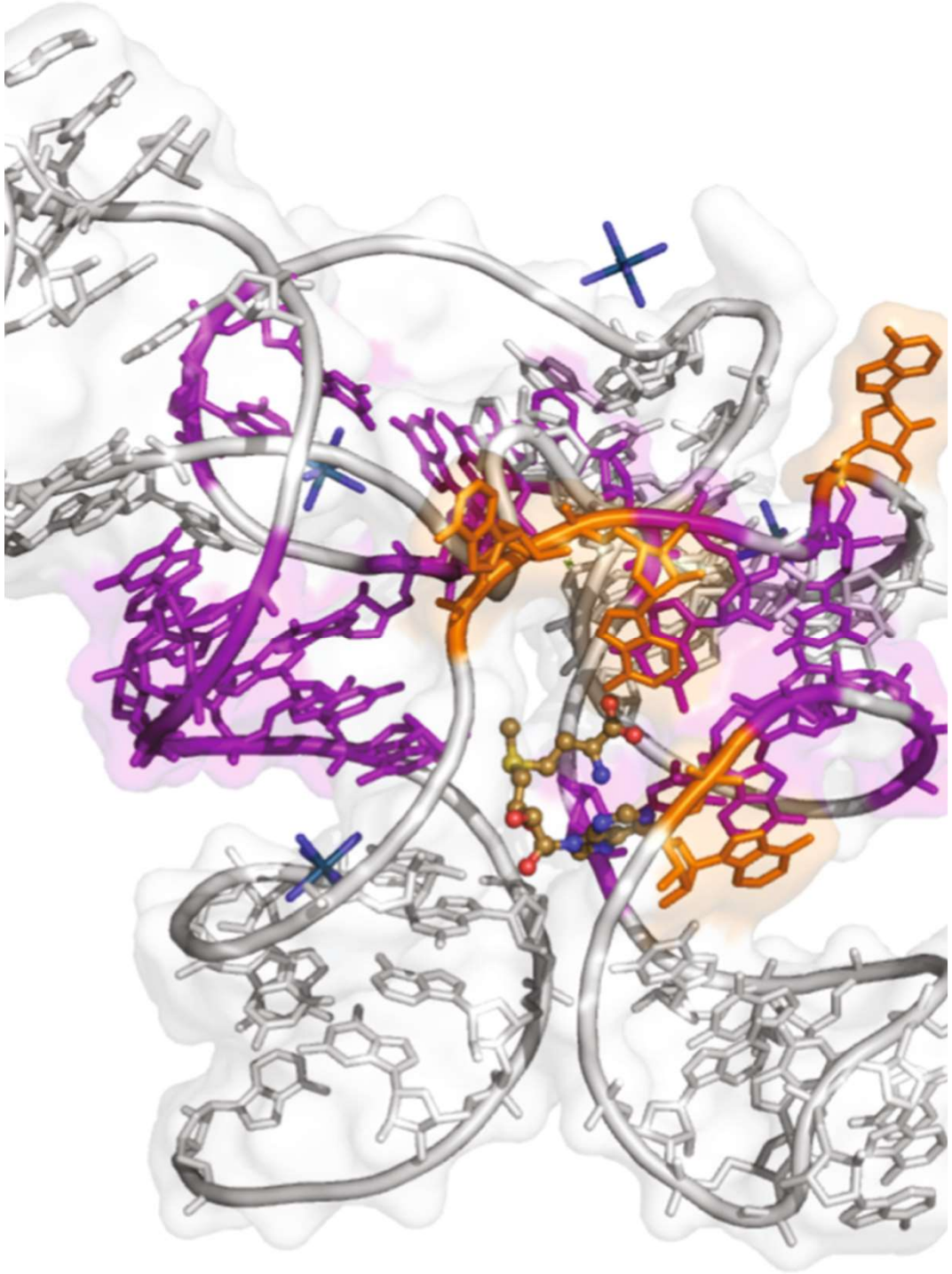


Probing into RNA structures

20180303

Literature Seminar

Yun-wei Xue

Contents

1. Introduction

2. Hydroxyl radical footprinting

3. LASER (main paper)

(Light Activated Structural Examination of RNA)

1.1 Roles of RNA in life

1. Translation from DNA to protein

   (mRNA, tRNA, rRNA)

2. RNA interference

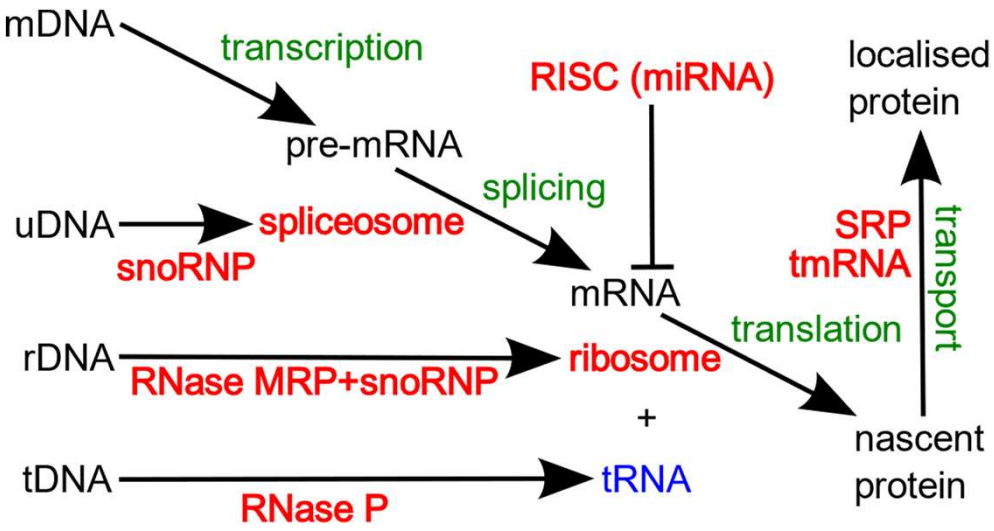
   (miRNA, siRNA, shRNA)

3. Other ncRNAs acting in regulation,

   RNA processing, reverse

   transcription, …

   (snoRNA, piRNA, dsRNA, …)



1) Non-coding RNA https://en.wikipedia.org/wiki/Non-coding_RNA#cite_note-MorrisKV-4 (accessed Feb 28, 2018).

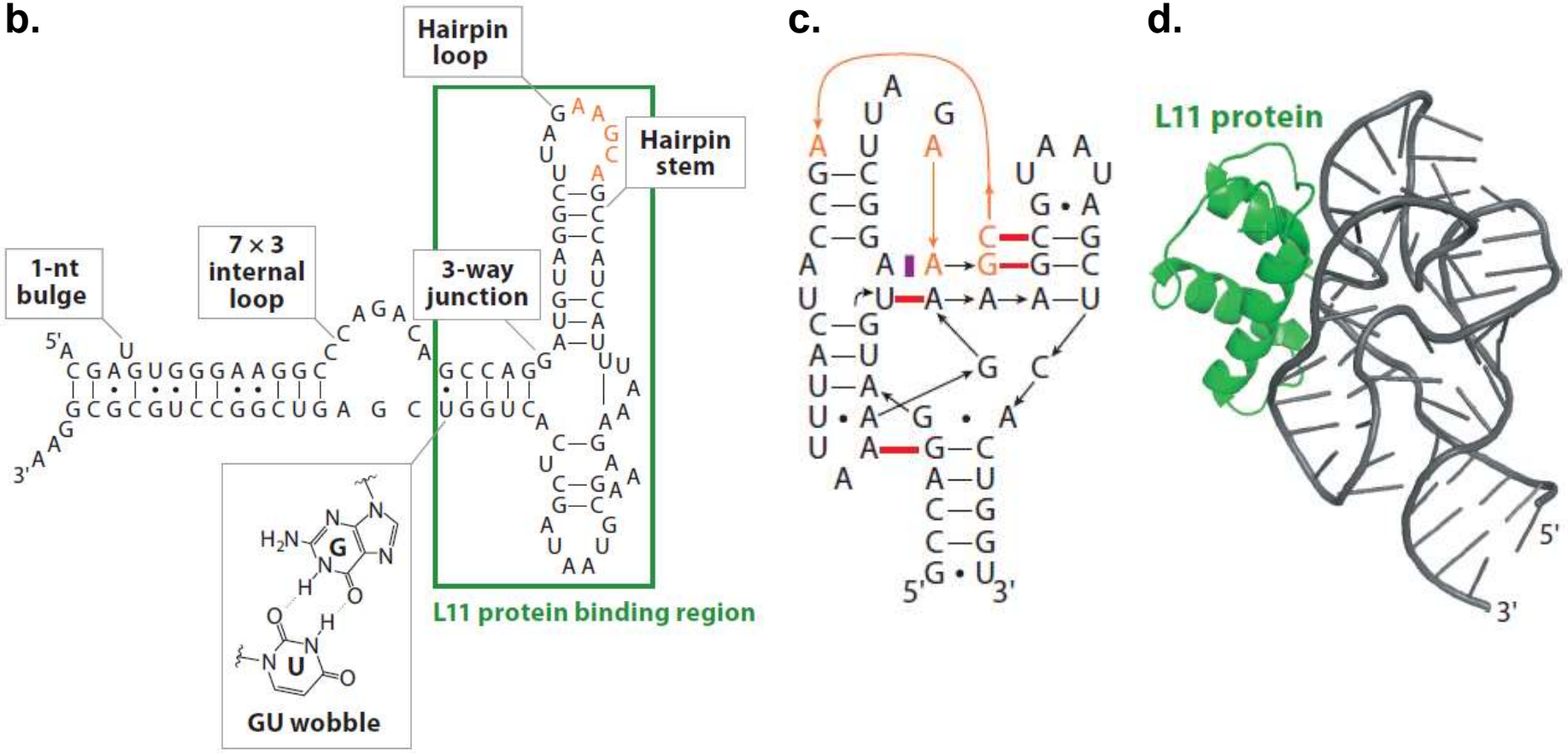
1.2 Overview of RNA structures

1. Single-strand (in most cases, different from DNA)
2. Complicated secondary and tertiary structure
3. Variable (not static)due to different surroundings

   (*in vitro* / *in vivo*)

1.3 Examples of complicated RNAs
—GTPase center RNA (PDB ID: IQA6)

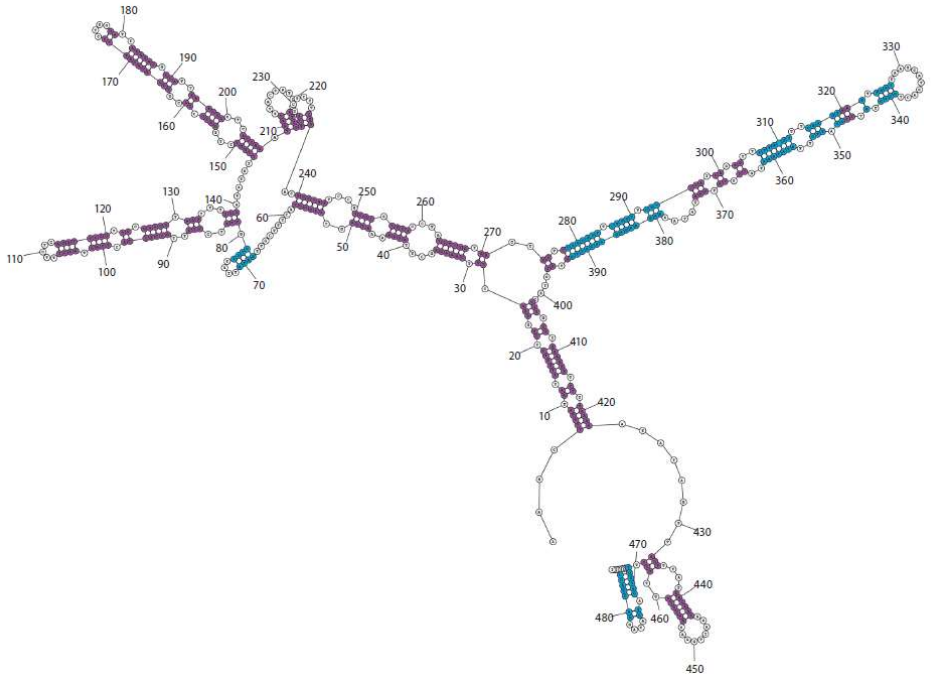
a. **5'**ACGAUGUGGGAAGGCCCAGACAGCCAGGAUGUUGGCUUAGAAGCAGCCAUCAUUUAAAGAAAGCGUAAUAGCUCACUGGUCGAGUCGGCCUGCGCGGAA**3'**



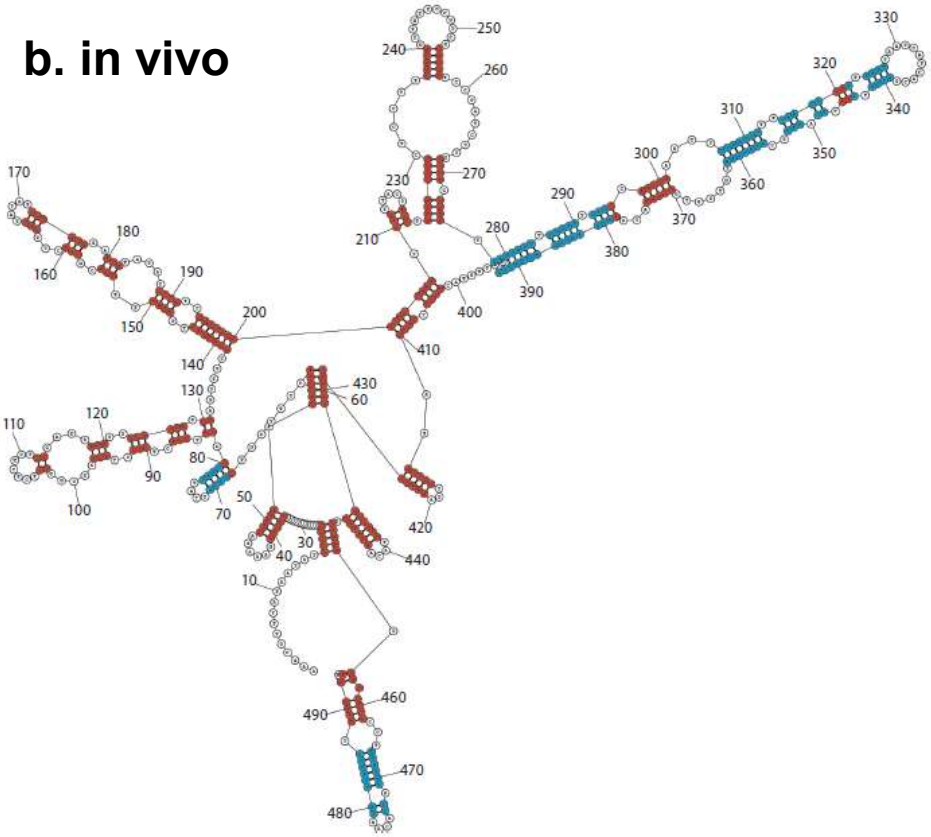
1) Bevilacqua, P. C. *Annual Review of Genetics* **2016**, *50* (1), 235–266.

