Expanding the versatility of RNA hacking technology by G supply Staple oligomer

([1] *Faculty of Advance Science and Technology, Kumamoto University,* [2]*StapleBio Inc.*) ○Tomoki Kida,[1] Yousuke Katsuda,[1,2] Yua Hasegawa,[1] Miko Kato,[1] Mahiro Ohtani,[1] Shin-ichi Sato, [1,2] Yusuke Kitamura,[1] Toshihiro Ihara,[1]

Keywords: *Nucleic acids medicine; RNA G-quadruplex; RNA hacking*

Nucleic acid medicines facilitate the expeditious design of seed molecules and hold promise for the development of medicines to treat rare diseases. Among these, small interfering RNA (siRNA), which leverages RNA interference (RNAi), forms a complex with Argonaute proteins to degrade target mRNAs, thereby providing a powerful gene-silencing tool. Despite its versatility in targeting a wide range of genes, siRNA suffers from reduced silencing efficacy when non-natural nucleic acids are incorporated and raises concerns about off-target effects due to unintended interactions with non-target mRNAs.

We previously described the development of RNA hacking (RNAh), a new technology that induces the formation of RNA G-quadruplexes (rG4) on target mRNAs using short nucleic acids named Staple oligomer. The rG4 structures induced by Staple oligomers inhibit the ribosomal translation process. Staple oligomer, in repressing protein expression level, differ from existing nucleic acids medicine mechanisms like siRNA in that they do not rely on endogenous enzyme such as Argonaute. This independent mechanism allows them to maintain their gene-silencing efficacy even in cases where non-natural nucleic acids are incorporated as components of the Staple oligomer. While this provides a significant advantage in terms of efficacy, there remains a challenge regarding the number of genes RNAh can target effectively. Bioinformatics analysis indicated that the previously reported Staple oligomers are applicable to only about 65% of all genes, indicating a significant limitation. To expand the applicability of RNAh, it is necessary to develop methods for targeting a broader range of mRNAs.

In this presentation, we are going to report G-tracts supply Staple oligomer (Gs Staple oligomer), which have been designed with the objective of enhancing the versatility of RNAh. Gs Staple oligomer incorporate G-tract sequences that are essential components of rG4, thereby reducing the sequence constraints of target mRNAs (**Fig. 1a**). Furthermore, we demonstrated that Gs Staple oligomer, designed for *m*TRPC6 mRNA, exhibit gene silencing effects *in vivo* (**Fig. 1b**). In addition to these findings, we will report how modifying the number of linkers between the two G-tracts in the Gs Staple oligomer allows for the fine-tuning of the gene silencing effect.

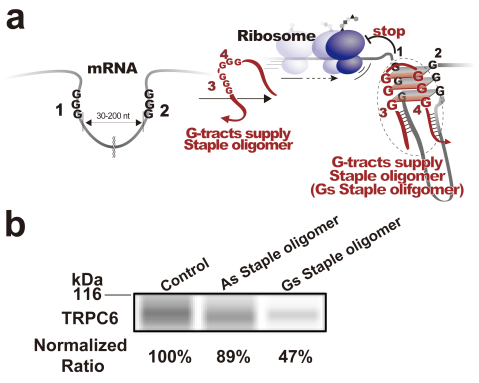

Fig. 1 a. Mechanism of action G-tracts supply Staple oligomer. **b.** Evaluation of protein expression levels of *m*TRPC6 *in vivo* by western blotting. As Staple oligomers are sequences in which G-tracts are replaced with adenine.