R&D Day

molecure

Fate can be altered

February 6, 2024

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Warsaw, February 6, 2024

Agenda

13.00-13.05 (5 min)
Welcome and Agenda
Katarzyna Mucha, CCGroup

(Part 1)
13.05 – 13.15 (10 min)
Introduction – Plans for 2024
dr Marcin Szumowski, CEO

13.15-13.30 (15 min)
OATD-01&OATD-02: 2024 the year of clinical trials
dr Samson Fung, CMO

(Part 2)
13.30 – 13.45 (15 min)
DUBs – an important group of targets for anticancer therapies
dr Zbigniew Zasłona, CSO

13.45-14.00 (15 min)
Future of the mRNA platform
dr Zbigniew Zasłona, CSO

14.00-14.20 (20 min)
MoleCuring diseases by targeting RNA - methods for identifying compounds
dr Joanna Sztuba-Solińska, Principal Scientist at Pfizer

14.20-14.40 (20 min)
Studying RNA conformations with DyRNA Thermometry and cryo-EM
dr Jakub Nowak, Max Planck Research Group JU

14.40-15.00
Concluding Remarks
dr Marcin Szumowski, CEO

15.00- 15.30 - Q&A & discussion panel

Molecure | Experienced company leadership

Marcin Szumowski
Chairman of the Board & CEO

Zbigniew Zasłona
Chief Scientific Officer

Samson Fung
Chief Medical Officer

Sławomir Broniarek
Chief Financial Officer

Barbara Dymek
R&D Operations Director

Molecure | Scientific support of experts

Paul Van der Horst, PhD

President of the Supervisory Board

Dr Van der Horst has a strong track record of biotech business development having acted as lead negotiator in over 20 licensing and M&A transactions and raised over $1.3 billion through capital market transactions during his career.

AGOMAB THERAPEUTICS Galápagos

Luke O'Neill, PhD

Member of the Scientific Advisory Board

A world expert on innate immunity and inflammation listed in the top 1% of immunologists in the world. Professor of Biochemistry in the School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute at Trinity College Dublin. Co-founder of Sitryx Ltd.

sitryx Trinity College Dublin, Coláiste na Tríonóide, Baile Átha Cliath, The University of Dublin

Nancy Van Osselaer, PhD

Member of the Supervisory Board and the Scientific Advisory Board

Dr Van Osselaer is an experienced global biopharmaceutical professional with 25-year-long experience leading drug development projects at pharmaceutical companies.

Kynexis Galápagos Johnson&Johnson

Bart Lambrecht, MD, PhD

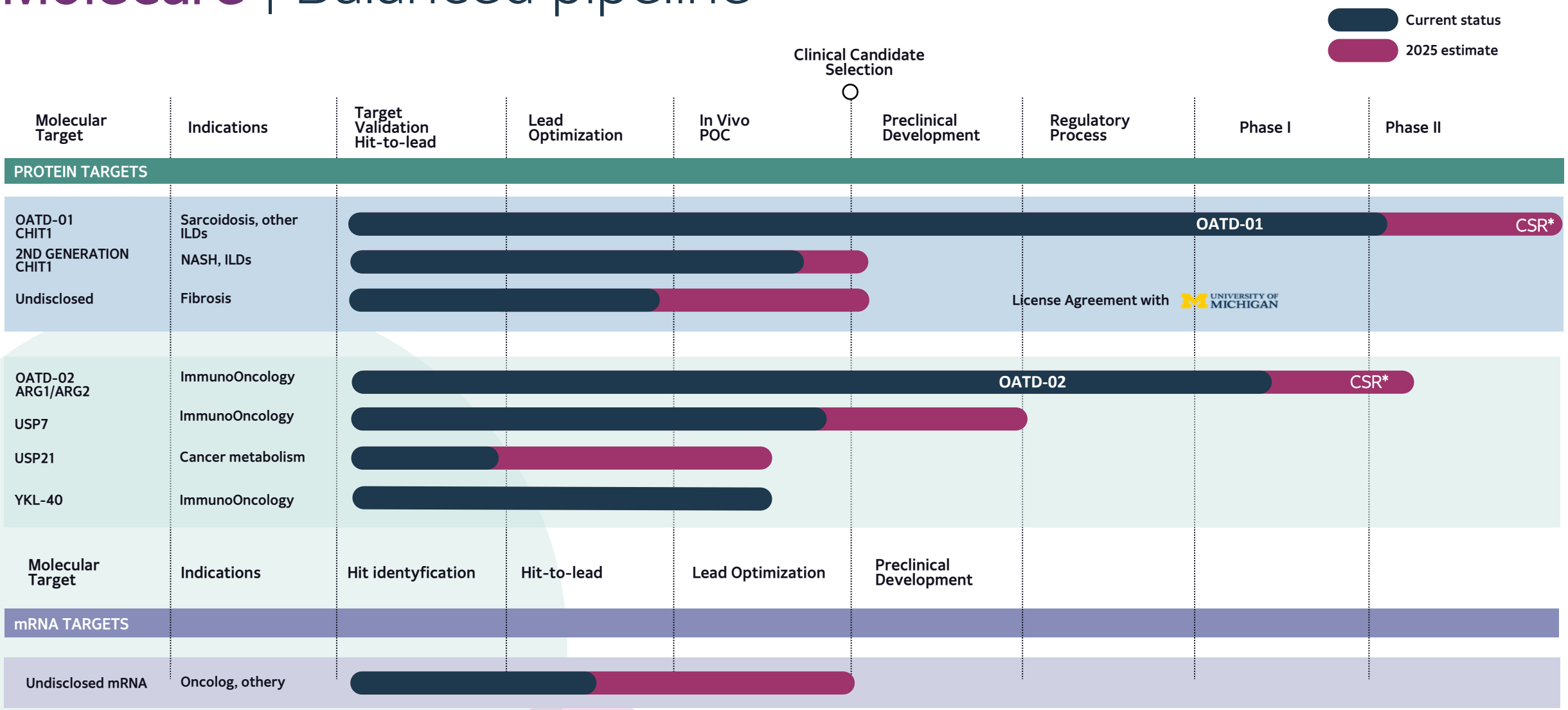
Member of the Scientific Advisory Board

Director of the VIB Inflammatory Disease Research Center in Gent. His team's research focuses on the role of dendritic cells and epithelial cells in stimulating immune responses in the lung.

VIB GHENT UNIVERSITY

2024
Plans and key upcoming catalysts

Molecure | Balanced pipeline



*Clinical Study Report

Molecure | Key near term milestones

OATD-01

- First patient dosed in the KITE Phase II PoC study (expected March)
- Interim data analysis by an independent unblinded committee after completion of dosing of approx. 50 patients with lung sarcoidosis (2024 / early 2025)

OATD-02

- Reaching the therapeutic dose (Ph2RD) (end of 2024 / early 2025)

AI

- Following the implementation of AI tools into our drug discovery process: nomination of the first preclinical development candidate generated by AI engine (2024/2025)

Partnering

- Signing a revenue generating collaboration or licensing agreement in one of the clinical programs or the mRNA platform (mid-late 2024).

OATD-01 & OATD-02
Clinical update

OATD-01 | Phase 2 in sarcoidosis

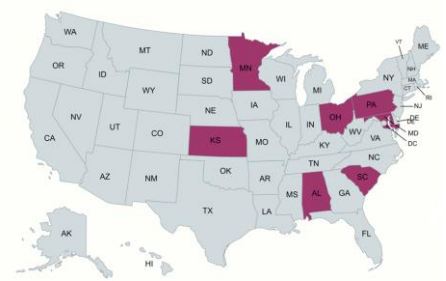
Double-blind, randomized, placebo-controlled multi-center study to assess the safety and efficacy of an oral inhibitor of CHIT1 (OATD-01) in patients with active pulmonary sarcoidosis.

FDA approval	Central Bioethic Committee (BC) approval	Application submissions in EU/UK	MHRA approval	FPFV (US/UK) / EU approval	FPFV (EU)	Intermediate checkpoint	LPLV (US & EU)
Jul 2023	Sep 2023	Sep 2023	Dec 2023	Q1/2 2024	Q2 2024	Q4 2024/Q1 2025	H1 2025

H2 2025
Results and CSR

Treatment goals

- Stop disease progression and organ damage
  - Quantification of the change in granulomatous inflammation
- Improve symptoms
  - Boost lung function/ prevent lung dysfunction
  - Forced Vital Capacity (FVC) and radiographic sarcoidosis
- Improve Quality of Life (QoL)



Each patient treated for 12 weeks

~90 male and female patients with active pulmonary sarcoidosis

20-30 outpatient sites in the EU and US

OATD-01 | current status

THE KITE STUDY

[Study Details | Efficacy and Safety Study of OATD-01 in Patients With Active Pulmonary Sarcoidosis | ClinicalTrials.gov]

Approval from the U.S. FDA and Central Bioethics Commission

Clinical trial in Europe:
- Approval of Medicines and Healthcare products Regulatory Agency (MHRA) in UK
- Resubmission for EU and Norway

Site initations process in US and UK in final stage

Grant application to PARP (FENG program) for a total amount of PLN 16m and NIH for 2.2m USD in review

Branding activities started (WASOG participation, established FSR collaboration, study design poster at ERS, social media campaign in US and EU ready to launch), Website in US

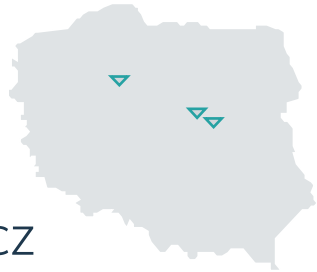
FDA's green light paved the way for Molecure to initiate the study in the United States becoming **only the second Polish biotech company** ever to do so

OATD-02 | Phase I FIH clinical trial

Design: Open-label single-arm dose-escalation monotherapy study (Bayesian design, 2.5-30mg)

Patient population (30-40 pts): Relapsed/refractory advanced and/or metastatic solid tumors

Location: 3 sites in Poland: Warsaw, Otwock, Bydgoszcz

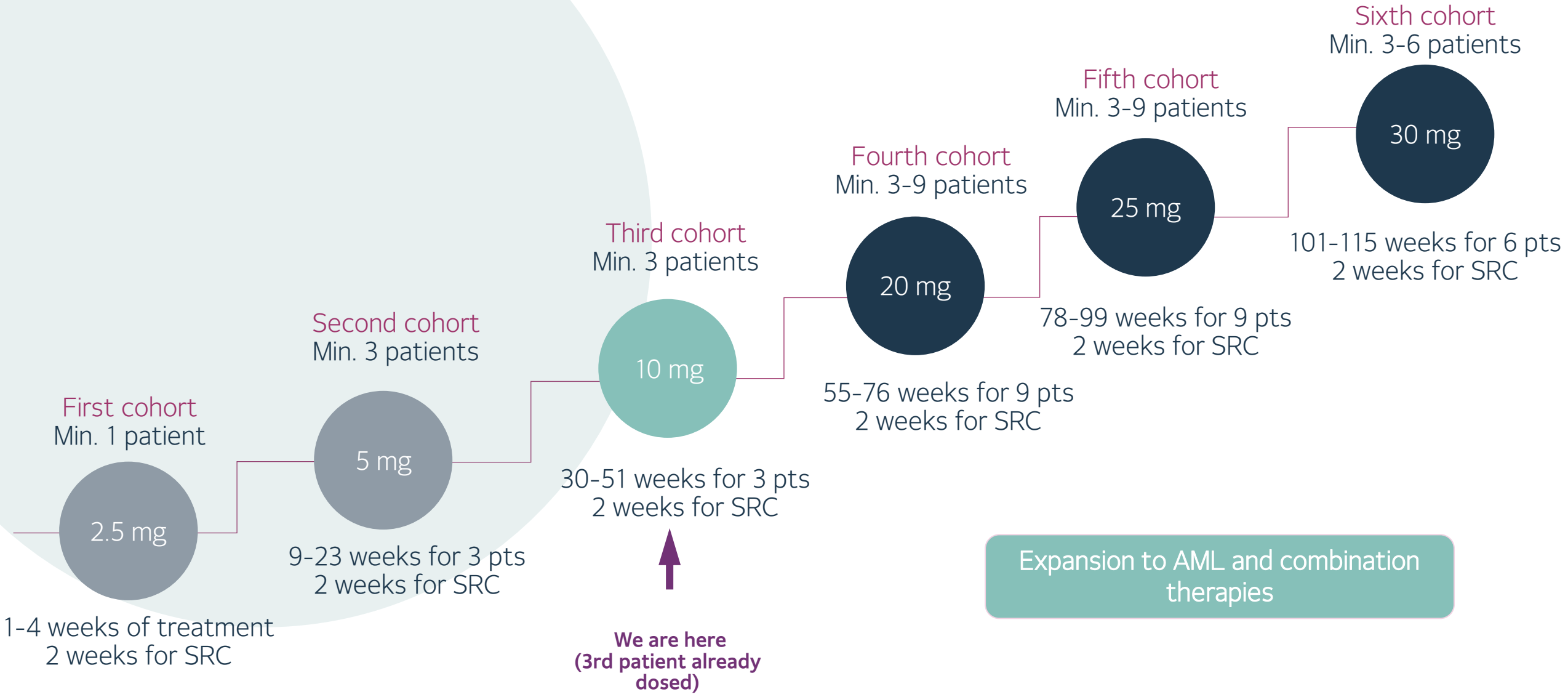


Study Duration: Approx. 2 years (Q1 2023 – approx. Q1 2025)

OATD-02 | Addressing cancers with unmet need:

- Pancreatic ductal cancer (advanced, inoperable)
- Metastatic colorectal cancer
- Serous Ovarian Cancer
- Renal Cell Cancer

OATD-02 | administered to the third cohort of patients



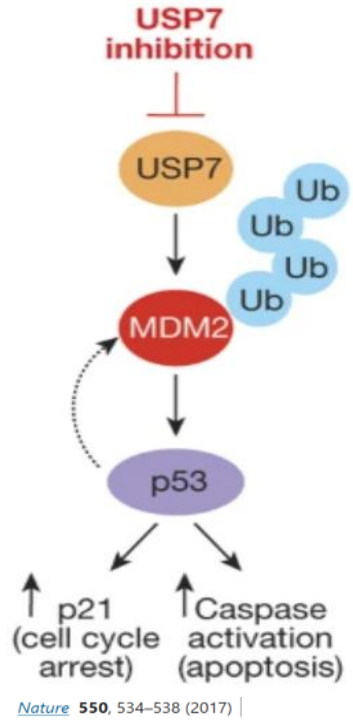
Expansion to AML and combination therapies

SRC – Safety Review Committee

Deubiquitinase (DUBs)
An important group of targets for anticancer therapies

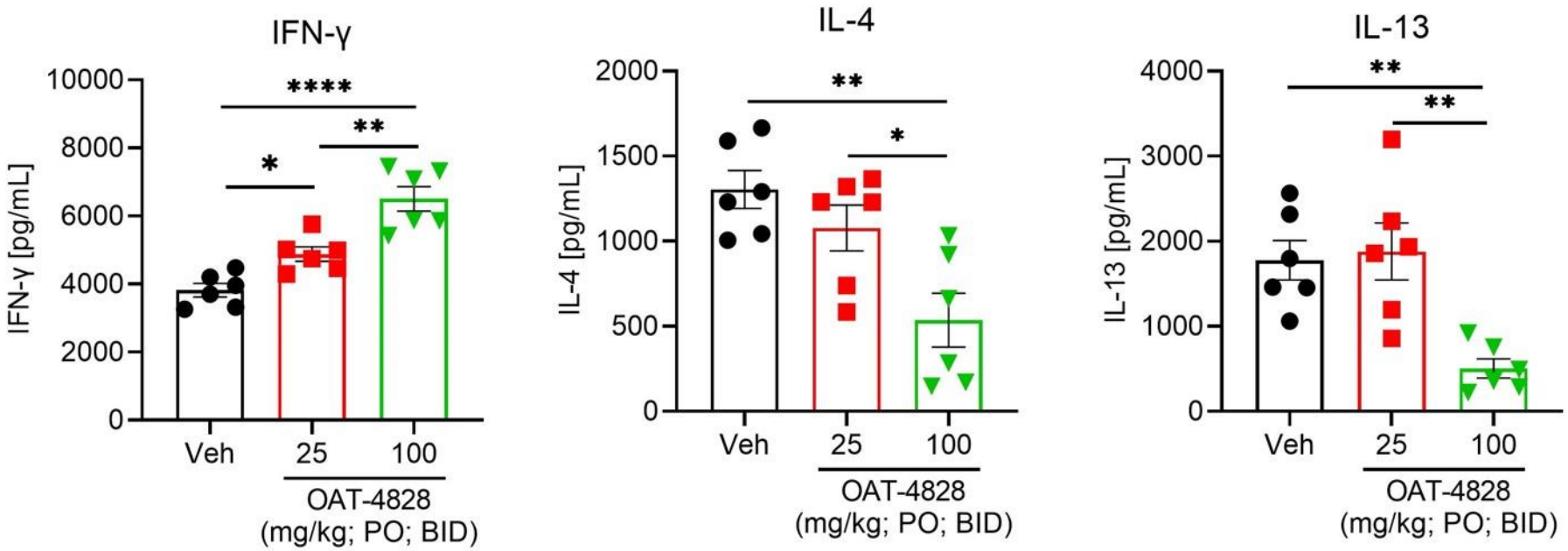
USP7i | stabilizes p53 and upregulates p21 expression – we have utlilzed this mechanism in a context of T cell activation

- The MDM2/MDMX-p53 circuitry plays a pivotal role in cell proliferation, cell cycle progression, apoptosis, and senescence
- USP7 directly interacts with MDM2 and MDMX, regardless of p53 status.
- USP7 inhibition promotes the degradation of MDM2 and MDMX, activates the p53 signaling
- Primary T cells used in the screening cascade to assess cellular effects of USP7i



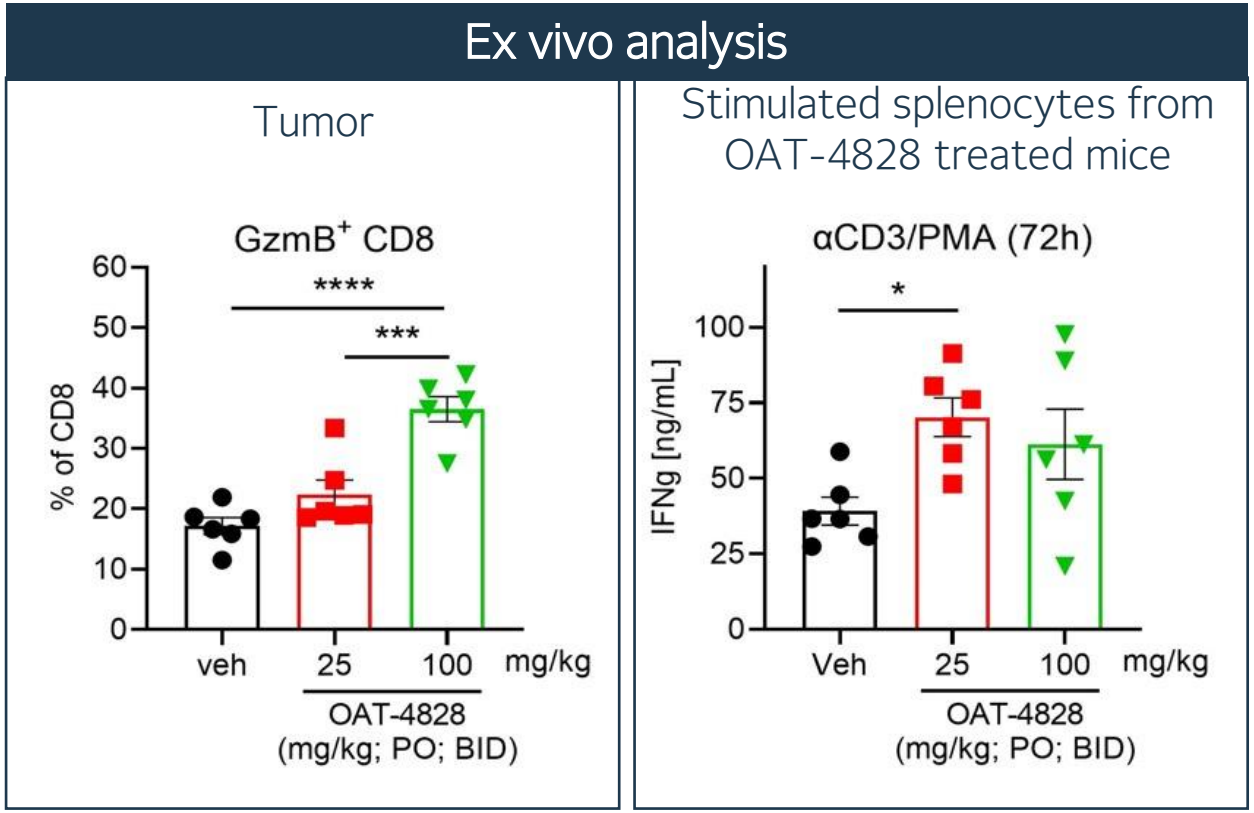
T-cell activation

USP7i | enhances anti-tumor CD4 T cell response

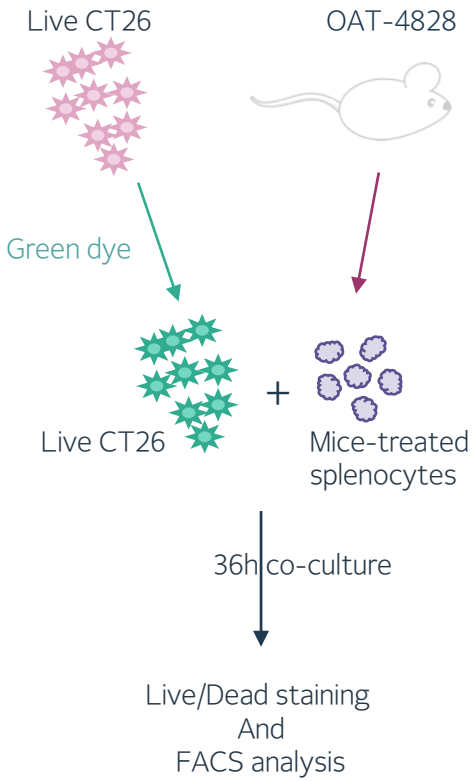


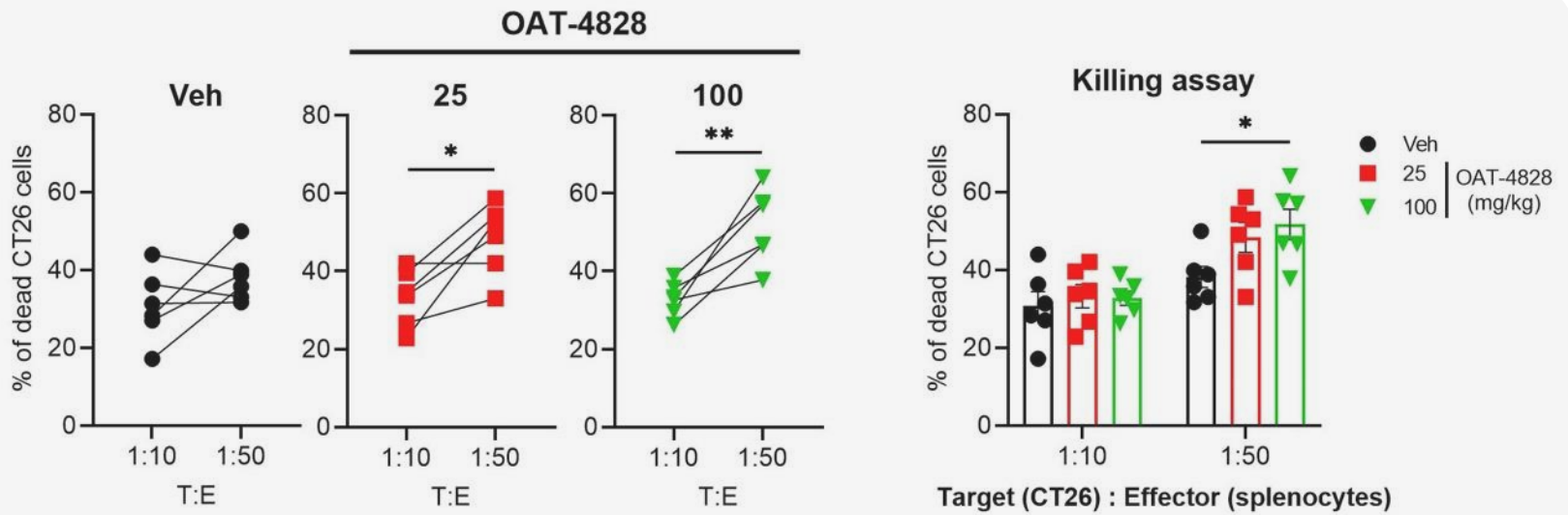
In isolated CD4 T cells from naive mice treated with USP7i and stimulated with CD3/CD28 for 3 days, the pre-harvest treatment led to an increase of IFNγ a marker of Th1 response, and a decrease of both IL-4 and IL-13, markers of Th2 response.

