and oxidation of three residues (Gln65-Tyr66-Gly67) located at the center of internal α-helix. The imidazolinone moiety of chromophore was exposed to the outside of the β-barrel through the characteristic water-filled channel. It is considered that oxygen molecules are converted to ROS with light induced energy transfer at chromophore, followed by ROS diffuse to outside of β-barrel through this channel.

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**Keywords: protein, structure**

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**Structure improvement and analysis of F16L lipase crystallized under microgravity environment**

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Previously we have solved the structure of wild type T1 lipase at 1.5Å which revealed that a unique $Na^{+}$-π interaction with Phe16. The 1.5 Å resolution map revealed that a specific atom tightly interacts with the aromatic π-system of Phe16 [1]. To further confirm the positive contribution of the side chain of Phe16 for the present cation-π interaction, we have also solved the crystal structure of mutant F16L T1 lipase at 1.8 Å, which were prepared and crystallized similarly to the wild-type enzyme. Although the resolution was slightly lower (1.8 Å), we found almost zero electron density in mutant F16L enzyme at the position corresponding to the metal ions in the wild-type enzyme. In order to improve the structure of F16L lipase, we send the protein to space under our National Angkasawan Program. Space crystallization was carried out using high-density protein crystal growth apparatus (HDPCG) utilizing vapor diffusion method and X-ray diffraction data was collected at SPring-8 BL41XU, Japan. Slight increase in crystal size and interface was observed. Higher resolution of 1.7 Å was obtained for space crystals with better quality electron-density map distributed over the entire F16L lipase space crystal structure.

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**Crystal structure of the engineered cross-linked complex between Fd and FNR**

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In photosynthetic organisms, Ferredoxin (Fd) and Fd-$NADP^{+}$ reductase (FNR) are redox partner proteins responsible for the conversion between $NADP^{+}$ and NADPH. The plant-type Fd is an electron carrier protein containing one [2Fe-2S] cluster, and FNR catalyzes the reduction of $NADP^{+}$ using two electrons donated from reduced Fds. Electron transfer between Fd and FNR requires the formation of transient Fd-FNR complex. Three X-ray crystal structures of Fd-FNR complexes from maize leaf and root tissues and the cyanobacterium *Anabaena* have been reported so far [1-3]. However, the specific interaction mode of Fd and FNR is largely different among the three known X-ray structures of Fd-FNR pairs, which exhibit distinct physicochemical and physiological properties. Understanding the mechanism of electron transfer between redox partners is a complex problem involving specific recognition between redox partners, subsequent conformational changes, and redox potentials of redox centers.

To investigate the relative contribution of these factors to the electron transfer between Fd and FNR, we introduced the specific disulfide bonds between maize leaf Fd and FNR by engineering cysteines into the two proteins resulting in 13 different Fd-FNR cross-linked complexes[4]. These variants display a broad range of activity to catalyze the NADPH-dependent cytochrome *c* reduction, categorized into three groups with more than 70% activity in comparison to wild-type Fd/FNR, with intermediate activity (7-46%), and with less than 3% activity. These distinctive electron transfer rate of Fd-FNR must be closely related to the distance and orientation of two redox centers, and environment around them. We have crystallized the two types of Fd-FNR cross-linked complexes, with high and intermediate activities. Furthermore, the X-ray diffraction data were collected at 2.7 Å, and 4.5 Å resolution, respectively, on the synchrotron-radiation beam line BL44XU at the SPring-8. The crystal structures of the two types of engineered cross-linked Fd:FNR complexes were solved by molecular replacement. We will describe the possible determinant to tune the electron transfer rate between the plant-type Fd and FNR based on two complex structures.

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**A single amino acid limits the substrate specificity of uridine-cytidine kinase**

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Nucleoside metabolism is one of important metabolic systems. Many anti-cancer and anti-viral drugs have been developed to act on nucleoside metabolic enzymes. Insight into the structure and mechanism of the enzymes can aid in the development of potent inhibitors. Uridine-cytidine kinase (UCK) catalyzes the phosphorylation of cytidine and uridine to CMP and UMP, respectively. However, mechanism of substrate specificity in UCK has not been investigated in detail. We found that the UCK homologue from Thermus thermophilus HB8 (ttCK) has the substrate specificity toward only cytidine. Activity of ttCK was inhibited by CTP, but not uridine, UMP, and UTP, could suggesting that uridine cannot bind to ttCK. In order to elucidate the reason for the exclusion of uridine by ttCK, we determined the X-ray crystal structures of ttCK in ligand-free form and in complex with CMP