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Electronic supplementary information
for

Effect of the stiffness of one-layer protein-based microcapsules on dendritic cell uptake and endocytic mechanism

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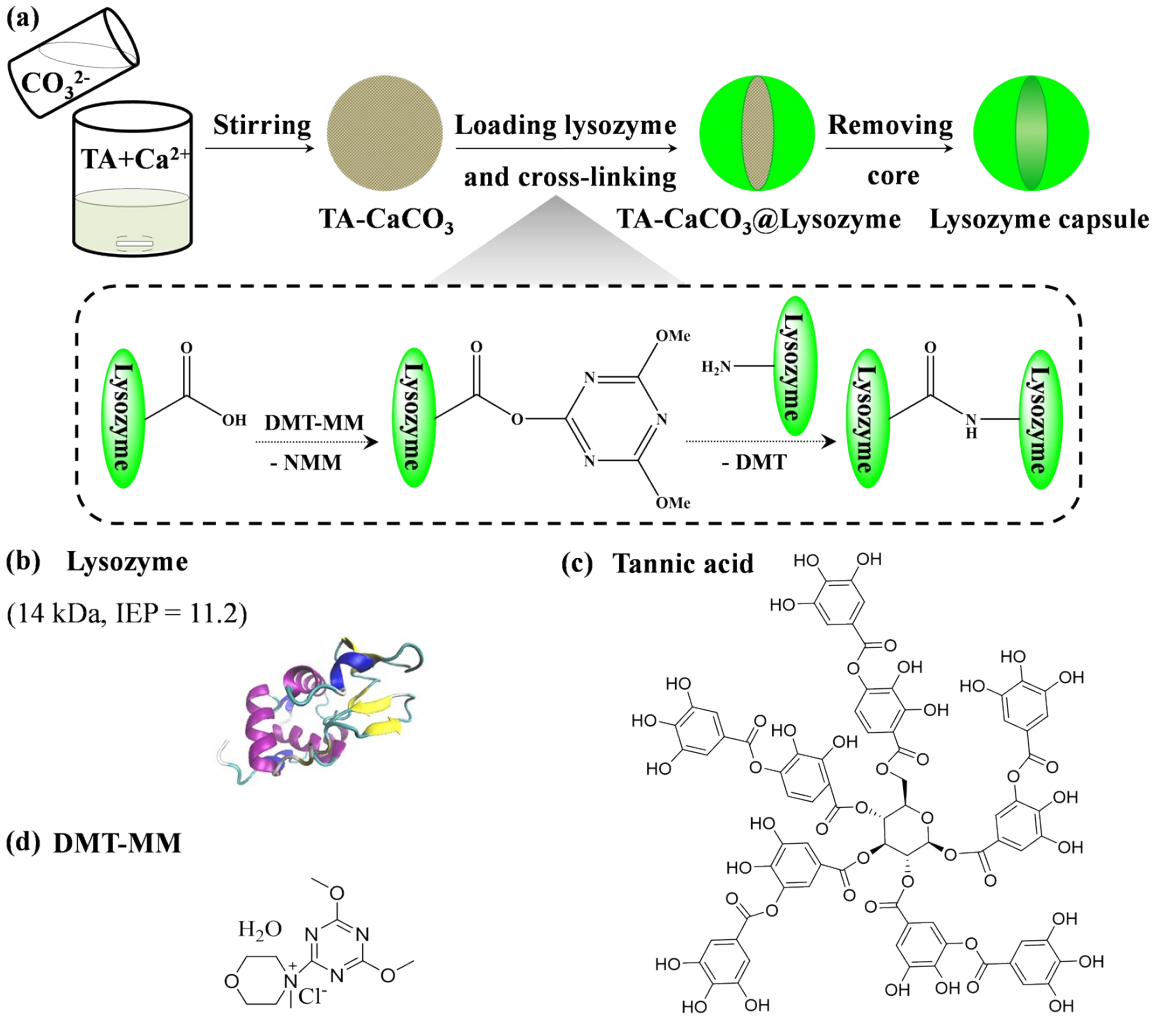

Figure S1. (a) Schematic illustration of the fabrication process of lysozyme capsules. Protein cross-linking mechanism via DMT-MM (insert section). (b) Cartoons of lysozyme molecule (1VDQ), which was referenced from the protein database files (http://www.rcsb.org). Molecular structures of (c) tannic acid (TA) and (d) 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl morpholinium chloride (DMT-MM).

