Electronic Supplementary Material (ESI) for Chemical Science
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Glutathione (GSH)-Decorated Magnetic Nanoparticles for Binding Glutathione-S-transferase (GST) Fusion Protein and Manipulating 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. ^{1}H NMR spectra were obtained on Varian Unity Inova 400 spectrometer, LC-MS on Waters Acquity ultra Performace LC with Waters MICROMASS detector, and TEM on Morgagni 268 transmission electron microscope.

Synthesis and characterization

Synthesis of **3**: dopamine (**1**, 0.382 g, 2 mmol) was added to maleic anhydride (**2**, 0.491 g, 5 mmol) in acetic acid. The reaction mixture was refluxed for 10 hours and further stirred overnight at room temperature. The product was dried by a rotary evaporator and subjected to HPLC purification. Compound **3** was obtained with 40% yield. ^{1}H NMR(CD_3OD): 6.75 (s, 2H) , 6.65 (d, 1H), 6.6 (d, 1H), 6.4 (dd, 1H), 3.6 (t, 2H), 2.7 (t, 2H).

Synthesis of **5:** Compound **3** (0.233 g, 1 mmol) in 5 mL methanol was dropwisely added to glutathione (**4**, 0.307 g, 1 mmol) in 4 mL PBS (pH=7.6). Then the mixture was stirred for 5 hours under nitrogen atmosphere. The reaction mixture was subjected to HPLC purification to afford **5** with 80% yield. ^{1}H NMR(CD_3OD): 6.65 (d, 1H), 6.6 (d, 1H), 6.4 (dd, 1H), 4.7 (m, 1H), 4.05 (m, 1H), 3.8 (m, 3H), 3.6 (t, 2H), 3.2-2.8 (m, 2H), 2.7 (m, 2H),2.6 (t, 2H), 2.5-2.3 (m, 2H), 2.3-2.1 (m, 2H). ^{13}C NMR (CD_3OD): 178.8-178.7, 176.7, 174.5, 172.7, 171.5, 146.2, 144.9, 130.7, 121.3, 117.0, 116.4, 54.3, 53.7-53.5, 41.9-41.2, 40.4, 37.0-36.7, 34.6, 33.6, 32.4, 27.0. MS: calc. M =540.15, obsvd. $(M-1)^-$ = 539.46.

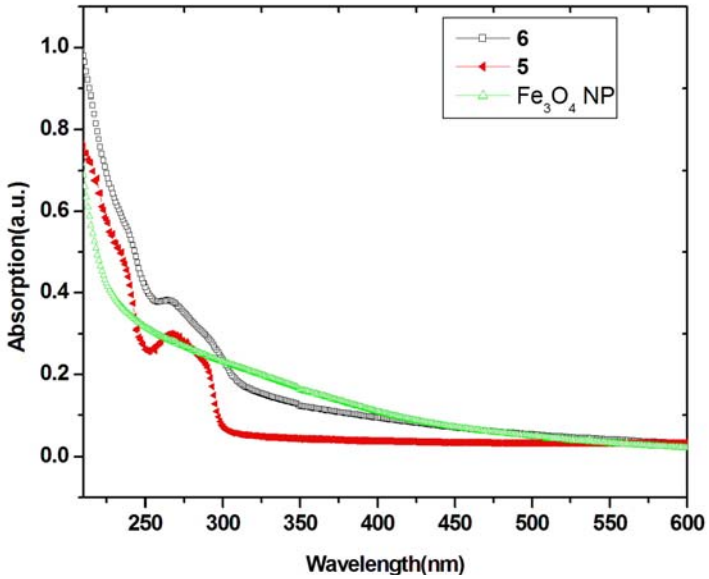

Figure S1. UV-Vis spectra of Fe_3O_4 nanoparticles, **5** and **6.**

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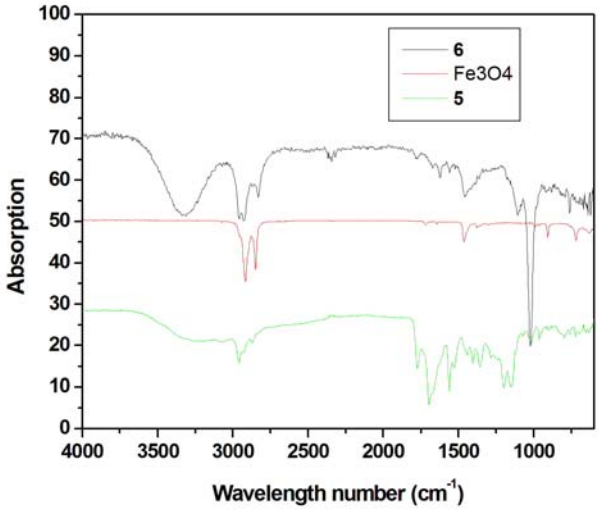

Figure S2. Infrared spectra of Fe_3O_4 nanoparticles, **5** and **6.**

Table S-1. The estimation of the number of **5** on the surface of the nanoparticle **6**.

