This Supplementary Information file replaces the version that was published on 15 February 2024. The following changes have been made: (i) in the "Materials and methods" section, "…red fluorescent channel at 530 nm for RhB…" has been revised to "red fluorescent channel at 561 nm for RhB", (ii) the starting position of the y-axis in Fig. S11 has been adjusted to 0 and (iii) minor typographical errors have been corrected throughout the file.

# Supporting Information

# Pickering emulsion templated proteinaceous microparticles as glutathione-responsive carriers for endocytosis in tumor cells

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## Experimental section

### Materials and methods

Zein (grade Z3625), fluorescein isothiocyanate (FITC), and FITC-dextran (70 kDa) were purchased from Sigma-Aldrich (USA). Glucose oxidase (GOx) was supplied by J&K Scientific Co., Ltd (China). Actin-tracker red-rhodamine, amplex red and thiazolyl blue tetrazolium bromide (MTT) were bought from Beyotime Biotechnology Co., Ltd (China). Tannic acid (TA), hydrochloric acid (HCl), ethanol (AR, >99.7%), and n-hexane were supplied by Sinopharm Chemical Reagent Co., Ltd (China). Dodecane, rhodamine B (RhB), citric acid, and glutathione (GSH) were acquired from Macklin Biochemical Co., Ltd (China). Calcium carbonate nanoparticles were purchased from Jiangsu Xianfeng Nanomaterials Technology Co., Ltd (China). Perylene, resorufin, and horseradish peroxidase (HRP) were obtained from Aladdin Chemical Reagent Company (China). Dulbecco's modified Eagle's medium (DMEM) and phosphate buffered saline (PBS) were acquired from HyClone Laboratories Co., Ltd (USA). Fetal bovine serum (FBS) was supplied by Gibco Co., Ltd (USA). 4',6-diamidino-2-phenylindole (DAPI) was purchased from Meilun Biotechnology Co., Ltd (China). All chemicals were used as received. Deionized water was used in all experiments.

The morphology of the microparticles was determined by scanning electron microscopy (SEM; Hitachi S-4800; Japan) and optical microscopy (Nikon, Ni-U Japan). The size distribution of microparticles was measured by dynamic light scattering technique (DLS; ZetaPALS, America). All fluorescence images were obtained by confocal laser scanning fluorescence microscopy (CLSM; Nikon AX, Japan), exciting the green fluorescent channel at 488 nm for FITC, red fluorescent channel at 561 nm for RhB, and blue fluorescent channel at 405 nm for Perylene. The size analysis of particles was calculated by Image J software. A fluorescence spectrometer (CARY Eclipse; Varian; America) was used to measure fluorescence intensity. The contact angle was conducted by video optical contact angle measuring instrument (OCA15EC, Dataphysics, Germany). The FT-IR analysis measurements were carried out with an infrared spectrometer (Nicolet iS50, Thermo Fisher Corp.) in the range of 500–4000 $cm^{-1}$ with a resolution of 4 $cm^{-1}$ and an accumulation of 32 scans.

### Synthesis of $CaCO_3$/zein microparticles

According to our earlier study, a similar method was used to make the hybrid microparticles. To begin, a specific quantity of zein powder was ultrasonically dissolved in a 70 vol% ethanol-water solution to create a 20 wt% zein stock solution. The oil phase was prepared by mixing 1 g of hydrophobic $CaCO_3$ nanoparticles with 20 mL of dodecane. Emulsification was carried out by adding 1 mL of the zein stock solution to 20 mL of the oil phase by sonication (10 min, 600W). A Pickering double emulsion that had already been created was then quickly transferred to a flask. Using reduced pressure rotary evaporation, the ethanol was evaporated while hybrid zein-based microparticles progressively formed. To get rid of the inner oil, the hybrid microparticles were rinsed with n-hexane several times. After vacuum drying at 50°C for 12 h, the microparticles were finally recovered.

### Preparation of $CaCO_3$/zein microparticles loaded with bioactives

According to the above-described procedures, firstly, adjust the zein solution to pH 7.0, then 800 μL of FITC-dextran or GOx solution (5 mg/mL) were diluted and subsequently added into zein solution with ultrasonic. Afterwards, following the steps above ultrasonic emulsification and rotary evaporation were performed.

### Construction of TA/zein microparticles

The prepared $CaCO_3$/zein microparticles were ultrasonically dispersed in the TA dispersion to explore the factors of proportions of TA and microparticles (1:1, 5:1, 10:1) on its dispersion in aqueous solution.

