Supplemental information:

Angiopep-2-Functionalized Nanoparticles Enhances Transport of Protein Drugs across Intestinal Epithelia by Self-Regulation of Targeted Receptors

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Methods

1. Cell viability

Alamar Blue assay was applicated to evaluate the effect of NPs on cell viability. Caco-2 cells were seeded in 96-well plates at the density of 8×10^3 cells/mL and cultured for 3 days. After removal of DMEM and washed by PBS, cells were incubated with blank NPs decorated by different molar

ratios of ANG (PEG NPs, 25%ANG NPs, 50%ANG NPs, 75%ANG NPs and 100%ANG NPs) at the concentrations of PLGA equivalent to 100, 200, 400, 600 and 800 μg/mL (150 μL/well) for 3 h. Then, cells were washed twice with cold PBS to separate extracellular NPs. Finally, Alamar Blue DMEM solution (10 μg/mL) was added into each well (150 μL/well) for 1 h and the absorption of each well was measured at Ex: 490 nm/ Em: 570 nm via a Varioskan Flash Multimode Reader. The cell viability was calculated by following formula:

$$Hemolysis\ ratio\% = \frac{A_{NPs} - A_{saline}}{A_{triton} - A_{saline}} \times 100$$

2. LDH release study

Caco-2 cells were seeded in 96-well plates at the density of 8×10^3 cells/mL and cultured for 3 days. After removal of DMEM and washed by PBS, cells were exposed to blank PEG NPs and 100%ANG NPs at the concentration of PLGA equivalent to 100, 200, 400, 600 and 800 μg/mL (150 μL/well) for 3 h. Finally, the supernatant was collected and LDH level was measured by a LDH assay kit.

Figures:

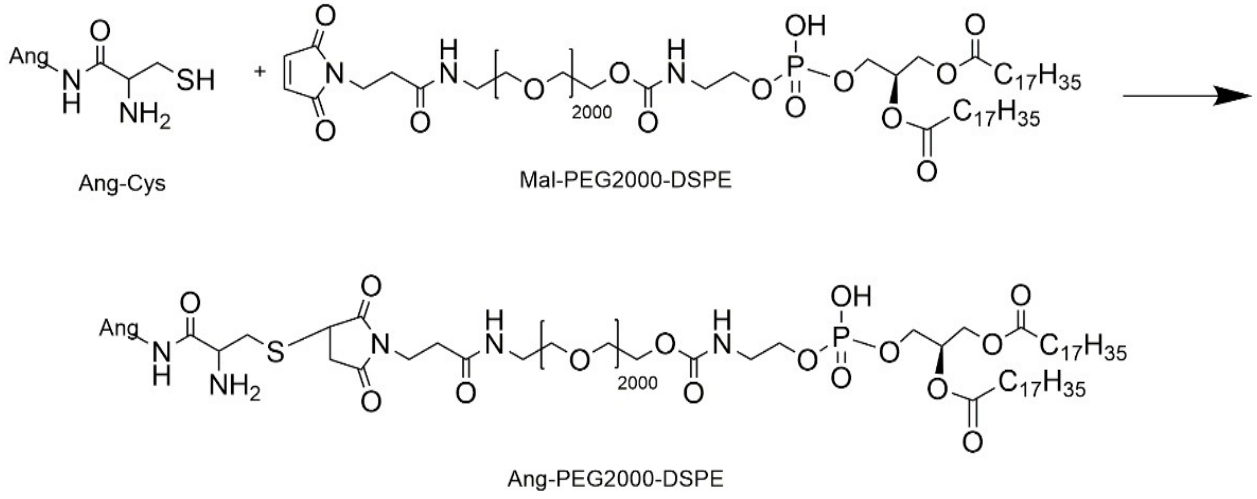

Figure S1. Synthesis reaction formula of DSPE-PEG_{2000}-ANG between the DSPE-PEG_{2000}-Mal and

Cys-Angiopep-2.

