Supplemental Materials

Quantitative Analysis of Multivalent Interactions of Carbohydrate-Encapsulated Gold Nanoparticles with Concanavalin A

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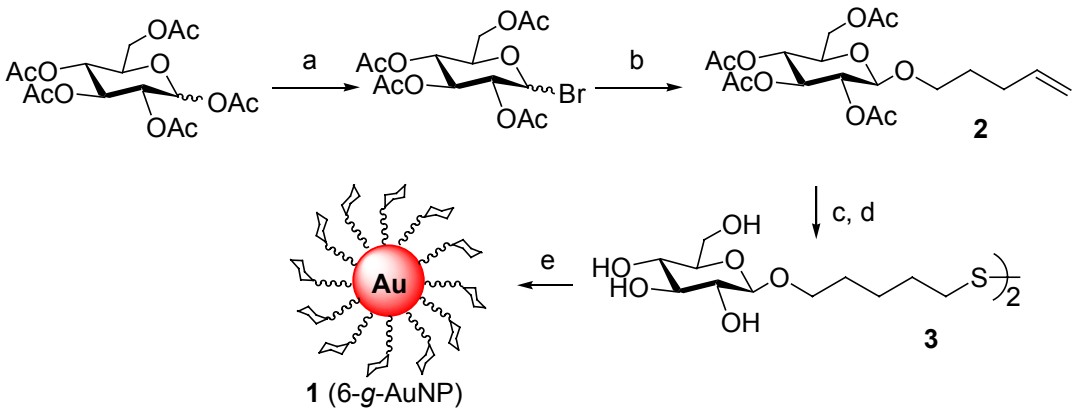
● Synthetic Schemes and NMR data:

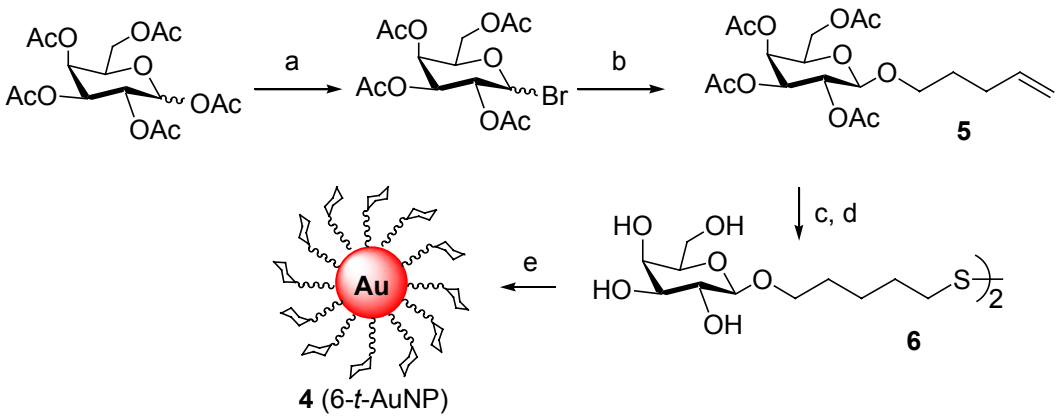
Synthesis of 6-*g*-AuNP



Keys: (a) HBr/HOAc, 84%. (b) 4-pentenyl alcohol, $Hg(CN)_2$, 87% (c) HSAc, AIBN, dioxane, 95%. (d) NaOMe(cat.), MeOH, 93%. (e) $HAuCl_4$, $NaBH_4$.

5-Thiopentyl β-D-glucopyranoside dimmer (3). ^{1}HNMR (400 MHz, CD_3OD) δ 1.50-1.55 (m, 2H), 1.62-1.69 (m, 4H), 2.51 (t, J = 7.8 Hz, 2H), 3.17 (dd, J = 8.8, 7.8 Hz, 1H), 3.33-3.35 (m, 3H), 3.56-3.59 (m, 1H), 3.70 (dd, J = 11.8, 5.3 Hz, 1H), 3.87 (m, 2H), 4.26 (d, J = 7.8 Hz, 1H); ^{13}CNMR (100 MHz, CD_3OD) δ 25.04, 26.00, 30.38, 35.14, 62.97, 70.80, 71.86, 75.30, 78.08, 78.31, 104.54.

