Supporting information

Enzymatic Protein Immobilization on Amino-functionalized Nanoparticles

Estimation of the number of proteins immobilized on nanoparticles:

The total mass of Fe_3O_4: m= 12 mg.

Assuming the Fe_3O_4 nanoparticle is a regular sphere, the mass of a single Fe_3O_4 nanoparticle: $m_{single} = \rho * V = 5.18\ g/cm^3 \times (4/3) \times \pi \times (9.62\ nm/2)^3 = 2.4 * 10^{-15}$ mg.

The total Fe_3O_4 nanoparticles is: N_{total} = 12 mg / $2.4 * 10^{-15}$ mg = $5 * 10^{15}$.

The molar amount of catechol-PEG5000-NH_2: n = 55 mg/5500 g/moL = 0.01 mmoL.

The concentration of catechol-PEG5000-NH_2: c = 0.01 mmoL /$5 * 10^{-3}$ L = 2 mM.

The number of catechol-PEG5000-NH_2: $N_{PEG} = 0.01\ mmoL * N_A * 10^{-3} = 6.02 * 10^{18}$.

The number of catechol-PEG5000-NH_2 on a Fe_3O_4 nanoparticle: $N_{particle}$ = $6.02 * 10^{18}$ / $5 * 10^{15} = 1.2 * 10^3$.

The number of eGFP-ELP_8-NGL on a Fe_3O_4 nanoparticle: $N_{protein} = 1.2 * 10^3 * 2 * 10^{-2}$ = 24.

Protein sequence

eGFP-ELP_8-NGL (MW: ~33 kDa)

MGHHHHHHMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKL
TLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQ
ERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNS
HNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNH
YLSTQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYKRSVPGVGVPGVGV
PGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGGLRSNGL

The fluorescence emission spectrum excited at 488 nm was shown in figure S7.

GL-eGFP (MW: ~29 kDa)

MGLHHHHHHGSMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATY
GKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEG
YVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYN
YNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLP
DNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYKRS

Supplementary Figures

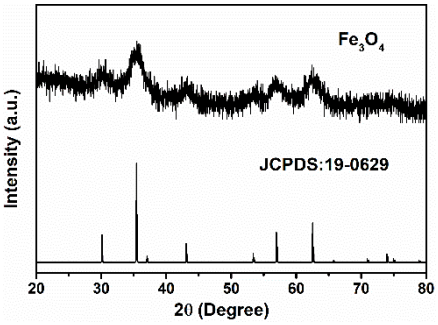

Figure S1. XRD patterns of the obtained Fe_3O_4 and standard diffraction peaks of Fe_3O_4 (JCPDS NO.19-0629).

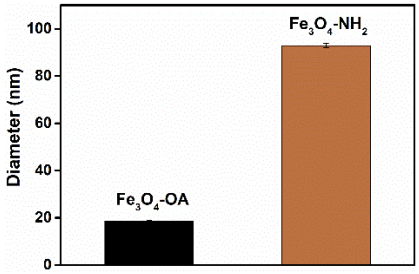

Figure S2. Average diameter of Fe_3O_4-OA and Fe_3O_4-NH_2 measured by DLS.

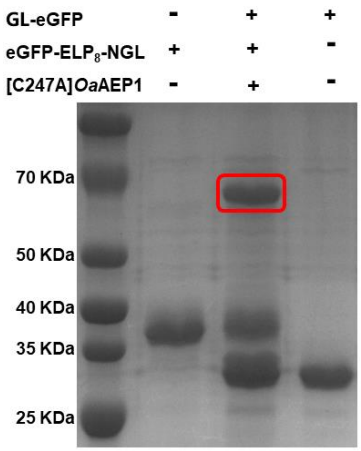

Figure S3. SDS-PAGE gel result of the ligation of eGFP-ELP_8-NGL with protein GL-eGFP by *Oa*AEP1.

