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Commentary and Perspective

Physico- and chemical biology using nanomanipulation and micromanipulation technologies

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Various nano- and micromanipulation technologies have provided novel strategies to elucidate nature in many scientific fields such as biophysics, physicobiology, and chemical biology. To highlight recent progress in this research area by biophysicists, cell biologists, and chemists in Japan, a symposium was organized during the 60th Annual Meeting of the Biophysical Society of Japan held in September 2022 titled "Physico- and chemical biology using nanomanipulation and micromanipulation technologies," organized by Akira Kitamura (Hokkaido University and Japan Agency for Medical Research and Development) and Ryo Iizuka (The University of Tokyo). It was ensured that the content of the symposium was not easily guessed by the participants based on the symposium title or the organizers' or speakers' names because one of the important objectives of the symposium was to gather participants with different research backgrounds, who would not join other conventional symposiums, and promote interdisciplinary collaboration. Therefore, the organizers invited first time and/or young speakers as well as regular speakers of the Biophysical Society in Japan. Cutting-edge research involving nanomanipulation and micromanipulation technologies with single-molecule sensitivity, chemical biology, optogenetics, and mechanistic measurements to understand and control cells and organisms were discussed. In addition, molecular and cellular biology research from a physicochemical perspective was articulated. Herein, we briefly introduce the topics discussed by the speakers.

Dr. Kaori Kuribayashi-Shigetomi (Inst. Adv. High. Edu., Hokkaido Univ.) discussed three-dimensional tumors using micro/nano processing technology. A well-known fact was stated while discussing this topic: "1 in 3 people die from cancer." However, the success rate of cancer drug discovery is extremely low. A major problem in achieving success is the lack of valid in vitro 3D human models that can be attached to a specific position on a dish for their reconstruction. Therefore, an adhesive system on the dish was established using micropatterning of nanostructures (i.e., a narrow scaffold on the chip) filled with non-adhesive 2-methacryloyloxyethyl phosphorylcholine polymer on the glass surface [1]. Using this system, microtumors evade attack from immune cells by covering dead cells on the tumor surface, thus resembling the dead cells. The shape and dynamic morphological changes of micro-tumors after treatment with anti-cancer drugs were successfully observed using this system; thus, it is possible to achieve a high throughput that can be applied to future new drug development.

Dr. Yukako Nishimura (IGM, Hokkaido Univ.) discussed the mechanism of force generation from actin filaments in adherent cells. Crosstalk between actin turnover and contractility in stress fibers is important to produce traction force at the focal adhesion spot between the plasma membrane and extracellular matrix. To visualize the actin dynamics and traction forces in a single stress fiber, micropillar arrays were employed. It is a powerful system for analyzing force

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generation by visualizing the degree of deformation of micropillars. Permeabilized cells were also used to observe the dynamics of actin filaments following treatment with various inhibitors of actin dynamics. It was found that a formin specific inhibitor, SMIFH2, blocks strong reduction in traction forces by inhibiting both formins and myosin 2 [2,3]. Therefore, the importance of crosstalk between actin turnover and contractility in stress fibers was communicated [2].

Mr. MD Monir Hossain (Grad. Sch. NanoLSI, Kanazawa Univ.) and his coauthors introduced nanoheating technology that enables spatiotemporal temperature control for the investigation of thermal effects on the subcellular microenvironment [4]. They designed a photothermal dye-based nanoheater that allowed the creation of a subcellular-sized heat spot with concurrent fluorescent thermometry. Specifically, three photothermal dyes were embedded into the polymeric particles. An individual nanoheater can be operated by a relevant near-infrared laser at 808, 855, and 980 nm. Three nanoheaters in live cells simultaneously produce multiple heat spots within a single cell. Furthermore, they observed biomolecular behavior after spot-heating manipulation.

Dr. Ryo Iizuka (Grad. Sch. of Sci., The Univ. of Tokyo) evaluated the physicochemical properties of biomolecules using microdroplets. First, he presented a detection mode for enzymatic activity in microdroplets based on their deformability. Deformability-based microdroplet sorting was achieved through a microfluidic channel with two grooved rails on the ceiling. Using the microfluidic channel, microdroplets were successfully sorted in agarose that was hydrolyzed by bacterial cells. Second, the effect of microenvironments created by microdroplets on polynucleosome condensation was demonstrated. Therefore, they suggested that the nucleus of eukaryotic cells not only compartmentalizes genomic DNA but is also involved in higher-order condensation of DNA.

