Supporting Information

Nanoparticles with Surface Feature of Dendritic Oligopeptides as Potential Oral Drug Delivery System

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Author Contributions

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Supplementary Methods

Mucin adhesion assay

For the measurement of mucin-particle aggregation, according to our previously reported methods[1], freshly prepared NPs were dispersed in porcine mucin solution at 2.0% (m/v), then vortexed at 100 rpm, and incubated for 2 h at 37 °C. The mixture was centrifuged at 3000 rpm for 5 min, and 100 μL of the supernatant was withdrawn. Then the supernatant was treated with 100 μL of DMSO and fluorescence intensity was measured using a Varioskan Flash multimode reader.

Preparation and characterization of FITC-Ins loaded NPs

The preparation of FITC-Ins loaded NPs was similar with that of drug-free NPs. Briefly, 16.0 mg PLGA, 3.2 mg DSPE-PEG2000 and 3.2 mg soybean lecithin were fully dissolved in 1.0 mL of dimethyl sulfoxide (DMSO), forming an organic phase, then FITC-Ins (dissolved in DMSO) was mixed with the organic phase as previously described. Then, FITC-Ins loaded P NPs (FITC-Ins-P NPs) and insulin loaded Glu-APD NPs (FITC-Ins-Glu-APD NPs) were obtained via the self-assembly nanoprecipitation method. The unloaded FITC-Ins was removed by ultrafiltration and the NPs were re-dispersed in SF-DMEM or PBS for further use. The drug loading (DL%) was determined by ultrafiltration method as previously described. Briefly, the NPs dispersion was placed in an ultrafiltration tube (MWCO: 100 kDa), centrifuged at 5000 rpm and 4 °C for 20 min to separate the unloaded free FITC-Ins. Then, the NPs were dissolved in DMSO and the loaded FITC-Ins was quantified by fluorescence intensity using a Varioskan Flash multimode reader. DL% was calculated according to the following equation:

$$DL\% = \frac{the\ weight\ of\ FITC-Ins\ loaded\ in\ NPs}{total\ weight\ of\ NPs} \times 100$$

Cellular uptake study and Transepithelial Transport Study of FITC-Ins loaded NPs

To quantify the cellular uptake of FITC-Ins loaded NPs, according to our previously reported methods[2], Caco-2 cells in logarithmic growth phase were cultured in a 96-well plate. The cells were incubated with FITC-Ins loaded NPs (20 μg/mL of FITC-Ins) and SF-DMEM as control for 3 h, then rinsed with ice cold PBS. Then 0.1 mL of DMSO was added to destroy cells. The fluorescence intensity was measured by Varioskan Flash multimode reader and the number of cells was estimated via Alamar Blue assay.

For the transepithelial transport study of FITC-Ins loaded NPs, Caco-2 cells were seeded into a 24-well plate containing Transwell chamber (pore size 0.4 μm, area 0.33 cm2) at 3×10^4 cells/well, and cultured for 21 days. Cells with a TEER value higher than 800 Ω were selected for the experiment. Prior to the experiment, the selected wells with Transwell chambers were balanced by pre-warmed SF-DMEM at 37 °C for 30 min. After removing the SF-DMEM, 200 μL of FITC-Ins loaded NPs (100 μg/mL of FITC-Ins) was added to the apical chamber, and 800 μL of SF-DMEM was added to the basolateral chamber. At each time point (0, 0.25, 0.5, 1, 2, 4, and 6 h), 80 μL of the sample was withdrawn from basolateral chamber, and same volume of pre-warmed SF-DMEM was supplemented. The amount of transported NPs was quantified by detecting the fluorescence intensity. The apparent permeability coefficient (Papp) was calculated using the following equation:

$$Papp = \frac{dQ}{dt} \times \frac{1}{A \times C_0}$$

where dQ/dt is the transported rate of FITC-Ins loaded NPs from apical chamber to basolateral chamber, C_0 is the initial concentration of FITC-Ins loaded NPs, and A is the membrane area (cm^2).

