BCHM 421/422 – 2022-23 – Jia Lab Project #2

Project Outline: Eukaryotic Elongation Factor 2 (eEF2) is responsible for binding the ribosome and promoting elongation of the protein peptide from the ribosomal A site to P site. Phosphorylation of Thr56 by its regulatory kinase eEF2K, inhibits this binding and subsequently, protein translation in eukaryotic cells. eEF2K is a calmodulin-dependent alpha kinase. Whereas hundreds of conventional protein kinases share similar sequences and motifs, eEF2K does not share a similar sequence, yet does have a similar bi-lobed fold. Selective inhibition of eEF2K, a key regulatory target, has been promising in different cancer types and neurological diseases. Moreover, targeting an alpha kinase may ensure that hundreds of other key kinases are not disrupted in the cell. To date, the Jia lab has expressed and purified a custom eEF2K construct for structural determination and biochemical characterization. The 421/422 student will work on a range of experiments including crystallization, X-ray diffraction, activity and inhibition assays, site-directed mutagenesis etc. Determination of the X-ray structure will allow the project to proceed with structure-based drug design to derive an eEF2K inhibitor with high efficacy.

Supervisor: Dr. Zongchao Jia

Project Title: Structural Investigations and Characterization of an Atypical Alpha-Kinase, Eukaryotic Elongation Factor 2 Kinase

Project Goals: Structural determination of eEF2K, eEF2, and other related proteins. Inhibitor design and validation, targeting eEFK2. Analysis of eEF2K biochemical and biological properties.

Experimental Approaches:

- X-ray crystallography
- ATP-radiolabeled/kinase assays
- Molecular Dynamics/Computer simulations
- Protein expression and purification
- Western Blotting
- Other cell biology related experiments (e.g. fluorescent microscopy)

References:

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2. Kaul, G., Pattan, G., and Rafeequi, T. (2011) Eukaryotic elongation factor-2 (eEF2): its regulation and peptide chain elongation. *Cell Biochemistry and Function*. 29, 227–234
3. Piserchio, A., Long, K., Lee, K., Kumar, E. A., Abzalimov, R., Dalby, K. N., and Ghose, R. (2021) Structural dynamics of the complex of calmodulin with a minimal functional construct of eukaryotic elongation factor 2 kinase and the role of Thr348 autophosphorylation. *Protein Sci*. 30, 1221–1234

4. Pigott, C. R., Mikolajek, H., Moore, C. E., Finn, S. J., Phippen, C. W., Werner, J. M., and Proud, C. G. (2012) Insights into the regulation of eukaryotic elongation factor 2 kinase and the interplay between its domains. *Biochem J*. 442, 105–118
5. Liu, R., and Proud, C. G. (2016) Eukaryotic elongation factor 2 kinase as a drug target in cancer, and in cardiovascular and neurodegenerative diseases. *Acta Pharmacol Sin*. 37, 285–294