Literature Report

Reporter: Liu Weiwei

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RESEARCH ARTICLE

Optical manipulation of subcellular protein translocation using a photoactivatable covalent labeling system

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Kazuya Kikuchi

Graduate School of Engineering, Osaka University

- 1994年于日本东京大学获得博士学位，并在UCSD和Scripps研究所进行博士后研究
- 东京大学研究助理，并晋升为副教授
- 2005年 大阪大学教授

- 用于荧光成像和磁共振成像的分子成像探针的开发、体内成像和单分子细胞成像

Shin Mizukami

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研究功能分子的设计及其在观察和控制生物学功能中的应用

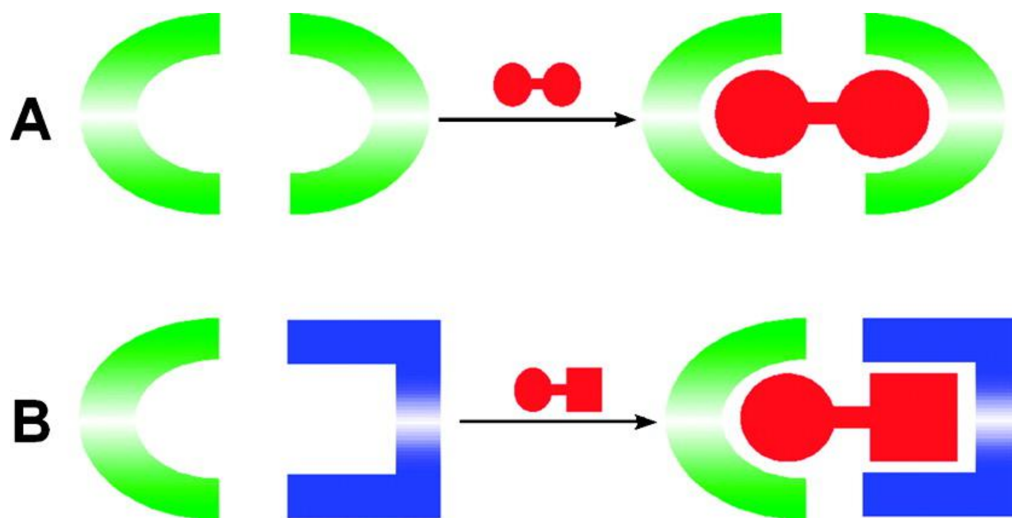
研究方向：

- 可视化细胞中的生物分子动力学（Bioimaging）
- 细胞内和体内分子功能的光操纵（光药理学，光化学生物学）
- 阐明蛋白质功能并开发新的功能蛋白质

Introduction

光遗传学技术是近年来在生命科学领域飞速发展的一种新型技术，它是利用光来远程控制基因改造过的靶向细胞。光信号在时间和空间上能被快速精确地输送到目标细胞，因此光遗传学技术可以对细胞的活动进行精准的时空控制。

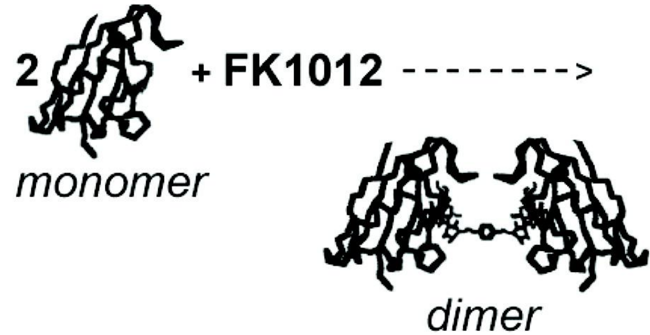
化学诱导蛋白质二聚(CID)是一种使用外源配体来调控蛋白功能和结构变化的策略，可用于操纵活细胞中的蛋白质-蛋白质相互作用，以前所未有的精度和时空分辨率控制蛋白质功能。在大多数情况下，目标蛋白质与蛋白标签融合，而小分子二聚体将两个标签配体连接在两端。

