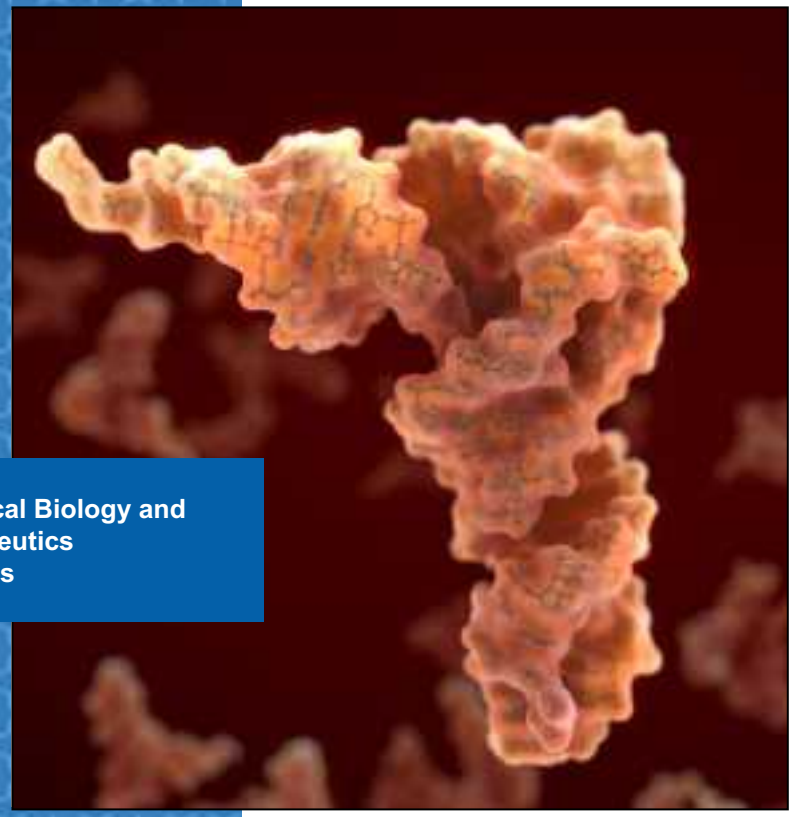
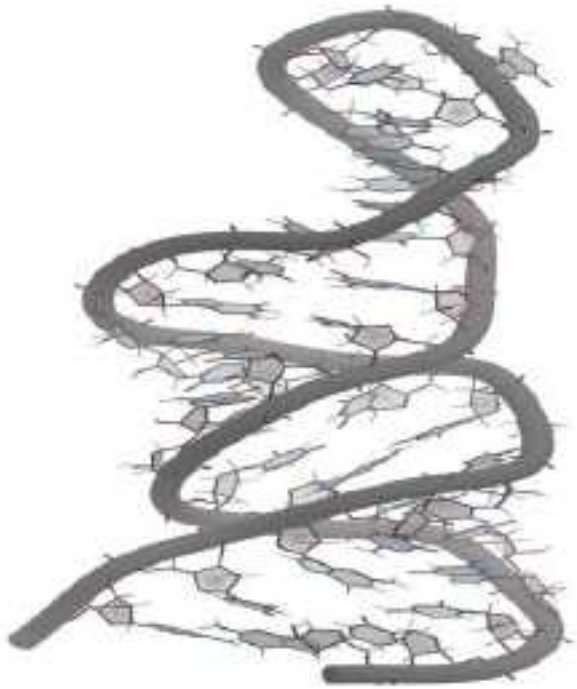
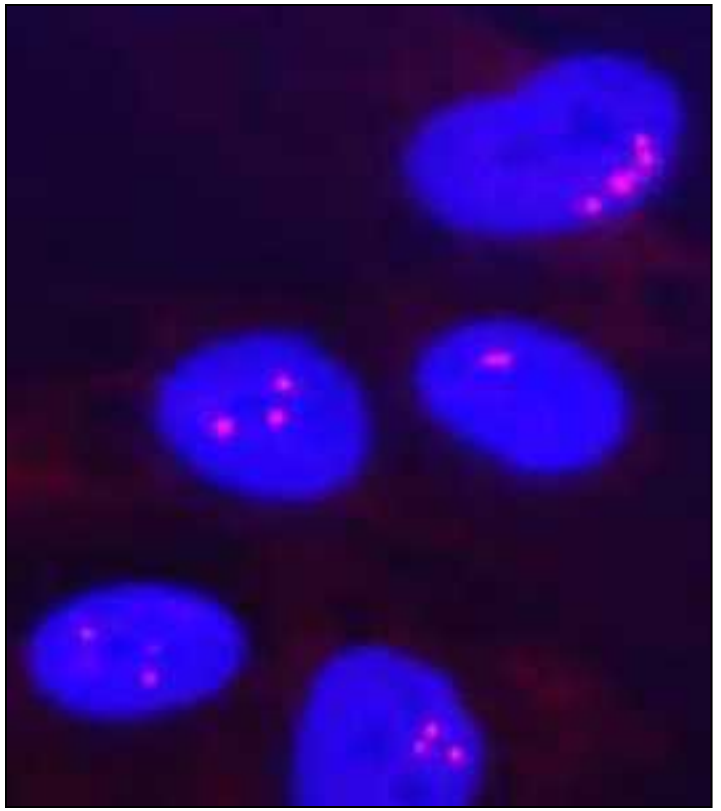






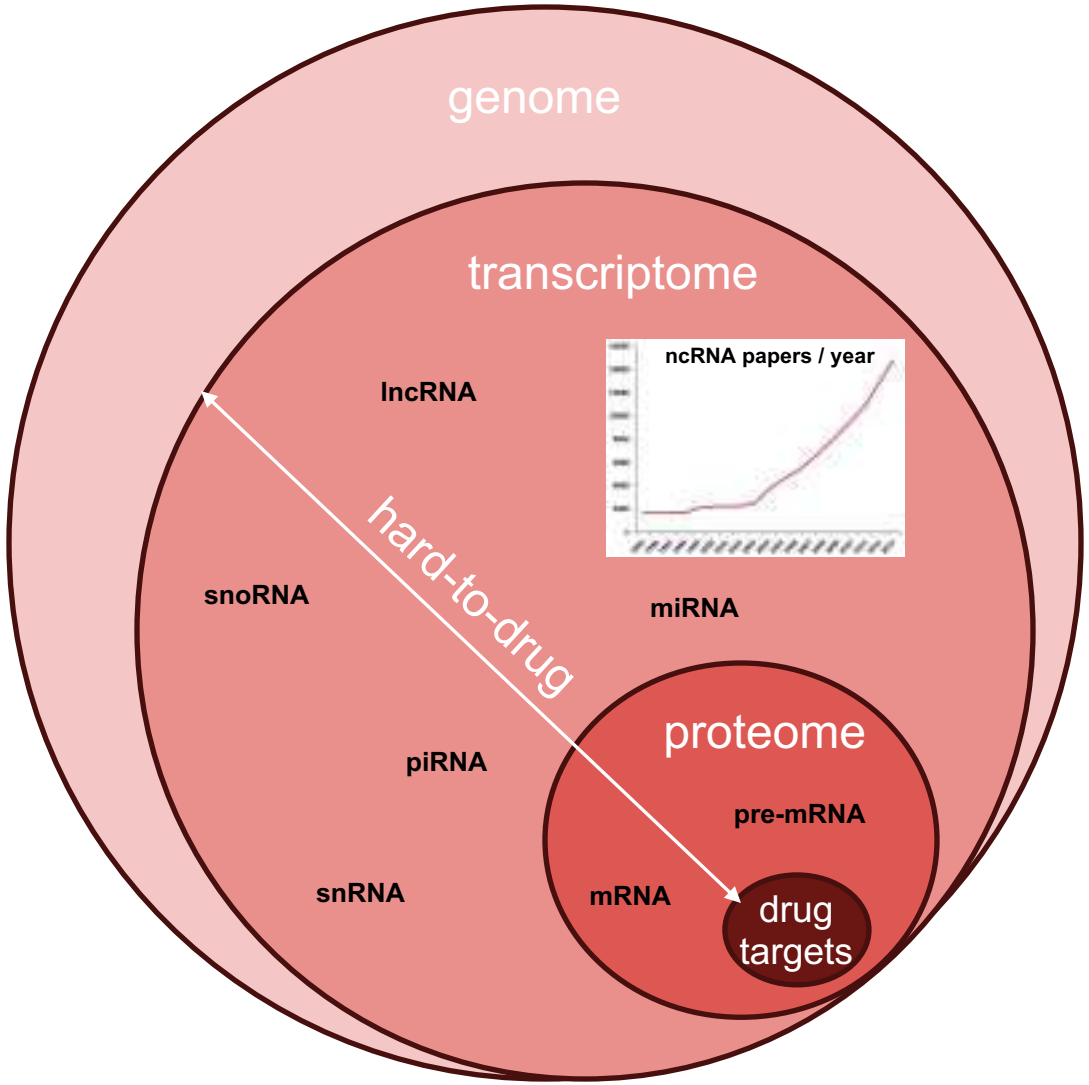
Chemical Biology and Therapeutics
Novartis

