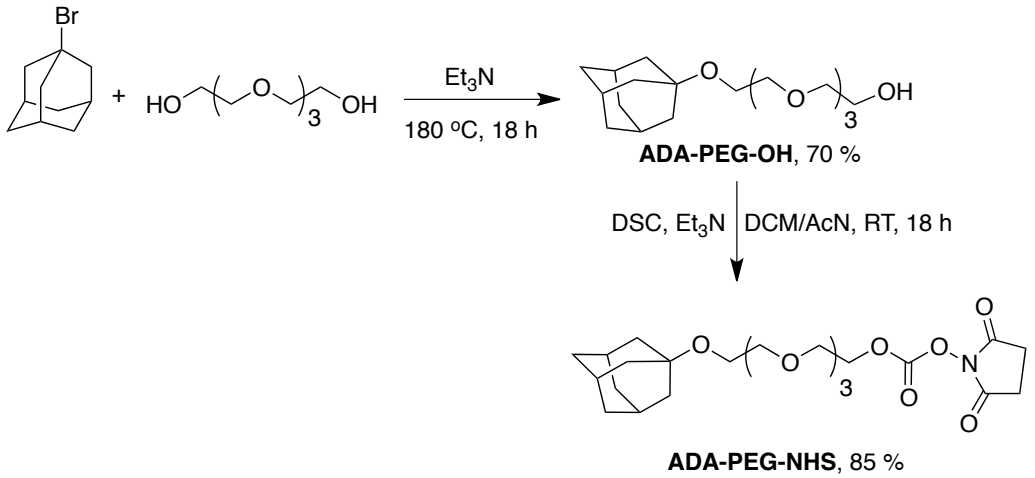
Supporting Information

General

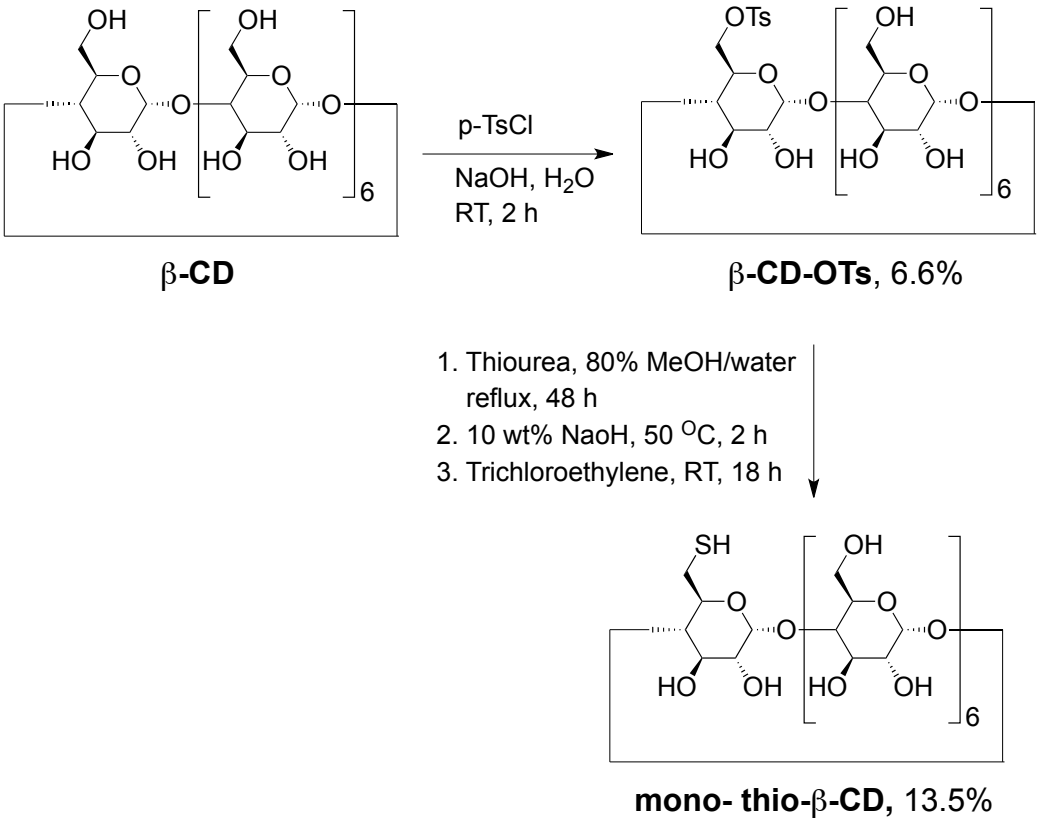
All chemicals and solvents were purchased from commercial suppliers and used as received. Unless otherwise stated, the chemicals and solvents were purchased from Sigma-Aldrich. Mono-thio-β-CD was synthesized by following previously reported procedure.[1,2] Bovine serum albumin (BSA), 10x phosphate buffered saline (PBS) liquid concentrate, ethylene diaminetetraacetic acid (EDTA), sulfosuccinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC), *N*-succinimidyl 3-(2-pyridyldithio)-propionate (SPDP), dithiothreitol (DTT) were purchased from Thermo Fisher Scientific. VivoTag 680 (VT680) was purchased from PerkinElmer. Fluorescein isothiocyanate (FITC), TO-PRO-1 iodide, Amine-functionalized Quantum Dot 655, Amine-functionalized magnetic beads (Dynabeads M-270 Amine) were purchased from Invitrogen. BD Fix Buffer and BD Perm/Wash Buffer were purchased from BD Biosciences. Dulbecco's Modified Eagle Medium (DMEM), Trypsin/EDTA, and Hank's balanced salt solution (HBSS) were purchased from Mediatech. Fetal bovine serum (FBS) was purchased from ISC BioExpress.



Scheme S1. Synthetic scheme for the preparation of ADA-polyethylene glycol succinimidyl ester (ADA-PEG-NHS).[3] DSC: disuccinimidyl carbonate; Et_3N: triethyl amine; DCM: Dichloromethane; AcN: acetonitrile.

Synthesis of ADA-PEG-OH. Tetraethylene glycol (83.3 ml, 0.482 mol) was placed in a round-bottom flask. The flask was heated to 60°C and triethyl amine (10 ml, 0.072 mol) was added. After stirring the solution for 5 minutes at 60°C, bromoadamantane (5.0 g, 0.023 mol) was added. The reaction mixture was then heated to 180°C and stirred for 18 hours. After cooling at room temperature (RT), 100 ml of dichloromethane (DCM) was added to the reaction mixture. The DCM layer was then washed with 2M HCl (4 X 30 ml) and a saturated NaCl solution. The DCM layer was subsequently dried over Na_2SO_4, and evaporated to dryness to afford a pale yellow-brown viscous liquid. This product was used in the subsequent step without further purification. Yield 70%. ^{1}H NMR (400 MHz, $CDCl_3$): 3.71-3.57 (m, 16H), 2.76 (t, ^3J = 6 Hz, 1H), 2.11 (m, 3H), 1.72-1.55 (m, 12H).

Synthesis of ADA-PEG-NHS. ADA-PEG-OH (0.500 g, 1.52 mmol) was dissolved in 10 ml of DCM/acetonitrile (1:1, v/v) before subsequent addition of disuccinimidyl carbonate (1.170 g, 4.57 mmol) and triethyl amine (0.636 ml, 4.57 mmol). The reaction was allowed to stir at RT for 18 hours. After evaporation of the solvent, the crude product was dissolved in excess DCM. The organic component was washed with 1M HCl and saturated NaCl solution. After drying over Na_2SO_4, the organic layer was concentrated under vacuum. The crude product was subsequently purified using a SiO_2 column (eluent: hexane/ethyl acetate (1:1, v/v)). The isolated product was a colorless viscous liquid. Yield 85%. ^{1}H NMR (400 MHz, $CDCl_3$): 4.41 (t, ^3J = 4.6 Hz, 2H), 3.74 (t, ^3J = 4.6 Hz, 2H), 3.62-3.53 (m, 12H), 2.78 (m, 4H), 2.09 (m, 3H), 1.69-1.52 (m, 12H). MS (electrospray ionization mass spectrometry: ESI-MS) calculated: 469.23, found: 470.29 $[M+H]^+$.



Scheme S2. Synthetic scheme for the preparation of **mono-thio-β-CD**.

Synthesis of β-CD-OTs. β-Cyclodextrin (β-CD, 17.22 g, 15 mmol) was initially dissolved in 200 mL 1% NaOH solution at RT. p-toluene sulfonyl chloride (p-TsCl, 2.9 g, 15 mmol) was then dissolved in 11 mL acetonitrile, and added drop wise to the stirring colorless β-CD solution over 25 minutes, in which time a fine white suspension gradually formed. The reaction mixture was stirred for a further 2 hours at RT. The precipitate was subsequently filtered, preserved and the filtrate was acidified to pH 2-3 using 1M HCl. Following acidification, the solution was allowed to form a precipitate by maintaining at 4°C overnight. The second precipitate was then combined with the first precipitate and re-crystalized from water in order to obtain spectroscopically pure product. The product (β-CD-OTs) obtained was a white solid. Yield 6.6%. ^{1}H NMR (400 MHz, DMSO-d_6): 7.71 (d, ^{2}J = 6.8 Hz, 2H), 7.39 (d, ^{2}J = 7.6 Hz, 2H), 5.82-5.61 (m, 14H), 4.80-4.73 (m, 7H), 4.50-4.15 (m, 6H), 3.61-3.18 (m, 40H), 2.39 (s, 3H).

