Synergistic Interactions Are Prevalent in Catalytic Amyloids

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Experimental procedures

Peptide Synthesis

The peptides were synthesized by manual Fmoc solid-phase synthesis at elevated temperature using Rink amide resin and previously reported protocols.[1] Cleavage was achieved by treatment with a mixture of trifluoroacetic acid (TFA)/H_2O/triisopropylsilane (TIPS) (95:2.5:2.5, v/v) for 2 hours at room temperature. The crude peptides were precipitated and washed thrice with cold *tert*-Butyl methyl ether, then purified on a preparative reverse phase HPLC system (Varian ProStar 210) with C4 preparative column (Phenomenex), using a linear gradient of solvent A (0.1% TFA in water) and solvent B (90% CH_3CN, 10% H_2O, 0.1% TFA). The peptides were lyophilized, and their identities were confirmed by MALDI-TOF mass spectrometry (Bruker Autoflex III Smartbeam MALDI-TOF mass spectrometer). The purity of the peptides was evaluated on a Shimadzu Prominence UFLC or Agilent 1260 Infinity II HPLC instrument with an analytical Zorbax Eclipse XDB-C18 column (4.6 mm x 150 mm).

Preparation of the peptide stocks and solutions.

The purified and lyophilized peptides were dissolved in 10 mM hydrochloric acid to make a 1.0 mM, pH 2 stock solution. The concentration of the stock solutions was determined on an Agilent 8453 UV-Vis spectrophotometer using absorbance at 214 nm. Extinction coefficients of the peptides were calculated using literature values.[2] The pH 8 stock solution of the peptides was prepared by mixing pH 2 peptide stock with buffer containing 25 mM Tris (pH 8) and 0.1 mM or 1 mM ZnCl2. Zn(II) solution was prepared by dissolving zinc chloride in water to a concentration of 50 mM and this solution was further diluted to 0.1 mM or 1 mM. The pH 2 stock solutions were stable for at least a week and the pH 8 solutions were prepared immediately before experiments.

Kinetic assay of pNPA hydrolysis.

Kinetic measurements were carried out at 25 °C on a BioTek Eon Microplate Reader using 96-well plates. p-Nitrophenol acetate solutions in buffer were freshly prepared by dilution of a 0.1 M acetonitrile stock to a final concentration of 195 µM, 375 µM, 570 µM and 750 µM in 25 mM Tris, 1 mM Zn, pH 8.0. Substrate solutions of variable concentrations were added to Zn(II)-peptide pH 8 solution (100 µM for isolated peptides, 50 µL) or Zn(II) in buffer. The final acetonitrile content was 2% in all reaction mixtures. Once these components were mixed, absorbance of the product (p-nitrophenol) at 405 nm was monitored. Kinetic parameters were obtained by fitting the data to the Michaelis–Menten equation: $v_0 = kcat[E]_0[S]_0/(K_M+[S]_0)$, where v_0 was derived from the initial linear portion of the reading and the extinction coefficient of the product (16,600 M-1 cm-1).[3]

Peptide mixing at pH 2.

Stock solutions of peptides were prepared by dissolving lyophilized peptides in 10 mM HCl to the final concentration of 4 mM. Two stocks were mixed to produce 50 µL samples of peptides at various ratios (0, 8, 16, 24, 32, 40, 48, 55, 70, 85 and 100%). To each of these samples, 1.8 mL of 25 mM Tris (pH 8) containing 0.1 mM $ZnCl_2$ was added. 150 µL of the *p*NPA substrate solution was added to 50 µL of the final peptide mixture and hydrolytic activity was measure immediately, after 24 and 48 hours, as described above. Final concentrations in the reaction mixture were: 25 µM peptide, 195 µM substrate, 0.1 mM $ZnCl_2$ and 25 mM Tris (pH 8). The data were fit to a probabilistic binomial binary model that assumes random mixing of two species and an active species that requires two strands:

$$(v_0)_{obs} = (v_0)_{AA}(X_A)^2 + (v_0)_{BB}(1 - X_A)^2 + (v_0)_{AB}2(1 - X_A)X_A \quad \text{(S1)}$$

where $(v_0)_{obs}$ is the observed rate of the reaction ; X_A is the molar fraction of peptide A; $(v_0)_A$, $(v_0)_B$ and $(v_0)_{AB}$ are the observed rates for the reaction catalyzed by the homomer of A, the homomer of B and the heteromers of A and B, respectively.