Enabling Modulation of RNA Biology in Human Disease with Small Molecules

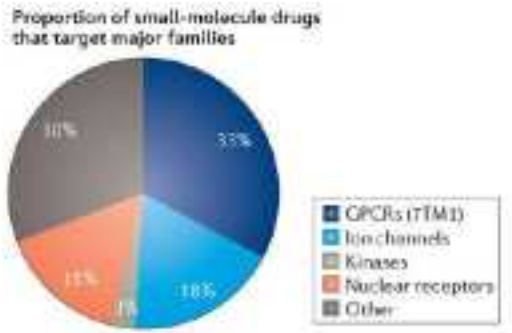
Razvan Nutiu on behalf of the team

NOVARTIS

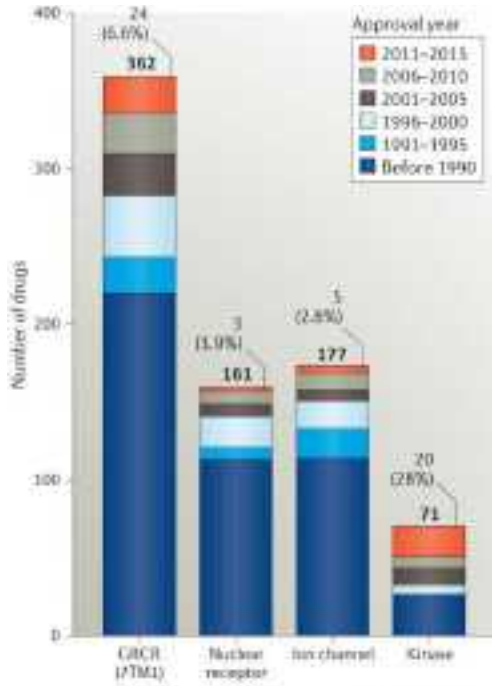
Need for innovation



~1500 FDA approved small molecules
~ 750 targets

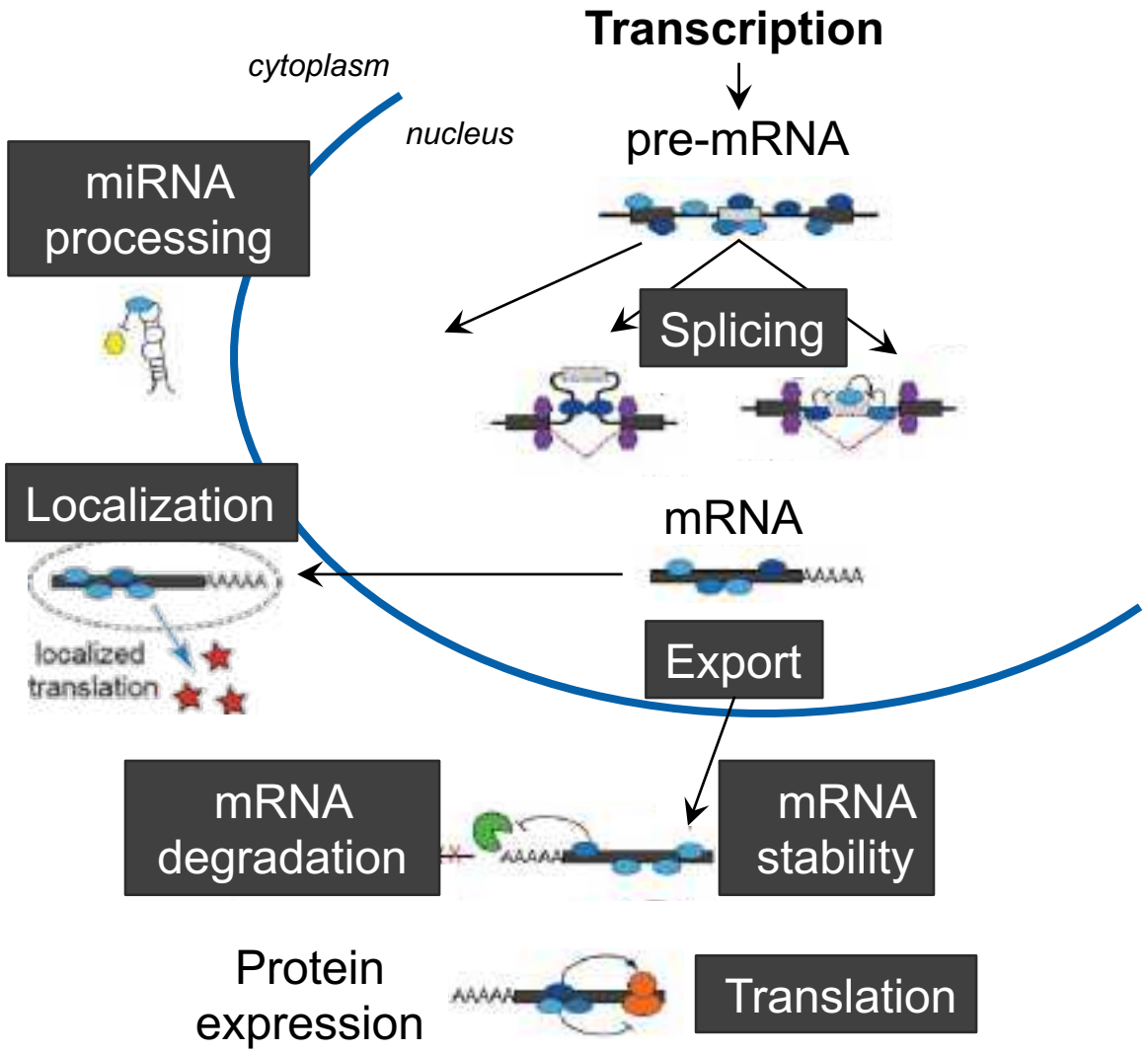


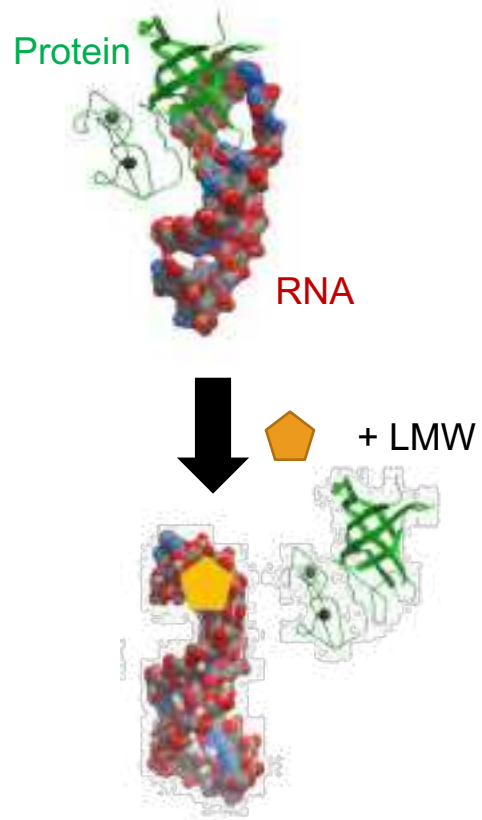
Santos el al, 2017



Nature Reviews | Drug Discovery

RNA Biology – potentially vast therapeutic target space





RNA biology is controlled by interactions of RNA structures with RNA binding proteins

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RNA is structurally diverse

Every RNA has a different 'personality'

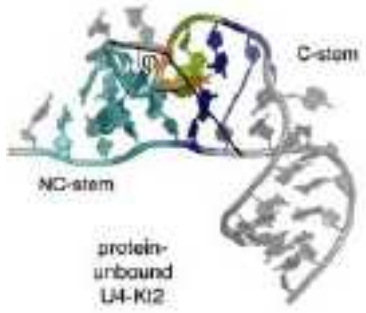
Human tRNA
Itoh et al, N.A.R. 2009

HCV IRES pseudoknot
Berry et al, Structure 2011

SAM riboswitch
Batey et al, Nature 2006

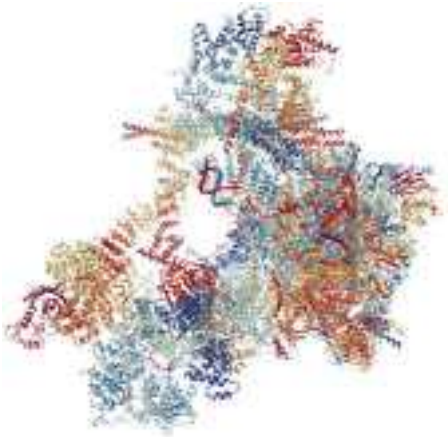
Tetrahymena ribozyme
Golden et al, Science 1998

ZN finger / RNA
Lu et al, Nature 2003



Human spliceosomal U4 snRNA
Falb et al, N.A.R. 2010

Group II intron lariat
Robart et al, Nature 2014



Spliceosome C complex Cryo-EM
Zhan et al, Science 2018

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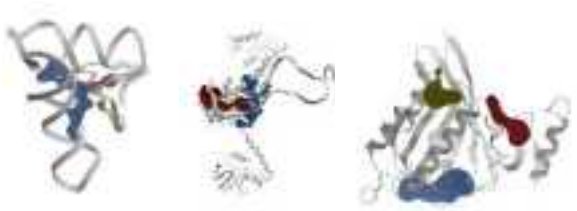
RNA space is ligandable

Pocketome Computational Analysis

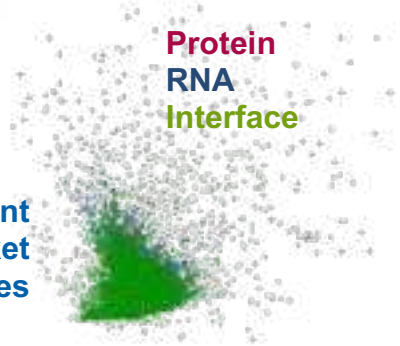


RNA
2GIS
~500

Interface
RNA/Protein
3PLA
~400

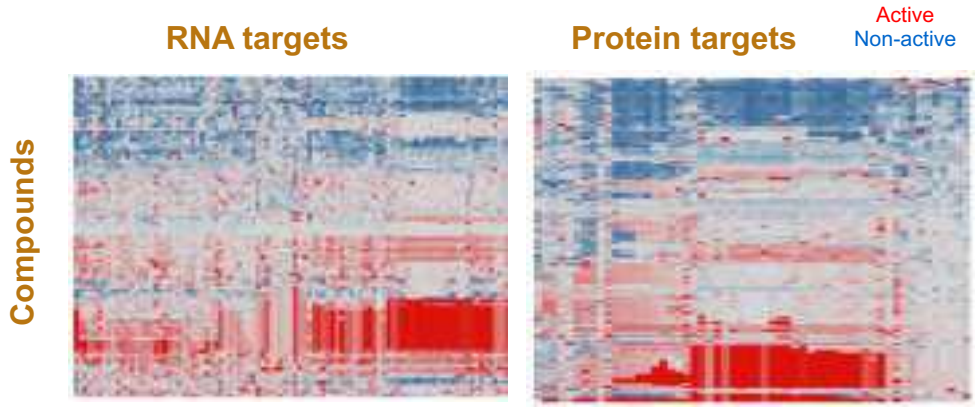
Protein
3MJH
~1000s

Analysis of putative binding pockets for structures across PDB.



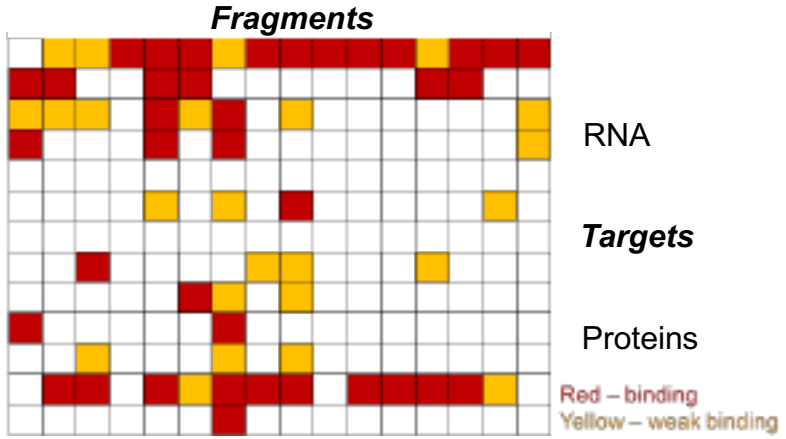
Principal component analysis of pocket properties

Virtual screening results similar to protein targets



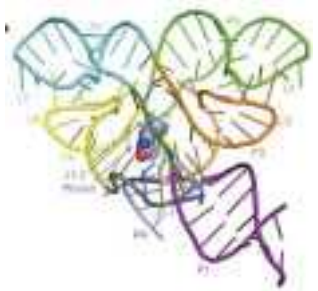
Fragment NMR Screens for RNA

- >1000 fragments vs 6 structurally different RNAs
- RNA binders show selectivity vs other RNAs or proteins



Early examples prove the concept

Branaplam / SMN2 ex. 7
Novartis



Ribocil / FMN riboswitch
Merck

Chemical Biology & Therapeutics

NOVARTIS

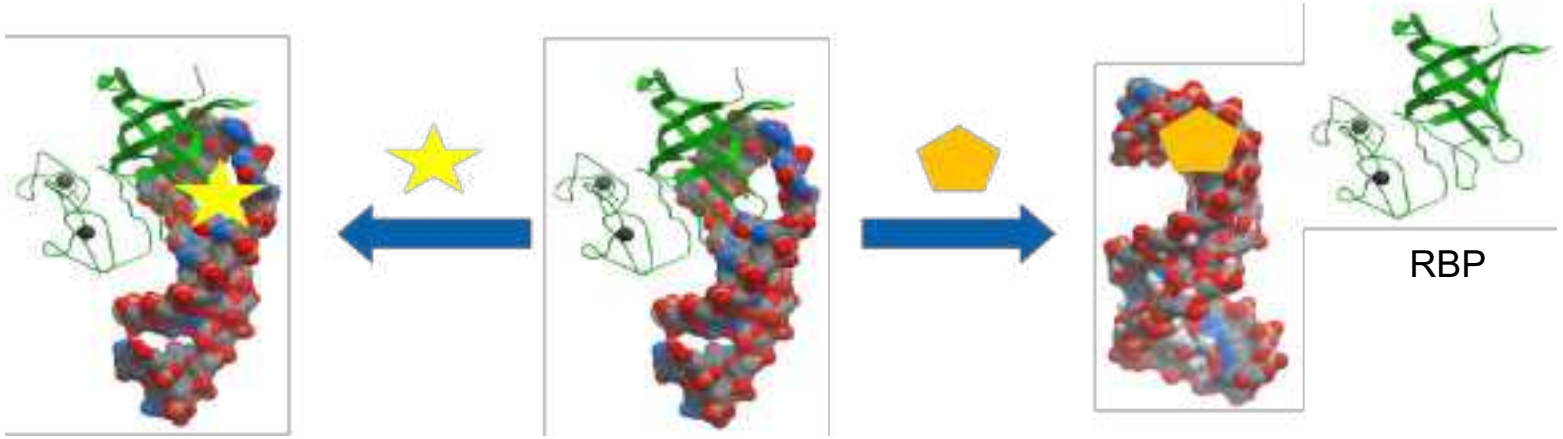
RNA biology as therapeutic space

RNA biology is relevant to disease and drug discovery

- mRNA regulation controls protein production
- RNA mutations, editing and modifications influence protein outcomes
- ncRNAs have biological function and could drive disease

