Supplementary Material (ESI) for Chemical Communications

Gold Nanoparticle-Based Competition Colorimetric Assay for Detection of Protein-Protein Interactions

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Supporting Information

Synthetic procedures and spectroscopic data for mannopyranoside, overlaid UV-vis spectra of Man-GNPs in the presence of individual tested protein, MALDI-TOF-MS spectra, Job's plot, Hill plot as well as the binding isotherm of ConA in the presence of different amount of BS-I with the curve fitting to obtain the binding constant.

Experimental Section

Synthesis of Man-GNP: To 100 mL of 32-nm gold nanoparticle solution prepared according to the Frens method[1] was added 200 L of 1.0 mM thiol-derivatized mannopyranoside (see Figure S1). Self-assembly was facilitated by leaving the solution for a period of 15 h. The suspension was centrifuged at 7000 rpm with Eppendorf 5810R centrifuge for 15 min at 10 ºC and the resulting modified nanoparticles were then resuspended in 0.01% aqueous sodium citrate solution to give an

approximate concentration of 0.29 nM. Based on elemental analysis, there are 9000 mannopyranosides per gold nanoparticle.[2]

UV-vis absorption measurements: Absorption spectra were recorded 25 °C on a Hewlett-Packard 8453A diode array spectrometer under the control of a Pentium PC running the manufacturer-supplied software package. The concentration of the proteins is made to 1 mg/mL by dissolving them in 0.01 M PBS (pH=7.4) buffer containing 0.1mM $CaCl_2$, and $MnCl_2$. Addition of 20 L individually tested protein (1 mg/mL) to 2 mL Man-GNP (0.29 nM), the absorption spectrum was recorded, respectively, after 30 min of incubation time (see Figure S2).

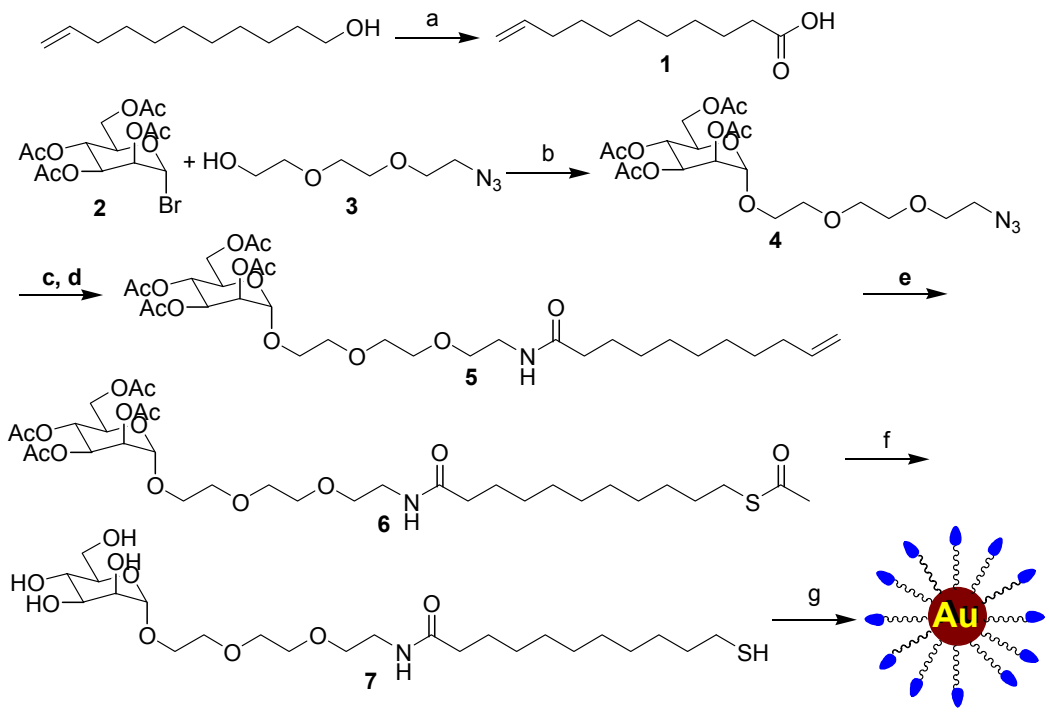
Competition colorimetric assay: (a) The same series of proteins with comparable concentrations of ConA were added to the preformed Man-GNPs (0.29 nM)/ConA (98 nM) solution, respectively and the resulting absorption spectra were recorded. Or (b) ConA (98 nM) was first mixed with the tested protein having comparable concentrations for 5 min at 4 °C followed by the addition of Man-GNPs (0.29 nM). After 30 min incubation, the absorption spectrum was recorded (see text Figure 2 and Table S1).

