Literature Report

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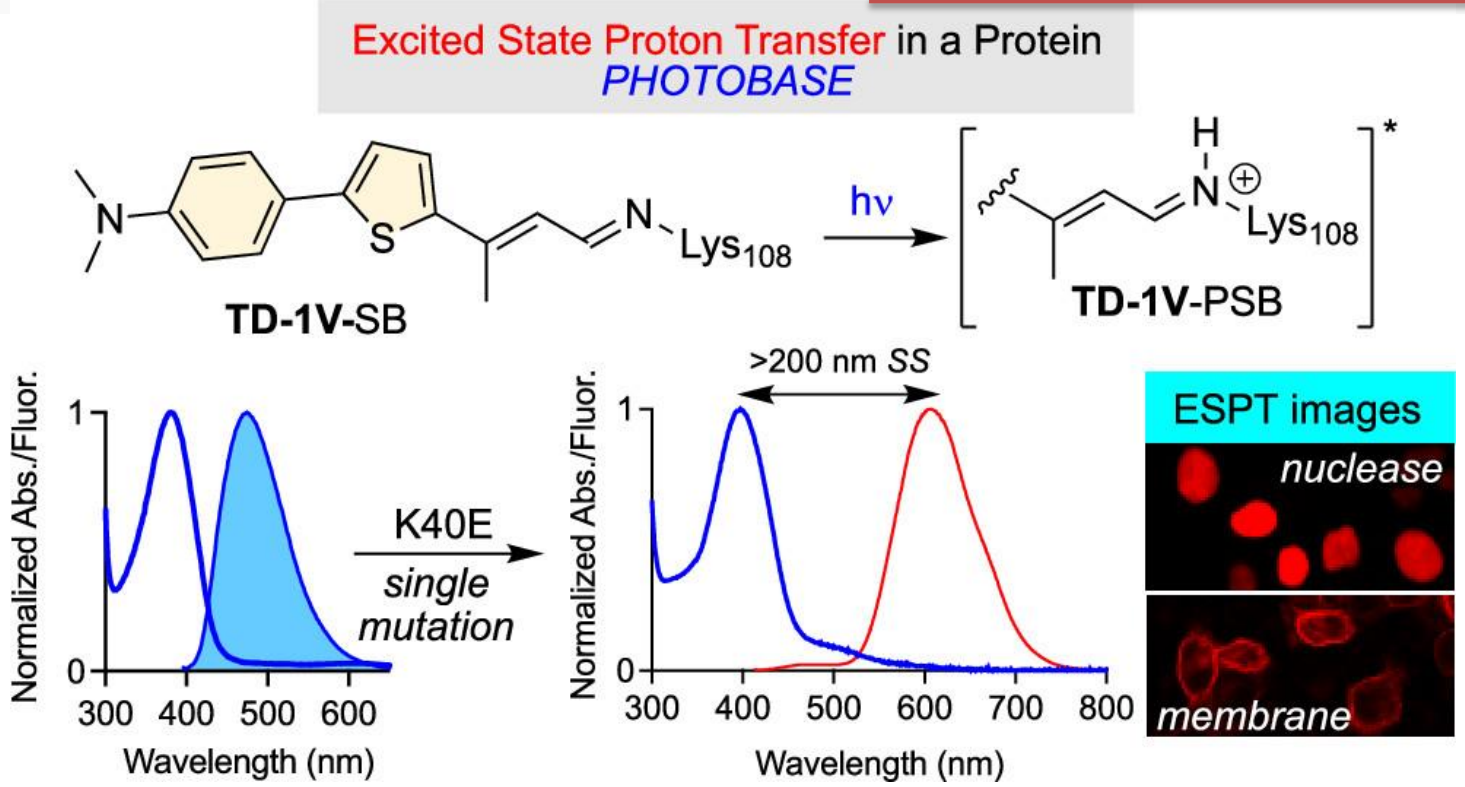
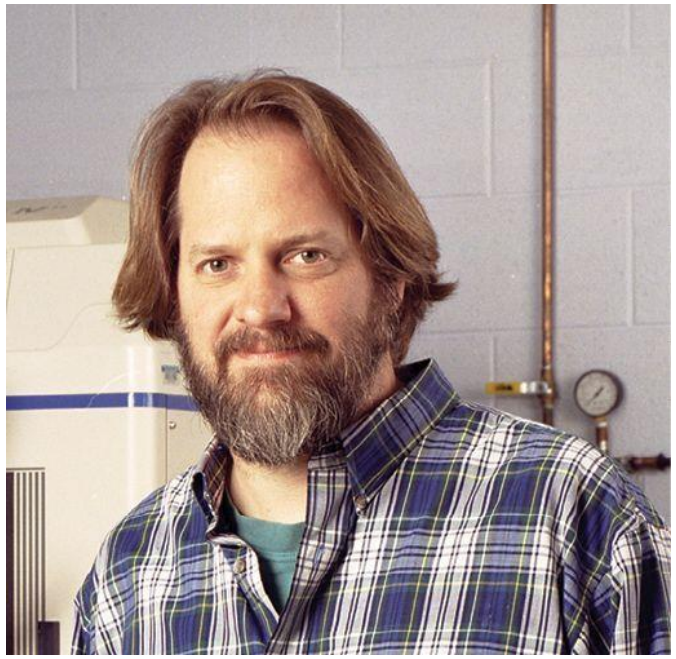
Article

Design of Large Stokes Shift Fluorescent Proteins Based on Excited State Proton Transfer of an Engineered Photobase

Elizabeth M. Santos,[‡] Wei Sheng,[‡] Rahele Esmatpour Salmani, Setare Tahmasebi Nick, Alireza Ghanbarpour, Hadi Gholami, Chrysoula Vasileiou, James H. Geiger,* and Babak Borhan*





James Geiger
Professor
Michigan State University
Department of Chemistry

Research interests

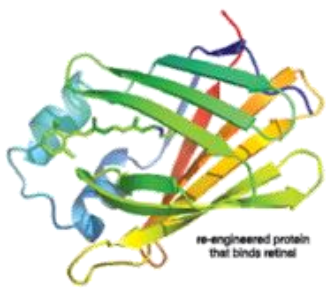
- Structural Biology Using X-Ray Crystallography

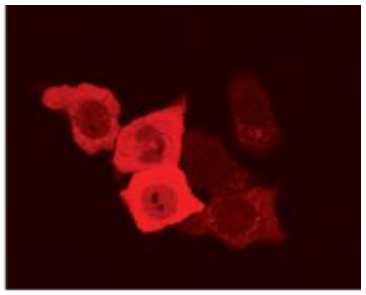
1. Structure and mechanism of enzymes.
2. Visualizing rhodopsin mimics at atomic resolution.
3. Engineering domain swapped dimers and the creation of new allosteric proteins.