Nanoparticle	Fe_3O_4
Average size (nm)	6
Total weight of particles (mg)	10
Total # of particles	$2.1x10^{16}$
Total weight of **5** (mg)	0.9
Total # of **5**	$1.0x10^{18}$
# of **5** per particle	48

Investigation of binding condition

We optimized the condition of using **6** to bind GST protein from an *E. coli* lysate by sequentially changing a range of variables and additives: glycerol, Triton X-100, pH, and salt concentration. The following conditions were found to be optimal: no additives, phosphate buffer, pH 6.6, and no salt. We observed that the binding capacity would be much lower when we used the condition suggested by most commercial sources (PBS, pH 7.6) that sell microbeads. Incubation of lysate with **6** for 1-2 hours; magnetic attraction: 10 minutes; washes instantaneous; elution for 1 hour.

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Expression of the fusion proteins and analysis

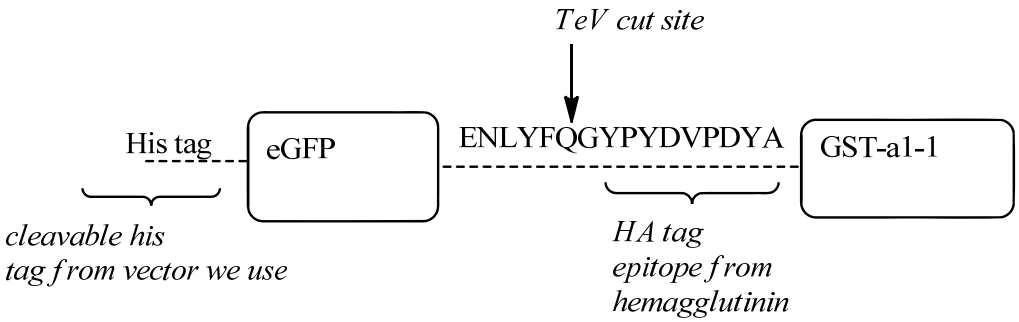

Figure S3. Illustrated domains of His_6GFP-TeV-HA-GST fusion protein.

This construct was cloned into a pET 28a vector and verified by sequencing/fluorescence/activity. Envisioning that a medium length linker (16 amino acid residues) would reduce domain-domain interaction, we designed a linker consisting of both a TEV protease (a highly specific cysteine protease from the tobacco etch virus) recognition sequence, to allow liberation of the POI under mild conditions, and an HA sequence (an epitope derived from hemagglutinin for which highly specific/sensitive antibodies are available), which would allow us to show that the protein we elute is indeed the fusion protein. This construct has several advantages: cleavable GFP; HA gives unambiguous identification with high sensitivity; medium linker means domains do not interact; GFP is on N-terminus so it will not block the GSH binding site (although GST is often substituted on either end).

GST α-1 (from pOXO4-1) was PCR amplified using the following primers: (forward) GTA TTT CCA GGG CTA CCC GTA CGA CGT TCC GGA CTA CGC G*AT GGC AGA GAA GCC CAA GCT* (reverse) CTC AGC TTC CTT TCG GGC TTT GTT A*TT AAA ACC TGA AAA TCT TCC TTG CTT C*; this product was amplified using the following primers: (forward) ATC ACT CTC GGC ATG GAC GAG CTG TAC AAG GAA AAC TT*G TAT TTC CAG GGC TAC CCG TAC* and the reverse primer from before. eGFP was amplified successively using the following forward and reverse primers: (forward each time) TGG TGC CTC GTG GTA GCC AT*A TGG TGA GCA AGG GCG*; (reverse 1) CGG AAC GTC GTA CGG GTA GCC CTG GAA ATA CAA GTT TTC *CTT GTA CAG CTC GTC CAT GCC*; (reverse 2) AAG TAG TGG AGC TTG GGC TTC TCT GCC ATC GCG TAG TC*C GGA ACG TCG TAC GGG TAG*. Italics indicate homology with template.

