# Supporting information

## Complying the Physiological Functions of Golgi Apparatus for Secretory Exocytosis Facilitated Oral Absorption of Protein Drugs

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**Supplementary information includes:**

Supplementary methods.

Supplementary SchemeS1. Synthetic routes of DSPE-PEG-Cys and DSPE-PEG-R8.

Supplementary Figures:



Supplementary Tables:

## Supplementary methods

### *In vitro* cytotoxicity study

The cytotoxicity of NPs and chemicals on Caco-2 cells was investigated by the Alamar Blue method. Caco-2 cells were seeded in 96-well plates at $1 \times 10^4$ cells/well and cultured for 36 h. Different concentrations of NPs (100-400 μg/mL of PLGA) or tested chemicals was added respectively. After incubated for 3 h, the NPs and chemicals were removed. The cells were washed by PBS and incubated with Alamar Blue solution (10 μg/mL) for another 1 h. The fluorescence intensity was measured by multimode reader.

### Colloidal stability study

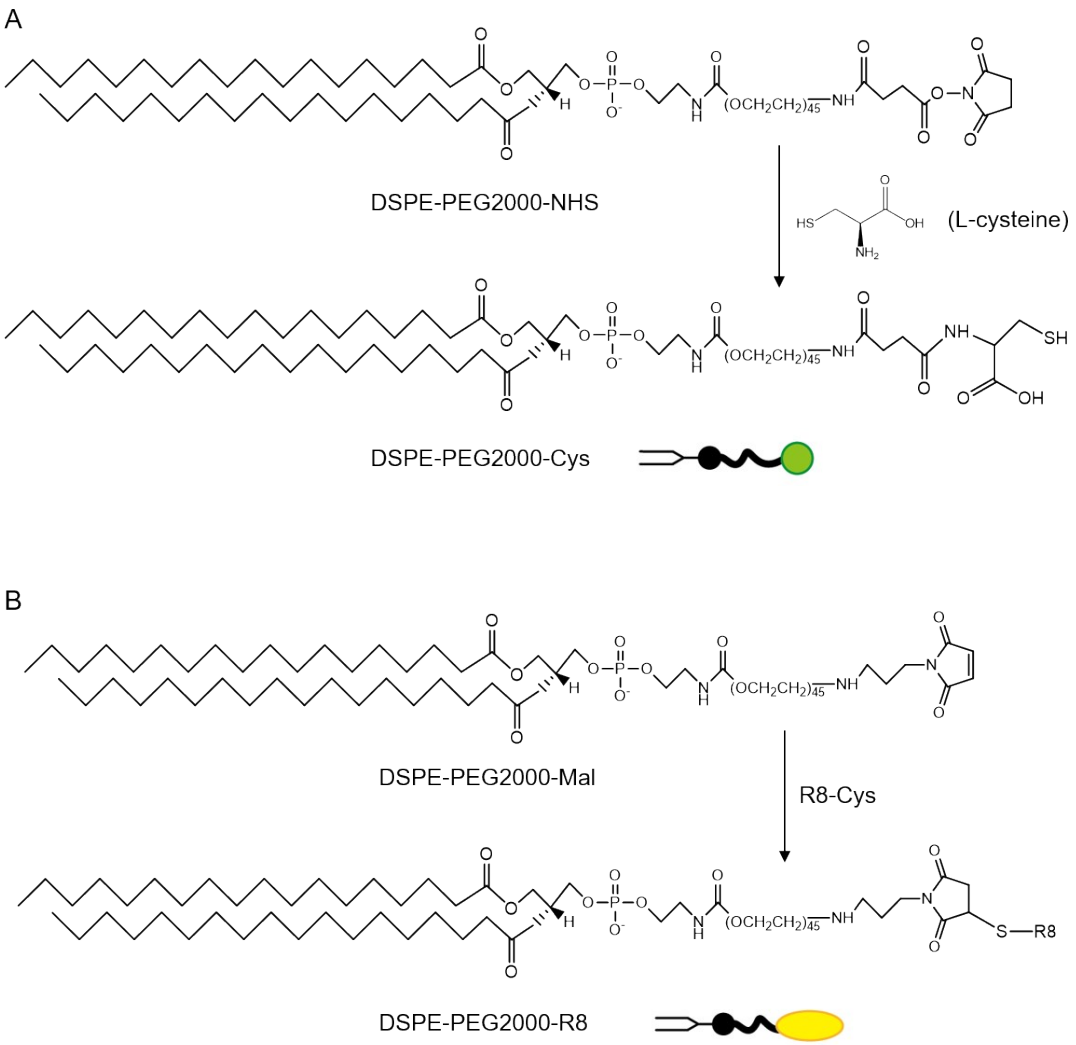
To investigate the colloidal stability of NPs in mimic gastrointestinal environment, NPs were suspended in simulated gastric fluids (SGF, with 0.32% (w/v) pepsin) and simulated intestinal fluids (SIF, with 1% (w/v) trypsin) at 37℃ with gentle shaking. At predetermined time intervals, the particle size of NPs was measured by Malvern Zetasizer NanoZS90 analyzer (Malvern Instruments Ltd, UK).

