Supporting Information:
Cascade autohydrolysis of Alzheimer's Aβ peptides

Martin Wolfram,[1,*] Manish K. Tiwari,[1,2] Ming Li,[3] Tue Hassenkam,[4] Morten J. Bjerrum,[1] and Morten Meldal[1,*]

[1]*Department of Chemistry, University of Copenhagen, Universitetsparken 5, 2100 Copenhagen, Denmark*

[2]*Current address: Novozymes Innovation Campus, Novozymes A/S, Biologiens Vej 2, 2800Kongens Lyngby*

[3]*The Danish Hydrocarbon Research and Technology Centre, Elektrovej, 2800 Kongens Lyngby, Denmark*

[4]*Globe Institute, Section for Geobiology, Copenhagen University, Øster Voldgade 5-7, 1350Copenhagen K, Denmark*

E-mail: meldal@chem.ku.dk

Phone: +45 21 30 82 99

martin.wolfram@nano.ku.dk

Phone: +45 27 20 23 55

List of Figures

List of Tables

Experimental section

General remarks

NMR data were recorded on a Bruker 500 MHz spectrometer at 298 K with d6–DMSO as internal standard. High resolution mass spectrometry (HRMS) was performed on a Bruker SolariX XR spectrometer with α-Cyano-4-hydroxycinnamic acid (α-CHCA) as matrix for MALDI-TOF measurements. ESI-MS were recorded on a Micromass Global Ultima instrument or a Bruker Solarix ICR instrument, including HRMS. Fluorescence plate reader experiments were conducted on a instrument SpectraMax® M2E or a SpectraMax® i3x instrument, Molecular Devices. Fluorescence microscopy images were acquired on an Olympus wide-field fluorescence IX73 inverted microscope equipped with a 4× objective (0.16 numerical aperture) and 10× objective (0.4 numerical aperture), and an X-Cite 120Q excitation light source. FRET fluorescence was detected using 320/14 nm excitation filter and a 420/38 nm emission filter.

Materials

All standard and unusual amino acids as well as coupling reagents used for this work were purchased either from Chem-impex Inc., BaChem GmbH or Iris GmbH. Aβ_{1-28} and Aβ_{1-40} was purchased from CASLO ApS, Denmark. Aβ_{1-42} was purchased from Bachem Holding AG. Standard chemicals and solvents have been purchased from Sigma Aldrich (Merck Chemicals) or VWR. PEGA resin was purchased from Polymer Laboratories Ltd.

Analytical Methods

Liquid chromatography/mass spectrometry (LC/MS)

LC-MS analysis of peptide material and their autocleavage mixtures was performed on a Dionex UltiMate® 3000 (Thermo Scientific) instrument equipped with an Acclaim RSLC

120 C_{18} column (2.2 μM, 120 Å, 2.1 × 100 mm) coupled to a Bruker microTOF-QIII mass spectrometer. A linear gradient of CH_3CN in H_2O with 0.1 % formic acid was used, running from 5 % to 100 % CH_3CN, 0.5 mL/min over 10 minutes. UV-signal absorbance was recorded at 214, 225, 250 and 275 nm. Because of the salt content in the monitoring samples, eluate injection to the ESI mass detector was delayed for 60 seconds using a software controlled diverter method. The LC-MS data analysis was conducted with the Compass DataAnalysis software (version 5.0, Bruker). For UV-trace quantification, LC-UV chromatograms were processed via 1 – 2 × baseline subtraction (flatness parameter 0.8 – 0.9). The baseline-corrected chromatograms were smoothed with the Savitzky Golay algorithm of the analysis software. Supplementary HPLC and LC-MS analysis of peptides and their hydrolysis samples was conducted on a Waters Acquity™ UPLC system with a Xevo G2-S QTOF mass spectrometer (Waters Inc.). A linear gradient of CH_3CN in H_2O with 0.1% formic acid was used, running from 5 % to 100 % CH_3CN, 0.7 mL/min over 5 minutes. UV-signal absorbance was recorded with a diode array detector 214 - 300 nm. Because of the salt content in the monitoring samples, spray injection of eluate to the QTOF mass detector was delayed for 60 seconds using a software controlled diverter method. LC-UV chromatograms were processed via instrument software MassLynx™ (version 4.1, Waters)

Thioflavin T (ThT) assay

The ThT assay was used for detection of fibril formation of peptides **1a** – **3** in solution by periodically monitoring of the ThT fluorescence enhancement over time as described previously.[1] The increase of fluorescence (I_F at λ_{em} = 486 nm) occurs through binding of the ThT chromophore to amyloid fibrils via π-stacking interactions with their hydrophobic surface side-chain groove.[2] Synthetic peptides **1a** – **3** were prepared at 60 - 130 μM in weak phosphate buffer at 10 mL reaction stock volume. Aliquots were used for addition of ThT (100 – 150 μM). Replica aliquots from these mixed solutions were distributed over a 96 well-plate. The formation of amyloid fibrils was monitored through fluorescence enhancement of

fibril-bound ThT in 5 – 20 mM phosphate buffer (pH 7.5 and 8.2) with excitation at 440 nm and emission recorded at 486 nm on a SpectraMax® M2E or a SpectraMax® i3x instrument, Molecular Devices. Dual fluorescence assays were run in a similar fashion to monitor the FRET fluorescence of **1a** derivatives at $\lambda_{ex/em}$ 320/420 nm (Figure S18E and Figure S21A). Aβ_{1-28} was dissolved in Milli-Q water and the concentration was determined using UV-Vis absorbance method as described previously.[1]

Atomic force microscopy (AFM)

Bright-field microscope images on AFM samples were conducted on a Zeiss axiotech microscope working in DIC mode. For AFM imaging we used a Cypher from Asylum (now Oxford instruments) equipped with a standard AC240 silicon tip from Olympus with a spring constant around 2 nN/nm and a resonant frequency around 70 kHz. Images were at least 512 × 512 pixels and aimed for a resolution of 1 nm per pixel or less. The scanning was performed in ambient conditions at 1 Hz. The height of the rings (thickness) was measured using the section analysis tool in the Igor pro control software for the AFM. **Surfaces:** Mica was purchased from SPI Supplies (Mica grade v-4). Scotch tape was used to repeatedly cleave the mica until a complete cleavage was achieved (usually 3 – 5 ×). **Samples:** The samples were prepared in several ways: 1: A mixture with products was placed on freshly cleaved mica for 30 seconds to allow polymers to adhere, then flushed with ultra-pure deionized water (Milli-Q). The surface was dried under a stream of nitrogen gas, then examined by atomic force microscopy. 2: The solution was allowed to dry on the mica surface and then studied in optical microscopy. 3: The solution was allowed to dry on the mica surface and then rinsed with ultra-pure deionized water (Milli-Q). The surface was dried under a stream of nitrogen gas, then examined by atomic force microscopy.

Circular dichroism spectroscopy (CD)

Measurements in the far-UV region (190 – 260 nm) were conducted using a 0.1 cm path length quartz cuvette on a J-815 JASCO spectropolarimeter. All measurements were performed at 25 ℃. Stock aliquots of peptide **1a** or f0(**1a**) were diluted to 50 – 110 μM in 10 mM phosphate buffer, pH 7.4. The spectra shown in Figure S6A, Figure S18C and Figure S25A,D are derived from an average of 6 scans, and the optical activity is reported as ellipticity machine units (millidegrees). During monitoring of CD, aliquot samples were collected and subjected LC-MS analysis at 20 ℃ without further dilution.

Dynamic light scattering (DLS)

Dynamic light scattering measurements were carried out on a custom-made instrument. The instrument uses as a light source a 150 mW Nd:Yag laser (wavelength 532 nm) from ADLAS, and scattered light was collected at a scattering angle of 90° by a single mode optical fibre setup feeding into a photon counting photomultiplier tube connected to a digital autocorrelator, ALV-5000/EPP (ALV, Germany). The measured homodyne autocorrelation functions were fitted using the regularized fit option in the ALV-Correlator Software version 3.0 supplied with the autocorrelator. Samples were prepared at 0.8 – 1 mL at a molar weight concentration of 100 – 200 μM from diluted concentrated stock solutions of $>$ 1 mM peptide dissolved in aqueous NaOH (2 – 10 mM). Dilute samples were 3 $\times$ filtered over a syringe filter (0.2 μm). The walls of the sample vial was washed 5 $\times$ with Milli-Q water before inserting the sample solution. DLS was monitored at 25 ℃, over a period of 60 – 100 minutes, and each of the 3 – 5 autocorrelation functions was obtained as an average of 20 autocorrelation functions recorded over 60 seconds. The result of the fitting procedure is a size distribution where the sizes are weighted by their light scattering power. The light scattering power of a molecular species is proportional to the mass concentration times the molar mass of the species. This means that high molar mass species (like, e.g. aggregates) even at a low mass concentration will still be prominent in the size distribution. The results of DLS analysis

are consistent with our assumption that the applied stock solution recipes afforded largely monomeric peptide and some low molecular weight (LMW) oligomers (Figure S19B,C).

Experimental procedures and Assay description

Solid-phase peptide synthesis

Notes on preparative procedures. The preparation of pure **1a** via solid-phase peptide synthesis was challenging because trifluoroacetic acid (TFA)-mediated side-chain deprotection resulted in active ester formation between nitro-tyrosine and TFA anhydride. We repeatedly obtained a 1/1 to 3/2 mixture of **1a** and Lys_{16} – TFA-acylated derivative **1b** after basic workup (Figure S1A and Figure S3A). To circumvent this problem we reduced the initial peptide loading on the $PEGA_{800}$ resin (0.02 mmol/mL) to 0.01 mmol/mL through partial linker cleavage with neat propylamine and subsequent extraction of released side-chain protected peptide **1c** (Figure S1D). Reducing the peptide loading on solid support prior to TFA treatment seemingly could diminish intermolecular $Y(NO_2)$ TFA-active ester transfer reaction due to reduced encounter between side-chains of neighboring peptides in the resin matrix. Following this improved procedure, we obtained higher yields of **1a** upon TFA mediated side-chain deprotection and subsequent HMBA-linker cleavage Figure S1A. The crude peptide materials obtained from synthesized batches of **1a** were subjected to further purification by HPLC (Figure S1B,C). This procedure allowed access to **1a**, **1c** and **1d** in high purity (Figure S1D,F).

Linker coupling

Peptide synthesis was performed with attachment to the base labile HMBA linker on the $PEGA_{800}$ resin,[3] using Fmoc-based SPPS strategy and the standard TBTU activation procedure for amide coupling on solid support, including subsequent HMBA esterfication with Fmoc-glycine via MSNT-activation.[4] **Synthesis sequence:** $PEGA_{800}$ resin (1 g, 0.2 mmol/g,

10 mL) was swelled in DMF and treated with a solution of 4-hydroxymethyl-benzoic acid (HMBA, 3 equiv.), TBTU (2.9 equiv.), and NEM (4 equiv.) in DMF after 5 minutes of pre-activation. After 2 hours the resin was washed with DMF (5×), DCM (5×). For subsequent esterfication of the HMBA-linker the resin was treated with a mixture of Fmoc-Gly-OH (3 equiv), being activated for three minutes with MSNT (3 equiv), and MeIm (6 equiv.) in 7 mL DCM. The MSNT-coupling procedure was repeated once.

General Procedure for Peptide Couplings

TBTU-mediated couplings were performed by dissolving the amino acid (3 equiv.) in an appropriate amount of DMF ($\sim$ 30 % of resin volume), followed by the addition of *N*-ethylmorpholine (NEM, 4.0 equiv.) and TBTU (2.9 equiv.) for subsequent OBt-active ester formation. The resulting solution was allowed to react for 3 minutes and then added to the swollen $PEGA_{800}$ resin and allowed to react for 2 hours under continuous mixing via syringe nitrogen bubbling, followed by subsequent washing of the resin with DMF (5×). To verify that full conversion of the free amine had taken place, the resin was checked by the Kaiser test.[5]

General Procedure for Linker Attachment on Solid Phase

Attachment of the linker to the amino-functionalized resin ($PEGA_{800}$) was carried out by activation of HMBA (3.0 equiv.) with NEM (3.0 equiv.), and TBTU (2.95 equiv.) in a minimal amount of DMF for 3 minutes before adding this mixture to the pre-swelled $PEGA_{800}$ resin. Upon 2 hours of reaction the mixture was washed off from the resin with DMF (5×).

General Procedure for Fmoc Deprotection

The Fmoc-group was removed by reaction with 20 % piperidine in DMF for 5 minutes, and the resin was washed with DMF (3×), followed further addition of 20 % piperidine in DMF. After 5 minutes the resin was again washed with DMF (5×). In order to minimize diketopiperizine formation during Fmoc deprotection before the third coupling the piperidine treatment was adopted with two times 2 minutes under continuous flow and DMF washing in between.

General procedure for side-chain deprotection

The resin was swelled in methylene chloride and treated with TFA 95 % (5 % water/TIS, 1:2) for 15 minutes. The mixture was washed out with DCM (3×) and the TFA treatment was repeated 2× 45 minutes with DCM washing in between. For neutralization the resin was washed with DCM (5×), DMF (3×), 2 % DIEA/DMF (3×) and again DMF (3×).

General Procedure for Cleavage of Resin-Supported Peptides

After side-chain deprotection and subsequent washing steps the resin was washed with water (5×). In order to cleave the peptide from the base labile HMBA linker the resin was treated with a 10–15 times volume excess of 5 % triethylamine/water for 2 hours. After filtration over a solid phase extraction column and washing the resin with the same volume 70 % acetonitrile/water the combined extracts were lyophilized and the obtained peptide material was characterized by HPLC-MS and high resolution MALDI-TOF.

Assembly of D-amino acid peptide, compound 4 and its f0 fragment

The peptides were synthesized twice on a custom build MPM-Continuous Flow Synthesizer on a 3 mL column of $PEGA_{800}$ resin (0.4 mmol/g, 0,12 mmol) from Agilent Technologies using DMF for peptide couplings and washing processes. During the first assembly aliquots 800 μL

were removed at the stage of f4 and f0. The resin was coupled with 4-hydroxymethylbenzoic acid using COMU as a coupling reagent and 4-ethylmorpholine for deprotonation. The linker was esterified in DCM with Fmoc-Gly-OH using MSNT for activation and N-methyl imidazole as a catalyst. The Fmoc-group was generally removed with 20 % piperidine in DMF (10 min) and peptide coupling times (COMU 3 eqv., 15 % Oxyma and 3 eqv. Fmoc-D-amino acid, preactivation for 3 min) were 60 min until residue 6 and 3h for the rest of the assembly to account for peptide aggregation observed as resin shrinkage at residue 7 and 8. An aliquot of 1.5 mL of the final resin was used in the deprotection. The Fmoc-group was removed from the peptide with piperidine /DMF and the resin was washed with DMF and DCM and dried with a flow of nitrogen. The dry resin was treated with 95 % aqueous TFA for 1.5 h and carefully washed with DCM, DMF, 2 % piperidine in DMF, then with DMF and water. The resin was covered in water and 400 μL of 1 M aqueous NaOH was added. After 1 h the reaction solution was collected and the resin was washed with 5 mL of water. The combined aqueous phase was neutralized with 1M HCl whereby the peptide precipitated as a gel. Upon lyophilization the yield of assembly, deprotection and cleavage was 73 mg of peptide containing 18 mg of NaCl (more than 50 % of the theoretical yield of peptide). The product (0.4 mg) was dissolved by addition of 10 μL TFA then 1 mL water and analyzed by LC-MS (see Figure S12). $[M+H]^+$ (1682,8655), $[M+2H]^{2+}$ (841.9724), $[M+3H]^{3+}$ (561.6560). The product was essentially pure except for the presence of NaCl. A small aliquot ($\sim$ 5 mg) was purified by preparative HPLC to provide pure salt free peptide for autoproteolysis studies. Fragment f0 of peptide **4** was obtained in a similar fashion.

Peptide purification

Peptides **1a** – **4** were purified by RP-HPLC on a Gilson 215 liquid handler using a 10 μm 19 $\times$ 150 mm XTerra Prep RP18 column. A 25 minute gradient from 13.5 % to 76.5 % CH_3CN in H_2O with 0.1 % TFA and a flow of 15 mL/min was used. Detection of signal was performed by a Gilson 170 diode array measuring the absorbance of UV-light at 210 and

254 nm. The identity of the used peptide material were identified by their high-resolution mass via LC-MS analysis (Figure S1C,D). Lyophilization of concentrated peptide solutions, either obtained from HMBA-linker cleavage procedure or isolate HPLC eluate fractions was conducted on a high vacuum freeze trap.

Stock preparation recipes for HPLC purification

Peptide 1a: A 1:2 mixture of **1a**/**1b** (5.5 mg) was partially dissolved in 500 μL of 50 % acetonitrile/water. After addition of 200 μL PB (100 mM) most of the material dissolved. Upon addition of 100 μL urea solution (4.5 M) and 400 μL Milli-Q water the resulting clear solution was run through a PTFE syringe filter (0.45 μm) and subjected to preparative HPLC purification (injection loading: 4.2 mg/mL). The isolated fraction of **1a** eluate was lyophilized affording 2 mg of colorless material with $>$ 95 % purity (Figure S1B).

Peptide 2: The crude material of peptide **2** (12.4 mg) were poured into 400 μL 50 % acetonitrile/water and 400 μL urea solution (14 M) added. The resulting mixture quickly became a stiff gel. Addition of 30 μL aqueous NaOH (0.1 M) was followed by dilution with 200 μL 50 % acetonitrile/water affording a highly viscous solution. Addition of 300 μL PB (100 mM), pH 7.6 resulted a clear solution. This solution was run through a syringe filter (0.45 μm) and 1 mL was subjected to preparative HPLC purification (injection loading: 9.3 mg/mL). The eluate of the isolated **2** HPLC fraction was subsequently lyophilized, affording 6 mg of colorless powder. Analysis by LC-MS confirmed high purity of the obtained peptide material (Figure S1D).

Peptide 3: The crude material of peptide **3** (9.7 mg) mixed with 400 μL urea solution (4.5 M) and 400 μL PB (100 mM), pH 7.6. After addition of 500 μL 50 % acetonitrile/water material still remained poorly soluble. Upon addition of 250 μL aqueous NaOH (0.1 M) the mixture became a clear solution. This solution was run through a syringe filter (0.45 μm) and 2 mL were subjected to preparative HPLC purification (injection loading: 6.4 mg/mL). The HPLC eluate of the isolated main fraction was subsequently lyophilized, affording 4

mg of colorless powder. Analysis by LC-MS confirmed high purity of the obtained peptide material (Figure S1D).

LC-MS sample preparation

Method A: An aliquot of an incubated hydrolysis sample (50 – 150 μM peptide loading) was 1:10 diluted in 70 % acetonitrile/water at 20 ℃ in a standard LC-MS vial. **Method B:** An aliquot of an incubated hydrolysis sample (50 – 150 μM peptide loading) was 1:2 or 1:4.5 diluted in neutral Milli-Q water in a capped standard LC-MS vial. **Method C:** An undiluted aliquot of an incubated hydrolysis sample (50 – 150 μM peptide loading) was transferred to an LC-MS vial with a glass insert and measured on the Waters Acquity™ UPLC system with a Xevo G2-S QTOF mass spectrometer (Waters Inc.) at 10 ℃ (tempered autosampler chamber) (Figure S7A,C,E). After 30 – 60 minutes at 10 ℃ the sample was 1:4.5 diluted in Milli-Q water at 20 ℃ and subjected to further LC-MS analysis (Figure S9) on the UltiMate® 3000 (Thermo Scientific) instrument (Bruker microTOF-QIII mass spectrometer).

Stock recipes for autohydrolytic amyloid

Preparation of an activated stock solution: Peptide **1a** (7.5 mg, 4.1 mmol) was dissolved in 500 μL sterile DMSO. To the resulting orange solution 40 μL of fresh PB pH 7.8 (100 mM) and 3 μL of urea solution (1 M) were subsequently added and the mixture was diluted with 4 mL Milli-Q water. The solution became turbid and showed pH 7.4. Balancing the pH toward 7.65 and obtaining a clear yellow solution required addition of 40 μL NaOH (0.1 M) and 10 μL HCl (0.1 M) at 21 ℃. After adding 1 mL of Milli-Q water, the solution remained clear but showed some chunks of undissolved starting material. The solution was filtered through a 0.2 μm supor® membrane filter affording a clear stock solution with final concentrations: c(**1a**) = 0.75 mM in 0.7 mM PBS with 0.5 mM urea at pH 7.8 (Figure S2B,C).

Peptide **2** (3.3 mg, 1.88 mmol) was dissolved in 200 μL DMSO after addition of 10

μL aqueous NaOH (0.25 M). Dilution with 300 μL Milli-Q resulted in gel formation. The gel became again a solution after addition of 10 μL aqueous NaOH (0.25 M) followed by ultrasonication for one minute (see Table S1, stock entry III).

Peptide **2** (1.1 mg, 0.63 mmol) was dissolved in 200 μL DMSO after addition of 20 μL aqueous NaOH (0.1 M). To the resulting clear solution 10 μL of aqueous urea (3.4 M), 100 μL PB (100 mM), pH 7.6 and 200 μL Milli-Q were subsequently added (see Table S1, stock entry V).

Stock solution recipe for activated f0(1a) and cross-seeding conditions: Peptide f0(**1a**) (7.6 mg, 5.71 mmol, yellow lyophilised powder) was dissolved in DMSO (200 μL) DMSO followed by addition of 150 μL aqueous NaOH (100 mM). The orange solution was diluted with 150 μL Milli-Q and stored at 4 ℃. Dilution aliquots for ACR activation experiments were prepare in the following sequence: 50 μL stock were diluted in 90 μL PB (100 mM) and 10 μL Milli-Q with 1 w% sodium azide followed by addition of 1.65 mL Milli-Q. Replicates of this dilution stock were stored in the fridge for 7 – 12 hours and at 20 ℃ prior to 1:3 dilution in Mill-Q containing protease inhibitor cocktail and sodium azide (0.05 w%). The resulting solution (100 μM peptide f0(**1a**)) was kept for 10 minutes at 20 ℃ before incubation succeeded for 1 - 3 weeks at 4 ℃. Cross-seeding with aliquots of this stock solution provided ACR in Aβ_{1-28} (Fig. S15E(iii)) and Aβ_{1-42} (Fig. S35B-D). Final f0-activation samples were produced through 1:2 dilution in Milli-Q (10 – 20 ℃) followed by incubation for 7 – 14 days at 20 ℃ in the dark prior for direct use in cross-seeding ACR experiments. In parallel, replicates of these samples (7 – 10 d at 20 ℃) were incubated for 1 – 4 d at 37 ℃ and kept at room temperature thereafter which resulted in activation with subsequent progression of f1 $\rightarrow$ f6 (Fig. S26B).

Stock solution recipe for largely monomeric peptide: Peptide **1a** – **3** (1 – 4 mM) were dissolved in 60 – 100 % DMSO, acetonitrile or Milli-Q water with 5 – 20 mM aqueous NaOH, respectively (Table S1). Dilute samples derived from fresh stock solution in 10 mM aqueous NaOH showed mainly monomer among low molecular weight oligomers after being

incubated for several hours at 21 – 25 ℃ as confirmed by dynamic light scattering (DLS) (Figure S19). Circular dichroism (CD) confirmed random coil assembly for these stock conditions conditions (Figure S18C, blue CD spectra and Figure S25D).

Stock preparation of Aβ_{1-40} and Aβ_{1-42} in monomeric form and conditions for sample dilution Aβ solutions were freshly prepared by the dissolving lyphilised peptide in hexafluoroisopropanol (1.5 – 2 mg/mL HFIP) in a low protein binding Eppendorf tube. The solution was evaporated under argon flow and the procedure was repeated by redissolving the peptide film again in HFIP and evaporate with argon. The peptide film was then dissolved in 10 mM NaOH to a concentration of 1.5 – 2 mg/mL. After ultracentrifugation at 4 ℃ the supernatant of this solution was collected and diluted 1:5 in 125 mM HEPES buffer pH 7.4 for concentration determination by tyrosine UV-absorbance as described elsewhere.[1] Samples for aggregation assays and ACR screenings were prepared from the 10 mM NaOH peptide stock solution (290 – 460 μM) through 1:10 (1:20) dilution in PB (10 mM) pH 7.0 resulting a final pH 7.33 – 7.42.)