Babak Borhan
Professor
Michigan State University
Department of Chemistry

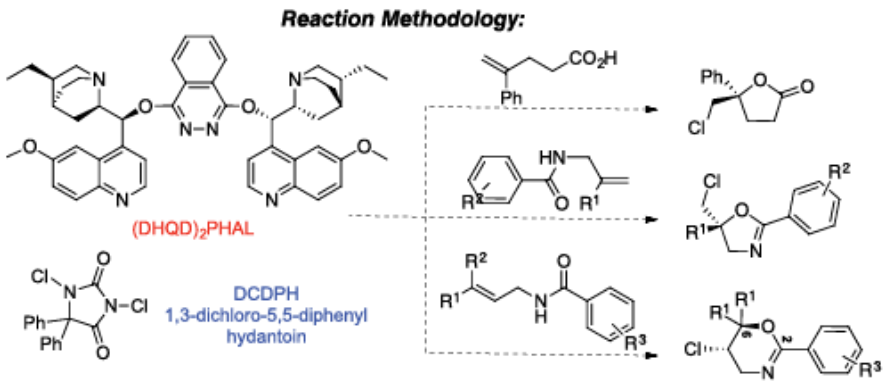
Research interests

- ➢ Bioorganic Chemistry
  - Design protein mimics

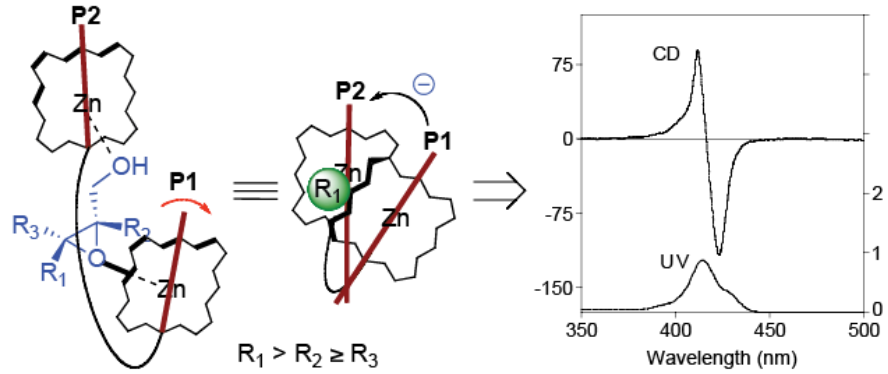




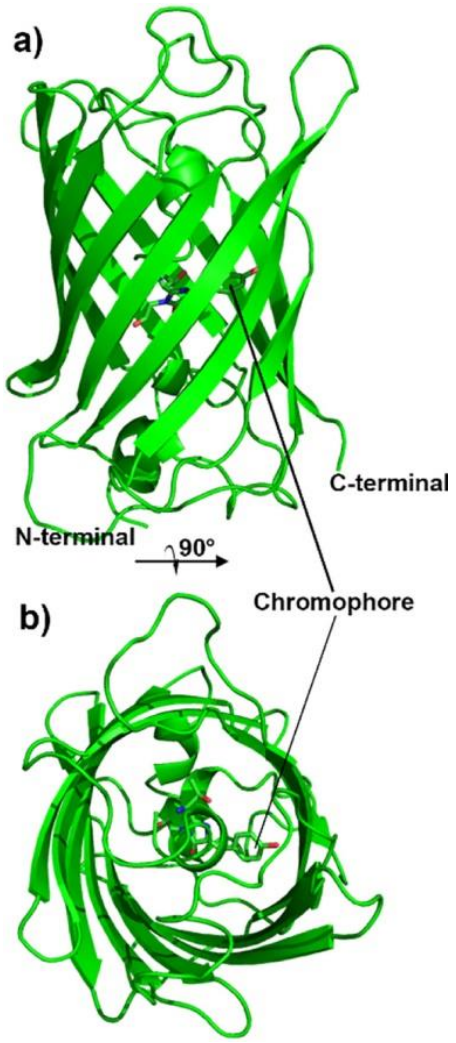
- ➢ Synthetic Chemistry
  - Develop new reactions



- ➢ Organic Spectroscopy
  - Develop host/guest systems for absolute stereochemical determination of chiral compounds