### Enzymatic stability of insulin

To evaluate the protection of NPs on insulin, the enzymatic stability of insulin was investigated. INS PEG NPs, INS 25%R8+75%Cys NPs and free insulin solution were incubated in SIF (with trypsin, 40 μg/mL), making the final concentration of insulin at 200 μg/mL. 100 μL of samples was withdrawn at each time point (0, 1, 2, 4 h) and mixed with 100 μL DMSO containing 2% trifluoroacetic acid to terminate the enzymatic interaction. Finally, the amount of insulin was tested by high performance liquid chromatography (HPLC).

### Statistical Analyses

Student's t-test or one-way analysis of variance (ANOVA) was used for statistical analyses. All data were presented as the mean ± SD. Experiments were performed in triplicate if not specified. Differences at P values $< 0.05$ were considered to be statistically significant.




**Scheme S1** Synthetic routes of (A) DSPE-PEG-Cys and (B) DSPE-PEG-R8.

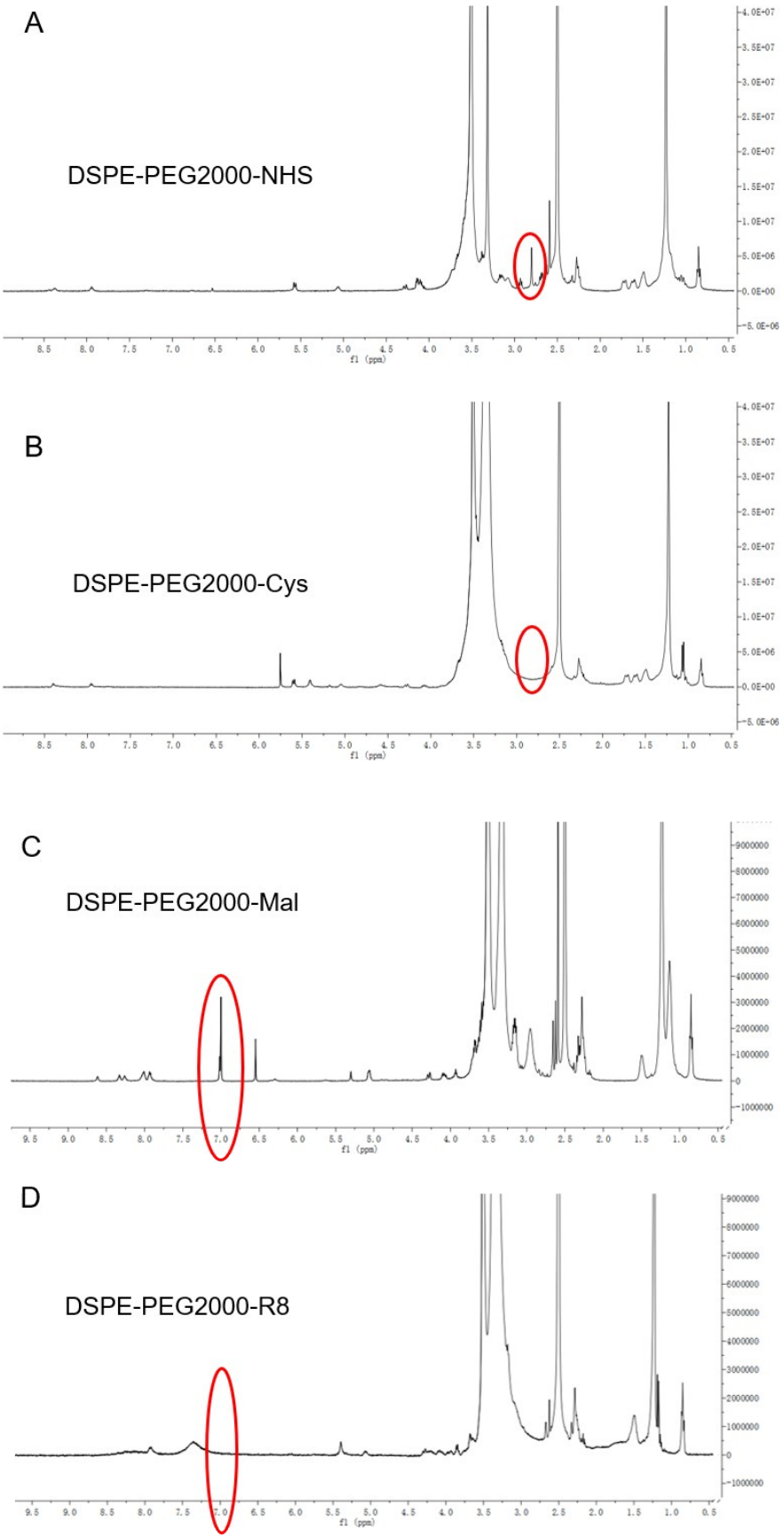



**Fig. S1** $^{1}$H-NMR spectra of (A) DSPE-PEG-NHS, (B) DSPE-PEG-Cys, (C) DSPE-PEG-Mal and (D) DSPE-PEG-R8.

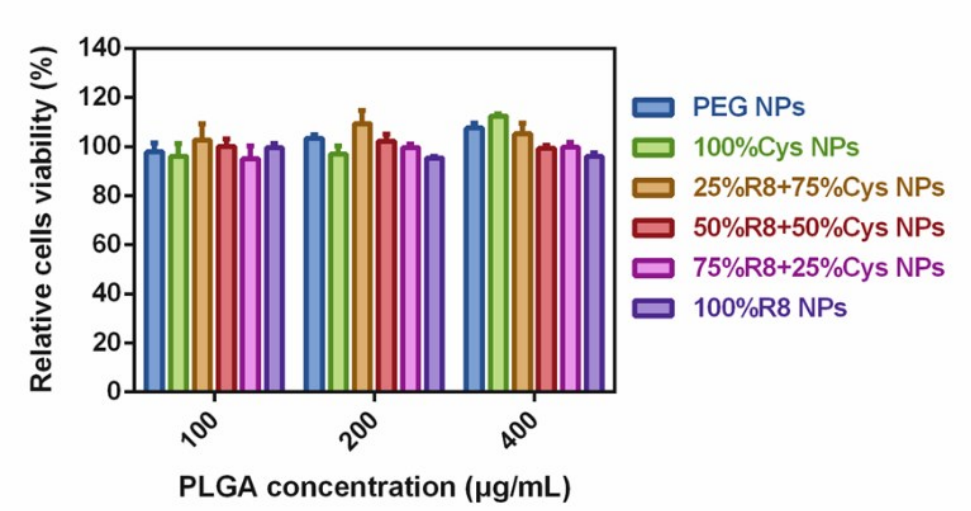



**Fig. S2** Caco-2 cells viability after treatment with various concentrations of NPs in different concentrations via Alamar Blue method (n = 3).

