Supplementary Information

Spin Coating Mediated Morphology Modulation in Self Assembly of Peptides

Nandini Bhandaru^{†#*}, Gagandeep Kaur[‡], Apurva Panjla^{‡,} and Sandeep Verma^{†‡*}

[†]Center for Nanosciences and [‡]Department of Chemistry, Indian Institute of Technology-Kanpur, Kanpur-208016, Uttar Pradesh, India [#]Present address: Department of Chemical Engineering, BITS Pilani, Hyderabad, Telangana 500078, India

Corresponding authors: <u>*nandini@hyderabad.bits-pilani.ac.in</u>, <u>*sverma@iitk.ac.in</u>

Table 1: *The name and the molecular structure of the diphenylalanine peptide analogues used in this work.*

Molecular structure	Code
	Boc-FF-OMe
	NH ₂ -FF-OH
	Boc-FF-OH
	NH ₂ -FF-OMe

Synthesis of Boc-FF-OMe (N-tert-butyloxycarbonyl-di-L-Phenylalanine methyl ester) -A solution of compound N-tert-butyloxycarbonyl L-Phenylalanine (3g, 1eq.) in dry DCM (50 mL) was cooled to 0 °C, and 1-hydroxybenzotriazole (1.9g, 1.3 eq) was added, followed by 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (2.82 g, 1.2 eq). After half an hour, a clear solution is obtained, then L-Phenylalanine methyl ester hydrochloride salt (2.68 g, 1.1 eq.) was added to it, followed by N-methylmorpholine (1.49 ml, 1.2 eq.). The reaction mixture was stirred for 12 h. The crude compound was acidified under an ice-cold condition with 1N HCl to pH 2-3 and extracted with (3 x 15 mL) dichloromethane. The organic layer was washed with 10% NaHCO₃ solution (3 x 10mL), and finally with saturated brine solution (2 x 10 mL), followed by drying of the organic layer over anhydrous sodium sulphate. Dichloromethane was evaporated to get the crude compound (2.2 g), which was further purified with a silica gel column (0-4 % CH₃OH gradient in CH₂Cl₂) to give protected compound N-tertbutyloxycarbonyl di-L-Phenylalanine methyl ester as a white solid, Rf value is 0.65 (10% methanol in dichloromethane, 4.1 g, 80.87 % yield). ¹H NMR: (400 MHz CDCl₃, 25°C, TMS) δ(ppm) 1.32 (s, ^{*t*}Boc 9H); 2.97 (m 4H), 3.6 (s, 3H), 4.70 (m, 1H), 4.85 (m, 1H), 6.19 (bs, 1H), 6.91 (bs, 1H, -NH), 7.11- 7.23 (m, 10H, aromatic H). ¹³C NMR (100 MHz, CDCl₃, 25°C, TMS) δ (ppm) = 28.80, 37.84, 38.07, 51.0, 55.40, 55.83, 80.06, 126.78, 126.94, 128.27, 128.37, 129.20, 129.36, 136.60, 136.75, 155.20, 169.98, 172.64.

Synthesis of Boc-FF-OH (N-tert-butyloxycarbonyl-di-L-Phenylalanine) - To a stirring solution of N-tert-butyloxycarbonyl-di-L-Phenylalanine methyl ester (1g, 2.3 mmol) in 10 ml methanol, sodium hydroxide (2.5 eq.) solution in 1ml water was added. The solution was kept for stirring for 2 hours. The crude compound was dissolved in methanol and passed through cation exchange resin.

Synthesis of NH₂-FF-OMe (L-Phenylalanine-L-Phenylalanine methyl ester) - To a stirring solution of N-tert-butyloxycarbonyl-di-L-Phenylalanine (1g, 3 mmol) in 10 ml dichloromethane, triflouroacetic acid was added. The solution was kept for stirring for 2 hours. The solvent was then evaporated under vacuum and washed with diethyl ether resulting in white solid. The crude solid was then dissolved in methanol and passed through anion exchange resin and evaporated under reduced pressure to obtain pure white solid.

Synthesis of NH₂-FF-OH (L-Phenylalanine-L-Phenylalanine) - To a stirring solution N-tert-butyloxycarbonyl-di-L-Phenylalanine (500 mg, 4.2 mmol) in 10 ml dichloromethane, triflouroacetic acid was added. The solution was kept for stirring for 2 hours. The solvent was

then evaporated under vacuum and washed with diethyl ether resulting in white solid. The crude solid was then dissolved in methanol and passed through anion exchange resin and evaporated under reduced pressure to obtain pure white solid.



Fig. S1: Optical microscope images of Boc-FF-OMe deposited on a glass substrate by dropcasting (0 RPM) of 100 μ l for 1 mg/ml solution with slow drying at room temperature. The images were taken at different locations of the footprint of the drop. The scale bar in each image corresponds to 100 μ m.



Fig. S2: Optical microscope images of Boc-FF-OMe deposited on a glass substrate by spin coating at 500 RPM, $C_P = 1$ mg/ml. The images were taken at different locations on the substrate. The scale bar in each image corresponds to 50 μ m.



Fig. S3: *XRD spectrum of the peptide assemblies on a glass substrate: (A) Boc-FF-OMe peptide drop cast and spin coated at different RPM. (B) NH*₂-*FF-OH peptide fibers and flakes.*



Fig. S4: SEM images of Boc-FF-OMe peptide assemblies obtained from spin-coating a solution with $C_p = 1$ mg/ml at different spinning speeds. (A) Drop casting of the solution with slow drying at room temperature results in long fibrils with random distribution. (B) 500 RPM, short fibrils. (C) 1500 RPM, peptide globules. The scale bar in each image corresponds to 1 μ m.