Electronic Supplementary Material (ESI) for Chemical Science

Magnetic Nanoparticles for Direct Protein Sorting inside Live Cells

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Supporting information

Materials and instruments

Chemical reagents were purchased from Sigma-Aldrich Co., and solvents from Fisher Scientific Inc. Microscopy was carried out on a Marianas Spinning Disk Confocal Microscope.

Synthesis and characterization

6 nm iron oxide nanoparticles were synthesized using a modified procedure:[1] 2 mmol of $Fe(acac)_3$, 10 mmol of 1,2-hexadecanediol, 6 mmol of oleic acid, 6 mmol of oleylamine, and 20 mL of benzyl ether were mixed in a glove box under an argon atmosphere. The mixture was heated to 200 °C for 2 h and then heated to reflux (300 °C) for 1 h. The black-colored mixture was cooled to room temperature after removing the heat source. The iron oxide nanoparticles were washed with ethanol and re-dispersed in hexane.

Surface modification of the iron oxide nanoparticles using dopa-GSH ligand: 1 mL of chloroform solution containing ~5 mg of iron oxide nanoparticles was added into 1 mL of the chloroform solution containing ~12 mg of dopa-GSH ligand**.** These mixtures were mixed by vigorous stirring and kept stirring for about 24 hours. The addition of hexane to the dispersion produces brown precipitates. The product was washed using hexane following a magnetic harvest and the supernatant was discarded. The final product was purified by repeating the above procedures two more times. The average diameter of the particles is similar to that of as-prepared iron oxide nanoparticles.[2]

Surface modification of the iron oxide nanoparticles using dopa-TMP ligand: 1 mL of chloroform solution containing ~5 mg of iron oxide nanoparticles was added into 1 mL of the methanol solution containing ~10 mg of dopa-TMP ligand. These two solutions were mixed by vigorous stirring and kept stirring for about 24 hours. After dried using a rotary evaporator, the mixture was stirred in phosphate buffer (pH = 6.0) for another day. The product was washed using hexane following a magnetic harvest and the supernatant was discarded. The final product was purified by repeating the above procedures two more times. The average diameter of the particles is similar to that of as-prepared iron oxide nanoparticles.[3]

Expression of the fusion proteins and analysis

Bicistronic constructs were cloned from two previously reported plasmids, GFP-TEV-HA-GST[2] and eDHFR-HA-GFP[3].

Expression of DsRed_IRES_GFP-$(ser,gly)_5$-eDHFR-HA:

DsRed_IRES-GFP was PCR amplified from XX (Elledge lab) using the following primers TTTAGTGAACCGTGGATCCACC*ATGGCCTCCTCCGAGAAC* and GCCGGACCCTGAACCCGATCCTGAGCCGGACCCTGA*CTTGTACAGCTCGTCCATGC*

C. This PCR product (1) was PCR amplified using the following primers AGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTGG*TTTAGTGAACCGTGGAT CCACC* and CGATCTACCGCTAACGCCGCAATCAGACTGATCAT*GCCGGACCCTGAACCC* to give product 2. eDHFR was PCR amplified from eDHFP-HA-GFP using the following primers TCAGGGTCCGGCTCAGGATCGGGTTCAGGGTCCGGC*ATGATCAGTCTGATTGCGGC* and ACGGCCAGTGCCAAGCTA*TTACCGCCGCTCCAGAATC*. This product (3) was PCR amplified using the following primers to give PCR product 4 TGGACGAGCTGTACAAG*TCAGGGTCCGGCTCAG* and AAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGT*ACGGCCAGTGCCAAG CTA*. Products 2 and 4 were PCR ligated and the crude product was PCR amplified using the following primers. This product was PCR ligated into Not1 linearized pMIR report. Viable clones were verified by sequencing, fluorescence, and activity.

