Docket #: S15-015

Platform for Engineering Molecular Sensor RNA Devices

Researchers in Prof. Christina Smolke's laboratory have developed a robust, high-throughput directed evolution platform to design and discover RNA devices that can sense and respond to a diverse range of target ligands (e.g., chemicals, metabolites, proteins, cell-specific signals) in real-time. This automated system uses a robotic platform to measure activities of hundreds of thousands of sequences from libraries of functional RNA sequences (aptamers coupled with genetic control elements) in the absence or presence of the molecule of interest under identical conditions. The selection assay uses ligand molecules in their natural state (not immobilized) and does not require any prior knowledge of the ligand structure. Because the aptamers and their control elements are jointly optimized and function through conformational changes in their tertiary structure, the resulting programmable RNA-based sensors are more sensitive to their target ligands with better activation ratio than previous RNA devices. These molecular sensors can then be utilized to sense and respond to the presence of other molecules in a wide range of end-user applications, such as synthetic biology, cell or gene therapy, research or diagnostics.

Stage of Research

The inventors have validated their high-throughput platform to build biosensors for diverse ligand. The resulting RNA devices performed better than traditional (secondary structure) RNA devices in terms of gene silencing, activation ratio, and ligand sensitivity.

Related Inventions

Compositions and methods for regulation of gene expression with and detection of folinic acid and folates (Stanford Docket S14-300)
Synthetic ribozyme-based architecture for fast-switching, robust gene regulation (Stanford Docket S15-170)

Applications

- **RNA-based molecular biosensors and gene switches** - high-throughput platform to generate novel RNA devices that sense and respond to other molecules

Advantages

- **Robust, scalable, high-throughput process:**
  - rate of ~2 hours/cycle and less than 1 week total elapsed time to develop a new sensor to a target of interest
  - solution-based process can be automated and implemented on a robotic platform (no complex separation steps)
  - readily parallelizable – measures activities of hundreds of thousands of sequences from RNA device libraries in the absence or presence of ligands
- **Selection for diverse molecules in natural state:**
  - target ligand is not immobilized
  - can sense unidentified or unisolated targets – no structural or detailed knowledge of targets are needed
  - generates new aptamers in context with switching components such that they work together
- **Advantages of RNA devices** created with this system:
  - fast switching based on conformational changes in tertiary structure of RNA molecule
  - better ligand sensitivity, dynamic range, gene silencing, and activation ratio compared with than traditional RNA devices

Publications

- Townshend, Brent, et al. A multiplexed, automated evolution pipeline enables scalable discovery and characterization of biosensors. Nature communications 12.1 (2021): 1-15.
- Townshend Brent, et al. High-throughput cellular RNA device engineering. *Nature Methods.* 12 (2015): 989–994

Patents

- Published Application: WO2017030659
- Published Application: 20180223274
- Published Application: 20200362333
- Issued: 10,689,642 (USA)
- Issued: 11,293,020 (USA)

Innovators

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