Self-assembly of diphenylalanine backbone homologues and their combination with functionalized carbon nanotubes

Bhimareddy Dinesh,[a] Marco A. Squillaci,[b] Cécilia Ménard-Moyon,[a] Paolo Samorì,[b] Alberto Bianco*[a]

Electronic Supplementary Information

Experimental details

All reagents were obtained from commercial suppliers and used without further purification. Purified multi-walled carbon nanotubes (MWCNT) were purchased from Nanostructured & Amorphous Materials Inc. (Houston, USA). Regular MWCNTs used in this study were 95% pure, stock No. 1240XH. Outer average diameter was between 20 and 30 nm, and length between 0.5 and 2 µm before oxidative treatment. Transmission electron microscopy (TEM) was performed on a Hitachi600 microscope with an accelerating voltage of 75 kV and at different magnifications. Pictures were taken using a CCD high-resolution camera AMT. The thermogravimetric analyses (TGA) were performed using a Mettler Toledo TGA 1 instrument using platinum pans and lids with a ramp of 10 °C·min^{-1} under N_2 with a flow rate of 50 mL·min^{-1} from 100 to 900 °C.

The *N*-Boc protected β and γ diphenylalanines (Boc-β^3(*R*)Phe-β^3(*R*)Phe-OH (**1**) and Boc-γ^4(*R*)Phe-γ^4(*R*)Phe-OH (**2**)) were synthesized as previously reported.[1] The MWCNTs were first oxidized by acid treatment,[2] then treated with Boc-monoprotected diaminotriethyleneglycol (TEG) followed by deprotection in acidic conditions to have ammonium-functionalized MWCNTs (MWCNT-TEG-NH_3^+). The MWCNT-TEG-NH_3^+ were derivatized with succinic anhydride. The peptides were conjugated to respective N- and C-terminals. The deprotections were done followed by neutralizations to obtain MWCNT-TEG-β^3(*R*)Phe-β^3(*R*)Phe-NH_2 (**3**), MWCNT-TEG-β^3(*R*)Phe-β^3(*R*)Phe-OH (**4**) and MWCNT-TEG-γ^4(*R*)Phe-γ^4*R*)Phe-NH_2 (**5**). The peptide loadings were determined by TGA.

To avoid any pre-aggregation, fresh stock solutions of the peptides were prepared for each experiment. The fresh stock solutions of **1** and **2** were prepared by dissolving the lyophilized forms of the peptides in 1,1,1,3,3,3-hexafluoro-2-propanol (HFP, Sigma-Aldrich) to a concentration of 100 mg/mL. For the self-assembly of the individual peptides, the beta and

gamma diphenylalanine peptides were diluted in 50% ethanol to several different concentrations. The CNT-peptide conjugates were dispersed in 50% ethanol by sonicating in a water bath for few minutes until visibly uniform solution. It should be noted that due to the highly volatile nature of the solvent, the experiments are sensitive to small changes in the concentration of the peptides.

Transmission Electron Microscopy (TEM). A drop containing 6 µL of the peptides solution, incubated at room temperature (RT) over 10 h was placed on 300-mesh copper grid, covered by carbon-stabilized Formvar film (Electron Microscopy Sciences). The peptide solutions were allowed to dry on the grid at RT. After the solvent evaporation, the pH-dependent and CNT-peptide conjugate sample grids were immersed in water wells for about 20 min to remove salts.
The CNT samples were dispersed in water by sonication, and the dispersions were drop-casted onto carbon-coated copper grids which were subsequently dried. The TEM analyses were performed using a Hitachi H7500 microscope (Tokyo, Japan) with an accelerating voltage of 80 kV, equipped with an AMT Hamamatsu camera (Tokyo, Japan). Length and diameter measurements were performed using NIH ImageJ software

Scanning Electron Microscopy (SEM). A drop containing 20 µL of the peptides solution was deposited on micro cover slips or thin glass slides kept over aluminum stub and dried at RT for 12 h. A thin layer of gold-platinum was sputtered on the samples using a Balzers SCD030 Coater (Bal-Tec, Leica Microsystems). SEM images were taken using a Hitachi S800 (Hitachi, Tokyo, Japan) operating at 5 kV.

High Resolution Scanning Electron Microscopy (HR-SEM). 100 mL of the peptide **1** at 25 mg/mL and 40 mL of the peptide **2** at 40 mL solutions were placed on ultraflat P-doped silicon with native oxide layer. The dried peptides images were recorded using an FEI Quanta 250 FEG instrument operating a 5 kV.

Atomic Force Microscopy (AFM). The samples were deposited on freshly cleaved mica substrate to disperse the aggregates and avoid the formation of too thick structures and imaged by Veeco Dimension 3100 AFM running with Nanoscope IV controller, under ambient conditions in tapping mode.

Fourier Transform Infrared Spectroscopy (FT-IR). All the infrared spectra were recorded in PerkinElmer Spectrum spectrometer with deuterated triglycine sulfate (DTGS) detector using the KBr disk technique.

