THE PURE SYSTEM FOR ARTIFICIAL CELLS. Takuya Ueda, Department of Medical Genome Sciences, Graduate School of Frontier Sciences, The University of Tokyo, FSB-401, 5-1-5 Kashiwanoha, Kashiwa, Chiba Prefecture, 277-8562 Japan, ueda@k.u-tokyo.ac.jp

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Introduction: The ultimate goal of biology is to obtain the answer to the question, "what is life?" We have two approaches to reach the goal, analytical and synthetic approaches. In the last century molecular biology had afforded a number of powerful tools to analyze molecular mechanism occurring in living organisms and is still extending our vast knowledge on life. One of outcomes achieved through the approach of reductionism is undoubtedly the huge database of genome sequences of various species. The accumulating blue-prints of life will allow us to proceed the other approach, synthetic biology. Based on the gene information, we could synthesize a set of proteins encoded on a particular genome. Such possibility leads us to challenge the synthesis of cell or life.

Experimentals Results and Discussion: To address this objective we first reconstituted cell-free translation system from translation factors individually purified from over-expressed *E. coli* cell and named the system PURE system[1]. We are addressing to create cell-like system in test tube by combining PURE system with lipid-bilayer. In addition to DNA, RNA and protein, lipid is indispensable for the cell, due to several properties, such as compartmentalizing ability, substance exchange, DNA replication, energy production, etc. Thus, cell-like system should be comprised of gene expression machinery and functional membrane. Whereas the development of gene expression system had been realized by the PURE system, the creation of biologically active membrane *in vitro* has not been established so far.

We first made an attempt to create energy-generating liposome by expressing the genes corresponding to ATPase subunits onto lipid membrane using the PURE system supplemented with liposome. We succeeded in construction of membrane insertion system using the PURE system and membrane fraction and efficient integration of membrane protein into lipid bilayer was observed [2]. Furthermore, preliminary results indicate that Fo subunit of ATPase can be synthesized onto the proteoliposome as active form and F_1 assembly can be fulfilled simply by adding a set of ATPase genes. Through this approach, complete synthesis of ATPase on liposome from template DNA by PURE system was focused and efficient ATP generation system derived by the proton gradient across the liposome membrane was achieved.

The reproduction of gene-expression system *in vitro* is also necessary to generate an artificial cell. To address this objective, the reconstitution of ribosome has been challenged and we succeeded in the efficient assemble of 30S ribosomal subunit in the presence of the factors involved in biosynthesis of ribosome. The reconstitution of ribosome will be discussed.

References: [1] Shimizu et al. (2001) *Nat Biotechnol, 19,* 751-755. [2] Kuruma Y. et al. (2005) *Biotechnol Prog*, 21, 1243-1251. [3] Kuruma Y. et al. (2012) *Biochemical Journal*, 442, 631-638.