Supporting information

Selective protein purification by PEG-IDA-functionalized iron oxide nanoparticles

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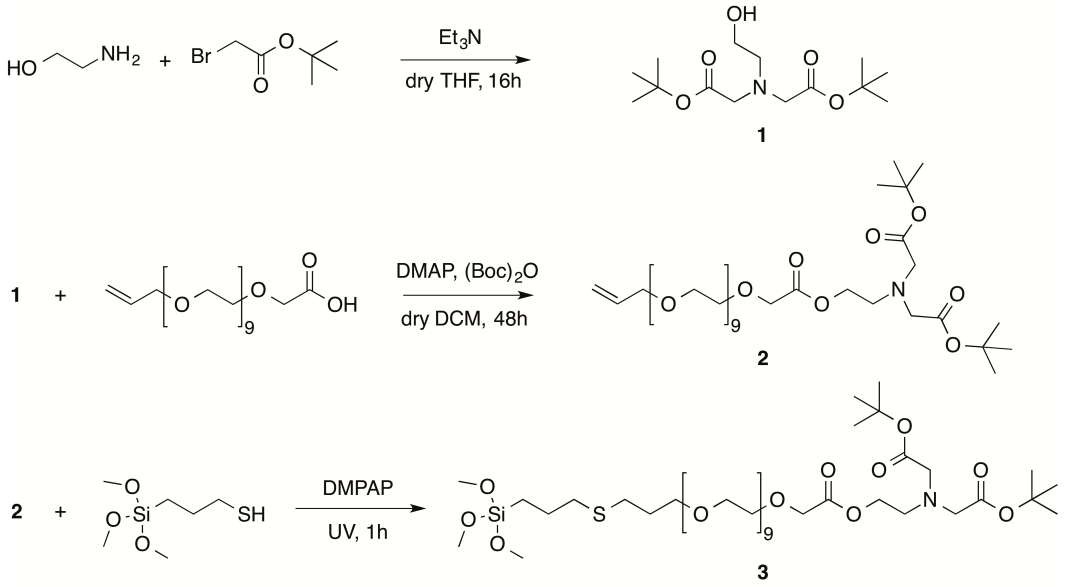

Experimental

Materials and methods

2,2-dimethoxy-2-phenylacetophenone (DMPAP, 99%), 4-dimethylaminopyridine (DMAP, >99%), ethanolamine (>99%), sulfuric acid (97%), trifluoro acetic acid (TFA, 99%) and mercaptopropyltrimethoxysilane (95%) were purchased from Sigma Aldrich. Allyl-PEG10-OH was obtained from Polysciences, Inc. Triethylamine and potassium dichromate were ordered at Janssen Chimica. *Tert*-butyl bromoacetate (99%), nickel(II+)chloride hexahydrate (97%), sodium sulfate (99%) and di-*tert*-butyl dicarbonate (Boc_2O, 99%) were purchased at Acros Organics. Toluene was ordered from Fisher Chemical. Diethylether was obtained from VWR.

All ultrasonication steps were performed in a Branson 5510 sonicator bath. Fourier transform infrared (FTIR) spectra were measured using a Bruker Alpha FT-IR spectrometer equipped with a Platinum ATR module. UV–Vis Spectrometry was performed on a Perkin Elmer Lambda 900 spectrometer. Prior to the measurement, all samples were centrifuged for 60 minutes at 15000 rpm, 4°C in an Eppendorf 5425R centrifuge to remove any possible nanoparticle background.

Ligand synthesis

N,N-Bis[(*tert*-butoxycarbonyl)methyl]-2-ethanolamine (**1**): *Tert*-butyl bromoacetate (12.6 mmol, 1.86 mL, 2.1 eq) and triethyl amine (13.2 mmol, 1.84 mL, 2.2 eq) were added to 10 mL of dry tetrahydrofuran. While stirring at room temperature, ethanolamine (6 mmol, 0.362 mL, 1 eq) was added slowly. After stirring for 16 hours, 50 mL of diethylether was added. The reaction mixture was washed with a saturated sodium hydrogencarbonate solution (2x 50 mL). This aqueous layer was extracted with diethylether (2x 50 mL), after which all ether fractions were combined. After washing with a saturated sodium chloride solution (2x 50 mL), the organic layer was dried with anhydrous sodium sulphate and concentrated under reduced pressure. The raw product was purified further by column chromatography (silica, heptane/diethylether, 2/1 to 1/1). This yielded a yellow liquid (1.19 g, 4.17 mmol, 70%, 100% purity). Rf: 0.2; ^{1}H NMR (300 MHz, $CDCl_3$): (ppm) 1.47 (s, 18H), 2.88 (t, 2H), 3.47 (s, 2x2H), 3.53 (t, 2H).