1.3 Examples of complicated RNAs
—mRNA RCI2A

a. in silico



b. in vivo



1) Bevilacqua, P. C. *Annual Review of Genetics* **2016**, *50* (1), 235–266.

1.4 Parameters that affect RNA structure

1. Cations conc.

   (Mg^{2+}/Na^{+} ,tend to promote folding of RNA)

2. pH

3. Compatible solute

4. Crowding

5. Environment in living cells

   (Interaction with proteins or small molecules)

1) Bevilacqua, P. C. *Annual Review of Genetics* **2016**, *50* (1), 235–266.

1.5 Methods used for probe RNA

Table 1 Properties of some useful ribo-endonucleases

Enzyme	Specificity	Phosphates	Reference
RNase T_1	G	2', 3'	79
RNase CL 3	C	2', 3'	79
RNase U_2	A>G	2', 3'	79
RNase A	C, U	2', 3'	80
RNase Phy M	A, U	2', 3'	81
RNase T_2	Single strand	2', 3'	79
E. coli RNase I	Single strand	2', 3'	82
Nuclease S_1	Single strand	5'	83
Mung bean nuclease	Single strand	5'	84
Micrococcal nuclease	Single strand	3'	85
RNase V_1	Double strand	5'	86

control in 2.3 Example 1 (*in vitro*)

Table 2 Chemical probes used for nucleic acid structure determination

Probe	Specifity	Cleavage	Reference
Dimethylsulfate (DMS)	Adenine N1	No	90
Dimethylsulfate (DMS)	Cytosine N3	Yes	90
Dimethylsulfate (DMS)	Guanine N7	Yes	90
Ethylnitrosourea (ENU)	Phosphates	Yes	91
Diethylpyrocarbonate (DEP)	Adenine N7	Yes	92
CMCT[a]	Guanine N1	No	93
CMCT[a]	Uracil or thymine N3	No	93
Kethoxal[b]	Guanine N1–N2	No	93
Bromoacetaldehyde	Adenine N1–N6	No	94
Osmium tetroxide	Pyrimidine C5–C6	Yes	95
Potassium permanganate	Pyrimidine C5–C6	Yes	96
2:1,1,10-Phenanthroline-Cu^{2+}	Single strand	Yes	97
EDTA-Fe^{2+}	Solvent accessible regions	Yes	98, 99
Methidiumpropyl-EDTA-Fe^{2+}	Double strand	Yes	100
Uranyl acetate	Nonselective (light-activated)	Yes	101
$Rh(phen)_2phi^{3+}$[c]	Tertiary interaction sites (light-activated)	Yes	102, 103

[a] 1-Cyclohexyl-3-(2-morpholinoethyl) carbodiimide methane-p-toluene sulfonate
[b] β-Ethoxy-α-ketobutyraldehyde
[c] Bis(phenanthroline)(phenanthrenequinonediimine)-rhodium(III)

2.2-1 Fenton reaction of iron(Ⅱ) EDTA with hydrogen peroxide

Methods still in frequent use:

1. Dimethylsulfate (DMS) alkylation
2. Selective hydroxyl acylation (SHAPE)
3. Hydroxyl radical footprinting*
4. Ribo-endonucleases (e.g. RNase T)

1) Jaeger, J. *Annual Review of Biochemistry* **1993**, *62*(1), 255–287.

Contents

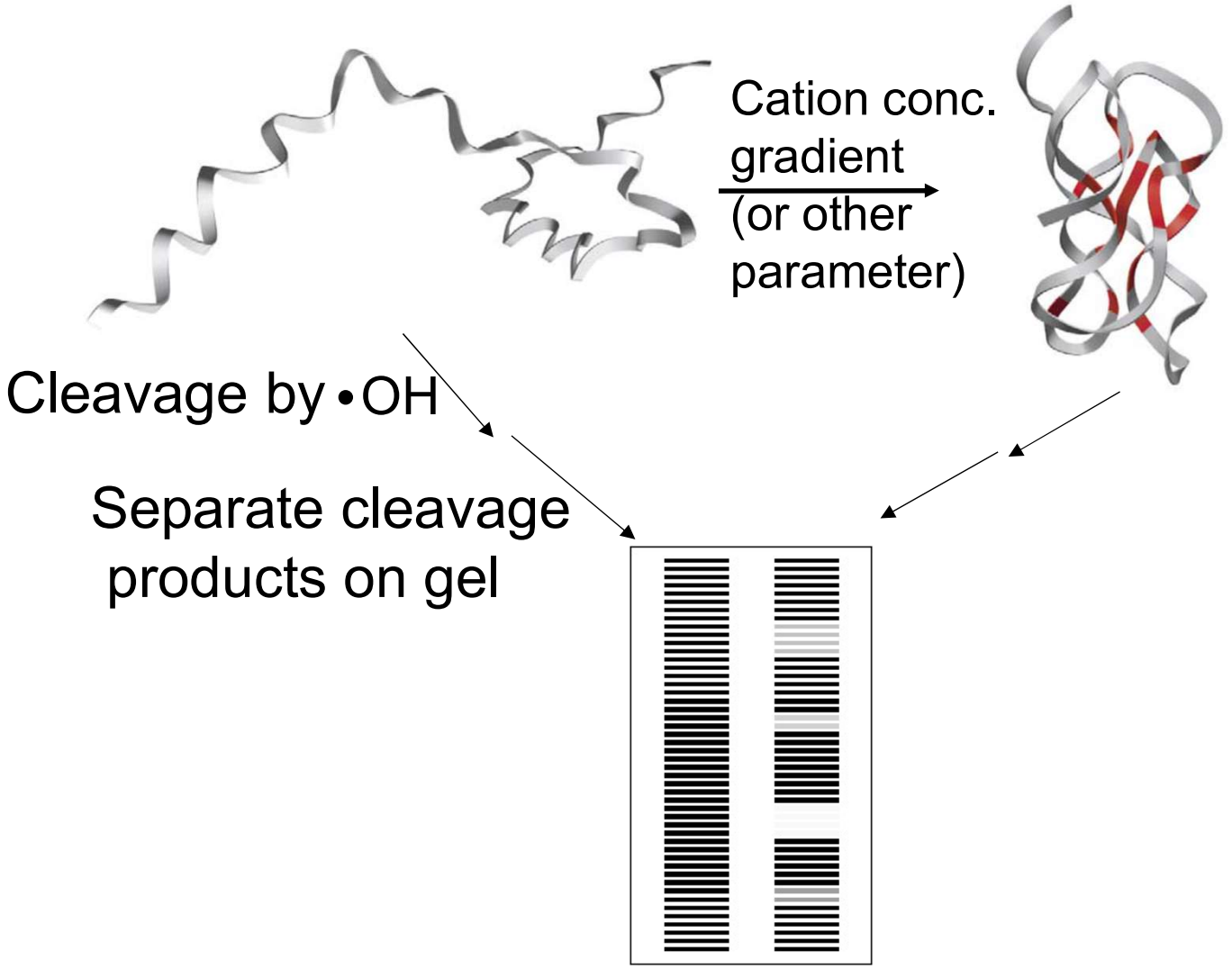
1. Introduction

2. Hydroxyl radical footprinting

3. LASER (main paper)

(Light Activated Structural Examination of RNA)

2.1 Overview of Hydroxyl radical footprinting of RNA



1) Tullius, T. D. *Current Opinion in Chemical Biology* **2005**, *9* (2), 127–134.

2.2 Method for generating •OH

*1. Fenton reaction of iron(Ⅱ) EDTA with hydrogen peroxide (common)

$$[Fe(II)(EDTA)]^{2-} + H_2O_2 \longrightarrow [Fe(III)(EDTA)]^{-} + {\bullet}OH + OH^{-}$$

2. Homolytic dissociation of peroxynitrous acid

$$ONOO^{-} + H^{+} \longrightarrow ONOOH$$

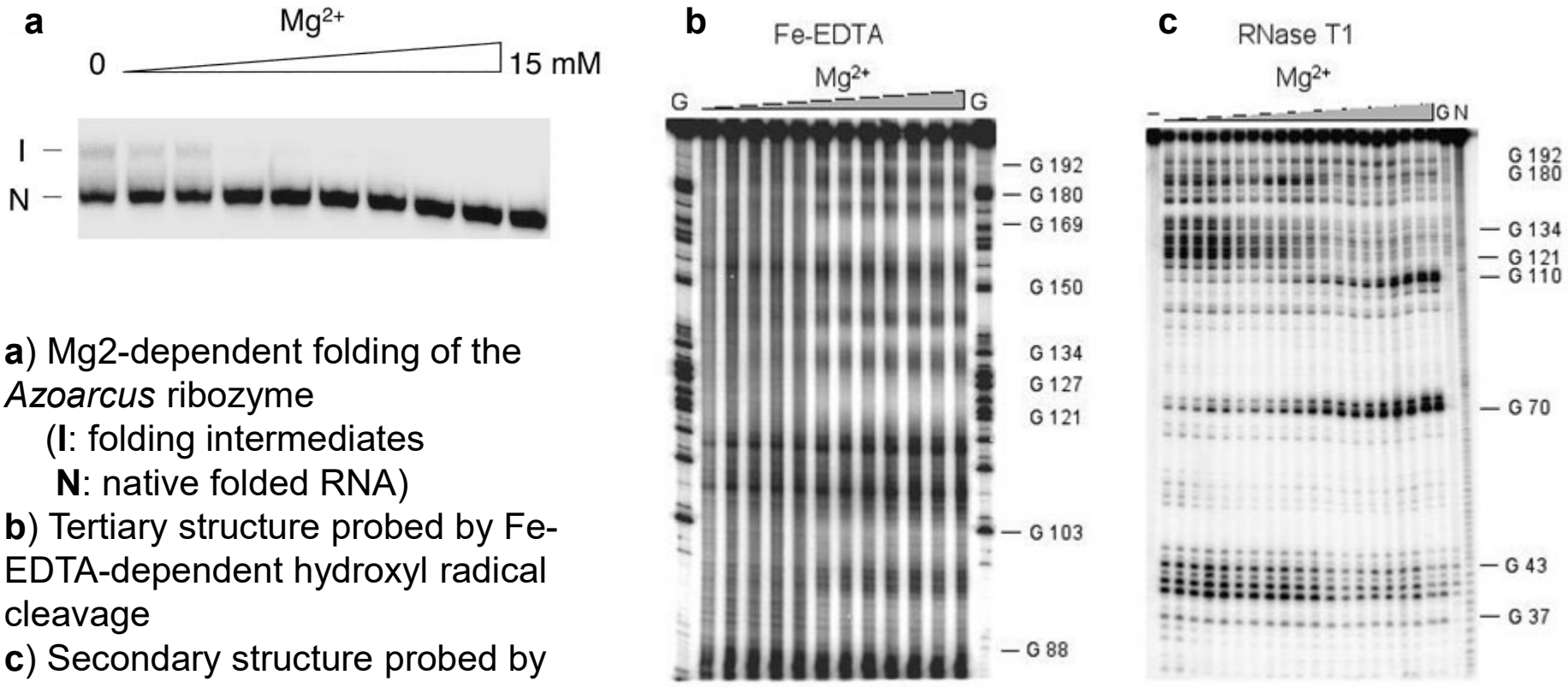
$$ONOOH \longrightarrow NO_2{\bullet} + {\bullet}OH$$

3. Synchrotron radiolysis of water (applied to *in vivo* use)

1)Tullius, T. D. *Current Opinion in Chemical Biology* **2005**, *9* (2), 127–134.
2) Adilakshmi, T. *Nucleic Acids Research* **2006**, *34* (8).

2.3 Example 1. (*in vitro*)

Target: *Azoarcus* group I ribozyme (195 nt)



a) Mg2-dependent folding of the *Azoarcus* ribozyme
(**I**: folding intermediates
N: native folded RNA)
b) Tertiary structure probed by Fe-EDTA-dependent hydroxyl radical cleavage
c) Secondary structure probed by RNase T1

1) Rangan, P. *Proceedings of the National Academy of Sciences* **2003**, *100* (4), 1574–1579.