研究背景

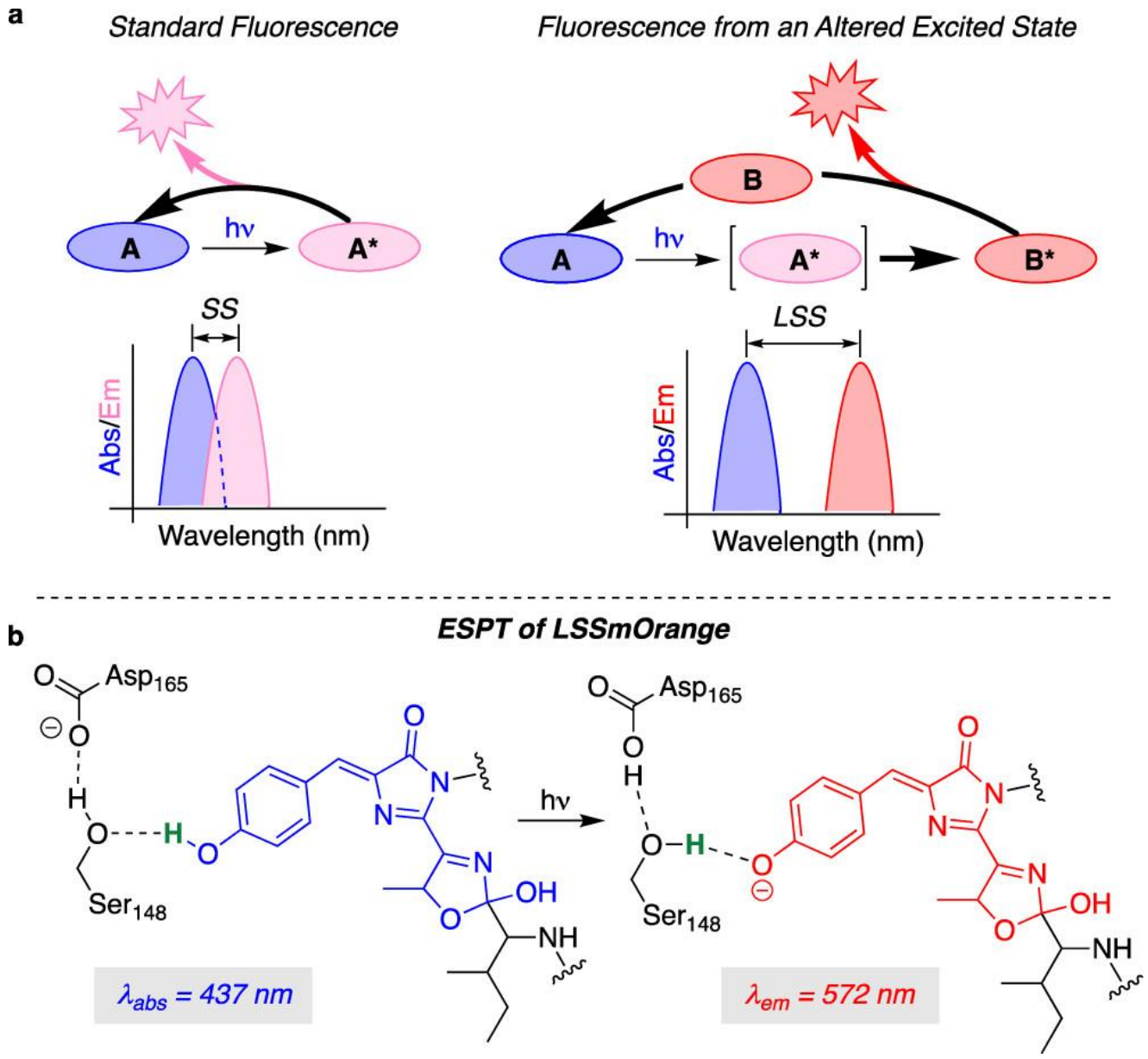


- 荧光蛋白优化目标：高的光稳定性、长波长发射、高荧光量子产率(QY)、高摩尔消光系数(ε)和大斯托克斯位移(≥100 nm)。

优点：
减少自吸收和内滤效应产生的干扰，有利于单激发多色成像实验的进行。

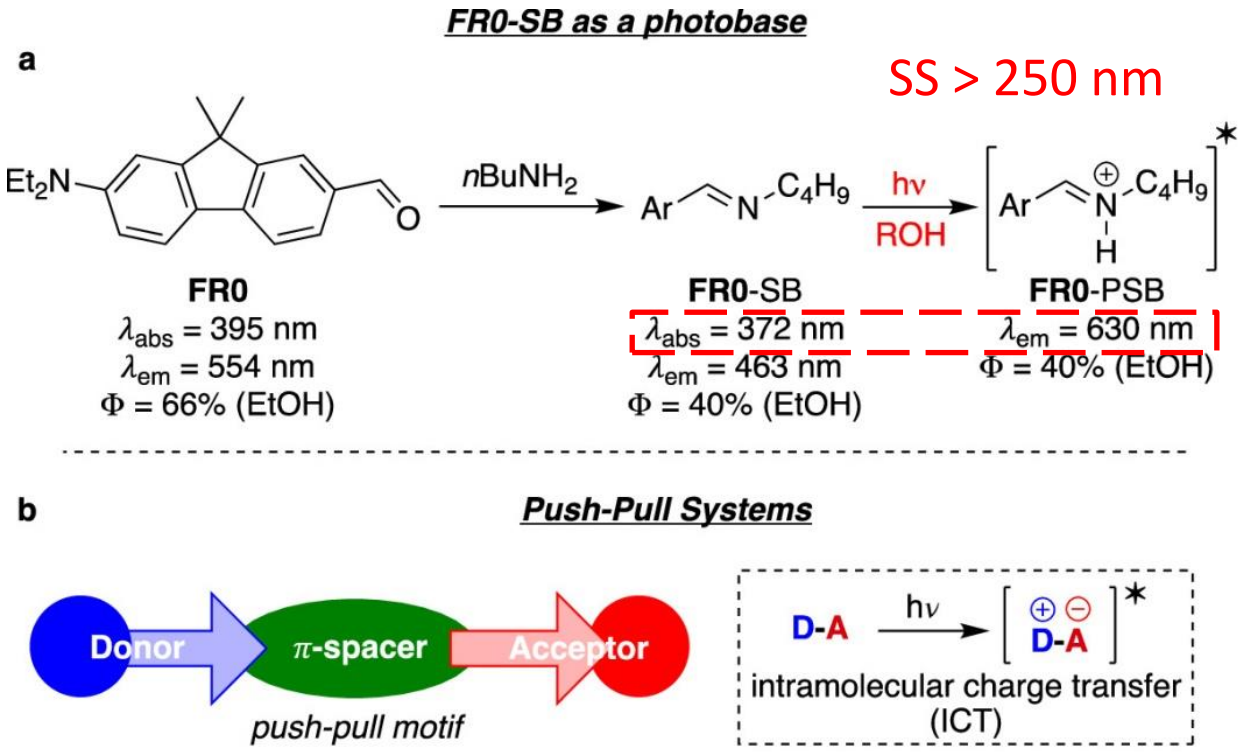
Adv Colloid Interface Sci, 2020, 285, 102286

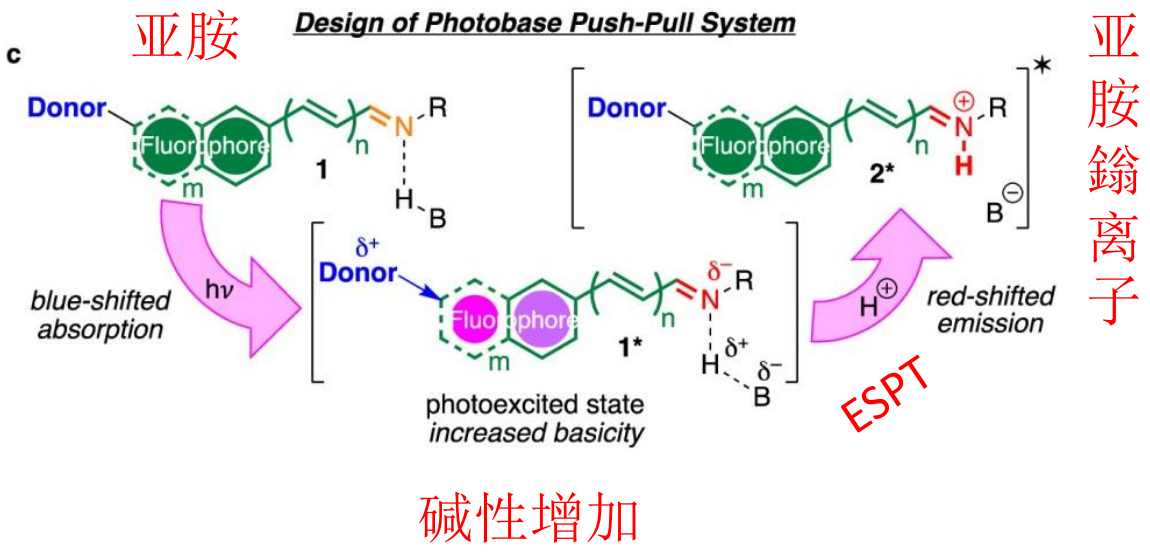
研究背景



成功关键是在酚羟基附近放置氢键受体以促进ESPT

设计策略



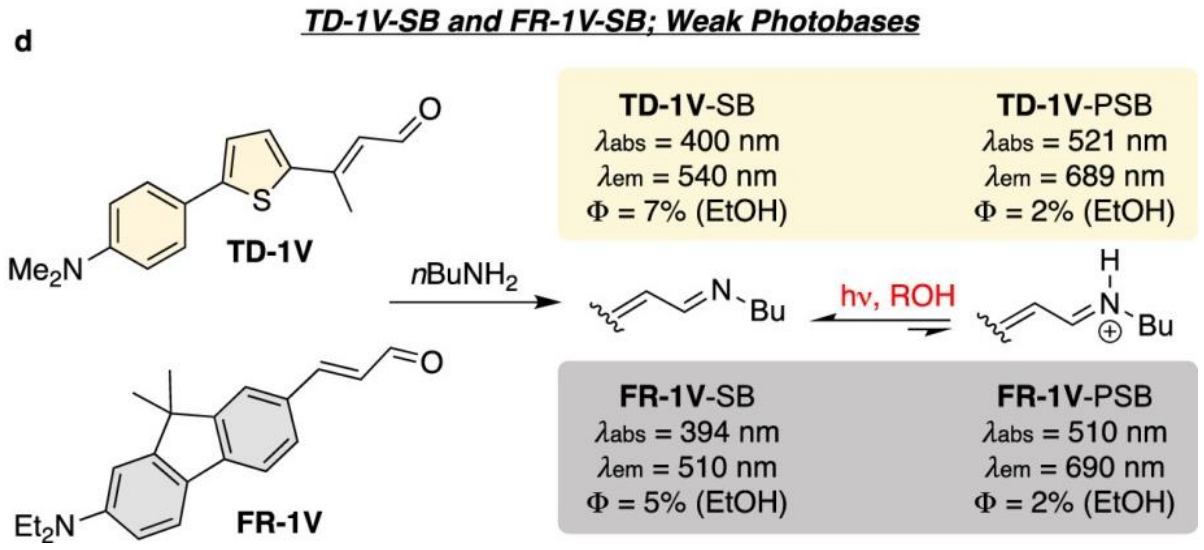


如果弱酸或弱碱在光激发下表现出其酸性（$pK_a^* < pK_a$）或碱性（$pK_a^* > pK_a$）的增加，那么就称之为光酸或光碱。

荧光染料初选

如何实现荧光蛋白的选择性？

1. 形成的亚胺系统在质子溶剂中表现出弱的ESPT。
2. 加速蛋白质/荧光团相对于非特异性亚胺形成的动力学。
3. 控制目标蛋白结合口袋中所得亚胺的 pK_a 。



