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Photocleavable proteins that undergo fast and efficient dissociation†

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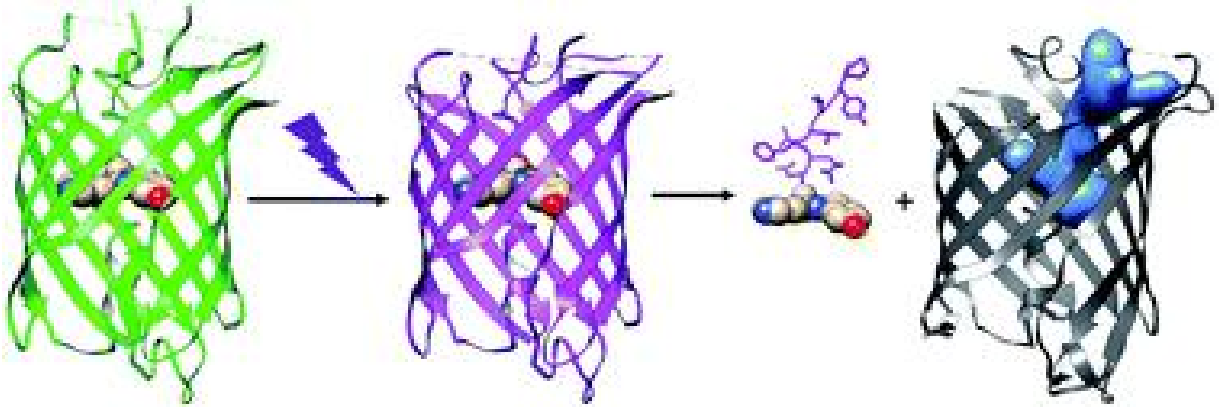

2nd gen = faster and more efficient!

Corresponding author

Robert E. Campbell

University of Alberta , CA Professor at Chemistry
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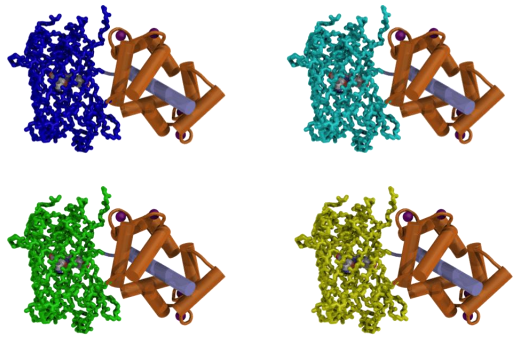
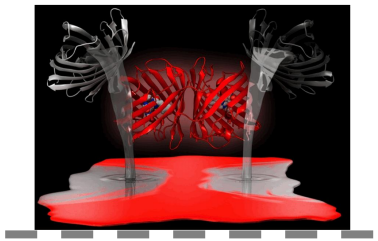
Education:
University of British Columbia - Ph.D., Chemistry
Universiy of California, San Diego - Postdoctoral Fellow, Preceptor: Roger Tsien
University of Alberta - Assistant Professor 2004-2009(Tenure)

Research Interests:

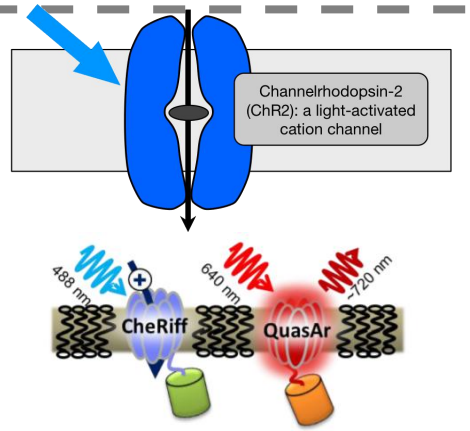
Optogenetic reporters for cell biology and neuroscience

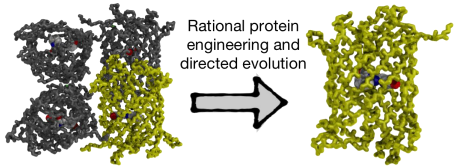


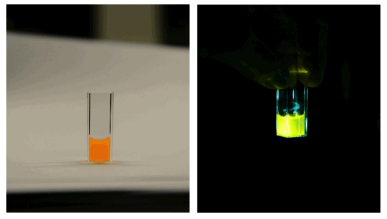


Dimerization-dependent fluorescent proteins (ddFPs) biosensors

Expanding the toolbox of spectrally orthogonal optogenetic actuators and reporters

