# Supporting Information

## "Decoration of Superparamagnetic Iron Oxide Nanoparticles with $Ni^{2+}$: Agent to Bind and Separate Histidine-Tagged Proteins"

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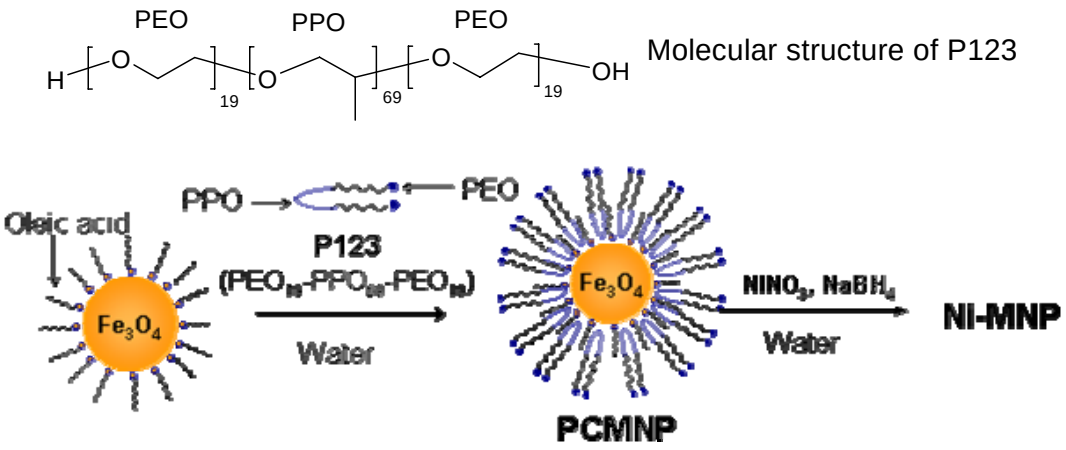
***General consideration.*** Any reagent including $FeCl_3$, $NiNO_3$, $NaBH_4$, Sodium Oleate (TCI), Oleic acid (Aldrich), and P123 (BASF) was used as purchased without any purification. His-tagged GFP and normal mouse $IgG_1$ conjugated by PE-Cy5 were purchased from Santa Cruz Biotechnology and Upstate, respectively. Photoluminescnet spectra were gathered on a Perkin-Elmer LS 50B with an excitation wavelength of 400nm (for GFP) and 630nm (for Cy5). Analyses of transmission electron microscopy (TEM), Scanning Transmission Electron Microscopy (STEM) and Energy Dispersive X-ray Spectroscopy (EDX) were conducted with JEOL JEM-2010 and JEOL JEM 2100F. Scanning tunneling microscopy (SEM) was carried out with LEO SUPRA 55 (Carl Zeiss, Germany). Magnetic properties of nanoparticles were measured using superconducting quantum interference device (SQUID) magnetometer (Quantum Design, MPMS5XL), which is equipped with a 5 T superconducting magnet. X-ray Photoelectron Spectroscopy was obtained using K-Alpha (Thermo Electron, U.K.)

***Preparation of pluronic copolymer-coated magnetic nanoparticle (PCMNP).*** Iron oxide nanoparticles having 11nm of core size were synthesized through the previously reported procedure. 10 mg of iron oxide nanoparticle and 100 mg of P123 were mixed into 5 ml $CHCl_3$ solution. After shaking the reaction solution for several minutes, solvent was evaporated with rotary evaporator generating black film on a bottom of flask. . Reaction flask was placed onto hot oil bath (at 150 °C), connected to vacuum line, annealed for 1 hr. After cooling to room temperature, 4 ml of water was poured and sonicated for 10 min, affording dark-brown suspension with slight amount of white floating matters. Filtration with syringe filter (Cellulose acetate, 0.20 μm of pore size, MFS) gave transparent suspension. Twice repetition of purification process by addition of 15 ml of water, centifugation (13,000 rpm, 20 °C, 30 min), and removal of

supernatant gave highly concentrated suspension of PCMNP.

***Preparation of Ni immobilized magnetic nanoparticle (Ni-MNP).*** 5 ml aqueous solution of $NaBH_4$ (2.5 mg) was added to an aqueous suspension containing 5 mg of iron oxide nanoparticle and 2.5 mg of $NiNO_3$ and shaken at room temperature. Within 1 hr, a dark brown precipitate appeared, indicating the formation of Ni-MNP. Resulting solid of Ni-MNP was isolated by magnetic decantation and purification process was repeated three times by addition of water, centrifugation, and removal of supernatant.




