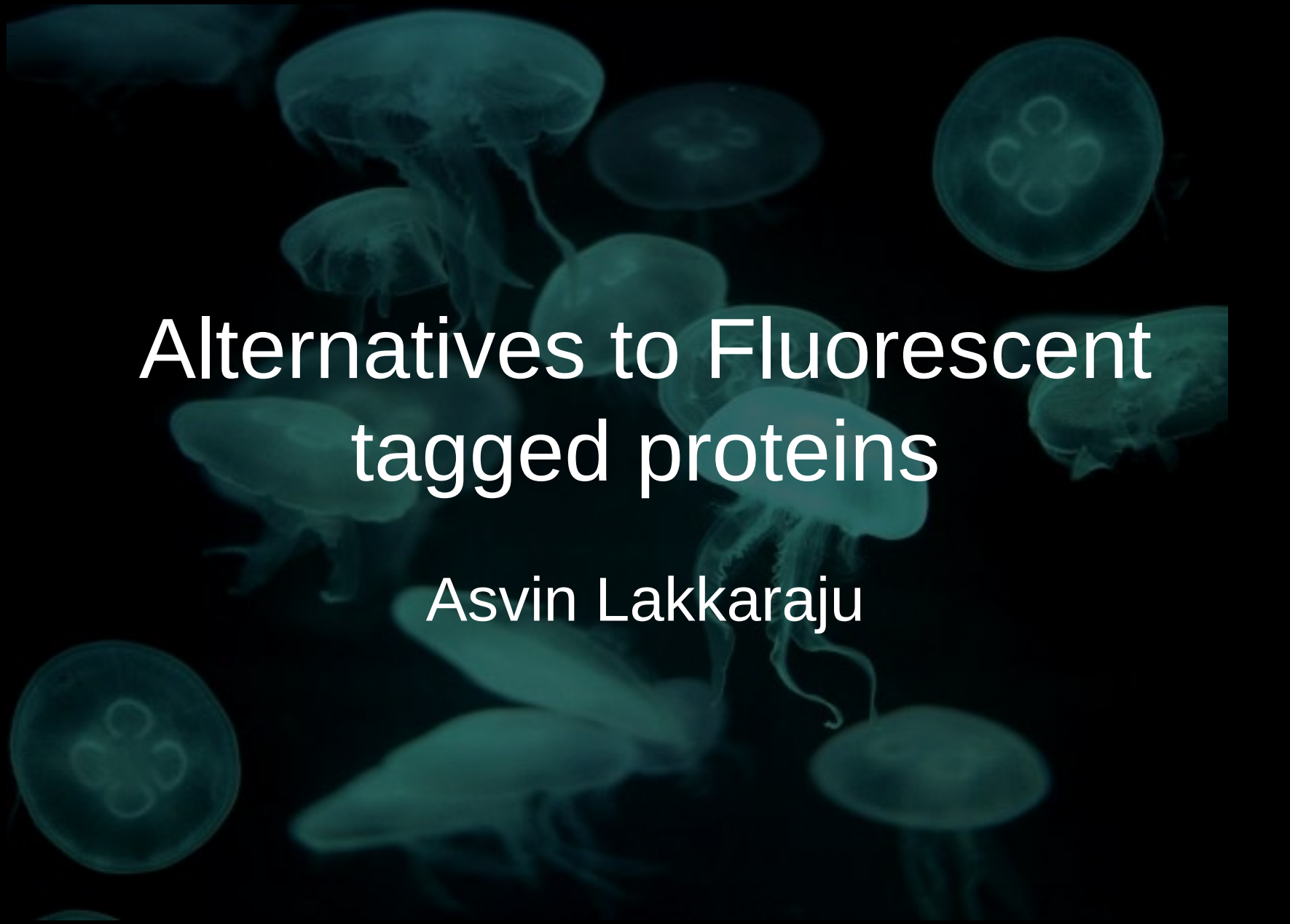


Alternatives to Fluorescent tagged proteins

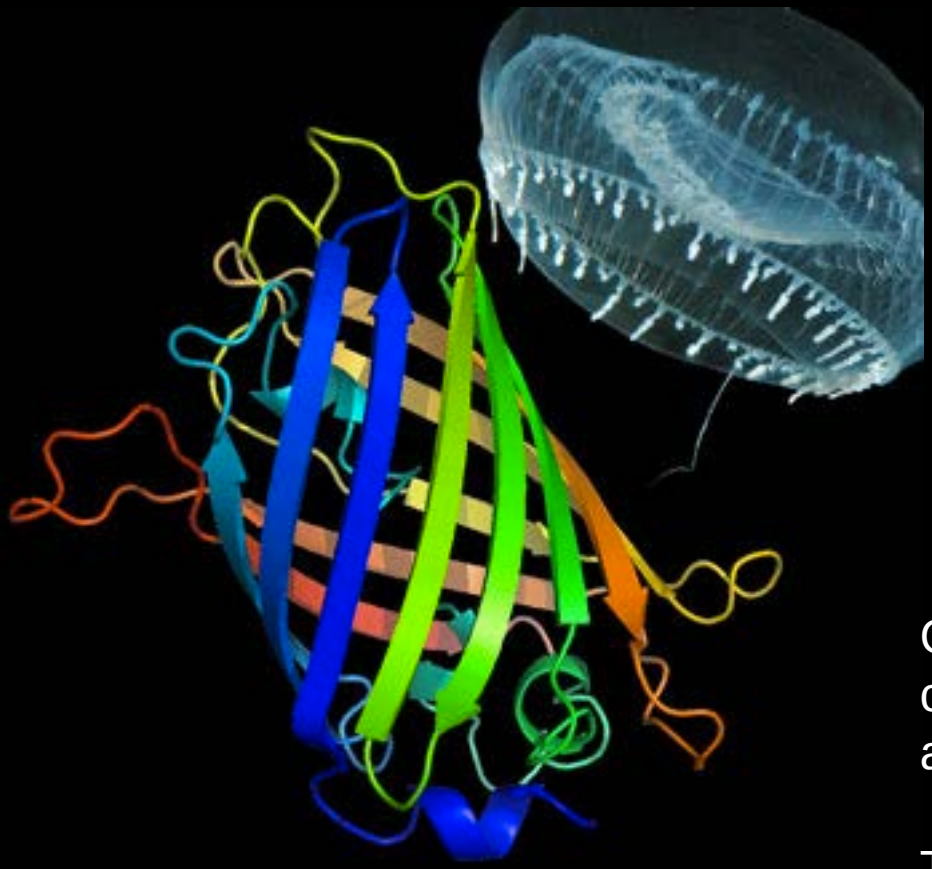
Asvin Lakkaraju

3 Fundamental Questions

What do biological molecules do?

Where are they located?

How do they behave and how are they regulated?



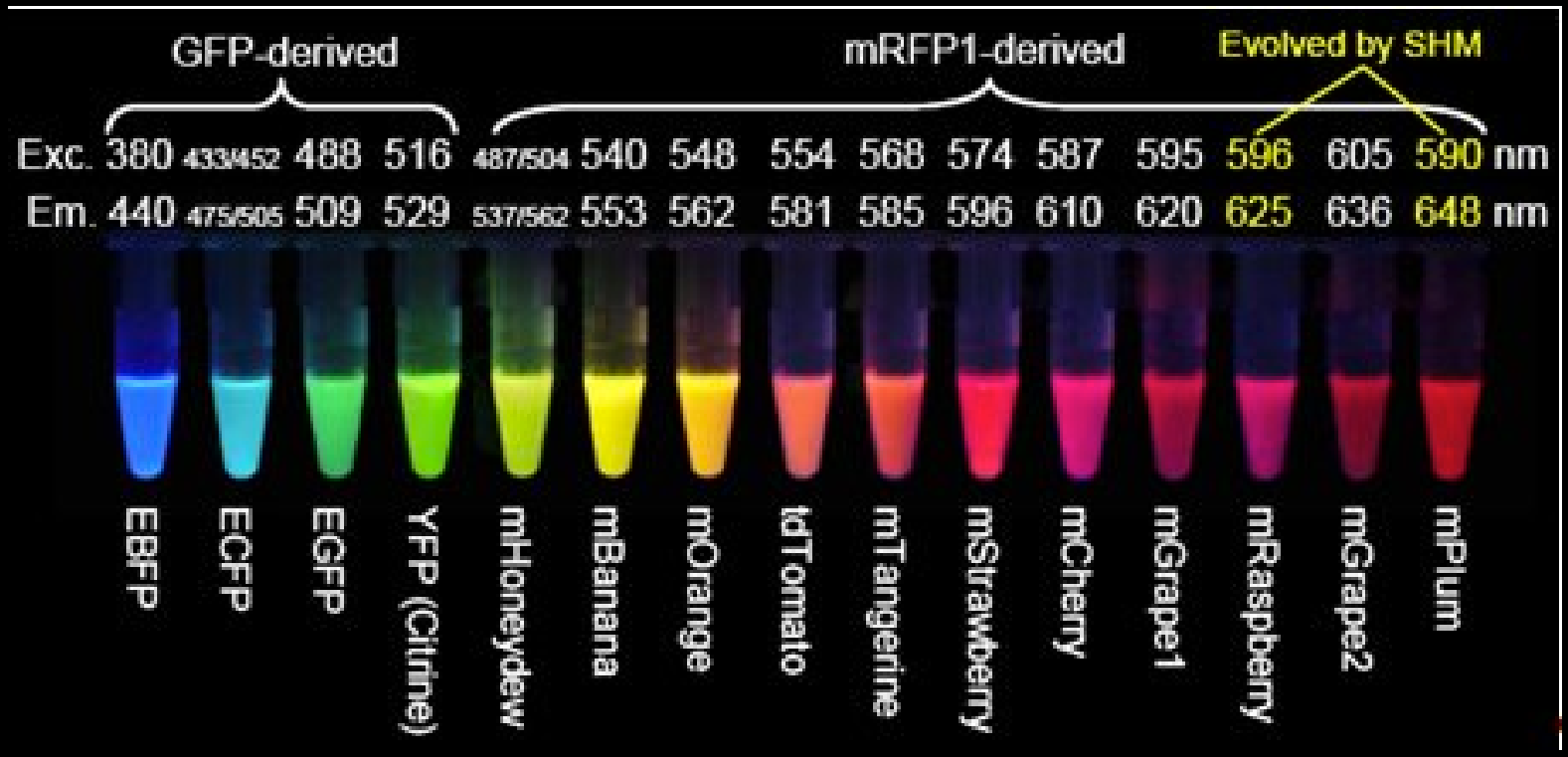
GFP

O.Shimomura

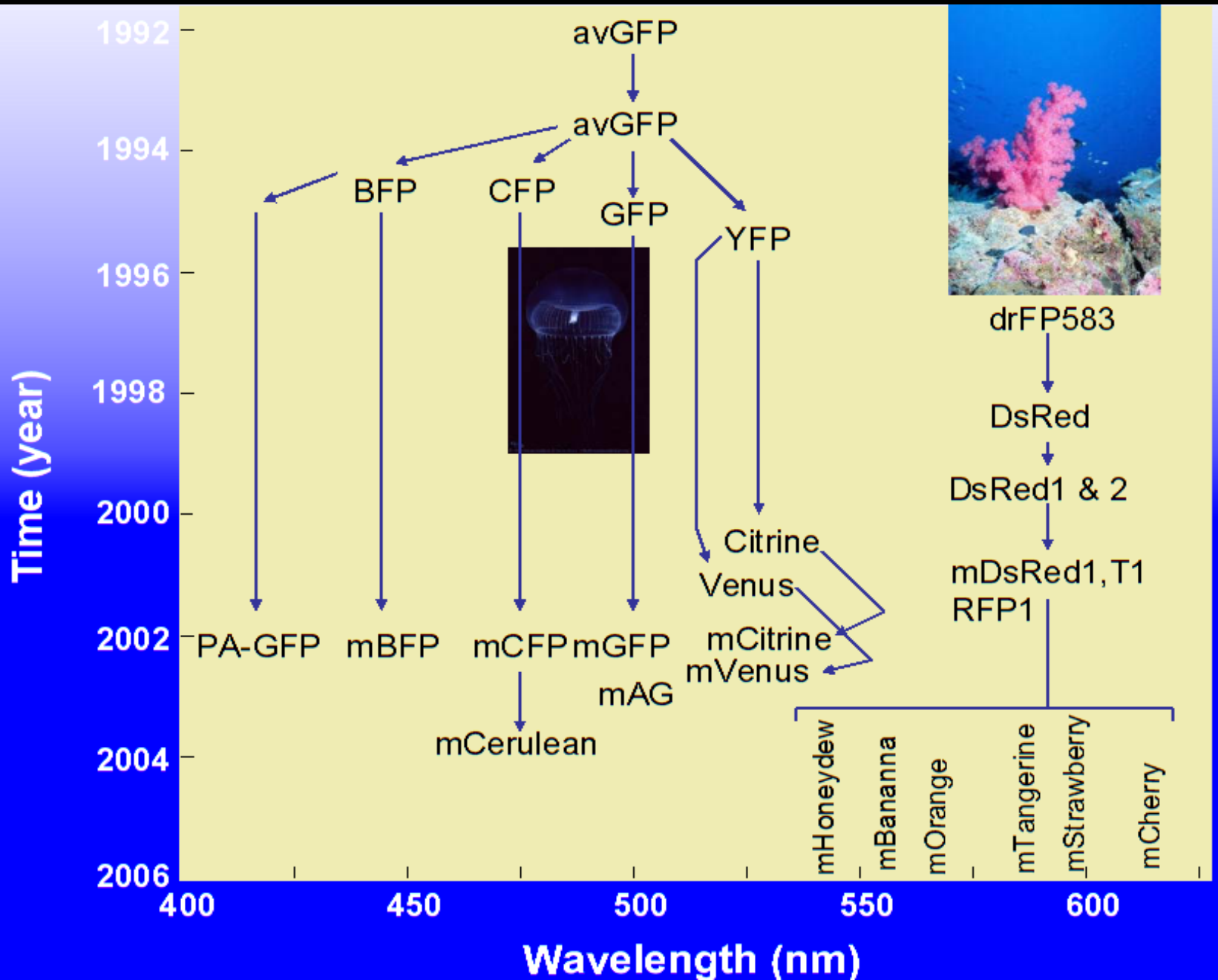
GFP was first discovered in jellyfish and can now be found in cell biology labs across the world.

This glowing protein has given us a whole new way to examine the inner workings of cells, providing insights into a range of diseases

