# Structural and Functional Studies on Phosphorylase Kinase using Cryo-Electron Microscopy

Li Z[1]*, Schenk A[2], Nadeau OW[3], Carlson G[3], Walz T[2], Vénien-Bryan C[1]

[1]*IMPMC, UMR7590, CNRS,UPMC,MNHN,IRD Paris, France*
[2]Department of Cell Biology, Harvard Medical School, Boston, USA
[3]*Department of Biochemistry and Molecular Biology, University of Kansas Medical center, Kansas, USA*

We are interested in the structure of Phosphorylas Kinase (PhK), one of the most complex kinase. It is composed of four types of subunits $(\alpha\beta\gamma\delta)_4$, with a total MW of 1.3 MDa. PhK integrates signals to catalyze the conversion of inactive glycogen phosphorylase b (GPb) to active glycogen phosphorylase a (GPa) and subsequent glycogen degradation. This either provides energy to sustain muscle contraction or, in liver, results in glucose provision to the brain and other tissues. PhK is a potential target for controlling glucose levels in the diseased state such as diabetes.

Our main objective is to understand how the large regulatory $\alpha$ and $\beta$ subunits modulate the activity of the catalytic $\gamma$ subunit in the context of the quaternary structure of the $(\alpha\beta\gamma\delta)_4$ complex, with the ultimate goal of understanding how PhK is regulated. The X-ray crystal structure of the catalytic $\gamma$ subunit is known, but how the large complex of $\alpha$ and $\beta$ subunits together with the calmodulin domain $\delta$ act to restrain the kinase in the inactive state until stimulation is the topic of this project.

The enzyme is purified from rabbit. We are have taken images with a direct electron detection camera (Gatan K2 Summit) on a 200KV FEG FEI Microscope. We are performing single particle image analysis using various software.