Synthesis of mono-thio-β-CD. β-CD-OTs (1 g, 0.77 mmol) was dissolved in 50 ml 80%

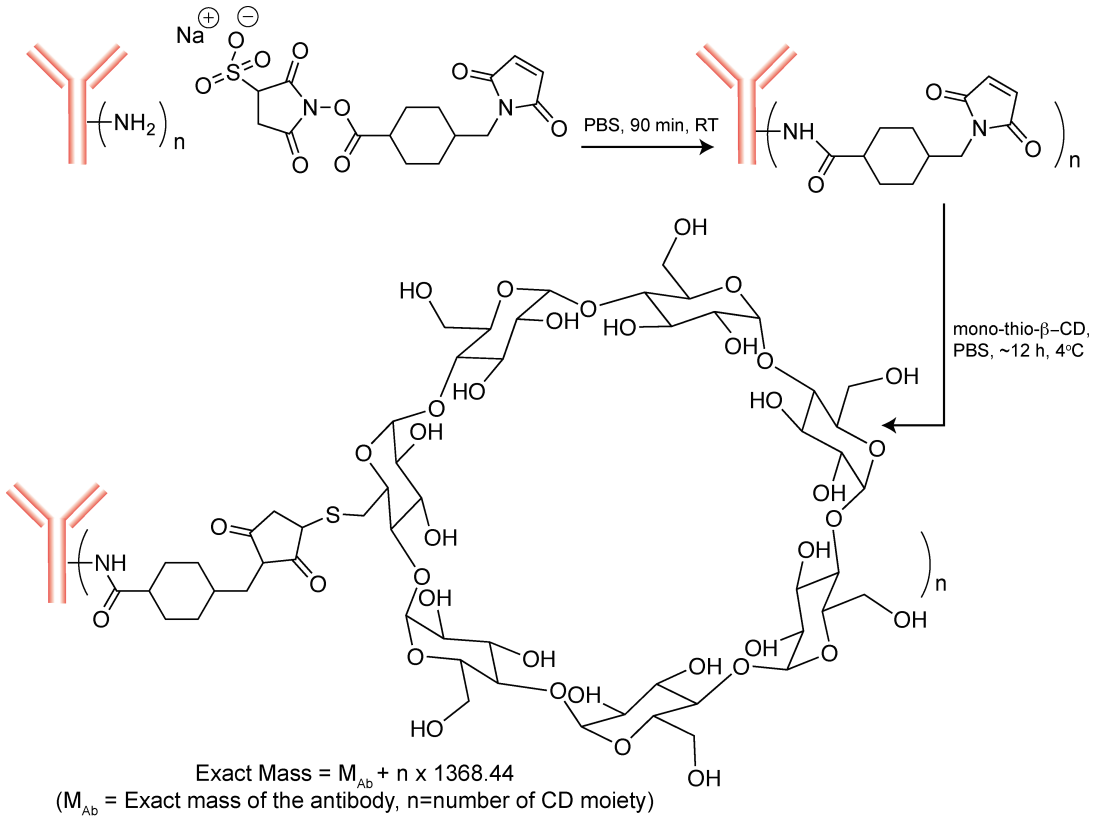
methanol/water (v/v). Thiourea (1 g,13.13 mmol) was added and the reaction mixture heated to reflux for 48 hours. The solvent was then removed and 15 ml methanol added to the residue, before stirring for 1 hour. After filtering, the residue was added to 35 ml of 10 wt % NaOH solution. The mixture was stirred at 50 °C for 2 hours and subsequently adjusted to pH 2 with 10 wt % HCl. Trichloroethylene (2.5 ml, 27.78 mmol) was then added to the solution. The mixture was allowed to stir overnight at RT. The resulting white precipitate was collected via suction filtration. After washing with water, the crude product was purified by re-crystalizing from 1:1 ethanol/water (v/v). Yield 13.5%. ^{1}H NMR (400 MHz, DMSO-d_6): 5.80-5.61 (m, 14H), 4.87-4.83 (m, 7H), 4.55-4.47 (m, 6H), 3.73-3.30 (m, 38H), 2.97-2.75 (m, 2H), 2.07 (t, ^{3}J = 8 Hz, 1H). MS (ESI-MS) calculated: 1150.35, found: 1149.21 $[M-H]^-$.

Synthesis of MFNPs. Cross-linked dextran-coated iron oxide particles were used for the magnetic labeling of cells. These particles are ~27 nm in hydrodynamic diameter (3 nm iron oxide core) and have a relaxivity value of 70 $mM^{-1}[Fe]\ s^{-1}$. The synthesis of amine-terminated probes and their conjugation with fluorescent molecules (FITC and VT680) were both done using a previously described protocol.[4] Each fluorescent conjugate had ~8 dye molecules and ~90 amine groups available for further conjugation.

To attach ADA to the nanoparticles, MFNPs (0.5 mg Fe) were mixed with ADA-PEG-succinimidyl-ester (0.234 mg) in PBS solution (1.25 mL, pH 8) containing 10% sodium bicarbonate (0.1 M) for 4 hours. ADA-MFNPs were subsequently purified using membrane filtration (Millipore Amicon, MWCO 30,000) and Sephadex G-50 (GE Healthcare), with PBS as the eluent buffer. Measurement of the hydrodynamic diameter (~27 nm) by dynamic light scattering showed that there was no aggregation of nanoparticles following conjugation, although a change in zeta potential (from +3.96 mV to -2.07 mV) indicated that the nanoparticle surface had been modified. The number of ADA on a single

nanoparticle was ~63, as determined by using the *N*-succinimidyl-3-(2-pyridyldithio)-propionate (SPDP) conjugation and cleavage method.[5]

For CD conjugation, MFNPs (0.5 mg) were first mixed with sulfosuccinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC; 0.18 mg) in 1.25 mL PBS solution (pH 7.2) for 3 hours. SMCC-MFNPs were then purified using membrane filtration (Millipore Amicon, MWCO 30,000) and Sephadex G-50 (GE Healthcare), with PBS as the eluent buffer. The purified maleimide-activated MFNPs were subsequently mixed with the mono-thio-β-CD solution (0.3 mg) in 4 mL PBS solution (1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.2) and the reaction proceeded overnight at 4°C. After the incubation, conjugates were purified using membrane filtration and Sephadex G-100, with PBS as the eluent buffer.



Scheme S3. Synthetic scheme for the antibody modification with **mono-thio-β-CD**.

Antibody modifications. The following antibodies were used in the experiments: anti-HER2 antibodies (Trastuzumab; Genentech) for HER2/*neu* markers, anti-EpCAM (Human EpCAM/TROP1 monoclonal antibody, Mouse IgG 2a/b; R&D Systems) for epithelial cell adhesion molecule (EpCAM) markers, anti-EGFR (Cetuximab; ImClone Systems) for epidermal growth factor receptor (EGFR) markers, and anti-CK18 (Cytokeratin 18, Axxora) for cytokeratin markers. To attach CD to the antibodies, each antibody (0.25 mg) was mixed with sulfosuccinimidyl 4-(maleimidylmethyl)cyclohexane-1-carboxylate (sulfo-SMCC) (72.8 μg) for 90 minutes at room temperature before being purified by gel filtration using PBS (1 mM EDTA, pH 7.2). The maleimide-activated antibodies were then mixed with mono-thio-β-CD (0.19 mg) in 1 mL PBS solution (1 mM EDTA, pH 7.2). The reaction proceeded overnight at 4°C, after which the conjugates were purified using both membrane filtration and gel filtration, with PBS as the eluent buffer. The formation of CD-antibody conjugates (CD-Abs) was verified by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (Figure S1). The number of CD moieties per antibody, estimated from the change in molecular weight, was 18-20.

Matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry characterization of CD-Ab conjugates. MALDI-TOF was used to quantify the amount of CDs conjugated to each antibody. The CD-modified antibodies (CD-Abs) were dissolved and concentrated to 1 mg/mL in Milli-Q water using a 0.5 mL microconcentrator (Millipore Amicon; MWCO: 30K). Sinapinic acid (1 mg, Thermo Fisher Scientific) was dissolved in acetonitrile (70 μL, Sigma-Aldrich) and water with 0.1% trifluoroacetic acid (TFA, 30 μL, Sigma-Aldrich). The antibody solution (0.5 μL) was deposited onto the MALDI plate and mixed with the MALDI matrix (0.5 μL) before being allowed to dry at RT.

