Examining the Bacterial Methionine Transporter Utilizing Soluble Lipid Bilayer Systems

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Introduction

- The phospholipid bilayer present in both eukaryotes and prokaryotes regulates the cell's acquisition of nutrients and excretion of waste.
- Integral membrane proteins mediate the movement of molecules across the cell membrane. Investigating the mechanistic properties of these proteins can help us understand how substrates are being transported.
- ABC (ATP-Binding Cassette) transporters constitute a superfamily of transmembrane transporters. Both importers and exporters of this family change conformation via the binding and hydrolysis of ATP to facilitate active transport[1].

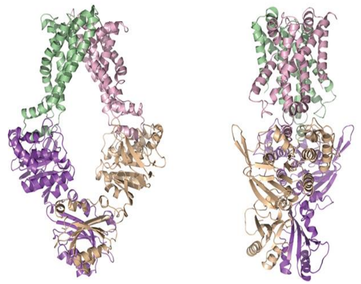

Figure 1. MetNI ABC Transporter[4]

Background

- The study of ABC transporters is significant in human disease treatment; for example, several ABC transporters have been implicated in multidrug-resistance[2], and a mutation in an ABC transporter has been found in cystic fibrosis patients[3].
- The structure of the E. coli methionine importer, called MetNI, has been solved by x-ray crystallography[4]. Functional characterization has shown that methionine is not only the substrate, but is also an allosteric inhibitor of transporter activity[1].
- Amphipathic detergents have been traditionally used to isolate and purify these transport proteins for structural and functional studies. Recent work suggests that these conditions may not accurately mimic *in vivo* conditions. A novel system, called nanodiscs, allows for the characterization of membrane proteins in a lipidic bilayer[5].

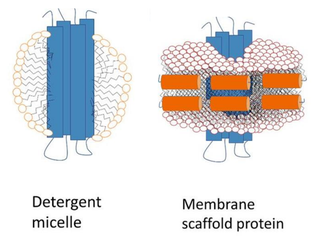

Figure 2. Schematic for detergent-solubilized transporter vs. reconstituted in nanodiscs[5]

Questions

- **Will transporters isolated using detergent differ significantly in activity compared to activity measured of transporters reconstituted in nanodiscs? If so, how are they different?**
- **Will our results justify in using nanodiscs over detergent to prepare transporters for structural and functional studies?**

Methods & Results

1) Transformation and Large-Scale Expression of Membrane Scaffold Protein (MSP) Component

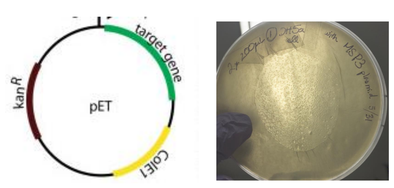

Figure 3. Transformation of membrane scaffold protein (MSP3) into BL21 E. coli cells. Colonies were chosen for antibiotic resistance to kanamycin[6].

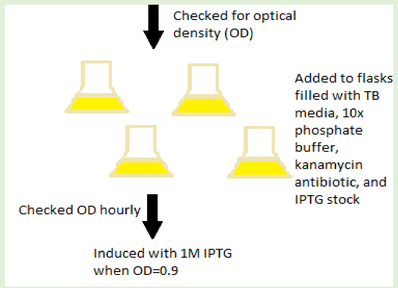

Figure 4. Schematic for large-scale expression

2) Purification

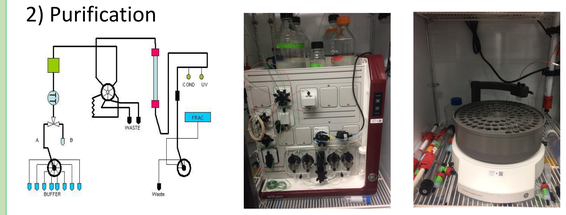

Figure 5. Experimental setup (left to right): schematic of FPLC[7], FPLC, and fraction collector

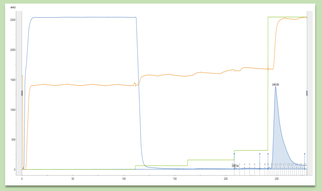

Figure 6. Chromatogram of column wash. The blue peak is purified sample containing target MSP protein with the poly-His tag.