The final products of each two step sequence (which have homology and can aneal together/prime a PCR reaction of each other) were mixed 1:1 and PCR amplified (no primers). The resulting mixture was PCR amplified using the following primers: (forward) ATG GGC AGC AGC CAT CAT CAT CAT CAT CAC AGC AGC GGC CTG GTG CCT CGT GGT AGC CAT; (reverse) TAT GCT AGT TAT TGC TCA GCG GTG GCA GCA GCC AAC TCA GCT TCC TTT CGG GCT TTG TTA to give predominantly one band on a 1% agarose gel. This product was used as a "mega primer" in a PCR reaction using ECOR1 linearized

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pET28a vector as a template. This crude sample was used to transform, *E. coli* (XL10) and resulting colonies were analyzed by colony PCR and sequenced. Clones 22 and 19a were verified to be correct.

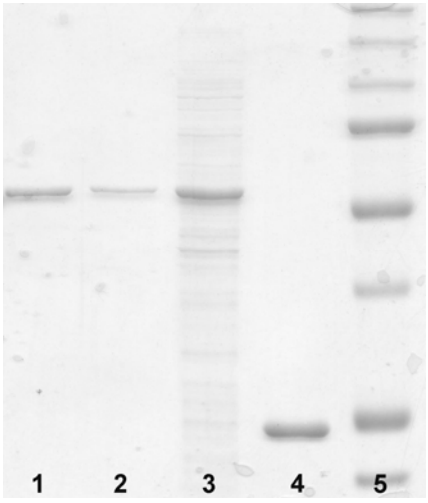

Figure S4. SDS/PAGE analysis of His_6GFP-TeV-HA-GST after purification. The lanes are: lane 1: 5 μg of GFP-TEV-HA-GST; lane 2: 2.5 μg GFP-TEV-HA-GST; lane 3: *E. coli* lysate overexpressing GFP-TEV-HA-GST; lane 4: 8μg GST-a1; lane 5: Molecular weight marker.

The recombinant protein (His_6GFP-TeV-HA-GST) has a predicted weight of 57 KD (GST around 23 KD) and more than doubled the weight of GST by adding GFP to it. The GST-GFP protein was expressed in BL21 (DE3) cells as follows: plasmid 22 was transformed into BL21 (DE3) chemically competent cells by heat shock (42^{o}C 1 minute allowed to rest on ice for 4 minutes and grown for 1 hour in Luria broth for 1 hour) and plated on Luria broth/agar Kanamycin (25 μg/mL) plates. A single transformant was picked from the plate and added to Luria broth/50μM kanamycin and grown at 37^{o}C overnight. This culture was diluted into a 1 L culture of Luria broth/50 μg/mL kanamycin and grown at 37^{o}C to an OD of 1. This was cooled to 16^{o}C and induced with 500 μM IPTG for 18 hours. Cells were pelleted by centrifugation (6000 rpm) and resuspended in lysis buffer (50mM phosphate pH 7.6, 50 mM NaCl, 10% glycerol). Cells were lysed by sonication. Cell debris was removed by centrifugation (14000 RPM) and the GFP-GST was purified by sephadex-glutathione affinity chromatography: elution 15 mM GSH, 50mM Tris, pH 8.0, 50 mM NaCl; final protein dialyzed (50 mM Tris, pH 8.0, 50 mM NaCl, 15% glycerol) such that GSH concentration at femtomolar levels.

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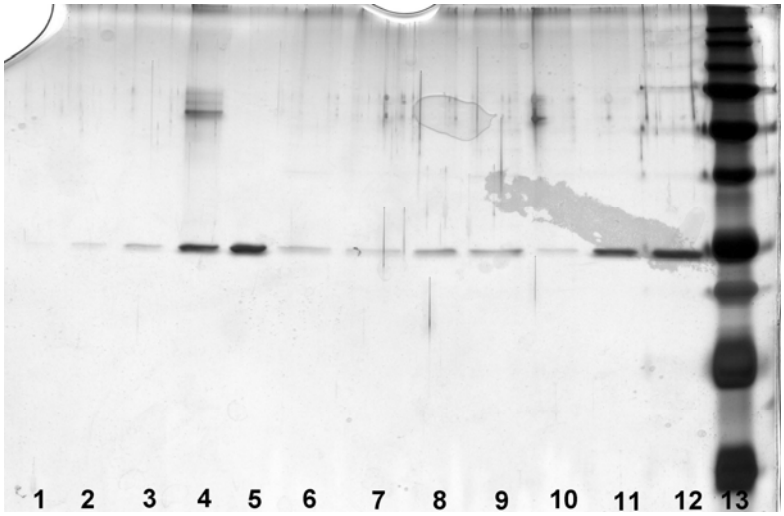

