

Supporting information for

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Salvador Ventura<sup>1\*</sup>

## **Minimalist Prion-Inspired Polar Self-Assembling Peptides**

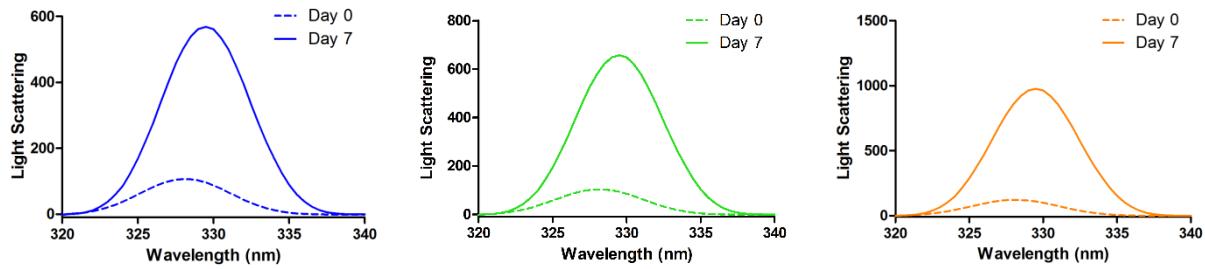
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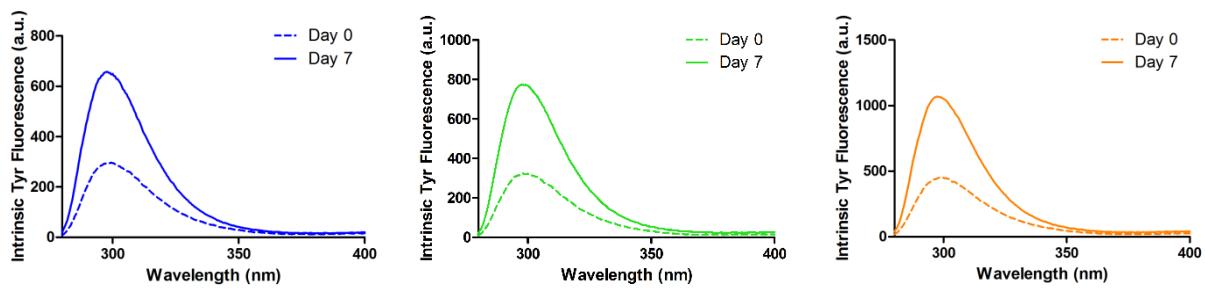
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**This PDF file includes Supporting Figures S1 to S16 and Tables S1 and S3.**

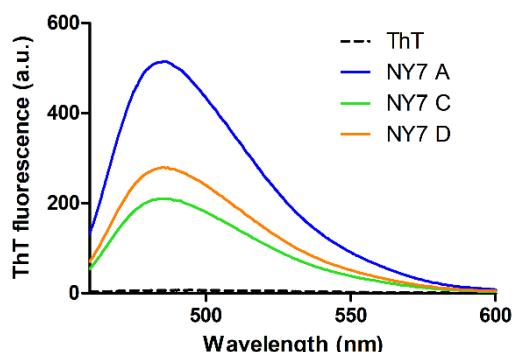
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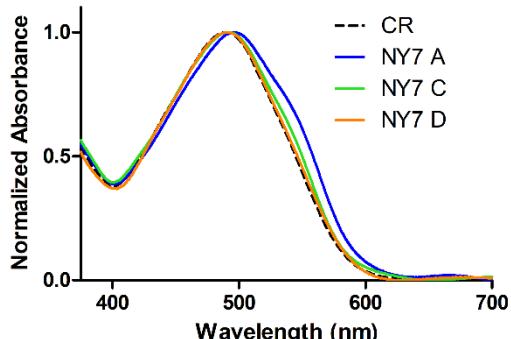
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C

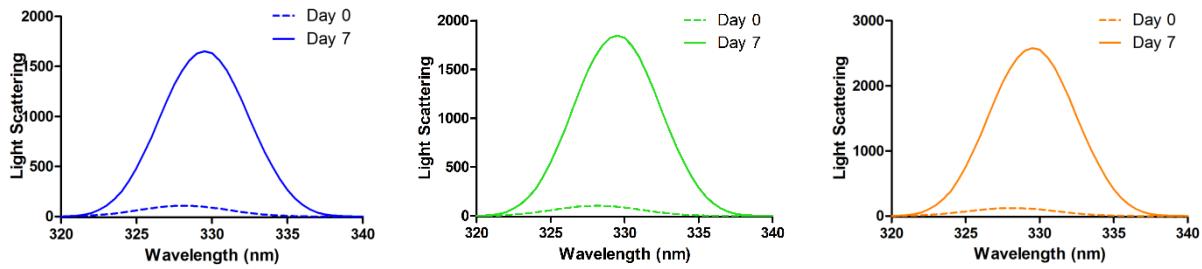


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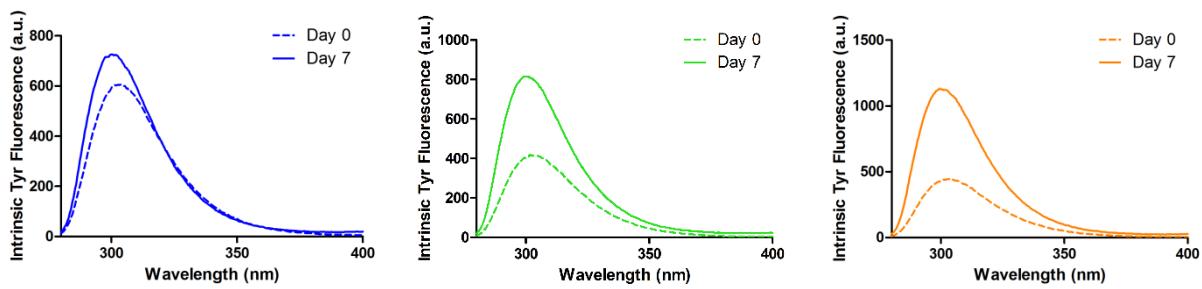


**Figure S1. NY7 peptide biophysical characterization in different buffers.** NY7 peptide was prepared in the following buffers: buffer A: 100 mM potassium phosphate, pH 6.0 (blue); buffer C: 100 mM potassium phosphate, pH 6.0, 150 mM NaCl (green); buffer D: 100 mM potassium phosphate, pH 7.0, 150 mM NaCl (orange) at a final concentration of 250  $\mu$ M. Peptide aggregation was analysed at 0 (dashed line) and 7 days (solid line) of incubation: (A) NY7 peptide synchronous light scattering. (B) Intrinsic tyrosine fluorescence. Tinctorial properties of incubated peptide: (C) Th-T and (D) CR binding assays in the absence (dashed line) and in the presence (solid lines) of NY7.

