Simple one-pot separation of histidine-tagged proteins using NTA-functionalized gold nanostars

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Abstract

Nanoparticle-based protein separation methods rely on a specific interaction between a target protein and a ligand, and a way to specifically separate it from the medium. In this sense, magnetic nanoparticles are normally employed based on their ability to be specifically separated using a strong magnetic field. This Project is a follow-up based on previous published work by our research group using magnetic Ni-NTA coated gold nanostars (AuNS) for protein purification [1]. However, considering that magnetic gold nanostars are difficult to synthesize and require a magnetic component, this work focus on the development of an easier method for protein separation using centrifugation, targeting histidine-tagged proteins. As a proof of concept, histidine-tagged *Pf*HRPII (his-*Pf*HRPII), a histidine rich protein present in the blood of patients infected with malaria, was chosen as target, with the objective of purifying it by solely using a centrifugation procedure. To achieve that, Nickel-Nitrilotriacetic Acid (Ni-NTA), was employed as capping agent and ligand based on its specific interaction with histidine tags. Recombinant protein expression was performed in *E. coli* BL21 (DE3) transformed with a pET-his-*Pf*HRPII construct and induced by IPTG. Gold nanoparticles were synthesized and used as seeds to produce gold nanostars by a seed-mediated growth method adapted by Yuan et al. [2]. Nanostars were functionalized with Ni-NTA and 11-Mercapto-1-undecanol (MU), (AuNS-Ni-NTA-MU) and were used to purify his-PfHRPII from cell lysate. Nanostars functionalized with 11-Mercaptoundecanoic acid (MUA) (AuNS-MUA) were used as a negative control. Average nanoparticle concentration was accessed by Nanoparticle Tracking Analysis, and their UV-Visible spectra were analyzed to evaluate the quality of the synthesis. Agarose gel electrophoresis was used to evaluate the functionalization yield. Molecular weight and purity of the recombinant protein were accessed by SDS-PAGE. The purification procedure using AuNS-Ni-NTA-MU showed promising results when compared to a negative control of AuNS-MUA, and a high percentage of contaminants were removed. Colorimetric and Surface Enhanced Raman Spectroscopy (SERS) assays using gold nanostars-based detection on Western transfers are underway to evaluate the presence of the purified his-*Pf*HRPII. Once completed, this work will make possible both a expedite purification procedure and facile detection of histidine-tagged proteins by membrane staining and SERS.

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