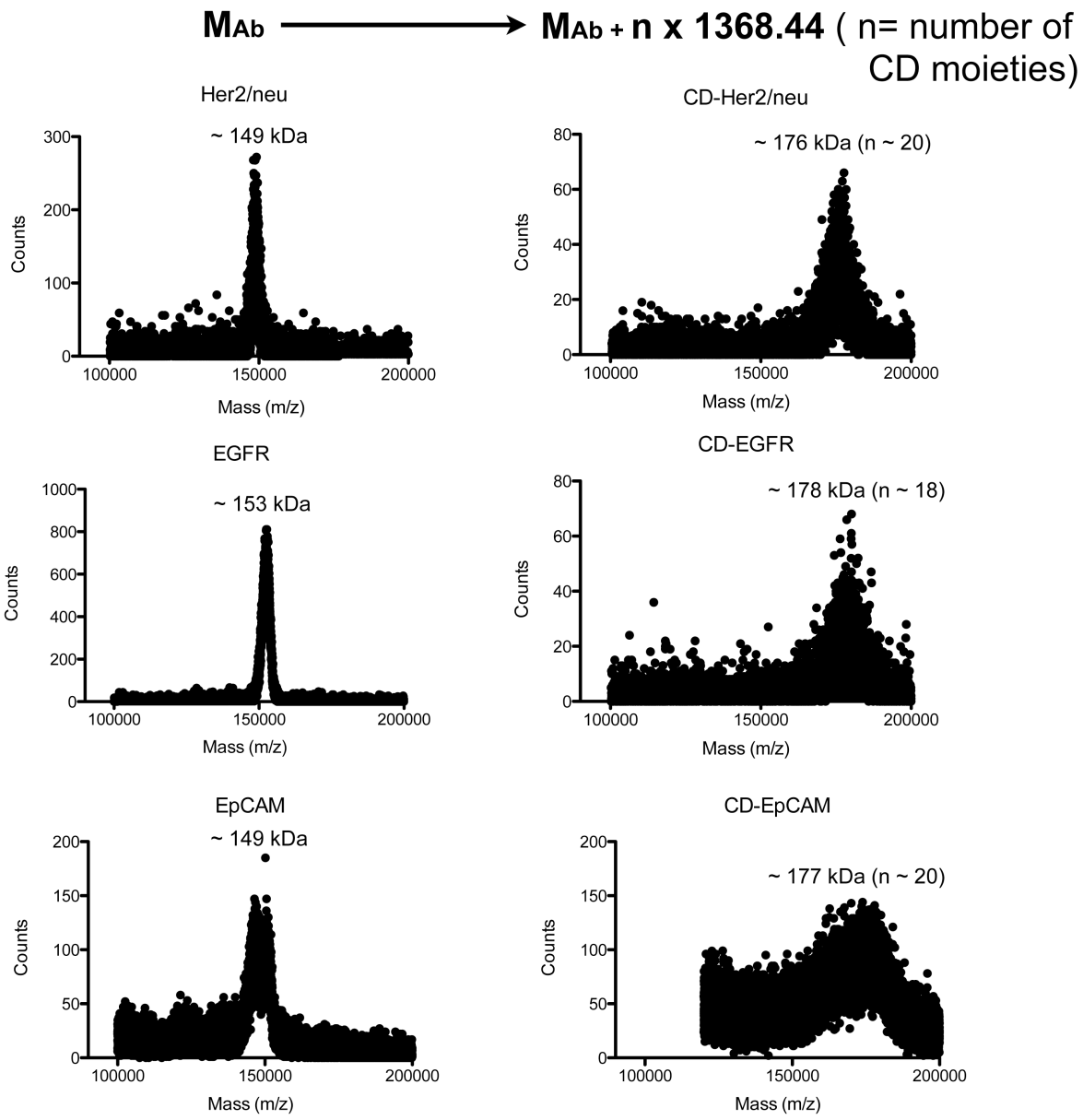


Figure S1. Characterization of CD-Ab conjugate formation via MALDI-TOF mass spectrometry analysis. MALDI-TOF mass spectrometry data showing the increase in molecular mass following CD conjugation. The difference in mass between modified and unmodified antibodies was used to calculate the number of CD moieties loaded onto a single antibody. As shown in Scheme S3, conjugation of each CD moiety added 1368.44 Da mass to the mass of the antibody. Based on this calculation, the number of CD moieties per antibody was estimated ~18-20.

Characterization of the binding efficiency of CD-Ab conjugates. SK-BR-3 cells were incubated with either unmodified primary antibody or cyclodextrin modified primary antibody (CD-Ab conjugates),

before being labeled with secondary antibodies. FITC-labeled Goat anti-Human IgG (Invitrogen) was used as the secondary antibody for both HER2/*neu* and EGFR (epidermal growth factor receptor), along with their immunoconjugates. FITC-labeled Rat anti-Mouse IgG 2a/b (BD Biosciences) was used as the secondary antibody for epithelial cell adhesion molecule (EpCAM), along with its immunoconjugates. Primary antibodies were omitted for control samples and the labeled cells were analyzed using flow cytometry.

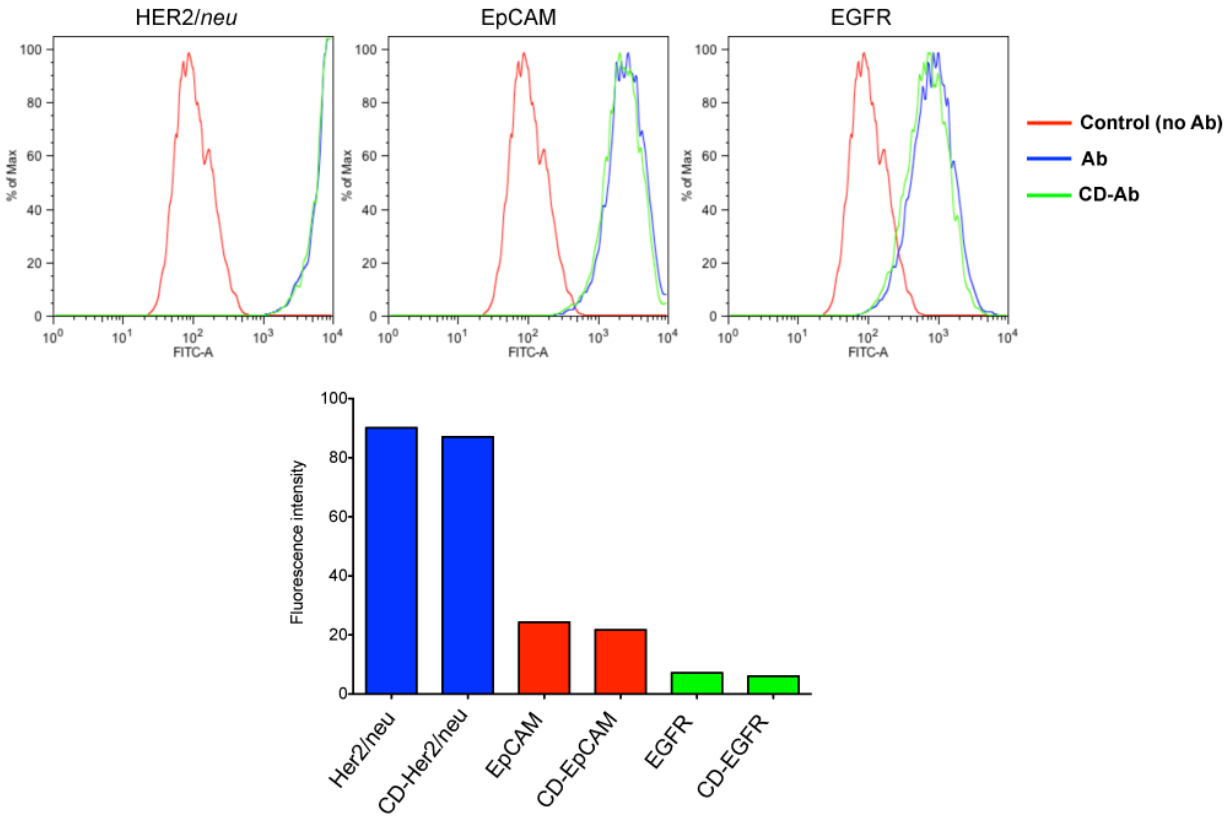


Figure S2. Flow cytometry analysis data showing the extent to which antibodies retain their ability to recognize targets after modification. SK-BR-3 cells were targeted with modified and unmodified antibodies. Negligible differences in fluorescence intensity (from flow cytometry analysis) between CD-modified antibodies and unmodified antibodies were observed after secondary antibody staining.

Synthesis of direct conjugate Ab-MFNPs. MFNPs (0.25 mg) were mixed with sulfo-SMCC (90 µg) for 3 hours at room temperature. In the meantime, antibodies (0.5 mg) were reduced with dithiothreitol

(DTT; 3x molar excess) in PBS (1 mM EDTA, pH 8) for 60 minutes. The purified MFNP-SMCCs were then mixed with the reduced antibodies overnight at 4°C and purified with Sephadex G-100 in PBS. The number of antibodies conjugated to the probes was subsequently quantified using absorbance measurements and the bicinchoninic (BCA) protein assay (Thermo Fisher Scientific). Each Ab-MFNP probe contained approximately 5 antibodies.

Synthesis of avidin-conjugated MFNPs. To conjugate avidin to the nanoparticles, MFNPs (0.5 mg) were first mixed with sulfo-SMCC (0.18 mg) in 1.25 mL PBS solution (pH 7.2) for 3 hours. Concurrently, 1 mg Neutravidin (Thermo Fisher Scientific) was mixed with 2-iminothiolane (4.6 ug) in 1 mL PBS solution (1 mM EDTA, pH 7.2) for 1 hour and purified by gel filtration. SMCC-MFNPs were purified using membrane filtration and Sephadex G-50, with PBS as the eluent buffer. The purified maleimide-activated MFNPs were subsequently mixed with the Neutravidin solution in 4 mL PBS solution (1 mM EDTA, pH 7.2) and the reaction proceeded overnight at 4°C. After the incubation, conjugates were purified using membrane filtration and Sephadex G-100, with PBS as the eluent buffer.