2.4 Example 2. (*in vivo*)

Target: 16S rRNA(1,542 nt) in *E. coli*

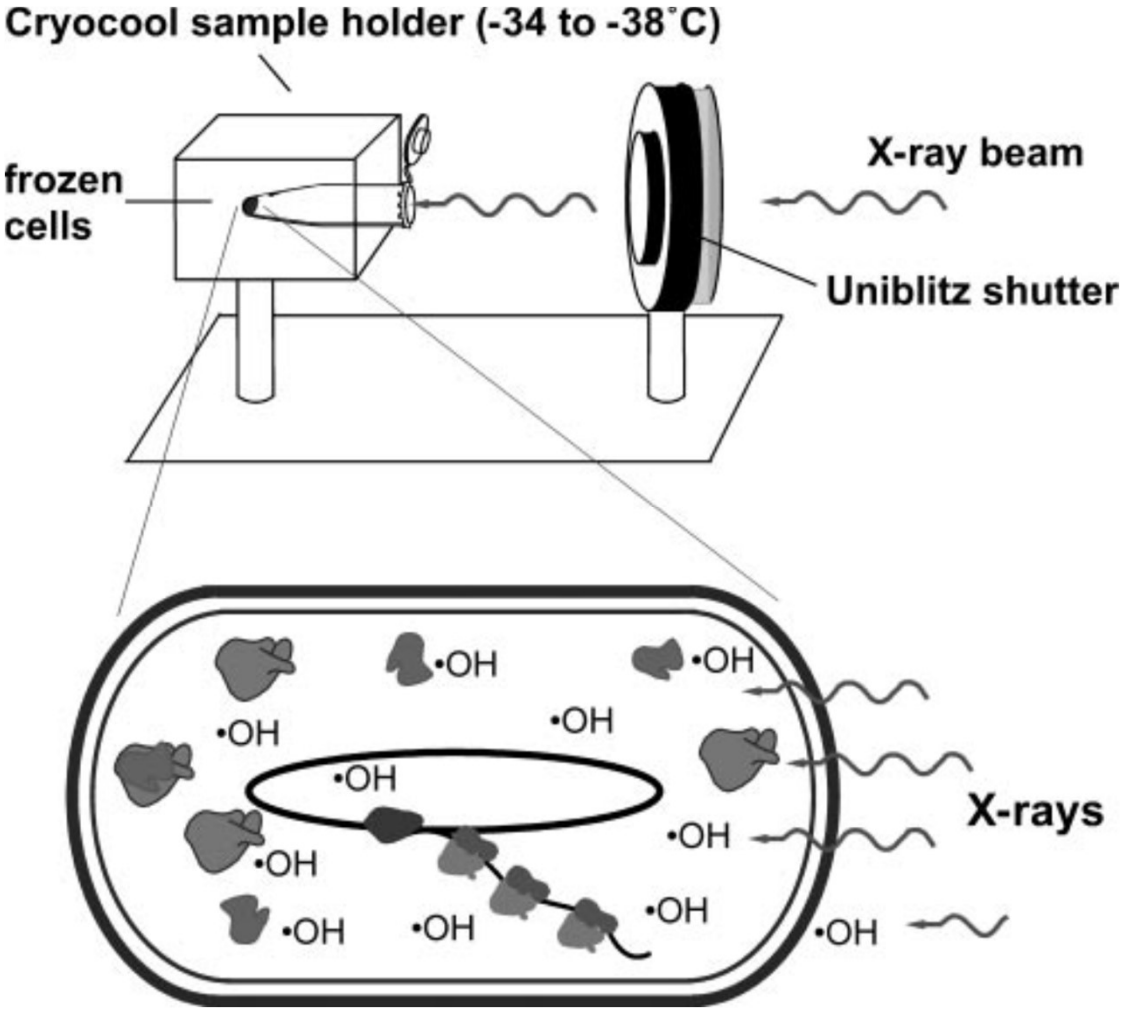
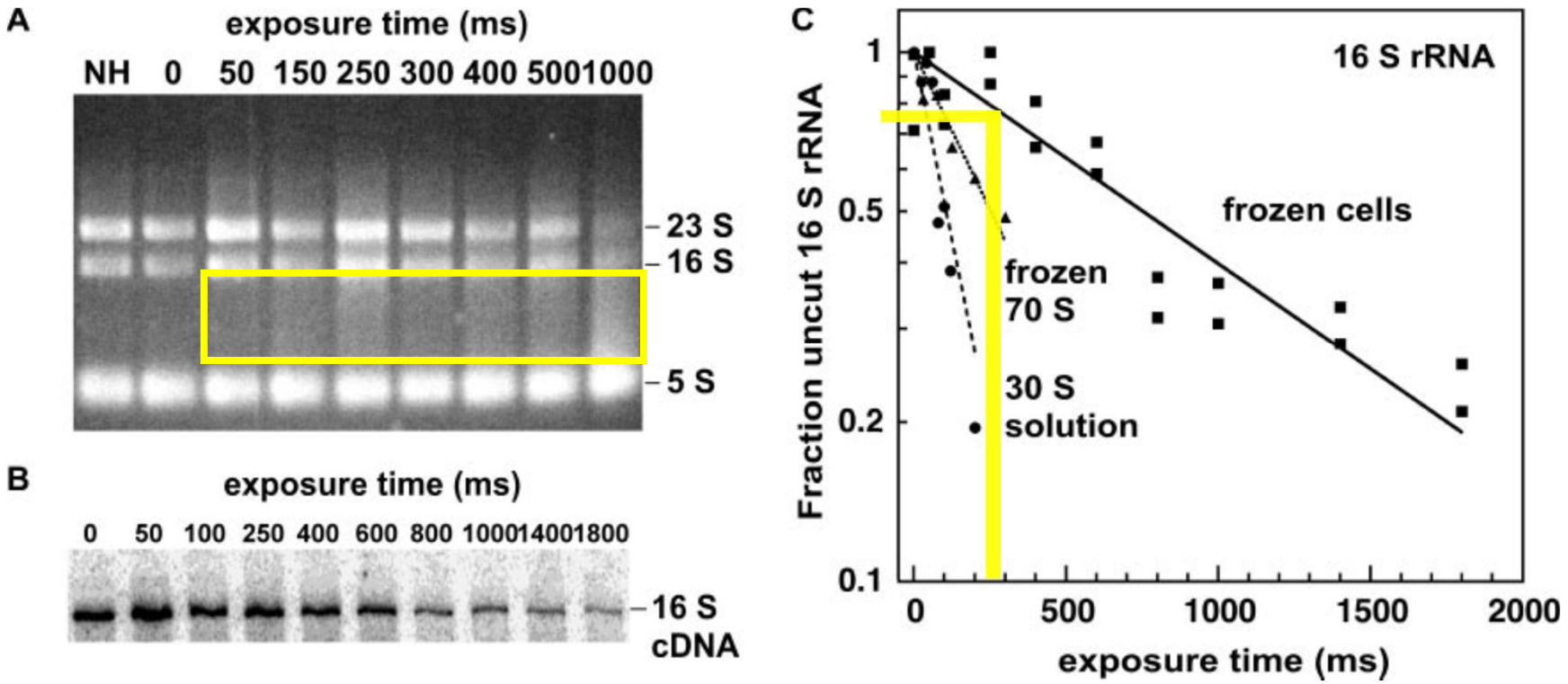


Diagram for *in vivo* hydroxyl radical footprinting
Absorption of X-rays by water in the sample generates ●OH, which cleaves accessible regions of the RNA backbone.

1) Adilakshmi, T. *Nucleic Acids Research* **2006**, *34*(8).

2.4 Example 2. (*in vivo*)

Target: 16S rRNA(1,542 nt) in *E. coli*



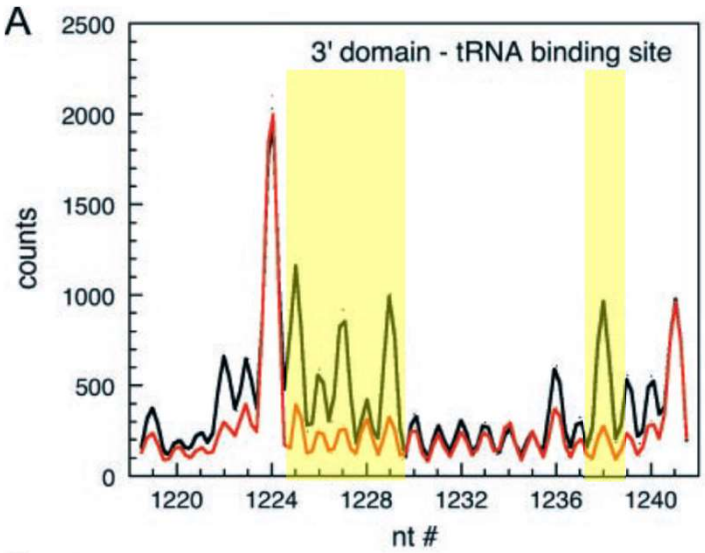
Determination of the optimal X-ray dose: **A**) Agarose gel electrophoresis of total cellular RNA **B**) Full-length cDNA of 16S rRNA from irradiated cells **C**) X-ray dose response curves for hydroxyl radical footprinting

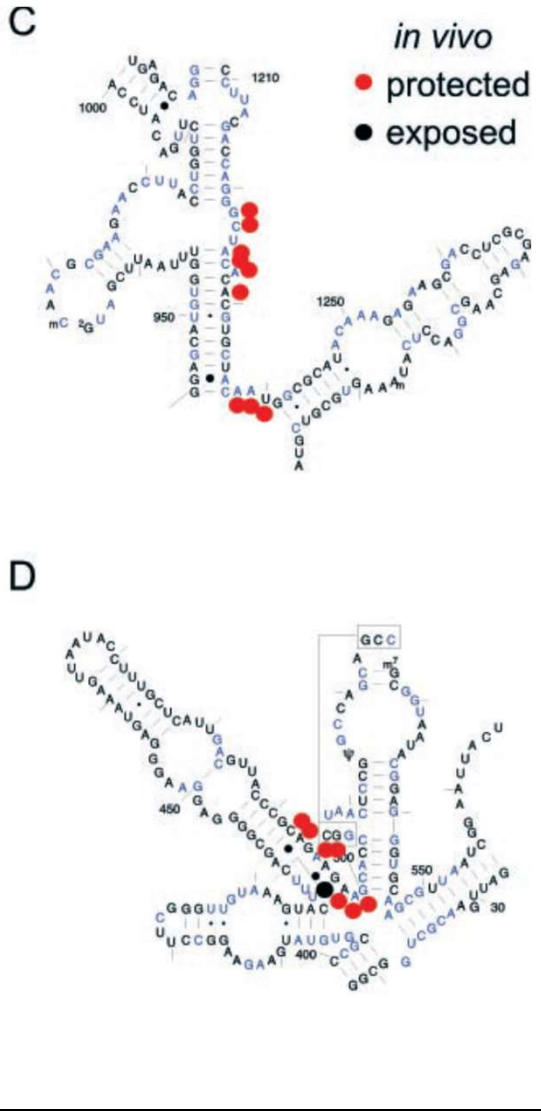
---

1) Adilakshmi, T. *Nucleic Acids Research* **2006**, *34*(8).

2.4 Example 2. (*in vivo*)

Target: 16S rRNA(1,542 nt) in *E. coli*





Comparison of *in vivo* and *in vitro* footprinting of 16S rRNA : **A**) nt 1218-1242 in the 16S 3' domain. **B**) nt 483-508 in the 5' domain. **C&D**) Differences in cleavage protection.

Red lines: in vivo(250 ms)
Black lines: in vitro (30 ms)

Red circles:
greater protection in vivo than in vitro
Black circles:
enhanced cleavage in vivo

1) Adilakshmi, T. *Nucleic Acids Research* **2006**, *34*(8).

2.5 Problems remain to be solved

*1. Limited use in cells due to experimental challenges

2. Lack in fundamental physical or quantum mechanical theory (hard to be rigorously calculated)

3. Unable to be applied in supercoiled structure (supercoiled DNA)

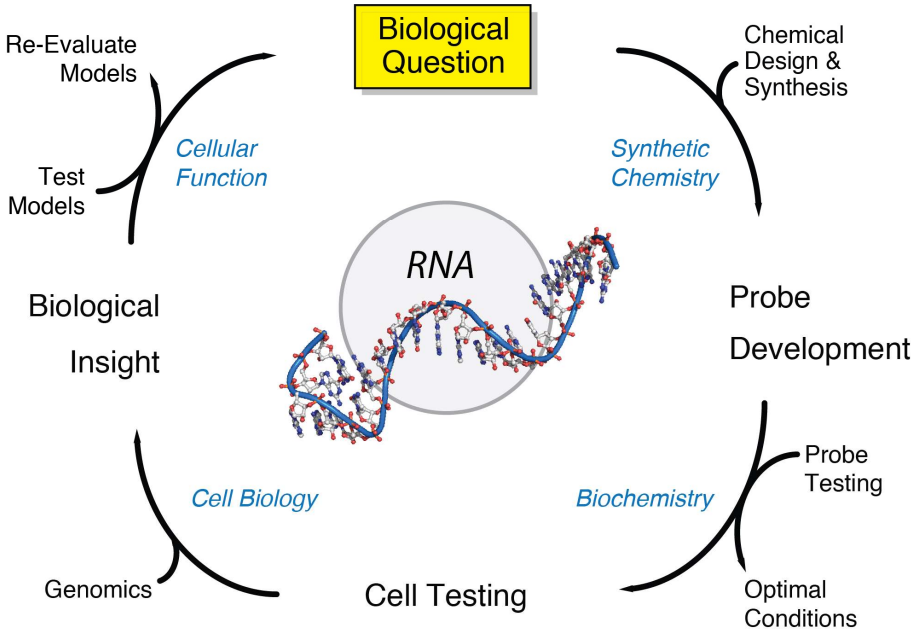
1) Tullius, T. D. *Current Opinion in Chemical Biology* **2005**, *9* (2), 127–134.
2) Spitale, R. C. *Nature Chemical Biology* **2018**, *14* (3), 325–325.

Contents

1. Introduction
2. Hydroxyl radical footprinting
3. LASER (main paper)

(Light Activated Structural Examination of RNA)

Robert Spitale



2000-2004, B.S. in Biology, B.S. in Chemistry
(S.U.N.Y. College at Fredonia, Prof. Mark E. Janik, synthetic organic/medicinal chemistry)

2004-2009 , Ph.D. in Chemistry
(University of Rochester, Joseph E. Wedekind, ncRNAs)

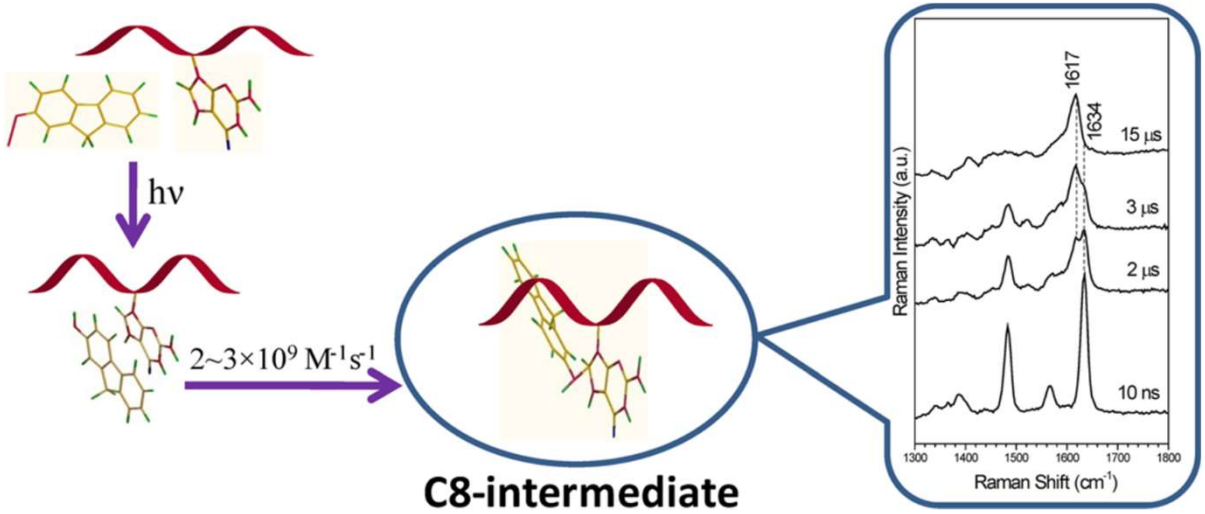
2010-2014, Postdoctoral Fellow
(Stanford University, Howard Y. Chang, Cancer and ncRNAs)

7/1/2014-Present, Assistant Professor
(University of California, Irvine)

