Supporting Information

Functionalization of multiwall carbon nanotubes and their pH-responsive hydrogels with amyloid fibrils

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Experimental Section

Materials

BioPURE-beta lactoglobulin (lot JE 003-6-922, from 23-05-2005) was obtained from Davisco Foods International, Inc. (Le Sueur, MN) and purified according to the reference [S1]. The typical protocol was the following: The protein aqueous solution (10 wt %) was adjusted to pH 4.6 and then centrifuged at 15000 rpm over a period of 15 min at 20 °C. The supernatant was adjusted to pH 2 and filtered through a 0.22 μm Millipore filter. To remove traces of ions, the filtered protein solution was dialyzed first against pH 2 MilliQ water and second against MilliQ water, using a Spectra-Por Dialysis Membrane 1, with a MWCO of 6000−8000 Da (Spectrum Laboratories, Inc., CA). Multi-walled carbon nanotubes (MWNTs), synthesized by chemical vapor deposition method and followed by HCl demineralization, were purchased from Sigma. According to the specification, their bundle size is 6-13 nm × 2.5-20 μm with an average wall thickness of 7-13 graphene layers.

Preparation of amyloid fibrils

Preparation of long β-Lactoglobulin fibers was carried out, in short, as follows:[S1] after purification, β-lactoglobulin was dissolved into water and adjusted to 2 wt% and pH 2. Then the solution was kept at 90 °C for at least 6 h to form long individual fibrils, which was confirmed by atomic force microscopy (AFM), electron transmission microscopy and dynamic light scattering (DLS).

Functionalization of MWNTs

MWNTs were chemically functionalized via a diazonium reaction in water. Typically, Sulfanilic acid (4 equiv per 20 mg MWNT C) was dissolved into 20 ml millipore water at 80 °C. Then the solution was added into a 100 ml round-bottom flask equipped with a condenser, which contained MWNTs (20 mg). After the flask was kept at 80 °C with stirring in an oil bath for 20 min, isoamyl nitrite (3 equiv per MWNT C) was then dropped in slowly. The mixture was stirred vigorously at 80 °C for 20 h. After cooling down to room temperature, the mixture was filtered though a filter of pore size of 0.22 μm. The filter cake was washed with deionized water and acetone until the filtrate was clear. The product was re-dispersed in DMF to remove any remaining unreacted organics and filtered again with a pore size of 0.22 μm.

Non-covalent functionalized MWNTs were prepared as follows: First, pyrene sulfonic acid was dissolved into water (20 ml) upon heating to give a 1 wt % solution. 20 mg pristine MWNTs were added into this solution, followed by vigorously stirring for 12h and ultrasonication for 30 min. Then the suspension was filtered on a filter (pore size of 0.22 μm). The filter cake was washed with deionized water and acetone until the filtrate was clear. To remove the trace of free pyrene sulfonic acid, the product was added into hot water (80 °C) and mixed for 5h. The filtration was repeated until the filtrate was clear.

Preparation of hybrid hydrogel

Functionalized MWNTs were added into water (pH 7) and stirred for 12 h and then sonicated for 5 min to obtain a homogenous and stable dispersion. The pH 7 solution of amyloid fibrils was prepared by tuning the pH 2 solution of amyloid fibrils via 1M NaOH solution. Then both the solutions were mixed to get a homogenous mixture (the final concentration is 0.8 wt% amyloid fibrils and 0.35 wt% MWNTs). In order to test the pH-responsive behavior of the hybrid hydrogels and bring the mixture pH from 7 to 2, a predetermined amount of HCl solution (1 M) was used, added quickly to the solution with very mild stirring; stirring was then arrested and

then the mixture was let resting for several minutes before measuring the gel properties. Vigorously stirring was avoided to prevent destroying the network formed.

Characterization

The Fourier Transform Infra Red (FTIR) spectra were recorded at Varian FTIR system in transmission mode, using KBr pellets within the wavelength range of 600 to 4000nm. Thermogravimetric analysis (TGA) was performed on a TGA Instrument (TA 2950). After the sample (2~5 mg) was loaded, a temperature scan was performed at a heating rate of 20 °C min^{-1} under nitrogen protection.

Transmission electron microscopy (TEM) images were obtained with a Philips TEM (CM 20) instrument operating at a voltage of 100 kV. A drop of the diluted solution (0.1−1 wt % final concentration) was casted onto a carbon support film on a copper grid. The excess solution was removed after 30 s using a filter paper. Contrast to electrons was achieved by negative staining by adding a droplet of uranyl acetate solution 1 wt % onto the grid, over a period of 15 s, after deposition of β-lactoglobulin aggregates solution. Any excess of staining agent was removed again by a filter paper.

