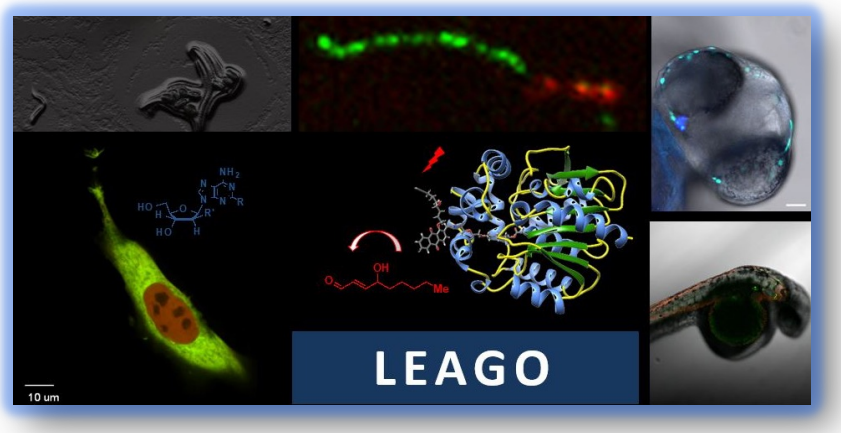
Welcome to CH-313: Chemical Biology

Prof. Yimon Aye https://leago.epfl.ch/



Laboratory of Electrophiles And Genome Operation

Lecture Week 6:

Chemical Biology tools to interrogate the transcriptome / RNAs of the living systems

2023 Oct 24th (Room: BS 270): 10:15 am – noon

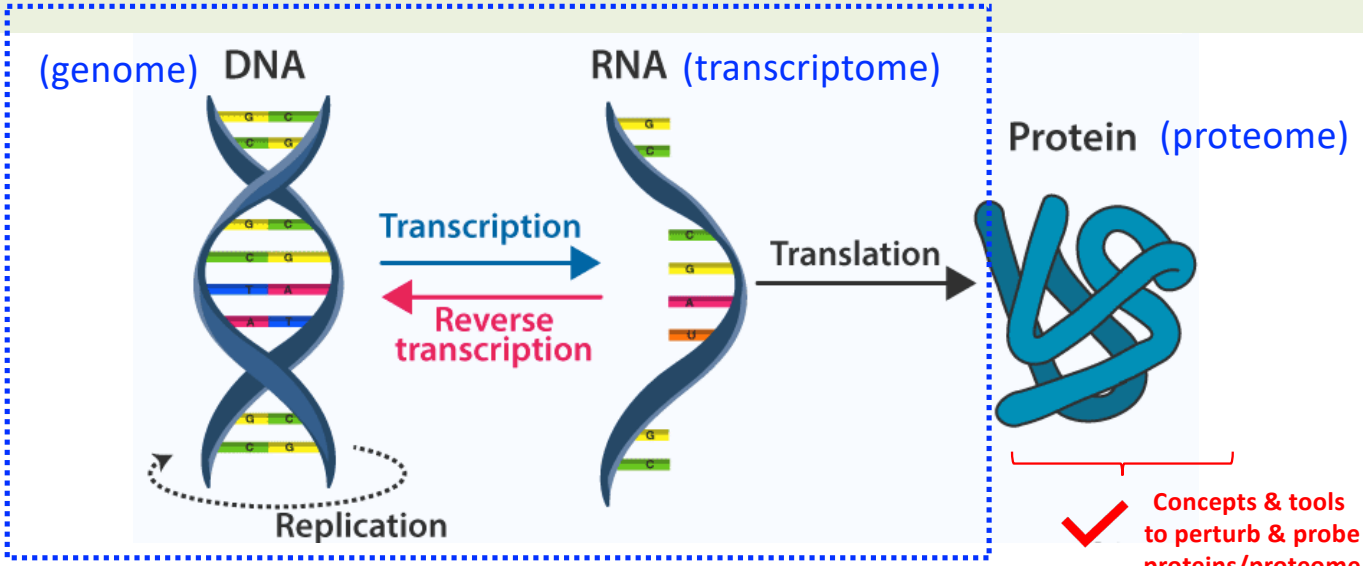
https://moodle.epfl.ch/course/view.php?id=15521

Part I: Chem Bio Toolsets

Chemical biology tools

Week	Date	Topic	Notes
1	19th Sept	Intro + Enzyme inhibitors as drugs	
2	26th Sept	Tools modulating enzyme activity/protein function	PSet 1
3	3rd Oct	Genetic vs. pharmacological perturbation methods	
4	10th Oct	Quantitative mass spectrometry: concepts & tools	PSet 2
5	17th Oct	Leveraging quant. Proteomics for target profiling	
6	24th Oct	Tools interrogating the genome & transcriptome	PSet 3
7	31st Oct	… topic above continues	
8	7th Nov	Tools interrogating proteome-level processes	PSet 4

Central Dogma: DNA to RNA to Protein



Over the next few weeks:

- ✓ DNA-based tools
- **TODAY's TOPIC** → **RNA aptamers: e.g., Spinach technology**
- **Genetic code expansion (GCE) and related technologies**

Fundamental concepts:

- – cell cycle and DNA replication, and nucleotides
- – fluorescence-based quantitative readouts (e.g., flow cytometry); photocrosslinking
- – basic principles of fluorophores
- – sensors, reversibility, and binding affinities
- – ribosomal translation
- – unnatural amino acids, including mRNA-display/target profiling concepts

Biological Questions (often unsolvable/not easily solvable using conventional tools)

Week 5 lecture

- How do we **interrogate specific cell-cycle stages** ✓
- How can we **monitor DNA replication rate in cellular context** ✓
- How do we **map changes in DNA-protein interactions in response to specific perturbation** ✓

This lecture

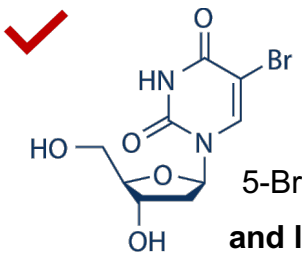
- How do we **track and report cellular mRNAs in real time** ⇦

