2019-2023 MEXT Grant-in-Aid for Scientific Research on Innovative Areas
"Non-equilibrium-state molecular movies and their applications (Molecular Movies)"
Molecular Movies International Symposium 2022

International Symposium Abstract

12th /May 5:00pm- 9:10pm JST（CET 12th/May 10:00am- 2:10pm, CST 12th/May 4:00pm- 8:10pm）

Mechanism and dynamics of fatty acid photodecarboxylase

Prof. Ilme Schlichting (MPI for Medical Research)

Light is important for organisms from all domains of life, serving as an energy resource or carrier of information initiating intra- or intercellular signaling. Photosensitive proteins, endowed with a light-absorbing chromophore, enable this. Detailed insights, including the initial events on the ultrafast time scale, were obtained by various forms of spectroscopy and computation. However, direct structural information necessary to understand the underlying molecular mechanisms has been inaccessible until recently. The unique properties of X-ray free electron lasers open the sub-ps time domain for time-resolved crystallography using small crystals that can be efficiently photolyzed, thus providing access to the long sought-after excited state and intermediate structures.

Photodecarboxylation is a well-established reaction in chemistry; however, no photo¬enzymatic equivalent was known until the discovery of Fatty Acid Photodecarboxylase (FAP), a flavin containing photoenzyme [1]. The enzymatic mechanism was investigated in detail by a large interdisciplinary consortium [2]: decarboxylation occurs directly upon reduction of the photo-excited flavin by the fatty acid substrate. Along with flavin reoxidation by the alkyl radical intermediate, a major fraction of the cleaved carbon dioxide unexpectedly transforms in 100 ns into another species, assigned to be bicarbonate based on IR-spectroscopy. Despite a great deal of insight into the catalytic mechanism and the role of two strictly conserved residues for substrate stabilization and functional charge transfer [2], a number of questions remain. To address these, including the nature of the transiently generated CO2-derivative we performed a follow-up time-resolved serial femtosecond crystallography experiment at SwissFEL spanning time-delays from ns to ms after photoexcitation. The results will be discussed.

[1] Sorigué et al An algal photoenzyme converts fatty acids to hydrocarbons, Science 357: 903-907 (2017) [2] Sorigué et al Mechanism and dynamics of fatty acid photodecarboxylase, Science 372, eabd5687 (2021)

Fixed target serial crystallography for studying protein dynamics

Dr. Alke Meents (DESY)

For serial X-ray crystallography highly reliable sample delivery requiring only small amounts of sample has remained one of the bottlenecks. Among other methods, fixed target sample delivery, where the microcrystals are immobilized on a solid support and then systematically scanned through the X-ray beam, is one of the most powerful methods. For the method of fixed-target serial crystallography we have developed the Roadrunner goniometer, which allows collecting a complete serial X-ray data set at 1 kHz sample exchange rate in less than 2 minutes. In combination with very low background scattering levels achievable with our approach this method is ideally suited to study and protein dynamics and enzyme reactions in a highly efficient and reliable fashion.

Using the Roadrunner goniometer we have performed several time resolved diffraction experiments at both synchrotron sources utilizing the polychromatic 'pink' X-ray beam and at XFEL sources. Different pumping schemes have been applied to trigger protein motion and reactions. These include optical laser pumping experiments of the photoactive yellow protein PYP and temperature jump experiments induced with ns-duration infrared laser pulses . The presentation will provide an overview of the method followed by a few recent application examples.

Three routes to molecular movies

Prof. Helmut Grubmüller (MPI for Multidisciplinary Sciences)

We will discuss three examples from the fields of atomistic simulation, cryo-electron microscopy, and serial crystallography illustrating how molecular movies can be achieved. The first example shows how non-equilibrium atomistic simulations can solve biological problems even for systems as large as whole microtubules. In particular, we will address the question how, despite very similar structure of the tip, microtubules switch between growing and shrinking phase. Our results show that and why the primary steps of microtubule tip flaring differ kinetically between GTP and GDP loaded states. In the second example, we used non-equilibrium atomistic

simulations of shock freezing of whole solvated ribosomes to quantify how much of the physiological temperature structural heterogeneity and dynamics is preserved in the cryogenic electron microscopy samples. The simulations also revealed which processes contribute to the reduction of structural heterogeneity during shock freezing. In our third example we applied a rigorous Bayesian approach to structure determination of single proteins by single molecule femtosecond XFEL diffraction (serial crystallography). Using synthetic data and a hierarchical Gaussian mixture approach, we will demonstrate that near-atomistic resolution should be possible even for small proteins.

