“Molecular Movies” International Symposium 2023
高速分子動画 国際シンポジウム 2023 要旨集

Program_プログラム

Abstract_要旨

Organizer

Grant-in-Aid for Scientific Research on Innovative Areas “Non-equilibrium-state molecular movies and their applications (Molecular Movies)”

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1. Oral Session Program

Thu. 30th November				
	Speaker	Affiliation	Title	Chair
12:00-13:00	Set up posters			
13:10-13:20	So Iwata	Kyoto University	Opening Remarks	
13:20-13:55	Shigeki Kiyonaka	Nagoya University	Molecular-targeted chemogenetics, a next-generation technique for manipulating cell-surface receptors	Nagasawa
13:55-14:30	Yoshitaka Bessho	RIKEN SPring-8 Center/ University of Tokyo	Time-resolved crystallography of ultrafast light-driven DNA repair by photolyases	Park
14:30-15:05	Jiangyun Wang	Chinese Academy of Sciences	Molecule movies enabled by genetic code expansion (Online)	Kiyonaka
Break (15:05-15:15)				
15:15-15:50	Osamu Miyashita	RIKEN R-CCS	Integrative/Hybrid Modeling Approaches for Dynamic Structural Biology	Shinoda
15:50-16:25	Ana-Nicoleta Bondar	University of Bucharest	Water-mediated hydrogen-bond networks for signal relay in G Protein Coupled Receptors (Online)	Miyashita
16:25-17:00	Gerhard Hummer	Max Planck Institute of Biophysics	Molecular simulations in the era of AI and exascale computing (Online)	Shoji
Break (17:00-17:10)				
17:10-17:45	Takeshi Murakawa	Osaka Medical and Pharmaceutical University	Molecular movie of the catalytic reaction of bacterial copper amine oxidase conducted by mix-and-inject serial femtosecond X-ray crystallography	Kimura
17:45-18:20	Valérie Panneels	Paul Scherrer Institut	Ultrafast structural changes direct the first molecular events of vision (Online)	Nango
18:20-18:55	Christopher Schofield	University of Oxford	Time-resolved studies on the mechanisms of oxygenases and related enzymes (Online)	Nagano
18:55-19:00	So Iwata	Kyoto University	Closing Remarks	
Dinner (19:30-21:00)				
21:00-22:30	Poster Session			
22:30-	Remove posters			

2. Short-Talk Session Program

Fri. 1st December				
Room	Event hall	403	404	405
	A01_Structural biology	A01_Structural biology	B01_Molecular Movie Platform Design＆C01_Spectroscopy	C01_Computational Chemistry
Chair	Iwata	Park	Yamamoto／Kubo	Miyashita
9:00-9:10	Masahiro Fukuda	Michihiro Suga	Hiroaki Matsuura	Kiyoshi Yagi
9:10-9:20	Toshiaki Hosaka	Atsuhiro Shimada	Akihiro Suzuki	Mitsuo Shoji
9:20-9:30	Ritsu Mizudori	Junpei Yamamoto	Tetsunari Kimura	Duy Phuoc Tran
9:30-9:40	Kota Katayama	Yasufumi Umena	Yosuke Mizuno	Akio Kitao
9:40-9:50	-	Sam-Yong Park	Yuji Furutani	Ayori Mitsutake
9:50-10:00	-	-	Tetsuro Katayama (Online)	-
	A01_Chemical biology	A01_Structural biology		C01_Computational Chemistry
Chair	Kiyonaka	Nagano		Shoji
10:00-10:10	Takuya Terai	Toru Nakatsu		Shigehiko Hayashi (Online)
10:10-10:20	Takushi Shimomura	Takehiko Tosha		Junichi Ono
10:20-10:30	Tomohiro Doura	Eiichi Mizohata		Sriram Srinivasa Raghavan
10:30-10:40	Tsuyoshi Araya	Shingo Nagano		Keiko Shinoda
Coffee Break & Photo Shoot（10:40-11:15)				

3. 新学術領域「高速分子動画」領域会議プログラム(Closed)

12 月 1 日（金）			
	講演者	所属	タイトル
11:15-11:21	岩田　想	京都大	はじめに／構造生物の結果
11:21-11:27	清中　茂樹	名古屋大	高速分子動画撮像に向けたケミカル光制御技術の開発
11:27-11:33	南後　恵理子	東北大	時分割実験のための多様な反応誘起システムの開発
11:33-11:39	久保　稔	兵庫県立大	時間分解分光で観るタンパク質の反応ダイナミクス
11:39-11:45	宮下　治	理化学研究所	分子シミュレーションによるタンパク質化学反応ダイナミクスの解明
11:45-12:00	質疑応答・アドバイザーコメント		