Supplementary figures

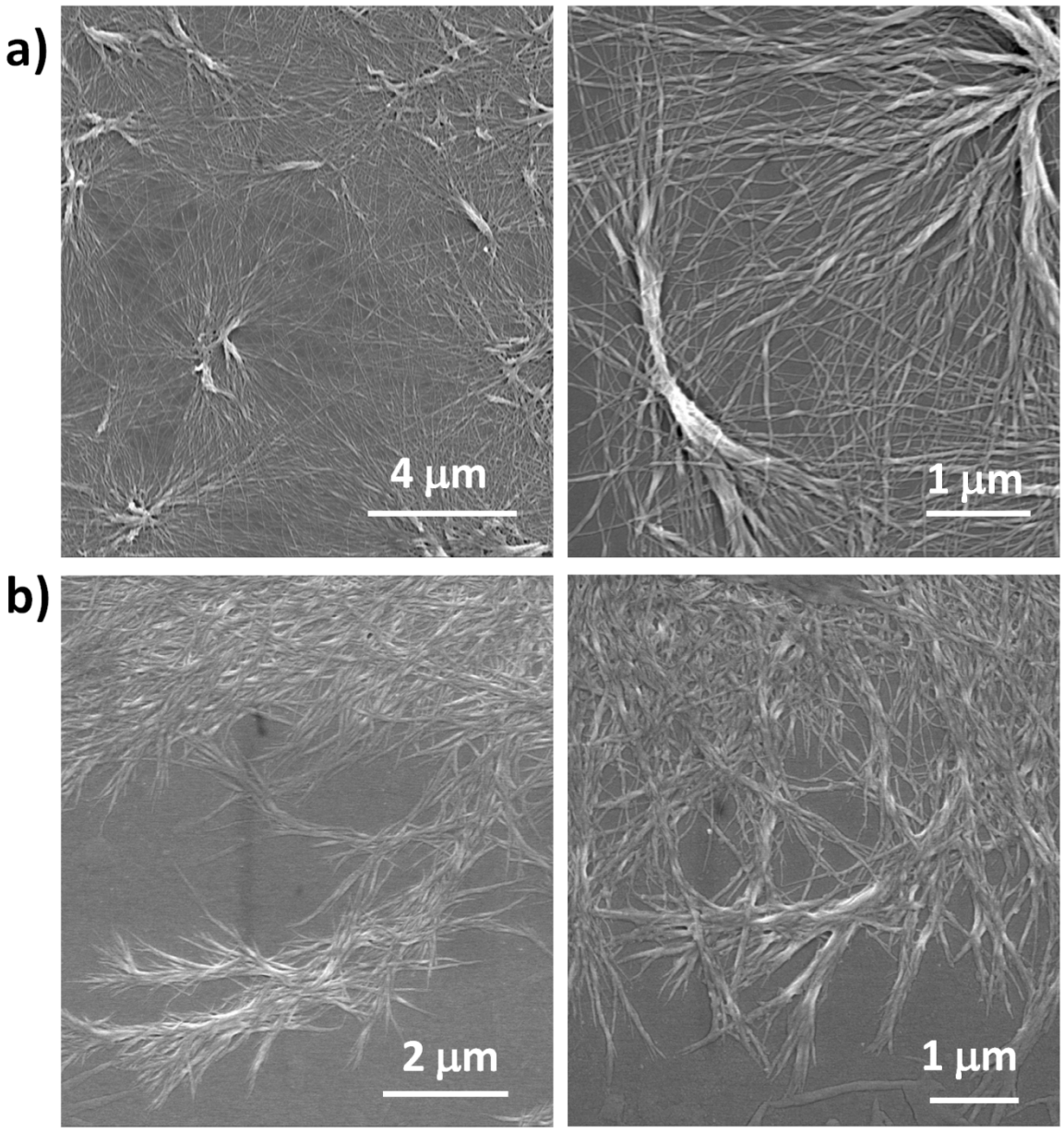

Figure S1: SEM images of aged assemblies of peptide **1** (a) and **2** (b) analyzed after 30 days. The morphologies of both peptides remain unchanged.

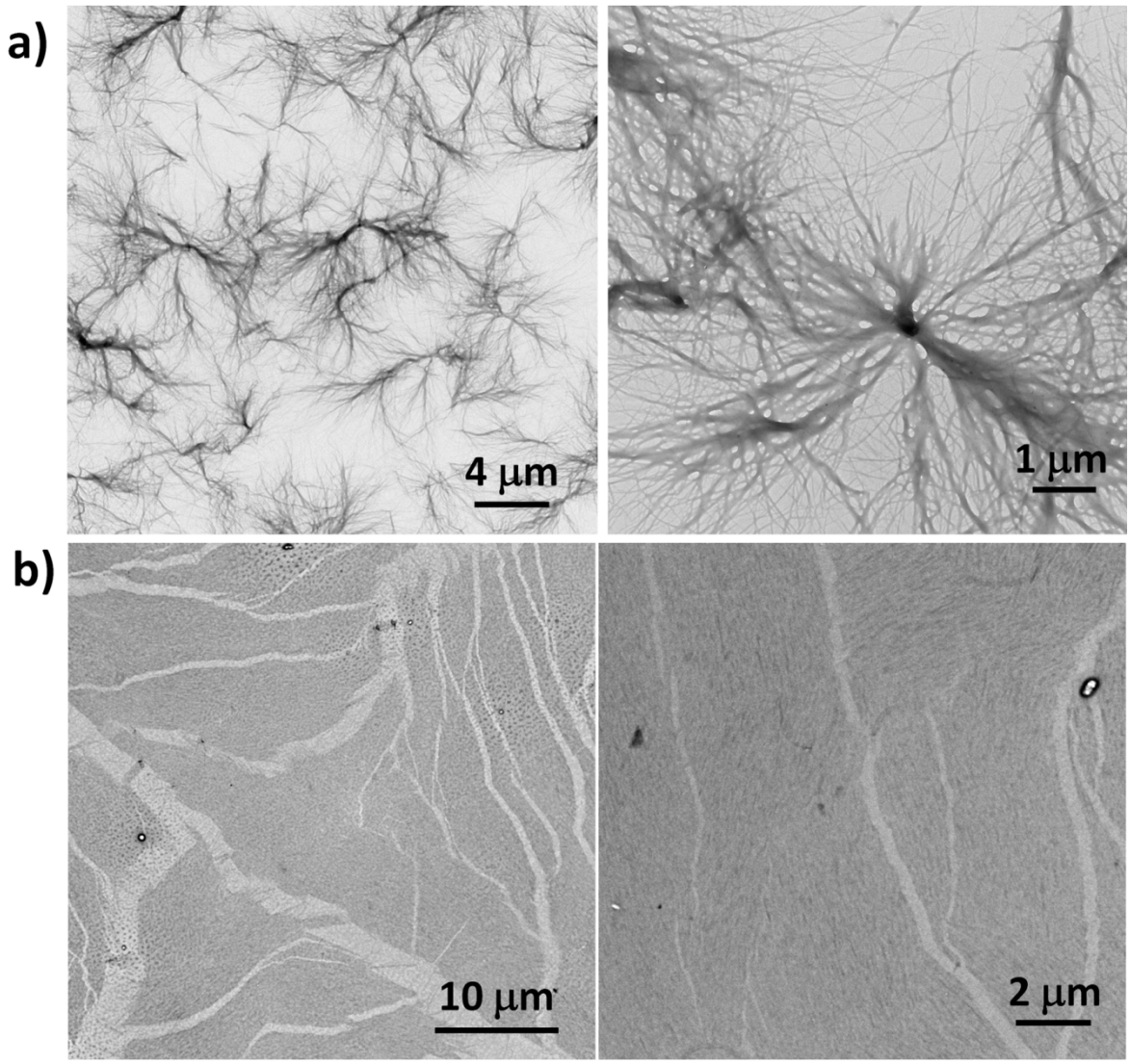

Figure S2: TEM images of the peptides **1** (a) and **2** (b). The peptides were immediately drop cast upon mixing in methanol and allowed to air dry

