Literature Report

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ARTICLES

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Orthogonal fluorescent chemogenetic reporters for multicolor imaging

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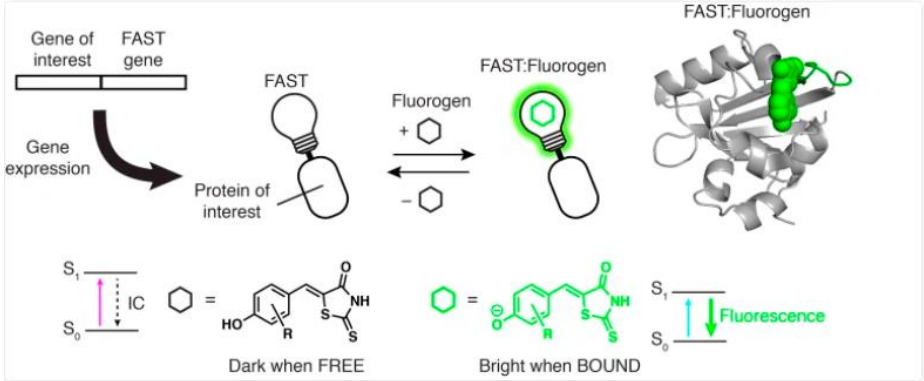
Arnaud Gautier

Professor of Chemistry at Sorbonne University, Paris, France – Investigator in the Laboratory of Biomolecules

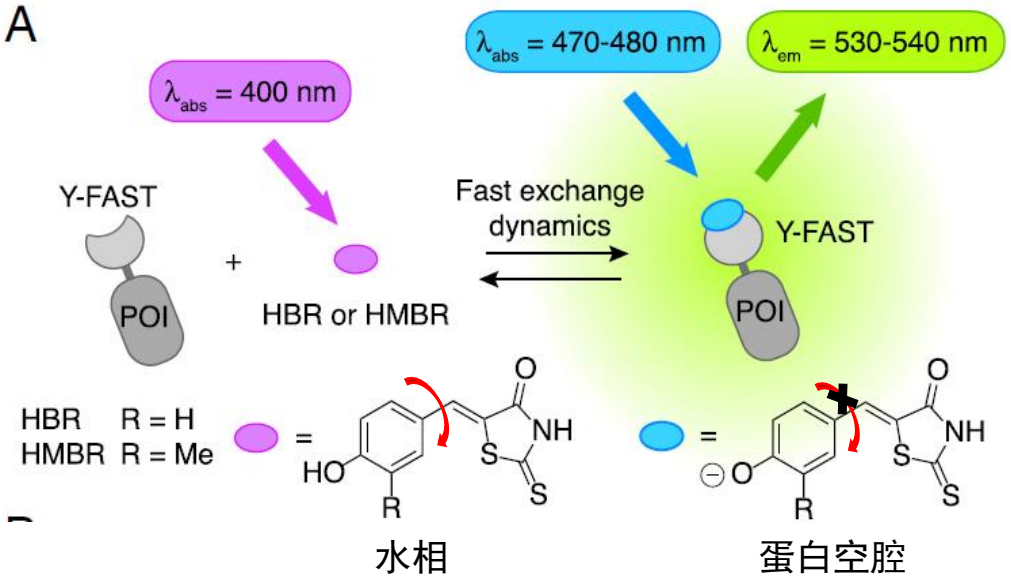
Inventing new tools for observing biomolecules and dynamic biochemical events in live cells and tissues.

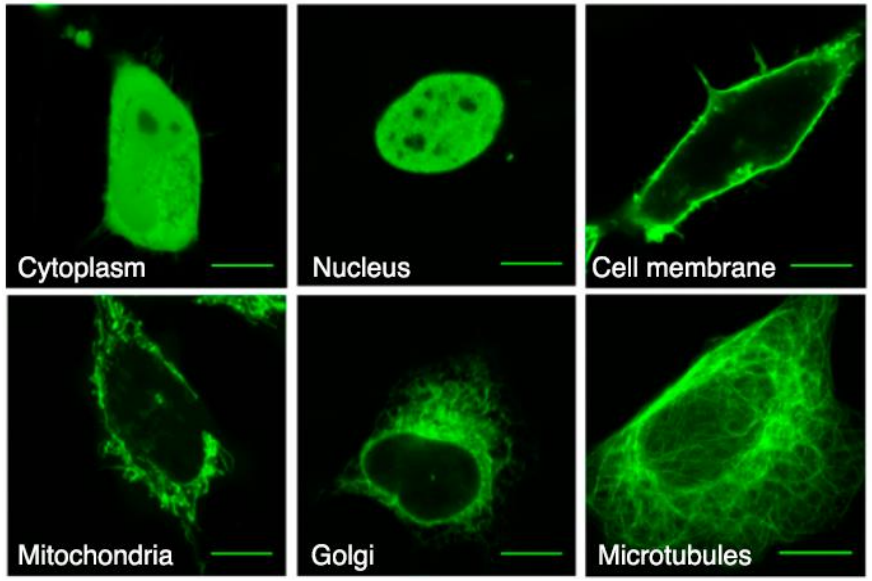
Protein tags: SNAP-, Halo-
FAST



previous work

Yellow Fluorescence-Activating and absorption-Shifting Tag (Y-FAST)

