Electronic Supplementary Material (ESI) for Soft Matter

Supplementary Information for

Self-assembly and Fibrillization of a Fmoc-functionalized polyphenolic amino acid

Abhijit Saha, Sreenath Bolisetty, Stephan Handschin and Raffaele Mezzenga*

ETH Zurich, Food & Soft Materials Science, Institute of Food, Nutrition &Health,

Schmelzbergstrasse 9, LFO, E23, 8092 Zürich, Switzerland.

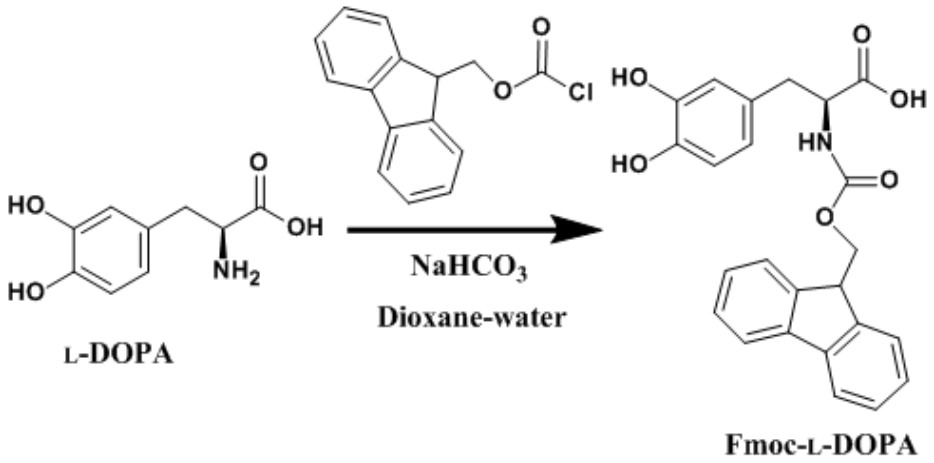
E-mail: raffaele.mezzenga@hest.ethz.ch

Materials and Methods:

9-Fluorenylmethyloxycarbonyl chloride (Fmoc-Cl) and 3,4-dihydroxy-L-phenylalanine (L-DOPA) were purchased from TCI Deutschland GmbH. $NAHCO_3$, Na_2SO_4 and solvents were purchased from Merck, Germany. All the materials were used without further purification.

Synthesis of N-(9-fluorenylmethyloxycarbonyl)-3,4-dihydroxy-L-phenylalanine (Fmoc-L-DOPA):

Fmoc-L-DOPA was synthesized according to a reported procedure.[1] L-DOPA (1 g, 0.005 mol) was dissolved in dioxane-10% $NaHCO_3$ (15 mL, 1:2) and the solution was stirred on an ice bath for 15 min before the addition of 9-fluorenylmethyloxycarbonyl chloride (Fmoc-Cl) (1.71 g, 0.005 mol) in dioxane (5 mL) over 30 min. The reaction was kept under stirring first on an ice bath for two hours and then at room temperature for 18 hours. The cream colored suspension was added to a separatory funnel and was washed with ether (3x40 mL). The aqueous layer was cooled on an ice bath and acidified with 6 N HCl to pH 3. This aqueous solution was extracted with ethyl acetate (3x40 mL). The combined organic layers were washed with brine, and dried over anhydrous Na_2SO_4. Solvent was removed under reduced pressure to yield a light brown, foamy solid. The crude Fmoc-L-DOPA was loaded onto a silica gel column and eluted with CH_2Cl_2/MeOH= 10/1 to get Fmoc-L-Dopa, 1.02 g as white solid (product yield 48%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ ppm 8.76 (br s, 1H), 7.88 (d, 1H), 7.68-7.27 (m, 8H), 6.69-6.60 (m, 2H), 6.51-6.49 (m, 1H), 4.22-4.02 (m, 3H), 2.91-2.87 (m, 1H), 2.72-2.66 (m, 1H). Mass Spec. calculated for $C_{24}H_{22}NO_6$: m/z (M+H)=420.14, found: 420.11



Scheme1: Synthesis of Fmoc-L-DOPA

Ref: 1 M. J. Sever and J. J. Wilker, Tetrahedron, 2001, 57, 6139-6146

Sample preparation:

Samples were prepared by taking the appropriate amount of Fmoc-L-DOPA and three different pH conditions (pH 2, 5 and 7) where achieved by adding dil. HCl or dil. NaOH solutions and then the mixtures were heated to dissolve. Then the samples were incubated at 25 °C. At pH 2, turbidity appeared which vanished gradually with time. After incubating the samples overnight at 25 °C, studies were performed.

Polarized optical microscopy:

POM was performed under cross polarized light using a Zeiss Axioskop 2 mot light microscope with a Plan-Neofluar 10x/0.3 NA objective. All pictures were collected with a Hamamatsu C5810 CCD camera.

Circular Dichroism (CD) Spectroscopy:

All the CD spectra were taken in a JASCO CD spectrophotometer (model J-815) equipped with a peltier element for temperature variation experiments in a 1 mm cuvette. The scan range was 375-290 nm at the rate of 100 nm/min.

