Fluorescent RNA aptamers mimicking green fluorescent protein

technical journal club
14/10/2014

Manuela Pfammatter

fluorescence spectroscopy

RNA aptamers

RNA mimics of green fluorescent protein (GFP)   *Paige et al., Science, 2011*

fluorescence imaging of cellular metabolites   *Paige et al., Science, 2012*

structural basis of the Spinach fluorescence   *Huang et al., Nat Chem Biol, 2014*
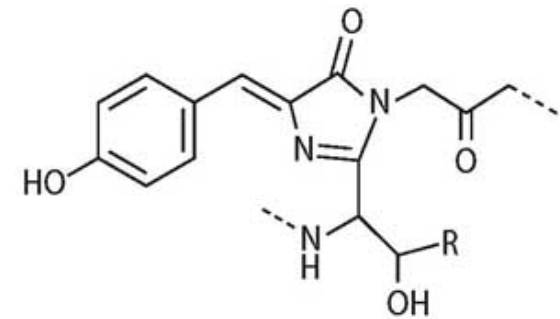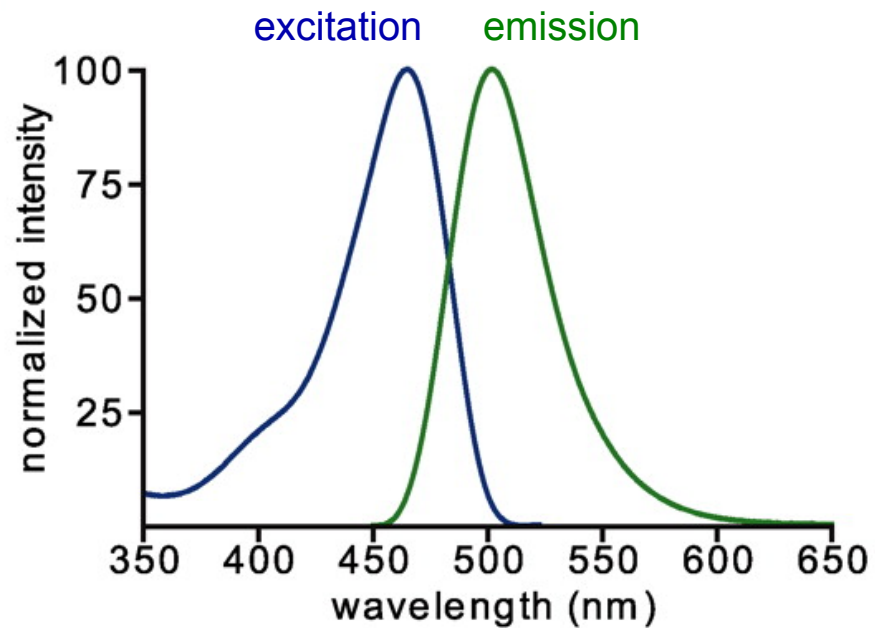
conclusion & outlook
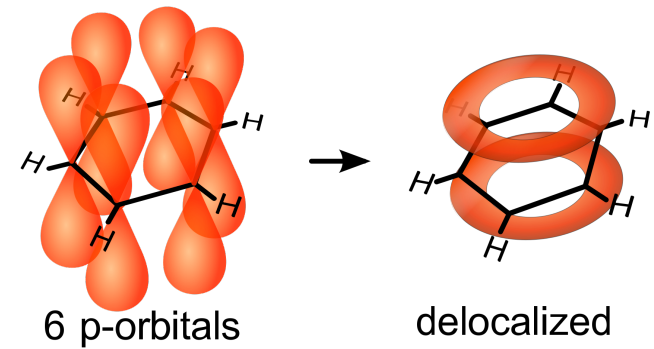
Fluorescence Spectroscopy – Jablonski diagram

property of a compound to absorb light of a certain energy and to rapidly re-emit light of lower energy through the radiative loss of energy

Fluorescence Spectroscopy

excitation   emission

(1) GFP fluorophore

6 p-orbitals → delocalized
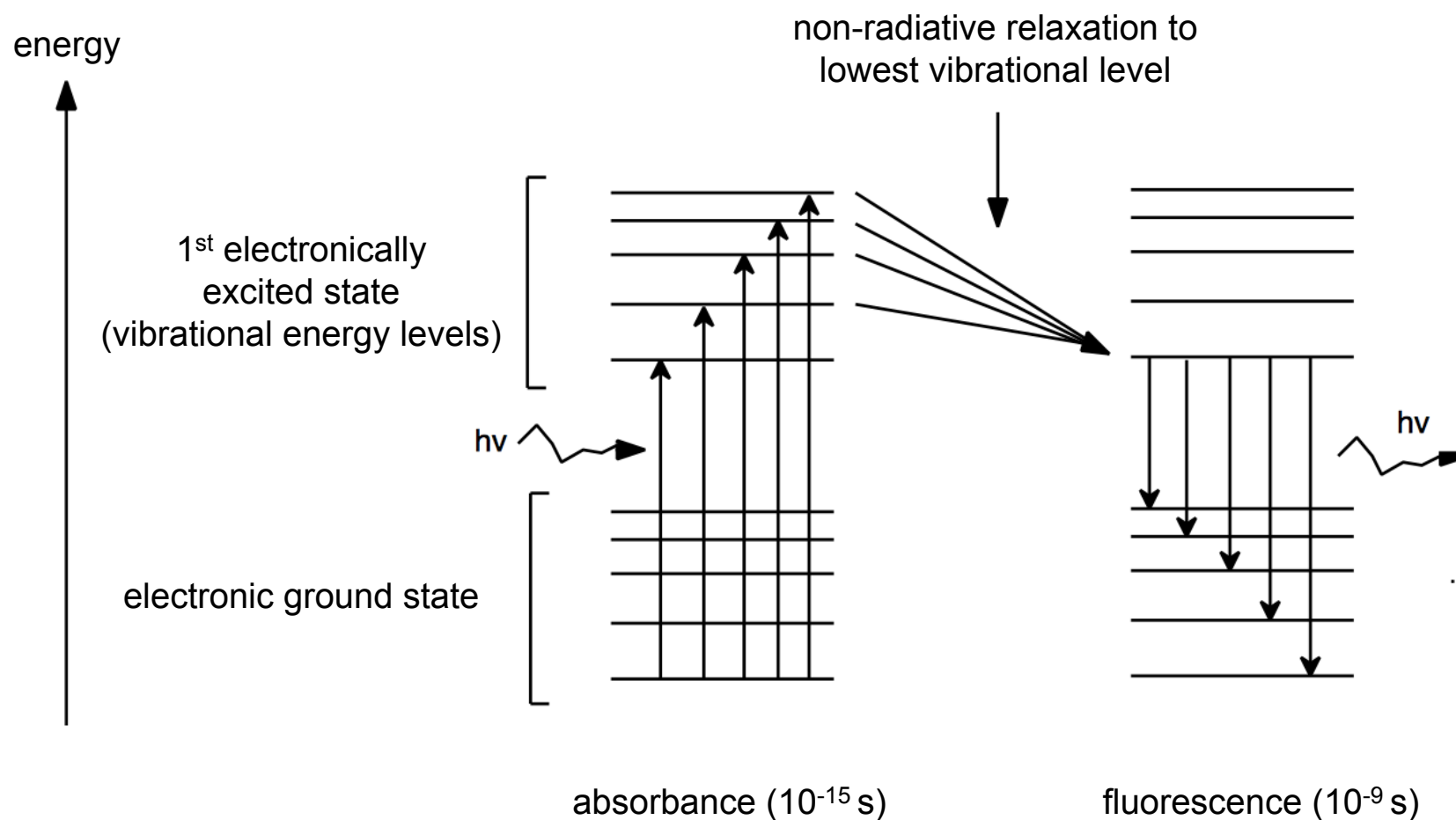
organic fluorophores are planar systems with delocalised π-electrons
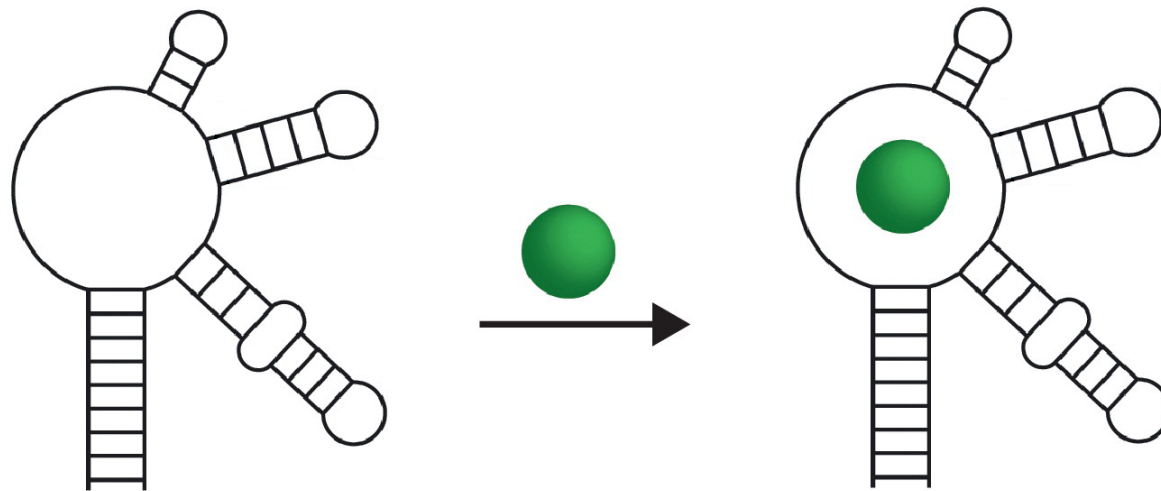
Fluorescent **RNA aptamers** mimicking green fluorescent protein
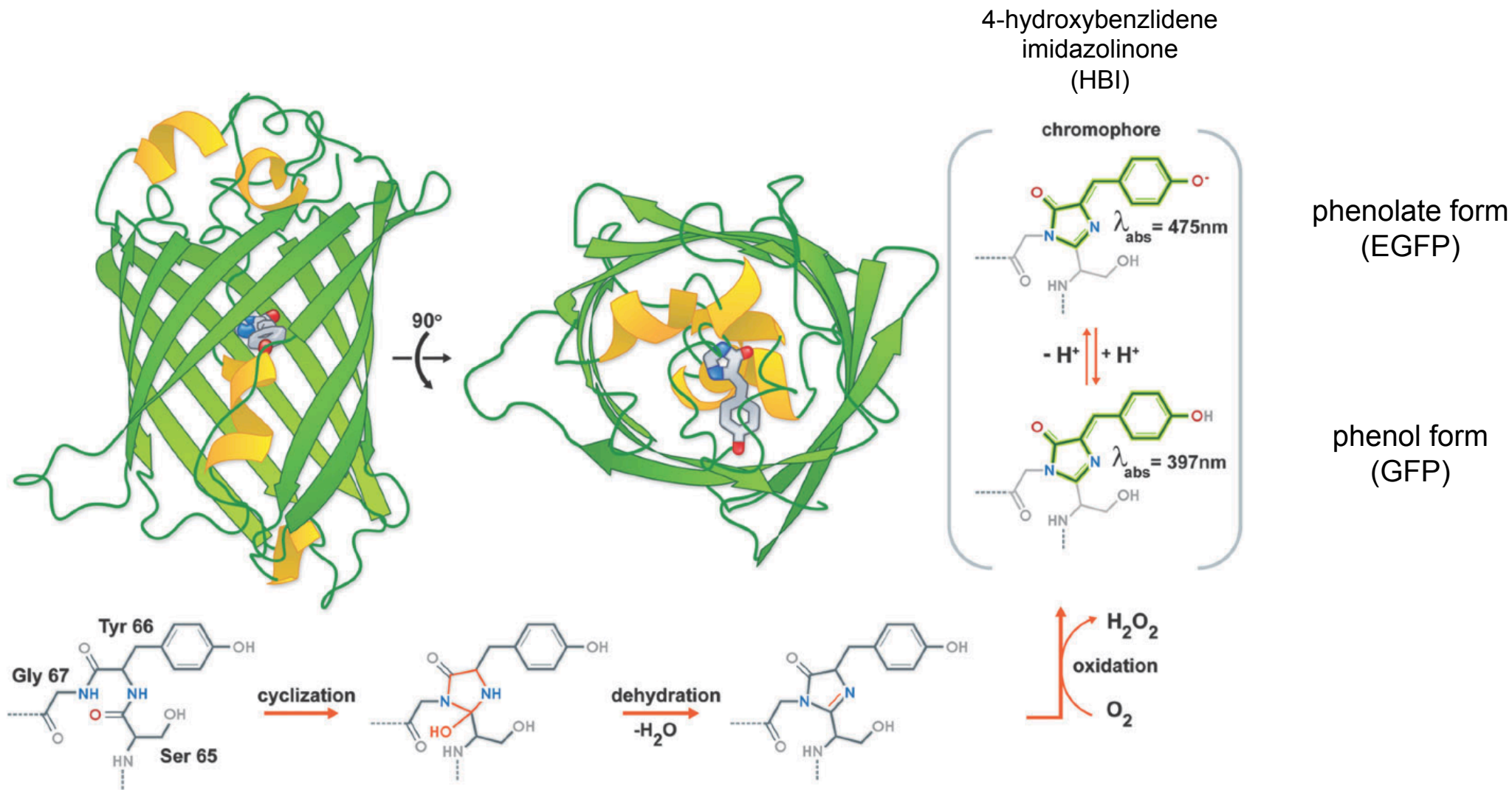
RNA aptamer

