Supporting Information:

Fluorinated amino acids in amyloid formation: a symphony of size, hydrophobicity, and α-helix propensity

Ulla I.M. Gerling[†], Mario Salwiczek[†], Cosimo D. Cadicamo[†], Holger Erdbrink[†], Constantin Czekelius[†], Stephan L. Grage[§], Parvesh Wadhwani[§], Anne S. Ulrich[§‖], Malte Behrends[‡], Günter Haufe[‡], Beate Koksch[†]*

[†] Department of Chemistry and Biochemistry, Freie Universität Berlin, Takustrasse 3, 14195 Berlin, Germany

[§] Karlsruhe Institute of Technology (KIT), Institute for Biological Interfaces (IBG-2), P.O. Box 3640, 76021 Karlsruhe, Germany

[‖] Karlsruhe Institute of Technology, Institute of Organic Chemistry, Fritz-Haber-Weg 6, 76131 Karlsruhe, Germany

[‡] Organisch-Chemisches Institut, Westfälische Wilhelms Universität Münster, Corrensstraße 40, 48149 Münster, Germany

Content:

1. Names, sequences, and identification of synthesized peptides by ESI-ToF mass spectrometry.
2. CD spectra of fluorinated and non-fluorinated **P1** variants.
3. CD spectra of K*Xaa* peptides that were used to calculate the α-helix propensity.
4. Complete version of the ThT fluorescence staining assays for substituted position 13 and 14 and the triple substitutions.
5. Kinetics of amyloid formation under agitated conditions for substituted position 13.
6. Oligomerization of **K*Xaa*** peptides.
7. Oligomerization of the fluorinated and non-fluorinated **P1** variants.
8. TEM-Images of P1 and the Single MfeGly variants.

Beate.Koksch@fu-berlin.de

Table S1: Names, sequences and identification of synthesized **P1** variants by ESI-ToF mass spectrometry.

Name	**Sequence**	observed $[M+3]^{3+}$	calculated $[M+3]^{3+}$
P1	Abz-LK**V**ELEKLKSEL**VV**LKSELEKLKSEL	1048.6149	1048.6177
P1_TfeGly 13	Abz-LKVELEKLKSEL-**TfeGly**-VLKSELEKLKSEL	1061.9436	1061.9582
P1_TfeGly 14	Abz-LKVELEKLKSELV-**TfeGly**-LKSELEKLKSEL	1061.9501	1061.9582
P1_DfeGly 13	Abz-LKVELEKLKSEL-**DfeGly**-VLKSELEKLKSEL	1055.9345	1055.9615
P1_DfeGly 14	Abz-LKVELEKLKSELV-**DfeGly**-LKSELEKLKSEL	1055.9358	1055.9615
P1_MfeGly 13	Abz-LKVELEKLKSEL-**MfeGly**-VLKSELEKLKSEL	1049.9629	1049.9649
P1_MfeGly 14	Abz-LKVELEKLKSELV-**MfeGly**-LKSELEKLKSEL	1049.9597	1049.9649
P1_Leu 13	Abz-LKVELEKLKSEL-**Leu**-VLKSELEKLKSEL	1053.2982	1053.2895
P1_Leu 14	Abz-LKVELEKLKSELV-**Leu**-LKSELEKLKSEL	1053.2890	1053.2895
P1_TfeGly 3,13,14	Abz-LK-**TfeGly**-ELEKLKSEL-**TfeGly**-**TfeGly**-LKSELEKLKSEL	1088.5842	1088.6392
P1_Leu 3,13,14	Abz-LK-**Leu**-ELEKLKSEL-**Leu**-**Leu**-LKSELEKLKSEL	1062.6406	1062.6333
P1_(SS)-TfV 14	Abz-LKVELEKLKSELV-**(*SS*)-TfV**-LKSELEKLKSEL	1066.6173	1066.6315
P1_(SR)-TfV 14	Abz-LKVELEKLKSELV-**(*SR*)-TfV**-LKSELEKLKSEL	1066.6172	1066.6315

Table S2: Names, sequences and identification of synthesized **K *Xaa*** peptide variants by ESI-ToF mass spectrometry.

