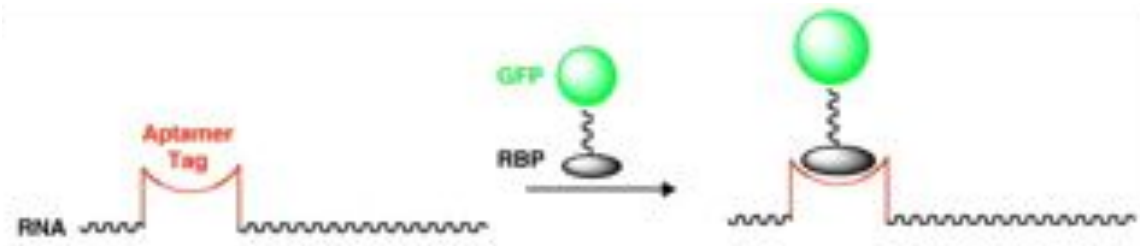
RNA Mango: a bright, high affinity complex for RNA labeling and tracking

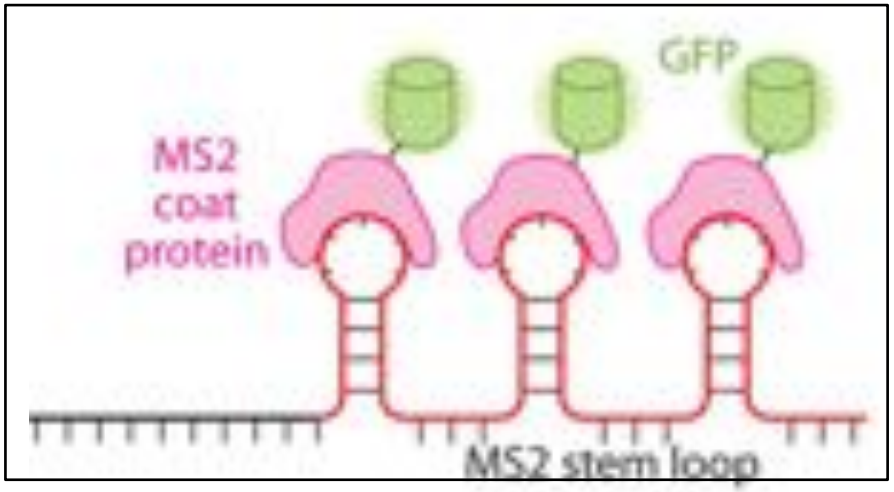
Peter J. Unrau
Simon Fraser University

Fluorescent *in vivo* RNA imaging

MS2 stem-loop/coat protein



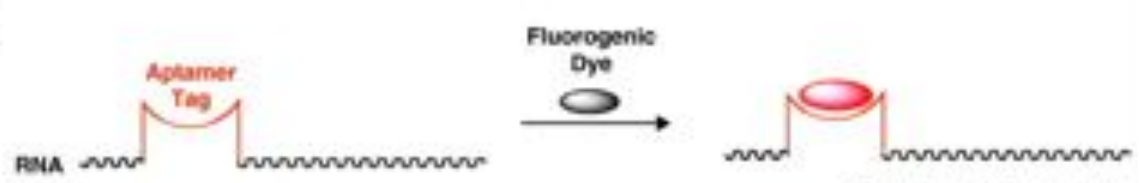
Armitage, *Current Opinion in Chemical Biology*. **15** (2011)



Bao G. *et al.*, *Annu. Rev. Biomed. Eng.* **11** (2009)

Fluorescent *in vivo* RNA imaging

Aptamer/Fluorophore pair



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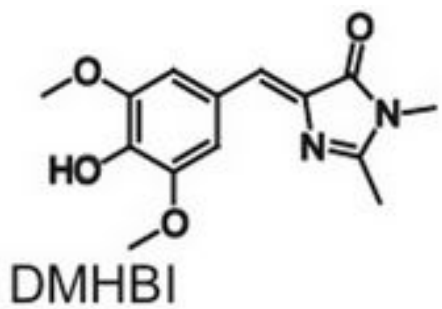
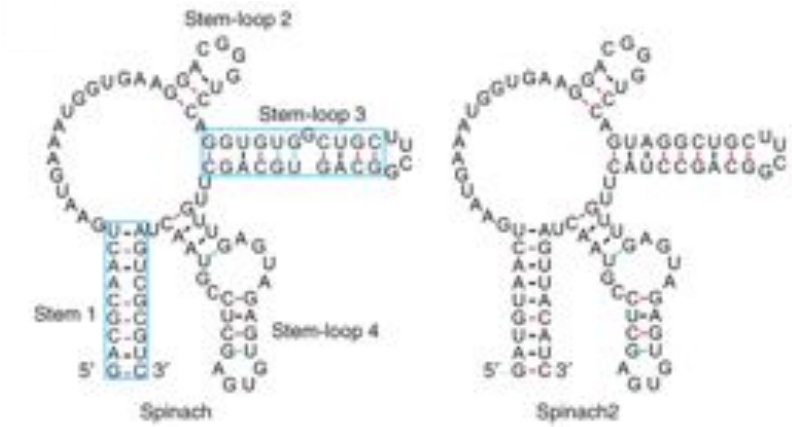
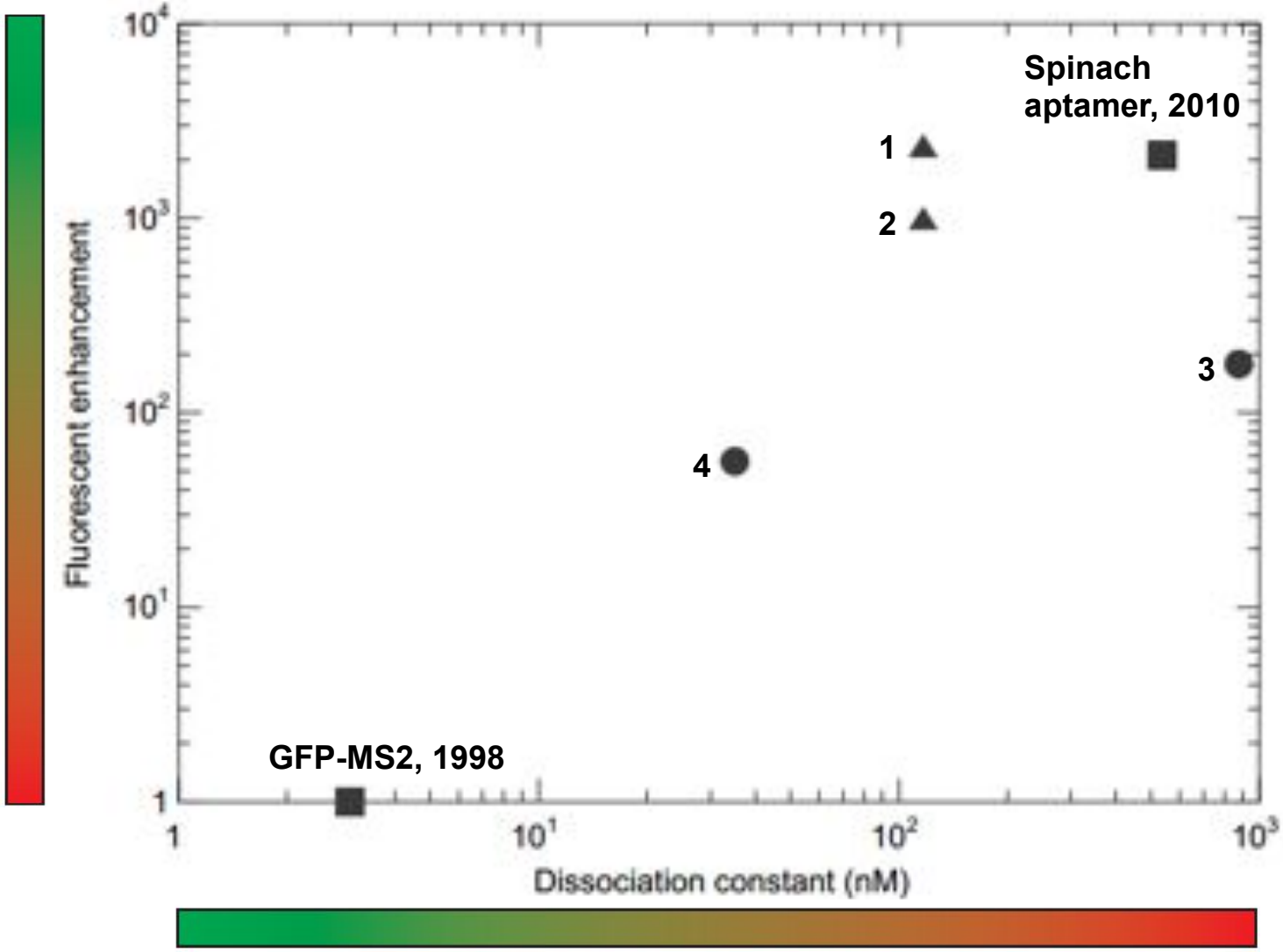


GFP Fluorophore
Paige *et al.*, *Science*. **10** (2011)



Strack *et al.*, *Nature Methods*. **10** (2013)

An optimal RNA tracking system

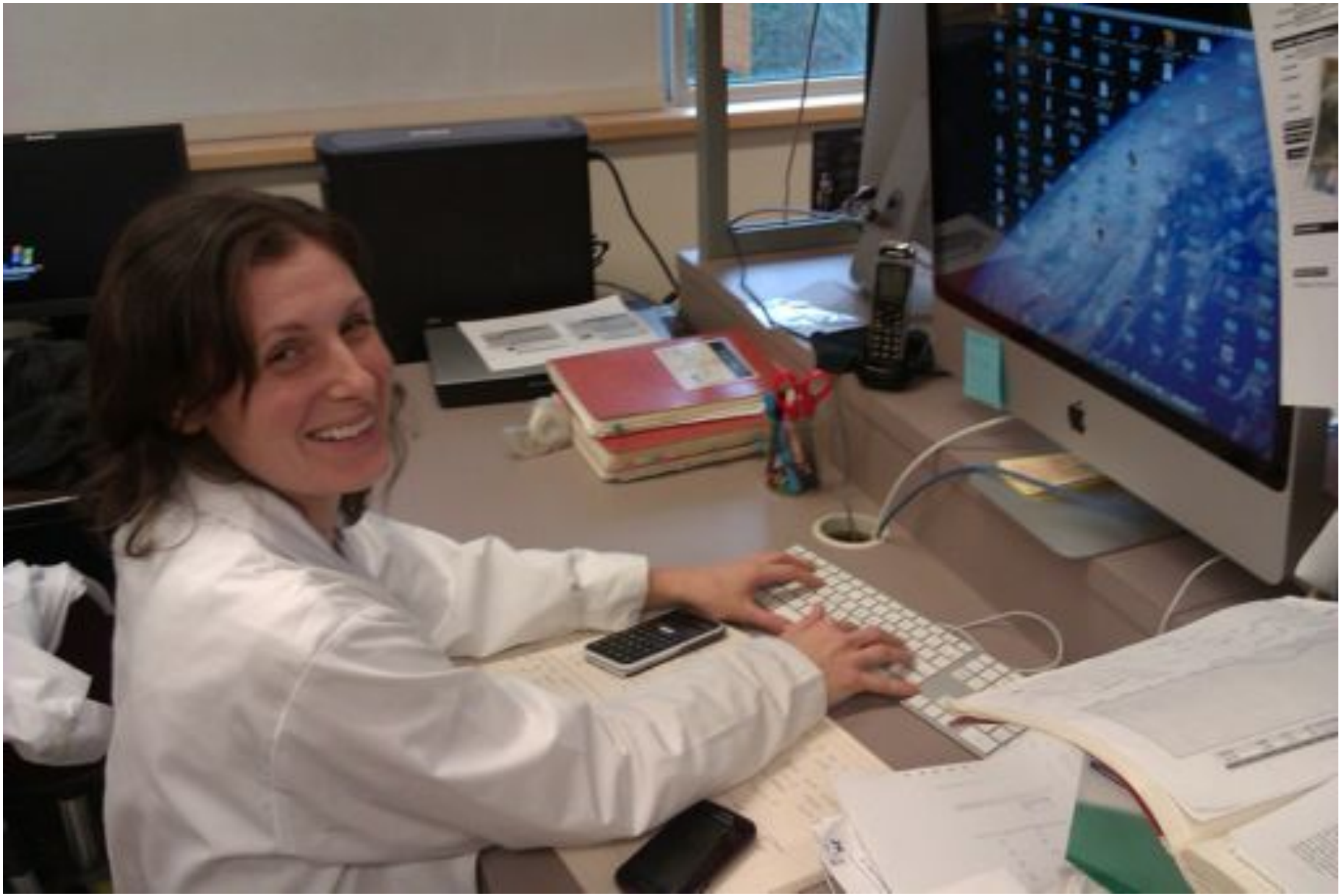


▲ Toxic to cells

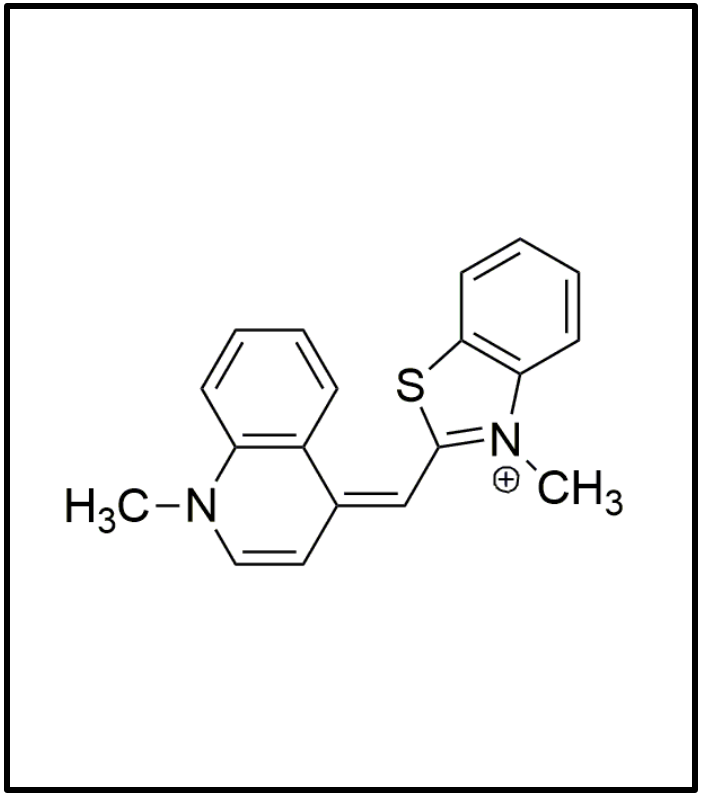
● Poor membrane permeability

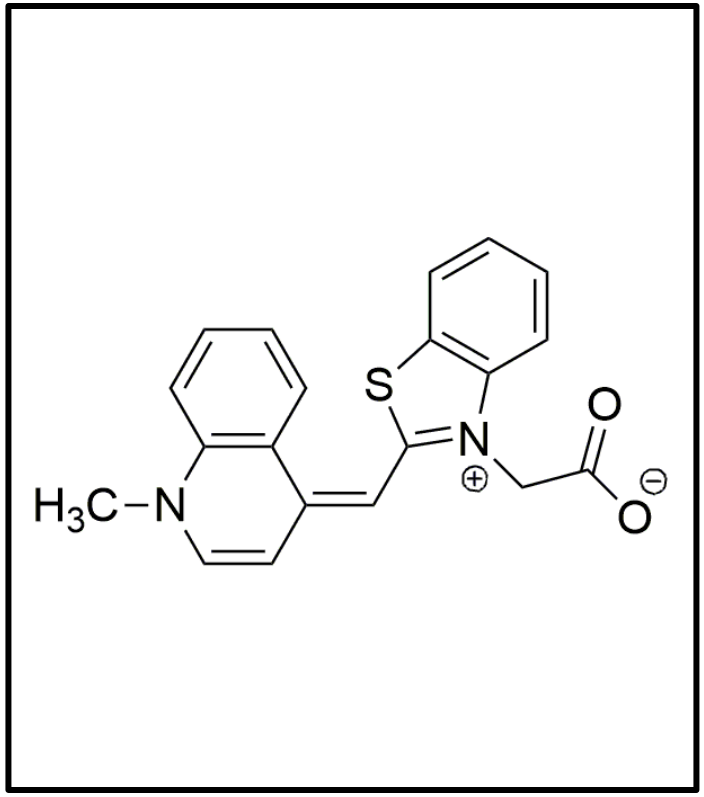
1. Malachite green, 2003
2. Dimethylin dole red, 2008
3. Hoechst 33258: DNA, 2008
4. Hoechst 33258: RNA 2007

