HTS platforms for diverse RNA-targeted drug discovery applications

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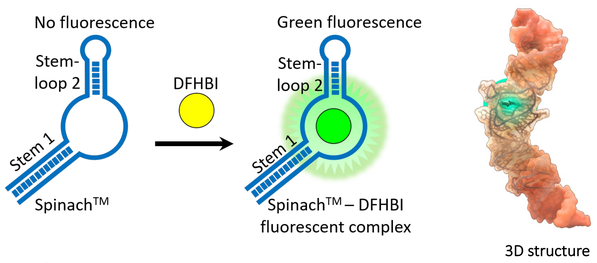
ABSTRACT

Recent advances in drug discovery and the COVID-19 pandemic demonstrates the importance of developing RNA-based vaccines and RNA-targeting therapeutics for human diseases. Nusinersen and risdiplam are two first-in-class spinal muscular atrophy drugs that restored functional motor neuron proteins by targeting RNA splicing. The COVID-19 vaccines demonstrated that mRNA can be used to generate highly efficacious vaccines at unprecedented speed. Advances in RNA structure modeling now allows precise small molecule modulation of RNAs that encode previously undruggable protein targets. The accumulation of a large body of clinical data validating the efficacy of these interventions has prompted significant R&D investments in RNA-focused drug discovery and therapeutic development. However, early-stage hit discovery has been hindered by current assay technologies designed to suit protein targets but not RNA targets. Lucerna, Inc. is leveraging its fluorescent aptamer technology (Spinach™) to enabling target validation and high-throughput screening (HTS) platforms for the purpose of accelerating new RNA-focused drug discovery. Specifically, we have developed a real-time RNA imaging platform that can track mRNA therapeutic delivery, measure RNA half-life, and assess RNA target engagement in cells. Additionally, we have developed the following HTS platforms for identifying hits against specific RNA-focused pathogenic mechanisms: (1) an HTS assay that directly measures changes in transcript levels caused by small molecule modulator of pyruvate kinase mRNA splicing (a key cancer metabolism regulator), (2) an HTS assay that identifies small molecules and/or antisense oligonucleotides that bind the iron-responsive element of α-synuclein and modulate its protein translation in Parkinson's Diseases, and (3) a cellular assay that reports transcriptional activity changes in the presence of RNA Pol III inhibitors and RNA degraders. These HTS assay platforms overcome several major issues in existing RNA-targeted screening technologies such as throughput, use of protein reporters, sequence/structure specificity, and the use of systems that do not accurately represent the natural cellular environment, to name a few. In summary, the Spinach™ technology is an RNA-specific platform that can target diverse disease mechanisms and has the potential to greatly accelerate the discoveries of many first-in-case therapeutics.

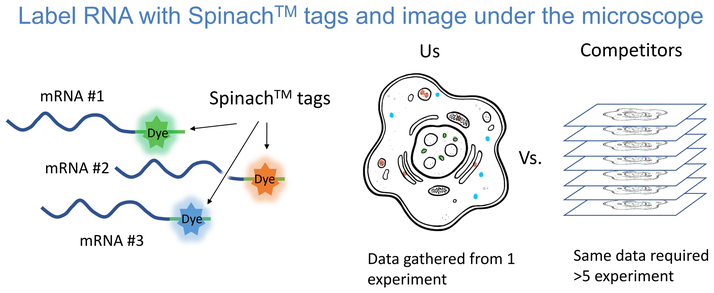
PLATFORM CONCEPT



Spinach™ technology:

- Consists of RNA aptamers that form specific 3D structures that bind and turn on the fluorescence of otherwise non-fluorescent dyes, such as DFHBI.
- RNA versions of fluorescent proteins.
- Different RNA-dye pairs emit different colors: Broccoli, Corn, Spinach, Squash
- Genetically encodable for RNA imaging in cells and can be engineer into sensors for HTS assay applications.