Allyl-PEG-IDA-*t*Bu (**2**): Allyl-PEG_{10}-OH was first oxidized to introduce the carboxylic acid. Potassium dichromate (17 mmol, 5 g) was dissolved in 21 ml of water, to which sulfuric acid (98%, 3.9 mL, 73 mmol) was added. This red mixture was slowly added to a solution of allyl-PEG_{10}-OH (6 mmol, 3 g) in 150ml of acetone. After 4 hours of stirring at room temperature, the mixture had turned green and was filtered. All acetone was removed under reduced pressure and 100 ml of water was added. This solution was extracted with dichloromethane (DCM, 3x 75 mL). After drying of sodium sulphate, the organic layer was concentrated under reduced pressure to obtain the carboxylic acid-terminated allyl PEG molecule.
To allyl-PEG-COOH (1 mmol, 514 mg, 1 eq), dissolved in 5ml of dry DCM, was added product **1** (1 mmol, 289 mg, 1 eq), DMAP (0.05 mmol, 6 mg, 0.05 eq) and di-*tert*-butyl dicarbonate (1.1 mmol, 240 mg, 1.1 eq).[22] This mixture was stirred for 48 hours at room temperature, after which 15 ml of DCM was added. The organic phase was washed with a hydrogen chloride (1 M, 50 mL), sodium hydrogen carbonate (saturated, 2x 50 ml) and sodium chloride (saturated, 3x 25 mL) solution. Afterwards the DCM layer was dried over sodium sulphate and concentrated under reduced pressure. The product (**2**) was obtained as a pale yellow liquid (584 mg, 0.74 mmol, 74%, 95% purity). ^{1}H NMR (300 MHz, $CDCl_3$): (ppm) 1.45 (s, 18H), 3.04 (t, 2H), 3.46 (s, 2x2H), 3.65 (m, 36H), 4.03 (d, 2H), 4.17 (s, 2H), 5.15-5.30 (m, 2H), 5.87-5.96 (m, 1H).

Thiol-ene click chemistry

The siloxane end-group was clicked onto the allyl group of molecule **2** based on the procedure published by Tucker-Schwartz *et al.*[23] A mixture of molecule **2** (0.1 mmol, 78.5 mg) and allyl-PEG_{10}-OH (0.9 mmol, 450 mg) was added to mercapto propyl trimethoxysilane (1 mmol, 185 L) and DMPAP (0.05 mmol, 12.8 mg). After adding 0.5 ml of DCM to increase the volume (better illumination), the mixture was illuminated with 360 nm UV-light during 1 hour.[24] The resulting product (**3**) was used without further purification.

Nanoparticle functionalization

Oleic acid coated iron oxide (magnetite) nanoparticles, as synthesized by Park *et al.*, were functionalized with freshly prepared siloxane-PEG-IDA-*t*Bu (**3**) and siloxane-PEG_{10}-OH (10/90 ratio) ligands.[9] The nanoparticles (50 mg) were dispersed in 25 ml of toluene, to which triethylamine (1.25 mL), the clicked siloxanes (0.5 mmol in total) and water (25 μL) was added.[12] This mixture was ultrasonicated for 5 hours. Heptane (50 ml) was added to destabilize the nanoparticles, which were magnetically attracted and washed with acetone and heptane. Finally the particles were dispersed in DCM (10 mg/mL).
Since the IDA group of the ligand is still protected and hence not water soluble, a deprotection reaction was performed.[25] TFA (0.5 mL) was added to the nanoparticle dispersion (1mL) and this mixture was placed on a shaker for 2 hours. The particles precipitated during this process, and where easily collected by decanting the liquid phase. After washing five times with acetone, the functionalized nanoparticles were dispersed in MilliQ (5 mg/mL).

Nickel loading

5 mL of the deprotected nanoparticles, dispersed in water, was mixed with a 5 mL of a 100 mM $NiCl_2$ solution and this mixture was shaken for 30 minutes. After magnetic separation, the particles were washed with MilliQ, weighed and dispersed in MilliQ (5 mg/mL).

Bacteria culture
His-tagged DsRed was expressed in an *E. coli* JM109 culture in LB medium using the pRSet B expression vector. The cells were harvested by centrifugation at 5000 RPM in a Sorvall (R) Evolution RC centrifuge and resuspended in TN buffer. The cells were mechanically lysed using a Sim-Aminco French (R) pressure cell press and centrifuged at 8000 RPM in a Sorvall (R) primo R centrifuge to remove cell debris.
The DsRed control purification was performed using commercial agarose beads functionalized with nickel-NTA (Qiagen) packed in a gravity column in a traditional bench-top protocol.

