Orthogonal signal amplification circuit composed of acyclic nucleic acid for RNA visualization

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Keywords: Hybridization chain reaction; Orthogonality; RNA detection

By utilizing a simple concept of toehold-mediated strand displacement, hybridization chain reaction (HCR) circuit enables signal amplification targeting nucleic acids. However, application of DNA-HCR *in vivo* is limited due to unintended cross-hybridization and degradation by nuclease.

Previously, our group has demonstrated high orthogonality of left-handed *acyclic* D-threoninol nucleic acid (D-*a*TNA) against D-DNA and D-RNA.[1] Serinol nucleic acid (SNA), composed symmetric linker with no helical preference, could act as interface that enabled activation of D-*a*TNA circuit by D-RNA (figure 1a).[2]

We report a novel HCR circuit composed of D-*a*TNA. Since D-threoninol cannot be recognized by nuclease, high enzymatic resistance has been confirmed. Because of strong base-paring interaction, *a*TNA hairpin with short stem (7-mer) ensured clear ON-OFF control of the HCR circuit. Right-handed *acyclic* L-threoninol nucleic acid (L-*a*TNA) HCR circuit was also designed, and high orthogonality between D- and L-*a*TNA HCRs was confirmed by activation of each *a*TNA HCR via corresponding input strand. Finally, D-*a*TNA HCR was applied to RNA-dependent signal amplification system via SNA-interface (figure 1b). Incorporation of C3-spacer into SNA-interface solved inhibition of activating D-*a*TNA HCR by a propagation of helicity, achieving fast signal amplification (data not shown). This work represents the first example for conducting heterochiral HCR circuits, which is potential for direct visualization of RNA *in vivo*.

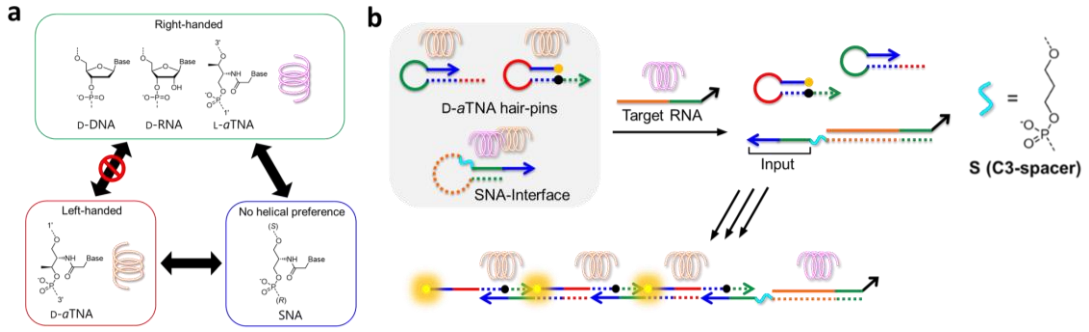

Figure 1 (a) Chemical structures, helicities, and hybridization compatibilities of DNA, RNA and acyclic XNAs. (b) Schematic illustration of SNA-mediated D-*a*TNA HCR triggered by RNA.

1) Murayama, K.; Kashida, H.; Asanuma, H. *Chem. Commun.* **2015,** *51*, 6500-6503. 2) Murayama, K.; Nagao, R.; Asanuma, H. *ChemistrySelect* **2017**, *2*, 5624–5627.