Biophysical research in Okazaki, Japan

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Abstract

This commentary summarizes the recent biophysical research conducted at the National Institute for Basic Biology, the National Institute for Physiological Sciences, and the Institute for Molecular Science in Okazaki, Japan.

Keywords National Institute for Basic Biology (NIBB) · National Institute for Physiological Sciences (NIPS) · Institute for Molecular Science (IMS) · National Institutes of Natural Sciences (NINS) · Okazaki

Interuniversity research institutes in Okazaki City

Okazaki, located at the center of Aichi Prefecture in Japan, is a city where scientists from around the world come to conduct collaborative research and attend conferences hosted by three research institutes: the National Institute for Basic Biology (NIBB) that promotes studies in the field of biology, the National Institute for Physiological Sciences (NIPS) that focuses on the mechanisms of the function of life, and the Institute for Molecular Science (IMS) that serves as a core research facility for molecular science. These three institutions, organized as members of the National Institutes of Natural Sciences (NINS), are nonprofit interuniversity

research organizations that provide the research community with opportunities for various types of joint research projects.

The aim of this commentary is to summarize the recent biophysical research conducted at these three institutes, and to share this overview with international colleagues as well as biophysical societies. For more details or activities in other fields, we refer the reader to additional information available on the following websites:

NIBB: <http://www.nibb.ac.jp/en/>

NIPS: <https://www.nips.ac.jp/eng/>

IMS: <https://www.ims.ac.jp/en/>

NINS: <https://www.nins.jp/en/>

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Biophysical research at the NIBB

The NIBB was founded in 1977 to promote and stimulate collaborative research in basic biology. NIBB covers a wide range of biological research fields, including biophysics. Current biophysical research at NIBB is mostly divided between two subjects, namely state-of-the-art live-cell fluorescence imaging combined with perturbation, and mechanobiology in morphogenesis, including embryonic development.

The former includes live-cell imaging of whole mouse embryos with light sheet–based fluorescence microscopy (LSFM), which has been widely used by researchers at various universities in Japan. LSFM enables live imaging of gastrulation of mouse embryos (Takao et al. 2012) and high-speed amoeboid movements (Ichikawa et al. 2013) (Fig. 1, left). In addition, perturbation techniques such as infrared laser–evoked gene operator (IR-LEGO) (Kamei et al. 2009; Hasugata et al. 2018) and optogenetic tools (Uda et al. 2017) were developed at NIBB. These perturbation techniques allow for precise control of gene expression and cell signaling with high spatial and temporal resolutions.

The latter research subject, namely mechanobiology research conducted at NIBB, is characterized by an approach that combines biophysical theory and experimentation. The

following are good examples of recent mechanobiology research achievements at NIBB: analysis of three-dimensional epithelial fold pattern formation observed in the mouse oviduct (Koyama et al. 2016) and intercellular signal propagation and its role in collective cell migration (Aoki et al. 2017) (Fig. 1, right). The mechanical properties of embryogenesis in *Xenopus* (Inoue et al. 2016) and wound healing (Kondo et al. 2018) in cultured monolayer cells have also been quantitatively analyzed by experiments and computer simulation at NIBB.

Biophysical research at the NIPS

The research mission of the NIPS is to understand the mechanisms of the function of life, especially those of humans. Guided by this mission, various studies have been conducted at the institute ranging from the molecular and cellular levels up to the system level. Biophysical research is also an important aspect of the NIPS mission and is undertaken within the five laboratories described below as well as in other labs.

