Supplementary Information

Nanoparticles composed of tea polysaccharide-complexed cationic vitamin B_{12}-conjugated glycogen derivative

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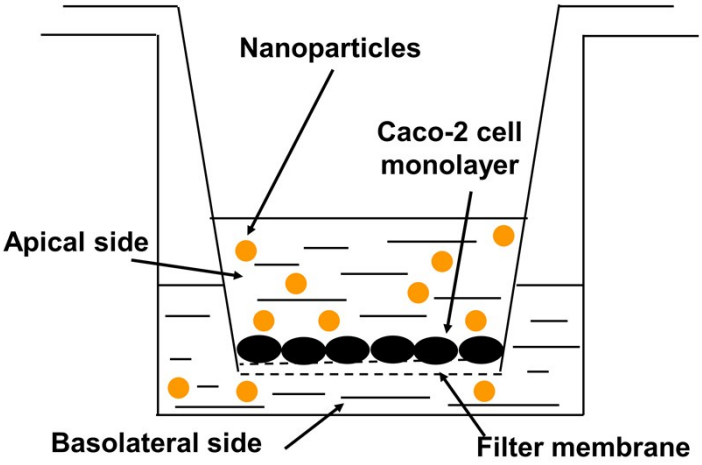
Contents

1. Model of Caco-2 cell monolayers



Scheme S1. Monolayer cell model of Caco-2 cells (Nanoparticles transferred from the apical side to the basolateral side).

2. Determination of degree of substitution of VB_{12} residues of the VB_{12}-DETA-Gly derivative using UV-vis spectroscopy

The UV-vis measurements were performed on a UV-vis spectrophotometer (Lambda750, PerkinElmer Corporation, Waltham, MA, USA). The standard VB_{12} aquous solutions (1~50 μg/mL) were preapred by dissoving VB_{12} in distilled water and used to obtian a working curve (Fig. S1, Eq. (S1)). Fig. S2 shows UV-vis spectrum of VB_{12}-DETA-Gly-II derivative. The degree of substitution of VB_{12} residues on the glycogen chain, defined as the number of VB_{12} residues per glucose unit of glycogen, was determined to be 0.6% according to Eq. (S1).

$$A = -1.9\times10^{-3}C + 2.7\times10^{-2} \quad (R^2=0.9999) \qquad \text{(S1)}$$

where A and C are aborbance and concentration of VB_{12} at λ=360 nm.

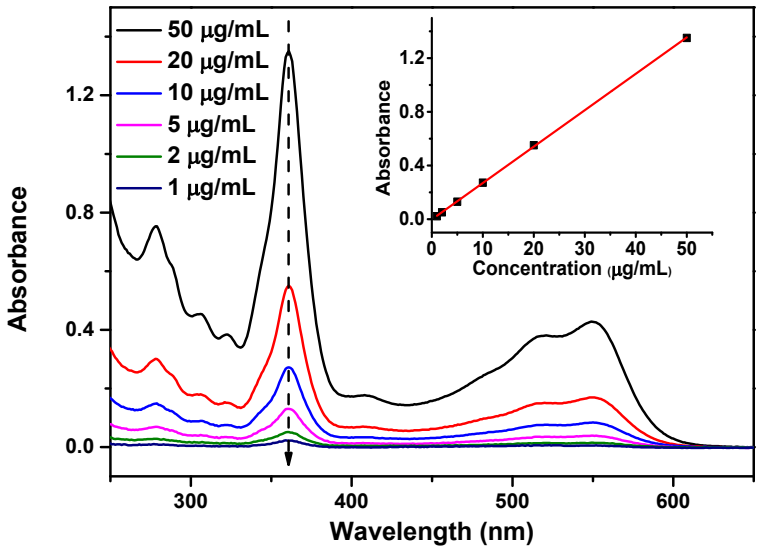


Fig. S1. UV-vis spectra of VB_{12} and standard working curve of VB_{12} in water (λ=360 nm).