Magnetic separation
The nickel loaded nanoparticles (3 mg, 600 µL) were mixed with pure cell lysate and shaken for 45 minutes. After magnetic attraction (NdFeB magnet, 30x30x15mm), the supernatant was collected (sample: *supernatant*). The particles were washed with 1 mL of TN buffer (100 mM Tris, 300 mM NaCl, pH 7.4, sample: *W1*) and 1 mL of TNI buffer (100 mM Tris, 300 mM NaCl, 10 mM imidazole, pH 7.4, sample: *W2*) and finally dispersed in 0.5 mL Tris buffer. The His-tagged proteins were released from the nanoparticles by adding 0.5 mL of Tris/imidazole buffer (100 mM Tris, 300 mM NaCl, 1M imidazole) and shaking for 15 minutes. After magnetic attraction, the protein fraction was collected (sample: *elution*). Finally, the particles were washed once more with 1 mL of Tris buffer (*W3*). To reuse the nanoparticles, they were dispersed in 600 µL of Tris buffer.

Gel electrophoresis
The purity of the fractions was verified by SDS-PAGE (Biorad Protean Mini Tetra, 1 mm gel) using a two-part gel consisting of a 4% acrylamide stacking gel and a 12% acrylamide resolving gel. Fragment length was determined using a commercial product (Thermo Scientific PageRuler Prestained Protein Ladder). The running time for a gel was about an hour at 35 mA and the results were visualized by staining with a Coomassie Brilliant Blue R 250 solution.

^{1}H-NMR spectra

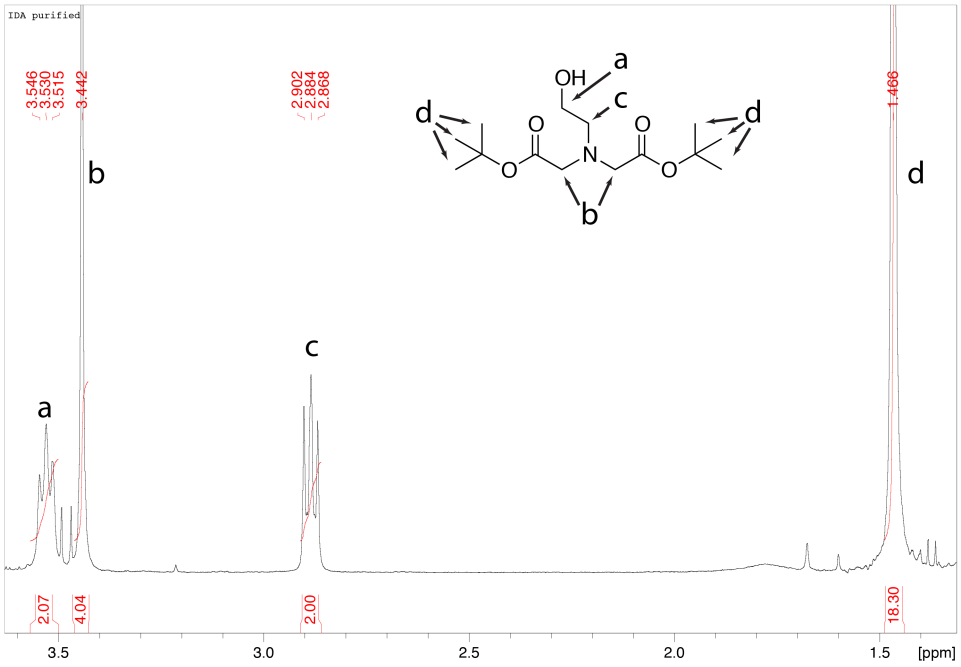

Figure S1: ^{1}H-NMR spectrum of N,N-Bis[(*tert*-butoxycarbonyl)methyl]-2-ethanolamine (**1**).

