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Supporting Information

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Multi-Targeting Macrocyclic Peptides as Nanomolar Inhibitors of Self- and Cross-Seeded Amyloid Self-Assembly of α-Synuclein

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Experimental section

Peptides, peptide synthesis, and proteins

All peptides, i.e. MCIPs, **4Ala-2b**, ISMs, IAPP, and IAPP or αSyn segments (see Supporting Tables 1 and 2), and their N^{α}-terminal fluorescein-labeled analogs (Fluos-peptide) (all C-terminal amides) were synthesized using Fmoc-based solid phase synthesis (SPPS) on Rink resin and purified via RP-HPLC using previously described protocols and chemicals.[1] Briefly, double or triple couplings were performed using Fmoc-protected amino acids (3-fold molar excess) and as coupling reagents N,N,N′,N′-tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) or 2-(7-aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) (3-fold molar excess) for selected couplings and N,N-diisopropylethylamine (DIEA) (4.5-fold molar excess) in N,N-dimethylformamide (DMF). Cysteines were coupled using 1-hydroxybenzotriazole (HOBt) and diisopropylcarbodiimide (DIC). N^{α}-terminal fluorescein-labels were introduced by coupling 5(6)-carboxyfluorescein (3-fold molar excess) using (HATU (3-fold molar excess) and DIEA (4.5-fold molar excess) in DMF (double coupling). In the case of the SPPS of IAPP, pseudoproline dipeptides (3-fold molar excess) were used at specific sequence positions and coupled using HATU (3-fold excess) and DIEA (4.5 fold excess); SPPS, cleavage of protecting groups/from the resin, disulfide bridge formation, and RP-HPLC purification were performed as described.[1e, 1f] Disulfide bridge formation of the MCIPs and **4Ala-2b** was performed in aq. 0.1 M NH_4HCO_3 solution containing DMSO (40%) (stirring for ~1.5 h).[1g] SPPS was performed manually for sequence parts containing N-methyl amino acids and either manually or with a CS336X peptide synthesizer (CSBio) for the rest of the sequence parts or peptides containing no N-methyl amino acids. RP-HPLC purifications were preformed using Nucleosil 100 C18 (Grace) or Reprosil Gold 200 C18 columns (Dr. Maisch) according to previously developed protocols.[1d-g, 2] Peptide purity was verified by MALDI-TOF mass spectrometry (MS); results are in Tables S1 and S2 (in Supporting Information). Peptide stock solutions were freshly made in 1,1,3,3,3,3-hexafluoro-2-isopropanol (HFIP) on ice; concentrations were determined by peptide weight and/or the BCA assay and by UV spectroscopy in the case of IAPP and fluorescein-labeled peptides as described.[1d-g] Stock solutions of IAPP and fluorescein-labeled peptides in HFIP were filtered over 0.2 mm filters just before the determination of their concentration.[1d, 1f] Peptides were applied from their HFIP stocks following HFIP evaporation with N_2 and reconstitution with assay buffer.

Recombinant human αSyn was produced as described before[3] or was purchased from Eurogentech (Anaspec) (catalog number AS-55555 with an additional Gly residue at the N-terminus) or from AlexoTech (Catalog Number AS-600-100). αSyn aliquots were prepared in ddH_2O, frozen immediately, lyophilized, and freshly reconstituted with assay buffer for each

experiment. Of note, freshly made αSyn solutions at the low μM concentration range used in our studies consisted predominantly of non-toxic monomers based on CD spectroscopy, SEC, ThT binding, the MTT reduction assay, and TEM (Fig. 2a, 3c, 3f, 3g, and Supporting Fig. S3a, S15b). Aβ42 used for the preparation of Aβ42 fibrils (fAβ42) (for the αSyn cross-seeding assays) was synthesized and treated as described.[1a] Bovine insulin used for the studies of the effects of the MCIPs on insulin amyloid self-assembly was purchased from Sigma-Aldrich and aliquoted as described.[4]

Thioflavin T (ThT) binding assays

Effects of peptides on αSyn self- and cross-seeded amyloid self-assembly were studied by a combination of the ThT binding assay with TEM and the MTT reduction assay according to previously developed protocols.[1a, 1e-h] The ThT binding assays were performed based on a previously published protocol of the Hoyer group.[5] Solutions used for the assays of amyloid self-assembly of αSyn alone (3 μM) or its mixtures with various peptide amounts (as indicated) with or w/o preformed (cross-)seeds (10% or 1% as indicated) or related controls (including peptides alone) were made in 20 mM sodium phosphate buffer (pH 6.0) containing 50 mM NaCl (abbreviated "ThT assay buffer") and ThT (40 μM) in 96-well black MTPs (FluoroNunc/Thermo Fisher Scientific) and incubated ("aged") for up to 7 days at 37°C with shaking at 800 rpm in a MTP shaker (Thermoshaker TPS-4H; 4 More Labor). Of note, each MTP well contained one glass bead (2 mm). ThT fluorescence was measured at 486 nm (excitation at 450 nm) using the Multilabel reader VictorX3 (Perkin Elmer Life Sciences) at the indicated time points with short shaking (1 min) before measuring. In each assay, solutions were prepared in 3 technical replicates (n=3 wells) and the ThT fluorescence signal of the buffer was subtracted from all samples. Data is means (±SD) of 3 independent assays (n=3 wells each) if not stated otherwise.

Preformed seeds of IAPP fibrils (fIAPP) were prepared by incubating IAPP (128 μM) in ThT assay buffer for 24 h (RT; no shaking); fibril formation was verified by TEM (Fig. 2h (inset)). αSyn fibrils (fαSyn) were prepared by incubating αSyn (69 μM in ThT assay buffer in a MTP well containing a glass bead (2 mm) for 7 days (at 37°C & 800 rpm); their formation was verified by TEM (Fig. 2e (inset)). Preformed fαSyn seeds were sonicated for 1 min prior to their use. Preformed seeds of Aβ42 fibrils (fAβ42) were prepared by incubating synthetic Aβ42 for 7 days at 37°C as described.[1a] Following centrifugation (20000 g for 10 min), pellets were suspended in (αSyn) ThT assay buffer and used for the αSyn cross-seeding assays as described below.

Solutions used for the incubations were made as follows: (1) To study effects of the peptides on (unseeded) αSyn amyloid assembly, freshly made αSyn (3 μM) or αSyn/peptide mixtures (αSyn/peptide molar ratios as indicated) or control solutions in ThT assay buffer containing 40

μM ThT were incubated for 7 days as mentioned above (37°C & 800 rpm). Of note, peptides were preincubated in ThT assay buffer for 6 h prior mixing with αSyn and ThT to ensure complete dissolution. (2) To study effects of the peptides on αSyn amyloid self-assembly (cross-)seeded with preformed fαSyn or fIAPP, freshly made αSyn (3 μM), the corresponding αSyn/peptide mixtures (αSyn/peptide molar ratios as indicated) with or w/o fαSyn (10% (0.3 μM)), fIAPP (10% (0.3 μM) or 1% (0.03 μM) as indicated), or control solutions (e.g. seeds alone) were incubated in ThT assay buffer containing 40 μM ThT for 7 days as mentioned above (at 37°C & 800 rpm). Of note, peptides with or w/o fαSyn or fIAPP were preincubated in ThT assay buffer (for 6 h, RT) prior to mixing with αSyn and ThT as under (1). (3) To investigate the (cross-)seedability of the αSyn/peptide hetero-complexes by fαSyn, fIAPP, or fAβ42 seeds, peptides (αSyn/peptide molar ratios as indicated) were preincubated (6 h, RT) with αSyn (3 μM) in ThT assay buffer prior to mixing with fibril seeds to allow for hetero-complex formation. Following addition of fαSyn or fIAPP seeds (both at 10% (0.3 μM)) or of fAβ42 (20% (0.6 μM)), solutions were incubated in ThT assay buffer containing 40 μM ThT for 7 days as mentioned above (at 37°C & 800 rpm).

Effects of **2b** and **2e** on amyloid self-assembly of insulin were studied using an earlier developed protocol.[4, 6] Briefly, solutions containing insulin (10 μM) alone (freshly made from its lyophilized aliquots) or its mixtures with each of the peptides in water/HCl (pH 2) (100 μM) and ThT (20 μM) were incubated in sealed 96-well MTPs at 60°C for several days. ThT binding was determined at the indicated time points after placing the MTP on ice for 5 min and a short (5 sec) gentle automatic shaking by measuring fluorescence emission at 486 nm (excitation at 450 nm) using the Multilabel reader VictorX3 (Perkin Elmer Life Sciences) as for the αSyn-related studies as well.

Assessment of PC12 cell damage by the MTT reduction assay

PC12 cells were obtained from DSMZ (German Collection of Microorganisms and Cell Cultures GmbH) (DSMZ number: ACC 159) and were cultured and plated as described.[1h] Effects of MCIPs and controls on formation of cell-damaging αSyn assemblies were studied by the MTT reduction assay using solutions from the ThT binding assays aged for 0 h, 24 h, 48 h, or 7 days (at 37°C & 800 rpm as under "ThT binding assays") according to previously developed protocols.[1a, 1f-h] Briefly, aliquots of ThT binding assay solutions were diluted with cell medium at various incubation time points and added to the PC12 cells at the indicated final concentrations of αSyn and peptides (IC_{50} values at 100 nM αSyn; 7 day-aged solutions). Following incubation with the cells for ~20 h (37°C, humidified atmosphere with 5% CO_2), cells were incubated with MTT (0.92 mg/ml) for ~2 h at 37°C. Following addition of 10% SDS in 20 mM HCl (pH 4.5) and overnight incubation, generated formazan was quantified by its

absorbance at 570 nm with a Multilabel reader VictorX3 (Perkin Elmer Life Sciences); for 100% MTT reduction the absorbance of untreated cells and for 0% MTT reduction the absorbance of Triton X-100-treated cells was used.

Transmission electron microscopy (TEM)

Aliquots (10 μl) of solutions to be examined, including solutions used for ThT binding and MTT reduction assays, were applied at the indicated incubation time points on formvar/carbon-coated grids (3 min). Grids were washed with ddH_2O and stained with aqueous 2% (w/v) uranyl acetate solution for 1 min.[7] Examination of the grids was performed using a JEOL 1400 Plus electron microscope at 120 kV.

Far-UV CD spectroscopy

CD measurements were performed using a Jasco 715 spectropolarimeter and spectra (average of 3 spectra) were recorded between 200 and 250 nm, at 0.1 nm intervals, with a response time of 1 sec as previously described.[1g] For the CD studies on structures and effects of αSyn/MCIP hetero-complexes on αSyn self-assembly (Fig. 3c,d), freshly made solutions of αSyn alone (1 μM), MCIP alone (10 μM), and αSyn/MCIP mixtures (1/10) in ThT assay buffer were made and incubated for 48 h at 37° C to mimic the experimental conditions of the ThT binding assays. CD spectra were recorded at the 0 h incubation time point (after a 20 min preincubation at 37° C) and at the 48 h incubation time point. Of note, the spectra of the MCIPs alone are not shown in Fig. 3c,d as they were as the previously reported ones.[1g] The CD concentration dependence studies of **2b** and **2e** (5-50 μM) were performed in freshly made peptide solutions in aqueous 10 mM sodium phosphate buffer (pH 7.4) containing 1% HFIP as previously described.[1g] The spectrum of the buffer alone was subtracted from all CD spectra.

Fluorescence spectroscopic titrations

Fluorescence spectroscopic titration studies were performed using a Jasco FP-6500 fluorescence spectrophotometer and previously established protocols.[1b, 1d-f, 1h, 7] Briefly, excitation was at 492 nm and emission spectra were recorded between 500 and 600 nm. For all experiments, freshly prepared stocks of peptides and their fluorescently labeled analogs in HFIP were used while αSyn stocks were in aqueous 10 mM sodium phosphate buffer (pH 7.4). Measurements were performed in freshly made solutions containing synthetic N^{α}-terminal fluorescein-labeled IAPP (Fluos-IAPP), IAPP(8-28) (Fluos-IAPP(8-28)), MCIPs (Fluos-**2e** & Fluos-**2b**), or **4Ala-2b** (Fluos-**4Ala-2b**) (1, 5, or 10 nM as indicated) alone or with various amounts of the binding partner (αSyn, αSyn segments, MCIPs, or IAPP) in aqueous 10 mM sodium phosphate buffer (pH 7.4) containing 0.5% HFIP (for binding to αSyn or its segments)

or 1% HFIP (for binding to IAPP, IAPP(8-28), and MCIPs). Measurements were performed within 2-5 min after solution preparation. Under these experimental conditions, Fluos-peptides were mostly monomeric (Supporting Fig. S9a,b and references 1d-h).[1d-h] Apparent (app.) K_d values were calculated as described using 1/1 binding models which in the case of the αSyn/MCIP interactions were in accordance to the 1/1 αSyn/MCIP ratio required for full inhibition of amyloid self-assembly and cytotoxicity.[1a, 1b, 1d-h, 7] However, due to the self-assembly propensities of the examined peptides/proteins and their multi-site binding features more complex models may also apply. App. K_ds are means (±SD) of three binding curves derived from 3 titration assays.

Sedimentation assays

Solubilities of MCIPs **2b** and **2e** were determined by a previously developed sedimentation assay.[1e, 1f] Briefly, **2b** (500 μM and 1 μM) and **2e** (1 mM) were incubated in aq. 10 mM sodium phosphate buffer (pH 7.4) at room temperature for 7 days. Following centrifugation at the indicated time points peptide amounts in supernatants and pellets were quantified by the BCA protein assay. The peptide amount in a non-centrifuged sample was also quantified to determine the total amount; non-specific binding (NSB) corresponded to the amount which remained in the tube after removing the solution and was 5-20% of total.

Cross-linking, SDS-PAGE, and Western blot (WB) analysis

Cross-linking studies were performed in combination with NuPAGE (Thermo Fisher Scientific) and WB based on a previously developed assay system.[1a, 1b, 1g, 1h] Briefly, for characterizing αSyn homo- and αSyn/MCIP hetero-assemblies, αSyn alone (10 μM), αSyn/MCIP mixtures (1/5), and MCIPs alone (50 μM) were prepared in ThT assay buffer. Following incubation for 30 min, solutions were cross-linked by adding 25% aqueous glutaraldehyde (Sigma-Aldrich) (2 min). Following treatment with 2 M $NaBH_4$ (in 0.1 M NaOH, 20 min), cross-linked complexes were precipitated with 10% aqueous trichloroacetic acid (4°C), centrifuged (10 min, 12000 g), and pellets were dissolved in NuPAGE LDS-sample buffer (w/o reducing agent) boiled for 5 min, and subjected to gel electrophoresis (10-20% Tricine gels) with Tricine SDS running buffer according to the manufacturer's (Invitrogen) recommendations. Equal amounts of αSyn (4.3 μg) and MCIPs (2.5 μg for **2b** and **2e** and 2.2 μg for **4Ala-2b**) were loaded in the different lanes and prestained protein size markers (from 3.5 to 260 kDa (Invitrogen)) were run in the same gels.

