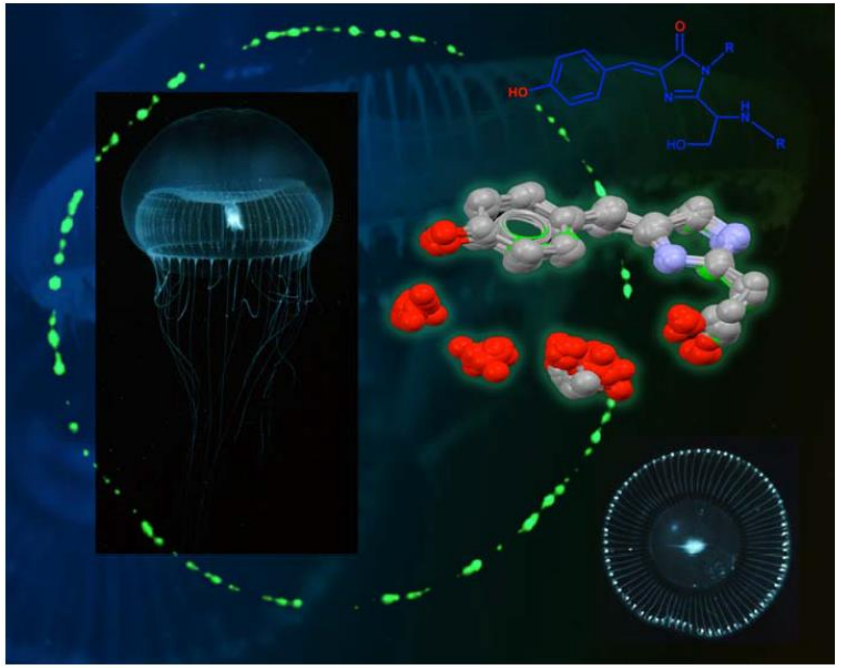
# •Fluorescent Protein

- 1.Photochemistry in FPS
- 2.Super resolution with FPS
- 3.Sensors based on FPS---2016.12.14
- 4.Red FPS
- 5.Other FPS



Reporter: Qinglong Qiao
Date: 2020.10.22

nature chemical biology

ARTICLES

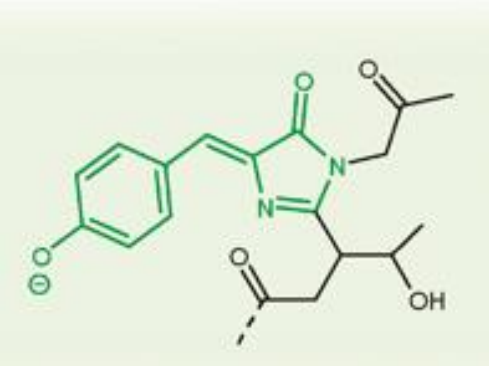
https://doi.org/10.1038/s41589-020-0641-7

Check for updates

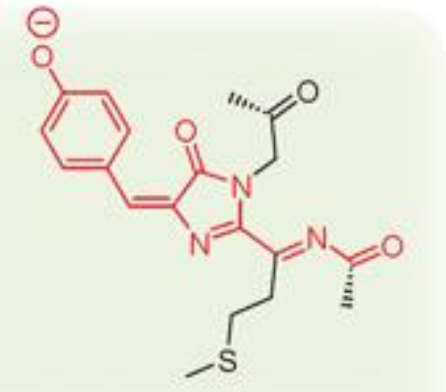
# A general strategy to red-shift green fluorescent protein-based biosensors

