

BCHM 421/422 – 2018/2019

Project Outline:

Supervisor: Dr. Graham Côté

Project Title: Functional and structural studies on the α -kinase family of protein kinases

Keywords (3-5):

1. **Protein kinases**
2. **Protein phosphorylation**
3. **Signal transduction**
4. **Protein regulatory mechanisms**
5. **Protein structure and function**

Project Goals: The human genome encodes 520 protein kinases, all of which regulate cellular processes by phosphorylating proteins on serine, threonine or tyrosine residues. Seven of the human protein kinases are termed α -kinases and differ significantly from other protein kinases in terms of their structure and enzymatic properties (1-4). This project will involve studies on the structure and function of α -kinases, including MHCK-A, which inhibits myosin II contractile activity, eEF2K, which inhibits protein synthesis and ALPK1, whose function is not known. We will investigate the regulatory properties of these α -kinases, and in particular the function of accessory domains that may interact with the α -kinase domain to promote or inhibit catalytic activity.

Experimental Approaches:

1. DNA cloning and site-directed mutagenesis
2. Expression of proteins in bacteria
3. Protein purification using affinity chromatography and ion exchange chromatography
4. ATPase assays and protein phosphorylation assays.
5. Protein-protein interaction assays using gel filtration, pull-down methods and isothermal titration calorimetry
6. There will be an opportunity to learn how to crystallize proteins and if successful, to help solve a protein structures by X-ray crystallography.

References:

1. Ye Q, Crawley SW, Yang Y, Côté GP, & Jia Z (2010) Crystal structure of the α -kinase domain of Dictyostelium myosin heavy chain kinase A. *Science Signaling* 3(111):ra17.
2. Yang Y, Ye Q, Jia Z, & Côté GP (2015) Characterization of the catalytic and nucleotide binding properties of the α -kinase domain of *Dictyostelium* myosin-II heavy chain kinase A. *J. Biol. Chem* 290(39):23935-23946.
3. Ye Q, *et al.* (2016) Structure of the Dictyostelium Myosin-II Heavy Chain Kinase A (MHCK-A) α -kinase domain apoenzyme reveals a novel autoinhibited conformation. *Sci Rep* 6:26634.

4. Middelbeek J, Clark K, Venselaar H, Huynen MA, & Van Leeuwen FN (2010) The alpha-kinase family: an exceptional branch on the protein kinase tree. *Cell Mol. Life Sci* 67:875-890.