Gels were blotted onto nitrocellulose membranes using an XCell II Blot Module blotting system (Invitrogen) and membranes were blocked by overnight incubation (10°C) with 2% BSA in TBS-T (20 mM Tris, 150 mM NaCl, 0.05% Tween-20). To detect αSyn containing bands, membranes were incubated with a rabbit anti-human αSyn antibody (Sigma-Aldrich,

SAB4502829, Lot: 210582) (1:1000) in 0.5% BSA in TBS-T (2 h at RT or overnight at 10°C) and thereafter with donkey anti-rabbit-HRP antibody (GE Healthcare, NA934, Lot: 16836138) (1:5000) in 0.5% BSA in TBS-T (2 h at RT or overnight at 10°C). Western blots were developed using Super Signal West Dura Extended Duration Substrate (Thermo Scientific, Cat. No. 34075) and imaged using a LAS-4000 mini imager (Fujifilm).To detect **2e**- or **2b**-containing bands, membranes were stripped by incubating with stripping buffer consisting of 2% SDS and 100 mM β-mercaptoethanol in 50 mM Tris (pH 6.8) (40 min at 60°C and 60 min at RT).[1a] After washing with TBS-T, membranes were blocked with 2% BSA in TBS-T (3 h, RT). Detection of **2b** and **2e** was done by incubation (2 h at RT or overnight at 10°C) with mouse anti-**2e** antibody (Clone MCP2E 26C3), which was produced at Helmholtz Center Munich as described below, and with goat anti-mouse-HRP antibody (Abcam, ab6789, Lot: GR3257574-1) (1:10000) in 0.5% BSA in TBS-T as secondary antibody (2 h at RT or overnight at 10°C). Of note, the anti-**2e** antibody recognized **2b** as well and did not bind with αSyn (Fig. 3e); the anti-αSyn antibody did not bind to **2b** and **2e** (data not shown). Densitometric quantification of WB band intensities was performed by using ImageJ, background signal subtraction, and normalization relative to the strongest band.

Generation of monoclonal antibodies against 2e

Thirteen week old female Balb/c wild type mice (Charles River) were immunized subcutaneously (s.c.) and intraperitoneally (i.p.) with a mixture of 50 µg ovalbumin-coupled 2e dissolved in a mixture of 200 µl PBS containing 5 nmol CpG2006 (TIB MOLBIOL) and 200 µL incomplete Freund's adjuvant (Sigma-Aldrich). Animal procedures were approved by the local Animal Use and Care Committee with approval by the local authorities of Upper Bavaria, Germany (reference number ROB-55.2Vet-2532.Vet_03-22-25) in accordance with European and German animal welfare regulations. After 12 weeks, a boost without Freund's adjuvant was given i.p. and s.c. 3 days before fusion. Fusion of the myeloma cell line P3X63-Ag8.653 with the mouse immune spleen cells was performed using polyethylene glycol 1500 according to standard procedure. After fusion, the cells were plated in 96-well plates with medium consisting of RPMI 1640 with stable glutamine (RPMI-STA, Capricorn) supplemented with 20% fetal calf serum, 1mM pyruvate, 1x non-essential amino acids and HAT media supplement (Hybri-Max, Sigma-Aldrich). Hybridoma supernatants were screened 10 days later in a flow cytometry assay (iQue, Intellicyt; Sartorius) using N-terminal biotinylated **2e** captured on streptavidin beads (PolyAN) and incubated for 90 min with hybridoma supernatant and Atto-488-coupled isotype-specific monoclonal rat-anti-mouse IgG secondary antibodies. Antibody binding was analyzed using ForeCyt software (Sartorius). Positive supernatants were further validated by dot blot and Western blot. Hybridoma cells from selected supernatants were

cloned by limiting dilution to obtain stable monoclonal cell lines. Our assays (Fig. 3e) were performed with hybridoma supernatant of clone MCP2E 26C3 (mouse IgG2a/ĸ).

Size exclusion chromatography (SEC) and ESI-MS of collected peaks

For the SEC studies a Superdex 75 10/300 GL column (GE Healthcare) and a Dionex UltiMate 3000 device (Thermo Fisher Scientific) were used.[1a] Elution buffer was the ThT assay buffer, the flow rate was 0.5 ml/min, and protein/peptide detection was at 214 nm. The column was calibrated with a gel filtration protein standard (Bio-Rad, Cat. No. 151-1901) (Supporting Fig. S13a). The retention time (t_R) of αSyn monomers was determined by injecting αSyn (3 μM) freshly dissolved in 6 M GdnHCl in 0.1 M NH_4HCO_3 (pH 8.4) (Supporting Fig. S13b). Of note, αSyn monomers eluted earlier than expected based on the applied globular protein standard likely due to their disordered structure consistent with previous reports.[3, 8] For the analysis of αSyn/MCIP hetero-complexes, freshly made solutions (400 μl) of αSyn alone (3 μM), **2e** alone (30 μM), and αSyn/**2e** mixtures (1/10) in ThT assay buffer were loaded onto the column. Peaks were collected, lyophilized, and analyzed by liquid-chromatography electrospray ionization mass spectrometry (LC-ESI-MS). For sample analysis, a Dionex UltiMate 3000 HPLC system (column: MSPac DS-10 (10 x 2.1 mm, 5 μm) (Thermo Scientific)) coupled to a LCQ Fleet mass spectrometer (Thermo Scientific) with a heated ESI source was used. The mass spectrometer was run in positive mode collecting ion-trap scans at scan rate “normal” from 300 to 2000 m/z. Spectra were visualized using XCalibur 2.2 SP1.48 (Thermo Scientific) and deconvoluted using MagTran 1.02 (Amgen Inc.) implementing the ZSCORE algorithm.[9]

Slot blot analysis

Effects of MCIPs on kinetics of formation of cytotoxic (A11-reactive) αSyn oligomers (Fig. 3g) were studied by Slot blot analysis in combination with ThT binding, MTT reduction assays, and TEM (see Fig. 2a-c, Fig. S15). Slot blot analysis was performed using a PR648 Slot blot blotting manifold (Hoefer) with nitrocellulose membrane based on a previously described protocol.[10] Briefly, solutions containing αSyn (3 μM) alone, **2e** (3 μM) alone, and their mixture (1/1) in ThT assay buffer were incubated for 7 days (37°C, 800 rpm) in MTPs as for the ThT binding assay and added (200 μl each) at the indicated incubation time points to the slots which had been pre-wetted with ThT assay buffer. Solutions were left in the slots for 2 h (RT) to allow for binding with the membrane; thereafter, they were removed with vacuum and slots were washed with ThT assay buffer (1x100 μL). The membrane was washed 3x5 min with TBS-T and blocked with 2% BSA in TBS-T overnight at 10°C. Then, the membrane was incubated with a rabbit A11 antibody[11] (Invitrogen, AHB0052, Lot: WH329538) (1:1000 in 0.5% BSA in TBS-T) (overnight at 10°C) and, following washing with TBS-T (3x5 min), incubated with donkey anti-rabbit-HRP antibody (GE Healthcare, NA934, Lot: 16836138) (1:5000 in 0.5% BSA in TBS-T) 2h at RT or overnight at 10°C. Following washing with TBS-T (3x5 min), the membrane was

developed using Super Signal West Dura Extended Duration Substrate (Thermo Scientific, Cat. No. 34075) and imaged with a LAS-4000 mini imager (Fujifilm). Densitometric quantification of band intensities (Supporting Fig. S15a) was performed by ImageJ following subtraction of the signal intensity of the background from each sample.

Dot blot assays

Binding of **2b**, **2e** and **4Ala-2b** to fIAPP or fαSyn (Fig. 3h) was studied by dot blot analysis performed according to a previously described protocol.[1a] Briefly, fIAPP was prepared by incubating an IAPP solution (128 µM) in 50 mM sodium phosphate buffer, pH 7.4, containing 100 mM NaCl and 0.5% HFIP for 24 h at RT (fIAPP confirmed by ThT and TEM).[1a] fαSyn were prepared by incubating αSyn (69 µM) in ThT assay buffer at 37°C & 800 rpm for 7 days as for the ThT binding assay (confirmed by ThT and TEM; data not shown). fIAPP (20 µg) or fαSyn (10 µg) were spotted on nitrocellulose membranes. Membranes were washed with TBS-T, blocked with 5% milk in TBS-T overnight at 10°C, and washed again with TBS-T. Then, membranes were incubated with solutions of N-terminal fluorescein-labeled peptides (Fluos-peptide) (1.5 µM) in 50 mM sodium phosphate buffer, pH 7.4, containing 100 mM NaCl and 0.5% HFIP (for fIAPP binding studies) or in ThT assay buffer (for fαSyn binding studies) overnight at 10°C.[1a] To control for fibril autofluorescence, membranes containing spotted fIAPP or fαSyn were incubated in parallel with buffer alone.[1a] Following washing with TBS-T, bound peptides were visualized with a LAS-4000 mini imager equipped with a suitable fluorescence filter (Fujifilm). Densitometric quantification of dot intensities was performed by ImageJ; dot intensities of the different dot blots were normalized relative to the buffer, the intrinsic fluorescence of each peptide, and the highest dot intensity (set to 100%).

Studies using peptide arrays

To identify the αSyn binding sites for its interactions with MCIPs and IAPP, we used synthetic peptide arrays. Peptide arrays containing IAPP or αSyn decamers covering full length IAPP or αSyn and positionally shifted by one residue were synthesized on a modified cellulose membrane support using stepwise SPOT synthesis protocols and a MultiPep RSi (Intavis) peptide synthesizer as previously described.[1d, 10, 12] Thereafter, arrays were immobilized on glass slides according to the manufacturer's instructions and processed and developed using previously described protocols.[1d, 10, 12] Briefly, glass slides blocked for 4 h at RT with 1% BSA in TBS-T and incubated with synthetic N^{α}-terminal fluorescein-labeled **2e** (Fluos-**2e**) or IAPP (Fluos-IAPP) (1 µM in 1% BSA in TBS-T) or with recombinant Biotin-labeled M1C-αSyn (Biotin-αSyn; Cys1(side-chain)-labeled M1C-αSyn via thiol-maleimide-chemistry) (0.5 µM in 1% BSA in TBS-T) overnight (at 10°C) followed by washing with TBS-T.[3] Bound peptides were

visualized by using a LAS-4000 mini instrument as follows (Fujifilm): In the case of fluorescein-labeled peptides, visualization was based on their fluorescence readout. In the case of Biotin-αSyn, visualization was performed via enhanced chemiluminescence (ECL) following incubation (2h at RT) of the glass slides with streptavidin-POD antibody (Roche Diagnostics, 11089153001, Lot: 56790500) (1:1000 in 1% BSA in TBS-T) and development by the SuperSignal West Dura Extended Duration Substrate (Thermo Scientific, Cat. No. 34075) as described.[1d, 1h] For quantification (Supporting Fig. S21, S23), the optical density of each spot was determined using ImageJ software. For each array, the highest optical density value was defined as 100%, while the optical density value of the negative control spot was defined as 0% and relative intensity of each spot was determined by using the following formula:

$$Rel.intensity = \frac{optical\ density\ (spot) - optical\ density\ (neg.control, 0\%)}{highest\ optical\ density\ (100\%) - optical\ density\ (neg.control, 0\%)}$$

Binding regions corresponded to clusters of at least 4 strong binding decamers defined as indicated in the legends of Supporting Fig. S21 and S23. Of note, results of densitometric analyses were consistent with the results of the visual inspection of the arrays.

Fluorescence polarization assays

Fluorescence polarization (FP) assays were performed using a Jasco FP-8550 fluorescence spectrophotometer. Excitation was at 492 nm and emission was recorded at 522 nm. Bandwidth for excitation and emission was set at 5 nm and time response at 0.5 s. Measurements were performed within 2-5 min upon solution preparation. To study interactions of the three identified αSyn key segments αSyn(1-14), αSyn(34-52), and αSyn(87-105) with fIAPP, we measured FP of freshly made Fluos-fIAPP alone (100 nM) in aqueous 10 mM sodium phosphate buffer (pH 7.4) containing 0.5% HFIP and its mixtures with each of the 3 segments (1.5 μM). Fluos-fIAPP was made by incubating Fluos-IAPP (20 μM) in aqueous 10 mM sodium phosphate buffer (pH 7.4) containing 0.5% HFIP for 48 h; solutions were sonicated for 30 seconds prior to their use for FP assays; the presence of fibrils was verified by TEM (not shown).

Preparation and characterization of αSyn oligomers for the *ex-vivo* hippocampal LTP measurements

αSyn oligomers for the hippocampal LTP measurements were prepared based on a previously reported protocol.[13] Briefly, lyophilized αSyn was suspended at 2 mg/ml (138 μM) in ddH_2O and incubated under continuous shaking for 5 days at 37°C in a Thermomixer (Eppendorf) at 1400 rpm. Solutions were centrifuged at 22000g for 15 min, the resulting supernatant (oligomers) was lyophilized. The concentration of αSyn oligomer solutions (supernatants) was determined using the BCA assay (Pierce). Supernatant fractions containing the oligomers were

lyophilized, kept at -60°C, and reconstituted with aCSF just prior to the LTP measurements. In addition, assemblies present in supernatants and pellets of the αSyn oligomer preparations were characterized by TEM, the ThT binding assay, the MTT reduction assay, and a dot blot assay to assess A11-reactivity using above or previously described protocols (see Supporting Figure S19).[1a] Briefly, TEM grids were prepared and imaged as described under TEM. ThT binding was determined in aliquots of solutions containing αSyn (1.7 μM) monomers (freshly made) and supernatant (oligomer) or pellet (fibril) fractions in ThT assay buffer containing 20 μM ThT as described under "ThT binding assays". For determination of A11-reactivity, solutions containing mostly αSyn monomers and the supernatants or re-suspended pellets (69 μM in ddH_2O) were spotted on a nitrocellulose membrane (as described under "Dot blot assays") and the membrane was blocked with 2% BSA in TBS-T overnight at 10°C and incubated with rabbit A11 antibody and developed as described under "Slot blot assays". For the assessment of the cell-damaging effects of αSyn oligomer-containing fractions and the effects of the MCIPs, lyophilized supernatant fractions were dissolved in ThT assay buffer alone (αSyn oligomers 3 μM) or in ThT assay buffer containing **2b** or **2e** (10-fold). Following dilution with cell medium, αSyn oligomers or their mixtures with **2b** (or **2e**) were added to the cultured PC12 cells plated in MTPs (αSyn, 300 nM) and incubated with the PC12 cells for ~20 h as described under "MTT reduction assays". MTT reduction was assessed as described under "MTT reduction assays".

Hippocampal long-term potentiation (LTP) measurements (*ex vivo*)

LTP measurements were performed as previously described.[1a, 1g, 14] 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% O_2 and 5% CO_2 according to protocols approved by the ethical committee on animal care and use of the government of Bavaria Germany (animal ethics approval 4-016-18) and were performed according to the German animal protection law. Ethics oversight by ethical committee on animal care and use of the government of Bavaria (Regierung von Oberbayern, ROB). 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 $NaHCO_3$, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 25 mM D-glucose, and 1.25 mM NaH_2PO_4 (pH 7.3) and was bubbled with 95% O_2 and 5% CO_2. Field excitatory postsynaptic 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; 100Hz/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. αSyn oligomers (175 nM) (made as

described in the previous chapter), their mixtures with MCIPs (1/10), or MCIPs alone (1.75 µM) were freshly dissolved in aCSF and applied to the slices 60-90 min before HFS. Responses were measured for 60 min after 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%). For statistical comparisons, we performed non-parametric testing with Mann-Whitney U tests or a Kruskal-Wallis test. We did not perform a correction for multiple comparisons, which is acceptable as for instance stated by Rothman[15] and present uncorrected p-values in Fig. 4a-c.