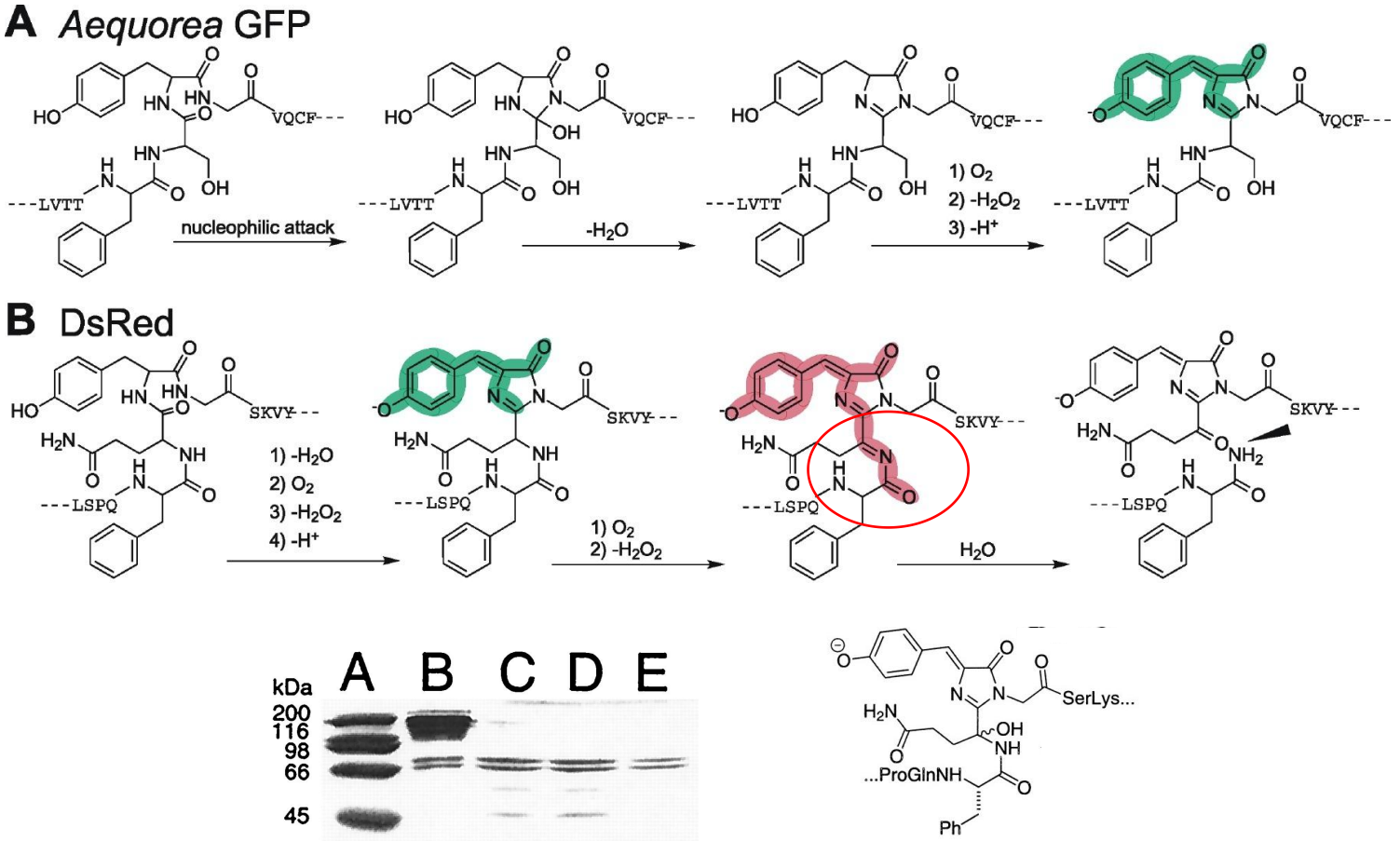


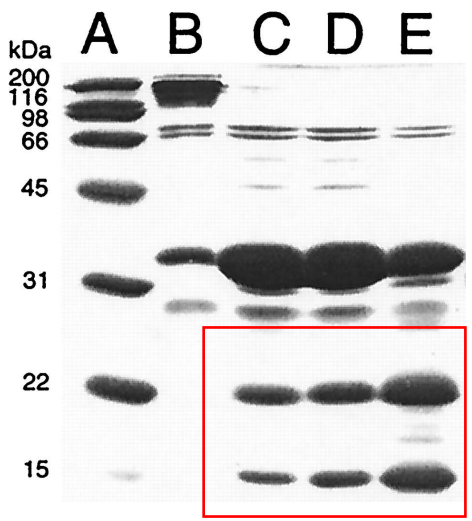


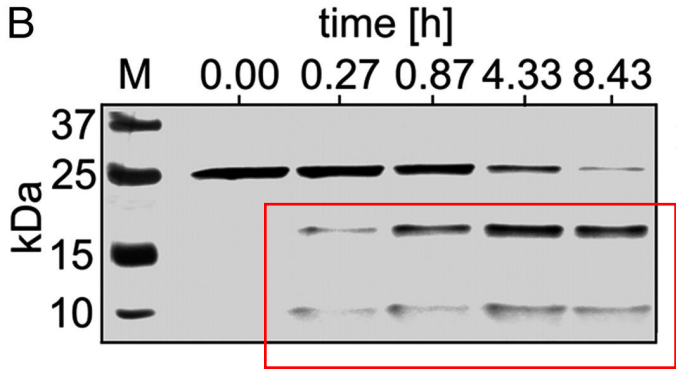


Engineering new fluorescent proteins

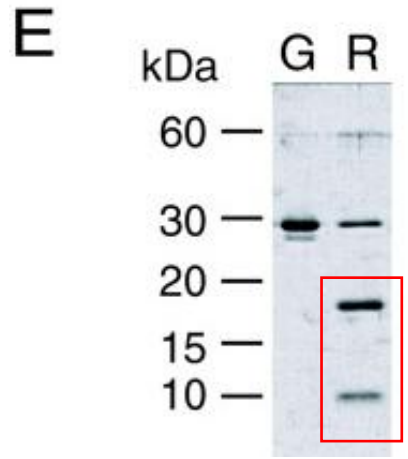
Photo-Induced Peptide Cleavage in Red Fluorescent Protein







mEos 2



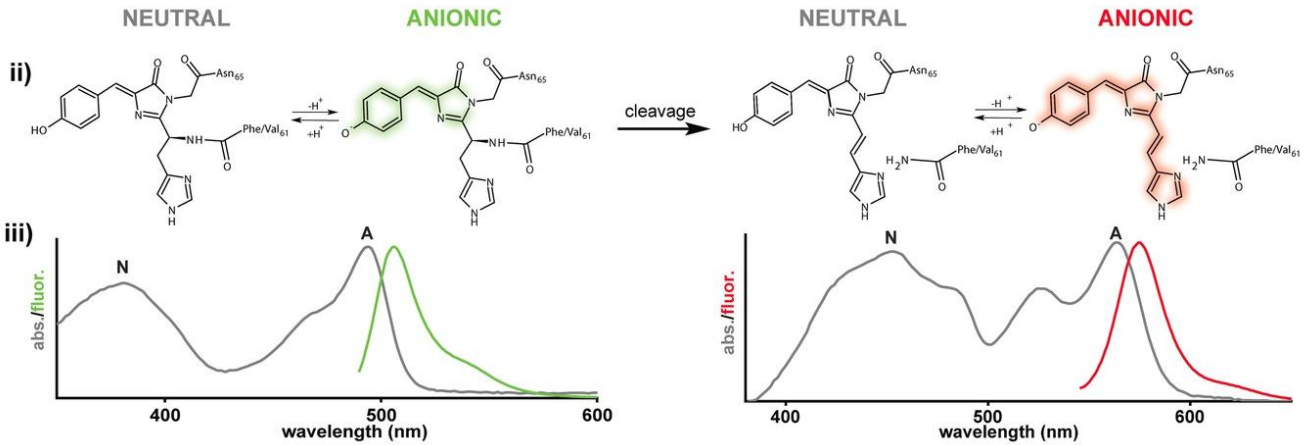
Kaede

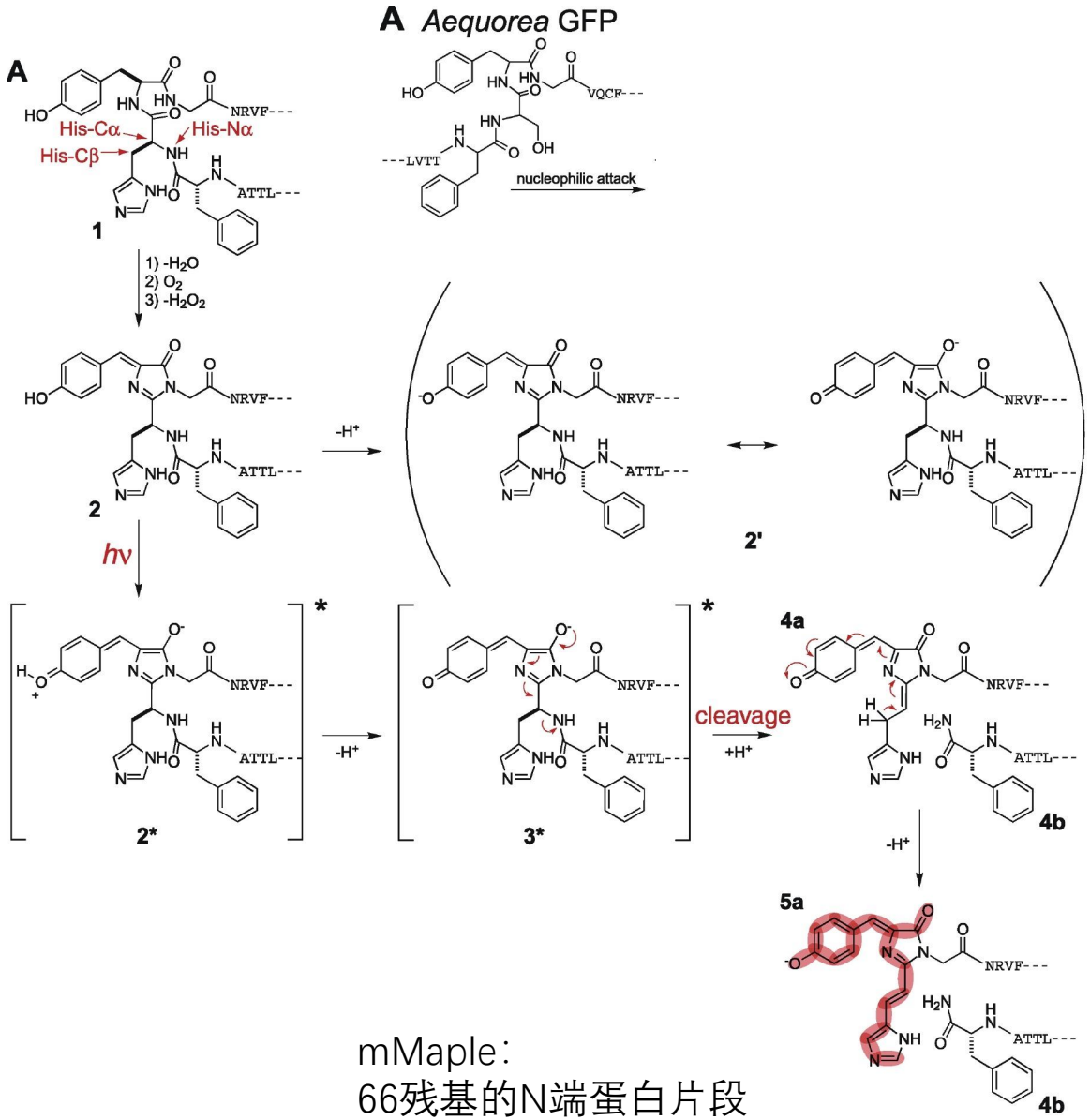
Molecular Cell, 2003, 12, 1051-1058.
Proc Natl Acad Sci, 2002, 99 (20) 12651-12656
Proc Natl Acad Sci, 2000, 97, 11990-11995