USP7i | increases cytotoxicity of CD8+ T cells in tumor and spleen by increasing the expression of Granzyme B in the tumors and INFg in stimulated splenocytes



Increased killing of CT26 cells by effector cells from USP7i treated mice

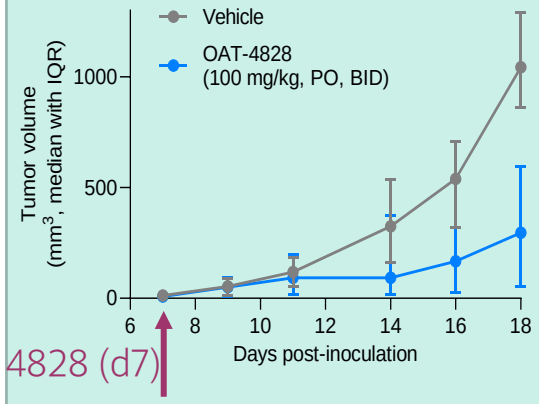


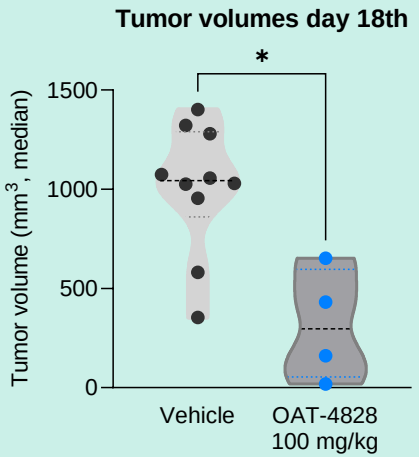


Effector cells isolated from the spleen of mice treated with USP7i for 20 days bear the potential to induce cell death ex vivo, in fresh CT26 cells in the absence of other treatment

USP7i | efficacy in in vivo experiments

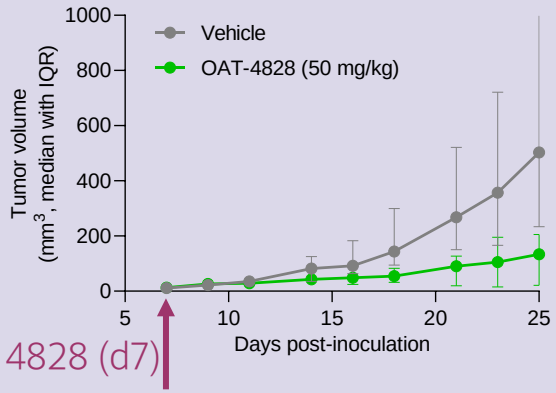
B16F10 melanoma

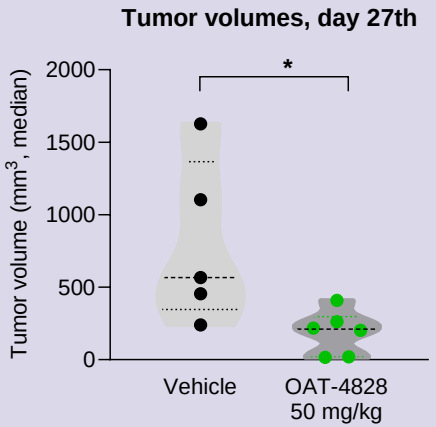




TGI 72% (100 mg/kg)

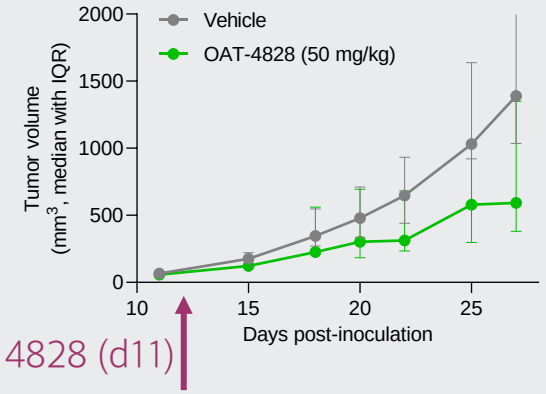
E0771 breast

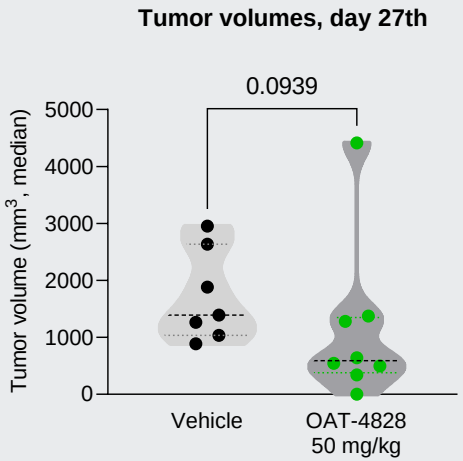




TGI 62% (50 mg/kg)

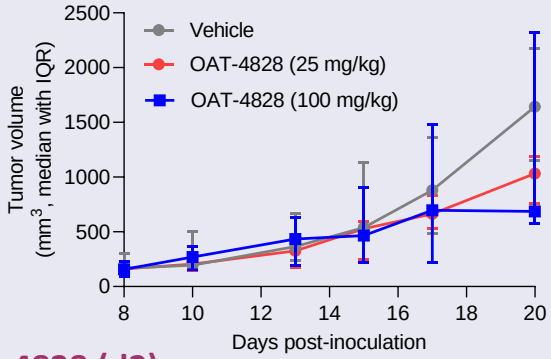
A20 lymphoma

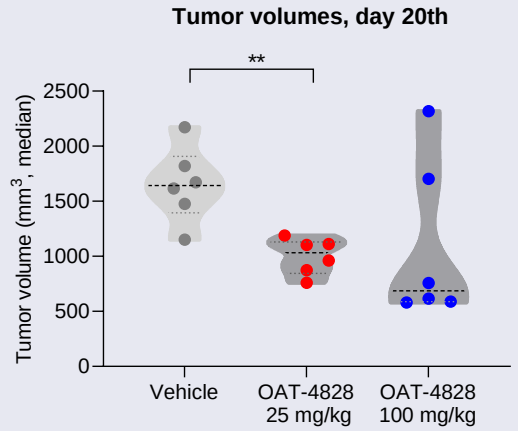




TGI 57% (50 mg/kg)

CT26 colon

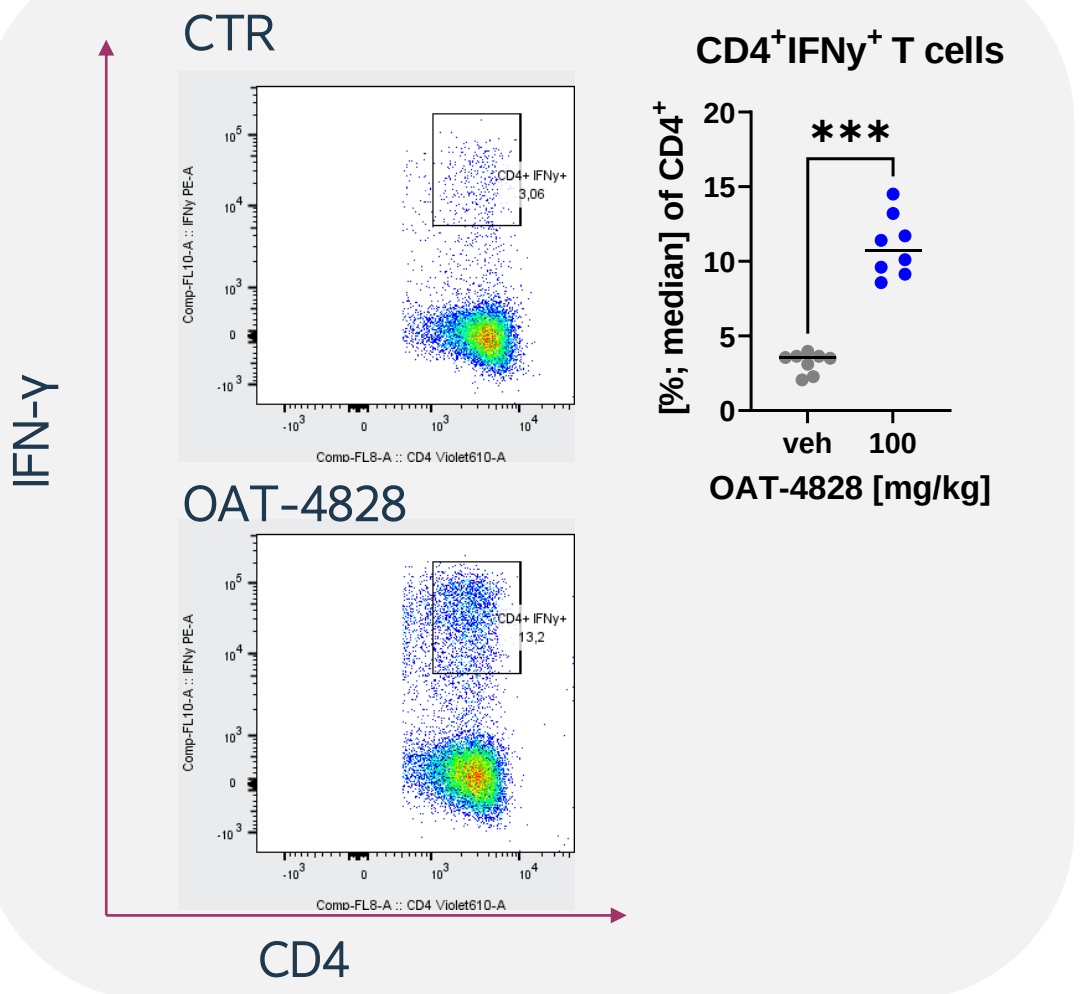


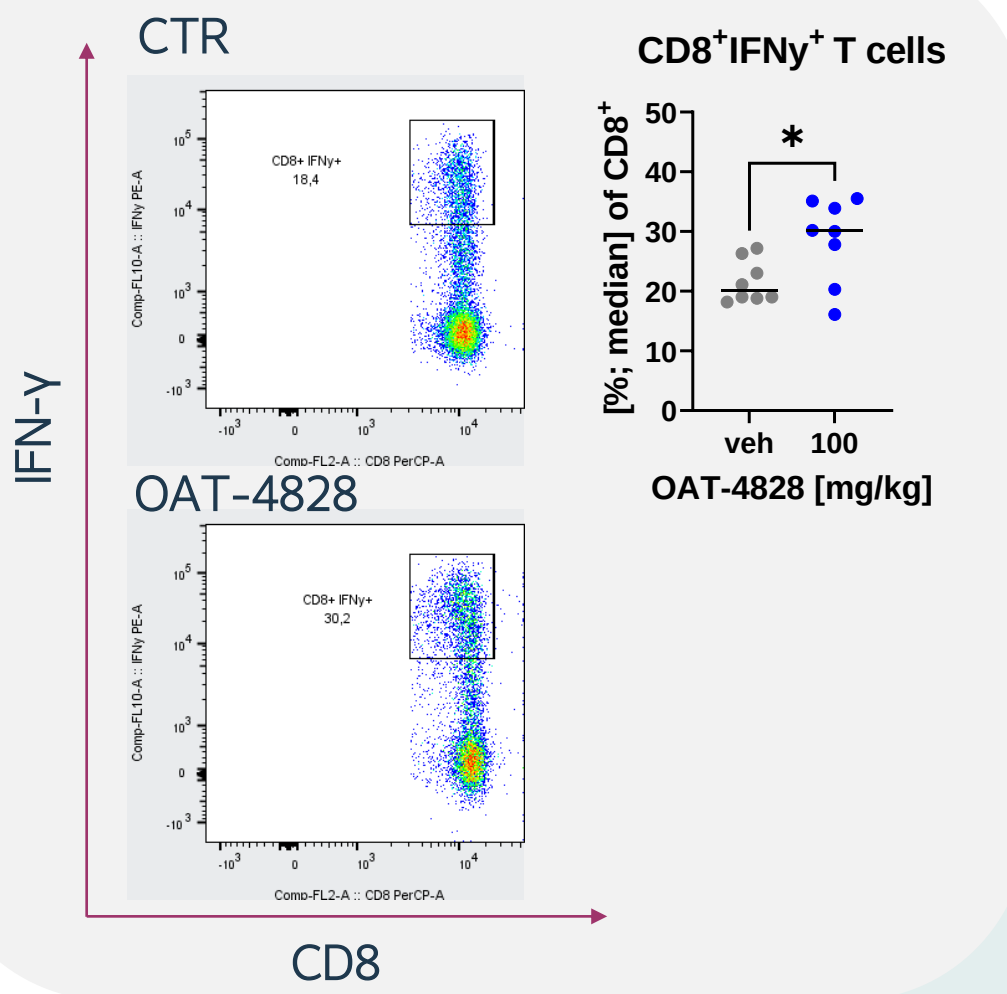


TGI 37% (25 mg/kg)
TGI 58% (100 mg/kg)

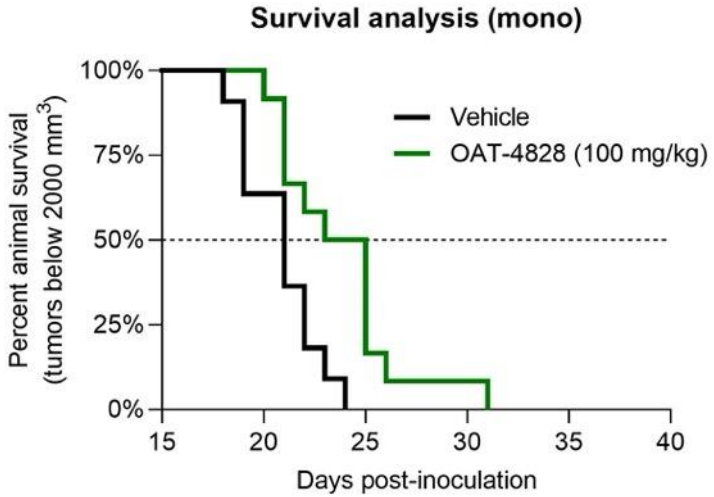
USP7i | strongly enhances IFN-γ production in vivo

B16F10 melanoma

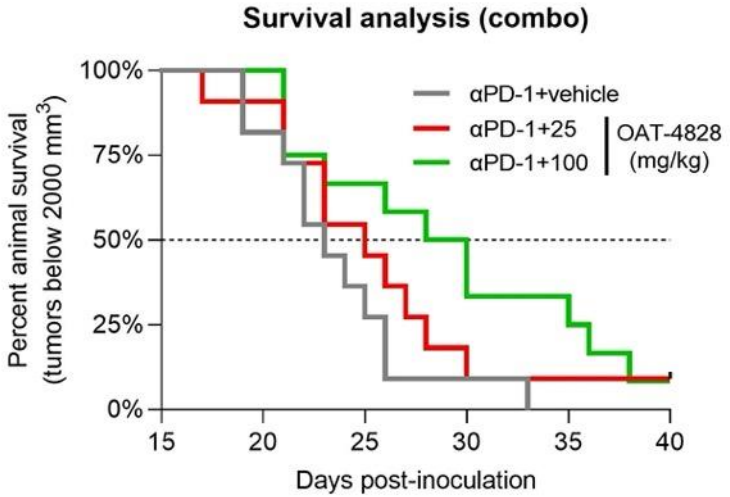




Significant survival increase in CT26 model after USP7i treatment in combination with αPD-1



	Vehicle	OAT-4828 100 mg/kg
Median survival	21 days	24 days
Statistical significance (Mantel-Cox test)		** $p = 0.0059$

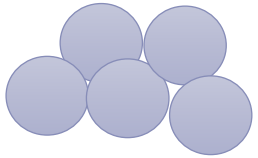


	αPD-1	αPD-1 + OAT-4828 (25 mg/kg)	αPD-1 + OAT-4828 (100 mg/kg)
Median survival	23 days	25 days	29 days
Statistical significance (Mantel-Cox test)		ns	* $p = 0.0198$

USP7i increases the survival of animals by 14% vs. control (stand alone) and by 26% in combination with an aPD-1 vs. aPD-1 alone at 100 mg/kg PO, BID.

USP7i | boosts human T cells activation and proliferation

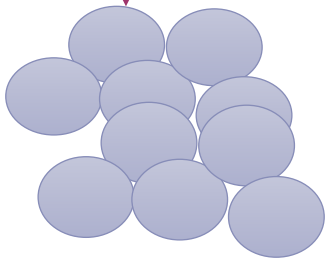
human CD3+ T cells



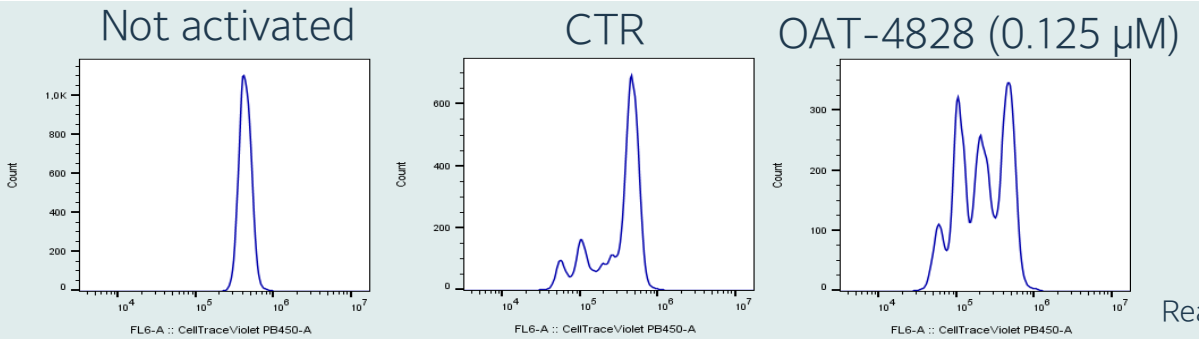
+ 1h OAT-4828

washed

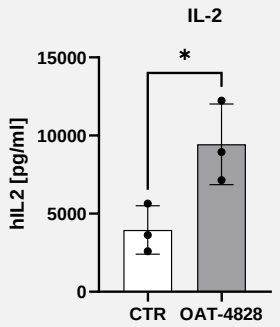
αCD3 + αCD28

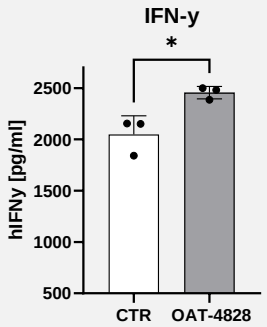


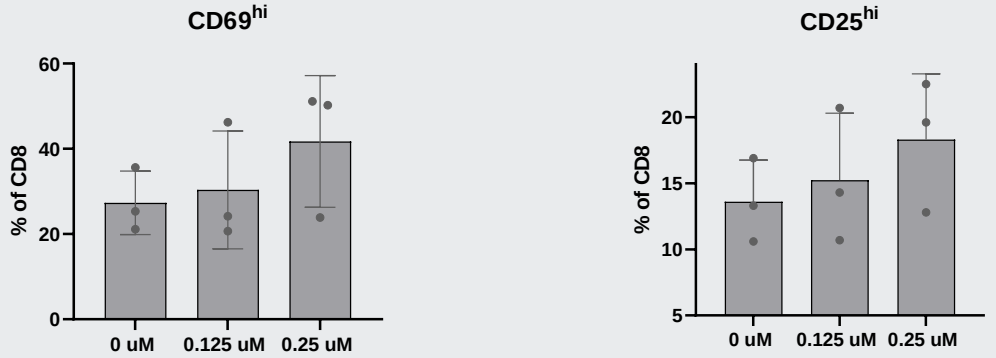
Read out after 24 hrs

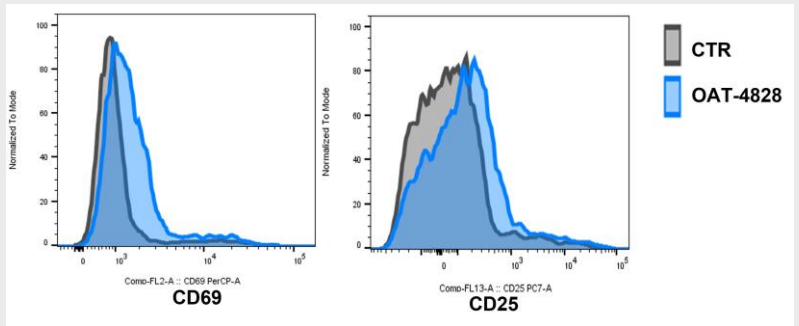


Read out after 72 hrs









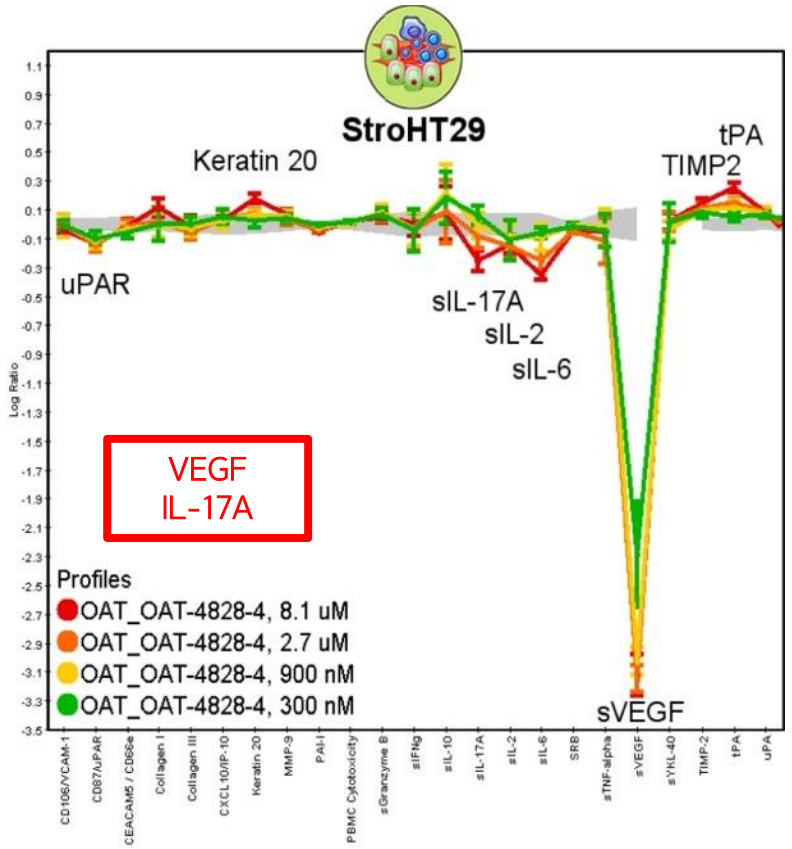
Read out after 24 hrs

Human primary co-cultures validates anti-tumor and immunomodulatory properties of USP7i

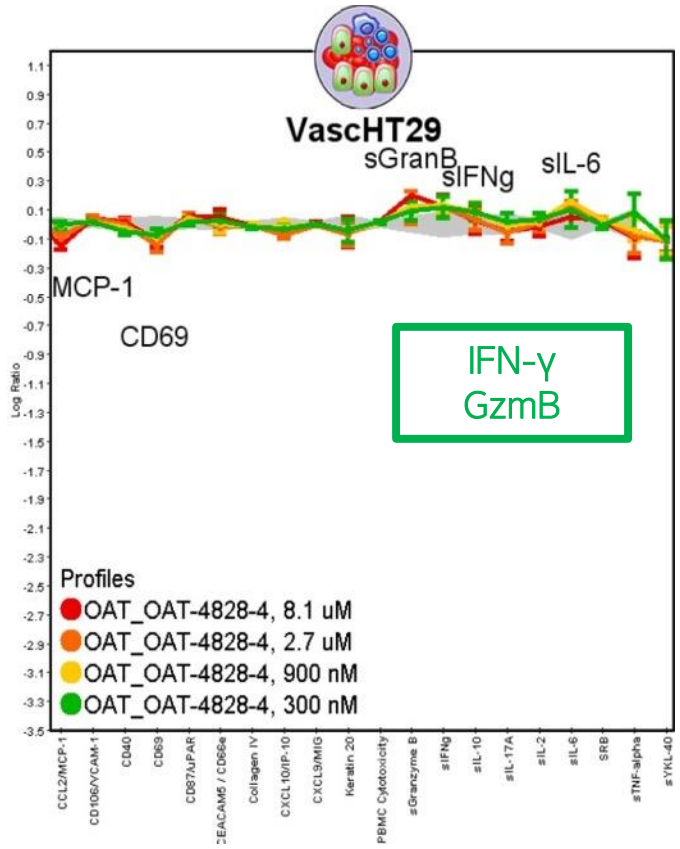
In human primary co-culture of fibroblasts (stromal model) or venular endothelial cells (immune model) with PBMC and colorectal cancer cells, upregulation of GzmB and INFg is observed, as in the animal models as well as a downregulation of IL 2, 6 and 17 and VEGF (ELISA of soluble proteins)

We confirmed that USP7i increases the expression of cytotoxic effectors in human cells and hypothesize that USP7i could influence the inhibition of neovascularization of the tumors

Fibroblasts
+ PBMCs + HT-29 (CRC)



Venular endothelial cells
+ PBMCs + HT-29 (CRC)



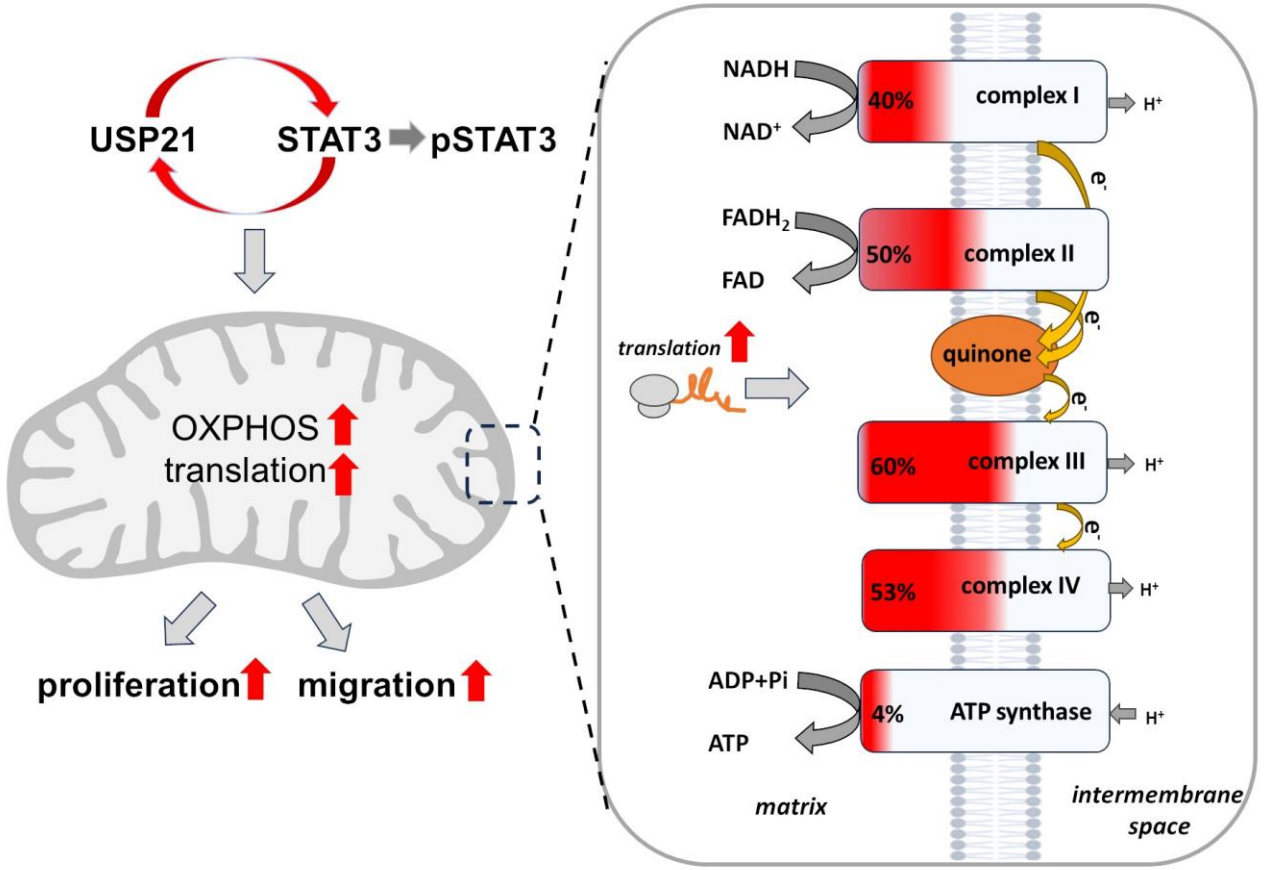
Competitive landscape

No USP7 has entered clinical development stage yet

The main, active competitors, based on patent searches and business intelligence include:

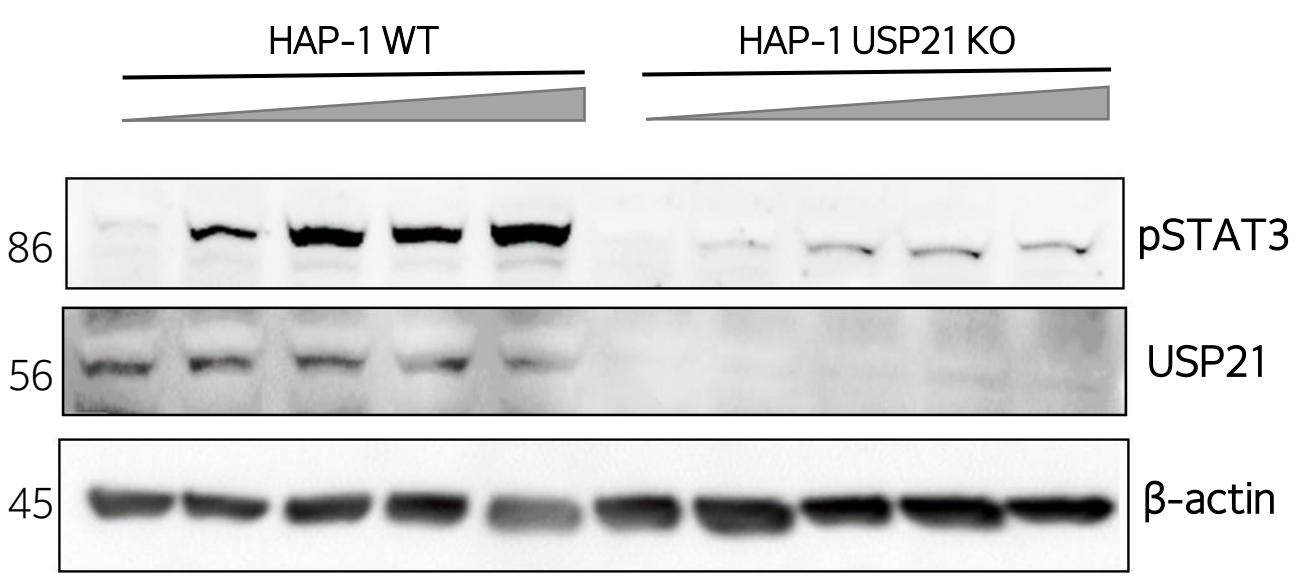
- RAPT Therapeutics - terminated
- Ubiquigent
- Forma Therapeutics
- Servier
- Hybrigenics
- Almac Discovery
- Shouyao Holdings
- Schrödinger, Inc

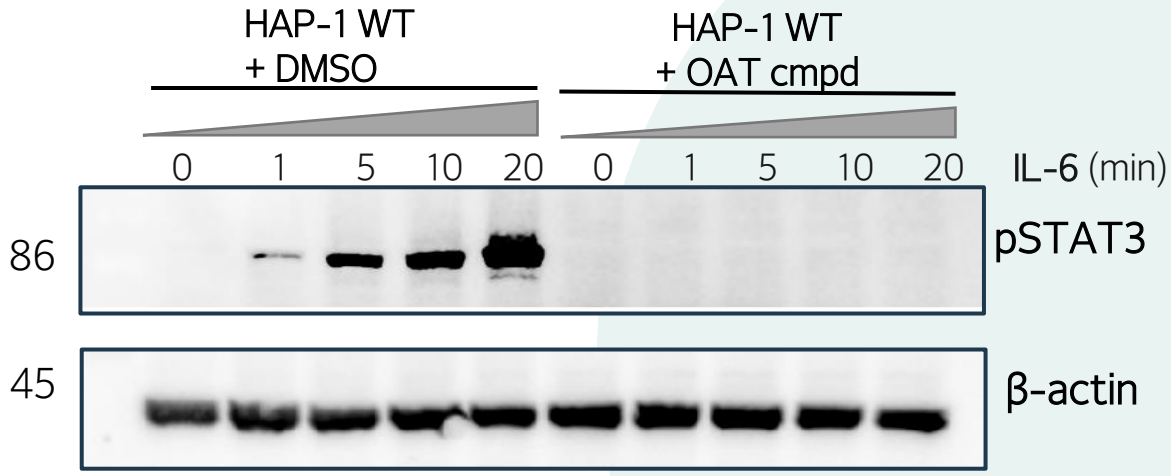
USP21 | attractive therapeutic target



USP21 controls STAT3 activation cancer cell metabolism. Genes of mitochondrial respiration chain complexes regulated by USP21 are marked in red. USP21 drives intracellular ATP production used by cancer cells for proliferation and migration

