# SUPPORTING INFORMATION

# Glyconanoparticles for the plasmonic detection and discrimination between human and avian influenza virus

María J. Marín,<sup>‡a</sup> Abdul Rashid,<sup>‡b</sup> Martin Rejzek,<sup>b</sup> Shirley A. Fairhurst,<sup>b</sup> Stephen A. Wharton,<sup>c</sup> Stephen <sup>10</sup> R. Martin,<sup>c</sup> John W. McCauley,<sup>c</sup> Thomas Wileman,<sup>d</sup> Robert A. Field<sup>\*b</sup> and David A. Russell<sup>\*a</sup>

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<sup>a</sup> School of Chemistry, University of East Anglia, Norwich Research Park, Norwich, Norfolk NR4 7TJ, UK. E-mail:

d.russell@uea.ac.uk

<sup>15</sup> <sup>b</sup> Department of Biological Chemistry, John Innes Centre, Norwich Research Park, Norwich, Norfolk NR4 7UH, UK. Email: rob.field@jic.ac.uk

<sup>c</sup> MRC National Institute of Medical Research, Mill Hill, London, NW7 1AA, UK

<sup>d</sup> Norwich Medical School, University of East Anglia, Norwich Research Park, Norwich, Norfolk NR4 7TJ, UK

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## **General methods**

Commercial reagents were obtained from Acros, Aldrich, Alfa Aesar and Fluka and used without further purification. *N*-Boc aminopentanoic acid (valeric acid) was purchased from Sigma Aldrich. TLC was performed on precoated silica plates (Merck 60 F254, 0.25 mm) containing a fluorescence indicator. Compounds were <sup>3</sup> visualised under UV (254 nm) and by heating after dipping the TLC plate in a solution of 5 % H<sub>2</sub>SO<sub>4</sub> in ethanol, in a solution of ninhydrin (200 mg) in butanol (95 mL) and acetic acid (10 %, 5 mL) or in 15 % aqueous sulfuric acid saturated with cerium(IV) sulfate. Gel chromatography was performed on TSK HW40S gel using a XK16/40 column. Flash column chromatography was performed on silica gel (Biotage KP-SIL 60A, 40–63 µm). Standard column chromatography was performed on silica gel (Fluka 60, 63-200 µm). NMR spectra were recorded on a <sup>10</sup> Bruker spectrometer: <sup>1</sup>H NMR spectra recorded at 400 MHz were referenced to  $\delta_{\rm H}$  7.26 for CDCl<sub>3</sub> or  $\delta_{\rm H}$  3.34 for CD<sub>3</sub>OD; <sup>13</sup>C NMR spectra recorded at 100 MHz were referenced to  $\delta_{\rm C}$  77.0 for CDCl<sub>3</sub> or  $\delta_{\rm C}$  49.05 for CD<sub>3</sub>OD. Chemical shifts of NMR signals recorded in D<sub>2</sub>O are reported with respect to the methyl resonance of internal acetone at  $\delta_{\rm H}$  2.22 ppm and  $\delta_{\rm C}$  30.89 ppm, respectively. Assignments were made with the aid of COSY and HSQC experiments. Multiplicity of signals in <sup>13</sup>C NMR spectra was determined from HSQC spectra.

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#### Synthesis of trivalent ligand 1

#### Synthesis of 3-Aminopropyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside



3-Azidopropyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside **4**<sup>1</sup> (6.5 g, 15.1 mmol) and 10 % Pd-C (200 mg) in ethyl acetate (EtOAc, 50 mL) were stirred under an atmosphere of hydrogen for 6 h, at which point TLC showed a complete conversion to a slower moving product ( $R_r = 0.1$ , dichloromethane/methanol 3:1). The catalyst was removed by filtration of the suspension through a plug of Celite, which was washed with ethyl <sup>10</sup> acetate and the combined filtrate was concentrated *in vacuo* to yield a colourless oil. The residue was subjected to column chromatography on silica gel (dichloromethane/methanol, stepwise gradient 10:1, 5:1) to give the corresponding aminopropyl glycoside (5.8 g, 95 %). <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta = 6.04$  (bt, 1H, NH), 5.41 (dd, 1H, H4, <sup>3</sup>J<sub>3,4</sub> = 3.4 Hz, <sup>3</sup>J<sub>4,5</sub> = 1.1 Hz), 5.20 (dd, 1H, H2, <sup>3</sup>J<sub>1,2</sub> = 7.9 Hz, <sup>3</sup>J<sub>2,3</sub> = 10.4 Hz), 5.04 (dd, 1H, H3, <sup>3</sup>J<sub>2,3</sub> = 10.4 Hz, <sup>3</sup>J<sub>3,4</sub> = 3.4 Hz, <sup>3</sup>J<sub>4,5</sub> = 1.1 Hz), 5.20 (dd, 1H, H2, <sup>3</sup>J<sub>1,2</sub> = 7.9 Hz, <sup>3</sup>J<sub>2,3</sub> = 10.4 Hz), 5.04 (dd, 1H, H3, <sup>3</sup>J<sub>2,3</sub> = 10.4 Hz, <sup>3</sup>J<sub>3,4</sub> = 3.4 Hz), 4.45 (d, 1H, H1, <sup>3</sup>J<sub>1,2</sub> = 7.9 Hz), 4.08 - 4.22 (m, 2H, H6), 4.00 (m, 1H, Ha), 3.92 (m, 1H, H5, <sup>3</sup>J<sub>4,5</sub> = 1.1 Hz, <sup>3</sup>J<sub>3,6</sub> = 6.6 Hz), 3.58 (m, 1H, Ha), 3.47 - 3.38 (m, 1H, Hc), 3.34 - 3.20 (m, 1H, Hc), 2.17, 2.08, 2.06, 1.99 (4s, 12H, CH<sub>3</sub>CO), 1.92 - 1.72 (m, 2H, Hb); <sup>13</sup>C NMR (CDCl<sub>3</sub>) from HSQC:  $\delta = 102.2$  (d, 1C, H1), 70.9, (d, 1C, C5), 70.6 (d, 1C, C3), 69.2 (t, 1C, Ca), 68.8 (d, 1C, C2), 66.9 (d, 1C, C4), 61.2 (t, 1C, C6), 37.7 (t, 1C, Cc), 29.1 (t, 1C, Cb), 20.7 (4xq, 3C, 4xCH<sub>3</sub>CO-); m/z (MALDI<sup>+</sup>) 405.90 [M+H]<sup>+</sup>; HR-MS calcd for C<sub>17</sub>H<sub>28</sub>NO<sub>10</sub><sup>+</sup> [M+H]<sup>+</sup> 406.1708, found 406.1707.