short strand of oligonucleotides

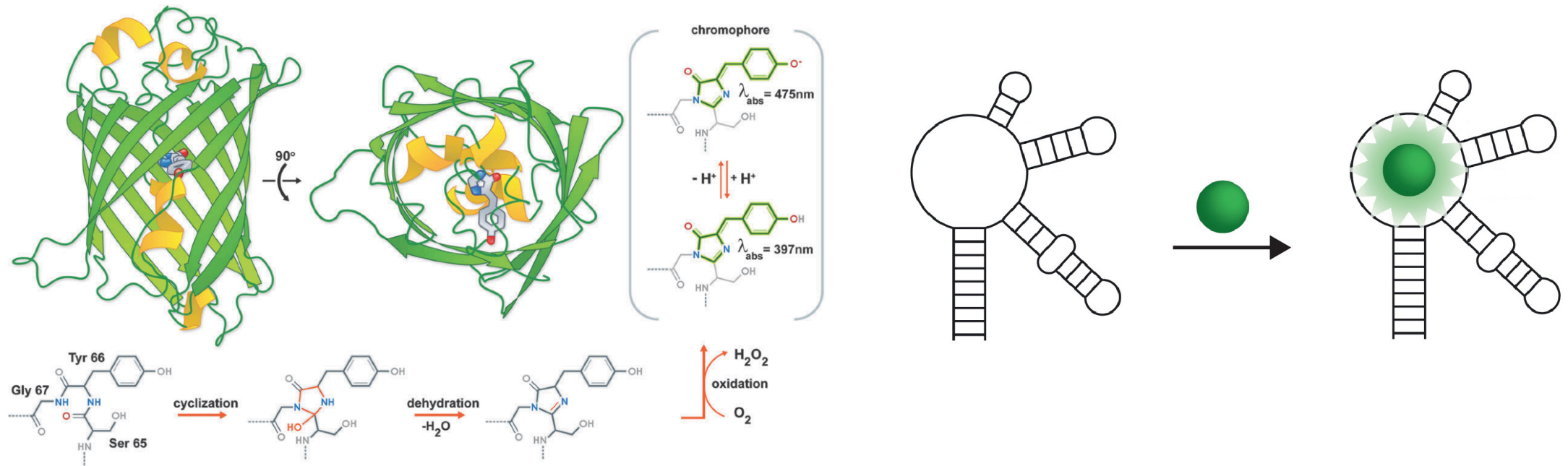fold into a well-defined three-dimensional structure

high affinity and specificity for their target molecules

Fluorescent RNA aptamers mimicking **green fluorescent protein**

Fluorescent RNA aptamers mimicking green fluorescent protein

conditional, specific activation of fluorophore

non-cytotoxic, membrane permeable, non-interfering

brightness, sensitivity and photostability

RNA Mimics of Green Fluorescent Protein

Jeremy S. Paige,[1] Karen Y. Wu,[1] Samie R. Jaffrey[1,2]*

[1]Department of Pharmacology, Weill Medical College, Cornell University, New York, NY 10065, USA.
[2]Tri-Institutional Program in Chemical Biology, Weill Medical College, Cornell University, New York, NY 10065, USA.

generation of RNA aptamers that bind fluorophores resembling the fluorophore in GFP

1   fluorophore

HBI   DMHBI   DMABI   2-HBI   DFHBI
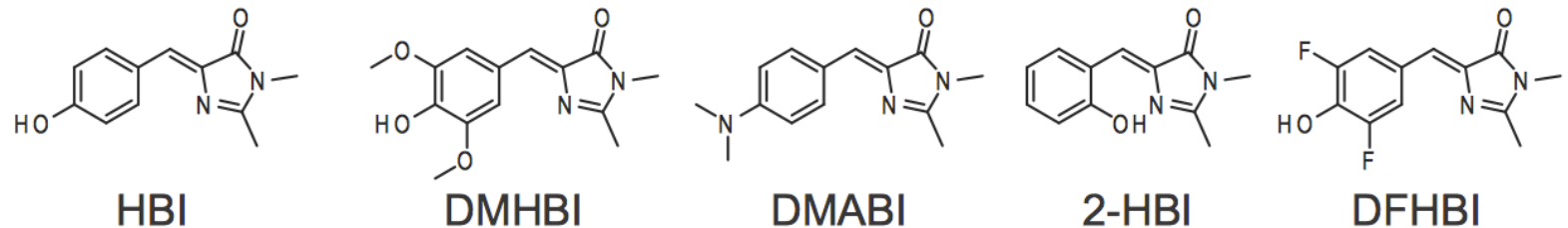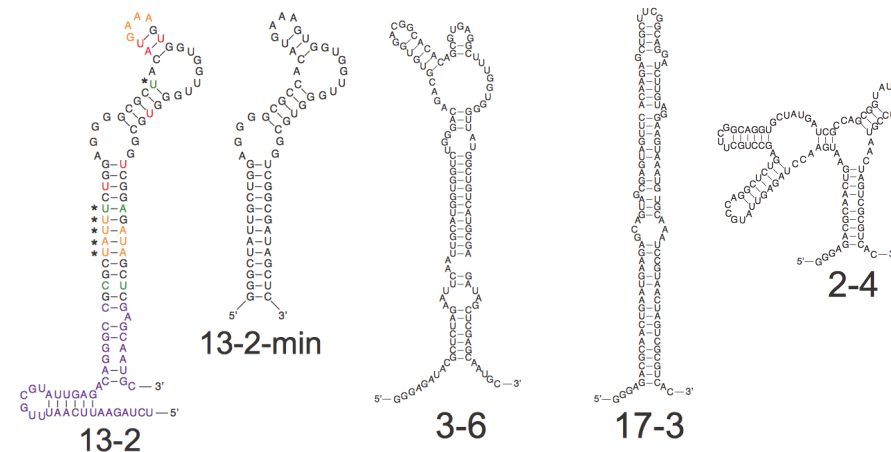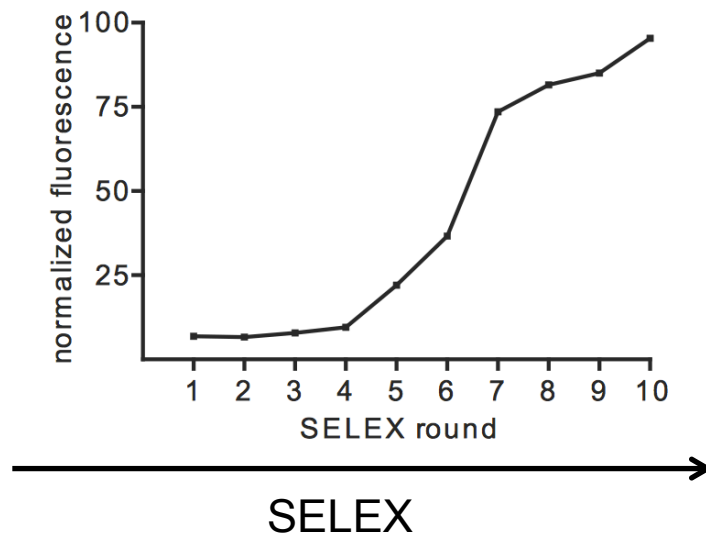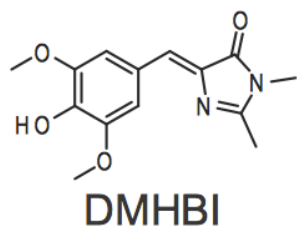
2   RNA aptamer   systematic evolution of ligands by exponential enrichment (SELEX)

