OptoBioTechnology Research Center
Nagoya Institute of Technology

International Symposium on "Optobiotechnology"

5 (Tue.) – 6 (Wed.) February 2019

at the conference hall (4th building), Nagoya Institute of Technology

OB+RC OptoBioTechnology Research Center

Nagoya Institute of Technology

Address | OptoBioTechnology Research Center, Nagoya Institute of Technology
Showa-ku, Nagoya 466-8555, Japan
NIT URL | http://www.nitech.ac.jp

OptoBioTechnology Research Center, Nagoya Institute of Technology

International Symposium on “Optobiotechnology”

February 5 - 6, 2019

Nagoya Institute of Technology (Nagoya, Japan)

Plenary Lecturers

Oded Béjà (Israel Institute of Technology, Israel)

Peter Hegemann (Humboldt University, Germany)

Ryoichiro Kageyama (Kyoto University, Japan)

Osamu Nureki (The University of Tokyo, Japan)

Krzysztof Palczewski (University of California, Irvine, USA)

Yoshinori Shichida (Ritsumeikan University, Japan)

Invited Speakers

Takehisa Dewa (Nagoya Institute of Technology, Japan)

Akimasa Hirata (Nagoya Institute of Technology, Japan)

Kota Katayama (Nagoya Institute of Technology, Japan)

Masae Konno (Nagoya Institute of Technology, Japan)

Shuji Ogata (Nagoya Institute of Technology, Japan)

Tetsuo Soga (Nagoya Institute of Technology, Japan)

Satoshi P. Tsunoda (Nagoya Institute of Technology, Japan)

Daichi Yamada (Nagoya Institute of Technology, Japan)

Tatsuyuki Yoshii (Nagoya Institute of Technology, Japan)

Organizer

Hideki Kandori (Nagoya Institute of Technology, Japan)

Welcome address

Experimental life science combined with optotechnology

In August 2013, Nagoya Institute of Technology (NIT) established a new center named OptoBioTechnology Research Center. The center sets the goal to create a new field of industry, based on the optical manipulation technologies in bioscience, as represented by optogenetics. One of the most fast-growing techniques is now indispensable in the brain science, in which the optogenetic control of neural activity elucidates the brain function at the molecular level, as well as in the neural network systems. We elucidate the molecular mechanisms of energy conversion between light and biomaterials, which is yet fully understood, by using advanced analytical tools. The most promising seeds shall be identified through the basic research, and we will grow them to contribute the society to improve the quality of life.

On behalf of OptoBioTechnology Research Center of NIT, I sincerely welcome all of you to this International Symposium, which is the 5th Anniversary Memorial Symposium of the center. I am pleased that outstanding researchers in the "Light & Life" field agreed to come and give Plenary Lectures, which leads to the symposium success. You will also see the research activities on "Light & Life" in NIT from invited talks and poster presentations.

I hope all the participants enjoy this symposium and get new inspirations.

Hideki Kandori
Center Director,
OptoBioTechnology Research Center,
Nagoya Institute of Technology

Member List, OptoBioTechnology Research Center

Optoscience Department

Yuji Furutani (Department of Life Science and Applied Chemistry, NIT)

Shuji Ogata (Department of Physical Science and Engineering, NIT)

Shingo Ono (Department of Physical Science and Engineering, NIT)

Norihiro Shida (Department of Life Science and Applied Chemistry, NIT)

Optoenergy Technology Department

Tomohiko Inomata (Department of Life Science and Applied Chemistry, NIT)

Hideki Kandori (Department of Life Science and Applied Chemistry, NIT)

Katsuhiko Ono (Department of Life Science and Applied Chemistry, NIT)

Tetsuo Soga (Department of Electrical and Mechanical Engineering, NIT)

Optomedical Technology Department

Takehisa Dewa (Department of Life Science and Applied Chemistry, NIT)

Akimasa Hirata (Department of Electrical and Mechanical Engineering, NIT)

Kota Katayama (Department of Life Science and Applied Chemistry, NIT)

Akiko Obata (Department of Life Science and Applied Chemistry, NIT)

Selected Publications and Awards, OptoBioTechnology Research Center

2014

O. P. Ernst, D. T. Lodowski, M. Elstner, P. Hegemann, L. S. Brown, H. Kandori,
"Microbial and animal rhodopsins: Structures, functions, and molecular mechanisms"
Chem. Rev. 114, 126-163 (2014).

Comprehensive review article on rhodopsins; 395 citations (Dec. 27, 2018).

2015

H. E. Kato, K. Inoue, R. Abe-Yoshizumi, Y. Kato, H. Ono, M. Konno, S. Hososhima, T. Ishizuka, M. R. Hoque, H. Kunitomo, J. Ito, S. Yoshizawa, K. Yamashita, M. Takemoto, T. Nishizawa, R. Taniguchi, K. Kogure, A. D. Maturana, Y. Iino, H. Yawo, R. Ishitani, H. Kandori, O. Nureki,
"Structural basis for Na^+ transport mechanism by a light-driven Na^+ pump"
Nature 521, 48-53 (2015).

Structure/function study of *Krokinobacter eikastus* rhodopsin 2 (KR2).

Hideki Kandori, Molecular Science Society Award（分子科学会賞）

2016

M. Konno, Y. Kato, H. E. Kato, K. Inoue, O. Nureki, H. Kandori,
"Mutant of a light-driven sodium ion pump can transport cesium ions"
J. Phys. Chem. Lett. 7, 51-55 (2016).

Creation of Cs^+ pump by light, which was reported by many newspapers.

K. Inoue, S. Ito, Y. Kato, Y. Nomura, M. Shibata, T. Uchihashi, S. P. Tsunoda, H. Kandori,
"Natural light-driven inward proton pump"
Nat. Commun. 7, 13415 (2016).

Discovery and characterization of a light-driven inwardly-directed proton pump.

2017

S. Gulati, B. Jastrzebska, S. Banerjee, Á. L. Placeres, P. Miszta, S. Gao, K. Gunderson, G. P. Tochtrop, S. Filipek, K. Katayama, P. D. Kiser, M. Mogi, P. L. Stewart, K. Palczewski,
"Photocyclic behavior of rhodopsin induced by an atypical isomerization mechanism"
Proc. Natl. Acad. Sci. USA 114, E2608-E2615 (2017).

Dr. Katayama’s study in USA, whose manuscript was published after his return to Japan.