Shen Zhang and Hui-wang Ai

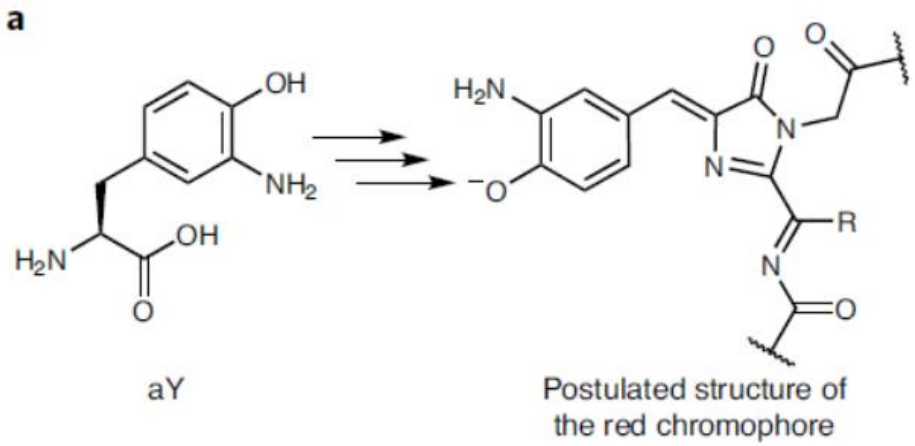
EGFP
484 nm; 507 nm



mKate
588 nm; 635 nm



This Work



# Fluorescent proteins and fluorophores

下村修
- 发现并提纯水母素
- 发现GFP

Douglas Prasher
- 克隆并测序水母素基因
- 克隆并测序GFP基因

GFP基因赠予

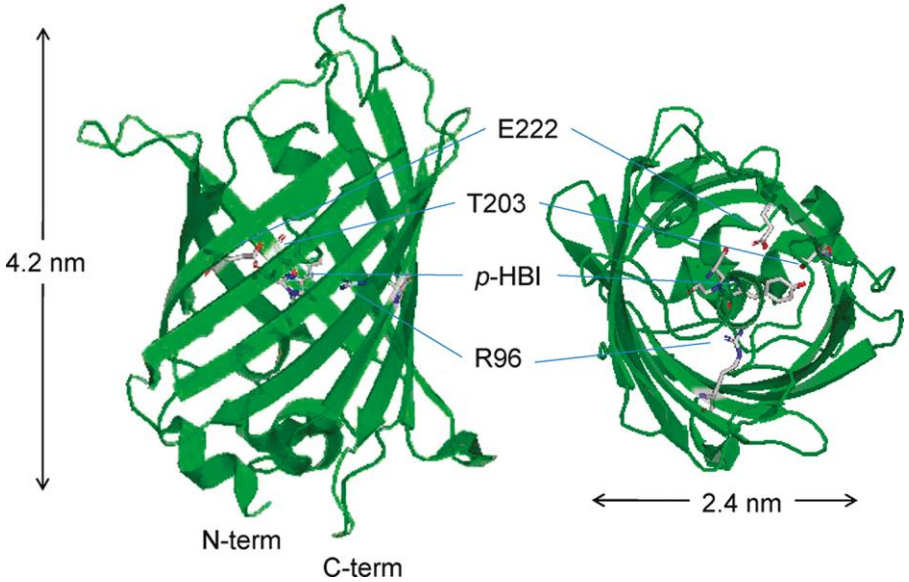
GFP基因赠予

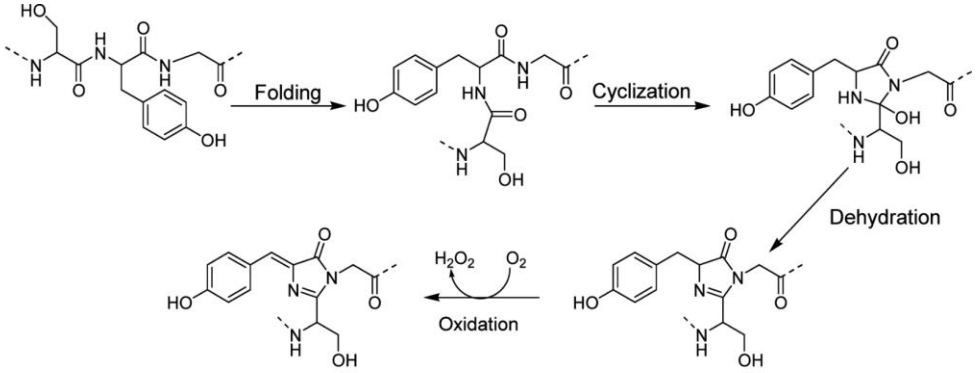
Martin Chalfie
- 首次用GFP在线虫里监测基因表达和蛋白定位

Ghia Euskirchen
- Chalfie的研究生
- 首次在细菌里表达发绿荧光的GFP

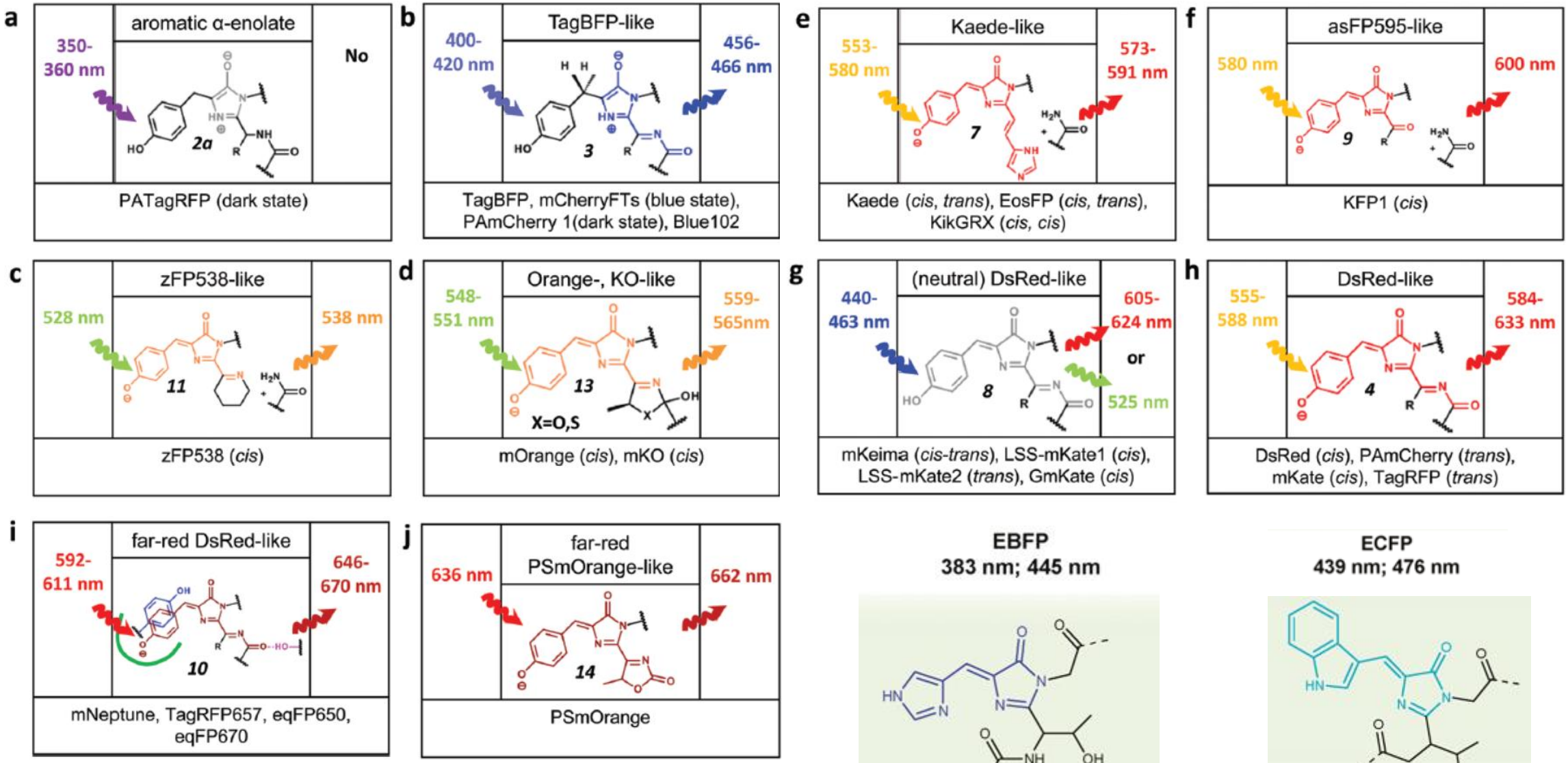
钱永健
- 在酵母中表达GFP
- 研制出加强版的GFP和其它颜色的荧光蛋白

Roger Heim
- 钱永健的博士后
- 做具体的GFP改造工作



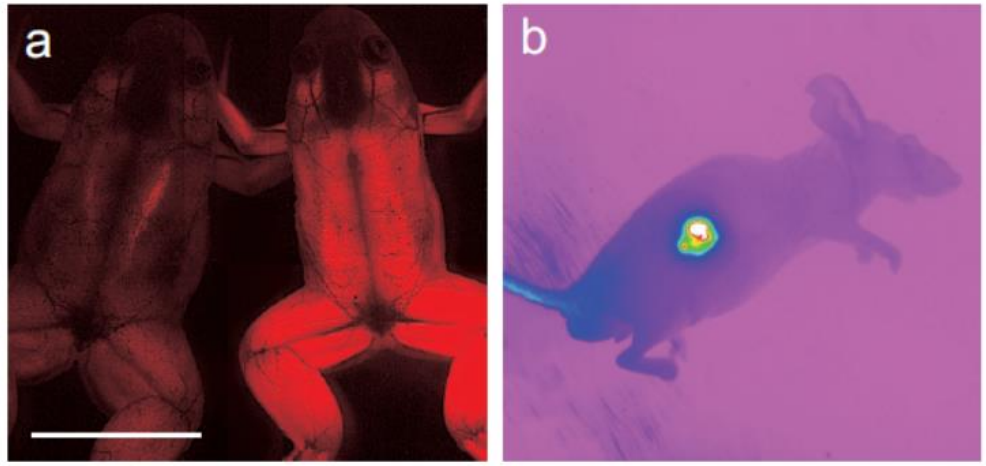



# Beyond the rainbow



# Beyond the rainbow: new fluorescent proteins brighten the infrared scene

1. reduced phototoxicity
2. decreased autofluorescence
3. enhanced tissue penetration

