Supporting Information for *Chemical Communications*

Enzymatically triggered amyloid formation: an approach for studying peptide aggregation

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I. Experimental Section

Peptide Synthesis

Peptides were synthesized on SyroXP-I peptide synthesizer (Multi-SynTech GmbH) according to standard Fmoc/tBu chemistry using TBTU/HOBt and preloaded Fmoc-Leu-Wang resin (0.64 mmol g^{-1}; Novabiochem). Fmoc-Ser(PO(OBzl)OH)-OH (Bachem) was activated with HATU/DIEA and coupled manually to the resin. DIEA was added in 3-fold excess with respect to the amino acid and HATU. The reaction time was extended to 6 h. Peptides were N-terminally labelled with anthranilic acid (Abz). A mixture of DBU and piperidine (2% each) in DMF was used for Fmoc deprotection. Peptides were cleaved from the resin by treatment with 2 mL TFA/TIS/H_2O (95/2.5/2.5) for 3 h following by precipitation with cool diethyl ether. Purification was carried out by preparative reversed phase HPLC on a Knauer Smartline system (Knauer GmbH) equipped with a Luna^{TM} C8 (10 µm, 250×21.20 mm) column (Phenomenex) running with ACN/0.1% TFA and water/0.1% TFA gradient at 20 mL min^{-1}. Purified peptides were characterized by analytical HPLC (Fig. S1) and HRMS (ESI-TOF): PP m/z: 786.7197 $[M+4H]^{4+}$ (calcd.: m/z: 786.7195); p10 m/z: 806.7088 $[M+4H]^{4+}$ (calcd.: m/z: 806.7110).

Sample Preparation

Peptide concentrations were estimated by UV spectroscopy on a Cary 50 UV/Vis spectrometer (Varian) using the absorption maximum at 320 nm of Abz. A calibration curve was recorded using different concentrations of H_2N-Abz-Gly-OH·HCl (Bachem). Lyophilized peptide was dissolved in hexafluoroisopropanol (HFIP) (~ 2 mg/mL) and sonicated for 15 minutes to dissolve all aggregates. 50 µL of this stock solution was dried under argon flow and 1 mL phosphate buffer (100 mM, pH 7.4) was added. This peptide solution was transferred into a disposable Plastibrand® cuvette (Brand GmbH) with 1 cm path length and a UV spectrum was recorded. After calculation of the peptide concentration the stock solution was aliquoted into glass vials and the HFIP was removed under argon flow. The samples were stored at -20 °C prior to use.

For CD, HPLC and TEM analysis p10 was dissolved in 50 mM Tris/HCl buffer, pH 7.5 containing 20 units of λ-PPase (EC 3.1.3.16, New England Biolabs) and 1 mM $MnCl_2$ immediately before measurements to yield 100 µM concentration. For ThT binding assay samples additionally contained 10 µM HPLC purified ThT (Aldrich). PP samples were prepared analogously, except that they did not contain enzyme and $MnCl_2$.

CD spectroscopy

CD spectra were recorded on a Jasco-715 spectropolarimeter (Jasco GmbH) at 25 °C (Jasco PTC-348W1 peltier thermostat) using 0.1 cm Quartz Suprasil® cuvettes (Hellma). Spectra were averaged over three scans (240-190 nm, 0.5 nm intervals, 1 nm bandwidth, 1 s response time) and background corrected. Elipticity was normalized to concentration (c/mol L^{-1}), number of residues (n = 27, including the N-terminal label Abz) and

path length (l/cm) using equation 1, where θ_{obs} is the measured ellipticity in mdeg and [θ] the mean residue ellipticity in 10^3 deg cm^2 dmol^{-1} residue^{-1}.

$$[\theta] = \theta_{obs} / (10000 \cdot l \cdot c \cdot n) \quad (1)$$

Analytical HPLC analysis

Samples were analyzed on a VWR-Hitachi Elite LaChrome system (VWR International GmbH) equipped with a Capcell Pak C18 (5 µm, 250×4.6 mm) column (Shiseido) running with water/0.1% TFA (solvent A) and ACN/0.1% TFA (solvent B) gradient (20-70% B, 50 min) at 1 mL min^{-1}. Chromatograms were recorded at 220 nm and peaks corresponding to PP and p10 were integrated.

