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In-Situ Assembled ZIF Superstructures via an Emulsion-Free Soft-Templating Approach

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1. Experimental Section

Materials. All the chemicals and solvents were used as received without further purification. $ZnSO_4 \cdot 7H_2O$, $CoSO_4 \cdot 7H_2O$ and 2-Methyl-imidazole were purchased from sigma Aldrich. Bovine serum albumin (BSA), Catalase, Horseradish peroxidase (HRP), lysozyme, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), N-hydroxysuccinimide (NHS), Rhodamine B isothiocyanate and Fluorescein isothiocynate (FITC) were purchased from sigma Aldrich. Methanol was procured from Merck.

Preparation of ZIF-8 Colloidosome:

2-Methyl-imidazole (5 mmol) was dissolved in 15 mL methanol and added to (0.5 mmol) $ZnSO_4 \cdot 7H_2O$ methanolic solution (15 mL) while stirring at room temperature. The reaction mixture was stirred for 1hr. A white product was then separated by centrifugation and washed with water followed by methanol 2-3 times and dried in a vacuum oven.

Preparation of ZIF-67 Colloidosome:

To a stirred solution of $CoSO_4 \cdot 7H_2O$ (0.5mmol) in methanol (15 mL) was added 2-Methyl-imidazole (5mmol) methanolic solution (15 mL) and stirred for 1hr at room temperature. A blue product was then separated by centrifugation and washed with methanol 2-3 times and dried in a vacuum oven.

Preparation of FITC or Rhodamine B labelled Proteins

HRP, BSA, Catalase, Lysozyme (5 mg) dissolved in 10 mL of PBS (pH 7) was mixed with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) (5 mg) and N-hydroxysuccinimide (NHS) (2.5 mg) and stirred for 4 h. Then the mixture was treated with FITC or Rhodamine B isothiocyanate (100 µg) for another 24 h in the dark. The resulting solution was purified by PD-10 Desalting column (GE healthcare, Illinois, United States) for removing the remaining EDC, NHS, and FITC or Rhodamine B isothiocyanate.

Preparation of proteins encapsulated ZIF-8 Colloidosome

In this study, we encapsulated proteins into hollow ZIF-8 colloidosomes via the diffusion method. Briefly, 0.5 ml of the stock solution (1mg/ml) of model proteins (0.5 mg of BSA, Albumin, HRP, Lysozyme and Catalase) was prepared followed by the addition to 5mg of ZIF-8 colloidosomes and sonicated for 10 minutes then stirred at 100rpm for 48 hr. Particles were centrifuged at 10,000 rpm for 10 minutes and washed by trypsin followed by SDS to ensure the de-adsorption of proteins onto the colloidosomes surface. Then, we washed our system with water and sonicated it three times for 10 minutes each.

Calculation of Encapsulation Efficiency (%EE) and Loading Capacity (%LC).

Encapsulation Efficiency was calculated using Bradford Assay

%EE = [(protein added - Free "unentrapped protein")/protein added] *100

%LC = [Entrapped protein/nanoparticles weight] * 100

Table S1. Encapsulation efficiency (%EE) and loading capacity (%LC) of proteins

Protein	Encapsulation Efficiency (%EE)	Loading Capacity (%LC)
0.5g mg Albumin	98.84 ± 1.13	9.88 ± 0.11
0.5 mg FITC-BSA	99.14 ± 0.14	9.91 ± 0.01
0.5 mg HRP	99.74 ± 0.14	9.97 ± 0.01
0.5mg BSA	99.34 ± 0.1	9.93 ± 0.01
1 mg BSA	97.14 ± 1.27	19.71 ± 0.13

Table S2. Encapsulation efficiency (%EE) and loading capacity (%LC) of different ratios of BSA:ZIF-8 colloidosomes.

BSA: ZIF-8 Colloidosome	Encapsulation Efficiency (%EE)	Loading Capacity (%LC)
1:10	99.34 ± 0.1	9.93 ± 0.01
1:5	97.14 ± 1.27	19.71 ± 0.13
1:3	96.34 ± 0.9	32.86 ± 0.21
1:1	24.04 ± 2.26	62.02 ±1.13

Release Profile of encapsulated protein @ ZIF-8 in PBS pH 7

2ml of PBS pH 7 was added to 1mg of Albumin-FITC@ZIF-8 colloidosomes and sonicated to disperse the particles. Samples were centrifuged at 10,000 rpm for 10 minutes and the absorbance for Alb-FITC in supernatant was measured using fluorescence spectrophotometer at ex.495 nm em. 525 nm at each time point.

