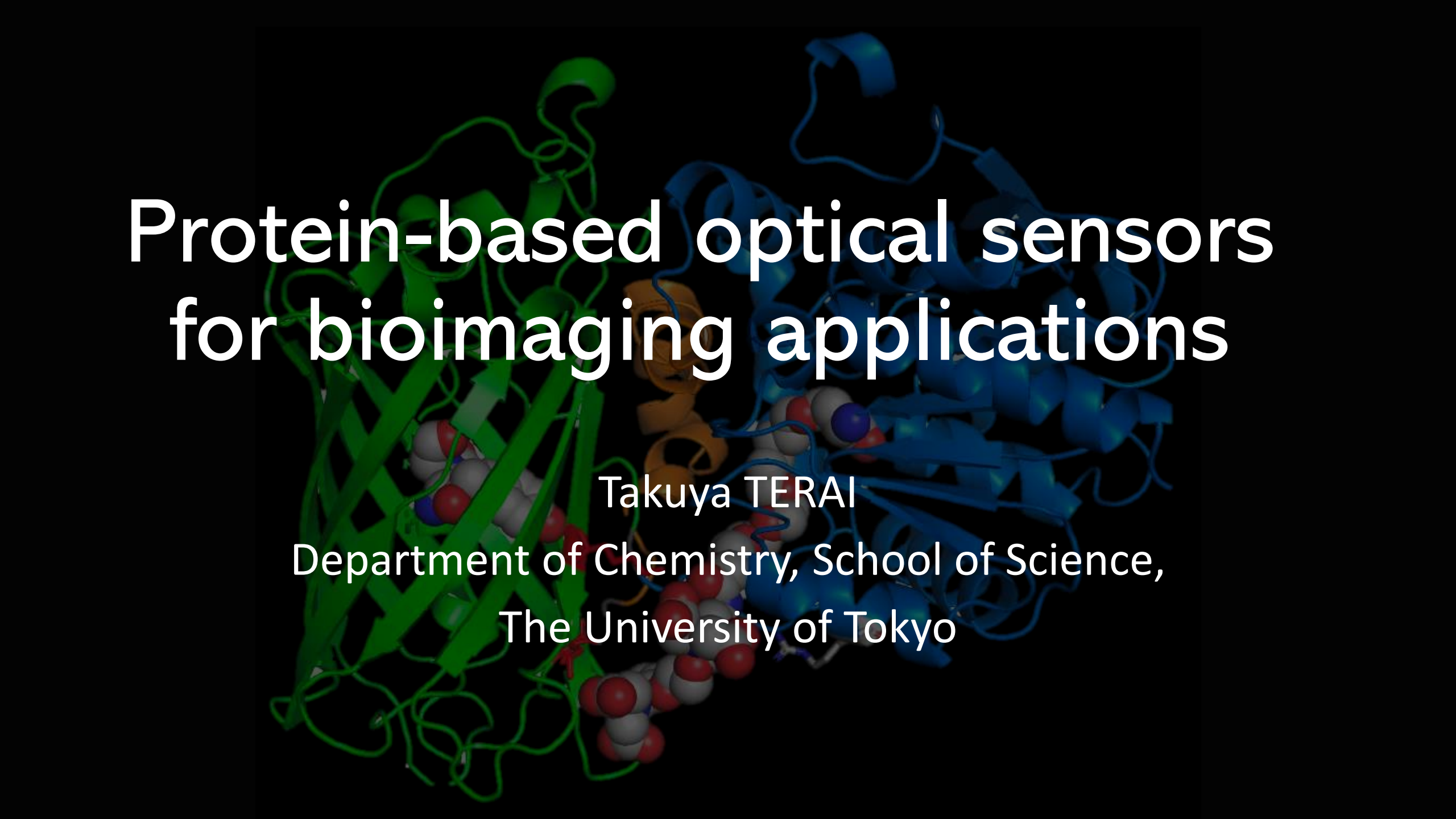


Protein-based optical sensors for bioimaging applications

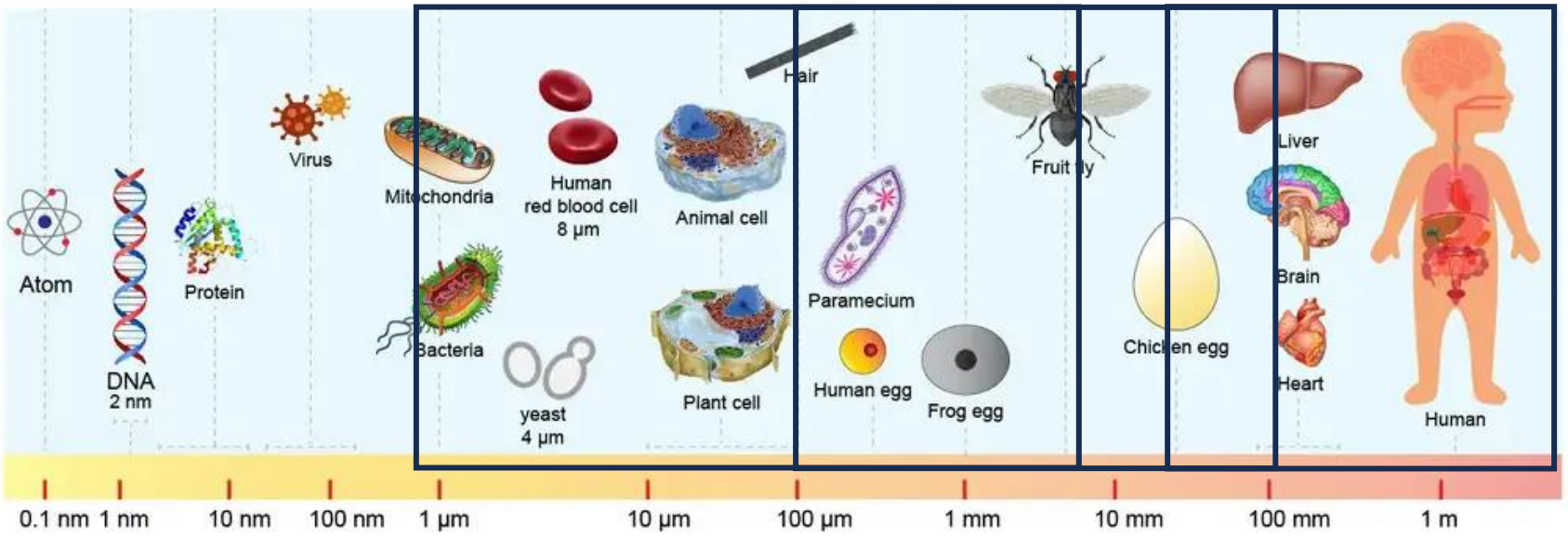
Takuya TERAI

Department of Chemistry, School of Science,

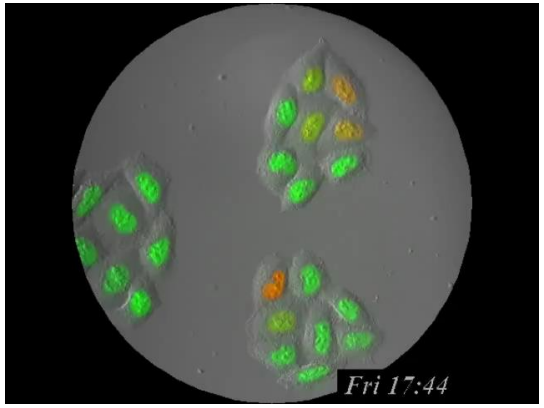
The University of Tokyo

Bio(medical) imaging

What we want to see in biology

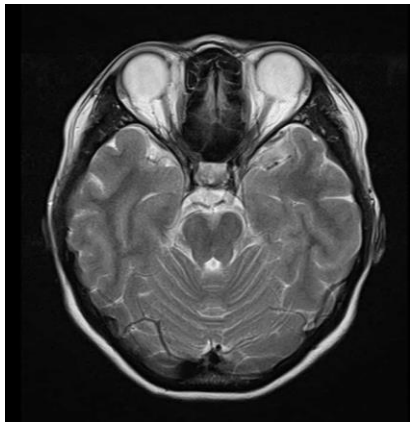


Fluorescence (cell)



