

**Experiment title:**

Structural analysis of membrane proteins, blue-light receptors and the biosynthesis machineries of non-ribosomal peptide antibiotics

Experiment number:

MX-659

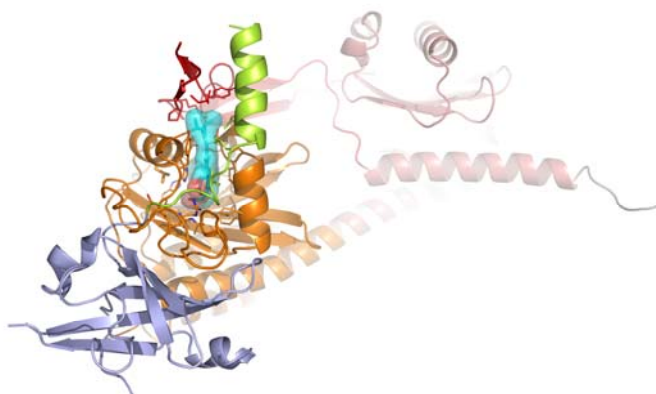
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Names and affiliations of applicants (* indicates experimentalists):**Essen, Lars-Oliver****Maillet, Jo****Psakis, Georgios****Kress, Daniel****Report:**

We collected native X-ray data for the sensory module of the plant-like phytochrome cph1 at 2.2x2.7 Angstrom resolution. Together with previous MAD-data this best cph1 dataset obtained so far is part of a publication on phytochrome signaling to be published in PNAS.

Sensory module of cph1:

The structure shows a tandem-GAF domain arrangement, where both GAF domains (orange, red) contribute to the formation of the bilin-binding site (cyan)



For the class II photolyase from *Methanosarcina barkeri* we collected first datasets from three different crystal forms at 2.0-2.7 Angstrom resolution. The first structure of a class II photolyase could be then solved by combining molecular replacement and DM-averaging and revealed a different organisation of the surface regions responsible for DNA-binding. First microspectroscopic measurements were performed in parallel for the photolyases and the cph1 phytochrome at the Cryobench.

For the termination module of the surfactin biosynthesis machinery, SrfA-C (MW ~ 145 kDa), we collected a 2.6 Angstrom native dataset and performed extensive screening on SeMet-crystals of SrfA-C. One of these prescreened crystals provided 2.8 Angstrom MAD data at the SLS a day later that allowed to solve and refine the first structure of a complete NRPS module at 2.6 Angstrom resolution. The structure shows remarkable insights into domain-domain interactions which are crucial for the assembly-line mechanism of non-ribosomal peptide synthetases (Ms. in preparation). A 1.8 Angstrom dataset was collected for the arginine-beta-hydroxylase VioC that is involved in the biosynthesis of viomycin. Its structure was solved by molecular replacement using our recent structure of the asparagine-hydroxylase AsnO and showed the substrate bound with tartrate and ion in the active site. One 1.8 Angstrom dataset was collected for the ferritin-like protein complex MrgA from *B. subtilis* that was complexed to cobalt ions, but we could not observe electron density for bound metal ions.

Overall, 135 crystals were screened for diffraction (projects: phytochrome, SrfAC, VioC, MrgA, LDL, NiCoT, FlhA, photolyases, glutaconyl-CoA-decarboxylases), of which several prescreened crystals were used for subsequent data collection at the SLS.