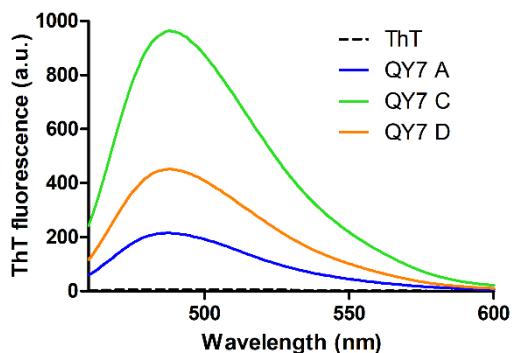
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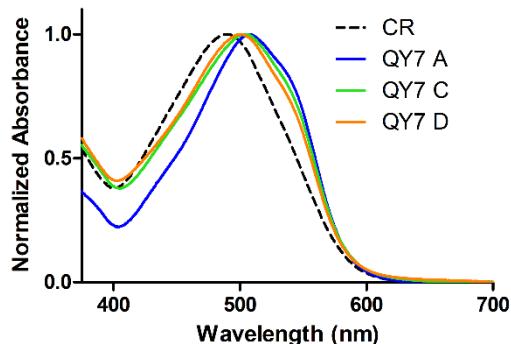
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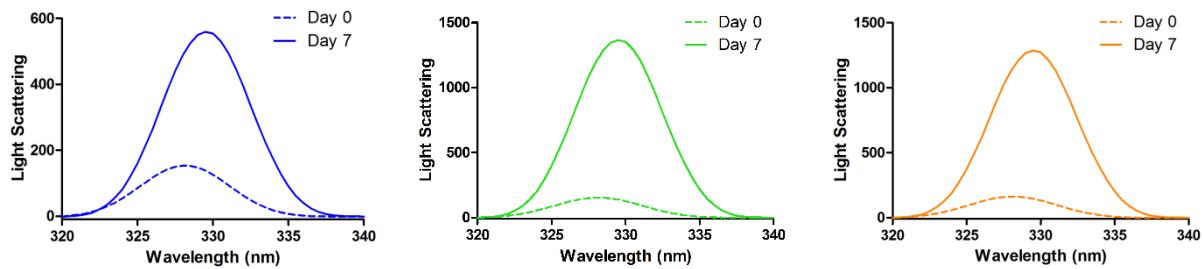
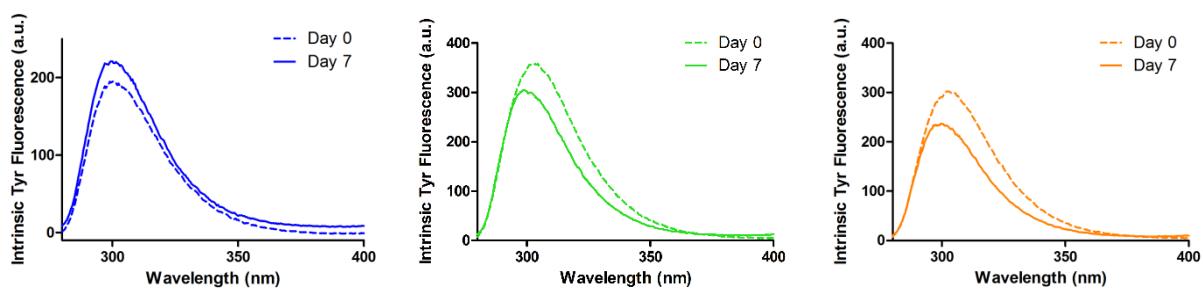
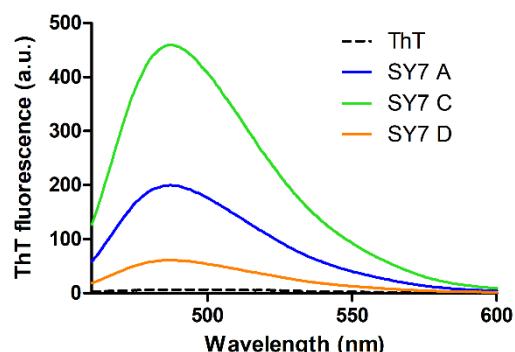
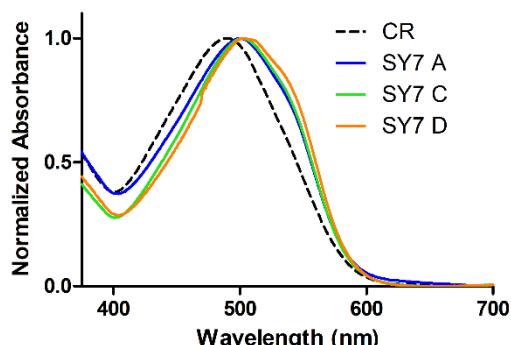
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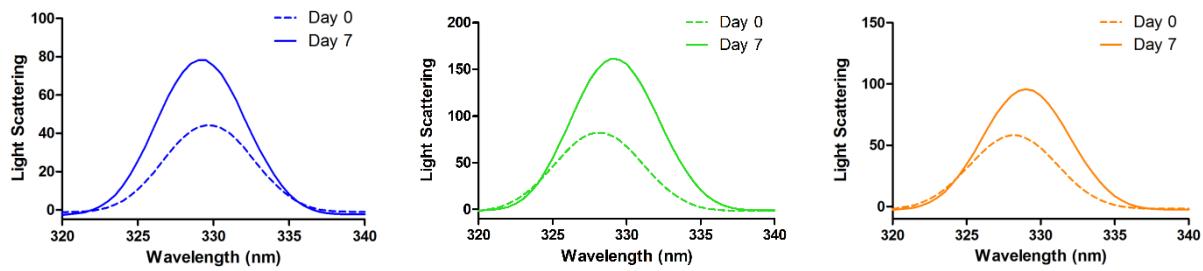
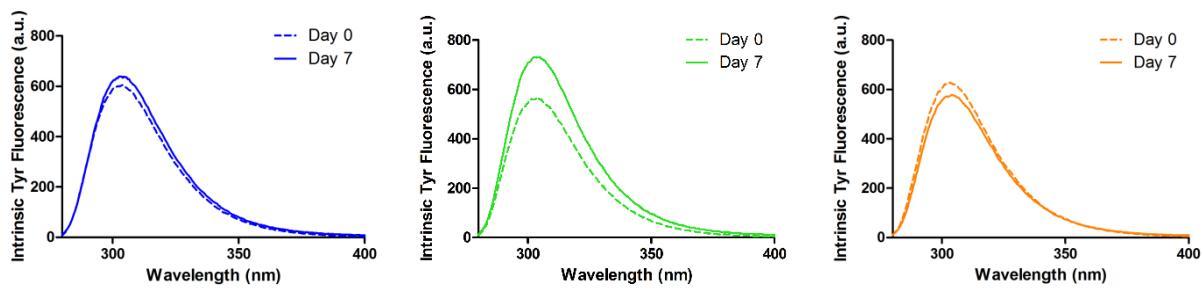
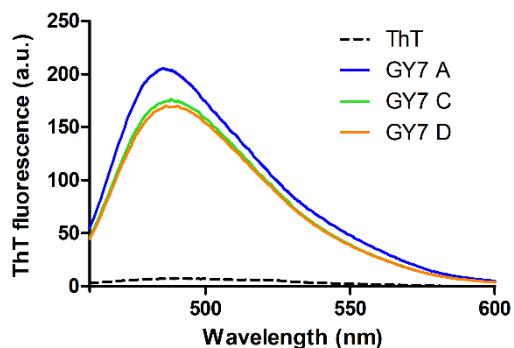
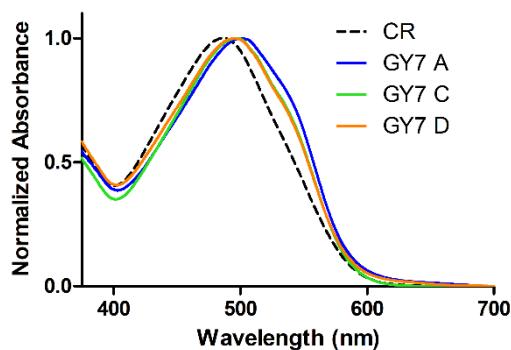
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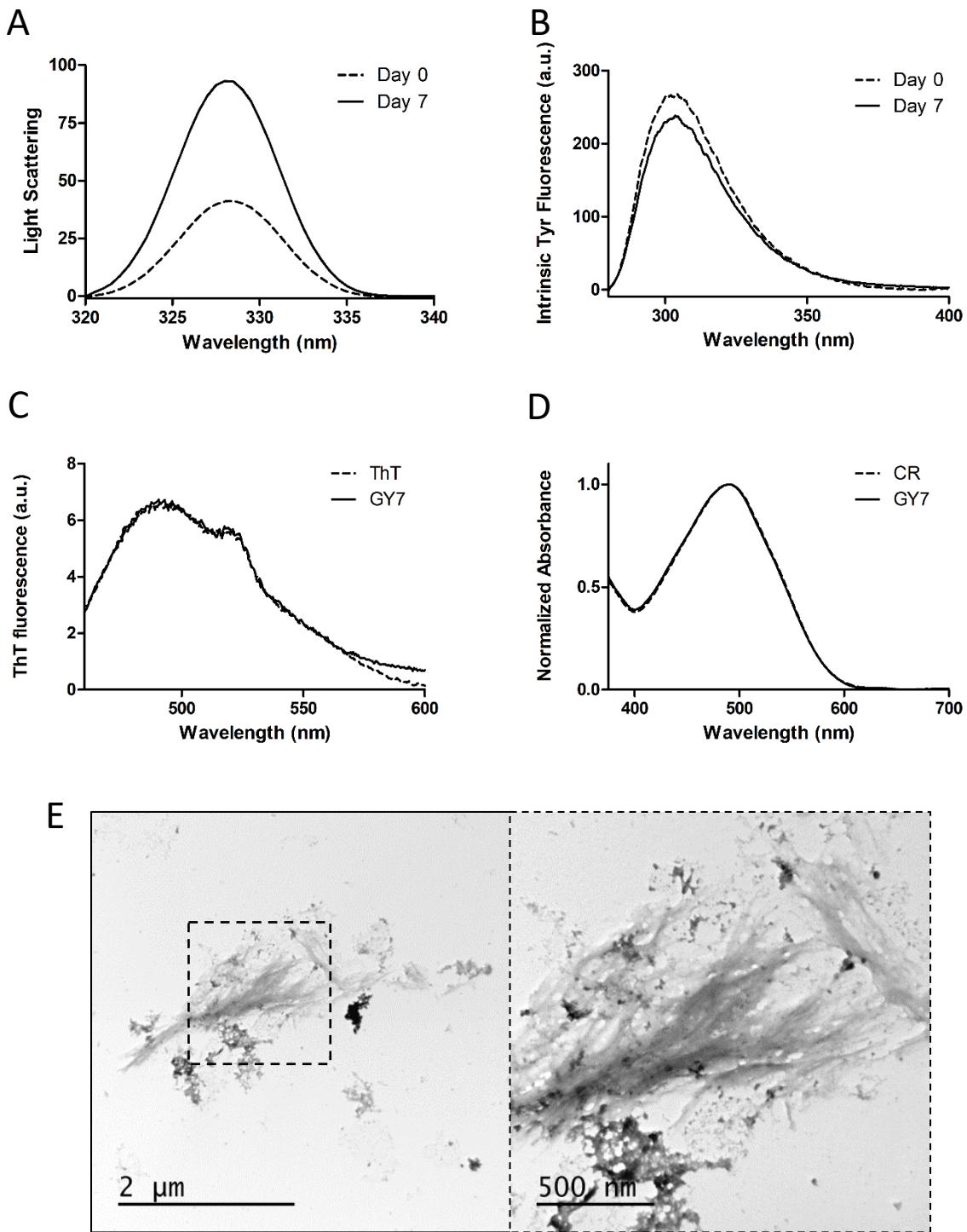
**Figure S2. QY7 peptide biophysical characterization in different buffers.** QY7 peptide was prepared in the following buffers: buffer A: 100 mM potassium phosphate, pH 6.0 (blue); buffer C: 100 mM potassium phosphate, pH 6.0, 150 mM NaCl (green); buffer D: 100 mM potassium phosphate, pH 7.0, 150 mM NaCl (orange) at a final concentration of 250  $\mu$ M. Peptide aggregation was analysed at 0 (dashed line) and 7 days (solid line) of incubation: (A) QY7 peptide synchronous light scattering. (B) Intrinsic tyrosine fluorescence. Tinterior properties of incubated peptide: (C) Th-T and (D) CR binding assays in the absence (dashed line) and in the presence (solid lines) of QY7.