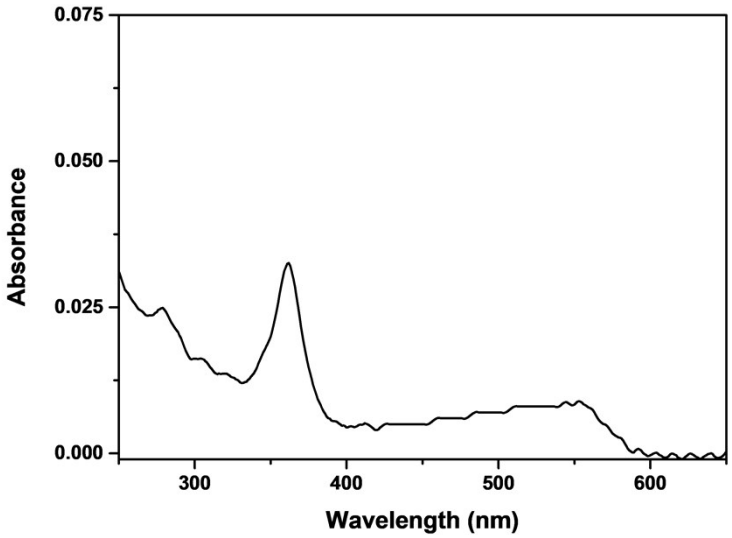
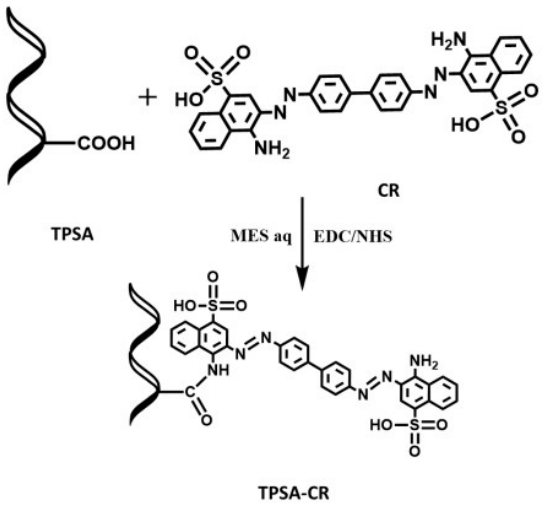


Fig. S2. UV-vis spectrum of VB_{12}-DETA-Gly-II derivative.

3. Synthesis of Congo red-conjugated TPSA (CR-TPSA) derivative

The Congo red-conjugated TPSA (CR-TPSA) derivative was synthesized using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) as coupling reagents at room temperature (Scheme S2). EDC·HCl and NHS were purchased form Sigma-Aldrich Co. (St Louis, MO, USA). Congo red and, 2-morpholinoethanesulfonic acid (MES) were bought from Aladdin Reagent Co. Ltd

(Shanghai, China). The TPSA solution was prepared by dissolving TPSA (440 mg, containing 0.25 mmol of saccharide units) in 5 mL of MES buffer solution (pH = 4, composed of 0.1 mol/L MES and 0.5 mol/L NaCl). The EDC and NHS mixture solution (5 mL, composed of 0.15 mol/L EDC and 0.15 mol/L NHS) was added to the TPSA solution with stirring for 1 h. After the Congo red/MES solution (5 mL, 0.15 mol/L) was added to the reaction system, the reaction was allowed to proceed at room temperature for 24 h. The reaction mixture was neutralized by the dropwise addition of ammonia water (25%, v/v), dialyzed against distilled water in a dialysis bag (molecular weight cutoff 14 kDa) for 72 h, and lyophilized to yield the solid CR-TPSA product. The chemical structure of the CR-TPSA derivative was analyzed using ^{1}H NMR spectroscopy in D_2O on a Varian Inova 500NB NMR spectrometer (Varian Inc., Palo Alto, CA, USA) at 25 °C. The degree of substitution of Congo red residues on the TPSA chain, defined as the number of Congo red residues per saccharide unit of TPSA, was determined using UV-vis spectroscopy on a UV-vis spectrophotometer (Lambda750, PerkinElmer Corporation, Waltham, MA, USA), PerkinElmer Corporation, Waltham, MA, USA).



Scheme S2. Synthesis route of the CR-TPSA derivative (CR: Congo red).

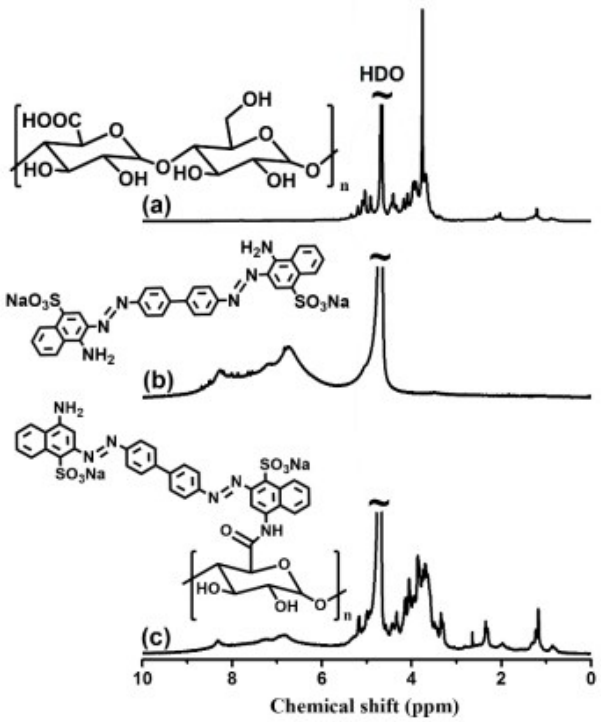


Fig. S3. ^{1}H NMR spectra of (a) TPSA, (b) Congo red and (c) the CR-TPSA derivative (solvent: D_2O).