Effects of the peptides on αSyn toxicity in α-Syn overexpressing postmitotic dopaminergic LUHMES neurons

A previously established cell model of αSyn toxicity in αSyn overexpressing dopaminergic postmitotic Lund human mesencephalic (LUHMES) neurons was used.[16] Briefly, LUHMES cells (ATCC, catalogue number: CRL-2927) were cultivated (~100.000 cells/cm²), differentiated into a postmitotic dopaminergic phenotype, and transduced to overexpress αSyn *via* addition of adenoviral vectors encoding wild type αSyn (on day 1 after the begin of differentiation) as described.[16] Following removal of the virus-containing medium and a cell-wash step (PBS), peptides (10 nM in cell medium) were added to the cells (on day 2 after the begin of differentiation) and cell death in peptide-treated or untreated transduced cells and in untransduced control cells and GFP-transduced cells (control for adenovirus effects) was quantified by the lactate dehydrogenase (LDH) release assay which was performed 6 days after transduction as described.[16] Data in Fig. 4d are presented as “relative LDH release” (% of LDH release of untreated cells overexpressing αSyn which was set to 100%); background values were measured in wells containing medium but no cells and their averages were subtracted from all conditions.

Supporting Tables S1 and S2

Supporting Table S1. Sequences, abbreviations, and molecular weights (M) of synthesized and studied peptides as determined by MALDI-TOF-MS. All peptides have a free N-terminal amino group and are C-terminal amides. IAPP contains a disulfide bridge between Cys residues 2 and 7 while **2b**, **2e** and **4Ala-2b** contain a disulfide bridge between their N- and C-terminal Cys residues.[1g]

Abbreviation	Sequence	$[M+H]^+$ calculated[a]	$[M+H]^+$ found[a]
IAPP	KCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTY	3901.89	3901.49
IAPP(8-28)[1c]	ATQRLANFLVHSSNNFGAILS	2259.20	2259.57
IAPP(8-28)-GI[1c]	ATQRLANFLVHSSNNF(NMe)GA(NMe)ILS	2287.23	2286.61
R3-GI[1b]	ATQRLANFLVHRRRNF(NMe)GA(NMe)ILS	2467.43	2467.62[1g]
2b[1g]	CGFLGGRRRGFGGIGGC	1694.88	1695.27
2e[1g]	cGflGGrrrGfGGIGGc	1694.88	1695.04
4Ala-2b[1g]	CGAAGGRRRGAGGAGGC	1458.65	1459.12
αSyn(1-14)	MDVFMKGLSKAKEG	1539.80	1539.85
αSyn(34-52)	KEGVLYVGSKTKEGVVHGV	1985.12	1985.71
αSyn(87-105)	SIAAATGFVKKDQLGKNEE	2005.07	2005.25

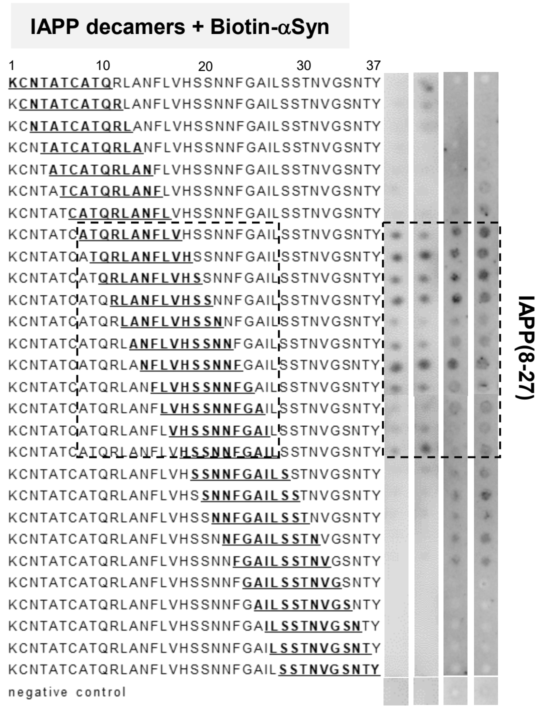
[a] M, monoisotopic mass

Supporting Table S2. Sequences, abbreviations, and molecular weights (M) of synthesized and studied N^{α}-terminal fluorescein-labeled peptides (Fluos-peptide) by MALDI-TOF-MS. All peptides are C-terminal amides. Fluos-IAPP contains a disulfide bridge between Cys residues 2 and 7 while Fluos-**2b**, Fluos-**2e** and Fluos-**4Ala-2b** contain a disulfide bridge between their N- and C-terminal Cys residues.

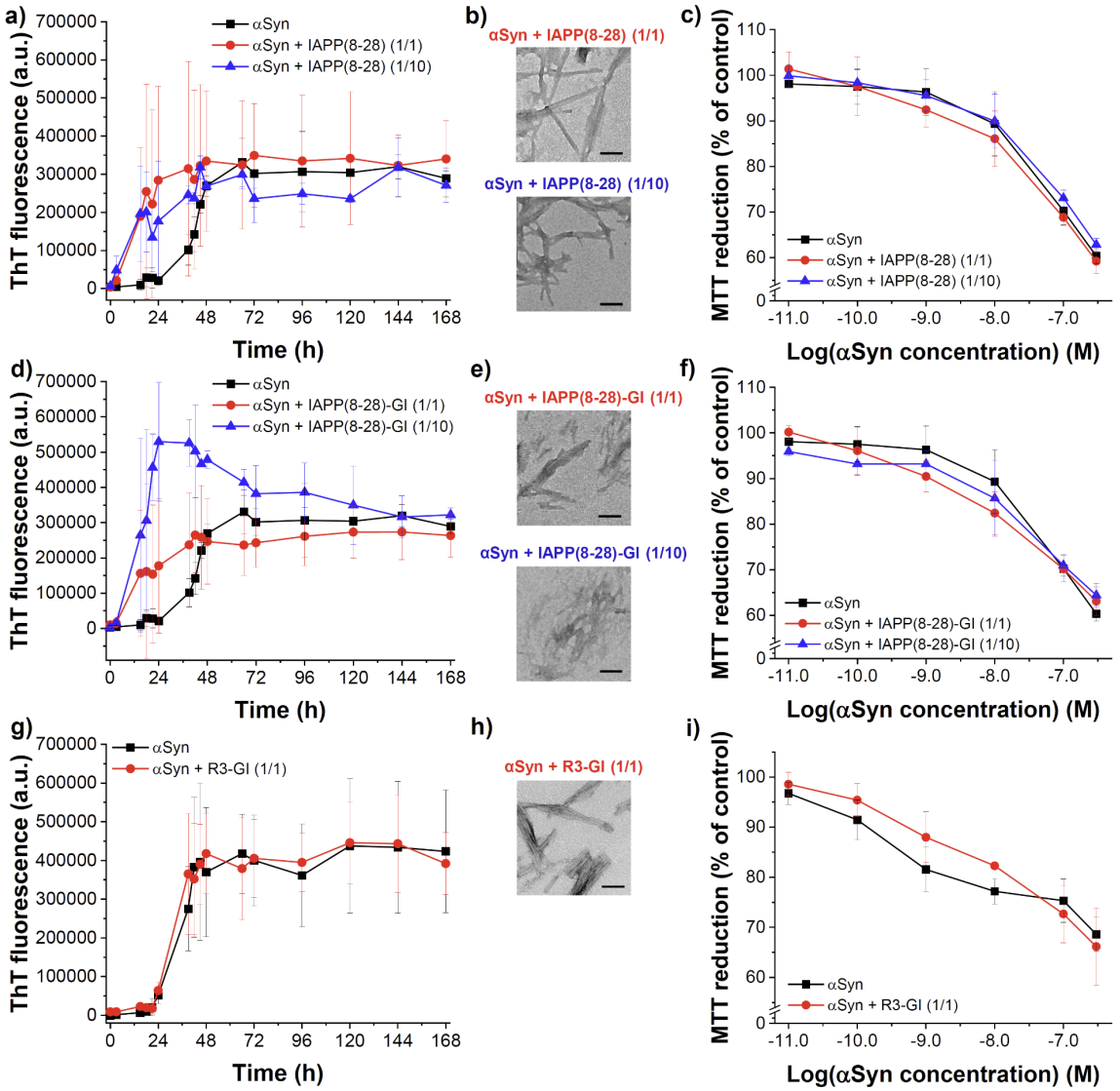
Abbreviation	Sequence	$[M+H]^+$ calcu-lated[a]	$[M+H]^+$ found[a]
Fluos-IAPP[1f]	Fluos-KCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTY	4260.21	4259.81
Fluos-IAPP(8-28)[1c]	Fluos-ATQRLANFLVHSSNNFGAILS	2617.52	2617.84
Fluos-2b[1g]	Fluos-CGFLGGRRRGFGGIGGC	2053.20	2052.89
Fluos-2e[1g]	Fluos-cGflGGrrrGfGGIGGc	2053.20	2053.19
Fluos-4Ala-2b[1g]	Fluos-CGAAGGRRRGAGGAGGC	1816.97	1817.01
Fluos-αSyn(1-14)	Fluos-MDVFMKGLSKAKEG	1898.12	1898.79
Fluos-αSyn(34-52)	Fluos-KEGVLYVGSKTKEGVVHGV	2343.44	2343.45
Fluos-αSyn(87-105)	Fluos-SIAAATGFVKKDQLGKNEE	2363.39	2363.41

[a] M, monoisotopic mass

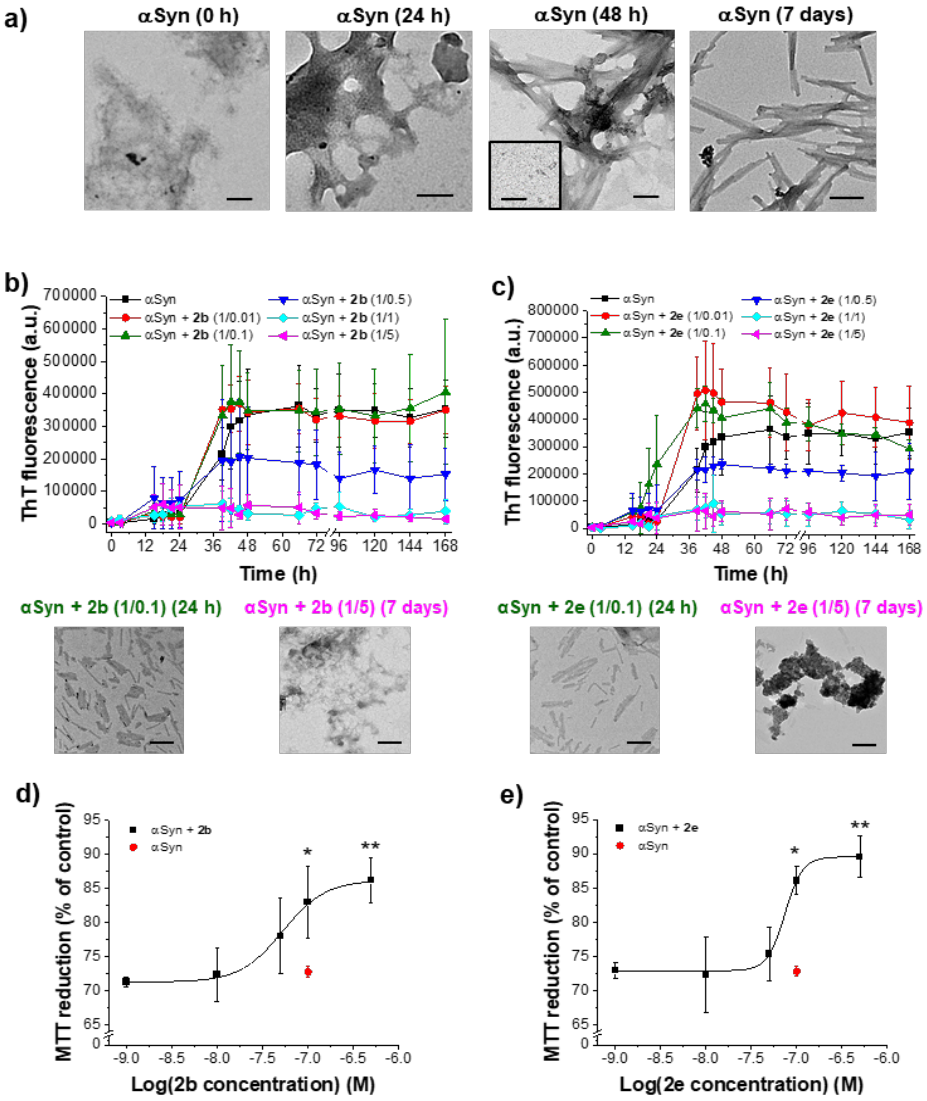
Supporting Figures S1-S25



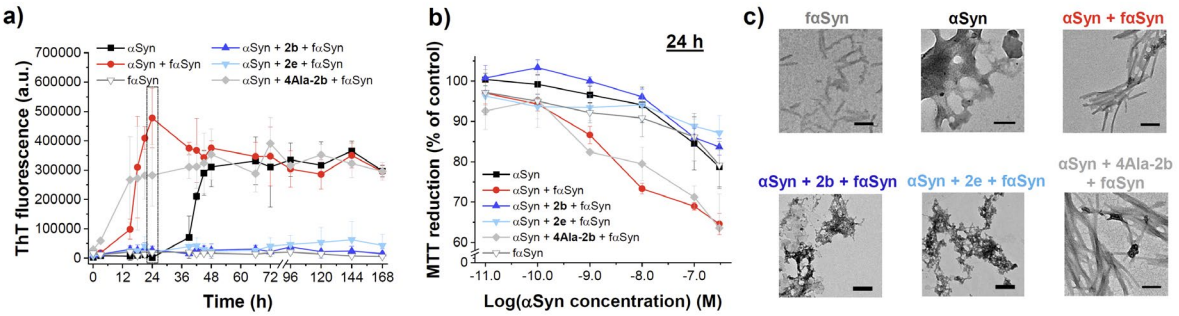
Supporting Figure S1. Identification of IAPP regions that interact with αSyn using peptide arrays. Shown are 4 arrays consisting of IAPP decamers covering full-length IAPP and positionally shifed by one residue (bold, underlined) following incubation with Biotin-αSyn (0.5 μM) and visualization of bound peptide by ECL. The dashed squares indicate the IAPP(8-27) region containing the IAPP decamers which bound Biotin-αSyn. Data is from 2 independent incubations/ developments of 2 arrays synthesized in parallel.



