Supporting Information for:

Nanoscale Assembly of Functional Peptides with Divergent Programming Elements

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S1. Spectroscopic data........................................................................................ S2
S2. HPLC traces.................................................................................................... S18
S3. Rheology data for 2a and 2b and photographs of 2b................................. S19
S4. TEM micrographs........................................................................................ S19
S5. AFM images.................................................................................................. S24
S6. Full-atom MD simulations of tripeptides..................................................... S28
S7. DLS data....................................................................................................... S30
S8. FT-IR spectra............................................................................................... S31
S9. XRD data..................................................................................................... S31
S10. Aβ(1-42) inhibition data............................................................................ S38
S11. Thioflavin T fluorescence on tripeptides................................................... S39
S1. Spectroscopic data (NMR and ESI-MS)

1a: L-Pro-L-Phe-L-Phe

![Chemical structure of 1a](image)

**Fig. S1.** Chemical structure of 1a.

**1H NMR** (500 MHz, DMSO-\(d_6\)) \(\delta\) 8.68 (d, \(J = 8.5\) Hz, 1H, NH), 8.46 (d, \(J = 7.8\) Hz, 1H, NH), 7.36 – 6.93 (m, 10H, Ar), 4.58 (ddd, \(J = 9.7, 4.2\) Hz, 1H, \(\alpha\)CH), 4.44 (ddd, \(J = 8.5, 5.2\) Hz, 1H, \(\alpha\)CH), 4.09 – 3.94 (m, 1H, \(\alpha\)CH), 3.21 – 2.97 (m, 4H, 2 x ProCH\(_2\), 2 x \(\beta\)CH\(_2\)), 2.91 (dd, \(J = 13.9, 9.2\) Hz, 1H, \(\beta\)CH\(_2\)), 2.72 (dd, \(J = 13.9, 10.2\) Hz, 1H, \(\beta\)CH\(_2\)), 2.25 – 2.12 (m, 1H, ProCH\(_2\)), 1.92 – 1.64 (m, 3H, ProCH\(_2\)). **13C NMR** (125 MHz, DMSO-\(d_6\)) \(\delta\) 173.1, 171.1, 168.4 (3 x CO); 137.9, 137.8, 129.6, 129.5, 128.6, 128.5, 126.9 (Ar); 59.2, 54.6, 54.0 (3 x \(\alpha\)C); 46.2 (C-Pro); 37.9, 37.1 (2 x \(\beta\)C), 30.0, 23.9 (2 x C-Pro). **MS (ESI)** m/z 410.1 (M+H\(^+\)) \(\text{C}_{23}\text{H}_{27}\text{N}_{3}\text{O}_{4}\) requires 409.2.

**Fig. S2.** \(^1\)H-NMR spectrum of 1a.
Fig. S3. $^{13}$C-NMR spectrum of 1a.

Fig. S4. ESI-MS spectra of 1a in the positive (left) and negative (right) ion mode.
1b: D-Pro-D-Phe-D-Phe

Fig. S5. Chemical structure of 1b.

$^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 8.70 (d, $J = 8.5$ Hz, 1H, NH), 8.47 (d, $J = 7.9$ Hz, 1H, NH), 7.38 – 7.02 (m, 10H, Ar), 4.72 – 4.51 (m, 1H, $\alpha$CH), 4.45 (ddd, $J = 8.9, 5.0$ Hz, 1H, $\alpha$CH), 4.04 (ddd, $J = 8.5, 6.6$ Hz, 1H, $\alpha$CH), 3.21 – 2.98 (m, 3H, 2 x ProCH$_2$, 2 x $\beta$CH$_2$), 2.91 (dd, $J = 13.9, 9.2$ Hz, 1H, $\beta$CH$_2$), 2.73 (dd, $J = 13.9, 10.1$ Hz, 1H, $\beta$CH$_2$), 2.26 – 2.16 (m, 1H, ProCH$_2$), 1.90 – 1.66 (m, 3H, ProCH$_2$). $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 173.1, 171.1, 168.4 (3 x CO); 137.9, 137.9, 129.6, 129.5, 128.6, 128.5, 126.9 (Ar); 59.1, 54.6, 54.0 (3 x $\alpha$C); 46.2 (C-Pro); 37.9, 37.1 (2 x $\beta$C); 30.0, 23.9 (2 x C-Pro). MS (ESI) m/z 410.2 (M+H)$^+$ C$_{23}$H$_{27}$N$_3$O$_4$ requires 409.2.

