SUPPLEMENTARY INFORMATION

Amyloid fibrils from organic solutions of amphiphilic dipeptide

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METHODS

Peptide Synthesis and Characterization

The synthesis and chemical characterization of TFA·FF-Fmoc was reported in a previous work.[S1]

Peptide samples preparation

Peptide containing solutions (25 or 100 μL) were prepared from 5 mg/mL stocks using HFIP, DMF and DMSO as solvent. The peptide concentration was reduced by adding milli-Q water or MeOH as co-solvent, to a given stock solution. More specifically, peptide concentrations of 4, 2, 1, 0.5, 0.25 and 0.1 mg/mL were obtained using 4:1, 4:6, 1:4, 1:9, 1:19 and 1:49 solvent:co-solvent ratios, respectively. Finally, 10 or 20 μL aliquots were placed on microscope coverslips stored in the lab (room temperature) until dryness.

Scanning electron microscopy (SEM)

SEM studies were performed in a Focussed Ion Beam Zeiss Neon 40 scanning electron microscope operating at 5 kV and equipped with an EDX spectroscopy system. Samples were mounted on a double-side adhesive carbon disc and sputter-coated with a thin layer of carbon to prevent sample charging problems.

Atomic Force Microscopy (AFM)

Topographic AFM images were obtained using either a Dimension 3100 Nanoman AFM or a Multimode, both from Veeco (NanoScope IV controller) under ambient conditions in tapping mode. AFM measurements were performed on various parts of the morphologies, which produced reproducible images similar to those displayed in this

work. Scan window sizes ranged from 3×3 μm^2 to 40×40 μm^2. In order to obtain detailed information about individual structures, AFM images were taken from regions at the edges of the corresponding aggregates.

Optical microscopy

Morphological observations were performed using a Zeiss Axioskop 40 microscope. Micrographs were taken with a Zeiss AxiosCam MRC5 digital camera.

S1. D. Martí, E. Mayans, A. M. Gil, A. Díaz, A. I. Jiménez, I. Yousef, I. Keridou, C. Cativiela, J. Puiggalí and C. Alemán, *Langmuir*, 2018, **34**, 15551

Table S1. Summary of the results obtained in this work.

HFIP:MeOH	[Peptide] mg/mL	Outcome
1:4	1	Dense bundles of amyloid fibrils
1:9	0.5	Poorly defined bundles of amyloid fibrils
1:19	0.25	Poorly defined bundles of amyloid fibrils
DMF:water		
4:6	2	Mixture of twisted and straight microfibers
1:4	1	Mixture of twisted and straight microfibers
1:19	0.25	Stacked braid-like microstructures
1:49	0.10	Stacked braid-like microstructures
DMF:KCl(aq)		
4:1	4	Branched-like structures
4:6	2	Branched-like structures
1:9	0.5	Branched-like structures
1:19	0.25	Branched-like structures
DMF:MeOH		
1:4	1	Branched structures connected by networks of thin amyloid fibrils.
1:9	0.5	Branched structures connected by networks of thin amyloid fibrils.
DMSO:water		
4:6	2	Irregular structures
1:4	1	Irregular structures
1:9	0.5	Branched-like structures resembling spherulitic-like organizations
1:19	0.25	Branched-like structures resembling spherulitic-like organizations
DMSO:KCl(aq)		
24:1	4.8	Branched-like structures resembling spherulitic-like organizations
4:1	4	Branched-like structures resembling spherulitic-like organizations

4:6	2	Irregular structures
1:4	1	Irregular structures
DMSO:MeOH		
4:6	2	Irregular structures
1:4	1	Irregular structures
1:9	0.5	Irregular structures