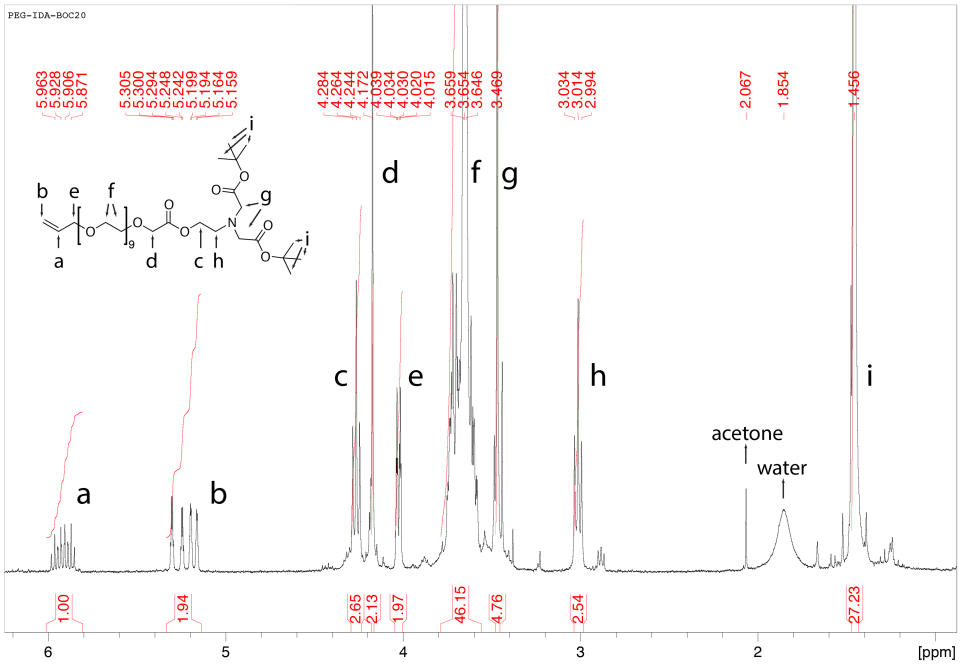

Figure S2: ^{1}H-NMR spectrum of allyl-PEG-IDA-*t*Bu (**2**).

Transmission electron microscopy

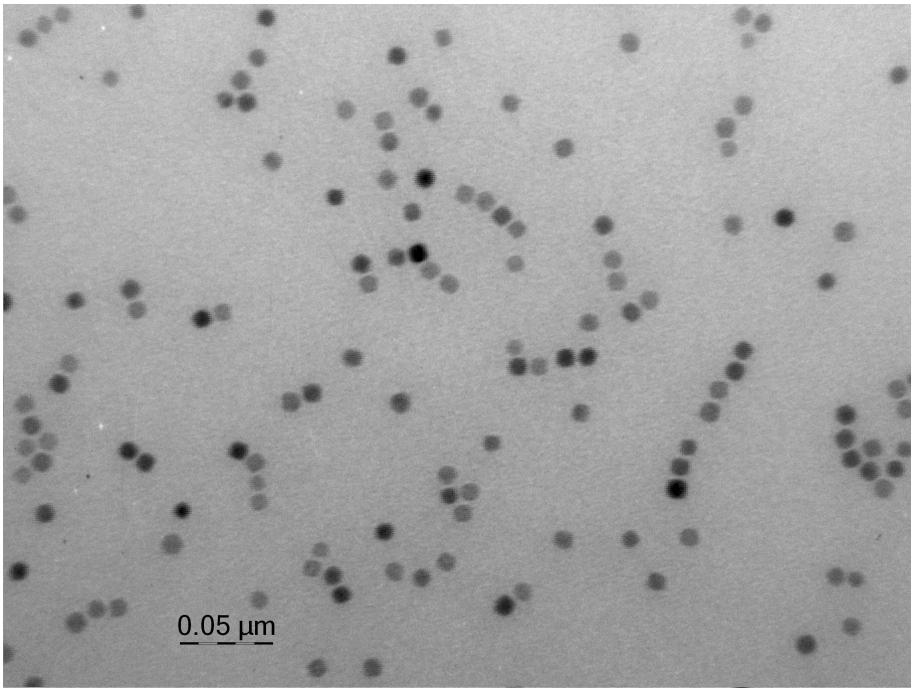

Figure S3: Transmission electron microscopy (TEM) photo of the magnetite nanoparticles (8.6±0.6nm). Their size was determined by ImageJ software.

