Characterization of the C2 Domain of the Cellulose Synthase Interacting Protein 1

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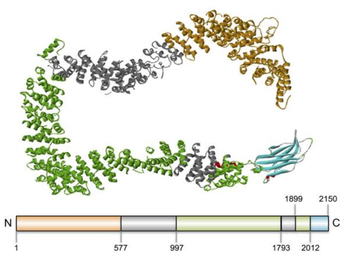
Motivation

Cellulose, which is derived from β 1-4 polymerized glucose, constitutes a major part of the plant cell wall. Stemming from the abundance of biomass with copious amounts of cellulose, this carbohydrate has been proposed as a potential source of biofuels. In order to optimize cellulose production, the pathway for the production of cellulose must be detailed and understood. Cellulose is synthesized by the cellulose synthesis complex (CSC), which is brought to the cell wall by microtubules and helper proteins such as cellulose interacting protein 1 (CSI1). Unfortunately, as of today, there is no reported structure for the individual proteins that comprise the CSC or the accessory protein, CSI1.

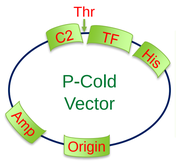
Project Goal

In order to understand the CSC, the enzyme's path to the cell wall was investigated. It is known that microtubules help coordinate the CSC position in the plasma membrane with the help of CSI1; however, the exact mechanism of this interaction is unknown (1,2). To fill this knowledge gap, the CSI1 complex was recombinantly expressed and induced in *Escherichia coli* BL21 cells. Once upscaled, the protein was purified in a series of affinity and size exclusion chromatography steps. Both the full CSI1 protein and the CSI1-C2 domain were purified, but the main goal for this project was to analyze the C2 domain, the microtubule interacting domain, of the protein. The goal was to characterize the C2 domain's physicochemical properties as well as to understand the protein's structure and oligomeric state with small angle X-ray and neutron scattering techniques. The long term goals are to use single particle cryo-electron microscopy to obtain an atomistic model of the cellulose synthesis complex.



Computational model of CSI1 from reference 1

Methods



1

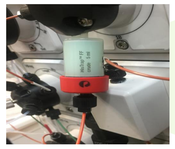
Transformation of *E. coli*

- Add P-Cold plasmid to E. coli suspension cells
- Heat shock cells (45 sec) to uptake plasmid into cells
- Grow cells in Luria-Bertani (LB) at 37 °C for 18 h

2

Protein production

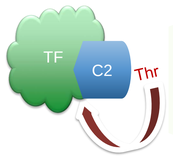
- Inoculate 1 L LB media with *E. coli* culture
- Induce cells at an OD 0.6 - 0.8 with 1 mM IPTG
- Grow overnight at 18 °C, harvest cells, store at -80 °C



3

Chromatography

- Lyse cells and centrifuge to separate cell debris
- Apply supernatant to Ni affinity column
- Remove impurities by size exclusion chromatography



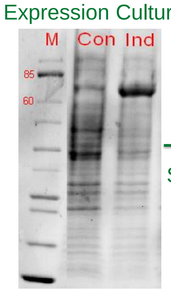
4

Removal of expression tag

- Add Factor Xa or Thrombin to protein solution
- Incubate at room temperature overnight
- Purify the CSI1-C2 domain using Ni column

Results

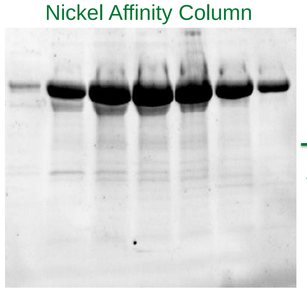
Expression Culture



Purify

Step #3

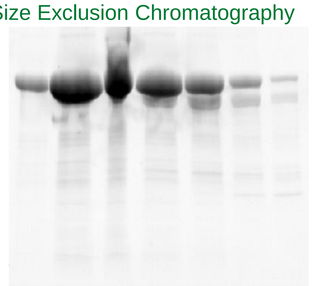
Nickel Affinity Column



Purify

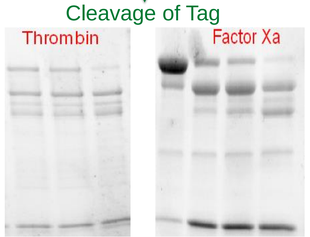
Step #3

Size Exclusion Chromatography

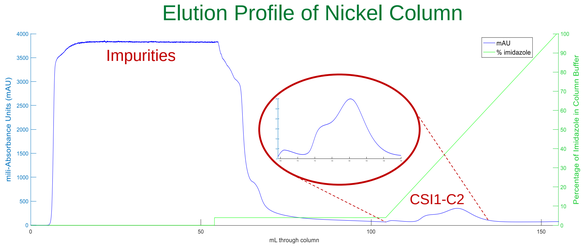


Step #4

Cleavage of Tag



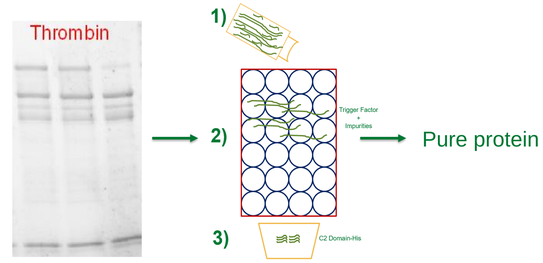
Elution Profile of Nickel Column



Next Steps

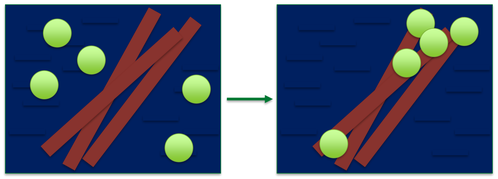
Nickel column chromatography:

- Separate CSI1-C2 domain from trigger factor tag



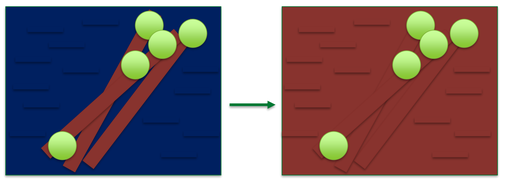
Microtubules binding assay:

- Verify the CSI1-C2 domain interaction with the microtubule



Small angle neutron scattering:

- Compare the structure of deuterated CSI1-C2 domain, bound and unbound, to microtubules using contrast matching SANS



Acknowledgments

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References

1. Lei, L., Singh, A., Bashline, L., Li, S., Yingling, Y. G., & Gu, Y. (2015). CELLULOSE SYNTHASE INTERACTIVE1 Is Required for Fast Recycling of Cellulose Synthase Complexes to the Plasma Membrane in Arabidopsis. *The Plant Cell*, *27*(10), 2926–2940. http://doi.org/10.1105/tpc.15.00442
2. Li, S., Lei, L., Somerville, C. R., & Gu, Y. (2012). Cellulose synthase interactive protein 1 (CSI1) links microtubules and cellulose synthase complexes. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(1), 185–190. http://doi.org/10.1073/pnas.1118560109

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