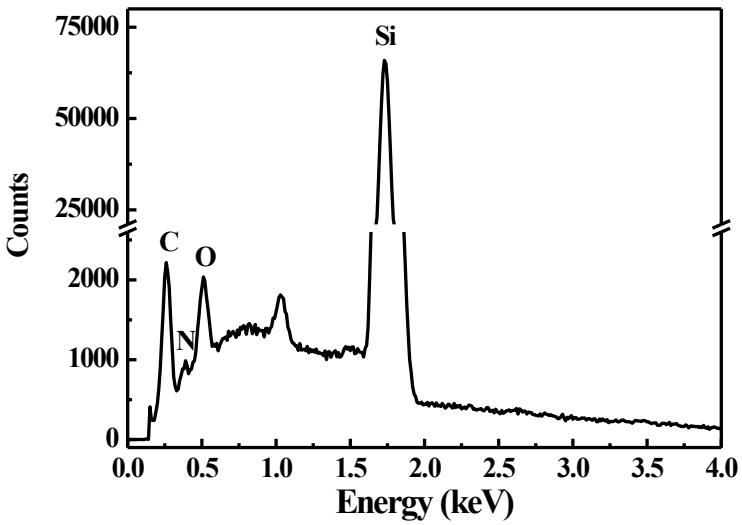

Figure S2. EDX analysis of lysozyme capsules.

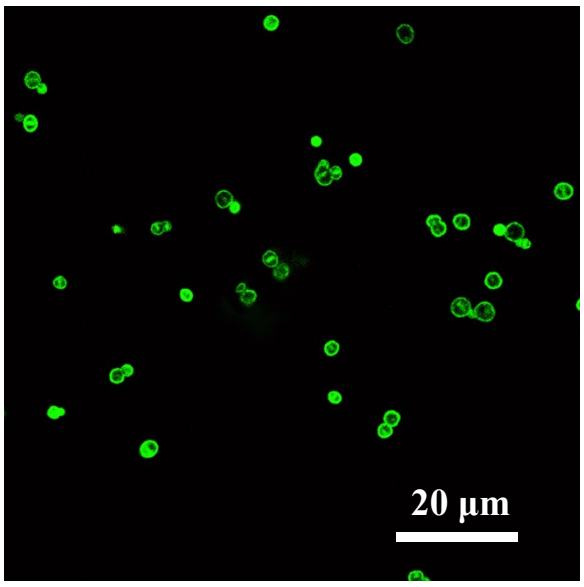

Figure S3. LSCM images of FITC-lysozyme capsules incubated with aqueous solution (pH 7.4) for three months at 4°C.

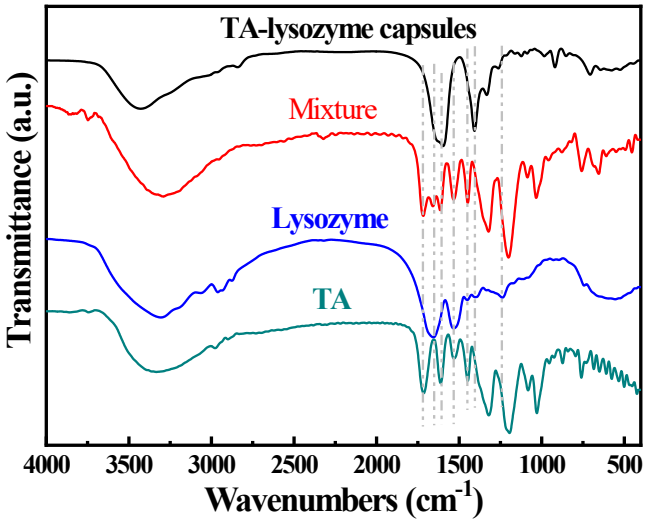

Figure S4. FT-IR spectra of TA, lysozyme, mixture of TA and lysozyme, and lysozyme capsule, respectively.

