ELECTRONIC SUPPORTING INFORMATION

Hierarchical Self-Assembly of Di-, Tri- and Tetraphenylalanine Peptides Capped with Two Fluorenyl Functionalities: From Polymorphs to Dendrites

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DESCRIPTION OF ALL INTERMEDIATES OBTAINED IN THE PEPTIDE SYNTHESIS

Boc-L-Phe-OFm. White solid, mp 130°C. $[\alpha]_D^{20}$: –27.1 (c = 0.39, MeOH). IR (KBr) ν 3348, 1738, 1682 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.43 (s, 9H), 2.97–3.10 (m, 2H), 4.15 (t, 1H, J = 6.9 Hz), 4.42 (m, 2H), 4.66 (dd, 1H, J = 13.9 Hz, J = 6.2 Hz), 4.97 (d, 1H, J = 8.0 Hz), 7.09–7.55 (m, 11H), 7.76–7.78 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.44, 38.48, 46.83, 54.65, 67.28, 80.14, 120.20, 125.16, 125.22, 127.22, 127.35, 128.03, 128.74, 129.44, 136.10, 141.44, 143.63, 155.23, 172.07. HRMS (ESI) $C_{28}H_{29}NO_4Na$ $[M+Na]^+$: calcd. 466.1989, found 466.1996.

Boc-L-Phe-L-Phe-OFm. White solid, mp 159°C. $[\alpha]_D^{20}$: –23.9 (c = 0.31, MeOH). IR (KBr) ν 3338, 3304, 1737, 1665 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.42 (s, 9H), 2.95–3.07 (m, 4H), 4.14 (t, 1H, J = 6.8 Hz), 4.38 (dd, 2H, J = 10.4 Hz, J = 6.9 Hz), 4.46 (dd, 1H, J = 10.7 Hz, J = 6.9 Hz), 4.85 (dd, 1H, J = 13.2 Hz, J = 6.2 Hz), 4.97 (m, 1H), 6.34 (d, 1H, J = 7.5 Hz), 6.94–7.00 (m, 2H), 7.16–7.57 (m, 14H), 7.79 (dd, 2H, J = 7.5 Hz, J = 3.3 Hz).^{13}C NMR ($CDCl_3$, 100 MHz) δ 28.36, 38.12, 38.35, 46.73, 53.51, 55.77, 67.31, 80.35, 120.21, 125.09, 125.12, 127.10, 127.31, 127.36, 128.03, 128.06, 128.72, 128.79, 129.36, 129.48, 135.68, 136.61, 141.42, 143.46, 143.52, 155.40, 170.92, 171.12. HRMS (ESI) $C_{37}H_{38}N_2O_5Na$ $[M+Na]^+$: calcd. 613.2673, found 613.2676.

Boc-L-Phe-L-Phe-L-Phe-OFm. White solid, mp 192°C. $[\alpha]_D^{20}$: –27.4 (c = 0.34, MeOH). IR (KBr) ν 3425, 3296, 1731, 1695, 1652 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.37 (s, 9H), 2.85–3.04 (m, 6H), 4.12 (t, 1H, J = 6.7 Hz), 4.25–4.31 (m, 1H), 4.34–4.45 (m, 2H), 4.54 (dd, 1H, J = 13.9 Hz, J = 7.0 Hz), 4.74 (dd, 1H, J = 13.5 Hz, J = 6.4 Hz), 4.82 (m, 1H), 6.22 (m, 1H), 6.39 (d, 1H, J = 7.4 Hz), 6.96–7.52 (m, 21H), 7.76 (dd, 2H, J = 7.3 Hz, J = 3.7 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) 28.34, 37.96, 38.07, 38.14, 46.74,

53.77, 54.33, 55.79, 62.79, 80.57, 120.18, 125.11, 125.16, 127.16, 127.33, 127.36, 128.02, 128.04, 128.76, 128.85, 129.31, 129.41, 135.78, 136.29, 136.52, 141.41, 143.52, 143.56, 155.55, 170.22, 171.05, 171.27. HRMS (ESI) $C_{46}H_{47}N_3O_6Na$ $[M+Na]^+$: calcd. 760.3357, found 760.3361.

Boc-L-Phe-L-Phe-L-Phe-L-Phe-OFm. White solid, mp 211°C. $[\alpha]_D^{20}$: –11.9 (c = 0.35, DMSO). IR (KBr) ν 3291, 1748, 1695, 1645 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.35 (s, 9H), 2.79–3.18 (m, 8H), 4.16–4.24 (m, 2H), 4.36–4.46 (m, 2H), 4.54 (dd, 1H, J = 12.0 Hz, J = 5.7 Hz), 4.70 (m, 1H), 4.80–4.90 (m, 2H), 6.40 (m, 1H), 6.61 (m, 1H), 6.74 (m, 1H), 6.93–7.85 (m, 28H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.33, 37.36, 37.67, 37.82, 37.99, 46.77, 53.81, 54.33, 54.53, 56.19, 67.39, 80.82, 120.13, 125.23, 125.28, 126.96, 127.18, 127.28, 127.33, 127.97, 128.69, 128.94, 129.14, 129.22, 129.31, 129.38, 136.08, 136.23, 136.80, 141.41, 143.68, 155.80, 170.36, 170.47, 171.23, 171.76. HRMS (ESI) $C_{55}H_{56}N_4O_7Na$ $[M+Na]^+$: calcd. 907.4048, found 907.4041.

Fmoc-L-Phe-L-Phe-OFm (Fmoc-FF-OFm). White solid, mp 195°C. $[\alpha]_D^{20}$: –19.8 (c = 0.34, DMSO). IR (KBr) ν 3300, 1734, 1698, 1645 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 2.80–3.01 (m, 4H), 4.03–4.14 (m, 2H), 4.15–4.44 (m, 5H), 4.73 (dd, 1H, J = 13.7 Hz, J = 6.3 Hz), 5.14 (m, 1H), 6.08 (m, 1H), 6.77–6.86 (m, 2H), 7.02–7.51 (m, 20H), 7.68–7.72 (m, 4H).^{13}C NMR ($CDCl_3$, 100 MHz) δ 37.99, 38.38, 46.76, 47.20, 53.55, 56.03, 67.25, 67.31, 120.14, 120.22, 125.08, 125.14, 125.23, 127.22, 127.36, 127.88, 128.06, 128.09, 128.73, 128.87, 129.31, 129.51, 135.56, 136.31, 141.43, 143.43, 143.51, 143.84, 155.95, 170.42, 171.04. HRMS (ESI) $C_{47}H_{40}N_2O_5Na$ $[M+Na]^+$: calcd. 735.2829, found 735.2799.