Expression of DsRed_IRES_GFP-TEV-HA-GST:

GFP-TEV-HA-GST was PCR amplified using the following primers *ATGGTGAGCAAGGGCGAG* and AAAACGACGGCAGTGCCAAGCTA*TTAAAACCTGAAAATCTTCCTTGCTTC* to give PCR product 5. DsRed_IRES-GFP was amplified using the following primers TTTAGTGAACCGTGGATCCACC*ATGGCCTCCTCCGAGAAC* and CTTGTACAGCTCGTCCATGCCGA to give PCR product 6. 6 and 5 were PCR ligated to give 7 which was PCR amplified using the following primers: AGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTGG*TTTAGTGAACCGTGGAT CCACC* and AAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGT*ACGGCCAGTGCCAAG CTA.* This product was PCR ligated into NOT1 linearized pMIR report. Viable clones were verified by sequencing, and fluorescence.

For pre-incubation of cells with nanoparticles then transfection

Cells were grown under the standard conditions (37 ^{o}C in a 5% CO_2 humidified atmosphere) in complete media [DMEM supplemented with 10% Fetal Bovine Serum, 1x glutamine, and 1x penicillin/streptomycin (all Hyclone)] in 96 well plates. At 20-30% confluence, nanoparticles were added at the stated concentration (25 μg/ml). Cells were allowed to grow under standard conditions. At the stated time, media were aspirated, and 0.25% trypsin was added directly. After 5 minutes, cells were replated on a glass backed tissue culture dish (In vitro Scientific) and grown overnight. At this point (approximately 35-65% confluence), cells were transfected with the required plasmid (Mirus 2020® used as per manufacturer's instructions). Cells were allowed to grow for another day. Cells were observed using confocal microscopy, which usually last several hours.

For addition of nanoparticles to transfected cells

Cells were grown under standard conditions in complete media in glass backed tissue culture dishes. At 40-50% confluence, cells were transfected with the required plasmid (Mirus 2020®) and cells were grown for 6 hours. At this point nanoparticles were added at the stated concentration (25 μg/ml). Cells were allowed to grow under standard conditions for 24 hours.

After this time, cells were observed using confocal microscopy, which usually last several hours.

Activity of the expressed GFP-(ser,gly)$_5$-eDHFR-HA

COS-1 cells were grown to 60% confluence in 6 well plates. At this point, one set of 3 wells was left untreated and the other set of 3 wells was transfected with DsRed_IRES_GFP-(ser,gly)$_5$-eDHFR-HA and grown for one more day. The cells were then harvested and pelleted. The cells were lysed using freeze thaw (3 cycles) in a buffer containing 100 mM of sucrose, 50 mM of Phosphate pH 7.5, and 150 mM of NaCl. The lysates (transfected and non transfected) were both clarified by centrifugation at 12000 x g for 10 minutes and then normalized for protein content by Bradford assay. Equal amounts of total lysate were then assayed for DHFR activity by measuring the decrease in absorption at 340 nm, corresponding to the conversion of NADPH to NADP/dihydrofolate to tetrahydrofolate as per Thillet et al.[4]