Mr. Yassine Sabek (Grad. Sch. of Sci. and Eng., Soka Univ.) and his collaborators' research focus was the function of small G-proteins (Ras), such as the stimulation of downstream pathways, cell proliferation, and differentiation among others that may promote tumorigenesis. They studied the control of the Ras function using photochromic molecular devices. Calmodulin was used as an ionochromic molecular device to control Ras with Ca^{2+} ions [5]. A calmodulin fusion protein with an inhibitory peptide for the Ras and M13 calmodulin target peptide (CAM-I-M13) was designed and prepared using the *E. coli* expression system. CAM-I-M13 exhibited different inhibitory activities against Ras GTPases in the presence and absence of Ca^{2+}. The Ca^{2+}-dependent reversible binding of CAM-I-M13 was also demonstrated using these recombinant proteins.

Dr. Yasuhiro Hirano (Grad. Sch. of Front. Biosci., Osaka Univ.) introduced 12× modified expansion microscopy (mExM) [6]. The kinetochore is essential for efficient chromosome segregation during mitosis and is assembled on centromeres through dynamic processes involving numerous kinetochore proteins. However, the orientation of the kinetochore proteins remains elusive because of the limitation of fluorescence microscopy resolution, despite super-resolution microscopies enabling us to resolve the 50−100 nm structure. They obtained a diffraction-limited resolution in the expanded sample that corresponds to ~30 nm resolution in the original sample under conventional microscopy; however, using mExM, they found that constitutive centromere association network proteins, CENP-T and CENP-C, formed a different structure in the kinetochore. Furthermore, he summarized the advantages and disadvantages of mExM. In particular, there is no objective lens as efficient as that used in the mExM for the problem of sample distortion.

Dr. Akihiro Kishimura (Fac. of Eng., Kyusyu Univ.) discussed the regulation method of the liquid-liquid phase separation state using polyion complexes and their relevance to biological systems; for example, biomolecular condensates or membraneless organelles was intensively discussed. Their project aimed to control the micro- and macroscopic structures of complex coacervates by using bottom-up molecular technology [7,8]. Thus, they developed a method for microstructural control of complex coacervates based on synthetic-polypeptide-based block copolymers, which are typically composed of charge-neutral polymers, PEG, and poly(aspartic acid)s [9-11]. Next, they attempted to obtain hierarchical structures and successfully developed novel (sub)micron-sized structures. In addition, unique multiphase coacervates were developed, in which the arrangement of different droplets was controlled.

Dr. Akira Kitamura (Fac. of Adv. Life Sci., Hokkaido Univ.) introduced the history and applications of chromophore-assisted light inactivation (CALI), which enables the destruction of the protein of interest through reactive oxygen species (produced by photosensitizer fluorescent probes) [12]. Furthermore, he demonstrated the photophysical and photochemical reaction system of a photosensitizer and provided an understanding of the wavelength selectivity of photosensitizers for CALI using fluorescence correlation spectroscopy [13] and fluorescence lifetime measurements.

Accordingly, in this symposium, interdisciplinary researchers discussed new methods for manipulating biomolecules and observing their functions and structures, particularly in cells. The speakers elaborated on "control systems" at various levels, from the molecules, aggregates, single cells, and clusters of cells to the freedom of science that makes it possible to use these systems. Such "control systems" is one of the best strategies to understand the underlying complicated biological processes, providing detailed information, which is generally difficult, if not impossible, to obtain using conventional methods. To put the ideas into practice, an effective interdisciplinary collaboration among researchers, such as synthetic chemists, microfabrication engineers, and microscopic specialists, is often required. In the future, the collaboration between applied physicists and life scientists will produce more scientific achievements. We hope that this symposium will serve as a foundation for such collaboration. Needless to say, we would like to start collaborative research

among the symposiasts. Also, we hope that this recognition was shared by the audience and that this review will be helpful to those who were unable to attend this symposium.

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