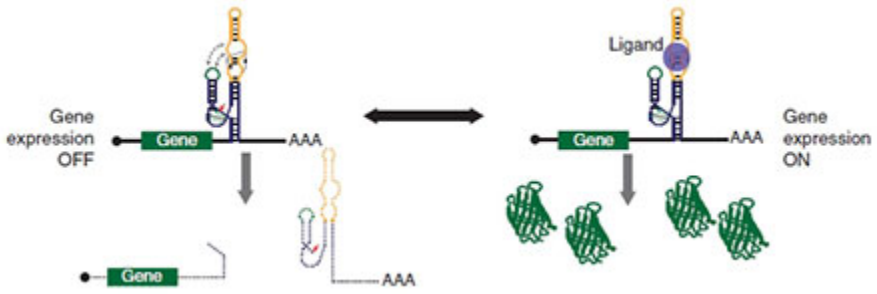
Docket #: S15-170

Synthetic ribozyme-based architecture for fast-switching, robust gene regulation

Researchers in Prof. Christina Smolke's laboratory have engineered an architecture for a sensitive, robust RNA device that can control gene expression through fast-acting tertiary interactions with a range of ligands. This system integrates a ligand-binding RNA sequence with the self-cleaving sequence of a hammerhead ribozyme. The resulting synthetic ribozyme can be inserted into a user-specified gene that allows users to control genes or complex gene circuits through ligands that deactivate cleavage activity (and thereby allow expression of the gene attached to the RNA device). Because the system relies on ligand interactions with the tertiary structure of the ribozyme, it is a faster acting switch with a wider dynamic range than previous RNA devices (which relied on secondary ribozyme structure). In addition, it is not limited to natural protein-binding RNA sequences for ligand binding. This technology could be used as a gene switch to program cell behavior for research, gene therapy, cell therapy or cell-based biomanufacturing.



RNA device regulatory mechanism. *The RNA device is encoded into the 3' UTR of a gene, such that device cleavage results in transcript destabilization and reduced expression levels. Binding of ligand (blue circle) to the RNA device disrupts tertiary interactions required for self-cleavage, thereby stabilizing the transcript and upregulating gene expression.*

Stage of Research
The inventors have determined consensus sequences that enable ligand-responsive tertiary interactions and demonstrated that the resulting devices perform better than previous devices in terms of gene silencing, activation ratio, and ligand sensitivity. They have also used these tools to build a library of biosensors for diverse ligands.

Applications

- **Gene switching** - controlled gene expression to modulate complex gene circuits, with end user applications such as:
  - gene therapy
  - cellular therapy
  - cellular systems for biomanufacturing
- **Research** - molecular level control for in vitro biochemical assays to elucidate sequence-structure-function relationships

Advantages

- **Fast switching** - activated through the tertiary structure of the ribozyme, resulting in faster actuation than previous technologies which rely on secondary structure
- **Wide dynamic range/activation ratio**
- **High ligand sensitivity**
- **Variety of ligand options** - RNA device is not limited to any particular type of ligand (i.e., small molecule, peptide, protein, oligonucleotide)

Publications

- Townshend, B., Kennedy, A. B., Xiang, J. S., & Smolke, C. D. "High-throughput cellular RNA device engineering." *Nature Methods* 12, 989–994 (2015).

Patents

- Published Application: WO2017003726
- Published Application: 20180187192
- Published Application: 20200277601
- Issued: 10,513,702 (USA)

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