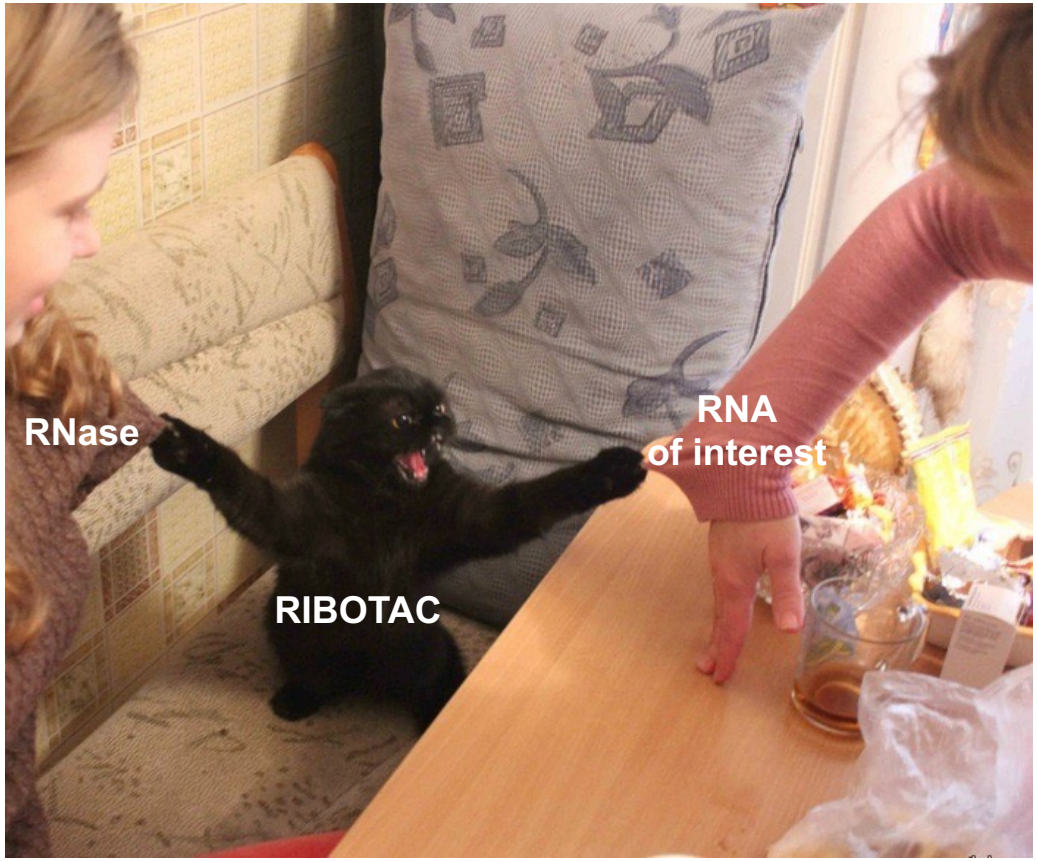
Targeted RNA Degradation by Small Molecule Chimera



25.02.08

Literature Seminar

Junhao Fu

Contents

1. Targeting RNAs for development of a new therapy
2. Main topic

Article

Programming inactive RNA-binding small molecules into bioactive degraders

https://doi.org/10.1038/s41586-023-06091-8

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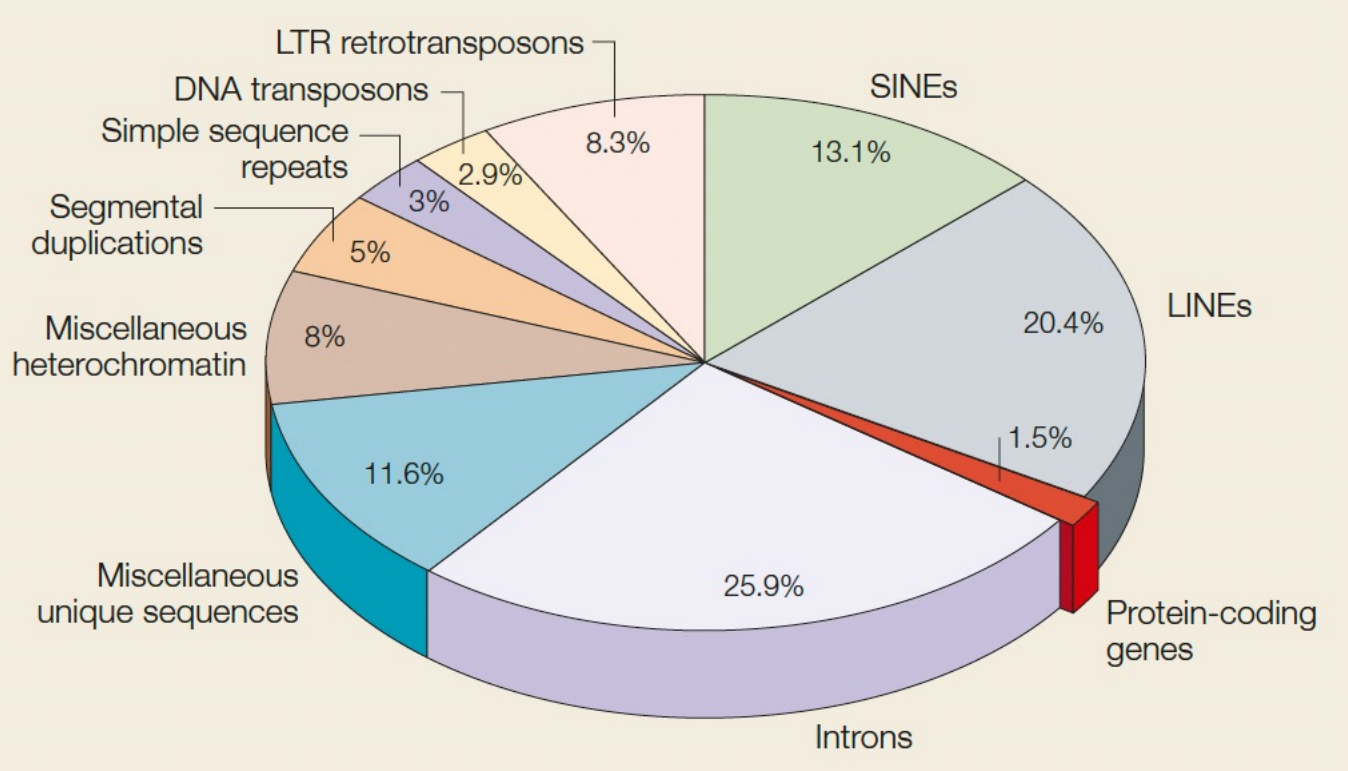
Yuquan Tong[1,9], Yeongju Lee[1,9], Xiaohui Liu[1,9], Jessica L. Childs-Disney[1,9], Blessy M. Suresh[1], Raphael I. Benhamou[1], Chunying Yang[2], Weimin Li[2], Matthew G. Costales[1], Hafeez S. Haniff[1], Sonja Sievers[3,4], Daniel Abegg[1], Tristan Wegner[5], Tiffany O. Paulisch[5], Elizabeth Lekah[1], Maison Grefe[1], Gogce Crynen[6], Montina Van Meter[7], Tenghui Wang[1], Quentin M. R. Gibaut[1], John L. Cleveland[2], Alexander Adibekian[1], Frank Glorius[5]✉, Herbert Waldmann[3,4,8]✉ & Matthew D. Disney[1]✉

Potential of RNAs as Drug Targets

composition of human genome:



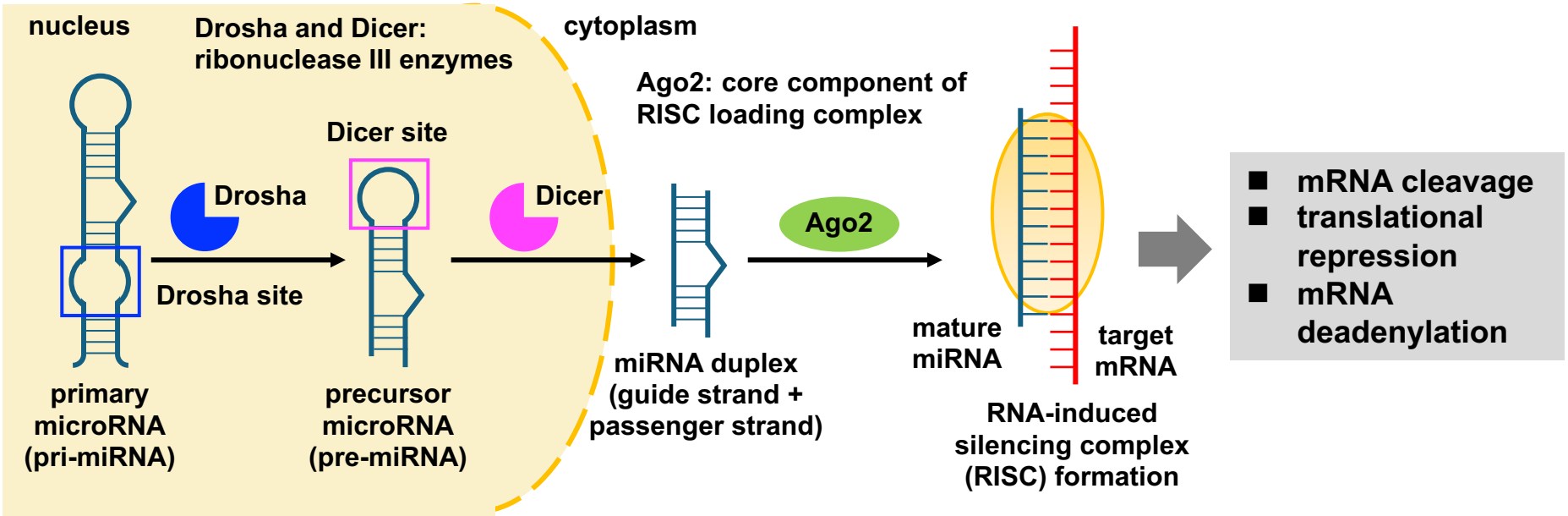
- **only 1.5% of the human genome is protein-coding**
- **non-coding functions such as epigenetic regulation and cellular signaling of RNA are also important**
- **potential of RNAs as new drug targets (>100-fold greater number than protein drug targets)**

[1] Gregory, T. R. *Nat. Rev. Genet.* **2005**, *6*, 699-708.

micro RNAs: Potential Targets for Novel Therapies

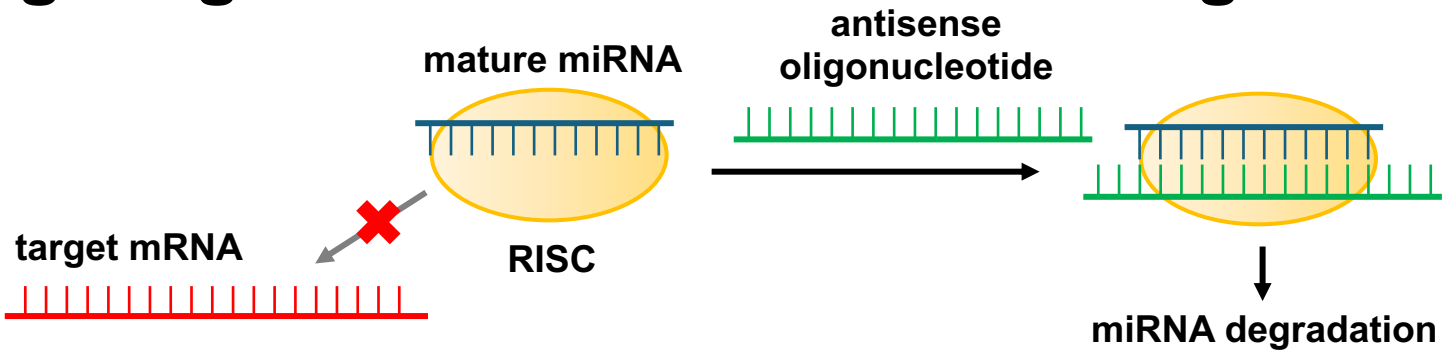
- RNAs
  - messenger RNAs (mRNAs)
  - non-coding RNAs (ncRNAs)
    - ribosomal RNAs (rRNA)
    - transfer RNAs (tRNA)
    - microRNAs (miRNA)
    - others (snRNA, snoRNA, etc.)

- small single-stranded consisting of 20-25 nucleotides
- evolutionarily conserved
- gene expression regulator binding to mainly the 3'-untranslated regions of specific mRNAs
- involves in diseases such as cancer and autoimmune disorders

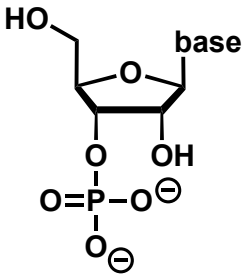


[1] see also: 170513_LS_Kai_Kitamura
[2] Winter, J.; Jung, S.; Keller, S.; Gregory, R. I.; Diederichs, S. *Nat. Cell Biol.* **2009**, *11*, 228-234.