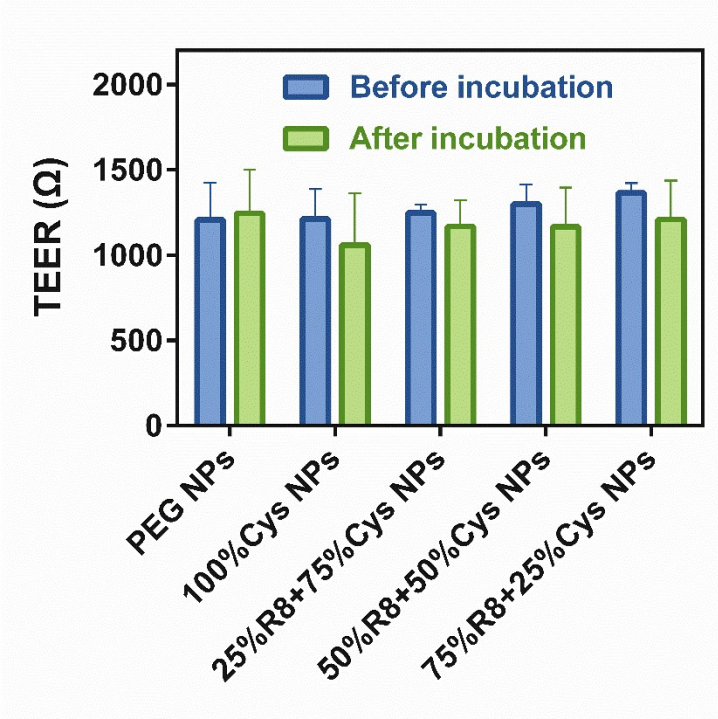



**Fig. S3** TEER values of Caco-2 cell monolayers before and after NPs incubation (n=3).

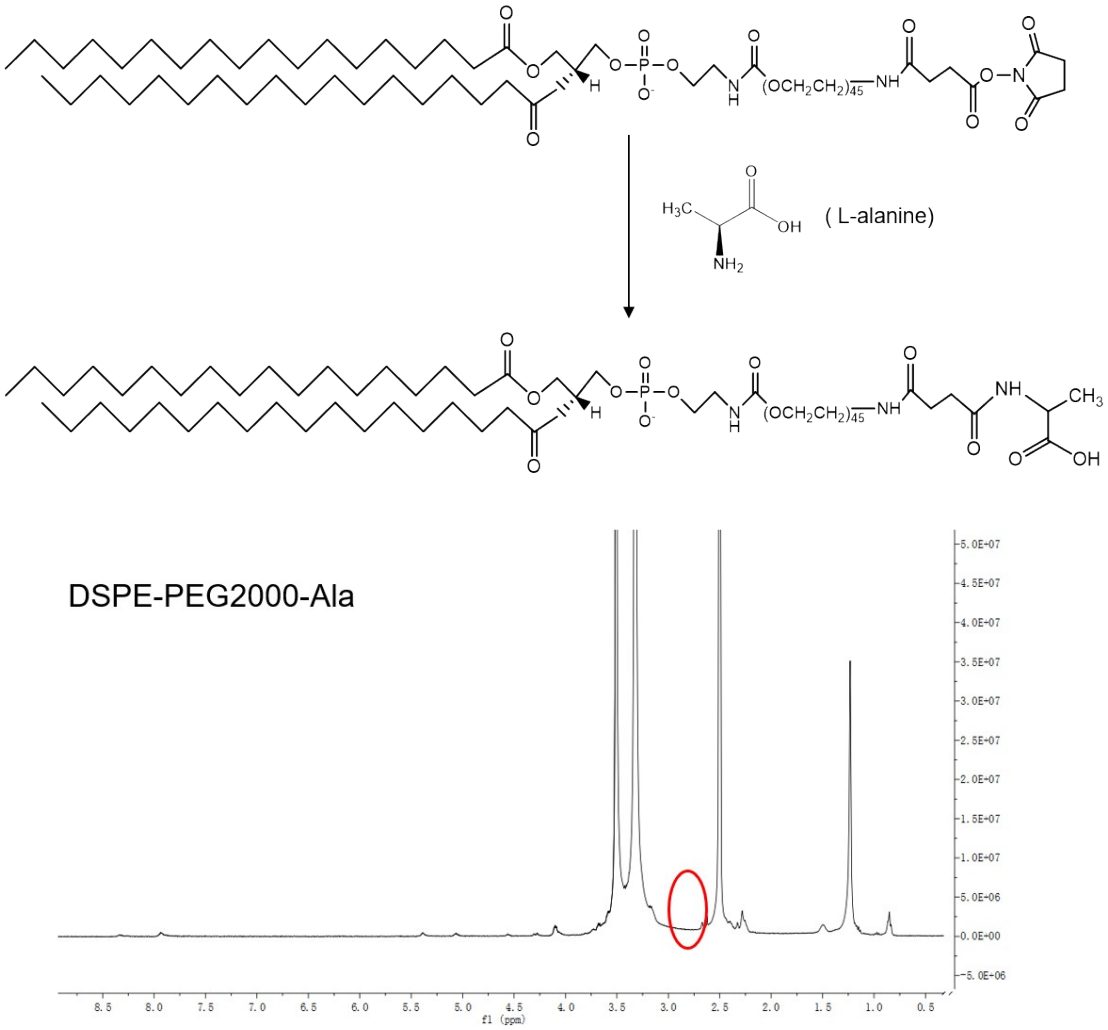



**Fig. S4** Synthesis of DSPE-PEG-Ala and $^{1}$H-NMR spectra of DSPE-PEG-Ala.