### In vitro release of TA/zein microparticles

FITC-dextran was encapsulated in the TA/zein microparticles. The release percentage of FITC-dextran upon GSH (0, 5, 10 mM) with time was measured by fluorescence spectrometer under 525 nm of emission wavelength.

**Catalytic activity of GOx@TA/zein microparticles**

The reaction cocktail was prepared using Amplex red (25 μM) dissolved in DMSO, HRP solution (25 μM), and glucose solution (0.25, 0.5, 1, 2.5, 5, 10 mM) for 30 min. To assay the activity of the microparticles, the reaction mixture (150 μL) was placed in a well of a 96-well plate, then the microparticles' dispersion (50 μL, 0, 1, 2.5, 5, 10, 25, 50 μg/mL) was added, and the changes in absorbance at 570 nm were monitored by a TECAN plate reader (infinite F200, Swiss).

**Cell Culture**

The HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 100 mg/mL penicillin, 100 mg/mL streptomycin, and 10% fetal bovine serum (FBS). The cells were incubated at 37°C under a humidified atmosphere of 5% $CO_2$ and 95% air.

**Cellular Uptake of TA/zein microparticles**

HeLa cell suspensions were inoculated in laser confocal culture dishes at a density of $1\times10^5$ cells per well. The culture dishes were incubated in the incubator for 24 h. After the cells were plastered for 24 h, the culture medium was removed, and FITC-dextran-loaded microparticles were added and incubated for 3 h, 6 h, 12 h, and 24 h, respectively. The culture medium was aspirated and discarded, followed by 3 x wash with PBS. The cells were then fixed with 4% paraformaldehyde solution for 20 minutes, followed by 3 x wash with PBS. After that, the cell nuclei were stained with DAPI for 30 minutes, followed by 3 x wash with PBS. Subsequently, the cell membranes were labeled with actin-tracker red-rhodamine for 30 minutes, followed by another 3 x wash with PBS. The samples were then observed using confocal laser scanning microscopy (CLSM).

**MTT assay**

HeLa cell suspension was inoculated into 96-well culture plates with a density of $1\times10^4$ cells per well in 100 μL of culture medium. The inoculated cells were placed in a cell incubator for 24 h, and the medium in each well was discarded after adherence. Then 100 μL of medium containing GOx-loaded TA/zein microparticles of different concentrations was added, and the culture was continued for 24 h. After 24 h, 20 μL 5 mg/mL MTT serum-free medium was added to each well, and the culture was continued. 4 h later, the medium in each well was carefully absorbed and discarded, 100 μL DMSO was added, and the crystals were fully dissolved by shaking for 10 min. The absorbance value of each well was measured at the absorption wavelength of 490 nm in a microplate tester (infinite F200, Swiss).

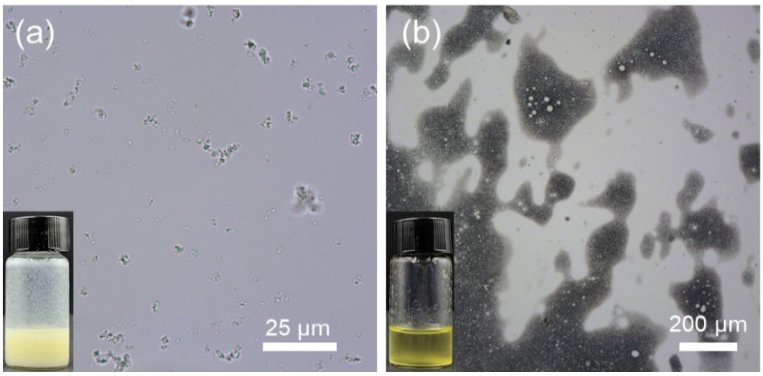


**Fig. S1** Photographs and optical microscope images of emulsions stabilized by hydrophobic $CaCO_3$ nanoparticles and zein (a), only zein (b).