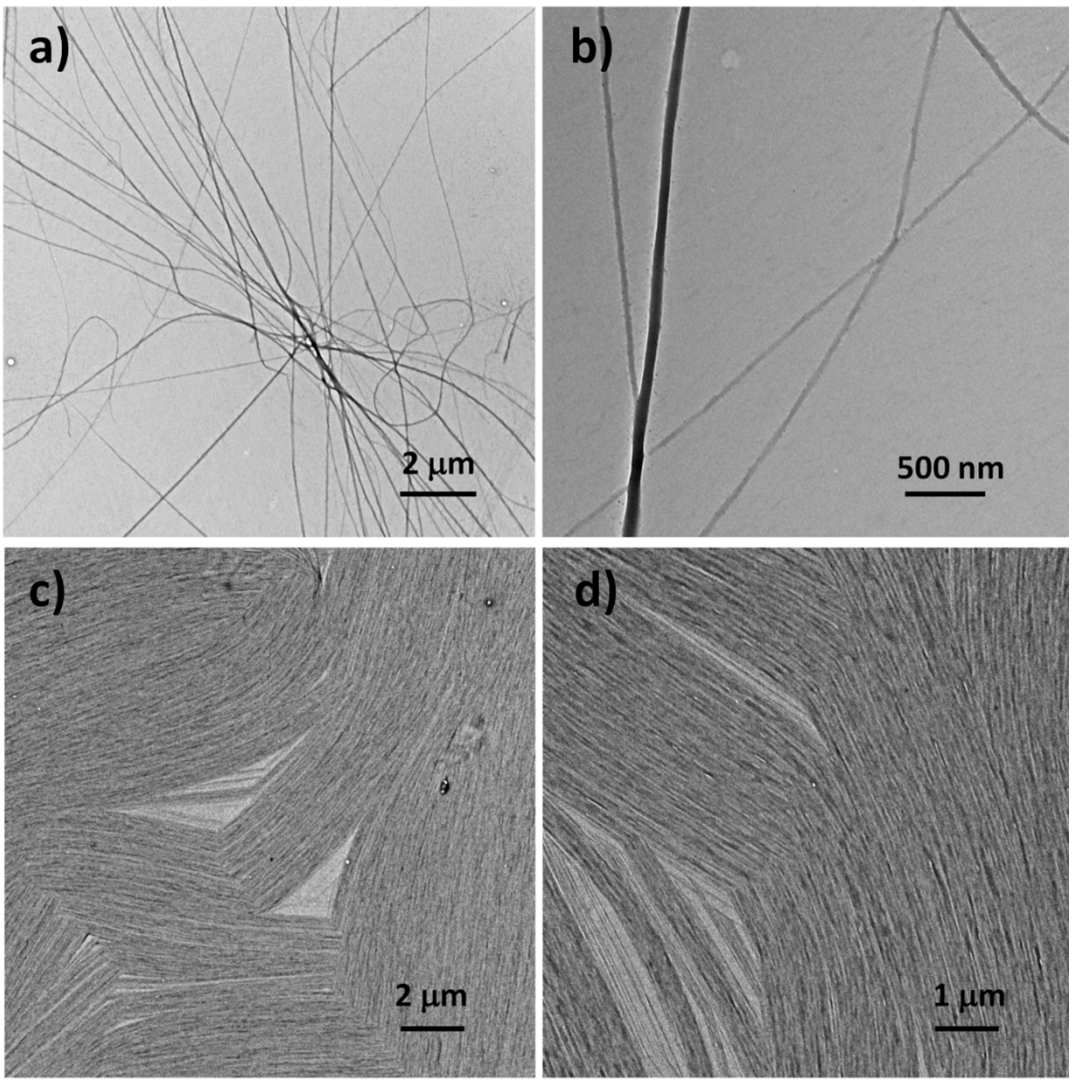

Figure S3: TEM images of nanofibrils of **1** (a, b) and **2** (c, d) formed in water at 1 mg/mL. The fibrillar structures indicate the independence of the self-assembly changing the solvent from organic media to aqueous solutions.

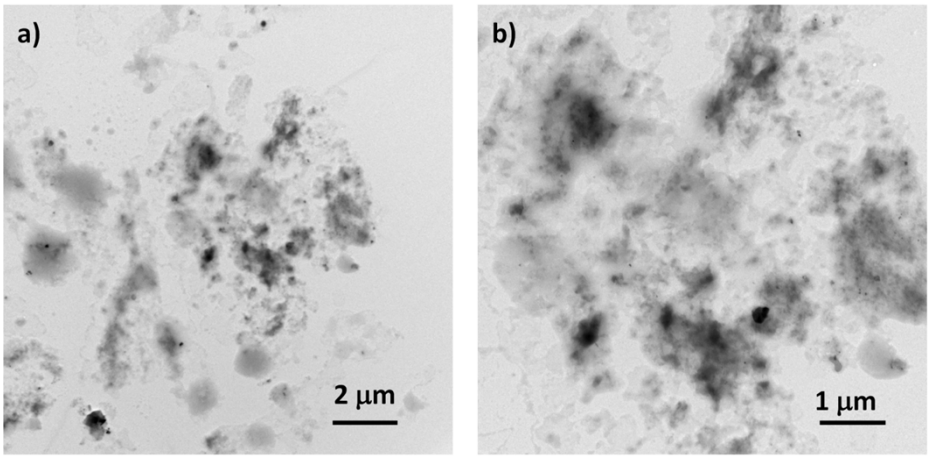

Figure S4: TEM images of β^3(*R*)Phe-β^3(*R*)Phe showing the complete absence of fibrillar structures support the importance of N-capping (Boc-protection) to promote their formation. Panel (b) is a magnification of panel (a).

