Short talk Session Abstract

May 13th (Fri) 1:30pm -4:00pm　Room : Main Office Building Lecture Hall

＜A01_Structural biology＞

Elucidation of cancer signal transduction mechanism using photo-controllable Ras on an atomic scale.

Shima Fumi (Kobe University)

Ras cancer-driver mutations result in reduction of intrinsic GTP hydrolysis rate, thereby preventing active Ras-GTP/inactive Ras-GDP conversion. Consequently, active Ras-GTP is constitutively enriched in cells, leading to tumorigenesis. Thus, Ras is a quite promising target for anti-cancer drug development, however, dedicated efforts to directly target clinically dominant Ras mutations for decades still have not yielded therapeutic efficacy. Lack of the information on structural dynamics at atomic level upon GTP hydrolysis process of natural GTP-bound Ras is presumed one of such obstacles.
Here, to elucidate the structural dynamics of natural GTP-bound Ras, we conducted time-resolved structural analysis on GTP hydrolysis of Ras by SACLA, SPring-8 and NMR, using photo-controllable substrate of Ras, "caged-GTP". We identified bona fide "novel allosteric regions" which play essential roles for GTP-hydrolysis initiation. The results suggested that conformational changes in these regions triggers Ras-GTP inactivation. Our achievements may provide valuable and scaffold information that may overcome the obstacles on developing Ras inhibitors.

The structures of catalytic intermediates of cytochrome c oxidase

Shimada Atsuhiro (Gifu University)

Cytochrome c oxidase (CcO), the terminal oxidase in a cell respiration chain, reduces a dioxygen to two water molecules coupled with pumping protons across a membrane. Spectroscopic analyses have been proposed that CcO passes through 6 intermediate states during the complete reduction reaction of a dioxygen. To understand the reaction mechanism of CcO, our group determined these 6 catalytic intermediate structures of bovine heart CcO by X-ray crystallography. Based on the determined structures, I propose the unidirectional proton-transport mechanism driven by each electron donation from cytochrome c to the dioxygen bound to the dioxygen reduction site of CcO.

Extrapolated difference Fourier map is an illustrative method to analyze light-induced structural changes in a photosynthetic membrane protein

Suga Michihiro (Okayama University)

An X-ray free-electron laser allows us to capture structural snapshots of enzymatic reactions with an extremely high temporal resolution. The most successful method to trigger the reaction is a pump-probe experiment of light-sensitive proteins. However, a problem often encountered is the inefficiency of the sample being excited, which results in a combined electron density map derived from both excited and unexcited structures. Using an extrapolated difference Fourier map, we analyzed the light-induced structural changes of photosystem II. The calculated extrapolated map showed structural features of the higher Si-state while those of the lower Si-state disappeared. The movement of Glu189 of D1 protein, which is a typical structural change in the S2-to-S3 state transition, was estimated to be about 1-Å while it was 0.5-Å in the previous analysis. Therefore, this method is especially effective when the excited structure is unknown, or there are minor differences between the excited and ground structures.

Strategic approach towards cone pigment structure determination

Ohashi Sayaka (Nagoya Institute of Technology)

Color vision is achieved by three cone pigments, blue, green, and red. Each cone pigment consists of a different opsin protein bound to a common chromophore, 11-cis-retinal; differential chromophore-protein interactions allow preferential absorption at a selected range of wavelengths. Structural determination of cone pigments is needed for a precise understanding of spectral tuning. The principle obstacle to solving the structures is their innate instability in detergent micelles and crystal packing.
Here, we demonstrate successful optimization for the purification and stabilization of primate green cone pigment (MG) for further structural determinations. The 1st screening crystallization of purified MG gives some promising crystal images under dime-red light.

Vibrational spectroscopic study of G protein-coupled receptor

Katayama Kota (Nagoya Institute of Technology)

IR spectroscopy is one of excellent methods for analyzing structural changes related to function of membrane protein. Recently, we have attempted to use Attenuated Total Reflection (ATR)-Fourier Transform IR (FTIR) spectroscopy with combining a two-liquid exchange system to study the conformational changes in muscarinic acetylcholine receptor (M2R) that are induced by

ligand binding. And, we have successfully measured the systematic ligand binding-induced difference ATR-FTIR spectroscopy on ligands with four different efficacies (agonist, partial agonist, antagonist, and inverse agonist). By monitoring the C=O stretch of amide-I band, distinct conformational changes were observed among the agonist, partial agonist, and antagonist, from which the degree of vibrational band change correlated with the functional results of G-protein activity in the cells.

Reconsideration of hydrolysis reaction mechanism by lysozyme-NAG complex crystal structure analysis

Tanaka Ichiro (Ibaraki University)

Though the mechanism of lysozyme hydrolysis has been proposed for 50 years, but various reaction mechanisms are still being discussed. In order to trace the intermediate structure during reaction in a natural system as much as possible, crystallization was carried out under conditions using antifreezing agents in a pH away from the optimum one. Then, the X-ray and neutron structure data of the complex with the reaction products such as NAG3 and NAG4 were obtained to be considered as a system to infer the structure during the reaction. In the poster, a new conformation structure of the short chain NAG2 complex resulting from hydrolysis reaction during crystallization and the state of protonation of NAG3 and NAG4 complexes will be presented.