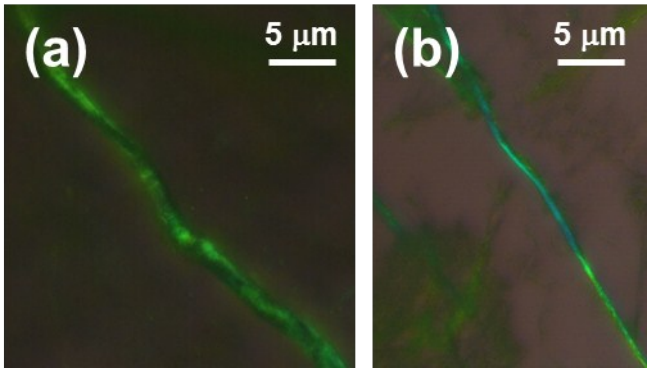

Figure S1. Polarized optical microscope images of Congo red stained structures obtained from 1 mg/mL TFA·FF-Fmoc solutions in (a) 1:4 HFIP/MeOH and (b) 1:4 DMF/MeOH at room temperature.

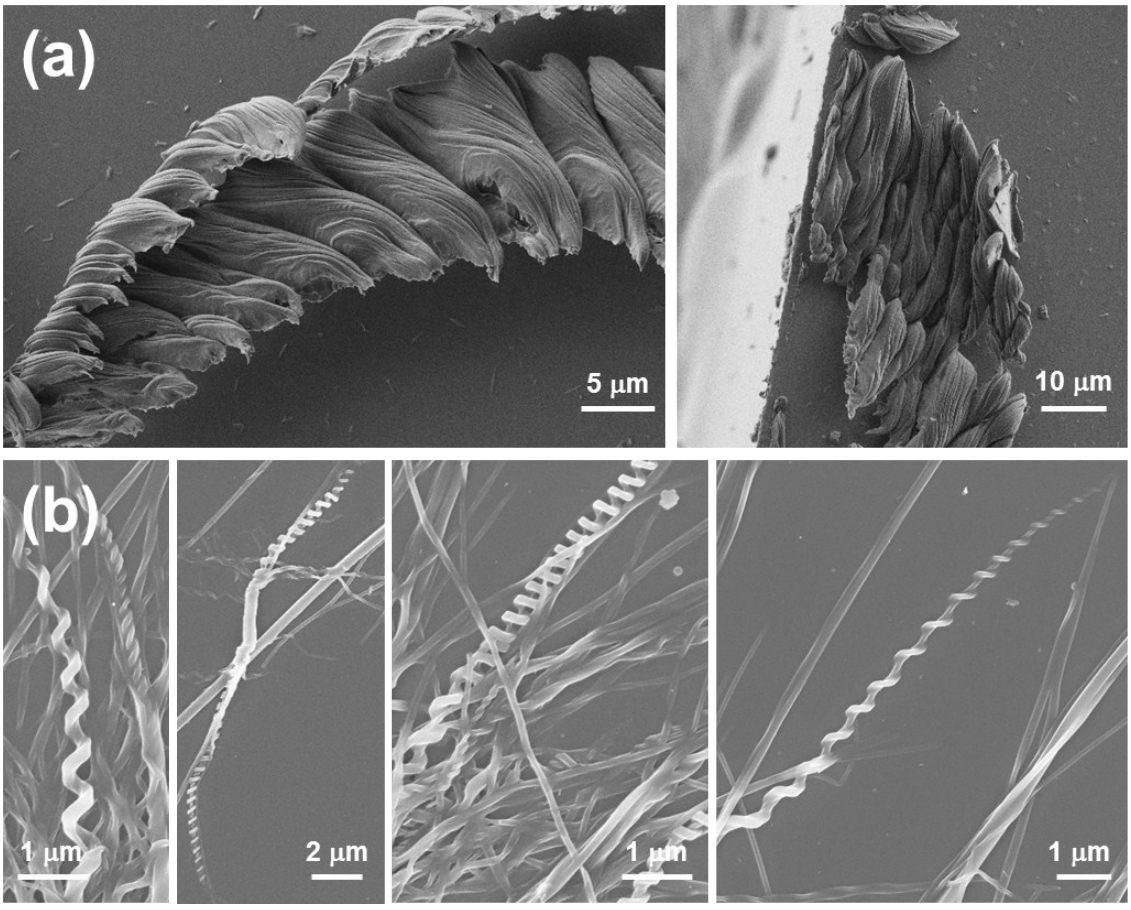

Figure S2. Representative SEM micrographs of (a) stacked braid-like microstructures located at the coverslips edges derived from 0.1 and 0.5 mg/mL (left and right, respectively) TFA·FF-Fmoc solutions in 1:49 and 1:9 DMF/water at room temperature; and (b) twisted and straight microfibers derived from 1.0 and 2.0 mg/mL (left and right, respectively) TFA·FF-Fmoc solutions in 1:4 and 4:6 DMF/water at room temperature.

