Magnetic glyconanoparticles as a versatile platform for selective immunolabelling and imaging of cells

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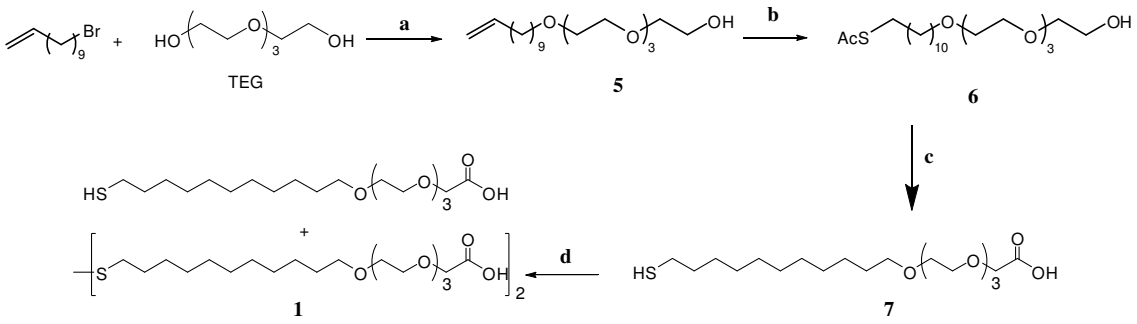
Synthesis of the amphiphilic linker 22-mercapto [2,5,8,11-tetraoxadocosan]-1-oic acid (SH-C_{11}-TEG-CO_2H) and its oxidized product 23,23'-Dithio bis [2,5,8,11- tetraoxadocosan]-1-oic acid .



Scheme1. Reagents and conditions. a) NaOH (50%), 100ºC, 16h. b) AcSH, AIBN, THFdry, reflux, 3h . C) (1) Jones reagent, acetone, 30 min. D) (i) MeONa, MeOH, 15h (ii) amberlite IR-120.

Undecen-1-en-11-yl tetra(ethylene glycol) (5).

A mixture of tetraethylenglycol (TEG) (35.6 g, 183.5 mmol) and NaOH (50%) (2.7 ml, 43.7 mmol) were stirred for 30 min at 100 ºC. The reaction was cooled to room temperature and 11-Br-undecene (10.2 g, 43.7 mmol) was added. The reaction was heated at 100ºC and left overnight under stirring. The mixture was diluted with CH_2Cl_2 (20 ml), washed with water (40 ml) and extracted with hexane (3 x 40 ml). The hexane fractions were collected, dried over Na_2SO_4, filtered and the solvent evaporated at reduced pressure. The

crude product (16 g) was purified by flash column chromatography on silica gel (EtOAc) to give **5** (10.46 g, 69%) as a yellow oil.

Rf = 0.31 (EtOAc). **^{1}H RMN** ($CDCl_3$, 500 MHz) 1.26-1.36 (m, 12H); 1.57-1.65 (m, 2H); 2.01 (dd, *J* = 14.4, 6.9 Hz, 2H). 3.12 (bs, 1H), 3.43 (t, *J* = 6.8 Hz, 2H); 3.54-3.67 (m, 14H); 3.68-3.73 (m, 2H); 4.90 (dd, *J* = 10.2, 9.1, 1H), 4.97 (ddd, *J* = 17.1, 3.4, 1.6, 1H); 5.78 (ddt, *J* = 16.9, 10.2, 6.7, 1H).

1-(Thioacetylundec-11-yl)tetra(ethylene glycol) (6).

To a solution of **5** (9.6 g, 27.7 mmol) in dry THF, AcSH (10.49 g, 138 mmol) and AIBN (cat.) were added. The mixture was stirred under reflux for 3 hours. The reaction was diluted with 30 ml of EtOAc and pH neutralised with a saturated solution of $NaHCO_3$. The organic phase was washed with brine, dried over Na_2SO_4 and the solvent removed at reduced pressure. The residue was purified by flash column chromatografy on silica gel (EtOAc:hexane 9:1 to EtOAc) to obtain **6** as a colourless oil (7.8 g, 67%). **Rf** = 0.28 (EtOAc). **^{1}H RMN** ($CDCl_3$, 500 MHz) 1.25-1.36 (m, 14H); 1.53-1.67 (m, 4H); 2.12 (s, 3H); 2.65 (t, *J* = 7.3 Hz, 2H); 3.26 (t, *J* = 6.8 Hz, 2H); 3.55-3.71 (m, 16H).