Synthesis of biotin-conjugated antibodies. To biotinylate the antibodies, EZ-Link NHS-LC-Biotin (33 uL, 10 mM, Thermo Fisher Scientific) was added to the antibody solution (0.25 mg, 217 uL) in PBS (pH 7.2) and incubated for 1 hour at room temperature. The biotinylated antibodies were purified using gel filtration with PBS as the eluent buffer.

Synthesis of ADA-conjugated QDs. To react QDs with ADA, a 2 μM solution of QDs was prepared in PBS containing 10% $NaHCO_3$ (0.1 M). 400 μl of the QD solution was mixed with ADA-PEG-succinimidyl-ester (0.138 mg in 20 μl dimethylformamide/DMF) and the conjugation was continued for 4 hours at RT. The ADA-modified QDs were then purified using centrifugal filtration (Millipore

Amicon; molecular weight cut off/MWCO: 30K).

Synthesis of ADA conjugated magnetic beads. To react amine-containing magnetic beads (~2.8 μm) with ADA, $2x10^8$ magnetic beads were dispersed in 1.375 ml of PBS containing 10% $NaHCO_3$ (0.1 M). The magnetic bead solution was mixed with ADA-PEG-succinimidyl ester (0.234 mg in 68 μl DMF) and the conjugation was continued for 4 hours at RT. The ADA-modified magnetic beads were subsequently purified using a permanent magnet.

Surface plasmon resonance (SPR) measurements. SPR measurements were performed on a Biacore T100 instrument using a Sensor Chip CM5 (carboxymethylated dextran matrix immobilized on a gold surface). Surfactant P-20 and immobilization reagents (amine coupling kit) were purchased from GE Healthcare. Binding and stability were measured at 25°C. PBS pH 7.4 containing 0.005% P-20 surfactant (PBS-P) was used as the dilution and running buffer.

Immobilization of anti-glutathione S-transferases (GST) to the sensor surface: Carboxyl groups on the dextran matrix were activated with a solution of *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide and *N*-hydroxysuccinimide (1:1 EDC/NHS; 480 second injection at 10 μL/min) followed by a solution of anti-GST (18 μg/mL in acetate buffer, pH 5.0; 420 second injection at 10 μL/min). The remaining NHS-ester groups on the sensor surface were quenched with ethanolamine (420 second injection at 10 μL/min). Using this general method, anti-GST was immobilized on flow cells 1 (FC1) and 2 (FC2) to attain binding levels of 15262 response units (RU) and 13459 RU, respectively.

Preparation of GST-cyclodextrin conjugate (CD-GST): A solution of sulfo-SMCC (20 μL, 22 mM in water) was added to GST (50 μL, 1 mg/mL in PBS) and shaken at RT for 1 hour. Excess reagent was removed using a spin desalting column (7K MWCO) equilibrated with PBS. The recovered filtrate was immediately treated with mono-thio-β-CD (100 μL, 1.6 mM in water) and shaken overnight at 4ºC. The

protein conjugate was then purified using a spin desalting column (7K MWCO) equilibrated with PBS-P to afford the desired CD-GST (165 µL, 0.3 mg/mL). To verify that the anti-GST conjugate still retained its target recognition capabilities, a solution of CD-GST (17 µg/mL in PBS-P) was injected over FC1 (600 second injection at 7 µL/min) to provide 1070 RU of captured CD-GST. Surface-bound CD-GST was removed with two short pulses (30 second at 30 µL/min) of regeneration solution (10 mM glycine, pH = 2.0).

The binding kinetics between ADA-MFNPs and immobilized CD-GST: Reference FC1 was prepared by capturing 960 RU of GST (5 µg/mL in PBS-P injected for 420 second at 5 µL/min). On sample FC2, the settings for each cycle (capture/binding/regeneration) were as follows: i) capture = CD-GST injection (0.4 µg/mL), contact time 60 seconds at 5 µL/min (capture levels ranged from 80-88 RU); ii) binding = sample injection, contact time 120 seconds at 60 µL/min followed by a dissociation time of 120 seconds; iii) regeneration = surface regeneration with 2 x 20 second pulses of regeneration solution at 30 µL/min. ADA-MFNP binding was characterized in step (ii) by running multiple cycles and measuring binding at various concentrations (1:2 dilution series ranged from 15.6 to 500 ng ADA-MFNP/mL). The resulting binding curves were double-reference subtracted and fitted to a one-to-one binding model.

Complex stability was assessed by monitoring the signal decay after flowing 60-120 second pulses of high pH, low pH and high salt solutions over immobilized conjugates, using the manual run protocol.

Stability of the FC1 (anti-GST/GST) complex: With a flow rate of 10 µL/min over 120 seconds, a solution of GST (10 µg/mL) was injected over pre-immobilized anti-GST. Capture was monitored by a rise in response to approximately 1120 RU. At t = 120 seconds, the mobile phase was switched to running buffer. From t = 187 - 247 seconds, a solution of ADA-MFNP (5 µg/mL) was injected. No binding to the reference FC was detected. At t = 247 seconds, the flow was switched to running buffer.

From t = 315 - 375 seconds, an acetate solution (10 mM, pH = 4.0) was injected. From t = 443 - 503 seconds, a solution of NaCl (0.5 M) was injected. From t = 570 - 630 seconds, a solution of $NaHCO_3$ (0.1 M pH = 8.5) was injected. No complex (anti-GST/GST) decay was detected after the injections.

Stability of the FC2 (GST-CD/ADA-MFNP) complex: With a flow rate of 10 µL/min over 180 seconds, a solution of ADA-MFNPs (5 µg/mL) was injected over the pre-immobilized anti-GST/GST-CD complex. ADA-MFNP capture was determined by a rise in signal to approximately 990 RU. At t = 180 seconds, the mobile phase was switched to running buffer. From t = 254 - 374 seconds, a solution of NaCl (0.5 M) was injected. From t = 450 - 570 seconds, a solution of $NaHCO_3$ (0.1 M pH = 8.5) was injected. From t = 644 - 764 seconds, an acetate solution (10 mM, pH = 4.0) was injected. From t = 956 - 1076 seconds, a solution of NaOH (0.1 mM, pH 9.6) was injected. No complex (GST-CD/ADA-MFNP) decay was detected after the injections.

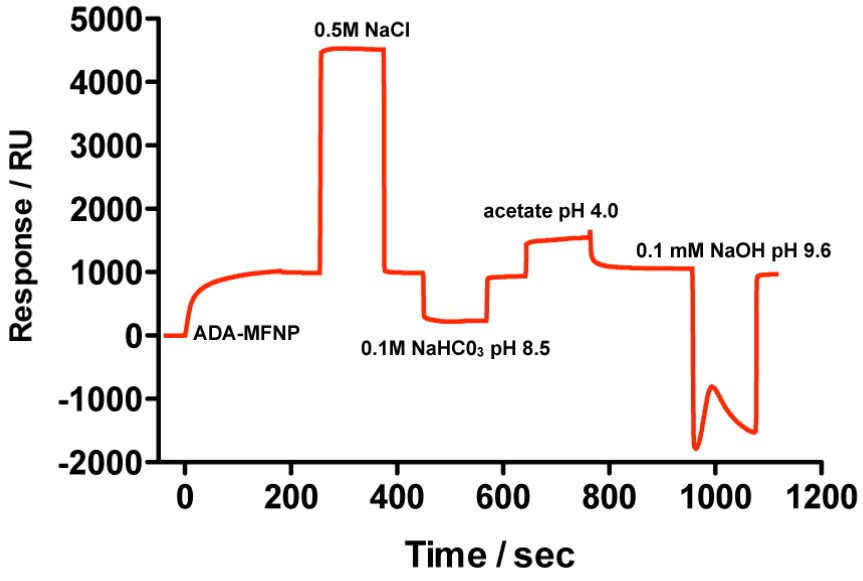


Figure S3. Stability of the CD-ADA complex under different conditions as measured by surface plasmon resonance (SPR). The stability of the complexes was measured under conditions of high salt, high pH and low pH. SPR data showed negligible dissociation of the CD-ADA complex under these conditions.