ThT-Fluorescence Spectroscopy

Fluorescence measurements were carried out on a luminescence spectrometer LS 50B (Perkin Elmer) using a 1cm Quartz Suprasil® cuvette (Hellma). Spectra were recorded from 470 nm to 520 nm after excitation at 450 nm (ex. slit 5 nm, em. slit 20 nm) and background corrected. Fluorescence signal at 485 nm was normalized (final fluorescence intensity at the endpoint of the kinetic trace was set to 100 %) and fitted to the Chapman, 4 parameter sigmoidal equation using SigmaPlot 10.0 software. The squared correlation coefficient R^2 was greater than 0.996 (p10) and 0.998 (PP). From the fitted data the midpoints of aggregation (times at which 50 % of the sample was aggregated) and lag times (points of intersection of a straight line running through the point of inflection with the abscissa) were calculated.

TEM

Aliquots of the solutions (6 µL) were placed for 60 s on glow-discharged (60 s plasma treatment at 8 W in a BALTEC MED 020) carbon-coated collodium support films covering 400-mesh copper grids (BAL-TEC, Lichtenstein). After blotting and negative staining with phosphotungstic acid (PTA, 1%), the grids were left to air-dry. TEM images were recorded with a Philips CM12 transmission electron microscope (FEI company, Oregon, USA) at 100 kV accelerating voltage and at primary magnification 58000x on Kodak SO-163 negative film by using a defocus of 0.9 nm. Image J (version 1.38x, Wayne Rasband, USA) was used for the determination of the diameter of peptide fibers.

II. Additional chromatographic and spectroscopic data

Analytical HPLC of pure PP and p10

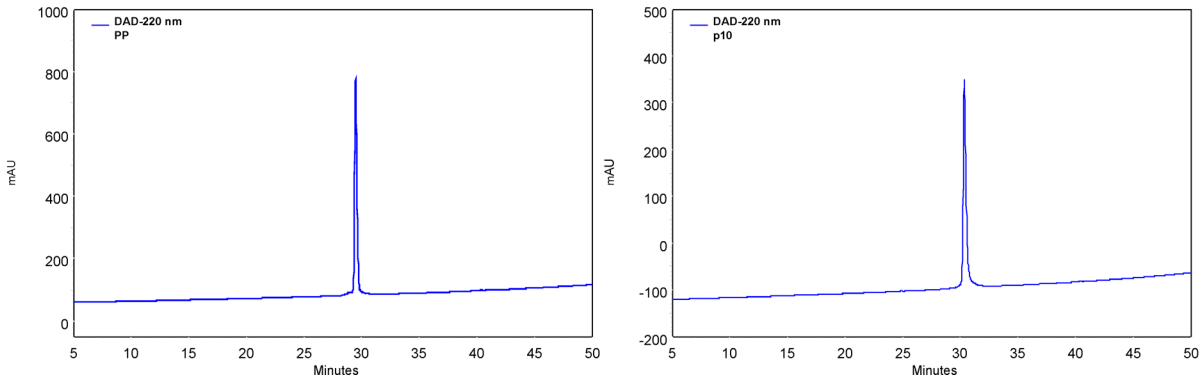

Fig. S1 Analytical HPLC of and PP (left) p10 (right).

