Novel Mass Spectrometry-Based Footprinting Method for RNA Higher Order Structure

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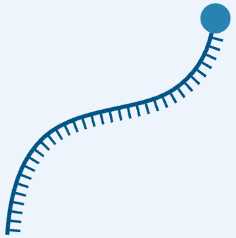
Washington University in St. Louis

Pfizer

CASSS MS
2024/09/10

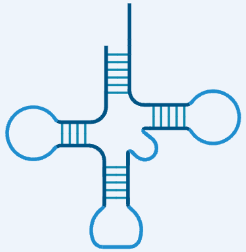
Diversity of RNA types

mRNA



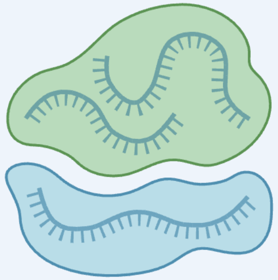
Encodes proteins

tRNA



Acts as adaptor between mRNA and amino acids

rRNA

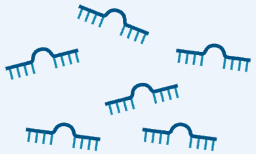


Forms the ribosome

snoRNA

Facilitates chemical modification of RNAs

miRNA

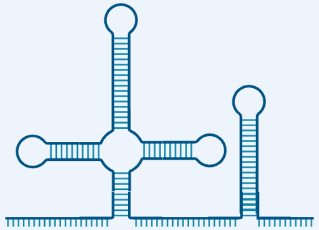


Regulates gene expression

siRNA

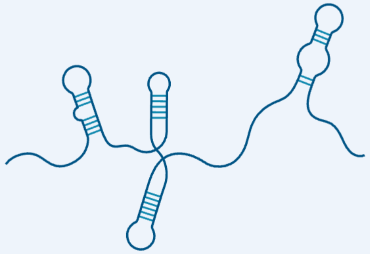
Silences gene expression

snRNA



Functions in various nuclear processes (e.g. splicing)

lncRNA

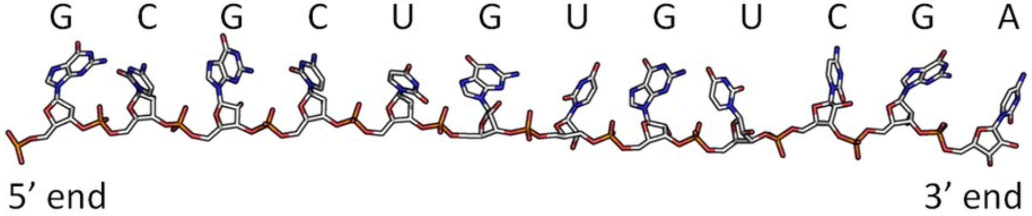


Regulates gene expression

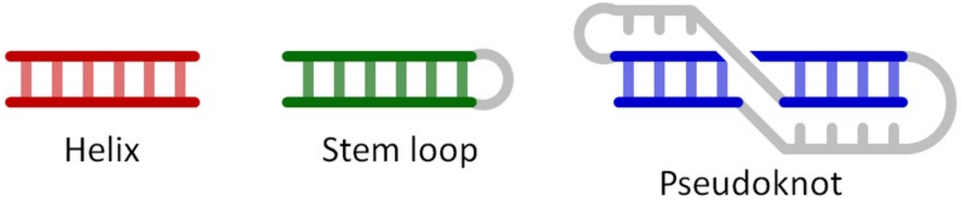
Suleiman, A. A.; Al-Chalabi, R.; Shaban, S. A. *Mol. Biol. Rep.* **2024**, *51* (1), 107.

Structure of RNA

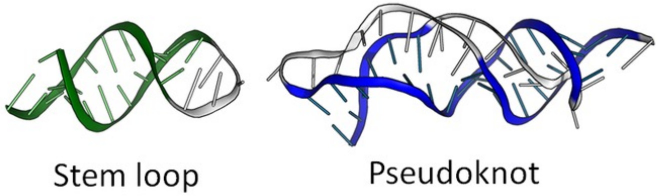
Primary



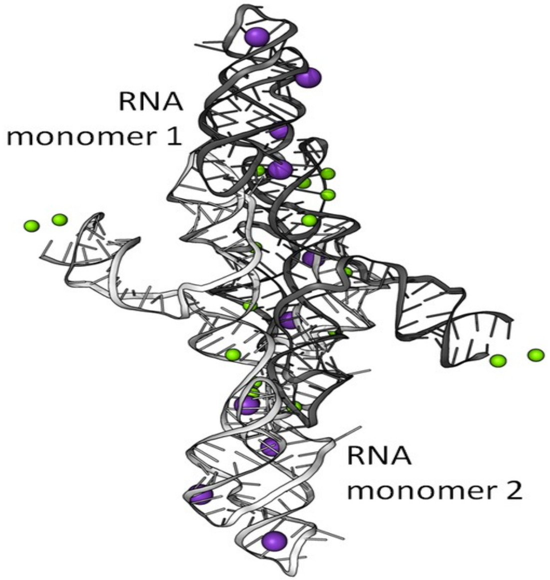
Secondary



Tertiary



Quaternary



https://en.wikipedia.org/wiki/Nucleic_acid_quaternary_structure

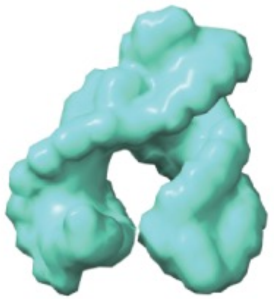
Current landscape of RNA structure determination

Direct RNA structure methods

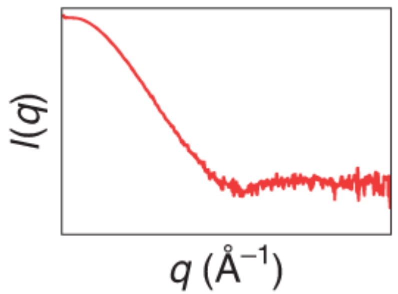
- NMR
- X-ray Crystallography
- SAXS
- Cryo-EM

Indirect RNA structure methods

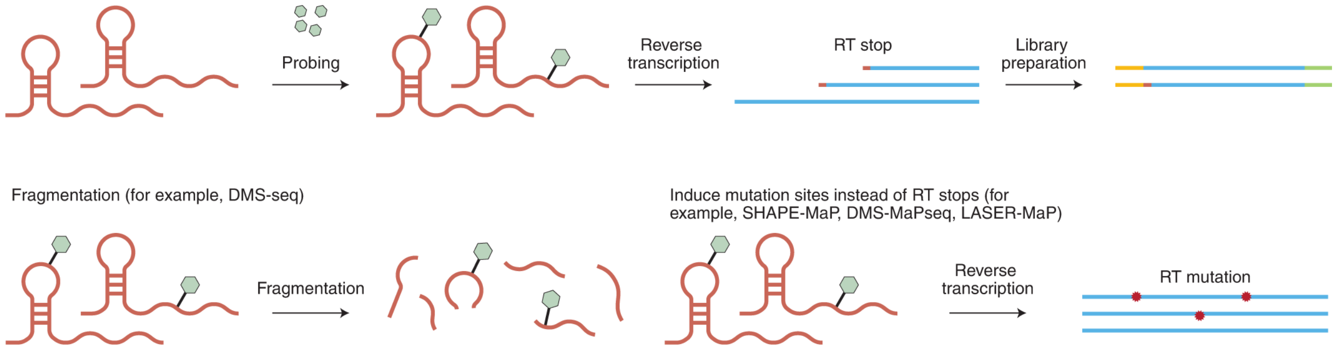
- Enzymatic-Based Footprinting
- Chemical-Based Footprinting
- Proximity Ligation-based Method



Density map



Scattering profile



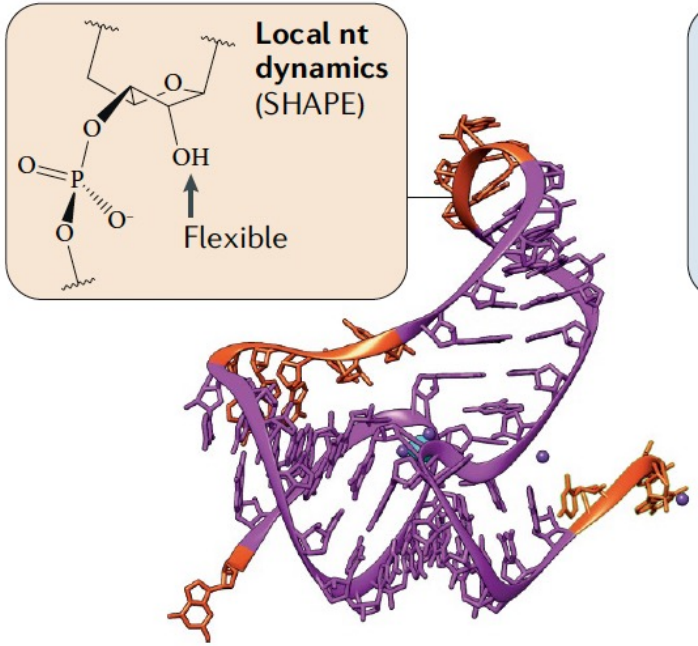
Zhu, Y.; Zhu, L.; Wang, X.; Jin, H. *Cell Death Dis.* **2022**, *13* (7), 644.
Wang, XW.; Liu, CX.; Chen, LL. *Nat Chem Biol.* **2021**, 17, 755–766