Spinach & related technologies

- How do we **sense and report endogenous metabolites in living systems** ⇦

Representative Chemical Biology Toolsets to Perturb & Probe cellular RNA/DNA

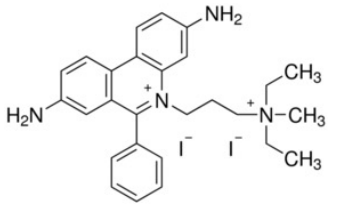
BrdU: ✓



5-Bromo-2'-deoxyuridine

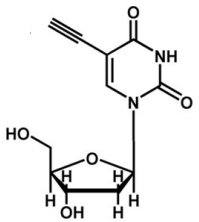
and likewise CldU and IdU

Intercalators: ✓



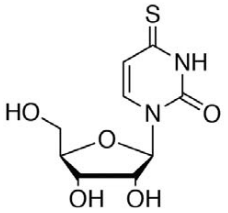
PI (propinium iodide)

EdU(alkyne): ✓



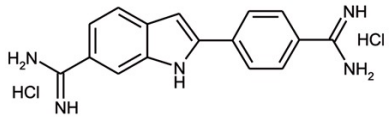
PAR-CLIP probe:

technique not covered in this class

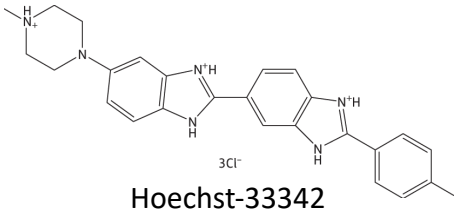


4-thiouridine (s^4U)

and similarly, 6SG and so on



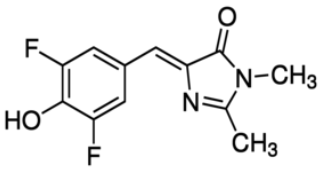
DAPI



Hoechst-33342

'Spinach' probes: ⇦

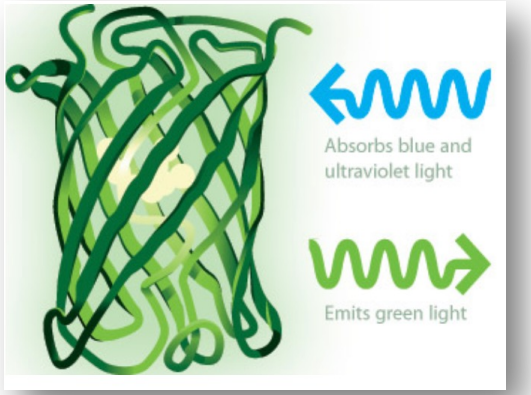
e.g., DHFBI:



The Nobel Prize in Chemistry 2008

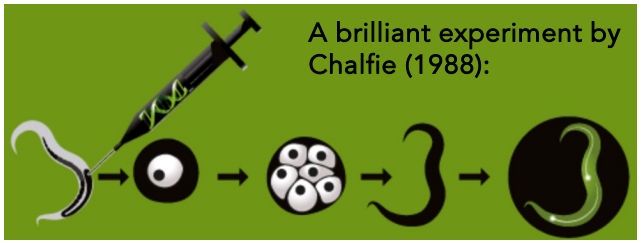
A brief intro to fluorescent proteins

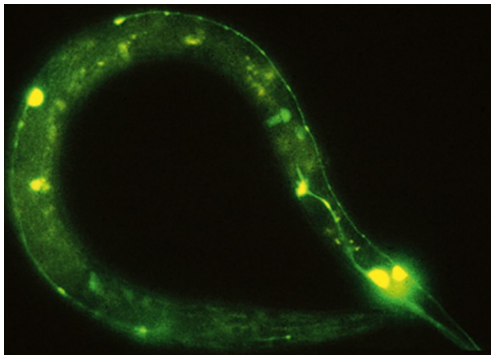
An unexpected catch for Shimomura
(1960s)



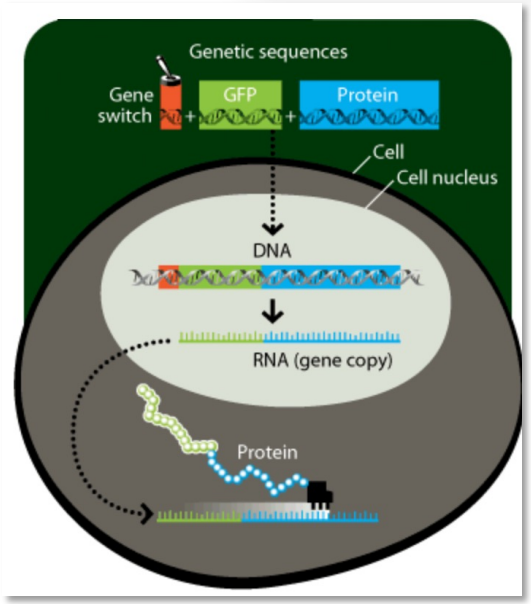
Osamu Shimomura Martin Chalfie Roger Tsien

Tsien creates a palette with all the colours of the rainbow (1990s)

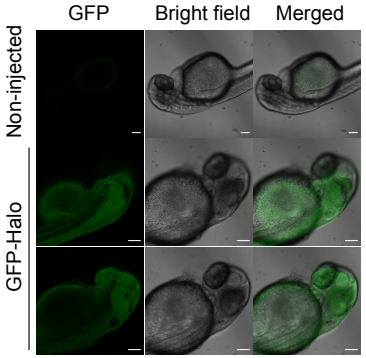




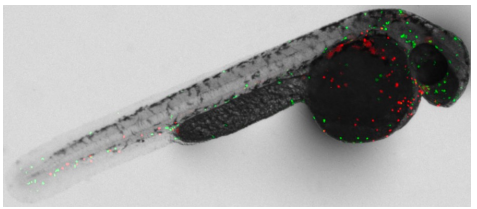
By introducing the DNA wherein a tissue-specific promoter drives the expression of GFP gene, the green fluorescence is observed selectively in certain types of neurons where the protomer is switched on



GFP: a green guiding star for biosciences



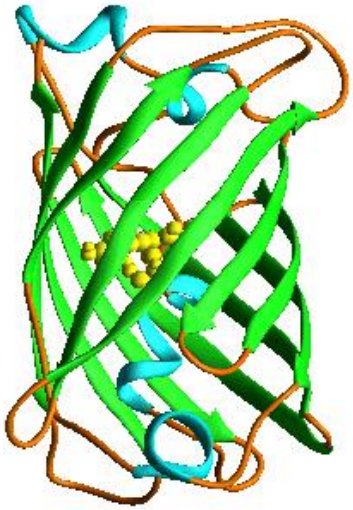
Zebrafish embryos expressing GFP-tagged Halo protein
2017 *Nature Chem Biol* 13 333-338