4. Poster Session Program

Thu. 30th November / 9:00pm- JST / Lobby

	名前	所属	ポスタータイトル
1	荒谷　剛史	京都大学医学研究科	Crystal structure of photoresponsive ligand bound adenosine A2A receptor
2	安東　智大	京都大学理学研究科	イクオリンの生物発光過程についての理論的研究
3	伊藤　朱音	京都大学医学研究科	アンタゴニスト結合型ヒスタミンH4受容体の立体構造解析
4	井上　始	名古屋大学工学研究科	化学遺伝学的手法を用いたセロトニン5-HT2A受容体に対するAntagonistの親和性制御
5	梅名　泰史	名古屋大学シンクロトロン光研究センター	Fast time-resolved structural analysis of light-response in photosynthetic antenna protein Phycocyanin
6	江尻　智森	京都大学理学研究科化学専攻	アニオンポンプロドプシン NpHR のスイッチング機構解明に向けた理論的研究
7	戎　唯良汰	東京大学理学系研究科	Cryo-EM structure analysis of Schizorhodopsins from mesophilic archaea
8	王　雨竹	東京大学大学院理学系研究科	藍色光を受容するチャネルロドプシンの構造及び機能解析
9	小野　純一	Waseda University	Hybrid in silico drug study based on quantum molecular dynamics with virtual screening and docking
10	片山　耕大	名工大院工	Identifying functional hotspot residues for activation in M2 muscarinic receptor (M2R)
11	北尾　彰朗	東京工業大学生命理工学院	Molecular mechanisms of structure support and smooth rotation in the bushing/rod complex of bacterial flagella
12	木村　哲就	神戸大学大学院理学研究科	Stepwise calcium binding in photoluminescence protein; aequorin, revealed by the time-resolved spectroscopy
13	近藤　匠	名古屋大学工学研究科	作用薬の機能転換を利用した代謝型グルタミン酸受容体1の活性制御
14	志甫谷　渉	東京大学理学系研究科	Cryo-EM sructure of the zeaxanthin-xanthorhodopsin complex
15	島田　敦広	岐阜大学応用生物科学部	XESとXRDの同時測定法を用いた、シトクロム酸化酵素の反応機構解明の試み
16	下村　拓史	生理学研究所　神経機能素子研究部門	Characterization of a light-gated K+ channel generated by introducing an photo-isomerizing unnatural amino acid
17	庄司　光男	筑波大CCS	Overview of our research collaborations performed in the research area of "molecular movies"
18	Duy Phuoc Tran	東京工業大学・生命理工学院	How the mGLU1 receptor dimer be activated: a molecular dynamics study
19	菅　倫寛	岡山大学異分野基礎科学研究所	Real-Time Structural Changes during the Light-induced Water Oxidation of Photosystem II Caught by Time-Resolved Crystallography
20	鈴木　啓文	名古屋大学工学研究科	光薬理学によるアデノシンA2A受容体のサブタイプ選択的な活性化
21	Sriram Srinivasa Raghavan	RIKEN	ResiDEM: Analytical Tool for Isomorphous Difference Electron Density Maps Utilizing Dynamic Residue Identification via Density Clustering.
22	鈴木　明大	北海道大学電子科学研究所	Low background graphene sample holder for XFEL-based crystallography and imaging
23	田口　真彦	東北大学多元物質科学研究所	Understanding of protein functional expression using hybrid QM/MM free energy method