In Dr. Yoshihiro Kubo's lab, studies on the structure–function relationship of ion channels using an in vitro expression system are performed in order to elucidate their functional mechanisms. In particular, the Kubo lab is interested in the

Fig. 1 Biophysics in National Institute for Basic Biology (NIBB). Left: Shown here are images of the gastrulation of mouse embryos and amoeboid movements obtained by light sheet–based fluorescence microscopy (LSFM). Right: The upper figure is the folding pattern of the mouse oviduct epithelial sheet obtained by computer simulation. The lower figure shows ERK MAP kinase activity and phase contrast in wound healing of cultured monolayer cells. Figure courtesy of Dr. Shigenori Nonaka (NIBB) and Dr. Hiroshi Koyama (NIBB)

stoichiometry and gating of voltage-gated K^+ channel complexes, the effects of drugs on G protein-coupled inward rectifier K^+ channels (Chen et al. 2019), the regulation of two-pore Na^+ channel 3 by PIP2 and voltage (Shimomura and Kubo 2019, Fig. 2), and the voltage-dependent gating of the ATP-gated receptor channel P2X₂. They use various methods including electrophysiological recordings and optophysiological analyses such as single-molecule imaging, Förster resonance energy transfer (FRET) analysis, and voltage-clamp fluorometry.

Transient receptor potential (TRP) ion channels are key thermosensitive molecules that regulate thermosensation and nociception. This superfamily of proteins is the subject of extensive studies by Dr. Makoto Tominaga's research group.

To elucidate the molecular mechanisms by which TRP channels are either activated or inactivated below or above a particular temperature threshold, the group utilizes a variety of experimental techniques including cell biology, biochemistry, and patch-clamp/calcium imaging (Saito et al. 2019). Results obtained with these different approaches are often compared with behavioral analyses of mice lacking thermosensitive TRP channels, thereby permitting the interpretation of experimental results in an integrative manner (Takayama et al. 2017).

Dr. Kazuyoshi Murata operates a series of electron microscopes (EM) complementarily to visualize medical and biological targets that span the molecular to cellular scales. While a high-voltage EM (H-1250 M: 1 MV) is designed for observing microorganisms and cells, a phase-contrast cryo-EM

Biophysics in NIPS

Structure-function study of ion channels

Thermo-sensing mechanisms

High-resolution structures by electron-microscope

Super-resolution two-photon STED (TP-STED) imaging

Two-photon fluorescence life-time imaging (2pFLIM)

Fig. 2 Biophysics in National Institute for Physiological Sciences (NIPS). Shown here are representative examples of the biophysical research activities. Upper left: Structure-function study of two-pore Na^+ channel (Shimomura and Kubo 2019). Upper middle: Thermo-sensing mechanisms by TRP channels and the evolutionary changes (Saito et al.

2019). Upper right: High-resolution structure analysis of virus capsid by cryo-EM. Lower left: Two-photon STED (TP-STED) imaging of α -tubulin in COS-7 cells (Ishii et al. 2019). Lower right: Two-photon fluorescence lifetime imaging (2pFLIM) of the activity of synaptic molecules

(JEM2200FS; 200 kV) is equipped with a Zernike phase plate to achieve high-resolution structural analyses of membrane proteins (Tsunoda et al. 2018) and viruses (Okamoto et al. 2018). These advanced EMs and analytical techniques are shared with research communities, including the biophysics community, through joint research projects.

Dr. Tomomi Nemoto is a specialist in intravital and super-resolution imaging utilizing two-photon microscopy. Recently, his research team introduced stimulated emission depletion (STED) to two-photon microscopy, where transmissive liquid crystal devices convert the STED laser light beam into an optical vortex (Otomo et al. 2018). The microscope that the Nemoto team developed currently boasts superior spatial resolution below 100 nm and has been used to observe the finer structures of microtubule networks as well as presynaptic protein clusters without unwanted photobleaching effects (Ishii et al. 2019). The group is committed to applying their microscopes and methodologies to in vivo observation of physiologically or biologically essential targets.

Two-photon fluorescence lifetime imaging microscopy (2pFLIM) allows pixel-by-pixel mapping of the fluorescence lifetime. This method is advantageous especially for detecting FRET in subcellular compartments of living cells located deep within tissues, such as in brain slices. To visualize protein activity and protein-protein interactions in neurons, Dr. Hideji Murakoshi has been developing novel fluorescent proteins, FRET sensors, and optogenetic tools (Murakoshi et al. 2017, 2019). Using these probes, they have imaged the activity of various signaling molecules within the dendritic spines of hippocampal neurons. Interestingly, these molecules have specific spatiotemporal activity patterns in dendritic spines that regulate synaptic plasticity.

