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Supplementary Information

Multivalent Gold Glyconanoparticles with Enhanced Binding to the Anti-Viral Lectin Cyanovirin-N

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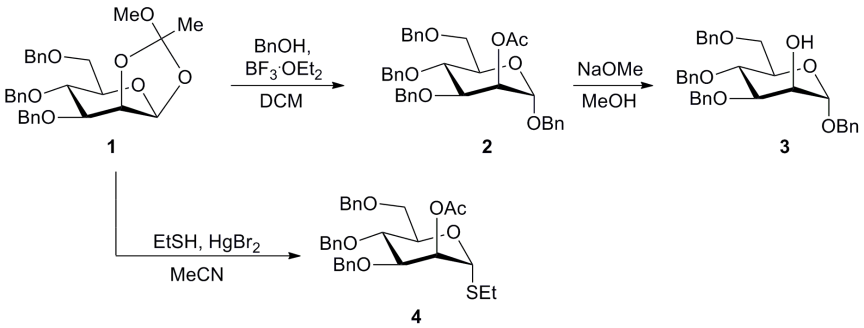
1. Experimental section

Materials. Hydrogen tetrachloroaurate (III) hydrate ($HAuCl_4 \cdot XH_2O$, 99.9%-Au) was purchased from Strem Chemicals (Newburyport, MA). Sodium citrate was obtained from Mallinckrodt. Tween 20 was obtained from TCI America. Anthrone (97%), and bovine serum albumin (BSA) were purchased from Sigma-Aldrich. All water was treated via a Milli-Q ultrapure water purification system. Dialysis tubing (G-Biosciences Tube-O-dialyzer, 15K, medium) was purchased from VWR International.

Syntheses of glycosyl acceptor 3 and glycosyl donor 4



Scheme 1S. Synthesis of compounds **3** and **4**.

2-*O*-acetyl-1,3,4,6-tetra-*O*-benzyl-α-D-mannopyranoside (2). Compound **1**[1] (1.01 g, 2 mmol) and benzyl alcohol (1.08 g, 10 mmol) were mixed in dry DCM (15 mL) with 4Å molecular sieves. The reaction flask was cooled down to 0 °C and then $BF_3 \cdot Et_2O$ was added dropwise. After the addition, the reaction mixture was allowed to warm up to ambient temperature and kept under nitrogen atmosphere for 2 hours. After completion of the reaction, monitored by TLC, the reaction mixture was washed with saturated $NaHCO_3$, brine, and extracted with DCM. The combined organic phase was dried over Na_2SO_4 and then evaporated under reduced pressure. The crude product was purified by

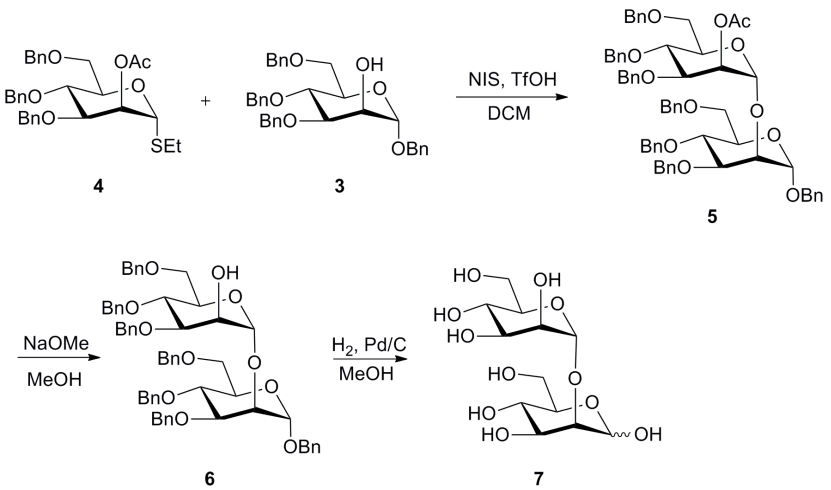
flash column chromatography using hexane/EtOAc (10:1 v/v) as the solvent system and compound **2** was obtained in 96% yield (1.12 g) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.14 (s, 3 H, C(O)C*H*$_3$), 3.70 (dd, 1 H, *J* = 1.6 and 10.5 Hz, H-6b), 3.81 (dd, 1 H, *J* = 4.1 and 10.5 Hz, H-6a), 3.85 (m, 1 H, H-5), 3.91 (t, 1 H, *J* = 9.2 Hz, H-4), 4.03 (dd, 1 H, *J* = 3.5 and 9.2 Hz, H-3), 4.88-4.45 (m, 8 H, 4 × C*H*$_2$Ph), 4.94 (d, 1 H, *J* = 1.5 Hz, H-1), 5.42 (dd, 1 H, *J* = 1.5 and 3.5 Hz, H-2), 7.40-7.11 (m, 20 H, 4 × C$_6$*H*$_5$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 21.27, 68.96, 69.00, 69.48, 71.77, 72.02, 73.62, 74.51, 75.38, 78.46, 97.26, 127.75, 127.80, 127.87, 127.94, 128.07, 128.09, 128.24, 128.48, 128.54, 128.60, 137.01, 138.12, 138.39, 138.50, 170.61. Data were in agreement with those reported in the literature.[2]

