Electronic supplementary information (ESI)

Synthesis and cell-surface binding of lectin-gold nanoparticle conjugates

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1. Additional tables: table S1 and S2

2. Synthesis of mPEG-SH stabilized gold nanoparticles

3. Additional Figures S1-S5

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1. Additional tables

Table S1 Carbohydrate specificities of the lectins.

Lectins	Abbreviation	Binding specificity[a]	Name of lectin-GNP conjugates
Ricinus Communis Agglutinin I	RCA 120	Galβ(1,4)GlcNAcβ1	RCA@GNP
Concanavalin A	Con A	Branched and terminal mannose, terminal GlcNAc	Con A@GNP
Sambucus Nigra Lectin	SNA	α-2,6 sialic acid	SNA@GNP
Soybean Agglutinin	SBA	Terminal GalNAc	SBA@GNP
Maackia Amurensis Lectin II	MAA II	α-2,3 sialic acid	MAA II@GNP
Griffonia simplicifolia II	GS II	Terminal GlcNAc	GS II@GNP

[a] The binding specificity is obtained from manufacturer's introduction.

Table S2 The properties of lectin-GNP conjugates.

Lectin-GNP conjugates	Maximum absorption wavelength/nm	Diameter/nm TEM[a]/DLS[b]	Total number of lectin molecules per particle[c]
citrate@GNP	518	13.7±1.9/18.64±0.21	-
RCA@GNP	524	14.7±2.2/48.31±0.12	17
Con A@GNP	524	14.3±1.8/32.39±0.50	21
SNA@GNP	524	14.3±2.2/25.91±1.0	20
SBA@GNP	524	14.3±1.8/33.78±0.79	17
MAA II@GNP	524	13.8±2.0/32.18±0.68	19
GS II@GNP	524	13.7±2.0/27.83±0.20	17

[a] The diameter of nanoparticle was measured by TEM; [b] The diameter of nanoparticle was measured by DLS; [c]and we found that the total number of lectin molecules per particle was about 20, that means single GNP may react with several glycosyl residues on cellular surface.

Total number of lectin molecules per particle was estimated by fluorescent method. Here, the fluorescent dye (FTIC or FC) conjugated lectins were used for synthesizing the lectin-GNP conjugates. After purified by centrifugation, and resuspended in water, a 150 μL aliquot of the purified lectin-GNP conjugate solution (14.4 nM) was reacted with dithiothreitol (DTT, 150 μL, 0.2 M in phosphate buffered saline (PBS), pH 7.5) in dark cabinet at room temperature for 12 h, respectively. In this step, all particle-bound lectins were released by a ligand exchange reaction with DTT, which

led to the precipitation of the particles [S1,S2]. Subsequently, the samples were stirred by ultrasound for 5 min, and centrifuged at 12 000 g for 30 min. The fluorescence intensity of the supernatant solution was measured by a spectrofluorometer. The concentration of particle-bound lectin was calculated by corresponding calibration curve from pure FITC/FC conjugated lectin.

2. Synthesis of mPEG-SH stabilized gold nanoparticles

The mPEG-SH stabilized GNPs were prepared as follows: 13 nm citrate stabilized GNPs were incubated with mPEG-SH at various molar ratios (GNP: mPEG-SH= 1:2000, 1:4000, 1:5000, 1:10000, 1:50000 and 1:100000, respectively) in 1.8 mM potassium carbonate for 1 h, respectively. Then, the particles were purified by repeated centrifugation (9600 g for 30 min, 3 times), and resuspended in water. Then the stabilities of mPEG-SH stabilized GNPs were tested by changing solution ionic strengths. The experimental result demonstrates that the mPEG-SH stabilized GNPs have good stabilities while the molar ratio of mPEG-SH to GNP is above 10000: 1 (see Fig. S1). Therefore, the molar ratio of 10000:1 (mPEG-SH : citrate stabilized GNP) was selected for synthesizing mPEG-SH stabilized GNPs (mPEG@GNPs). The interactions of mPEG@GNPs with cells were used as control experiments.