Bioluminescence (mouse)

MRI (human brain)



https://rsscience.com/scale-of-biology-cell-size/: Cell, 2008, 132, 487-498; Lab Animal, 52, s247–257 (2023); https://www.melbourneradiology.com.au/diagnostic-imaging/mri-scan-brain/

Three components for bioimaging

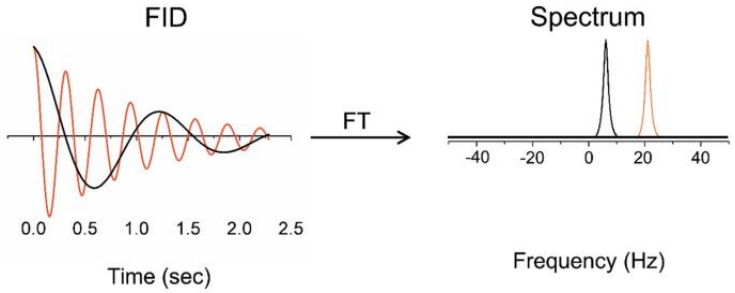
Fluorescence microscope

MRI@ETHZ

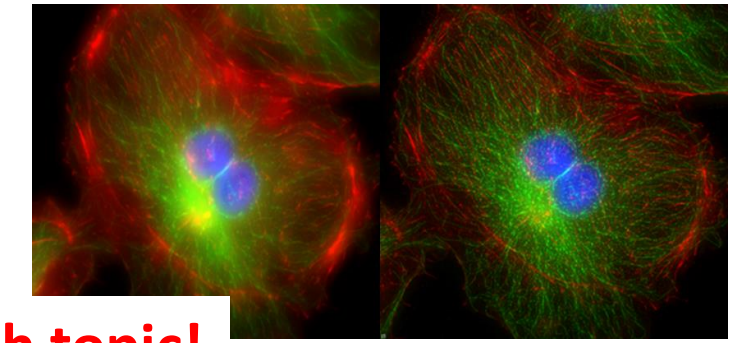
Hardware (machine)

Software (data analysis)

Wetware (molecules)

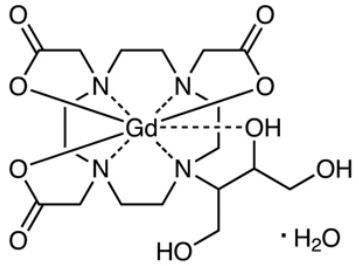


Fourier transformation of MR signal

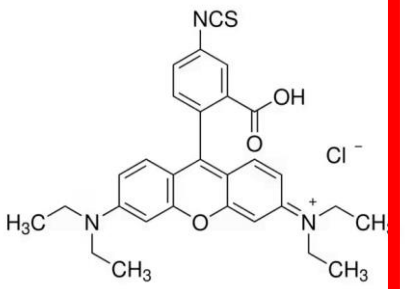


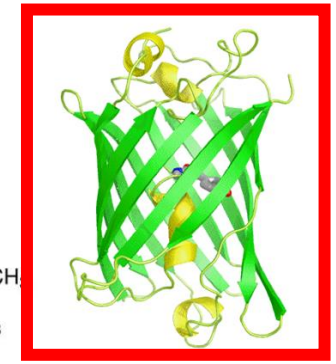
on of fluorescence images

Our main research topic!



Gd^{3+} contrast agent (for MRI)



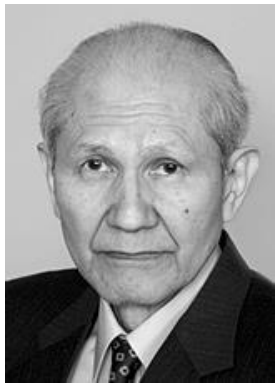


Fluorescent molecules/proteins (for fluorescence imaging)

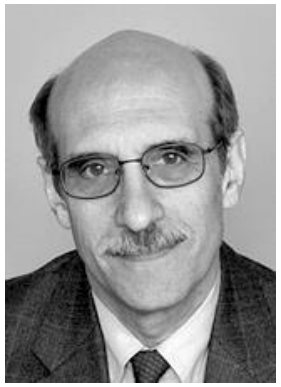
https://www.olympus-lifescience.com/ja/microscopes/inverted/ix83/; A Graduate Course in NMR Spectroscopy (Springer); Biology Imaging core website at Washington University at Saint Louis

Discovery and application of GFP

In 2008, the Nobel prize in Chemistry was awarded to the three scientists for “the discovery and development of the green fluorescent protein, GFP”

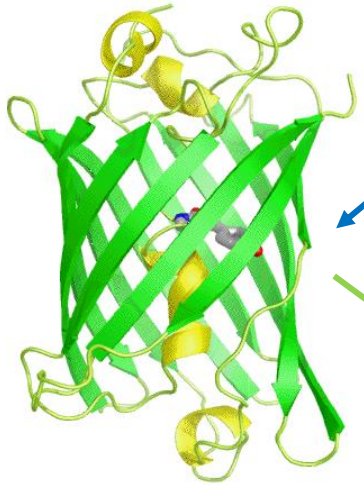


Osamu Shimomura



Martin Chalfie

Roger Y. Tsien



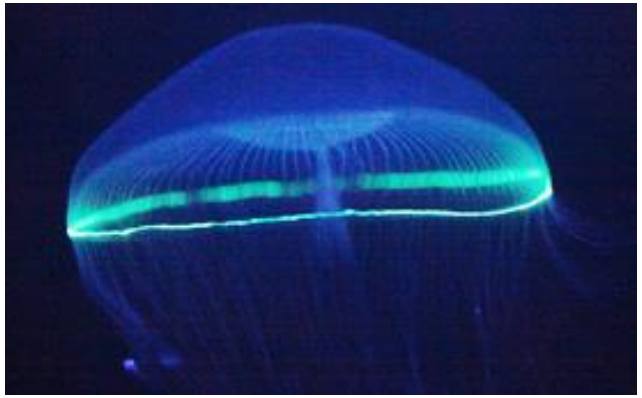
Excitation light

Ex. 395 nm (wt GFP)

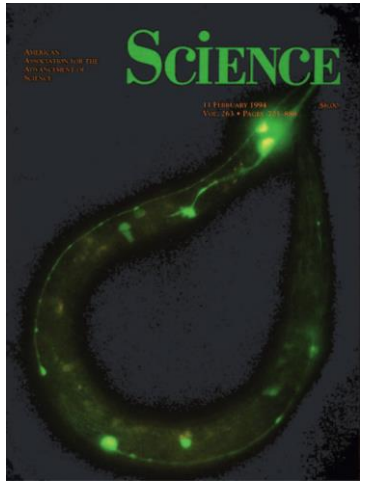
Fluorescent light (emission)

Em. 509 nm

GFP (PDB 1EMA)

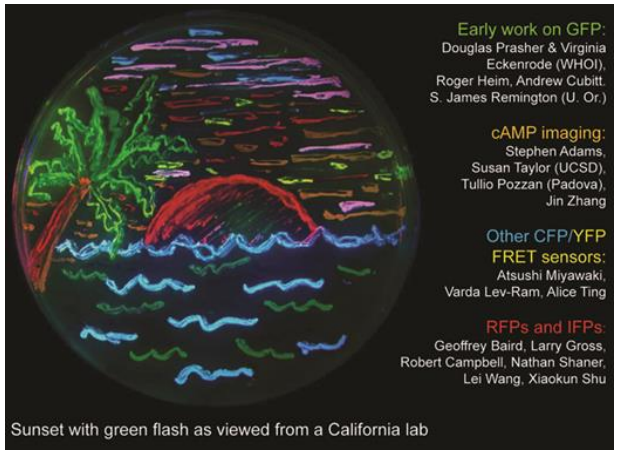


Fluorescent jerry fish
(*Aequorea victoria)*



Science, 263, 802 (1994)

