Electronic Supplementary Information

In Situ Formation of Polymer-Protein Hybrid Spherical Aggregates from (Nitrilotriacetic Acid)-End-Functionalized Polystyrenes and His-Tagged Proteins

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Gene cloning of the His-tagged lipases

The lipase genes (GenBank accession numbers AAC73578 and CAD00528) were amplified using the bacterial genomic DNA of *Escherichia coli* K-12 and *Listeria monocytogenes*. The genomic DNA samples were available from American Type Culture Collection. The forward and the reverse primers contained *Bam*HI and *Pst*I restriction sites, respectively, just before and after each open reading frame. After a standard PCR reaction was performed, the PCR products were cloned into the pCR2.1-TOPO vector (Invitrogen) and then sequenced to ensure a correct gene

amplification. Restriction enzyme-digested fragments were ligated into pET21-a plasmid linearized with the same enzymes to produce the expression plasmids pET21a-21H and pET21a-83H. *E. coli* JM109 cells were transformed by electroporation (Gene Pulser Xcell; Bio-Rad) with the plasmids, according to the procedures previously reported.[1]

Expression and purification of His-tagged enzymes

E. coli cells were grown at 37°C in 100 mL of Luria-Bertani medium supplemented with ampicillin (100 μg/mL) until an OD_{600} of 0.6 and then induced with different concentrations of isopropyl β-D-1-thiogalactopyranoside (IPTG). After 16 h of cultivation at 18°C, the cells were harvested by centrifugation at 9,800 *g* for 15 min and resuspended in Native IMAC lysis buffer (300 mM KCl, 50 mM KH_2PO_4, 5 mM imidazole, pH 8.0, Bio-Rad). The resuspended cells were lysed with sonication (VCX 750; Sonics & Materials Inc., USA) and centrifuged at 12,000 *g* for 30 min at 4°C. The supernatants were filtered through a 0.45 μm filter and used as a source for intracellular soluble proteins. Three histidine-tagged enzymes were purified by immobilized metal affinity chromatography (IMAC) using the Profinia protein purification system (Bio-Rad) according to the manufacturer's instructions. The purification procedure using the Bio-Scale Mini Profinity IMAC cartridge (1 mL, Bio-Rad) and the Bio-Scale Mini Bio-Gel1 P-6 desalting cartridge (10 ml, Bio-Rad) were adopted. The enzyme purity was estimated by denaturing sodium dodecyl sulfate-polyacrylamide gel electrophoresis in eluted fractions using 10% polyacrylamide gels. The protein concentrations were determined using the Bradford assay kit (Bio-Rad) with bovine serum albumin as a standard. The purity of the sample was shown on SDS-PAGE and quantified by the microfluidics-based electrophoresis system (Experion and Quantity One software; Bio-Rad).

Characterization of polymer (NTA-PS, M_n~21,800)

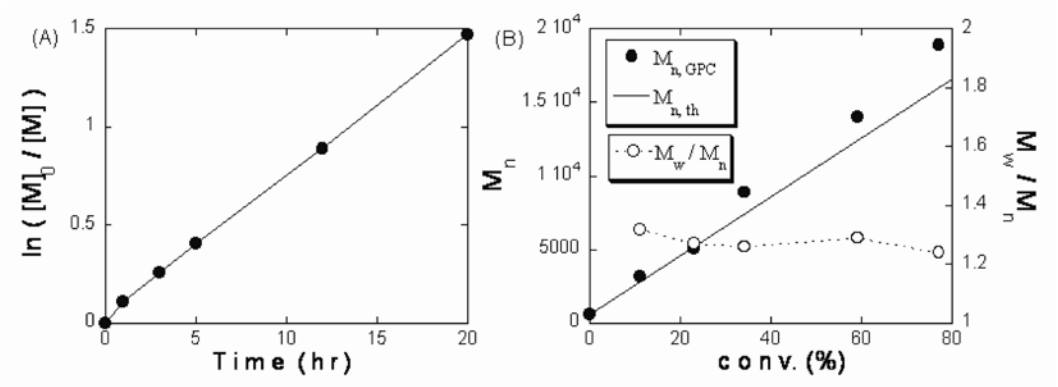

Fig. S1 Results of polymerization: (A) First-order kinetic plot, (B) M_n and M_w/M_n evolution on monomer conversion.