Name	**Sequence**	observed $[M+2]^{2+}$	calculated $[M+2]^{2+}$
K Ala	Ac-YGGKAAAAKA-**Ala**-AAKAAAAK-NH_2	851.4915	851.4920
K Leu	Ac-YGGKAAAAKA-**Leu**-AAKAAAAK-NH_2	872.5213	872.5155
K Val	Ac-YGGKAAAAKA-**Val**-AAKAAAAK-NH_2	865.5041	865.5077
K MfeGly	Ac-YGGKAAAAKA-**MfeGly**-AAKAAAAK-NH_2	867.4920	867.5285
K DfeGly	Ac-YGGKAAAAKA-**DfeGly**-AAKAAAAK-NH_2	876.4890	876.5235
K TfeGly	Ac-YGGKAAAAKA-**TfeGly**-AAKAAAAK-NH_2	885.4880	885.5185
K (SS)-TfV	Ac-YGGKAAAAKA-**(*SS*)-TfV**-AAKAAAAK-NH_2	892.4904	892.5285
K (SR)-TfV	Ac-YGGKAAAAKA-**(*SR*)-TfV**-AAKAAAAK-NH_2	892.4919	892.5285

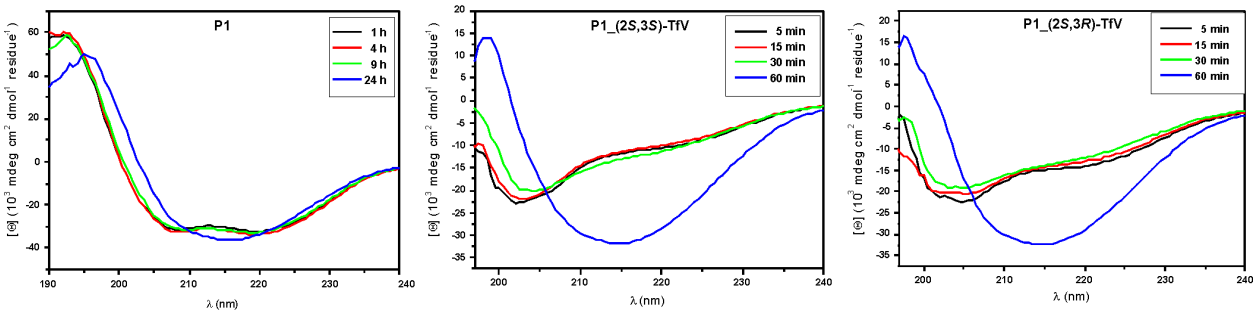

Figure S1. CD spectra of **P1** and the two **TfV** variants (100µM each) at different time points of incubation in 10 mM phosphate buffer at pH 7.4.