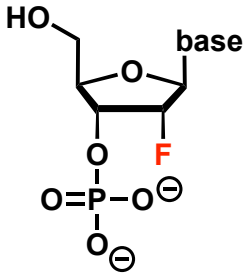
Targeting miRNAs with Antisense Oligonucleotides



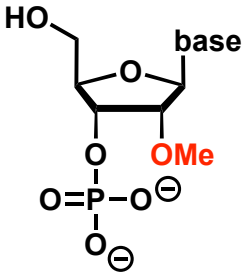
- high complementarity to single strand miRNA through Watson-Crick base pairing
- poor cell permeability and instability to serum nuclease – chemical modification
- best suited for targeting unstructured regions



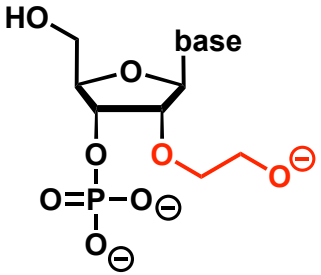
chemical modification



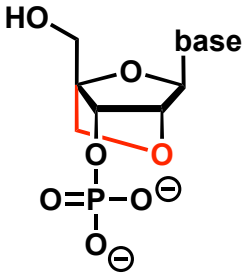
2'-F



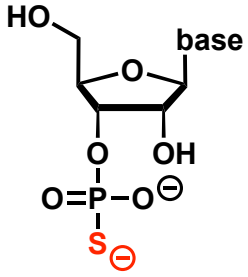
2'-OMe



2'-MOE



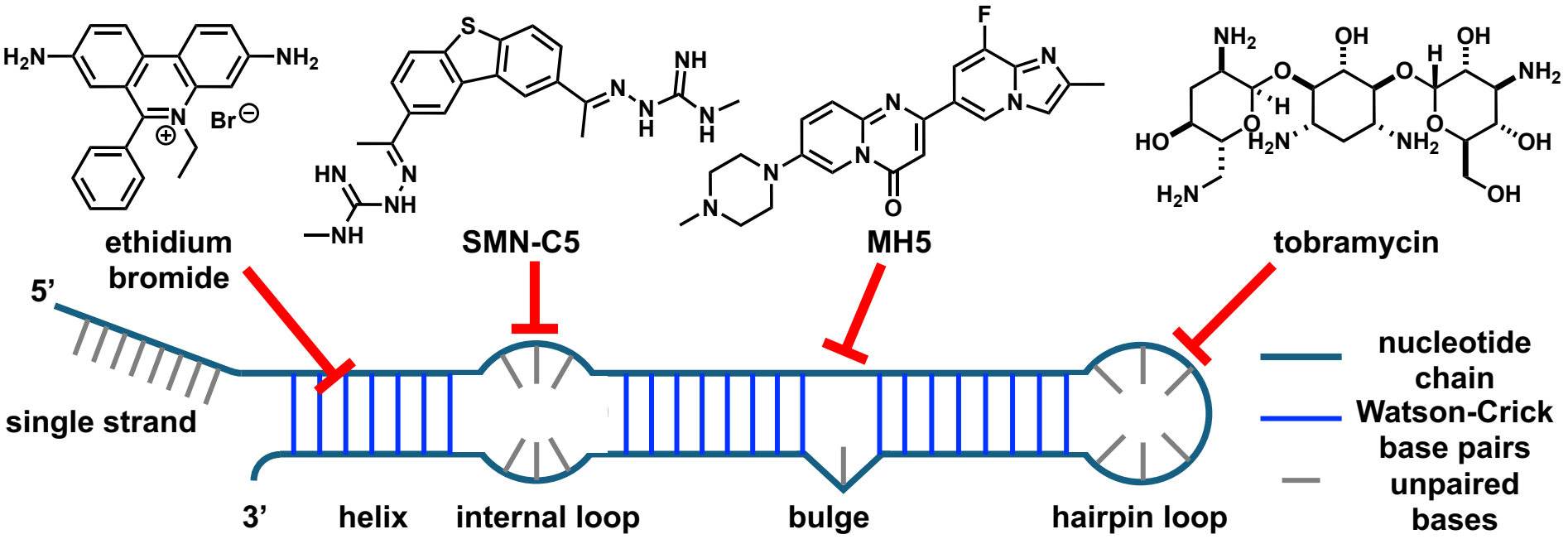
locked nucleic acid (LNA)

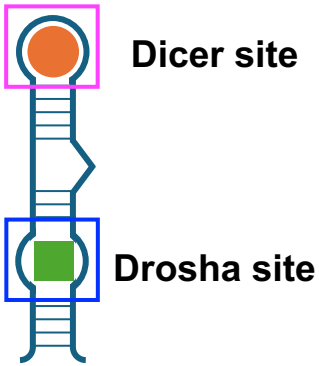


phosphorothioate

[1] Li, Z.; Rana, T. M. *Nat. Rev. Drug Discov.* **2014**, *13*, 622-638.

Targeting miRNAs with Small Molecules





- preference of highly structured regions (appropriate binding pockets)
- mainly through π-stacking and hydrogen bonding (salt bridge) – extended aromatic ring and cationic moiety
- inhibiting the processing and maturation of miRNA by occupying functional sites of pri- and pre-miRNAs (Drosha and Dicer sites)
- binding with non-functional sites may not elicit biological effect

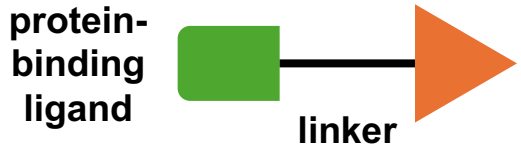
programming inactive RNA-binding small molecules into bioactive degraders

[1] Chen, S.; Mao, Q.; Cheng, H.; Tai, W. *J. Med. Chem.* **2024**, *67*, 16002–16017.

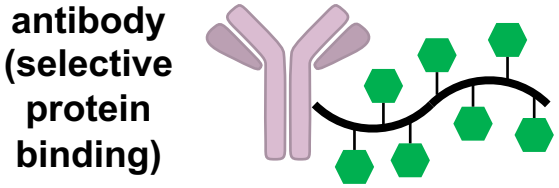
Targeted RNA Degradation Strategy

- targeted protein degradation: PROTAC,[1] molecular glue, LYTAC[2]

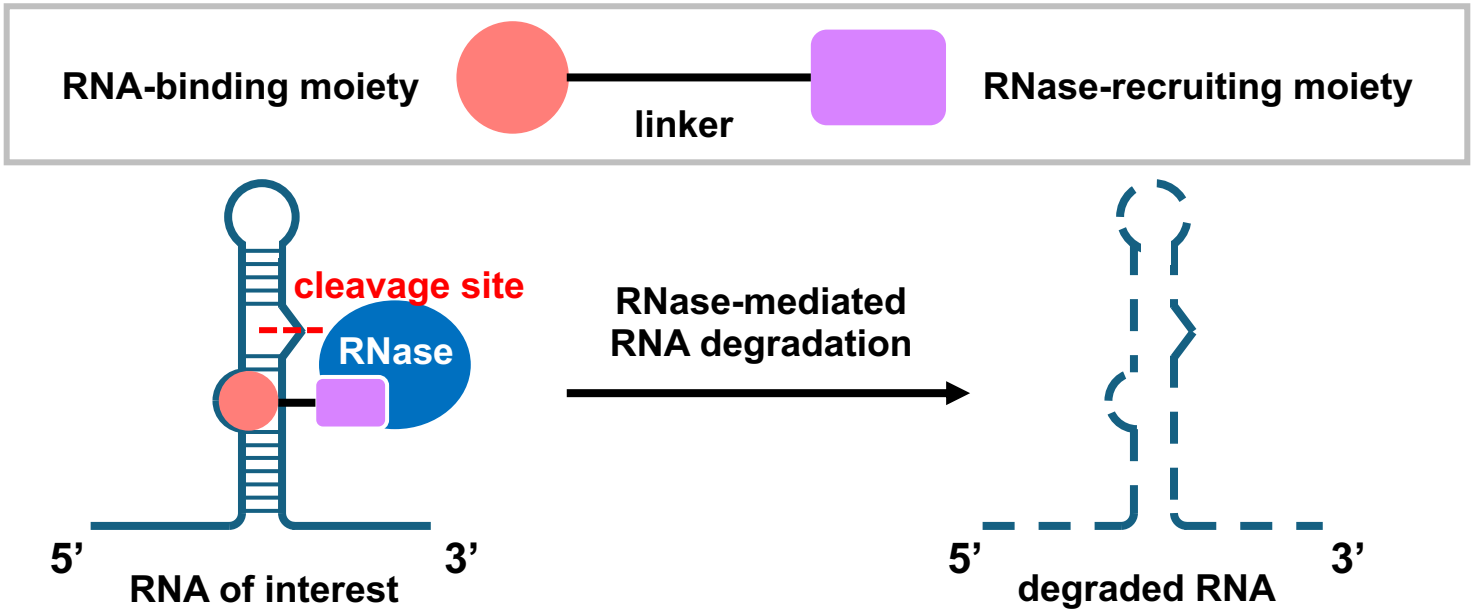
proteolysis-targeting chimera (PROTAC):



lysosome-targeting chimera (LYTAC):



- What about targeted RNA degradation? — ribonuclease-targeting chimera (RIBOTAC)[3]



- selective binding of RNAs with intricate secondary and tertiary structures
- selective degradation of RNAs of interest

[1] Zhao, L.; Zhao, J.; Zhong, K.; Tong, A.; Jia, D. *Signal Transduct. Target. Ther.* **2022**, *7*, 113.
[2] Banik, S. M.; Pedram, K.; Wisnovsky, S.; Ahn, G.; Riley, N. M.; Bertozzi, C. R. *Nature* **2020**, *584*, 291–297.
[3] Costales, M. G.; Matsumoto, Y.; Velagapudi, S. P.; Disney, M. D. *J. Am. Chem. Soc.* **2018**, *140*, 6741–6744.

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1997: B.S., chemistry
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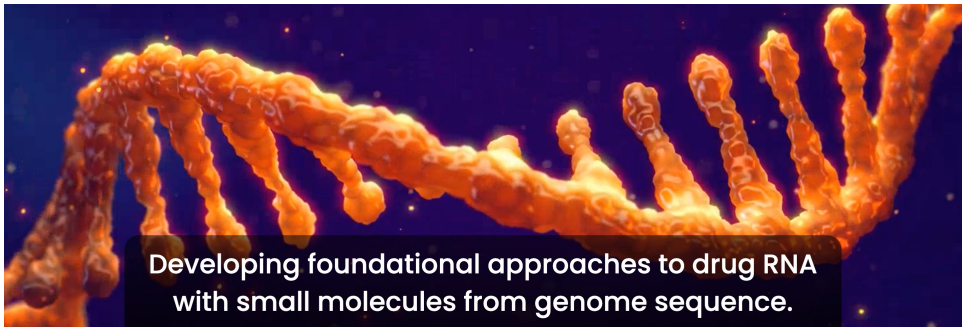
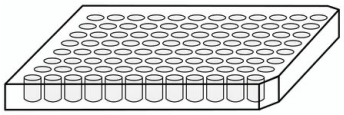
1999: M.S., chemistry
@University of Rochester
2003: Ph.D., biophysical chemistry
@University of Rochester (Prof. Edwin L. Turner)
2002-2005: postdoctoral fellow
@ETH (Prof. Peter H. Seeberger)
2005-2010: Assistant Professor
@University at Buffalo, New York
2010-present: Professor, Department of Chemistry
@the Scripps Research Institute

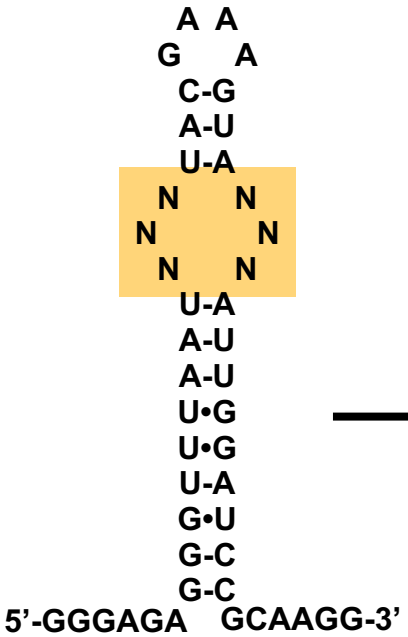
research field: RNA disease-related medicinal chemistry
research topics:
1. development of small molecules targeting disease-related non-coding RNAs
2. development of small molecules targeting mRNAs encoding 'undruggable' proteins



Flow of this Research

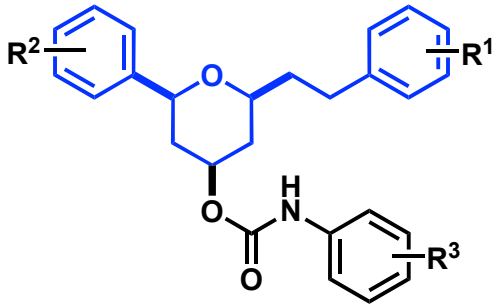


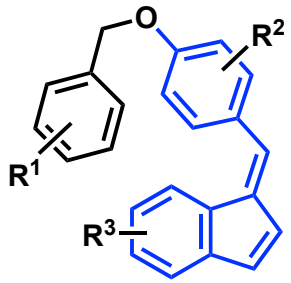
natural product-inspired small molecule library (*n* = 15,000)

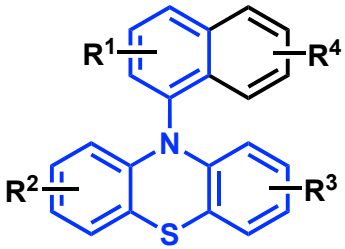


3D-fold RNA library (*n* = 4,096)

library-versus-library screening







…

RNA-binding scaffolds

biologically inert interactions

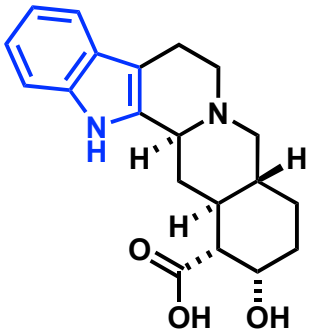
design and application of RIBOTACs

miRNA-RIBOTAC (pre-miR-155)