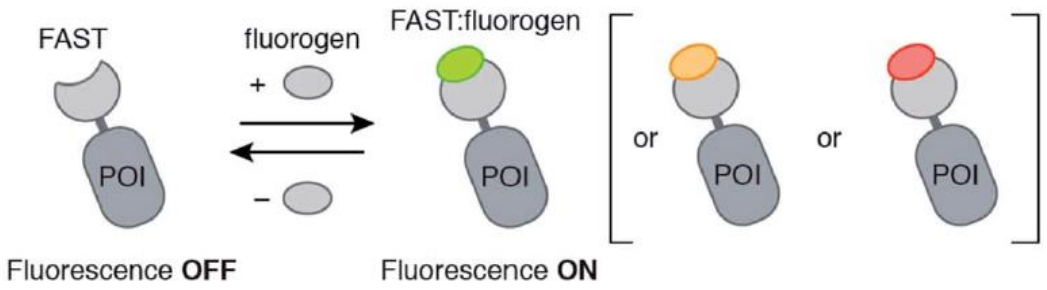


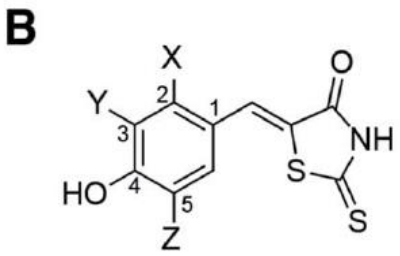


Compound	λ_{abs}, nm	λ_{em}, nm	ε, $M^{-1}\cdot cm^{-1}$	ϕ, %	K_D, μM	$10^{-7} \times k_{ON}$, $M^{-1}\cdot s^{-1}$	k_{OFF}, s^{-1}
HBR (pH 6.8)*	397	470	33,000	0.02			
HBR (pH 10.1)*	449	545	34,500	0.3			
Y-FAST:HBR (pH 7.4)	467	527	44,000	9	0.62 ± 0.05	3† (2.9 ± 0.4‡)	17† (8.5 ± 1.2‡)
HMBR (pH 5.8)§	401	480	29,500	0.04			
HMBR (pH 10.5)§	461	561	33,500	0.2			
Y-FAST:HMBR (pH 7.4)	481	540	45,000	33	0.13 ± 0.01	6.3 ± 0.9	6.3 ± 0.7