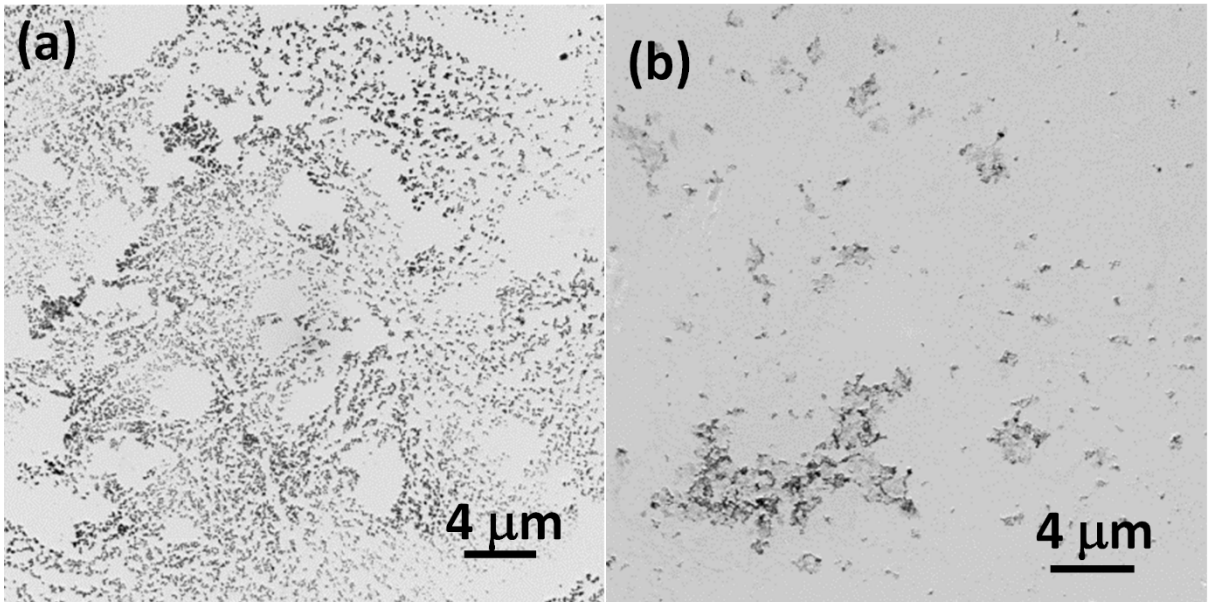

Figure S5 TEM images of peptides **1** (a) and **2** (b) after changing pH.

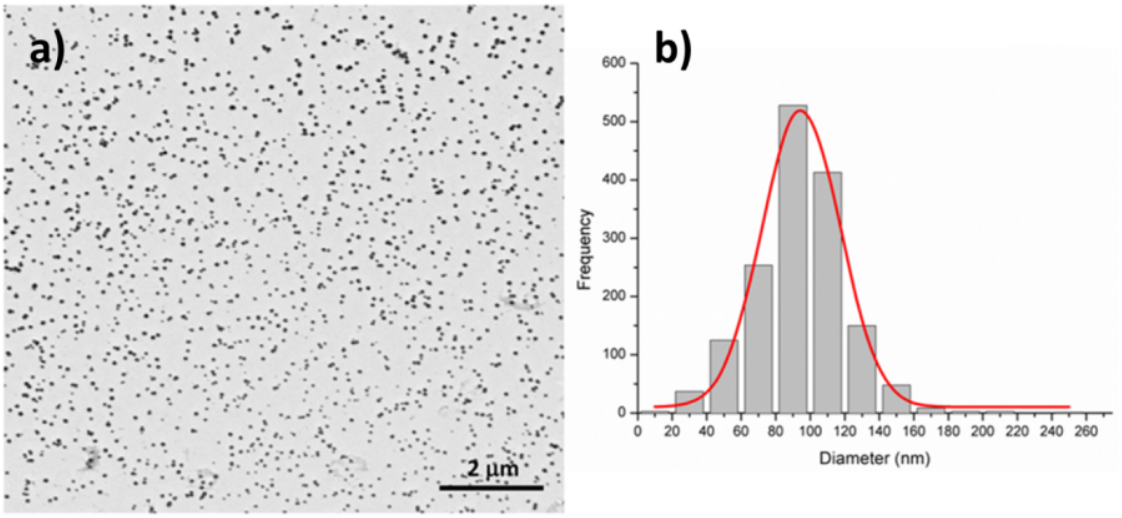

Figure S6: Average size distribution (b) of the peptide nanospheres formed by peptide **1** after changing pH. About 2000 nanospheres were measured from TEM images to calculate the distribution (a).