C. elegans expressing GFP

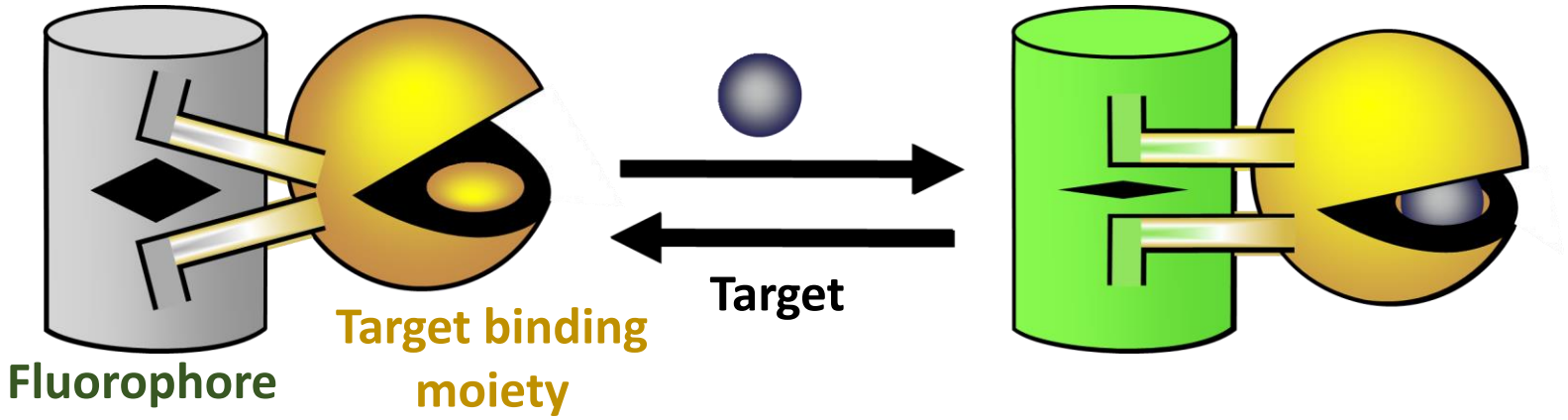


A palette of fluorescent proteins

https://www.nobelprize.org/prizes/chemistry/2008/; *Nature* **440**, 280 (2006); *Angew. Chem. Int. Ed.* 2009, 48, 5612; https://global.canon/ja/technology/kids/mystery/m_01_08.html

Fluorescent sensors for bioimaging

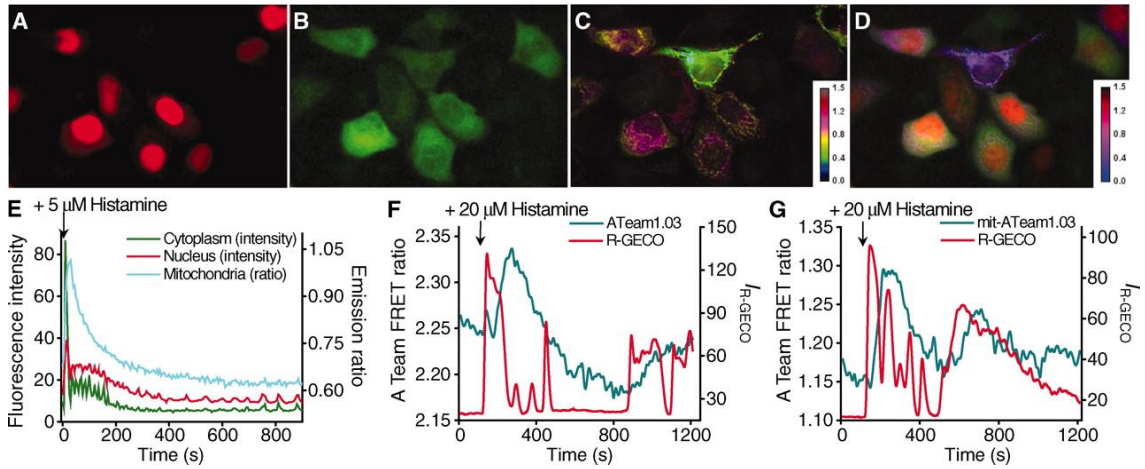
Fluorescent probe (biosensor, indicator)



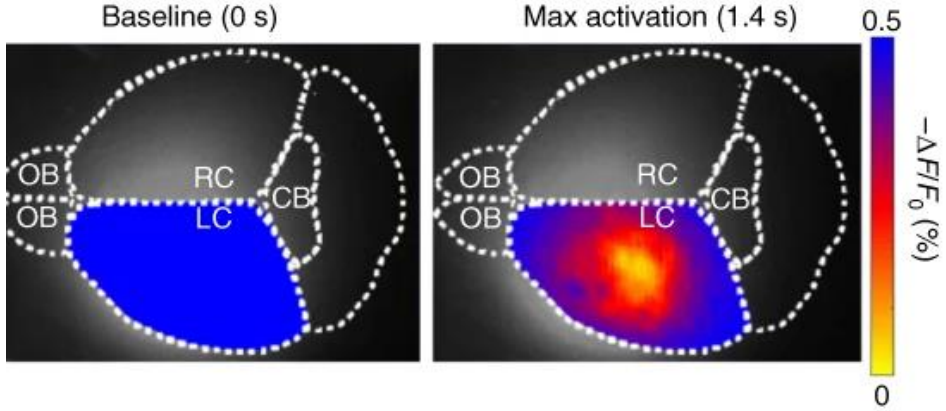
A molecule (synthetic, protein, or others) that reacts with the analyte and changes its fluorescence properties (intensity, wavelength).

Ca^{2+} imaging in cells/organisms

B/G/R-GECO1

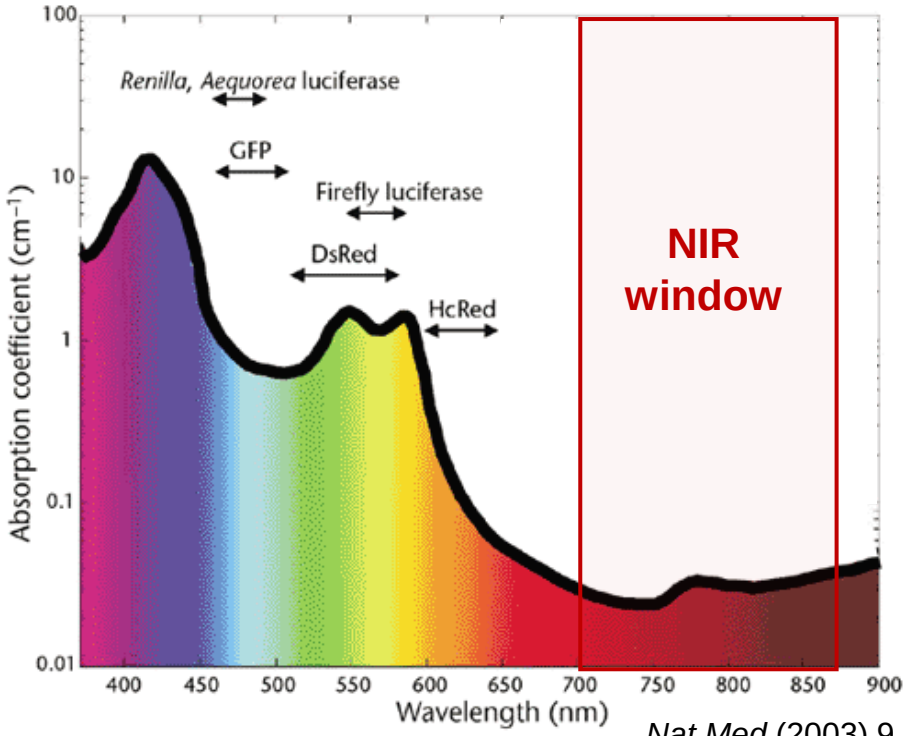


NIR-GECO1

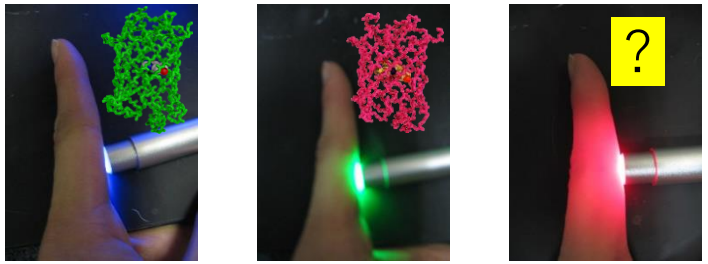


Science, **2011,** 333, 1888; *Nat. Methods,* **2019,** 16, 171.