＜A01_Chemical biology＞

Generation of photo-switchable potassium channels by incorporation of the azobenzene-based unnatural amino acid

Shimomura Takushi (National Institute for Physiological Sciences)

Incorporation of genetically encoded unnatural amino acids (UAAs) is a powerful technique to provide a variety of unconventional functions to target proteins. We successfully introduced phenyalanine-azobenzene (Pab), a UAA that isomerizes in response to ultraviolet and visible light, into two potassium channels with different activation mechanisms, bacterial KcsA and human Kv1.2. Both mutant channels, in which Pab was introduced into their stimulant receptor region, became photo-switchable. Depending on the position of the introduction, their channel activities were increased or decreased by ultraviolet light, and reversed by visible light. These results indicate that photo-switchable UAAs may confer photosensitivity to a variety of proteins.

Next Generation Biosensors Enabled by High-speed Visualization of Dynamic Mechanisms

Campbell Robert (The University of Tokyo)

Genetically encoded biosensors based on the jellyfish green fluorescent protein (GFP) have revolutionized modern neuroscience research. However, only a few of them have been highly optimized because there is almost complete lack of understanding of the mechanisms by which these biosensors actually operate. To address this issue, we propose to utilize time-lapse X-ray Free Electron Laser (XFEL) techniques and caged ligands to reveal the dynamic response mechanisms of the biosensors for the first time. The obtained information will establish general principles that will guide the future development of high-performance biosensors.

＜B01_ Molecular Movie Platform Design＞

Reducing background noise of X-ray crystallography data through improved sample environment

Suzuki Akihiro (Hokkaido University)

The capability to detect very weak signals like diffuse scattering from protein crystals and Bragg diffraction from sub-micron crystals will bring new knowledge about molecular dynamics. To realize the measurements with a higher signal-to-noise ratio, reducing the background levels from solvent, air, optics, and sample holders is necessary. Therefore, we are developing a vacuum measurement system collaborating with the B01 Yamamoto group at the SPring-8 RIKEN beamline. In addition, creating sample holders using graphene is underway. In this presentation, We will report the recent experimental results at SPring-8 and the progress of primary studies to develop an ultra-low background graphene sample holder.

Development of in-vacuum diffractometer for microcrystallography at SPring-8

Matsuura Hiroaki (RIKEN)

Recent developments in SR or XFEL facilities enable structural determination from um-sized protein microcrystals. To realize structure analysis from further smaller (sub-um-sized) crystals, a high S/N ratio observation of weak diffraction is required. As the crystal size becomes smaller, background scattering from the air becomes more severe resulting in loss of signals. Therefore,

we have been developing an in-vacuum diffractometer to avoid the scatter from the air and enable observation of weak signals. Furthermore, we introduce a SiN grid for the sample mount to further reduce background scattering. Here the current progress on our in-vacuum diffractometer will be presented.

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＜C01_ Computational Chemistry and Spectroscopy＞

Microspectroscopic systems for time-resolved measurements of protein microcrystals

Kimura Tetsunari (Kobe University)

Time-resolved spectroscopy is important to complement the understanding the time-resolved crystallography. The novel time-resolved spectroscopic systems have been developed by equipping the microfluidics mixer with microspectroscopy, allowing us to investigate the time-courses of product-formation and substrate-binding. These systems could be applied both solution and microcrystal samples because the substrate diffusion is induced by the hydrodynamic focusing of the solution either with or without microcrystals. The chemical changes in substrate molecules or proteins investigated by the time-resolved spectroscopy would clarify the molecular mechanism.

Cis-trans reisomerization preceding reprotonation of the retinal chromophore in the schizorhodopsin photocycle

Mizuno Misao (Osaka University)

Schizorhodopsin (SzR) is a newly discovered rhodopsin family of light-driven inward proton pumps. The photocycle of SzR is initiated by photoisomerization of the retinal chromophore similarly to that of conventional outward proton-pumping rhodopsins while it contains multiple M intermediates. We measured time-resolved resonance Raman spectra of SzR AM_5_00977, called SzR4, and explored the chromophore structures of two M intermediates. The observation demonstrated that the retinal chromophore of SzR4 undergoes cis-trans reisomerization preceding reprotonation at the Schiff base in the retinal chromophore. The sequence of structural changes is essential for proton uptake from the extracellular side.

Time-Resolved Spectroscopy for Tracking DNA Repair by Photolyase

Kubo Minoru (University of Hyogo)

Photolyases are the flavoenzymes that use blue light to repair UV-induced DNA damages, such as cyclobutene pyrimidine dimer (CPD) and (6-4) photoproduct (6-4PP). The photorepair mechanism of CPD has been well understood; however, that of 6-4PP remains still elusive and receives attention as one of the targets for "Molecular Movie" researches. A key question under debate on the mechanism is whether or not two photons are required for the photorepair of 6-4PP, which may be rather intriguing in photochemistry. We here employed time-resolved spectroscopic techniques, and obtained a result suggesting the repair processes involving two photons.