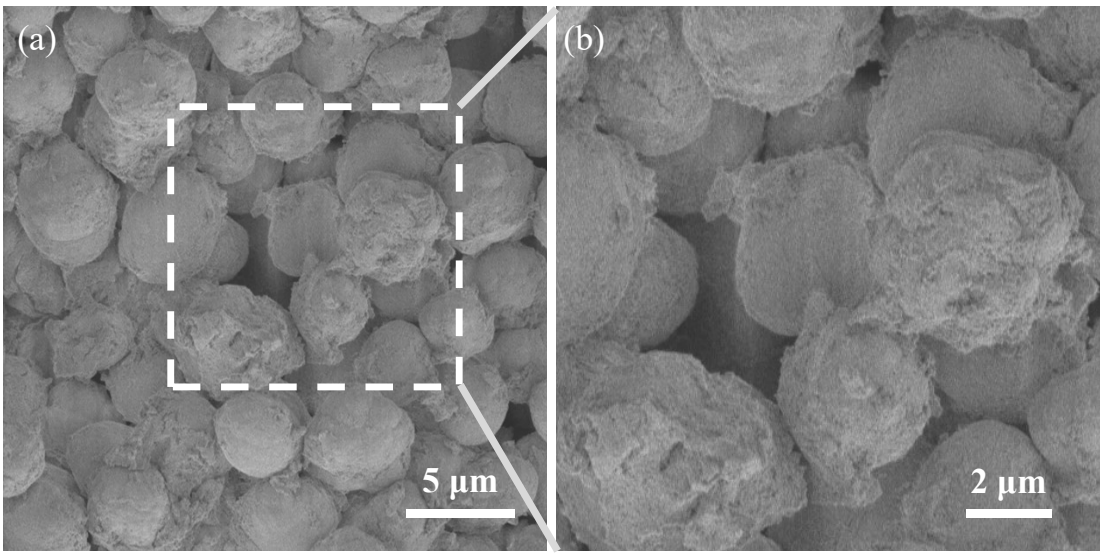

Figure S5. (a) Overview and (b) magnified SEM images of TA-200/$CaCO_3$ particles.