22-(Thioacetyl)-2,5,8,11-tetraoxadocosan-1-oic acid (7).

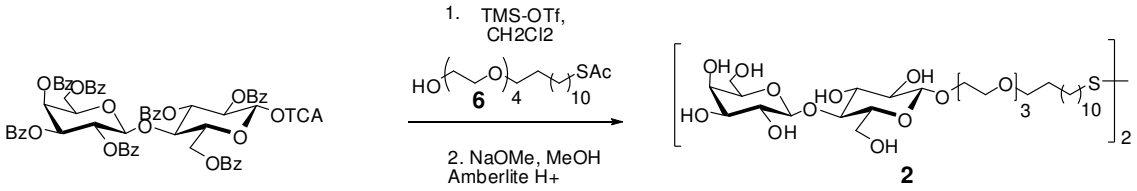
[1-(Methylcarbonyl)thio]undec-11-yl]tetra(ethylene glycol) **6** (1 g, 2.37 mmol) was dissolved in 5 mL acetone and Jones reagent was added drop by drop until red colour persisted. Then, the reaction was allowed to continue for 30 min and it was subsequently stopped with 2-propanol, diluted with ethyl acetate, washed twice with water, and dried in the rotatory evaporator. The purification was performed by column chromatography (CH_2Cl_2: MeOH 19:1) and product **7** was obtained as a syrup (69% yield). **^{1}H NMR** (500 MHz, $CDCl_3$), δ 1.12-1.36 (m, 14H), 1.43-1.66 (m, 4H), 2.30 (s, 3H), 2.84 (t, *J* = 7.3 Hz, 2H), 3.40 (t, *J* = 6.8 Hz, 2H), 3.49-3.82 (m, 12H), 4.00 (s, 2H).

22-Mercapto [2,5,8,11- tetraoxadocosan]-1-oic acid and 23,23'-Dithio bis [2,5,8,11- tetraoxadocosan]-1-oic acid (1).

The protected product **7** (0.712 g, 1.8 mmol) was dissolved in 34 mL methanol and sodium methoxide (0.088 g, 1.6 mmol) was added. The solution was stirred for 6 hours and then the pH was neutralized with Amberlite IR120 H^+. The

reaction was filtered, dried in the rotary evaporator and washed several times with diethyl ether. The final product **1** was obtained as a mixture of thiol and disulfide (39:61). (82% yield). ^{1}H NMR (500 MHz, $CDCl_3$), δ 1.30-1.70 (m, 18H), 2.50 (t, *J* = 7.0 Hz, 2H thiol), 2.69 (t, *J* = 7.0 Hz, 2H disulfide) 3.50 (t, *J* = 14.0 Hz, 2H), 3.60-3.80 (m, 12H), 4.15 (s, 2H disulfide), 4.19 (s, 2H thiol).: **HR-MS** (pos., ionization phase MeOH) m/z: 416.2213 $[M + 2Na]^{+2}$ ($C_{38}H_{72}O_{12}S_2Na_2$ requires 416.2209).

Synthesis of lactose neoglycoconjugate 2.



Scheme 2. Synthesis of neoglyccoconjugate **2**.

11,11-Dithiobis[3,6,9,12-tetraoxaundecanyl-(*ß*-D-galactopyranosyl)(1→4)-ß-D-glucopyranoside (2).