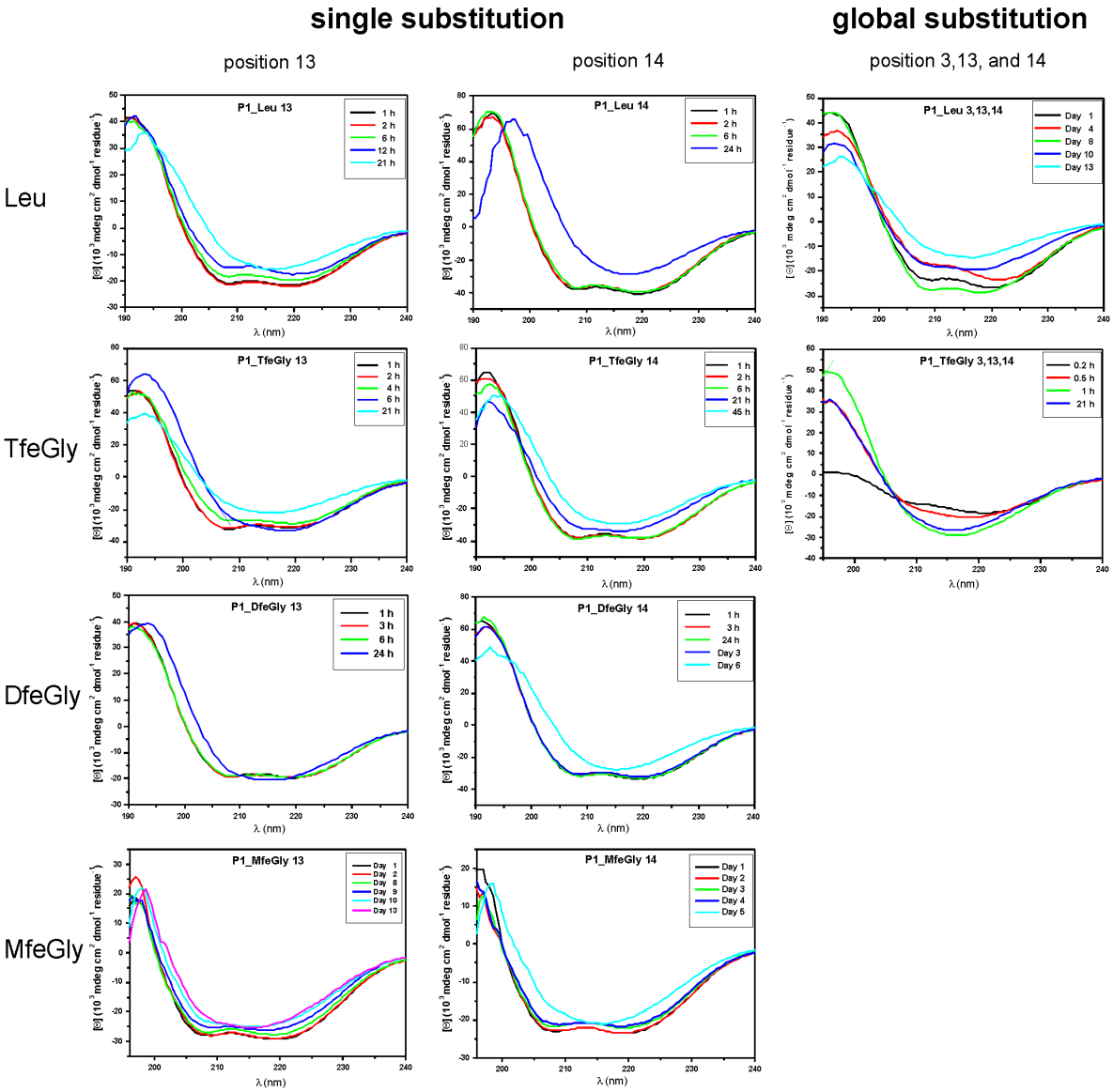

Figure S2. CD spectra of fluorinated and non-fluorinated **P1** variants (100 µM each) after different periods of incubation in 10 mM phosphate buffer at pH 7.4.

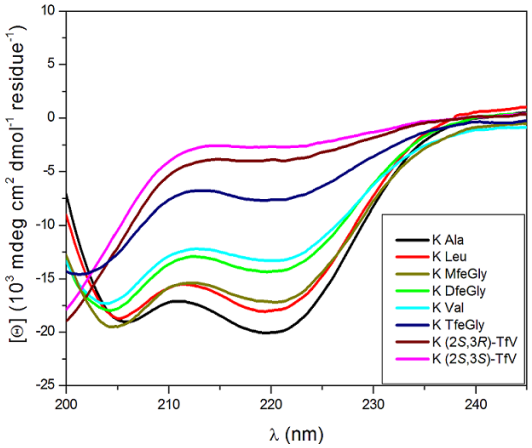

Figure S3. CD spectra of the **K *Xaa*** peptides at pH 7 in 1 mM phosphate, borate, and citrate with 1 M NaCl at 0°C. The displayed spectra represent the mean of three independent measurements at concentrations of 80µM, 50µM, and 30µM. The fractional helical content (f_{helix}) was calculated from mean residue molar ellipticity at 222 nm.

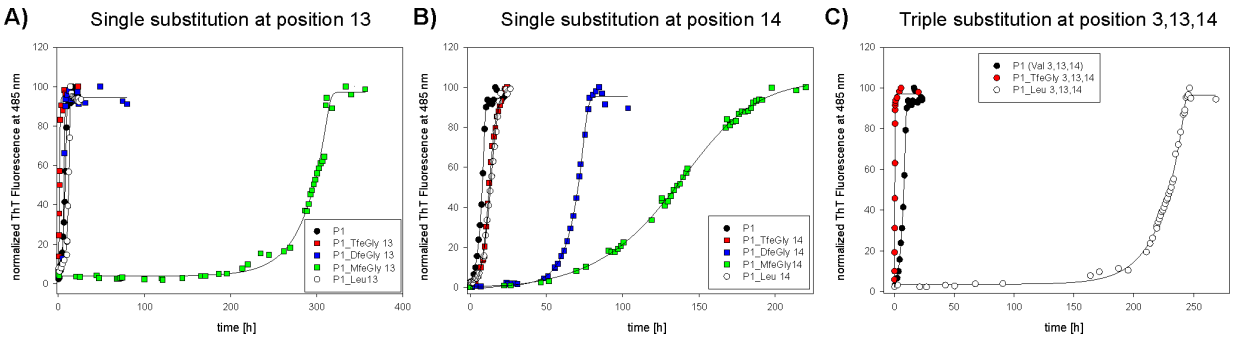

Figure S4. Complete version of ThT fluorescence staining assay for peptides variants compared to P1 (●) and the Leu containing reference peptides (○). Single substitution of valine 13 (A) and valine 14 (B) with fluorinated analogues of L-amino butyric acid: TfeGly (■), DfeGly (■), and MfeGly (■). (C) Simultaneous substitution of all three valine residues with TfeGly (●), and Leu (○).