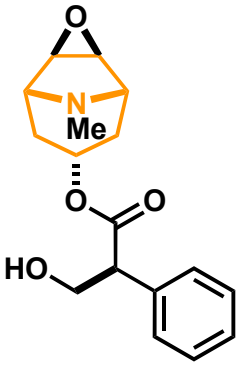
mRNA-RIBOTAC (*MYC*)

selective RNA degraders

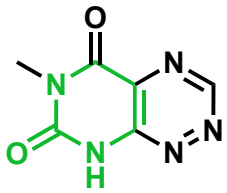
Small Molecule Library Inspired by Natural Products



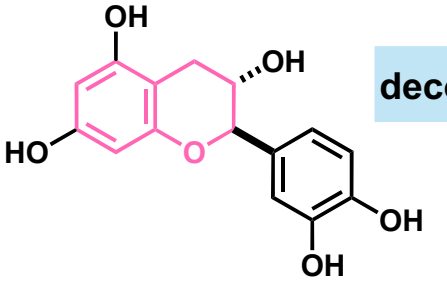
yohimbinic acid



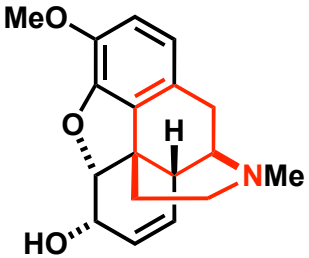
scopolamine



reumycin



catechin



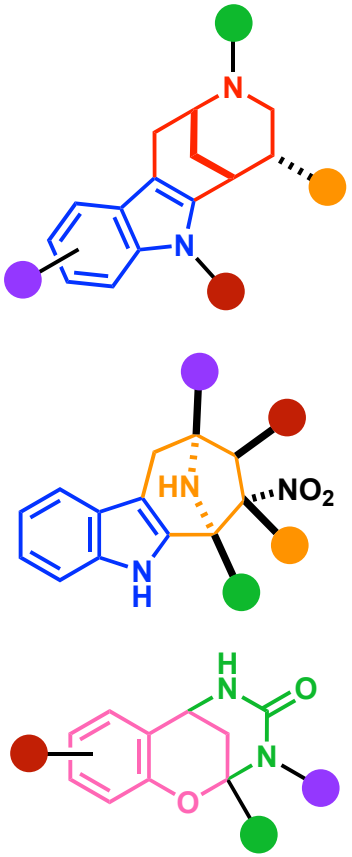
codeine

natural products

deconstruction

natural product fragments

recombination



natural product-inspired small molecule library (*n* = 15,000)

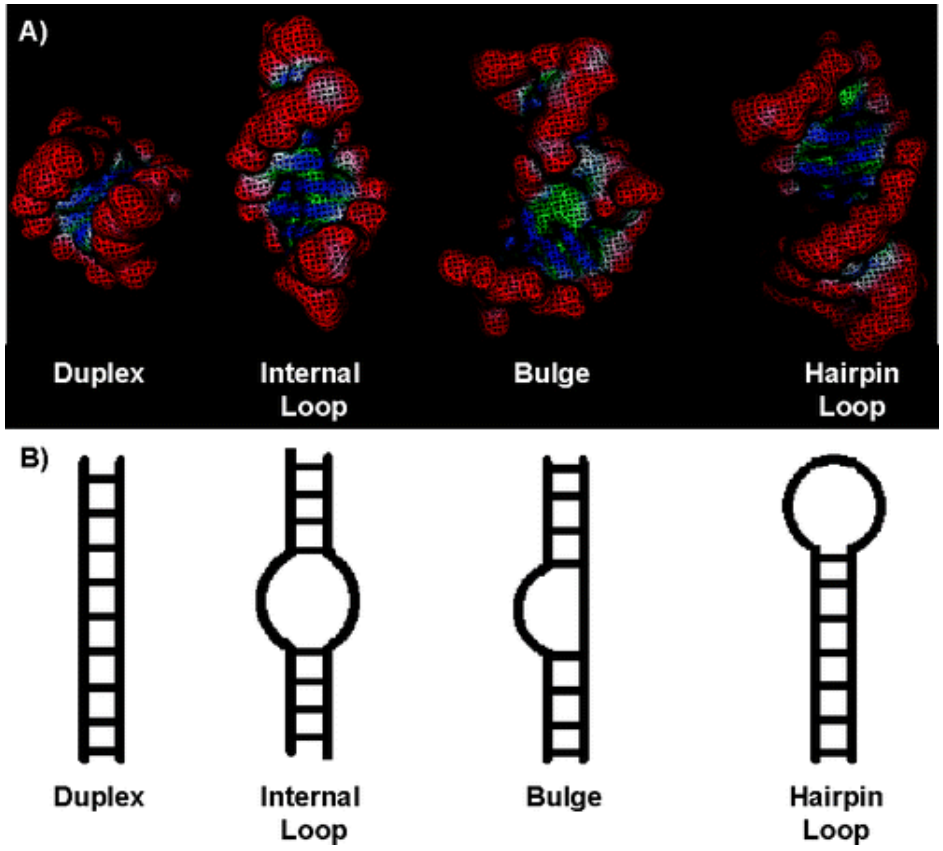
- reservoir of biologically active molecules
- evolutionary constraints: conserved scaffolds

- structural diversity
- expansion of biological activity

[1] Grigalunas, M.; Brakmann, S.; Waldmann, H. *J. Am. Chem. Soc.* **2022**, *144*, 3314-3329.

3D-Fold RNA Library

secondary structures of RNA:



N = A, C, U, or G
library capability:
4^6 = 4,096

A A
G A
C-G
A-U
U-A
N N
N N
N N
U-A
A-U
A-U
U•G
U•G
U-A
G•U
G-C
G-C
5'-GGGAGA GCAAGG-3'

U-A
A C
C C
C U
5'…U-A…3'
3 × 3
internal loop

U-A
A C
C C
A-U
5'…U-A…3'
2 × 2
internal loop

U-A
A-U
C C
A-U
5'…U-A…3'
1 × 1
internal loop

U-A
A-U
C-G
A-U
5'…U-A…3'
fully paired

- **RNA helix: deep and narrow major grooves, shallow minor grooves[1]**
- **internal loops, bulges, and hairpin loops[1] – possible binding sites, highly abundant in cellular RNA**

[1] Thomas, J. R.; Hergenrother, P. J. *Chem. Rev.* **2008**, *108*, 1171–1224.

Screening of RNA-Small Molecule Interaction

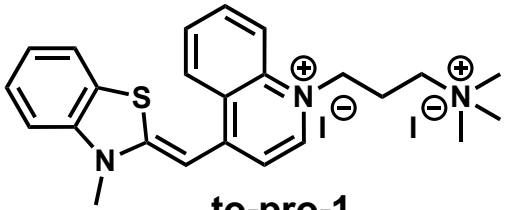
3D-fold RNA library (*n* = 4,096)

pseudo-natural product library (*n* = 15,000)

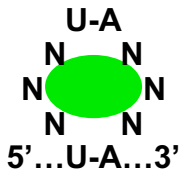
screening of 61,440,000 interactions

344 hits

to-pro-1 replacement assay:

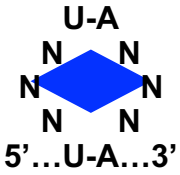


to-pro-1
nucleic acid dye



to-pro-1 bound with RNA

ligand displacement

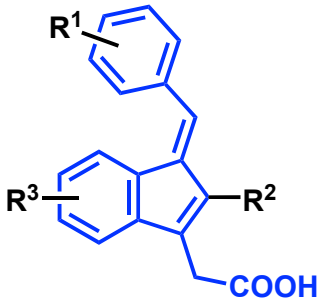


+

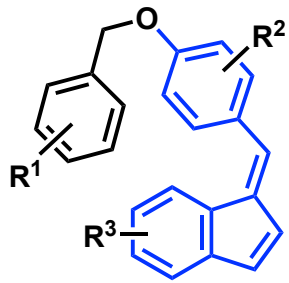
free to-pro-1

reduced fluorescence

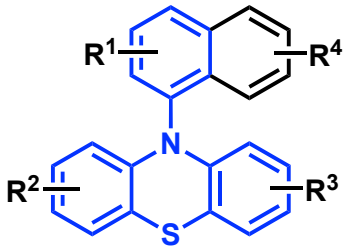
newly discovered RNA-binding scaffolds



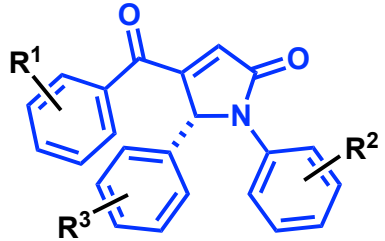
class II, 31 hits (9.0%)



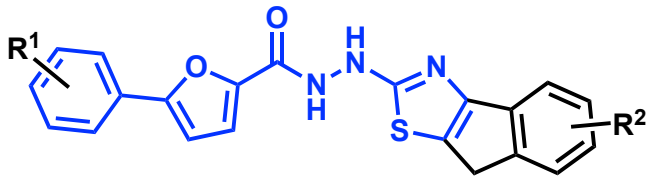
class IV, 15 hits (4.4%)



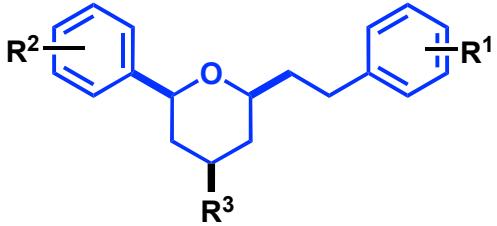
class V, 12 hits (3.5%)



class VI, 10 hits (2.9%)

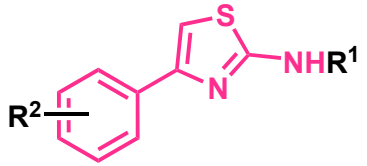


class VII, 10 hits (2.9%)



class VIII, 10 hits (2.9%)

known RNA-binding scaffolds

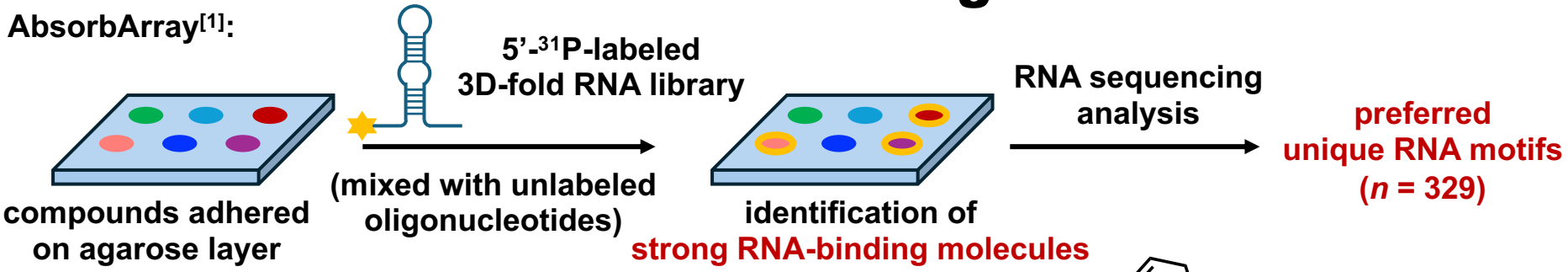


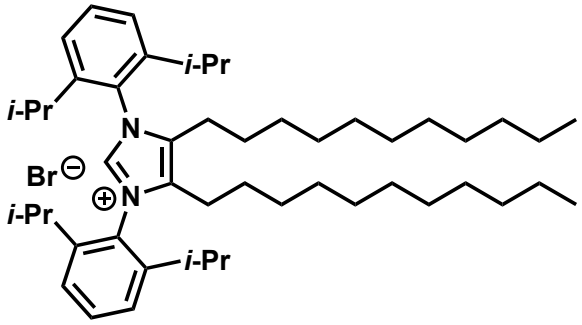
class I, 77 hits (22.4%)

other classes

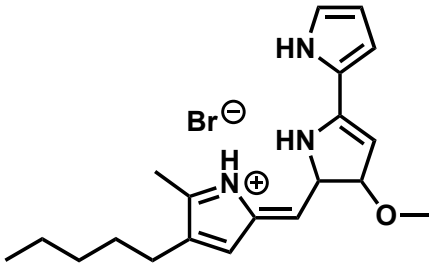
[1] Tran, T.; Disney, M. D. *Nat. commun.* **2012**, *3*, 1125.

