Expression. Characterization, and Crystallization of the *E. coli* Acetate Transporter YaaH

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Introduction

Protein crystallography is the science of elucidating the atomic resolution structure of macromolecules. Scientific research strongly suggests that the function of a protein directly depends on its corresponding structure, thus the 3-D structure of a protein can answer questions regarding what the mechanism of a protein. The structure guided development of therapeutics is one of the practical applications of a high resolution protein structure.

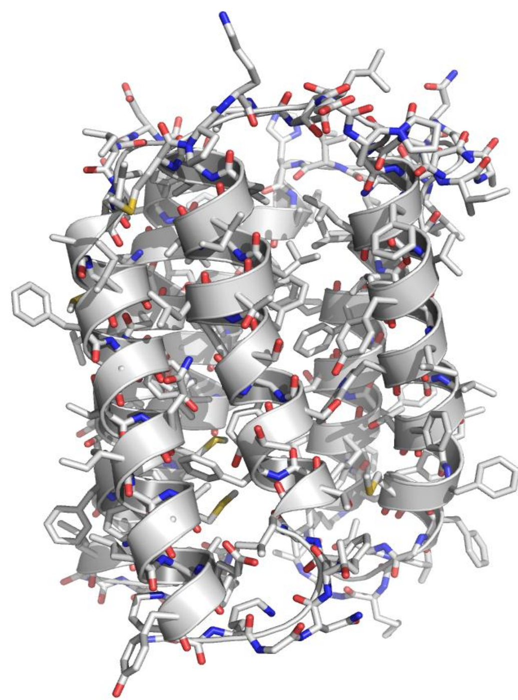

Figure 1: Possible 3-D structure of YaaH

In this work, we are seeking to solve the first structure of a member of GPR1/FUN34/YaaH superfamily of proteins, which are integral membrane proteins involved in the transport of acetate, ammonia, and possibly other small carboxylates. These transport proteins are involved in nutrient intake, waste secretion, and environmental adaptation. They have been found to be important in the survival of some pathogenic fungi such as *Candida albicans.* By inhibiting the activity of this transporter, it is possible to discover a new class of anti-fungals targeting one of the most troublesome fungal pathogen that afflict people. Here, we present our work on the E. *coli* YaaH, a prototype of this superfamily of membrane transporter. Our goal is to characterize and describe the expression, purification, function, and the crystal structure of YaaH in order to gain a clear understanding of the structure and function of this protein.

Experimental Methods

Transformation and expression:

- Expression plasmid was constructed by inserting the YaaH gene (567 bp in length) into vector pE-SUMO Amp[1] (see Figure 2).
- The plasmid is propagated in *E. coli.*

Protein Extraction and Purification:

- Pelleting of transformed cells via centrifugation
- Lysis of cells via sonication
- Solubilization of the protein with detergents
- Purification via Ni-NTA chromatography and cleaving of His tag
- Purification using size exclusion column chromatography.

Protein Crystallization:

- Testing different solvent conditions for production of protein crystals [2]
- Optimization of crystals based on initial results

X-Ray crystallography:

- X-Ray diffraction methods used to obtain a diffraction data to determine the structure of the protein.