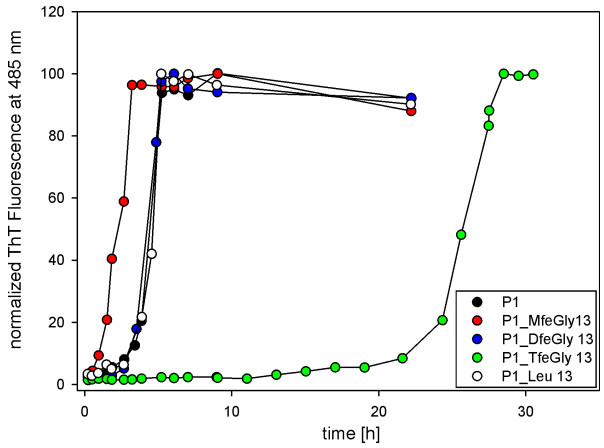

Figure S5. ThT fluorescence staining assay under constantly agitated conditions for P1 (●) and the variants containing TfeGly (●),DfeGly (●),MfeGly (●) and Leu (○)in position 13.

Oligomerization of K*Xaa* peptides

The monomeric oligomerization state has been determined applying size exclusion chromatography (SEC) in combination with static light scattering (SLS). Obtained results indicate monomeric oligomerization of the peptides. Single peaks ware found for all peptide variants with SEC containing species with a molecular weight comparable to theoretical monomer masses (*table S3, figure S6*). The reported values and spectra are the mean of three individual measurements. The sample concentration was 80 µM for each peptide.

Table S3: Theoretical end experimental determined mol masses of **K*Xaa*** peptide variants.

Peptide	theoretical monomer mass [Da]	SEC/SLS determined mass [Da]
K TfeGly	1771	1950 ± 220
K DfeGly	1752	1948 ± 236
K MfeGly	1733	1909 ± 226
K Val	1729	2029 ± 143
K (2S,3S)-TfV	1782	1998 ± 183
K (2S,3R)-TfV	1783	1919 ± 243