K. Katayama, Y. Nonaka, K. Tsutsui, H. Imai, H. Kandori,
"Spectral tuning mechanism of primate blue-sensitive visual pigment elucidated by FTIR spectroscopy"
Sci. Rep. 7, 4904-4914 (2017).

The first structural study of primate blue-sensitive protein, being reported by many newspapers.

Akimasa Hirata, JSPS Award & Japan Academy Medal（日本学術振興会賞＆日本学士院学術奨励賞）

2018

A. Pushkarev, K. Inoue, S. Larom, J. Flores-Uribe, M. Singh, M. Konno, S. Tomida, S. Ito, R. Nakamura, S. P. Tsunoda, A. Philosof, I. Sharon, N. Yutin, E. V. Koonin, H. Kandori, O. Béjà,
"A distinct abundant group of microbial rhodopsins discovered using functional metagenomics"
Nature 558, 595-599 (2018).

Discovery of heliorhodopsin through the collaboration with an Israel group.

Y. S. Kim, H. E. Kato, K. Yamashita, S. Ito, K. Inoue, C. Ramakrishnan, L. E. Fenno, K. E. Evans, J. M. Paggi, R. O. Dror, H. Kandori, B. K. Kobilka, K. Deisseroth,
"Crystal structure of the natural anion-conducting channelrhodopsin GtACR1"
Nature 561, 343-348 (2018).

H. E. Kato, Y. S. Kim, J. M. Paggi, K. E. Evans, W. E. Allen, C. Richardson, K. Inoue, S. Ito, C. Ramakrishnan, L. E. Fenno, K. Yamashita, D. Hilger, S. Y. Lee, A. Berndt, K. Shen, H. Kandori, R. O. Dror, B. K. Kobilka, K. Deisseroth,
"Structural mechanisms of selectivity and gating in anion channelrhodopsins"
Nature 561, 349-354 (2018).

Spectroscopic contribution to the structural analysis by Stanford group.

H. Kandori, K. Inoue, S. P. Tsunoda,
"Light-driven sodium-pumping rhodopsin: a new concept of active transport"
Chem. Rev. 118, 10646-10658(2018).

This protein can actively transport Na^+ without initial binding, which is very unusual.

2019

Hideki Kandori, Chemical Society of Japan (CSJ) Award（日本化学会賞）

Program

Schedule and Oral Presentations

February 5 (Tue)

8:50 Opening Remark: Hiroyuki Ukai (President; Nagoya Institute of Technology, Japan)

Session 1 Light & Life: Animal Rhodopsins

Chair: Yuji Furutani (Nagoya Institute of Technology, Japan)

9:00 Plenary Lecture: Krzysztof Palczewski (University of California, Irvine, USA)
"Chemistry that converts light into vision"

9:45 Plenary Lecture: Yoshinori Shichida (Ritsumeikan University, Japan)
"Opsin evolution: Exploring the effects of mutations"

10:30 Invited Talk: Kota Katayama (Nagoya Institute of Technology, Japan)
"Structural basis for elucidating spectral tuning mechanism of cone pigments"

Session 2 Light & Life: Microbial Rhodopsins

Chair: Shinya Tsukiji (Nagoya Institute of Technology, Japan)

11:00 Plenary Lecture: Oded Béjà (Israel Institute of Technology, Israel)
"Type-1 & 3 rhodopsins: The search for new microbial rhodopsins using metagenomics"

11:45 Plenary Lecture: Osamu Nureki (The University of Tokyo, Japan)
"Light-gated opening mechanism of channelrhodopsin and structure-guided development of optogenetics tools"

12:30 Invited Talk: Masae Konno (Nagoya Institute of Technology, Japan)
"Molecular properties of new type microbial rhodopsins"

12:50-13:50 Lunch

Session 3 Light & Life: Optogenetics

Chair: Masahiko Hibi (Nagoya University, Japan)

13:50 Plenary Lecture: Peter Hegemann (Humboldt University, Germany)
"Two component optogenetics"

14:35 Plenary Lecture: Ryoichiro Kageyama (Kyoto University, japan)
"Oscillatory control of somitogenesis and neurogenesis: imaging and control of gene expression by light technology"

15:20 Invited Talk: Satoshi P. Tsunoda (Nagoya Institute of Technology, Japan)
"Enzyme rhodopsins, potential optogenetics tools for modulating intracellular cyclic-nucleotide levels"

15:40 Invited Talk: Daichi Yamada (Nagoya Institute of Technology, Japan)
"FTIR study of photoactivated cyclase"

16:00 Invited Talk: Tatsuyuki Yoshii (Nagoya Institute of Technology, Japan)
"Optochemical control of protein localization in living cells using synthetic organic molecules"

Photograph

16:20-18:30 Poster Session

16:45-17:15 Odd numbers

17:30-18:00 Even numbers

19:00 Banquet

KOUYOUEN (Sapporo Nagoya Brewery) 浩養園 052-741-0211

http://www.kouyouen.jp/index.html

February 6 (Wed)

Session 4 General Light & Life

Chair: Tomohiko Inomata (Nagoya Institute of Technology, Japan)

10:00 Invited Talk: Shuji Ogata (Nagoya Institute of Technology, Japan)
"Exotic Behavior of Water Around the Anti-Freeze Protein: Large-Scale Molecular Dynamics Simulations"

10:25 Invited Talk: Tetsuo Soga (Nagoya Institute of Technology, Japan)
"Effect of third component in bulk-heterojunction organic solar cell"

10:50 Invited Talk: Takehisa Dewa (Nagoya Institute of Technology, Japan)
"Functional Extension and Linkage of Photosynthetic Biohybrid Light–Harvesting/Reaction Center Complexes in Artificial Systems"

11:15 Invited Talk: Akimasa Hirata (Nagoya Institute of Technology, Japan)
"Multiscale modeling of electrostimulation"

11:40 Closing Remark: Hideki Kandori (Center Director; Nagoya Institute of Technology, Japan)

Poster Presentations

P01 Spectroscopic analysis of homo-oligomerization of AtCRY2
Kazuya Agata, Daichi Yamada, Hideki Kandori

P02 Characteristic of Dye Sensitized Solar Cell using PEDOT:PSS Counter Electrode on the Transparent Conductive Oxide
Masaya Ando, Yurie Murata, Shinya Kato, Naoki Kishi, Tetsuo Soga

P03 Molecular Dynamics Simulation of Water around Amino Acids of the Anti-freeze protein
Hayato Demura, Shuji Ogata, and Yasuhiro Kajima