**Scheme S1**. Procedure for the preparation of Ni-MNP

***Investigation of binding and separation of His-tagged proteins with Ni-MNP.*** Ni-MNP nanoparticles (100 μg) were added to protein solution containing His-tagged GFP or normal mouse $IgG_1$ conjugated by PE-Cy5 in PBS (50 μg/ml, 100 μl) and incubated with shaking for 30 min. Ni-MNP nanoparticles were isolated from supernatant by using the attraction with small magnet, redispersed into imidazole solution (0.1 g/ml, 1ml), and incubated with shaking for 30 min to release proteins captured by nanoparticles. Experiment for the separation of His-tagged proteins from untagged proteins were

carried out with the mixture solution of His-tagged GFP and normal mouse $IgG_1$ conjugated by PE-Cy5 (5 μg/ml for each).

In order to find the ratio between Ni-MNP and His-tagged GFP (number of His-tagged GFP captured by Ni-MNP), 10 μg of his-tagged GFP was reacted with Ni-MNP with variation on the amount of Ni-MNP from 17 μg to 100 μg. When 100 μg, 67 μg, and 40 μg of Ni-MNP were used, the most of his-tagged GFP was found to be captured by the Ni-MNP. And, the reaction with 23 μg ($1.1 \times 10^{-8}$ mol) and 17 μg ($7.8 \times 10^{-9}$ mol) of Ni-MNP resulted in 66.5 % ($2.2 \times 10^{-7}$ mol) and 40.7 % ($1.3 \times 10^{-7}$ mol) of protein capturing was found, respectively. From this, the number of captured GFP per Ni-MNP is estimated as 17 ~ 22.

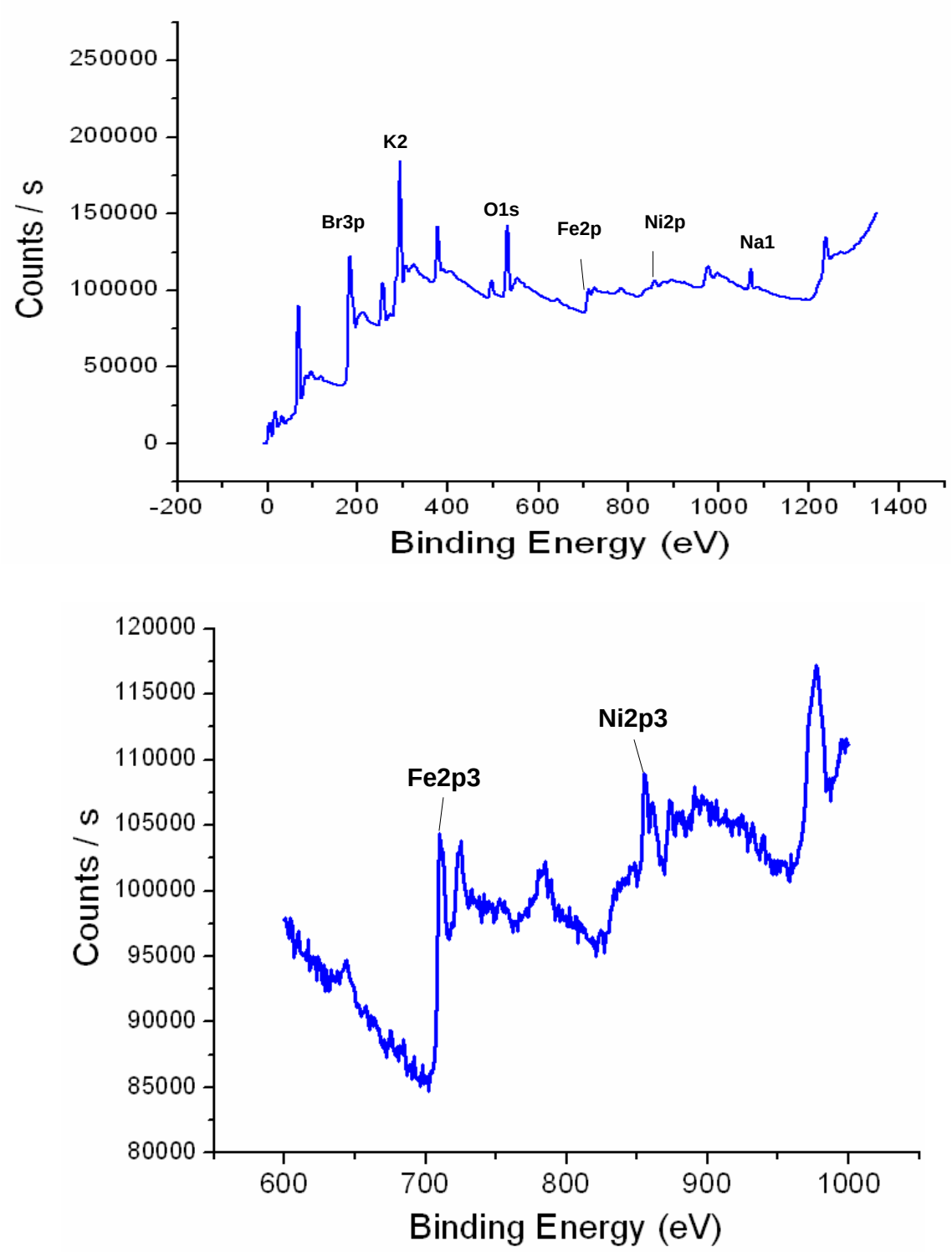
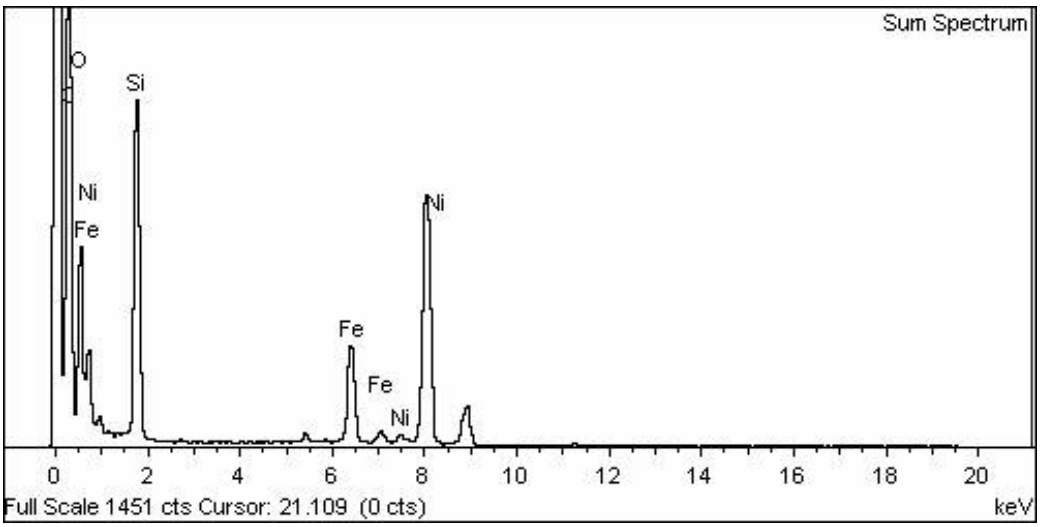
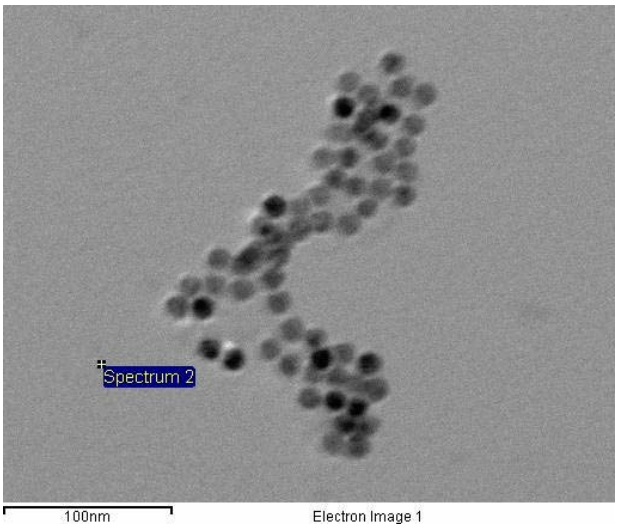