Bulk rheology measurement was performed on a TA Instruments AR2000 rheometer with cone-plate geometry (40mm diameter, 2°). Frequency sweep measurements were carried out from 0.01 to 100 Hz with the controlled strain amplitude of 1%. The temperature was controlled at 25 ± 0.1°C.

The conductivity of the solution was measured using a conductivity meter (Hanna Combo pH/EC/TDS/Temp tester, HI98130).

Solution Conductivity

In order to confirm that the hydrogel was not originated from the network of NTs, which could potentially take place via strong mutual π–π interactions, the pH dependence of conductivity was measured. As shown in Figure S2, the conductivity of amyloid fibril solution (0.8 wt%)

increases from pH 7 to 2. The increased conductivity resulted from the charges on amyloid fibrils, β-lactoglobulin monomers remaining in the solution, and the ions added to change the pH. For functionalized MWNTs (0.35 wt%), due to their sulfonated groups and intrinsic conductive nature (electrical conductivity of about 1.85×10^3 S/cm along the long axis) [S2], the conductivity of the solution at pH 2 &7 is much higher than Millipore water but lower than amyloid fibrils, which suggested that MWNTs did not form an interconnected network at this concentration. For their hybrids, the conductivity is higher than amyloid fibrils at pH 7 but lower at pH 2. At pH 7 both the MWNTs and amyloid fibrils possess negative charges. At pH 2, however, the negative charges on MWNTs were neutralized by amyloid fibrils and/or β-lactoglobulin monomers, resulting in a lower conductivity.

References:

[S1] Jung J. M.; Savin, G.; Pouzot, M.; Schmitt, C.; Mezzenga, R. Structure of Heat-Induced-Lactoglobulin Aggregates and their Complexes with Sodium-Dodecyl Sulfate. *Biomacromolecules* **2008**, *9*, 2477-2486. Jung, J. -M.; Mezzenga, R. Liquid Crystalline Phase Behavior of Protein Fibers in Water: Experiments versus Theory. *Langmuir* **2010**, *26*, 504-514.

[S2] Y. Ando, X. Zhao, H. Shimoyama, G. Sakai, K. Kaneto, Physical properties of multiwalled carbon nanotubes. *International Journal of Inorganic Materials* **1999**, *1*, 77.