Maxim Igaev, Lars V. Bock, Steffen Schultze, Helmut Grubmuller

Resolving gating and allosteric modulation in ion channels through simulations and small-angle neutron scattering

Prof. Erik Lindahl (Stockholm University)

Pentameric ligand-gated ion channels (pLGICs) perform electrochemical signal transduction in organisms ranging from bacteria to humans. In addition to their normal gating cycle, pLGICs are highly sensitive to allosteric modulation where small compounds such as barbiturates, benzodiazepines or alcohols influence the gating kinetics by binding in separate sites, either in the transmembrane or extracellular domain. Despite a wealth of new experimental structures, it has been challenging to understand the gating kinetics, in particular since the channels rapidly undergo transitions to a desensitized nonconducting state rapidly after opening. I will present our recent combined experimental and computational work on a number of prokaryotic and eukaryotic pLGICs from the team, and how we are trying to combine low-resolution experimental techniques such as SANS (small-angle neutron scattering) with simulations to model channels under realistic conditions. In addition, I will show how we have been able to resolve structures in all separate functional states, their state-specific interactions with lipids, and not least how we are beginning to understand the properties of the desensitized state.

Bioorthogonal Protein Activation in Space and Time

Prof. Peng Chen (Peking University)

Employing small molecules or other chemical means to modulate the function of an intracellular protein of interest, particularly in a gain-of-function fashion, remains highly desired but challenging. In this talk, I will introduce a "genetically encoded chemical decaging" strategy that relies on our recently developed bioorthogonal cleavage reactions to control protein activation with high spatial and/or temporal resolution in living systems. These reactions exhibit high efficiency and low toxicity for decaging the chemically "masked" lysine or tyrosine residues on intracellular proteins, allowing the gain-of-function study of individual enzymes within living cells and mice. Most recently, with the assistance of computer-based design and screening, we further expanded our method from "precise decaging" of enzyme active-sites to "proximal decaging" of enzyme pockets. This new method, termed Computationally Aided and Genetically Encoded Proximal Decaging" (CAGE-prox) (CAGE-prox), showed general applicability for switching on the activity of a broad range of proteins under living conditions. I will end by showcasing exciting applications of our CAGE-prox technique on: i) constructing orthogonal and mutually exclusive kinase signaling cascades; ii) temporal caspase activation for time-resolved profiling of proteolytic events upon apoptosis; and iii) on-demand activation of bacterial effectors as potential protein prodrugs for cancer therapy. Finally, by coupling with the proximity-labeling enzymes that have been used for subcellular targeting, we further developed a spatial-temporal resolved proteomics strategy for subcellular proteome profiling in living cells.

Visible/near-infrared-light photorelease: How far can we go with one-photon absorption?

Prof. Petr Klán (Masaryk University)

Photoactivatable compounds, also called caged compounds, are those which, upon photoactivation, irreversibly release a species possessing required physical, chemical, or biological qualities. Short-wavelength UV radiation is not compatible with many biological and medical applications because it can induce adverse side-reactions. Photorelease induced by red or NIR light is most desirable as the tissue absorption is limited by the absorption of hemoglobin below 600 nm and absorption of water over 900 nm.

Only a few known photoactivatable (caged) molecules can be activated directly by visible/NIR light because the delivered excitation energy is in principle too low for a covalent bond cleavage. In the past years, we have introduced several new chromophores absorbing in the region of 600−1100 nm that can release biologically relevant species, for example, H2S or CO as gaseous signaling molecules. The design, photoreaction mechanisms, spectroscopy and biological applications of these systems will be presented.

13th /May 8:00am-12:05pm JST (CDT 12th/May 6:00pm-10:05pm, EDT 12th/May 7:00pm-11:05pm)

Controlling the Fate and Function of Proteins with Proximity Photopharmacology

Prof. Dirk Trauner (New York University)

Photopharmacology endeavors to control biological function with synthetic photoswitches that can be attached covalently or non-covalently to their targets - or nearby. I will discuss potential applications of photopharmacology in biology and medicine, in particular with respect to controlling signal transduction and targeted protein degradation. I will make a case that "Proximity Photopharmacology" is a particularly effective strategy to control the fate and function of proteins, with an emphasis on applications in neuroscience.

Mix-and-Inject Serial Crystallography

Prof. Marius Schmidt (University of Wisconsin-Milwaukee)

The characterization of biomedically relevant enzymes is essential for the treatment and prevention of life-threatening diseases. With time-resolved X-ray crystallography enzymes in action can be captured in real time. Free Electron Lasers for hard X-rays (XFELs) are powerful tools to investigate micron sized crystals on all time scales (1-2). To initiate reactions in enzyme crystals, diffusion of substrate can be employed (3). For the investigation of biomedically important enzymes we used an approach called “mix-and-inject serial crystallography”(3,4) to trigger reactions by diffusion of substrate. We demonstrate that the approach is feasible using the M. tuberculosis β-lactamase (BlaC). BlaC promotes broad-scale antibiotic resistance by chemically inactivating β-lactam antibiotics. We characterize the structure of the enzyme-substrate

complex and that of a reaction intermediate along the catalytic pathway of the BlaC reaction with the antibiotic ceftriaxone (5). In the times of the COVID-19 pandemic, these investigations become disproportionally more relevant to (i) aid the design and discovery of new inhibitory compounds that affect the function of essential enzymes to prevent the outbreak of life-threatening diseases, and (ii) contribute to the development of MISC as an applicable method at XFELs, to be used for the structural characterization of reactions in biologically significant molecules (6).