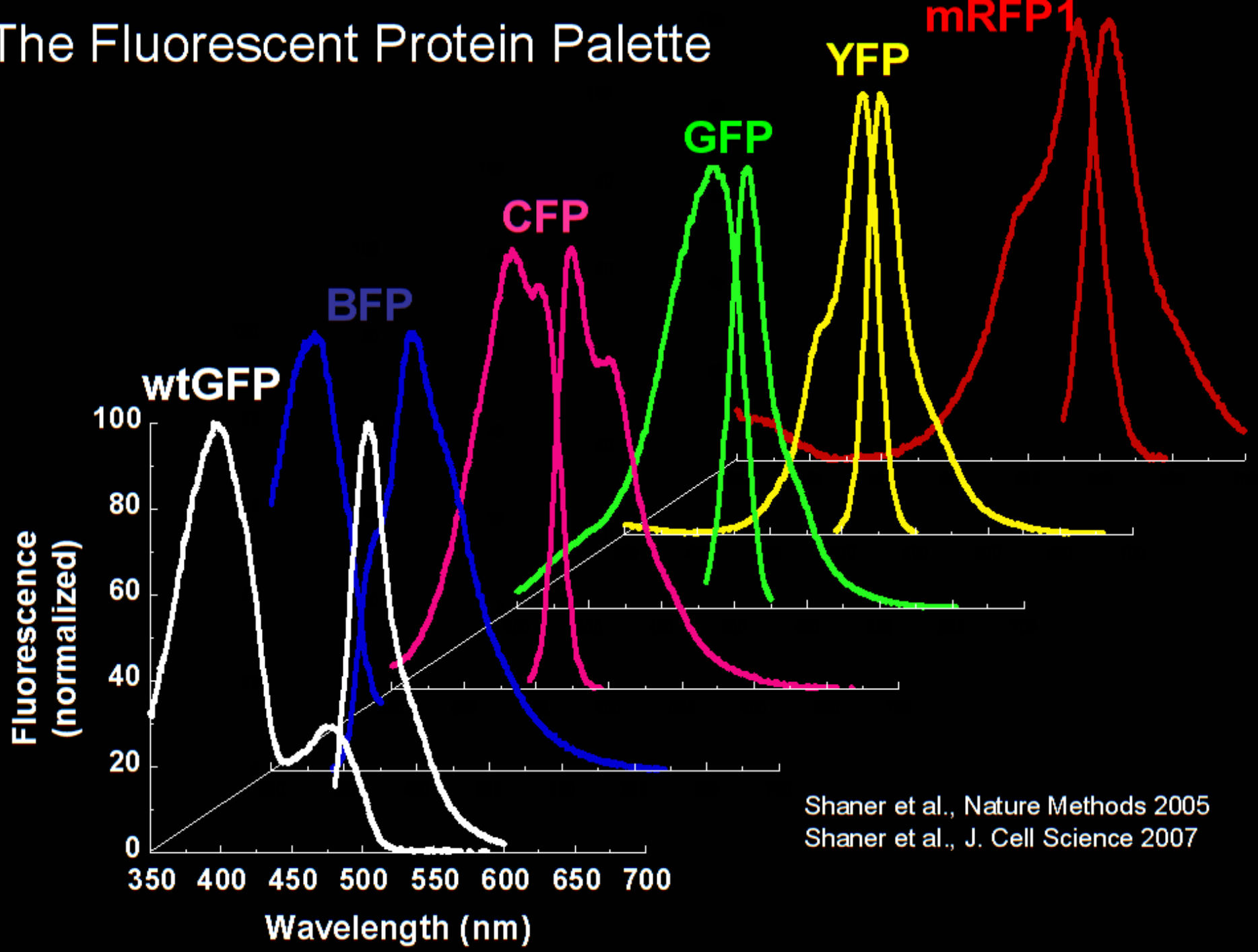
Flavors of GFP



Overview of Fluorescent proteins

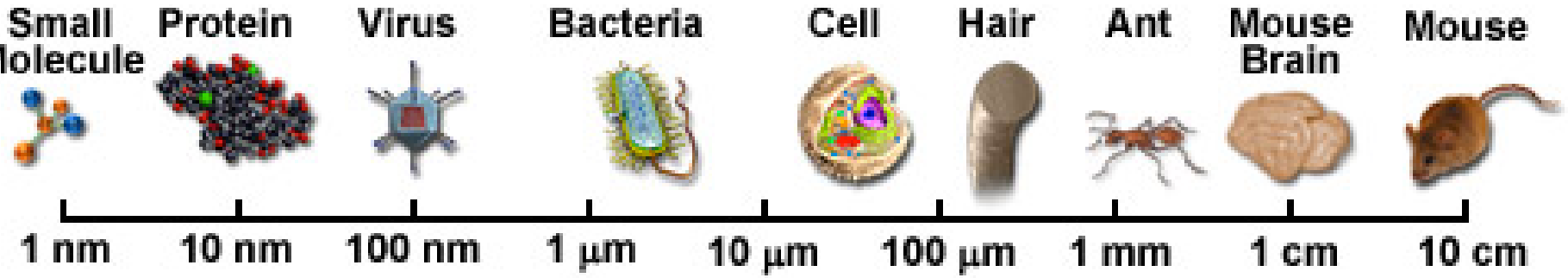


The Fluorescent Protein Palette



Shaner et al., Nature Methods 2005
Shaner et al., J. Cell Science 2007

Spatial Resolution of Biological Imaging Techniques



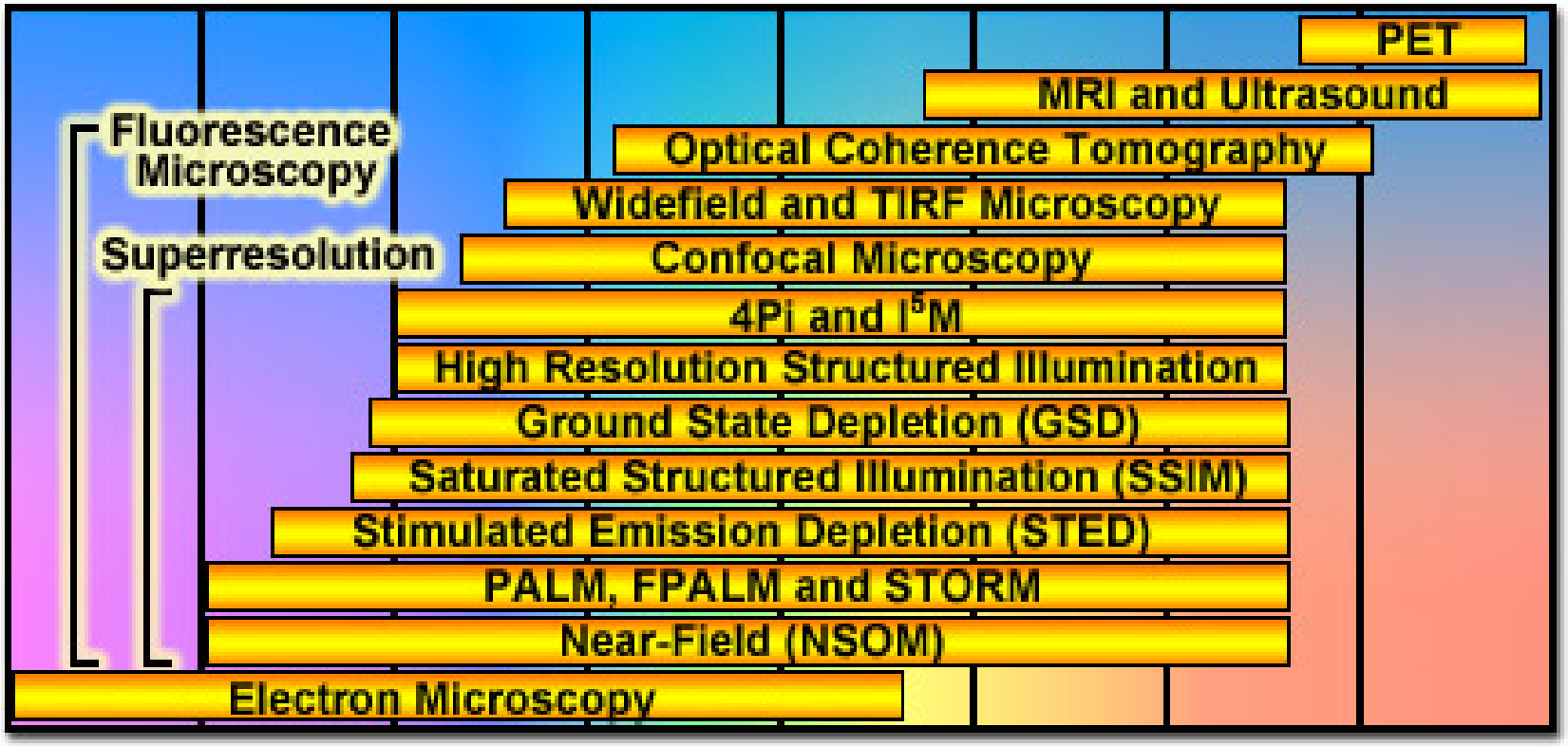


Figure 1

Goals of Microscopy

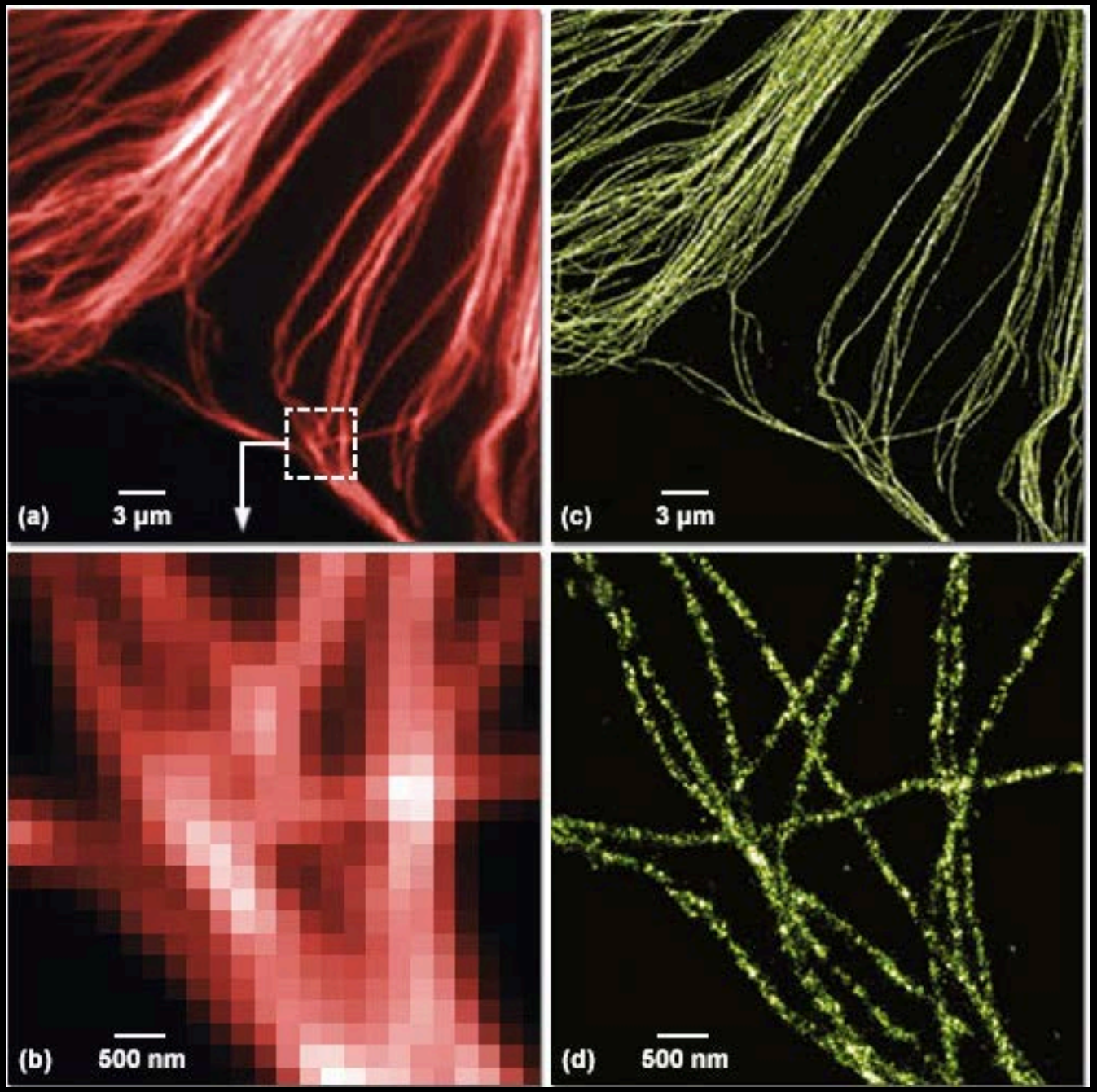
- Three-dimensional imaging with high spatial resolution
- High temporal resolution (<10−3 s)
- Low phototoxicity for prolonged imaging (>106 s) of single cells and biological tissues
- Single-molecule sensitivity
- Simultaneous observation of multiple molecular targets
- Nonperturbing labeling strategies
- Imaging of proteins under control of native genomic promoters.

Methodologies

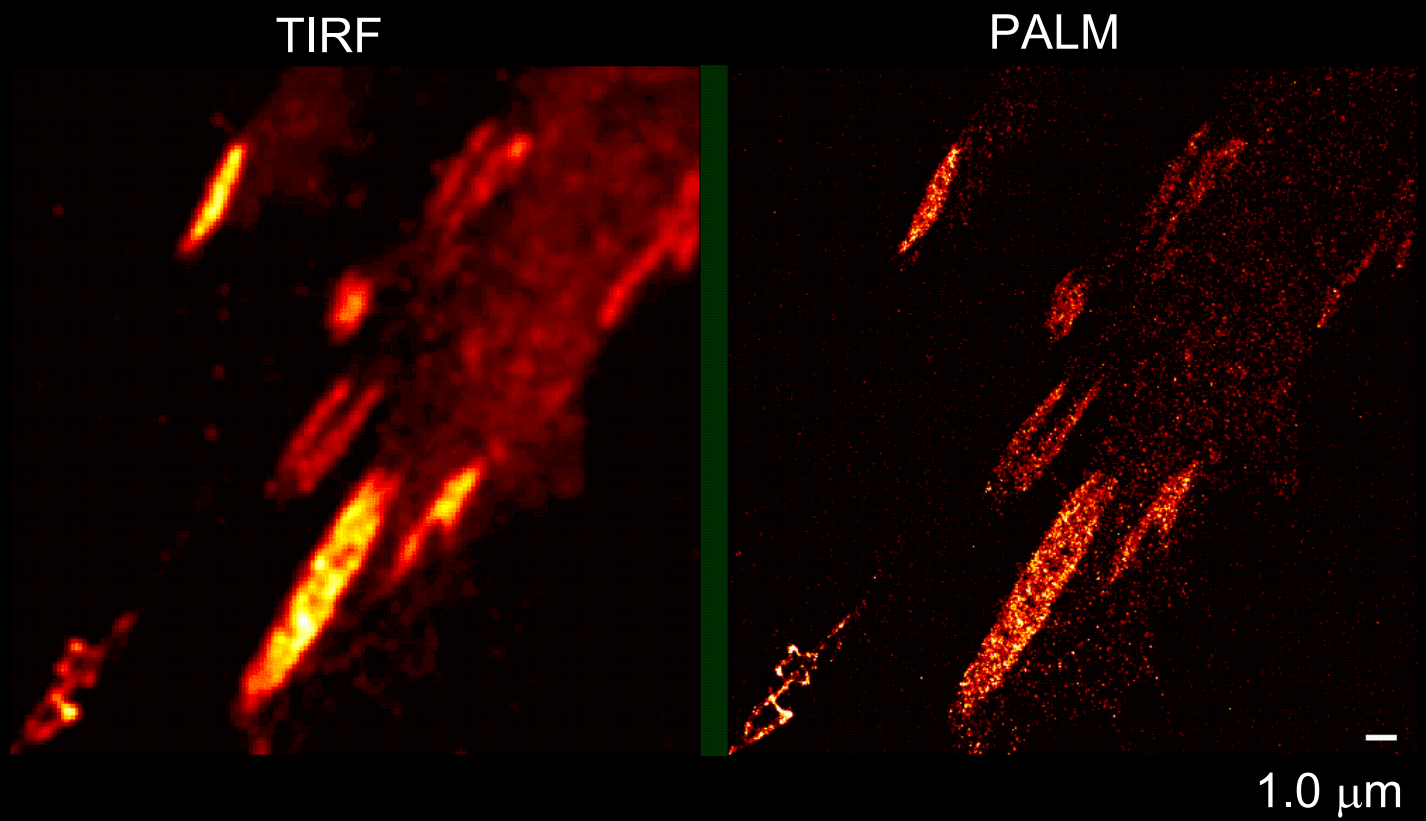
• **Wide-field microscopy:** Diffraction-limited lateral resolution (~250 nm) and diminished contrast due to illumination and detection of probes above and below the optical focus. Fluorescent probes should be bright and photostable.

• Laser-scanning and spinning-disk confocal microscopy: Improved axial resolution (~800 nm) due to rejection of out-of-focus fluorescence emission. Moderately intense and periodic illumination increases phototoxicity and requires particularly photostable bright probes.

• Total internal reflection fluorescence microscopy (TIRF): An exponentially decaying evanescent wave propagates ~200 nm into the sample, enabling single-molecule imaging/tracking. Probes should be bright, photostable and resistant to ‘dark-state’ photoconversion for prolonged particle tracking.



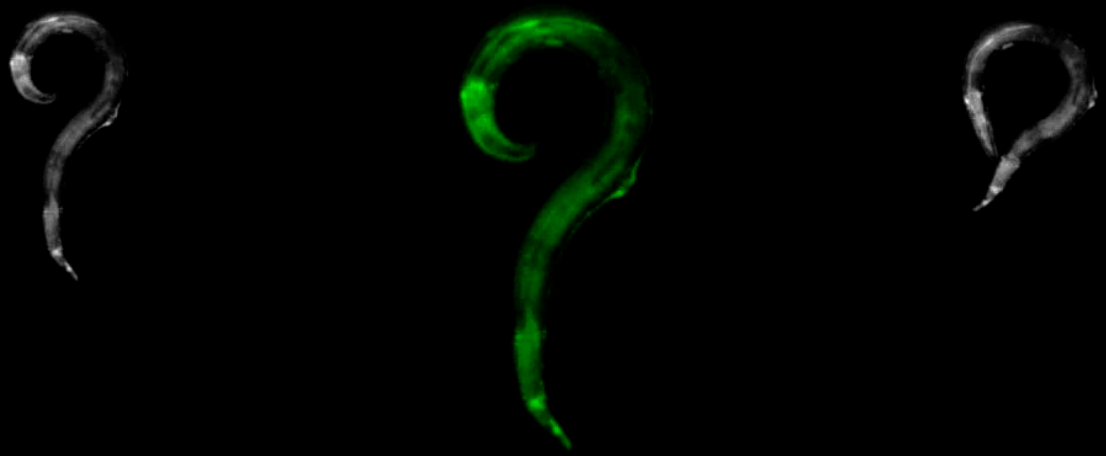
Microtubules imaged
By STORM



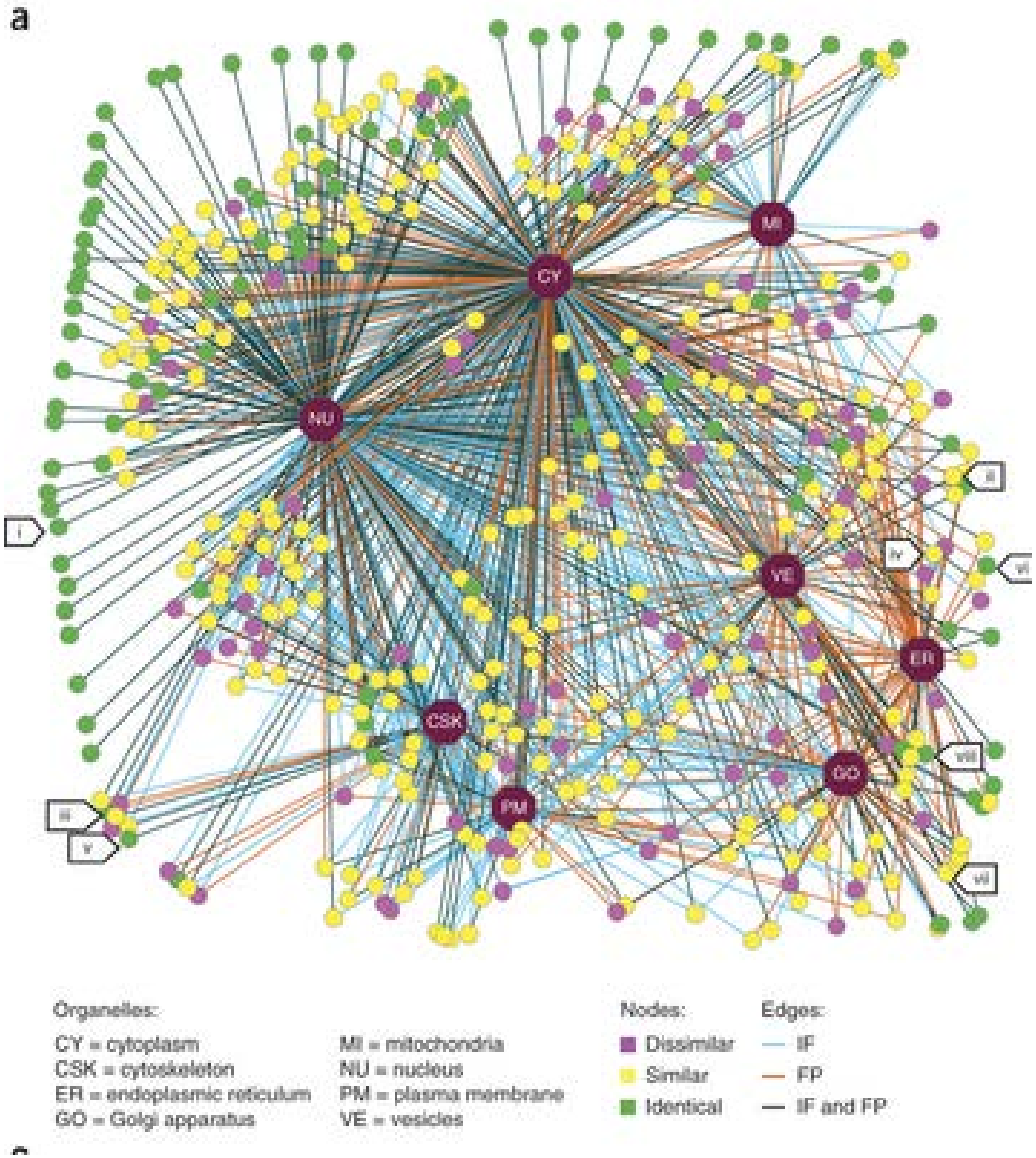
Focal adhesions, FoLu cell, dEosFP-tagged vinculin

- Being able to observe processes as they happen within the cell by light microscopy adds a vital extra dimension to our understanding of cell function.

