Supporting information

Ovalbumin as an Outstanding Pickering Nano-stabilizer for High Internal Phase Emulsions

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1. Experimental Section

1.1. Characteristization of OVA

The particle size distribution and hydrodynamic diameter (D_h) of OVA (from chicken egg; 95%, Sigma Aldrich) were determined using the dynamic light scattering (DLS) technique. DLS analysis was conducted at a fixed angle of 173° using a Zetasizer Nano-ZS instrument (Malvern Instruments Ltd., Malvern, Worcestershire, UK) equipped with a 4 mW He-Ne laser (633 nm wavelength) at 25 °C. All the tested protein solutions were filtered using a 0.22 μm filter, prior to determination. The dispersion medium was set as water. And the ξ-potential of the protein was measured in an electrophoresis cell (Model DTS 1060C, Malvern Instruments Ltd.) using the same Nano-ZS instrument connected with a multipurpose autotitrator (model MPT-2, Malvern Instruments Ltd.) at 25 °C.

The relative surface hydrophobicity (H_o) was evaluated by fluorescence spectroscopy using 1-anilinonaphthalene-8-sulfonate (ANS^-; Sigma Aldrich) as a fluorescent probe, using a F-7000 fluorescence spectrophotometer (Hitachi Co. Ltd, Japan), according to the methods of Delahaije et al. [1] and Xiong et al. [2], with slight modifications. In brief, for each operation, 50 μL of 8 mM ANS^- solution were added into 5 mL of protein solution. The mixed solution was excited at 385 nm, and the emission spectrum was scanned from 400 to 650 nm (25 °C). Then relative surface hydrophobicity could be evaluated by the area of the fluorescence spectrum of each protein, which was corrected via subtracting the area of the fluorescence spectrum of corresponding protein solution without ANS^-. The area of the fluorescence spectrum was expressed using ave (S_3) calculated by FL solutions software (Hitachi Co. Ltd, Japan).

The hydrodyanmic size, ζ-potential and surface hydrophobicity, of OVA were summarized in Supplemenatary **Table S1**.

References

[1] R. J. Delahaije, P. A. Wierenga, N. H. van Nieuwenhuijzen, M. L. F. Giuseppin, H. Gruppen, *Langmuir* **2013,** *29*, 11567.

[2] W. Xiong, Y. Wang, C. Zhang, J. Wan, B. R. Shah, Y. Pei, B. Zhou, J. Li, B. Li, *Ultrason. Sonochem.* **2016**, *31*, 302.

Supplementary Table S1.
Some physicochemical parameters of OVA. Hydrodynamic diameter (D_h), ζ-potential and surface hydrophobicity (H_o) of OVA are presented.

Protein type	D_h (nm)	ζ-potential (mV)	H_o (c = 0.5 wt%)	H_o (c = 0.1 wt%)
OVA	5.15 ± 1.27	-20.6 ± 0.9	2306 ± 83	578 ± 32

Data are reported as means ± S.D. (n =3).