Detection of protein-protein interaction with SDS-PAGE and MALDI-TOF-MS: The above-mentioned competition assay solutions were transferred to vials and centrifuged. The sinked protein-bound nanoparticles were isolated and washed with deionized water several times to remove the residual protein solution. Some of the particles were loaded to 12 % SDS-PAGE and separated by gel electrophoresis followed by staining the gel with Coomassie Bule. Some of the protein-bound nanoparticles were mixed with matrix (sinapinic acid in 0.01% TFA, 50% $CH_3CN_{(aq)}$), and directly placed on the MALDI-TOF plate. A Bruker Biflex MALDI-TOF-MS was employed to analyze the sample (see Figure S3).

Surface Plasmon Resonance measurement: Biacore X (Biacore, Piscataway, NJ) was used in the conventional injection mode for the immobilization and characterization of the interaction. ConA was

immobilized on a research-grade CM5 chip (Biacore, Uppsala, Sweden) using standard amine coupling. Four concentrations of BS-1 (from 0.5 to 4.0 μM) in phosphate buffered saline (PBS) containing 0.1 mM $MnCl_2$ and 0.1 mM $CaCl_2$ were passed over the ConA-labeled chip at a rate of 20 μL/min at 25 °C (see text Figure 5).

Synthetic Scheme 1



(a) Jones reagent, Acetone, 98%; (b) $Hg(CN)_2$, $HgBr_2$, 4 A MS, CH_3CN; (c) PPh_3, THF, H_2O; (d) **1**, DCC, DMAP, HOBt, CH_2Cl_2, 45%; (e) AcSH, AIBN, MeOH, 365 nm, 81%; (f) NaOMe, MeOH, 80%; (f) 32-nm Gold nanoparticles

Synthesis of linker-containing mannopyranoside

ω-Undecylenyl alcohol was oxidized by Jones reagent giving rise to undec-10-enoic acid (**1**). D-Mannopyranose (Man) was activated by using acetyl chloride, and treated with 8-azido-3,6-dioxa-1-octyl-ol in the presence of mercuric cyanide and mercuric bromide to provide the

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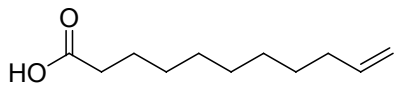
glycosylated peracetate product, which was subsequently reduced to 2-[2-(2-Aminoethoxy)-ethoxy]ethoxyl 2,3,4,6-*tetra*-*O*-acetyl-α-mannopyranoside under Staudiger reaction condition. Condensation of the resulting amine with **1** in the presence of DCC yielded compound **5**. The terminal olefin was converted to thioacetate through radial type addition with thiolacetic acid in the presence of AIBN under photolytic conditions. Saponification of OAc and SAc groups was effected by stirring with sodium methoxide in dry methanol at room temperature for 3h to afford the corresponding thiol (**7**). Compounds **1**-**7** were fully characterized by IR, MS, HRMS, ^{1}H and ^{13}C NMR spectra.