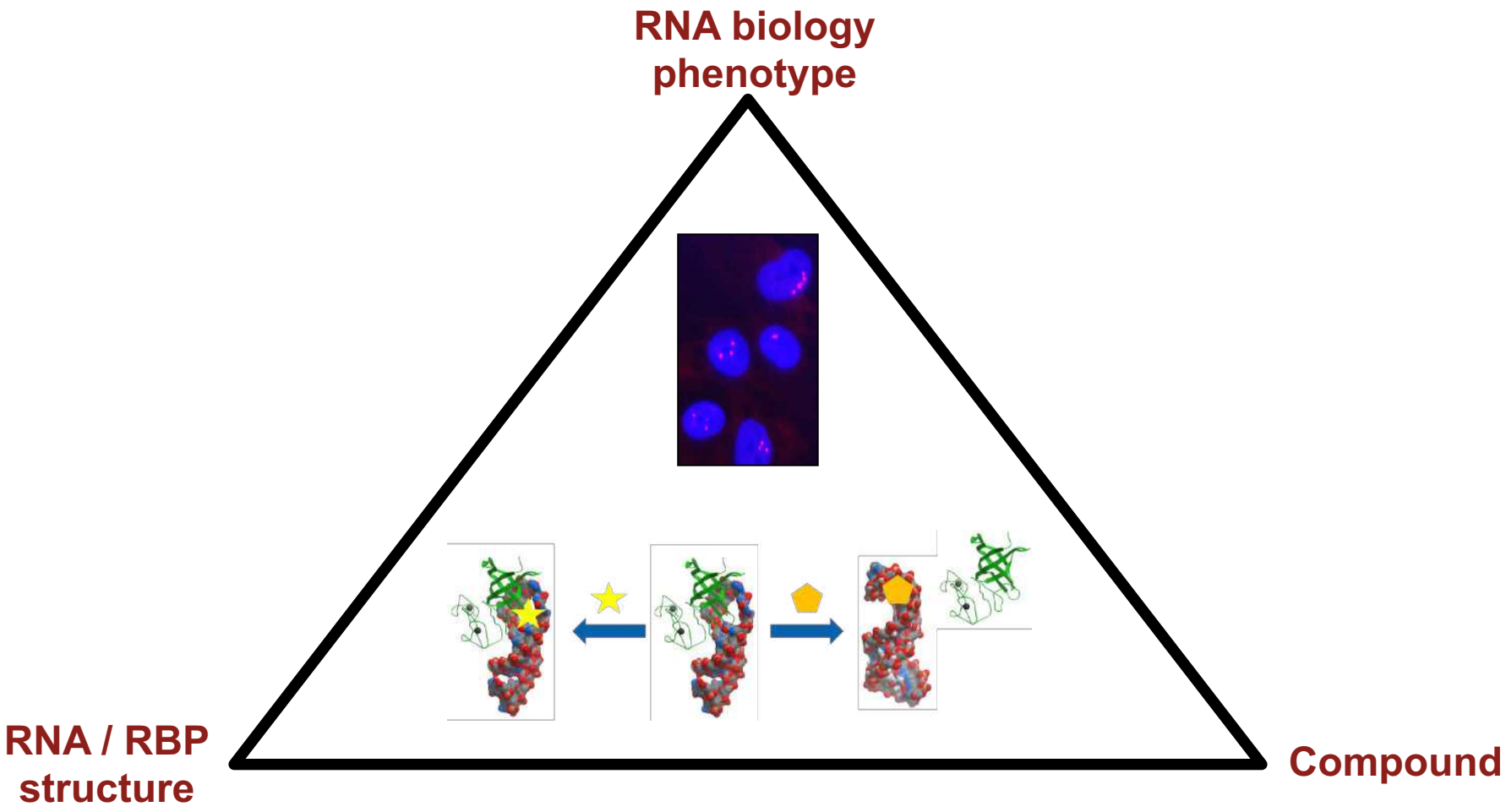
RNA and RNA / protein complexes have structure

RNA and RNA/protein complexes can be targeted by LMW to alter regulation



Unlocking RNA biology as target for LMW would considerably reduce the undruggable space

Drug discovery in RNA biology space



SMA paves the way: LMI070 & RG7916

LMI070 (Novartis)
Stabilizes RNA/protein interaction

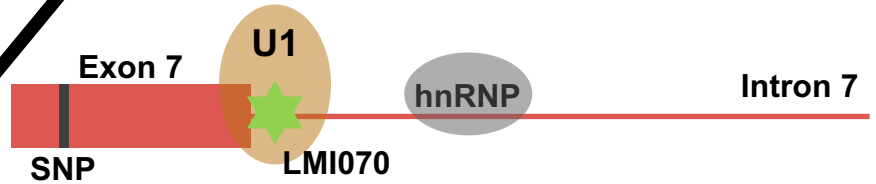
RG7916 (PTC, Roche)
RNA structure modulation

RNA biology phenotype

Splicing correction exon 7
Increase SMN2 protein

Exon 7 inclusion

Cell based screens
MedChem



LMI070

RNA / RBP structure

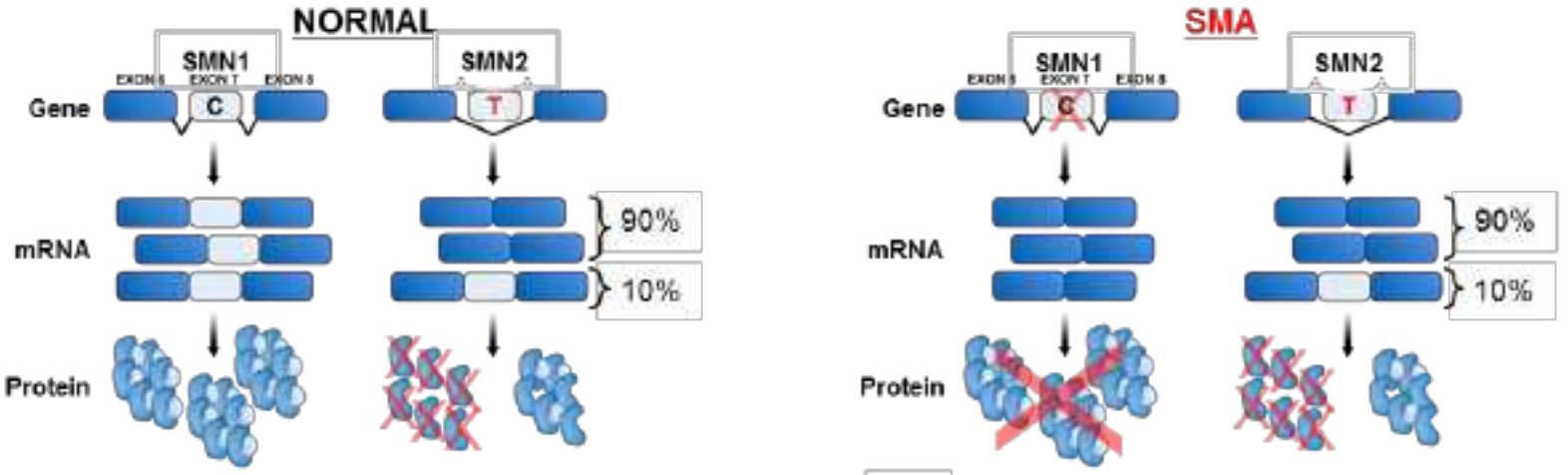
Compound

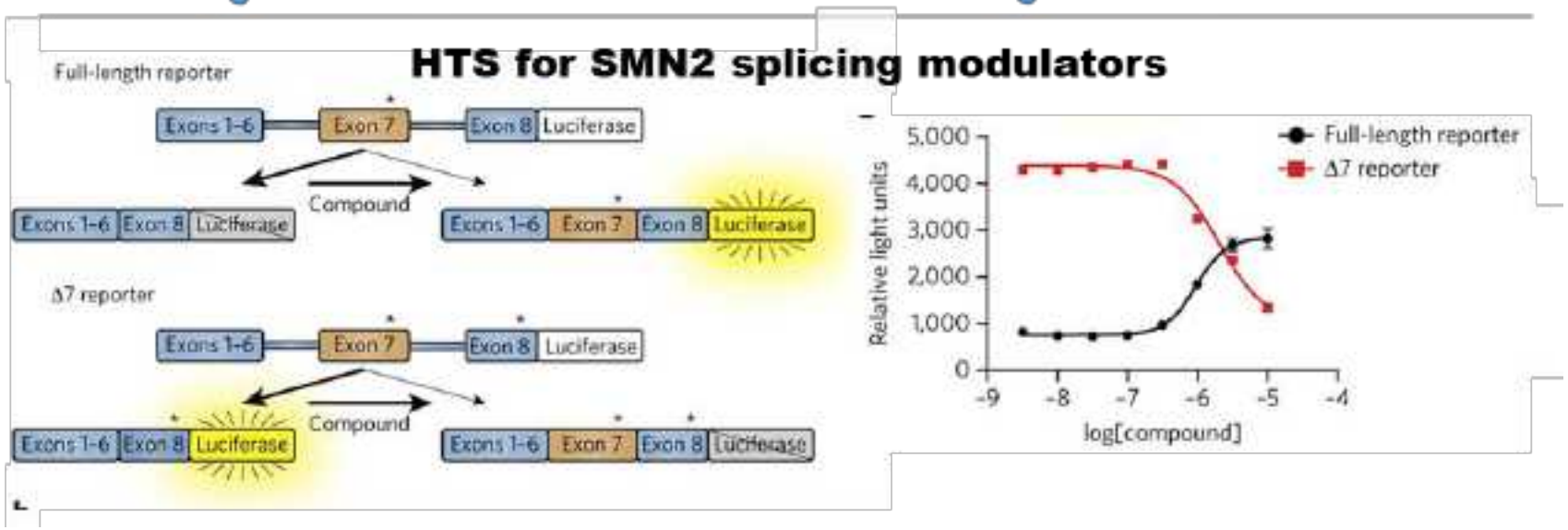
U1 / pre-mRNA 5' splice site

Target ID

Spinal Muscular Atrophy

Therapeutic rationale for a splice modulator

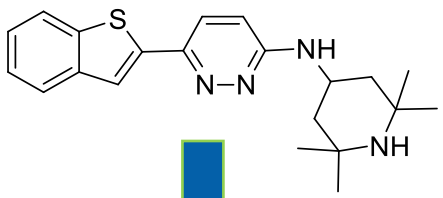




MedChem optimization leads to LMI070

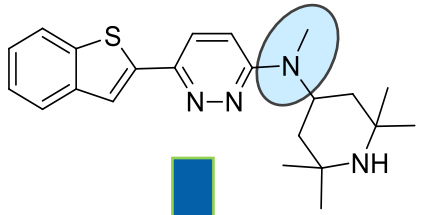
Cheung et al, JMC; 2018

Phenotypic hit



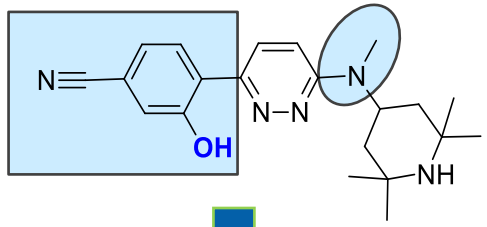
EC_{50} = 600 nM
cLogP = 5.5
Poor brain distribution
%F low, CL = High

- Retained activity
- Improved exposure, distribution to brain



EC_{50} = 500 nM
cLogP = 5.5
Good brain distribution
%F high, CL = High

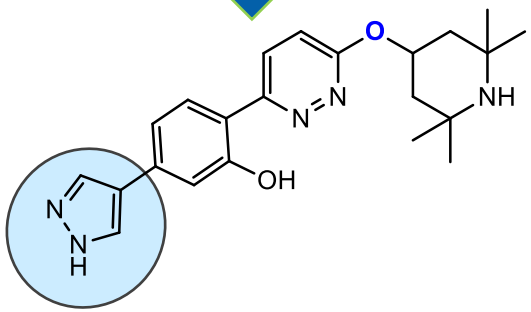
- Improved activity
- Reduced cLogP
- Reduced CL
- Good exposure and distribution to brain



EC_{50} = 30 nM
cLogP = 3.4
Good brain distribution
%F moderate, CL = Low

Early tool for in vivo validation

- High Amax
- Good exposure, %F, and distribution to brain
- Reduced off-target activity

