

PROGRESS REPORT

Project title: Molecular Mechanism of Cystinosis

Persons work on the project: Xue Guo, Research Fellow; Liang Feng, Mentor

Date: 02/29/2016

Objectives and specific aim:

In this study, our goal is to decipher the molecular mechanism of membrane transporters on lysosome that play critical roles in pathogenesis and therapy of cystinosis. We aim to obtain their atomic structures and studying their transport function in a well-defined *in vitro* system. The result will address the following questions: (1) How membrane transporters specifically recognize and transport their substrate; (2) How disease-causing mutations affect transport activities.

The specific aims are:

1. Determine the high resolution structure of membrane transporters by X-ray crystallography
2. Characterize the transport function in a well-defined system.

Executive overview of progress: Suitable for public disclosure

Obtaining sufficient quantities of well-behaved recombinant protein is a bottle neck for structural studies on membrane proteins. The hydrophobic nature of the membrane proteins makes them difficult to work with. They require a proper environment of lipid or detergent micelle for maintaining structural integrity and function. Furthermore, expression host often have dramatic effect on the folding, proper membrane insertion or function of membrane proteins. We have systemically analyzed the most widely used expression system, including *E. coli*, yeast, insect cells and mammalian cells to evaluate the expression and biochemical behavior of the transporter proteins. To increase the likelihood of finding a protein suitable for structural studies, homologs that can be used as faithful template to understand human transporter function were screened and promising candidates were identified.

To obtain milligram quantity of the recombinant protein for structural and functional studies, we chose the expression system that has the highest yield to cost ratio. We then systematically compared factors that might affect the level of protein expression, including temperature, time, the strength of transcription initiation and variations among different clones. Furthermore, since the choice of detergent has profound effects on protein stability and their crystallization ability, we evaluated a panel of the most commonly used detergents for their ability to stabilize transporter protein in solution and identified detergents suitable for extraction and purification. Our work led to the establishment of large-scale expression and purification scheme, as well as the identification of conditions that maintain the protein stability. Our future plan is to identify conditions that may facilitate structural studies and carry out crystallization trials.