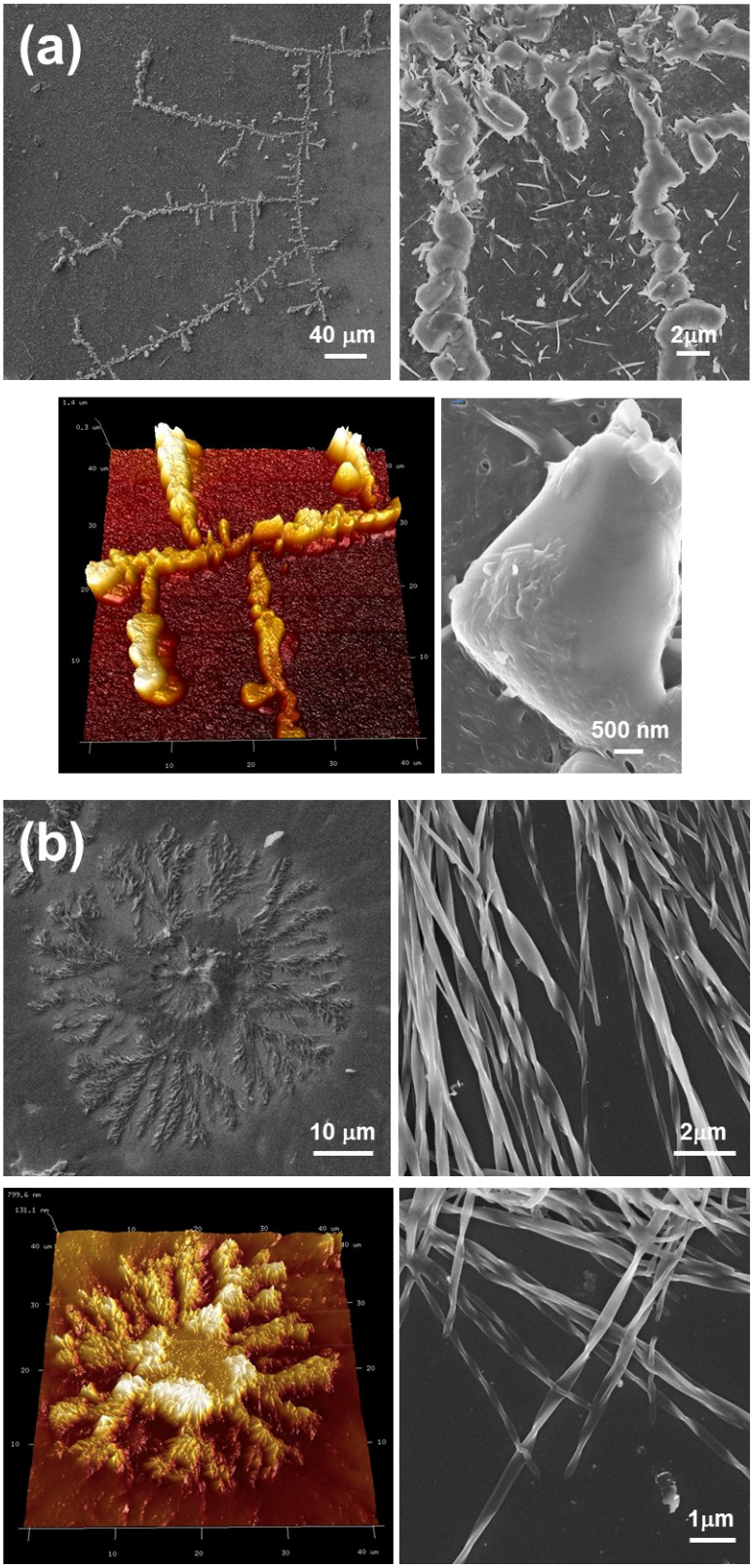

Figure S3. Representative SEM micrographs and AFM images (40×40 μm^2) of the branched-like structures derived from (a) 0.5 mg/mL TFA·FF-Fmoc solutions in 1:9 DMF/50 mM KCl(aq) solutions at room temperature; and (b) 4.0 mg/mL TFA·FF-Fmoc solutions in 4:1 DMF/50 mM KCl(aq) solutions at room temperature.

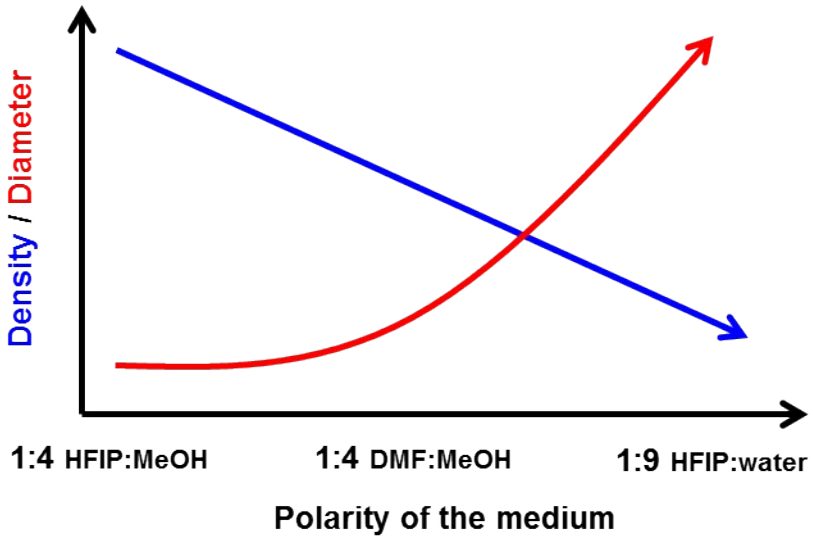

Figure S4. Sketch representing the variation of the density (amount of fibrils per surface area) and diameter of amyloid fibrils against the polarity of the medium. The most polar medium, which consisted in 1:9 HFIP:water, exhibited the lowest density and the highest diameter (reference 13). In contrast, the least polar medium, which was 1:4 HFIP:MeOH, showed the highest density of fibrils and the lowest diameter.