3. Additional Figures S1-S5

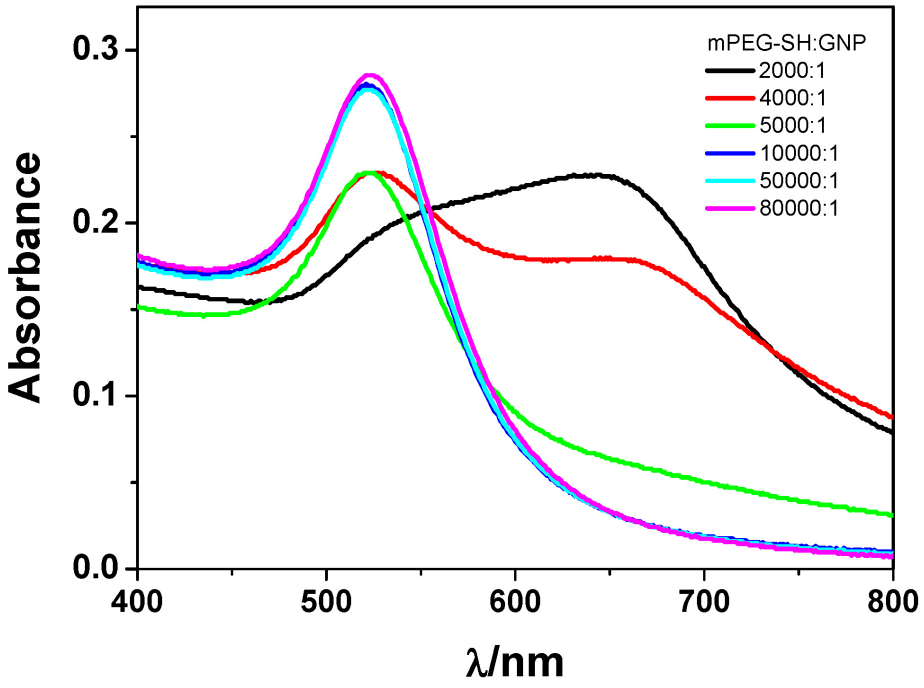

Fig. S1 The UV-visible spectra of mPEG@GNPs (3.6 nM) in the presence of 0.5 M NaCl in the solution. The molar ratio of mPEG-SH to GNP is 2000:1, 4000: 1, 5000: 1, 10000: 1, 50000: 1 and 80000: 1 in the reaction mixture of mPEG@GNP synthesis, respectively.

