Literature Report

Reporter: Zhou Xuelian

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ARTICLE

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Resonance energy transfer sensitises and monitors in situ switching of LOV2-based optogenetic actuators

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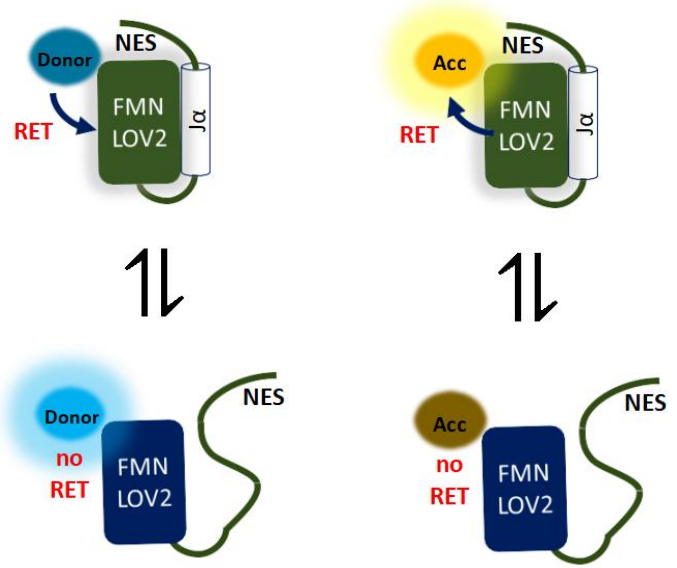
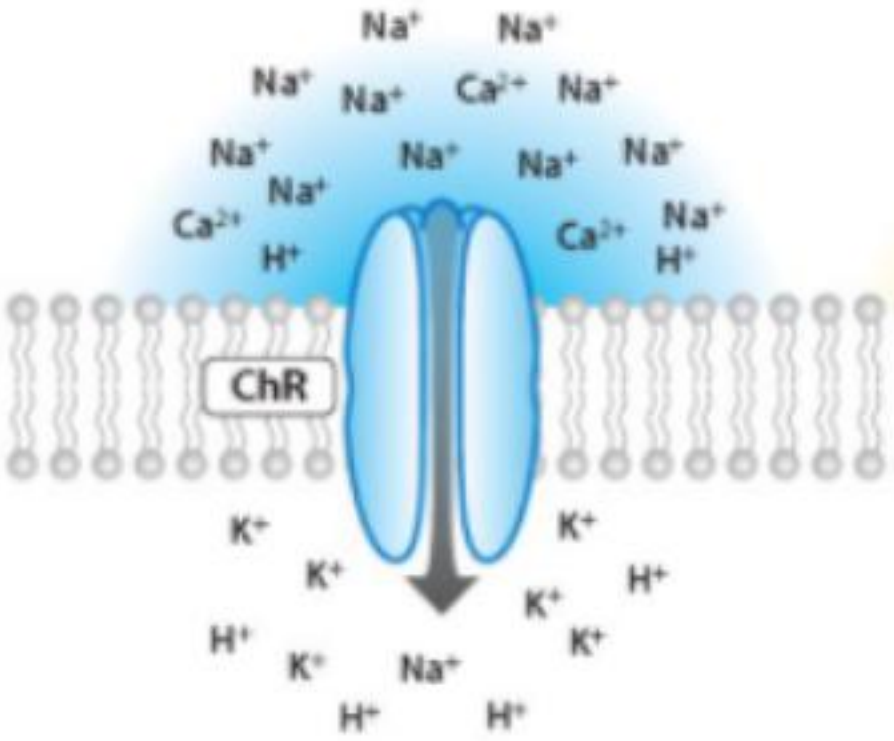
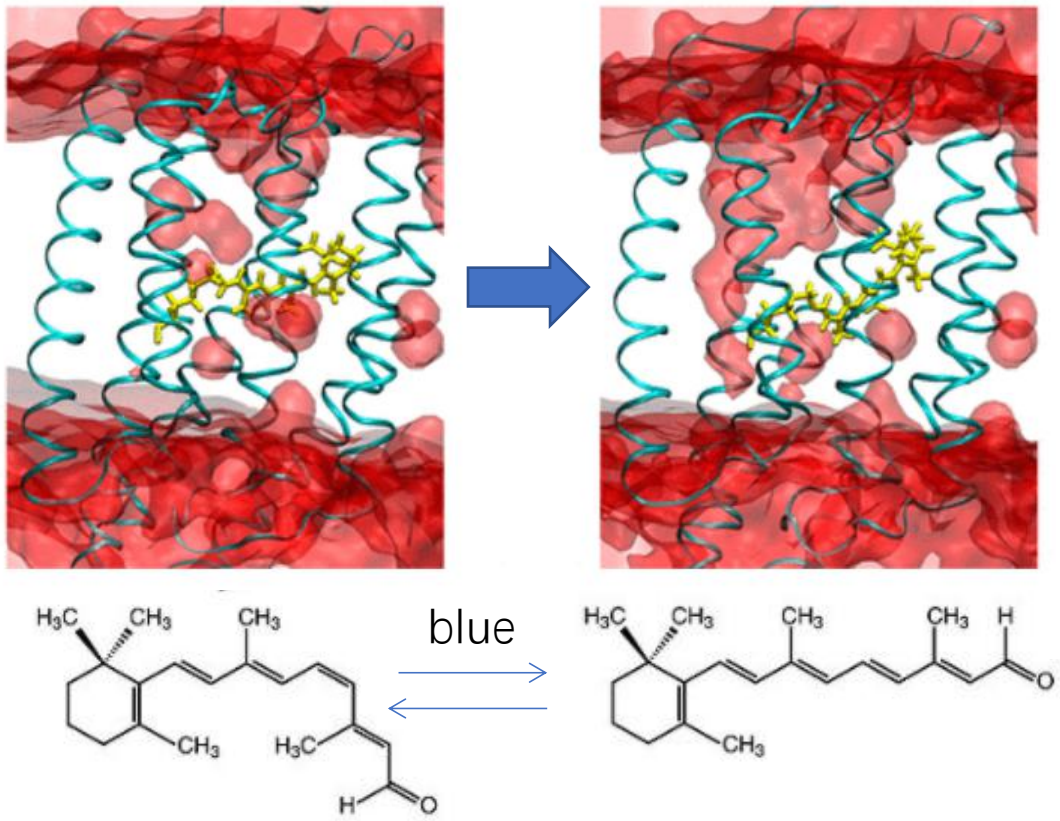
光遗传学和光学测定；细胞信号、成像；单细胞方法；神经科学；蛋白质工程等。



光遗传学（optogenetics）

光遗传学是整合了光学、基因操作技术、电生理等多学科交叉的生物工程技术。通过将对光响应敏感的光感受器比如光敏蛋白等通过基因融合引入细胞，利用光控系统精确调控神经元等细胞活动，可以在亚细胞水平定位蛋白活动、调控蛋白功能等。