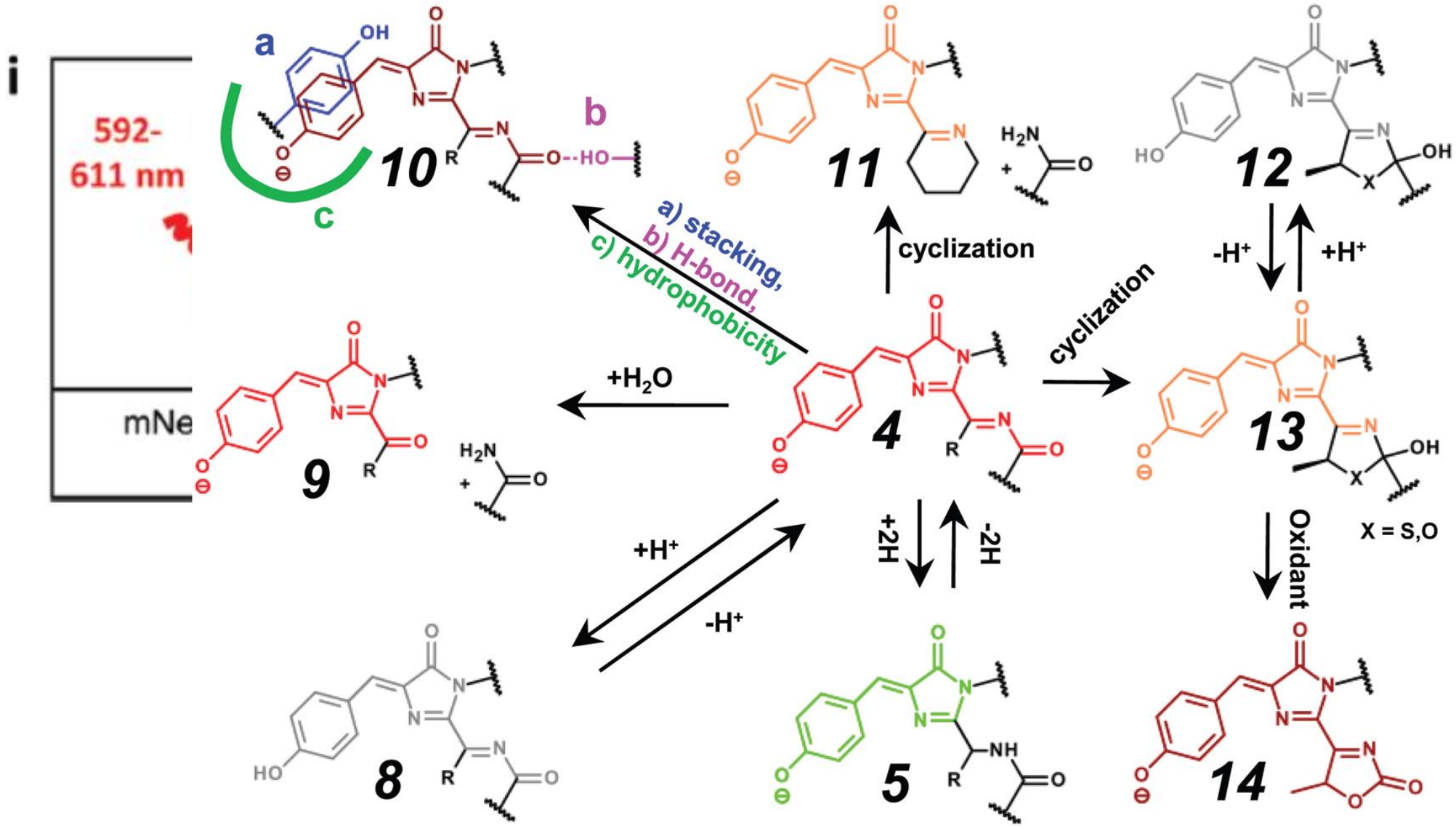



1. small dynamic ranges
2. mislocalization
3. undesired photoconversion

**Table 1** | Key characteristics of far-red fluorescent proteins

| | mCherry | Katushka | RFP639 | E2-Crimson | mNeptune |
|---|---|---|---|---|---|
| Excitation peak (nm) | 587 | 588 | 588 | 605 | 599 |
| Emission peak (nm) | 610 | 635 | 639 | 646 | 649 |
| Fluorescence quantum yield | 0.22 | 0.34 | 0.18 | 0.12 | 0.18 |
| Molar extinction coefficient ($M^{-1}$ $cm^{-1}$) at excitation maximum | 72,000 | 65,000 | 69,000 | 58,500 | 57,500 |
| Brightness[a] (a.u.) | 15,840 | 22,100 | 12,420 | 7,080 | 10,350 |
| Molar extinction coefficient ($M^{-1}$ $cm^{-1}$) at 635 nm | 1,000 | 1,700 | ~2,000 | 12,640 | 7,900 |
| Quantum yield | 0.04 | 0.07 | ~0.05 | 0.03 | 0.05 |

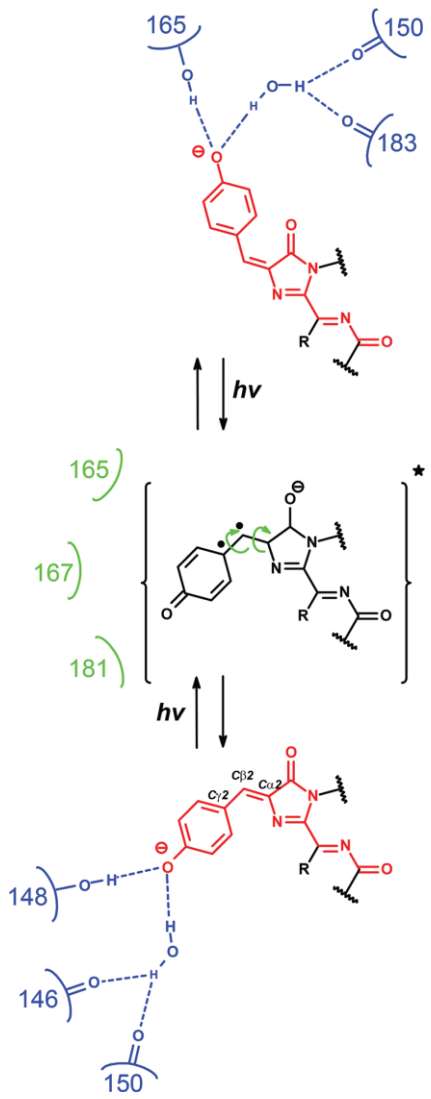
# DsRed and Derived fluorescent protein



# DsRed

*Nat. Methods* 7, 821–830 (2010)

*PNAS* 115, 11294-11301 (2018)



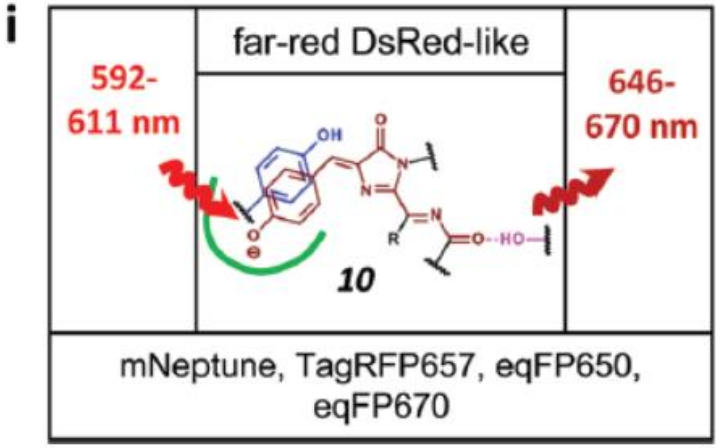
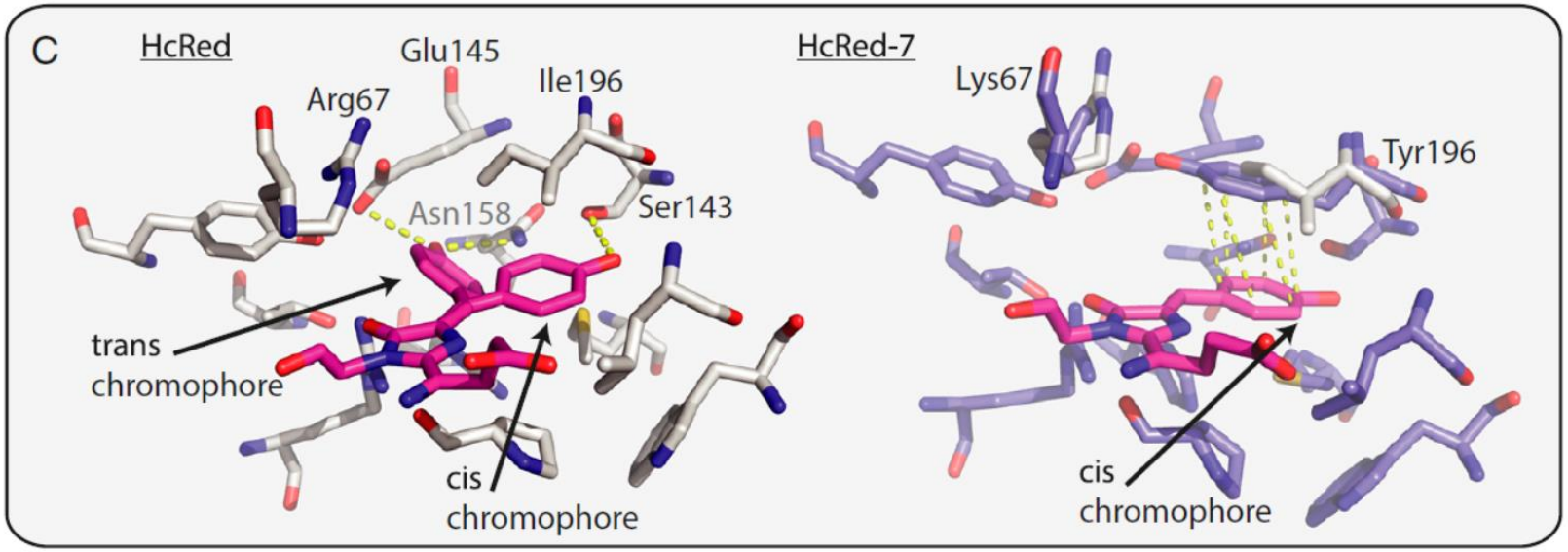




**Table 1** | Key characteristics of far-red fluorescent proteins

| | mCherry | Katushka | RFP639 | E2-Crimson | mNeptune | eqFP650 | eqFP670 |
|---|---|---|---|---|---|---|---|
| Excitation peak (nm) | 587 | 588 | 588 | 605 | 599 | 592 | 605 |
| Emission peak (nm) | 610 | 635 | 639 | 646 | 649 | 650 | 670 |
| Fluorescence quantum yield | 0.22 | 0.34 | 0.18 | 0.12 | 0.18 | 0.24 | 0.06 |
| Molar extinction coefficient ($M^{-1}$ $cm^{-1}$) at excitation maximum | 72,000 | 65,000 | 69,000 | 58,500 | 57,500 | 65,000 | 70,000 |
| Brightness[a] (a.u.) | 15,840 | 22,100 | 12,420 | 7,080 | 10,350 | 15,600 | 4,200 |
| Molar extinction coefficient ($M^{-1}$ $cm^{-1}$) at 635 nm | 1,000 | 1,700 | ~2,000 | 12,640 | 7,900 | 4,300 | 15,700 |
| Quantum yield in infrared (700–900 nm) | 0.04 | 0.07 | ~0.05 | 0.03 | 0.05 | 0.07 | 0.03 |
| Brightness in infrared[b] (a.u.) | 40 | 119 | ~100 | 379 | 395 | 301 | 471 |
| Photostability, widefield[c] (s) | 601 | ND | ND | 19 | 216 | 190 | 1,289 |
| Photostability, confocal[c] (s) | 48 | 77 | ND | 14 | 29 | 67 | >700 |
| pKa | 4.5 | 5.5 | ND | ND | 5.8 | 5.7 | 4.5 |
| Reference | 9 | 2 | 10 | 8 | 6 | This work | This work |



