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Commentary and Perspective

Bottom-up creation of cell-free molecular systems: Basic research toward social implementation

Izuru Kawamura[1], Ryuji Kawano[2], Tomoaki Matsuura[3]

[1] *Graduate School of Engineering Science, Yokohama National University, Yokohama, Kanagawa 240-8501, Japan*
[2] *Department of Biotechnology and Life Science, Tokyo University of Agriculture and Technology (TUAT), Koganei, Tokyo 184-8588, Japan*
[3] *Earth-Life Science Institute, Tokyo Institute of Technology, Meguro-ku, Tokyo 152-8550, Japan*

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Research on the bottom-up creation of cells has progressed substantially, resulting in the development of reconstituted molecular systems that mimic various cellular functions and properties. However, bottom-up construction of molecular systems aimed at applied and socially relevant goals has seldomly been pursued. Here, we focus on advanced technologies including cell-free protein synthesis, bacterial degradation of oil and fatty acids, and high-throughput single-cell screening toward socially relevant goals. In a symposium at the 61st Annual Meeting of the Biophysical Society of Japan, held on November 15, 2023, five researchers will be invited to present current research topics, including applied and social development, based on the fundamental research on unique molecular systems. This paper summarizes the research topics going to be discussed at the symposium.

The protein synthesis using recombinant elements (PURE) system is a cell-free protein synthesis system developed by Yoshihiro Shimizu et al [1] that is reconstituted solely from essential elements of the *Escherichia coli* translation system. The system, well-known for its high controllability, has been commercially available worldwide for 20 years as the PURE system and has made significant contributions to the advancement of synthetic biology. Professor Shimizu (RIKEN Center for Biosystems Dynamics Research), one of our invited speakers, will discuss the history and recent achievements of the PURE system [2,3] and the impact of societal implementation. Professor Tomoaki Matsuura (Earth-Life Science Institute, Tokyo Institute of Technology) the leader of "Bottom-up Creation of Cell-free Molecular Systems (Grant-in-Aid for Transformative Research Areas (A))", will describe the optimization of PURE system by incorporating additional components and altering their concentrations. He will also introduce research on incorporating polyphosphate kinase 2 class III enzymes to improve and alter the energy regeneration system of the PURE system [4]. Additionally, he will also introduce applications using PURE system; artificial cell assemblies and in vitro directed evolution of membrane proteins [5,6].

The development of single-cell analysis techniques using microfluidic devices and the technological innovations in next-generation sequencing have made it possible to unravel the heterogeneity within cell populations and analyze "rare cells". Professor Tomoko Yoshino's research group (Tokyo University of Agriculture and Technology) has focused their research on circulating tumor cells (CTCs), a cell population with very low absolute numbers, and has contributed to the cancer diagnosis and treatment, as well as the understanding of metastatic mechanisms. At this symposium, Professor Yoshino will present recent advancements in the development and clinical application of a single-cell gene analysis platform for CTCs [7–9]. Various applications beyond CTC analysis, such as the analysis of rare microorganisms in environmental samples and the screening of mutant strains will also be introduced.

Biomanufacturing is the process of using biological systems such as enzymes, microorganisms, and cells to produce fuel, chemicals, and nutrients from renewable resources. Biomanufacturing is gaining attention as a means of addressing

Corresponding author: Izuru Kawamura, Graduate School of Engineering Science, Tokiwadai 79-5 Hodogaya-ku, Yokohama, Kanagawa 240-8501, Japan. ORCID iD: https://orcid.org/0000-0002-8163-9695, e-mail: kawamura-izuru-wx@ynu.ac.jp

societal issues such as environmental and resource depletion, while promoting economic growth. To fully harness the potential of biomanufacturing, it is pivotal to source materials that can be "manufactured" from a vast array of microorganisms present on Earth. Professor Masahito Hosokawa (bitBiome, Inc., Waseda University) and his team possess the world's largest uncultured microbial genome database, comprising 1.2 billion genes, achieved via decoding microbial genomes at the single-cell level [10–12]. He will introduce the next-generation of biomanufacturing using a large database based on the integration of synthetic biology-AI-robotics technology.

The two microorganisms isolated by Prof. Katsutoshi Hori (Nagoya University), *Burkholderia arbol*is KH-1 and *Yarrowia lypolitica* KH-2, rapidly degrade all types of animal and plant fats (including trans fatty acids) in wastewater because of their high lipase activity [13–15]. The microbial products made by the Friend Microbe Inc. affiliated with Nagoya University, have been commercially available "Mibiocon" for treating high-concentration oil and fat in wastewater. At this symposium, Professor Hori will introduce latest research findings and the research strategy for implementing them in the field of biophysics from the perspective of an entrepreneurial scientist.

In conclusion, this symposium will provide an opportunity to advance biophysical research and share technical pathways and discussions to connect new biotechnologies with social implementation. We believe that this symposium will motivate and provide expertise to researchers from other fields to accelerate the social implementation of their research.

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