1,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranoside (3). Compound **2** (720 mg, 1.2 mmol) was dissolved in MeOH (3 mL) and NaOMe (64.80 mg, 1.2 mmol) was added. The reaction mixture was stirred under nitrogen atmosphere at ambient temperature for 1 hour. After the completion of the reaction, monitored by TLC, the mixture was further stirred with added Amberlyst 15 for 10 minutes, after which the solvent was evaporated under reduced pressure. The crude product was then purified by flash column chromatography using hexane/EtOAc (4:1 v/v) as the solvent system and compound **3** was obtained in 97% yield (629 mg) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.70 (dd, 1 H, *J* = 1.2 and 10.7 Hz, H-6b), 3.77 (dd, 1 H, *J* = 4.2 and 10.7 Hz, H-6a), 3.91-3.81 (m, 2 H, H-4, H-5), 3.93 (dd, 1 H, *J* = 3.2 and 8.8 Hz, H-3), 4.08 (dd, 1 H, *J* = 1.6 and 3.2 Hz, H-2), 4.85-4.46 (m, 8 H, 4 × C*H*$_2$Ph), 5.02 (d, 1 H, *J* = 1.6 Hz, H-1), 7.40-7.14 (m, 20 H, 4 × C$_6$*H*$_5$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 68.57, 69.06, 69.23, 71.44, 72.17, 73.61, 74.48, 75.31, 80.43, 98.57, 127.70, 127.82, 127.96, 127.98, 128.04,

128.09, 128.20, 128.46, 128.49, 128.55, 128.66, 137.31, 138.06, 138.41. Data were in agreement with those reported in the literature.[3]

Ethyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (4). Compound **1** (2.02 g, 4 mmol) was dissolved in MeCN (20 mL) and 4Å molecular sieves were added. The mixture was stirred for 1 h before ethanethiol (930 mg, 15 mmol) and $HgBr_2$ (72 mg, 0.2 mmol) were added. The suspension was stirred at 60 °C under nitrogen atmosphere for 24 hours. After completion of the reaction, monitored by TLC, the reaction mixture was diluted with DCM, filtered through celite, washed with 5% NaOH, dried over Na_2SO_4 and then concentrated under reduced pressure. The crude product was purified by flash column chromatography using hexane/EtOAc (8:1 v/v) as the solvent system and compound **4** was obtained in 72% yield (1.54 g) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.28 (t, 3 H, J = 7.3 Hz, SCH_2CH_3), 2.16 (s, 3 H, $C(O)CH_3$), 2.70-2.53 (m, 2H, SCH_2CH_3), 3.69 (dd, 1 H, J = 1.7 and 10.6 Hz, H-6b), 3.84 (dd, 1 H, J = 4.2 and 10.6 Hz, H-6a), 3.97-3.87 (m, 2 H, H-3, H-4), 4.16 (m, 1 H, H-5), 4.90-4.43 (m, 6 H, 3 × CH_2Ph), 5.32 (d, 1 H, J = 1.5 Hz, H-1), 5.43 (dd, 1 H, J = 1.5 and 2.6 Hz, H-2), 7.40-7.12 (m, 15 H, 3 × C_6H_5). ^{13}C NMR (125 MHz, $CDCl_3$): δ 15.03, 21.31, 25.64, 68.98, 70.73, 71.97, 72.01, 73.55, 74.70, 75.31, 78.74, 82.59, 127.15, 127.74, 127.77, 127.92, 127.99, 128.31, 128.46, 128.58, 128.74, 137.85, 138.35, 138.54, 170.59. Data were in agreement with those reported in the literature.[4]

Synthesis of Man2 (7)



Scheme S2. Synthesis of **Man2** (**7**).

