Decoration of superparamagnetic iron oxide nanoparticles with Ni^{2+}: agent to bind and separate histidine-tagged proteins†

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The decoration of iron oxide nanoparticles with Ni^{2+} ions provided the superparamagnetic nanoparticles with a binding site for His-tagged proteins, allowing their selective binding and convenient separation from a multi-component solution with an appropriately applied magnetic field.

Magnetic nanoparticles, of which superparamagnetic iron oxide (SPIO) is a representative example, have been used for some time in various biomedical and biological applications such as magnetic resonance imaging (MRI) contrast enhancement, hyperthermia, and gene and drug delivery.[1–3] Recently, they have been successfully employed in the development of highly sensitive and highly selective techniques for the magnetic separation and manipulation of cells and proteins.[4] Because of their unique and superior properties originating from their small size and large surface area, such as their easy penetration of biomembrane systems, fast and rich binding of biomolecules, and reversible and controllable attraction, magnetic nanoparticles have many advantages over conventional micron-sized beads or resins, especially in the specific separation of low numbers of target molecules.[5] For instance, Xu and coworkers modified magnetic nanoparticles with capturing agents such as nitriloacetic acid (NTA) and vancomicine and successfully demonstrated their ability to select and separate biological targets at low concentrations and with high specificity.[6] Very recently, Hyeon and one of the authors helped develop NiO coated Ni nanoparticles as a novel agent to bind and magnetically separate histidine-tagged (His-tagged) proteins.[7] The high affinity of the NiO shell, spontaneously generated through air oxidation, provided a convenient means of purifying His-tagged proteins without any effort in order to synthesize and conjugate NTA derivatives on substrate materials. However, it is necessary to improve their stability against oxidation in an aqueous environment. While the core/shell structure of the Ni/NiO nanoparticles is stable in powder form, it is subject to further oxidation and slowly loses its magnetic characteristics in aqueous dispersion within 7 days.

In order to extend the applicability of this approach, while taking advantage of its convenience, our research efforts have been devoted to developing protein capturing nanoparticles with high stability against oxidation. In this work, we treated biocompatibly modified superparamagnetic iron oxide nanoparticles with Ni^{2+} ions to provide them with a binding site for His-tagged proteins. Herein, we report a novel and facile method of immobilizing Ni^{2+} on iron oxide nanoparticles and demonstrate their successful utilization for separating and purifying His-tagged proteins from a multicomponent solution.

The superparamagnetic iron oxide nanoparticles stabilized by oleic acid were prepared using a previously reported procedure.[8] In order to provide them with water compatibility and minimize the non-specific binding of biomolecules, the iron oxide nanoparticles were coated with Pluronic copolymer (P123, PEO_{19}–PPO_{69}–PEO_{19}) by mixing them with P123 in $CHCl_3$ solution, evaporating the solvent, and annealing at 150 °C *in vacuo*. The isolation of the Pluronic copolymer coated magnetic nanoparticles (PCMNPs) was accomplished by repeated cycles of ultracentrifugation and dispersion in water. An aqueous dispersion of the PCMNPs was then reacted with $NiNO_3$ and $NaBH_4$ under vigorous shaking at room temperature, leading to the formation of a dark brown precipitate within 1 h. The precipitate was concentrated and isolated by magnetic decantation and washed with water three times.

Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) did not reveal any discernable changes after the treatment with $NiNO_3$ and $NaBH_4$ (Fig. 1a, b). However, energy dispersive spectroscopy analysis revealed the obvious presence of Ni near the surface of the iron oxide nanoparticles (Fig. 1d). X-ray photoelectron spectroscopy also showed a peak at 855 eV

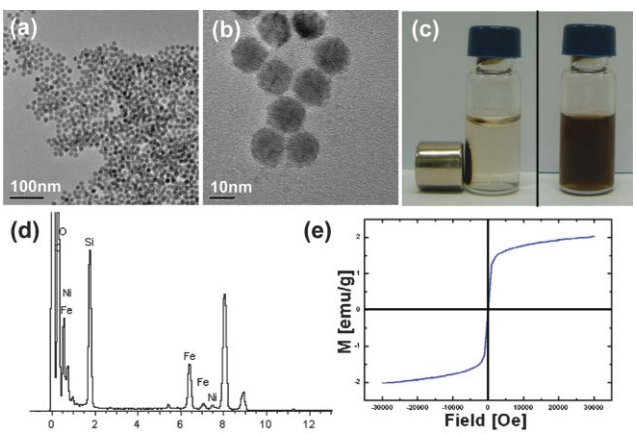

Fig. 1 TEM images of Ni-MNP (a, b). (c) Pictures showing magnetic attraction of Ni-MNP. (d) An EDX spectrum obtained by fixing the position of the electron beam on Ni-MNP. (e) A hysteresis loop showing superparamagnetic characteristics of Ni-MNP.

