Investigating the mechanism of the *E. coli* ATP-binding cassette transporter MetNI

Matthew Foronda, Kha Andy Tran, Janet G. Yang

UNIVERSITY OF SAN FRANCISCO

Introduction

- ABC (ATP-binding cassette) transporters have become a topic of research as mutations in them can lead to a number of diseases such as cystic fibrosis, Stargardt's disease (vision loss), and even development of drug-resistant tumors.[1]
- These transporters utilize ATP hydrolysis to transport molecules across a membrane against their concentration gradient.
- Mechanisms for these transporters are being researched as their understanding could bring vital clues to finding cures for their associated diseases.
- We are using a bacterial amino acid transporter to understand how these proteins function

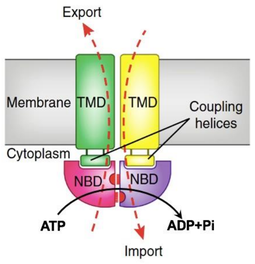

Figure 1. General structure of an ABC transporter.[2]

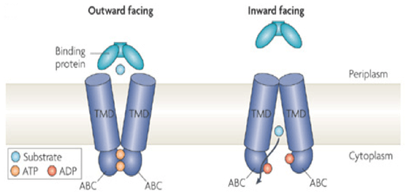

Figure 2. ABC transporter and substrate binding protein in inward and outward facing conformations.[3]

Research Goal

Our work looks into defining the mechanism of the bacterial methionine transporter MetNI by looking into its binding affinity with substrate binding protein MetQ. There are two roles proposed for the MetQ protein. MetQ could either bind substrate and deliver it to the transporter (canonical model) or it could bind to the transporter and create a pathway for substrate to enter the transmembrane pathway (non-canonical model).

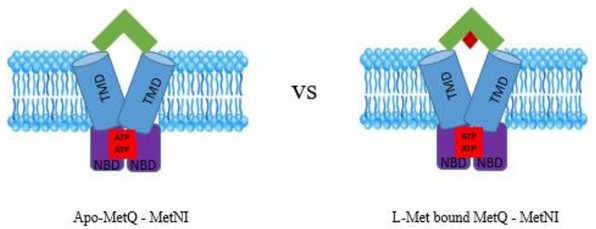

Figure 3. The binding affinity of apo-MetQ and L-Met bound MetQ to ATP-bound MetNI were compared.

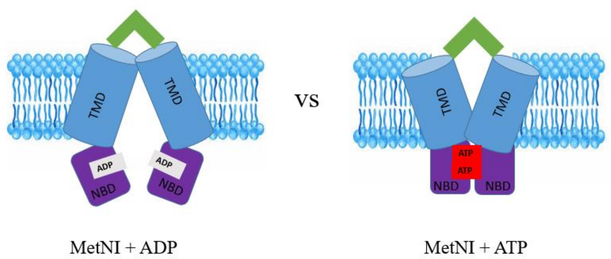

Figure 4. The binding affinity of ADP-bound MetNI and ATP-bound MetNI to both apo-MetQ and L-Met bound MetQ were compared.

Methods

- Wild-type MetNI and MetNI E166Q, a mutant that can bind but not hydrolyze ATP, was purified using high performance liquid chromatography
- ATPase assays were performed on wild-type MetNI and MetNI E166Q to confirm the mutant's inability to hydrolyze ATP
- Wild type MetQ and MetQ N229A were purified and labeled with fluorescein 5- maleimide
- Using 20 nM labeled MetQ and varying concentrations of MetNI, the change in polarization values make it possible to determine the dissociation constant (k_d) of the MetQ-MetNI complex in different nucleotide and substrate environments.

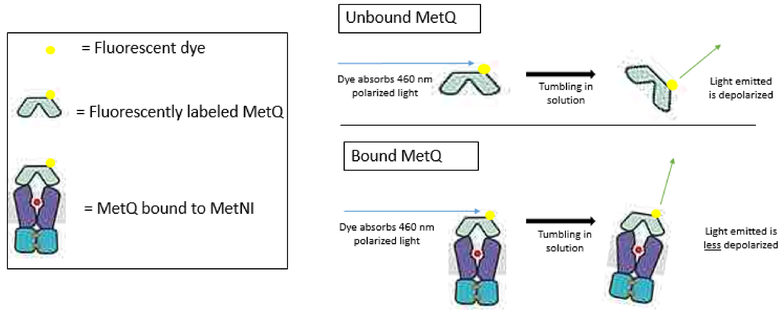

Figure 5. When MetQ binds to MetNI, its anisotropy value increases as the complexed molecule is larger and will tumble less in solution. Using the difference in anisotropy, assays can determine the binding affinity of MetQ to MetNI in different nucleotide and substrate environments.