Fmoc-L-Phe-L-Phe-L-Phe-OFm (Fmoc-FFF-OFm). White solid, mp 212°C. $[\alpha]_D^{20}$: –20.9 (c = 0.33, DMSO). IR (KBr) ν 3311, 3287, 1727, 1692, 1647 cm^{-1}. ^1H NMR

(DMSO-d_6, 400 MHz) δ 2.62–3.00 (m, 6H), 4.05–4.22 (m, 5H), 4.33–4.44 (m, 2H), 4.50 (dd, 1H, J = 13.5 Hz, J = 6.3 Hz), 4.56–4.65 (m, 1H), 7.10–7.43 (m, 23H), 7.50 (d, 1H, J = 8.7 Hz), 7.57–7.68 (m, 4H), 7.83–7.90 (m, 4H), 8.10 (d, 1H, J = 8.1 Hz), 8.59 (d, 1H, J = 7.2 Hz).^{13}C NMR (DMSO-d_6, 75 MHz, 60ºC) δ 36.49, 36.93, 37.51, 46.27, 46.53, 53.76, 54.33, 56.09, 65.65, 66.10, 120.06, 121.41, 125.24, 125.36, 126.17, 127.07, 127.31, 127.62, 127.78, 127.98, 128.34, 128.95, 128.98, 129.26, 129.40, 137.01, 137.44, 138.12, 139.43, 140.65, 140.79, 142.58, 143.49, 143.52, 143.70, 143.79, 155.63, 170.46, 171.30, 172.59. HRMS (ESI) $C_{56}H_{49}N_3O_6Na$ $[M+Na]^+$: calcd. 882.3514, found 882.3510.

Fmoc-L-Phe-L-Phe-L-Phe-L-Phe-OFm (Fmoc-FFFF-OFm). White solid, mp 243°C. $[\alpha]_D^{20}$: –10.5 (c = 0.36, DMSO). IR (KBr) ν 3398, 3278, 1740, 1689, 1668, 1639 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.61–3.00 (m, 8H), 4.03–4.22 (m, 5H), 4.31–4.44 (m, 2H), 4.48–4.54 (m, 2H), 4.56–4.62 (m, 1H), 7.00–7.43 (m, 28H), 7.52 (d, 1H, J = 8.8 Hz), 7.57–7.68 (m, 4H), 7.82–7.90 (m, 4H), 7.99 (d, 1H, J = 7.5 Hz), 8.17 (d, 1H, J = 7.8 Hz), 8.57 (d, 1H, J = 7.5 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, 60ºC) δ 36.45, 37.23, 37.37, 37.43, 46.13, 46.43, 53.35, 53.43, 53.48, 55.86, 65.57, 65.93, 119.73, 119.79, 121.04, 124.84, 124.88, 124.96, 125.88, 125.92, 126.29, 126.77, 126.89, 127.32, 127.47, 127.64, 127.71, 128.03, 128.66, 128.87, 128.90, 136.76, 137.23, 137.27, 137.78, 140.44, 140.53, 143.29, 143.50, 155.37, 170.33, 170.70, 170.82. HRMS (ESI) $C_{65}H_{58}N_4O_7Na$ $[M+Na]^+$: calcd. 1029.4198, found 1029.4164.

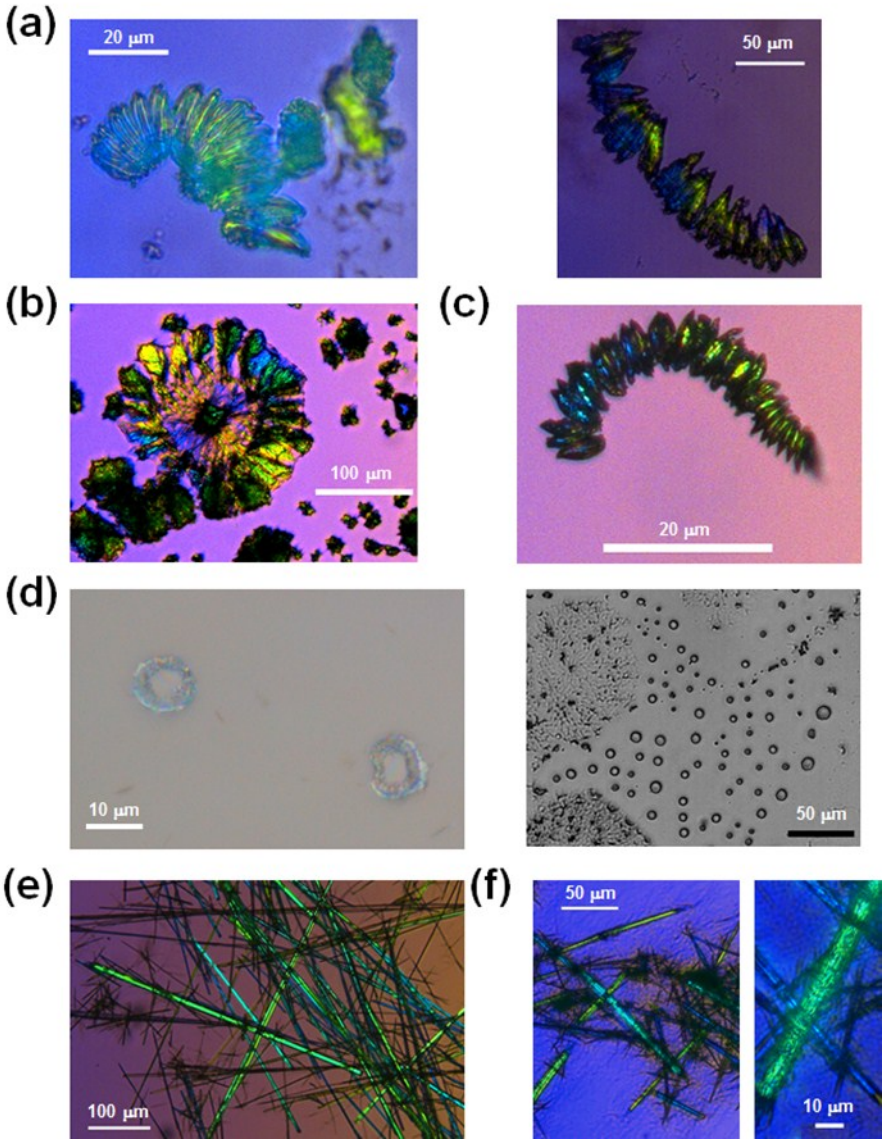

Figure S1. Representative optical micrographs illustrating the influence of the co-solvent, the temperature and the peptide concentration in the morphology of Fmoc-FF-OFm self-assemblies: (a) Stacked braids obtained in 11:14 HFIP:water at 4 ºC using a peptide concentration of 2.2 mg/mL and sonicating the solution; (b) Braids stacked in a spherulitic-like structure obtained in 24:1 HFIP:water at 4 ºC using a peptide concentration of 4.8 mg/mL; (c) Stacked braids obtained in 1:49 HFIP:MeOH at 4ºC using a peptide concentration of 0.087 mg/mL; (d) Doughnut-like microstructures obtained in 4:1 HFIP:water at 4 ºC using peptide concentrations of 4 mg/mL; (e) Tubes obtained in 4:1 HFIP:^{i}PrOH at room temperature using a peptide concentration of 4 mg/mL; and (f) Tubes obtained in 4:1 HFIP:acetone at 4 ºC using a peptide concentration of 3.44 mg/mL.

