Supplementary information

Selective recognition of protein isoforms using functionalized gold nanoparticles

Kaimin Chen,[ab] Subinoy Rana,[a] Daniel F. Moyano,[a] Yisheng Xu,[a] Xuhong Guo*[b] and Vincent M. Rotello*[a]

[a] *Department of Chemistry, University of Massachusetts at Amherst, Amherst, MA 01003, USA. E-mail: rotello@chem.umass.edu*
[b] *State Key Laboratory of Chemical Engineering, School of Chemical Engineering, East China University of Science and Technology, Shanghai 200237, China.*

Materials:

Bovine β-lactoglobulin variants (BLGA, BLGB) were purchased from Sigma-Aldrich (Lot no. 097K7010 and 048K7003, respectively) and were used as received. Gold nanoparticles (NPs) were synthesized following previous report.[1] The NP core size was ~2 nm and the hydrodynamic size was ~10 nm with various cationic ligands grafted on the surface. The number of ligands attached to the gold core was ~100. Sodium chloride, sodium phosphate (monobasic, dibasic), standard sodium hydroxide, and hydrochloric acid solutions were purchased from Fisher Scientific. Milli-Q water was used throughout all the experiments.

Dynamic light scattering:

Dynamic light scattering (DLS) studies were carried out at 25 °C using a Malvern Zetasizer Nano ZS instrument. The measurement angle was 173° (backscatter). Data were analysed by the "multiple narrow modes" (high resolution) based on non-negative-least-squares (NNLS). Samples (1 mL) at pH 5.5 were taken from a mixture of NP and BLGA/BLGB solution with molar ratio of 1:4 and

equilibrated for 5 min before the measurements. For all the cases 3 measurements were recorded, each measurement consisting of 11 runs (33 data points).

Isothermal titration calorimetry:

Isothermal titration calorimetry (ITC) was performed in a MicroCal VP-ITC calorimeter. BLGA/BLGB solutions of 20 μM were prepared in 5 mM phosphate buffer with pH 5.5, stirred and degassed for 10 min. The reference cell was filled with the same buffer. The NPs dissolved in 5 mM phosphate buffer (8 μM) were titrated into BLGA/BLGB solution (20 μM) in the sample cell to obtain high signal-to-noise ratio. Automated addition of 5 μL titrant were continued for 55 injections with an interval time of 300 s. Acquisition time between two data points was set to 2 s. For BLGA/BLGB and NP blank titrations, 5 mM phosphate buffer was titrated into BLGA/BLGB solution, or NPs were titrated into 5 mM phosphate buffer. ITC data were fitted by Origin 7.0 program (MicroCal, Northampton, MA, U.S.A.).

Detailed analysis of the thermograms was carried out using certain assumptions about the BLG-NP interactions. The DLS results indicated that soluble complexes are formed after incubating BLG and NPs. This is also supported by the observation of optical clarity after completion of the ITC experiment. Such uniformity of complexation fits well with the assumption that every nanoparticle possesses *n* identical and independent binding sites. Every binding event is assumed to follow the same equilibrium:

$$\mathbf{site + BLG \rightleftharpoons complex} \qquad (1)$$

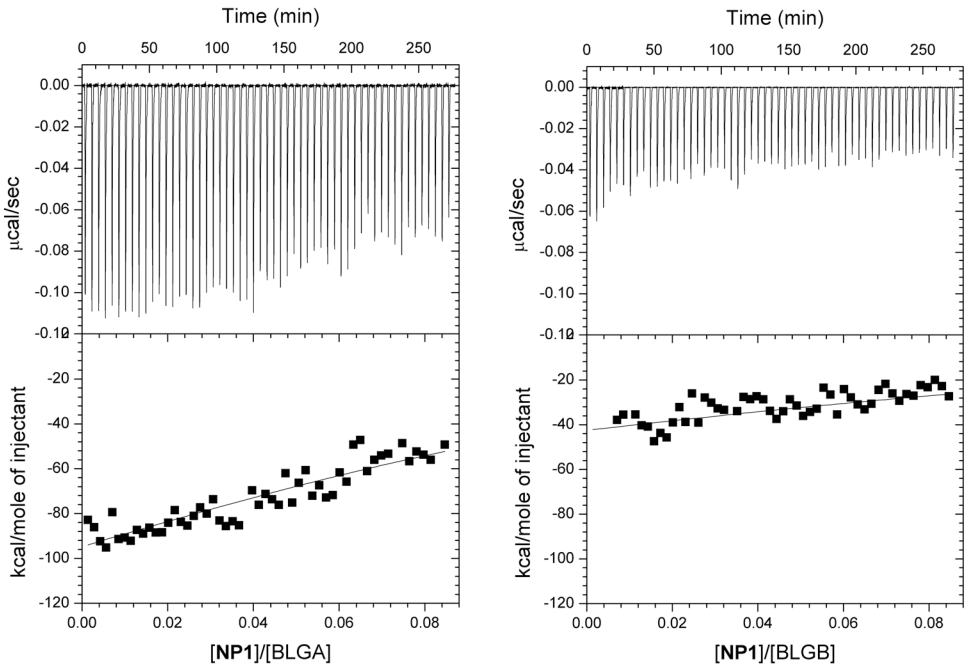
in which "complex" stands for an occupied binding site on a nanoparticle, with a binding affinity proportional to K_b. The total heat change (Q) at any point in the titration is assumed to be proportional to the concentration of the complex, *i.e.* $Q = \alpha[\text{complex}]$. α is a constant for equivalent, independent sites. The molar concentration of total binding sites ($[\text{site}]_{tot}$) should be $n[\text{NP}]_{tot}$, where $[\text{NP}]_{tot}$ represents the total concentration of the nanoparticle. The binding constant can be written as,

