

Target Capture SELEX (TACS) enables the design of U1 snRNP riboswitches for conditional gene expression control in mammalian cells

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Background:
U1 snRNP is a key ribonucleoprotein that orchestrates spliceosome assembly and prevents premature intronic polyadenylation (pA). Redirecting U1 snRNP to specific mRNAs enables downregulation of target gene expression with therapeutic potential. To achieve conditional control, U1-based riboswitches (RS) have been engineered by coupling U1 recognition sites with tetracycline (TC)-responsive aptamers.

Methods:
We combined U1-guided inhibition of pA with TC-dependent aptamers to generate drug-responsive U1 RS. In parallel, we developed a modified capture SELEX strategy, termed Target Capture SELEX (TACS), in which the RS expression platform itself acts as the docking sequence for ligand-dependent RNA enrichment. This approach enables the unbiased discovery of aptamers whose conformational change directly affects U1 RS functionality.

Results:
Optimized U1 RS achieved up to 100-fold inhibition and 30-fold TC-mediated induction of target gene expression in cell culture. Using TACS, we identified an improved TC-responsive U1 RS with enhanced activity both in vitro and in vivo. Structural analyses revealed the molecular mechanisms underlying switching capacity. Furthermore, TACS enabled the identification of U1 RS responsive to alternative ligands such as Acyclovir and Raltegravir, even without prior knowledge of binding motifs.

Conclusion:
TACS provides a robust platform for the generation of functional U1-based riboswitches responsive to diverse small molecules. This technology enables precise, drug-dependent regulation of gene expression, with proof-of-concept demonstrated in CAR-T cells, and holds promise for broad therapeutic applications.