Fig. S6. $^1$H-NMR spectrum of 1b.
Fig. S7. $^{13}$C-NMR spectrum of 1b.

Fig. S8. ESI-MS spectra of 1b in the positive (left) and negative (right) ion mode.
2a: d-Pro-L-Phe-L-Phe

Fig. S9. Chemical structure of 2a.

$^{1}H$ NMR (500 MHz, DMSO-$d_6$) $\delta$ 8.72 (d, $J$ = 9.0 Hz, 1H, NH), 8.58 (d, $J$ = 7.9 Hz, 1H, NH), 7.37 – 7.11 (m, 10H, Ar), 4.70 (ddd, $J$ = 10.9, 9.3, 3.7 Hz, 1H, $\alpha$CH), 4.46 (ddd, $J$ = 9.0, 5.0 Hz, 1H, $\alpha$CH), 4.04 (ddd, $J$ = 7.6 Hz, 1H, $\alpha$CH), 3.14 – 3.00 (m, 4H, 2 x ProCH$_2$, 2 x $\beta$CH$_2$), 2.92 (dd, $J$ = 14.0, 9.3 Hz, 1H, $\beta$CH$_2$), 2.65 (dd, $J$ = 13.7, 11.0 Hz, 1H, $\beta$CH$_2$), 2.04 (ddd, $J$ = 14.4, 7.9 Hz, 1H, ProCH$_2$), 1.71 (m, $J$ = 14.3, 7.3 Hz, 1H, ProCH$_2$), 1.60 – 1.41 (m, 1H, ProCH$_2$), 1.21 (ddd, $J$ = 14.6, 7.2 Hz, 1H, ProCH$_2$). $^{13}C$ NMR (125 MHz, DMSO-$d_6$) $\delta$ 173.2, 171.2, 168.0 (3 x CO); 138.0, 137.8, 129.8, 129.5, 128.7, 128.4, 126.9, 126.8 (Ar); 59.4, 54.1, 53.9 (3 x $\alpha$C); 45.9 (C-Pro); 38.6, 37.0 (2 x $\beta$C); 30.9, 23.6 (2 x C-Pro). MS (ESI) m/z 410.2 (M+H)$^+$ C$_{23}$H$_{27}$N$_3$O$_4$ requires 409.2.

Fig. S10. $^{1}H$-NMR spectrum of 2a.
Fig. S11. $^{13}$C-NMR spectrum of 2a.

Fig. S12. ESI-MS spectra of 2a in the positive (left) and negative (right) ion mode.
Fig. S13. Chemical structure of 2b.

2b: L-Pro-D-Phe-D-Phe

Fig. S14. 1H-NMR spectrum of 2b.
Fig. S15. $^{13}$C-NMR spectrum of 2b.

Fig. S16. ESI-MS spectra of 2b in the positive (left) and negative (right) ion mode.
3a: L-Pro-D-Phe-L-Phe

![Chemical structure of 3a.](image)

Fig. S17. Chemical structure of 3a.

**$^1$H NMR** (500 MHz, DMSO-$d_6$) δ 8.66 (dd, $J = 8.7$ Hz, 2H, NH), 7.31-7.22 (m, 4H, Ar), 7.22-7.11 (m, 4H, Ar), 7.09-7.04 (m, 2H, Ar), 4.76-4.64 (m, 1H, αCH), 4.49 (ddd, $J = 10.0$, 8.6, 4.6 Hz, 1H, αCH), 4.04 (dd, $J = 7.7$ Hz, 1H, αCH), 3.12 (ddd, $J = 11.5$, 5.8 Hz, 1H, ProCH$_2$), 3.09 – 2.98 (m, 2H, ProCH$_2$, βCH$_2$), 2.92 – 2.77 (m, 1H, βCH$_2$), 2.71 (dd, $J = 13.6$, 3.7 Hz, 1H, βCH$_2$), 2.39 (dd, $J = 13.5$, 10.7 Hz, 1H, βCH$_2$), 2.13 – 1.93 (m, 1H, ProCH$_2$), 1.71 (m, $J = 14.5$, 7.3 Hz, 1H, ProCH$_2$), 1.61 – 1.44 (m, 1H, ProCH$_2$), 1.23 (m, $J = 44.2$, 22.1 Hz, 1H, ProCH$_2$).