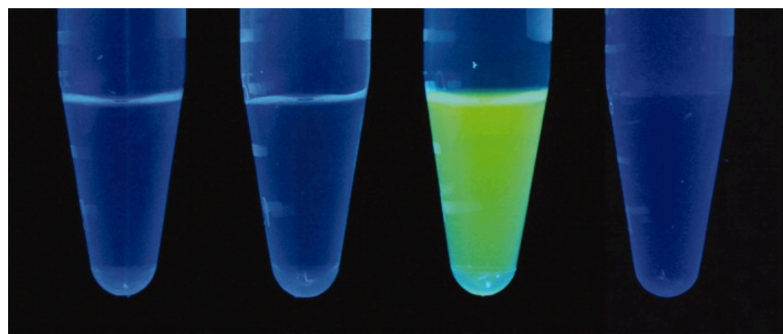# Systematic Evolution of Ligands by EXponential enrichment SELEX

production of oligonucleotides (single-stranded DNA or RNA) that specifically bind to a target ligand
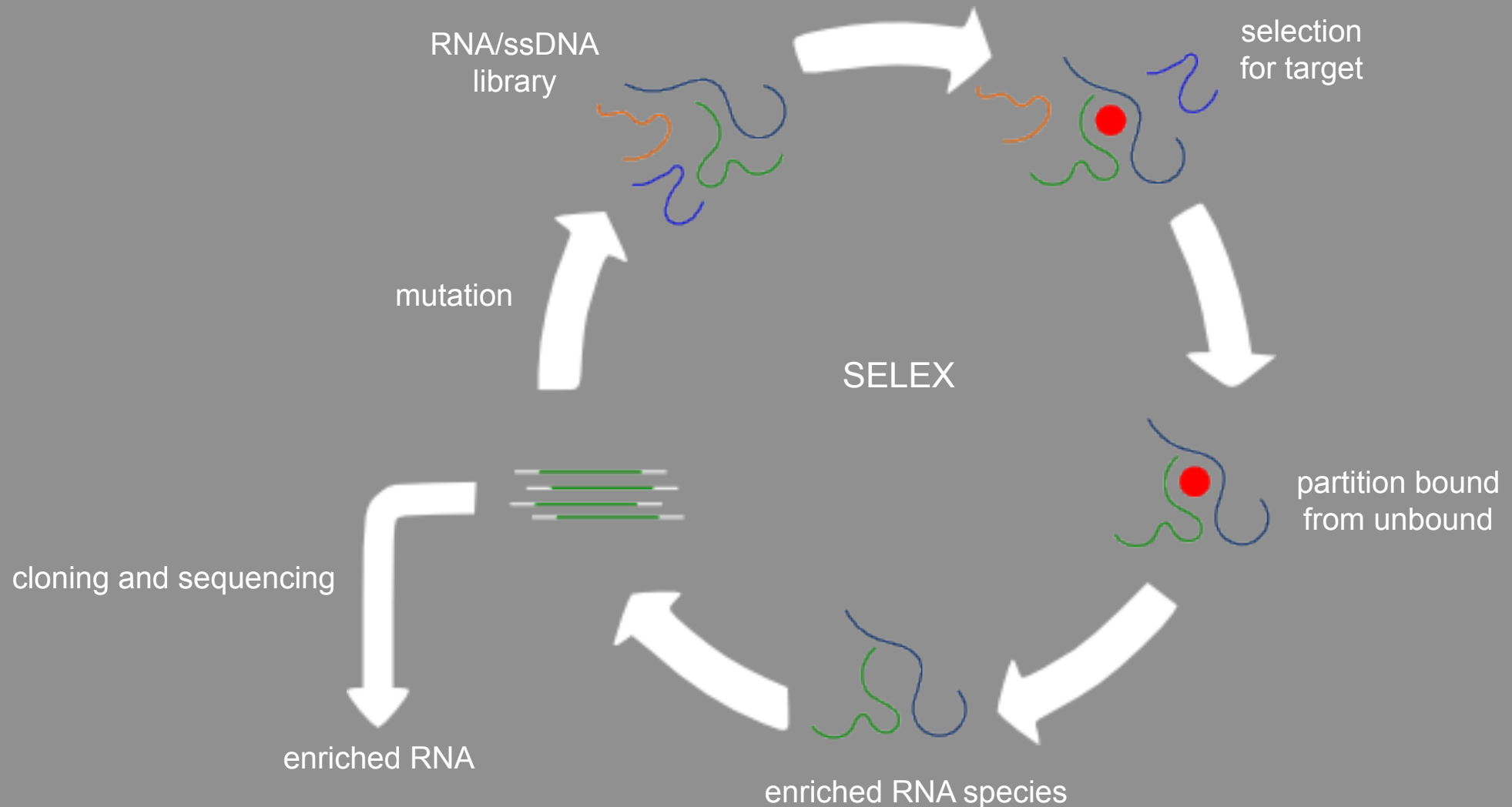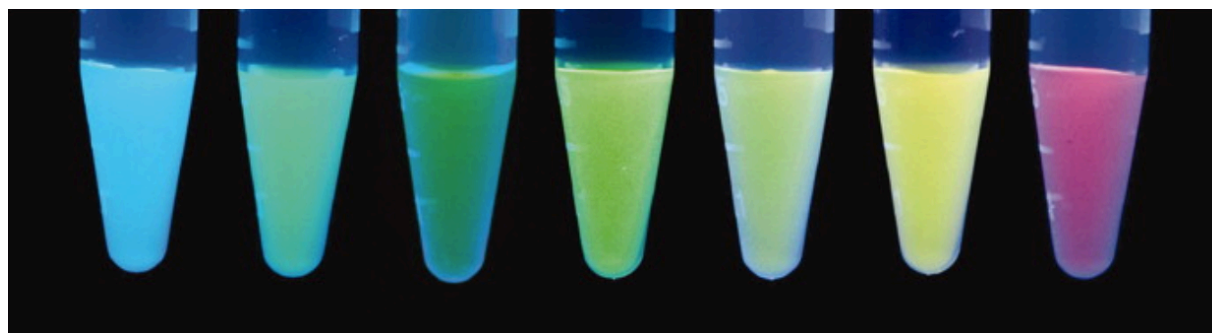
SELEX of fluorescent RNA aptamers

DMHBI

13-2   13-2-min   3-6   17-3   2-4

GFP-like RNA-fluorophore complexes spanning the visible spectrum

DMHBI   13-2 RNA   DMHBI +   DMHBI +
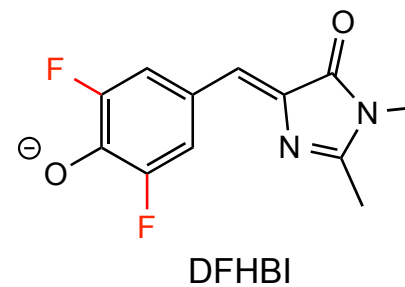   13-2 RNA   control RNA

Paige et al., Science, 2011

spectral properties of RNA-fluorophore complexes

A

DMHBI
DFHBI
DMABI
2-HBI

B

2-4
24-2
11-3
13-2
3-6
17-3
6-8

C

2-4
24-2
11-3
13-2
3-6
17-3
6-8

GFP

phenol

$\lambda(ex)_{max} \sim 400$ nm
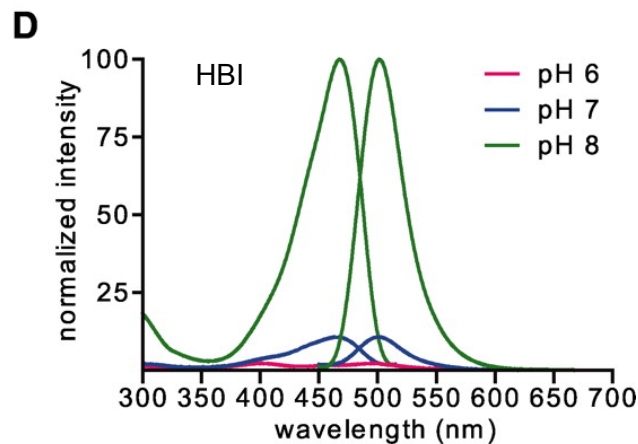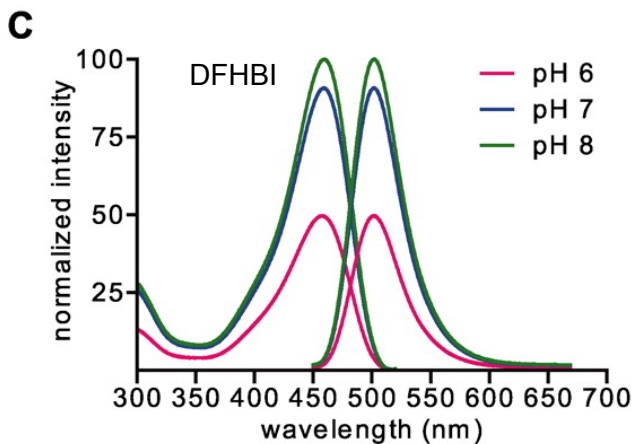
EGFP

