Supplemental Information

One-Step Facile Synthesis of N-acetylglucosamine-Functionalized Gold Nanoparticles for Direct Visual and Light-Scattering Detection of Lectin from Wheat Germ

Erqun Song,* Jingrong Li, Hong Wei and Yang Song*

Key Laboratory of Luminescence and Real-Time Analysis of the Ministry of Education, College of Pharmaceutical Sciences, Southwest University, Chongqing , 400715, China.

Tel: +8615826022758; E-mail: eqsong@swu.edu.cn; songyangwenrong@hotmail.com

EXPERIMENTAL SECTION

Reagents: Hyaluronic acid (HA, MW: 34,600) were obtained from Shandong Dongchen Bioengineering Holding CO, Ltd.; wheat germ agglutinin (WGA), peanut agglutinin(PNA), ulex europaeus agglutinin(UEA), dolichos biflorus agglutinin(DBA) were purchased from Vector company; $HAuCl_4$ were obtained from Tianjin Guangfu Chemical Research Institute.

Instruments: Rayleigh light scattering (RLS) spectra were obtained using a Hitachi F-7000 fluorescence spectrophotometer(Japan); absorption spectra were obtained using a Shimadzu UV-2450 ultraviolet spectrophotometer(Japan); the nanoparticles size analyzed by using dynamic light scattering instrument (Malver Zetasizer nanozs, Britain) and dark-field light scattering images form Olympus BX-51 microscope (Japan), and transmission electron microscopy imaging by Shimadzu H-7500 TEM (Japan)

Synthesis of N-acetylglucosamine functionalized gold nanoparticles (NAG-AuNPs): 15 mL of 1% (W/V) HA in 0.1 mol/L phosphate buffer (NaH_2PO_4, Na_2HPO_4, pH 11) was mixed with 500 μL $HAuCl_4$ (4 mol/L), then the mixture was stirred for 30 min at 50 ºC until the solution color changed to pink. After cooled down to room temperature, the products were subsequently purified via ultra-filtration, and stored at 4 ºC.

Quantification of the NAG molecules on each AuNP: The number of NAG

molecules on each AuNP was determined based on the classical Elson and Morgan method [1, 2] with some mild modification [3, 4].

The details are described as follows: Firstly, two reagents, Ehrlich's reagent (0.8 g of p-Dimethylaminobenzaldehyde dissolved in 15 mL of 96% ethanol and 15 mL of 12 M hydrochloric acid) and acetylacetone reagent (3.5 mL of acetylacetone dissolved in 50 mL of 0.5 M sodium carbonate solution), were prepared. A series of aqueous solution of glucosamine hydrochloride (5mL, with different concentrations) mixed with freshly prepared acetylacetone reagent (1mL) and heated for 25min in a boiling water-bath. Then the mixture were cooled down to room temperature in ice water, and treated with Ehrlich's reagent and 96% ethanol (4mL) in a water bath with 60 ºC for 1 hour. Finally, the absorbance at 525 nm of the resultant red solution was measured to give a standard curve for the glucosamine hydrochloride (see Figure S2).

For the sample of NAG-AuNPs, the glucosamine hydrochloride needs to be firstly released from the nanoparticles by treatment with hydrochloric acid (2M) at 100 ºC for 12h. Once collected from the supernatants, the glucosamine hydrochloride was subjected to quantitative analysis by the procedure mentioned above. The calculation of the number of NAG molecules is based on the following equation:

$$N = n_{(NAG)} / n_{(AuNPs)}$$

In the equation, the "*N*" stands for the number of NAG on each gold nanoparticle, while the "*n*" means for the mole quantity.

Binding assay of the NAG-AuNPs with lectins: Lectins (WGA, UEA, PNA and DBA) with different quantities were first mixed with 200 μL of NAG-AuNPs (4 nM) solution, and then the mixture was diluted to 400 μL with 10 mM phosphate buffered saline (NaH_2PO_4, Na_2HPO_4, 10 mM NaCl, pH 6.5), following incubated at 37 ºC for 100 min with continuous shaking. The color and spectra changes of the samples were observed using a digital camera or a UV-vis spectrophotometer and fluorescence spectrophotometer.