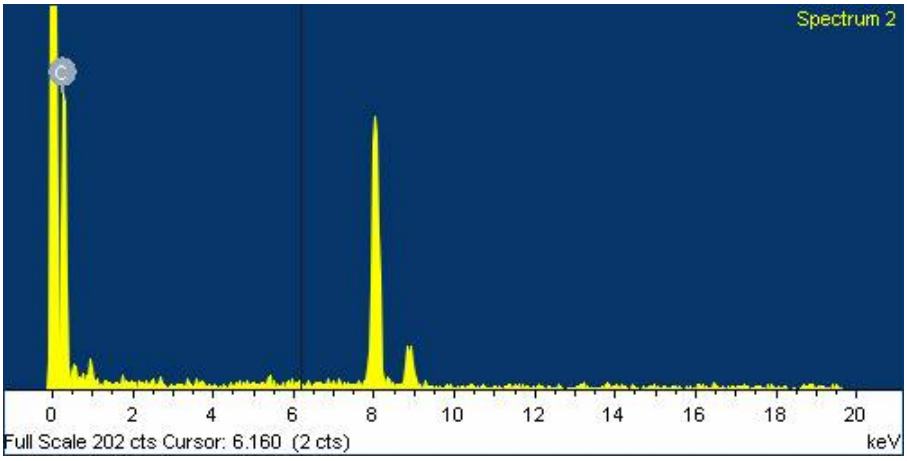

**Figure S1**. X-ray Photoelectron Spectroscopy (XPS) of Ni-MNP (KBr pellet)




| Element | Peak Area | Area Sigma | k factor | Abs Corrn. | Weight% | Weight% Sigma | Atomic% | |
|---|---|---|---|---|---|---|---|---|
| C K | 7417 | 177 | 2.208 | 1.000 | 53.27 | 0.77 | 69.67 | |
| O K | 2857 | 120 | 1.810 | 1.000 | 16.82 | 0.63 | 16.52 | |
| Si K | 5971 | 136 | 1.000 | 1.000 | 19.42 | 0.46 | 10.86 | |
| Fe K | 2619 | 96 | 1.170 | 1.000 | 9.97 | 0.36 | 2.80 | |
| Ni K | 128 | 49 | 1.245 | 1.000 | 0.52 | 0.20 | 0.14 | |
| Totals | | | | | 100.00 | | | |