phenolate

$\lambda(ex)_{max} > 400$ nm
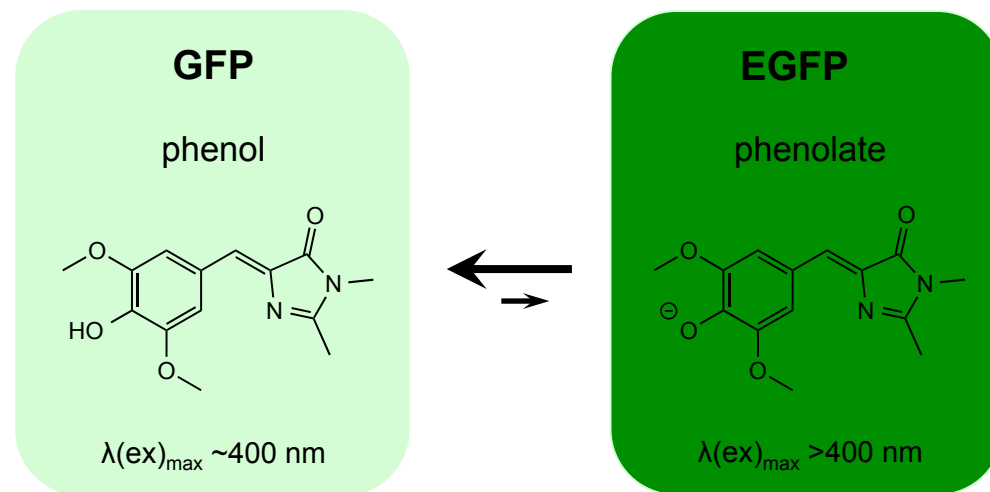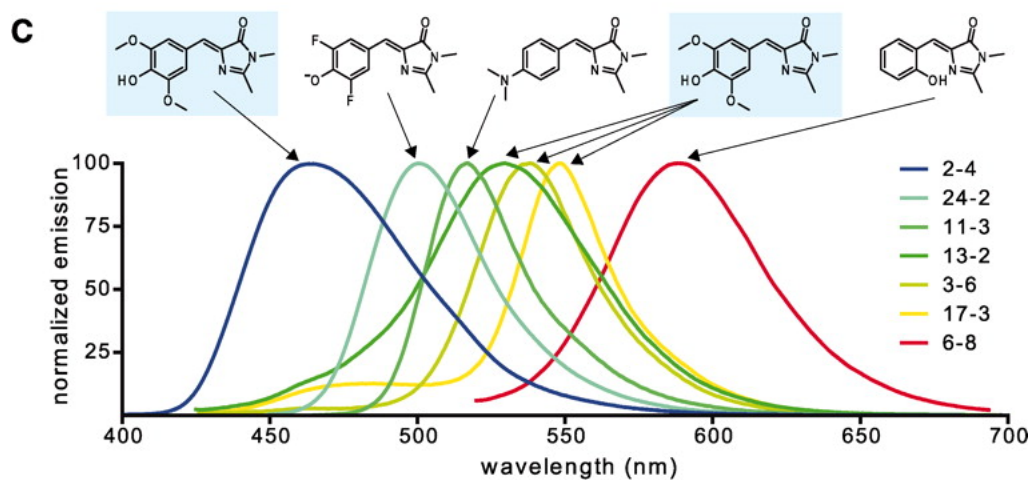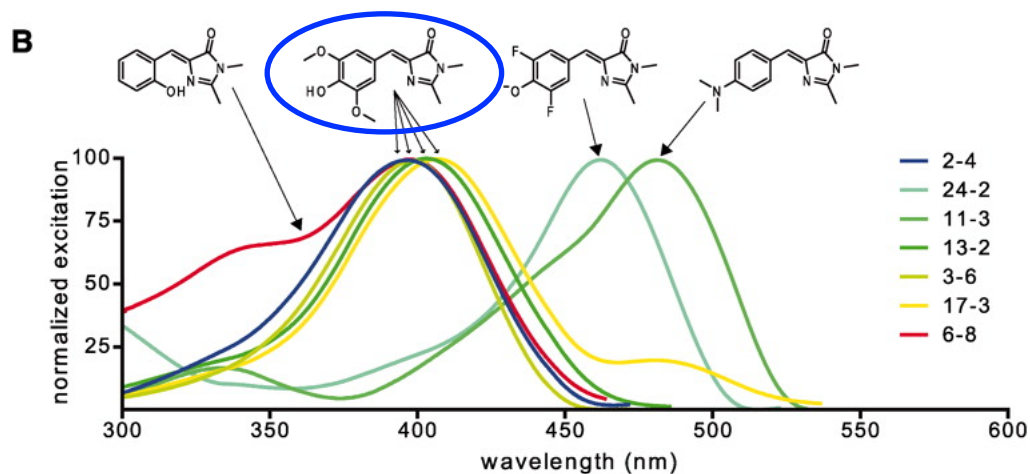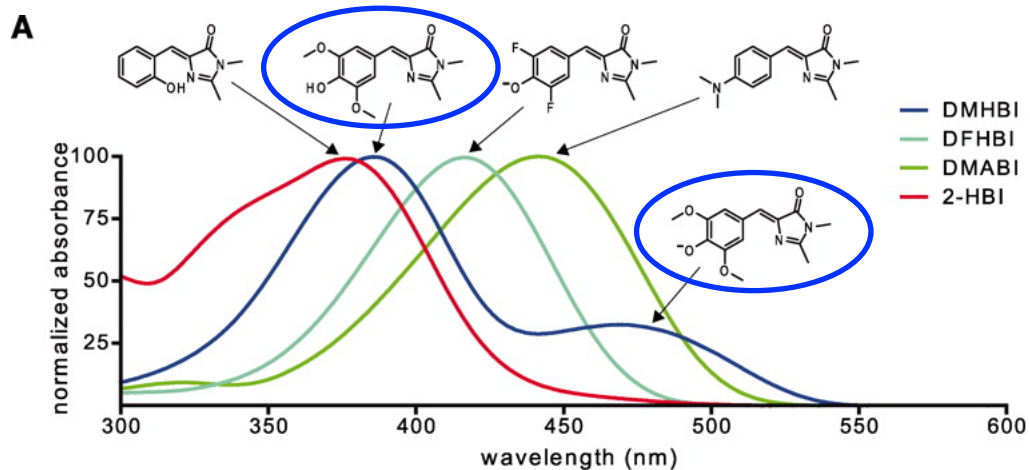
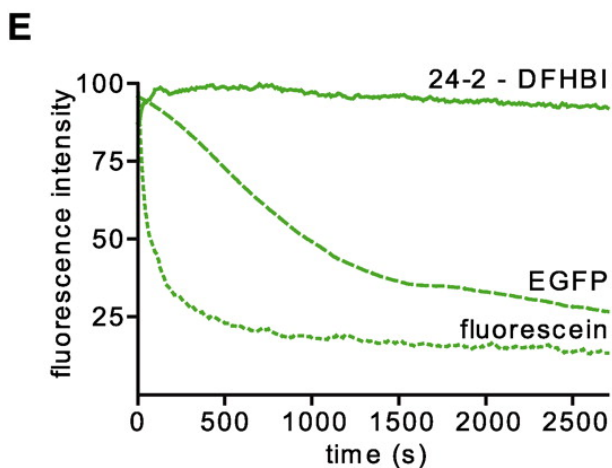increase acidity of phenolic –OH group
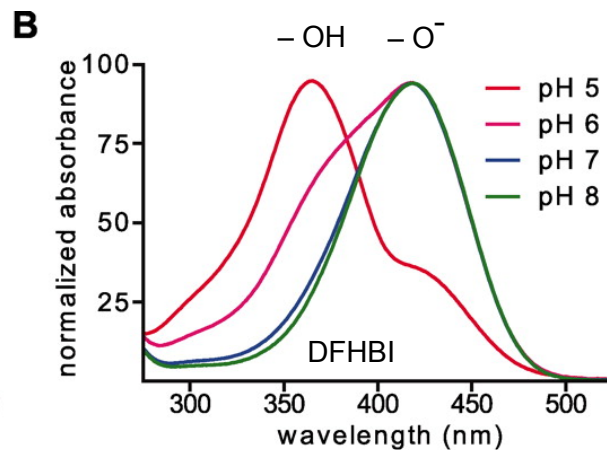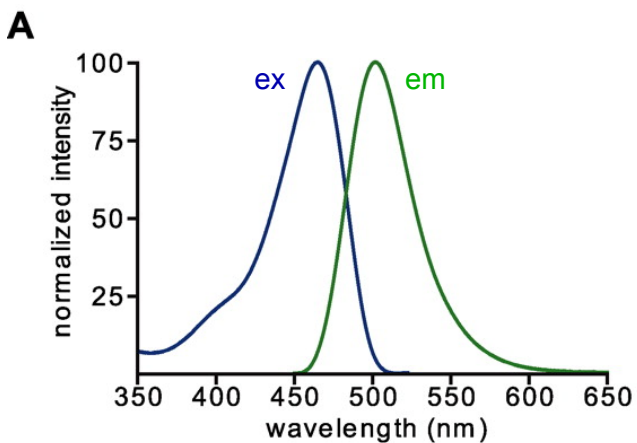
DFHBI

photophysical properties of
24-2–DFHBI complex

24-2 selectively recognises the
phenolate form of DFHBI

–> EGFP-like

negligible photobleaching

EGFP-like fluorescent RNA
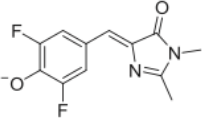24-2–DFHBI complex ("Spinach")

Paige et al., Science, 2011

photophysical properties of
24-2–DFHBI complex

Fluorophore	Extinction coefficient ($M^{-1}cm^{-1}$)	Fluorescence quantum yield	Brightness
Aequorea GFP[c]	27,600	0.79	100
EGFP[c]	55,000	0.60	151
DFHBI	11,864	0.0007	0.04
24-2 (Spinach)	24,271	0.72	80

Brightness (extinction coefficient × quantum yield) is reported relative to *Aequorea* GFP.