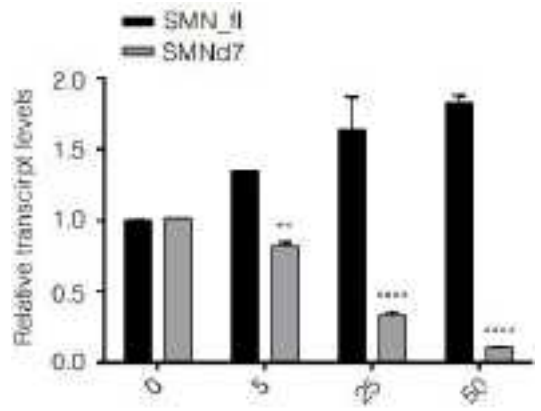


LMI070 - Branaplam
EC_{50} = 20 nM, 3.7 fold
Favorable PK, brain:plasma > 1

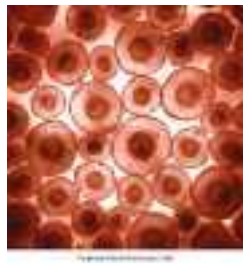
Clinical compound

LMI070 increases SMN2 levels in SMA patient PBMCs and iPS derived-neurons

SMA Patient PBMCs

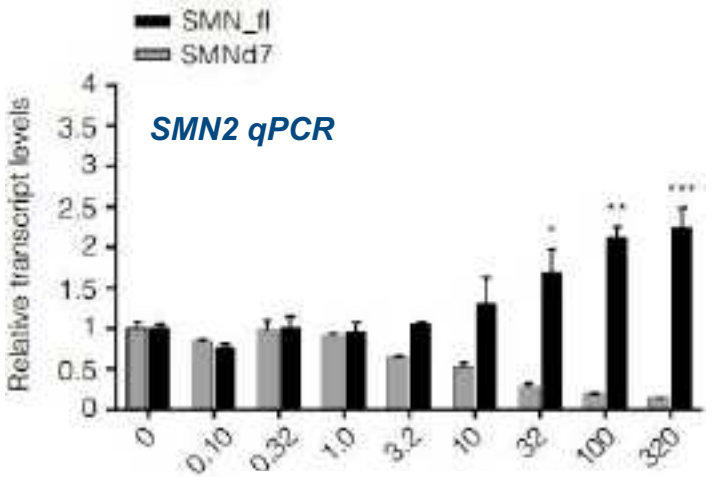


LMI070

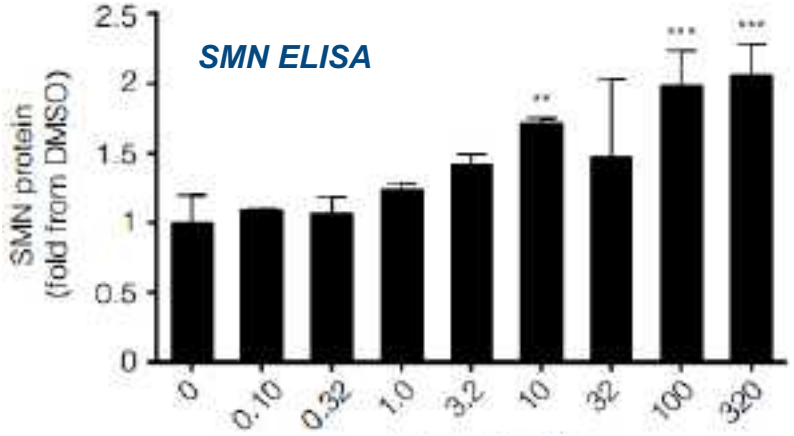


PBMCs

SMA Patient iPS-derived neurons



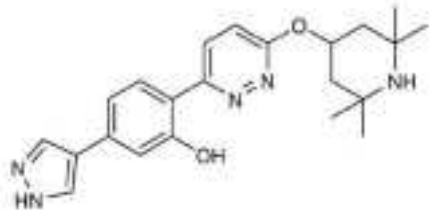
LMI070



LMI070

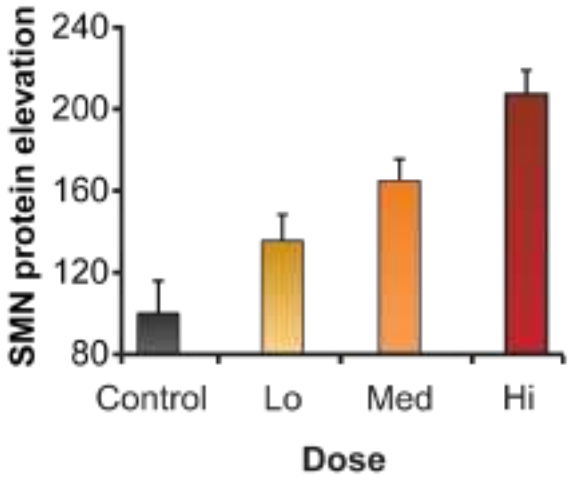
LMI070 is efficacious in the SMNΔ7 model

Dose-dependent effects on SMN protein, body weight and survival

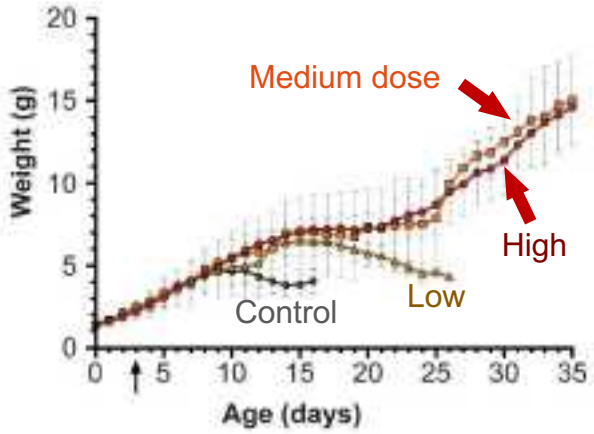


LMI070 (NVS-SM1)

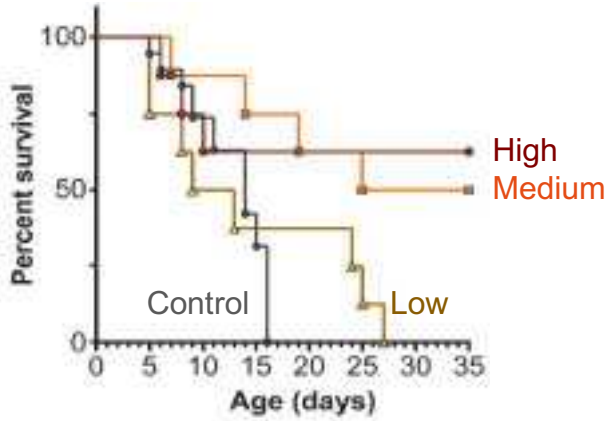
CNS SMN protein



Body weight



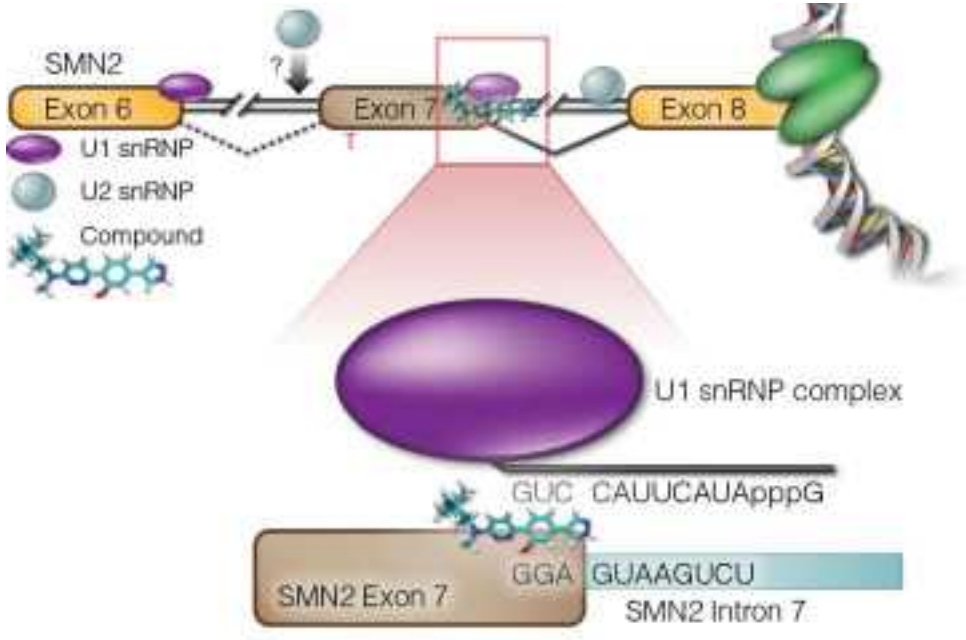
Survival

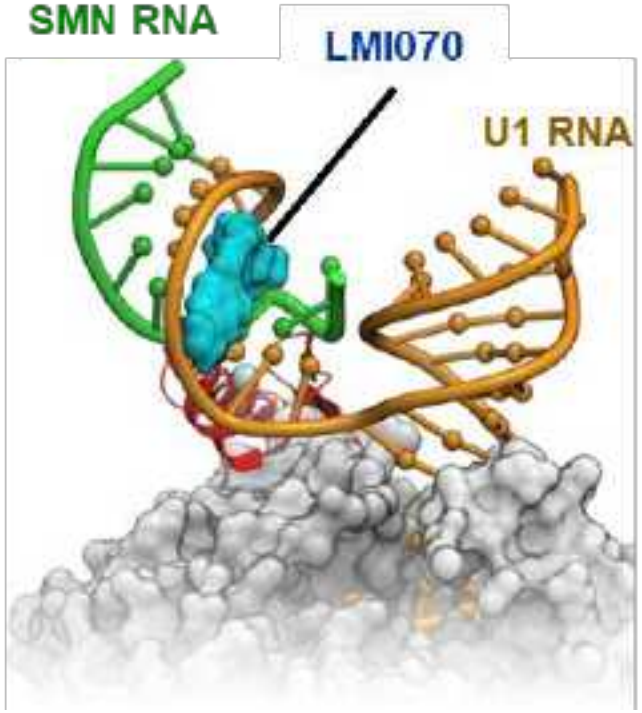


Model for mechanism of action of LMI070

Mechanistics

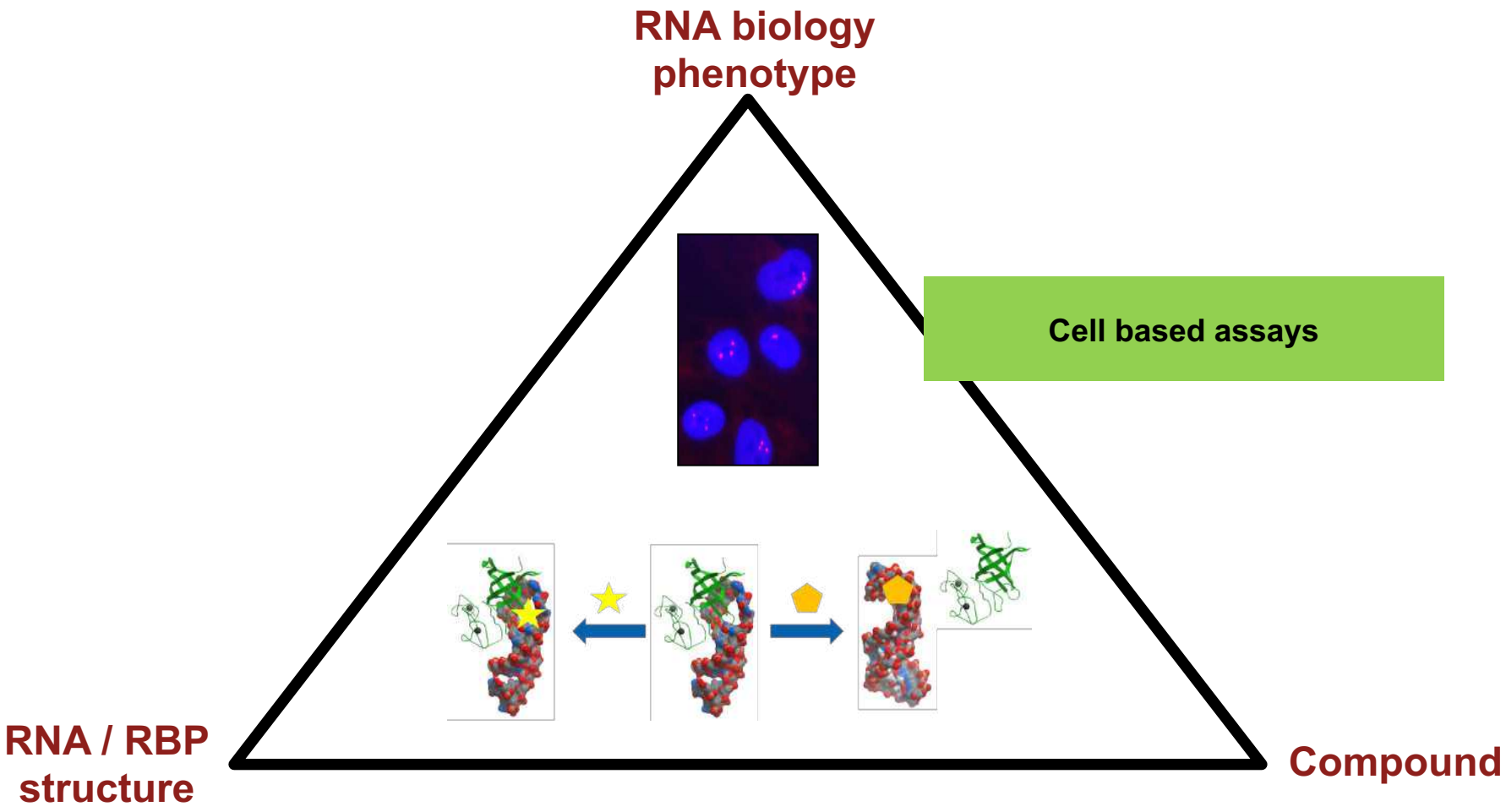
- Mutational analysis and splice site swap
- SPR and NMR