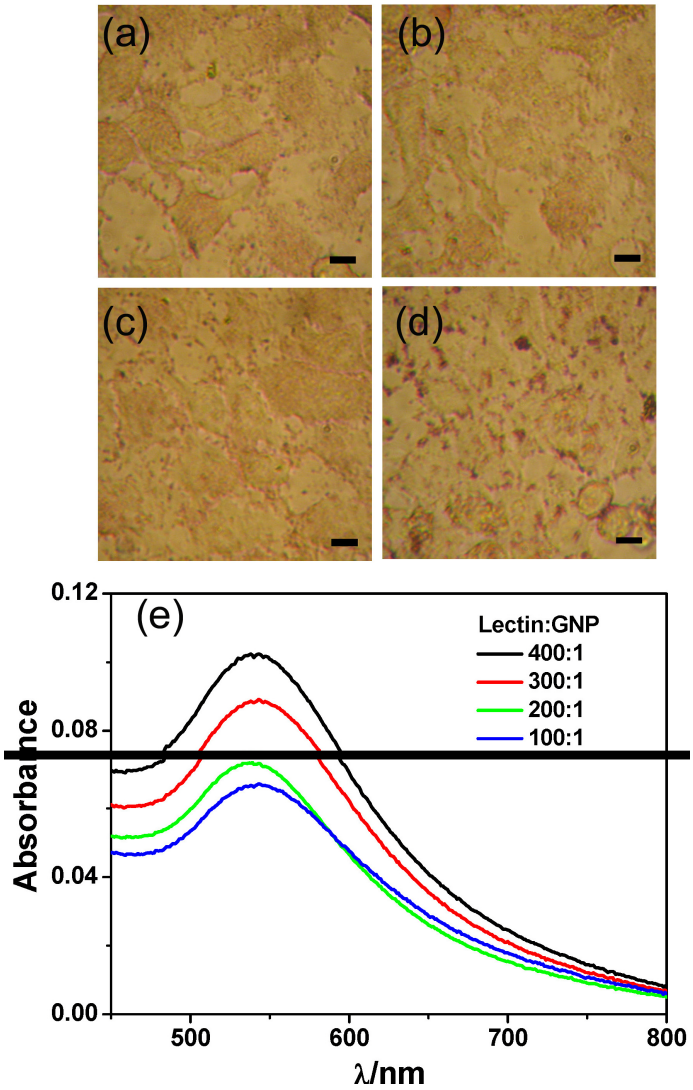

Fig. S2 Bright-field microscopic images (a-d) and UV-visible spectra (e) of HeLa cells after incubated with 5.76 nM of Con A@GNP for 1 h at 37 ºC, respectively. Scale bar: 10 μm for all images. The concentration of cells was 7.5 × 10^5 cells/mL. The molar ratio of lectin to GNP is 100:1 (a), 200: 1 (b), 300: 1 (c), and 400: 1 (d) in the reaction mixture of Con A@GNP synthesis, respectively. The molar ratios of lectin to NHS-PEG-S-S-PEG-NHS (2:1), and mPEG-SH to GNP (10000: 1) are fixed in the reaction mixture of Con A@GNP synthesis. The concentration of GNP is 6 nM in the reaction mixture of Con A@GNP synthesis.

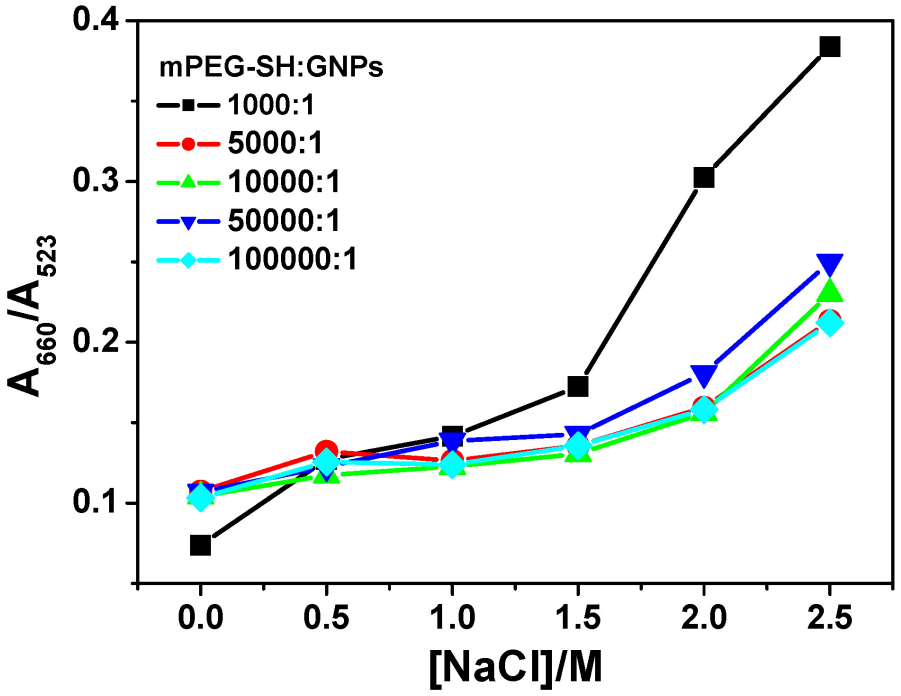

Fig. S3 The effect on stability of Con A@GNP by changing solution ionic strength. The molar ratio of mPEG-SH to GNP is 1000:1, 5000: 1, 10000: 1, 50000: 1, and 100000: 1 in the reaction mixture of Con A@GNP synthesis, respectively. The molar ratios of lectin to NHS-PEG-S-S-PEG-NHS (2:1), and lectin to GNP (300: 1) are fixed in the reaction mixture of Con A@GNP synthesis. The concentration of GNP is 6 nM in the reaction mixture of Con A@GNP synthesis.

The monodispersing GNP has maximum SPR absorption band at 523 nm, and the GNP aggregate has maximum SPR absorption band at 660 nm. Therefore, the increasing A_{660}/A_{523} value means that the stability of Con A@GNP is decreased, *i.e.* the Con A@GNP forms aggregates in the solution.[S3,S4]