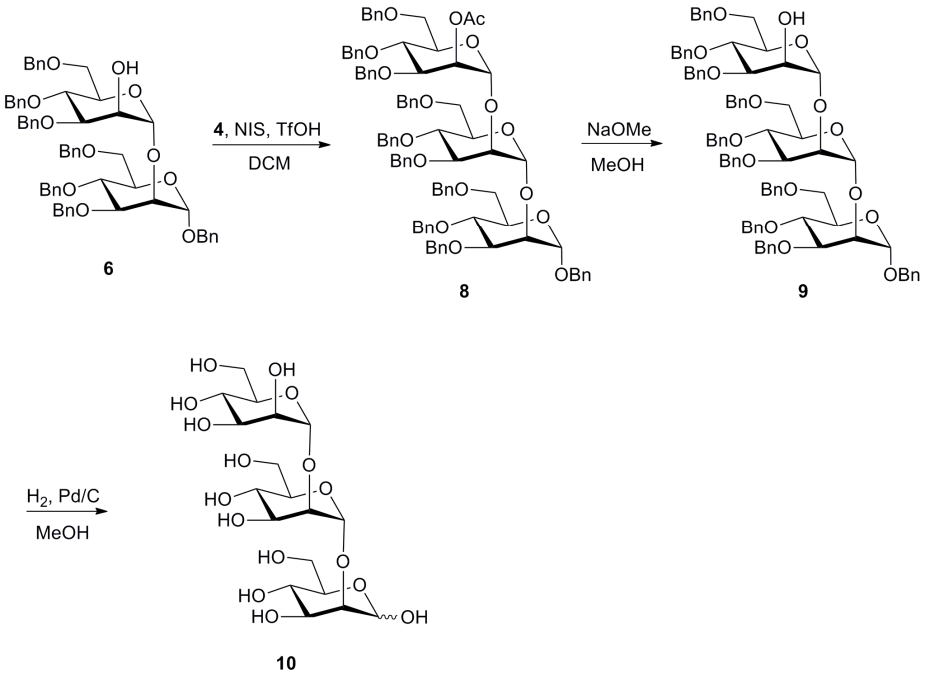
2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-1,3,4,6-tetra-*O*-benzyl-α-D-mannopyranoside (5). A solution of compounds **3** (176 mg, 0.32 mmol) and **4** (204 mg, 0.38 mmol) in dry DCM was stirred for 0.5 h with 4Å molecular sieves under nitrogen atmosphere at -10 °C. NIS (216 mg, 0.96 mmol) was added and then a solution of TfOH in dry DCM (7.5 mg, 0.05 mmol, 10 µL/ 1 mL) was added dropwise. Stirring was continued at the same temperature for another 0.5 h and the acid was neutralized with saturated aqueous $NaHCO_3$ solution, $Na_2S_2O_5$(s), brine, and extracted with DCM. The combined organic phase was dried over Na_2SO_4 and then evaporated under reduced pressure. The crude product was purified by flash column chromatography using solvent system hexane/EtOAc (8:1 v/v) giving compound **5** in 78% yield (253 mg) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.11 (s, 3 H, C(O)CH_3), 3.55 (dd, 1 H, J = 1.7 and 10.7 Hz), 3.90-3.65 (m, 7 H), 3.99-3.92 (m, 2 H), 4.03 (dd, 1 H, J = 1.7 and 2.9 Hz, H-2), 4.90-4.31 (m, 14 H, 7 × CH_2Ph), 4.95 (d, 1 H, J = 1.7 Hz, H-1), 5.06 (d, 1 H, J = 1.6 Hz, H-1'), 5.54 (dd, 1 H, J = 1.6 and 3.5 Hz, H-2'), 7.36-7.10 (m, 35 H, 7 × C_6H_5). ^{13}C NMR

(125 MHz, $CDCl_3$): δ 21.28, 68.88, 69.01, 69.16, 69.40, 71.93, 72.09, 72.22, 72.26, 73.47, 73.56, 74.45, 74.83, 75.08,75.20, 75.32, 78.32, 79.82, 98.16, 99.77, 127.56, 127.63, 127.67, 127.73, 127.79, 127.89, 127.93, 127.95, 128.03, 128.22, 128.31, 128.41, 128.45, 128.48, 128.53, 137.42, 138.18, 138.36, 138.53, 138.64, 138.67, 170.27. Data were in agreement with those reported in the literature.[5]