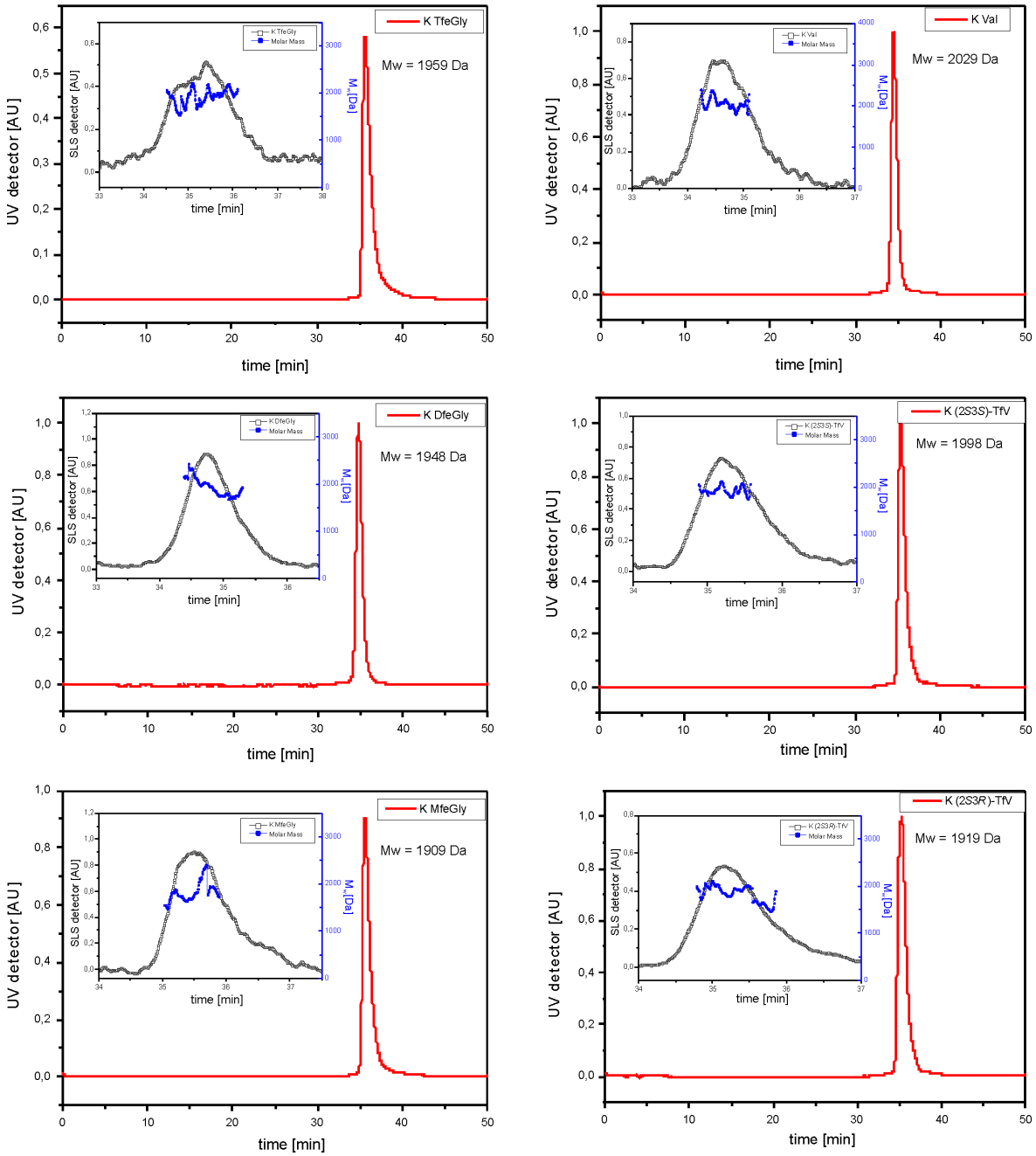

Figure S6. SEC/SLS chromatograms of **K*Xaa*** peptides. Single peaks were detected with UV at 230 (red). Corresponding rayleigh ratio (black) and molar mass distribution (blue) indicate monomeric species for all peptide variants.

Oligomerization of P1 variants

The dimeric oligomerization state of the initial coiled coil starting structure has been determined applying size exclusion chromatography (SEC) in combination with static light scattering (SLS). Obtained results reveal dimeric oligomerization for all investigated peptide variants. Single peaks ware found for all peptide variants with SEC containing species with a molecular weight comparable to theoretical dimer masses (*Table 1, Figure S7*). The reported values and spectra are the mean of three individual measurements. The sample concentration was 1000 µM for each peptide.