Fig. S3 shows the ^{1}H NMR spectra of TPSA, Congo red and the CR-TPSA derivative. The resonance peaks in the range from 6.0 to 10.0 ppm assigned to the protons of Congo red residues appeared in the ^{1}H NMR spectrum of the CR-TPSA derivative (Fig. S3c), which was in the absence in the ^{1}H NMR spectrum of TPSA (Fig. S3a), when compared with that of Congo red (Fig. S3b). This confirmed that the Congo red residues were conjugated with the TPSA chains.

The UV-vis spectra of Congo red were obtained on a UV-vis spectrophotometer used in Section 1. The standard Congo red aquous solutions (1~50 μg/mL) were preapred by dissoving Congo red in distilled water and used to obtian a working curve (Fig. S4a, Eq. (S2)).

$$A = 2.1\times10^{-2}C + 4.3\times10^{-2} \quad (R^2=0.9969) \qquad \text{(S2)}$$

where A and C are aborbance and concentration of Congo red at λ=498 nm.

Fig. S4b shows UV-vis spectrum of the CR-TPSA derivative. The degree of substitution of Congo red residues on the TPSA chain, defined as the number of Congo red residues per monosaccharide unit of TPSA, was then determined to be 0.02

according to Eq. (S2).

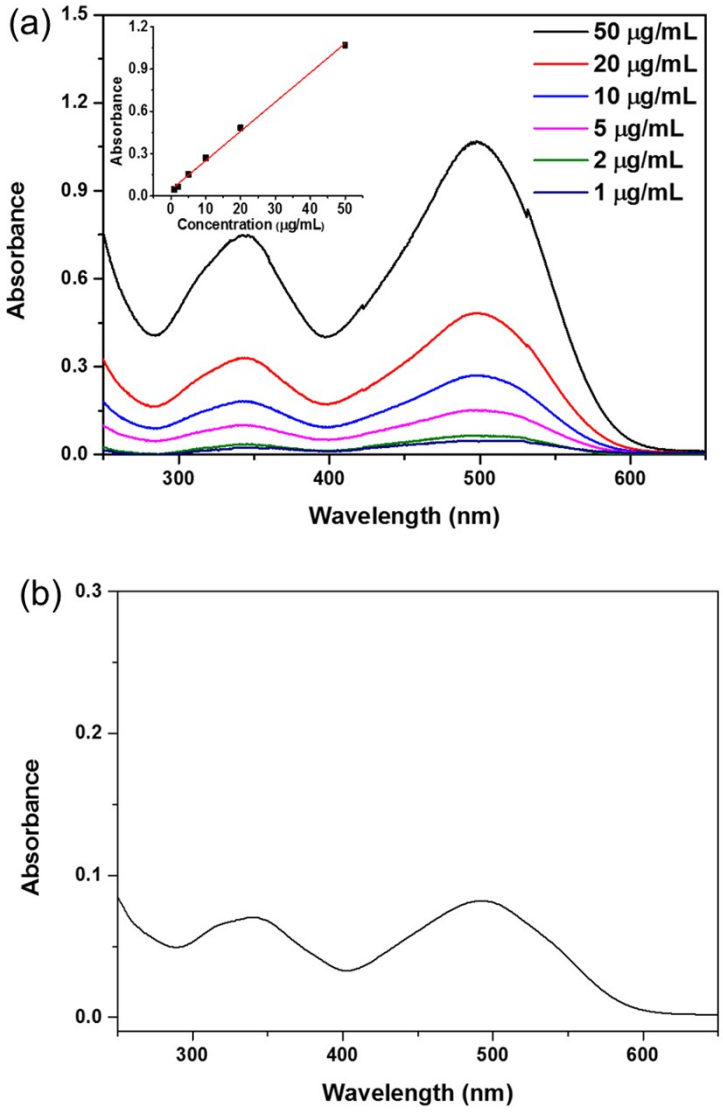
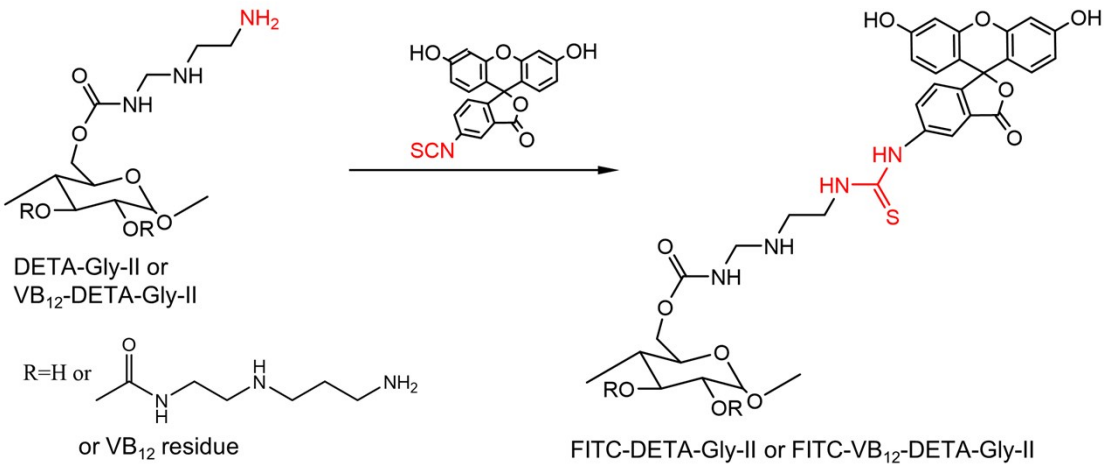