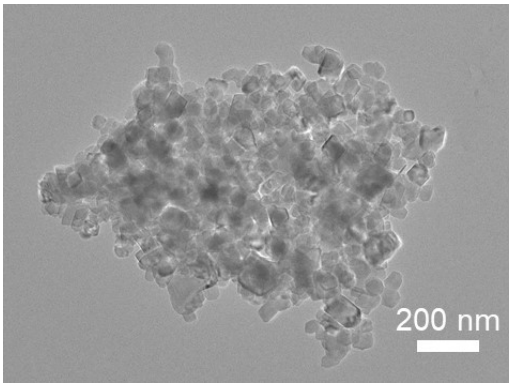


**Fig. S2** TEM image of the $CaCO_3$/zein microparticle.

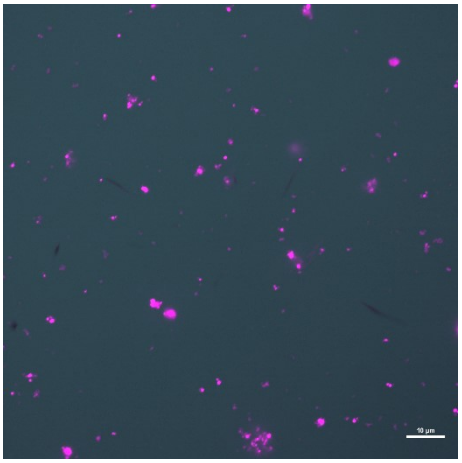

**Fig. S3** CLSM images of the dispersion of the $CaCO_3$/zein microparticles: zein and oil phases were separately labelled with rhodamine B (red channel) and perylene (blue channel).

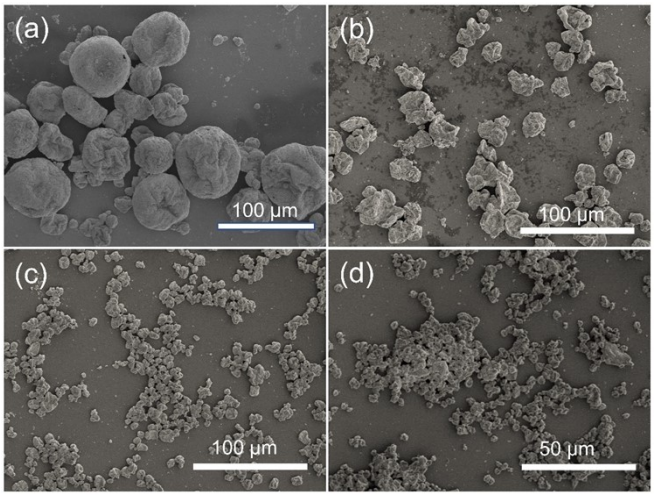


**Fig. S4** SEM images of the $CaCO_3$/zein microparticles were obtained with varying concentrations of particulate stabilizers: 0.5 wt% (a), 1 wt% (b), 2.5 wt% (c), and 5 wt% (d).

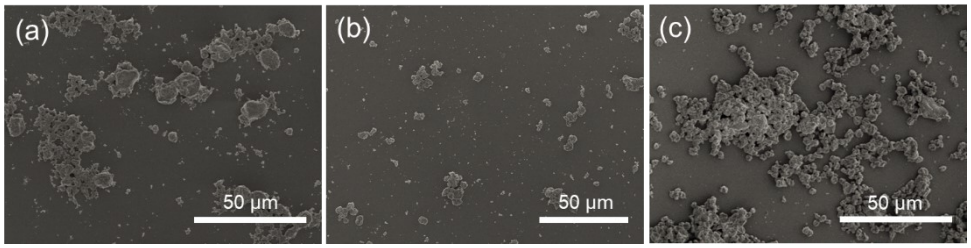


**Fig. S5** SEM images of the $CaCO_3$/zein microparticles were obtained with a homogenizing speed of 17000 rpm (a), as well as under different ultrasonic powers: 200 W (b) and 600 W (c).