2-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-1,3,4,6-tetra-*O*-benzyl-α-D-mannopyranoside (6). Compound **5** (209 mg, 0.21 mmol) was dissolved in MeOH (3 mL) and NaOMe (11 mg, 0.21 mmol) was added. The reaction mixture was stirred under nitrogen atmosphere at ambient temperature for 3 hours. After completion of the reaction, as detected by TLC, the mixture was further stirred with added Amberlyst 15 for 10 minutes, after which the solvent was evaporated under reduced pressure. The crude product was then purified by flash column chromatography using hexane/EtOAc (3:1 v/v) as the solvent system and compound **6** was obtained in 92% yield (188 mg) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.56 (dd, 1 H, J = 1.7 and 10.7 Hz), 3.74-3.64 (m, 2 H), 3.92-3.75 (m, 6 H), 3.97 (dd, 1 H, J = 2.9 and 9.2 Hz), 4.07 (dd, 1 H, J = 1.3 and 2.4 Hz, H-2), 4.13 (dd, 1 H, J = 1.5 and 3.0 Hz, H-2'), 4.88-4.29 (m, 14 H, 7 × CH_2Ph), 5.00 (d, 1 H, J = 1.3 Hz, H-1), 5.14 (d, 1 H, J = 1.5 Hz, H-1'), 7.38-7.14 (m, 35 H, 7 × C_6H_5). ^{13}C NMR (125 MHz, $CDCl_3$): δ 68.68, 69.16, 69.20, 69.44, 71.67, 72.27, 72.31, 73.49, 73.47, 73.57, 74.51, 74.98, 75.14, 75.16, 75.30, 79.91, 80.17, 98.34, 101.28, 127.54, 127.61, 127.71, 127.75, 127.80, 127.85, 127.90, 127.99, 128.02, 128.13, 128.44, 128.46, 128.50, 128.60, 128.62, 137.49, 138.16, 138.41, 138.43, 138.56, 138.59, 138.78. Data were in agreement with those reported in the literature.[5]

2-*O*-α-D-mannopyranosyl-D-mannopyranose (Man2, compound 7). To a solution of compound **6** (104 mg, 0.11 mmol) in degassed methanol was added Pd/C (10%, 40 mg), and the mixture was stirred at ambient temperature under hydrogen (1 atm) for 3 days. After completion of the reaction, monitored by TLC, the reaction mixture was filtered through celite, concentrated, and further washed with distilled water. After removal of water, compound **7** was obtained in 88% yield (33 mg) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.90-3.58 (m, 9 H), 3.97-3.91 (m, 2 H), 4.06 (dd, 1 H, J = 1.5 and 3.1 Hz, H-2'), 5.03 (d, 1 H, J = 1.5 Hz, H-1'), 5.37 (d, 1 H, J = 0.8 Hz, H-1). ^{13}C NMR (125 MHz, $CDCl_3$): δ 60.91, 61.02, 66.82, 67.00, 69.94, 70.28, 72.42, 73.20, 79.09, 92.51, 102.15. Data were in agreement with those reported in the literature.[5]

Synthesis of Man3 (10)



Scheme S3. Synthesis of **Man3** (**10**).