Supplementary Results

Cellular uptake and trans-epithelial transport study of FITC-Ins loaded NPs

To better study the cellular behaviors of loaded insulin, we further investigated the cellular uptake and trans-epithelial transport using FITC-marked insulin (FITC-Ins). Firstly, FITC-Ins loaded P NPs (FITC-Ins-P NPs) and FITC-Ins loaded Glu-APD NPs (FITC-Ins-Glu-APD NPs) were obtained via the self-assembly nanoprecipitation method. Characterizations of the NPs are shown in Table S2. Then, in vitro cellular uptake and transepithelial transport of FITC-Ins loaded NPs were conducted on Caco-2 cell models. As shown in Fig. S10A, the cellular uptake of FITC-Ins-Glu-APD NPs was 4.74-fold and 5.38-fold higher than that of FITC-Ins-P NPs and free FITC-Ins, respectively. Meanwhile, the accumulated amount and apparent permeability coefficient (Papp) value of transported FITC-Glu-APD NPs were significantly higher than that of free FITC-Ins and FITC-Ins-P NPs after incubation for 6 h (Fig. S10B and Fig. S10C). The trends of cellular uptake and trans-epithelium transport between DiI-loaded NPs and FITC-Ins loaded NPs were similar, suggesting that introduction of Glu-APD could increase the cellular uptake and trans-epithelium transport of loaded drugs.

Table S1. Reagents with different functions used in the cell studies and their concentrations.

Reagents	Concentrations	Functions
NaN_3	1.0 mM	Inhibitor of energy-dependent pathway
M-β-CD	1.0 mM	Inhibitor of caveolae-mediated pathway
Lovastatin	10 μg/mL	Inhibitor of caveolae -mediated pathway
Filipin	500 nM	Inhibitor of caveolae -mediated pathway
Rottlerin	10 μM	Inhibitor of macropinocytosis
Amiloride	12 μg/mL	Inhibitor of macropinocytosis
Chlorpromazine	30 μM	Inhibitor of clathrin-mediated pathway
Hypertonic sucrose	0.4 M	Inhibitor of clathrin-mediated pathway
L-Glu	100 mM	Competitive inhibitor of AAT
L-Lys	100 mM	Competitive inhibitor of AAT
Gly-Sar	100 mM	Competitive substrate of PepT1
Brefeldin A	25 μg/mL	The Golgi apparatus/ Endoplasmic reticulum related inhibitor
Monencin	33 μg/mL	The Golgi apparatus related inhibitor
Nocodazole	10 μM	The lysosome related inhibitor
chloroquine	100 μM	The lysosome related inhibitor

Table S2. Characterization of FITC-Ins-P NPs and FITC-Ins-Glu-APD NPs (n = 3).

Samples	Size (nm)	PDI	Zeta potential (mV)	DL%
FITC-Ins-P NPs	138.63 ± 19.89	0.291 ± 0.090	-26.87 ± 1,97	18.00 ± 0.06
FITC-Ins-Glu-APD NPs	167.43 ± 37.19	0.229 ± 0.018	5.78 ± 0.33	12.74 ± 0.16