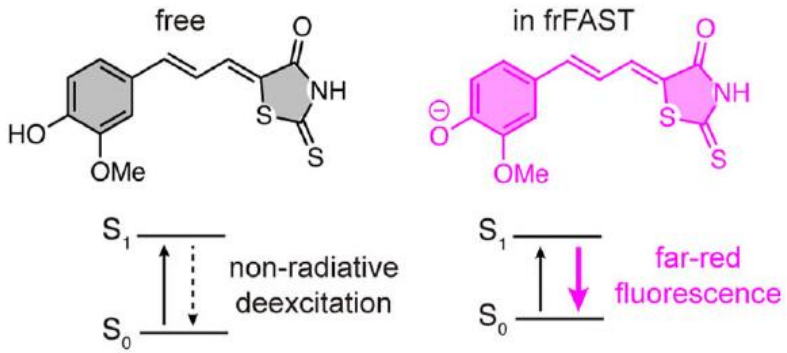
PNAS-2016-497

previous work





λ_{em} = 540-600 nm

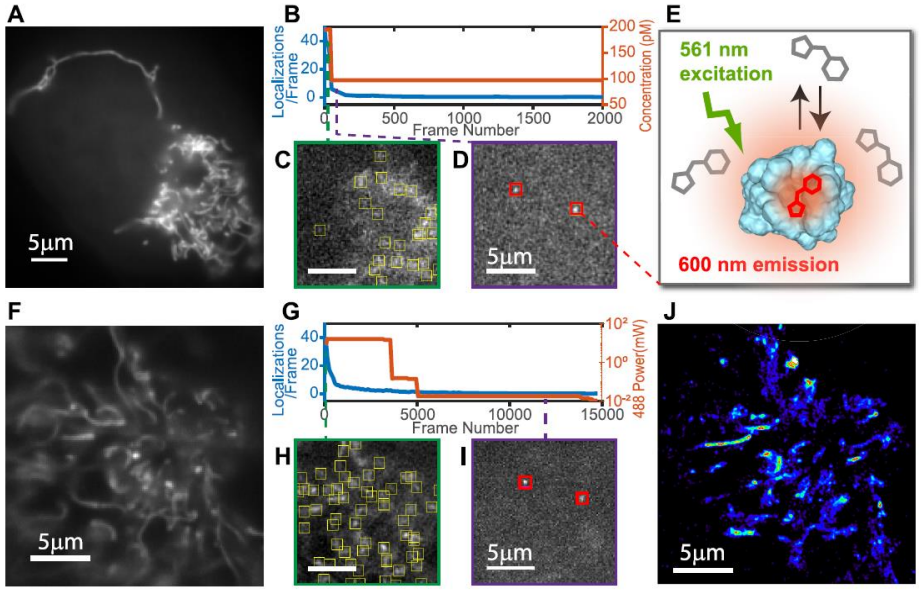


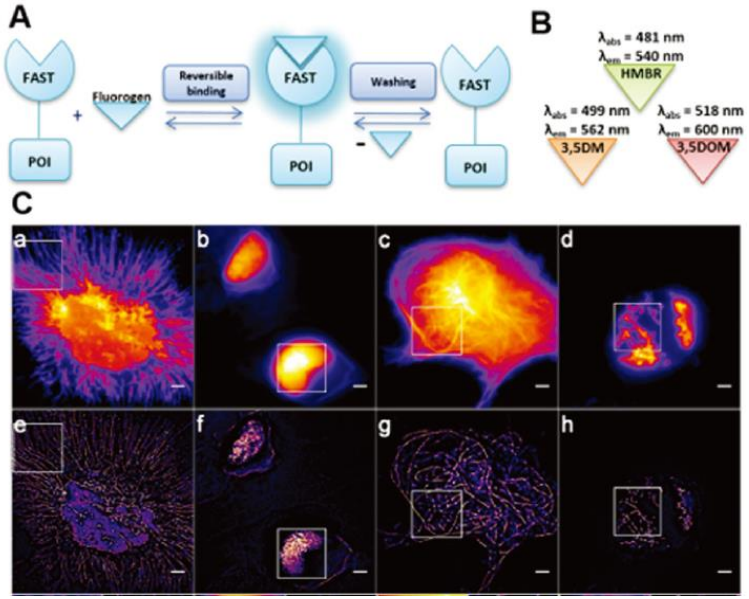
λ_{em} = 670 nm

of interest). (B) Structures of the fluorogens discussed in this study: HMBR (X = H, Y = Me, Z = H), HBR-3E (X = H, Y = Et, Z = H), HBR-2,5DM (X = Me, Y = H, Z = Me), HBR-3,5DM (X = H, Y = Me, Z = Me), HBR-3OM (X = H, Y = OMe, Z = H), HBR-3OE (X = H, Y = OEt, Z = H), and HBR-3,5DOM (X = H, Y = OMe, Z = OMe). (C–E) The absorption (dashed line, left axis) and emission (solid line, right axis) spectra of the free fluorogens (grey

Chem. Sci-2017-5598

Angew. Chem-2020-2



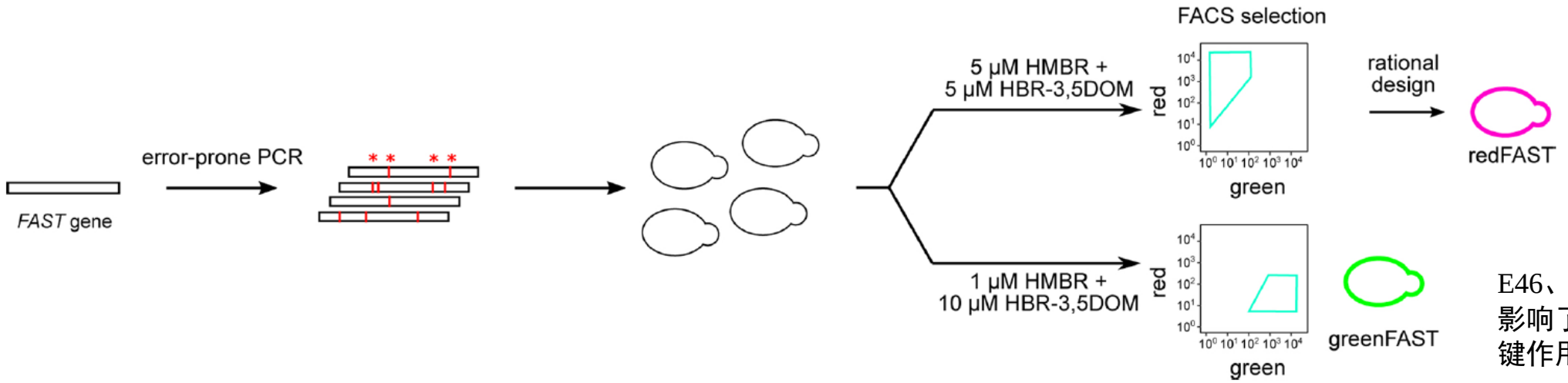


ACS Chem. Biol-2019-1115

Nanoscale-2019-3626

蛋白筛选

error-prone PCR :是在采用DNA聚合酶进行目的基因扩增时，通过调整反应条件，如提高镁离子浓度、加入锰离子、改变体系中四种的dNTPs浓度或运用低保真度DNA聚合酶等，来改变扩增过程中的突变频率，从而以一定的频率向目的基因中随机引入突变，获得蛋白质分子的随机突变体。