	名前	所属	ポスタータイトル
24	田中　達基	東京大学理学系研究科	Structural basis for the highly sensitive channelrhodopsin GtCCR4
25	永澤　秀子	岐阜薬科大学	生理活性作用を有するデカリン生成のための化学的および酵素的分子内Diels-Alder環化付加反応の立体制御機構の解明
26	Nipawan Nuemket	JASRI	Towards Elucidating the Structural Dynamics of Animal and Microbial Rhodopsin using Time-Resolved Crystallography at XFEL and Synchrotron
27	Basudev Maity	Tokyo Institute of Technology	Observation of a CO release reaction within protein crystal.
28	長谷川　和也	JASRI	SPring-8 BL41XUにおける時分割構造解析環境の構築
29	馬場　清喜	高輝度光科学研究センター	Non-cryogenic X-ray crystallography under various ambient conditions using the humid air and glue-coating (HAG) method
30	原　隆史	名古屋大学工学研究科	ループ工学に基づいたドーパミン受容体の活性制御
31	日野　智也	鳥取大学工学研究科	プレートスキャンシステムを用いたTRPV3の新規微結晶化条件の探索
32	福田　昌弘	東京大学 先進科学研究機構	Structural and functional analyses of pump-like channelrhodopsins
33	藤原　孝彰	東北大学多元物質科学研究所	テルペン環化酵素CotB2の室温結晶構造解析
34	保坂　俊彰	理研　BDR	Synthesis of photocaged L-tyrosine containing proteins through an Escherichia coli cell-free protein synthesis system for time-resolved structural analysis
35	松浦　滉明	理化学研究所　放射光科学研究センター	Development of an in-vacuum diffractometer for protein micro-crystallography
36	松岡　佑真	名古屋大学工学研究科	細胞外ループに着目したケモジェネティクスによるアデノシンA2A受容体の自在な制御
37	水鳥　律	名古屋工業大学大学院　工学専攻	Crystal structure of V2HeR3, a Viral heliorhodopsin transporting proton
38	水野　秀昭	Department of Chemistry, KU Leuven	Decarboxylation via two-photon absorption process results in efficient LSSmOrange photoconversion
39	水野　陽介	名古屋工業大学	Structural features of Lumi intermediate on a primate blue-sensitive visual pigment revealed by FTIR
40	光武　亜代理	明治大学理工学部物理学科	Analysis of protein simulations using relaxation mode analysis and 3D-RISM theory.
41	宮崎　育実	東京大学大学院理学系研究科	A caged lactate for light-induced biosensor activation
42	八木　清	理研CPR	非断熱QM/MM分子動力学計算法の 開発と応用
43	山元　淳平	大阪大学大学院基礎工学研究科	Time-resolved serial femtosecond crystallography of light-induced structural changes of a bifunctional cryptochrome
44	横井　駿	Department of Physics, School of Science and Technology, Meiji University / Department of Structural Biology, School of Medicine, Stanford University	Structural and Computational Insight into Dynamics and Intermediate State in Activation of Orexin 2 Receptor
45	Luo Fangjia	JASRI	Time-resolved structure analysis enhanced by mixing and improved liquid injectors
46	長谷川　颯人	鳥取大学工学研究科	立体選択的なDiels-Alder反応を行う酵素の生成物結合型結晶構造とその反応過程の分子動画撮影に向けた取り組み

2023.11.29

5. 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)

Matthew Rodrigues[1], Thomas Gruhl[1], Tobias Weinert[1], Schertler group[1,2], Nango group[3], Neutze group[4], Nogly group[2], SwissFEL group[5], SACLA group6, Standfuss group[1], Gebhard Schertler[1,2], Valérie Panneels[1].

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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 J. 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.

6. Short-Talk Session Abstract

<A01_Structual biology>

Fri. 1st December / 9:00 -9:40am JST / Event Hall

Light energy conversion mechanism of photosensitive membrane proteins revealed by cryo-electron microscopy

Masahiro Fukuda (The University of Tokyo)

In nature, a variety of light-sensitive proteins exist and convert light energy into various types of work. Channelrhodopsins (ChR) are representative photosensitive membrane proteins that can convert light energy into ion transport through cell membranes. Although ChRs are widely used as an optogenetic tool in fields such as neuroscience, there are still many unknown points on the molecular mechanism of ChRs. In this talk, the molecular mechanism of recently discovered unique ChRs will be presented.

Synthesis of photocaged L-tyrosine containing proteins through an Escherichia coli cell-free protein synthesis system for time-resolved structural analysis

Toshiaki Hosaka (RIKEN Center for Biosystems Dynamics Research)

Genetically encoded caged amino acids can be used to control the dynamics of protein activities and cellular localization in response to external cues. In this study, we have established a protein preparation system that introduces photocaged Tyr at arbitrary positions using an Escherichia coli cell-free protein synthesis system. Using this system, we prepared lysozyme and homoisocitrate dehydrogenase with photocaged Tyr, and crystallographic study showed that the site-specifically incorporated photocaged Tyr were degraded to tyrosine by light irradiation on the crystals. Thus, this cell-free protein synthesis of proteins with the photocaged Tyr could facilitate time-resolved structural analysis of proteins, including medically important membrane proteins.

