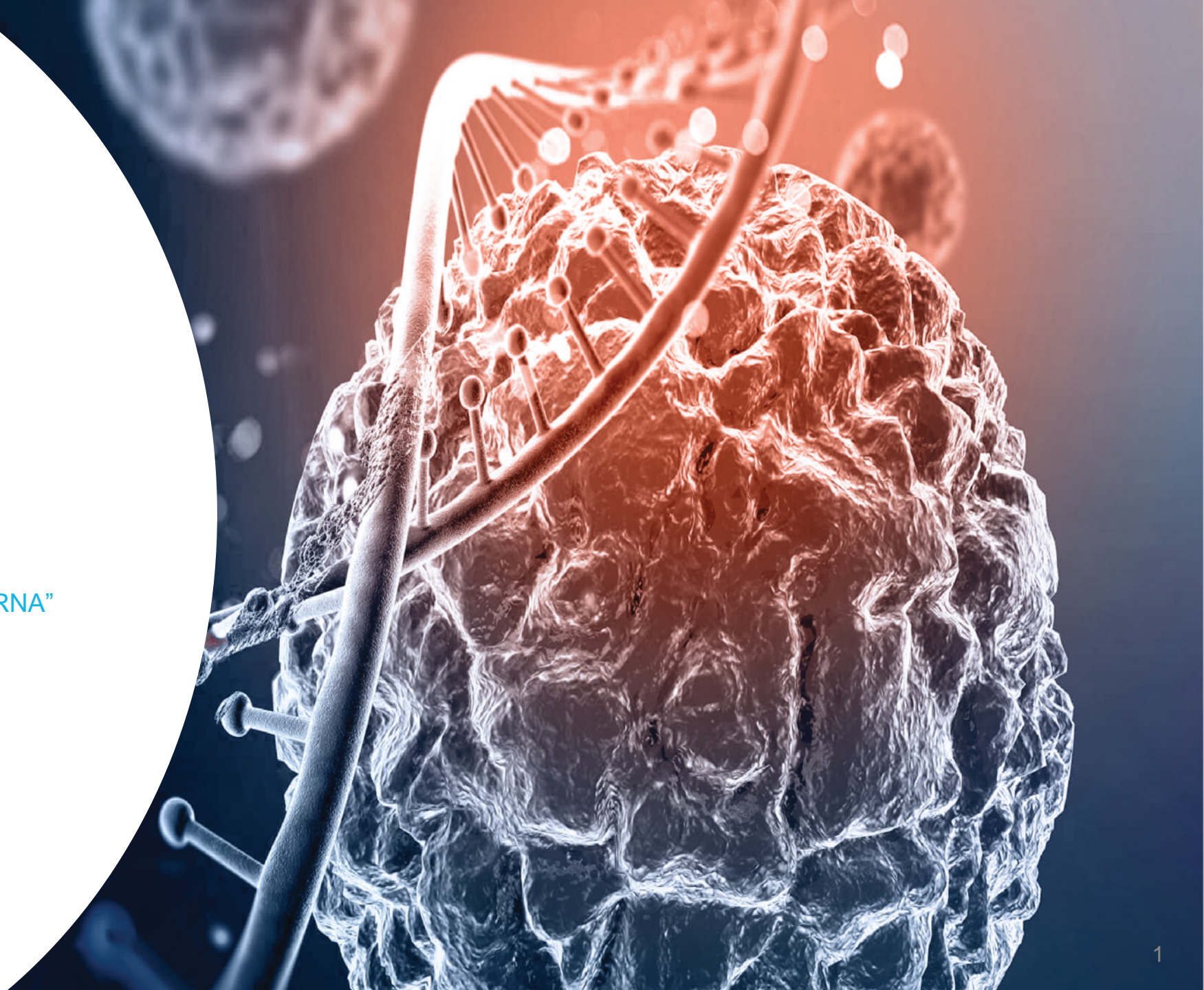
Technology Capability Presentation

"New toolkits to see, study, and drug RNA"

Non-confidential information only

www.lucernatechnologies.com



About us

Who

Lucerna, Inc. is a biotech company with a proprietary fluorescent aptamer technology developed from Dr. Samie Jaffrey's laboratory at Cornell University.

How

Spinach™ is the RNA version of green fluorescent protein (GFP). It is genetically encodable with proven utility for imaging RNA in living cells and measuring cellular biomolecules in a HTS assay format.

What

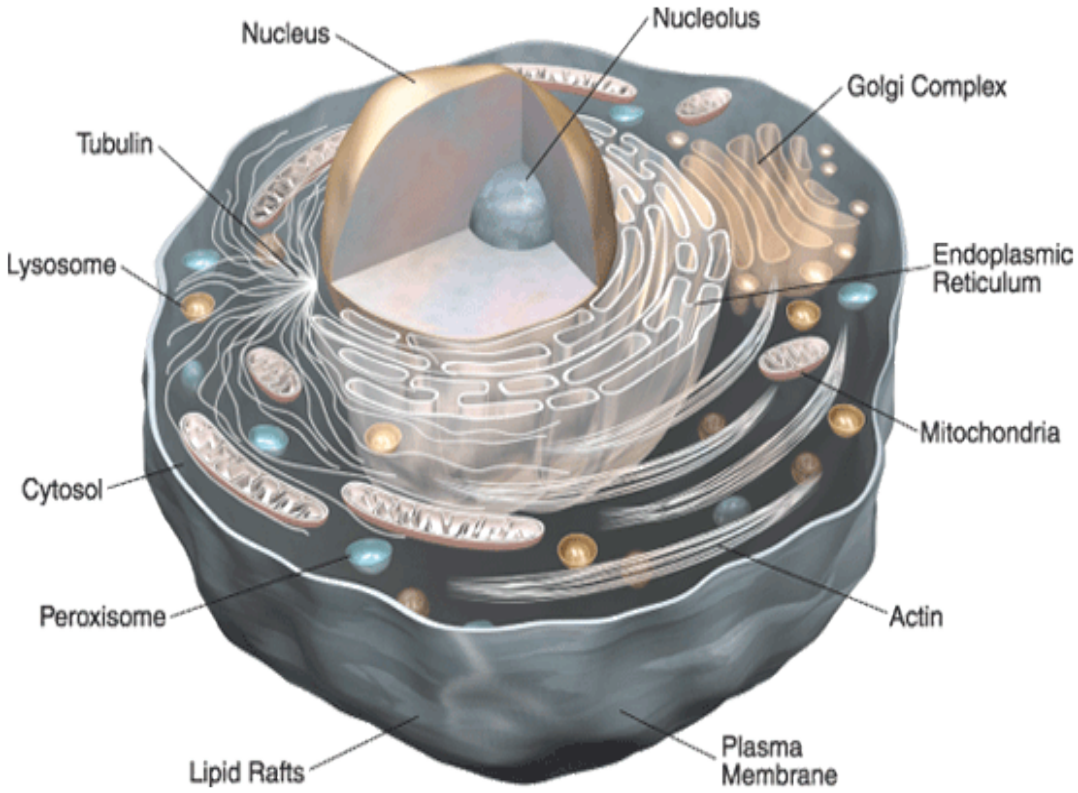
The **Spinach™ Technology** has highly versatile applications:

- **Drug Discovery** – Modular platform, mix-and-read, HTS assays
- **RNA Imaging** – Live-cell imaging of endogenous mRNAs and non-coding RNAs
- **R&D Toolkits** – Customized RNA tools for molecular detection and diagnostics

New

We have now achieved **single-molecule imaging resolution** and has developed a high-throughput compatible **RNA splicing assay** for drug screening.

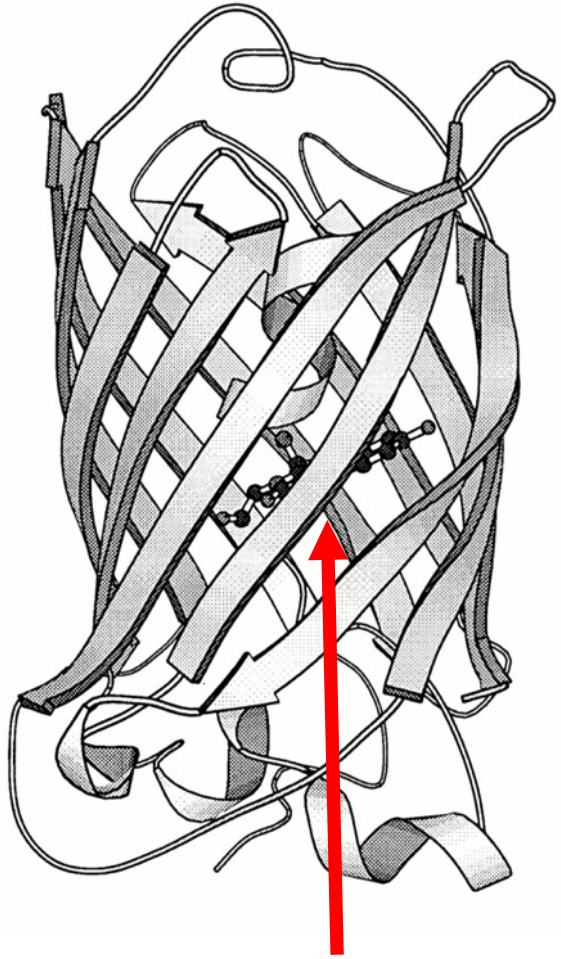
There are many unanswered questions in RNA biology



- How does RNA move in subcellular compartments?
- Where are specific RNAs located in cells?
- What is the timing and intracellular path of newly transcribed RNA?
- What structures are involved in:
  - RNA interference
  - RNA modifications
  - RNA degradation
  - Toxic RNA (trinucleotide-repeat)
  - Noncoding RNA signaling
  - RNA splicing
- How does RNA folding influence RNA functions?

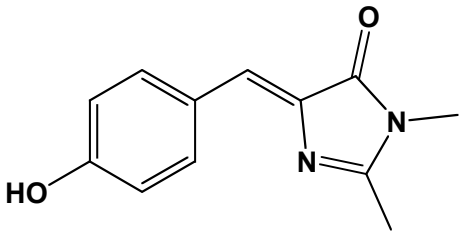
Spinach: RNA mimic of green fluorescent protein (GFP)