Photo-Induced Peptide Cleavage in the Green-to-Red Conversion of a Fluorescent Protein

Background

Kaede



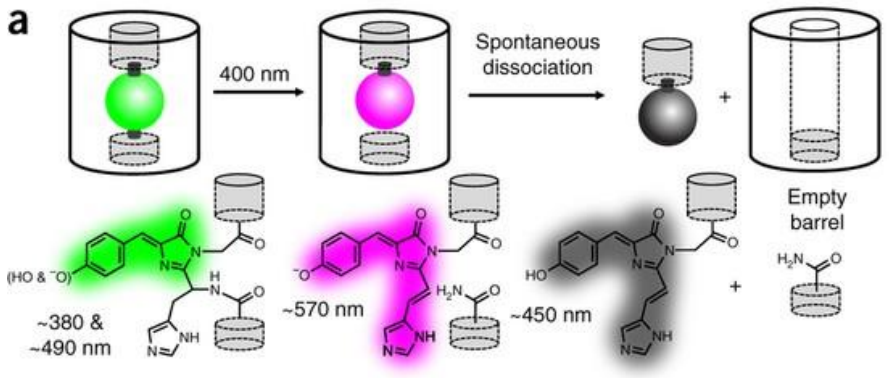


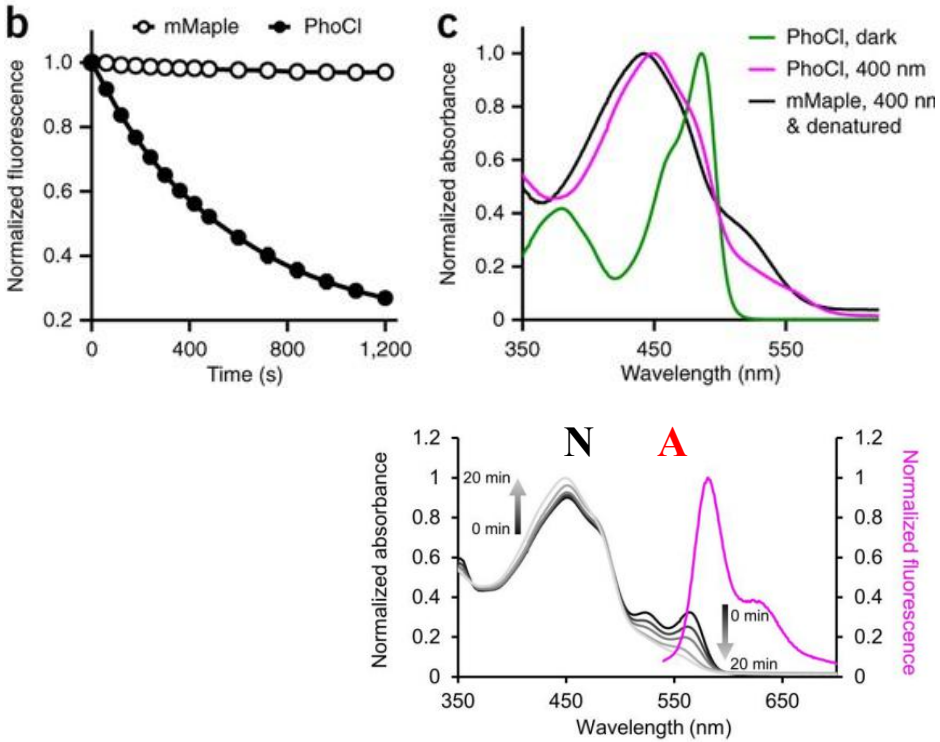
Molecular Cell, 2003, 12, 1051-1058.
Angew.Chem.Int.Ed., 2017, 56, 11634-11639.

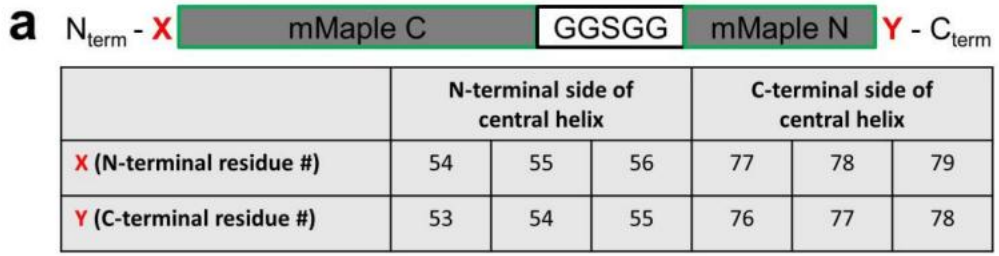
mMaple:
66残基的N端蛋白片段
+166个残基的C端片段

Photocleavable Fluorescent Protein (PhoCl1)

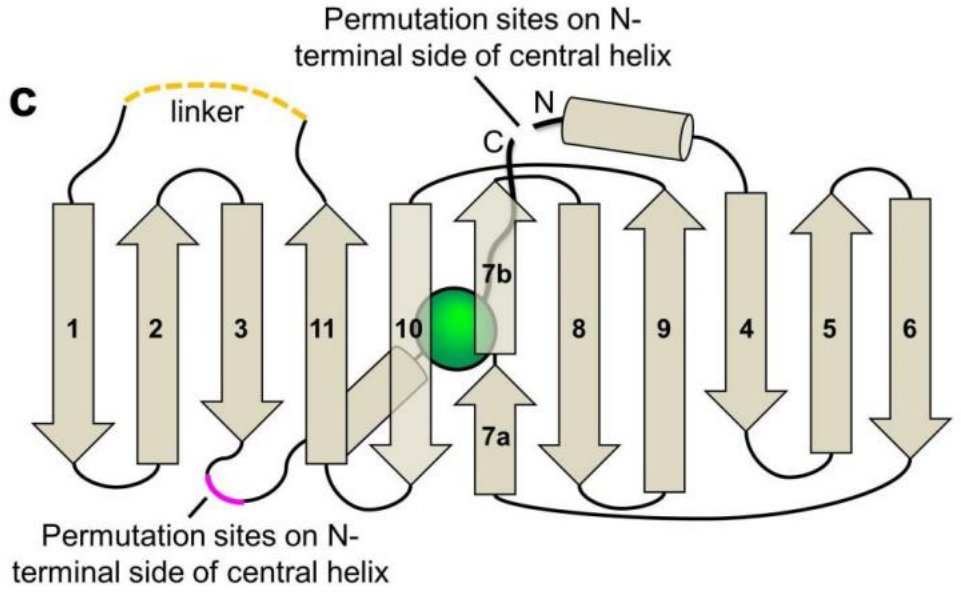
10残基+220残基

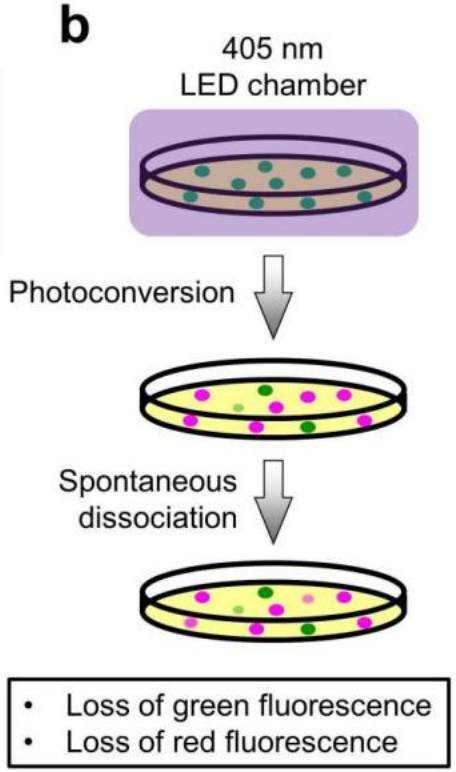






	N-terminal side of central helix			C-terminal side of central helix		
X (N-terminal residue #)	54	55	56	77	78	79
Y (C-terminal residue #)	53	54	55	76	77	78