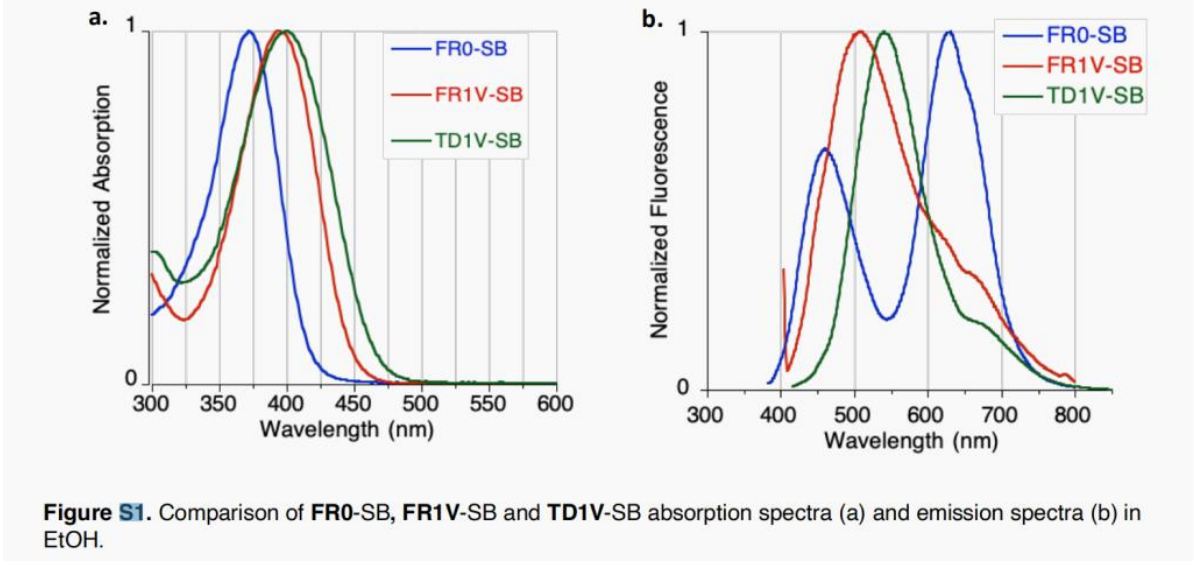
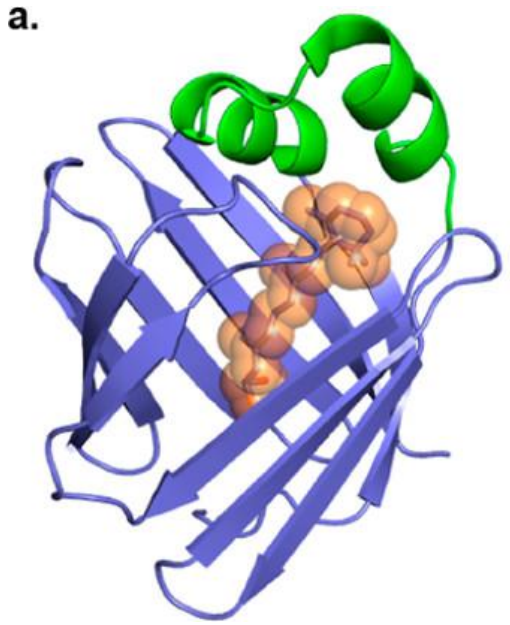


Figure S1. Comparison of **FR0**-SB, **FR1V**-SB and **TD1V**-SB absorption spectra (a) and emission spectra (b) in EtOH.

蛋白质突变体筛选



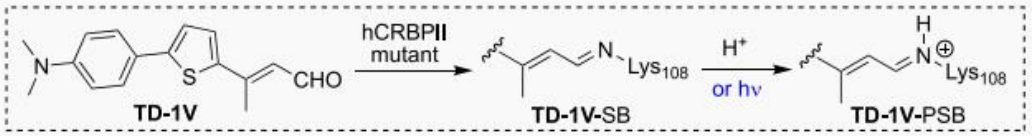
人类细胞视黄醇结合蛋白 II（hCRBPII）

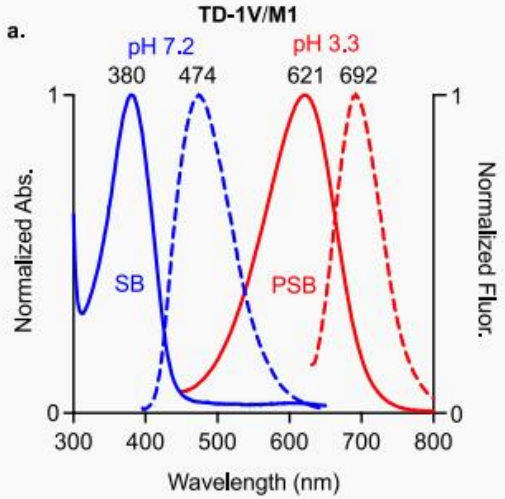
- 小的、具有高表达率和出色结构弹性的胞质蛋白。
- 该蛋白质众多突变体的结构稳定。

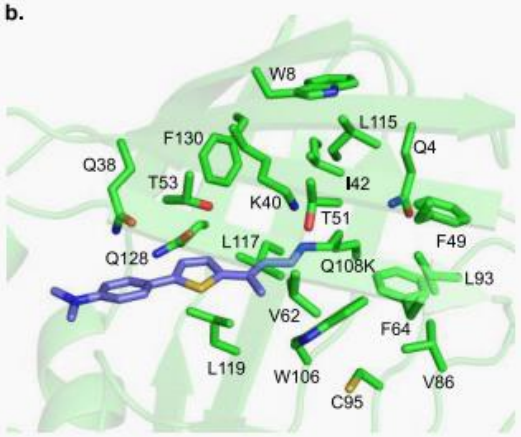
对hCRBPII蛋白质突变体的要求：

1. 含有活性位点赖氨酸残基，与荧光染料结合形成亚胺。
2. 含有为ESPT过程提供质子的氨基酸残基，且位置妥当，既可以避免亚胺在基态被质子化，又可以促进蛋白腔中高水平ESPT的发生。

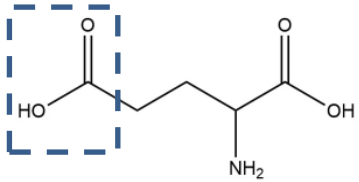
蛋白质突变体筛选







引入谷氨酸残基作为质子供体。



谷氨酸（E）

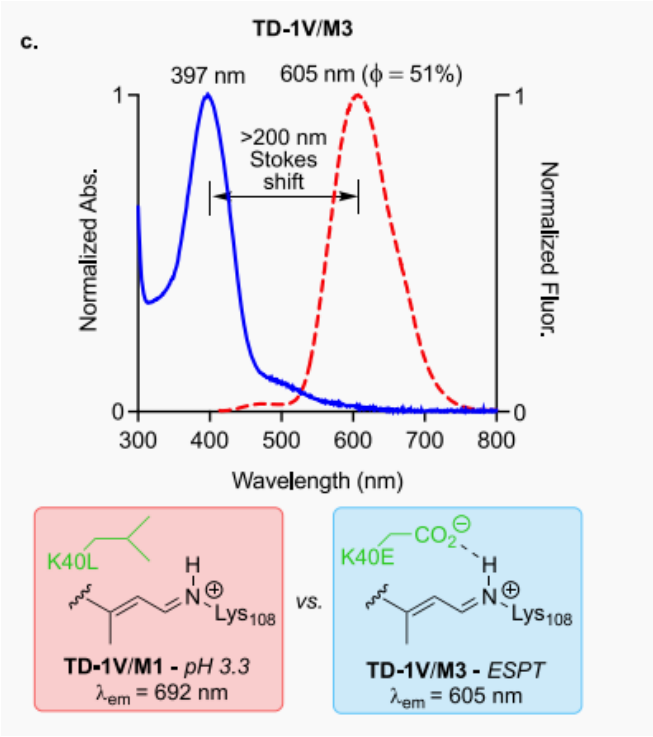
Table 1. Spectroscopic Properties TD-1V/hCRBPII Mutant Complexes