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Synthesis of 3-(N-Azidopentyloxycarbonyl)aminopropyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside



<sub>s</sub> To a solution of 3-Aminopropyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (5.5 g, 13.5 mmol) and 6azidohexanoci acid NHS ester<sup>2</sup> (3.4 g, 13.5 mmol) in dry dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>, 50 mL) was added triethylamine (Et<sub>3</sub>N, 4.1 mL, 30 mmol) and the reaction mixture was stirred at room temperature for 3 h, at which point TLC showed the reaction to be complete (product  $R_f = 0.6$ , ethyl acetate). The solvent was removed in vacuo and the resulting residue was purified by silica column chromatography (stepwise gradient,  $_{10}$  ethyl acetate/hexane 50:50, 100:0) to give the corresponding amide as a syrup (5.0 g, 68 %). <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$ = 5.94 (s, 1H, NH), 5.41 (d, J<sub>3,4</sub> = 3.5 Hz,1H, H-4), 5.20 (dd, J<sub>1,2</sub> = 8.0 Hz, J<sub>2,3</sub> = 10.5 Hz, 1H, H-2), 5.04 (dd, J<sub>2,3</sub> = 10.5 Hz, J<sub>3.4</sub> = 3.5 Hz, 1H, H-3), 4.45 (d, J<sub>1.2</sub> = 8.0 Hz, 1H, H-1), 4.16 (m, 2H, H6a,H6b), 4.00 (m, 1H, OCHHCH<sub>2</sub>CH<sub>2</sub>NH-), 3.92 (t, J 6.6 Hz, 1H, H-5), 3.58 (m, 1H, H- OCHHCH<sub>2</sub>CH<sub>2</sub>NH-), 3.45(m, 1H, OCH<sub>2</sub>CH<sub>2</sub>CHHNH-), 3.28 (m, 3H, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NHNH, NHCO(CH<sub>2</sub>)<sub>4</sub>CH<sub>2</sub>N<sub>3</sub>), 2.21 (m, 2H, NHCOCH<sub>2</sub>(CH<sub>2</sub>)<sub>4</sub>N<sub>3</sub>), 2.17, 2.08, 2.06, 2.00 (4s, 15 each 3H, 4xCOCH<sub>3</sub>), 1.78 (m, 2H, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NH-), 1.51 (m, 4H, NHCOCH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>N<sub>3</sub>, NHCO(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 1.43 (m, 2H, NHCO(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>N<sub>3</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  = 172.84, 170.39, 170.19, 170.08, 169.93 (CO.(CH<sub>2</sub>)<sub>5</sub>N<sub>3</sub>, 4xCOCH<sub>3</sub>), 101.42 (C-1), 70.82 (C-3), 70.64 (C-5), 69.32 (C-2), 68.92 (C-4), 66.98 (OCH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>NH-), 61.20 (C-6), 51.29 (NHCO(CH<sub>2</sub>)<sub>4</sub>CH<sub>2</sub>N<sub>3</sub>), 37.55 (NHCOCH<sub>2</sub>(CH<sub>2</sub>)<sub>4</sub>N<sub>3</sub>), 36.24 (O(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>NH-), 29.30, 28.63, 26.41, 25.22 (OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NH-, NHCOCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 20.83, 20.68, 20.57 (4xCOCH<sub>3</sub>); m/z (MALDI<sup>+</sup>) 567.14  $_{20}$  [M+Na]<sup>+</sup>; HR-MS calcd for C<sub>23</sub>H<sub>36</sub>N<sub>4</sub>NaO<sub>11</sub><sup>+</sup> [M+Na]<sup>+</sup> 567.2273, found 567.2262.

Synthesis of 3-(N-Azidopentyloxycarbonyl)aminopropyl 2,3,4-tri-O-acetyl-β-D-galactopyranoside (5)



s To a solution of 3-(N-Azidopentyloxycarbonyl)aminopropyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (3.0 g, 5.5 mmol) in dry methanol (20 mL) was added 1 M sodium methoxide-methanol (NaOMe-MeOH, 0.25 mL) and the reaction was stirred at room temperature for 1 h. The mixture was then neutralized with Amberlite IR 120  $(H^{+})$  resin, the solution filtered and the filtrate concentrated *in vacuo* to a gum. TLC (ethyl acetate) indicated absence of starting material. The residue was taken up in dimethylformamide (DMF, 10 mL), the solution was 10 cooled to 0 °C and tert-butyldimethylsilyl chloride (TBDMS-Cl, 1.1 g, 7.5 mmol) was added. The reaction mixture was stirred at room temperature for 4 h after which the DMF was removed in vacuo. The residual oil was taken up in a mixture of pyridine (Pyr, 10 mL) and acetic anhydride (Ac<sub>2</sub>O, 10 mL) and the reaction mixture was stirred at room temperature overnight. Solvents were then removed in vacuo, the resulting residue was taken up in CH<sub>2</sub>Cl<sub>2</sub> (50 mL), washed with water and the organic extract was dried (MgSO<sub>4</sub>) and concentrated in  $_{15}$  vacuo to give the acetylated TBDMS ether, [R<sub>f</sub> = 0.6 (ethyl acetate/hexane 3:1)]. This material was dissolved in 10 % trifluoroacetic acid (TFA) in 80 % aqueous acetic (aq. AcOH) mixture (10 mL), allowed to stand at room temperature for 1 h until TLC showed the complete disappearance of starting material (product TLC:  $R_f = 0.3$ , ethyl acetate) and solvents were removed in vacuo. The residue was subjected to column chromatography on silica (ethyl acetate/hexane, stepwise gradient 5:1, 3:1, 1:0) to give primary alcohol 5 as a syrup (1.8 g, 65 % <sup>20</sup> over 4 steps). <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  = 6.12 (bs, 1H, NH), 5.26 (dd, 1H, H2, <sup>3</sup>J<sub>1,2</sub> = 7.9 Hz, <sup>3</sup>J<sub>2,3</sub> = 10.3 Hz), 4.97 (dd, 1H, H3,  ${}^{3}J_{2,3}$  = 10.3 Hz,  ${}^{3}J_{3,4}$  = 3.2 Hz), 4.43 (d, 1H, H1,  ${}^{3}J_{1,2}$  = 7.9 Hz), 4.3 (m, 2H, H6), 4.06 (d, 1H, H4,  ${}^{3}J_{3,4}$  = 3.2 Hz), 3.98 (m, 1H, Ha), 3.66 - 3.53 (m, 1H, Ha), 3.49 - 3.29 (m, 2H, Hc), 3.28 (t, 2H, Hi, <sup>3</sup>J<sub>h,i</sub> = 6.8 Hz), 2.22 (t, 2H, He, <sup>3</sup>J<sub>ef</sub> = 7.5 Hz), 2.09, 2.07, 2.05 (3s, 9H, CH<sub>3</sub>C(O)), 1.86 - 1.73 (m, 2H, Hb), 1.71 - 1.59 (m, 4H, Hf, Hh), 1.45 - 1.37 (m, 2H, Hg); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  = 173.2, 170.9, 171.1, 171.0 (4xs, 4C, 3xCH<sub>3</sub>*C*O-, Cd), 101.2 (d, 1C, H1), 72.9 (d, 1C, C3), 69.1 (dt, 3C, C2, Ca), 61.9 (t, 1C, C6), 51.1 (t, 1C, Ci), 37.6 (t, 1C, Cc), 36.2 (t, 1C, Ce), 29.1 (t, 1C, Cb), 28.4 (t, 1C, Cf), 26.3 (t, 1C, Cg), 25.2 (t, 1C, Ch), 20.8 (3 x q, 3C, 3 x *C*H<sub>3</sub>C(O)); m/z (MALDI<sup>+</sup>) 525.01 [M+Na]<sup>+</sup>; HR-MS calcd for C<sub>21</sub>H<sub>34</sub>N<sub>4</sub>NaO<sub>10</sub><sup>+</sup> [M+Na]<sup>+</sup> 525.2167, found 525.2163.

