Electronic Supplementary Information

Amyloidogenesis highlighted by designed peptides forming supramolecular self-assemblies

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Figure S1. Chemical structures of peptide edifices and corresponding precursors.

**Synthesis of cyclic decapeptide 3** (Figure S2). The linear peptide R(Pmc)-K(Dde)-R(Pmc)-P-G-R(Pmc)-K(Dde)-R(Pmc)-P-G was first built up automatically (Advance Chem Tech 348 Ω peptide synthesizer) on Fmoc-Gly-Sasrin® resin (500 mg, 0.69 mmol.g⁻¹) and cyclized (415...
mg, 1.59.10^{-4} \text{ mol}) in DMF (0.5 \text{ mmol.L}^{-1}) under high dilution using PyBOP (1.2 \text{ eq.}) and DIPEA. The white solid powder obtained after precipitation and washing in diethyl ether was solubilized in a solution of 2% of hydrazine in DMF to remove Dde protecting groups. The cyclopeptidic intermediate \textbf{1} (272 mg, 1.21.10^{-4} \text{ mol}) was then obtained after precipitation and washing in diethyl ether (76% yield from the linear peptide). To a solution of compound \textbf{1} (272 mg, 1.21.10^{-4} \text{ mol}) in DMF (0.01 \text{ mol.L}^{-1}), were added Boc-Ser(tBu)-OH (3 eq), PyBOP (3 eq.) and DIPEA. The mixture was stirred for 2 h at r.t. Then Boc, tBu and Pmc protecting groups were removed using a solution of TFA/TIS/H_2O (95:2.5:2.5) (0.01 \text{ mol.L}^{-1}). After 2 h, the solvent was evaporated and the crude compound \textbf{2} (145 mg, 7.08.10^{-5} \text{ mol}) was obtained by precipitation with diethyl ether as white solid powder with 59% yield. HPLC t_R = 4.9 min. ESI-MS calc 1362.8, found 1362.5.

To a solution of compound \textbf{2} (5 mg, 2.44.10^{-6} \text{ mol}) in H_2O/CH_3CN (1:1) (0.01 \text{ mol.L}^{-1}) was added NaIO_4 (20 eq.). The reaction was stirred for 20 min at r.t. and immediately purified by RP-HPLC (C18 Nucleosil® column, 5-100% B in 30 min). This procedure was realized 8 times to afford compound \textbf{3} (21 mg, 1.19.10^{-5} \text{ mol}) as a white powder with 61% yield. HPLC t_R = 5.0 min. ESI-MS calc 1300.7, found 1300.4.

Figure S2. Synthesis of the cyclodecapeptide \textbf{3}. (a) Piperidine/DMF (1:4); (b) Fmoc-Xaa-OH (2 eq.), PyBOP (2 eq.), DIPEA (3-4 eq.), DMF; (c) TFA/CH_2Cl_2 (1:99), 10 min (three times); (d) PyBOP (1.2 eq.), DIPEA (3-4 eq.), DMF (0.5 \text{ M}), 1 h; (e) Hydrazine/DMF (2:98), 2 h, 76% from the linear form of \textbf{1}; (f) i) Boc-Ser(tBu)-OH (3 eq.), PyBOP (3 eq.), DIPEA, DMF (10^{-2} \text{ M}), 2 h; ii) TFA/TIS/H_2O (95:2.5:2.5), 2 h, 59% from \textbf{1} (2 steps); (g) NaIO_4 (20 eq.), H_2O/CH_3CN (1:1), 61%.

\textbf{Synthesis of peptide 4} (Figure S3). The GGCA\beta_{16-37}Y_{20}K_{22}K_{24}C peptide sequence was synthesized automatically (Applied Biosystems) by solid phase synthesis on NovaSyn® TG Sieber resin (300 mg, 0.19 mmol.g^{-1}). Peptide on resin (5.7.10^{-5} \text{ mol}) was then solvated in 10 mL of DMF and the pH was adjusted with DIEPA to pH 8-9. 2-(1-ethoxyethylideneaminooxy)acetic acid (2 eq.), and PyBOP (2 eq.) were added to the resin
solution. The mixture was stirred for 1 h at r.t. Peptide was then solvated in DMF and iodine (20 eq.) was added. The peptide was released from the resin using 10 mL cleavage solution of TFA/H₂O/TIS (95:2.5:2.5). The mixture was stirred for 2 h at r.t then 10 eq. of NH₄I was added and the mixture was stirred for another 30 min. The crude free peptide 4 was obtained as a white powder (142 mg, 4.34×10⁻⁵ mol) and then purified by RP-HPLC (C18 Nucleosil® column, 5-100% B in 30 min) affording pure peptide 4 (15.1 mg, 4.62×10⁻⁶ mol) as a white powder with 8% overall yield from the resin. HPLC tᵣ = 8.3 min. ESI-MS calc 2698.4, found 2699.1.