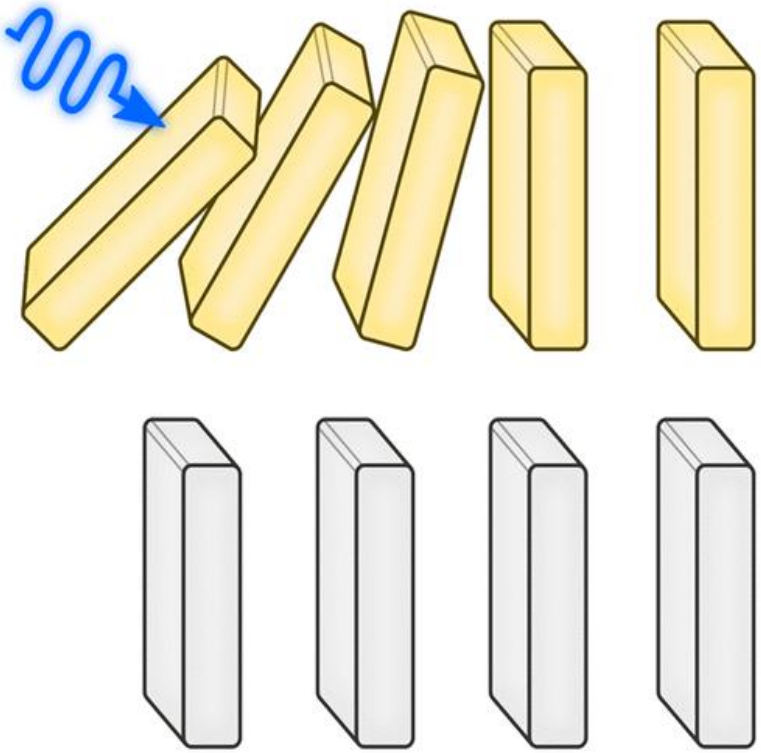


Peter Hegemann, Science, 2002, 296: 2395-8

Karl Deisseroth, Annu Rev Biomed Eng, 2014, 16: 103-129

J Phys Chem Lett. 2019, 10, 2862–2868

光感受器

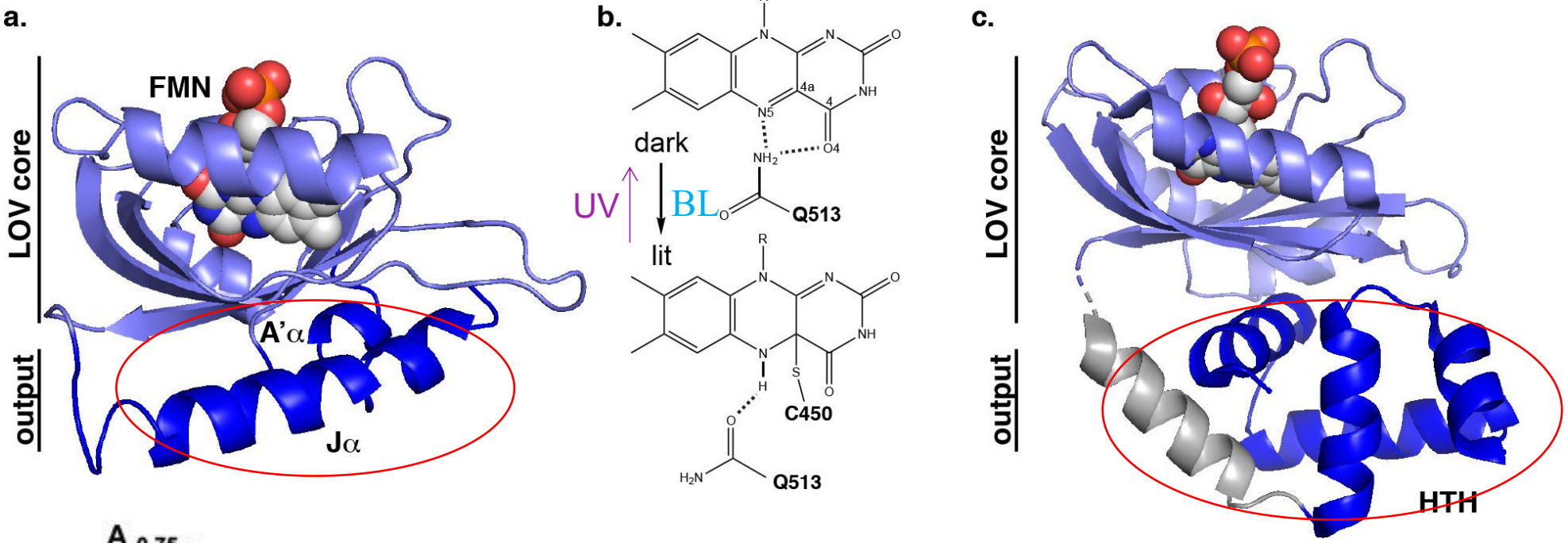


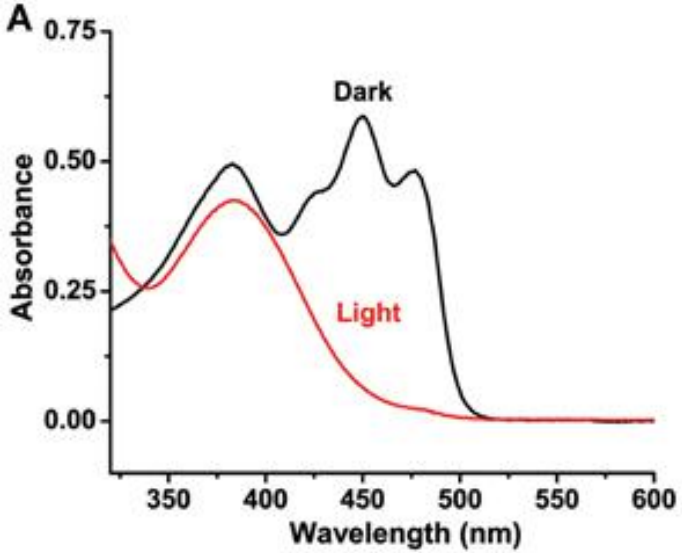
Opto system	Ta1/2	Td1/2	λon (nm)	λoff (nm)	Size (aa)	Chromophore	Mechanism
ChR2	0.2 ms	15 ms	470–480	–	737	Retinal*	Ion channel
Bphp1 PpsR2	3–30 s	15 min (dark)	740–780	640–660	BphP1:732 PpsR2:465	Biliverdin*	Dimerization
CRY2 CIBN	10s (90%)	12 min	420–490	–	CRY2:498 CIBN:170	FAD*	Dimerization
pMag nMag	1.5 s	6.8 s	<500	–	pMag:150 nMag:152	FMN*	Dimerization
AsLOV2	Seconds	30–50 s	<500	–	143	FAD*	Caging
PhyB PIF	1.3 s	4 s	650	760	PhyB:908 PIF:100	Phycocyanobilin	Dimerization
CRY2 (Oligo)	30 s	5.5 min	420–490	–	498	FAD*	Dimerization
UVR8 COPI	Hours	Irreversible	280–315	–	UVR8:440 COPI:675	*	Dimerization

