interests.

Additional information

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