Literature Report

Reporter: Zhou Xuelian

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ARTICLES

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

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Education:

Tsinghua University, Beijing - B.S. Chemistry (2003)
University of Alberta, Edmonton - Ph.D. Chemical biology (2008)
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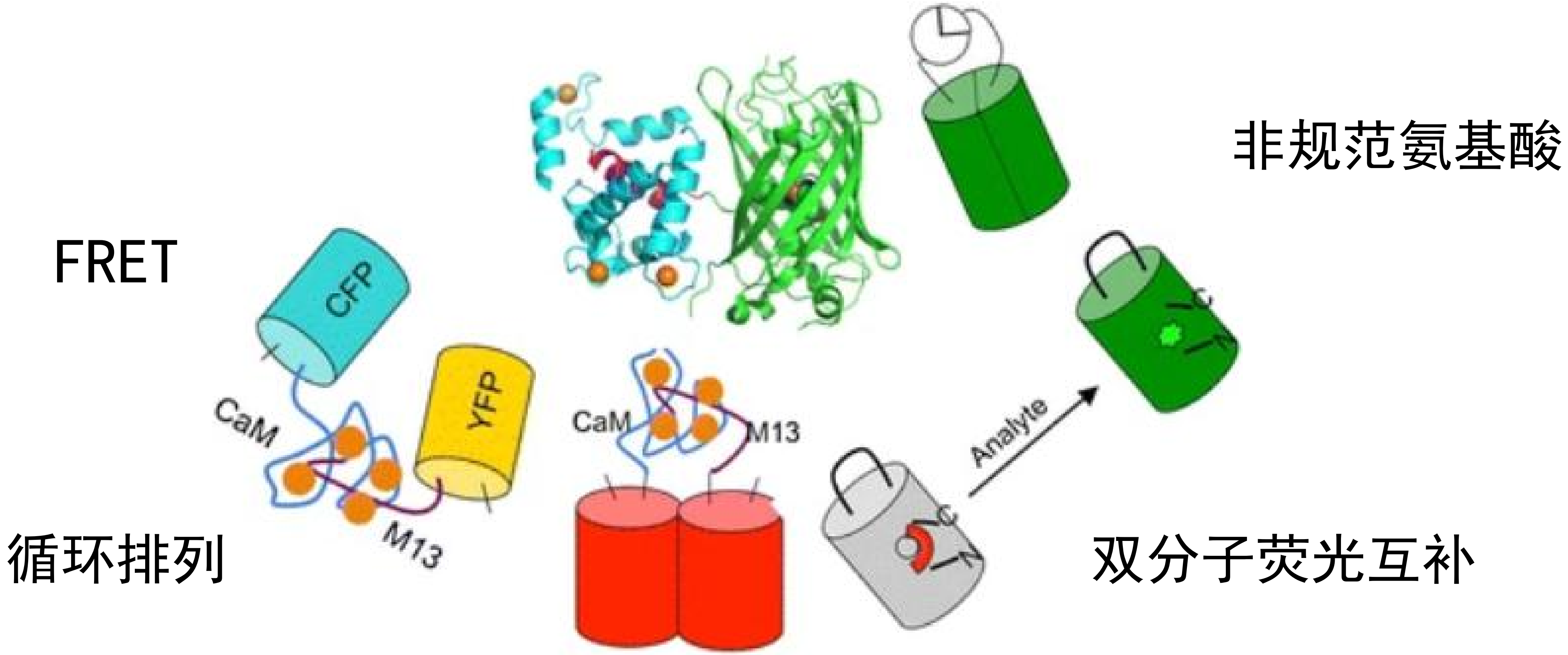
University of California, Riverside, CA - Associate Professor (2011-2017)
University of Virginia, Charlottesville - Associate Professor (2017- present)