USP21 | First lead-stage USP21i compounds inhibit STAT3 phosphorylation





Pharmacological inhibition of usp21 results in blockage of STAT3 phosphorylation - an undruggable target and a crucial pathway driving cancer cell biology

Competitive landscape

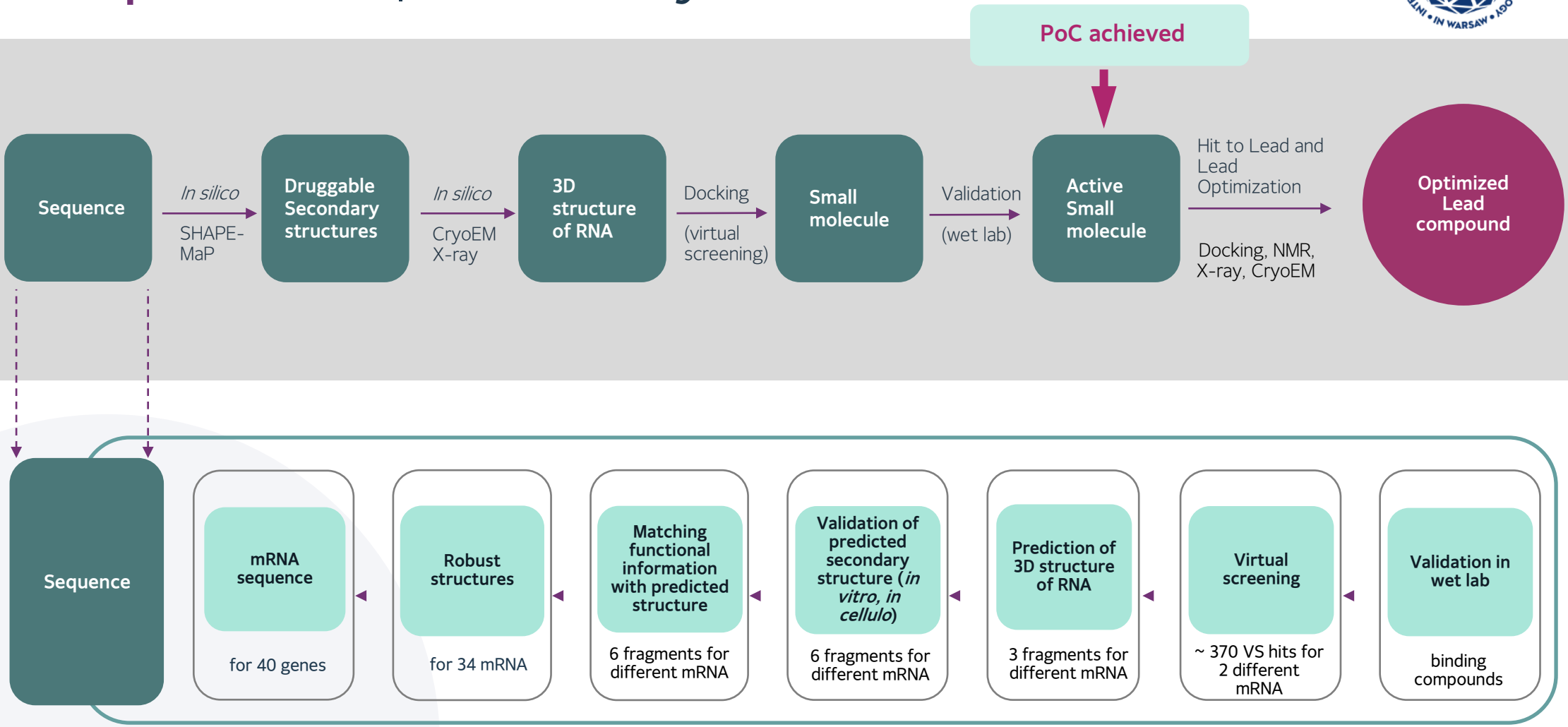
The main, active competitors based on patent searches and business intelligence include:

- Mission Therapeutics
- Bayer

Our small molecule mRNA discovery platform

with potential to disrupt direct mRNA targeting approaches

mRNA platform | discovery workflow



mRNA platform | druggable regions selection

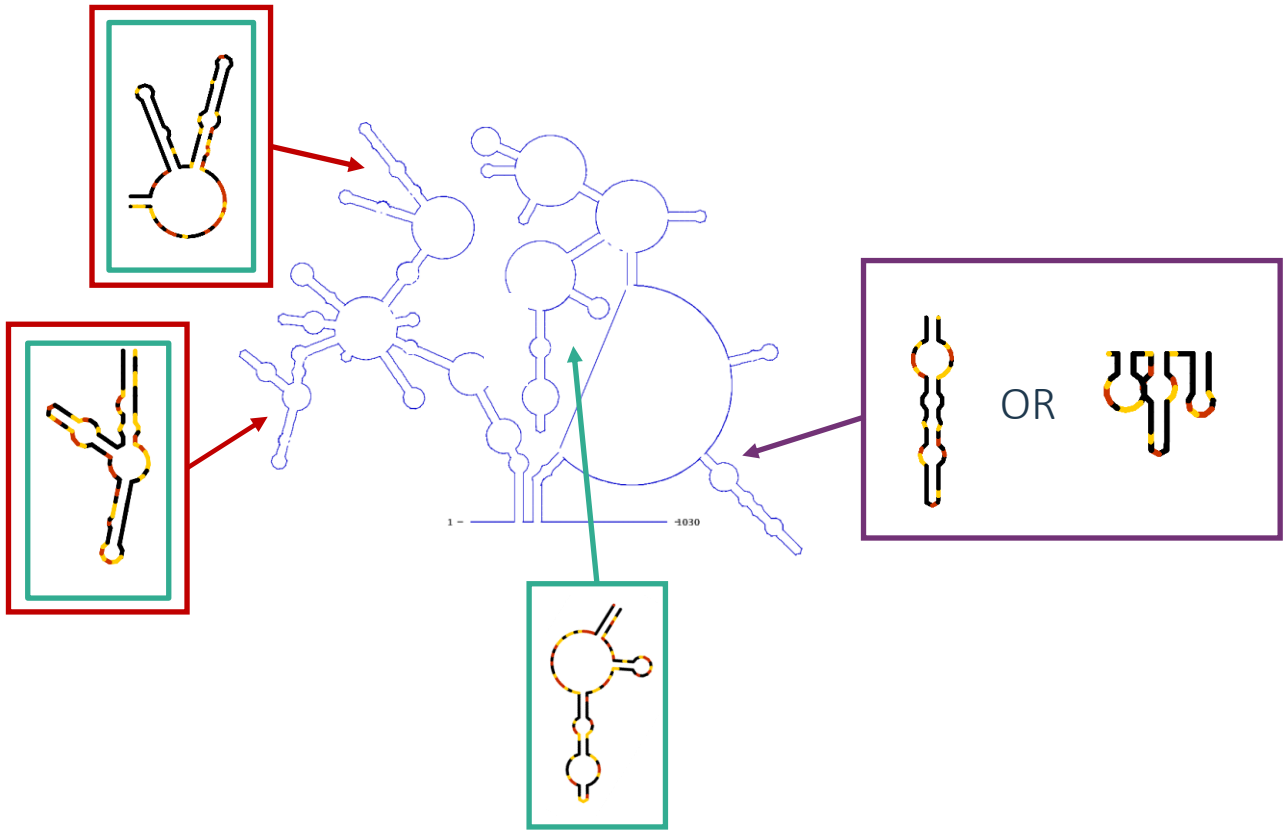
Druggable Secondary structures

Robust structures:
SHAPE-MaP, prediction

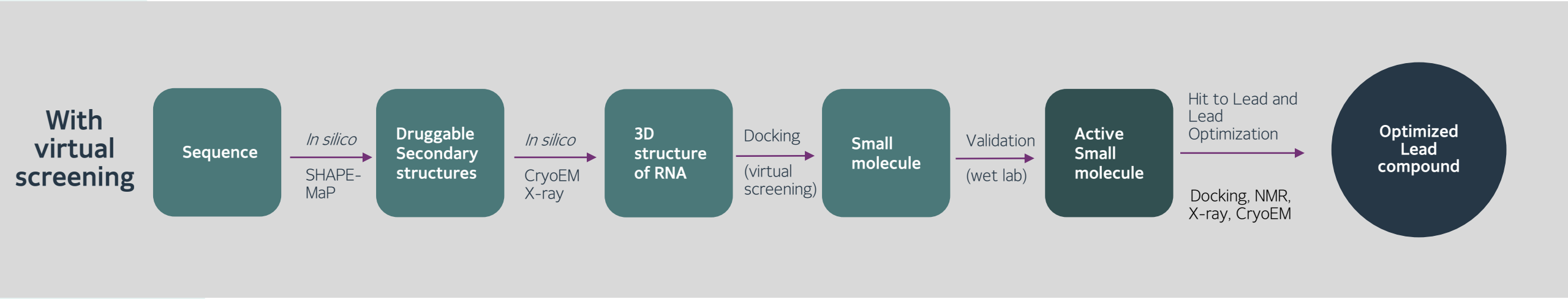
Functionaly:
- literature
- omics data
- verification experiments

Conservation

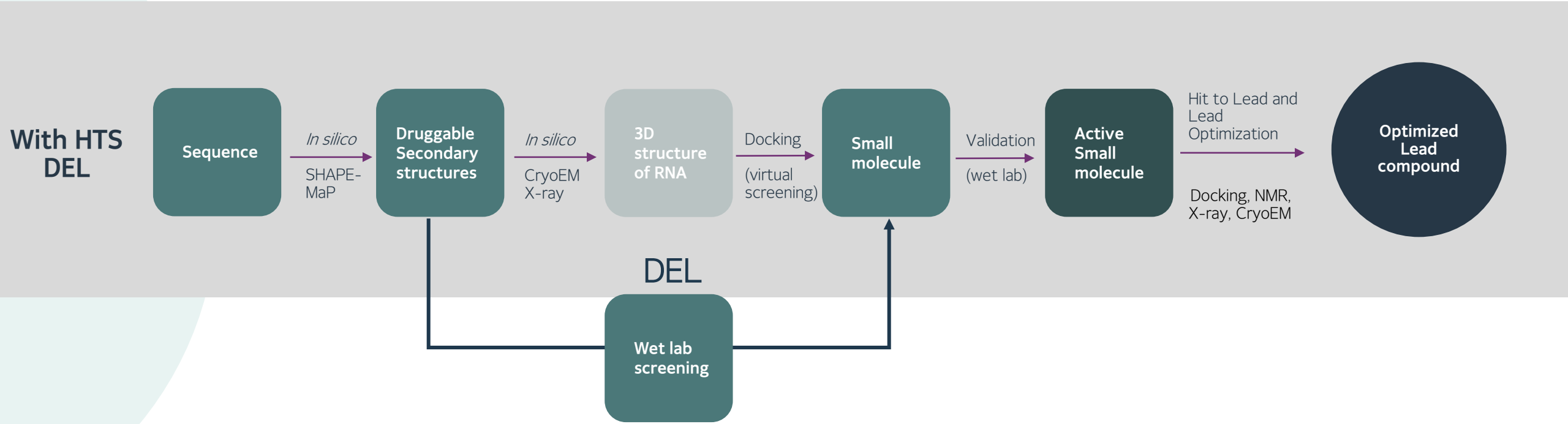
RNA isoforms analysis



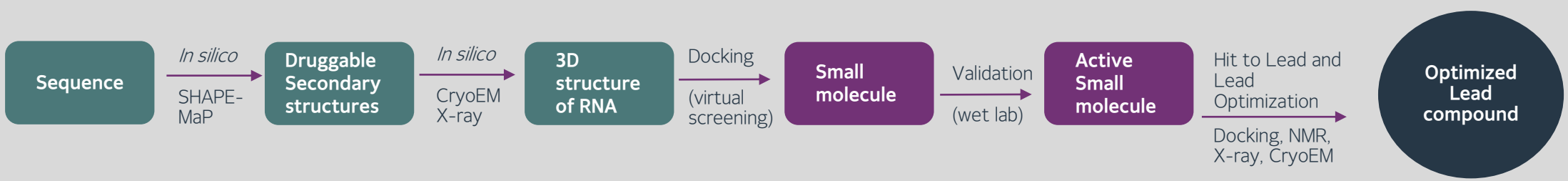
mRNA platform | two ways to the target



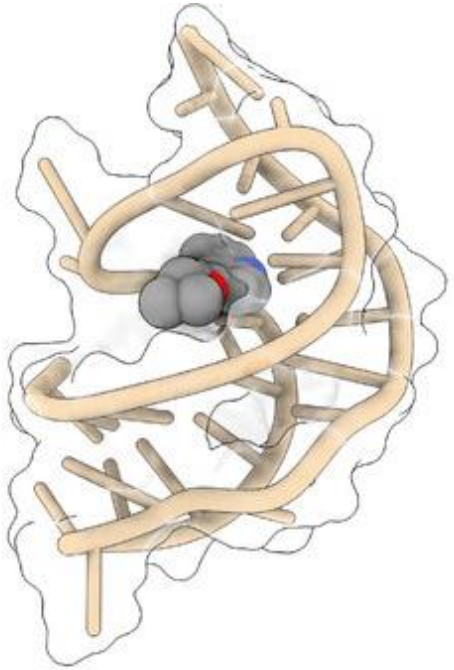
mRNA platform | two ways to the target



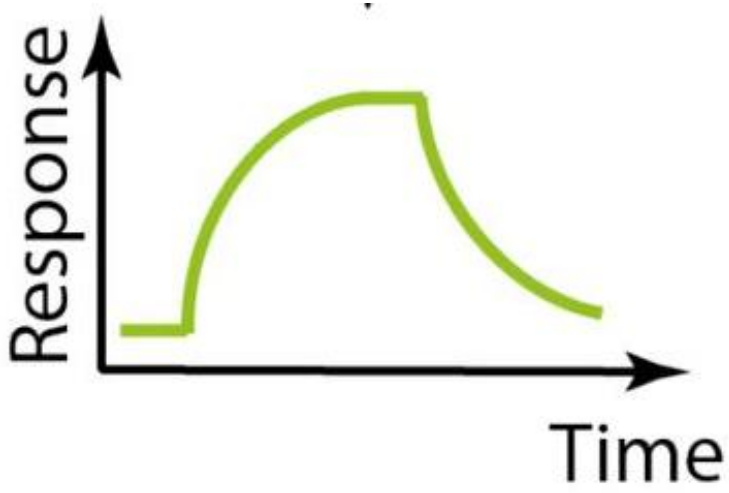
mRNA platform | verification of binding in wet lab



Small molecule
bound to an RNA target



Evalution of binding
in biophysical test e.g. SPR



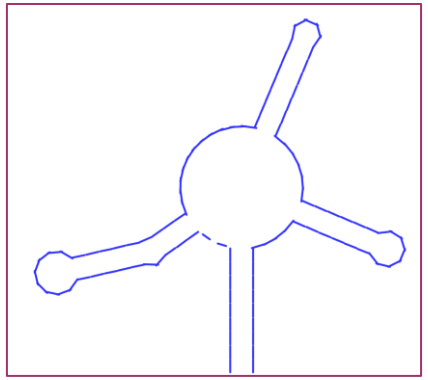
PoC

First milestone achieved

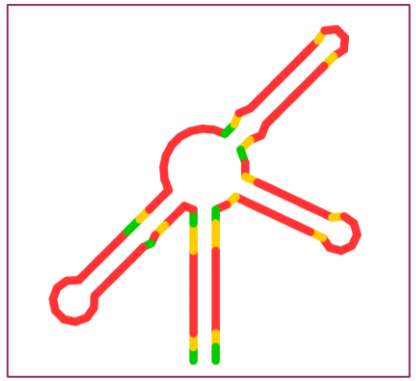
mRNA platform | first step - druggable region selection

Region with a stable structure

Predicted robust structure



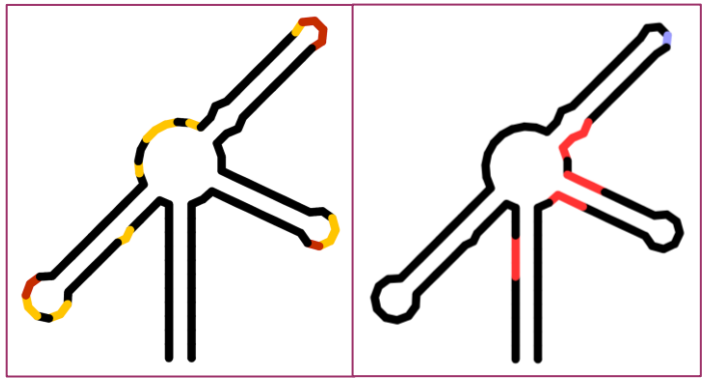
Shannon entropy



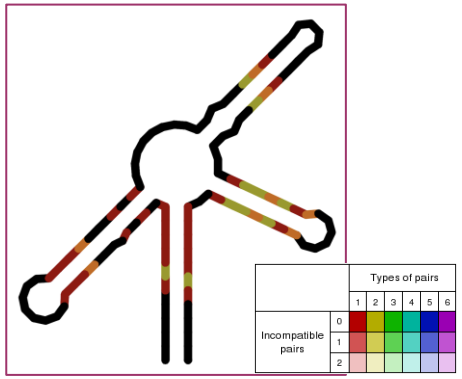
SHAPE-MaP

In vitro

In vitro – in cellulo

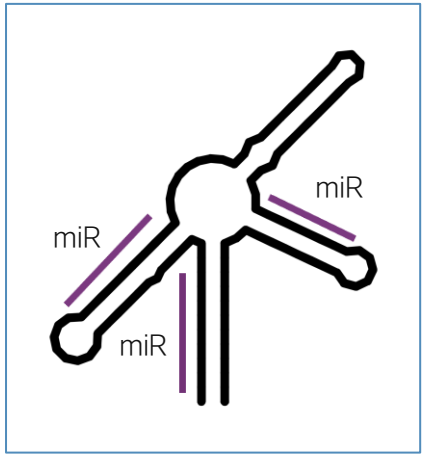


Conservation

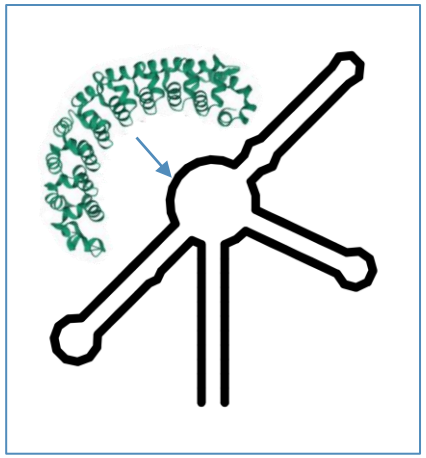


Functionality

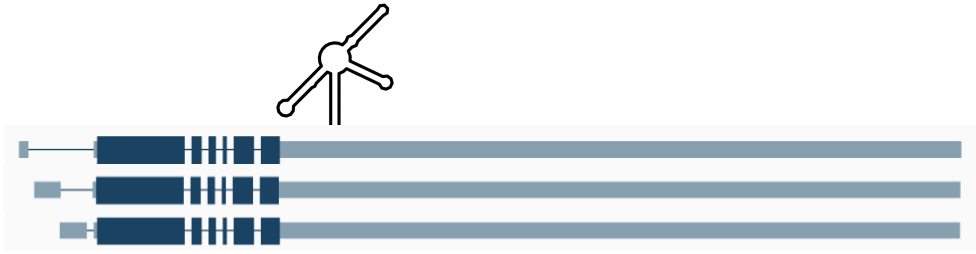
Publications



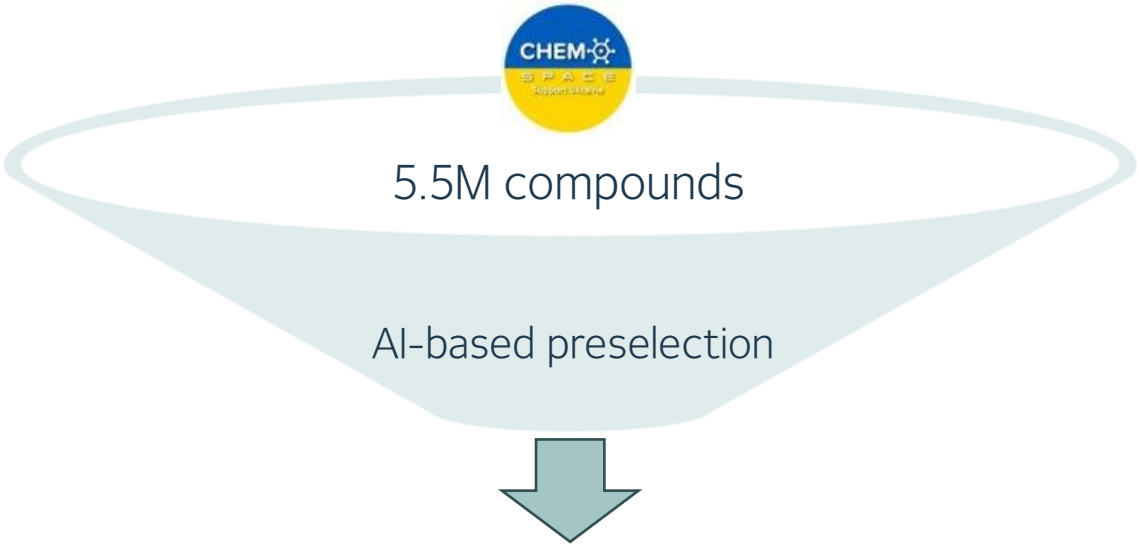
CLIP-seq



Protein coding RNA isoforms
from patient's tissues and cell lines contain the target region



mRNA platform | wet lab screening cascade – biophysical tests



500k
RNA-Targeted Library

VS → 376 VS HITs

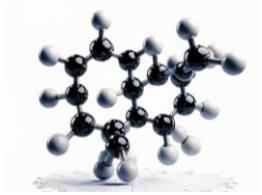
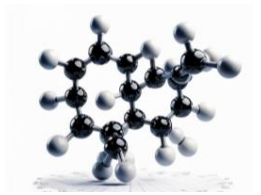
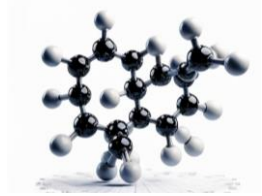
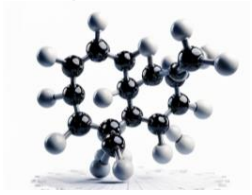
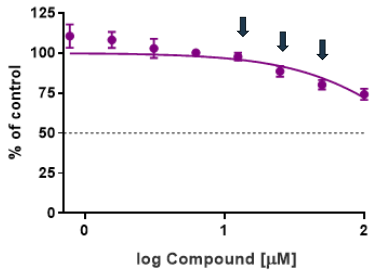
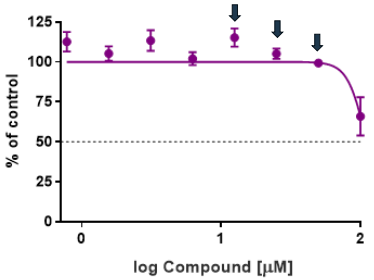
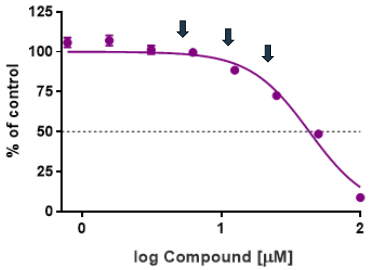
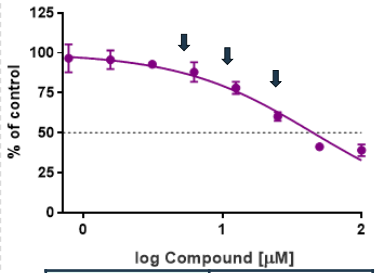
SPR →

~ 30 HITs with K_D < 100 µM
5 Different chemotypes
6 HITs with K_D ≤ 10 µM

mRNA platform | preliminary MedChem analysis of the properties of the obtained HITs

- No PAINs
- No aggregation
- No redox
- Molecular Weight < 500 g/mol
- LogP < 5
- PSA < 100 $Å^2$
- No solubility issues in assay
- Reasonable IP space
- No safety concerns (no hERG, no CYP inhibition, no genotoxicity) – In silico predictions
- No reactive functional groups or problematic moieties in the chemical structure

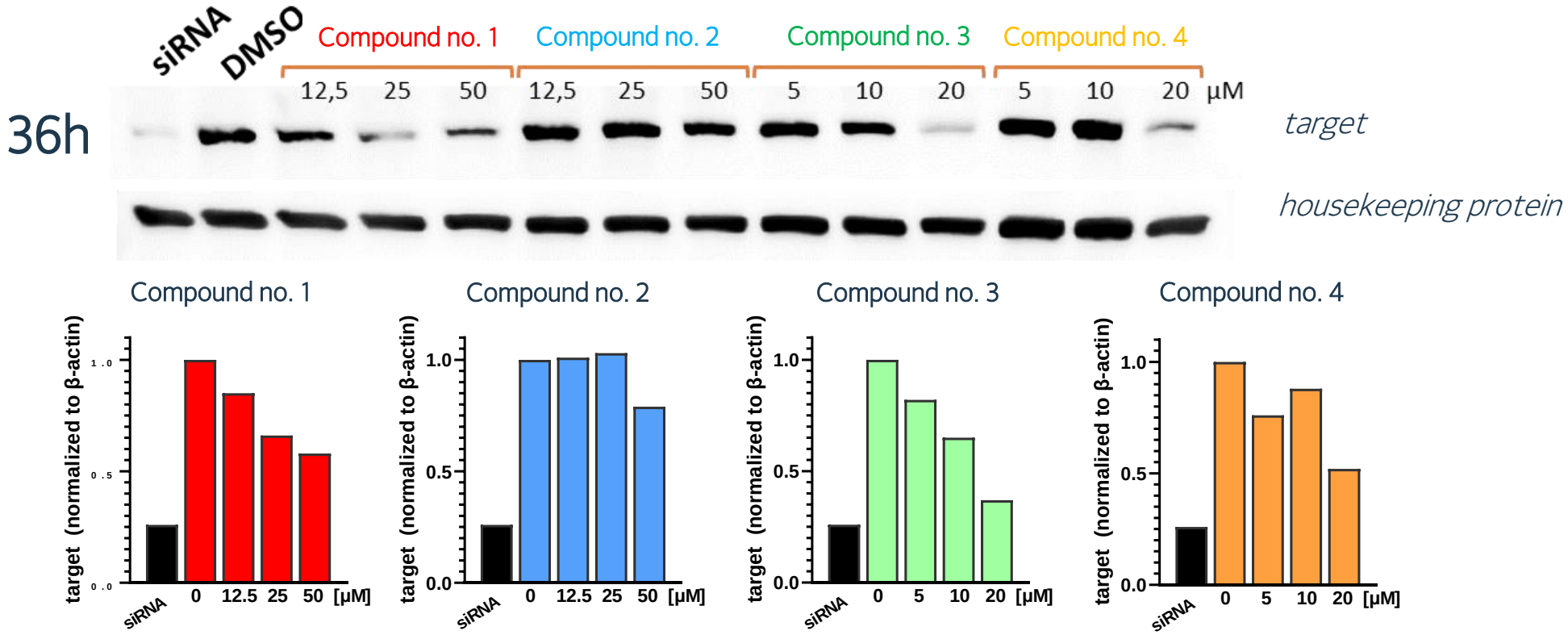
mRNA platform | wet lab screening cascade – cellular tests (cytotoxicity)

Compound no. 1	Compound no. 2	Compound no. 3	Compound no. 4
			
SPR K_D = 11 µM	SPR K_D = 31 µM	SPR K_D = 5 µM	SPR K_D = 1.3 µM
Compound no. 1	Compound no. 2	Compound no. 3	Compound no. 4
			