Near infrared (NIR) Ca^{2+} sensors

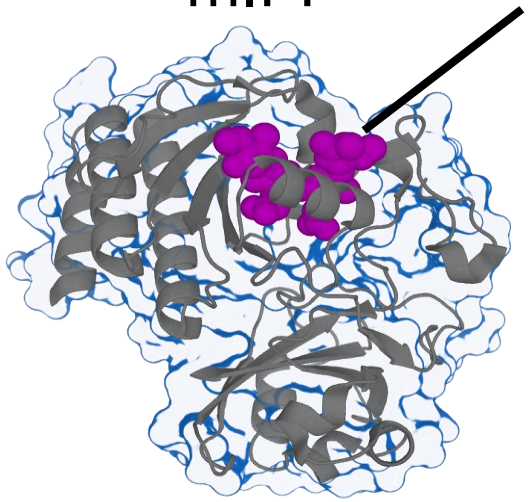


Nat Med (2003) 9, 123



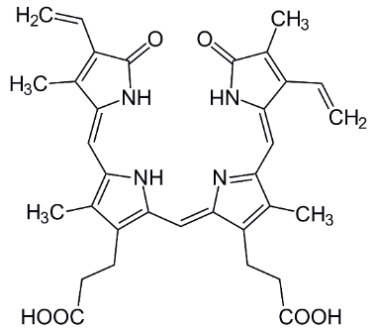
Short WL (blue) → **Long WL (red)**

mIFP



ε = 82,000 $M^{-1}cm^{-1}$; ϕ = 8%
Ex 683 nm; Em 704 nm

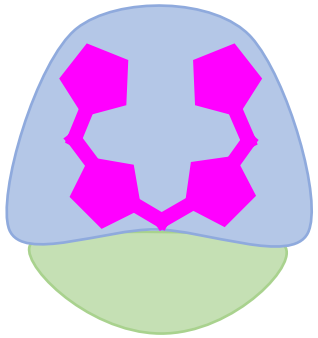
Biliverdin (BV)



Nat Methods, **2015,** 12, 763

- Ubiquitous in mammalian tissues
- An intermediate in *heme* catabolism

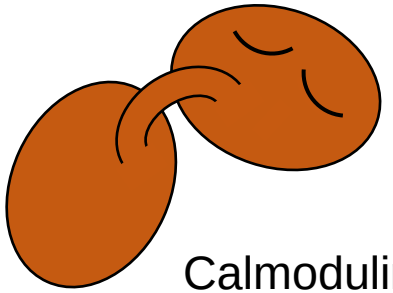
||



Fluorophore

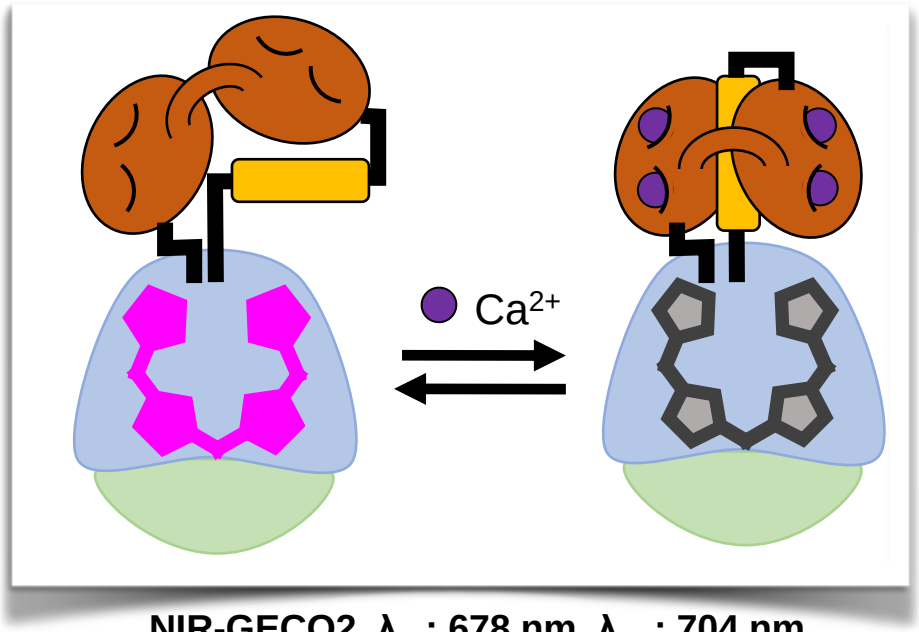
+

Target binding moiety



+ RS20

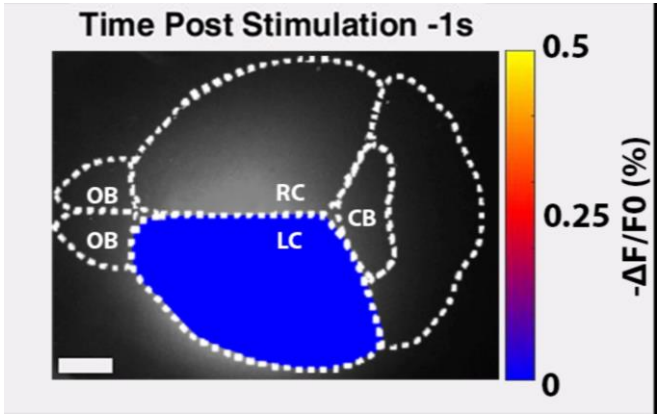
Development of NIR-GECO1/2



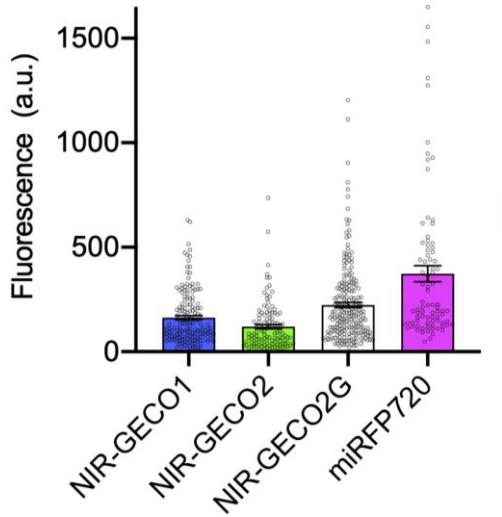
NIR-GECO2 λ_{ex}: 678 nm, λ_{em}: 704 nm