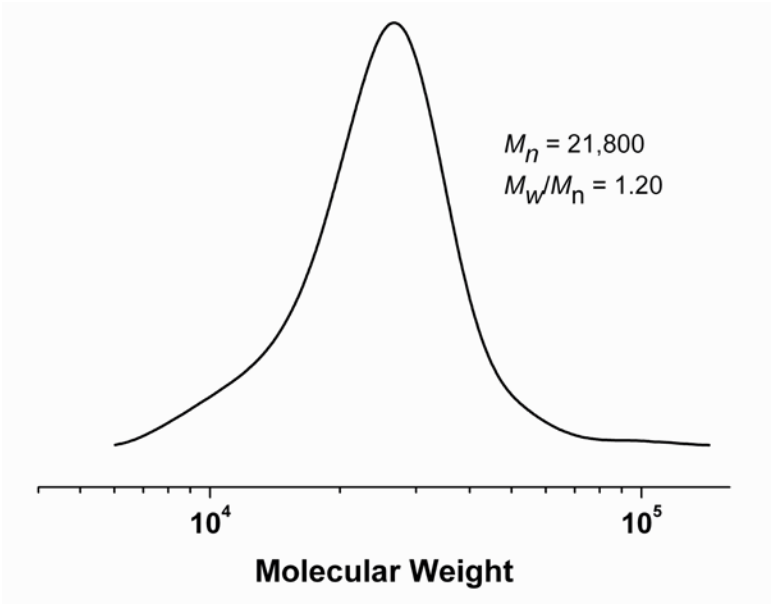

Fig. S2 Gel permeation chromatogram traces of NTA-PS.