荧光和生物发光成像；用于成像、诊断和治疗的基因和蛋白质工程；
合成生物学与化学生物学

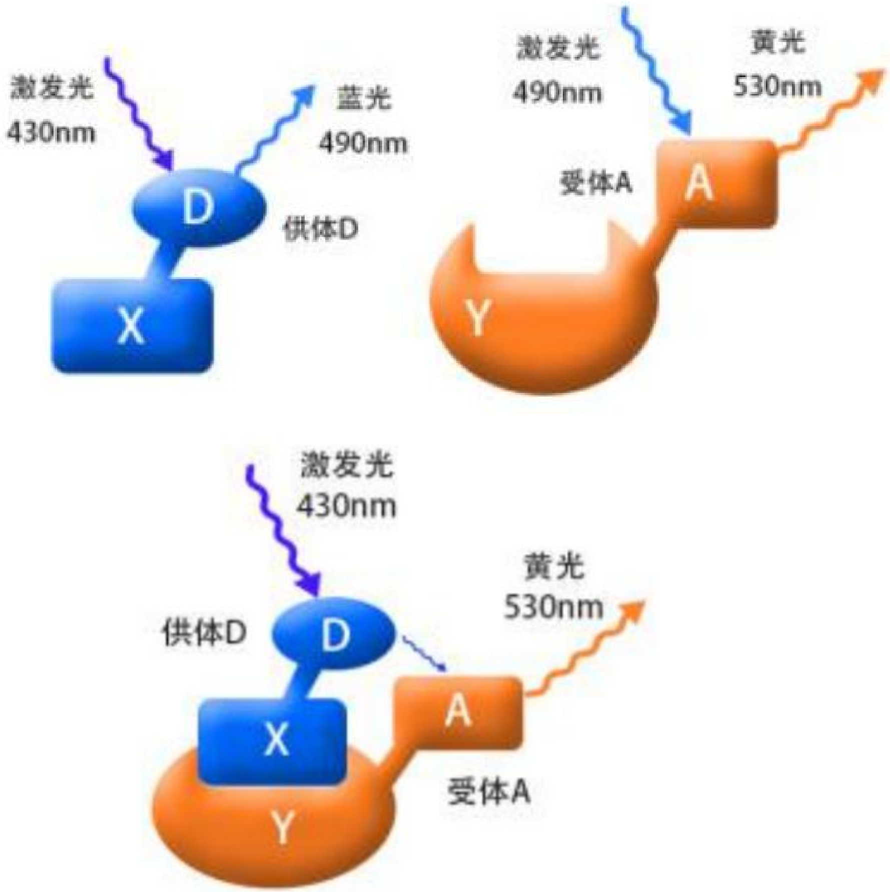
基于FP的生物传感器



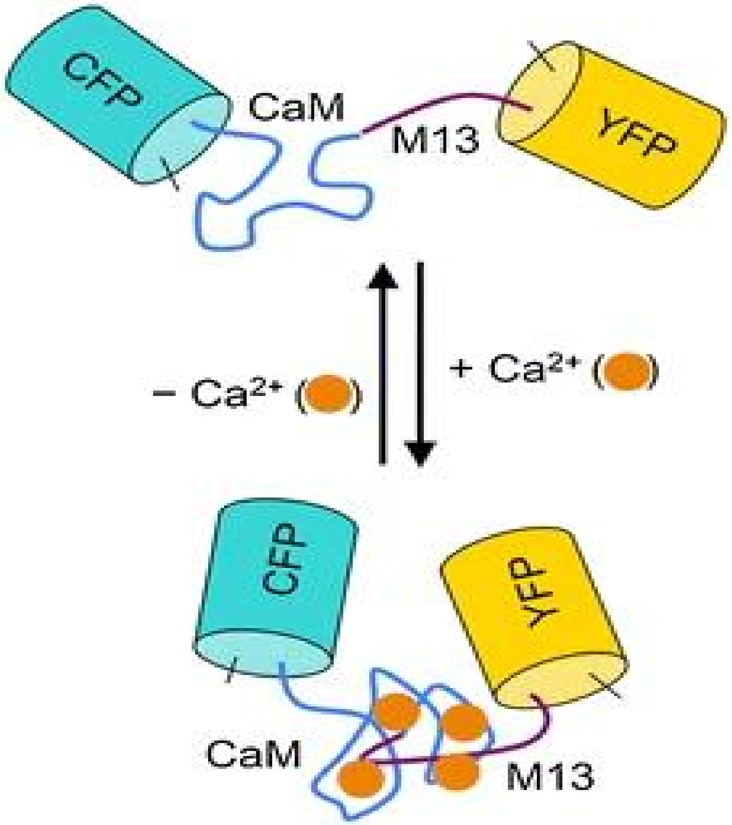
Fluorescent protein based sensors

基于FP的生物传感器——FRET

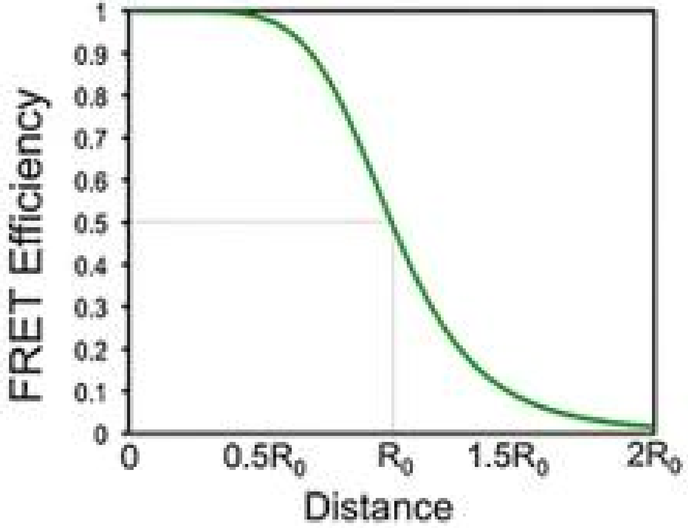
双分子型



单分子型

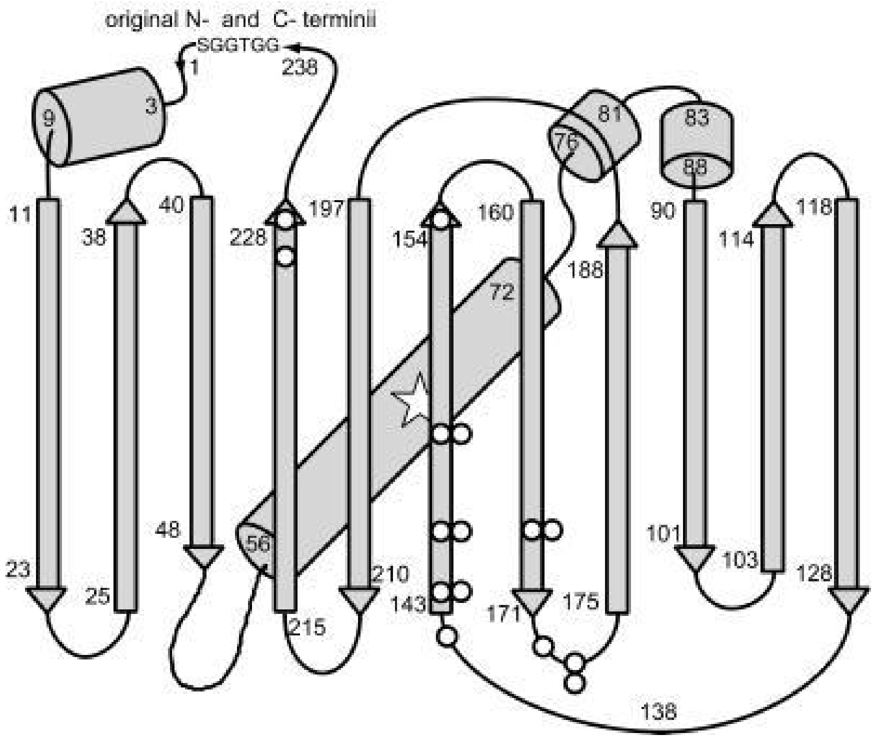


Cameleon

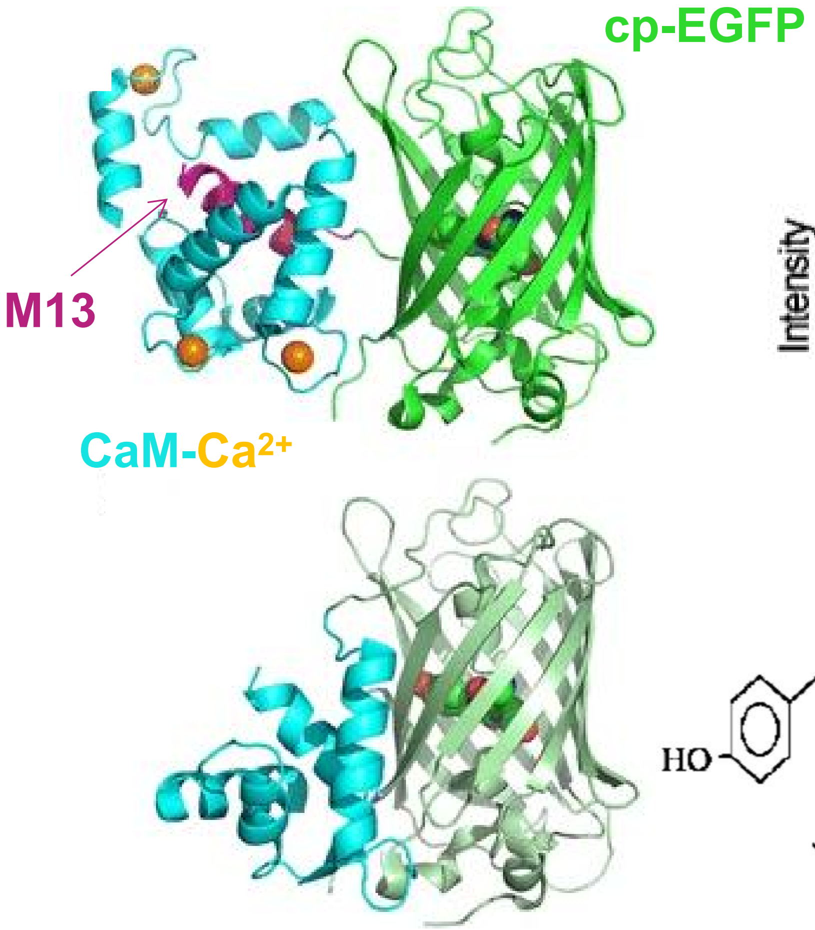


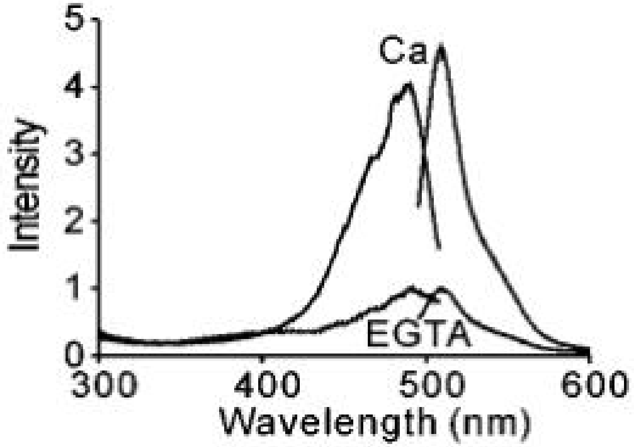