2-*O*-(2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-1,3,4,6-tetra-*O*-benzyl-α-D-mannopyranoside (8). A solution of compounds **6** (74 mg, 0.076 mmol) and **4** (54 mg, 0.1 mmol) in dry DCM was stirred for 0.5 h with 4Å molecular sieves under nitrogen atmosphere at -10 °C. NIS (56 mg, 0.25 mmol) was added and then a solution of TfOH in dry DCM (1.97 mg, 0.013 mmol, 10 μL/ 1 mL) was added dropwise. Stirring was continued at the same temperature for another 0.5 h and the acid was neutralized with saturated aqueous $NaHCO_3$ solution, $Na_2S_2O_5$(s), brine, and extracted with DCM. The combined organic phase was dried over Na_2SO_4 and then evaporated under reduced pressure. The crude product was purified by flash column chromatography using hexane/EtOAc (6:1 v/v) as the solvent system and compound **8** was obtained in 79% yield (87 mg) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.15 (s, 3 H, C(O)C*H*$_3$), 4.16-3.52 (m, 17 H), 4.91-4.28 (m, 20 H, 10 × C*H*$_2$Ph), 5.04 (d, 1 H, *J* = 1.5 Hz, H-1), 5.08 (d, 1 H, *J* = 1.6 Hz, H-1''), 5.21 (d, 1 H, *J* = 1.6 Hz, H-1'), 5.56 (dd, 1 H, *J* = 1.6 and 2.9 Hz, H-2''), 7.46-7.11 (m, 50 H, 10 × C$_6$*H*$_5$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 21.32, 68.87, 68.95, 69.23, 69.45, 69.52, 72.03, 72.04, 72.11, 72.20, 72.28, 72.34, 73.42, 73.47, 73.49, 74.40, 74.86, 75.03, 75.14, 75.24, 75.30, 78.31, 79.45, 79.64, 98.38, 99.54, 100.85, 127.54, 127.57, 127.58, 127.63, 127.67, 127.69, 127.71, 127.78, 127.81, 127.87, 127.91, 127.96, 127.98, 128.13, 128.31, 128.37, 128.43, 128.47, 128.54, 137.53, 138.20, 138.35, 138.51, 138.56, 138.61, 138.62, 138.74, 170.28. Data were in agreement with those reported in the literature.[6]

2-*O*-(2-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-1,3,4,6-tetra-*O*-benzyl-α-D-mannopyranoside (9). Compound **8** (71 mg, 0.049 mmol) was dissolved in MeOH (1 mL) and NaOMe (2.7 mg, 0.05 mmol)

was added. The reaction mixture was stirred under nitrogen atmosphere at ambient temperature overnight. After completion of the reaction, monitored by TLC, the mixture was further stirred with added Amberlyst 15 for 10 minutes, after which the solvent was evaporated under reduced pressure. The crude product was then purified by flash column chromatography using hexane/EtOAc (3:1 v/v) as the solvent system and compound **9** was obtained in 77% yield (53 mg) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.08-3.42 (m, 18 H), 4.79-4.19 (m, 20 H, 10 × CH_2Ph), 4.95 (d, 1 H, *J* = 1.6 Hz, H-1''), 5.05 (d, 1 H, *J* = 1.3 Hz, H-1), 5.13 (d, 1 H, *J* = 1.6 Hz, H-1'), 7.32-7.04 (m, 50 H, 10 × C_6H_5). ^{13}C NMR (125 MHz, $CDCl_3$): δ 68.69, 69.10, 69.22, 69.46, 69.57, 71.73, 72.03, 72.16, 72.26, 72.39, 72.50, 73.42, 73.44, 73.48, 74.46, 75.00, 75.12, 75.19, 75.30, 75.51, 79.56, 80.06, 80.14, 98.37, 101.06, 101.11, 127.53, 127.63, 127.71, 127.74, 127.77, 127.81, 127.85, 127.92, 127.99, 128.02, 128.15, 128.42, 128.45, 128.48, 128.53, 128.60, 137.54, 138.21, 138.36, 138.48, 138.57, 138.67, 138.73. Data were in agreement with those reported in the literature.[6]

2-*O*-(2-*O*-α-D-mannopyranosyl-α-D-mannopyranosyl)-D-mannopyranose (Man3, compound 10). To a solution of compound **9** (43 mg, 0.03 mmol) in degassed methanol Pd/C (10%, 45 mg) was added, and the mixture was stirred at ambient temperature under hydrogen (1 atm) for 4 days. After completion of the reaction, monitored by TLC, the reaction mixture was filtered through celite, concentrated, and further washed with distilled water. After removal of water, compound **10** was obtained in 94% yield (14 mg) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.13-3.56 (m, 18 H), 5.04 (d, 1 H, *J* = 1.2 Hz, H-1'), 5.29 (d, 1 H, *J* = 1.3 Hz, H-1''), 5.37 (d, 1 H, *J* = 0.6 Hz, H-1). ^{13}C NMR (125 MHz, $CDCl_3$): δ 60.90, 61.02, 66.79, 67.00, 69.91, 69.95, 70.30, 72.43, 73.19,

78.53, 79.32, 92.46, 100.53, 102.21. Data were in agreement with those reported in the literature.[6]

Protein cloning, expression, and purification. Protein was expressed from a synthetic gene using pET26b(+) (Novagen; Madison, WI) and *Escherichia coli* BL21(DE3) as expression vector and host strain, respectively. The CVN^{Q50C} mutant coding sequence was created using the QuikChange XL II site-directed mutagenesis (Stratagene) kit with the CV-N wild-type gene and two forward/reverse primers:

5'-GTTGACGGTTCCCTGAAATGGTGCGGTTCCAACTTCATCGAAACC-3'

5'-GGTTTCGATGAAGTTGGAACCGCACCATTTCAGGGAACCGTCAAC-3'.