Synthesis of 3-(N-Azidopentyloxycarbonyl)aminopropyl 2,3,4-tri-O-acetyl-6-O-trifluoromethanesulfonyl-β-Dgalactopyranoside (6)



To a stirred solution of primary alcohol **5** (0.2 g, 0.4 mmol) in dry  $CH_2CI_2$  (10 mL) at 0 °C under nitrogen was added pyridine (126 µL, 1.5 mmol), followed by triflic anhydride ( $Tf_2O$ , 140 µL, 1 mmol). The reaction was stirred at 0 °C for 30 min when TLC showed complete conversion to product ( $R_f$  = 0.7, ethyl acetate).  $CH_2CI_2$  (25 mL) was added, the organic solution was washed with 1 M HCl (25 mL), dried (MgSO<sub>4</sub>) and concentrated *in vacuo* to approximately 10 mL. The resulting solution was applied to a silica column (1 cm x 2 cm) and the product was washed through with ethyl acetate. The eluted fraction was mixed with DMF (2 mL) and the ethyl acetate removed *in vacuo* to leave the product **6** in a DMF solution for subsequent use. [NB - triflate **6** rapidly decomposes on concentration to dryness].

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#### Synthesis of 3-(N-Azidopentyloxycarbonyl)aminopropyl S-(methyl 5-acetamido-3,5-dideoxy-D-glycero- $\alpha$ -D-

galacto-2-nonulopyranosylonate)-(2-6)-6-deoxyl-6-thio- $\beta$ -D-galactopyranoside (8)



To a stirred solution of known per-N,O-acetylated sialic acid thioacetate  $7^3$  (0.15 g, 0.3 mmol) in DMF (2 mL) at 0 °C under nitrogen was added diethylamine (Et<sub>2</sub>NH, 0.5 mL, 19 mmol). After 15 min a solution of triflate 6 in DMF (vide supra) was added at 0 °C and the reaction was stirred for 2 h. DMF was then removed in vacuo, the resulting residue was taken up in  $CH_2Cl_2$  (25 mL), washed with water, dried (MgSO<sub>4</sub>) and concentrated to <sup>10</sup> dryness. The residue was subjected to column chromatography on silica (stepwise gradient ethyl acetate, ethyl acetate/methanol 95:5) to give the acetylated thioglycoside intermediate as a glass.  $R_f = 0.3$  (ethyl acetate/methanol 95:5); m/z (MALDI<sup>+</sup>) 1014.31 [M+Na]<sup>+</sup>, calcd for  $C_{41}H_{61}N_5NaO_{21}S$  [M+Na]<sup>+</sup> = 1014.36. To a solution of the acetylated intermediate (0.12 g, 120 µmol) in dry methanol (2 mL) was added 1 M sodium methoxide-methanol (50 µL) and the reaction was stirred at room temperature for 1 h. The reaction mixture <sub>15</sub> was neutralized with Amberlite IR 120 ( $H^{\dagger}$ ) resin, the solution was filtered and the filtrate was concentrated *in* vacuo to a solid. This material was purified by TSK gel chromatography (water, flow rate 0.5 mL/ min) to give de-O-acetylated azide **8** as a white solid (40 mg, 25 % over 3 steps). <sup>1</sup>H NMR (D<sub>2</sub>O):  $\delta$  = 4.27 (d, 1H, H1', <sup>3</sup>J<sub>1,2</sub> = 7.9 Hz), 3.89 (d, 1H, H4',  ${}^{3}J_{3',4'}$  = 3.4 Hz,  ${}^{3}J_{4',5'}$  = 1.1 Hz), 4.84 - 3.60 (m, 7H, H4, H6', H7, H8, Ha), 3.81(s, 3H, CH<sub>3</sub>CO), 3.54 (dd, 2H, H3',  ${}^{3}J_{2',3'}$  = 10.0 Hz,  ${}^{3}J_{3',4'}$  = 3.4 Hz), 3.48 (dd, 1H, H5',  ${}^{3}J_{4',5'}$  = 1.1 Hz,  ${}^{3}J_{5',6'}$  = 8.7 Hz), 3.40 <sub>20</sub> (dd, 1H, H2', <sup>3</sup>J<sub>1',2'</sub> = 7.9 Hz, <sup>3</sup>J<sub>2',3'</sub> = 10.0 Hz), 3.26 (t, 2H, Hi, <sup>3</sup>J<sub>h,i</sub> = 6.8 Hz), 3.21 (m, 2H, Hc), 3.0 - 2.83 (m, 2H, H9), 2.76 (dd, 2H, H3<sub>eq</sub>, <sup>2</sup>J<sub>3ax,3eq</sub> = 12.8 Hz, <sup>3</sup>J<sub>3eq,4</sub> = 4.7 Hz), 2.22 (t, 2H, He, <sup>3</sup>J<sub>e,f</sub> = 7.3 Hz), 1.96 (s, 3H, CH<sub>3</sub>CO.NH), 1.80

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(m, 1H, H3<sub>ax</sub>), 1.70 (m, 2H, Hb), 1.59 - 1.44 (m, 4H, Hf, Hh), 1.42 - 1.26 (m, 2H, Hg); <sup>13</sup>C NMR (D<sub>2</sub>O):  $\delta$  = 176.8, 174.9, 171.0 (3s, 4C, CH<sub>3</sub>C(O)O, C2, CH<sub>3</sub>C(O)NH), Cd), 102.8 (d, 1C, H1'), 75.0, 73.8 (dd, 2C, C7, C8), 72.8 (d, 1C, C3'), 70.4(d, 1C, C2'), 68.9 (d, 1C, C2, C4'), 68.0 (d, 1C, C5'), 67.7 (t, 1C, Ca), 67.5 (d, 1C, C4), 63.0 (t, 1C, C6'), 51.5 (q, 1C, CH<sub>3</sub>C(O)O), 51.1 (t, 1C, Ci), 39.9 (t, 1C, C3), 36.1 (t, 1C, Cc), 35.6 (t, 1C, Ce), 29.0 (t, 1C, C9), 28.4 (t, <sup>5</sup> 1C, Cb), 27.6 (t, 1C, Cf), 25.3 (t, 1C, Cg), 24.9 (t, 1C, Ch), 21.9 (q, 1C, CH<sub>3</sub>CONH); m/z (MALDI<sup>+</sup>) 720.16 [M+Na]<sup>+</sup>, HR-MS calcd for C<sub>27</sub>H<sub>47</sub>N<sub>5</sub>NaO<sub>14</sub>S [M+Na]<sup>+</sup> 720.2737, found 720.2740.