Characterization

Powder X-ray diffraction (PXRD) patterns were collected at room temperature on a Bruker D2 phaser powder diffractometer using Cu Kα radiation (λ = 1.5418 Å). Scanning electron microscopy (SEM) was performed on FEI Nova Nano and Magellan electron microscope. Transmission electron microscopy (TEM) was conducted on FEI Titan-ST electron microscope. Thermo gravimetric analysis (TGA) measurements was carried out on a Q5000 (TA Instruments) in a nitrogen atmosphere at a heating rate of $5^{o}C\ min^{-1}$. Fourier transform infrared (FTIR) spectra were recorded using a Thermo Scientific spectrometer (Nicolet iS10). Nitrogen adsorption–desorption isotherms were acquired using a Microactive 4.0 ASAP 2420 instrument and pore size distribution were measured using NLDFT (Tarazona approach on cylindrical pores). The confocal laser scanning microscopy (CLSM) images were acquired on Leica TCS SP8, Leica micro-system using an oil dipping objective (60X).

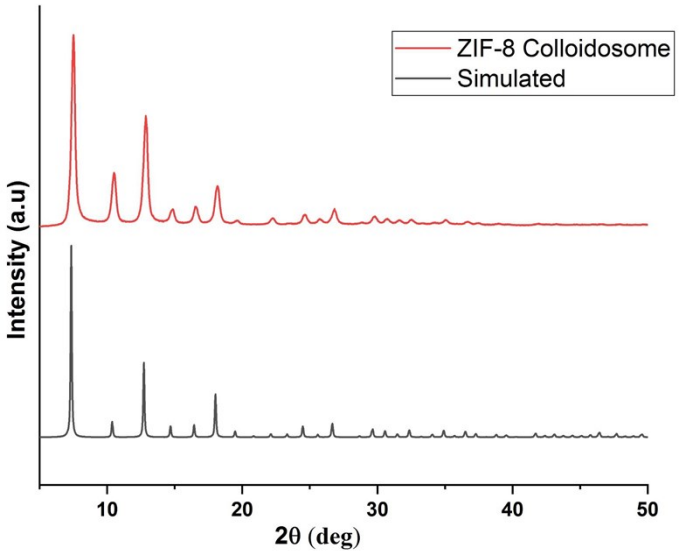

Figure S1. PXRD pattern of the ZIF-8 colloidosome experimental (red) and simulated (gray).

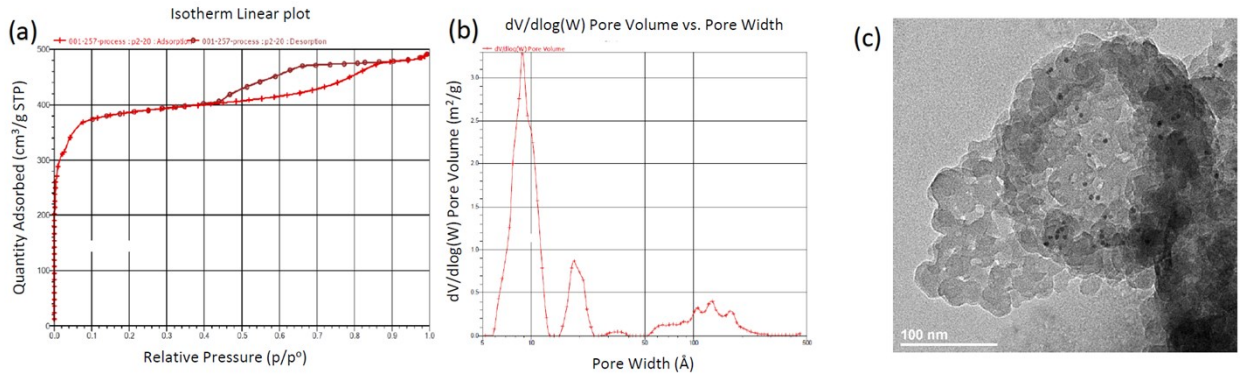

Figure S2. (a) N_2 isotherm at 77 K (b) Pore size distribution of ZIF-8 colloidosomes using NLDFT (c) TEM image of gold nanoparticles (5nm) @ZIF-8 colloidosomes.