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corresponding to the Ni2p3 binding energy, which confirms that Ni exists as Ni(II) having affinity to polyhistidine.[9] Based on this observation, it is presumed that Ni(II) was implanted or absorbed on the iron oxide nanoparticle surface through a process including the formation of tiny Ni(0) nanoparticles or the deposition of metallic Ni(0) species at the iron oxide surface through the reduction by $NaBH_4$ and subsequent re-oxidation into NiO or Ni(II) species in an aqueous environment.

After dispersing the Ni immobilized magnetic nanoparticles (Ni-MNPs) in water by shaking, vortexing, or sonication, they can be easily attracted within several minutes by placing a small magnet on the side of the vessel (Fig. 1c). The magnetic measurement of the Ni-MNPs shows that they exhibit a saturated magnetization value of 2.0 emu g^{-1} and superparamagnetic behavior at 298 K. Their superparamagnetic properties were preserved for more than one month, even in the aqueous dispersion (Fig. 1d).

The protein separation efficiency of the Ni-MNPs was investigated through their reaction with His-tagged GFP protein. In a typical experiment, the Ni-MNPs were incubated with His-tagged GFP for 30 min at room temperature and then separated from the supernatant by applying a magnetic force. The binding of His-tagged GFP on the nanoparticles could be monitored by measuring the diminution of the fluorescent emission from the solution. The fluorescent emission intensity of the supernatant solution isolated from the reacted nanoparticles showed a 99% decrement from that of the starting solution, indicating the efficient binding and separation of His-tagged GFP. The incubation of the His-tagged GFP captured nanoparticles with concentrated imidazole solution resulted in the dissociation of the proteins from the nanoparticles, resulting in 92% recovery of the fluorescent emission intensity (Fig. 2). When the Ni-MNPs were retrieved from the imidazole solution and recycled for the next reaction, a 93% decrement and 87% recovery and 83% decrement and 75% recovery of the emission intensity were obtained in the second and third cycles, respectively, confirming their reusability and long-term stability. By variation of the amount of Ni-MNP, the number of His-tagged GFP captured by a Ni-MNP was estimated as 17–22. As a control experiment, the PCMNPs were reacted with His-tagged GFP under almost identical conditions and separated by centrifugation. In this experiment, it was found that very little protein was bound to the PCMNPs and only a 6% decrement in the fluorescent emission intensity was observed from the supernatant, confirming that the binding of His-tagged GFP to

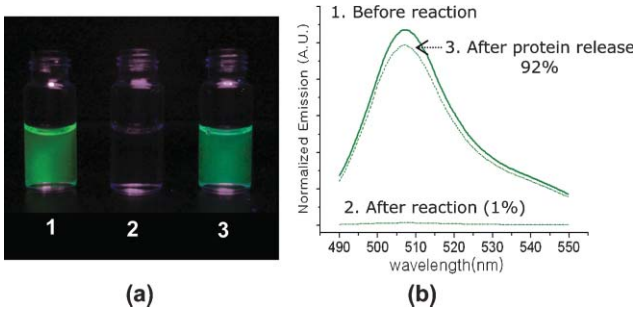


Fig. 2 (a) Fluorescent images from the solutions of His-tagged GFP (1) before and (2) after treatment with Ni-MNPs and (3) after treating protein-bound Ni-MNPs with imidazole solution. (b) Fluorescent spectra showing the change of emission intensity of the solution.