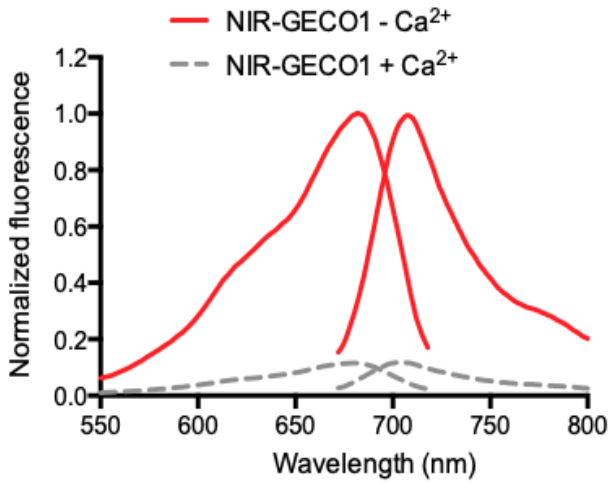
Ca^{2+} binding domain

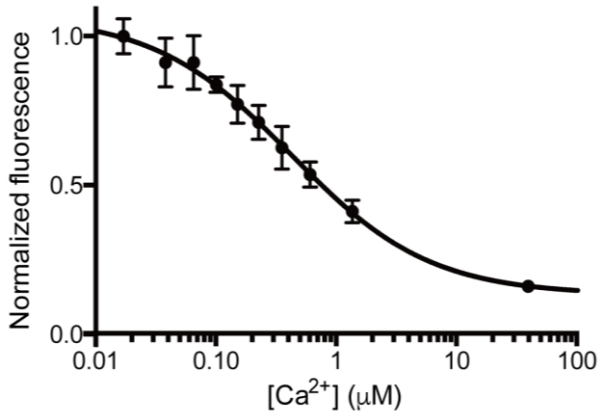
NIR-GECO1 in mouse brain



Brightness of NIR-GECOs







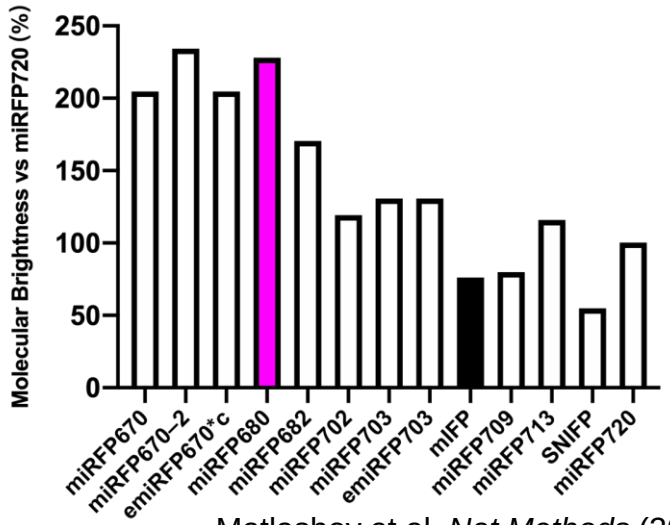
The sensor was not bright because

1. **mIFP is dim itself.**
2. **Affinity to BV needs improvement.**

Nat. Methods, **2019,** 19, 171 (NIR-GECO1); *PLoS Biol.*, **2020,** 18, e3000965 (NIR-GECO2)

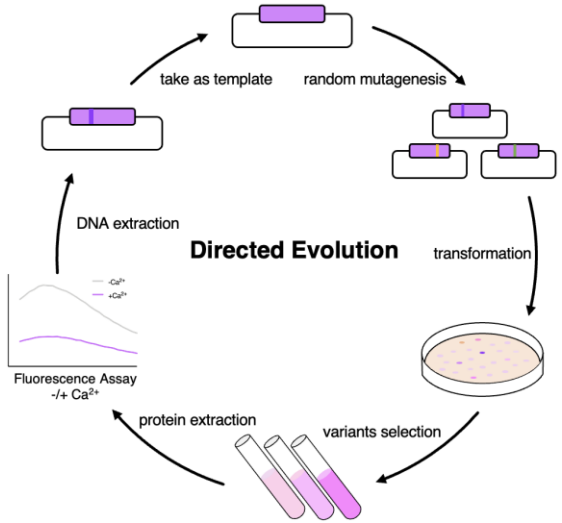
Development of NIR-GECO3

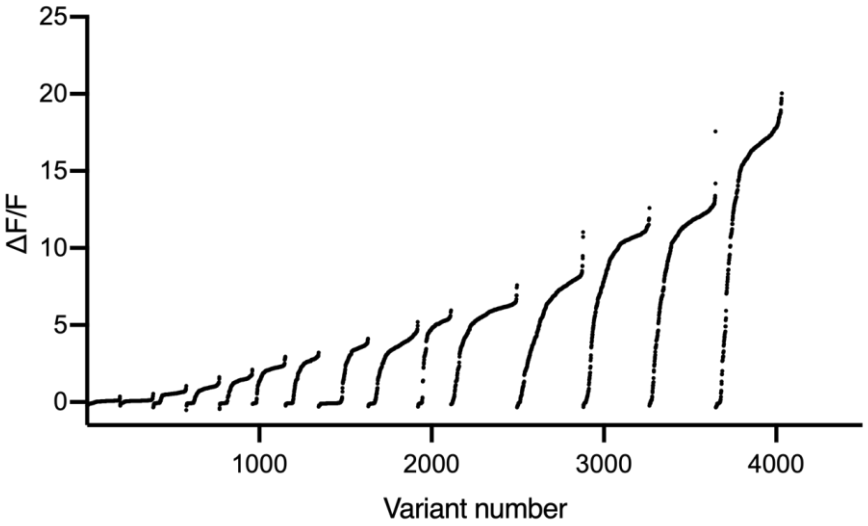
1. Use of brighter FP as a scaffold



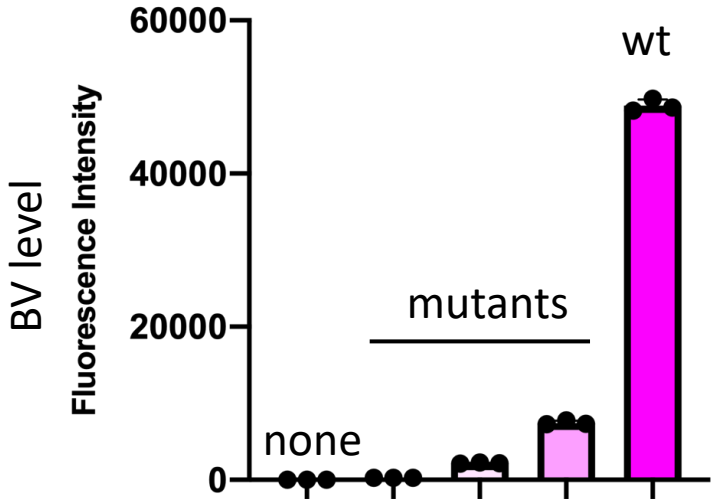
Matlashov et al. *Nat Methods* (2020) 11, 239

2. Directed evolution

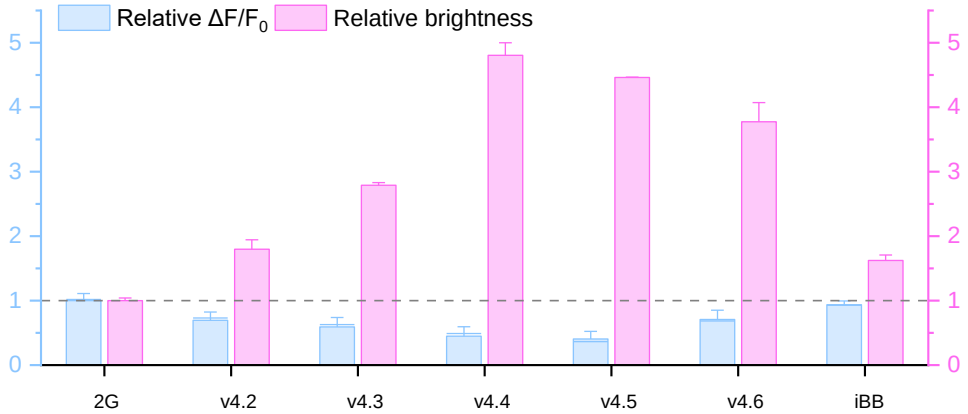




3. Screening with HO-1 mutant



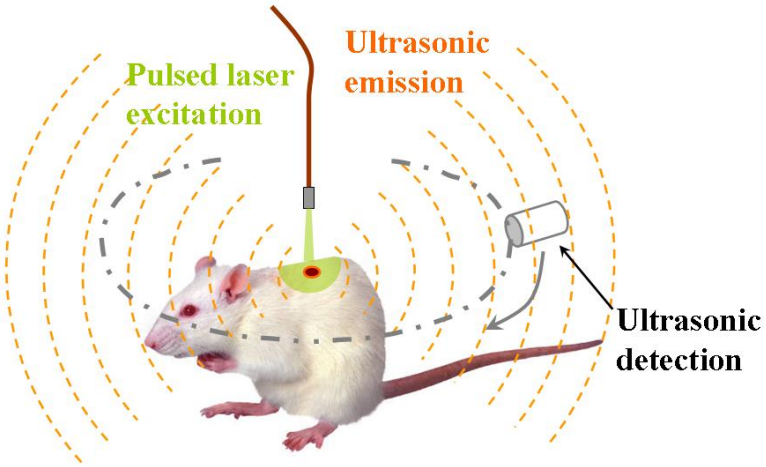
4. Evaluation in mammalian cells



With Prof. Pen Zou
(Pekin University)