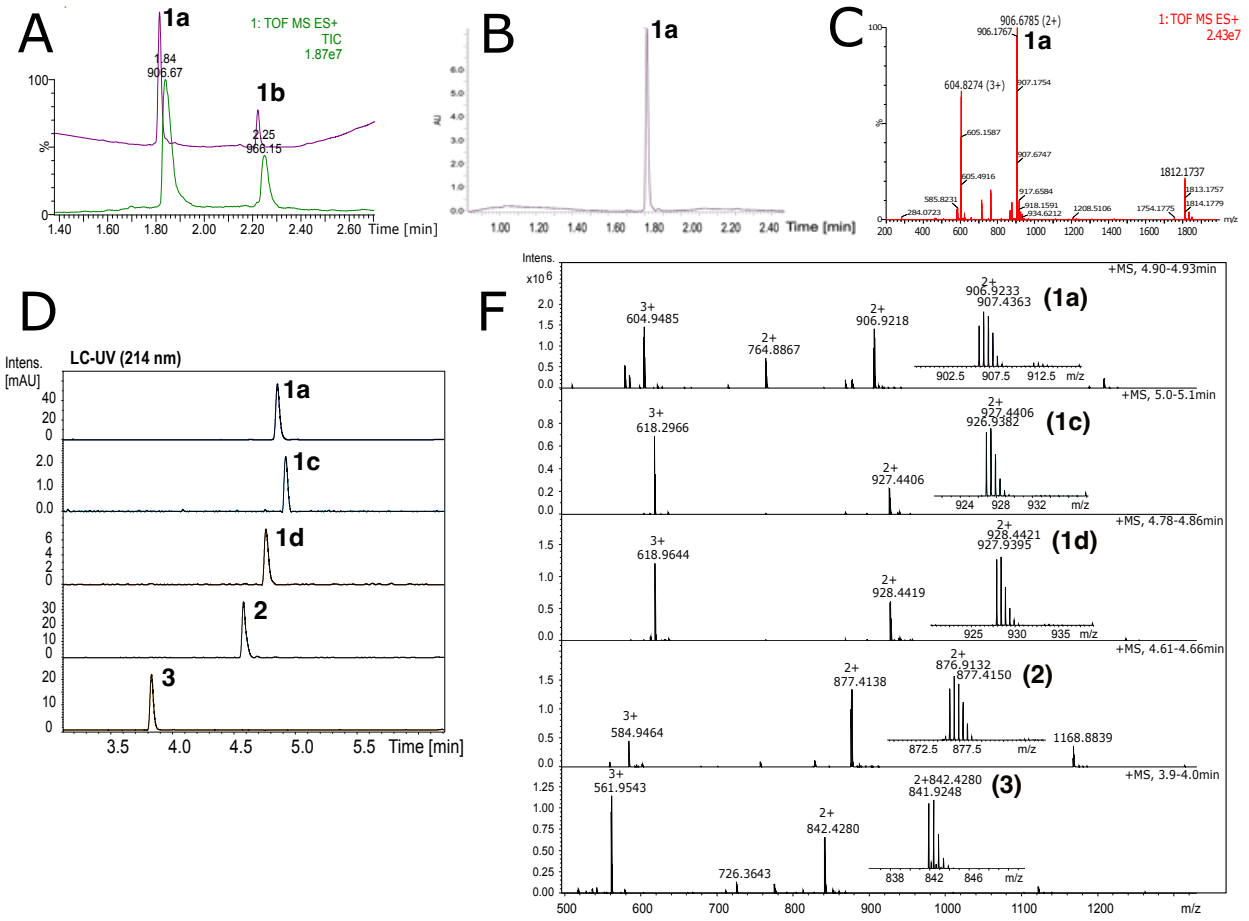

Figure S1. Mass spectroscopic characterization of peptide starting materials. (**A**) Stacked UPLC-MS chromatorgam of the crude peptide mixture **1a**/**1b**. Purple trace: UV-trace (214 – 300 nm diode arry absorption), green trace: total ion current (TIC) chromatogram for positive mode detection. The TIC peaks are annotated with the monoisotopic mass (m/z) of their doubly charged mass peak and retention time. MS/MS sequence identification of **1b** is shown in Figure S3A, revealing **1b** as Lys_{16}-TFA-acylated derivative of **1a** that appears as a stable sodium ion complex in ESI-MS. (**B**) UV-chromatogram for **1a** after preparative HPLC purification of the mixture. (**C**) Corresponding mass spectrum of **1a** recorded on a Waters Acquity™ UPLC system with a Xevo G2- QTOF mass spectrometer (Waters Inc.). (**D**) Stacked LC-chromatograms (UV-214 nm) for peptide starting materials **1a** – **3** recorded on a Bruker Ultimate® 3000 instrument. (**E**) Stacked mass spectra were obtained from high resolution ESI-MS. The doubly charged mass peak of the peptides are displayed as enlarged inserts. Peptide sequences: **1a** (Abz-Pra-HQKLVFFAEDVY(NO_2)G), **1c** (Abz-Pra-HQKLVFFAEDVY(NO_2)G-propylamide), **1d** (Abz-Pra-HQKLVFFAEDVY(NO_2)G-ethanolamide), **1a** (Bz-Pra-HQKLVFFAEDVYG), **3** (VH-HQKLVFFAEDVGG)

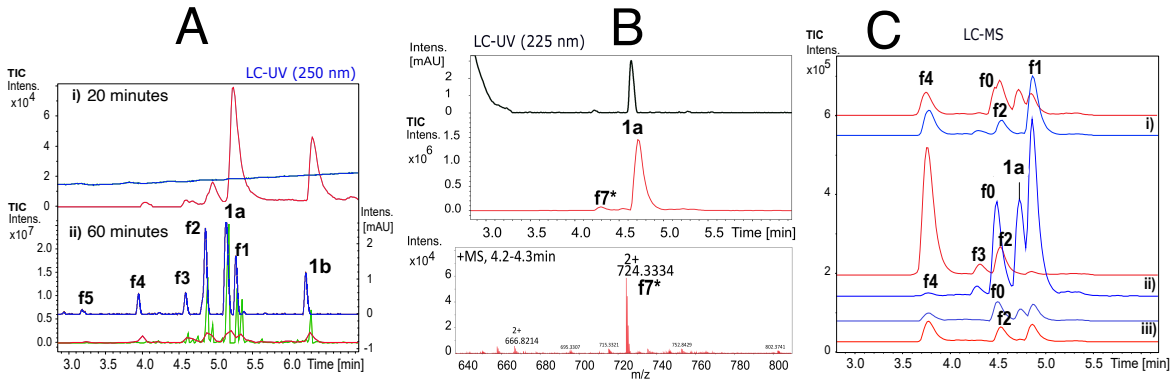

Figure S2. Screening for palladium assisted peptide bond cleavage and identification of autohydrolysis. (**A**) Stacked LC-MS chromatograms (red (MS-TIC), green (MS/MS-TIC), blue (UV-250nm)) for a hydrolysis of Abz-Pra-HQKLVFFAEDVY(NO_2)G (**1a**) (150 μM in PB (10 mM), pH 7.8) containing a palladium catalyst. After incubation for 4 days at 37 °C followed by 3 days at 20 °C, LC-MS samples were obtained through 1:10 dilution in 70 % acetonitrile/water. (i) 20 minutes upon sample dilution; (ii) 10-fold higher sample injection volume after 60 minutes. Hydrolysis fragments were identified by their high-resolution mass and the MS/MS (m/z = 500 – 2000) fragmentation pattern of autocleavage fragments f1 and f2. (Figure S3A-C).(**B**) LC-MS chromatogram (black (UV-225 nm), red (MS-TIC)) of a freshly prepared stock solution (1 h at 20 °C) of **1a** (0.75 mM in 0.7 mM PBS with 0.5 mM urea and 9 % DMSO at pH 7.8) showed a trace of the autocleavage fragment Abz-Pra-HQKLVFFAED (f7*) already 2 h upon 100-fold dilution in 70 % acetonitrile/water (see report on Aβ_{1-40} autocleavage by Hosia et al.[6]) as indicated by doubly charged mass peak (m/z = 724.3334^{2+}) in the mass spectra below. Furthermore, tiny traces of the f1 (m/z = 1204) and f2 (m/z = 1091) fragments were detected. The undiluted stock solution remained unchanged over a period of two years storage at 20 °C. (**C**) Right upon preparation, dilute aliquots of this stock solution were used in a hydrolysis assay for testing cleavage activity of a palladium catalyst (1 mol%) in phosphate buffer (PB), pH 7.6. Sample conditions: (i) **1a** (74 μM) PB (10 mM), (ii) (152 μM) PB (5 mM), (iii) (38 μM) PB (10 mM). Incubation conditions: 3 days at 20 °C, followed by 1:4 dilution in Milli-Q and continuous incubation for 3 days at 37 °C. LC-MS samples were 1:10 diluted in Milli-Q. MS-TIC chromatograms of negative controls without Pd-catalyst (red traces) are stacked with the traces of their respective catalyst containing samples (blue MS-TIC).

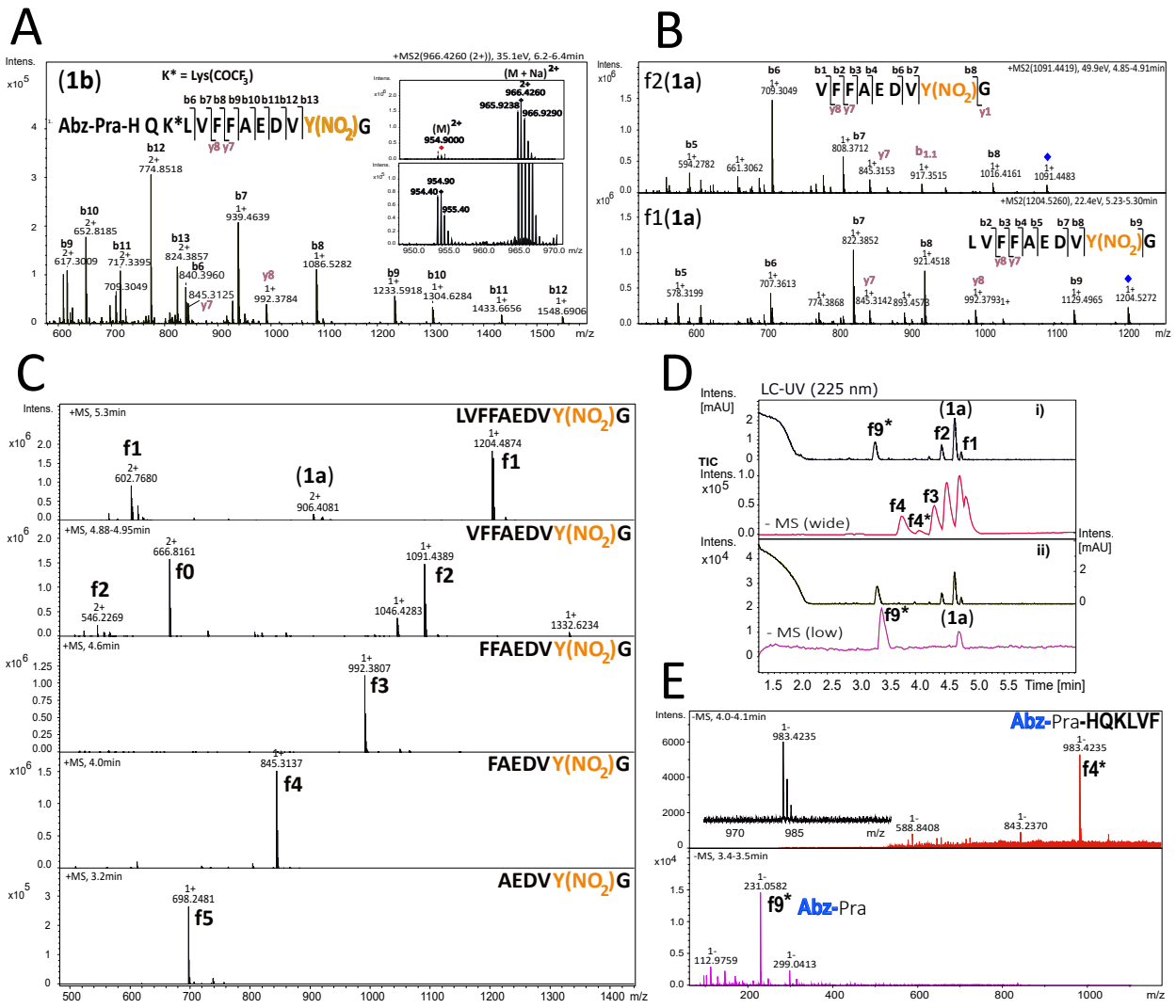

Figure S3. Mass spectroscopic identification of **1a** autocleavage fragments. (**A**) MS/MS fragmentation spectrum of peptide **1b** suggests tight binding of a sodium ion to the TFA-acylated Lysine residue (K*) (see mass peak insert in (A)) in fragments of the *b*-series (fragment mass peak + Na). (**B**) MS/MS spectra of autocleavage fragments f1(**1a**) and f2(**1a**) with assignment of their *b*, *y* - fragmentation series. (**C**) Stacked mass spectra with fragment assignment according to consecutive aminopeptidase processing of *C*-terminal autocleavage fragments f0 (KLVFFAEDVY(NO_2)G) toward f5 (AEDVY(NO_2)G). (**D**) LC-MS analysis of a hydrolysis sample of **1a** (100 μM, PB (10 mM), pH 7.6) after 4 days incubation at 37 °C followed by 1:10 dilution in 70 % acetonitrile/water: (i) Stacked LC-MS chromatograms (red (MS-TIC), black (UV-225 nm)) for negative mode at wide mass range and (ii) at low mass range (TIC: magenta). (**E**) Mass spectra of fragment f4* (Abz-Pra-HQKLVF) and f9* (Abz-Pra).

Preparation of autoproteolytic amyloid assemblies

Stock solutions of **1a** – **3** were prepared at room temperature by dissolving 5 – 7 mg peptide/mL in either DMSO, aqueous acetonitrile or Milli-Q water together with 10 – 20 mM NaOH in sterilized 1.5 mL Eppendorf© vials. A variety of working stock solutions were obtained through subsequent dilution of the mother stock in Milli-Q water and/or phosphate buffer (Table S1). Buffers, Milli-Q water and the peptide stock solutions contained sufficient amounts of sodium azide and a commercial protease inhibitor cocktail without EDTA (cOpmlete™, Merck chemicals). Hydrolysis samples were diluted to a final peptide concentration of 50 – 100 μM in 10 mM phosphate buffer (pH 7.4 – 7.6) containing 0.02 w% NaN_3. Diluted samples were filtered over syringe (PTFE, d = 0.2 μM) and subsequently incubated at 37 ℃ in a closed, dark environment (multi-column heating mantle). Consecutive LC-MS screening (1 – 3 × a week) indicated start of the ACR after 6 - 14 days incubation at 37 ℃ (Table S2).

Table S1. Recipes for stock solutions

*P*eptide	Stock entry	c[A] (mM)	*c*[B1] (mM)	*c*[B2] (mM)	pH	co-solvent %	t_1 (d)	t_2 (d)	Figure references
1a/**1b**	I	9.6	0	15	> 10	42 % ACN	1	-	S6A
1d	II	8.6	0	0	-	100 % DMSO	0	-	S6C and S22A
1a (**2**)	III	1.0 (3.7)	0	10 (14)	-	32 (38) % DMSO	0	-	S7A(G)
1a	IV	2	0	10	-	99.8 % DMSO	2	-	S7C,E
2	V [a]	1.2	20	3.8	8.0	37 % DMSO	0	-	main Figure 3B,C
1a – **3**	VI	1.6 – 2	0	10	-	99.8 % DMSO	0	-	S9A,B
1a	VII [b]	0.66	10	3.3	8.4	33 % DMSO	-	48	S9E
f0(**1a**)	VIII	1.8	0	10	> 12	35 % ACN	0	-	S10 and S15A,D
f0(**1a**)	IX [c]	0.52	10	1.3	7.8	2 % DMSO	27	27	S11A,B
f0(**1a**)	X	1.1	0	2	-	-	0	-	S11D,E and S25
f0(**4**)	XI	0.86	0	8	-	-	0	-	S13
f0(**1a**)	XII [d]	0.1	1.7	0.25	7.6	0.36 % DMSO	76	-	S15E(iii)
$A\beta_{1-28}$	XIII	0.6 (0.7)	0	0	7.0	-	0	-	S15 (S17)
2	XIV	1.16	0	12	-	50 % ACN	48	-	S18A-C
3	XV	2.6	0	10	> 12	66 % ACN	0	-	S18
1a	XVI [e]	0.73	20	1.8	7.8	13 % ACN	4 (0)	-	S18A,C,E (S9F)
1a	XVII	4	0	9.1	-	64 % ACN	0	-	S19 and S25C
3	XVIII [b]	0.6	10	3.3	8.4	33 % DMSO	-	48	S20A
2 (**3**)	XIX	8.2 (7.0)	0	37.5	> 12	62.5 % DMSO	1	-	S20A,D and S22D,F
1a	XX	0.5	0	2.5	-	-	0	-	S25D-H
$A\beta_{1-40}$	XXI	0.33	0	10	11	0	0	-	S27 and S31
$A\beta_{1-40}$	XXII	0.29	0	10	11	0	0	-	S28 and S33
$A\beta_{1-40}$	XXIII	0.31	0	10	11	0	0	-	S27
$A\beta_{1-42}$	XXIV	0.46	0	10	11	0	0	-	S35

Notes. *c*[A]: peptide concentration in stock solution; *c*[B1]: initial buffer concentration (phosphate buffer); *c*[B2]: initial concentration of aqueous NaOH; co-solvent: dimethylsulfoxide (DMSO) and acetonitrile (ACN); t_1: storage time at 4 °C prior to the use in the referenced assay; t_2: incubation time at 20 °C. (*a*) Peptide stock solution contained 64 mM urea. (*b*) Peptide stock solution contained 5 mM urea. (*c*) Obtained through 1:20 dilution of stock solution (11 mM f0(**1a**) in aqueous NaOH (28 mM) with 40 % DMSO) in PB (10 mM), pH 7.6. (*d*) Obtained through 1:36 dilution of stock solution (11 mM f0(**1a**) in aqueous NaOH (28 mM) with 40 % DMSO) in PB (5 mM), pH 7.6. After 7 h storage at 20 °C followed by 10 h at 4 °C the final working stock was obtained by further 1:3 dilution in Milli-Q water. (*e*) Derived from stock XVII through 1:5 dilution in PB (20 mM), pH 7.4.

Table S2. Hydrolysis conditions for repeat assays and kinetic properties of the ACR

Peptide	Stock entry	c[A] (μM)	c[B] (mM)	pH	DMSO %	t_1 (d)	t_2 (d)	Hydrolysis[(a)] t_1	Hydrolysis[(a)] t_2	t_{lag} (h)	$t_{1/2}$ (h)	Figure ref.
1a/**1b**	I	96	10	7.6	0	-	18	-	+++	n.a.	n.a.	S6A
1d	II	86	5	7.6	0	8	21	-	+++	n.a.	n.a.	S6C
1a	III [(b)]	100	10	7.6	3.2	9 (9)	-	+ (++)	n.a.	96	160 (72)	S7A(B)
2	III [(b)]	158	10	7.6	1.7	7 (5)	-	+ (++)	n.a.	96	144 (72)	S7G
1a	IV	100	5	7.6	5	24	-	+++	n.a.	280	60	S7C
1a	IV	69	10	7.8	3.4	22	-	+++	n.a.	160	72 - 96	S7E
3	VI [(c)]	100	10	7.8	5	35	3	-	++	n.a.	60 - 72	S9A
1a	VI [(c)]	100	10	7.8	5	35	9	-	+	216	n.a.	S9B
1a	IV [(c)]	69	10	7.8	3.4	4	2	-	+++	24	12	S9C
1a/f0	XVI/VIII [(d)]	104/30	10	7.7	0	2	12	-	+++	72	30 - 36	S9F
f0(**1a**)	VIII [(e)]	90	5	7.4 – 7.8	0	7	6	++	-	168	36 - 48	S10B,C
f0(**1a**)	IX	55	5	7.6	0.2	-	20	n.a.	++	340	20	S11A
f0(**1a**)	IX	55	5	7.6	0.2	-	16	n.a.	++	216	24	S11B
f0(**1a**)	X	110	10	7.4	0	7	-	++	n.a.	120	48	S11D,E
f0(**4**)	XI	86 – 115	10	7.6	0	15	0	++	n.a.	n.a.	n.a.	S13A
Aβ_{1-28}/f0(**1a**)	XIII/VIII [(f)]	30/5	10	7.4	0	7	5	-	+++	240	13	S15A
Aβ_{1-28}	XIII [(d)]	50	10	7.6	0	-	35	n.a.	++	n.a.	160 - 200	S17A
Aβ_{1-28}	XIII [(d)]	40	0	7.0	0	16	18	n.a.	++	n.a.	n.a.	S17B
Aβ_{1-28}	XIII [(d)]	25	10	7.6	0	-	16	n.a.	+	384	n.a.	S17D(i)
3	XV [(g)]	170	10	7.6	0	14	18	-	+	n.a.	-	S18A,D
3	XVIII	50	8	7.6	0	6	21	-	+	n.a.	-	S20A,B,F
2	XIX	60	10	7.8	3.1	7	-	+	n.a.	144	36 - 60	S22F
1a	XX [(h)]	50	10	7.4	0	14	2	+	+++	336	24	S25D-F
1a/f0	XX/X	45/12	10	7.4	0	14	-	+++	n.a.	144	84	S25G

Notes. c[A]: peptide concentration in reaction stock; concentration ratios in f0-cross-seeding experiments are written respectively according to c[A]/c[f0]; c[B]: initial buffer concentration (phosphate buffer); t_1: incubation time at 37 °C; t_2: incubation time at room temperature (20 – 22 °C), t_{lag} : Latency of ACR initiation under constant incubation conditions or estimated lag-phase for onset of ACR upon induced environmental change (dilution, temperature, pH). If applicable, the observed half-times ($t_{1/2}$) of the ACR are annotated as approximate values by considering additional delays during LC-MS monitoring due to sample preparation and varying queue times during high-throughput screenings. Based on the relative fragment abundance (LC-UV trace intensities), $t_{1/2}$ can represent the half-life of ACR primary autocleavage or downstream autoprocessing as well as the half-time for complete consumption of the parent peptide. (*a*) Extent of hydrolysis: +++ 95 %, ++ 40 – 95 %, + 20 – 40 %, – 0 %. Notes on screening conditions: Stock entries I – XX refer to those described in Table SS1. Reaction samples derived from dilution of these stocks and were further treated as indicated: (*b*) ACR progression was monitored during continuous incubation at 37 °C upon 1:4.5 dilution of a preincubated reaction stock aliqout. (*c*) Monitoring of ACR at 20 °C upon downstream 1:4.5 dilution in Milli-Q. (*d*) Monitoring of ACR at 20 °C upon 1:2 dilution in Milli-Q. (*e*) After 3 days incubation at 20 °C the initial pH 8.2 PB (5 mM) of the sample was adjusted to pH 7.8 (17 mM) and kept 3 days at 20 °C prior to subsequent incubation at 37 °C. (*f*) ACR cross-seeding with activated f0(**1a**) and subsequent LC-MS monitoring at 20 °C. (*g*) long-term ACR monitoring upon 1:2 dilution with periodic changes of incubation temperature comprising further downstream dilution for LC-MS screening at 20 °C. (*h*) Spontaneous ACR at 20 °C without downstream dilution of the preincubated sample.

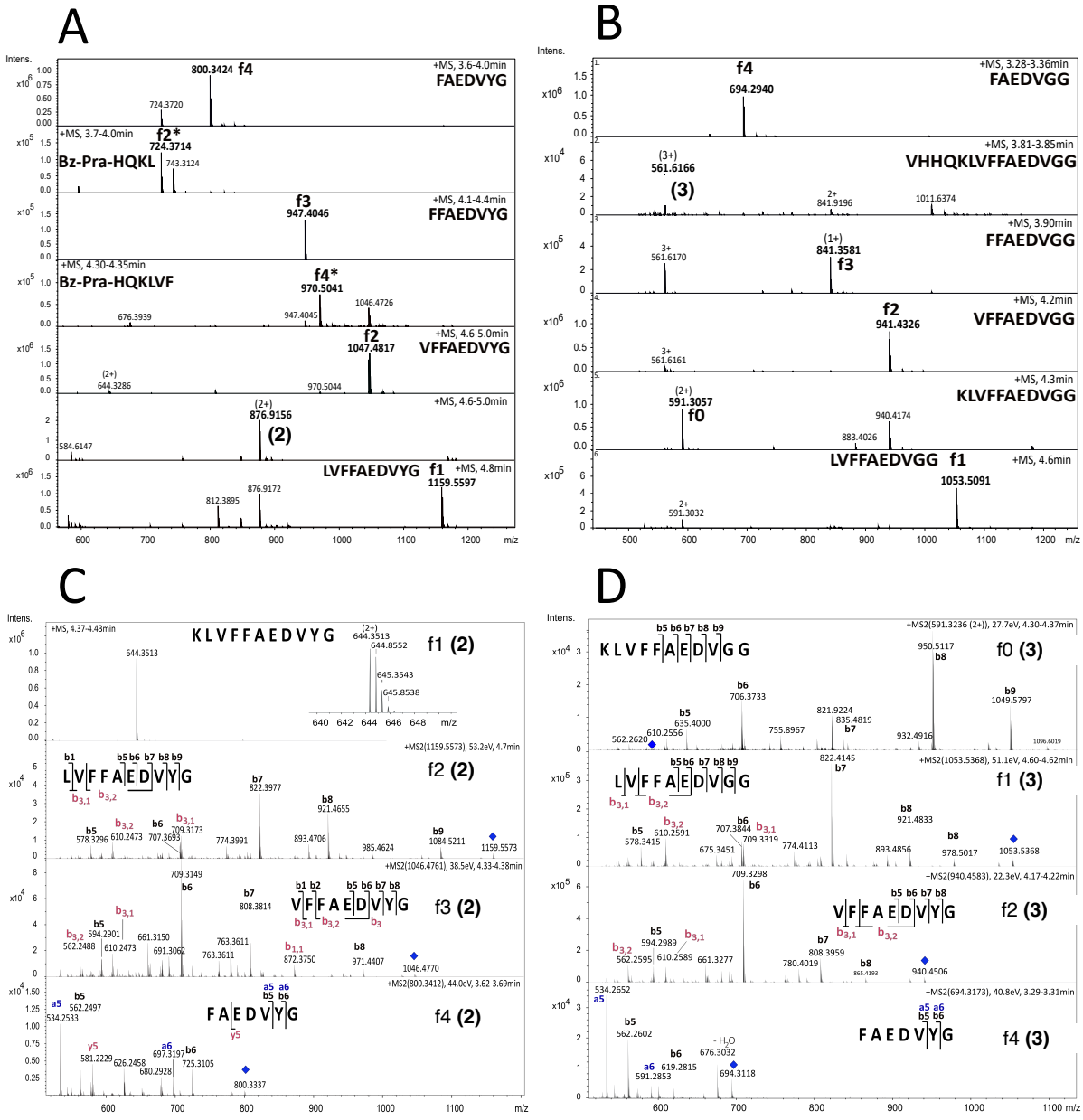

Figure S4. Mass spectroscopic identification of autocleavage fragments. (**A**) Stacked mass spectra (ESI-MS) of autocleavage fragments observed for peptide **2**. (**B**) Stacked mass spectra (ESI-MS) of autocleavage fragments observed for peptide **3**. (**C**) Stacked ESI-MS/MS spectra of *C*-terminal autocleavage fragments of peptide **2**. (**D**) Stacked ESI-MS/MS spectra of *C*-terminal autocleavage fragments of peptide **3**. Fragment identification is annotated according to the a, b, y - fragment series nomenclature. Annotated retention times correspond to the peak appearance in LC-MS recorded on a Dionex UltiMate® 3000 (Thermo Scientific) instrument.