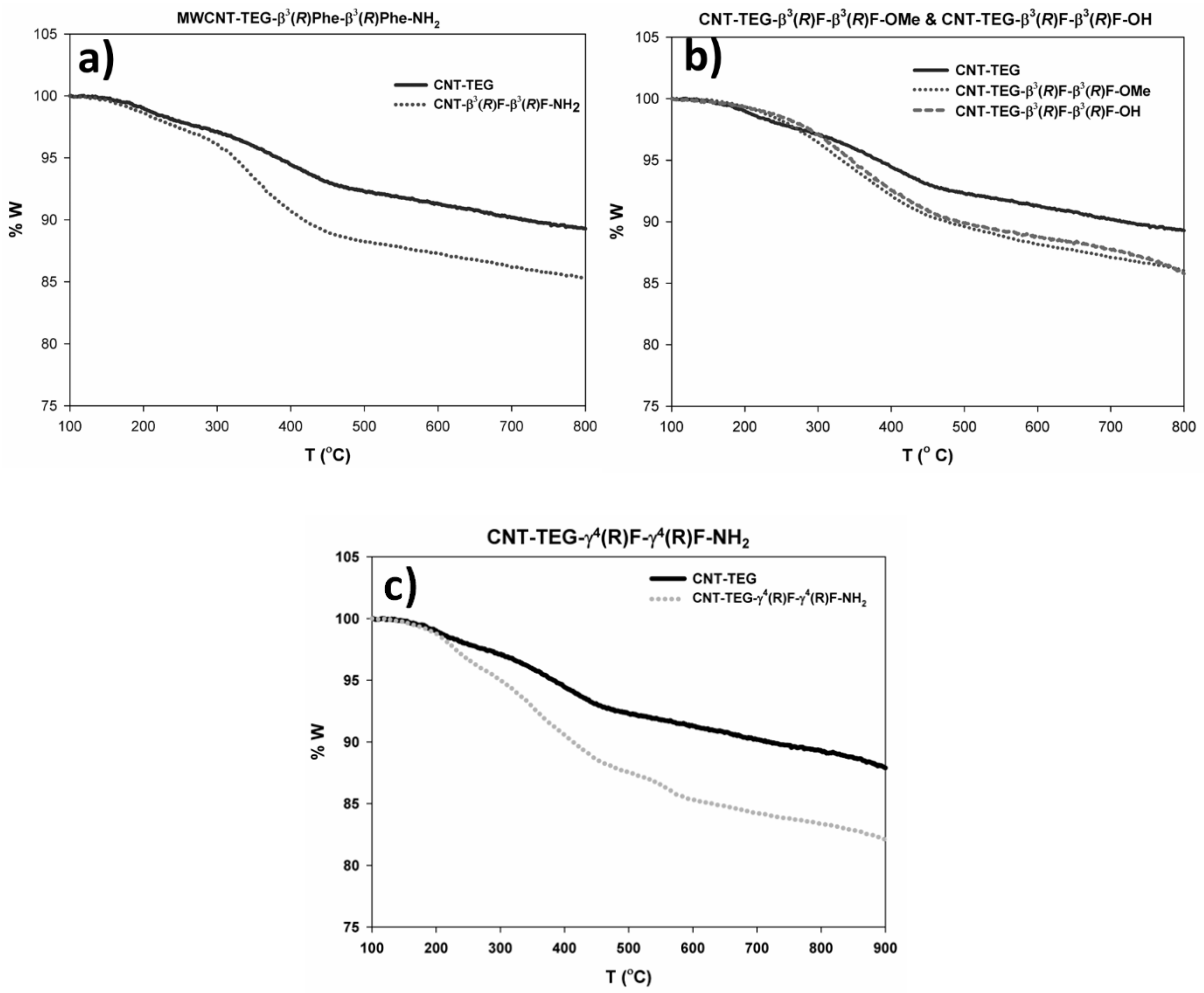

Figure S7: TGA profiles of MWCNT-TEG-NH_3^+, *N*-terminal free CNT-peptide conjugate **3** (a) and **5** (c), and *C*-terminal free peptide-CNT conjugate **4** (b), and its precursor under N_2 atmosphere with a ramp of 10 °C/min. Weight loss in a) is 4 % for **3**, and c) is 4.8 % for **5**, corresponding to a peptide loading on CNTs of 126 μmol/g and 84 μmol/g respectively. Weight loss in b), before and after hydrolysis of methyl ester is 2.7 % and 2.4 %, respectively, corresponding to a peptide loading on CNTs of 63 μmol/g and 57 μmol/g. The weight loss was calculated at 500°C.

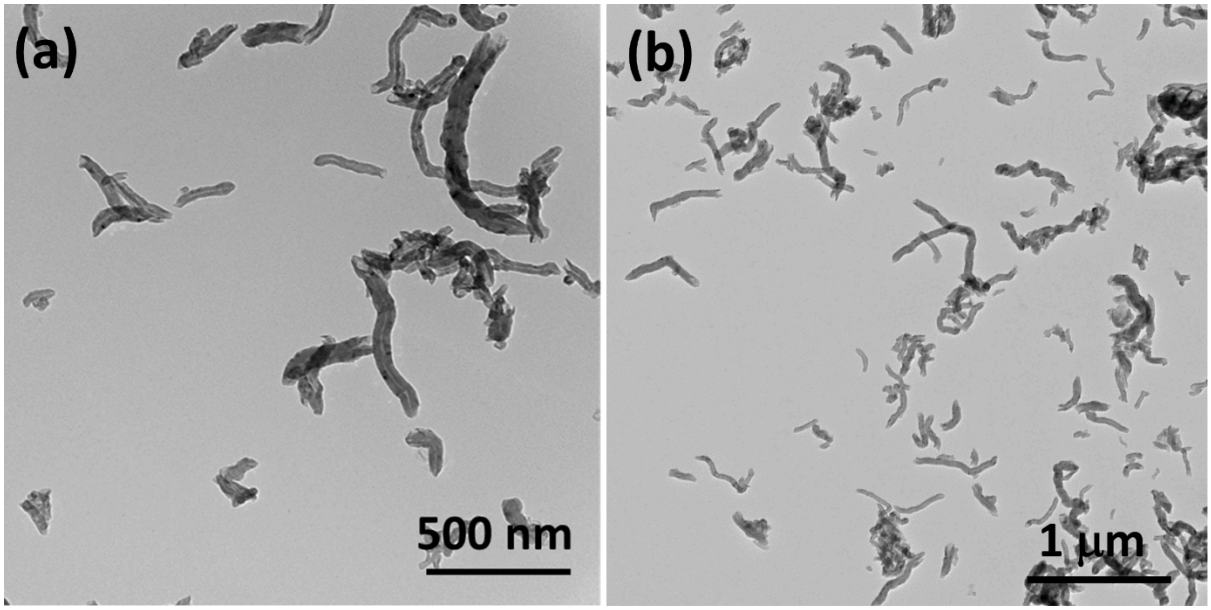

Figure S8: TEM image of CNT-peptide conjugate **4** (a), showing no assembly or changes in the CNT morphology in comparison to the nanotube precursor, MWCNT-TEG-NH-$COCH_2CH_2COOH$ (b).