Cell labeling and detection. Human cancer cells (SK-BR-3, MCF-7, MDA-MB-231, A431, and HCT-116) and NIH/3T3 fibroblasts were cultured in Dulbecco's Modified Eagle Medium (DMEM), supplemented with fetal bovine serum (FBS; 10%), penicillin and streptomycin (1%), and L-glutamine (1%). Cell lines were maintained at 37°C in a humidified atmosphere containing 5% CO_2. At confluence, the cells were washed, trypsinized and resuspended in PBS solution containing 2% FBS and 1% bovine serum albumin (BSA; C-PBS). In a typical labeling experiment, 10^5 cells were incubated in C-PBS containing CD-Abs (10 μg/mL) for 20 minutes at room temperature. Following aspiration and centrifugation, the cells were mixed with ADA-MFNP (40 μg/mL). The cells were then fixed using BD Fix Buffer (BD Biosciences) for fluorescence and magnetic measurements. Control samples were prepared in the same manner, but were incubated without the antibody conjugates. Flow cytometry measurements were performed using a BD LSR II flow cytometer, and mean fluorescence intensity was determined using FlowJo software. Changes in transverse relaxation rate (ΔR_2) were measured using a previously described miniaturized nuclear magnetic resonance system. The sample volume per measurement was 1 μl. Carr-Purcell-Meiboom-Gill pulse sequences were used with the following parameters: echo time (TE) = 4 milli-seconds; repetition time (TR) = 6 seconds; number of 180° pulses per scan = 500; number of scans = 8. All measurements were performed in triplicate and the data are presented as mean ± standard error. The measured ΔR_2 values were then converted to cellular relaxivity Δr_2 (R_2 divided by cell concentration), a value which is proportional to the number of MFNPs per cell. These values were used to determine the expression levels of target markers by taking the ratio $\Delta r_2^{mAb}/\Delta r_2^{ø}$, where Δr_2^{mAb} and $\Delta r_2^{ø}$ are cellular relaxivities for marker-specific and control MFNPs, respectively.

For the labeling of live cells and for microscopy imaging, cells were grown to confluency in an 8-well chamber slide. After washing the cells with Hank's balanced salt solution (HBSS), the cells were

labeled as described above, but using cell culture medium instead of C-PBS. The cells were then fixed and permeabilized with BD Fix Buffer and BD Perm/Wash Buffer for 20 minutes before incubating with a solution of TO-PRO-1 iodide (1 μM in BD Perm/Wash Buffer) for 15 minutes.

For labeling intracellular markers using ADA-QDs, the cells were initially fixed and permeabilized with BD Fix Buffer and BD Perm/Wash Buffer for 20 minutes. The cells were further incubated with C-PBS blocking solution containing the BD Perm/Wash Buffer for an additional 30 minutes. The permeabilized cells were labeled with anti-CK18-CD (10 μg/mL) for 20 minutes at room temperature. After washing away excess antibodies, ADA-QDs were added to the cells and incubated for 20 minutes at room temperature. Following washes to remove unbound QDs, Vectashield mounting medium containing 4',6-diamidino-2-phenylindole (DAPI; Vector Labs) was added to the samples prior to imaging with confocal microscopy.

For magnetic cell sorting, $25x10^4$ SK-BR-3 cells were mixed with $25x10^4$ MDA-MB-231 cells in C-PBS. Mixed cells were then incubated with CD-Abs (10 μg/mL) for 20 minutes at room temperature. Following centrifugation and aspiration, the cells were mixed with ADA conjugated magnetic beads ($125x10^5$ beads) for 20 minutes. A permanent magnet was then used to isolate the cells attached to the magnetic beads. After isolation, both the fractions (isolated and remaining) were stained with FITC-labelled secondary antibody for HER2/*neu.* The cells were then fixed using BD Fix Buffer (BD Biosciences) for flow cytometry analysis. Fluorescence images were taken after staining the nuclei with DAPI.

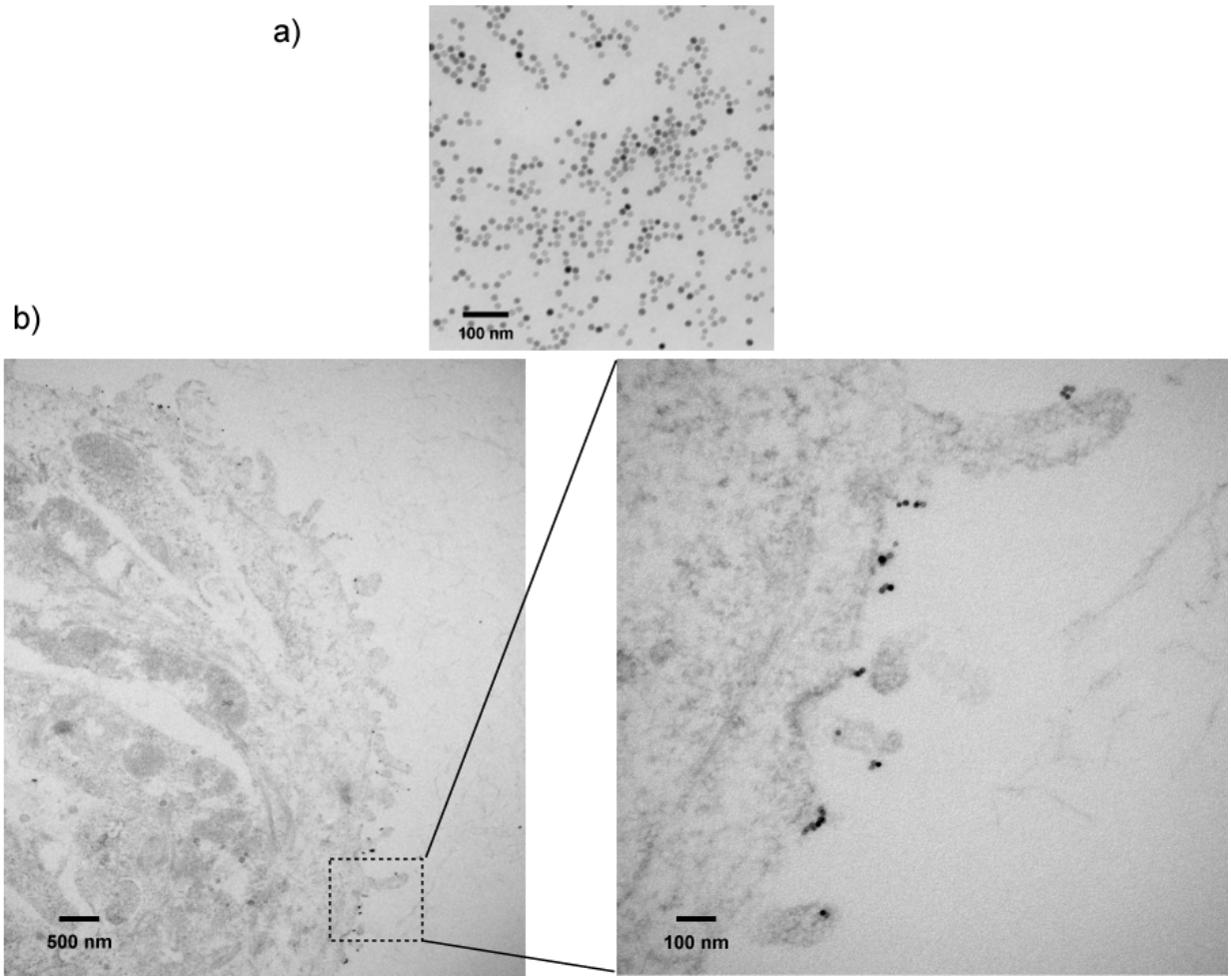


Figure S4. Transmission electron microscopy (TEM) images showing nanoparticle localization within the cellular membrane. a) TEM images of ADA-conjugated nanoparticles (~16 nm manganese-doped iron oxides) used for cell labeling. b) Cross-section TEM images of SK-BR-3 cells labelled with ADA-conjugated nanoparticles targeted to cell surface associated-HER2/*neu* marker. Localization of the nanoparticle within the cellular membrane demonstrated its labeling of the cell surface marker.

