Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information (ESI)

New biocompatible thermo-reversible hydrogels from PNiPAM-decorated amyloid fibrils

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Experimental Section

BioPURE-β-lactoglobulin (lot JE 003-6-922, from 23-05-2006) was obtained from Davisco Foods International, Inc. (Le Sueur, MN) and purified according to reference [S1]. Anthraquinone-2-sulfonic acid sodium salt, N-isopropylacrylamide (NiPAM) and N,N'-Methylenebis(acrylamide) (BIS) were obtained from Aldrich-Sigma. NiPAM was purified by recrystallization from a mixture of benzene and hexane (3/7, v/v) before further use.

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 $^{\circ}$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). The fibril solution was further dialysed to remove the remaining unreacted monomers.

To prepare PNiPAM coated fibrils, a predetermined amount of anthraquinone-2-sulfonic acid sodium salt aqueous solution (0.058 wt%) and NiPAM and BIS (2.02 and 0.187 wt%, respectively) aqueous mixtures were dropped sequentially into 0.1 wt% fibril solutions accompanied by continuous stirring. Then the polymerization was carried out under N_2 for 60 min by exposing the solution to UV light (Power: 40W, wavelength: 420 nm)

Atomic force microscopy (AFM) was performed in an intermittent mode at ambient conditions at a scan rate of 2 Hz. Silicon nitride cantilevers with a typical tip radius below 10 nm were used. The AFM samples were prepared by depositing 20 µl solutions onto freshly cleaved mica and incubating for 30 s. Then the sample was rinsed with millipore water and dried with air for measurements. Light scattering experiments were performed using a 3D cross correlation Spectrometer (LS instruments) equipped with a HeNe laser (λ = 632.8 nm). The solution was filled into a cylindrical glass tube of 10 mm inner diameter. The vial was placed in the centre of a cylindrical vat filled with an index-matching fluid, cis-trans decahydronaphthalene (decalin). The temperature of the index-matching fluid was controlled between 0~50 °C (± 0.1 °C). For

dynamic light scattering (DLS), the time-averaged intensity correlation function was measured with the constant scattering angle (θ = 90°). The sol-gel transition was obtained by recording the temperature dependence of total scattered light at θ = 90°. Diffusing wave spectroscopy (DWS) was performed on a DWS Research Lab (LS Instruments GmbH) in transmission geometry. The beam laser of wavelength λ = 683.2 nm was focused to the sample cell (5.0 mm length). The scattered light was collected and cross-correlated with a digital photodiode correlator (APD Avalanche Photodiode). The Rheo lab software was used for data collection. When specified, polystyrene particles (640 nm) (Polysciences Europe GmbH) were added as tracking probe particles. Bulk rheology was performed on a ARES 2000 rheometer with cone-plate geometry (20 mm diameter, 2°). Temperature sweep measurement was carried out at temperature gradient 1 °C / 5 min at a fixed frequency 1 rad/s.

Preparation of PNiPAM/decorated amyloid fibrils β-lactoglobulin is the major whey protein present in cow's milk, which makes it easily accessible and inexpensive. By denaturation and dialysis, this globular protein yields pure, long, thin and highly charged amyloid fibrils at pH 2. The positively charged fibrils are then further available to ionic complexation to sulphated short surfactants. Here we exploit ionic supramolecular interactions to complex the fibrils with a negatively charged photoinitiator, anthraquinone-2-sulfonic acid sodium salt (AQS). Upon addition of NiPAM monomers in water and exposing the solution to UV light, free radical polymerization of NiPAM into PNiPAM occurs, yielding β-lactoglobulin fibrils partially decorated by PNiPAM layers. The presence of additional N,N'-Methylenebis(acrylamide) (BIS) in solution, was used to allow chemical cross-linking of PNiPAM brushes into compact layers (see Scheme S1).

