Ribosomal incorporation of cyclic γ-amino acids into peptide libraries for drug screening

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γ -amino acids are found in some natural bioactive peptides and play important roles in inducing unique secondary structures of peptides to form stable helices, and improving proteolytic stability of peptides. Especially, cyclic γ-amino acids (cγAA, Figure) are ideal building blocks to stabilize structures of peptides and improve their binding affinity against target proteins because of their constrained structures. Therefore, incorporation of cγAAs into a random peptide library and its application to in vitro selection of active ligands would be a powerful approach for discovering novel peptide drugs. However, the diversity of peptide libraries prepared by conventional chemical synthesis is generally limited to only 10^6. On the other hand, those prepared by ribosomal synthesis can achieve more than 10^{13} diversities and are applicable to in vitro screening methodologies.[1]

Recently, our group reported the ribosomal incorporation of cγAAs into model peptides using a reconstituted translation system assisted by flexizyme technology, referred to as the Flexible In vitro Translation (FIT) system.[2] This system allows for construction of peptide libraries containing nonproteinogenic amino acids with vast variety and for in vitro selection of peptides in combination with the mRNA display technique, Random nonstandard Peptides Integrated Discovery (RaPID) system. Here, by optimizing the reaction conditions in FIT system, we succeeded in incorporating cγAAs into peptide libraries, which were applied to the RaPID selection against Interferon-gamma receptor 1. As a selection result, peptides containing cγAA were obtained with strong binding affinity against the target protein (K_D = up to 38.7 nM).

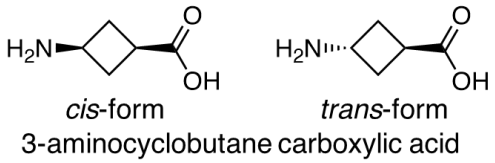

Figure Cyclic γ-amino acids used in this study

[1] Yamagishi, Y.; Shoji, I.; Miyagawa, S.; Kawakami, T.; Katoh, T.; Goto, Y.; Suga, H. *Chem. Biol.* **2011**, *18*, 1562.
[2] Katoh, T.; Suga, H. *J. Am. Chem. Soc.* **2020**, *142*, 4965.