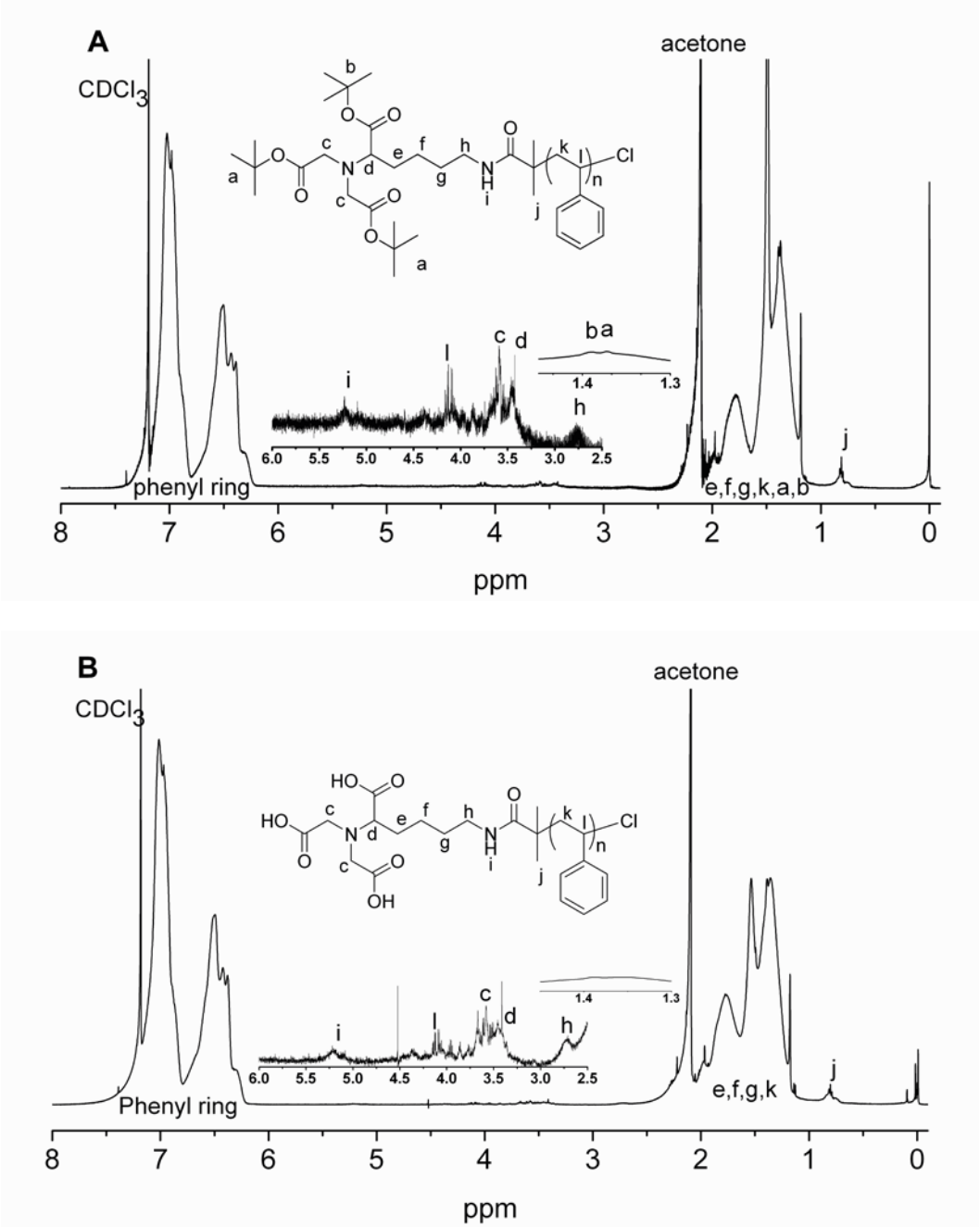

Fig. S3 (A) ^{1}H NMR spectra of α–(p-NTA)-polystyrene, $CDCl_3$ 100%, and (B) α-(NTA)-polystyrene, mixed solvent system ($CDCl_3$/MeOH-d_4 = 99/1 by vol. %).

Characterization of polymer (NTA-PS, M_n~4900)

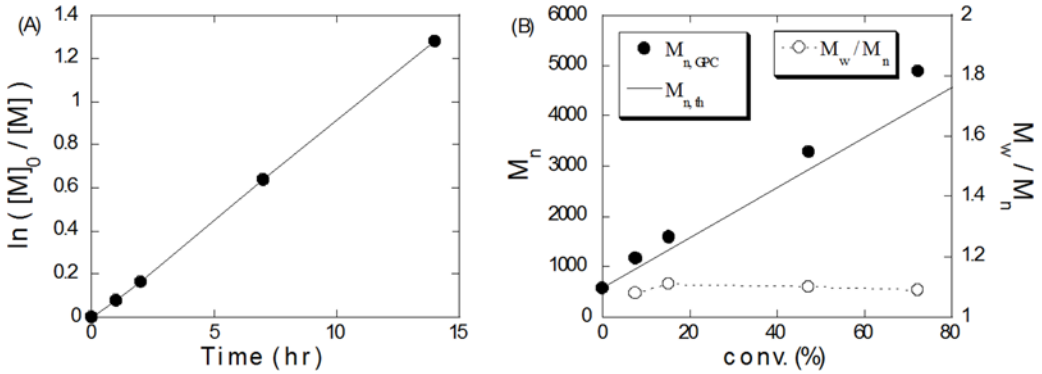

Fig. S4 Results of polymerization: (A) first-order kinetic plots, (B) M_n and M_w/M_n evolution on monomer conversion.

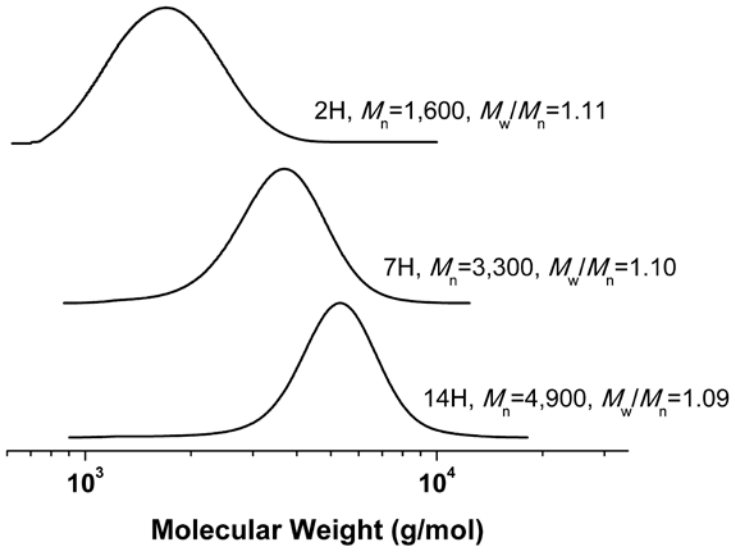

Fig. S5 Gel permeation chromatogram traces of NTA-PS.