CD spectroscopy.

The CD spectra were collected on the Jasco J-715 CD spectrometer, using a step scan mode (4 sec averaging time) averaging three runs, using a quartz cuvette with a 0.1 cm path length. Samples were prepared as follows: 25 µL peptide stock (to the final concentration of 25 µM) and 275 µL buffer (25 mM TRIS pH 8 with or without 0.5 mM $ZnCl_2$) were mixed together and spectra were taken immediately, after 24 and 48 hours. Care was taken so the sample absorbance never exceeded 1.5 at all wavelengths to produce reliable ellipticity values.

Thioflavin T (ThT) assay.

Fluorescence spectra were obtained on a Cary Eclipse (Agilent) fluorescence spectrophotometer operating in the steady-state mode at 25°C with emission and excitation band width set to 5 nm. The measurements were taken using a quartz cuvette with 5 mm excitation and 5 mm emission path lengths. Samples were excited at 440 nm and emission was monitored between 460 and 600 nm. Samples were prepared by mixing peptide (1 mM stock at pH 2, 40 µL) with buffer (25 mM Tris, pH 8 and 1 mM ZnCl2) and then ThT (0.5 mM, 10 µL) was added to a final volume of 200 µL and the samples were vortexed. Final concentration of peptide and ThT were at 200 µM and 25 µM, respectively.

Transmission electron microscopy.

Peptide stocks were diluted 10-fold to an approximate concentration of 100 µM 25 mM TRIS (pH 8) and 0.1 mM $ZnCl_2$. Sample aliquots of 10 µl were adsorbed for 2-5 minutes onto formvar/carbon-coated, 200-mesh copper grids (Ted Pella). Grids were then washed briefly with water and stained with one 10 µl drop of 2% (w/v) uranyl acetate. After drying, samples were viewed with a JEOL-JEM 2100F Field Emission electron microscope at an acceleration voltage of 200 kV. Electron micrographs were recorded on a Gatan OneView 4K CCD camera.

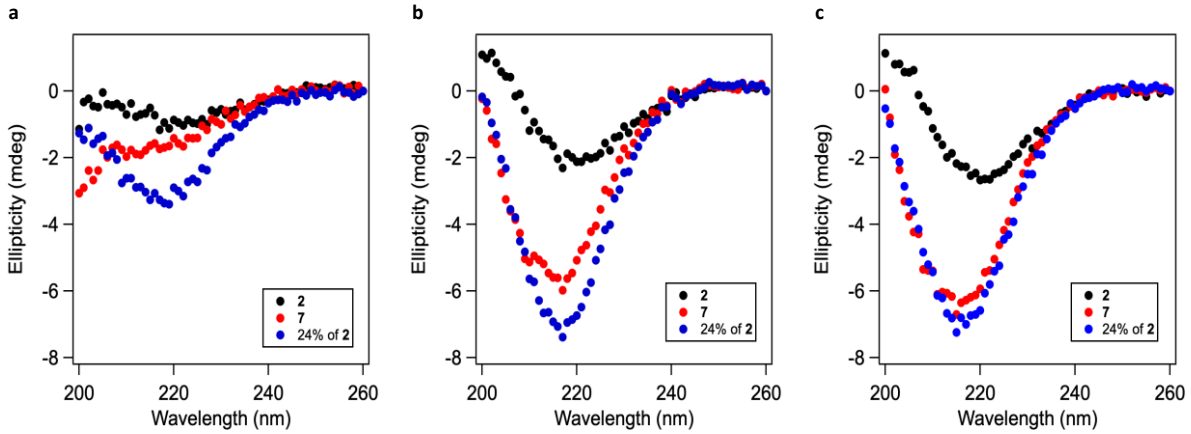

Figure S1. Circular dichroism spectra of **2**, **7** and the mixture of the two peptides after incubating the fibrils for 0 (a), 24 (b) and 48 (c) hours at room temperature. Conditions: 25 µM peptide, 0.1 mM $ZnCl_2$ and 25 mM Tris (pH 8).