GFP's protein structure



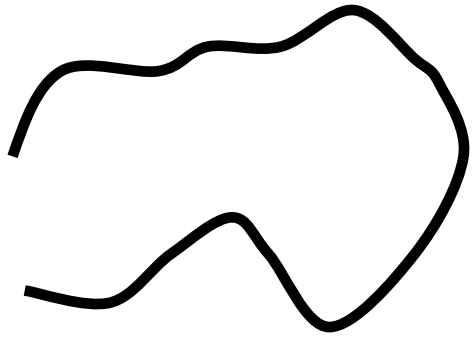
Chromophore enclosed in β-barrel

Chromophore



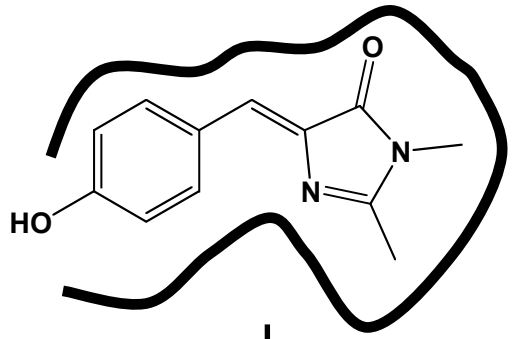
Non-fluorescent

RNA aptamer

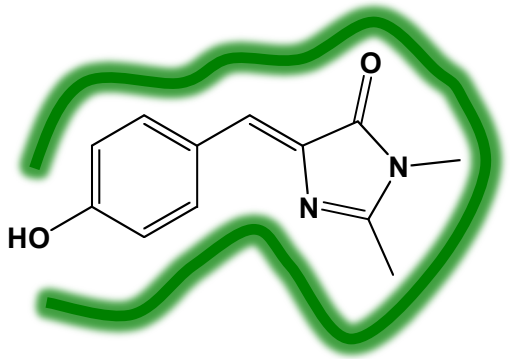


Non-fluorescent

RNA-chromophore complex



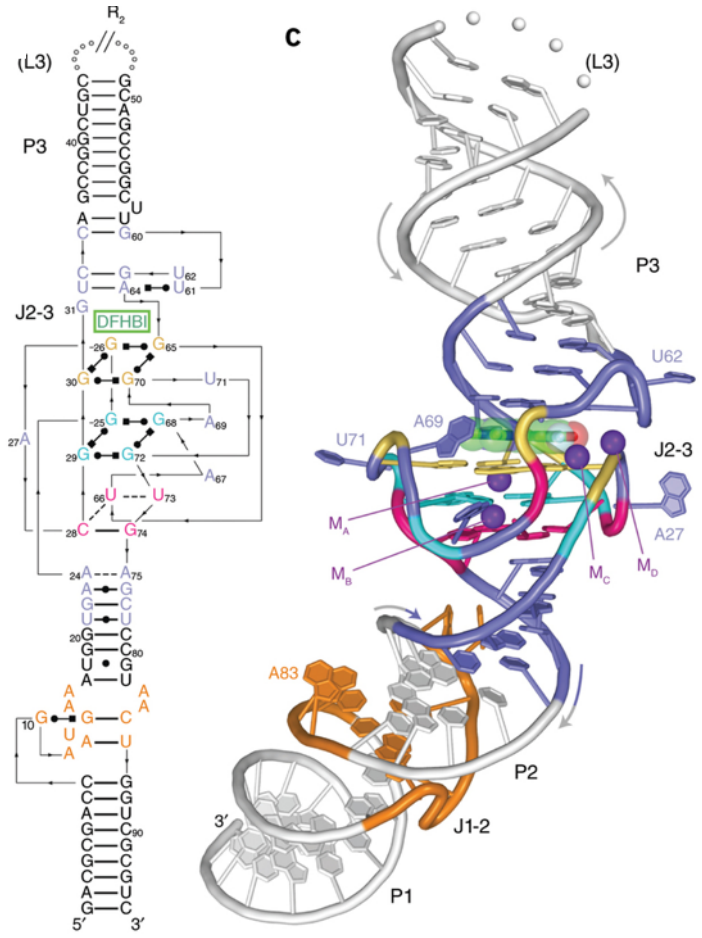
$h\upsilon$



Fluorescent

Genetically encodable fluorescent reporter

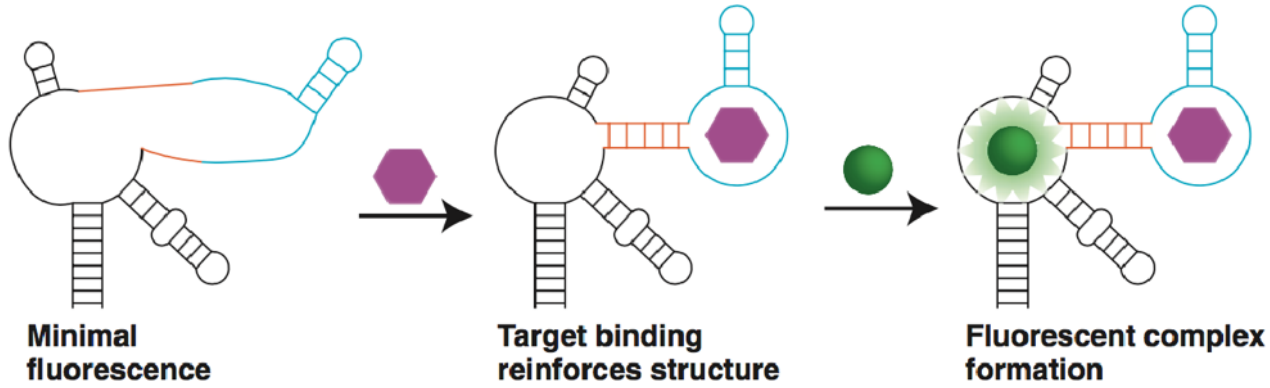
A

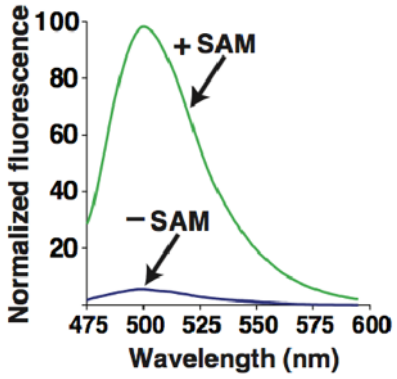


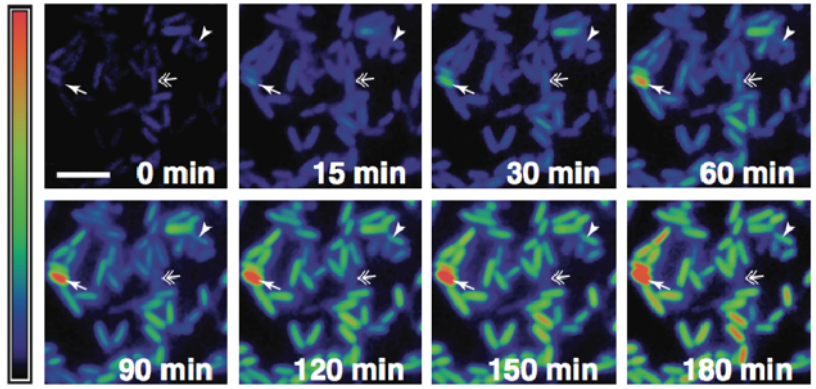
Spinach/Broccoli is an aptamer that contain a G-quadruplex and a base triple

B

Spinach can be fused with S-adenosylmethionine (SAM) riboswitch to create a reporter that detects intracellular SAM concentration

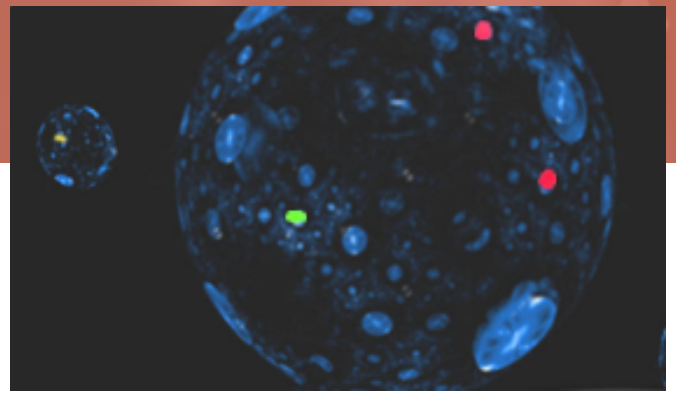






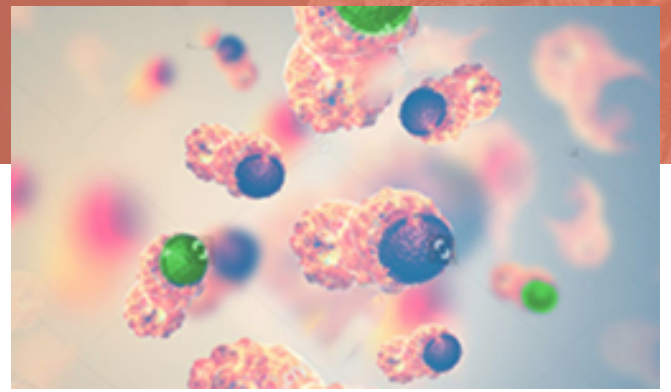
Capability outline

RNA imaging tools



- Single-molecule RNA imaging
- Transcriptional activity sensors

Cell-based HTS assays

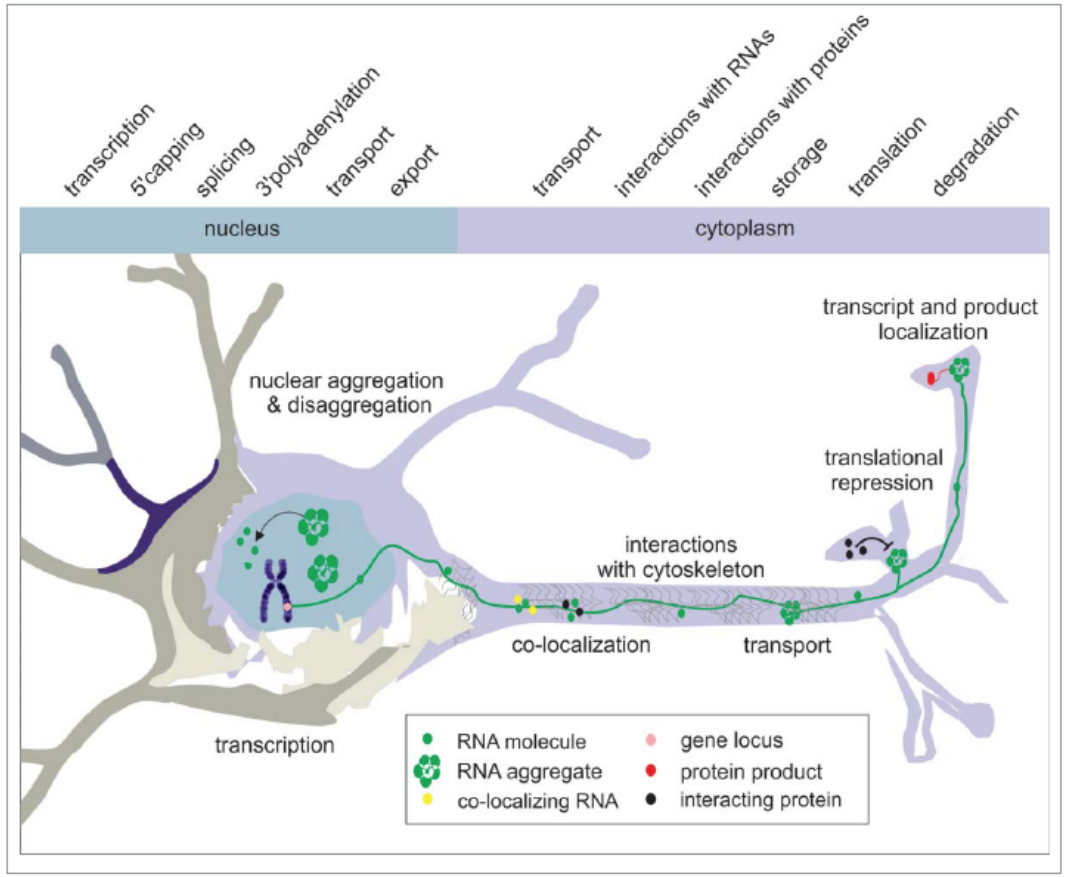


- Trinucleotide repeat assays
- miRNA-targeting assays

In vitro HTS assays

- RNA splicing assays
- circRNA detecting assays

Application #1: RNA imaging



Problem

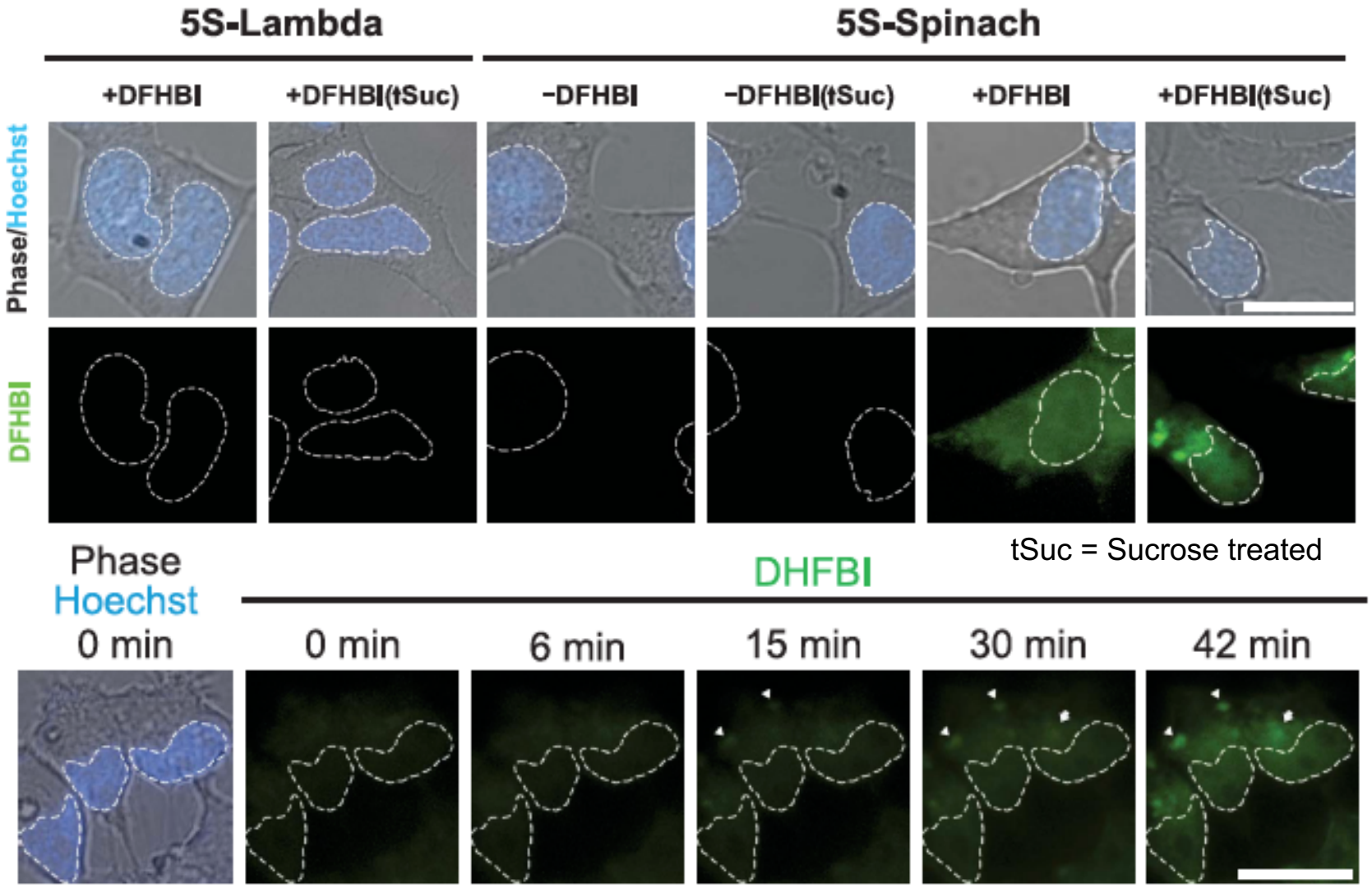
- Hybridization-based methods are very sensitive but mostly work in fixed cells
- Plasmid-based methods require tagging with fluorescent proteins, which are large, irreversible, and can mis-localize RNA transcripts.