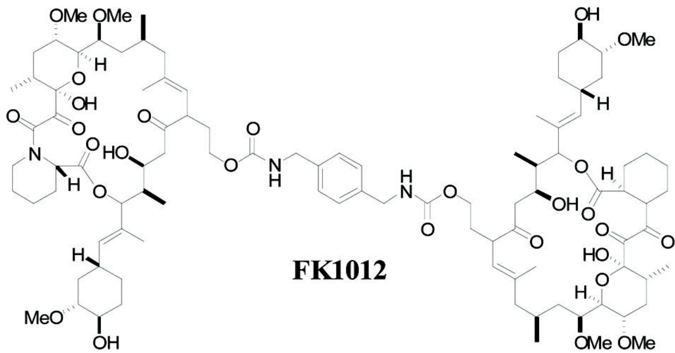


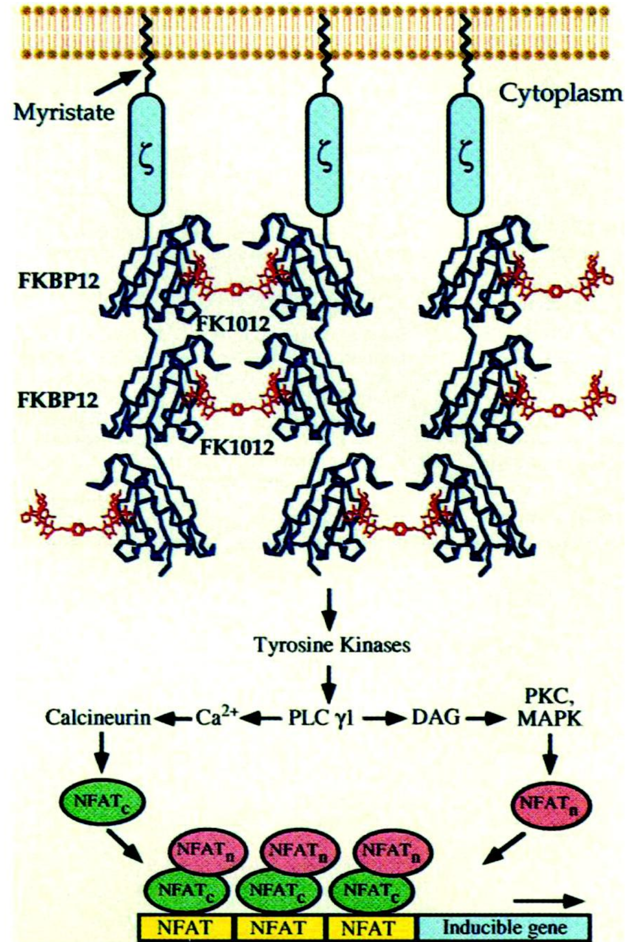
在对称配体的存在下，两种蛋白质可以结合在一起形成同型二聚体;使用非对称配体，可以将两种不同的蛋白质结合在一起形成异二聚体。