FTIR spectra

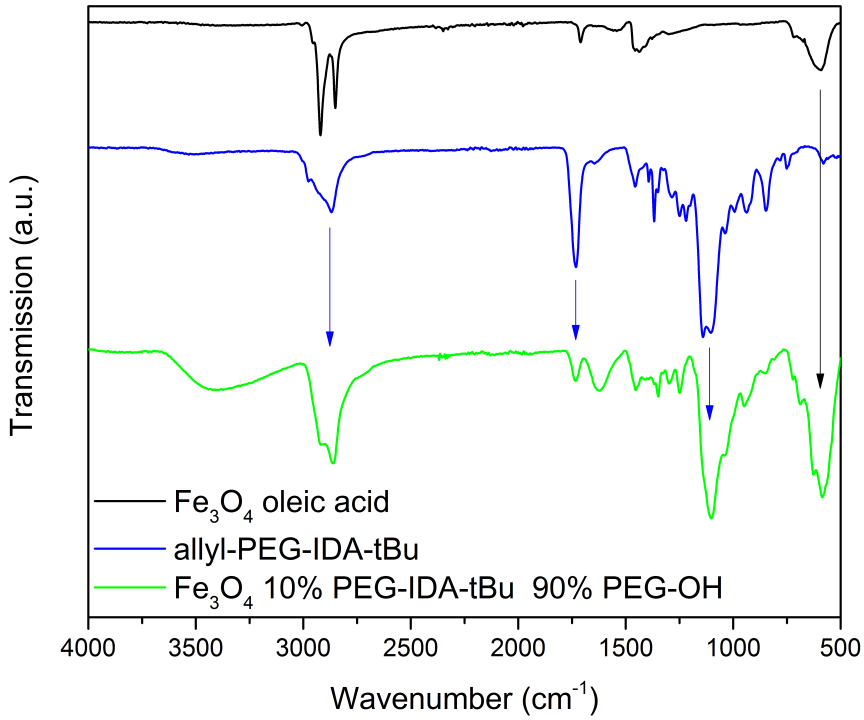

Figure S4: The FTIR spectra confirm a successful functionalization of the iron oxide nanoparticles. In the green spectrum, the Fe-O vibration at 580 cm^{-1} and the characteristic peaks of the ligand (1150 cm^{-1}, PEG/Si-O; 1740 cm^{-1}, ester; 2850-2920 cm^{-1}, C-H) can clearly be seen. The strong C-H vibrations (2850-2920 cm^{-1}) of the initial oleic acid ligand (black curve) are replaced by the slightly broader peak of the PEG C-H vibrations.

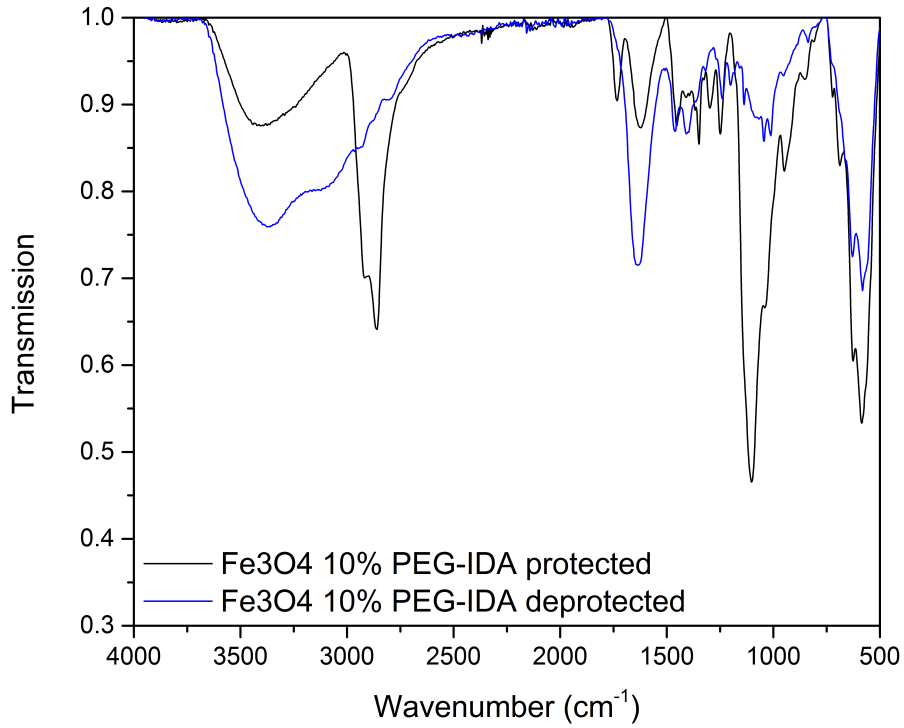

Figure S5: The Fourier transform infrared spectrum of the protected and deprotected nanoparticles have some distinct differences. Firstly, the ester peak at 1740 cm^{-1} disappears after deprotection (blue curve). Secondly, the symmetric and asymmetric stretching of the carboxylate becomes visible at 1630 and 1414 cm^{-1} respectively.