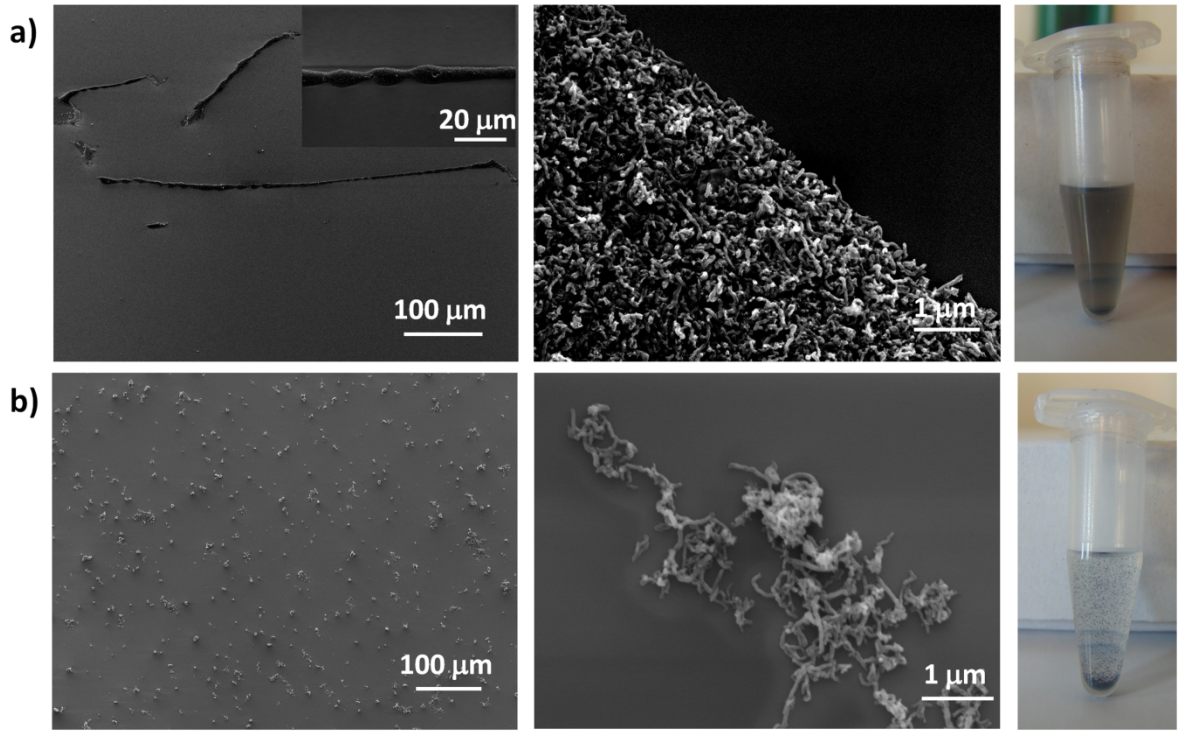

Figure S9. SEM images showing rope-like morphology of CNT-peptide conjugate **3** at different magnifications and photograph of the dispersion of **3** (a). SEM images of protonated CNT-peptide conjugate **3** showing dispersed CNTs showing disruption of the assembly. Photograph of the dispersion after adding diluted HCl (b).

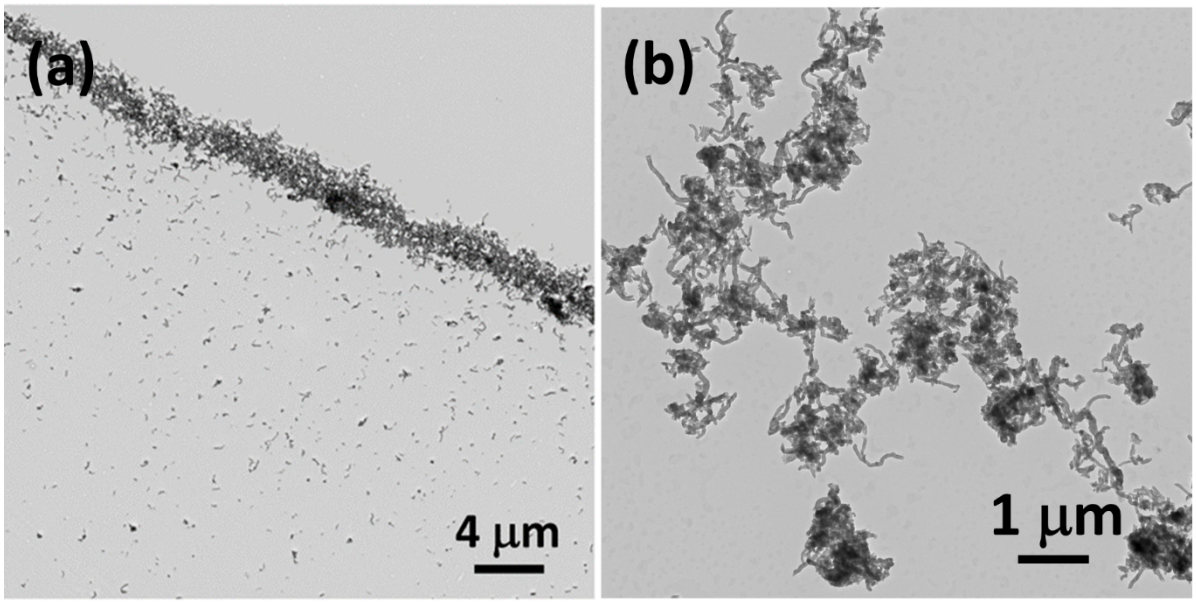

Figure S10. TEM image of CNT-peptide conjugate **3** (a) showing the aggregation that is in agreement with SEM analysis. TEM image of CNT-peptide conjugate **5** (b) showing different morphology with aggregated clusters.