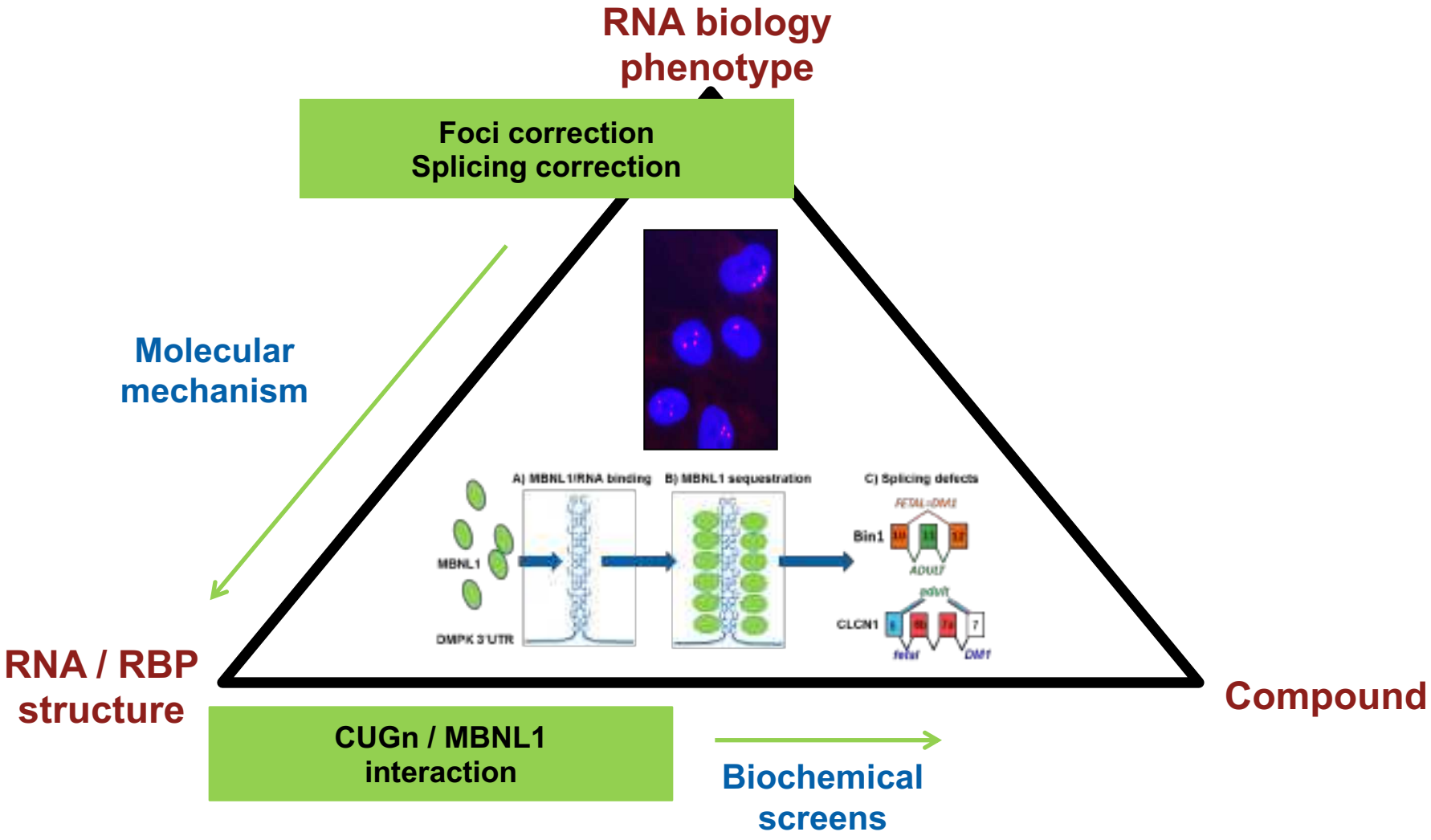


Palacino et al, Nat Chem Biol; 2015

Drug discovery in RNA biology space



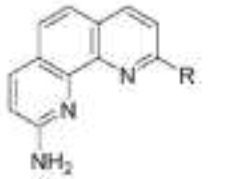
DM1: Myotonic Dystrophy Type 1



Search for assays and tool compounds

- Fragments by MB, NMR, SPR
- MedChem
- Literature
- 50k pilot

DAP

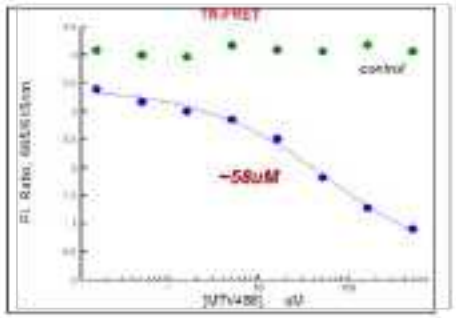


= NH_2 (NVP-MTV486)
= OH (NVP-MTV485)

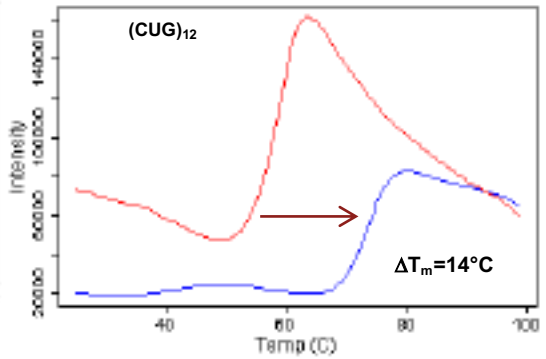
Inactive!

Nakatani et al, 2016

FRET CUG12/MBNL1

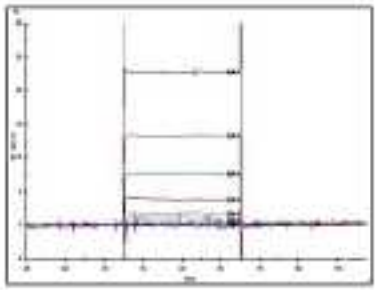


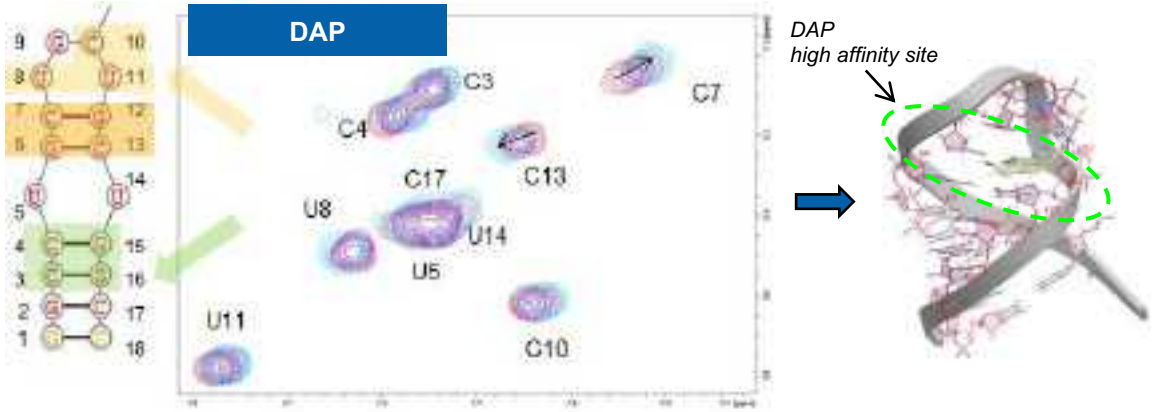
Molecular beacon



DAP binding confirmed in FRET, NMR, SPR, molecular beacons

DR w/ $(CUG)_{12}$ in SPR





Minimal CUG repeat NMR model

DAP – LMW tool compound

- positive results in all assays
- data suggests mechanism of binding
- productive binding mode
- Not selective against cTNT

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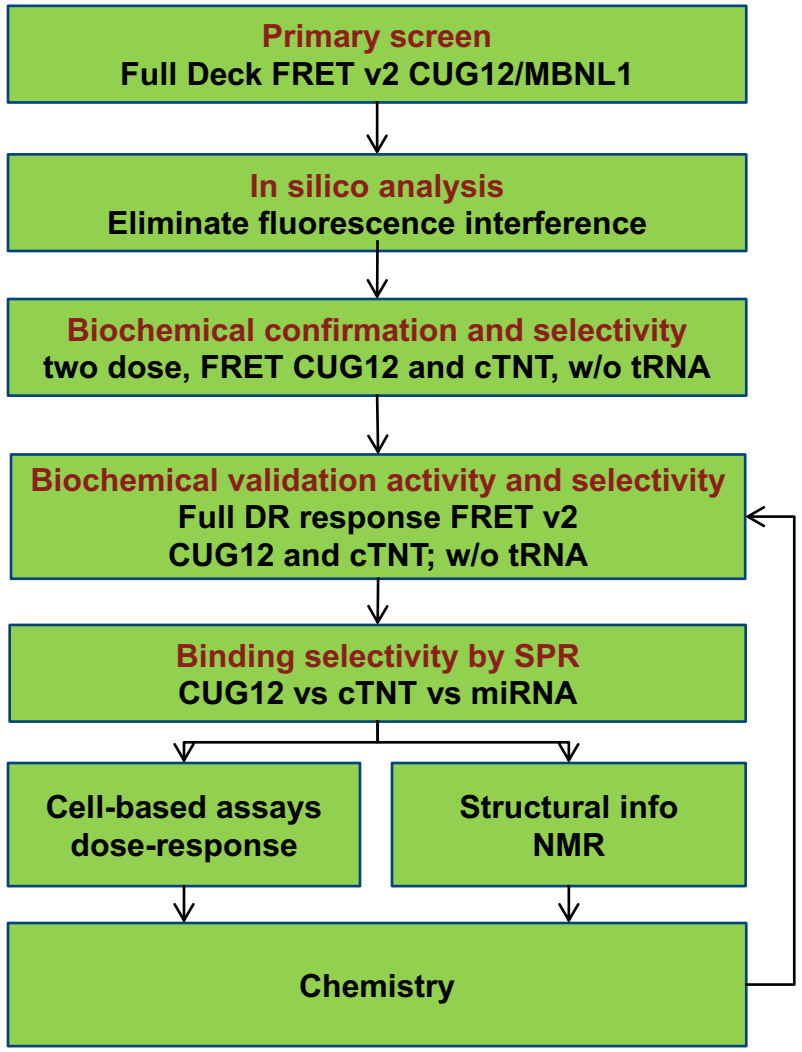
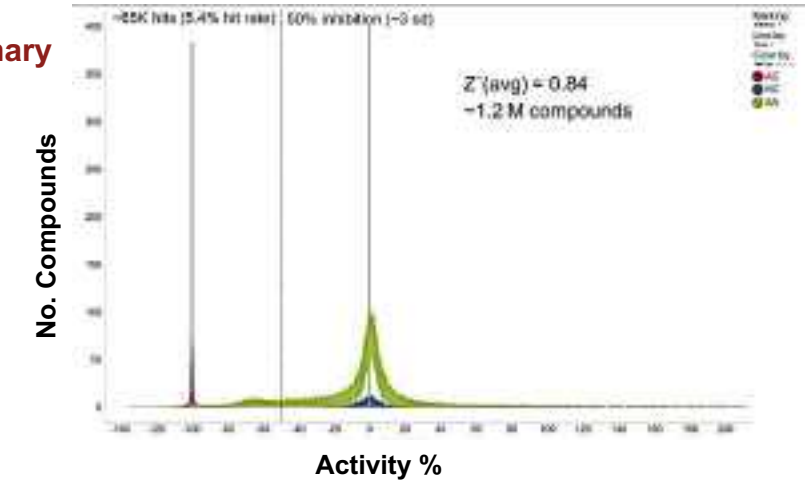
High Throughput Screening Campaign

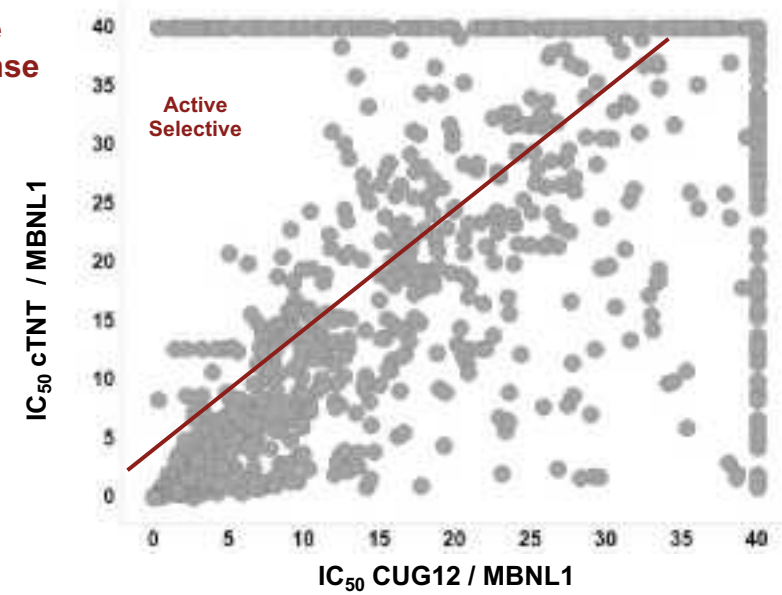
Integrated Flow chart for the Biochemical and Cell Based assays