E46、R52残基发生突变，影响了与底物的结合的氢键作用力

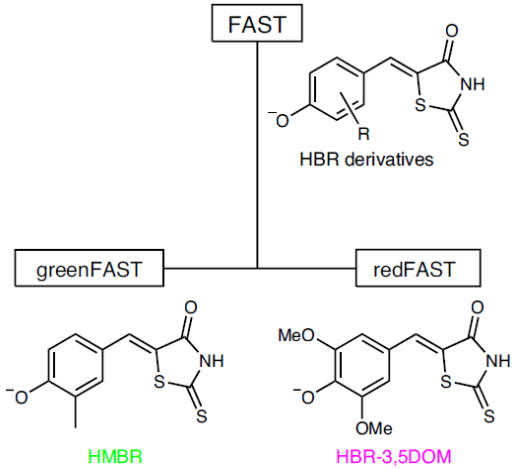
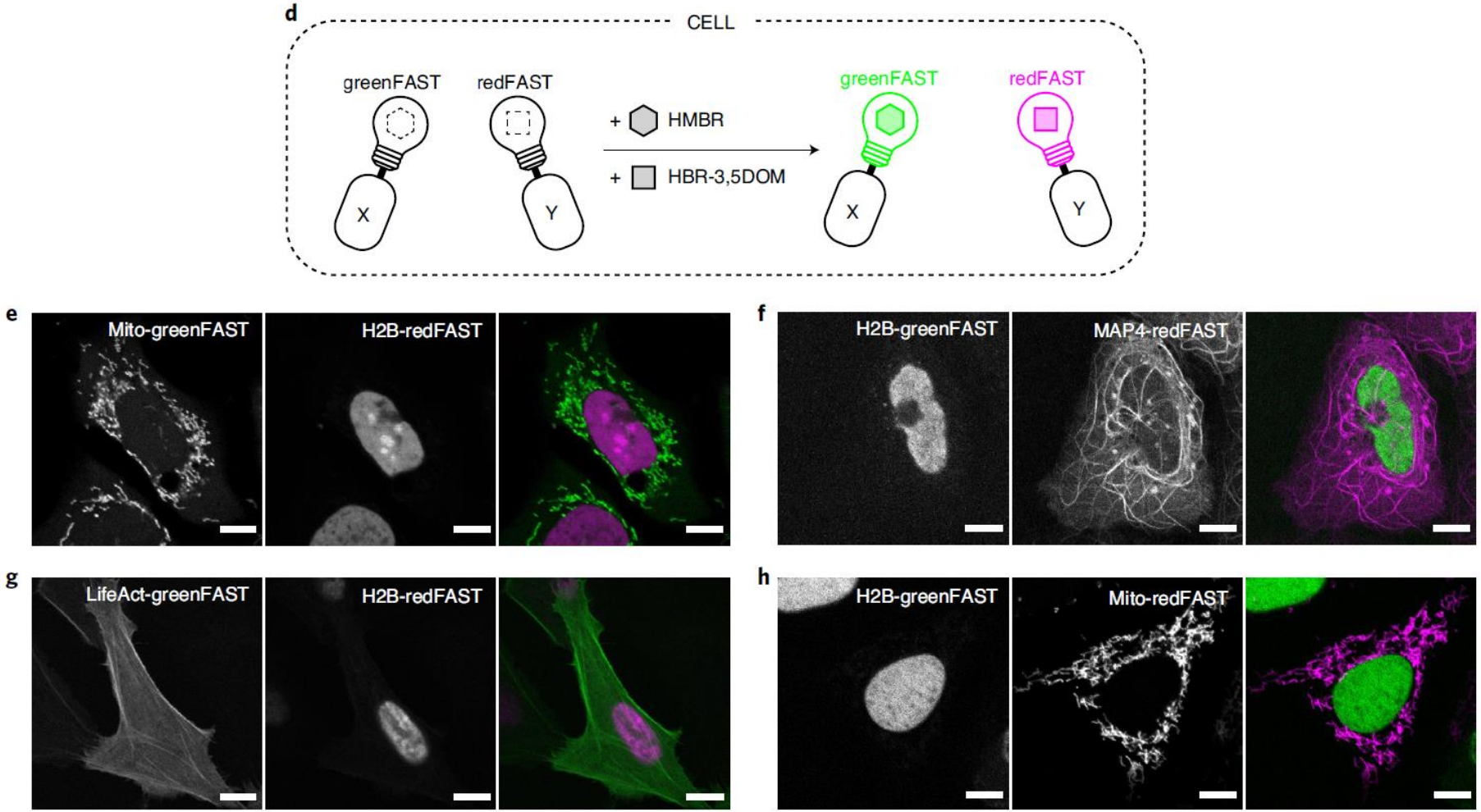


Table 1 | Physicochemical characteristics of FAST, iFAST, greenFAST and redFAST with HMBR and HBR-3,5DOM in PBS pH 7.4