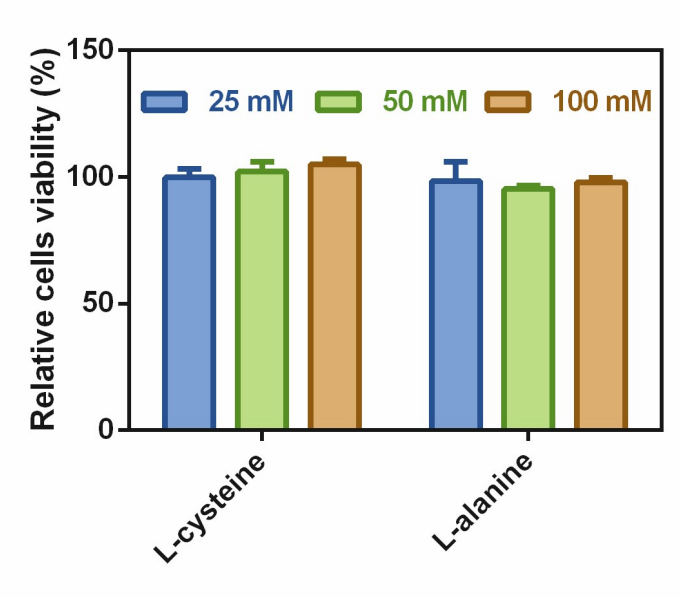



**Fig. S5** Caco-2 cells viability after treatment with various concentrations of L-cysteine and L-alanine in different concentrations via Alamar Blue method (n = 3).