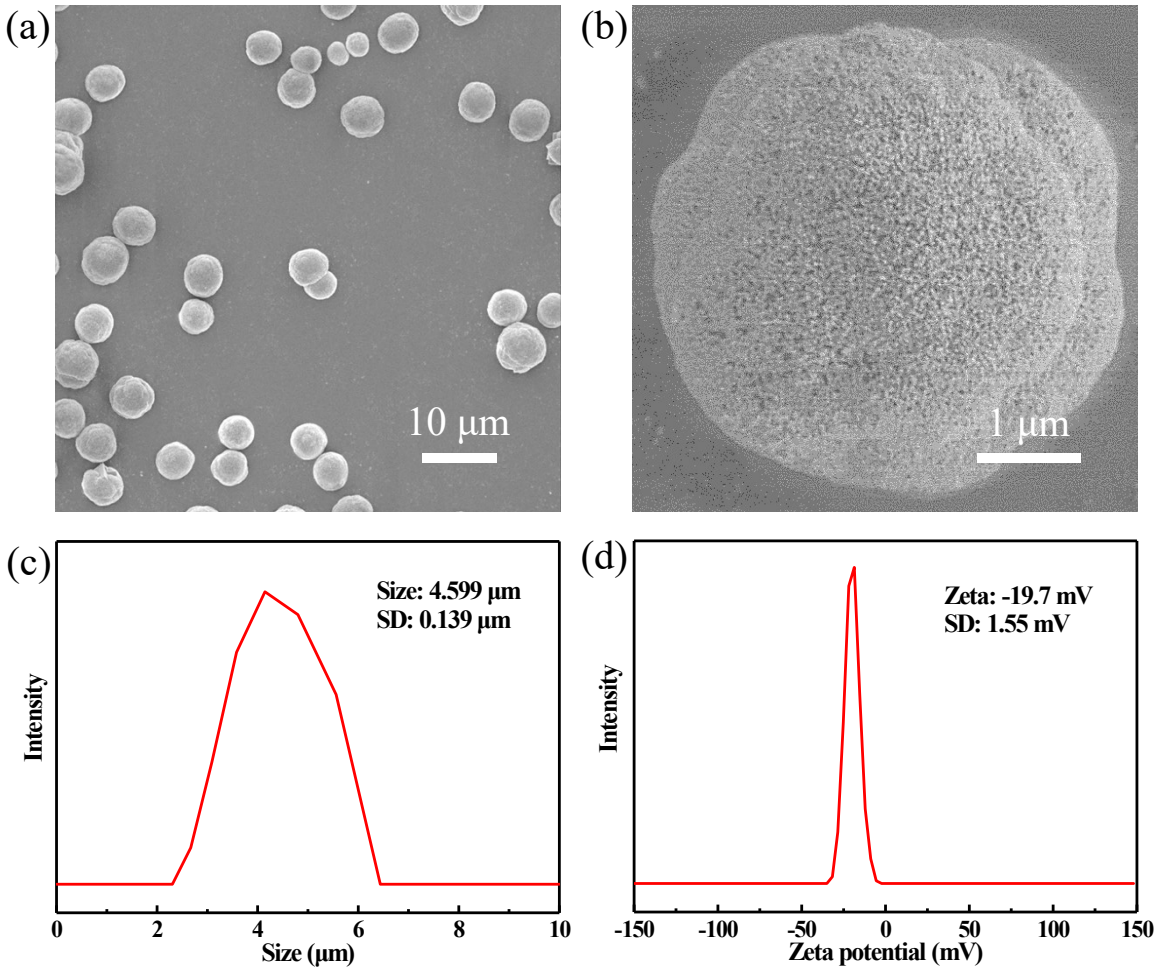

Figure S6. (a) Overview and (b) magnified SEM images of TA-50/$CaCO_3$ particles; (c) the size distribution and (d) zeta potential of TA-50/$CaCO_3$ particles at pH 7.0.

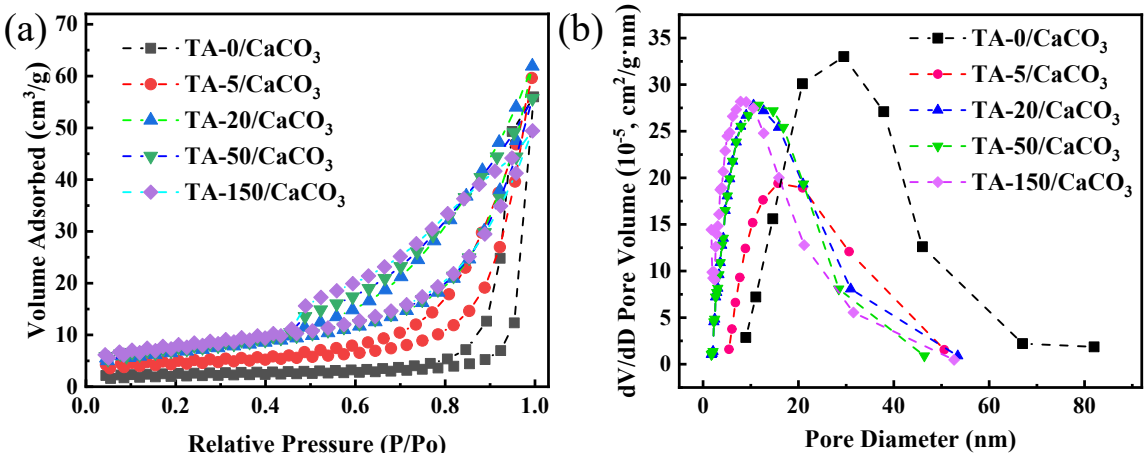

Figure S7. (a) N_2 adsorption-desorption isotherms of TA/$CaCO_3$ microparticles; (b) BJH average pore size distributions of TA/$CaCO_3$ particles.