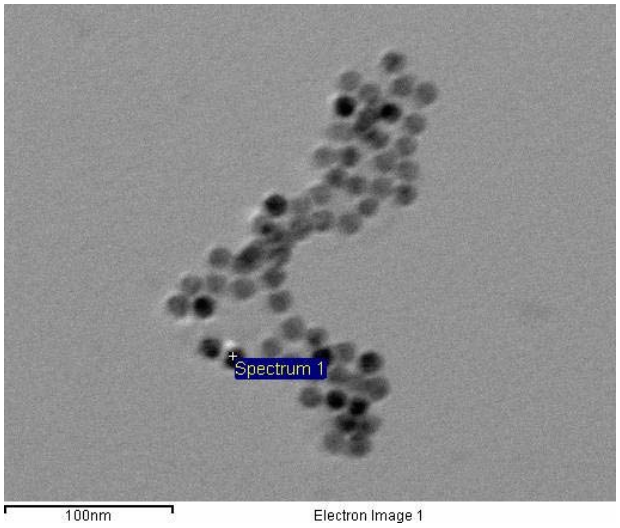
**Figure S2**. Energy dispersive spectroscopy of Ni-MNP obtained during TEM measurement

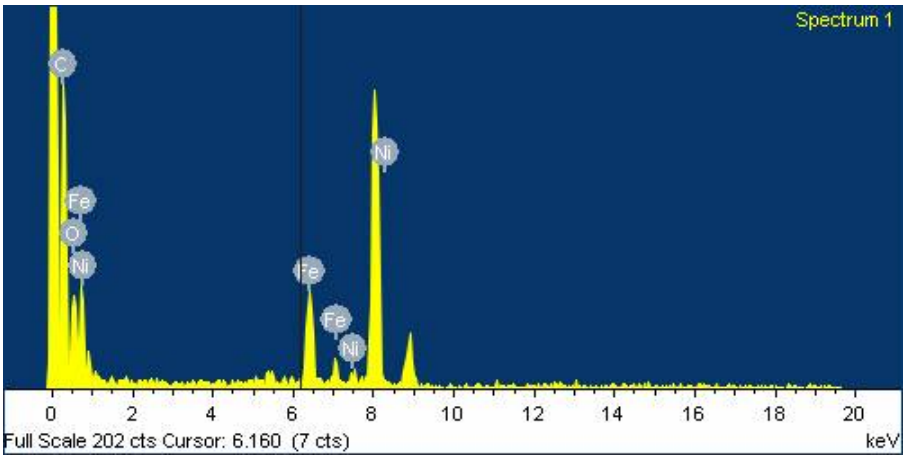



| Element | Weight% | Atomic% |
|---|---|---|
| C K | 100.00 | 100.00 |
| Totals | 100.00 | |

**Figure S3**. Energy dispersive spectroscopy with focus on carbon coated TEM grid.




| Element | weight% | Atomic% | |
|---|---|---|---|
| C K | 62.93 | 79.58 | |
| O K | 15.30 | 14.53 | |
| Fe K | 19.35 | 5.26 | |
| Ni K | 2.42 | 0.62 | |
| Totals | 100.00 | | |