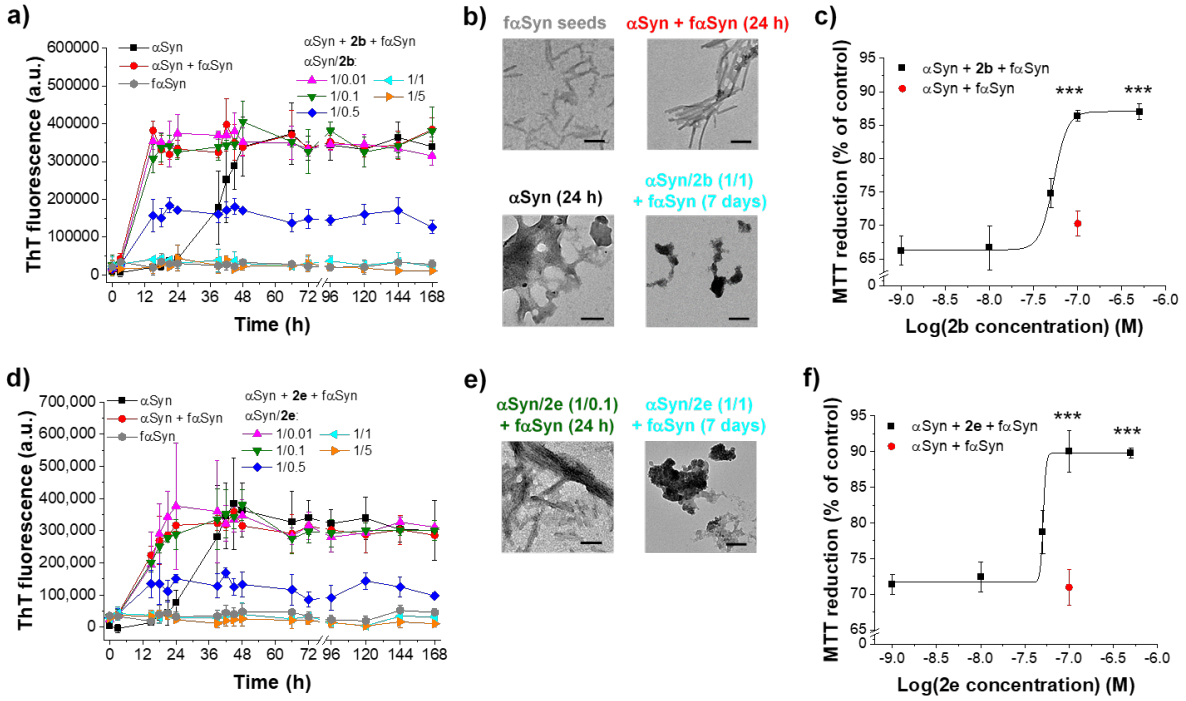
Supporting Figure S2. IAPP(8-28) and its non-amyloidogenic linear peptide analogs IAPP(8-28)-GI and R3-GI are unable to interfere with αSyn amyloid assembly and cell-damaging effects. a,d,g) Fibrillogenesis of αSyn (3 μM) alone and in the presence of IAPP(8-28) (αSyn/peptide 1/1 or 1/10) (a), IAPP(8-28)-GI (αSyn/peptide 1/1 or 1/10) (d), and R3-GI (αSyn/peptide 1/1) (g) as determined by the ThT binding assay (means (±SD), 3 assays with n=3 wells each). b,e,h) TEM images of solutions (24 h-aged) from (a), (d), and (g) as indicated (scale bars, 100 nm). c,f,i) Effects of αSyn alone and its mixtures (7 day-aged) with IAPP(8-28) (c), IAPP(8-28)-GI (f), and R3-GI (i) on PC12 cell viability. Solutions from the ThT binding assays of (a), (d), and (g) were added to the cells at the indicated final concentrations and cell viability was determined by the MTT reduction assay (means (±SD), 3 assays with n=3 wells each).



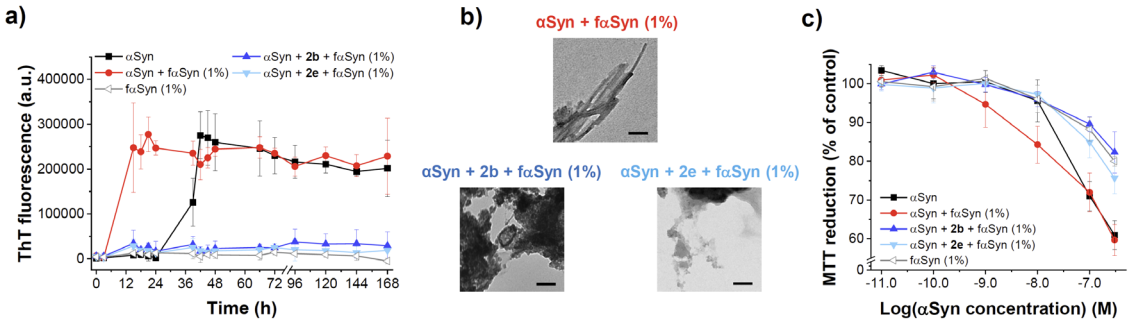
Supporting Figure S3. Concentration-dependence of the inhibitory effects of **2b** and **2e** on αSyn fibrillogenesis (a-c) and related cell-damaging effects (c,d). a) Kinetics of αSyn fibrillogenesis as followed by TEM confirm that fibrils which were absent in 0 and 24 h-aged but major species in 48 h-aged αSyn solutions consistent with the ThT binding assay results. αSyn solutions (3 μM) from a ThT binding assay (Fig. 2a) were examined by TEM at the indicated incubation time points; scale bars 100 nm. Of note, the TEM image of 7 day-aged αSyn is also shown in Fig. 2b but is included here for comparison. b,c) Top, fibrillogenesis of αSyn (3 μM) alone and in the presence of various amounts of **2b** (b) or **2e** (c) as determined by the ThT binding assay (means (±SD), 3 assays with n=3 wells each). Bottom, TEM images of αSyn co-incubations with **2b** or **2e** from (b) and (c) at αSyn/peptide molar ratios of 1/0.1 (no inhibition) or 1/5 (inhibition) and incubation time points of 24 h or 7 days as indicated (scale bars, 100 nm). d,e) Determination of IC_{50} values of effects of **2b** and **2e** on formation of cell-damaging αSyn aggregates. Aged solutions (7 days) of αSyn (100 nM) alone or its mixtures with several amounts of **2b** (solutions from (b)) (d) or **2e** (solutions from (c)) (e) were added to PC12 cells at the indicated final concentrations and cell damage was determined by the MTT reduction assay. Data of αSyn alone are also shown for comparison (red symbols). Data and IC_{50} values (Table 1) means (±SD) from 3 assays with n=3 wells each. Statistical significance of the differences of the effects of αSyn/peptide mixtures at 1/1 and 1/5 ratios (plateau values) *versus* αSyn was determined using one-way ANOVA with Tukey test (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001; p values <0.05 were considered significant).



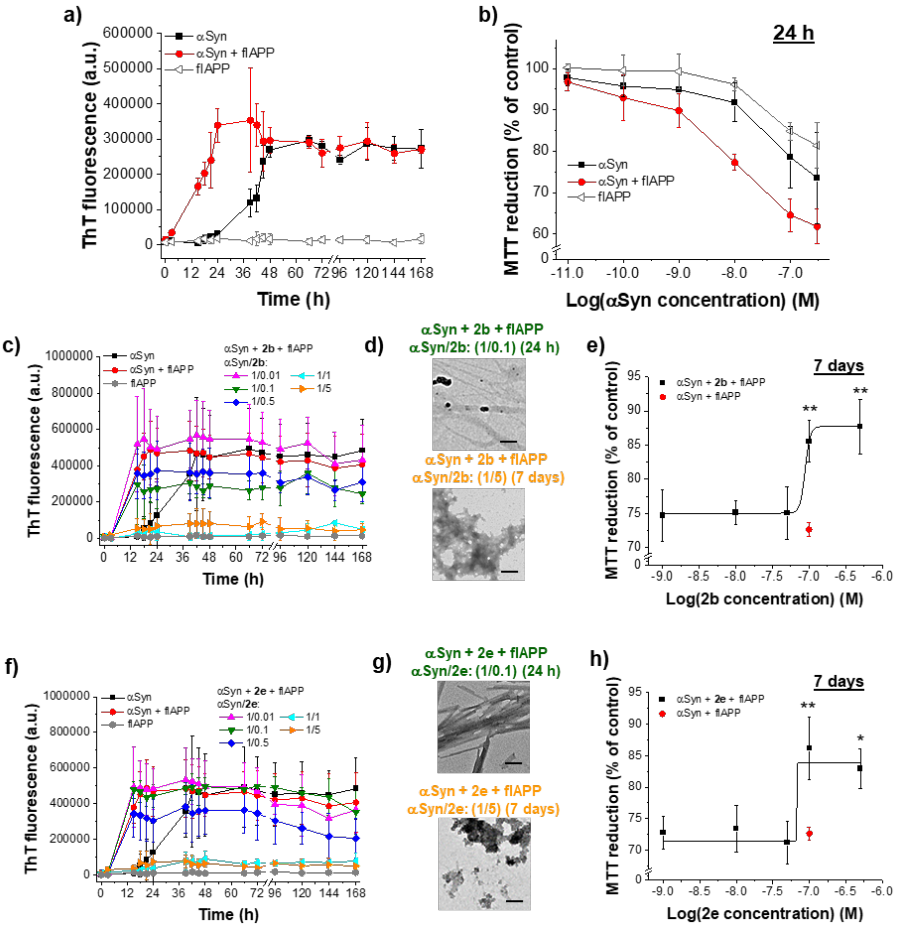
Supporting Figure S4. Seeding of αSyn with fαSyn (10%) results in acceleration of formation of both αSyn fibrils (a,c) and cell-damaging assemblies (b) and is fully suppressed in the presence of **2b** and **2e** (a-c) whereas **4Ala-2b** is unable to inhibit. a) Fibrillogenesis of αSyn (3 μM) alone, or seeded by fαSyn (10%), or seeded with fαSyn (10%) in the presence of **2b**, **2e** (3 μM), or **4Ala-2b** (150 μM) as determined by ThT binding (means (±SD), 3 assays with n=3 wells each). Data of Supporting Fig. S4a is also shown in Fig. 2d but it is presented here as well to accompany data shown in (b) and (c) for which the 24 h aged solutions of Fig. 2d were used; 24 h incubation time point indicated by a rectangle. b) Formation of cell-damaging assemblies in 24 h-aged solutions of αSyn (3 μM) alone, or seeded by fαSyn (10%), or seeded with fαSyn (10%) in the presence of **2b**, **2e** (3 μM), or **4Ala-2b** (150 μM). Solutions of (a) (24 h-aged) were added to PC12 cells at the indicated final concentrations and cell damage was determined by the MTT reduction assay (means (±SD), 3 assays, n=3 wells each). c) TEM images of solutions from (a) aged for 24 h and of the applied fαSyn seeds as indicated (scale bars, 100 nm).



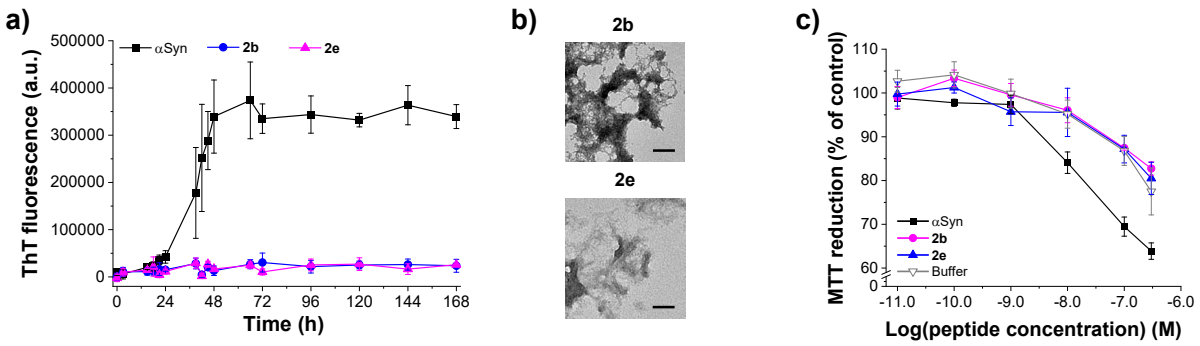
Supporting Figure S5. Concentration-dependence of inhibitory effects of **2b** and **2e** on fαSyn-seeded αSyn amyloid assembly and cytotoxicity (fαSyn, 10%). a,d) Fibrillogenesis of αSyn (3 μM) alone, αSyn seeded by preformed fαSyn, and αSyn seeded by fαSyn in the presence of different amounts of **2b** (a) or **2e** (d) (αSyn/peptide molar ratios as indicated) was followed by the ThT binding assay (means (±SD), 3 assays with n=3 wells each). Data of fαSyn alone is also shown for comparison. b,e) TEM images of solutions from (a) or (d) as indicated (scale bars, 100 nm). c,f) Determination of the IC_{50} values of effects of **2b** (c) or **2e** (f) on formation of cytotoxic αSyn aggregates. Solutions of fαSyn-seeded αSyn (100 nM) alone or its mixtures with several amounts of **2b** (c) or **2e** (f) (7 day-aged; solutions from (a) and (d)) were added to PC12 cells at the indicated final concentrations and cell damage was assessed by the MTT reduction assay. Data of fαSyn-seeded αSyn are also shown (red symbols). Data and IC_{50} values (Table 1) means (±SD) from 3 assays with n=3 wells each. Statistical significance of differences of the effects of αSyn/peptide mixtures at 1/1 and 1/5 ratios (plateau values) *versus* fαSyn-seeded αSyn was determined using one-way ANOVA with Tukey test (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001; p values <0.05 were considered significant).