BEFORE **AFTER**



Correlation between FP and IF

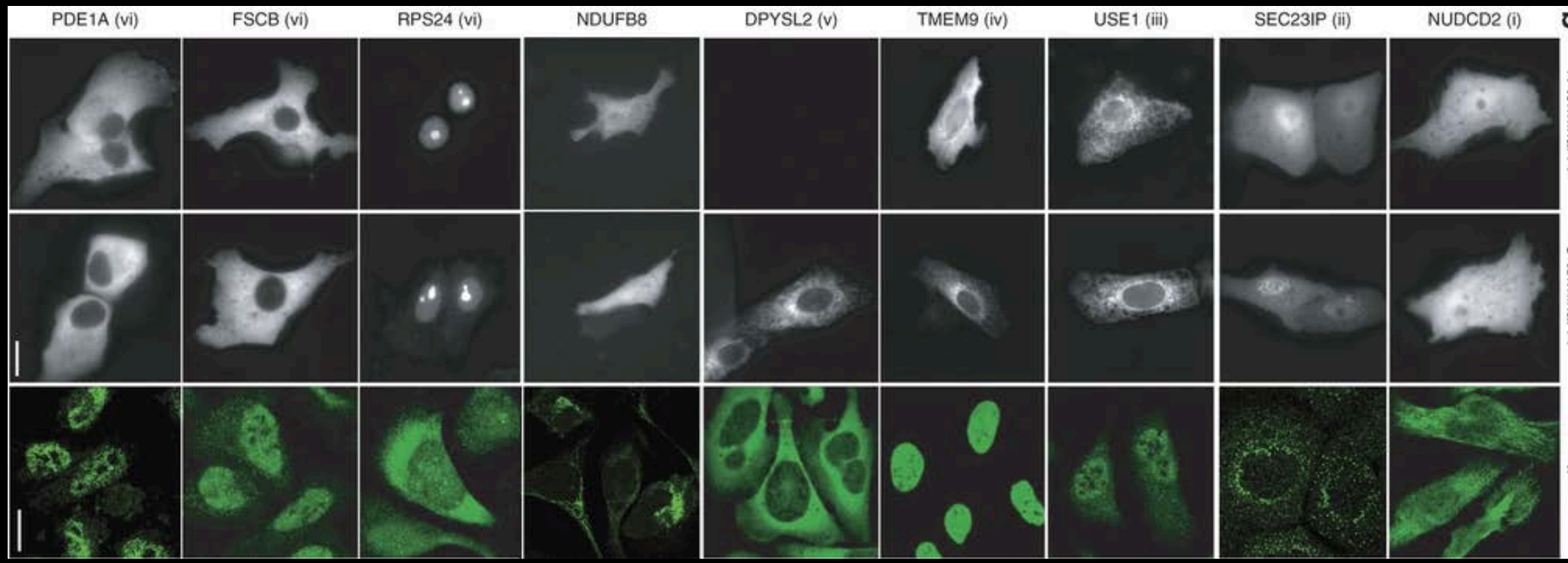


500 proteins were monitored.

Discrepancies in localization
Depending on the technique used

Stadler et al; Nature Methods 10, 315–323 (2013)

Location of Tag altersLocalization of FP



Stadler et al; Nature Methods 10, 315–323 (2013)

Problems

Phototoxicity

Over-Expression

Thermodynamic Stability

Oligomerization

Alternatives?

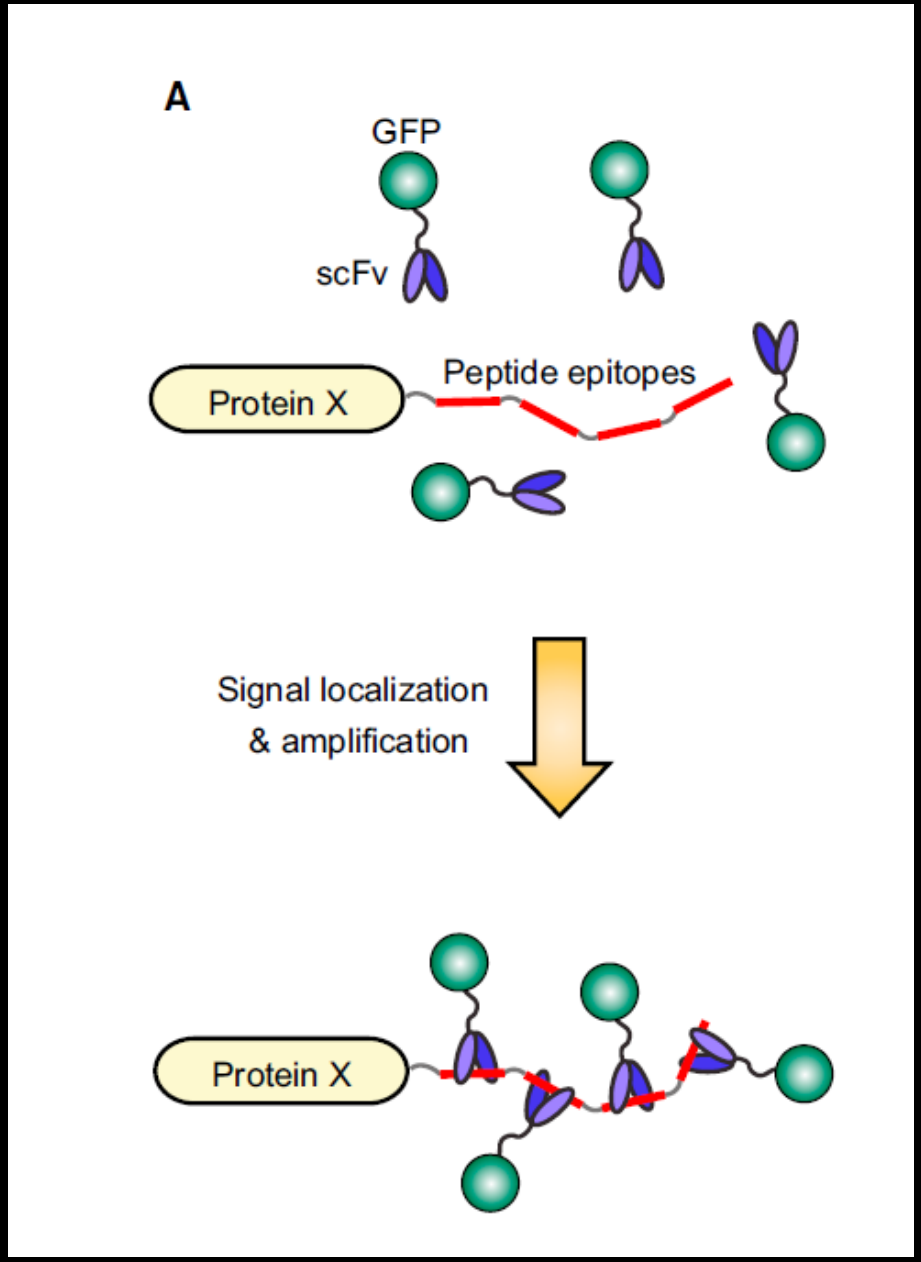
1) Self labeling Technologies: SNAP Tag, CLIP Tag, Halo Tag…
2) Protein tagging system.
3) Innoucuos tags

Resource

A Protein-Tagging System for Signal Amplification in Gene Expression and Fluorescence Imaging

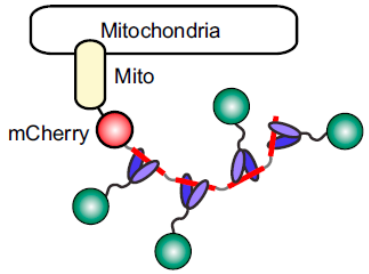
Marvin E. Tanenbaum,[1,2] Luke A. Gilbert,[1,2,3,4] Lei S. Qi,[1,3,4] Jonathan S. Weissman,[1,2,3,4] and Ronald D. Vale[1,2,*]
[1]Department of Cellular and Molecular Pharmacology, University of California, San Francisco, San Francisco, CA 94158, USA
[2]Howard Hughes Medical Institute, University of California, San Francisco, San Francisco, CA 94158, USA
[3]Center for RNA Systems Biology, University of California, Berkeley, Berkeley, CA 94720, USA
[4]California Institute for Quantitative Biomedical Research (QB3), San Francisco, CA 94158, USA
*Correspondence: vale@ucsf.edu
http://dx.doi.org/10.1016/j.cell.2014.09.039

ANTIBODY PEPTIDE LABELING STRATEGY

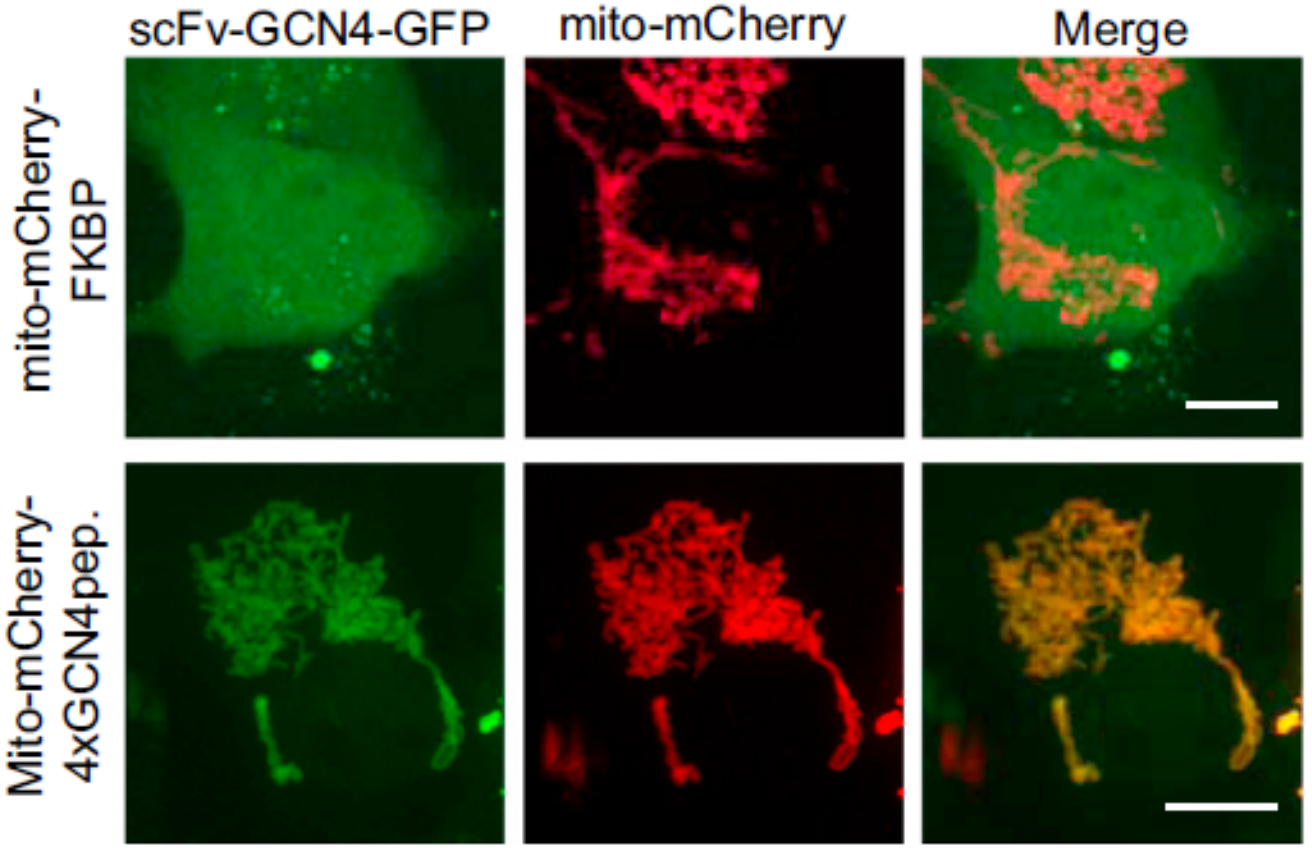


Identification of antibody peptide pair

B

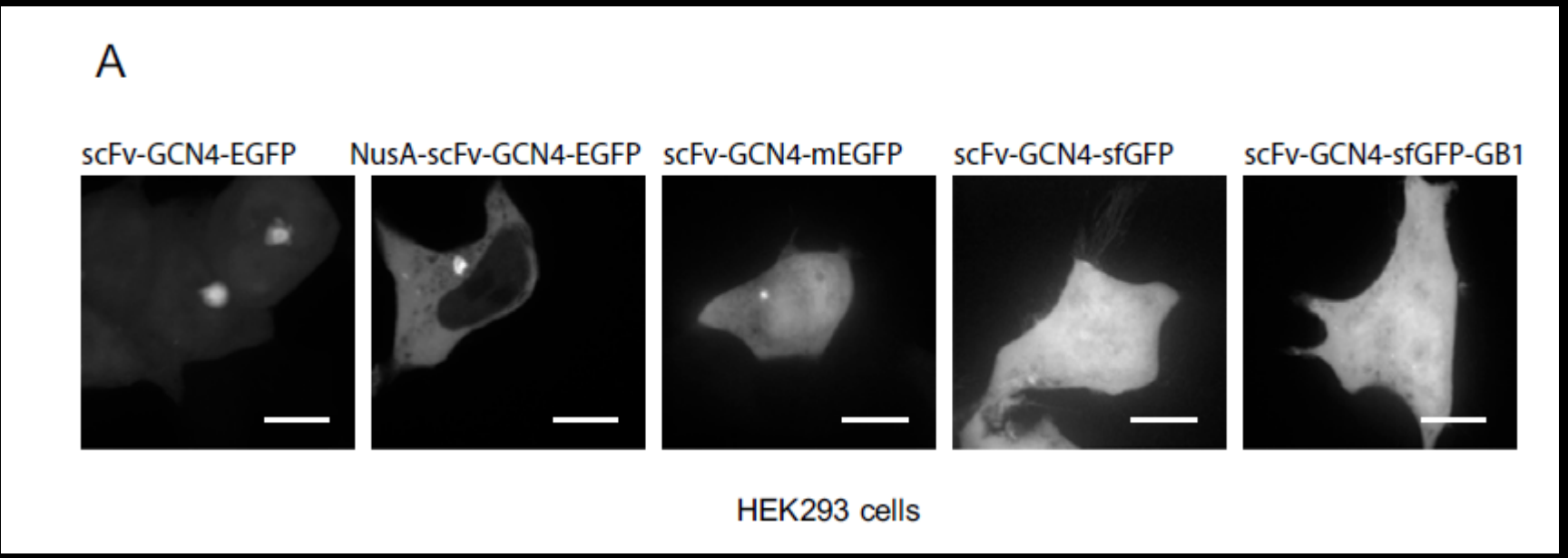


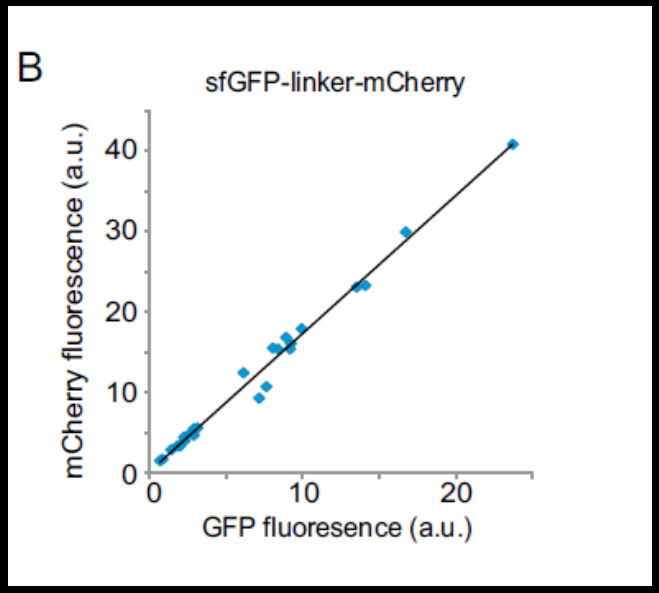
Library of scFv targeting various epitopes



How to make the antibody optimal?