Chem. Rev. 2018, 118, 21, 10659–10709

LOV结构域

FMN黄素核苷酸在各种代谢蛋白质和酶中作为必需的核心辅助因子，对呼吸等生物氧化过程的电子传递起着重要的作用。



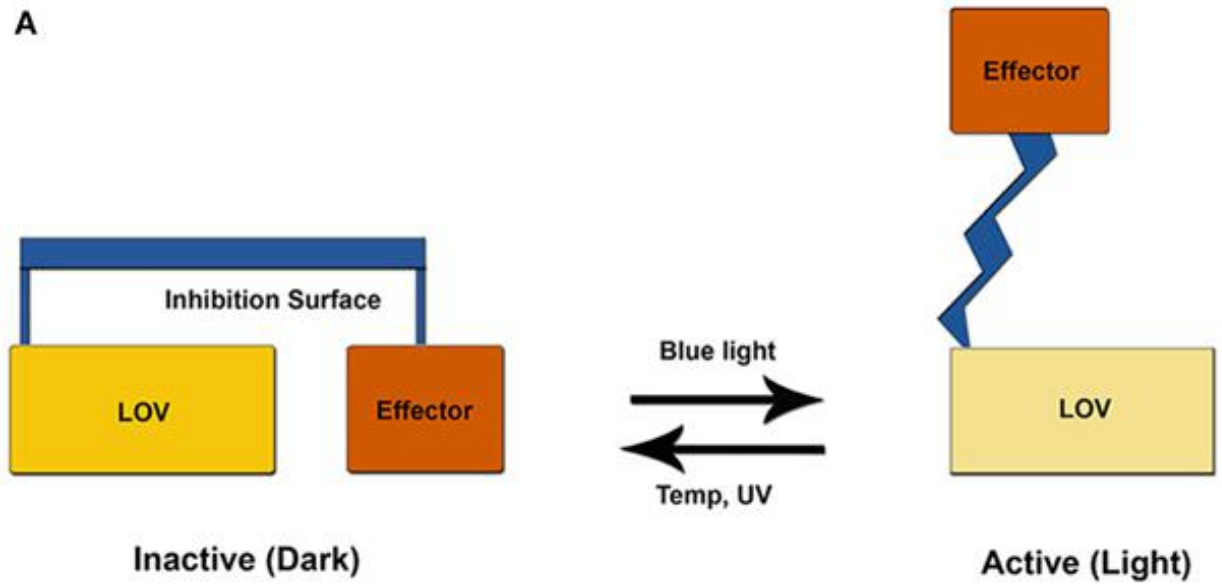


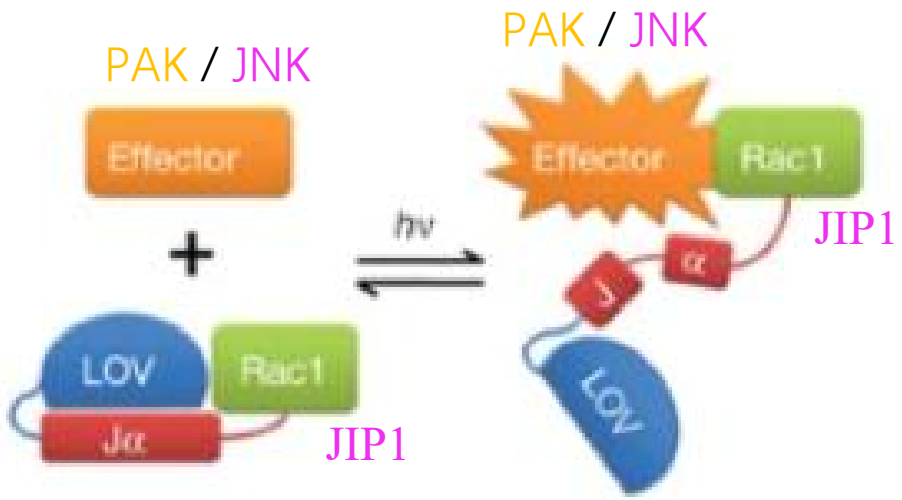
Chem Rev 2018, 118, 21, 10659–10709

CURR OPIN CHEM BIOL 2015, 27, 39-45

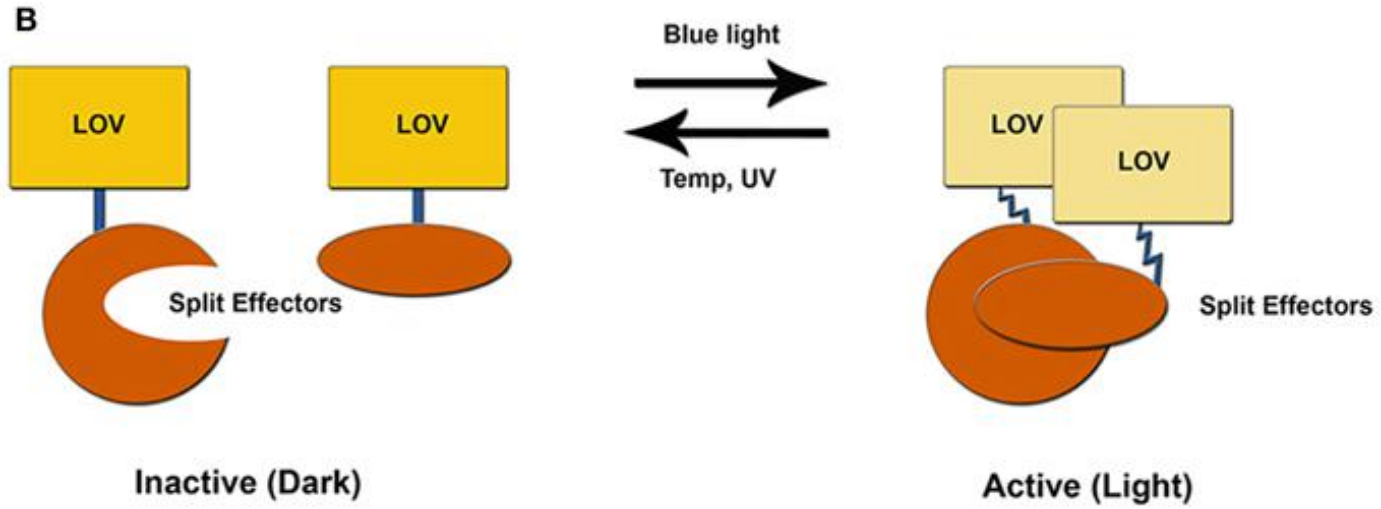
LOV应用——光遗传学致动器

细胞迁移、光遗传学抑制剂optoJNKi



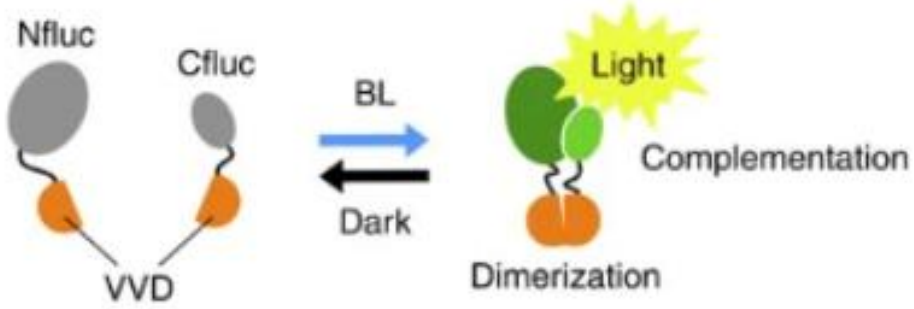


Nat Commun 8, 15017 (2017).



Front. Mol. Biosci. 2:18 (2015)

PPI

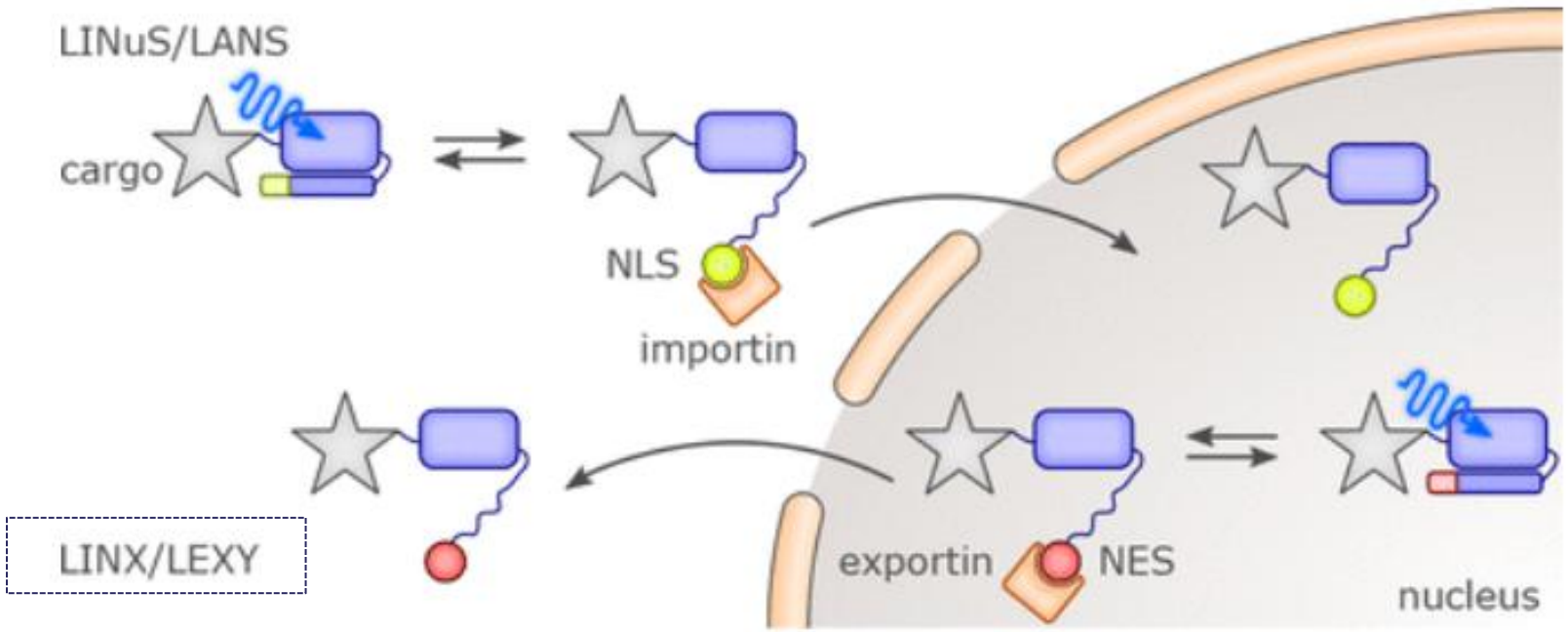


Nat Commun 6, 6256 (2015).

Nature 461, 104–108 (2009)