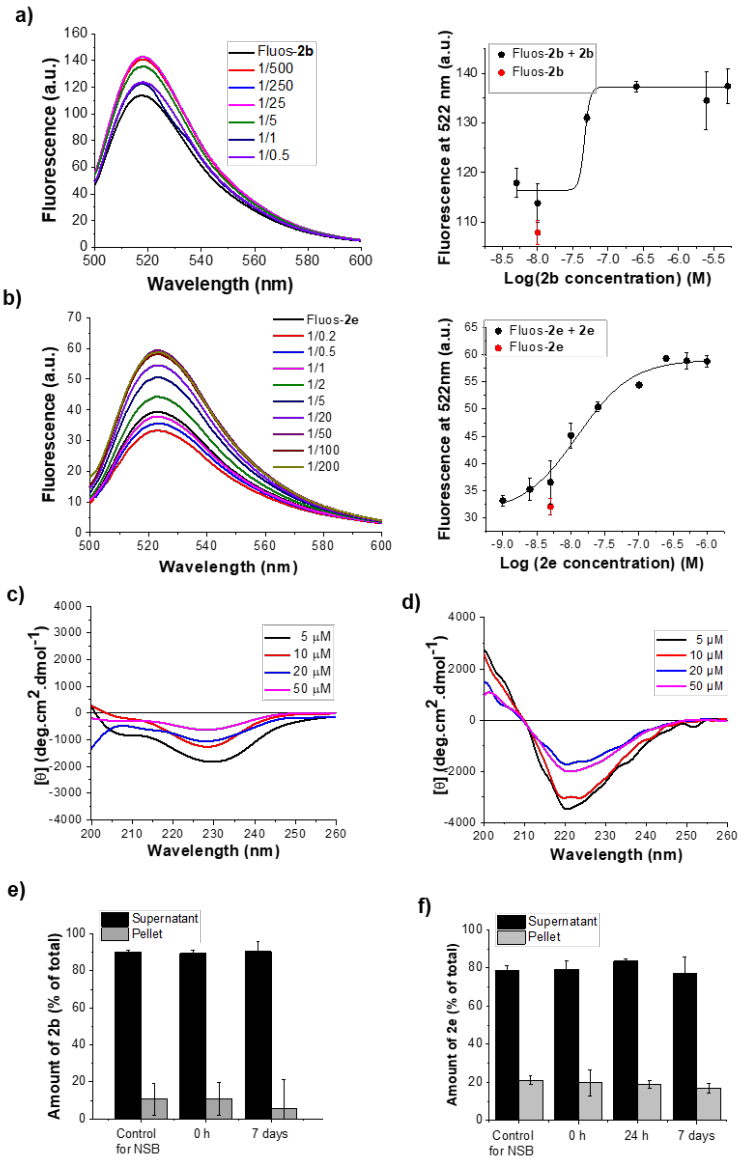
Supporting Figure S6. Effects of **2b** and **2e** on amyloid assembly and cell-damaging effects of fαSyn-seeded αSyn (fαSyn, 1%). a) Fibrillogenesis of αSyn (3 μM), fαSyn-seeded αSyn, and fαSyn-seeded αSyn in the presence of **2b** and **2e** (3 μM) followed by ThT binding assay (means (±SD), 3 assays with n=3 wells each). Data of fαSyn seeds alone is also shown. b) TEM images of solutions from (a) aged for 24 h (αSyn + fαSyn seeds; mostly fibrils) or 7 days (seeded αSyn mixtures with peptides; mostly amorphous aggregates) (scale bars, 100 nm). c) Effects of αSyn, αSyn seeded by fαSyn, and αSyn seeded by fαSyn (1%) in the presence of **2b** and **2e** (1/1) on PC12 cell viability. Aged solutions from (a) (7 day-aged) were added to PC12 cells at the indicated final concentrations and cell viability was determined by the MTT reduction assay (means (±SD), 3 assays with n=3 wells each).



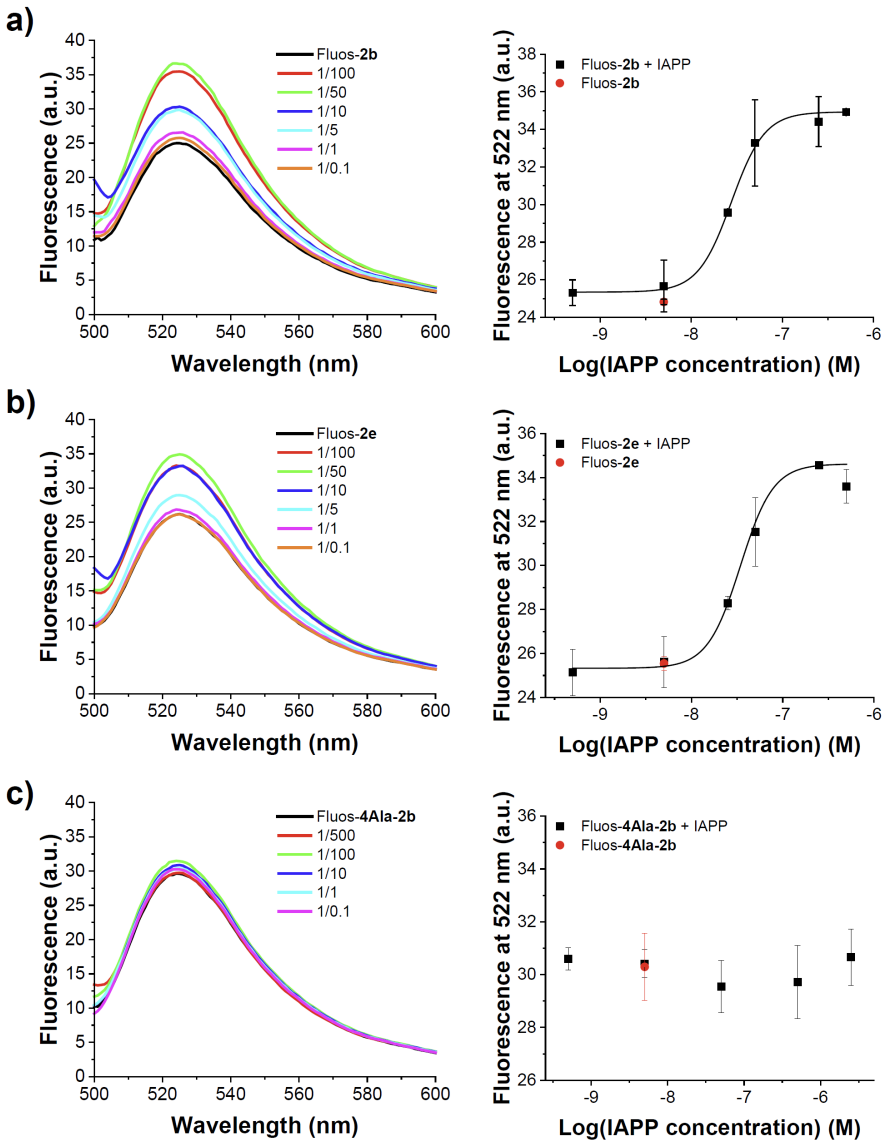
Supporting Figure S7. flAPP-mediated cross-seeding of αSyn amyloid self-assembly and formation of cell-damaging assemblies (a,b) and concentration dependence of the inhibitory effects of **2b** and **2e** (c-h). a) Fibrillogenesis of αSyn (3 μM), αSyn cross-seeded by preformed flAPP (10%), and flAPP used for cross-seeding (control) determined by the ThT binding assay (means (±SD), 3 assays, n=3 wells each). Of note, data shown in (a) is also shown in Fig. 2g; data is shown here as well to accompany the cell viability data shown in (b). b) PC12 cell viability after treatment with solutions from (a) (or Fig. 2g) aged for 24 h (final concentrations as indicated) determined by the MTT reduction assay (means (±SD), 3 assays (3 wells each)). The difference between the cytotoxicity of 24 h-aged αSyn alone and flAPP-cross-seeded αSyn indicates that flAPP cross-seeds both αSyn fibrillogenesis and formation of cell toxic assemblies. c,f) Fibrillogenesis of αSyn (3 μM) alone, αSyn cross-seeded by flAPP (10%), and αSyn cross-seeded by flAPP in the presence of various amounts of **2b** (c) or **2e** (f) as indicated determined by the ThT binding assay (means (±SD), 3 assays, n=3 wells each). (d,g) TEM images of aged solutions from (c) and (f) as indicated show fibrils as major species at the non-inhibitory αSyn/peptide molar ratio of 1/0.1 and amorphous aggregates as major species at the inhibitory ratio of 1/5 (scale bars, 100 nm). e,h) Determination of IC_{50} values of the effects of **2b** (e) and **2e** (h) on flAPP-mediated cross-seeding of αSyn cytotoxicity. Solutions of flAPP-cross-seeded αSyn (100 nM) alone or its mixtures with several amounts of **2b** (e) or **2e** (h) (7 day-aged) were added to PC12 cells and cell damage was assessed by the MTT reduction assay. Data of flAPP-cross-seeded αSyn is also shown (red symbols). Data and IC_{50} values (see Table 1) means (±SD) from 3 assays with n=3 wells each. Statistical significance of differences of the effects of αSyn/peptide mixtures at 1/1 and 1/5 ratios (plateau values) *versus* flAPP-seeded αSyn was determined using one-way ANOVA with Tukey test (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001; p values <0.05 were considered significant).



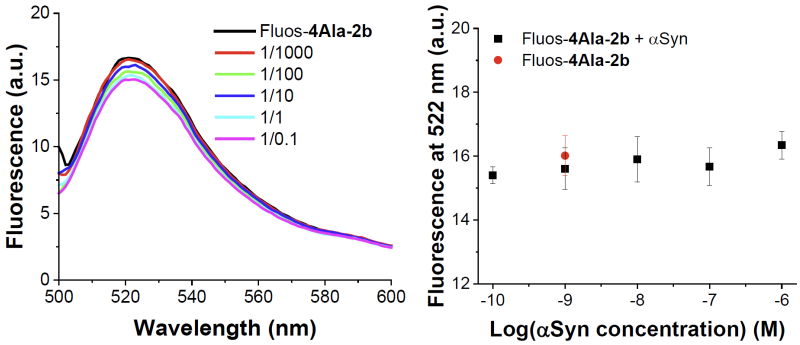
Supporting Figure S8. Peptides **2b** and **2e** are non-amyloidogenic and non-cytotoxic at least up to 200-fold higher concentrations than the IC_{50} values of their effects on αSyn cytotoxicity. a) Fibrillogenesis of αSyn (3 μM) and of **2b** or **2e** alone (15 μM) as determined by the ThT binding assay (means (±SD), 3 assays (3 wells each)). b) TEM images of solutions of MCIPs (7 day-aged) from (a) as indicated (scale bars, 100 nm). c) PC12 cell viability after treatment with (7 day-aged) αSyn, **2b**, and **2e** solutions from (a) and buffer alone as determined by the MTT reduction assay (means (±SD), 3 assays (3 wells each)).



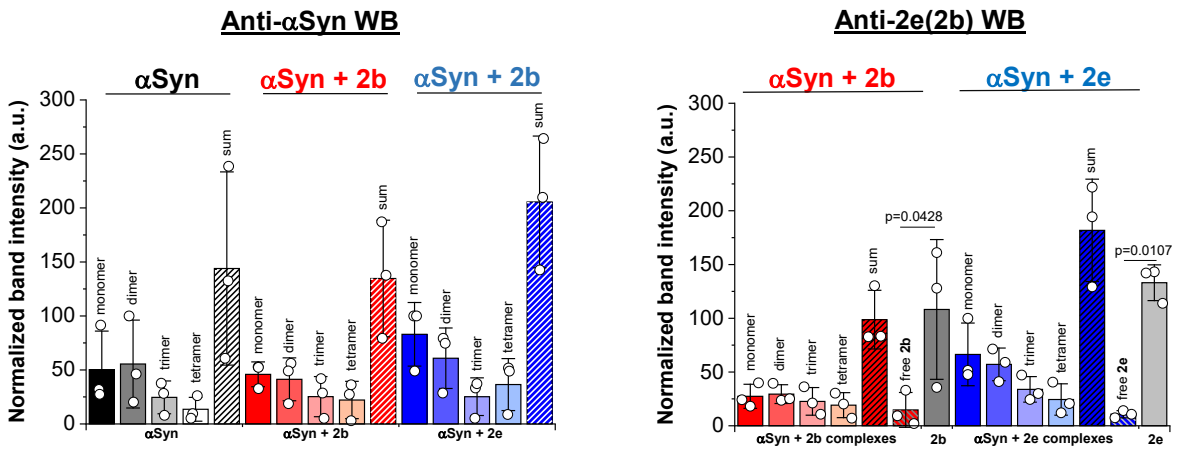
Supporting Figure S9. Self-assembly of MCIPs **2b** and **2e** into soluble oligomers as studied by fluorescence spectroscopic titrations (a,b), CD spectroscopy (c,d), and a sedimentation assay (e,f). (a & b) Left, fluorescence emission spectra of Fluos-**2b** (10 nM) (a) or Fluos-**2e** (5 nM) (b) alone or with varying amounts of **2b** (a) or **2e** (b) (Fluos-peptide/peptide molar ratios as indicated). Right, binding curves; determined app. K_d values of self-assembly were 43 (±3) nM (**2b**) and 11.6 (±1.8) nM (**2e**) (data means (±SD) of 3 titration assays; data of Fluos-peptide alone is shown for comparison). (c,d) Far-UV CD spectra of **2b** (c) and **2e** (d) at various different concentrations (5-50 µM) are shown as indicated (pH 7.4). Loss of signal with increasing concentration indicates oligomerization; no turbidity or precipitation was observed. (e,f) Solubility of **2b** at 500 µM (e) and **2e** at 1 mM (f) (aq. buffer, pH 7.4) as determined by a sedimentation assay. Peptides were incubated for 7 days and following centrifugation peptide amounts in supernatants and pellets were quantified by the BCA assay. Data indicates that **2b** is soluble up to a concentration of at least 500 µM and **2e** up to a concentration of at least 1 mM (NSB 5-20% of total amount); data means (±SD) from 3 assays.



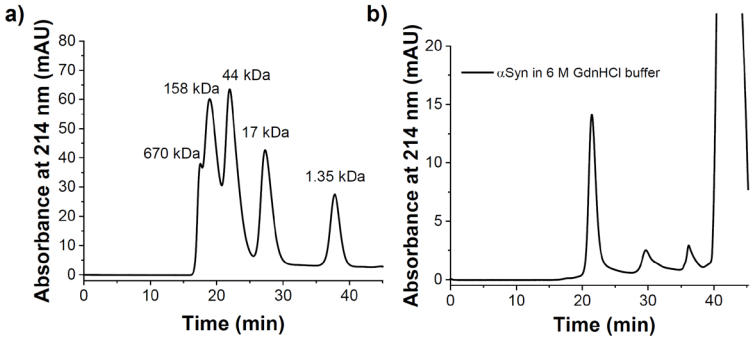
Supporting Figure S10. Fluorescence spectroscopic titration studies on the interactions of **2b**, **2e**, and **4Ala-2b** with IAPP. a-c) Left, fluorescence emission spectra of Fluos-**2b** (a), Fluos-**2e** (b), and Fluos-**4Ala-2b** (c) (5 nM) alone or with varying amounts of IAPP; Fluos-peptide/IAPP molar ratios as indicated. Data representative from one assay out of three. Right, binding curves (a,b) or plot of the fluorescence emission values at 522 nm of the Fluos-**4Ala-2b**/IAPP mixtures (c) which is indicative of no interaction (IAPP≤2.5 μM); data of Fluos-peptide alone is also shown for comparison. Data is means (±SD) of 3 titration assays; determined app. K_ds are in Table 2.