**A****B****C****D**

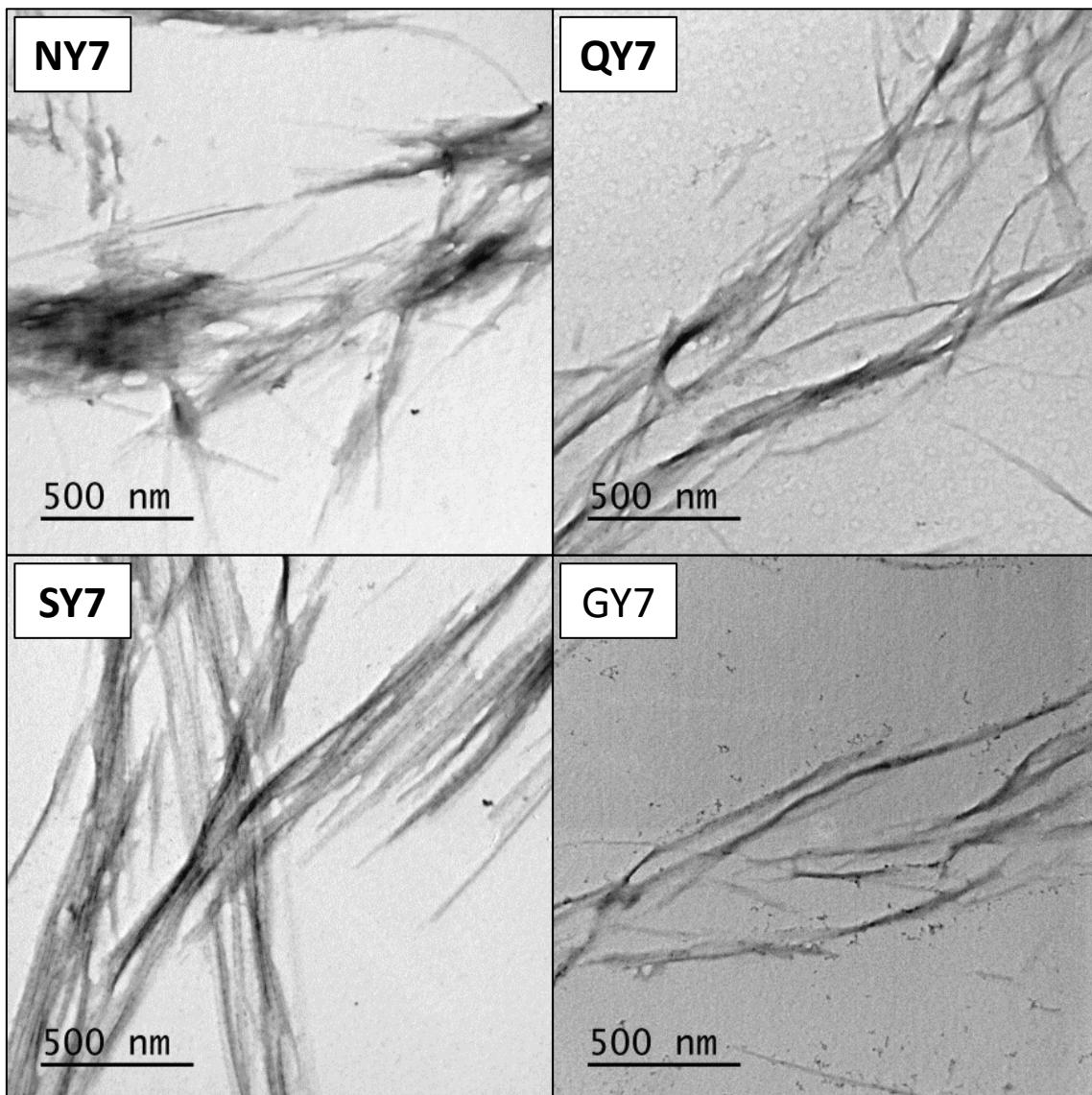
**Figure S3. SY7 peptide biophysical characterization in different buffers.** The peptide was prepared in the following buffers: buffer A: 100 mM potassium phosphate, pH 6.0 (blue); buffer C: 100 mM potassium phosphate, pH 6.0, 150 mM NaCl (green); buffer D: 100 mM potassium phosphate, pH 7.0, 150 mM NaCl (orange) at a final concentration of 250  $\mu$ M. Peptide aggregation was analysed at 0 (dashed line) and 7 days (solid line) of incubation: (A) SY7 peptide synchronous light scattering. (B) Intrinsic tyrosine fluorescence. Tinctorial properties of incubated peptide: (C) Th-T and (D) CR binding assays in the absence (dashed line) and in the presence (solid lines) of SY7.