Figures

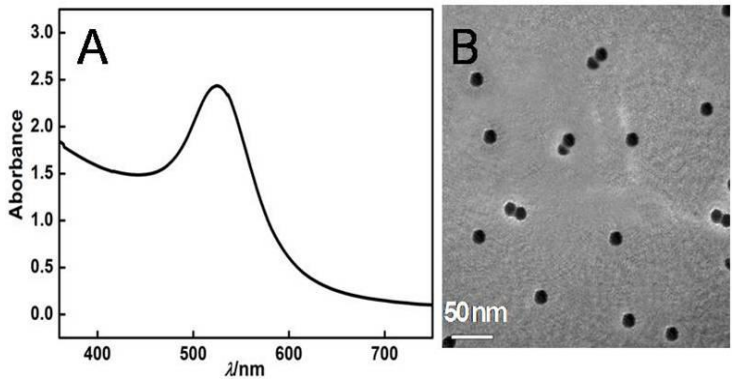

Figure S1 Absorption spectrum (A) and TEM image (B) of NAG-AuNPs

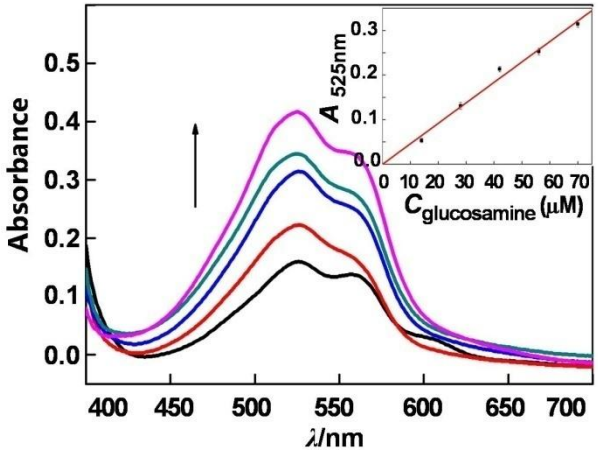

Figure S2 Absorption spectra of the glucosamine derivative obtained by Elson &Morgan's method. Insert: standard curve of absorbance at 525 nm as the function of glucosamine concentration.

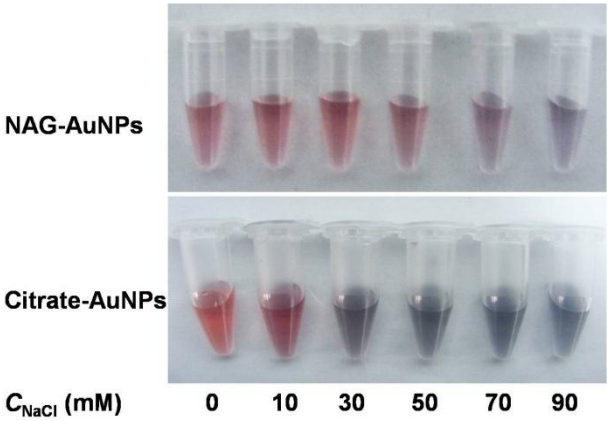

Figure S3 Investigation of NAG-AuNPs withstanding salt-induced aggregation. Citrate-AuNPs were analyzed in parallel as a control.

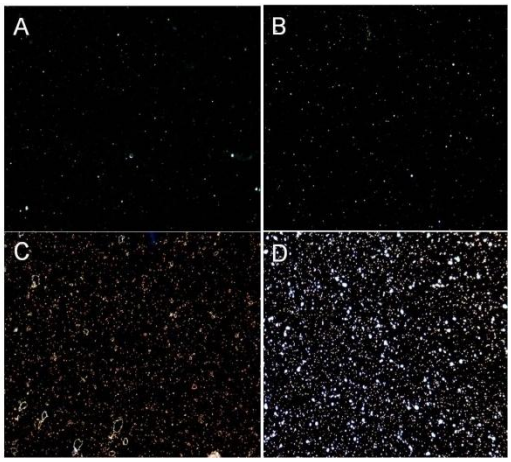

Figure S4 Dark-field light scattering images (×40) of AuNPs in the absence (A) and presence of WGA (B, C and D). B: [WGA]=5 nmol/L; C: [WGA]=40 nmol/L; D: [WGA]=100 nmol/L

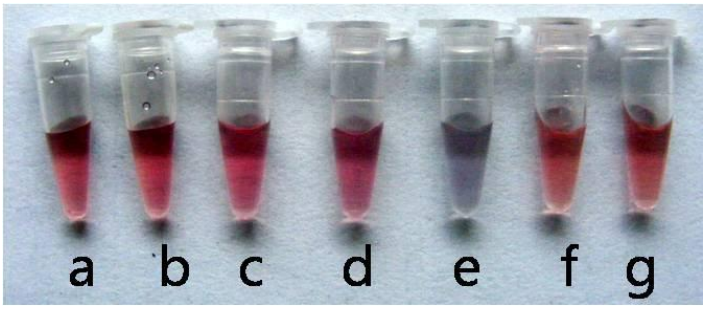

Figure S5 Photographs of NAG-AuNPs suspension in the absence (a) and presence of 100 nM different lectins: (b) UEA, (c) PNA, (d) DBA, (e) WGA; (f) and (g): citrate AuNPs only and citrate AuNPs with 100nM of WGA respectively.

References:

1. Elson L. A., Morgan W. T. J. A colorimetric method for the determination of glucosamine and chondrosamine. *Biochem. Journal.*, 1933, **27**, 1824-1828.
2. Morgan W. T. J., Elson L. A. A colorimetric method for the determination of N-acetylglucosamine and N-acetylchrondrosamine. *Biochem. Journal.*, 1934, **28**, 988-995.
3. Reissig, J. L., Strominger, J. L., Leloir, L. F. A modified colorimetric method for the estimation of N-acetylamino sugars. *J. Biol. Chem.*, 1955, **217**, 959-966.
4. Robinson A., Fang J. M., Chou P. T., Liao K.W., Chu R. M., Lee S. J. Probing lectin and sperm with carbohydrate-modified quantum dots. *ChemBioChem*, 2005, **6**, 1899-1905.