Life Science :


"Rupinasu" - *Lupinus*

To resolve three-dimensional structures of biomacromolecules, macromolecular crystallography (MX) is an essential and powerful method, which provides static but precise structures of molecules. As synchrotron beamlines advances, crystals structures can be analyzed from diverse aspects. Last year we reported the first results from micro-focus beamline BL32XU in which its microbeam, which reaches 1 μm, was effectively applied to microcrystals and ill-diffracting crystals. This year BL41XU has been upgraded by installing new optical components that produce a high-flux beam with various beam dimensions of 3 to 50 μm and a new pixel array X-ray detector for ultrafast data collection up to 100 Hz. These improvements will promptly contribute to high-throughput and high-precision data collection. Moreover, we have established a simultaneous measurement technique using ultraviolet and visible absorption spectroscopies, and a post-crystallization treatment using humid air. With the X-ray free electron laser facility, SACLA, serial femtosecond crystallography has been established for vast number of tiny crystals, and diverse macromolecular crystals are analyzed in the SPring-8 campus. Due to these cutting-edge resources, many outstanding results have been reported in 2013. Here we summarize into three subjects in structural biology: rational drug design, molecular recognition, and dynamic structure analysis.

Rational drug design is a key technology to tackle diseases. Cycles of biological assays and computational estimations improve drugs. MX provides detailed images of the drug binding environment on the molecular surface of proteins, including strong and specific interactions between small molecules and proteins. Often efforts to treat serious endemics such as neglected tropical diseases are limited because drug development is expensive. For example, the pathogen that causes African sleeping sickness is a trypanosoma protozoa transmitted by an insect, the tsetse fly. Shiba *et al.* resolved the structure of a protozoan drug target, TAO in a complex with a drug candidate. Their finds will accelerate fine-tuning of the drug. Additionally in cancer research, one of the *termini ad quem* is the establishment of molecularly targeted therapy. Although the Ras protein is a very promising target for the therapy, a pharmacophore (a possible drug binding pocket) has yet to be been identified. Kataoka *et al.* are the first to find a pharmacophore in the crystal structure and biochemical assay and are currently developing novel anti-cancer drugs.

Structural Biology

Molecular recognition among molecules larger than drugs is essential to achieve more complicated biological mechanisms. Selenium is a micronutrient incorporated as an unusual amino acid, selenocysteine, which has a strong redox activity that is useful for various catalyses. Itoh *et al.* determined the structures of the homododecameric SelA protein that produces the amino acid. Cooperation of the ring-like oligomeric assembly is essential to recognize the substrate and to convert catalysis of sulfur to selenium. Peroxisomes are organelles, which contribute to cell development, are involved in catabolism of fatty acids, etc. In the organelle assembly, matrix proteins are recognized with those signal peptides and translated into the organelle by the protein receptors, peroxins. Pan *et al.* have determined the structure of peroxins; in this case molecular recognition by peroxins helps tag the matrix for organelle assembly. Toll-like receptors, a key player in an innate immune system, recognize ligand molecules derived from pathogenic organisms. Upon ligand recognition and biding, Tanji *et al.* found that the double-ring structure of the dimeric receptor is rearranged and activated.

Molecular motion expands the functional ability of elastic protein molecules. Dynamical structural changes of proteins affect the affinity toward other molecules via molecular recognition, produce mechanical or chemical forces, etc. Although the MX technique only resolves a static photograph, a series of snapshots for different states can be used to construct a molecular movie. Na^+,K^+-ATPase generates a concentration gradient of sodium and potassium ions across a cell membrane. This potential energy and voltage are essential to drive molecular devices and transduce signals. Toyoshima *et al.* determined that its structure in the ADP-binding E1 state shows three sodium ions, and suggested that this high affinity state is an induced sequential structural transition by an allosteric effect initiated by the first sodium binding. V-ATPase also shows a rotatory movement as an ion pump transporting protons across the membrane due to the energy of ATP hydrolysis. Suzuki *et al.* determined the structure of V-ATPase in three different states and finely depicted the machinery of the molecular motor. Because multi-drug transporters allow drug resistance to pathogens and cancer cells, it is a considerable target for drug development. Through two structural snapshots, Tanaka *et al.* showed the dynamical open/close movement of the transporter. Nyirenda *et al.* determined the mobile region in the C-terminal domain of oligosaccharyltransferases, which are key enzymes in protein glycosylation, by MX and investigated the dynamical properties in detail by a further NMR study. They revealed that this mobility is essentially efficient scanning and recognition of the glycosylated signal peptide.

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