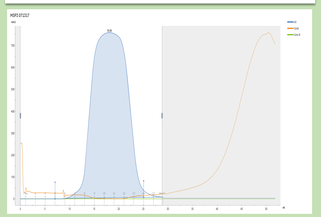

Figure 7. Chromatogram of sample desalting. Peak fractions were collected and saved for SDS-gel.

3) SDS-PAGE

Figure 8. Bio-Rad SDS gel equipment

Figure 9. SDS-PAGE. Samples for lanes 2 and 4 were collected during the purification process. Different volumes of the concentrated sample of purified protein after desalting are in lanes 6 and 7.

4) ATPase Assay

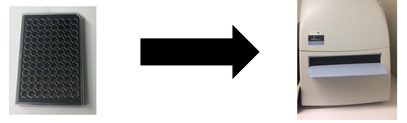

Figure 10. Standard 96-well plate and Perkin Elmer Envision 2104 plate reader

V_{max}^{app} ($ADP \cdot min^{-1}$)	K_m^{app}MgATP (nM)	k_{cat} (min^{-1})
432 ± 80	619 ± 200	4 ± 1

Table 1. Kinetic data for detergent-solubilized MetNI. Average values for V_{max}, K_m, and k_{cat} from three independent ATPase assays of WT MetNI transporter

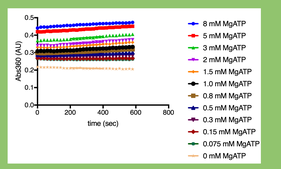

Figure 11. Raw data from ATPase assay. The Enzchek Assay kit from Molecular Probes was used to measure ADP production as a function of time.

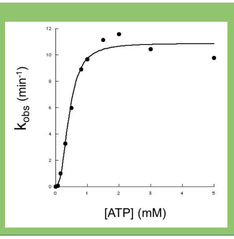

Figure 12. MetNI data fit to a Michaelis-Menten model. Representative data shown here were fit with a Hill coefficient of = 1.8.

Acknowledgements

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- Phong Nguyen and Jeff Lai provided the plasmids for membrane scaffolding proteins.
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Conclusion

Overall, this project yielded two important advancements:

(1) the purification of membrane-scaffolding protein MSP3

(2) successful measurement of MetNI ATPase activity in detergent

This work sets the foundation for the next step, assaying ATPase activity in MetNI nanodiscs. We are currently optimizing the protocol for nanodisc reconstitution. Our ultimate goal is to compare the turnover rate of detergent-solubilized MetNI to that of MetNI reconstituted into nanodiscs.

References

1. Yang, J. G., & Rees, D. C. (2015). The allosteric regulatory mechanism of the Escherichia Coli MetNI methionine ATP binding cassette (ABC) transporter. Journal of Biological Chemistry, 290(14), 9135–9140.
2. Boumendjel, A., Boutonnat, J., & Robert, J. (Eds.). (2009). ABC Transporters and Multidrug Resistance. Hoboken, NJ, USA: John Wiley & Sons, Inc.
3. Mendoza, J. L., & Thomas, P. J. (2007, December). Building an understanding of cystic fibrosis on the foundation of ABC transporter structures. Journal of Bioenergetics and Biomembranes.
4. Kadaba N.S., Kaiser J.T., Johnson E., Lee A.,and Rees D.C. (2008). The high-affinity E. coli methionine ABC transporter: structure and allosteric regulation. Science, 321(5886):250-3.
5. Hardy, D., Bill, R. M., Jawhari, A., & Rothnie, A. J. (2016). Overcoming bottlenecks in the membrane protein structural biology pipeline. Biochemical Society Transactions, 44(3), 838–844. I.G. Denisov, S.G. Sligar, Nanodiscs in Membrane Biochemistry and Biophysics, Chem. Rev. 117 (2017).
6. Schlegel, S., Rujas, E., Ytterberg, A. J., Zubarev, R. A., Luirink, J., & de Gier, J. W. (2013). Optimizing heterologous protein production in the periplasm of E. coli by regulating gene expression levels. Microbial Cell Factories, 12(1).
7. Technology Facility, Department of Biology at University of York. Protein Production Equipment. https://www.york.ac.uk/biology/technology-facility/protein-production/pp-equipment/pp-akta-prime/#tab-1.