The glycosidation reaction with trichloroacetimidate as donor was carried out as previously described. [S1] TMSOTf (18 μL, 0.072 mmol) was added to a solution of trichloroacetimidate derivate of lactose (0.433 g, 0.36 mmol) and the acceptor **6** (0.23 g, 0.54 mmol) of dry CH_2Cl_2 (8.7 mL) at 0º C. The mixture was stirred at this temperature and monitorized by TLC (hexane:EtOAc 1:1). After 3h 30min, the pH was adjusted to 7 with addition of Et_3N (50 μL), the solvent was removed under reduced pressure and the residue purified by flash column (hexane:EtOAc 1:1) affording the glycosilated product as a syrup. Yield 30%. **^{1}H NMR** (500 MHz, $CDCl_3$): δ 1.15-1.60 (m, 16H); 2.24 (s, 3H); 2.78 (t, *J* = 7.5 Hz, 2H); 3.20-3.90 (m, 18H); 4.17 (t, *J* = 9.5 Hz, 1H); 4.40 (dd, *J* = 12.0, 4.0 Hz, 1H); 4.51 (dd, *J* = 12.0, 1.5 Hz, 1H); 4.73 (d, *J* = 8 Hz, 1H); 4.79 (d, *J* = 8 Hz, 1H); 5.28 (dd, *J* = 10.5, 3.5 Hz, 1H); 5.37 (dd, *J* = 10.0, 8.0 Hz, 1H); 5.64 (m, 2H); 5.72 (t, *J* = 9.5, 1H); 7.00-8.00 ppm (m, 35H). The previous glycosylated product (150 mg, 0,0283 mmol) was treated with 1 N

methanolic solution of NaOMe (2 mL). The mixture was stirred for 3 hours under argon atmosphere and left for 24 hours without the argon atmosphere to complete the oxidation of the thiol group to the corresponding disulfide. After 24 h, TLC (CH_2Cl_2: methanol 8:3) showed the appearance of a new product (Rf_0.7). The reaction was neutralised with Amberlist IR-120 H^+, filtered and evaporated to dryness. Yield 90% **^{1}H NMR** (500 MHz, D_2O): δ 1.30-1.50 (m, 12H); 1.57 (m, 2H); 1.67 (q, *J* = 7.5 Hz, 2H); 2.48 (t, *J* = 7.5 Hz, 2H, thiol); 2.68 (t, *J* = 7.5 Hz, 2H, disulfide); 3.25-4.00 (m, 30H); 4.33, 4.35 (dd, *J* = 9.5, 8.0 Hz, 1H); **^{13}C NMR** (500 MHz, D_2O): δ 21.5, 23.2, 26.0, 26.2, 28.5, 28.7, 29.3, 29.6, 29.8, 30.0, 30.9, 35.5, 39.0, 55.2, 60.2, 61.0, 68.5, 68.7, 69.8, 70.9, 71.1, 72.6, 72.8, 74.3, 74.8, 75.4, 78.5, 102.2, 103.0 ppm; MALDI-TOF: m/z: 726.3955 [M + 2Na]$^{+2}$ ($C_{62}H_{118}O_{30}S_2Na_2$ requires 726.3472); 727.3987 [M + Na]$^+$ ($C_{31}H_{60}O_{15}SNa$ requires 727.3550).

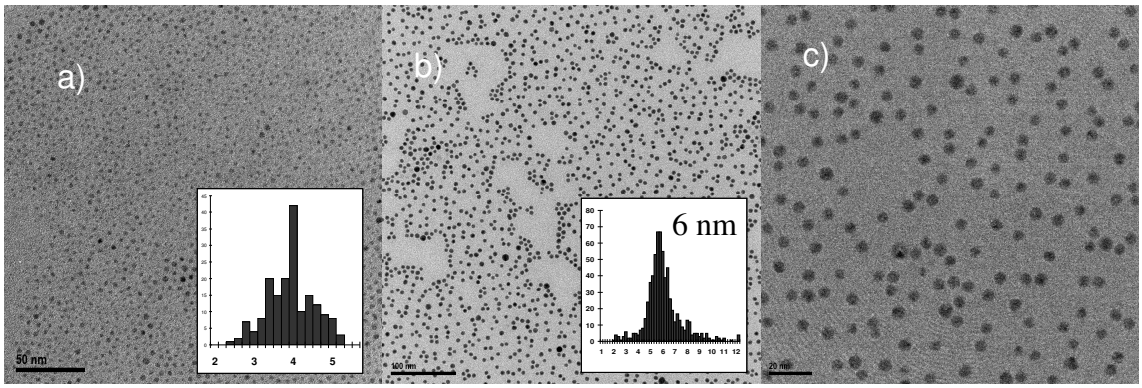


Figure 1S. TEM images of a) Fe_3O_4(oleic) nanoparticles (4 nm) and b) and c) Fe_3O_4@Au(oleic) nanoparticles (6 nm).