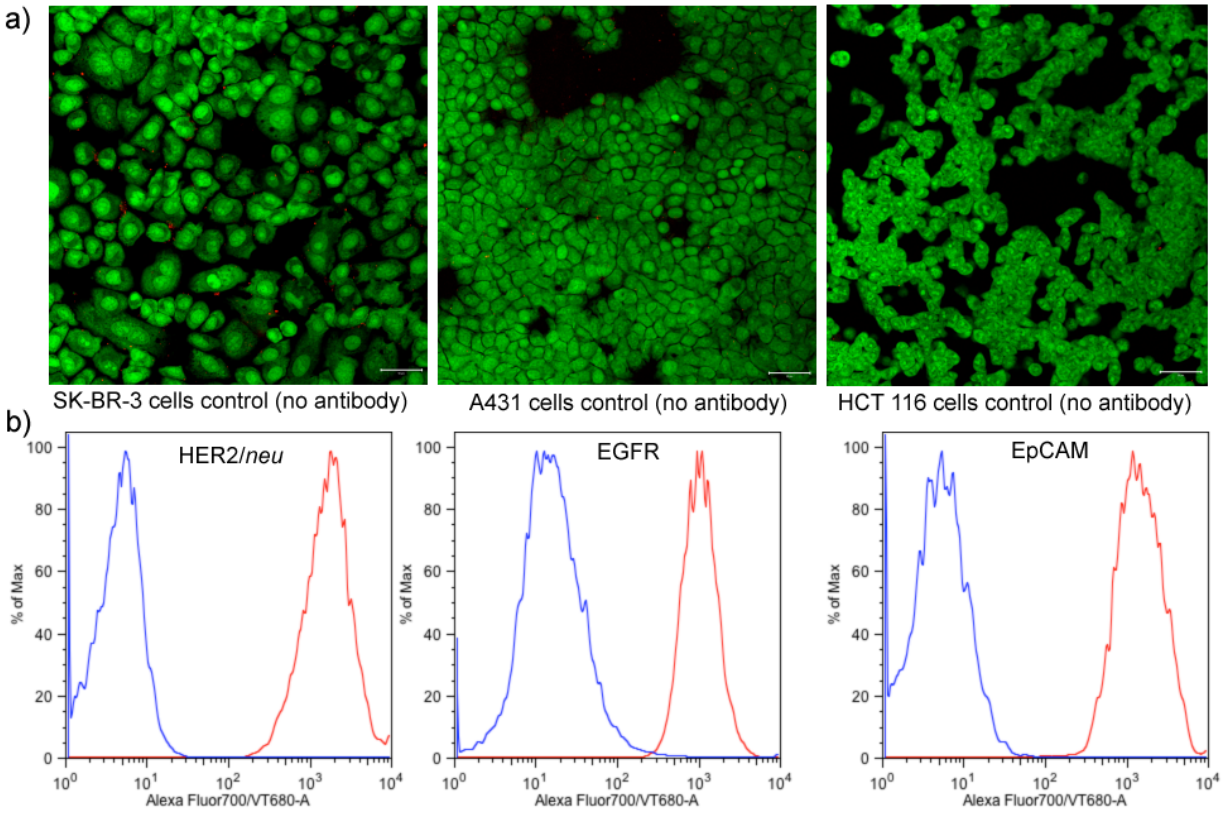


Figure S5. Control experiments of non-targeted cells. a) Confocal microscopy images of SK-BR-3, A431 and HCT-116 cells incubated with ADA-MFNPs in the absence of respective CD-Abs. Images were captured at using similar settings as those for labelled cells. Cell nuclei were stained using TO-PRO-1 (green fluorescence). Note the very low number of nonspecific interactions between cells and ADA-MFNPs as negligible VT-680 fluorescence was observed from the cells. Scale bar 50 µm. b) Flow cytometry analysis of both labelled cells and controls. Blue traces represent controls (non-targeted cells) and red traces represent labelled cells (targeted with CD-Abs and coupled with ADA-MFNPs).

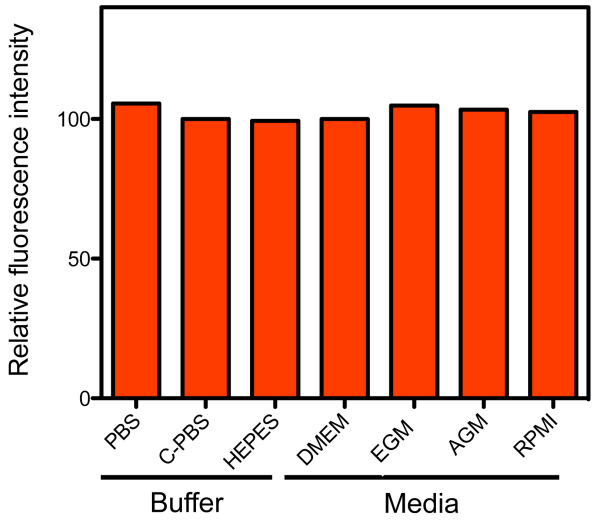


Figure S6. Labeling efficiency of **ADA-MFNP**s in various biological medium. In this experiment, SK-BR-3 cells were targeted with CD-HER2/*neu* primary antibody and then labeled with nanoparticles using CD-ADA supramolecular method. Incubation with ADA-MFNPs for cellular binding was carried out in different buffers and various serum containing cell culture media. Performance of the labeling experiment was assessed using flow cytometry. Signal for the buffers was normalized against the signal from C-PBS buffer and the signal for the media was normalized against the signal from DMEM media.

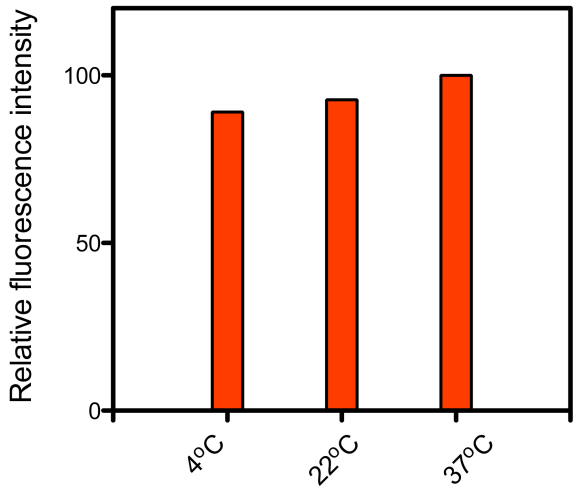


Figure S7. Labeling efficiency at different temperatures. Cell labeling was performed using ADA-MFNPs at different temperatures. The negligible difference in fluorescence signal (as determined by flow cytometry) over a range of temperatures served to demonstrate the effectiveness of this labeling method. Fluorescence signal was normalized against the signal from 37 °C experiment.

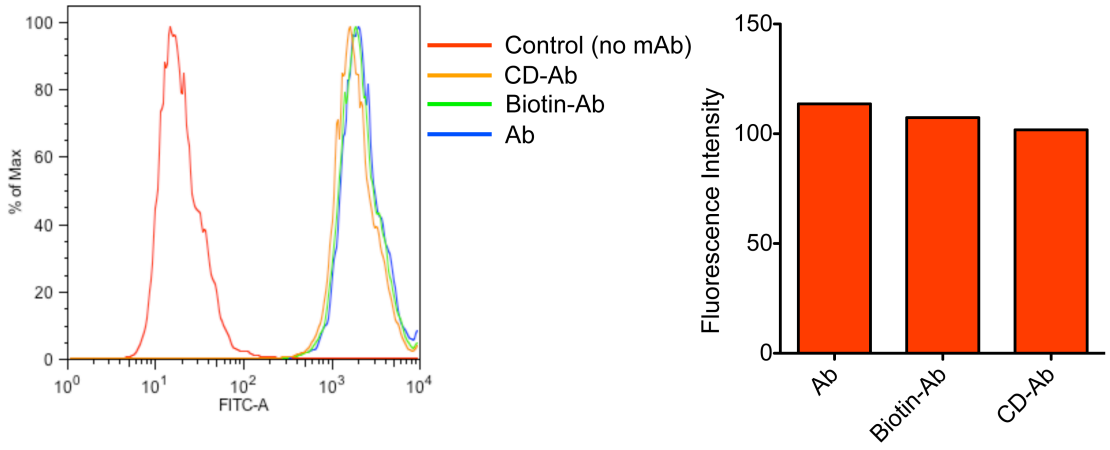


Figure S8. Flow cytometry analysis showing the extent to which different antibodies binds to the cell surface. For this purpose, SK-BR-3 cells were initially incubated with HER2/*neu* primary antibody conjugates for 20 minutes. Afterwards, the cells were fluorescently stained with secondary antibody. Flow cytometry analysis showed similar fluorescence intensity from the cells targeted with different antibodies (i.e. unmodified Ab, CD-Ab and Biotin-Ab), thus eliminating the possibility of difference in uptake rates and/or lower uptake of the firstly administered primary antibody conjugates.

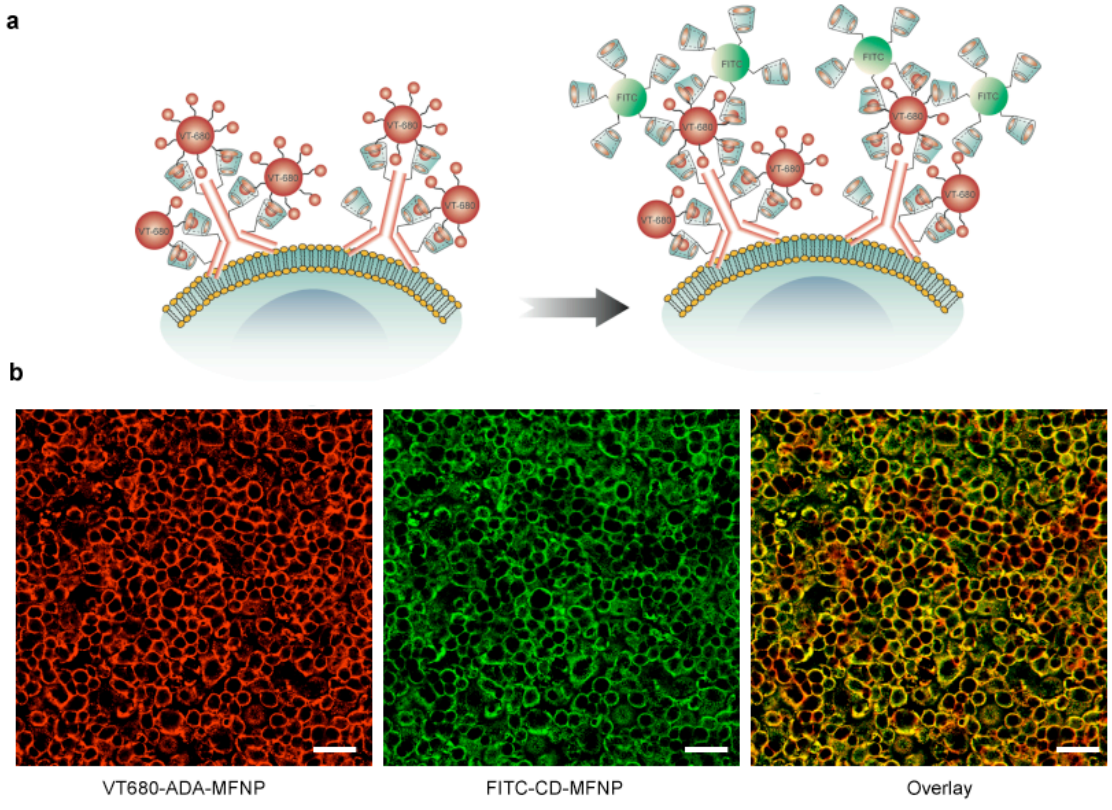


Figure S9. Signal amplification strategy by successive labeling of biomarkers. a) Schematic showing the strategy for signal amplification based on layer-by-layer nanoparticle immobilization approach. To test this strategy, we used MFNP constructs

encoded with different fluorophores, VT680 for ADA-MFNPs and fluorescein isothiocyanate (FITC) for CD-MFNPs, and used them to label HER2-targeted SK-BR-3 cells. b) Confocal microscopy images showed the formation of a second nanoparticle layer (FITC-CD-MFNP) from the binding with the first VT680-ADA-MFNP layer. Co-localization of the two colors confirmed the successive labeling of the specific structural component. Scale bar 50 μm.