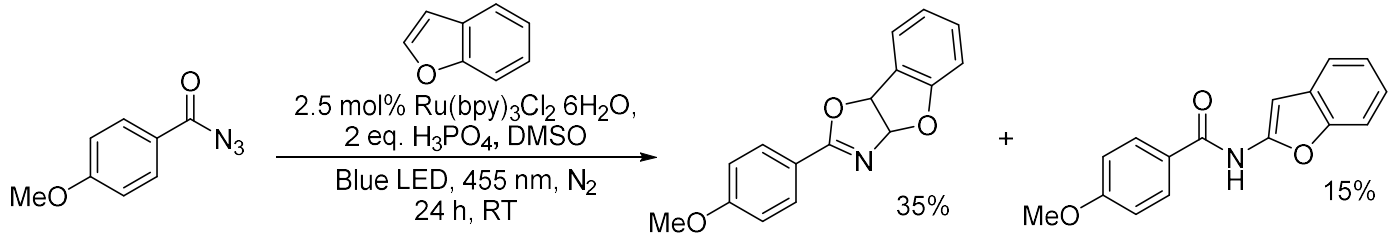
3.1 Design of chemical probe

Former results:

a) Aryl nitrenium ions: *react with solvent-accessible regions of adenosine (A) and guanosine (G) in DNA to form C8 adducts on picosecond time scales.*



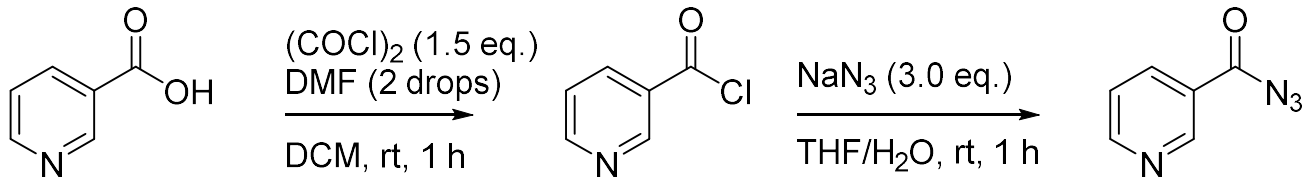
b) Aroyl nitrenium ions: *form single-site amidation products with electron-rich heteroarenes*



1) Phillips, D. L. *J. Org. Chem.* **2014**, *79* (8), 3610–3614.
2) Hadad, C. M. *J. Am. Chem. Soc.* **2009**, *131* (32), 11535–11547.
3) König, B. *Chemical Science* **2015**, *6* (2), 987–992.

3.1 Design of chemical probe

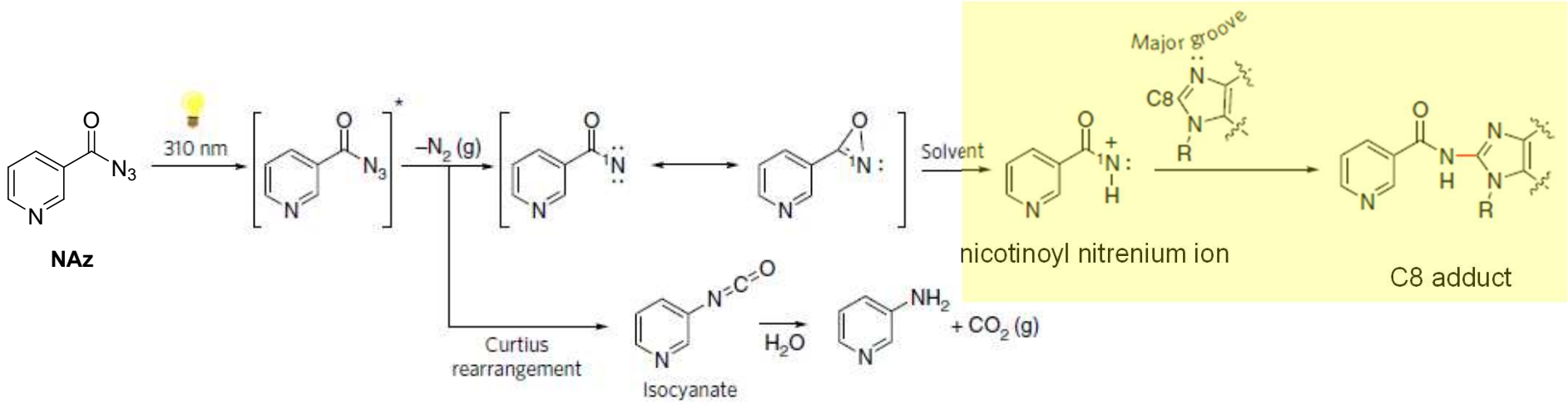
Designed probe with its synthetic route:



nicotinic acid

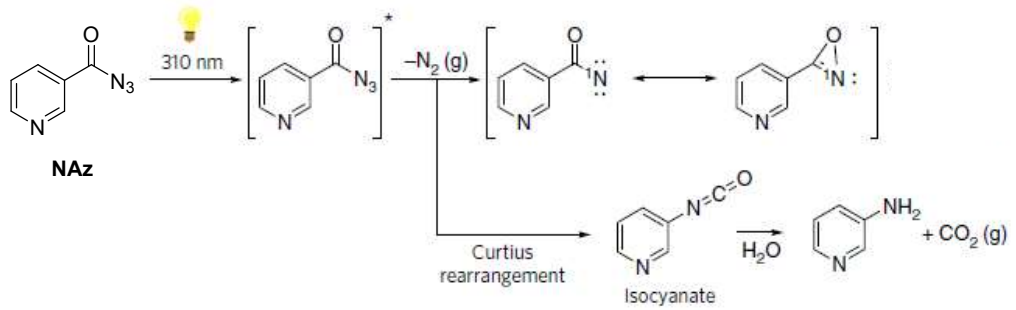
nicotinoyl azide ≡ **NAz**

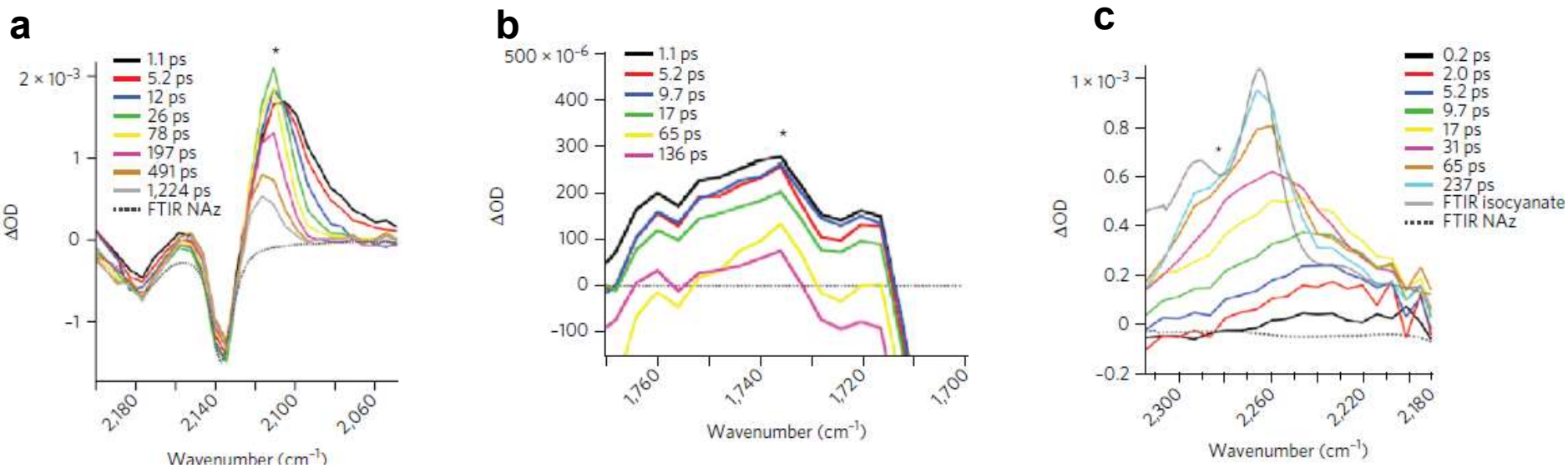
Working mechanism:



1) Spitale, R. C. *Nature Chemical Biology* **2018**, *14* (3), 325–325.

3.2 Excitation pathway of NAz

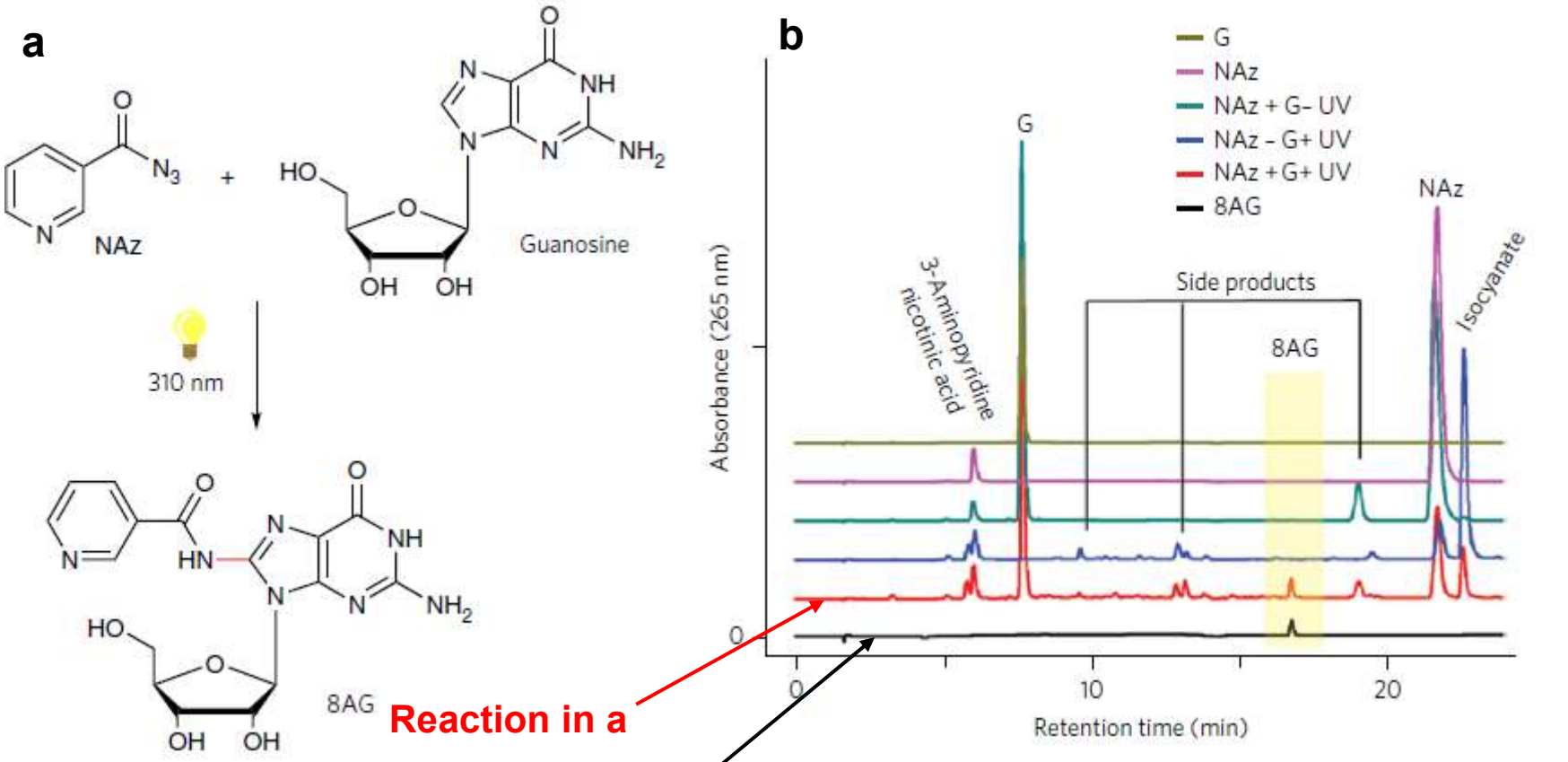




fs-TRIR spectra of **a**) the single state photoexcitation of NAz
b) nitrene formation from higher singlet excited state
c) formation of isocyanate after NAz photoexcitation

1) Spitale, R. C. *Nature Chemical Biology* **2018**, *14* (3), 325–325.

3.3 Examination using synthesized 8AG



Reaction in a

Synthesized **8AG** in 10 steps from Guanosine

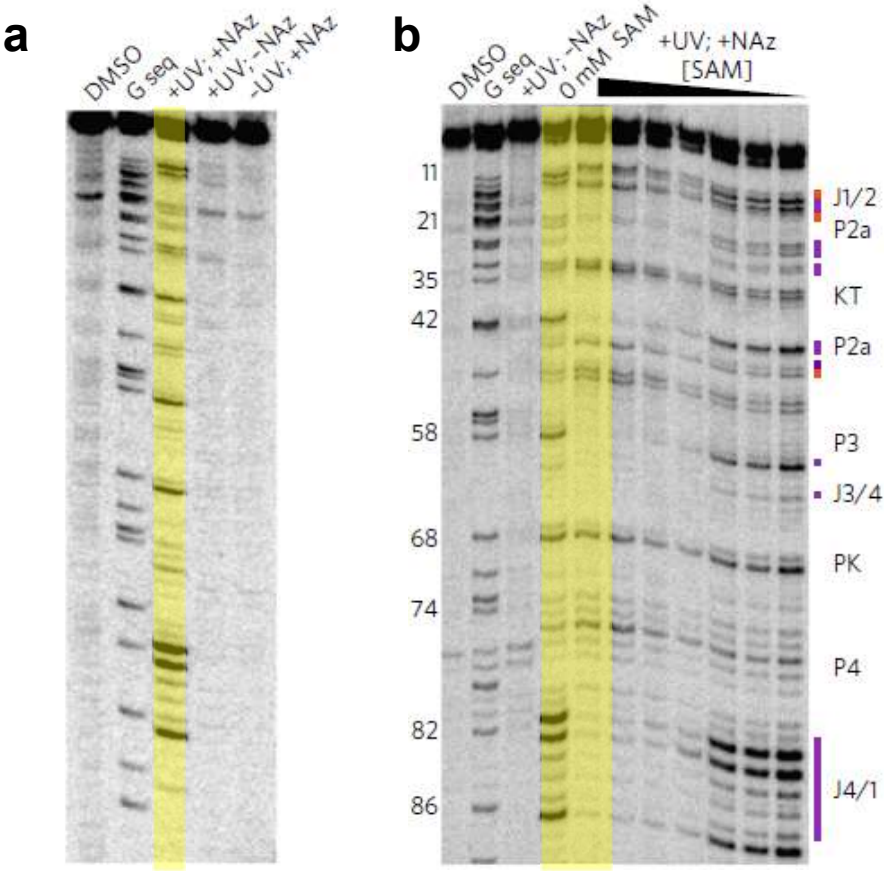
a) Reaction of NAz with guanosine to yield **8AG**
b) HPLC analysis of the reaction depicted in **a**.