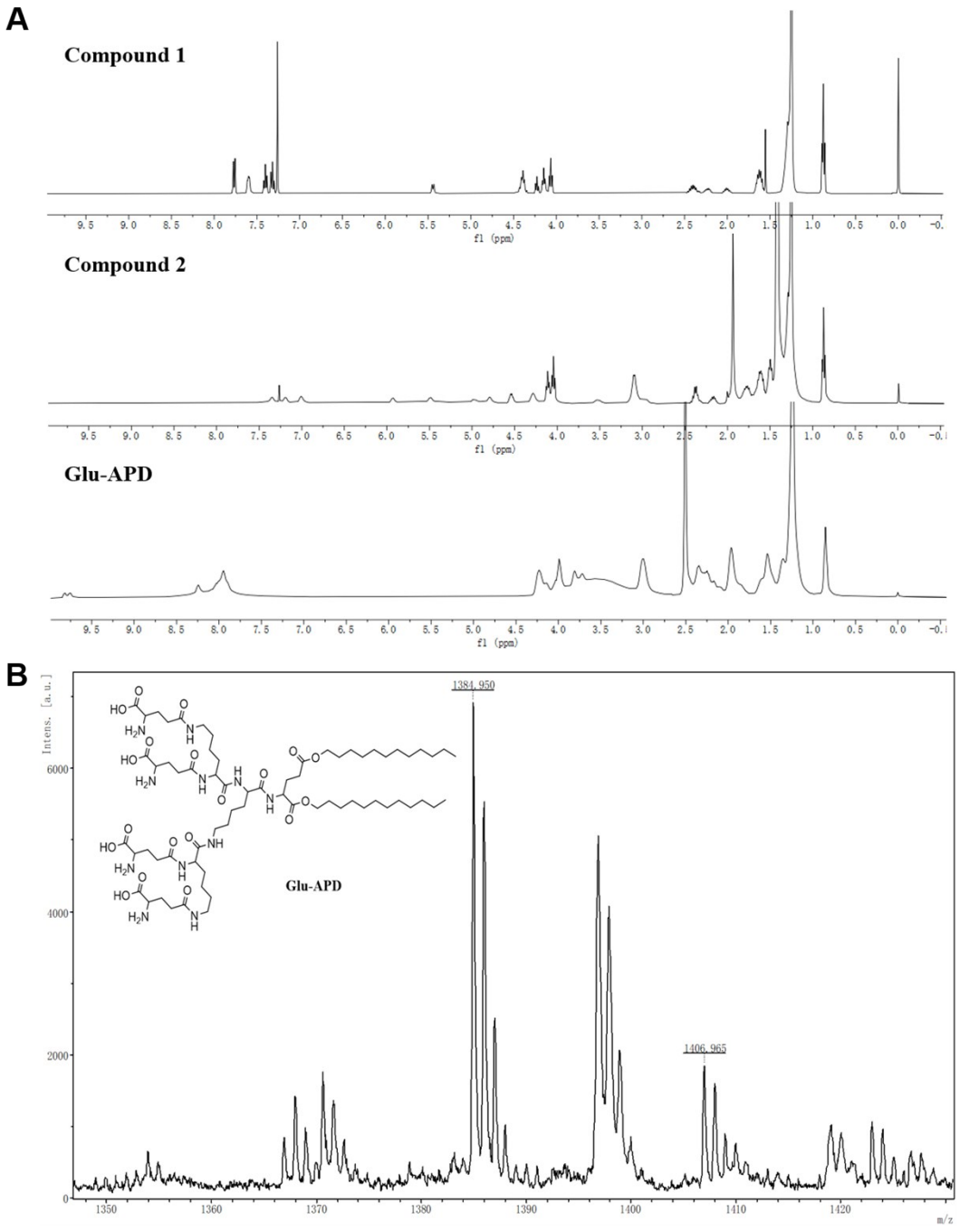

Fig. S1. (A) ^{1}H-NMR spectra of compound 1, compound 2 and Glu-APD. (B) MALDI-TOF MS spectrometry of Glu-APD.

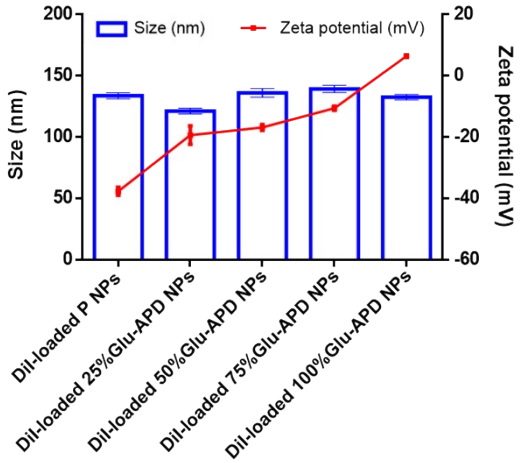

Fig. S2. The sizes and zeta potentials of NPs after loaded with DiI. Error bars represent SD (n = 3).

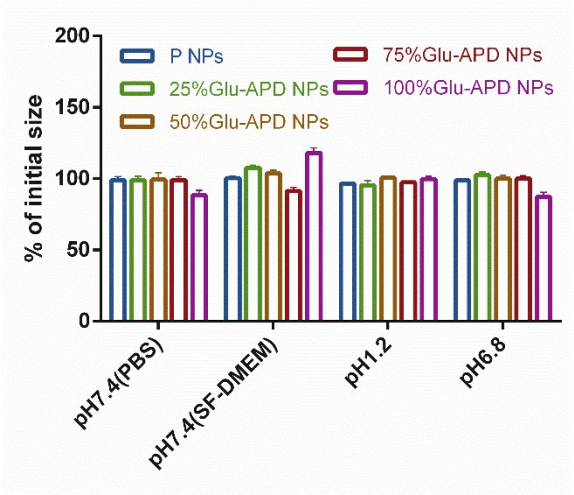

Fig. S3. Stability of five kinds of NPs after incubation in pH 1.2 for 3 h, in pH 6.8 and 7.4 (PBS and SF-DMEM) for 6 h. Results were expressed as a percentage of the size of initial NPs. Error bars represent SD (n = 3).

