

Supplementary Methods

Dynamic Light Scattering (DLS) experiments

DLS experiments were performed using a Malvern nanozetasizer (Malvern, UK). The samples were placed in a disposable cuvette and held at 278 K. Three different samples were prepared: 1) sample 1 containing a solution of 10 mM phosphate buffer, pH 7.4; 2) sample 2 prepared by dissolving ZnCl_2 in water of MILLIQ purity at a final concentration of 100 μM ; 3) sample 3 obtained by adding 100 μM ZnCl_2 (final concentration) to the 10 mM phosphate buffer solution, pH 7.4. For each sample, five measurements with 11 sub-runs using the multimodal mode were performed. Then, the average hydrodynamic diameter (nm) was calculated from the correlation function using the ZS Xplorer Malvern software (version 3.2.0.84).

Size Exclusion Chromatography (SEC) experiments

For analytical SEC, $\text{A}\beta$ from Bachem and recombinantly expressed ^{19}F - $\text{A}\beta$ were incubated in hexafluoroisopropanol (HFIP) for at least 30 minutes at 20 °C and then aliquoted, lyophilized and stored dry for a few days at 20 °C (or longer at -80 °C). For the quantitative SEC experiments, the peptides were first repurified via RP-HPLC, so that un-labelled and ^{19}F -labelled $\text{A}\beta$ could be aliquoted in HFIP according to their chromatographic peak areas. All quantifications were done according to absorption at $\lambda = 215 \text{ nm}$, because fluorination seems to affect the aromatic absorption, so that the ratio $A_{\lambda=276\pm 6 \text{ nm}}/A_{\lambda=215\pm 5 \text{ nm}}$ in HPLC was increased by 42% for ^{19}F - $\text{A}\beta$. Each aliquot of roughly 100 μg $\text{A}\beta$ was dissolved at $c = 100 \mu\text{M}$ in either 6 M guanidinium, 30 mM Tris, pH = 7.4, or 10 mM sodium phosphate, pH = 7.4, or 10 mM sodium phosphate, pH=7.4 and then 100 μM ZnCl_2 (1:1) added from a 10 mM stock solution. $\text{A}\beta$ was dissolved through pipetting and short vortexing, while not incubating for any longer than 5 minutes at 20 °C, and all samples in sodium phosphate buffer (with/without Zn) were then centrifuged at $15,000 \times g$ for 15 min at 4 °C. Without further incubation (< 5 min) the samples were applied onto a Superdex 75 10/300 (~23.5 mL) column (Cytiva). In case of the qualitative measurements (Fig S19 A,B), 0.2 mL supernatant was injected into a 500 μL sample loop; while in case of the second experiment (quantitative measurements shown in Fig S19 C-H), the supernatants were 10-fold diluted in 6 M guanidinium, 30 mM Tris, pH = 7.4 and 1.6 mL of this dilution was injected into a 5 mL sample loop. The chromatographies were performed at a flow rate of between 0.4 and 0.5 mL/min (10 mM sodium phosphate, pH = 7.4, degassed under vacuum and 0.2 μm filtered [Sartorius 18407-50-N]), using an ÄKTA Pure chromatography system (Cytiva) at 20 °C. Chromatograms were evaluated (including peak integration), using UNICORN 7.8 Evaluation (Cytiva). The potential error in the peak area results was estimated to be roughly $\pm 10\%$, being the sum of an estimated injection volume error of $\pm 5\%$ ($\pm 0.08 \text{ mL}$ for an injection of 1.6 mL using a 3 mL syringe with 0.1 mL graduation marks), an estimated error of $\pm 4\%$ for initial aliquotation (including quantification via HPLC, equal aliquotation and lyophilization in HFIP), an estimated pipetting error of $\pm 0.5\%$ for dissolving and diluting, and an estimated final peak integration error of $\pm 0.5\%$. Due to the short incubation time for dissolving the $\text{A}\beta$, there could be an additional error for slight individual differences in the degree of dissolution of the samples in sodium phosphate buffer.

Supplementary Results

Dynamic Light Scattering (DLS)

Total scattering intensity of samples 1 (10 mM phosphate) and 2 (100 μ M ZnCl₂) measured at a right angle to incident light was small and stable over time: it was 43.68 ± 5.56 kcps (kilo counts per second) for sample 1 and 20.63 ± 3.54 kcps for sample 2. After addition of ZnCl₂ to phosphate buffer (sample 3, 100 μ M ZnCl₂ in 10 mM phosphate), the total scattering intensity increased to 69.06 ± 8.35 kcps, approximately equal to the sum of the values obtained for samples 1 and 2. The total scattering intensity of sample 3 slightly rose to about 111 kcps after 3 hours and 127 kcps after 24 hours and remained nearly stable afterwards up to 6 days. The obtained size distributions for sample 3 did not show any significant zinc phosphate particle formation larger than 1 nm in hydrodynamic radius (Fig. S12). To note, the measured hydrodynamic radius of non-fluorinated (Fig. S7) and fluorinated A β 40 (Fig. 1G) here is around 1.9 nm.

Size Exclusion Chromatography (SEC)

In order to monitor the size distribution of A β peptides in their initial state, as well as upon Zn addition, un-labelled A β (Bachem) and ¹⁹F-labelled A β (from recombinant expression) were analyzed by SEC. Apart from the A β monomer peak, no differently sized species could be reproducibly detected for any sample in 10 mM sodium phosphate, pH = 7.4 with or without Zn (1:1), probably due to dissociation of reversible aggregates upon equilibrium shift during chromatographic separation, and exclusion of large insoluble aggregates (Fig. S19 A,B). In another set of SEC measurements, optimized for quantification, the loss in soluble A β was determined by integration of the detected monomer peaks (Fig. S19 C-H). A control of 1.6 mL uncentrifuged A β , dissolved and diluted in buffer with 6 M guanidinium, yielded a monomer peak with an area of 92.99 mL $\times$ mAU at λ = 215 nm at an elution volume of 13.6 mL, which is similar to the elution of A β at roughly 15 mL, as reported by Lattanzi *et al.*¹. The supernatants of un-labelled and ¹⁹F-labelled A β dissolved in 10 mM sodium phosphate, pH = 7.4 yielded peak areas of 86.3 % and 88.8 % of the control, respectively; and with Zn added (1:1) 77.3 % and 64.3 % of the control, respectively (Fig. S19 I). The loss of A β in the samples with Zn can be considered significant, compared to the control of A β in guanidinium. The differences between un-labelled A β (synthetic) and ¹⁹F-labelled A β (recombinant) are considered insignificant. In short, while exchanging A β oligomers of 20 – 60 kDa mass could not be observed in SEC, our chromatograms show reduced amounts of soluble A β for samples with equimolar Zn added. The loss in soluble A β in SEC is in close agreement with the calculations based on NMR data. No significant differences could be observed between un-labelled and ¹⁹F-labelled A β in SEC.

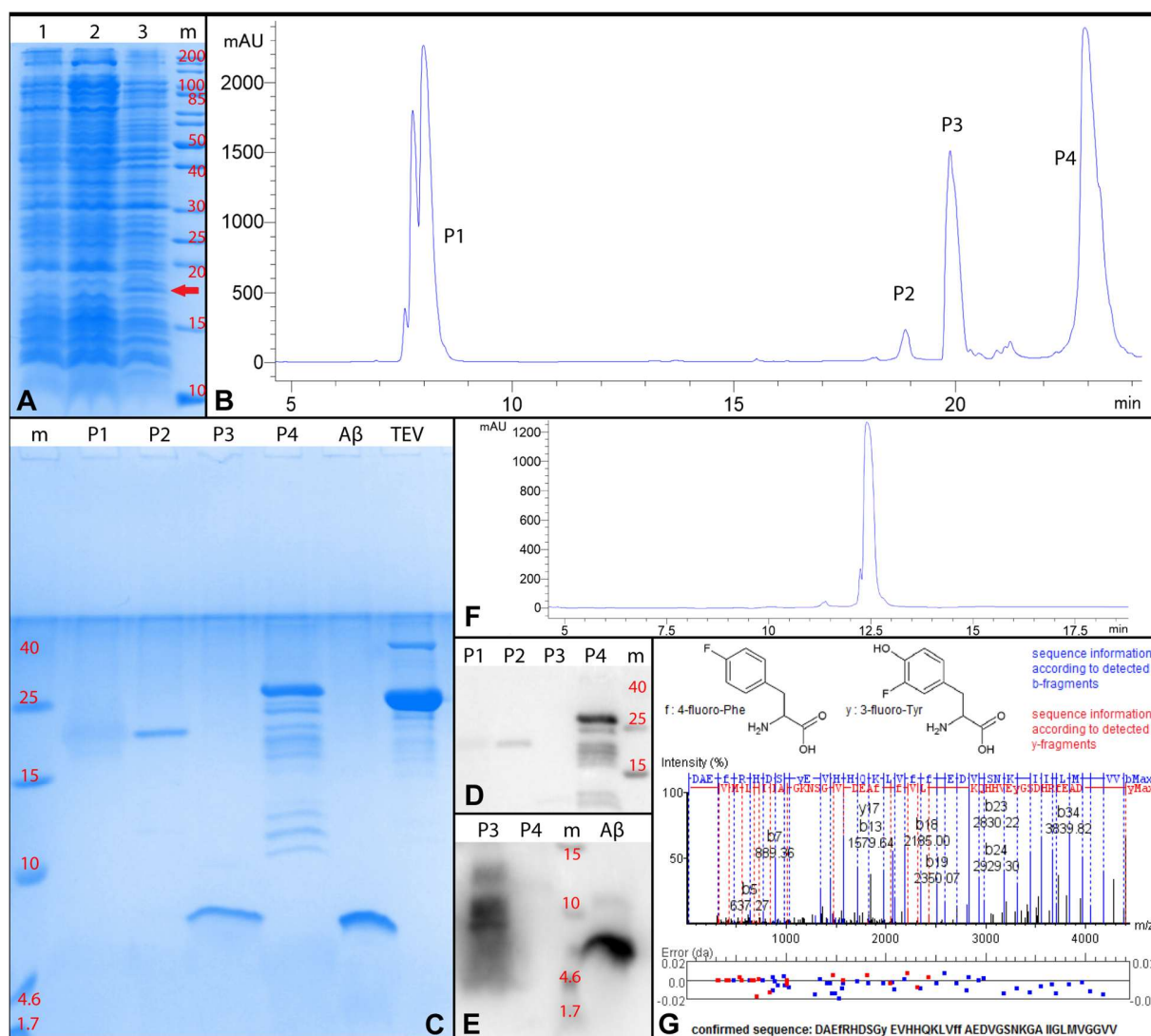
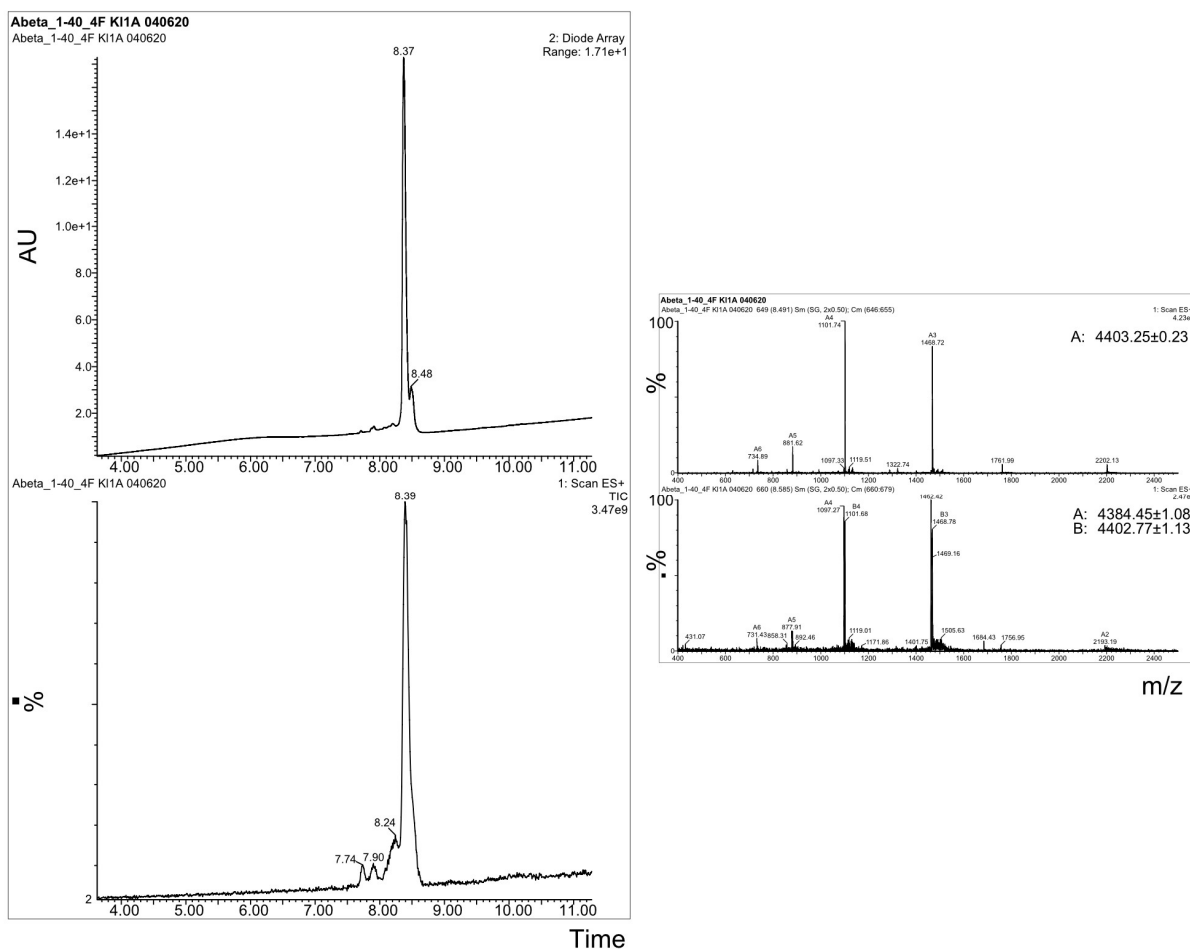


