Purification and Initial Characterization of a Hypothetical Membrane Protein

UNIVERSITY of HOUSTON
BIOLOGY AND BIOCHEMISTRY

Stewart Fannin, Jonathan Rangel, William Widger, Mehmet Sen

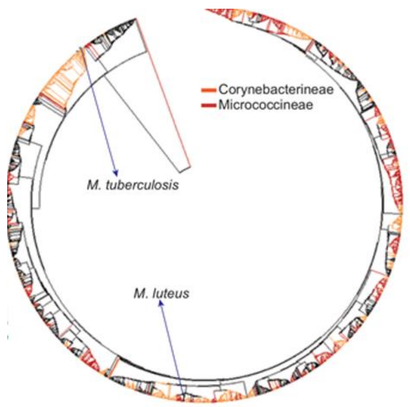
University of Houston department of Biology and Biochemistry

SEN LAB

Abstract

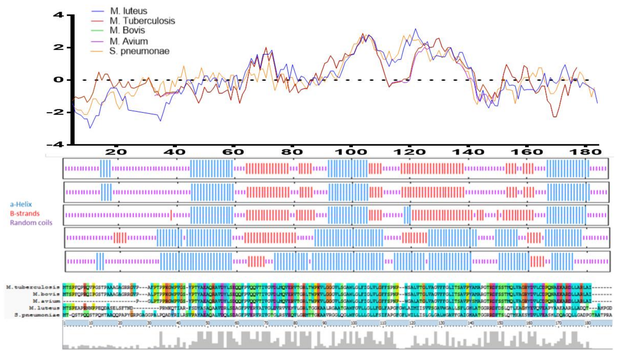
Comparatively few structures of membrane proteins have been determined. Approximately 1% of structures in the protein database (PDB) belong to membrane proteins, despite the fact that 20-30% of predicted proteins and 50% of known drug targets being membrane proteins. This discrepancy is largely due to the difficulties presented by purifying and working with hydrophobic structures such as the transmembrane domains of membrane proteins. Previous data from our lab has identified a number proteins that are upregulated in the dormant state of *Micrococcus luteus* including a previously uncharacterized membraned protein (Hyp730). In this study we have used Ginzu and ROBETTA server structure prediction, to computational predict the structure of Hyp730 based. We also Identified a method of purifying Hyp730 using a mono Q anion exchange column. Once the protein was purified, we used CD spectroscopy to identify the secondary structure composition of Hyp730. The CD data corroborates our predicted structure, though further experiments will still be required fully elucidate the structure.

Phylogenetic Analysis

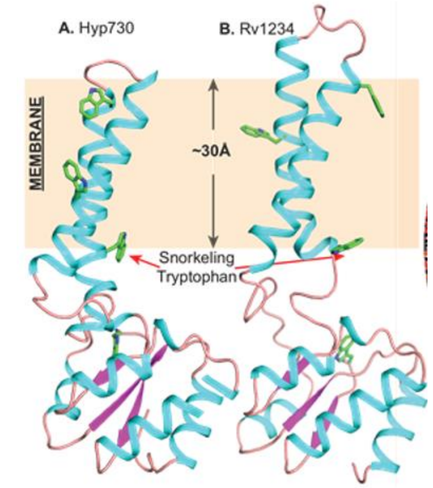


Phylogenic analysis of Hyp730 shows it to be conserved throughout the phylum actinobacteria, and ancient phylum of G/C rich gram positive bacteria. None of the homologs have been structurally or functionally characterized, however previous data would seem to indicate a role in dormancy. Actinobacteria contains a number of pathogenic bacteria including those in the genera *Corynbacterium, Mycobacterium,* and *Nocardia*. As such characterizing these proteins could yeild valuable insights into potential clinical treatments for these diseases. .