P04 Infrared spectroscopy on membrane proteins for studying their molecular mechanisms
Yuji Furutani

P05 Characterization of high light-sensitive cation channels from *Guillardia theta*
Shoko Hososhima, Hideki Kandori, Satoshi Tsunoda

P06 Artificial Siderophore Fe Complexes-modified Substrates: Application to Microbial Sensors and Reactors
Tomohiko INOMATA, Suguru ENDO, Hiroki IDO, Tomohiro OZAWA, and Hideki MASUDA

P07 Three-dimensional mapping of Eu^{2+} ions and Eu^{3+}ions doped CaF_2 single crystal by measurement of multi-photon luminescence
Hiroaki Ito, Shusaku Terakawa, Toru Asaka, Akihiro Yamaji, Shunsuke Kurosawa, Akira Yoshikawa, Shingo Ono

P08 Metal binding to the voltage-gated proton channel studied by ATR-FTIR spectroscopy combined with MD/QC calculations
Masayo Iwaki, Kohei Takeshita , Hiroko X. Kondo, Kengo Kinoshita, Yasushi Okamura, Yu Takano, Atsushi Nakagawa and Hideki Kandori

P09 Fast Computation of Temperature and Water Loss in Human Models for Simultaneous Exposure to Ambient Heat and Solar Radiation
T. Kamiya, K. Hasegawa, and A. Hirata

P10 Spectroscopic study of microbial rhodopsin with deprotonated retinal Schiff base at neutral pH
Chihiro Kataoka, Keiichi Inoue, Kota Katayama, Oded Béjà, Hideki Kandori

P11 Energy Transfer of LH2-Fluorophore Conjugates Using a Mutant of Light Harvesting

Complex (LH2)
Daiji KATO, Akari GOTO, Masaharu KONDO, Yusuke YONEDA, Hiroshi MIYASAKA, Yutaka NAGASAWA, Takehisa DEWA

P12 Development of Vacuum Ultraviolet Light Detector Using Electric Property of CaF_2-metal Interface
Seiya Kato, Kentaro Suzuki, Jun Otani, Masahiko Kase, Shingo Ono

P13 Reconstitution of zinc protoporphyrin IX with transmembrane cytochrome b and its photochemical functions
Hiroki Kojima, Yoko Kondo, Masaharu Kondo, Masaki Ihara, Takehisa Dewa

P14 Synthesis and Properties of N-Boc-Pyrrole Derivatives as p-Type Semiconductors
Takuya Matsuoka, Yuya Makino, Yuta Imaeda, and Katsuhiko Ono

P15 Molecular characterization of heliorhodopsins from marine giant virus
Ritsu Mizutori, Masae Konno, Keiichi Inoue, Oded Béjà, Hideki Kandori

P16 Synthesis and Absorption Properties of Bis(dioxaborin) Derivatives
Yoshitaka Mori, Fumiyasu Ishikawa, and Katsuhiko Ono

P17 Study on the regulatory mechanism of absorption-wavelength of rhodopsins and construction of red-shift mutants for optogenetics
Yuta Nakajima, Keiichi Inoue, Hideki Kandori

P18 Protein Adsorption Behaviors on Calcium-salt Particles
Chiaki Otsuka, Hirotaka Maeda, Akiko Obata, Toshihiro Kasuga

P19 Electrospun fibermats with protein-loading ability for tissue engineering
Y. Ozeki, M. Iguchi, T. Mizuno, A. Obata, T. Kasuga.

P20 Molar Concentration Precursors Effect on the Optical and Photovoltaic Properties of BiOI Films Prepared by SILAR
Anissa A. Putri S. Kato, N. Kishi and T. Soga

P21 The correlation between the hydrogen bonding network and FAD redox states in photolyase/cryptochrome family
Yui Sakai, Daichi Yamada, Hideki Kandori

P22 Synthesis and Absorption Properties of (1,3-Diketonato)boron Difluoride Derivatives with π-Extended Triphenylamine
Yuki Sakura, Keisuke Banno, and Katsuhiko Ono

P23 FTIR study of primate green-sensitive cone visual pigment at >100 K

Takuma Sasaki, Kota Katayama, Rei Abe-Yoshizumi, Hiroo Imaiand Hideki Kandori

P24 Theoretical study of multiple proton transfer reaction in DNA base pair
Tsugutake Kato, Norihiro Shida

P25 Study of cation channelrhodopsin Gt_CCR4 for optogenetics
Shunta Shigemura, Shoko Hososhima, Hideki Kandori, Satoshi Tsunoda

P26 Identification of Novel Family in Microbial Rhodopsin: Heliorhodopsin (HeR) and Mutational Analysis of Heliorhodopsin 48C12
Manish Singh, Keiichi Inoue, Alina Pushkarev, Akihiro Otomo, Yasuhisa Mizutani, Oded Béjà, and Hideki Kandori

P27 Evaluation of cell penetration properties of the series of cationic PG-surfactants
Natsumi Sumito

P28 3D flexible processing of organic materials by femtosecond laser
Yoshiki Tanaka, Fumihiro Itoigawa, Hironori Suzuki, Hiroshi Nakao, Shingo Ono

P29 Unique channel kinetics of anion channelrhodopsin GtACR1
Rintaro Tashiro, Hideki Kandori, Satoshi Tsunoda

P30 The evaluation of Ce^{3+} ion distribution in Ce^{3+}:$LiCaAlF_6$ single crystal by multiphoton luminescence and the effect on lasing properties
Shusaku Terakawa, Hiroaki Ito, Marilou Cadatal-Raduban, Takafumi Hirata, Shingo Ono

P31 FTIR analysis of hydrogen bonding network in the extracellular side of a light-driven sodium pump KR2
Sahoko Tomida, Shota Ito, Keiichi Inoue, Hideki Kandori

P32 Photoreaction Mechanism of Enzymatic Rhodopsin Rh-PDE revealed by Infrared Spectroscopy
Masahito Watari, Tatsuya Ikuta, Daichi yamada, Wataru Shihoya, Kazuho Yoshida, Satoshi Tsunoda, Osamu Nureki, Hideki Kandori

P33 Gaining function of microbial rhodopsin without retinal-binding lysine
Yumeka Yamauchi, Masae Konno, Daichi Yamada, Kei Yura, Keiichi Inoue, Oded Béjà and Hideki Kandori