Figure S1. Recombinant production of fluorinated Aβ(1-40). **A**, Tris/Glycine SDS-PAGE of expression of NANP19-Aβ(1-40) in *E. coli* [1: in 2YT, no induction; 2: in defined medium, no induction; 3: in defined medium after induced expression; m: marker – PageRuler™ Unstained Protein Ladder]. **B**, Preparative HPLC chromatogram after TEV protease cleavage. **C-E**, Analysis of HPLC peaks P1-P4 by Tris/Tricine SDS-PAGE (**C**), anti-6His ["His.H8"] western blot (**D**), and anti-Aβ ["IC16"] western blot (**E**), with synthetic Aβ(1-40) [Bachem] and recombinant TEV protease (6His-tagged) as references [m: Spectra™ Multicolor Low Range Protein Ladder]. **F**, HPLC chromatogram of purified F-Phe, F-Tyr-Amyloid β (1-40). **G**, UHPLC-coupled ESI mass spectrometry-based sequence analysis of purified F-Phe, F-Tyr-Amyloid β (1-40). Shown is the tandem mass spectrum (MS-MS) of the most abundantly detected peptide species in the sample, which can be identified as the desired Aβ(1-40) peptide with all 4 fluorinations (at Phe4, Tyr10, Phe19 and Phe20). Species with less than 4 fluorinations were also found in the sample.



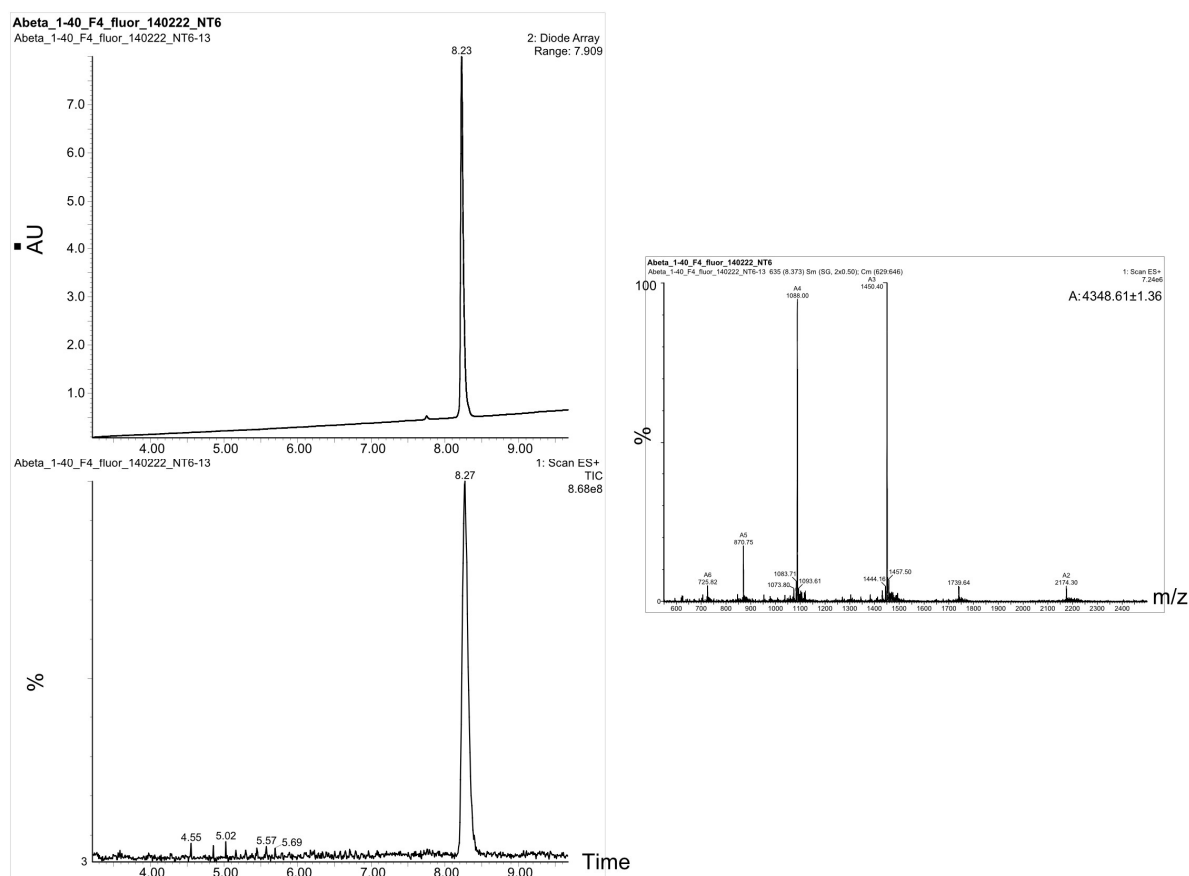


Figure S3. HPLC chromatogram and mass spectrometry analysis of chemically synthesized fluorinated A β (1-40) at Phe4.

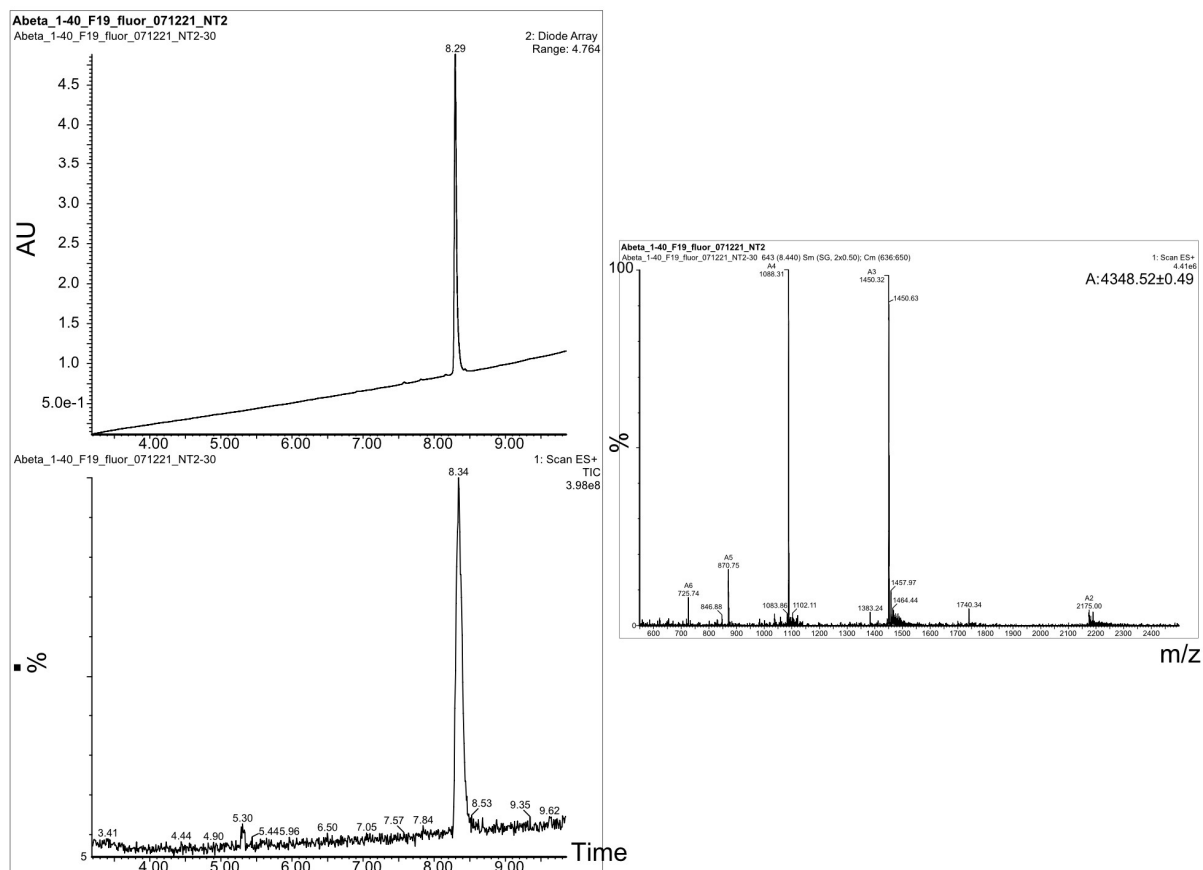


Figure S4. HPLC chromatogram and mass spectrometry analysis of chemically synthesized fluorinated A β (1-40) at Phe19.

