Report

Project MX-1161: Structural studies on retinoblastoma protein

1. Project objectives.

The project aims to get a structural information on retinobastoma protein and its conformational changes upon ligand binding or phosphorylation.

2. Results.

Full-length Rb contains three recognized structural domains, the N-terminal domain (RbN), the central Rb pocket domain and the C-terminal domain (RbC). The structures of the individual subdomains are known, however how the different domains align and interact to form the full size molecule is not clear.

To answer this question, the fragments of Rb containing either RbN and Rb pocket domains or Rb Pocket and RbC domains have been studied by SAXS. Due to the fact that RbN and Rb pocket domains have structural homology, from the previously obtained *ab initio* models of Rb fragment containing RbN and Rb pocket domains it was impossible to determine the domains orientation within the molecule. To overcome this difficulty, maltose binding protein (MBP) has been fused to either the N-terminus or to C-terminus of the Rb fragments containing RbN and Rb pocket domains, and the fusion proteins have been expressed, purified and studied by SAXS. The previous SAXS experiment allowed to obtain the orientation of the RbN domain. However, the orientation of the Rb pocket domain still remained unknown. The current SAXS experiment has been performed using an Rb construct containing RbN, Rb Pocket domains and C-terminal MBP-fusion. However, additional experiments are needed on Rb fragments containing C-terminal MBP-fusion, RbN and Rb pocket domains due to the fact that this sample reveals a concentration dependant dimerization and additional measurements with lower sample concentrations are necessary to obtain the accurate data on the Rb pocket domain orientation. Moreover, the sample containing Rb pocket and RbC domains has been studied by SAXS, however, additional measurements are needed to obtain the accurate data for this sample and finalize the *ab initio* model.