Primary



Dose Response



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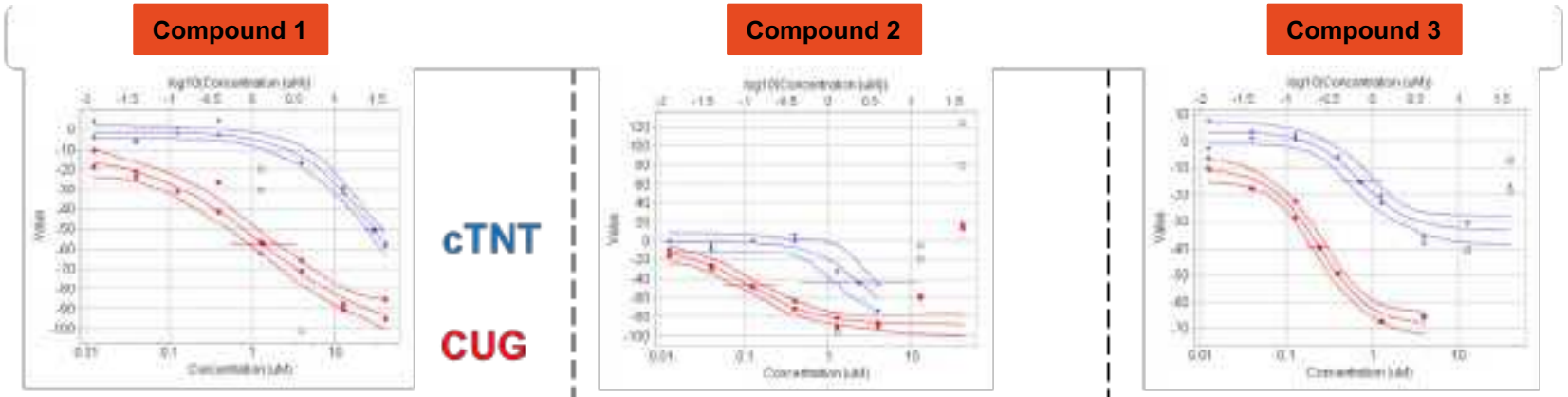
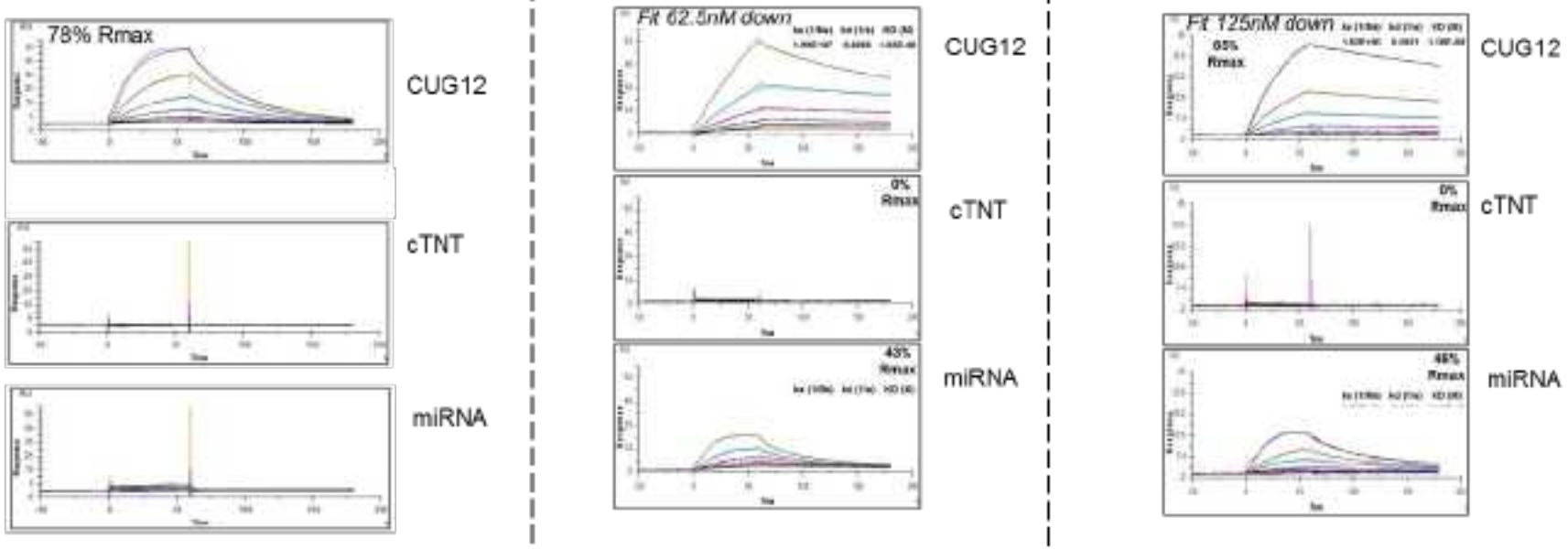
Series 1 compounds bind CUG repeats selectively



Surface plasmon resonance confirms selective RNA binding



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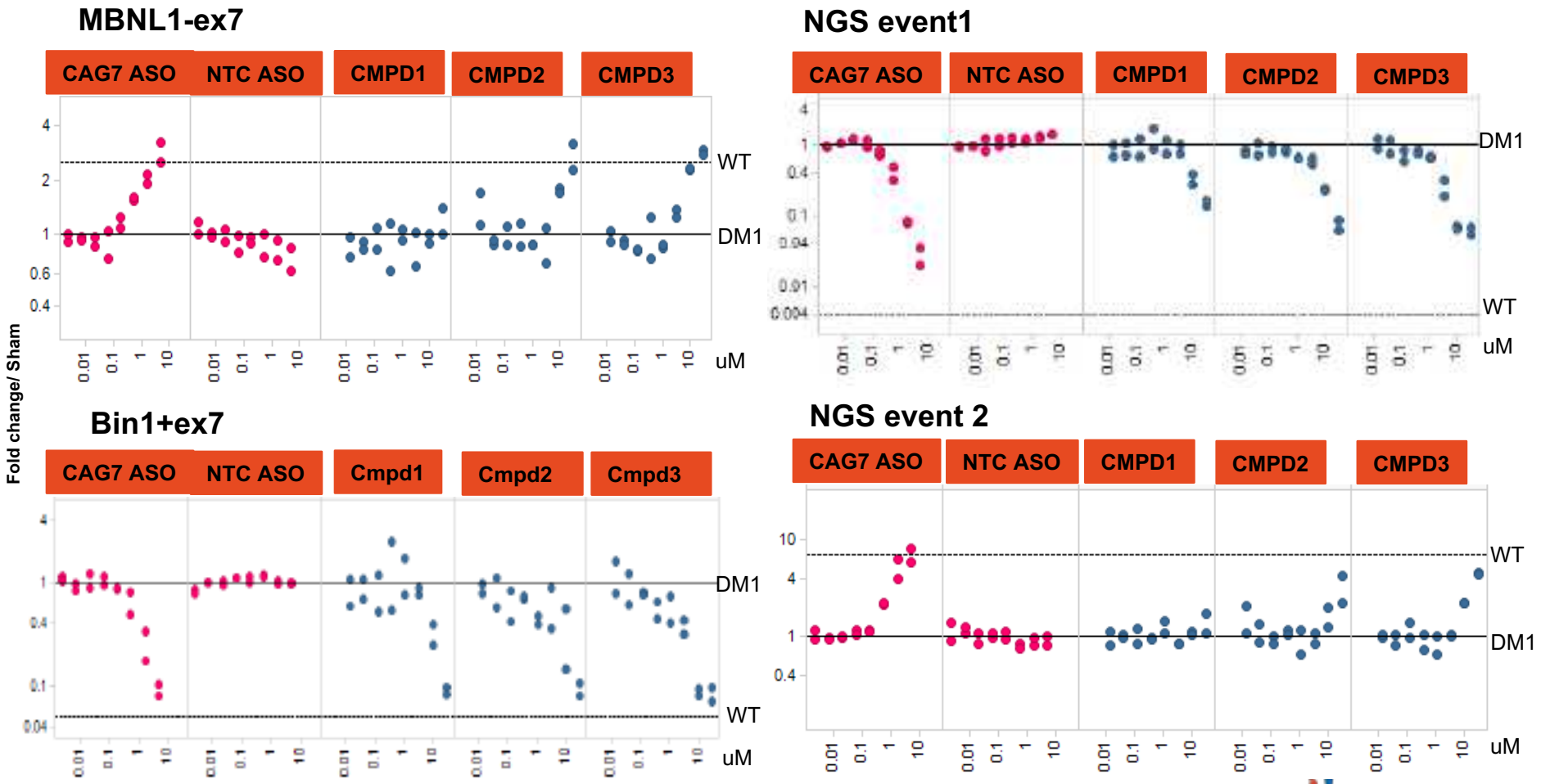
Series 1 compounds correct splicing

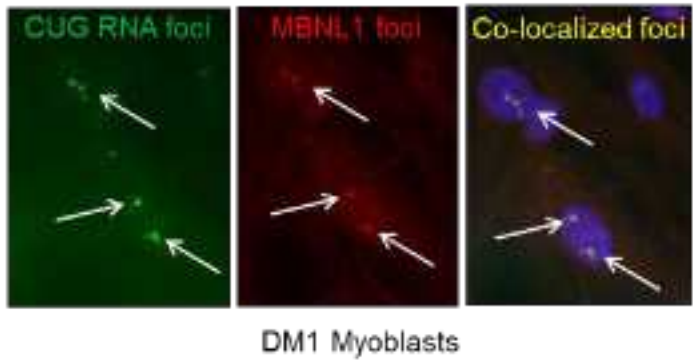
Literature → Splicing panel ← In-house NGS

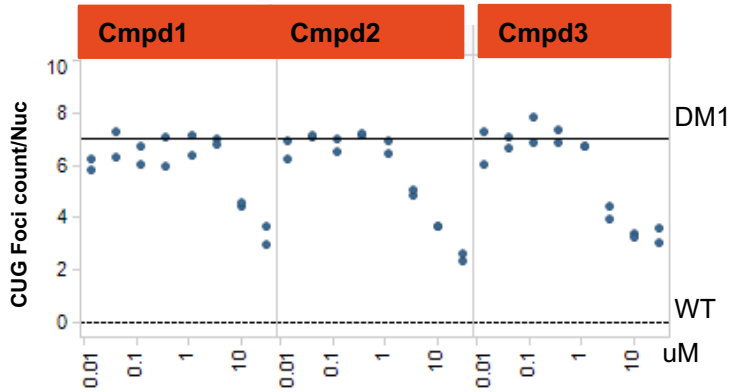
- Compounds
- Steric blocking antisense oligo