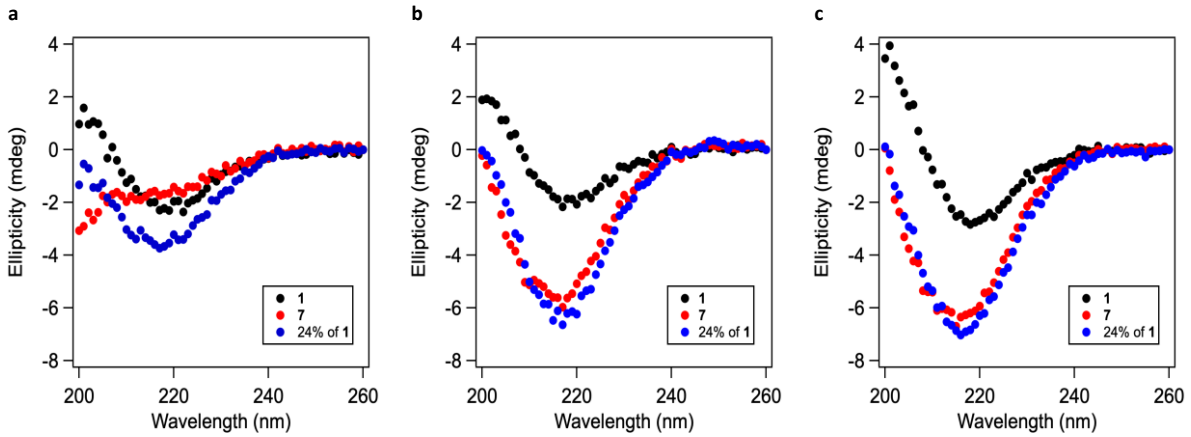

Figure S2. Circular dichroism spectra of **1**, **7** and the mixture of the two peptides after incubating the fibrils for 0 (a), 24 (b) and 48 (c) hours at room temperature. Conditions: 25 µM peptide, 0.1 mM $ZnCl_2$ and 25 mM Tris (pH 8).

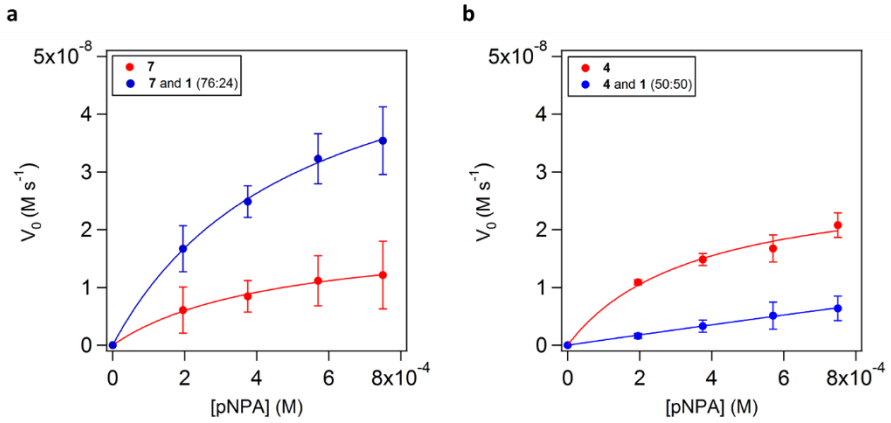

Figure S3. Kinetic profiles for a) a 76:24 mixture of **7** and **1** compared to **7** and b) a 50:50 mixture of **4** and **1** compared to **4**, both at 0 h Experimental conditions: 25 mM Tris, pH 8.0, 25 µM peptide, 0.1 mM Zn^{2+}.