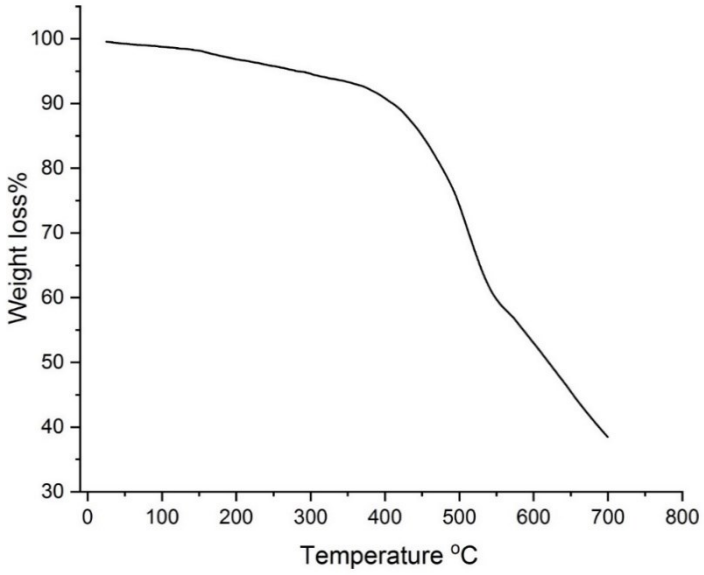

Figure S3. TGA of ZIF-8 colloidosome.

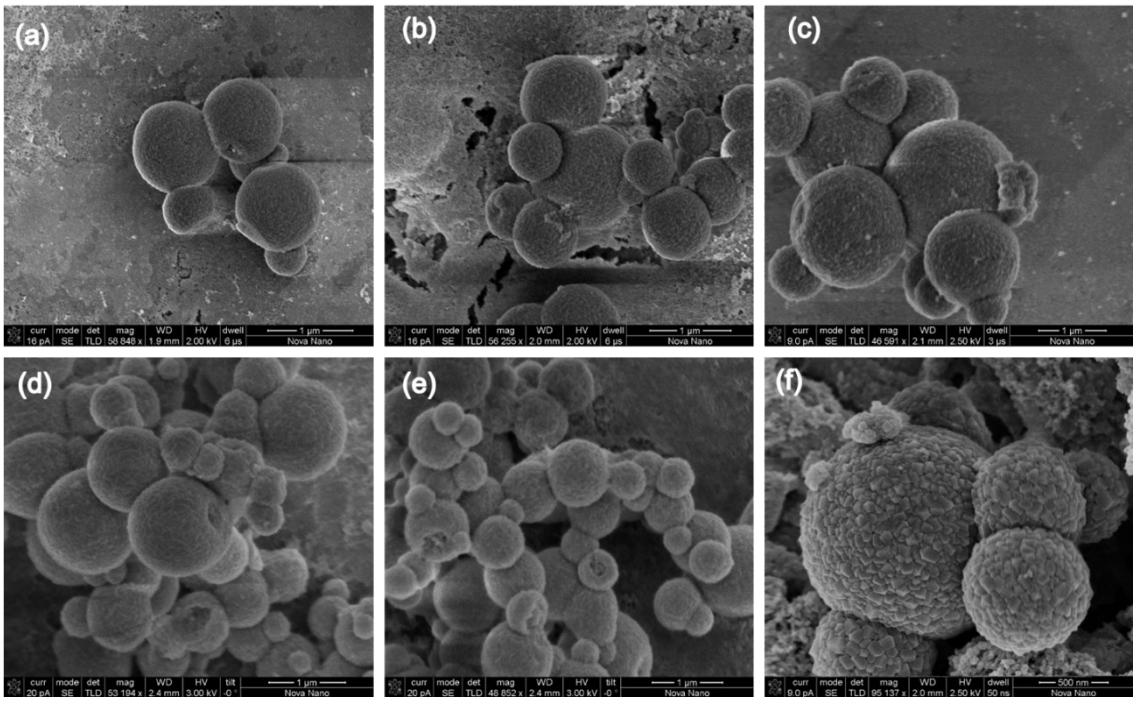

Figure S4. SEM images of the stirred product after (a) 10 min, (b) 20 min, (c) 30 min, (d) 40 min, (e) 50 min and (f) 60 min.