Fig. S4. (a) UV-vis spectra of Congo red and standard working curve of Congo red in distilled water (λ=498 nm). (b) UV-vis spectrum of the CR-TPSA derivative in distilled water.

4. Synthesis of the FITC-labeled DETA-Gly and VB_{12}-DETA-Gly derivatives

For study of phagocytosis of Caco-2 cells to DETA-Gly-II and VB_{12}-DETA-Gly-II derivatives and the VB_{12}-DETA-Gly-II/TPSA nanoparticles, the FITC-labeled derivatives, named FITC-DETA-Gly-II and FITC-VB_{12}-DETA-Gly-II, were synthesized following the method used in the literature (Scheme S3).[1] FITC was purchased from Dalian Meilun Biological Technology Co. Ltd (Dalian, China). Briefly, The DETA-Gly-II solution was prepared by dissolving the DETA-Gly-II

derivative (100 mg, 0.34 mmol) in 20 mL of distilled water. After the FITC/DMSO solution (5 mg, 13 μmol) was added to the DETA-Gly-II solution with stirring, the reaction was allowed to proceed at room temperature for 5 h. The reaction mixture was dialyzed against distilled water in a dialysis bag (molecular weight cutoff 14 kDa) for 72 h, and lyophilized to yield the solid FITC-DETA-Gly-II product. Following the same method, the FITC-VB_{12}-DETA-Gly-II derivative was synthesized using the VB_{12}-DETA-Gly-II derivative and FITC.



Scheme S3. Synthesis route of the FITC-DETA-Gly-II or FITC-VB_{12}-DETA-Gly-II derivative.

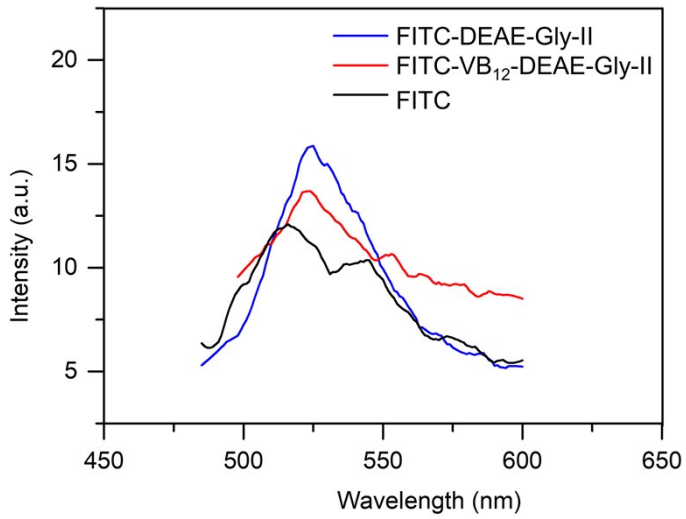


Fig. S5 Fluorescence spectra of the FITC-DETA-Gly-II and FITC-VB_{12}-DETA-Gly-

II derivatives and FITC in distilled water (λ_{Ex}=460 nm).

The fluorescence properties of the FITC-DETA-Gly-II and FITC-VB_{12}-DETA-Gly-II derivatives were studied using fluorescence spectroscopy in distilled water (Fig. S5). Fluorescence measurements were carried out on a RF-5301PC fluorescence spectrometer (Shimadzu Corporation, Kyoto, Japan). The excitation wavelength was set at 460 nm, and the emission spectra were recorded over a scanning wavelength range of 480–600 nm. Both slit widths of the excitation and emission were set at 5.0 nm.

References

1 D. Vllasaliu, R. Exposito-Harris, A. Heras, L. Casettari, M. Garnett, L. Illum and S. Stolnik, Tight junction modulation by chitosan nanoparticles: Comparison with chitosan solution, *Int. J. Pharm.*, 2010, **400**, 183–193.