**A****B****C****D**

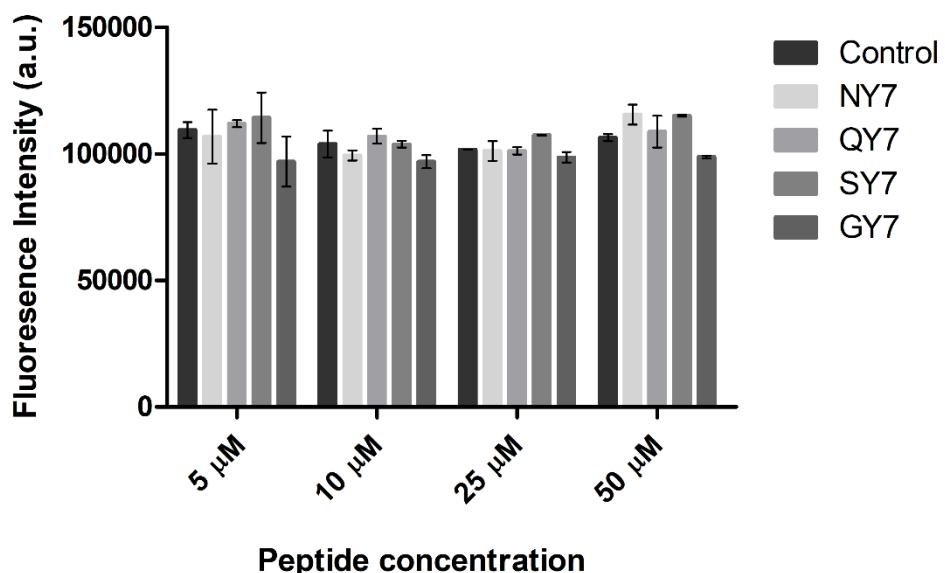
**Figure S4. GY7 peptide biophysical characterization in different buffers.** GY7 peptide was prepared in the following buffers: buffer A: 100 mM potassium phosphate, pH 6.0 (blue); buffer C: 100 mM potassium phosphate, pH 6.0, 150 mM NaCl (green); buffer D: 100 mM potassium phosphate, pH 7.0, 150 mM NaCl (orange) at a final concentration of 500  $\mu$ M. Peptide aggregation was analysed at 0 (dashed line) and 7 days (solid line) of incubation: (A) GY7 peptide synchronous light scattering. (B) Intrinsic tyrosine fluorescence. Tinterior properties of incubated peptide: (C) Th-T and (D) CR binding assays in the absence (dashed line) and in the presence (solid lines) of GY7.



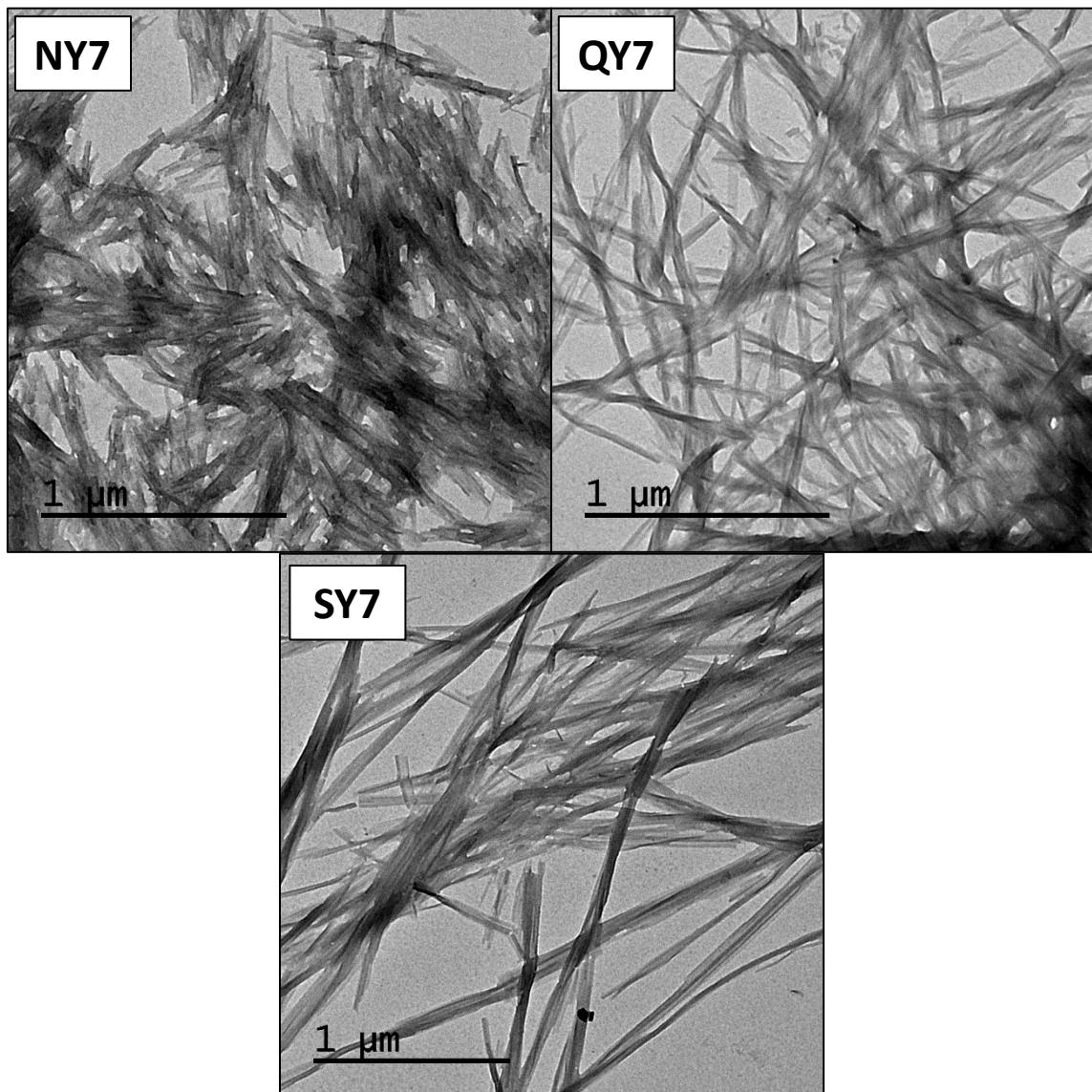
**Figure S5. GY7 peptide biophysical characterization.** GY7 peptide was prepared at 250  $\mu$ M in buffer 100 mM phosphate pH 7, and aggregation was analysed at 0 (dashed line) and 7 days (solid line) of incubation: (A) Synchronous light scattering; (B) Intrinsic tyrosine fluorescence. Tinterior properties of incubated peptide was assessed: (C) Th-T and (D) CR binding assays in the absence (dashed line) and in the presence (solid lines) of GY7. Fibril morphology of incubated peptide was assessed: (E) Representative TEM micrographs.