# Dipyrromethenes in our lab

## A far-red fluorescent protein evolved from a cyanobacterial phycobiliprotein

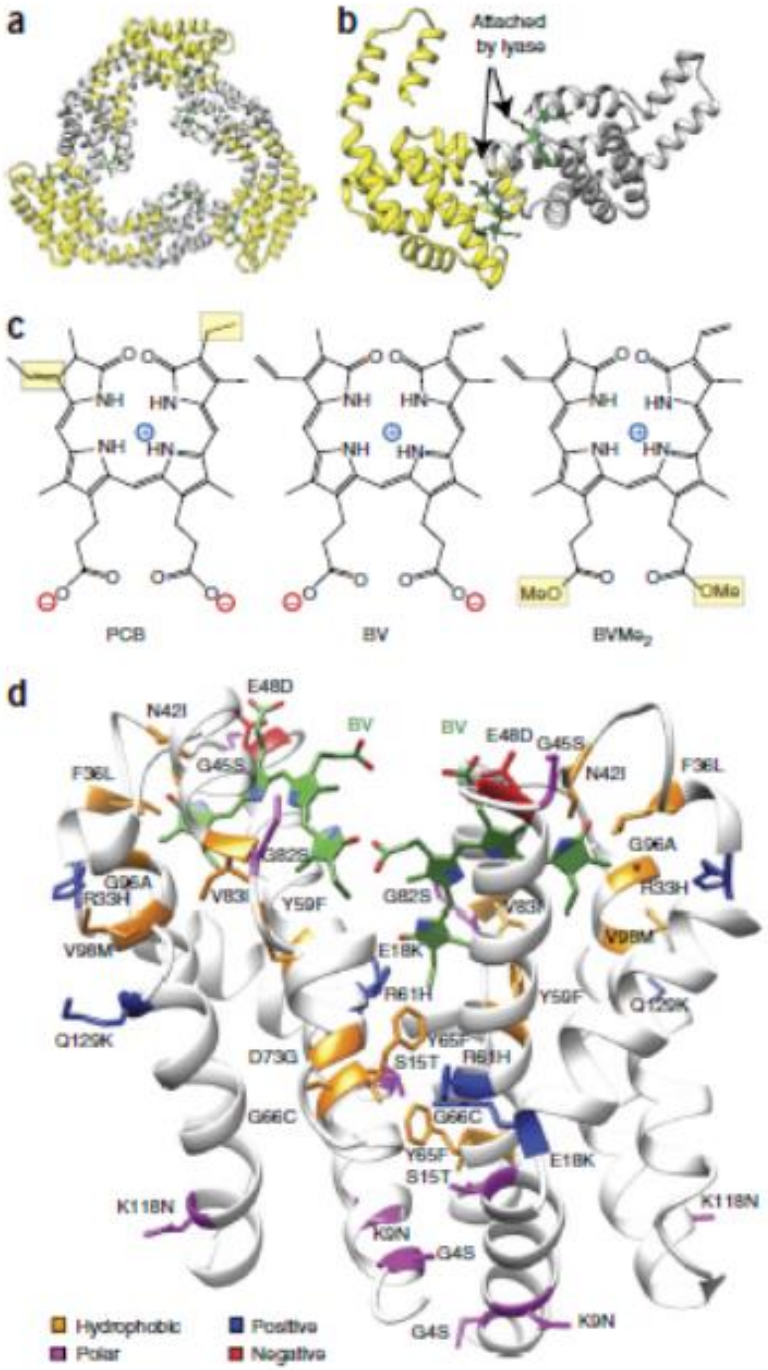
Erik A Rodriguez[1,7], Geraldine N Tran[2,7], Larry A Gross[3], Jessica L Crisp[1], Xiaokun Shu[4,5], John Y Lin[6] & Roger Y Tsien[1,3]

**Table 1** | Biophysical properties of FPs and Cy5

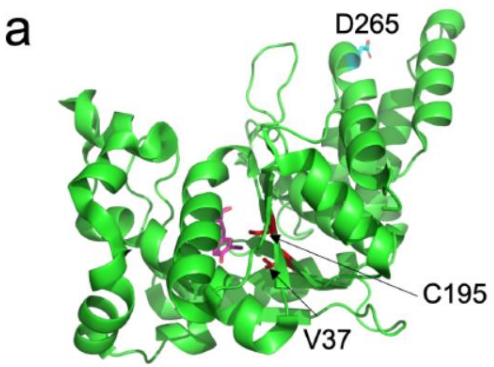
| Fluorescent molecule | Excitation maximum (nm) | Emission maximum (nm) | Extinction coefficient ($M^{-1}cm^{-1}$/ chromophore) | Quantum yield (%) | *In vitro* photostability $t_{50\%}$ (s)[a] | Mammalian cell photostability $t_{50\%}$ (s)[a] | Stoichiometry | Chromophore maturation or attachment $t_{50\%}$ (min) | Protein stability $t_{50\%}$ (h)[b] | Molecular brightness relative to eGFP (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| eGFP | 488[c] | 507[c] | 56,000[c] | 60[c] | 110[c] | 560 | Monomer | 25[c] | 21 | 100 |
| mCherry | 587[c] | 610[c] | 72,000[c] | 22[c] | 96[c] | 89 | Monomer | 15[c] | | 47 |
| mCardinal | 604[d] | 659[d] | 87,000[d] | 19[d] | 730[d] | | Monomer | 27[d] | | 49 |
| smURFP + BV | 642 | 670 | 180,000 | 18 | 300 | 570 | Dimer | 39 | 33 | 96 |
| smURFP + $BVMe_2$ | 646 | 672 | 65,000 | 12 | | 340 | Dimer | | 35 | 23 |
| TDsmURFP + BV | 642 | 670 | 170,000 | 18 | 190 | | Tandem dimer | | | 91 |
| iRFP670 | 643[e] | 670[e] | 114,000[e] | 11[e] | | 290[e] | Dimer | | | 37 |
| Cy5 | 649 | 670 | 250,000 | 25 | 22 | | NA | | NA | 186 |
| IFP1.4 | 684[f] | 708[f] | 92,000[f] | 7[f] | 8.4[f] | 70[e], 50[g] | Weak dimer | 114[g] | 4.4[g] | 19 |
| IFP2.0 | 690[h] | 711[h] | 86,000[h] | 8[h] | | | Weak dimer | | | 20 |
| iRFP713 | 690[g] | 713[g] | 98,000[g] | 6.3[e], 5.9[g] | | 960[e], 450[g] | Dimer | 168[g] | ~4.4[g] | 18 |

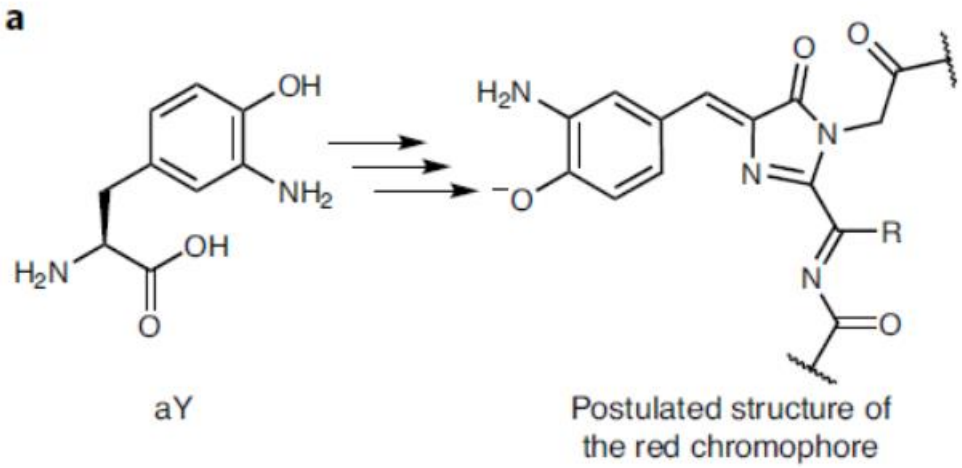
Photostability, time to bleach 50% from an initial emission rate of 1,000 photons per s. NA, not applicable. [a]Determined as described in ref. 3. [b]Determined as described in Online Methods. [c]Data from ref. 3. [d]Data from ref. 29. [e]Data from ref. 17. [f]Data from ref. 15. [g]Data from ref. 16. [h]Data from ref. 18.