**Figure S4**. Energy dispersive spectroscopy with focus on Ni-MNP.

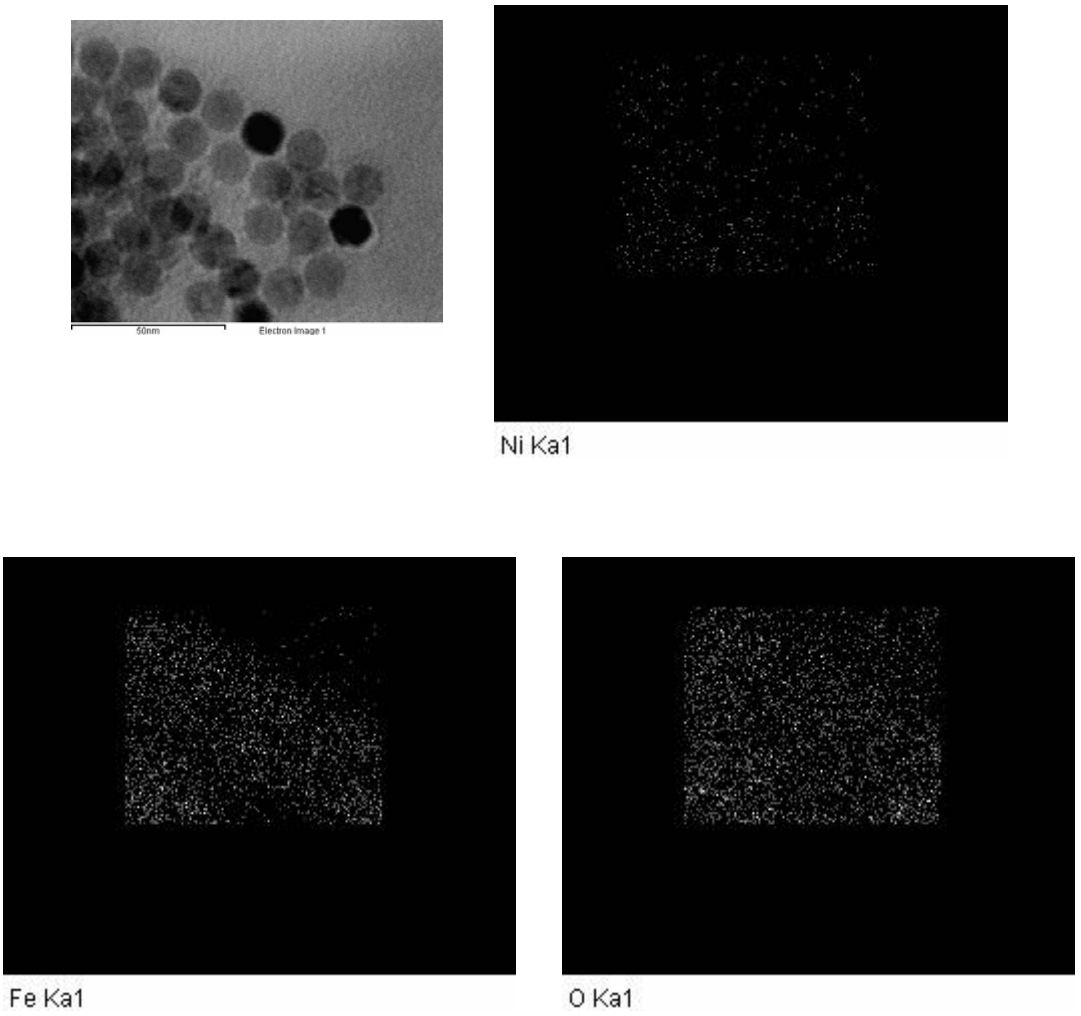



**Figure S5**. Electron energy loss