

BCHM 421/422 – 2020/2021

Supervisor: Dr. Graham Côté

Project Title: Studies on the alpha-kinase family of protein kinases

Keywords (3-5):

1. Protein kinases
2. Enzyme assays
3. X-ray crystallography
4. Protein phosphorylation

Project Goals:

The human genome encodes more than 500 protein kinases which play key roles in regulating cellular processes by phosphorylating substrate proteins on serine, threonine and tyrosine residues. Seven of the human protein kinases belong to the atypical alpha-kinase family and differ significantly from other protein kinases in terms of their structure and enzymatic properties (1, 2). This project will involve studies on the structure and function of three human alpha-kinases, termed ALPK1, ALPK2 and ALPK3, which play key roles in regulating the innate immune response in neutrophils and other immune cells (3). The project will investigate the mechanisms regulating the catalytic activity of these kinases, and in particular the possibility that activity is controlled by formation of a complex between a regulatory domains and the α -kinase domain.

Experimental Approaches:

1. DNA cloning and site-directed mutagenesis
2. Expression and purification of proteins from bacteria
3. ATPase assays and protein phosphorylation assays.
4. Protein-protein interaction assays using gel filtration, pull-down methods and isothermal titration calorimetry
5. Protein crystallization and X-ray crystallography.

References:

1. Ye Q, *et al.* (2016) Structure of the Dictyostelium Myosin-II Heavy Chain Kinase A (MHCK-A) alpha-kinase domain apoenzyme reveals a novel autoinhibited conformation. *Sci Rep* 6:26634.
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. Zhou P, *et al.* (2018) Alpha-kinase 1 is a cytosolic innate immune receptor for bacterial ADP-heptose. *Nature* 561(7721):122-126.