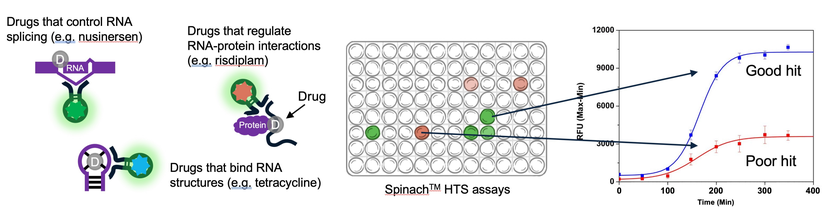
Label RNA with Spinach™ tags and image under the microscope



Applications

- Real-time imaging of mRNA therapeutic delivery
- RNA degrader drug discovery
- Circular RNA quantification
- Transcription inhibition drug discovery

Sensors that report drug interaction with RNA targets

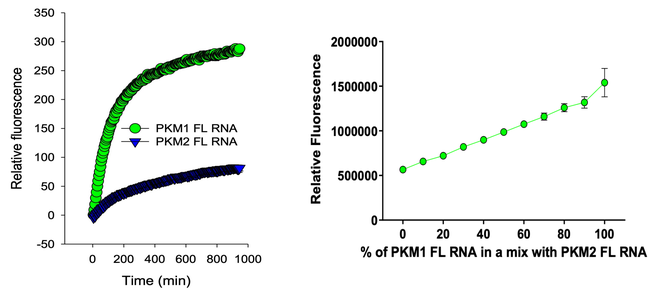


Applications:

- RNA splice modulator drug discovery
- RNA structure drug discovery
- RNA-protein drug discovery *in development*

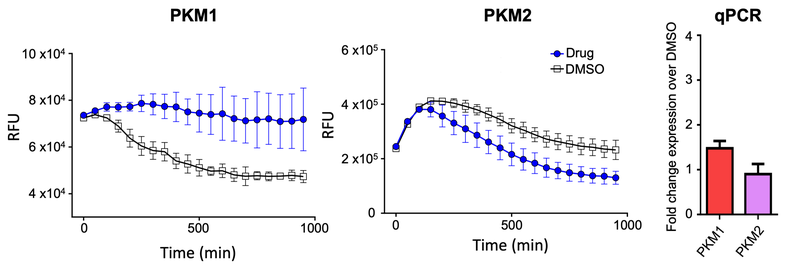
RNA SPLICING

Measures wild-type and alternative splice isoform levels



- Pyruvate kinase splice isoforms PKM1 (Ex8/9) and PKM2 (8/10) regulates cancer metabolism. Isoform switching from PKM2 to PKM1 reverts oncogenic effects.
- Sensors discriminate between PKM1 and PKM2 RNA within a large dynamic range.
- Significant fluorescence changes are detected in mixtures with only 10% splicing changes.

Detects drug-induced changes of endogenous RNA

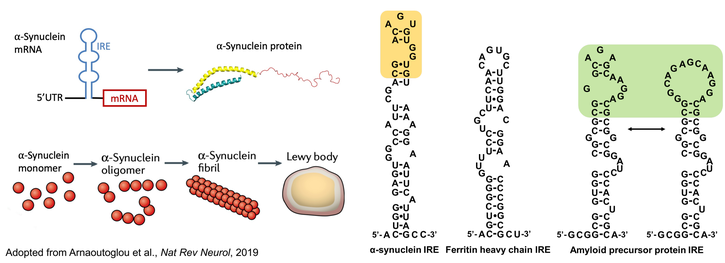


PKM splice HTS assay:

- PKM1 and PKM2 sensors detect splice changes in cells treated for 24 hours with oleanolic acid (drug).
- Fluorescence assay signals correlate with qPCR results.
- Sensors can be engineered to target other spliced isoforms.

RNA STRUCTURE

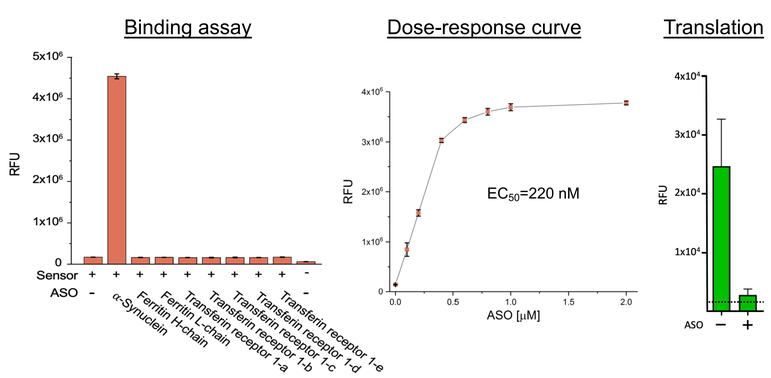
Some RNA structures are critical regulators of protein expression



Adopted from Arnaoutoglou et al., *Nat Rev Neurol*, 2019

- α-synuclein (SNCA) is the key protein in Parkinson's Disease pathology.
- 5'UTR of SNCA contains an iron-response element (IRE) that regulates its translation.
- IREs are stem-loop structures in the 5' or 3' UTR of genes involved in iron metabolism and regulate protein translation.