LOV应用——光遗传学致动器

核易位

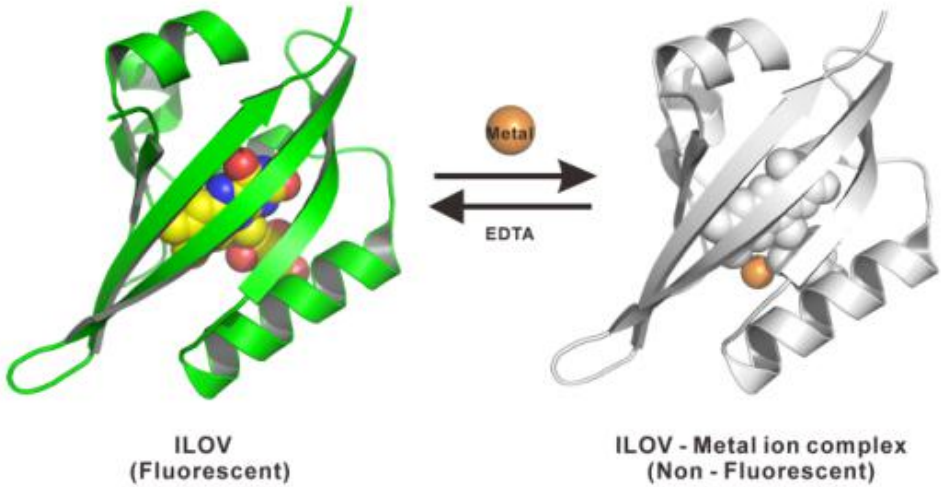


importin、exportin: 核蛋白。通过与特定的识别序列（NLS、NES）结合，将蛋白质分子从细胞的细胞质/核转运到细胞核/质。

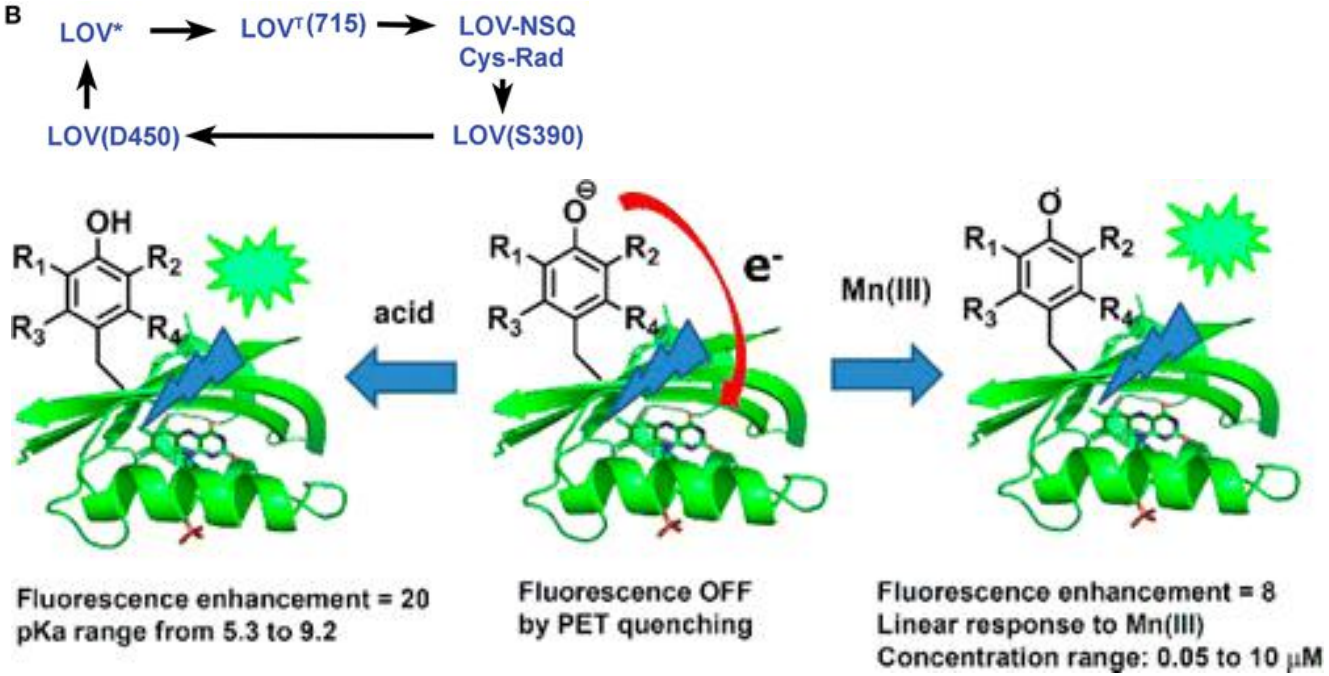
NLS: 核定位序列
将蛋白从细胞质通过核孔运输到细胞核
NES: 核输出序列
将蛋白从细胞核通过核孔运输到细胞质

Nat Commun 5, 4404 (2014)

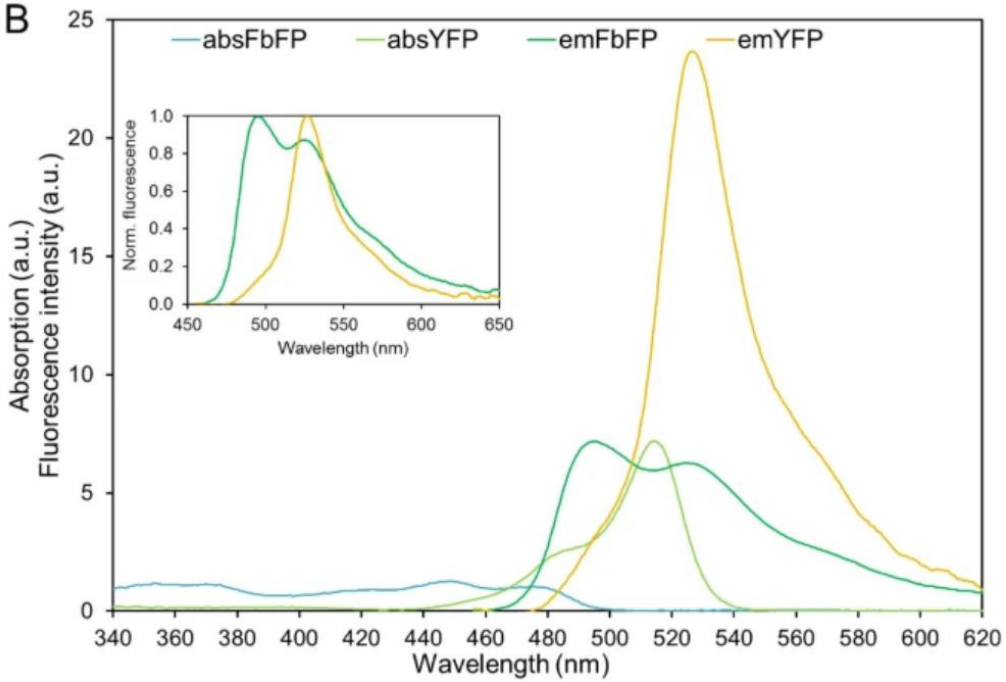
LOV应用——FbFP



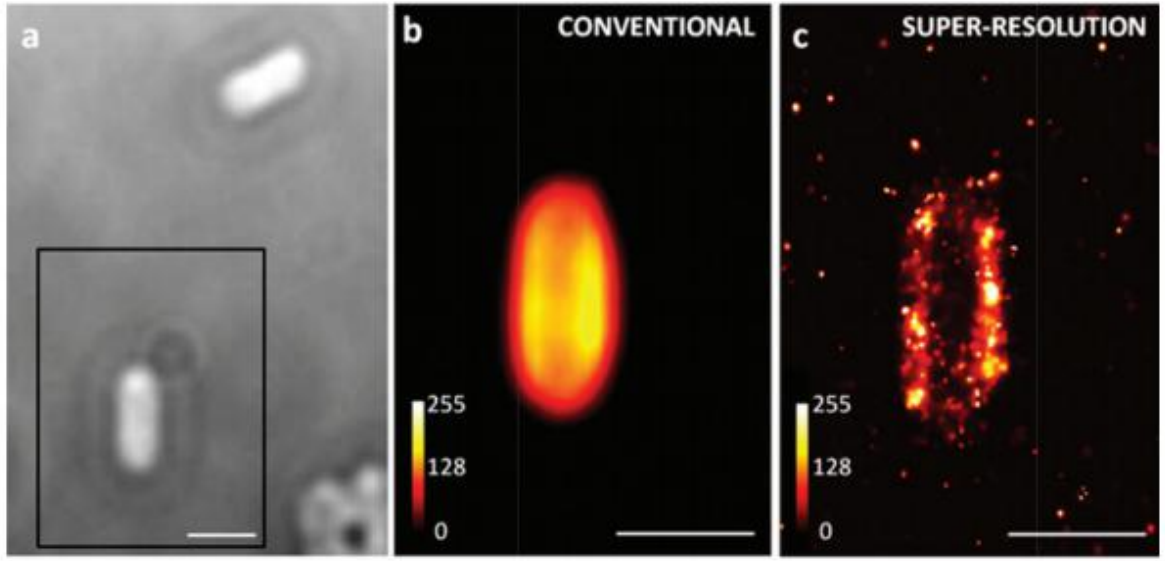
J Microbiol Biotech. 25 (2014).503-510



J. Am. Chem. Soc. 2014, 136, 38, 13094–13097



BMC Biol (2012), 10, 28



Biophy J (2013) 104, 2,672a

asLOV2光致动器特点

光遗传学致动器可以使用我们自定义的光程序通过光感应受体在时空上来控制特定的分子活性，操纵细胞的活动和功能。

1、遗传编码

2、空间分辨率

3、光敏性

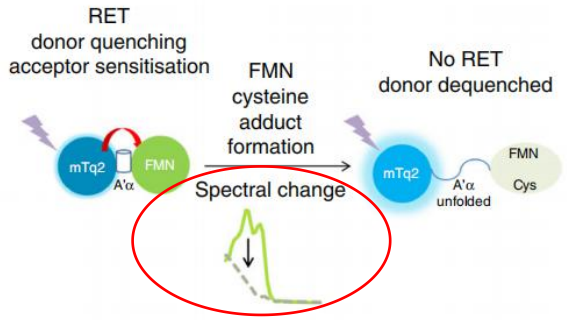
4、时间分辨率

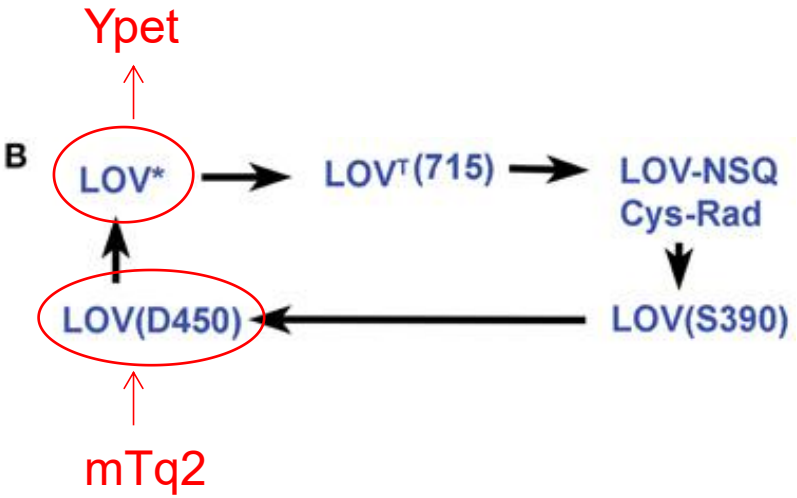
BUT

1、蓝光

2、光致动器开关的实时原位检测，激活弛豫特性的精确定量

Results——RET设计





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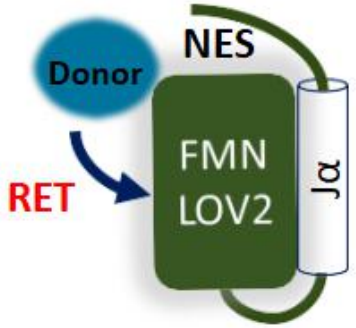