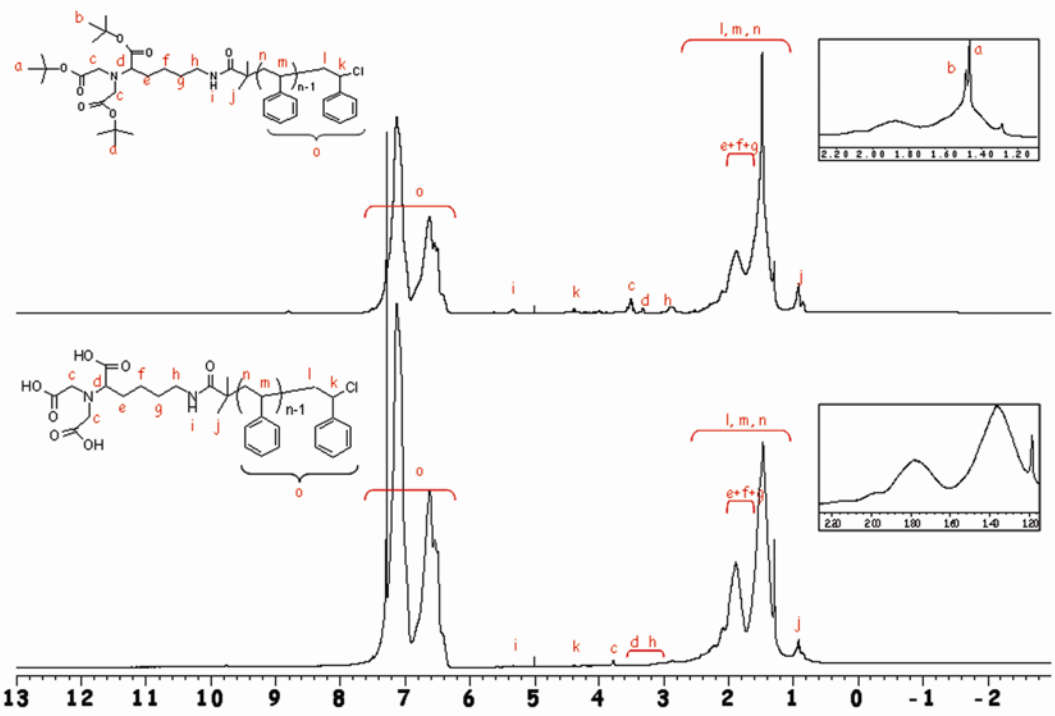

Fig. S6 ^{1}H NMR spectra of α–(p-NTA)-polystyrene, (M_n, GPC = 4,900; M_w/M_n = 1.09) and α-(NTA)-polystyrene, (M_n, GPC = 4,700; M_w/M_n = 1.40).

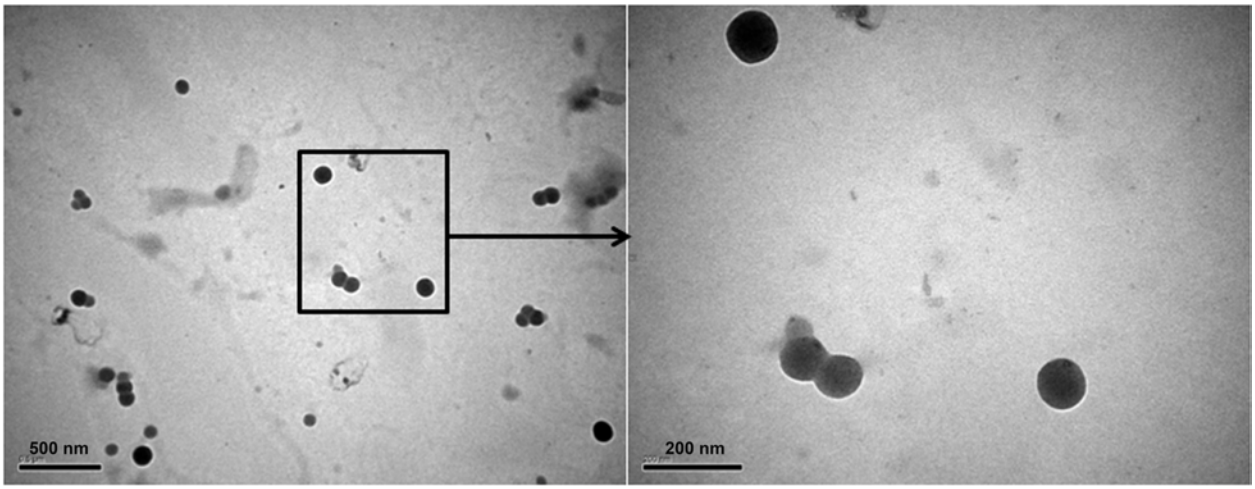

Fig. S7 Polymer-protein hybrid aggregates obtained from nickel complexed (nitrilotriacetic acid)-end-functionalized polystyrene (Ni-NTA-PS, M_n~21,800) with His_6-GFP in water/DMF (DMF 4 vol %): TEM images (A) at lower resolution, and (B) at higher resolution.