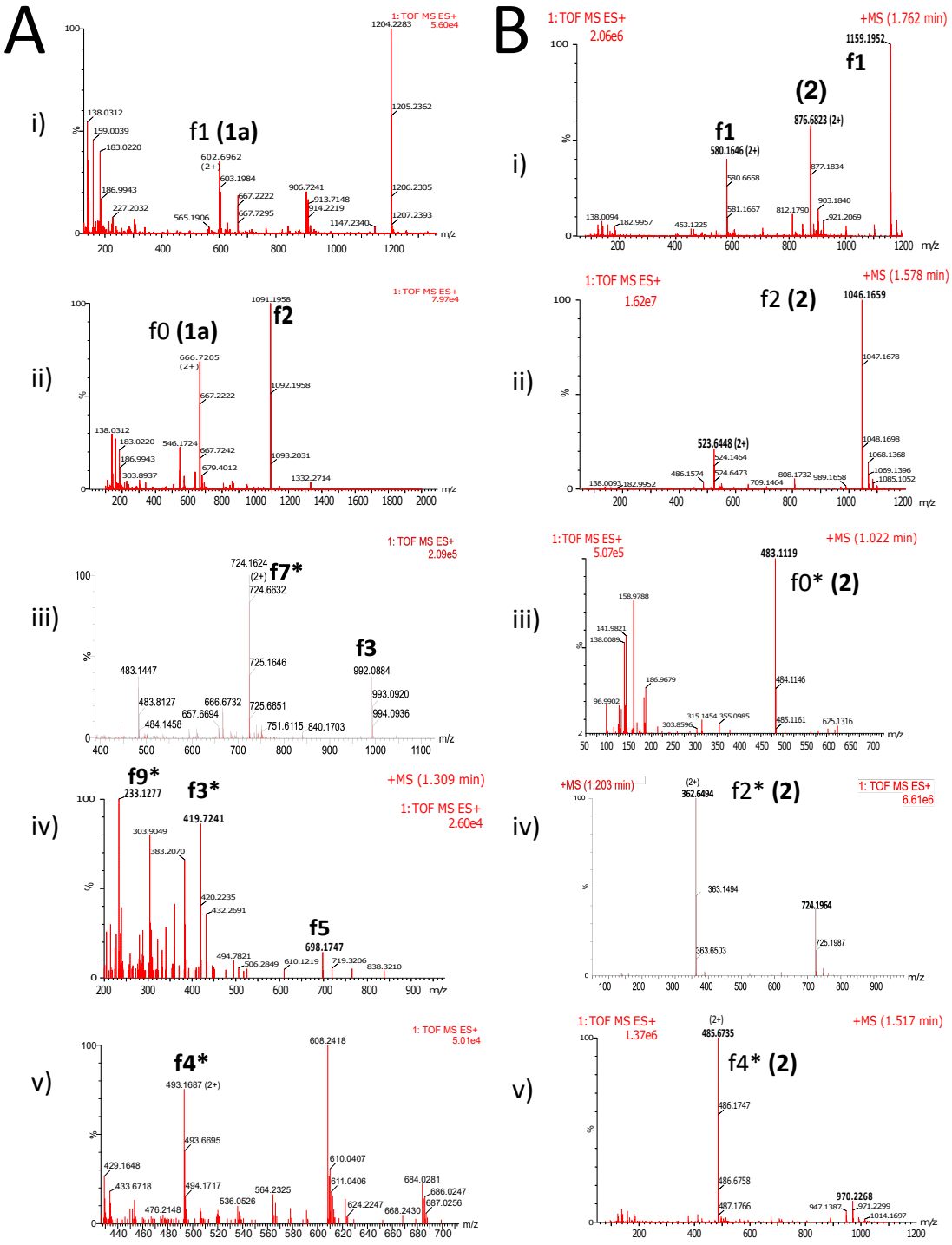

Figure S5. LC-MS fragment assignment. (**A**) Autocleavage fragments of **1a**: (i) f1 (LVFFAEDVY(NO_2)G), (ii) f0 (KLVFFAEDVY(NO_2)G) and f2 (VFFAEDVY(NO_2)G), (iii) f3 (FFAEDVY(NO_2)G) and f7* (Abz-Pra-HQKLVFFAED), (iv) f3* (Abz-Pra-HQKLV), f9* (Abz-Pra) and f5 (AEDVY(NO_2)G), (v) f4* (Abz-Pra-HQKLVF). (**B**) Autocleavage fragments of **2**: (i) f1 (LVFFAEDVYG), (ii) f2 (VFFAEDVYG), (iii) f0* (Bz-Pra-HQ), (iv) f2* (Bz-Pra-HQKL), (v) f4* (Bz-Pra-HQKLVF). All depicted MS data were acquired on a Waters Acquity™ UPLC system with a Xevo G2-S QTOF mass spectrometer(Waters Inc.).

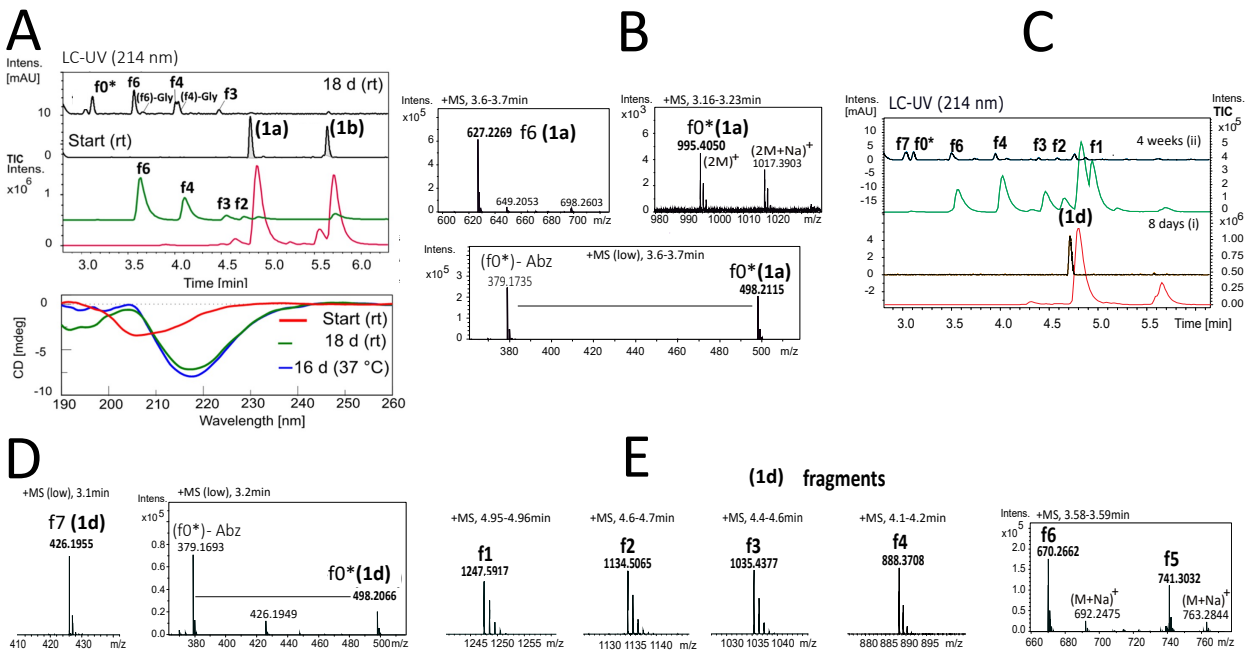

Figure S6. ACR at room temperature and identifcation of general ACR fragmentation pattern. (**A**) Top panel: Stacked LC-MS chromatograms (red and green (MS-TIC), black (UV-214 nm)) of autoproteolytic peptide mixture **1a**/**1b** (100 μM) in PB (10 mM), pH 7.6 at 20 ℃ (stock entry I, Table S1). Bottom panel: Overlaid CD for start of incubation (red CD) and after 18 days at 20 ℃ (green CD). The sample showed transition from random coil to β-sheet signature alike its replicate sample being incubated at 37 ℃ (blue CD) with LC-MS showing complete autohydrolysis (green MS-TIC, top panel). (**B**) Mass spectra of fragment EDVY(NO_2)G (f6(**1a**)) and Abz-Pra-HQ (f0*(**1a**)). (**C**) Stacked LC-MS traces of a repeat experiment (stock entry II, Table S1) using peptide **1d** (86 μM in PB (5 mM), pH 7.7 incubated for (i) 8 days at 37 ℃ followed by (ii) 21 days at 20 ℃. (**D**) MS spectra of fragment VY(NO_2)G-propylamide (f7(**1d**)) and Abz-Pra-HQ (f0*(**1d**)). (**E**) Assignment of **1d** autocleavage fragments to their mass peaks (+MS) with respective elution times as they appear in the LC-MS chromatogram.

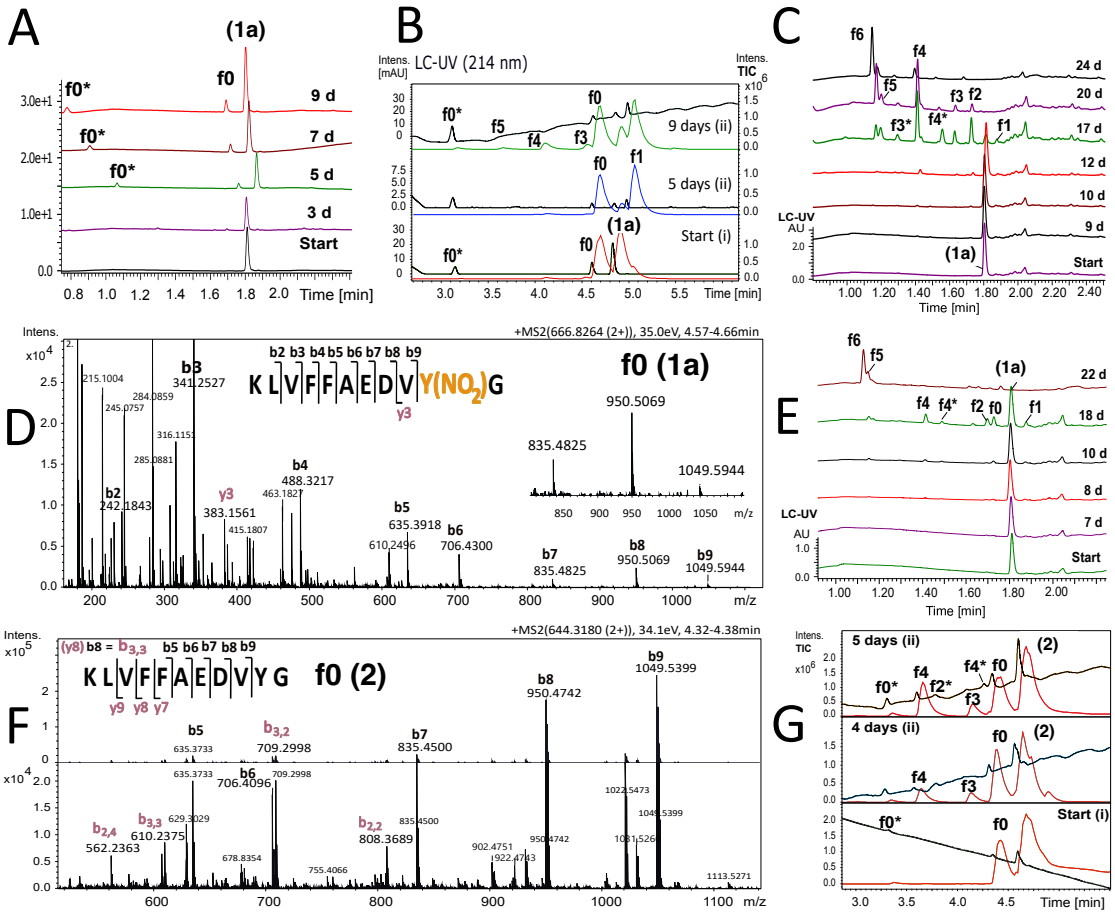

Figure S7. Identification of primary autocleavage fragments and kinetic trends of the ACR at 37 °C. (**A**) Stacked UPLC-chromatograms (colored traces) indicate autohydrolysis of **1a** (100 μM) in PB (10 mM), pH 7.6 containing 3.2 % DMSO (stock entry III, Table S1) at 37 °C. (**B**) Stacked LC-MS chromatograms (black (UV-214 nm), colored (MS-TIC)) of an aliquot of sample (**A**) after 9 days at 37 °C and (i) subsequent 1:4.5 dilution in Milli-Q water. (ii) LC-MS monitoring of dilute sample during subsequent incubation at 37 °C for another 9 days. (**C**) Stacked LC-UV traces for autohydrolysis of **1a** (100 μM, PB (5 mM), pH 7.6 containing 5 % DMSO) at 37 °C (for stock conditions see entry IV in Table S1). Annotated autocleavage fragments are listed in the MS spectra shown in Figure S5A. (**D**) LC-MS/MS sequencing of f0(**1a**). The *b*,*y* – fragmentation series of f0(**1a**) are shown in the ESI-MS/MS spectra (positive low mass range detection mode). (**E**) Stacked LC-UV chromatograms of a repeat reaction with **1a** (68 μM, PB (3.3 mM) pH 7.6 with 3.4 % DMSO) at 37 °C (for stock conditions see entry IV in Table S1). (**F**) LC-MS/MS sequencing of f0(**2**). The *b*,*y* – fragmentation series of f0(**2**) are shown in the ESI-MS/MS (positive wide mass range detection mode). (**G**) Stacked LC-MS-chromatograms (black (UV-214 nm), black (MS-TIC)) of a repeat reaction parallel to (**B**) using peptide **2** (158 μM, PB (10 mM), pH 7.6 containing 1.7 % DMSO). For stock conditions see entry III in Table S1. (i) LC-MS after 7 days at 37 °C and subsequent 1:4.5 dilution in Milli-Q water. (ii) LC-MS monitoring of dilute sample during incubation at 37 °C.

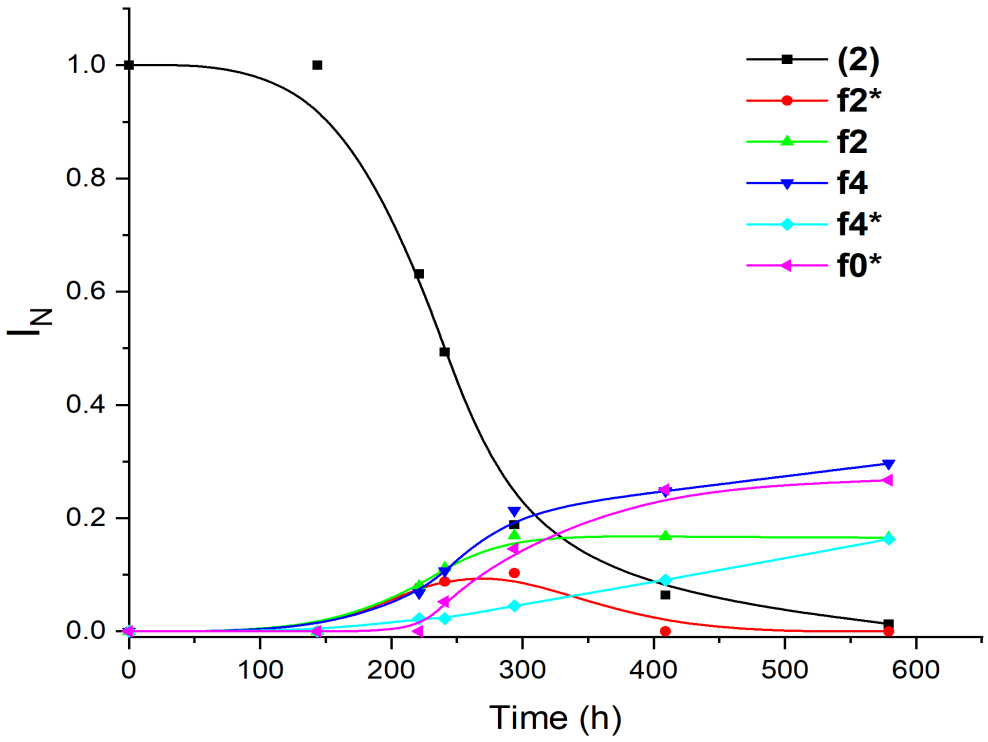

Figure S8. Kinetic analysis of the ACR. The kinetic curves represent plots of normalized LC-UV integral intensities (I_N) for ACR monitored by UPLC-MS. Sequence annotation for fragments are: peptide 2 (Bz-Pra-HQKLVFFAEDVYG), f2* (Bz-Pra-HQKL), f2 (VFFAEDVYG), f4 (FAEDVYG), f4* (Bz-Pra-HQKLVF) and f0* (Bz-Pra-HQ). The result shows that the changing composition of autoproteolytic aggregates can alter reactivity of the reaction. Clearly there are several pathways in the processing the most prominent early event being cleavage into f2 and f2*. Fragment f2* is then further cleaved probably leading to the formation of f0*. Fragment f0 is not observed and f0* is therefore derived with a delay mainly from f2* by caboxypeptidase activity. Direct cleavage at the Phe-Phe bond to give f4* is a slow process. Initially formed f4* is probably also subject to *C*-terminal processing to f2* and f0*. Therefore, f4 which is an end product is formed faster and does not correspond to peak intensities of f4*. It therefore, from the kinetic analysis seems that the autoprocessing is a result of a combination of endo- and carboxypeptidase activity of the peptide aggregates. It should be noted that the composition of these aggregates changes with the progress of the autoproteolysis and this change can influence the kinetics of processing with time.

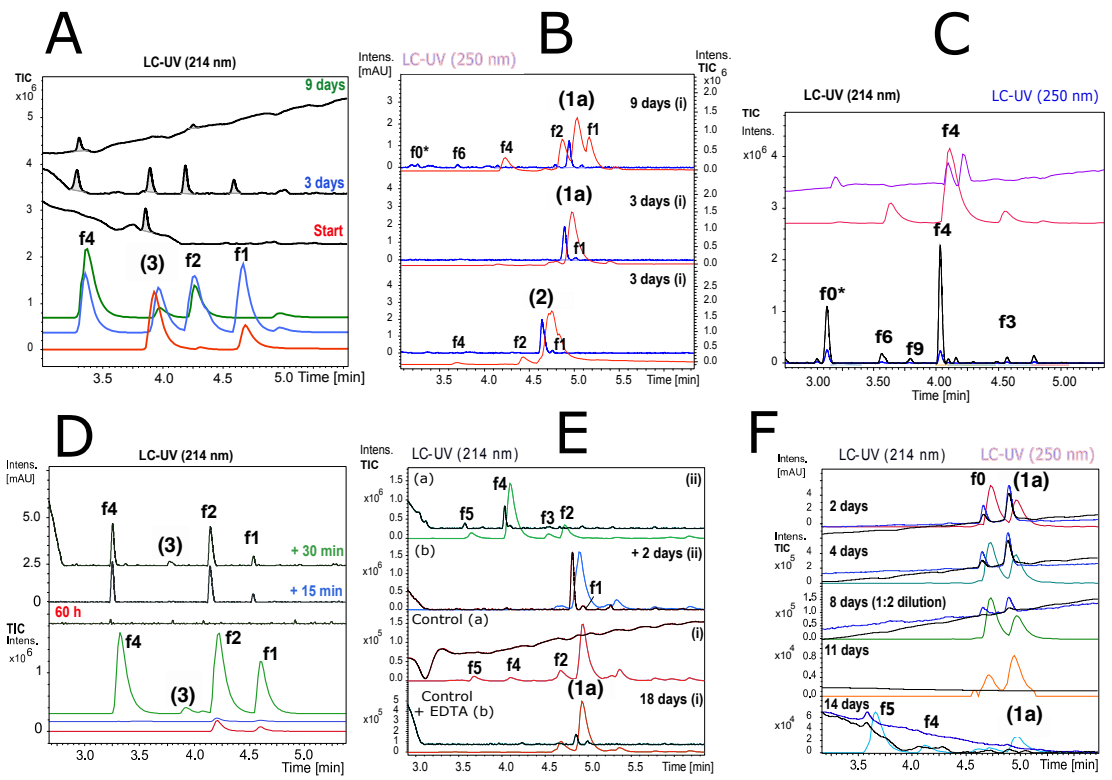

Figure S9. Aggregation behavior and downstream autohydrolysis upon dilution. (**A**) LC-MS monitoring (colored traces (MS-TIC), black traces (UV-214 nm)) of preincubated reaction stock solution of **3** (for conditions see Table S2, entry VI) upon 1:4.5 dilution in Milli-Q at 20 °C. (**B**) LC-MS chromatograms (red (MS-TIC), blue (UV-250 nm)) for parallel control reactions of peptide **1a** and **2** were conducted under the same conditions as described in (**A**) followed by 1:4.5 dilution in Milli-Q water (i) and continuous incubation at 20 °C. (**C**) Stacked LC-MS chromatograms (MS-TIC (wide mass range): red, MS-TIC (low mass range): magenta, UV-214nm: black trace). Conditions: **1a** (69 μM, PB (3.3 mM), pH 7.6 with 3.4 % DMSO) was incubated for 4 days at 37 °C (see stock conditions in Table S1, Entry IV). A retrieved aliquot showed complete hydrolysis upon 1:4.5 dilution in Milli-Q within 2 days at 20 °C while the undiluted reaction stock at 37 °C remained intact (Figure S7E). (**D**) After 4 days incubation at 37 °C an aliquot from a parallel repeat reaction with **3** (65 μM, PB (3.3 mM), pH 7.6 with 3.4 % DMSO) showed disappearance of LC-UV signal (aggregation) upon 1:4.5 dilution in Milli-Q. During high-throughput LC-MS screening the sample was analyzed again after 60 hours at 20 °C in three successive runs with different mass acquisition modes (colored MS-TIC chromatograms). Reappearance of the LC-UV(214 nm) trace indicated disaggregation and downstream autohydrolysis. (**E**) Stacked LC-MS chromatograms (colored (MS-TIC), black (UV-214 nm) for an independent repeat reaction (for stock conditions see Table S1, Entry VII): (a) with **1a** (100 μM, PB (10 mM), pH 7.8 with 5 % DMSO) and the replicate (b) with additional 200 μM EDTA ligand. Conditions: (i) 18 days incubation at 37 °C (ii) After subsequent 1:4.5 dilution in Milli-Q LC-MS was recorded after two more days incubation at 20 °C. (**F**) Stacked LC-MS chromatograms (colored (MS-TIC), black (UV-214 nm), blue (UV-250 nm)) of a mixture of **1a**/f0 (104/30 μM in PB (10 mM), pH 7.7) after two days incubation 37 °C and subsequent 1:2 dilution in Milli-Q water (Table S2, entry XVI/VIII). After 6 more days at 20 °C further 1:2 dilution triggered aggregation (disappearance of UV-trace) and downstream hydrolysis.

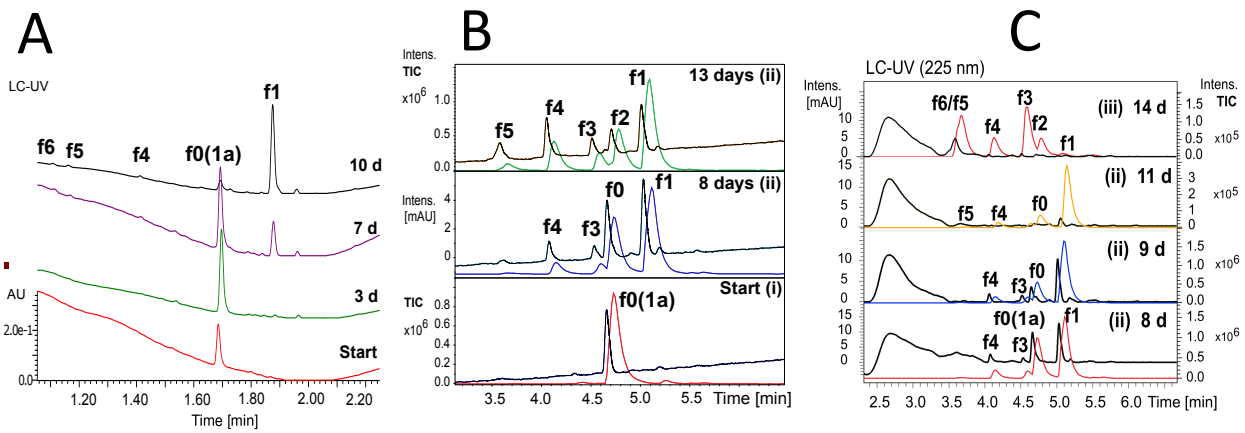

Figure S10. LC-MS monitoring of repeat reactions for f0-ACR activation. (**A**) f0(**1a**) (90 μM) in PB(10 mM), pH 7.8 showed no hydrolysis at 37 ℃ over a period of two weeks (for stock conditions see Table S1, entry VIII). However, a retrieved aliquot, which was subsequently 1:2 diluted in Milli-Q water showed downstream ACR at 20 ℃ (stacked UV-chromatograms in color according to their time of acquisition). (**B**) Stacked LC-MS chromatograms (colored (MS-TIC), black (UV-214 nm)) of an independent repeat reaction (f0(**1a**) (90 μM) in PB (5 mM)) under modified activation conditions (stock entry VIII, Table S1). (i) After three days incubation at 20 ℃ the initial pH 8.2 of the sample was adjusted to pH 7.8 (17 mM) and the sample (78 μM) was kept three more days in the dark at 20 ℃ prior to subsequent incubation (ii) at 37 ℃. In parallel, retrieved aliquots from the pre-activated reaction stock (after pH adjustment) were kept at 20 ℃ and further used for ACR-cross-seeding in Aβ_{1-28} (as shown in Figure S15A). (**C**) A replicate was subjected to the same incubation conditions as in (**B**) but pH was adjusted to pH 7.4 PB (14 mM). An aliquot was retained at 20 ℃ after pH adjustment and used for cross-seeding hydrolysis in Aβ_{1-28} (Figure S15D).