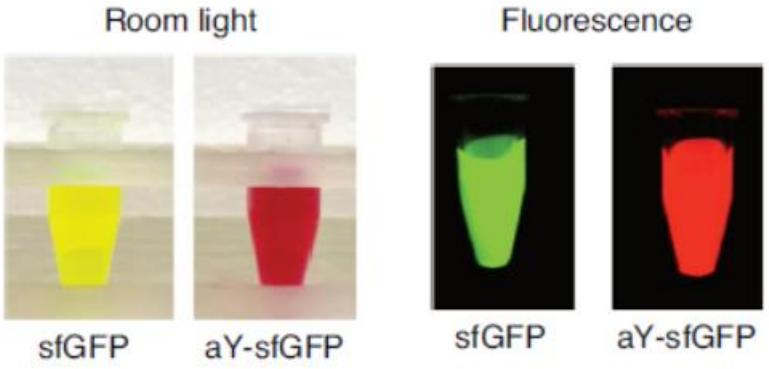
*Nat. Methods* 13, 763–769 (2016)

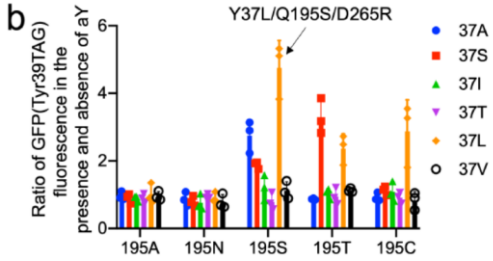


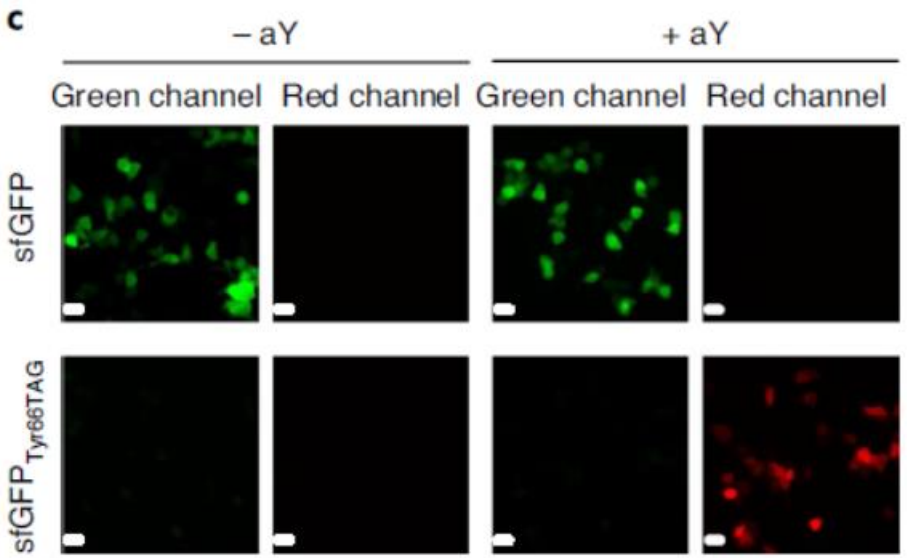
# This paper: A general strategy to red-shift green fluorescent protein-based biosensors

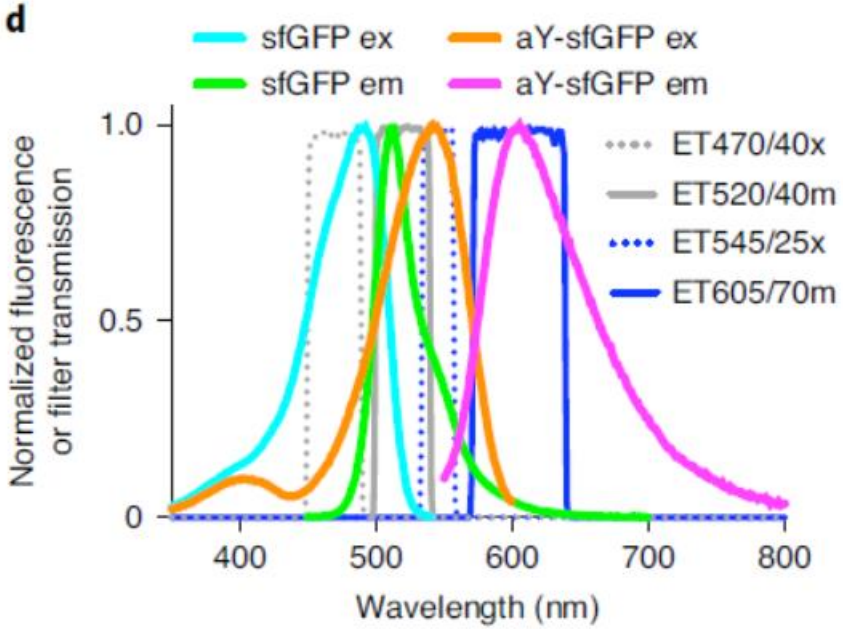


# Improved properties

**Table 1 | Photophysical properties of the selected FPs**

| | Chromophore-forming residues | Normal proteins | | | | Proteins with aY-derived chromophores | | | | Brightness ratios[e] |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $\lambda_{ex}$[a] (nm) | $\lambda_{em}$[b] (nm) | $\varepsilon$ ($mM^{-1}$ $cm^{-1}$)[c] | $\varphi$[d] | $\lambda_{ex}$[a] (nm) | $\lambda_{em}$[b] (nm) | $\varepsilon$ ($mM^{-1}$ $cm^{-1}$)[c] | $\varphi$[d] | |
| **sfGFP** | TYG | 485 | 510 | 85.6 | 0.70 | 541 | 605 | 125.1 | 0.43 | 89.8% |
| **cpsGFP**[f] | TYG | 488 | 510 | 73.1 | 0.66 | 527 | 615 | 88.5 | 0.40 | 73.4% |
| **mTFP1** | AYG | 462 | 492 | 65.6 | 0.81 | 514 | 581 | 85.5 | 0.50 | 80.5% |
| **cpYFP**[g] | GYG | 503 | 515 | 76.3 | 0.58 | 569 | 609 | 82.6 | 0.38 | 70.9% |
| **Citrine** | GYG | 516 | 529 | 75.3 | 0.75 | 565 | 624 | 95.0 | 0.45 | 75.7% |

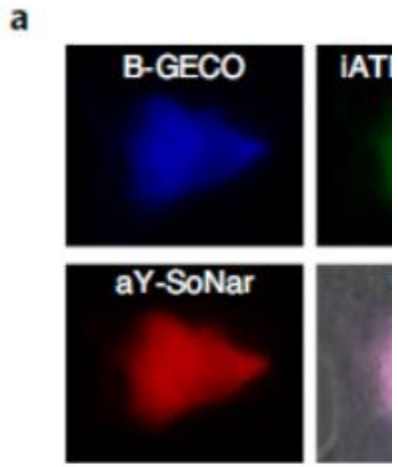
[a]Wavelength of the excitation peak. [b]Wavelength of the emission peak. [c]Extinction coefficient. [d]Quantum yield. [e]Molecular brightness (defined as $\varepsilon \times \varphi$) of an aY-modified protein presented as the percentage of the molecular brightness of the corresponding normal protein. [f]Circularly permuted sfGFP variant previously used to derive the hydrogen sulfide sensor, hsGFP[33]. [g]Circularly permuted EYFP variant previously used to derive the hydrogen sulfide sensor, cpGFP-*p*AzF[23].