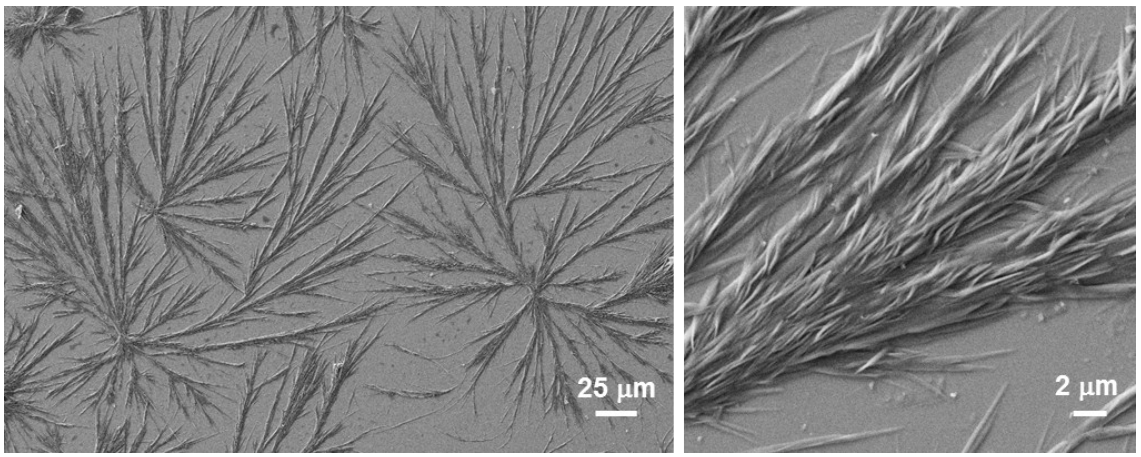

Figure S5. Representative SEM micrographs of branched-like microstructures derived from 0.5 mg/mL TFA·FF-Fmoc solutions in 1:9 DMSO/water at room temperature.

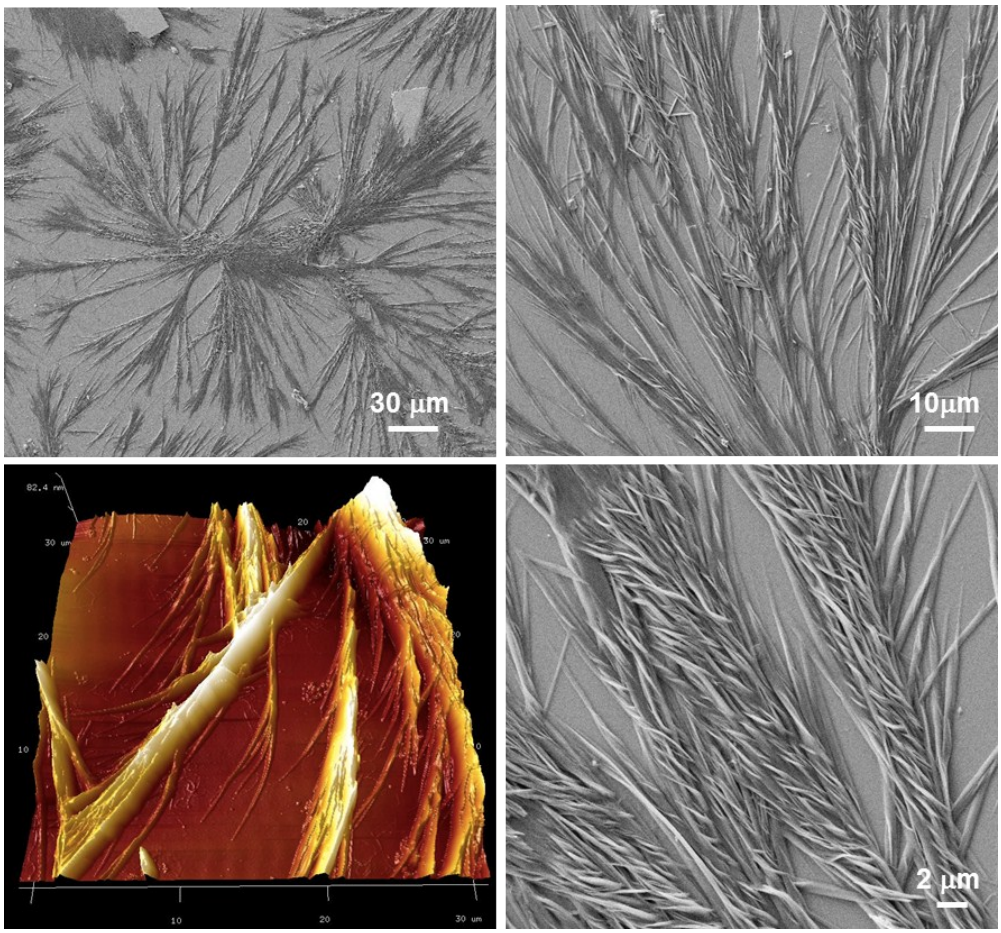

Figure S6. Representative SEM micrographs and AFM image (30×30 μm^2) of branched-like microstructures derived from 4.8 mg/mL TFA·FF-Fmoc solutions in 24:1 DMSO/50 mM KCl(aq) solutions at room temperature.

