1-1-2019

Structure-Function Analysis of Inorganic Pyrophosphatase from Thermus thioreducens

Joseph Ng
University of Alabama in Huntsville

Structure-Function analysis of Inorganic Pyrophosphatase from *Thermus thioreducens*

Faculty proposal for the Research or Creative Experience for Undergraduate 2018

1. Dr. Joseph D. Ng, Professor

Department of Biological Sciences, Shelby Science and Technology Center Rm 369J

Email: NGJ@UAH.EDU, Phone: (256) 824-6166

Proposal Identifier: **RCEU19-BYS-JDN-01**

2. Project Description. **The goal** of the proposed RCEU study is to biochemically characterized the family I archaeal Inorganic Pyrophosphatase (IPPase) from *Thermus thioreducens*. The study of this enzyme is important for antibiotic discovery. The crystallographic structure of the *T. thioreducen* IPPase has already been determined by our laboratory in over five crystal forms. These structures include IPPase bound to Mg^{+2}, Ca^{+2}, Br^{-}, and SO_2^{-3} in the active site showing the presence and absence of the non-hydrolyzed and hydrolyzed pyrophosphate. Even though there is structural information on IPPase, there is still not a complete understanding of its catalytic and biochemical parameters. **The objectives** are to 1) determine the specific catalytic activity of the enzyme under different temperatures, pH and ionic concentration as well as the binding capacity of its ligands to determine K_d and K_m values; and 2) conduct functional and mutagenesis studies based on structural information. Kinetic parameters of wild type and mutants will be measured as a function of different metal binding with variations in pH and temperature. Using gene synthesis and mutagenesis techniques previously developed in our laboratory, we will alter proton networks by mutating targeted residues that are determined to be functionally important and consequently disrupt or enhance biochemical activities. This project seeks to identify structural factors that may be uniquely observed among thermophilic proteins and determine how these factors can contribute to thermal stability.

3. Student Duties, Contributions and Outcomes. In an iterative process from gene modification to protein structure the student will investigate the functional consequence of altering targeted residues thought to be important for IPPase activity

and assembly. The catalytic site contains a series of acidic side chains involved with substrate binding and catalysis as revealed by the X-ray crystallographic model. The **specific duties** will include doing site directed or regional mutation studies to examine the catalytic effect of the hydrogen coordination within 10 weeks. Sequence modification procedures will be employed by techniques published by the Dr. Ng's laboratory using PCR-based gene synthesis and *in vivo* homologous recombination allowing quick subcloning and mutagenesis. Residues of interest will be mutated and the newly reconstructed protein will be expressed by methods developed in the PI's laboratory. The **tangible contributions** the students will make include a poster, slide presentation and abstract in the Alabama Academy of Science conference book. Any publishable data will be included in a formal submission to a peer-reviewed journal that will include the student's name among the author list.

The **specific outcomes** projected for the student include 1) Experience in high through-put protein purification and handling; 2) Proficiency in site-directed mutagenesis**;** 3) Fundamental knowledge in enzymology; and 4) Experience in macromolecular modeling

4. <u>Faculty Requirements and Mentorship.</u> Student applicants should be in good academic standing with a GPA of 3.0 or better and must be at least a rising sophomore. Required coursework includes BYS119 and BYS120 or their equivalents. It is preferable, but not required, that the student has taken BYS363. The student is expected to not have any outside employment and preferably not take any classes while performing RCEU activities. Dr. Ng will serve as the primary mentor to the student. Current technicians and research associates (postdoctoral fellow) in the Ng lab will assist the student and research associates in his/her technical training. They will also be available during the work period to answer any questions or respond to any concerns that the student may have. Reports will be submitted on a weekly basis summarizing the progress of the experiment. The student will also attend weekly team meetings to discuss the progress of the experiment with Dr. Ng and the rest of his laboratory group.

5. Related references

- Pusey, M., Barcena, J., Morris, M., Singhal, A., Yuan, Q. and **Ng, J.D**. (2015). Trace fluorescent labeling for protein crystallization. *Acta Crystallogr Sect F Struct Biol Cryst Commun.* 71:806-814.
- **Ng, J.D.,** Baird, J.K., Coates, L., Garcia-Ruiz, J.M., Hodge, T.A. and Huan, S. (2015). Large volume protein crystal growth for Neutron Macromolecular Crystallography. *Acta Crystallogr Sect F Struct Biol Cryst Commun.* 71:358-370.
- Chu, X.Q., Gajapathy, M., Weis, K., Mamontov, E., **Ng, J.D.** and Coates, L. (2012). Dynamic Behavior of Oligomeric Inorganic Pyrophosphatase Explored by Quasielastic Neutron Scattering. *The Journal of Physical Chemistry* 116:9917-9921.
- García-Ruiz, J.M. and **Ng, J.D.** (2012). Inorganic pyrophophatase crystals from *Thermococcus thioreducens* for X-ray and neutron diffraction. *Acta Crystallogr Sect F Struct Biol Cryst Commun* 68:1482-1487.
- Hughes, R. C., **and Ng, J.D.** (2007). Can Small Laboratories Do Structural Genomics? Crystal Growth & Design **7:**2226-2238.
- Marsic, D., R. Hughes, M. Byrne-Steele, and **Ng, J.D. (**2008). PCR-based gene synthesis to produce recombinant proteins for crystallization. BMC Biotechnology **8:**44.

6. Prior Awardees

1) 2017 Recombinant purification and crystallization of Inorganic Pyrophosphatase

Tangible contribution: Research results were shown as a poster presentation as well as an internal department student seminar talk.

Specific outcomes: Student was able to purify and crystallize recombinant proteins. In addition, the student learned basic biochemical laboratory including SDS-PAGE analysis, column chromatography and large-scale fermentation.

2) 2014 Transcriptome analysis of white blood cell samples from Post-Traumatic Stress Disorder patients.

Tangible contribution: Research results were shown as a poster presentation as well as a report given to the Rensselaer Polytechnic Institute (RPI) and the Naval Reserve Officers Training Corps (NROTC).

Specific outcomes: The student obtained 1) Experience in high through-put RNA purification and handling 2) Fundamental knowledge of immunology and 3) Experience in bioinformatics analysis of gene sequences related to expression.