MS9-O2 Time-Resolved Serial Femtosecond Crystallography On Photoswitchable Fluorescent Proteins

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Reversibly photoswitchable fluorescent proteins (RSFP) are essential tools in advanced fluorescence nanoscopy of live cells (such as PALM and RESOLFT). They can be repeatedly toggled back and forth between a fluorescent (*on*) and a non-fluorescent (*off*) state by irradiation with light at two different wavelengths. Mechanistic details of photoswitching, in particular on the ultra-fast photochemical time scale, remain largely unknown. Our consortium combines time-resolved serial femtosecond crystallography (TR-SFX) at X-ray free electron lasers, ultrafast absorption spectroscopy in solution and simulation methods to study *off*-to-*on* photoswitching intermediates in two RSFP on the picosecond to microsecond time scale. Two major bottlenecks had to be passed before TR-SFX could be conducted, *i.e.* the production of well-diffracting microcrystals in large quantities and efficient inline pre-illumination to photoswitch RSFP microcrystals from the *on* to the *off* state prior to injection. First pump-probe TR-SFX experiments were conducted at both the LCLS and SACLA that, together with time-resolved absorption spectroscopy, provide first insight into a possible sequence of events involved in photoswitching.

Keywords: time-resolved serial femtosecond crystallography, XFEL, microcrystals, fluorescent proteins, chromophore

MS9-O3 Changes in metal coordination are required to regulate activity of bacterial phosphodiesterases, implicated in c-di-GMP regulated biofilm dispersal

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Many chronic infections are underpinned by the ability of bacteria to transition to the biofilm life-style, which is up to 1000 fold more tolerant to antibiotics. Both biofilm formation and dispersal are controlled by the secondary messenger bis-(3'-5') cyclic dimeric guanosine monophosphate (c-di-GMP); high levels of c-di-GMP are associated with biofilm formation, while a reduction in c-di-GMP induces biofilm dispersal[(1)]. Future pharmaceutical strategies may interfere with these mechanisms. We look at biofilm dispersal, studying c-di-GMP hydrolysis by bacterial phosphodiesterases of the EAL type.

Structures of the EAL type enzymes are readily observed in the substrate bound state (c-di-GMP). These structures pose the question of full enzyme activation; while dimerisation is known to play a key role, many of the structures are observed as dimers[(2,3)]. We present an analysis of these dimers and show that dimerisation alone is insufficient for activation.

Our study of the *Pseudomonas aeruginosa* protein MorA demonstrates that dimerisation is linked with reorganisation of the catalytic site, by unwinding of a helical segment (figure 1)[(4)]. However, this structure is again observed in the substrate bound state. We now present further evidence from phosphodiesterase structures in complex with the hydrolysis product 5'-phosphoguanylyl-(3'-5')-guanosine. Observed differences in metal coordination in the catalytic centre may represent the final layer of enzyme activation. Understanding the full catalytic potential of EAL-type phosphodiesterases is required to explore this class of enzymes in drug design.

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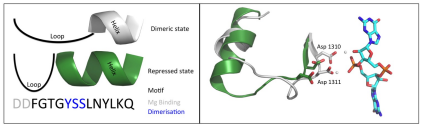

Figure 1. EAL domain dimerisation induces a change in the length of the R-helix; this brings a pair of aspartic acid side chains (Asp 1310 and Asp 1311) closer to the catalytic centre. These amino acids are involved in metal coordination and thus required for catalytic activity.

Keywords: EAL domain, cyclic-di-GMP signalling, phosphodiesterase, metal-ion catalysis, biofilm

MS9-O4 pH Dependent conformational change of bound maltose observed in the crystal of β-amylase at room temperature.

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β-Amylase catalyzes the liberation of maltose from the non-reducing ends of α-1, 4-glucan such as starch and glycogen. In contrast to α-amylase, β-amylase produces β-anomeric maltose, and is classified as an inverting enzyme. In soybean β-amylae (SBA), the hydrolysis of the α-1, 4-glucosyl linkage is proceeded by two catalytic residues, Glu186 (acid) and Glu380 (base). The enzyme has two mobile loops, flexible loop (residue 96-103) and inner loop (residue 341-345) near the active site. The conformation of these loops change from open to closed form and from apo to product form, respectively, during enzyme action. In this paper, we are intended to determine the structural changes of SBA/maltose complex in a different pH media. In order to control pH correctly, we have determined the crystal structure at room temperature to avoid the undesirable effect of freezing and cryo-protectant such as glycerol. SBA was expressed in expression system of *E. coli*, and was purified and crystallized by a hanging-drop vapor diffusion against 1 ml of the bottom solution containing 45% saturated ammonium sulfate, 0.1 M sodium acetate buffer, pH 5.4. The obtained crystals were packed in glass capillaries after soaked with 200mM maltose in the different pH buffer (0.1 M acetate, PIPES or Tris buffer, pH 3-9), containing 45% saturated ammonium sulfate. The diffraction data sets were collected at BL26B1/B2 beam-lines in SPring-8. Each crystal data was collected with 98-100 % completeness and R_{merge} of 0.04-0.05 up to 1.6-1.7 Å resolution. The models were refined with SHELXL program including protein anisotropic B-factors. The refined models contains one molecules of SBA comprising 492 amino acid residues, 3-7 sulfate ions and 403-450 water molecules with R = 0.12-0.13 and R_{free} = 0.14-0.16. At pH 5.4, two maltose molecules were located at the subsites -2~-1 and +1~+2 with α-anomer boat form at subsite -1, whereas α/β-anomer chair forms were clearly found at pH 4.0 (Fig. 1). This indicates that the glucose residue at subsite -1 is distorted to boat form by the deprotonation of a protein residue with p*K*a = 4.5 in the active site. The pH dependency of the conformational change of maltose in mutant proteins, K295A and T342V were also analyzed. It is suggested that the sugar conformational change from chair to boat form occurs by the increased nucleophilicity of the catalytic water near subsite -1.