Goal

Genetically encodable fluorescent tag that can be inserted into any RNA of interest and expressed in live cells for real-time RNA imaging

LUCERNA™

Live-cell imaging of Spinach tagged 5S rRNA

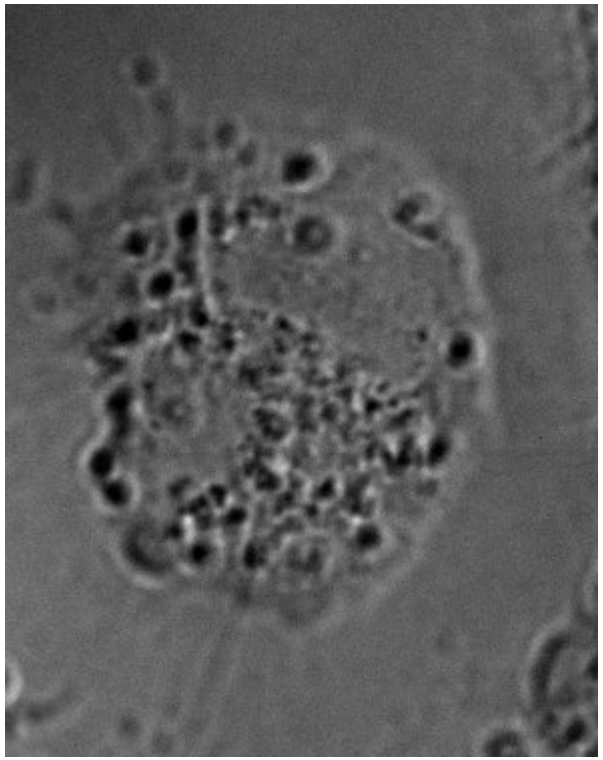


Paige *et al*., Science, 2011.

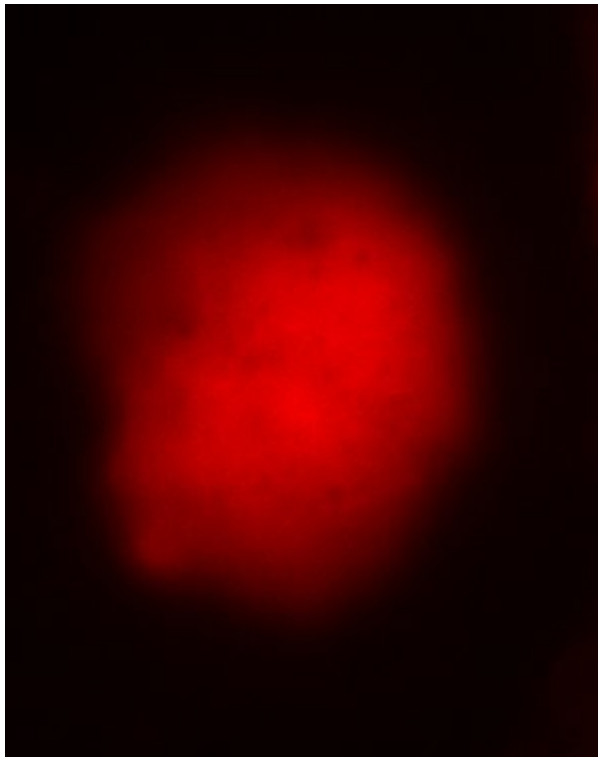
Live-cell imaging of single molecule mRNA