Dynamic light scattering:

0.25 wt% Fmoc-L-DOPA samples at pH 2 were studied at 25 °C and 70 °C by light scattering using a LS Instruments machine equipped with He–Ne Laser emitting a polarized light beam at 632.8 nm. The dynamic light scattering measurements were performed at a fixed angle of 90^0 by averaging 3 runs of 600 s each. In dynamic light scattering, the time correlation function (TCF) of the scattered intensity is analyzed by the CONTIN method.

Transmission Electron Microscopy Measurements (TEM):

Imaging was done on carbon coated copper grids for transmission electron microscopy (TEM), which were previously glow discharged for 45 s (Emitech K100X, GB). 3 µl of the dispersion was placed on them and after waiting 1 min for settlement, excess liquid was drained off with a piece of filter paper and the grids were transferred on a droplet of 2 % uranyl acetate, immediately blotted dry and placed on a second droplet for 15 s and blotted dry again. Dried grids were examined by bright field TEM (FEI, model Morgagni, NL) operated at 100 kV.

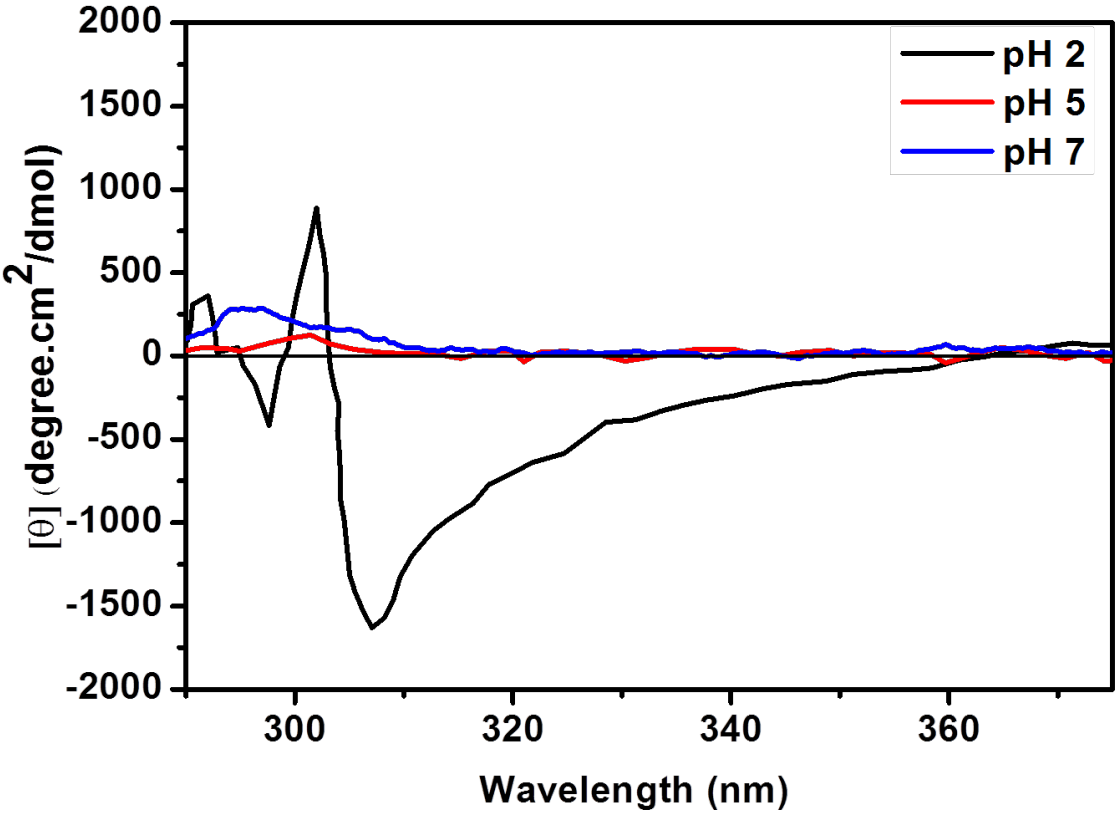
A FEI CM12 microscope (FEI Eindhoven, NL) was used for the Cryo-TEM (Cryogenic Transmission Electron Microscopy) imaging to characterize the fibers in their native state. 400 mesh copper grids with lacey carbonfoil (Quantifoil Micro Tools GmbH, Germany), were glow discharged (Emitech K100X, GB) for 45s. 2.5 µl of sample solution were applied onto the grid and the excess of the dispersion was removed by a blotting paper for two seconds. Liquid ethane cooled to -175 °C was used for sample vitrification. The vitrified sample was cryo-transferred into the microscope and continuously cooled during the imaging process at -180 °C. Micrographs were recorded under low dose conditions using a Gatan 794 CCD camera operating the microscope at bright field mode, at 100 kV acceleration voltage.