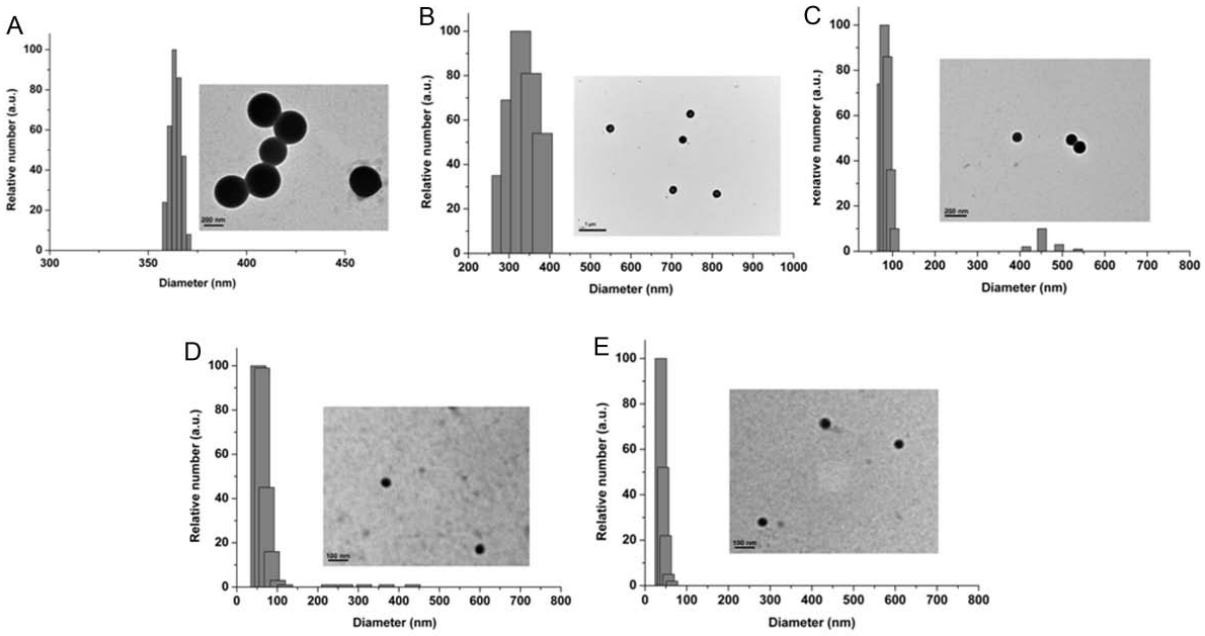

Fig. S8 Effect of polymer (Ni-NTA-PS, M_n~4,900) and protein concentration (His_6-GFP) on the size of polymer-protein hybrid aggregates: DLS data and TEM images for (A) 0.125 mg Ni-NTA-PS and 540 µg His_6-GFP, (B) 0.0625 mg Ni-NTA-PS and 270 µg His_6-GFP, (C) 0.03125 mg Ni-NTA-PS and 135 µg His_6-GFP, (D) 0.003125 mg Ni-NTA-PS and 13.5 µg His_6-GFP, and (E) 0.001562 mg Ni-NTA-PS and 6.75 µg His_6-GFP. Polymer and His_6-GFP were dissolved in 0.1 mL DMF and 2.5 mL water (pH 7.4) respectively. And the addition rate of polymer

solution to protein solution was 0.01 mL/h. Polymer solution was prepared by stepwise dilution from higher concentration.