Figure S5. SDS/PAGE analysis (by silver staining) of the affinity of 6 to GST: 4 ng (lane 1), 20 ng (lane 2), 40 ng (lane 3), 200 ng (lane 4), 400 ng (lane 5) of pure GST in 20μL PBS buffer; first (lane 8), second (lane 7), and third (lane 6) washes of the nanoparticles by a PBS buffer (50 mM); lane 10 and lane 9 are elutions by 30 mM and 100 mM GSH solutions , respectively; GST solution before (lane 12) and after (lane 11) incubating with 6; and molecular weight marker (lane 13).

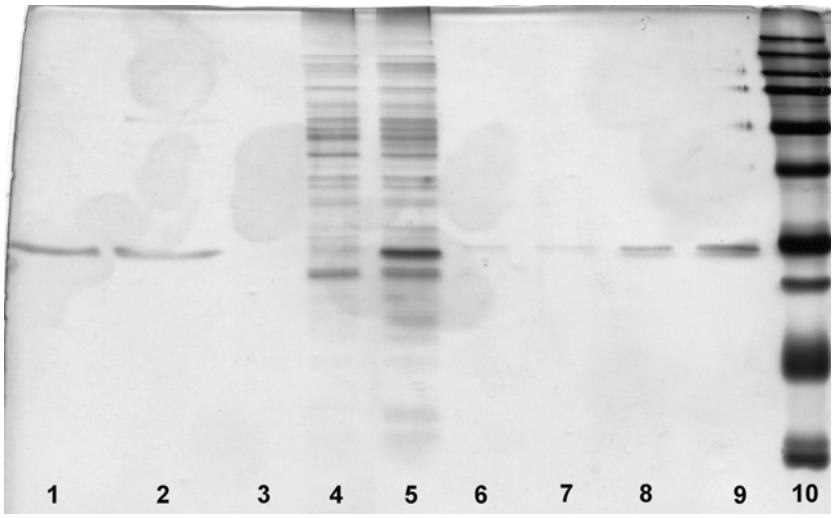

Figure S6. SDS/PAGE analysis (by silver staining) of the binding of **6** to GST in an *E. coli* lysate (1/3 of total volume in each lane).The lanes are: lane 1*:* elution overnight by GSH; lane 2: elution 2 hours by GSH (300mM GSH in 500 mM Tris, 150 mM NaCl, pH 8.0); lane 3: 1st wash; lane 4: lysate after the removal of **6**; lane 5: cell lysate; lane 6: 50 ng, lane 7: 100 ng, lane 8: 200 ng, lane 9: 400 ng of GST; lane 10: molecular weight marker.

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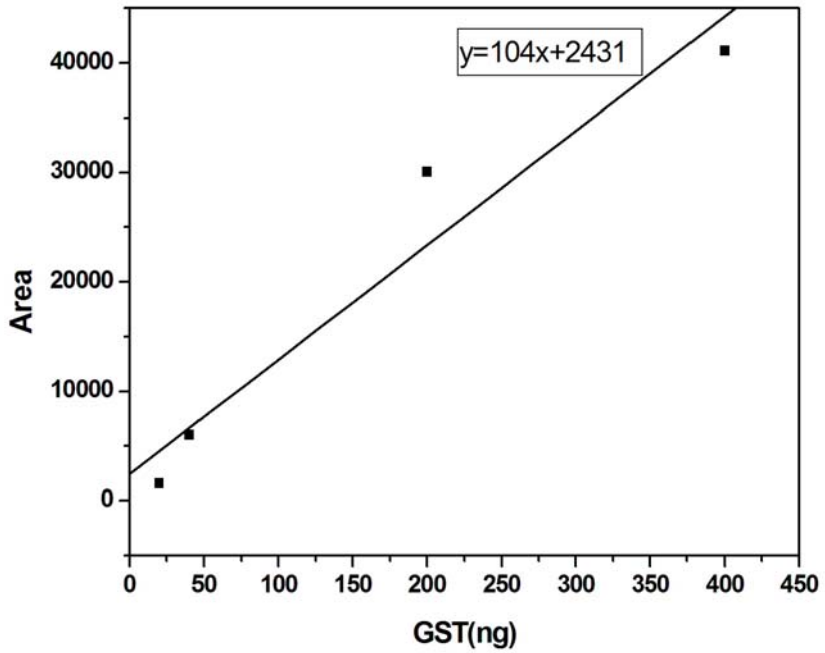

Figure S7. Calibration curve of GST calculated by processing the standards in Fig S4 lanes 2-5 using Image J software.

