Growth and Purification of a Designed Zn(II) Binding Metalloprotein

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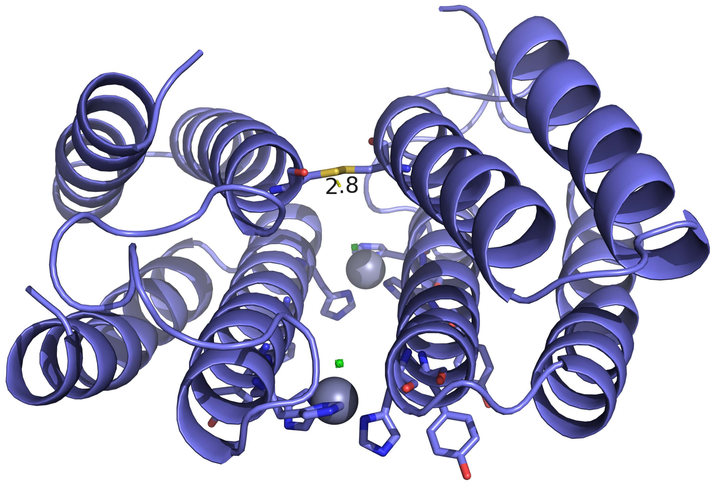
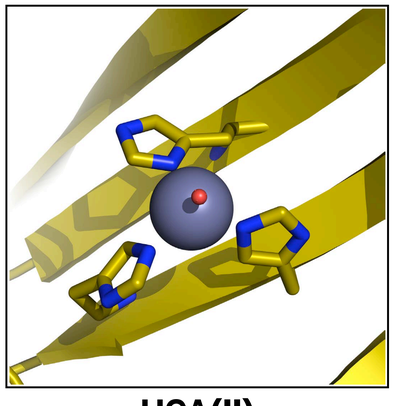
Abstract

Enzymes in nature take advantage of the robust functionality of metal ions to catalyze a diverse set of reactions that are a vital part of life. These enzymes are able to use protein secondary and tertiary structures to achieve extremely high reaction rates in aqueous environments at ambient temperatures and pressures. The creation of artificial enzymes is a promising area of research because it allows us to use inspiration from nature to create faster, more environmentally friendly catalysts while also providing insight into naturally occurring systems as we inch closer to the ability to recreate them.

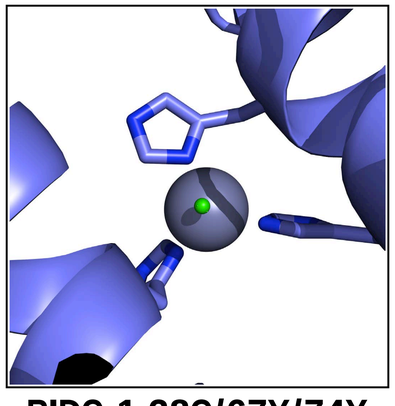
RIDC-1 38C/67Y/74Y Model



Here we present RIDC-1 38C/67Y/74Y. This protein takes advantage of a previously designed interface between monomers of cytochrome cb_{562} in order to help stabilize the engineered Zn(II) binding sites. The tyrosine residues were mutated in to remove a metal binding aspartate and open the coordination sphere of Zn(II) and to prevent other monomers from interfering with dimer formation. Finally, a disulfide crosslink was added to further stabilize the two Zn(II) binding sites. We hypothesize that since these metal binding sites resemble the active sites from other Zn(II) enzymes, most notably Human Carbonic Anhydrase II (HCAII), that we can use this protein as an artificial enzyme for hydrolysis and the reversible hydration of CO_2.



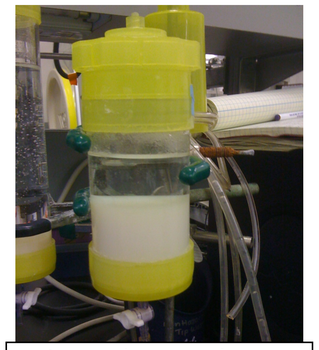
HCA(II)



RIDC-1 38C/67Y/74Y

Methods

This is the cell pellet we collected after growing our protein in E. Coli BL21. Their red color comes from the heme and is an indication that our protein expressed well.