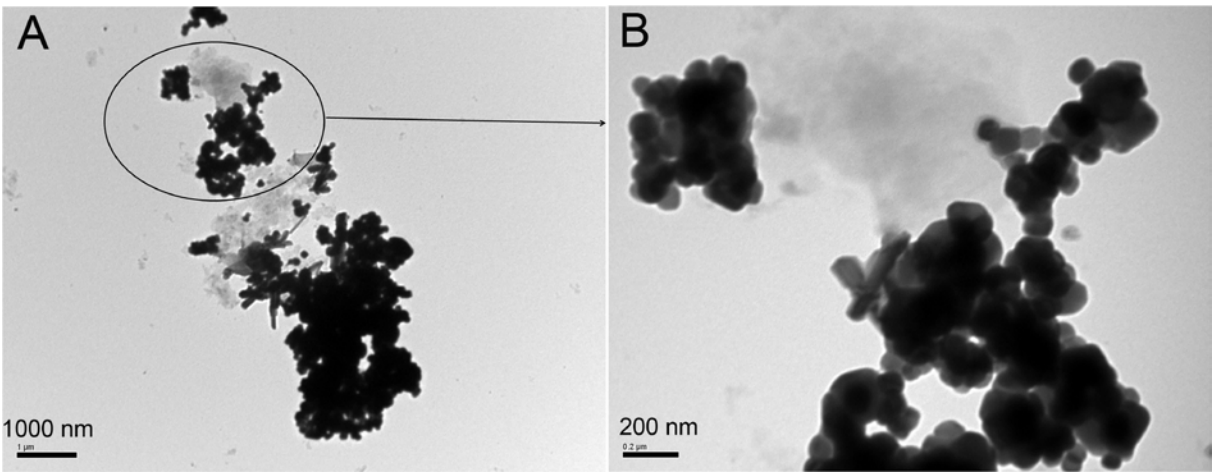

Fig. S9 TEM images of polymer-protein hybrid aggregates obtained from nickel complexed (nitrilotriacetic acid)-end-functionalized polystyrene (Ni-NTA-PS, M_n~21,800) with His_6-GFP in water/DMF (DMF 4 vol %) after removal of DMF by dialysis (2 day): (A) at lower resolution, and (B) at higher resolution.

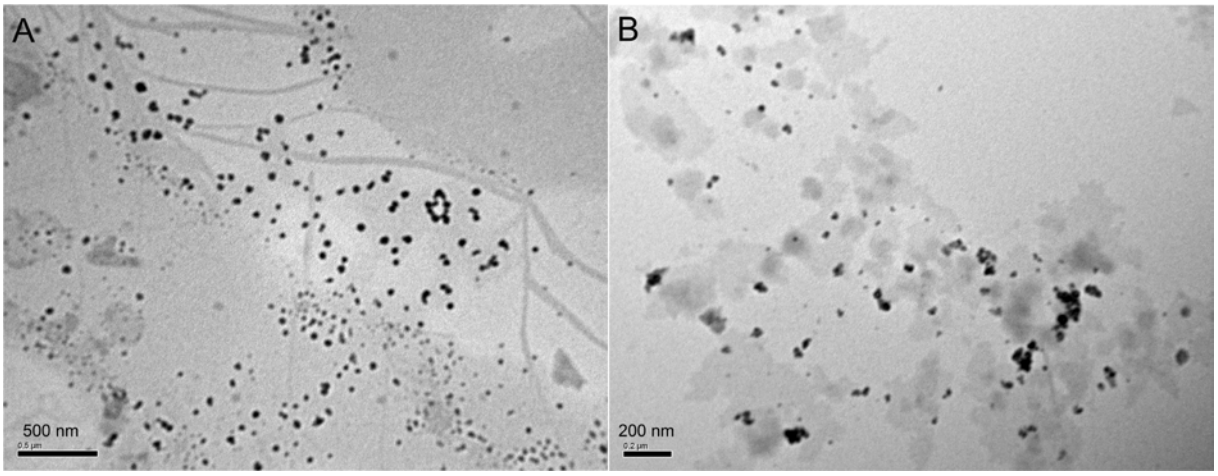

Fig. S10 TEM images after addition of excess imidazole (250 mM) to the solution of polymer-protein hybrid aggregates obtained from nickel complexed (nitrilotriacetic acid)-end-functionalized polystyrene (Ni-NTA-PS, M_n~21,800) and His_6-GFP in water/DMF (DMF 4 vol %): (A) after 1 day of addition, and (B) after 6 days of addition. DMF was removed by dialysis before addition of imidazole.

References

1 E. Yu, M.-A. Kwon, M. Lee, J. Oh, J.-E. Choi, J. Lee, B.-K. Song, D.-H. Hahm and J. Song, *Appl. Microbiol. Biotechnol.*, 2011, **90**, 573.