For protein production, *E. coli* BL21(DE3) cells (Stratagene) were transformed with the CV-N variant containing vector. Cells were grown at 37 °C and induced with 1 mM isopropyl 1-thio-β-D-galactopyranoside for 3 h. Isotopic labeling was carried out by growing the culture in modified M9 minimal medium containing $[^{15}N]H_4Cl$ as sole nitrogen source. The expressed protein was isolated from the periplasmic fraction of the *E. coli* cells by twice heating (62 °C) and cooling (0 °C) the cell suspension in phosphate buffered saline (pH 7.4). After removal of insoluble material by centrifugation, the supernatant, containing soluble protein, was fractionated by gel filtration on Superdex 75 (HiLoad2.6 x 60 cm, Amersham Biosciences), equilibrated in 20 mM sodium phosphate buffer (pH 6.0). A similar protocol was used to purify CVN^{MutDB}. Both proteins were isolated as monomers. The purity and identity of the protein was assessed and verified by mass spectrometry and the structural integrity of the proteins was ascertained by recording a ^{1}H-^{15}N HSQC spectrum.

Fluorescent labeling. Purified CVN^{Q50C} protein (~100 μM) was incubated with an equimolar concentration of the Cy5 dye (GE Healthcare) in 20 mM sodium phosphate buffer, pH 7.4 , in the presence of 2 mM TCEP to protect the free cysteines from oxidation. The reaction was carried out for 2 hours at room temperature in the dark. The unreacted fluorophore was removed in two steps. First, the bulk of the free dye was removed by passage over a PD-10 desalting column (GE Healthcare) in pH 7.4 sodium phosphate buffer (20 mM). Dye-labeled protein fractions were collected and concentrated to ~15 μM using centriprep devices (Millipore). The labeling efficiency was about 95%, as evaluated by ESI - mass spectrometry. The structural integrity of the labeled protein was ascertained by recording a ^{1}H-^{15}N HSQC spectrum and the fluorescently labeled protein was found to be well structured.

Preparation of gold glyconanoparticles. AuNPs, 22 nm in diameter, were prepared and functionalized with PFPA-disulfide following the same procedure as described in a previous paper.[7] The subsequent carbohydrate coupling was carried out photochemically as reported previously.[8] Briefly, a solution of PFPA-functionalized AuNPs in acetone (10 nM) mixed with an aqueous solution of Man2 or Man3 (1 mM) was irradiated for 10 min with a 450-W medium pressure Hg lamp (Hanovia) with a 280-nm long-path optical filter. The resulting GNPs were then dialyzed overnight to remove excess carbohydrate. Before the binding experiments, the GNPs were incubated in 20 mM sodium phosphate buffer solution (pH 6.0), containing 0.01% NaN_3, 0.01% Tween 20 and 3% BSA for 1 hour, centrifuged, and placed in buffer without BSA until further use.

CVN binding assays. GNP-M3 (5 nM, 1.0 mL) was incubated in a solution of CVN^{Q50C} or CVN^{MutDB} in 20 mM sodium phosphate buffer solution (10 μM, 1.0 mL), pH 6.0, containing 0.01% NaN_3 for 1 hour with constant shaking. UV-vis spectra of the resulting solutions were recorded on a Perkin Elmer Lambda 45 UV-vis spectrophotometer, and each measurement was performed at least 3 times.