Anal Bioanal Chem (2015) 407:9-15

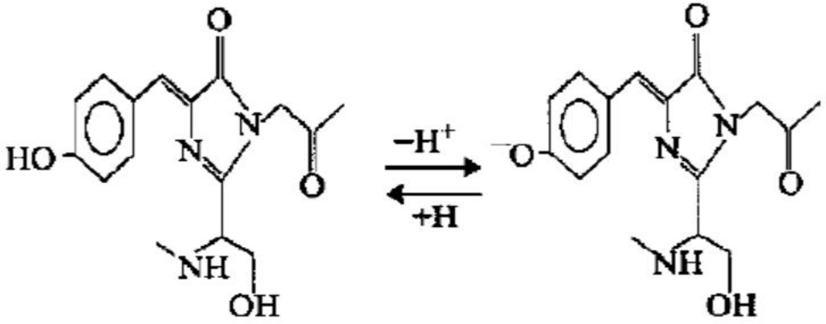
基于FP的生物传感器——循环排列

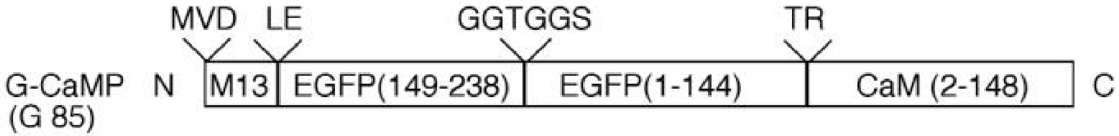


Proc Natl Acad Sci USA (1999) 96(20): 11241-11246.



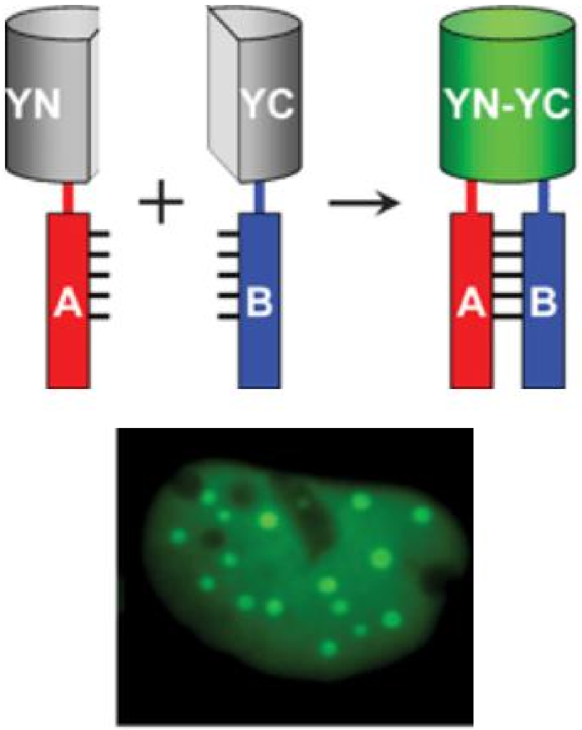


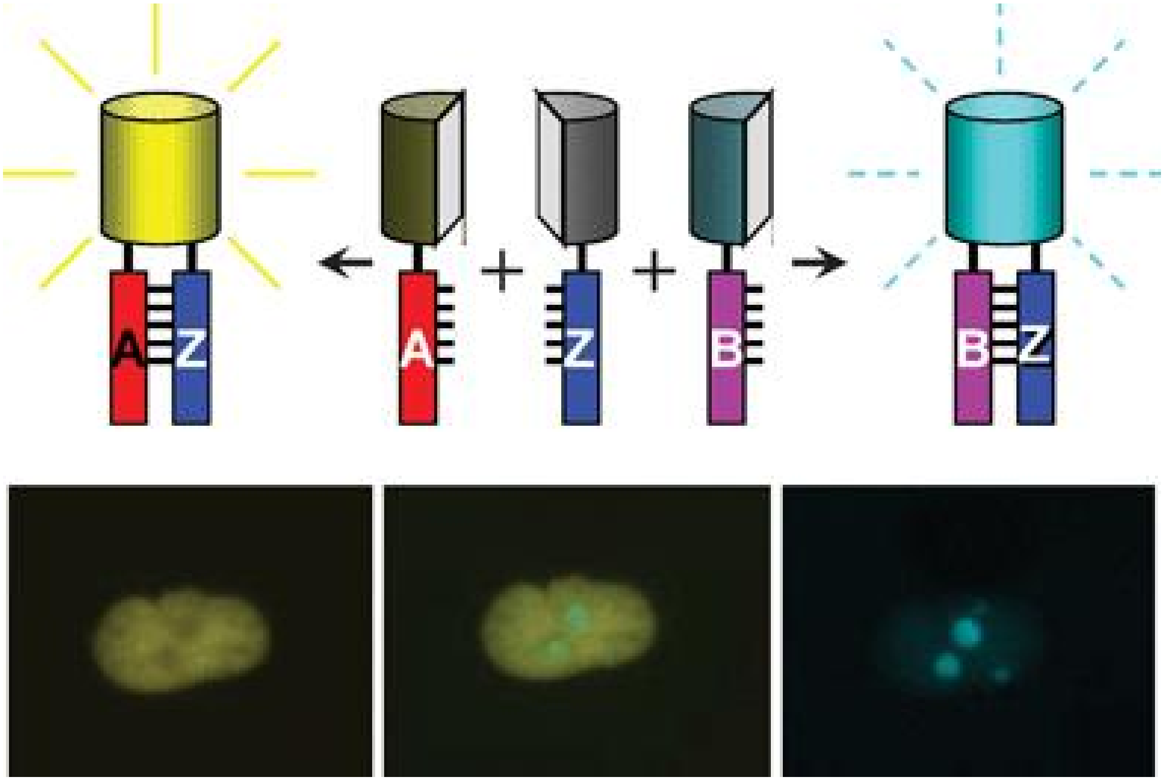




Nat Biotech (2001) 19: 137-141

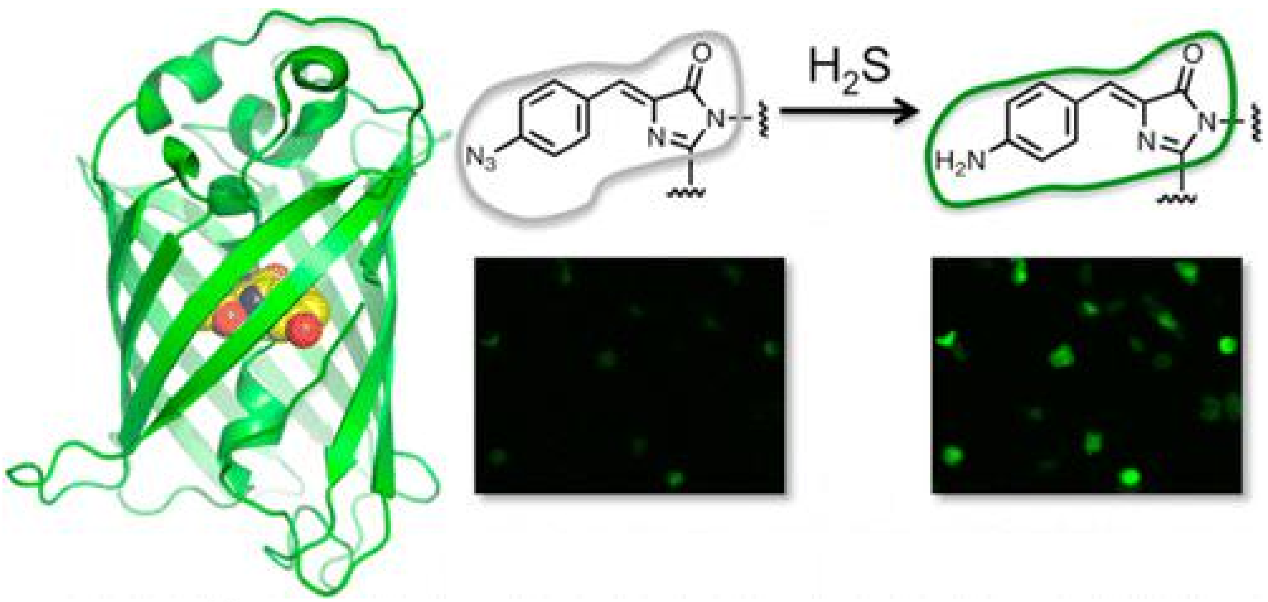
基于FP的生物传感器——双分子荧光互补(BiFC)