EC_{50} >100 µM	EC_{50} >100 µM	EC_{50} 43 µM	EC_{50} 44 µM

Resynthetized HITs

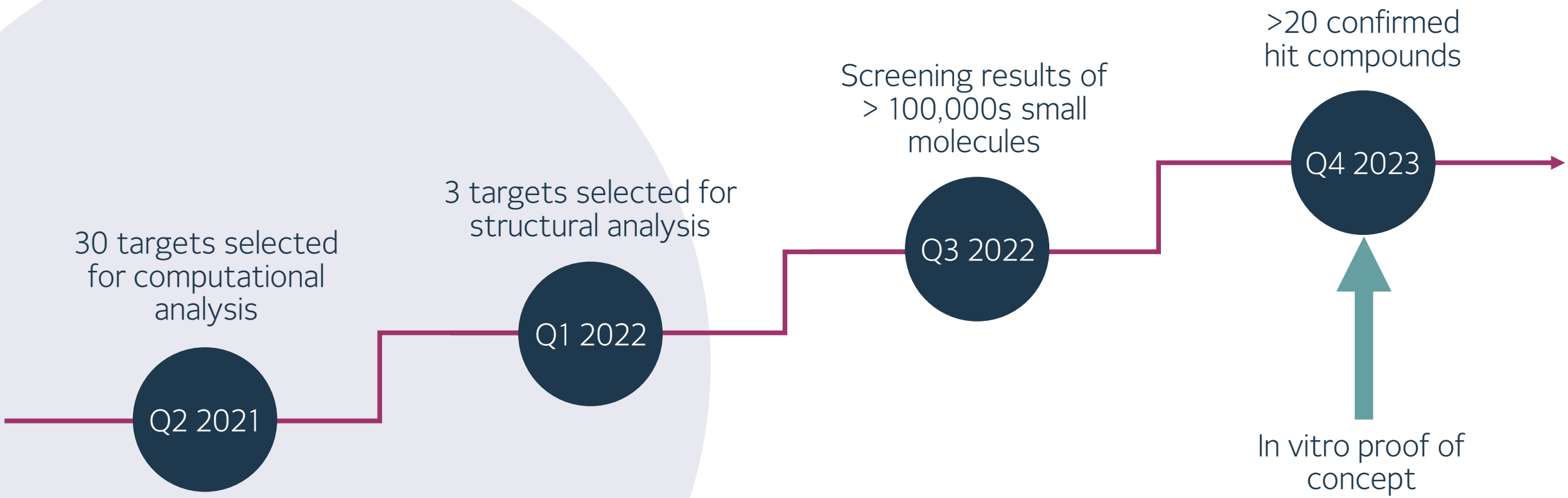
24h

mRNA platform | wet lab screening cascade – cellular tests (target protein level)



Compound no. 1,3 and 4 causes downregulation of protein target in cells

mRNA platform | our path to success in discovering RNA targeting small molecules



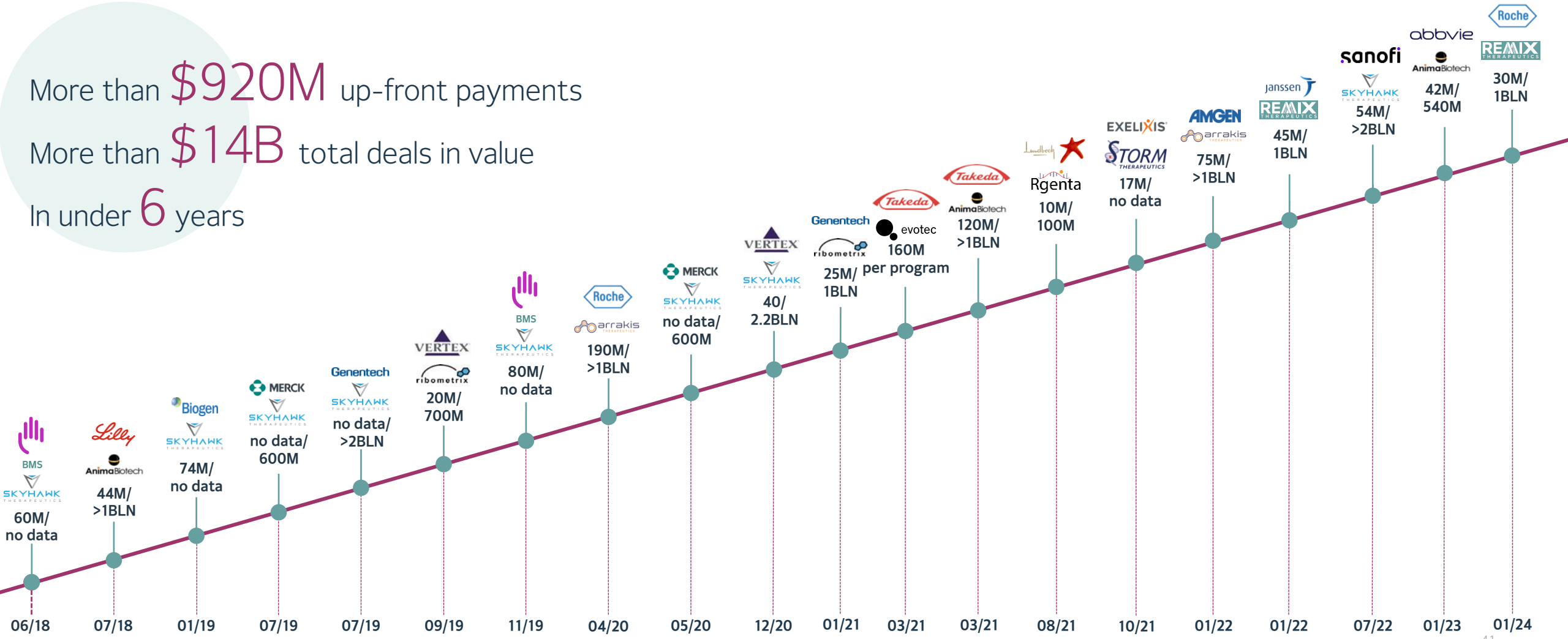
The RNA space is advancing rapidly

Targeting RNA with small molecules has led to multiple significant biotech / big pharma partnerships

More than $920M up-front payments

More than $14B total deals in value

In under 6 years



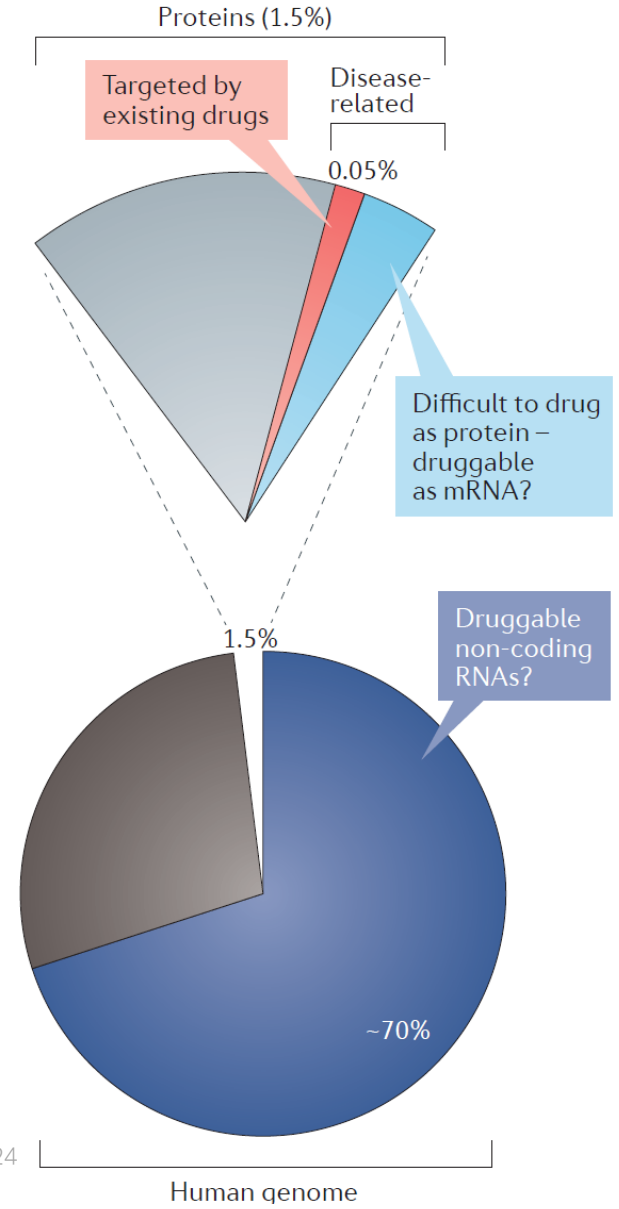
mRNA platform | multiple collaborations with RNA experts

Field of cooperation		
RNA bioinformatics		**Prof. Janusz Bujnicki**, head of the Laboratory of Bioinformatics and Protein Engineering (LBIB) at the International Institute of Molecular and Cell Biology (IIMCB) in Warsaw.
RNA secondary structure prediction using SHAPE		**Joanna Sztuba-Solińska, PhD,** Principal Scientist at Pfizer (expert in the field of using experimental methods to determine the 2D structure of RNA molecules)
RNA forecast		**Michael T. Wolfinger, PhD** (professor of bioinformatics at the University of Freiburg, expert in the field of development and utilization of bioinformatics methods for RNA secondary structure prediction, and identification of evolutionarily conserved RNA regions).
RNA protein networks		**Chase Weidmann, PhD** (assistant professor at the University of Michigan, expert in the field of long-range RNA interactions and RNA-protein interaction investigation).

MoleCuring diseases by targeting RNA - methods for identifying compounds

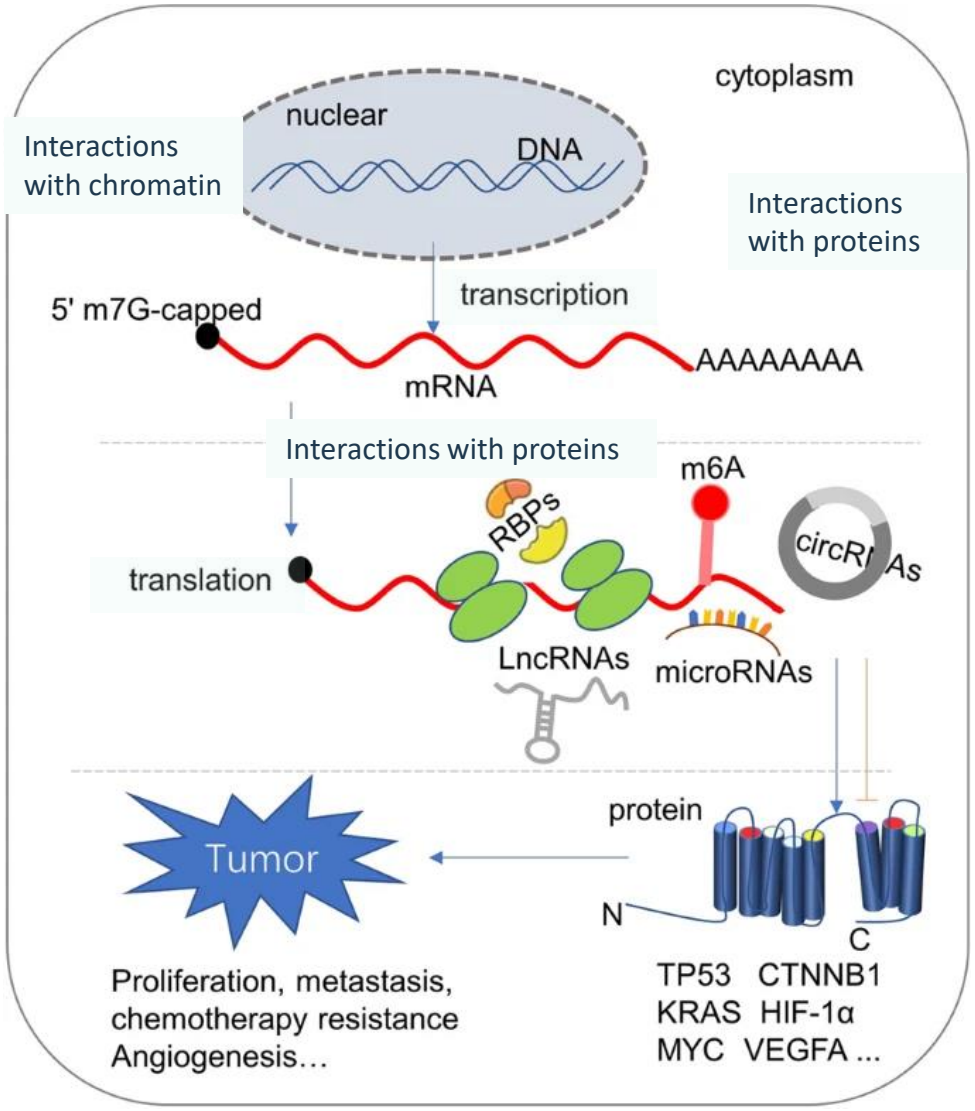
Joanna Sztuba-Solińska, PhD

Why target RNA?



- Most drugs on the market target enzymes (1.5% of human genome ~ 20,000 proteins)
- Within 20,000 proteins encoded roughly 12% is disease related = 3,000 proteins
- Currently approved drugs interact with ~ 700 proteins (0.05 of the genome)
- Mutations, drug resistance
- We have no other choice but to look for other targets!

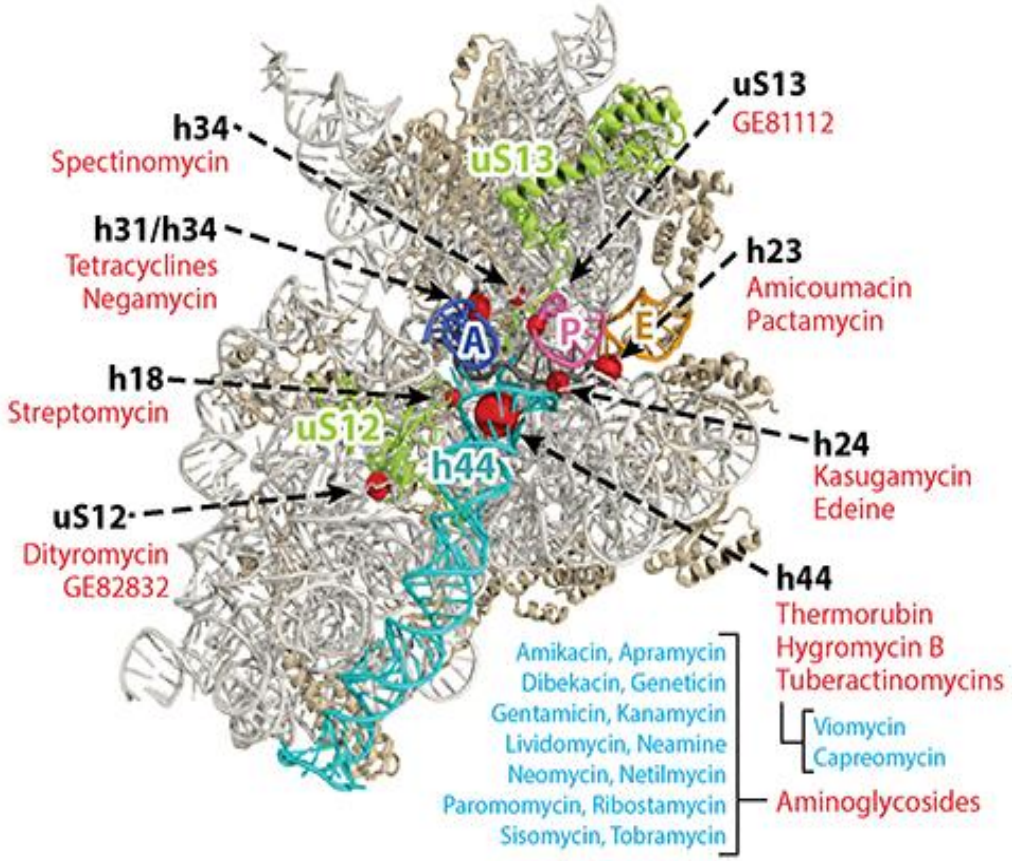
RNA regulates myriad of biological processes!



- Transcription of other RNAs and self
- Degradation of other RNAs and self
- Translation = protein expression
- Interaction with chromatin
- Localization of other RNAs, proteins
- Sensing of metabolites, ions, temperature change

Why is RNA lagging behind?

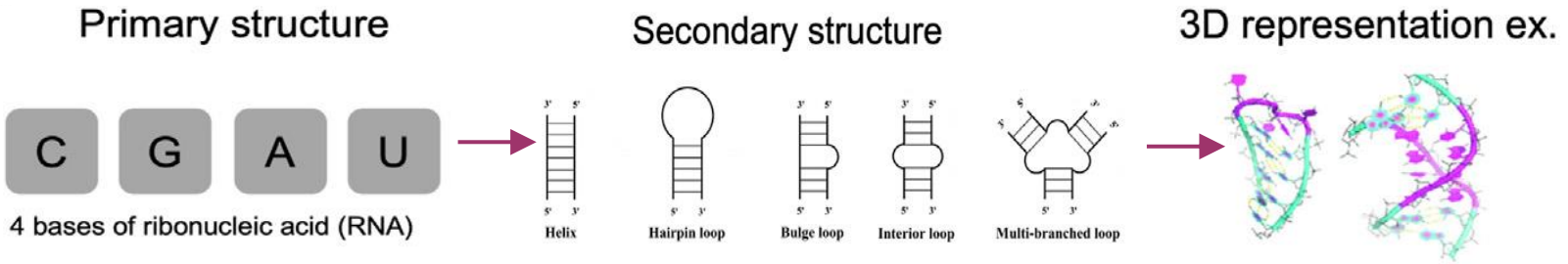
- Viewed as a simple messenger (1947, Boivin and Vendrely)
- 14 years later, mRNA was isolated for the first time (Brenner, Jacob, Meselson)
- The discovery of regulatory RNAs began to change that view (T. Cech and S. Althman, 1980)
- 1980 - first FDA-approved antibacterial drugs targeting rRNA – antibiotics!
- RNA sequencing technologies developed only recently!



Major antibiotic binding sites on the 30S rRNA
doi: 10.1146/annurev-biochem-062917-011942

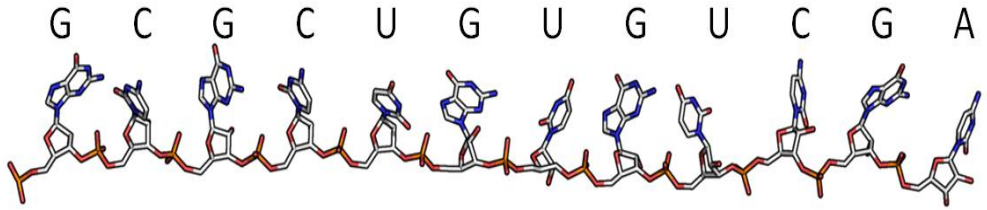
RNA is a challenging target

Challenge 1: "simplicity" of RNA molecule

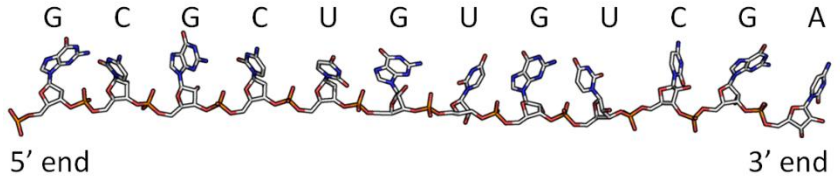


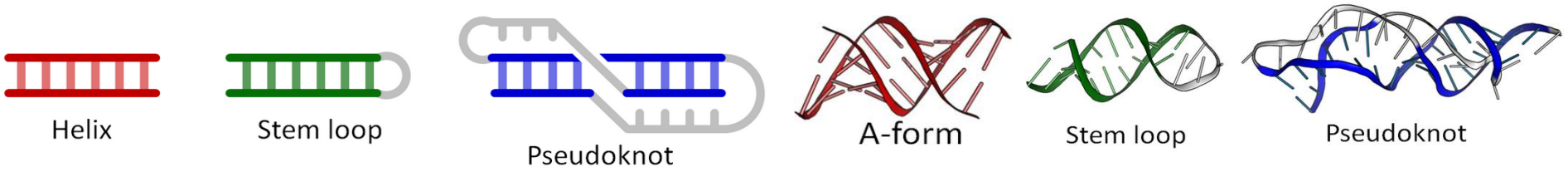
Challenge 2: most RNAs exist not as static folds but as ensembles of structurally and temporally dynamic molecule

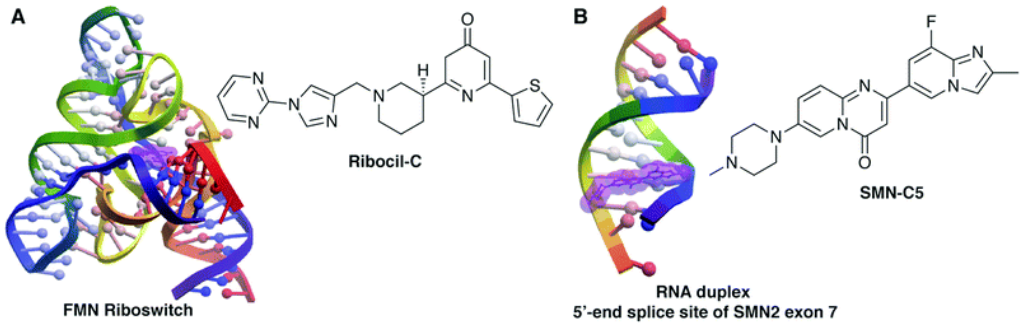
Challenge 3: the polyanionic backbone of RNA makes it difficult to form a deep hydrophobic pocket that would provide a favorable site for ligand binding

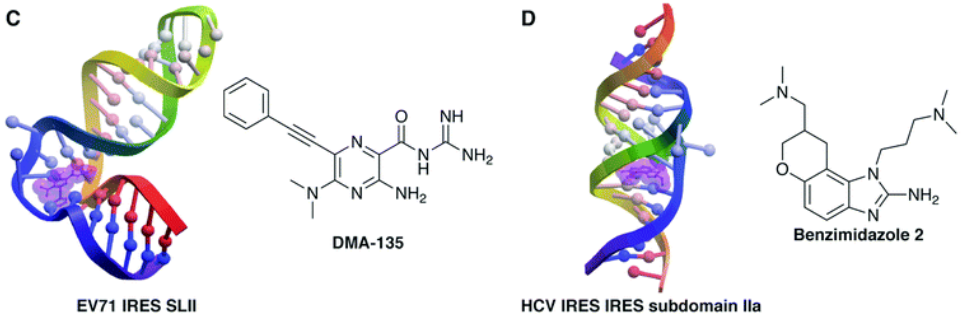


What makes RNA druggable?









Structures in human mRNAs regulate key biological processes

Synucleozid - binds an A bulge in the IRE of SNCA mRNA, inhibiting translation (Parkinson)

IRE (iron-responsive element) - translational regulation

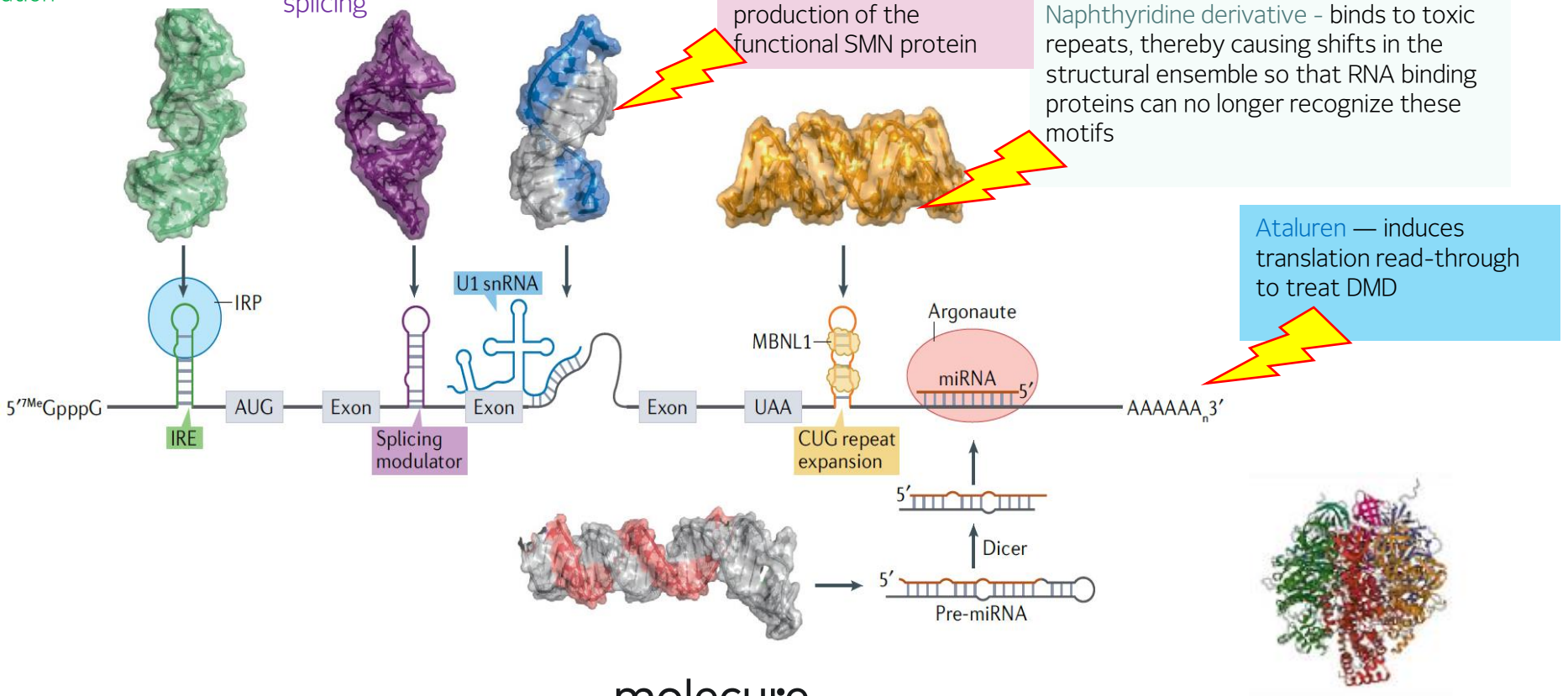
Branaplam – leads to the inclusion of an extra exon in the *HTT*. This extra exon makes the mRNA molecule unstable, and it ends up getting rapidly destroyed through

splicing modulators - alternative pre-mRNA splicing

Risdiplam (Evrysdi) - stabilizing *SMN2* and U1 snRNA = *SMN2* exon 7 is included, increased production of the functional SMN protein

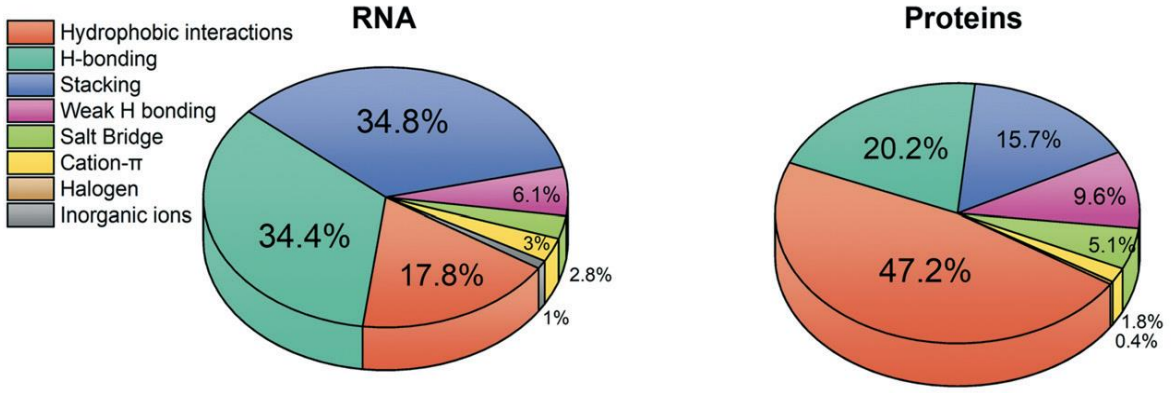
Naphthyridine derivative - binds to toxic repeats, thereby causing shifts in the structural ensemble so that RNA binding proteins can no longer recognize these motifs