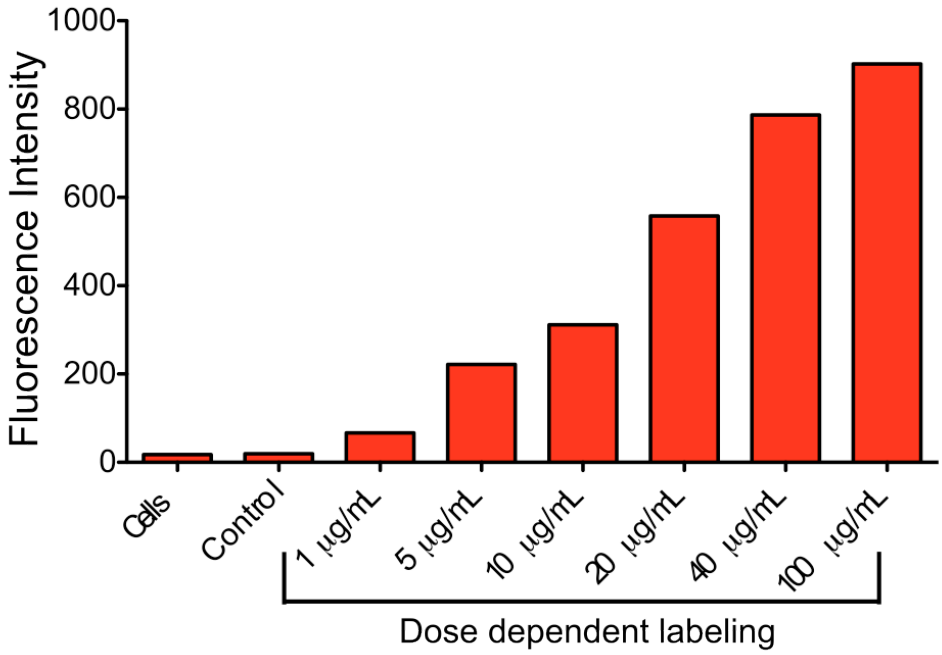


Figure S10. Flow cytometry analysis for quantification of the non-specific binding of FITC-CD-MFNP and dose-dependent labeling of VT680-ADA-MFNP with FITC-CD-MFNP. Here, control represents the experiment where CD-Ab targeted cells were incubated with FITC-CD-MFNP (~40μg/mL) without the intermediate conjugation of VT680-ADA-MFNP layer. Observation of the negligible fluorescence signal from the control experiment demonstrates the low non-specific binding of FITC-CD-MFNP. Dose-dependent labeling of VT680-ADA-MFNP with FITC-CD-MFNP was tested by incubating VT680-ADA-MFNP conjugated cells with different concentration of FITC-CD-MFNP. A dose dependent increase in fluorescence signal, saturating ~40μg/mL was observed from this experiment.

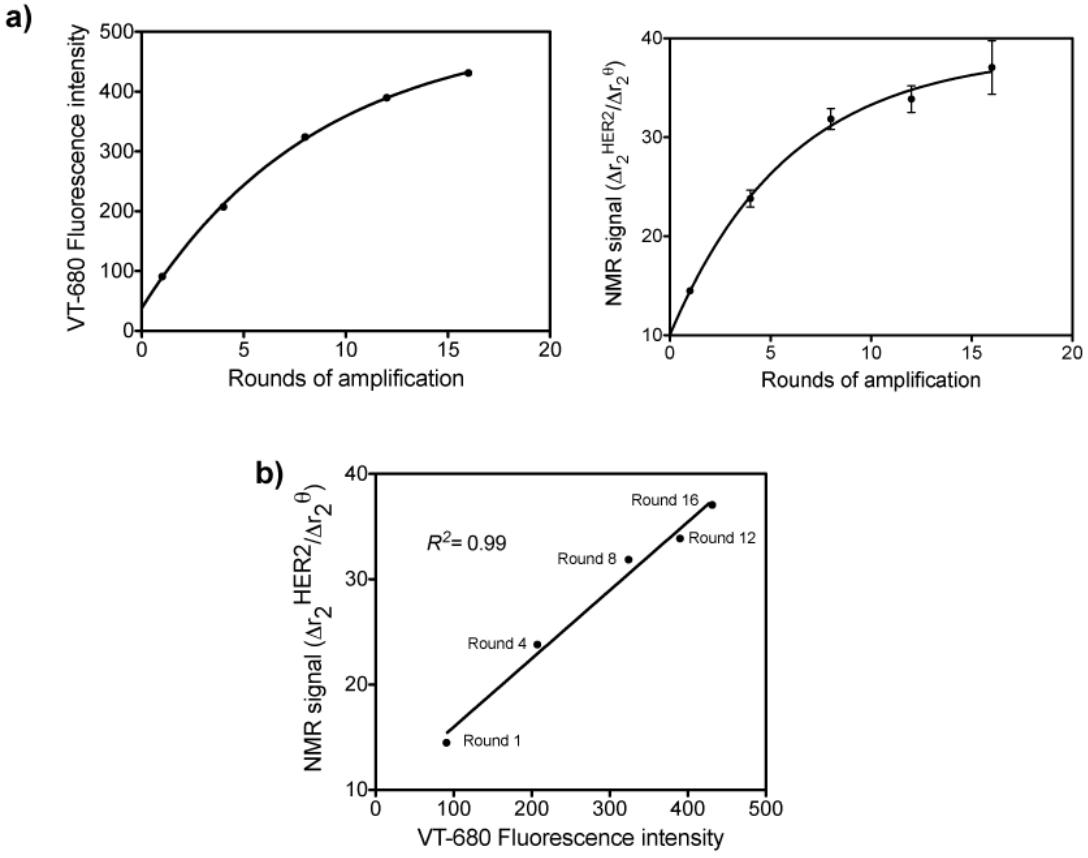


Figure S11. Effect of sequential rounds of amplification on the overall signal. a) Sequential rounds of amplification were quantified using both flow cytometry and μNMR analysis. With each round of labeling (ADA-MFNPs and CD-MFNPs), fluorescence and the magnetization increased. The signal almost reached saturation after 16 sequential rounds of amplification steps. b) Direct correlation was observed between fluorescence and NMR measurements.

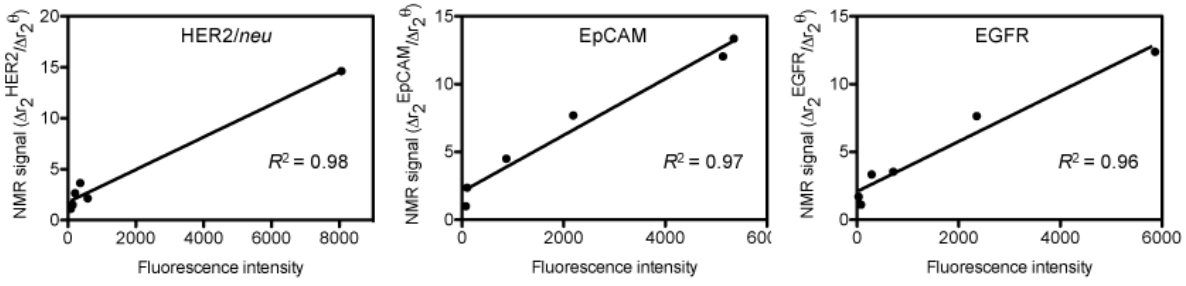


Figure S12. Correlation between μNMR and marker expression level. Expression levels of different markers were measured with flow cytometry using secondary antibody staining. As seen in the plots, there was a direct correlation between μNMR and fluorescence measurements.