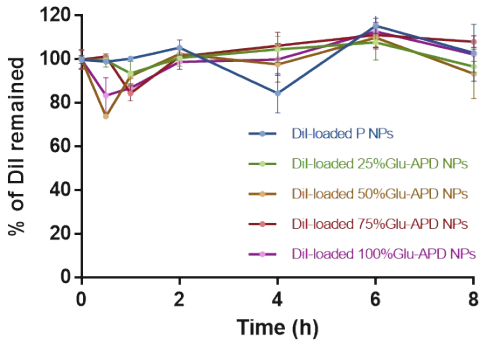

Fig. S4. Release profile of DiI-loaded NPs in PBS (pH 7.4). Error bars represent SD (n = 3).

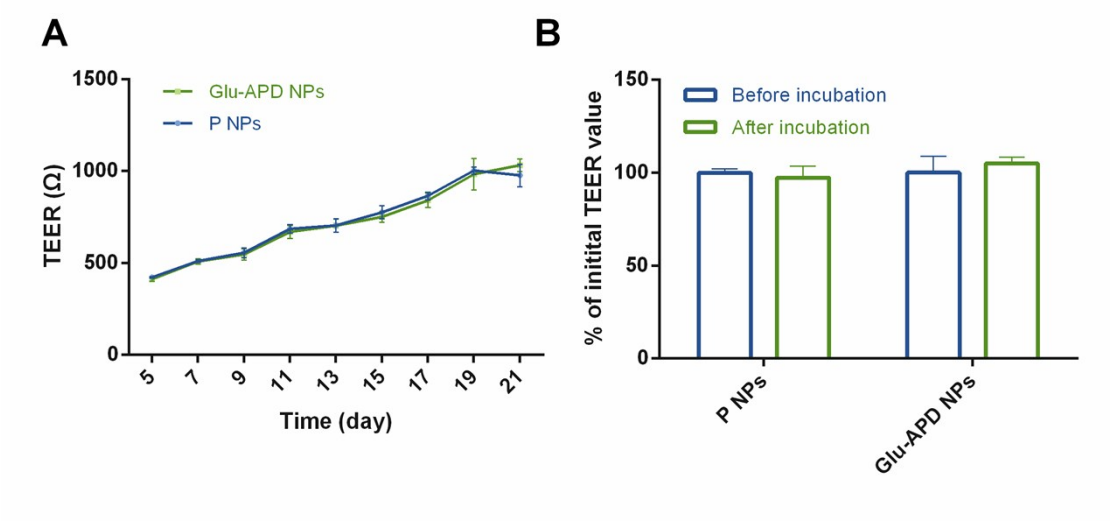

Fig. S5. (A) The TEER values were monitored for 21 days with Caco-2 cells seeded on 24-well Transwell plates. (B) The TEER values of Caco-2 cell monolayers before and after co-incubation with P NPs and Glu-APD NPs. Error bars represent SD (n = 3).

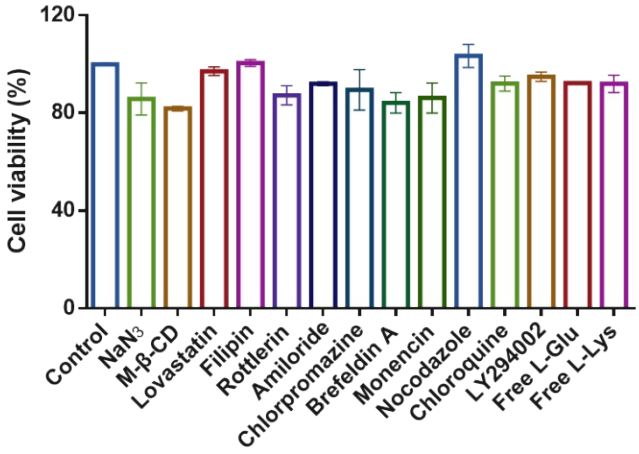

Fig. S6. The Caco-2 cell viability after incubation with tested reagents. The cells incubated with PBS were employed as a negative control and normalized for 100% cell viability. Error bars represent SD (n = 3).