Lena Dolgosheina: RNA Mango Selection



Thiazole Orange (TO1)

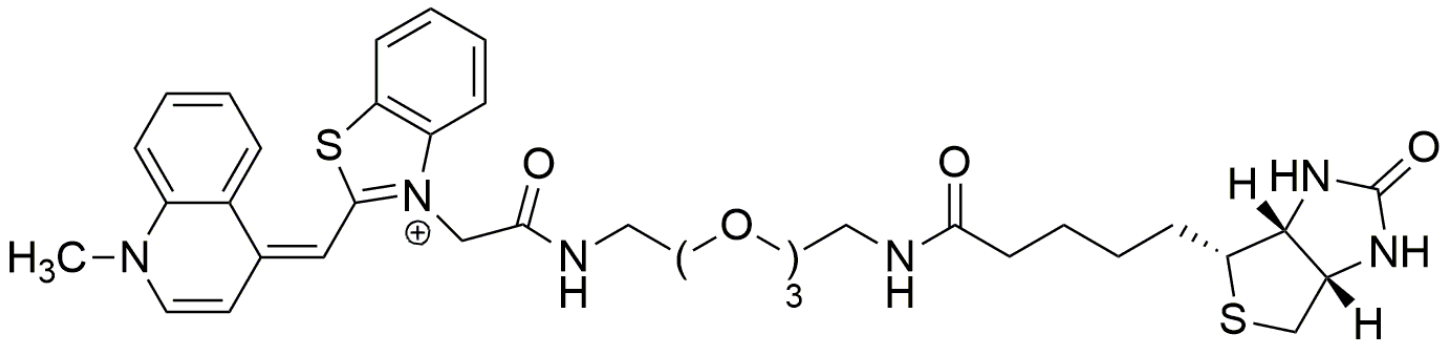




- Thiazole Orange
- Non-toxic
- Membrane-permeable
- Strongly fluorescent when bound to nucleic acid

- Substituent on benzothiazole nitrogen impedes intercalation
- Carboxylate allows attachment of bulky groups

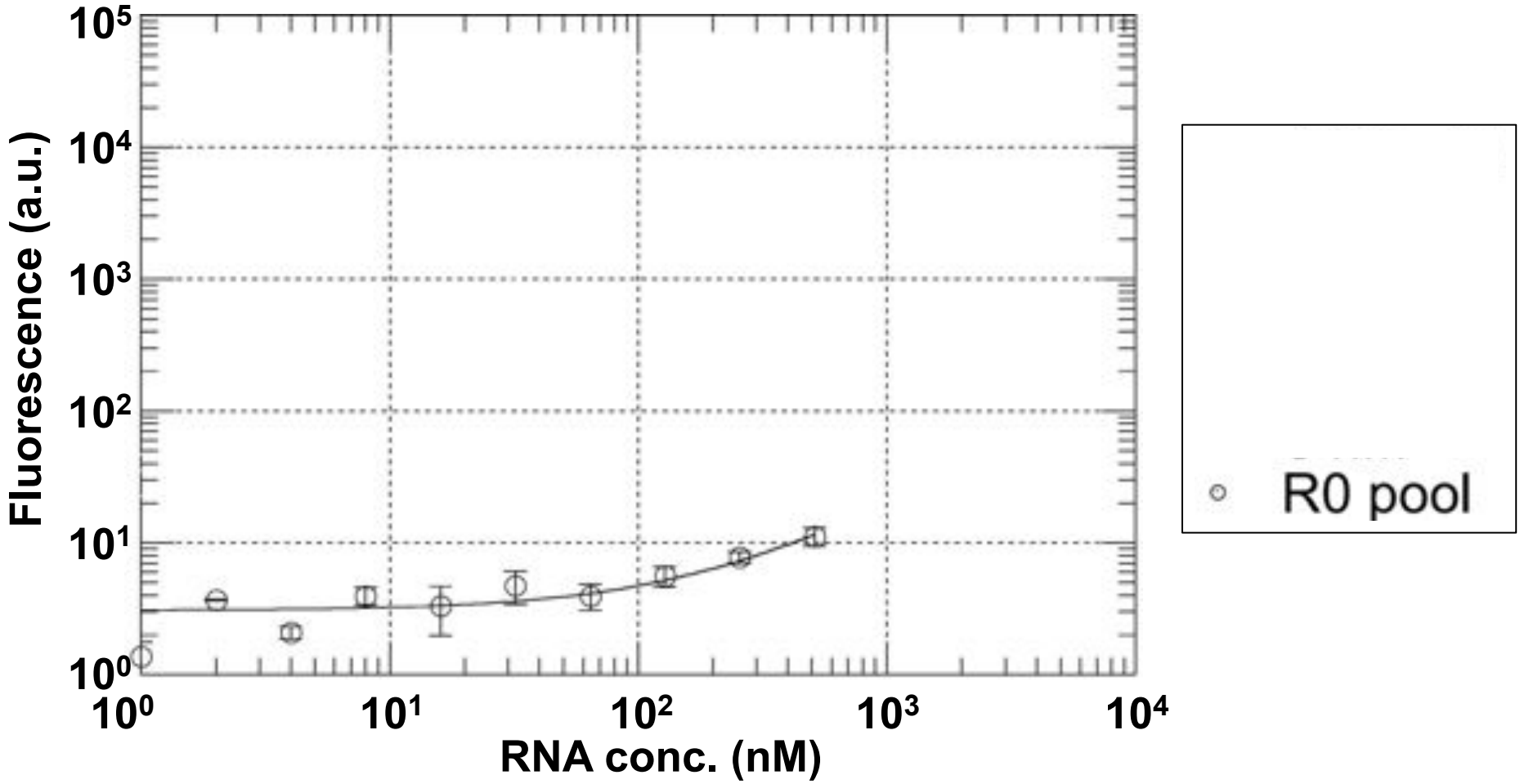
Working dye – TO1-Biotin



TO1-Biotin

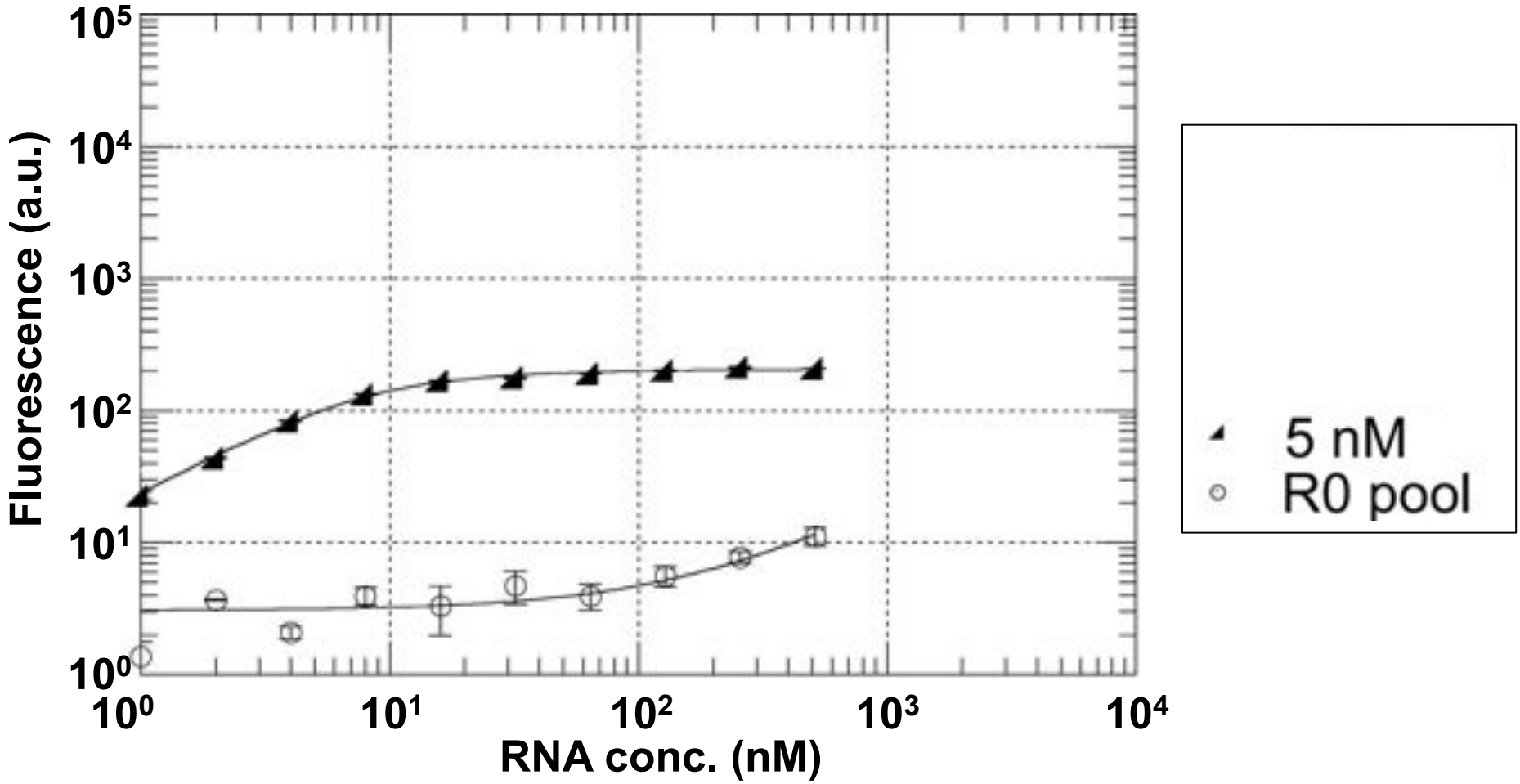
- **Natural nucleic acid intercalation is perturbed by 7-fold**
- **Biotin suitable for *in vitro* selection + RNA complex purification**

Best isolate: RNA Mango



Best isolate: RNA Mango

K_d = 3.4 ± 0.6 nM



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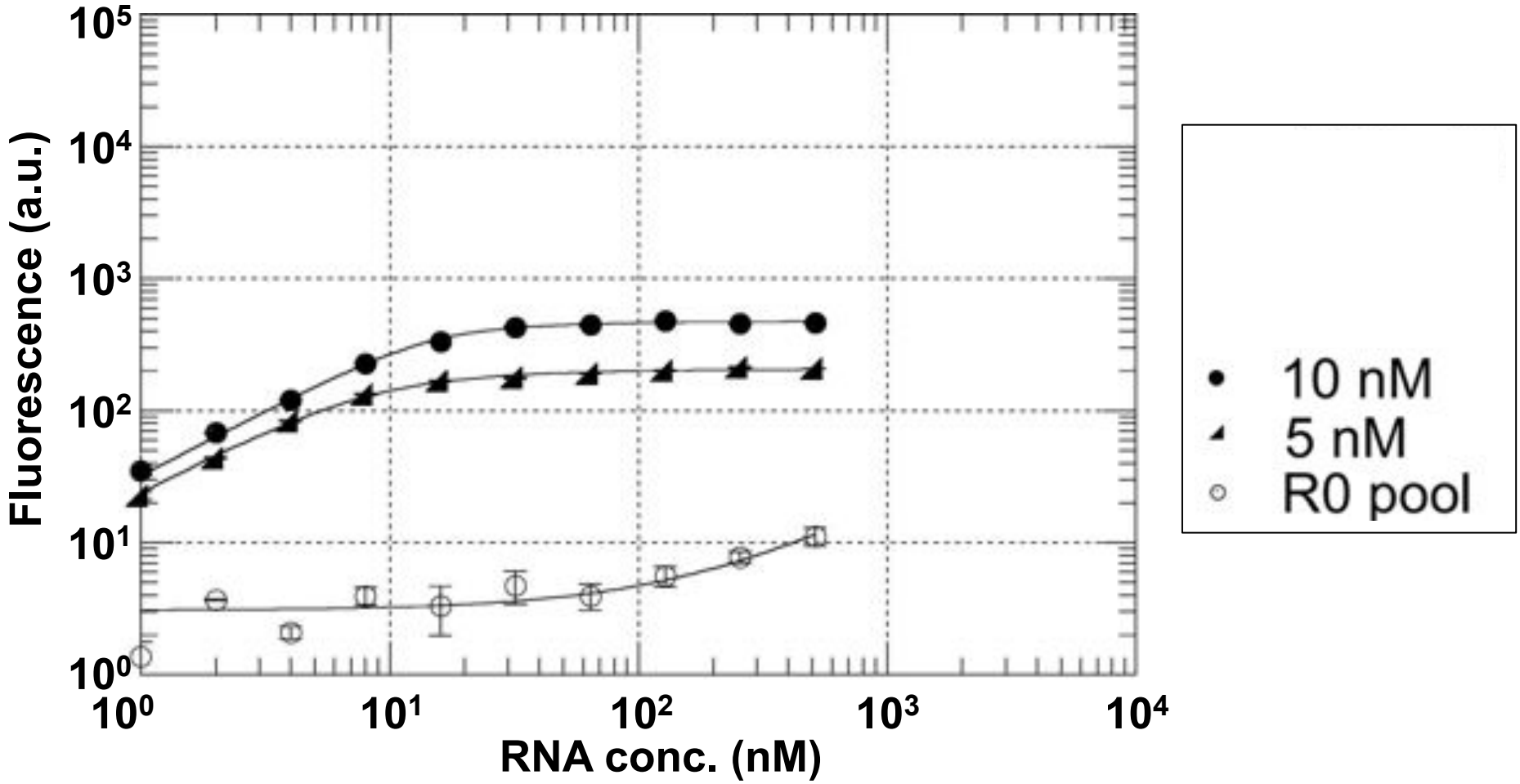

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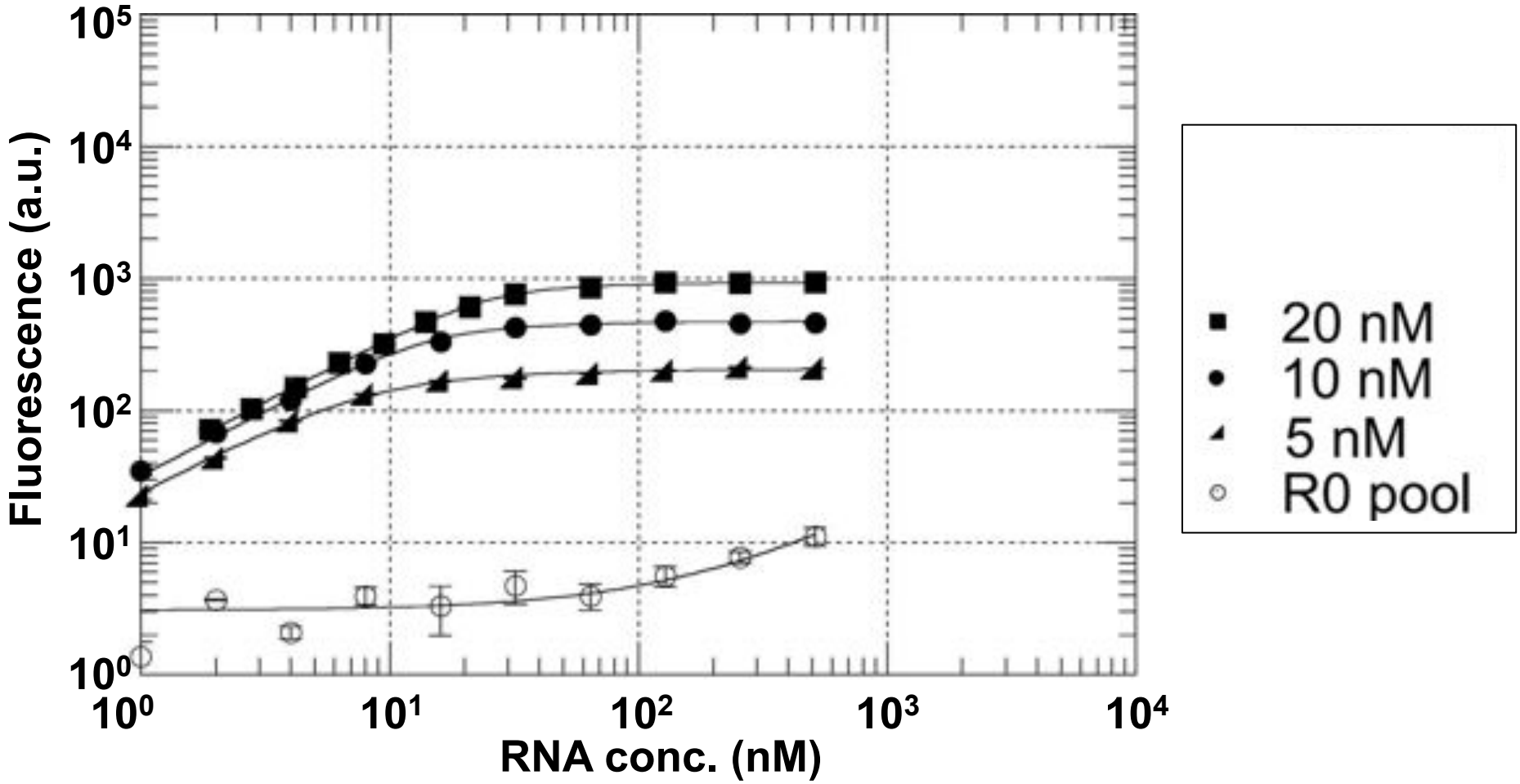