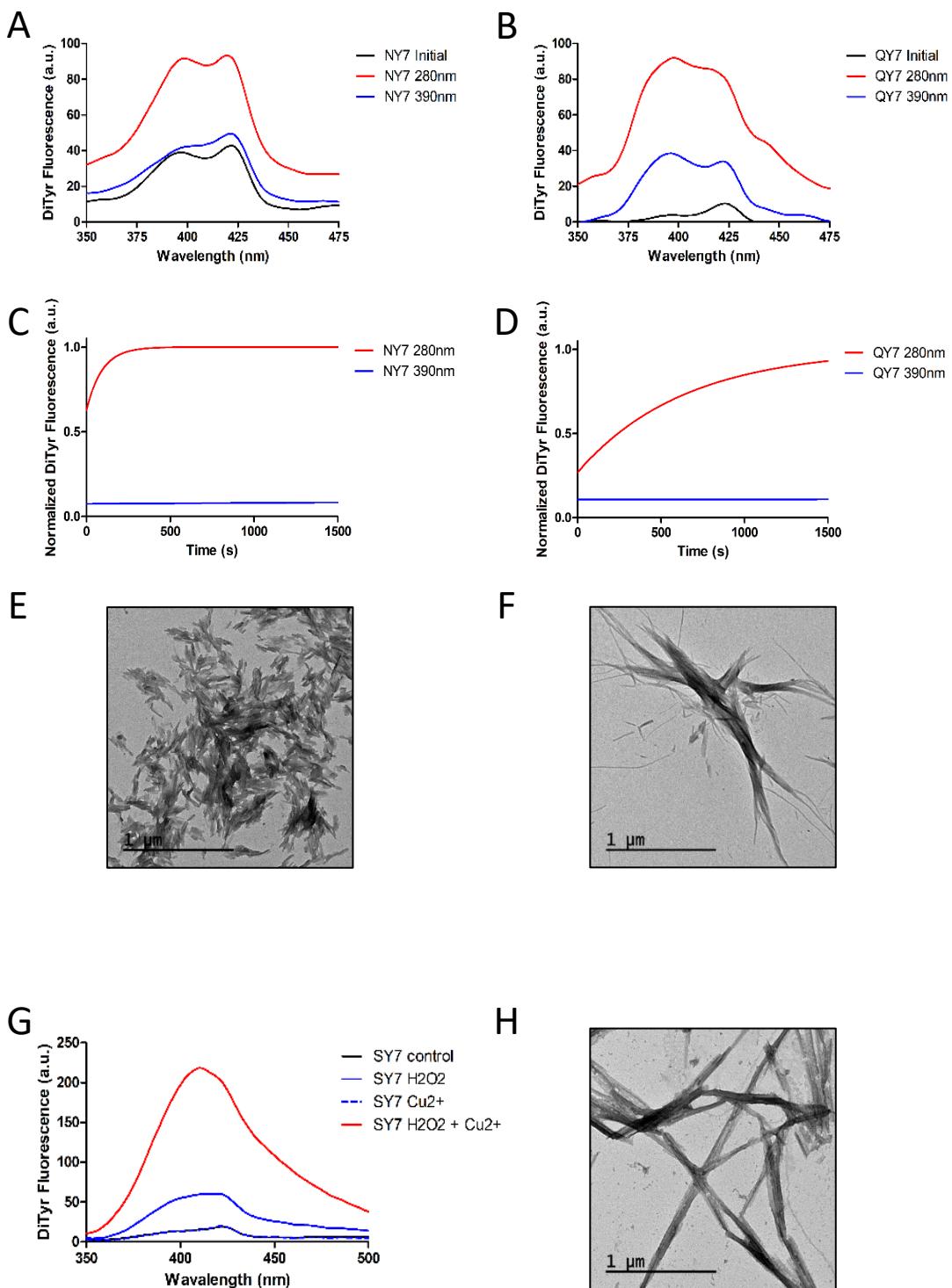
**Figure S6. TEM micrographs of the aggregated NY7, QY7, SY7 and GY7 peptides at high magnification.**



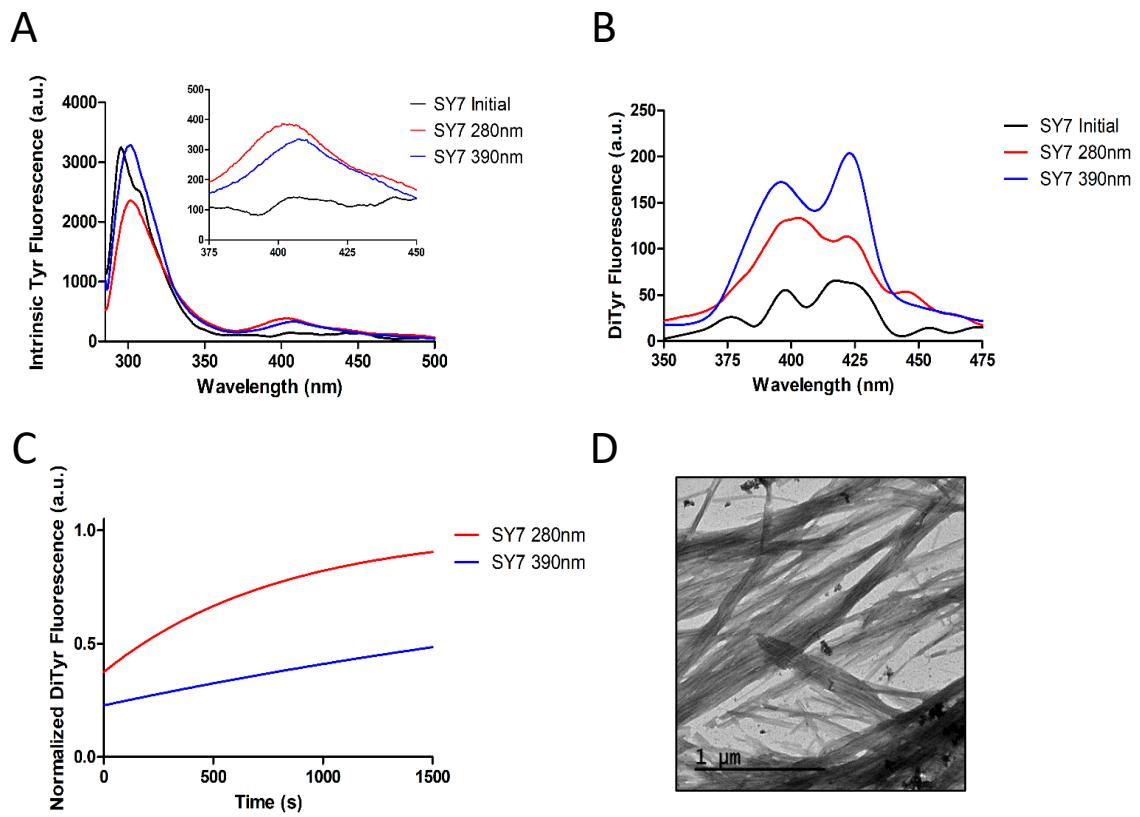
**Figure S7. Cytotoxicity of the aggregated peptides.** Cell viability was assessed adding incubated peptides at 5, 10, 25 and 50  $\mu\text{M}$  final concentration to cultured SH-SY5Y cells and cytotoxicity was compared relative to cells treated with buffer 100 mM phosphate pH 7.0. Data correspond to the average value from two independent experiments and bars correspond to standard error of the mean. For all peptides, Two-way ANOVA statistical test was performed, with a P-value  $>0.05$ .



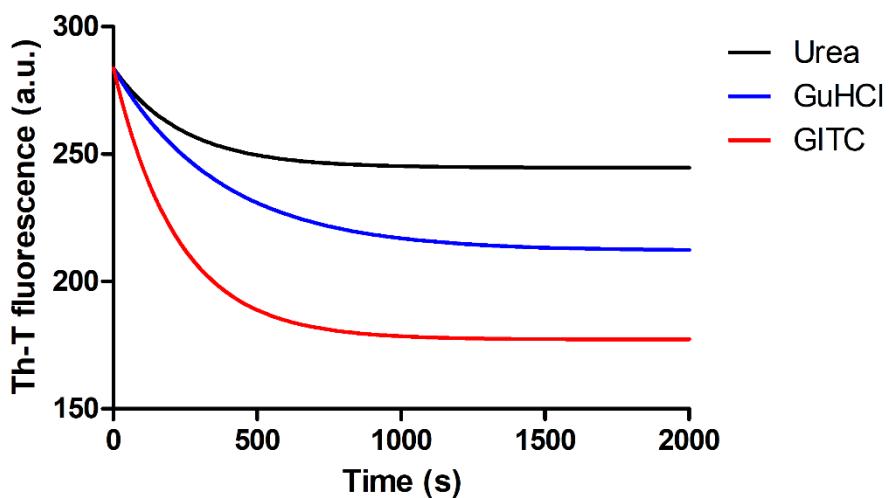
**Figure S8. TEM micrographs of NY7, QY7 and SY7 fibril meshes.**