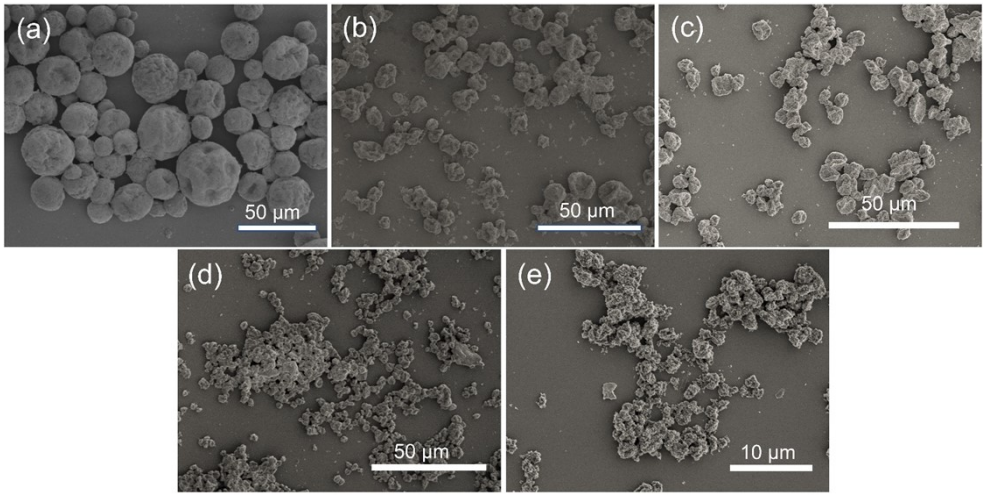


**Fig. S6** SEM images of the $CaCO_3$/zein microparticles were obtained with varying volume ratios of (ethanol/water) to oil: 0.5 (a), 0.33 (b), 0.2 (c), 0.1 (d), and 0.05 (e).

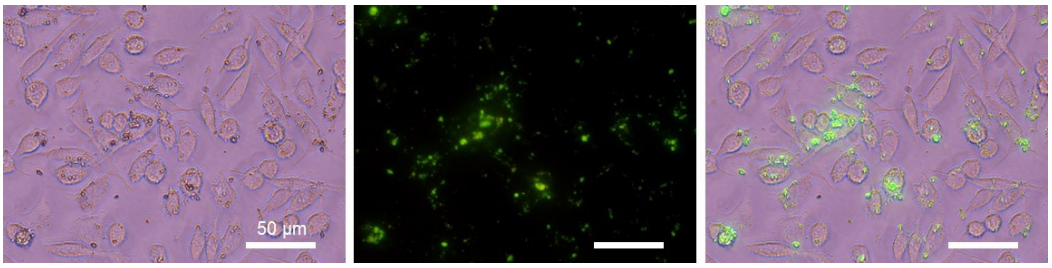


**Fig. S7** Fluorescence microscope images of the $CaCO_3$/zein microparticles after HCl treatment cultured with HeLa cells.

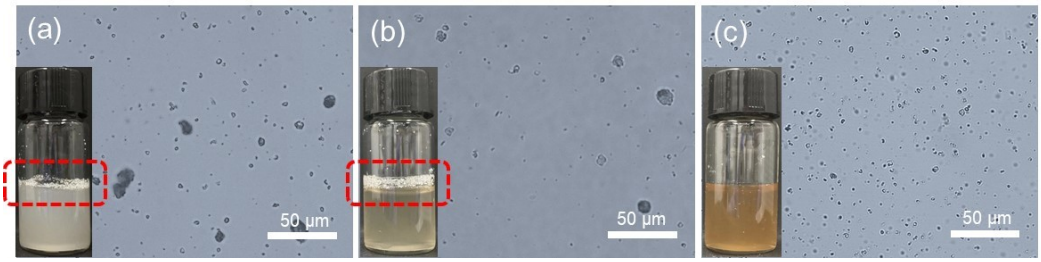


**Fig. S8** Optical microscope images of the $CaCO_3$/zein microparticles treated by different ratios of tannic acid to the microparticles: 1:1 (a), 5:1 (b), and 10:1 (c).