Chem. Rev. 2010, 110, 6, 3315–3336
Angew. Chem. Int. Ed. 2012, 51, 8166 – 8176

Introduction





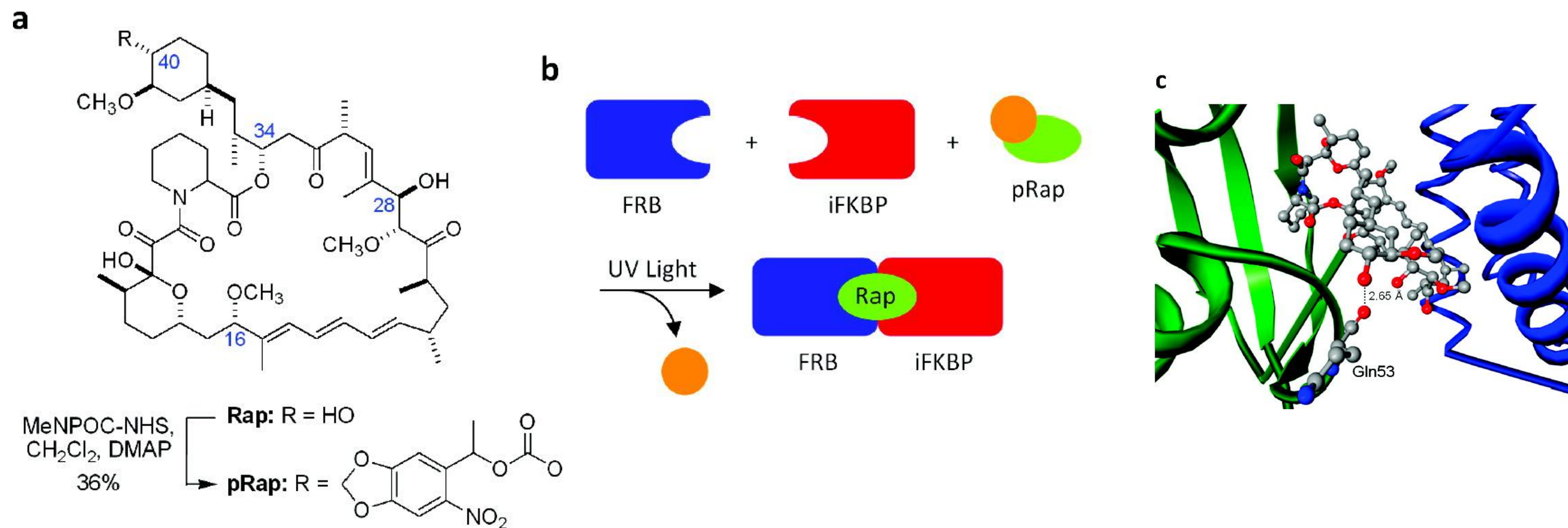


由小分子引发的化学诱导二聚化的概念是由Schreiber于1993年提出的。他们发现免疫抑制药FK1012的二价衍生物显示可逆地二聚体化其蛋白质靶标FK1012结合蛋白（FKBP）。最重要的是，FK1012可用于驱动生物学功能。

Science 1993, 262, 1019

Introduction

最为经典的是以天然产物雷帕霉素或其类似物为配体的方法。雷帕霉素通过促进FKBP12与FRB（FKBP-雷帕霉素结合蛋白） 结合来进一步诱导含有融合有FKBP和FRB结构域的蛋白质复合物的二聚。