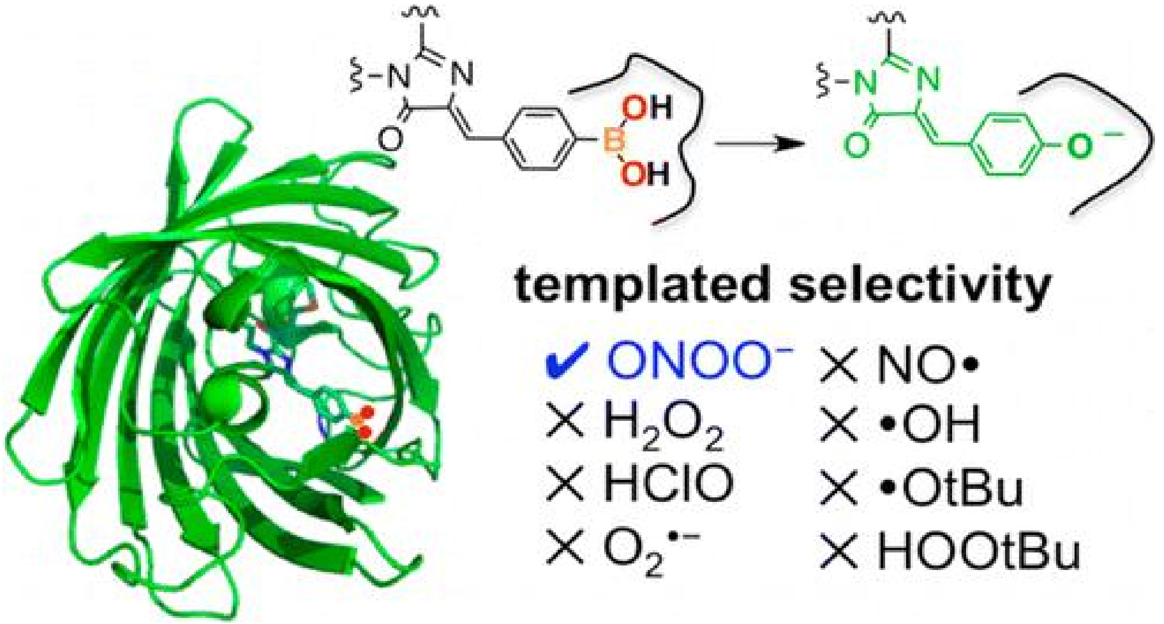


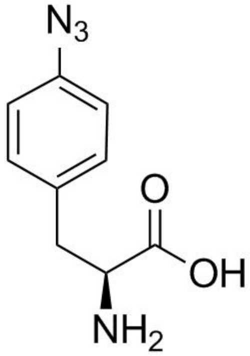


Chem Soc Rev (2009) 38, 2876-2886

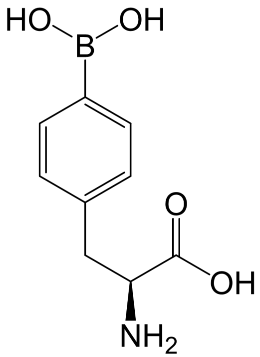
基于FP的生物传感器——非规范氨基酸ncAAs







pAzF

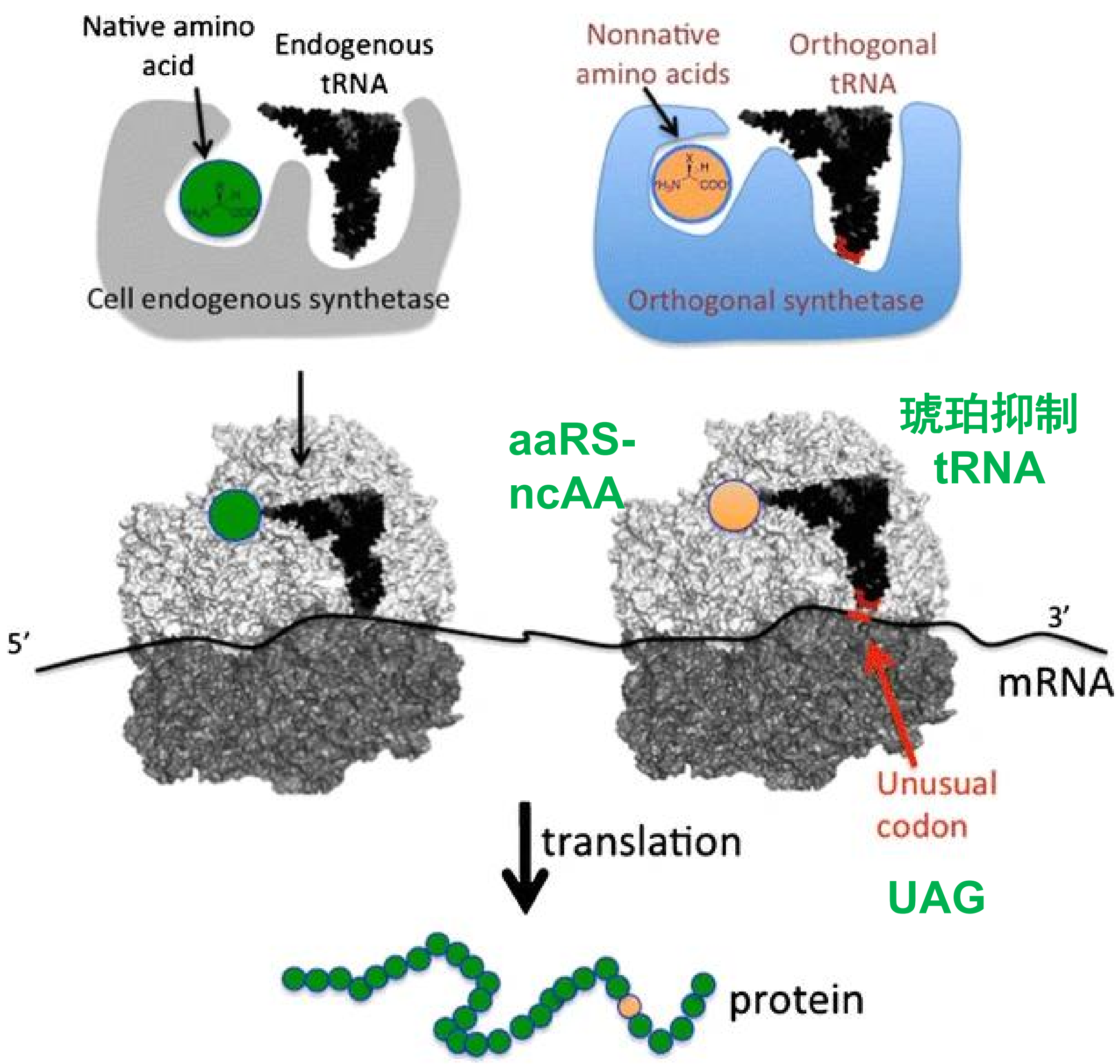


pBoF

J Am Chem Soc (2013) 135(40): 14940-14943

J Am Chem Soc (2012) 134(23): 9589-9592

遗传密码扩展技术



遗传密码扩展技术：利用不寻常的密码子（无义密码子或四碱基密码子）实现蛋白质中非规范氨基酸(ncAAs) 的定点引入。

作用元件:、特异性识别不寻常密码子的tRNA (suppressor tRNA) 及特异性识别ncAA和相应tRNA的氨酰 tRNA 合成酶(aminoacyl-tRNA synthetase, **aaRS**)，这些元件在蛋白质翻译过程中必须保证正交性从而使 ncAAs 被引入到蛋白质的特定位置上。

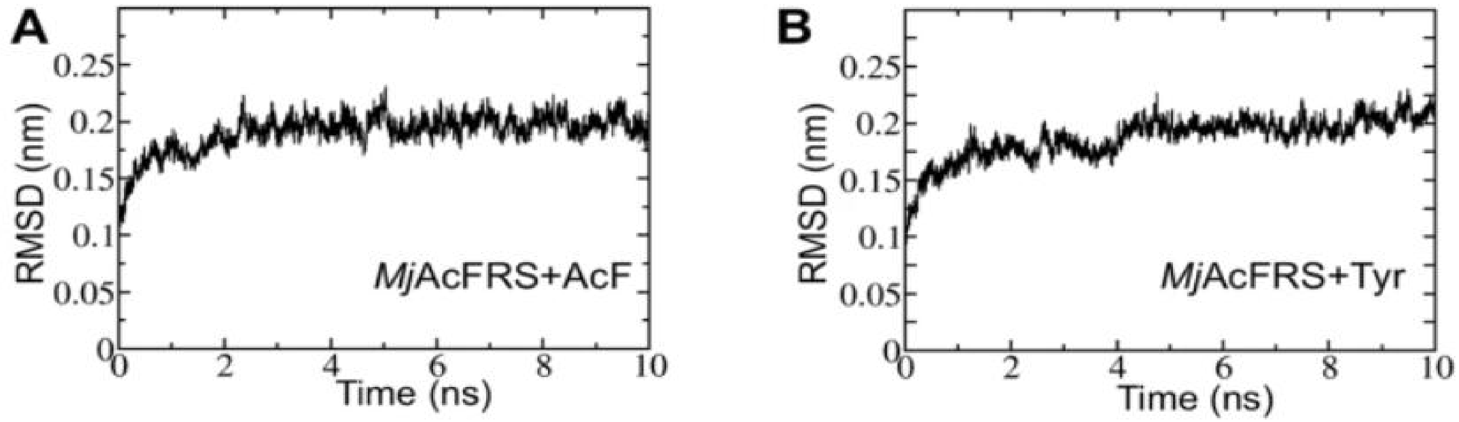
正交性：aaRS不应识别细胞内源性tRNA和氨基酸，并且tRNA不得与任何内源性aaRS发生作用，只能特异性识别不寻常密码子。这种**tRNA /合成酶对**被称为**正交对**。

Chem Biol (2009) 16(3): 323–336

遗传密码扩展技术

正交

Proteins		*Mj*AcFRS		*Mj*IoYRS	
Amino acids		AcF	Tyr	IoY	Tyr
Energy Scores	AutoDock Vina (kcal/mol)	−7.09 [1.15][a]	−6.15	−6.85 [1.05][a]	−6.54
	ROSETTA (REU)	−10.72 [1.20][a]	−8.94	−16.12 [1.44][a]	−11.21