Optoacoustic imaging of Ca^{2+}

Optoacoustic imaging

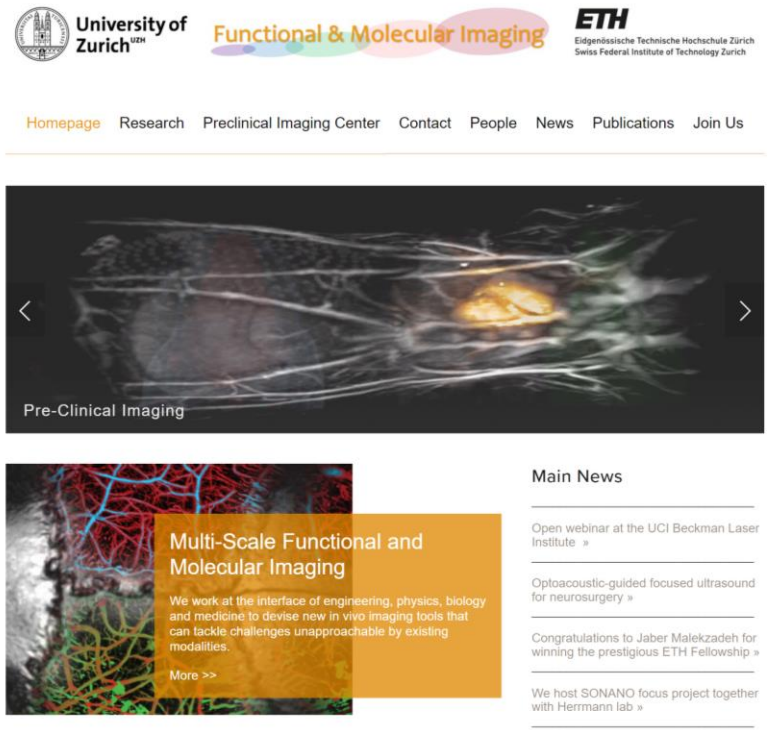


Excitation of light-absorbing molecules

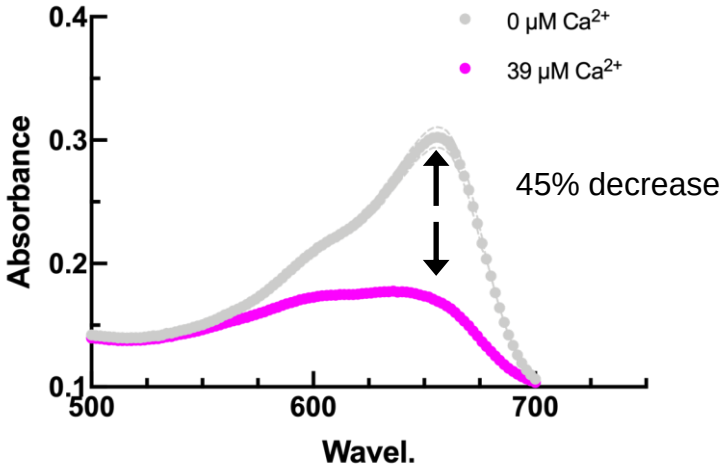
↓

Energy is converted to ultrasound wave

Razansky Lab @ETHZ/UZH



Absorbance change of NIR-GECO



↓

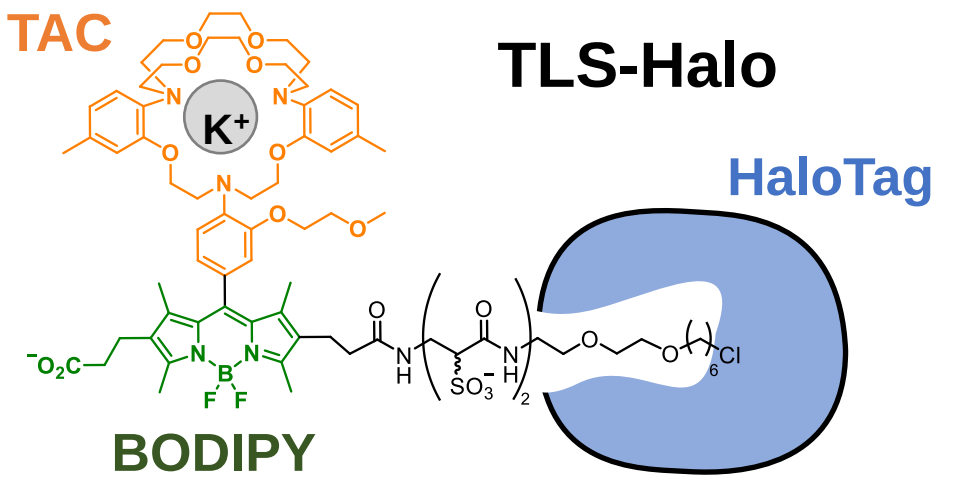
Change of optoacoustic signal?

For optoacoustic imaging, the sensor should be non-fluorescent but change its absorbance, in response to the target. We are derivatizing NIR-GECO for such purpose, in collaboration with the Razansky lab.

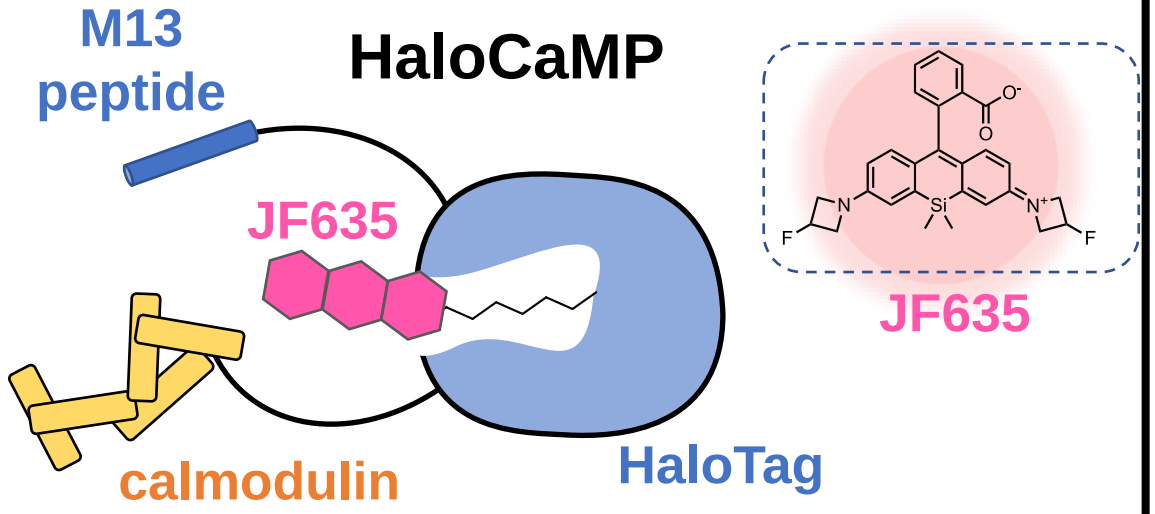
https://en.wikipedia.org/wiki/Photoacoustic_imaging; https://www.razanskylab.org/

Chemigenetic sensors

Chemigenetic = chemical compound + genetically encoded protein



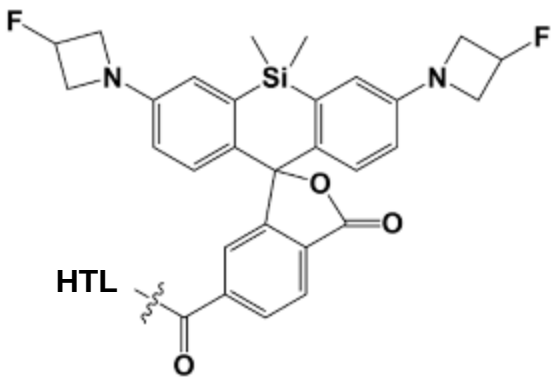
Anal. Chem., **2016**, 88, 2693



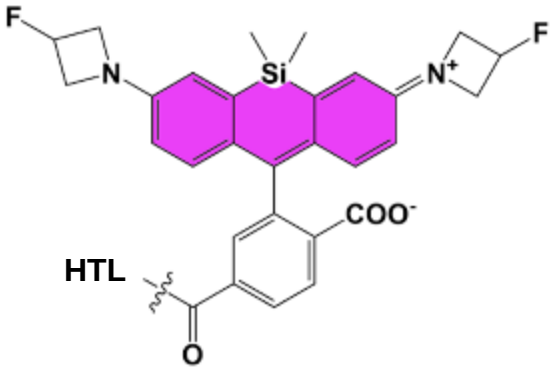
Nat. Chem. Biol., **2021**, 17, 718

Synthetic molecule	**Binder**	**Protein**
Synthetic molecule	**Fluorophore**	**Synthetic molecule**
Protein	**Scaffold**	**Protein**
Selective localization	**Pros**	**NIR fluorescence**
Tedious synthesis **No directed evolution**	**Cons**	**Limited targets** **Incomplete optimization**