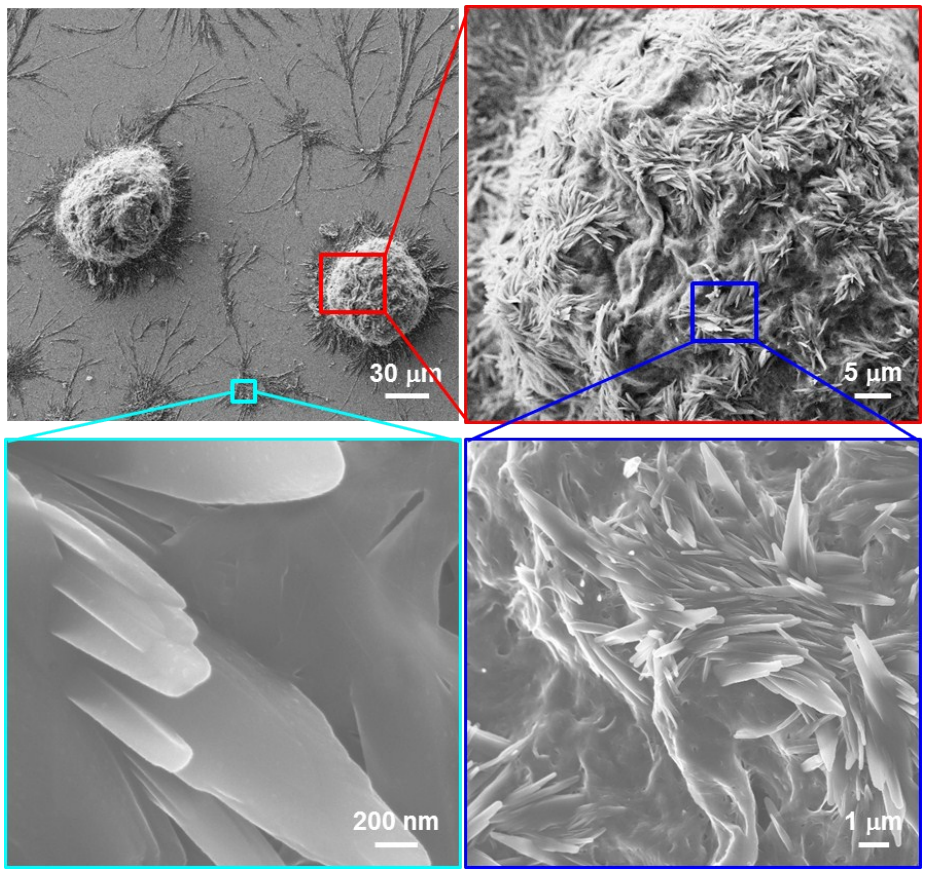

Figure S7. Representative SEM micrographs of poorly defined microstructures derived from 1 mg/mL TFA·FF-Fmoc solutions in 1:4 DMSO/MeOH solutions at room temperature.

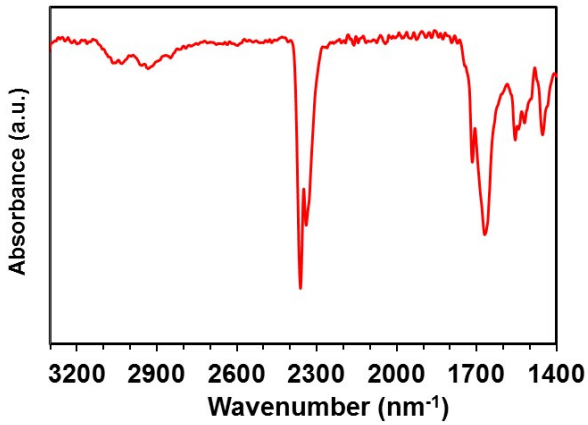

Figure S8. FTIR spectrum of the fibres derived from 1 mg/mL TFA·FF-Fmoc solutions in 1:4 HFIP/MeOH Typical amide bands indicative of hydrogen bonding interactions are detected at 3321, 3065, 1669, and 1555 cm^{-1}, which correspond amide A, amide B, amide I and amide II bands. These results are consistent with the antiparallel β-sheet structure predicted by DFT calculations.

