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.