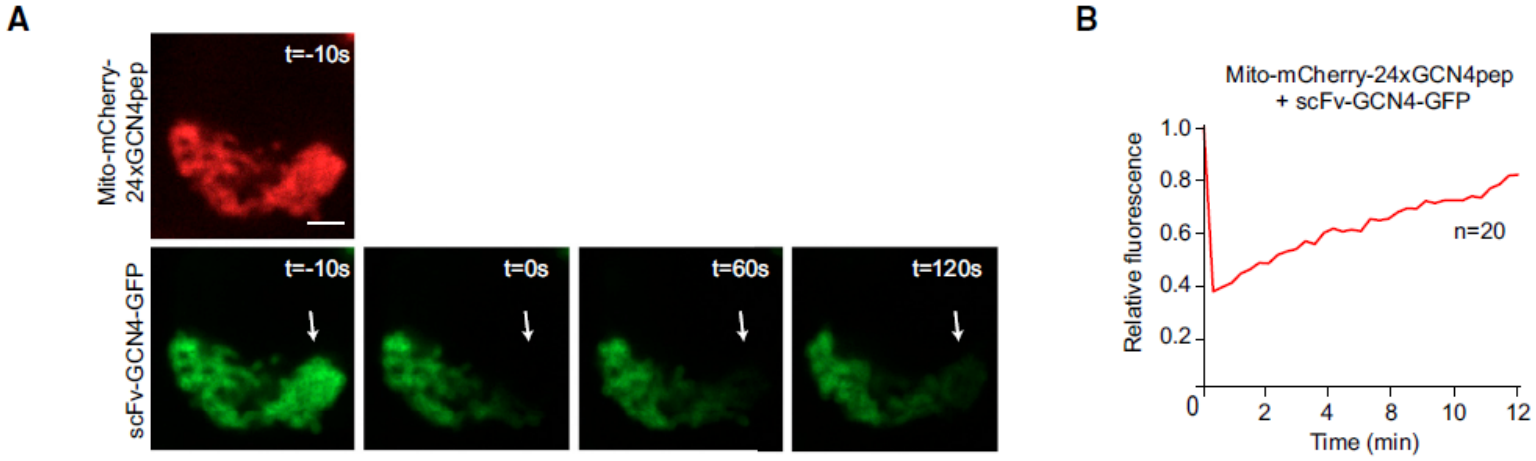
Goal: Solve the aggregation issues





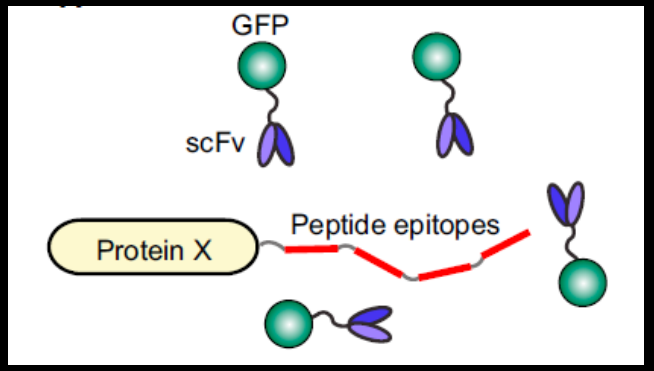
Goal: Obtain a version that does not Aggregate in the cells

Stability of GCN4 antibody-Peptide binding



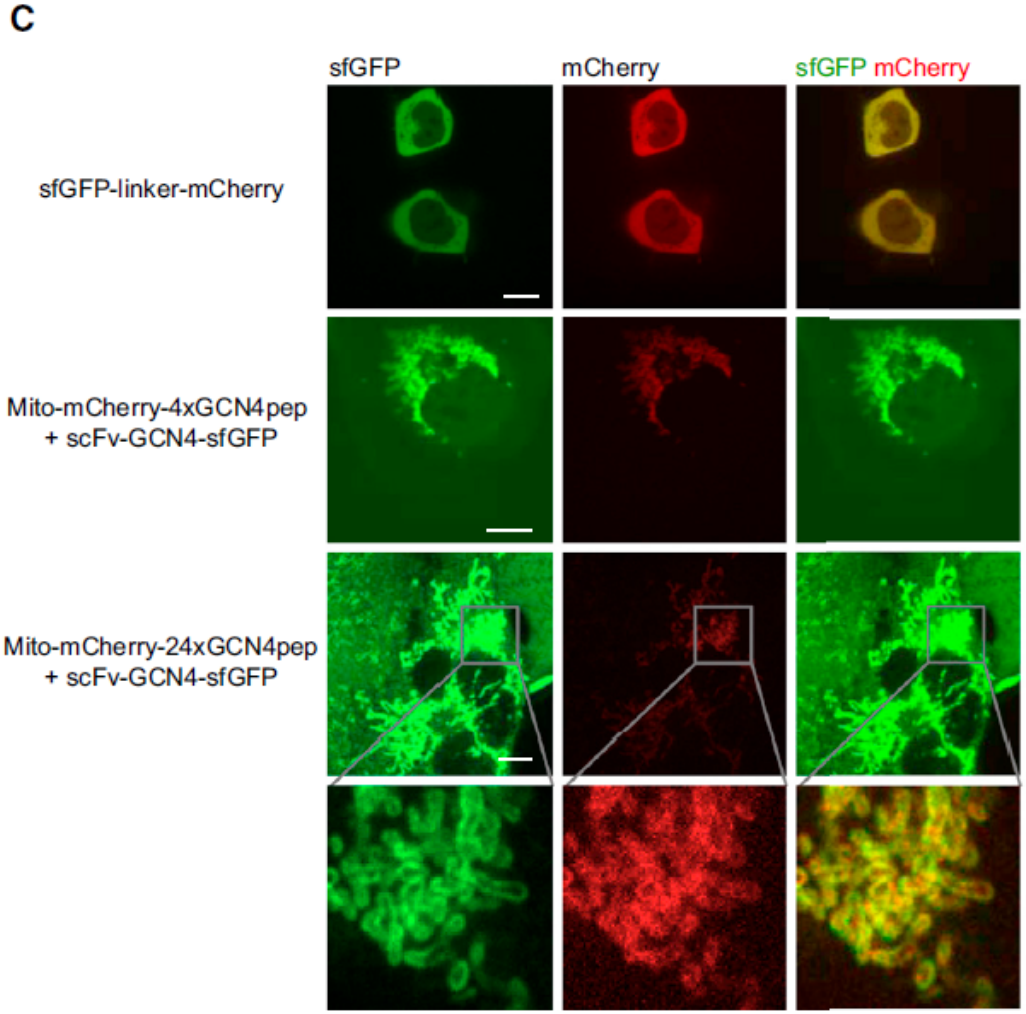
FRAP analysis

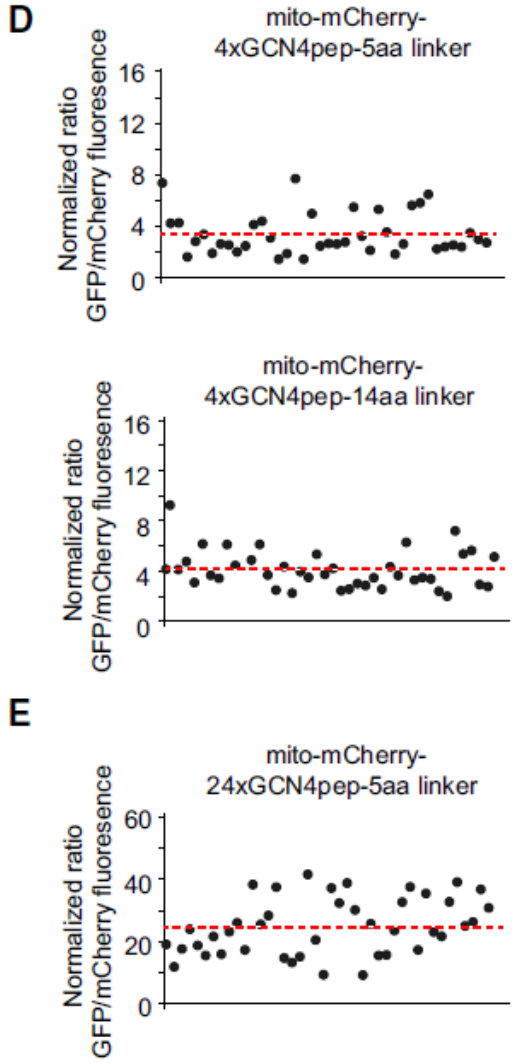
What is the optimal linker size between the peptides?



Short Linker : GGSGG

Long Linker : GGSGGSGGSGGSGG





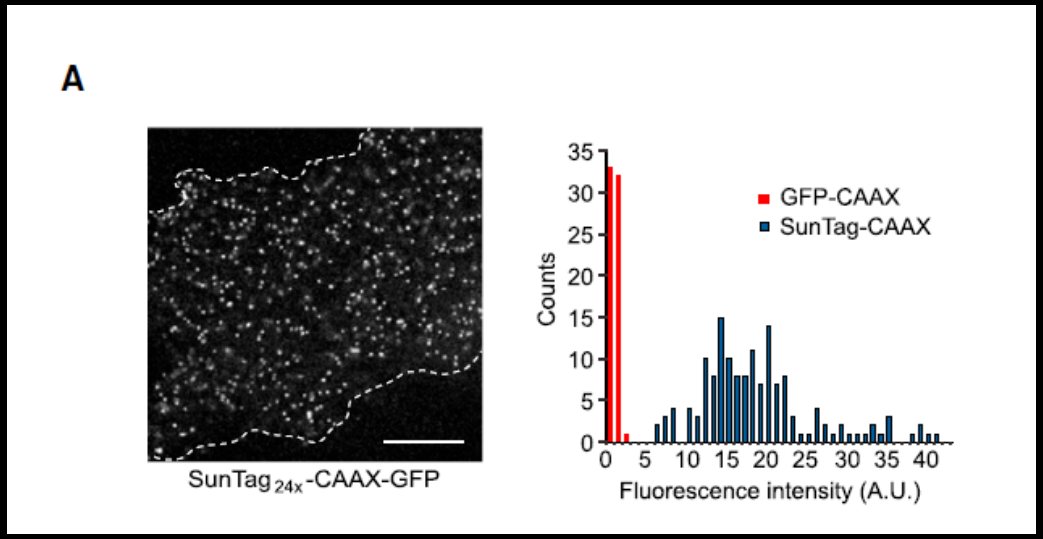
5 aa linker is sufficient for optimal expression: Average GFP:mCherry-24.

A molar ratio of 4 when everything is bound was observed.

24 GFP molecules were bound to 24x GCN4

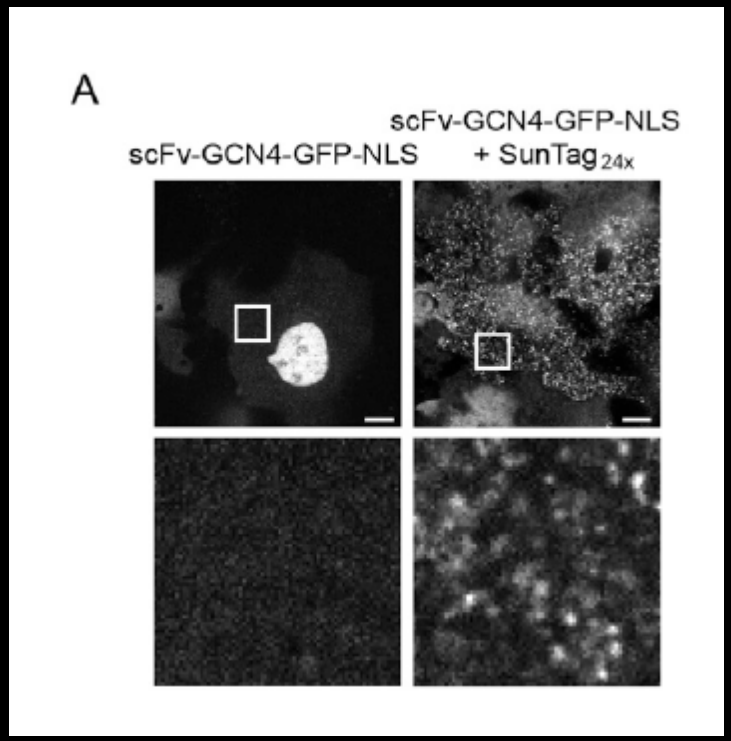
Applications of SuN Tags

Organellar localizations



Plasma Membrane.

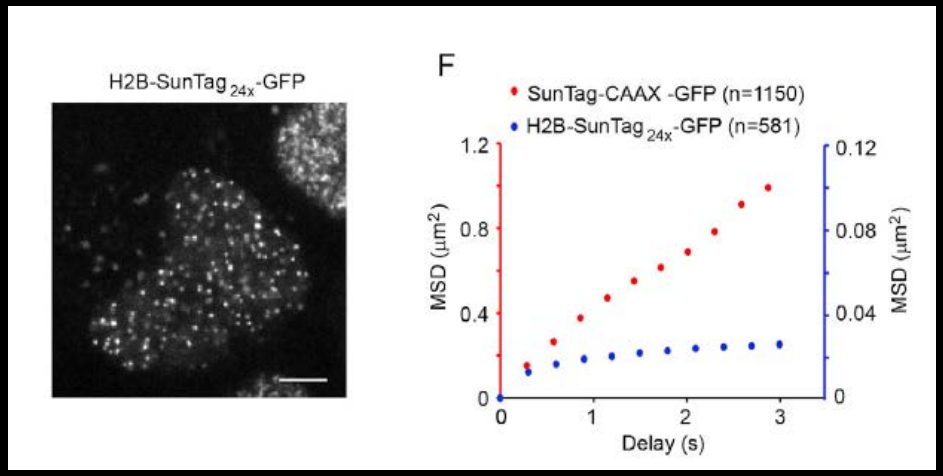
Slow diffusion on PM

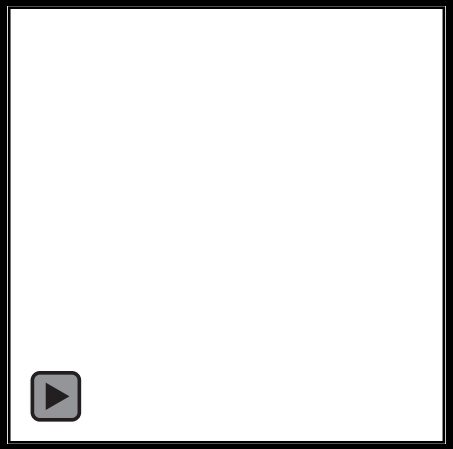


Nucleus

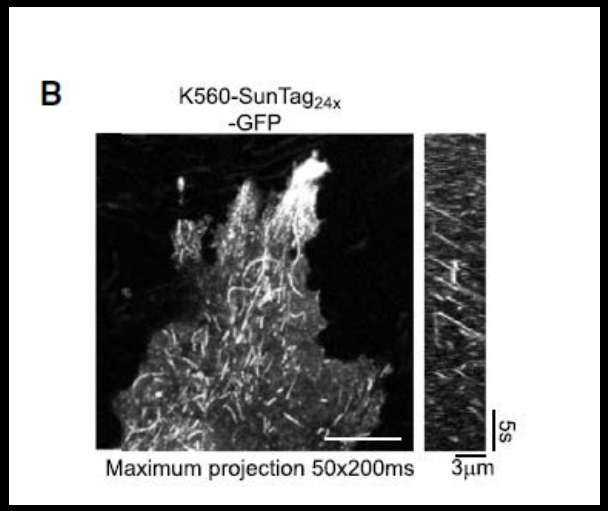
U2OS cells were transfected (from left to right) with SunTag24x-CAAX-GFP, SunTag24x-GFP, NLS-SunTag24x-GFP, and mito-mCherry-SunTag24x-GFP

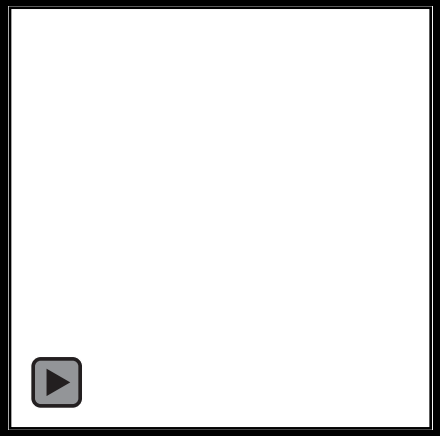
Chromatin Dynamics



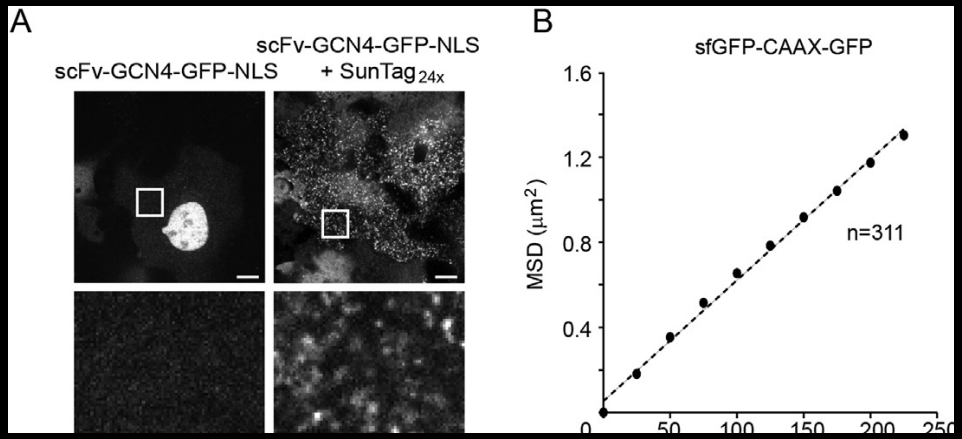


Chromatin dynamics reveal that the punctae exhibit slow and highly confined Diffusion in the nucleus suggesting that H2B is bound to the DNA.