AutoDock Vina：成本低，快；部分合成酶的交叉作用可能无法区分。

ROSETTA：当所有估计的界面能一起分析时，无明显的阈值来区分有利、不利的相互作用。

MD-MM：计算量大，成本高，不适合大批量筛选。

MM：相较于MD-MM成本低、精确度高。

		ΔE_{vdw} [a]	ΔE_{ele} [a]	$\Delta \acute{g}_{PS}$ 一个	ΔG_{SASA} [a]	$\Delta G_{总}$ [a], [b]
Mj AcFRS + AcF	MD-MM / PBSA	−30.56±0.27	−26.12±0.30	44.45±0.22	−3.11±0.01	−15.35±0.22
	直接MM / PBSA	−30.08	−27.73	43.49	−3.24	−17.56
Mj AcFRS + Tyr	MD-MM / PBSA	−25.61±0.25	−23.46±0.30	41.68±0.21	−2.79±0.01	−10.17±0.23
	直接MM / PBSA	−23.72	−25.99	43.31	−2.92	−9.33

Sci Rep (2015), 5:12632

Introduction

nature chemical biology

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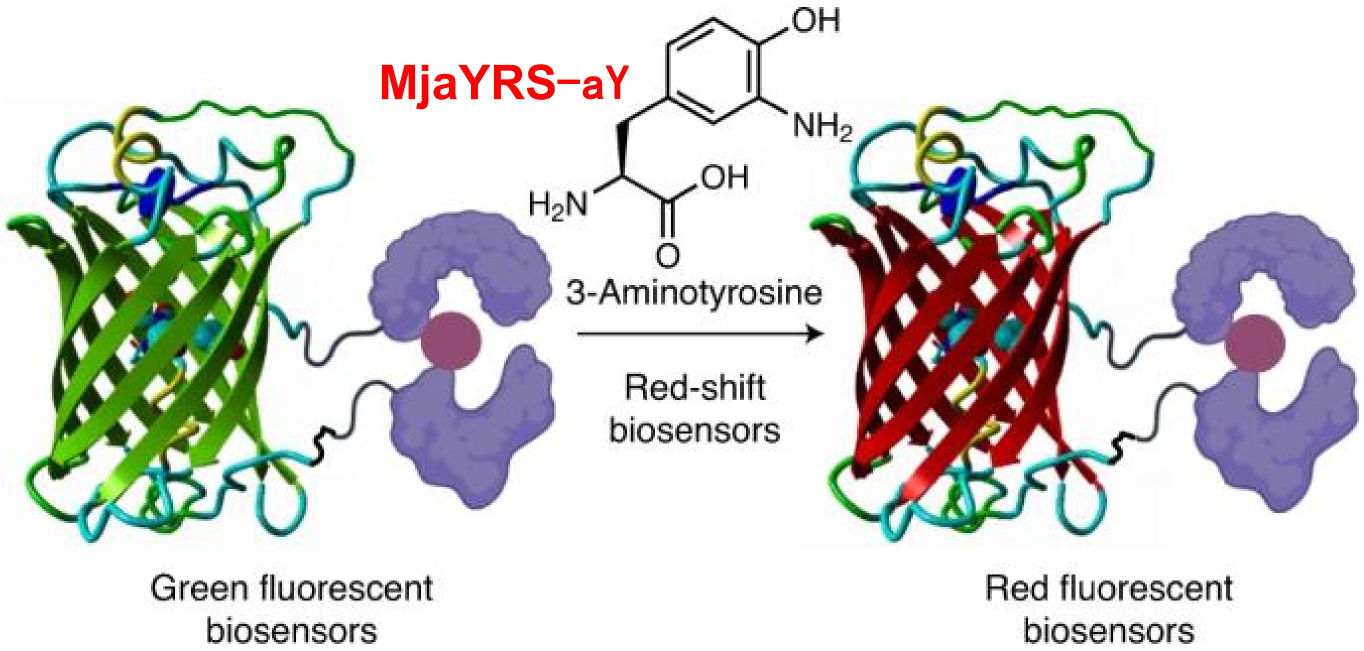
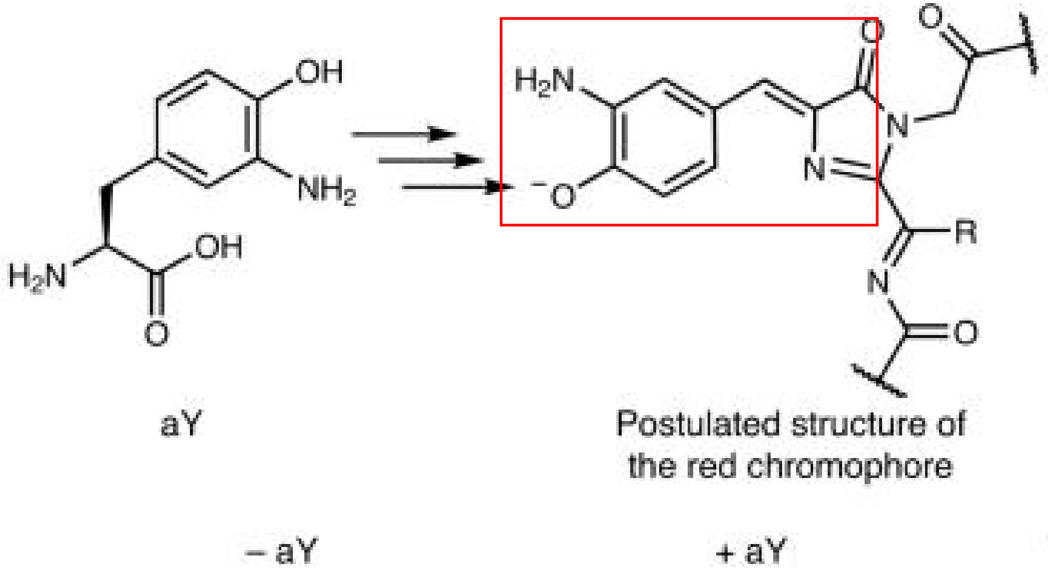




Design——大肠杆菌中aY的遗传编码

MjTyrRS突变体（3-碘酪氨酸）

↓ AutoDock 筛选

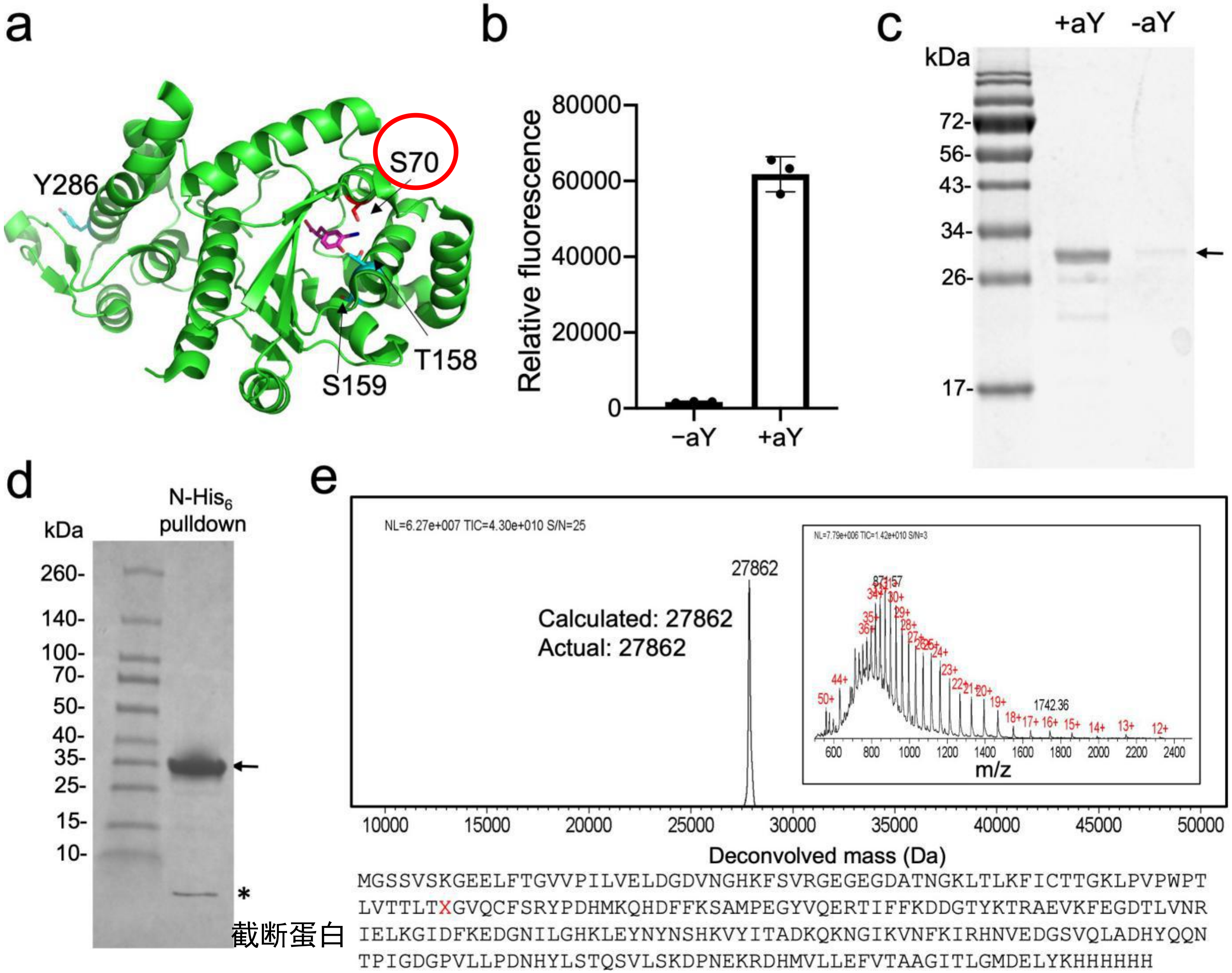
MjaYRS

↓ +琥珀抑制tRNA

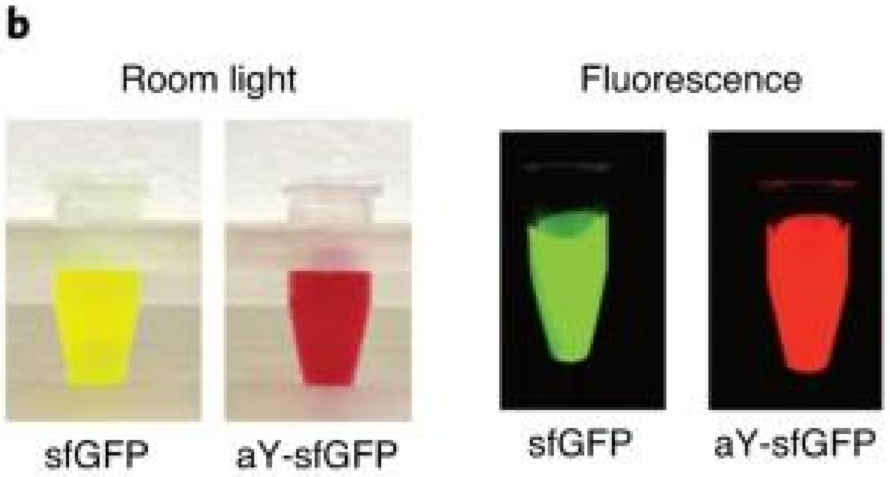
sfGFP(残基66位酪氨酸
突变为琥珀密码子)