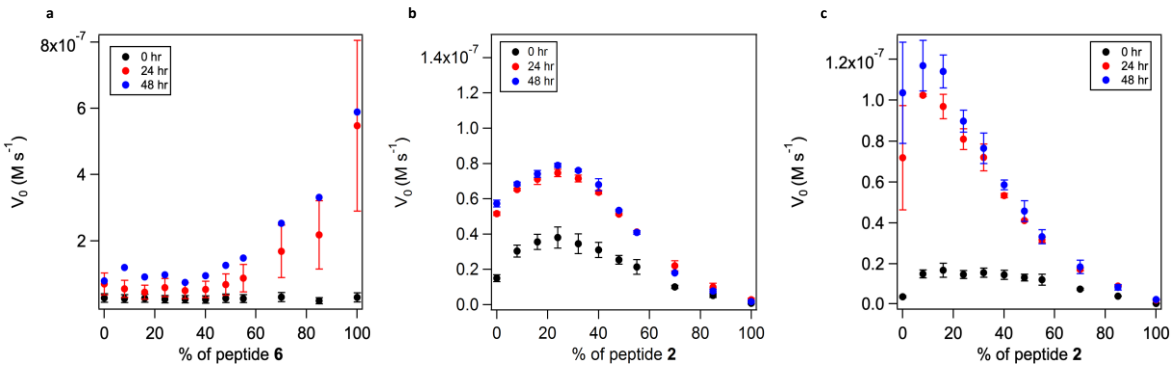

Figure S4. Mixing plots for a) **6** and **5**, b) **2** and **7** and c) **6** and **2**. Conditions: 25 μM peptide, 0.1 mM $ZnCl_2$ and 25 mM TRIS (pH 8).

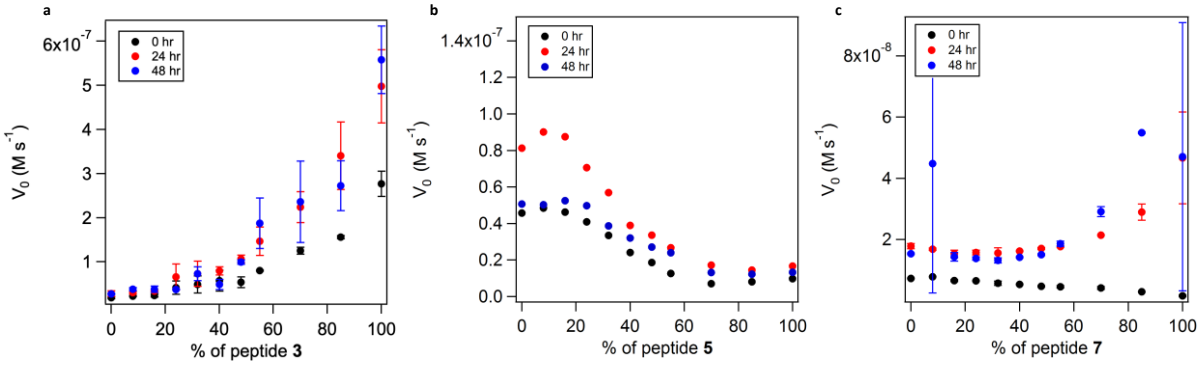

Figure S5. Mixing plots for a) **3** and **2**, b) **5** and **3**, c) and **7** and **5**. Experimental conditions: 25 mM Tris, pH 8.0, 25 μM total peptide, 0.1 mM Zn^{2+}, 195 μM pNPA.

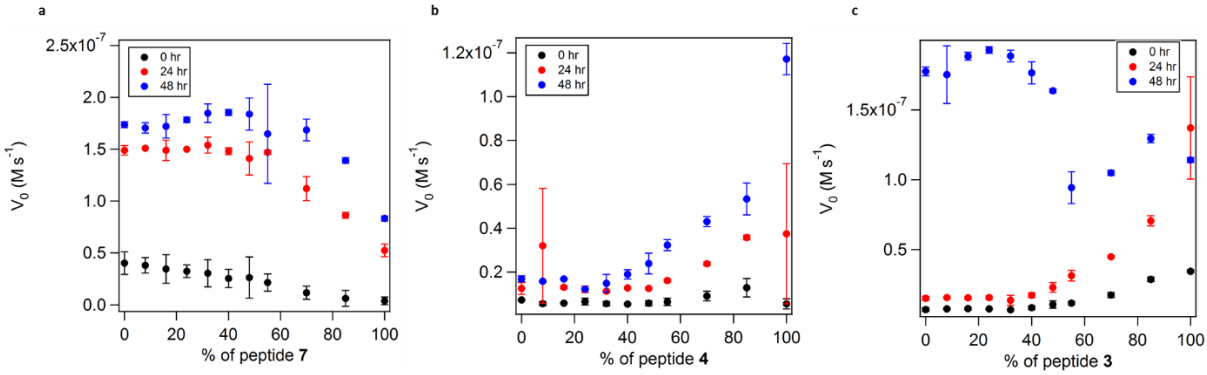

Figure S6. Mixing plots for a) **6** and **7**, b) **4** and **5**, and c) **3** and **6**. Experimental conditions: 25 mM Tris, pH 8.0, 25 μM total peptide, 0.1 mM Zn^{2+}, 195 μM pNPA.