Images

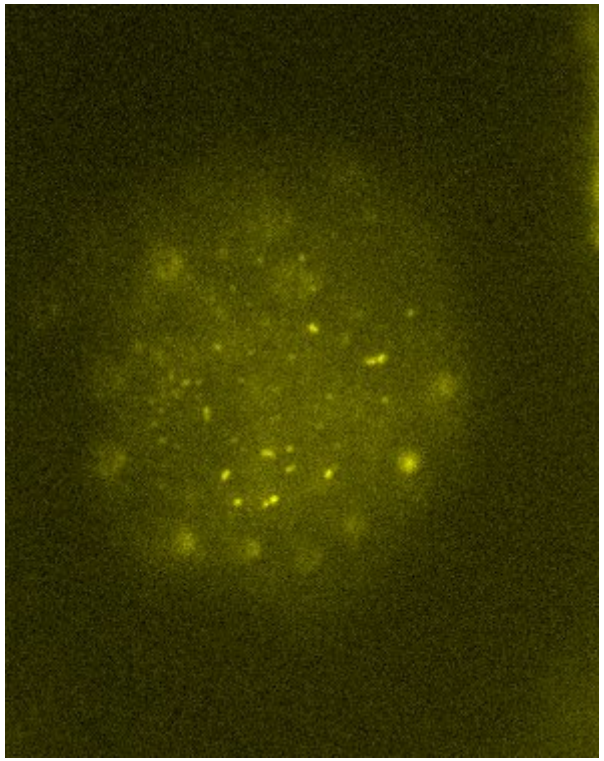
Movie



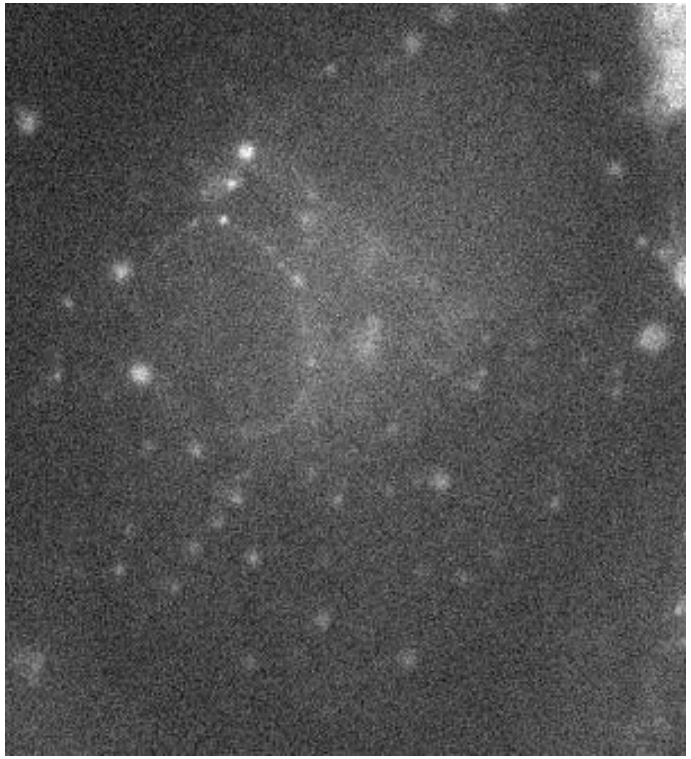
Bright field



mCherry

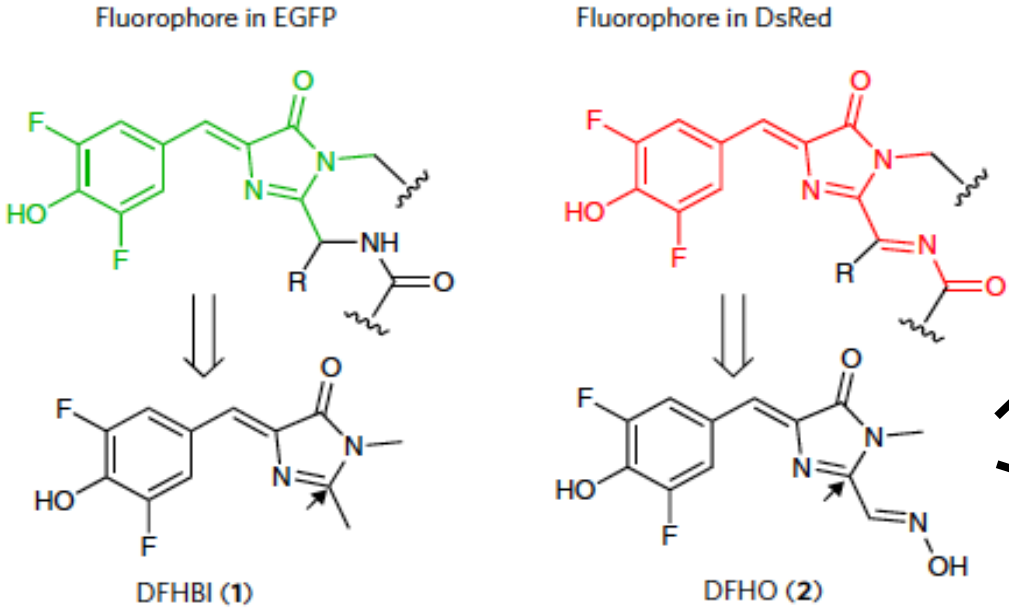


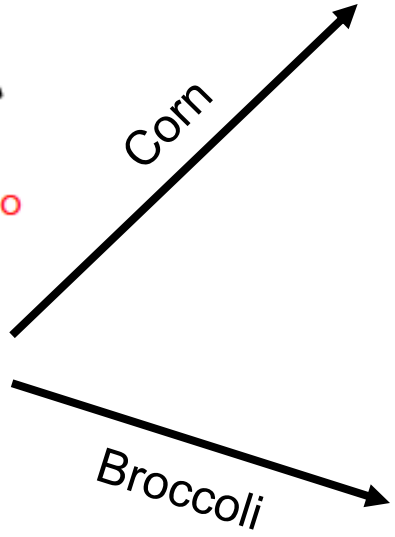
BI/Broccoli

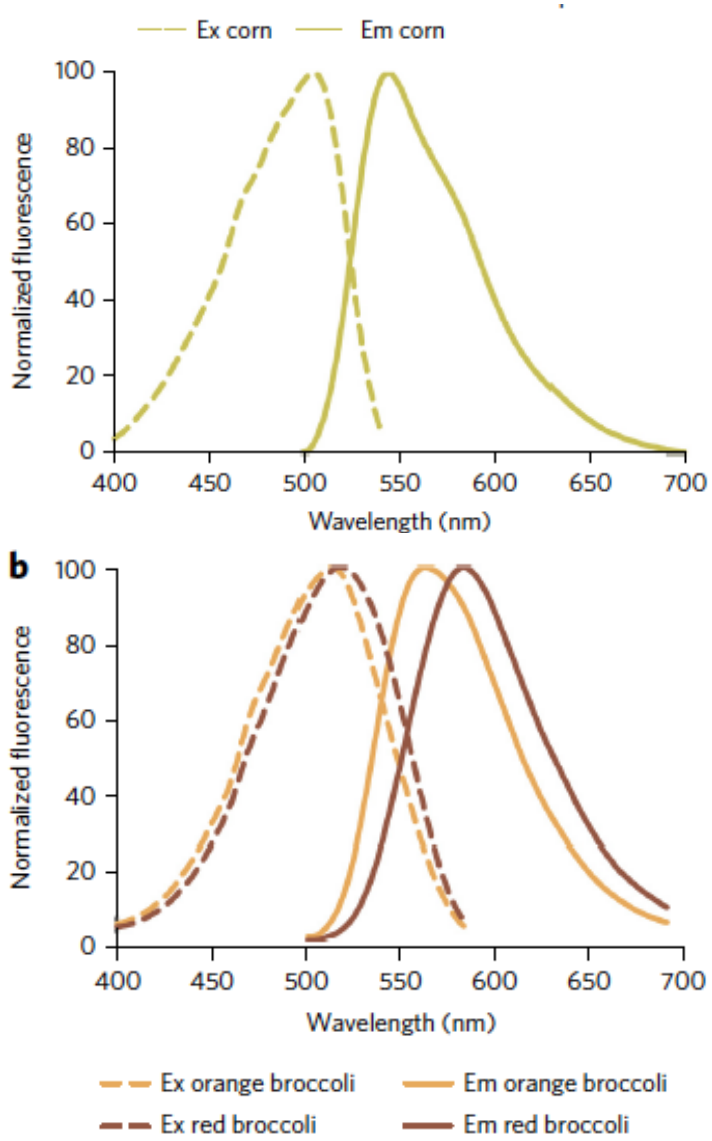


BI/Broccoli

DFHO: RNA mimic of RFP

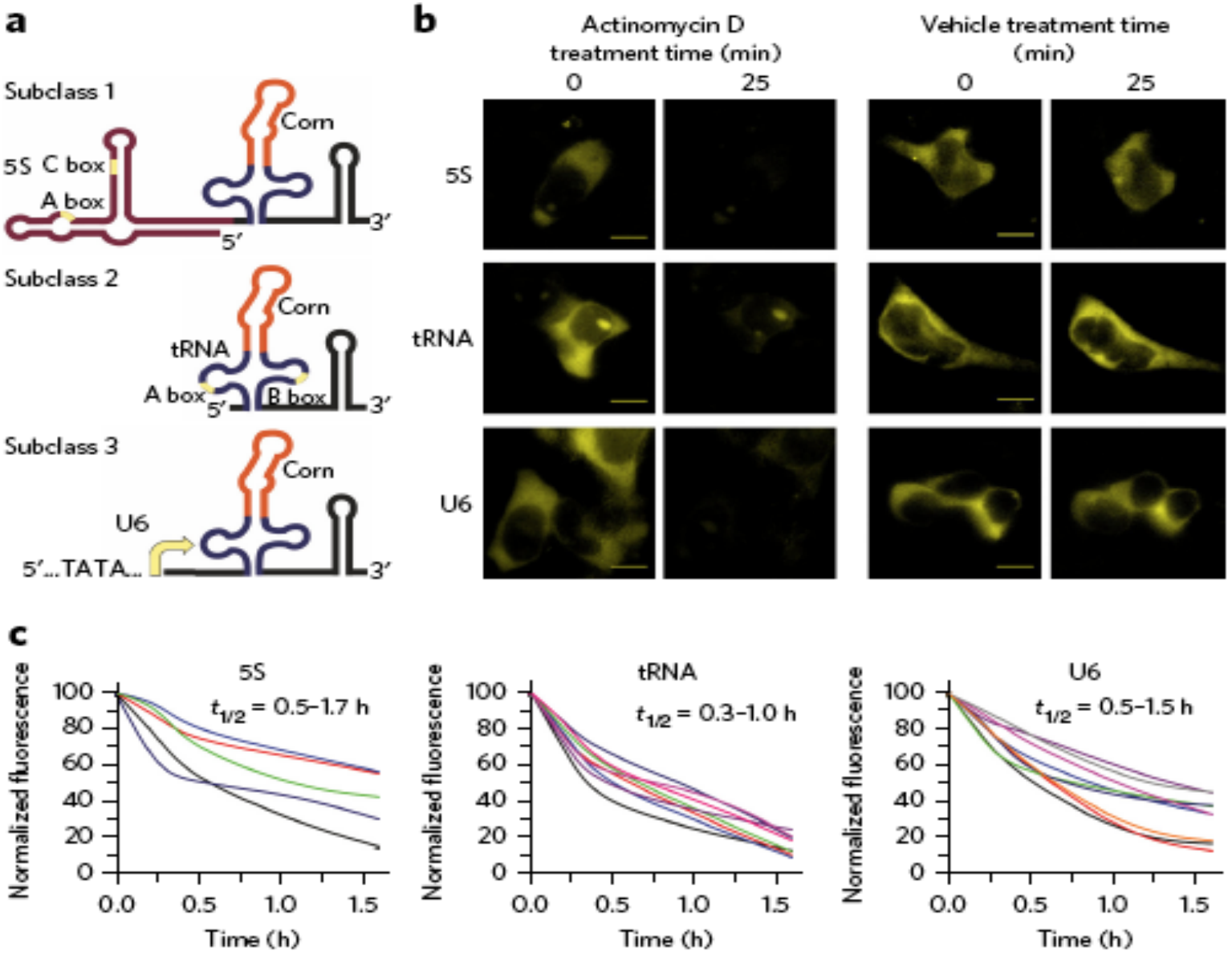






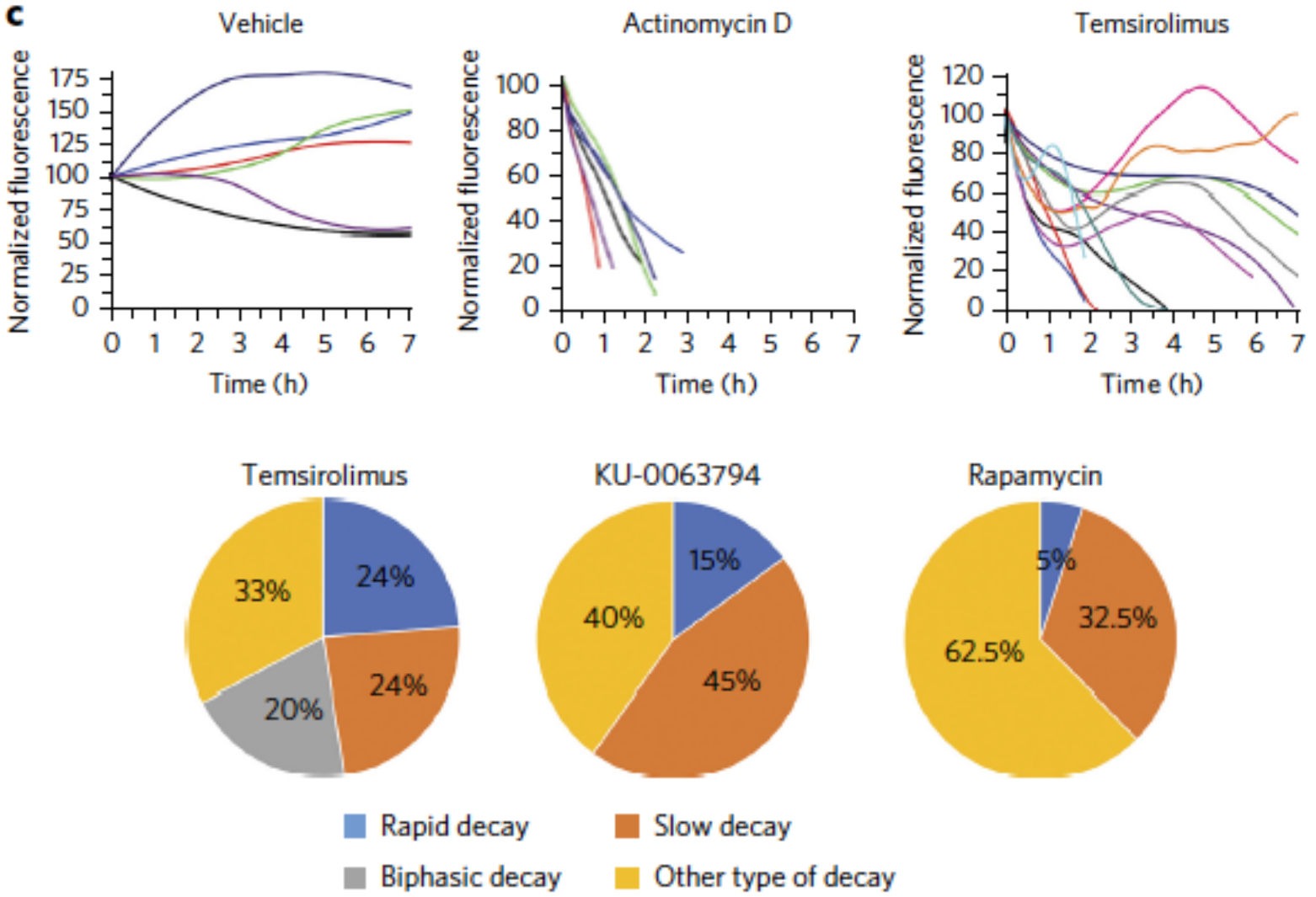
Song et al., Nat Chem Bio, 2017

Measuring RNA Pol III promoter activities in single cells



Song et al., Nat Chem Bio, 2017

mTOR inhibitors exhibit incomplete Pol III inhibition



Song et al., Nat Chem Bio, 2017

Application #2: Cell-based HTS assays

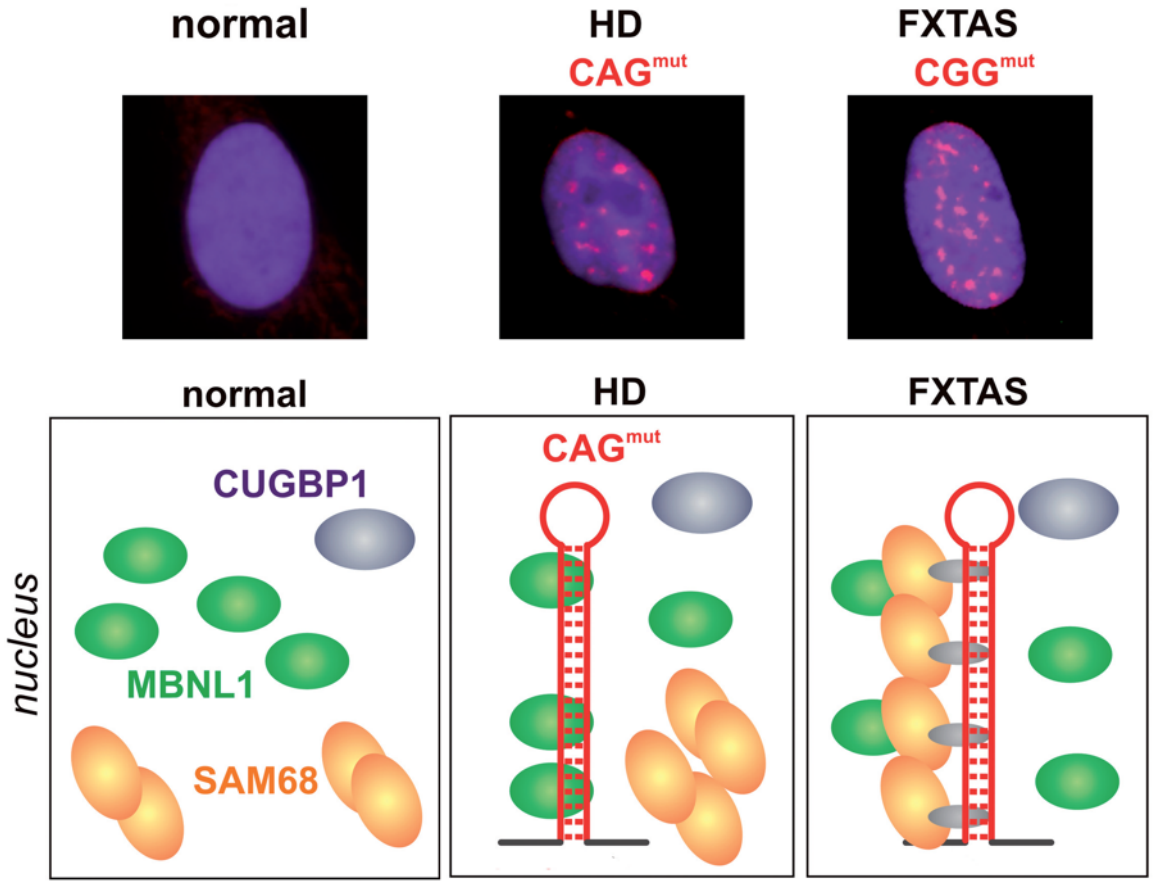
Problem

Target-based screens allow faster SAR optimization but often identify hits with cell toxicity and permeability issues. Phenotypic screens identify hits *in vivo* but require target and/or mechanism deconvolution.