Results

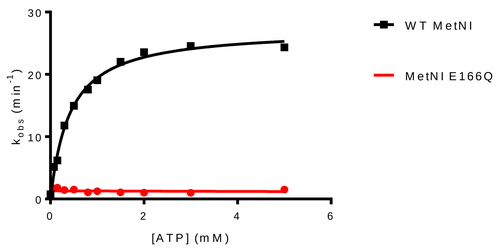

Figure 6. ATPase assays of wild-type MetNI and MetNI E166Q exhibit the loss of ATPase activity by the MetNI mutant.

- By losing a crucial amino acid responsible for ATP hydrolysis, the MetNI E166Q mutant is locked in its ATP-bound form with ATP present.

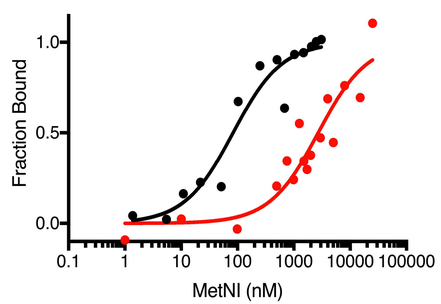

Figure 7. Anisotropy curves of MetQ N229A and MetQ wild-type with varying concentrations of ATP-bound MetNI show a distinct difference in binding affinity between an outward-facing transporter, apo-MetQ and L-met bound MetQ.

- k_d of apo MetQ - MetNI in ATP is 83 nM
- k_d of L-met bound MetQ – MetNI in ATP is 2706 nM
- These results are consistent with published results utilizing thermophoresis titration, validating the fluorescence anisotropy method

Results continued

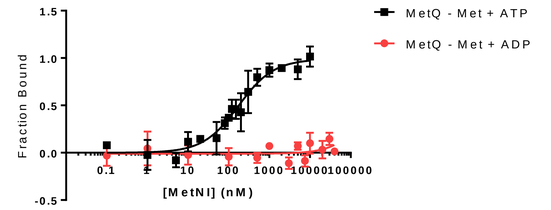

Figure 8. When MetNI is bound to ADP, there is little to no complex formation with apo MetQ.

- For both apo-MetQ and L-met bound MetQ, there is no complex formation below 20 μM with ADP-bound MetNI

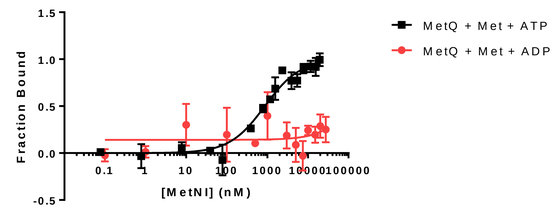

Figure 9. As is the case for apo-MetQ, MetNI bound to ADP does not form a complex with L-met bound MetQ.

- MetQ binding must occur when ATP is bound but before hydrolysis occurs, unbinding once the transporter switches to an inward-facing conformation

Future Work

- Use fluorescence anisotropy to determine the binding affinity of L-Methionine to the C2 domains of the transporter
- Reconstitute MetNI in lipid nanodiscs
- Compare nanodisc and detergent-solubilized MetNI binding affinities with MetQ to test whether or not lipid environment has an effect on transporter binding affinity

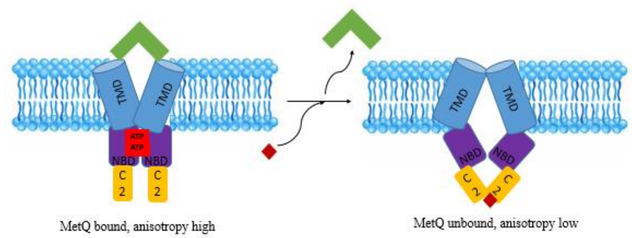

Figure 10. The C2 domains inhibit transport activity from occurring by locking the transporter in the inward-facing conformation when L-methionine is bound. Using fluorescence anisotropy the binding affinity of L-methionine to the C2 domains will be determined.

References

1. Norouzi S., Valokala M.G., Mosaffa F., Zirak M.R., Zamani P., Behravan J. Crosstalk in cancer resistance and metastasis. *Critical Review in Oncology/Hematology.* 2018. 132:145-153
2. Rees DC, Johnson E, Lewinson O. ABC transporters: The power to change. Nature reviews Molecular cell biology. 2009;10(3):218-227. doi:10.1038/nrm2646.
3. 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.

Acknowledgements

This research is funded by the Faculty Development Fund of the University of San Francisco and the Whitehead Fellowship. A special thanks to the Narlikar and Agard labs at UCSF for allowing us to use their space and resources.