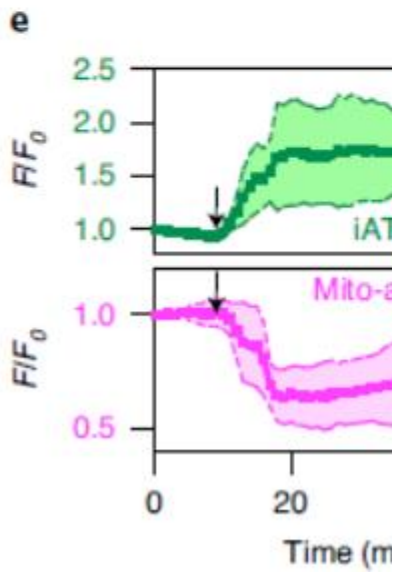
# Sensors

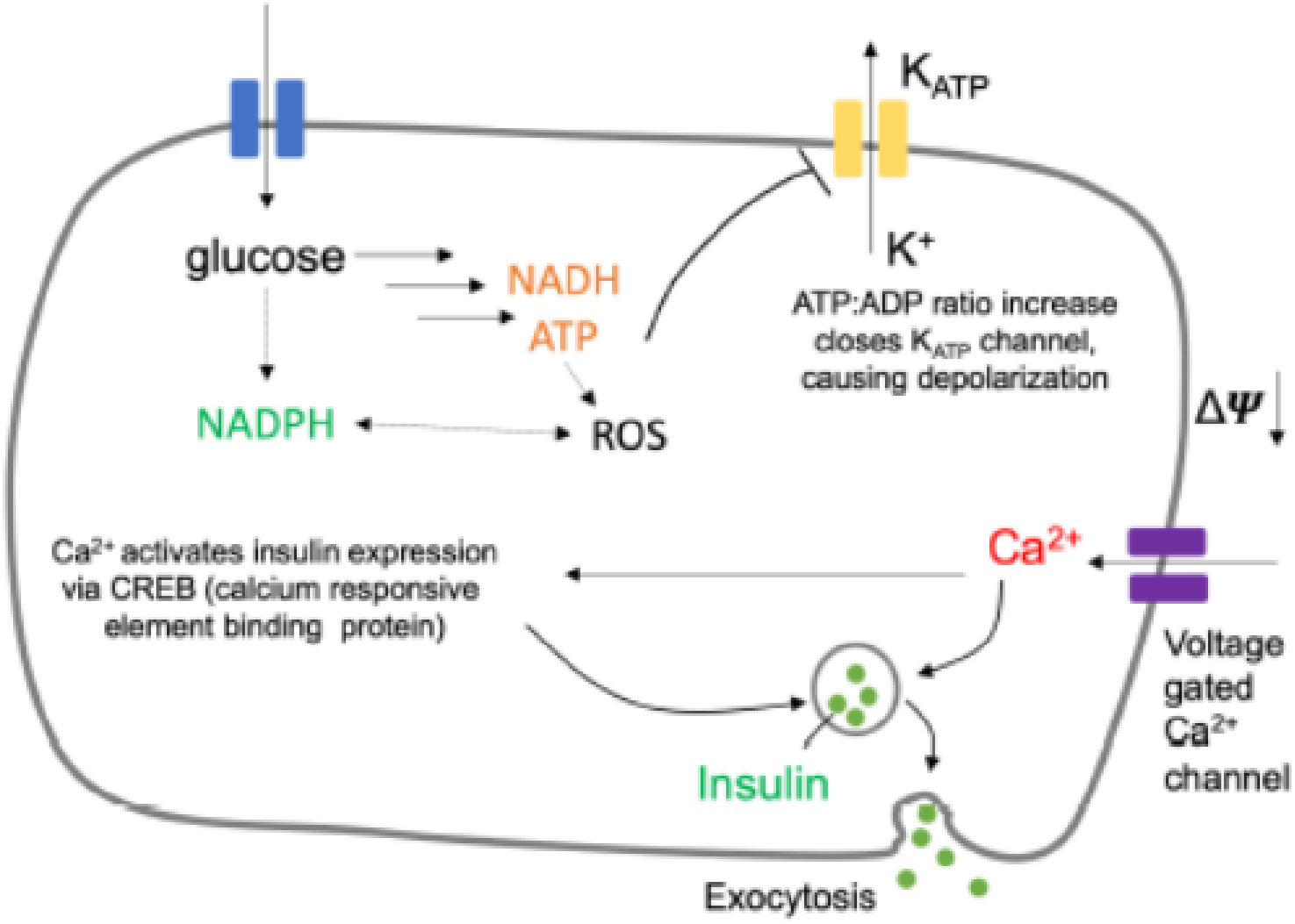
**Table 2 | Photophysical properties of the tested genetically encoded biosensors and their aY-modified variants**

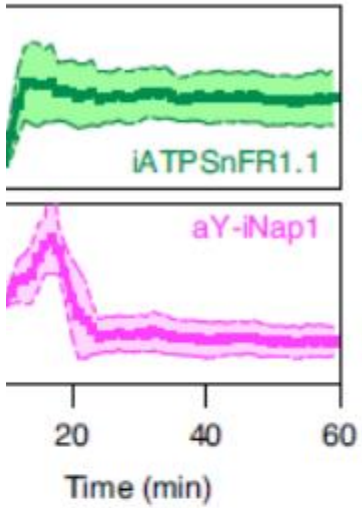
| Biosensor | Analytical target | $\lambda_{ex}$[a] (nm) | $\lambda_{em}$[b] (nm) | Dynamic Range[c] (%) | $\varepsilon$[d] ($mM^{-1}$ $cm^{-1}$) | | $\varphi$[e] | |
|---|---|---|---|---|---|---|---|---|
| | | | | | − | + | − | + |
| G-GECO1 (ref. [34]) | $Ca^{2+}$ | 497 | 512 | 1,983 | 4.7 | 68.1 | 0.26 | 0.38 |
| aY-G-GECO1 | $Ca^{2+}$ | 528 | 594 | 1,566 | 7.7 | 79.9 | 0.14 | 0.22 |
| ZnGreen1 (ref. [35]) | $Zn^{2+}$ | 474 | 500 | 2,531 | 24.8 | 1.3 | 0.48 | 0.35 |
| aY-ZnGreen1 | $Zn^{2+}$ | 520 | 585 | 915 | 34.7 | 4.8 | 0.28 | 0.20 |
| iGluSnFR[36] | glutamate | 488 | 510 | 257 | 9.6 | 32.6 | 0.58 | 0.60 |
| aY-iGluSnFR | glutamate | 528 | 600 | 270 | 12.2 | 42.7 | 0.46 | 0.49 |
| iGABASnFR[37] | GABA | 502 | 513 | 143 | ND[f] | ND[f] | ND[f] | ND[f] |
| aY-GABASnFR | GABA | 541 | 602 | 92 | ND[f] | ND[f] | ND[f] | ND[f] |
| dLight1.2 (ref. [38]) | dopamine | 496 | 513 | 194 | ND[f] | ND[f] | ND[f] | ND[f] |
| aY-dLight1.2 | dopamine | 545 | 603 | 117 | ND[f] | ND[f] | ND[f] | ND[f] |
| SoNar[39] | $NAD^+$/NADH | 497 | 512 | 257 [2,532][g] | 7.6 | 23.0 | 0.44 | 0.51 |
| aY-SoNar | $NAD^+$/NADH | 544 | 604 | 426 [2,656][g] | 6.5 | 28.6 | 0.29 | 0.35 |
| iNap1 (ref. [40]) | NADPH | 497 | 513 | 82 [273][g] | 22.6 | 13.1 | 0.50 | 0.43 |
| aY-iNap1 | NADPH | 545 | 604 | 614 [11,735][g] | 27.6 | 4.4 | 0.41 | 0.36 |
| PercevalHR[41] | ATP | 498 | 513 | 155 [364][g] | 14.6 | 35.6 | 0.28 | 0.28 |
| aY-PercevalHR | ATP | 545 | 604 | 117 [650][g] | 18.4 | 40.5 | 0.21 | 0.21 |
| iATPSnFR1.1 (ref. [42]) | ATP | 493 | 513 | 106 | 22.6 | 43.9 | 0.36 | 0.37 |
| aY-iATPSnFR1.1 | ATP | 541 | 608 | 75 | 29.1 | 50.9 | 0.28 | 0.28 |

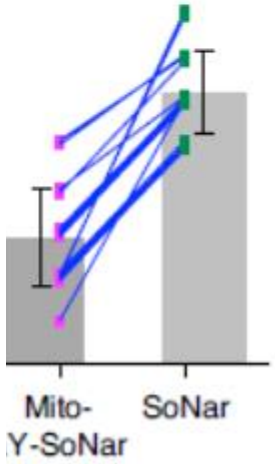
# Imaging of metabolic dynamics in pancreatic β-cells