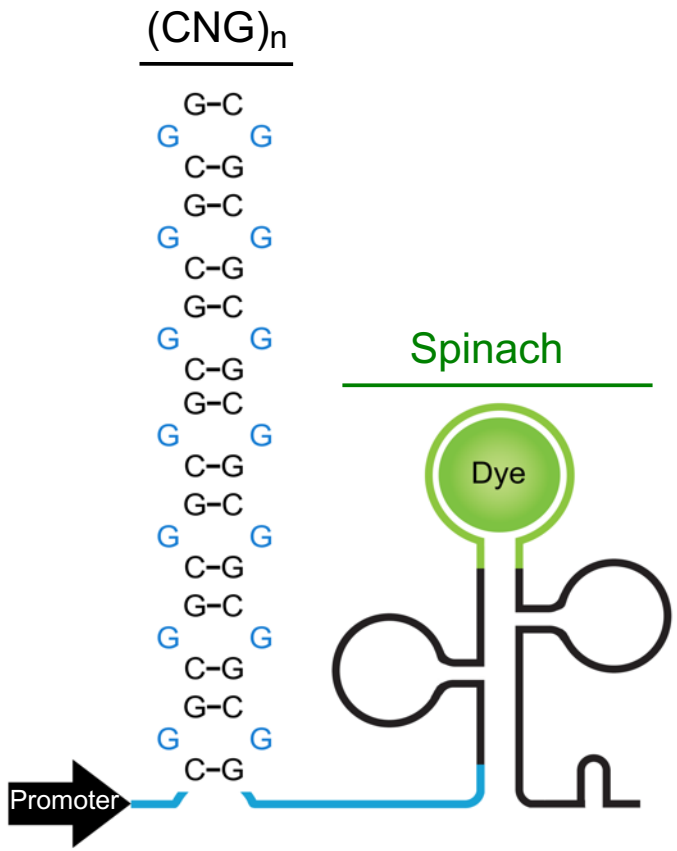
Goal

In vivo target-based fluorescent sensors for cell-based drug screens

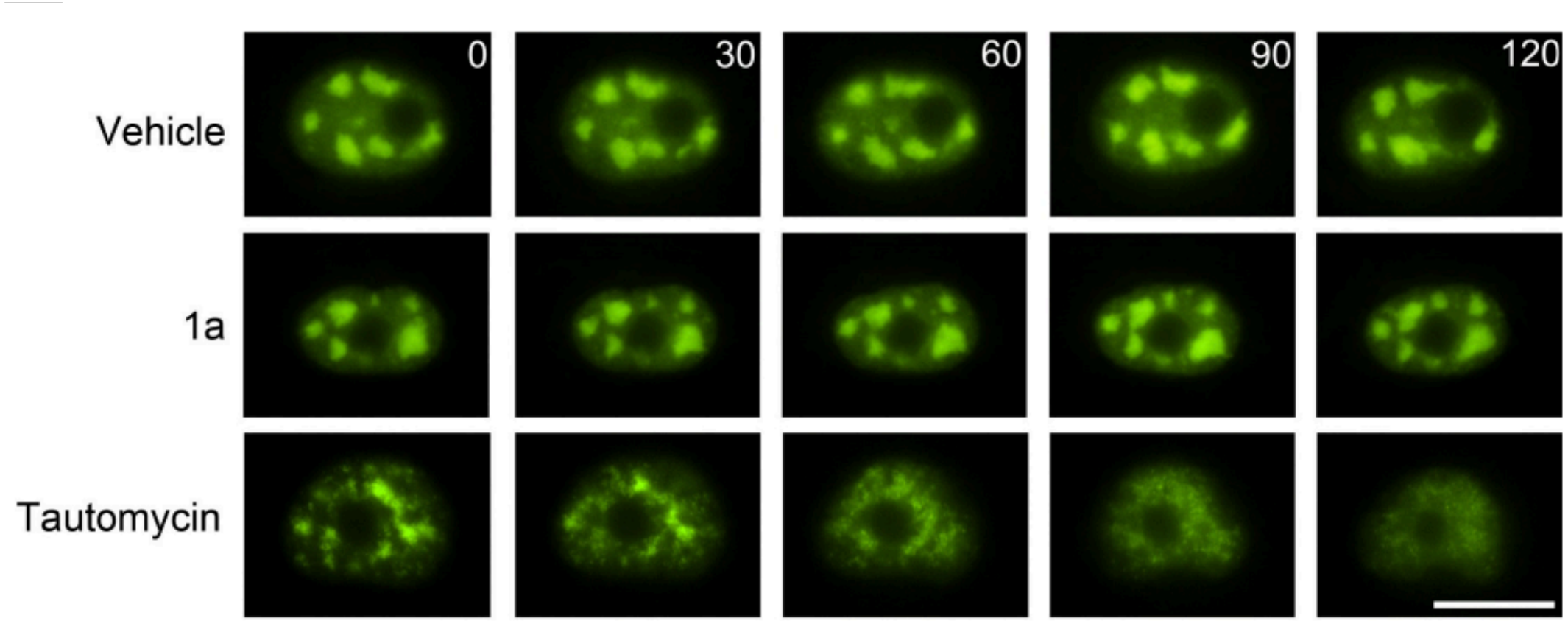
Imaging toxic trinucleotide repeat RNA in mammalian cells



$(CNG)_n$-Spinach reporter:



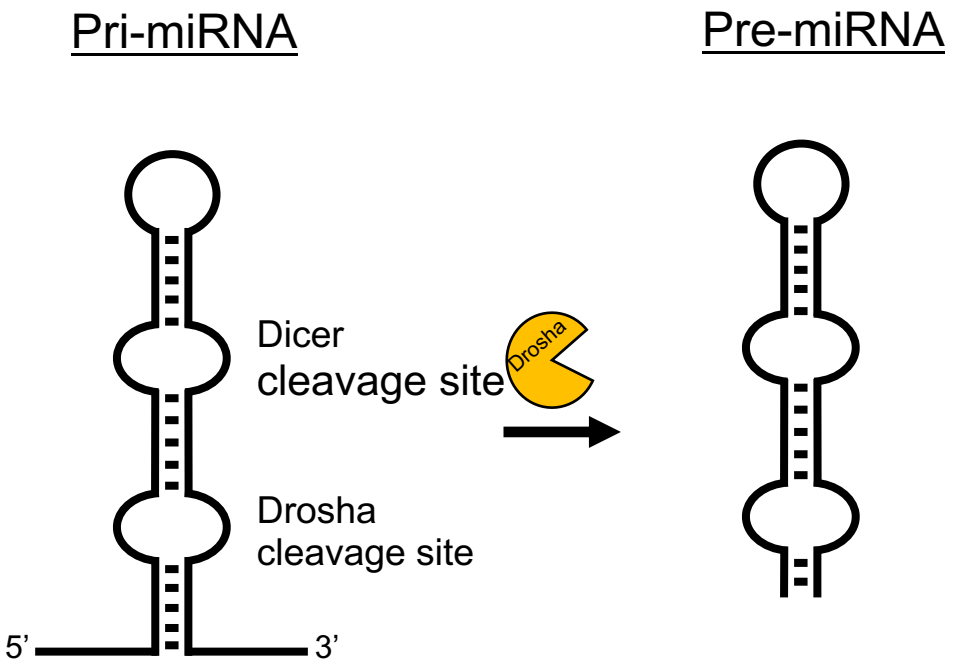
Expanded CGG-repeat reporter form toxic RNA foci that is reversible



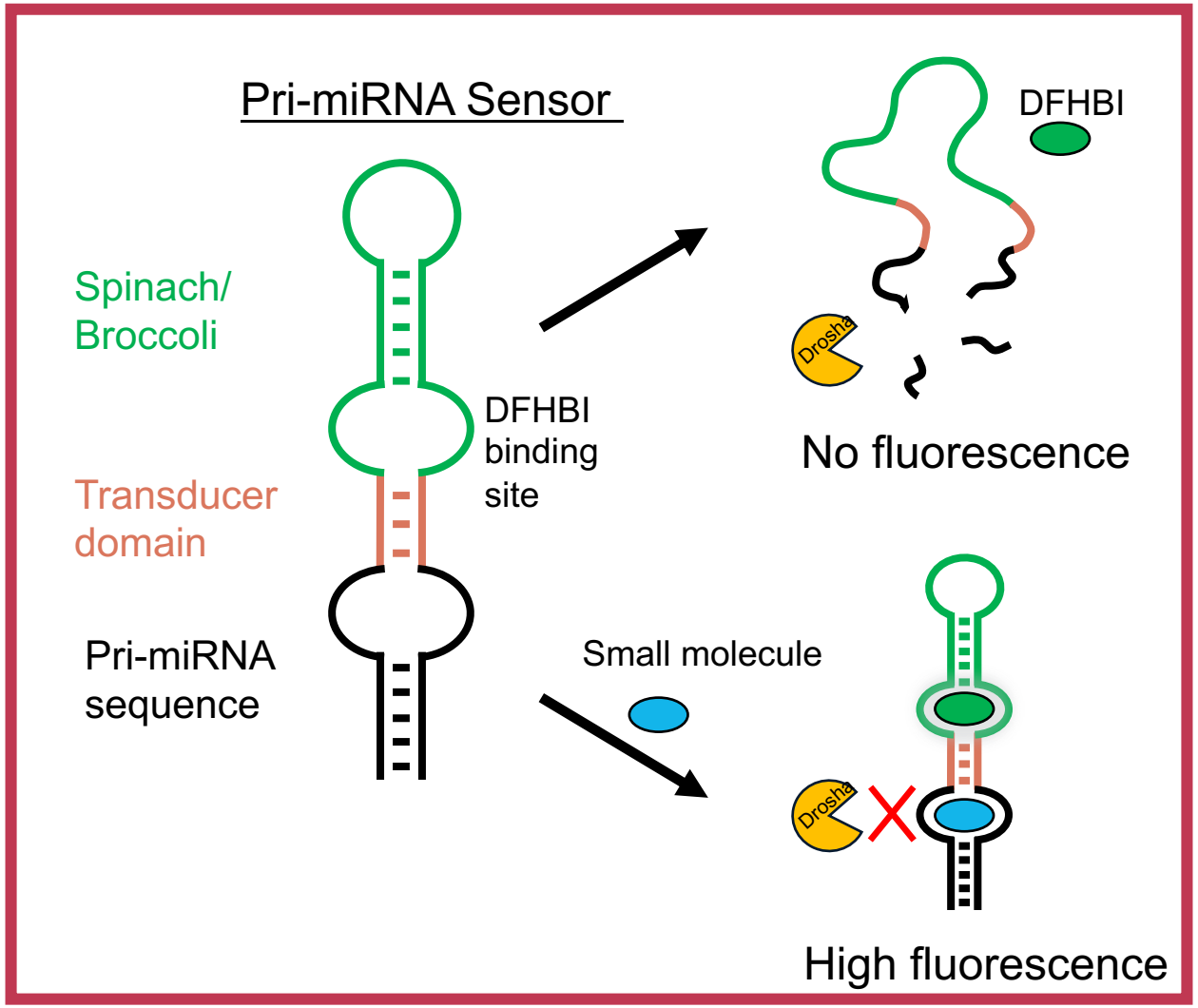
Tautomycin reverses CGG RNA aggregation

Strack et al., Nat Method, 2013

Detection of miRNA-targeting small molecules

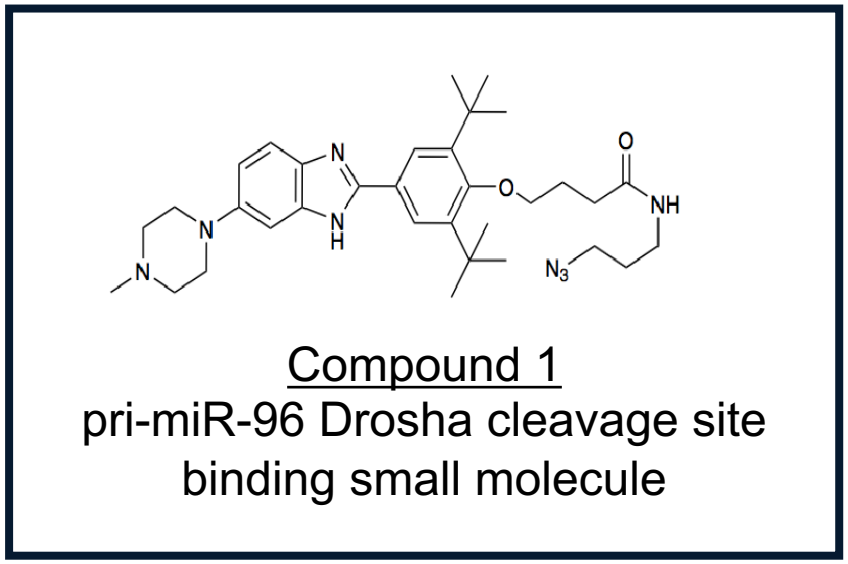


First step in the miRNA processing pathway is the cleavage of miRNA transcript (pri-miRNA) by Drosha. Small molecules that block Drosha cleavage site will prevent miRNA expression