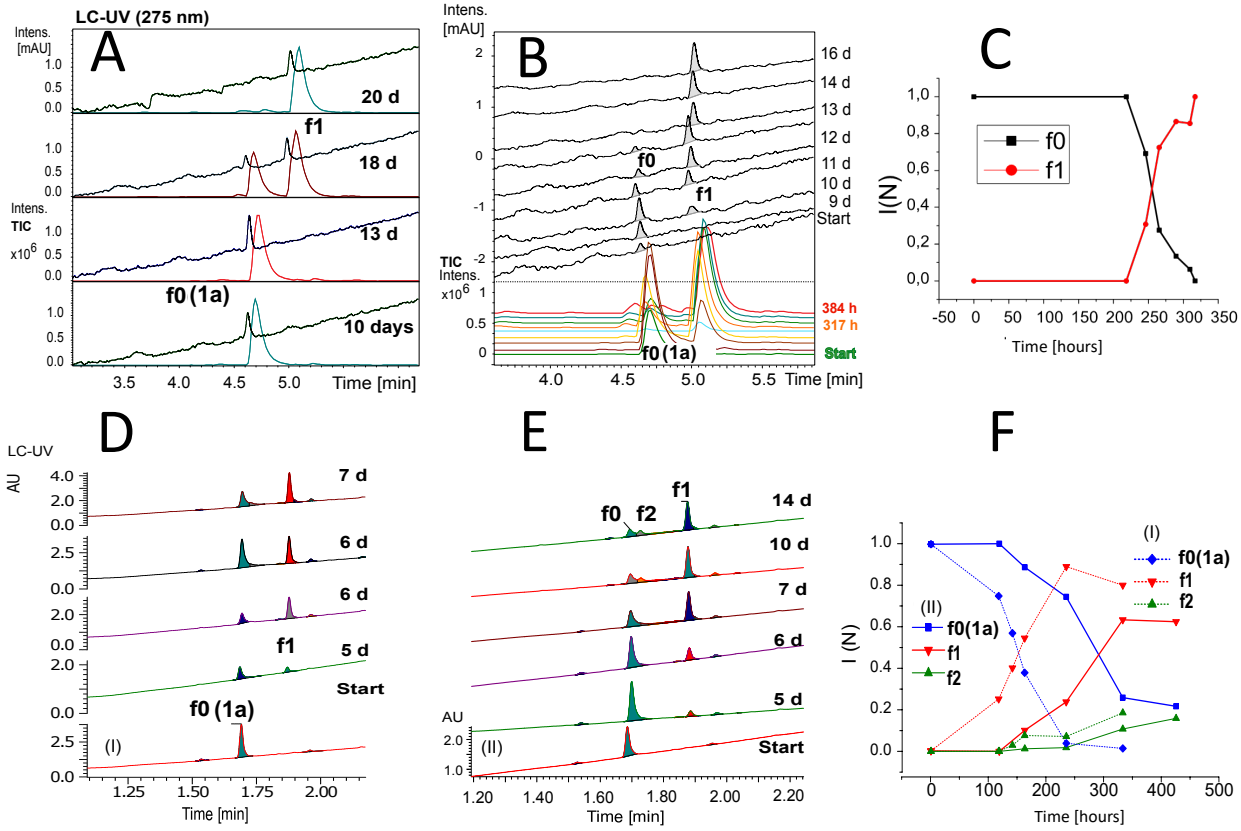

Figure S11. LC-MS monitoring of ACR kinetics of the primary autocleavage fragment f0(**1a**) (KLVFFAEDVY(NO_2)G). (**A**) Stacked LC-MS chromatograms (MS-TIC: colored traces, UV-275 nm: black traces) represent the kinetic profile of autohydrolysis of f0(**1a**) at 20 ℃. The sample (55 μM) was derived from a partially aggregated stock solution (stock entry IX, Table S1) upon 1:20 dilution in PB (5 mM), pH 7.6. The initial stock solution was stored for three weeks at 4 ℃.(**B**) Kinetic profile (colored traces (MS-TIC), black traces (UV-275 nm)) of a repeat reaction for f0-ACR at 20 ℃. Conditions: A replica mother stock solution as described under (**A**) was stored for three weeks at 20 ℃ prior to 1:20 dilution in PB (5 mM). (**C**) Kinetic plot of normalized LC-UV (275 nm) integrals for f0/f1 – autocleavage as shown in (**B**). (**D**) In an independent repeat experiment, incubation samples were derived from freshly prepared stock (see stock entry X, Table S1). Conditions: f0(**1a** (110 μM in PB (10 mM), pH 7.4) was incubated in the dark at 37 ℃. Aliquots (I) were collected periodically after 5 days and subjected to UPLC-MS monitoring at 20 ℃ (stacked UV-chromatograms). A duplicate of an aliquot retrieved after 6 days incubation showed altered fragment intensity ratio when measured first after 2 hours resting period at 20 ℃ suggesting disaggregation taking place. (**E**) Stacked UPLC chromatograms of replicate sample (II), containing additionally 0.05 w% sodium azide. (**F**) Kinetic profile for f0/f1 – autocleavage as shown in (**D**) (dotted colored lines) and (**E**) (straight colored lines).

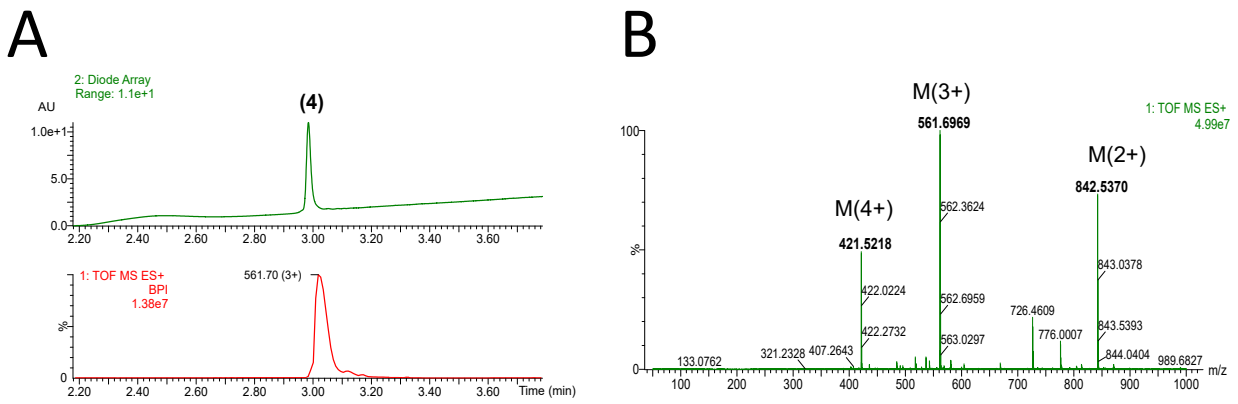

Figure S12. Analysis of purified D-amino acid peptide Aβ_{12-25}–gly. (**A**) LC-MS chromatogram (green LC-UV trace) of peptide **4** (vhhqklvffaedvgg) after HPLC purification. Base peak ion (BPI) chromatogram (red). (**B**) Mass spectra of peptide **4**.

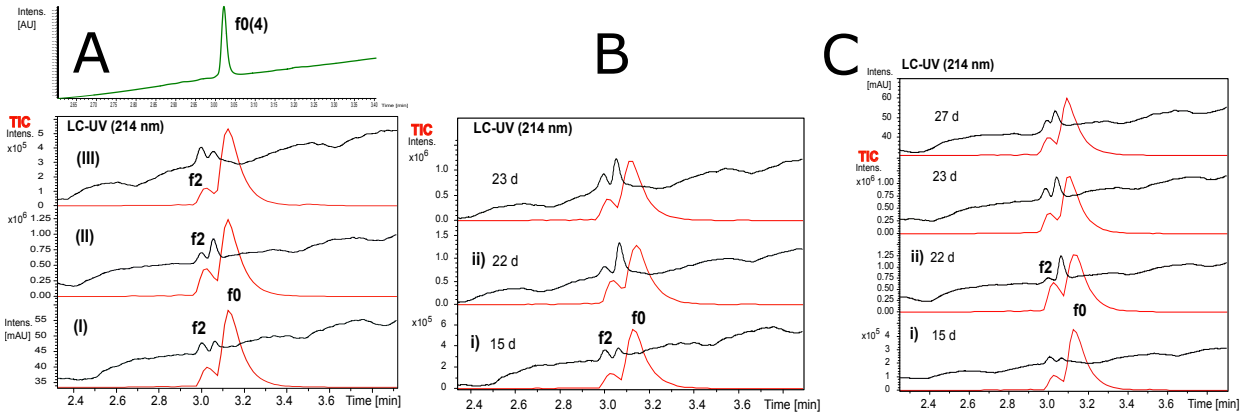

Figure S13. Autohydrolysis of D-amino acid peptide [Aβ_{16-25}]–gly (f0(**4**)). (**A**) HPLC-chromatogram (top) of purified Aβ_{16-25}–Gly (klvffaedgvgg). Below stacked LC-MS traces (red (MS-TIC), black (UV-214 nm)) show autohydrolysis of f0 to f2 in parallel repeat samples (I) – (III) after 15 days incubation under different conditions. Stock solution: Aβ_{16-25}–gly (0.86 mM in 8 mM $NaOH_{aq}$). (I) 115 μM peptide in PB (10 mM) pH 7.6 was incubated at 37 ℃ and subsequently 1:2 diluted in Milli-Q water after 15 days. (II) Replicate (115 μM) incubated for 9 days at 20 ℃ followed by 6 days at 37 ℃. (III) 86 μM in PB (10 mM) pH 7.6 incubated 15 days at 37 ℃. (**B**) After 15 days at 37 ℃ (i), sample (I) was 1:2 diluted in MilliQ water and kept at 20 ℃. (**C**) A parallel repeat reaction with f0(**4**) (43 μM in PB (10 mM) pH 7.6) was incubated (i) for 15 days at 37 ℃ and (ii) subsequently monitored by LC-MS (UV-214 nm: black traces, MS-TIC: red traces) at 20 ℃.

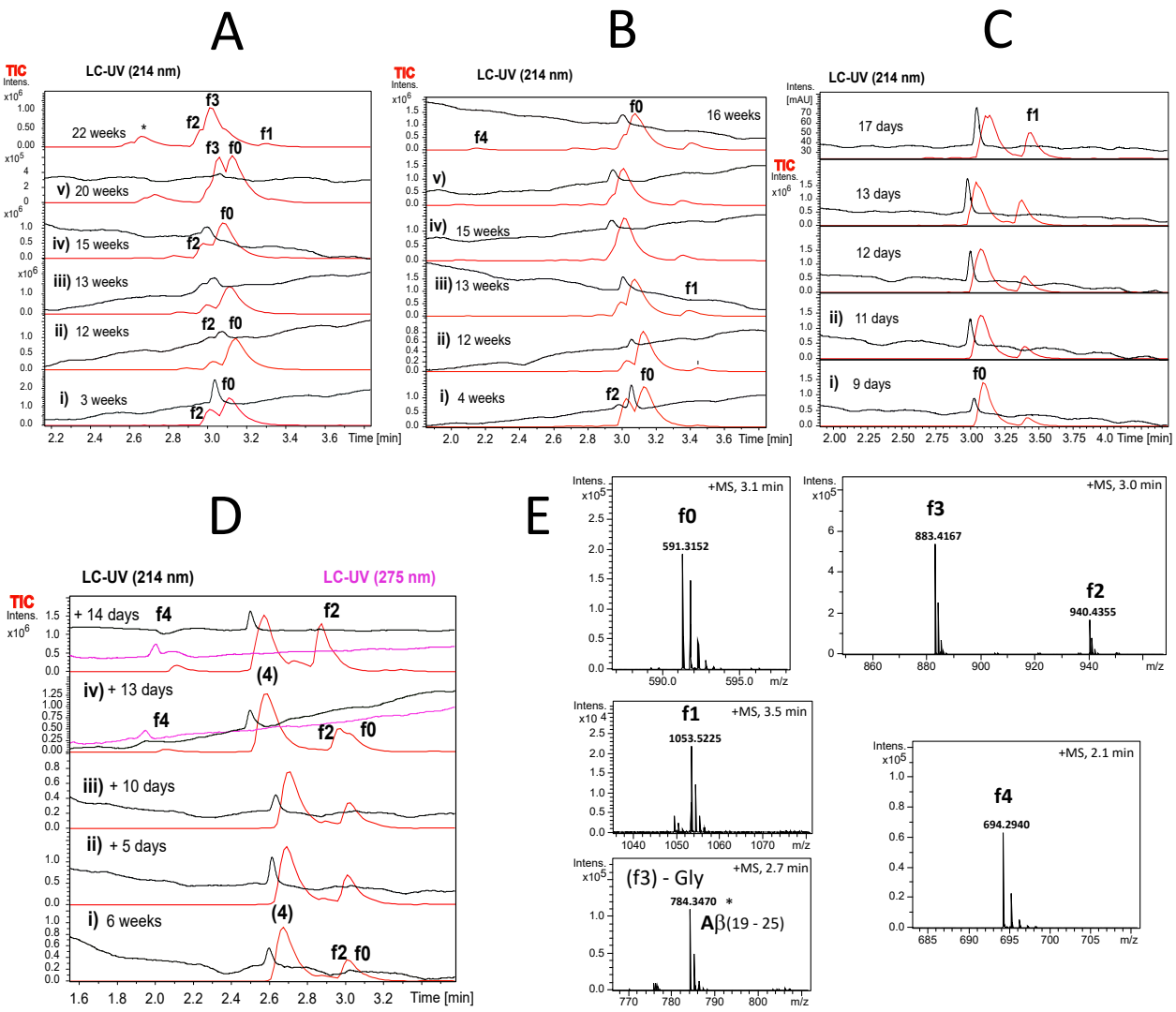

Figure S14. LCM-MS monitoring of conditional autohydrolysis of D-amino acid Aβ_{16-25}–gly. (**A**) Stacked LC-MS chromatograms (MS-TIC: red trace, UV-214 nm: black trace) of reaction sample (I) after three weeks incubation with occasional adjustment of pH to 7.6 – 7.8 and T changed between 20 ℃ and 37 ℃ as described in Figure S13A. (i) Upon 1:2 dilution in PB (10 mM) pH 7.6 the reaction was set to 20 ℃. (ii) After 9 more weeks temperature was changed between 20 – 37 ℃ every 3 days (iii). (iv) 1:2 dilution with adjustment to pH 7.8 was followed by resting period of 5 weeks at room temperature (v) the reaction sample was incubated for two days at 37 ℃ followed by LC-MS analysis at 20 ℃. (**B**) Stacked LC-MS chromatograms (MS-TIC: red, UV-214 nm: black) of reaction sample (II) (Figure S13A) as compared to (**A**) but without dilution and pH adjustment. Sample (II) showed significant, yet less processing than sample (I). (**C**) Reaction starting with f0(4) (100 μM in PB (10 mM) pH 7.48), (i) diluted 1:4 after 9 d at 37 ℃ and analyzed by LC-MS. (ii) Analyzed after 11 d at 37 ℃ and then (iii)-(vi) periodically at 20 ℃. (**D**) Peptide **4** (50 μM) in PB (10 mM, pH 7.6) was mixed with 25 mol% of the reaction mixture described in Figure S13C. pH changed from 7.6 to 8.46 during 6 weeks at 20 – 37 ℃ without showing hydrolysis. (i) Analysis after 5 days at 37 ℃, pH 7.75 and (ii) after 13 days at 37 ℃. (iii) Periodic analysis of this sample maintained at 20 ℃. (**E**) Mass peaks $[M+2H]^{2+}$ for f0(**4**) starting material and $[M+1H]^{+}$ for observed autocleavage fragments f1, f2, f3 and Aβ_{19-25} (*).

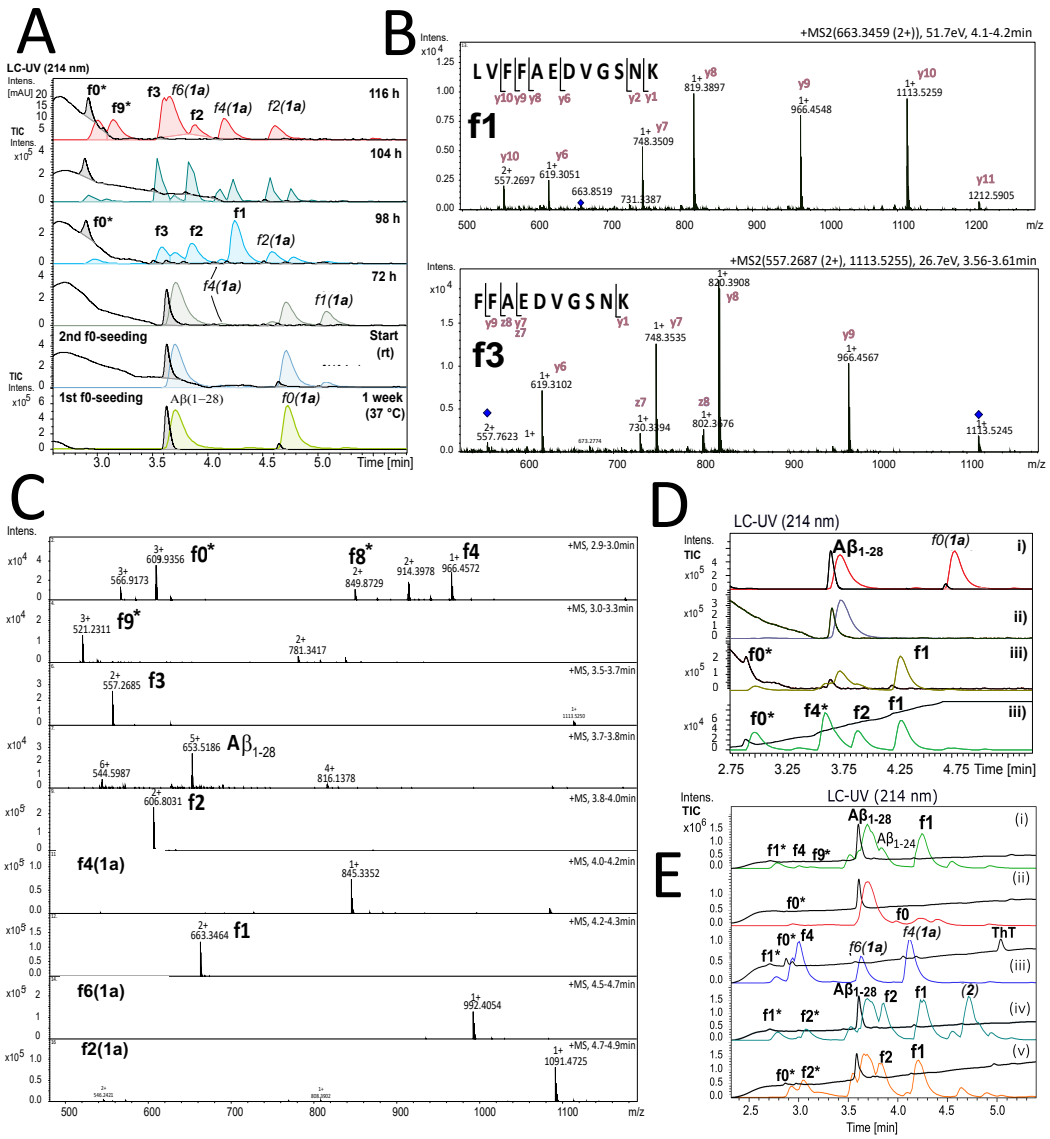

Figure S15. ACR seeding in Aβ_{1-28} via f0-cross-catalysis. (**A**) Aβ_{1-28} (30 μM in PB (10 mM), pH 7.6) was treated with an aliquot of f0(**1a**) (5 μM) (Table S1, entry IX) and incubated for 8 days at 37 °C (no hydrolysis). A second cross-seeding with activated f0(**1a**) (Figure S10B) induced hydrolysis, followed by LC-MS at 20 °C (UV-214 nm: black traces, MS-TIC: colored traces). (**B**) MS/MS sequencing of autocleavage fragments f1 and f3. (**C**) Stacked mass spectra, with assignment of Aβ_{1-28}-derived autocleavage fragments. (**D**) LC-MS chromatograms (black (UV-214 nm), colored (MS-TIC)) of repeat experiments: (i) Replicate of the sample shown in (**A**), without the second f0-cross-seeding. (ii) Repeat sample of Aβ_{1-28} (30 μM in PB (10 mM) pH 7.4) after 10 d 37 °C followed by 1:2 dilution in Milli-Q and 14 d at 20 °C. (iii) An aliquot of (ii), retrieved after 7d at 20 °C, received 30 mol% of activated f0(**1a**) (Figure S10C). Hydrolysis was detected after 4 days (iii) and 5 days (green MS-TIC chromatogram). (**E**) Set of repeat reactions: (i) Aβ_{1-28} (50 μM) in Milli-Q water after 11 weeks at 20 °C. (ii) Aβ_{1-28} (25 μM) in PB (10 mM), pH 7.6 after 11 weeks at 20 °C. (iii) Aliquot of (ii), retrieved after 10 weeks, that received additional f0(**1a**)-cross-seeding (7.5 μM) (see stock entry XII, Table S1) and ThT (40 μM). The mixture was incubated for 24 h at 37 °C followed by 6 days at 20 °C. (iv) Aβ_{1-28} (25 mM) in PB (5 mM), with 10 mol% of peptide **2** after 7 weeks 20 – 37 °C. (v) Aβ_{1-28} (25 μM) in PB (5 mM) after 7 weeks incubation at 20 – 37 °C.