1) Spitale, R. C. *Nature Chemical Biology* **2018**, *14* (3), 325–325.

3.4 NAz probes purine in folded RNA (*in vitro*)

Target: SAM- I riboswitch (PDB ID: 2GIS)

an RNA that binds S-adenosyl methionine (SAM)



a) Denaturing gel electrophoresis demonstrating the formation of adducts in the presence of NAz and light (**unfolded data**)

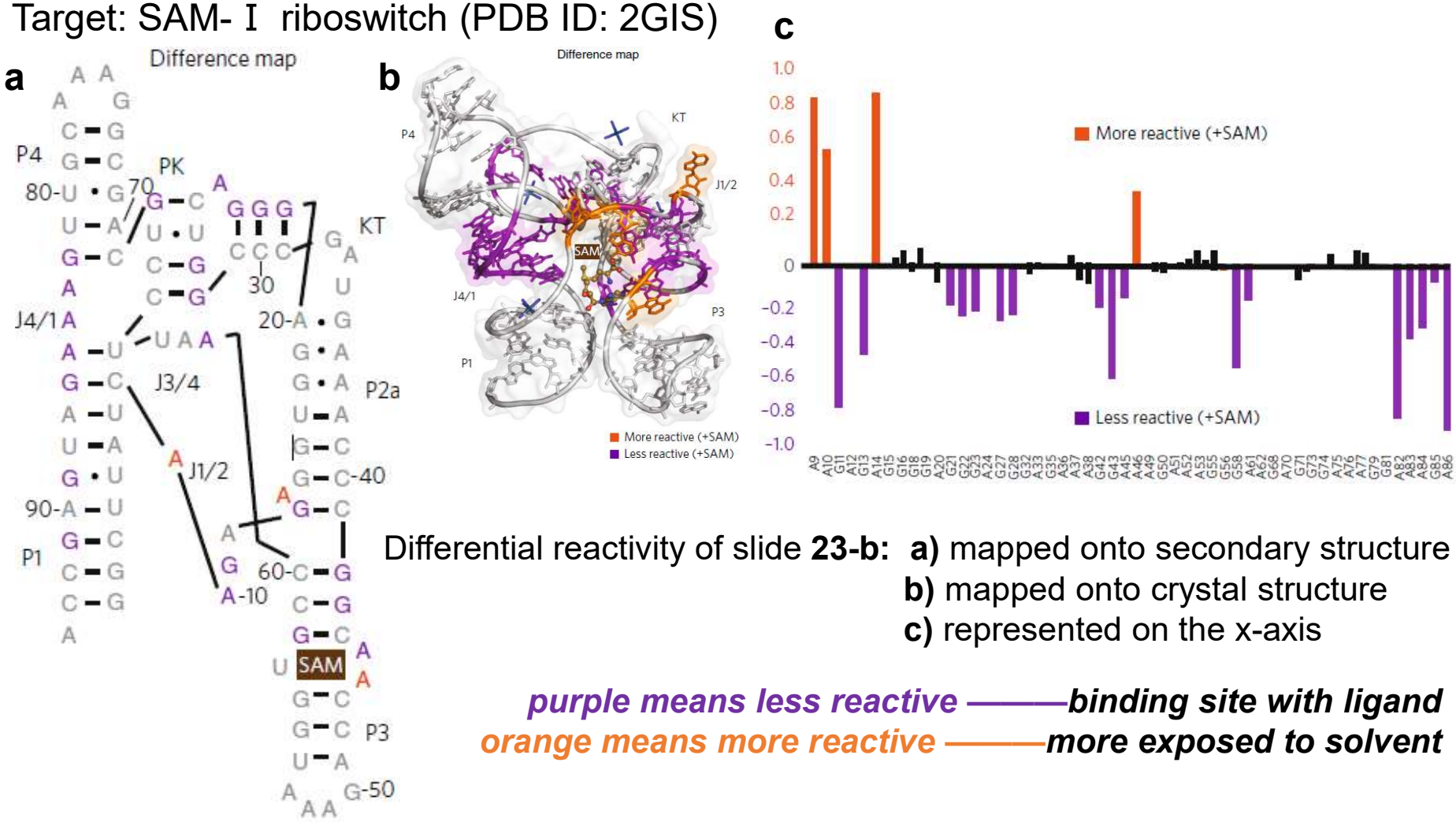
b) NAz probing of SAM-I-RNA with increasing amount of SAM

unfolded SAM-I riboswitch —a titration of SAM→ folded SAM-I riboswitch

1) Spitale, R. C. *Nature Chemical Biology* **2018**, *14* (3), 325–325.

3.4 NAz probes purine in folded RNA (*in vitro*)

Target: SAM- Ⅰ riboswitch (PDB ID: 2GIS)



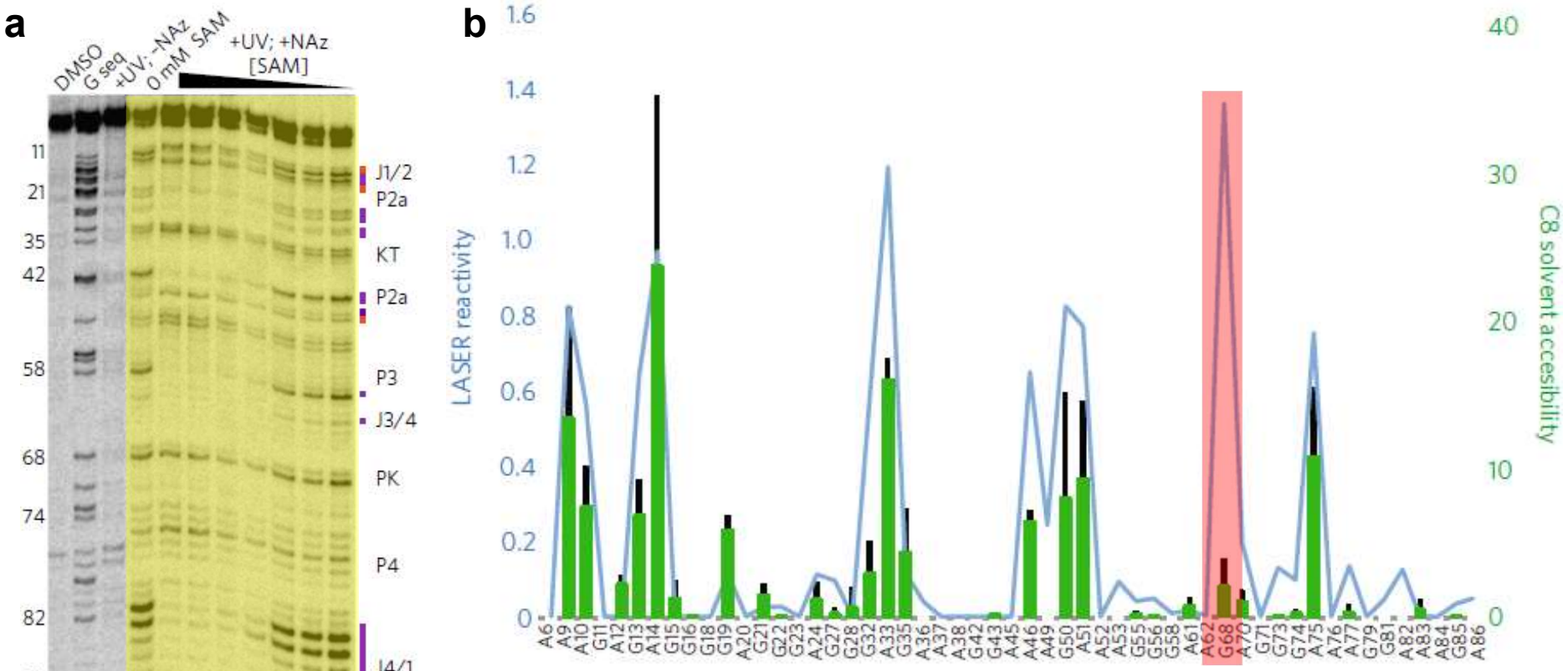
Differential reactivity of slide **23-b:** **a)** mapped onto secondary structure
b) mapped onto crystal structure
c) represented on the x-axis

purple means less reactive ———binding site with ligand
orange means more reactive ———more exposed to solvent

1) Spitale, R. C. *Nature Chemical Biology* **2018**, *14* (3), 325–325.

3.4 NAz probes purine in folded RNA (*in vitro*)

Target: SAM- I riboswitch (PDB ID: 2GIS)



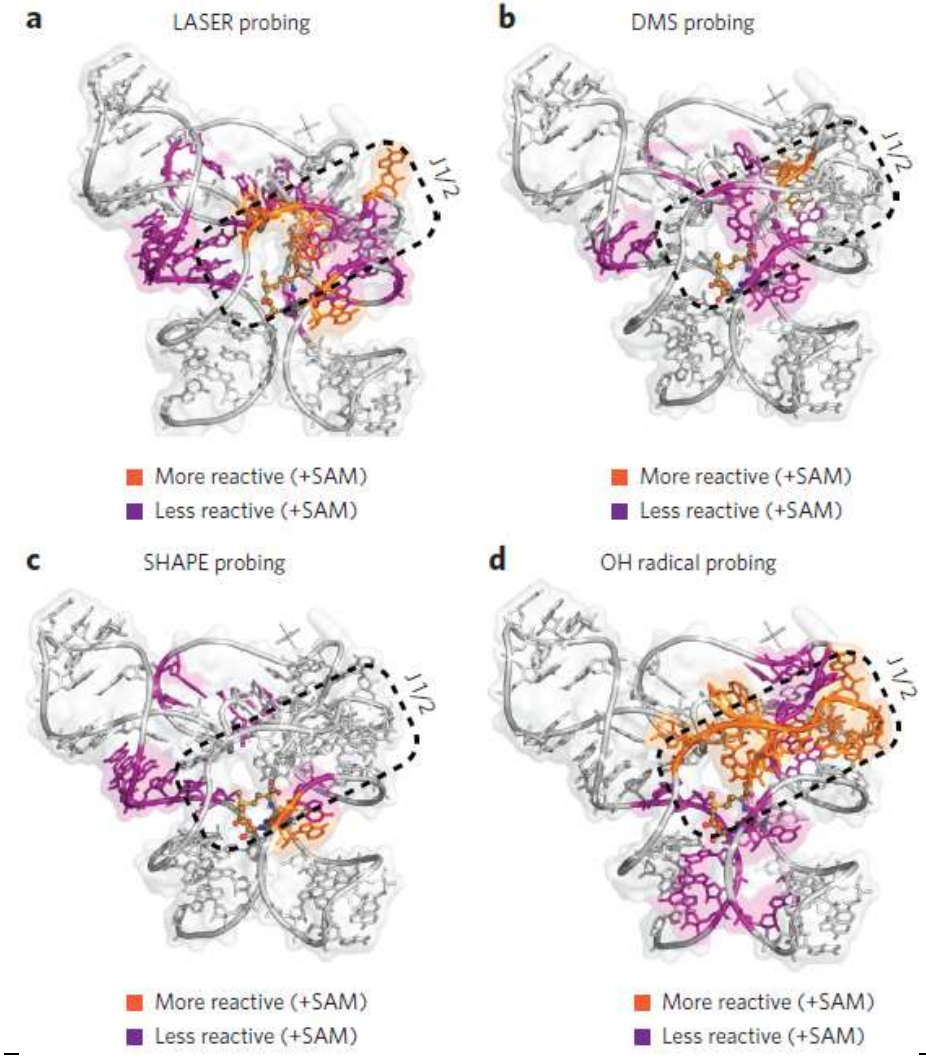
a) NAz probing of SAM-I-RNA with increasing amount of SAM (same as slide 23-b)

b) Blue lines: LASER reactivity derived from band intensity of **a**
green bars: Computed solvent accessibility with PyMOL

1) Spitale, R. C. *Nature Chemical Biology* **2018**, *14* (3), 325–325.

3.5 Comparison with other chemical probes (*in vitro*)

Target: SAM- I riboswitch (PDB ID: 2GIS)



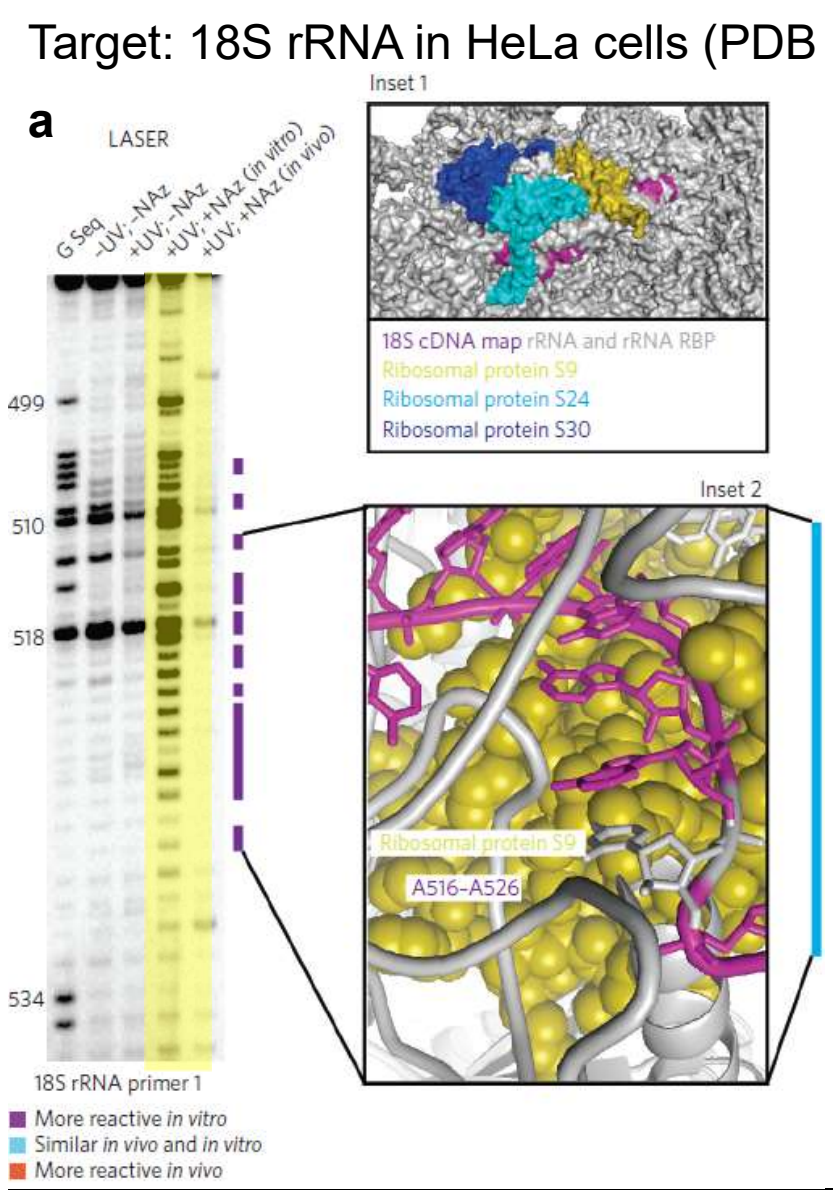
The SAM-I crystal structure mapped with
a) LASER
b) DMS (dimethylsulfate)
c) SHAPE (selective hydroxyl acylation)
d) Hydroxyl radical probing
(discussed in section 2)

purple means less reactive
orange means more reactive
grey means no apparent difference