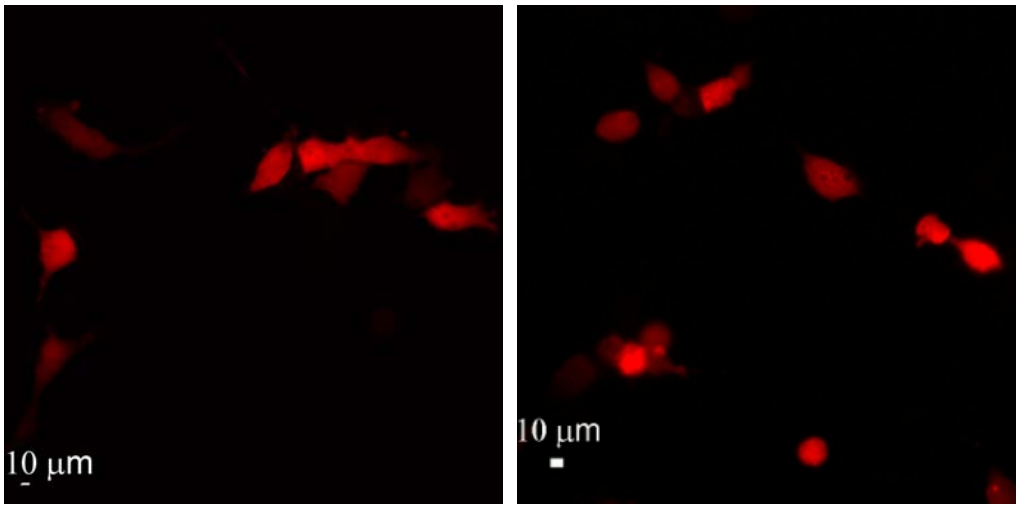

Figure S1. The COS-1 cells incubated with **1** for seven days and transfected with a plasmid expressing DsRed under a CMV promoter (left); the COS-1 cells incubated with **2** for seven days and transfected with a plasmid expressing mRFP-GST under a CMV promoter (right).

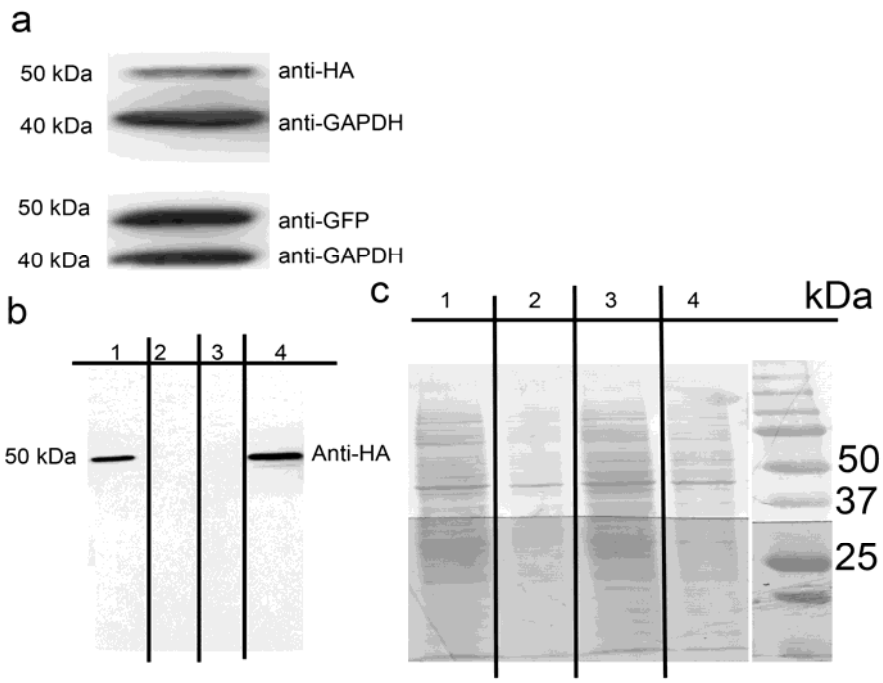

Figure S2. (a) COS-1 cells were transiently transfected with DsRed_IRES_GFP-eDHFR-HA and after 24 hours, cells were lysed and the clarified lysates were analyzed by western blot for (upper) HA and GAPDH (as a loading control) and (lower) GFP and GAPDH (as a loading control). (b) COS-1 cells were transfected with either DsRed-IRES_GFP-TEV-HA-GST (lane 1), untransfected (lanes 2 and 3) or DsRed_IRES_GFP-eDHFR-HA (lane 4) for 24 hours. Clarified lysates were analyzed by anti HA. (c) membrane in (b) was stained with Coomassie.