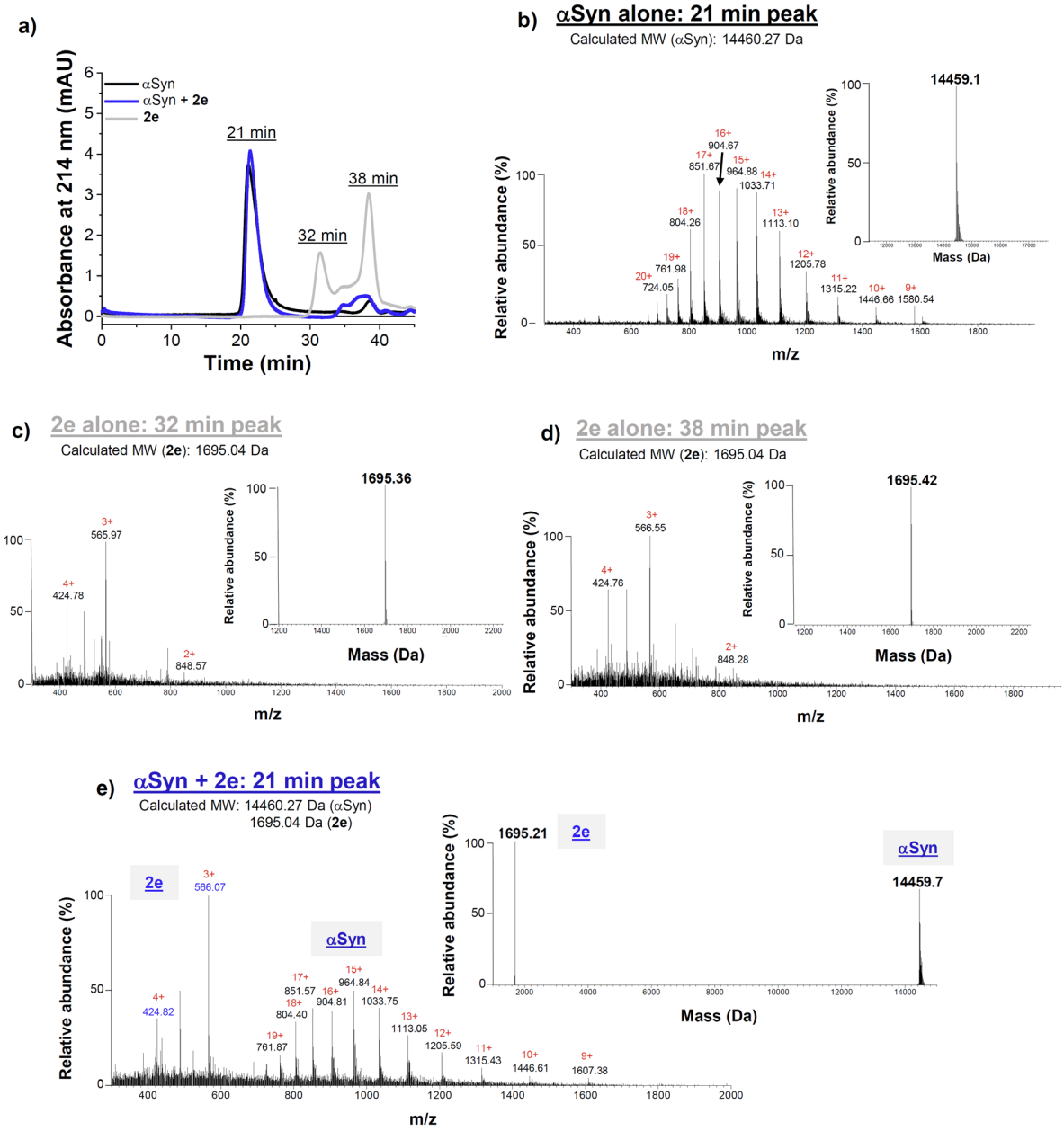
Supporting Figure S11. Fluorescence spectroscopic titration studies on the interaction of Fluos-**4Ala-2b** with αSyn. Left, fluorescence emission spectra of Fluos-**4Ala-2b** (1 nM) alone and with varying amounts of αSyn (Fluos-peptide/αSyn molar ratios as indicated). Data representative from one assay out of three. Right, plot of the fluorescence emission values at 522 nm of the Fluos-peptide/αSyn mixtures indicates no interaction between αSyn and **4Ala-2b** (αSyn≤1 μM); data of Fluos-peptide alone are also shown for comparison. Data is means (±SD) of 3 titration assays.



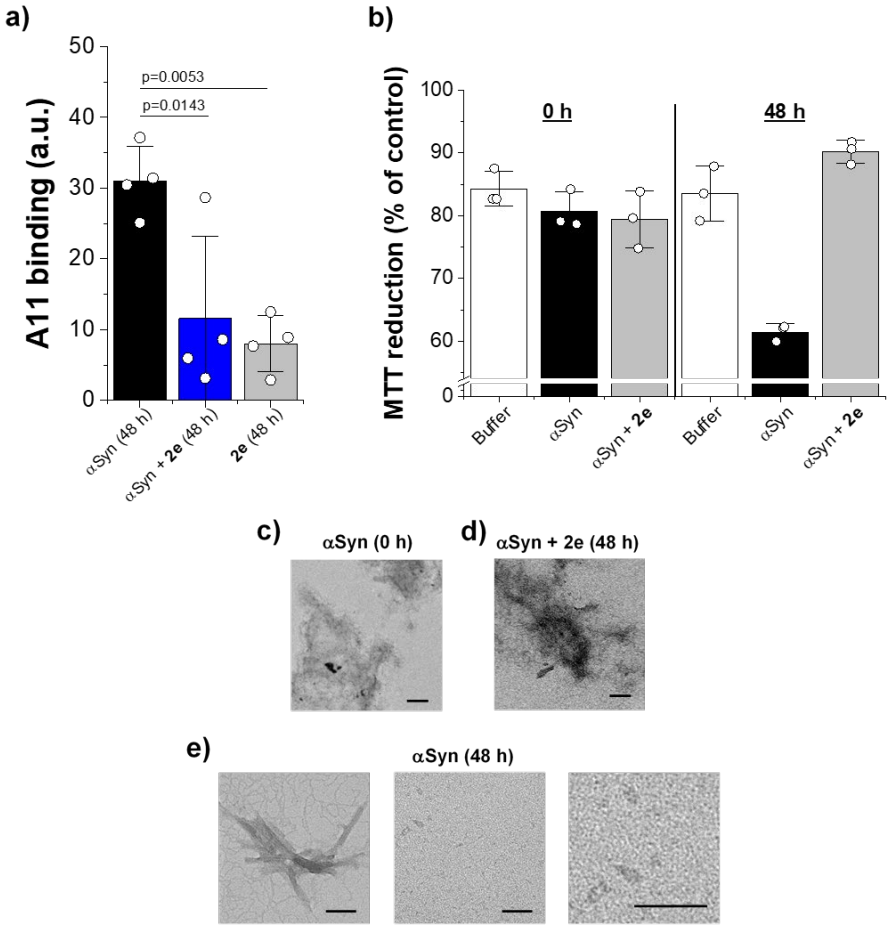
Supporting Figure S12. Densitometric quantification and statistical analysis of bands of αSyn, **2b**, and **2e** in Western blots (WBs) originating from the cross-linking assay shown in Fig. 3e and additional two cross-linking assays (data not shown). Left, normalized intensity of bands corresponding to αSyn monomers and its various different self-/co-assembly states (as indicated) in cross-linked solutions of αSyn alone or its mixtures with **2b** or **2e** in WBs developed with the anti-αSyn antibody; the sums of all bands for αSyn alone and its mixtures with each of the peptides are also shown for comparison. Data is means (±SD) from 3 WBs with samples from 3 different cross-linking assays. No statistically significant differences between the band intensities of αSyn monomers & different assembly states (or their sums) in αSyn alone and its mixtures were observed (n=3, one-way ANOVA with Tukey test; p-values not shown; p values <0.05 were considered significant). Right, normalized intensity of bands corresponding to the various different αSyn co-assembly states (as indicated) in cross-linked solutions of mixtures of αSyn with **2b** or **2e** in WBs developed with the anti-**2e**(**2b**) antibody. The sums of all bands for the mixtures αSyn with each of the two peptides are also shown as indicated. The bars “free **2b**” or “free **2e**” correspond to the “remaining” peptide amounts, i.e. peptide which was not incorporated in the αSyn/**2b**(**2e**) hetero-complexes. Data is means (±SD) from 3 WBs with samples from the same 3 cross-linking assays as for the left part of the figure. No statistically significant differences between the band intensities corresponding to the different αSyn/peptide co-assembly states (or sums thereof) were found (p-values indicating no statistical significance are not shown; p values <0.05 were considered significant). However, a statistically significant difference ($p<0.05$) was found between the band intensities of the sums of the bands corresponding to αSyn/**2b**(**2e**)-containing hetero-complexes and the bands corresponding to “free” **2b** or **2e** as indicated (n=3, one-way ANOVA with Tukey test; p-values as indicated) consistent with significant amounts of the two peptides co-assembling with αSyn.



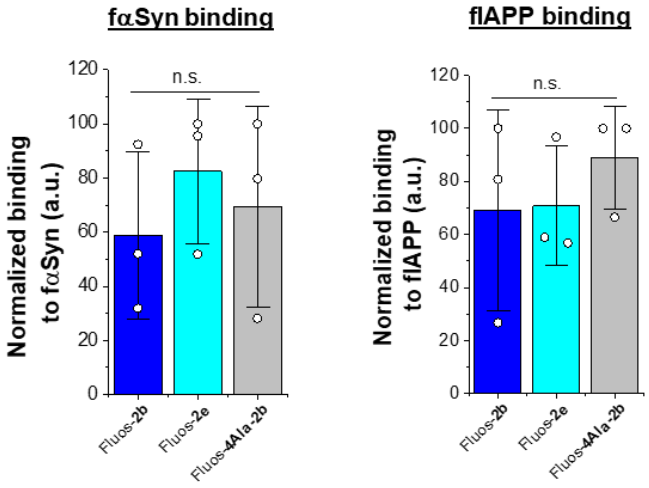
Supporting Figure S13. Calibration of the SEC column (Superdex 75 10/300 GL) used for studies shown in Fig. 3f and Supporting Fig. S14 and determination of the retention time (t_R) of αSyn monomers. a) Chromatogram of the gel filtration standard; MWs as indicated. b) Chromatogram of αSyn monomers (αSyn, 3 µM) in aq. 6 M GdnHCl; the major fraction of αSyn elutes at 21 min; the peak at 40-45 min is due to GdnHCl.



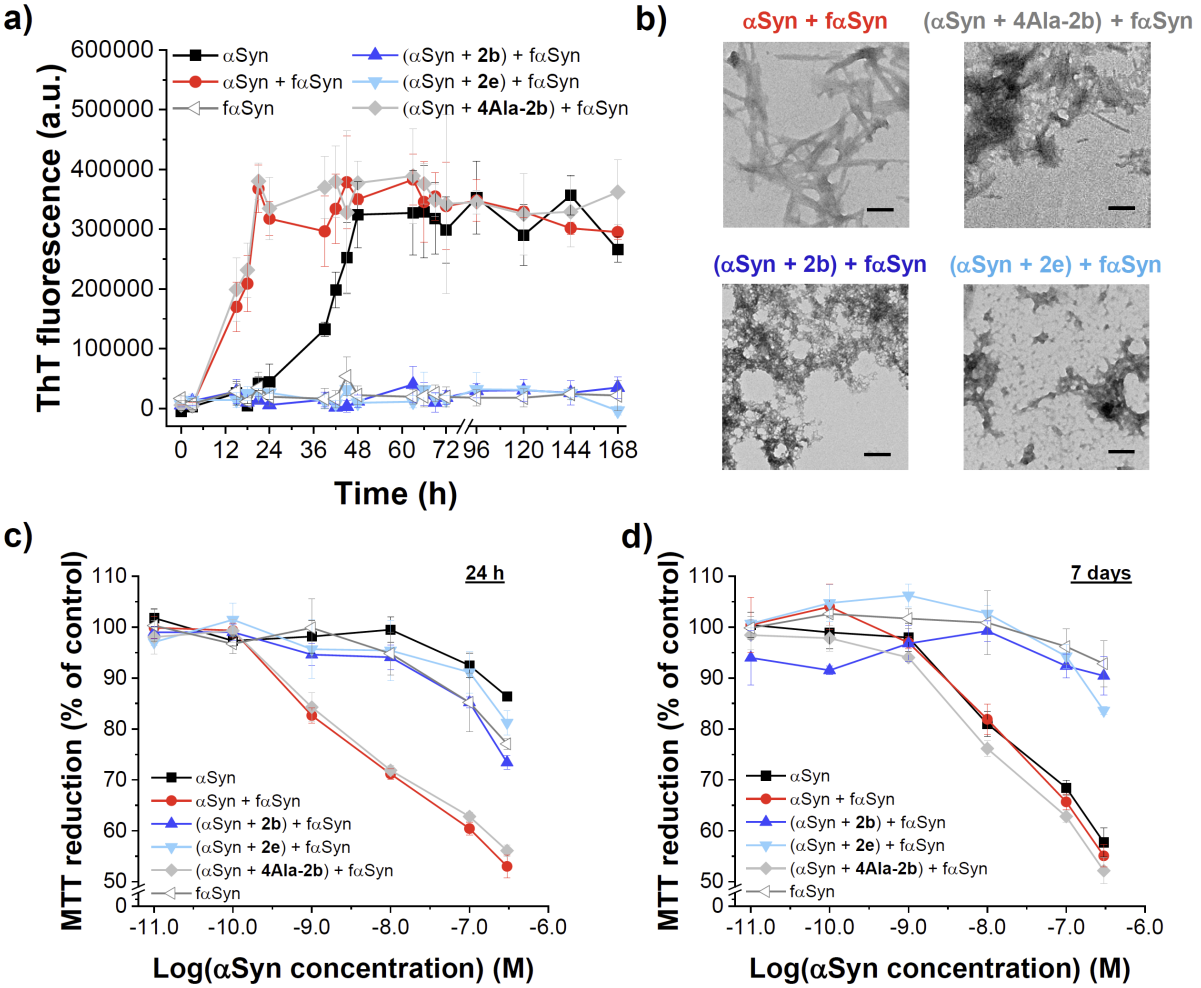
Supporting Figure S14. ESI-MS analysis of peaks collected from SEC of αSyn alone, **2e** alone, and the αSyn/**2e** mixture (from Fig. 3f) provides evidence for αSyn/**2e** hetero-complex formation. a) Chromatograms of αSyn (3 μM), **2e** (30 μM), and the αSyn/**2e** (1/10) mixture (also shown in Fig. 3f); retention times (t_R) of peaks collected for ESI-MS analysis are shown above the peaks. b-d) ESI-MS spectra of main peaks of αSyn (b), **2e** (c,d), and the αSyn/**2e** mixture (e) (see also inset of Fig. 3f); in (e) determined m/z shown with black (αSyn) or blue (**2e**) letters; charge states shown with red letters. Insets, deconvoluted spectra; the determined mass is shown; calculated average mass (MW) as indicated.



