

## **PROGRESS REPORT**

**Project title:** Molecular Mechanism of Cystinosis

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

**Date:** March 2016 – Aug 2016

### **Objectives and specific aim:**

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

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**

One major challenge of this project is to obtain milligram quantity of the recombinant protein for structural and functional studies. From test expressions and small-scale studies, we have identified conditions that enabled the expression and purification of the transporter protein.

Now we are taking a protein engineering approach to systematically evaluate parameters that could help to improve the yield, stability and crystallizability of the protein. These parameters are often linked, and it is important to find the optimal combination. Particularly, transporter proteins are dynamic, and flexible parts of the protein can interfere with crystallization because protein molecules need to pack in a highly ordered manner in three dimensions. We are exploring targeted mutations/truncations that might potentially improve the homogeneity of the protein. A large number of constructs were tested at a small scale to evaluate the biochemical behavior of the protein. Those with improved properties were then subject to larger scale purification in the presence of different detergents.