Cancer cell detection in mouse blood. SK-BR-3 cells were trypsinized, washed with PBS solution and inoculated into 0.5 ml of mouse blood. Red blood cells were lysed by centrifuging blood samples (including the SK-BR-3 cells) for 10 minutes at 2,500 rpm, removing the supernatant, and adding 0.5 ml of ACK lysing buffer (Lonza). Lysis buffer addition and centrifugation steps were repeated until complete lysis of red blood cells was achieved. Once lysis was complete, the cell pellet was resuspended in C-PBS, and incubated with 10 μg/ml of CD-Abs (targeted to HER2/*neu)* for 20 minutes at RT. Cells were then washed twice and incubated with 40 μg/ml ADA-MFNPs in C-PBS at RT. The cells were fixed for subsequent flow cytometry and μNMR measurements.

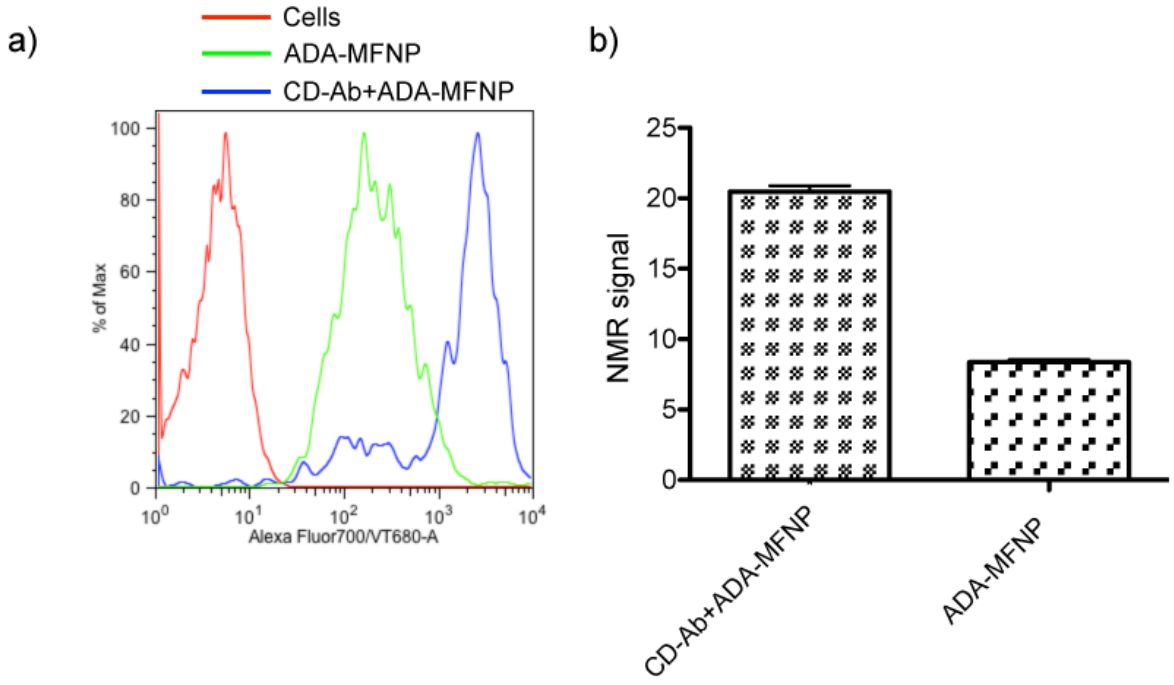


Figure S13. Labeling and detection of cancer cells in blood. a) Flow cytometry analysis showed increased fluorescence in the CD-Ab-targeted sample. b) μNMR measurements showed a significant difference in magnetization between targeted cancer cells labelled with ADA-MFNPs and control sample.

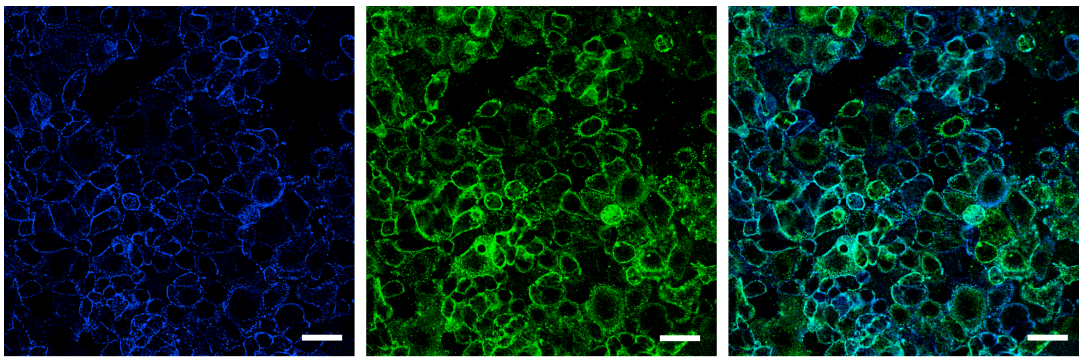


Figure S14. Confocal microscopy images showing multiplexed labeling of two surface markers on the same cell. In SK-BR-3 cells, CD-ADA method was used to label EpCAM receptors and avidin-biotin method was used to label of HER2/*neu* receptors. SK-BR-3 cells were first targeted with CD modified EpCAM antibody and biotinylated HER2/*neu* antibody. After primary targeting EpCAM receptors (~$1X10^6$ receptors/cell) were labeled with VT-680 conjugated ADA-MFNP (pseudo colored blue) through CD-ADA supramolecular interactions and HER2/*neu* receptors (~$2X10^6$ receptors/cell) were labeled with FITC conjugated avidin-MFNP (pseudo colored green) through avidin-biotin interactions. Scale bar, 50 μm.

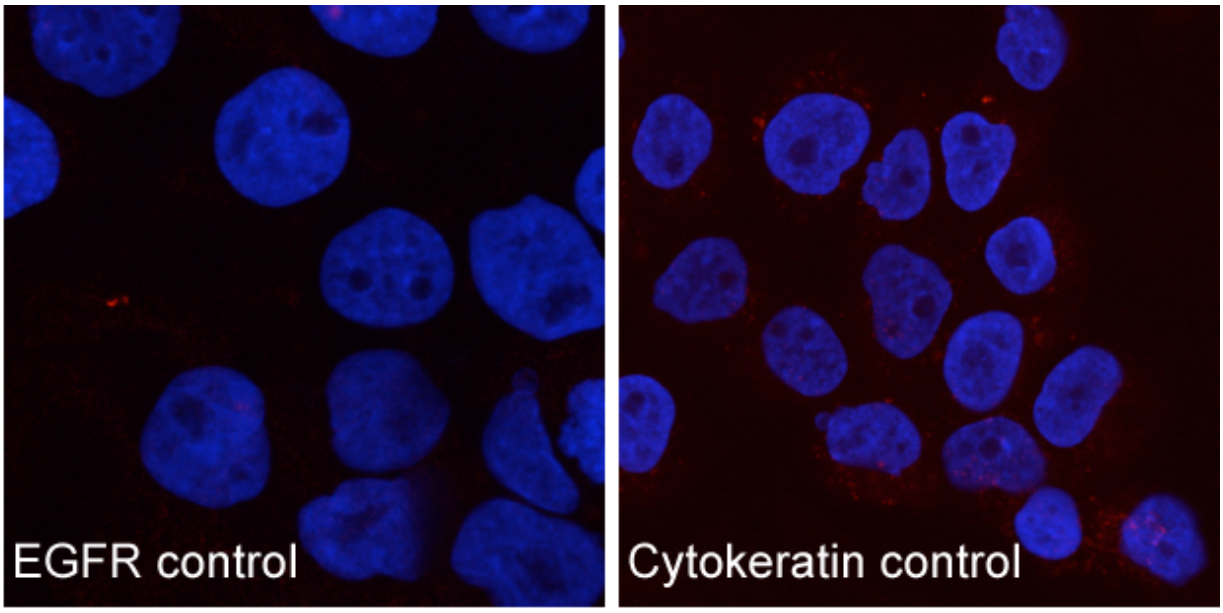


Figure S15. Control (confocal microscopy) experiments for QD labeling. Confocal microscopy images of A431 and SK-BR-3 cells incubated with ADA-QDs in the absence of primary targeting (with respective CD-Abs).

Supporting References:

1. D. Vizitiu, C. S. Walkinshaw, B. I. Gorin, G. R. J. Thatcher, *J. Org. Chem.* **1997**, *62*, 8760 –8766.
2. B. Jing, X. Chen, X. D. Wang, Y. R. Zhao, H. Y. Qiu, *ChemPhysChem* **2008**, *9*, 249 – 252.
3. A. Mulder, S. Onclin, M. Pe´ter, J. P. Hoogenboom, H. Beijleveld, J. ter Maat, M. F. Garcı´a-Parajo´, B. J. Ravoo, J. Huskens, N. F. van Hulst, D. N. Reinhoudt, *Small* **2005**, *1*, 242 – 253.
4. M. J. Pittet, F. K. Swirski, F. Reynolds, L. Josephson, R. Weissleder, *Nat. Protoc.* **2006**, *1*, 73 – 79.
5. L. Josephson, C. H. Tung, A. Moore, R. Weissleder, *Bioconjugate Chem.* **1999**, *10*, 186-191.
6. Full author list for the main text reference 13:
   D. W. Lee, K. M. Park, M. Banerjee, S. H. Ha, T. Lee, K. Suh, S. Paul, H. Jung, J. Kim, N. Selvapalam, S. H. Ryu, K. Kim, *Nat. Chem.* **2011**, *3*, 154–159.