Preferred Motifs of RNA-Binding Small Molecules

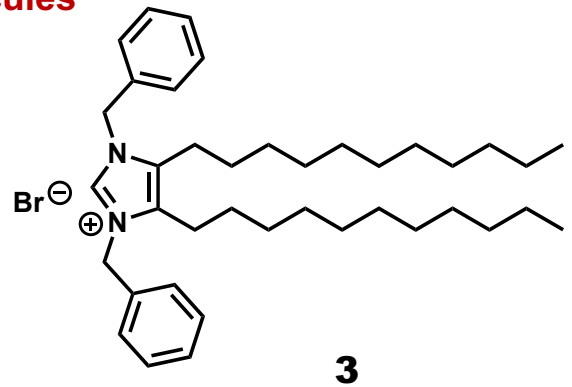




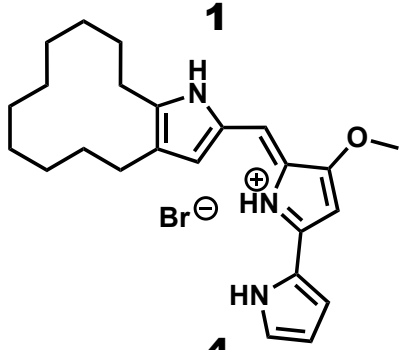
1



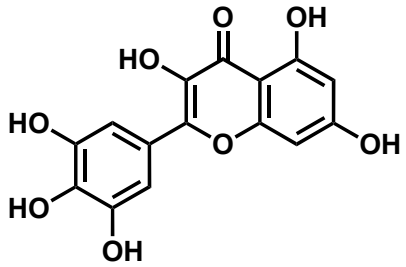
2



3



4



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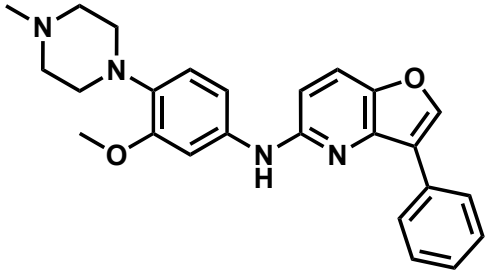


6

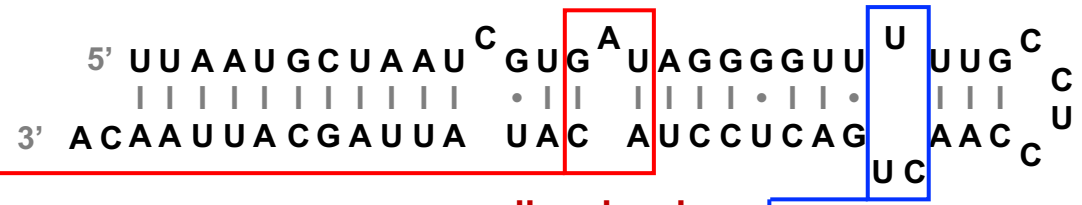
- majorly azolium salts (**1**, **3**, and **4**)
- preference towards 3 × 3 and 2 × 2 internal loops (>90%)
- 13 unique motifs presented in 6% of human miRNAs – possible targets of **1**-**6**
- less than 30% present in Drosha or Dicer sites – mostly biologically inactive interactions (70%)

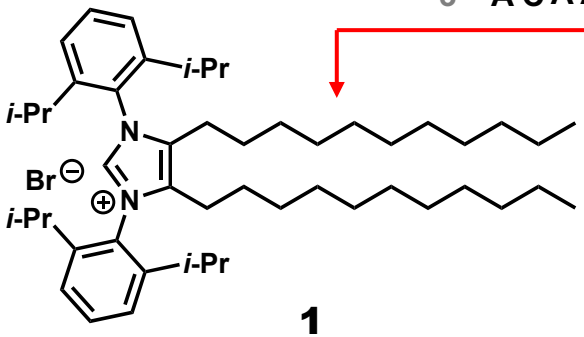
[1] Velagapudi, S. P.; Costales, M. G.; Vummidi, B. R.; Nakai, Y.; Angelbello, A. J.; Tran, T.; Haniff, H. S.; Matsumoto, Y.; Wang, Z. F.; Chatterjee, A. K.; Childs-Disney, J. L.; Disney, M. D. *Cell Chem. Biol.* **2018**, *25*, 1086–1094.

Targeted miRNA Degradation – miR-155

- target gene expression regulator[1]
- related with cell proliferation, apoptosis, cancer cell migration, and inflammation, etc.

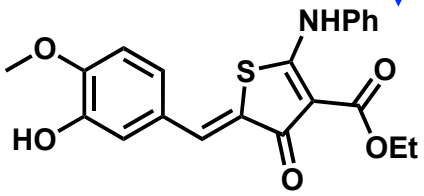
sequence of pre-miR-155:





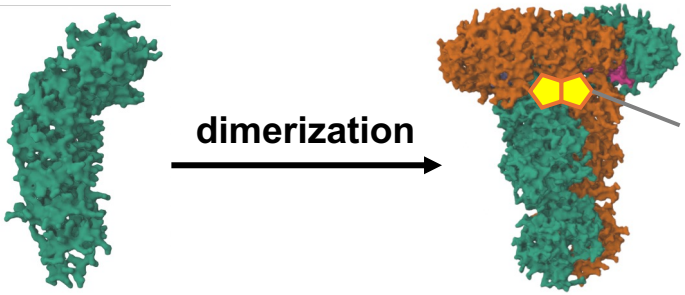
1

k_D = 1.1 μM against pre-miR-155



known RNase L recruiter[2]

endoribonuclease RNase L[3]:



RNase L monomer
inactive
(PDB: 1WDY)

RNase L crossed homodimer
active
(PDB: 4OAV)

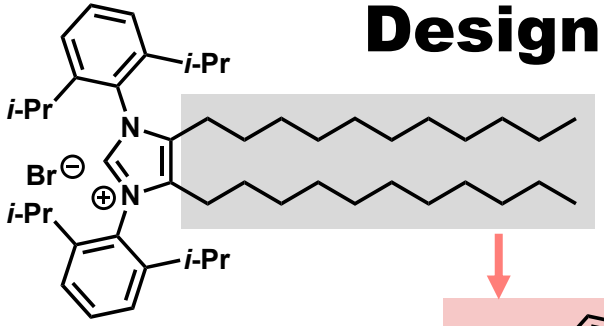
2′,5″-linked oligoadenylates (2-5 A) or RNase-activating small molecules

preferential cleavage sites:

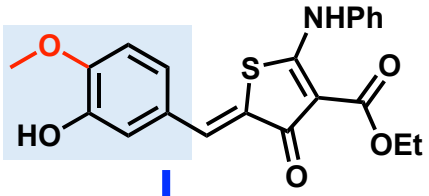
UNN patterns with unpaired Us

[1] Faraoni, I.; Antonetti, F. R.; Cardone, J.; Bonmassar, E. *Biochim. Biophys. Acta, Mol. Basis Dis.* **2009**, *1792*, 497–505.
[2] Costales, M. G.; Aikawa, H.; Li, Y.; Childs-Disney, J. L.; Abegg, D.; Hoch, D. G.; Pradeep Velagapudi, S.; Nakai, Y.; Khan, T.; Wang, K. W.; Yildirim, I.; Adibekian, A.; Wang, E. T.; Disney, M. D. *Proc. Natl. Acad. Sci. USA* **2020**, *117*, 2406–2411.
[3] Han, Y.; Donovan, J.; Rath, S.; Whitney, G.; Chitrakar, A.; Korennykh, A. *Science* **2014**, *343*, 1244–1248.

Design of pre-miR-155-RIBOTAC

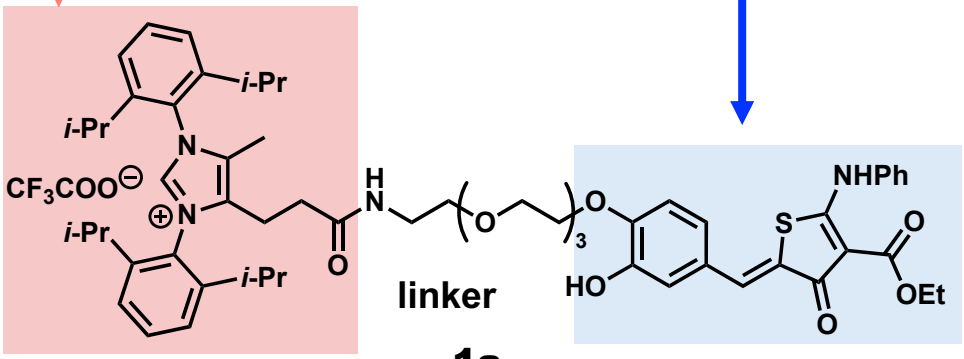


n-dodecyl chains unimportant for binding affinity



***p*-substitution of methoxy important for RNase L-recruiting ability**

pre-miR-155 binding moiety

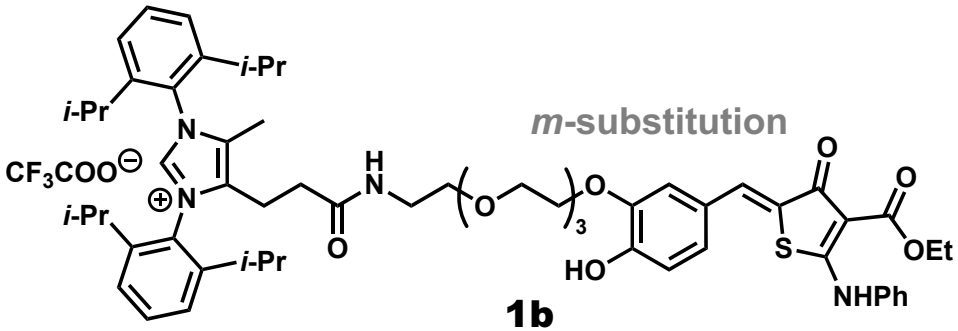


linker

RNase L recruiting moiety

1a

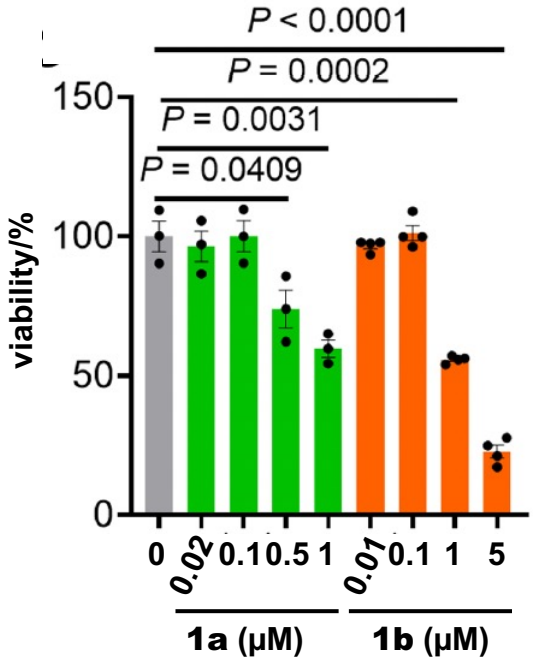
K_D = 2.2 μM against pre-miR-155



m-substitution

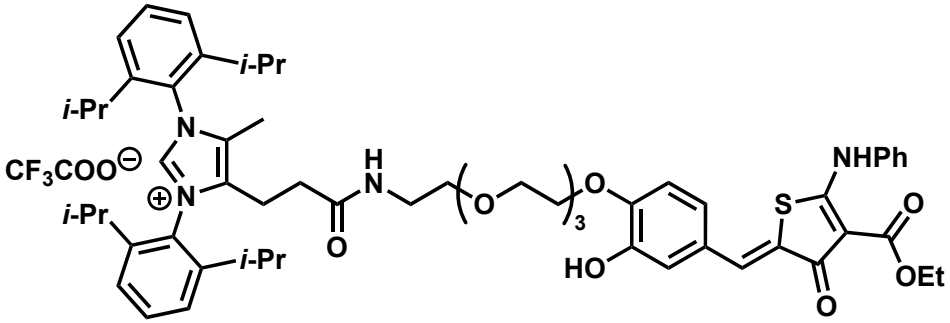
1b

regioisomer with lower RNase L recruiting activity

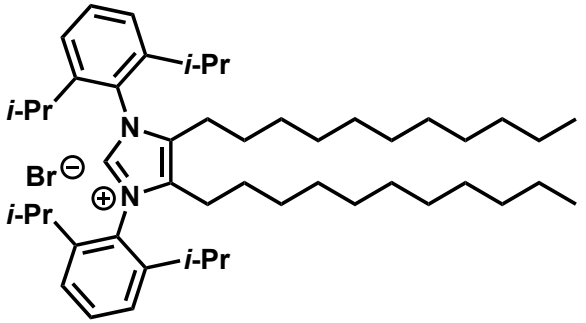


- retained binding affinity towards pre-miR-155
- low toxicity towards breast cancer MDA-MB-231 cells

Dose-Dependent Degradation of pre-miR-155

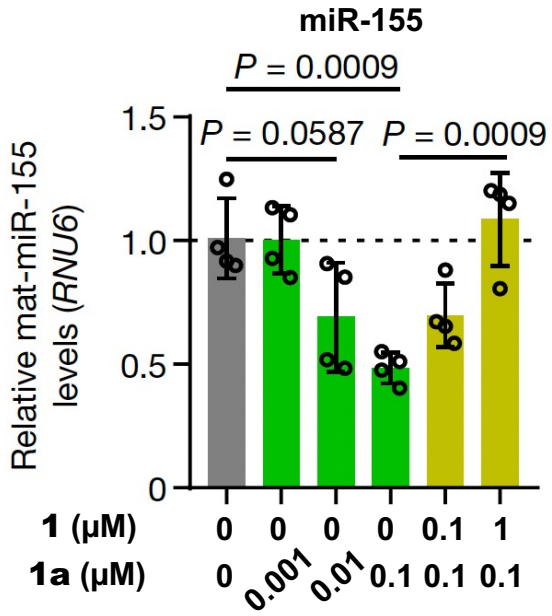


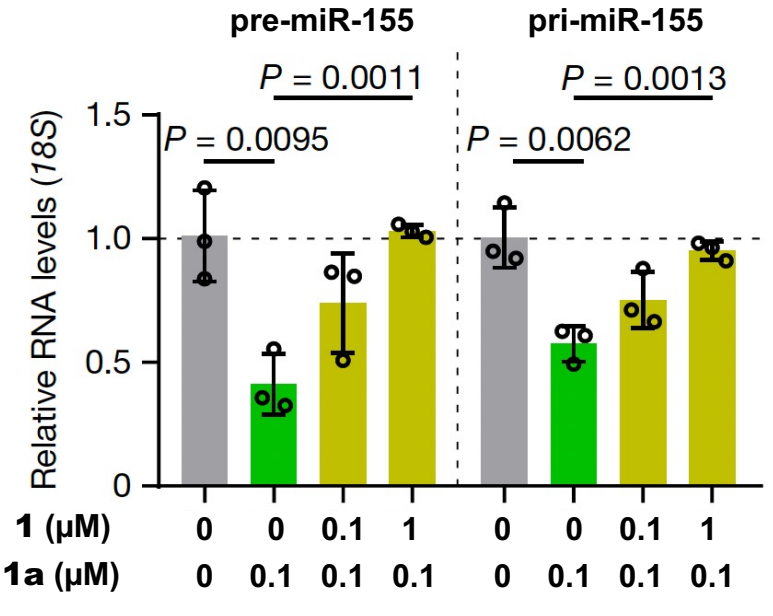
1a



1

- **RNA sequencing in MDA-MB-231 cells:**

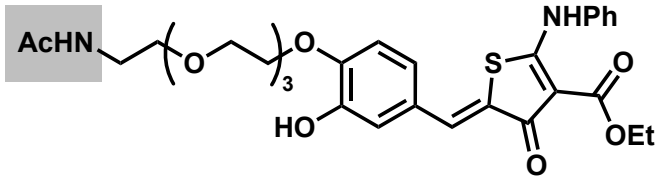




*RNA level was measured using quantitative PCR with reverse transcription (RT-qPCR).