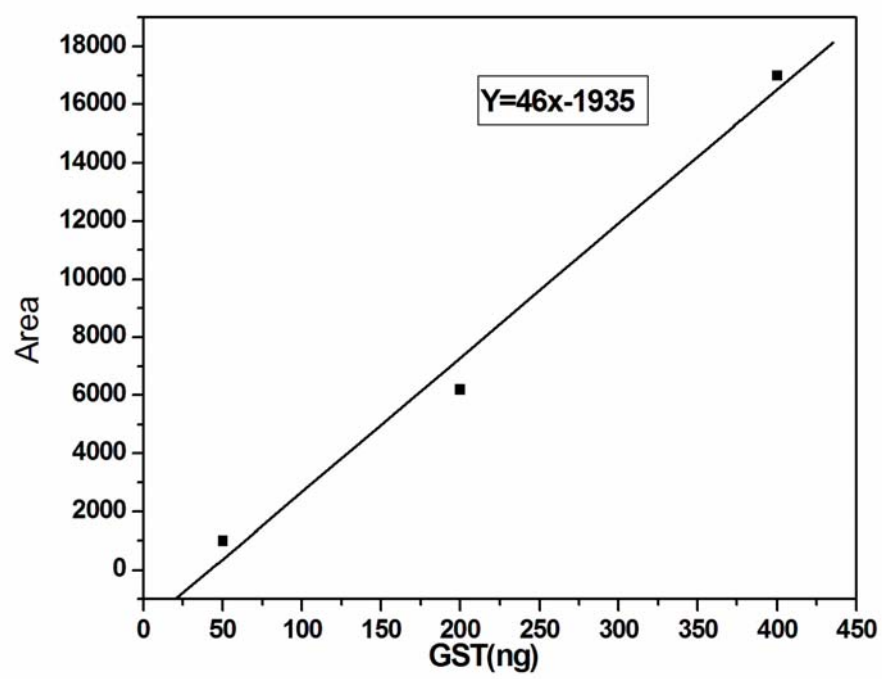

Figure S8. Calibration curve of GST calculated by processing the standards in Fig S5 lanes 6, 8, and 9 using Image J software.

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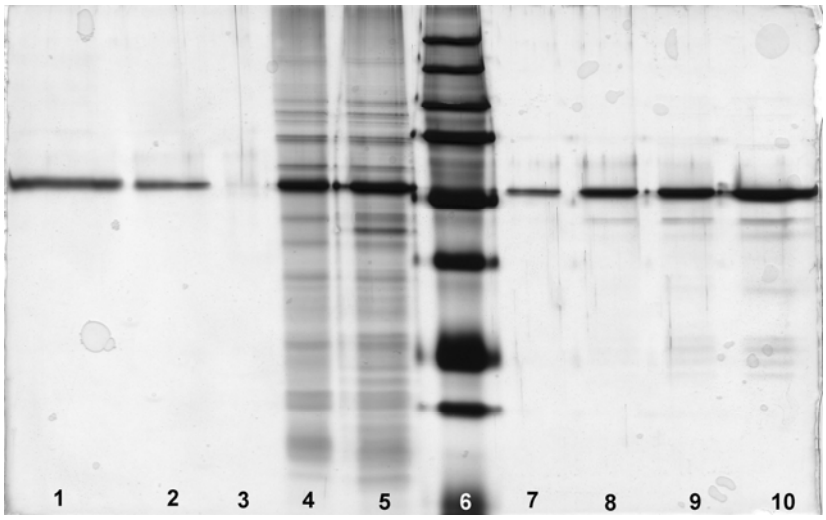

Figure S9. SDS/PAGE analysis (by silver staining) of the binding of **6** to GFP-TEV-HA-GST in an *E. coli* lysate (lane 2, 1/10 of total volume; the other lanes, 1/3 of total volume in each lane): lane 1: elution by GSH (1/3 of total volume) , lane 2: elution by GSH (1/10 of total volume) (300 mM GSH, 500 mM Tris, pH 8.0, 150 mM NaCl); lane 3: 1st wash; lane 4: lysate after the removal of **6**; lane 5: cell lysate; lane 6: molecular weight marker.; lane 7: 100 ng, lane 8: 500 ng, lane 9: 1000 ng, lane 10: 2000 ng of GFP-TEV-HA-GST.