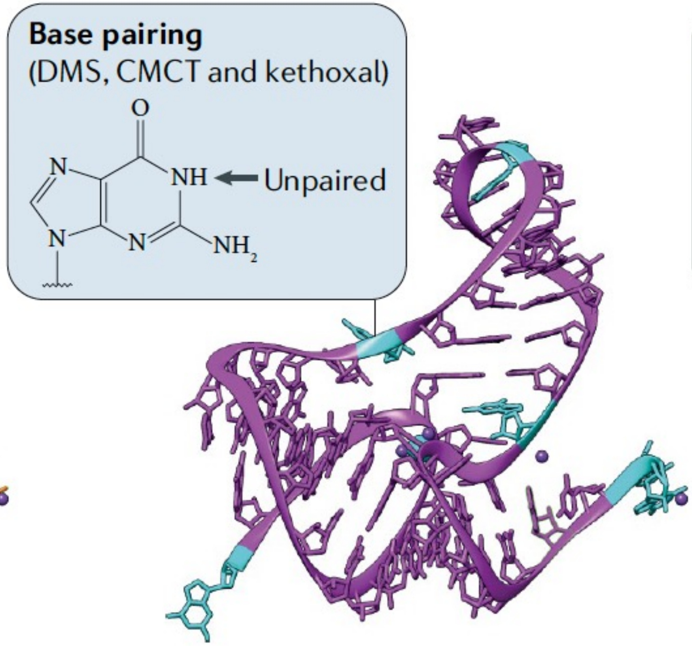
A palette of chemical probes to interrogate many layers of RNA structure

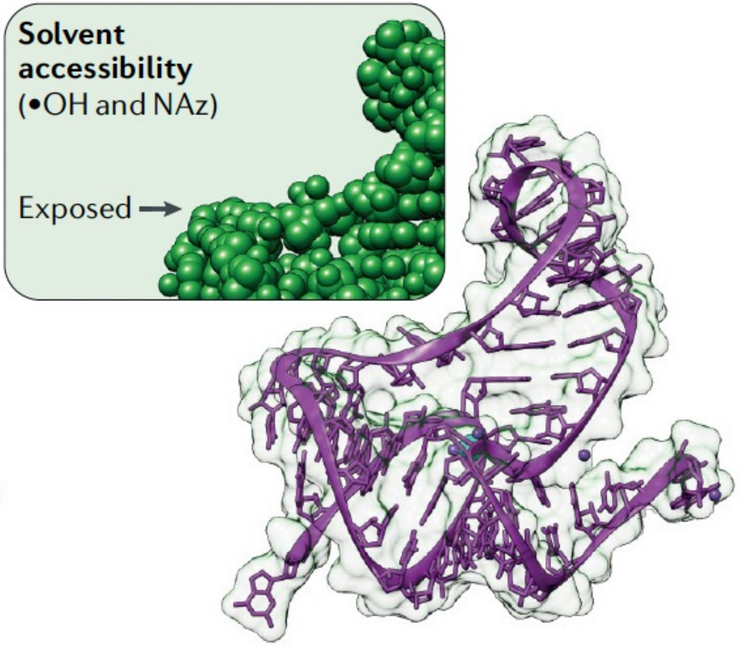
Local dynamics

Base paring

Solvent accessibility







SHAPE: BzCN (s)
SHAPE: 1M7 (s)

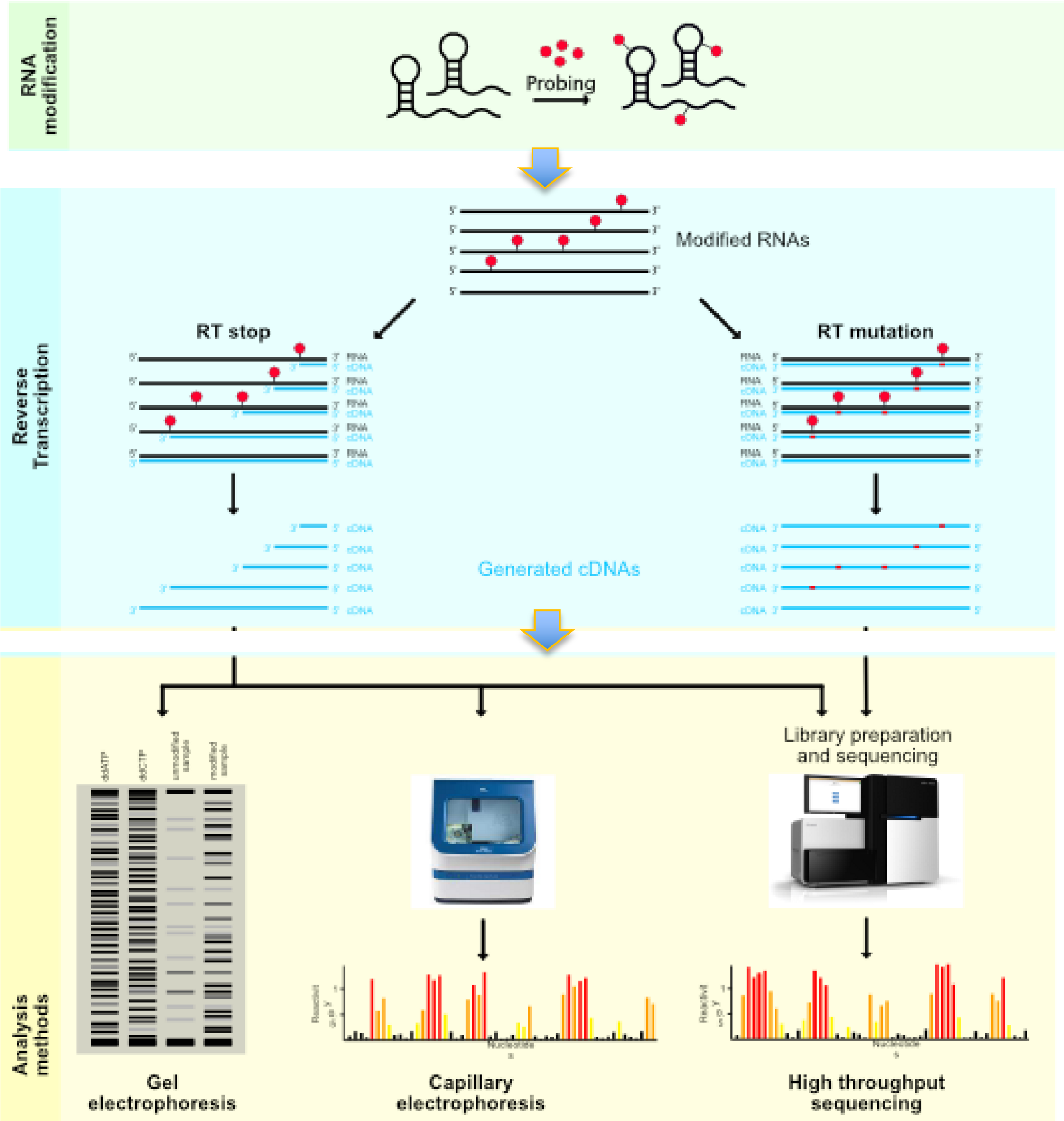
DMS (GN7,AN1,CN3) (mins)
DEPC (mins)

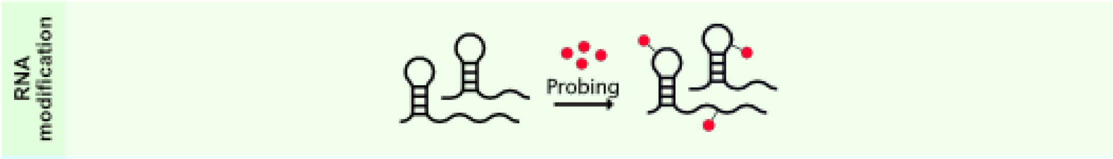
·OH (radical based) Fenton; (s)
X-ray; (ms)