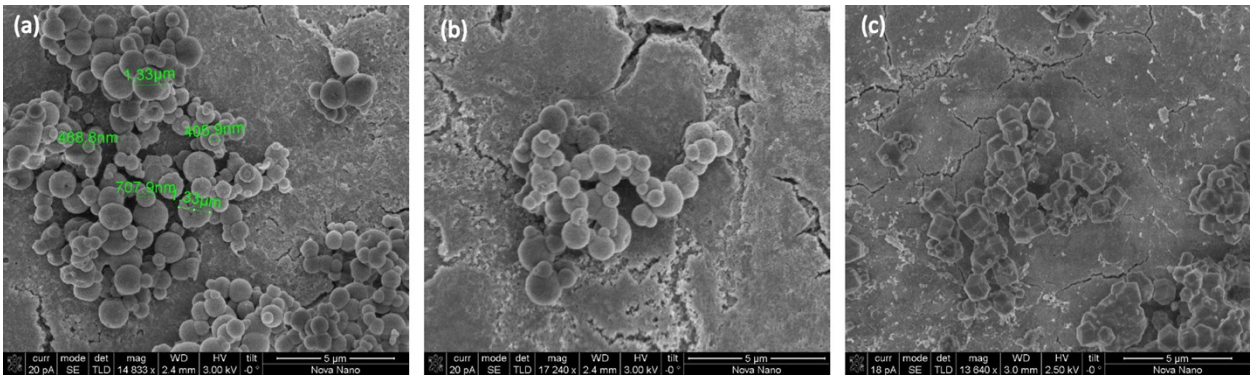

Figure S5. SEM images of the ZIF-8 colloidosomes with Metal- Ligand ratio (a) 1:8, (b) 1:6 and (c) 1:5.

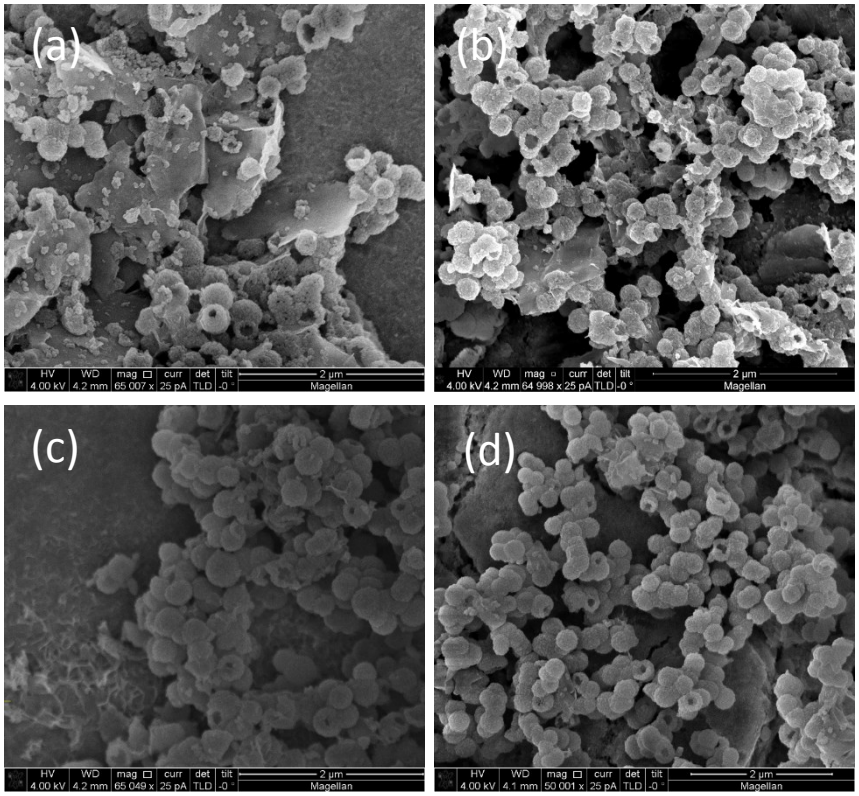

Figure S6. SEM images of the ZIF-8 colloidosomes with Metal- Ligand ratio (a) 1:12, (b) 1:14, (c) 1:16 and (d) 1:18.