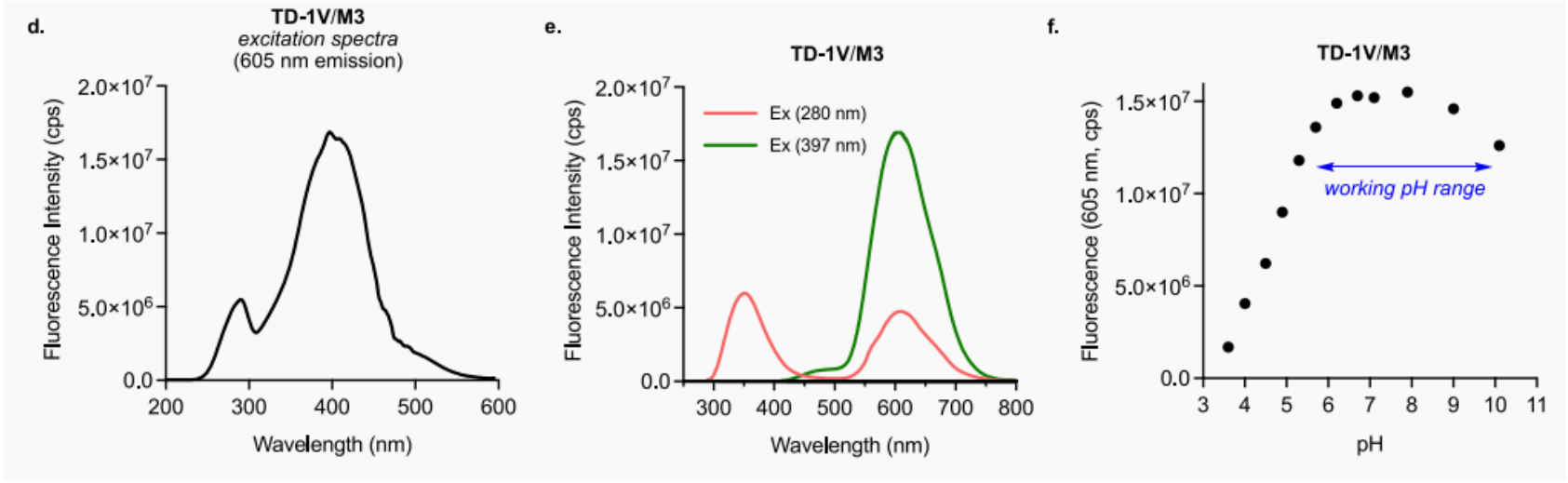
mutant	hCRBPII mutant[a]	λ_{abs}	$\lambda_{em\ (SB)}$	$\lambda_{em\ (PSB)}$	Φ^b	Φ_{ESPT}
M1	Q108 K:K40L:T53A:R58L:Q38F:Q4F	379	474	n.d.	0.03	<0.02
M2	Q108 K:K40L:T53A:R58L:**Q38E**:Q4F	389	485	n.d.	0.05	<0.02
M3	Q108 K:**K40E**:T53A:R58L:Q38F:Q4F	397	n.d.	605	0.51	>0.99
M4	Q108 K:K40L:T53A:R58L:Q38F:Q4F:**I42E**	406	n.d.	603	0.41	0.97
M5	Q108 K:K40L:T53A:R58L:Q38F:Q4F:**T51E**	399	n.d.	602	0.12	0.86
M6	Q108 K:K40L:**T53E**:R58L:Q38F:Q4F	387	480	n.d.	0.04	<0.02
M7	Q108 K:K40L:T53A:R58L:Q38F:Q4F:**V62E**	386	474	598	0.08	0.27
M8	Q108 K:K40L:T53A:R58L:Q38F:Q4F:**L117E**	391	486	653	0.13	0.73
M9	Q108 K:K40L:T53A:R58L:Q38F:Q4F:**L119E**	384	479	n.d.	0.05	0.13
M10	Q108 K:K40L:T53A:R58L:Q38F:Q4F:**Q128E**	385	473	n.d.	0.05	<0.02
M11	Q108 K:**K40E**:T53A:R58W:Q38F:Q4F:Y19W	392	n.d.	621	0.43	>0.99
M12	Q108 K:**K40D**:T53A:R58L:Q38F:Q4F	393	481	639	0.34	0.81

[a]20 μM protein and 0.5 equiv **TD-1V** at pH 7.2. [b]Absolute quantum yield was measured on a Quantaurus-QY. Not detected (n.d.).