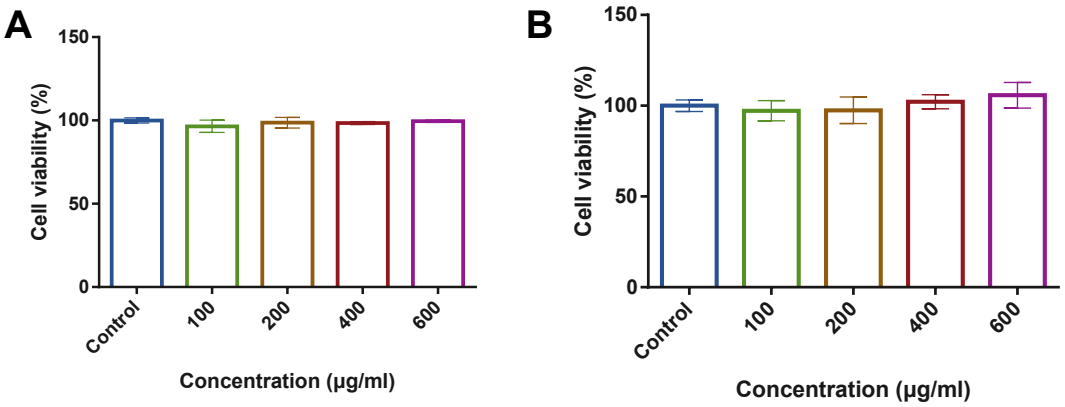

Fig. S7. The Caco-2 (A) or E12 (B) cell viability after treatment with P NPs at the PLGA concentrations of 100-600 μg/ml. Error bars represent SD (n = 3).

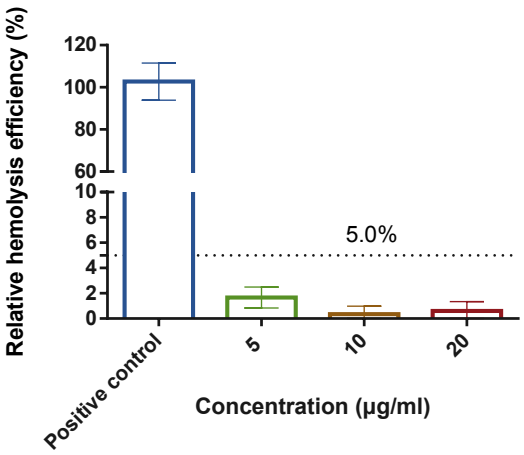

Fig. S8. Hemolysis efficiency following exposure of erythrocyte to P NPs for 2 h. Error bars represent SD (n = 3).

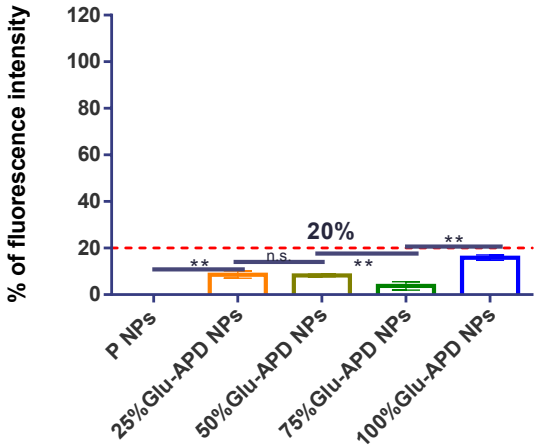

Fig. S9. Mucin adhesion assay. Adhesion was measured as fluorescence intensity of adhesion of NPs. Error bars represent SD (n = 3). **p < 0.01, one-way ANOVA.