Synthesis of N-(tert-Butyloxycarbonyl)-N-(butanoyl)tris[(propargyloxy)methyl] aminomethane (9)



Known tri-*O*-propargyl-trishydroxymethylaminomethane<sup>4</sup> (0.15 g, 0.5 mmol) was dissolved in 80 % aqueous trifluoroacetic acid (1 mL) and the solution allowed to stand at room temperature for 15 min after which the <sup>15</sup> solvents were removed *in vacuo*. The residue was co-evaporated with ethanol and then toluene to give a yellow oil (0.1 g). This oil was taken up in dry DMF (0.5 mL) and added to a mixture of commercial *N*-Boc aminopentanoic acid (86 mg, 0.4 mmol), HATU (2-(1H-7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate) (0.2 g, 0.5 mmol), *N*-methylmorpholine (0.1 g, 1 mmol) in DMF (1 mL). The reaction was stirred at room temperature overnight, the DMF removed *in vacuo*, the resulting residue was taken up in  $^{20}$  CH<sub>2</sub>Cl<sub>2</sub> (25 mL), washed with water, dried (MgSO<sub>4</sub>) and concentrated to dryness. The residue was purified by column chromatography on silica (stepwise gradient, ethyl acetate/hexane 5:95, 25:75, 40:60) to yield compound **9** as a syrup (0.14 g, 78 %). R<sub>f</sub> 0.7 (ethyl acetate/hexane 1:1). <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  = 5.71 (brs, 1H, NH), 4.65 (brs, 1H, NH), 4.15 (s, 6H, 3 x OCH<sub>2</sub>CECH), 3.84 (s, 6H, NHC(CH<sub>2</sub>O)<sub>3</sub>-), 3.13 (m, 2H, NHCH<sub>2</sub>-), 2.44 (s, 3H, 3 x OCH<sub>2</sub>CECH), 2.19 (t, *J*<sub>HH</sub> = 7.2 Hz, 2H, -CH<sub>2</sub>CONH-), 1.65 (m, 2H, -NHCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>-), 1.52 (m, 2H, -NHCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>-),

1.48 (s, 9H,  $3xCCH_3$ ); m/z (MALDI<sup>+</sup>) 435.04 [M+H]<sup>+</sup>; HR-MS calcd for  $C_{23}H_{35}N_2O_6^+$  [M+H]<sup>+</sup> 435.2490, found

435.2490.

#### s Synthesis of N-Boc-protected trivalent methyl ester (10)



To a stirred solution of azide **8** (10 mg, 14 µmol) and tri-propargyl ether **9** (2 mg, 4.6 µmol) in *tert*-<sup>10</sup> butanol/water 1:1 (tBuOH/H<sub>2</sub>O, 2 mL) was added premixed aqueous 1 M copper sulphate (CuSO<sub>4</sub>, 1 µL, 1 µmol) and 1 M sodium ascorbate (NaAsc, 2 µL, 2 µmol). The reaction mixture was stirred at 50 °C for 2 h and solvents were removed *in vacuo*. The resulting residue was taken up in water (0.5 mL), filtered through a 0.2 µm syringe filter and the solution was applied to a TSK gel column (water, 0.5 mL/ min). The tri-clicked product **10** was obtained as a white solid (5 mg, 43 %). <sup>1</sup>H NMR (600 MHz; D<sub>2</sub>O):  $\delta$  = 7.63 (s, 3H, *CH*NN), 4.09 (t, 6H,Hi, <sup>15</sup> <sup>3</sup>J<sub>h,i</sub> = 6.7 Hz), 3.99 (d, 3H, H1', <sup>3</sup>J<sub>1',2'</sub> = 7.9 Hz), 3.64 (d, 3H, H4', <sup>3</sup>J<sub>3',4'</sub> = 3.4 Hz ), 3.60 - 3.30 (m, 21H, H4, H7, H8, H6', Ha), 3.54 (s, 9H, CH<sub>3</sub>CO-), 3.29 (dd, 6H, H3',  ${}^{3}J_{2',3'} = 9.8$  Hz,  ${}^{3}J_{3,4} = 3.4$  Hz), 3.23 (d, 3H, H6,  ${}^{3}J_{6,7} = 9.0$  Hz), 3.16 (dd, 3H, H2',  ${}^{3}J_{1',2'} = 7.9$  Hz,  ${}^{3}J_{2',3'} = 9.8$  Hz), 2.94 (m, 6H, Hc), 2.6 - 2.7 (m, 6H, H9), 2.67 (t, 2H, Ht,  ${}^{3}J_{s,t} = 6.7$  Hz), 2.48 (dd, 6H, H3<sub>eq</sub>,  ${}^{2}J_{3e,3a} = 12.8$  Hz,  ${}^{3}J_{3e,4} = 4.5$  Hz), 1.89 (t, 6H, He,  ${}^{3}J_{e,f} = 6.7$  Hz), 1.85 (t, 2H,Hq,  ${}^{3}J_{s,t} = 7.2$  Hz), 1.71 (s, 3H, CH<sub>3</sub>CO-), 1.56 - 1.53 (2xm, 9H, H3<sub>ax</sub>, Hh), 1.55 (m, 3H), 1.47 (m, 6H, Hb), 1.28 (m, 6H, Hf), 1.17 (m,  ${}^{5}$  2H, Hq), 1.07 (m, 2H, Hs), 1.07 (m, 6H, Hg);  ${}^{13}$ C NMR (D<sub>2</sub>O): δ = 176.4, 174.9, 174.0, 171.0 (4xs, 8C, CH<sub>3</sub>CO-, Cd, Cp, Cu), 143.9 (s, 3C, Ck), 124.8 (d, 3C, Cj), 102.9 (d, 3C, C1'), 94.0 (s, 3C, C2), 83.1 (s, 1C, Cv), 73.7 (d, 3C, C7 or C8), 72.7 (d, 3C, C3'), 70.9 (d, 3C, C4), 70.4 (d, 3C, C2'), 69.0 (2xd, 6C, C2, C4'), 68.1 (d, 3C, C6), 67.7 (t, 3C, Ca), 67.6 (d, 3C, C8 or C7), 59.6 (2xt, 6C, C6', CI), 53.6 (q, 3C, CH<sub>3</sub>C(O)O), 50.2 (t, 3C, Ci), 40.0 (t, 3C, C3), 39.4 (t, 1C, Ht), 36.1 (t, 3C, Cc), 35.7 (t, 1C, Cq), 35.5 (t, 3C, Ce), 29.1 (2xt, 6C, C9, Ch), 28.4 (2xt, 4C, Cb, Cs), 25.0 (t, 3C, Cg),  ${}^{10}$  24.7 (t, 3C, Cf), 22.0 (t, 1C, Cr); m/z (MALDI<sup>+</sup>) 2591.19 [M+Cu]<sup>+</sup>, calcd for C<sub>104</sub>CuH<sub>175</sub>N<sub>17</sub>O<sub>48</sub>S<sub>3</sub> [M+Cu]<sup>+</sup> = 2591.33.