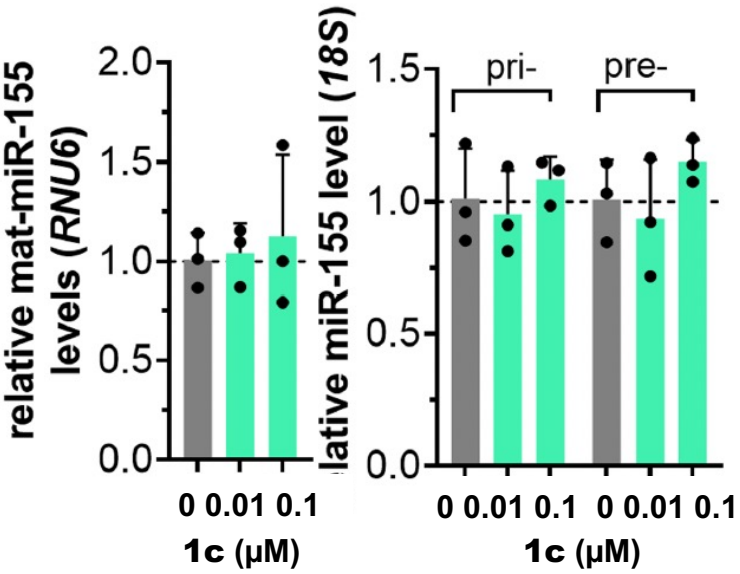
- **dose-dependent degradation of three species of miR-155 by treatment of 1a: inert interaction into degrading activity**
- **degradation of RNAs rescued by competition by 1: retained binding site**

Pre-miR-155 Binding-Dependency of the Degradation

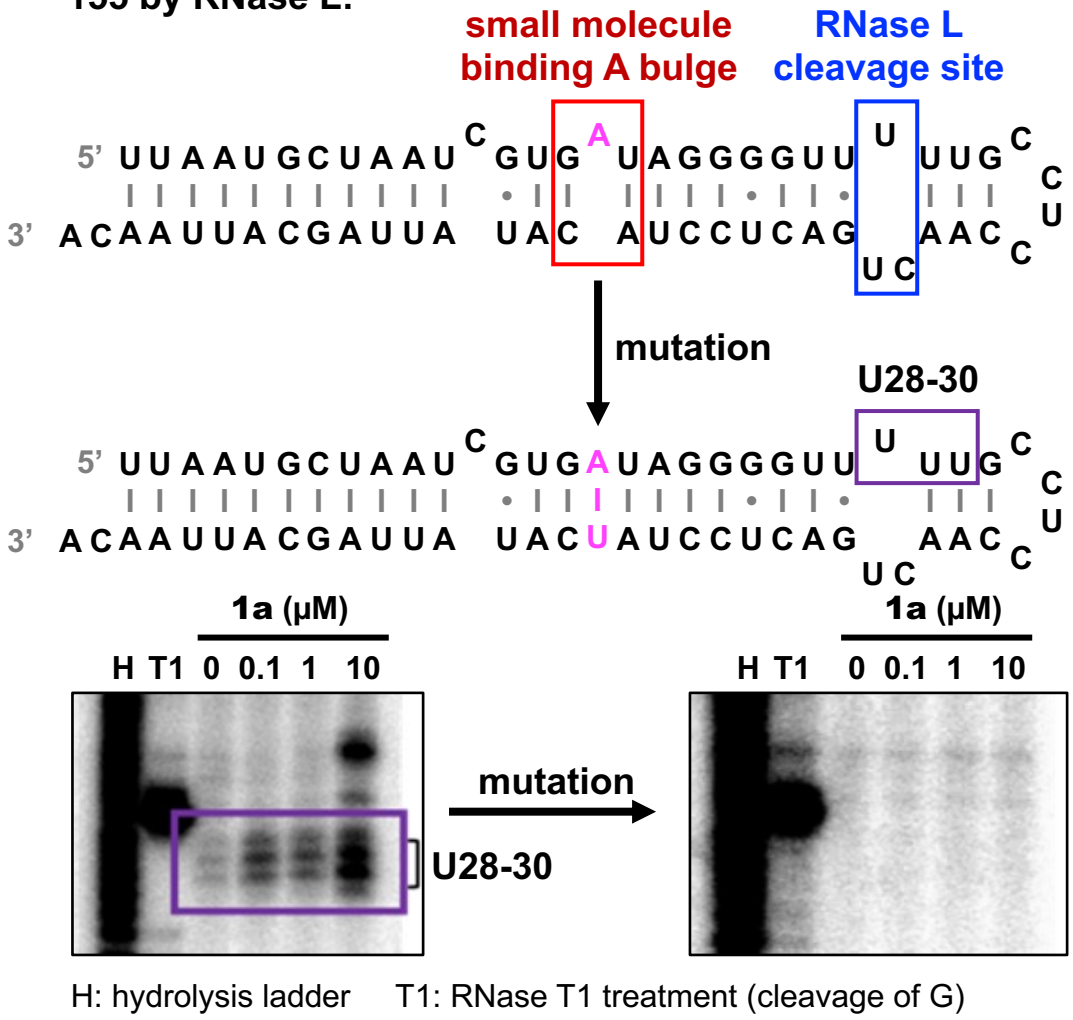


1c
non-pre-miR-155 binder

- **RNA sequencing in MDA-MB-231 cells:**



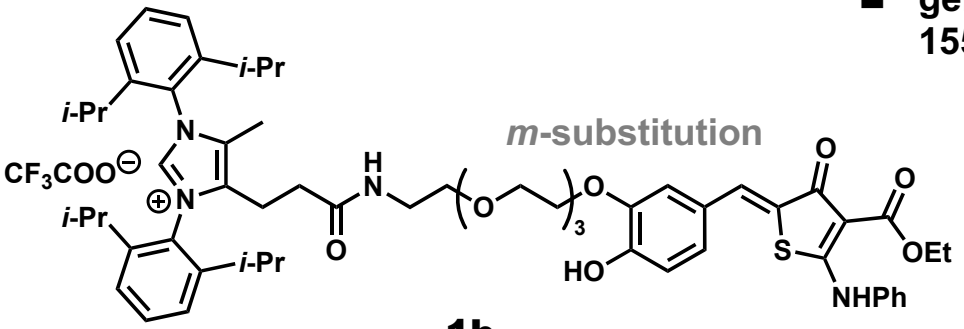
- **gel autoradiogram of the cleavage of 5'-[^{32}P]-pre-miR-155 by RNase L:**



H: hydrolysis ladder T1: RNase T1 treatment (cleavage of G)

- **pre-miR-155 binding moiety** of RIBOTAC required for **degradation of pre-miR-155**
- **binding site A bulge** of RNA required for **degradation of pre-miR-155**
- degradation of pre-miR-155 is **pre-miR-155 binding-dependent**

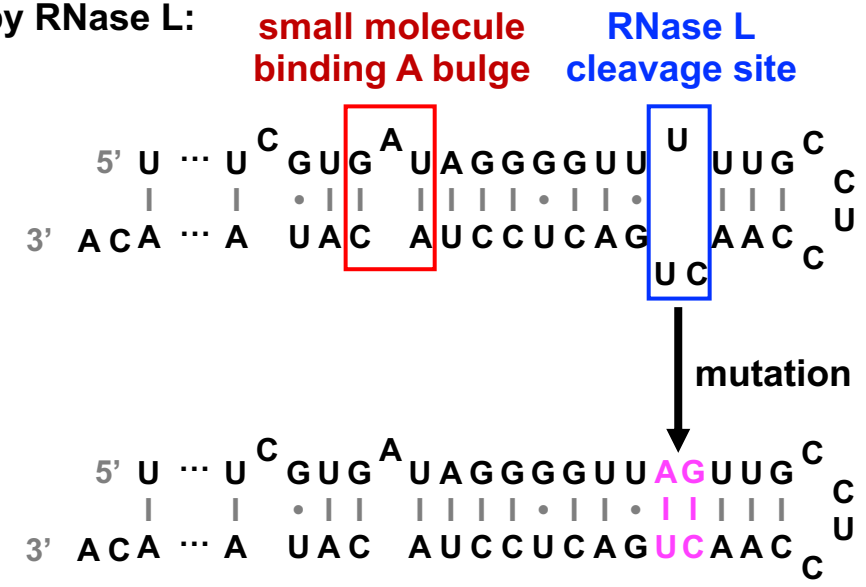
RNase L-Dependency of the Degradation



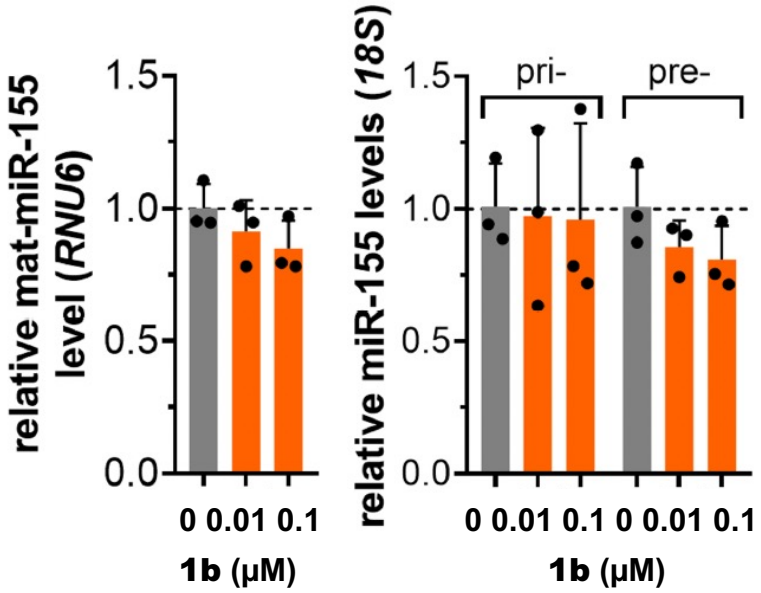
1b

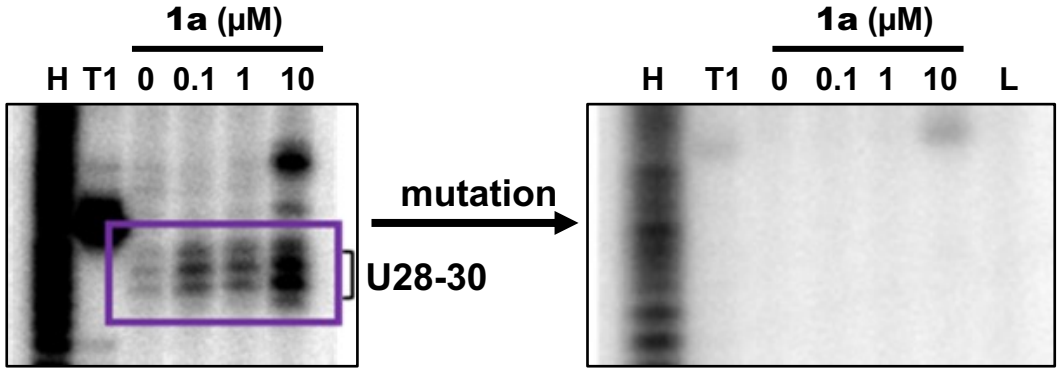
low RNase L recruiting activity

- gel autoradiogram of the cleavage of 5'-[^{32}P]-pre-miR-155 by RNase L:



- RNA sequencing in MDA-MB-231 cells:



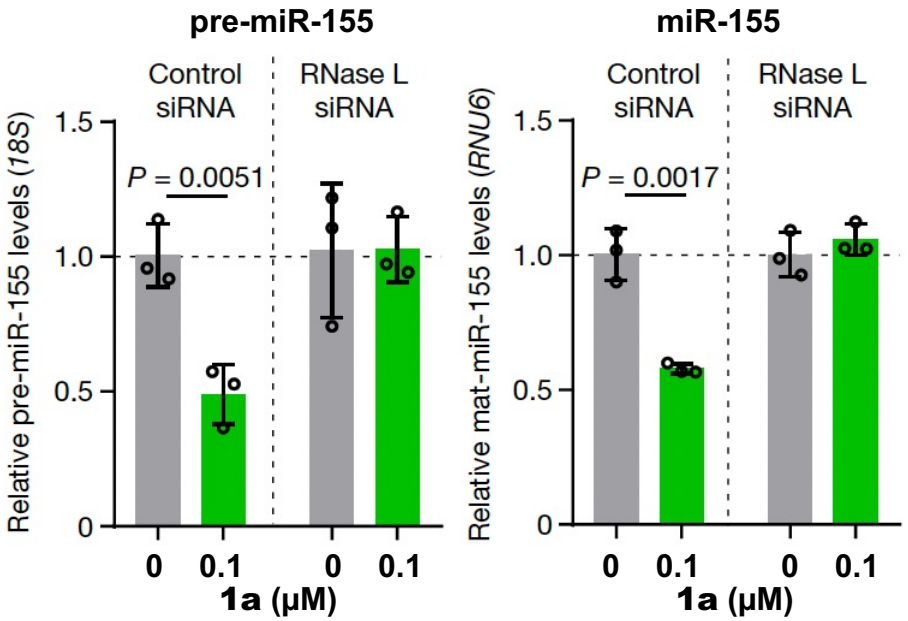


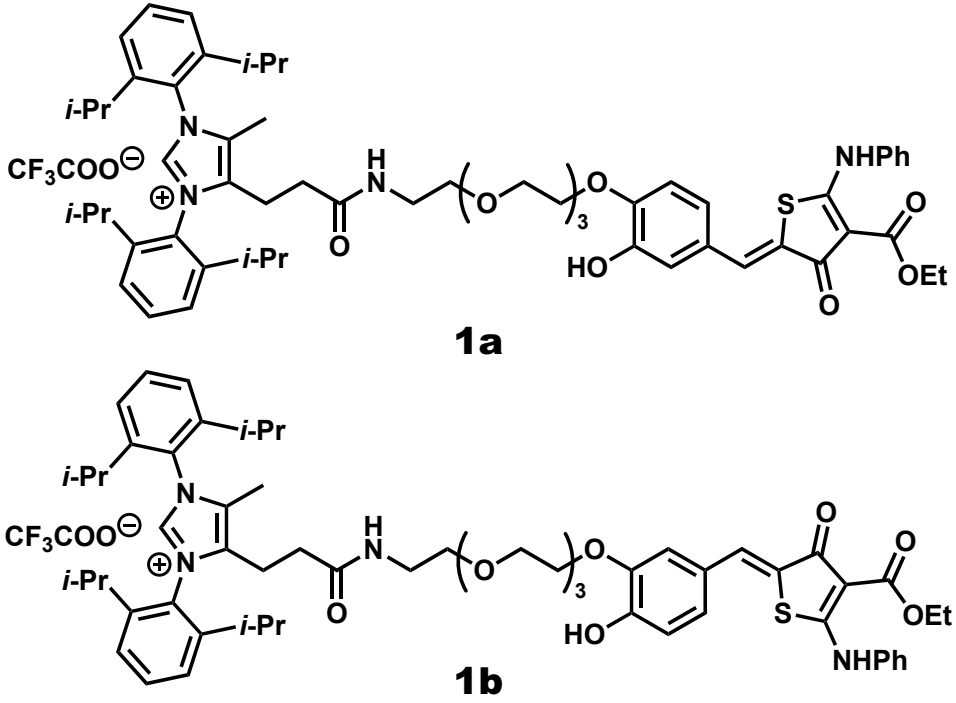
H: hydrolysis ladder T1: RNase T1 treatment (cleavage of G)
L: RNase L treatment

- RNase L recruiting moiety of RIBOTAC required for degradation of pre-miR-155
- RNase L cleavage site U bulge of RNA required for degradation of pre-miR-155
- degradation of pre-miR-155 is RNase L-dependent

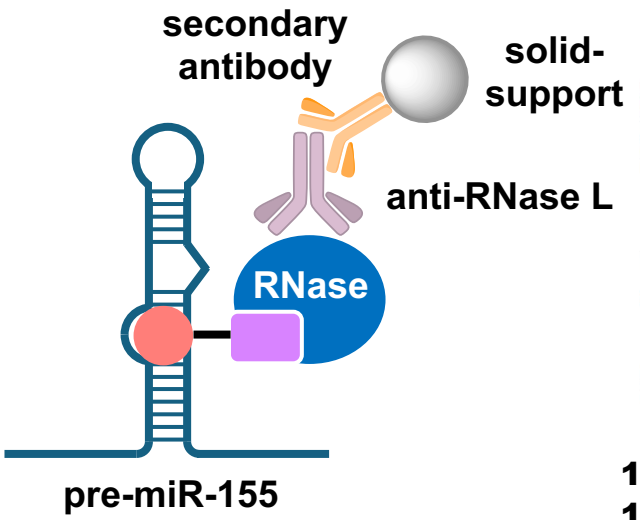
Mode of Action of Pre-miR-155-RIBOTAC

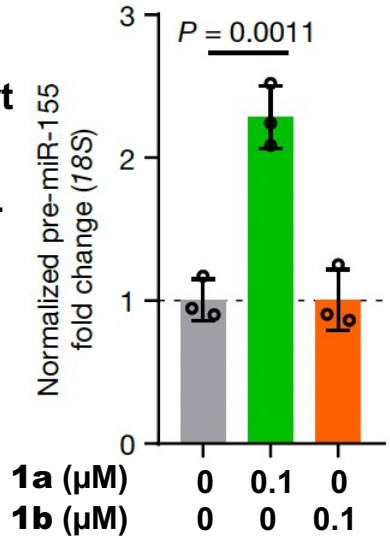
- knockdown of RNase L:





- immunoprecipitation of pre-miR-155:



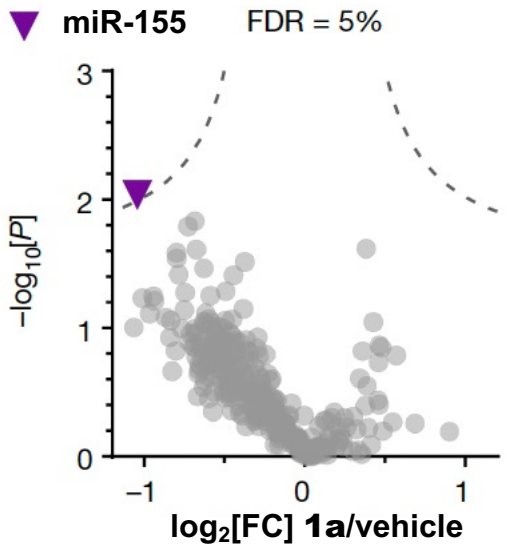


- knockdown of RNase L ablated degradation of miR-155 by **1a**
- formation of pre-miR-155-**1a**-RNase L complex

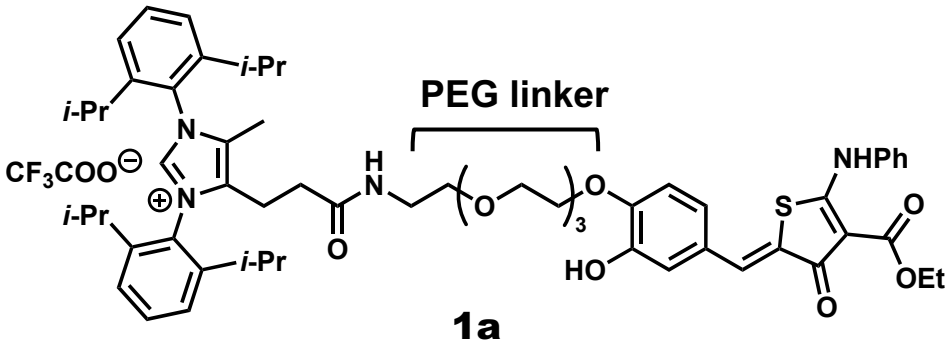
direct engagement of pre-miR-155 and RNase L in the 1a-induced RNA degradation

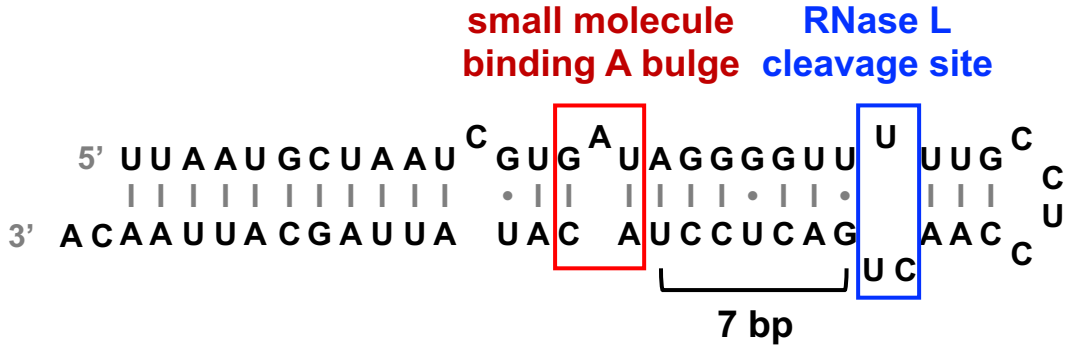
miRNA Selectivity of Pre-miR-155-RIBOTAC

- RT-qPCR profiling of 373 miRNAs in MDA-MB-231 cell line:



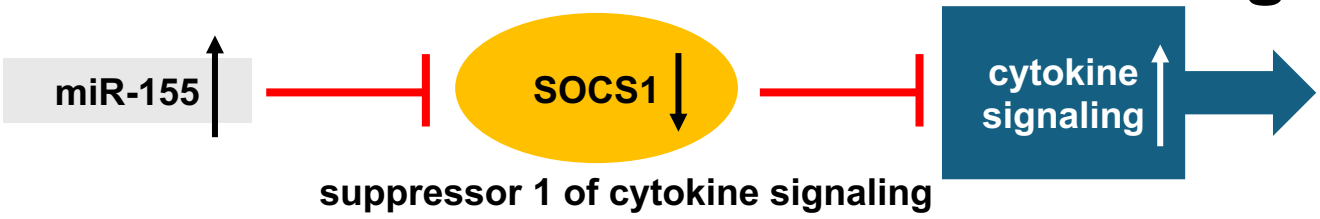
- selective attenuation of miR-155 level over other miRNAs
- no effect on miR-18a, miR-101-1, and miR-1226 with the same A bulges – miR-155 sequence selective





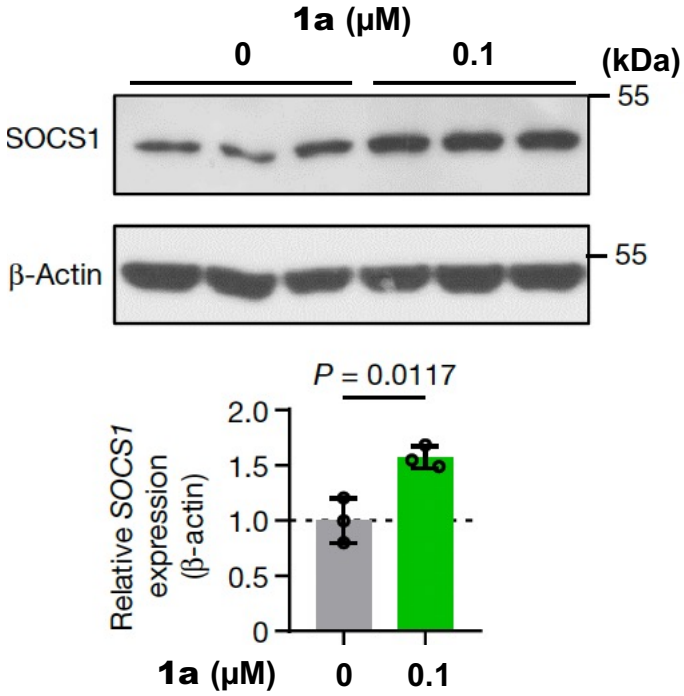
- unique distance (7 bp) between binding and cleavage sites may account for the sequence selectivity
- selectivity could be programmed by modulating the linker length of the RIBOTAC

Effect of RIBOTAC on Pre-miR-155 Target Protein

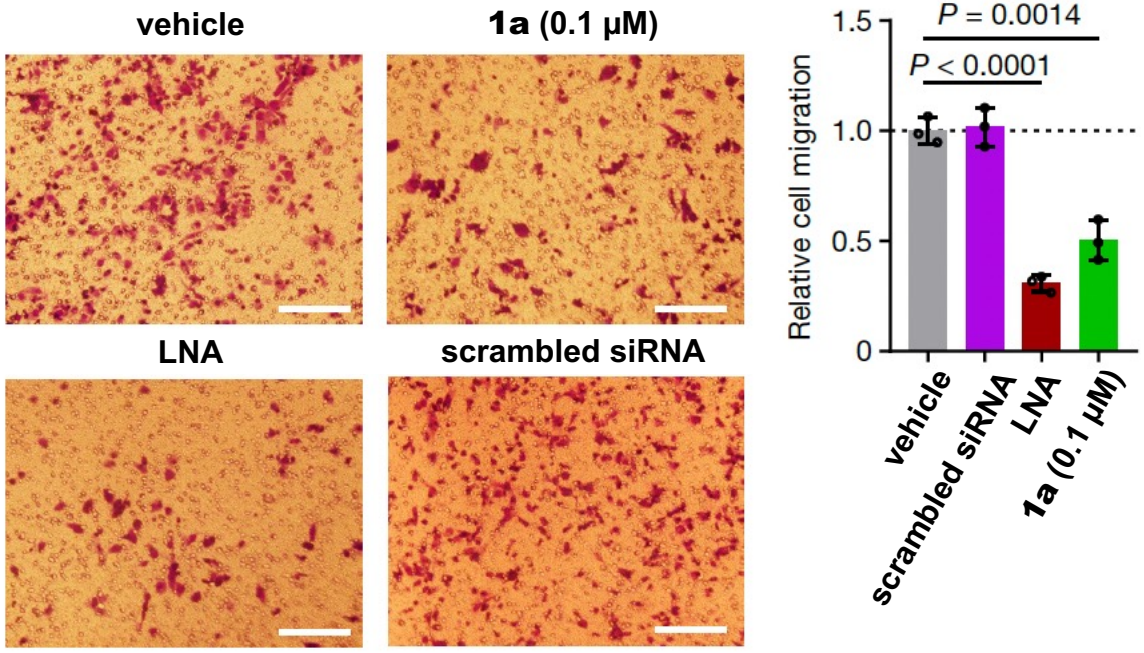


upregulated miR-155 caused downregulation of SOCS1, contributing to cancer cell migration