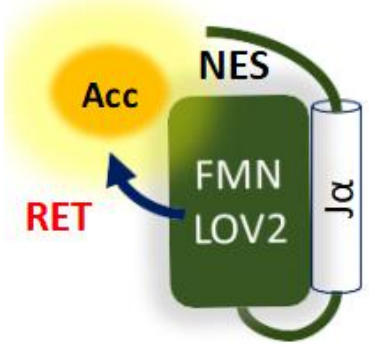

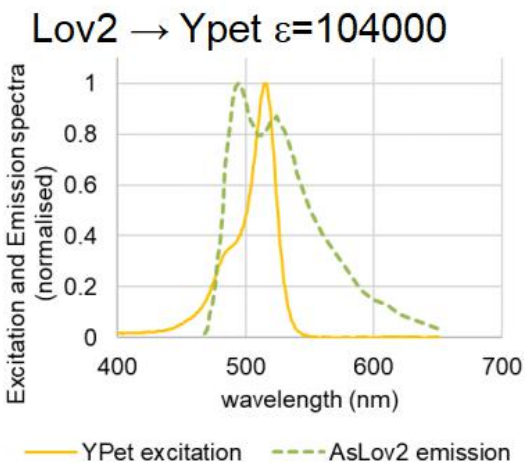

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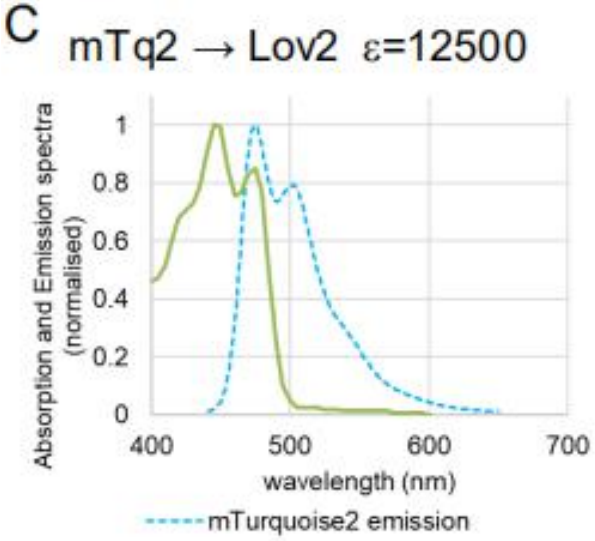
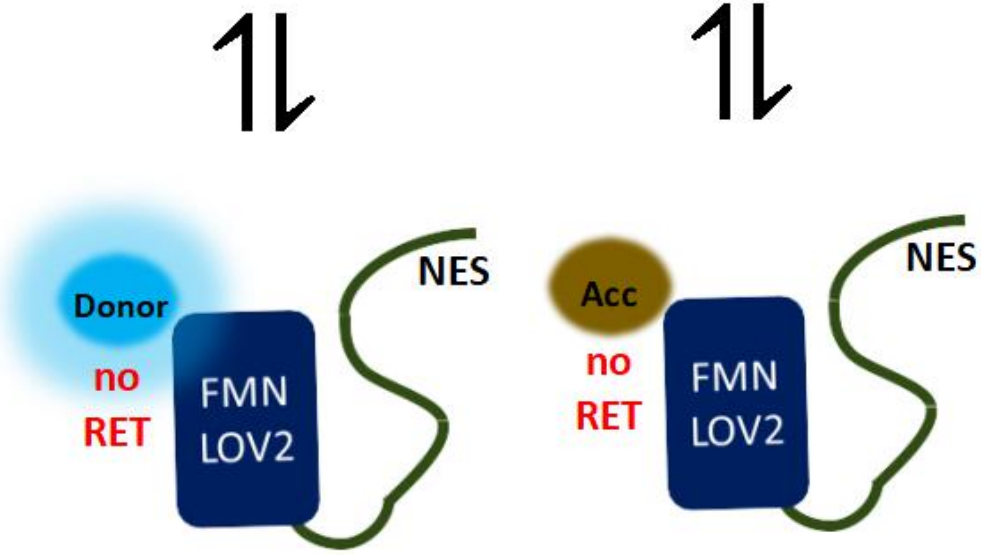
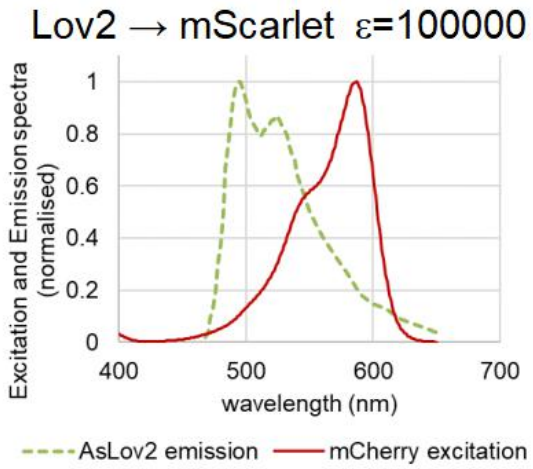
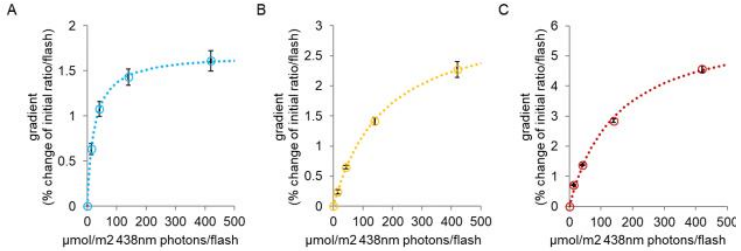
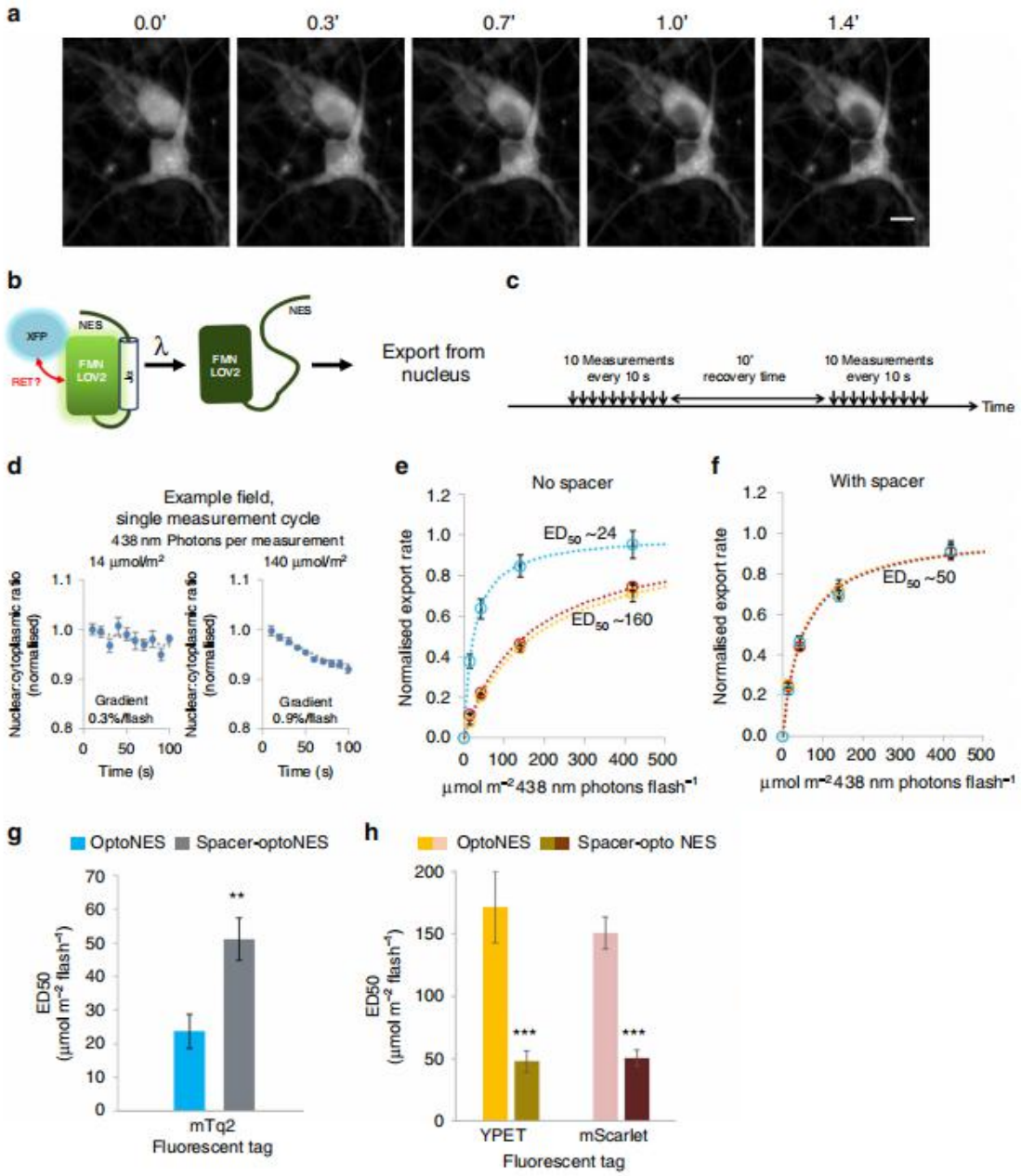