**$^{13}$C NMR** (125 MHz, DMSO-$d_6$) δ 173.4, 170.7, 168.2 (3 x CO); 138.0, 137.8, 129.8, 129.7, 128.6, 128.3, 126.9, 126.8 (Ar); 59.4, 54.0, 53.9 (3 x αC); 45.9 (C-Pro); 38.8, 37.6 (2 x βC); 30.0, 23.7 (2 x C-Pro). **MS (ESI)** m/z 410.1 (M+H)$^+$ C$_{23}$H$_{27}$N$_3$O$_4$ requires 409.2.

Fig. S18. $^1$H-NMR spectrum of 3a.
Fig. S19. $^{13}$C-NMR spectrum of 3a.

Fig. S20. ESI-MS spectra of 3a in the positive (left) and negative (right) ion mode.
3b: D-Pro-L-Phe-D-Phe

Fig. S21. Chemical structure of 3b.

$^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 8.70 d, $J = 8.4$ Hz, 1H, NH), 8.68 (d, $J = 9.0$ Hz, 1H, NH), 7.29 – 7.11 (m, 8H, Ar), 7.11 – 7.00 (m, 2H, Ar), 4.70 (ddd, $J = 10.6, 9.4, 3.8$ Hz, 1H, aCH), 4.50 (ddd, $J = 10.2, 8.6, 4.6$ Hz, 1H, aCH), 4.05 (dd, $J = 7.7$ Hz, 1H, aCH), 3.10 – 3.01 (m, 3H, 2 x ProCH$_2$, βCH$_2$), 2.84 (dd, $J = 13.7, 10.2$ Hz, 1H, βCH$_2$), 2.70 (dd, $J = 13.6, 3.7$ Hz, 1H, βCH$_2$), 2.38 (dd, $J = 13.6, 10.8$ Hz, 1H, βCH$_2$), 1.71 (m, $J = 14.5, 7.4$ Hz, 1H, ProCH$_2$), 1.59 – 1.47 (m, 1H, ProCH$_2$), 1.24 (m, $J = 14.7, 7.3$ Hz, 1H, ProCH$_2$). $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 173.3, 170.7, 168.0 (3 x CO); 137.9, 137.7, 129.7, 129.6, 128.6, 128.4, 127.0, 126.8 (Ar); 59.5, 54.0, 53.8 (3 x αC); 45.9 (C-Pro); 38.8, 37.6 (2 x βC); 30.0, 23.6 (2 x C-Pro). MS (ESI) m/z 410.2 (M+H)$^+$ C$_{23}$H$_{27}$N$_3$O$_4$ requires 409.2.

Fig. S22. $^1$H-NMR spectrum of 3b.
Fig. S23. $^{13}$C-NMR spectrum of 3b.

Fig. S24. ESI-MS spectra of 3b in positive (left) and negative (right) ion mode.
4a: L-Pro-L-Phe-D-Phe

Fig. S25. Chemical structure of 4a.