spectroscopy of Ni-MNP.

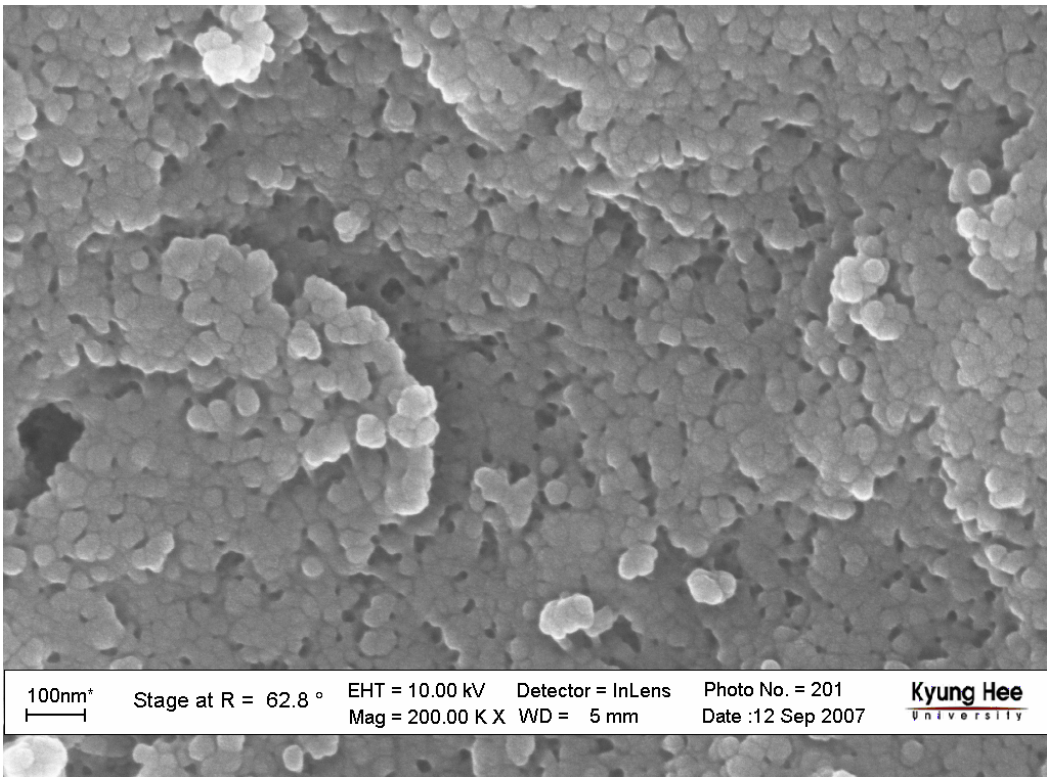
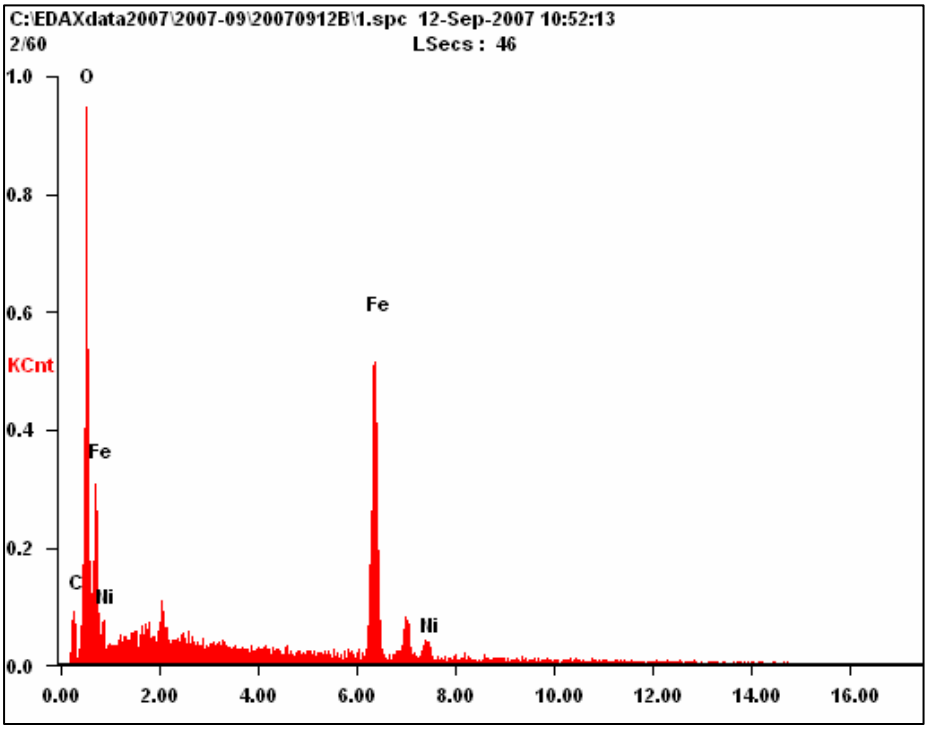
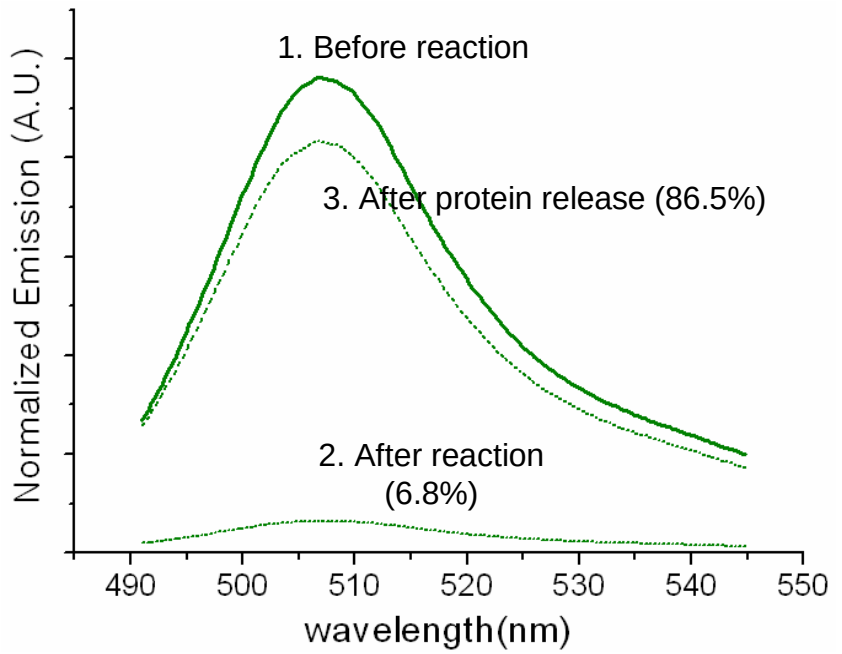