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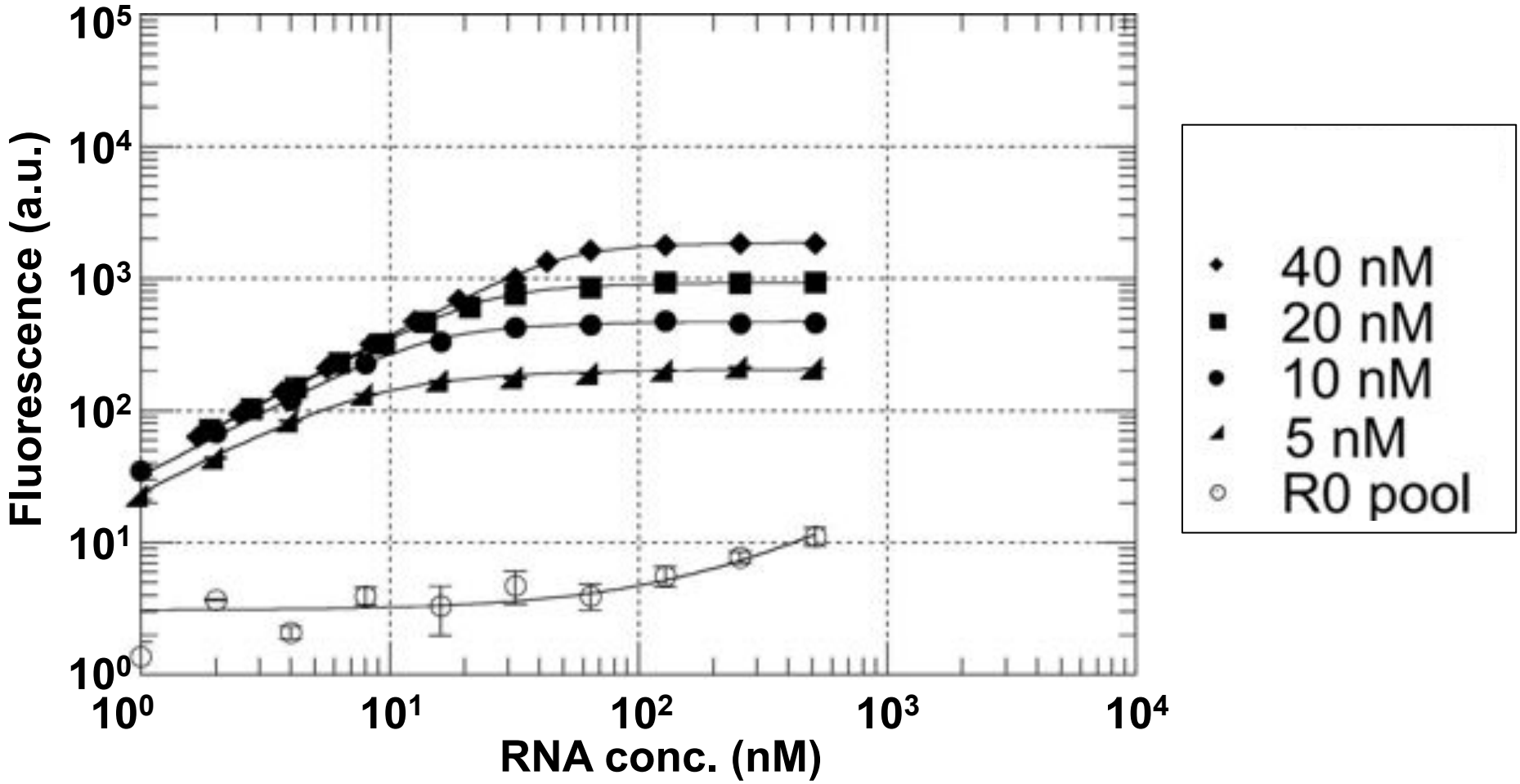

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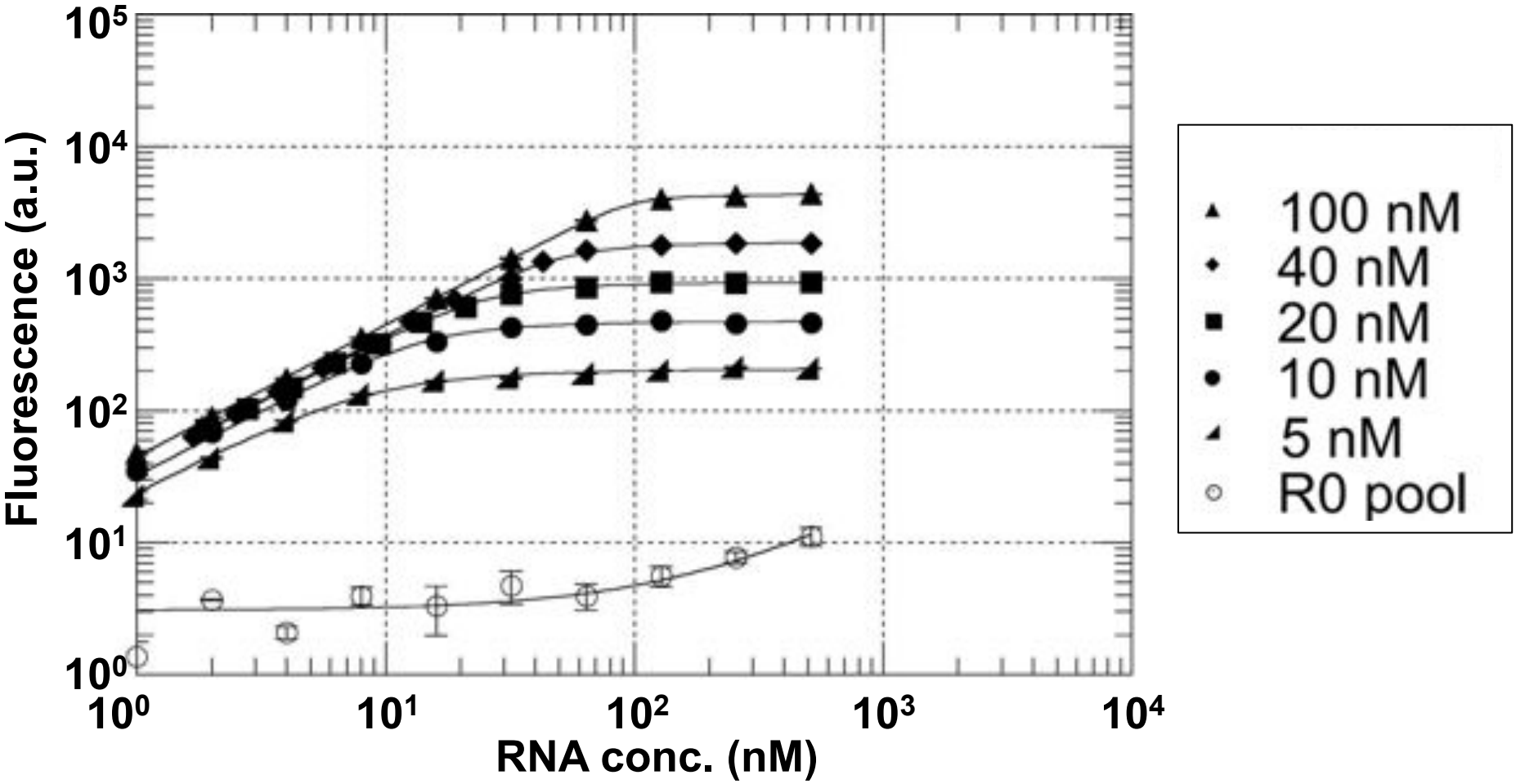

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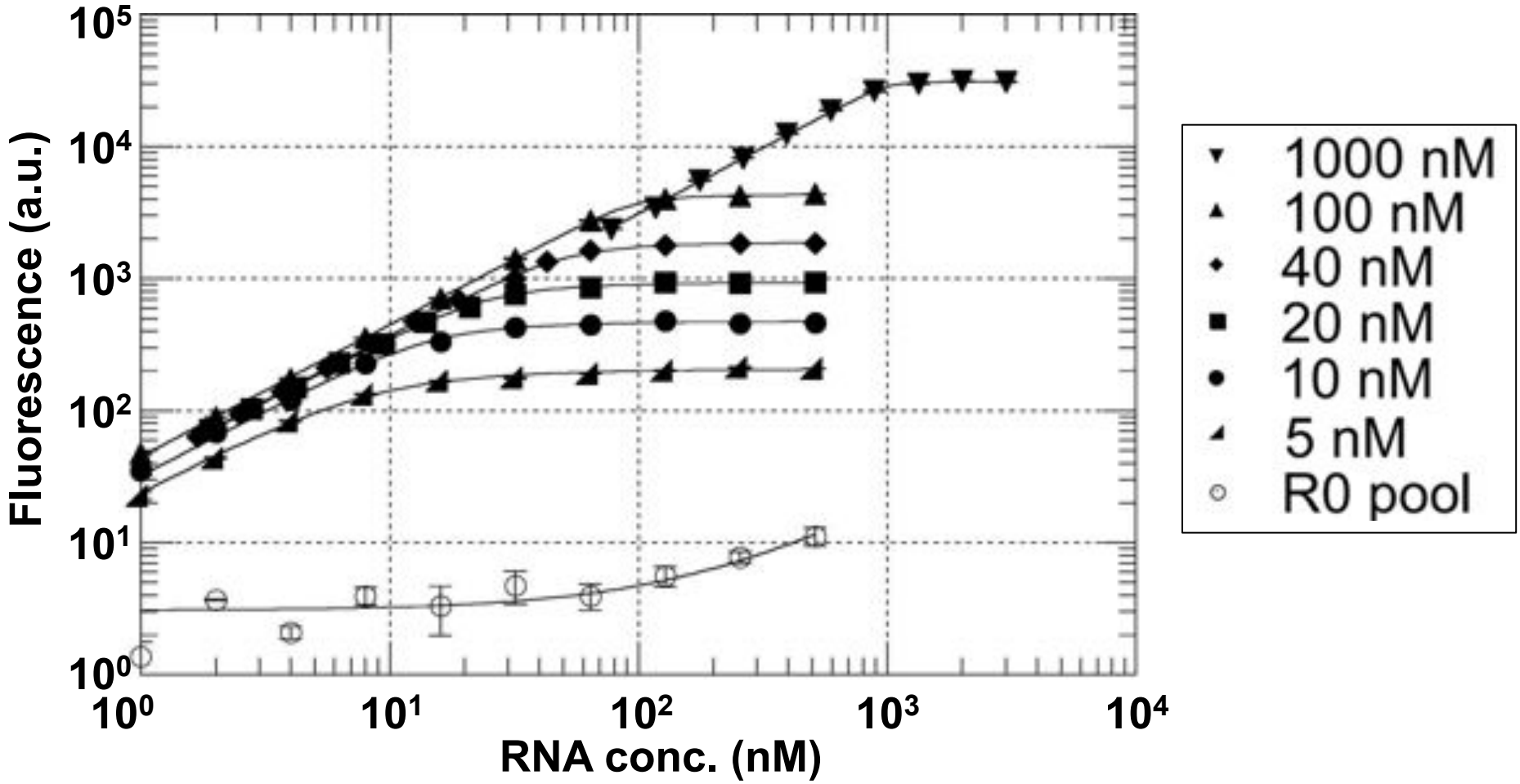
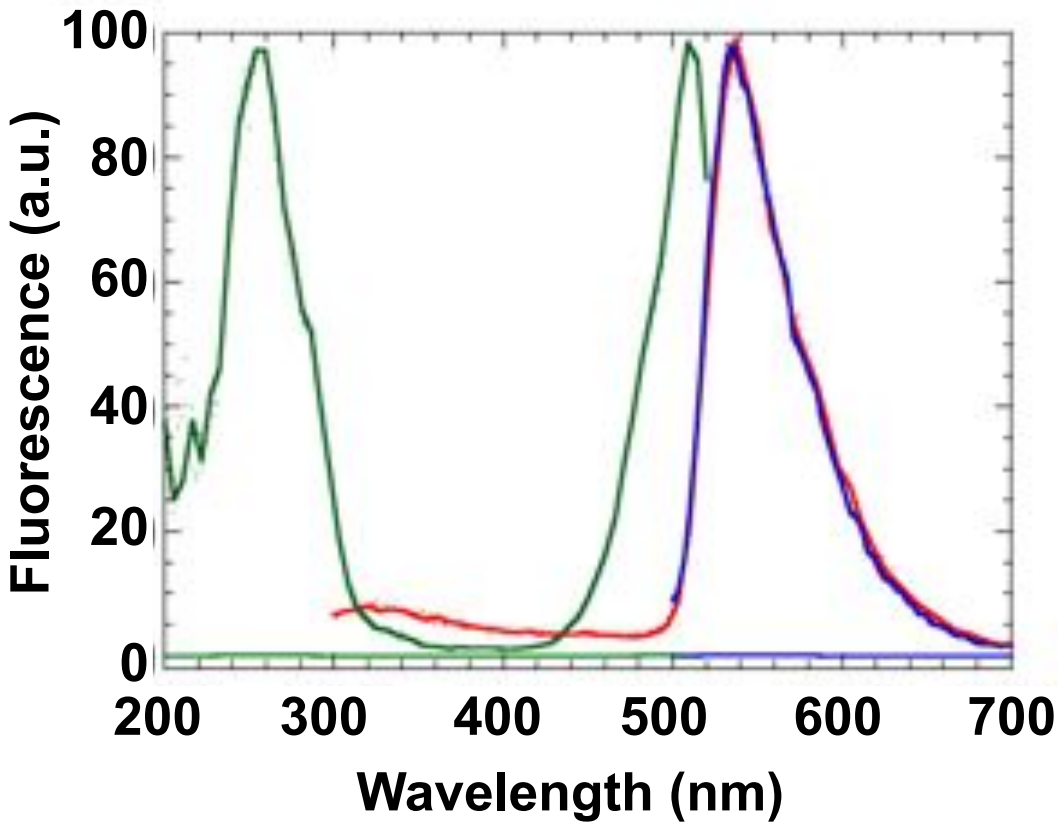


