1P001 高エネ機構フォトンファクトリーにおける創薬等支援基盤プラットフォーム事業による構造生物学研究の支援と高度化
Promotion of the Platform for Drug discovery, Informatics, and Structural life science (PDIS) project at Photon Factory in KEK

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Platform for Drug discovery, Informatics, and Structural life science (PDIS) is a five-year national project started FY2012. KEK has been selected as one of the institutes / universities participating in the project. In the project, KEK supplies protein crystallization, synchrotron X-ray beamtime, diffraction data collection and structure determination activities. PDIS will accept numerous applications of interest in the fields of basic science, medical, and pharmaceutical researches. All Japanese scientists can apply to use resources open for the PDIS project. In addition to the support, we promote technology development in the project.

1P002 海産無脊椎動物由来溶血性レクチン CEL-III の結晶化
Crystallization of the pore forming toxin CEL-III from marine invertebrate, *Cucumaria echinata*

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CEL-III is a hemolytic lectin isolated from the sea cucumber *Cucumaria echinata*. This lectin binds to the cell surface carbohydrate chains and associates to form transmembrane pores. We prepared the pore-forming oligomer using rabbit erythrocytes. The oligomer was solubilized with n-dodecyl- β -D-maltoside (DDM) from rabbit erythrocytes and purified by lactose-fixed affinity column chromatography. The purified oligomer was homogeneous on SDS-PAGE and used to crystallization. The crystals of pore-forming oligomer were obtained by vapor diffusion method.

1P003 イエロープロテインの 150 ピコ秒時間分解能ラウエ構造解析
Time-resolved Laue crystallography of photoactive yellow protein with 150psec time resolution

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In order to understand the molecular mechanism of signal transduction in photoactive yellow protein (PYP), we investigated its structural dynamics with time-resolved Laue crystallography. We acquired 1.65 Å; diffraction data over 10 decades of time with ~150 psec time resolution, the highest time resolution achieved thus far. The time-resolved diffraction data could be decomposed into 4 intermediates (pR0, pR1, pR2, pB0) plus the unphotolyzed ground state (pG). The chromophore in pR0 is highly contorted, with its carbonyl oriented perpendicular to the phenolate plane. This strained cis state is short-lived (~600 ps lifetime), and transitions reversibly to pR1, a planar cis state. The structural changes observed as PYP advances through its photocycle will be described.

1P004 トマトモザイクウイルス複製タンパク質と阻害因子 Tm-1 の複合体形成機構の解明
Interaction mechanism of Tomato mosaic virus replication protein and the resistance factor Tm-1

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Tm-1 gene is a resistance gene of tomato against Tomato mosaic virus (ToMV). Tm-1 binds the helicase domain of ToMV replication proteins (ToMV-Hel) and inhibits its RNA replication. However, little is yet known about the interaction mechanism of ToMV-Hel and Tm-1. In this study, we determined the crystal structures of ToMV-Hel and N-terminal domain of Tm-1 complex. Furthermore, the binding energies of Tm-1 with ToMV-Hel were measured by isothermal titration calorimetry under various conditions. The asymmetric unit of the crystal contains a tetrameric complex in a 2:2 stoichiometry, comprising of a Tm-1 dimer and two monomeric ToMV-Hel. The complex formation is enthalpically driven and requires an ATP analog or ADP.

1P005 真菌由来 TRP チャネル制御領域への Ca²⁺イオン結合の結晶学的解析
Crystallographic analysis of the Ca²⁺-binding sites in the regulatory-bundling region of the fungus TRP channel

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TRPGz is a fungus TRP channel responding to osmotic upshock. Previously, we identified the crucial region for the osmotic responses in the cytosolic region of TRPGz, and revealed that the interprotomer helix-bundle formation by the region is critical for the response. In this study, we analyzed the divalent-cation binding to this region. Crystallographic analyses revealed the binding of Ca²⁺ and other cations with the similar ionic radii of Ca²⁺ at the interprotomer interfaces, while no significant binding or binding at a different position was observed in the case for other cations with different ionic radii from Ca²⁺. The results suggested the existence of the Ca²⁺-specific binding sites in the region and their regulatory roles for the osmotic responses.

1P006 分裂酵母由来の MAP キナーゼによりリン酸化される RNA 結合タンパク質 Nrd1 の構造解析
Structural studies of RNA-binding protein Nrd1, a fission yeast MAPK target RNA binding protein

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Negative regulator of differentiation 1 (Nrd1) is known as a negative regulator of sexual differentiation in fission yeast. Further, Nrd1 binds and stabilizes the Cdc4 mRNA which encodes a myosin II light chain, and thereby suppressing the cytokinesis. Pmk1 (yeast MAPK) phosphorylates Nrd1 resulting in markedly reduced RNA binding activity. The mechanism by which Pmk1 regulates the RNA binding activity of Nrd1 is unknown. In an effort to delineate the relationship between Nrd1 structure and function, we prepared each RRM (RNA recognition motif) of Nrd1. The structure of the second RRM of Nrd1 has been determined (Kobayashi et al., *BBRC*, in press). Furthermore, we will discuss usage of split intein and paramagnetic relaxation enhancement for the full-length Nrd1.