ESRF	**Experiment title:** Protein function in the light of conformational changes caused by Ca^{2+}-binding: Physarum polycephalum myosin RD and drug binding by calmodulin	**Experiment number**: LS 1885
Beamline: ID 29	**Date of experiment**: from: 20 / 04 / 2000 to: 21 / 04 / 2001	**Date of report**: 28 / 02 / 2007
Shifts: 3	**Local contact(s)**: G. Leonard	*Received at ESRF:*

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Report:

Aim: The experiment was a continuation of our previous project (LS 1756).

The Structure of the Complex of Calmodulin with KAR-2 [1]

3-(β'-Chloroethyl)-2',4'-dioxo-3,5'-spiro-oxazolidino-4-deacetoxyvinblastine (KAR-2) is a potent antimicrotubular agent that arrests mitosis in cancer cells without significant toxic side effects. In this study we demonstrate that in addition to targeting microtubules, KAR-2 also binds calmodulin, thereby countering the antagonistic effects of trifluoperazine. To determine the basis of both properties of KAR-2, the three-dimensional structure of its complex with Ca^{2+}- calmodulin has been characterized both in solution using NMR and when crystallized using X-ray diffraction.
Heterocorrelation (^{1}H-^{15}N heteronuclear single quantum coherence) spectra of ^{15}N-labeled calmodulin indicate a global conformation change (closure) of the protein upon its binding to KAR-2. The crystal structure at 2.12 Å resolution reveals a more complete picture; KAR-2 binds to a novel structure created by amino acid residues of both the N- and C-terminal domains of calmodulin (Figure 1). Although first detected by X-ray diffraction of the crystallized ternary complex, this conformational change is consistent with its solution structure as characterized by NMR spectroscopy.

It is noteworthy that a similar tertiary complex forms when calmodulin binds KAR-2 as when it binds trifluoperazine, even though the two ligands contact (for the most part) different amino acid residues. These observations explain the specificity of KAR-2 as an anti-microtubular agent; the drug interacts with a novel drug binding domain on calmodulin. Consequently, KAR-2 does not prevent calmodulin from binding most of its physiological targets.

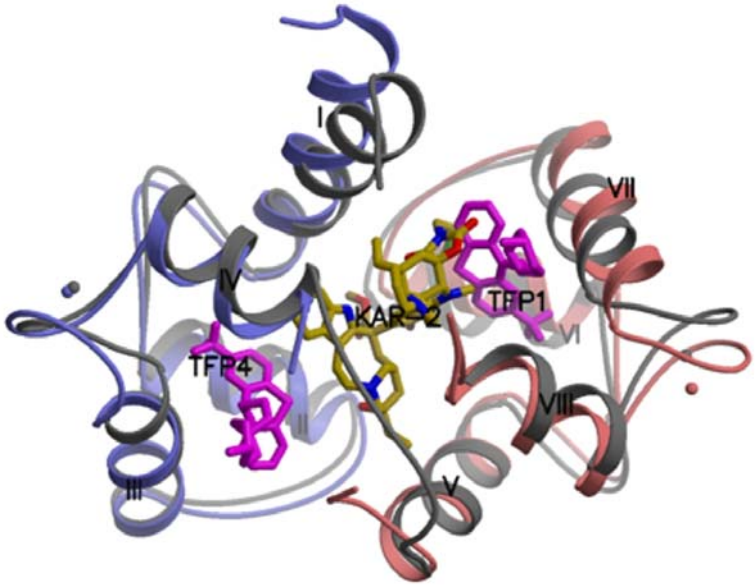

Figure 1. KAR-2 and TFP bind to different binding sites of calmodulin. Superposition of calmodulin-KAR-2 and calmodulin-4TFP complex. Helices of calmodulin are numbered, N-terminal domain blue, C-terminal domain red.

References:

1 Horvath I, Harmat V, Perczel A, Palfi V, Nyitray L, Nagy A, Hlavanda E, Naray-Szabo G, Ovadi J. "The structure of the complex of calmodulin with KAR-2: a novel mode of binding explains the unique pharmacology of the drug."
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