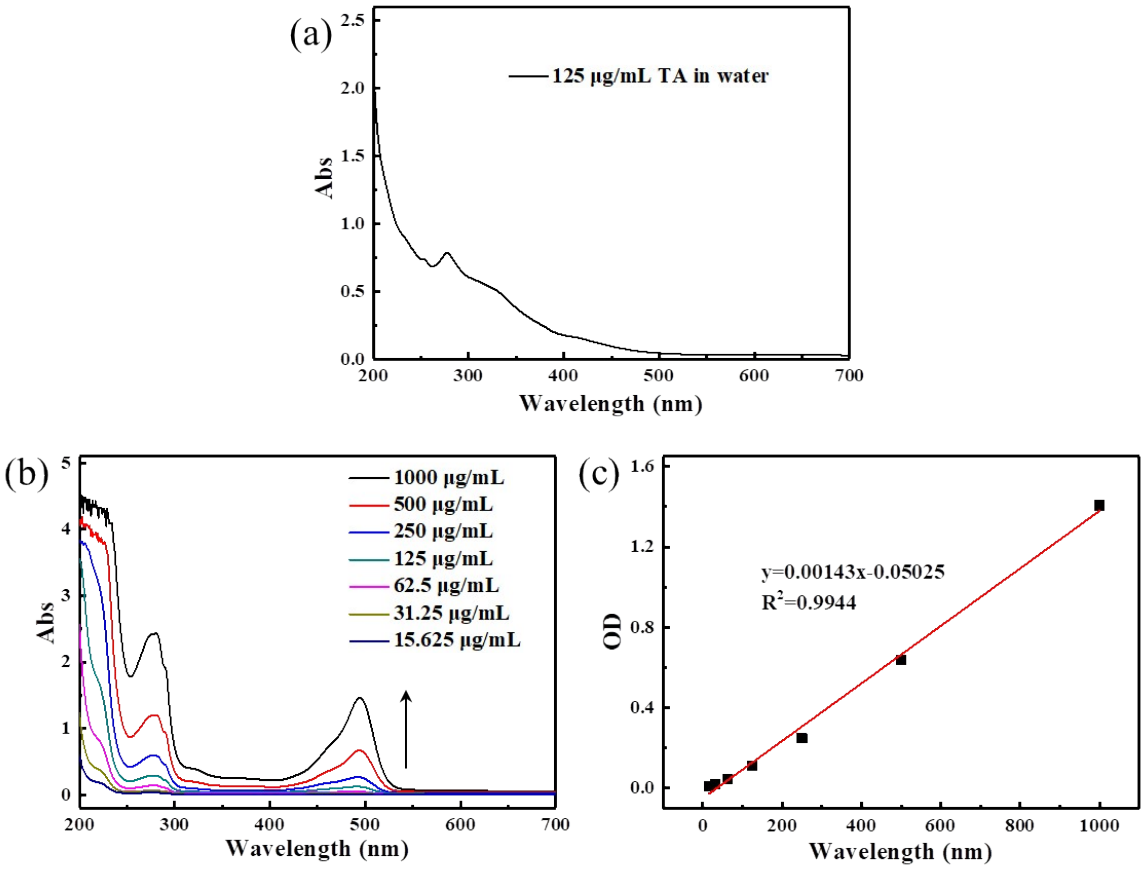

Figure S8. UV-vis spectra of TA in water (a, 125 μg/mL) and FITC-lysozyme with diluted solutions (b, 15.625-1000 μg/mL). (c) The standard cure of lysozyme at 494 nm (R^2 = 0.9944).

