Experimental Methods

Materials

Hen egg white lysozyme (HEWL) was purchased from J&K Scientific Ltd. ThT and bovine serum albumin (BSA) were obtained from Aladdin. ANS was purchased from Macklin and HCl was purchased from Sinopharm Chemical Reagent. Copper mesh was obtained from Electron Microscopy China. Phosphotungstic acid was purchased from Bide Pharmatech Ltd. Above chemicals were used without any further purification.

Instruments

UV-vis spectra were measured on a Varian VARY 100 Bio UV-vis spectrophotometer, with a light range length of 1 cm. Photoluminescence (PL) spectra were recorded on a Shimadzu RF-5301PC spectrofluorophotometer. The electron microscope photos were taken with an H-7650 Hitachi microscope operating at 80 kV.

Preparation of protein solutions

Photophysical properties were measured directly after dissolving different masses of BSA or HEWL with deionized water.

Preparation of HEWL fibrillar aggregates

Protein solutions (20 mg/mL) were prepared in deionized water (pH 2.2) (pH adjusted with HCl), and aliquots were incubated at 65 °C for 7 days. The effect of protein gelation was eliminated by centrifugation at 12,000 rpm for 15 minutes and the supernatant was taken for testing.

CL assay

CL was used to detect amyloid aggregation, and the treated supernatant was taken to test the fluorescence directly (20 mg/mL), with excitation fixed at 365 nm. The slit widths were 10 & 5 nm when λ_{ex} =280, 300 nm of aqueous HEWL and BSA (20 mg/mL). Other excitation and emission slit widths were both set at 10 nm.

ThT assay

For monitoring the growth of HEWL fibrils, we performed ThT fluorescence assays in a mixture of 0.8 mg/mL incubated solutions and 40 μ M ThT, with excitation fixed at 440 nm and emission at 482 nm. The excitation and emission slit widths were both set at 10 nm.

ANS binding assay

Emission spectra of ANS were recorded between 400 nm and 600 nm, using an excitation wavelength of 370 nm. Aliquots of the incubated mixtures were not diluted (final concentration of 20 mg/mL) containing 20 μ M ANS. Similarly, when probing the thermal denaturation of HEWL, the protein concentration was 20 mg/mL, while the ANS concentration was 20 μ M. Excitation and emission slit widths were both set at 5 nm.

Transmission electron microscopy

Incubation samples were diluted to 0.1 mg for Transmission electron microscopy. 10 μ L of sample was adsorbed onto copper 300 mesh grid, previously covered by carbon-coated film. Absorb the solution with filter paper after ten minutes. Then, a drop of 2% phosphotungstic acid solution was added for 10 mins. Absorb the solution with filter paper and leave to dry naturally at least 30 minutes at room temperature. The samples were observed by an H-7650 Hitachi microscope operating at 80 kV.





Figure S1. A) Photographs of aqueous HEWL under UV light at different concentrations:0, 0.01, 0.1, 1, 4, 20 mg/mL;B) Photographs of aqueous BSA under UV light at different concentrations:0, 0.01, 0.1, 1, 4, 20 mg/mL.



Figure S2. PL spectra of ANS in aqueous HEWL at different temperature.



Figure S3. TEM photo of HEWL amyloid fibrils at 7 days x20,000.