P34 Terahertz Antireflective Tapered Structures Fabricated by Femtosecond Laser Processing
Xi Yu, Seiya Kato, Michiharu Onta, Bae Jongsuck, Shingo Ono

P35 Boron β-Ketoiminate Dye Sensitizers Containing Linear D-π-A Structures
Fumina Yumioka and Katsuhiko Ono

P36 Silver-doped bioactive glasses with cotton-wool-structure for wound dressing
T. Zenji, E. Norris, G. Poologasundarampillai, J R. Jones, A. Obata, T. Kasuga

Oral Presentation

Chemistry that Converts Light into Vision

Krzysztof Palczewski
Center for Translational Vision Research; Gavin Herbert Eye Institute
Department of Ophthalmology, University of California, Irvine, CA 92697-4375

The retina converts light into an electrical signal through a series of biochemical steps collectively referred to as phototransduction. This signal is eventually relayed to the visual cortex of the brain, where visual perception occurs. Photoreceptor cells are able to respond to light throughout our lives because they have the ability to regenerate proteins as well as a light-sensitive chromophore. The long-term objective of our research is to elucidate the molecular reactions involved in phototransduction, including those directly involved in the regenerative capability of photoreceptor cells. Phototransduction serves as a prototype for a multitude of G protein-mediated signal transduction events initiated by activation of G protein-coupled receptors (GPCRs) and thus understanding of this process is broadly applicable to other signal transduction cascades. Although outnumbered more than 20:1 by rod photoreceptors, cone cells in the human retina mediate daylight vision and are critical for visual acuity and color discrimination. Originating in the early 1900s, past research has begun to provide insights into cone ultrastructure but has yet to afford an overall perspective of cone cell organization. Mutations in the rod and cone genes encoding are among the main causes of blinding diseases in humans. We ultimate goal to advance our understanding of the molecular basis of vision and to develop strategies to stop progression of human retinal diseases using animal models. Pharmacologic interventions to save vision are now within reach due to a significantly improved understanding of these chemical transformations.

Opsin evolution: Exploring the effects of mutations

Yoshinori Shichida
Research Organization for Science and Technology,
Ritsumeikan University, Kusatsu, Shiga 525-8577, JAPAN

Vison plays a very important role in our daily life and a considerable part of our brain is utilized for visual information processing. Animals evolved from simple forms, and our visual function is also the product of countless iterations resulting in a highly complex system. Visual function probably first emerged from an ancestral G protein-coupled receptor which used a molecule (retinal) that can absorb light as a ligand. The original agonist was probably all-*trans*-retinal which can undergo an isomerization reaction by light and would change the receptor into a low activity state. Then the receptor evolved so that the isomerized retinal molecule can function as an inverse agonist. Finally, the receptor may have evolved to preferentially bind to the inverse agonist. Now a days, with the excellence of light as an information medium, the opsin receptor has diversified tremendously, supporting light receiving functions of most animals.

Unlike the eyes of insects and molluscs (squid and octopus), vertebrate eyes are thought to be organs that originally only perceived light and darkness, such as the circadian rhythm, rather than organs that mediated vision. They evolved into elaborative visual organs during the transition from protochordates to vertebrates, and further evolved into the eyes of primates including humans through the nocturnal evolutionary period of mammals. What kind of changes happened to opsins during these processes, and what kind of visual functions were induced by the changes in opsins?

Evolution and diversification of opsin can be thought as mutational experiments conducted by Nature. Using recent genetic engineering techniques, it is possible to reproduce opsin changes in the laboratory and to estimate how the opsin's molecular properties changed during evolution. Additionally, it may be possible to create opsins with molecular properties consistent with our specific purpose based on opsin evolution. In this talk, I will outline the mutational experiments we are doing from the viewpoint of opsin evolution and review the evolution toward human eyes.

Structural basis for elucidating spectral tuning mechanism of cone pigments

Kota Katayama
Department of Life Science and Applied Chemistry, Nagoya Institute of Technology
OptoBio Technology Research Center, Nagoya Institute of Technology

Vitamin A is adequately distributed within the body to maintain the biological function of retinoids in the peropheral tissues and the production of visual chromophore, 11-*cis*-retinal, in the eye. One of the mysteries in our vision is that humans recognize color by use of a single chromophore molecule (11-*cis*-retinal), meaning that the chromophore is identical even between blue-absorbing and red-absorbing sensors. Thus, 11-*cis*-retinal is versatile photo-sensitive nano-machine in the eye. Humans have two different types of retinal containing light-sensitive proteins expressed in the retina, rhodopsin (Rh) achieving twilight vision and three cone pigments, which mediate color vision. Each different chromophore-protein interaction allows preferential absorption of selected range of wavelengths. While the structural basis for photoreaction and signal transduction of Rh has been well understood by determination of its atomic revel structure, structural studies of cone pigments lag far behind those of Rh, mainly because of difficulty in sample preparation and lack of suitable methods in structural analysis.

We thus attempted to express monkey cone pigments in HEK293 cell lines for structural analysis using light-induced difference Fourier-transform infrared (FTIR) spectroscopy at 77K. The first structural information successfully elicited from the highly accurate spectra for each cone pigment showed that the retinal chromophore is structurally similar between Rh and cone pigments, but hydrogen-bonding network around the retinal chromophore is entirely different between them. In addition, some spectral differences are observed between cone pigments, including protein-bound water molecules. These differences could be interpreted to play a role in spectral tuning.

Structural determination of cone pigments is also needed for a precise understanding of spectral tuning. The principle obstacle to solving the structures is their innate instability in detergent micelles. Here, we demonstrate successful optimization for the expression by insect cells (Sf9), purification, and stabilization with thermally stable site-directed mutation of primate cone pigments for further structural determinations.