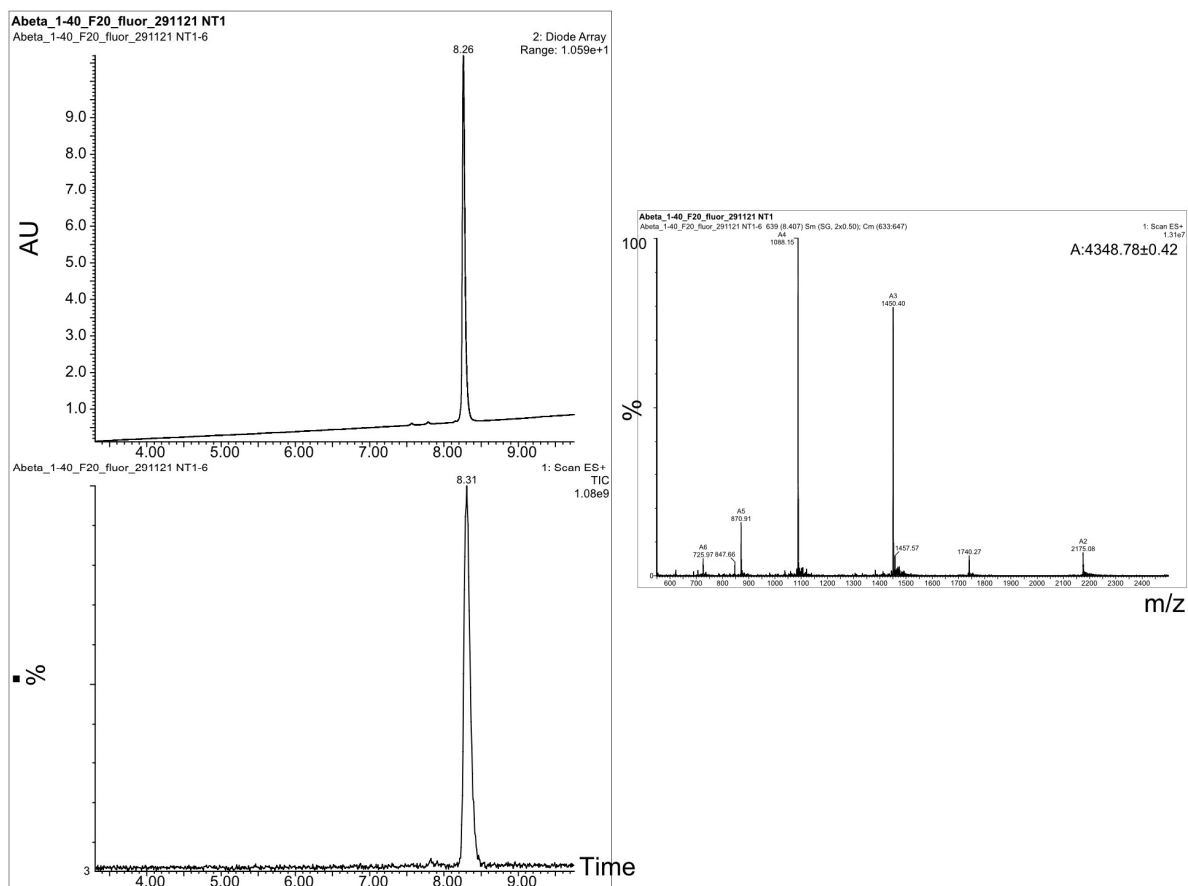


Figure S5. HPLC chromatogram and mass spectrometry analysis of chemically synthesized fluorinated A β (1-40) at Phe20.

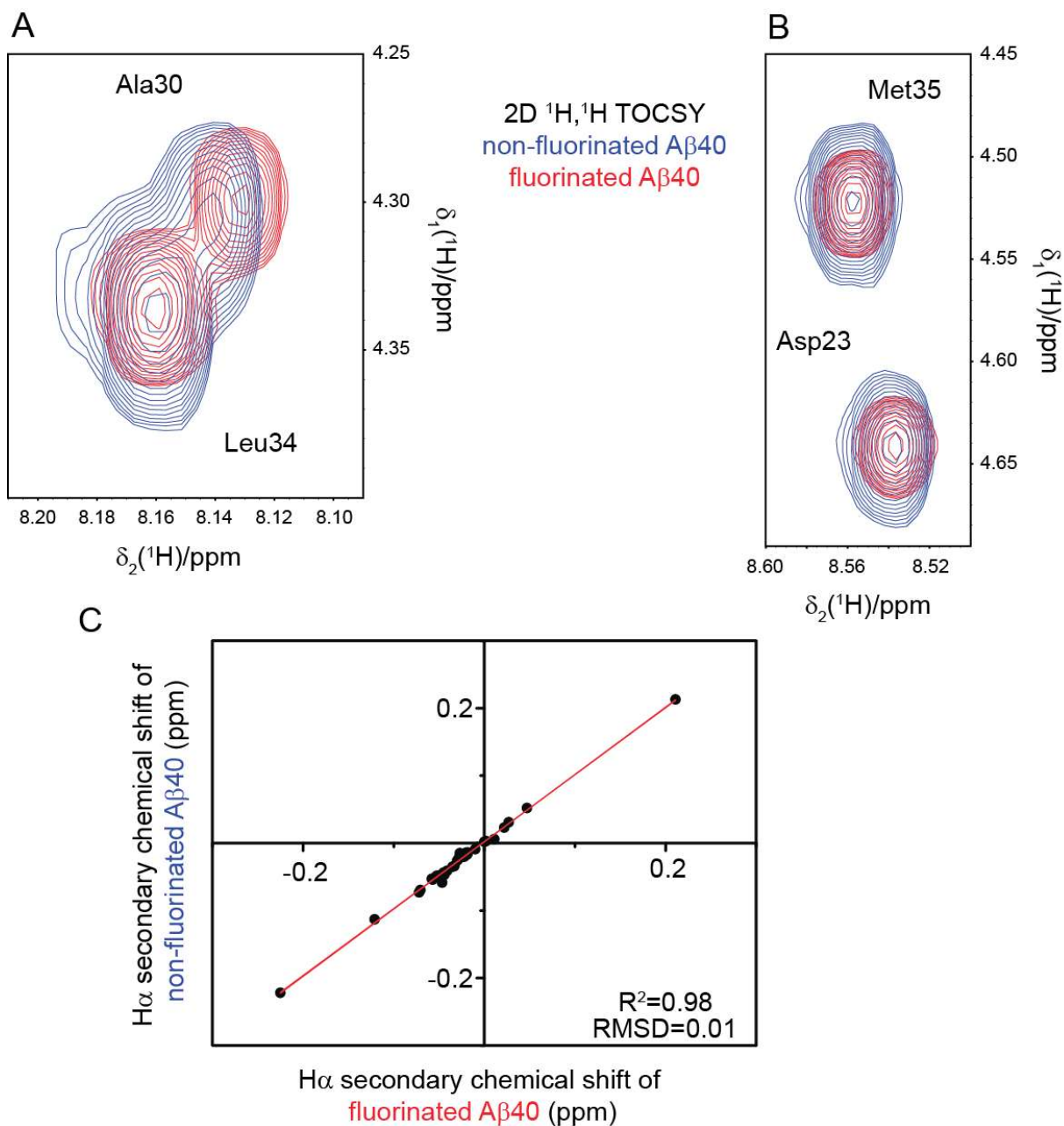


Figure S6. The overlaid 2D $^1\text{H}, ^1\text{H}$ TOCSY spectra of non-fluorinated (blue) and fluorinated A β 40 (red) showing as examples the HN/H α cross-peaks of residues Ala30 and Leu34 (**A**) and Asp35 and Met35 (**B**). In (**C**), the strong correlation between the secondary H α chemical shifts ($R^2=0.98$) indicate that ^{19}F labeling of aromatic side chains of the three phenylalanine (Phe4, Phe19, Phe20) and Tyr10 residues does not alter backbone conformation of A β 40. The H α secondary chemical shifts of fluorinated residues were not included in the analysis. The regression line is shown in red and the root mean square deviation (RMSD) is reported.

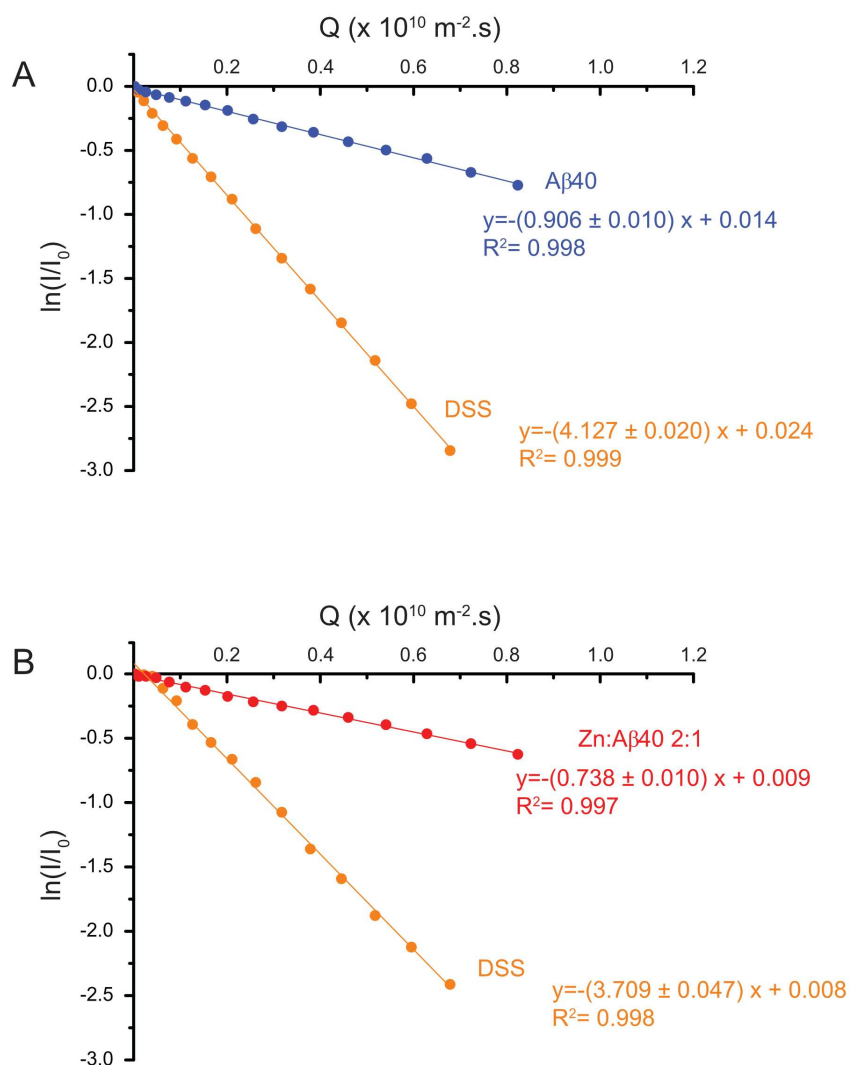


Figure S7. Pulse field gradient (PFG)-NMR based diffusion coefficients of non-fluorinated Aβ40 (100 μM), measured in the absence (**A**) or presence (**B**) of ZnCl₂ (200 μM, in 10 mM phosphate). The gradient-dependent decay in the relative intensity of NMR signals (in methyl region) is shown, with the slope of the fitted lines representing diffusion coefficients (in 10⁻¹⁰ m².s⁻¹ unit). An internal reference DSS was also measured and served as a reference for the hydrodynamic radius (*R_h*) calculation of Aβ40. Addition of Zn ion causes a decrease in diffusion coefficient of DSS, potentially reflecting a viscosity increase. Upon addition of Zn ion, the apparent *R_h* of Aβ40 increases from 1.87±0.02 nm to 2.06±0.04 nm, an increase by 10±2 %.

