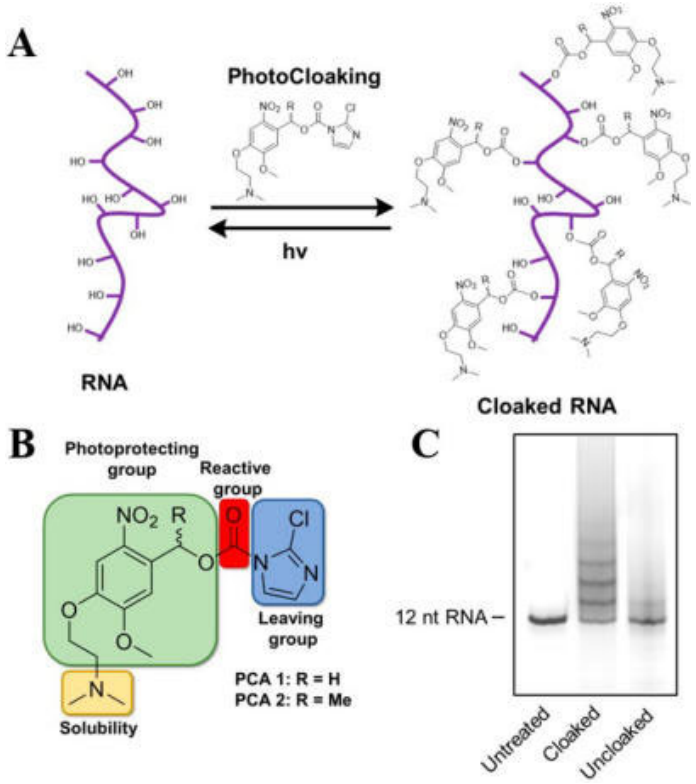
Docket #: S17-479

Photoreversible Acylation Reagents for Controlling RNA

Researchers at Stanford have developed light-cleavable cloaking agents to control and study RNA activity in vitro and in vivo. Their simple, one-step postsynthetic technique for switching RNA structure and function ON/OFF utilizes novel acylating agents that can "cloak" RNA by adding photoreversible blocking groups. The cloaked RNA cannot fold, hybridize or perform other biological functions. Later, the RNA can be easily switched on ("uncloaked") with short-wave light, reverting the RNA to its native, active form so that its function can be analyzed. Unlike other photocaging technologies, **these reagents and methods do not require any special synthesis or equipment**. Another major advantage is this method **can be used to cloak any RNA, regardless of length**, enabling studies of long, biologically relevant molecules (mRNA, lncRNA etc.) that are difficult or impossible to synthesize via solid-state oligonucleotide synthesis. Use of the new approach on a transcribed 237 nt RNA aptamer has demonstrated the utility of this method to switch on RNA folding in a cellular context, and underlines the potential for application in biological studies.



Photocloaking agents and cloaking strategy. *(A) Schematic of the photocloaking strategy; polyacylation of 2'-OH groups in an existing RNA blocks RNA structure and function, while photoexposure (365 nm) removes these blocking groups. (B) Structures of PCA reagents 1 and 2. (C) Gel electrophoretic analysis of untreated, cloaked, and uncloaked RNA.*

Stage of Research

The inventors photocaged a transcribed a 237 nt Broccoli RNA aptamer and demonstrated the ability of this acylating agent to switch on RNA folding in a cellular context by exposing cells to light. In this experiment, cloaked RNA showed no measurable signal above background while subsequent light exposure restored aptamer function. This method showed ON/OFF behavior that was comparable or better than conventional photocaged phosphoramidites.

Applications

- **RNA drug development** - evaluating effects of therapeutic RNA candidates with precise temporal control of RNA function
- **Biology/biochemistry research:**

    - study function of particular RNA molecules in cells (e.g, gene expression, catalysis and cell signaling)
    - stabilize RNA for future experimental use

Advantages

- **Convenient, simple techniques:**
    - reagents and methods do not require any special synthesis or equipment
    - one-step photoprotection
    - postsynthetic labeling of RNAs that are difficult or impossible to synthesize via solid-state oligonucleotide synthesis
- **Large RNA molecules:**
    - cloaking can be performed on any RNA, regardless of length (not limited to short, synthetic RNAs)
    - ability to cloak messenger RNA and other, long, biologically relevant RNAs
    - demonstrated on photocaged RNA aptamer
- **Non-toxic** - compatible with in vivo applications

Publications

- W.A. Velema, A.M. Kietrys, and E.T. Kool RNA Control by Photoreversible Acylation *J. Am. Chem. Soc.* 2018, 140, 3491-3495.

Patents

- Published Application: 20190264205
- Issued: 11,572,557 (USA)

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