Chemigenetic K^+ sensor



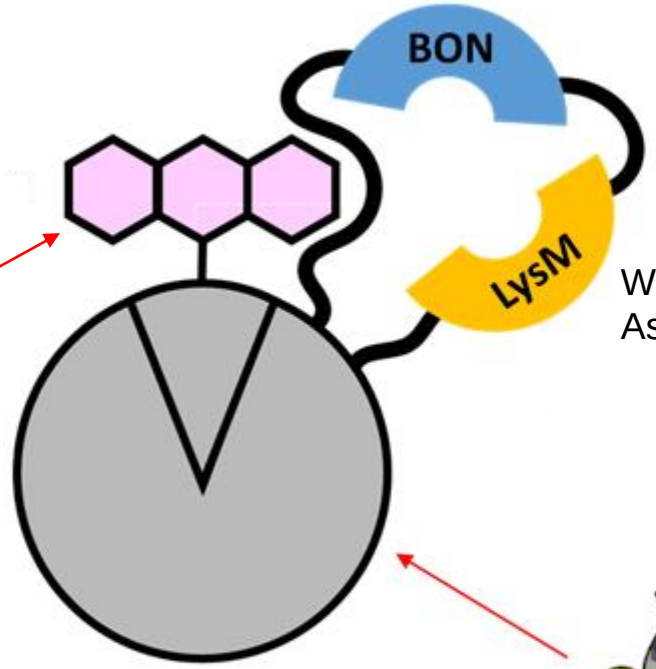
Dim state (Lactone)



Bright state (Zwitterion)

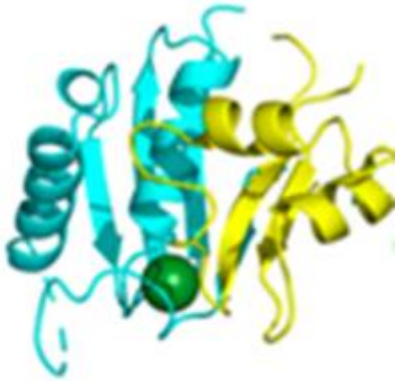
Grimm, J. B., *et al*, *Nat. Methods*, **2017**, *14*, 987-994.

Janelia Fluor (JF_{635}) **Dye** as the **fluorophore** (+ HTL= HaloTag ligand)

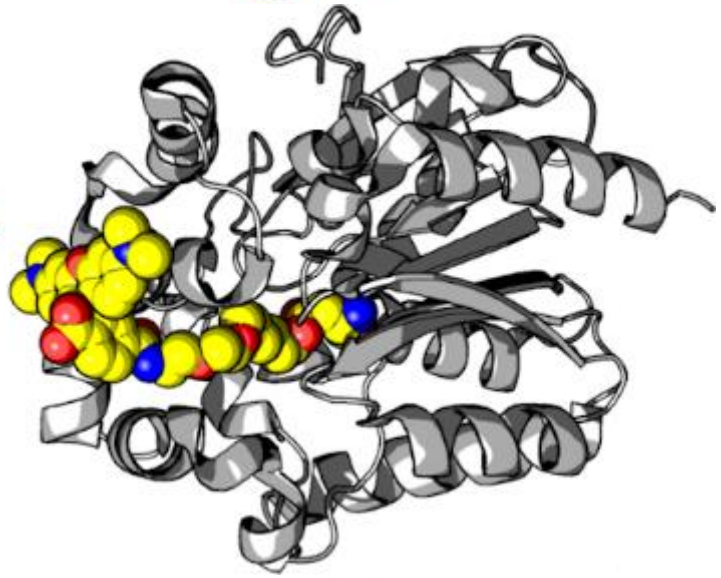


Split HaloTag **Protein** as the scaffold

Kbp **Protein** as the **receptor**



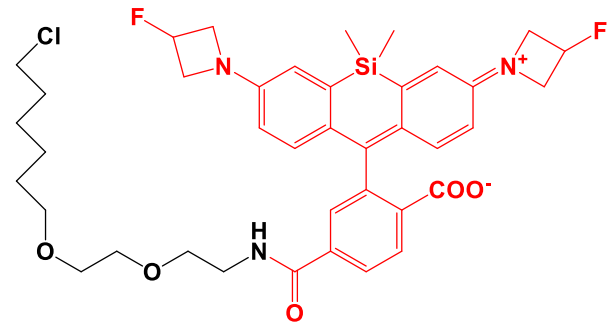
Wu, S. Y., *et al*, *PLoS Biology*, **2022**, *20,* e3001772.
Ashraf, K. U., *et al, Structure*, **2016**, *24*, 741–749.



G. V. Los, *et al, ACS Chem. Biol.,* **2008**, *3*, 373-382.

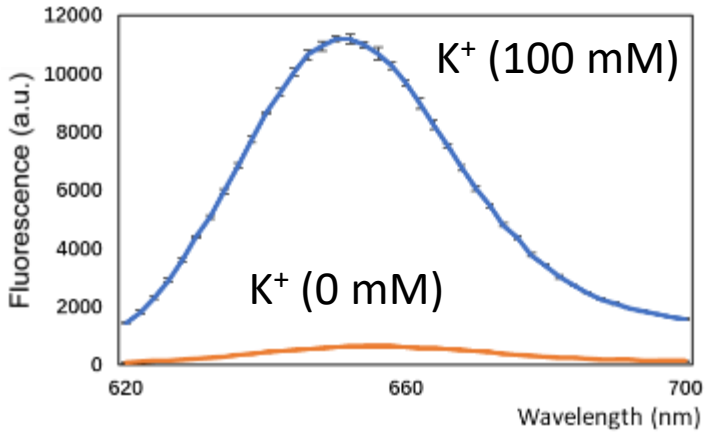
Performance of the sensor

In vitro data (JF_{635})	Halo-Kbp4.2	HaloTag only
K_d (mM)	35.9±5.4	
$\Delta F/F_{0max}$	16.39	-0.02
Quantum Yield [K^+(100 mM)/K^+(-)]	0.72/0.52	0.63/0.64

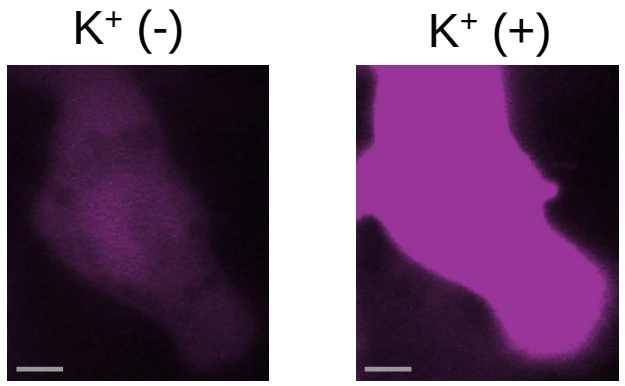


JF_{635} - HTL

Emission spectra

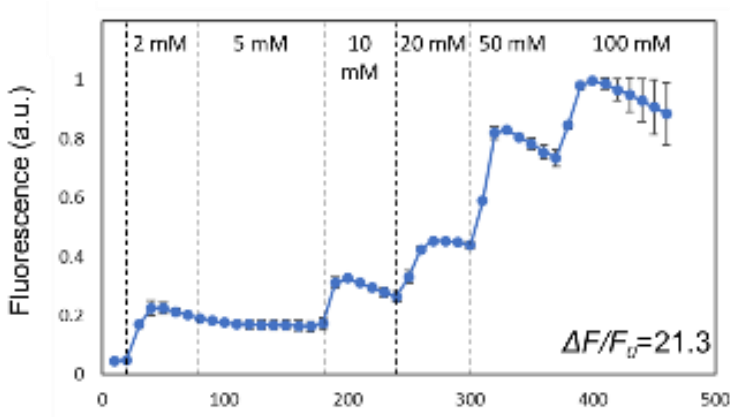


HeLa cell imaging



(digitonin permeabilized)