- SOCS1 expression analysis in MDA-MB-231 cells:



- migration assay in MDA-MB-231 cells :



LNA: locked nucleic acid which selectively targets miR-155[1]

- upregulation of SOCS1 by **1a**-induced pre-miR-155 degradation
- inhibition of cell migration by **1a**-induced pre-miR-155 degradation

→ potential cancer cell migration inhibitor

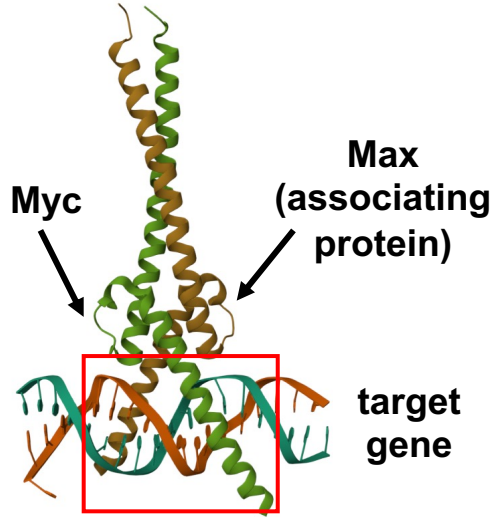
[1] Zhang, Y.; Roccaro, A. M.; Rombaoa, C.; Flores, L.; Obad, S.; Fernandes, S. M.; Sacco, A.; Liu, Y.; Ngo, H.; Quang, P.; Azab, A. K.; Azab, F.; Maiso, P.; Reagan, M.; Brown, J. R.; Thai, T.-H.; Kauppinen, S.; Ghobrial, I. M. *Blood* **2012**, *120*, 1678–1686.

Targeted mRNA Degradation – *MYC*

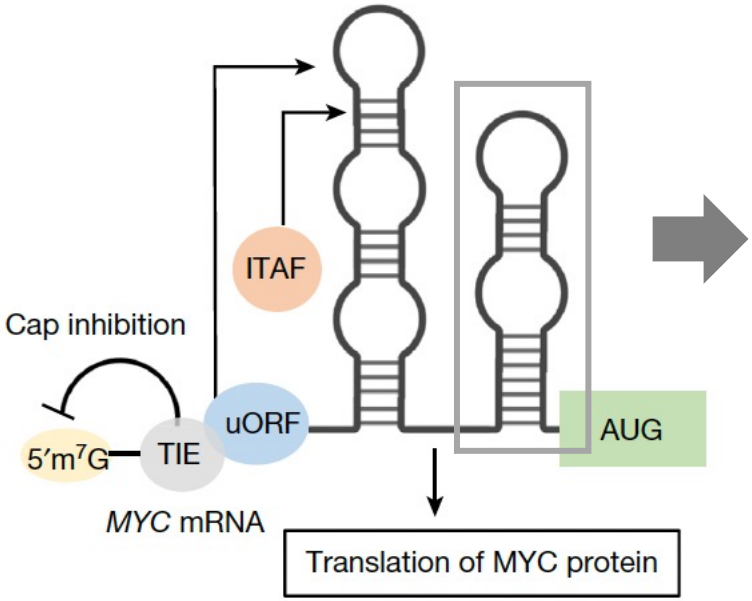
proto-oncogene *MYC* (*c-Myc*) [1]:

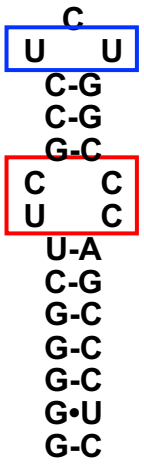
- transcription factor **regulating 15% of all genes**
- **constitutively expressed in cancer** – increased expression of many genes
- associates **cell growth** and **proliferation**
- **'undruggable'** – absence of suitable pocket for high-affinity binding

indirect inhibition of Myc through **RIBOTAC** towards its mRNA



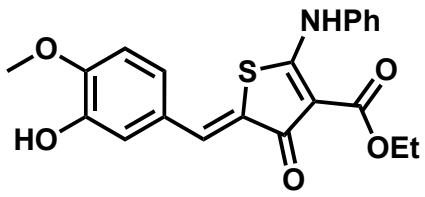
internal ribosome enter site (IRES) of mRNA of *MYC*:



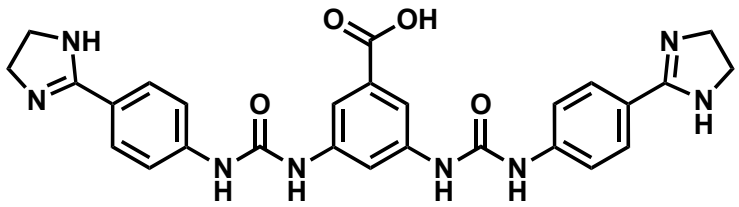


possible RNase L cleavage site

7-binding internal loop



RNase L recruiter



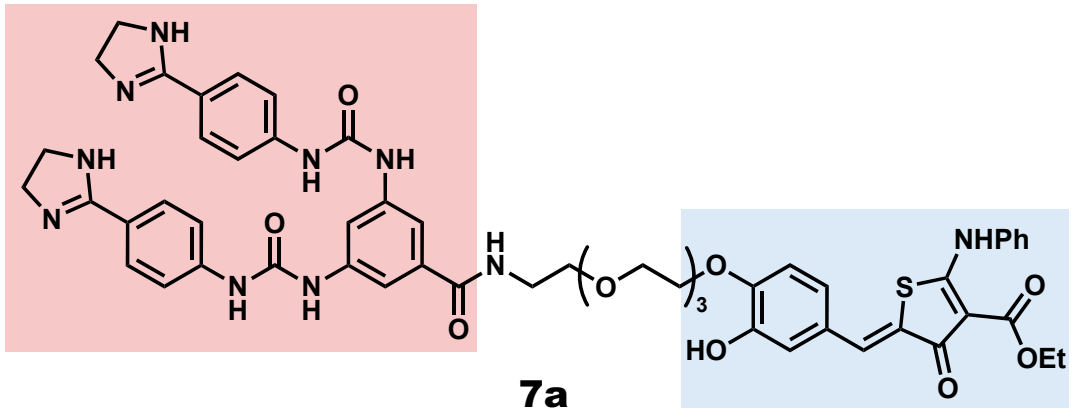
7

k_D = 2.3 μM (biologically inert binding)

[1] Dang, C. V. *Cell* **2012**, *149*, 22-35.

Design of MYC-RIBOTAC

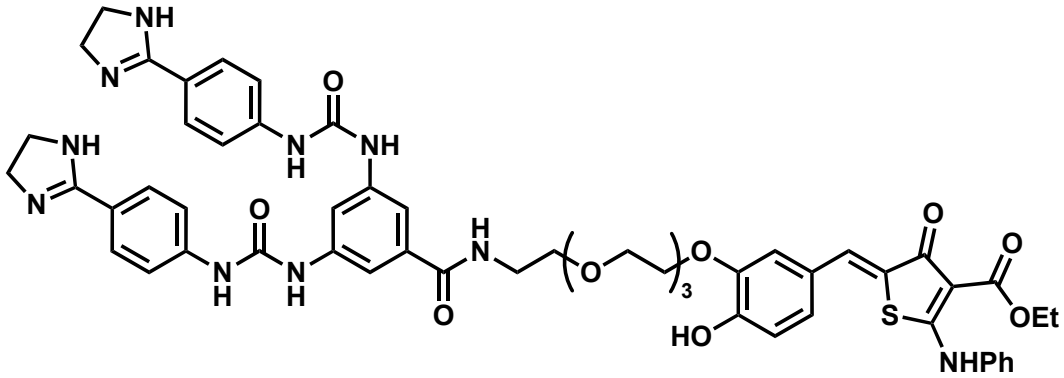
MYC mRNA-binding moiety



RNase L-recruiting moiety

7a

k_D = 1.1 μM



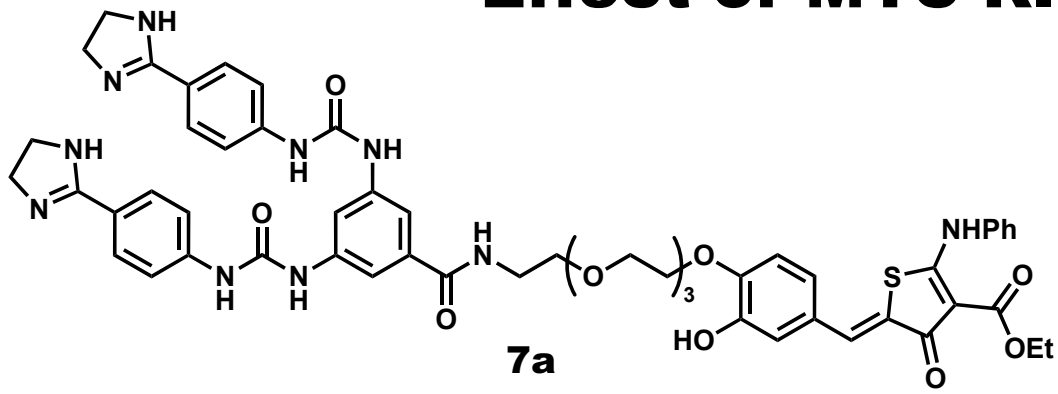
7b

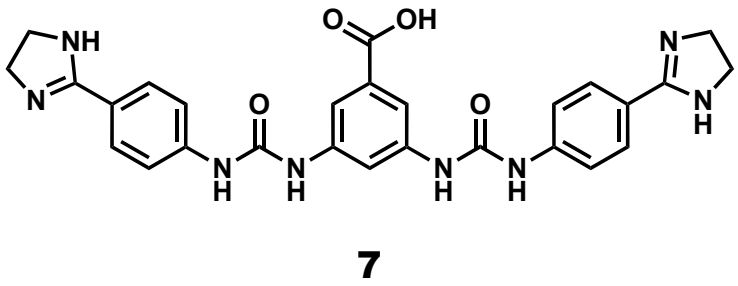
regioisomer with lower RNase L-recruiting activity

k_D = 0.9 μM

- PEG linker connecting an *MYC* mRNA-binding moiety and a RNase L-recruiting moiety
- binding affinity towards IRES of *MYC* mRNA retained

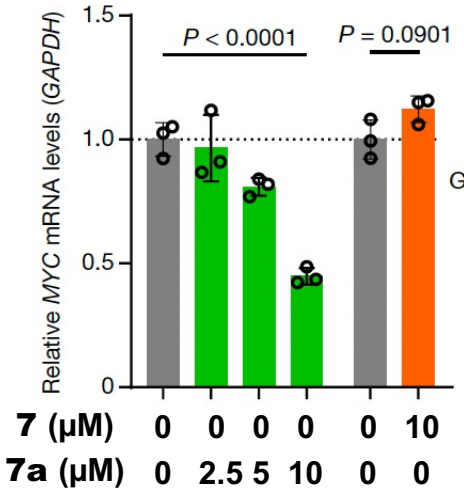
Effect of MYC-RIBOTAC



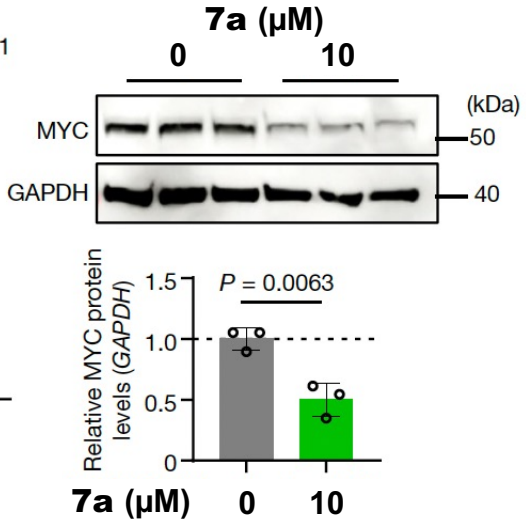


treatment of cervical cancer HeLa cells with MYC-RIBOTAC:

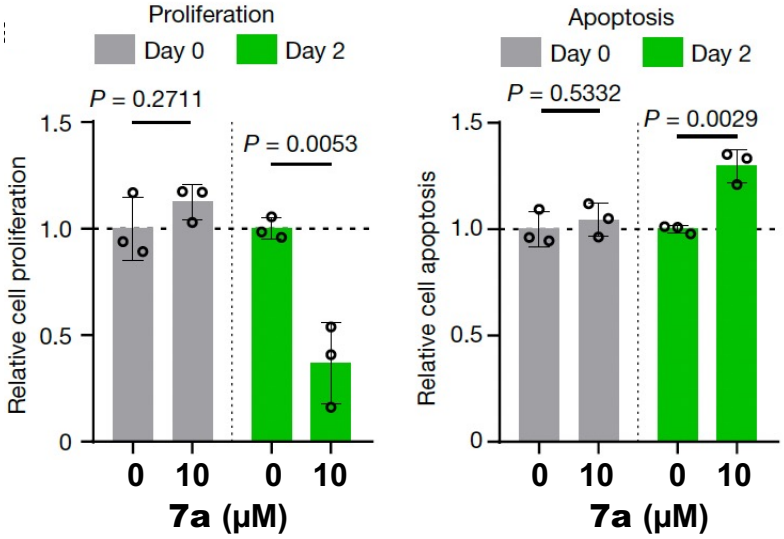
- ***MYC*** **mRNA level:**



- **Myc protein level:**



- **proliferation and apoptosis:**

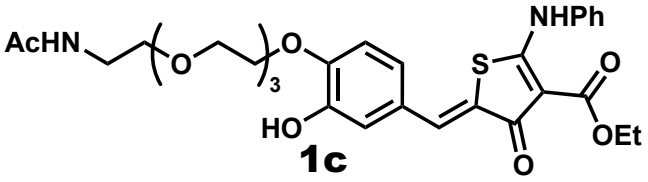


- dose-dependent degradation of ***MYC*** mRNA level by treatment with **7a**
- correspondingly reduced Myc protein level