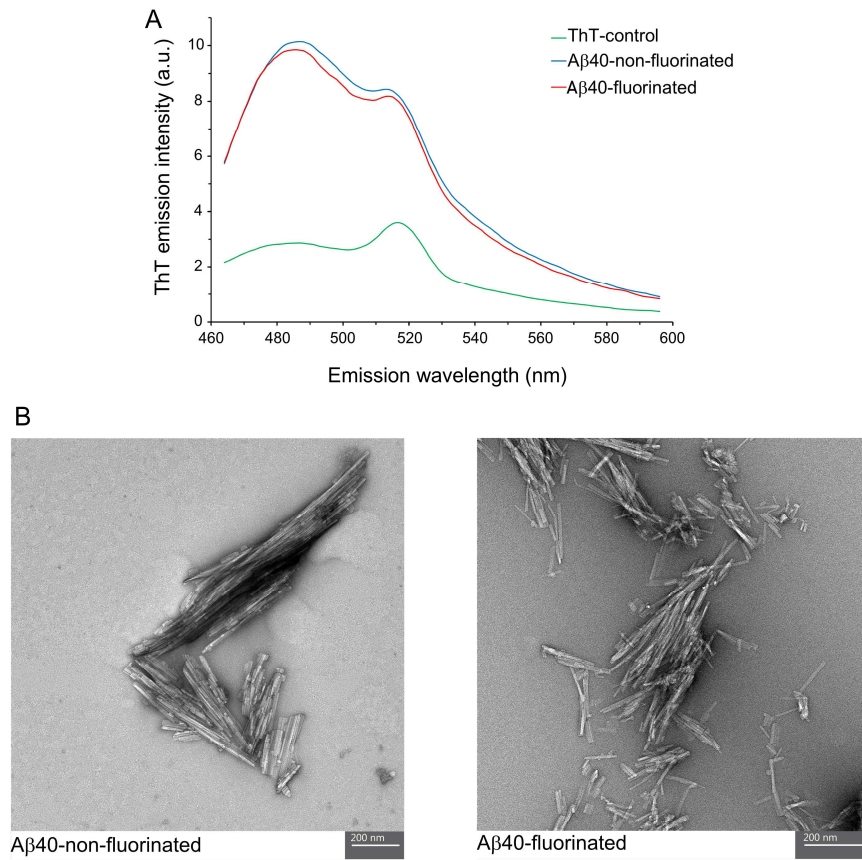


Figure S8. Fluorinated A β 40 (at Phe4, Tyr10, Phe19 and Phe20) is capable of forming Thioflavin T (ThT)-reactive amyloid fibrils, similar to non-fluorinated A β 40. **A**, ThT fluorescence emission spectra, without (ThT-control) or with added non-fluorinated or fluorinated A β 40 incubated for 48 hours in an aggregation-promoting condition (37 °C, agitation). **B**, Transmission electron microscopy (TEM) images of non-fluorinated or fluorinated A β 40, after 48 hours of incubation in the above-mentioned aggregation-promoting condition.

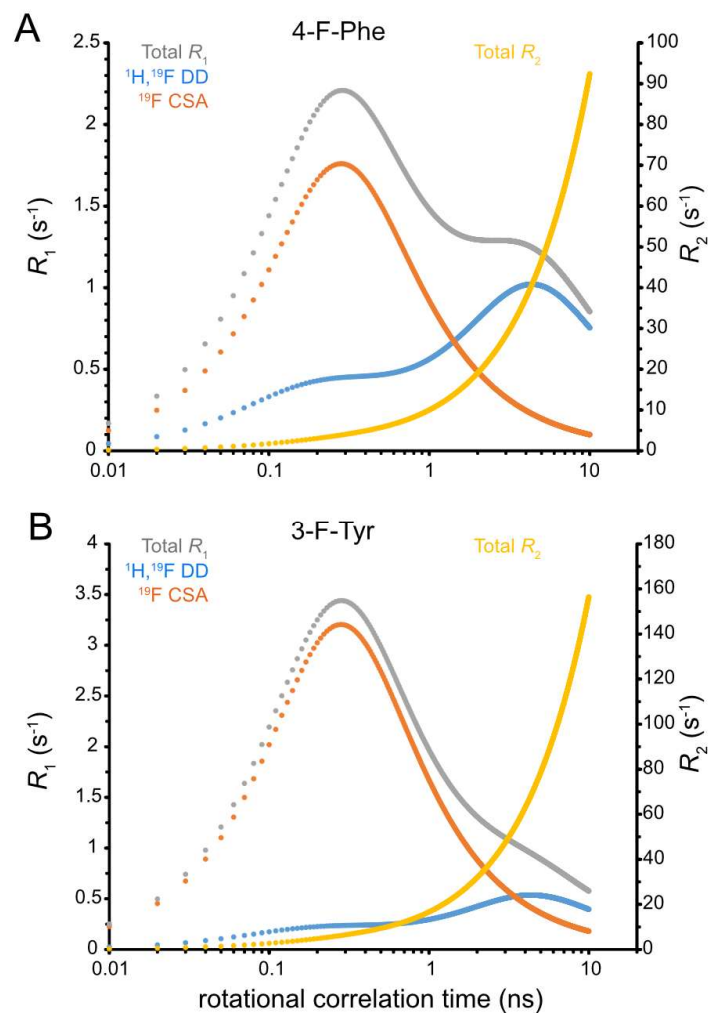


Figure S9. Simulated ^{19}F longitudinal (R_1) and transverse (R_2) relaxation rates of 4-F-Phe (**A**) and 3-F-Tyr (**B**) in dependence of rotational correlation time (τ_c). The $^1\text{H}, ^{19}\text{F}$ dipole-dipole (DD) and ^{19}F chemical shift anisotropy (CSA) relaxation-mediated parts of total R_1 are shown. The simulations were based on a reduced ^{19}F CSA of 57.5 ppm for 4-F-Phe and 77.6 ppm for 3-F-Tyr. All intra-residue protons within 4 Å from fluorine were included in the simulation.

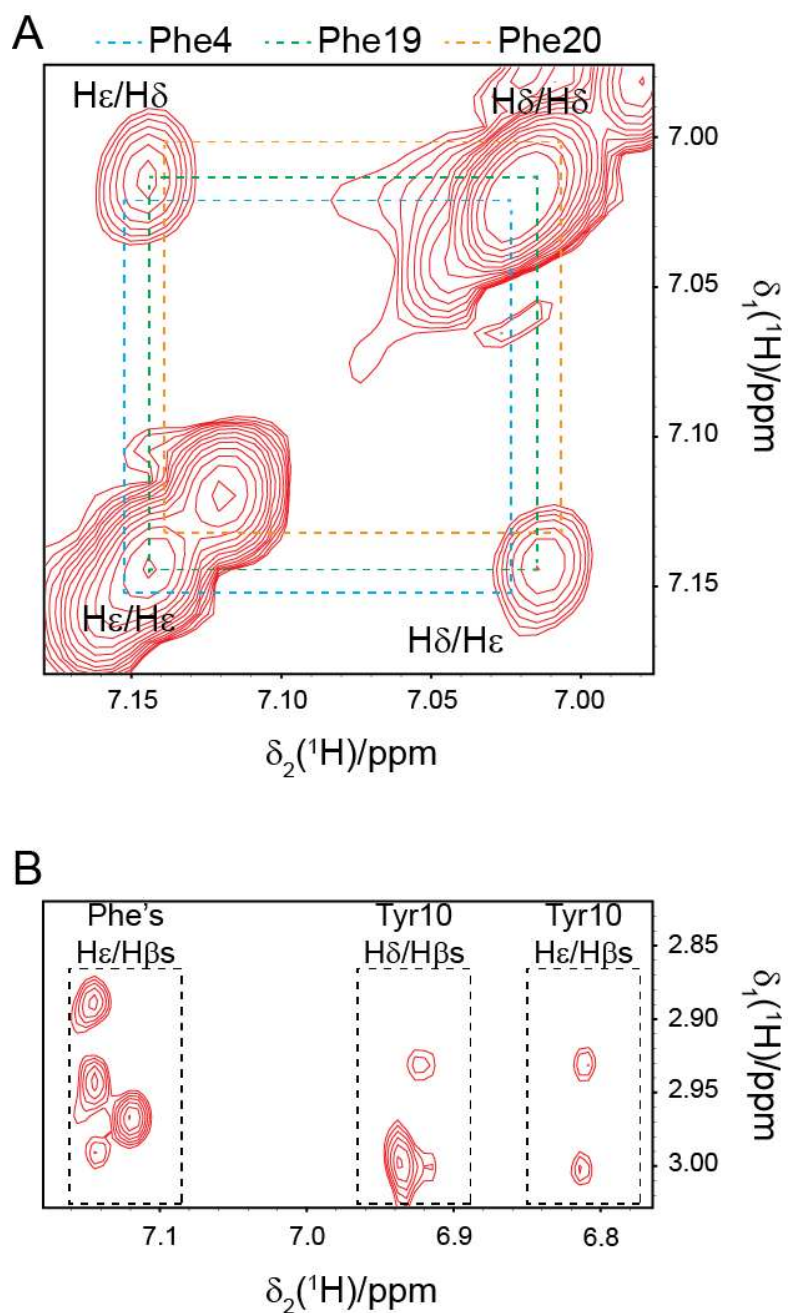


Figure S10. Portions of the 2D ^1H , ^1H NOESY spectrum of fluorinated A β 40, showing NOE H δ /H ϵ cross-peaks (**A**) and between aromatic and H β (**B**) protons of Phe and Tyr residues. The NOESY spectrum was acquired at 600 MHz proton Larmor frequency. The diagonal and NOE cross peaks have the same sign, indicating a rotational correlation time (τ_c) longer than ~ 0.3 ns for the aromatic rings.

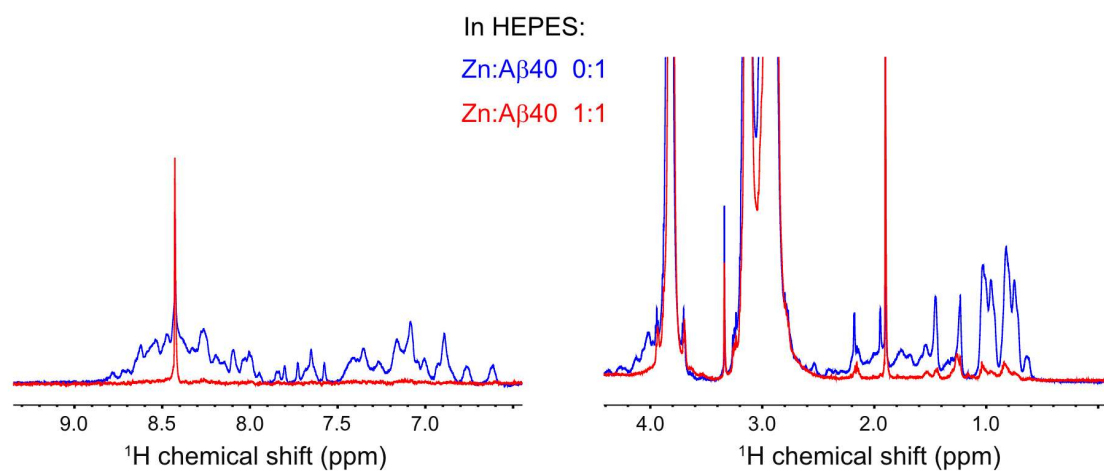


Figure S11. 1D ¹H NMR spectra of non-fluorinated Aβ40 peptide (0.4 mg/mL) without or with 1:1 molar ratio of ZnCl₂ in 12.5 mM HEPES, pH 7.4. The NMR signals in the amide (left) or aliphatic (right) regions are almost completely lost.