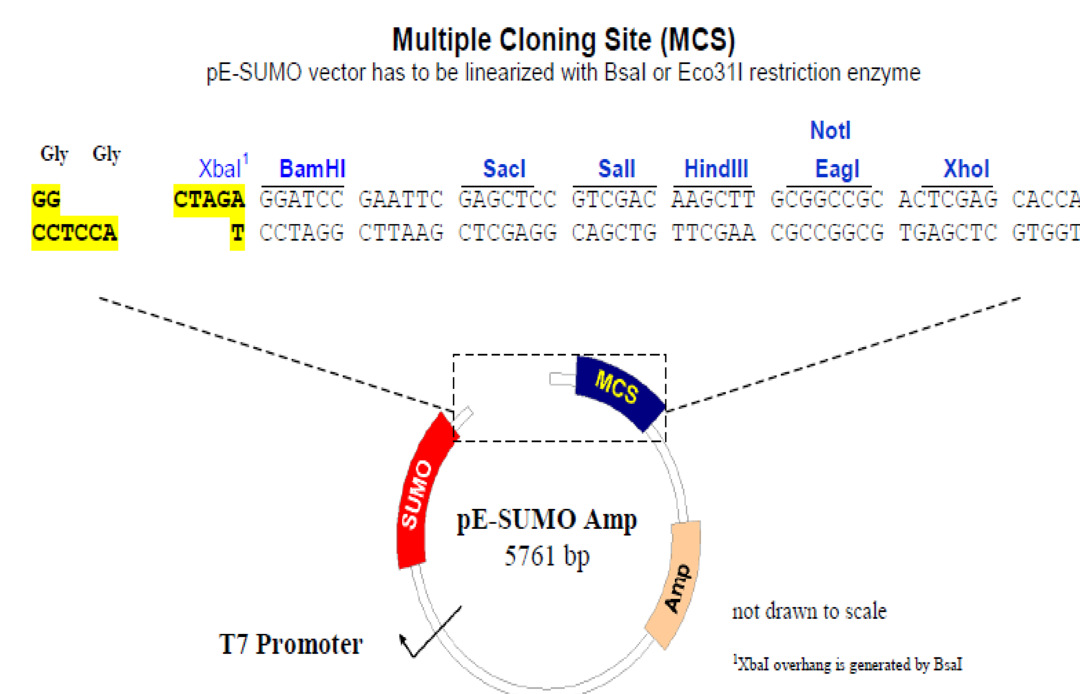

Figure 2: Plasmid construct of pE-SUMO Amp'

Results

Yaah exists as a stable hexameric complex:

- The protein in SDS runs at ~130kDa rather than the expected 20kDa in SDS PAGE gel indicating that YaaH is running as a homo-oligomer. (See figure 3B)
- This conclusion was confirmed by size exclusion chromatography which suggested high oligomeric state. (See figure 3A)
- It was also concluded that the oligomeric state is very stable since it maintains this structure even in SDS.
- The protein crystallized in an hexameric space group, with the theoretical asymmetric unit containing 20kD, suggesting the protein is a homo-hexamer.