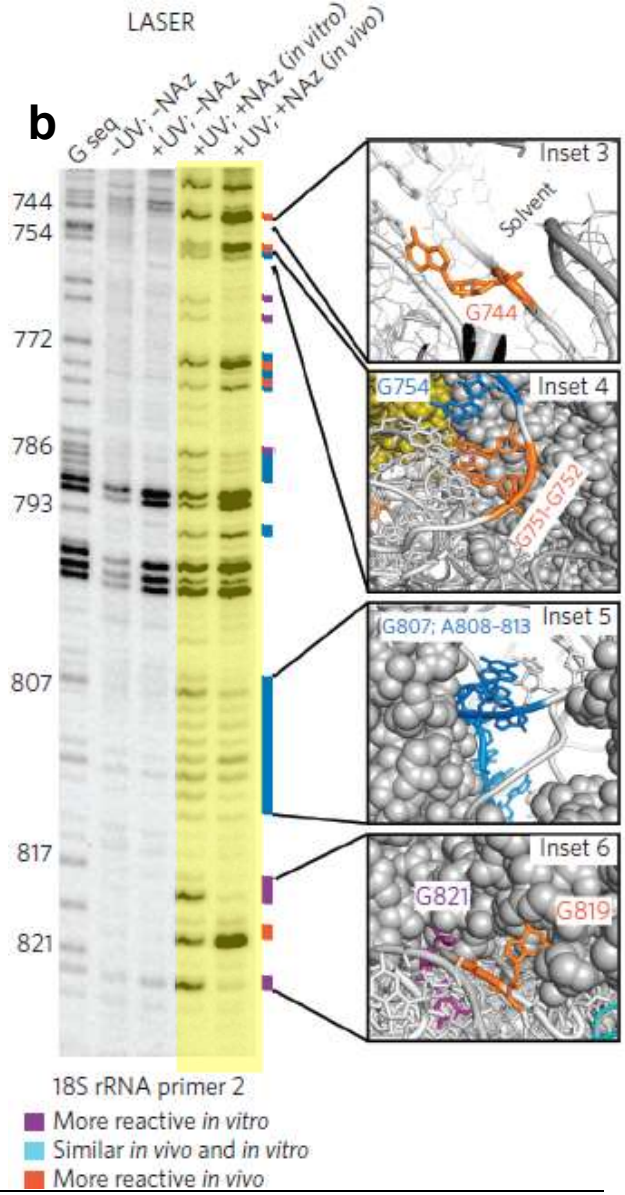
1) Spitale, R. C. *Nature Chemical Biology* **2018**, *14* (3), 325–325.

3.6 NAz probes purine in living cells (*in vivo*)

Target: 18S rRNA in HeLa cells (PDB ID: 4V6X)



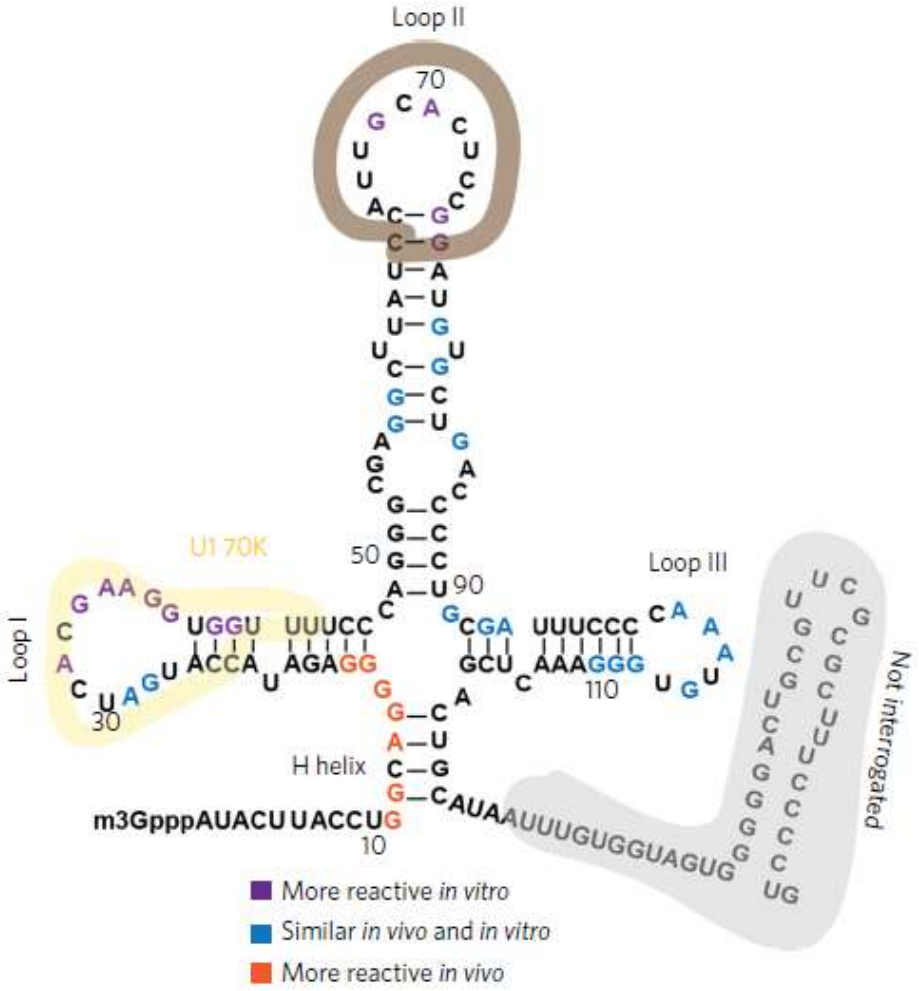
a) LASER probing into section 1 of 18S RNA
b) LASER probing into section 2 of 18S RNA



1) Spitale, R. C. *Nature Chemical Biology* **2018**, *14* (3), 325–325.

3.7 LASER probing of the U1 snRNP inside living cells (*in vivo*)

Target: U1 snRNP in HeLa cells (PDB ID: 4PKD)



Secondary structure of the U1 snRNP

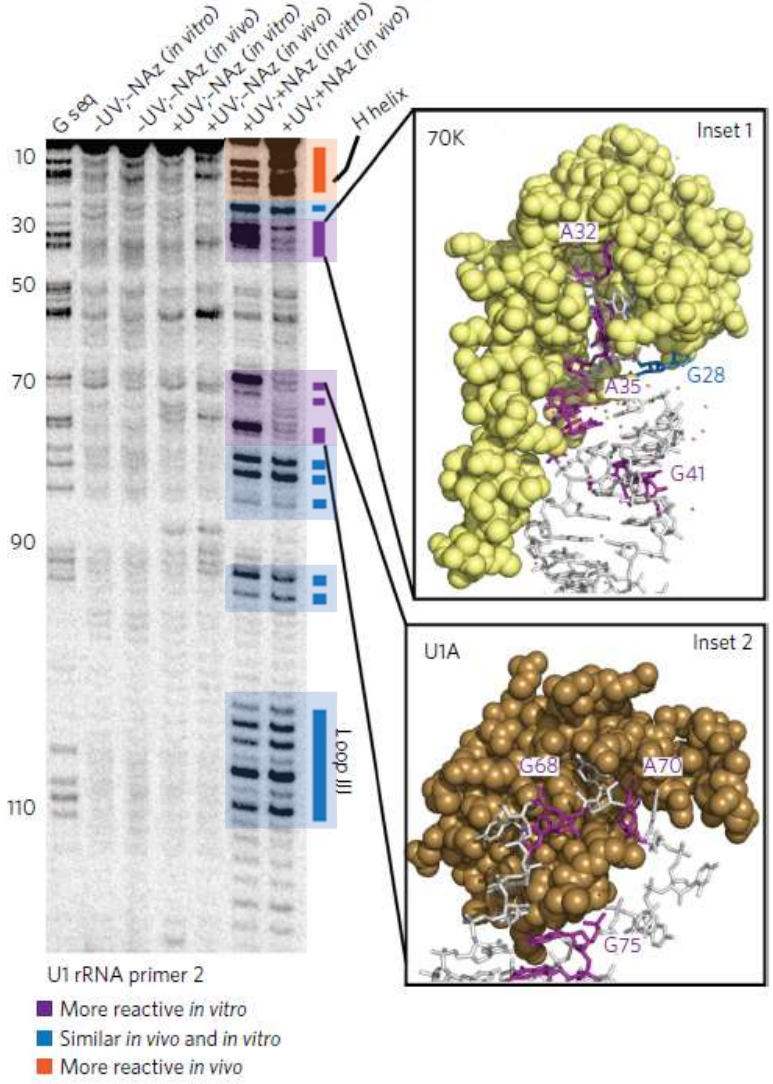
(Utilize lower expressed RNAs inside living system in order to test the limit of this new method LASER)

1) Spitale, R. C. *Nature Chemical Biology* **2018**, *14* (3), 325–325.

3.7 LASER probing of the U1 snRNP inside living cells (*in vivo*)

Target: U1 snRNP in HeLa cells (PDB ID: 4PKD)

Denaturing gel electrophoresis of LASER on U1 snRNP & close up of residues that have differential NAz probing and their relationship to the U1 and 70K proteins.

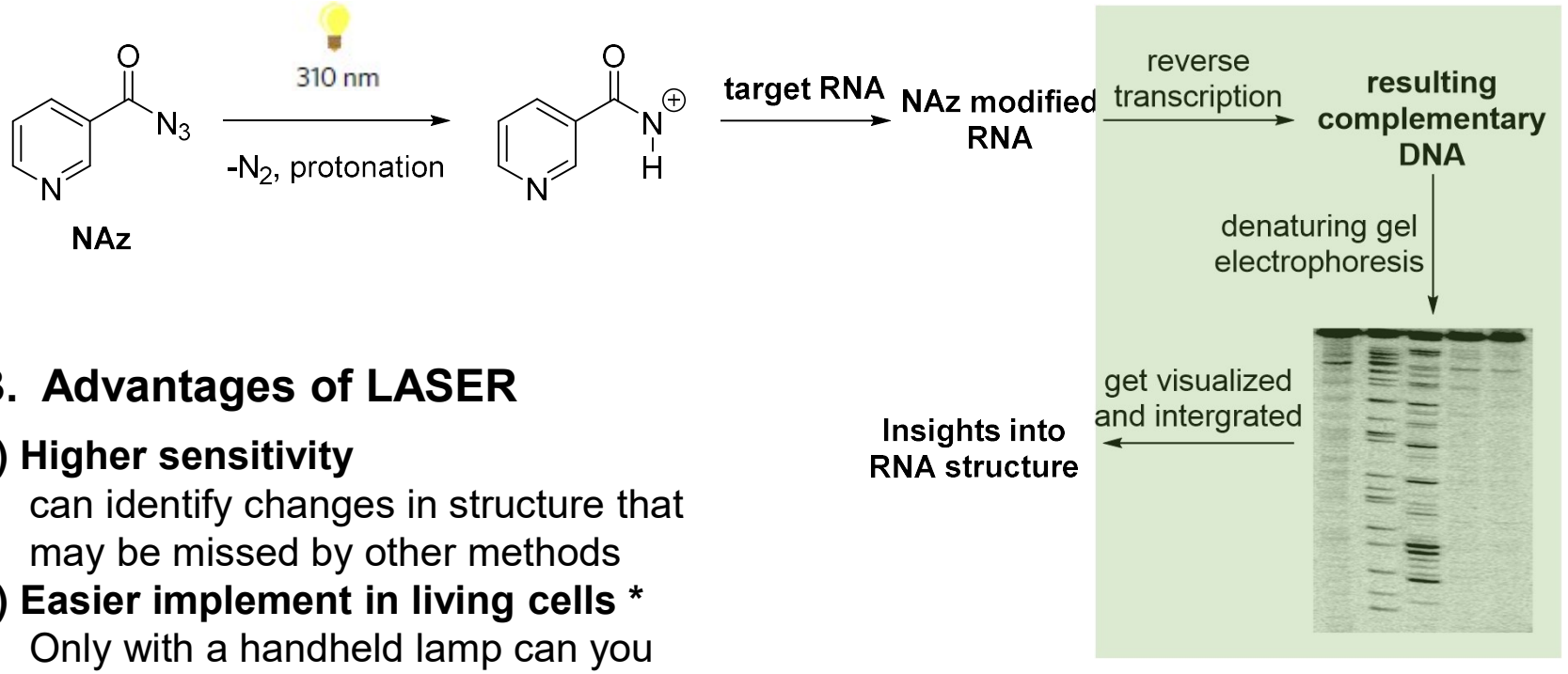


purple means more reactive *in vitro*
blue means similar reactivity
orange means more reactive *in vivo*

1) Spitale, R. C. *Nature Chemical Biology* **2018**, *14* (3), 325–325.

Summary

A. Overall working proposal of LASER



B. Advantages of LASER

1) Higher sensitivity

can identify changes in structure that may be missed by other methods

2) Easier implement in living cells *

Only with a handheld lamp can you measure unique structural motifs of RNA in its native environment (also works when treating RNAs of lower expression)

1) Spitale, R. C. *Nature Chemical Biology* **2018**, *14* (3), 325–325.