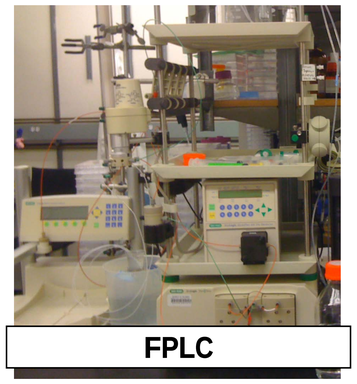


Ion exchange column

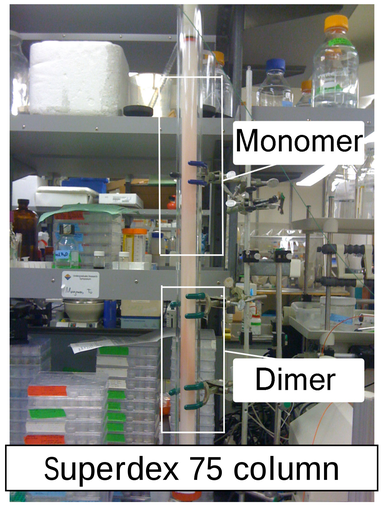
Ion Exchange Chromatography

We used a combination of positively and negatively charged columns to purify our protein based on charge. We controlled the overall charge of our protein by changing the pH. Once our protein was stuck to the column, we ran a gradient of NaCl to remove it. The gradient helps ensure separation from other proteins that were attracted to the column as well.

This is our Fast Protein Liquid Chromatography (FPLC) machine that runs our protein through our specified columns and measures the protein's salt concentration and absorption of ultra-violet light then graphs it in a computer program.



FPLC

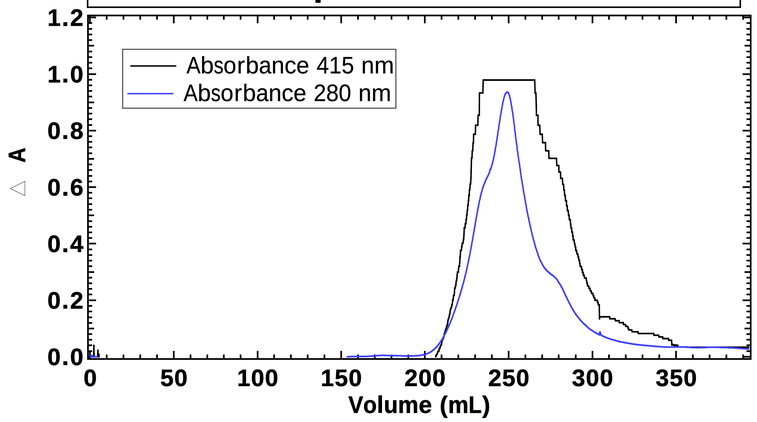


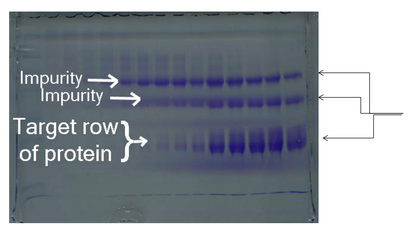
Superdex 75 column

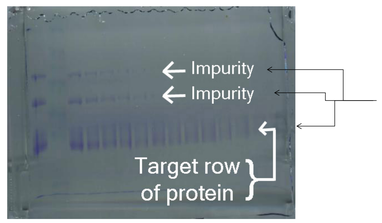
Size Exclusion Chromatography

This is a Superdex 75 column. It used to separate proteins based on molecular weight (3-70 kDa). The protein was concentrated and 3 mL of 2 mM protein was placed on the column. Two distinct bands appeared and these were analyzed using the UV/Vis detectors on the FPLC and gel electrophoresis.

Size Exclusion Chromatography (SEC)
20 mM Tris pH 8.8 w/ 200 mM NaCl





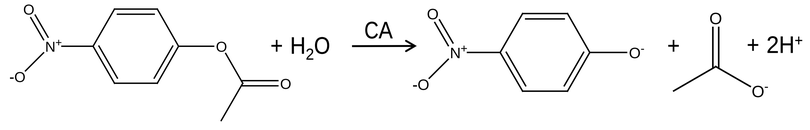


We used non-reducing SDS-PAGE to analyze the fractions from the FPLC. The gels show that while our protein is present as a dimer, it also seems to interact with other proteins in solution through disulfide crosslinks and/or protein-protein interactions (PPIs) most likely due to the added tyrosines.

Conclusions

The added cysteine and tyrosine residues have complicated the purification of our protein due to their ability to allow our protein to interact strongly with other protein impurities. The normal process by which our protein is purified consisting of two ion exchange columns and a size exclusion were not enough to purify our mutated protein. The next step will be to use the ion exchange columns again but this time in the presence of a reducing agent so that there are do disulfide bonds and to vary the pH in order to interfere with any unwanted PPIs that may be caused by the tyrosine residues. Once our protein is finally pure we can try to get a crystal structure in the presence of Zn(II) and try an enzymatic assay to test its activity.

para-Nitrophenyl Acetate (pNPA) Hydrolysis



A_{max} = 274 nm

A_{max} = 410 nm