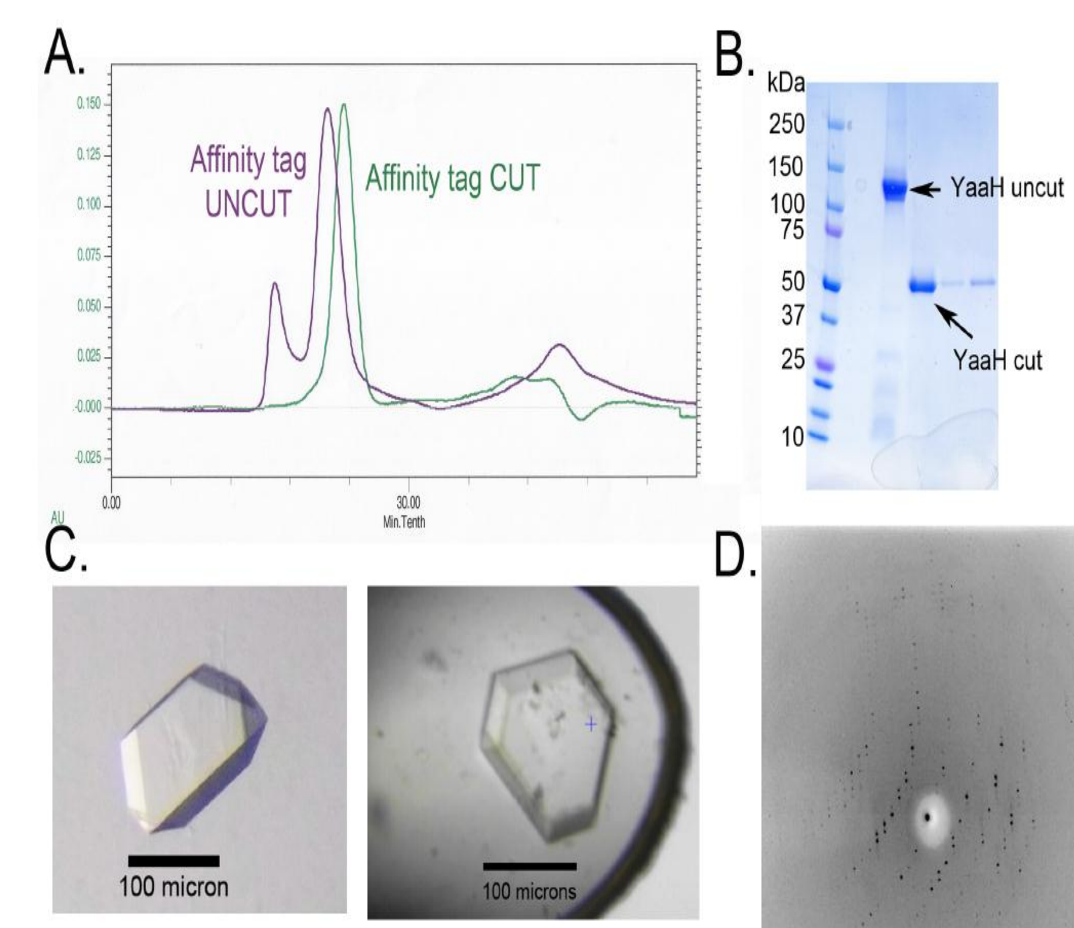

Figure 3: A) Size exclusion chromatograms (Superdex) of YaaH. The purple trace is the sample that still has the SUMO affinity tag attached while the green trace is that of the YaaH after the affinity tag has been cleaved. B) SDS PAGE gel of YaaH. This gel suggests that the protein runs as an oligomer even in SDS. The protomer size is 20kDa. C) Representative crystals of YaaH. D) representative diffraction pattern

Conclusions

Based on our work thus far we have promising preliminary results and we are hoping to do more work to elucidate the structure. Currently, the highest diffracting crystals up to 2.6A resolution.

Possible future directions will be to conduct growth tests using YaaH knockout and mutants, and proteoliposome transport assays to further characterize the function of YaaH in E. Coli. Through our efforts we hope to solve the structure in order to determine the selectivity and gating of this transporter. Our future results could provide structure-based development of anti-fungal drugs specifically targeting the acetate YaaH orthologs in yeast.

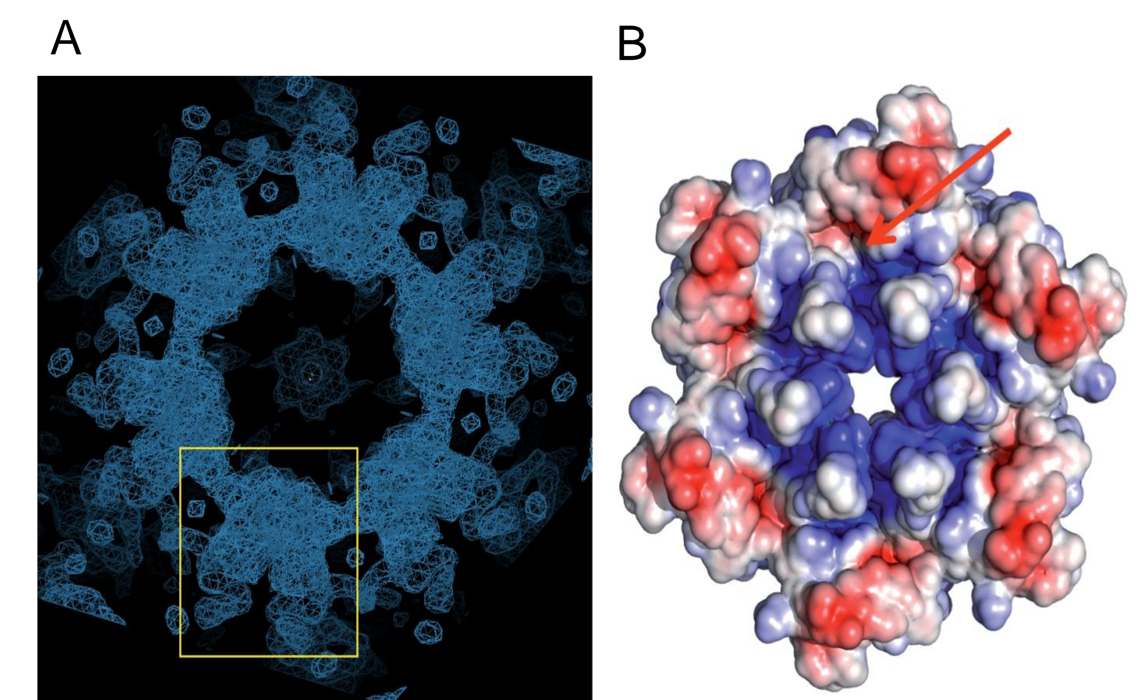

Figure 4: A) Electron density map of YaaH based on preliminary data B) Urea channel surface diagram

References

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3) Strugatsky, David, et al. "Structure of the proton-gated urea channel from the gastric pathogen Helicobacter pylori." *Nature* 493.7431 (2012): 255-258.

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