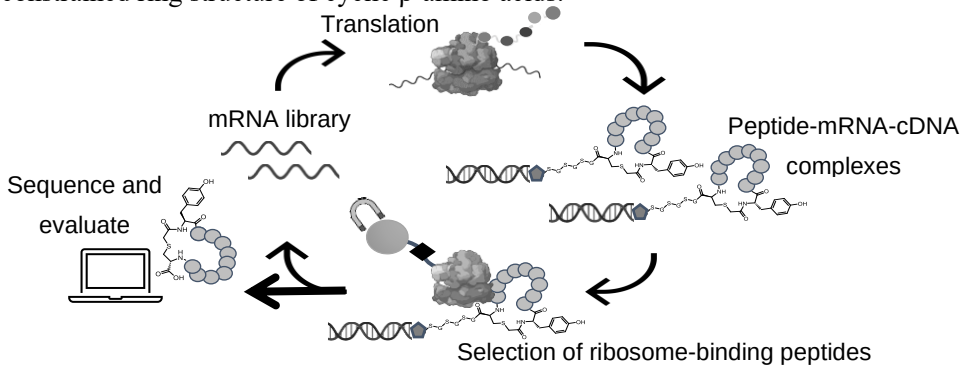
In vitro selection of antibiotic peptides that inhibit the bacterial ribosome

(*[1]The University of Tokyo Graduate School of Science Department of Chemistry, [2]Institut Européen de Chimie et Biologie (IECB)*) ○Rei Takahashi,[1] Takayuki Katoh,[1] Axel Innis,[2] Hiroaki Suga[1]

Keywords: Molecular biology; Drug discovery; Antibiotics; Peptides; Bacterial ribosome

The ribosome is a complex molecule found in living organisms responsible for translating genetic information encoded in mRNA into proteins. Although ribosomes exist in both prokaryotes and eukaryotes, there are structural differences that can be ideal target locations for an effective antibiotic – thus, the bacterial ribosome is a target for more than half of the antibiotics available today.[1] Traditional discovery platforms heavily screened soil-derived antibiotics which led to the discovery of numerous antibiotics used in clinical settings today; however, this once successful platform is now unreliable as the speed of resistance is much faster than the discovery of new antibiotics classes.[1] This research explores *de novo* discovery of macrocyclic peptides as a new platform for antibiotic discovery and development by utilizing an mRNA display-based method called the Random nonstandard Peptide Integrated Discovery (RaPID) that can achieve up to a 10^{13} chemical diversity.

To conduct RaPID screening, a peptide library with ClAc-L/D-Tyr at the N-terminus, a repeat of six to fifteen random α-amino acid region, and a free cysteine at the C-terminus were constructed. Macrocyclization was achieved through nucleophilic substitution of the thiol of a free cysteine residue with the initiator chloroacetyl moiety. Several rounds of RaPID screening were conducted to enrich the diverse libraries to identify the high-affinity ribosome-peptide binders. Through the biochemical evaluation of candidate antibiotic peptides, five peptides were found that exhibited successful binding and inhibitory activity. To explore more potent antibiotic peptides, we are currently attempting to incorporate cyclic β-amino acids as previous work from our lab has shown increased binding affinity to the target of interest due to the constrained ring structure of cyclic β-amino acids.[2]



[1] Lin, J.; Zhou, D.; Steitz, T. A.; Polikanov, Y. S.; Gagnon, M. G. *Annu. Rev. Biochem.* **2018**, *87* (1), 451–478. [2] Katoh, T., Sengoku, T., Hirata, K. *et al. Nat. Chem.* **2020,** *12,* 1081–1088.