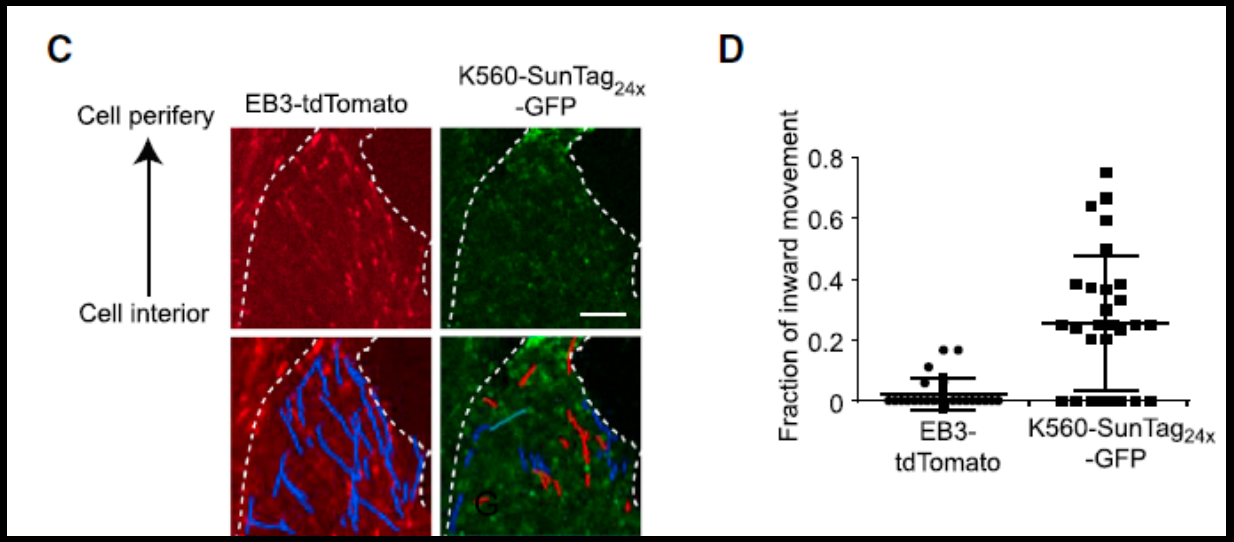




Previous imaging attempts with GFP had issues with low signals



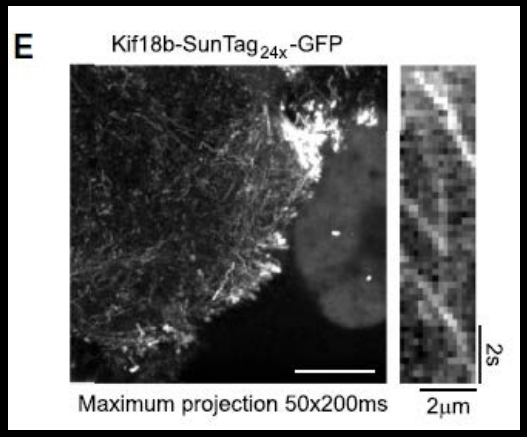
Monitoring the + ends of Microtubules

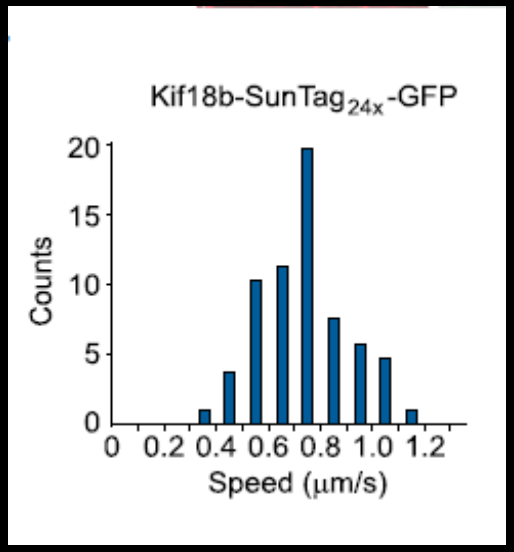


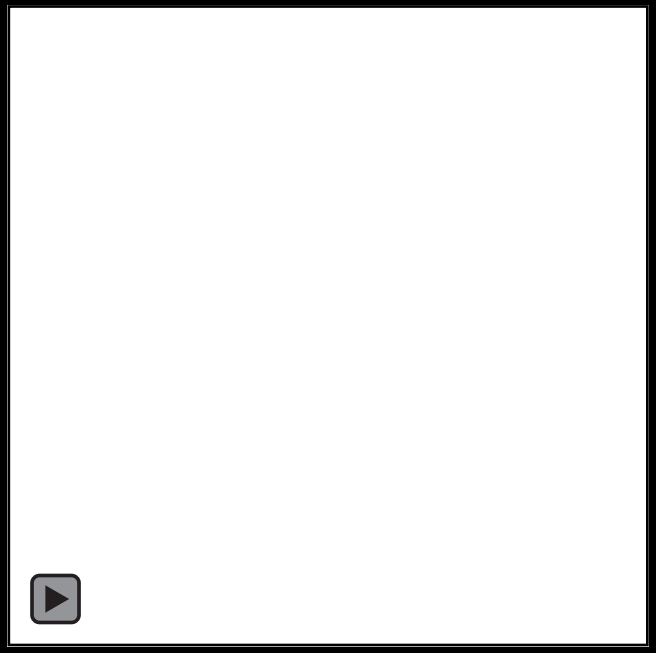
EB3td tomato marks + ends of microtubules

- End protein fused to Sun Tag

Cytoskeletal proteins with uncharacterized motility

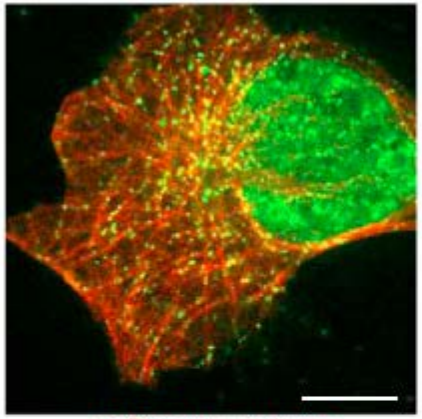






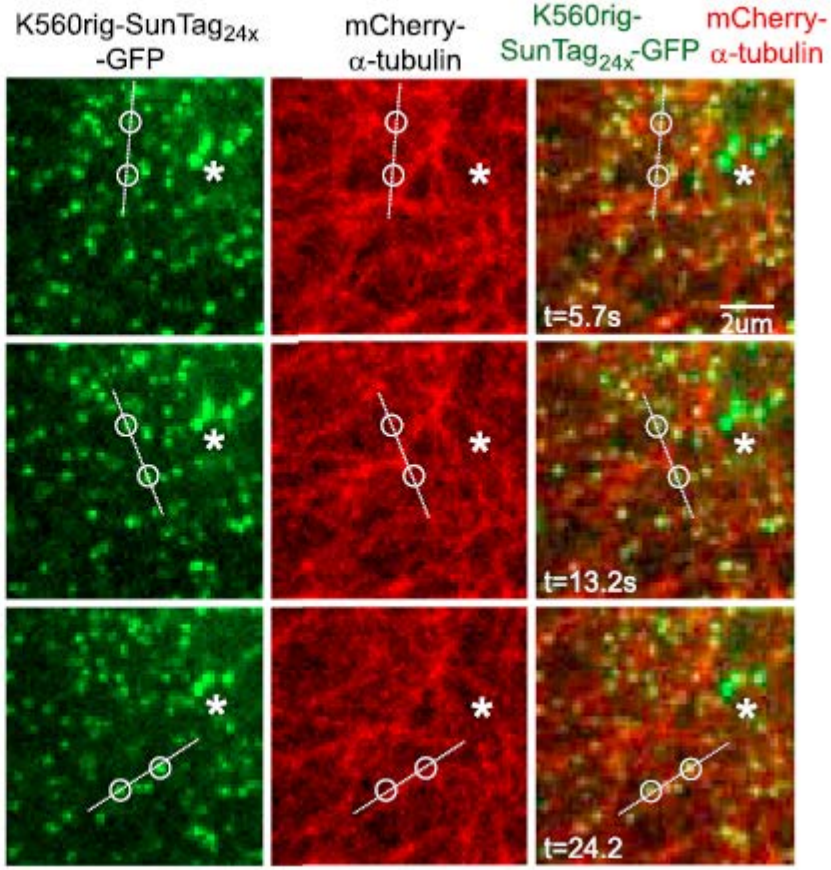
Do Kif18b molecules move along microtubules: No

G



mCherry-α-tubulin
K560rig-SunTag$_{24x}$-GFP

H

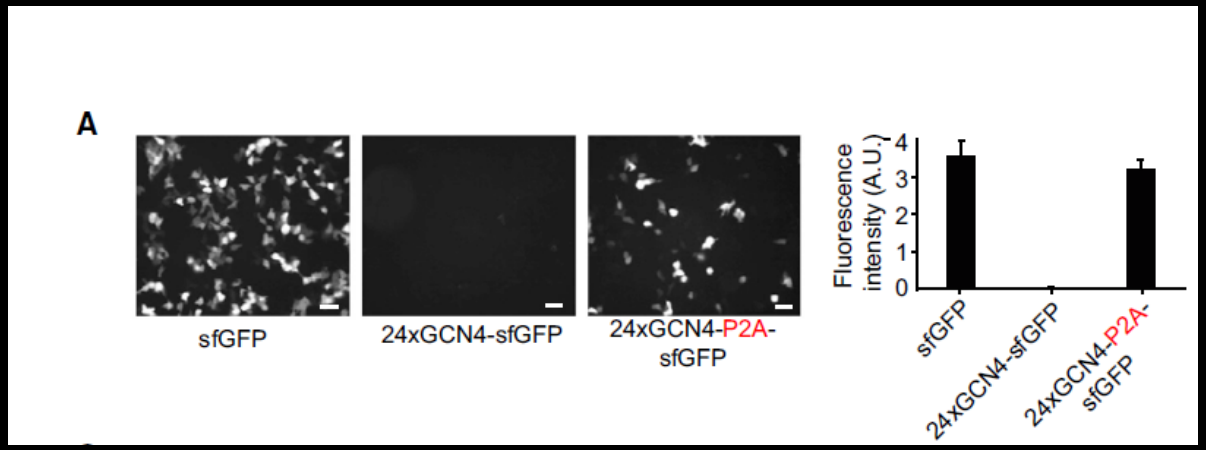


Optimizing the SuN tag for higher protein expression

Only a few hundred copies are made: Why and how can this be improved?

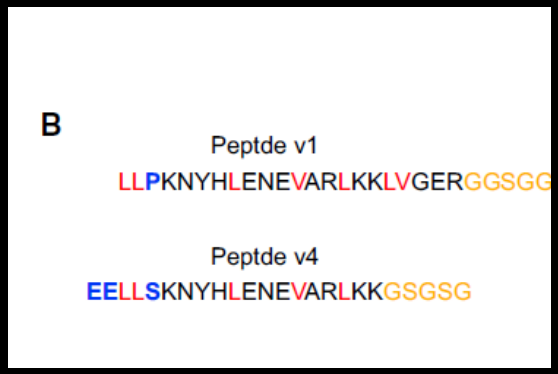
Lower stability of Sun Tag results in low expression

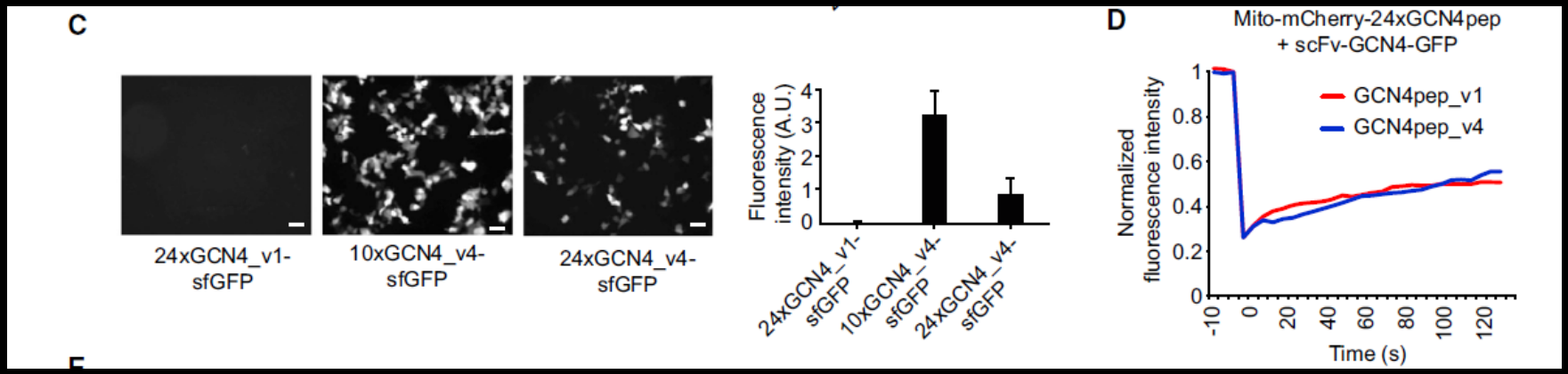
Only few hundred copies are made



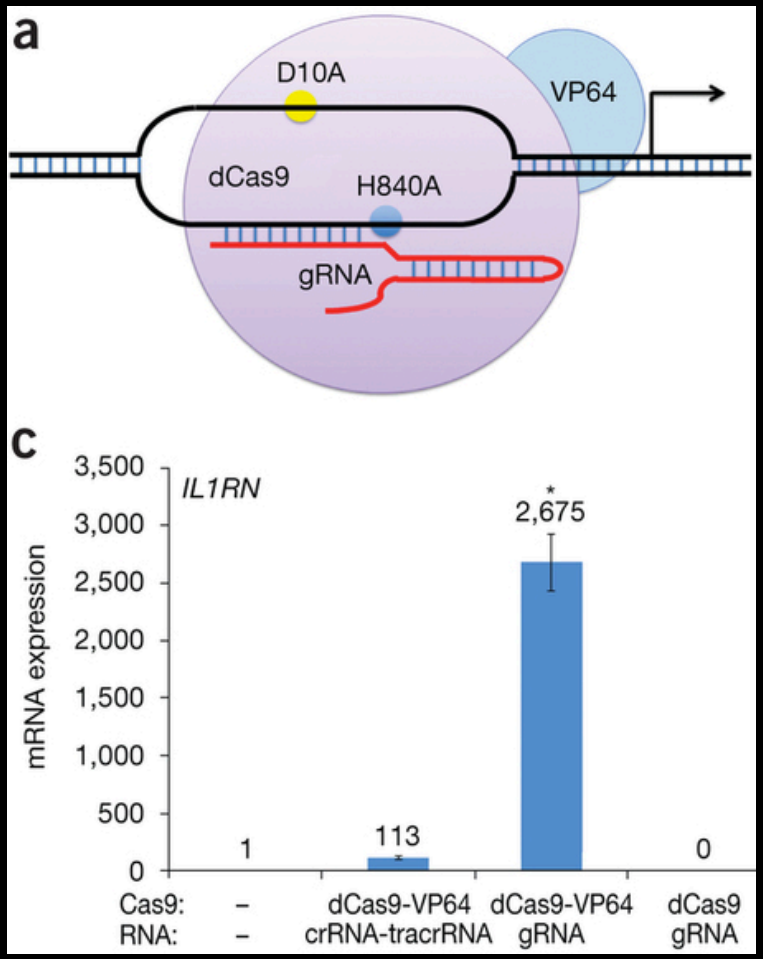
mRNA synthesis is not affected suggestion stability of the protein is the issue

Reduced hydrophobic residues and increased alpha helical propensity
Enhanced stability