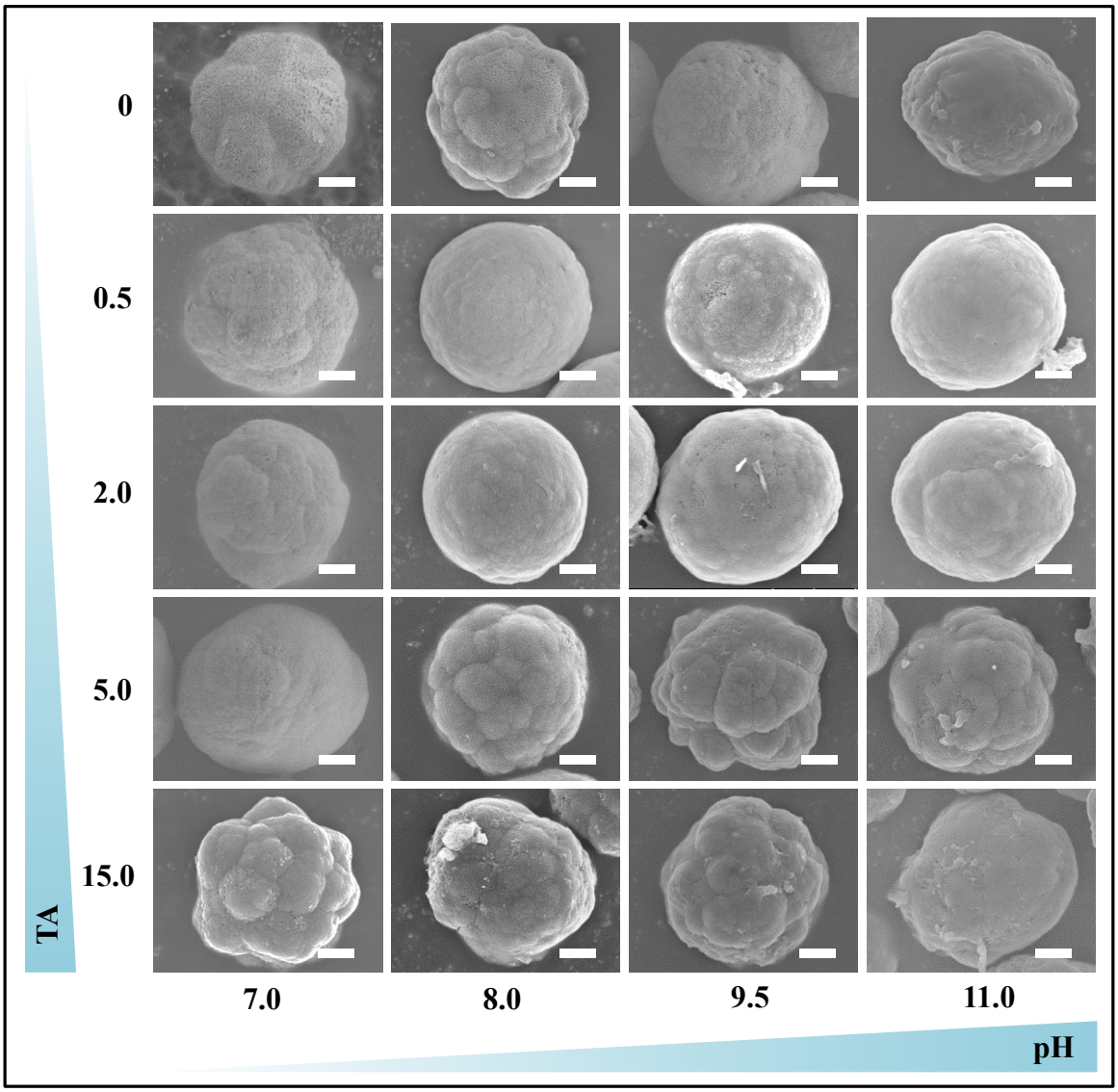

Figure S9. SEM images of lysozyme adsorption on particles at different doping contents of TA and pH values of lysozyme solution. All scale bars are 1 μm.

