Development and optimization of strand displacement based conditional small interfering RNAs for operation inside mammalian cells

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A longstanding goal for nucleic acid nanotechnology[1, 2] and biomolecular computing[3] is the development of conditionally activated oligonucleotide therapeutics that can detect and respond to cellular expression of specific genes[2, 3].

Nucleic acid switches based on toehold mediated strand displacement[4] have executed logic operations and detected RNA transcripts in both bacteria[5] and mammalian cells[2, 6], but the conditional activation of oligonucleotide drugs by RNA transcripts in mammalian cells has not been convincingly demonstrated. Significant challenges include poorly suppressed background drug activity, weak ON state drug potency, input and output sequence overlap, high design complexity, short device lifetimes (< 24 hours) and high required device concentrations (> 10 nM).

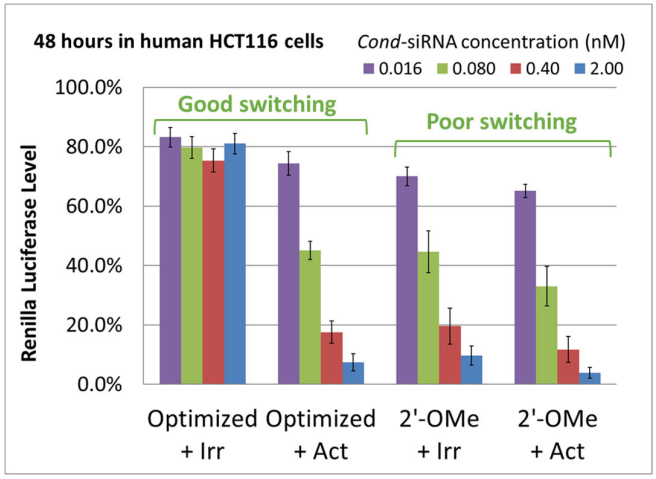

Figure 1: RNAi activity of *Cond*-siRNAs with fully optimized sensors or 2'-OMe only sensors in human cells expressing irrelevant (Irr) or correct (Act) RNA inputs. Data is from three biological replicates. Expression levels of the target (Renilla Luciferase) are normalized to cells with no transfected siRNA.

We have now overcome all of these problems to develop a programmable, *conditionally activated* small interfering RNA (*Cond*-siRNA). These simple riboswitches can maintain their integrity over days in the mammalian cytosol, and detect RNA transcripts from specific **input genes** via toehold mediated strand displacement. Upon input detection, they can release potent RNAi triggers[7] silencing specified **target genes** with *completely independent sequences* from the input. We tested the switching activity of dozens of *Cond*-siRNA variants in human adherent cells to identify necessary and sufficient chemical modification motifs that allow good device performance over diverse input: output combinations. Some optimized *Cond*-siRNAs achieved more than 90% silencing of target genes (protein expression versus baseline) in cells expressing sequence-matched RNA transcripts, and strongly suppressed background RNAi activity (< 25% knockdown) in cells expressing mismatched inputs (e.g. Fig. 1).

Our findings provide a set of clear and simple guidelines for substantively improving the performance of strand displacement switches in live mammalian cells. The *Cond*-siRNA technology provides a plausible platform for gene expression activated RNAi smart drugs.

References

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