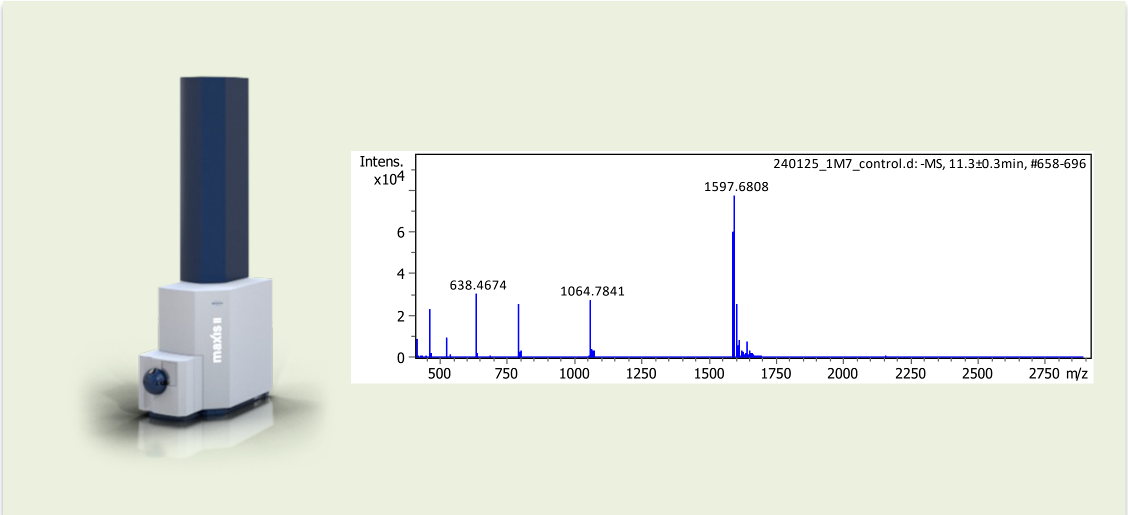
Naz (radical based) UV 310nm; (ps)

Strobel, E. J.; Yu, A. M.; Lucks, J. B. *Nat. Rev. Genet.* **2018**, *19* (10), 615–634.

Chemical probe for RNA footprinting





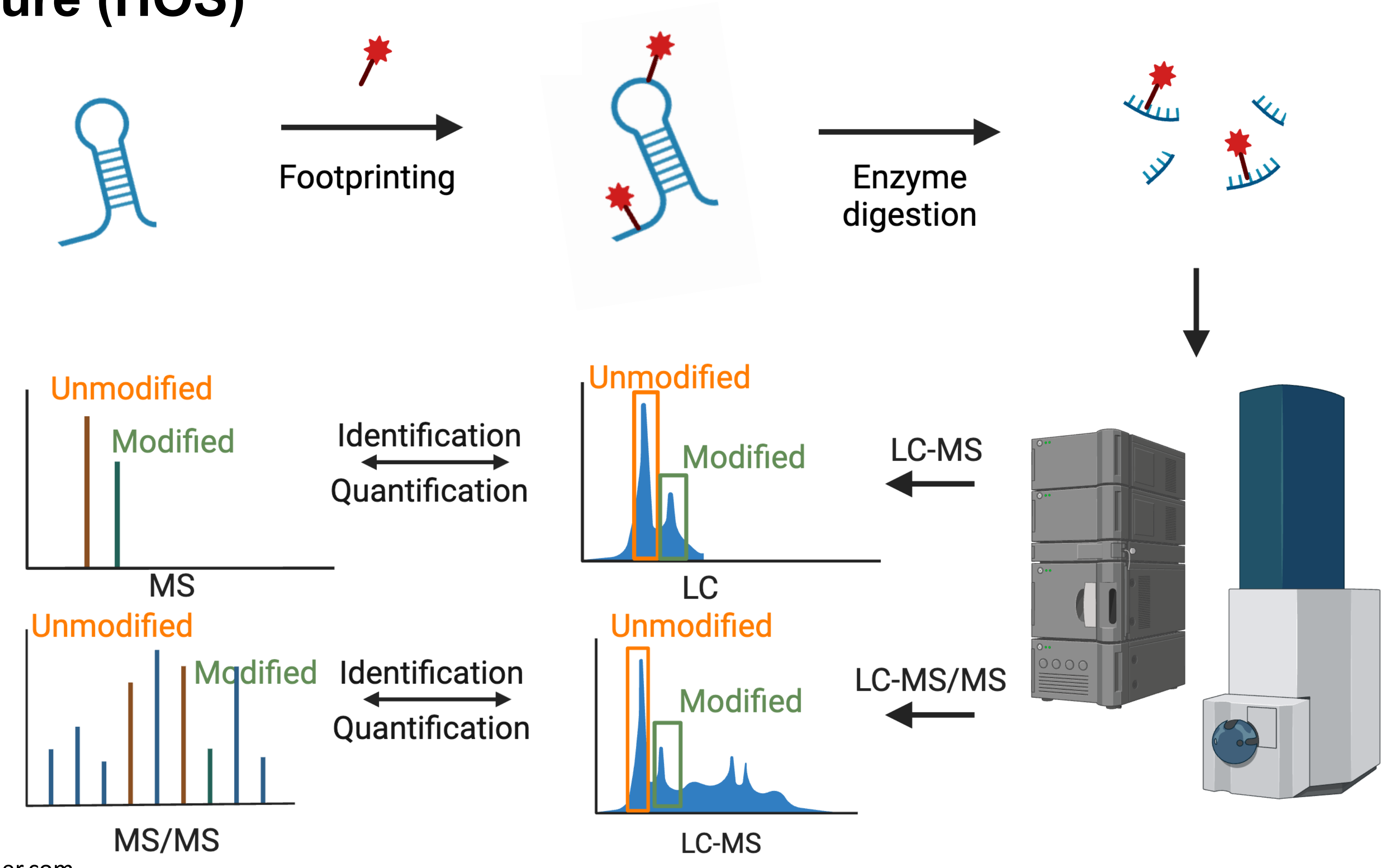


Gilmer, O.; Quignon, E.; Jousset, A.-C.; Paillart, J.-C.; Marquet, R.; Vivet-Boudou, V. *Viruses* **2021**, *13* (10), 1894.

Goal

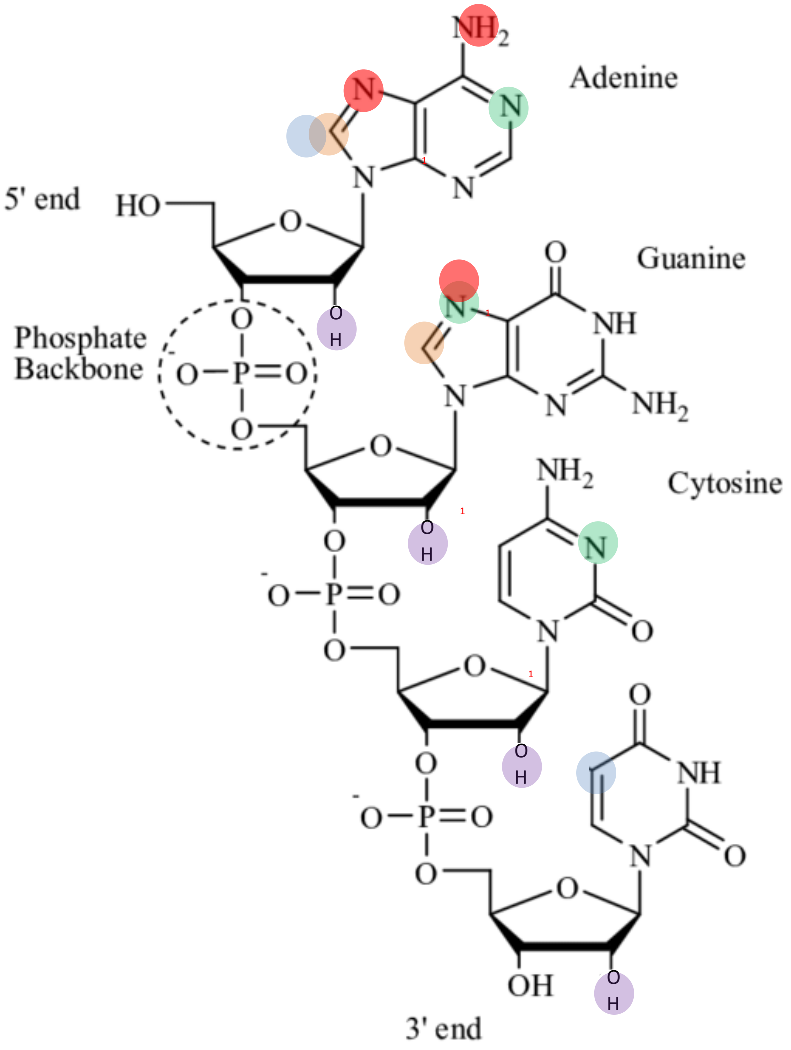
Develop a mass spectrometry- based footprinting approach for RNA higher-order structure (HOS)

Workflow:

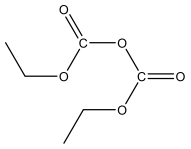


Created with BioRender.com

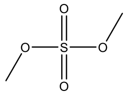
Reagent candidates



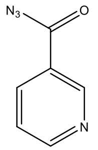
- Base-specific probe
  - Dimethyl sulfate (DMS)
  - Diethyl pyrocarbonate (DEPC)
  - Nicotinoyl Azide (NAz)
  - Hydrogen peroxide (OH radical)
  - 4-(Trifluoromethoxy)benzyl bromide (Carbocations (R_3C^+))
- Sugar-phosphate targeting probe
  - 1-Methyl-7-nitroisatoic anhydride (1M7)
  - Benzoyl Fluoride (Base; 2’-OH)
  - Hydrogen peroxide (OH radical)
  - 4-(Trifluoromethoxy)benzyl bromide (Carbocations (R_3C^+))



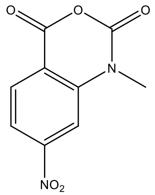
DEPC



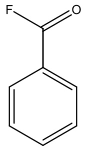
DMS



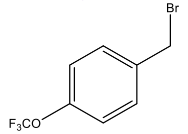
NAz



1M7

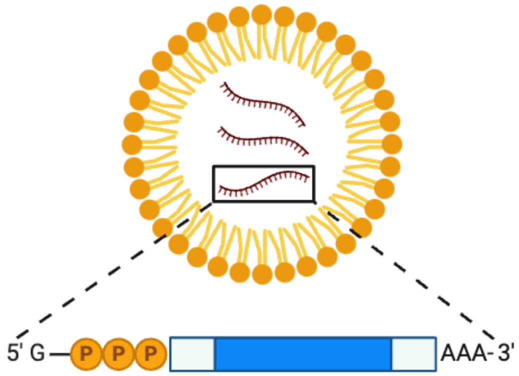


Benzoyl fluoride



4-(Trifluoromethoxy)benzyl bromide

Analytes



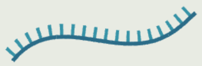
Small Oligo

10mer

ACAAAACAAG

Small Oligo

12mer



AAAUUUGGGCCC

PreQ1 Riboswitch

33mer

CUGGGUCGCAGUAACCCCAGUUAACAAAACAAG

Preliminary results of screening reagent candidates

RNA (10mer): ACAAAACAAG

Reagent	Molar ratio	Laser	Reaction time	Mass shift (Da)	Modification level
DMS	50000	no	1.5 mins 10 mins	14	7% 51%
1M7 (SHAPE)	10000	no	1.5 mins	178	75%
DEPC	10000	no	10 mins	72; 62	86%
BF	5000	no	10 mins	104	7%
FPOP(H_2O_2)	2000	yes	laser	16	14%
TBF (carbocation)	10000	yes	laser	174	7%

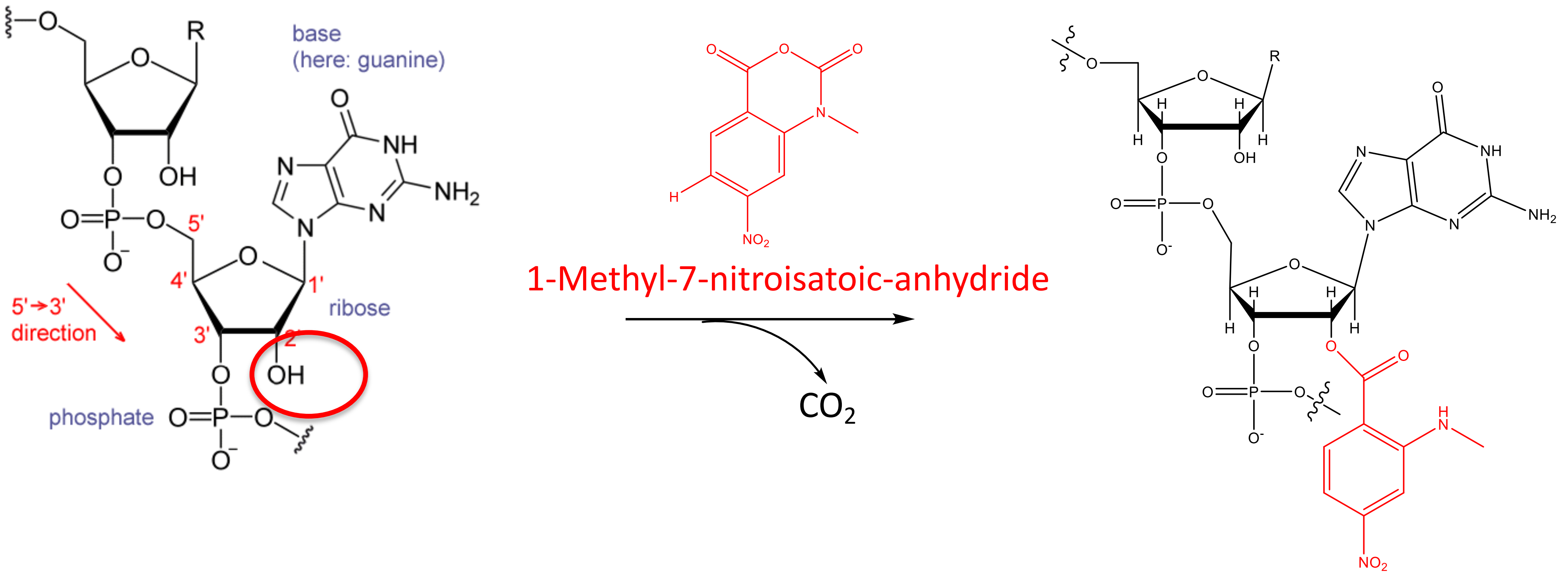
RNA (12mer): GGGUUUCCCAAA

Reagent	Molar ratio	Laser	Reaction time	Mass shift (Da)	Modification level
DMS	50000 10000 5000	No	10 mins	14	47% 41% 32%
1M7 (SHAPE)	10000 5000 1000	no	1.5 mins	178	75% 36% 20%
BF	10000 5000 2000	no	10 mins	104	7% 4% 4%
FPOP(H_2O_2)	2000	yes	laser	16	14%

$$\text{Modification Level} = \frac{EIC\ Area_{\ modified}}{EIC\ Area_{\ Unmodified} + EIC\ Area_{\ modified}}$$

1-Methyl-7-nitroisatoic-anhydride (1M7) labeling mechanism

1M7 is a SHAPE (Selective 2′ Hydroxyl Acylation analyzed by Primer Extension) reagent



10mer from PreQ1: ACAAAACAAG

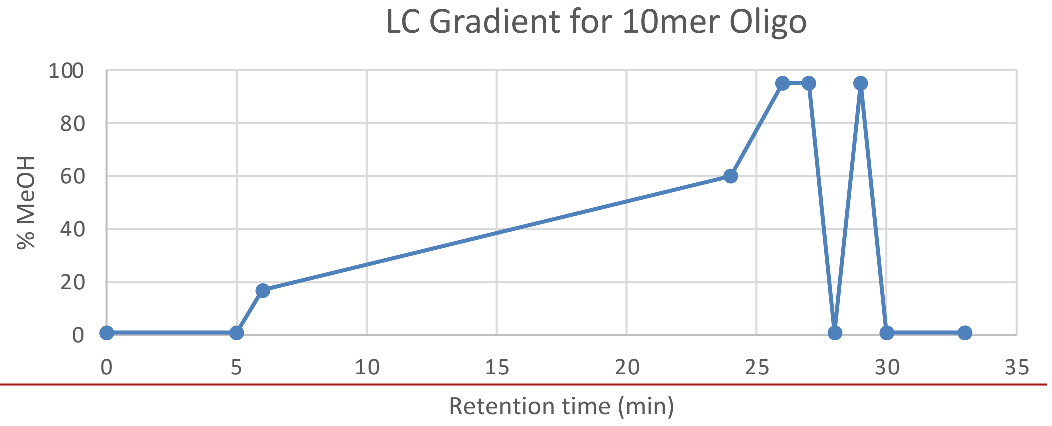
M_{RNA} + $C_8H_6N_2O_3$

(Mass shift in Mass spectrum: M_{RNA} + **178**)