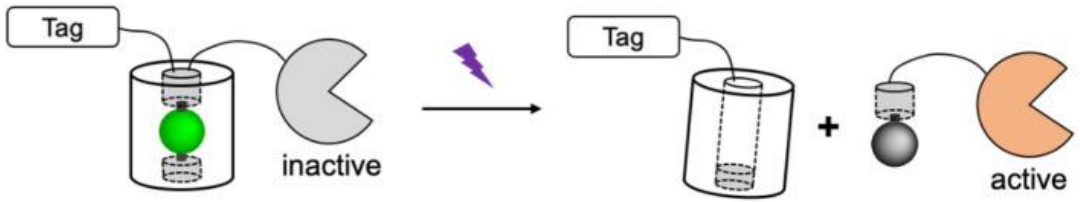




Nature Methods, 14, 391–394 (2017)

Applications of PhoCl1

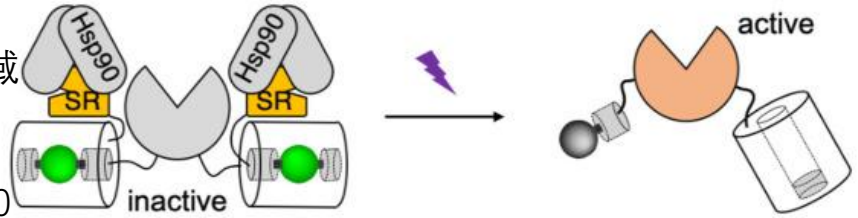
(i) Manipulation of protein localization



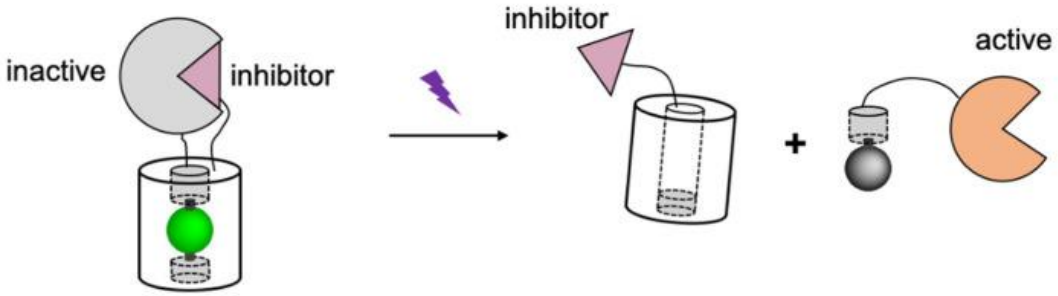
(ii) Regulation of protein via steric effect

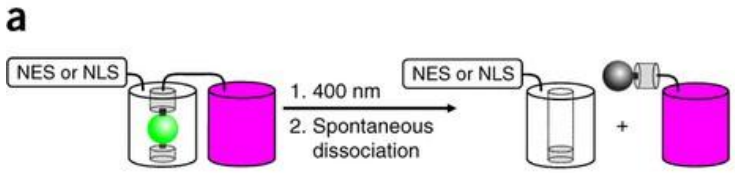
(SR)类固醇受体 结构域

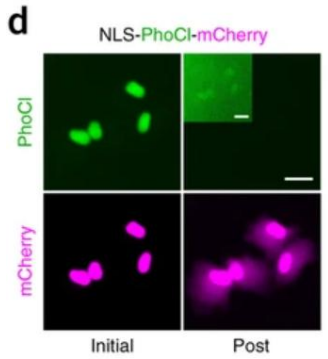
(Hsp90) 热休克蛋白 90

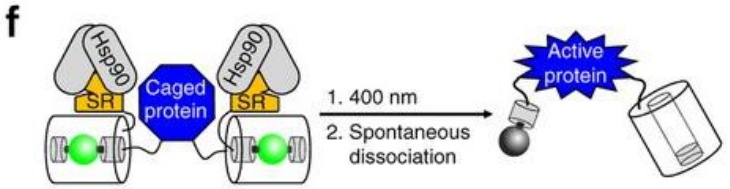


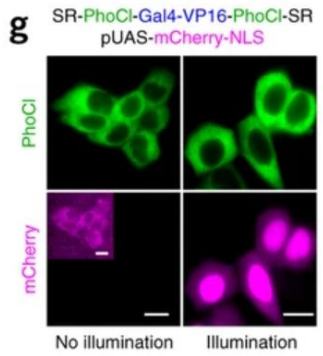
(iii) Light-induced releasing of inhibitor