TXRF spectrum

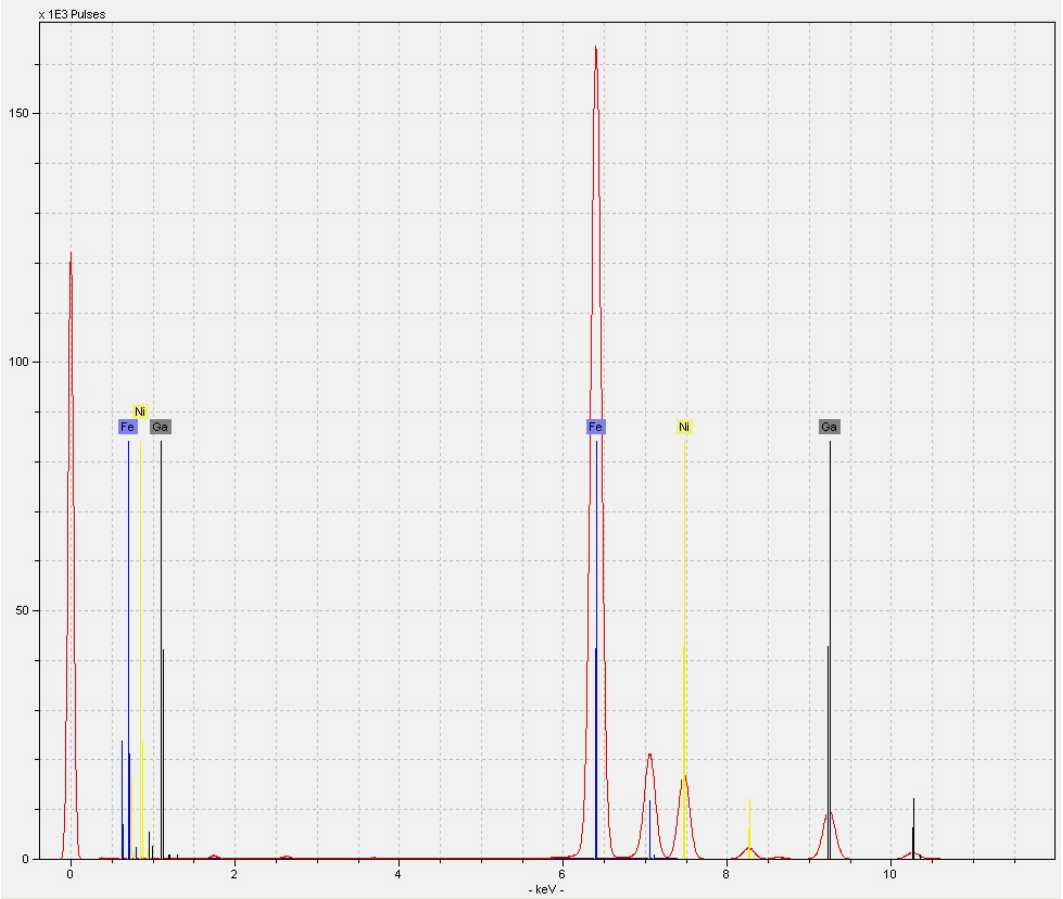

Figure S6: The TXRF spectrum of the iron oxide nanoparticle, functionalized with PEG-IDA and loaded with Ni(II+) ions clearly shows the signal of iron (blue), from the core of the particle, and nickel (yellow). The gallium peak (gray) serves as an internal standard for concentration determination.

UV-Vis data

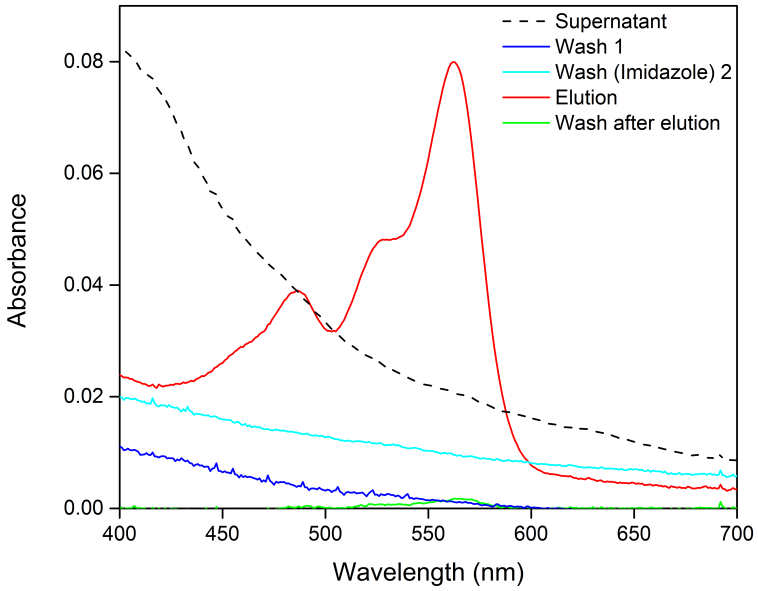

Figure S7: The UV-Vis spectra of the different obtained fractions can clearly show the presence of the fluorescent protein (562 nm). DsRed is present neither in the supernatant nor the washing steps (1-2). In the elution fraction, on the other hand, a large concentration of DsRed can be seen. The final washing step (after the elution) shows a very small peak at 562 nm, indicating a minor presence of fluorescent proteins.

