

Nucleic Acids Chemistry beyond the Watson-Crick Double Helix (75): Development of RNA-ligand pairs for multicolor RNA imaging in cells

(¹FIBER, Konan University, ²Sun Yat-sen University, ³FIRST, Konan University) ○Tamaki Endoh¹, Jia-Heng Tan,² Shuo-Bin Chen², Naoki Sugimoto^{1,3}

Keywords: RNA; Aptamer; Selection; Imaging; Broccoli

Recent successes in RNA selection toward chemicals that mimic chromophore of fluorescent protein has intensified development of light-up RNA aptamers, which drastically enhance fluorescence of small chemicals. For examples, Spinach, Broccoli, and their derivatives, which interact with a 3,5-difluoro-4-hydroxybenzylidene imidazolinone (DFHBI), enabled real-time imaging of RNA in living cells.¹

Based on a structural analysis, the binding pocket of DFHBI in Spinach was revealed to consist of U-A-U triad and G-quartet. DFHBI is sandwiched by the triad and quartet interacting with a nucleobase connecting the triad and the quartet.² Here, we envisioned that the basic structure core of the binding pocket would be suitable scaffold for accommodating various chemicals as well as DFHBI. Especially, G-quadruplex ligands, some of which show unique light-up properties, likely be accommodated with relatively high affinity because the core contains G-quartet unit. We have recently demonstrated a simple optimization technology of the light-up aptamer by using RNA-capturing microsphere particles (R-CAMPs), which immobilize DNA and RNA clones of identical sequence on the surface of the same particle.³ In this study, we tried simultaneous selection of light-up aptamers, which emit fluorescence in different colors, by using a mutated Broccoli and a mixture of G-quadruplex ligands and DFHBI as an initial RNA library and target ligands, respectively (Figure). Two of three light-up aptamers selected successfully functioned in cells. In addition, by combinational use with another RNA aptamer targeting malachite green, we have succeeded triple color imaging of different RNAs in cells.

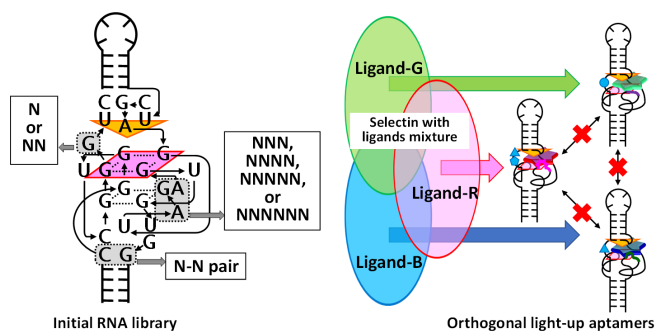


Figure. Schematic illustration of RNA derivatization and selection of orthogonal light-up aptamers.

1) a) S. R. Jaffrey *et al.*, *Science*, **2011**, 333, 642. b) S. R. Jaffrey *et al.*, *J. Am. Chem. Soc.*, **2014**, 136, 16299. 2) A. R. Ferré-D'Amaré *et al.*, *Nat. Struct. Mol. Biol.*, **2014**, 21, 658. 3) a) N. Sugimoto *et al.*, *Small*, **2019**, 15, 1805062. b) T. Endoh and N. Sugimoto, *Anal. Chem.*, **2020**, 92, 7955.