Supporting Information

About the mechanism of ultrasonically induced protein capsule formation

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Experimental Section

Materials: Bovine serum albumin (BSA, > 95%) was purchased from Alfa Aesar (Germany). Toluene (≥ 99,5%) was purchased from Th. Geyer (Germany). Dithiothreitol (DTT, ≥ 99%) was purchased from Carl Roth (Germany). All chemicals were used without further purification.

Preparation of protein microcapsules: The microcapsules were prepared by following Suslick's method.[1] In a cylindrical vessel, 1.4 mL toluene was layered over 2.1 mL of a 5% w/v BSA solution. The used ratio of aqueous/organic phase was 3:2. A high-intensity ultrasonic horn with a tip diameter of 2 mm was placed at the oil-water interface. To maintain the temperature below 30°C during ultrasonication, the vessel was positioned in an ice bath. The solutions were sonicated for 3 minutes at an acoustic power of $\sim$ 200 W cm^{-2}. Simultaneously, the solutions were mixed with a magnetic stirrer. The obtained microcapsules were dialyzed against distilled water with pH of 6.2 ± 0.3 using a dialysis tube with a cutoff of 1000 kDa (Spectrum Labs Spectra/Por Dialysis Membrane Biotech CE) to remove residual chemicals and fragments of broken microcapsules.

Mixing experiments by the use of a Vortex mixer: In a cylindrical vessel, 1.4 mL toluene was layered over 2.1 mL of a 5% w/v BSA solution. The used ratio of aqueous/organic phase was 3:2. The solutions were shaken by a Vortex mixer for 3 minutes at moderate intensity.

Fluorescence Microscopy: Fluorescence Microscopy images were taken by a Leica DMi8 microscope and the resulted images were processed via Leica Application Suite X software provided by Leica.

Cryo Scanning Electron Microscopy: Characterization with cryo scanning electron microscopy was done on a S-4800 (Hitachi) with an acceleration voltage of 2 keV.

Sodium dodecylsulfate polyacrylamide gel electrophoresis: Intermolecular disulfide bonds should form covalent oligomers that can be verified by their size and should be cleaved by reducing agents. Thus, the samples were analyzed by reducing and non-reducing sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE). The sample of empty capsules as well as one of pristine BSA as control were mixed with non-reducing buffer (ROTILoad 2, Carl Roth) with or without addition of DTT (100 mM) and separated using a gradient gel (4 - 15 %, Bio-Rad) at constant current of 25 mA. Each lane of SDS-PAGE assay was loaded with the same amount (13.5 µg) of abovementioned samples. Afterwards, the gel was stained with Coomassie Brilliant Blue R-250 (Applichem). A protein ladder (ROTIMark TRICOLOR XTRA, Roth) was used to determine the size of the samples.

Cleavage of the disulfide bonds with DTT for Raman spectroscopy: The protein microcapsules were prepared as described before. A 1 M DTT solution was added and the mixture was stirred for 10 minutes. Subsequently, the solution was dialyzed again against distilled water. BSA was treated the same way.

Raman spectroscopy: All Raman data was acquired using a Witec Alpha 300 Raman microscope system (Witec, Ulm, Germany) equipped with a spectrograph (600 gr/mm grating) and an Andor DU401A-BR-DD-352 CCD camera (Andor, Concord, Massachusetts, USA). An Olympus MPlanFL-N 100x objective (N.A. = 0.9, Shinjuku-ku, Tokyo, Japan) and 488 nm and 532 nm excitation lasers were utilized during the measurements. Before measurements, the power density was adjusted to 3.7×10^4 W/cm^2 for the 488 nm laser (the estimated spot size is 1.32 µm). The dried samples were placed on empty microscope slides and slightly pressed and flattened using a spatula. Slides were put under the microscope and the signal was acquired by measuring single point spectra: The sample of dried BSA-toluene-capsules cleaved with DTT was measured using 10 s integration time and averaging over 100 spectra, the dried BSA sample with 12 s and 20 spectra averaging and the dried, non-cleaved BSA-toluene-capsule sample with 8 s and 30 spectra averaging. The toluene spectrum was also recorded using 1 s integration time and averaging 40 spectra and it was used as an internal standard to check the instrument calibration over the measurement range.