\[ \text{\textsuperscript{1}H NMR (500 MHz, DMSO-d\textsubscript{6}) } \delta 12.88 \text{ (s, 1H, OH), 8.64 \text{ (d, } J = 8.6 \text{ Hz, 1H, NH), 8.57} \text{ (d, } J = 8.4 \text{ Hz, 1H, NH), 7.32} - 7.12 \text{ (m, 8 H, Ar), 7.11} - 7.07 \text{ (m, 2H, Ar), 4.59 (ddd, } J = 9.7, 4.1 \text{ Hz, 1H, } \alpha \text{CH), 4.48 (ddd, } J = 10.0, 8.5, 4.6 \text{ Hz, 1H, } \alpha \text{CH), 4.04 (dd, } J = 8.4, 6.8 \text{ Hz, 1H, } \alpha \text{CH), 3.21} - 3.04 \text{ (m, 3H, 2 x ProCH\textsubscript{2}, } \beta \text{CH\textsubscript{2}}, 2.83 \text{ (dd, } J = 13.8, 10.1 \text{ Hz, 1H, } \beta \text{CH\textsubscript{2}}, 2.72 \text{ (dd, } J = 13.8, 4.0 \text{ Hz, 1H, } \beta \text{CH\textsubscript{2}}, 2.53} - 2.44 \text{ (m, 1H, } \beta \text{CH\textsubscript{2}}, 2.31} - 2.18 \text{ (m, 1H, ProCH\textsubscript{2}}, 1.92} - 1.67 \text{ (m, 3H, 3 x ProCH\textsubscript{2}}). \text{\textsuperscript{13}C NMR (125 MHz, DMSO-d\textsubscript{6}) } \delta 173.2, 170.7, 168.3 \text{ (3 x CO); 137.9, 137.8), 129.7, 129.6, 128.6, 128.5, 127.0 (Ar); 59.2, 54.6, 53.8 (3 x } \alpha \text{C); 46.1 (C-Pro); 38.1, 37.5 (2 x } \beta \text{C); 30.0, 23.9 (2 x C-Pro). MS (ESI) m/z 410.2 (M+H)\textsuperscript{+} C\textsubscript{23}H\textsubscript{27}N\textsubscript{3}O\textsubscript{4} requires 409.2. \]

Fig. S26. \textsuperscript{1}H-NMR spectrum of 4a.
Fig. S27. $^{13}$C-NMR spectrum of 4a.

Fig. S28. ESI-MS spectra of 4a in the positive (left) and negative (right) ion mode.
**4b: D-Pro-D-Phe-L-Phe**

![Chemical structure of 4b](image)

**Fig. S29.** Chemical structure of 4b.

**1H NMR** (500 MHz, DMSO-d$_6$) δ 12.92 (s, 1H, OH), 8.65 (d, $J = 8.6$ Hz, 1H, NH), 8.57 (d, $J = 8.4$ Hz, 1H, NH), 7.31 – 7.13 (m, 8H, Ar), 7.13 – 7.06 (m, 2H, Ar), 4.59 (ddd, $J = 9.6$, 4.1 Hz, 1H, αCH), 4.48 (ddd, $J = 10.0$, 8.5, 4.6 Hz, 1H, αCH), 4.05 (dd, $J = 8.2$, 6.9 Hz, 1H, αCH), 3.21 – 3.04 (m, 3H, 2 x ProCH$_2$, βCH$_2$), 2.83 (dd, $J = 13.7$, 10.1 Hz, 1H, βCH$_2$), 2.72 (dd, $J = 13.8$, 4.0 Hz, 1H, βCH$_2$), 2.49 (m, $J = 13.8$, 11.6 Hz, 1H, βCH$_2$), 2.31 – 2.19 (m, 1H, ProCH$_2$), 1.98 – 1.65 (m, 3H, ProCH$_2$). **13C NMR** (125 MHz, DMSO-d$_6$) δ 173.3, 170.8, 168.3 (3 x CO); 137.9, 137.8, 129.7, 129.6, 128.6, 128.5, 126.9, 126.8 (Ar); 59.2, 54.6, 53.8 (3 x αC); 46.1 (C-Pro); 38.1, 37.5 (2 x βC); 30.0, 23.9 (2 x C-Pro).

**MS (ESI)** m/z 410.1 (M+H)$^+$ C$_{23}$H$_{27}$N$_3$O$_4$ requires 409.2.

![1H-NMR spectrum of 4b](image)

**Fig. S30.** 1H-NMR spectrum of 4b.
**Fig. S31.** $^{13}$C-NMR spectrum of 4b.

**Fig S32.** ESI-MS spectra of 4b in the positive (left) and negative (right) ion mode.
S2. Analytical LC data for full series of Pro-Phe-Phe tripeptides

**Fig S33.** Analytical LC traces for each pair of enantiomers with Pro-Phe-Phe sequence. Retention times (Rt): 13.5 min (1a/b); 13.9 min (2a/b); 14.6 min (3a/b); 14.2 min (4a/b).
S3. Rheological data for the hydrogel 2a and 2b.

Fig. S34. A) Stress sweep for 2a (24 mM). B) Frequency sweep for 2a (24 mM). C) Frequency sweep for 2b (24 mM). D) Photograph of 2b hydrogel (24 mM). E) Photograph of 2b insoluble aggregates after heating the hydrogel to 85 °C and cooling it down to RT. By contrast, if the hydrogel was heated to 40 °C, no aggregates were formed and the hydrogel disassembled into a clear solution reversibly, as indicated in the MS.