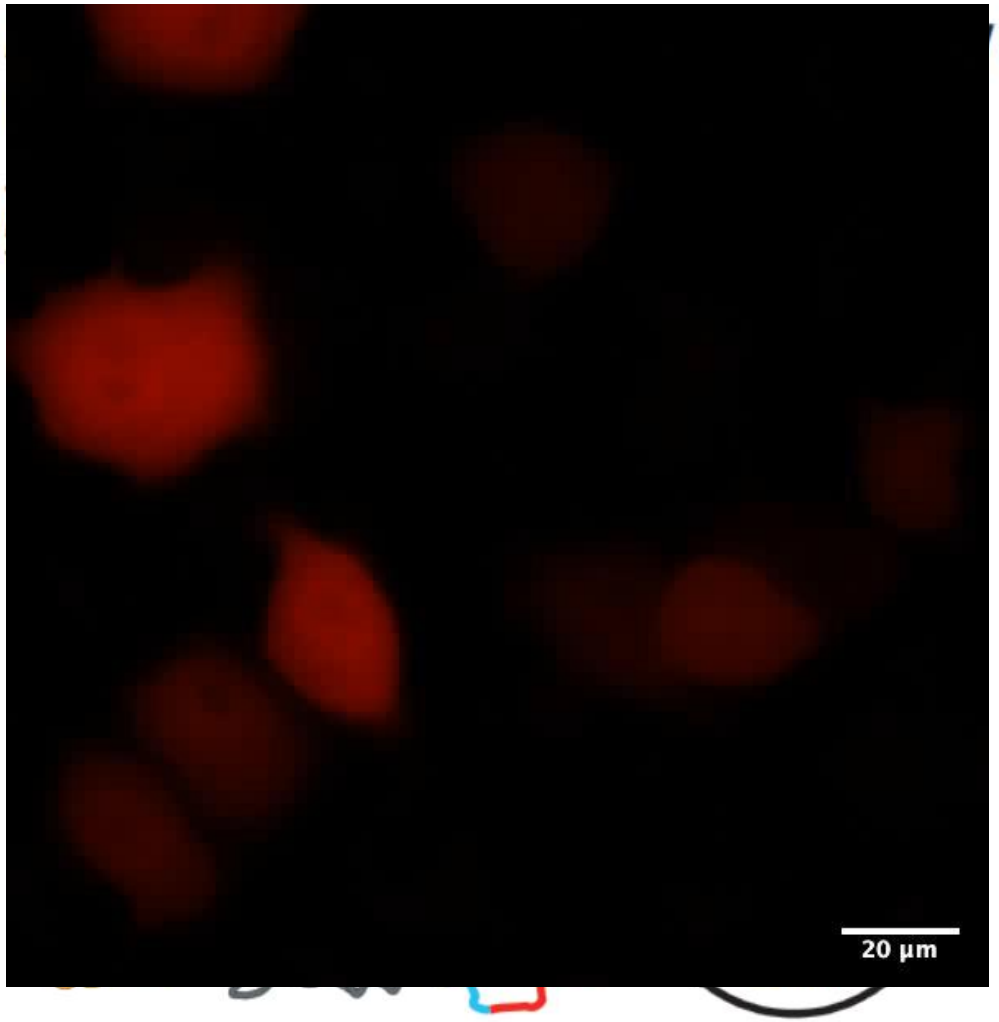



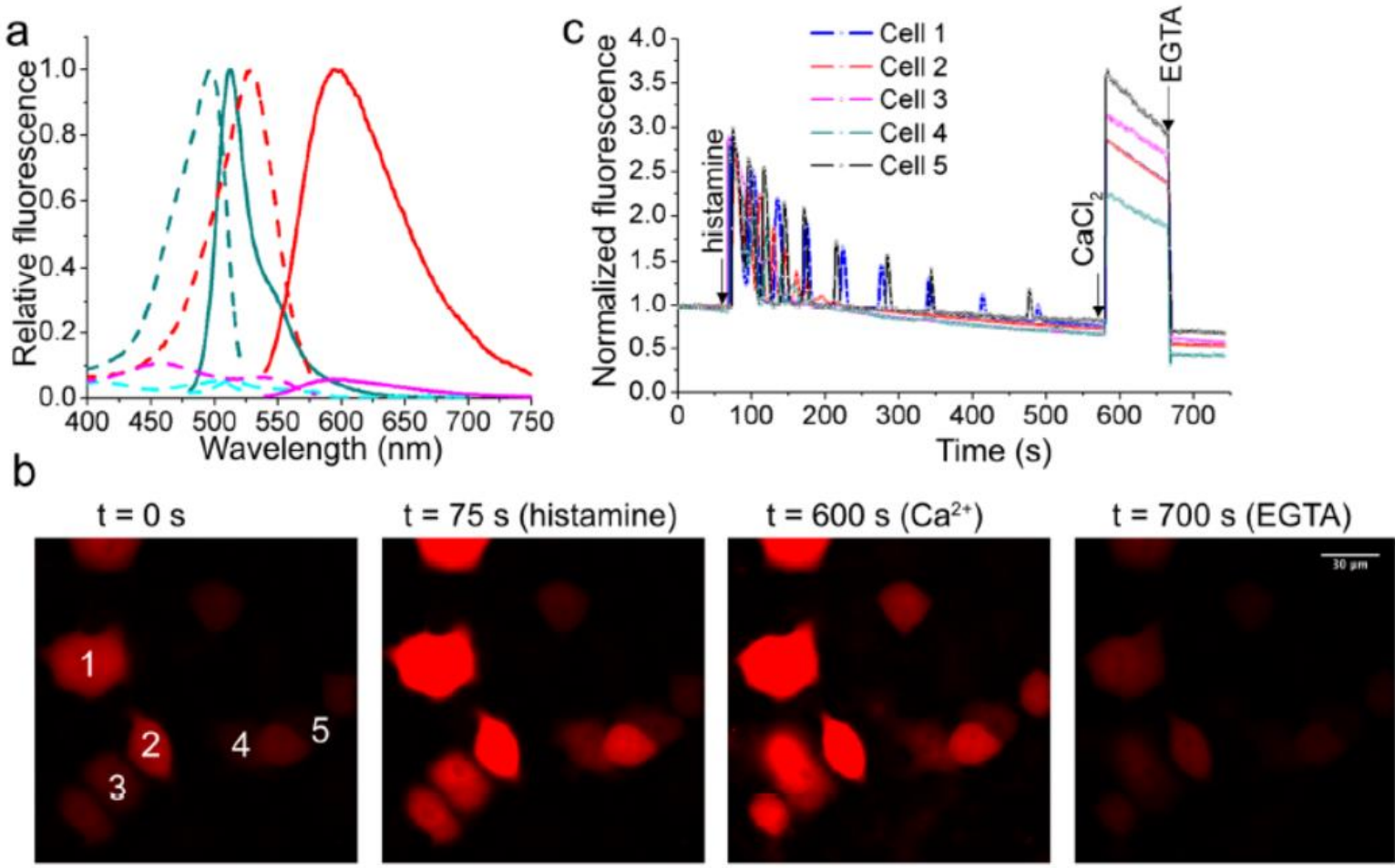



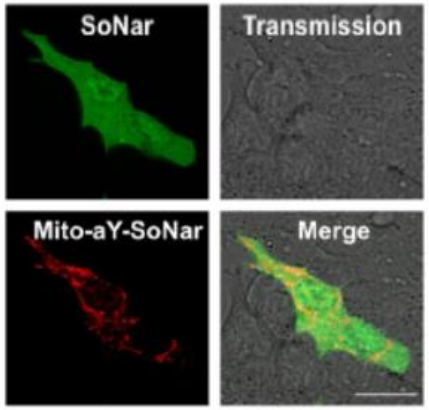
# $Ca^{2+}$ Indicators



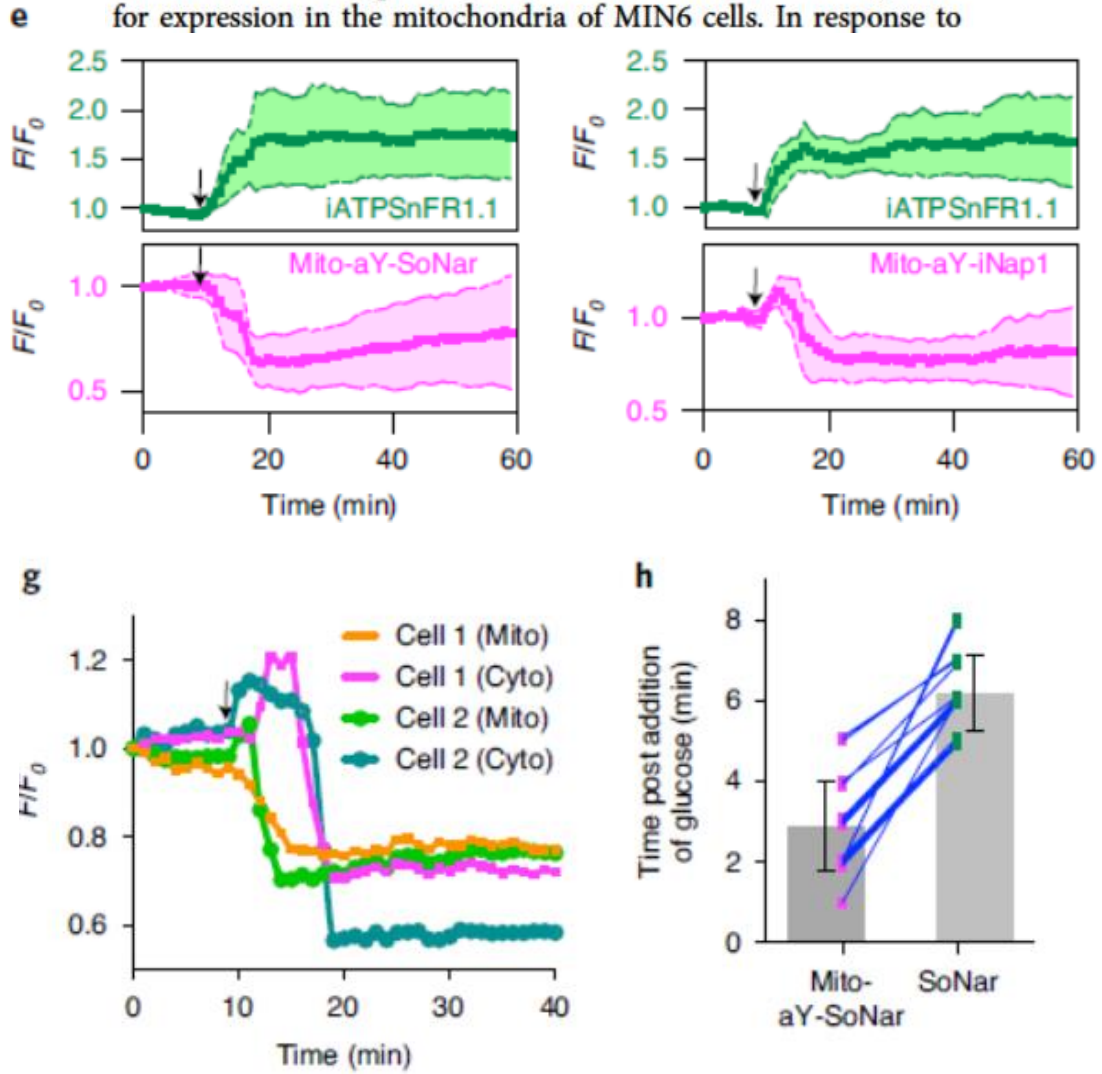



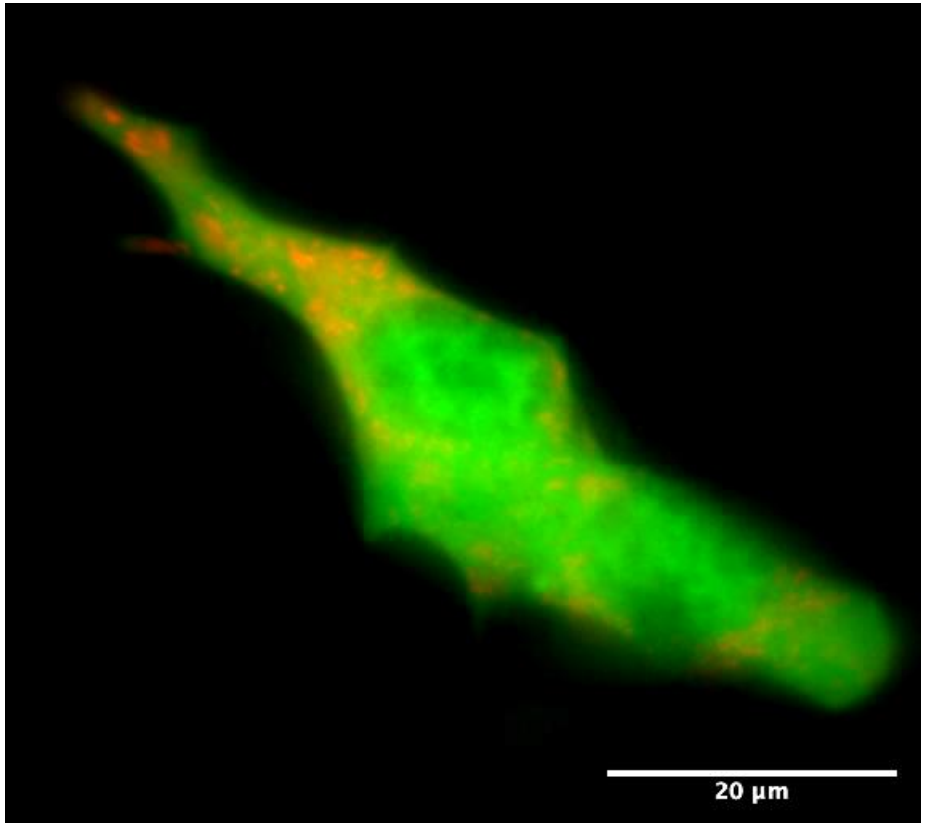

*Science* 333, 1888–1891 (2011).

# dual-color images of MIN6 cells co-expressing SoNar and mitochondrial