Crystal structure of V2HeR3, a Viral heliorhodopsin transporting proton

Ritsu Mizutori (Nagoya Institute of Technology)

Heliorhodopsins (HeRs), a recently discovered rhodopsin family, are widely present in archaea, bacteria, unicellular eukaryotes, and giant viruses, but their function remained unknown. In 2022, we reported that a viral HeR from Emiliania huxleyi virus 202 (V2HeR3) is a light-activated proton transporter. Comprehensive mutagenesis study using electrophysiological experiment revealed some amino acids together with the retinal Schiff base forming the proton transporting gate. Here, we show the 2.1 Å resolution structure of V2HeR3. Structural and spectroscopic analyses reveal V2HeR3 specific water-mediated hydrogen bond network in both extracellular and cytoplasmic sides. These structural elements are believed to enable proton selective transport functions.

Identifying functional hotspot residues for activation in M2 muscarinic receptor (M2R)

Kota Katayama (Nagoya Institute of Technology)

The M2 muscarinic receptor (M2R) is a prototypical G-protein-coupled receptor (GPCR) that serves as a model system for understanding both ligand recognition and activation of GPCR. Here, we identify the mechanisms governing M2R activation flexibility using vibrational spectroscopy in combination with mutagenesis and organic synthetic chemistry, focusing on the native agonist acetylcholine. From these studies, we found that the exquisite distance between acetylcholine and Asn404, one of the amino acids that constitute the ligand binding site, is important for M2R activation, and that the N404Q mutant exhibits partial activity-like conformational changes. Thus, these results strongly indicate that Asn404 is a hotspot residue in M2R activation.

<A01_Chemical biology>

Fri. 1st December / 10:00 -10:40am JST / Event Hall

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

Takuya Terai (The University of Tokyo)

Fluorescent protein (FP)-based biosensors are powerful tools in biology research, but only a few biosensors have excellent performance. A major limitation for their development is the lack of understanding of the mechanisms by which they operate. To address this issue, we are trying to capture a Molecular Movie of a crystallized FP biosensor that responds to a ligand released by light illumination. We have synthesized photocaged lactate and validated its performance. We have got grown crystals of lactate biosensors in their apo states, though the quality may need further improvement The latest progress will be described in the presentation.

A light-gated K+ channel generated by introduction of a photo-isomerizing unnatural amino acid

Takushi Shimomura (National Institute for Physiological Sciences)

The introduction of genetically encoded unnatural amino acids (UAAs) allows us to confer a variety of functions on target proteins. We introduced phenylalanine-azobenzene (Pab), a photo-isomerizing UAA, into KcsA, a pH-dependent K+ channel. Screening of the Pab position for introduction resulted in a mutant that can switch its activity with high efficiency by ultraviolet and visible light. Protein purification of the Pab mutant showed a quality similar to that of wild-type. Detailed analysis of the KcsA Pab mutant reveals the relationship between pH and light dependence. These results suggest that Pab introduction may be a useful method to confer light dependence to various proteins.

Development of adenosine A2A receptor-selective photo-switchable ligands

Tomohiro Doura (Nagoya University)

Subtype-selective photo-switchable ligands are considered a kind of powerful molecular tools for photopharmacology or optochemogenetics of G-protein coupled receptors (GPCRs). Here we report two kinds of adenosine A2A receptor (A2AR)-selective ligands with photo-switching properties. One A2AR-selective ligand is termed photoAd(vio) which is isomerized to the cis-isomer by ultraviolet (UV) light irradiation. Because the cis-isomer of photoAd(vio) activates A2AR selectively, photoAd(vio) allows to control A2AR in living cells with high spatiotemporal resolution. The other A2AR-selective ligand is named photoAd(blue). The cis-isomer of photoAd(blue) is isomerized to the trans-isomer which activates A2AR selectively by blue light irradiation. The thermally stable trans-isomer of photoAd(blue) is expected to be exploited in the structural analysis of A2AR-selective photo-switchable ligands.

Crystal structure of photoresponsive ligand bound adenosine A2A receptor

Tsuyoshi Araya (Kyoto University)

The rational synthetic expansion of photoresponsive ligands is important for the development of G protein-coupled receptors (GPCRs) photopharmacology. In this study, we synthesized the novel photoresponsive and receptor selective ligand photoNECA(blue) for adenosine A2A receptor and determined the crystal structure of the ligand-bound receptor complex. This complex structure could explain the binding mode and the photoresponsive mechanism of photoNECA(blue). And we mentioned the possibility that the photoresponsive ligand could be applied not only to photopharmacology but also to the dynamic structural analysis of GPCRs from this study.