Electrophoretic Mobility Measurements:

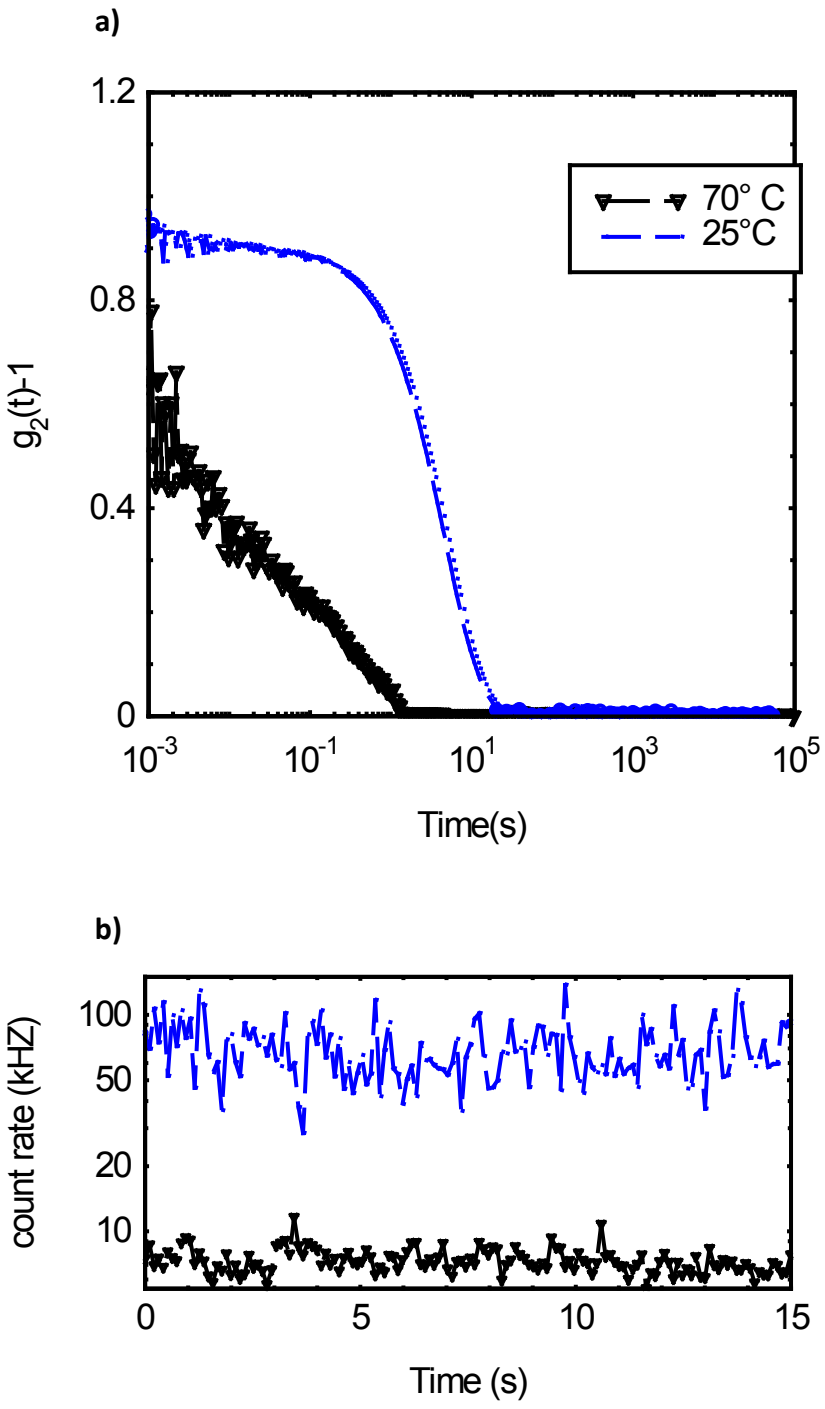
Electrophoretic mobilities of 0.1 wt% Fmoc-L-DOPA at three different pH conditions (pH 2, 5 and 7) were determined by the Zetasizer Nano ZS dynamic light scattering device (Malvern Instruments, Worcestershire, U.K.). Samples were inserted in folded capillary plastic cells (Malvern Instruments), equipped with two metal electrodes at each end of the capillary. Colloidal particle motion was measured by laser light scattering in a pulsed electric field.

Small-Angle X-ray Scattering (SAXS) and Wide-Angle X-ray Scattering (WAXS):