General Method: All reagents and starting materials were obtained from commercial suppliers (Acros, Aldrich, Sigma and Merck) and were used without further purification. IR spectra were recorded on a Nicolet 550 series II spectrometer. ^{1}H and ^{13}C NMR spectra were recorded using either a Varian Mercury Plus 400 or Bruker Avance 400 spectrometer. The proton and carbon chemical shifts are given in ppm using $CDCl_3$ (δ_H 7.24 and 77.0) as internal standard. High resolution mass spectra were recorded with a JEOL-102A mass spectrometer. Analytical TLC (silica gel, 60F-54, Merck) and spots were visualized under UV light and/or phosphomolybdic acid-ethanol. Flash column chromatography was performed with Kiesegel 60 (230-400 mesh) silica gel (Merck). All proteins are purchased from Sigma and used without further purification.

Experimental procedures:



Undec-10-enoic acid (1)

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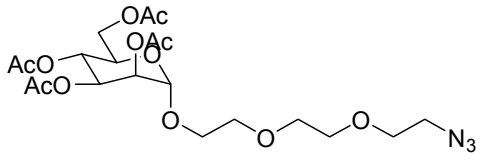
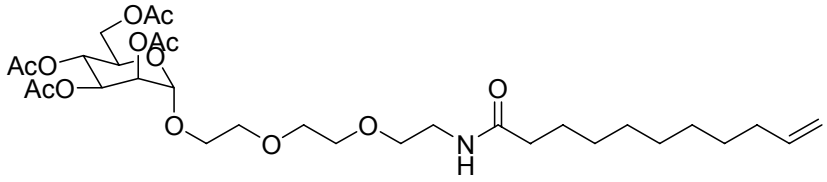
Undec-10-en-1-ol (3.0 g, 17.6 mmol) was dissolved in 20 mL of acetone and cooled to 0 °C. Jones reagent (2.7 M H_2SO_4, 2.7 M CrO_3; 16.0 mL, 44.0 mmol) was added dropwise. The reaction was stirred at 0 °C for 0.5h, quenched with isopropyl alcohol (12 mL), and stirred for 10 min. The precipitate was filtered, and the filtrate was concentrated. The resulting residue was extracted with EtOAc and washed with 5% citric acid (×3), H_2O (×3) and brine (×1). The organic phase was dried over anhydrous $MgSO_4$, filtered, and concentrated. The product **1** (3.23 g, 98%) was obtained as an oil. TLC (Hex/EtOAc (4:1)) R_f = 0.33. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 5.77 (m, 1 H), 4.93 (q, J = 10.2 Hz, 2 H), 2.33 (t, J = 7.2 Hz, 1 H), 2.02 (q, J = 7.2 Hz, 2 H), 1.63-1.59 (m, 2 H), 1.40-1.18 (m, 12 H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 179.7, 138.6, 113.8, 34.4, 34.1, 29.6, 29.5, 29.4, 29.3, 29.2, 25.0; IR (neat) 3416, 3323, 2925, 2859, 1646, 1553, 1467, 1135, 1062 cm^{-1}. FAB-HRMS calcd for $C_{11}H_{20}O_2$ (M^++1) 185.1542, Found 185.1542.



8-Azido-3,6-dioxa-1-octyl2',3',4',6'-tetra-*O*-acetyl-α-D-manno-pyranoside (4)

Compound **2** and **3** were synthesized as previously reported.[3,4] To a solution of compound **3** (2.1 g, 12.0 mmol) in 40 mL of CH_3CN was added $Hg(CN)_2$ (3.0 g, 12.0 mmol), $HgBr_2$ (4.3 g, 12.0 mmol), 4 A MS (5.0 g). Compound **2** (5.9 g, 14.4 mmol) in 10 mL of CH_3CN was added dropwise to the reaction mixture at room temperature for 5 h. It was filtered and concentrated. The residue was redissolved in 100 mL of CH_2Cl_2 and washed with 1% $KI_{(aq)}$(×2), 10% $NaHCO_{3(aq)}$ (×2) and brine. The organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated. The product **5** (3.0 g, 50%) was obtained as an oil after silica gel column chromatography eluted with Hex/EtOAc (7/3). TLC (Hex/EtOAc (1:1)) R_f