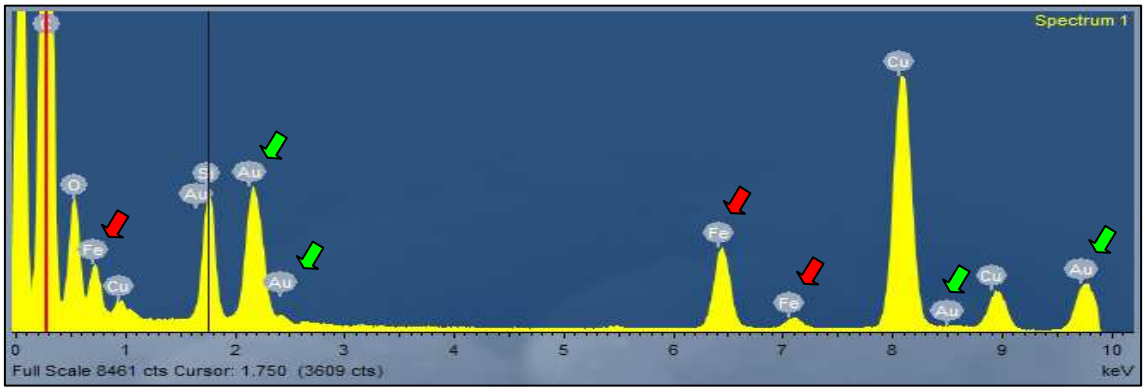


Figure 2S. EDX spectra of Fe3O4@Au (lactose)$_{50}$ (Linker)$_{50}$ nanoparticles: green arrows (Au peaks) and red arrows (Iron peaks).

Table 1S. ICP results: Fe and Au composition of 6.0 nm magnetic glyconanoparticles.

Au	**54 ± 1 (% in weight)**
Fe	**3.6 ± 0.8 (% in weight)**

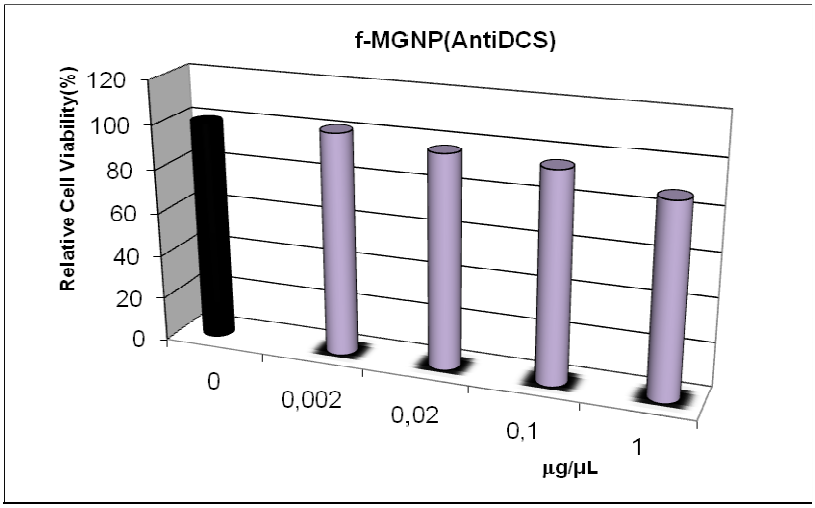


Figure 3S. Viability assay of *anti*-DC-SIGN@protG-MGNPs with Raji+ cells.

Assesment of ligands 1 and 2 on MGNPs.