Type-1 & 3 rhodopsins: The search for new microbial rhodopsins using metagenomics

Oded Béjà[1], Johannes Oppermann[2], Jonas Wietek[2], Peter Hegemann[2], Keiichi Inoue[3], Hideki Kandori[3,4]

[1]Faculty of Biology, Technion – Israel Institute of Technology, Haifa, Israel; [2]Institute for Biology, Experimental Biophysics, Humboldt-Universität zu Berlin, Berlin, Germany; [3]Department of Life Science and Applied Chemistry, Nagoya Institute of Technology, Nagoya, Japan; [4]OptoBioTechnology Research Center, Nagoya Institute of Technology, Nagoya, Japan

Many organisms capture or sense sunlight using rhodopsin pigments. These rhodopsins are currently divided to two distinct protein families: type-1 (microbial rhodopsins) and type-2 (animal rhodopsins). Type-1 and type-2 rhodopsins show little or no sequence similarity to each other, as a consequence of extensive divergence from a common ancestor or convergent evolution of similar structures. Searching metagenomes from the *Tara* Oceans expedition, new anion channelrhodopsins (type-1) were detected that form a distinct family compared to the 3 known cation and anion channelrhodopsin families. The novel rhodopsins show an unprecedented desensitization of the initial peak current to almost zero activity in continuous light. In addition, and this time using functional metagenomics, a previously unknown diverse family, heliorhodopsins (type-3), which are distantly related to type-1 rhodopsins was discovered. The orientation of heliorhodopsins in the membrane is opposite to that of type-1 or type-2 rhodopsins, with the N-terminus facing the cell cytoplasm. In addition, heliorhodopsins show photocycles longer than 1 second, suggestive of light sensory activity. In my lecture I will discuss the potential of metagenomics for future discoveries of new rhodopsin activities.

Molecular mechanism of channelrhodopsin and structure-guided development of useful optogenetics tools

Osamu Nureki

Department of Biological Sciences, Graduate School of Science, The University of Tokyo, 2-11-16 Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan

Abstract

Channelrhodopsins (ChRs) are light-activated cation channels that mediate cation permeation across the cell membrane upon light perception. We first solved the high-resolution crystal structure of ChR (C1C2), and based on the structure, many ChR variants are made and utilized in optogenetics. We designed by QM/MM simulation and developed blue-shifted variant. Among these variants, red-shifted (650 nm) channelrhodopsins are particular important, because the longer wavelength light allows penetration into deeper tissues. However, the molecular mechanism of the red-shifted absorption has not been elucidated so far. Here we report the crystal structure of the most red-shifted channelrhodopsin, Chrimson, at 2.6 Å resolution. The structure revealed unique molecular architectures to achieve its highly red-shifted absorbance; hydrophobicity around the retinal Schiff's base and the biased distribution of the polar residues and the rigidity in the retinal binding pocket. The structural comparison revealed that Chrimson has several structural features that resemble the light-activated proton pump, bacteriorhodopsin, while retaining the similarity to other channelrhodopsins around the ion channel pore, indicating that Crimson is a primitive variant during molecular evolution from the prokaryotic ancestors. Based on these mechanistic insights, we engineered ChrimsonSA, a mutant with an about 20 nm red-shifted maximum activation wavelength and accelerated closing kinetics. When expressed in hippocampal neurons, ChrimsonSA allowed selective action potential generation with red light, and thus it is ideally suited for dual color optogenetic applications. To uncover the mechanism how ChR opens the channel upon perception of blue light, we performed time-resolved crystallography using XFEL in SACLA. I will talk about the light activation mechanism of ChR.

References

1. "Crystal structure of the red light-activated channelrhodopsin Chrimson" K. Oda, J. Vierock, S. Oishi, S. Rodriguez-Rozada, R. Taniguchi, K. Yamashita, J. S. Wiegert, T. Nishizawa, P. Hegemann and O. Nureki.
   Nat. Commun. *in press*.

2. “Atomistic design of microbial opsin-based blue-shifted optogenetics tools”H. E. Kato, M. Kamiya, S. Sugo, J. Ito, R. Taniguchi, A. Orito, K. Hirata, A. Inutsuka, A. Yamanaka, A. D. Maturana, R. Ishitani, Y. Sudo, S. Hayashi and O. Nureki.
   Nat. Commun. **6**, 7177 (2015). doi: 10.1038/ncomms8177.
3. H. E. Kato, K. Inoue, R. Abe-Yoshizumi, Y. Kato, H. Ono, M. Konno, T. Ishizuka, M. R. Hoque, S. Hososhima, H. Kunitomo, J. Ito, S. Yoshizawa, K. Yamashita, M. Takemoto, T. Nishizawa, R. Taniguchi, K. Kogure, A. D. Maturana, Y. Iino, H. Yawo, R. Ishitani, H. Kandori and O. Nureki “Structural basis for Na^+ transport mechanism by a light-driven Na^+ pump”
   Nature 521*, 48-53 (2015) DOI 10.1038/nature14322.*
4. “H. E. Kato, F. Zhang, O. Yizhar, C. Ramakrishnan, T. Nishizawa, K. Hirata, J. Ito, Y. Aita, T. Tsukazaki, S. Hayashi, P. Hegemann, A. D. Maturana, R. Ishitani, K. Deisseroth and O. Nureki “Crystal structure of the channelrhodopsin light-gated cation channel”
   Nature **482**, 369-374 (2012).

Molecular properties of new type microbial rhodopsins

Masae Konno
([1]Grad. Sch. Eng., NIT, [2]OBTRC, NIT)

Microbial rhodopsin is the photoreceptive membrane protein which binds an all-*trans* retinal as a chromophore to a conserved Lys residue. Microbial rhodopsins show various functions upon retinal photoisomerization, such as light-driven ion transporter, light sensor and light-activated enzyme. Here, our recent studies on new types of rhodopsins will be presented.

Heliorhodopsins (HeRs) were found by functional metagenomic analysis[1]. Their amino-acid sequences are far different from typical microbial rhodopsins. We investigated molecular properties of 48C12, the first-discovered HeR, by biophysical methods. While 48C12 showed no ion transport activity, a long photocycle with multiple-proton transfer processes was observed suggesting that 48C12 works as a light-sensor. The laser flash photolysis with a pH-indicator and FTIR spectroscopy provided more insights about the proton transfer process related to M-state.

Schizorhodopsins (SzRs) were discovered from Asgardaeota archaea which is the closest extant relative of eukaryotes to date and metagenomic scaffolds from putative bacteria. Multiple sequence alignments showed that SzRs is present at the phylogenetically intermediate position between type-1 rhodopsins and HeRs. Upon light-illumination SzRs showed a strong inward proton-pumping activity. To reveal the mechanism of inward proton-transport, we conducted site-directed mutational analysis of one of SzRs, AM_5S_00009. Some of the mutants for the residues expected to be located on the proton transport pathway abolished proton transport activities. Based on these results, we will discuss the mechanism of active inward proton-transport.