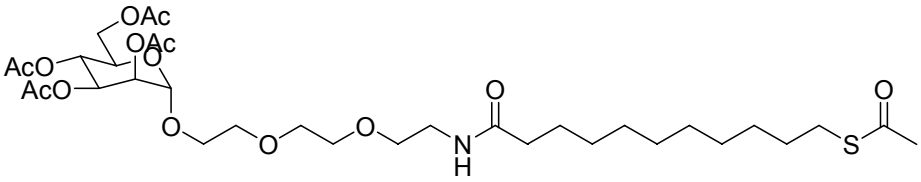
= 0.30. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 5.30-5.19 (m, 3 H), 4.80 (d, J = 1.6 Hz, 1 H), 4.22 (m, 1 H), 4.05-3.96 (m, 2 H), 3.75 (m, 1 H), 3.65-3.37 (m, 9 H), 3.33 (t, J = 5.3 Hz, 2 H), 2.08 (s, 3 H), 2.03 (s, 3H), 1.98 (s, 3 H), 1.91 (s, 3 H). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 170.6, 169.9, 169.8, 169.6, 97.6, 70.6, 70.5, 69.9, 69.9, 69.4, 68.9, 68.2, 67.2, 66.0, 62.3, 50.5, 20.7, 20.6, 20.6, 20.5. IR (neat) 2937, 2880, 2113, 1750, 1438, 1373, 1223, 1138, 1087, 1050, 981, 937, 921, 602 cm^{-1}. FAB-HRMS calcd for $C_{20}H_{31}N_3O_{12}$ (M^++1) 506.1986, found 506.1983.



Compound (5)

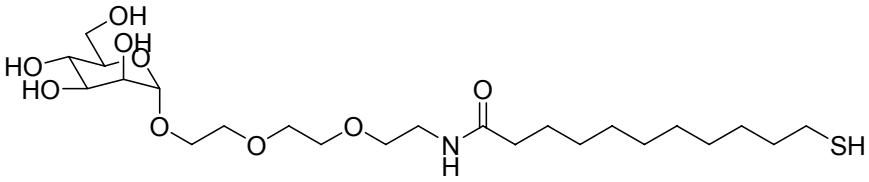
To a solution of compound **4** (1.26 g, 2.5 mmol) in 20 mL of THF was added PPh_3 (0.77 g, 2.7 mmol) and H_2O (67.0 μL, 3.7 mmol). The mixture was stirred at room temperature for 8 h. THF was removed and added CH_2Cl_2 (20 mL), compound **1** (0.69 g, mmol), HOBt (0.34 g, 2.5 mmol), a catalytic amount of DMAP, and TEA (1.0 mL). The reaction mixture was stirred at room temperature for additional 10 h and was then filtered through a pad of celite. The filtrate was concentrated and the resulting residue was dissolved in 70 mL of EtOAc and washed with 5% citric acid (×3), 10% $NaHCO_3$ (×3) and brine. The organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated. The product **5** (0.65 g, 45%) was obtained as an oil after silica gel column chromatography eluted with EtOAc/CH_2Cl_2 (2/3). TLC (CH_2Cl_2/EtOAc (1:1)) R_f = 0.30. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.11 (s, 1 H, NH), 5.70 (m, 1 H), 5.35-5.15 (m, 3 H), 4.94-4.80 (m, 2 H), 4.79 (d, J = 1.5 Hz, 1 H), 4.21 (d, J = 5.1, 12.4, 1 H), 4.08-3.96 (m, 2 H), 3.76 (m, 1 H), 3.68-3.51 (m, 7H), 3.48-3.46 (m, 2 H), 2.09 (s, 3 H), 2.03 (s, 3 H), 1.98-1.90 (m, 8 H), 1.56-1.54 (m, 2 H), 1.35-1.19 (m, 12 H). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 172.4, 169.8, 169.2, 169.1, 1688, 138.5, 113.7, 97.3, 70.5, 70.1, 69.9, 69.8, 69.4, 68.9, 68.3, 67.3, 65.9, 62.3, 39.2, 36.8,

33.9, 29.5, 29.5, 29.2, 29.0, 25.9, 21.1, 21.0, 20.9. IR (neat) 2932, 2866, 1759, 1666, 1228,1142, 1096, 1043, 771 cm^{-1}. FAB-HRMS calcd for $C_{31}H_{52}NO_{13}$ (M^++1) 646.3438, found 646.3445.