## Synthesis of the deprotected mercaptobutyrate derivative 1 (trivalent ligand 1)



To a solution of N-Boc-protected trivalent methyl ester 10 (15 mg, 6 µmol) in water (1 mL) was added 1 M sodium hydroxide (NaOH, 10 µL) and the reaction was allowed to stand at room temperature for 12 h. The solution was neutralized with Amberlite IR-120 ( $H^{+}$ ), filtered and freeze dried to give a white solid which was treated with 80 % aqueous trifluoroacetic acid (100 µL) for 15 min. The solvent was evaporated in vacuo and s the residue was taken up in 0.5 M sodium bicarbonate/ethanol 1.5:1 (2 mL). To this solution was added  $\gamma$ thiobutyrolactone (125 µL, 1.4 mmol) and dithiothreitol (DTT) (0.1 g, 0.7 mmol) and the reaction was heated at 50 °C for 2 h. The solvents were removed in vacuo, the resulting residue was taken up in water (0.5 mL), filtered through a 0.2 µm syringe filter and chromatographed on TSK gel (water, 0.5 mL/ min) to yield the product **1** as a white solid (6 mg, 41 % over 3 steps). <sup>1</sup>H NMR ( $D_2O$ ):  $\delta$  = 7.85 (s, 3H, CHNN), 4.48 (bs, 6H, HI), <sup>10</sup> 4.33 (t, 6H, Hi,  ${}^{3}J_{hi}$  = 6.9 Hz), 4.26 (d, 3H, H1',  ${}^{3}J_{1',2'}$  = 7.9 Hz), 3.93 (d, 3H, H4',  ${}^{3}J_{3',4'}$  = 3.4 Hz ), 3.39 (dd, 3H, H2',  ${}^{3}J_{1',2'} = 7.9 \text{ Hz}, {}^{3}J_{2',3'} = 10.0 \text{ Hz}), 3.18 (t, 6H, Hc, {}^{3}J_{b,c} = 6.6 \text{ Hz}), 2.87 (dd, 6H, H9), 2.73 (dd, 3H, H3_{eq}, {}^{2}J_{3e,3a} = 12.2$ Hz,  ${}^{3}J_{3e,4} = 4.5$  Hz), 2.60, 2.38, (2 x t, 4H, Hq-Ht or Hv-x), 2.14 (t, 6H, He,  ${}^{3}J_{e,f} = 7.6$  Hz), 1.85 - 1.77 (m, 6H, Hh), 1.71 (m, 6H, Hb), 1.71 - 1.65 (m, 3H, H3<sub>ax</sub>), 1.52 (m, 6H, Hf), 1.22 - 1.13 (m, 6H, Hg); <sup>13</sup>C NMR (D<sub>2</sub>O):  $\delta$  = 125.6 (d, 3C, CHNN), 102.5 (d, 3C, C1'), 70.4(d, 3C, C2'), 68.7 (d, 3C, C4'), 63.2 (t, 3C, Cl), 49.9 (t, 3C, Ci), 36.3 (t, 3C, 15 Cc), 35.3 (t, 3C, Ce), 29.2 (t, 3C, C9), 28.7 (t, 3C, Ch), 28.2 (t, 3C, Cb), 24.8 (t, 3C, Cg), 24.5 (t, 3C, Cf); m/z  $(MALDI^{+})$  2527.78  $[M+K]^{+}$ , calcd for  $C_{100}H_{168}KN_{17}O_{47}S_4 [M+K]^{+} = 2527.85$ .

# Synthesis of PEG ligand 2

20

25

HO O O SH

The S-acetyl derivative of PEG ligand **2**, purchased from Quanta Biodesign, was deacetylated with catalytic methoxide immediately prior to use.

#### Synthesis of monovalent ligand 3



The synthesis of the monovalent Neu5Ac thioglycoside **3** is detailed in Scheme S1.

<sup>5</sup> Scheme S1. Synthesis of monovalent Neu5Ac thioglycoside **3**. a) lodoacetic anhydride, Et<sub>2</sub>O; b) 1. NaOMe/MeOH, -40 °C and 2. Amberlite IR-120 (H<sup>+</sup>), -40 °C; c) **12**, DIPEA, DCM; d) NaOMe/MeOH, r.t.; e) 1 M NaOH, r.t.; f) TFA, DCM, r.t.; and g) γ-thiobutyrolactone, aq. NaHCO<sub>3</sub>/EtOH, DTT, 50 °C.

Part of the aliphatic side chain of the alkyl thioglycoside of Neu5Ac **3** was synthesised starting from *N*-Boc-2,2'-<sup>10</sup> (ethylenedioxy)bis(ethylamine) **11**, prepared from the corresponding diamine following a published procedure.<sup>5</sup> The mono-*N*-Boc-protected diamine **11** was reacted with iodoacetic anhydride to give the corresponding iodoacetamide **12** in 70 % yield. Compound **12** was then used for the formation of the thioglycoside **13**. The synthesis of the monovalent Neu5Ac thioglycoside **3** started with the known  $\alpha$ thioacetate **7**.<sup>3, 6</sup> Subsequent chemoselective de-*S*-acetylation under low temperature Zemplén conditions <sup>15</sup> followed by low temperature quenching by acidic (H<sup>+</sup>) resin generated the corresponding  $\alpha$ -configured Neu5Ac thiol,<sup>3, 7</sup> which was used directly in the next step. Alkylation of the thiol with iodoacetamide **12** in dichloromethane in the presence of Hunig's base<sup>8</sup> gave thioglycoside **13** in 65 % yield over two steps. An alternative diethylamine-promoted de-*S*-acetylation of thioacetate **7** in DMF, as reported by Bennett and co-workers,<sup>9</sup> also afforded the desired thioglycoside **13** when performed in the presence of the iodoacetamide **12**. However, the isolation of **13** was complicated by the presence of a side product with a very similar  $R_f$  on s silica gel. According to the <sup>1</sup>H NMR this impurity was diethylamine coupled with the iodoacetamide **12**. Global deprotection and subsequent reaction of the resulting free amine with  $\gamma$ -thiobutyrolactone in a buffered (pH  $\sim$  9.0) ethanolic solution<sup>10</sup> afforded the sodium salt of **3**, which upon acidification gave the desired monovalent Neu5Ac thioglycoside **3** in 84 % yield over 4 steps. Starting from sialic acid, the desired thioglycoside of Neu5Ac, **3**, was prepared in 10 synthetic steps with a 34 % overall yield.

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#### Synthesis of t-butyl 2-(2-(2-(2-iodoacetamido)ethoxy)ethoxy)ethylcarbamate (12)

Iodoacetic anhydride (555 mg, 1.57 mmol) was dissolved in absolute diethylether (10 mL) and the solution was added to a stirred solution of *t*-butyl 2-(2-(2-aminoethoxy)ethoxy)ethylcarbamate (**11**) (299.6 mg, 1.21 mmol) is in absolute diethylether (10 mL) under nitrogen atmosphere and with exclusion of light. The mixture was allowed to stir for 60 min at room temperature and the formation of product was followed by TLC ( $R_f$  = 0.67, ethyl acetate/methanol 20:1). The volatiles were evaporated *in vacuo* and the residue was subjected to column chromatography on silica gel (first ethyl acetate then ethyl acetate/methanol 20:1) to give pure **12** (353.5 mg, 70 %) as a pale yellow oil. <sup>1</sup>H NMR (400 MHz; CDCl<sub>3</sub>): δ = 8.09 and 6.77 (2 bs, 1H, C-N rotamers <sup>20</sup> NHC(O)CH<sub>2</sub>), 5.55 and 5.01 (2 bs, 1H, C-N rotamers NHC(O)O), 3.66 (s, 2H, H<sub>2</sub>1), 3.57 (bs, 4H, H<sub>2</sub>5, H<sub>2</sub>6), 3.51 (t, 4H, <sup>3</sup>J<sub>3,4</sub> = <sup>3</sup>J<sub>7,8</sub> = 4.4 Hz, H<sub>2</sub>4, H<sub>2</sub>7), 3.43-3.39 (m, 2H, H<sub>2</sub>3), 3.29-3.22 (m, 2H, H<sub>2</sub>8), 1.40 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>CO); <sup>13</sup>C NMR (100 MHz; CDCl<sub>3</sub>): δ = 167.6 (s, 1C, C2), 156.3 (s, 1C, C9), 79.5 (s, 1C, (CH<sub>3</sub>)<sub>3</sub>CO), 70.7, 70.5, 70.4, 69.6 (4 x t, 4C, C4, C5, C6, C7), 40.6 (t, 1C, C8), 40.4 (t, 1C, C3), 28.7 (3 x q, 3C, (CH<sub>3</sub>)<sub>3</sub>CO), -0.3 (t, 1C, C1); m/z (Cl<sup>+</sup>) 434 ([M+NH<sub>4</sub>]<sup>+</sup>, 12%), 417 ([M+H]<sup>+</sup>, 68), 217 (100); HR-MS calcd for C<sub>13</sub>H<sub>26</sub>IN<sub>2</sub>O<sub>5</sub><sup>+</sup> [M+H]<sup>+</sup> 417.0881, found 417.0882.