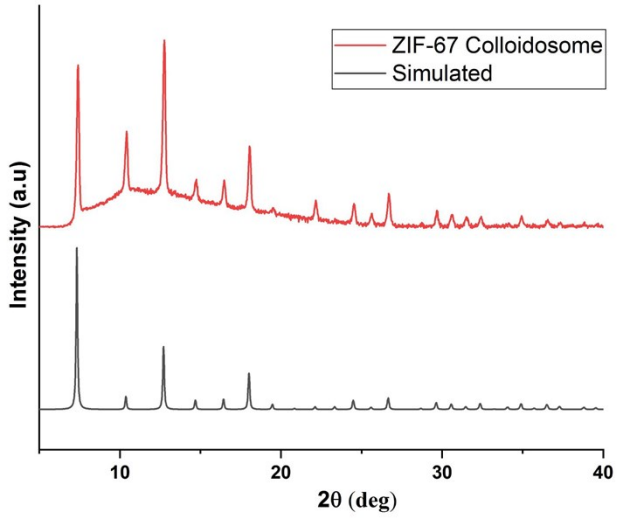

Figure S7. PXRD pattern of the ZIF-67 colloidosome experimental (red) and simulated (gray).

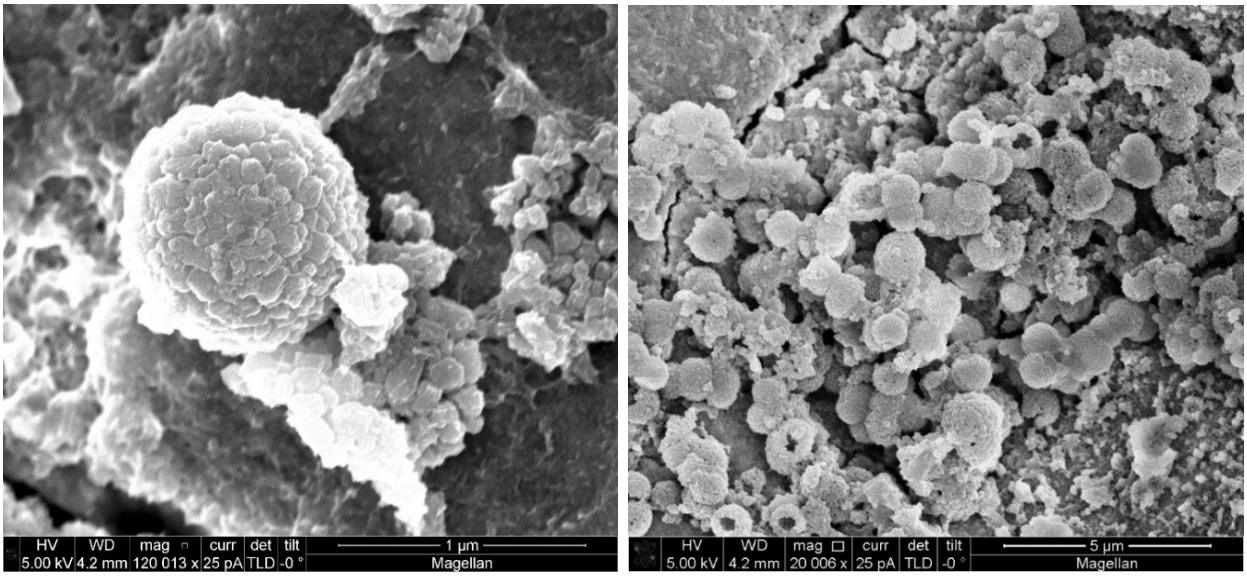

Figure S8. SEM images of the ZIF-67 colloidosomes.