As general procedure described previously by Templeton *et al.* [S1] was used. NaCN (2 mg) was added to a solution of magnetic glyconanoparticles (1 mg) in water (500 µl) at room temperature. The mixture was stirred until burgundy colour disappeared. After decantation, the supernatant was lyophilized and the resultant residue was dissolved in 600 µl of D_2O and ^1H-NMR spectrum was registered without further purification (Fig. 4S).

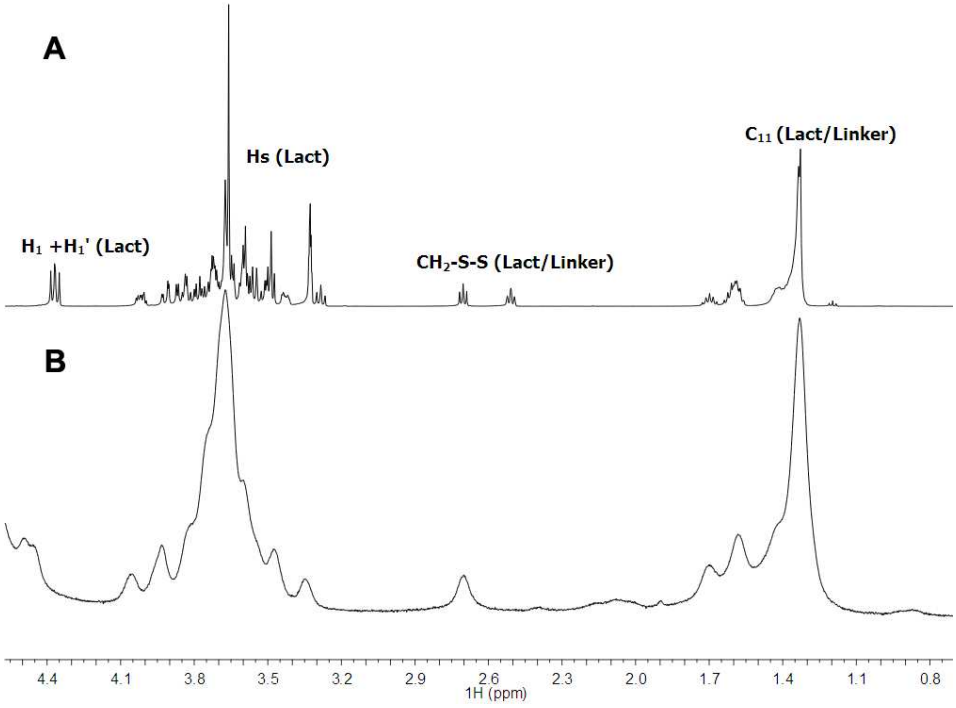


Fig. 4S. (A) ^{1}H-NMR spectrum in D_2O of the ligands mixture (**1** and **2**) in the supernatant after ligand exchange and **(B)** ^{1}H-NMR spectrum in D_2O of released ligands after treating magnetic glyconanoparticle with NaCN. Broad signals were observed probably because of the presence of magnetic traced (Fe) in the NMR solution.

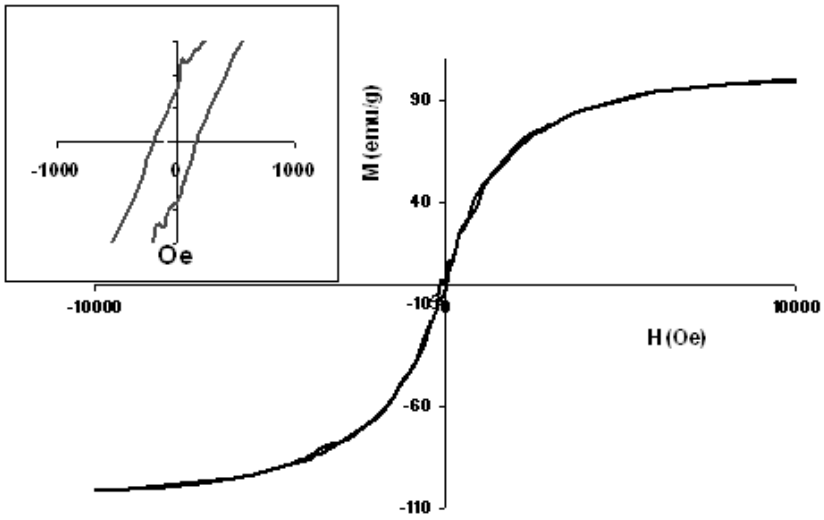


Fig. 5S. Magnetization (emu per gram of Fe) versus applied field curve measured at 300K of the MGNPs. Inserted image: the corresponding hysteresis curve measured at 5K.

