Oral Session Abstract

Thu. 30th November / 1:10pm- 7:00pm JST / Event Hall

Molecular-targeted chemogenetics, a next-generation technique for manipulating cell-surface receptors

Prof. Shigeki Kiyonaka (Nagoya University)

Cell surface receptors transmit extracellular information into cells. Spatiotemporal regulation of receptor signaling is crucial for cellular functions, and dysregulation of signaling causes various diseases. Thus, it is highly desired to control receptor functions with high spatiotemporal resolution. Conventionally, genetic engineering or chemical ligands have been utilized to control the receptor function. As the alternative, chemogenetics has been recently proposed, in which target proteins are genetically engineered to interact with a designed chemical partner with high selectivity. The engineered receptor can dissect the function of one receptor member among a highly homologous receptor family in a cell-specific manner. In most cases, engineered receptors can be activated by designer ligand instead of the endogenous ligand. However, neither cellular responses nor the timing of activation may reflect the physiological responses. To overcome the shortcomings of the current techniques, we have recently proposed "molecular-targeted chemogenetics" which allows the cell-type specific control of target endogenous receptors without affecting original receptor function. In this talk, I will present recent progress about "molecular-targeted chemogenetics" and its applicability for molecular movie studies.

Time-resolved crystallography of ultrafast light-driven DNA repair by photolyases

Dr. Yoshitaka Besho (RIKEN SPring-8 Center/ University of Tokyo)

DNA photolyases are flavoenzymes that repair UV-induced DNA pyrimidine dimer damage, such as cyclobutane pyrimidine dimers (CPD). Blue light activates the enzyme through the photoreduction of its flavin adenine dinucleotide (FAD) cofactor. In the process of photoactivation, two light-triggered single-electron photoreduction steps

convert the oxidized chromophore (FADox), via the radical semiquinone state (FAD•-and its subsequently protonated form FADH•), to the reduced hydroquinone state (FADH-). To clarify the photoreduction mechanism of photolyase, we used the femtosecond pulses of the SACLA X-ray Free-Electron Laser (XFEL) as a probe. The damage-free crystal structure of Methanosarcina mazei CPD photolyase revealed the exact arrangement of the water molecule clusters adjacent to the U-shaped FAD cofactor. By using time-resolved crystallography, we found the reaction intermediates in the light-dependent reduction of photolyase, at atomic resolution. We could observe time-dependent buckling and twisting in the isoalloxazine ring of the FAD cofactor in different redox states. In addition, our results uncovered the molecular repair mechanism for CPD-containing DNA. Our 3D molecular movies showed how the protein environment of redox cofactors organizes multiple electron and proton transfer events in an ordered fashion. At this symposium, we will report our latest results.

Molecule movies enabled by genetic code expansion

Prof. Jiangyun Wang (Chinese Academy of Sciences)

One of the primary objectives in chemistry research is to observe atomic motions during reactions in real time. Although X-ray free-electron lasers (XFELs) have facilitated the capture of reaction intermediates using time-resolved serial femtosecond crystallography (TR-SFX), only a few natural photoactive proteins have been investigated using this method, mostly due to the lack of suitable phototriggers. Here we report the genetic encoding of a xanthone amino acid (FXO), as an efficient phototrigger, into a rationally designed human liver fatty-acid binding protein mutant (termed XOM), which undergoes photo-induced C–H bond transformation with high selectivity and quantum efficiency. We solved the structures of XOM before and 10–300 ns after flash illumination, at 1.55–1.70 Å resolutions, and captured the elusive excited-state intermediates responsible for precise C–H bond activation. We expect that most redox enzymes can now be investigated by TR-SFX, using our method, to reveal reaction intermediates key for their efficiency and selectivity.

Integrative/Hybrid Modeling Approaches for Dynamic Structural Biology

Dr. Osamu Miyashita (RIKEN R-CCS)

Understanding the complex mechanisms that govern protein functions depends on knowledge of their structures and dynamics. While experimental techniques are essential for such studies, computational methods are equally important. Experimental data are limited in resolution and require computer simulations to obtain a detailed picture of protein dynamics. Emerging technologies that generate large and complex data sets also pose challenges. On the other hand, computer simulations are limited in accuracy due to computational cost. Therefore, the integration of computational simulations and experimental data is essential.

In this presentation, we discuss two integrative modeling approaches. One example is the use of molecular dynamics (MD) simulations to interpret time-resolved serial femtosecond crystallography (TR-SFX) data. This method supports the construction of structural models from complex TR-SFX data by combining structure sampling based on MD and consistency measurements between models and data. The second example concerns the analysis of cryo-electron microscopy image datasets. Here, our goal is to obtain a complete conformational ensemble of the biomolecules in the sample by integrating computational simulations with image datasets, leading to a better understanding of protein dynamics.

The integration of computational methods and experimental data is a powerful tool in the elucidation of protein structure and dynamics. Through these illustrative integrative modeling approaches, we hope to demonstrate their potential and discuss further extensions in the field of protein research.