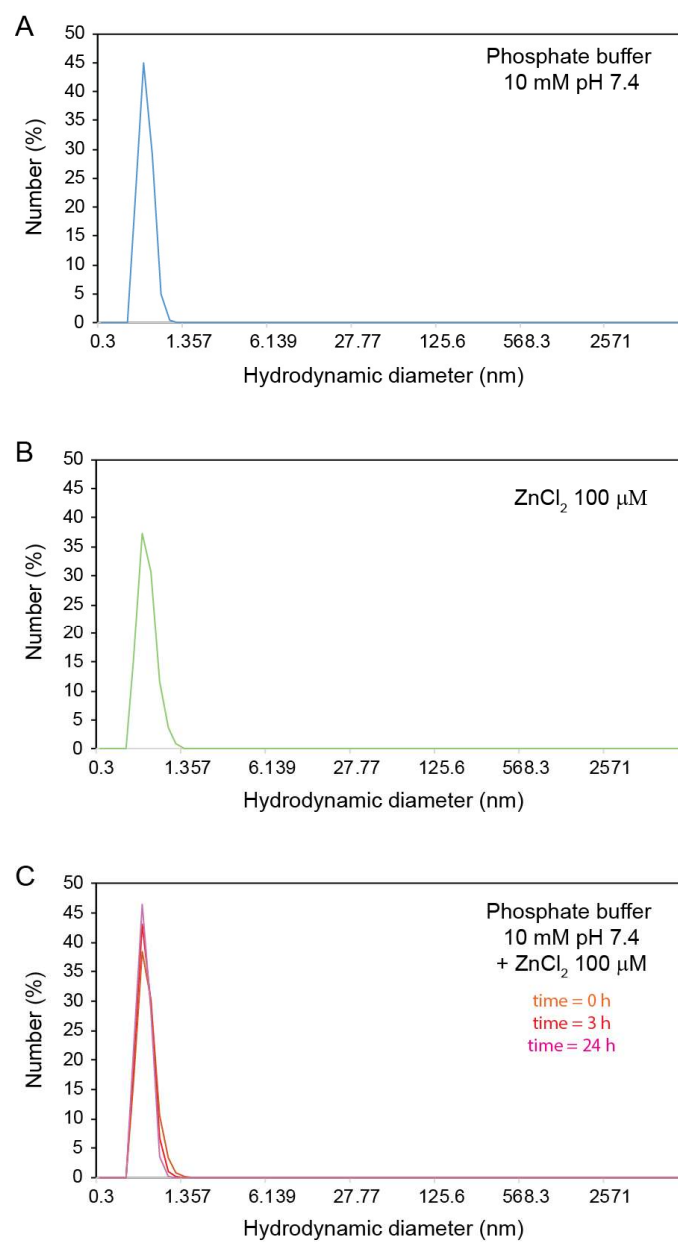


Figure S12. Dynamic light scattering measurement of **(A)** phosphate buffer (10 mM, pH 7.4) solution, **(B)** 100 μM ZnCl_2 solution and **(C)** phosphate buffer (10 mM, pH 7.4) upon addition of 100 μM ZnCl_2 , immediately (orange) and after 3 (red) and 24 (magenta) hours. The obtained size distributions do not show any significant particle formation larger than ~ 1.4 nm (hydrodynamic) diameter upon mixing of phosphate and ZnCl_2 solutions. As a comparison, the PFG-NMR diffusion-based hydrodynamic diameter of non-fluorinated and fluorinated $\text{A}\beta 40$ measured here is about 3.8-3.9 nm.

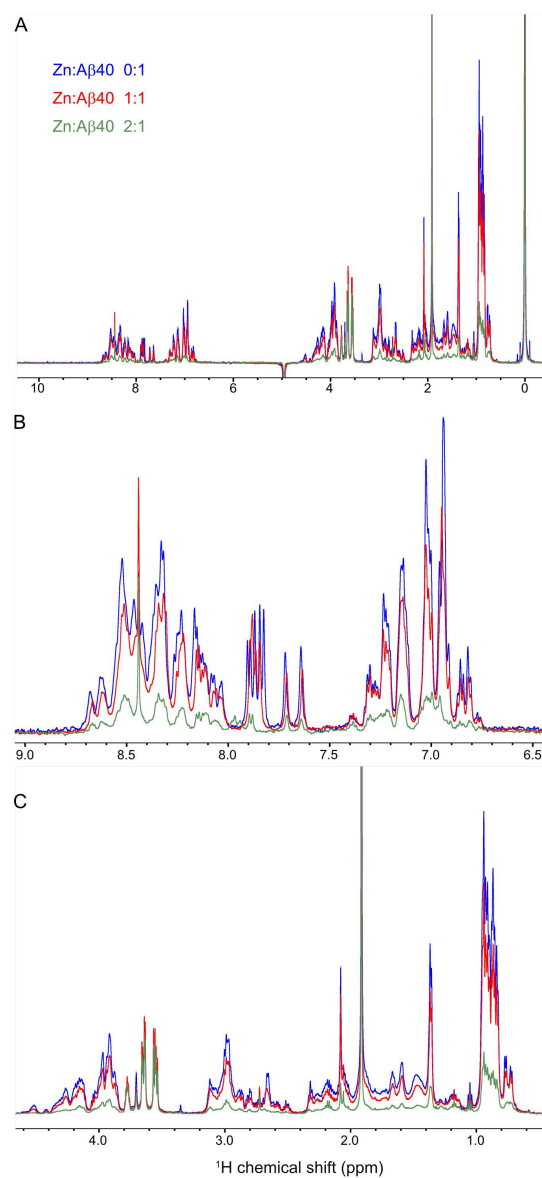


Figure S13. 1D ^1H NMR spectra of fluorinated $\text{A}\beta_{40}$, without (blue) and with added zinc ion at Zn: $\text{A}\beta_{40}$ ratios of 1:1 (red) and 2:1 (green), shown for the whole spectrum (**A**), the amide and aromatic (**B**) and the aliphatic and methyl (**C**) regions. The general intensity reduction indicates Zn-induced conversion of monomeric $\text{A}\beta_{40}$ into NMR-invisible $\text{A}\beta_{40}$ aggregates.

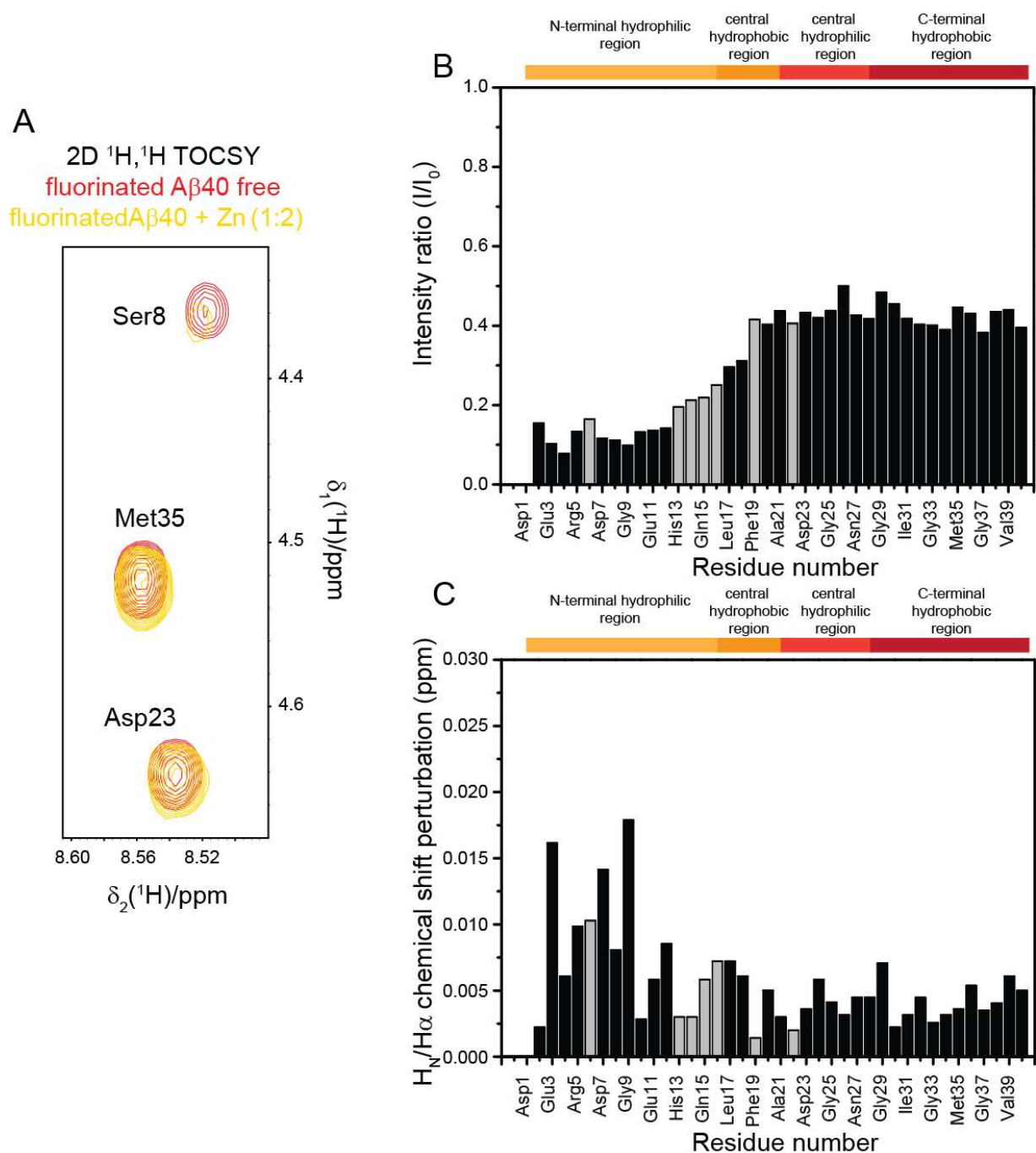


Figure S14. Interaction of fluorinated A β 40 with zinc ion (Zn), probed through chemical shift and intensity perturbation analyses. **A**, The overlaid 2D $^1\text{H}, ^1\text{H}$ TOCSY spectra of fluorinated A β 40, without (red) and with (yellow) Zn (Zn:A β 40 2:1). **B**, Zn-induced perturbation in the intensity of H_α - H_N TOCSY cross-peaks, showing a general intensity reduction which is more pronounced in the N-terminal region of A β 40. The general loss of signal intensity indicates a Zn-induced conversion of monomeric A β 40 to NMR-invisible aggregates. **C**, Combined $\text{H}_\text{N}, \text{H}_\alpha$ chemical shift perturbation analysis of A β 40 upon addition of 2 equivalent of Zn. In panels (**B**) and (**C**), the domains of A β 40 (N-terminal hydrophilic region, central hydrophobic region, central hydrophilic region and C-terminal hydrophobic) are shown at top and residues with overlapped $\text{H}_\text{N}/\text{H}_\alpha$ cross-peaks are highlighted in light grey.