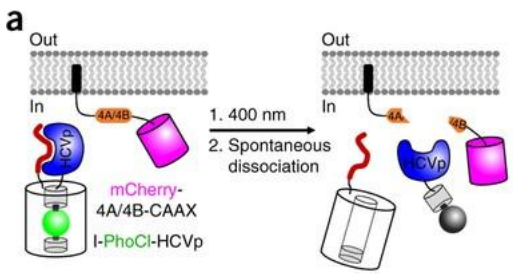


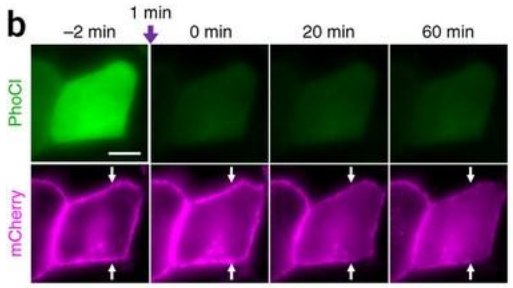


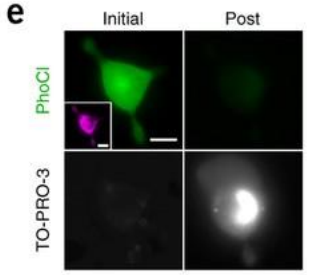






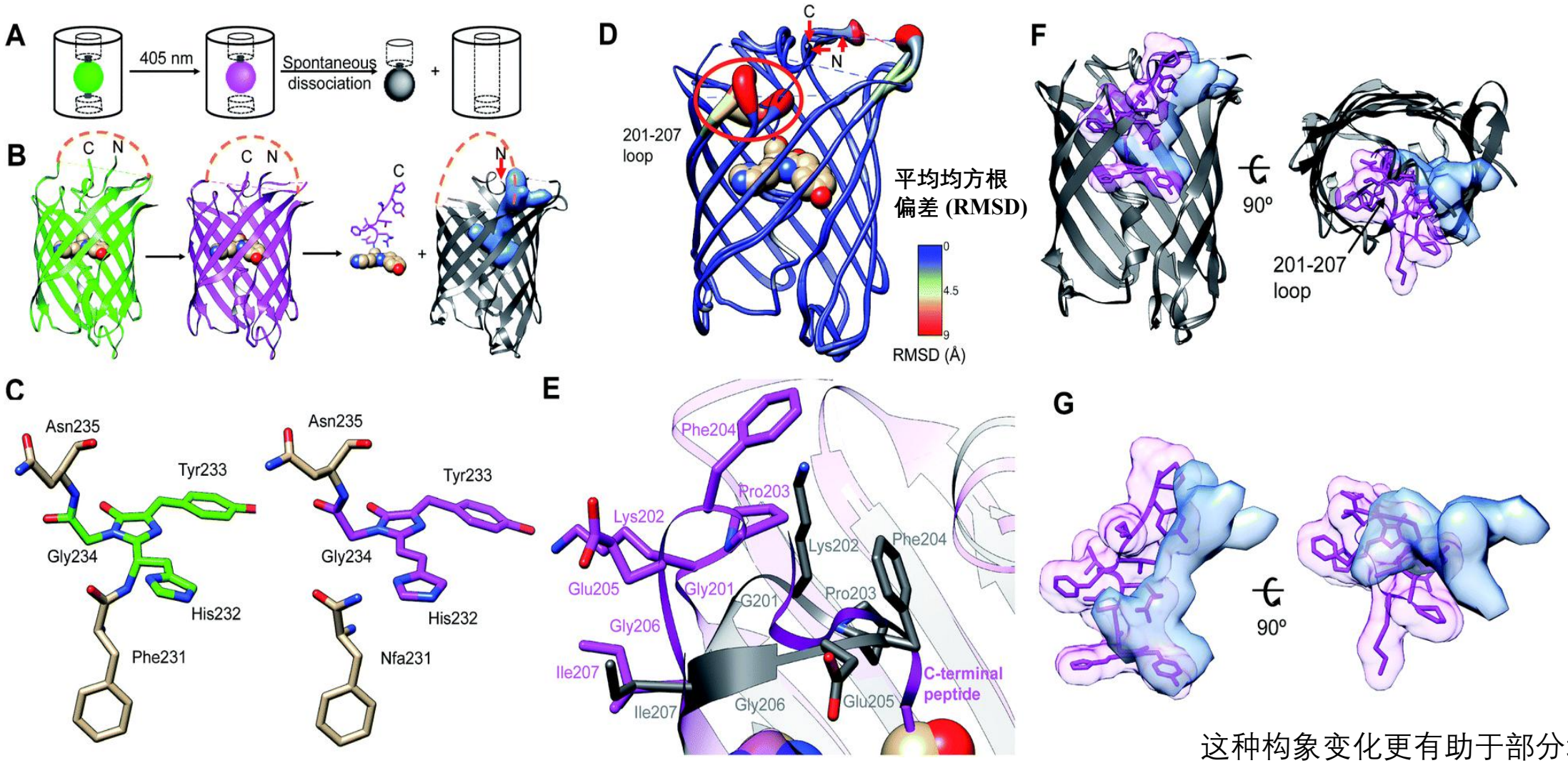






Nature Methods, 14, 391–394 (2017)
Int J Mol Sci. 2020, 21(18): 6522.

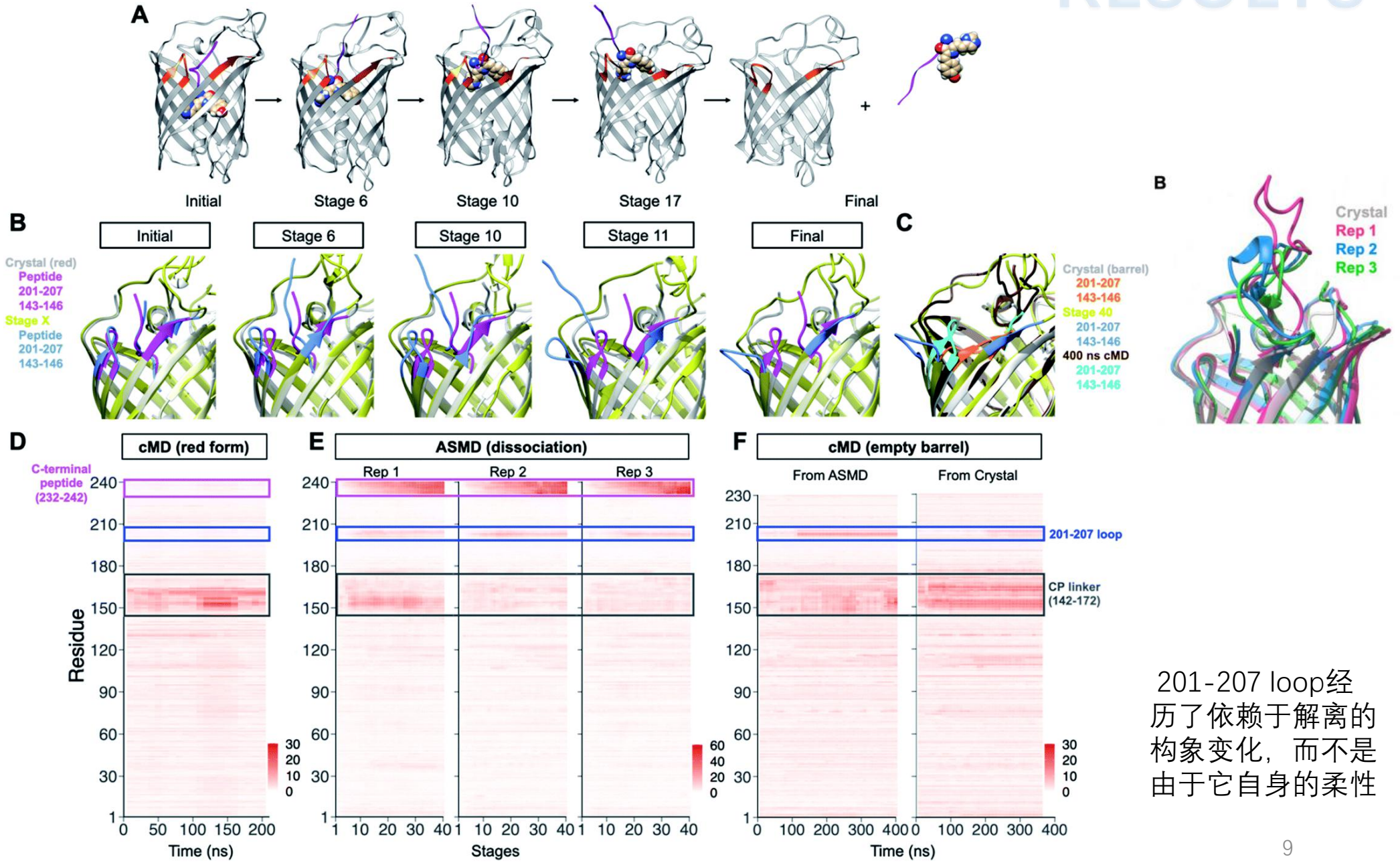
Structural changes associated with photoconversion and dissociation