Experimental condition of 1M7 labeling

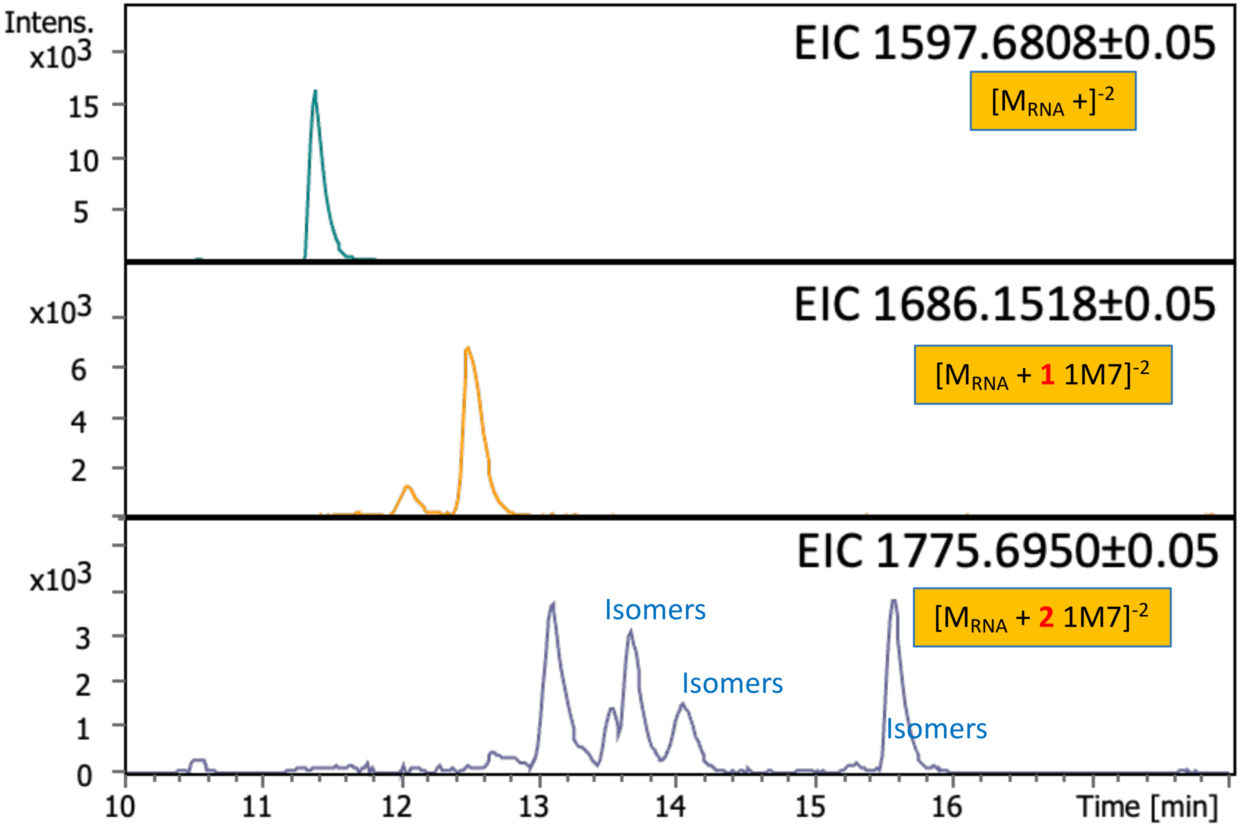
RNA Oligo conc. (µM)	1M7/10mer (molar ratio)	Reaction Temp. (°C)	Buffer, solvent	Time (min)	Shaking speed (rpm)
1	10,000	37	10% DMSO in PBS	1.5	200
1	5,000	37	10% DMSO in PBS	1.5	200
1	1,000	37	10% DMSO in PBS	1.5	200

- Quench the reaction by loading to the LC column immediately after 1.5 min-incubation.
- Inject 50 pmol of 10 mer for LC-MS analysis

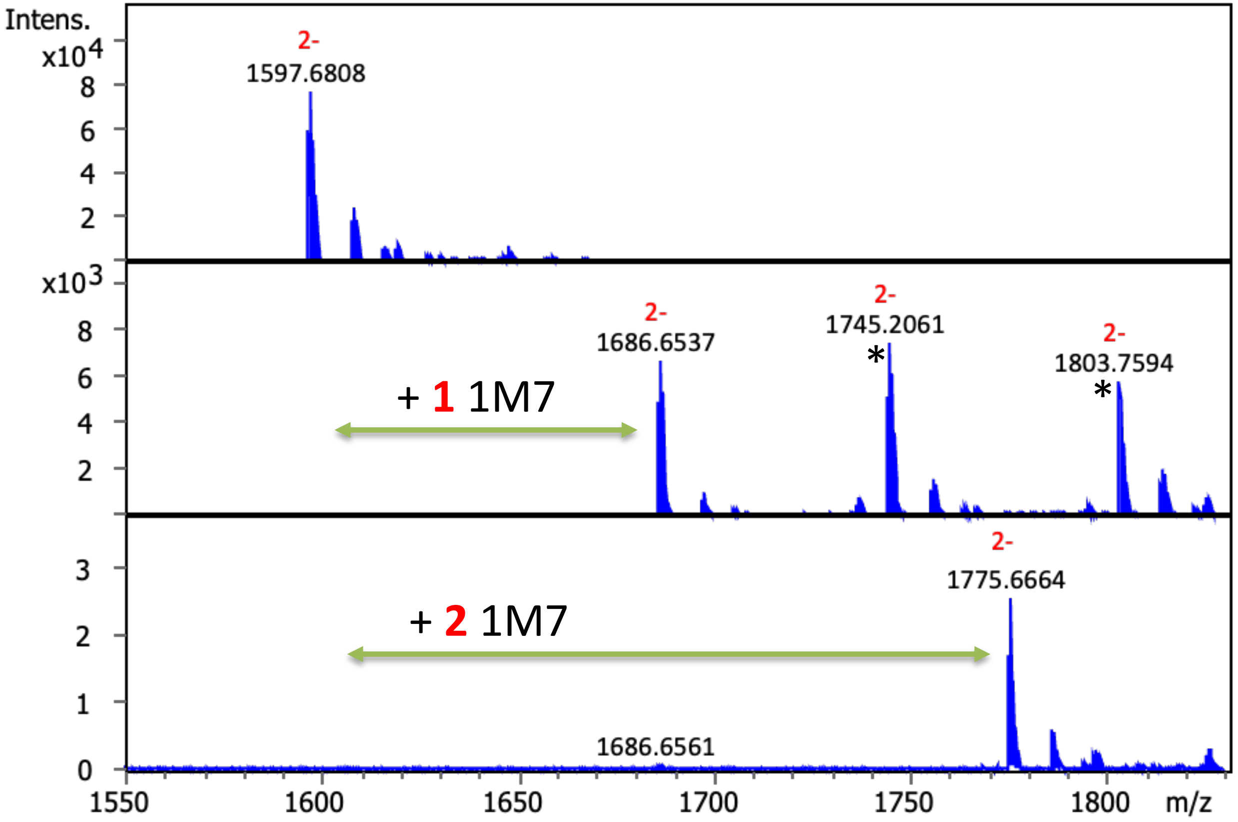


LC and MS of 1M7 labeling

Chromatogram (EIC)



Mass spectra



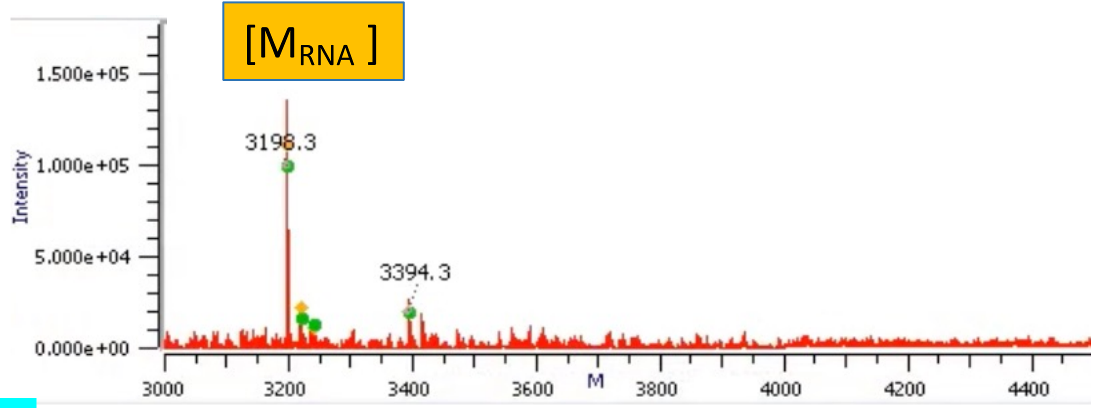
* : Oxidated RNA adduct Triethylamine (TEA)

10mer Oligonucleotide, ACAAAACAAG
10000 molar ratio, 37 °C, 1.5 min,

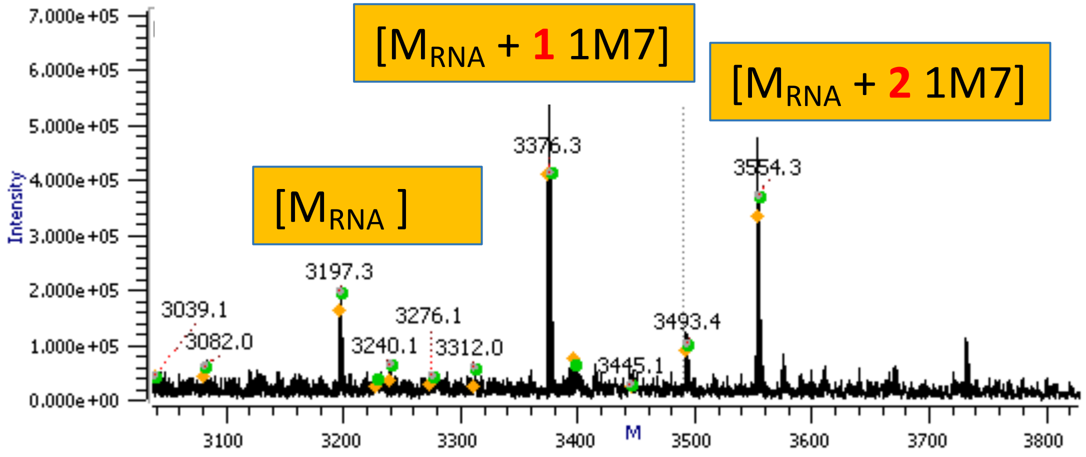
Modification level as a function of 1M7 amount