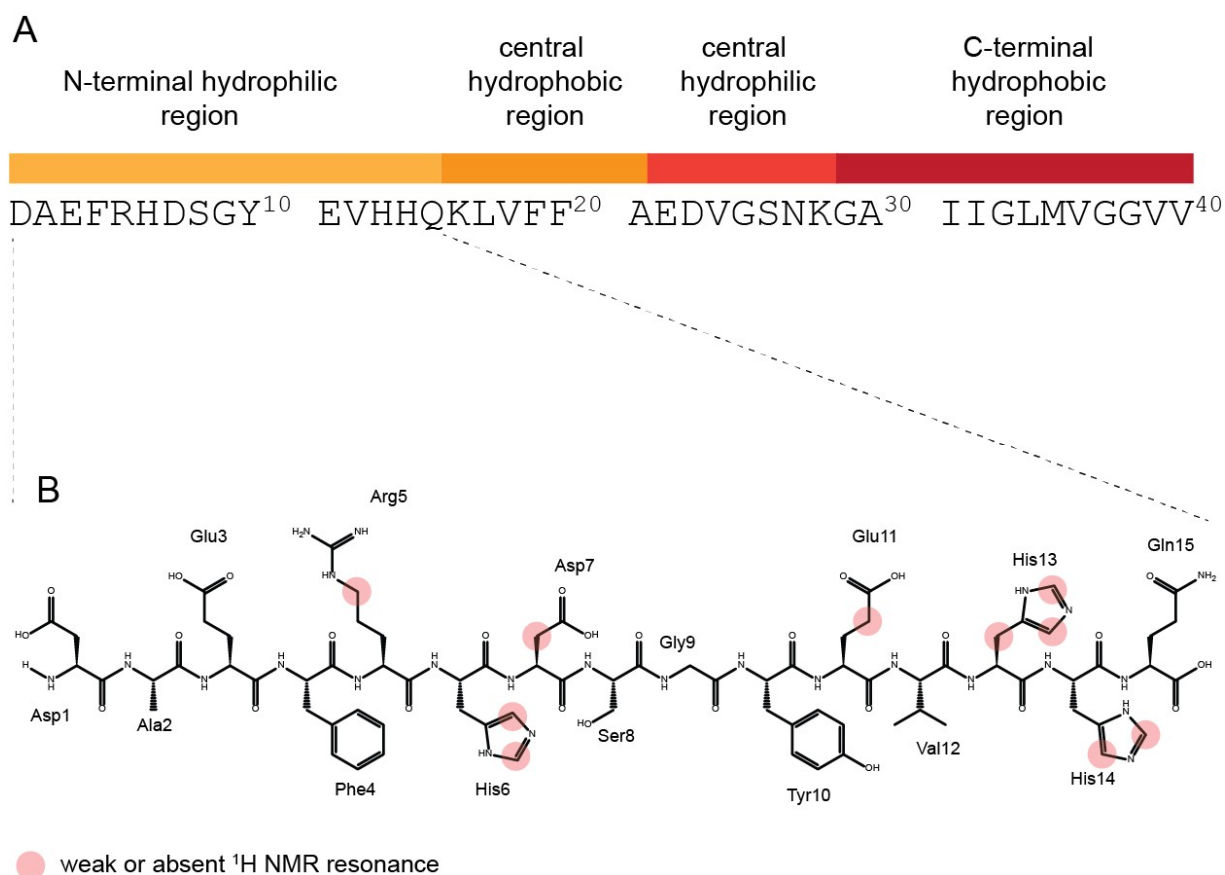


Figure S15. Deciphering of the zinc ion (Zn)-binding site of fluorinated Aβ40. **A**, Aβ40 primary sequence in which the N-terminal hydrophilic, the central hydrophobic, the central hydrophilic and the C-terminal hydrophobic regions are highlighted. **B**, Mapping of protons on their corresponding heavy atoms of the residues, located within the metal-binding region in the N-terminus of Aβ40, showing weak or absent NMR signals upon addition of 2 equivalent of Zn (red circle).

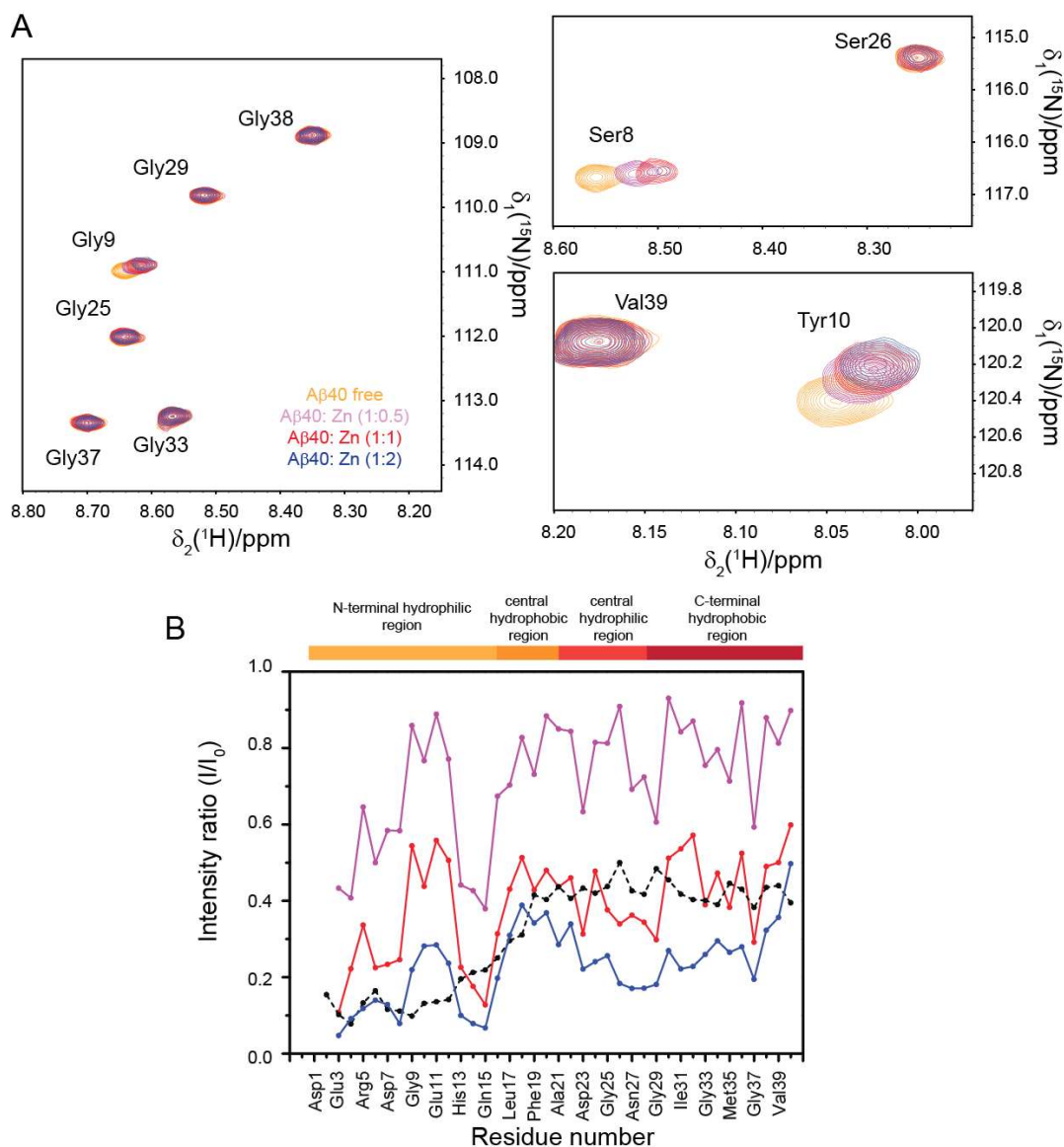


Figure S16. ^{15}N , ^1H HSQC spectra of 100 μM A β 40 in 10 mM Tris-HCl buffer at pH 7.4, 278 K, before (yellow) and after addition of ZnCl_2 at molar ratios (A β 40:Zn) of 1:0.5 (magenta), 1:1 (red) and 1:2 (blue). **A**, Portions of the overlaid ^{15}N , ^1H HSQC spectra showing Zn-induced peak displacements for residues Ser8, Gly9 and Tyr10 in the N-terminal region of A β 40. **B**, Zn-induced perturbation in the intensity of H_N -N cross-peaks upon addition of different Zn equivalent(s) in 10 mM Tris-HCl buffer at pH 7.4. The corresponding intensity profile of A β 40 upon addition of 2 equivalents of Zn in 10 mM phosphate buffer (pH 7.4) is also reported (the black line). The intensity reduction profiles observed in Tris-HCl buffer (at Zn:A β 40 ratios of 1:1 and 2:1) are comparable to that obtained for the peptide in phosphate buffer.

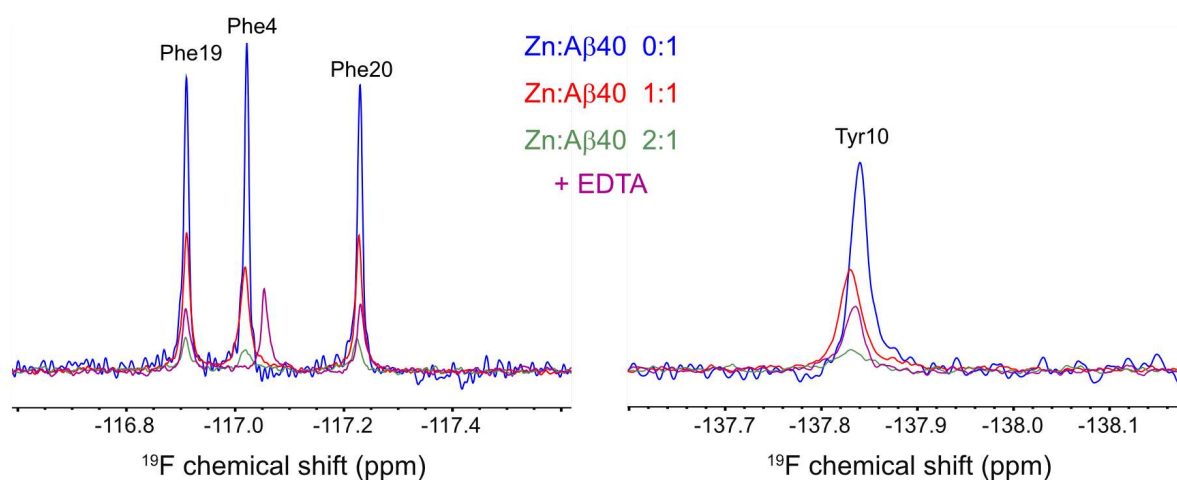


Figure S17. 1D ^{19}F NMR spectra of recombinantly produced fluorinated A β 40 in ^{19}F -Phe (left) and ^{19}F -Tyr (right) regions, in the absence or presence of zinc ion at Zn:A β 40 ratio of 1:1 or 2:1, showing significant zinc ion concentration-dependent intensity reduction for all residues. The intensity reduction is more pronounced for residues Phe4 and to a lower extent, Tyr10. After addition of a chelating agent (EDTA), the intensities of ^{19}F signals are partially recovered. The observed change in the chemical shift of Phe4 is probably due to a slight pH change after addition of 5 mM EDTA and the resultant alteration in the protonation state of the nearby His6's side chain.

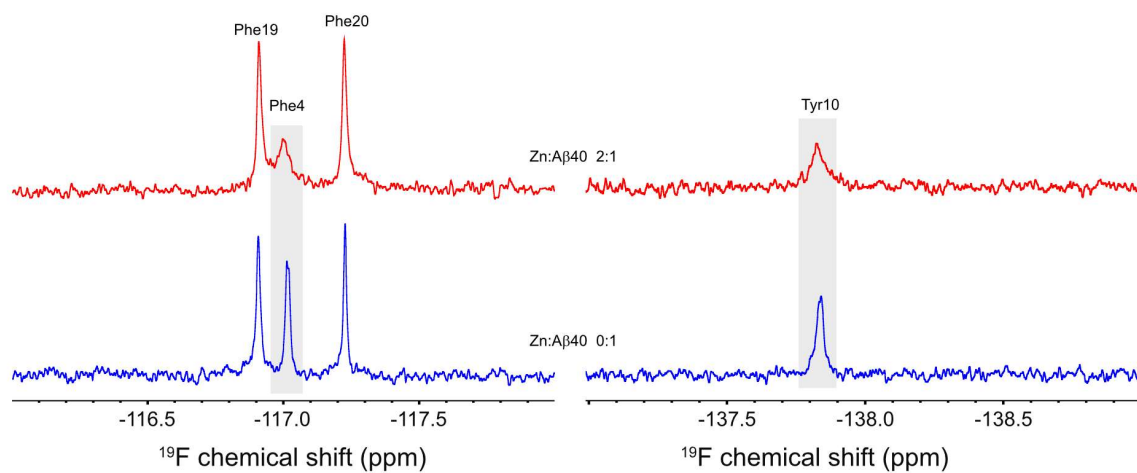


Figure S18. 1D ^{19}F spectra of chemically synthesized fluorinated A β 40 with ^{19}F labels incorporated at positions Phe4, Tyr10, Phe19 and Phe20 before and after addition of zinc ion. The ^{19}F signals of Phe4 and Tyr10 show clear intensity reduction after addition of metal ion.