这种构象变化更有助于部分填充小肽片段解离产生的疏水空隙，从而来稳定空桶结构。

Molecular dynamics (MD) simulation of the dissociation process

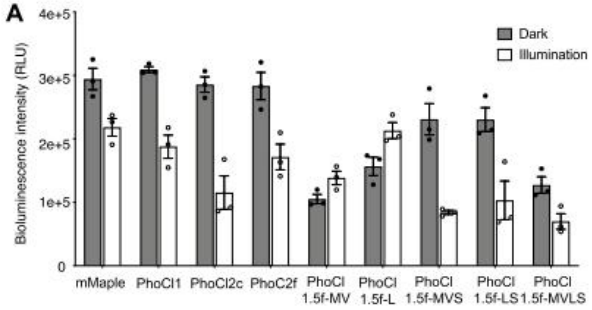
RESULTS



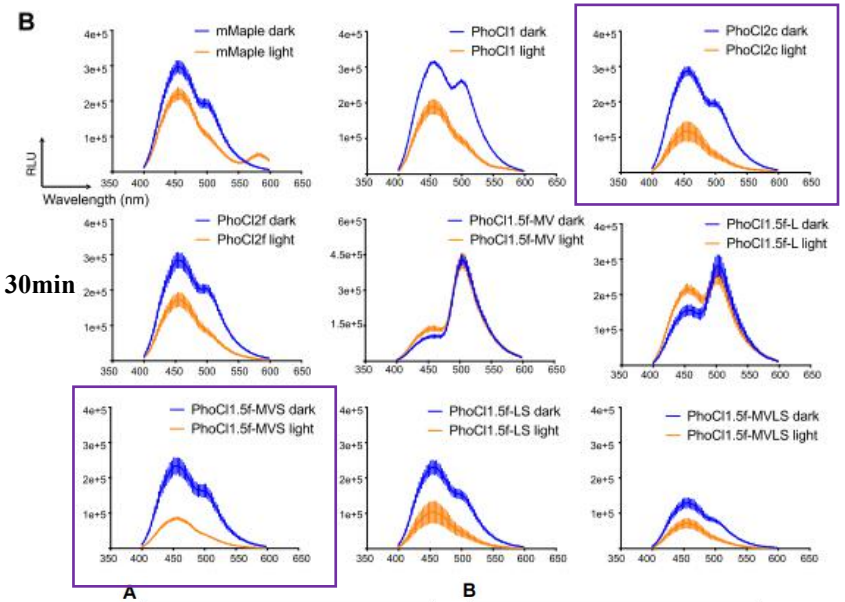
201-207 loop经历了依赖于解离的构象变化，而不是由于它自身的柔性

Engineering and characterizations of PhoCl2 variants

RESULTS



生物发光筛选



after 30min

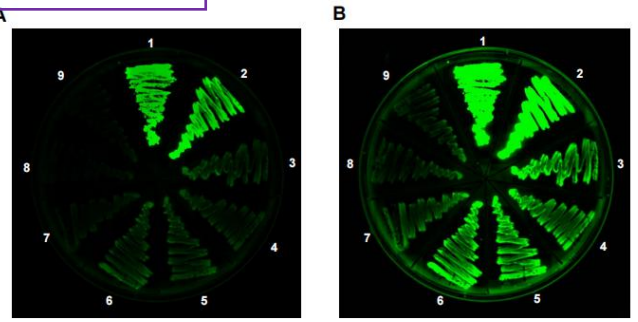
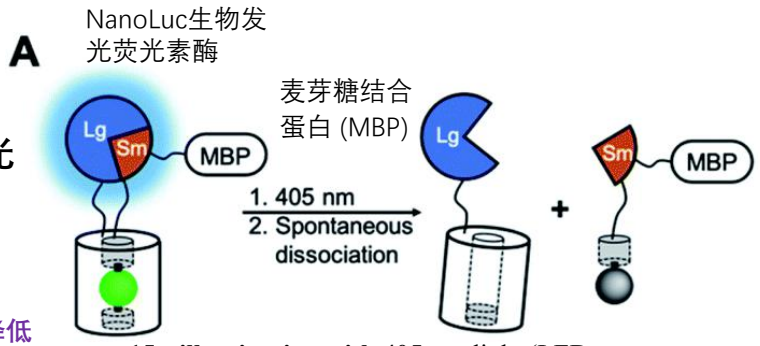
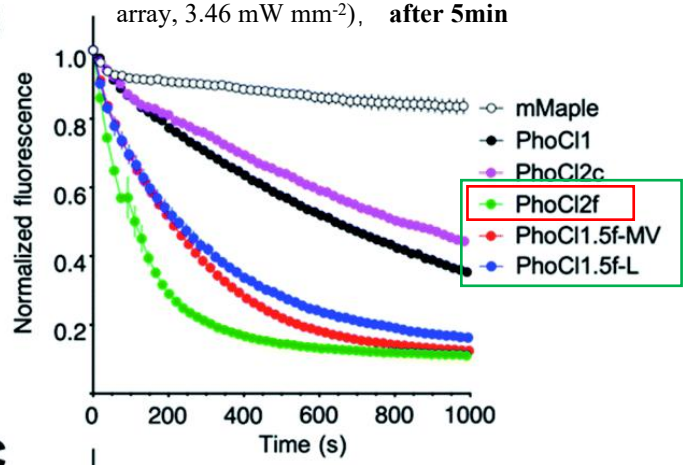


Fig. S9 Green fluorescence image of *E. coli* expressing PhoCl variants on agar media in a Petri dish. 1: mMaple, 2: PhoCl1, 3: PhoCl2c, 4: PhoCl2f, 5: PhoCl1.5f-MV, 6: PhoCl1.5f-L, 7: PhoCl1.5f-MVS, 8: PhoCl1.5f-LS, 9: PhoCl1.5f-MVLS. (B) is the same image as (A) with 5× increased contrast.

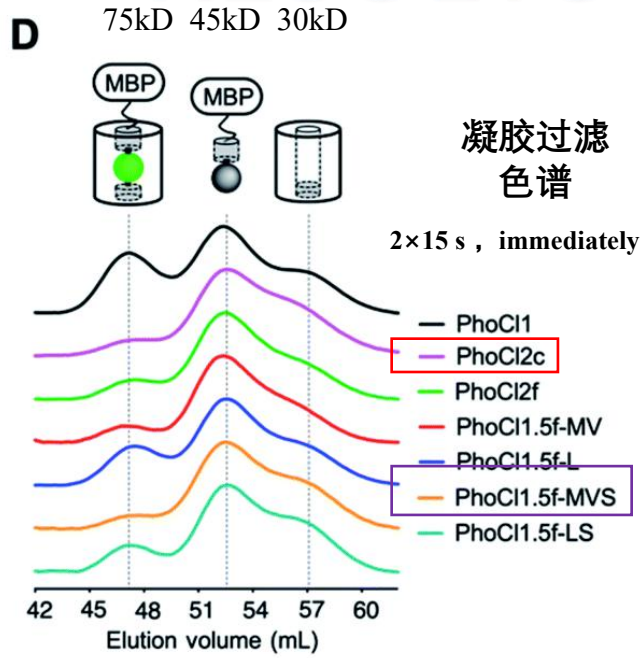


60%降低

15 s illumination with 405 nm light (LED array, 3.46 mW mm^{-2}), **after 5min**

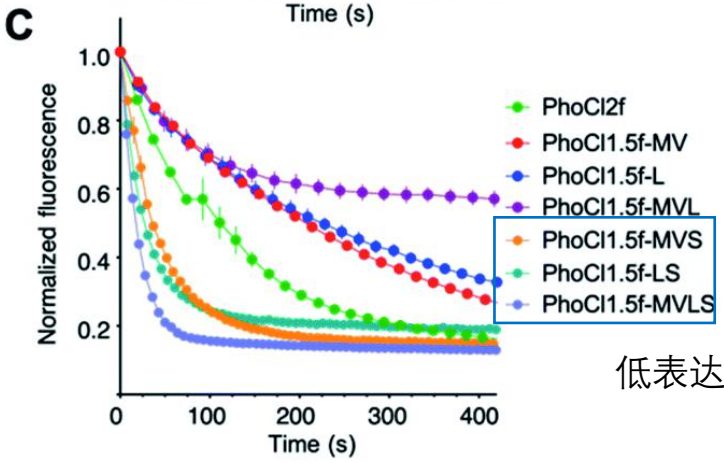


201-207 loop 附近突变

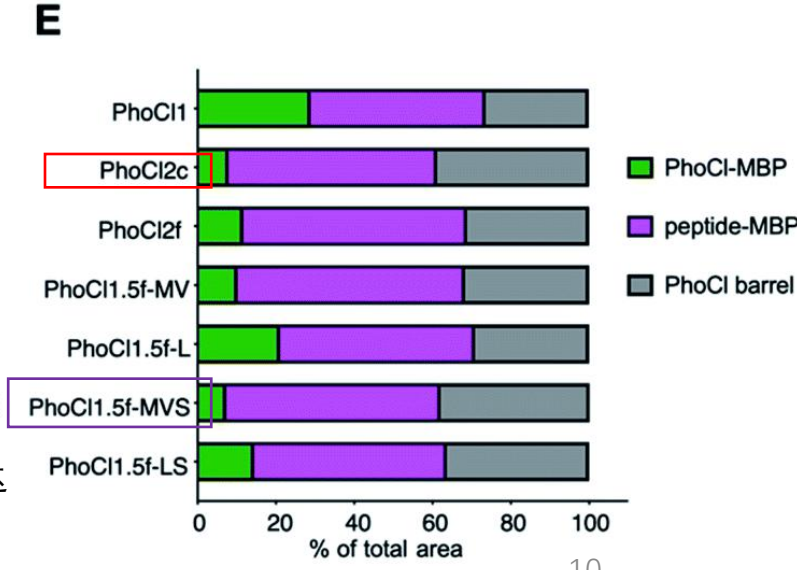


凝胶过滤色谱

2×15 s，immediately



低表达



Engineering and characterizations of PhoCl2 variants

Table S3. Summary of mutations in PhoCl2 variants.