Ataluren — induces translation read-through to treat DMD



RNA binding pockets have unique properties

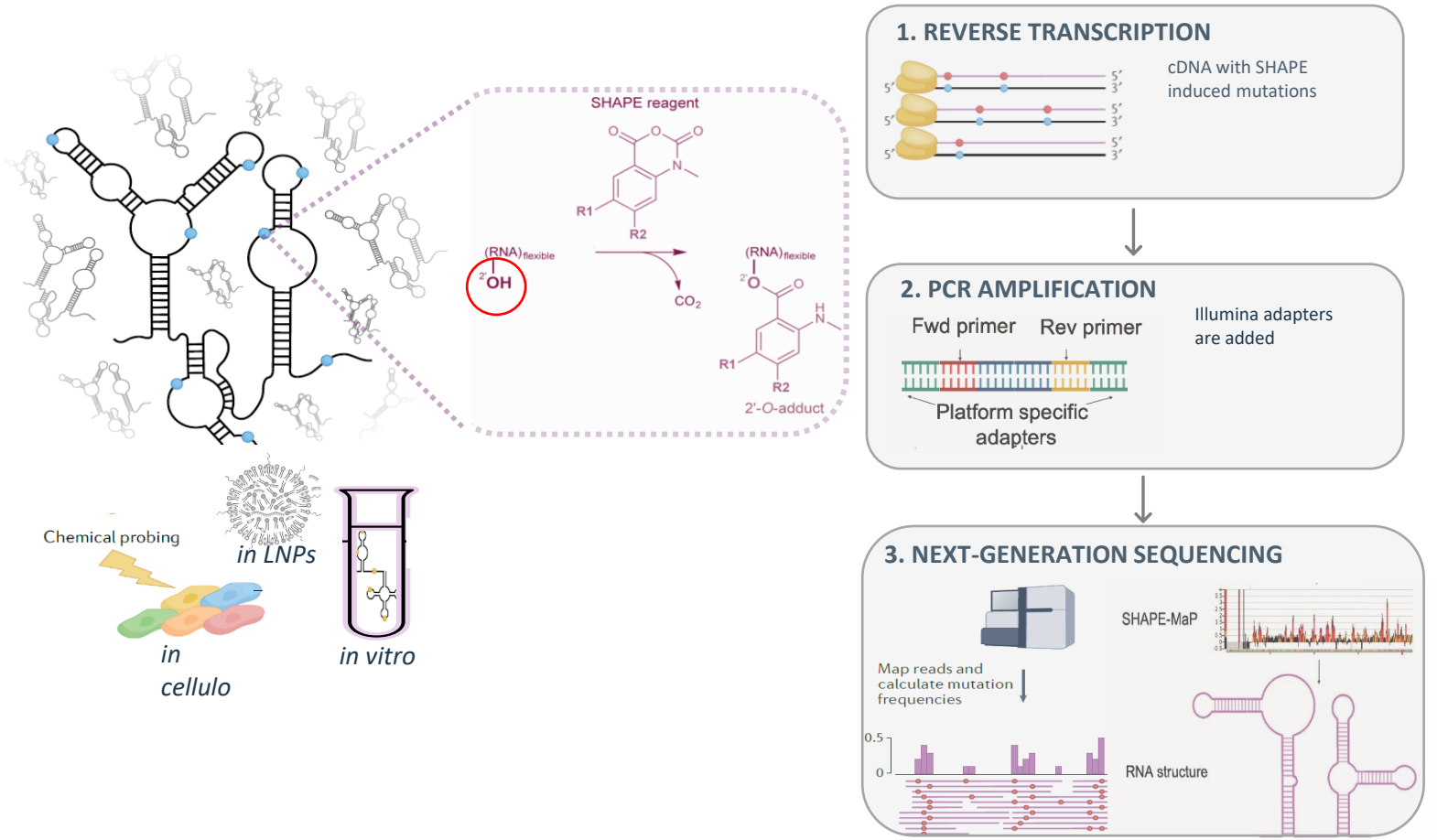
- similar propertied to protein binding pockets, i.e., volume, buriedness (lack of solvent exposure),
- recognition by small molecules is driven by:
  - ✓ stacking interactions of nucleobases (34.8%)
  - ✓ hydrogen bonding (34.4%) – RNA binding pockets have wide variety of hydrogen bond acceptors and donors, both in the sugar–phosphate backbone and nucleobases
  - ✓ hydrophobic contacts (17.8%) – guanine is the most hydrophobic of the natural bases
  - ✓ Ions – Mg^{2+} and K^{+} may mediate the binding



Distribution of molecular interactions between RNA – the totality of small molecules, Protein–small molecules.

doi: 10.1039/d0md00167h

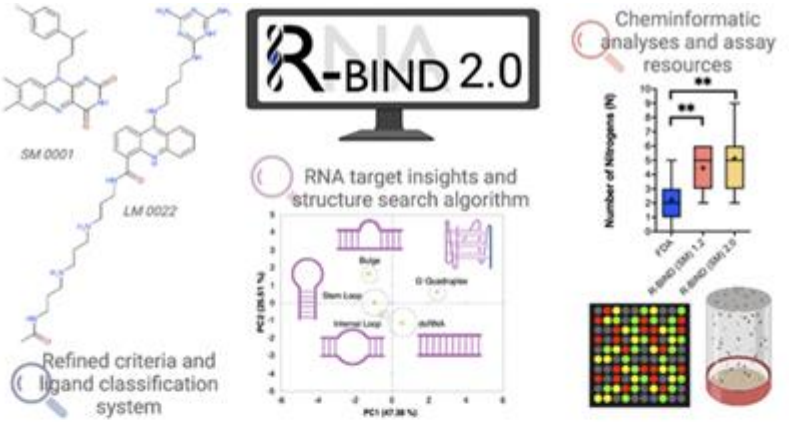
Good RNA targets must have high information content

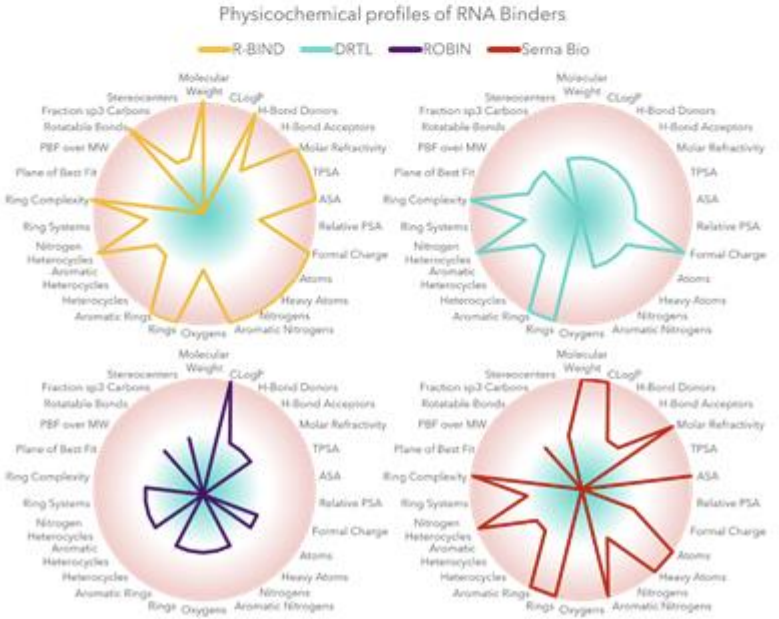


↑ information content = ↑ structural complexity that specifies targets uniqueness (selectivity)

What about ligands aka small molecules?

- RNA-binding small molecules have unique properties:
  - ✓↓ octanol–water partition coefficients than protein-binding compounds (*measure of lipophilicity)*
  - ✓↑ topological polar surface areas (*drug absorption*)
  - ✓↑ hydrogen bond donors and acceptors
  - ✓↑ heteroatom-containing aromatic
  - ✓rod-like shape

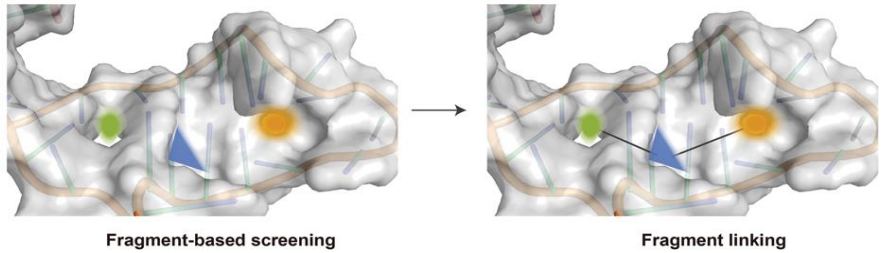




Strategies for RNA targeting
Framework 1: *RNA motifs used as modules for ligand binding*

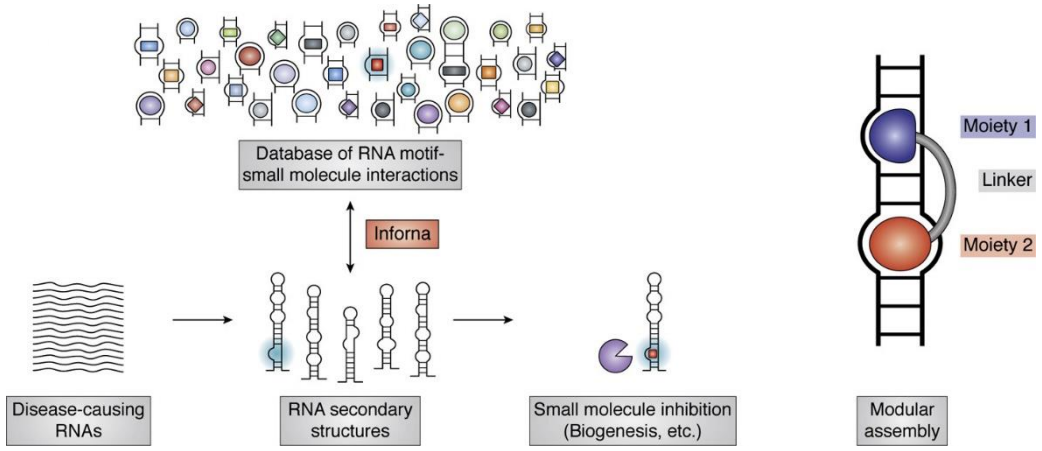
Focused on modules of RNA structure that can be used as units in designing selective small molecules

1. Hergenrother lab - discovered wedge-shaped compounds binding RNA bulges and other compounds binding to octaloops.



Fragment library is experimentally or computationally screened against an RNA target. Identified fragments are linked or optimized for higher affinity and specificity

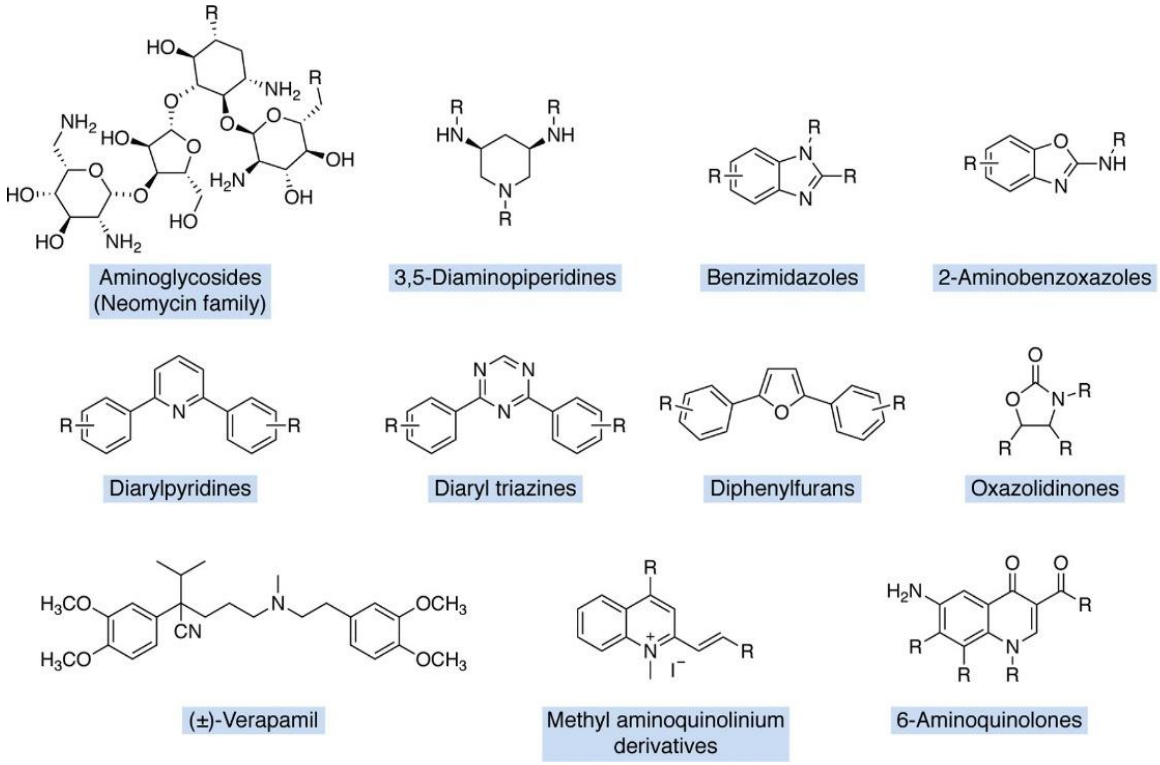
2. Disney lab - Large scale approach (Inforna) includes a randomized library of RNA motifs, e.g., internal loops that bind SM, and their modular assembly.



Strategies for RNA targeting
Framework 2: *RNA-biased chemical space*

RNA molecule is considered **as a whole unique target** that forms defined binding pockets that can be target by specifically designed small molecule

1. **Scaffold-based synthesis** - molecular scaffold known to interact with an RNA molecule is further diversified to produce analogs that are optimized for specific RNA structures

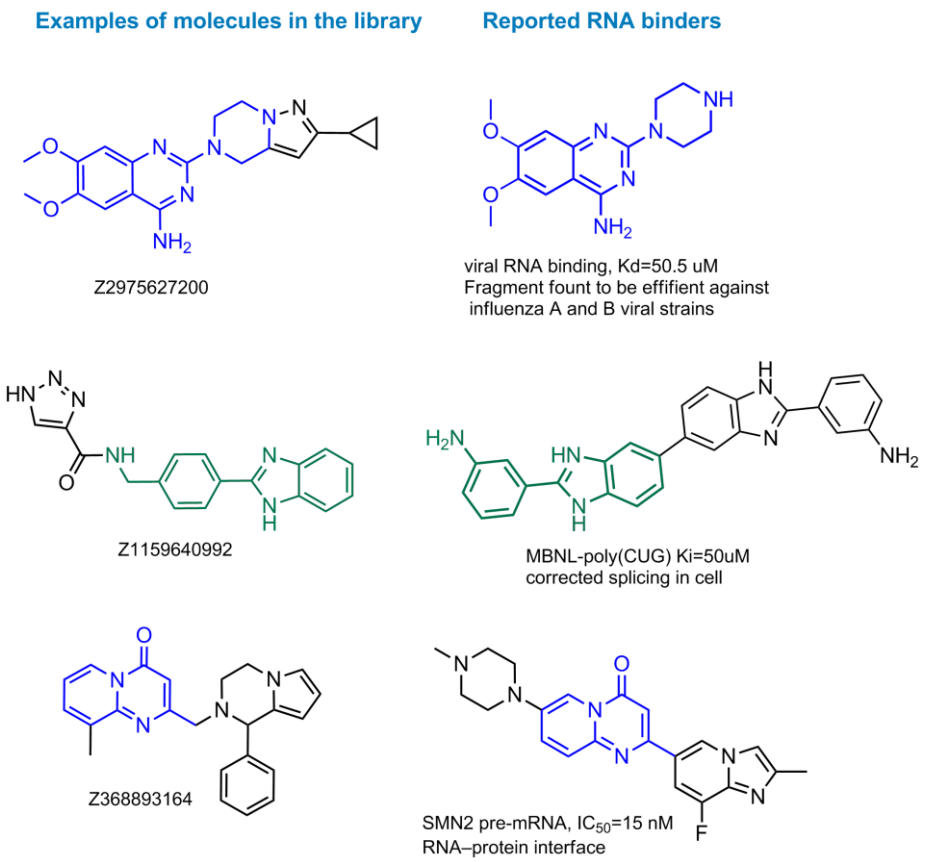


Example of small-molecule classes that have been pursued through scaffold-based synthesis. R groups represent substituents used to diversify the central core scaffold.

Strategies for RNA targeting
Framework 2: *RNA-biased chemical space*

2. RNA-biased libraries –

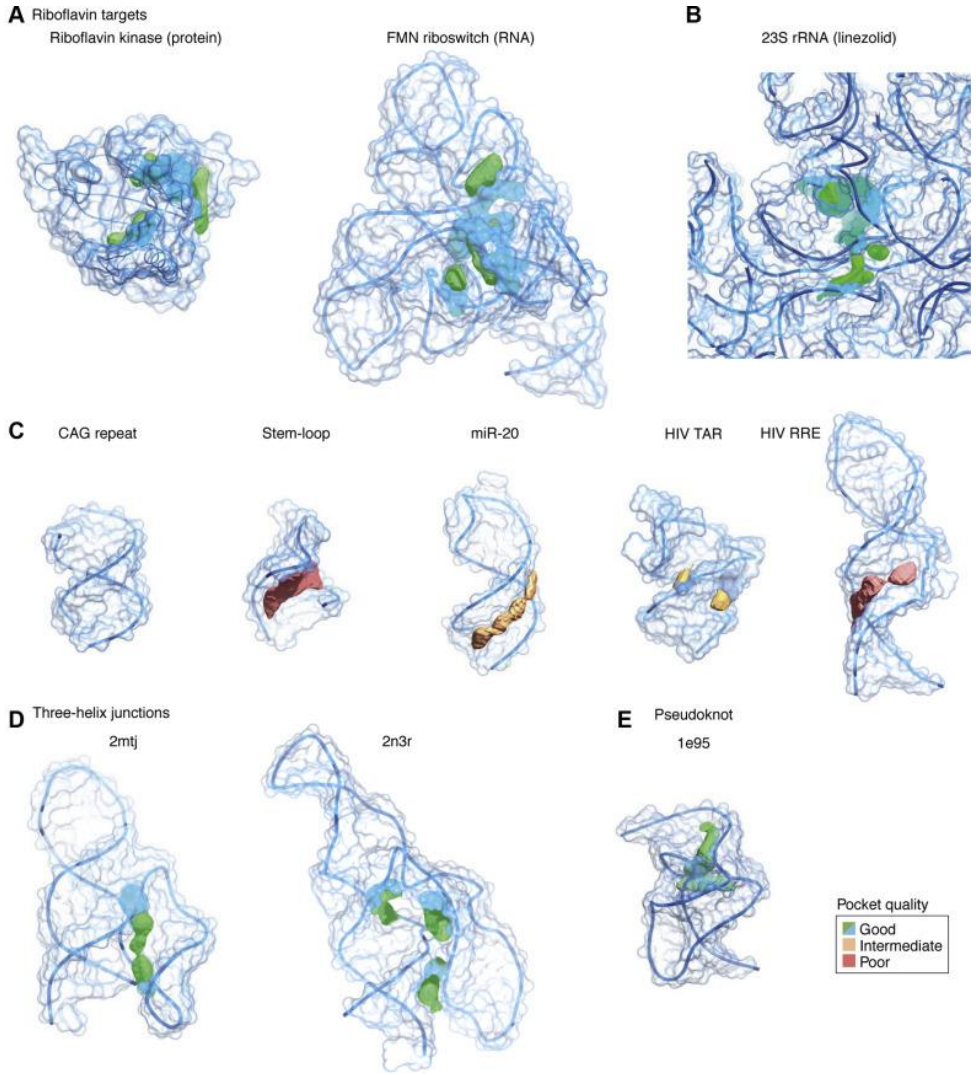
- focused on studying structural properties of RNA-binding molecules and using these properties to design RNA-biased libraries
- These guiding principles can facilitate the future design of selective ligands targeted toward therapeutically relevant RNAs (Deep learning approaches)



Strategies for RNA targeting

Framework 3: *RNA-targeted small molecules may look like typical drugs*

- Traditional medicinal chemistry approaches are applied to RNA targets, i.e., Lipinski's and Veber's rules
- Determinants for RNA binding are sufficiently similar to those for protein targeting to warrant use of established medicinal chemistry libraries,
- Focus is taken away from the uniqueness of RNA and redirected to its similarity to proteins, i.e., searching for protein-like binding pockets in higher level folding RNA structures



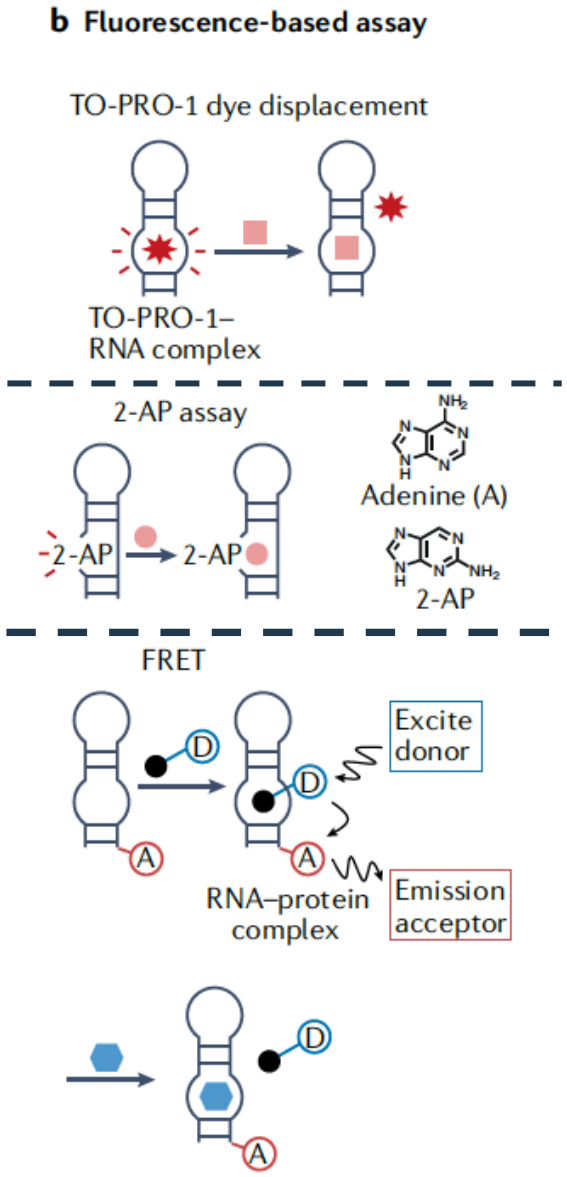
Screening and validating assays

Fluorescence-based assays

✓**Displacement of a fluorescent dye or compound by** a small molecule, e.g., TO-PRO-1

✓**2-aminopurine** – 2-AP is incorporated into the target RNA; its fluorescence depends on the microenvironment, which changes upon small-molecule binding. The position of 2-AP in RNA must be carefully chosen to ensure a strong signal

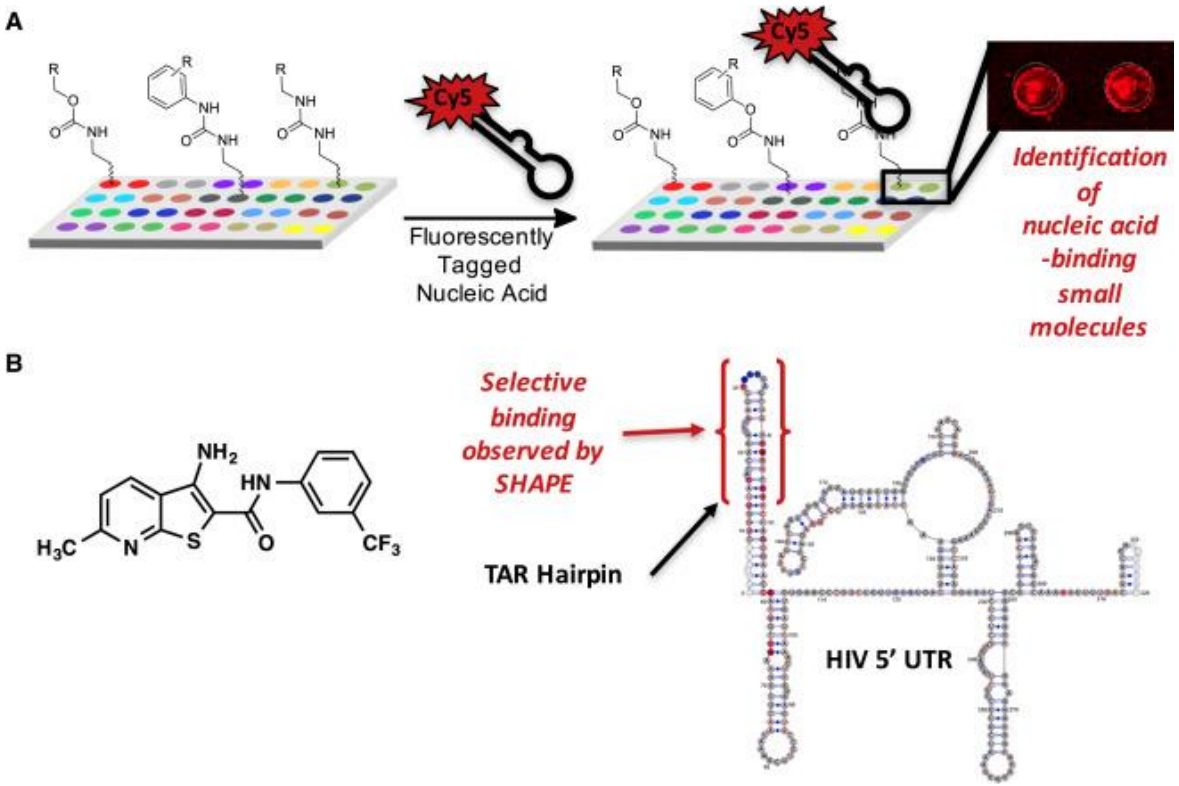
✓**FRET** – labels on the RNA and protein are FRET pairs, and disruption or inhibition of the complex formation reduces the observed FRET signal. Small molecule can bind either RNA or protein, thus, additional investigations needed. Developed for HIV TAR/Tat system.



Screening and validating assays

Small molecule microarray

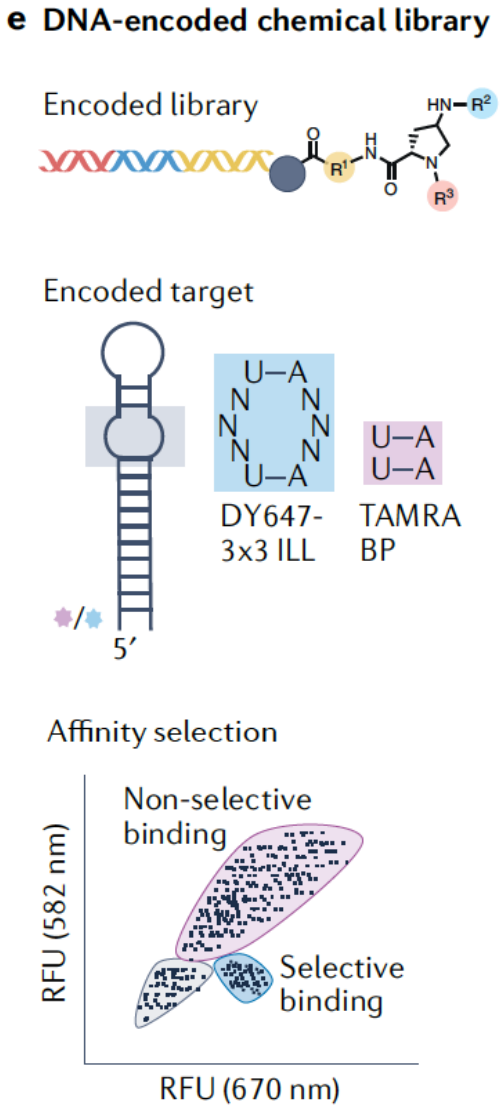
- A library of 20,000 small molecules (primary, secondary alcohols, amines) are printed onto a functionalized glass surface.
- Then, the array is incubated with a Cy5-labeled target RNA molecule as well as another Cy5-labeled control RNA, irradiated and scored for the increase in fluorescence.



Screening and validating assays

DNA-encoded compound libraries

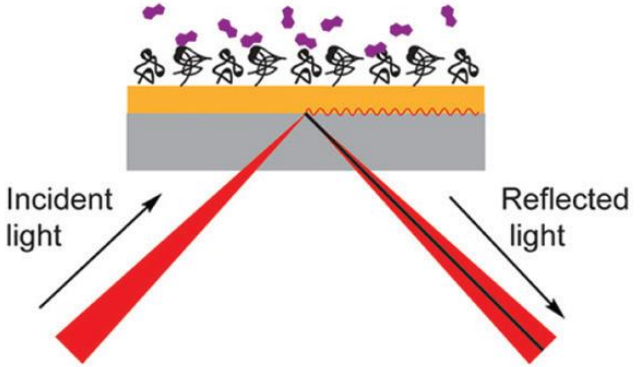
- ✓ small molecules are synthesized on beads, and each compound is encoded with a DNA tag.
- ✓ Compound-beads are screened for binding to a fluorescently labeled target, often in the presence of an off-target that is differentially labeled (ctrl)
- ✓ A counter-screen can be completed by using an RNA in which the desired binding site has been mutated.
- ✓ Beads that bind the desired target but not the off-target are sorted by flow cytometry
- ✓ Deep sequencing of the beads identifies the binding compound.