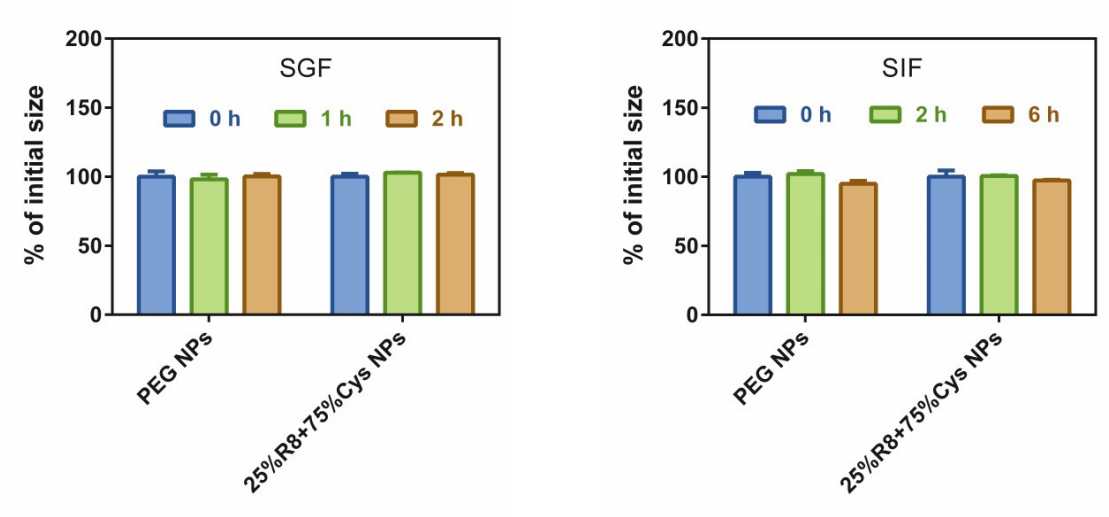



**Fig. S6** Colloidal stability of NPs in SGF and SIF (n = 3).

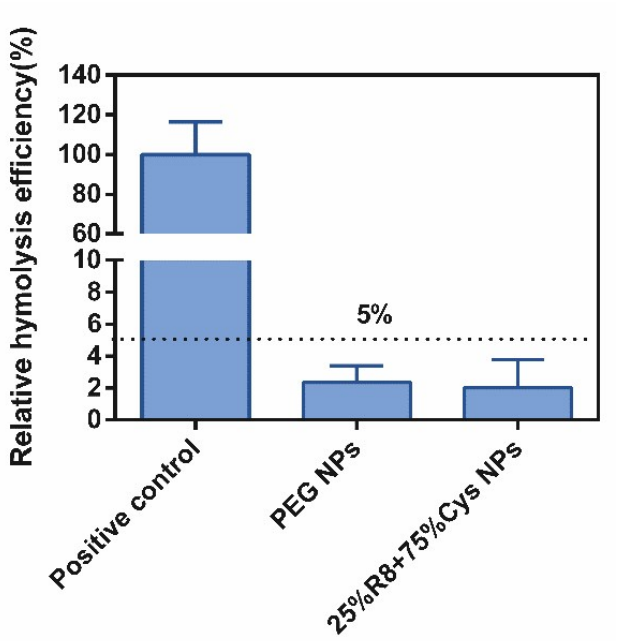



**Fig. S7** Hemolysis rate of NPs after incubated with erythrocyte for 2 h (n = 3).

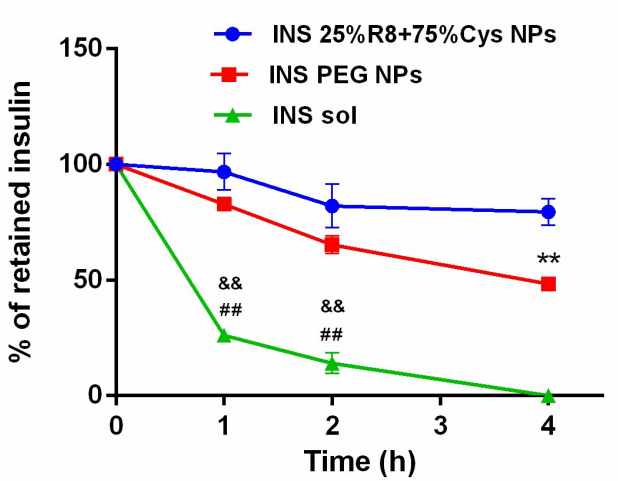



**Fig. S8** The percentage of remained insulin in SIF with trypsin (n = 3). **p< 0.01, $^{\#\#}$P< 0.01 vs INS 25%R8+75%Cys NPs, $^{\&\&}$P< 0.01 vs INS PEG NPs.

**Table S1** Characterization of Ala NPs.

| Sample | Size (nm) | PDI | Zeta potential (mV) |
|---|---|---|---|
| Ala NPs | 89.0±3.7 | 0.225±0.053 | -26.76±0.76 |

**Table S2** Characterization of insulin-loaded NPs.

| Sample | Size (nm) | PDI | Zeta potential (mV) | EE% | DL% |
|---|---|---|---|---|---|
| PEG NPs | 86.2±7.9 | 0.287±0.005 | -26.03±1.80 | 69.12±6.51 | 11.53±1.27 |
| 25%R8+75%Cys NPs | 103.5±5.2 | 0.229±0.043 | -19.93±0.45 | 74.44±10.61 | 11.38±2.34 |