Although the conserved Lys residue is considered to be essential for the rhodopsin function, many rhodopsin-like proteins without conserved Lys residue (Rh-noK) are present in nature. The presence of Rh-noK suggests that it has some physiological role. Recently, we succeed in a functional recovery of Rh-noK using site-directed mutagenesis. *E. coli* cells expressing SAMEA2621536_1986778_5.1 (SAMEA5.1) Rh-noK exhibited no color, because of no retinal binding. When retinal-binding Lys was introduced to the corresponding position of SAMEA5.1, the cells showed a visible color. Additional mutation of the Schiff base counterion site conferred a proton pumping activity. Success of gaining function, the visible color and proton pumping activity, suggests that Rh-noK conserves a characteristic structure of microbial rhodopsins.

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Two component optogenetics

Peter Hegemann, Ulrike Scheib, Matthias Broser, Yinth Andrea Bernal Sierra
Humboldt-Universität zu Berlin, Germany

The recently discovered photoreceptor family of enzyme rhodopsins comprises a number of Rhodopsin-cyclases (RhGC/ACs) and Rhodopsin-phosphodiesterases (RhPDEs). These are rhodopsins that are either directly connected to an enzyme as realized in fungal class of Blasticladiomycetes (Avelar et al. 2014 Curr.Biol.) or the coupling is indirect via a His-kinase and a Response Regulator as in case of the Histidine Kinase Rhodopsins of Chlorophyte algae (Luck et al. 2012).

We characterized two recombinant Rhodopsin-cyclases from *Blastocladialla emersonii* (BeRhGC) (Scheib et al. 2015 Sci Signal.) and *Catenaria anguillulae* (CaRhGC) (Scheib et al. 2018 Nat. Comm.) and have shown that both enzymes are completely inactive in the dark and are active upon illumination. The enzymatically active state is formed within 10 to 40 ms after a flash and decays within 100 to 500 ms depending on the species. The cyclases are highly selective for GTP whereas ATP is acting as a competitive inhibitor. Coexpression of RhGC with a cGMP-channel from olfactory neurons and other sources in Xenopus oocytes , ND cells, HEK cells or neurons generating a large photocurrent upon illumination and conferring a high light sensitivity to the cell of interest. We are currently working on a cGMP-sensitive potassium channel in order to use our Two-Component Approach for rhodopsin-based hyperpolarization in the neurosciences and cell biology as we recently exemplified by combining the flavin-based photoactivated cyclase bPAC with the small cAMP-activated K-channel Sthk (Bernal-Sierra et al. 2018 Nat. Comm).

In parallel we work on Chlamydomonas Histidine kinase rhodopsins (HKRs) from *Chlamydomonas reinhardtii* (Luck and Hegemann 2017 J.Plant Phys.; Luck et al. 2012 JBC) and *Ostreococcus tauri* (Luck & Hegemann unpublished). Both are synthesized in the cell as blue-green sensitive rhodopsins but are converted upon illumination into a UVA-sensitive species (Rh-UV) with maximal absorption near 380 nm. The thermal stability of the Rh-UV is very high but varies between many minutes to hours but it can be photochemically back-converted upon illumination with UVA light. We propose that these HKRs developed in algae for measuring the ratio between UVA and Blue light and to adapt cellular processes to this ratio accordingly. We are currently deleting the 8 HKRs in *C.reinhardtii* by using a Cas9-based reverse genetics (Greiner et al. 2017 Plant Cell) to study the cellular function by comparing development and gene expression in wild type and different deletion strains.

Oscillatory control of somitogenesis and neurogenesis: imaging and control of gene expression by light technology

Ryoichiro Kageyama
(Kyoto University)

Somites, precursors for vertebrae, ribs, and skeletal muscles, are periodically formed by segmentation of the anterior parts of the presomitic mesoderm (PSM). In the mouse embryo, this periodicity is controlled by the segmentation clock gene *Hes7*, which exhibits synchronous oscillatory expression in the PSM. Without such Hes7 oscillations, all somites and their derivatives are severely fused, but the detailed mechanism of synchronous Hes7 oscillations still remains to be analyzed. We found that the Notch ligand Delta-like1 (Dll1) expression oscillates in the PSM, and that when Dll1 expression was accelerated or delayed by shortening or elongating the Dll1 gene, not only Dll1 but also Hes7 oscillations became dampened, leading to severe fusion of somite. Thus, the appropriate timing of Dll1 expression is critical for the oscillatory networks. We also developed an optogenetic approach and found that intracellular and intercellular periodic inputs of Notch signaling entrain intrinsic oscillations by frequency tuning and phase shifting. Thus, the oscillation dynamics are transmitted through Notch signaling, thereby synchronizing the population of oscillators. We now developed a real-time imaging system for analysis of Hes7 expression dynamics at a single cell resolution, using a new fluorescent reporter, and found that the Notch signaling modulator Lfng regulates synchronized Hes7 oscillations by adjusting the coupling delays.

Oscillatory gene expression is not unique to the segmentation clock but is observed in many cell types, including neural stem cells. In these cells, the cell fate determination factors Ascl1/Mash1, Hes1, and Olig2, which regulate the fate choice of neurons, astrocytes, and oligodendrocytes, respectively, are expressed in an oscillatory manner. However, in each differentiation lineage, one of the factors becomes dominant and is expressed in a sustained manner. We used optogenetics to control expression of Ascl1/Mash1, and found that although sustained Ascl1/Mash1 expression promotes neuronal fate determination, oscillatory Ascl1/Mash1 expression activates proliferation of neural stem cells. Thus, the multipotent state correlates with oscillatory expression of several fate-determination factors, whereas the differentiated state correlates with sustained expression of a single fate-determination factor.

In my lecture, I will discuss the functional significance of gene expression dynamics in developmental processes such as somitogenesis and neurogenesis.

Session 3 : Light & Life; Optogenetics #3

Enzyme rhodopsins, potential optogenetics tools for modulating intracellular cyclic-nucleotide levels

Satoshi Tsunoda[1, 2], Kazuho Yoshida[2], Leonid Brown[3], Hideki Kandori[2,4]

[1] JST PRESTO, [2]Life Science and Applied Chemistry, Graduate School of Engineering, Nagoya Institute of Technology, [3]Department of Physics, University of Guelph, [4]JST CREST

Function of microbial rhodopsins involve ion pump, ion channel, light sensor for phototaxis response. Recent studies revealed novel rhodopsins with enzymatic functions such as guanylate cyclase (Rh-GC) and phosphodiesterase (Rh-PDE)(1–3). Rh-GC functions as a tightly light-regulated guanylate cyclase whereas only a weak light dependency was demonstrated in Rh-PDE when studied in heterologous expression systems. These molecules could be genetically targeted into various types of cells, serving as optogenetics tools for optical control of intercellular cyclic nucleotide-associated signal transductions.
To get deeper insights into the light-activation mechanism of Rh-GC and Rh-PDE, we here investigate the spectroscopic property, enzymatic kinetics, and evaluate the light-dependent activity in mammalian cells. Mutation studies were performed to identify critical residues for enzymatic activation.