S4. TEM micrographs.

Fig. S35. TEM micrograph of 1a amorphous aggregate (left) and selected area (red circle) electron diffraction (SAED, right) revealed it was amorphous.
Fig. S36. TEM micrograph of 1a microcrystals.
**Fig. S37.** TEM micrographs of 2a fibrils forming nanotapes (detail) with plot profiles compatible with the presence of $1.6 \pm 0.2$ nm-wide individual fibrils. The area in the yellow box is enlarged below: with (left) or without (right) yellow lines to guide the eye.

**Chart S1.** Fibril size distribution for 2a (counts = 150) with mean diameter of individual (n=1) fibril corresponding to $1.6 \pm 0.2$ nm, two fibrils (n=2) corresponding to $3.2 \pm 0.3$ nm, and three fibrils (n=3) corresponding to $4.6 \pm 0.4$ nm.
Fig. S38. TEM micrograph of 2a nanotapes forming the gel.

Fig. S39. TEM micrograph of 3a nanoparticles.
**Fig. S40.** TEM micrograph detail of 3a rare instances of short, thin fibrils, with (left) or without (right) yellow lines to guide the eye.

**Chart S2.** Fibril size distribution for 3a (counts = 25, due to rare instances).
Fig. S41. TEM micrograph of 4a nanotapes.

S5. AFM images

Fig. S42. AFM image of 1a aggregates.
Fig. S43a. AFM image of 2a nanotapes showing they arise from the lateral association of elongated structures with 1-2 nm step heights.
**Fig. S43b.** Additional AFM images of 2a nanotapes.

**Chart S3.** Height profile shown in Fig. S43b (right) confirmed 1-2 nm step heights.

**Fig. S44.** AFM images of 3a nanoparticles.
Chart S4. Height profile shown in Fig. S44 (right) showed homogeneous nanoparticle size (confirmed to be $8 \pm 3$ nm on 100 measures).

Fig. S45. AFM images of 4a nanotapes, with height profiles showing 1-2 nm steps.
S6. Full-atom MD simulations of tripeptides.

Generation of tripeptide and Aβ structural models. Models of zwitterionic tripeptides were built upon the experimental structure (2a) or generated using the AmberTools19 package\textsuperscript{1} and the VMD1.9.3 software\textsuperscript{2} through in-house tcl scripts (3a). The initial coordinates of the protein were taken from the NMR structure with PDB ID 2NAO,\textsuperscript{3} namely from the chain A of the corresponding pdb file.

MD simulations. Multi-copy MD simulations of the self-assembly process for 216 2a and 3a tripeptides were performed as described previously.\textsuperscript{4} To further investigate the molecular determinants of 2a fibrillization, an additional set of simulations was performed with 1000 such peptides. The initial structures were generated by placing the center of mass of the peptide repeatedly on the 15 Å-spaced points of a 10x10x10 (2a) or 6x6x6 (2a, 3a) grid. Initial orientations of peptides were randomized, and the systems were solvated with water molecules.

To investigate the molecular interactions occurring in solvent between 3a and Aβ, we performed a MD simulation of Aβ and 20 tripeptides in water solution. In this case, due to the net electrostatic charge carried by the protein, the system was neutralized by adding 13 K\textsuperscript{+} and 10 Cl\textsuperscript{-} ions.

All the simulations were performed as follows. First, three consecutive restrained structural optimizations (up to 25,000 steps) were performed in the presence of harmonic restraints (k = 1 kcal mol\textsuperscript{-1} Å\textsuperscript{-1}) applied to: a) all non-hydrogenous atoms of the system; b) backbone atoms; c) C\textsubscript{α} atoms. Reference structures at steps b) and c) were the final ones from the previous step. Next, up to 50,000 cycles of unrestrained optimization were performed. Each system was then heated to 310 K in 1 ns via constant-pressure-temperature (NTP) MD simulations, followed by an equilibration phase of 10 ns. Starting from the equilibrated structure, multiple MD simulations were performed for each system (see Table S1). Pressure and temperature were set to 1 atm and 310 K (after the equilibration phase) using the isotropic Berendsen barostat\textsuperscript{5} and the Langevin thermostat,\textsuperscript{6} respectively. A time step of 2 fs was used for all the simulation steps but the production runs, where it was set to 4 fs after hydrogen mass repartitioning.\textsuperscript{7} Periodic boundary conditions were employed, and electrostatic interactions were estimated using the Particle Mesh Ewald scheme with a cutoff of 9.0 Å for the short-range evaluation in direct space and for Lennard-Jones interactions (with a continuum model correction for energy and pressure).