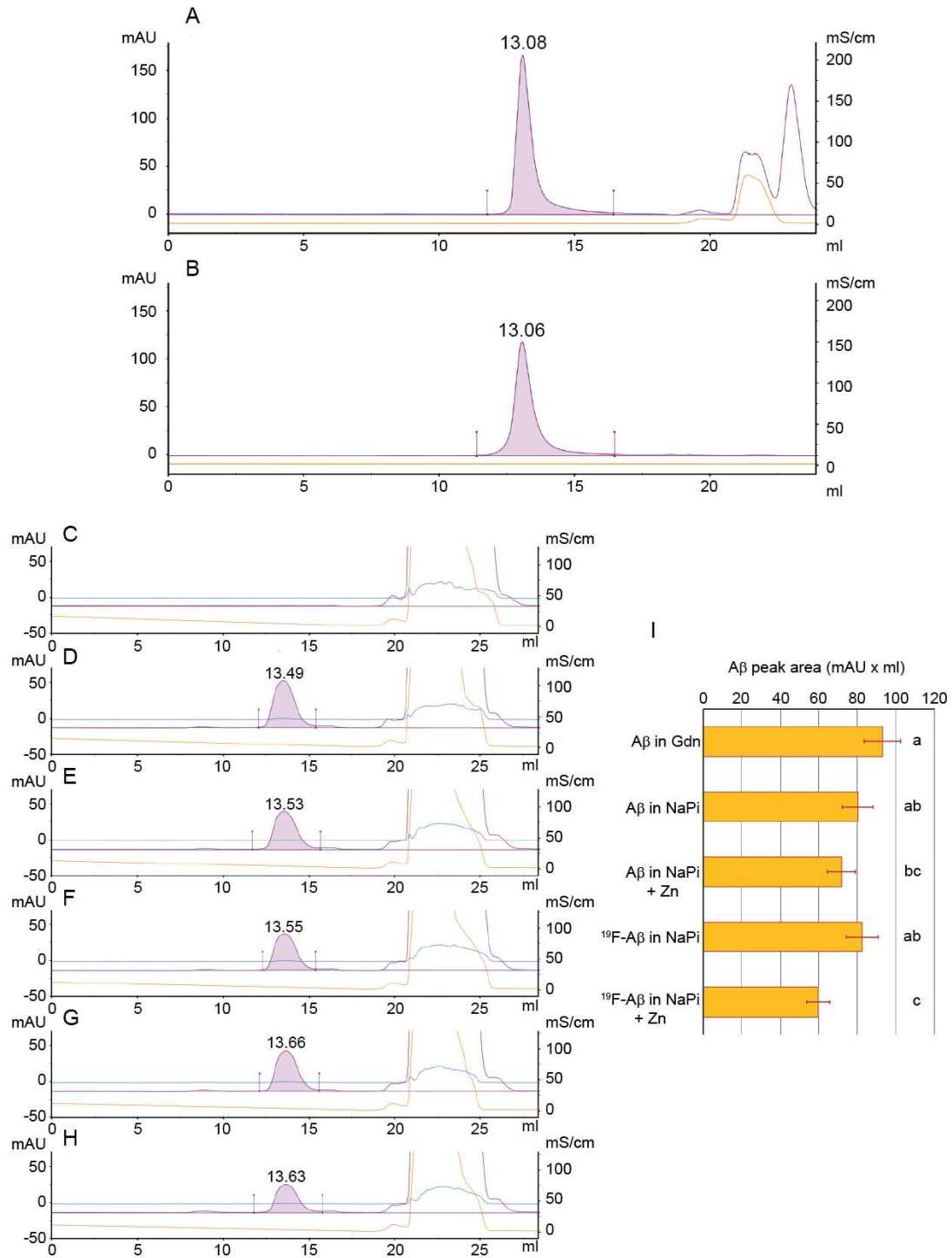
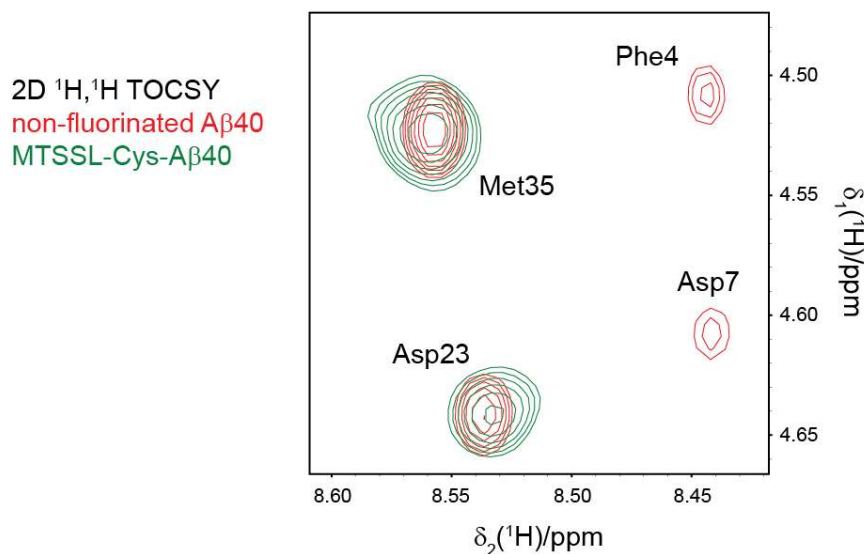


Figure S19. A,B, Size exclusion chromatograms of 0.2 mL supernatant of 100 μM Aβ (synthetic, Bachem). Absorption at 215 nm shown in purple (with baseline) and conductivity in orange. The highlighted peak areas correspond to monomeric Aβ. The numbers above indicate the retention volume (in mL). Oligomers are not detected. **A,** control: Aβ dissolved in 6 M guanidinium, 30 mM Tris, pH = 7.4. **B,** Aβ in 10 mM sodium phosphate, pH = 7.4 plus 100 μM Zn. **C-H,** Quantitative size exclusion chromatograms of 1.6 ml Aβ solutions, 10-fold diluted in 6 M guanidinium hydrochloride

(Gdn). Absorption at 215 nm shown in purple (with baseline), absorption at 276 nm in blue, and conductivity in orange. The highlighted peak areas correspond to monomeric A β and were integrated as they are shown in panel (I). The numbers above indicate the retention volume (in mL). **C**, guanidinium control: 2 mL buffer with 6 M guanidinium. **D**, Non-fluorinated A β dissolved in buffer with 6 M guanidinium. **E-H**, supernatant of A β dissolved in 10 mM sodium phosphate, pH = 7.4; non-fluorinated (**E**, **F**) and fluorinated (**G**, **H**) A β ; without (**E**, **G**) and with (**F**, **H**) equimolar Zn. **I**, A β peak areas from quantitative SEC of soluble A β shown in panels (**C-H**). The error bars indicate an estimated error of $\pm 10\%$. The letters next to error bars indicate significant or insignificant differences: areas sharing common letters do not differ significantly.

A



B

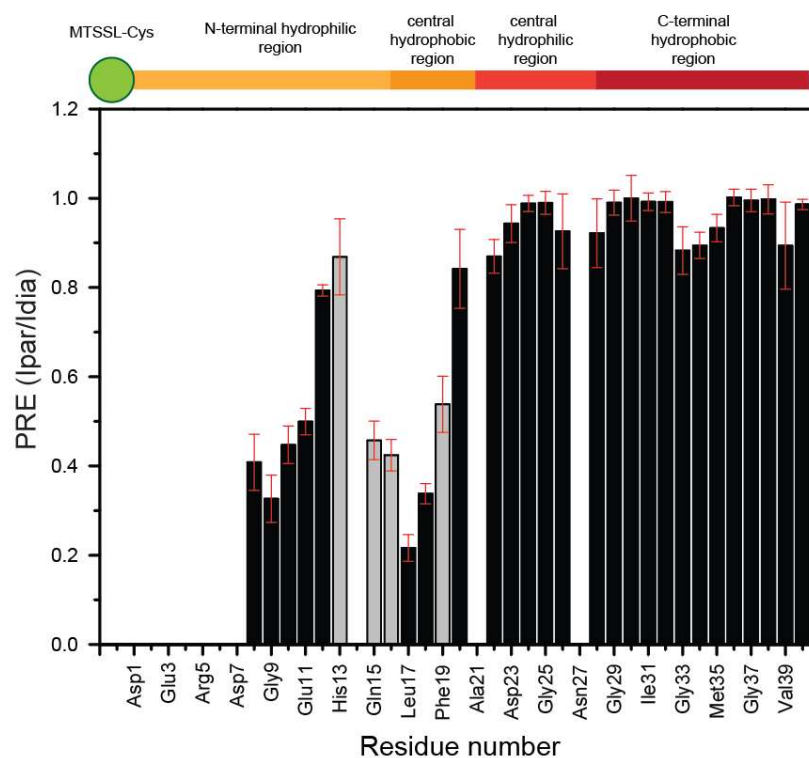


Figure S20. Paramagnetic Relaxation Enhancement NMR (PRE-NMR) analysis of MTSSL-Cys-A β 40. **A**, Portion of the 2D $^1\text{H}, ^1\text{H}$ TOCSY spectrum of diamagnetic A β 40 (red) and paramagnetic MTSSL-Cys-A β 40 (green). **B**, H_N/H_α PRE intensity ratio ($I_{\text{par}}/I_{\text{dia}}$) plot of A β 40 with the MTSSL paramagnetic spin label incorporated at position Cys0, showing complete disappearance of resonances belonging to residues Asp1-Asp7, adjacent to the nitroxide spin label, and significant intensity reduction of residues Ser8-Tyr10. In addition, a significant intensity reduction was detected for the residues Gln15-Phe19, indicating that this segment is spatially close to the MTSSL paramagnetic probe within the A β 40 conformational ensemble. In panel **B**, the domains of A β 40 (N-terminal hydrophilic region, central hydrophobic region, central hydrophilic region and C-terminal hydrophobic) and the MTSSL position (green fully circle) are shown at top.

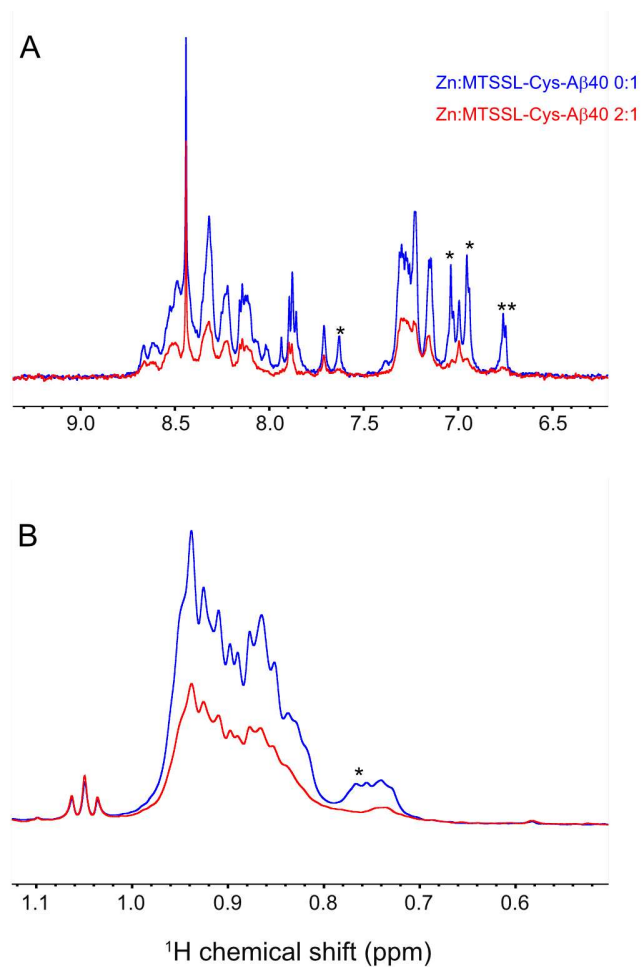


Figure S21. 1D ^1H NMR spectra of the MTSSL-Cys-A β 40, in the absence (blue) or presence (red) of zinc ion, confirming zinc ion binding to the peptide. **A**, the amide and aromatic region of the spectrum, showing a general signal intensity reduction, which is more pronounced for the signals of His6/13/14 and Gln15 side chains (marked by *) and Tyr10 side chain (marked by **). **B**, the methyl region of the spectrum, showing a general intensity reduction for the signals of peptide methyl groups. The signal of Val12 which exhibits a more pronounced intensity reduction is highlighted by *.