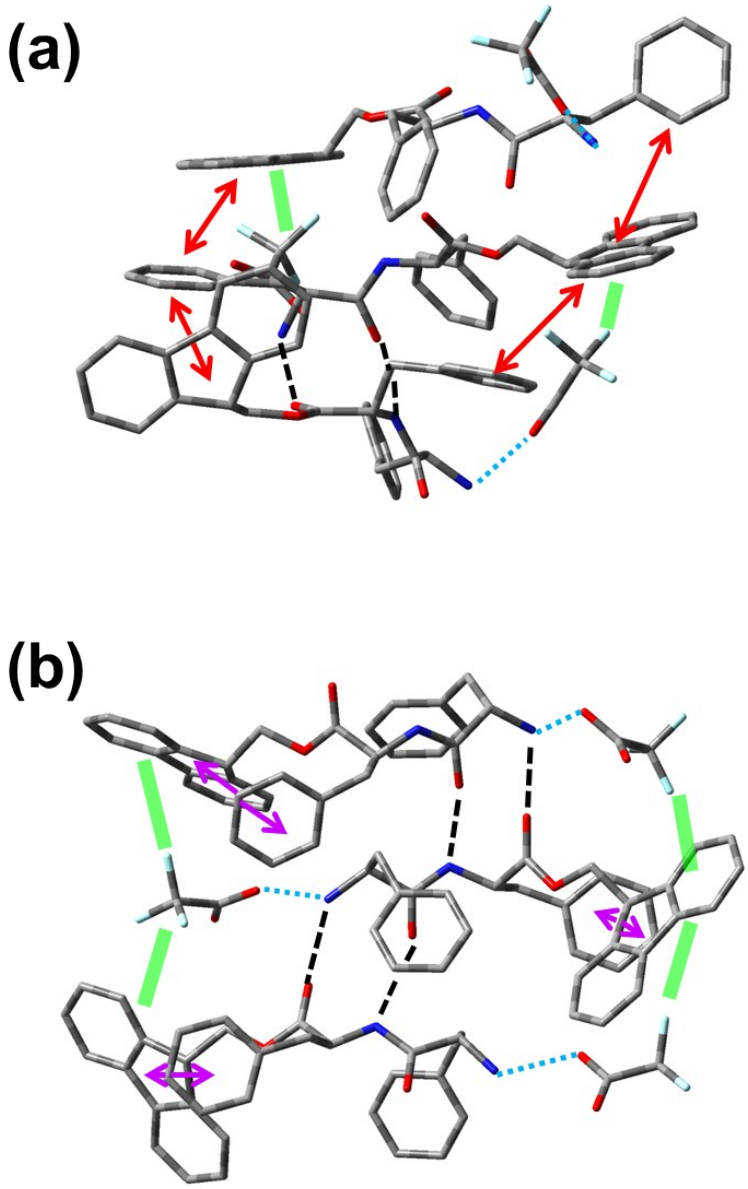

Figure S9. Antiparallel assembly predicted for three TFA·FF-Fmoc strands arranged in conformation (a) A and (b) B using M06L/6-31G(d,p) calculations. Interactions are identified as follows: N–H···O hydrogen bond (black dashed line), electrostatic COO^{-} ···$^{+}NH_3$ (blue dotted line), intermolecular π-π stacking (red double arrow), intramolecular π-π stacking (purple double arrow), and CF_3···π (green thick line).