The AMBER force fields parm14SB\textsuperscript{8} was employed to model the Aβ peptide and the tripeptides, while the TIP3P\textsuperscript{9} model was used describe water molecules, and the parameters for the ions were taken from ref. 10. The TIP3P (transferable intermolecular potential with 3 points) model describes a water molecule as three interaction sites corresponding to the oxygen and the two hydrogen atoms, linked by rigid bonds. Oxygen and hydrogens bear fraction charges amounting to -0.834 and 0.417, respectively. Coulomb interactions are present between all intermolecular pairs of charges, while Van der Waals interactions are described by a single Lennard-Jones term between oxygen.
Despite its simplicity, this model is still largely employed in the simulation of biomolecules, in view of its computational efficiency and of the reasonable thermodynamic and structural description provided for liquid water without the need for three-body corrections.

Table S1. Details of the full-atom MD simulations performed for this work.

| Simulation       | N<sub>pep</sub> | N<sub>waters</sub> | T<sub>simulation</sub> | c<sub>pep</sub> [M] | N<sub>replicas</sub> |
|------------------|-----------------|--------------------|-----------------------|---------------------|---------------------|
| 2a self-assembly | 216             | ~45,500            | 1 µs                  | 0.25                | 4                   |
| 2a self-assembly | 1000            | ~201,000           | 1.5 µs                | 0.25                | 4                   |
| 3a self-assembly | 216             | ~45,500            | 1 µs                  | 0.25                | 4                   |
| 3a/Aβ interaction| 20              | ~10,000            | 250 ns                | n.c.                | 1                   |

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**Fig. S46.** Assembly of 1,000 DLL 2a peptides as resulting from 1.5 microsecond long MD simulations in water solution. Six replicas were run. The surface colored by atom type (C, N, O in grey, blue and red, respectively) is the one in the primary simulation box. That in grey extends along the x direction, while the two other rod-like assemblies represent adjacent images.

**S7. DLS data**

**Fig. S47.** Representative DLS data of 3a samples.
S8. FT-IR spectra.

![FT-IR spectra](image_url)

**Fig S48.** ATR-FT-IR spectra of tripeptide enantiomers 1a-4a. Spectra were recorded using 24-hours aged samples. Numbers indicated the most relevant signals in the amide I region.

S9. XRD data

**Compound 1a** (CCDC 2021319)

Needle-shaped single crystals of the peptide were collected with a loop, cryoprotected by dipping the crystals polyethylene glycol with average molecular weight 200 g/mol, PEG 200, and stored frozen in liquid nitrogen. Crystal were mounted on the diffractometer at the synchrotron Elettra, Trieste (Italy), beamline XRD1, using the robot present at the facility. Temperature was kept at 100 K by a stream of nitrogen on the crystal. Diffraction data were collected by the rotating crystal method using synchrotron radiation, wavelength 0.70 Å, rotation interval 2°/image, crystal-to-detector distance of 85 mm. A total of 90 images were collected. Reflections were indexed and integrated using the software Mosflm,¹ space group $P2_1$ was determined using POINTLESS.² The resulting data set was scaled using AIMLESS.³ Phase information were obtained by direct methods using the software SHELX-T.⁴ Refinements cycles were conducted with SHELXL-18,⁵ operating through the WinGX GUI,⁶ by full-matrix least-squares methods on $F^2$. 