Protein	HMBR					HBR-3,5DOM					$K_{d,HMBR}/K_{d,HBR-3,5DOM}$
	K_d (μM)	λ_{abs} (nm)	λ_{em} (nm)	ϕ	ε (mM^{-1}cm^{-1})	K_d (μM)	λ_{abs} (nm)	λ_{em} (nm)	ϕ	ε (mM^{-1}cm^{-1})	
FAST[7,8]	0.13	481	540	0.23	45	0.97	516	600	0.31	39	0.1
iFAST[9]	0.07	480	541	0.22	41	0.41	516	600	0.40	38	0.2
greenFAST	0.09	478	544	0.23	40	16.2	516	597	~0.29	ND	0.005
redFAST	12	502	554	~0.18	ND	1.3	556	603	0.29	43	9.2

多色成像

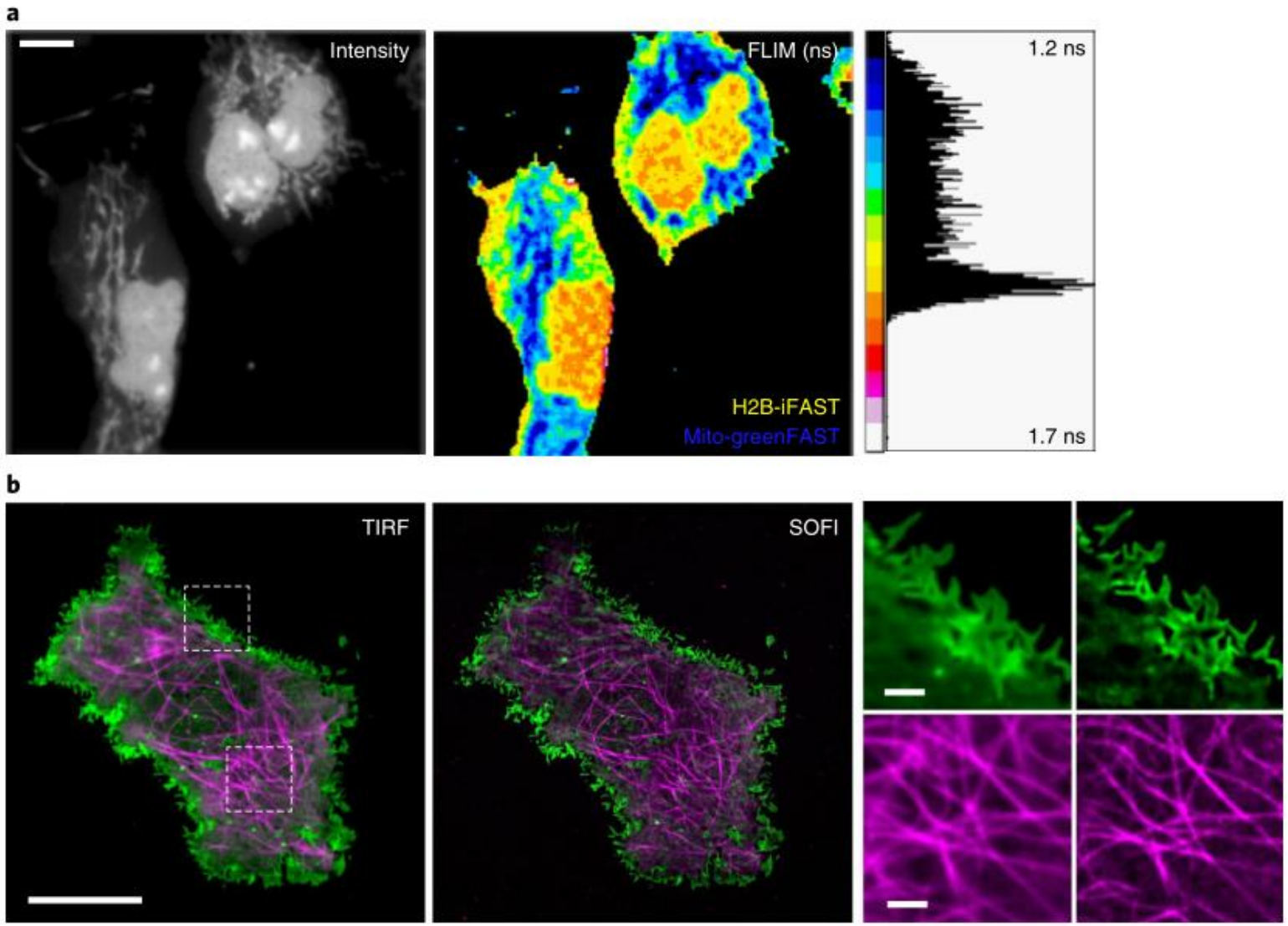


Two-color imaging of greenFAST and redFAST fusions in cells. **e**, Representative micrograph (n = 4 from one experiment) of U2OS cells expressing mito-greenFAST and H2B-redFAST. **f**, Representative micrograph (n = 10 from two experiments) of U2OS cells expressing H2B-greenFAST and MAP4-redFAST. **g**, Representative micrograph (n = 16 from three experiments) of U2OS cells expressing LifeAct-greenFAST and H2B-redFAST. **h**, Representative micrograph (n = 4 from one experiment) of U2OS cells expressing H2B-greenFAST and mito-redFAST. **e**–**h**, Cells were labeled with 5 µM of HMBR and 10 µM of HBR-3,5DOM. Scale bars, 10 µm.