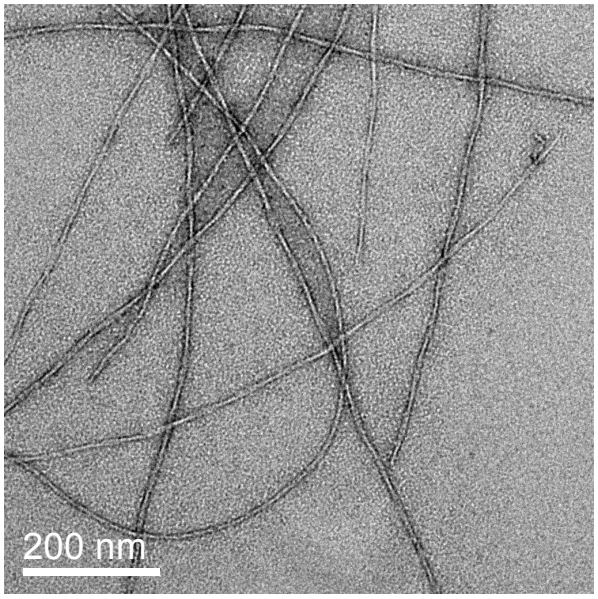

Figure S1. TEM image of amyloid fibrils formed at pH 2

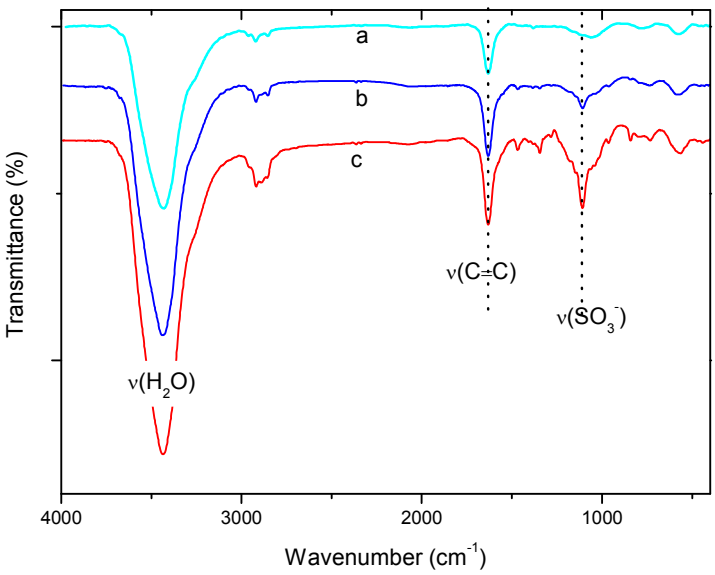

Fig. S2. FTIR spectra of pristine MWNTs (a), non-covalently functionalized MWNTs (b) and covalently functionalized MWNTs (c). The line at 1627 cm−1 was assigned to the C═C stretching mode. 1111 cm−1, representing the SO2 stretching mode in sulfonate $SO3^{-}$ group.

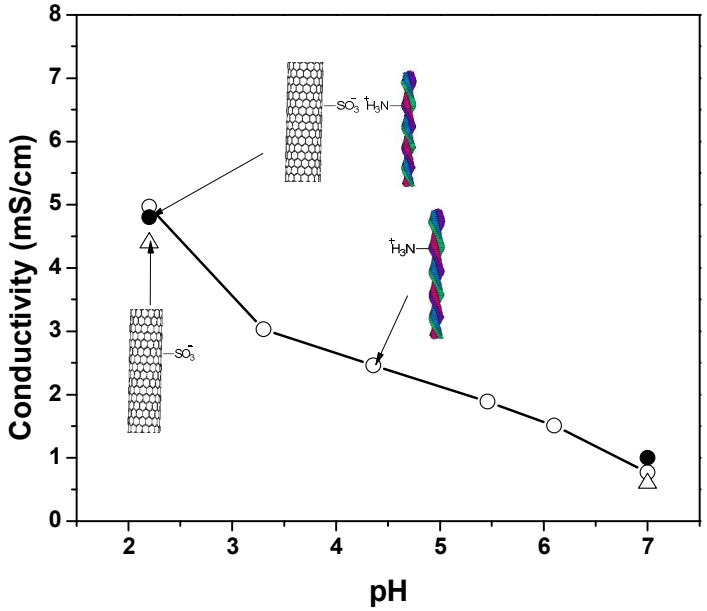

Fig. S3. Conductivity of MWCNs-amyloid dispersions as a function of pH. Amyloid fibrils (○), functionalized MWNTs (△) and hybrids (●)

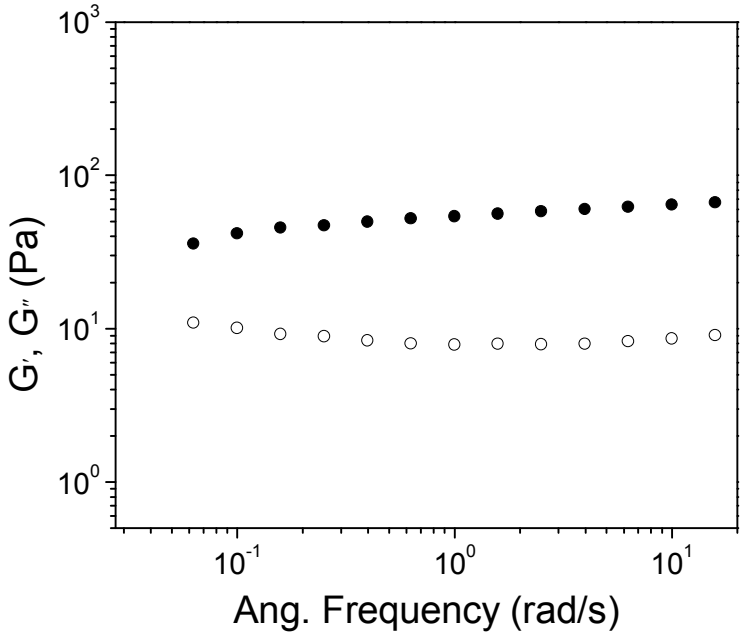

Fig. S4. Frequency dependence of the storage modulus (G', filled symbols) and loss modulus (G", open symbols) for hybrid hydrogels containing 0.8 wt% amyloid fibrils and 0.35 wt% non-covalently functionalized MWNTs at pH 2.