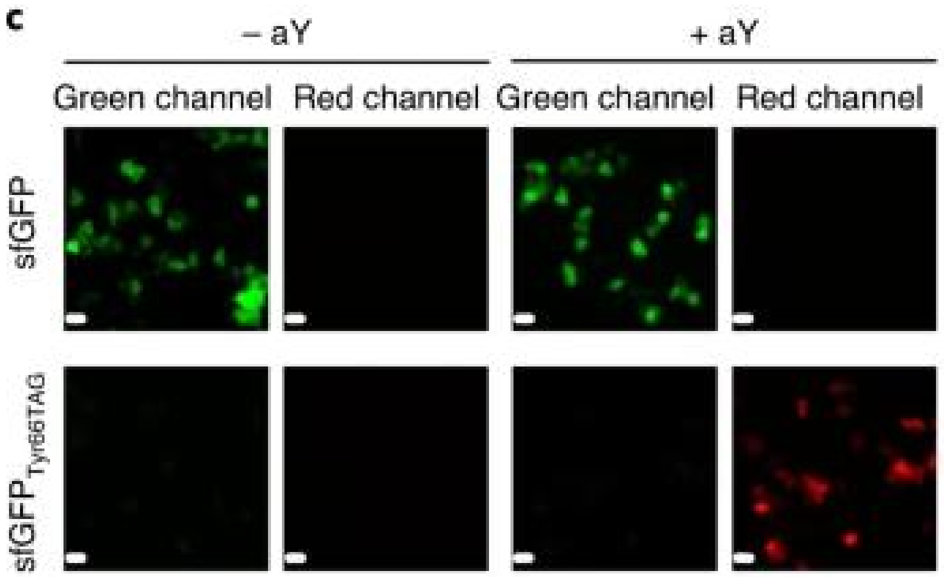
↓ +aY

aY-sfGFP
荧光增强、高产率的
全长蛋白、高特异性

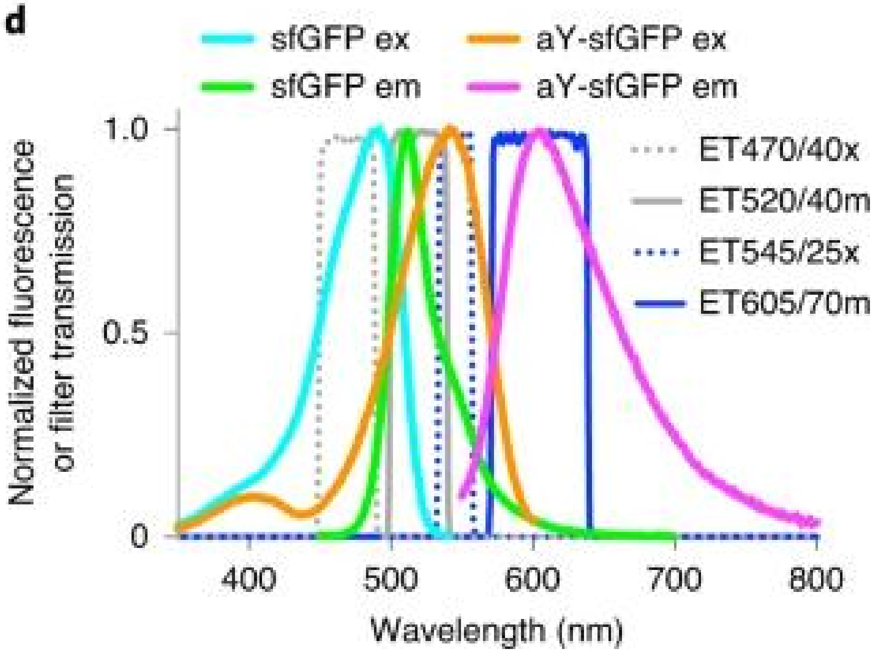


Results——aY引起的FPs和基于FP生物传感器的红移





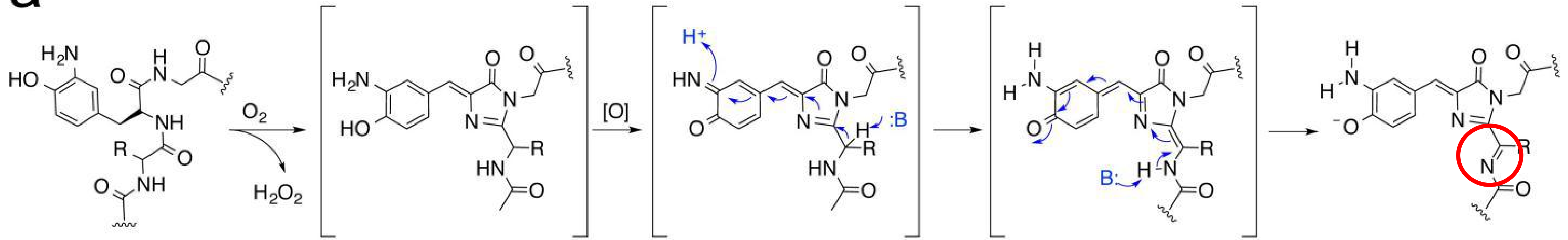
HEK 293T



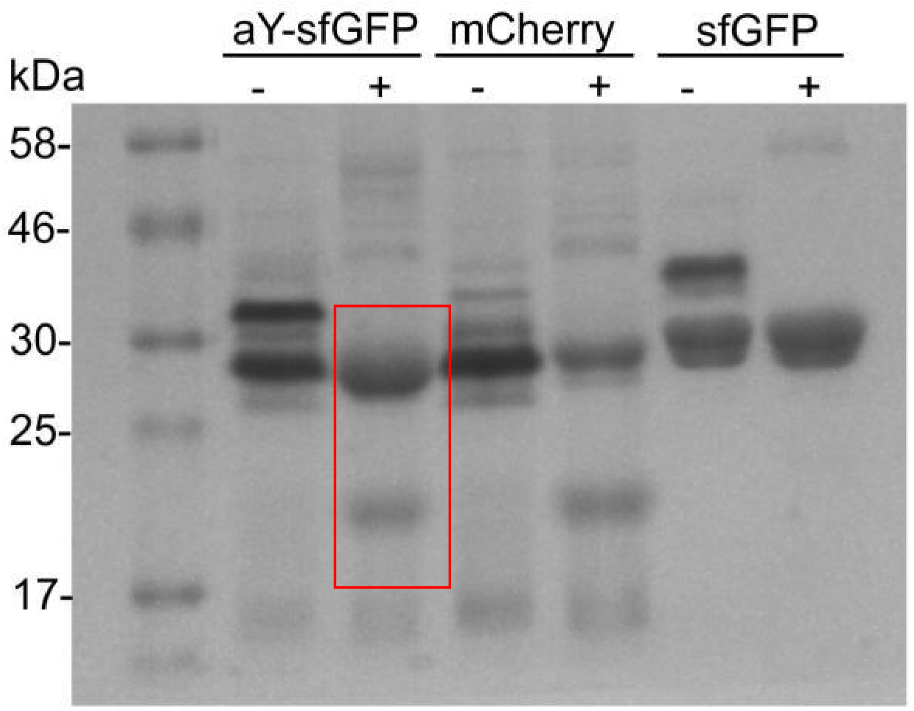
56
95

Results——aY引起的FPs和基于FP生物传感器的红移

a

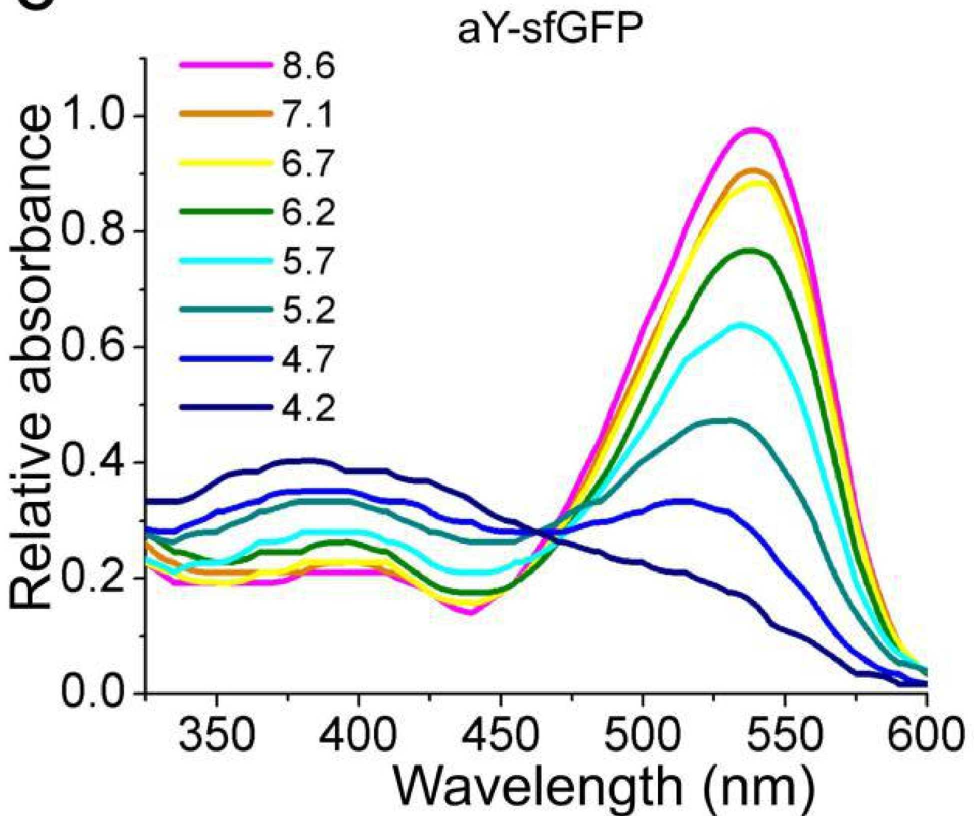


b



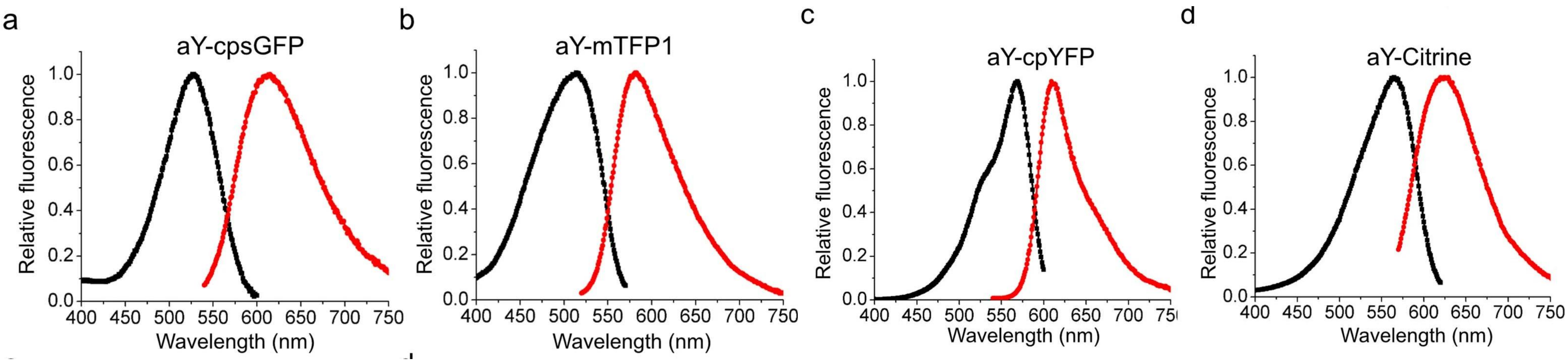
c

水解



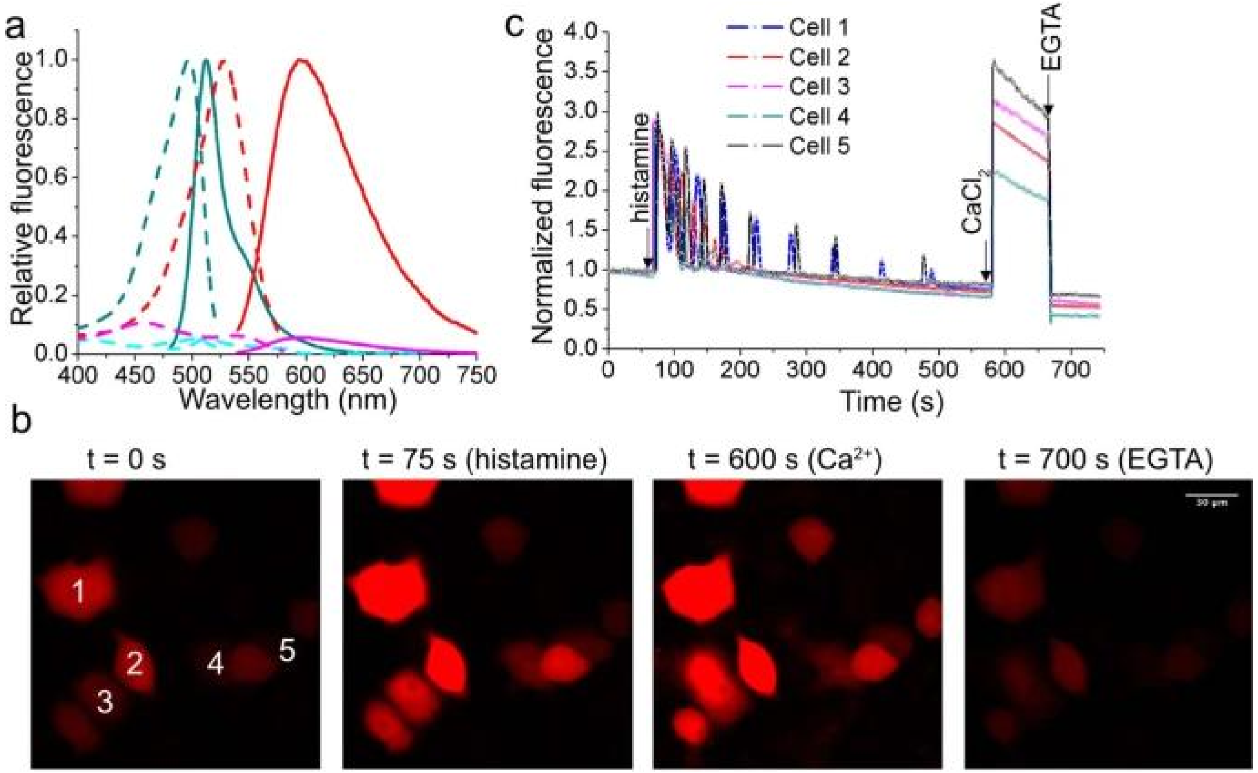
Results——aY引起的FPs和基于FP生物传感器的红移

	Chromophore-forming residues	Normal proteins				Proteins with aY-derived chromophores				Brightness ratios[e]
		λ_{ex}[a] (nm)	λ_{em}[b] (nm)	ε (mM^{-1} cm^{-1})[c]	φ[d]	λ_{ex}[a] (nm)	λ_{em}[b] (nm)	ε (mM^{-1} cm^{-1})[c]	φ[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%