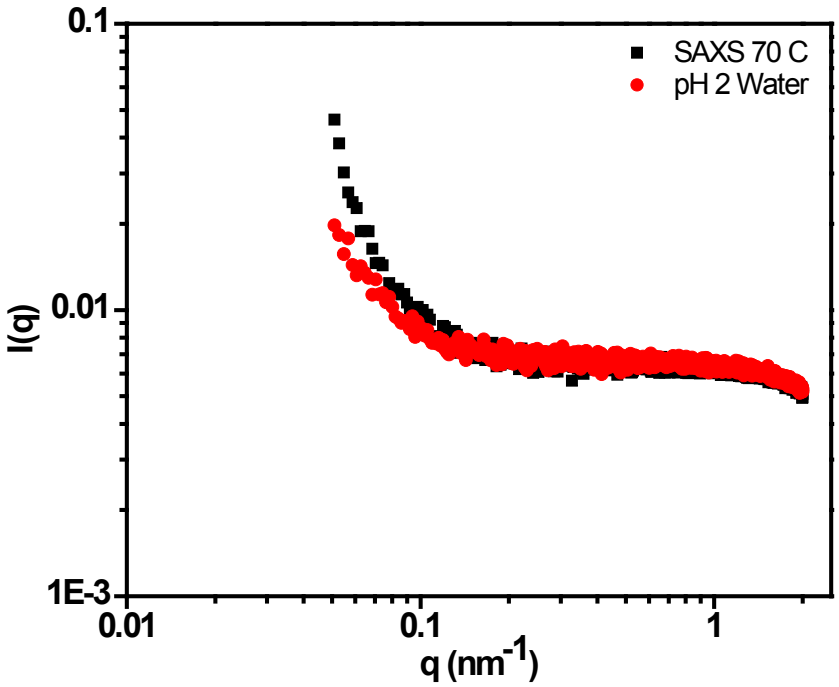
Small Angle X-ray Scattering experiments were performed using a microfocused Rigaku X-ray Ni-filtered Cu Kα X-ray radiation (0.154 nm). The applied voltage and filament current were 45 kV and 0.88 mA, respectively. The samples were held in 1.5 mm quartz X-ray capillaries and exposed to the radiation collimated with a set of three pinholes. The data were collected in a two-dimensional argon filled detector and azimuthally averaged to yield one-dimensional intensity versus scattering vector q. The collected data were further corrected for the transmission and the solvent background. The wide angle X-ray data are collected on an image plate and further scanned by an image reader (Supp. Fig. 10).



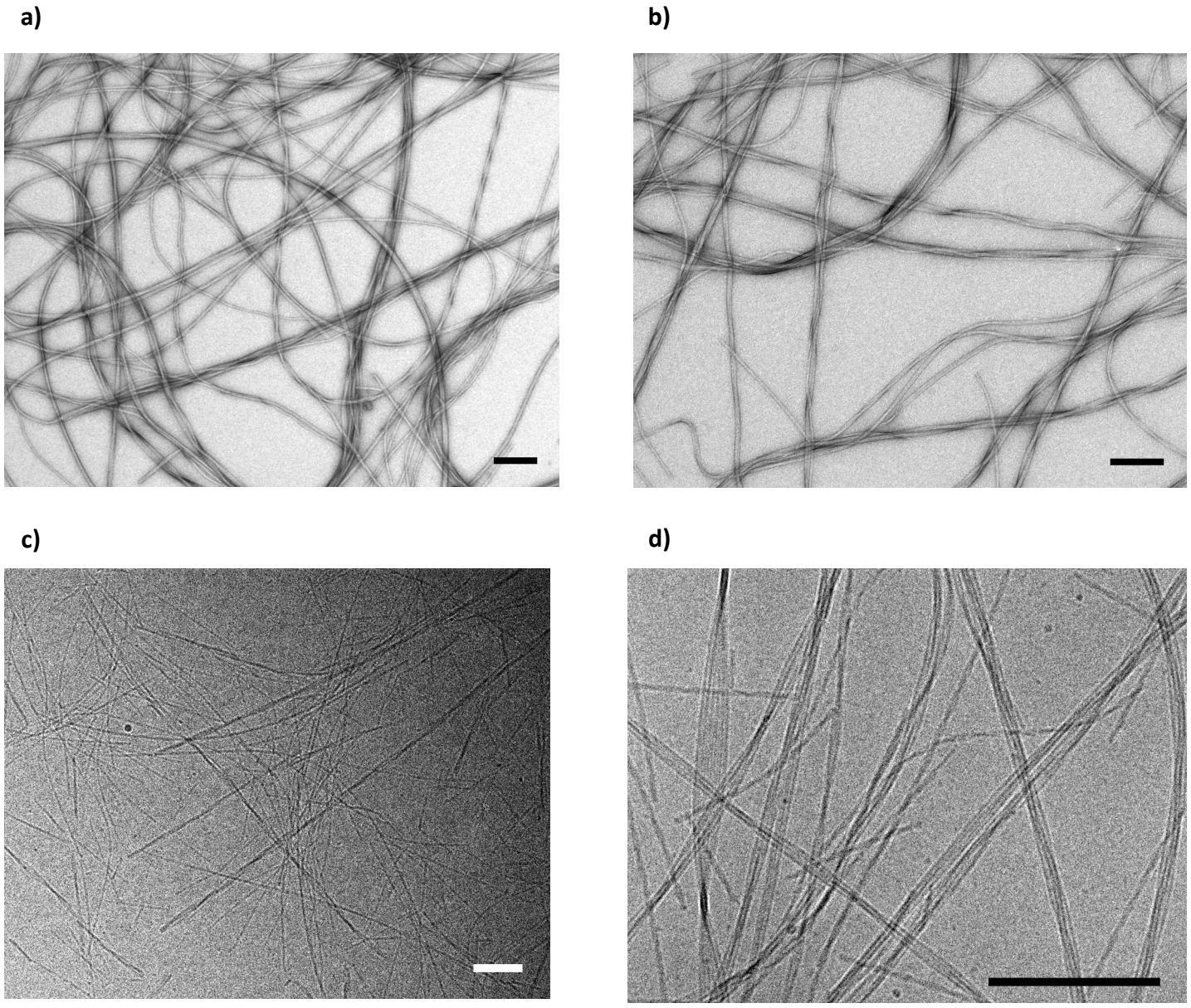
Supporting Figure 1: CD spectra of 0.1 wt% of Fmoc-L-DOPA at indicated pH conditions at 25 °C