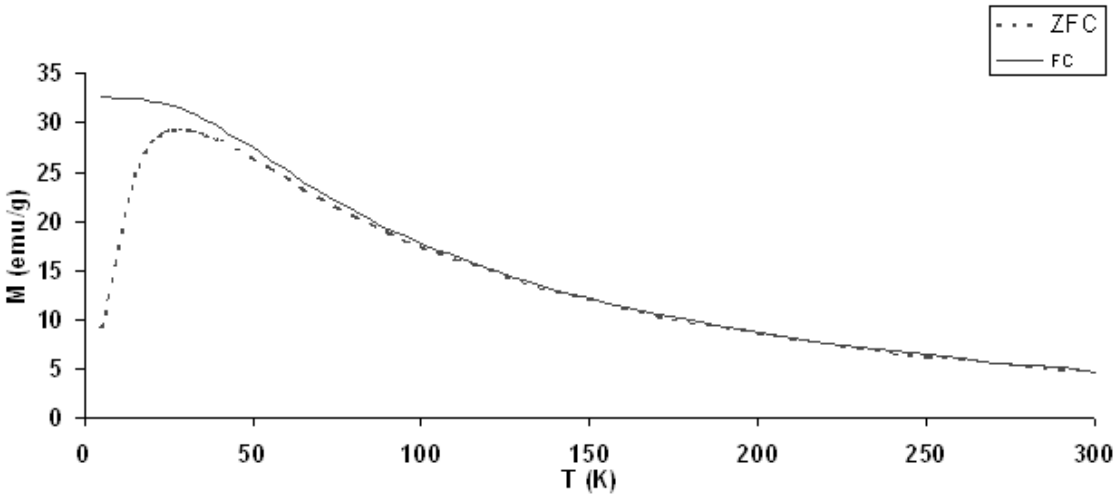


Fig. 6S. Field-cooled (FC) and Zero-field-cooled (ZFC) magnetizations of MGNPs versus temperature measured in a field of 100 *Oe*.

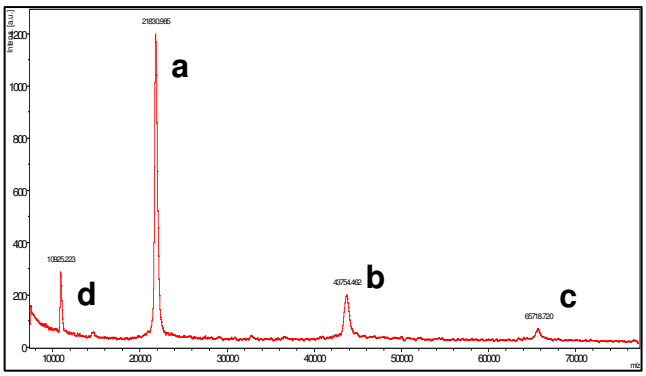


Fig. 7S. MALDI-TOF analysis of protein G-MGNP conjugate: peaks **a** (21830), **b** (10925), **c** (65718) and **d** (43754) can be assigned to protein G; dimmer protein G, trimmer protein G and double charged protein G, respectively.