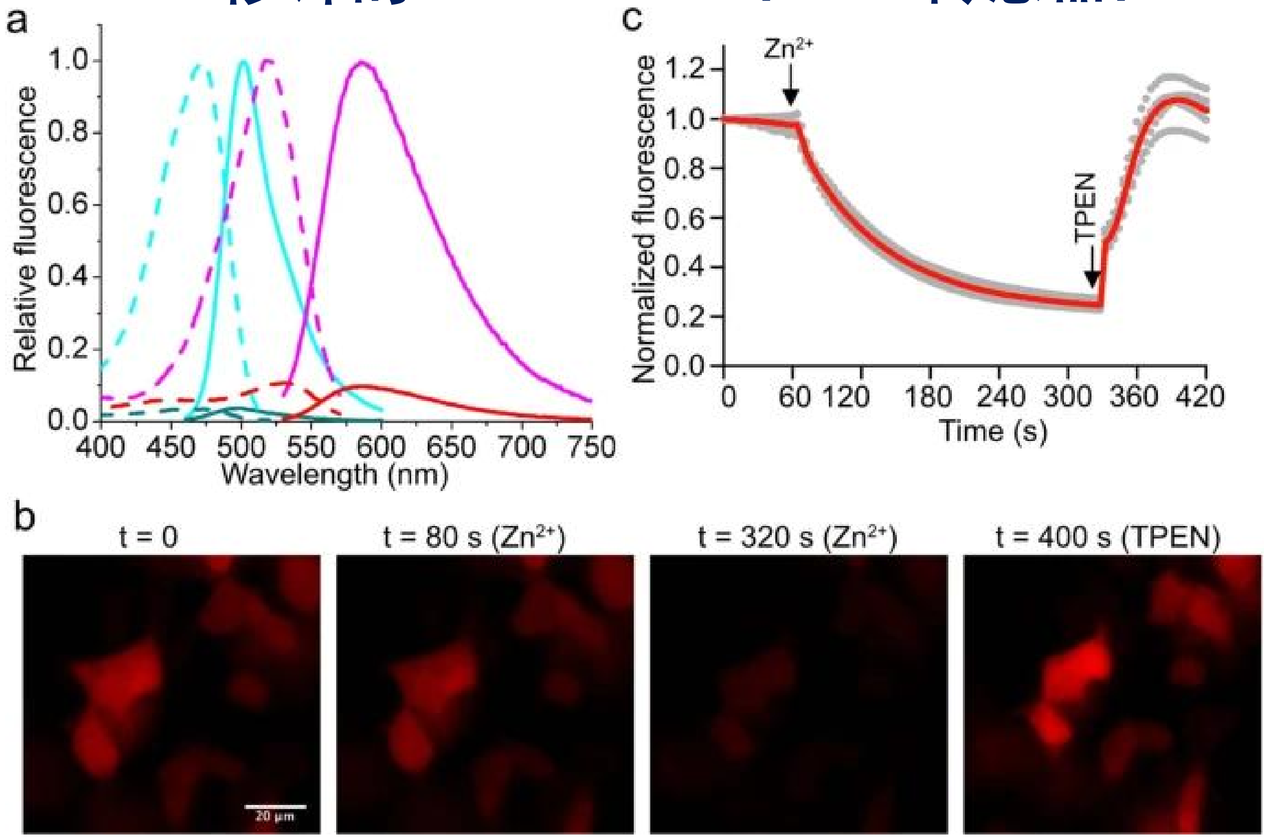


Results——aY引起的FPs和基于FP生物传感器的红移

aY修饰的G-GECO1（Ca 2+传感器）

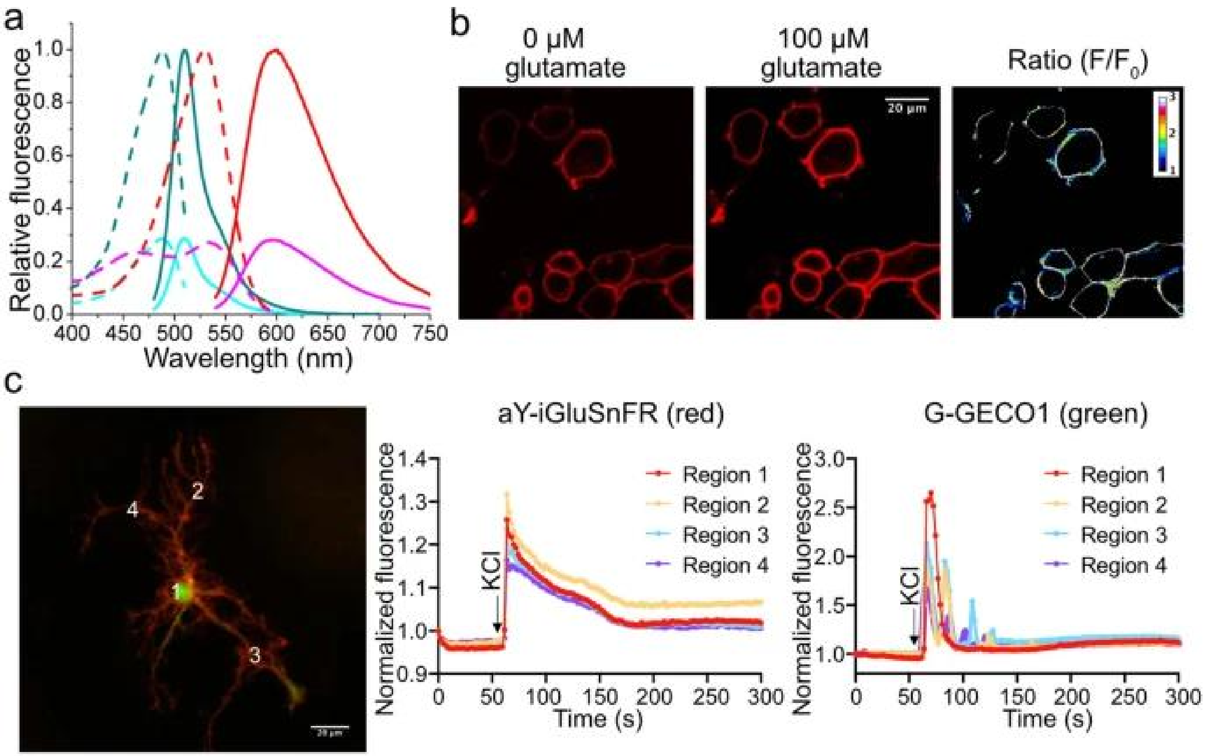


aY修饰的ZnGreen1（Zn 2+传感器）

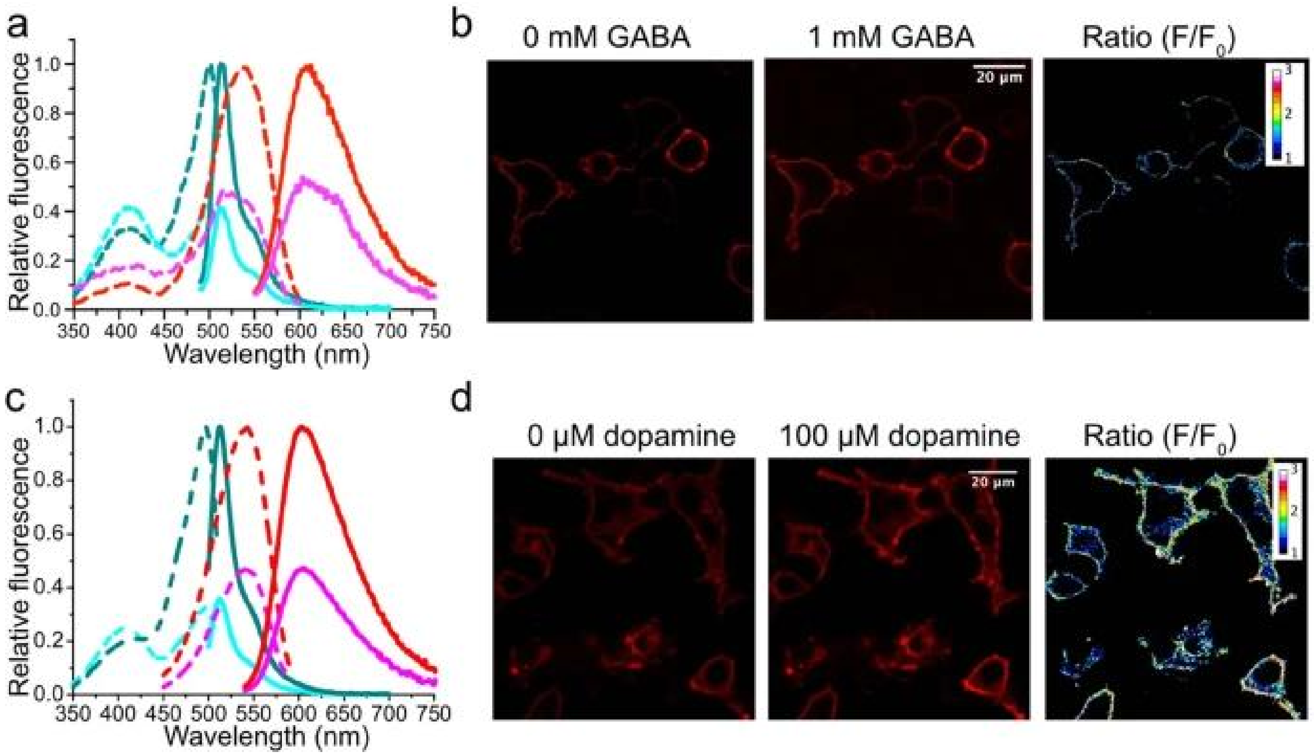


Results——aY引起的FPs和基于FP生物传感器的红移

aY修饰的iGluSnFR（谷氨酸传感器）

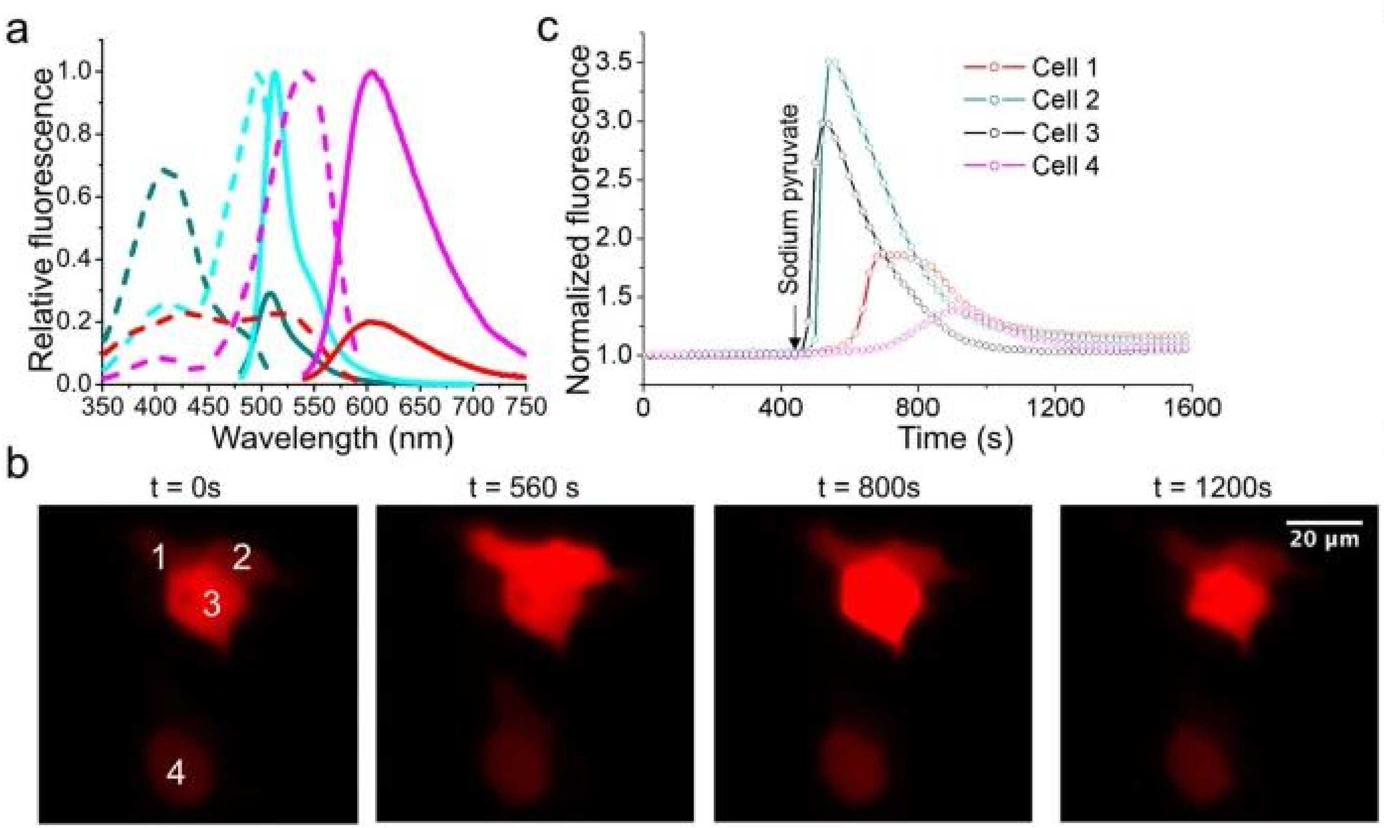


aY修饰iGABASnFR（a，b，GABA传感器）和dLight1.2（c，d，多巴胺传感器）

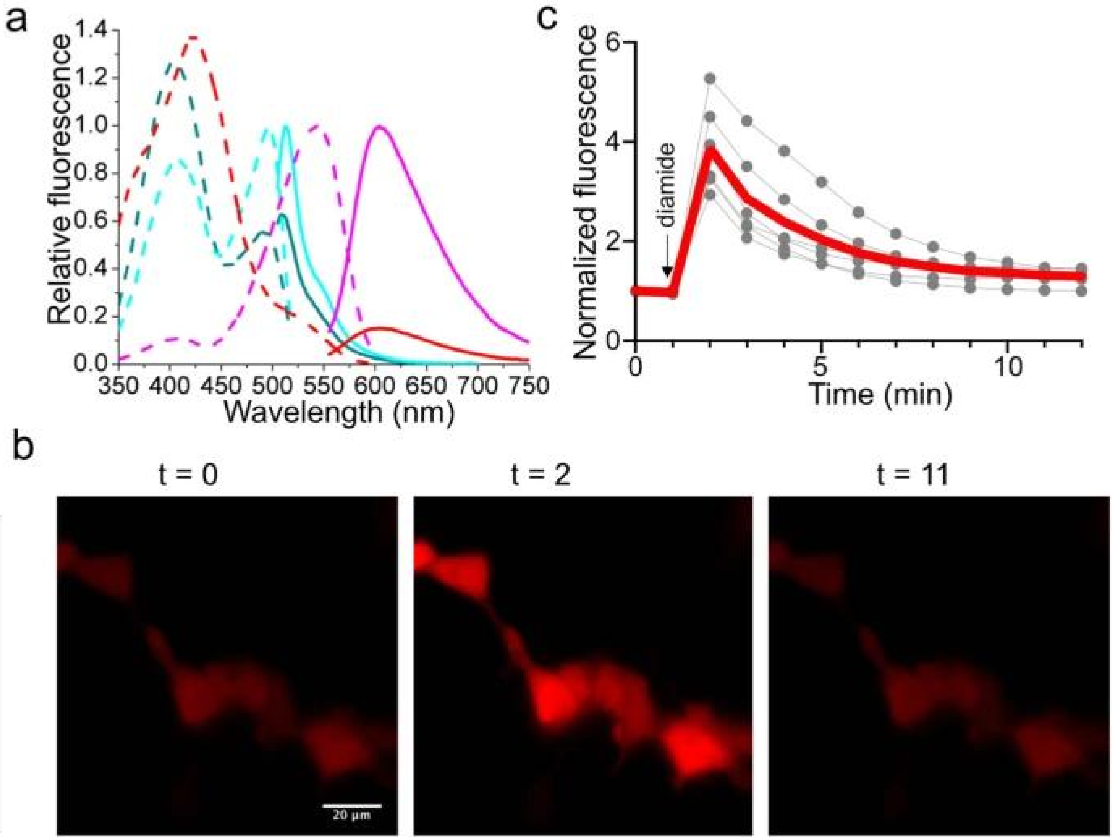


Results——aY引起的FPs和基于FP生物传感器的红移

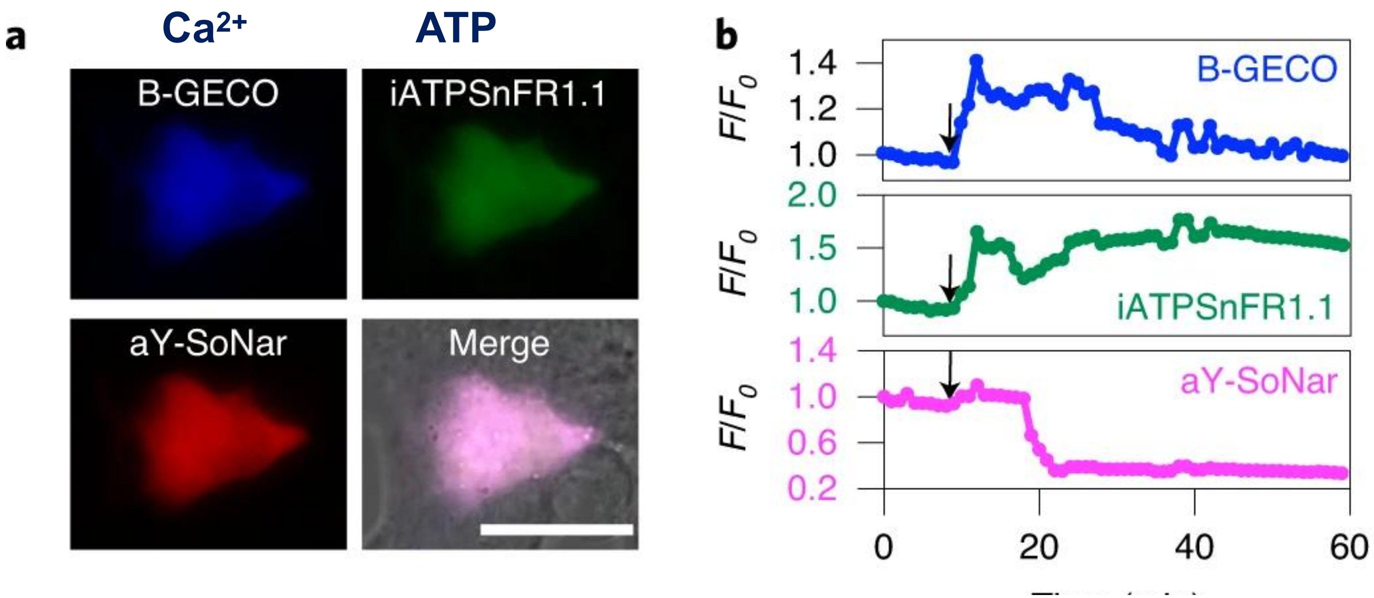