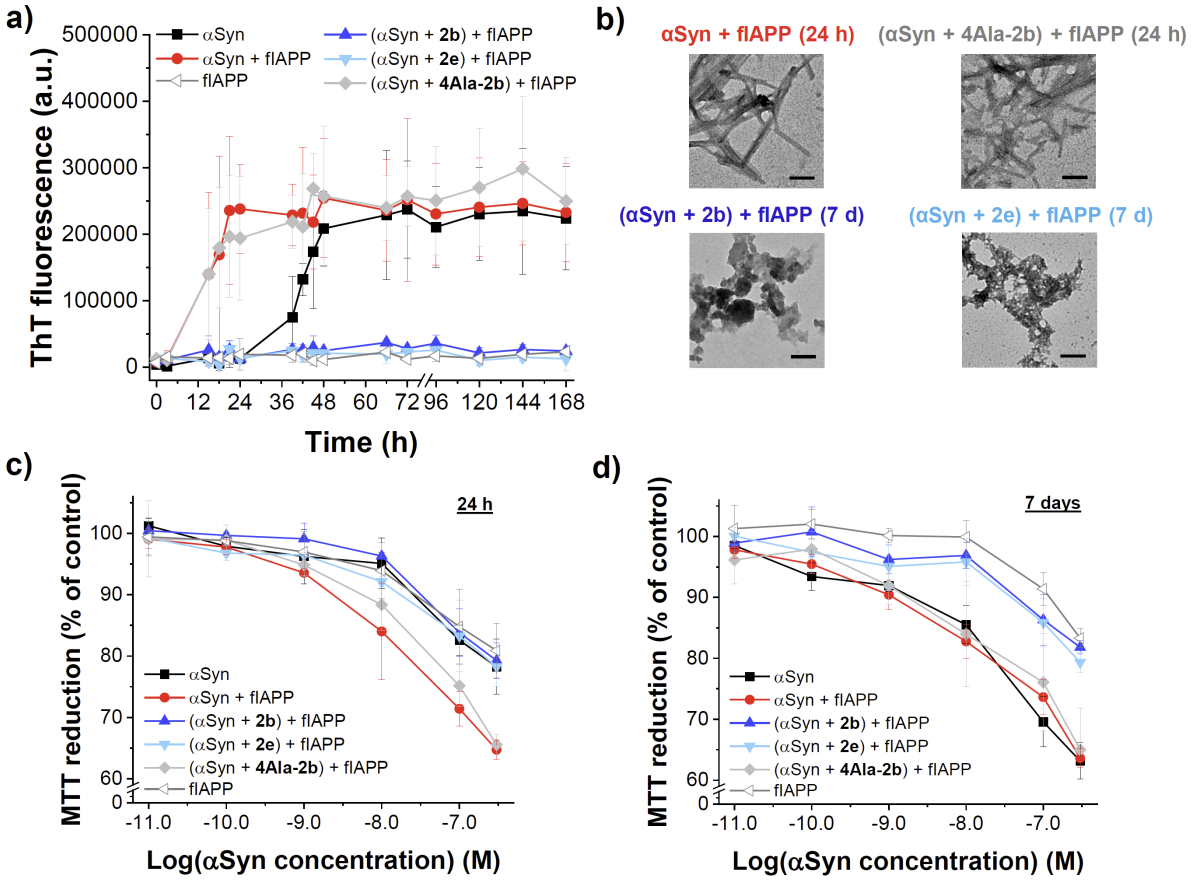
Supporting Figure S15. Confirmation of the presence of cytotoxic oligomers in A11-reactive 48 h-aged αSyn solutions (3 μM) and of their absence in non-A11-reactive αSyn (0 h-aged) and αSyn/**2e** (1/1; 48 h-aged) solutions. a) Densitometric quantification of A11-reactive species in solutions analyzed by the slot blot shown in Fig. 3g and by additional 3 slot blots (data not shown) reveals statistically significant amounts of A11-reactive oligomers in 48 h-aged αSyn alone in comparison to 48-h aged αSyn/**2e** (1/1) mixtures. Data means (±SD) from 4 slot blots analyses of 4 different sample preparations (n=4, one-way ANOVA with Tukey test; p-values as indicated; p values <0.05 were considered significant). b) Effects of 0 h- or 48 h-aged solutions containing buffer alone, αSyn alone, and αSyn/**2e** (1/1) on PC12 cell viability assessed by the MTT reduction assay (αSyn, 300 nM). Left side, data of 0 h-aged solutions; right side, data of 48 h-aged solutions (made as the solutions of Fig. 3g) as indicated. Data is means (±SD) from one representative assay out of 2 (n=3 wells each). c-e) TEM micrographs indicate amorphous aggregates as major species in 0 h-aged αSyn solutions (c) and in 48 h-aged αSyn/**2e** mixtures (d), and mixtures of oligomers and fibrils in 48 h-aged αSyn solutions (e) (solutions from (b)) (scale bars, 100 nm).



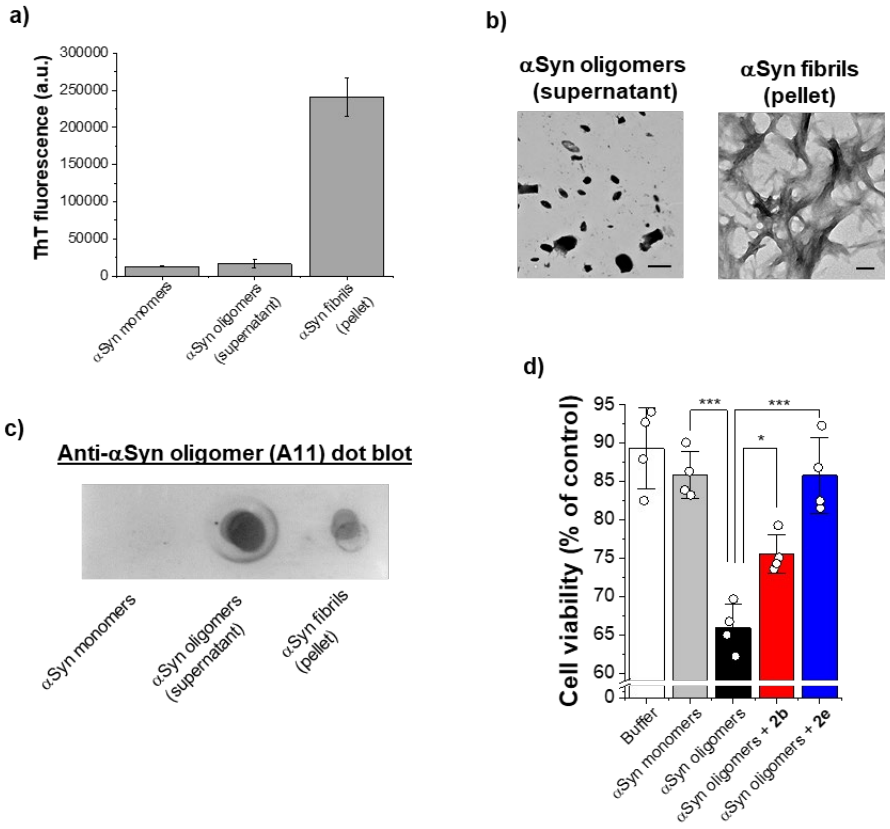
Supporting Figure S16. Binding of Fluos-**2b,** Fluos-**2e** and Fluos-**4Ala-2b** to αSyn fibrils (fαSyn) or IAPP fibrils (fIAPP) as assessed by densitometric quantification of dot intensities of the dot blot assay of Fig. 3h and two additional dot blots (data not shown). The data indicate no statistically significant (n.s.) differences between the three peptides. Data is means (±SD) from 3 dot blot analyses (each of them from a different sample preparation) (n=3, one-way ANOVA and Tukey-test). P-values not shown; p values <0.05 were considered significant.



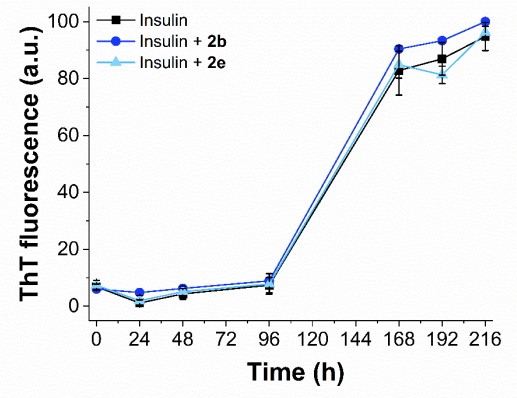
Supporting Figure S17. Formation of αSyn/**2b**(or **2e)** hetero-complexes protects αSyn from becoming seeded by preformed fαSyn. Addition of seed amounts of fαSyn to preformed αSyn/**2b**(or **2e)** hetero-complexes did not accelerate amyloid self-assembly and formation of cell-damaging aggregates but accelerated amyloid self-assembly when added to αSyn alone or αSyn/**4Ala-2b** mixtures. a) Fibrillogenesis of αSyn (3 μM), αSyn seeded by fαSyn (10%), and preformed αSyn/**2b**(or **2e)** hetero-complexes (1/1) following addition of fαSyn (10%) seeds as followed by the ThT binding assay. The effects of the addition of fαSyn seeds (10%) to mixtures of αSyn (3 μM) with the negative control **4Ala-2b** (150 μM) are also shown for comparison; the applied fαSyn seeds alone (controls) were studied in parallel. Data means (±SD) from one representative assay (n=3 wells) out of two. b) TEM images of 24 h-aged solutions from (a) as indicated reveal fibrils as major species in αSyn alone or αSyn/**4Ala-2b** seeded by fαSyn and amorphous aggregates as major species in αSyn/**2b**(or **2e**) hetero-complexes seeded with fαSyn seeds (scale bars, 100 nm). c,d) Effects on PC12 cell viability of 24 h-aged (c) or 7 day-aged (d) αSyn (3 μM), αSyn seeded by fαSyn, and αSyn/**2e**(or **2b**) (1/1) or αSyn/**4Ala-2b** (1/50) mixtures seeded with fαSyn (10%) seeds as determined by the MTT reduction assay; aged solutions from (a) were used. Effects of applied fαSyn seeds alone (controls) were studied in parallel. Data means (±SD) from one representative assay (n=3 wells) out of two.



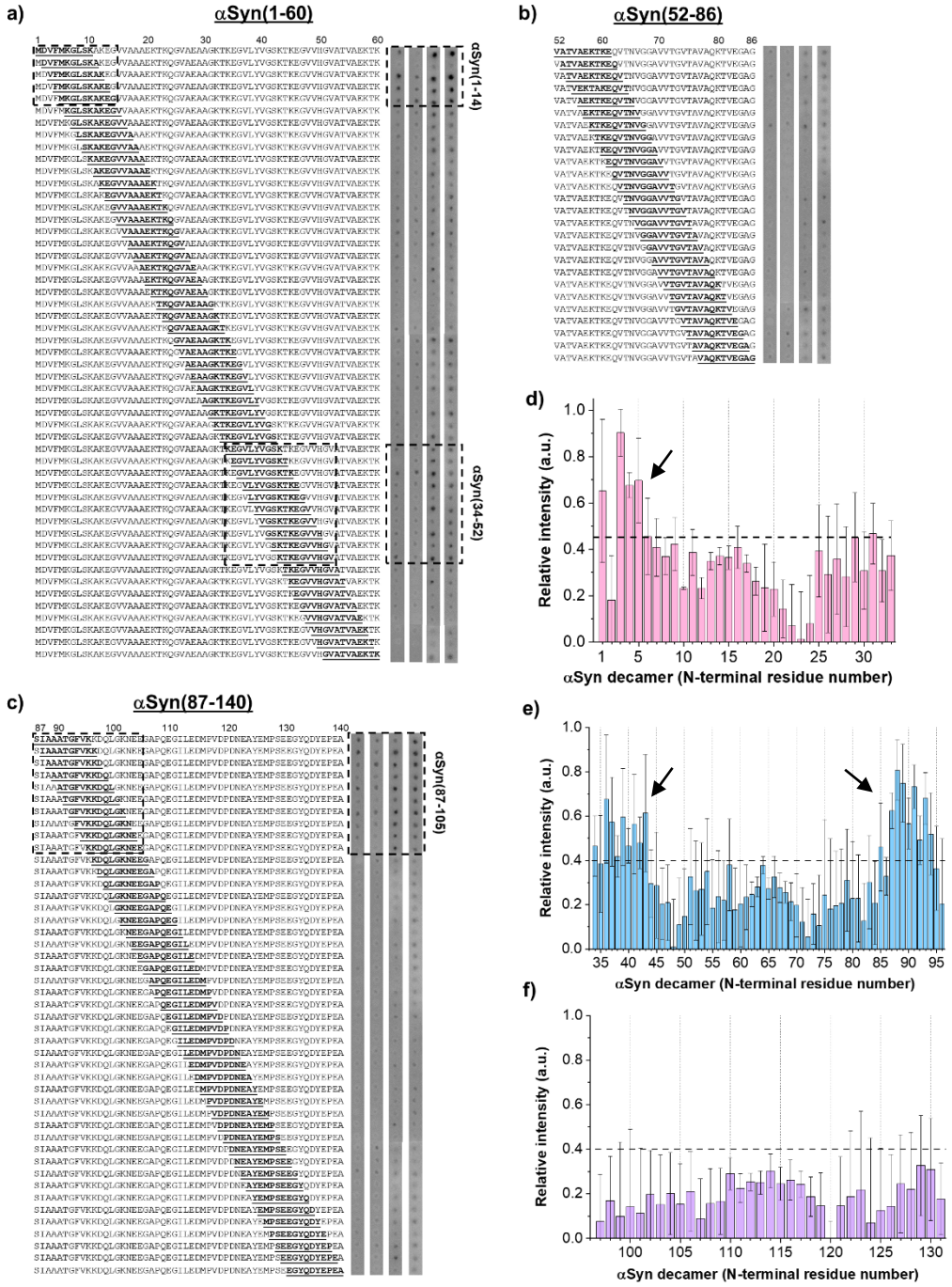
Supporting Figure S18. Formation of αSyn/**2b**(or **2e)** hetero-complexes protects αSyn from becoming cross-seeded by fIAPP seeds. Addition of seed amounts of fIAPP to preformed αSyn/**2b**(or **2e)** hetero-complexes did not accelerate amyloid self-assembly and formation of cell-damaging aggregates but accelerated amyloid self-assembly when added to αSyn alone or αSyn/**4Ala-2b** mixtures. a) Fibrillogenesis of αSyn (3 μM), αSyn seeded by fIAPP (10%), and preformed αSyn/**2b**(or **2e**) hetero-complexes (1/1) following addition of fIAPP seeds (10%) as followed by the ThT binding assay. The effects of addition of fIAPP seeds (10%) to mixtures of αSyn (3 μM) with the negative control **4Ala-2b** (150 μM) are also shown for comparison; applied fIAPP seeds alone (controls) were studied in parallel. Data means (±SD) from 3 assays (n=3 wells each). b) TEM images of aged solutions from (a) as indicated reveal fibrils as major species in αSyn alone or αSyn/**4Ala-2b** cross-seeded by fIAPP and amorphous aggregates as major species in αSyn/**2b**(or **2e**) hetero-complexes seeded with fαSyn seeds (scale bars, 100 nm). c,d) Effects of 24 h-aged (c) or 7 day-aged (d) αSyn (3 μM), αSyn seeded by fIAPP, and αSyn/**2b**(or **2e**) (1/1) or αSyn/**4Ala-2b** (1/50) mixtures seeded with fIAPP (10%) seeds on PC12 cell viability as determined by the MTT reduction assay; aged solutions from (a) were used. Effects of applied fIAPP seeds alone (controls) were studied in parallel. Data means (±SD), 3 assays (n=3 wells each).