Dynamic light scattering: The intensity weighted mean diameters (Z-average) of pristine monomeric BSA, empty BSA capsules and BSA chemically cross-linked with glutaraldehyde (GA) were measured by a Zetasizer Nano ZS (Malvern Panalytical). The samples were diluted and filtered with a 0.2 µm PVDF membrane. BSA was chemically cross-linked by shaking a mixture of 1 mg/ml BSA and 0.5% glutaraldehyde overnight.

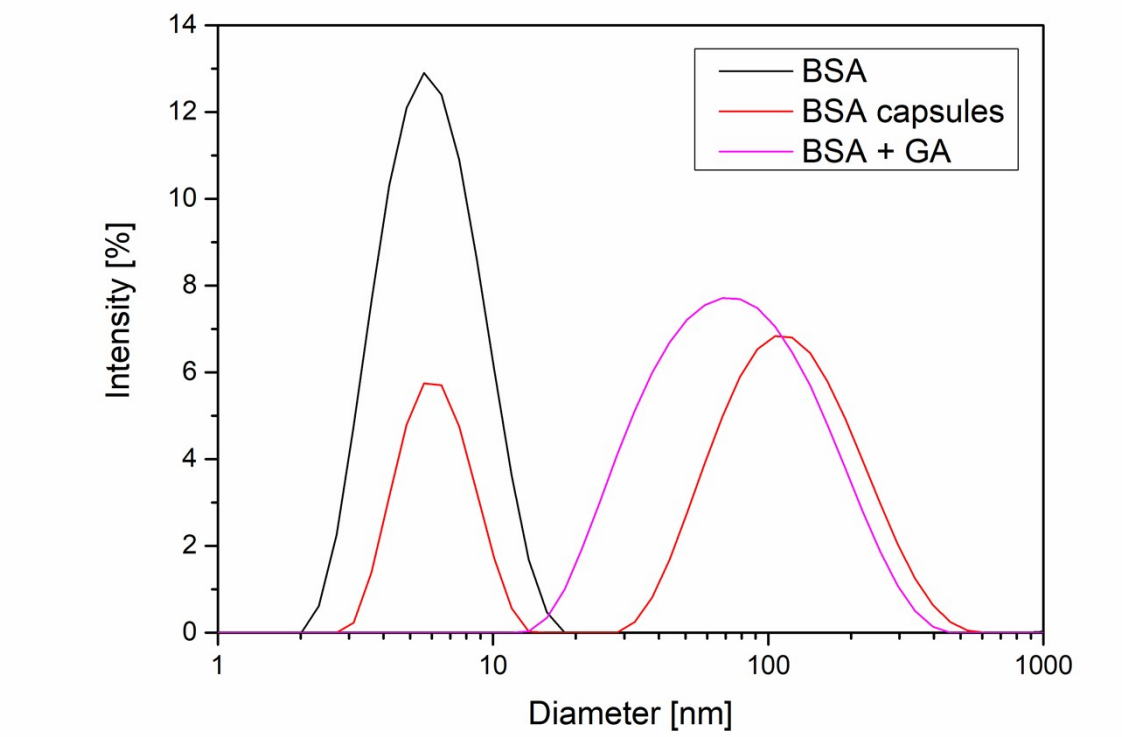

Figure S1. Results of DLS measurements for pristine monomeric BSA, empty BSA capsules and BSA chemically cross-linked with glutaraldehyde (GA).

References

1 K. J. Liu, M. W. Grinstaff, J. Jiang, K. S. Suslick, H. M. Swartz and W. Wang, *Biophys. J.*, 1994, **67**, 896.