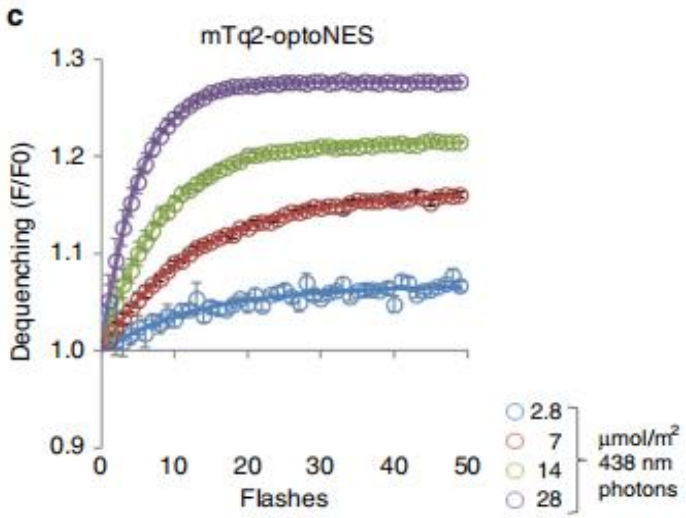


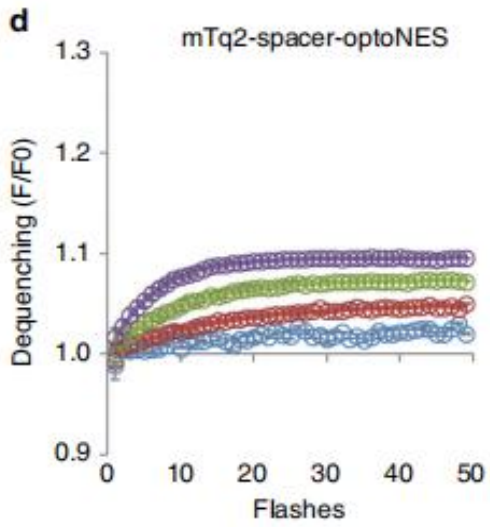
Results——optoNES光敏性

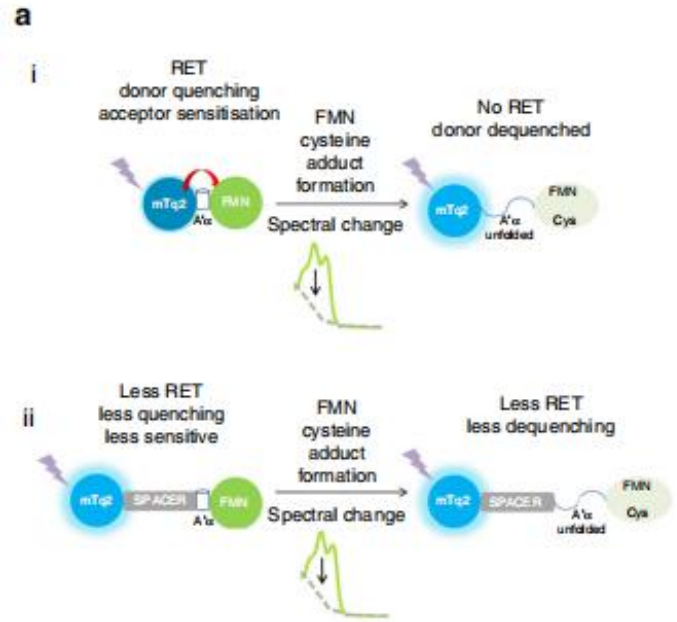


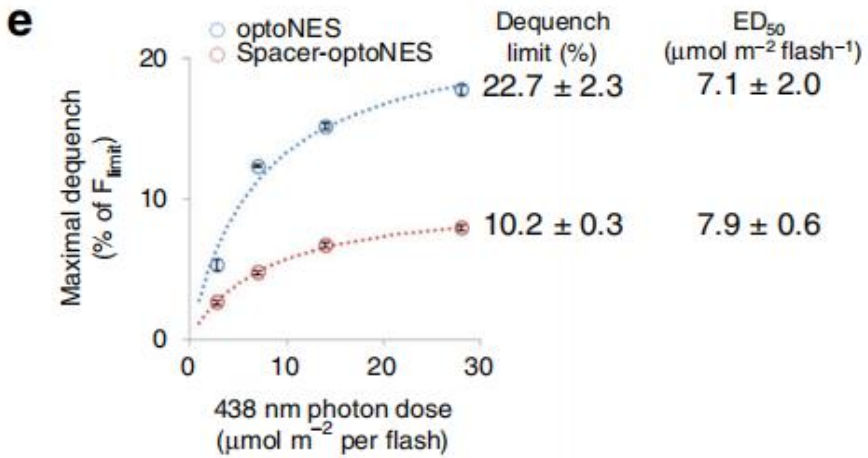


Results——细胞中LOV2转换的可视化和定量

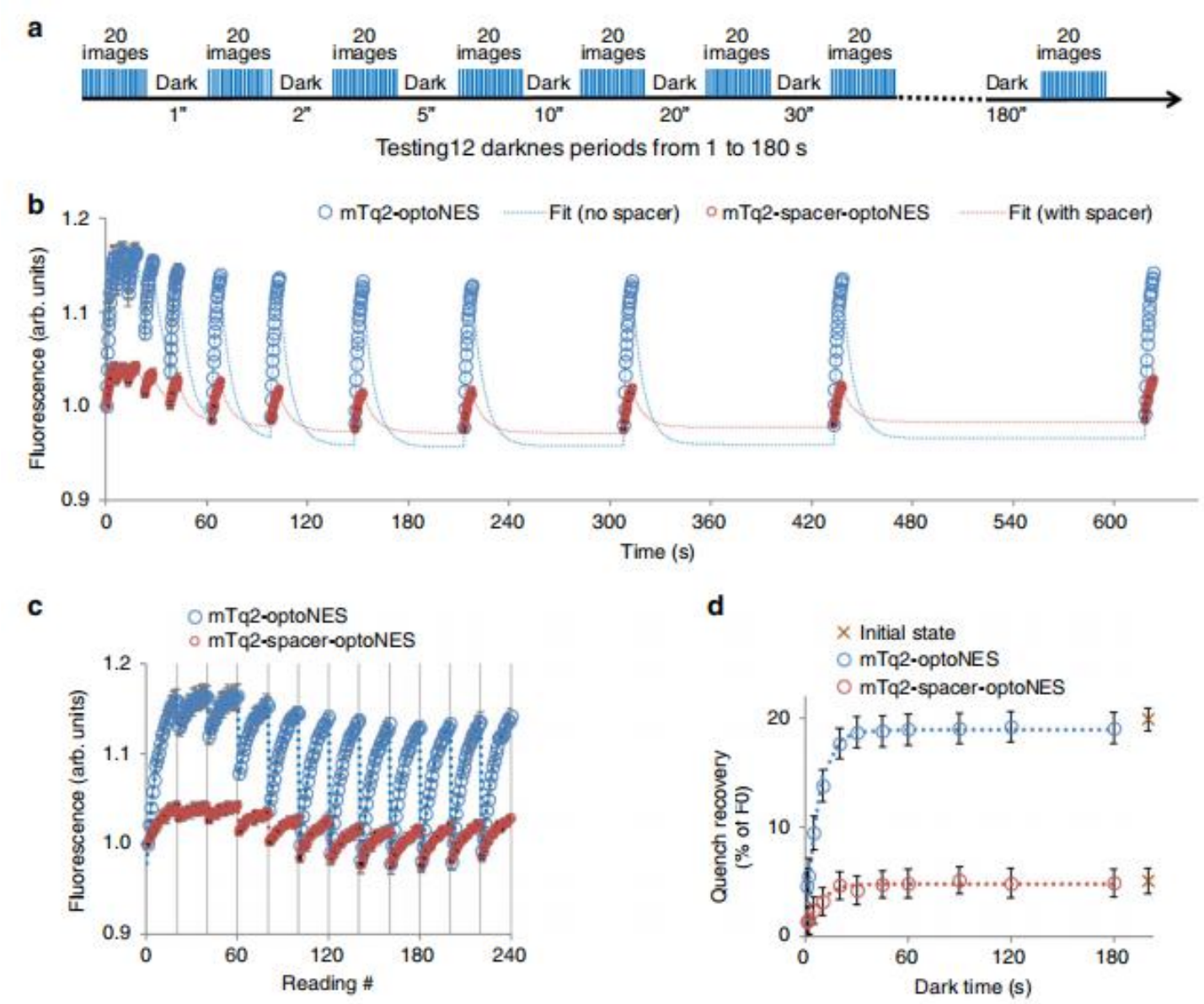








Results——细胞中LOV2弛豫的可视化和定量



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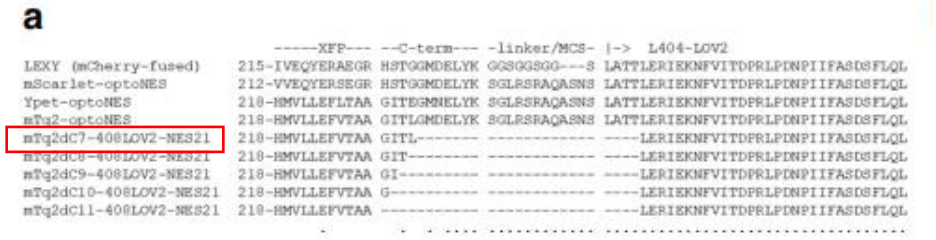
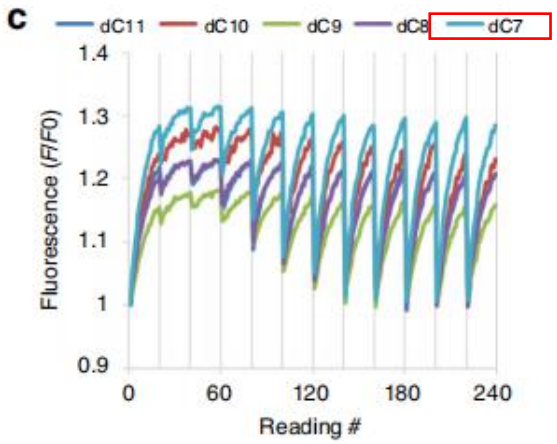
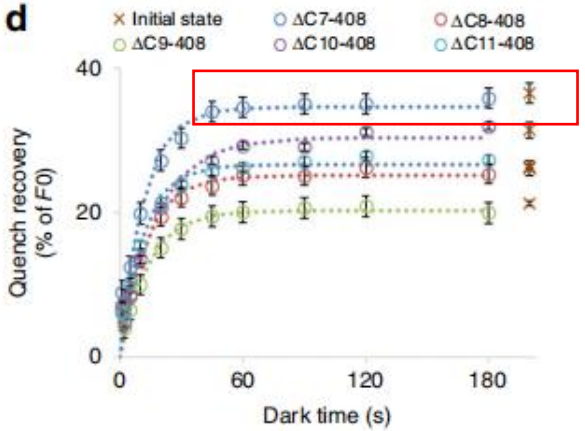
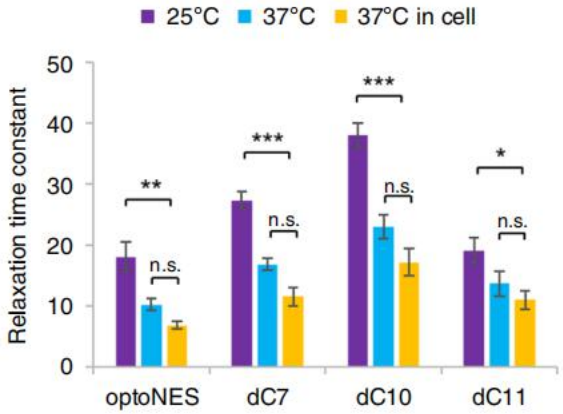
Results——RET优化