Comparison of the folding behavior of PP with and w/o enzyme

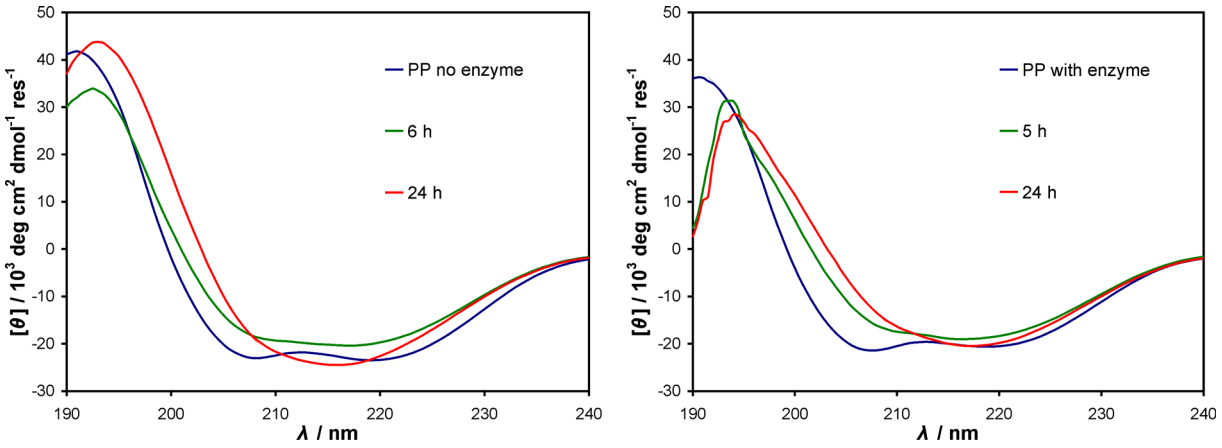

Fig. S2 CD spectra of 100 μM PP in 50 mM Tris/HCl buffer, pH 7.5 without (left) and with enzyme (right). Sample with enzyme contained 1 mM $MnCl_2$.

Comparison of the ThT fluorescence with and w/o enzyme

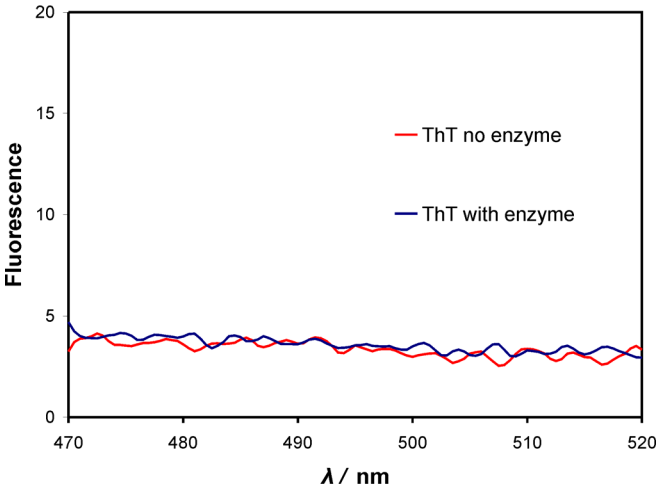

Fig. S3 Fluorecsence of 10 μM ThT in 50 mM Tris/HCl buffer, pH 7.5 with and w/o enzyme. Sample with enzyme contained 1 mM $MnCl_2$.

TEM of PP's fibers under the conditions of enzymatic reaction

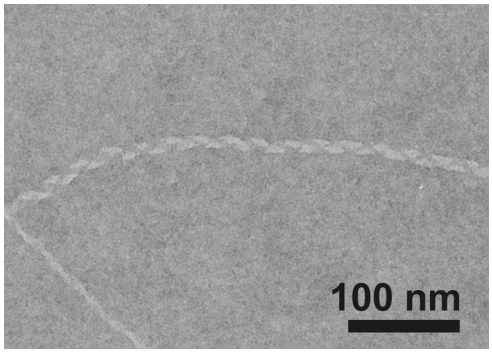

Fig. S4 TEM micrographs showing fibers of 100 μM PP in 50 mM Tris/HCl buffer, pH 7.5 containing enzyme and 1 mM $MnCl_2$. PP was incubated overnight at 25 ºC.