1. Pande K, et al. (2016) Science 352:725
2. Pandey S, et al. (2020) Nature methods 17:73
3. Schmidt M (2013) Advances on Condensed Matter Physics (2013):1.
4. Kupitz C, et al. (2017) Struct Dyn 4:044003.
5. Olmos JL, Jr., et al. (2018) BMC Biol 16:59.
6. Schmidt M (2020) Crystals 10.

Time-resolved serial femtosecond crystallography of microbial rhodopsins

Prof. Eriko Nango (Tohoku University)

Light-driven ion-pumping rhodopsins actively transport ions across the cell membrane. Although recent time-resolved serial femtosecond crystallography (TR-SFX) studies have revealed structural changes and ion transfer mechanisms in light-driven cation pumping rhodopsins, it has remained elusive until recently how conformational changes pump an anion to achieve unidirectional ion transport in anion-pumping rhodopsins. In this study, TR-SFX data of Nonlabens marinus rhodopsin-3 (NM-R3) from a marine bacteria were collected at a time-point of 10 μs and 1 ms after photoexcitation. The structural analysis revealed conformational alterations during ion transfer and after ion release. In addition, a pump–probe TR-SFX of bacteriorhodopsin was performed using a belt conveyor type device. The TR-SFX data of bacteriorhodopsin bicelle crystals revealed structural changes that differed from previous results of bacteriorhodopsin crystals retrieved from the lipidic cubic phase. In the symposium, I will present time-resolved studies of these two microbial rhodopsins.

Classical and QM/MM simulations of "molecular movies" for understanding the functions of biomolecular machines

Prof. Qiang Cui (Boston University)

In this talk, we will discuss recent developments and applications of QM/MM methods to biological systems, especially "biomolecular machines" that carry out important functions such as nucleic acid modification and energy transduction. We emphasize the importance of balancing computational efficiency and accuracy for these mechanistic analyses, for which both systematic improvement of an approximate (semi-empirical) DFT method and integration with machine learning techniques is worthwhile. If time permits, I'll also discuss briefly our perspective in combining experiments, classical molecular simulations and machine learning to probe complex protein functions such as allostery.

Unification of molecular movies and large-scale quantum molecular dynamics

Dr. Junichi Ono (Waseda University)

Proton transfers correlated with structural changes play a vital role in biological function. One of the most representative examples is a light-driven proton pump, bacteriorhodopsin (BR). In BR, at least five consecutive proton transfers on the photocycle achieve the unidirectional active proton translocation. In 2016, molecular movies of structural changes on the photocycle in BR have been successfully captured using time-resolved SFX at XFEL in SACLA, which unambiguously show the hydrogen-bond network for the primary proton transfer from the protonated Schiff base to Asp85 via Wat452 and Thr89 as the putative pathway. Although this state-of-the-art experiment has invoked technological innovation in various fields, the direct observation of proton transfers has not been accomplished due to the limitation of the spatio-temporal resolution. Here, large-scale quantum molecular dynamics (QMD) simulations were performed on the basis of the molecular movie of BR with focusing on the primary proton transfer, where all the atoms were treated quantum-mechanically. It was found that the proton relay occurs via the deprotonation of Wat452, followed by the reprotonation of the resultant hydroxide ion from the protonated Schiff base. This study demonstrates that the large-scale QMD simulations compensate for the molecular movies for revealing the microscopic mechanisms of proton transfers.

SLIPT: a chemical approach for controlling protein localization and cell signaling

Prof. Shinya Tsukiji (Nagoya Institute of Technology)

Controlling protein function in cells with synthetic small molecules is a key component of chemical biology. The self-localizing ligand-induced protein translocation (SLIPT) technique is a novel chemical approach we developed for controlling protein localization and cell signaling in living mammalian cells. This approach uses synthetic molecules, termed self-localizing ligands (SLs), which are designed to spontaneously localize to specific subcellular regions in mammalian cells. SLs bind their target proteins and relocate (tether) them rapidly from the cytoplasm to their targeting sites in a "single ligand-single protein" manner. In this symposium, I will present the basic principle, current applications, and future directions of the SLIPT approach in chemical biology, synthetic biology, and structural biology.

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.

May 13th (Fri) 1:30pm -4:00pm　Room : YCU 2F Library

＜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.