aY-SoNar

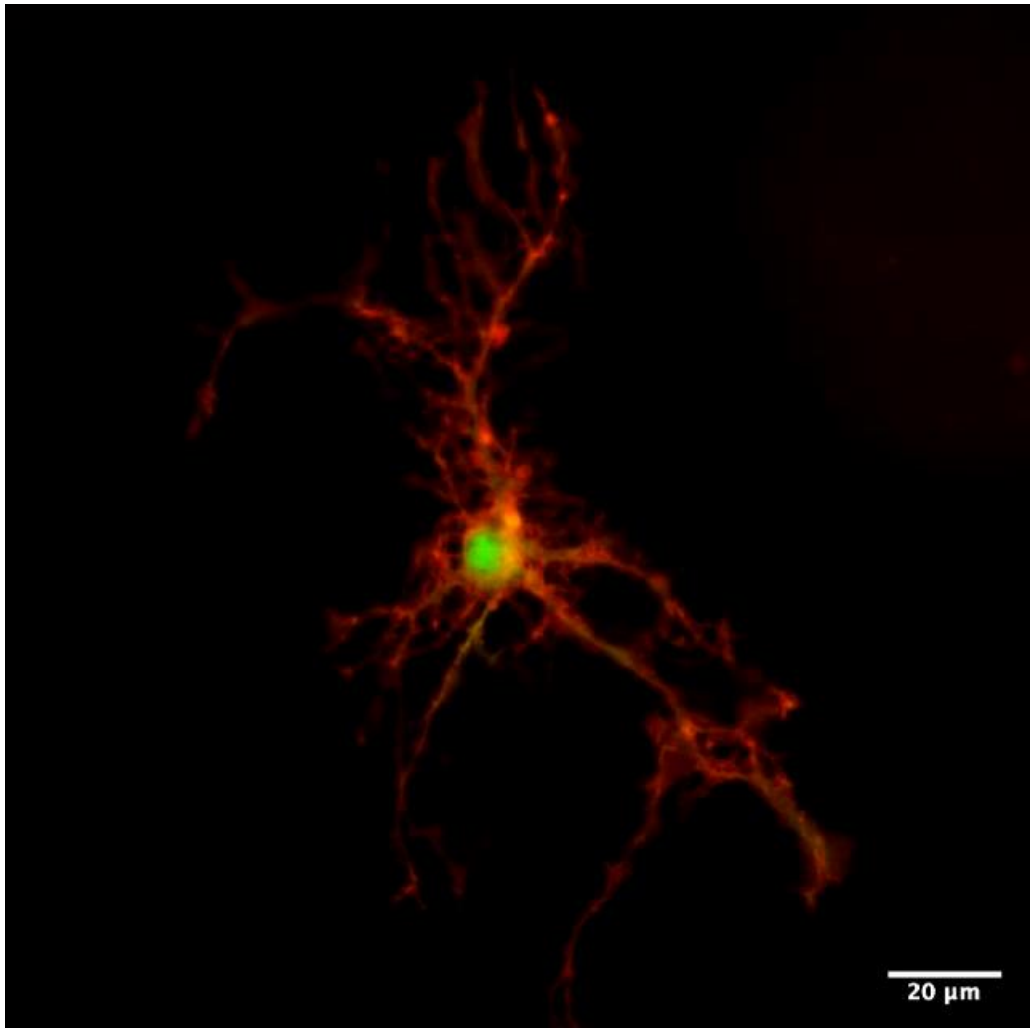



Because NAD+/NADH and NADPH are compartmentalized and mitochondria play a central role in metabolism, we next fused aY-SoNar or aY-iNap1 to a mitochondrial localization sequence for expression in the mitochondria of MIN6 cells. In response to



# $K^+$



# 3Q

## 3Q

### 3Q

#### 3Q