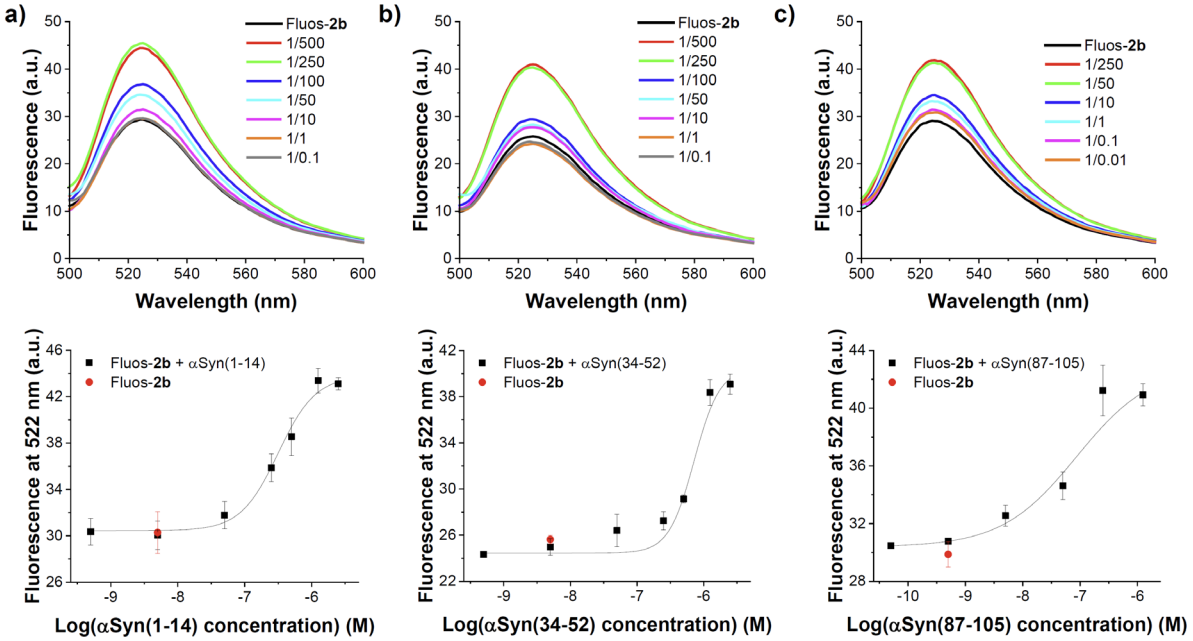
Supporting Figure S19. Confirmation of the presence of cell-damaging αSyn oligomers in the solutions used for studying effects of MCIPs on hippocampal synaptic LTP impairment mediated by αSyn oligomers in mouse brains *ex vivo* (a-d) and their reduction in the presence of peptides **2b** and **2e** (d) (see also Fig. 4a-c). αSyn oligomers were prepared according to a previously described protocol.[13] Following a 5-day aging of αSyn (138 μM) at 37°C, αSyn oligomers were separated from fibrils by centrifugation and supernatants were used for the LTP impairment studies. The presence of cytotoxic oligomers in the supernatant fraction was confirmed by the ThT binding assay (a), TEM (b), dot blot with the anti-oligomer antibody A11 (c), and the MTT reduction assay (d); for comparison, freshly dissolved αSyn (αSyn monomers) or buffer alone and pellet fractions (fibrils) were studied as well. Effects of **2b** and **2e** on cytotoxic αSyn oligomers were assessed by the MTT reduction assay (d). a) ThT binding properties of αSyn monomers and of the supernatant (αSyn oligomers) and pellet (αSyn fibrils) fractions (αSyn 1.7 μM) as indicated. Data is means (±SD) from 3 αSyn oligomer preparations. b) TEM micrographs of supernatant (αSyn oligomers) and pellet (αSyn fibrils) fractions; scale bars 1 μm and 100 nm, respectively. c) Dot blot analysis of A11 antibody binding of supernatant (αSyn oligomers) and pellet (αSyn fibrils) fractions as compared to αSyn monomers (5 μg each); a representative dot blot (n=2) is shown. d) Quantification of cell-damaging effects of αSyn oligomers (supernatant fraction) and their mixtures with MCIPs (1/10) in comparison to αSyn monomers and buffer control by the MTT reduction assay in PC12 cells. Significantly increased cytotoxicity was found in solutions containing αSyn oligomers alone (300 nM) as compared to solutions containing αSyn monomers or mixtures of αSyn oligomers with **2b** or **2e** consistent with the results of the *ex vivo* LTP impairment assay (Fig. 4a-c). Data means (±SD) of 4 assays (n=3 wells each) with 3 αSyn oligomer preparations; statistical analysis by one-way ANOVA with post-hoc Tukey test (*p<0.05; ***p<0.001; p-values not shown; p values <0.05 were considered significant).



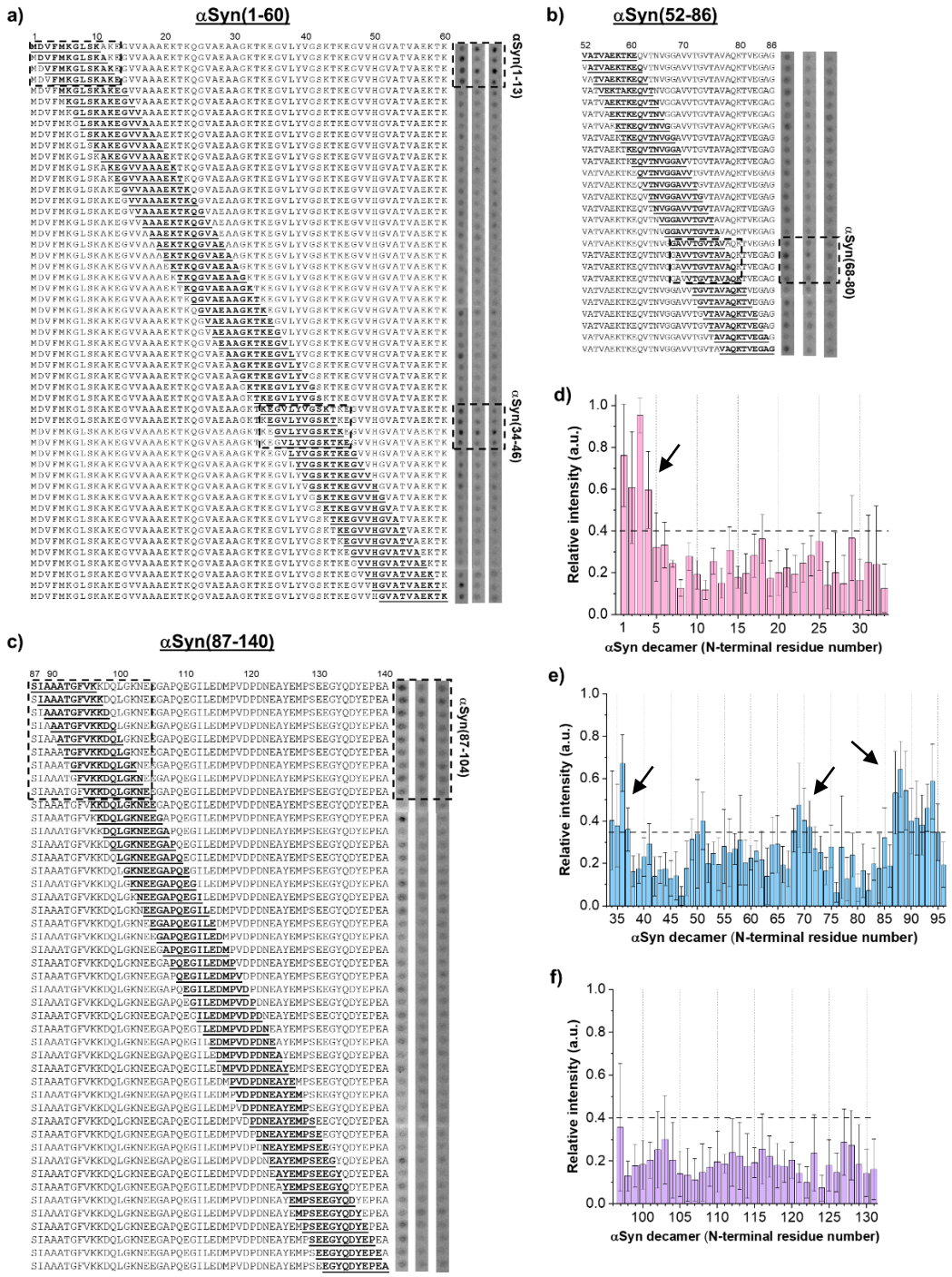
Supporting Figure S20. Peptides **2b** and **2e** do not inhibit amyloid self-assembly of insulin. Fibrillogenesis of insulin (10 μM) alone or in the presence of **2b** and **2e** (10-fold) followed by the ThT binding assay; data means (±SD) of 3 assays for insulin alone and insulin/**2e** mixture and mean of 2 assays (insulin/**2b** mixture).



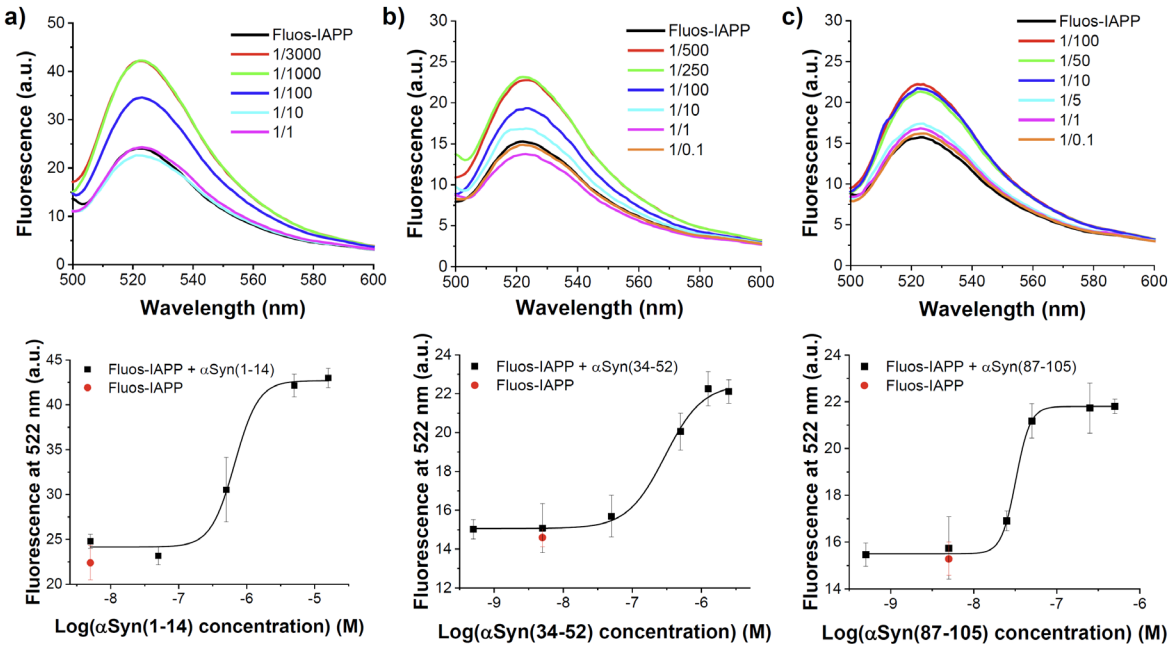
Supporting Figure S21. Peptide array data underlying the identification of the 3 key αSyn regions mediating its interaction with **2e** (see also Fig. 5a). a-c) Shown are 4 arrays containing αSyn decamers (bold, underlined) covering αSyn(1-60) (a), αSyn(52-86) (b), and αSyn(87-140) (c) following incubation with Fluos-**2e** (1 μM) and visualization of bound peptide by fluorescence. Identified Fluos-**2e** binding clusters are indicated by dashed rectangles. Data from 2 independent incubations with Fluos-**2e** of 2 arrays synthesized in parallel. d-f) Densitometric quantification of Fluos-**2e** bound to the decamers of the arrays shown (a)-(c); relative intensities are means (±SD) of 4 arrays. Fluos-**2e** binding clusters (arrows) were defined as αSyn regions corresponding to at least 5 consecutive decamers with at least 80% of them exhibiting relative intensities >0.40 or 0.45 (lower threshold values indicated by the dashed vertical line).



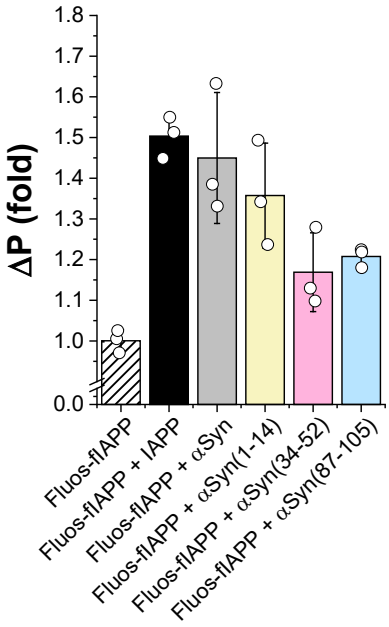
Supporting Figure S22. Determination of the affinities of interactions of Fluos-**2b** with key αSyn segments αSyn(1-14), αSyn(34-52), and αSyn(87-105) by fluorescence spectroscopic titrations. a-c) Top, fluorescence emission spectra of Fluos-**2b** (5 nM) alone and its mixtures with various amounts of αSyn(1-14) (a), αSyn(34-52) (b), and αSyn(87-105) (c); Fluos-**2b**/peptide molar ratios as indicated. Data representative from one assay out of 3. Bottom, binding curves; data of Fluos-peptide alone is also shown for comparison. Data means (±SD) of 3 titration assays; determined app. K_d values are in Table 2.



Supporting Figure S23. Peptide array data underlying the identification of αSyn regions which interact with IAPP (see also Fig. 5e). a-c) Shown are 3 arrays containing αSyn decamers (bold, underlined) covering regions αSyn(1-60) (a), αSyn(52-86) (b), and αSyn(87-140) (c) following incubation with Fluos-IAPP (1 μM) and visualization of bound peptide by fluorescence. Identified Fluos-IAPP binding clusters αSyn(1-13), αSyn(34-46), αSyn(68-80), and αSyn(87-104) are indicated by dashed rectangles. Data from 2 independent incubations with Fluos-IAPP (arrays synthesized in parallel). d-f) Densitometric quantification of Fluos-IAPP bound to the decamers of arrays shown in (a)-(c); data means (±SD) of 3 arrays. Fluos-IAPP binding clusters (arrows) were defined as αSyn regions corresponding to at least 4 consecutive decamers with relative intensities >0.35 or 0.40 (lower threshold values indicated by the dashed vertical line).



Supporting Figure S24. Determination of the affinities of interactions of Fluos-IAPP with the 3 αSyn segments found to interact with the MCIPs by fluorescence spectroscopic titrations. a-c) Top, fluorescence emission spectra of Fluos-IAPP (5 nM) alone and its mixtures with various amounts of αSyn(1-14) (a), αSyn(34-52) (b), and αSyn(87-105) (c); Fluos-IAPP/peptide molar ratios as indicated. Data from 1 representative titration assay out of 3. Bottom, binding curves; the data of Fluos-IAPP alone is also shown for comparison. Data is means (±SD) of 3 titration assays; determined app. K_d values in Table 2.



Supporting Figure S25. The 3 key αSyn segments found to interact with **2e**, **2b**, and IAPP bind fIAPP as well. Change of fluorescence polarization (ΔP) of Fluos-fIAPP (100 nM) (normalized to 1.0) following addition of IAPP (positive control), αSyn, and αSyn segments αSyn(1-14), αSyn(34-52), and αSyn(87-105) (1.5 μM). Data means (±SD) of 3 binding assays performed with 2 different Fluos-fIAPP preparations.

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