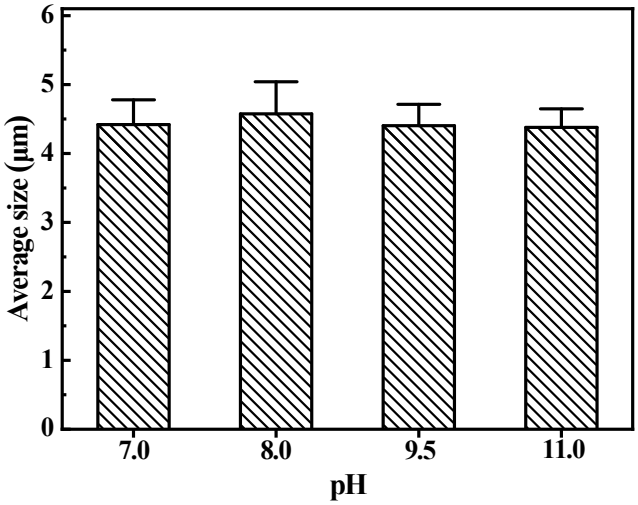

Figure S10. The average size of lysozyme capsules prepared under pH 7.0, 8.0, 9.5, and 11.0. For SEM images of capsule, 100 capsules were measured and their average size was calculated in each group.

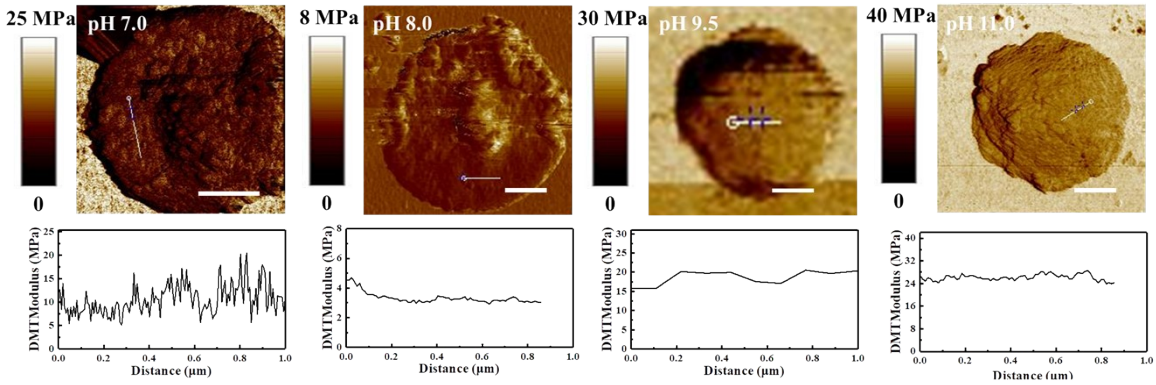

Figure S11. Modulus images and their corresponding sections of lysozyme capsules prepared at pH 7.0, 8.0, 9.5, and 11.0 and tested by AFM in water. All scale bars are 1 μm.