Enzymatic reaction medium has no influence on the folding of p10

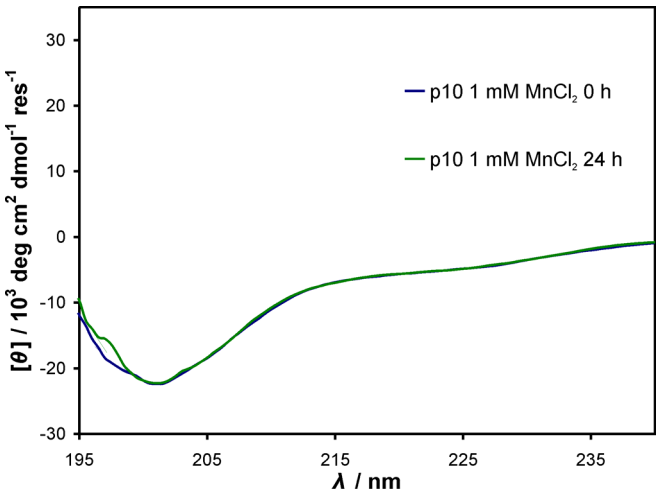

Fig. S5 CD spectra of 100 μM p10 in 50 mM Tris/HCl buffer, pH 7.5 containing 1 mM $MnCl_2$.

Elevated concentration

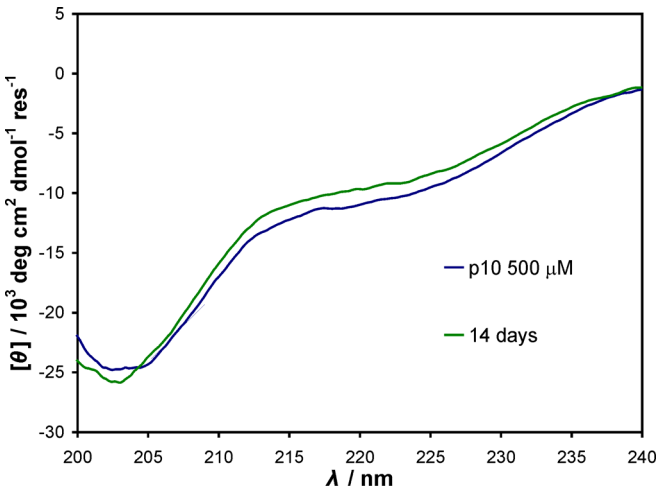

Fig. S6 CD spectra of 500 μM p10 in 10 mM Tris/HCl buffer, pH 7.4. Samples contained 0.025 % NaN_3.

Salt screening

We have previously shown that the unfolding of PP is due to electrostatic repulsions induced by the phosphate moiety.[1] These interactions can be diminished by the presence of charged co-solutes.[2]

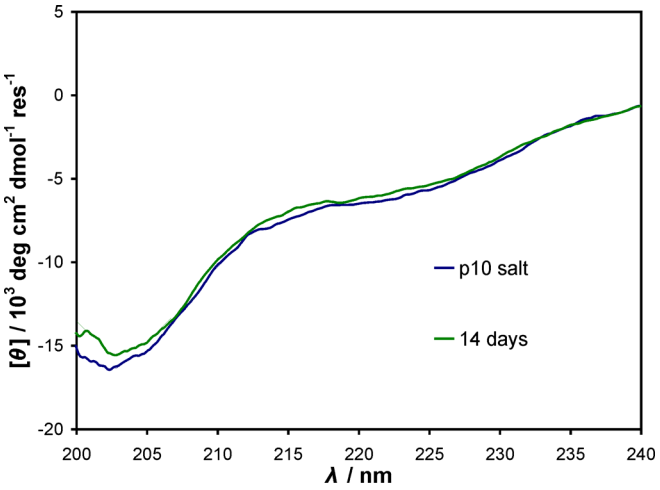

Fig. S7 CD spectra of 100 μM p10 in 10 mM Tris/HCl buffer, pH 7.4 containing 0.2 M NaCl. Samples contained 0.025 % NaN_3.

References

1 M. Broncel, J. A. Falenski, S. C. Wagner, C. P. R. Hackenberger and B. Koksch, *Chem. Eur. J.*, submitted.
2 K. Pagel, S. C. Wagner, R. Rezaei Araghi, H. von Berlepsch, C. Bottcher and B. Koksch, *Chem. Eur. J.*, 2008, **14**, 11442-11451.