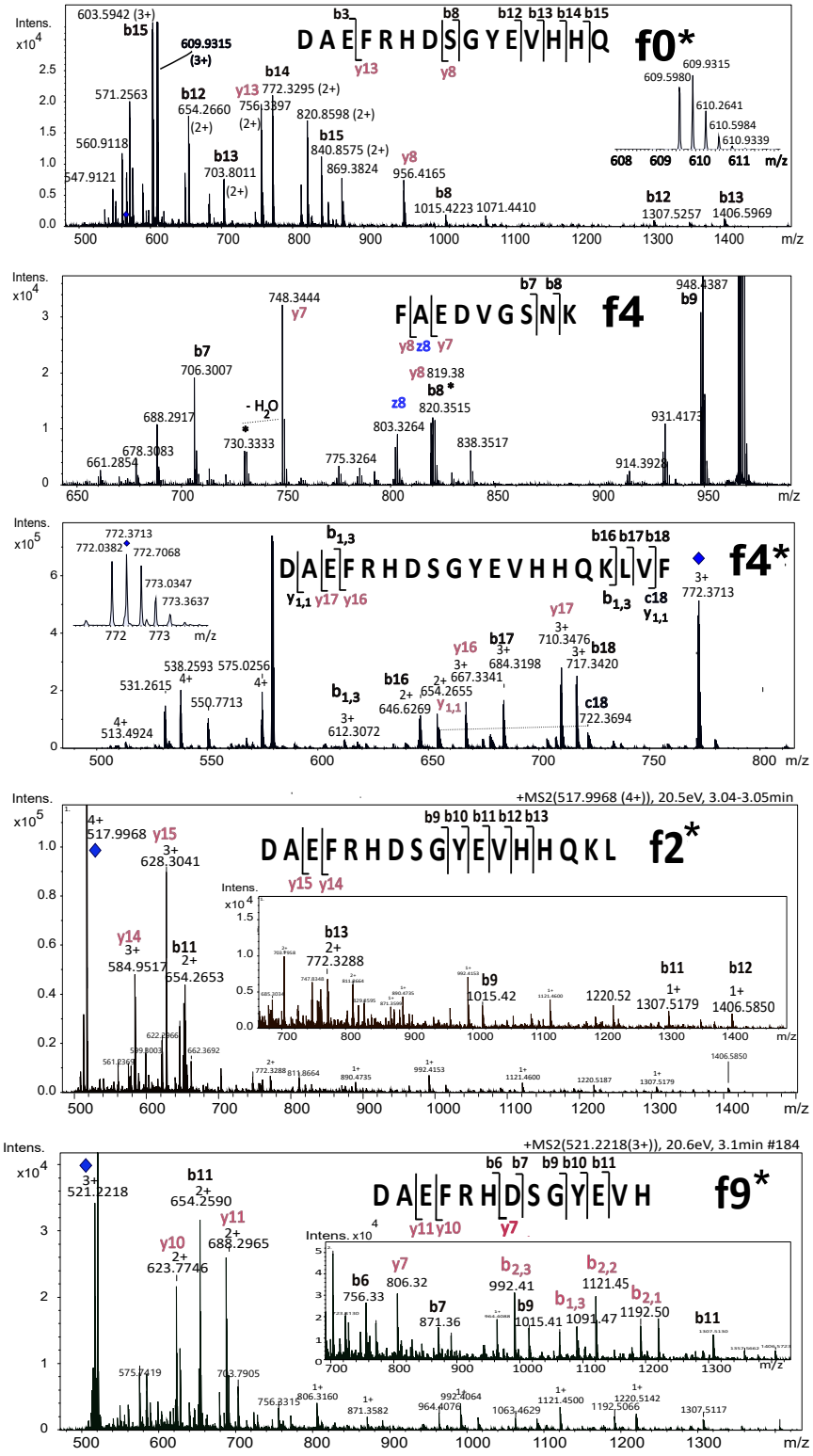

Figure S16. MS/MS sequencing of Aβ_{1-28} autocleavage fragments: f0* (Aβ_{1-15}), f4 (Aβ_{20-28}), f4* (Aβ_{1-19}), f2* (Aβ_{1-17}), f9* (Aβ_{1-13})

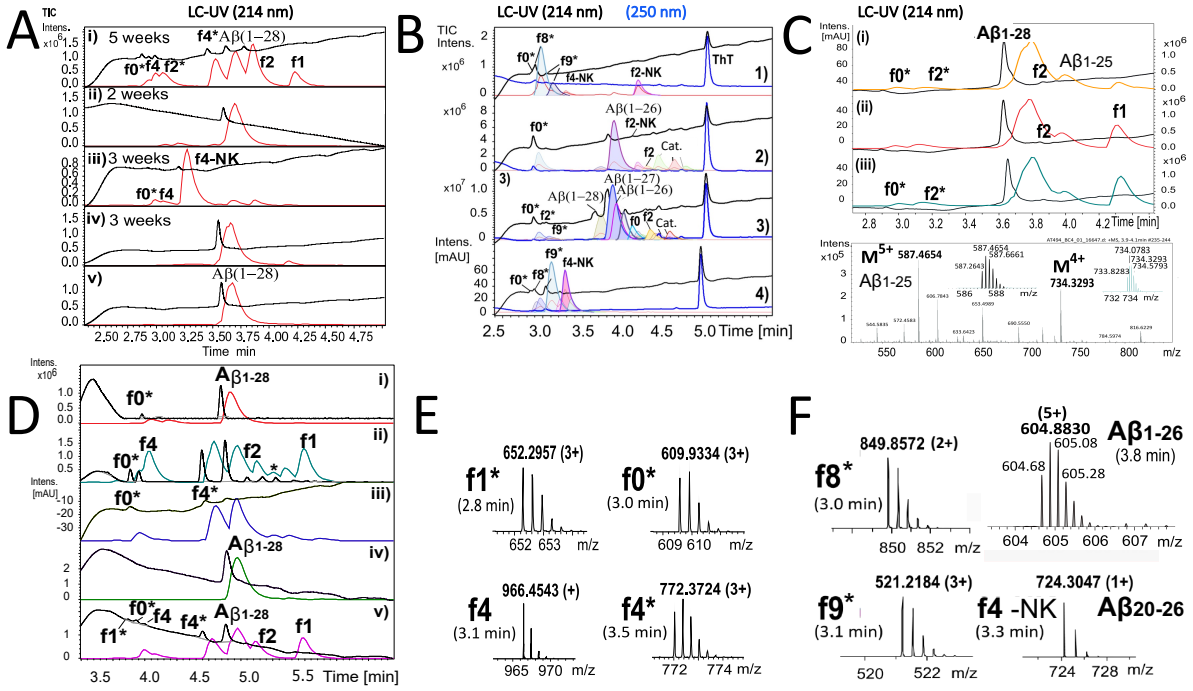

Figure S17. Autohydrolysis in Aβ_{1-28} and repeat assays with palladium peptide catalysts. (**A**) (i) Aβ_{1-28} (50 μM) in PB (10 mM), pH 7.6 was incubated 24 days at 20 ℃ prior to 1:2 dilution in Milli-Q water and 14 days succeeding incubation at 20 ℃. (ii) A replica aliquot stayed intact upon downstream dilution after 18 days at 20 ℃. (iii) A second replicate, retrieved after 23 days for subsequent 1:2 downstream dilution in Milli-Q water, showed instead weak fractions autocleavage fragments of f0*/f4/(f4)-NK with NK: Asn_{27}–Lys_{28}. (iv-v) Negative controls of Aβ_{1-28} (50 μM in neutral Milli-Q water (iv) and (v) 25 μM in PB (10 mM), pH 7.6 upon 1:2 dilution in Milli-Q water after 24 days at 20 ℃. (**B**) Stacked LC-MS spectra from 4 samples that were obtained from a ThT aggregation assay (data not shown) after three weeks incubation at changing temperature (23 – 37 ℃). Aβ_{1-28} (40 μM) in pure Milli-Q water (1),(4) and samples (2),(3) containing a palladium catalyst (Cat). (**C**) Stacked LC-MS traces (colored (MS-TIC), black (UV-214 nm)) from a set of parallel control samples after 7 days at 37 ℃ followed by 1:2 dilution in Milli-Q. MS spectra below shows mass peak for the autocleavage trace fragment A$\beta 1-25$. Stock conditions: (i) Aβ_{1-28} (52 μM in PB (10 mM), pH 7.6). (ii) Replicate containing a palladium catalyst. (iii) Replicate negative control in neutral Milli-Q water. (**D**) Stacked LC-MS traces (colored (MS-TIC), black (UV-214 nm)) of repeat reactions: (i) Aβ_{1-28} (25 μM in PB(10 mM), pH 7.6) after 16 days at 20 ℃, 2 hours upon dilution. (ii) Aβ_{1-28} (40 μM in neutral Milli-Q water with 200 mM urea and a palladium catalyst (*) after 8 days at 20 ℃. (iii) Aβ_{1-28} (15 μM) in neutral Milli-Q water containing a palladium catalyst after 11 days at 20 ℃ and subsequent 1:2 dilution. AFM imaging on this aging sample revealed thin fibrils after LC-UV signal had disappeared (Figure S23D). (iv) Undiluted mother stock. (v) Parallel repeat reaction of Aβ_{1-28} (25 μM in PB (5 mM), pH 7.6) containing a palladium catalyst after three weeks at 20 ℃ and subsequent 1:2 downstream dilution. (**E**,**F**) Molar mass peaks of the identified autocleavage fragments in Aβ_{1-28} ACR. f1* (Aβ_{1-16}), f0* (Aβ_{1-15}), f4 (Aβ_{20-28}), f4* (Aβ_{1-19}), f8* (Aβ_{1-14}), f9* (Aβ_{1-13}), Aβ_{1-26} and Aβ_{20-26}.

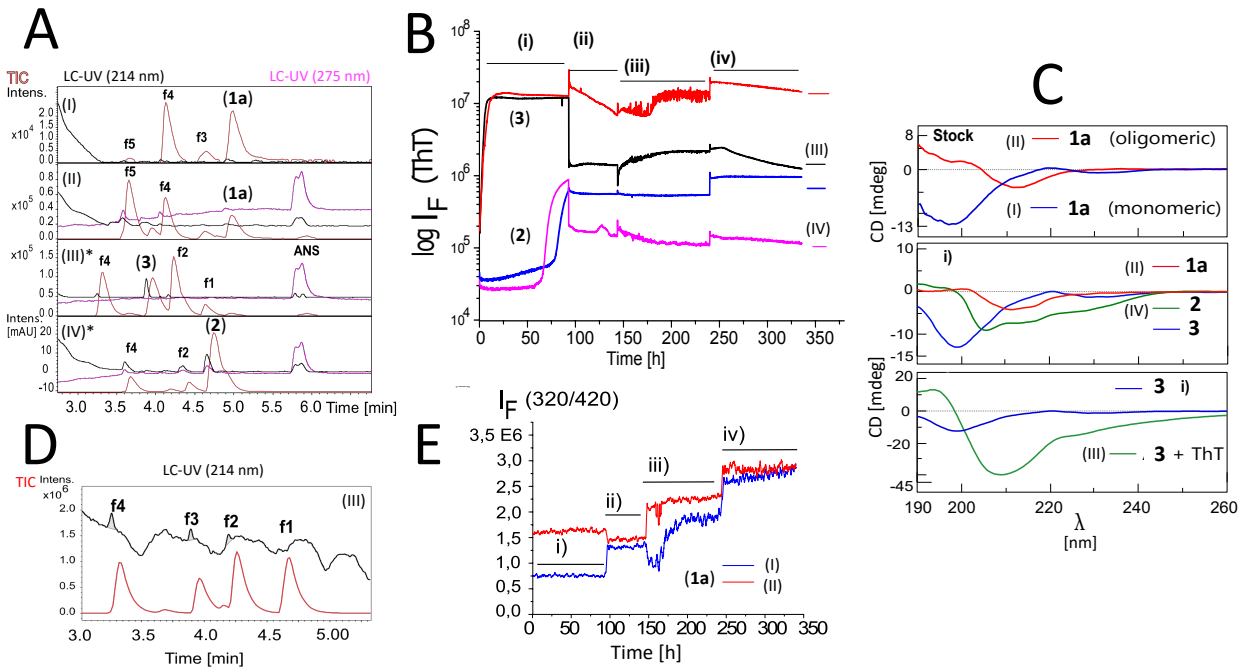

Figure S18. Spectroscopic characterization of aggregation properties and ACR progression of Aβ_{14-24} congeners. (**A**) Stacked LC-MS traces (black (UV-214 nm), magenta (UV-275 nm), red (MS-TIC)) of parallel reaction samples of **1a** – **3** obtained from a long-term plate reader assay with several fluorescent probes. Autohydrolysis was detected after a 5 weeks incubation period (i-iii) as obtained from assays shown (**B**) and (**E**). Samples (I) and (II) were derived from reaction stocks as annotated in (**C**) and (**E**). ANS: 8-Anilinonaphthalene-1-sulfonic acid. (**B**) ThT aggregation kinetics of peptide **2** (170 μM in PB (10 mM) pH 7.5, ThT (60 μM), stock entry XIV, Table S1) and **3** (170 μM in PB (18 mM), ThT (60 μM), stock entry XV, Table S1). Incubation conditions: (i) After shaking (5 sec) 4 d observation at 37 ℃ followed by 6 d at 20 ℃. (ii) 1:2 dilution in Milli-Q (red and blue graph), 1:3 dilution (black and magenta graph), respectively, and then 2 d observation at 37 ℃ followed by 16 d at 20 ℃. (iii) 4 d observation at 37 ℃. (iv) Shaking (10 sec) followed by 4 d observation at 37 ℃. (i-iv) No periodic agitation during consecutive fluorescence reading at 37 ℃. (**C**) Stacked CD spectra of **1a** (top panel): (I) Blue CD, derived from 1:2 dilution of a working stock solution (**1a** (220 μM) in PB (40 mM), pH 7.8, 8 % acetonitrile/water, stored for 4 d at 4 ℃) showed no secondary structure. (II) In parallel, 1:7.5 dilution of a hydrogel stock solution of (**1a**) (stored for 4 d at 4 ℃, see stock entry XVI, Table S1) to 97 μM in PB (20 mM), pH 7.4 showed β-sheet signature (red CD). Mid panel: Stacked CD-spectra from 1:2 diluted incubation samples of **1a** – **3**, obtained from plate reader assay (**B**) after 4 days incubation (i) at 37 ℃. Bottom panel: (i) **3** fibrillation sample with ThT (green CD) and replicate sample of **3** without ThT (blue CD). (**D**) LC-MS analysis (black trace (UV-214 nm), red trace (MS-TIC)) of an aliquot, retrieved from fibrillation sample (III) after 5 weeks incubation as annotated in (**B**), indicated spontaneous downstream autohydrolysis upon 1:4 dilution and further incubation at changing temperature (20 – 37 ℃) over two weeks. (**E**) Kinetic plot of FRET fluorescence intensity (I_F) of **1a**. Incubation conditions (i-iv) were the same as in (**B**). Sample (I) (110 μM) derived from random coil stock (blue CD in (**C**)) and sample (II) (104 μM) in 10 mM PB at pH 7.6) derived from stock with preformed β-sheet (red CD in (**C**)). *LC-MS on parallel replicate samples containing ANS dye.

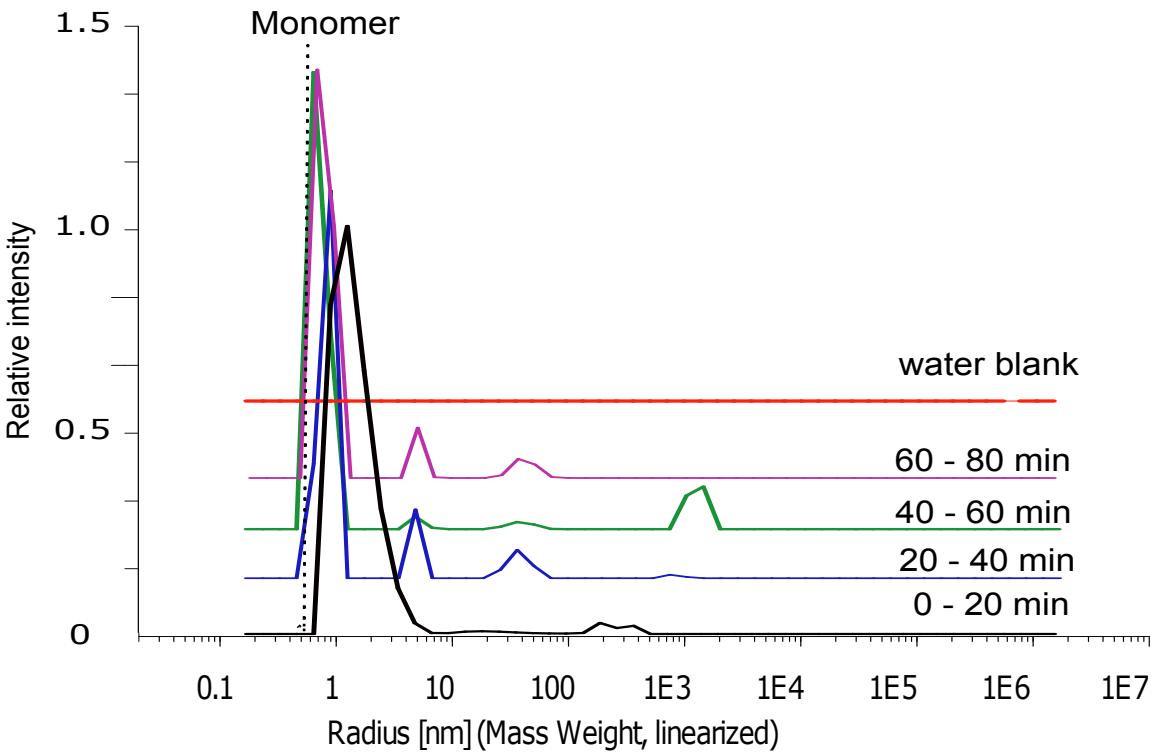

Figure S19. Dynamic light scattering on diluted stock solutions. Stacked distribution functions of the transformed autocorrelation function for DLS in the linear mass-weighted fitting mode on Milli-Q water (blank) and a sample of peptide **1a** diluted in phosphate buffer (PB). The average count rate for Milli-Q water was 4000 – 5000 counts per second. The average count rate for dilute screening samples was between 12000 – 20000 counts per second. Conditions: A freshly prepared stock solution of **1a** (4 mM) in 64 % acetonitrile/water with NaOH (9.1 mM) was diluted 1:40 in PB (10 mM, pH 7.4) and filtered 3 × over a syringe filter (0.22 μM, PTFE). DLS was monitored at 25 °C, over a period of 80 minutes in which the light scattering power was recorded in 20 cycles (a 60 seconds) for each acquisition of the autocorrelation function. The vertical black dotted line points to the expected hydrodynamic radius $R_{\mathrm{hyd}} = 0.5 \pm 0.1$ nm for the monomer (0.5 monomers per nm). The range for the respective hydrodynamic radius (R_{hyd}) is estimated from the value of $R_{\mathrm{hyd}} = 0.9 \pm 0.1$ nm for the Aβ_{1-40} monomer in water as previously reported by others.[7] The stacked size distribution functions (colored lines) show largely monomeric peptide and small populations of low molecular weight (LMW) oligomeric species around $R_{\mathrm{hyd}} \sim 4$ nm. Initial variations of the size distribution (R_{hyd}) could occur due to conformational fluctuations between monomers and dissociation of short-lived low molecular weight (LMW) oligomers. Aliquots of the stock solution were used for hydrolysis assays described in Figure S25C.

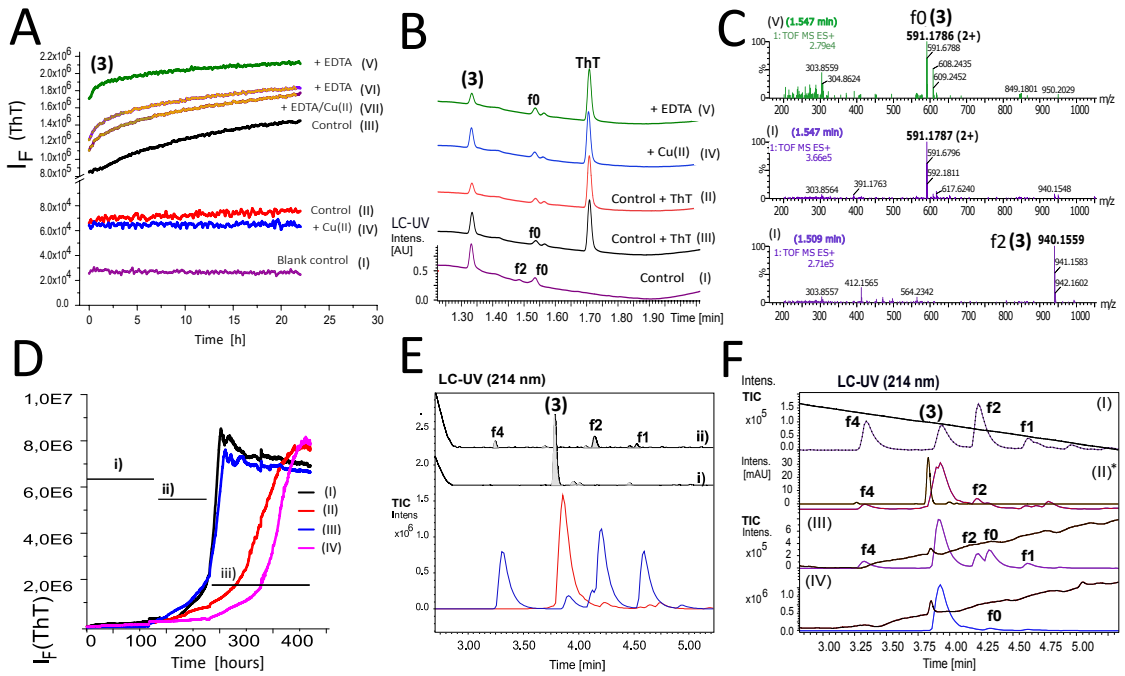

Figure S20. Detection of ACR autocleavage fragments in ThT - β-sheet fibrillation samples. (**A**) ThT fibrillation assay with peptide **3** (50 μM) in 10 mM PB (2.5 % DMSO), pH 7.6 (for stock conditions see entry XVIII, Table S1). Sample conditions: (I) negative control (no ThT), (II-III) replicates with ThT (146 μM), (IV) with additional Cu(II) acetate (2.5 μM), (V) ThT (180 μM) with EDTA (50 μM), (VI) with EDTA (100 μM), (VII) with Cu(II) acetate (2.5 μM) and EDTA (50 μM). Incubation conditions: After 7 days at 37 ℃ (3 seconds periodic orbital shaking after every 10 minutes) no ThT response was observed in any sample (data not shown). Continuous incubation for 10 hours at 20 ℃ and gradual warming to 30 ℃ (1 h), followed by 5 seconds orbital shaking, induced β-sheet fibril aggregation as indicated by rapidly increased ThT fluorescence. (**B**) Stacked LC-UV trace of undiluted ThT fibrillation samples (I) – (V) after 16 more days incubation at 20 ℃ showing little or no effect of Cu(II) and EDTA on primary autocleavage. (**C**) Mass spectra of sample (V) and (I) confirmed identity of the fragments f0 (KLVFFAEDVGG) and f2 (VFFAEDVGG). (**D**) An independent repeat experiment (stock entry XIX in Table S1) with peptide **3** (130 μM, 1.25 % DMSO, PB (10 mM), pH 7.6) afforded ThT fibrillation samples: (I) ThT (150 μM) control, (II) with 2.5 μM Cu(II) acetate, (III) with 7 μM Cu(II) acetate and (IV) with 45 μM EDTA. Incubation conditions: (i) 6 days at 30 ℃, (ii) 4 days at 37 ℃, (iii) 8 days at 30 ℃ without agitation. (**E**) Stacked LC-MS traces (colored (MS-TIC), black (UV-214 nm)) of a 1:4 diluted aliquot of fibrillation sample (II), red graph in (**D**). LC-MS was recorded two hours upon aliquot dilution (red MS-TIC chromatogram) at 20 ℃ (i) and again (ii) after 7 days incubation at 20 ℃ (blue MS-TIC-chromatogram). (**F**) Stacked LC-MS traces (colored (MS-TIC), black (UV-214 nm)) of fibrillation samples (I) and (II)*, retrieved from plate reader ThT assay (**D**), 8 days upon 1:4.5 dilution in Milli-Q and aliquots of (III) and (IV) stock samples, as shown in (**B**), 2 hours upon 1:4.5 dilution in Milli-Q. *LC-MS on parallel replicate sample containing ANS dye.

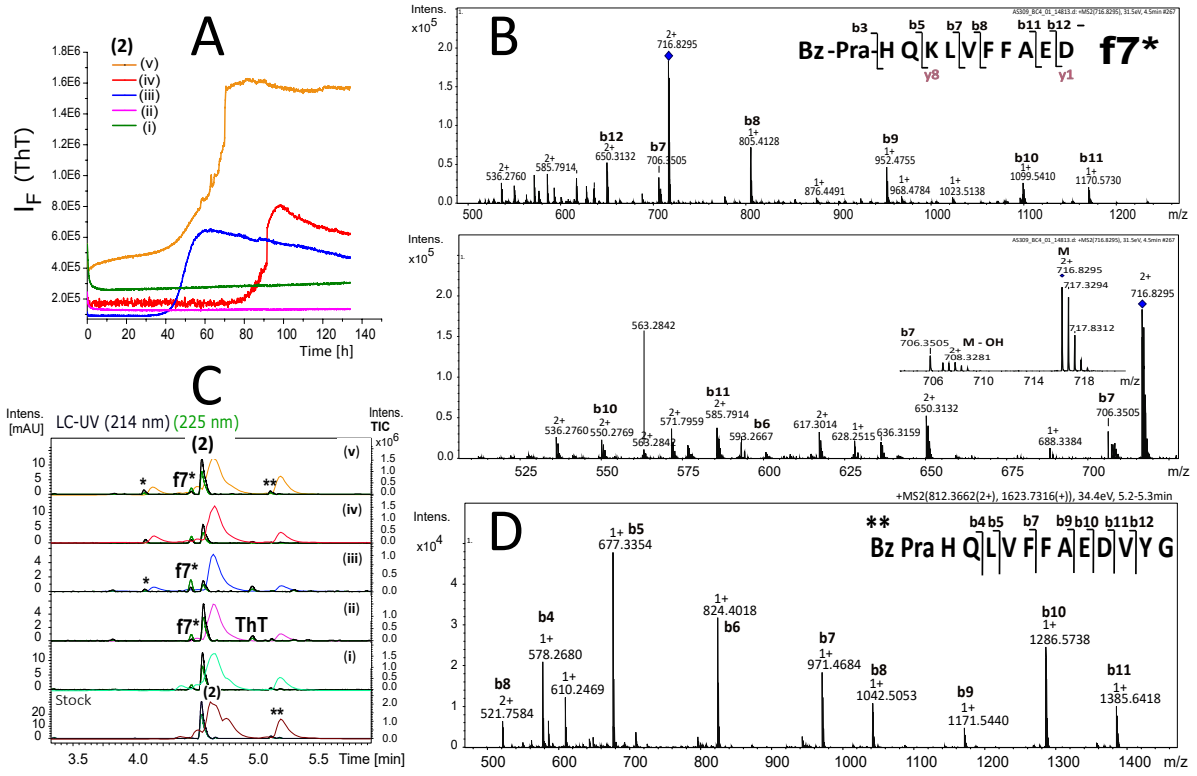

Figure S21. Cu(II) influence on β-sheet aggregation in **2** and identification of the f7*-autocleavage pathway. (**A**) Kinetic aggregation profiles (colored graphs (i) - (v)) for ThT fluorescence (λ_{ex} = 440 nm, λ_{em} = 486 nm) indicated moderate fibrillization of peptide **2** in the presence of Cu(II) ions. Assay conditions: Aliquotes from a freshly prepared stock solution of **2** (4.6 mg/mL in 50% acetonitrile/water; c(**2**) = 2.6 mM) were diluted 1:20 to a working stock concentration of 130 μM in PB (5 mM) at pH 7.8. The final screening samples were supplemented with ThT (100 μM) and additional Cu(II) acetate (45 μM). Sample conditions: (i) Negative control of **2** (60 μM) in PB (2.5 mM) without Cu(II), (ii) Negative control of **2** (30 μM) in PB (1.2 mM) without Cu(II), (iii) **2** (30 μM) in PB (1.2 mM) with Cu(II), (iv) **2** (60 μM) in PB (2.5 mM) with Cu(II), (v) **2** (118 μM) in PB (5 mM) with Cu(II). After 5 seconds orbital shaking at 23 ℃ the ThT flourescence was monitored at 37 ℃ without mechanical perturbation over a period of 6 days. Thereafter, aliqouts of (i)-(v) were diluted 1:4.5 in Milli-Q water and directly subjected to LC-MS analysis. (**B**) MS/MS sequencing of the main autocleavage fragment f7* (Bz-Pra-HQKLVFFAED) as obtained from LC-MS analysis. (**C**) Stacked LC-MS chromatograms obtained from dilute fibrillation samples. The product (*), annotated at 4.1 minutes elution time, is the HQKLVFFAEDVYG fragment as indicated by MS (Figure S22C). (**D**) MS/MS-fragment sequencing of impurity (**).