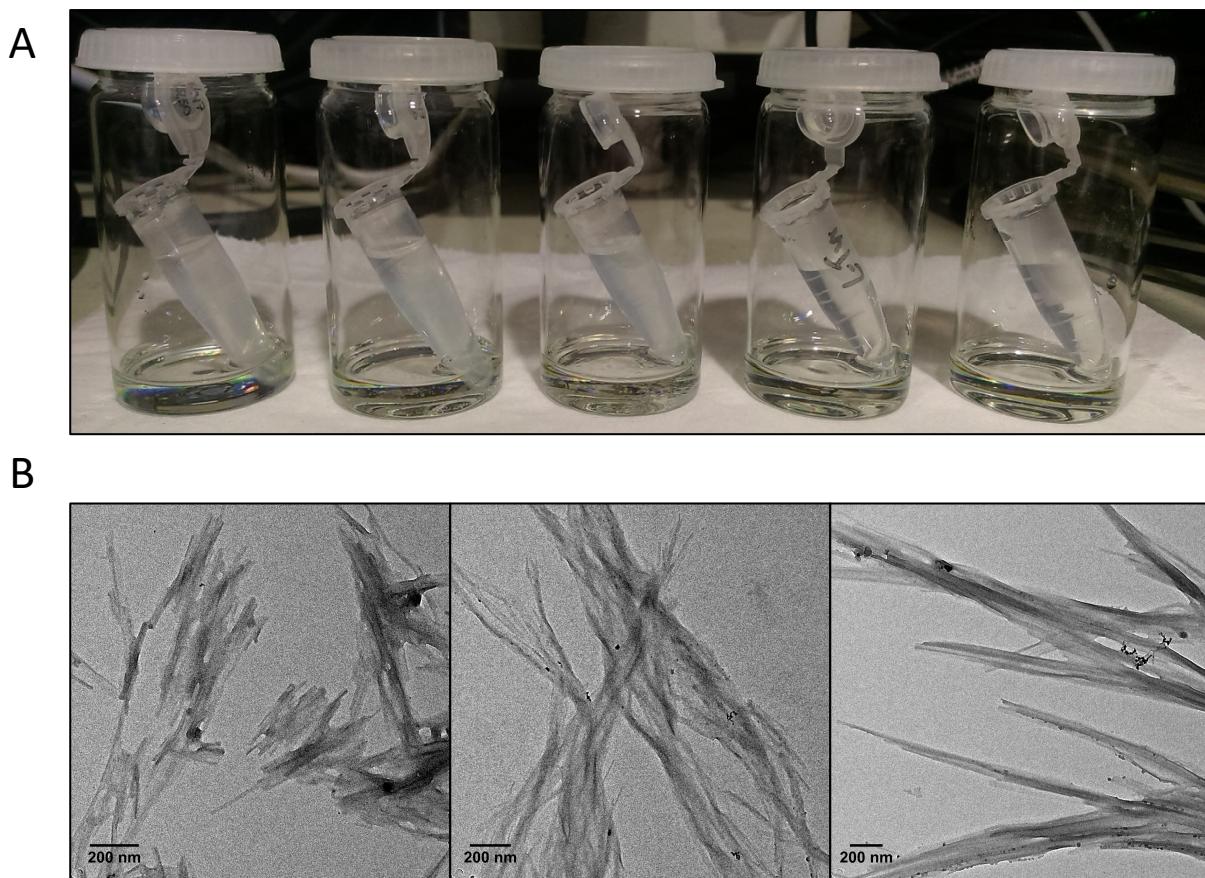
**Figure S9. NY7, QY7 and SY7 fibrils dityrosine cross-link and denaturation.** NY7, QY7 and SY7 fibrils were assembled at 250  $\mu$ M. NY7 and QY7 fibrils were cross-linked using UV-light. Measurements were performed before (black line), and after (red line) irradiation at 280 nm, a control was irradiated at 390 nm (blue line). (A and B) Fibrils spectra upon excitation at 320 nm. (C and D) Dityrosine formation kinetics of NY7 (C) and QY7 (D) fibrils irradiating at 280 nm (red line) or at 390 nm. (E and F) Representative TEM micrographs of NY7 (E) and QY7 (F) cross-linked fibrils. SY7 fibrils were chemically cross-linked. Measurements were performed with without treatment (black line), upon incubation with Cu<sup>2+</sup> and H<sub>2</sub>O<sub>2</sub> (red line), upon incubation with Cu<sup>2+</sup> only (dashed blue line) and upon incubation with H<sub>2</sub>O<sub>2</sub> only (solid blue line). (G) Fibrils spectra upon excitation at 320 nm. (H) Representative TEM micrograph of SY7 cross-linked fibrils.



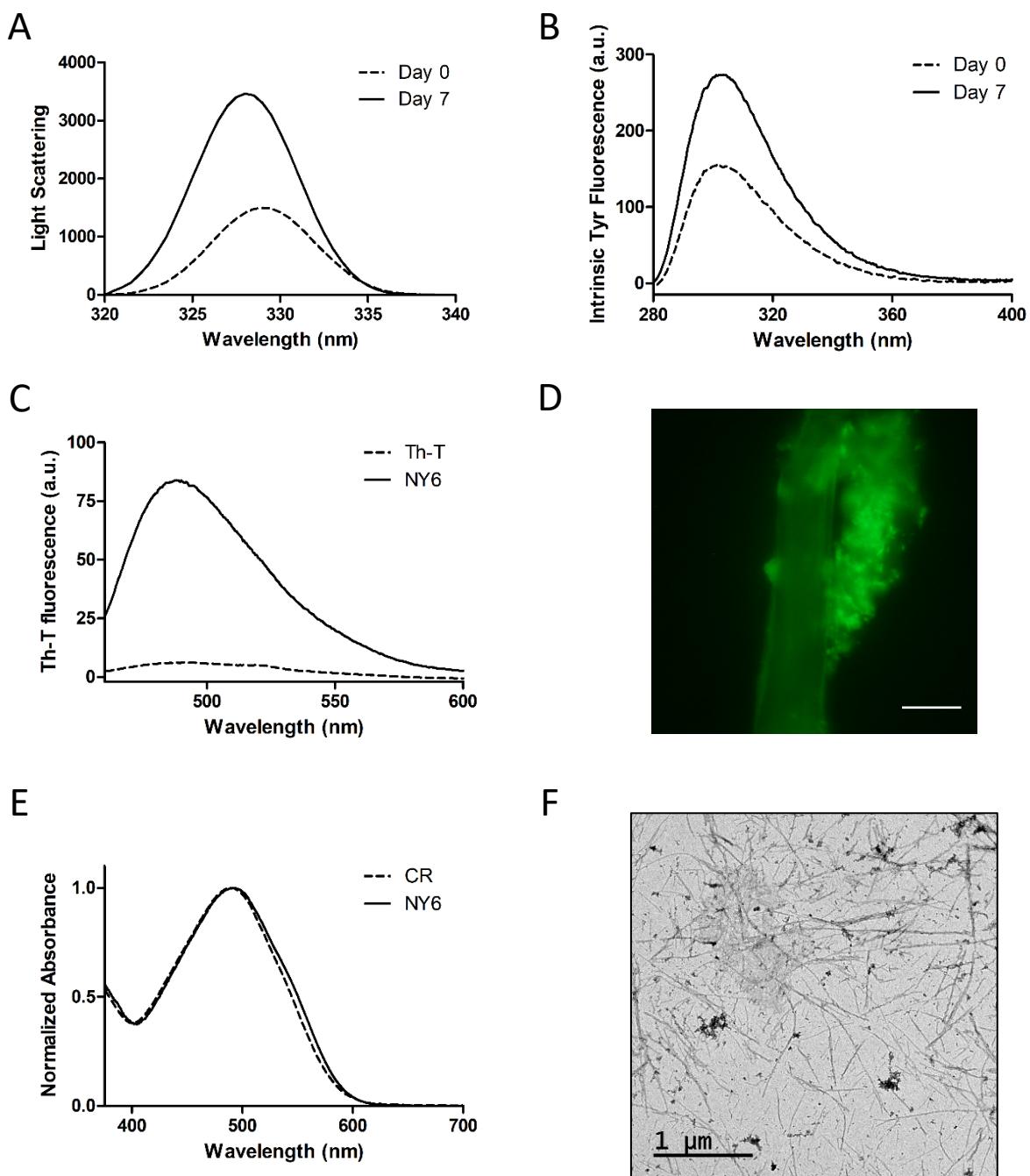
**Figure S10: SY7 dityrosine formation using UV light.** SY7 peptide fibrils assembled at 250  $\mu\text{M}$ . Measurements were performed before (dashed line), and after (solid black line) irradiation at 280 nm, a control was irradiated at 390 nm (blue line). (A) Fibrils spectra upon excitation at 274 nm. Insets show the dityrosine emission region (375-450 nm). (B) Fibrils spectra upon excitation at 320 nm (C) SY7 dityrosine formation kinetics irradiating at 280 nm (solid line) or at 390 nm (dashed line). (D) Representative TEM micrograph of SY7 cross-linked fibrils.