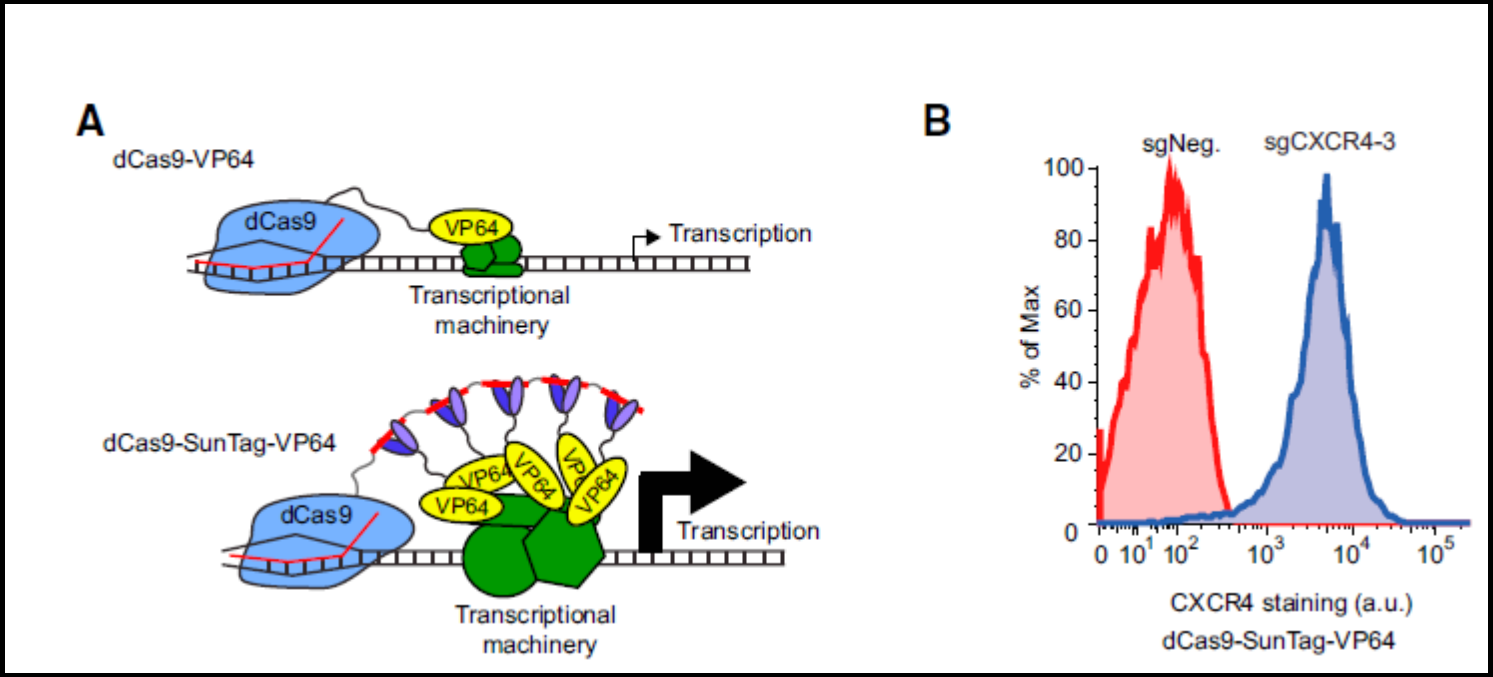




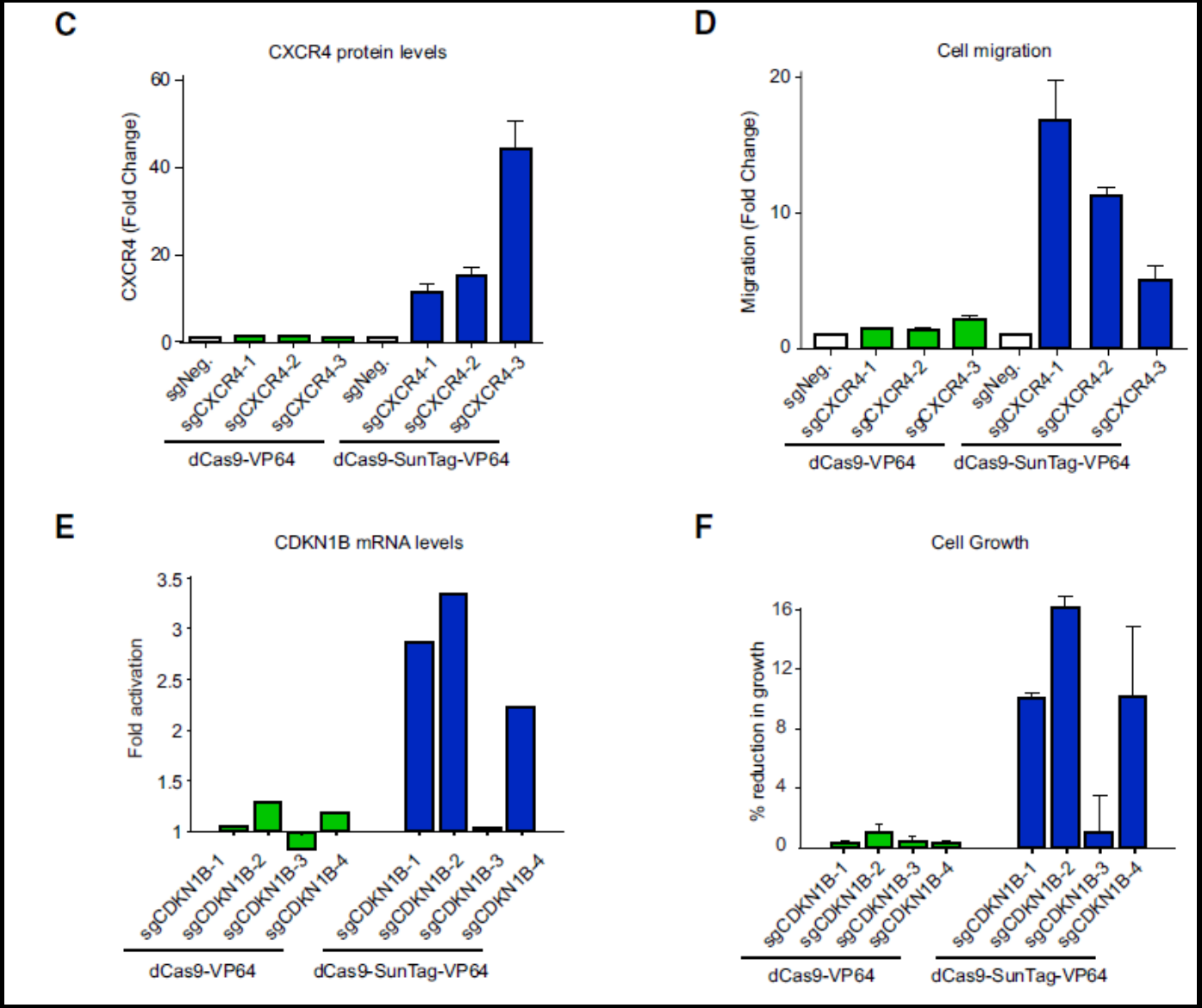
Activation of Transcription using Cas9-Sun Tag



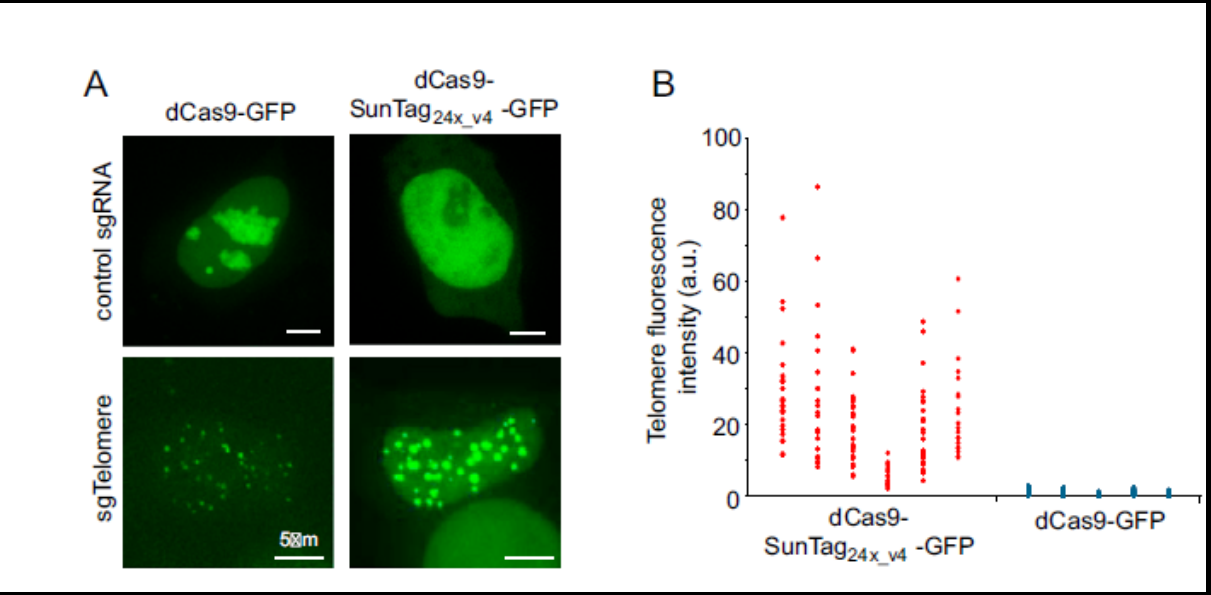
Sun Tag-CRISPR system



Applications of Sun Tag-CRISPR system



Sun Tags to monitor Telomeres



Summary

1) Can be used as alternatives for FP in imaging.

2) High resolution imaging in live cells.

3) Can be placed under genomic promoters: No need for huge overexpression.

4) Modulation of Gene expression: Genetic screens

Drawbacks:

1) Huge size of the complex that is formed: Altered kinetics?

2) Is this the best antibody pair?

Ron Vale Lab Plasmids

Create a link to this page

The Ron Vale Lab has deposited plasmids at Addgene for distribution to the research community. Addgene is a non-profit plasmid repository dedicated to improving life science research.

Learn more about research in the Ron Vale Lab.

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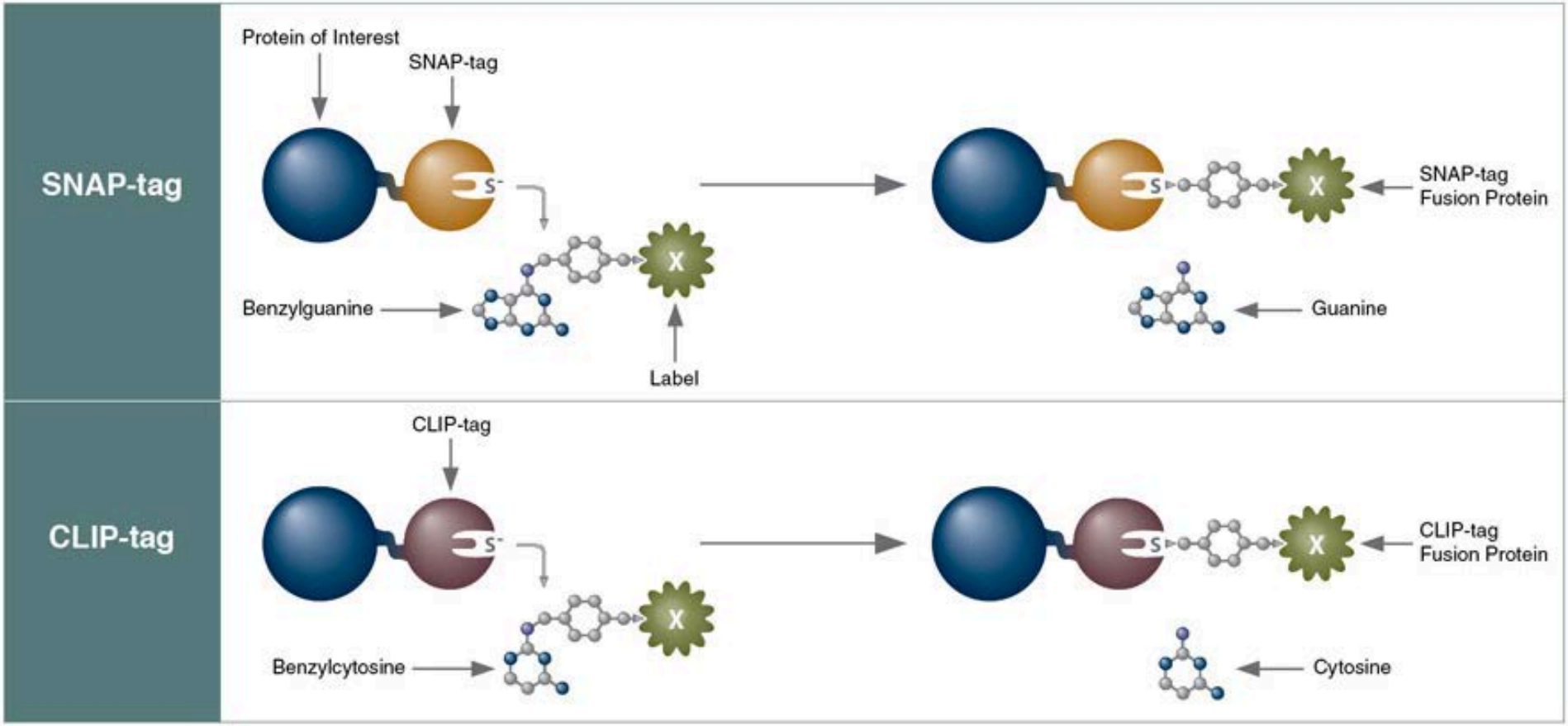
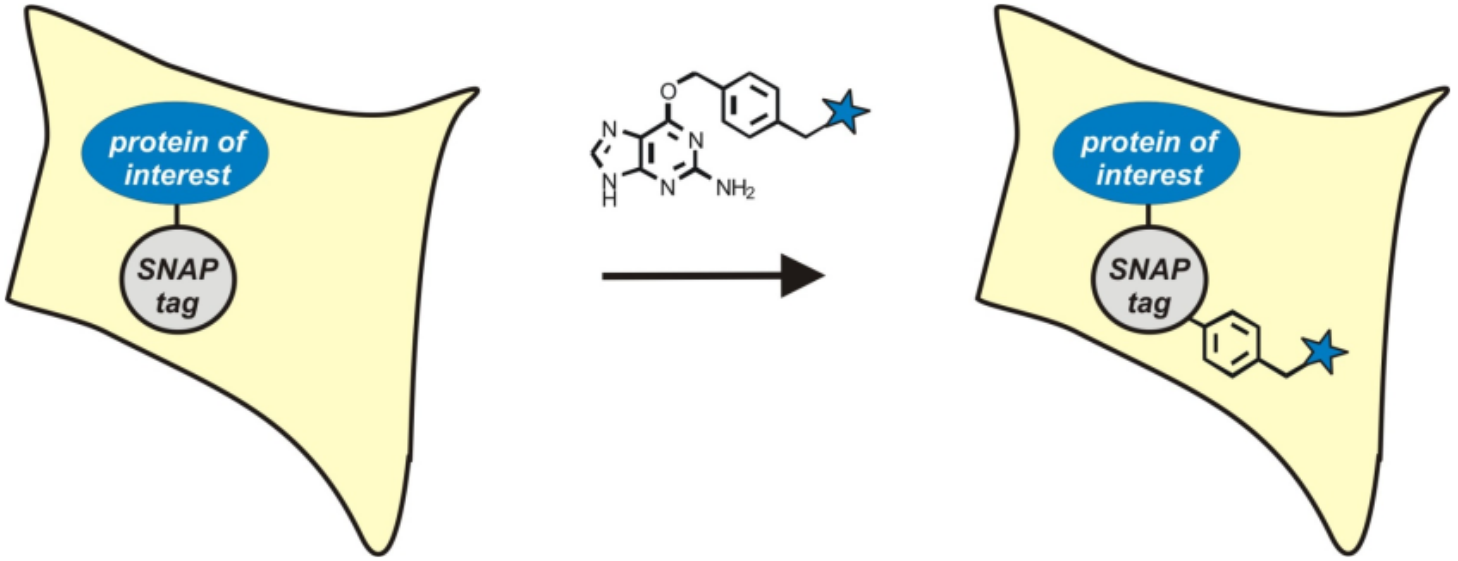
	60903	pHRdSV40-dCas9-10xGCN4_v4-P2A-BFP	Cas9 dead	Mammalian Expression, Lentiviral, CRISPR	*A Protein-Tagging System for Signal Amplification in Gene Expression and Fluorescence Imaging.* Cell. 2014 Oct 8. pii: S0092-8674(14)01227-6. doi: 10.1016/j.cell.2014.09.039.	Add to Cart
	60904	pHRdSV40-scFv-GCN4-sfGFP-VP64-GB1-NLS	scFv-GCN4	Mammalian Expression, Lentiviral	*A Protein-Tagging System for Signal Amplification in Gene Expression and Fluorescence Imaging.* Cell. 2014 Oct 8. pii: S0092-8674(14)01227-6. doi: 10.1016/j.cell.2014.09.039.	Add to Cart
	60906	pHR-scFv-GCN4-sfGFP-GB1-NLS-	scFv-GCN4	Mammalian Expression, Lentiviral	*A Protein-Tagging System for Signal Amplification in Gene Expression and Fluorescence Imaging.* Cell. 2014 Oct 8. pii:	Add to Cart

II. Chemical Indicators



SNAP-tag: A 20 kDa mutant of the DNA repair protein O6-alkylguanine-DNA alkyltransferase that reacts specifically and rapidly with benzylguanine (BG)

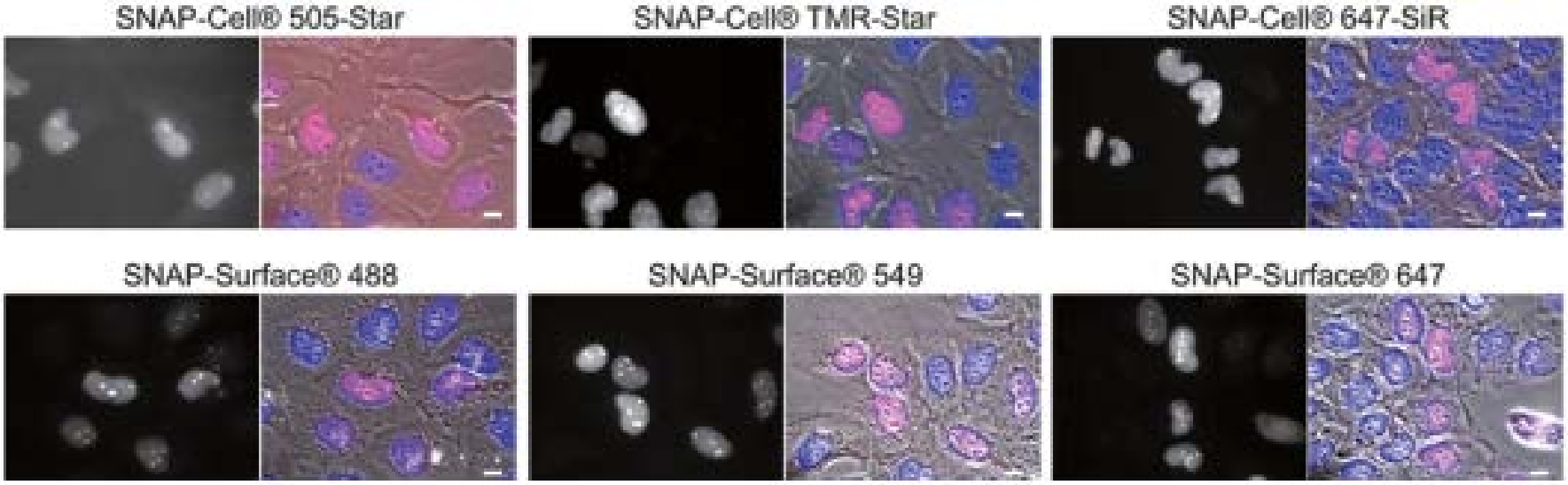
Labelling of SNAP-tag fusion proteins by benzylguanine (BG) derivatives



- 20 kD; monomeric
- High reaction rate (up to 10^5 sec^{-1} M^{-1})
- Reacts with large variety of BG derivatives