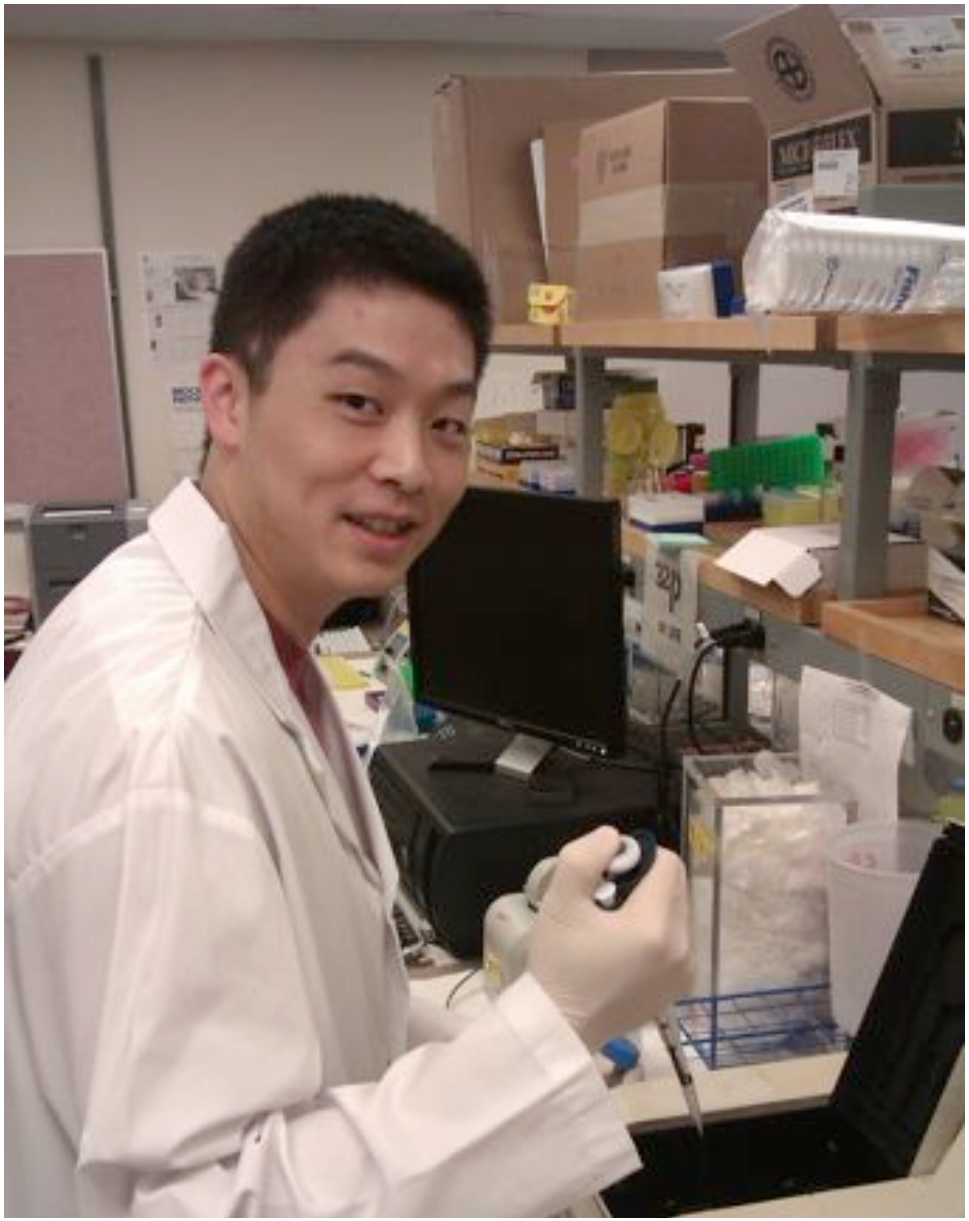
RNA Mango – Fluorescent properties

Fluorescent enhancement = 1100 ± 100

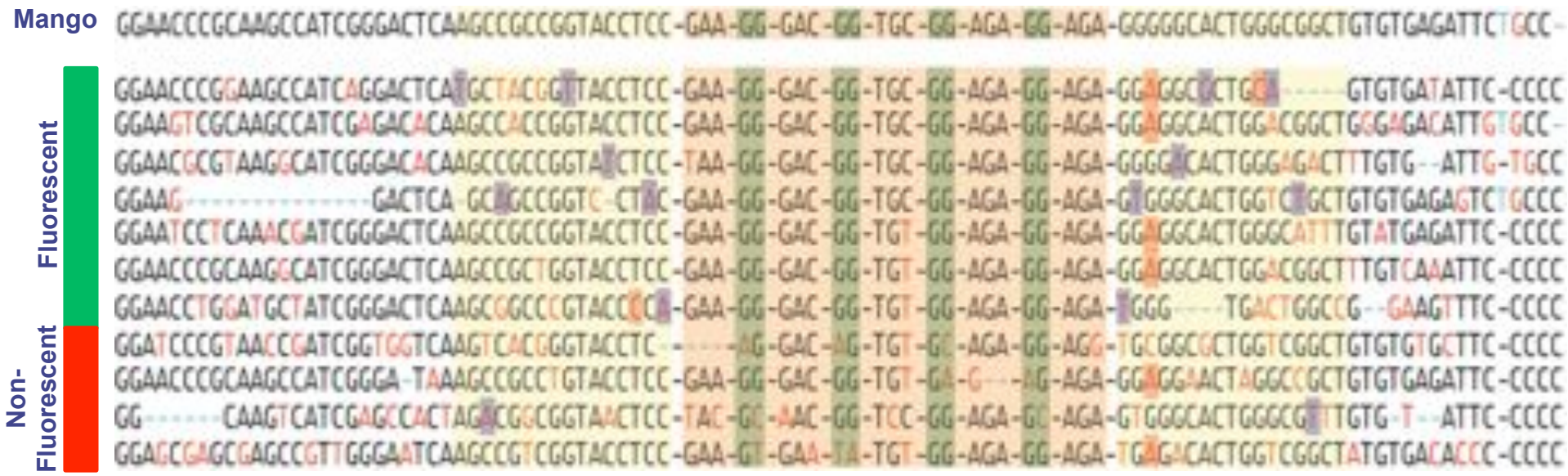


Excitation	260 nm	510 nm
Emission	535 nm	535 nm
ε	77,500 $M^{-1}cm^{-1}$	
$\Phi_{unbound}$	0.00012	
Φ_{bound}	0.14	
Brightness (B)	10,850 $M^{-1}cm^{-1}$	
B/B_{GFP}	0.5	