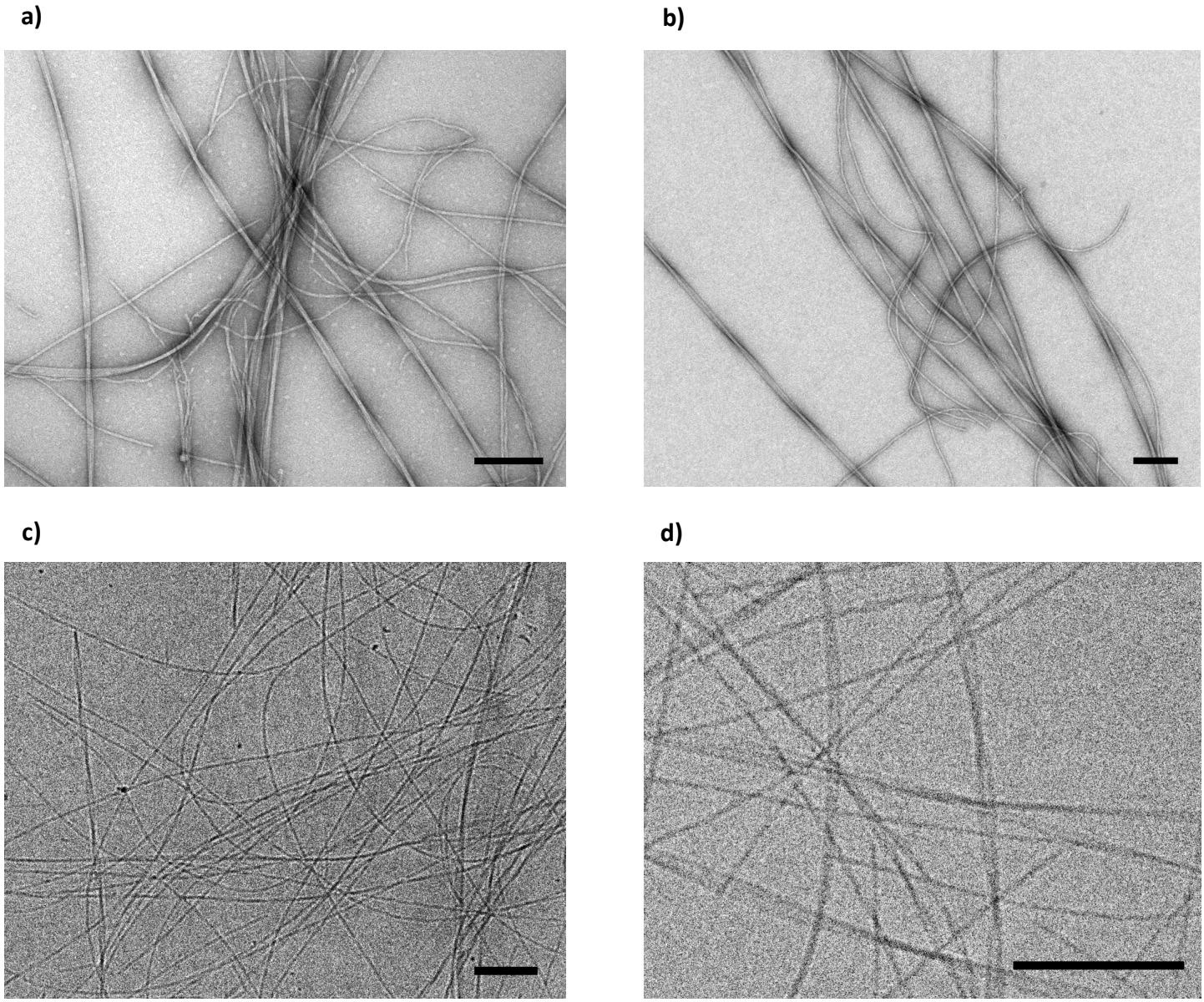
Supporting Figure 2: a) Time correlation function of Fmoc-L-DOPA at 25 °C and 70 °C (pH 2) and b) the decrease in scattering intensity with increase of the temperature due to the disassembly of self-assembled structures.