X₁ = G-S(ψMeMe pro)

Figure S3. Synthesis of the peptide 4. (a) Piperidine/NMP (1:4); (b) Fmoc-Xaa-OH (10 eq.), HBTU (10 eq.), DIPEA (20 eq.), NMP; (c) 2-(1-ethoxyethylideneamino)acetic acid (2 eq.), PyBOP (2 eq.), DIPEA (3-4 eq.), DMF; (d) I₂ (20 eq.), DMF; (e) TFA/H₂O/TIS (95:2.5:2.5), NH₄I (10 eq.), 8% from the resin.

The cyclic decapeptide 5 and the peptide 6 (Figure S1) were synthesized as previously described.¹ Synthetic Aβ₁-40 was prepared as previously described.²
Figure S4. Characterization of compound 2Lin by chromatography and mass spectrometry. RP-HPLC profile (a); ESI-MS analysis (b).
Figure S5. Characterization of compound 2Loop by chromatography and mass spectrometry. RP-HPLC profile (a); ESI-MS analysis (b).
Figure S6. Characterization of compound 4Loop by chromatography and mass spectrometry. RP-HPLC profile (a); ESI-MS analysis (b).
**Kinetics studies.** A concentration 25 µM of **4Loop**, **2Loop** and **2Lin** and 6 µM of **4Lin** were used for kinetic studies at 20°C. Fibril formation was monitored by the binding of Thioflavin T (10 µM), studying the fluorescence at 480 nm with excitation at 440 nm. The kinetic constants \( k_1 \) and \( k_2 \) were obtained using the Finke-Watzky (F-W) two-step mechanism of nucleation followed by autocatalytic surface growth.

\[
\begin{align*}
A & \xrightarrow{k_1} B \\
A + B & \xrightarrow{k_2} 2B
\end{align*}
\]

Using this mechanism (where A is the initial monomer and B (catalytic) aggregated form of peptide edifies past the critical nucleus size) we can extract from experimental data, two constants, \( k_1 \), which represents the nucleation process and \( k_2 \), which represents the extension of the fibre (Table S1). This model can be mathematically translated by the following equations:

\[
[A]_t = \frac{k_1 / k_2 + [A]_0}{1 + k_1 / k_2 \cdot [A]_0 \cdot \exp(k_1 + k_2 [A]_0) \cdot t}
\]

or

\[
[B]_t = [A]_0 - \frac{k_1 / k_2 + [A]_0}{1 + k_1 / k_2 \cdot [A]_0 \cdot \exp(k_1 + k_2 [A]_0) \cdot t}
\]

<table>
<thead>
<tr>
<th></th>
<th><strong>4Lin</strong></th>
<th><strong>4Loop</strong></th>
<th><strong>2Loop</strong></th>
<th><strong>2Lin</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_1 ) (min(^{-1}))</td>
<td>( 160 \times 10^{-3} \pm 2 \times 10^{-2} )</td>
<td>( 28 \times 10^{-3} \pm 4 \times 10^{-3} )</td>
<td>( 28 \times 10^{-3} \pm 4 \times 10^{-3} )</td>
<td>( 2.3 \times 10^{-3} \pm 4 \times 10^{-4} )</td>
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<tr>
<td>( k_2 ) (µM(^{-1}).min(^{-1}))</td>
<td>( 46 \times 10^{-3} \pm 5 \times 10^{-3} )</td>
<td>( 6.1 \times 10^{-3} \pm 7 \times 10^{-4} )</td>
<td>( 3.2 \times 10^{-3} \pm 4 \times 10^{-4} )</td>
<td>( 3.6 \times 10^{-3} \pm 4 \times 10^{-4} )</td>
</tr>
<tr>
<td>( t_{1/2} ) (min)</td>
<td>2.5</td>
<td>8.5</td>
<td>12.6</td>
<td>270.2</td>
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Table S1. Rate constants and half-life time values from fitting kinetic data with the F-W two-step mechanism.