aY修饰的SoNar（NAD⁺ / NADH传感器）



aY修饰的iNap1（NADPH传感器）



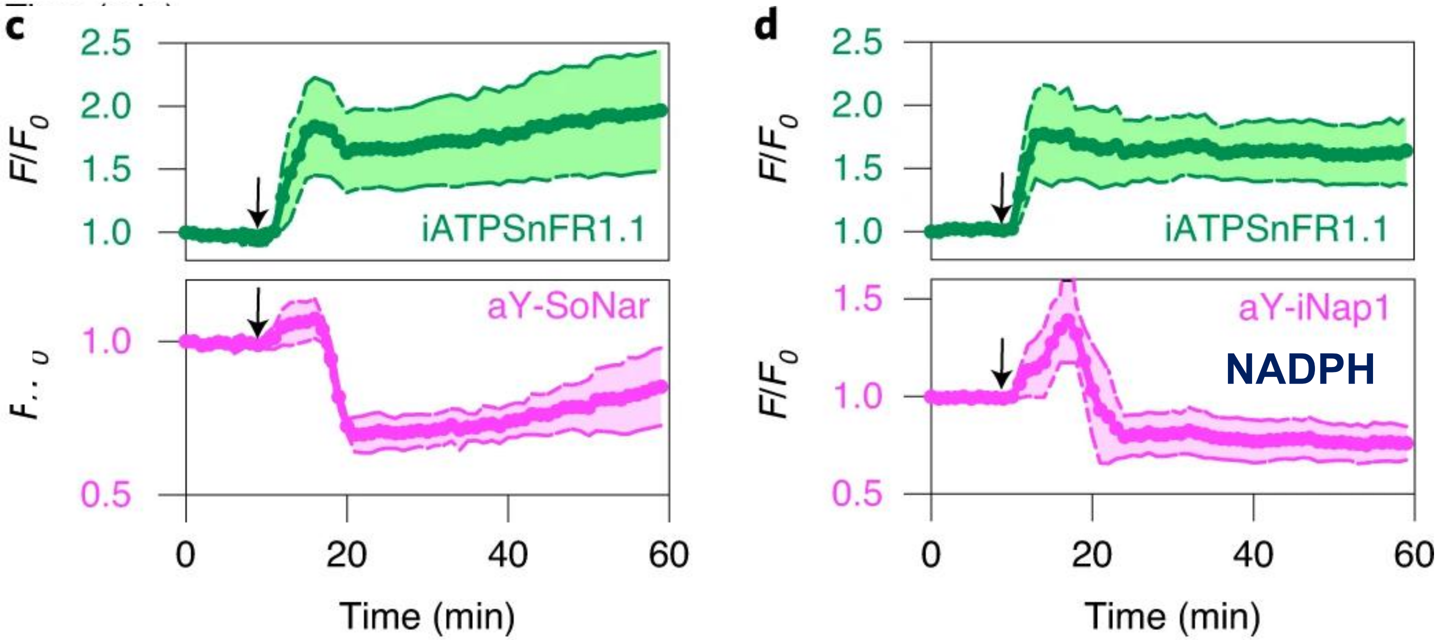
Results——MIN6胰腺β细胞代谢动力学的延时成像



Ca^{2+}、ATP⬆ 、NAD+/ NADH⬆ 后⬇

NAD+/ NADH

NADPH先⬇ 后⬆



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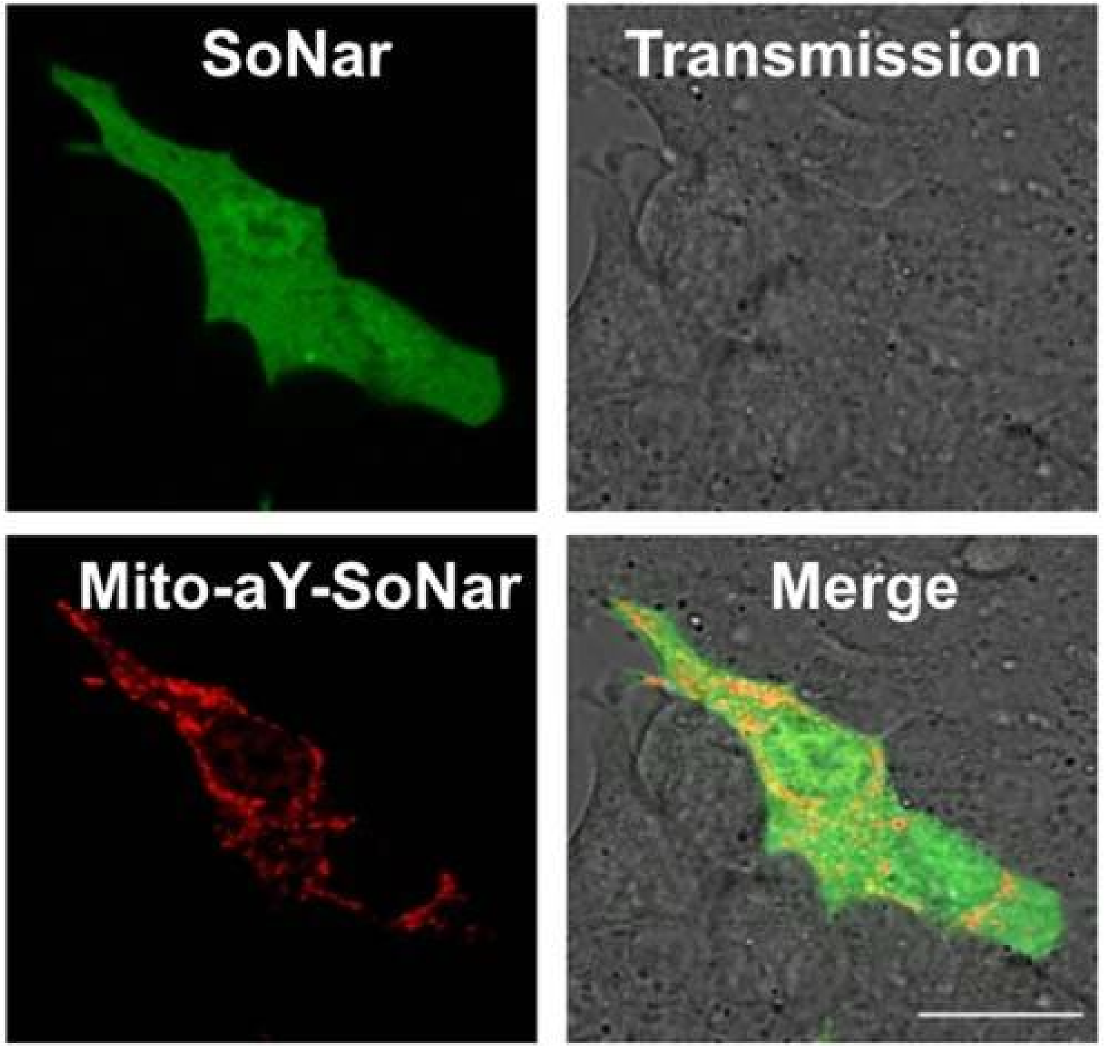
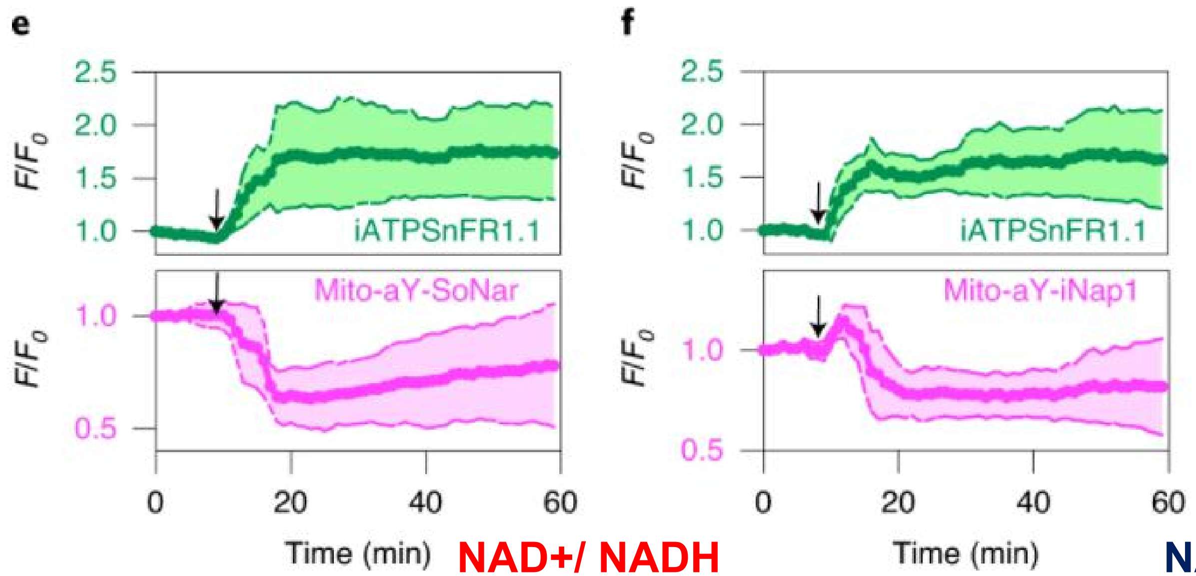
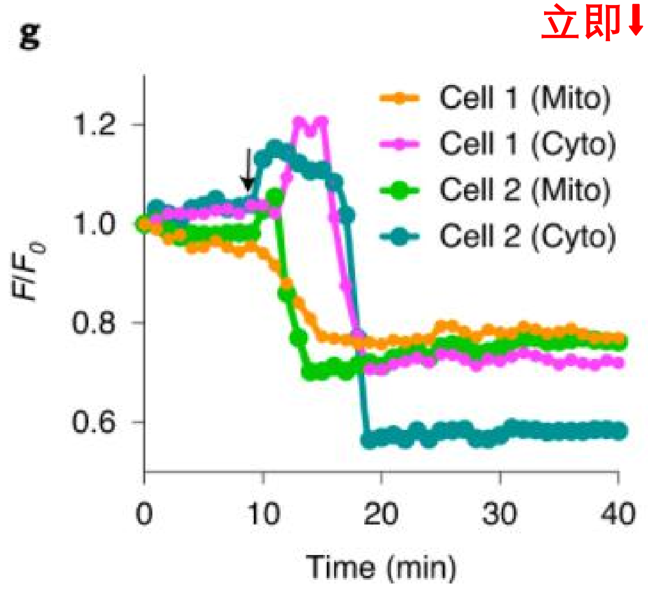




NAD+/ NADH
立即⬇

NADPH
先⬇ 后⬆



Cyto-SoNar、Mito-aY-SoNar

胞质NAD+/ NADH
的减少存在延迟，平
均延迟时间3.3Min

THANKS!