

# Science and Technology Group Annual Report FY2018

Yoko Nomura  
Science and Technology Associate

## 1 Introduction

I continued to engage in two independent research projects during FY2018, namely, engineering of catalytic RNAs (ribozymes) and investigations on natural fibers (silk and Bashofu).

Engineering of Catalytic RNAs: Coworkers and I developed a new scaffold based on a circularly-permuted structure of a natural catalytic RNA. In another project, Prof. Yokobayashi and I quantitatively evaluated >10,000 ribozyme sequences and discovered very small (18 nt) catalytic RNA motifs with RNA ligase activity. Apart from potential engineering applications, these small catalytic RNA motifs are of significant interest in the context of prebiotic evolution. Furthermore, as a member of Nucleic Acid Chemistry and Engineering Unit, I contributed to additional projects as a collaborator (2 other contributions).

Natural Fibers: I studied new fibroin binding peptides having antibiotic activity. Also, I aimed to apply these peptides for practical uses in the subtropical climate. In the Bashofu project, I characterized the fibers produced by the traditional process. Furthermore, CPR and I hosted various events at OIST and elsewhere to promote the the scientific findings to local and general public in collaboration with the Kijoka Bashofu Association.

## 2 Activities and Findings

### 1) Engineering of catalytic RNAs

#### Synthetic ribozyme scaffold for development of aptazymes and riboswitches (4, A1)

The ribozymes that have been exploited for genetic engineering have been limited to few naturally occurring sequences. We sought to expand the diversity of the ribozyme scaffolds which can be used to engineer synthetic ribozymes. We studied circularly-permuted variants of the pistol ribozyme class both in vitro and in vivo. We identified several synthetic ribozymes that are active in mammalian cells. We further used the ribozymes to engineer ligand-inhibited aptazymes which were used to control gene expression in mammalian cells. Notably, these riboswitches function as chemically inducible switches (ON switches) which are more difficult to engineer than OFF switches in mammalian cells.

#### Systematic minimization of RNA ligase ribozyme through large-scale design-synthesis sequence cycles (*manuscript submitted*)

Laboratory evolution experiments have yielded several classes of ligase ribozymes, but their minimal sequence requirements remain largely unexplored. We used large-scale DNA synthesis and high-throughput ribozyme assay enabled by deep sequencing to systematically minimize a previously laboratory-evolved ligase ribozyme. After designing and evaluating >10000 sequences, we identified catalytic cores as small as 18 contiguous bases that catalyze template-directed regiospecific RNA ligation. The fact that such a short sequence can catalyze this critical reaction suggests that similarly simple or even simpler motifs may populate the RNA sequence space which could have been accessible to the prebiotic ribozymes.

#### Other contributions

##### a) Photocaged guanine modulates riboswitch function by light (4, A2)

I designed and executed all experiments using mammalian cells in this work.

##### b) Self-powered RNA nanomachine driven by metastable structure. *In revision, Nucleic Acid Research.*

I executed all experiments for the publication.

##### c) New luciferase (4, A3)

A new luciferase mutant was engineered and characterized based on my protein engineering experiments in the previous appointment (UC Davis).

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d) Mentoring a rotation student in the Yokobayashi Unit

I mentored and trained a rotation student with a non-biochemistry background.

2) Natural fiber projects

Antibiotic silk fiber using peptides (Kakenhi project, 4. B1; oral presentation B1a)

I analyzed the NGS result from last year and found that the most enriched motif was VHWxxxQWWQPS. I individually fused 11 sequences with the antimicrobial peptide RRRWWW-NH<sub>2</sub> and I found that some of these peptides preserved sufficient antibacterial activity against both *E.coli* (gram negative) and *Staphylococcus epidermidis* (gram positive). I prepared antibacterial fibroin fiber using these peptides. It was expected that these peptides would show antibiotic activity on fibroin fibers. However the reproducibility was limited based on the extraction evaluation method. Subsequently, I tried direct-evaluations on the fibers under the subtropical climate, but due to unexpected failures of the thermo-hygrostat incubator, I could not complete this experiment in this year. I will try this experiment again after fixing this incubator.

Bashofu project

Research (4, B2a poster presentation): Bashofu is a typical traditional textile made from *Itobasho* (banana plant). I characterized materials before and after the traditional degumming process, a key step in Bashofu production last year (*JFST* 73(11), 317-326, 2017). This year, I characterized the fiber product made by this traditional process. The Imaging Section and I tried morphological observations using several microscopes: SEM, TEM, LM and AFM. I also performed preliminary tensile tests to characterize the mechanical properties of the Bashofu fiber. We plan to evaluate the samples from Kijoka Bashofu Association using by these instruments next year. I also started investigating the cooling mechanism of the Bashofu with a new collaborator at Japan Women's University.

Public outreach (in collaboration with CPR): We held the first scientific Bashofu exhibition accompanied by a symposium related to this research open to public, supported by Okigin-Furusato Promotion Fund (B2b). Furthermore, I also engaged in outreach activities to promote our Bashofu project (B2c1-4). I believe my Bashofu project has contributed significantly to promote OIST commitment to serve the local Okinawan communities. I also served as a member of an Okinawa prefectural project: "Raw Material Conservation of Okinawan Traditional Crafts".



Poster for OIST event  
Science of Bashofu

## 3 Collaborations

Prof. Yokobayashi (Nucleic Acid Chemistry and Engineering Unit)

Bashofu project collaborator: Kijoka Bashofu Association, University of the Ryukyus, OIST Imaging Section (Mr. Toshio Sasaki & Dr. Koji Koizumi), OIST Mechanical Engineering and Microfabrication Support Section (Dr. Hyung-Been Kan), Japan Women's University

## 4 Publications and other output

A1, Y. Nomura et al., International Symposium on Nucleic Acids Chemistry (Poster, Kyoto, Nov/7-9/2018).

A2, V. Dhamodharan, Y. Nomura, M. Dwidar, Y. Yokobayashi, *Chem. Commun.*54(48):6181-6183(2018).

A3, T. Pozzo, F. Akter, Y. Nomura, A.Y. Louie, Y. Yokobayashi, *ACS Omega*, Mar 31(3) 2628-2633(2018).

B1, Kakenhi Kiban C, FY 2016-2018 (4,290,000 yen in 3 years) Antibiotic silk fibroin using peptides.

B1a, Y. Nomura, The Japan Society of Home Economics, Kyushu Chapter (Oral, Miyazaki, Oct/6/2018)

B2a, Y. Nomura, The Japan Society of Home Economics, the 70<sup>th</sup> Conference (Poster, Tokyo, May 25-27/2018). ; B2b, Okigin-Furusato Promotion Fund (500,000 yen for Bashofu event).

B2c-1, Science Café (in Naha, Okinawa Prefectural Museum and Art Museum, April/21/2018).

B2c-2, Science Café (for press, in Tokyo, June/28/2018). B2c-3, Science Café, Junku-do in Naha, Science talk Oct (Oct/12/2018). B2c-4, Science Café, OIST Science Festival (Nov/17/2018).