Sensors reports drug interactions to specific RNA structures



SNCA IRE structure HTS assay:

- Consists of a Spinach™ sensor that emits fluorescence signal upon drugs binding to the SNCA IRE.
- Assay exhibits robust signal only in the presence of ASO that targets IRE sequences unique to SNCA and shown to inhibit SNCA translation.
- Sensors can be engineered to target other disease-modifying RNA structures.

RNA KINETICS

Transcription activity and RNA half-life reporters

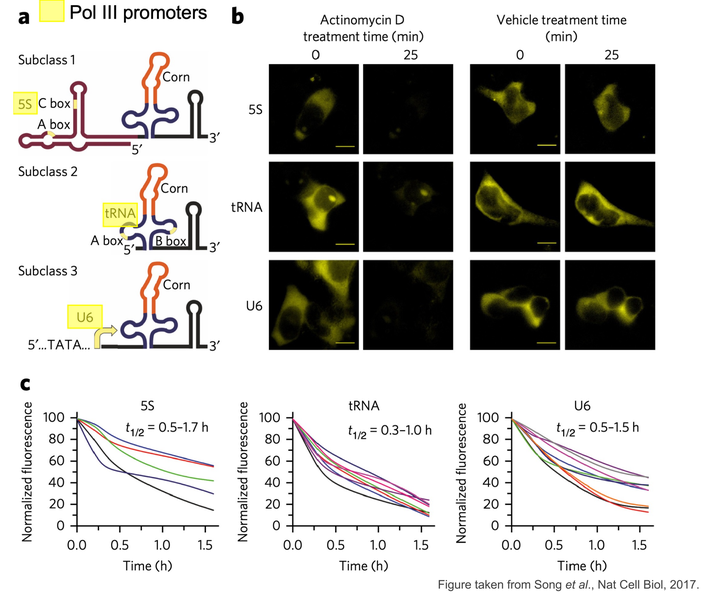


Figure taken from Song *et al.*, Nat Cell Biol, 2017.

- RNA polymerase III (Pol III) promoters transcribe small RNAs that coordinate cell growth and proliferation.
- Reporters containing Corn, a yellow fluorescent Spinach™ tag, can be used to monitor 5S, tRNA, U6 Pol III promoter activities in cells (**a**,**b**).
- In the presence of actinomycin D, a transcription inhibitor, reporter signals can be used to determine RNA half-life (**c**).

Whole-population and single-cell analysis of RNA repression

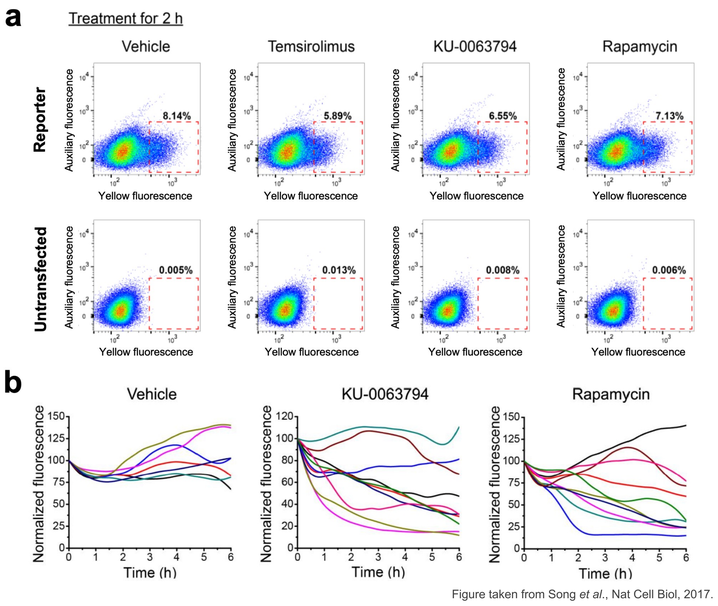


Figure taken from Song *et al.*, Nat Cell Biol, 2017.

Pol III transcription activity HTS assay:

- Flow cytometry analysis reveals the efficacy of whole-population RNA repression by different mTOR inhibitors (**a**).
- Single-cell tracking of reporter signals in HEK cells showed heterogenous responses to KU-0063794 and rapamycin treatment (**b**).
- Spinach™ tags can be used to track mRNA localization and stability in cells.

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