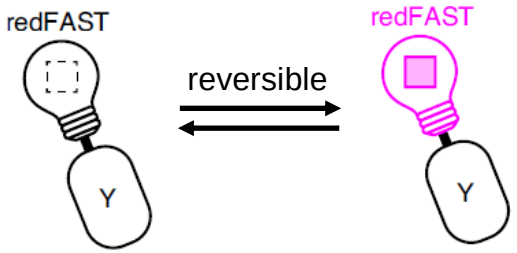
寿命成像及超分辨成像

寿命成像

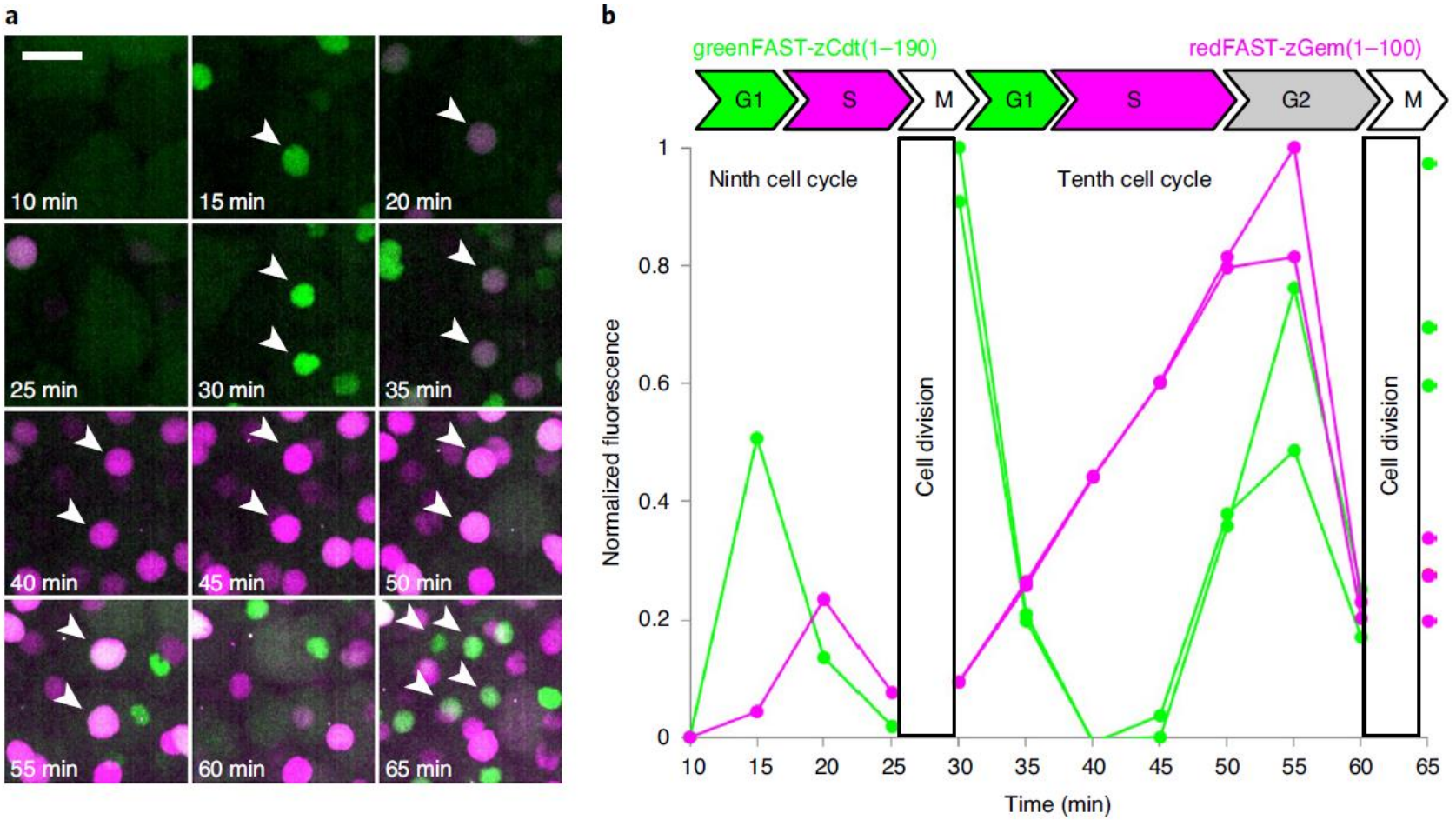
SOFI成像



GreenFAST and redFAST enable FlIM and SOFI imaging in live cells. a, Representative regular and FLIM micrographs (n = 6 from one experiment) of COS-7 cells expressing mito-greenFAST and H2B-iFAST labeled with 5 μM of HMBR. Scale bars, 5 μm. b, Representative averaged TIRF (left) and pcSOFI (right) micrographs (n = 17 from eight experiments) of COS-7 cells expressing lyn11-Skylan-S and MAP4-redFAST. Cells were labeled with 5 μM of HBR-3,5DOM. Scales bars, 10 μm (left) and 1 μm (right).