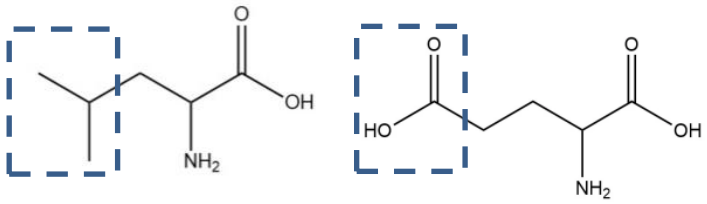
将距离亚胺氮原子10埃内的残基突变为谷氨酸获得的可溶性突变蛋白

蛋白质突变体筛选





280 nm处的吸收可能属于 hCRBPII中的Trp残基



亮氨酸（L） 谷氨酸（E）

正电荷沿π体系的共轭减少

蛋白质突变体筛选

Table 1. Spectroscopic Properties TD-1V/hCRBPII Mutant Complexes

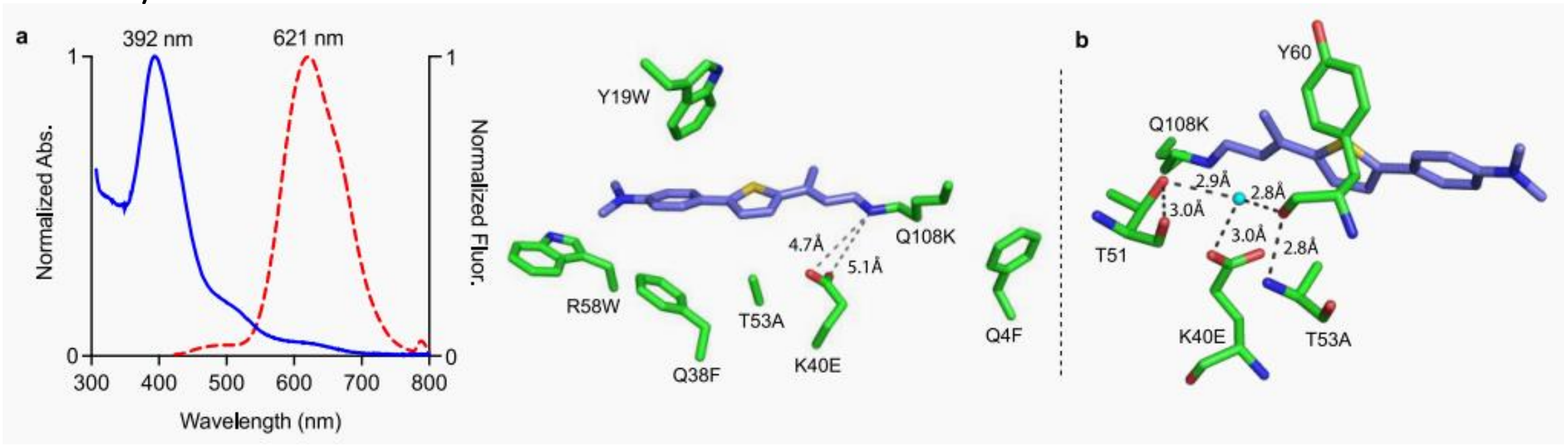
mutant	hCRBPII mutant[a]	λ_{abs}	$\lambda_{em\ (SB)}$	$\lambda_{em\ (PSB)}$	Φ^{b}	Φ_{ESPT}
M1	Q108 K:K40L:T53A:R58L:Q38F:Q4F	379	474	n.d.	0.03	<0.02
M2	Q108 K:K40L:T53A:R58L:**Q38E**:Q4F	389	485	n.d.	0.05	<0.02
M3	Q108 K:**K40E**:T53A:R58L:Q38F:Q4F	397	n.d.	605	0.51	>0.99
M4	Q108 K:K40L:T53A:R58L:Q38F:Q4F:**I42E**	406	n.d.	603	0.41	0.97
M5	Q108 K:K40L:T53A:R58L:Q38F:Q4F:**T51E**	399	n.d.	602	0.12	0.86
M6	Q108 K:K40L:**T53E**:R58L:Q38F:Q4F	387	480	n.d.	0.04	<0.02
M7	Q108 K:K40L:T53A:R58L:Q38F:Q4F:**V62E**	386	474	598	0.08	0.27
M8	Q108 K:K40L:T53A:R58L:Q38F:Q4F:**L117E**	391	486	653	0.13	0.73
M9	Q108 K:K40L:T53A:R58L:Q38F:Q4F:**L119E**	384	479	n.d.	0.05	0.13
M10	Q108 K:K40L:T53A:R58L:Q38F:Q4F:**Q128E**	385	473	n.d.	0.05	<0.02
M11	Q108 K:**K40E**:T53A:R58W:Q38F:Q4F:Y19W	392	n.d.	621	0.43	>0.99
M12	Q108 K:**K40D**:T53A:R58L:Q38F:Q4F	393	481	639	0.34	0.81

[a]20 μM protein and 0.5 equiv **TD-1V** at pH 7.2. [b]Absolute quantum yield was measured on a Quantaurus-QY. Not detected (n.d.).

M3有着相对较高的荧光量子产率和Φ_{ESPT}，但无法得到适合结构分析的衍射良好的晶体。

晶体结构分析

TD-1V/M11



Glu40 羧酸盐氧原子距离亚胺氮原子约 5 埃

Glu40 作为水分子的氢键供体参与其中

其他可电离残基的评估

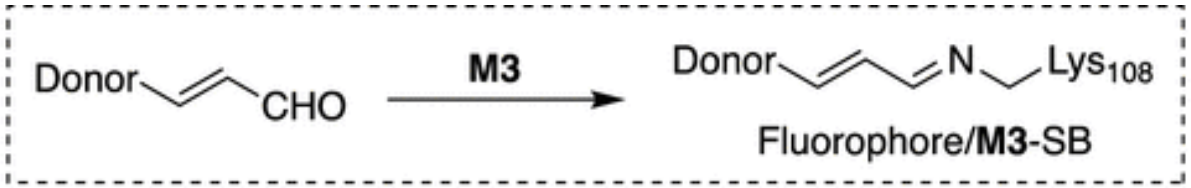
半胱氨酸
苏氨酸
组氨酸
赖氨酸
精氨酸
谷氨酰胺
酪氨酸

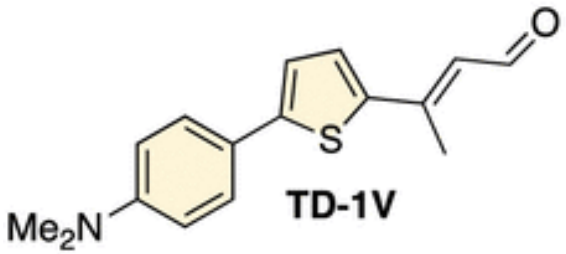
Table 2. ESPT of M3 Mutants at Position 40

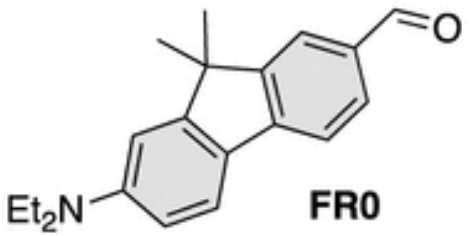
mutant	residue 40[a]	λ_{abs}	$\lambda_{em\ (SB)}$	$\lambda_{em\ (PSB)}$	Φ[b]	Φ_{ESPT}
M1	K40L	379	474	n.d.	0.03	<0.02
★**M3**	K40E	397	n.d.	605	0.51	>0.99
M13	K40C	383	480	n.d.	0.07	<0.02
M14	K40T	386	487	n.d.	0.13	<0.02
M15	K40H	393	477	642	0.09	0.50
M16	K40	389	491	661	0.08	0.40
M17[c]	K40R	392	492	653	-	0.43
M18	K40Q	388	488	663	0.11	0.11
M19	K40Y	393	495	674	0.04	0.14

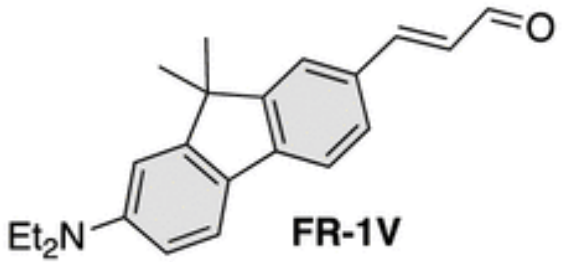
[a]20 μM protein and 0.5 equiv **TD-1V** at pH 7.2. [b]Absolute quantum yield was measured on a Quantaurus-QY. [c]The QY of **M17** could not be obtained due to its inherent instability. Not detected (n.d.).

