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# Method for massively-parallel screening of aptamer switches

Researchers at Stanford have developed a massively parallel screening strategy to screen for target-responsive molecular switches.

Target-responsive RNA- and DNA-based molecular switches have potential utility in a variety of technological applications. Such biosensors are typically based on aptamers that undergo a reversible structure-switching mechanism that is coupled to a detectable readout. Unfortunately, it remains a challenge to generate novel aptamer switches, because most aptamers assume a stably folded structure and do not undergo a binding-induced conformation change. Most approaches to engineer aptamer switches rely on rational design and require prior knowledge of aptamer structure. Such detailed structural characterization has only been achieved for a relatively small number of aptamers. Furthermore, even the most advanced modeling software fails to account for non-canonical base-pairing or can correctly predict three-dimensional folding, which can be critical for target recognition and binding.

**Stage of Research**
The inventors have developed a system for rapid and massively parallel screening of different switch scaffolds in a single experiment, circumventing the need for rational design. Building upon their recently developed non-natural aptamer array (N2A2) system, the method enables the synthesis and screening of an array of fluorophore-labeled anchored displacement strand (ADS) switch constructs directly onto the flow-cell of a modified Illumina MiSeq instrument. Target-responsive molecular switches are identified by sequential imaging of flow-cell with and without the target molecule, and signal from library members can be linked to their nucleotide sequence by the location of the signal. Imaging data from each ADS construct cluster reveals the presence of switches for which target binding results in increase (signal-on) or decreased (signal-off) fluorescence. This method thereby identifies

nucleic acid sequences that are able to act as target-responsive molecular switches. The inventors demonstrate the power of their method in identifying novel molecular switch sequences with a well-characterized ATP aptamer, including several sequences with non-canonical base-pairing. This approach bypasses the time-consuming process of structural analysis, rational design, and optimization by screening nearly every possible 10-nucleotide switch domain sequence in a single assay.

**Stage of Development**
Research – in vitro

## Applications

- Massively parallel screening of target-responsive nucleic acid-based molecular switches
- Screening strategy for converting aptamers to target-specific molecular switches

## Advantages

- Method could greatly accelerate the development of target-responsive molecular switches from existing aptamers without any a priori structural knowledge
- By covering the full range of available sequence space for the switch domain, screen can rapidly identify promising aptamer switch constructs that would be otherwise overlooked in rational design approaches
- Screening methods validated with natural DNA-based aptamers and aptamers containing chemically modified bases
- Methods do not need to start with a known aptamer sequence

## Patents

- Published Application: WO2023086335

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