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⁴ SHELX-T: A software for the direct methods, G. M. Sheldrick, Acta Crystallogr. Sect. A: Found. Crystallogr., 46 (1990) 467-473.
⁵ SHELXL-18: A software for the refinement of crystal structures, G. M. Sheldrick, Acta Crystallogr. Sect. C: Struct. Chem., 71 (2015) 3-8.
⁶ WinGX GUI: A graphical user interface for the SHELXL software, G. M. Sheldrick, J. Appl. Cryst., 36 (2003) 198-207.
Congruence of structure model and calculated electron density was inspected using the software Coot. The asymmetric unit contains a single molecule of the peptide. Hydrogen atoms were added at geometrically calculated positions and refined isotropically. All non-hydrogen atoms within the asymmetric unit have been refined with anisotropic thermal parameters. Unit cell parameters, scaling statistics, and refinement statistics are reported in Table S2.

**Compound 3a** (CCDC2026055)

A stick-shaped single crystal of the peptide was collected with a loop, cryoprotected by dipping the crystal in polyethylene glycol with average molecular weight 200 g/mol, PEG 200, and stored frozen in liquid nitrogen. The crystal was mounted on the diffractometer at the synchrotron Elettra, Trieste (Italy), beamline XRD1, using the robot present at the facility. Temperature was kept at 100 K by a stream of nitrogen on the crystal. Diffraction data were collected by the rotating crystal method using synchrotron radiation, wavelength 0.70 Å, rotation interval 1°/image, crystal-to-detector distance of 85 mm. A total of 180 images were collected. Reflections were indexed and integrated using the software Mosflm, space group P21 was determined using POINTLESS. The resulting data set was scaled using AIMLESS. Phase information were obtained by direct methods using the software SIR 2014. Refinement cycles were conducted with SHELXL-18, operating through the WinGX GUI, by full-matrix least-squares methods on F2. Congruence of structure model and calculated electron density was inspected using the software Coot. The asymmetric unit contains 10 crystallographically independent molecules of the peptide. In the residual electron density of the asymmetric unit, 12 water molecules were located, 7 of which in positions at full occupancy, 5 in positions statistically occupied in 50% of the unit cells. Restraints were applied to bond lengths and angles of the proline moiety of two crystallographically independent peptides, using the cards DFIX and DANG of the SHELXL-18 software, in particular when a statistical occupancy of two close positions was observed. In addition, restrains were applied to keeps similar values for anisotropic displacement parameters for adjacent atoms in the direction of bonds. Hydrogen atoms of the peptide molecules were added at geometrically calculated positions and refined isotropically. When a clear electron density could be observed, hydrogen atoms of the water molecules at full occupancy were added considering the hydrogen bonding pattern, and refined with restrains on bond lengths and angles, using the cards DFIX and DANG of the SHELXL-18 software. All the atoms,
except the hydrogen atoms, within the asymmetric unit have been refined with anisotropic thermal parameters. Unit cell parameters, scaling statistics, and refinement statistics are reported in **Table S2**.

**Compound 4b** (CCDC2021318)

A stick-shaped single crystal of the peptide was collected with a loop, cryoprotected by dipping the crystal in glycerol and stored frozen in liquid nitrogen. The crystal was mounted on the diffractometer at the synchrotron Elettra, Trieste (Italy), beamline XRD1, using the robot present at the facility. Temperature was kept at 100 K by a stream of nitrogen on the crystal. Diffraction data were collected by the rotating crystal method using synchrotron radiation, wavelength 0.70 Å, rotation interval 1°/image, crystal-to-detector distance of 85 mm. A total of 180 images were collected. Reflections were indexed and integrated using the XDS package, space group C2 was determined using POINTLESS, and the resulting data set was scaled using AIMLESS. Phase information were obtained by direct methods using the software SHELXS. Refinement cycles were conducted with SHELXL-14, operating through the WinGX GUI, by full-matrix least-squares methods on F2. Congruence of structure model and calculated electron density was inspected using the software Coot. The asymmetric unit contains a single molecule of the peptide and a molecule of water in a special position, with the oxygen atom located along the 2-fold symmetry axis, resulting in an occupancy of 50% in the asymmetric unit. During refinement, no restraints were applied on distances, angles or thermal parameters of the peptide or the water molecule. Hydrogen atoms of the peptide were added at geometrically calculated positions and refined isotropically, with thermal parameters dependent on those of the attached atom. All non-hydrogen atoms were refined with anisotropic thermal parameters. Unit cell parameters, scaling statistics, and refinement statistics are reported in **Table S2**.
Table S2: Crystallographic data.