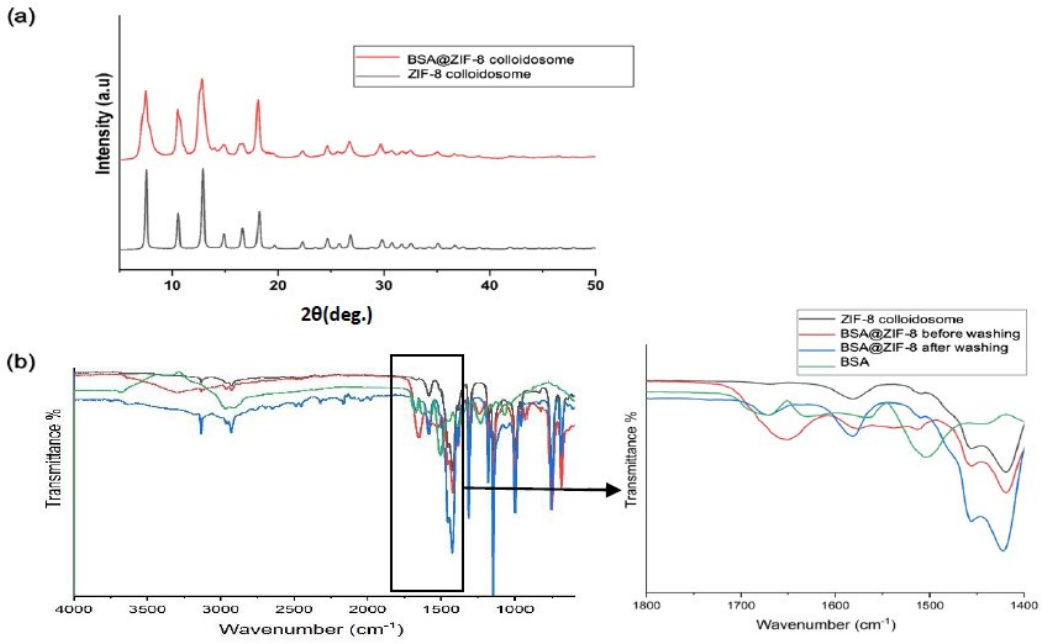

Figure S9. (a) PXRD of FITC-BSA@ZIF-8 colloidosome (b) IR spectra of ZIF-8 colloidosome (black), BSA@ZIF-8 before washing (red), BSA@ZIF-8 after washing (blue) and BSA (green).

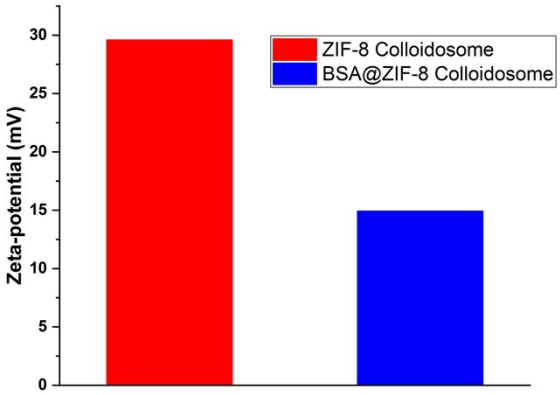

Figure S10. ζ-Potential of ZIF-8 colloidosomes and BSA encapsulated ZIF-8 colloidosomes.

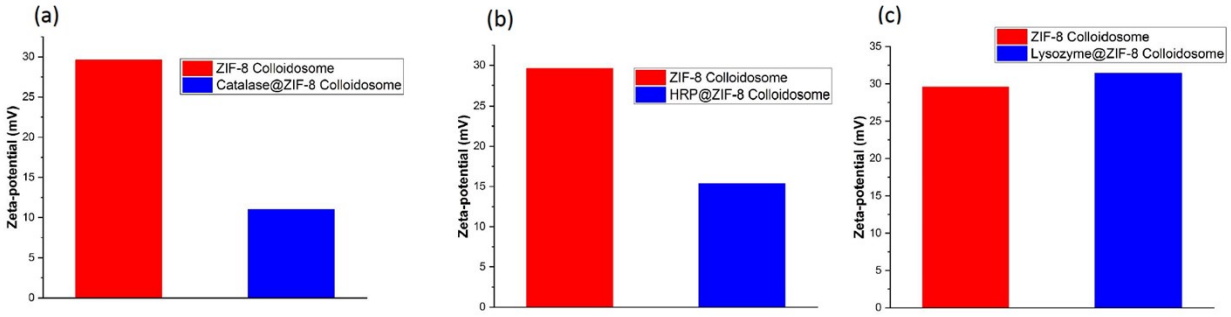

Figure S11. ζ-Potential of (a) Catalase, (b) HRP and (c) Lysozyme encapsulated ZIF-8 colloidosomes.

