

Crystallography Trials of Escherichia coli Nitrate Transporter, NarU

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Abstract : The stability of the protein in detergent-containing solution is the key for its successful crystallisation. Fluorescence-detection size-exclusion chromatography (FSEC) is a potential approach for screening monodispersity as well as the stability of protein in a detergent-containing-solution. In this present study, covalently linked Green Fluorescent Protein (GFP) to bacterial nitrate transporter, NarU from Escherichia coli was studied for pre-crystallisation trials by FSEC. Immobilised metal ion affinity chromatography (IMAC) and gel filtration were employed for their purification. The main objectives of this study were over-expression, detergent screening and crystallisation of nitrate transporter proteins. This study could not produce enough proteins that could realistically be taken forward to achieve the objectives set for this particular research. In future work, different combinations of variables like vectors, tags, creation of mutant proteins, host cells, position of GFP (N- or C-terminal) and/or membrane proteins would be tried to determine the best combination as the principle of technique is still promising.

Keywords : transporters, detergents, over-expression, crystallography

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