Reaction dynamics of light-driven protein studied by non-adiabatic QM/MM molecular dynamics simulations

Yagi Kiyoshi (RIKEN)

We develop non-adiabatic molecular dynamics (MD) method based on fast QM/MM calculations. QM/MM is a multiscale method that treats the reaction center by quantum chemical (QM) method and the biological environment by a classical force field (MM). The QM calculation of the electronic excited state, which is the computational bottleneck, will be highly parallelized to achieve high performance. In addition, we will develop a new method that extends the conventional MD calculation to multi-states, which can take into account non-adiabatic transitions between electronic states. The developed method will be applied to various rhodopsins (H+, Cl-, Na+ pumps) to reveal photochemical processes and the conformational changes.

Low-temperature UV-visible and FTIR spectroscopic studies on a UV sensitive visual pigment

Mizuno Yosuke (Nagoya Institute of Technology)

Animal visual pigments contain an 11-cis-retinal as common chromophore, which is usually bound to lysine residue via a protonated Schiff base (PSB), thereby absorbing visible region. In contrast, ultraviolet (UV) visual pigments uniquely contain an unprotonated SB. The key factors that modulates the differences of protonation state of the chromophore and photoreaction dynamics in UV pigments remain to be understood at molecular level.
Here, we address these questions by investigating photoreaction dynamics in the Siberian

hamster UV (SHUV) pigment. Light induced difference UV-visible and FTIR spectroscopies measured at 77 K reveal the protonation state of photo-intermediate state of SHUV. We discuss the structural changes of SHUV upon light absorption based on the spectral basis.

Analysis for Stability and Dynamics of Proteins using Molecular Dynamics Simulations

Mitsutake Ayori (Meiji University)

The analysis methods of molecular simulations are important to investigate the stability and dynamics of proteins. For stability and dynamics, we have applied 3D-RISM theory and relaxation mode analysis to protein systems and shown their effectiveness, respectively. 3D-RISM theory can calculate the distribution functions of solvents around proteins. Relaxation mode analysis can extract slow modes from the complicated motions of proteins. In the short talk, we introduce the results of our group's simulations for peptides and proteins.

Molecular Dynamics Simulations for Determination of the Characteristic Structural Differences between Inactive and Active States of Wild-type and Mutants of the Orexin 2 Receptor

Yokoi Shun (Meiji University)

We performed over twenty several microsecond-scale MD simulations of the wild-type and the mutants of the orexin 2 receptor (OX2R), which is classified as class A GPCRs. We introduced mutations that exhibited the stable inactive state and the constitutively active state in class A GPCRs to the OX2R. In these simulations, significant characteristic structural changes were observed in the V309(6.40)Y mutant. Here, we first show the results of the MD simulations and dynamics analysis using relaxation mode analysis (RMA), and then present the a suitable index for the quantitative evaluation of the active and inactive states of class A GPCRs. Finally, we discuss the structural advantages of TM7 inward movement for GPCR activation.

Analysis of free energy landscape and pathways of protein structural changes, dissociation and association

Kitao Akio (Tokyo Institute of Technology)

The X-ray crystallography was successful in observing structural changes of biological macromolecules with high resolution in both space and time, providing important information as the average over molecules in the sample. To provide complementary information on the behavior of a single molecule by molecular simulation, we generate ensembles of conformational changes using the PaCS-MD/MSM (parallel cascade selection molecular dynamics/Markov state model) method. The relationship between the ensemble-averaged information and the behavior of individual protein molecules and the free energy landscape and pathways involved in the conformational change and molecular binding are investigated.

Theoretical insights into the molecular mechanisms of dynamical biochemical reactions

Shoji Mitsuo (University of Tsukuba)

This presentation overviews my theoretical researches under the project of molecular movies. The subjects are (1) copper amine oxidase, (2) Mn complex binding in lysozyme, (3) resonance Raman spectra of hemoglobin, (4) C-phycocyanin, (5) heliorhodopsin and (6) 2-oxoglutarate dependent dioxygenase. Among these topics, we have recently advanced their theoretical analyese on (1)-(3). Using QM/MM method, their reaction mechanisms and structural changes are validated in collaboration with experimental groups. The theoretical results supported with SFX/X-ray structures are reported.

Theoretical study on molecular mechanism of an activation process of aequorin bioluminescence

Hayashi Shigehiko (Kyoto University)

Aequorin is a bioluminescent protein which binds coelenterazine as a light emitting molecule. A chemiluminescence process of coelenterazine with a molecular oxygen in the protein binding pocket is triggered by binding of calcium ions at EF-hands of the protein distant from the binding pocket. We theoretically investigate molecular mechanism of the chemiluminescence process in the protein by means of hybrid QM/MM molecular simulations. We found significant conformational changes of the binding pocket upon the binding of calcium ions which can regulate reaction free energy profile of dioxetanone formation prerequisite for the chemiluminescence.