<A01_Structual biology>

Fri. 1st December / 9:00 -10:40am JST / Room 403

Real-Time Structural Changes during the Light-induced Water Oxidation of Photosystem II Caught by Time-Resolved Crystallography

Michihiro Suga (Okayama University)

Photosystem II catalyzes water oxidation by capturing sunlight energy at the unique pair of Chlorophylls called P680 within a picosecond time scale that initiates a serial reduction/oxidation reaction between two plastoquinone, QA, and QB, a radical active tyrosine residue Yz, a catalytic manganese cluster, and substrate water molecules. This reaction is the so-called Si-state cycle catalyzed by the Mn4CaO5 cluster, which incorporates an extra oxygen O6 at the S3 state to form a possible di-oxygen. The structural changes of the metal cluster and its environment have been examined at the millisecond time range.

An Attempt at Elucidating the Reaction Mechanism of Cytochrome c Oxidase Using Simultaneous Measurement of XES and XRD

Atsuhiro Shimada (Gifu University)

Cytochrome c oxidase (CcO) has four essential redox metal sites crucial for its proton-pumping and O2 reduction reaction. It has been suggested that unidirectional proton-pumping across a membrane utilizes the structural changes of protein moieties driven by the redox changes of metal sites during the O2 reduction reaction. To elucidate the reaction mechanism of CcO, it is necessary to comprehend the correlation between the electron states of metal sites and the protein structure. For the sake of this objective, I endeavored to simultaneously acquire XRD data (atomic coordinates) and XES data (electron states of metal sites) using XFEL.

Photoresponse mechanism of a bifunctional cryptochrome

Junpei Yamamoto (Osaka University)

Photolyase/cryptochrome superfamily (PCSf) proteins are flavoproteins bearing flavin adenine dinucleotide (FAD) as a catalytic/photoreceptive center and involved in various biological functions such as DNA repair, signal transduction, entrainment of circadian rhythm, and so on. An animal-like cryptochrome from Chlamydomonas reinhardtii (CraCRY) is a unique protein responsible for both photolyase and cryptochrome functions. In this study, we intended to unravel dynamic behaviors of light-dependent DNA repair and FAD photoreduction via time-resolved serial femtosecond X-ray crystallography. Intermediate structures in the ns-ms region during the FAD photoreduction of CraCRY revealed molecular mechanism of unfolding of the C-terminal region.

Fast time-resolved structural analysis of light-response in photosynthetic antenna protein Phycocyanin

Yasufumi Umena (Synchrotron Radiation Research Center Division of Synchrotron Radiation)

The cyanobacterial antenna protein, phycocyanin (PC), captures and supplies light energy to the reaction center, photosystem II. PC is composed of alpha and beta subunits of Mw 20000 with three pigment molecules, phycocyanobilin (PCB). In this study, we investigated the light-energy transfer inside the PC in sub-picosecond using pump-probe serial femtosecond crystallography (pp-SFX) with XFEL. The fast transient-absorption spectra using a PC microcrystal provided proper time-points for time-resolved pp-SFX measurement. The pp-SFX after four picoseconds showed structural changes and laser intensity-dependent damages in PCBs at 1.6 Å resolution. We will discuss the results and current issues in this study.

Reaction mechanism of photoactivated adenylate cyclase OaPAC by dynamic structural analysis

Sam-Yong Park (Yokohama City University)

Cyclic-AMP is one of the most important second messengers, regulating many crucial cellular events in both prokaryotes and eukaryotes, and precise spatial and temporal control of cAMP levels by light shows great promise as a simple means of manipulating and studying numerous cell pathways and processes. The photoactivated adenylate cyclase (PAC) from the photosynthetic cyanobacterium Oscillatoria acuminatea (OaPAC) is a small homodimer eminently suitable for this task, requiring only a simple flavin chromophore within a BLUF domain.
The structure of OaPAC has been capturing dynamic structural changes as molecular movies on a wide range of time scales by structural analysis using synchrotrons.