Biophysical research at IMS

Molecular science is an interdisciplinary field of study that seeks to understand molecular functions and structures using physical and chemical techniques. This field covers a broad range of research topics including atoms/molecules in vacuo, in vitro, in vivo, in man-made devices, in the cosmos, and in silico. At IMS, there are 10 research groups in biophysics or biophysical chemistry including six experimental groups, three theoretical/computational groups, and one group that uses both experimental and theoretical approaches. They have gathered at IMS in order to address a difficult question: How do the characteristics of individual biomolecules lead to the expression of remarkable functions when they are assembled into molecular systems?

The group led by Dr. Shuji Akiyama focuses on the circadian clock system which is an endogenous time-measuring system that adapts to daily environmental alterations. Among clock-system components, some proteins have been

observed to undergo cyclical, 24-hour (circadian) changes in their structures and interactions, transferring rhythmic information upstream that functions at the molecular and cellular levels. In 2015, they elucidated the atomic-scale origins of clock slowness in the cyanobacterial circadian clock system (Abe et al. 2015). The Akiyama group is committed to improving our understanding of circadian clocks and other dynamic systems through the chemistry of the circadian rhythm (Ouyang et al. 2019), structure (Abe et al. 2015), and evolutionary diversity (Mukaiyama et al. 2019).

Molecular motors are a popular but competitive research topic in biophysics and best studied by Dr. Ryota Iino's group using single-molecule techniques. They recently succeeded in observing the *Enterococcus hirae* V₁-ATPase rotating in 120° steps with 40° and 80° substeps per round of ATP hydrolysis, and proposed a novel chemo-mechanical coupling mechanism distinct from that of the well-studied F₁-ATPase (Iida et al. 2019). The group is also actively pursuing development of advanced single-molecule techniques, such as multicolor, high-speed tracking of single biomacromolecules using alloy nanoparticles (Ando et al. 2019).

Dr. Koichi Kato and his colleagues have studied the assembly and disassembly dynamics of supramolecular machineries. The proteasome, a gigantic enzyme complex, has been their focus and characterized through the complementary use of atomic force microscopy, single-particle electron microscopy, and native-mass spectroscopy (Sekiguchi et al. 2019). This strategy of integrating various biophysical approaches has also been applied to immunoglobulin G and related molecules upon elucidating their dynamic conformational ensembles (Yanaka et al. 2019).

Dr. Shigetoshi Aono and his colleagues often use biophysical, biochemical, and crystallographic techniques in a complementary manner to elucidate the structure-function relationships of metal-containing sensor/signaling proteins (Muraki et al. 2016). Recently, the Aono research group determined the crystal structure of HypX, an enzyme that catalyzes the biosynthesis of carbon monoxide, and proposed a possible maturation mechanism for the hydrogen-sensing regulatory NiFe-hydrogenase (Muraki et al. 2019).

Dr. Nobuyasu Koga, who is skilled in both theory and experimentation, explores the fundamental working principles of protein molecules by synthesizing computationally designed proteins to test their design theory. Thus far, they have discovered a set of general rules for creating novel sequences that fold into *ideal* protein structures consisting of α -helices, β -strands, and loops (Koga et al. 2012), and extended their design rules in order to fine-tune the overall shape and size of the artificial proteins (Lin et al. 2015).

Biomacromolecules are often insoluble in aqueous solvents, and function in amorphous states even under physiological conditions. Dr. Katsuyuki Nishimura has been heavily involved in developing a solid-state NMR probe best suited

for direct observation of those targets. The device as well as analytical techniques that his lab developed has been successfully applied to the structural characterization of amyloid- β peptide oligomers in lipid bilayers (Yagi-Utsumi et al. 2016) as well as the characterization of model silk peptides (Naito et al. 2018).

