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"The RCEU Study Proposed Here Seeks to Biochemically Characterized the Family I Archaeal Inorganic Pyrophosphatase from Various Pathogenic Organisms (IPPase)"

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Faculty proposal for the Research or Creative Experience for Undergraduate (RCEU) Program (Summer 2017)

1. Faculty Mentor (participated previously in the RCEU program)

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2. Project Summary

The RCEU study proposed here seeks to biochemically characterized the family I archaeal Inorganic Pyrophosphatase from various pathogenic organisms (IPPase). The study of this enzyme is important for antibiotic discovery. The crystallographic structure of the *Thermus 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 proposed study seeks to determine the specific catalytic activity of the enzyme under different temperatures, pH and ionic concentration. In addition, the binding capacity of its ligands will determined acquiring Kd and Km values. It is also the aim of this study to 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. It is the goal of the project to identify structural factors that may be uniquely observed among thermophilic proteins and determine how these factors can contribute to thermal stability. We will correlate structural changes with any activity changes.

Benefits to the student:

1) Experience in high through-put protein purification and handling
2) Proficiency in site-directed mutagenesis
3) Fundamental knowledge in enzymology
4) Experience in macromolecular modeling

3. Student Prerequisites – Student applicants should be in good academic standing with a GPA of 3.0 or better. Required coursework includes BYS119 and BYS120 or their equivalents. It is preferable, but not required, that the student has taken BYS363.

4. Student Duties

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. Site directed or regional mutation studies will be employed to examine the catalytic effect of the hydrogen coordination. 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.

5. Mentor Supervision and Interaction

Dr. Ng will serve as the primary mentor to the student. Current technicians and research associates 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.

6. 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.