Compound (6)

To a solution of compound **5** (550.0, 0.85 mmol) in 20 mL of CH_3OH was added thiolacetic acid (0.40 mL, 5.10 mmol) and azobis(isobutylnitrile) (46 mg, 0.28 mmol). The reaction mixture was irradiated in a photochemical reactor (Rayonet reactor lamp, Southern New England Ultraviolet Co., model RPR-100, 365 nm) for 6 h under nitrogen atmosphere. The mixture was concentrated and purified with silica gel flash column chromatography eluted with EtOAc/CH_2Cl_2 (3/2) to yield yellowish oil (500.0 mg, 81%). TLC (CH_2Cl_2/EtOAc (2:3)) R_f = 0.33. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.13 (bs, 1 H, NH), 5.31-5.16 (m, 3 H), 4.81 (d, J = 1.5 Hz, 1 H), 4.21 (dd, J = 12.1, 4.8 Hz, 1 H), 4.08-3.96 (m, 2 H), 3.76 (m ,1 H), 3.67-3.52 (m, 7 H), 3.50-3.47 (m, 2 H), 3.39-3.37 (m, 2 H), 2.78 (t, J = 7.3 Hz, 2 H), 2.25 (s, 3 H), 2.13-2.10 (m, 5 H), 2.04 (s, 3 H), 1.97 (s, 3 H), 1.93 (s, 3 H), 1.60-1.40 (m 4 H), 1.35-1.15 (m, 12 H). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 195.1, 172.5, 169.9, 169.2, 169.2, 168.9, 97.4, 70.5, 70.1, 69.9, 69.9, 69.4, 69.0, 68.3, 67.3, 66.0, 62.4, 39.3, 36.8, 30.9, 29.7, 29.6, 29.5, 29.5, 29.3, 29.3, 29.0, 26.0, 21.2, 21.1, 21.0, 21.0. IR (neat) 2939, 2859, 1765, 1659, 1546, 1374, 1228, 1142, 1096, 1049 cm^{-1}. FAB-HRMS calcd for $C_{33}H_{55}NO_{14}S$ (M^++1) 722.3422, found 722.3417.



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Compound (7)

To a solution of compound **6** (445.0 mg, 0.62 mmol) in 10 mL of CH_3OH was added NaOMe (200.0 mg, 3.72 mmol) at room temperature for 3h. The mixture was neutralized by Dowex 50x8-400 resin. After filtration through a pad of celite, the filtrate was concentrated and the residue was purified with silica gel flash column chromatography eluted with MeOH/CH_2Cl_2 (2/8) to yield an oil (250.0 mg, 80%) TLC (CH_2Cl_2/MeOH (8:2)) R_f = 0.45. ^{1}H-NMR (CD_3OD, 400 MHz) δ 8.02 (bs, 1 H, NH), 4.81 (d, J = 1.5 Hz, 1 H), 3.88-3.80 (m, 3 H), 3.74-3.52 (m, 13 H), 3.39-3.32 (m, 2 H), 2.68 (t, J = 7.3 Hz, 2 H), 2.49 (t, J = 7.3 Hz, 1 H, SH), 2.20 (t, J = 7.5 Hz, 2 H), 1.70-1.56 (m, 4 H), 1.42-1.26 (m, 12 H); ^{13}C-NMR (CD_3OD, 100 MHz) δ 175.5, 101.4, 74.4, 72.4, 72.0, 71.5, 71.3, 71.2, 70.6, 68.5, 67.6, 62.9 ,40.5, 39.9, 37.2, 35.4, 30.8, 30.7, 30.6, 30.4, 29.7, 29.7, 27.3; IR (KBr) 3416, 3323, 2925, 2859, 1646, 1553, 1467, 1135, 1062 cm^{-1}; FAB-MS : m/z 512.3 (M^++1), 534.2 (M+Na^+); FAB-HRMS calcd for $C_{23}H_{45}NO_9S$ (M^++1) 512.2894, found 512.2885.