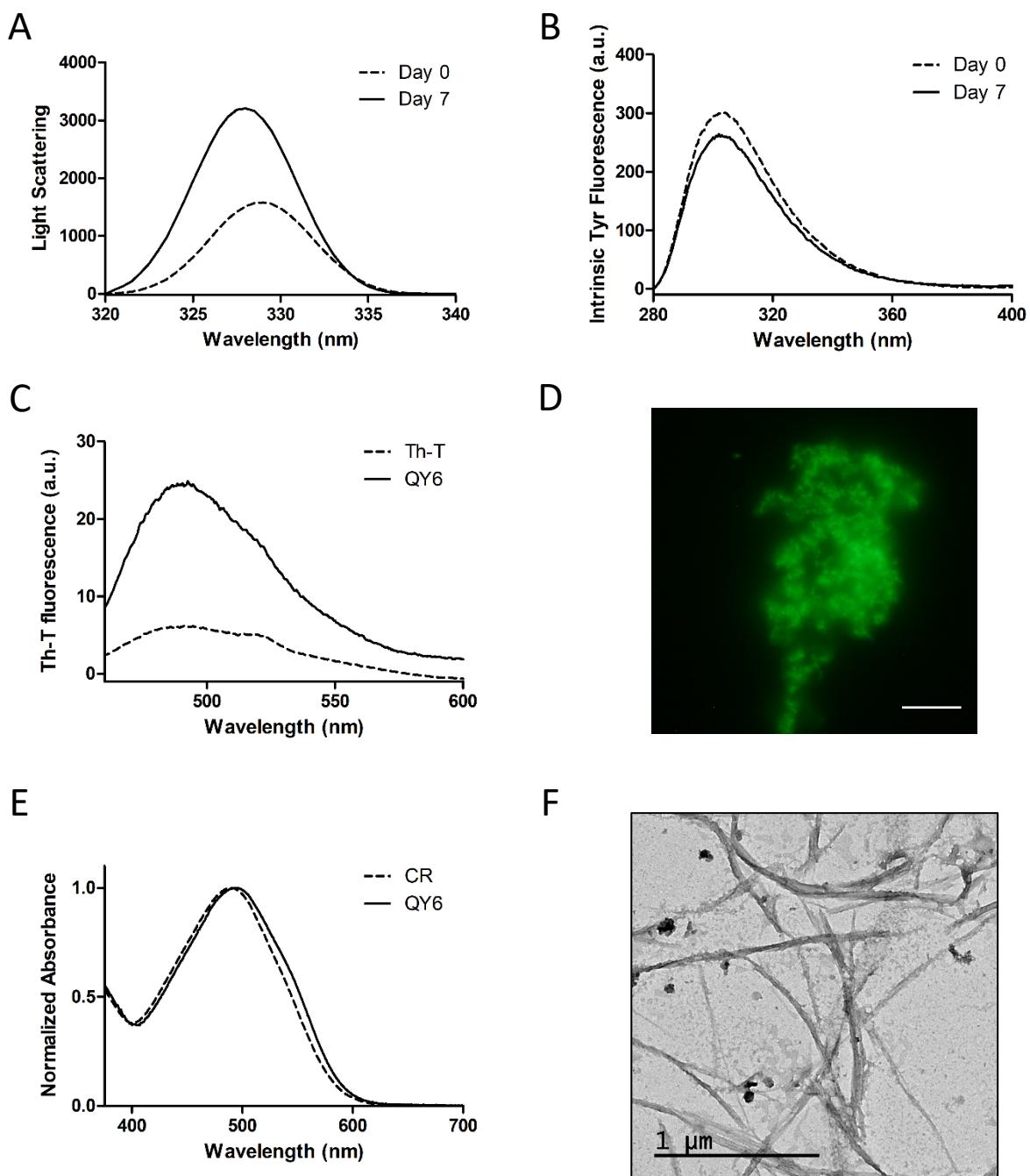
**Figure S11: Chemical denaturation of NY7 fibrils.** Disaggregation kinetics were performed with 3 M urea (black line), 4 M guanidinium hydrochloride (GuHCl) (blue line), and 2 M guanidinium isothiocyanate (GITC) (red line) and monitored following Th-T fluorescence.



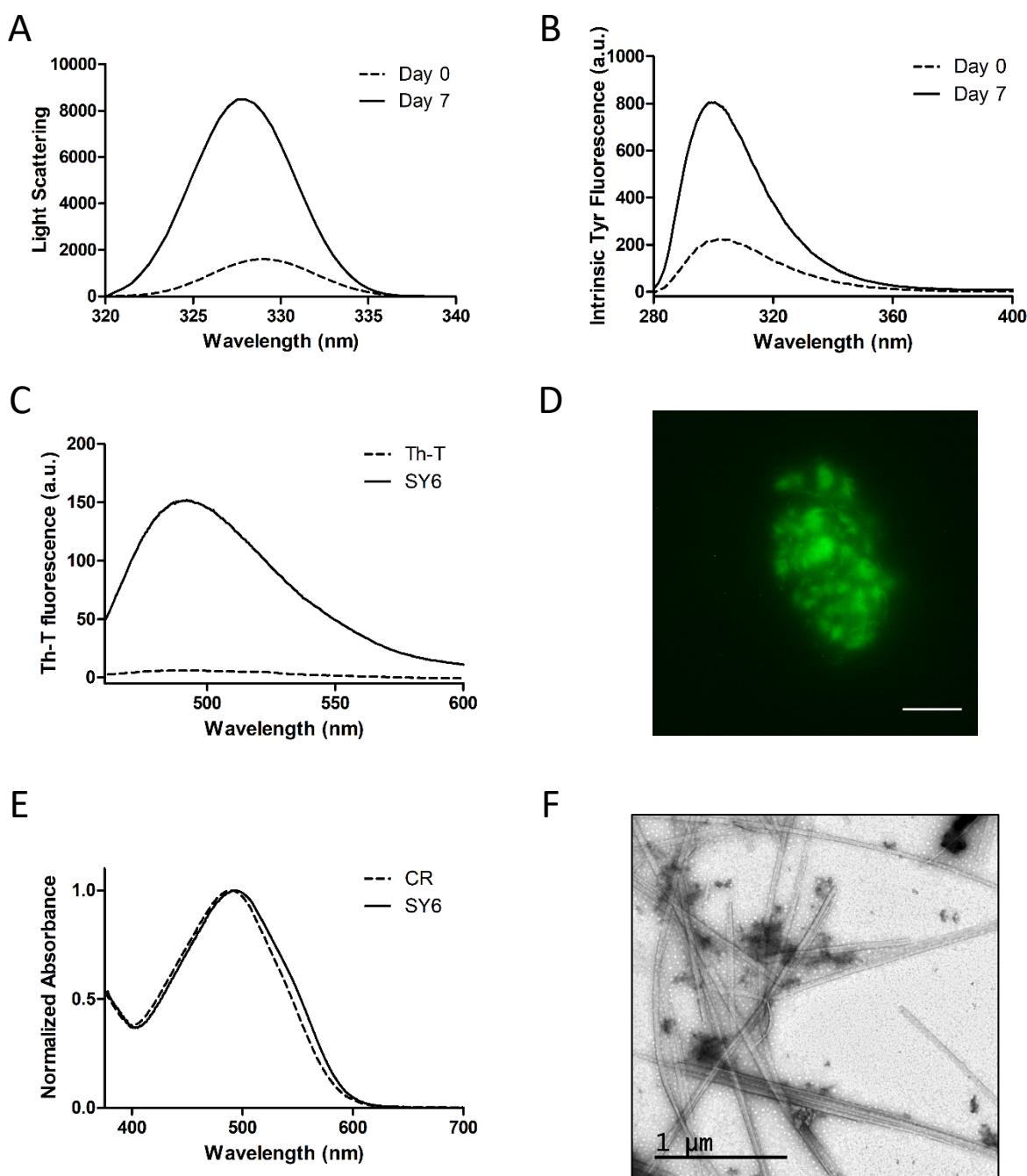
**Figure S12. NY7, QY7 and SY7 fibers performs as a bioelectrocatalyst.** (A) Set up for chemical polymerization of pyrrole using 4 mM CuCl<sub>2</sub> solution with 250 μM NY7 (the first starting from the left), QY7 (second), SY7 (third), L-tyrosine (forth) and only CuCl<sub>2</sub> (right) with distilled pyrrole vapour. The image corresponds to time 0 h. (B) TEM micrographs obtained after PPy formation (24 h) without any additional staining. From left to right: PPy-NY7, PPy-QY7 and PPy-SY7.