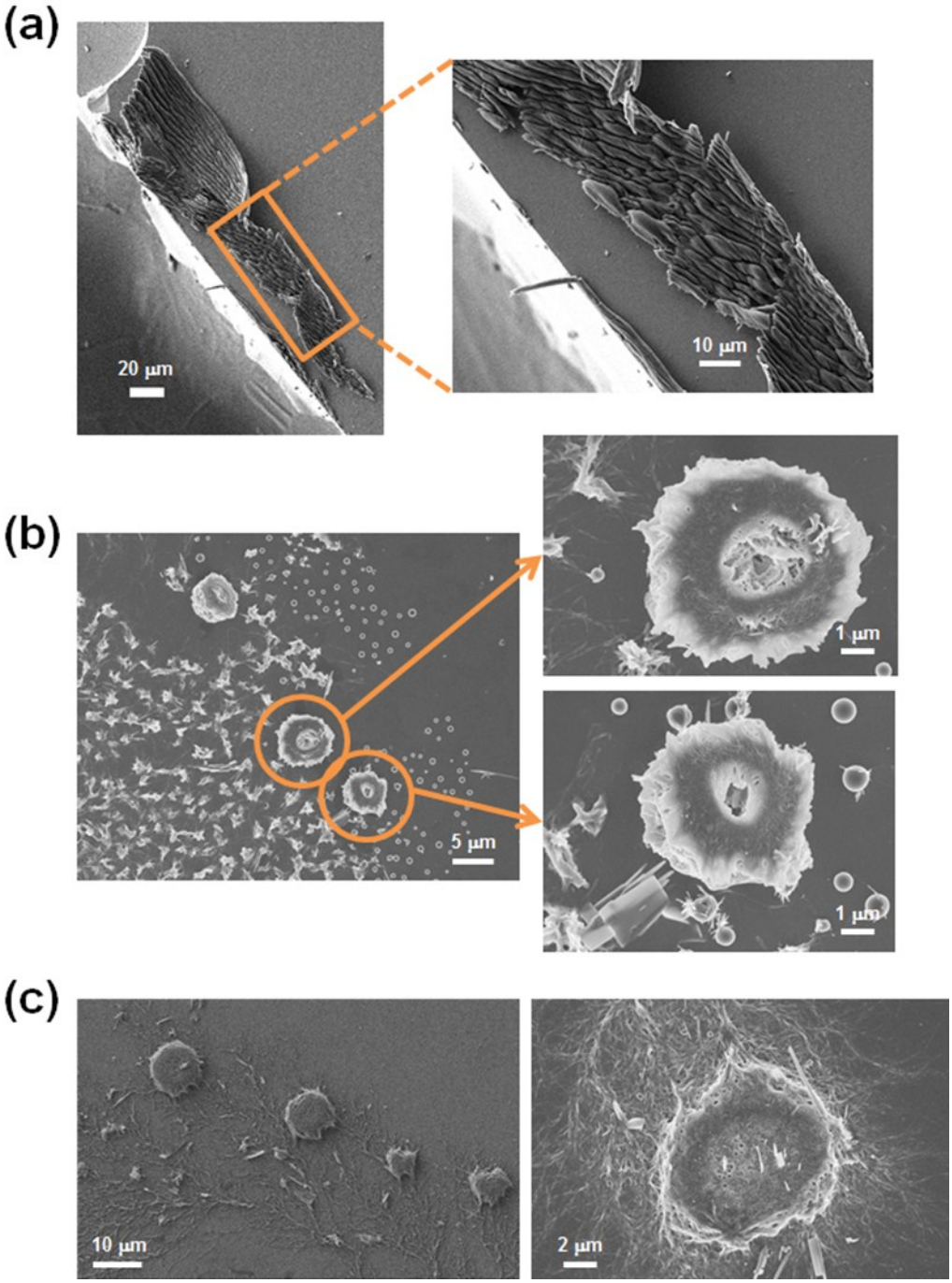

Figure S2. For Fmoc-FF-OFm, representative SEM micrographs of: (a) Stacked braids obtained in 1:99 HFIP:MeOH at 4ºC using a peptide concentration of 0.043 mg/mL; (b) Doughnuts-like microstructures obtained in 4:1 HFIP:water at 4 ºC using a peptide concentration of 4 mg/mL; and (c) Microstructures obtained in 4:1 HFIP:water at 4 ºC using a peptide concentration of 4 mg/mL that precede the formation of doughnuts-like assemblies.

