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SIMPLE ONE-POT SEPARATION OF HISTIDINE-TAGGED PROTEINS USING NTA-FUNCTIONALIZED GOLD NANOSTARS

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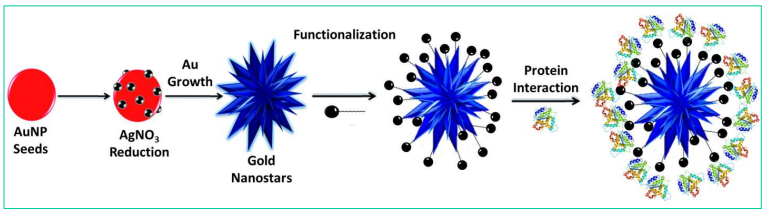
ABSTRACT

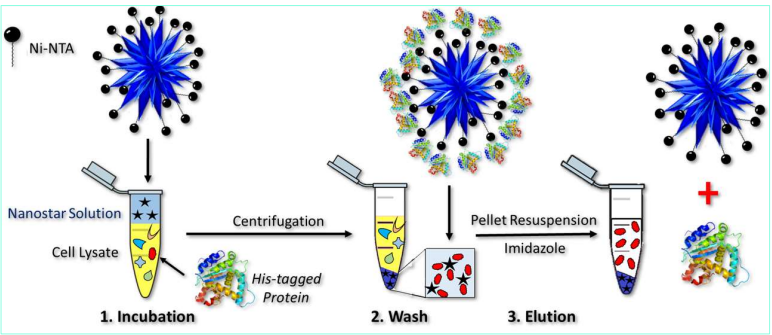
Nanoparticle-based protein separation methods rely on a **specific interaction between a target protein and a ligand**, and a way to specifically separate it from the medium. In this sense, magnetic nanoparticles are normally employed based on their ability to be specifically separated using a strong 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]. 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**. Gold Nanostars were chosen based on its high surface area and electric field enhancement due to its sharp tips and edges, and to further continue the project with the development of a detection system suitable with Surface Enhanced Raman Spectroscopy. 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. **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. Elution with 500 mM of Imidazole elutes the protein with contaminants, while a wash step with **30 mM Imidazole elutes the desired protein**, separating it from the contaminants eluted with 500 mM of Imidazole. 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.

Method

Nanostar Model

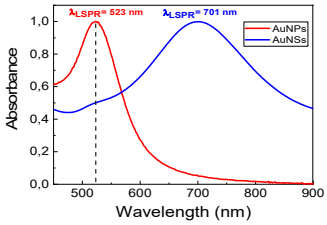


Purification Procedure



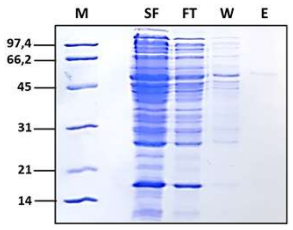
- Cell lysate containing the protein of interest is incubated with a solution of gold nanostars.
- To remove contaminants, the sample is then centrifuged and the pellet is ressuspended in water or buffer.
- **To elute the protein,** the solution is **centrifuged and ressuspended in Imidazole**.

Results



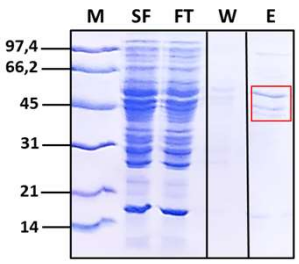
After synthesis, a red shift from 523 to 701 nm is observed, as well as the transverse contribution of the spherical component of the stars

Negative control: MUA-capped nanostar

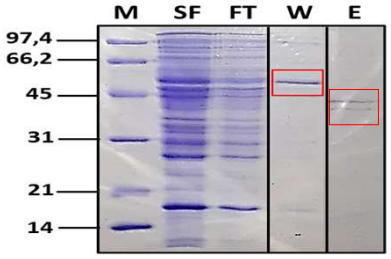


No specific pattern is eluted, lacks specific interaction with histidine tags

Purification with Nanostars functionalized with NTA and MU



Elution with 500 mM of Imidazole elutes the desired protein with 2 apparent contaminants



30 mM imidazole eluted the protein of interest and separated it from the contaminants

Legend:
M- Marker (kDa), SF- Soluble Fraction from cell lysate, FT- Flow through, W-Wash, E-Elution.

[Imidazole] mM		
500		Few contanimants
30	✓	No contaminants

Conclusions and Perspectives

- ❑ A method for purifying histidine-tagged proteins has been achieved, employing a centrifugation procedure
- ❑ Varying Imidazole concentration throughout the purification protocol separates contaminants from the desired protein fraction.
- ❑ Future perspectives envolve the use of purified protein in a colorimetric and SERS detection system, currently under development in our laboratory.

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REFERENCES

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