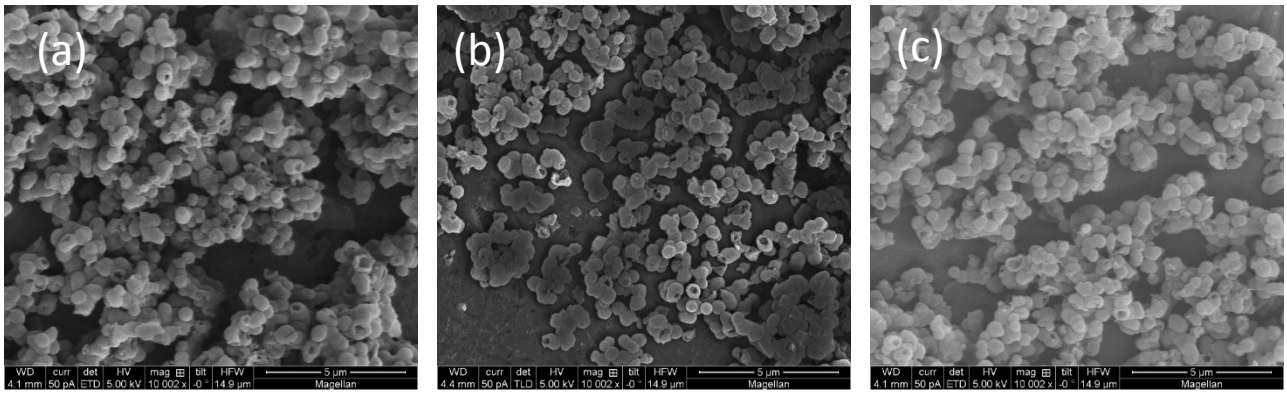

Figure S12. SEM images of (a) FITC-Catalase, (b) FITC-HRP and (c) FITC-Lysozyme encapsulated ZIF-8 colloidosomes.

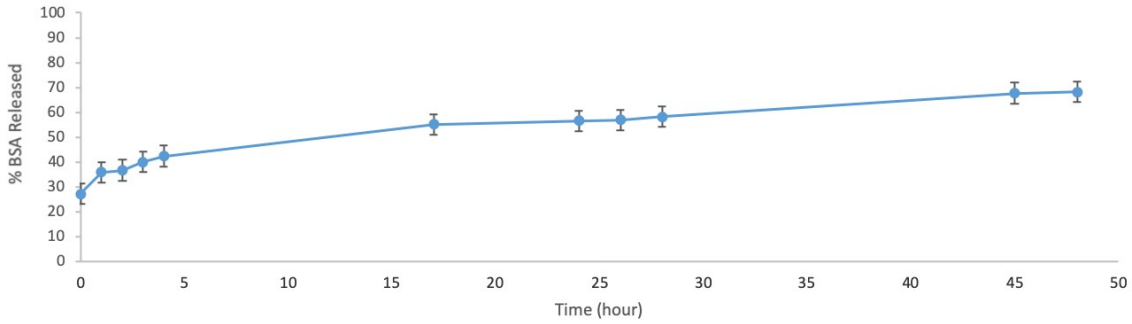

Figure S13. Leaching profile for BSA@ZIF-8 colloidosomes.

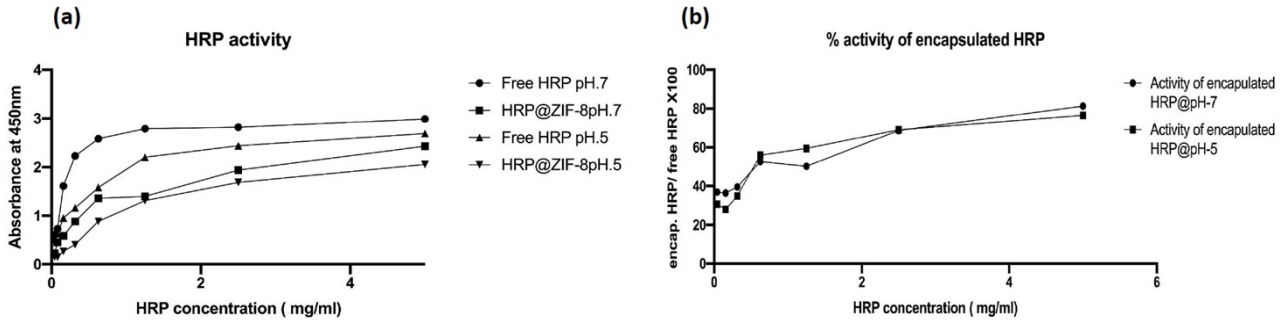

Figure S14. (a) Testing the activity of free and encapsulated enzyme at different concentrations and pH values (b) Activity percentage of encapsulated HRP at two different pH values.