24-2 selectively recognises the
phenolate form of DFHBI

–> EGFP-like

negligible photobleaching

development of EGFP-like fluorescent RNA: 24-2–DFHBI complex ("Spinach")

Imaging of RNA in *E. coli* using Spinach fluorescence

transformation of *E. coli* with plasmids expressing Spinach fused to RNA-stabilising element

incubation with DFHBI
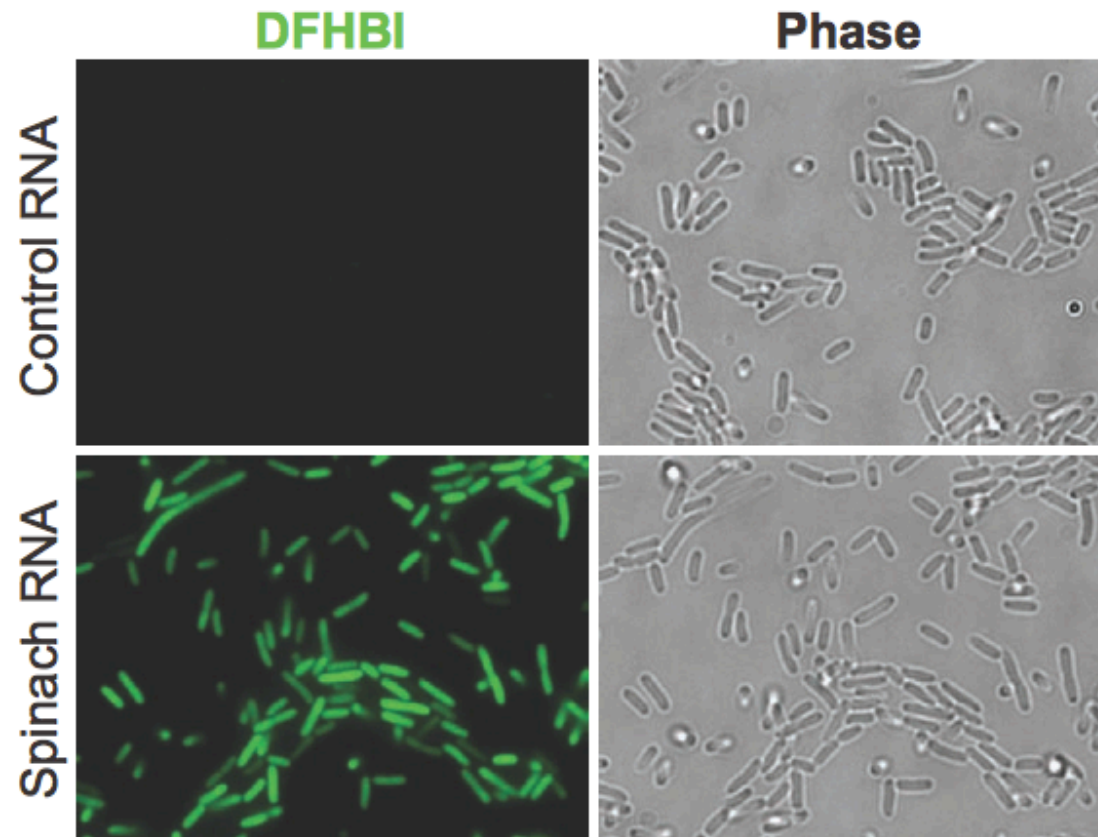
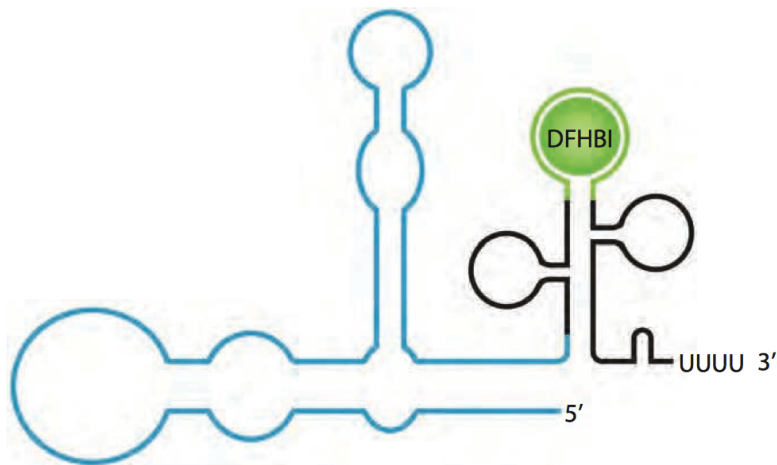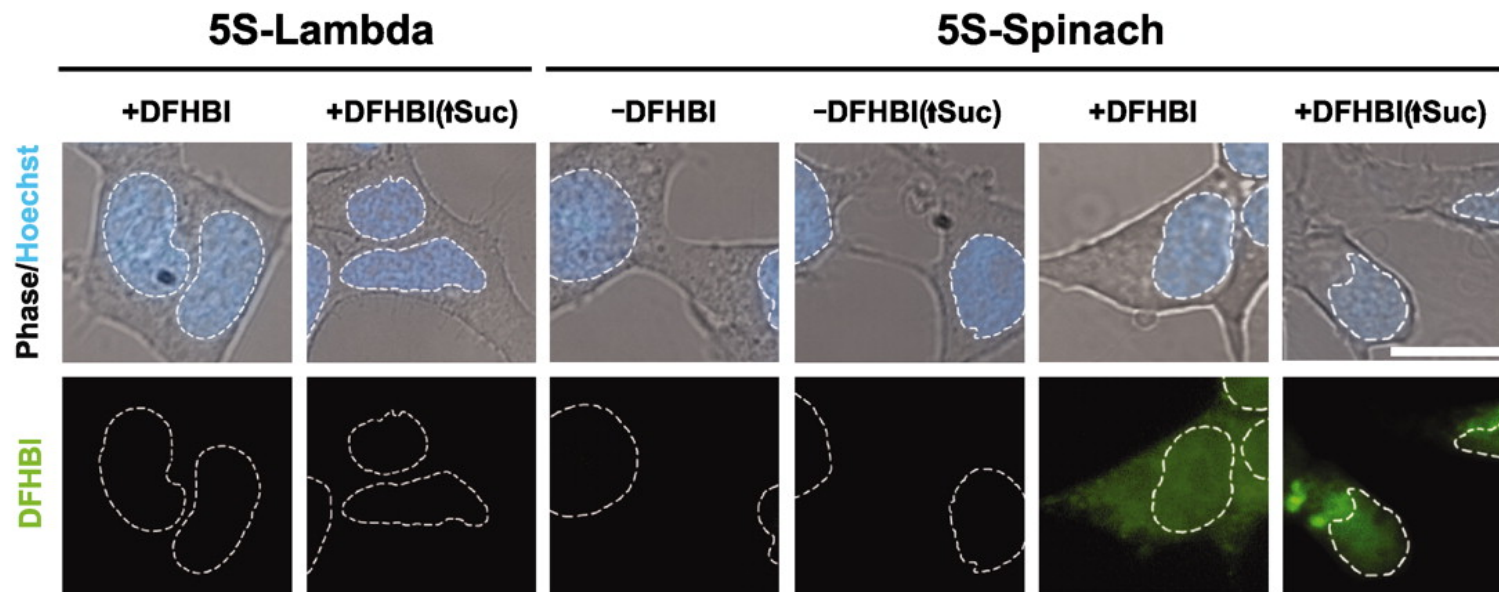# RNA dynamics in living mammalian cells monitored with Spinach fluorescence

fusion of Spinach to 5S

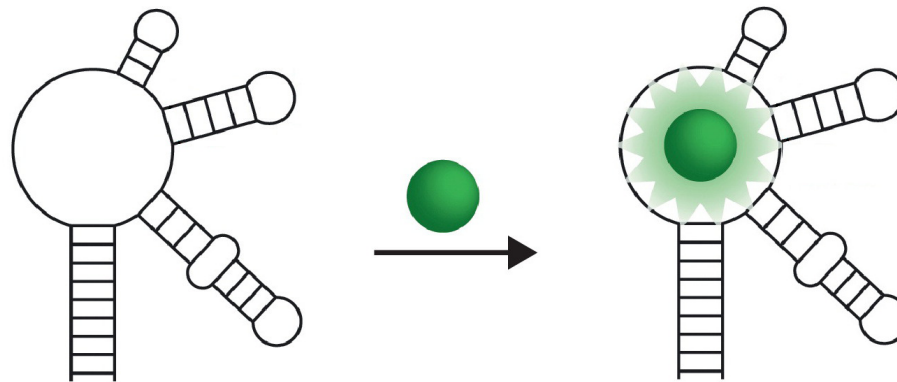transfection of construct into HEK293 T cells