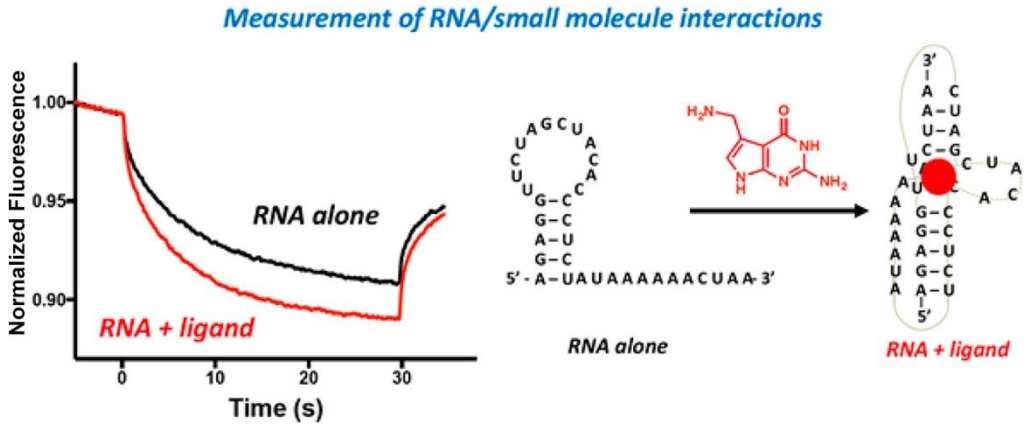
Screening and validating assays

Biophysical assays

- **surface plasmon resonance [SPR]** - directly measures changes in resonance angle or wavelength of a glass surface containing the immobilized RNA of interest upon addition of a small molecule
- **microscale thermophoresis [MST]** - measures the directed migration of a molecule and/or molecule–ligand complex along a temperature gradient, can be used to measure binding affinities using very small amounts of sample



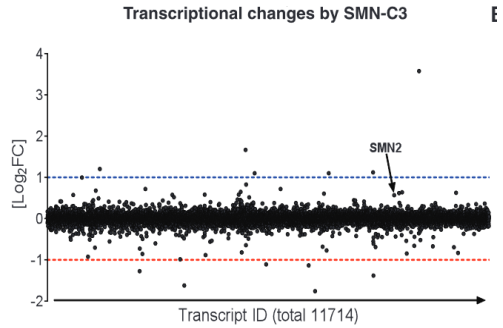
Output:
Binding
(K_d, K_{on}, K_{off})



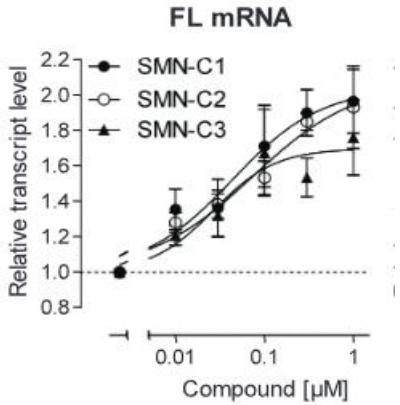
Screening and validating assays

Molecular assays

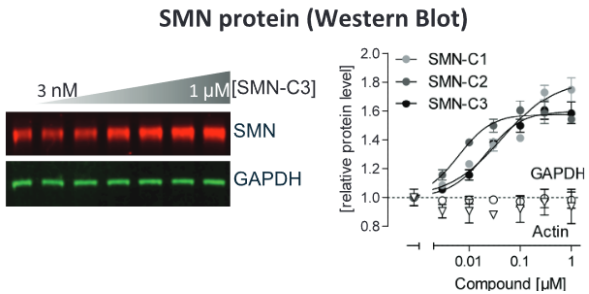
- **EMSA**- electrophoretic mobility shift assays
- **RNA-seq -** Differential expression analysis from RNA-Seq can also be used to quantitatively define on- and off-targets, as well as to study the downstream effects of compound treatment, i.e., downstream pathway analysis. A recent example includes utilizing RNA-Seq at an early time point in breast cancer cells treated with a compound that inhibited miR-515 biogenesis
- **RT-qPCR**
- **Western blot**



Difference in total transcript expression of SMN-C3 (500 nM) versus DMSO-treated SMA type I patient fibroblasts



RT-PCR analysis of SMN2 mRNAs. The effects of SMN-C1, SMN-C2, and SMN-C3 on FL and D7 mRNA levels in SMA type I patient fibroblasts are concentration-dependent.



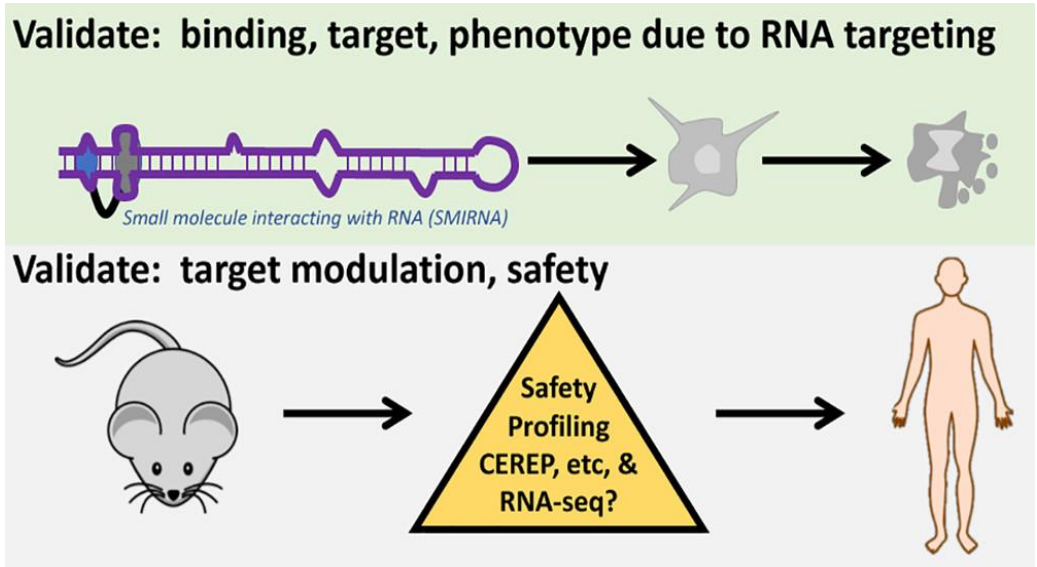
Western blot of SMN protein in SMA type I patient fibroblasts after 48 hours of continuous treatment with SMN-C3.

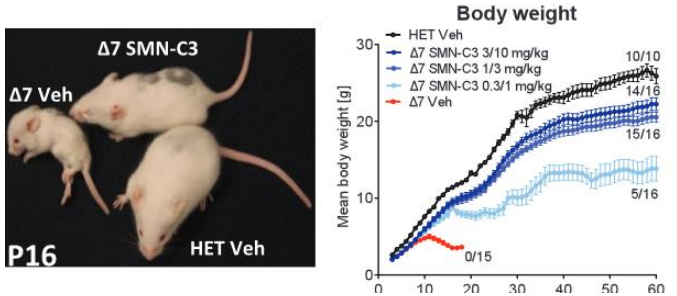
DOI: 10.1126/science.1250127

Screening and validating assays

Phenotypic screening

- Identification of compounds that affect pathways associated with a specific phenotype.
- Designed around a biological process, e.g., alternative splicing, translation
- No knowledge of the RNA structure or small molecule's mode of action is needed
- Based on luciferase or a fluorescent protein reporter assays
- Examples of successful application: risdiplam, branaplam (SMA treatment)





Appearance of a vehicle-treated D7 mouse (D7 Veh), a SMN-C3–treated D7 mouse (D7 SMN-C3), and a vehicle-treated heterozygous mouse (HET Veh).

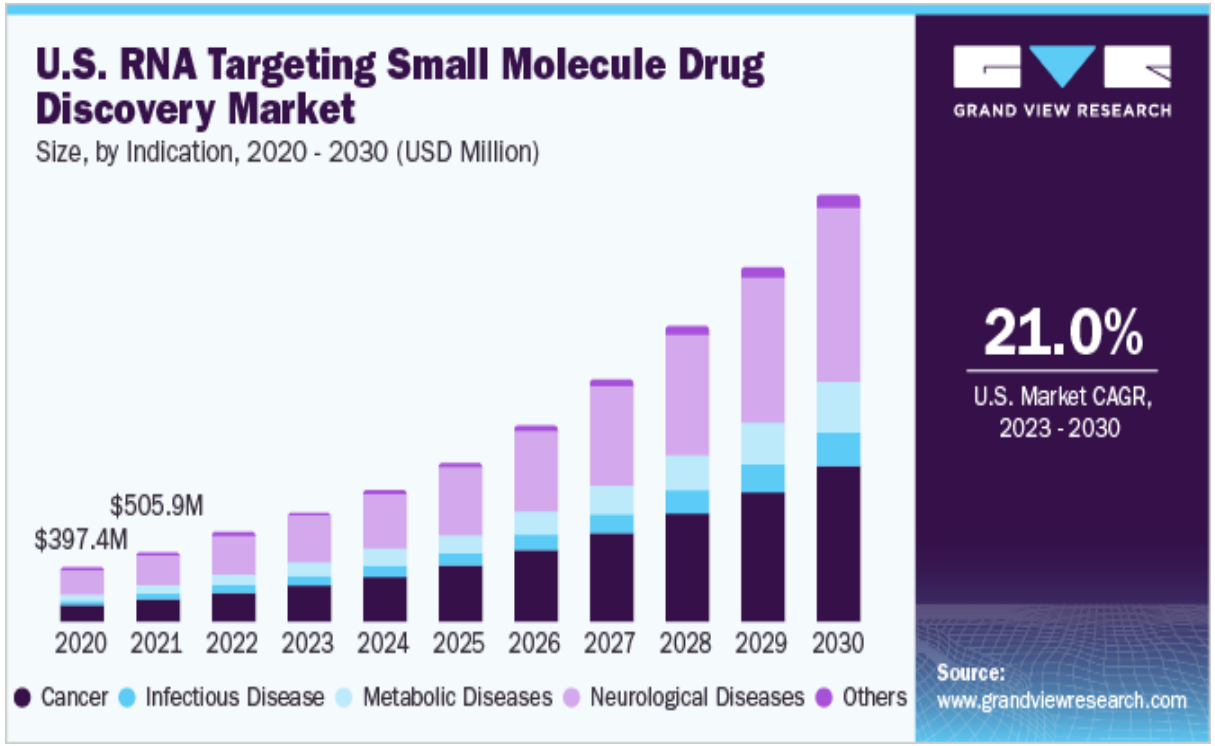
DOI: 10.1126/science.1250127

Thank you for your attention!

Table 1 | Biotech–pharma alliances involving small molecules targeting RNA biology

Target Company	Licensor	Indications	Terms	Date
Skyhawk Therapeutics	Merck	Autoimmune and metabolic diseases	$600 million (m) potential milestones per program	12 May 2020
Arrakis Therapeutics	Roche	Broad set of targets covering all of Roche's R&D areas	$190m up front plus milestones potentially worth several billion dollars	8 April 2020
Skyhawk Therapeutics	Celgene (now Bristol Myers Squibb)	Expansion of original agreement to include autoimmune disease, oncology and immuno-oncology	$80m up front, other terms not disclosed	12 November 2019
Ribometrix	Vertex Pharmaceuticals	Not disclosed	$20m up front and equity investment plus $700m in potential milestones	30 September 2019
Skyhawk Therapeutics	Genentech	Cancer and neurodegenerative diseases	Undisclosed up-front payment plus up to $2 billion in milestone payments and opt-in fees	16 July 2019
Skyhawk Therapeutics	Merck	Neurodegenerative diseases and cancer	$600m in potential milestones per program	8 July 2019
Skyhawk Therapeutics	Biogen	Expansion of original agreement to include additional neurological diseases	Not disclosed	8 July 2019
Skyhawk Therapeutics	Takeda (Tokyo, Japan)	Neurodegenerative diseases	Not disclosed	6 May 2019
Skyhawk Therapeutics	Biogen	Multiple sclerosis, spinal muscular atrophy and other neurological diseases	$74m up front plus undisclosed milestones	4 January 2019
Anima Biotech	Eli Lilly	Not disclosed	$30m up front, $14m research funding and $1.05 billion in milestones	23 July 2018
Skyhawk Therapeutics	Celgene (now Bristol Myers Squibb)	Neurological disease	$60m up front, other terms not disclosed	26 June 2018

Sources: Company websites; PR Newswire.

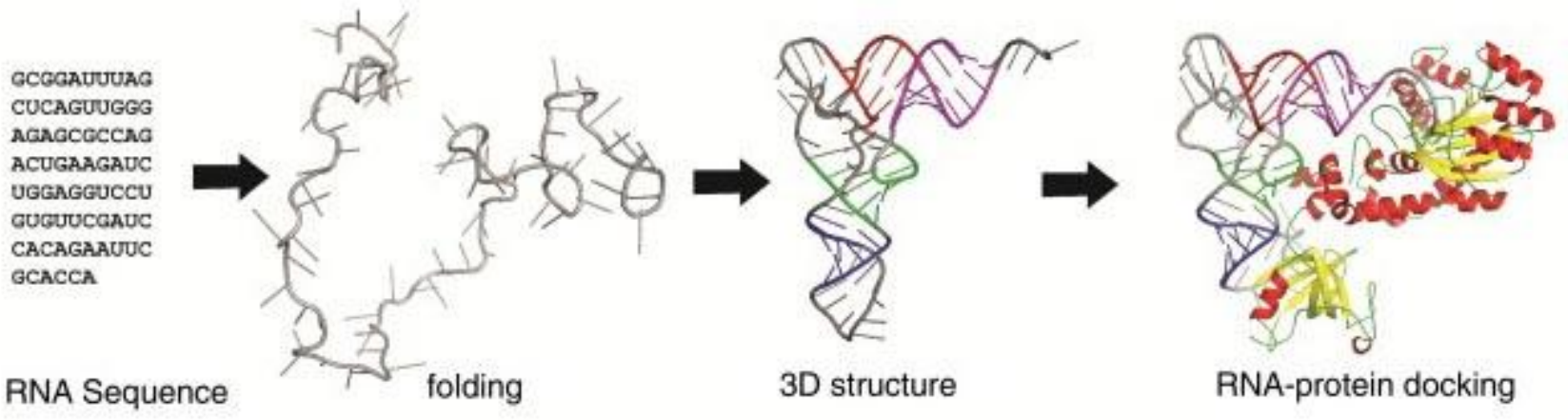


The global RNA targeting small molecule drug discovery market size was valued at USD 1.1 billion in 2022 and is expected to expand at a compound annual growth rate (CAGR) of 20.9% from 2023 to 2030

Studying RNA conformations with DyRNA Thermometry and cryo-EM

Jakub Nowak, PhD

What's do we know about structured RNA domains



~ 24.000.000 RNA sequences

> 4.000 non-coding RNA families (Rfam)

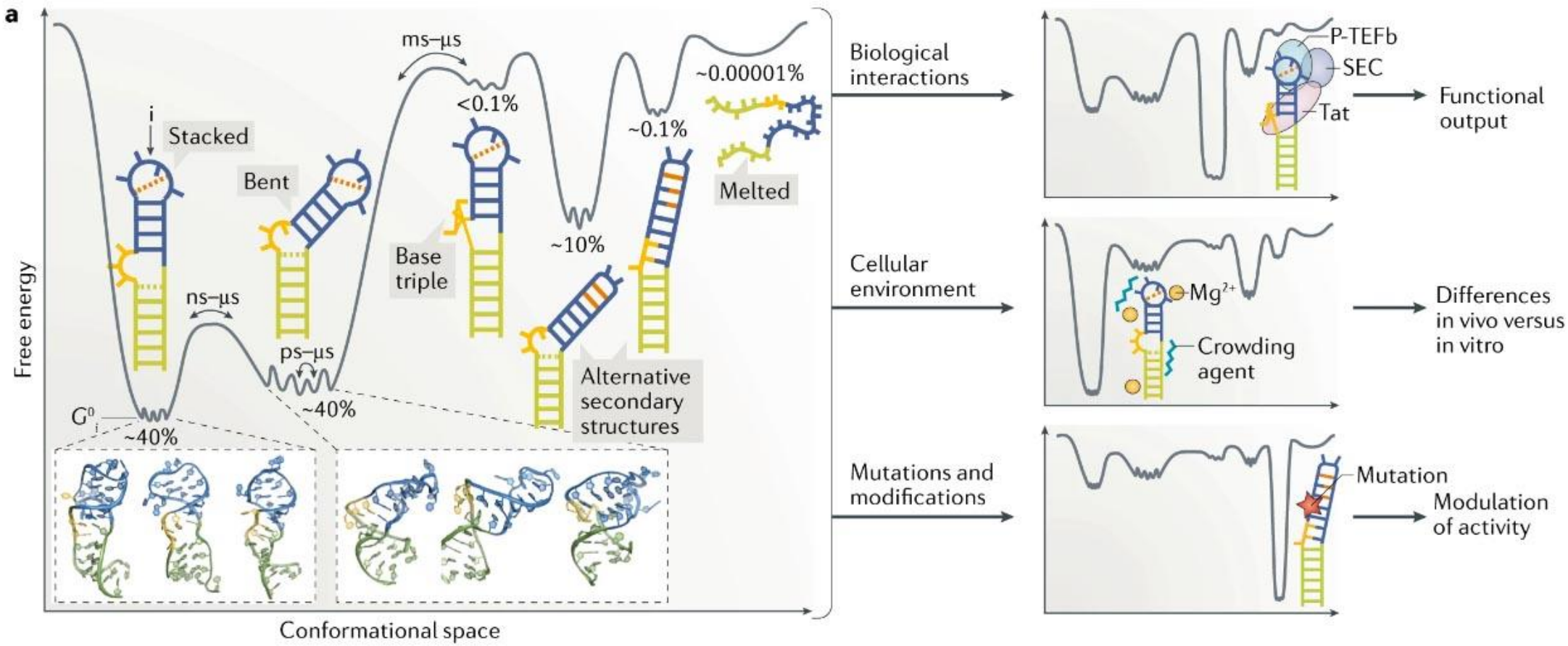
~ 150.000 RNA sequences with experimentally determined secondary structure

~ 5.400 known 3D structures of RNA and RNA-protein complexes

127 non-coding RNA families

3 RNA Cryo-EM structures below 4 Å resolution

Is there a single structure of a given RNA sequence

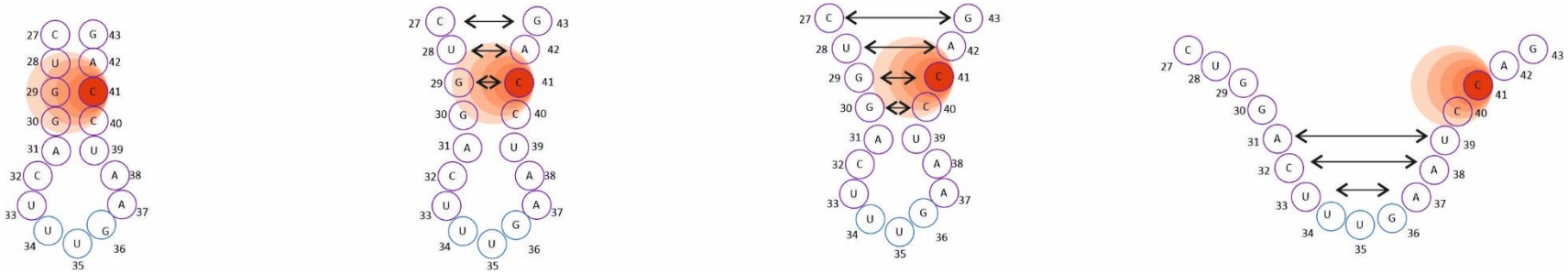


Ganser et al., Nat Rev Mol Biol 2019

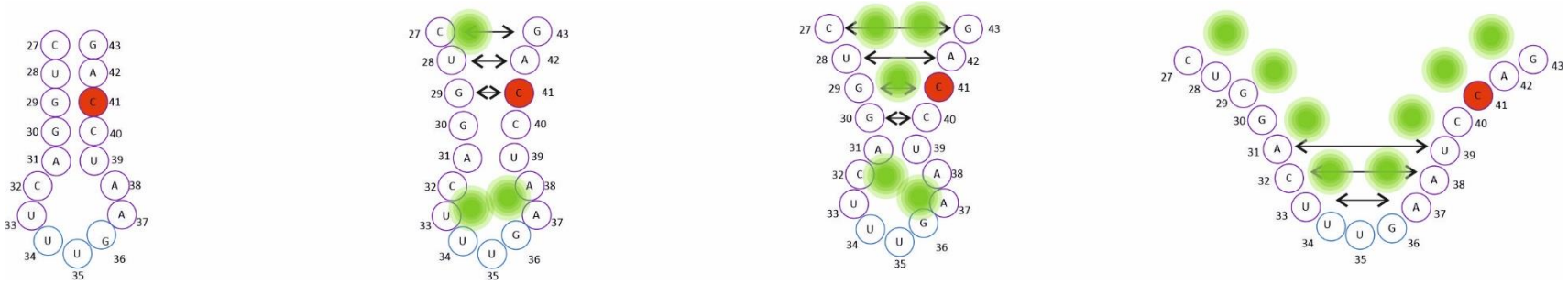
Fluorimetry based detection of conformational changes

External vs Internal fluorescence probes

inDyRNA Thermometry



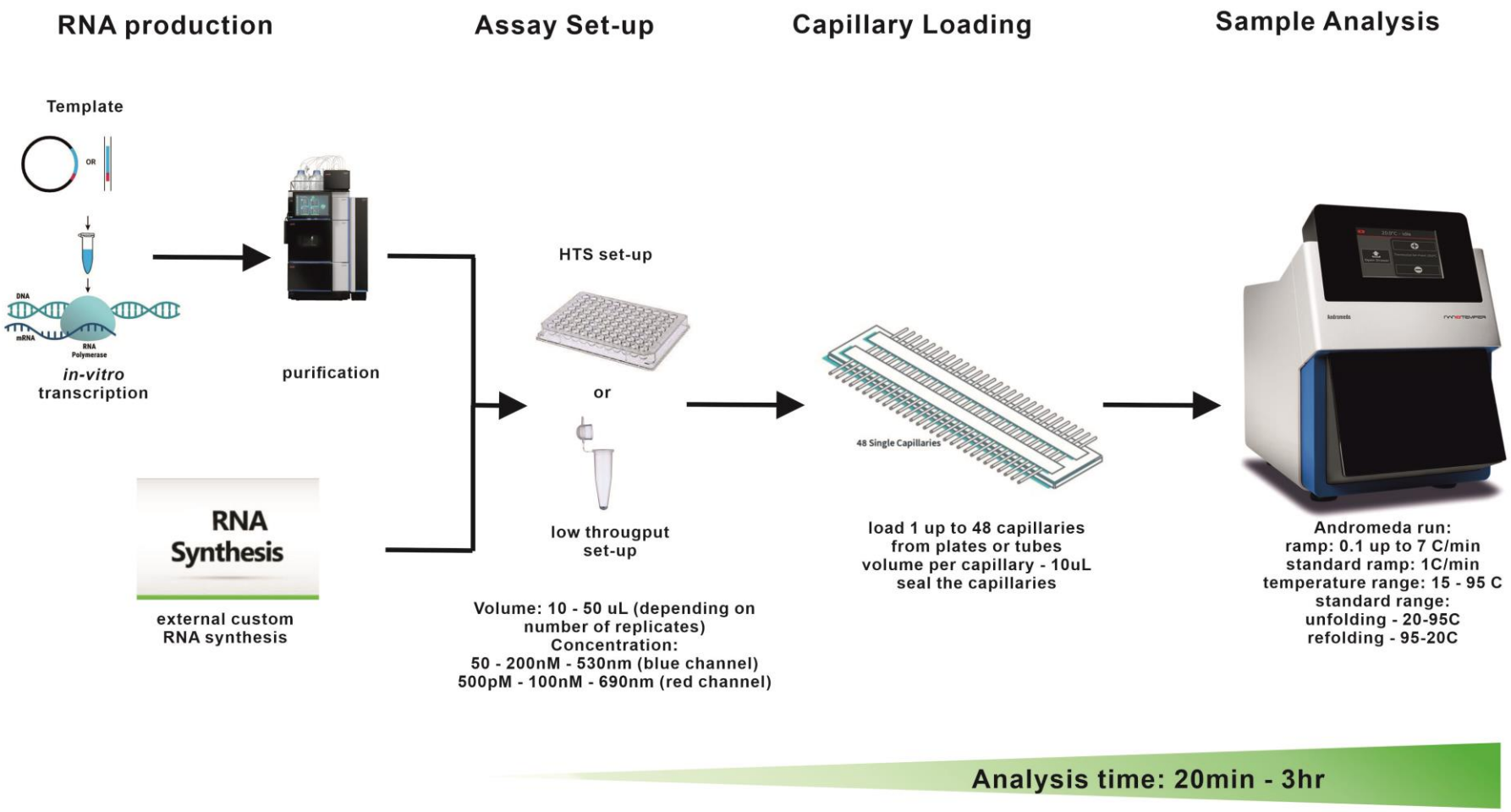
ExDyRNA Thermometry



Temperature

DyRNA Thermometry introduction

Standard experimental set-up



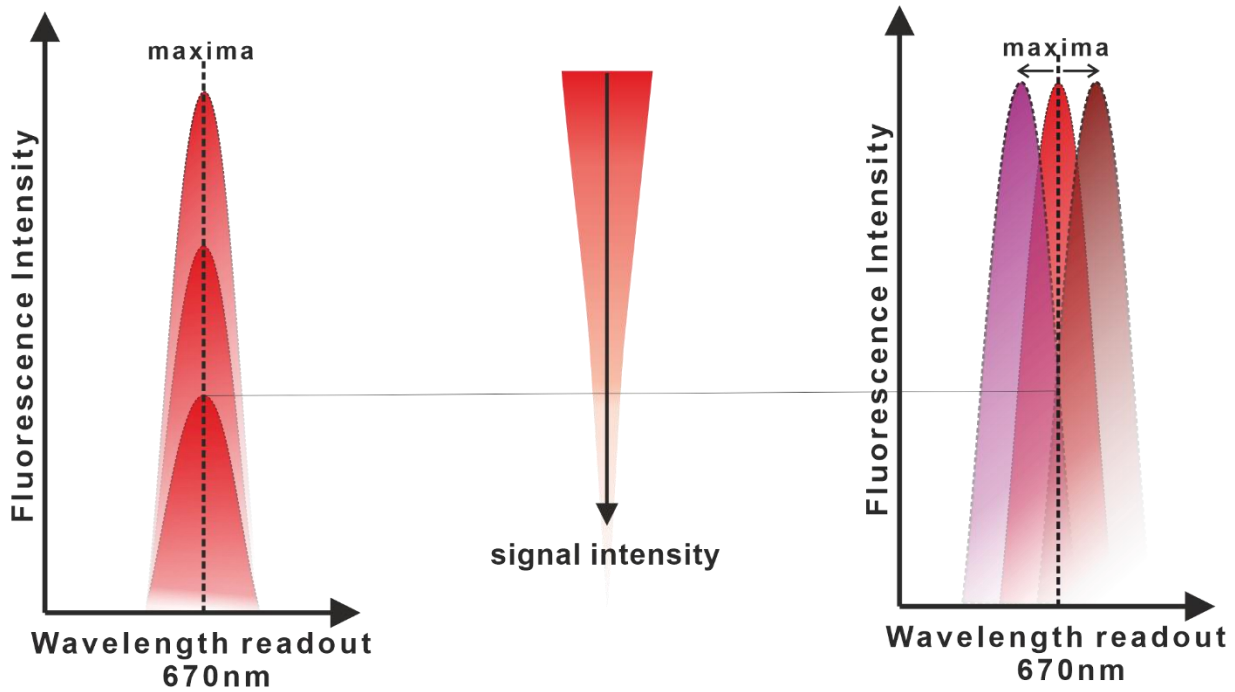
DyRNA Thermometry principle

Definition of TRIC effect

Physical components: $c\frac{\partial F}{\partial T}$

Quantum Yield

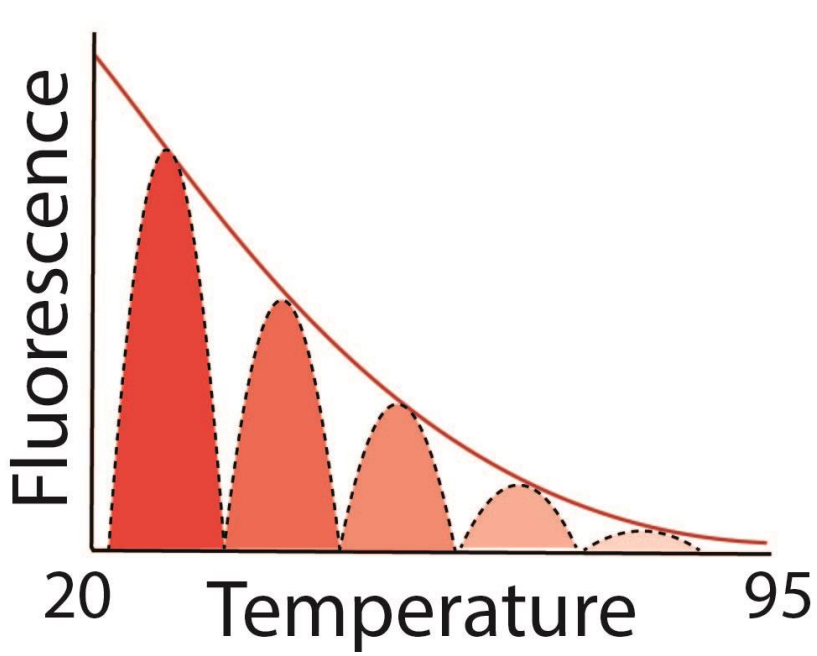
Peak wavelength (ie. Stoke shift)