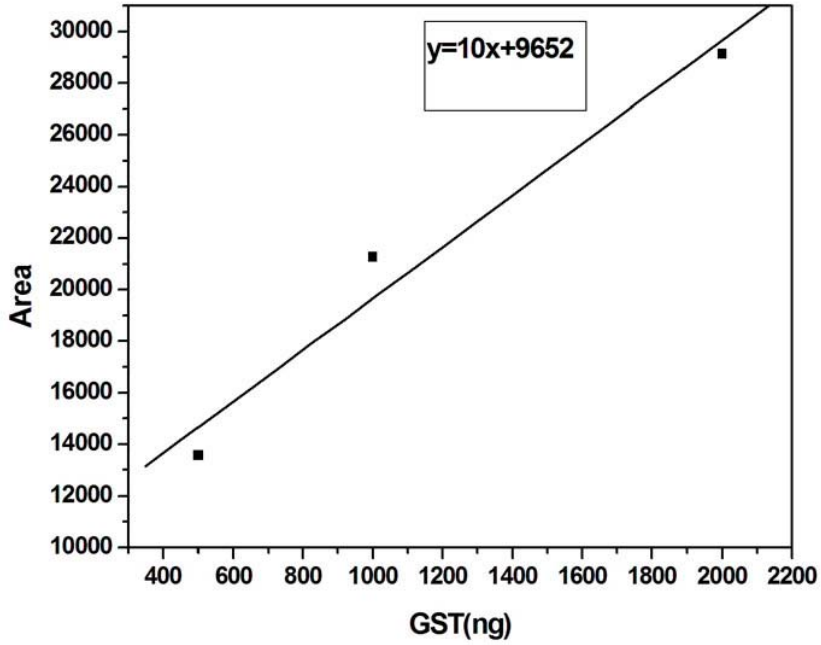

Figure S10. Calibration curve of GFP-TEV-HA-GST calculated by processing the standards in Fig S8 lanes 8, 9, and 10 using Image J software.

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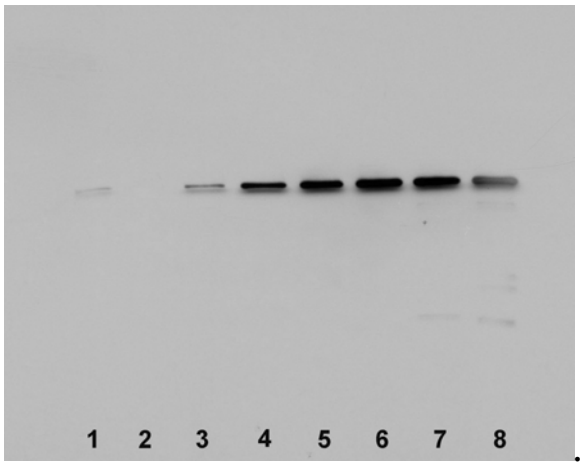

Figure S11. Anti-HA western blot: lane 1: Eluted protein; lane 2: 5ng; lane 3: 10ng; lane 4: 20 ng; lane 5: 50 ng; lane 6: 100 ng of GFP-TEV-HA-GST; lane 7: cell lysate diluted to 1:1000; lane 8: cell lysate diluted to 1:2000

Anti-HA western blot protocol: Protein was loaded onto a 12% denaturing PAGE gel. The resulting gel was transferred onto a polyvinyldifluoride membrane at 100V for 1.2 hours in Towbin buffer (14.4g glycine, 3.4 g Tris, 10% methanol, no pH adjustment). The membrane was blocked in 2% milk protein overnight in TBS Tween (100 mM Tris, 500 mM NaCl, pH 7.6, 0.5% Tween). Membrane was washed three times with TBS Tween then exposed to anti-HA [clone 3F10, Roche Diagnostics ® (3 μL of manufacturers recommended concentration into 15 mL)] in TBS Tween for 2 hours. Membrane was washed in TBS Tween twice for 10 minutes each then TBS for 15 minutes. The membrane was visualized using ECL plus (Amersham) and the bands were observed by exposure to film (HyBlotCL Denville Scientific).