development of Spinach, an EGFP mimicking fluorescent RNA aptamer

    conditional, specific activation of fluorophore DFHBI

    non-cytotoxic, membrane permeable, non-interfering

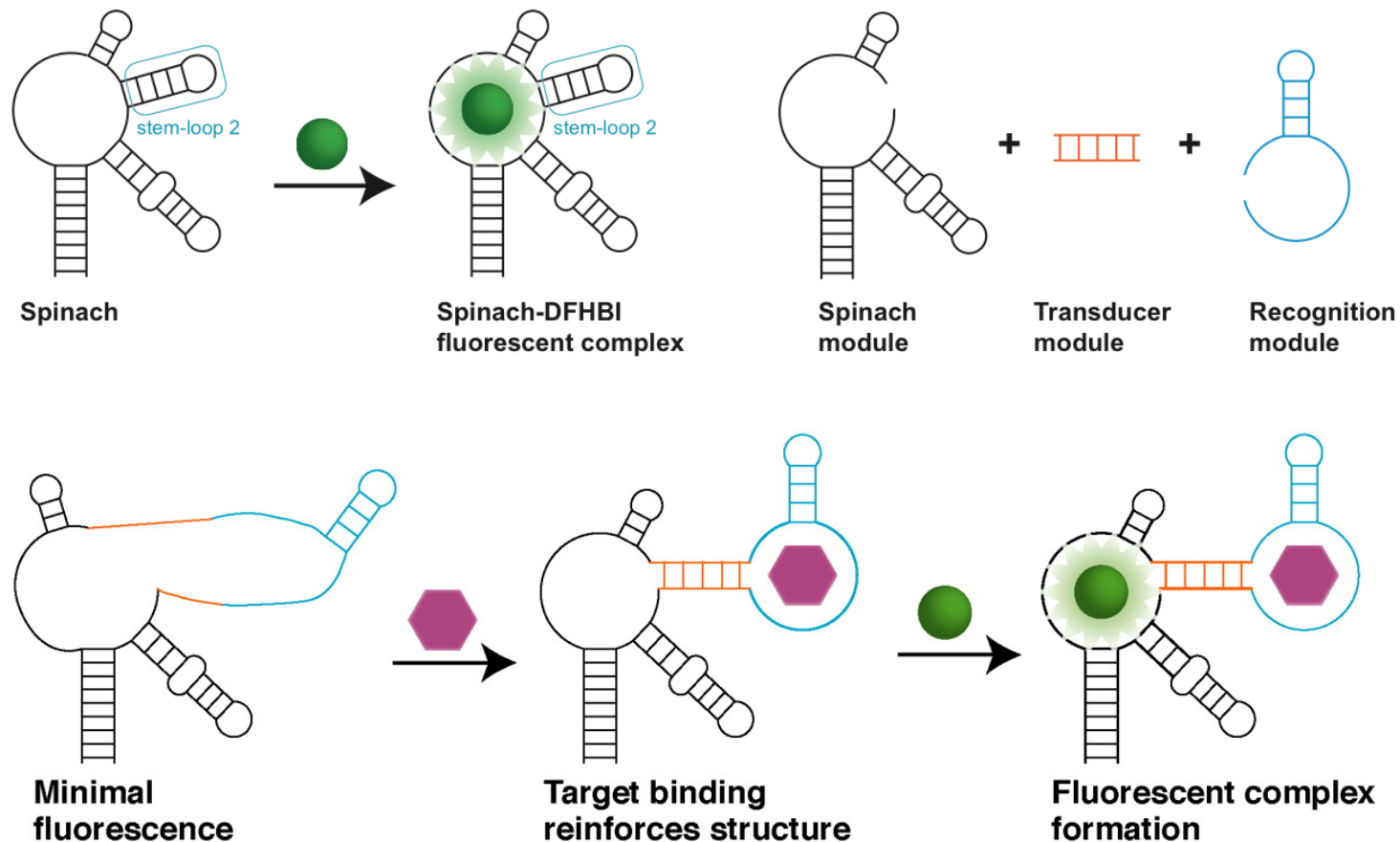   high photostability

application: tracking of RNA dynamics

Fluorescence Imaging of Cellular Metabolites with RNA

Jeremy S. Paige, Thinh Nguyen-Duc, Wenjiao Song, Samie R. Jaffrey*

Department of Pharmacology, Weill Medical College, Cornell University, New York, NY 10065, USA.
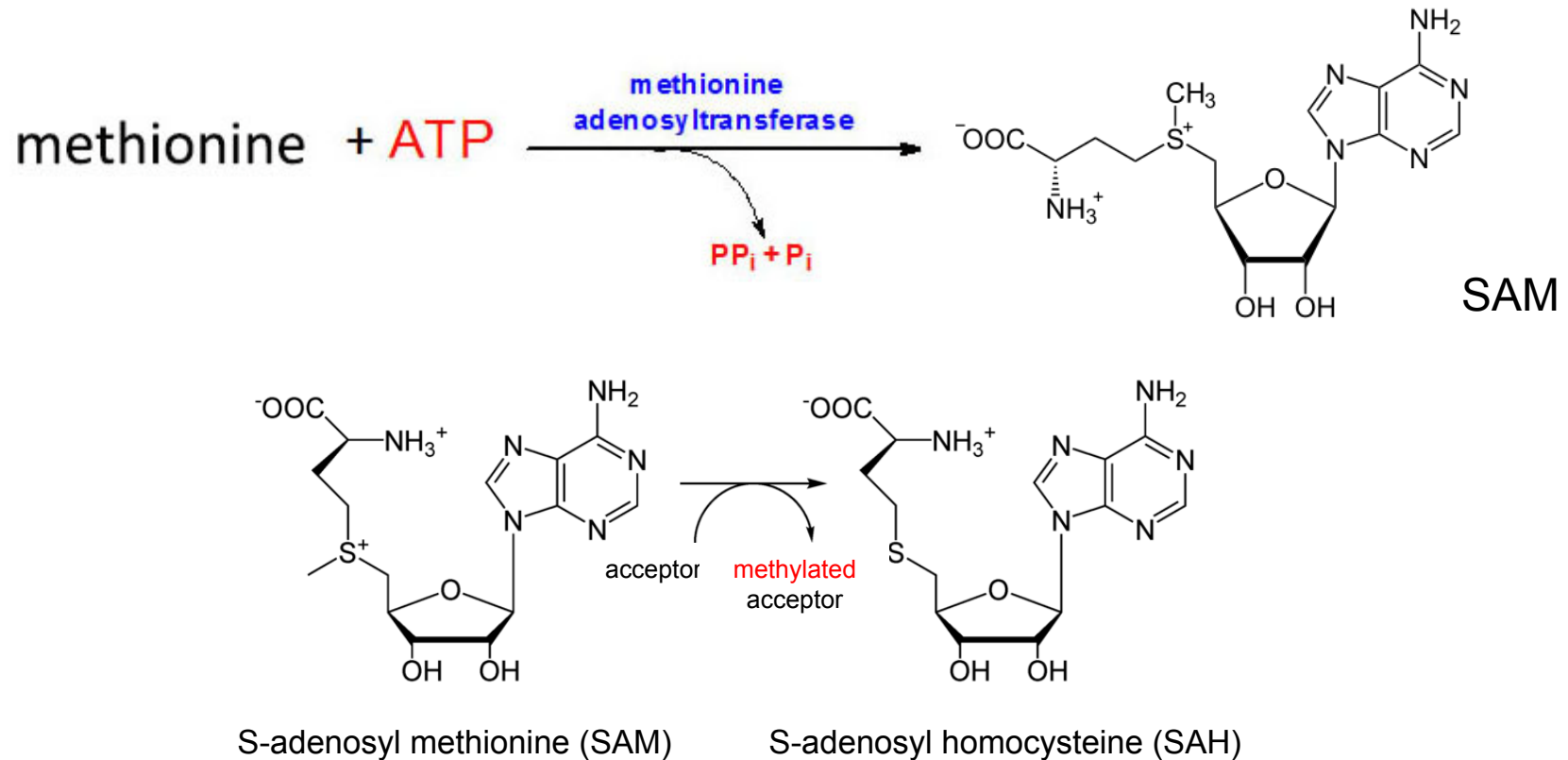
real-time imaging of cellular metabolites using Spinach-based sensors

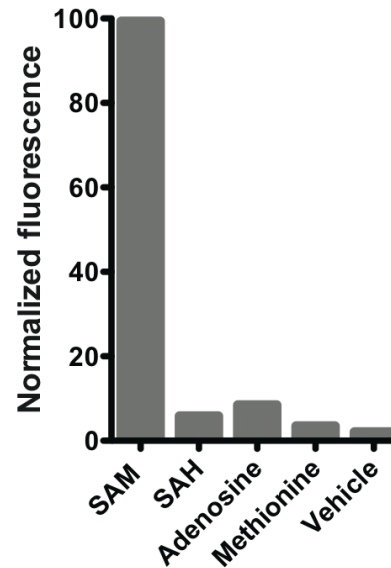# S-adenosyl methionine (SAM)

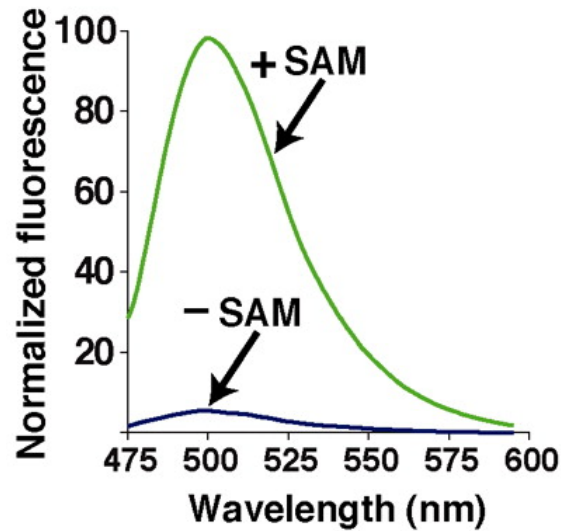S-adenosyl methionine (SAM)   co-substrate molecule involved in methyl group transfers