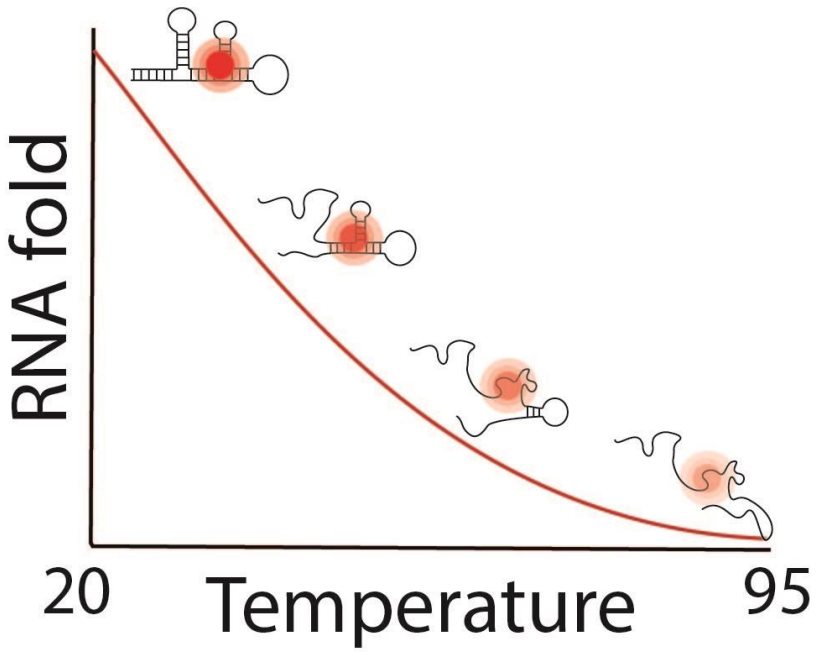


TRIC – temperature effect strongly dependent on fluorophore environment

DyRNA Thermometry principle

Combining temperature effect on fluorescence and stability of the RNA

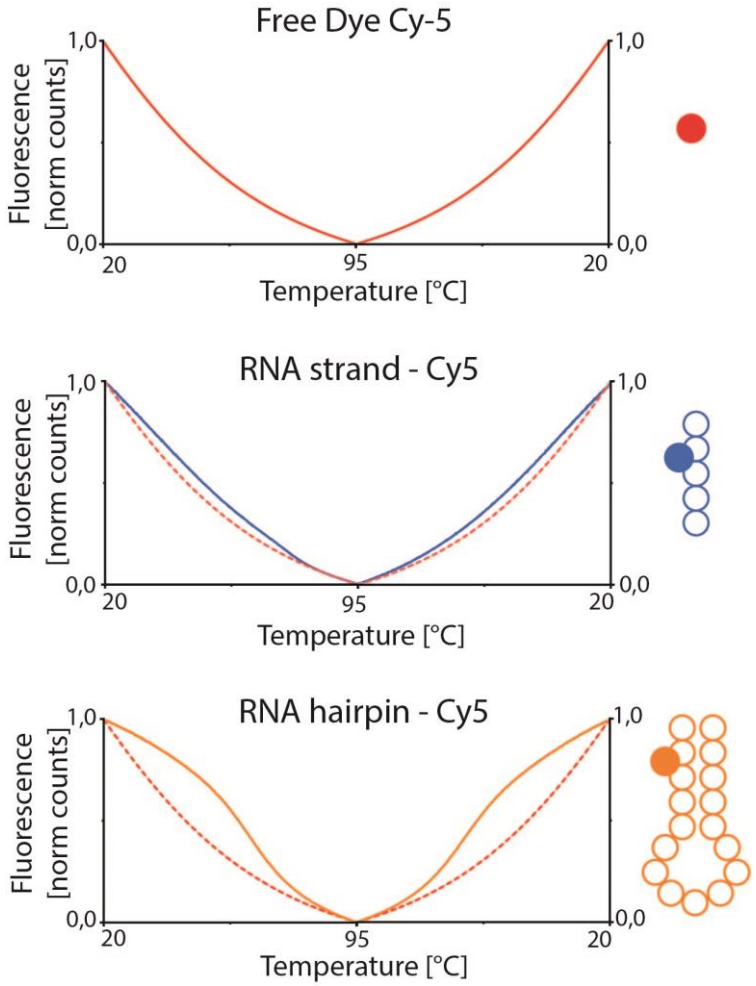




TRIC response coincide with the RNA unfolding

DyRNA Thermometry principle

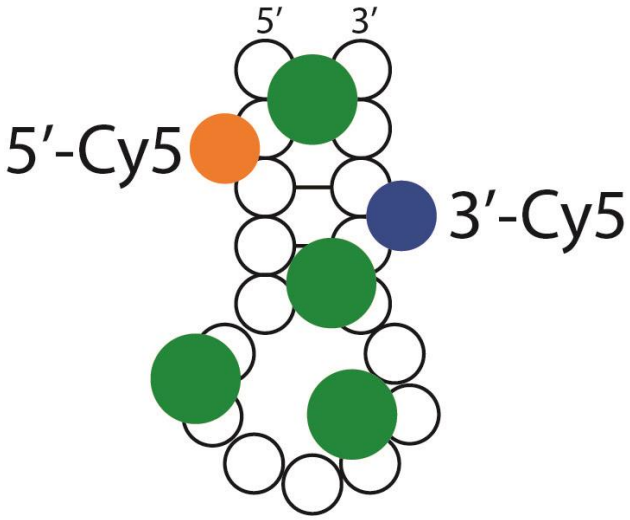
Combining temperature effect on fluorescence read-out and stability of the RNA



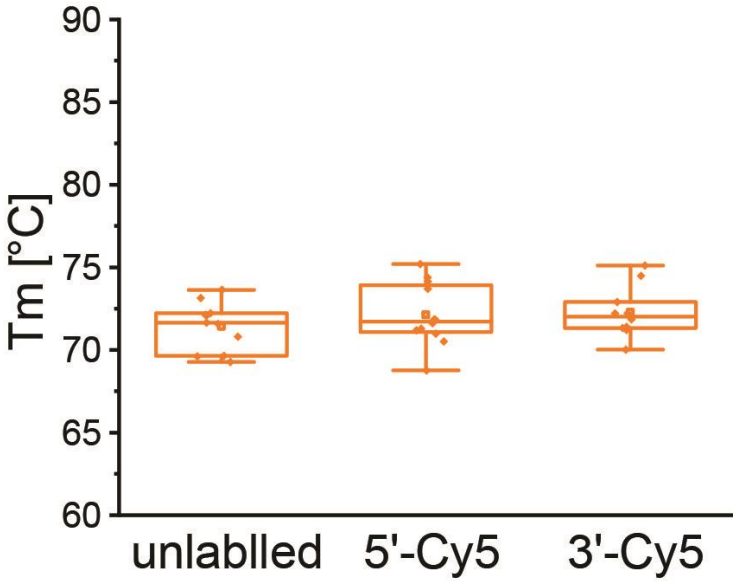
DyRNA Thermometry senses the stability of the RNA hairpin

inDyRNA Thermometry sensitivity

Global stability of unlabeled and an internally labeled hairpins



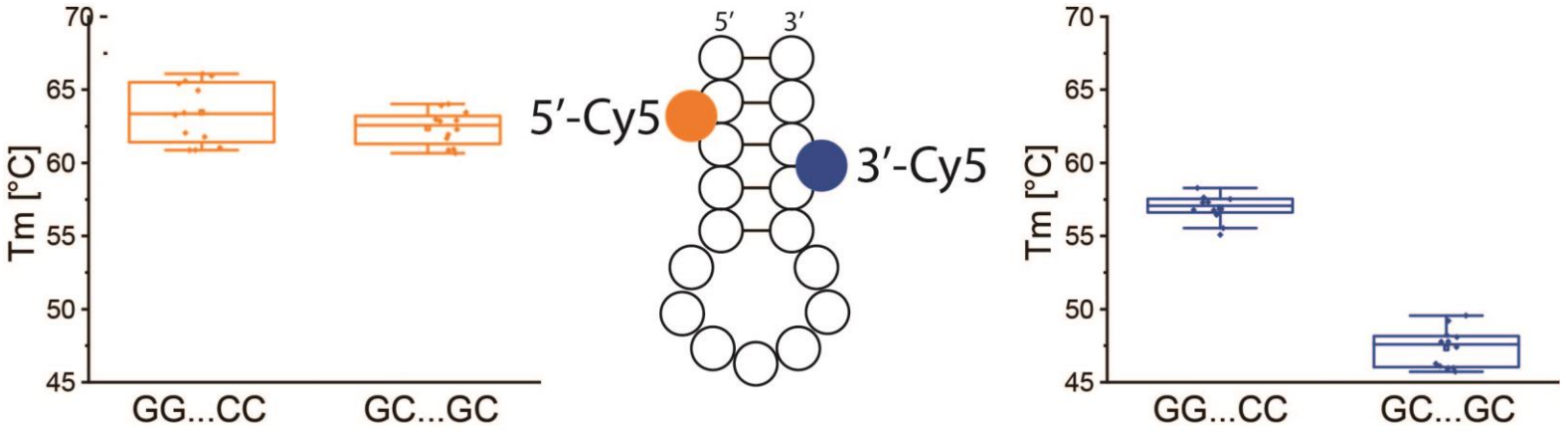
External fluorescent probe



Incorporation of the probe does not affect global stability readout

inDyRNA Thermometry sensitivity

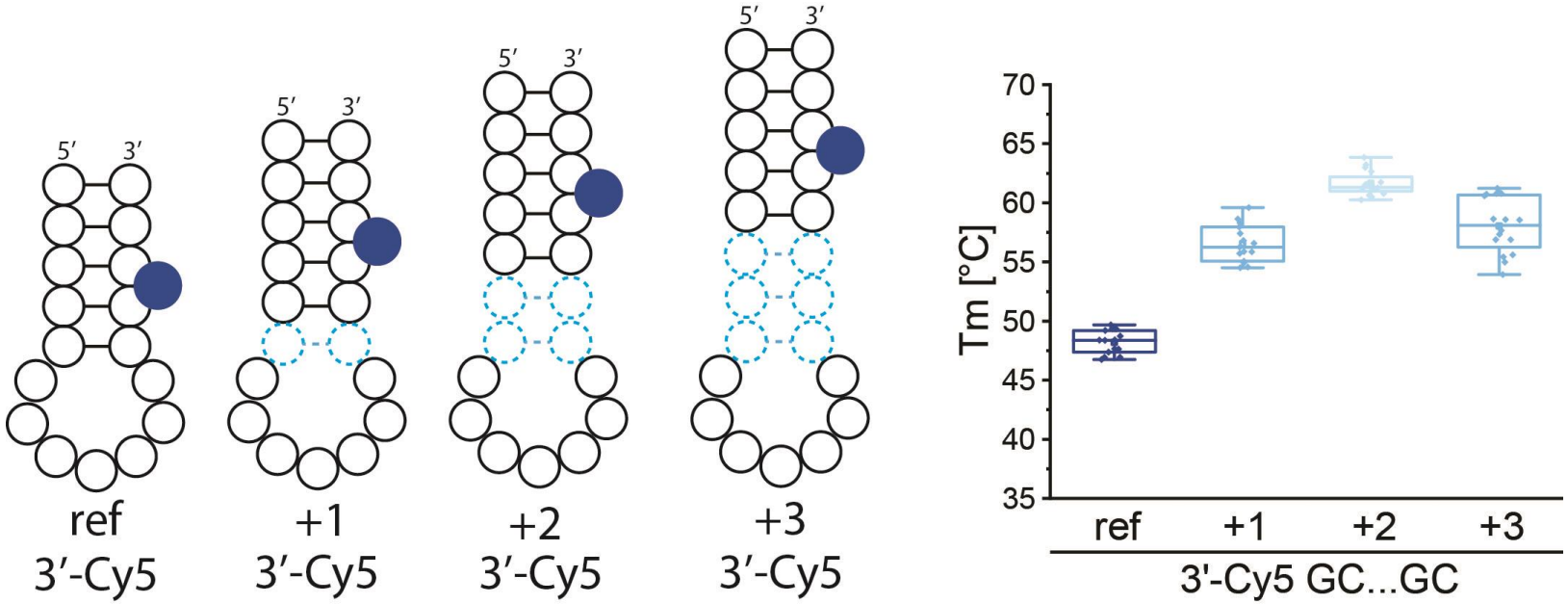
Label position effect



Position of the dye is linked to the stability readout

inDyRNA Thermometry sensitivity

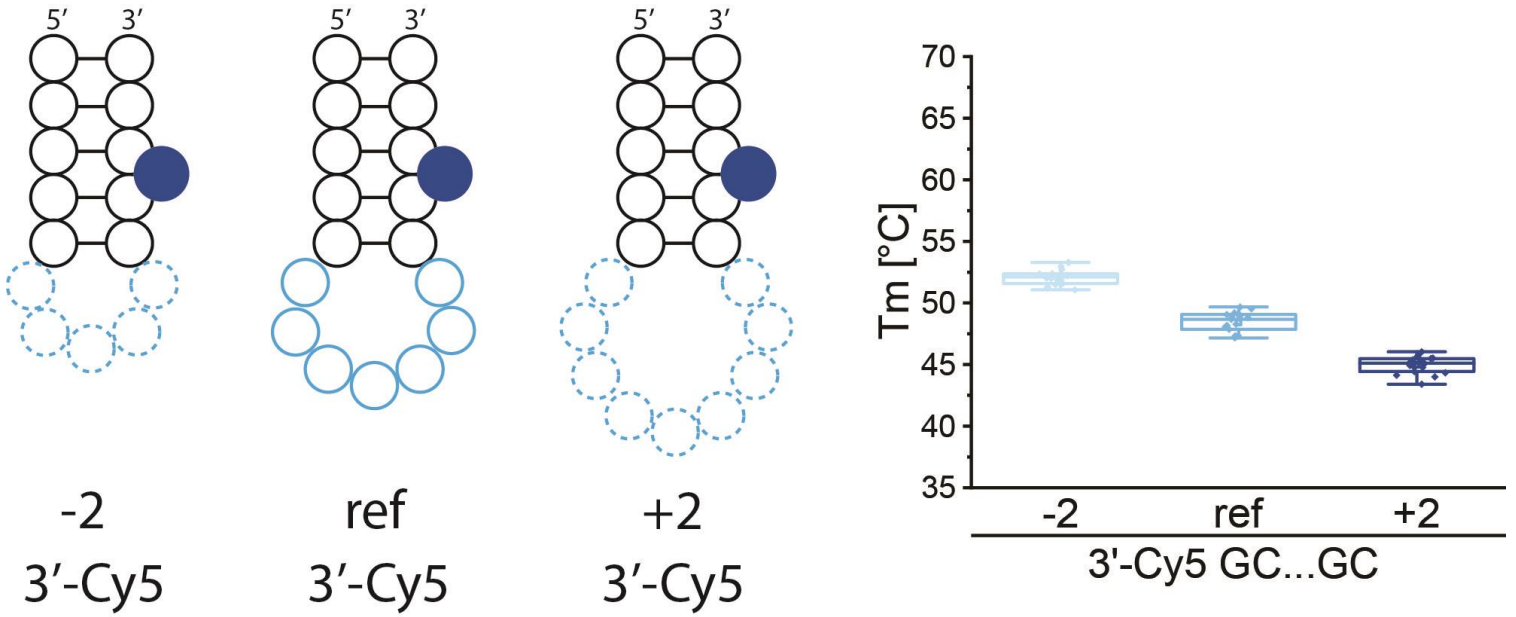
Stem extension effect



Extension of the probe-loop distance increases the stability

inDyRNA Thermometry sensitivity

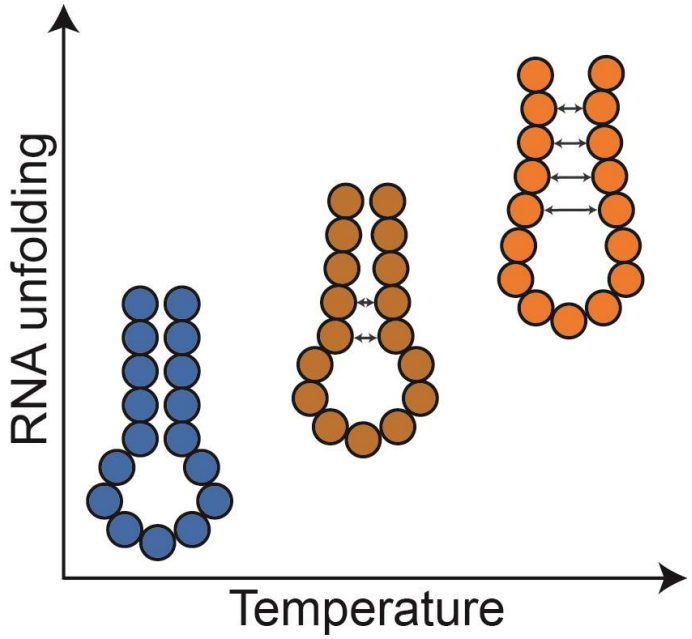
Loop size effect



Larger loop size is correlated with a decrease of the stability

inDyRNA Thermometry sensitivity

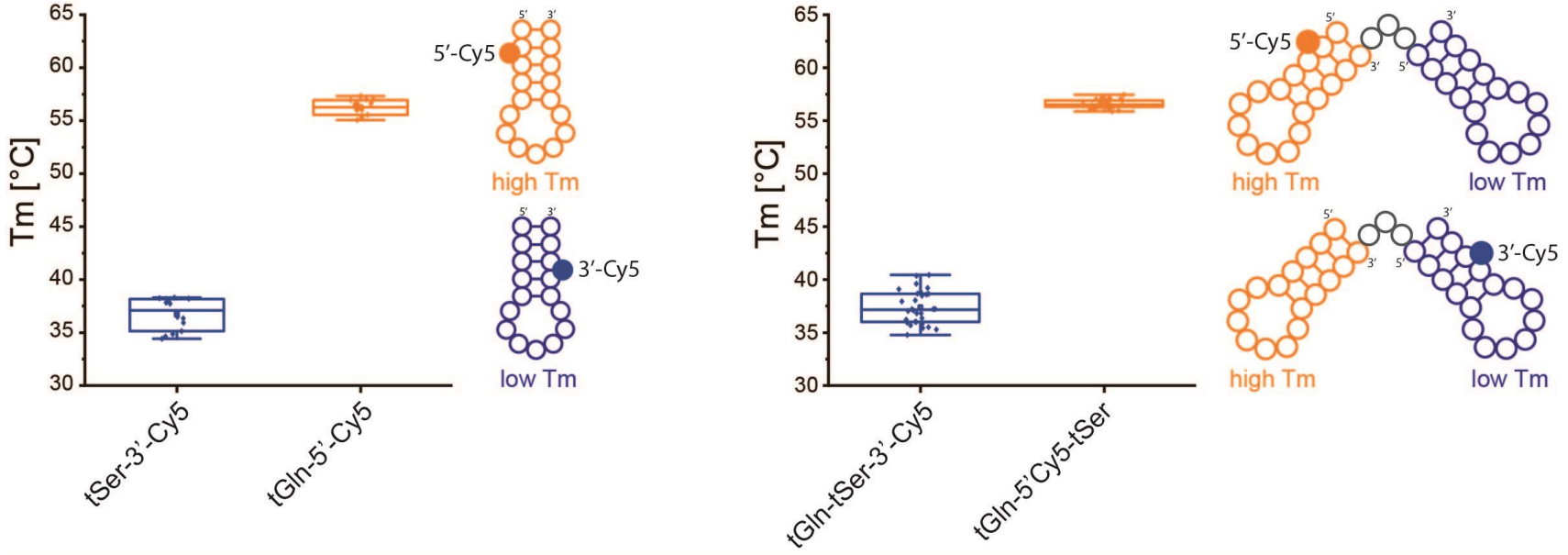
Sensing direction of hairpin opening



Extension of the distance between the loop and the probe shows increase in stability

inDyRNA Thermometry sensitivity

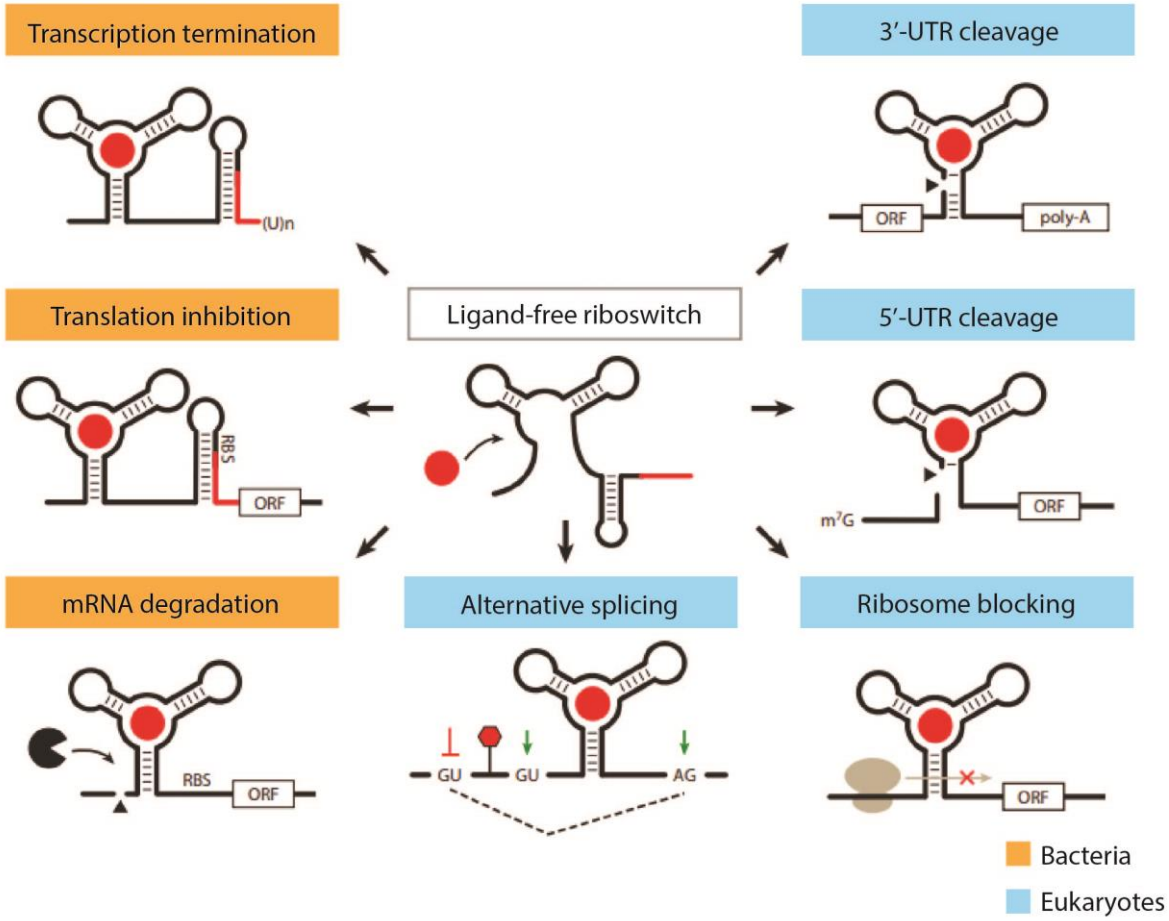
Global vs local stability sensing at the domain level



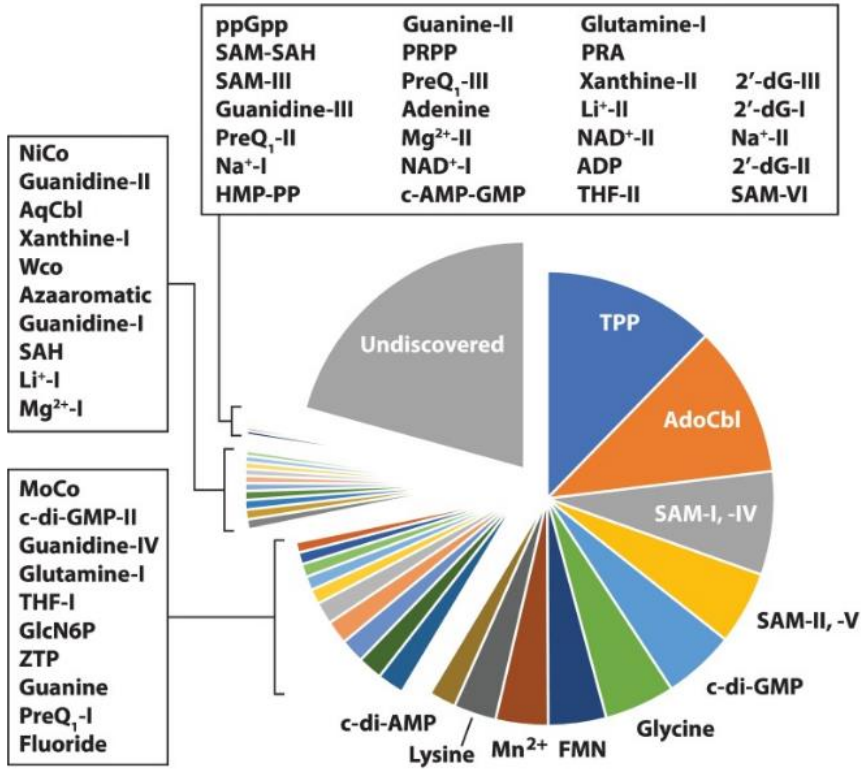
inDyRNA Thermometry allows for the specific local-domain probing of conformational changes

