ESRF	**Experiment title:** Calmodulin-sphingolipid complex	**Experiment number**: MX 825
Beamline: ID23-1	**Date of experiment**: from: 25 Jul 2008 to: 25 Jul 2008	**Date of report**: 27 January 2011
Shifts: 2	**Local contact(s)**: Juan Sanchez Weatherby	*Received at ESRF:*

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Report:

The main objective of the project was to explore the structural background of the antagonistic effect of sphingosilphosphorycholine (SPC) on calmodulin. We collected an 1.6 Å resolution dataset of calmodulin/SPC complex, which also helped to understand the possible two-step binding mechanism of SPC binding of calmodulin. Our report was published in the FASEB Journal in 2010.

Reference:

E. Kovacs, V. Harmat, J. Tóth, B.G. Vértessy, K. Módos, J. Kardos, Károly Liliom:
„Structure and mechanism of calmodulin binding to a signaling sphingolipid reveal new aspects of lipid-protein interactions ”
FASEB J **24:** 3829-3839 (2010) doi:10.1096/fj.10-155614

Abstract: Lipid-protein interactions are rarely characterized at a structural molecular level due to technical difficulties; however, the biological significance of understanding the mechanism of these interactions is outstanding. In this report, we provide mechanistic insight into the inhibitory complex formation of the lipid mediator sphingosylphosphorylcholine with calmodulin, the most central and ubiquitous regulator protein in calcium signaling. We applied crystallographic, thermodynamic, kinetic, and spectroscopic approaches using purified bovine calmodulin and bovine cerebral microsomal fraction to arrive at our conclusions. Here we present 1) a 1.6-Å resolution crystal structure of their complex, in which the sphingolipid occupies the conventional hydrophobic binding site on calmodulin; 2) a peculiar stoichiometry-dependent binding process: at low or high protein-to-lipid ratio calmodulin binds lipid micelles or a few lipid molecules in a compact globular conformation, respectively, and 3) evidence that the sphingolipid displaces calmodulin from its targets on cerebral microsomes. We have ascertained the specificity of the interaction using structurally related lipids as controls. Our observations reveal the structural basis of selective calmodulin inhibition by the sphingolipid. On the basis of the crystallographic and biophysical characterization of the

calmodulin–sphingosylphosphorylcholine interaction, we propose a novel lipid-protein binding model, which might be applicable to other interactions as well.

During our experiment we had time to collect data from cystals of other three proteins. These are still ongoing projects.

- Crystals of dynein light chain/peptide complex had one very long unit cell axis (possible hexagonal symmetry), and high mosaicity. Data processing failed. As a consequence we restarted crystallization with different protein constructs.
- MASP-2 the first enzyme of the complement activation has been long studied in our laboratory. It is a chymotrypsin type serine protease. The enzyme was co-crystallized with a highly specific peptide inhibitor, which was developed by Phage display technique, based on sunflower trypsin inhibitor scaffold. A 2.5 Å resolution dataset was collected. The structure does not contain the peptide, instead enzyme/product like contacts are established by symmetry equivalent molecules of MASP-2. This type of interaction is functionally relevant for MASP-2: it explores the intermolecular interactions of MASP-2 autoactivation, the first enymatic event of the lectin pathway of the complement (publication in preparation). Since then other crystal forms were also produced, but all contain the MASP-2/MASP-2 autoactivation complex instead of the MASP2/peptide complex.
- As a continuation of our previous project 14-U 831 we collected a new native dataset from Pyrococcus horikoshii acylpeptide hydrolase and successfully improved resolution to 1.6 Å. The strucutre revealed that in the applied crystallization conditions the noncovalently bound inhibitor molecules are released from the enzyme, so we continued crystalization with covalently bound inhibitors mimicking the substrate (the new crystals diffract to 1.9 Å at the home source, refinement is in progress).