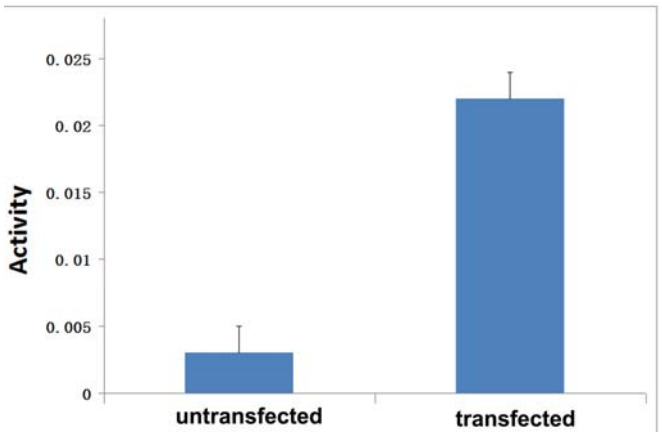

Figure S3. eDHFR activity of untransfected vs transfected cells indicated by the decrease of UV-Vis absorption at 340 nm per minute relative to background.

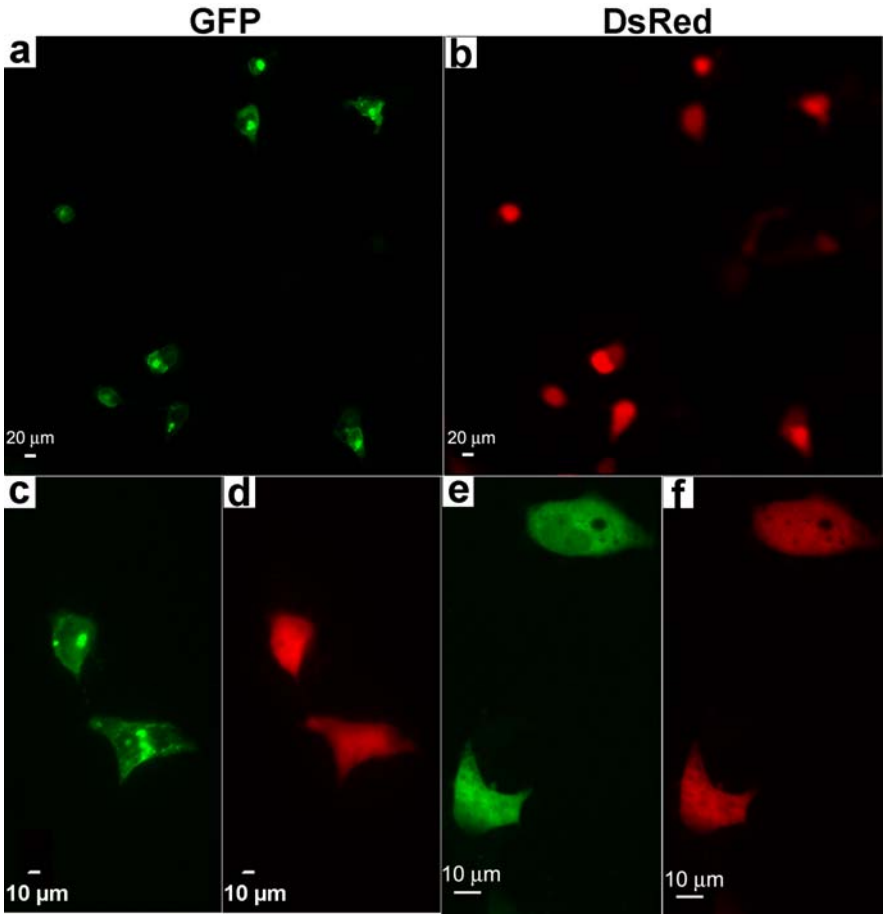

Figure S4. Fluorescence images of COS-1 cell (expressing DsRed/ GFP-TEV –HA-GST) incubated with **1** (a), (c) GFP channel, (b), (d) DsRed channel; without any nanoparticles (e) GFP channel, (f) DsRed channel.