DM1 myoblasts, qPCR data



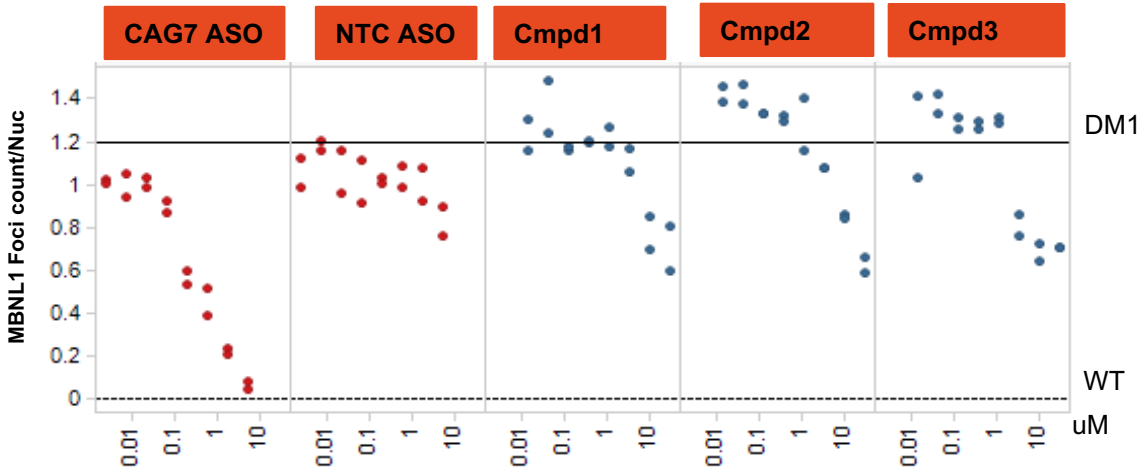
Series 1 compounds reduce CUG foci





- Compounds
- Steric blocking antisense oligo

RNA foci

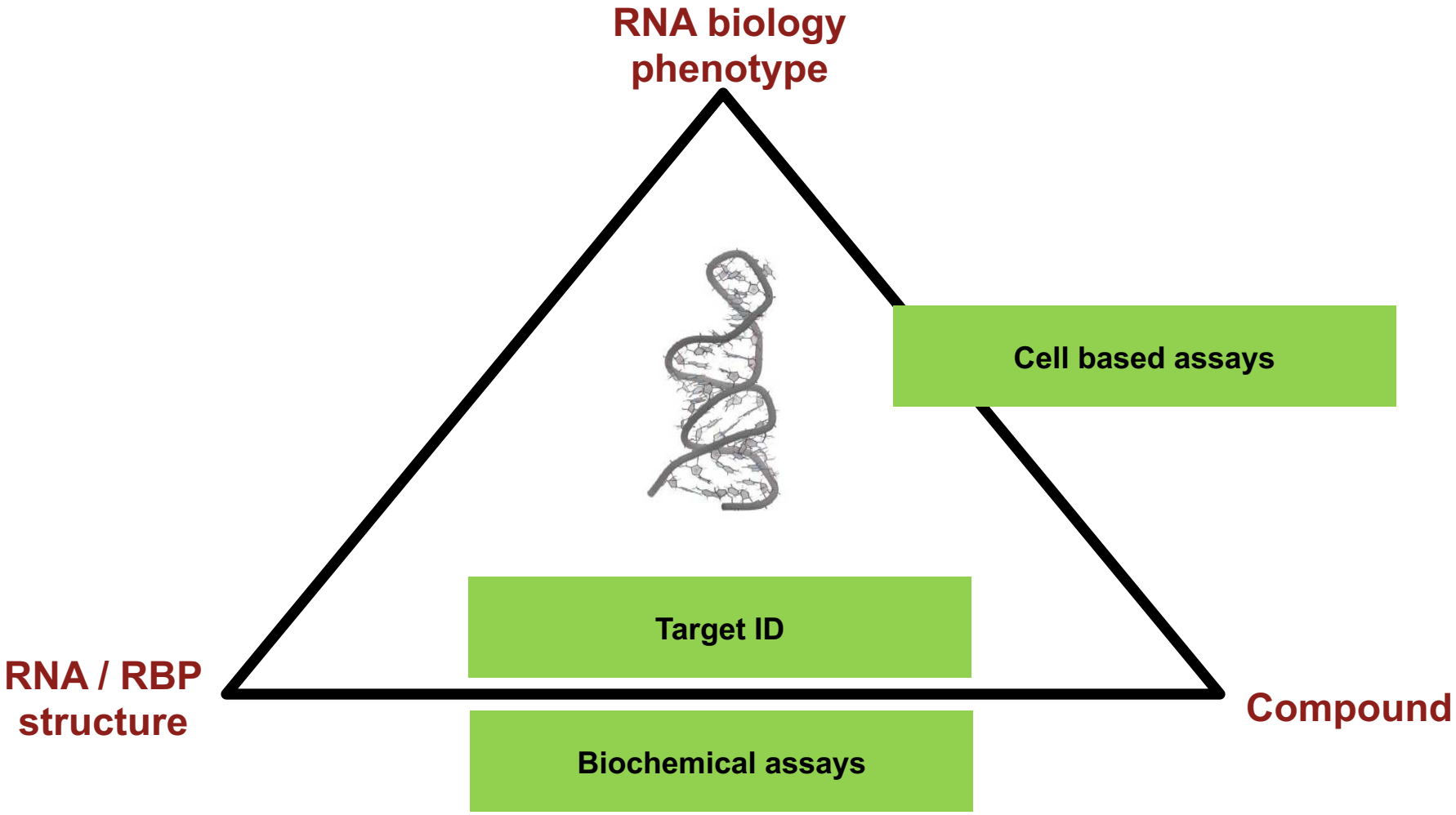


MBNL1 foci

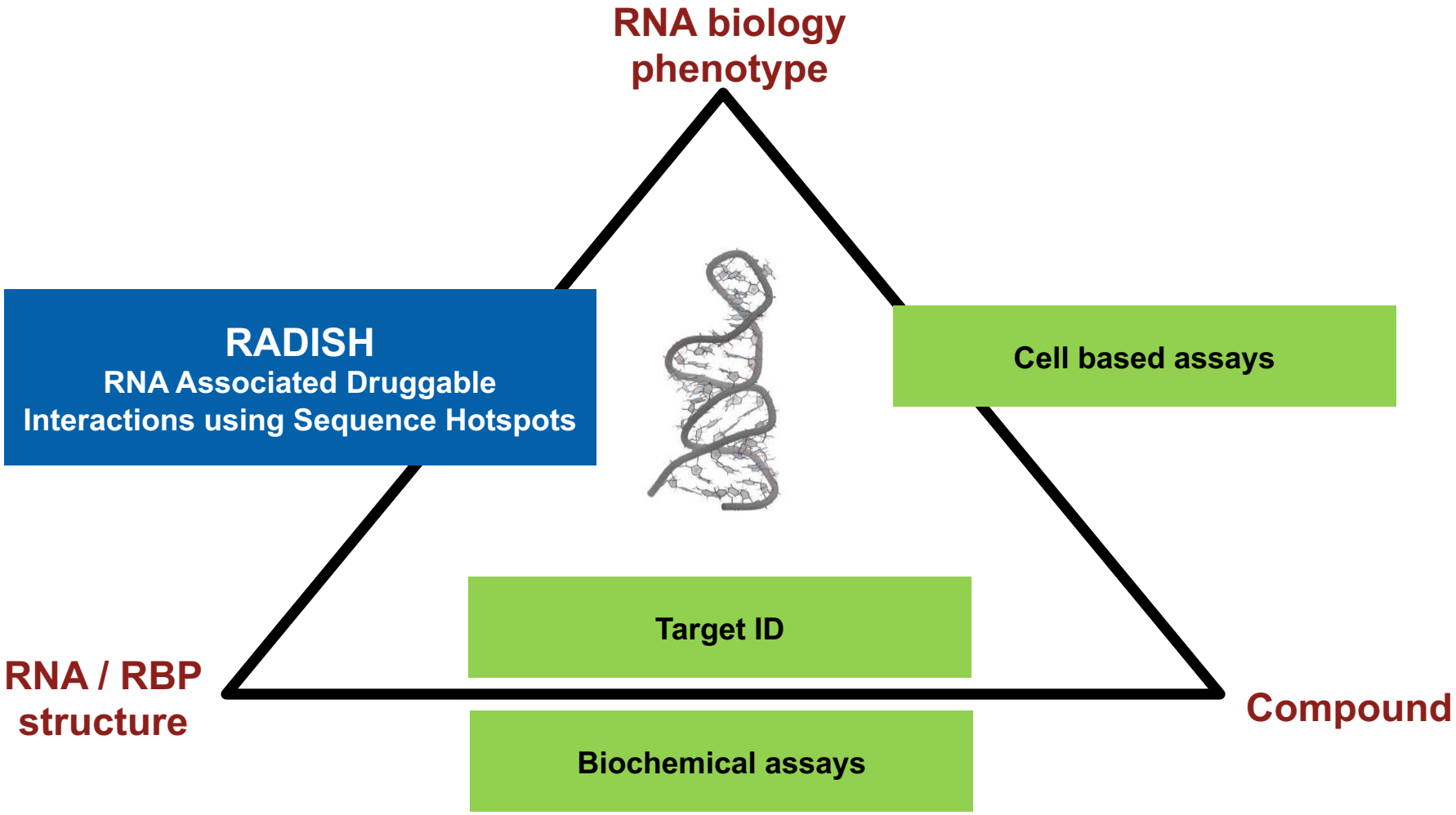
Series 1 -> MedChem

NOVARTIS

RNA Drug Discovery



RNA Drug Discovery



Reduce complex phenotypes to druggable RNA-PROTEIN molecular interactions

RNA biology phenotype

Map RNA active sites
RNA steric blocking & mutational analysis

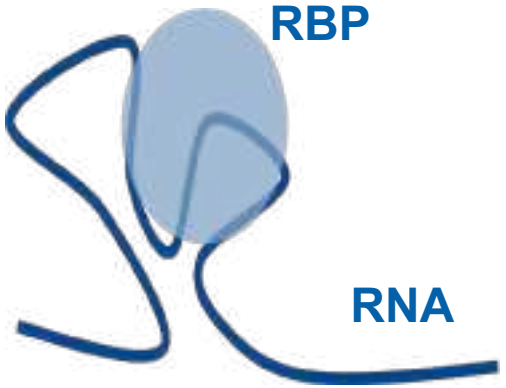
RADISH
RNA associated druggable interactions using sequence hotspots

Identify RNA binding proteins bound to RNA active sites
Genetic screening
RNA pulldowns followed by proteomics
eCLIP

Determine RNA local structure
SHAPE-MaP: chemical probing in vitro & in cell

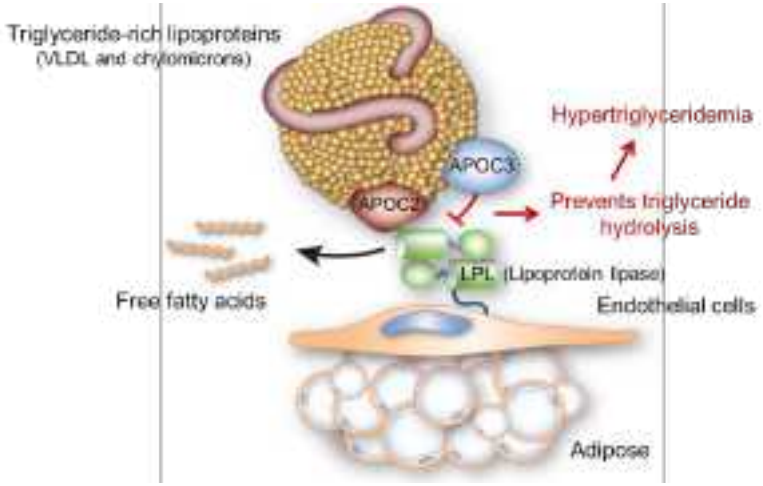
RNA / RBP structure

Computational biology
Public and in-house data analysis and integration



Model system: ApoC3

Apolipoprotein C3 modulates triglyceride-rich lipoprotein metabolism to regulate triglyceride levels

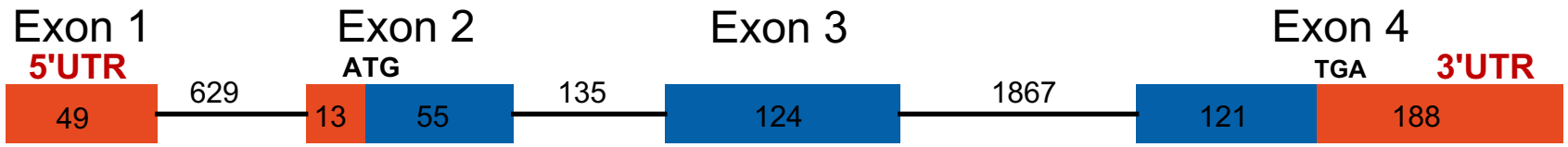


Disease Biology

- Highly validated target, human genetics
- Secreted protein – hard to drug

RNA Biology

- Very small gene ~3k genomic
- Expressed in liver: ENCODE data

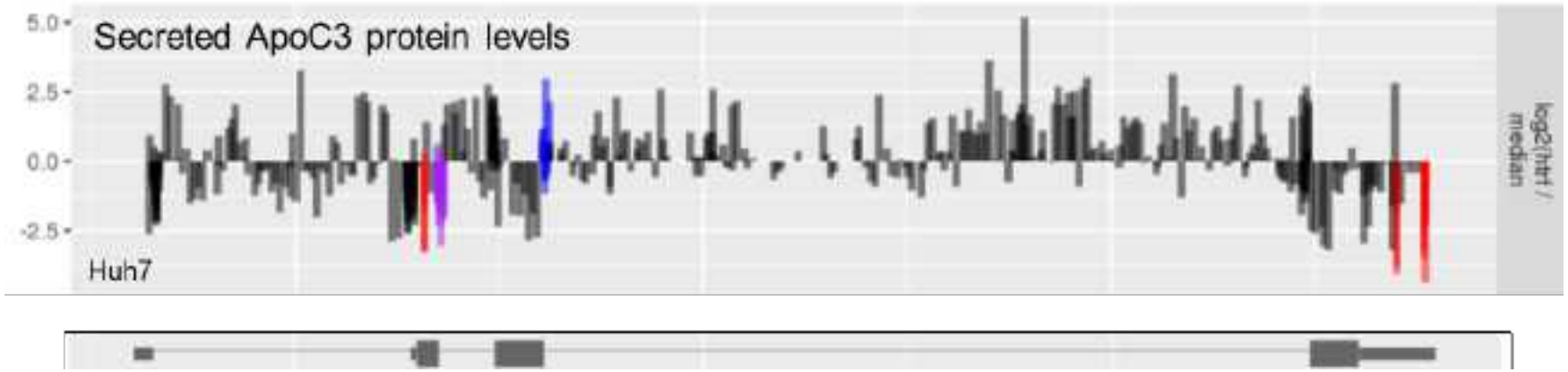


What are the critical RNA / protein interaction that control APOC3 mRNA fate?