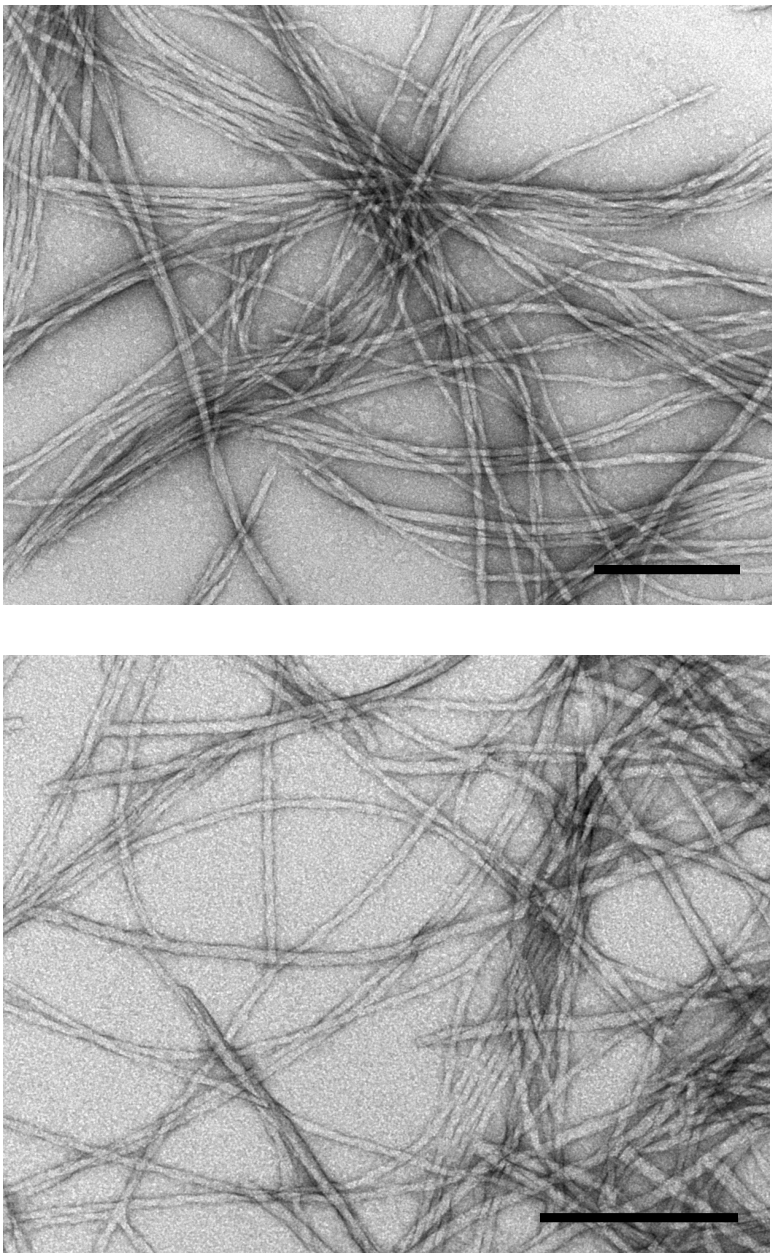
Supporting Figure 3: Comparison of the SAXS of 0.5 wt% of Fmoc-L-DOPA at pH 2 at 70 °C and the reference sample (pH 2 water at 70 °C).



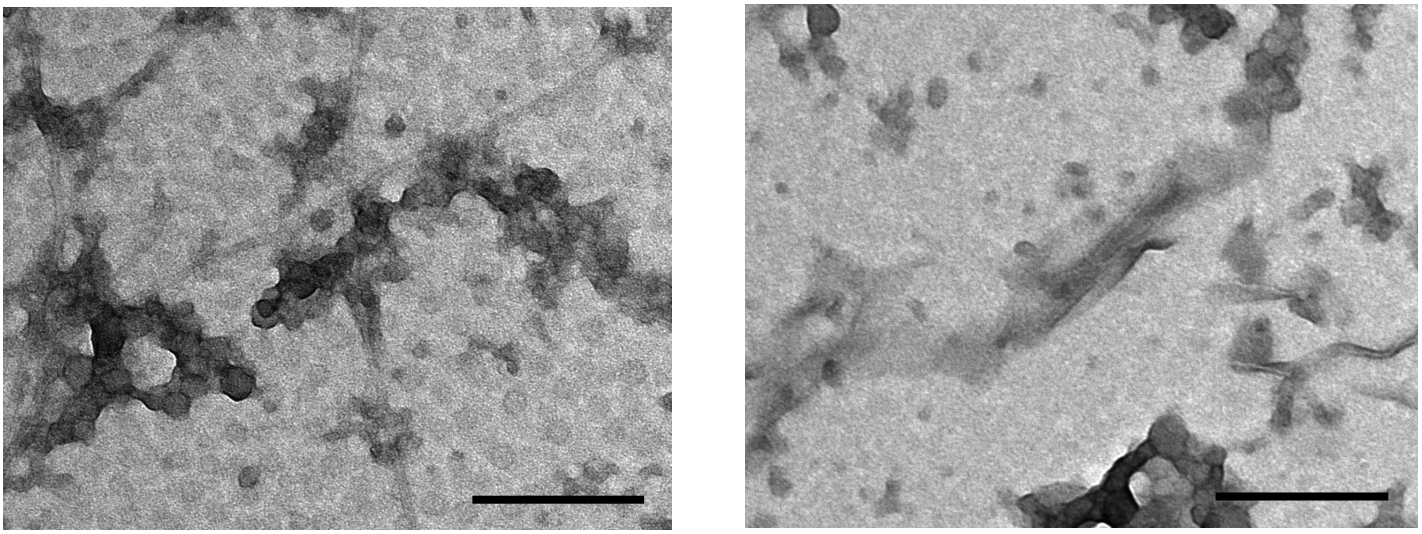
Supporting Figure 4: TEM (a,b) and Cryo-TEM (c,d) images of 0.1 wt% Fmoc-L-DOPA at pH 2 (scale bar is 200 nm).