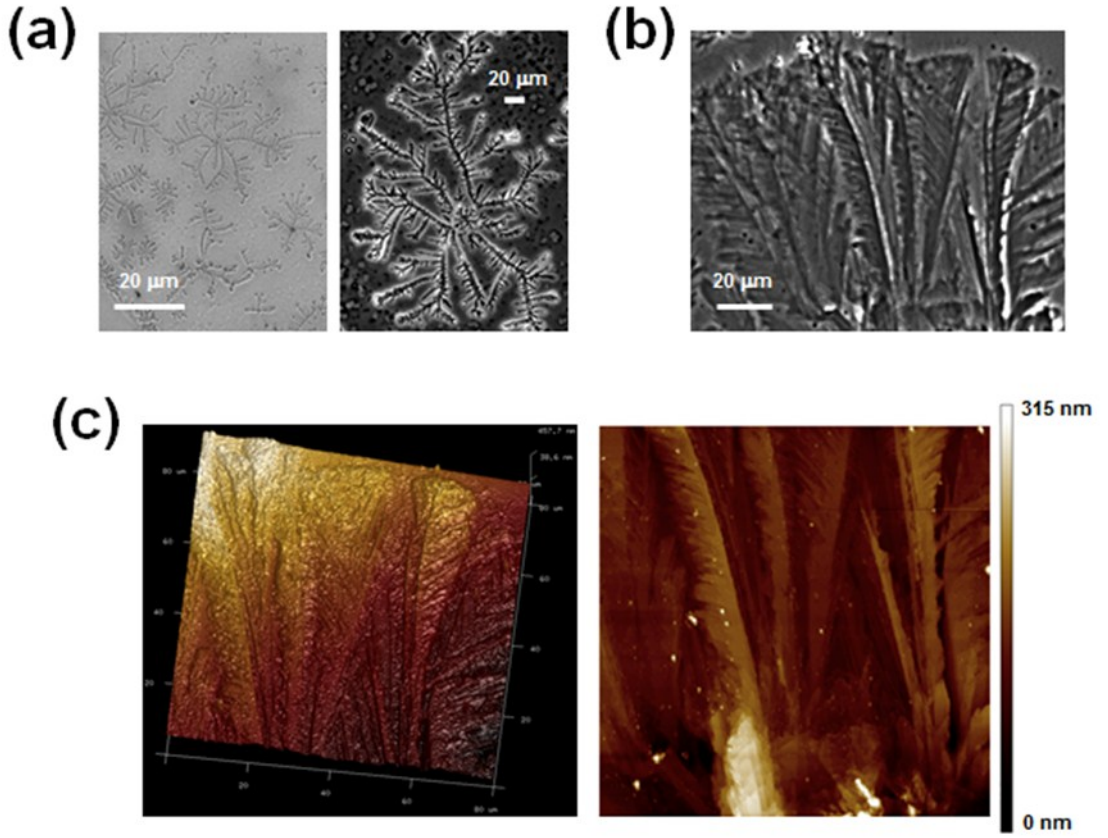

Figure S3. For Fmoc-FF-OFm assemblies, optical micrographs and AFM images of: tree-like dendritic microstructures obtained after sonication of the following solutions: (a) 2:3 HFIP:water at 4 ºC using a peptide concentration of 2 mg/mL; (b) and (c) 1:4 HFIP:MeOH at 4 ºC using a peptide concentration of 0.087 mg/mL. Windows of the AFM images displayed in (c) are 80×80 μm^2.

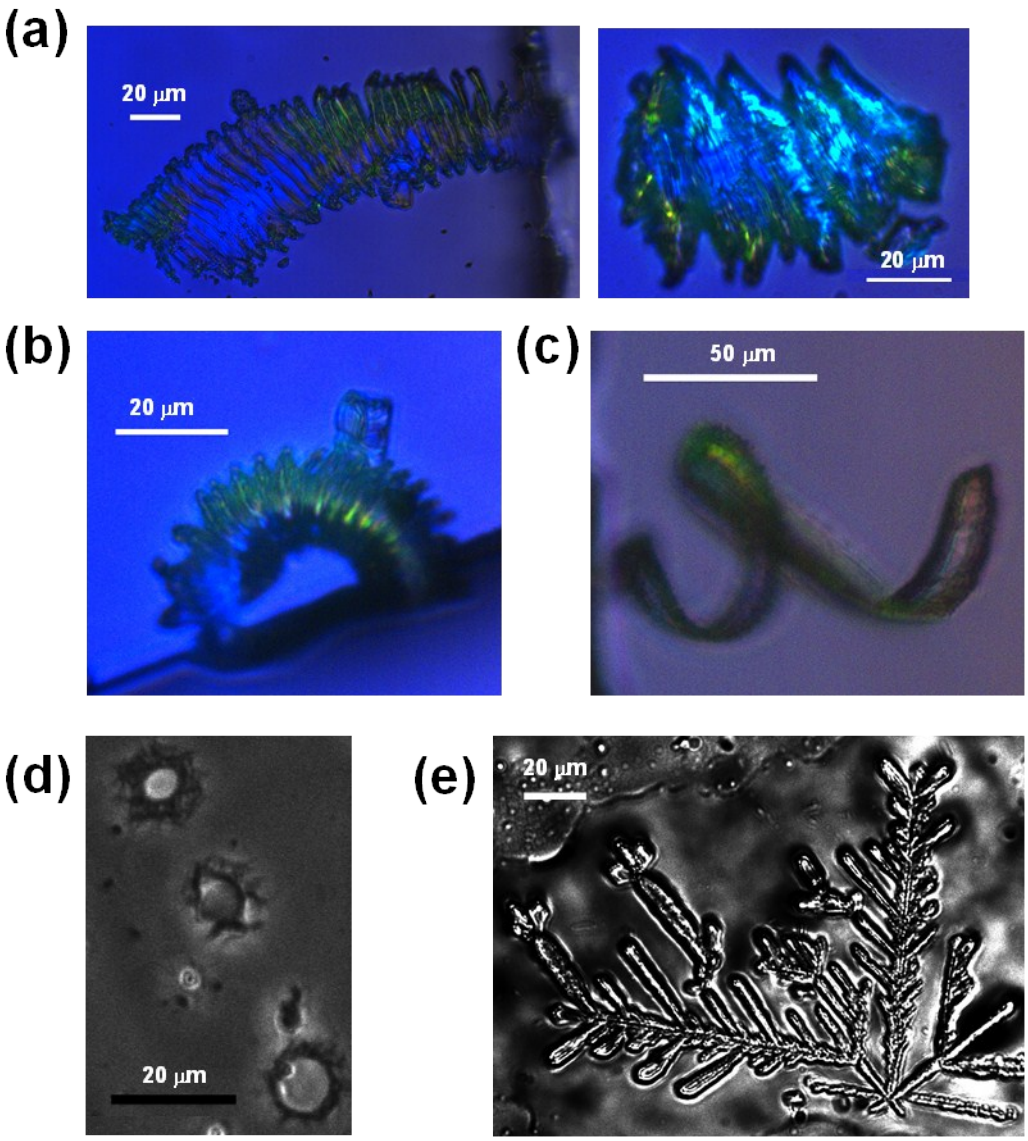

Figure S4. Representative optical micrographs illustrating the influence of the co-solvent, the temperature and the peptide concentration in the morphology of Fmoc-FFF-OFm self-assemblies: (a) Stacked braids obtained in 1:4 HFIP:water at 4 ºC using a peptide concentration of 1 mg/mL. The stacking between ultra-thin plates is reflected in the magnified images at the right; (b) Stacked platelets obtained in 1:49 HFIP:EtOH at 4 ºC using a peptide concentration of 0.1 mg/mL; (c) Corkscrew-like morphology obtained in 1:19 HFIP:EtOH at 4 ºC using a peptide concentration of 2 mg/mL; (d) Spherulitic craters obtained in 4:1 HFIP:water at 4 ºC using a peptide concentration of 4 mg/mL; (e) Dendritic-like structures obtained in 1:19 HFIP:EtOH at 4 ºC using a peptide concentration of 0.25 mg/mL.