Calcium binding induced structural changes required for the photoprotein Aequorin luminescence

Toru Nakatsu (Wakayama Medical University)

Aequorin is a Ca2+-binding photoprotein that was isolated from the luminous jellyfish Aequorea victoria and emits light by an intramolecular reaction upon binding with Ca2+. Ca2+ binds to three EF-hand motifs of Aequorin, showing a helix-loop-helix structure. Aequorin consists of apoaequorin and 2-peroxycoelenterazine, when Ca2+ binds aequorin, non-covalent bounded 2-peroxycoelenterazine decomposes into coelenteramide and CO2 to produce blue light. In this study, time-resolved X-ray crystallography of Aequorin using SACLA and SPring-8 will reveal in order to elucidate the order of calcium binding to EF-hand motifs and the mechanism of structural change associated calcium binding.

Anaerobic X-ray diffraction data collection using oxygen barrier film for study on nitric oxide reductase

Takehiko Tosha (University of Hyogo)

Bacterial nitric oxide reductase (NOR) is responsible for the elimination of cytotoxic NO. In order to elucidate the mechanism of NO decomposition at the heme/non-heme iron binuclear center of NOR, we designed experimental systems for anaerobic data collection of X-ray diffraction because the NOR-catalyzed reaction initiates the reaction of the reduced form of NOR and NO. To keep the anaerobic condition during the X-ray diffraction experiments, we focus on an oxygen barrier film to avoid the oxygen permeation. Ethylene vinyl alcohol copolymer-based oxygen barrier film showed a potential for anaerobic X-ray diffraction experiments.

A molecular movie of photoswitchable fluorescent protein

Eiichi Mizohata (Osaka University)

The photoswitchable green fluorescent protein, rsGamillus, presents unique properties distinct from existing photo-switchable fluorescent proteins. For example, the isomeric forms of its chromophore in on/off states are trans/cis, which is opposite to that of other photo-switchable fluorescent proteins. In this study, I report the successful capture of a molecular movie of the photo-isomerization reaction for off→on switching of the rsGamillus chromophore. This was achieved using time-resolved serial femtosecond crystallography (tr-SFX) with a nanosecond laser-based pump-probe technique, capturing the events from 10 nanoseconds to the microsecond timescale.

Structure and mechanism of a Diels-Alderase and a 2-oxoglutarate-dependent dioxygenase

Shingo Nagano (Tottori University)

A Diels-Alderase, Fsa2, which is involved in equisetin biosynthesis, produces a decalin

skeleton by stereo-selective 4+2 cyclization reaction. We determined the crystal structure of equisetin-bound Fsa2 and confirmed that the equisetin binding mode was consistent with the substrate binding mode previously predicted by a molecular dynamics simulation.

TauD, which hydroxylates taurine, is a non-heme iron- and 2-oxoglutarate-dependent dioxygenase. Metal-free and taurine-bound TauD microcrystals were mixed with FeSO4 solution to induce the enzyme reaction. The occupancy of the non-heme iron was time-dependently increased, confirming the possibility of non-heme iron reconstruction of crystalline TauD. In addition, the polder omit map with a delay time of 9.0 sec confirmed the electron density extending from the carbon atom to be hydroxylated in substrate taurine toward non-heme iron, suggesting that taurine was hydroxylated in the microcrystals.

< B01_Molecular Movie Platform Design>

Fri. 1st December / 9:00 -9:20am JST / Room 404

Development of an in-vacuum diffractometer for protein micro-crystallography

Hiroaki Matsuura (RIKEN SPring-8 Center)

Microbeams available at synchrotron and FEL facilities have enabled structure determination from tiny protein crystals. However, due to fewer unit cell copies in such tiny crystals, diffraction intensities become significantly weaker. This weak signal complicates the observation of scattering from sample crystals, particularly as background scattering from the air becomes prominent. We have been developing an in-vacuum diffractometer designed for micro-crystallography with crystals smaller than 1μm/side. Test experiments were conducted using cytidine crystals to compare data collection under atmospheric and vacuum conditions. Our findings indicate that data collection under vacuum conditions improves crystallographic statistics such as <I/σI> or R factors.

Low background graphene sample holder for XFEL-based crystallography and imaging

Akihiro Suzuki (Hokkaido University)

In the 2010s, X-ray free-electron laser (XFEL) facilities were constructed around the globe to pioneer new X-ray science by taking advantage of its highly brilliant and short radiation pulses. Single particle imaging of a protein that is difficult to crystallize was one of the driving forces behind the XFEL constructions. However, even after more than ten years in operation, detecting diffraction signals from a single particle is still a severe challenge. To improve the signal-to-noise ratio of diffraction signals, we have been developing a low-noise sample holder using graphene instead of liquid jets for sample delivery. In this presentation, we will present the current status of sample holder development and the latest result of the XFEL experiment.