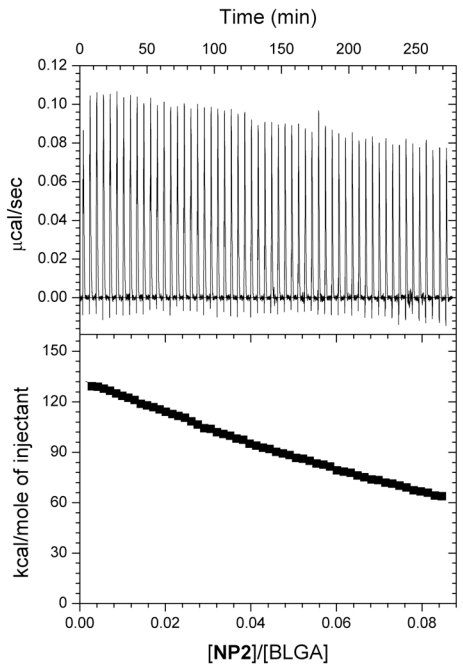
$$K_b = \frac{[\text{complex}]}{[\text{site}][\text{BLG}]} = \frac{Q/\alpha}{\left([\text{site}]_{tot} - Q/\alpha\right)\left([\text{BLG}]_{tot} - Q/\alpha\right)} \qquad (2)$$

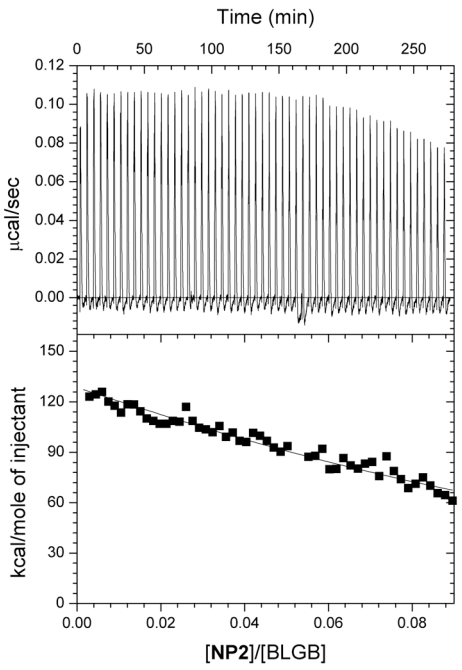
Solving for Q,

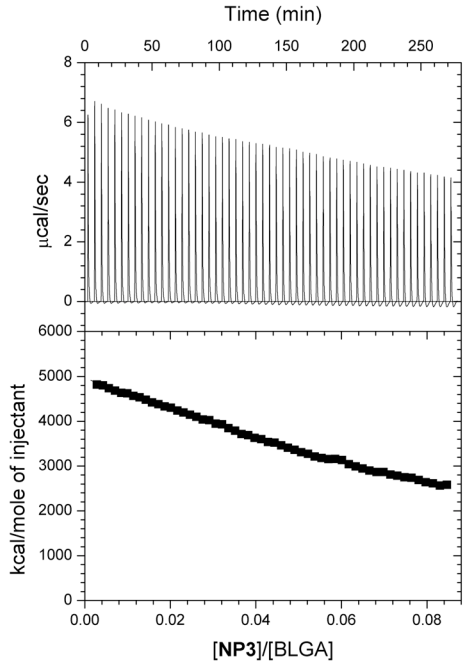
$$Q = \frac{\alpha}{2}\left[\left([\text{BLG}]_{tot} + n[\text{NP}]_{tot} + \frac{1}{K_b}\right) - \left\{\left([\text{BLG}]_{tot} + n[\text{NP}]_{tot} + \frac{1}{K_b}\right)^2 - 4n[\text{BLG}]_{tot}[\text{NP}]_{tot}\right\}^{1/2}\right]$$