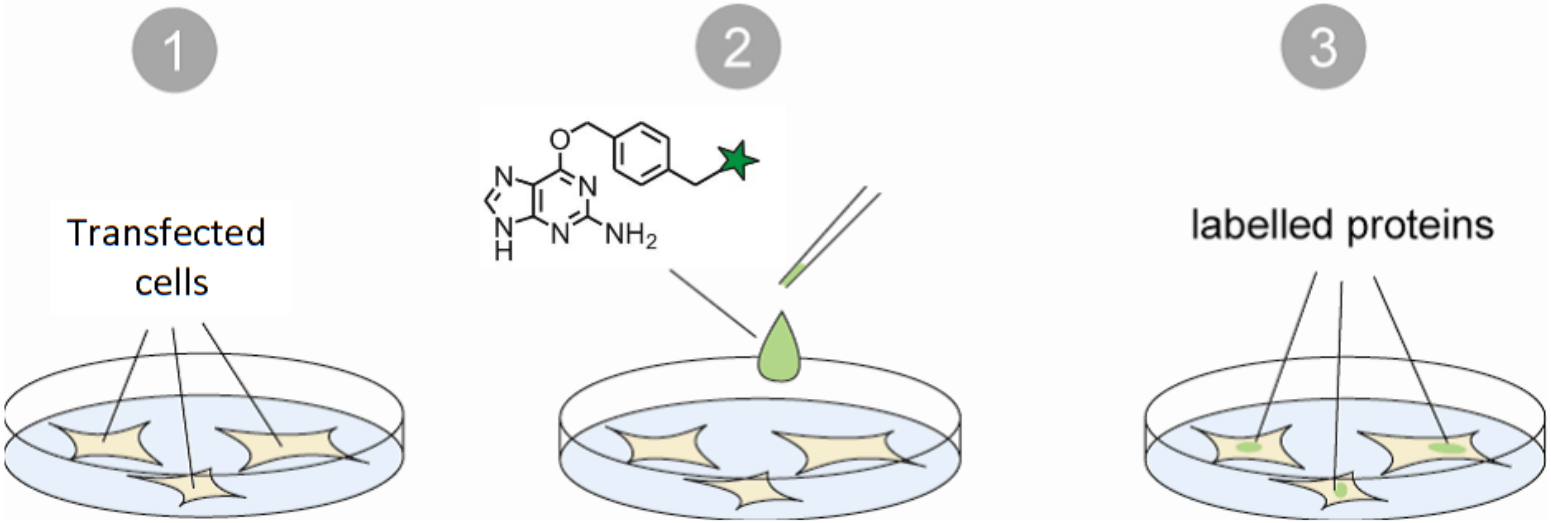
Reagents to label SNAP Tags

Cell permeable and Impermeable reagents



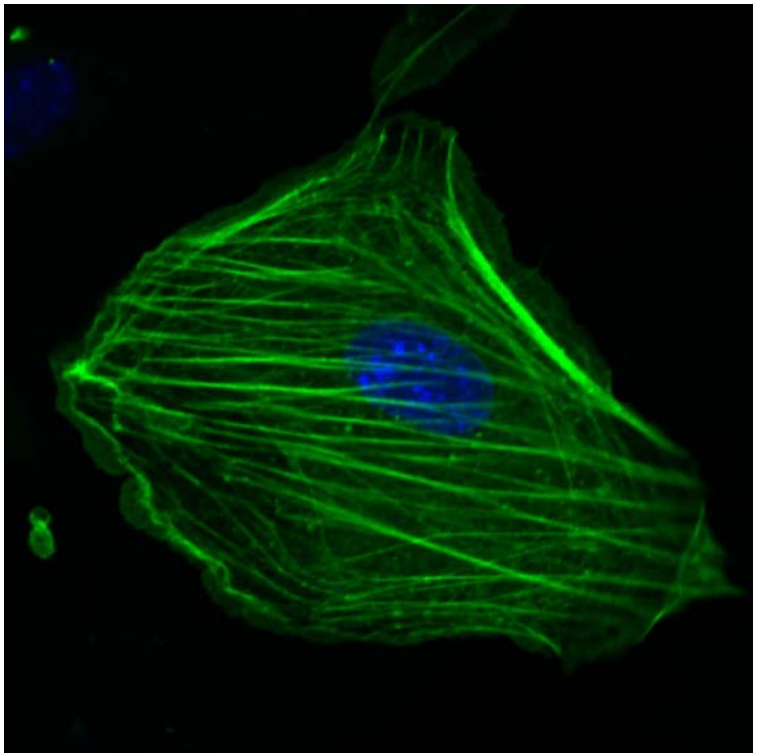
Methodology

Fluorescence labelling of SNAP-tag fusion proteins

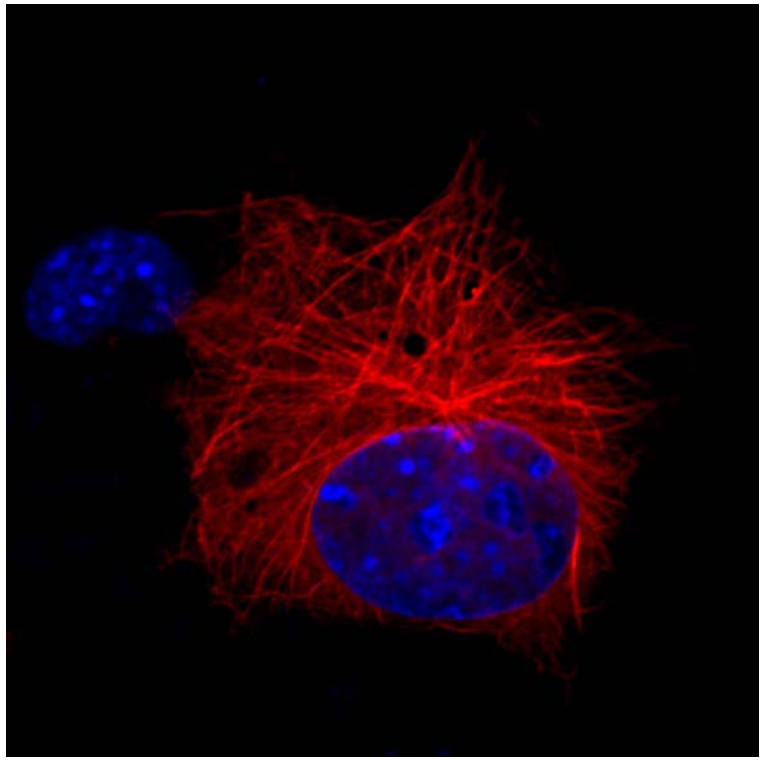


Incubation for >10 min
followed by washing

Live cell imaging with SNAP-tag fusion proteins



SNAP-actin



SNAP-β-tubulin

Images from Dr. Luo Sun, New England Biolabs

Applications

. Fluorescence microscopy

. Identification of multiprotein complexes using SNAP tag based FRET.

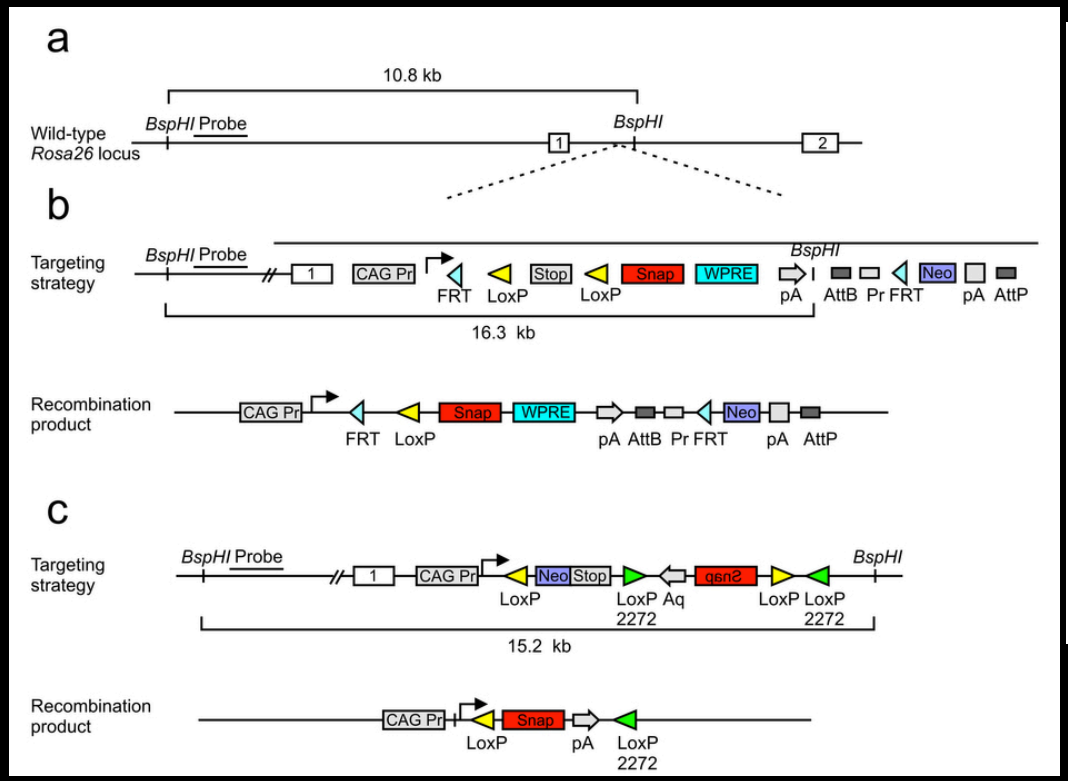
. In vivo half lives of proteins.

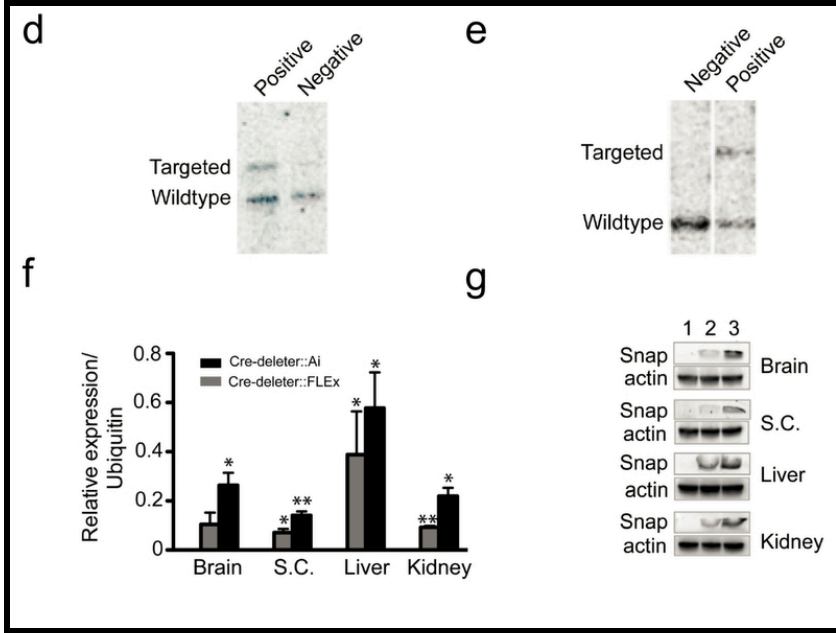
Can the applications of SNAP tag be transformed into in vivo?

Genetic targeting of chemical indicators *in vivo*

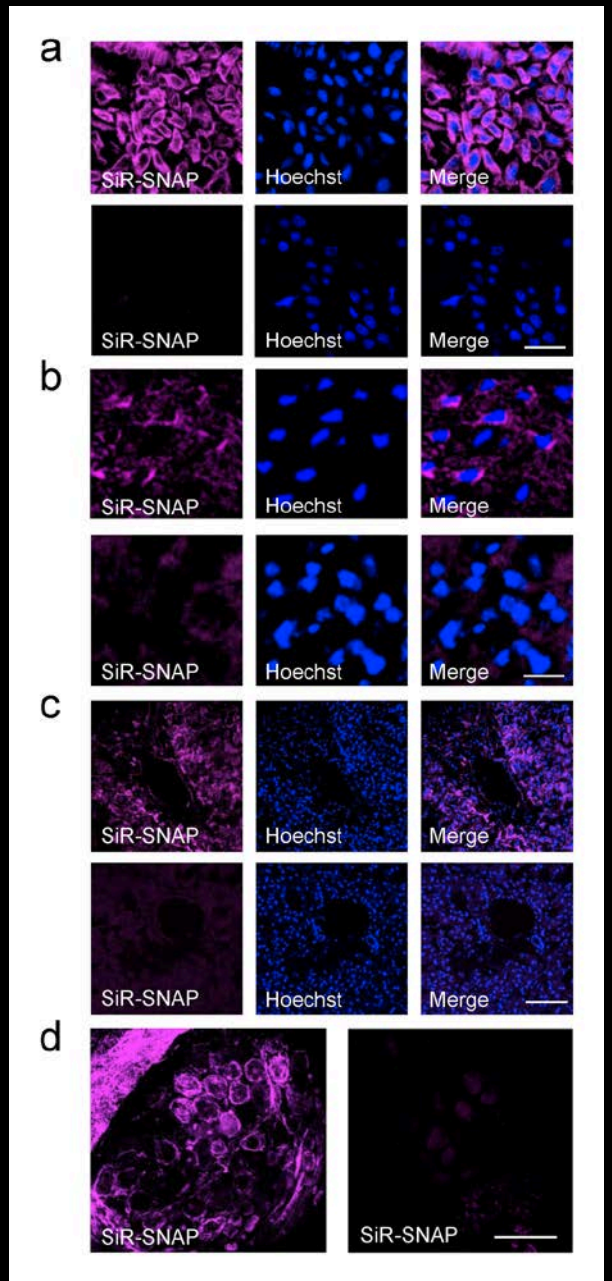
Guoying Yang[1,2,5], Fernanda de Castro Reis[1,5], Mayya Sundukova[1], Sofia Pimpinella[1], Antonino Asaro[1], Laura Castaldi[1], Laura Batti[1], Daniel Bilbao[1], Luc Reymond[3,4], Kai Johnsson[3,4] & Paul A Heppenstall[1,2]

Generation of SNAP-CAAX lines





Deleter-Cre induces robust expression of SNAP-CAAX tag



Skin

Brain slices

Liver slices

DRG

Samples were collected and after treated for 20 min with the Reagent before fixation.

Ex vivo and In vivo SNAP labeling

Brain slices (300µm)

Skin

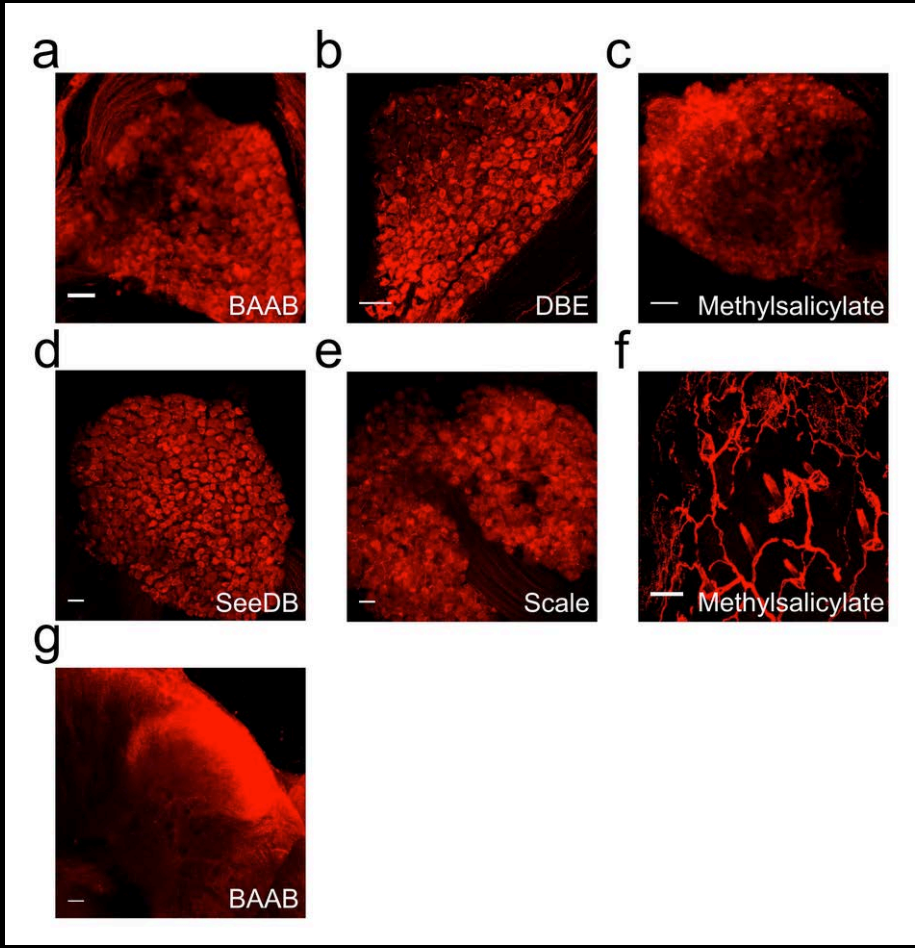
Hair follicles

Eye

Innervations of cornea

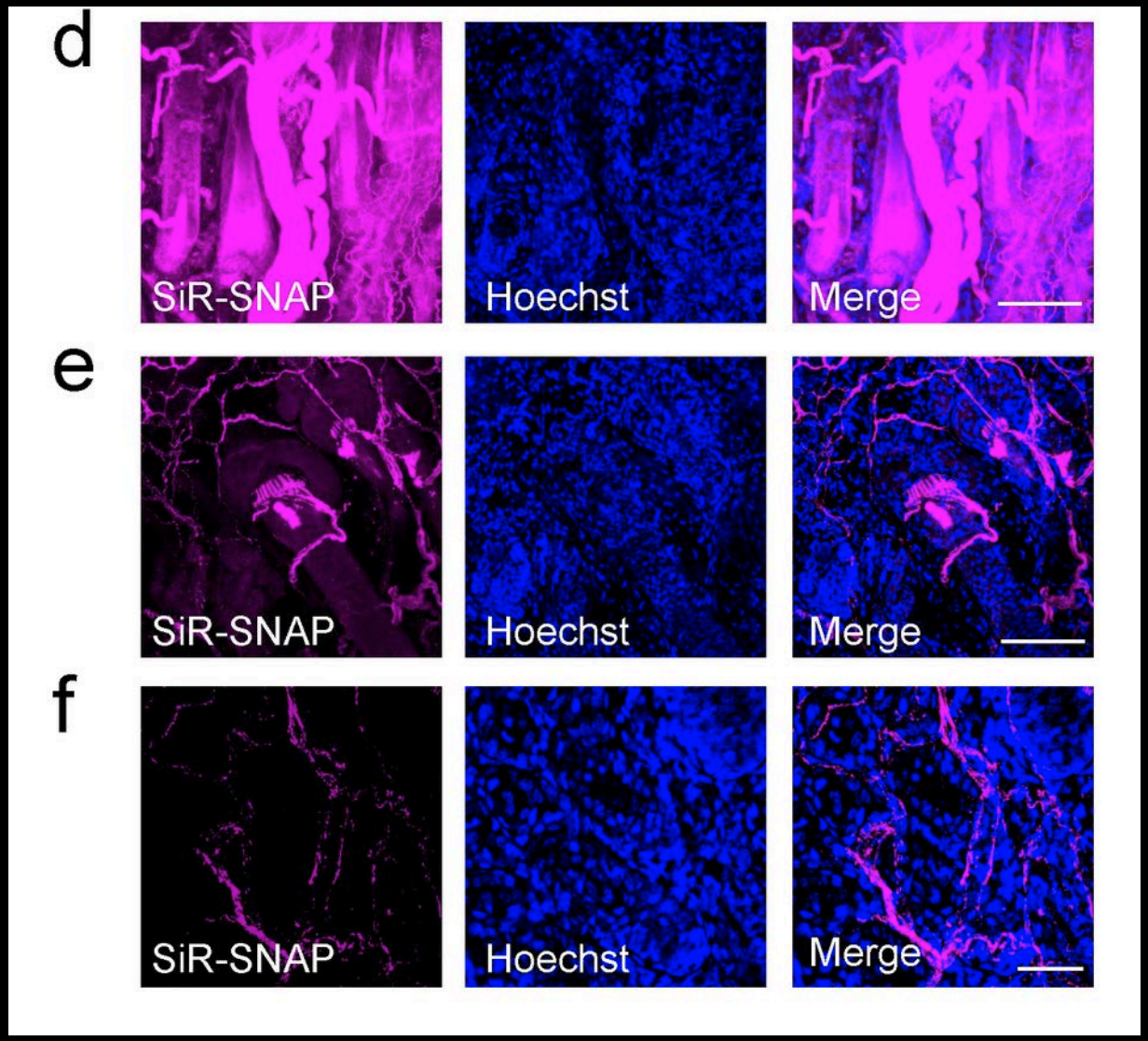
Reagent was injected in the eye and 8h later fixed and imaged

Optical clearance of SNAP labeled tissue



(a-e) TMR-Star labelled DRG from Avil-Cre::SNAPCaaX mice cleared with BAAB (a), DBE (b), methyl salicylate (c), SeeDB (d), or Scale (e). (f) TMR-Star labelled skin cleared with methylsalicylate. (g) TMR-Star labelled 1mm spinal cord slice cleared with BAAB. Scale bar 50μm.