Structural Prediction

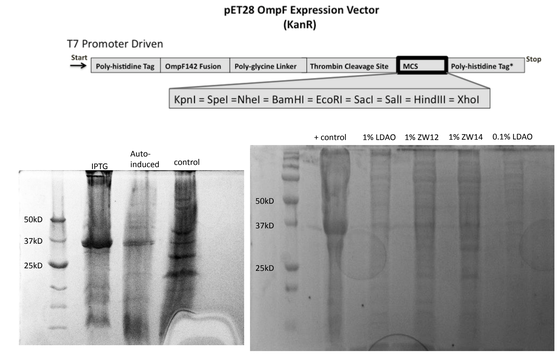


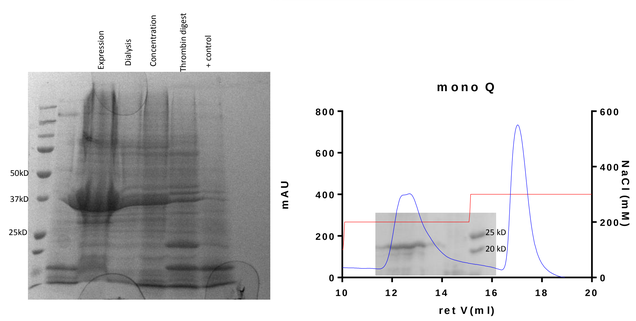
The sequences of Hyp730 and four homologs including the Rv1234 protein in *M. tuberculosis* were aligned using ClustalX sequence alignment software (C). This alignment data was then use to prepare an aligned Kyte-Doolittle hydrophobicity plot (A), as well as an aligned secondary structure prediction using the PHD method. This alignment shows a conserved hydrophobic region corresponding to two alpha helix domains. This combination indicates a putative transmembrane domain. .



Predicted structures where constructed for Hyp730 and Rv1234 using Ginzu comparative structural modeling (Shown) and the Robetta structure prediction server. The predicted structure indicates the presence of conserved Tryptophan residues at the edge of the transmembrane domain. Such 'snorkeling' Trp residues help to stabilize type I tansmembrane, and helps to validate this model. Furthermore The majority of the proteins used to construct the model structure where oxidoreductases and hydrolases. Furthermore *M. luteus* has been shown to undergo a loss a membrane potential and decreased redox balance in the dormant state (Sen & Widger unpublished). Which suggest a putative role for Hyp730 likely plays a role in regulating this change in redox balance.

Purification





The Hyp730 protein was co-expressed in BL21-*E.coli* as part of a fusion protein with a portion of the OmpF (Outer Membrane Protein F) protein, in order to facilitate easier expression of the membrane protein. Then expression was induce IPTG. After lysising the cells the protein was found in the inclusion bodies and needed solubilizes. Initial attempt to directly solubilize the protein in Non-denaturing detergents were unsuccessful. Ultimately Solubilization was achieved by first dissolving inclusion body in 6M Urea, then adding LDAO to a final concentration of 1% and dialyzing out the Urea. Once the protein was solubilized the OmpF tag was cleaved off by overnight Thrombin Digest. Finally Hyp730 was isolated from OmpF using a Mono Q anion exchange column, eluting the proteins by increasing the NaCl concentration in a stepwise fashion.

CD spectroscopy

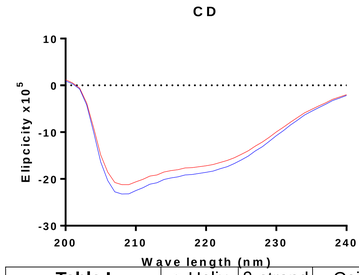


Table I	α-Helix	β-strand	Coil
Hyp730 (CD)	55%	8%	37%
Hyp730-Prediction	52.6%	8.6%	38.9%

Circular Dichroism Spectroscopy was used to analyze the secondary structure composition of the purified Hyp730 protein. The composition of the protein as measured by the CD was found to be primarily α-helical, and closely matches that the composition shown in our predicted model. Furthermore ability to measure the secondary with CD verifies that the protein has been properly refolded after denaturing it with urea during purification.

Discussion

This study constitutes the first step in characterizing an interesting new protein. While little is known about Hyp730 the data collected so far is a promising indication that plays role in the Redox balance associated with the dormant state of various bacteria, and this protein may even serve as a valuable drug target someday. The next steps towards this end will likely include using Trp fluorescence quenching to verify the prescence of snorkeling Trp, creating a more soluble construct for use in crystallization studies, creating conditional knockout strains in *M. luteus* in order to study to resulting phenotype and see if it verifies our hypothesized function for the protein, and potentially extending the study into the *M. tuberculosis* variant Rv1234.

References

1. Mali S, Mitchell M, Havis S, Bodunrin A, Rangel J, Olson G, Widger WR, Bark SJ. 2017. A proteomic signature of dormancy in the actinobacterium Micrococcus luteus. J Bacteriol 199:e00206-17. https://doi.org/10.1128/JB.00206-17.
2. Su P. et al 2013. High-yield membrane protein expression from E. coli using an engineered outer membrane protein F fusion, Protein Science vol 22:434—443