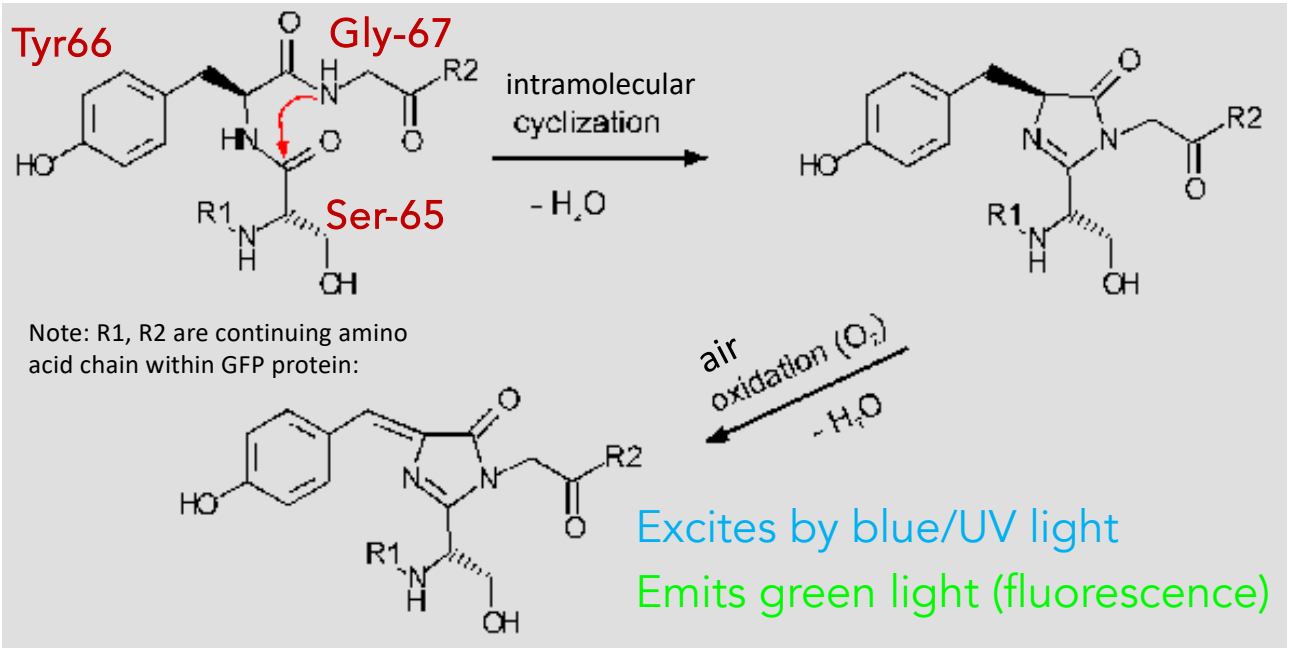
Macrophages and Neutrophils (2 types of innate Immune cells) marked by GFP and RFP
2021 *Nature Commun* 5736

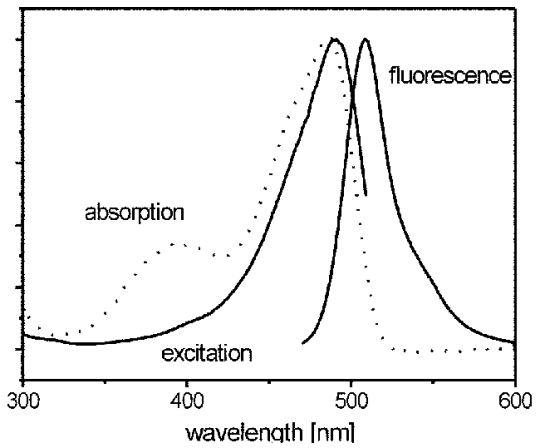
https://www.nobelprize.org/prizes/chemistry/2008/illustrated-information/

Chemistry underpinning GFP maturation

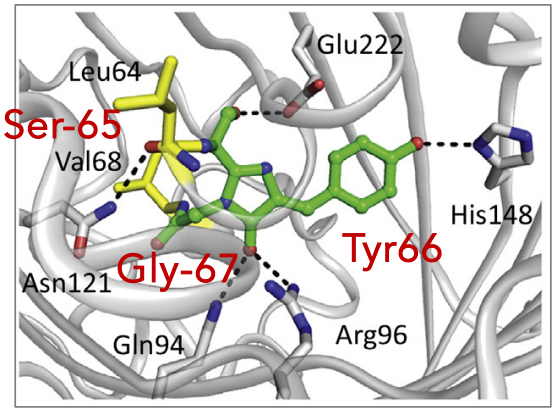


Q: what are the 3 amino acids involved in chromophore assembly?





←Q: comment on absorbance trace vs. excitation trace

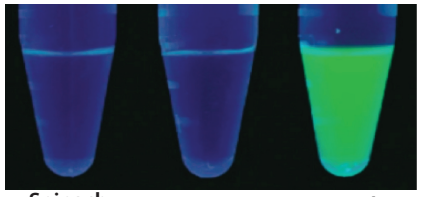


RNA aptamers

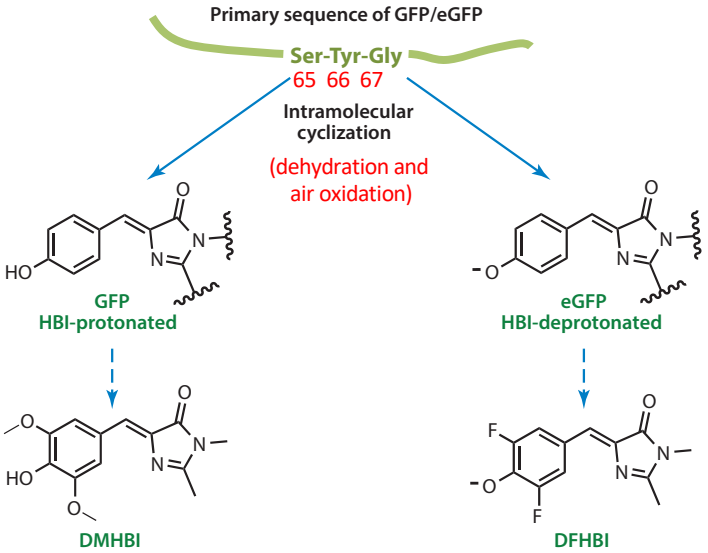
Structure and Mechanism of RNA Mimics of Green Fluorescent Protein

