Thursday, March 23, Session II

L6

AN EXTANT ENZYME WITH DUAL DEHALOGENASE–LUCIFERASE FUNCTION

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Haloalkane dehalogenases (HLDs, EC 3.8.1.5) are mostly microbial -hydrolase fold enzymes that cleave carbon-halogen bonds in diverse halogenated hydrocarbons. Interestingly, these enzymes exhibit significant sequence and structural similarity with *Renilla*-type luciferases (EC 1.13.12.5). These luciferases are decarboxylating monooxygenases that produce blue light through the oxidation of a luciferin (coelenterazine) [1,2]. Here, we identified several genes coding for putative dehalogenase/ luciferase-like enzymes in the draft genome of *Amphiura filiformis* [3], a luminous brittle star. Protein expression trials showed that some of these putative enzymes are expressed as soluble and properly folded proteins in *Escherichia coli*. Surprisingly, our biochemical experiments showed that one enzyme exhibits a dual function, catalyzing the hydrolytic dehalogenase reaction and the oxidation of coelenterazine followed by the emission of blue photons. Co-crystal structures reveal an as-yet-unseen mode of luciferin binding, which is radically dissimilar to that observed in *Renilla* luciferase and suggests a non-canonical, less-effective coelenterazine oxidation. Structure-based mutagenesis experiments support this unusual mode of catalysis. Our findings collectively support the hypothesis that coelenterazine-powered *Renilla*-type luciferases evolved from HLD-fold proteins.

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CRYSTAL STRUCTURE OF SMOLSTATIN – PROTEASE INHIBITOR FROM THE MYXOZOAN PARASITE *SPHAEROSPORA MOLNARI*

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The cystatin superfamily is a large group of cysteine protease inhibitors (including type 1 cystatins – stefins, type 2 cystatins – true cystatins and type 3 cystatins – kininogens), present in various organisms. Parasite cystatins are involved in the active parasitism in the host by supressing host immune responses. Thus, cystatins are critical for the interactions between host and parasite during the infection [1, 2].

Here, we structurally characterized stefin Smolstatin from *Sphaerospora molnari*, which is a myxozoan parasite of common carp *Cyprinus carpio*. From evolutionary point of view, myxozoans are parasites that stand at the base of the metazoan evolution, therefore structural information can bring insights into the evolution of the cystatin superfamily as well as it can contribute to the aquaculture field for studying host- parasite interactions [3]. Smolstatin is a 13.5 kDa large single domain protein, which consists of typical cystatin-like domain, but unlikely for stefins, it also carries a signal peptide.

Smolstatin was recombinantly produced, purified and crystallized using sitting drop vapour diffusion technique. Diffraction data were collected on BL14.1 at the BESSY II

electron storage ring operated by the Helmholtz-Zentrum Berlin [4]. Smolstatin crystallized as a domain swapped dimer. The crystal structure was determined by molecular replacement, refined and deposited to the PDB database under the accession code 8and.

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MASS PHOTOMETRY – REVOLUTIONARY BIOPHYSICAL CHARACTERIZATION OF SINGLE MOLECULES

Tomáš de Garay

Refeyn

Mass photometry is a novel bioanalytical technology that provides single-molecule mass measurements of biomolecules in their native state within minutes without the need for labelling, surface immobilization or big sample quantities. Mass photometry is based on interferometric scattering microscopy, measuring single molecules thanks to an unprecedented level of sensitivity. Its ease of use makes mass photometry the perfect tool for rapid assessments of sample purity and homogeneity, structural integrity or macromolecular interactions across biomolecules ranging from differently sized proteins to DNA and even small viruses, such as AAVs.

In this talk we will show how mass photometry can answer a wide range of questions in the structural biology and biochemistry fields and how it can be integrated with downstream structural biology approaches like CryoEM and crystallography.