Drug discovery, Medical & healthcare

# Synthetic small molecules for photocontrol of RNA structure and function

Department of Regulatory Bioorganic Chemistry, SANKEN (The Institute of Scientific and Industrial Research)

Associate Professor Chikara Dohno

Researchmap https://researchmap.jp/DC000016?lang=en

## Abstract

Transcribed single-stranded RNA folds to form various higher-order structures, which are responsible for the functions of RNA in cells. In order to control the functions of the target RNAs, we have been developing synthetic RNA-binding small molecules (ligands) that induce the conformational change of the RNA upon binding. We herein have created new photoresponsive RNA-binding ligands whose RNA-binding function can be turned ON and OFF by photoirradiation. The photoresponsive RNA-binding ligand allowed us to spatiotemporally control the higher-order structure and function of the ribozyme in cells by photoirradiation.

## Background & Results

We have developed a series of RNA binding small molecular ligands that induce the conformational change of the target RNA upon their binding. Since the RNA's higher-order structures are often responsible for the biological functions, the RNA binding ligands can modulate the RNA's function. Photocontrol is a useful technique to control the target of interest precisely by external light stimuli. By introducing the well-studied photochromic azobenzene unit into the RNA binding ligand, we successfully synthesized a photoresponsive RNA binding ligand named NCTA. NCTA reversibly undergoes photoisomerization from E to Z and Z to E configuration by 365 and 460 nm light, respectively. Z-NCTA binds to the specific RNA target, while E-NCTA does not; that is, RNA binding properties of NCTA are controlled by photoirradiation. We applied NCTA to control the structure and function of a hammerhead ribozyme, which is an RNA-cleaving enzyme made of RNA. Since proper folding of the ribozyme to form the higher-order structure is responsible for the catalytic RNA-cleaving activity, the ligand that can bind to the ribozyme and induce the conformational change can be a molecular switch for activation and inactivation of the ribozyme. Indeed, NCTA activates the ribozyme by 365 nm photoirradiation and inactivates the once-activated ribozyme by 460 nm light. The NCTA's photoswitching capability is effective in cells. In NCTA-treated cultured human cells, gene expression from engineered mRNA containing the ribozyme is reversibly controlled by photoirradiations.

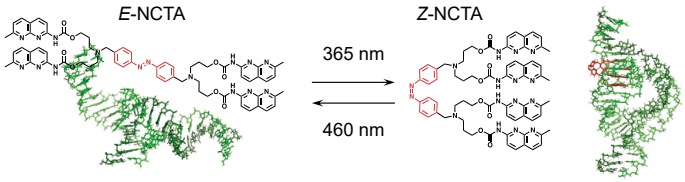


Figure 1. RNA binding ligand NCTA undergoes photoisomerization by 365 nm and 460 nm light.

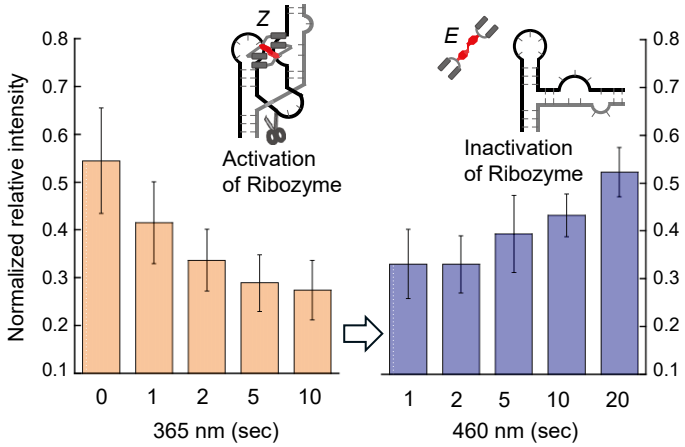


Figure 2. NCTA controls the gene expression level by activation (365 nm) and inactivation (460 nm) of ribozyme.

## Significance of the research and Future perspective

More than 70% of the human genome is transcribed to produce RNAs, while less than 5% of these RNAs encode proteins. Targeting RNA offers effective ways to modulate biological functions. Synthetic RNA binding ligands that can induce conformational changes of target RNA upon their specific binding are an important class of molecule to modulate functions arising from functional and structural RNAs. Photoresponsive RNA binding ligands allow us to control RNA functions in a spatial and temporal manner, which can be useful molecular probes to elucidate RNA functions and future photopharmacological drugs.

Patent

Treatise Dohno, Chikara et al. Photoswitchable molecular glue for RNA: reversible photocontrol of structure and function of the ribozyme. Nucleic Acids Res. 2023, 51, 9533-9541. doi: 10.1093/nar/gkad690
Dohno, Chikara; Kimura, Maki; Nakatani, Kazuhiko. Restoration of ribozyme tertiary contact and function by using a molecular glue for RNA. Angew. Chem. Int. Ed. 2018, 57, 506-510. doi: 10.1002/anie.201709041

URL

Keyword RNA, ribozyme, RNA-binding small molecule, synthetic biology