inDyRNA Thermometry validation with biological models

Function of riboswitches in cell biology



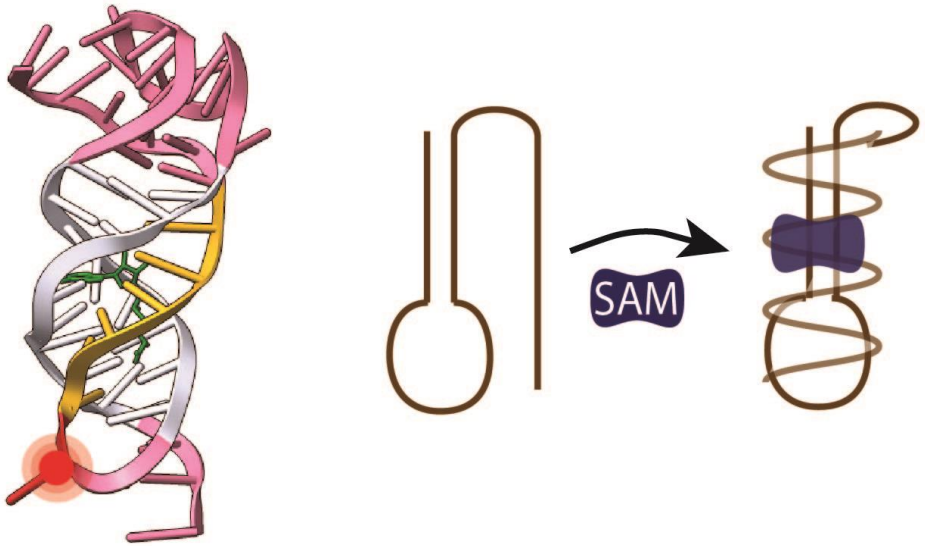
Hallber et al, Annu. Rev. Biochem. 2017

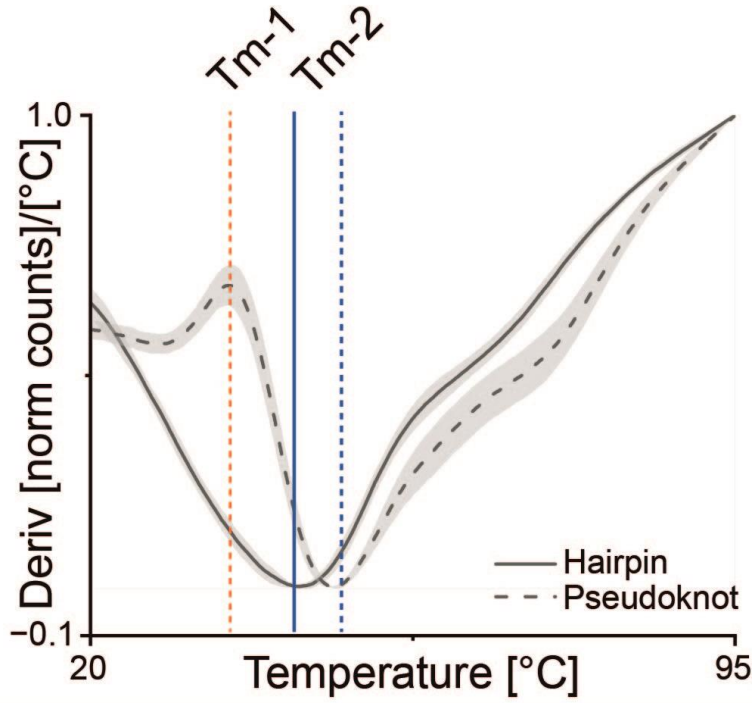


Kavita et al, Trends in Biochemical Sciences 2023

inDyRNA Thermometry validation with biological models

SAM-II Riboswitch conformational switch effect

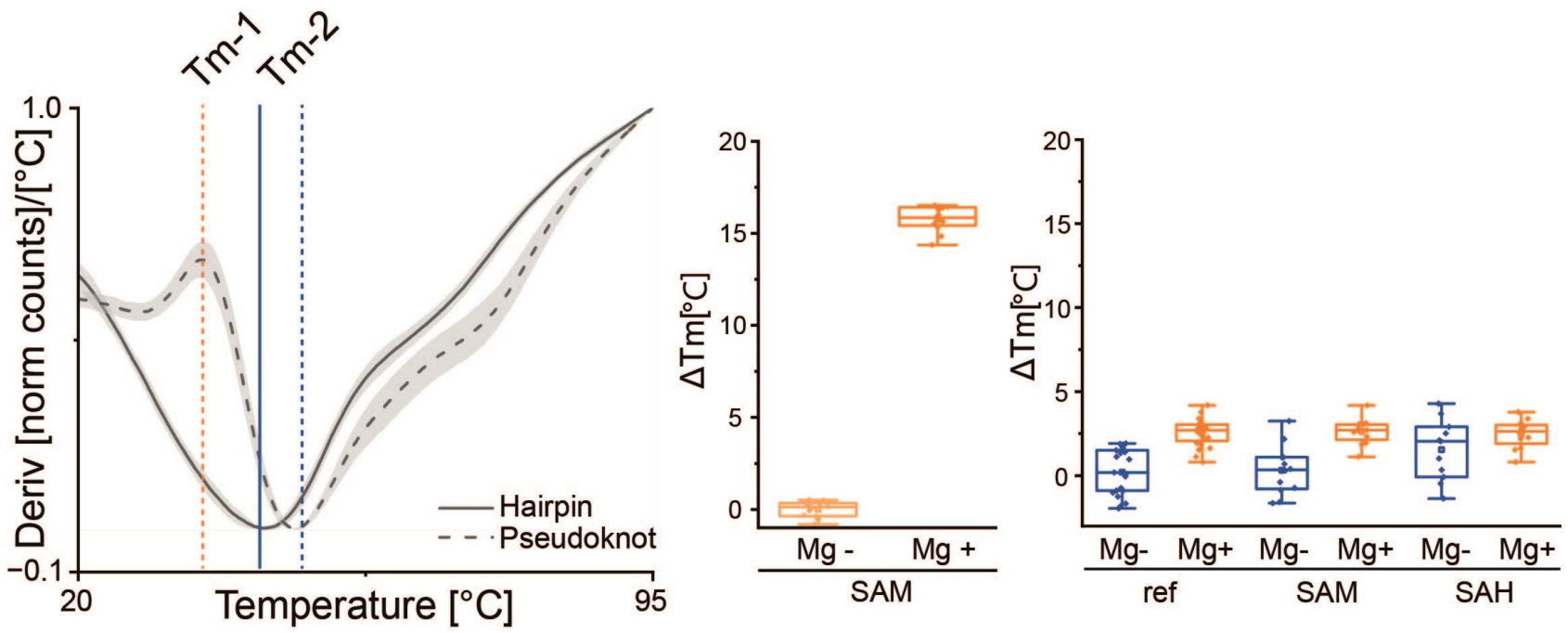




inDyRNA Thermometry could be used to monitor higher order conformation rearrangements – hairpin->pseudoknot

inDyRNA Thermometry validation with biological models

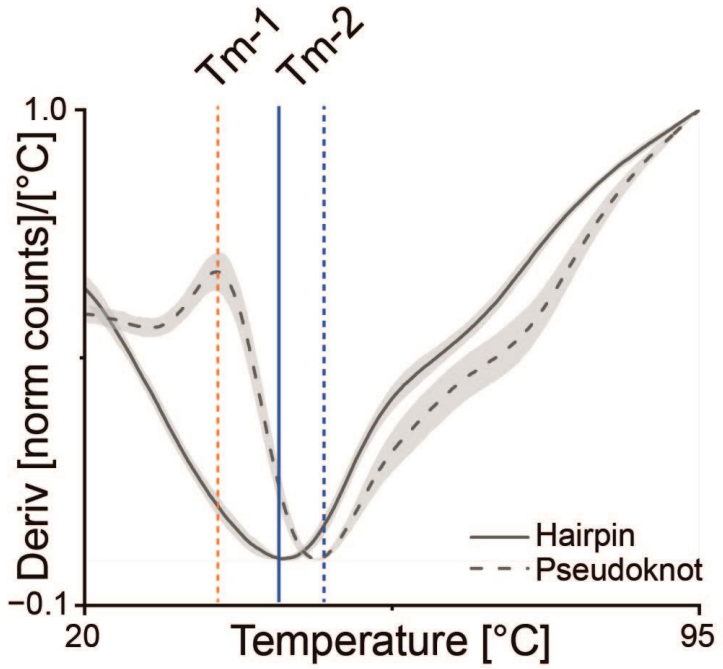
SAM-II Riboswitch conformational switch effect

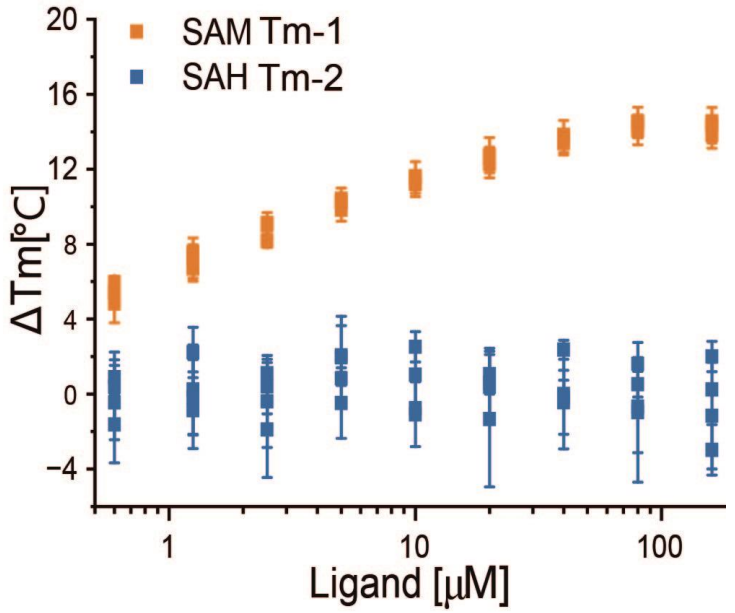


Core stability of the hairpin driven by Mg ions
Pseudoknot stability cooperation of Mg and SAM

inDyRNA Thermometry validation with biological models

SAM-II Riboswitch conformational switch ligand specificity

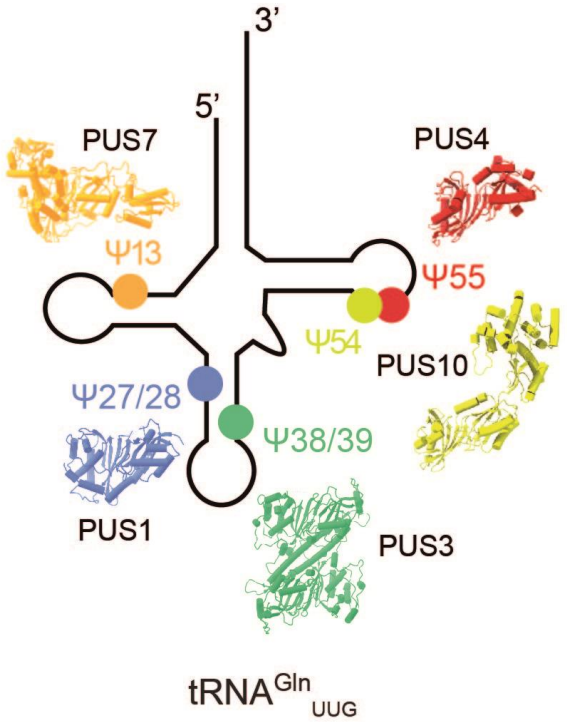


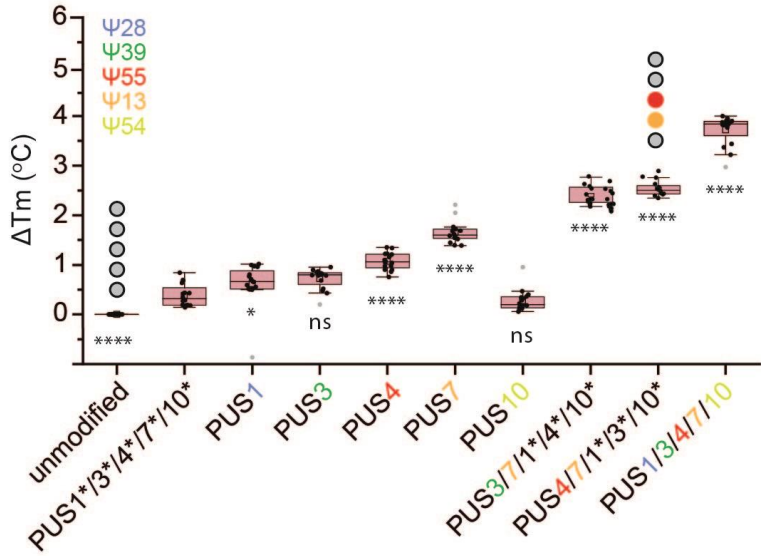


inDyRNA Thermometry could be used to screen for ligands introducing conformational switch

exDyRNA Thermometry analysis of tRNA modifications

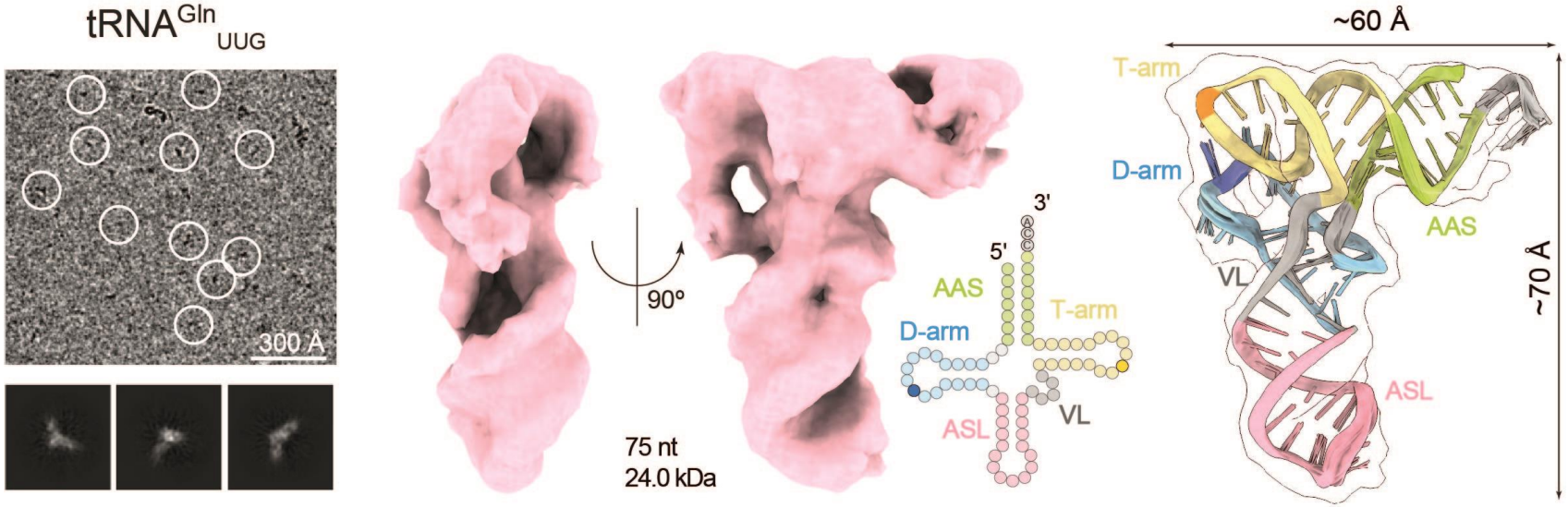
Position specific stabilization of tGln with pseudouridylation





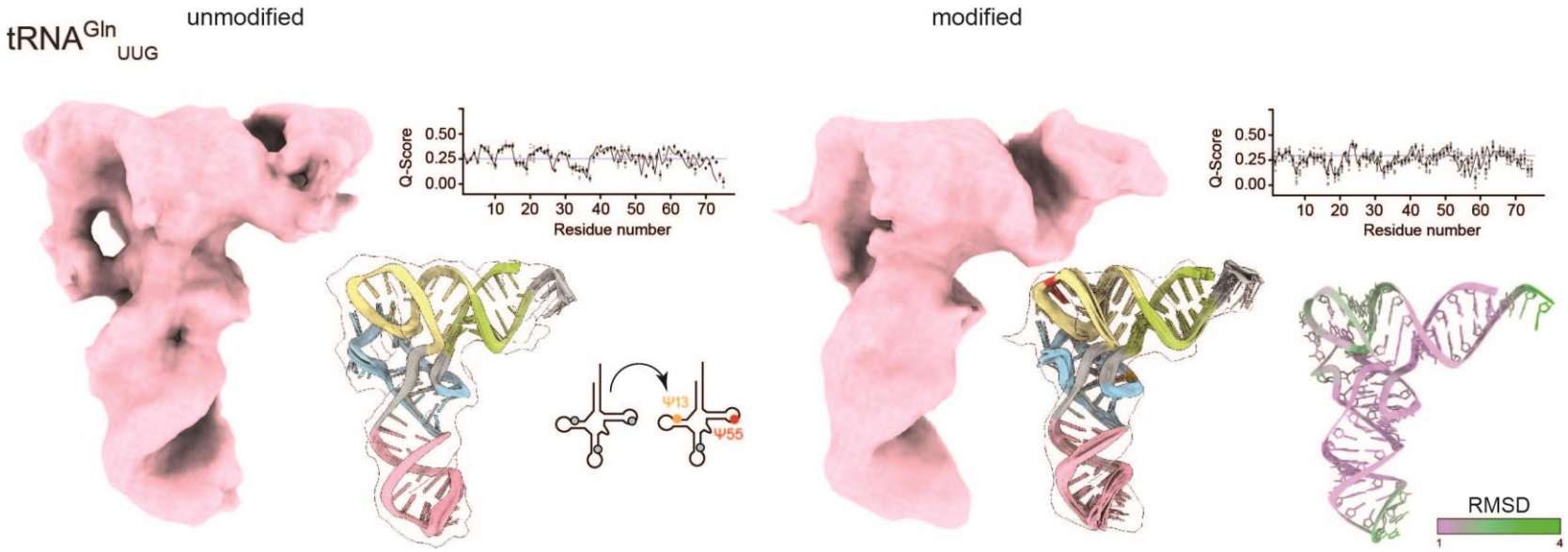
exDyRNA Thermometry identifies key Pus driven pseudouridylation spots

Cryo-EM structures of unmodified tRNAs



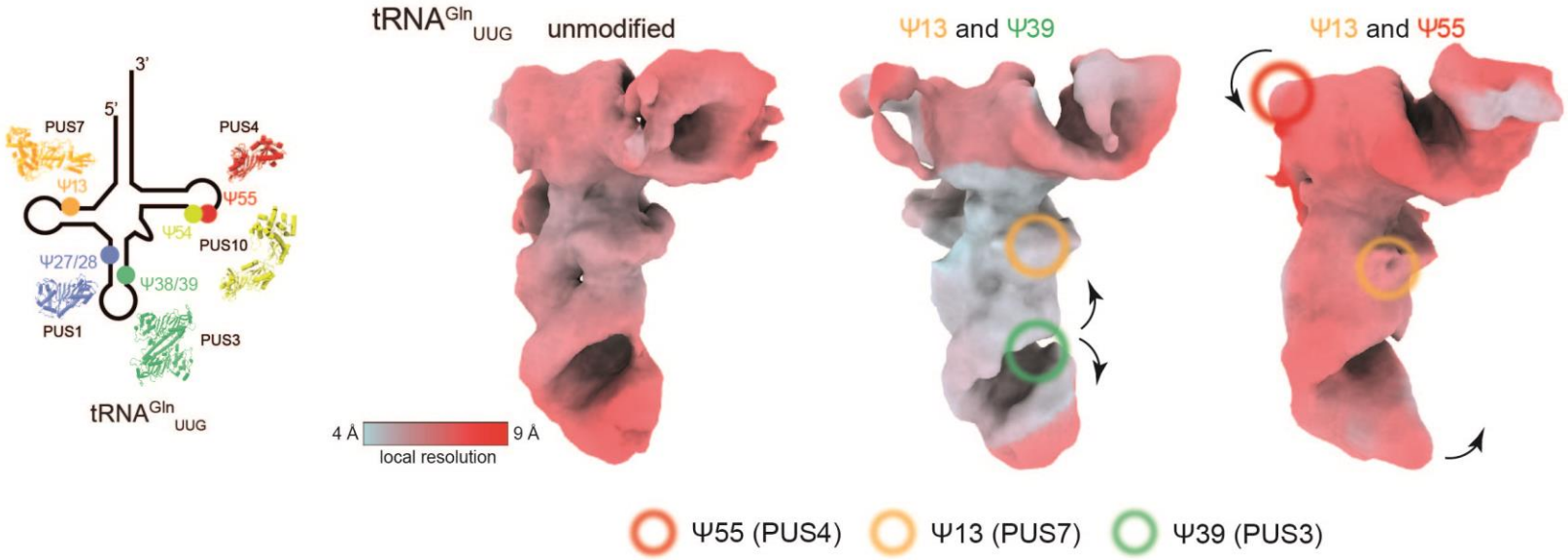
tGln cryo-EM density display characteristic for tRNA features

Cryo-EM structures of modified tRNAs



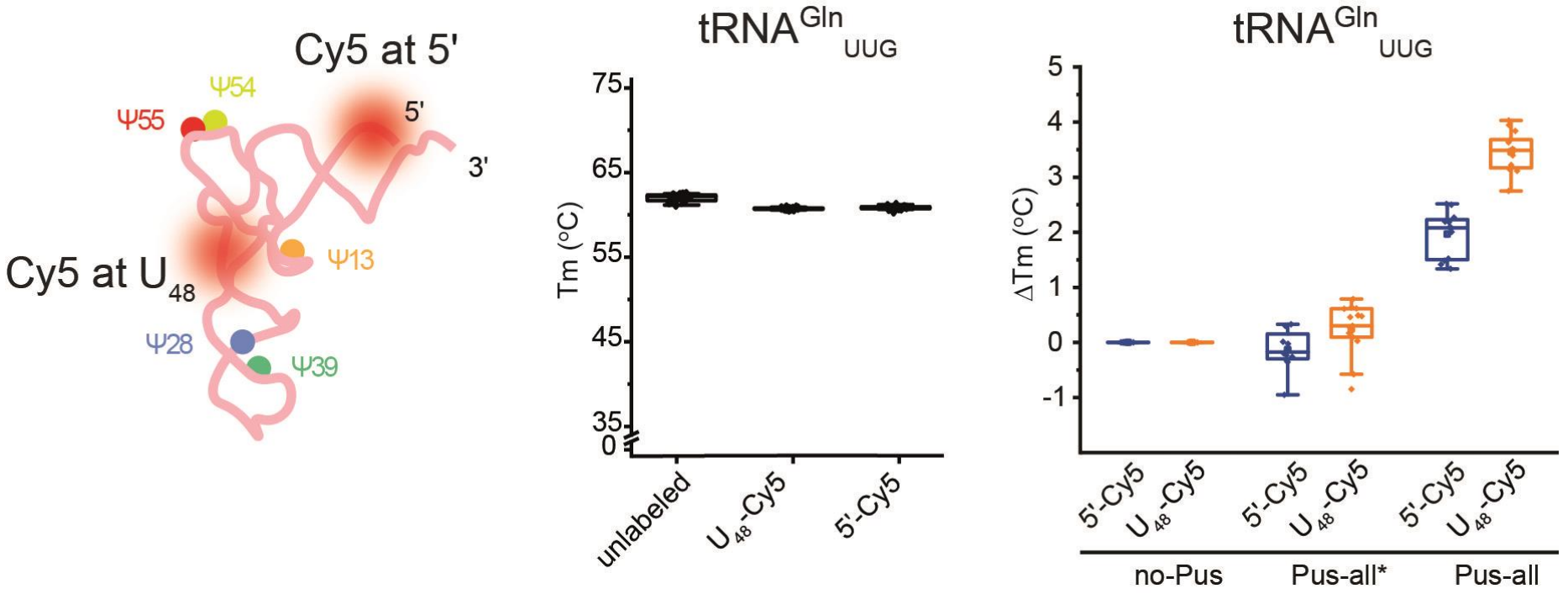
Introduction of Ψ leads to changes in the elbow region and distinct densities for the phosphates groups

Local structural stabilization of tRNAs by Ψ



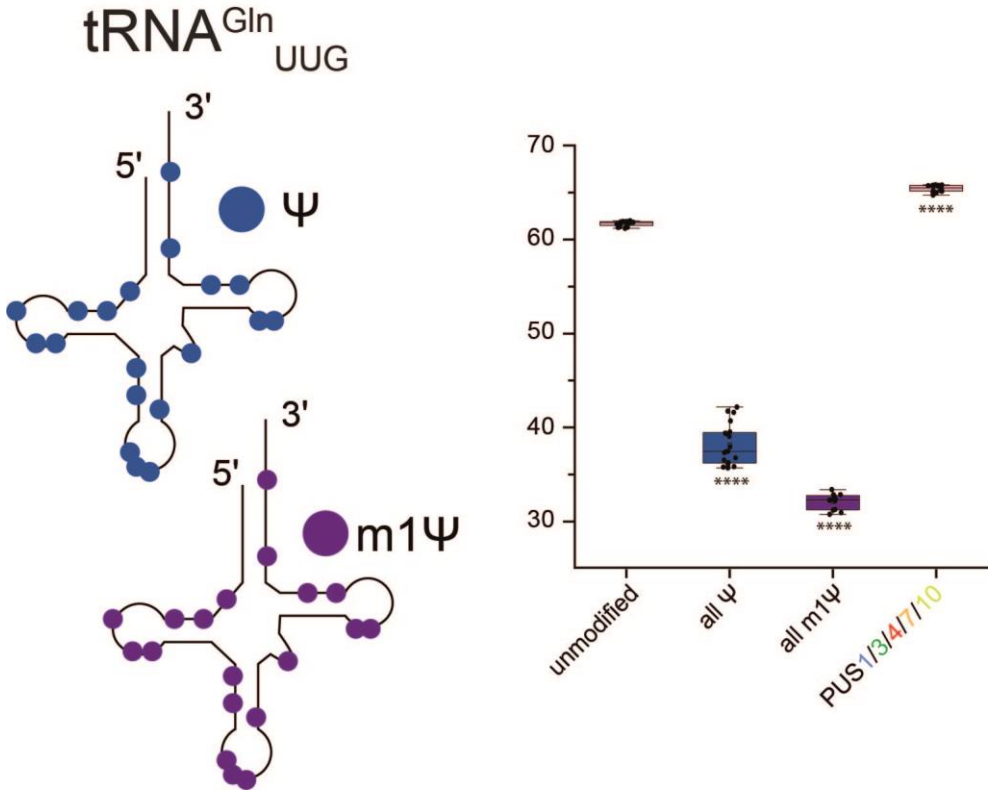
Ψ_{39} rigidifies the ASL
Ψ_{55} induces the compaction of the elbow region

inDyRNA Thermometry analysis of the Ψ local effects in tRNA



Ψ_{13} stabilizes the proximal nucleotides around the core of tRNA

inDyRNA Thermometry analysis of the Ψ local effects in tRNA



Complete Ψ pattern leads to high level of destabilization of tRNA conformation

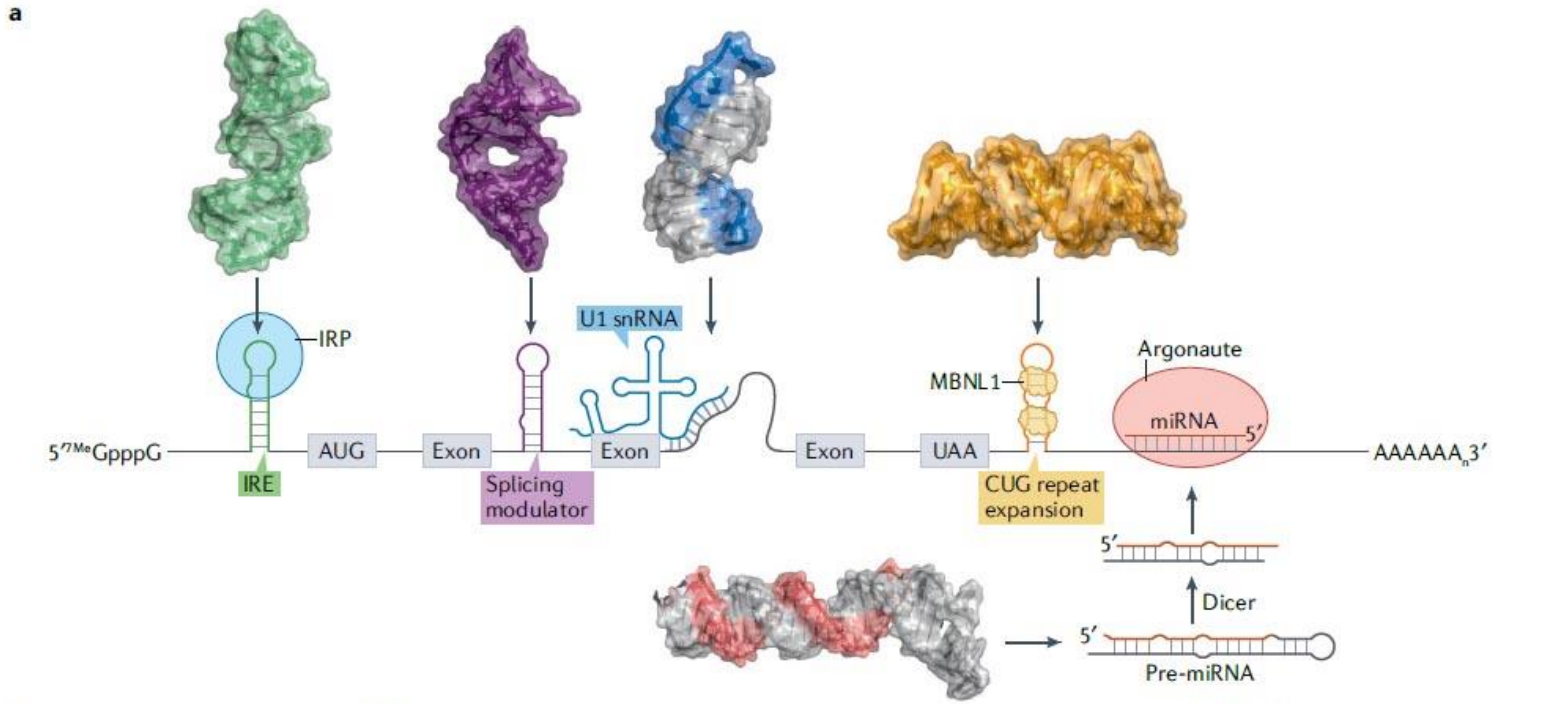
Summary

Conformational dynamics of RNA could be probed with a site-specific fluorescent labeling and the temperature gradient

- exDyRNA Thermometry global effect assessment
- inDyRNA Thermometry domain effect assessment

Pseuodouridylation placed at specific position in tRNA sequence leads to local stabilization effects measured with cryo-EM and DyRNA Thermometry

RNA domains as drug targets



Childs-Disney et al., Nat Rev Drug Discovery 2022

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Q&A