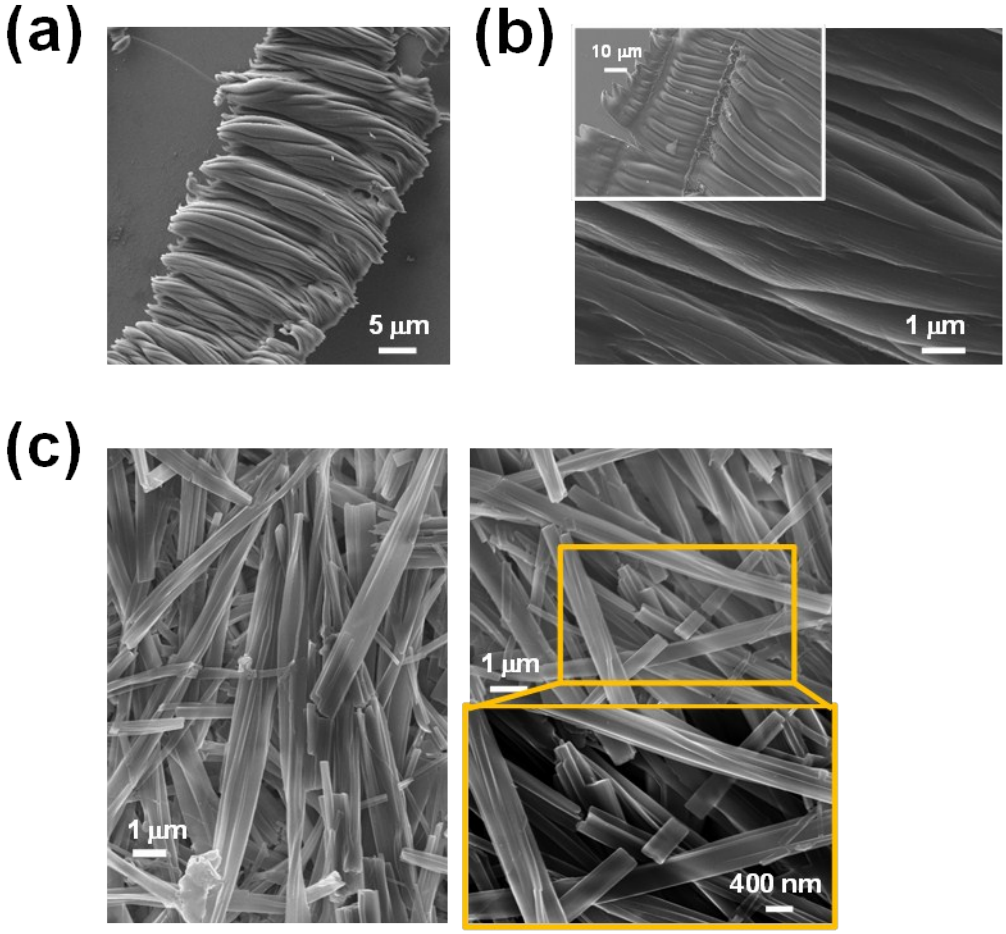

Figure S5. SEM micrographs for the following Fmoc-FFF-OFm assemblies: (a) Stacked braids obtained in 1:19 HFIP:EtOH at 4 ºC using a peptide concentration of 0.25 mg/mL; (b) Stacked braids obtained in 1:9 HFIP:^{i}PrOH at 4 ºC using a peptide concentration of 0.5 mg/mL; (c) Submicrotubes formed bundling nanotubes obtained in 38:11 DMF:MeOH at room temperature using a peptide concentration of 6.1 mg/mL.

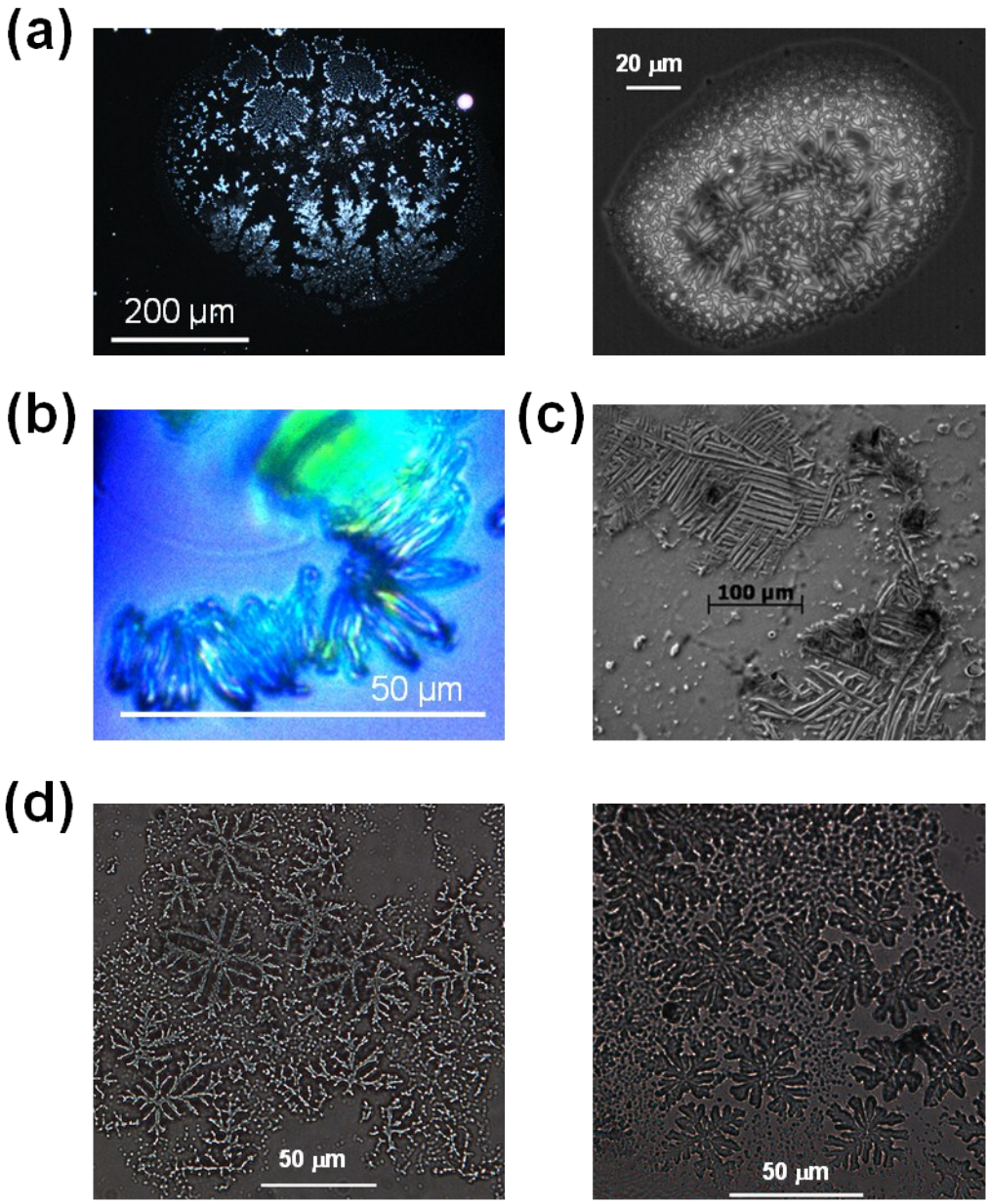

Figure S6. Representative optical micrographs illustrating the influence of the co-solvent, the temperature and the peptide concentration in the morphology of Fmoc-FFFF-OFm self-assemblies: (a) Ultra-thin dendritic structures obtained in 1:24 HFIP:water at 4 ºC using a peptide concentration of 0.2 mg/mL and after 20 min sonication. (b) Stacked braids obtained in 1:9 HFIP:EtOH at 4 ºC using a peptide concentration of 0.5 mg/mL; (c) Soft and meta-stable dendritic architectures obtained in 1:4 HFIP:EtOH at 4 ºC using a peptide concentration of 1 mg/mL; (d) Dendritic morphologies obtained in 1:4 HFIP:EtOH at room temperature using a peptide concentration of 1 mg/mL.