Isothermal Titration Calorimetry (ITC). ITC experiments of Man3 binding to CVN were performed using an ITC200 Microcalorimeter from Microcal, LLC. (Northampton, MA) in 50 mM sodium phosphate buffer, pH 7.5, 200mM NaCl, 0.02% NaN_3. The concentration of CV-N was 50 μM, and that of Man3 was 0.64 mM. In each individual experiment, ~38 μL of Man3 was injected through the computer-controlled 40-μL micro-syringe at an interval of 4 min into the protein solution in the same buffer (cell volume = 200 μL) while stirring at 350 rpm. Calorimetric titrations of Man2 binding to CVN were performed using a VP-ITC isothermal titration calorimeter (MicroCal, LLC; Northampton, MA). Titrations were carried out at 30°C in the same buffer as described above for Man3. A 35 μM CV-N solution was placed in the calorimeter cell (~1.44 mL active volume), stirred at 310 rpm, and 9-μL aliquots of 1.5 mM Man2 solution were added at 2 min intervals from a 295-μL stirring syringe. A total of 30 injections were performed. The experimental data were fitted to a theoretical titration curve using the software supplied by MicroCal. A standard two-site model was used with ΔH (enthalpy change, in kcal/mol), K_a (association constant, in M^{-1}), and N (number of binding sites) as the variables.

Fluorescence competition binding assays. The previously reported protocol was adapted,[7] as described below. A series of **GNP-M2** solutions were prepared by diluting a stock solution (10 nM) to concentrations between 5 nM and 1 x10^{-8} nM. A stock solution of Cy5-CVN (1.20 μM) was prepared in pH 6.0 sodium phosphate buffer (20 mM). To a solution of **GNP-M2** (1 mL) in a 1.5-mL microcentrifuge tube, Man2 (0.48 mM, 0.1 mL) and Cy5-CVN (1.2 μM, 0.1 mL) were added. The total volume of the final solution was 1.20 mL, and the concentrations of Man2 and Cy5-CVN were 40 μM and 100 nM, respectively. The solutions were shaken for 1 hour, and then centrifuged at 12,000 rpm for 30 min until all nanoparticles were completely pelleted at the bottom of the tube. The supernatants were taken out for fluorescence measurement using a PTI spectrofluorimeter (Photon Technology International). Excitation was at 649 nm and emission was recorded at 666 nm for analysis. The same procedure was followed for **GNP-M3,** except that Man3 was used as the competing ligand. Measurement for each concentration was repeated 5 times, and values were averaged.

K_{D1} and K_{D2}, apparent dissociation constants for GNP binding to the glycan-binding site on Domain A and Domain B of CVN^{Q50C}, respectively, were obtained from best-fitting the response curves, using a two-site competitive binding model with the equation in Figure 3b and KaleidaGraph software.

2. TEM image of gold nanoparticles

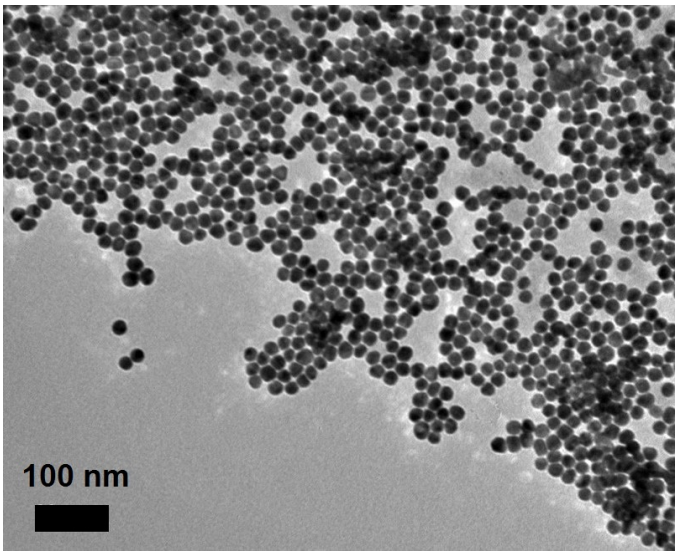


Fig. 1S TEM image of synthesized 22-nm gold nanoparticles.