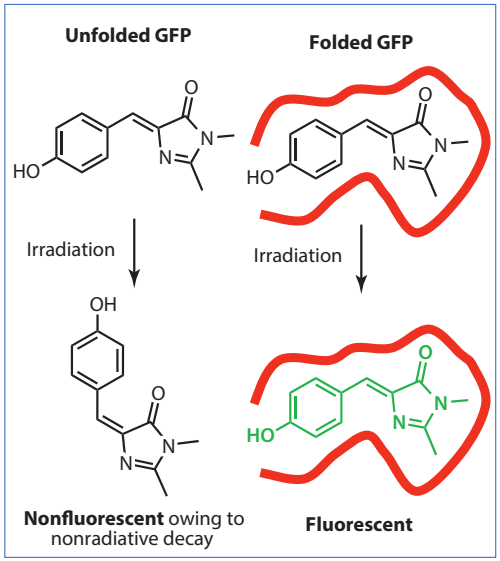
Mingxu You and Samie R. Jaffrey

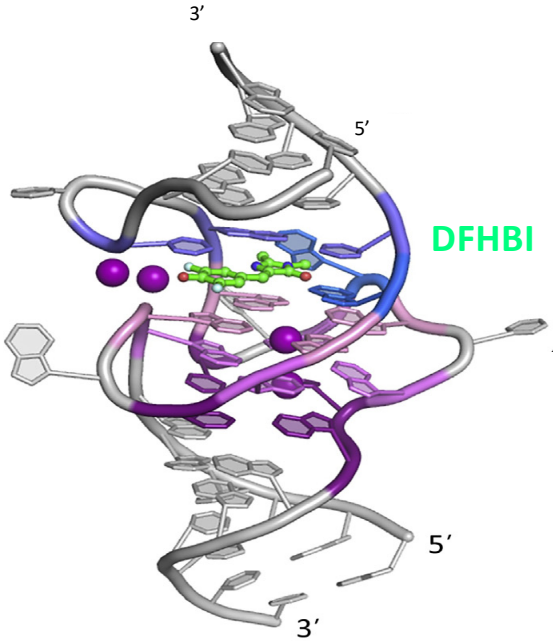
Annu. Rev. Biophys. 2015. 44:187–206



Biological aim: fluorescence proteins have revolutionize the way we can interrogate protein trafficking, protein associations, etc. How do we track cellular RNAs? Can we develop RNA mimics of GFP?





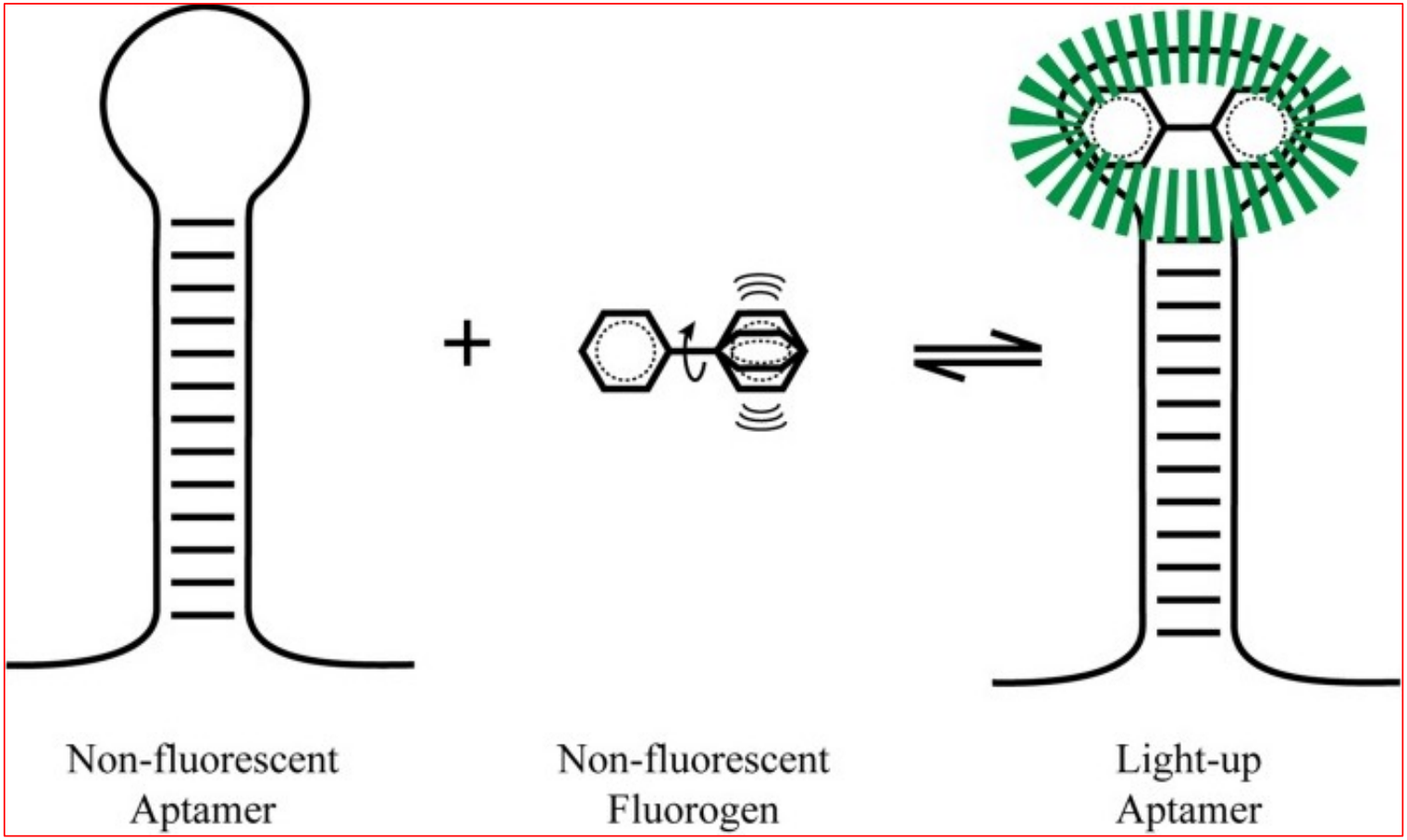


Model of Spinach—DFHBI complex:

Aptamers: short ssDNA/ssRNA engineered to 'selectively' bind target small- and biomolecules

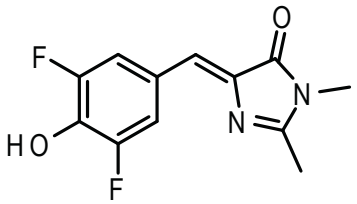
A critical requirement

Fluorophore must be in the dark state if unbound: How do we engineer to achieve this aspect?



First key application of fluorescent RNA aptamers: to visualize/track RNA in live cells

Spinach aptamer: RNA-mimic of GFP



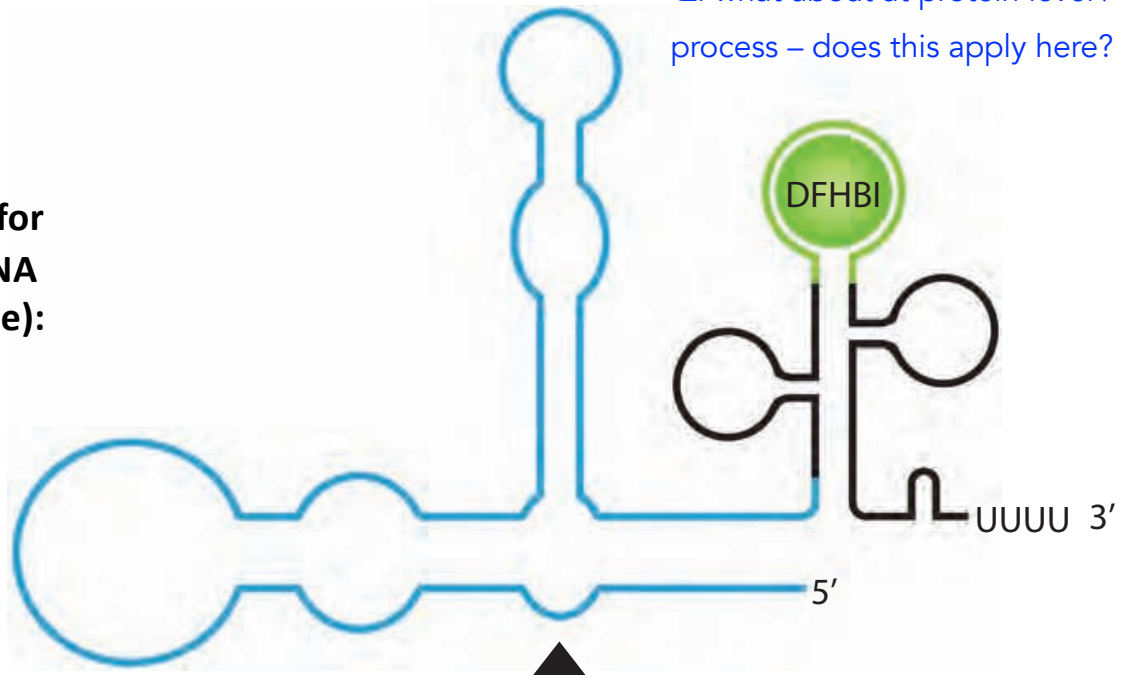
DFHBI

Example shown here is for tracking ribosomal (r)RNA (labeled as 5S rRNA gene):

Need to engineer/modify the RNA of interest such that we can introduce fluorophore-binding aptamer, here, DFHBI-binding aptamer called 'Spinach'

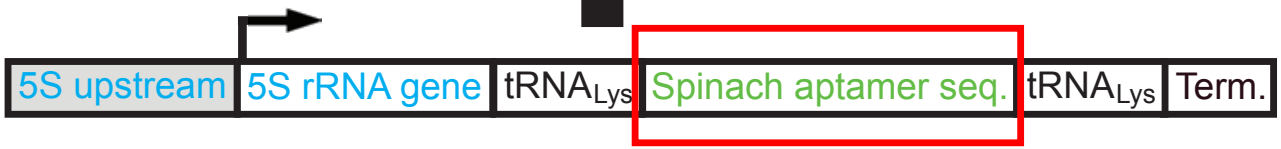
Q: what about at protein level / translation process – does this apply here?

At the RNA level:



← Q: what is this process?

At the DNA level:

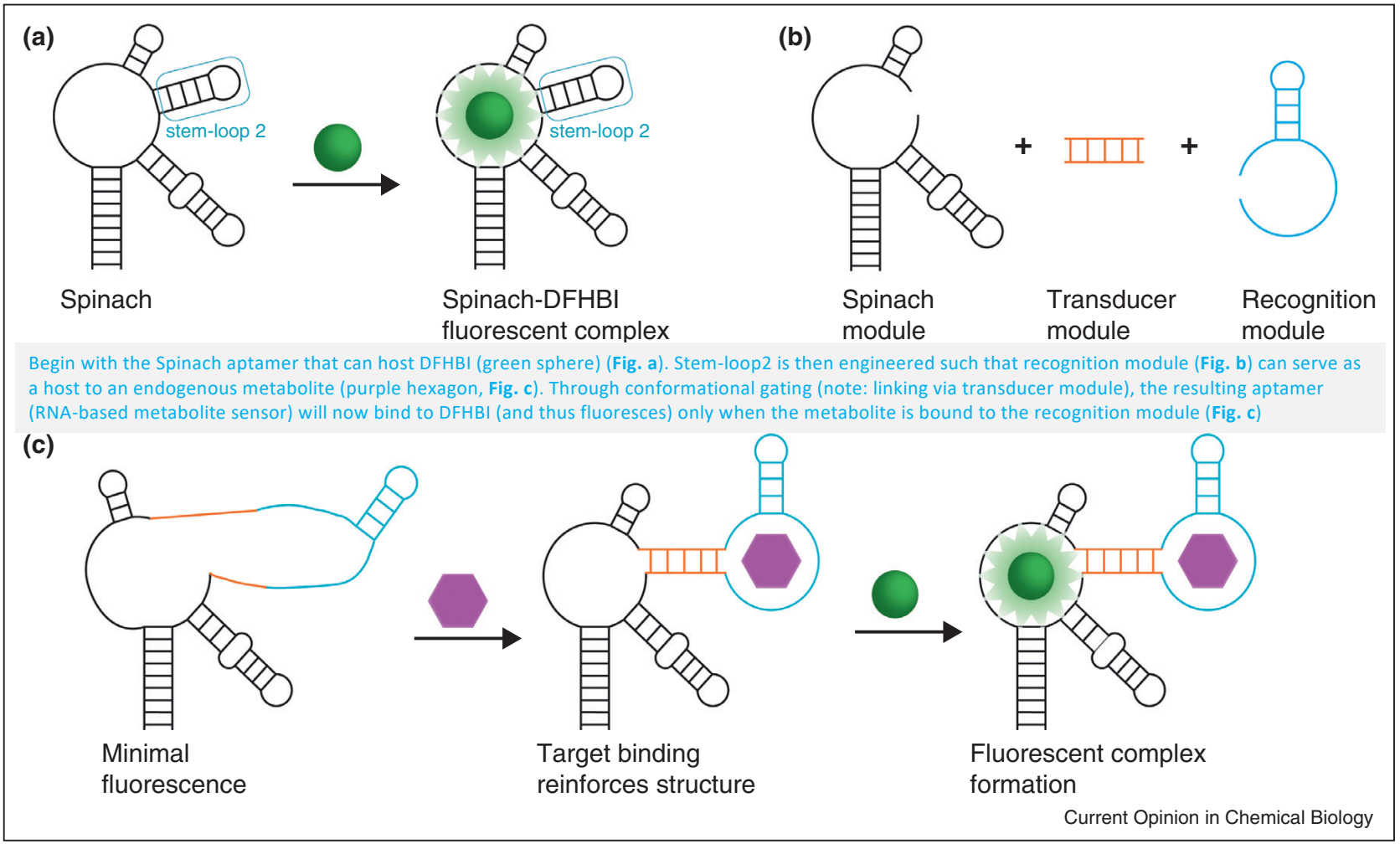


The other components integrated: namely, upstream, 'term' (termination), $tRNA_{Lys}$ sequences are, either the usual upstream / downstream components of an RNA encoding a gene, or stabilizing sequences (in the case of $tRNA_{Lys}$) that extends the cellular lifetime of engineered RNA construct..

Second key application of fluorescent RNA aptamers: to sense *endogenous* small-molecule metabolites

New approaches for sensing metabolites and proteins in live cells using RNA

Current Opinion in Chemical Biology 2013, **17**:651–655



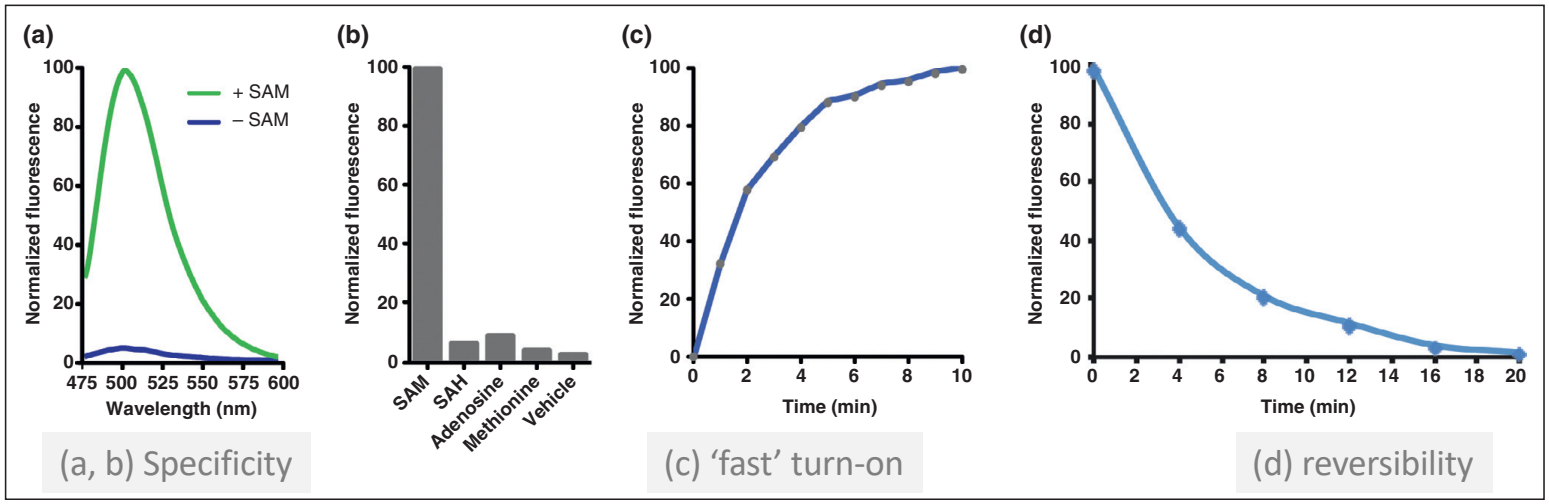
Begin with the Spinach aptamer that can host DFHBI (green sphere) (**Fig. a**). Stem-loop2 is then engineered such that recognition module (**Fig. b**) can serve as a host to an endogenous metabolite (purple hexagon, **Fig. c**). Through conformational gating (note: linking via transducer module), the resulting aptamer (RNA-based metabolite sensor) will now bind to DFHBI (and thus fluoresces) only when the metabolite is bound to the recognition module (**Fig. c**)

RNA-based sensor for the metabolite SAM – a case study

Question: Please help interpret the data in (**c**) and (**d**), given the following experiment set-up:
(**c**) Fluorescence was measured following addition of SAM to a solution containing the Spinach-based sensor and DFHBI;
(**d**) Fluorescence was measured after removal of SAM from the solution containing Spinach-based sensor, DFHBI, and SAM