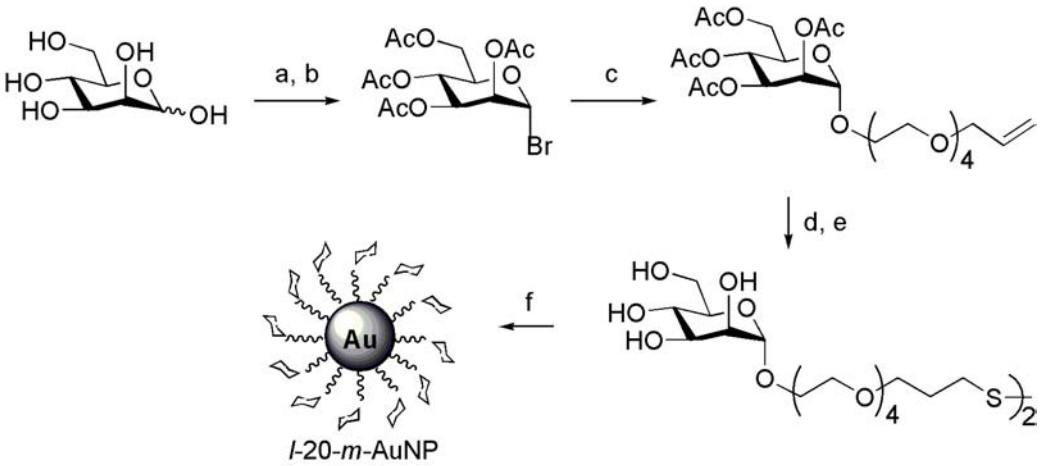
Synthesis of 6-*t*-AuNP



Keys: (a) HBr/HOAc, 85%. (b) 4-pentenyl alcohol, $Hg(CN)_2$, 80% (c) HSAc, AIBN, dioxane, 95%. (d) NaOMe(cat.), MeOH, 90%. (e) $HAuCl_4$, $NaBH_4$.

5-Thiopentyl β-D-galactopyranoside dimmer (4). ^{1}HNMR (400MHz, $CHCl_3$) δ 1.50-1.55 (m, 2H), 2.72 (t, J = 7.3 Hz, 2H), 3.43-3.47 (m, 1H), 3.52-3.65 (m, 17H), 3.63 (t, J = 9.6 Hz, 1H), 3.70-3.78 (m, 3H), 3.81 (dd, J =3.3, 1.7 Hz, 1H), 3.84 (dd, J = 11.7, 2.4 Hz, 1H), 4.76 (d, J = 1.6 Hz, 1H); ^{13}CNMR (400MHz, $CHCl_3$) δ 24.66, 24.74, 29.27, 33.61, 62.83, 71.48, 72.46, 72.72, 77.88, 78.34, 98.85.

Synthesis of *l*-20-*m*-AuNP



Keys: (a) Ac_2O, I_2, 90%. (b) HBr/HOAc, 80%. (c) 2-{2-[2-(2-allyloxy-ethoxy)-ethoxy]-ethoxy}-ethanol, $Hg(CN)_2$, 28% (d) HSAc, AIBN, dioxane, 74%. (e) NaOMe(cat.), MeOH, 83%. (f) $HAuCl_4$, Reducing reagent

tPEG 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (8). ^{1}HNMR (400MHz, $CHCl_3$)

δ 2.01 (s, 3H), 2.04 (s, 3H), 2.10 (s, 3H), 2.15 (s, 3H), 3.47 (dt, *J* = 11.2, 6.4 Hz, 1H), 3.52-3.65 (m, 16H), 3.70 (dt, *J* = 11.2, 6.4 Hz, 1H), 3.96 (d, *J* = 5.7, 1.2 Hz, 1H), 3.99 (ddd, *J* = 10.0, 5.2, 2.8 Hz, 1H), 4.11 (dd, *J* = 12.0, 2.8 Hz, 1H), 4.27 (dd, *J* = 12.0, 5.2 Hz, 1H), 4.80 (d, *J* = 1.6 Hz, 1H), 4.98-5.08 (m, 2H), 5.18 (dd, *J* = 10.7, 1.7 Hz, 1H), 5.27 (dd, *J* = 17.2, 1.7 Hz, 1H), 5.81-5.85 (m, 1H); ^{13}CNMR (100MHz, $CHCl_3$) δ 20.68, 20.70, 20.89, 20.93, 62.53, 62.63, 63.64, 66.25, 65.68, 68.37, 68.86, 69.09, 69.37, 70.18, 70.53, 70.58, 71.16, 72.20, 92.11, 117.19, 134.66, 169.86, 170.09, 170.26, 170.90.