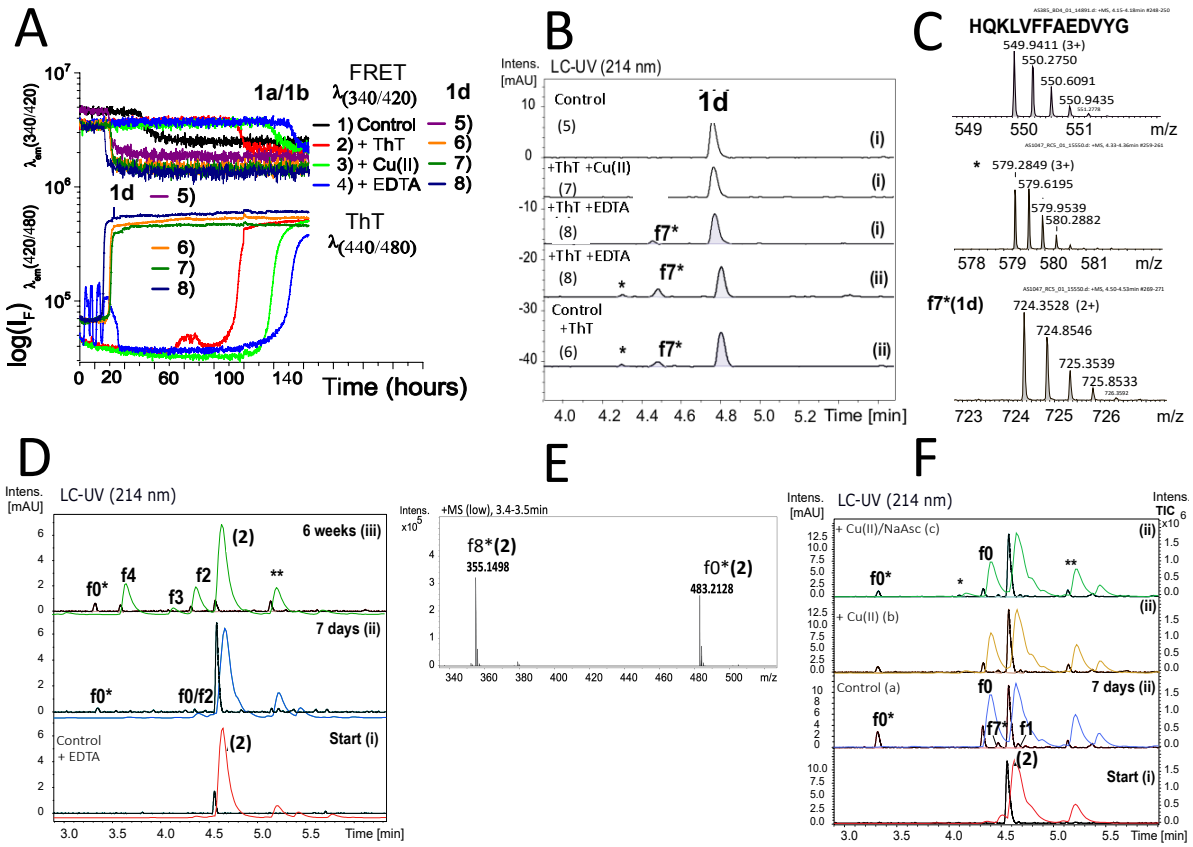

Figure S22. ThT aggregation assay and influence of copper trace metal and EDTA on autohydrolysis. (**A**) Kinetic aggregation assay via dual fluorescence probe (FRET - and ThT) of **1a**/**1b** (200 μM, PB 8 mM, pH 7.8) and **1d** (172 μM, PB 8 mM, pH 7.8). After initial 5 sec orbital shaking incubation succeeded at 30 ℃ over 6 days without mechanical agitation. Plotted kinetic graphs of FRET fluorescence: (1) and (5) controls without ThT; (2) and (6) replicates with ThT (153 μM); (3) and (7) with ThT and Cu(II) (2.3 μM); (4) and (7) with ThT and EDTA (45 μM). Respective ThT fluorescence is plotted below. (**B**) LC-MS analysis of samples (5)–(8) shown in (**A**) after 14 more days incubation at 37 ℃. (i) Stacked LC-chromatograms (UV-214 nm) of 1:4.5 diluted aliquots of **1d**. (ii) Controls both with and without EDTA showed increasing amounts of the f7*-autocleavage fragment (Abz-Pra-HQKLVFFAED) after one more week incubation at 21 ℃. (**C**) Mass peaks for fragments derived from *N*-terminal truncation toward the HQKLVFFAEDVYG derivatives of **2** (m/z = 549.94 (3+)) (as annotated with (*) in the LC-MS depicted in Figure S21C) as well as for **1d** (*) (m/z = 579.28 (3+)) and in addition, the mass peak of the f7*(**1d**) autocleavage fragment Abz-Pra-HQKLVFFAED (m/z = 724.35 (2+)). (**D**) Autohydrolysis of **2** (60 μM) in PB (5 mM), pH 7.8 with 3.1 % DMSO containing 52 μM EDTA (stock entry XIX, Table S1): black chromatograms (UV-214 nm) and colored traces (MS-TIC). (i) at 20 ℃ upon mixing, (ii) aliquot upon 1:4.5 dilution in Milli-Q after 7 days incubation at 37 ℃ and (iii) undiluted reaction stock after 5 more weeks incubation at 20 ℃. (**E**) MS spectra of fragments Bz-Pra-His-OH (f8*(**2**)) and Bz-Pra-His-Gln-OH (f0*(**2**)). (**F**) LC-MS of control reactions to parallel (**D**) for influence of copper on autocleavage of peptide **2** (60 μM) in PB (5 mM), pH 7.8 with 3.1 % DMSO). (i-ii) After 7 days at 37 ℃ (a) control without additive, (b) with additional 10 μM Cu(II) acetate and (c) with 10 μM Cu(II) acetate and 200 μM sodium ascorbate. (**) annotates an impurity as identified by MS/MS sequencing (Figure S21D).

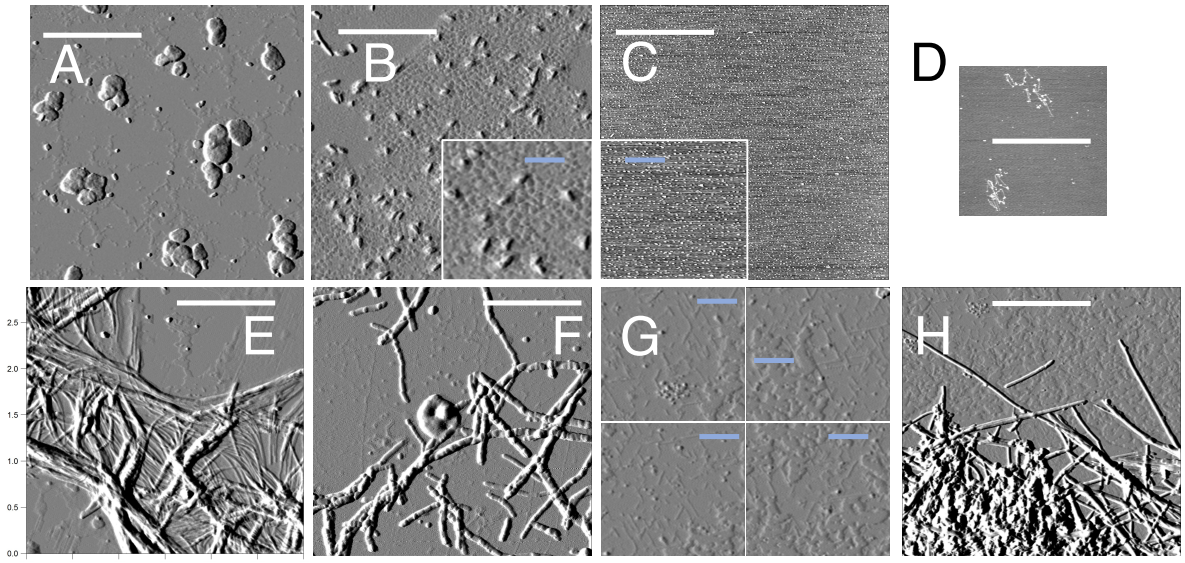

Figure S23. AFM imaging of aggregation samples. (**A**-**C**) Captured morphologies upon downstream aggregation derived from dilute LC-MS screening samples of **1a** that showed disappearance of UV-trace signal. (**D**) Fine fibrils obtained from a hydrolysis sample of Aβ_{1-28} (Figure S17D (iii)) were the UV-trace disappeared. (**E**,**F**) Different fibril samples of **3** were obtained after long-term maturation (4 – 8 weeks) without downstream dilution. (**G**) Zoomed images (blue bar represents 250 nm) of assemblies of nanotube-shaped objects captured at the outskirts of a fibrillation sample (**H**). The white bar in represents 1 μm.

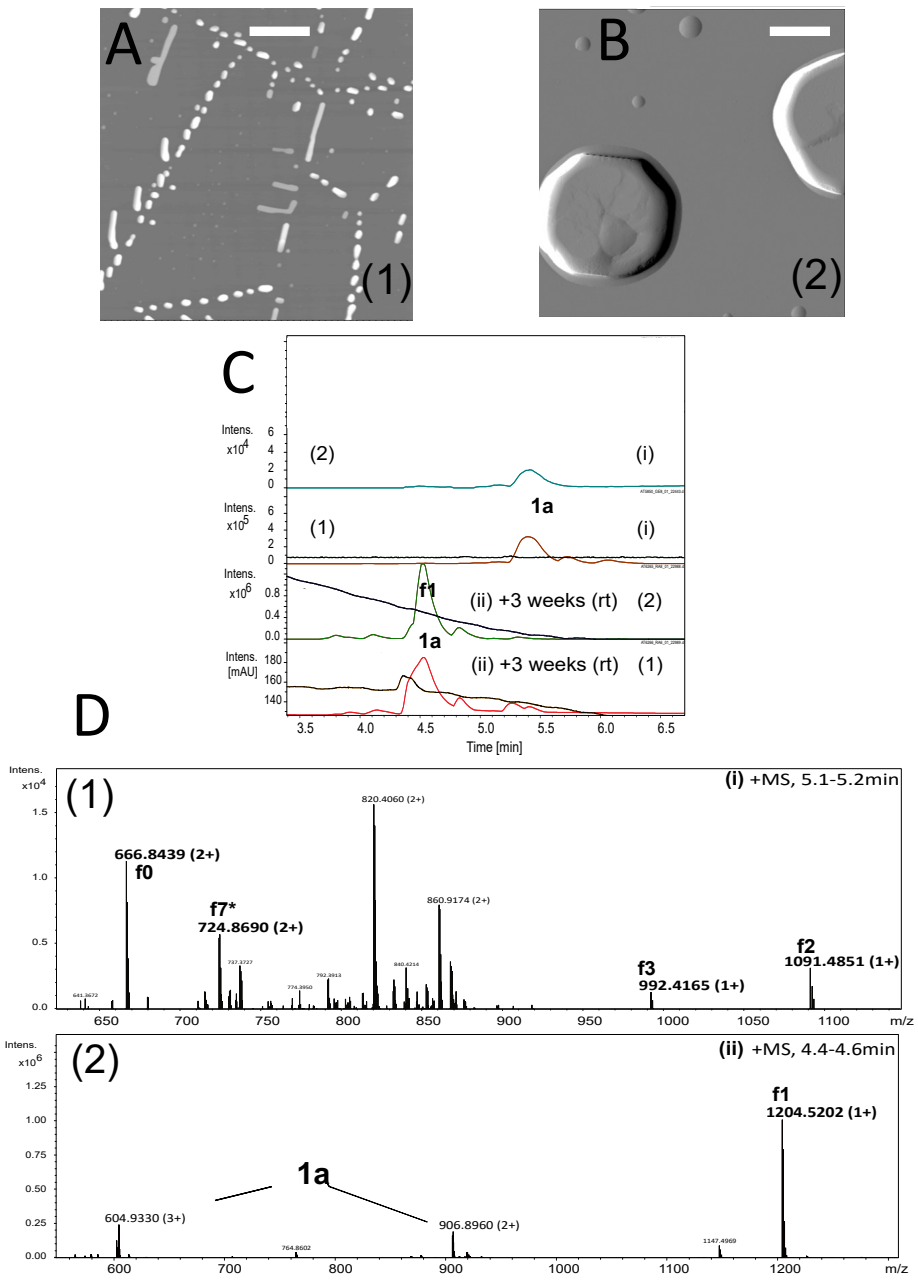

Figure S24. AFM imaging of aggregation samples. (**A**) AFM image on mica surface (white bar represents 1 μm). The sample (1) was derived from an LC-MS sample (**1a** that showed disappearance of LC-UV signal upon 1:4 dilution of a reaction stock aliquot in Milli-Q water. Initial sample conditions: **1a** (100 μM) in PBS (10 mM), pH 7.6) was incubated for 12 days at 37 ℃. (**B**) AFM image on mica surface (white bar represents 1 μm). The sample (2) was derived from an LC-MS sample that showed disappearance of LC-UV signal upon 1:4 dilution of a reaction stock aliquot in Milli-Q water. Initial sample conditions: **1a** (100 μM) in PBS (10 mM), pH 7.6) was kept 7 days at 20 ℃, thereupon 1:2 diluted with Milli-Q and incubated at 37 ℃ for 5 days. (**C**) Stacked LC-MS spectra (colored traces (MS-TIC)) for samples (1) and (2) upon dilution (i) and (ii) after three weeks at room temperature (black trace (UV-214 nm)). Elution times changed from (i) to (ii) due to change of solvent gradient in the LC-method. (**D**) Mass spectra with annotated autocleavage fragments in sample (1) upon dilution and sample (2) after 3 weeks.

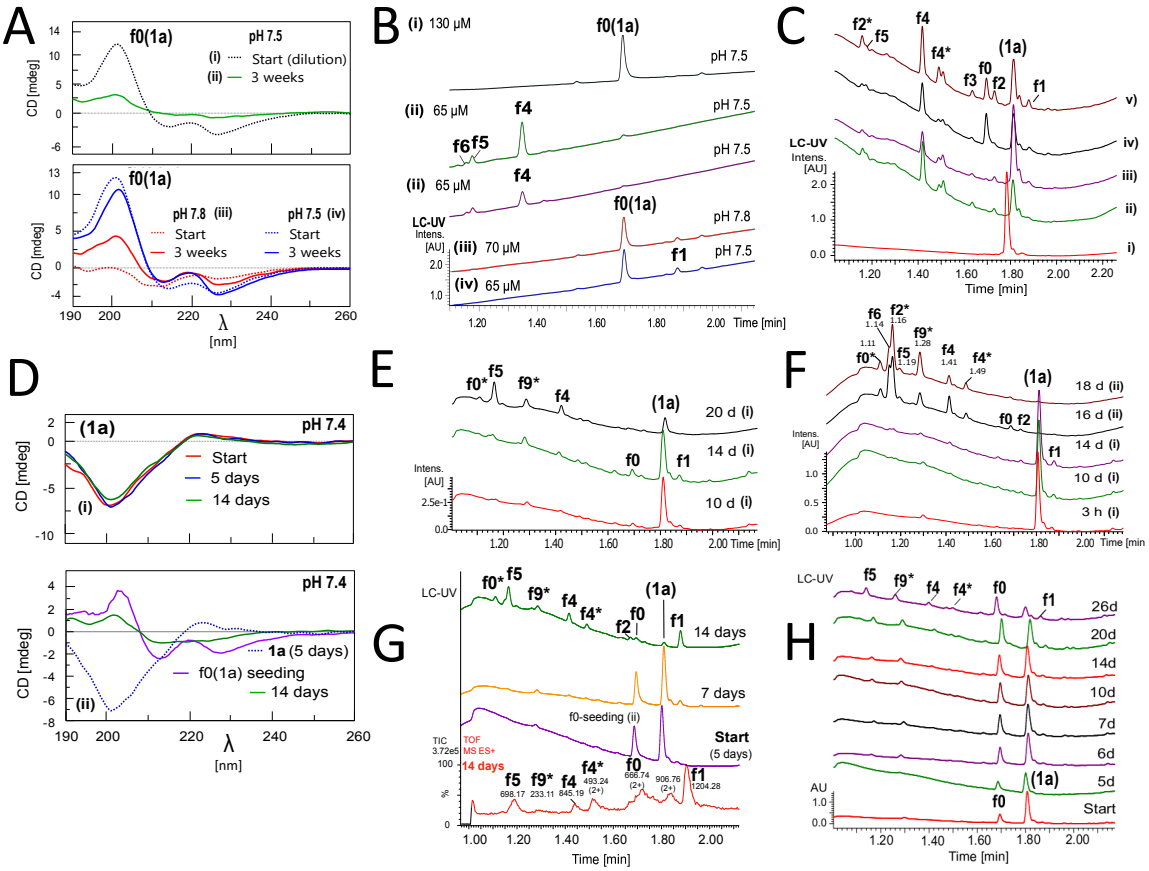

Figure S25. Secondary structure transitions in ACR-cross-seeding experiments and LC-MS analysis of CD samples. (**A**) CD-spectra (black dotted CD) of f0(**1a**) (65 μM in PB (10 mM) at pH 7.5) recorded right upon dilution (i) and (ii) after three more weeks incubation (green CD) at 37℃. (iii) Replicate sample at pH 7.8 (red CD). (iv) Replicate of (i) with adjustment from pH 7.8 to pH 7.5 (blue dotted CD). (**B**) Stacked LC-UV chromatograms for samples monitored by CD (i-iv) after three weeks incubation at 37 ℃. (i) Stock sample (ii) 1:2 diluted (green), 1:2 diluted duplicate (purple), (iii) control at pH 7.8 and (iv) control at pH 7.5. (**C**) Stack LC-UV chromatograms of samples analyzed by CD (see main text Figure 3J). All samples were incubated at 37 ℃ for an overall period of 4 weeks. Sample conditions: (i) **1a** (90 μM) in PB (7.5 mM), pH 7.5 after one week and (ii) after 4 weeks. (iii) Control with 2 μM Cu(II) acetate. (iv) After one week at 37 ℃, a retrieved aliquot of (i) was mixed with f0(**1a**) (as described in (**A**), CD (iv), 1d at 37 ℃) to final concentrations: **1a** (72 μM), f0(**1a**) (13 μM) in PB (11 mM). (v) In parallel, a retrieved aliquot of (iii) was equally treated with f0(**1a**) after one week at 37 ℃. (**D**) Overlaid CD-spectra: (i) **1a** (50 μM) in PB (10 mM), pH 7.4 over a period of two weeks incubation at 37 ℃ (Table S1, stock entry XX). (ii) An aliquot of f0(**1a**) (5 d at 37 ℃, Figure S11E) was mixed at 20 ℃ with an aliquot of (i) **1a** (5 d at 37 ℃, blue dotted CD). Sample condition upon mixing (purple CD): **1a** (45 μM) and f0(**1a**) (12 μM) in PB (10 mM), pH 7.4. Incubation succeeded for 9 more days at 37 ℃ (green CD). (**E**) Stacked LC-UV chromatograms for hydrolysis of **1a** control without f0-seeding (CD (i) in (**D**)) at 37 ℃. (**F**) After 14 days incubation at 37 ℃ an undiluted aliquot of (**E**) spontaneously hydrolyzed within two days at 20 ℃. (**G**) Stacked LC-UV chromatograms for CD sample (ii) as described in (**D**). (**H**) Stacked LC-UV chromatograms obtained from repeat experiment for CD monitoring at 37 ℃ (see main text Figure 3I). Conditions: Mixture of **1a** (45 μM) and f0(**1a**) (12 μM) in PB (10 mM), pH 7.4 derived from freshly prepared dilution samples at 20 ℃.

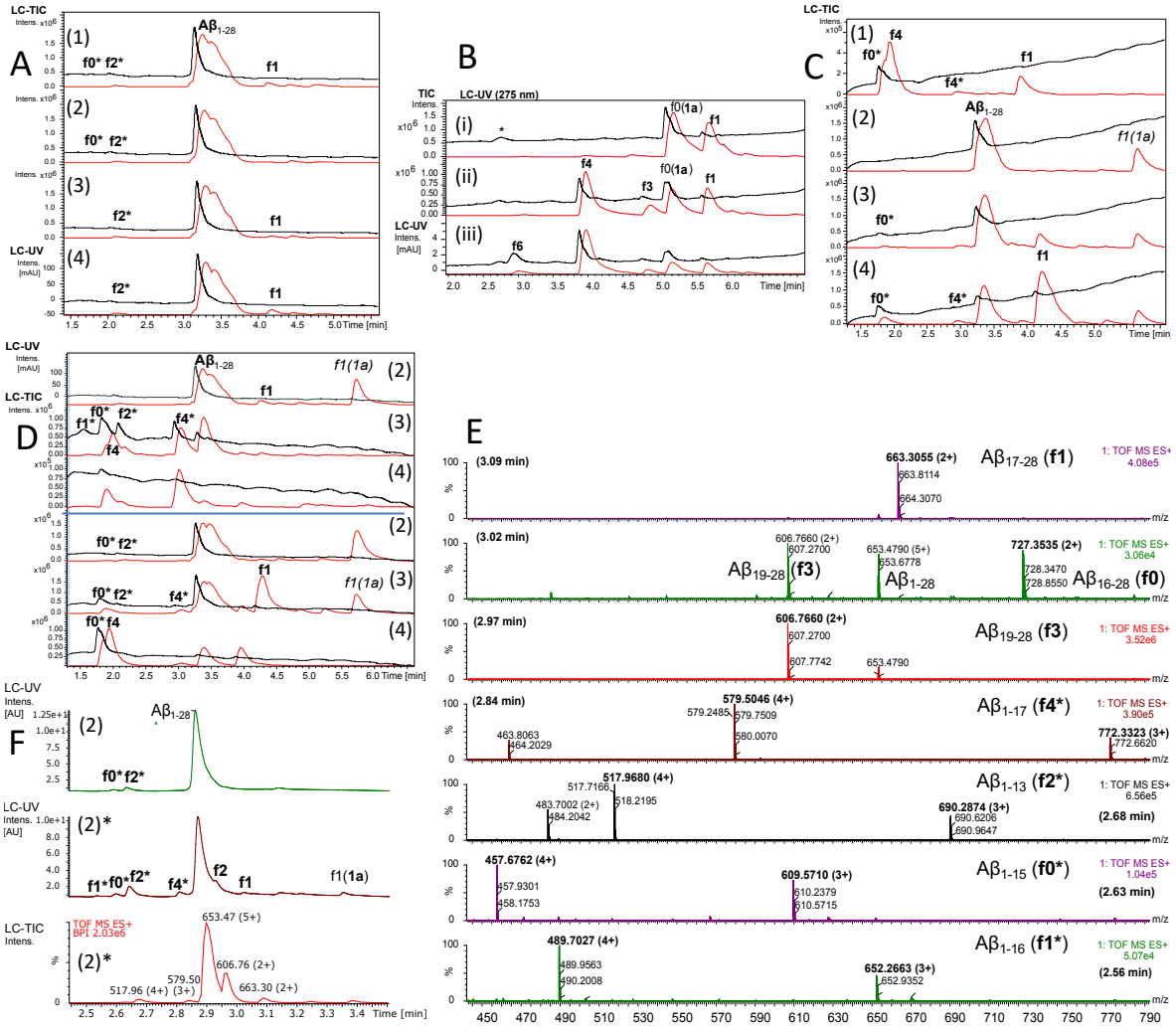

Figure S26. (**A**) Stacked LC-MS (red trace (MS-TIC), black trace (UV-214 nm)). Replicates of Aβ_{1-28} (27 μM) in PB (10 mM) pH 7.6 with additives: (1) with protease inhibitor cocktail, (2) with EDTA (220 μM), (3) 0.02 w% NaN_3 and (4) no additive, after 10 weeks incubation at 25 ℃. (**B**) Stacked LC-MS (red trace (MS-TIC), black trace (UV-214 nm)) of f0(**1a**) seeds prepared through 1:2 dilution of stock solution entry XII in Table S1 in Milli-Q. (i) f0(**1a**) (50 μM) in 1 mM PB pH 7.6 with 0.02 w% NaN_3 and protease inhibitor (*) after 6 weeks at 20 ℃ followed by 1 d at 37 and 5 more days at 20 ℃(ii) Replicate sample f0(**1a**) after 6 weeks at 20 ℃ followed by 1 d at 37 and 7 more days at 20 ℃. (iii) 1:1 mixture of (i) and (ii) 1 h upon mixing at 20 ℃. (**C**) LC-MS of replicates (1) – (4) as described in (**A**) after 6 weeks at 25 ℃ followed by f0(**1a**) cross-seeding with 18 mol% solution (**B**(i)) and subsequent incubation for 10 d at 37 ℃. (**D**) Stacked LC-MS traces of replicates of (2) – (4) as described in (**A**) with f0(**1a**) cross-seeding after 6 weeks followed by 3 weeks incubation at 25 ℃(stacked LC-MS above blue line) and 37 ℃ (stacked LC-MS below blue line). (**E**) Stacked mass spectra with denoted molpeaks of the major ACR fragments as they were recorded on the Waters Acquity™ UPLC system with a Xevo G2-S QTOF mass spectrometer. (**F**) Stacked UPLC-MS (green and brown traces (UV-diode array), red trace (MS-TIC)) of EDTA (220μM) containing replicates of (2) were retrieved after 10 weeks incubation at 25 ℃ (**A**) and one of them (2)* received 10 mol% f0(**1a**) cross-seeding (**B**(ii)). Both samples were vigorously shaken (3 sec) before incubation succeeded at 37 ℃ for 3 weeks.

