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

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1. INTRODUCTION

We are interested in the structural study 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 regulates glycogen metabolism and it 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 a and b subunits modulate the activity of the catalytic g 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 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.

2. RESULTS

2.1 Experimental conditions

We are using cryo-electron microscopy and single particle analysis to determine the 3D structure of the whole complex at a sufficiently higher resolution so we can identify the densities corresponding to the alpha-helix and beta-sheets from the EM map.
The enzyme is purified from rabbit, and kept in the inactivated state. The images are taken on direct electron detection camera (Gatan K2 Summit) on a 200KV FEG FEI Microscope. The programs and software that we are using for the image processing are SPIDER, EMAN, RELION and SPARX.

2.2 Results

We have imaged many particles and have selected so far about 26000 exploitable particles. We have created a first ab-initio model using SPARX and SIMPLE software. Then we have performed a 2D and 3D classification using RELION and our ab-initio model.

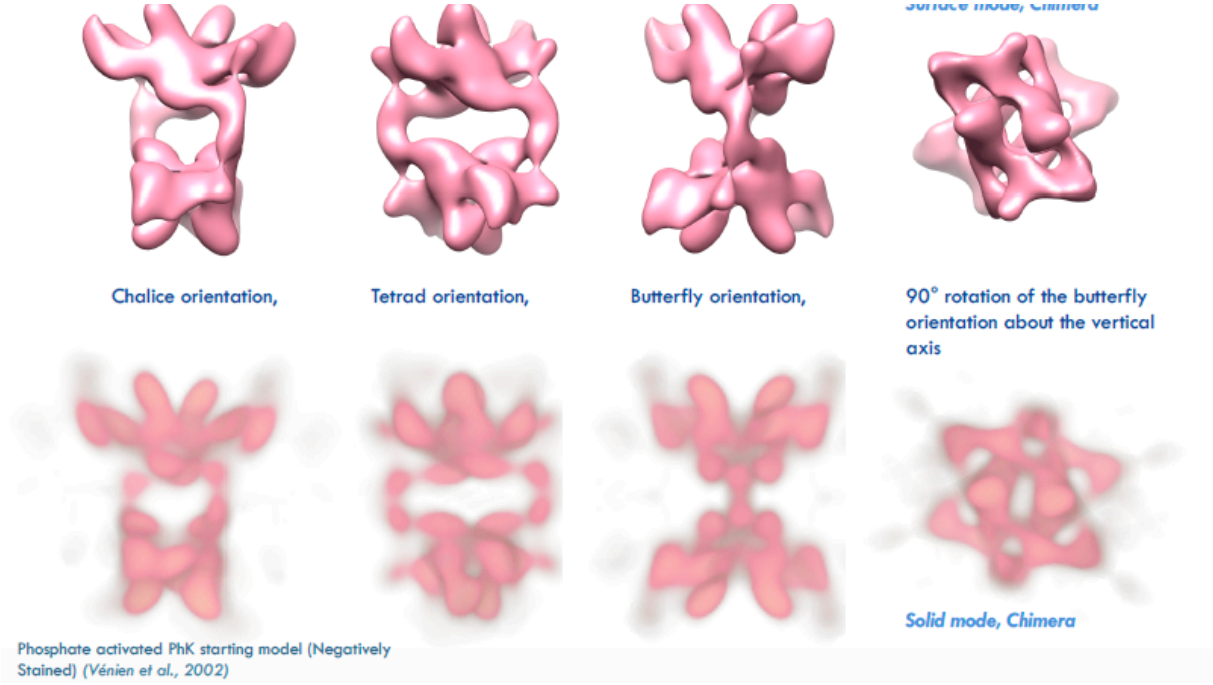

Figure 1. One class from the 3D classification performed with RELION

3. CONCLUSION

The results shows a variability in the inactive conformation, we are in the process of taking more images in order to better characterize the 3D conformation of the inactive state of PhK.

REFERENCES

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