**Figure S6**. Scanning tunneling microscopy of Ni-MNP.

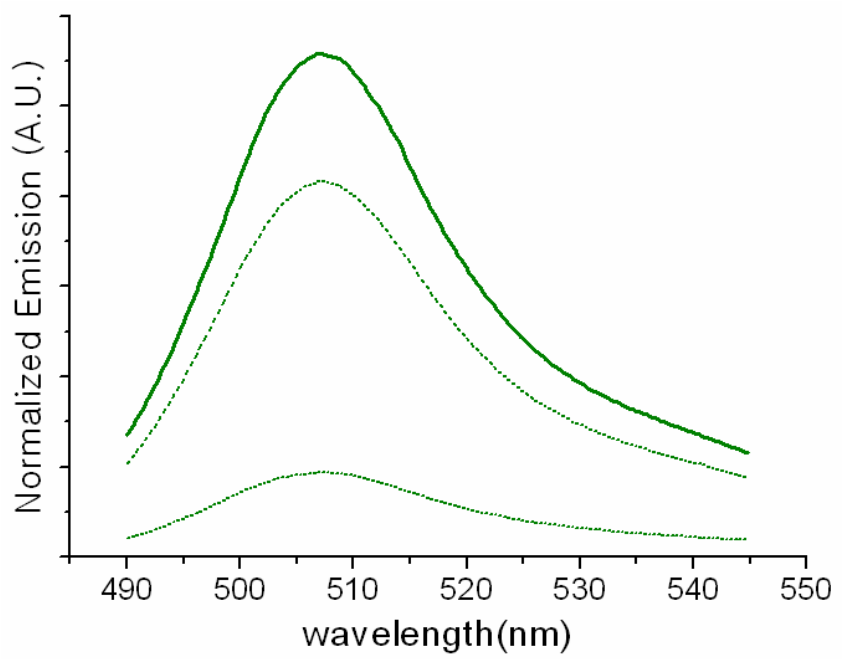



| Element | Wt % | At % |
|---|---|---|
| C K | 08.77 | 22.17 |
| O K | 20.96 | 39.79 |
| FeK | 63.46 | 34.51 |
| NiK | 06.80 | 03.52 |

**Figure S7.** Energy dispersive spectroscopy of Ni-MNP obtained during TEM measurement




**(a)**




**(b)**

**Figure S8.** Fluorescent spectra showing the change of emission intensity of the solution of first (a) and second (b) recycling experiment with Ni-MNP.

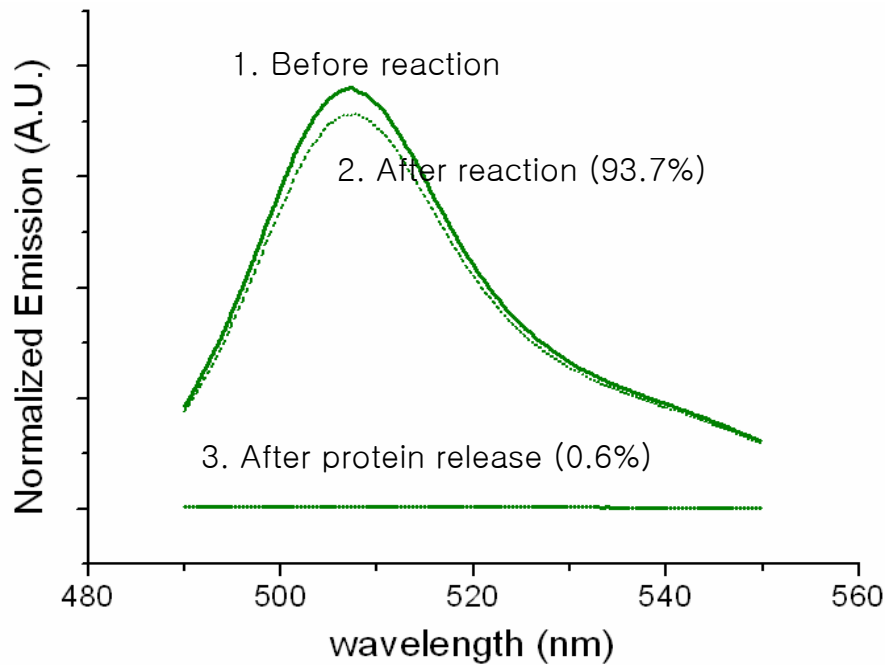
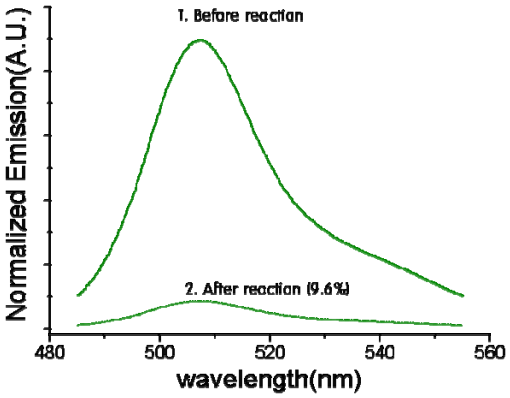
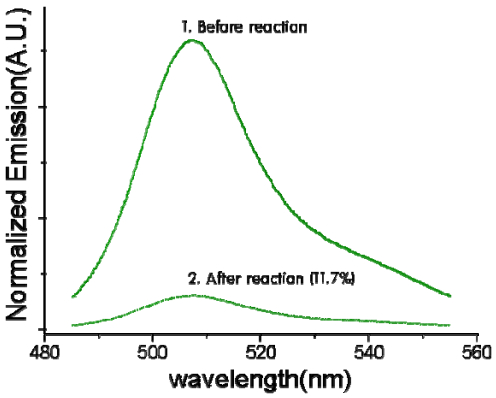