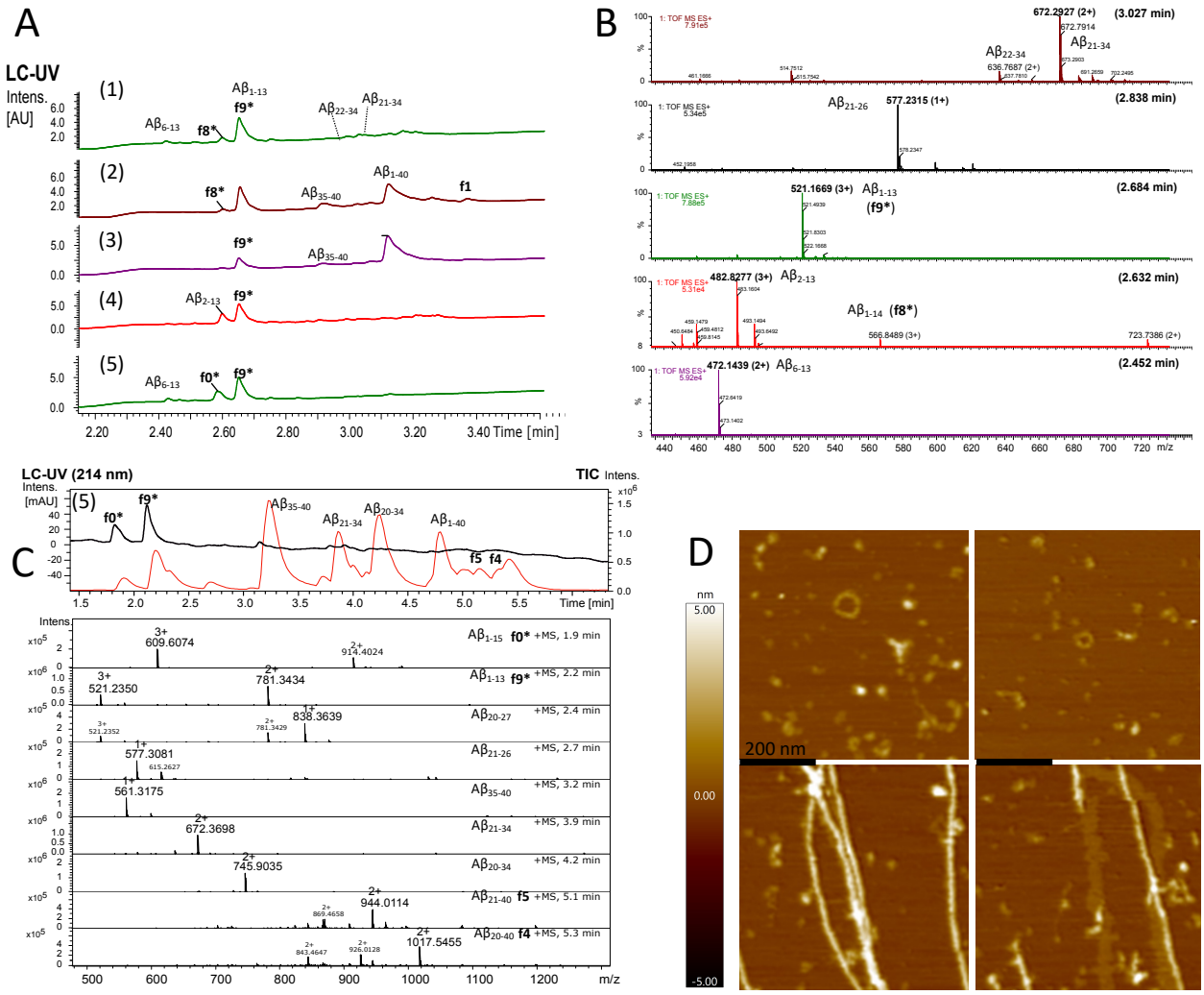

Figure S27. Repeat experiments for Aβ_{1-40} cascade hydrolysis and AFM imaging of aggregate morphologies. (**A**) Stacked UPLC traces of Aβ_{1-40} ACR samples: (1) Aβ_{1-40} (31 μM) in PB (10 mM) pH 7.4 after 3 d incubation at 25 ℃ followed by 6 d at 37 ℃ (stock entry XXIII in Tale S2). (2) Aβ_{1-40} (31 μM) in PB (10 mM) pH 7.4 after 21 d incubation at 25 ℃ (stock entry XXI in Tale S2). (3) Aβ_{1-40} (31 μM) in PB (10 mM) pH 7.4 after 13 d incubation at 25 ℃ followed by 6 d at 37 ℃ (stock entry XXIII in Table S2). (3) Aβ_{1-40} (31 μM) in PB (10 mM) pH 7.4 (vigorously shaken after sample dilution) was incubated 13 d at 25 ℃ followed by shaking and 6 d incubation at 37 ℃ (stock entry XXIII in Tale S2). (4) Aβ_{1-40} (31 μM) in PB (10 mM) pH 7.4 after 13 d incubation at 25 ℃ followed by 6 d at 37 ℃ (stock entry XXIII in Tale S2). (5) (33 μM) in PB (10 mM) pH 7.4 was incubated for 9 d incubation at 25 ℃ followed by vigorous shaking (3 sec) and 10 d at 37 ℃ (stock entry XXI in Table S2). (**B**) List of mass spectra with denoted fragment sequences of the molpeaks. (**C**) LC-MS traces (black (UV-214 nm), red (MS-TIC) of sample (5) as described in (**A**) after 4 d incubation at 37 ℃. Below stacked list of mass peaks shows denoted autocleavage fragments at their respection LC elution times. (**D**) AFM images of the sample as described in (**C**). The black bar represents a length of 200 nm.

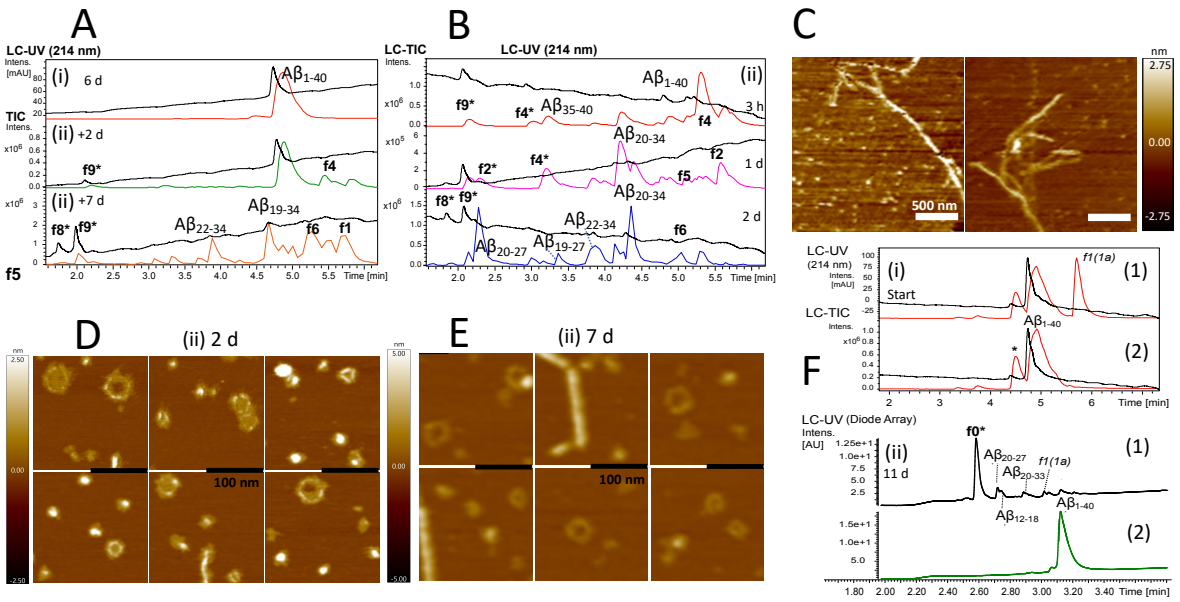

Figure S28. ACR cross-seeding in Aβ_{1-40} and AFM imaging aggregate morphologies. (**A**) Stacked LC-MS traces (UV-214 nm (black), MS-TIC (colored)) of (i) Aβ_{-40} (29.4 μM) in PB (10mM), pH 7.33 after at 25 for 6 d followed by 3 seconds vigorous shaking and measured after change to 37 (ii) 2 d and 7 d incubation. (**B**) LC-MS traces of the replicate that received additionally 20 mol% active seed solution of f0(**1a**) fragment composition as described in (Fig. S26B) followed by shaking (3 sec) and setting the reaction to 37 . (ii) Stacked LC-MS after addition of f0-seed solution and 3 hours incubation at 20 ℃, recorded after 1 d and 2 d at 37 ℃. Annotated fragments were identified by there high-resolution mass peaks and MSn fragment sequencing Fig. S32 and Fig. S29. AFM imaging of sample (**B**) after 2 d incubation at 37 ℃ one night at 20 ℃ show fibril bundles and particles (white size bar, 500 nm). (**D**) AFM imaging of sample (**A**) after 2 d incubation at 37 ℃ and one night at 20 ℃ show ring-segmented aggregates of discrete size distribution (20 – 50 nm). (**E**) AFM imaging of sample (**A**) after 7 d incubation at 37 ℃.(**F**) LC-MS traces (black (UV-214 nm), red (MS-TIC) of split aliquots tracked after (i) incubation for 5 d at 25 ℃ and followed by (ii) addition of f0/f1 seed solution to the replicate. Both samples were set to 37 ℃ 2 min after vigorous shaking (3 sec). Bottom stacked UPLC traces were recorded for both samples (i) and (ii) after incubation for 8 d at 37 ℃ followed by 6 d at 25 . (**E**) Stacked LC-MS (black (UV-214 nm), red (MS-TIC)) of replicate samples (1) and (2) of Aβ_{-40} (29.4 μM) in PB (10 mM) pH 7.4 (stock entry XXI in Table S2) after (i) 5 d at 25 ℃ sample (2) freshly receiving additional f0(**1a** seed solution (Fig. S26B(ii)). (ii) Stacked UPLC traces after additional incubation for 11 d at 37 ℃. Annotated autocleavage fragments of sample (2) are identified by their high resolution mass peaks listed in Fig. S36 and Fig.S37.

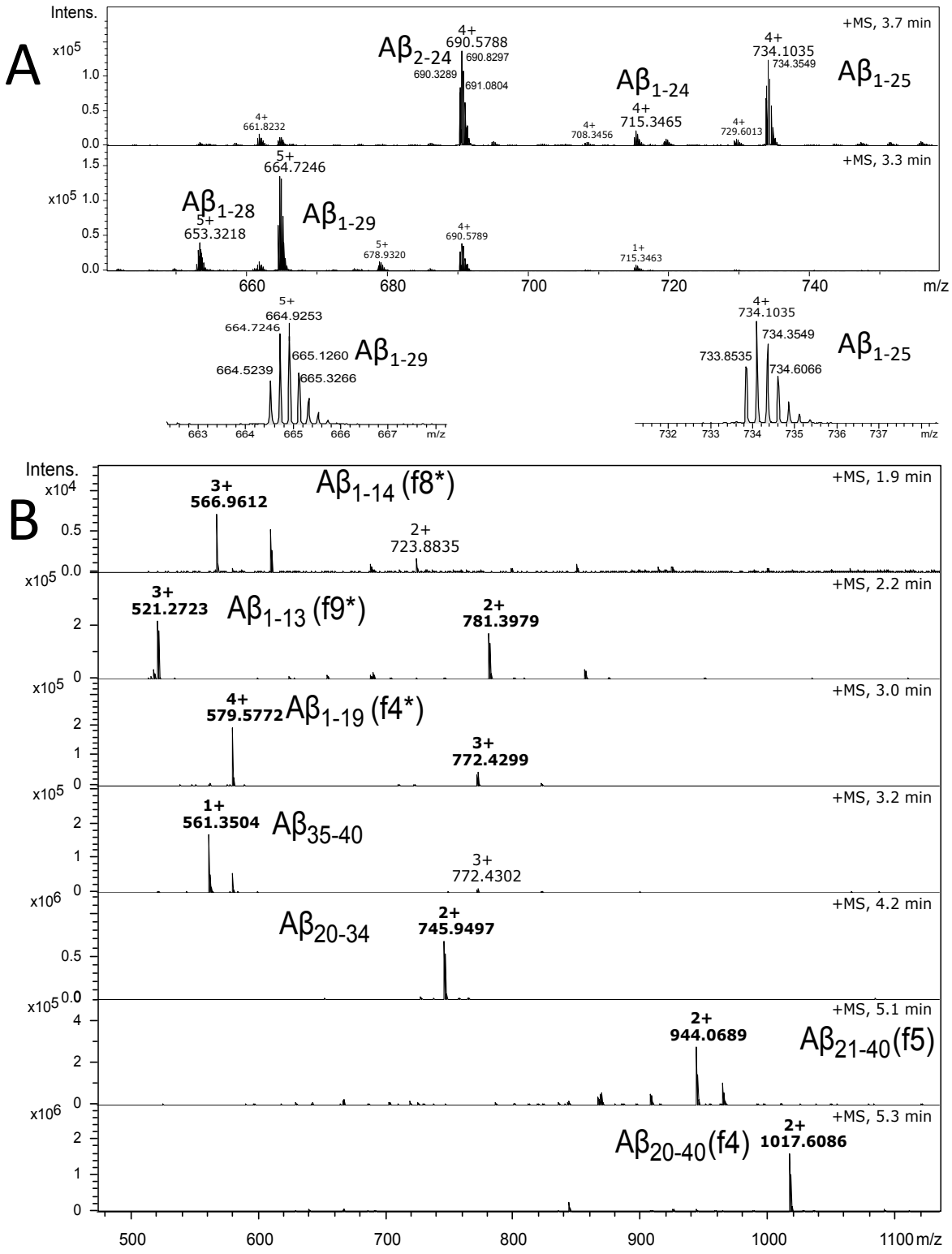

Figure S29. Mass list of Aβ_{1-40} ACR fragments. (**A**) Mass spectra of fragments commonly observed in small traces in the early aggregation phase (Fig. S28F(i)). (**B**) Stacked mass spectra of Aβ_{1-40} ACR fragments (Fig. S28A,B)

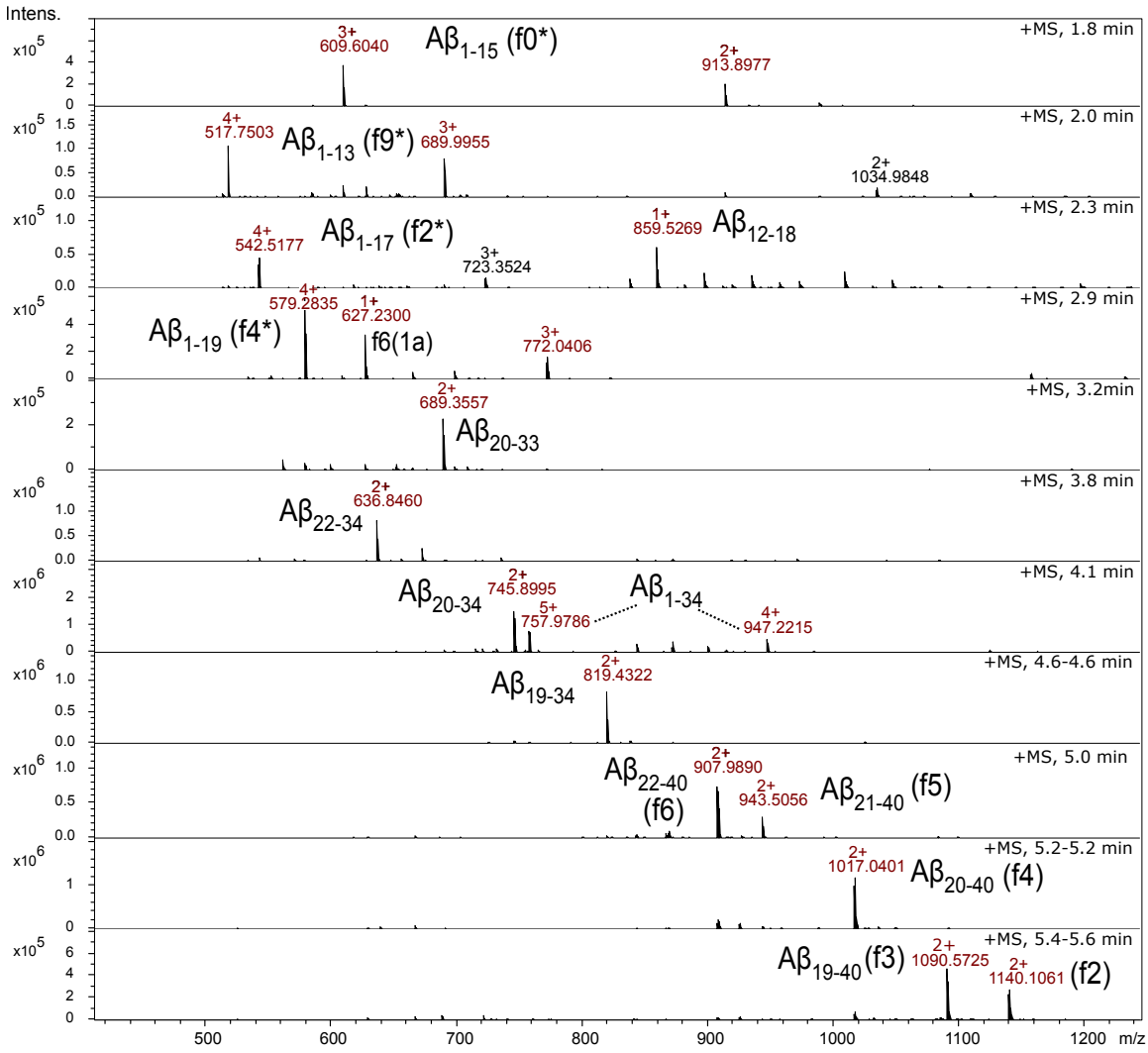

Figure S30. Stacked mass spectra of Aβ_{1-40} autocleavage fragments as they appear upon f0(**1a**) cross-seeding (Fig. S28B).

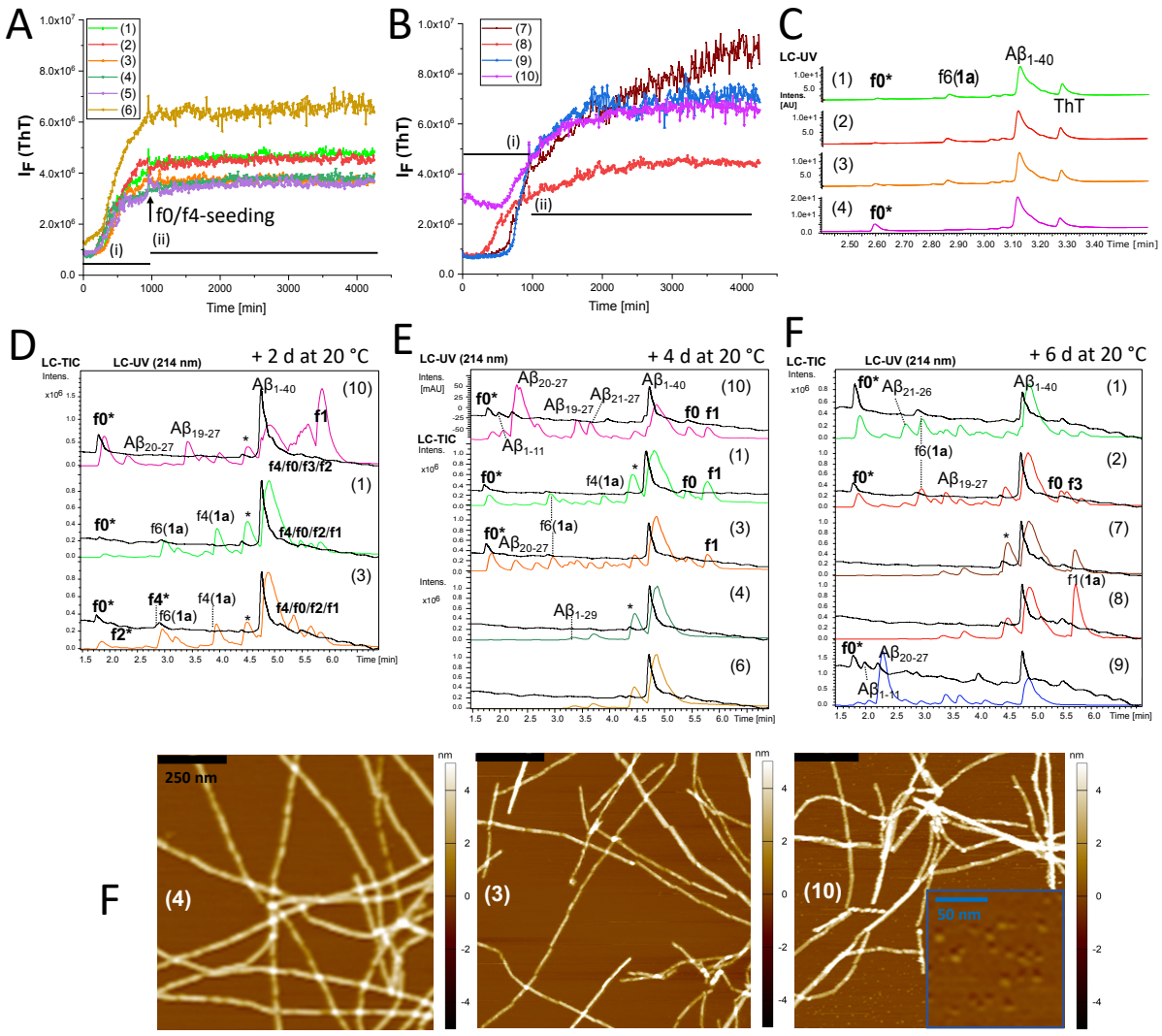

Figure S31. (**A**) Kinetic aggregation profiles of Aβ_{1-40} (33 μM in PB (10 mM), pH 7.4 with ThT (22 μM)) (stock entry XXI in Table S2). ThT fluorescence was excited at λ_{ex} = 420 nm and measured at λ_{em} = 500 nm. Replicate dilution samples (1) – (6) were kept 30 minutes at 22 ℃ prior to start of the plate reader assay. (i) Following gradual heating to 37 ℃ and 100 seconds orbital shaking the plate was periodically shaken (3 sec) every 10 minutes. (ii) After 16 h the plate was stopped for 5 minutes and replicates (1) – (3) received 15 mol% of a preactivated autohydrolytic seed mixture of fragments f6/f4/f1/f0(**1a**) (Fig. SS26B(iii)). After 100 seconds orbital shaking fluorescence reading continued with periodic shaking (3 sec) every 10 minutes for another two days. (**B**) Kinetic ThT aggregation profiles of replicates screened in parallel to (**A**) with (7) and (8) receiving 15 mol% f0/f1 (**1a**) (Fig. SS26B(i) and (9) and (10) with activated f4/f1/f0 (**1a**) (Fig. S26B(ii)) prior to the start of the plate reader assay. (**C**) Stacked LC-UV traces of fibrillation samples (1) – (4) as described in (**A**) were recorded after the assay finished. (**D**) Stacked LC-MS traces (colored (MS-TIC), black (UV-214 nm)) of fibrillation samples (**A**)–(**C**) after 2 d subsequent incubation at 20 ℃, (**E**) monitored after 4 d and (**F**) 6d at 20 ℃. Mass spectra of denoted fragments are shown in Fig. S32. (*) denotes the trace for mono-oxidised Aβ_{1-40}. (**F**) AFM images of samples (3), (4) and (10) as described in (**E**). The black bar represents 250 nm and the insert for sample (10) shows spherical-shaped oligomers occurring next to fibrils at 1:4 magnification.