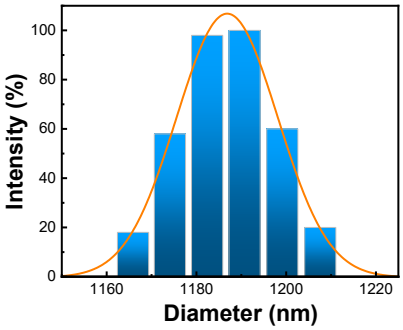


**Fig. S9** Size distribution of the TA/zein microparticles.

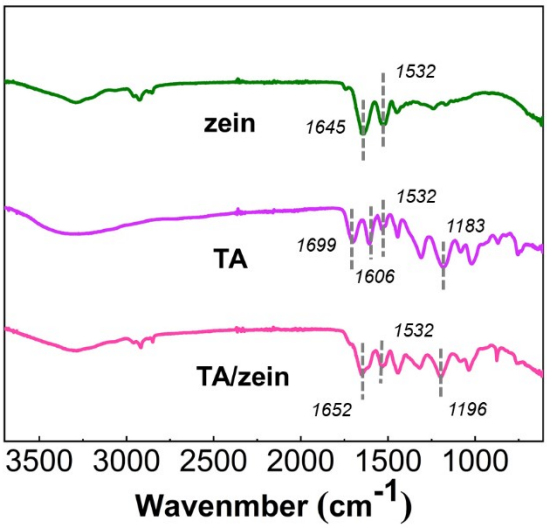


**Fig. S10** FT-IR spectra of zein powder, tannic acid powder, and the TA/zein microparticles.

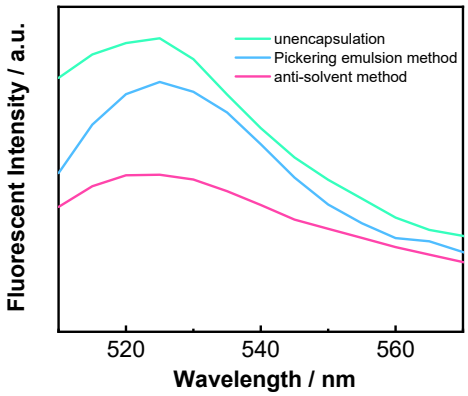


**Fig. S11** Fluorescence intensity of FITC-dextran fully released under different encapsulation methods.

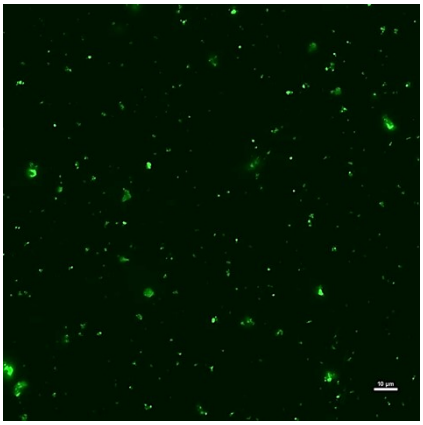

**Fig. S12** CLSM image of the TA/zein microparticles loaded with FITC-dextran.

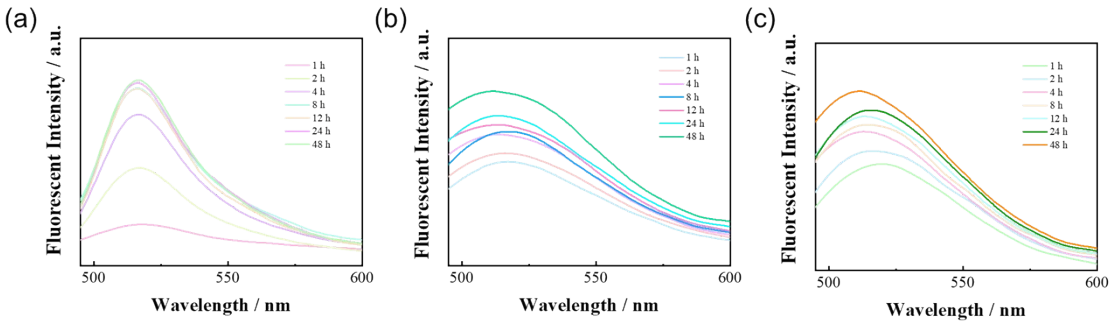


**Fig. S13** Fluorescence intensity versus time for the release of FITC-dextran from the TA/zein microparticles at different GSH concentrations: (a) 0 mM, (b) 5 mM, and (c) 10 mM.

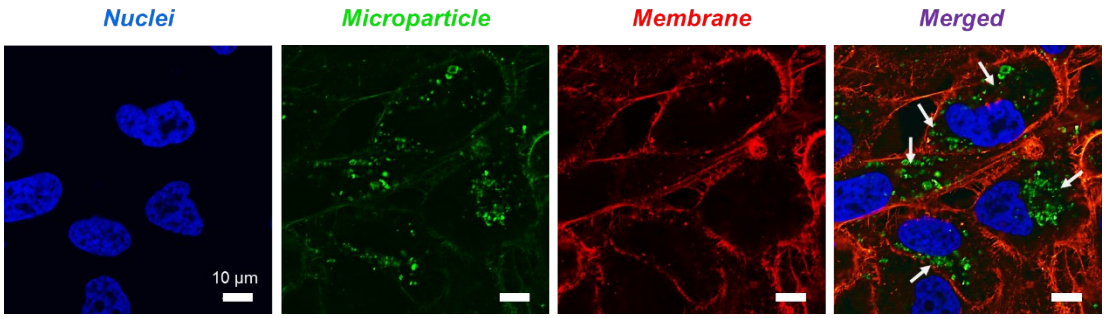


**Fig. S14** CLSM images of intracellular uptake of FITC-dextran-loaded microparticles by HeLa cells. Cells were counter-stained with DAPI (for nuclei) and actin-tracker red-rhodamine (for membrane).