Appendix

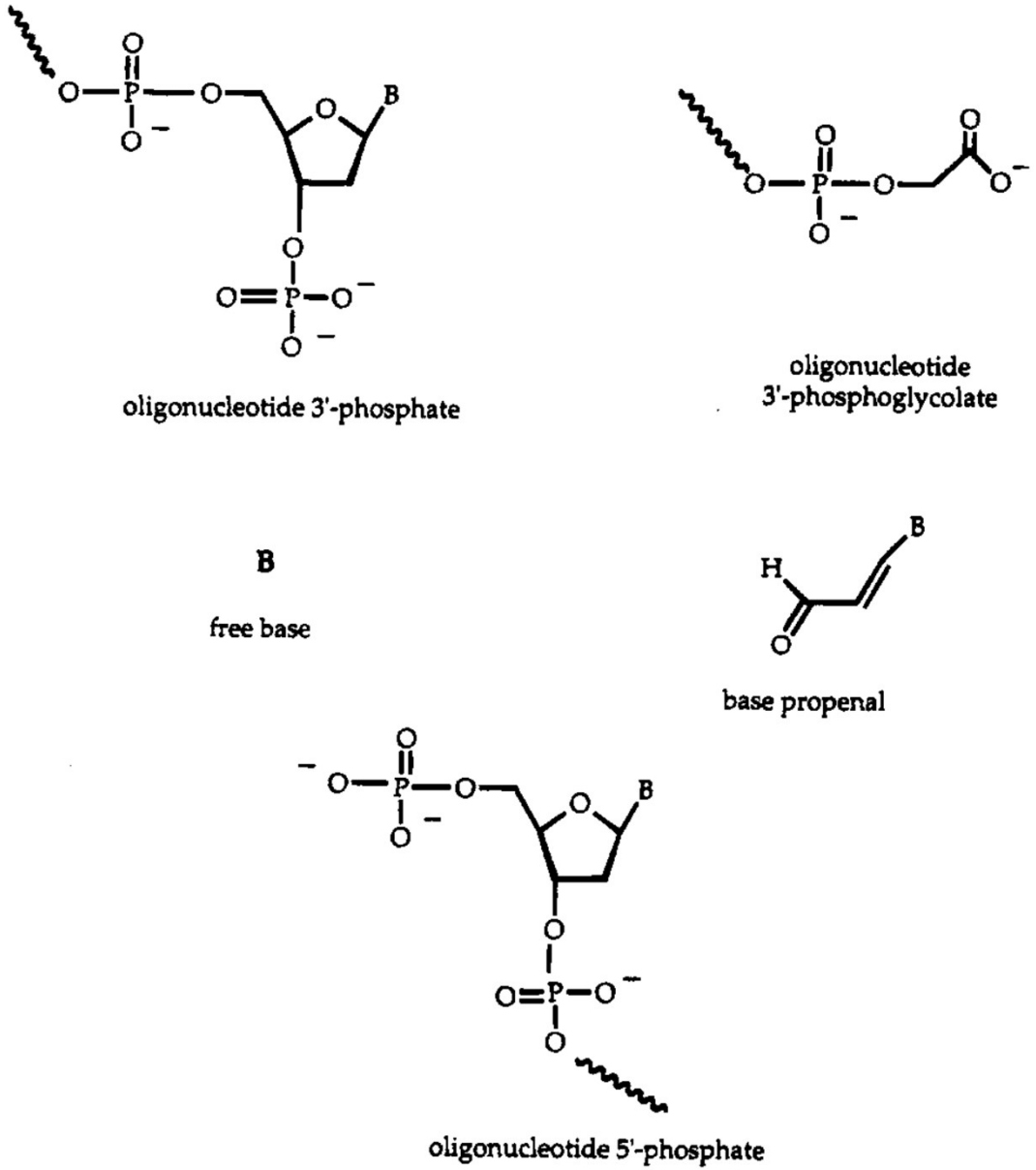
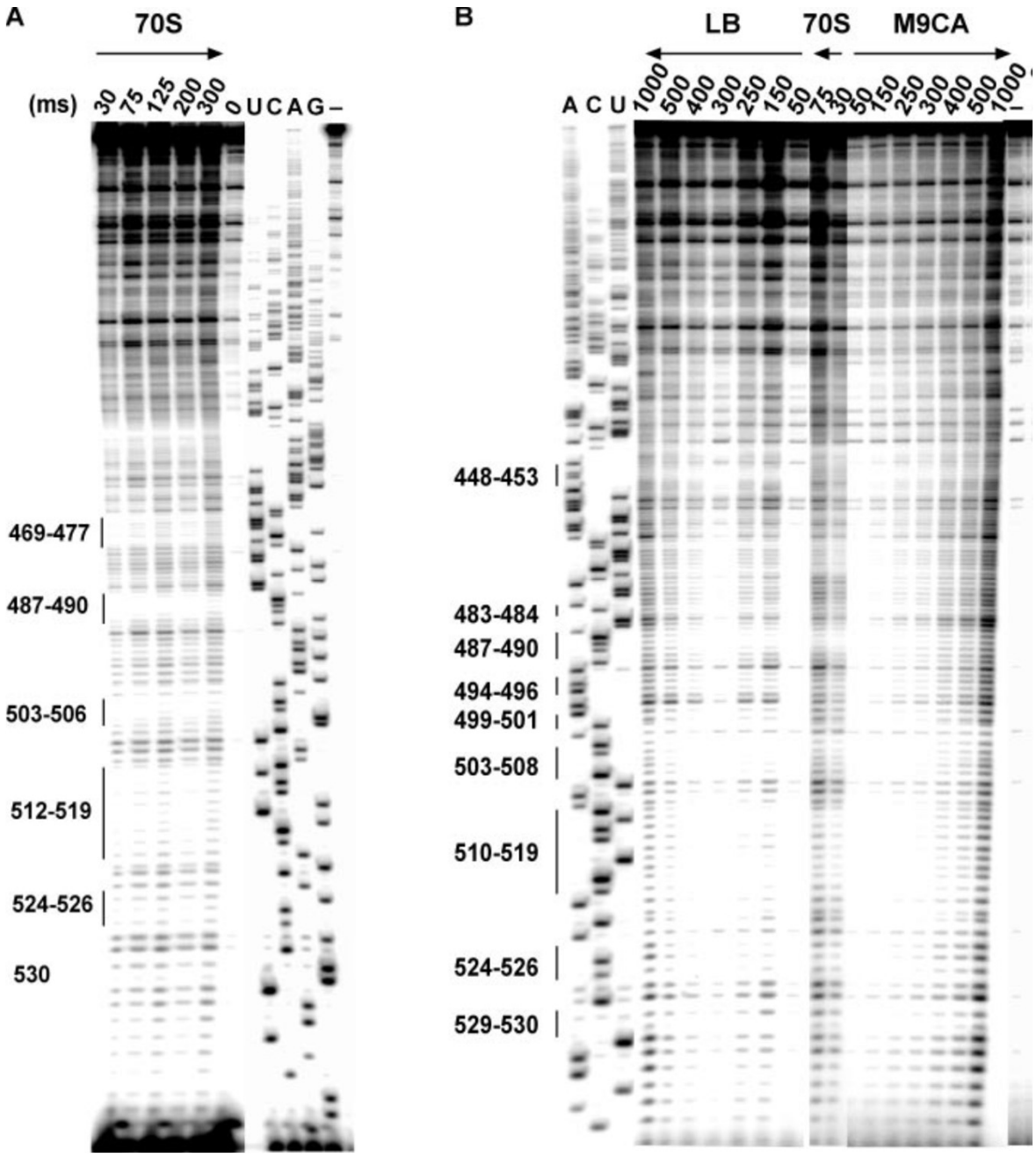
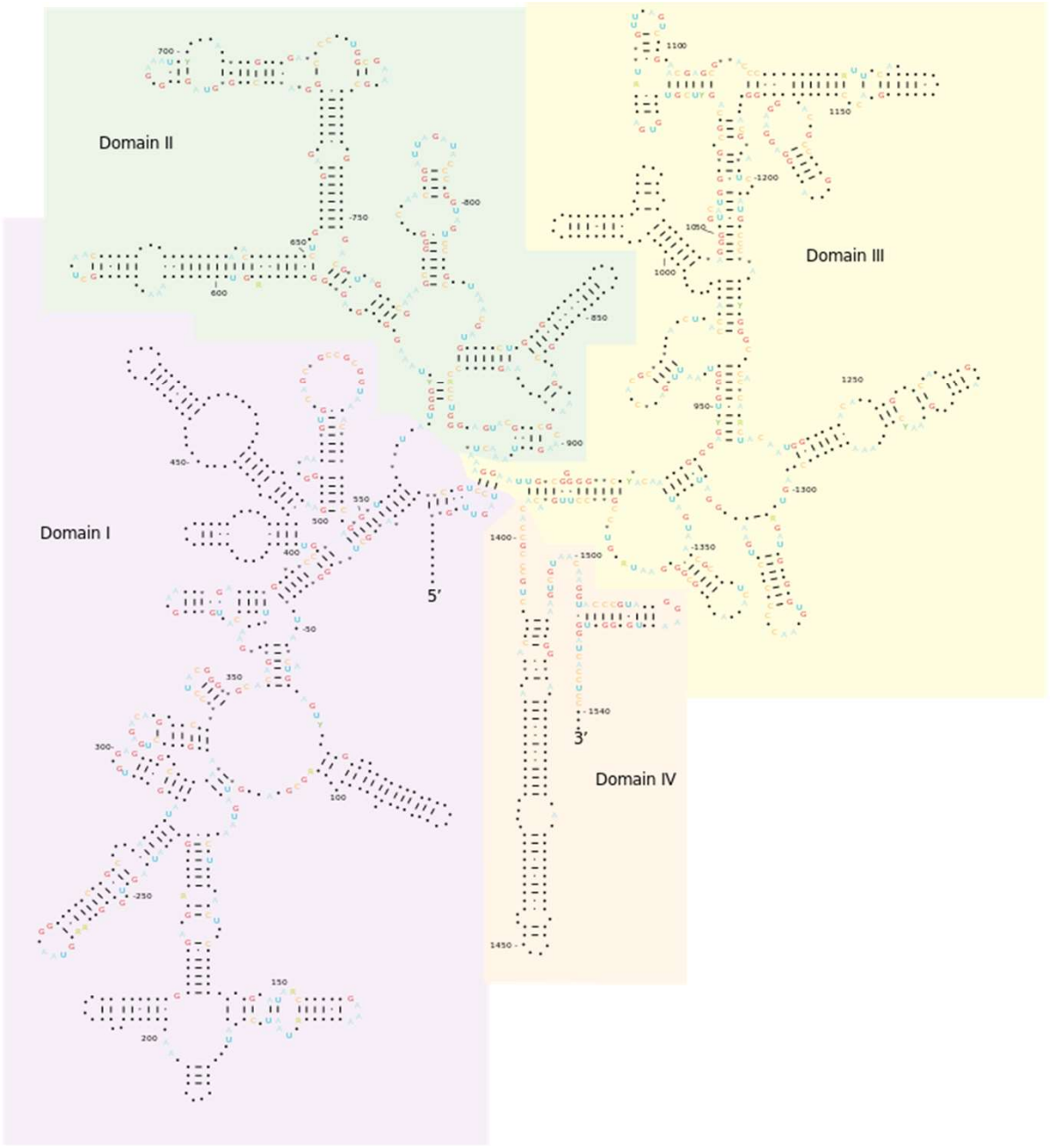


Figure 1. Products of DNA scission induced by the reaction of [Fe-(EDTA)]$^{2-}$ + H_2O_2 + ascorbate.

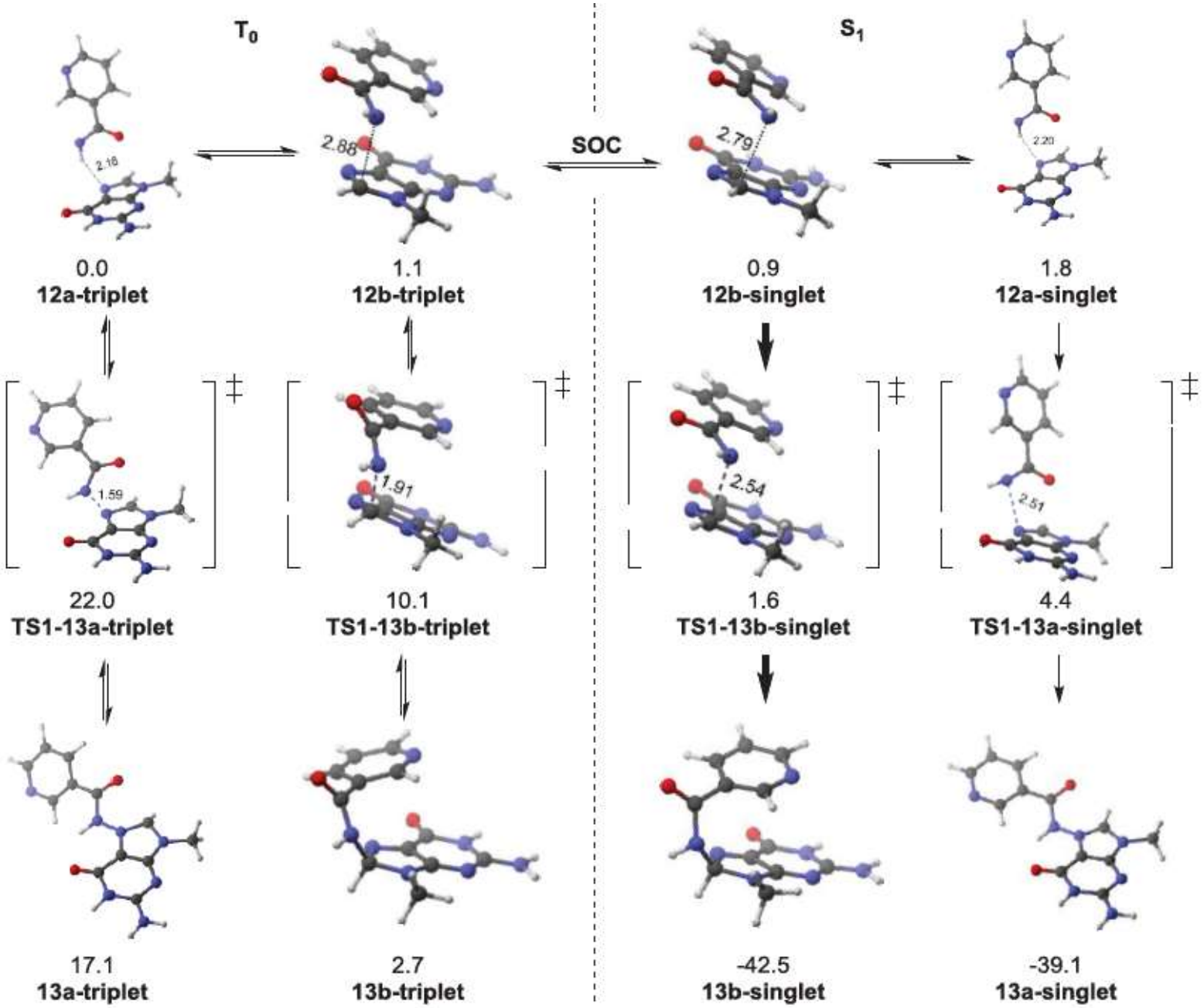
1) Pogozelski, W. K. *J. Am. Chem. SOC.* **1995**,117, 6428-6433



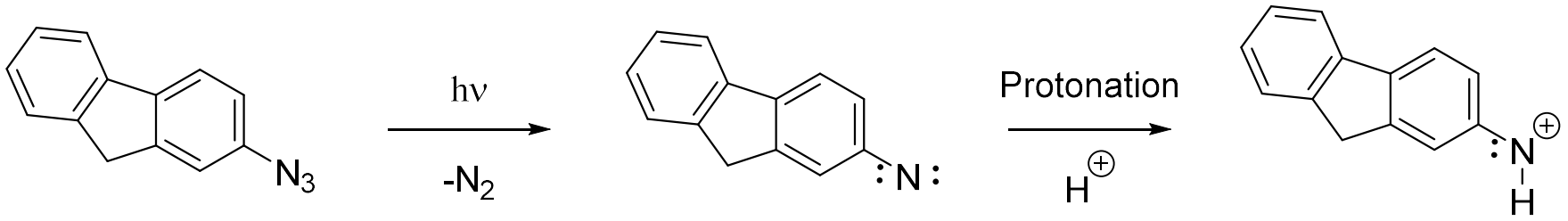
1) Adilakshmi, T. *Nucleic Acids Research* **2006**, *34*(8).



