Purification of the NCOA4 protein via liquid chromatography

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Background and Aim

The NCOA4 protein has been shown to bind to ferritin and plays an important role in Ferritinophagy [4], a form of autophagy, where NCOA4 acts as a cargo receptor and participates in ferroptosis. Recently NCOA4 has attracted increasing attention in cancer treatment with the idea that ferroptosis modulation via NCOA4 could be a potential treatment for some cancers. Ideally NCOA4 could be used to selectively induce ferroptosis in cancer cells for autophagy [1, 3]. The purpose of this research is to obtain a pure full length NCOA4 protein from a solubilized protein lysate. To achieve purification, an Akta Go liquid chromatography instrument is used to perform either protein affinity, size exclusion, or ion exchange chromatography experiment. In this research project we used primarily protein affinity with a His-Trap column to purify our His-Tag NCOA4 protein that we expressed in E. Coli bacteria before undergoing cell lysis. The cell lysate is then purified using a Ni-NTA His-Trap column chromatography, using a buffer exchange gradient elution. By measuring the UV absorbance of the eluted protein, we can observe the relative concentration of the protein in the eluted fraction. The eluted fractions are then run on an SDS-PAGE gel to determine purity and confirm the presence of the protein. By optimizing the purification process we can obtain a pure protein stock, this would facilitate the study of NCOA4 and its relationship with ferritin as a selective cargo receptor of ferritinophagy participating in ferroptosis.

Materials and Methods

- The His-tagged NCOA4 protein is overexpressed using E Coli cells.
- We previously developed a method to extract NCOA4 from protein exclusion bodies.
- The cell is lysed and protein dissolved and clarified in Buffer A: 50mM H_2PO_4, 150 mM NaCl, pH 8.0
- The protein solution is loaded into the Akta go machine using a 1ml His-Trap column where the NCOA4 protein binds.
- To elute the protein Buffer B: 50mM mM H_2PO_4, 150mM NaCl, 500 mM imidazole, pH 8.0.
- The eluted fractions containing the highest absorbance peak are then ran against 10% SDS PAGE gel to reach final conclusions on concentration and purity.

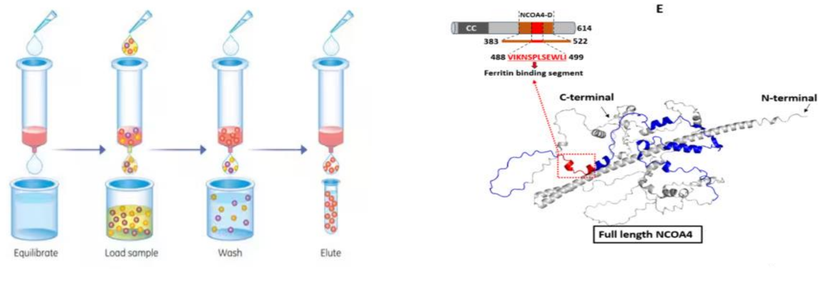
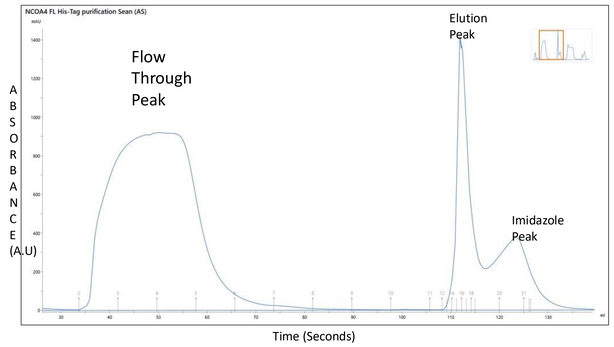


Figure 1 (Left). His-tagged NCOA4 clarified cell lysate is loaded onto a Ni^{2+} charged His-Trap column pre-equilibrated with buffer B. The histidine tag on NCOA4 coordinates with the immobilized nickel, while untagged proteins flow through. After washing with buffer A to remove nonspecific binders, elution is performed using a gradient of buffer B [3].
Figure 2 (Right). Depicts the NCOA4 full length protein. Which is solubilized from the cell lysate by suspending the pellet in buffer A with DNase, protease inhibitor and SDS and undergoing several rounds of sonication and centrifuging. Our lab was the first lab to achieve a solubilized NCOA4 FL protein [4].

Results



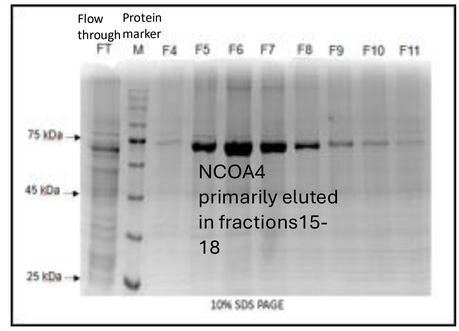


Figure 3 (Left). Shows the graph of the Akta purification of the NCOA4 protein using a His-Trap ion exchange column.
Figure 4 (Right). Shows the 10% SDS gel with a protein ladder marker M.

Conclusions

From the solubilized NCOA4 protein crude product we achieved a pure NCOA4 final product. Going forward we will use this NCOA4 to form a ferritin-NCOA4 complex which we will ship to our collaborative lab the University of Arizona where they will collect cryo-EM data on the NCOA4-ferritin complex sample that we shipped to them, to determine the structure of the complex. We also plan on performing thermo binding studies via ITC and investigating the role of ATP as an inhibitor on NCOA4 ferritin binding in our lab .

Discussion

- The graph displays the UV absorbance at 260nm recorded during purification of His-tagged NCOA4.
- The first peak represents the flow through fraction, where contaminants elute.
- The second peak corresponds to the elution of the purified NCOA4 protein.
- The vertical marks along the baseline indicate the individual fractions collected by the instrument.
- These fractions are subsequently analyzed by SDS-PAGE to verify the purity of each collection fraction.
- The gel contains a protein ladder in lane M that provides molecular-weight standards for comparison.
- Pooled fractions form the flow through which displays multiple impurity bands alongside residual protein at approximately 75 kDa.
- Fractions 5-11 show cleaner lanes, with the most concentrated NCOA4 appearing in fractions 5-8.
- Migration distance of each band relative to the ladder permits estimation of the molecular weights of the eluted proteins

Acknowledgments

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References

1. Le, Y., Liu, Q., Yang, Y. et al. The emerging role of nuclear receptor coactivator 4 in health and disease: a novel bridge between iron metabolism and immunity. Cell Death Discov. 10, 312 (2024). https://doi.org/10.1038/s41420-024-02075-3
2. Sun, K., Li, C., Liao, S., Yao, X., Ouyang, Y., Liu, Y., Wang, Z., Li, Z., & Yao, F. (2022). Ferritinophagy, a form of autophagic ferroptosis: New insights into cancer treatment. Frontiers in pharmacology, 13, 1043344. https://doi.org/10.3389/fphar.2022.1043344
3. Sigma Aldrich. (2025). NMR Chemical Shifts of Impurities. Merck, 1(1). https://www.sigmaaldrich.com/MX/en/technical-documents/technical-article/genomics/cloning-and-expression/blue-white-screening
4. Srivastava, A., Beyer, M., Hladun, C., Tardif, R., Arshad, A., Darie, C. C., ... & Bou-Abdallah, F. (2025). Characterization of the Iron–Sulfur Cluster in the NCOA4 Fragment (383–522) and Its Interaction with Ferritin. ACS Chemical Biology, 20(3), 731-745.