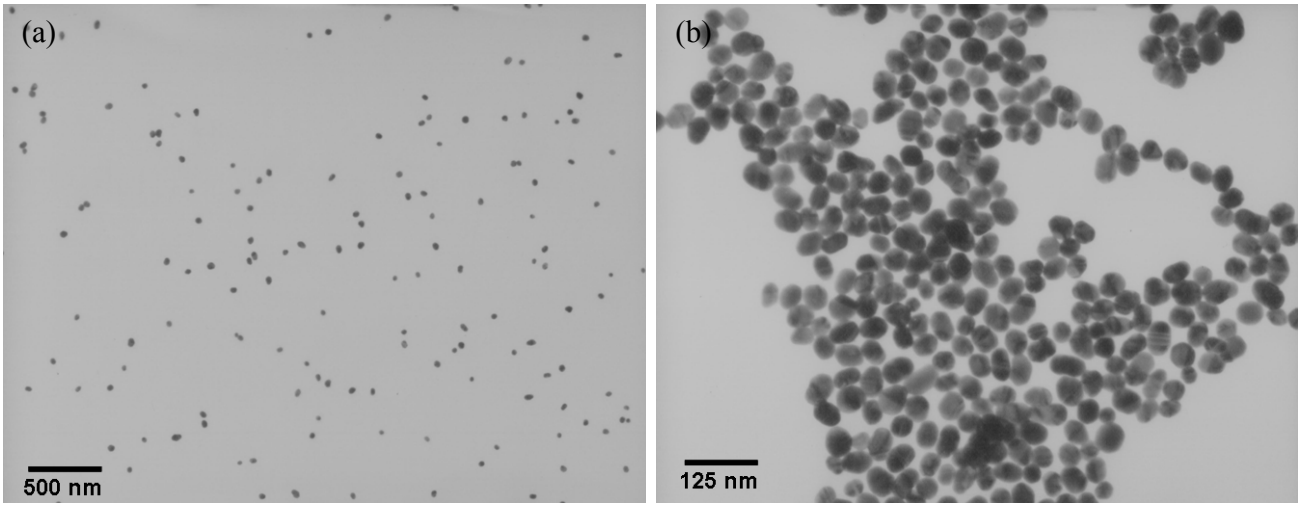

Figure S1. TEM photographs of 32 nm gold nanoparticles (a) Man-GNPs (b) In the presence of ConA, Man-GNPs form cross-linked aggregation mediated by ConA.

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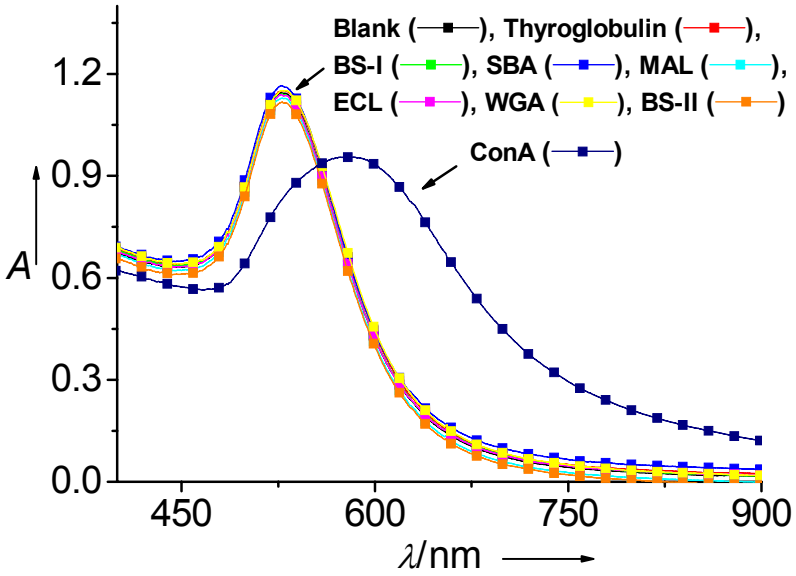

Figure S2. Overlaid absorption spectra of Man-GNPs in the presence of BS-I, SBA, MAL, ECL, WGA, BS-II, ConA and thyroglobulin. No detectable change was observed in the case of RNaseA, Trypsin inhibitor, BSA, and those spectra are omitted for the clarity.