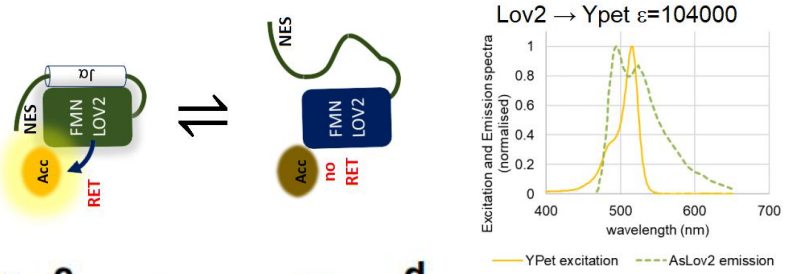


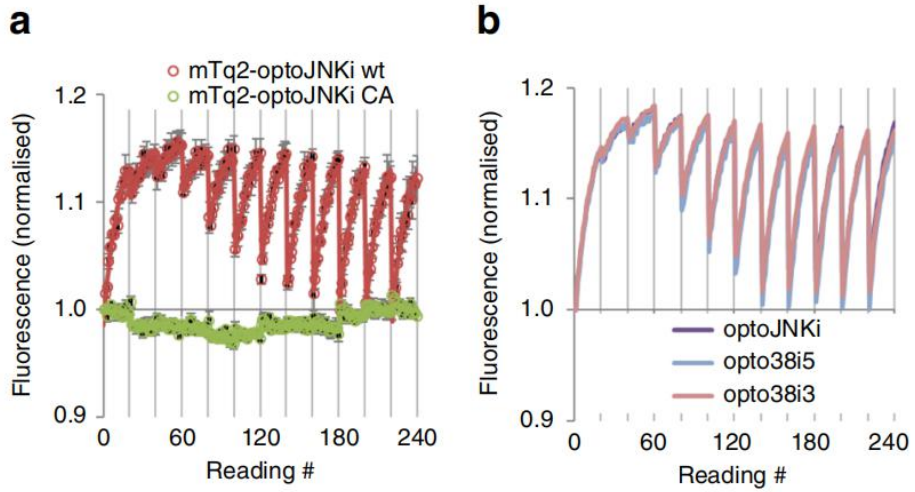


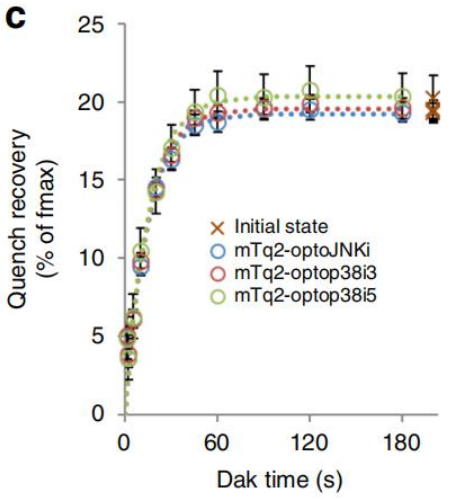


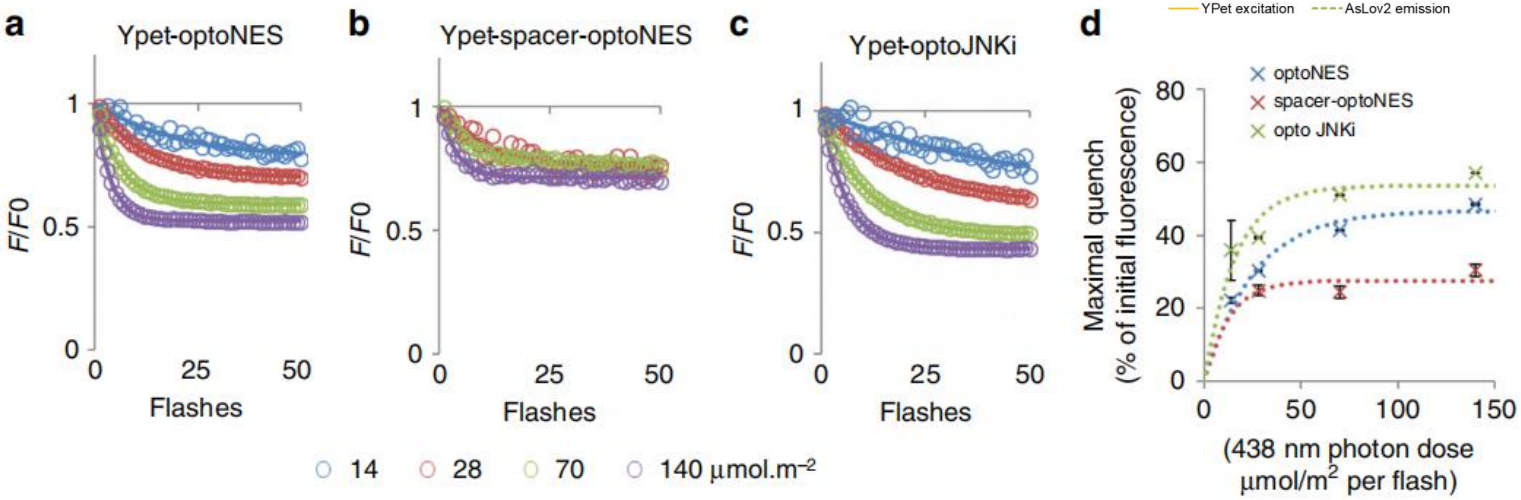


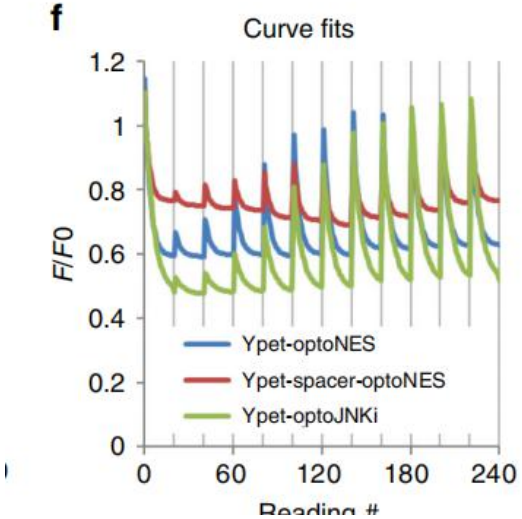
Results——其他光遗传学致动器

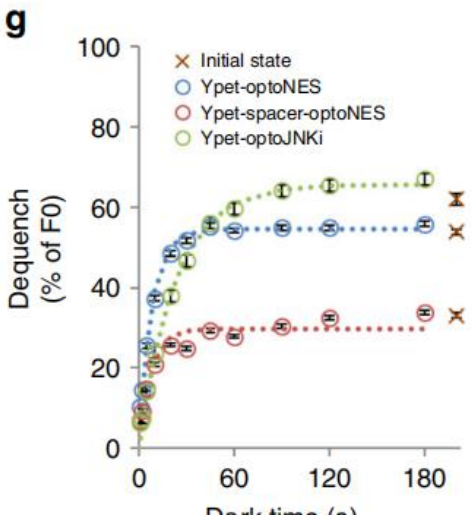




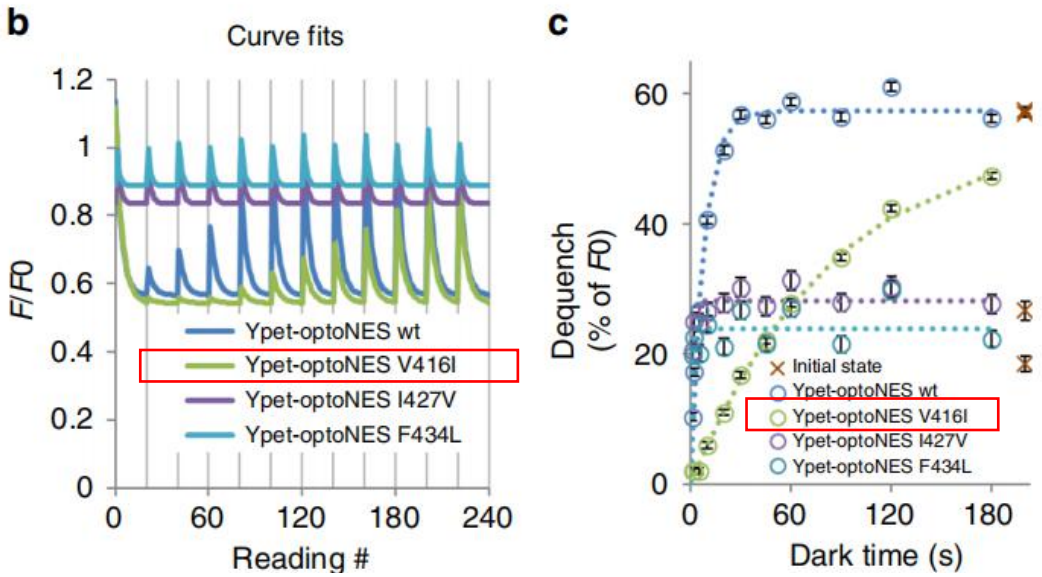