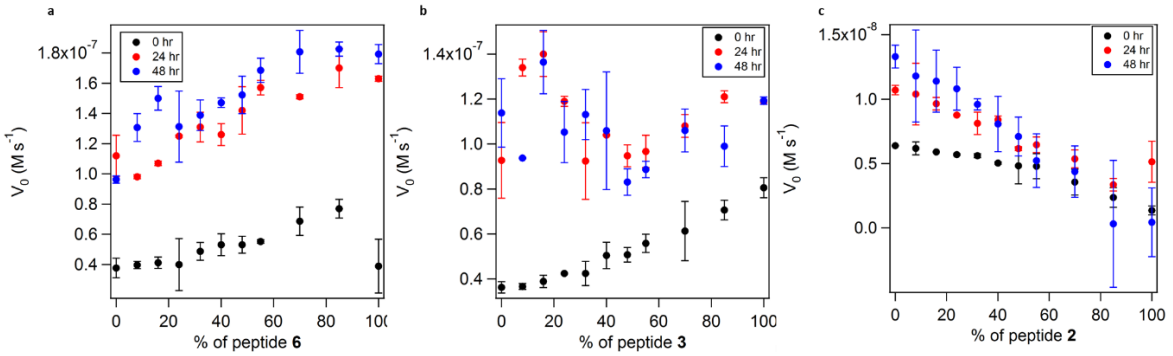

Figure S7. Mixing plots for a) **6** and **4**, b) **3** and **4** and c) **2** and **5**. Experimental conditions: 25 mM Tris, pH 8.0, 25 μM total peptide, 0.1 mM Zn^{2+}, 195 μM pNPA.

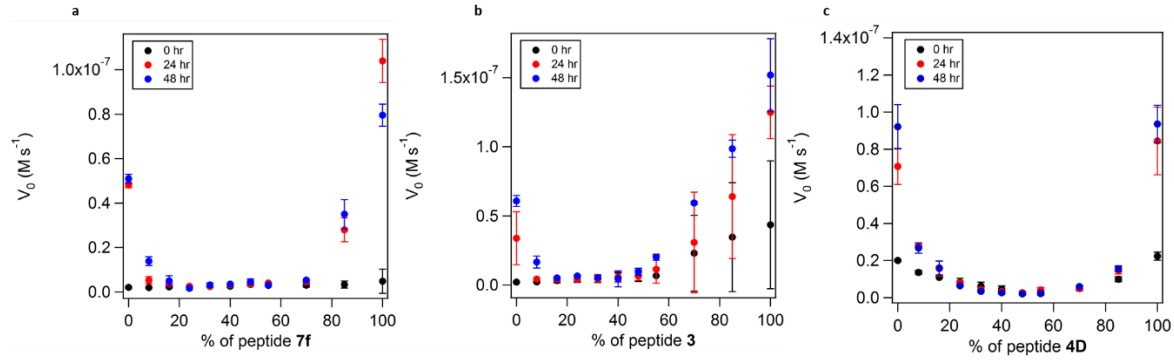

Figure S8. Mixing plots for a) **7D** and **7F,** b) **7D** and **3**, c) **4** and **4D.** Experimental conditions: 25 mM Tris, pH 8.0, 25 μM total peptide, 0.1 mM Zn^{2+}, 195 μM pNPA.