From Fig. S11, we can find that there are no non-specific bands in the lysate lanes. The anti-HA western blot proved that this is the GFP-TEV-HA-GST protein. HA is hemagglutinin from the influenza virus, so no native *E. coli* proteins will have this epitope.

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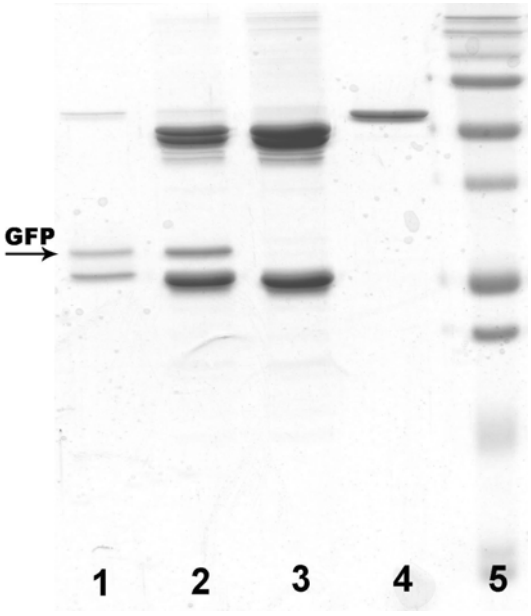

Figure S12. SDS/PAGE analysis (by coomassie staining): lane 1: 12 hours treatment (cleavage) of the conjugate of **6** and GFP-TEV-HA-GST with ProTEV protease; the solution after the removal of **6** (note new band around 28 kD, which is GFP); lane 2: pure GFP-TEV-HA-GST cleaved by ProTEV; lane 3: ProTEV protease; lane 4: pure GFP-TEV-HA-GST; lane 5: molecular weight marker.

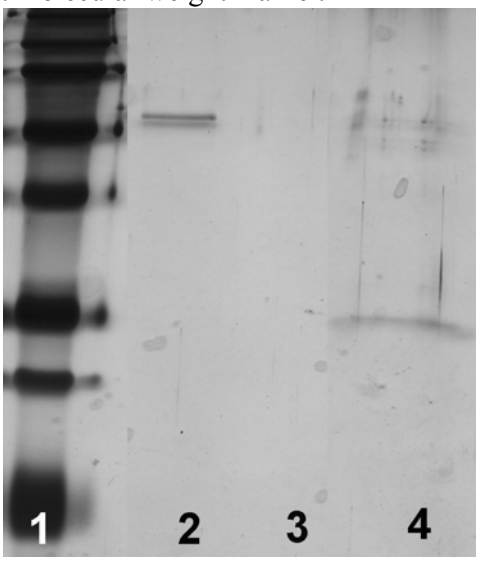

Figure S13. SDS/PAGE analysis (by silver staining): lane 1: molecular weight marker; lane 2: wash by 50 mM sodium phosphate buffer at pH 7.6; lane 3: final wash (no protein comes off); lane 4: after 12 hours treatment (cleavage) of the conjugate of **6** and GFP-TEV-HA-GST with ProTEV protease, then elution by 300 mM GSH to get pure GST.

The procedure for obtaining Figures S12 and S13: **6** pretreated with GFP-TEV-HA-GST pure protein following the optimized conditions. After several washes to get rid of all the GFP-TEV-HA-GST unbound to **6**, we treated the conjugate of **6** and GFP-TEV-HA-GST with ProTEV protease for 12 hours. Then we eluted the residue **6** by GSH (300 mM GSH, 500 mM Tris, pH 8.0, 150 mM NaCl) and got pure GST.

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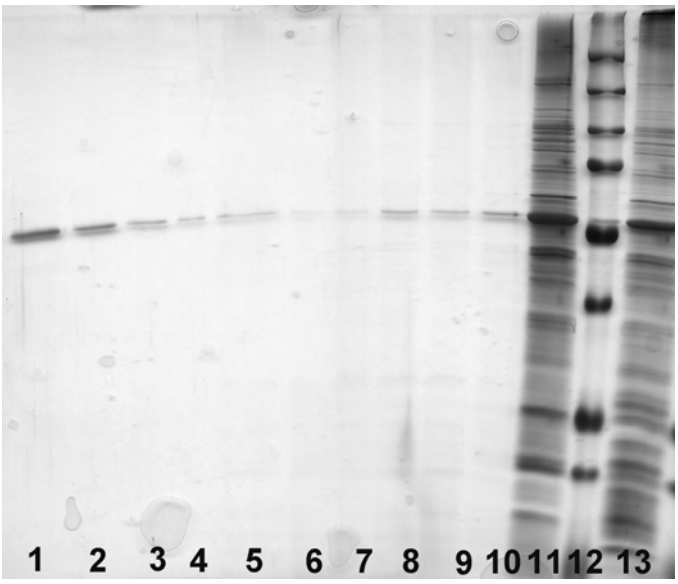