Deconvoluted MS

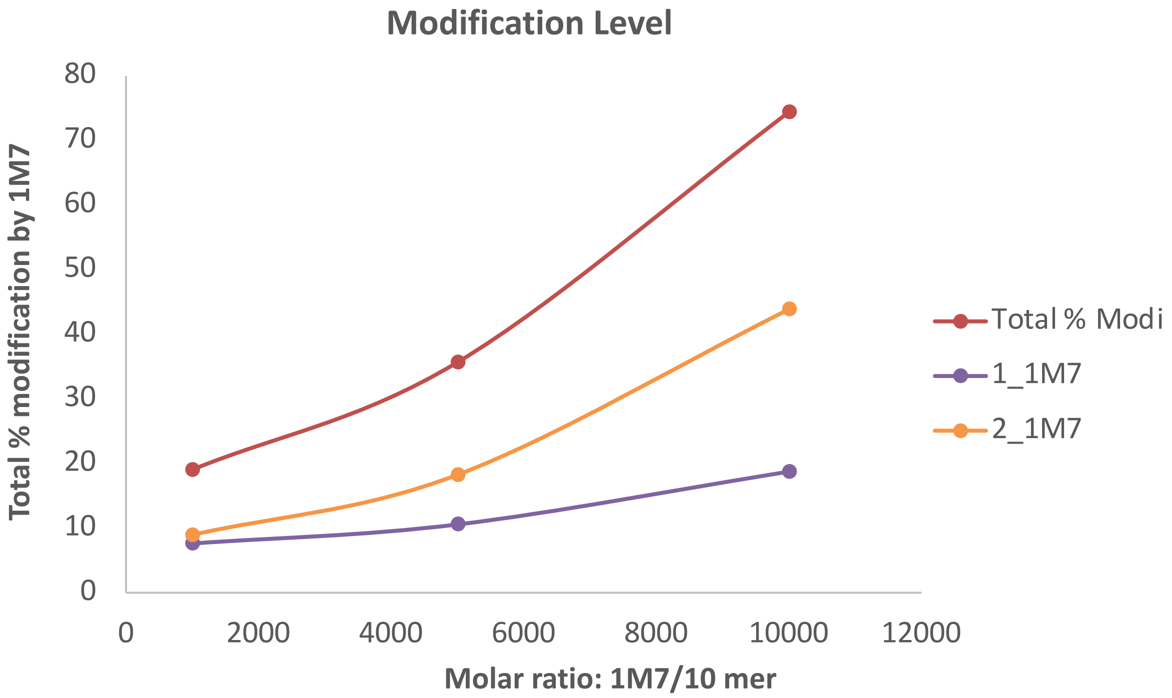
Unmodified



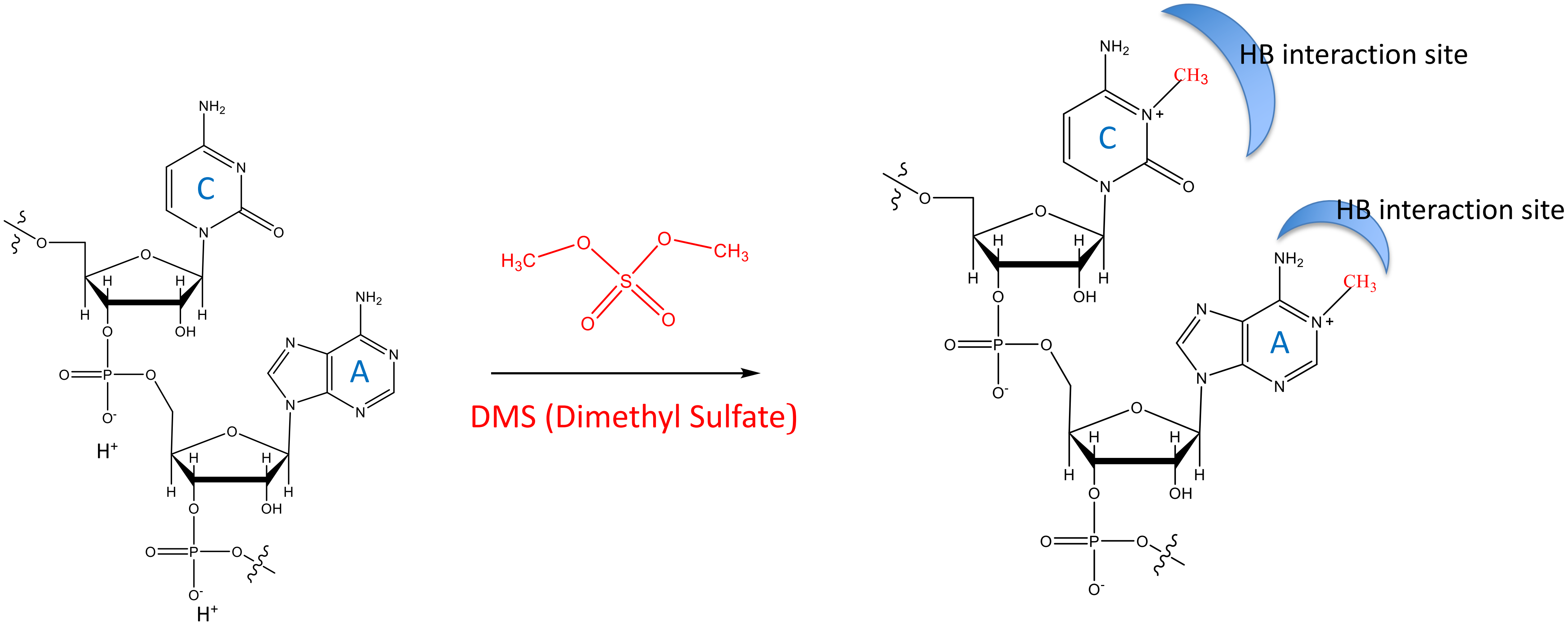
Modified



10mer Oligonucleotide, ACAAAACAAG
10000 molar ratio, 37 °C, 1.5 min,



DMS labeling mechanism



12mer: GGGUUUCCCAAA

M_{RNA} + CH_2

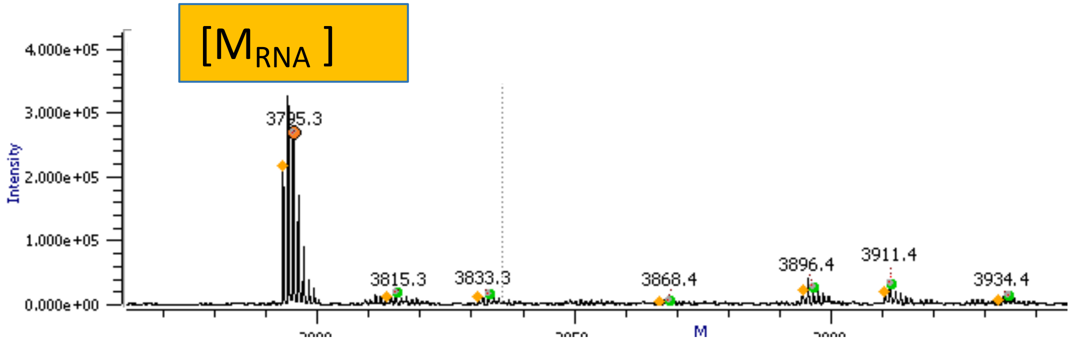
(Mass shift : $[M_{RNA}]$ + **14**)

