the phosphodiesterase active site of the Pseudomonas aeruginosa MorA, a bi-functional c-di-GMP regulator." FEBS Lett. 2014; 588 (24): 4631–6.

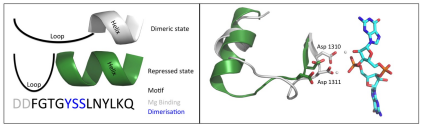

Figure 1. EAL domain dimerisation induces a change in the length of the R-helix; this brings a pair of aspartic acid side chains (Asp 1310 and Asp 1311) closer to the catalytic centre. These amino acids are involved in metal coordination and thus required for catalytic activity.

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.

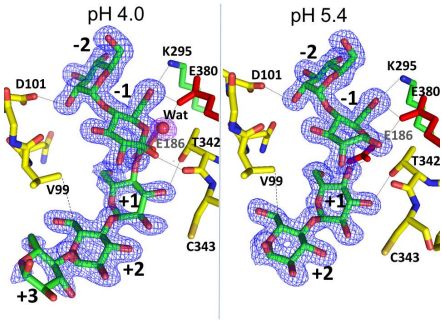

Figure 1. The effect of pH on the binding mode of maltose in the active site of SBA.

Keywords: β-amylase, capillary measurement

MS9-O5 Lipidic cubic phase injector is a viable crystal delivery system for time-resolved serial crystallography

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Serial femtosecond crystallography (SFX) using X-ray free-electron laser (XFEL) sources is a newly developed method with considerable potential for time-resolved pump probe experiments. I will present a lipidic cubic phase SFX structure of the light-driven proton pump bacteriorhodopsin (bR) to 2.3 Å resolution and a method to investigate protein dynamics with modest sample requirement. Time-resolved serial femtosecond crystallography (TR-SFX) with a pump-probe delay of 1 ms yields Fourier difference maps (F_o-F_o) compatible with the dark to M state transition of bR. Importantly the method is sample efficient and reduces sample consumption to a few milligrams of protein per collected time point. Accumulation of M intermediate within the crystal lattice is confirmed by time-resolved visible absorption spectroscopy. The impact of radiation damage free data collection using femtosecond XFEL pulses on the study of structural intermediates is discussed and compared with serial millisecond crystallography (SMX) at a synchrotron source. This study provides an important step towards characterizing the dynamics of complete photocycles of retinal proteins and demonstrates the feasibility of a sample efficient viscous medium jet in TR-SFX.

Nogly, P., Panneels, V., Nelson, G., Gati, C., Kimura, T., Milne C., Milathianaki, D., Kubo, M., Wu, W., Conrad, C., Coe, J. Bean, R., Zhao, Y., Bath, P., Dods, R., Harimoorthy, R., Beyerlein, K.R., Rheinberger, J., James, D., DePonte, D., Li, C., Sala, L., Williams, G., Hunter, M., Koglin, J.E., Berntsen, P., Nango, E., Iwata, So., Chapman, H.N., Fromme, P., Frank, M., Abela, R., Boutet, S., Barty, A., White, T.A., Weierstall, U., Spence, J., Neutze, R., Schertler, G. &Standfuss, J. *Lipidic cubic phase injector is a viable crystal delivery system for time-resolved serial crystallography.* (2016) under revision. Nogly, P., James, D., Wang, D., White, T. A., Zatsepin, N., Shilova, A., Nelson,