#### Synthesis of methyl (3',12'-diaza-15',15'-dimethyl-6',9'-dioxa-2',13'-dioxohexadecyl) 5-acetamido-4,7,8,9-

#### tetra-O-acetyl-3,5-dideoxy-2-thio-D-glycero- $\alpha$ -D-galacto-2-nonulopyranosidonate (13)

To a solution of methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2-S-acetyl-3,5-dideoxy-2-thio-D-glycero- $\alpha$ -D*galacto*-2-nonulopyranosidonate (7) (267 mg, 486 μmol) in absolute methanol (12.3 mL) cooled to -40 °C was s added a 1 M methanolic solution of MeONa (437μL, 437 μmol) under nitrogen atmosphere. The mixture was stirred for 30 min at -40 °C and then neutralised with Amberlite IR-120 H<sup>+</sup> resin with stirring for 15 min at -40 °C. The mixture was filtered and the filtrate was evaporated in vacuo to give the crude NeuAc thiol derived from 7. The residue was dissolved in dichloromethane (15 mL) and the resulting solution was added to a solution of the iodoacetamide 12 (240 mg, 577  $\mu$ mol) and N,N-diisopropylethylamine (254  $\mu$ L, 1457  $\mu$ mol) in <sup>10</sup> dichloromethane (15 mL). The mixture was stirred for 12 h at room temperature and the reaction was monitored by TLC. The mixture was diluted with dichloromethane (30 mL) and washed with water (10 mL). The organic layer was dried over MgSO<sub>4</sub>, filtered, evaporated and the residue was subjected to column chromatography on silica gel (7 g, ethyl acetate/hexane 1:10, then pure ethyl acetate, then pure dichloromethane to elute unreacted iodoacetamide 12 followed by stepwise gradient of dichloromethane/  $_{15}$  methanol 40:1, 30:1 and 20:1) to give pure **13** (251.2 mg, 65 % over 2 steps). R<sub>f</sub> = 0.58 (chloroform/methanol 10:1);  $[\alpha]_{D}^{25}$  +8.8 (c = 0.49, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz; CDCl<sub>3</sub>):  $\delta$  = 6.95 and 6.79 (bs, 1H, C-N rotamers NHC(0)CH<sub>2</sub>), 5.39 (bs, 1H, H7), 5.31-5.22 (m, 2H, H8, CH<sub>3</sub>C(0)NH), 4.99 (bs, 1H, NHC(0)OtBu), 4.81 (ddd, 1H,  ${}^{3}J_{4.5} = {}^{3}J_{4.3a} = 10.8$  Hz,  ${}^{3}J_{4.3e} = 4.2$  Hz, H4), 4.21 (dd, 1H,  ${}^{2}J_{9a.9b} = 12.4$  Hz,  ${}^{3}J_{9a.8} = 2.4$  Hz, H9a), 4.01-3.96 (m, 2H, H5, H9b), 3.76-3.70 (m, 1H, H6), 3.70 (s, 3H, COOCH<sub>3</sub>), 3.52-3.46 (m, 10H, H<sub>2</sub>4', H<sub>2</sub>5', H<sub>2</sub>6', H<sub>2</sub>7', H1'a, H3'a), <sup>20</sup> 3.40-3.33 (m, 1H, H3'b), 3.26-3.22 (m, 3H, H1'b, H<sub>2</sub>8'), 2.68 (dd, 1H, <sup>2</sup>J<sub>3e,3a</sub> = 12.8 Hz, <sup>3</sup>J<sub>3e,4</sub> = 4.2 Hz, H3e), 2.14, 2.09, 1.99, 1.97 (4 x s, 12 H, 4 x CH<sub>3</sub>C(O)O), 1.92-1.88 (m, 1H, H3a), 1.81 (s, 3H, CH<sub>3</sub>C(O)NH), 1.38 (s, 9H,  $(CH_3)_3CO$ ; <sup>13</sup>C NMR (100 MHz; CDCl<sub>3</sub>):  $\delta$  = 170.2, 169.9, 169.7, 169.2, 169.1, 167.6, 167.3 (7 x s, 8C, C2', COOCH<sub>3</sub>, 4 x CH<sub>3</sub>C(O)O, CH<sub>3</sub>C(O)NH), 155.0 (s, 1C, C9'), 81.1 (s, 1C, C2), 78.2 (s, 1C, (CH<sub>3</sub>)<sub>3</sub>CO), 72.9 (d, 1C, C6), 69.2, 68.7 (2 x t, 4C, C4', C5', C6', C7'), 68.4 (d, 1C, C4), 66.9 (d, 1C, C7), 65.9 (d, 1C, C8), 61.3 (t, 1C, C9), 52.2 (q, 25 1C, COOCH<sub>3</sub>), 48.2 (d, 1C, C5), 39.3 (t, 1C, C8'), 38.6 (t, 1C, C3'), 36.4 (t, 1C, C3), 31.5 (t, 1C, C1'), 27.4 (q, 3C,

 $(CH_3)_3CO)$ , 22.2 (q, 1C,  $CH_3C(O)NH$ ), 20.4, 19.9, 19.8 (3 x q, 4C, 4 x  $CH_3C(O)O$ ); m/z (ESI<sup>+</sup>) 818 ([M+Na]<sup>+</sup>, 100%), 796 ([M+H]<sup>+</sup>, 71), 696 (5); HR-MS calcd for  $C_{33}H_{54}N_3O_{17}S^+$  [M+H]<sup>+</sup> 796.3168, found 796.3168.

# Synthesis of 3',12'-diaza-6',9'-dioxa-2',13'-dioxo-17'-thiaheptadecyl 5-acetamido-3,5-dideoxy-2-thio-Ds glycero- $\alpha$ -D-galacto-2-nonulopyranosidonic acid (monovalent liqand 3)