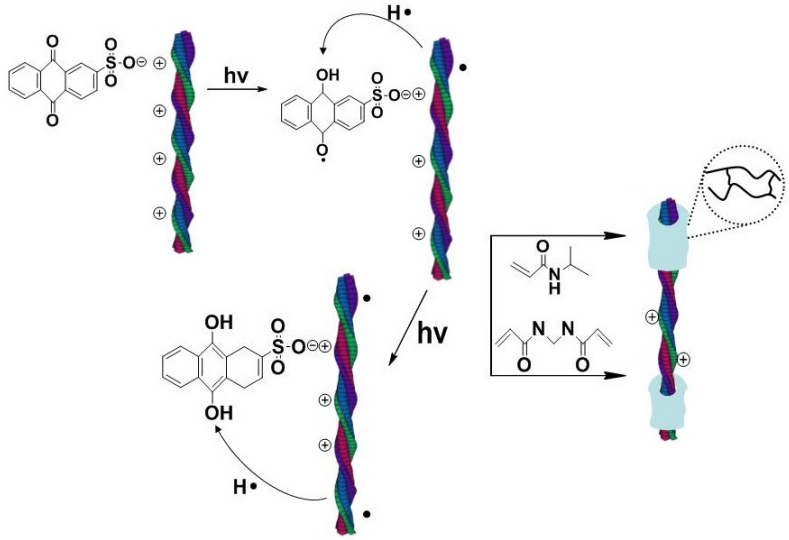
Photo-initiating mechanism The initiating mechanism likely occurs as follows: the triplet excited state of AQS employs a hydrogen atom from β-lactoglobulin or solvent to from a semianthraquinone radical and a photoinitiating radical. The semianthraquinone radical can further utilize a hydrogen atom to produce a hydroanthraquinone type structure and another photoinitiating radical (see Scheme S1).

Thickness of coated fibrils The thickness of the fibril was extracted from the AFM section profiles. As seen in Figure S2, along the contour length of one single fibril different heights are resolved.

Segregation of amyloid fibrils By increasing the monomers concentration from region III to region IV, it exists the possibility of partially coated fibrils associating together via N,N'-Methylenebis(acrylamide) cross-linkers. Thereby, separated pronounced fibril segregation quickly dominates (over 10^3 nm). The example of macroscopic segregation was given in Figure S3. Notably, segregation of fibrils occurs irreversibly in this regime via covalent bonds.

References:

[S1] J. M. Jung, G. Savin, M. Pouzot, C. Schmitt, R. Mezzenga, Biomacromolecules 2008, 9, 2477-2486.



Scheme S1. Schematic illustrations of coating amyloid fibrils by photo-initiating free radical polymerization of NiPAM and BIS.

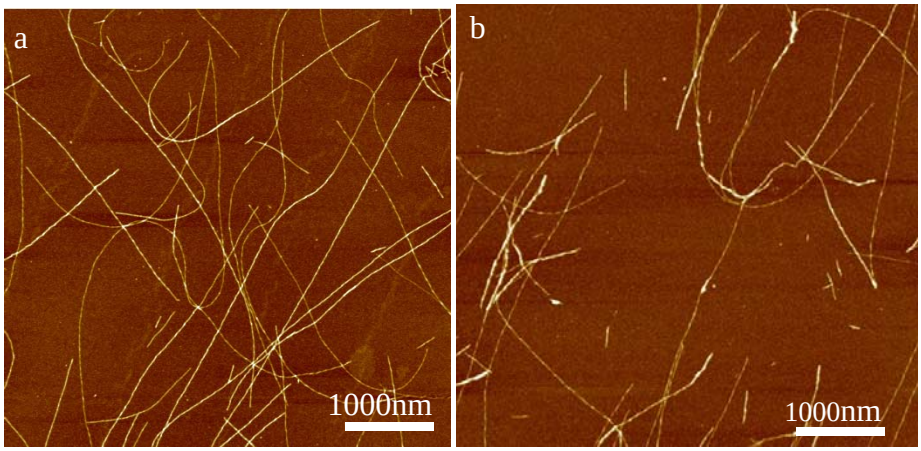


Figure S1. AFM images of uncoated fibrils (a) and coated fibrils (b)