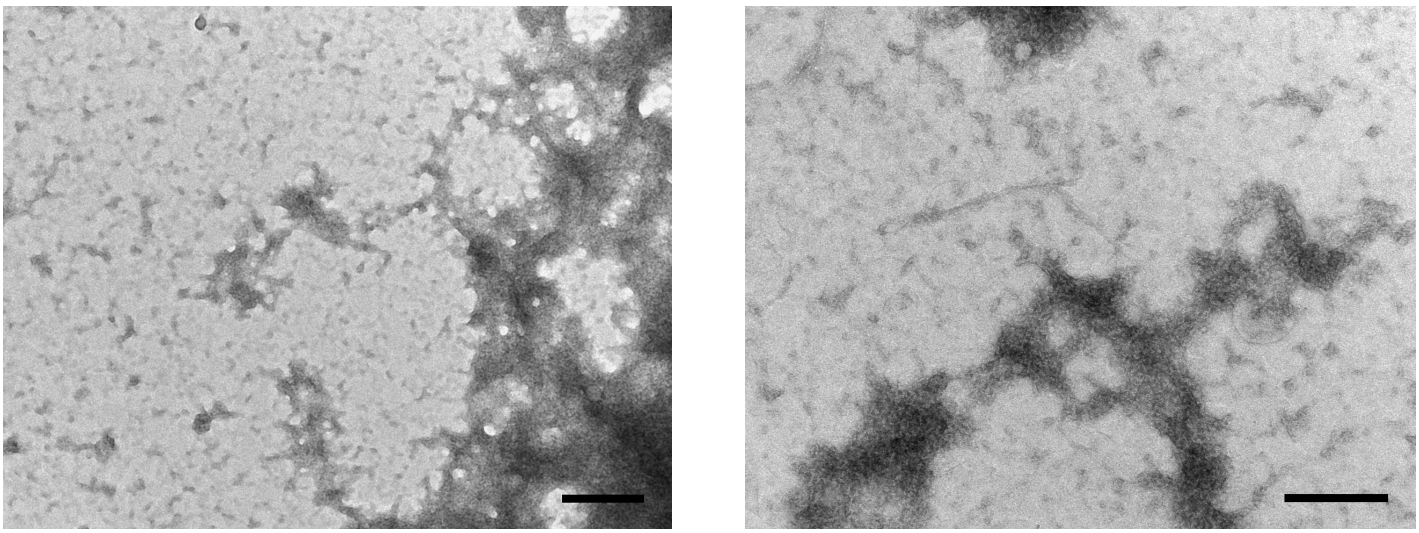
Supporting Figure 5: TEM (a,b) and Cryo-TEM (c,d) images of 0.25 wt% Fmoc-L-DOPA at pH 2 (scale bar is 200 nm).