Thio-tPEG 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside. ^{1}HNMR (400MHz, $CHCl_3$) δ1.14-1.47 (m, 2H), 2.01 (s, 3H), 2.04 (s, 3H), 2.10 (s, 3H), 2.15 (s, 3H), 2.34 (s, 3H), 2.89 (t, *J* = 7.2 Hz, 2H), 3.47 (dt, *J* = 11.2, 6.4 Hz, 1H), 3.52-3.65 (m, 18H), 3.70 (dt, *J* = 11.2, 6.4 Hz, 1H), 3.96 (d, *J* = 5.7, 1.2 Hz, 1H), 3.99 (ddd, *J* = 10.0, 5.2, 2.8 Hz, 1H), 4.11 (dd, *J* = 12.0, 2.8 Hz, 1H), 4.27 (dd, *J* = 12.0, 5.2 Hz, 1H), 4.80 (d, *J* = 1.6 Hz, 1H), 4.98-5.08 (m, 2H); ^{13}CNMR (100MHz, $CHCl_3$) δ 20.68, 20.70, 20.74, 20.93, 24.37, 62.53, 62.63, 63.64, 65.68, 66.25, 68.37, 68.86, 69.09, 69.37, 70.18, 70.53, 70.58, 71.16, 72.20, 92.11, 169.86, 170.09, 170.26, 170.90, 192.13.

tPEG α-D-mannopyranoside dimmer (9). ^{1}HNMR (400MHz, CD_3OD) δ 1.50-1.55 (m, 2H), 2.72 (t, *J* = 7.3 Hz, 2H), 3.43-3.47 (m, 1H), 3.52-3.65 (m, 17H), 3.63 (t, *J* = 9.6 Hz, 1H), 3.70-3.78 (m, 3H), 3.81 (dd, *J* = 3.3, 1.7 Hz, 1H), 3.84 (dd, *J* = 11.7, 2.4 Hz, 1H), 4.76 (d, *J* = 1.6 Hz, 1H); ^{13}CNMR (400MHz, CD_3OD) δ 24.68, 24.70, 33.74, 63.53, 63.63, 65.64, 66.68, 67.25, 69.37, 69.86, 70.09, 70.37, 71.18, 72.53, 72.58, 74.16, 75.20, 100.11.

Synthesis of Carbohydrate-Encapsulated Gold Nanoparticles.

An $HAuCl_4$ aqueous solution was added to a toluene solution in the presence of tetraoctylammonium bromide at room temperature. After stirring for 1 min, the organic layer was collected. The organic layer was then mixed with a freshly prepared reducing agent and methanol solution of carbohydrates (manno-, gluco- or galactopyranosides) with vigorous stirring. After stirring for 1 h, carbohydrate-AuNP were precipitated by centrifugation and then washed with methanol. The diameters of gold nanoparticles were controlled by reaction temperature and type of surfactant (see reference 14).

● Experimental Procedures:

Immobilized biosensor chip

Immobilization of ligand to the biosensor chip was preformed in BIAcore 3000 instrument. A mixture of 4-mercapto-1-butanol (Ligand **A**) and 5-thiopentyl α-D-mannopyranoside (Ligand **B**) in deionized water was injected into a flow cell with J1 biochip. The mixtures of Ligand **A** and Ligand **B** with various ratios were tested to generate the chips with considerably higher affinity to Con A. We found that, the Con A tetramer displayed a good binding profile with characteristic association, equilibrium, and dissociation phases, when the mixture with the ratio of 4:1 (Ligand **A**/**B**) were applied.

BIAcore assay

The experiments were carried out by following the procedures provided by the BIAcore software, version 3.0.