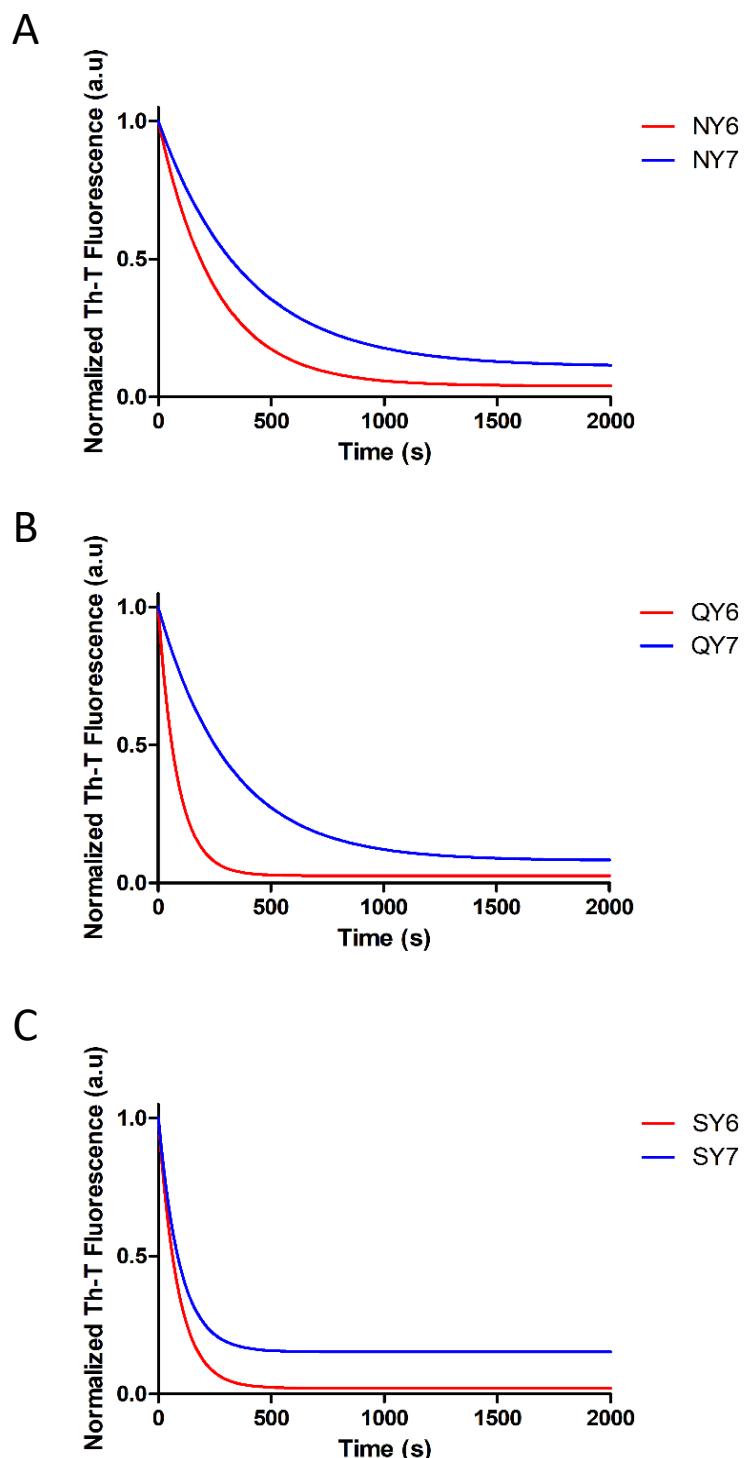
**Figure S13. NY6 peptide biophysical characterization.** NY6 peptide was prepared at 500  $\mu$ M in buffer 100 mM phosphate pH 7.0 at 25 °C and analysed at 0 (dashed line) and 7 days (solid line) of incubation: (A) Synchronous light scattering; (B) Intrinsic tyrosine fluorescence. (C) Th-T and (E) CR binding assays in the absence (dashed line) and in the presence (solid line) of NY6. (D) Fluorescence microscopy images of NY6 stained with Th-T. Scale bar corresponds to 20  $\mu$ m. (F) Representative TEM micrograph.



**Figure S14. QY6 peptide biophysical characterization.** QY6 peptide was prepared at 500  $\mu$ M in buffer 100 mM phosphate pH 7.0 at 25 °C and analysed at 0 (dashed line) and 7 days (solid line) of incubation: (A) Synchronous light scattering; (B) Intrinsic tyrosine fluorescence. (C) Th-T and (E) CR binding assays in the absence (dashed line) and in the presence (solid line) of QY6. (D) Fluorescence microscopy images of QY6 stained with Th-T. Scale bar corresponds to 20  $\mu$ m. (F) Representative TEM micrograph.



**Figure S15. SY6 peptide biophysical characterization.** SY6 peptide was prepared at 500  $\mu$ M in buffer 100 mM phosphate pH 7.0 at 25 °C and analysed at 0 (dashed line) and 7 days (solid line) of incubation: (A) Synchronous light scattering; (B) Intrinsic tyrosine fluorescence. (C) Th-T and (E) CR binding assays in the absence (dashed line) and in the presence (solid line) of SY6. (D) Fluorescence microscopy images of SY6 stained with Th-T. Scale bar corresponds to 20  $\mu$ m. (F) Representative TEM micrograph.



**Figure S16. Chemical denaturation of hexapeptides and heptapeptides.** Fibril disaggregation kinetics where analysed in buffer B in the presence of 2 M GITC, except for QY7 and QY6 fibrils, which were analysed in the presence of 3 M GITC. The final concentration of the fibrils in the assay was 125  $\mu$ M. The change of Th-T signal with time was monitored.

<i>ASSIGNMENTS</i>	<i>NY7</i>	<i>QY7</i>	<i>SY7</i>	<i>GY7</i>
<b>Intermolecular <math>\beta</math>-sheet (1615-1636 cm<sup>-1</sup>)</b>	<b>63%</b>	<b>51%</b>	<b>66%</b>	<b>36%</b>
<b>Disordered/Loops/Turns (1658-1662 cm<sup>-1</sup>)</b>	21%	38%	19%	42%
<b><math>\beta</math>-sheet (1675-1682 cm<sup>-1</sup>)</b>	<b>16%</b>	<b>11%</b>	<b>15%</b>	<b>22%</b>

**Table S1. Assignment and area of the secondary components of aggregated designed peptides in the amide I region of FT-IR spectra.** In bold,  $\beta$ -sheet contribution is indicated.

<b><i>MEASUREMENTS</i></b>	<b><i>NY7</i></b>	<b><i>QY7</i></b>	<b><i>SY7</i></b>	<b><i>GY7</i></b>
<b>Width of fibers</b>	12 to 18 nm	12 to 18 nm	12 to 18 nm	12 to 25 nm
<b>Length of fibers</b>	0.3 to 1.5 $\mu$ m	2 to 5 $\mu$ m	0.5 to 5 $\mu$ m	1 to 4 $\mu$ m

**Table S2. Measurement of width and length of fibers.** Measurements were performed using TEM micrographs at high magnification (x80) with ImageJ software.

<i>ELECTRON DIFRACTION PATTERN</i>	<i>Ag</i>	<i>NY7 + AgNO<sub>3</sub></i>	<i>QY7 + AgNO<sub>3</sub></i>	<i>SY7 + AgNO<sub>3</sub></i>
<b>Distances (Å)</b>	<b>2.367</b>	<b>2.450</b>	<b>2.429</b>	<b>2.419</b>
	<b>2.050</b>	<b>2.113</b>	-	<b>2.130</b>
	<b>1.450</b>	<b>1.466</b>	<b>1.489</b>	<b>1.490</b>
	<b>1.230</b>	<b>1.148</b>	<b>1.236</b>	<b>1.219</b>
	<b>0.941</b>	<b>0.974</b>	<b>0.918</b>	<b>0.856</b>

**Table S3. Interplanar distances obtained from electronic diffraction experiments.**  
 Comparison between the interplanar distances of metallic silver and peptide fibrils incubated in the presence of 1mM AgNO<sub>3</sub> for 24 h.