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FTIR study of photoactivated cyclase

Daichi Yamada
Nagoya Institute of Technology

Cyclic adenosine monophosphate (cAMP) 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 [1]. The photoactivated adenylyl cyclase from the photosynthetic cyanobacterium *Oscillatoria acuminata* (OaPAC) are powerful tools for optogenetics and for investigating signal transduction mechanisms in biological photoreceptors [2]. The OaPAC has two domains: N-terminal blue light using flavin (BLUF) domain and C-terminal adenylyl cyclases (AC) domain. The Structural changes of the BLUF domain lead to changes of the AC domain, although the underlying mechanism remains unknown.

Here, we attempted to monitor the structural changes of OaPAC for understanding the light-regulated mechanism by using FTIR spectroscopy. We obtained the temperature dependence light-induced difference spectra which are supposed to reflect structural changes of the enzyme domain in Amide I region, by comparing the results between OaPAC full length and without AC domain. Additionally, in order to investigate the structural changes due to enzyme activity from ATP to cAMP, we compared light-induced difference FTIR spectra of OaPAC full length in the absence and presence of ATP. In the photoreaction, differences were observed in the phosphate backbone reflected the change from ATP to cAMP, and the band considered as Asp residue necessary for enzyme activity were observed. We would like to discuss the light-regulated mechanism of OaPAC, based on these obtained results and together with mutation data.

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Optochemical control of protein localization in living cells using synthetic organic molecules

Tatsuyuki Yoshii[1, 2], Choji Oki[1], Shinya Tsukiji[1]
([1]Nagoya Institute of Technology, [2]JST-PREST)

Proteins rapidly and dynamically changes their localization during cell signaling. Thus, tools for rapid control of protein localization would arrow spatial and temporal manipulation of cell signaling and behavior. Chemically-synthesized molecule has potential to be a powerful tool because chemists can design and tune the property of photo-responsive group including wavelength and reversibility. However, opto-chemical control of protein localization without using chemically induced dimerization (CID) has not been explored due to the lack of the design to target small molecule to specific cellular organelle.

Here, we describe a protein translocation system by using photo-responsive, self-localizing ligand (pSLL). We designed a pSLL which consists of an organelle targeting motif and protein ligand protected by photo-removal group. We selected a lipopeptide motif to target the inner leaflet of plasma membrane (iPM). For the protein ligand, we chose trimethoprim (TMP) which binds to *Escherichia coli* dihydrofolate reductase (eDHFR). The amino group of the TMP was protected by nitroveratryloxycarbonyl (NVOC) group. The proof principle experiment was performed using Hela cells expressing eDHFR-mScarlet-i. When the pSLL was incubated with cells and irradiated with 405 nm light, rapid translocation of e eDHFR-mScarlet-i from the cytosol to the iPM was observed. We also applied this system to control Rac1 signaling in NIH3T3 cells by photo-induced translocation of Tiam1, a guanine nucleotide exchange factor for Rac1.

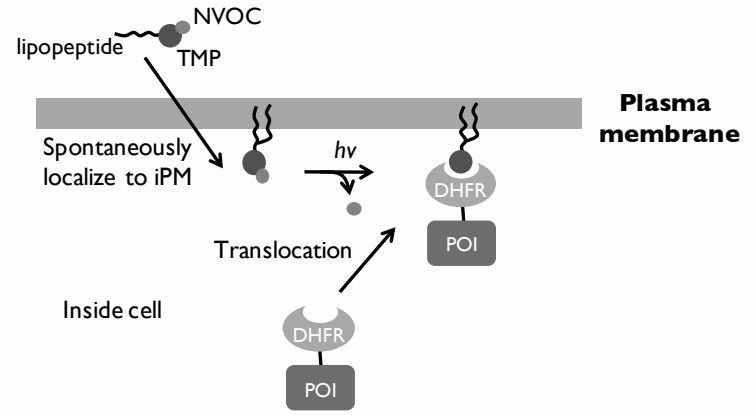

Figure 1. Opto-chemical control of protein localization using pSLL

Exotic Behavior of Water Around the Anti-Freeze Protein: Large-Scale Molecular Dynamics Simulations

Shuji Ogata[1] and Yasuhiro Kajima[2]
[1]Nagoya Institute of Technology
[2]Nagoya Zokei University

Various northern fishes, insects, and plants inhabiting under the sub-zero temperatures conditions synthesize a certain type of proteins to avoid freezing. These proteins are called anti-freeze proteins (AFP). They are believed to show the anti-freeze ability by binding to water-ice interfaces of emerged ice particles and thus inhibit their growth by Kelvin effect, resulting in melting point depression. However, neither detailed mechanism of the bindings nor the reasons why these proteins inhibit ice growth are known. The theoretical understanding of the anti-freeze mechanisms will aid in the proposal of, e.g., a novel snow melting agent.

Motivated by that, we perform two kinds of molecular dynamics simulations to unveil the mechanisms of type-I AFP. (i) One is large-scale (millions of atoms) simulations to see the ice growth after AFP's (PDB ID: 1j5b) are placed on the ice-water interface, which correspond to the situation where ice particles are formed in a supercooled water. We thereby confirm that the ice growth significantly slows down at a supercooled temperature (see Fig. 1). The AFP stabilizes most when the OH/CO groups face the water and the CH_3 groups fit within the trench structure of the ice surface. (ii) Another is medium-scale (tens of thousands of atoms) simulations to find the behaviors of water molecules around the amino residues that constitute the AFP. We thereby find that the microscopic structure of water molecules around the amino residues varies depending on the choice of the amino residues. Relation of the finding to the anti-freeze ability of the AFP will be explained in the presentation.