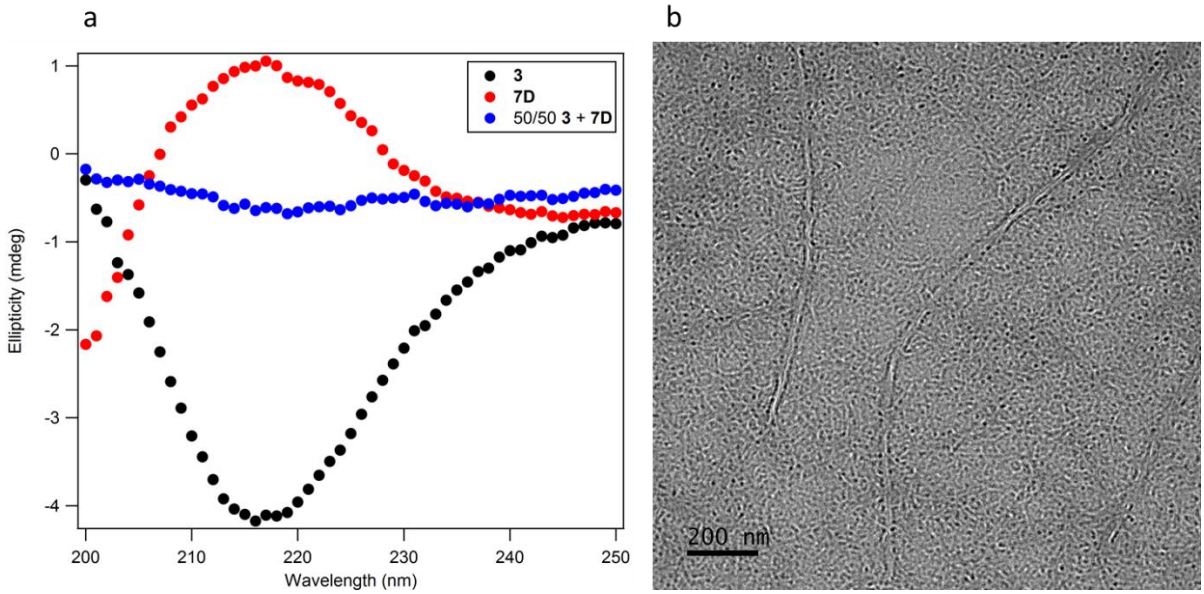

Figure S9. a) Circular dichroism showing the effect of mixing **3** and **7D** in an equimolar ratio. b) TEM micrograph of fibrils formed by 50:50 mixture of **3** and **7D**. Experimental conditions: 25 mM Tris, pH 8.0, 25 μM peptide, 0.1 mM Zn^{2+}, 195 μM pNPA.

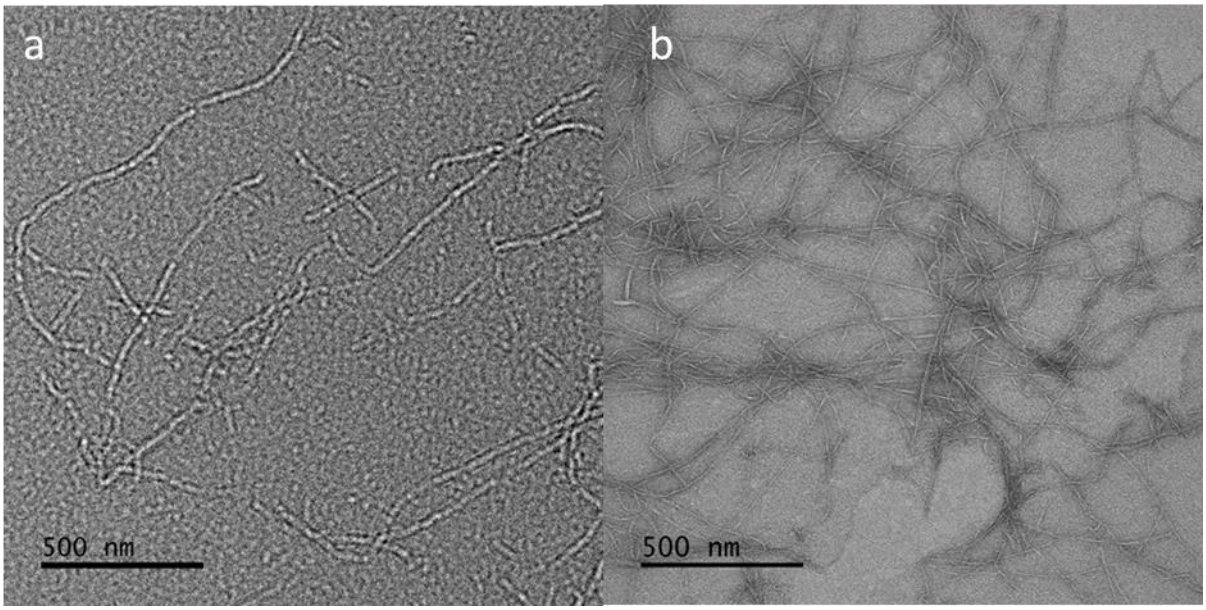

Figure S10. TEM micrographs of a) fibrils formed by 50:50 mixture of **4** and **2** b) fibrils formed by 76:24 mixture of **7** and **1.** Experimental conditions: 25 mM Tris, pH 8.0, 25 μM peptide, 0.1 mM Zn^{2+}.