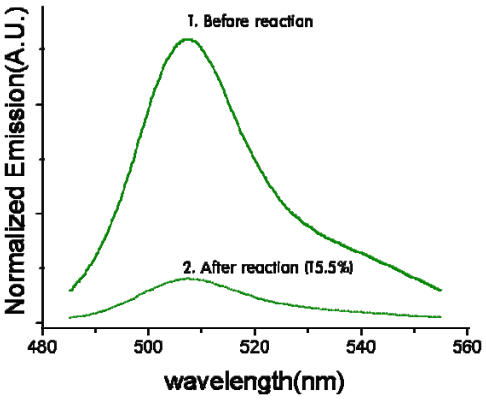

**Figure S9.** Fluorescent spectra showing the change of emission intensity of the solution of control experiment with PCMNP.



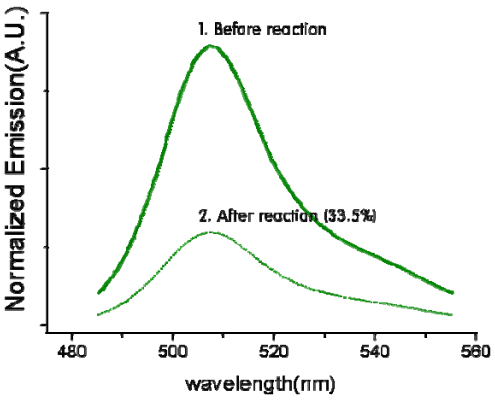

(a) with 100 ug of Ni-MNP



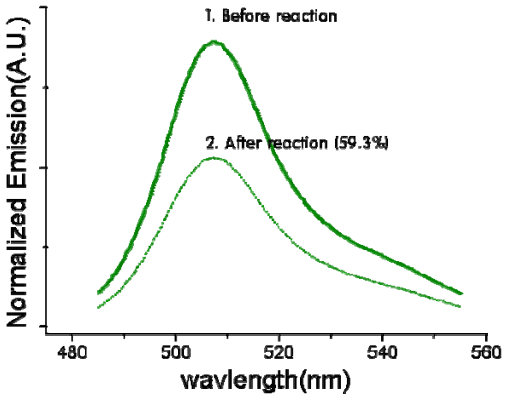

(b) with 67 μg of Ni-MNP




(a) with 40 ug of Ni-MNP




(b) with 23 μg of Ni-MNP




(a) with 17 ug of Ni-MNP

**Figure S10.** Fluorescent spectra showing the change of emission intensity in the protein capturing experiment with variation on the amount of Ni-MNP.

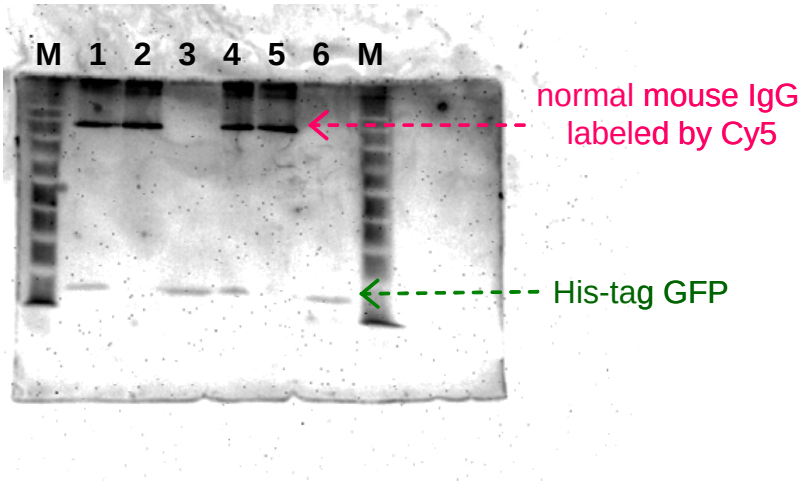



**Figure S11.** Electrophoresis analyses with the mixture solution containing His-tagged GFP and normal mouse IgG labeled by Cy5 (lane 1 and 4), the supernatant solution remained after removing His-tagged GFP bound Ni-MNPs (lane 2 and 5), and the imidazole solution containing His-tagged GFP proteins released from Ni-MNPs (lane 3 and 6). Lane M is molecular weight markers.