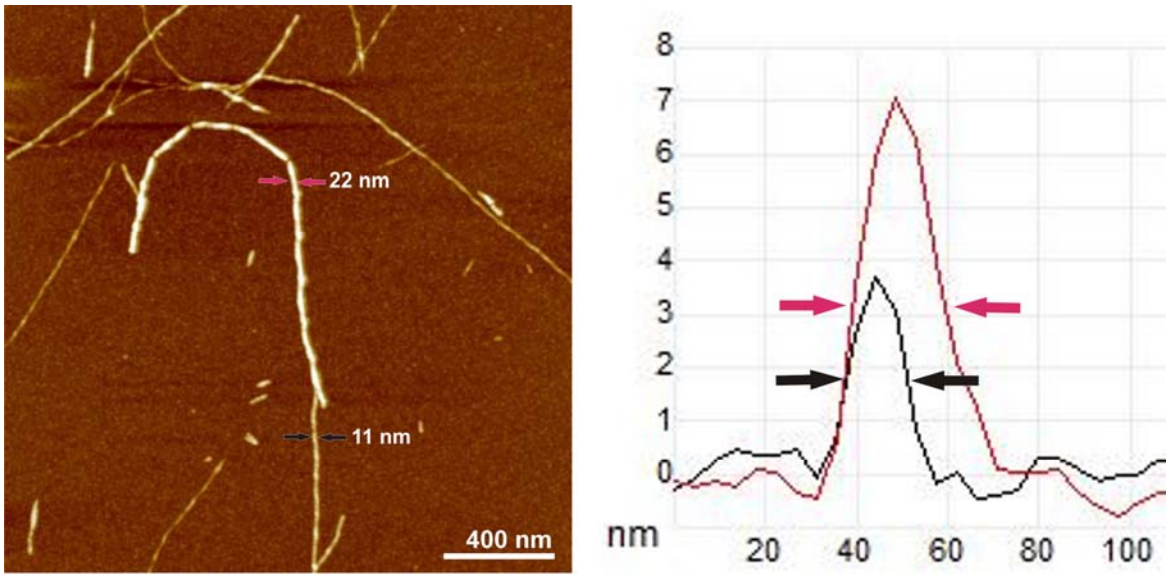


Figure S2. AFM images of coated fibrils and the section profiles. The thickness is taken as half-width of the fibrils.

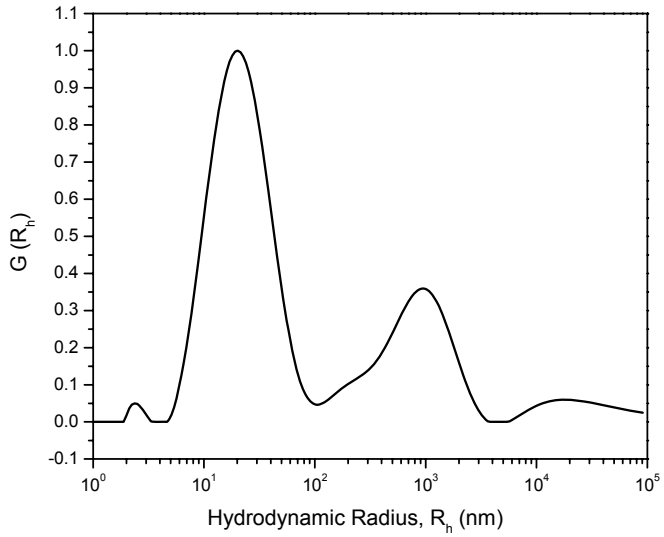


Figure S3. Hydrodynamic radius distribution of PNiPAM coated fibril clusters in region IV.