Dr. Kensuke Kurihara and his colleagues are pursuing methods for the *in vitro* construction of artificial cells using a supramolecular chemical approach. A good example of their approach is cell-mimicking giant vesicles (GV) that proliferate from generation to generation (Kurihara et al. 2015). Polystyrene beads encapsulated in the giant vesicles reveal a unique spatial distribution: smaller beads come closer to the vesicle (Natsume et al. 2019). They extended a typical statistical mechanics theory to explain this unique distribution as well as the frequent division of particle-containing vesicles.

Dr. Shinji Saito, who leads the theoretical chemistry research group at IMS, is deeply interested in heterogeneous fluctuations and dynamic behavior often observed in liquids and biomolecular systems. Based on detailed molecular dynamics simulations of Pin1 peptidyl-prolyl isomerase, they determined that conformational flexibility has a critical role to play; without conformational flexibility, the catalytic isomerization reaction never advances through ordinary thermal activation (Mori and Saito 2019). Their latest study is a careful and thorough investigation of the thermodynamic properties of supercooled water upon forming amorphous ice based on ultra-long computer simulations that last over 50 μ s (Saito and Bagchi 2019).

The free energy landscape of protein molecules appears funnel-like on a global scale but actually has many small rugged features and several local minima that limit a computational search across the vast conformational space. Dr. Hisashi Okumura has proposed a replica-permutation method (Itoh and Okumura 2013) as generalized-ensemble algorithms for overcoming this problem. While improving their simulation tools, they are committed to applying their method to various biological systems such as aggregates and fibrils of amyloid- β peptides (Itoh et al. 2019).

Dr. Kei-ichi Okazaki uses other techniques such as importance sampling and coarse-graining to overcome the problem of sampling of protein conformation. He worked on molecular dynamics simulations of the rotary molecular motor F_0F_1 -ATP synthase (Okazaki and Hummer 2015). His research interest has gradually broadened to include computational studies that target a series of transporters (Okazaki et al. 2019).

These experimental and theoretical groups are managed independently, but often work together to develop new concepts for explaining unique observations. For example, Dr. K. Okazaki collaborated with Dr. R. Iino to conduct atomic-scale simulation of unidirectional motions of chitinase (Nakamura

et al. 2018). Dr. H. Okumura and his colleagues worked with Dr. K. Kato's group to elucidate interface effects on the structural dynamics of amyloid- β peptides (Itoh et al. 2019), and also with Dr. S. Aono's group to perform molecular dynamics simulations using a newly solved crystal structure (Muraki et al. 2019). Dr. S. Saito and his colleagues collaborated with Dr. S. Akiyama's group on simulating the atomic-scale origins of slowness in the cyanobacterial circadian clock by focusing on the clock protein KaiC (Abe et al. 2015). While the IMS promotes inter-institute interactions of theory and experimentation as highlighted above, it also encourages joint research projects involving researchers affiliated with other institutes and universities so as to extend the existing biophysics research network.

Inter-institute collaboration and outlook

The Research Center for Computational Science (RCCS: <https://ccportal.ims.ac.jp/en>) is one of Okazaki's research facilities and has provided computational resources with academic researchers in molecular sciences and related fields, including biophysics. The Okazaki Institute for Integrative Bioscience (OIIB) was established as the other research facility from April 2000 to March 2017 to drive life-science research through synergy among the three institutes. In April 2018, OIIB was reorganized and renamed to the Exploratory Research Center on Life and Living Systems (ExCELLS: <https://www.excells.orion.ac.jp/en>) with the ultimate goal of addressing a fundamental question: What is life?

All of the organizations located in Okazaki are committed to promoting joint research projects as members of NINS. As a whole, they span the major scientific disciplines of physics, chemistry, biology, physiology, computational science, and biophysics, as one of the interplays between these disciplines and the expertise. We gladly welcome the opportunity to think about future directions and to develop close connections with international colleagues and societies of biophysics.

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Compliance with ethical standards

The authors declare that they have no conflict of interest. This article does not contain any studies with human participants or animal subjects performed by any of the authors.

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