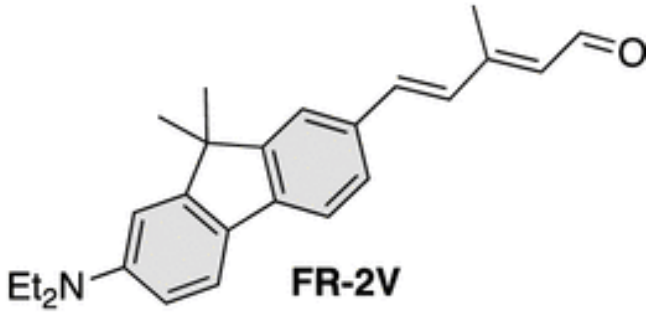
荧光染料扩展

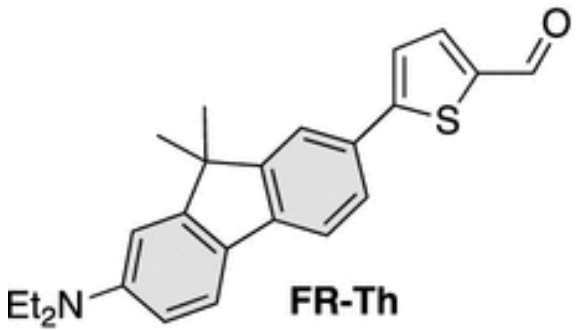




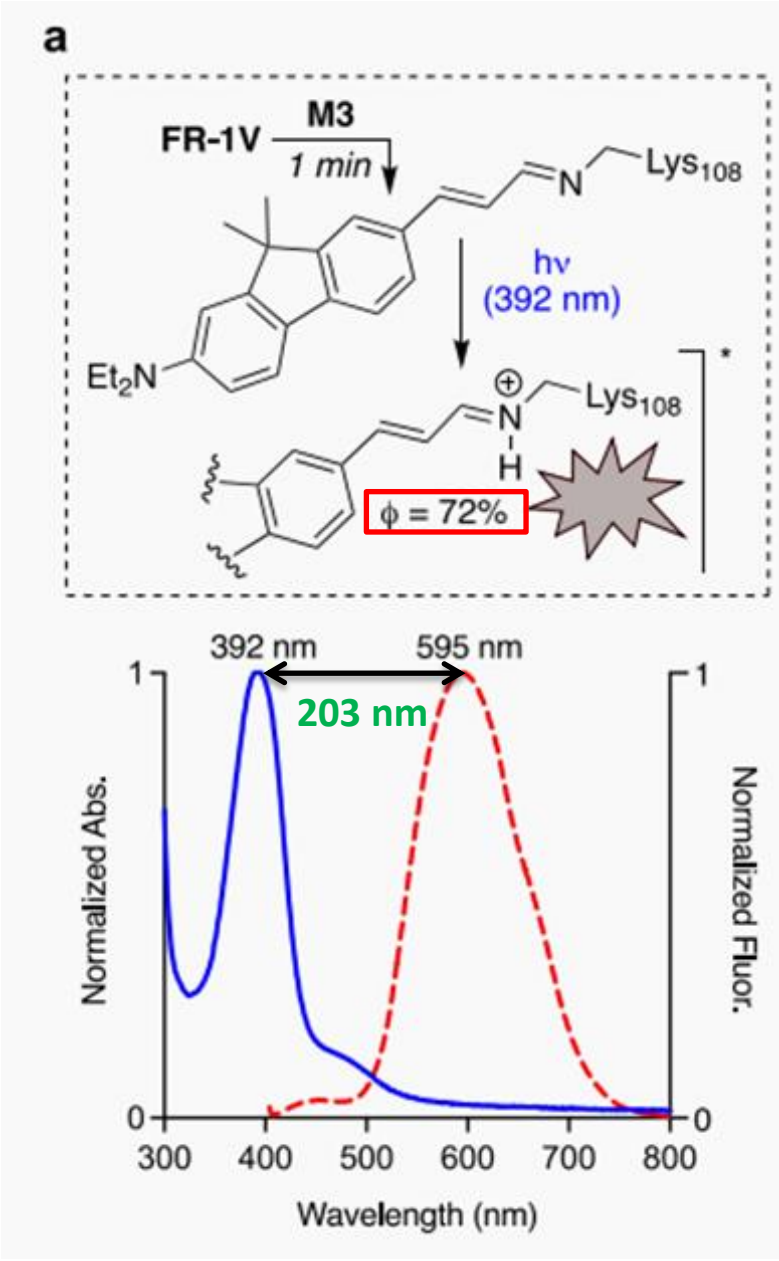








Fluorophore/**M3**-SB	ϕ_{ESPT}
TD-1V/M3-SB	>0.99
FR0/M3-SB	<0.02
FR-1V/M3-SB	>0.99
FR-2V/M3-SB	0.82
FR-Th/M3-SB	0.14



用于活细胞成像的荧光团选择

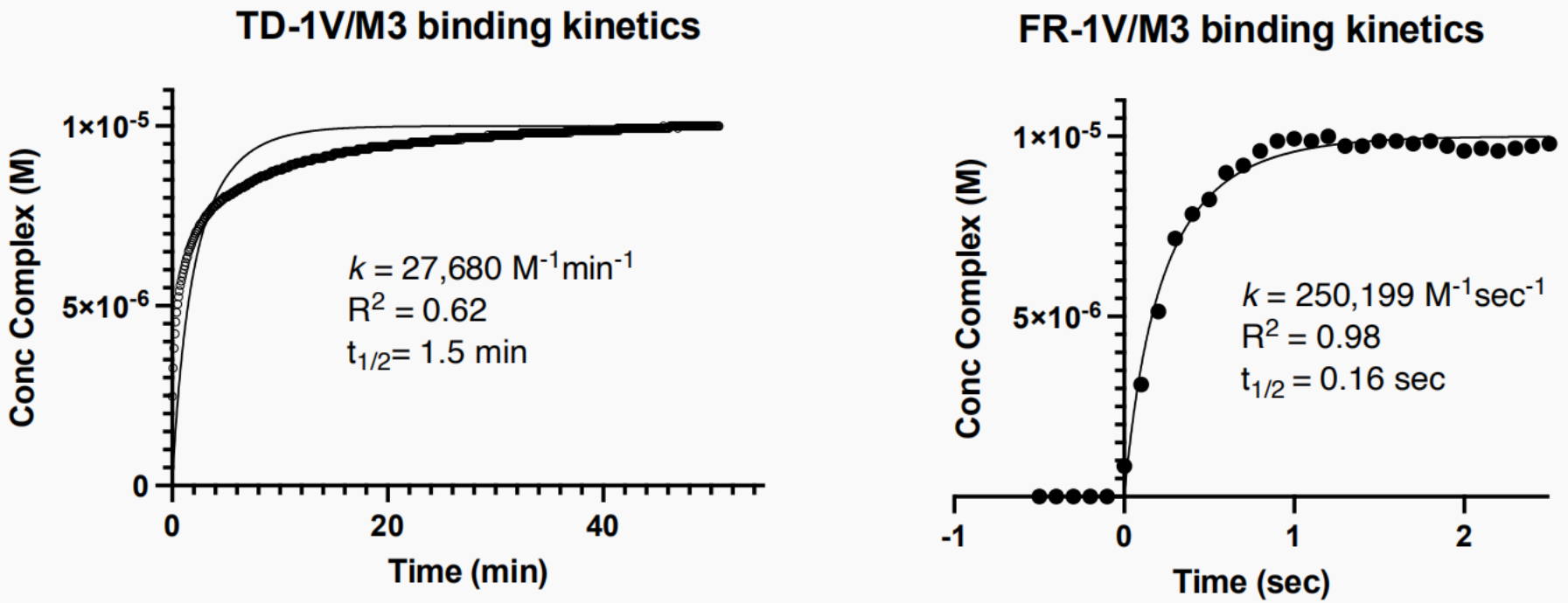


Figure S14. Rate of **TD-1V/M3 and FR-1V/M3** SB formation, fitted to 2nd order kinetics with 20 μM protein and 10 μM **TD-1V** or **FR-1V**.