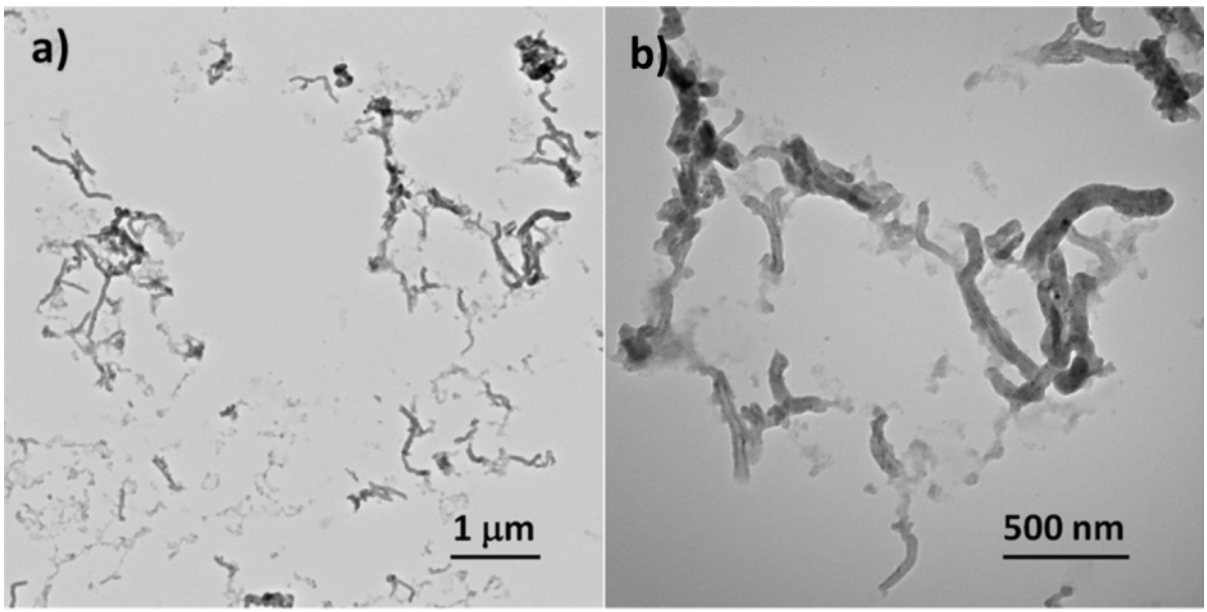

Figure S11. TEM image of co-assembly of peptide **2** and CNT-peptide conjugate **5** in 1:1 ratio showing the complete absence of assembly.

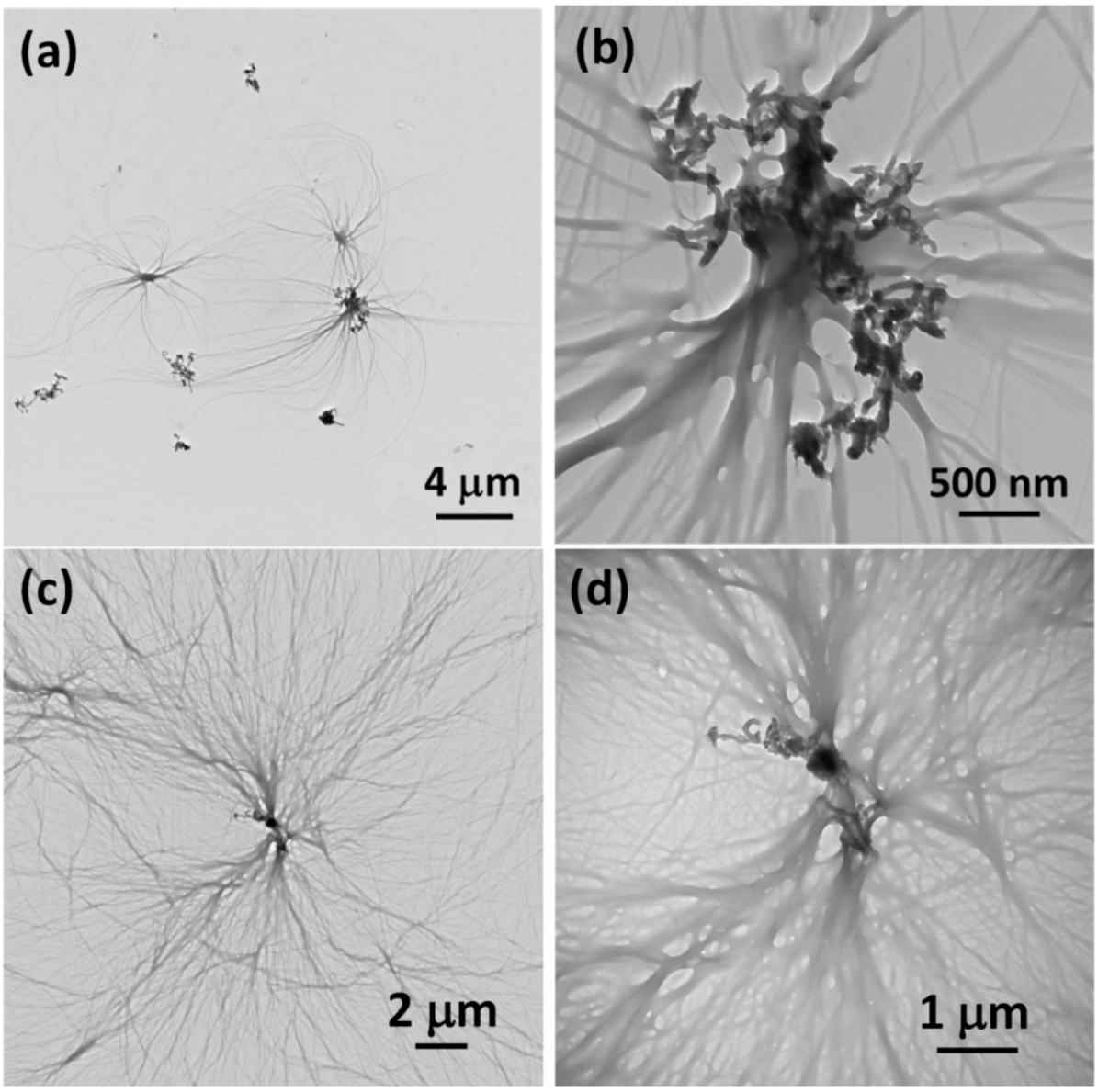

Figure S12: TEM images of the co-assemblies of: a) peptide **1** and CNT-peptide **3** showing tendency of the CNTs towards the peptide fiber formation; c) peptide **2** and CNT-peptide **5** showing dendritic morphology with altered morphology of the peptide **2** nanofibers. b) & d) are magnified images showing CNT-peptide conjugates **3** and **5** nucleated at the centre of peptide **1** and **2** nanofibers, respectively.