Water-mediated hydrogen-bond networks for signal relay in G Protein Coupled Receptors

Prof. Ana-Nicoleta Bondar (University of Bucharest)

G Protein Coupled Receptors (GPCRs) are seven-helical membrane proteins that mediate cell-signaling paths used by eukaryotic cells to communicate with their extracellular environment. Upon binding of an extracellular agonist ligand the GPCR changes conformation, becomes activated, binds and activates a cytoplasmic cytoplasmic G protein partner; this starts a cellular signaling cascade that ultimately leads to a

physiological response. We seek to understand the general principles of how GPCRs relay across the membrane changes in structure and fynamics. To this aim, we develop graph-based algorithms and graphical user interfaces that are particularly suitable to dissect dynamic water-mediated hydrogen-bond networks. We apply these algorithms to datasets of static GPCR structures, and to atomistic molecular dynamics simulations of GPCRs. The talk will present the principles of these algorithms, and focus on applications on GPCRs for which protonation states of the receptor and/or ligand are assential for function. The graph-based analyses identify dynamic water-mediated hydrogen-bond networks that extend throughout the receptors and relay structural perturbations across the membrane.

Molecular simulations in the era of AI and exascale computing

Prof. Gerhard Hummer (Max Planck Institute of Biophysics)

Rapid growth in raw computing power and advances in artificial intelligence are ushering in a new era in biomolecular modeling and simulation. On the one hand, a massive expansion in aggregate computing allows us to tackle ever larger biomolecular systems; on the other hand, the development of sophisticated artificial intelligence frameworks provides critical support for the design, operation, and analysis of these simulations. In my presentation, I will showcase our efforts to tackle the triple challenges of system size, complexity, and time scale. I will highlight our push towards cell-scale molecular simulations with a focus on the nuclear pore complex scaffold and its FG-nucleoporin permeability barrier. I will also describe our efforts to develop a self-learning AI framework to manage molecular dynamics simulations autonomously, with the aim to resolve seconds-scale dynamics in microsecond-scale simulations and reveal the mechanisms of biomolecular self-assembly and function.

Molecular movie of the catalytic reaction of bacterial copper amine oxidase conducted by mix-and-inject serial femtosecond X-ray crystallography

Dr. Takeshi Murakawa (Osaka Medical and Pharmaceutical University)

In order for enzymes to catalyze chemical reactions effectively and specifically, it is essential to optimize the active site environment at each reaction step, thereby reducing

activation energy and achieving reaction specificity. Studies based on this perspective require structural information with high time resolution. The present study aims at elucidating kinetically and structurally the precise catalytic mechanism of copper amine oxidase from Arthrobacter globiformis (AGAO) using mix-and-inject serial femtosecond X-ray crystallography (MISC).

The overall catalytic reaction of AGAO is composed of two half-reactions: the first reductive and the second oxidative half-reactions based on the redox state of a quinone cofactor, topa quinone (TPQ). The catalytic mechanism of this enzyme proceeds via a ping-pong bi-bi mechanism. In this study, AGAO microcrystals were prepared by combining micro-seeding and batch crystallization methods. MISC was conducted under anaerobic conditions to detect structural changes in the reductive half-reaction. We obtained several data sets at SACLA with different time delays after mixing the microcrystal solution of AGAO with the substrate solution. The data with a delay time of 1.0 s yielded only the structure of the semiquinone intermediate (TPQsq), the final product of the reductive half-reaction, indicating that the reductive half-reaction proceeds within 1.0 s in the crystal. In the symposium, we will discuss the catalytic mechanism of AGAO based on several structural data obtained at each delay time of MISC.

Ultrafast structural changes direct the first molecular events of vision

Dr. Valérie Panneels (Paul Scherrer Institut)

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Mammalian rhodopsin is our light receptor for vision. It belongs to the highly druggable G protein-coupled receptor family. It hosts the retinal chromophore which, like a switch, isomerizes in less than 200 femtoseconds upon photon absorption. This triggers sequential intramolecular changes in rhodopsin, initiating the signalling cascade generating in milliseconds vision events to the brain via the optic nerve. However, the intramolecular events transforming the rhodopsin resting state[1-2] (dark state) into the transducin-binding activated state[3-5] (Meta II) are not completely understood.
We now experimentally determined the ultrafast changes of native bovine rhodopsin at room temperature using time-resolved serial femtosecond crystallography, already successfully used for the proton pump bacteriorhodopsin[6-7], at SACLA and SwissFEL X-ray free electron lasers. Thousands of rhodopsin microcrystals grown in the dark are successively injected in the light of a pump laser and probed after various time-delays using an XFEL. After 1 picosecond, we observe a highly distorted all-trans retinal that has induced changes in its binding pocket while the excess energy of the absorbed 480 nm-photon dissipates inside rhodopsin through an anisotropic protein breathing motion towards the extracellular domain. Interestingly, some amino acids known to be key elements later in the transduction of the signal are involved in the ultrafast changes.

D.O.I.s [1]10.1016/j.jmb.2004.07.044 [2]10.1016/j.jmb.2004.08.090 [3]10.1038/nature09795 [4] 10.1038/nature09789 [5]10.1073/pnas.1114089108 [6]10.1126/science.aah3497 [7]10.1126/science.aat0094

Time-resolved studies on the mechanisms of oxygenases and related enzymes

Prof. Christopher Schofield (University of Oxford)

The lecture will describe time resolved crystallographic studies on the mechanisms of 2-oxoglutarate dependent oxygenases and related enzymes using both conventional and XFEL methods, as well as supporting data in solution. The results illustrate how XFEL studies can provide information on dynamic changes during catalysis that are not readily obtained by any other currently available method.