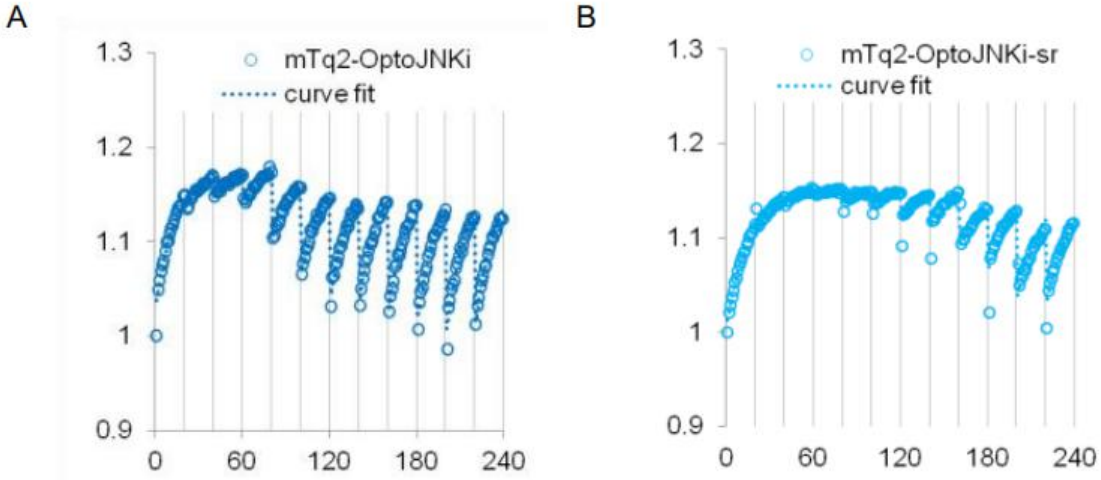






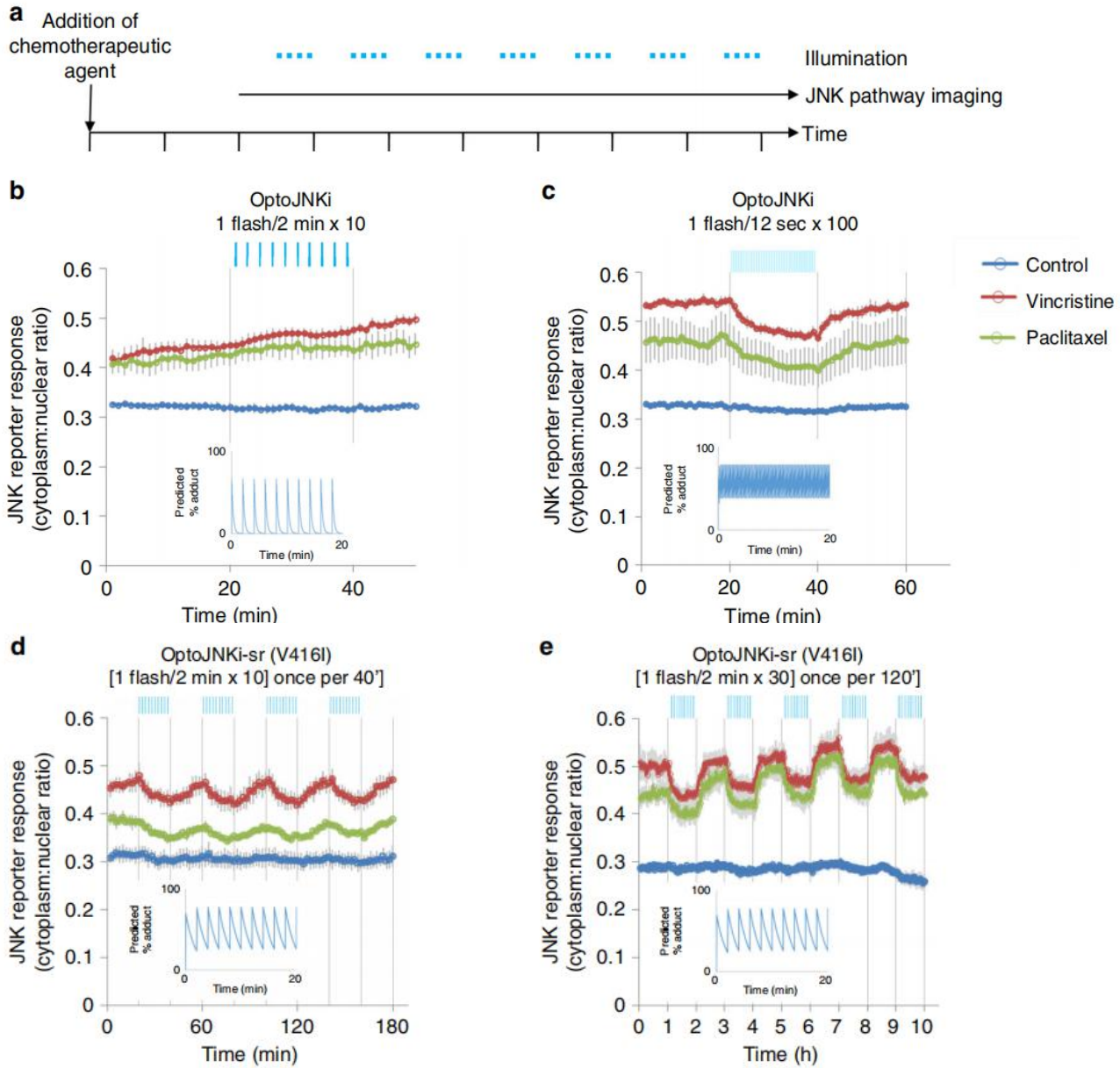
Results——原位弛豫时间的优化





Actuator	relaxation time constant in dark (s)	ED_{50} (μmol.m^{-2})	Dequench limit (%)	Sensitivity (μmol.m^{-2})
mTq2-OptoJNKi	15.5±3.2	2.9±0.9	15.9±1.3	179±40
mTq2-OptoJNKi-sr	109±23	0.4±0.1	12.6±1.3	154±44

Results——JNK信号通路动力学



THANKS!