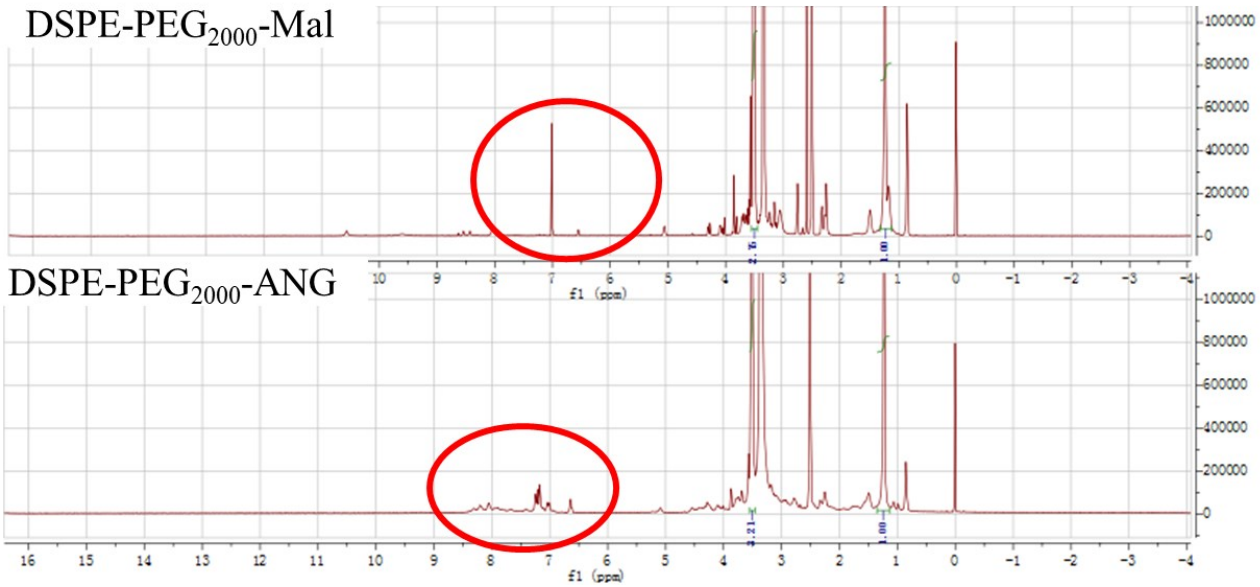

Figure S2. ^{1}H-NMR spectra of DSPE-PEG$_{2000}$-Mal and DSPE-PEG$_{2000}$-ANG.

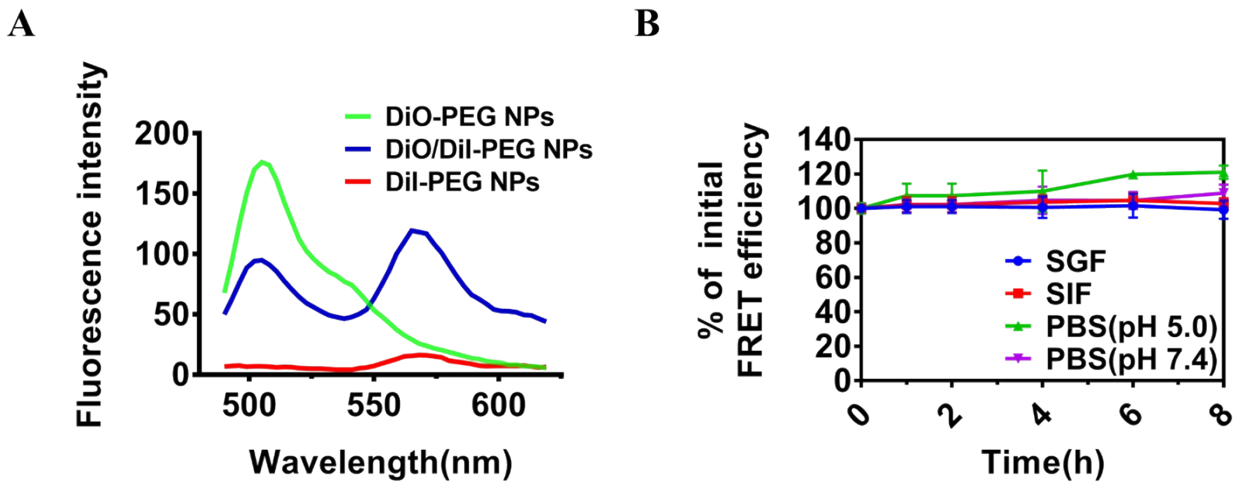

Figure S3. (A) FRET effects of DiI-PEG NPs, DiO-PEG NPs and DiO/DiI-PEG NPs; (B)FRET efficiency changes of fluorescence-labeled NPs after incubation with SGF (pH 1.2), SIF (pH 6.8), PBS (pH 5.0) and PBS (pH 7.4). Error bars represent SD (n = 3).