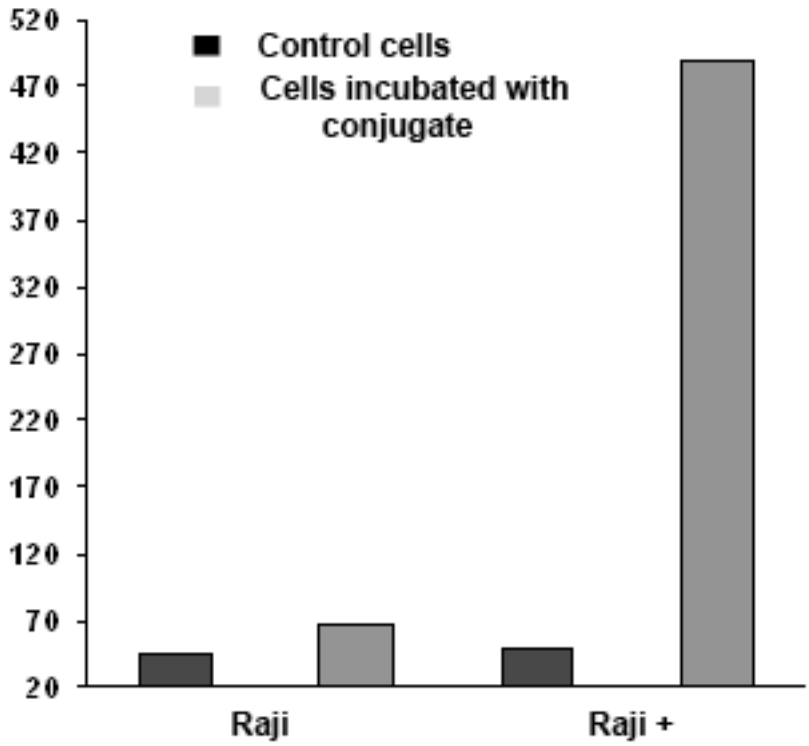


Figure 8S. Quantitative analysis of cellular labeling using flow cytometry. The mean fluorescence intensity of Raji+ cells, containing DC-SIGN receptor and labelled with the conjugate (*anti*-DC-SIGN@protG-MGNP), was much higher than the one from Raji cells.

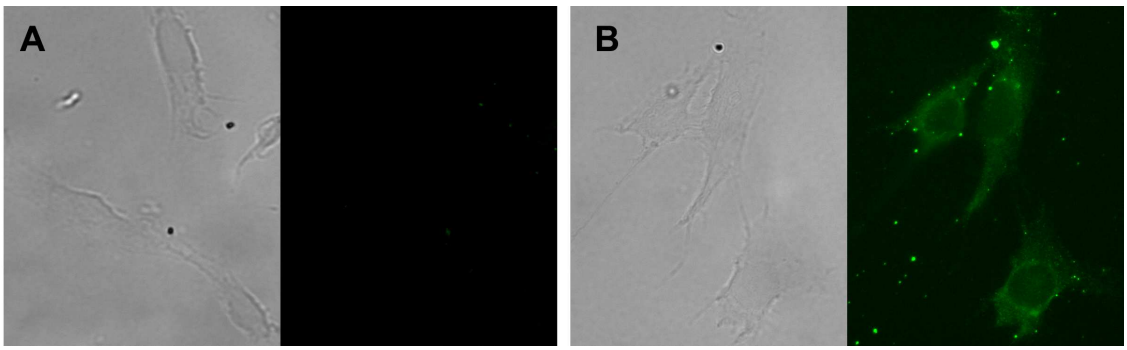


Figure 9S. A, Bright-field and fluorescence images obtained with C33 cells incubated with anti-DC-SIGN@ProtG-MGNPs and Alexa-Fluor488-secondary antibody (green fluorescence). **B**, Bright-field and fluorescence images obtained with C33 cells incubated with anti-CD71@protG-MGNPs.

No green fluorescent signal from the anti-DC-SIGN-MGNPs treated C33 cells is observed in anti-DC-SIGN, while intense green fluorescence is seen using anti-CD71.

References:

[S1]. Templeton, A. C.; Hostetler, M. J.; Kraft, C. T.; Murray, R. W.; *J. Am. Chem. Soc.,* **1998**, *120*, 1906.

[S2]. Ellervik, U.; Magnusson, G.; *Carbohydr. Res.* **1996***, 280*, 251-260.