In vivo SNAP labeling



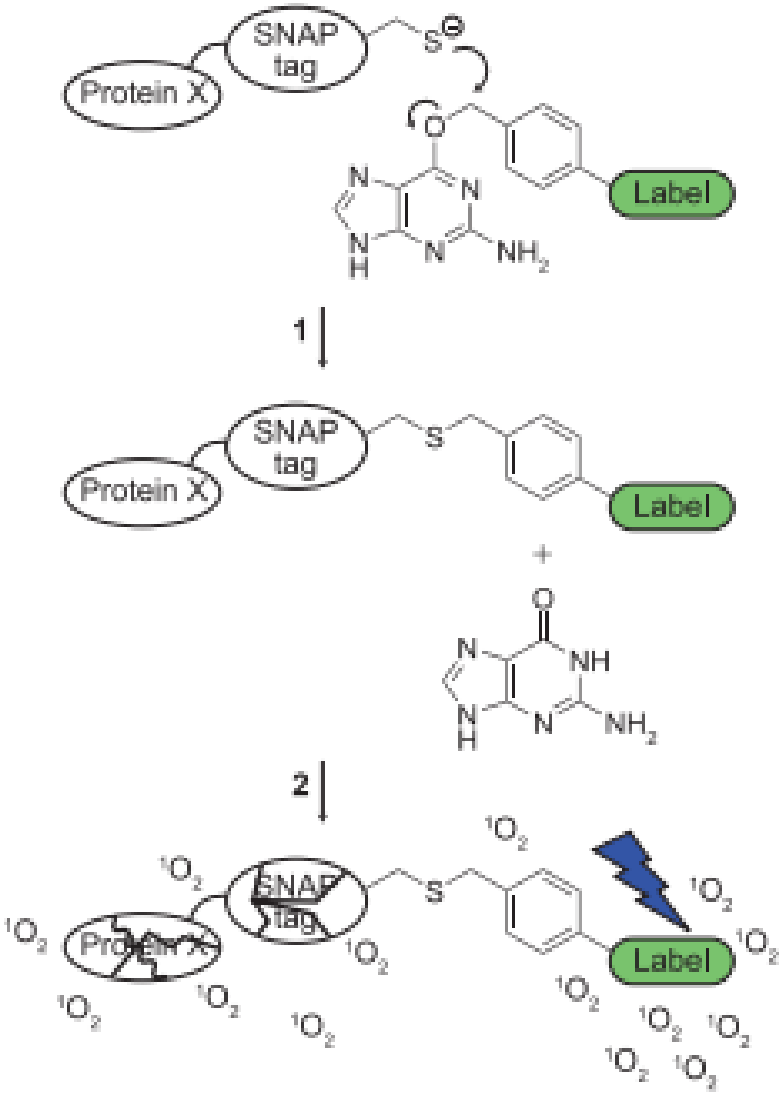
Avil-Cre mice vs SNAP CAAX

4h

8h

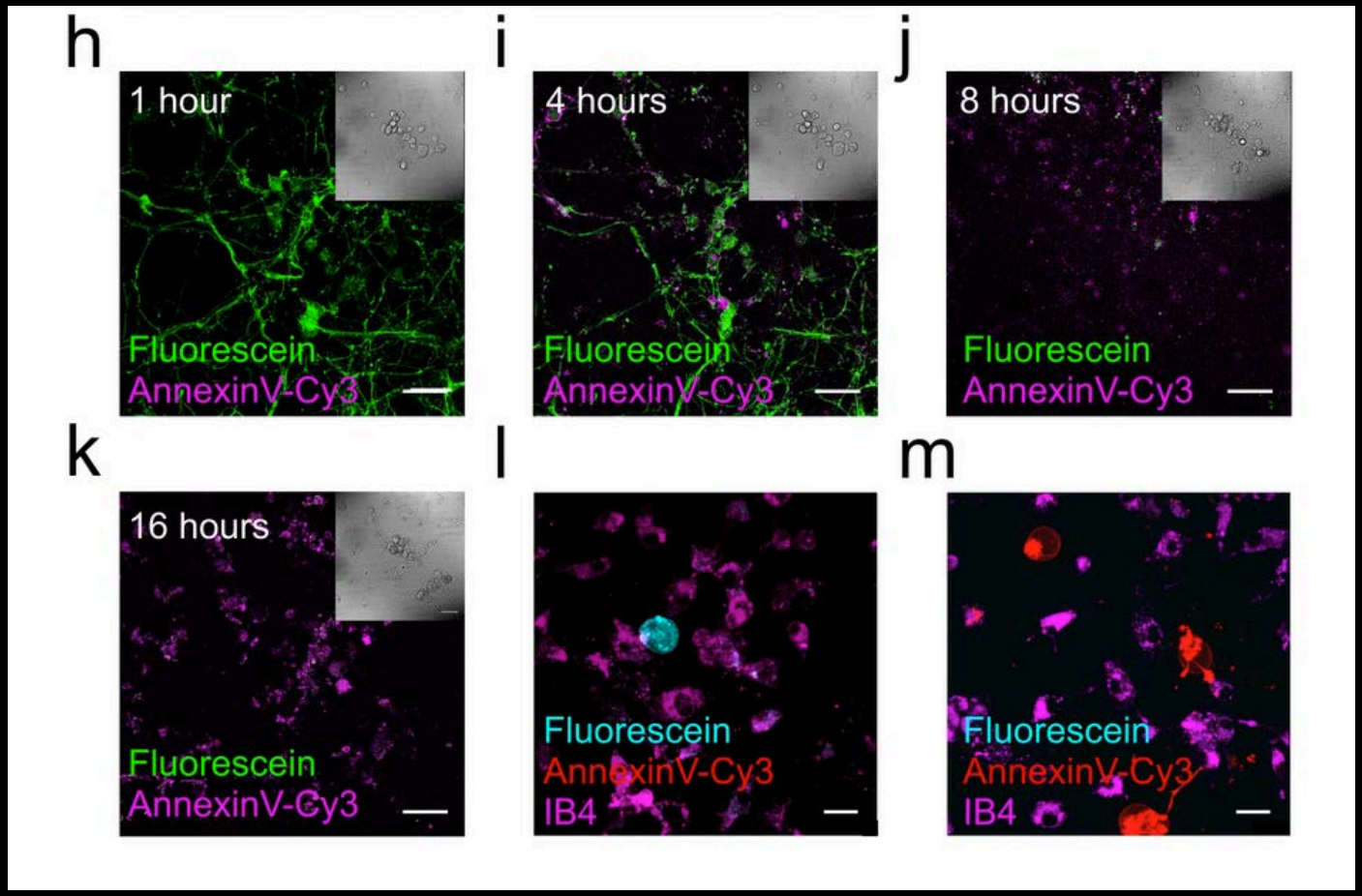
24h

Chromophore assisted light inactivation: CALI



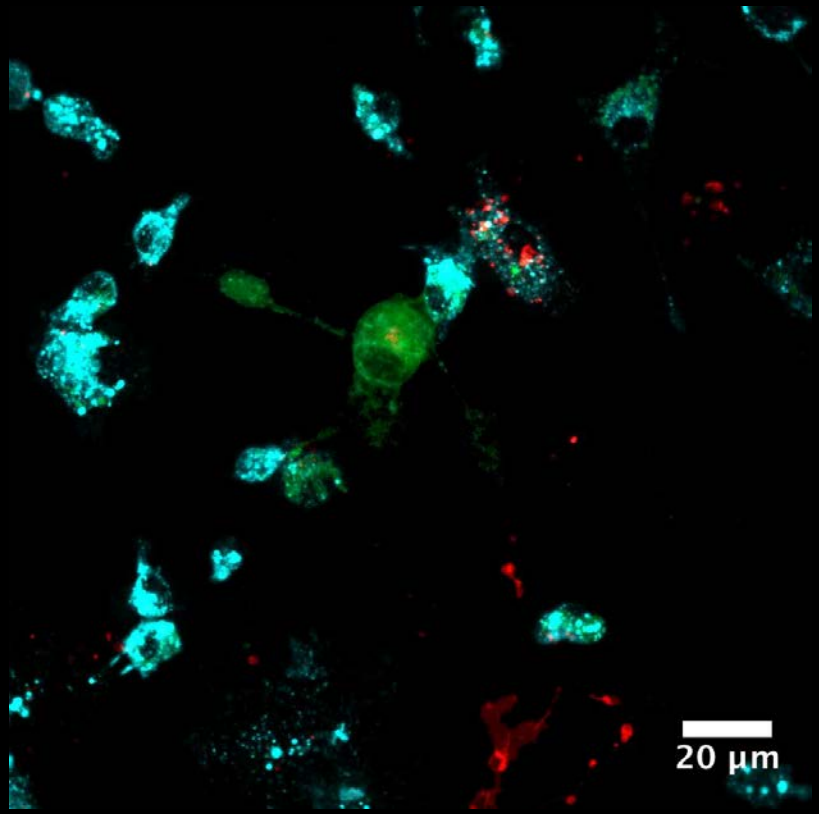
Fluorescein is usually used
For this

Application: Chromophore assisted light inactivation

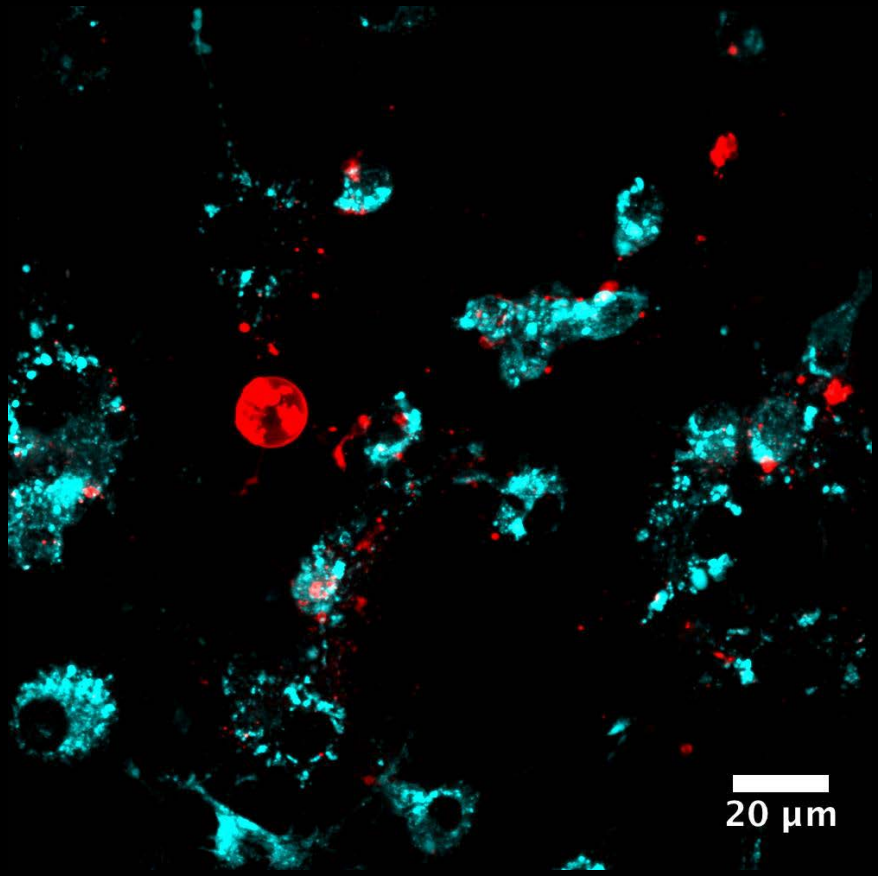


Once the bound chromophore is illuminated, it results in generation of ROS
And activation of apoptosis.

Non Illuminated



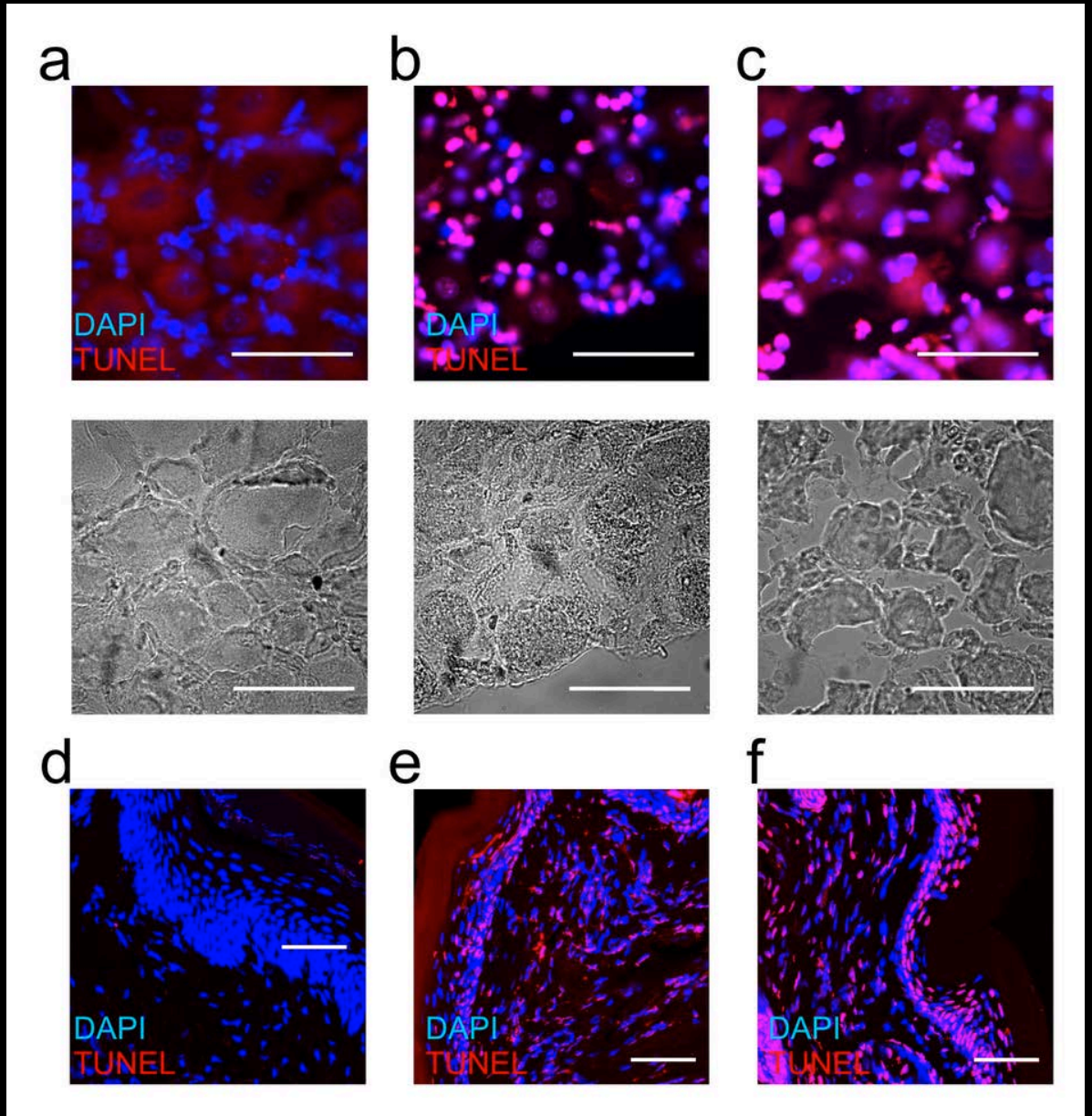
Non Illuminated



DRG/microglia co-cultures from Avil-Cre::SNAPCaaX mice labelled with BG-Fluorescein (neurons, green), IB4 (microglia, cyan) and AnnexinV-Cy3 (red) 24 hours post illumination. Microglia are engulfing dead neurons and neuronal debris.

In vivo CALI

DRG Skin Dnase 1 treated



Avil-Cre::SNAPCaaX mice

4 days post injections

Summary

1) Mice exressing SNAP tags as an alternative version to FP

2) Tissue specificity is maintained.

3) Tissue specific ablations possible by apoptosis.

4) More tests are needed: Cell permeable versions

Inntags: small self-structured epitopes for innocuous protein tagging

Maya V Georgieva[1,9], Galal Yahya[1,2,9], Laia Codó[3,4], Raúl Ortiz[1], Laura Teixidó[5], José Claros[6], Ricardo Jara[6], Mònica Jara[5], Antoni Iborra[5], Josep Lluís Gelpí[3,4,7], Carme Gallego[1,10], Modesto Orozco[4,7,8,10] & Martí Aldea[1,10]

Tags alter Cell Size!!!

Interactors of Tags

IT6 does not alter the localization

New generation of small ordered tags: Neutral and behave innocuos