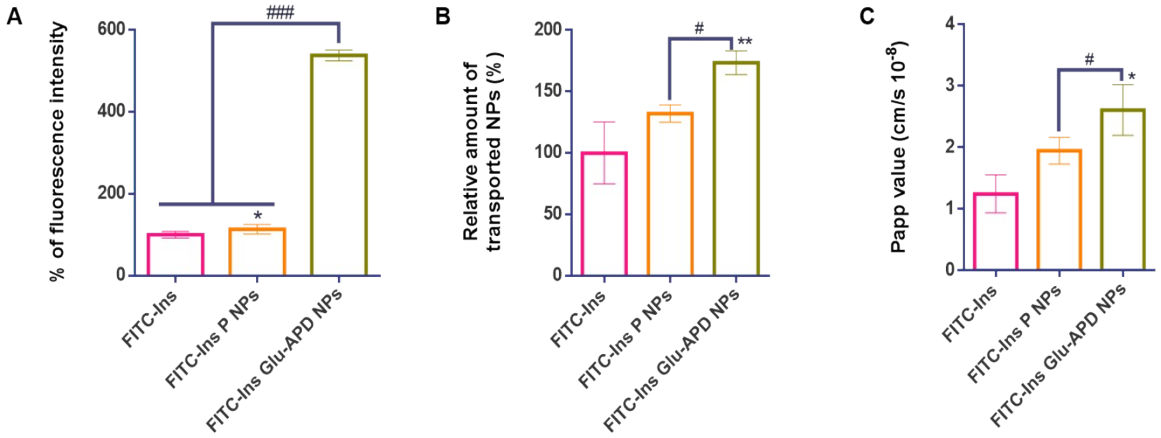

Fig. S10. (A) Relative fluorescence intensity of FITC-Ins on Caco-2 cells after incubation of FITC-Ins loaded NPs with different Glu-APD contents. (B) Relative amount of transported FITC-Ins loaded NPs across the Caco-2 cell monolayer after co-incubation for 6 h. (C) Papp values of FITC-Ins loaded NPs across the Caco-2 cell monolayer within the co-incubation for 6 h. Error bars represent SD (n = 3). *p < 0.05, **p < 0.01, versus free FITC-Ins group, #p < 0.05, ###p < 0.001, versus FITC-Ins-P NPs group.

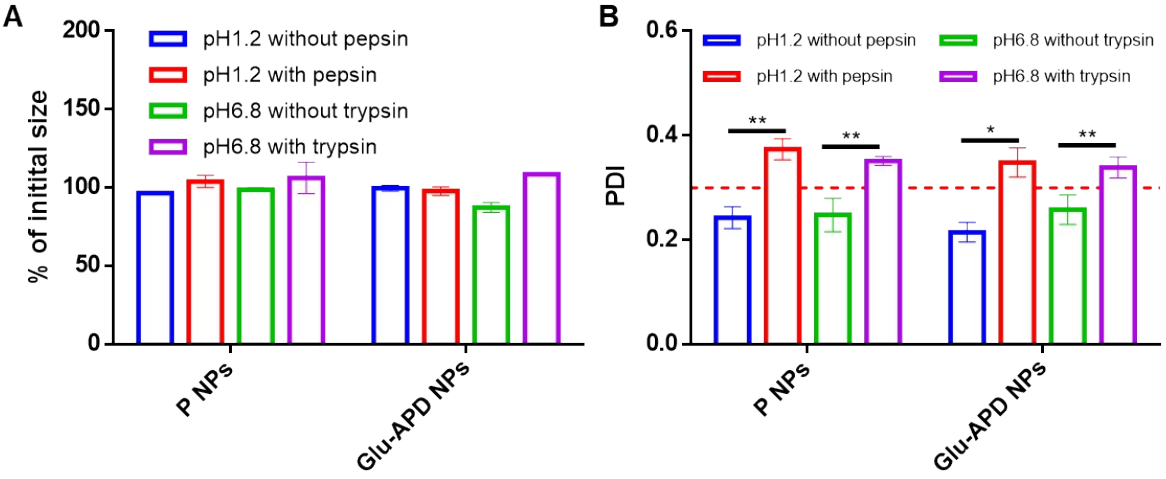

Fig. S11. Stability of both NPs after incubation at pH 1.2 for 3 h, and pH 6.8 for 6 h. (A) Results were expressed as a percentage of the size of initial NPs. (B) Results were expressed as PDI. Error bars represent SD (n = 3). **p < 0.01 and *p < 0.05 versus without pepsin or trypsin.

References

1. W. Shan, X. Zhu, W. Tao, Y. Cui, M. Liu, L. Wu, L. Li, Y. X. Zheng and Y. Huang, *ACS Appl. Mater. Interfaces*, 2016, **8**, 25444-25453.
2. M. Liu, J. Zhang, X. Zhu, W. Shan, L. Li, J. J. Zhong, Z. R. Zhang and Y. Huang, *Journal of Controlled Release*, 2016, **222**, 67-77.