HB: Hydrogen Bonding

Modification level as a function of DMS amount

Deconvoluted MS

Unmodified



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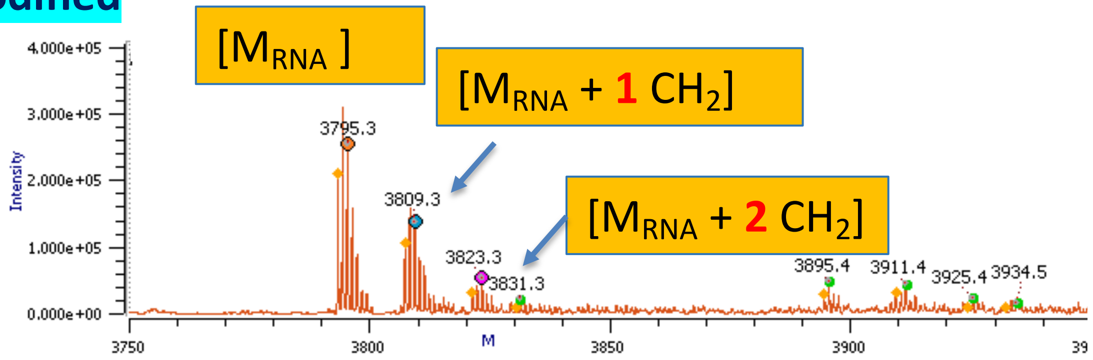
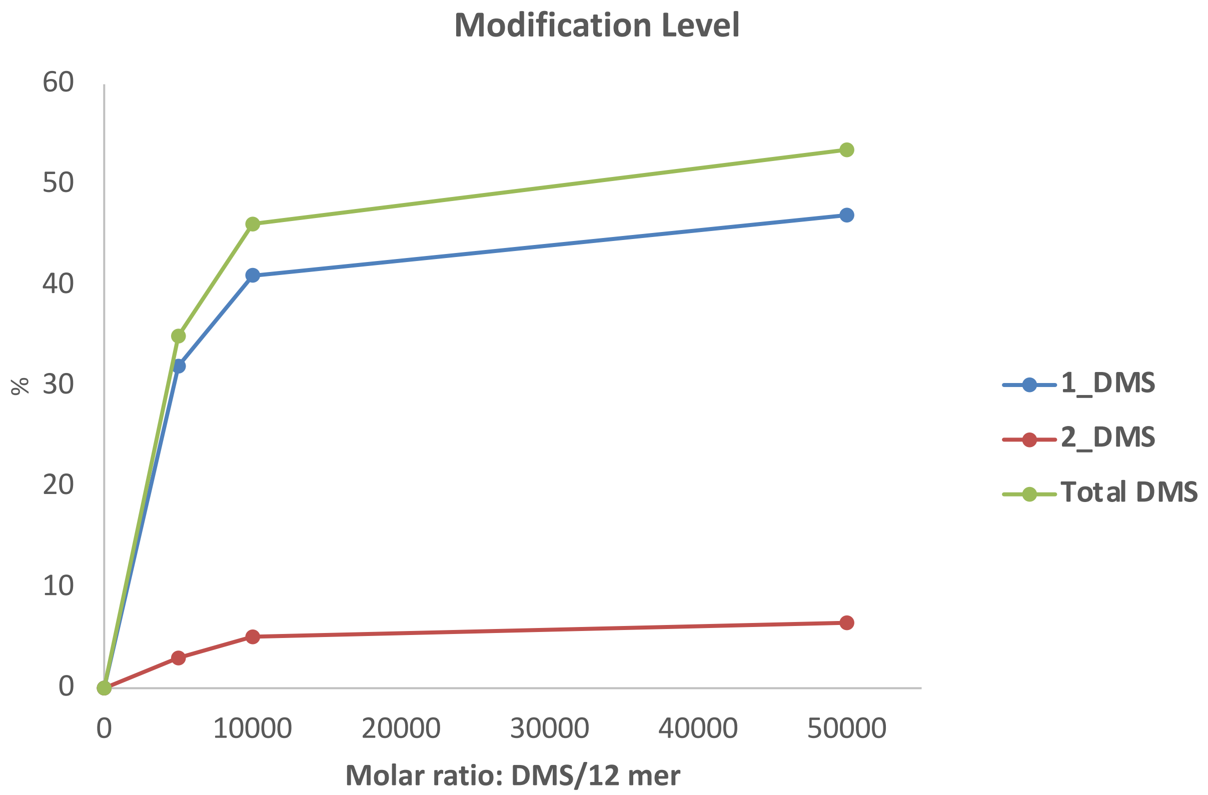
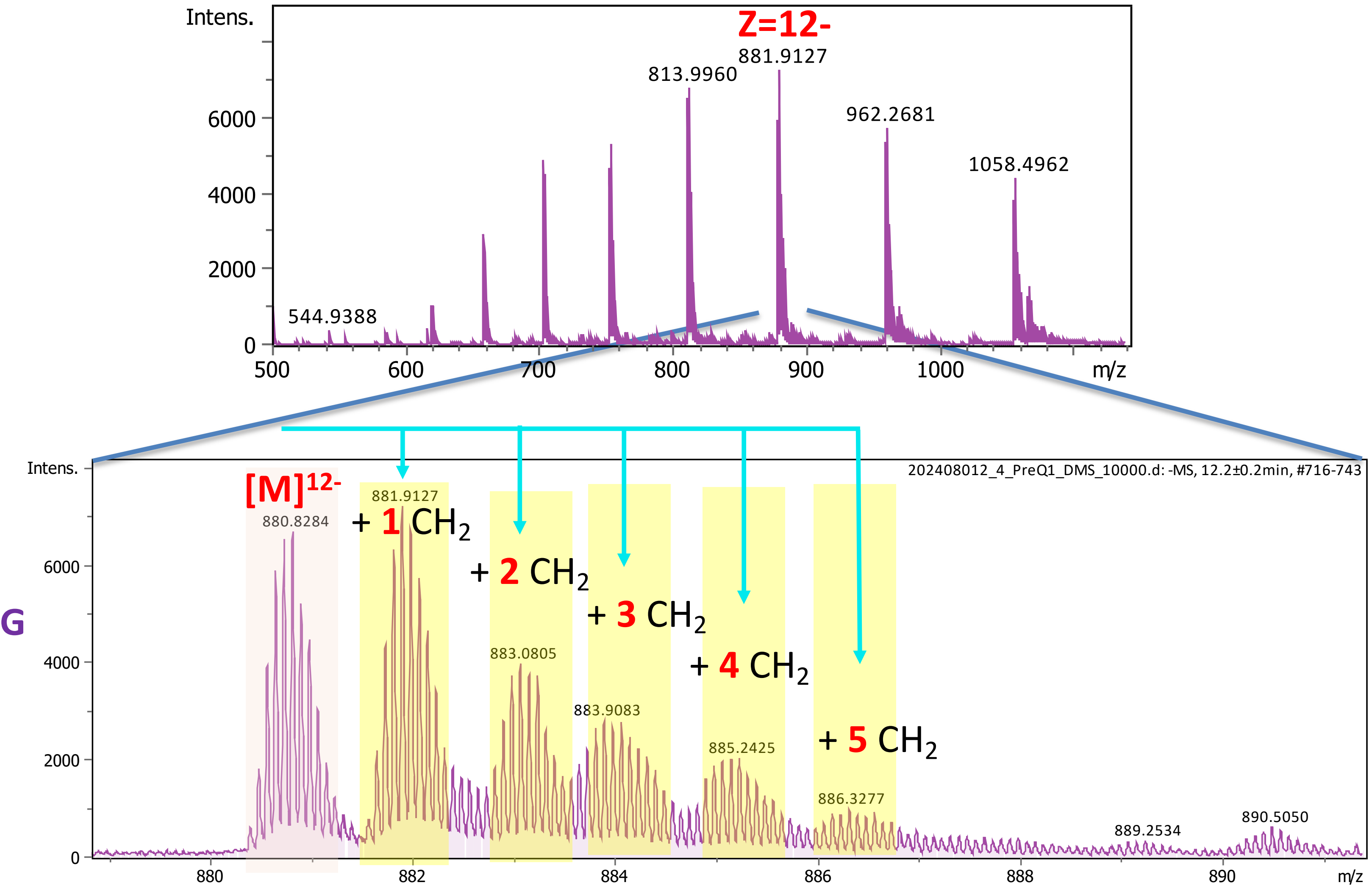


12mer Oligonucleotide, GGGUUUCCCAAA
10000 molar ratio, 37 °C, 10 min



DMS labeling of PreQ1 riboswitch



33 bases:
CUGGGUCGCAGUAACCCCAGUUAACAAAACAAG

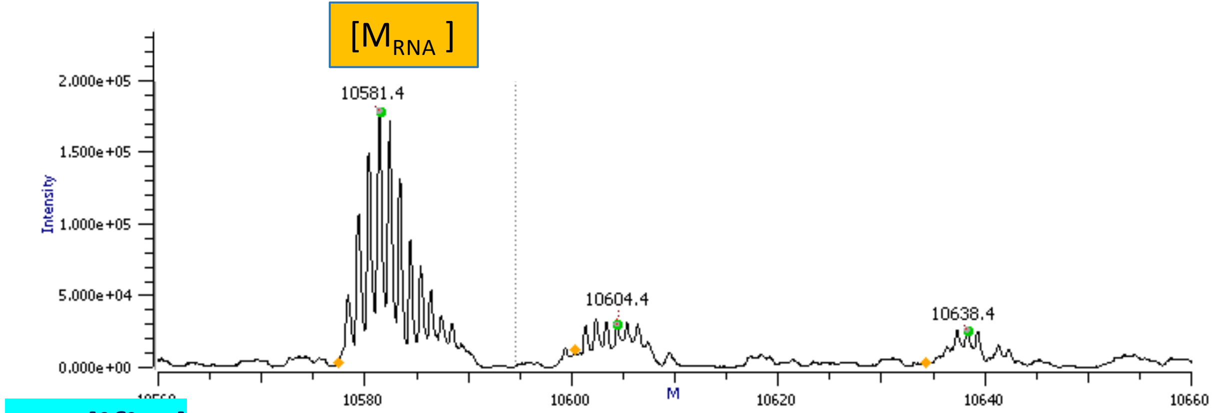
Reaction conditions:
10000 molar ratio, 37 °C, 10 min

Jenkins, J. L.; Krucinska, J.; McCarty, R. M.; Bandarian, V.; Wedekind, J. E. *J. Biol. Chem.* **2011**, *286* (28), 24626–24637.

