Supporting Information

Immunoassay of goat anti-human IgG antibody based on luminescence resonance energy transfer (LRET) between near infrared responsive $NaYF_4$:Yb,Er upconversion fluorescent nanoparticles and gold nanoparticles

Meng Wang [a], Wei Hou [a], Cong-Cong Mi [a], Wen-Xing Wang [a], Zhang-Run Xu [a], Hong-Hui Teng [a], Chuanbin Mao [b*], Shu-Kun Xu [a*]

[a] *College of Sciences, Northeastern University, Shenyang 110004, P.R. China, and* [b] *Department of Chemistry & Biochemistry, University of Oklahoma, 620 Parrington Oval, Room 208, Norman, Oklahoma 73019, U.S.A.*

1. Details for the flocculation test

The citrate stabilized Au NPs suspension synthesized in our work could be very stable for several months, due to the electrostatic repulsions between negatively charged Au NPs. However, when the salt (10% NaCl solution) was added into the suspension, Au NPs could become aggregated due to the interaction between the cations and the negative charges on the surface of Au NPs, which screens the electrostatic repulsions between Au NPs. As shown in Figure S1-a, when the salt was added, the maximum absorption peak not only decreased in intensity, but also showed

* Corresponding author. Tel.: 86 24 83681343, E-mail: xushukun46@126.com (S. K. Xu); Tel.: 1 405 325 4385, E-mail: cbmao@ou.edu (C. B. Mao).

a significant red shift. In addition, the suspension changed from transparent (red in color) to flocculated (dark blue in color), indicating that the large colloidal clusters were formed. When proteins (*e.g.* immunoglobulin G, antibody, *etc.*) are added to Au NPs suspension,, the proteins can be adsorbed to the surface of Au NPs, proventing the Au NPs from flocculation. A generally accepted view is that stabilization of Au NPs by an adsorbed protein is achieved when addition of salt fails to coagulate the Au NPs.

The minimal stabilizing protein concentration is defined by the lowest protein concentration required to avoid flocculation of Au NPs suspension, and a flocculation test was often carried out to determine the minimal stabilizing protein concentration. Typically, human IgG powder was diluted to 4.0×10^{-1} mg/mL with 1.0×10^{-2} mol·L^{-1} borate buffer solution (pH 8.0). 4.0×10^{-1} mg/mL human IgG with an increasing volume (0, 20, 30, 40, 50, 60, 70 μL) was added into the different tubes, and the total volume of each tube was adjusted to 1.0 mL by the addition of borate buffer solution. Subsequently, 2.0 mL colloidal gold was added into each tube and kept at 4 ^{o}C for 1 h. After 50 μL 10% NaCl solution was added into each tube and kept at 4 ^{o}C for 2 h, the Au NPs suspension changed from transparent (red in color) to flocculated (dark blue in color). UV-vis absorption spectrum of each colloid was recorded over the range of 400-800 nm at room temperature to determine the minimal stabilizing protein concentration.

As shown in Figure S1-a, when human IgG is added into the Au NPs suspension followed by the addition of salt, the UV-vis absorption of Au NPs shows little or no

red shift in wavelength, indicating that the human IgG protects the Au NPs from aggregation. Furthermore, the more human IgG is added into the Au NPs suspension, the less red shift of the UV-vis absorption it will be. With the increasing amount of human IgG added, the maximum UV-vis absorption (λ_{max}) of Au NPs suspension begins to blue shift and maintains constant when the quantity of human IgG is 16.0 μg (Figure S1-b), which indicates that 16.0 μg of human IgG is the minimal quantity needed to stabilize 2.0 mL of Au NPs suspension. Therefore, in the preparation of human IgG conjugated Au NPs, 20 mL of Au NPs suspension was stabilized by using 192 μg of human IgG (20% more than the minimum amount).

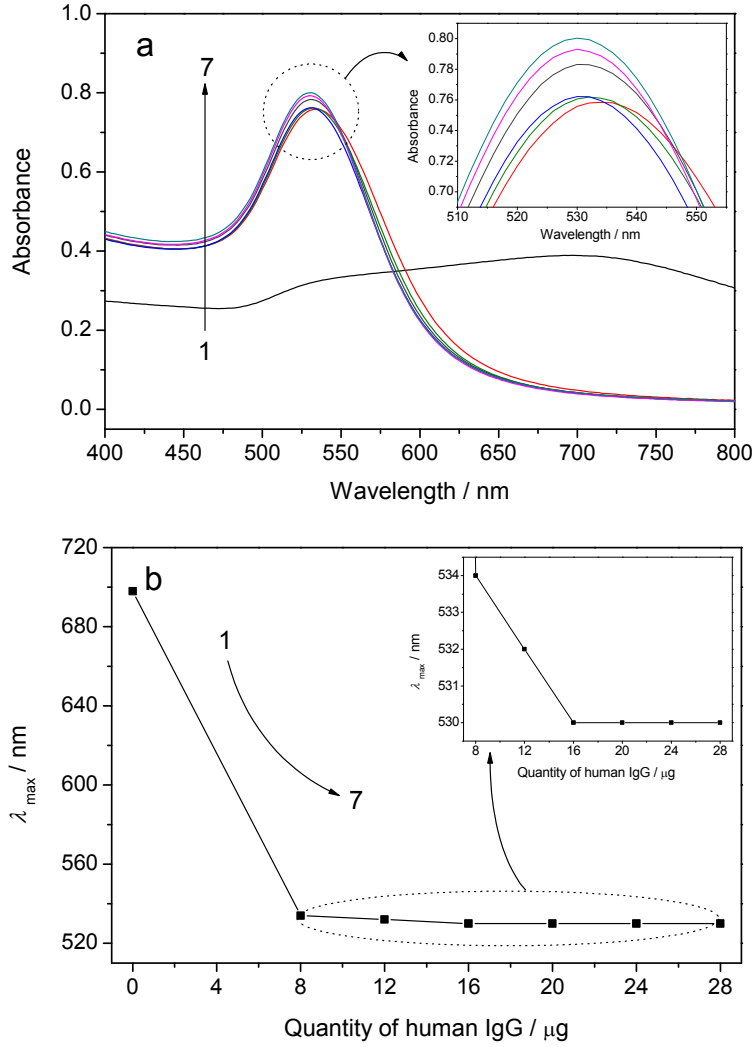

Figure S1. UV-vis absorption spectrum (a), and the peak positions corresponding to the maximum absorption (b) of Au NPs, conjugated with the increasing amounts of human IgG. The amount of human IgG corresponding to curve 1 to 7 is 0, 8, 12, 16, 20, 24, and 28 μg, respectively.

2. Data of XPS analysis

Table S1. Interrelated data of XPS analysis.

Name	Start BE	Peak BE	End BE	Height Counts	FWHM eV	Area (P) CPS. eV	Area (N)	At. %
C 1s	289	283.17	279.1	10189.92	1.79	21563.14	0.5	40.8
O 1s	536.8	530.58	525.1	25808.42	1.63	50403.22	0.41	33.51
Si 2p	105.5	101.47	97.5	3644.46	1.99	8042.22	0.22	18.39
N 1s	403.7	397.47	393	1247.97	1.59	3596.25	0.05	3.82
Cl 2p	201.8	195.99	193.3	411.99	0.38	1018.36	0.01	0.84
F 1s	693.1	687.72	683.4	2030.62	1.61	4044.46	0.02	1.83
Na 1s	1075.2	1069.49	1065.5	1216.96	0.19	2909.69	0.01	0.8