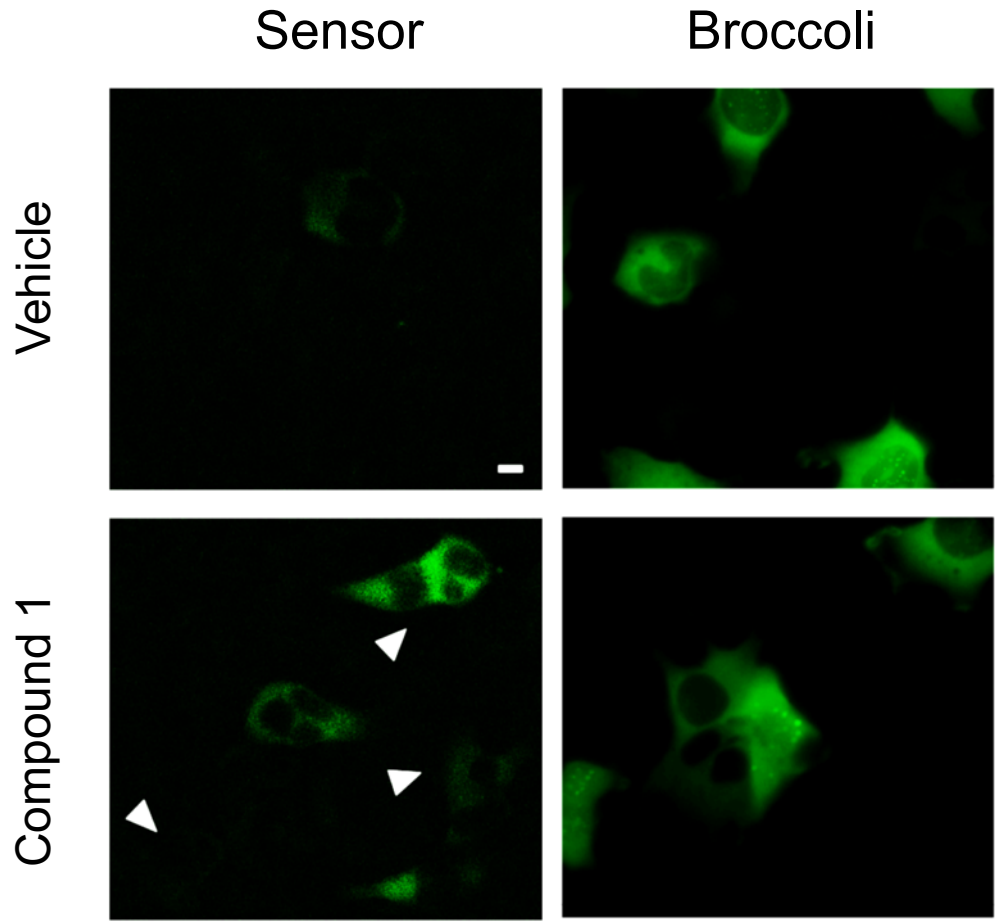


In vivo detection of compound 1 binding

Mammalian cells expressing the pri-miR-96 Broccoli sensor or Broccoli sequence alone



Compound 1
pri-miR-96 Drosha cleavage site binding small molecule



Application #3 – *In vitro* HTS assays

Problem

Many diseases are caused or affected by improper RNA splicing. It now has been shown that small molecules that modulate RNA splicing can be effective at reversing some disease phenotypes.

Drug screens are currently done using either splice mini-gene reporter or RT-qPCR.

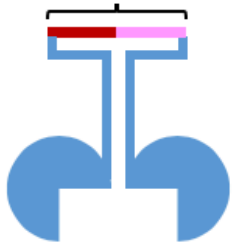
Goal

Mix-and-read, HTS compatible assay that allows direct detection of any endogenous RNA isoform of interest

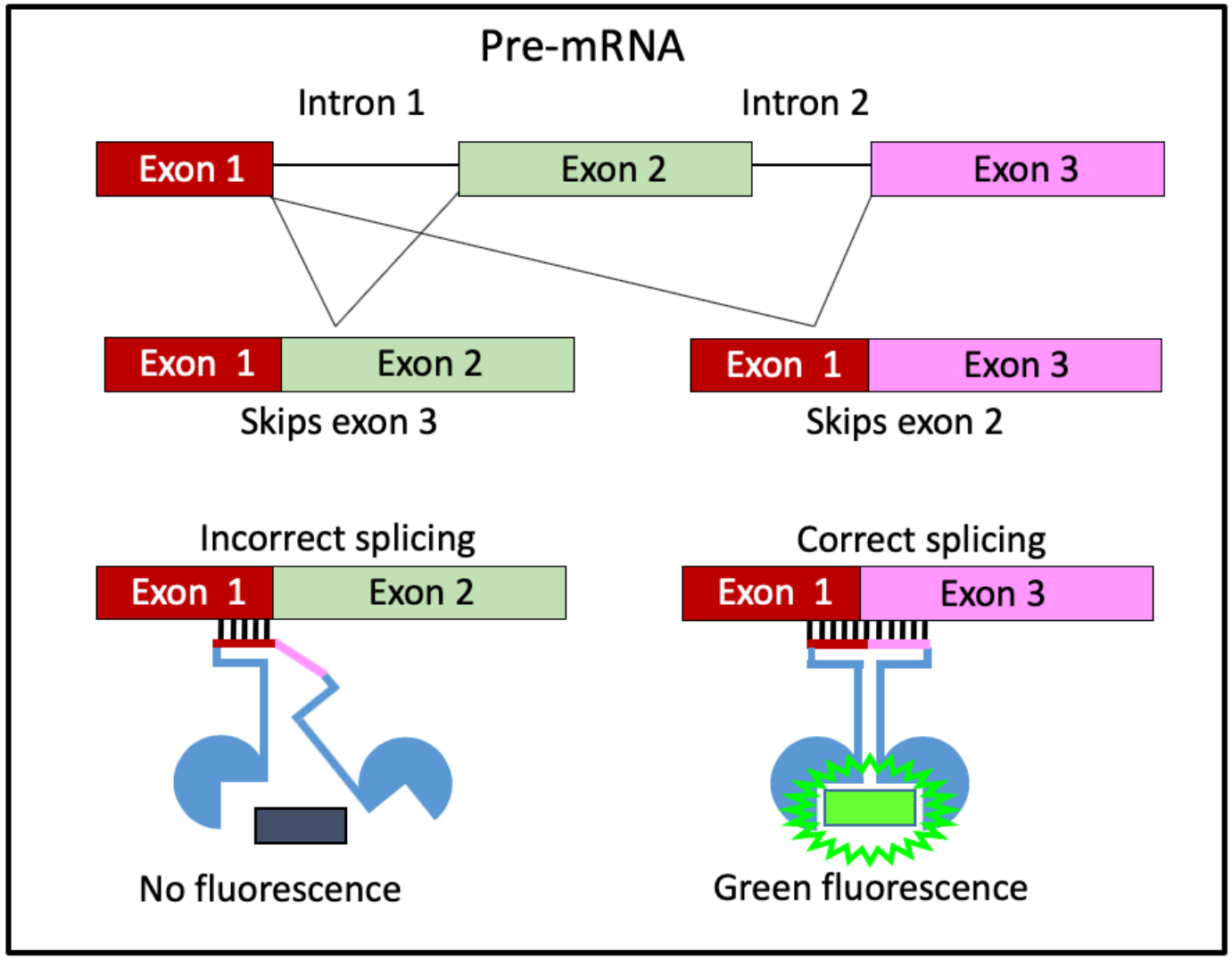
Splice sensor assay

Splice sensor:

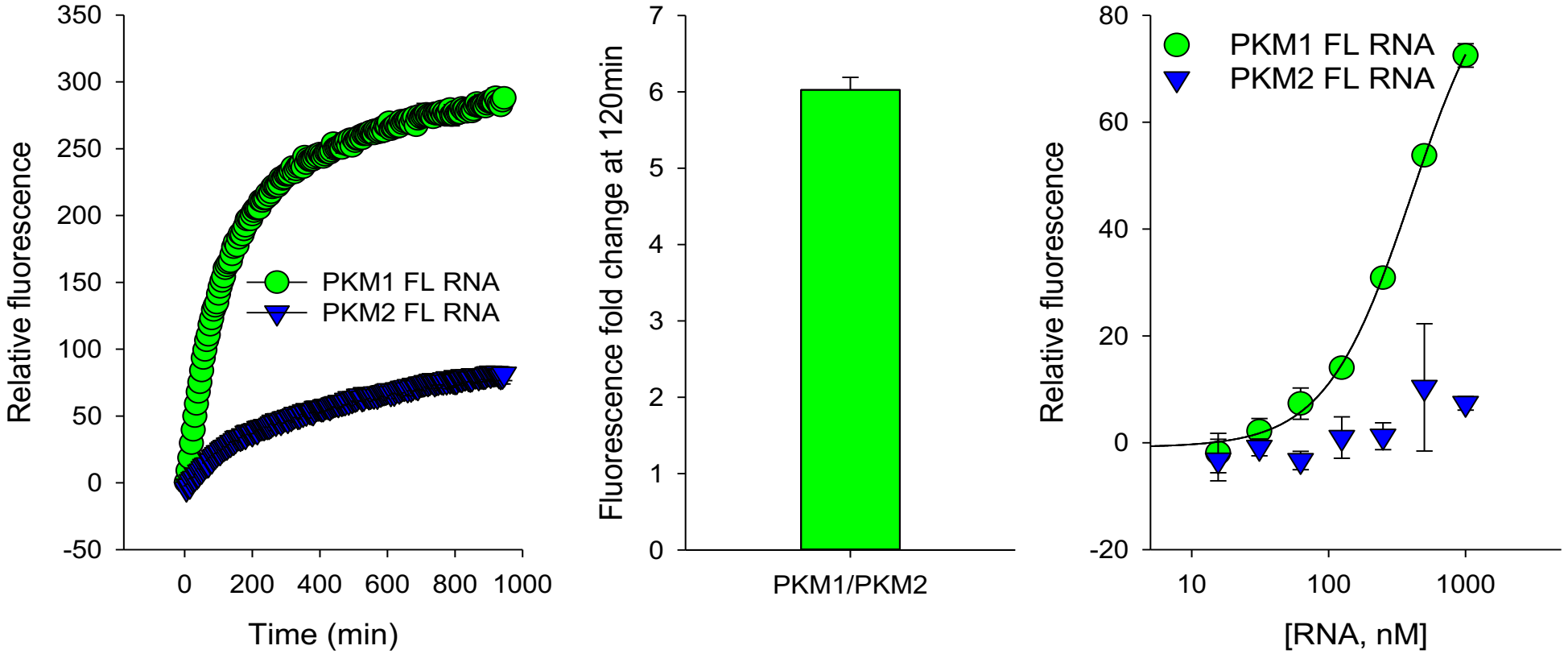
Target recognition region



Sensor dye:

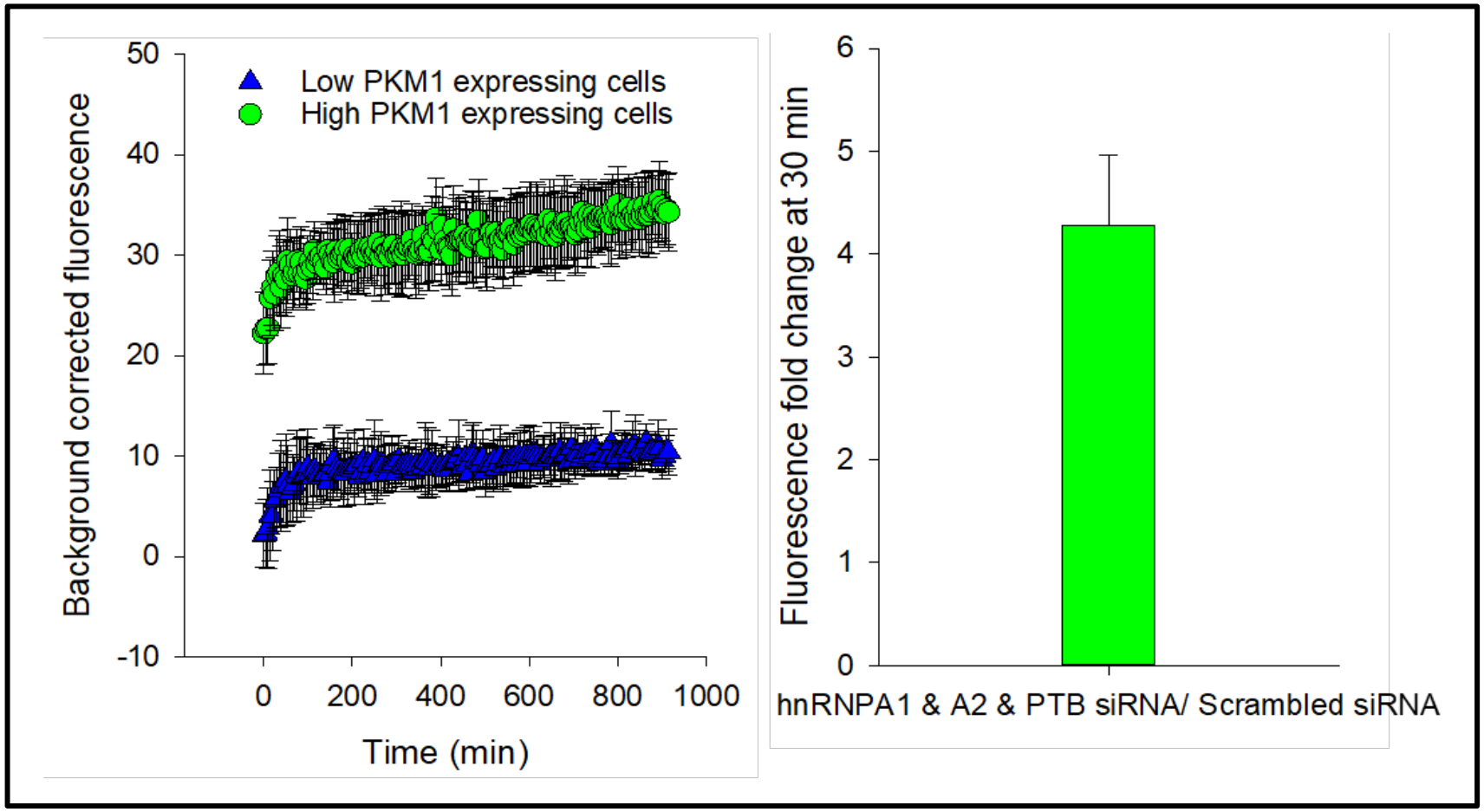


Splice sensors detecting pyruvate kinase isoforms



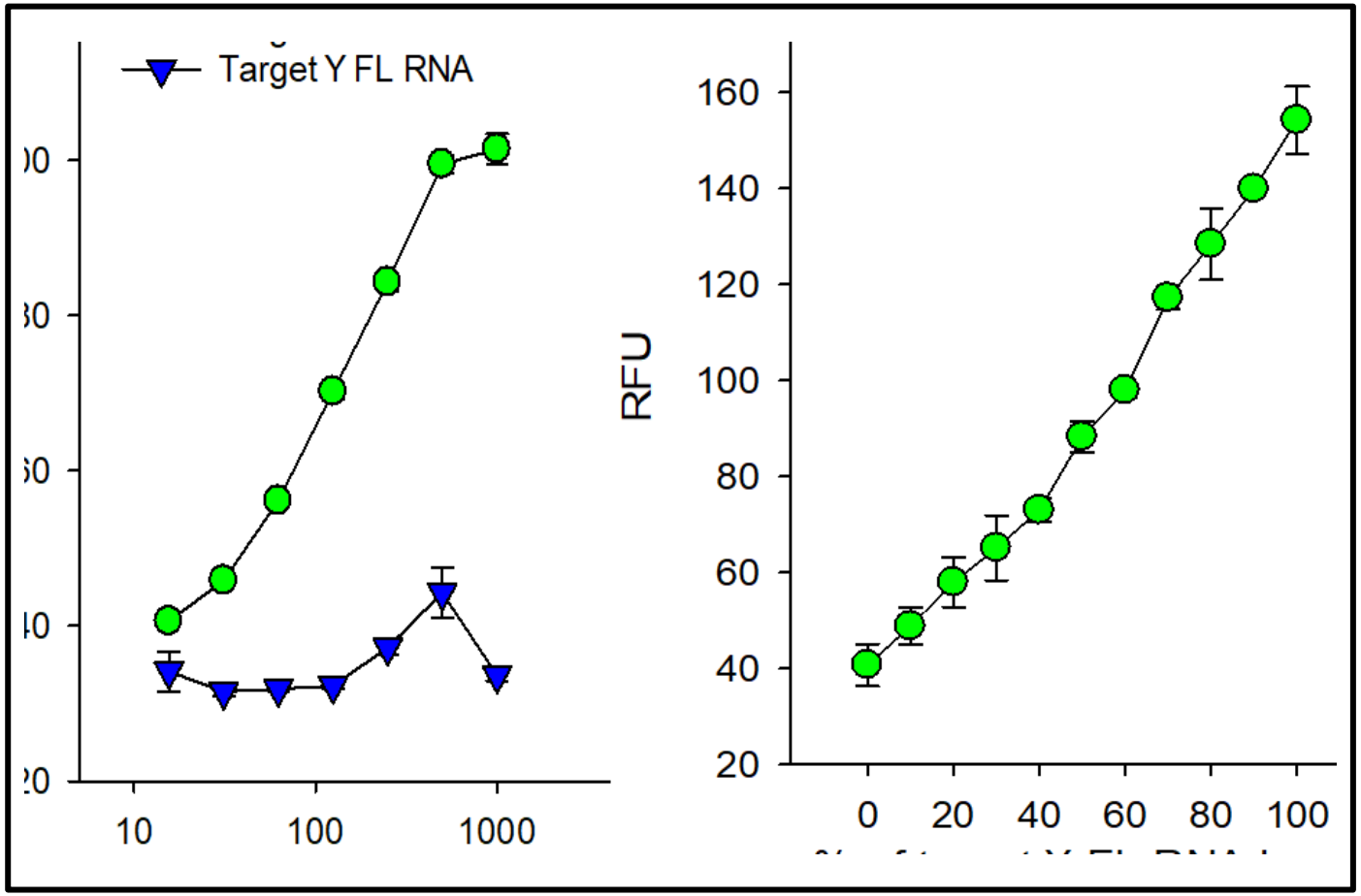
- PKM1 sensor exhibits ~6-fold specific fluorescence in the presence of PKM1 FL RNA.
- Sensor response is fast and stable for over 16h.
- Sensors discriminate between PKM1 and PKM 2 RNA at as low as 62.5 nM.

Sensor measurement is consistent with RT-qPCR data



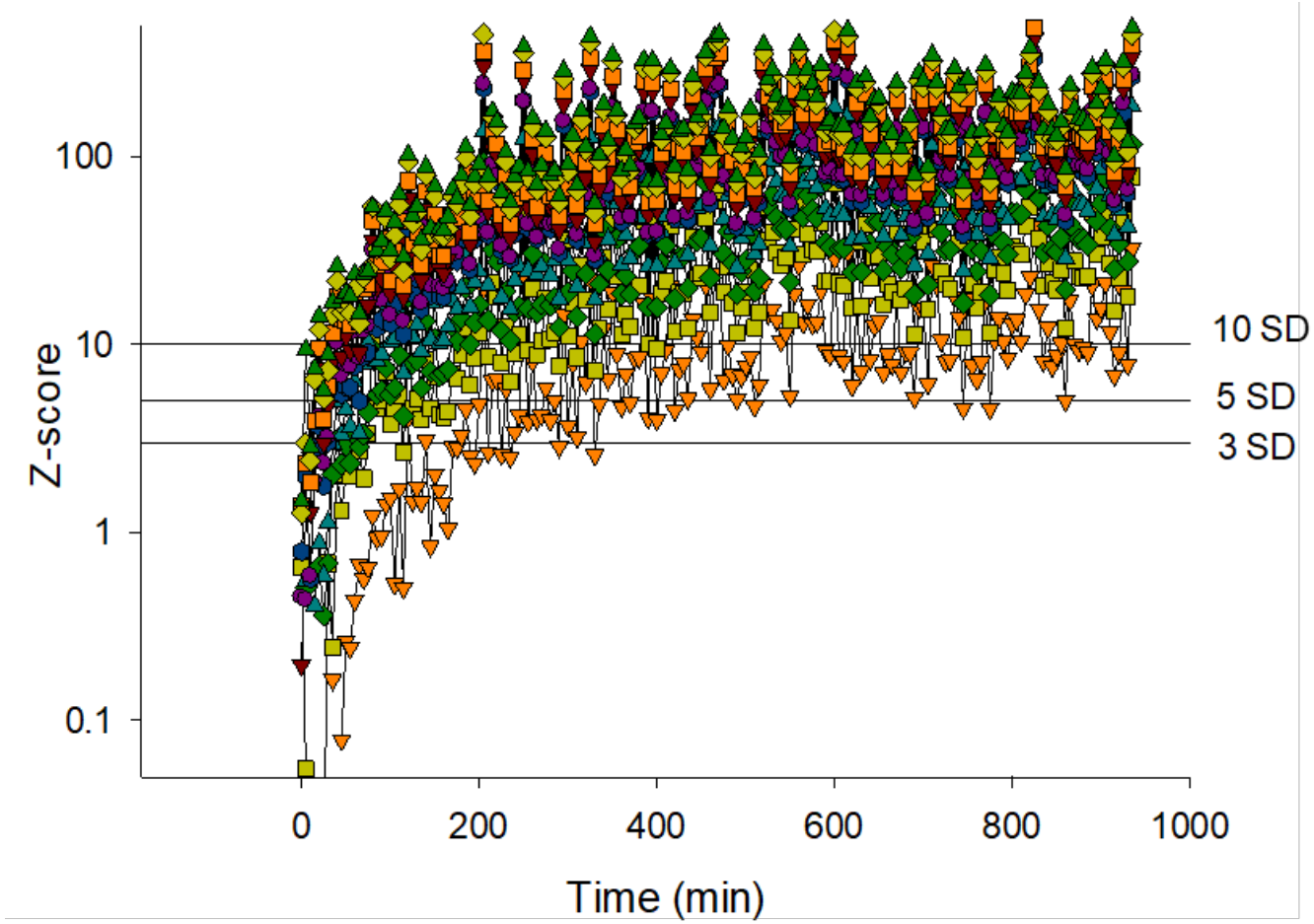
HEK293 cells were treated with hnRNPA1/A2/PTB siRNAs to induce PKM1 expression

CNS target sensors: (A pharma collaboration project)