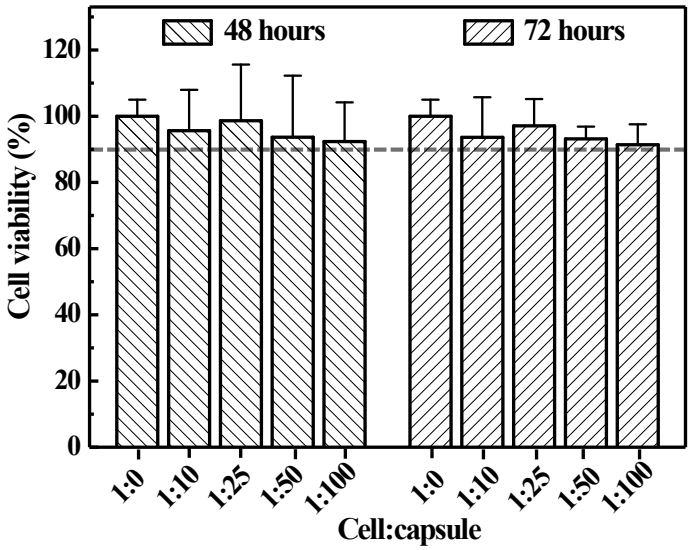

Figure S12. CCK-8 assay of lysozyme capsules on DC cells. Cells were incubated at the indicated capsule ratio for 48 h or 72 h. The results are average values with standard deviations (mean ± S.D., N =5).

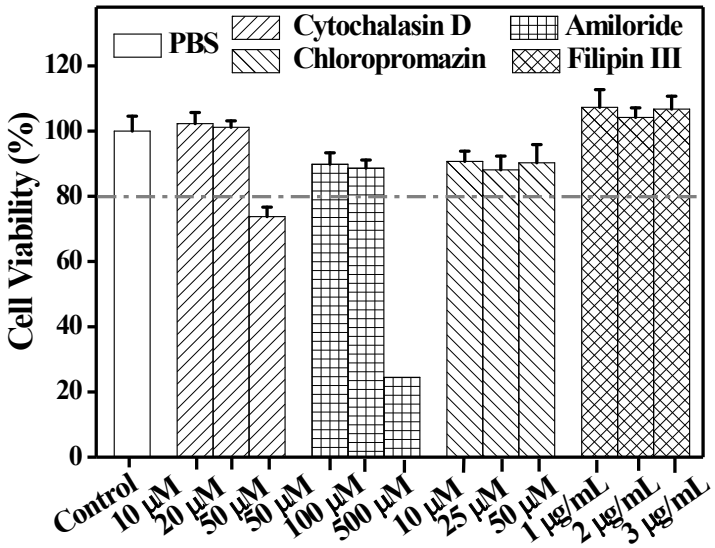

Figure S13. CCK-8 assay of inhibitors, including cytochalasin D, amiloride, chloropromazin, and filipin III at indicated concentrations on DC cells for 48 hours. The results are average values with standard deviations (mean ± S.D., N =5).

Table S1. Illustration of the different cellular organelles/structures studied for their co-localization with the FITC-lysozyme capsules as well as their associated uptake pathways. The antibody IgG (H+L) or markers used for staining as well as their concentrations (C_{final}) used are additionally presented.

Cellular structure	IgG/markers	C_{final}	Endocytic pathway	Co-localization
Phagolysosomes	Primary, rabbit anti-LAMP 1[a] antibody	1.5 μg/mL	Phagocytosis	Yes
	Secondary, Alexa Fluor 647-labeled goat anti-rabbit	1.3 μg/mL		
Clathrin-coated vesicles	Primary, rabbit anti-clathrin heavy chain antibody	7.5 μg/mL	Clathrin-mediated endocytosis	No
	Secondary, Alexa Fluor 647-labeled goat anti-rabbit	20 μg/mL		
Caveolin-coated lipid rafts	Primary, rabbit anti-caveolin-1 antibody	4 μg/mL	Caveolin-mediated endocytosis	No
	Secondary, Alexa Fluor 647-labeled goat anti-rabbit	3.5 μg/mL		
Nucleus	DAPI	50 μM	n/a.	n/a.

[a] Lysosomal-associated membrane protein 1.

Table S2. Illustration of the different habitation used to study their uptake pathways of lysozyme-based capsules.

Inhibitor	Concentration	Endocytic pathway	Quantification of inhibition
Cytochalasin D	20 μM	Phagocytosis	89.3~92.1%
Amiloride	50 μM	Micropinocytosis	60.2~75.5%
Chlorpromazine	10 μM	Clathrin-mediated endocytosis	4.9~47.2%
Filipin III	1 μg/mL	Caveolin-mediated endocytosis	2.8~40.7%