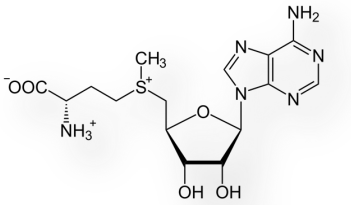
(c) Saturation is observed as binding equilibrium is reached as expected

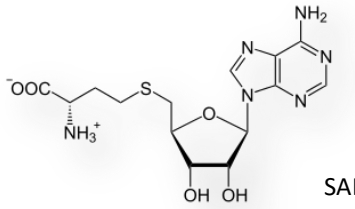
(d) Equilibrium is driven backwards to dissociated state due to dilution (likely "removal" of SAM (metabolite) is in practice triggered by rapid dilution, to perturb the ligand-binding equilibrium, Just like what we learned in class during Inhibitor topic lectures earlier on. Also it's worthwhile thinking about this data to Figure C in the slide above. Effectively you're going "backwards" so you get to the unstructured state of the aptamer once metabolite (purple hexagon) has dissociated and in that state the DFHBI (green circle) ligand can no longer bind to the aptamer since fluorescence signal decreases over time

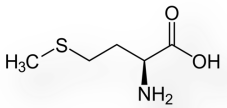


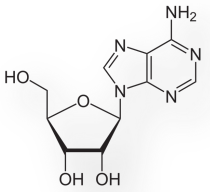
A Spinach-based sensor of SAM. **(a)** A SAM sensor shows minimal fluorescence in the absence of SAM, even in the presence of DFHBI. However, upon SAM addition, fluorescence is activated over 20-fold. **(b)** The SAM sensor is specifically activated by SAM, and not the closely related molecules S-adenyl histidine (SAH), methionine, or vehicle. **(c)**

These data are representative of the metabolite and protein sensors generated on the basis of Spinach, and are reproduced with permission from Ref. [5].

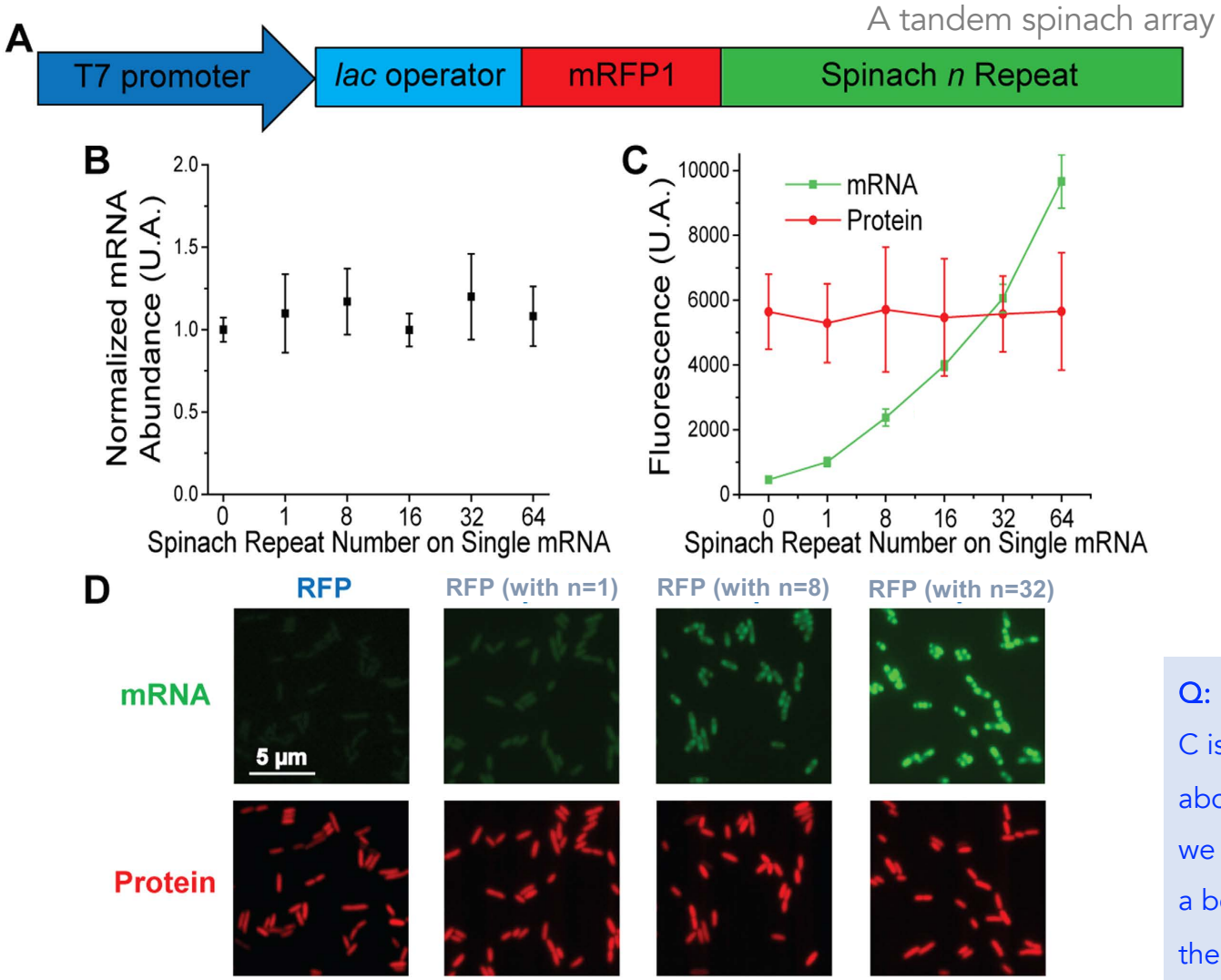

SAM (S-adenosyl methionine)


SAH (S-adenosyl homocysteine)


M (methionine)


A (adenosine)

A problem to think about in your own time: Do the data attest to the fact that more Spinach repeats give a higher signal to noise in detecting RNA?



E. coli cells were engineered to express the construct (shown in Fig. A) Note the presence of Spinach aptamer sequence; here they use a tandem repeat of Spinach aptamer sequence (where n is the number of monomeric unit).

[You may ignore T7 promoter and lac operon in this question, which are simply components that render gene transcription to be under temporal control (i.e., inducible system) in the presence of a small-molecule inducer known as IPTG].