活细胞成像

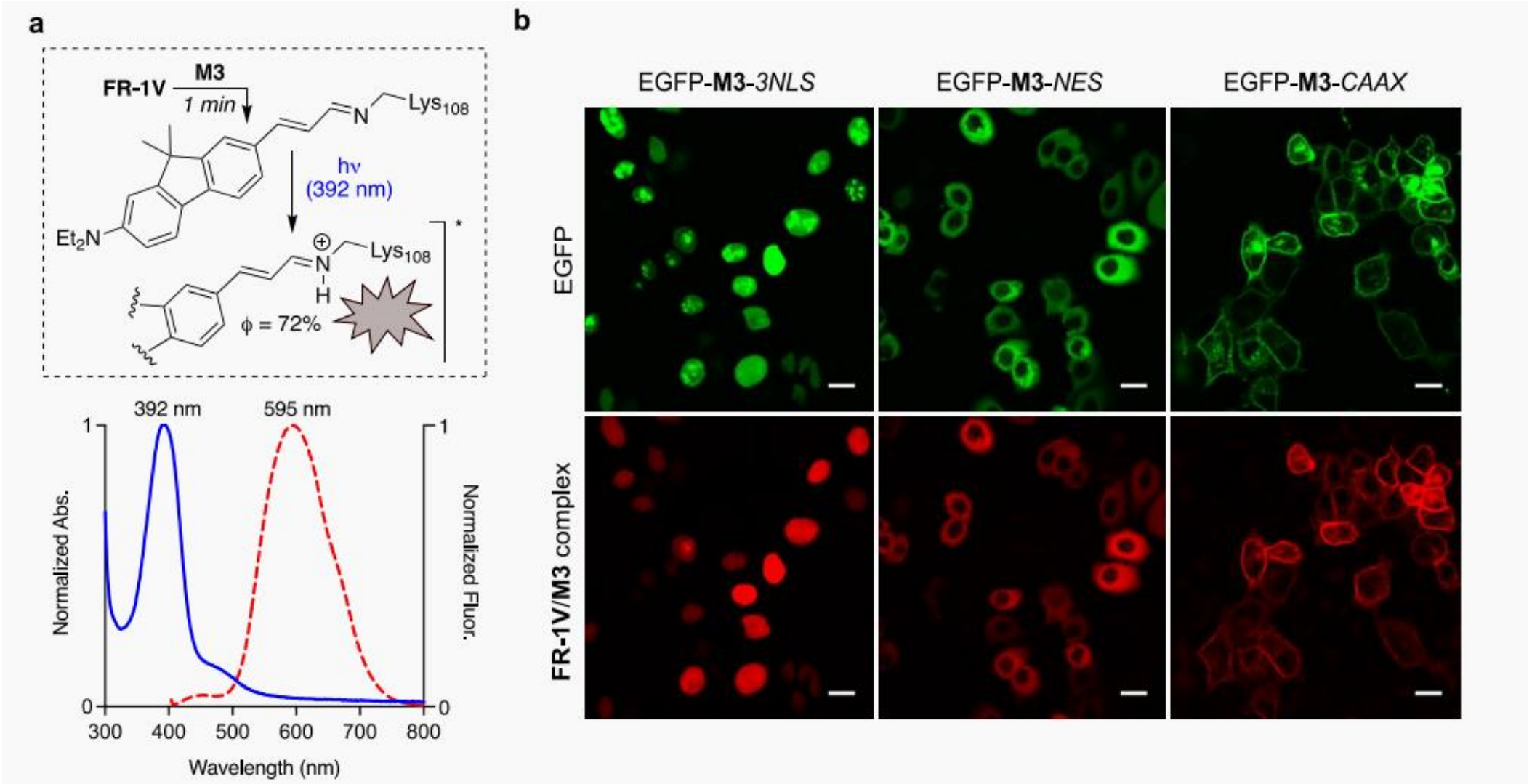
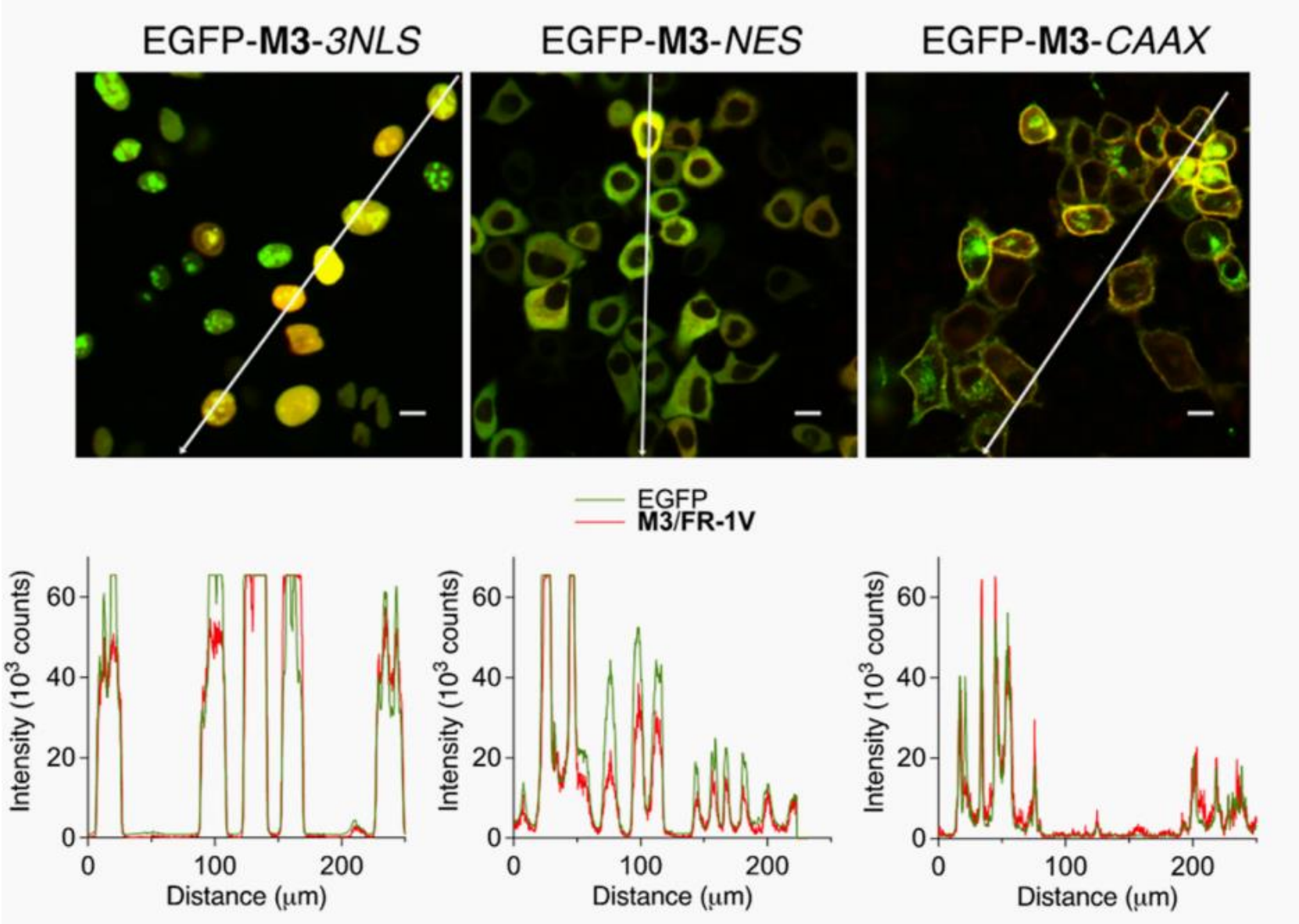


Figure 6. (a) **FR-1V** complexed with **M3** yields a SB in ground state (λ_{max} 392 nm, pK_a = 5.2). ESPT of the complex leads to emission at 595 nm; (b) compartmentalized **FR-1V/M3** imaging in live HeLa cells. NLS = nuclear localization sequence. NES = nuclear export sequence. CAAX = prenylation tag. Cells were stained with 500 nM **FR-1V** and incubated at 37 °C for 1 min. Cells were washed 3 times with DPBS before imaging. Scale bar, 10 μm.

活细胞成像


EGFP-M3-3NLS
EGFP-M3-NES
EGFP-M3-CAAX
EGFP
M3/FR-1V
Intensity (10^3 counts)
Distance (μm)
0
20
40
60
100
200

总结

- 合理设计并成功得到了基于光碱的LSSFR-1V/M3复合物
- FR-1V/M3的斯托克斯位移大于200 nm，荧光量子产率高达70%
- FR-1V/M3具有快速标记动力学、高亮度和染色特异性