The flow cell 1 with a surface composed only of pure Ligand **A** was used to obtain a background curve. As described above, the surface of flow cell 2 was immobilized by Ligand **A** and **B**. For evaluating Con A and carbohydrate interactions, response

derived from flow cell 1 were subtracted from those obtained from the flow cell 2 to obtain values of SPR response curves. Regeneration of the surface was accomplished by injection of H_3PO_4 (10 μL, 0.1 M) to remove bound Con A.

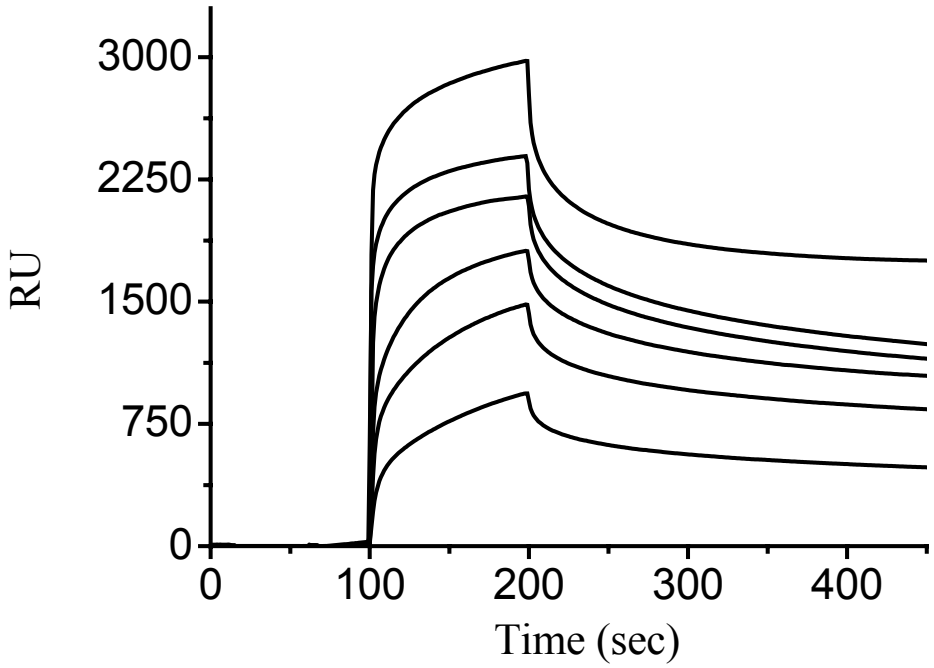
Measurement of binding affinity

Con A was dissolved in sample buffer (1.0 mL, 20 mM HEPES, pH 7.0, 90 mM NaCl with 1 mM $MnCl_2$ and $CaCl_2$) and then filtered by a syringe. Con A solution (50 μL) was injected over immobilized biosensor surface and allowed 260 seconds for dissociation and then followed by regeneration buffer. A set of SPR response curves (see the figure below) can be obtained after different concentrations of Con A solution were applied.