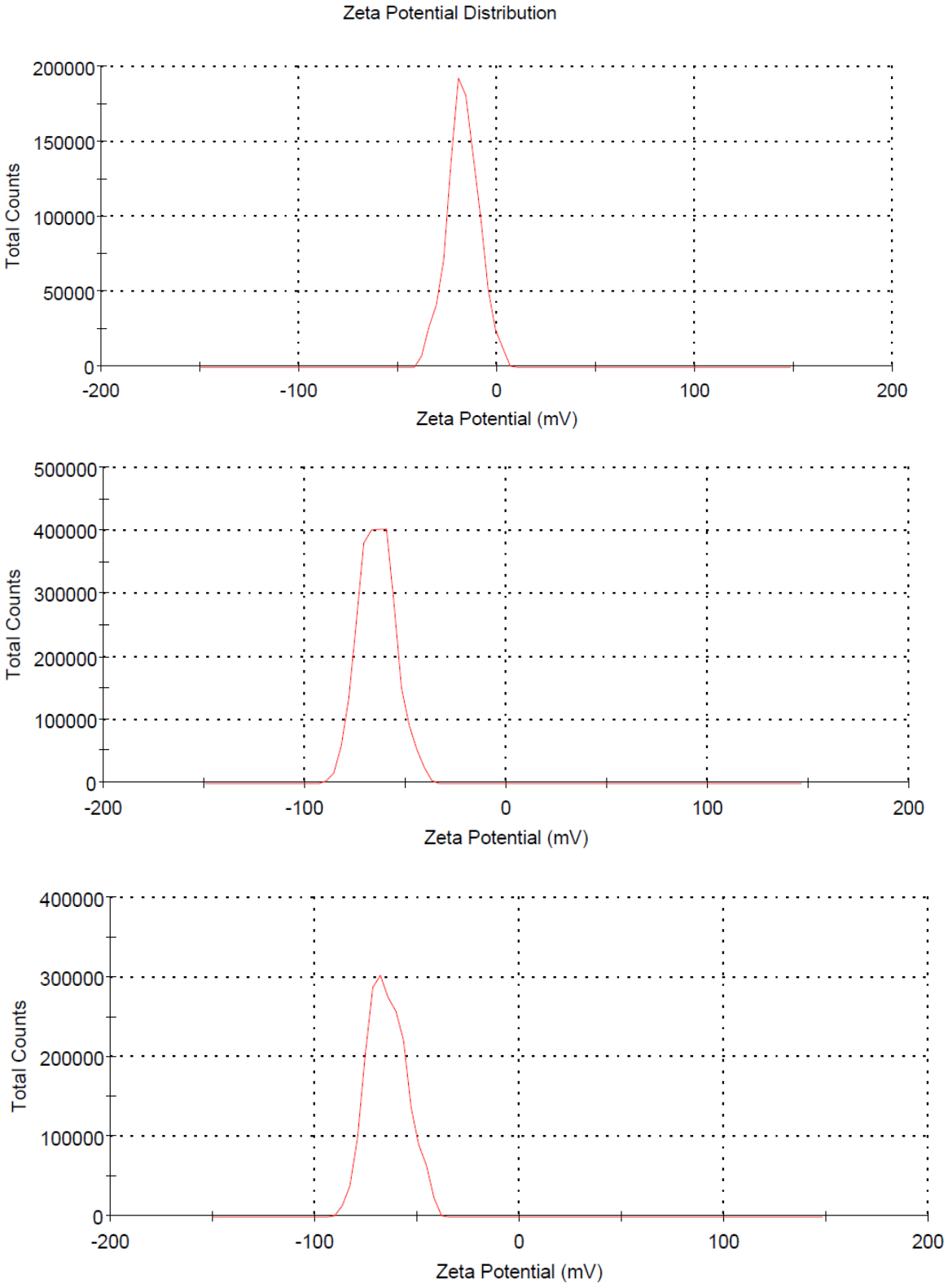
Supporting Figure 6: TEM images of 0.5 wt% Fmoc-L-DOPA at pH 2 (scale bar is 200 nm).



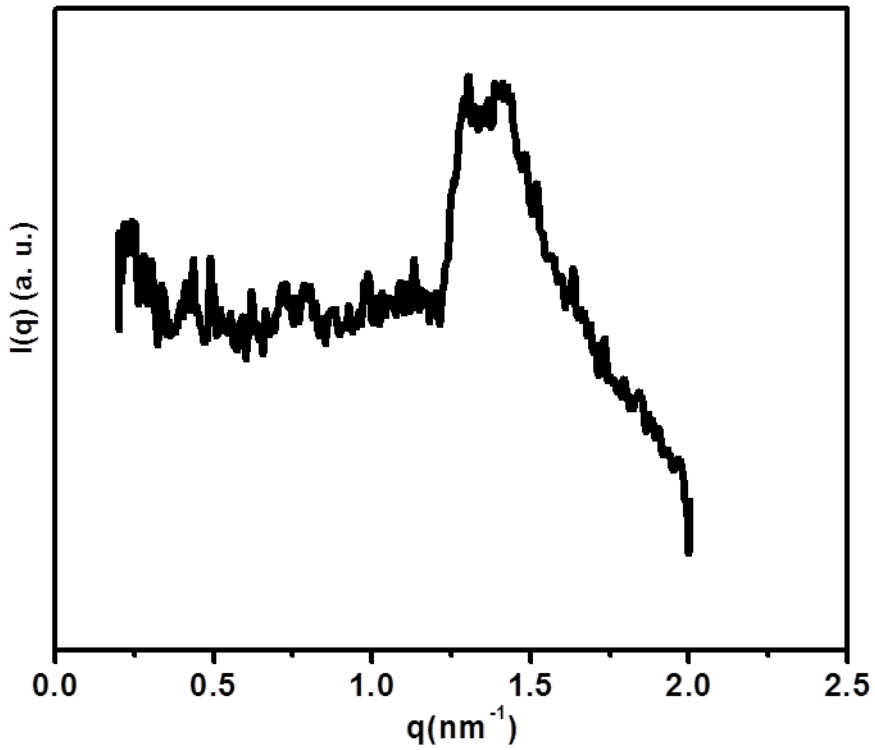
Supporting Figure 7: TEM images of 0.5 wt% Fmoc-L-DOPA at pH 5 (scale bar is 200 nm).



Supporting Figure 8: TEM images of 0.5 wt% Fmoc-L-DOPA at pH 7 (scale bar is200 nm).



Supporting Figure 9: Zeta potential measurements of 0.1 wt% Fmoc-L-DOPA at pH2 (top), pH 5 (middle) and pH 7 (below).



Supporting Figure 10: WAXS of Freeze-dried sample of 0.5 wt% of Fmoc-L-DOPA at pH 2.