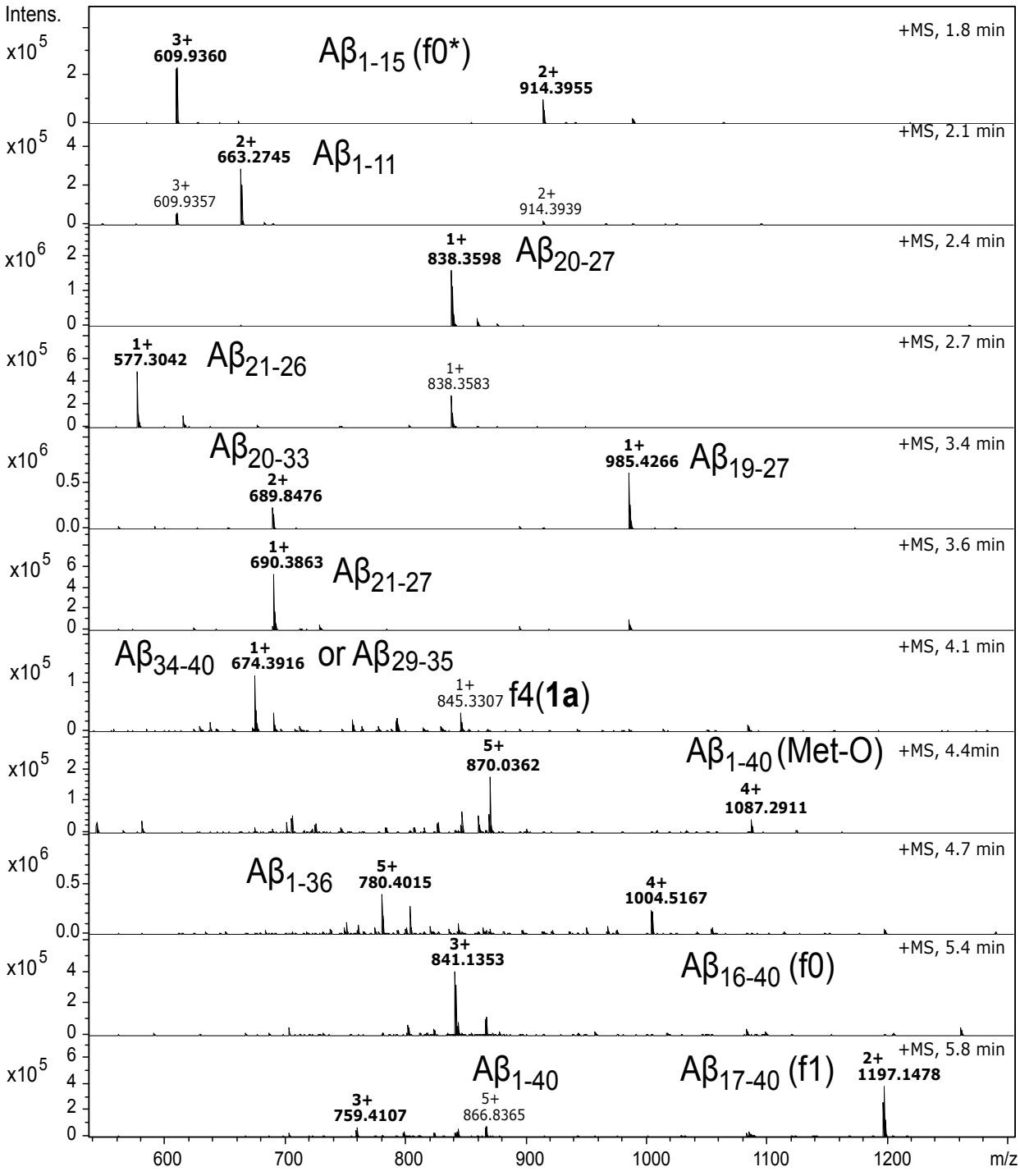

Figure S32. List of mass spectra with denoted molpeaks of Aβ_{1-40} autocleavage fragments that were identified by LC-MS (ESI positive mode) of the ACR-seeded fibrillation sample (10) as described in Fig. S31E.

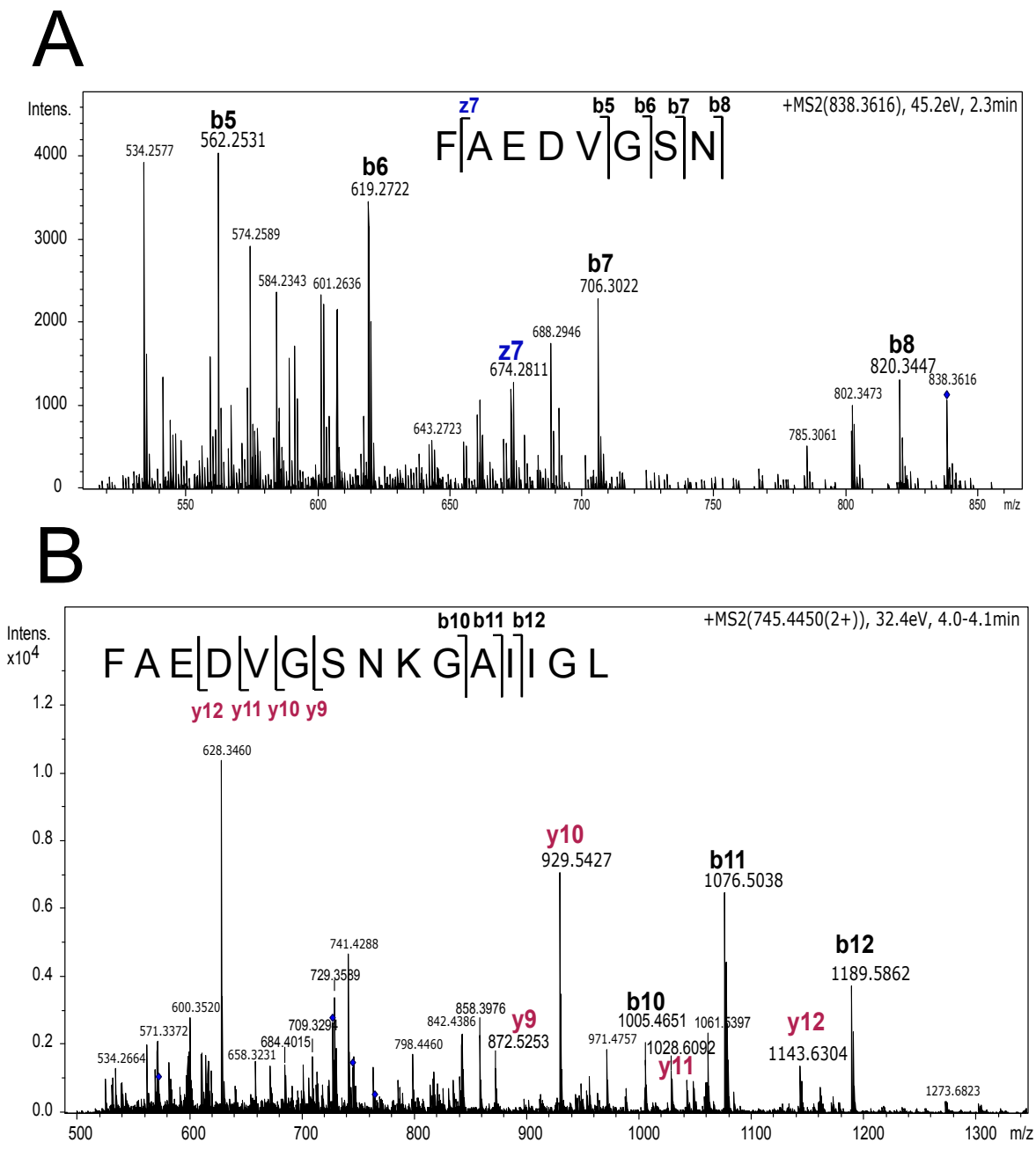

Figure S33. MSMS sequencing of abundant fragments of Aβ_{1-40} ACR as described Fig. S31E,F. (**A**) MSMS spectra of Aβ_{20-27} with denoted b–fragmentation series. (**B**) MSMS spectra of Aβ_{20-34} with denoted b - and y – fragmentation series

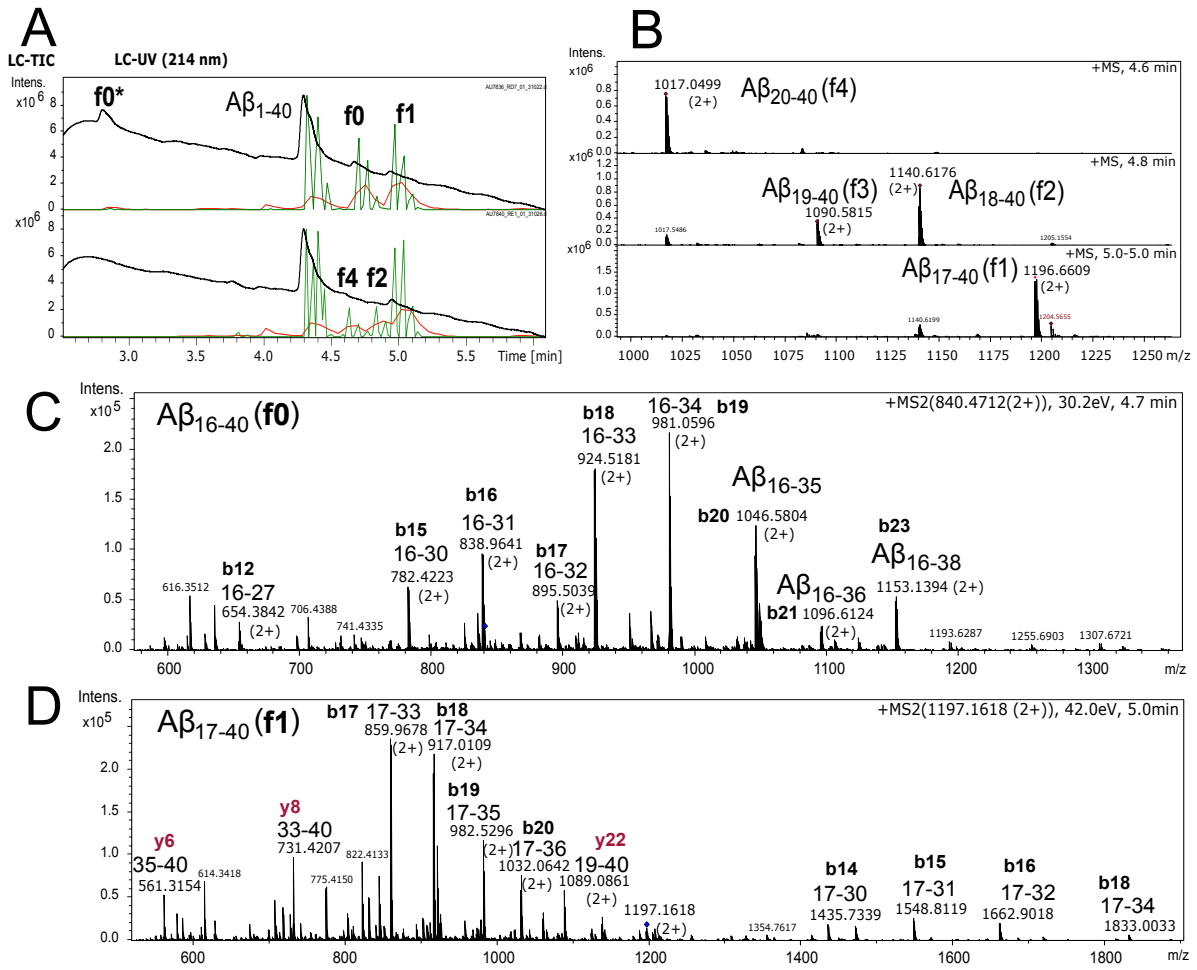

Figure S34. Identification of primary autocleavage fragments in f0-seeded Aβ_{1-40} ACR. (**A**) Stacked LC-MS/MS traces (black (UV-214 nm), red (MS-TIC), green (MSMS-TIC)) of A$\beta_{1\text{-}40}$ (30 μM) replicates (PB (10 mM) with 0.02 w% NaN_3 and protease inhibitor at pH 7.33) with additional 16 mol% f0(**1a**)-seed solution (Fig. S26B(ii)) after 6 d incubation at 37 °C (top) and 25 °C (bottom). (**B**) Stacked mass spectra with molpeak annotation of autocleavage fragment sequences. (**C**) MSMS spectra with denoted *b*–fragmentation series of Aβ_{16-40} (f0) and Aβ sequence indication. (**C**) MSMS spectra with denoted *b*– and *y*– fragmentation series of Aβ_{17-40} (f1) and Aβ sequence indication.

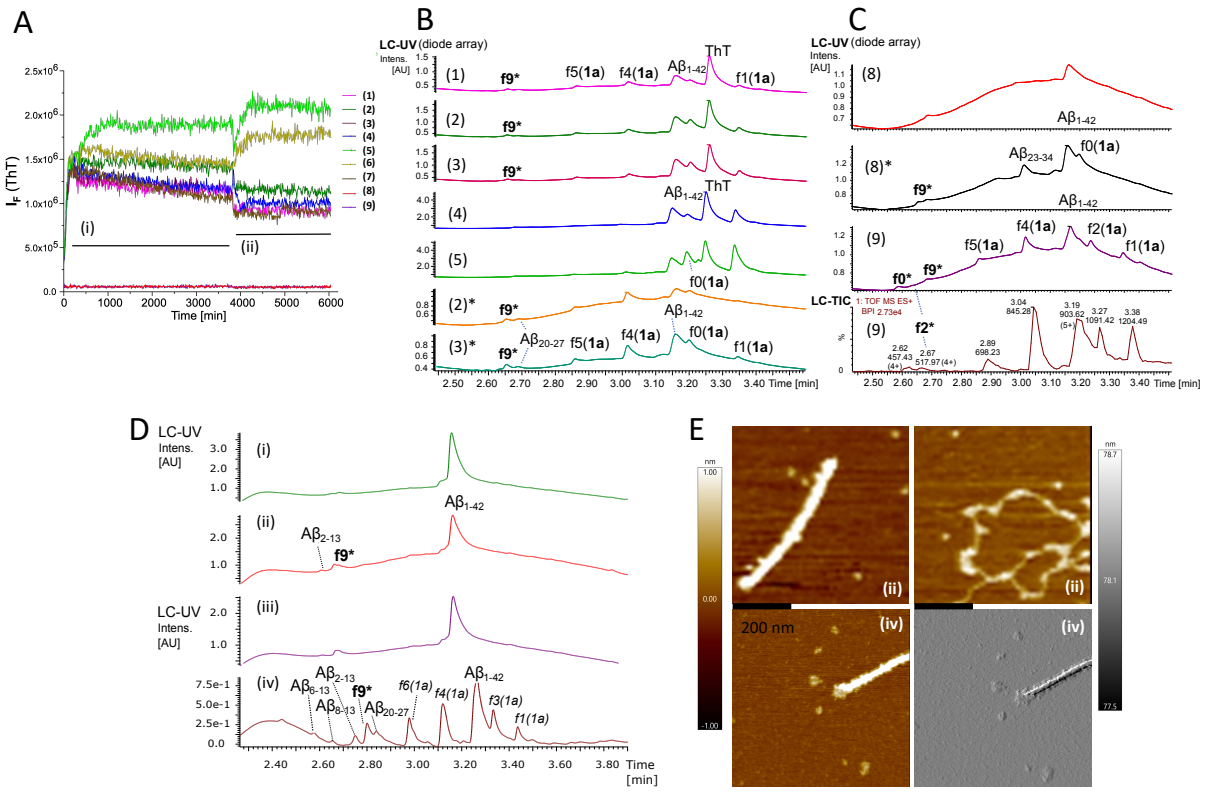

Figure S35. Influence of f0(**1a**) ACR-cross-seeding on Aβ_{1-42} aggregation samples. (**A**) Kinetic ThT aggregation profiles for ThT fluorescence (λ_{ex} = 420 nm, λ_{ex} = 500 nm). Replicates of Aβ_{1-42} (23 μM) in PB (10 mM), pH 7.4 with ThT (13.2 μM) (1) – (3) remained undisturbed at 20 °C for 2 h and received 20 mol% of f0(**1a**) seeding stock solution (Table S2, stock entry XII) prior to start of the plate reader assay at 37 °C. Replicate (4) remained unseeded. ThT containing replicates (5) – (7) received 40 mol% f0(**1a**) (derived from stock entry XII (Table S2) 1:2 diluted in Milli-Q and incubated 1 d at 20 °C). Replicates (2)* and (3)* did not contain ThT and received 20 mol% of f0(**1a**) seeding stock solution (Table S2, stock entry XII). (i) After 10 seconds orbital shaking the plate reader assay proceeded at 37 °C for 64 h without periodic agitation. (ii) After 10 seconds orbital shaking the plate read continued for another 36 h at 37 °C without periodic agitation. Subsequent LC-MS analysis showed no hydrolysis in any of the described fibrillation samples. Thereafter samples (1) – (9) were 1:3 diluted with Milli-Q. (**B**),(**C**) Stacked UPLC-traces of the samples described in (**A**) 3 weeks after dilution in Milli-Q and subsequent incubation at 25 °C. The brown LC-TIC trace in (**C**) corresponds to sample (9) with denoted elution times and masses of the fragments (Fig. S36). Base peak intensity (BPI). (**D**) Stacked UPLC-traces of replicate samples of Aβ_{1-42} (derived from stock entry XXIV, Table S2) after 14 d incubation without ThT. (i) Aβ_{1-42} (23 μM) in PB (10 mM) pH 7.4 with 2.5 % DMSO (variation of stock entry XXIV, Table S2: Aβ_{1-42} (460 μM) in 5 mM NaOH with 50 % DMSO) was kept 3 h at 20 °C prior to 14 d incubation at 37 °C. (ii) Replicate of sample (i) that was incubated for 3 h at 20 °C followed by vigorous shaking (3 sec) and incubation at 37 °C for 14 d without perturbation. (iii) Aβ_{1-42} (23 μM) in PB (10 mM) pH 7.4 after 14 d at 25 °C. (iv) Replicate of sample (ii) that received 25 mol % f0(**1a**) (derived from 1:2 diluted stock entry XII, Table S2; f0(**1a**) (50 μM) for 1 d at 20 °C) after 3 h at 20 °C which was followed by vigorous shaking (3 sec) and subsequent incubation for 14 d at 37 °C without perturbation. For fragment identification see Fig. S36. (**E**) AFM images of sample (ii) and (iv) as described in (**D**) after 14 d. The black bar represents a length of 200 nm.

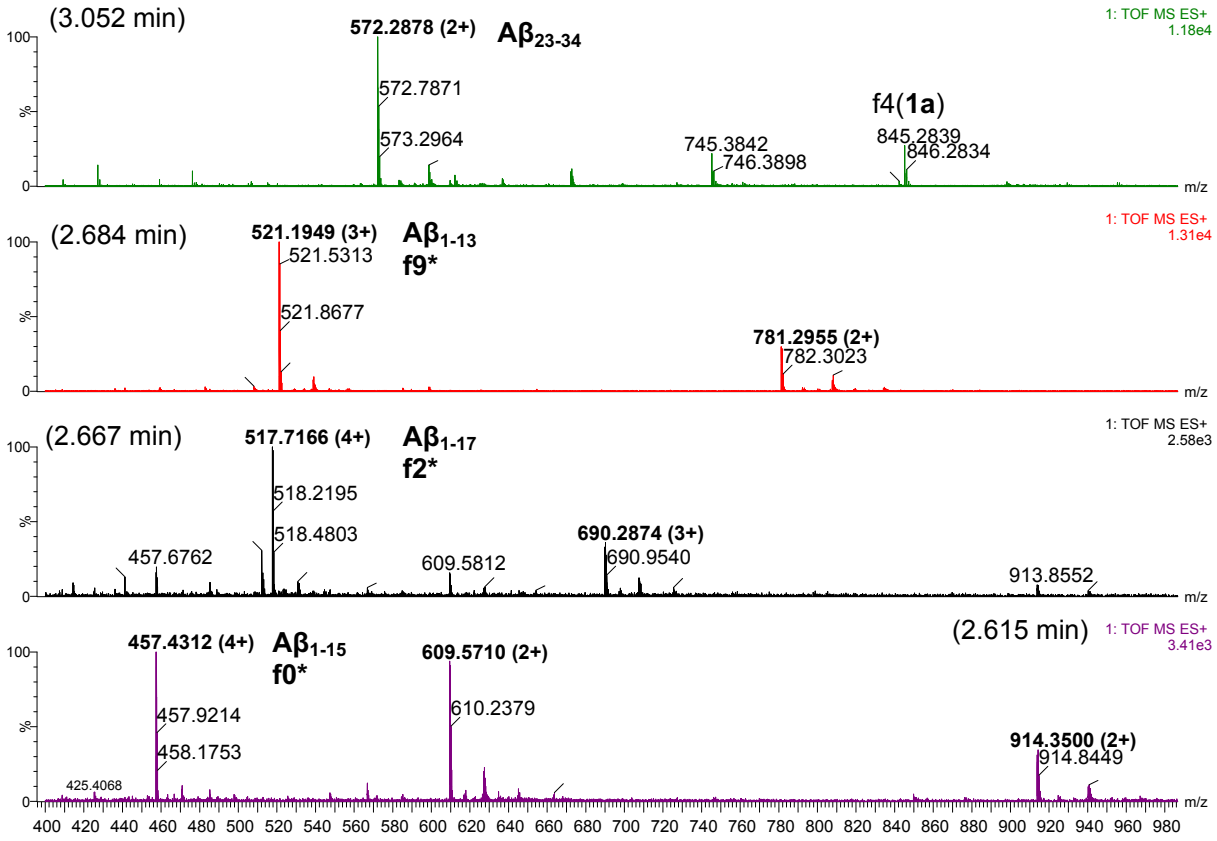

Figure S36. Mass list of Aβ_{1-42} autocleavage fragments derived from UPLC-MS analysis of samples described in Fig. S35B,C

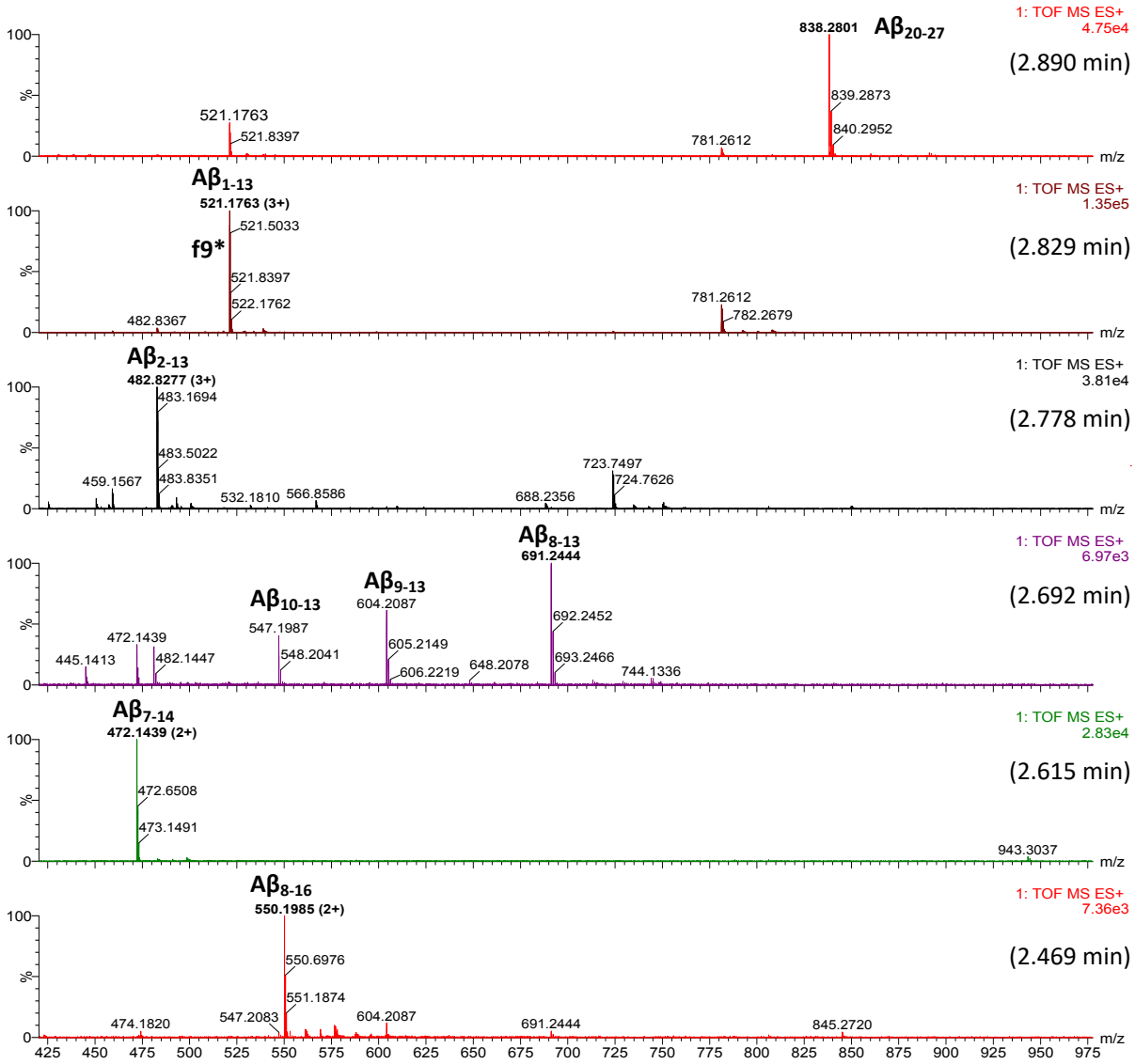

Figure S37. Mass list of Aβ_{1-42} autocleavage fragments derived from UPLC-MS analysis of sample (iv) as described in Fig. S35D

References

1. Somavarapu AK, Shen F, Teilum K, et al. The Pathogenic A2V Mutant Exhibits Distinct Aggregation Kinetics, Metal Site Structure, and Metal Exchange of the Cu2+–Aβ Complex. *Chem Eur J.* 2017;23(55):13591-13595.

2. Biancalana M, Koide S. Molecular mechanism of Thioflavin-T binding to amyloid fibrils. *Biochim Biophys Acta - Proteins and Proteomics.* 2010;1804(7):1405-1412.

3. Meldal M. Pega: a flow stable polyethylene glycol dimethyl acrylamide copolymer for solid phase synthesis. *Tetrahedron Lett.* 1992;33(21):3077-3080.

4. Tornøe CW, Christensen C, Meldal M. Peptidotriazoles on Solid Phase: [1,2,3]-Triazoles by Regiospecific Copper(I)-Catalyzed 1,3-Dipolar Cycloadditions of Terminal Alkynes to Azides. *J Org Chem.* 2002;67(9):3057-3064.

5. Kaiser E, Colescott RL, Bossinger CD, Cook PI. Color test for detection of free terminal amino groups in the solid-phase synthesis of peptides. *Anal Biochem.* 1970;34(2):595-598.

6. Hosia W, Griffiths WJ, Johansson J. Hydrolysis of the amyloid β-peptide (Aβ)1–40 between Asp23–Val24 produces non-aggregating fragments. An electrospray mass spectrometric study. *J Mass Spec.* 2005;40(2):142-145.

7. Lomakin A, Chung DS, Benedek GB, Kirschner DA, Teplow DB. On the nucleation and growth of amyloid beta-protein fibrils: detection of nuclei and quantitation of rate constants. *Proc Natl Acad Sci USA.* 1996;93(3):1125-1129.