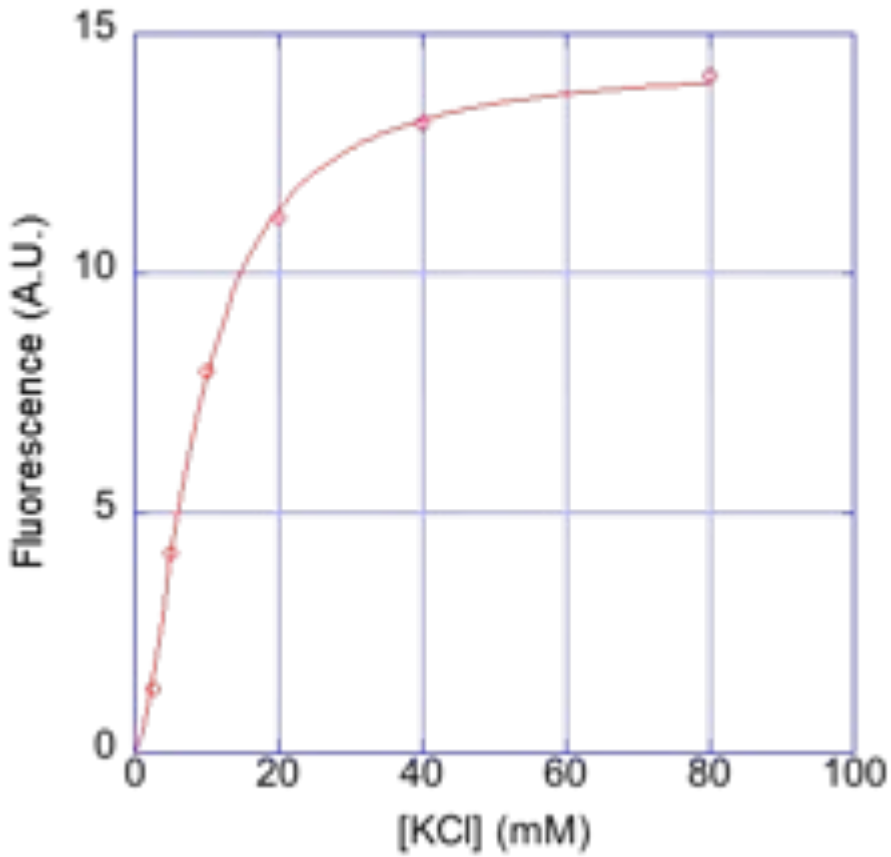
Sunny Jeng: RNA Mango Characterization

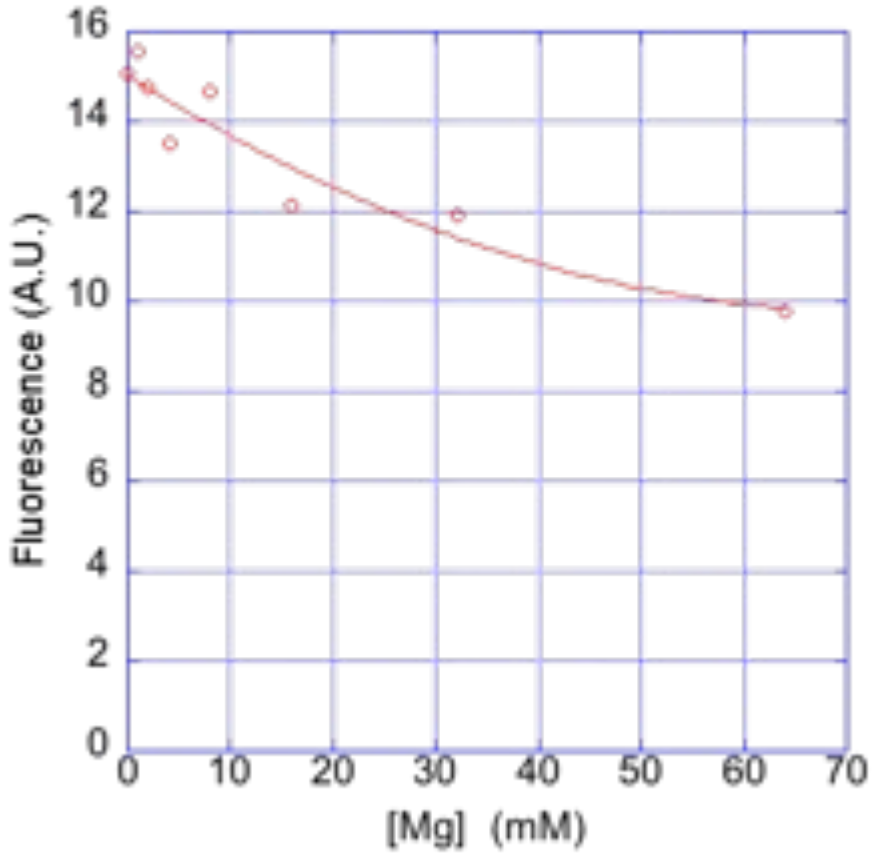


RNA Mango has a highly conserved core

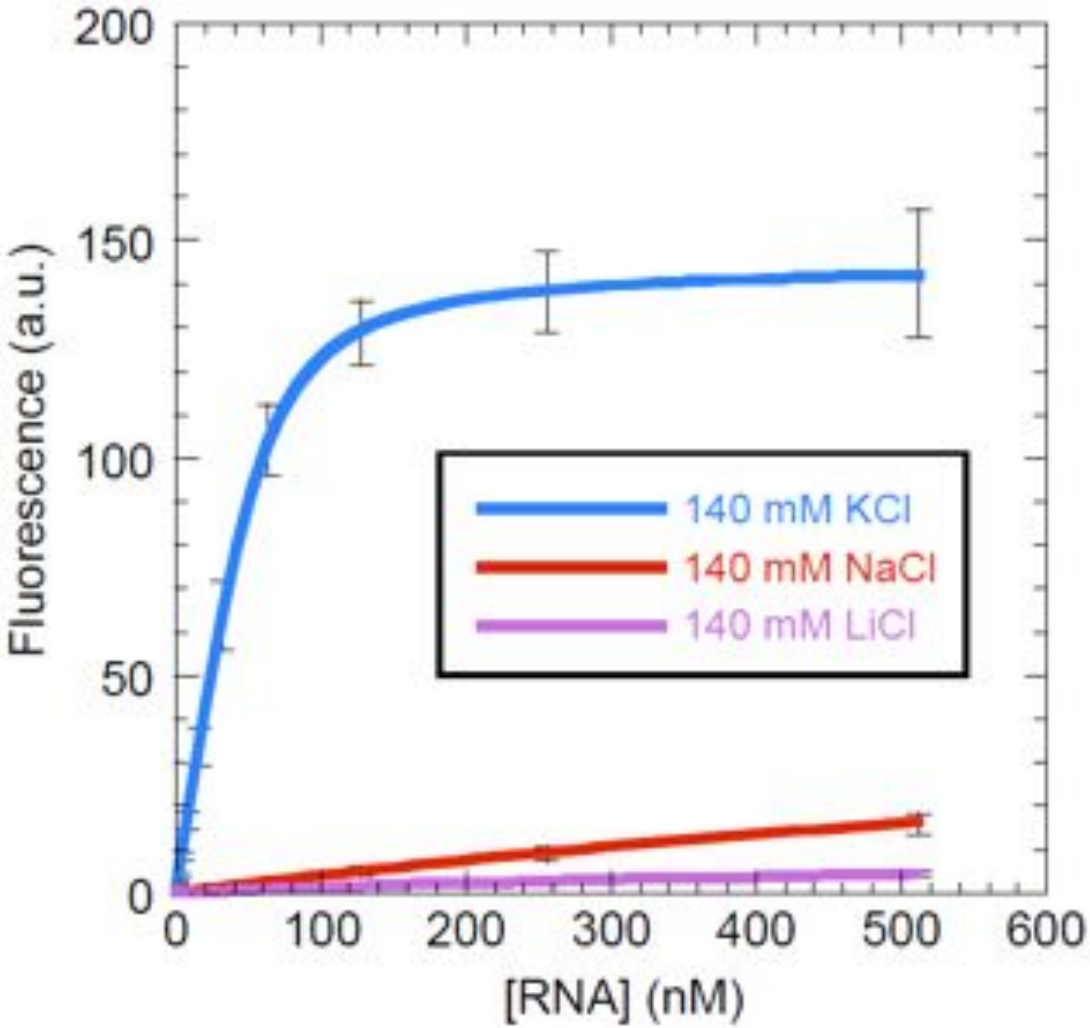


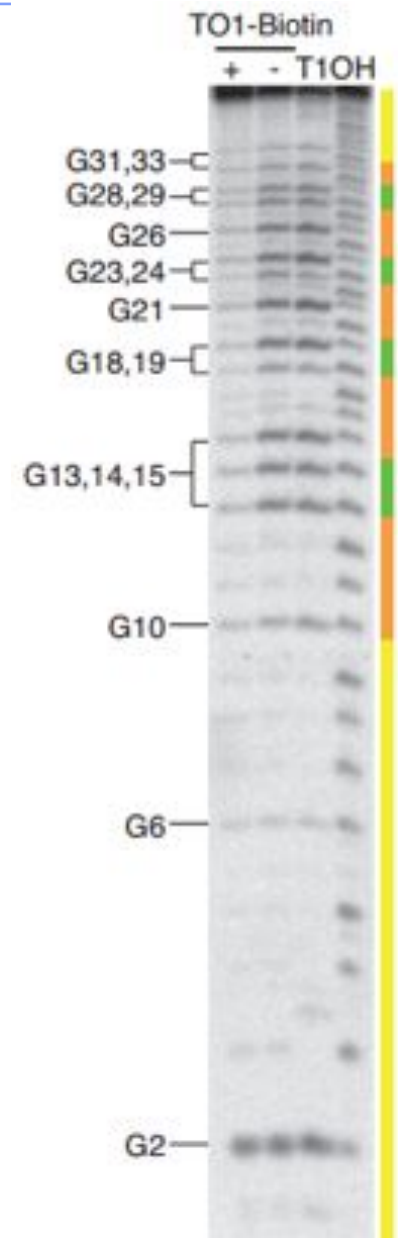
RNA Mango is potassium responsive



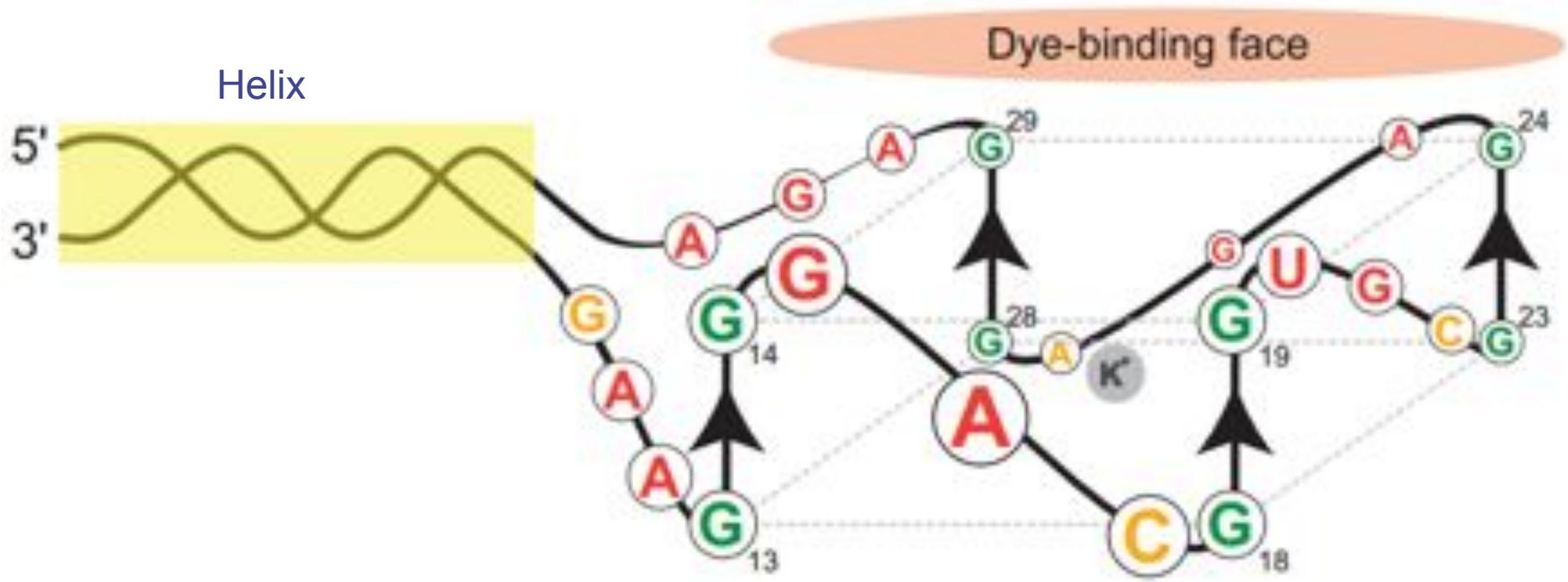


RNA Mango is a G-quadruplex



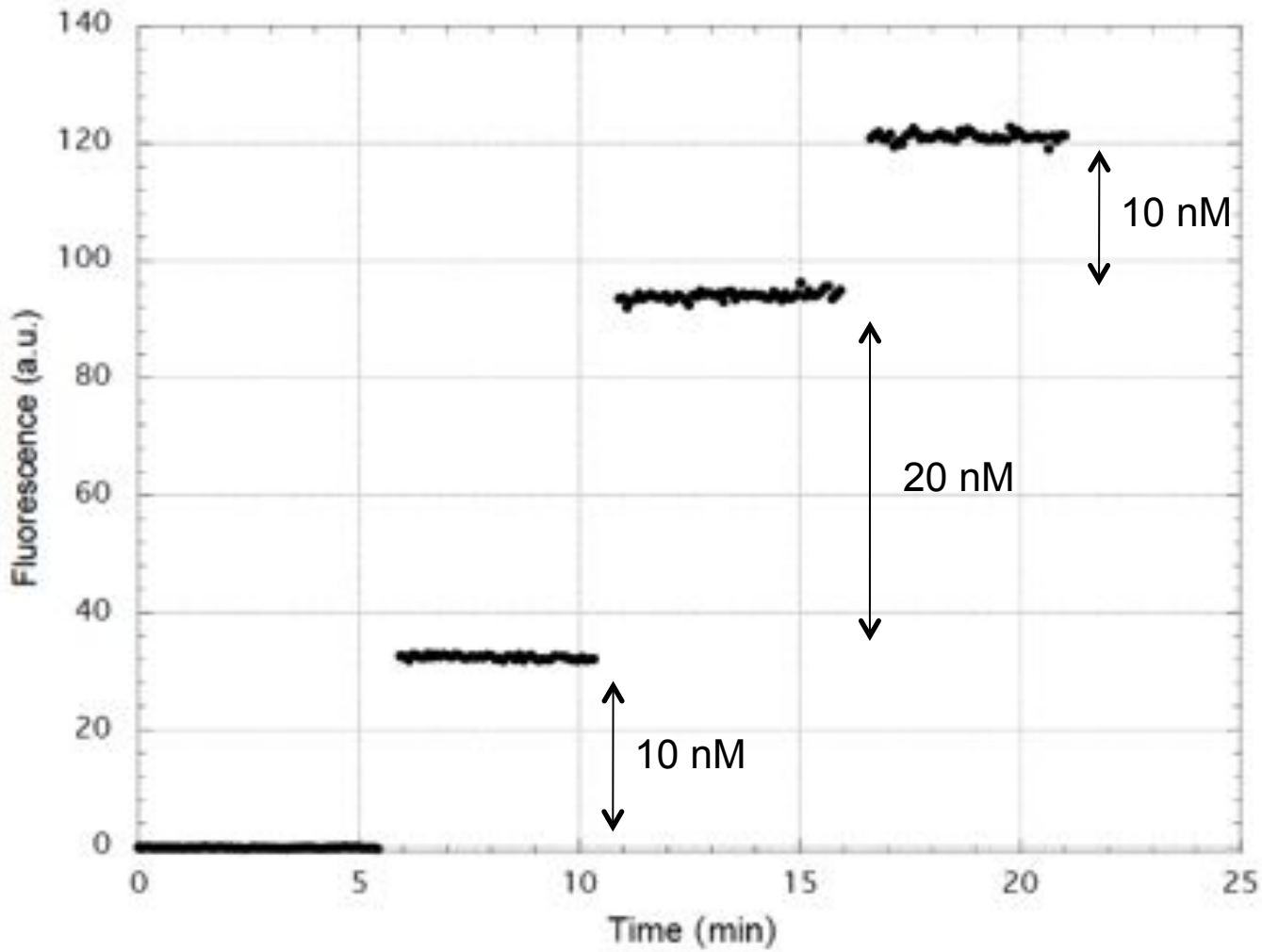


RNA Mango Structure

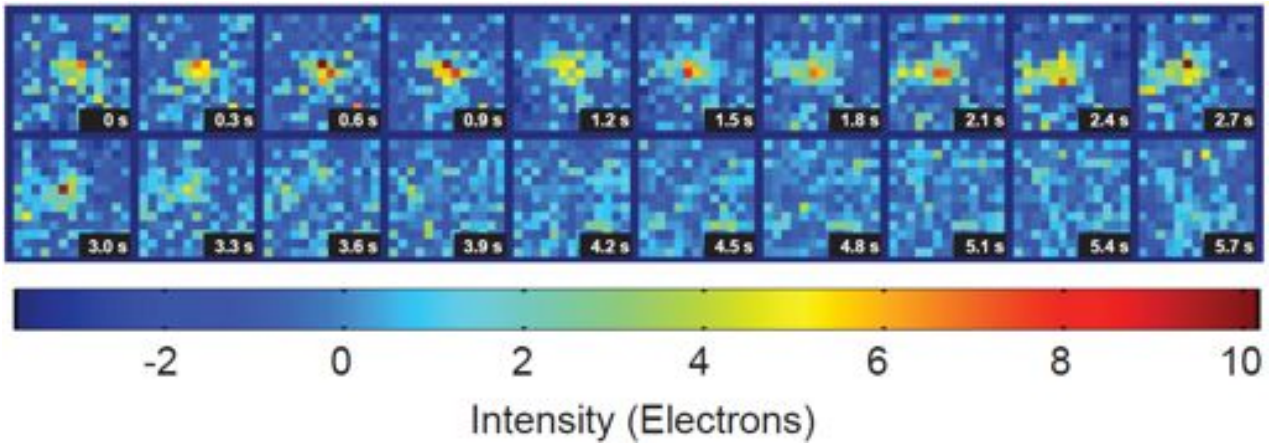


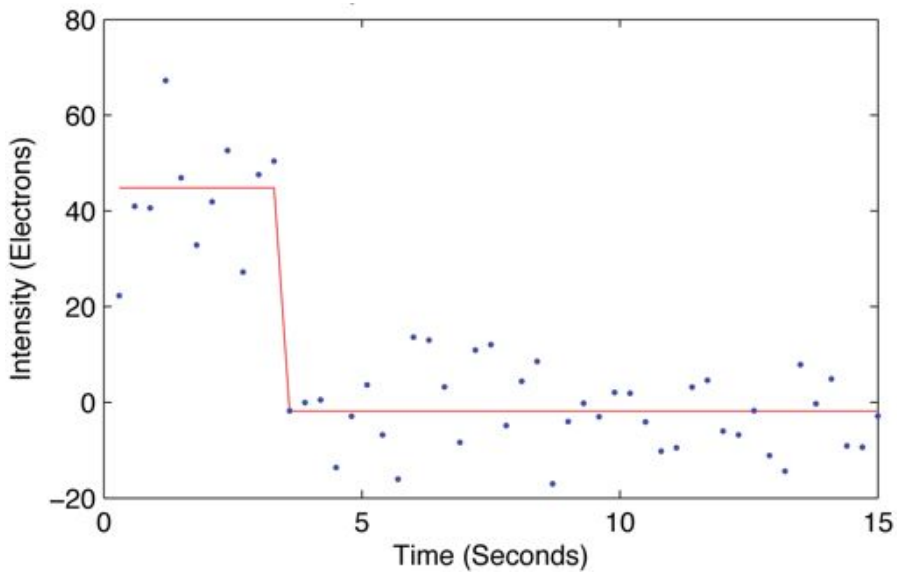
RNA MANGO APPLICATIONS

Rapid fluorophore binding



Single-molecule analysis

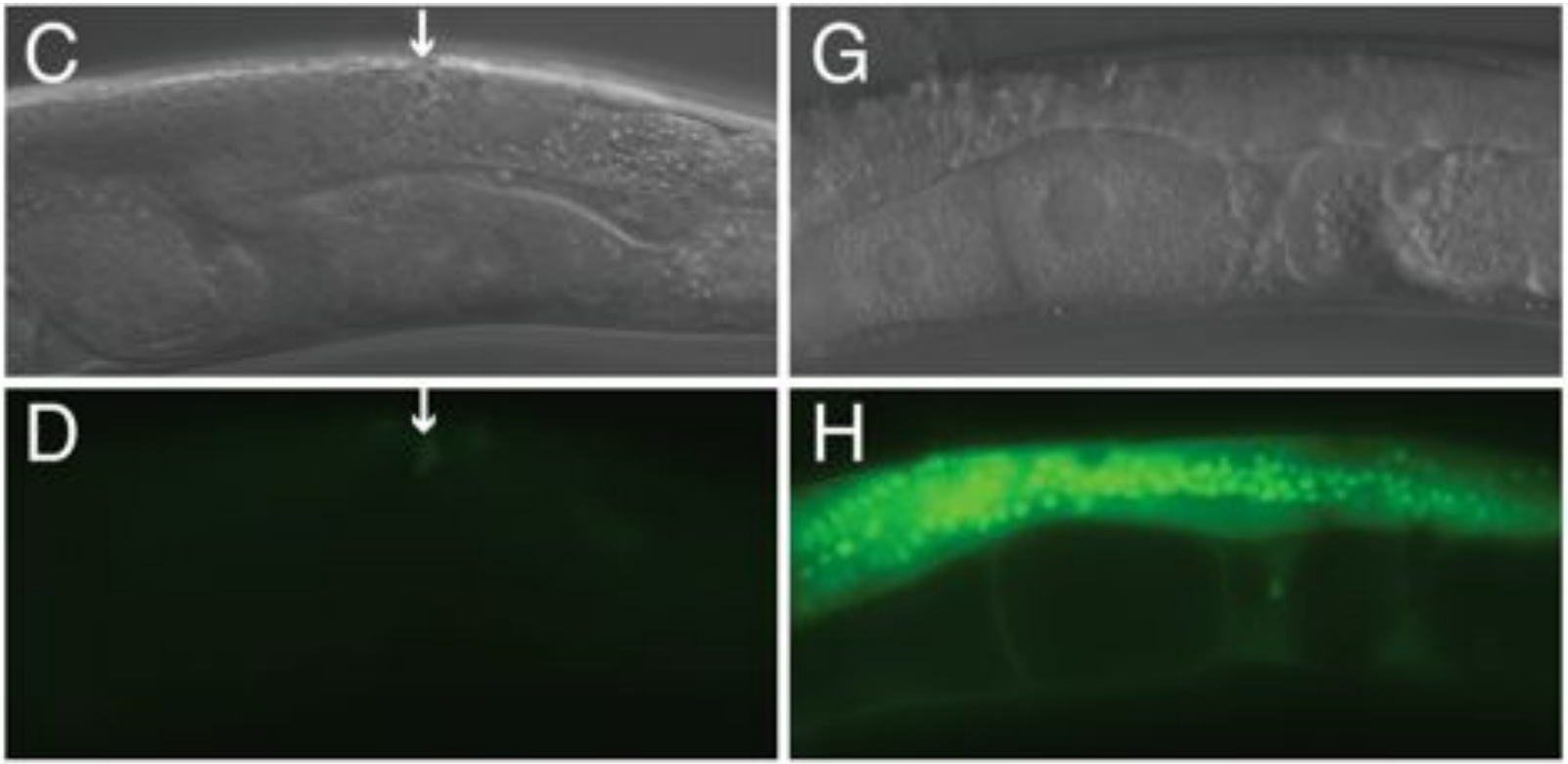




Paul Wiggins,
University of Washington

RNA Mango in *C. elegans*

100 µM	100 µM	TO1-Biotin
0 µM	100 µM	RNA Mango

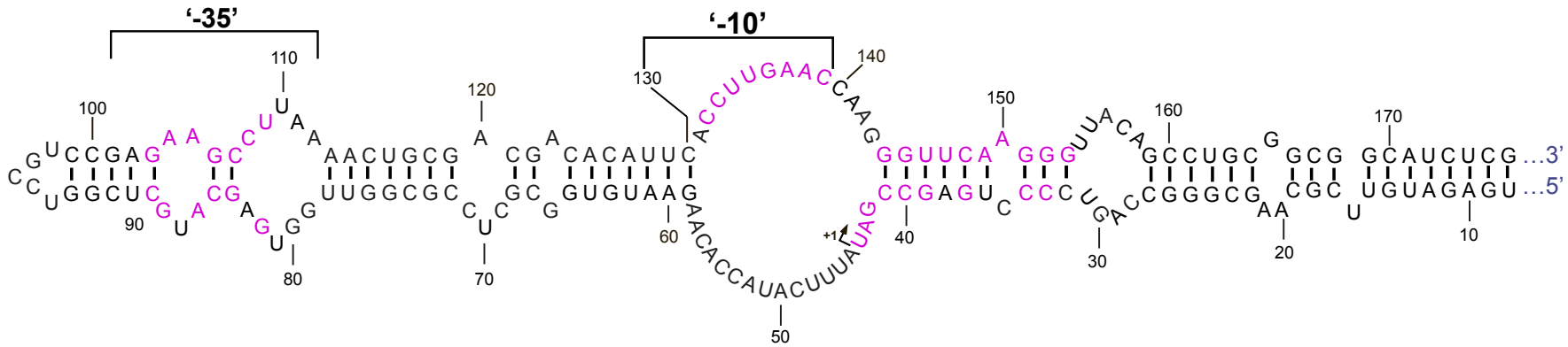


Nancy Hawkins,
Simon Fraser University

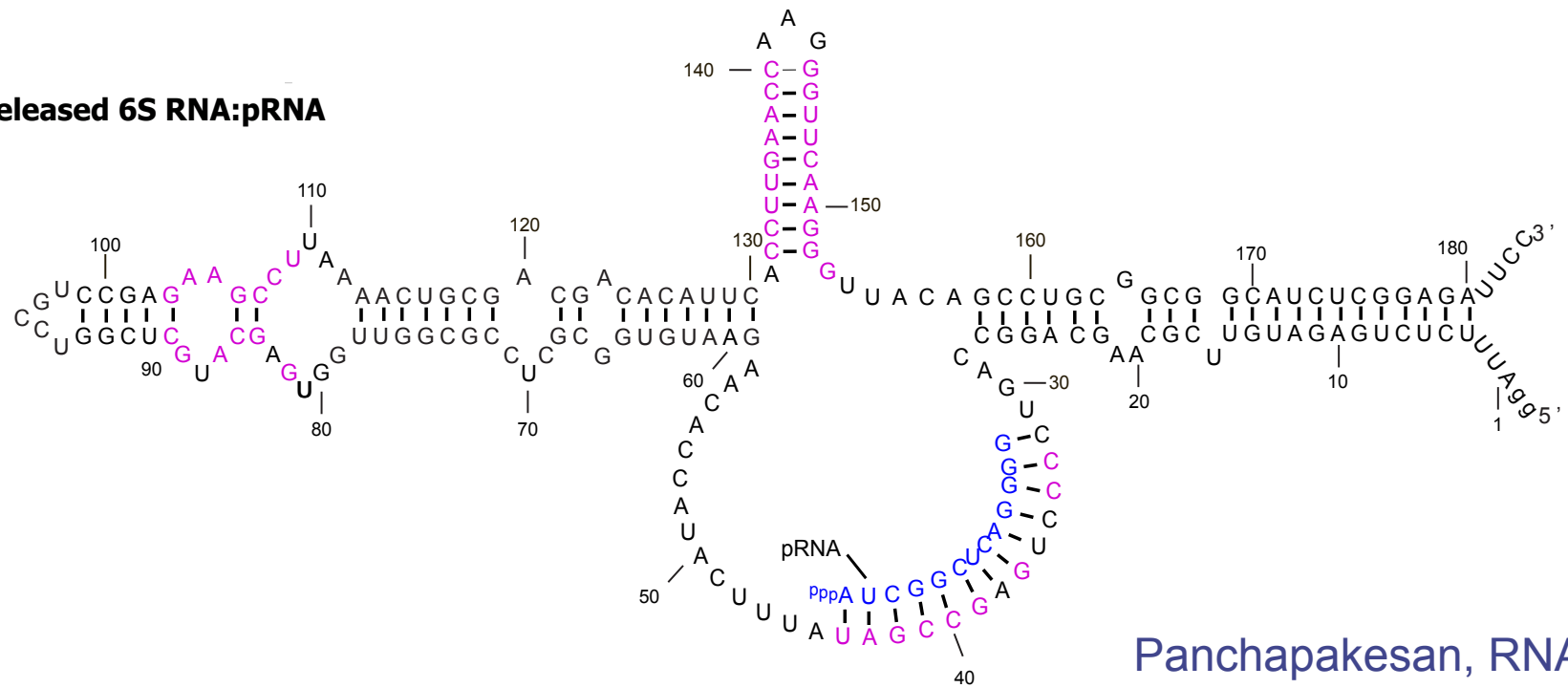
Shyam P: RNA Mango as a purification tag