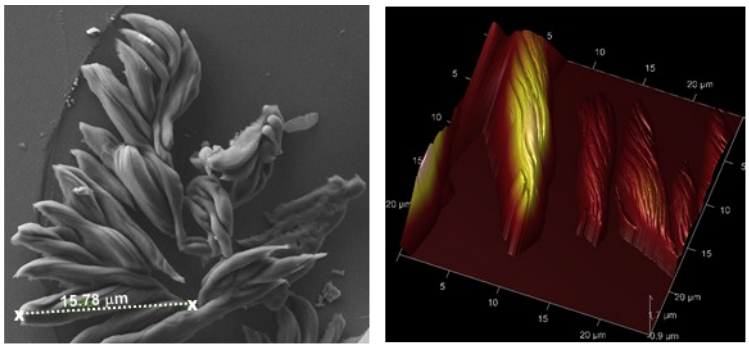

Figure S7. SEM micrographs and AFM images of Fmoc-FFFF-OFm assembled in stacked breads. This morphology is obtained in 1:99 HFIP:EtOH at 4 ºC using a peptide concentration of 0.05 mg/mL (25×25 μm^2 window for 3D AFM image).

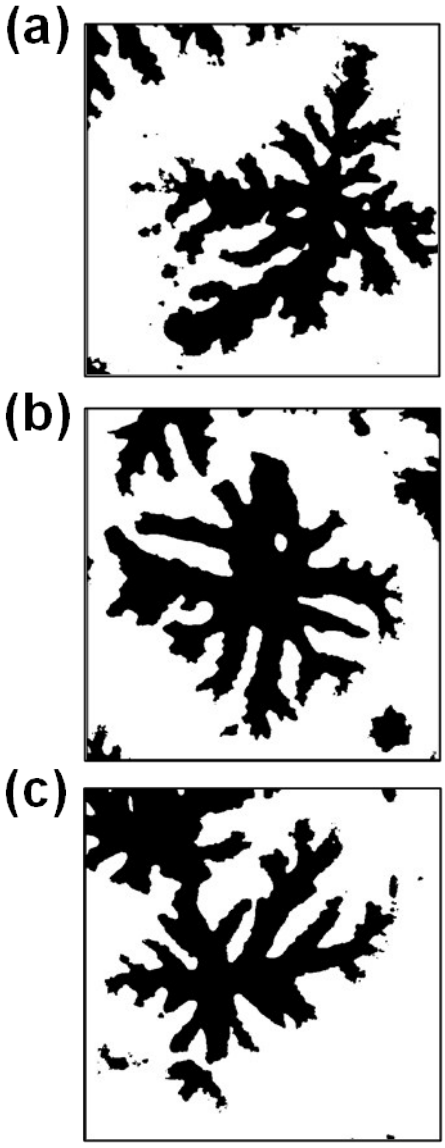

Figure S8. Black and white binary images of dendritic microstructures converted from 2D AFM images displayed in Figure 6, which were obtained for Fmoc-FFFF-OFm concentrations of (a) 0.5 mg/mL; (b) 1 mg/mL; and (c) 2 mg/mL.

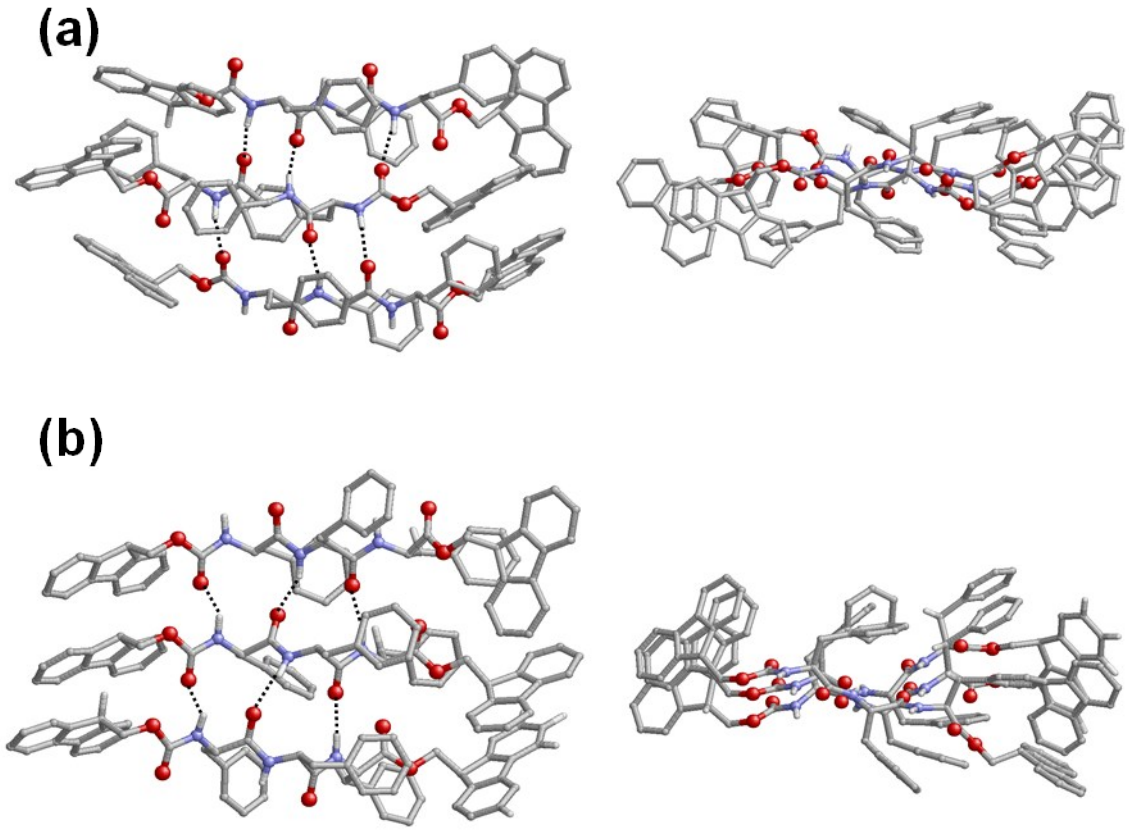

Figure S9. Lowest energy (a) antiparallel and (b) parallel assemblies predicted for three Fmoc-FFF-OFm strands using M06L/6-31G(d) calculations. Both lateral (left) and top (right) views are provided. Aliphatic and aromatic hydrogen atoms have been omitted for clarity while intermolecular hydrogen bonds in the β-sheet are represented by dashed lines. Relevant energetic information for these two assemblies is displayed in Table 1.