Timecourse of cell fluorescence



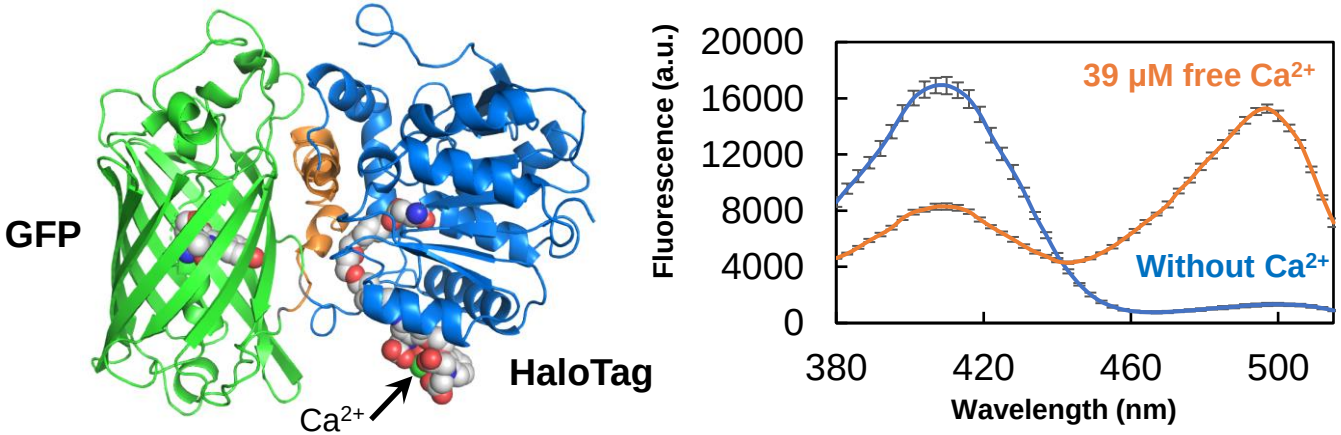
After directed evolution and protein engineering, the sensor showed >15 –fold fluorescence change in response to K^+, and it worked nicely in the cytosol of cells.

Summary

- In bio(medical) imaging, we often need molecular sensors (wetware).
- Fluorescent sensors based on proteins are useful tools to visualize targets in living cells or experimental animals.
- Directed evolution is a powerful technique to develop high-performance sensors.
- Chemigenetic approach is promising to create new interesting sensors.

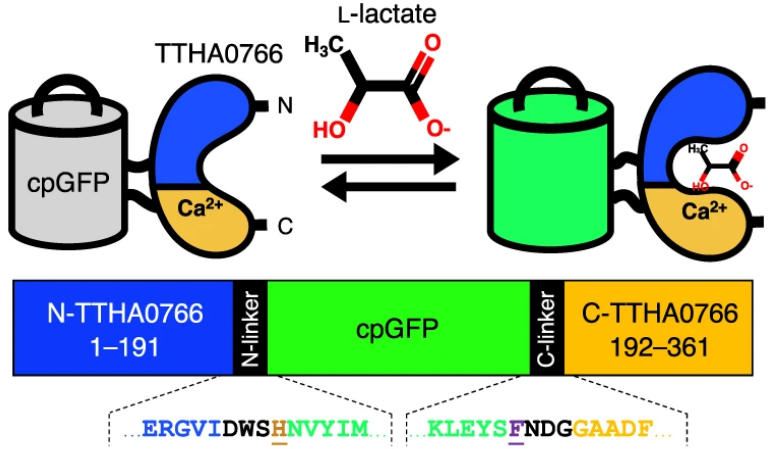
Other recent work in our lab

Another type of chemigenetic sensors



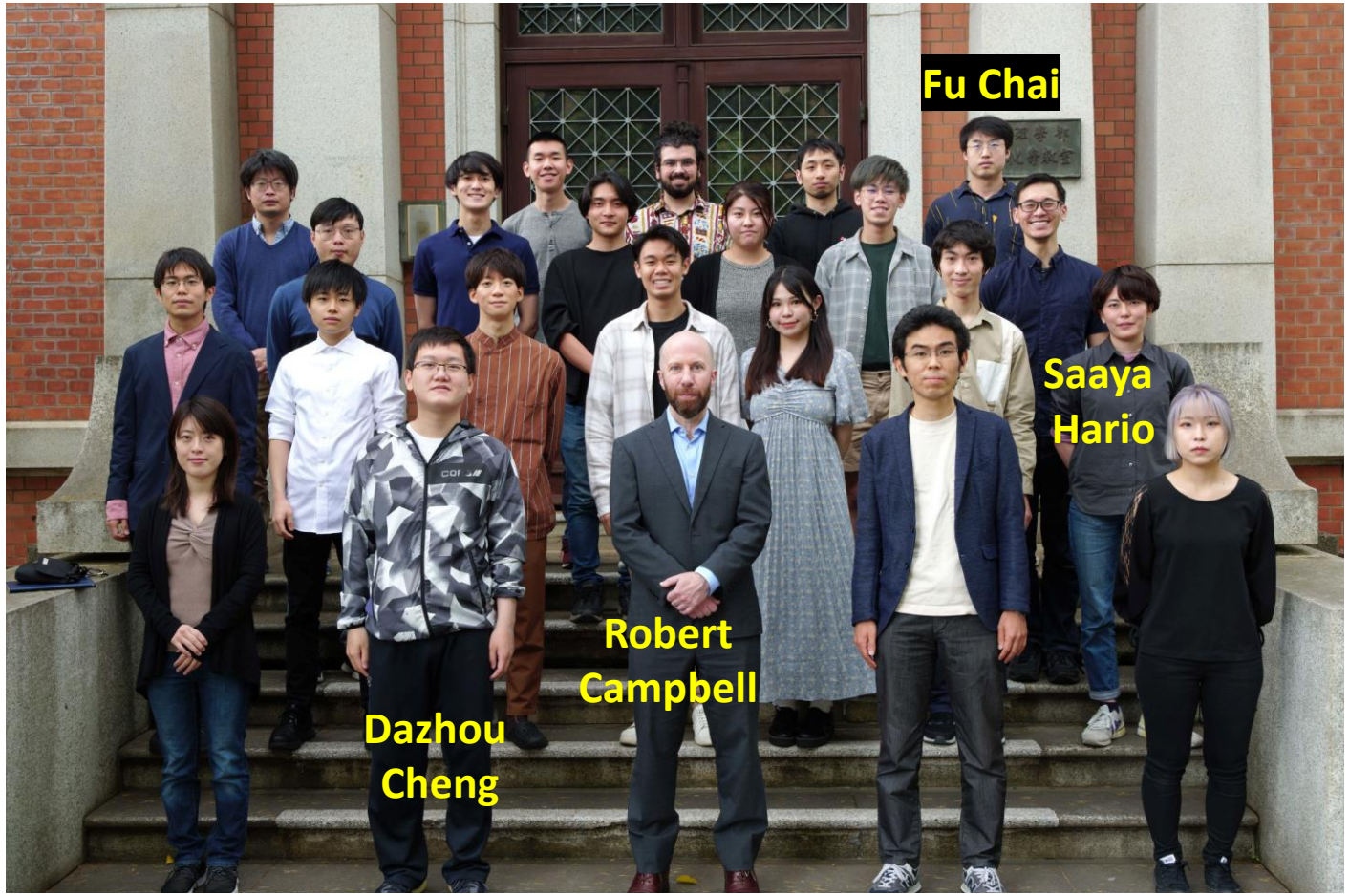
Nat. Chem. Biol., **2023**, 19, 38

Metabolite biosensors



Nat. Commun., **2021**, 12, 7058

Acknowledgements



ETHZ-UTokyo Strategic Partnership

JSPS

NIH National Institutes of Health
Turning Discovery Into Health

HFSP International Human Frontier Science Program Organization

公益財団法人 中谷医工計測技術振興財団
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