6S Regulatory RNA in *E. coli*

26



Released 6S RNA:pRNA

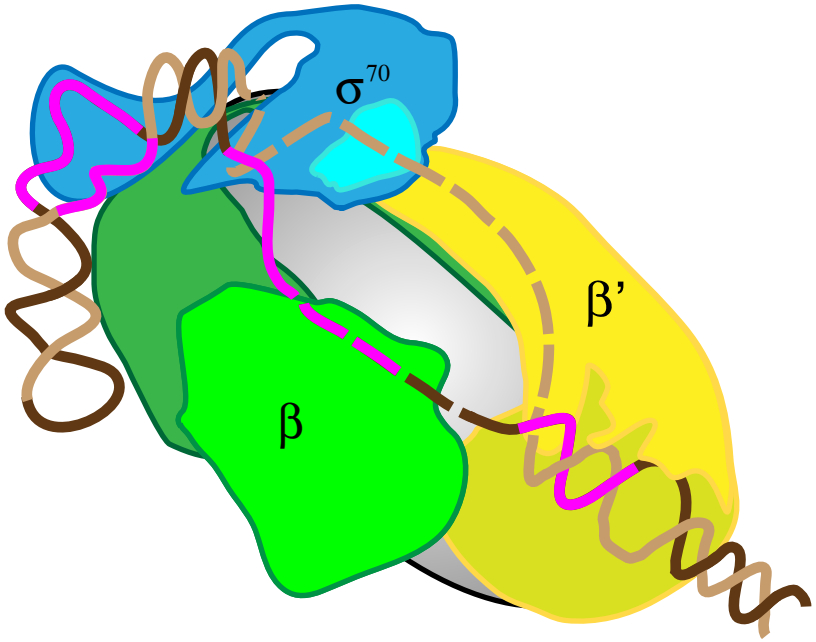


Panchapakesan, RNA, 2012

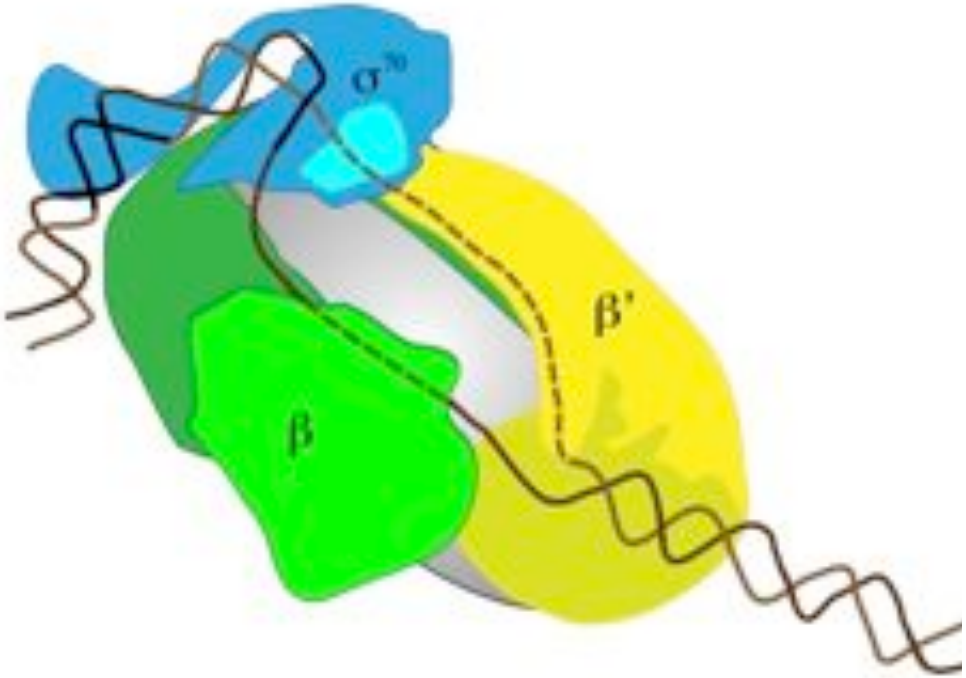
Commonalities with DNA dependent transcription

Open promoter complex

6S RNA dependent transcription



DNA dependent transcription

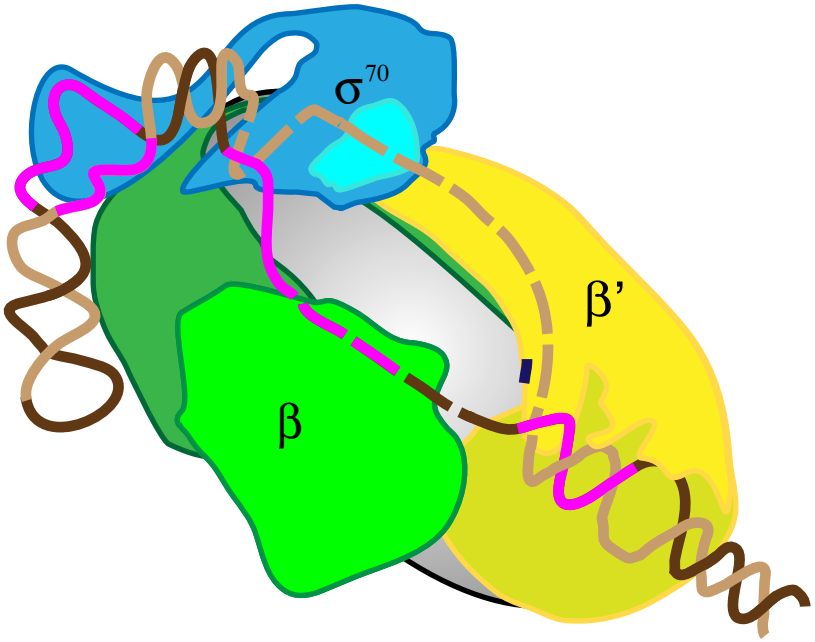


Panchapakesan, RNA, 2012

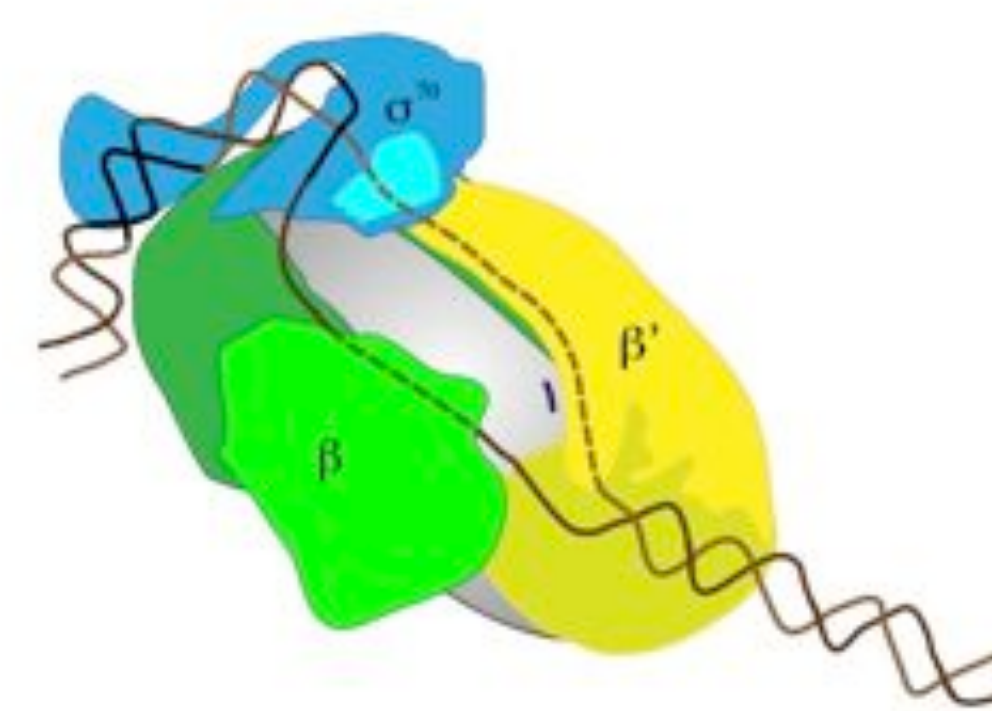
Commonalities with DNA dependent transcription

Transcription initiation

6S RNA dependent transcription



DNA dependent transcription

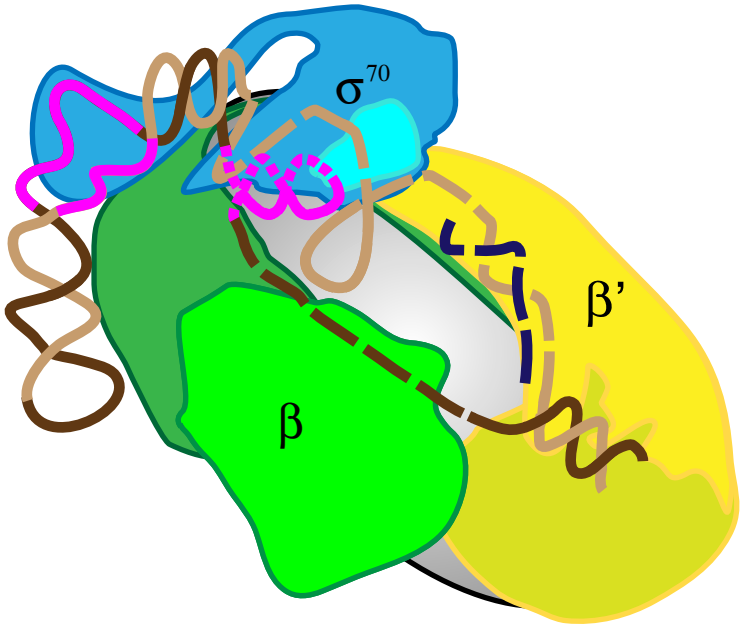


Panchapakesan, RNA, 2012

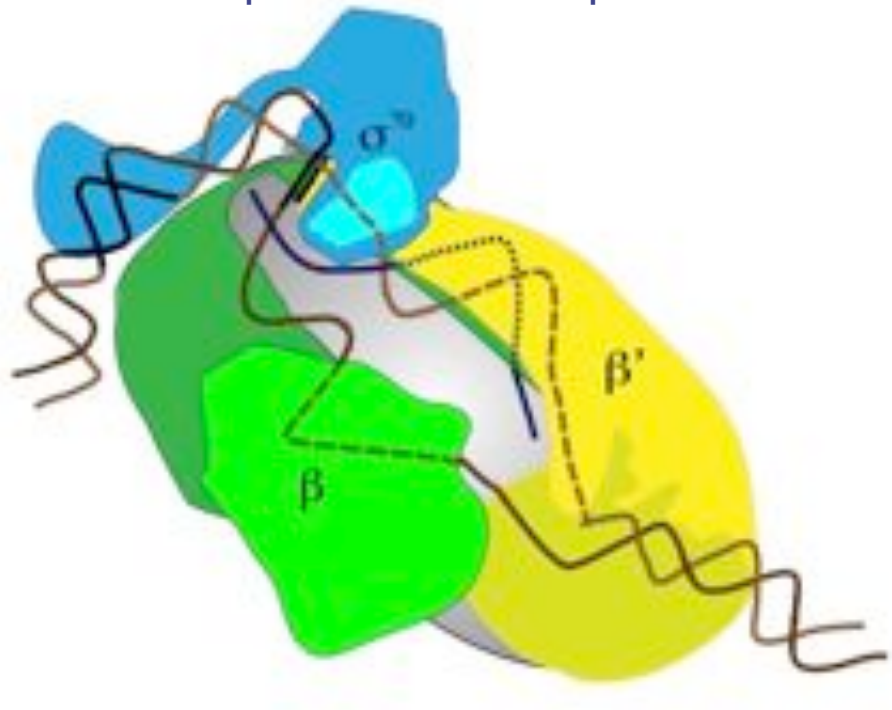
Commonalities with DNA dependent transcription

Template Scrunching

6S RNA dependent transcription



DNA dependent transcription

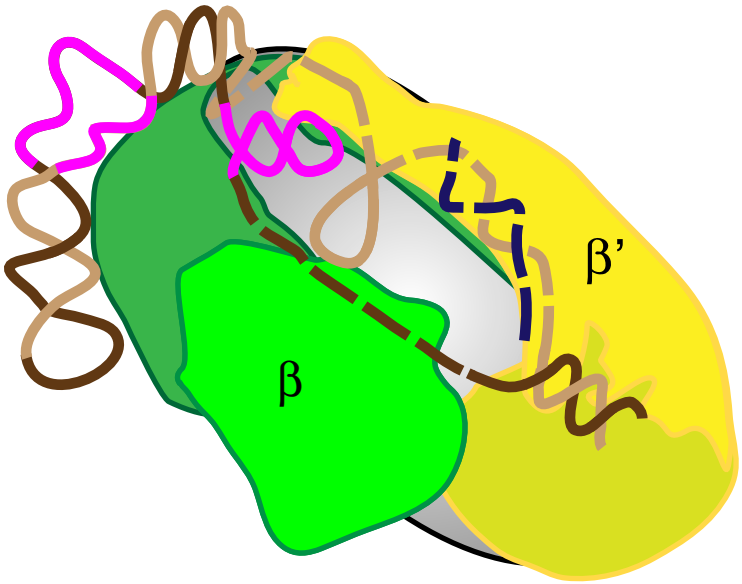


Panchapakesan, RNA, 2012

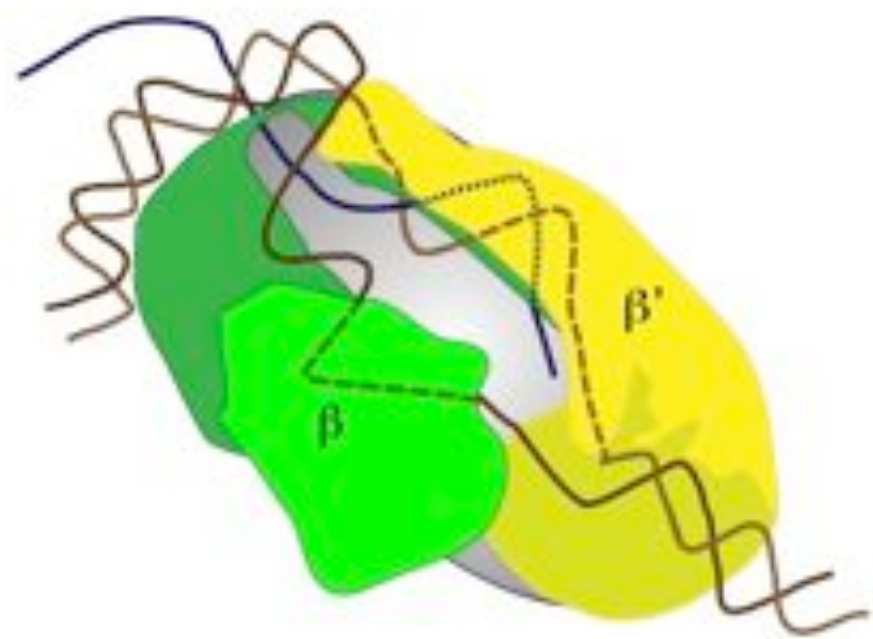
Commonalities with DNA dependent transcription