Table S1: Summary of protein-protein interactions results obtained from UV-vis studies empolying Man-GNP

proteins	Thyroglobulin	BS-I	SBA	MAL	ECL	WGA	BS-II	RNaseA	Trypsin inhibitor	BSA
Cognate substrates		α-gal α-galNAc	galNAc	Sialic acid	β-gal(1-4)glcNAc	$(glcNAc)_2$ NeuNAc	glcNAc			
ConA	+	+	+	+	-	-	-	-	-	-

+: Protein-protein interaction occurs. -: Protein-protein interaction does not occur.

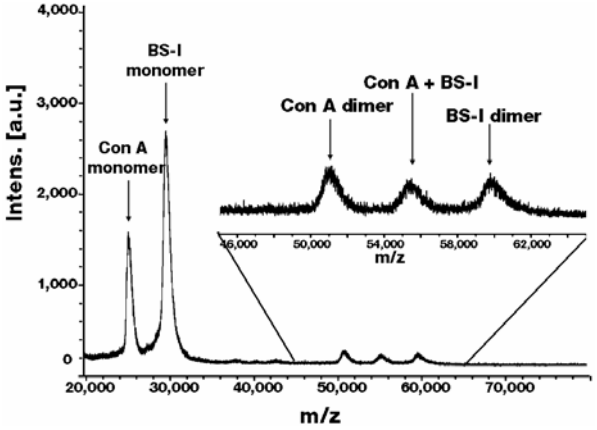

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Figure S3. MALDI-TOF mass spectrum of biomolecules adsorbed on the Man-GNPs in the case of mixing ConA and BS-I.

Fitting Equation:

$Y = K[L]^n/1+K[L]^n$

K: binding constant

L: concentration of BS-I

n: number of binding site

Y: percentage of signal change

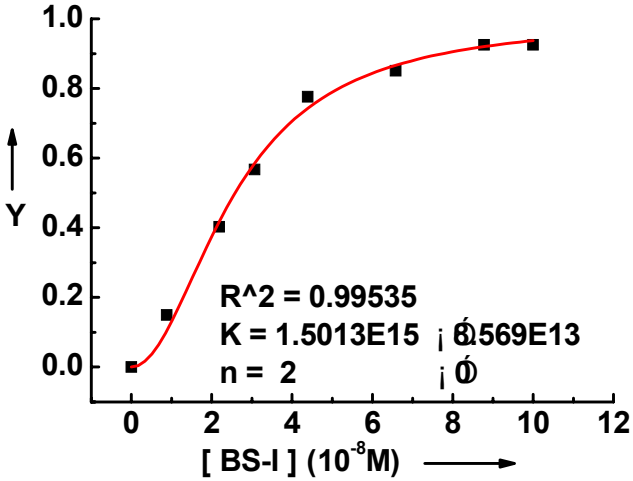

Figure S4. Binding isotherm of ConA titrated with different amount of BS-I in the presence of constant concentration of MAN-GNPs. The binding constant of ConA/BS-I based on the wavelength changes of the surface plasmon absorbance can be obtained by nonlinear regression based on the curve fitting equation shown at left hand side. The binding strength is calculated to be 1.5×10^{15}.