→

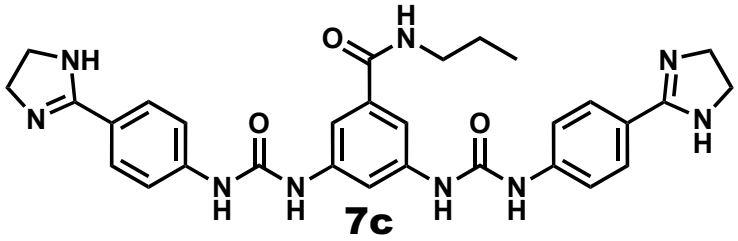
- suppressed proliferation and enhanced apoptosis
- potential anticancer activity of **7a**

Necessity of mRNA-Binding and RNase L Recruiting



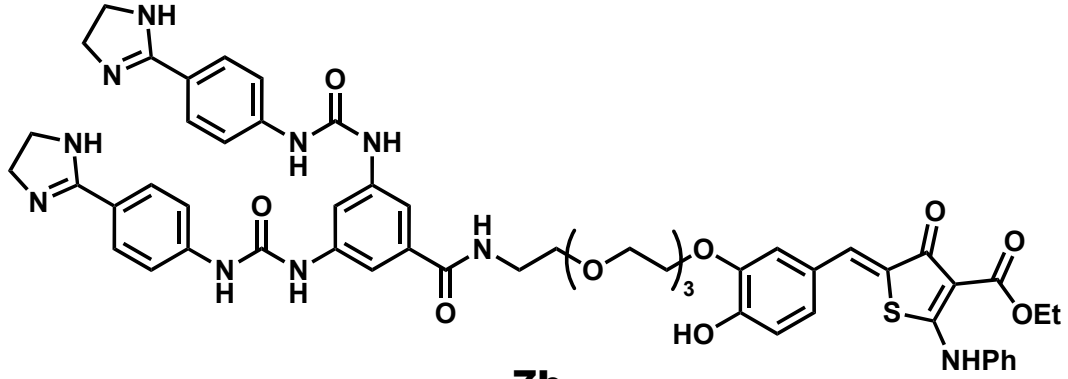
1c

non-*MYC* mRNA binder



7c

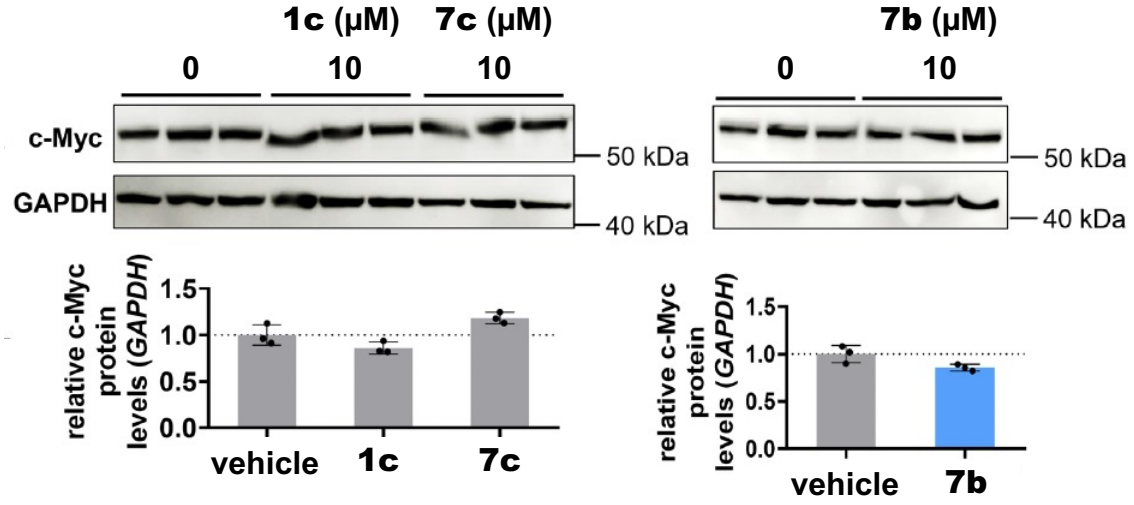
amide derivative of **7**



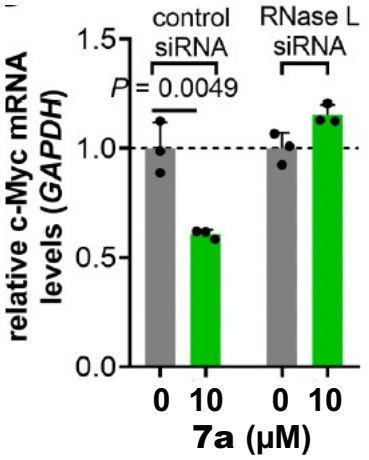
7b

regioisomer with lower RNase L-recruiting activity

- **Myc protein level of HeLa cells:**



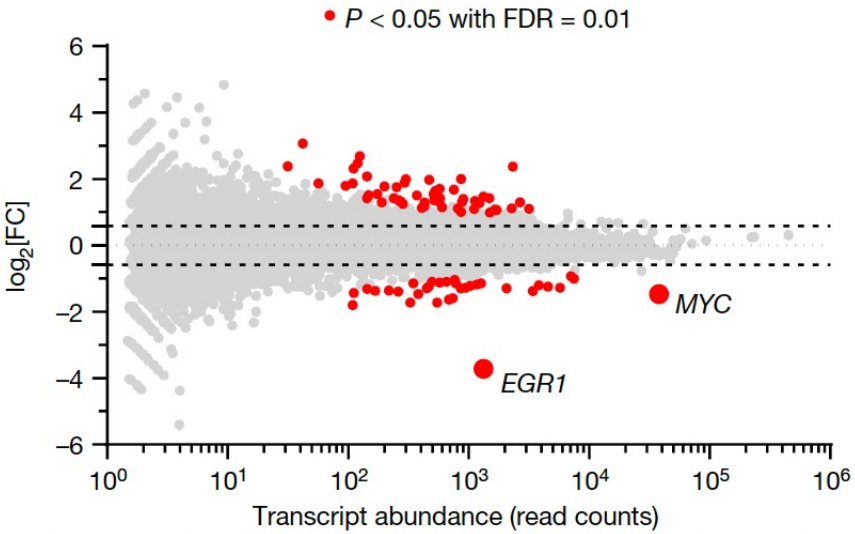
- **RNase L knockdown (HeLa cells):**



- ***MYC* mRNA binding required for degradation of Myc**
- **effective recruitment of RNase L required for degradation of Myc**

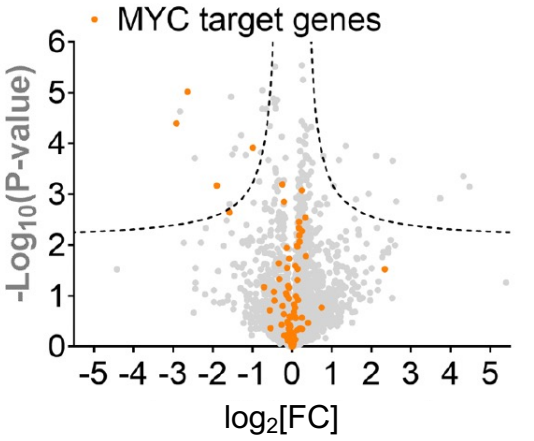
Selectivity of MYC-RIBOTAC

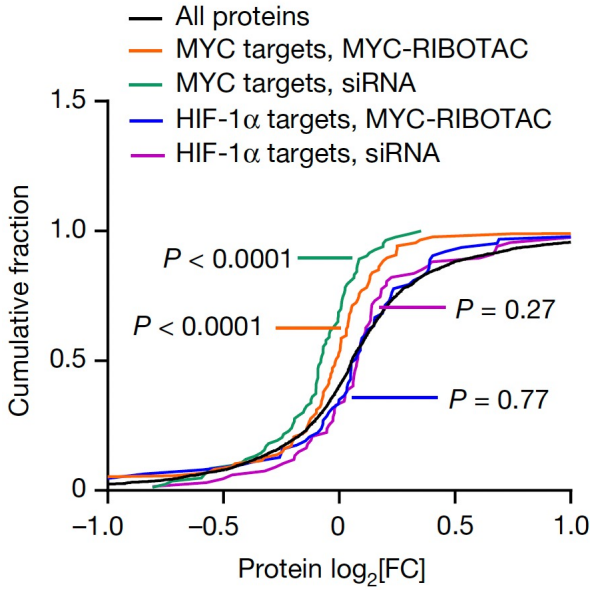
- **RNA-seq analysis in HeLa cells:**



- **84 (0.40%) out of 21,027 transcripts significantly affected (fold change > 1.5)**
- **transcription factor *EGR1* most significantly downregulated – a known down-stream target of MYC[1]**

- **proteomics analysis in HeLa cells:**

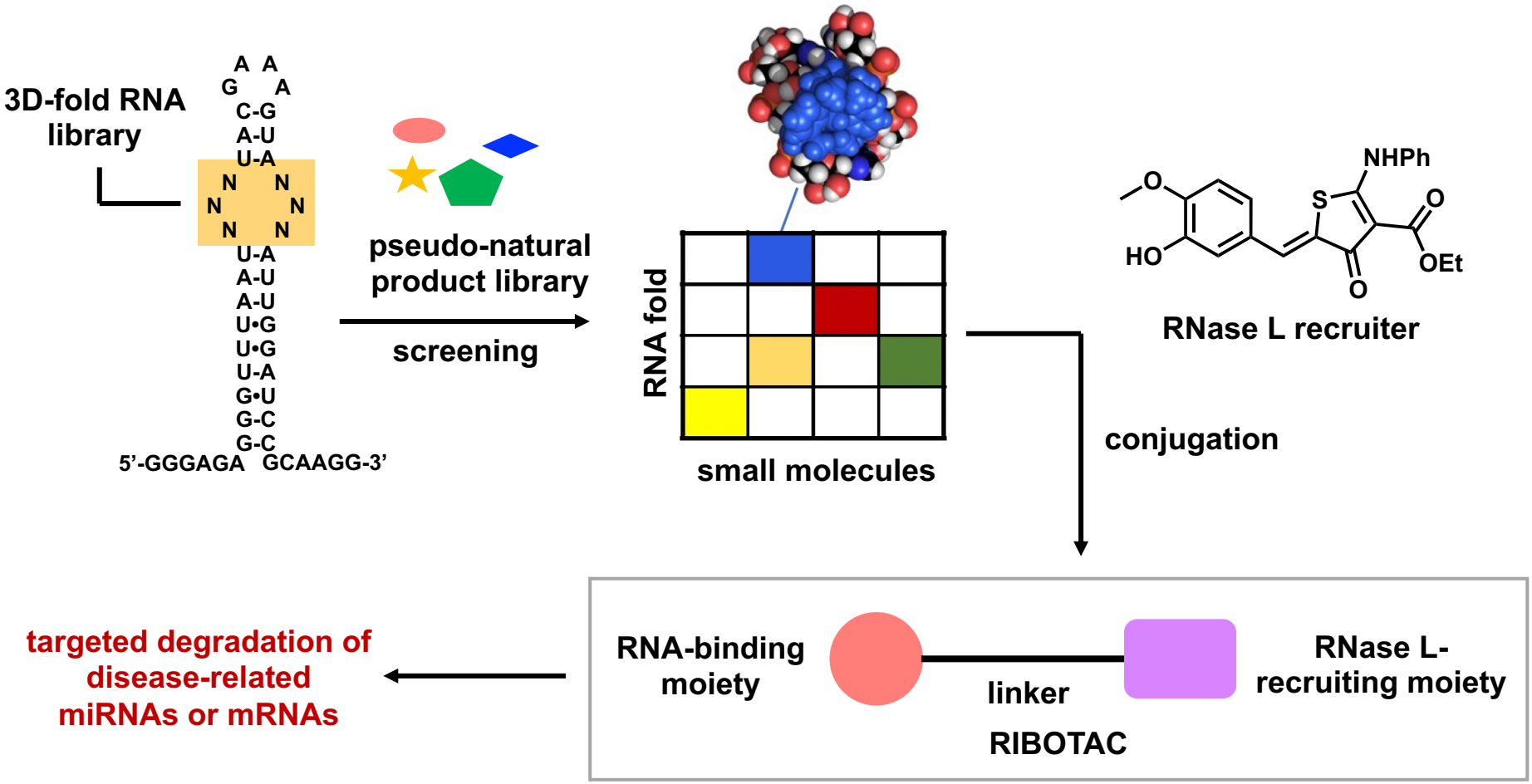




- **28 (1.0%) out of 2,769 proteins significantly affected**
- **4 direct down-stream targets of MYC down-regulated**
- **similar effect to MYC knockdown**
- **selective over another similar transcription factor HIF-1α**

[1] Boone, D. N.; Qi, Y.; Li, Z.; Hann, S. R. *Proc. Natl. Acad. Sci. USA* **2010**, *108*, 632–637.

Summary



- novel platform for programming inactive molecules for targeted biomolecule degradation
- potential for approaching 'undruggable' targets