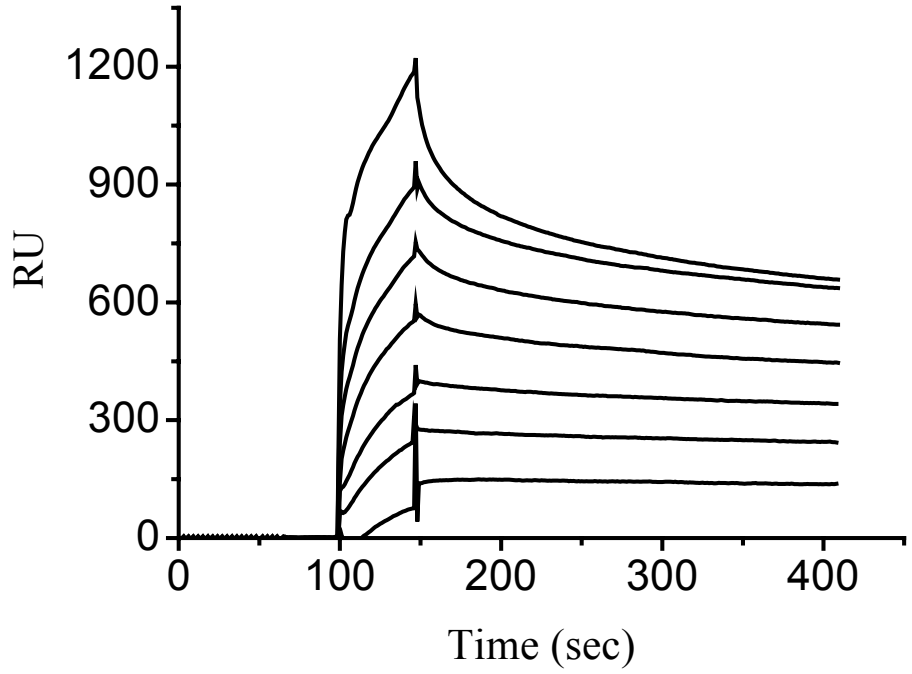
Competition experiments

Con A tetramer (0.5 μM) was first mixed with the inhibitor (αMeMan, 6-*m*-AuNP, *s*-20-*m*-AuNP, *l*-20-*m*-AuNP, 6-*g*-AuNP or 6-*t*-AuNP) and then the resulting mixture was pre-incubated for 1 hr before the injection. The mixture (50 μL) was then injected and the flow rate was controlled at 60 μL/minute. The equilibrium binding response values was collected at equilibrium binding portion of the curve (260 seconds post-injection). A set of SPR response curves (see the figures below) can be obtained after different concentrations of the inhibitor solution were applied. K_i value were determined by fitting the data into the equation: $f = [I]/([I] + K_i (1 + F/K_d)$, also see references 18 and 19.

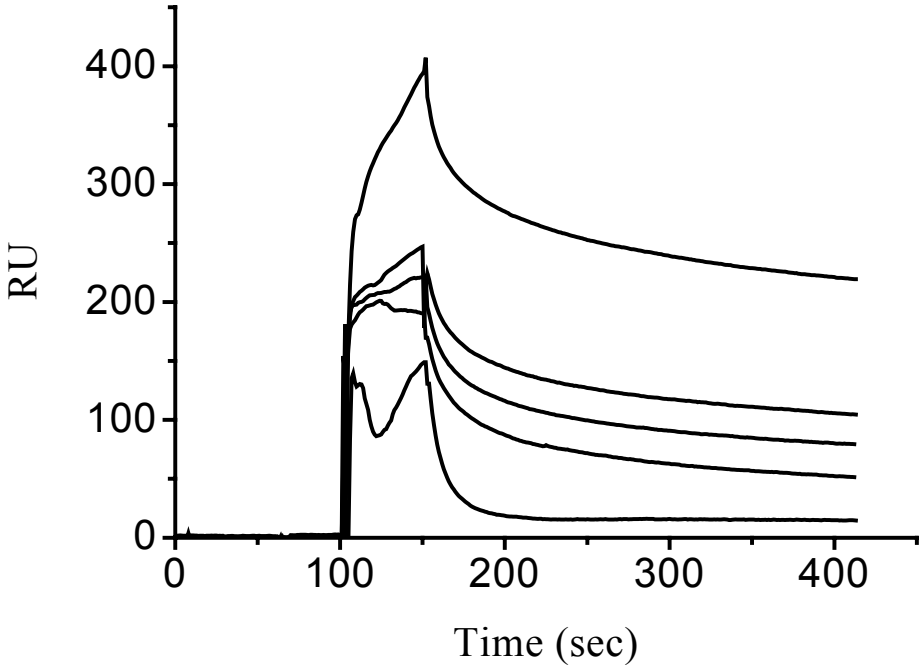
- **Typical SPR response curves in titration and competition assays.**



A set of curves for 6, 3, 1.5, 0.38, 0.19, 0.095 μM of Con A (top to bottom) were shown.



A set of inhibition curves for 0, 0.875, 1.75, 3.5, 7, 14, 28 mM of αMeMan (top to bottom) were shown.



A set of inhibition curves for 0, 0.006, 0.050, 0.101, 0.202 μM of 20-*l*-*m*-AuNP (top to bottom) were shown.