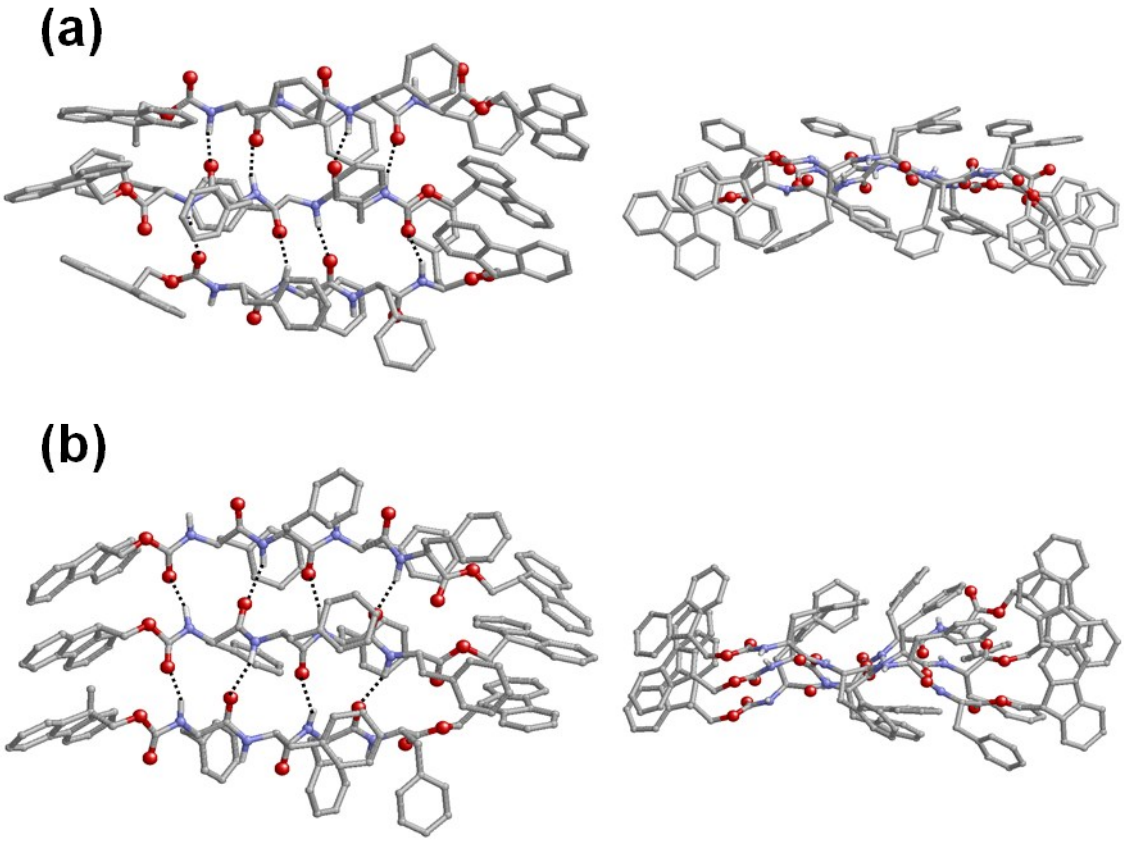

Figure S10. Lowest energy (a) antiparallel and (b) parallel assemblies predicted for three Fmoc-FFFF-OFm strands using M06L/6-31G(d) calculations. Both lateral (left) and top (right) views are provided. Aliphatic and aromatic hydrogen atoms have been omitted for clarity while intermolecular hydrogen bonds in the β-sheet are represented by dashed lines. Relevant energetic information for these two assemblies is displayed in Table 1.

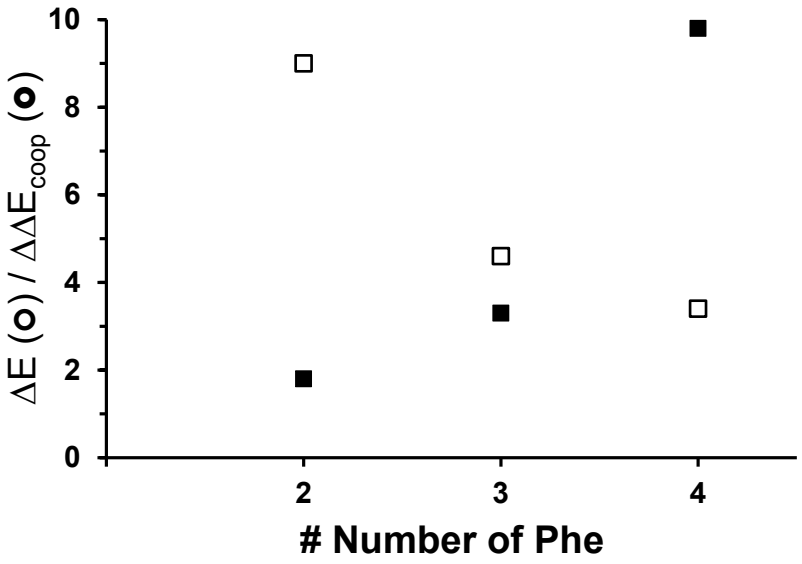

Figure S11. Relative energy between the parallel and antiparallel arrangements (ΔE in kcal/mol; filled squares) and difference between the cooperative energy of the parallel and antiparallel arrangements ($\Delta\Delta E_{coop}$ in kcal/mol, empty squares) against the number of Phe residues for the studied peptides.