细胞周期成像

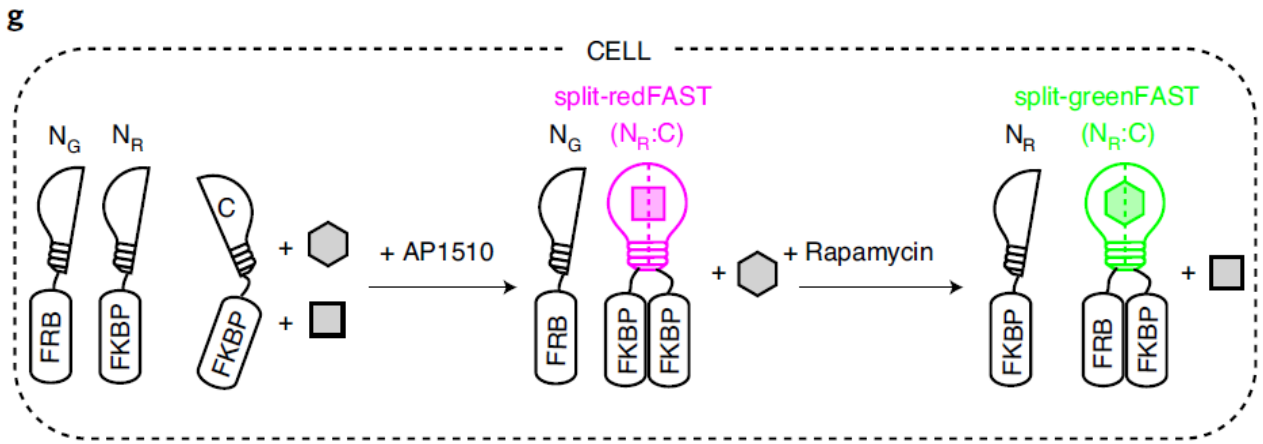


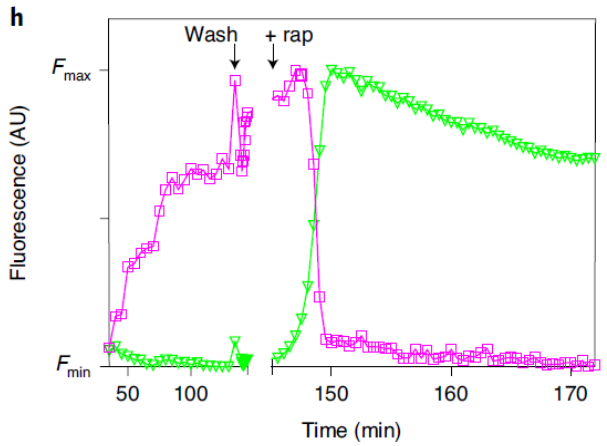
荧光泛素化细胞周期指示剂（缩写为FUCCI），这是一种基于荧光蛋白的探针，它将红色荧光蛋白（RFP）和绿色荧光蛋白（GFP）与不同的细胞周期因子Cdt1和geminin融合。这样，Cdt1和geminin就被泛素连接酶E3泛素化，继而降解。泛素连接酶的时间调节产生了Cdt1和geminin的双相周期。