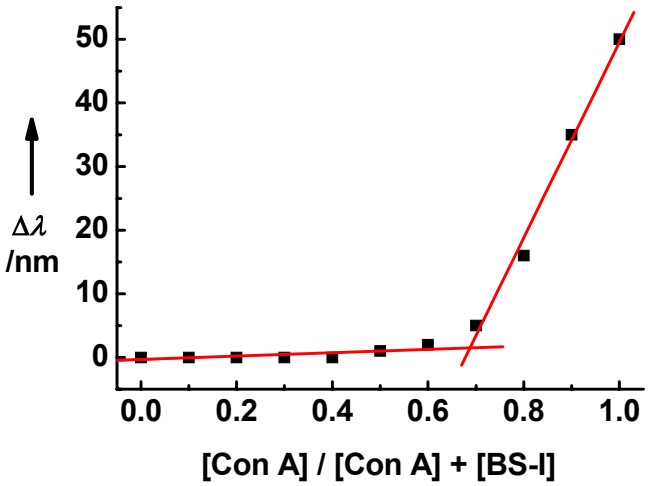

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Figure S5. Job's plot of ConA binding to BS-I based on gold nanoparticle-based competition colorimetric assay. X axis is the mole fractions of ConA. The crossing point of two lines corresponds to 0.68 at the X-axis, indicating the stoichiometry for the binding of BS-I to ConA is 1:2.

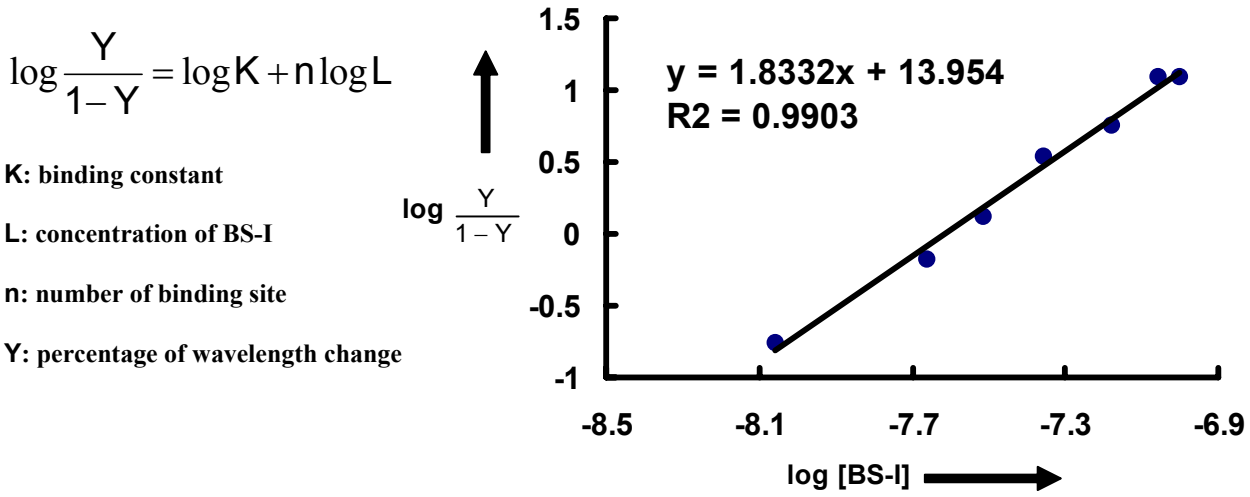

Figure S6. Hill plot of ConA binding to BS-I based on gold nanoparticle-based competition colorimetric assay. Y: percentage of wavelength change. The slope of the line is called Hill coefficient which is 1.8332 in our study. Since the magnitude of Hill coefficient is greater than 1, the binding of ConA/BS-I is cooperative.

Reference:

1. G. Frens, *Nature* 1973, *241*, 20-22.

2. Abad, J. M.; Mertens, S. F. L.; Pita, M.; Ferna´ndez, V.M.; Schiffrin, D.J. *J. Am. Chem. Soc.* **2005**, *127*, 5689-5694.

3. Sznaidman, M, L.; Johnson, S. C.; Crasto, C.; Hecht, S. M. *J. Org. Chem.* **1994**, *60*, 3942-3943.

4. Roy, B. C.; Santos, M.; Mallik, S.; and Campiglia, A.D. *J. Org. Chem.* **2003**, *68*, 3999-4007.