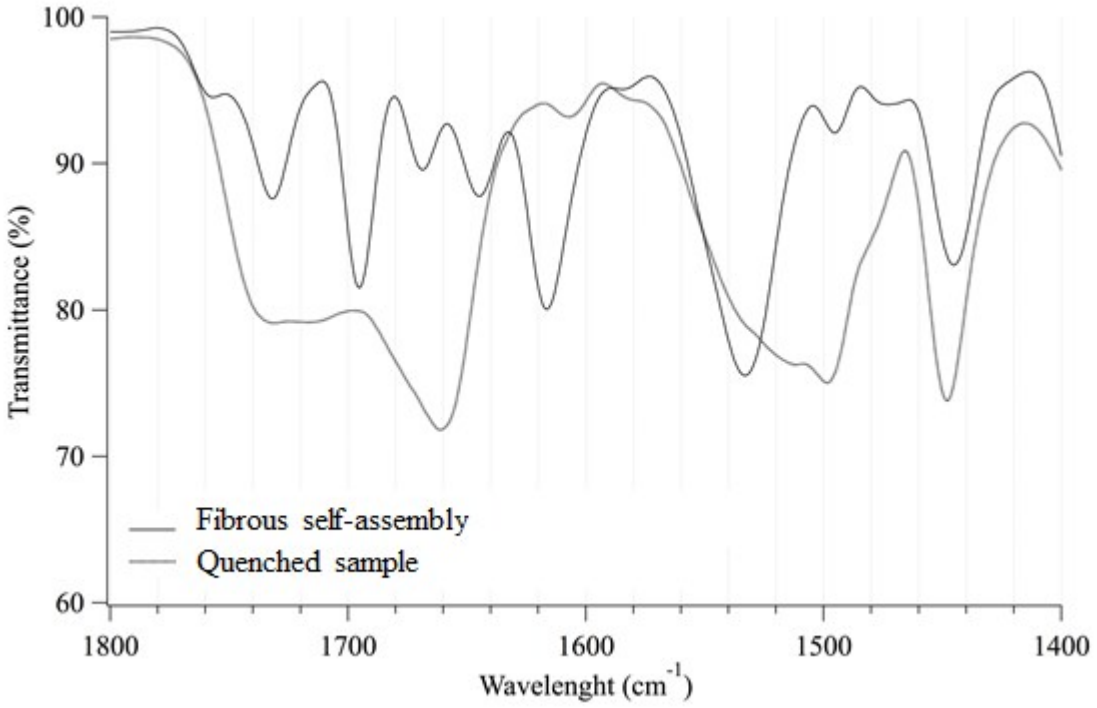

Figure S12. FTIR spectra of self-assembled and quenched Fmoc-FF-Fmoc samples.

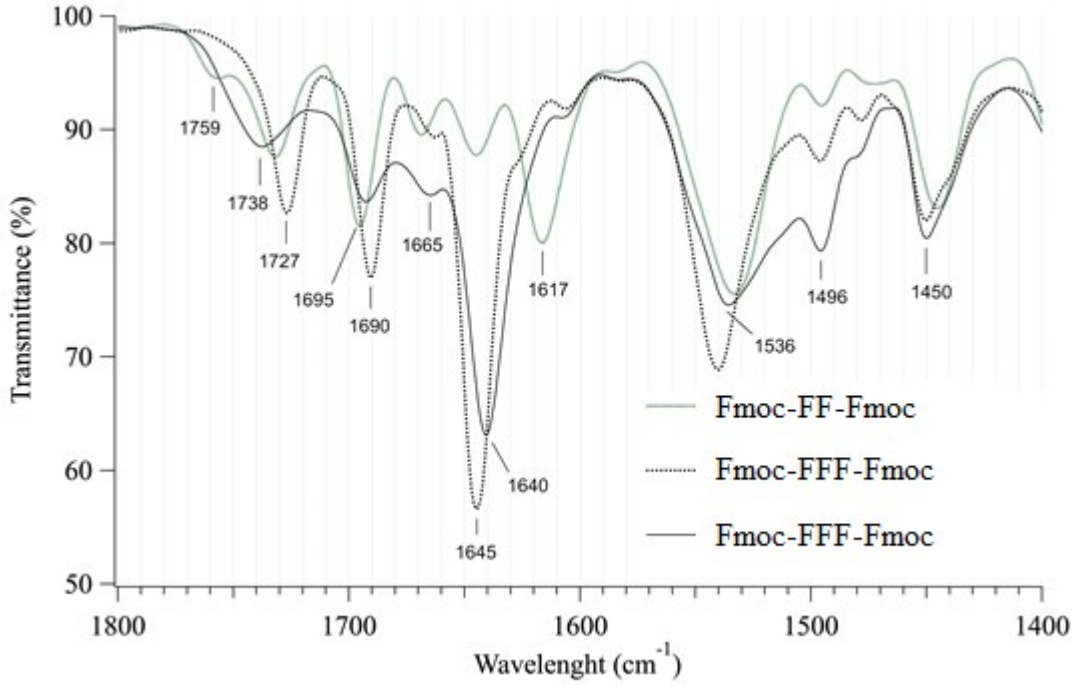

Figure S13. FTIR spectra of self-assembled Fmoc-FF-Fmoc, Fmoc-FFF-Fmoc and Fmoc-FFFF-Fmoc samples.

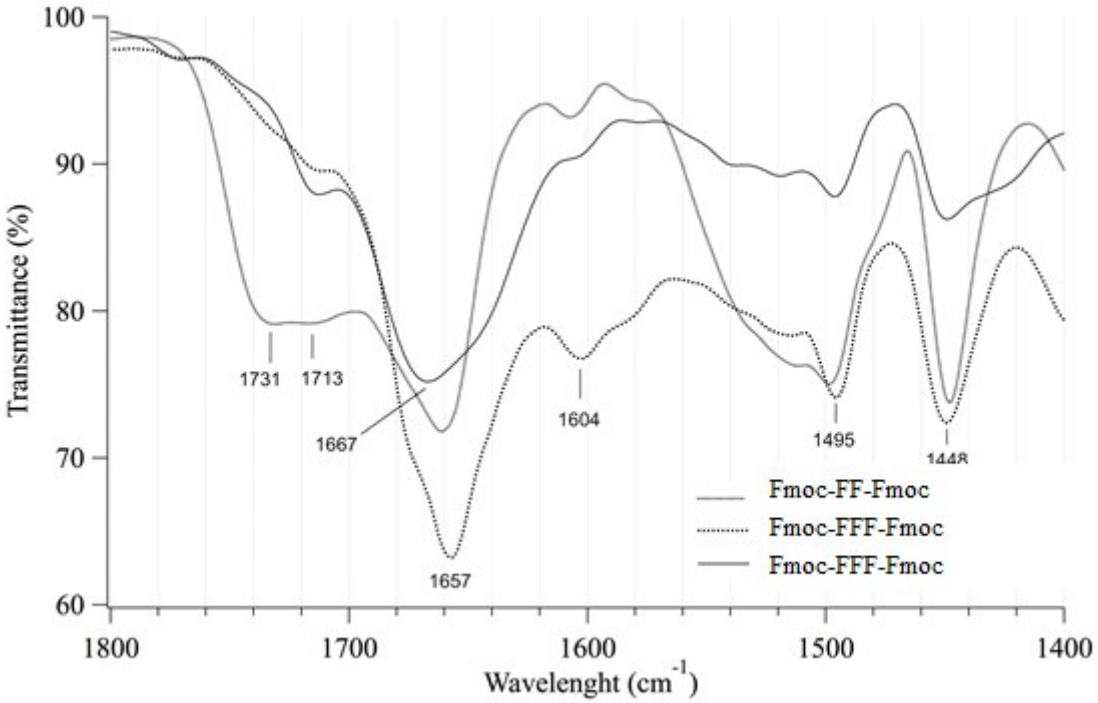

Figure S14. FTIR spectra of quenched Fmoc-FF-Fmoc, Fmoc-FFF-Fmoc and Fmoc-FFFF-Fmoc samples.