Q: First, interpret these data. (Note: the data in Fig. C is the quantitation of images in Fig. D). Then, think about why the data set in Fig. B is necessary before we can conclude that the tandem spinach array offers a better signal to noise in tracking mRNA, which is the authors' hypothesis.

From Taekjip Ha's lab SCIENTIFIC REPORTS | 5:17295 | DOI: 10.1038/srep17295

A problem to think about in your own time: Do the data attest to the fact that more Spinach repeats give a higher signal to noise in detecting RNA?

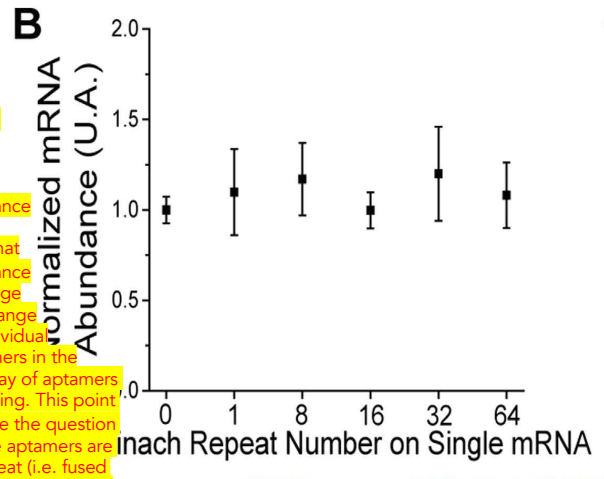
ANSWER KEY:
First it is important to appreciate that mRFP1 is protein (just like GFP) and spinach is RNA aptamer, so one is looking at protein level and mRNA levels, respectively, here…

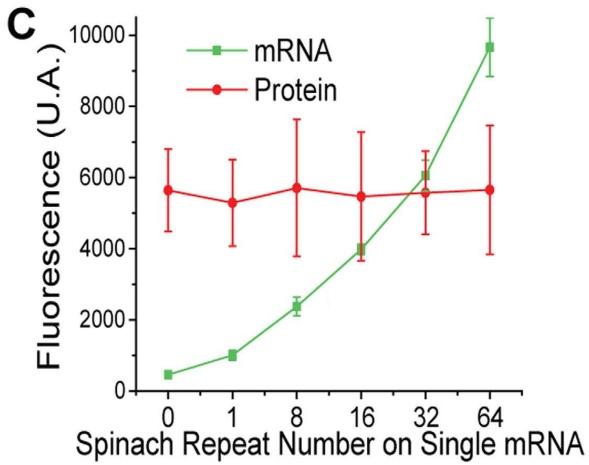
A tandem spinach array

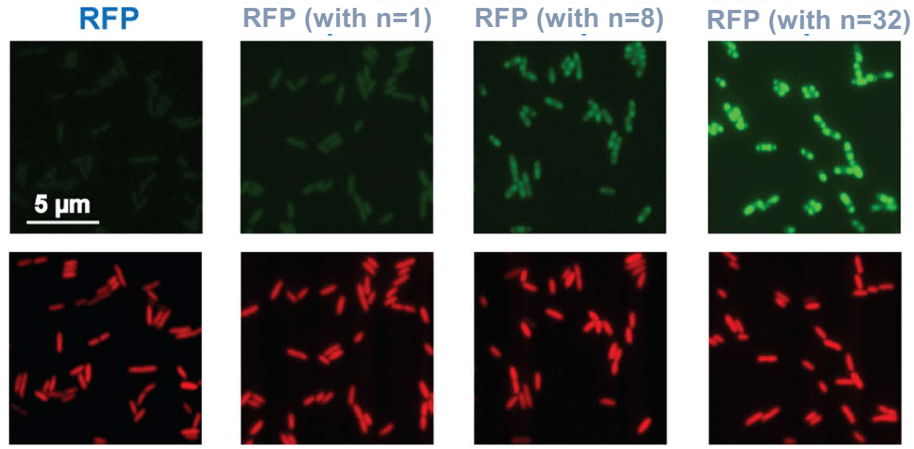
E. coli cells were engineered to express the construct (shown in Fig. A) Note the presence of Spinach aptamer sequence; here they use a tandem repeat of Spinach aptamer sequence (where n is the number of monomeric unit).

[You may ignore T7 promoter and lac operon in this question, which are simply components that render gene transcription to be under temporal control (i.e., inducible system) in the presence of a small-molecule inducer known as IPTG].

Validation
Shown in
B is critical
Because
If you read the
Plot axes in
B, we are
Measuring
mRNA abundance
And the data
Are showing that
mRNA abundance
Does not change
Even if you change
The no. of individual
Spinach aptamers in the
'polymeric' array of aptamers
you're expressing. This point
is obvious since the question
says that these aptamers are
in tandem repeat (i.e. fused
at the DNA level so remains
fused at the RNA level).
However it's still crucial to be
tested because if the mRNA
levels are to be increasing as
you increase the repeat
units, then one will end up
artificially reporting increase
in signal of DFHBI-Spinach
Complexes due to many
mRNA molecules…(as
opposed to increase in
signal that comes per a
single mRNA molecule)







Tandem array offers a better signal-to-noise
As you can see in Data C. RFP protein signal levels do not change (this also serves as an internal control since Both RFP and Spinach are driven by the same promoter). However, fluorescence signal (green) that comes from SPINACH-DHFBI complexes is rising as a function of repeat unit in this polymeric aptamer. And this Increase comes
As a direct
Result of
More
Spinach-DFHPI complexes per mRNA molecule
And not due
To having
Increased no. of
mRNA molecules
(this is shown
By validation experiment in B)

Q: First, interpret these data. (Note: the data in Fig. C is the quantitation of images in Fig. D). Then, think about why the data set in Fig. B is necessary before we can conclude that the tandem spinach array offers a better signal to noise in tracking mRNA, which is the authors' hypothesis.

From Taekjip Ha's lab SCIENTIFIC REPORTS | 5:17295 | DOI: 10.1038/srep17295

Learning outcomes (Week 6: CH-313 Chemical Biology - Synopsis)

- Fundamentals of fluorescent proteins vs. fluorescent RNAs
- Concepts underlying the design of fluorescent RNAs
- 2 major applications of fluorescent RNA aptamers: strengths & limitations and data interpretations