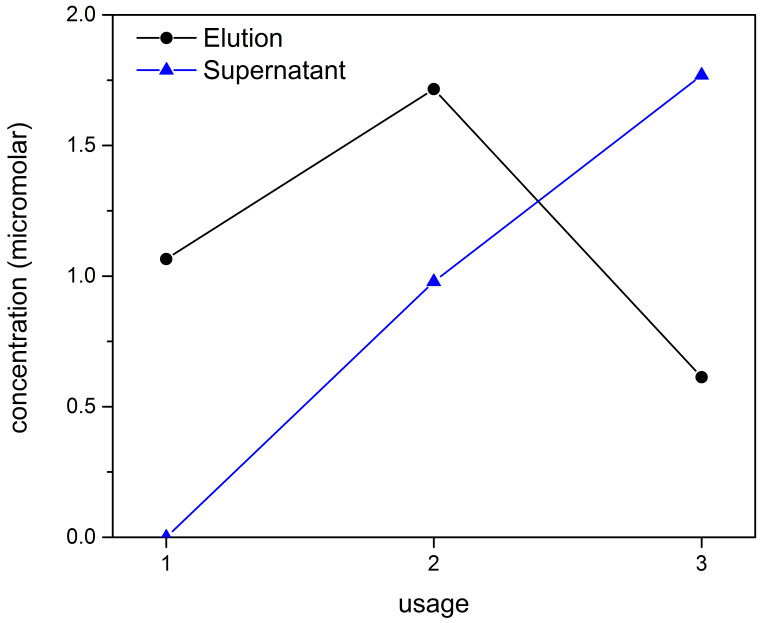

Figure S8: When the nanoparticles are reused for 3 consecutive purifications, the efficiency drops. The concentration of DsRed found in the supernatant increases gradually, while the recovered amount of DsRed (elution) decreases. Data from the absorbance measurements was used to obtain these values.

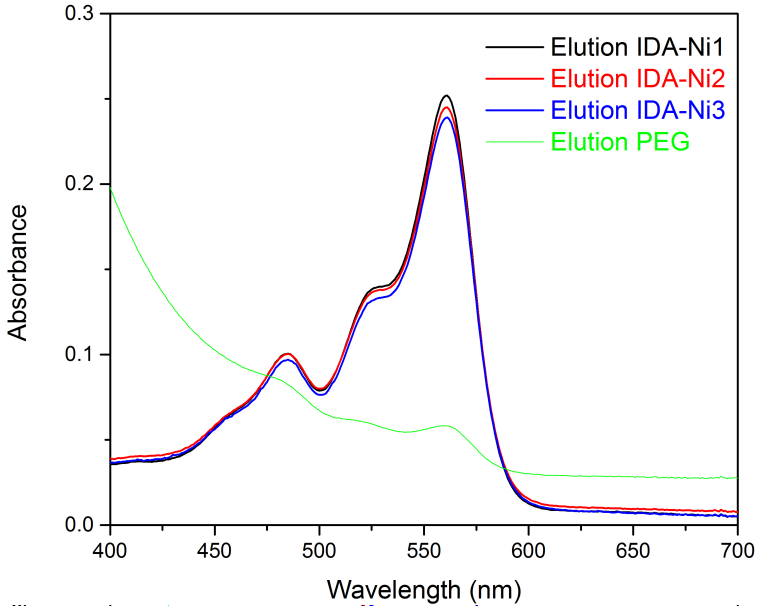

Figure S9: If the NP are reused for 3 consecutive purifications with an intermediate Ni(II+) reloading step, the efficiency remains constant. This is clear evidence that the particles can be reused multiple times. As a comparison, the elution from NP coated with PEG (without IDA or nickel, negative control) is shown as the green curve. No increased concentration of DsRed is visible, the spectrum corresponds to the initial cell lysate, albeit diluted. The concentration of the DsRed in the elution of IDA-Ni coated particles is approximately 150 micrograms/mL.