Modification level as a function of DMS amount

Deconvoluted MS

Unmodified



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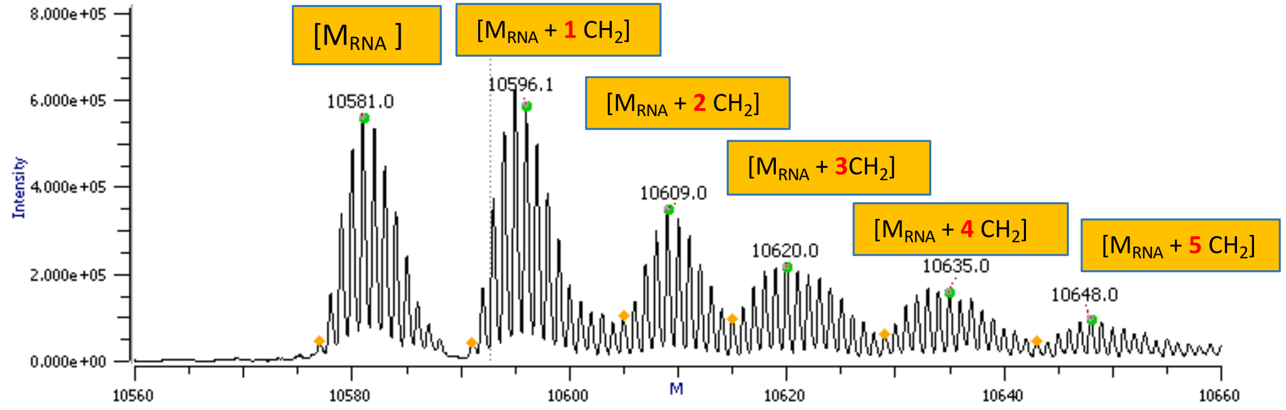
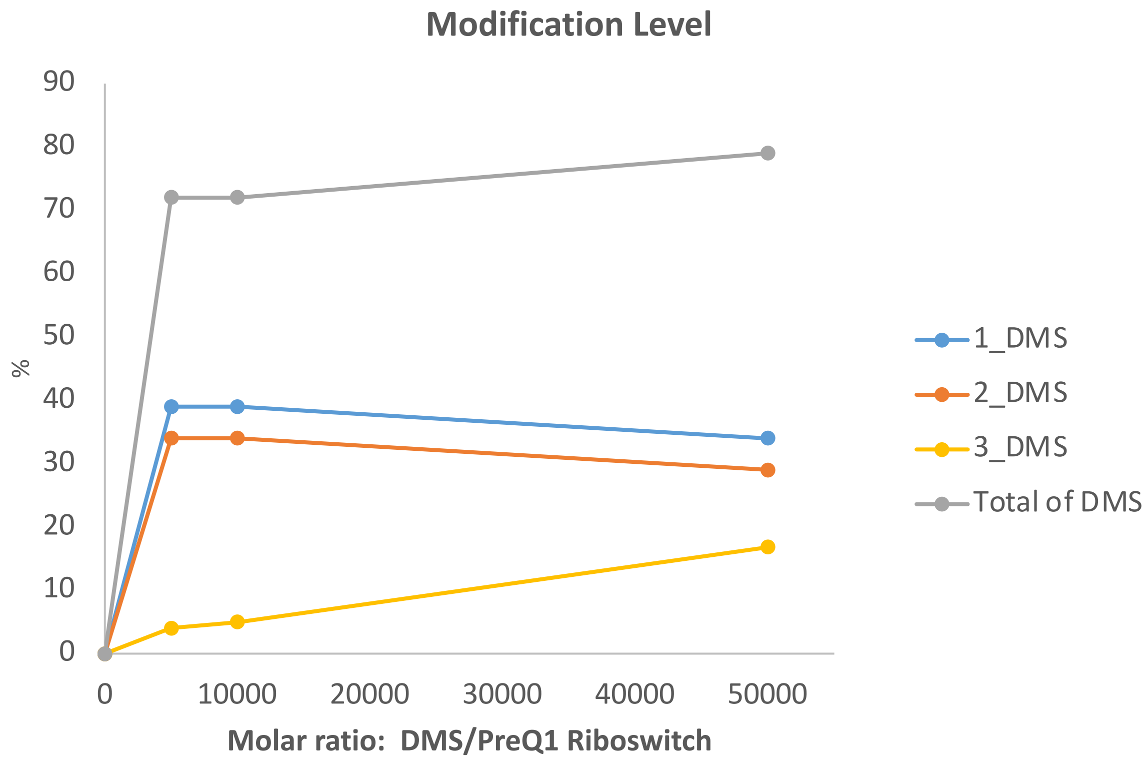




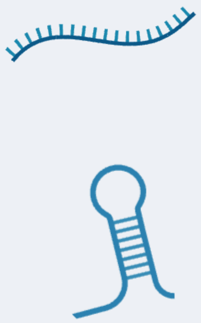
33mer PreQ1 Riboswitch
10000 molar ratio, 37 °C, 10 min

Conclusions:

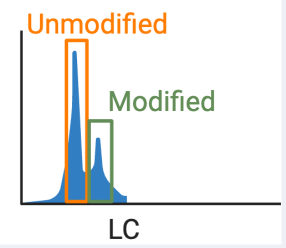
Reagents

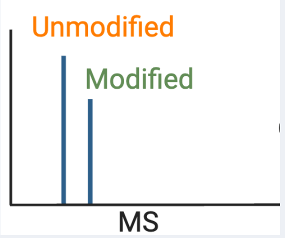
Local dynamics	SHAPE: 1M7 (1.5min)
Base paring	DMS (GN7,AN1,CN3) (10 mins) DEPC (10 mins)
Solvent accessibility	·OH (radical based) FPOP (µs) Naz (radical based) UV 310nm

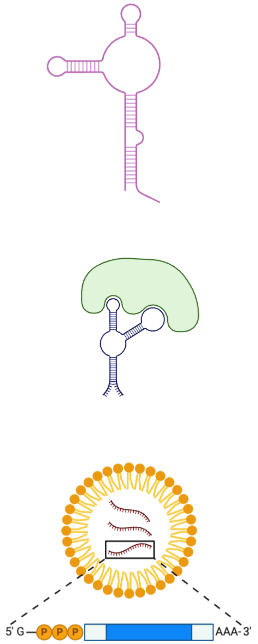
RNA

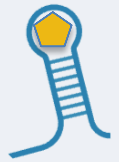


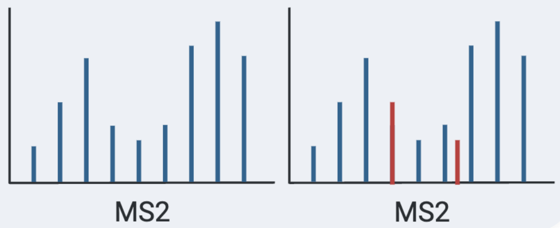
MS-Based Method











Conclusions:

1. Set up the MS-based footprinting for sub-nucleotide RNA structural characterization.
2. Develop labeling strategies for MS detection by using model oligonucleotides with several reagents.
3. Screen a series of chemical footprinting reagents, including backbone footprinting (hydroxyl radical, SHAPE reagent, benzoyl fluoride) and base-specific probes (dimethyl sulfate, diethylpyrocarbonate, nicotinoyl azide).
4. Define the most effective reagent for labeling is DEPC, then 1M7, and DMS. The modification levels are 86%, 75% and 51%, respectively, under comparable conditions.

Next steps:

- Test other reagents (e.g., carbocations) are also being tested.
- Use MS/MS to further elucidate the accurate site of modification.
- Apply to large-size RNA and RNA interacting with lipid nanoparticles under various conditions.

Acknowledgement

Pfizer

- Dr. Brian Gau
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- Don Rempel

- Dr. Nicole Wagner
- Dr. Jie Sun
- Wesley Wagner
- Xinyi Kuang
- Nolan McLaughlin
- Dr. Daryl Giblin

PROTEIN METRICS BRUKER

Thank you for your attention!