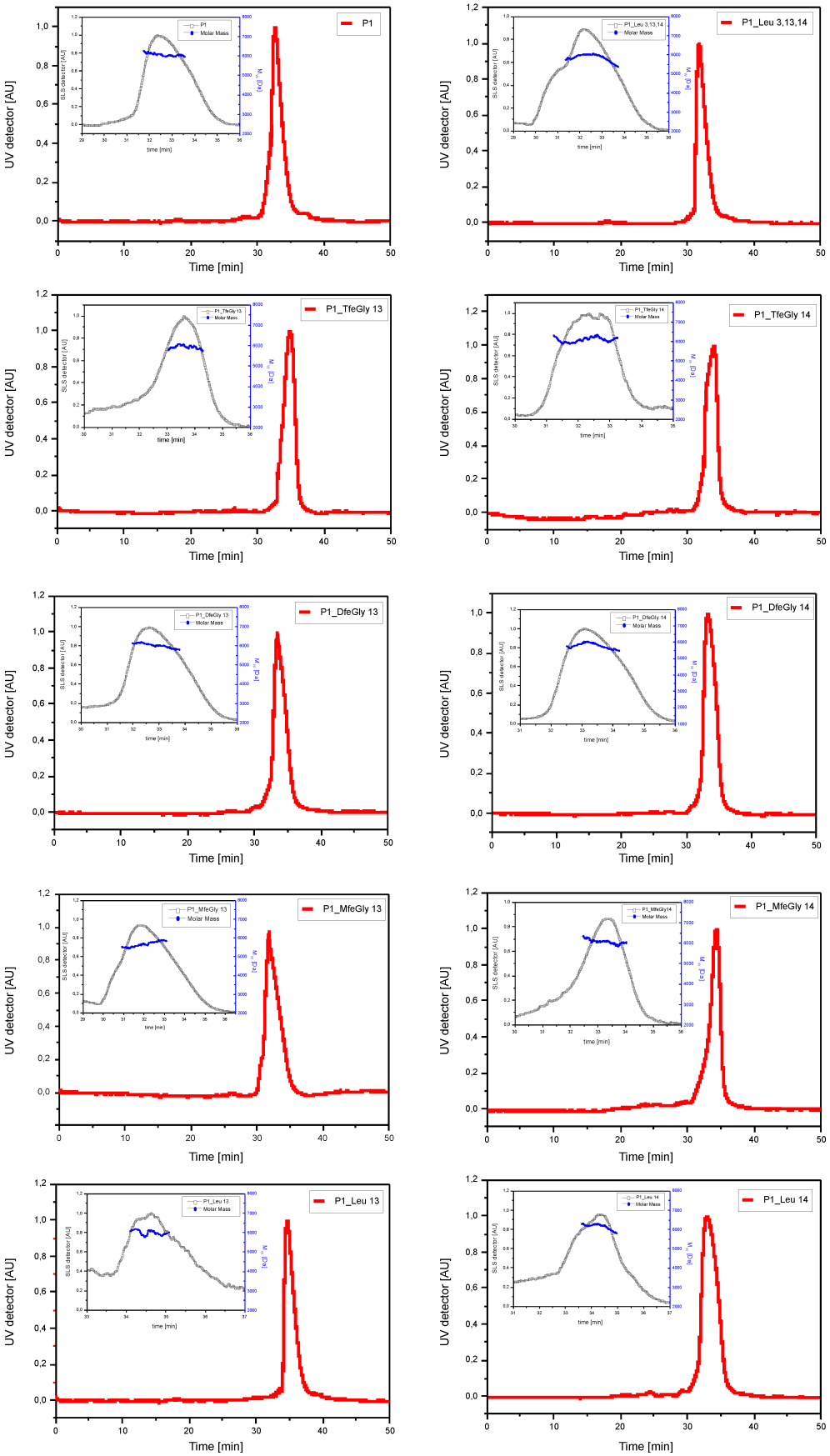

Figure S7. SEC/SLS chromatograms of **P1** and the fluorinated and non-fluorinated variants. Single peaks were detected with UV at 230 (red). Corresponding rayleigh ratio (black) and molar mass distribution (blue) show initial dimeric species for all peptides.

Transmission electron microscopy

Samples for staining electron microscopy were prepared by absorbing 7 µL aliquots of peptide solution to glow-discharged carbon-coated collodium films on 400-mesh copper grids. The grids were blotted, stained with 1% phosphotungstic acid (PTA) and air dried. TEM micrographs were taken using a Philips CM12 transmission electron microscope at a primary magnification of 58300× using a defocus of 0.6 µm.

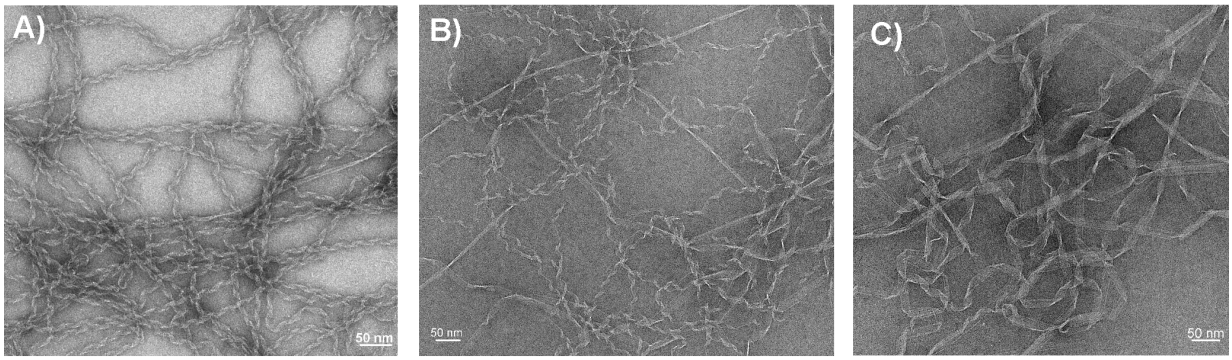

Figure S8. TEM micrographs of PTA stained fibrils of A) **P1**, B) **P1_MfeGly 13** and C) **P1_MfeGly 14**.