σ factor release

6S RNA dependent transcription

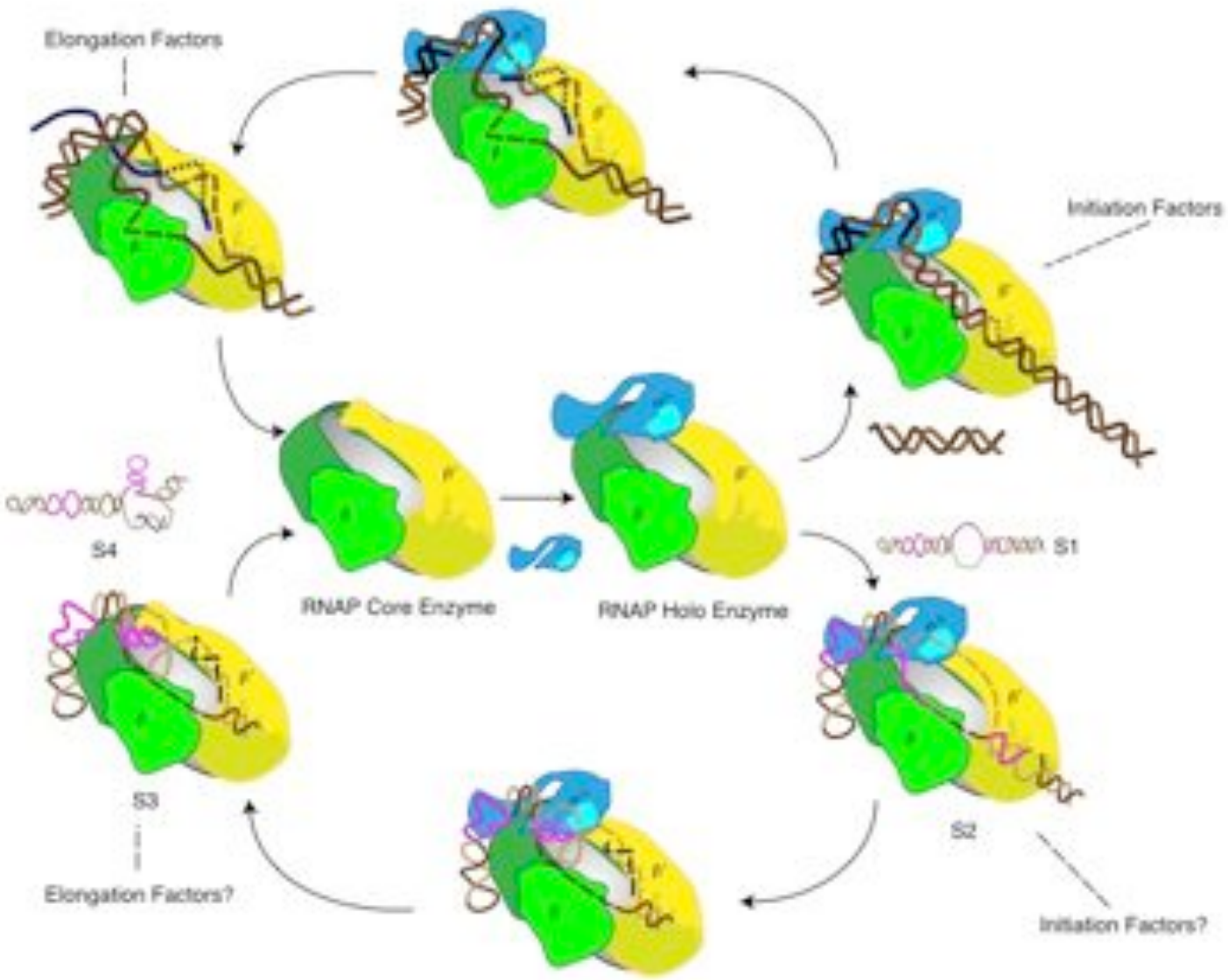


DNA dependent transcription



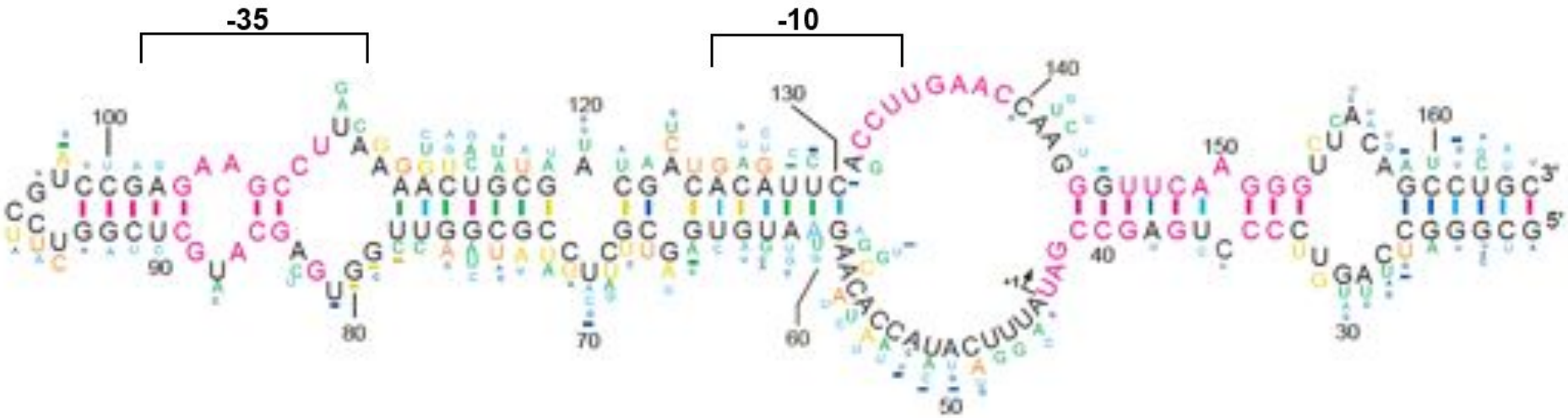
Panchapakesan, RNA, 2012

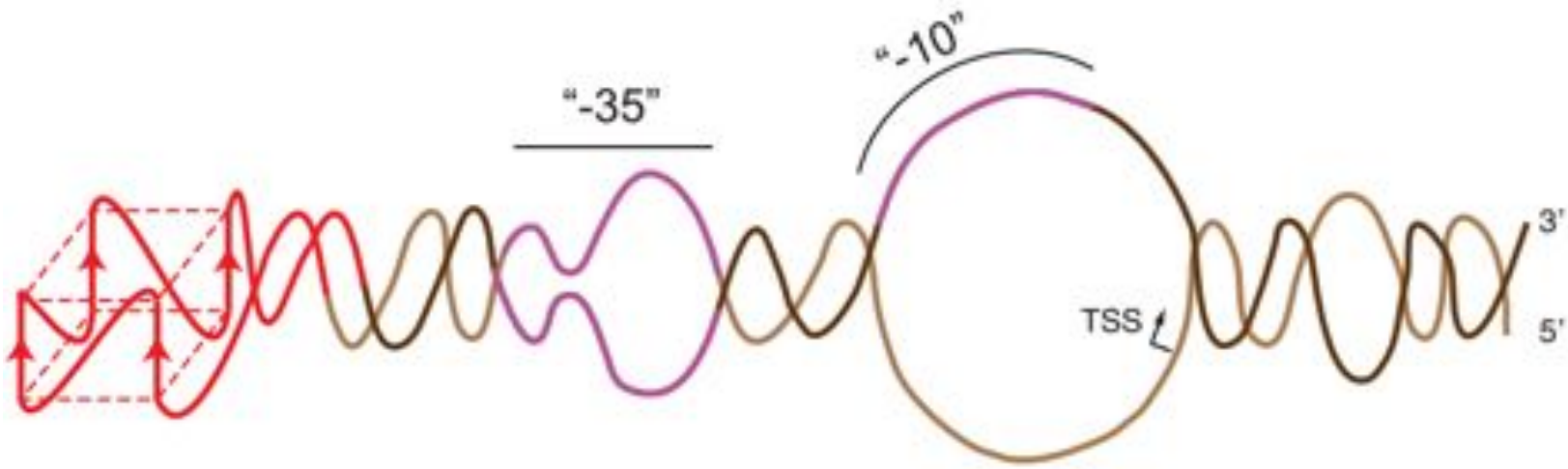
Commonalities with DNA dependent transcription