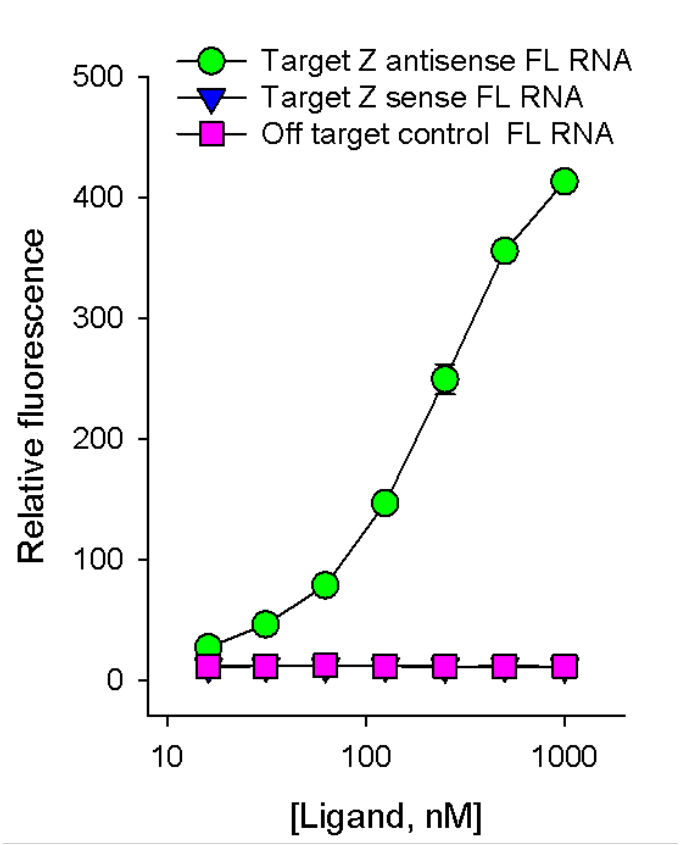


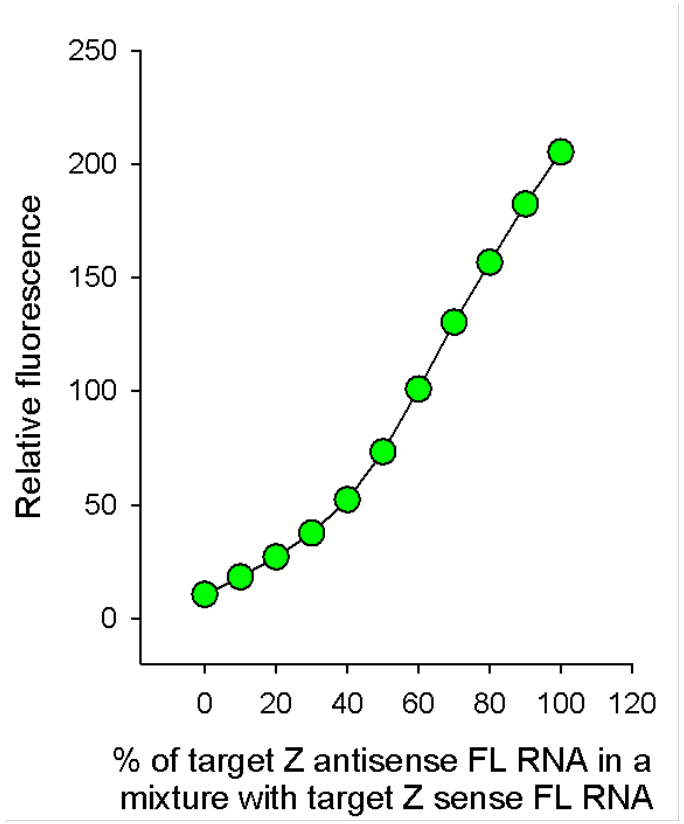
- Target X splice sensor shows a highly selective and dose-dependent increase in fluorescence in the presence of target X FL RNA
- Target X splice sensor is sensitive to as low as 15nM of target X FL RNA
- A statistically significant difference in fluorescence signal ($p<0.05$) can be observed even with a small (~10%) change in target X FL RNA in a mixture with target Y FL RNA

Target X sensor can detect 10% changes in splicing event



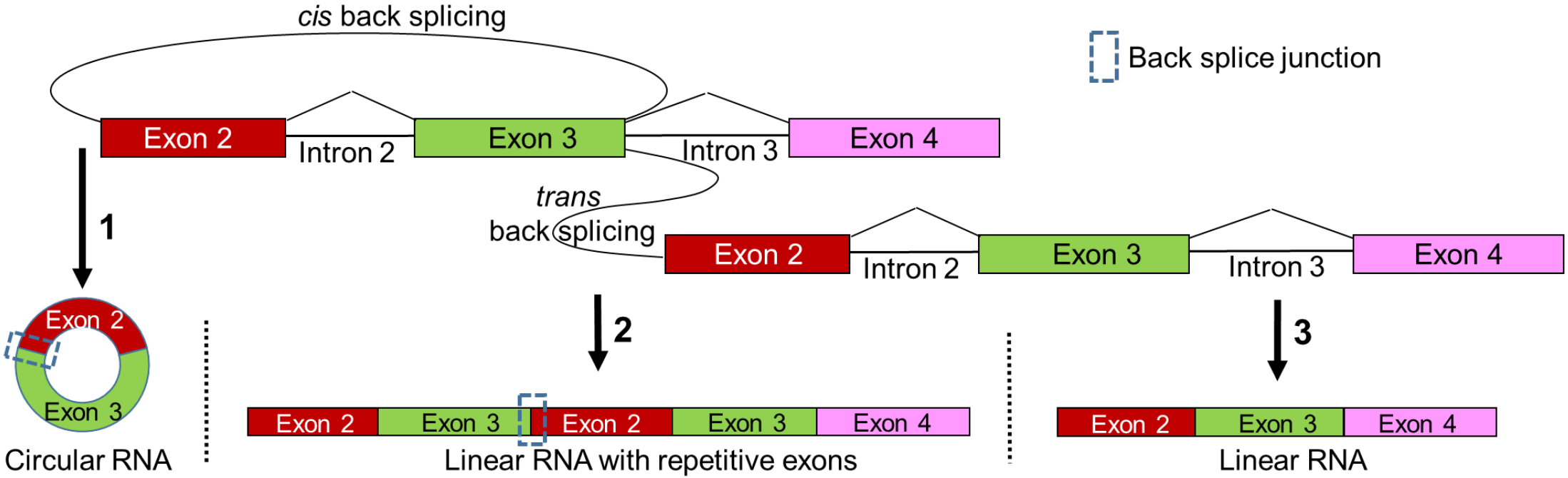
Intronic sensors: (Another collaboration project)





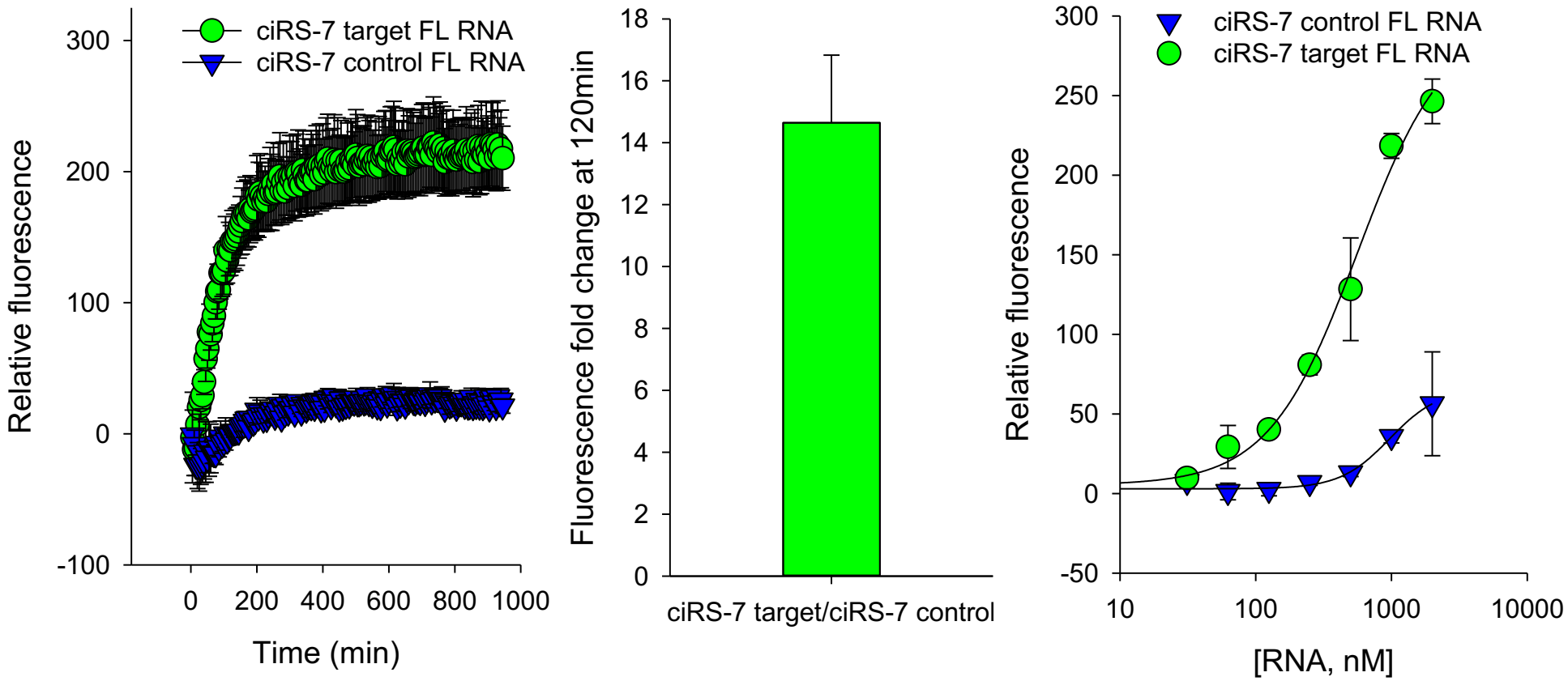
- Sensors were developed to detect the sense and antisense strands of an intronic RNA target.
- Sensor sensitivity is ~10 nM and 10% target changes in cell mixture experiments.

Circular RNA forms a unique splice site



- Circular RNAs (circRNAs) are a diverse family of covalently-closed, single-stranded RNA species that are formed by a special type of splicing called *cis* back splicing.
- Some circRNAs are now believed to be prognostic and diagnostic biomarkers

ciRS-7 circRNA sensor



- ciRS-7 sensor exhibits ~14-fold specific fluorescence in the presence of FL RNA.
- Sensor response is fast and stable for over 16h.
- Sensor sensitivity is as low as 62.5 nM.

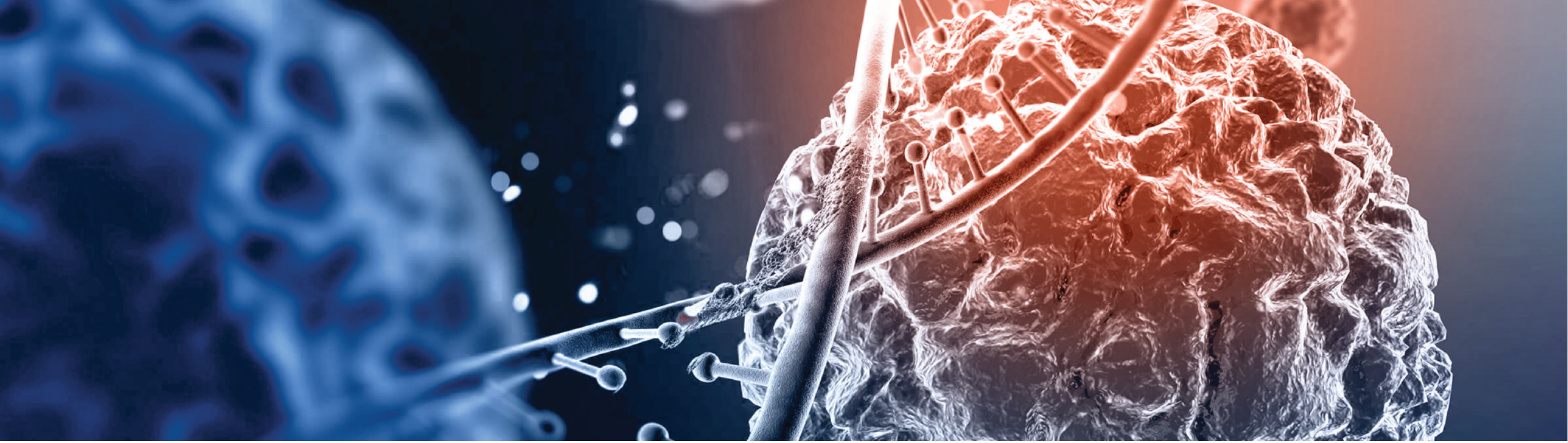
Summary

Current capabilities:

- Live-cell imaging of mRNA and Pol III transcripts
- *In vivo* target-based assays
- *In vitro* HTS assays targeting RNA splicing

Future plans:

- Endogenous labeling with CRISPR
- RNA structures and helicase HTS assays
- Metabolic engineering sensing
- RNA modification quantification
- RNA-protein interaction sensor



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