|                       | Compound 1a CCDC2021319 | Compound 3a CCDC2026055 | Compound 4b CCDC2021318 |
|-----------------------|---------------------------|--------------------------|--------------------------|
| Formula               | C_{23}H_{27}N_{3}O_{4}    | 10C_{23}H_{32}N_{3}O_{9}·9H_{2}O | C_{23}H_{27}N_{3}O_{4}·0.5H_{2}O |
| Temperature (K)       | 100                       | 100                       | 100                       |
| Wavelength (Å)        | 0.7                       | 0.7                       | 0.7                       |
| Crystal system        | Monoclinic                | Monoclinic                | Monoclinic                |
| Space group           | P 2₁                      | P 2₁                      | C 2                       |
| a (Å)                 | 5.321(1)                  | 13.280(2)                 | 27.040(5)                 |
| b (Å)                 | 11.574(2)                 | 22.700(4)                 | 5.550(1)                  |
| c (Å)                 | 17.058(3)                 | 39.530(5)                 | 19.320(4)                 |
| α (°)                 | 97.22(3)                  | 98.220(8)                 | 134.45(3)                 |
| β (°)                 | 90                        | 90                        | 90                        |
| γ (°)                 | 90                        | 90                        | 90                        |
| V (Å³)                | 1042.2(4)                 | 11794(3)                  | 2070(1)                   |
| Z, pcalc (g/cm³)      | 2, 1.305                  | 2, 1.201                  | 4, 1.343                  |
| μ (mm⁻¹)              | 0.086                     | 0.053                     | 0.090                     |
| F (000)               | 436                       | 4550                     | 892                       |
| Data collection θ range | 2.1 - 28.326             | 1.021 - 28.394            | 1.454 - 28.633            |
| Refl. Collected / unique | 6991 / 2504             | 65886 / 31222             | 14963 / 3014              |
| Rint                  | 0.062                     | 0.075                     | 0.065                     |
| Completeness (%)      | 89.4                      | 98.5                      | 98.9                      |
| Data/Restraints/Parameters | 2504 / 1 / 272         | 31222 / 216 / 2890       | 3014 / 1 / 297            |
| GoOF                  | 1.052                     | 1.001                     | 1.078                     |
| R1, wR2 [>2σ(I)]     | 0.0454 / 0.1176           | 0.0818 / 0.2111           | 0.0412 / 0.1046           |
| R1, wR2 all data      | 0.0483 / 0.1196           | 0.0889 / 0.2197           | 0.0451 / 0.1075           |

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**Fig. S49.** Ramachandran plot for the crystal structure of 1a and 4a (black circles).
**Fig. S50.** Inverted Ramachandran plot for the crystal structure of LDL 3a (orange squares refer to the 10 independent molecules).

**Fig. S51.** Crystal structure of 3a. Three snapshots show the position of water molecules that were superimposable in the structures crystallized from methanol/water or from phosphate buffer.
**Fig. S52.** Crystal structure of 4b (CCDC2021318). Strong electron density for the water molecule proved the very stable H-bonds that keep it between the peptide chains. Electron density was clearly visible also for the hydrogen atoms of the water and of the N-terminus.

**Fig. S53.** Superimposition of the 10 independent molecules of the crystal structure of 3a revealed the same conformation.
S10. Aβ(1-42) inhibition data.

**Fig. S54.** Thioflavin T assay at 48 hours revealed 3a was the most promising inhibitor, with analogous activity as the positive control D-Trp-Aib.

**Fig S55.** CD spectra of 15 μM Aβ(1-42) in the presence of 3a at the indicated concentrations (A) fresh and (B) after incubation for 24 hours at 37 °C.
S11. Thioflavin T fluorescence on tripeptides

**Fig. S56.** Thioflavin T fluorescence assay on peptides at 20 mM, except for 2a and 2b that were probed at the mge of 24 mM (at 20 mM the fluorescence was negligible). For reference, the Val-Phe-Phe sequence DLL and LDL peptides (black columns) were previously reported as the only gelators of the four stereoisomers (LLL, DLL, LDL, LLD) in S. Marchesan *et al.* *J. Mater. Chem. B* 2015, 3, 8123. Homochiral Pro-Phe-Phe (LLL or DDD) is not reported because of the presence of heterogeneous macroscopic aggregates at 20 mM that did not allow reproducible measurements.