Variants	Mutations
PhoCl1.5f-MV	Lys49Gln, Tyr117Cys, Val143Met, Ala144Val, Arg145Lys, Asn146Thr
PhoCl1.5f-L	Lys49Gln, Tyr117Cys, Arg145Lys, Asn146Thr, Lys202Leu
PhoCl1.5f-MVS	Lys49Gln, Tyr117Cys, Val143Met, Ala144Val, Arg145Lys, Asn146Thr, Ile207Ser
PhoCl1.5f-LS	Lys49Gln, Tyr117Cys, Arg145Lys, Asn146Thr, Lys202Leu, Ile207Ser
PhoCl1.5f-MVLS	Lys49Gln, Tyr117Cys, Val143Met, Ala144Val, Arg145Lys, Asn146Thr, Lys202Leu, Ile207Ser
PhoCl2f	Lys49Gln, Tyr117Cys, Arg145Lys, Asn146Thr, Ile207Ser
PhoCl2c	Lys49Gln, Cys99Gly, Tyr117Cys, Arg145Lys, Asn146Thr

PhoCl2f 1)减少空间位阻/增加解离肽附近桶的构象灵活性
——降低肽解离的能垒，更快的解离动力学

PhoCl2c 2)使完整的Phocl稳定性降低/增加空桶稳定性
——更有效的光解离

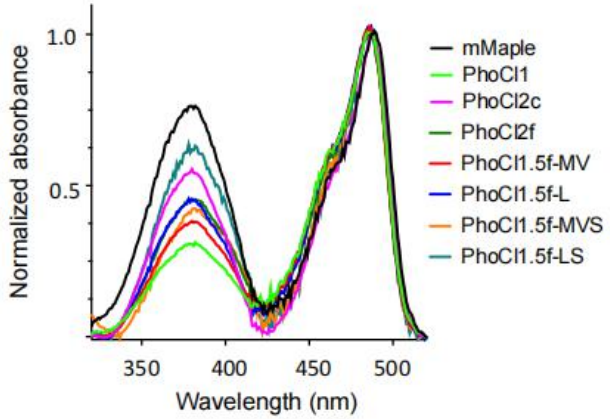


Fig. S12 Absorbance spectra of mMaple and PhoCl variants. Compared to PhoCl1, increased extinction coefficients at 405 nm were observed for the improved variants.

405 nm 处的消光系数提高——更有效的吸收
405 nm 光和更高效的光转换

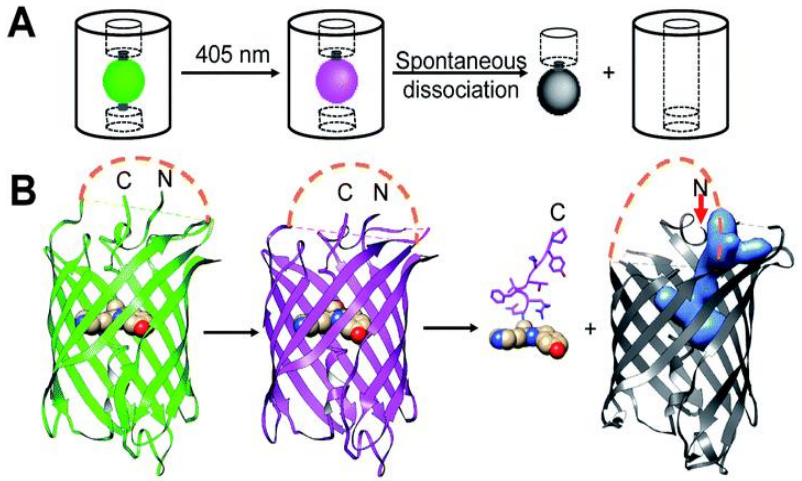
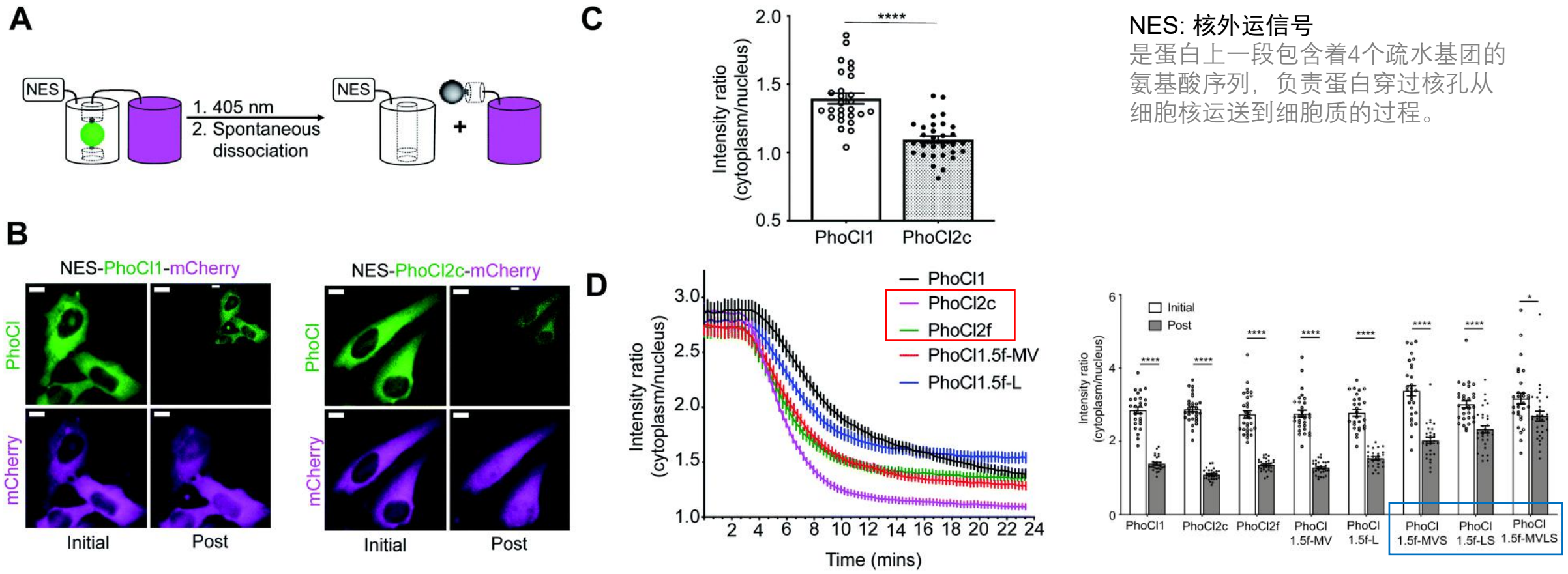


Table S6. Summary data of optogenetic manipulation of protein translocation assay with PhoCl variants.

Protein	Photoconversion Half Time (s)	Dissociation Half Time (s)
PhoCl1	75.5	241
PhoCl2c	62.7	114
PhoCl2f	78.1	135
PhoCl1.5f-MV	83.6	160
PhoCl1.5f-L	69.6	155

Plateau followed by one phase decay fit was used in both analyses. For fit of photoconversion, *R*-squared values range from 0.9900 to 0.9951. For fit of dissociation, *R*-squared values range from 0.7006 to 0.9040.