<C01_Spectroscopy >

Fri. 1st December / 9:20 -10:00am JST / Room 404

Stepwise calcium binding in photoluminescence protein; aequorin, revealed by the time-resolved spectroscopy

Tetsunari Kimura (Kobe University)

The photoluminescence protein; aequorin (AQ) has three Ca2+ binding sites with loop structure and the binding induces the transient emission of blue light. The stepwise Ca2+ binding has been proposed based on the time-resolved serial-femtosecond x-ray crystallography and serial soaking experiments. The comprehensive spectroscopic measurement is critical to evaluate the proposed mechanism. In this study, kinetic measurement of luminescence intensity along the AQ-Ca2+ binding and Ca2+ affinities were investigated by stopped-flow and isothermal titration calorimetry, respectively. Kinetic analysis clarified that the first Ca2+ binding induced the conformational change, which was followed by the second Ca2+ binding with higher affinity, indicating that the conformational change in the loop structure was necessary to accommodate Ca2+ ion, as proposed in structural biological experiments.

Structural features of Lumi intermediate on a primate blue-sensitive visual pigment revealed by FTIR

Yosuke Mizuno (Nagoya Institute of Technology)

Cone pigments are photoreceptors responsible for color vision. Lumi, an early photointermediate, of a primate blue pigment (MB) is deprotonated at the retinal Schiff base, which is an unusual reaction not seen in other visual pigments. Here, we report the structural features of Lumi of MB measured by FTIR spectroscopy. Site-directed mutagenesis study reveals that the Schiff base proton transfers to the E113, a counterion, coupled with local perturbation of α-helices. Hydrogen bond strengths of T118 and C211 together with internal water molecules change upon the formation of Lumi, and thus these hydrogen bond alterations might be key elements enabling early proton transfer reaction.

A Unique Molecular Property of a Sodium-Pumping Rhodopsin in Krokinobacter eikastus

Yuji Furutani (Nagoya Institute of Technology)

Microbial rhodopsins exhibit various functions such as light-driven ion pumps, light sensors, light-gated channels, and light-activated enzymes. In 2013, a sodium-pumping rhodopsin was found from Krokinobacter eikastus (KR2). Interestingly, a substrate sodium ion is not bound near the protonated Schiff base region, which is transiently incorporated into the protein moiety upon formation of the intermediate states. Thus, the molecular mechanism has been extensively studied by using various techniques. In this short talk, I will present our recent finding regarding importance of the covalent bond connecting the retinal chromophore in KR2 (S. Ochiai et al. Biochemistry 2023).

Excited state dynamics in a single protein crystal by using femtosecond transient absorption microscopy

Tetsuro Katayama (Tokushima University)

Energy transfer reactions are important in photosynthetic systems as natural systems and solar cells as artificial systems. In this study, femtosecond transient absorption microscopy was utilized to elucidate the energy transfer reaction behavior between dyes in a single crystal. In this presentation, we will discuss the details of the excited-state relaxation process between dyes from the viewpoint of polarization dependence.

< C01_Computational Chemistry>

Fri. 1st December / 9:00 -10:40am JST / Room 405

Development of non-adiabatic QM/MM molecular dynamics method

Kiyoshi Yagi (RIKEN Cluster for Pioneering Research)

We have developed a non-adiabatic molecular dynamics (MD) method based on QM/MM calculations. QM/MM is a multiscale method that treats the reaction center by quantum chemical (QM) method and the biological environment as a classical force field (MM). The QM calculation of the electronic excited state, which is the computational bottleneck, is highly parallelized to achieve high performance. In addition, QM/MM is combined with a surface hopping algorithm proposed by Zhu and co-workers, which takes into account non-adiabatic transitions between electronic states. The method is applied to photochemical processes of azobenzene in solution and bacteriorhodopsin.

Overview of our research collaborations performed in the research area of "molecular movies"

Mitsuo Shoji (University of Tsukuba)

We have performed collaborative researches under the research area of "molecular

movies". The target molecules and collaborators are (1) C-phycocyanin with Y. Umena (A01), (2) copper amine oxidase with T. Murakawa (A01), (3) heliorhodpsin with K. Katayama and H. Kandori (A01), (4) lysozyme with B. Maity and T. Ueno (B01), (5) 2-oxoglutarate-dependent dioxygenase with S. Nagano (A01) and (6) P450 sesamin with E. Mizohata (A01). We overview all of these research progresses, achievements and remaining issues.