1) Carl, R. Woese. *Microbiol Rev.* **1987** *51*(2) 221–271.

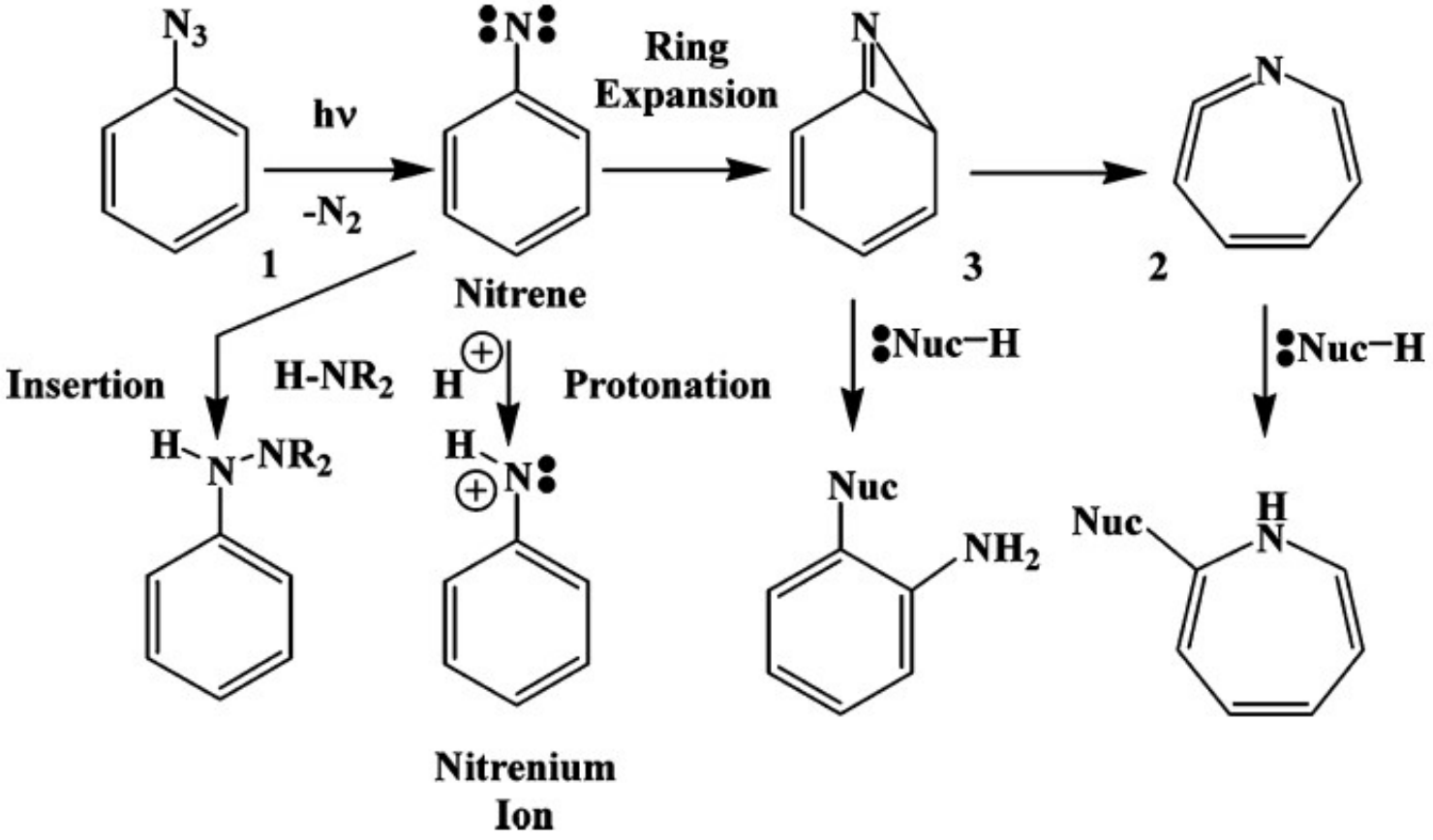


1) Spitale, R. C. *Nature Chemical Biology* **2018**, *14* (3), 325–325.

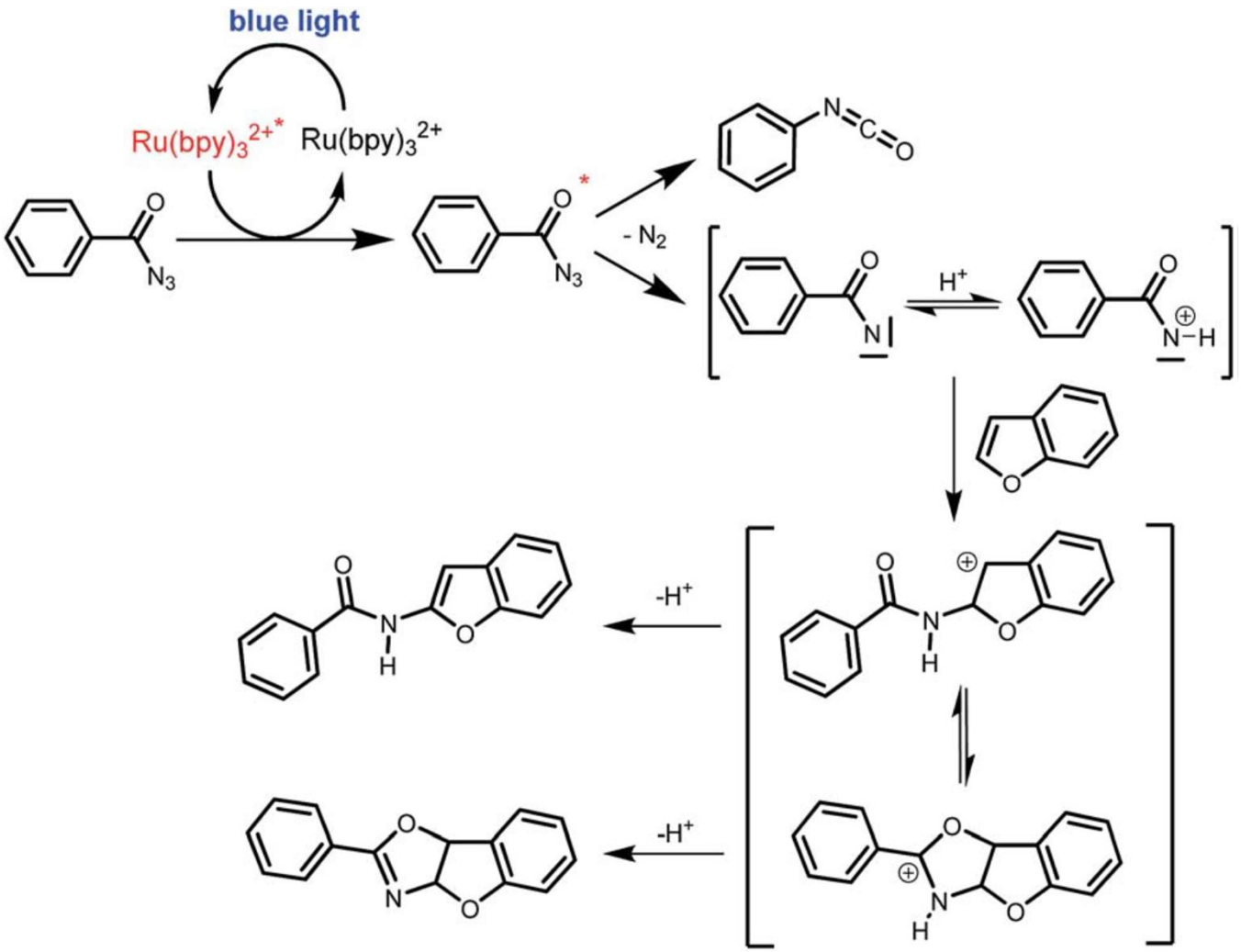


2-fluorenyl azide | 2-fluorenyl nitrene | 2-fluorenyl nitrenium ion

1) Hadad, C. M. *J. Am. Chem. Soc.* **2009**, *131* (32), 11535–11547.

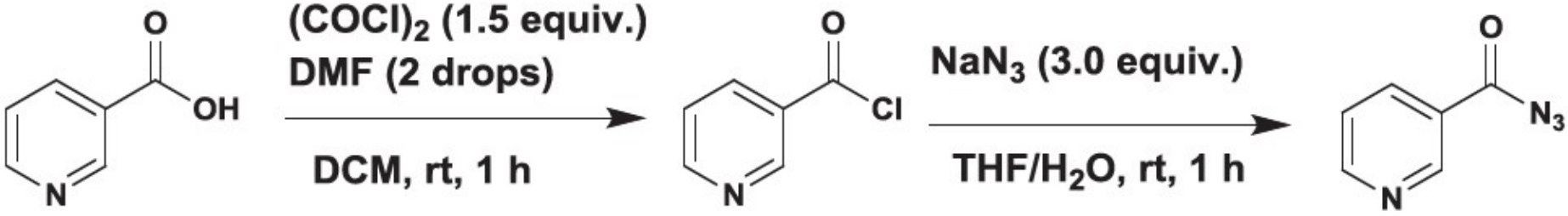


1) Phillips, D. L. *J. Org. Chem.* **2014**, *79* (8), 3610–3614.



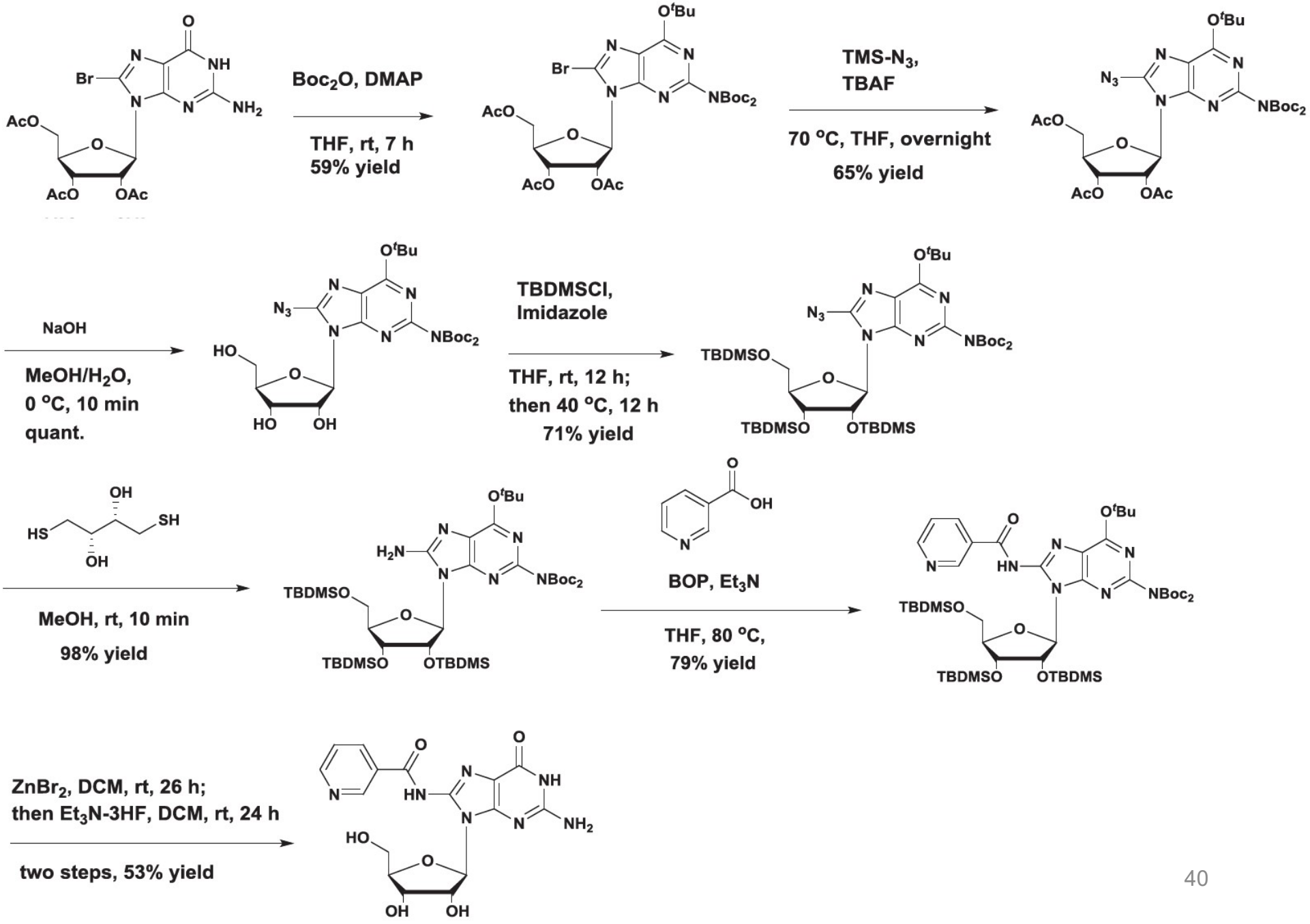
1) König, B. *Chemical Science* **2015**, *6* (2), 987–992.

Synthesis of NAz and the positive control 8AG



1) Spitale, R. C. *Nature Chemical Biology* **2018**, *14* (3), 325–325.

Synthesis of NAz and the positive control 8AG



What does the ‘S’ mean in like 16S or 18S rRNA?

S -> Svedberg unit :
Offer a measure of particle size based on the sedimentation rate.

Generally speaking, the bigger the number, the larger the size would be.