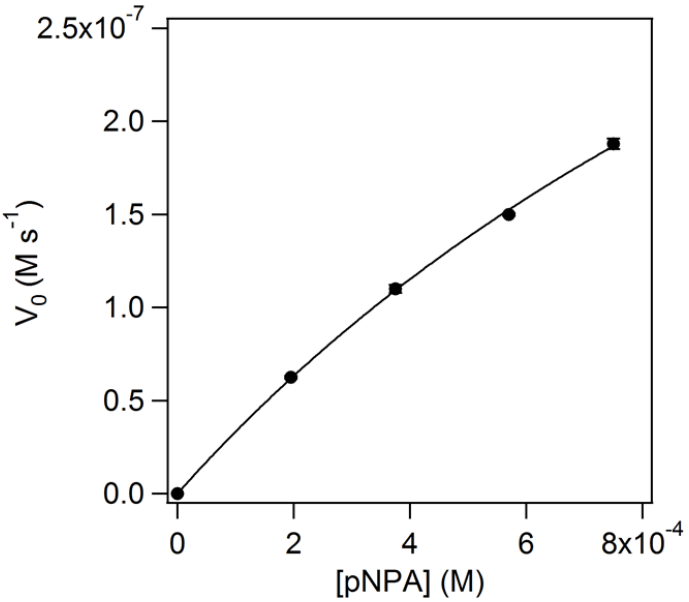

Figure S11. Kinetic profile for fibrils formed **7F** matured for 24 h. Experimental conditions: 25 mM Tris, pH 8.0, 25 μM peptide, 0.1 mM Zn^{2+}.

Table S1. Kinetic parameters for peptides. Values at 24 hours highlighted in bold.

Peptide	k_{cat} ($x10^{-2}$ s^{-1})		K_M (mM)		k_{cat}/K_M (M^{-1} s^{-1})	
7	0.15 ± 0.02	**2.5 ± 0.36**	0.45 ± 0.07	**1.5 ± 0.3**	3.4 ± 0.6	**16.6 ± 4.2**
7+ 1	0.96 ± 0.05	**4.9 ± 0.9**	0.5 ± 0.05	**1.0 ± 0.28**	18.7 ± 2.0	**47.0 ± 15.4**
4	0.2 ± 0.03	**0.66 ± 0.06**	0.35 ± 0.11	**0.5 ± 0.1**	6.6 ± 2.1	**12.1 ± 2.6**
4 + 1	n/a	**0.12 ± 0.03**	n/a	**1.0 ± 0.5**	n/a	**1.2 ± 0.5**
7F	**5.2 ± 0.5**		**1.84 ± 0.2**		**28 ± 4.1**	

Table S2. v_0/[E][S] values for the heterodimeric species obtained by fitting the experimental v_0 values to equation (S1).

Species	**Weighted average of activities, assuming no synergy, M^{-1} s^{-1}**	**v_0/[E][S], $M^{-1}s^{-1}$**
7 - 4	15.5 ± 6	35 ± 1.13
7 - 3	24 ± 5	45 ± 0.87

[1] I. V. Korendovych, A. Senes, Y. H. Kim, J. D. Lear, H. C. Fry, M. J. Therien, J. K. Blasie, F. A. Walker, W. F. DeGrado, *J. Am. Chem. Soc.* **2010**, *132*, 15516-15518.

[2] B. J. H. Kuipers, H. Gruppen, *J. Agric. Food Chem.* **2007**, *55*, 5445-5451.

[3] C. M. Rufo, Y. S. Moroz, O. V. Moroz, J. Stöhr, T. A. Smith, X. Hu, W. F. DeGrado, I. V. Korendovych, *Nat. Chem.* **2014**, *6*, 303-309.