Proposal for ENS-Lyon / Univ. Tokyo Internship Program

Project Title: Reconstitution of a gene expression system

The project will be conducted at: Laboratory of Biomolecules, Dept. of Medical Genome Sciences, Grad. Sch. of Frontier Sciences, The University of Tokyo, FSB-401, 5-1-5 Kashiwanoha, Kashiwa, Chiba Prefecture 277-8562 Japan

Project period: Sept. to Dec., 2014

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Key words: translation, ribosome, synthetic biology

Background and summary of the project:

1) Reconstitution of a protein synthesis system: We have reconstited the E. coli translation system in the test tube, which we named the PURE system. The PURE system of eukaryote (yeast) and mitochondria (human) are also under development. Based on these systems, we are studying the molecular basis of protein synthesis. 2) Synthetic biology by PURE system : Taking advantage of the rapid production of proteins of high quality using the PURE system, we are constructing the supermolecular complexes (ribosomes, ATP synthetase etc.), to ultimately create a biosystem in a test tube. 3) Creating polypeptides with new functions by PURE system : We have established the ribosome-display method using the PURE system, a method for identifying functional polypeptides from a pool of variants. Applying this technique, we are creating various proteins with new binding properties.

Techniques used: PCR, cell-free translations, protein purification, protein analysis

Related publications from our laboratory:

Niwa T *et al* (2012) Global analysis of chaperone effects using a reconstituted cell-free translation system. *Proc Natl Acad Sci U S A* 109(23): 8937-8942

Niwa T *et al* (2009) Bimodal protein solubility distribution revealed by an aggregation analysis of the entire ensemble of Escherichia coli proteins. *Proc Natl Acad Sci U S A* 106(11): 4201-4206

Tsuboi M, et al (2009) EF-G2mt is an exclusive recycling factor in mammalian mitochondrial protein synthesis. *Mol Cell* 35(4): 502-510

Shimizu Y et al (2001) Cell-free translation reconstituted with purified components. *Nat Biotechnol* 19(8): 751-755