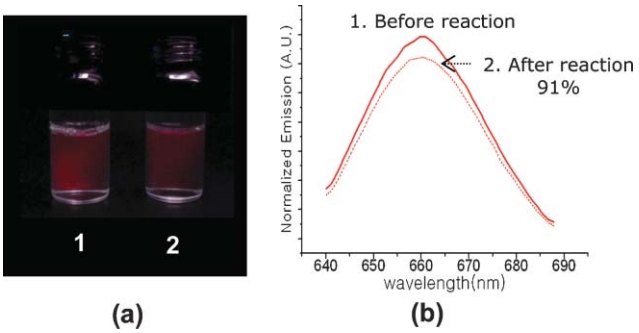
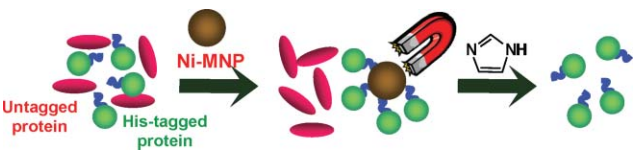


Fig. 3 (a) Fluorescent images from the solutions of normal mouse IgG labeled by Cy5 (1) before and (2) after treatment with Ni-MNPs. (b) Fluorescent spectra showing the change of emission intensity of the solution.

the Ni-MNPs occurs *via* the Ni^{2+} at the surface.[10] In order to check the nonspecific protein binding, normal mouse IgG, having no His-tag and labeled by red emitting Cy5, was reacted with the Ni-MNPs under almost identical conditions. In this reaction, only a 9% decrement in the fluorescent emission intensity was observed from the supernatant, indicating the limited binding of normal mouse IgG to the nanoparticles (Fig. 3).

In order to demonstrate the specific separation of His-tagged proteins from the multi-component solution, the Ni-MNPs were reacted with a mixture solution containing both His-tagged GFP and normal mouse IgG labeled by Cy5 (Scheme 1). When the Ni-MNPs were incubated with the mixture solution and then separated from the reaction solution, the supernatant exhibited a red emission. This indicates the selective removal of His-tagged GFP from the mixture solution initially showing a yellow emission. The His-tagged GFP was released by the treatment of the reacted nanoparticles with concentrated imidazole solution, generating a green emitting solution. The measurement of the fluorescent intensity revealed the efficient separation of His-tagged GFP from the mixture solution in 87% recovery yield (Fig. 4).



Scheme 1 Purification of His-tagged proteins from mixture solution.

In conclusion, we have demonstrated the synthesis of Ni immobilized superparamagnetic nanoparticles and their successful utilization to selectively separate specific proteins from a multi-component solution. The Ni^{2+} ions at the surface result in the affinity of His-tagged proteins to the superparamagnetic nanoparticles, thereby allowing them to be selectively and efficiently bound and removed from solution with an appropriately applied magnetic field. We believe that the approach presented herein provides a convenient way to bind biomolecules to nanomaterials and to produce novel biomedical nanomaterials.

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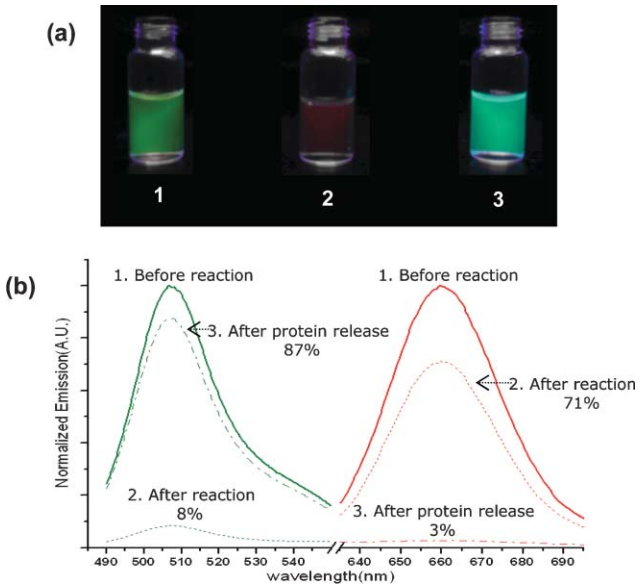

Fig. 4 (a) Fluorescent images taken with excitation light at 365 nm from the mixture solutions containing His-tagged GFP and normal mouse IgG labeled by Cy5 (1) before and (2) after treatment with Ni-MNPs and (3) after treating protein-bound Ni-MNPs with imidazole solution. (b) Fluorescent spectra showing the change of emission intensity of the solution.

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