Figure S14. SDS/PAGE analysis (by silver staining) of the binding of **6** (reused) to the GFP-TEV-HA-GST in an *E. coli* lysate: 200 ng (lane 1), 100 ng (lane 2), 50 ng (lane 3), 20 ng (lane 4) of pure GFP-TEV-HA-GST; lane 5 is elution by 100 mM GSH in 50 mM Tris, 150 mM NaCl, and pH 8.0 , 5th-1st washes of the nanoparticles by a 50 mM PBS buffer (lane 6-10), lysate after the removal of **6** (lane 11), molecular weight marker (lane 12), cell lysate (lane 13).

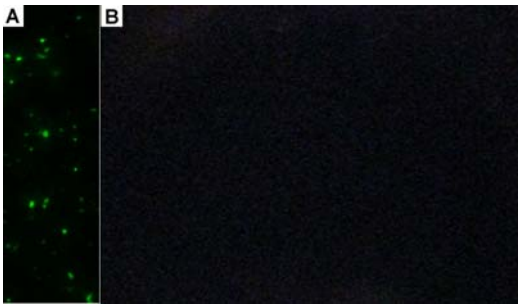

Figure S15. Fluorescent images of the nanoparticles (**6**) after being incubated with (A) GFP-TEV-HA-GST, (B) DHFR-GFP (using the same procedure as for A).

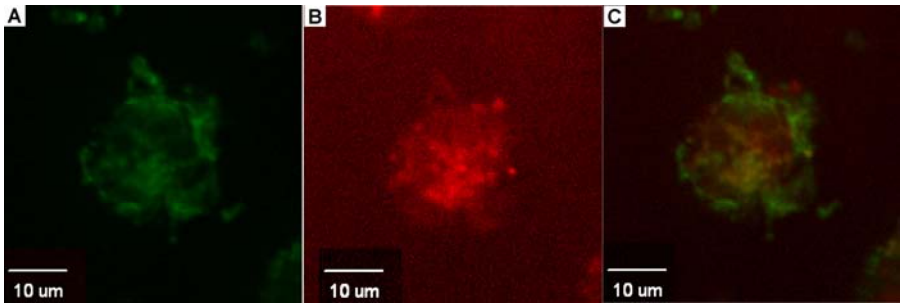

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Figure S16. (A, B, C) Confocal images of COS cell after incubated with the conjugate of **6** and GFP-TEV-HA-GST and RITC–Dextran for 12 hours. (A) GFP channel, (B) RITC channel, (C) overlay.

We added the conjugate **6** and GFP-TEV-HA-GST to the COS cell subsequently with 40μg/ml rhodamine B isothiocyanate(RITC)–Dextran and incubated for 12 hours. After the RITC-dextran permeated into the cell, after several washes to remove extracellular RITC-dextran, the confocal images show a distribution of the conjugate of **6** and GFP-TEV-HA-GST (green) in the cells.

Magnet-guided cell migration

For magnet-guided migration, the HeLa cells were grown to 80% confluence, in a 6 well plate in Dulbecco's Modified Minimal Essential Medium (Hyclone ®) supplemented with 10% fetal bovine serum and 1% glutamine (Sigma-Aldrich) (no antibiotics were added). At this point **6** pretreated with GFP-TEV-HA-GST as the optimized conditions were added to the cells and incubated overnight at 37^{o}C. After 12 hours, media was aspirated, and cells were washed 2 times with PBS (Hyclone) and then were treated with 0.25% trypsin (1mL, Gibco®) for 5 minutes and suspended in PBS (Hyclone®) supplemented with 2% fetal bovine serum. Cells were pelleted by centrifugation at 700g for 5 minutes; supernatant was aspirated and then cells were suspended in 50% trypan blue (Aldrich) solution in PBS and incubated at room temperature for 5 minutes. Cells were mounted on a hemocytometer and attracted by a magnet.