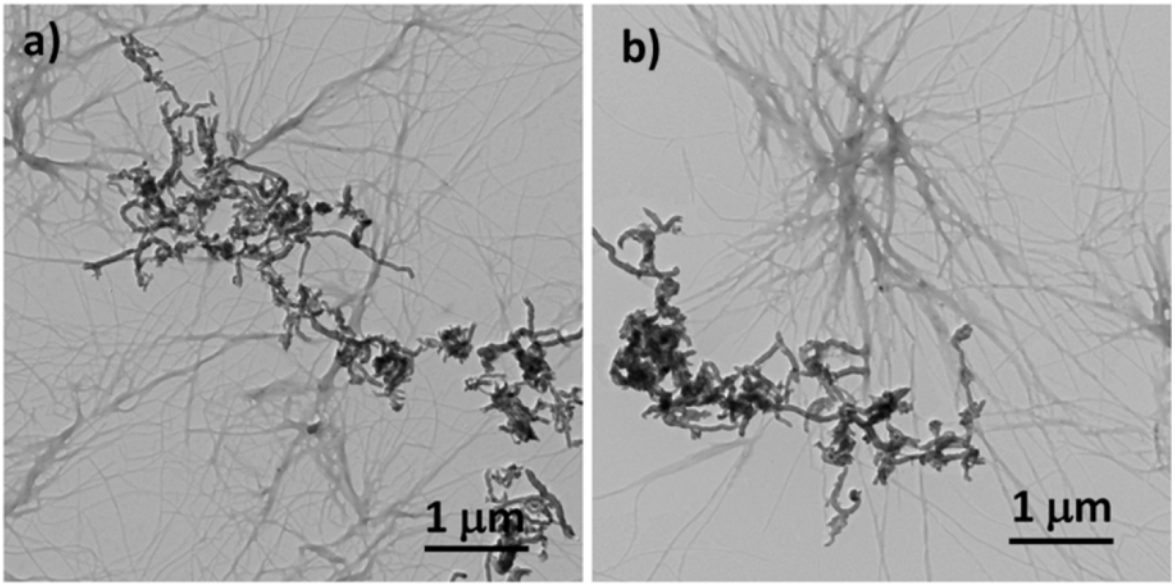

Figure S13: TEM images of co-assembly of the peptide **1** and MWCNT-TEG-NH_3^+. The morphology does not allow to prove the presence of a closed contact interaction between the peptide and CNT networks.

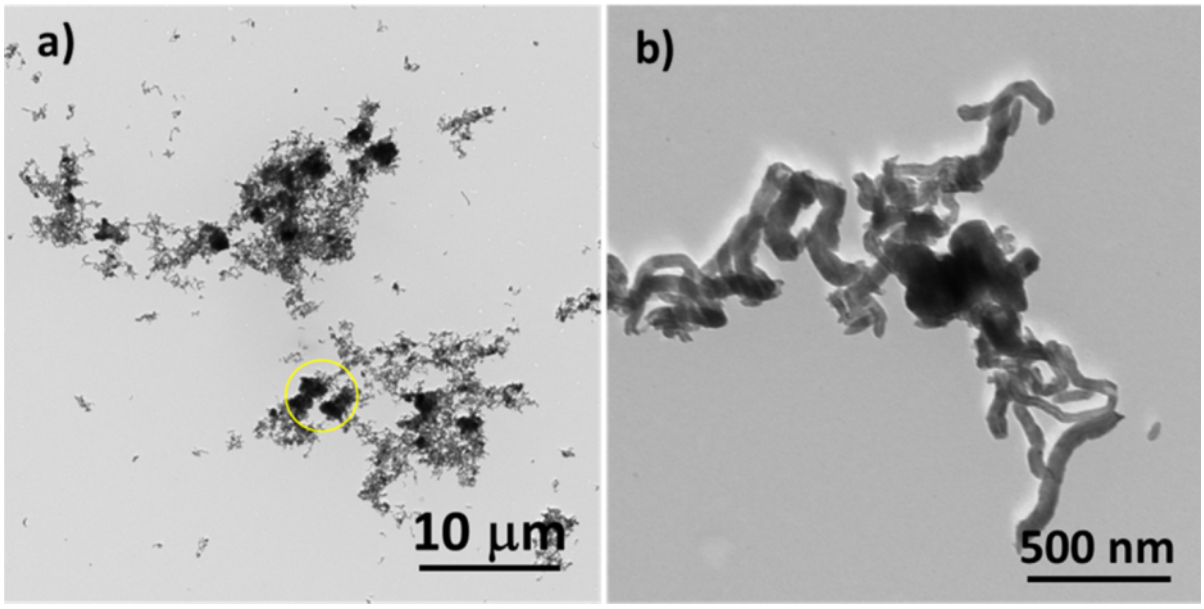

Figure S14: TEM image showing the formation of nanospheres (highlighted by the yellow circle) (a) of peptide **1** in the presence of CNT-peptide conjugate **3** at lower pH and magnified image of peptide **1** nanospheres and CNT-peptide **3** (b).

References

1. a) D. Seebach, M. Overhand, F. M. N. Kuhnle, B. Martinoni, L. Oberer, U. Hommel, H. Widmer, Helv. Chim. Acta 1996, 79, 913 – 941 a) K. Pluncin´ ska, B. Liberek, Tetrahedron 1987, 43, 3509 – 3517; b) C. Guibourdenche, D. Seebach, Helv. Chim. Acta 1997, 80, 1 – 13; c) M. Smrcina, P. Majer, E. Majerova, T. A. Guerassina, M. A. Eissenstat, Tetrahedron 1997, 53, 12867 – 12874; d) B. Dinesh, K. Basuroy, N. Shamala, P. Balaram, Tetrahedron 2012, 68, 4374 – 438; e) A. Bandyopadhyay, H. N. Gopi, Org. Lett. 2012, 14, 2770 – 2773

2. C. Gaillard, M. Duval, H. Dumortier and A. Bianco, J. Pep. Sci. 2011, 17, 139-142;