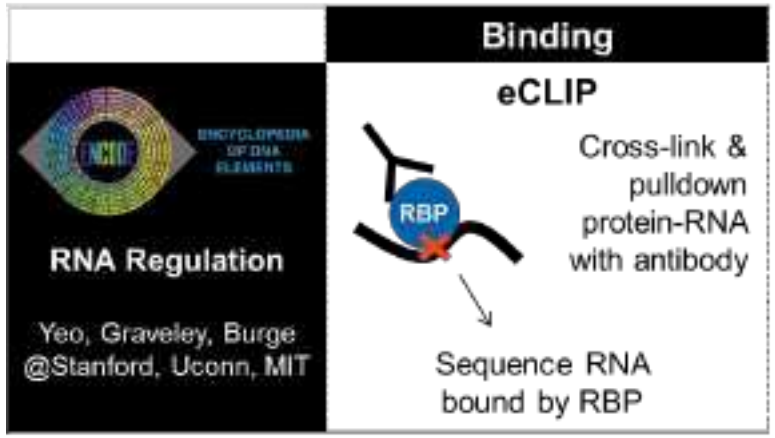
RNA active sites in APOC3 3′UTR

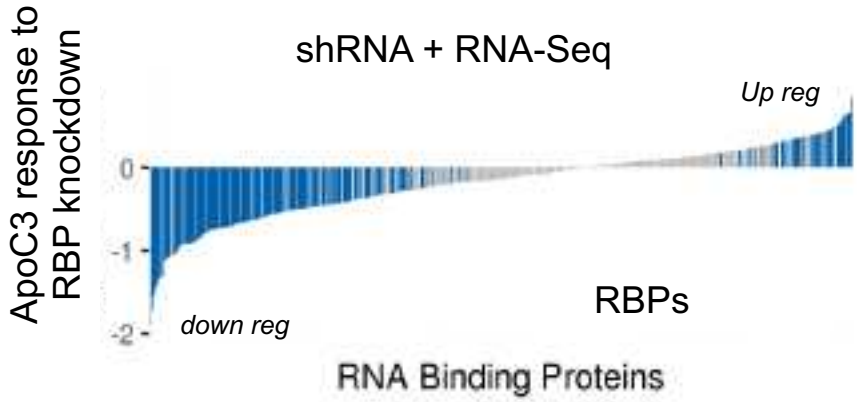
ASO walk: tile steric blocker ASOs over the entire gene

ASO walk on APOC3 UTR reveals a vast amount of regulation in all gene regions



Identify ApoC3 regulation by RBPs through genetic screening and eCLIP





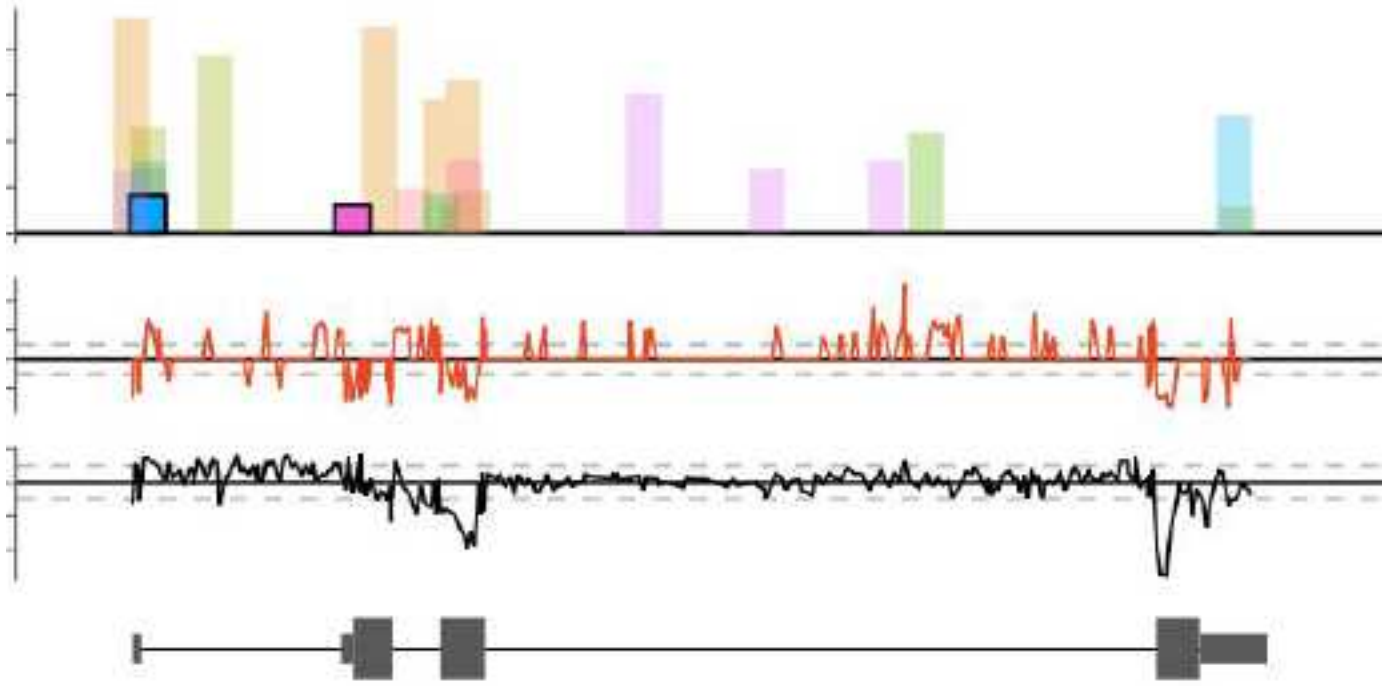
In-house (siRNA / qPCR)

1,947 predicted RBPs
8 siRNAs per gene

ApoC3 qPCR, secreted protein, and viability assays

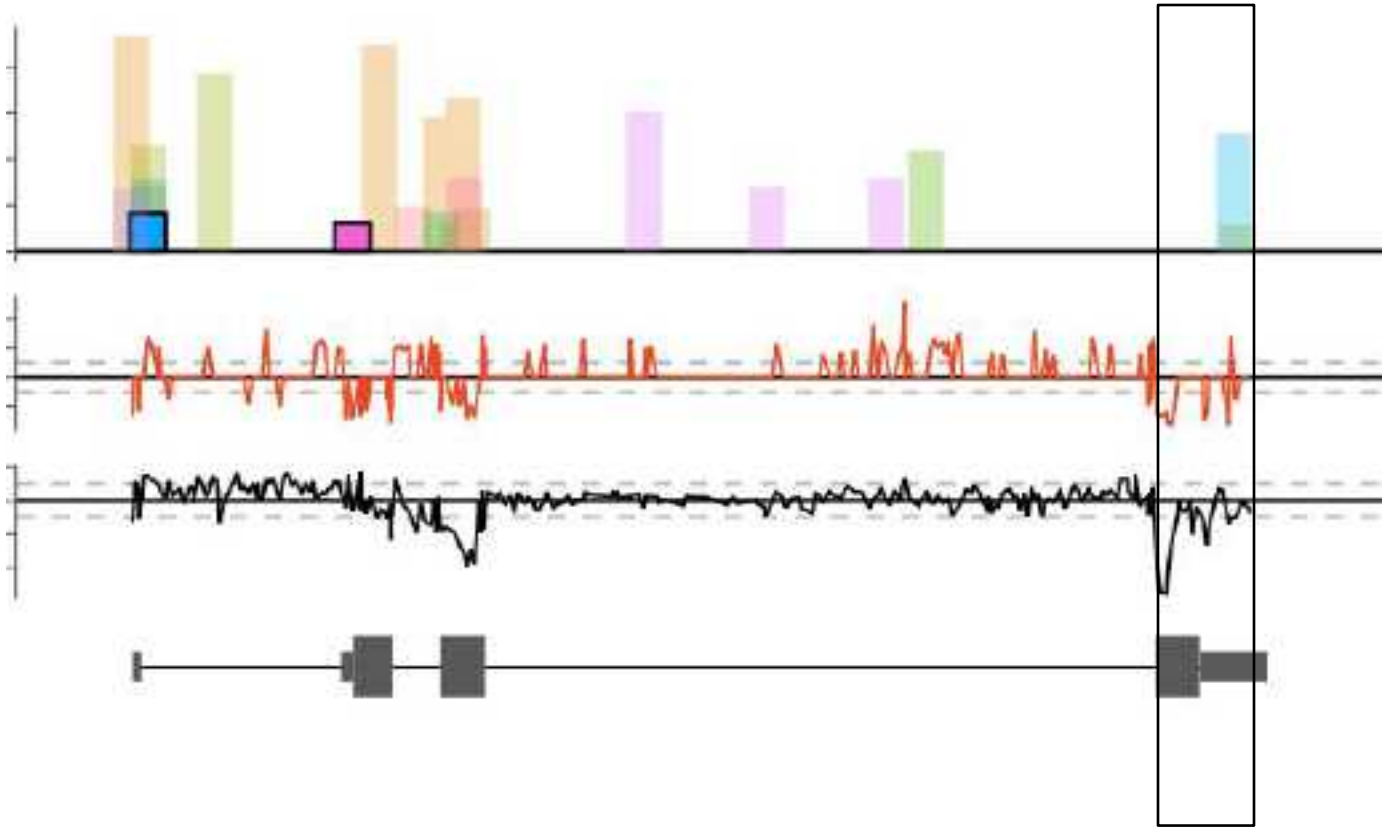
13 hits
2 with ENCODE data

Overlay of data



Lessons learned:
mRNA has multiple ‘active sites’ spread all around the gene
Many RBP binders don’t show phenotype on bound genes upon KD
Binding activity does not correlate with functional consequence upon binding

Overlay of data – 3’ UTR



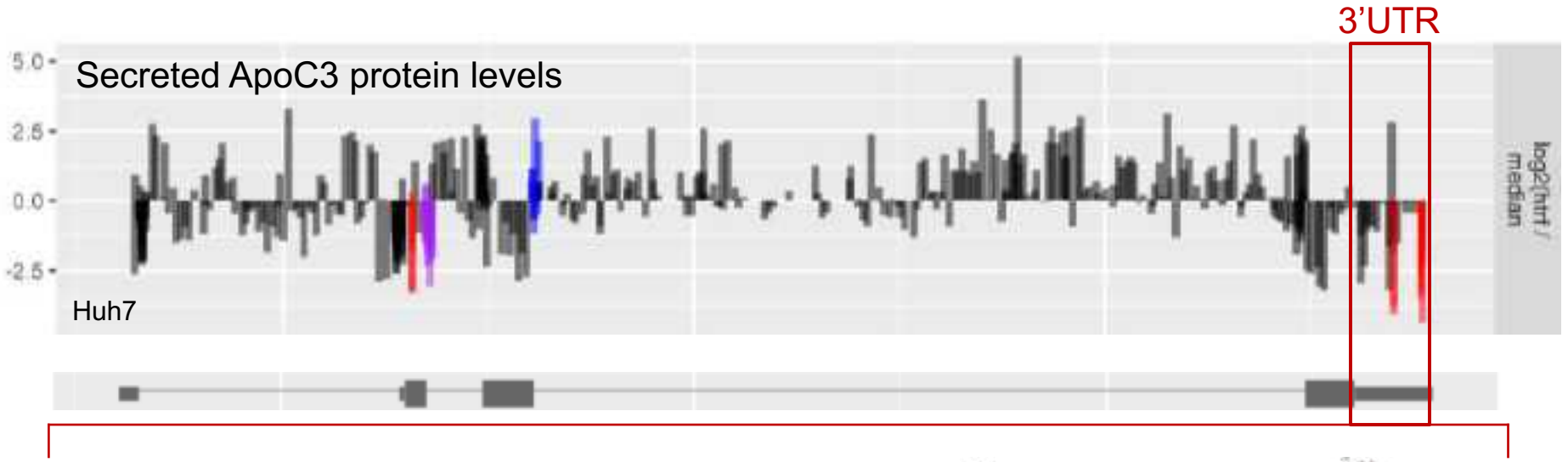
RBPs

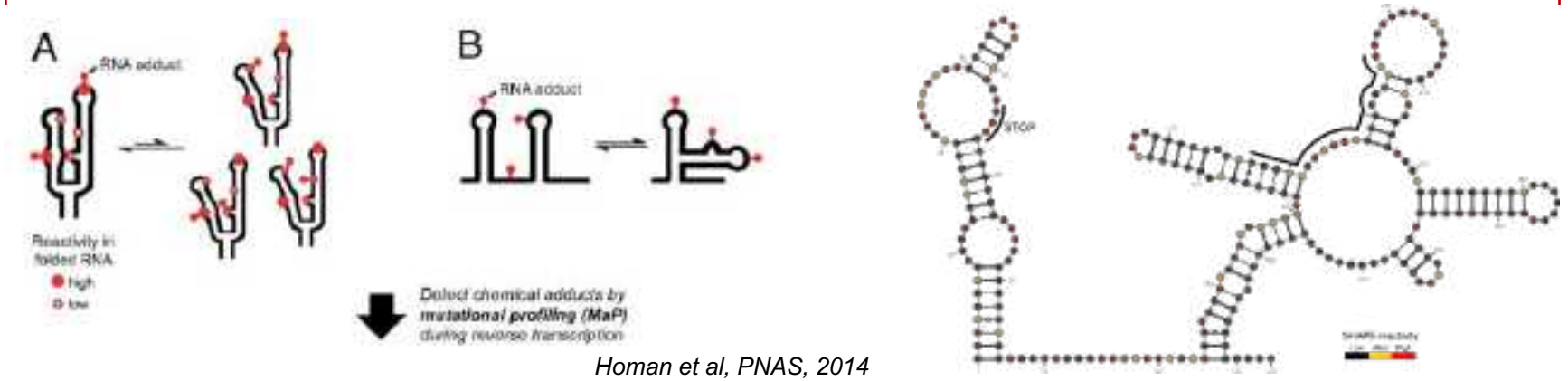
APOC3 protein

APOC3 mRNA

Mapping the structure of 3’ UTR

ASO binding in the 3’UTR reduces APOC3 mRNA and protein levels





Homan et al, PNAS, 2014

SHAPE-MaP generates structural information

SHAPE-MaP informed UTR structure

RNA Drug Discovery