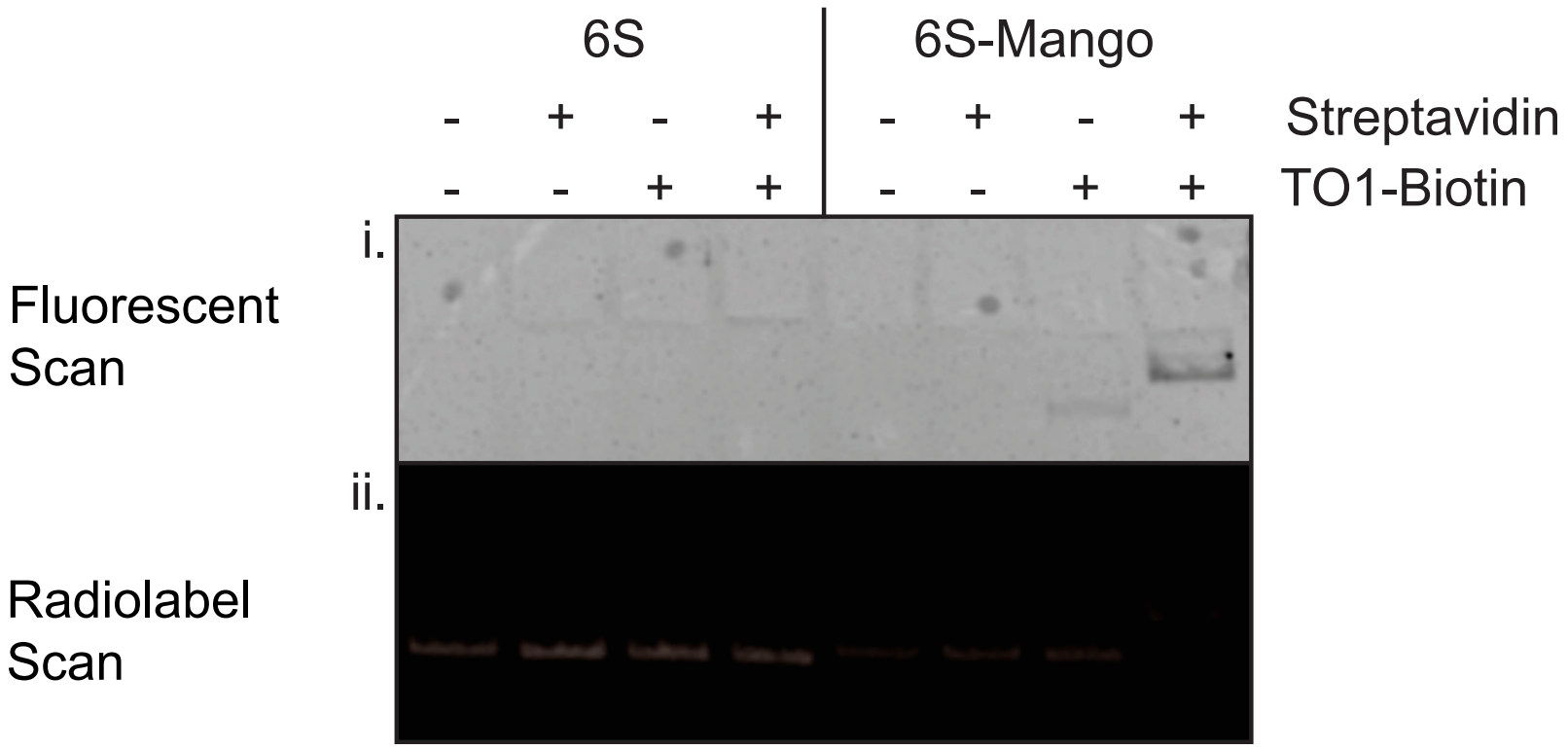
Panchapakesan, RNA, 2012

Tagging 6S RNA with RNA Mango



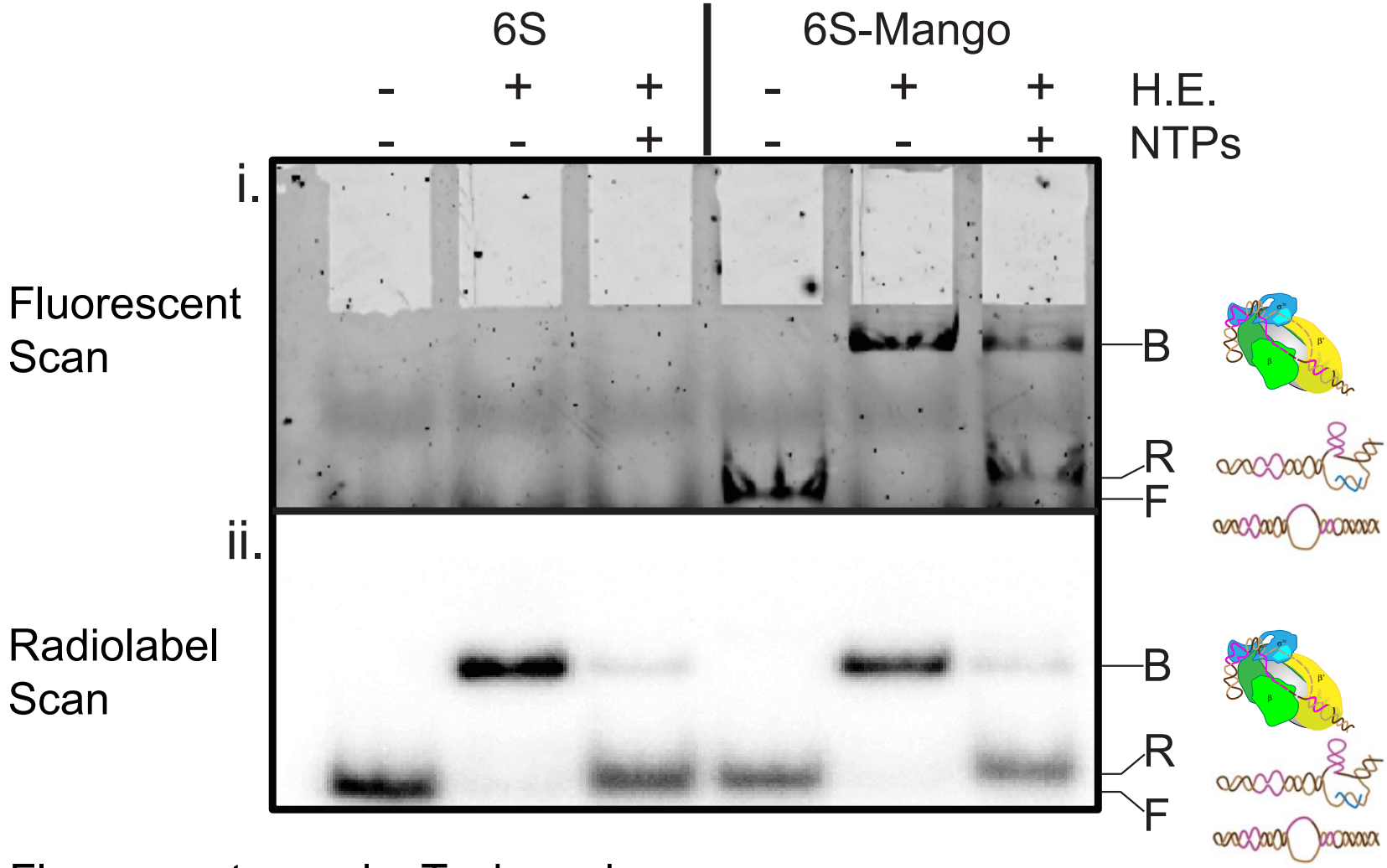


6S RNA Mango retains its dye binding and fluorescence inducing properties



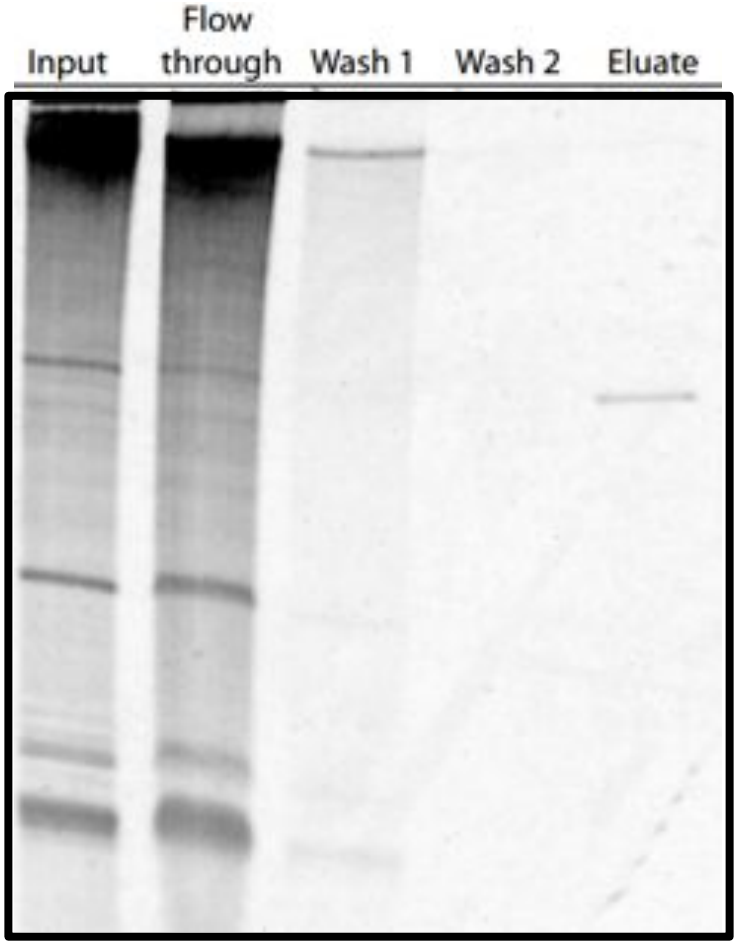
Imaged using a UV light box

6S RNA Mango retains its RNAP binding and pRNA synthesis properties



Fluorescent scan by Typhoon imager

Towards purification of native 6S RNA Mango complex from *E. coli*



RNA staining by SYBR Green

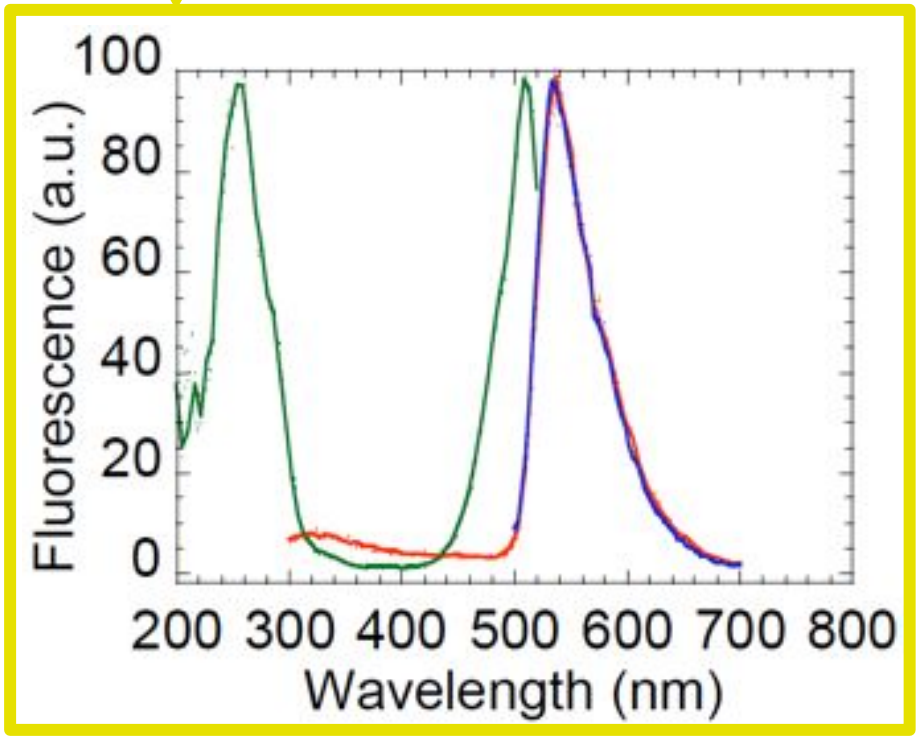
RNA MANGO SUMMARY

TO3-Biotin: A Red Mango

TO1-Biotin

+ - **RNA Mango**

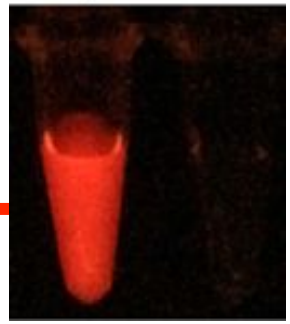
2 μM complex

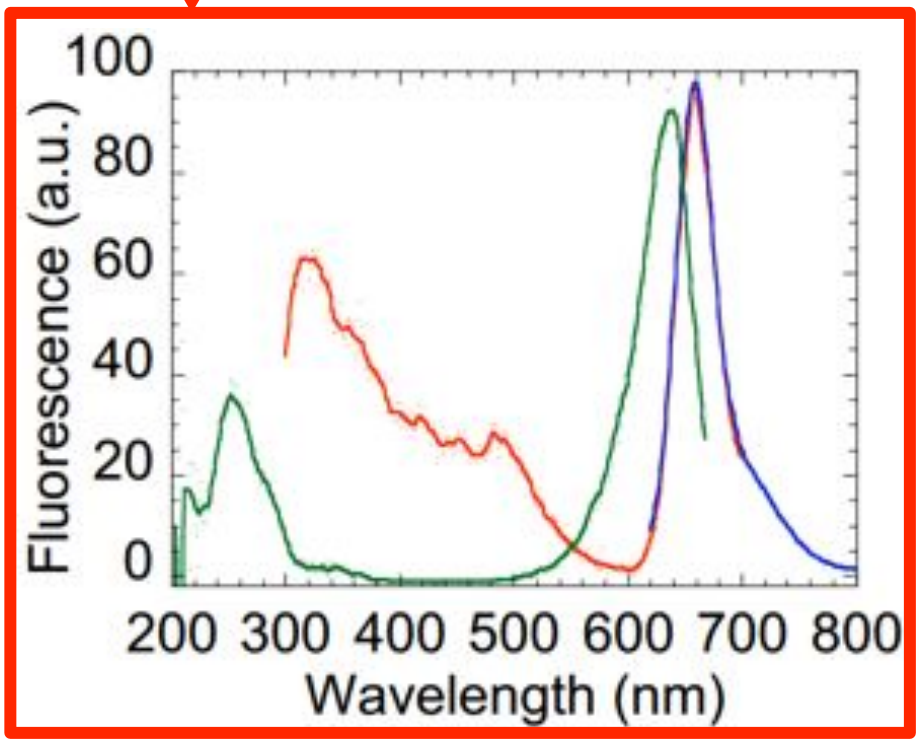


TO3-Biotin

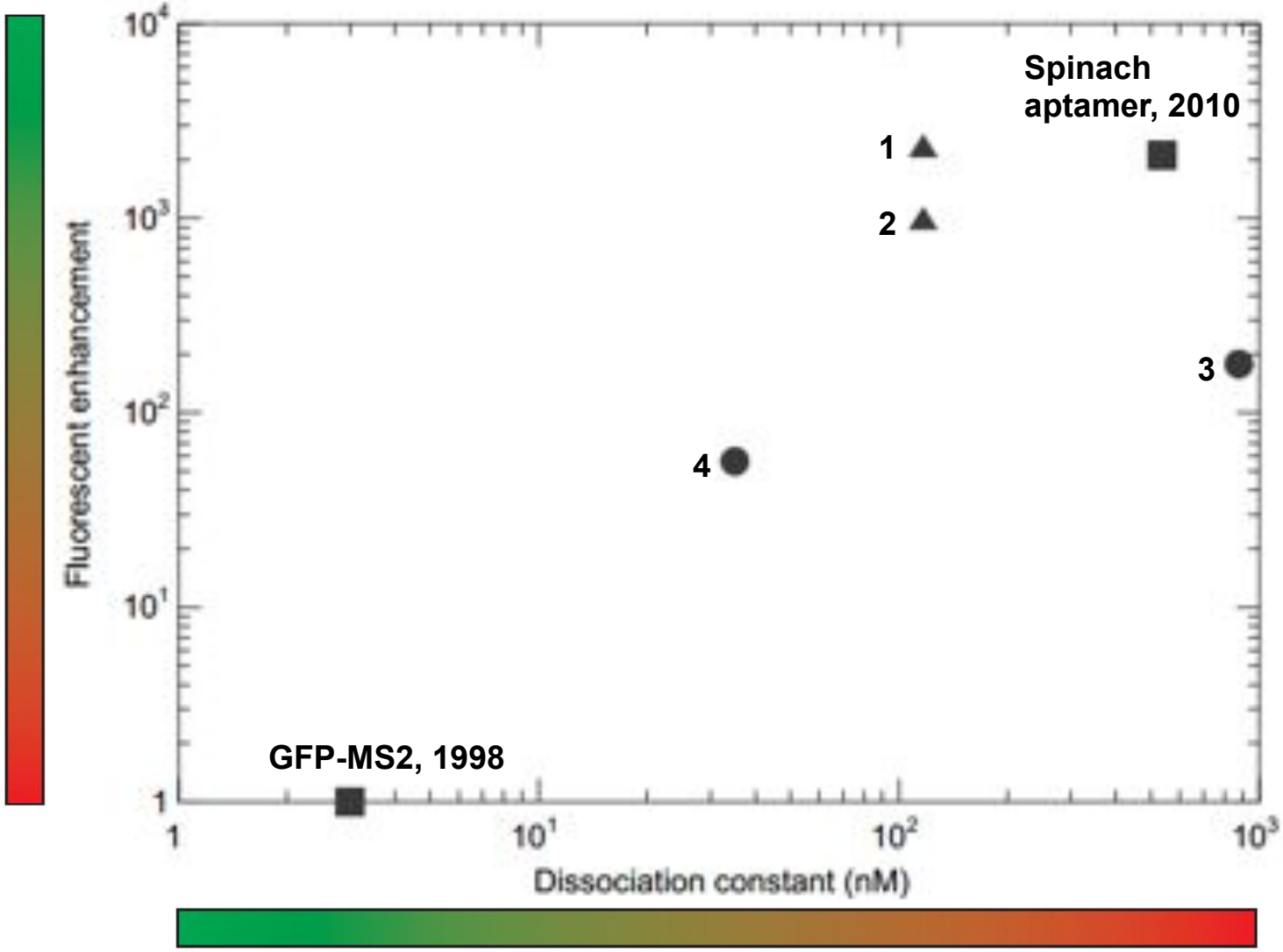
+ - **RNA Mango**

2 μM complex





An optimal RNA tracking system

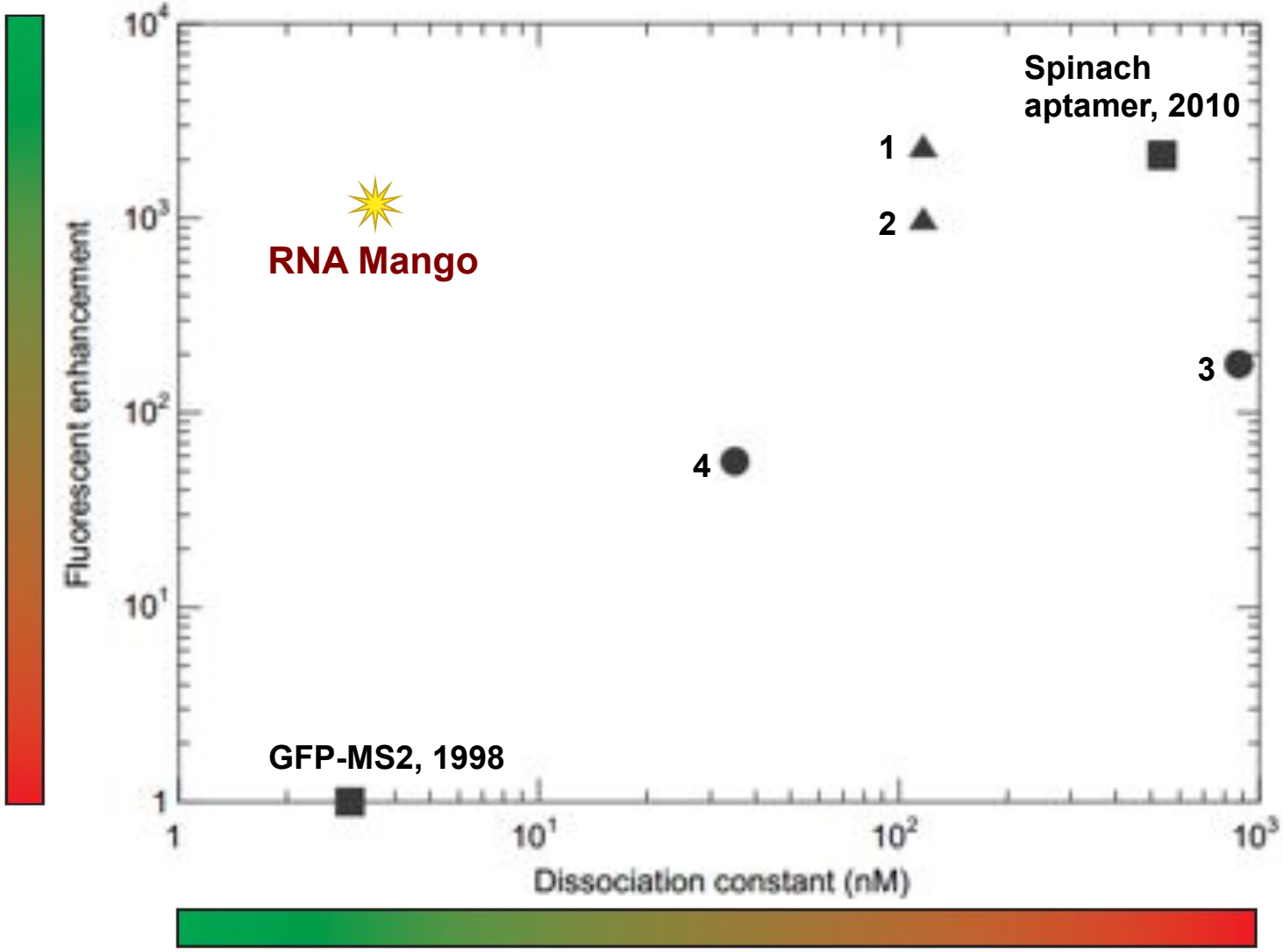


▲ Toxic to cells

● Poor membrane permeability

1. Malachite green, 2003
2. Dimethylin dole red, 2008
3. Hoechst 33258: DNA, 2008
4. Hoechst 33258: RNA 2007

An optimal RNA tracking system



▲ Toxic to cells

● Poor membrane permeability

1. Malachite green, 2003
2. Dimethylin dole red, 2008
3. Hoechst 33258: DNA, 2008
4. Hoechst 33258: RNA 2007

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Unrau lab members:

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- Razvan Cojocaru

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- Patrick Chen

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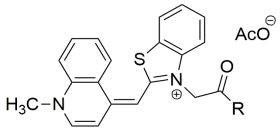
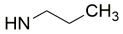
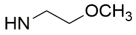
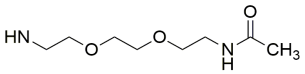
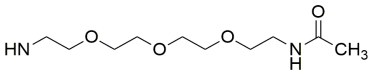
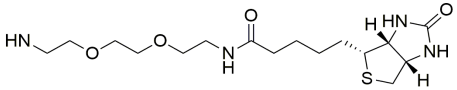
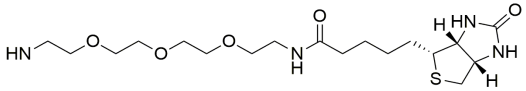
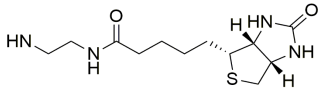
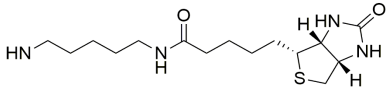
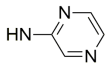
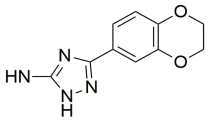
Nancy Hawkins

NSERC CRSNG

Michael Smith Foundation for Health Research

TO1 dye derivatives

Compound Number	 TO1-*N*-Acetamides, R =	K_D nM	F_E
1		6.4	230
2		6.8	220
3		3.4	440
4		4.2	600
5		2.4	1,200
6		3.2 ± 0.7	1,100
7		5.6	660
8		1.6	1,100
9		2.5	390
10		12	560