Thioglycoside 13 (178 mg, 224 µmol) was dissolved in absolute methanol (8.3 mL) and treated with 0.5 M NaOMe in methanol (966  $\mu$ L, 484  $\mu$ mol). After stirring for 1 h at room temperature (TLC monitoring, product R<sub>f</sub> = 0.08 (chloroform/methanol 10:1)), the solvent was removed in vacuo and then 1 M NaOH (1.72 mL, 1.72 mmol) was added to the residue. After stirring for 1.5 h, a 1:1 mixture of glacial acetic acid/ water (1.6 mL) was 10 added and then the volatiles were evaporated in vacuo. The residue was dissolved in dichloromethane (4.2 mL) and treated with trifluoroacetic acid (4.2 mL) at room temperature. After 3 h, the reaction mixture was concentrated to give the crude TFA salt of the amine derived from 13 that was used without further purification. The crude amine ( $\sim$  115 mg,  $\sim$  224  $\mu$ mol) was dissolved in a mixture of 0.5 M sodium bicarbonate (10.3 mL, pH  $\sim$  9.0) and ethanol (8.2 mL) and then dithiothreitol (173 mg, 1.12 mmol) and  $\gamma$ -thiobutyrolactone 15 (194 μL, 2.24 mmol) were added. The mixture was stirred overnight at 50 °C under nitrogen atmosphere. Using 1 M HCl the pH of the mixture was adjusted to 6.0. The mixture was concentrated in vacuo at 37 °C and the residue was freeze-dried. The residue was taken into methanol and filtered. The filtrate was evaporated and the residue was purified using column chromatography on silica gel (6 g, sample applied in methanol, chloroform/methanol 2:1, then chloroform/methanol/water 55:45:10) to give pure 3 (116 mg, 84 % over 4  $_{20}$  steps). The compound was stored neat at -20  $^{\circ}$ C under nitrogen atmosphere. R<sub>f</sub> = 0.58 (ethyl acetate/methanol/acetic acid/water 3:3:3:1); <sup>1</sup>H NMR (400 MHz;  $D_2O$ ):  $\delta = 3.85-3.79$  (m, 2H, H5, H9a), 3.71-3.61 (m, 11H, H4, H8, H9b, H<sub>2</sub>4', H<sub>2</sub>5', H<sub>2</sub>6', H<sub>2</sub>7'), 3.59-3.53 (m, 2H, H6, H7), 3.49-3.41 (m, 6H, H<sub>2</sub>1', H<sub>2</sub>3', H<sub>2</sub>8'), 2.79 (dd, 1H, <sup>2</sup>J<sub>3e.3a</sub> = 12.4 Hz, <sup>3</sup>J<sub>3e.4</sub> = 4.8 Hz, H3e), 2.56-2.52 (m, 2H, H<sub>2</sub>12'), 2.39-34 (m, 2H, H<sub>2</sub>10'), 2.01 (s, 3H, CH<sub>3</sub>C(O)NH), 1.92-1.76 (m, 3H, H3a, H<sub>2</sub>11'); <sup>13</sup>C NMR (100 MHz; CDCl<sub>3</sub>):  $\delta$  = 176.8, 175.6, 174.2, 172.5 (4 x s, 4C, 25 CH<sub>3</sub>C(O)NH, C1, C2', C9'), 86.1 (s, 1C, C2), 75.5 (d, 1C, C6), 72.4 (d, 1C, C8), 70.1, 70.0, 69.4, 69.3 (4 x t, 4C, C4',

C5', C6', C7'), 69.1 (d, 1C, C4), 68.7 (d, 1C, C7), 63.2 (t, 1C, C9), 52.2 (d, 1C, C5), 41.1 (t, 1C, C3), 39.9, 39.5 (2 x t, 2C, C3', C8'), 35.0 (t, 1C, C10'), 33.8 (t, 1C, C1'), 30.1 (t, 1C, C11'), 23.7 (t, 1C, C12'), 22.6 (q, 1C,  $CH_3C(O)NH$ ); m/z (ESI<sup>+</sup>) 660 ([M-H + 2Na]<sup>+</sup>, 100%), 638 ([M + Na]<sup>+</sup>, 21); m/z (ESI<sup>-</sup>) 614 ([M-H]<sup>+</sup>, 100%); HR-MS calcd for  $C_{23}H_{41}N_3NaO_{12}S_2^+$  [M + Na]<sup>+</sup> 638.2024, found 638.2028.

Synthesis of citrate coated gold nanoparticles, functionalised gold nanoparticles and virus detection

#### Reagents

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All reagents were of analytical grade, used as received and purchased from Sigma-Aldrich (UK) unless <sup>10</sup> specified. Millex GP syringe driven filter units (0.22 μm) and Amicon Ultra-4 centrifugal filter units (10,000 MW cut-off) were purchased from Millipore Corporation, USA. Inactivated viruses: X31 and RG14 and allantoic fluid (AF) virus X31 were provided by the WHO Collaborating Centre for Reference and Research on Influenza, Division of Virology, National Institute of Medical Research, UK.

#### 15 Instrumental methods

UV-Visible spectra were recorded using a Perkin Elmer Lambda 25 UV-Vis spectrometer at room temperature. Quartz cuvettes with a 1 cm path length were used. Transmission electron microscopy (TEM) images were obtained using a Jeol 2000EX transmission electron microscope, operating at 200 KV, by depositing samples on holey carbon film 300 mesh copper grids from Agar Scientific, UK.

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#### Synthesis of citrate stabilised gold nanoparticles

Water soluble gold nanoparticles were prepared *via* the citrate reduction method reported by Enüstün and Turkevich.<sup>11</sup> Briefly, aqueous solutions of HAuCl<sub>4</sub>·3H<sub>2</sub>O (12.5 mg, 32 µmol, in 100 mL) and sodium citrate tribasic dihydrate (50 mg, 168 µmol, in 50 mL) were prepared and heated to 60 °C. The sodium citrate solution <sup>25</sup> was rapidly added to the gold solution while stirring vigorously. The temperature was increased to 85 °C and

the solution was stirred for 2.5 h. A clear red gold nanoparticle solution was obtained that was cooled to room temperature and filtered through a Miller GP syringe driven filter unit (0.22  $\mu$ m). The particle concentration in the citrate stabilised gold nanoparticles solution was approximately 3 nM.

# s Synthesis of gold nanoparticles functionalised with trivalent ligand 1 and PEG ligand 2 (trivalent ligand

## 1:PEG functionalised gold nanoparticles)

Gold nanoparticles were functionalised with varying ratios of trivalent ligand **1** and PEG ligand **2**. Varying molar ratios of trivalent ligand **1** and PEG based ligand **2** (**Table S1**) were added to aliquots of freshly prepared gold nanoparticles (17 mL) and stirred for 60 h at room temperature to ensure self-assembly of the ligands onto the gold surface. The nanoparticle solution was centrifuged using Amicon Ultra-4 centrifugal filter units (10,000 MW cut-off) in a Sorvall Legend RT centrifuge for 10 min at 4,000x*g* to remove the excess trivalent ligand **1** and PEG ligand **2**. The centrifuged nanoparticles were resuspended in Tris buffer solution (17 mL, 10 mM, pH 7.6). The centrifugation process was repeated a total of two times.

% Trivalent ligand 1	Quantity of trivalent ligand 1 added (nmol)	% PEG ligand 2	Quantity of PEG ligand 2 added (nmol)
50	15.1	50	15.1
25	7.6	75	22.6
10	3.0	90	27.2
5	1.5	95	28.7
2	0.6	98	29.6

s Table S1. Molar ratios of trivalent ligand 1 and PEG ligand 2 added to the gold nanopa	articles.
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# Synthesis of gold nanoparticles functionalised with monovalent ligand 3 and PEG ligand 2 (monovalent

#### ligand 3:PEG functionalised gold nanoparticles)

Gold nanoparticles were functionalised with varying ratios of monovalent ligand **3** and PEG ligand **2**. Varying molar ratios of monovalent ligand **3** and PEG based ligand **2** (**Table S2**) were added to aliquots of freshly <sup>5</sup> prepared gold nanoparticles (17 mL) and stirred for 60 h at room temperature to ensure self-assembly of the ligands onto the gold surface. Excess ligands were removed as previously described for gold nanoparticles functionalised with trivalent ligand **1** and PEG ligand **2**.