Optogenetic control of protein localization by PhoCl2 variants in HeLa cells



NES: 核外运信号
是蛋白上一段包含着4个疏水基团的氨基酸序列，负责蛋白穿过核孔从细胞核运送到细胞质的过程。

Fig. 4 Optogenetic manipulation of protein translocation in HeLa cells. (A) Schematic of the NES-PhoCl-mCherry photocleavage. (B) Representative images of HeLa cells expressing NES-PhoCl-mCherry before and **after (15 min) photoconversion**. Conversion was performed with **10 s violet light pulses (395/40 nm, 2 mW mm^{-2}) every 15 s for 6 min.** Inset is the same image with 10× increased contrast. Scale bar, 10 μm. (C) Red fluorescence intensity ratios of cytoplasm to nucleus **at 15 min after photoconversion**. Ratios were calculated for single cells. Values are means ± SEM (n = 27 cells of PhoCl1, and n = 30 cells of PhoCl2c). ****P < 0.0001 by unpaired two-tailed t test (t (55) = 6.606). (D) Red fluorescence intensity localization ratios of cytoplasm to nucleus versus time. Values are means ± SEM (n = 27 cells of PhoCl1 and PhoCl1.5f-L, and n = 30 cells of the other variants).

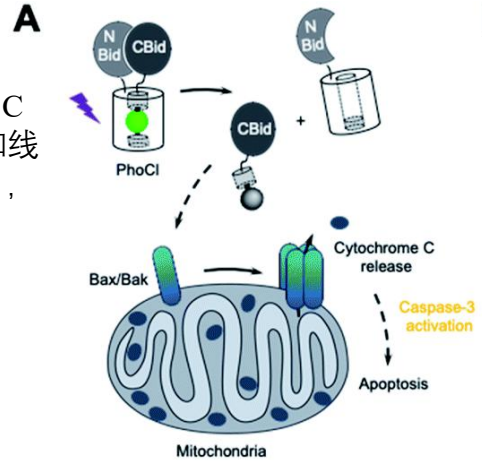
低表达

Optogenetic manipulation of cell apoptosis using PhoCl2c

RESULTS

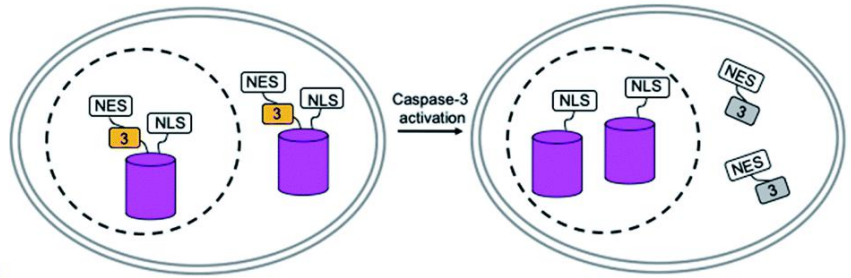
Bid： 促凋亡蛋白
功能：在细胞凋亡过程中会被caspase-8 水解酶切割，产物Bid C端结构域易位到线粒体，会增加线粒体膜通透性、释放细胞色素 c，细胞色素 c 会激活下游caspase-3蛋白酶解引起细胞凋亡。

caspase-8 酶切位点 (LQTDG)



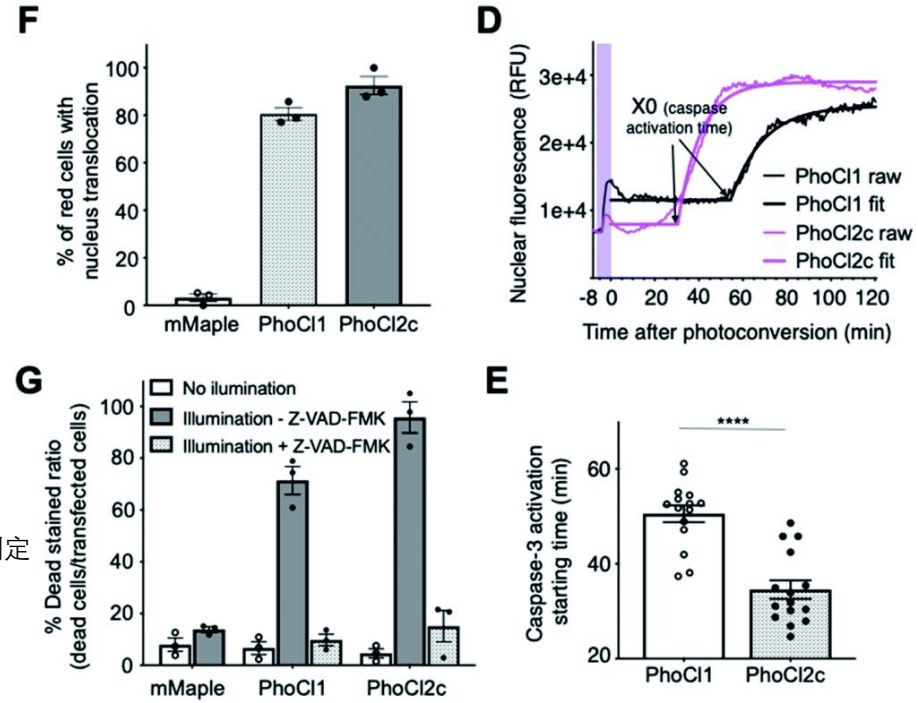
caspase-3识别序列

NES-DEVD-mCardinal-NLS



NES: 核外运信号
是蛋白上一段包含着4个疏水基团的氨基酸序列，负责蛋白穿过核孔从细胞核运送到细胞质的过程。

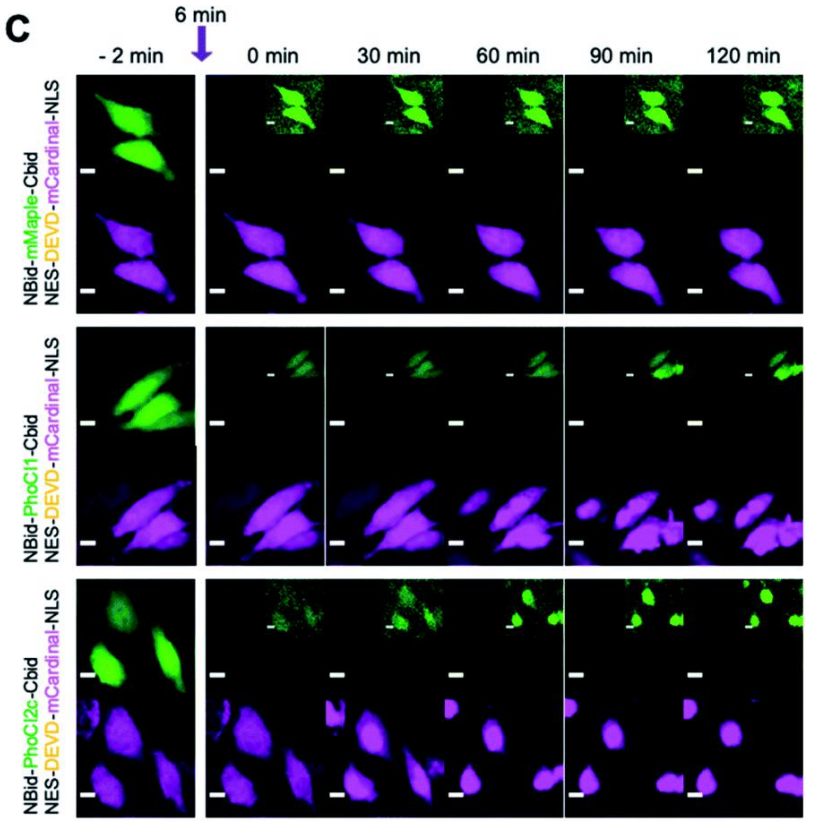
NLS：核定位信号
是蛋白质上的一个结构域，负责将大分子蛋白运进细胞核。



405 nm LED flood array with 15 s illumination, for 30 min at room temperature

DEAD 细胞活力测定染料： ethidium homodimer-1

Z-VAD-FMK: caspase 抑制剂



10 s violet light pulses (395/40 nm, 2 mW mm-2) every 15 s for 6 min

PhoCl green (490/15 nm ex; 525/50 nm em),
mCherry and DEAD stain (543/10 nm ex, 620/60 nm em)
mCardinal (605/50 nm ex; 670/50 nm em).

THANKS!