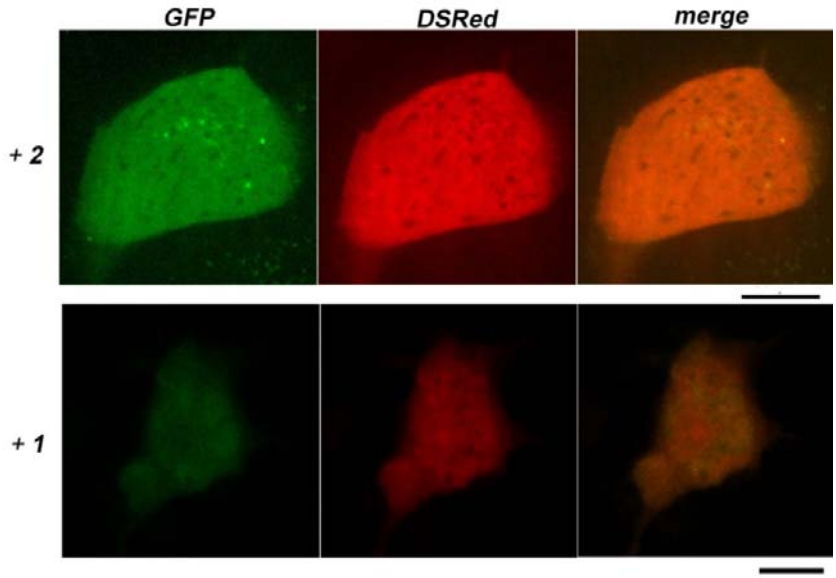

Figure S5. Direct protein sorting inside MCF-7 cells by TMP-decorated iron oxide nanoparticles (**2**). Fluorescence images of a MCF-7 cell, which simultaneously expresses DsRed and GFP-eDHFR-HA, incubated with **2** (top) or **1** (bottom, as the control) (scale bar = 10 µm). The cells were grown in a 96 well plate in complete media. 1 day after plating 25 µg/ml of **1** or **2** were added and the cells were grown for another five days. Then, the media were aspirated, and the cells were washed with PBS and trypsinized. Then, the whole portion was plated on a glass bottom dish, and the cells were grown for 1 day and then transfected with a bicistronic plasmid encoding DsRed and GFP-eDHFR-HA. The cells were visualized one day after the transfection.

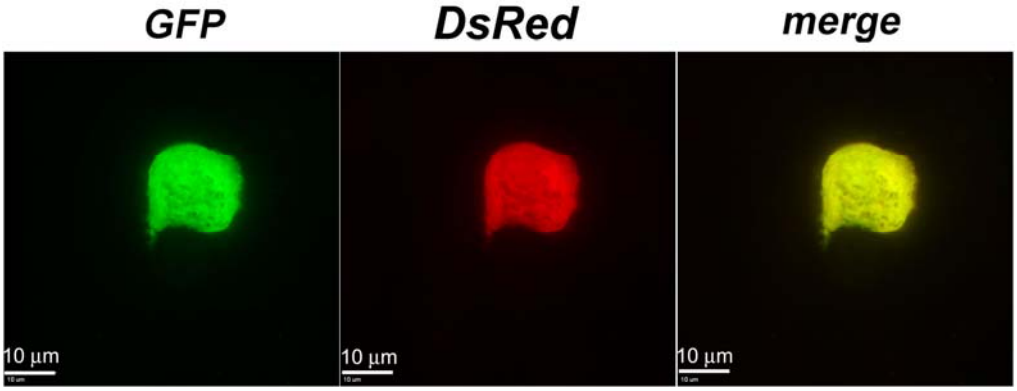

Figure S6. Fluorescence images of the MCF-7 cell (expressing DsRed/GFP-e DHFR-HA) without any nanoparticles. Both the green and red fluorescence are homogeneous.

Molecular mechanics (MM) calculations[5-7] were carried out to simulate the geometry of the pair of GST and GSH ligand, and eDHFR and trimethoprim ligand using the CHARMm Force Field[8] as implemented in the molecular modeling programs (Accelrys Inc., San Diego, CA, USA). From the optimized geometry of the pair of **1** and GST, **2** and eDHFR, there is still enough space for dopamine moiety to bind to the nanoparticle without causing significant steric effect. The iron oxide nanoparticles with 6 nm in diameter were built-up from the crystal structure of the Fe_3O_4.

References

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