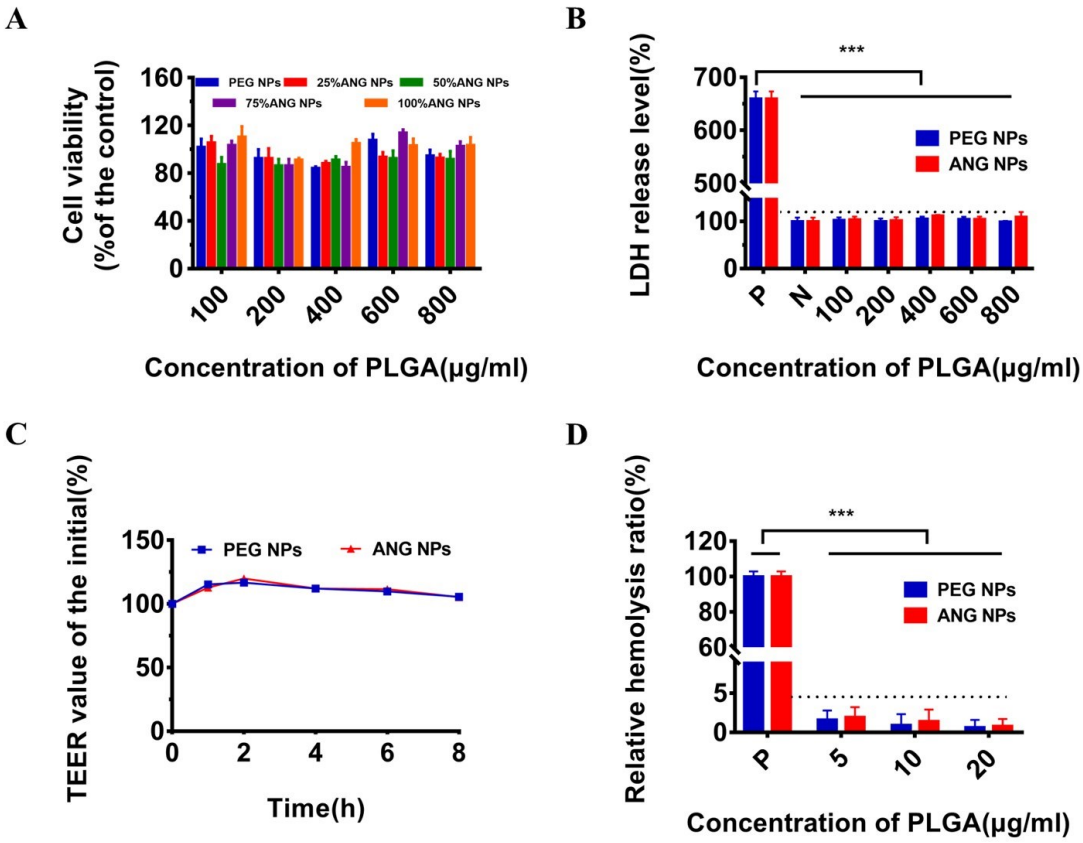

Figure S4 (A) Viability of Caco-2 cells after incubation with a series of NPs decorated by different molar ratios of ANG by Alamar Blue assay. Mean ± SD (n=6). (B) LDH release level of Caco-2 cells after incubation with PEG NPs and 100%ANG NPs. P and N respresent positive control (0.1% Triton) and negative control (PBS), respectively. Mean ± SD (n=6). (C) TEER change of Caco-2 cell monolayer before and after incubation with PEG NPs and 100%ANG NPs. Mean ± SD (n=3) (D) Relative hemolysis ratio after exposing erythrocyte to PEG NPs and 100%ANG NPs for 2 h. P represents positive control (0.1% Triton). Mean ± SD (n=6).

Tables:

Table S1. Size, PDI and zeta potential of dye-loaded NPs in water (n=3)

Samples	Size/nm	PDI	Zeta potential/mV
DiI PEG NPs	102.1±1.0	0.156	-4.97±1.33
DiI 25% ANG NPs	123.9±2.2	0.190	-23.63±1.34
DiI 50% ANG NPs	118±4.8	0.182	-22.47±2.19
DiI 75% ANG NPs	99.5±2.6	0.198	-6.99±3.27
DiI 100% ANG NPs	145.0±0.4	0.108	-13.17±0.22
DiO PEG NPs	105.5±1.4	0.140	-23.43±2.05
DiO100%ANG NPs	141.4±3.6	0.081	-7.29±0.84
DiR PEG NPs	86.2±0.2	0.213	-20.47±2.55
DiR 100%ANG NPs	110.2±0.8	0.160	-22.23±0.64

Table 1. Concentration and functions of chemicals used in endocytosis inhibition study

Endocytosis inhibitors	Concentration	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**
Chlorpromazine	**30 μM**	**Inhibitor of clathrin-mediated pathway**
Hypertonic sucrose	**0.4 M**	**Inhibitor of clathrin-mediated pathway**
Amiloride	**12 μg/mL**	**Inhibitor of macropinocytosis**

Table 2. Pharmacokinetic parameters of C6-PEG NPs and C6-ANG NPs in mice

Groups	Dose (mg/kg)	C_{max}(ng/mL)	$AUC_{0\text{-}24h}$(ng*h/mL)
C6-PEG NPs	**1**	**11.412 ± 4.486**	**40.862 ± 10.036**
C6-ANG NPs	**1**	**37.49±13.129**	**205.973 ±82.283**