Poster Presentation

MS101.P07

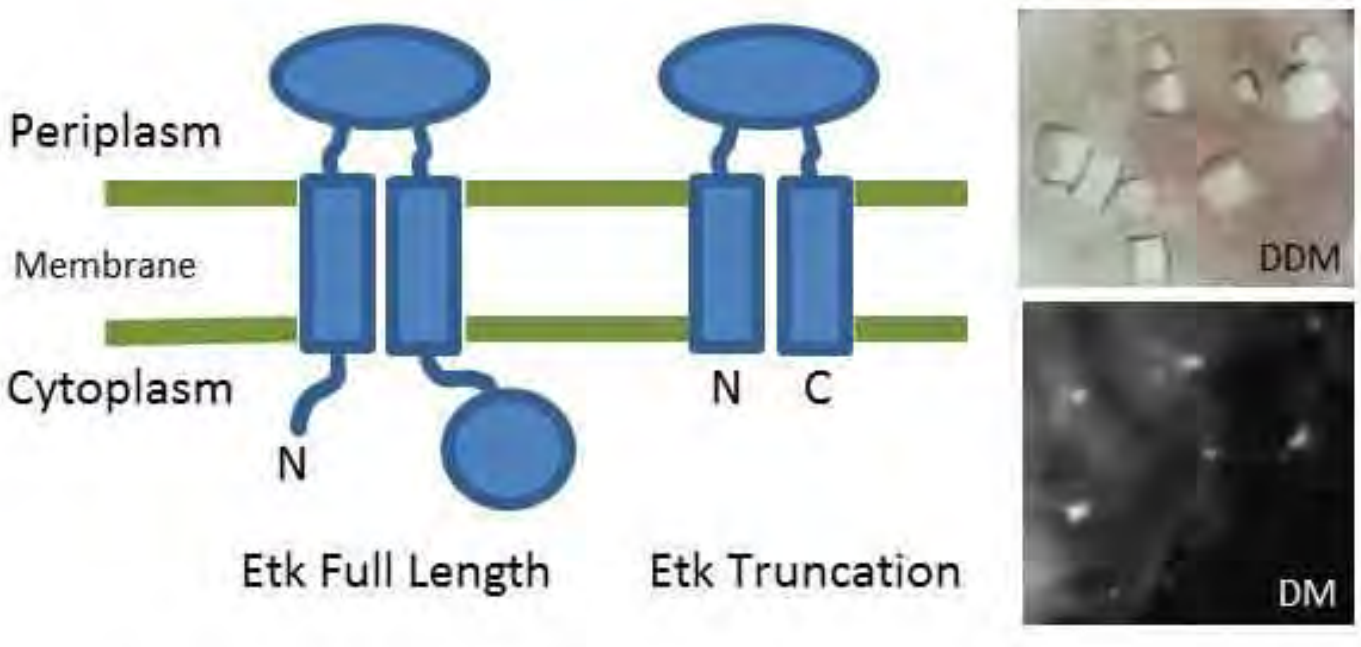
Biophysical characterization and crystallization of the membrane protein Etk

C. Prince[1], Z. Jia[1]

[1]*Queen's University, Department of Biomedical and Molecular Sciences, Kingston, Canada*

The inner membrane protein E. coli tyrosine kinase (Etk) is part of a large protein complex that assembles and exports capsular polysaccharide (CPS) in Gram-negative bacteria. Etk interacts with an outer membrane protein channel, YccZ (1), and regulates CPS export through autophosphorylation of a tyrosine cluster in its C-terminal tail. Previous work resulted in the structure of the isolated Etk C-terminal kinase domain (2). In the present study, the full-length protein has been purified and characterized in vitro. When purified in n-dodecyl-β-D-maltoside (DDM), Etk full length retains autophosphorylation activity, but is not suitable for crystallization because it severely aggregates and degrades. Using the main degradation product, a truncation containing the N-terminal domain (interacts with YccZ) and both transmembrane helices was designed. Truncated Etk does not further degrade and exists as a mixture of monomers and dimers when solubilized by five detergents as determined by size-exclusion chromatography and analytical ultracentrifugation. Crystals have been successfully grown when the protein is solubilized in DDM or n-decyl-β-D-maltoside (DM). The most promising crystals (DDM, 0.1 M MES pH 6.0, 1-5% PEG 3000, 20-30% PEG 200) have been reproduced and optimized with the assistance of a colorimetric assay (3). This assay relies on a reaction between 2,6-dimethylphenol, sulfuric acid, and the sugar head group of certain detergents to accurately quantify detergent in crystallization samples with minimal sample loss. Additive screening also revealed that MgCl2 improves crystallization. Optimization of this crystallization condition has significantly improved reproducibility of these crystals, but x-ray diffraction is limited to 6.5 Å. Current work is focused on reproducing and optimizing a second crystallization lead (DM, 0.1 M KH2PO4 pH 7.5, 32% PEG 400, 0.1 M KCl).

[1] *R. Collins, K. Beis, C. Dong, C. Botting et al. P. Natl. Acad. Sci. (2007) 104, 2390-2395,* ***[2]*** *D. Lee, J. Zheng, Y. She, Z. Jia, EMBO J. (2008) 27, 1758-1766.,* ***[3]*** *C. Prince, Z. Jia, Acta Cryst. D (2012) 68, 1694-1696.*



Keywords: Membrane Proteins, Biophysical Characterization, Crystallization