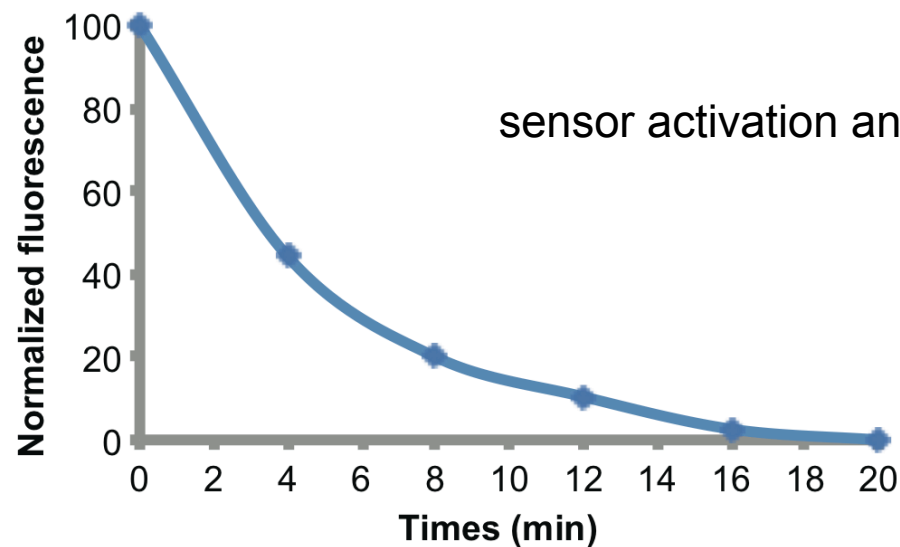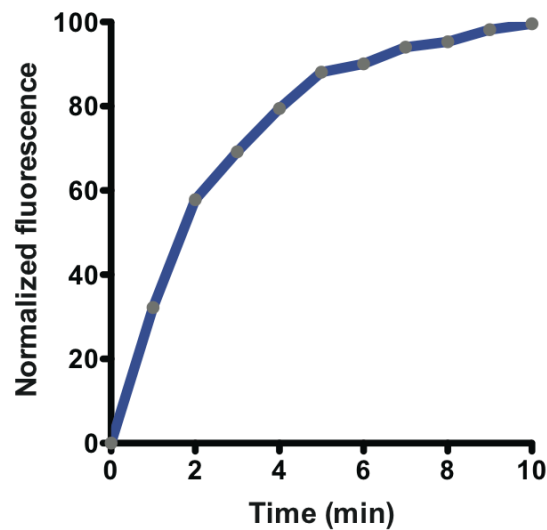

SAM levels have not been studied on single cell level

sensor activation upon SAM addition

20-fold increase in fluorescence upon SAM binding

high level of discrimination against highly related molecules

sensor activation and deactivation

Paige et al., Science, 2012

monitoring SAM metabolite dynamics

SAM sensor

| vehicle-treated | methionine-treated |

0 min — 180 min — 0 min — 180 min
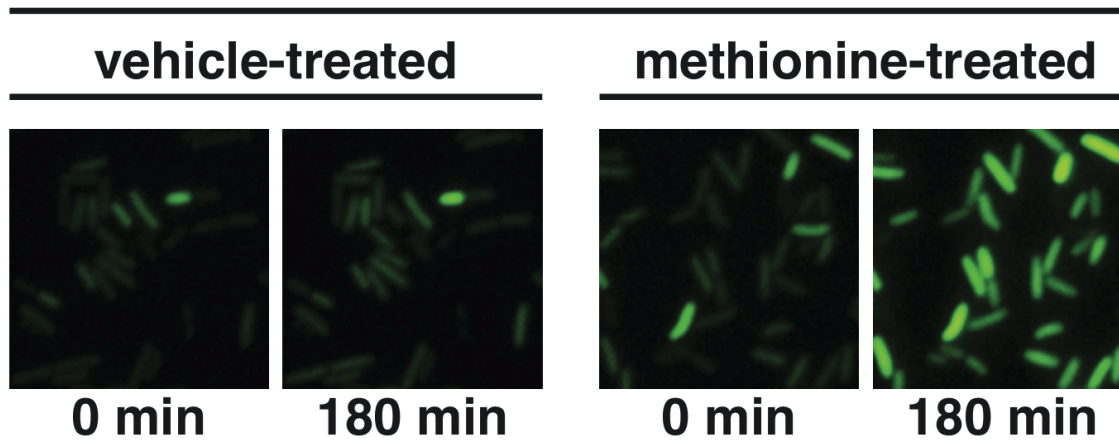
Met-treatment of Met-depleted cells

–> synthesis of SAM

0 min — 15 min — 30 min — 60 min

90 min — 120 min — 150 min — 180 min
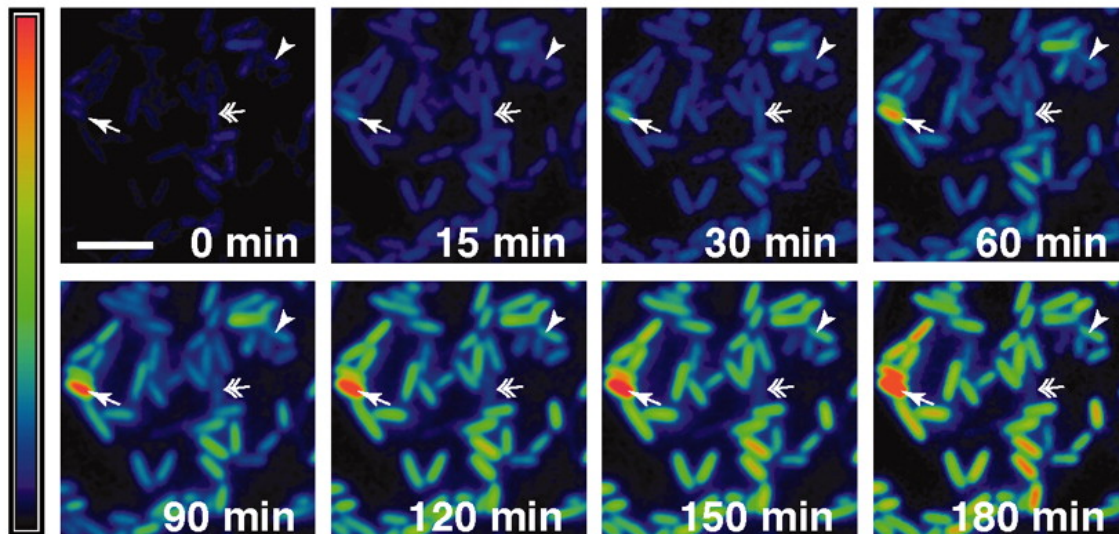
cell-to-cell variability in SAM biogenesis

Paige et al., Science, 2012

variability in SAM accumulation

Paige et al., Science, 2012
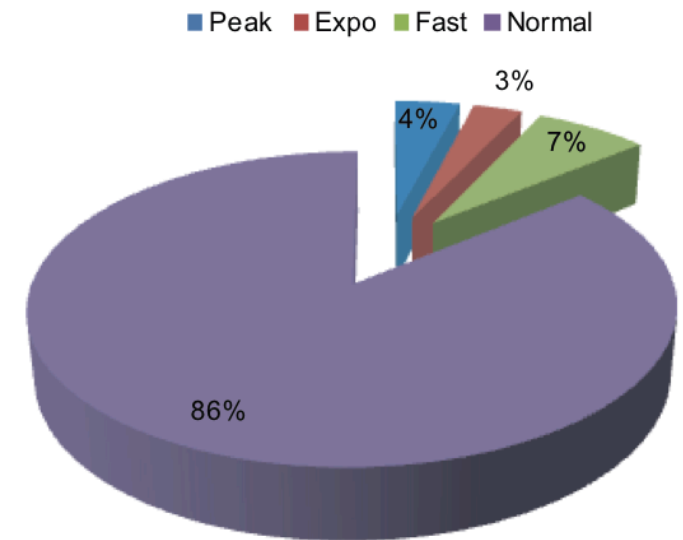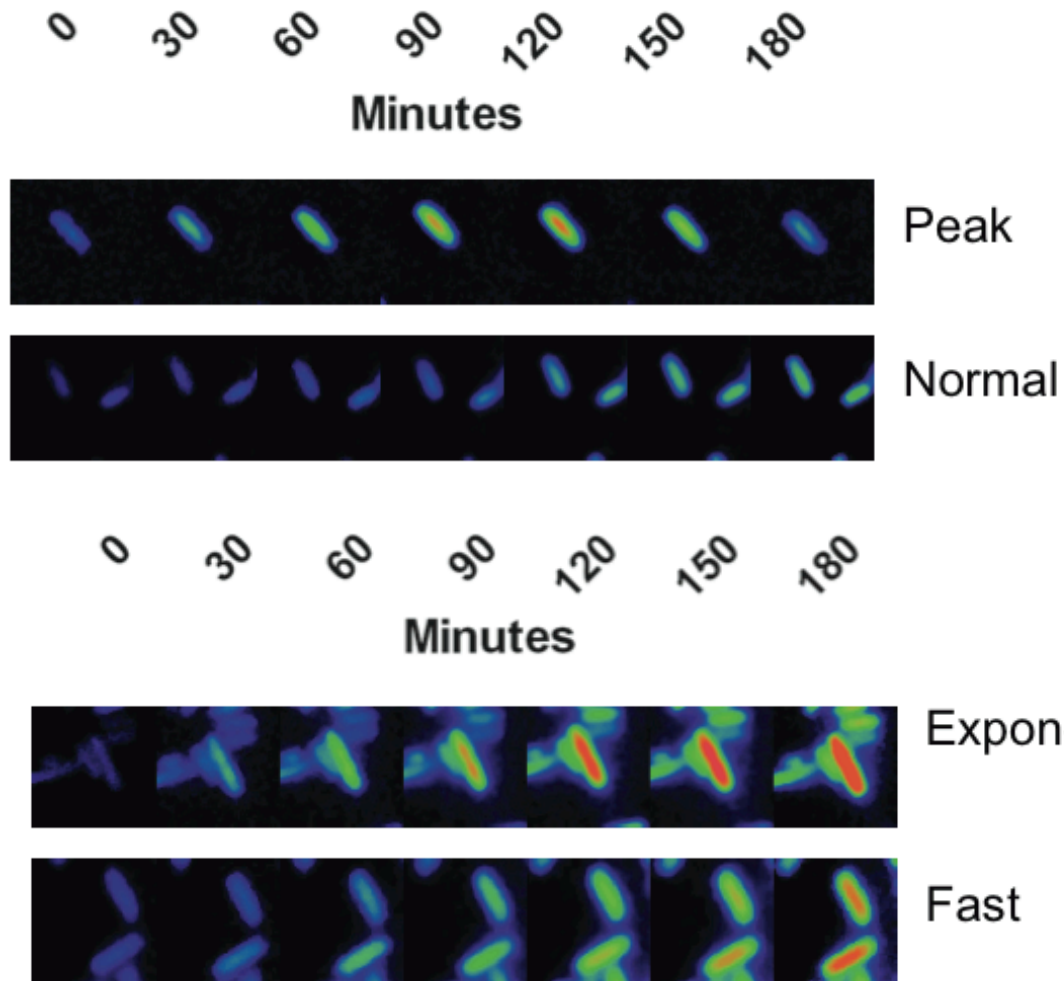
variability in SAM accumulation
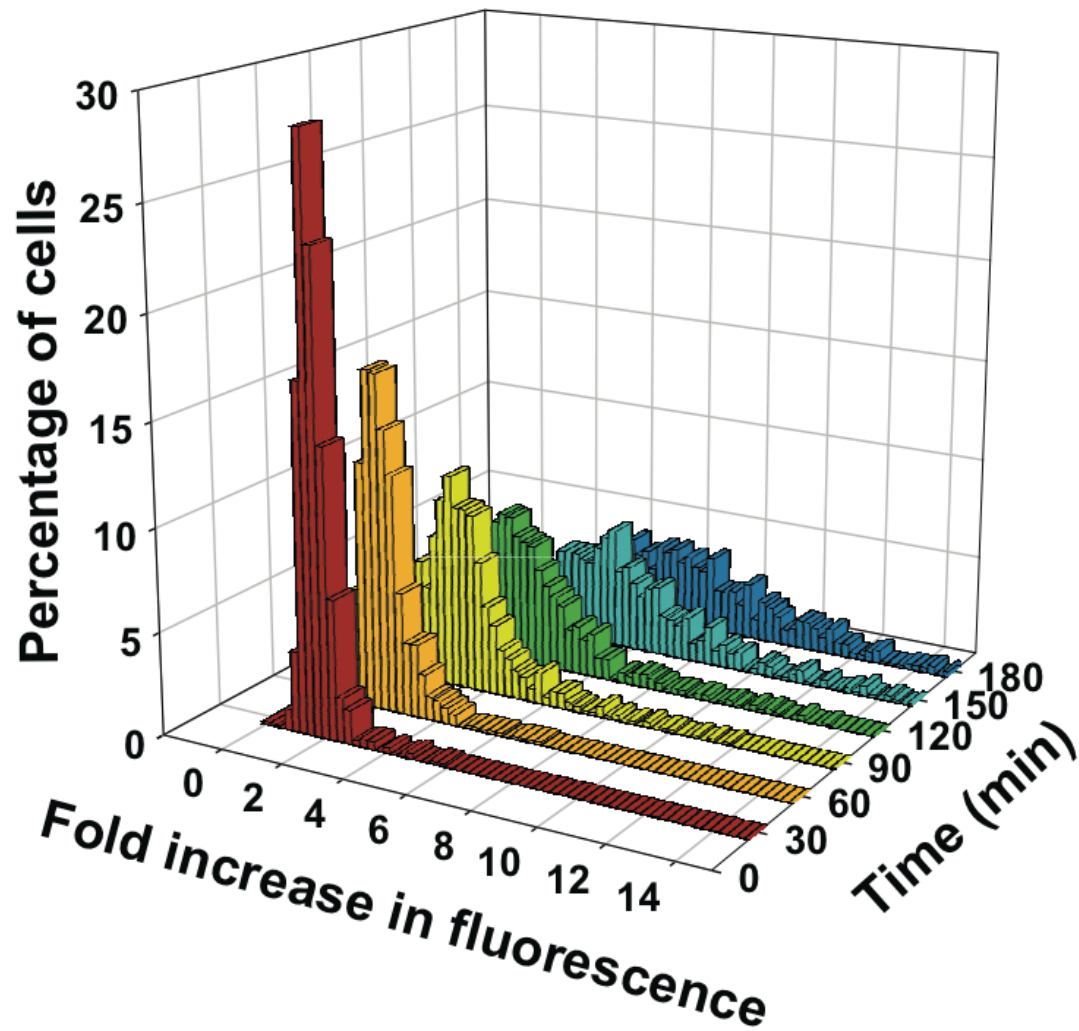
variability in SAM accumulation