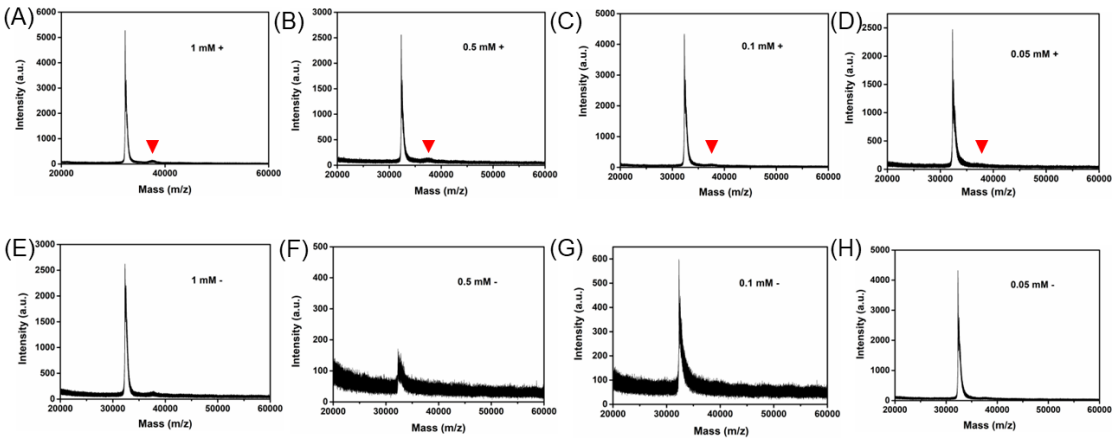

Figure S4. MALDI-TOF MS result of the ligation of eGFP-ELP$_8$-NGL with PEG5000-NH_2 (A, E 1 mM; B, F 0.5 mM; C, G 0.1 mM; D, H 0.05 mM) when *Oa*AEP1 was present (upper) and absent (bottom).

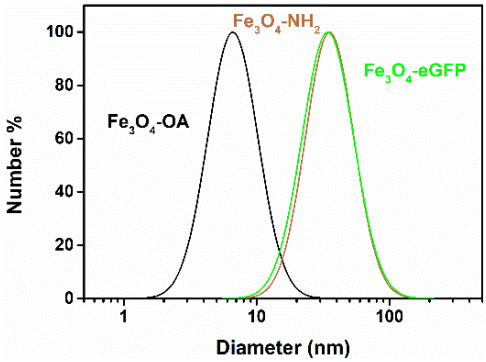

Figure S5. Hydrodynamic diameter distribution of Fe_3O_4-OA, Fe_3O_4-NH_2, Fe_3O_4-eGFP.

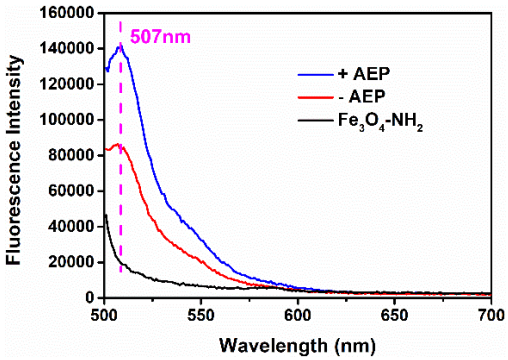

Figure S6. The fluorescence emission spectra of Fe_3O_4-NH_2 (black) and the obtained Fe_3O_4-eGFP complex when *Oa*AEP1 was present (blue) and absent (red).

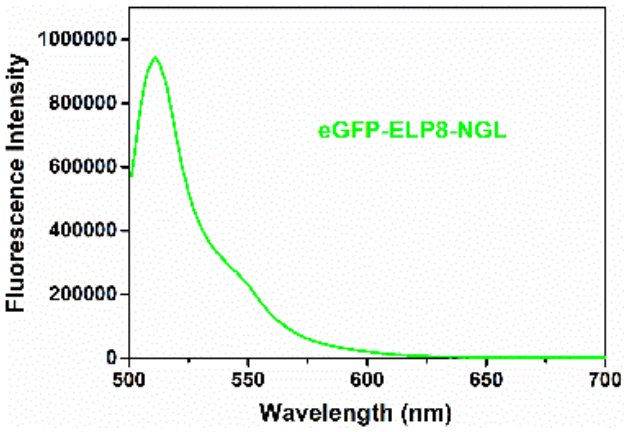

Figure S7. The fluorescence emission spectrum of target protein eGFP-ELP$_8$-NGL excited at 488 nm, which is same as free eGFP.