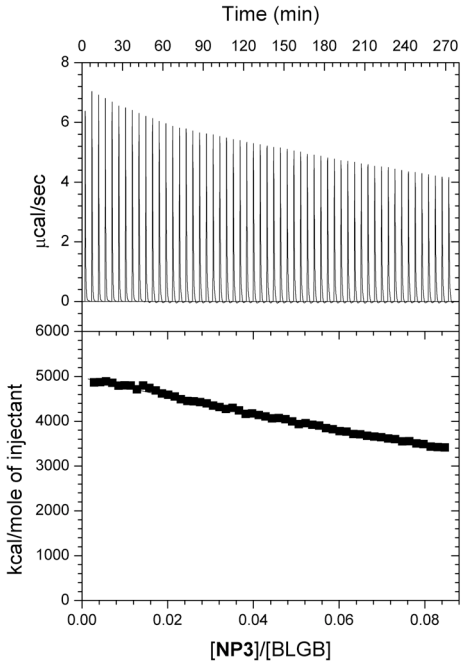
The integrated heat Q obtained from the raw titration plots are plotted for BLGA and BLGB versus $[\text{NP}]_{tot}$ in the following figures. Values of K_b obtained by fitting (Origin 7.0) to equation 3 are given in Table 2 (main text) along with calculating the values of ΔG and $T\Delta S$ following the relationships: $\Delta G = -RT\ln K_b$ and $T\Delta S = \Delta H - \Delta G$. ΔH was derived from the heat adsorption/release in the first injection.


Time (min)
µcal/sec
kcal/mole of injectant
[NP1]/[BLGA]
Time (min)
µcal/sec
kcal/mole of injectant
[NP1]/[BLGB]


Time (min)
µcal/sec
kcal/mole of injectant
[NP2]/[BLGA]


Time (min)
µcal/sec
kcal/mole of injectant
[NP2]/[BLGB]


Time (min)
µcal/sec
kcal/mole of injectant
[NP3]/[BLGA]


Time (min)
µcal/sec
kcal/mole of injectant
[NP3]/[BLGB]

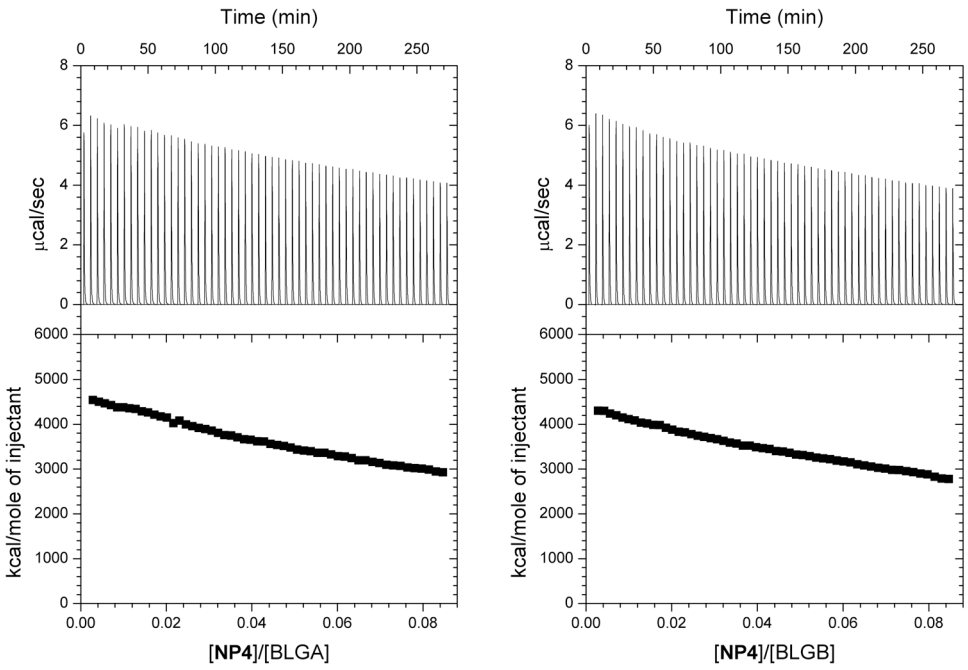


Fig. S1 Raw ITC thermogram and the integrated heat from each titration of NP with BLGA/BLGB proteins. The thermograms are blank corrected.

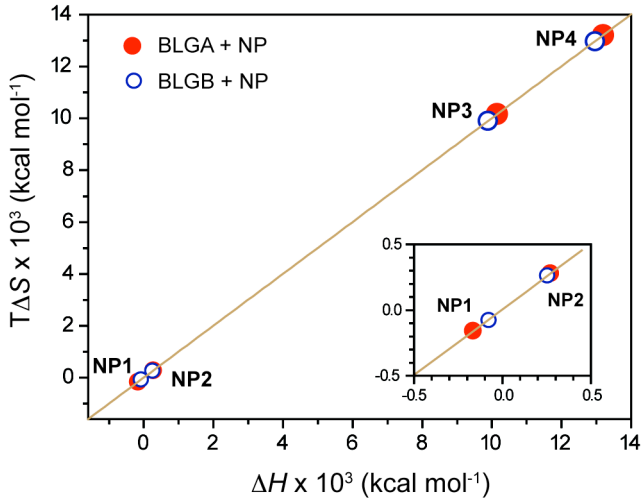


Fig. S2 Entropy (TΔS) vs. enthalpy (ΔH) plot for protein–NP complexes. Inset shows magnification of the correlation data for NP1 and NP2. Error bars are within symbol outlines

References:

1. C. C. You, O. R. Miranda, B. Gider, P. S. Ghosh, I. B. Kim, B. Erdogan, S. A. Krovi, U. Bunz and V. M. Rotello, *Nat. Nanotechnol.*, 2007, **2**, 318-323.