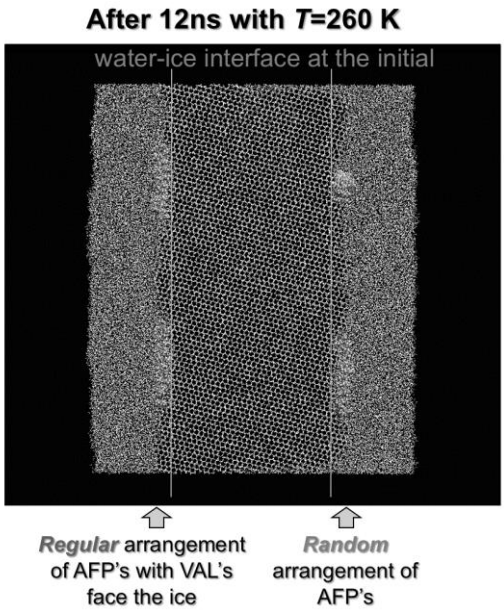

Fig. 1. Snapshot at 12 ns of the large-scale MD simulation of the ice-water interfacial system with 12 AFPs at 260 K. The yellow lines indicate the initial position of the ice-water interfaces.

Effect of third component in bulk-heterojunction organic solar cell

Tetsuo Soga, Naoki Kishi, Shinya Kato
Department of Electrical and Mechanical Engineering,
Nagoya Institute of Technology, Japan

Organic solar cells have attracted attention since bulk heterojunction was developed in the early 1990s. The advantage of organic solar cell is not only the high efficiency and low cost but also the new functions such as flexible, semi-transparent, light-weight, etc. Recently, the energy conversion efficiency higher than 17 % has been achieved by using tandem structure made of new polymer material [1], and more than 20 % efficiency is expected in the future [2].

In order to improve the efficiency of organic solar cell furthermore, a lot of approaches have been employed until now. However, there are still many unknown points to understand the operation of organic solar cell. Normally the organic solar cell is composed of donor material and acceptor material sandwiched between two electrodes (or electron/hole transport layer). One way to improve the performance is to dope third component in the donor/acceptor materials. This presentation describes the effect of addition of organic or inorganic third component materials on the photovoltaic properties of organic solar cells.

Regioregular poly(3-hexylthiophene-2,5-diyl) (P3HT) and [6,6]-phenyl-C61-butyric acid methyl ester (PCBM) were used as donor material and acceptor material, respectively. The active layer was a blend film of donor material and acceptor material which was prepared by spin coating on PEDOT-PSS (Poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate)) hole transport layer prepared on ITO-coated glass substrate. The Al electrode was evaporated on the active layer by thermal evaporation. The fundamental solar cell structure and fabrication procedure are reported in refs. 3 and 4. MEH-PPV (2-methoxy,5-(2′ethyl hex-yloxy)-PPV), polyethylene glycol, ZnO nanoparticle etc were used as third component. The effects of these third components on the photovoltaic properties of organic solar cell are described at the presentation

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Functional Extension and Linkage of Photosynthetic Biohybrid Light–Harvesting/Reaction Center Complexes in Artificial Systems

Takehisa Dewa
(Department of Life Science and Applied Chemistry, Graduate School of Engineering, Nagoya Institute of Technology)

Light-harvesting strategies are of crucial importance for the establishment of renewable energy production systems that can utilize solar energy with high efficiency. In terms of artificial photosynthesis, so called “photon flux density problem” should be overcome through development of light-harvesting systems that efficiently collect light energy to transfer it to an active site in a catalyst so as to provide high turnover frequency. In photosynthetic membranes, densely packed light-harvesting (LH) and reaction center (RC) complexes perform highly efficient energy conversion from light energy to chemical potential. Recently, we successfully extended light-harvesting ability of photosynthetic light-harvesting complex (LH2) from a purple bacterium through attachment of artificial fluorophores (Alexa Fluor 647: A647), which can expand the range of wavelength of light energy acquired. Femtosecond transient spectroscopy revealed that energy transfer from A647 to the intrinsic chromophores bacteriochlorophylls (BChls) occurs in sub picosecond to several dozen picoseconds time range [1]. When a hydrophobic fluorophore (Atto647N: AT647N) was used for the auxiliary chromophore attached to LH2 (LH2-AT647N), the energy transfer rate was significantly accelerated in the time range of sub picosecond to several picoseconds in a lipid bilayer environment. In this study, we addressed a combination of the biohybrid light-harvesting complex (LH2-AT647N) and a bacterial light-harvesting 1/reaction center core complex (LH1-RC) to show a cooperative effect of light harvesting and chemical reaction, i.e., charge separation and photocurrent generation, the latter of which results from the catalytic photocycle in RC. Recently, we have reported that LH1-RC from a purple photosynthetic bacterium (from *Rhodopseudomonas palustris*) effectively generates photocurrent when assembled with lipid bilayer on an ITO electrode [2]. When LH2-AT647N and LH1-RC (1/1 mixture) in lipid bilayer were placed on the ITO electrode, enhanced photocurrent was observed upon irradiation at 650 nm (absorption maxima of AT647) compared with a LH2/LH1-RC system. Action spectra were evident for the contribution of AT647N to photocurrent generation. The enhancement of photocurrent generation activity is due to increase in apparent absorption cross-section of LH1-RC.

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Multiscale Modeling of Electrostimulation

Akimasa Hirata
Dept. Electrical and Mech. Eng., Nagoya Institute of Technology, Japan

For the past 30 years, there has been significant progress in biophysical and electrophysiological modeling simulation techniques to evaluate electromagnetic field effects in the human body. Historically, the electromagnetic modeling techniques have been developed to clarify potential adverse health effect from the external electromagnetic field. However, most effort has been devoted into the macroscopic modeling, where the nerve activation was not considered. In addition, the phenomena one would like to know the response from external physical quantities. To understand the response from external physical quantity, three steps are needed.

The first step involves expressing a human body composed of the smallest elements (voxels) with a resolution of several millimeters based on medical images on the computer. The research necessity in this field is well summarized in Reilly and Hirata (*Phys. Med. Biol.* 2016). The second step involves the computation of the electromagnetic field and is achieved by assigning different electrical characteristics for each human tissue to each voxel/element constituting the model. The third step involves modeling the effect of the in situ electric current on the propagation of the neuronal signals. We included a new step that corresponds to the integration of local and remote circuits by synaptic modeling (Gomez-Tames and Hirata, *Int'l J. Neural Sys.* 2018).

In this study, we review the multiscale modeling of electrostimulation and resultant response with computational techniques. In particular, the brain stimulation and magnetophosphene from an external magnetic field are explained as computational examples. The limitation of the current status of the techniques is attributable to the synaptic effect which has not yet been well modeled even in typical biological response.