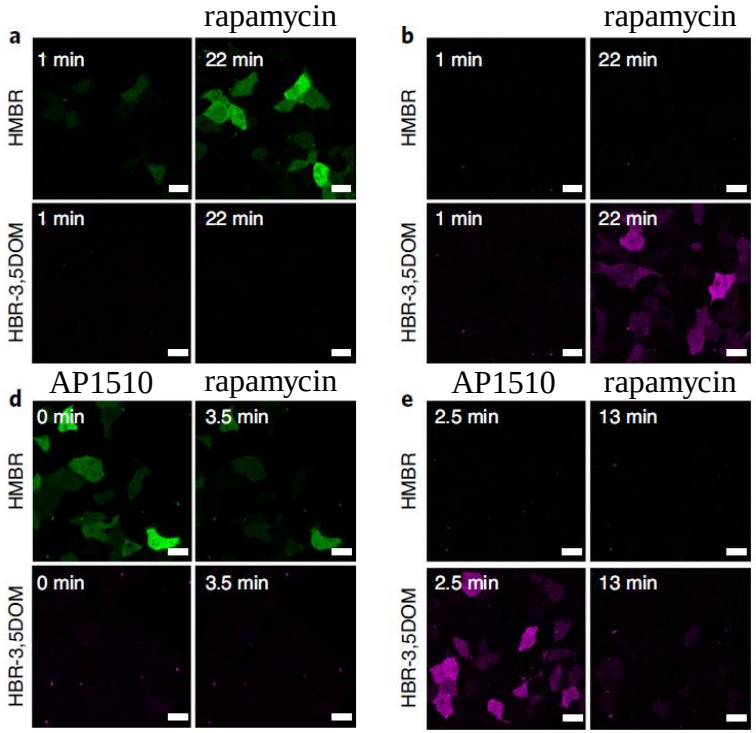
商业试剂限制：绿色荧光蛋白的成熟期太长，不能对早期的胚胎周期进行分析。

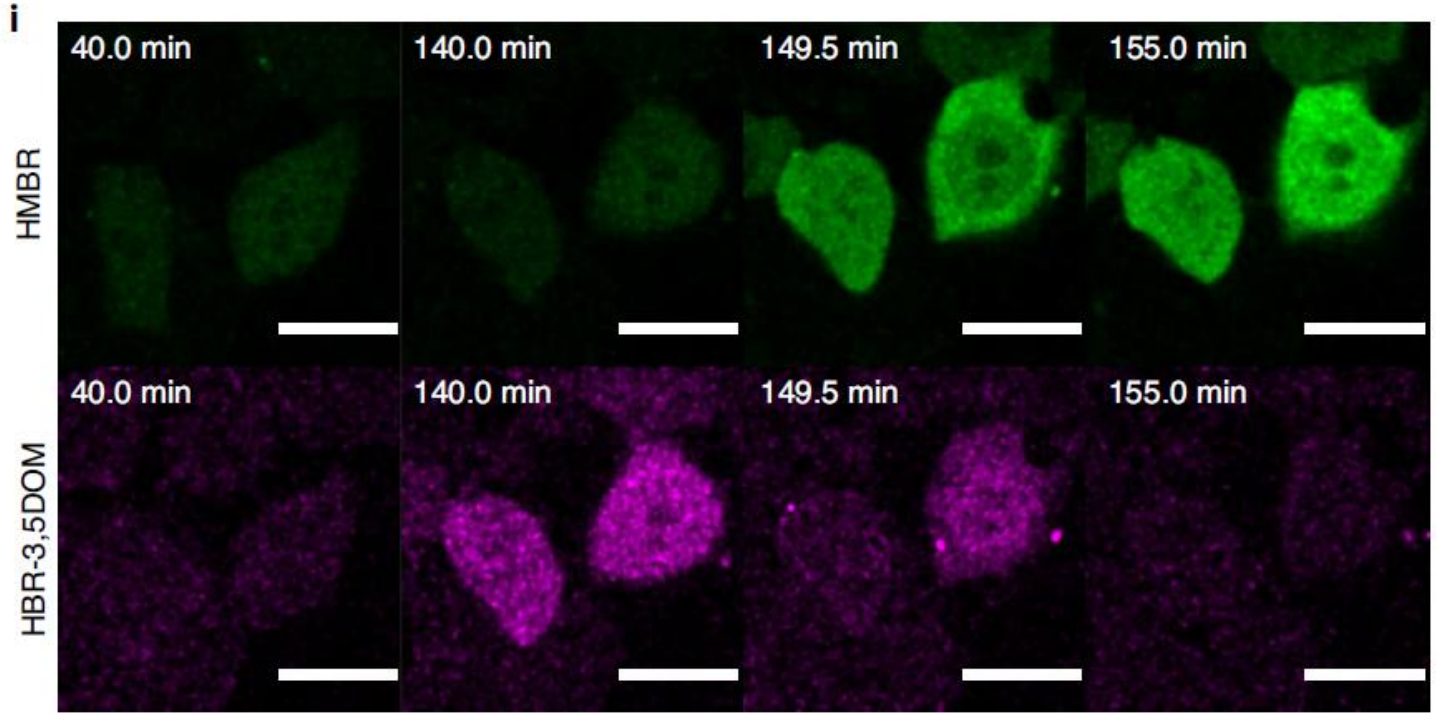
Cell cycle sensors based on greenFAST and redFAST. Zebrafish embryos were injected with redFAST-zGem(1–100)-P2A-greenFAST-zCdt1(1–190) mRNA at one-cell stage, and timelapse imaging was performed starting from 256-cell stage on embryos incubated with 5 μM of HMBR and 5 μM of HBR- 3,5DOM. **a**, Representative timelapse (*n* = 3 from three independent embryos) of single cells at high magnification. Scale bar, 20 μm. **b**, Corresponding quantification of fluorescence signal over time (cells with white arrows in **a**).

蛋白相互作用


g
CELL
N_G N_R
FRB
FKBP
C
FKBP
+ AP1510
split-redFAST
N_G (N_R:C)
FRB
FKBP
FKBP
+ Rapamycin
split-greenFAST
N_R (N_R:C)
FKBP
FRB
FKBP


h
Wash
+ rap
F_{max}
F_{min}
Fluorescence (AU)
50
100
150
160
170
Time (min)


a
rapamycin
1 min
22 min
HMBR
HBR-3,5DOM
b
rapamycin
d
AP1510
rapamycin
0 min
3.5 min
e
AP1510
rapamycin
2.5 min
13 min


i
40.0 min
140.0 min
149.5 min
155.0 min
HMBR
HBR-3,5DOM