–> population becomes more diverse

Paige et al., Science, 2012

development of sensors that detect a variety of small molecules

imaging of dynamic changes in metabolites

monitor cell-to-cell variation in intracellular metabolite levels

A

Minimal
fluorescence

Target binding
reinforces structure

Fluorescent complex
formation

ARTICLE
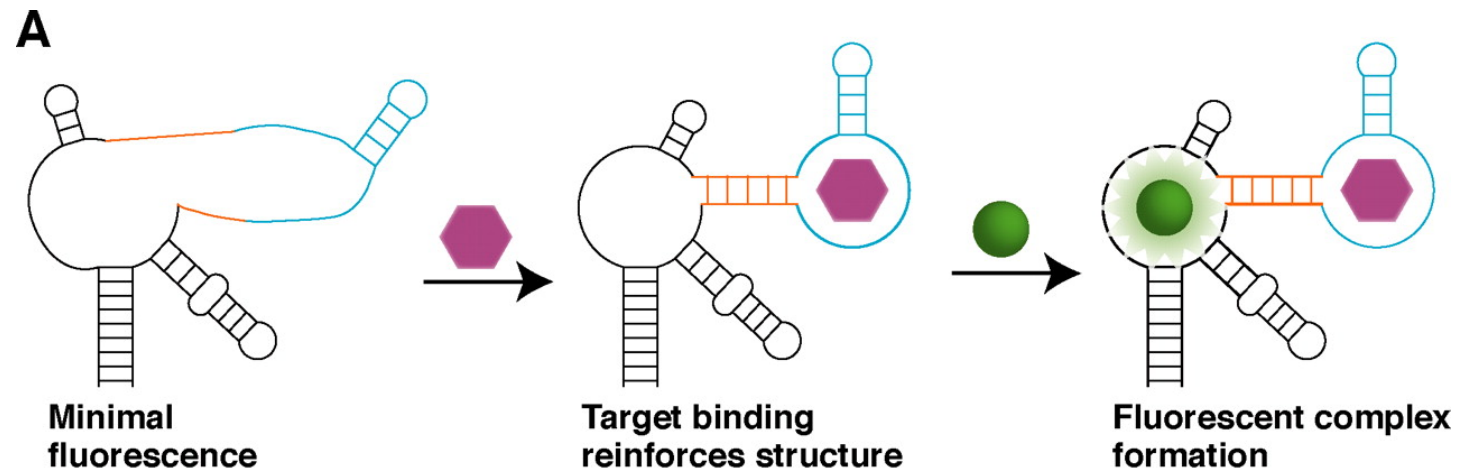
A G-quadruplex–containing RNA activates fluorescence in a GFP-like fluorophore

Hao Huang[1], Nikolai B Suslov[2,3], Nan-Sheng Li[2], Sandip A Shelke[2], Molly E Evans[2], Yelena Koldobskaya[1], Phoebe A Rice[2]* & Joseph A Piccirilli[1,2]*

[1]Department of Chemistry, University of Chicago, Chicago, Illinois, USA. [2]Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, Illinois, USA. [3]Present address: Takeda California, San Diego, California, USA.
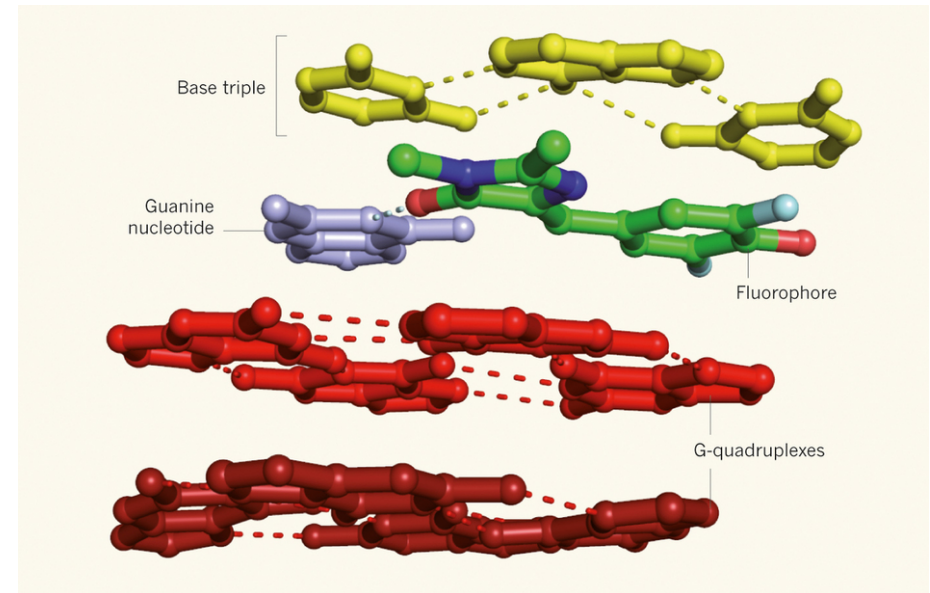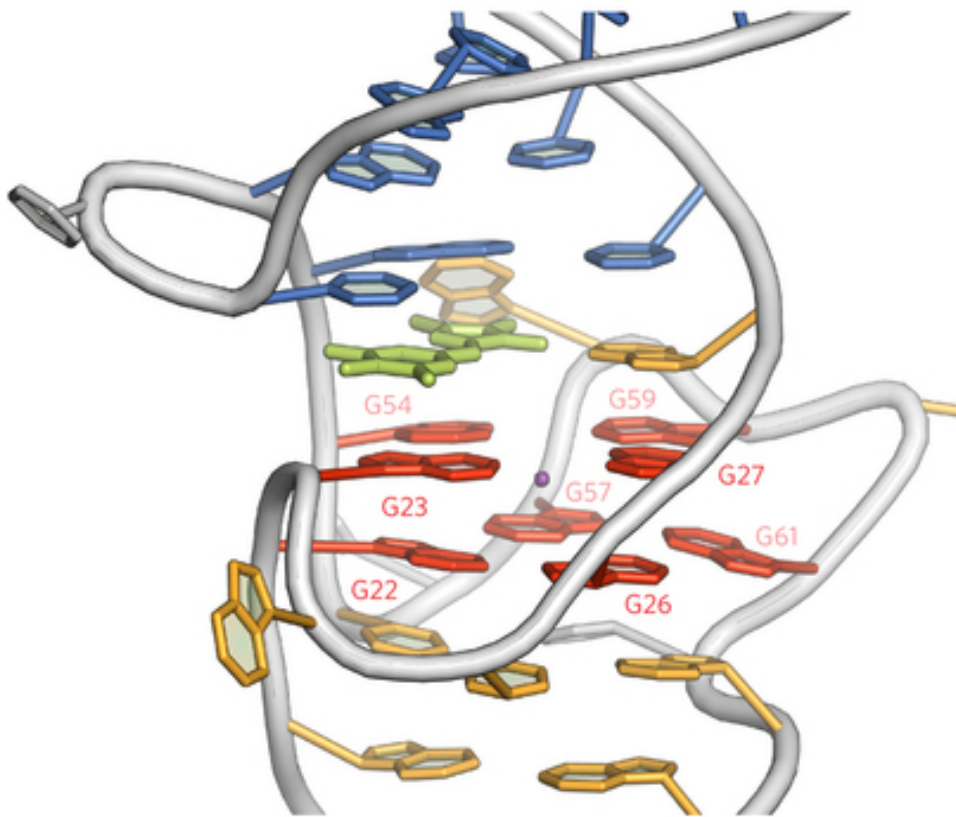
structure determination of Spinach RNA
in the presence and absence of bound DFHBI fluorophore

elongated structure containing two coaxially stacked helical stems

internal bulge = platform for DFHBI binding

Fluorophore binding site

two stacked G-quadruplexes

hydrophobic stacking platform for DFHBI binding

fluorophore in planar conformation fits into binding pocket   –> fluorescence

Huang et al., Nature Chem Biol, 2014

+   genetically encoded fluorophore with minimal background fluorescence

+   high photostability

+   small size, compactness   –>   reduced likelihood of interference with endogeneous RNA

–   reduced brightness

increase fluorophore stability and brightness

expand palette of aptamers –> variety in colour
simultaneous monitoring of several targets

visualisation of RNA dynamics and localisation   –>   elucidating RNA biology

simultaneous monitoring of the intracellular dynamics of several target metabolites

measurement of mRNA levels from engineered constructs in synthetic biology