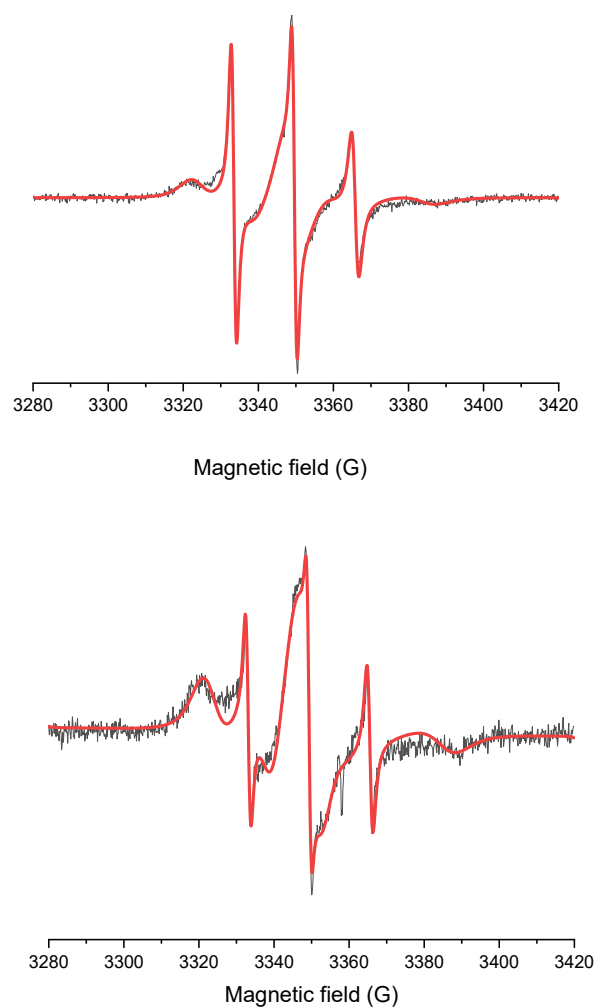


Figure S22. CW EPR spectra, black lines, for: **(Top)** MTSSL-Cys-A β : ^{19}F -A β (1:1) and **(Bottom)** MTSSL-Cys-A β : ^{19}F -A β : $[\text{Zn}^{2+}]$ (1:1:2). Red Lines: Easy-spin fitting of experimental data: 1st component, A (MHz): 42.3, 44.0, 50.0; g: 2.0035(8), 2.0066(1), 1.9991(1); 2nd component, A (MHz): 20.9, 19.8 105.3; g: 2.0039(8), 2.0053(1), 1.9993(6).

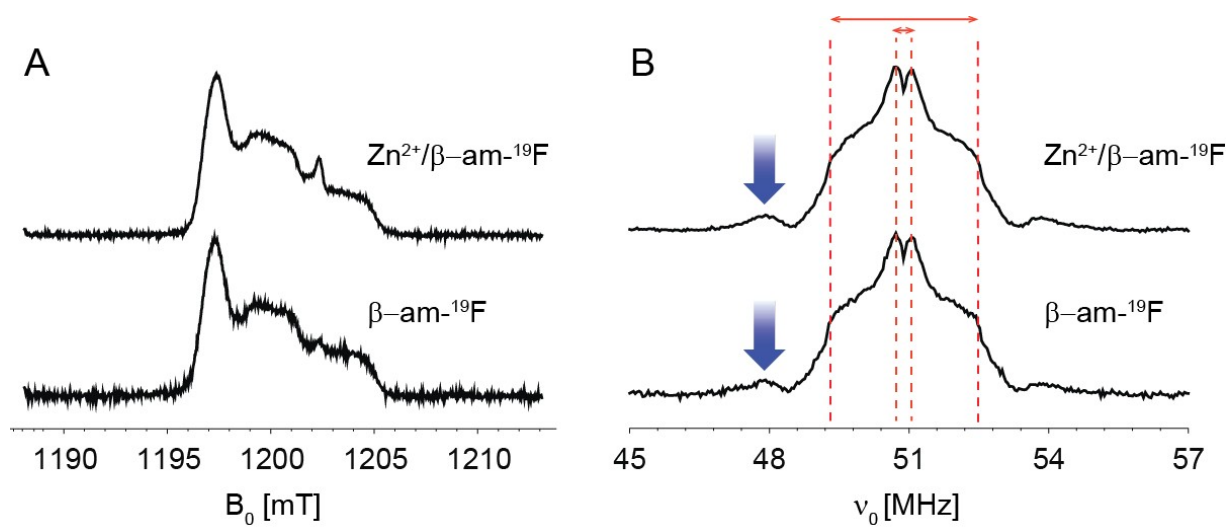


Figure S23. Echo-detected-field-swept (A) and Mims-ENDOR (B) spectra of 100 μM fluorinated A β 40 without or with equimolar ZnCl_2 , measured in 10 mM HEPES buffer, pH 7.4. Both spectra suggest that the presence of Zn^{2+} does not modify neither g-factors nor hyperfine coupling, in accord with observations in phosphate buffer (see Figs. 6 and S25).

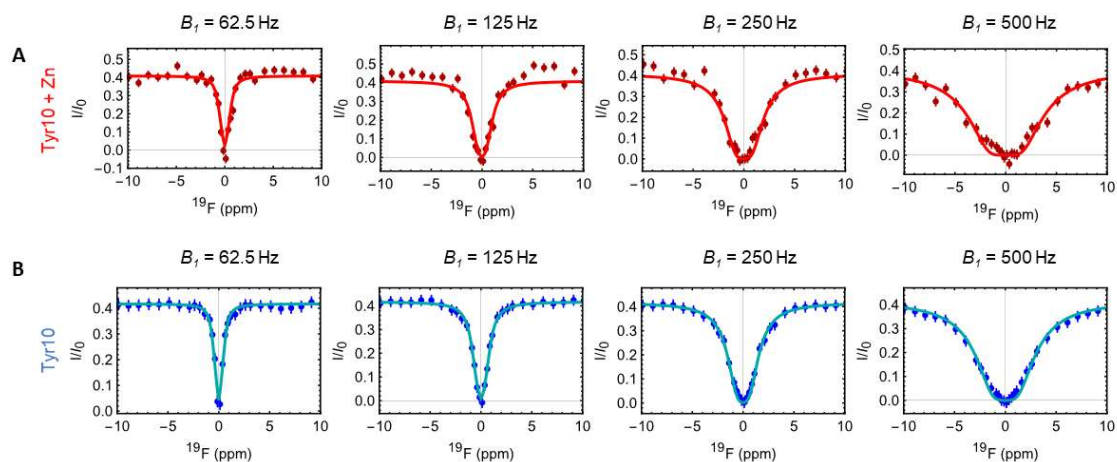


Figure S24. A, ^{19}F DEST profiles of fluorinated A β 40 in zinc-bound (red) form, for residue Tyr10, obtained at ^{19}F B_1 fields of 62.5, 125, 250 and 500 Hz. **B,** The free (blue) A β 40 profile is reproduced from Fig. 3B for visual comparison. The solid blue lines show a satisfactory global fit with a single-state (no exchange) model for Tyr10 in the free A β , based on which the ^{19}F relaxation rates of Tyr10 in free A β could be reliably extracted (see Fig. 3B and Table 1). The red solid lines show a fit with a two-state exchange model for Tyr10 of A β in the presence of zinc ion. This fit is suboptimal, suggesting that the exchange process for Tyr10 is more complex than a simple two-state exchange model.

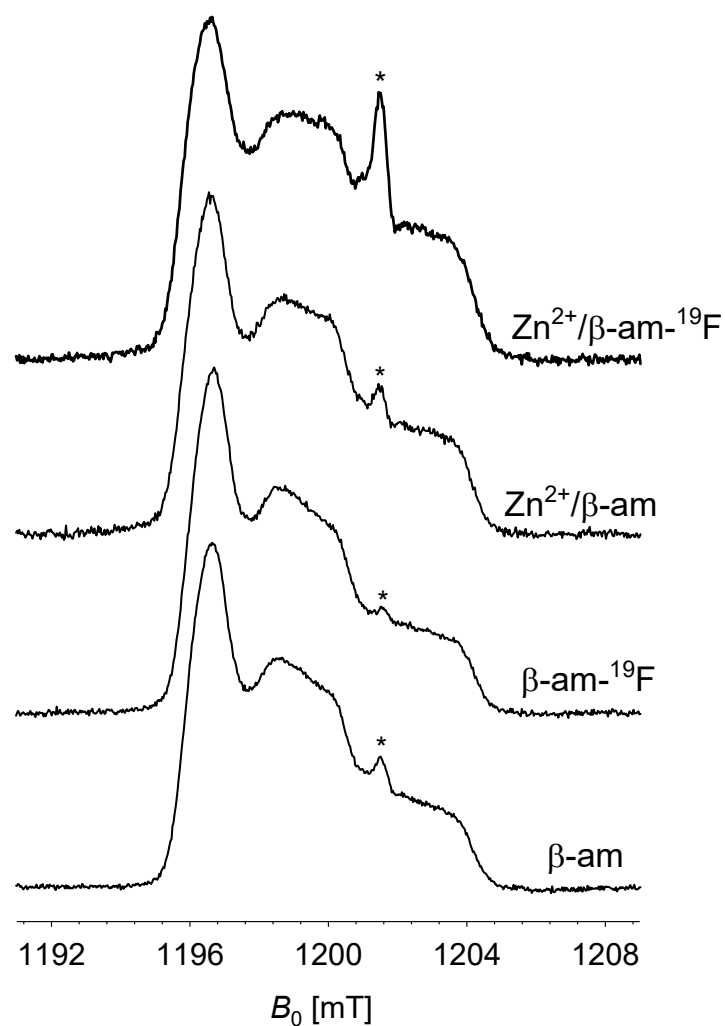


Figure S25. Q-band (34 GHz) Echo-Detected-Field-Swept (EDFS) for the four samples analyzed by Mims-ENDOR. (*) Signal from the background. Two-pulse (Hahn) echo detected Q-band (34 GHz) EPR spectra were collected with a $\pi/2-\tau-\pi-\tau$ -echo pulse sequence on a Bruker Eleksys-580 using an EN 5107D2 resonator and a HA8019 10 W amplifier. A temperature of 70 K was maintained with an Oxford liquid helium cryostat.

References

- 1 Lattanzi, V., Bernfur, K., Sparr, E., Olsson, U. & Linse, S. Solubility of A β 40 peptide. *JCIS Open* **4**, 100024 (2021).