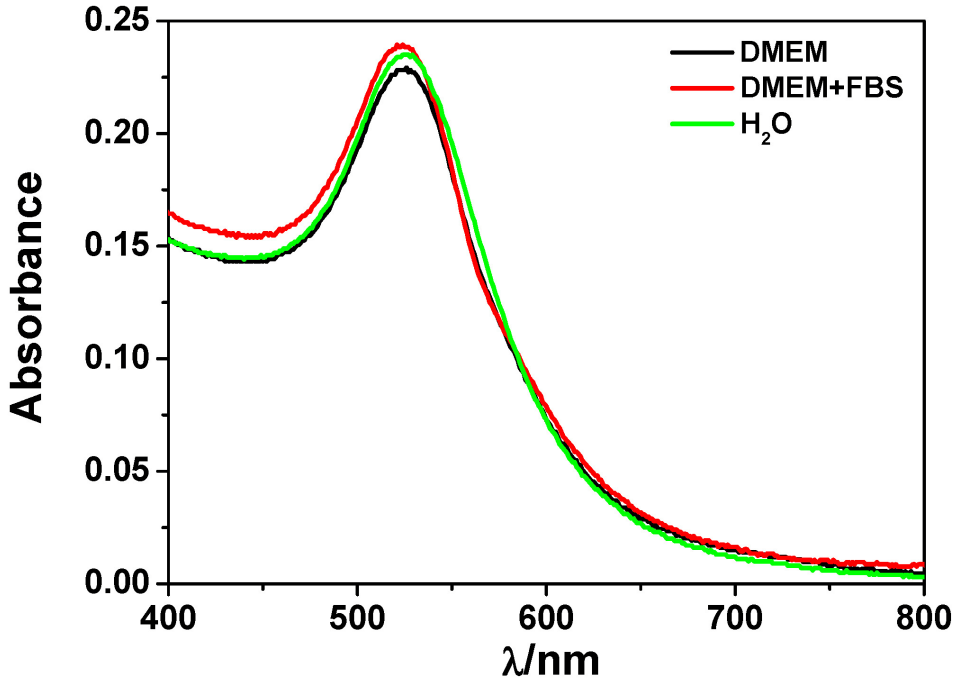

Fig. S4 UV-visible spectra of Con A@GNP dissolved in DMEM, cell culture medium (DMEM+10% FBS) and water. The concentration of Con A@GNP is 3.6 nM.

Here, no significant changes of spectra were observed.

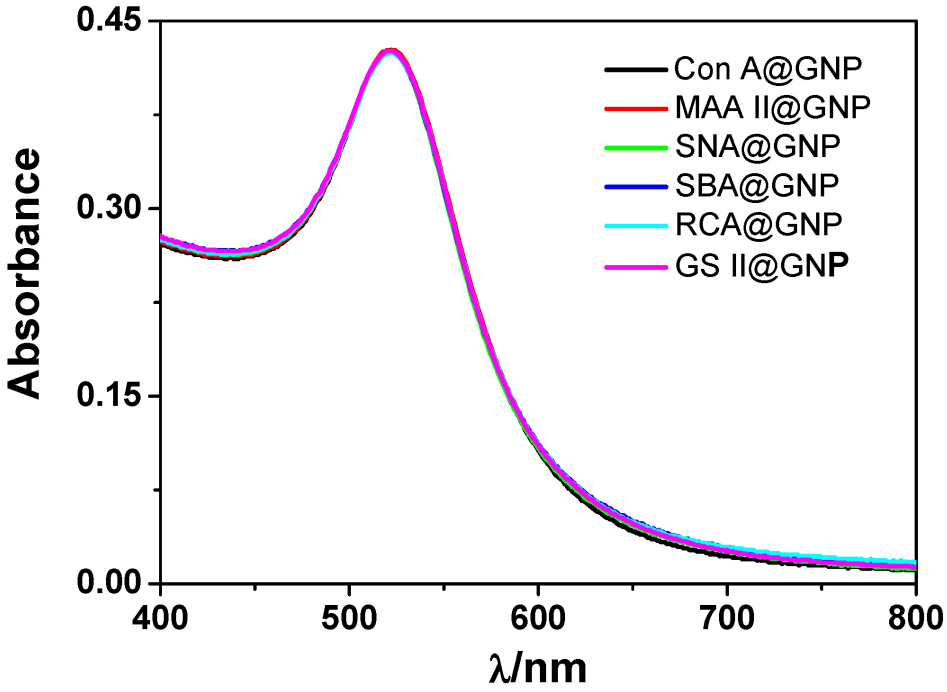

Fig. S5 UV-visible spectra of the six lectin-GNP conjugates. The concentration of the lectin-GNP conjugates is 4.0 nM.

References

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