How the mGLU1 receptor dimer be activated: a molecular dynamics study

Duy Phuoc Tran (Tokyo Institute of Technology)

We present in this study our recent progress in understanding the activation mechanism of the mGLU1 receptor. In collaboration with Kiyonaka group, we have figured out the mechanism of the activation of the mutations and ligands. In addition, we reconstructed the full-length structure of the mGLU1 dimer with Gi1 protein by molecular docking and simulation. The data show the differences in interaction interfaces between mGLU1 and the other mGLU receptors in sub-family.

Complementing molecular movie by analyzing free energy landscape, pathways, and flux of conformational change

Akio Kitao (Tokyo Institute of Technology)

We conducted molecular simulation of biomolecular systems to complement molecular movie experimentally determined. Using parallel cascade molecular dynamics (PaCS-MD) simulation and other computational techniques, we investigated conformational change of proteins by analyzing free energy landscape, transition pathways and flux. We report interfacial activation of Candida antarctica Lipase B and molecular mechanisms of structure support and smooth rotation in the bushing/rod complex of bacterial flagella.

Dynamical Analysis for Simulations of Orexin 2 Receptor Using Relaxation Mode Analysis

Ayori Mitsutake (Meiji University)

We have introduced and developed algorithms for protein simulation. For dynamics and stability, we introduced relaxation mode analysis and 3D-RISM theory, respectively. 3D-RISM theory estimates the solvation free energy. We use molecular dynamics simulations and these analyzes to study the inactive and active conformations of the orexin-2 receptor. From simulations of constitutively active mutants, we identified a good indicator for distinguishing between inactive and active conformations of class A GPCRs, the distance between Ile48.CA (3.46) and Tyr364.CA (7.53). From simulations of active and inactive structures in the presence and absence of ligands, we investigated characteristic amino acids that are thought to be important for activation of orexin-2 receptors.

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

Shigehiko Hayashi (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 and a proton exchange through hydrogen-bond network formed by the conformational changes which produces a stable intermediate for chemiluminescence.

Unification of molecular movies and quantum molecular dynamics

Junichi Ono (Waseda University)

Proton transfers associated with structural changes play a vital role in function. Recently, molecular movies of structural changes in biomolecules have been successfully captured using time-resolved SFX at XFEL. However, direct observation of proton transfers has not been accomplished because of the limitation of spatio-temporal resolution. Here, large-scale quantum molecular dynamics (QMD) simulations were performed on the basis of the molecular movies with focusing on the chemical reactions in rhodopsins, where all the atoms were treated quantum-mechanically. In this presentation, we discuss our results demonstrating that our QMD simulations compensate for the molecular movies to reveal the microscopic mechanisms of chemical reactions.

Resi-DEM: A Comprehensive Tool for Automated Density Extraction, Identification, and Analysis of Difference Densities in Isomorphous Maps, with Occupancy-Estimation for Conformer Fitting

Sriram Srinivasa Raghavan (RIKEN Center for Computational Science)

We will introduce Resi-DEM, an new tool designed to automate the identification of difference densities in isomorphous difference density maps, further associating them with relevant residues through a clustering approach. Utilizing the clustered difference density features, we estimated the occupancy of the triggered state population via the structure factor extrapolation technique. This clustering technique was employed to organize difference densities, enabling us to represent them as networks, facilitating the tracking of density changes over various time periods. Moreover, we employed molecular dynamics trajectory sampling to determine the most fitting conformation corresponding to the triggered data set. The results were validated using metrics such as the Real Space Correlation Coefficient (RSCC) and Real Space Difference Density Z score (RSZD).

Analysis on the dynamics of bacteriorhodopsin using MD simulation

Keiko Shinoda (The Institute of Statistical Mathematics)

In the "molecular movies", we have developed force fields for lipid molecules in the archaeal membrane and constructed an archaeal model membrane. Using this, we have carried out molecular dynamics (MD) simulations of bacteriorhodopsin. In this presentation, in addition to the previously reported results on the resting state and at 16ns after excitation of bacteriorhodopsin, I will report on the results at 760 ns and 2μs after excitation and present a comprehensive analysis on the dynamics of bacteriorhodopsin.