Table S2. Molar ratios of monov	alent ligand <b>3</b> and l	PEG ligand <b>2</b> added t	o the gold nanoparticles.
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% Monovalent ligand 3	Quantity of monovalent ligand 3 added (nmol)	% PEG ligand 2	Quantity of PEG ligand 2 added (nmol)
50	15.1	50	15.1
25	7.6	75	22.6
10	3.0	90	27.2
5	1.5	95	28.7
2	0.6	98	29.6

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### Synthesis of gold nanoparticles functionalised with PEG ligand 2 (PEG functionalised gold nanoparticles)

PEG ligand **2** (30.2 nmol) was added to a freshly prepared citrate stabilised gold nanoparticles solution (17 mL). The solution was stirred for 60 h at room temperature to ensure self-assembly of the ligand onto the gold surface. Excess ligands were removed as previously described for gold nanoparticles functionalised with 15 trivalent ligand **1** and PEG ligand **2**.

#### Optimised functionalisation of gold nanoparticles for the detection of human influenza virus

X31 virus (H3N2) (2.55  $\mu$ g/mL) was added to a sample of each of the synthesized gold nanoparticles including: citrate coated gold nanoparticles; trivalent ligand **1**:PEG functionalised gold nanoparticles (50:50, 25:75, 10:90,

5:95 and 2:98); and monovalent ligand **3**:PEG functionalised gold nanoparticles (50:50, 25:75, 10:90, 5:95 and 2:98). The samples were stirred at room temperature and the UV-Vis spectrum was recorded before addition of the virus and 0, 15, 30, 60 and 240 min after addition of the virus.

# s Colorimetric detection of X31 virus using trivalent ligand 1:PEG (25:75), monovalent ligand 3:PEG (25:75) and

### PEG functionalised gold nanoparticles

Increasing concentrations of X31 virus (from 0 to 3  $\mu$ g/mL) were added to a sample of the functionalised gold nanoparticles. The UV-Vis spectrum of each functionalised gold nanoparticle solution was measured before addition of the virus and 30 min after addition of the corresponding virus concentration.

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# Colorimetric detection of influenza X31 virus from allantoic fluid using trivalent ligand 1:PEG (25:75) functionalised gold nanoparticles

Increasing volumes of X31 virus from allantoic fluid (AF) (from 0 to 43.1 μL) were added to a sample of trivalent ligand **1**:PEG (25:75) functionalised gold nanoparticles (1000 μL). The UV-Vis spectrum of the sample <sup>15</sup> was measured before addition of the AF X31 virus and 30 min after addition of the corresponding volume. As control experiment of effect of dilution, the same measurements were repeated although adding increasing volumes of Tris buffer (from 0 to 47.1 μL) to a sample of trivalent ligand **1**:PEG (25:75) functionalised gold nanoparticles (1000 μL).

# <sup>20</sup> Colorimetric detection of avian RG14 virus using trivalent ligand 1:PEG (25:75) functionalised gold nanoparticles

Increasing concentrations of avian RG14 virus (H5N1) (from 0 to 6.8  $\mu$ g/mL) were added to a sample of trivalent ligand **1**:PEG (25:75) functionalised gold nanoparticles. The UV-Vis spectrum of the sample was measured before addition of the virus and 30 min after addition of each virus concentration.

# Discrimination between human X31 (H3N2) and avian RG14 (H5N1) influenza virus using trivalent ligand

# 1:PEG (25:75) functionalised gold nanoparticles

Each virus (6.8  $\mu$ g/mL) was added to a sample of trivalent ligand **1**:PEG (25:75) functionalised gold nanoparticles. The UV-Vis spectrum of each sample was measured after stirring the samples for 6 days at room

s temperature.

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# Supporting results



**Fig. S1.** Schematic representation of monovalent ligand **3**:PEG functionalised gold nanoparticles: gold s nanoparticles functionalised with monovalent ligand **3** (black) and PEG ligand **2** (blue).

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**Fig. S2. a)** Transmission electron micrograph (TEM) of a sample of trivalent ligand **1**:PEG (50:50) functionalised gold nanoparticles (the scale bar represents 100 nm) and **b)** size distribution of the trivalent ligand **1**:PEG (50:50) functionalised gold nanoparticles with a median value of  $16.4 \pm 1.6$  nm (n = 116 nm)



Fig. S3. Optimised trivalent ligand 1:PEG functionalisation ratio. UV-Vis spectra of different samples of functionalised gold nanoparticles before (black) and 0 min (red), 15 min (green), 30 min (blue), 60 min (cyan) and 240 min (magenta) after addition of virus X31 (2.55 μg/mL). The different stabilised gold nanoparticles are:
a) citrate coated gold nanoparticles and b) – f) trivalent ligand 1:PEG functionalised gold nanoparticles with trivalent ligand 1:PEG ratios: b) 2:98, c) 5:95, d) 10:90, e) 25:75 and f) 50:50.



**Fig. S4.** Optimised monovalent ligand **3**:PEG functionalisation ratio. UV-Vis spectra of different samples of functionalised gold nanoparticles before (black) and 0 min (red), 15 min (green), 30 min (blue), 60 min (cyan) and 240 min (magenta) after addition of virus X31 (2.55 μg/mL). The different functionalised gold s nanoparticles are monovalent ligand **3**:PEG functionalised gold nanoparticles with monovalent ligand **3**:PEG ratios: **a**) 2:98, **b**) 5:95, **c**) 10:90, **d**) 25:75 and **e**) 50:50.



**Fig. S5.** Comparison of trivalent ligand **1**:PEG (25:75), monovalent ligand **3**:PEG (25:75) and PEG functionalised gold nanoparticles. **a)** Extinction intensity at 525 nm of trivalent ligand **1**:PEG (25:75) (black), monovalent ligand **3**:PEG (25:75) (red) and PEG (blue) functionalised gold nanoparticles with varying concentrations of virus X31; and **b)** cuvettes containing trivalent ligand **1**:PEG (25:75) functionalised gold nanoparticles with different concentrations of virus X31. The extinction intensity was measured 30 min after addition of the virus.

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**Fig. S6.** Schematic representation of PEG functionalised gold nanoparticles: gold nanoparticles functionalised with PEG ligand **2**.

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**Fig. S7.** Dilution effect control. **a)** Variation of the UV-Vis spectrum of trivalent ligand **1**:PEG (25:75) functionalised gold nanoparticles with varying volumes of Tris buffer and **b)** extinction intensity at 525 nm of trivalent ligand **1**:PEG (25:75) functionalised gold nanoparticles with varying volumes of X31 virus from <sup>3</sup> allantoic fluid (black) and with varying volumes of Tris buffer (red). The extinction intensity was measured 30 min after addition of the virus.



**Fig. S8.** UV-Vis spectra of: **a)** trivalent ligand **1**:PEG (25:75) functionalised gold nanoparticles following addition of increasing concentrations (from 0 to 6.8 μg/mL) of RG14 (H5N1) (the UV-Vis spectrum was measured 30 min after addition of each virus concentration); and **b)** trivalent ligand **1**:PEG (25:75) functionalised gold <sup>5</sup> nanoparticles before (black) and 6 days after addition of the avian RG14 (H5N1) (red) and human X31 (H3N2) (green) influenza viruses (6.8 μg/mL).

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