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

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We are interested in the structure of Phosphorylase Kinase (PhK), one of the most complex kinase. It is composed of four types of subunits (αβγδ)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 α and β subunits modulate the activity of the catalytic γ subunit in the context of the quaternary structure of the (αβγδ)₄ complex, with the ultimate goal of understanding how PhK is regulated. The X-ray crystal structure of the catalytic γ subunit is known, but how the large complex of α and β subunits together with the calmodulin domain δ act to restrain the kinase in the inactive state until stimulation is the topic of this project.

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