3. DLS results

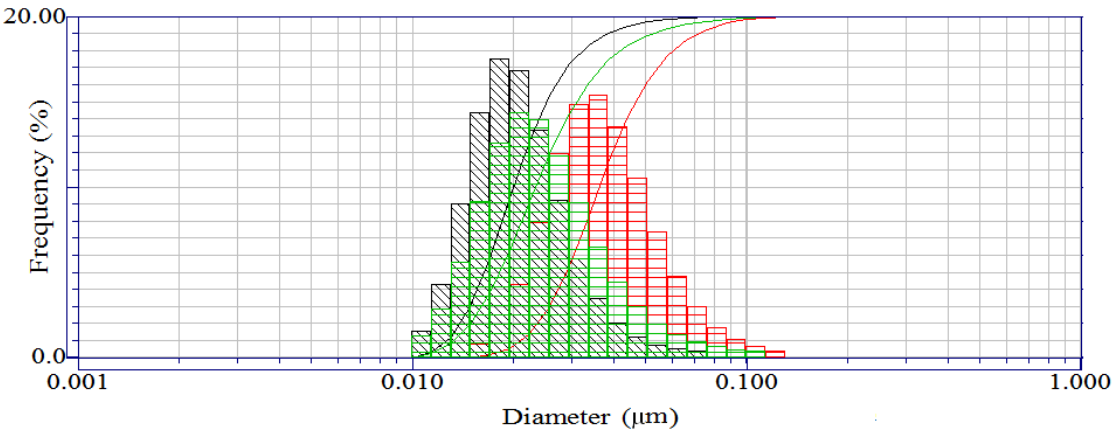


Fig. 2S DLS particle size distribution of **GNP-M3** (black bars), and after treating with CVN^{Q50C} (red bars) or CVN^{MutDB} (green bars).

4. ITC results

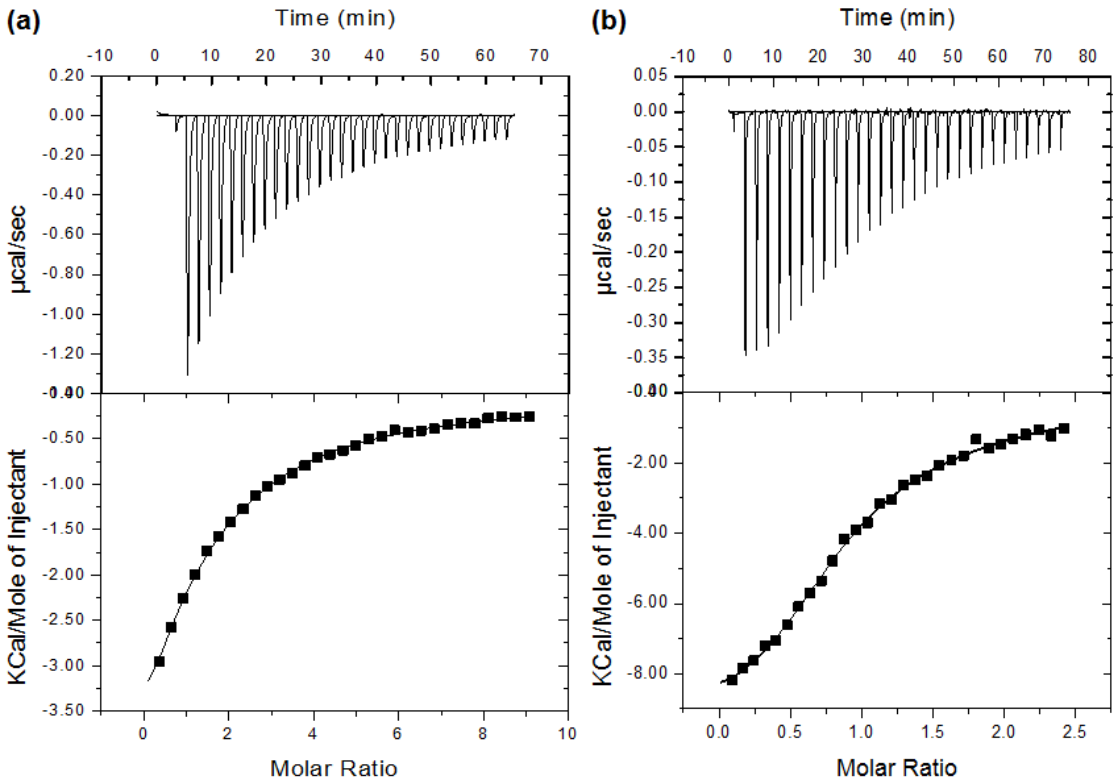


Fig. 3S Calorimetric titration of a) CVN^{Q50C} (35 µM) with Man2 (1.5 mM), and b) CVN^{Q50C} (50 µM) with Man3 (0.64 mM) at 30 °C. The raw data were obtained for 30 and 20 automatic injections, respectively**.** The integrated curves show experimental points (■) and the best fit (-). The buffer was 50 mM sodium phosphate, 200 mM NaCl, pH 7.5, containing 0.02% NaN_3.

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