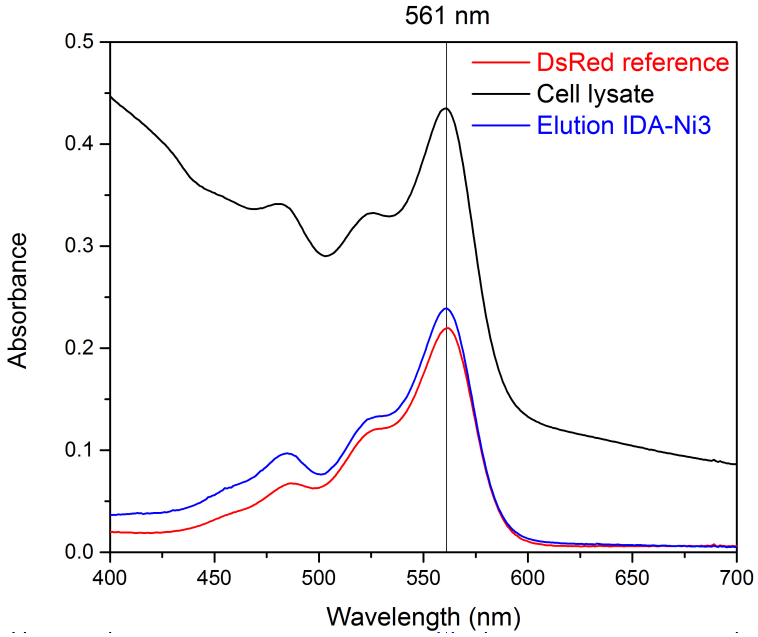

Figure S10: Comparison of the eluted DsRed versus the pure cell lysate and reference DsRed. The peak position is similar, indicating that no damage to the protein structure has occurred.

Gel electrophoresis

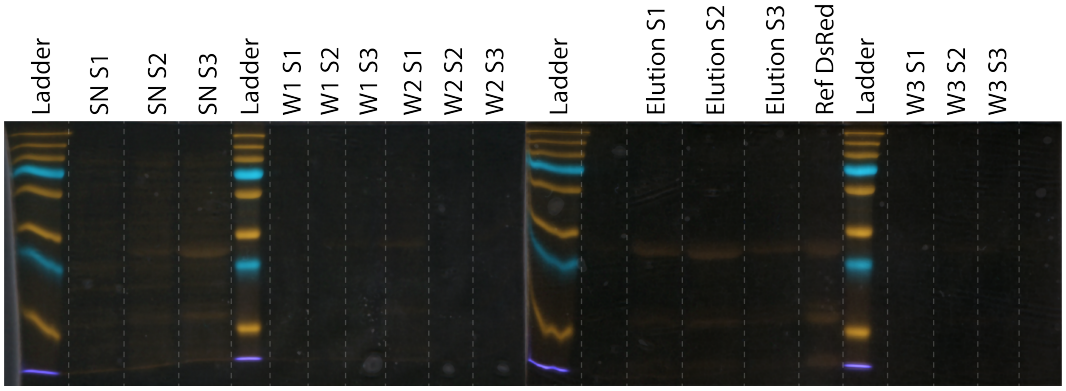

Figure S11: The gel electrophoresis of the obtained fractions gives a strong indication about the purity or presence of cellular debris. For clarity, the images of the gels were color-inverted. S1, S2 and S3 depict the reusing of the nanoparticles. One can clearly see that the supernatant fractions (SN1-3) contain a lot of different proteins, which correspond to the cellular debris. The washing fractions (W1 and W2), on the other hand show almost no bands (except small band in W2 S1). The recovery of the DsRed from the particles is visible in the elution samples. Except for the DsRed bands, no other bands can be seen, which indicates a high purity. Finally an extra washing (W3) of the eluted nanoparticles was performed, but only very little amounts of DsRed were recovered at this stage.

Fluorescence

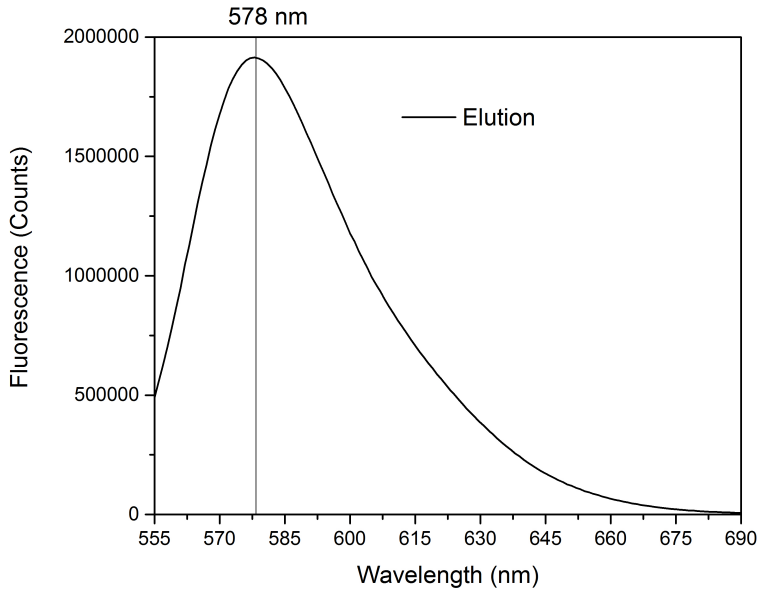

Figure S12: Fluorescence emission spectrum of an elution fraction, containing DsRed proteins. The sample was excited at 540nm and shows a clear emission peak at 578nm, which is typical for this protein.