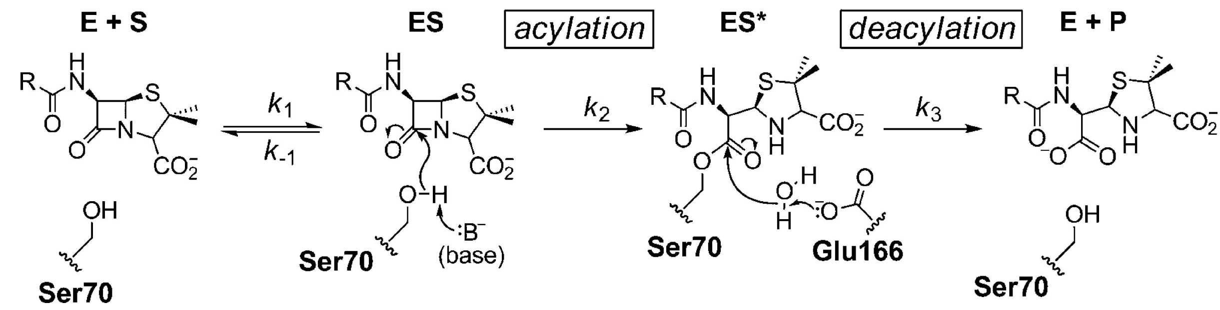


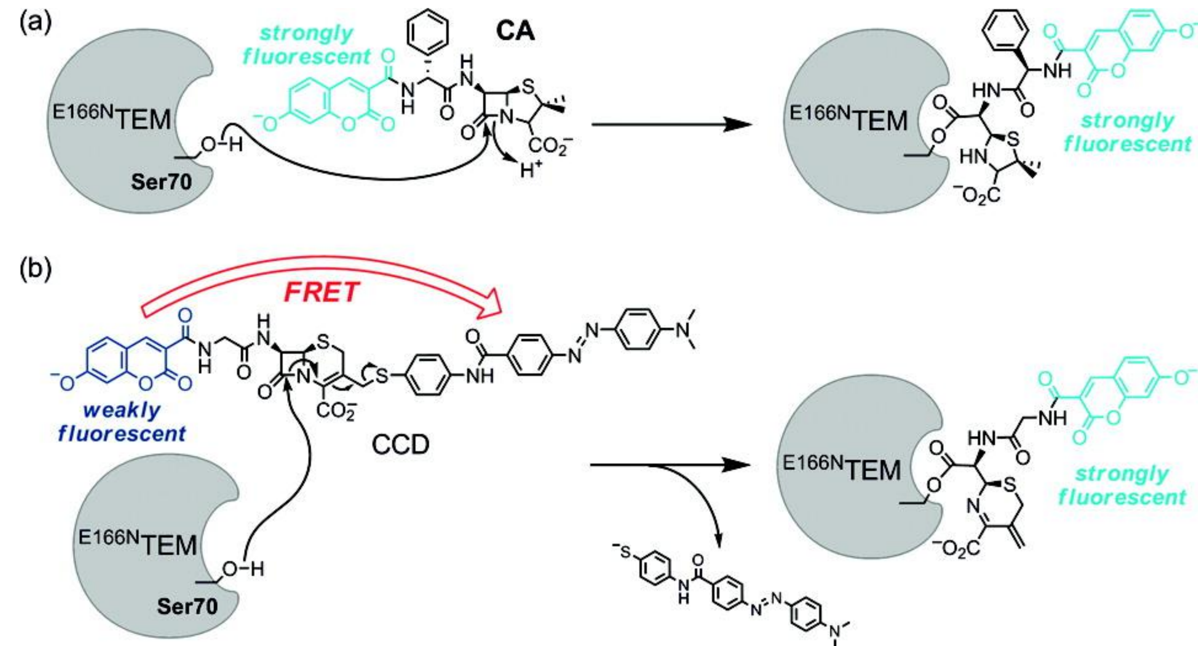
J. Am. Chem. Soc. 2011, 133, 3, 420–423

问题：这些CID系统中的大多数利用非共价蛋白质标记技术诱导蛋白质二聚。

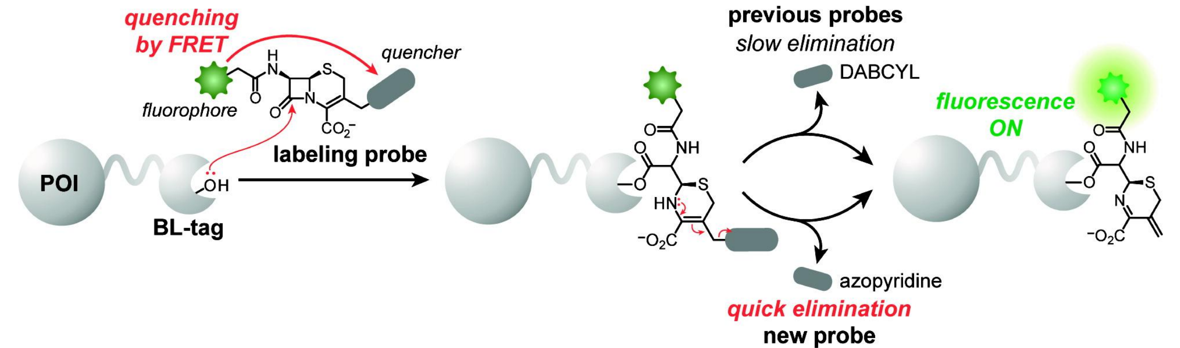
BL-tag system

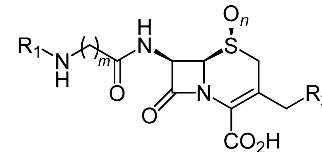
β-内酰胺酶是一种细菌酶，它水解含有β-内酰胺结构的抗生素（例如青霉素和头孢菌素），在真核细胞中没有内源性对应物





J. Am. Chem. Soc. 2009, 131, 5016–5017





	R_1	R_2	*m*	*n*
CCD			1	0
FCD			1	0
RCD			1	0

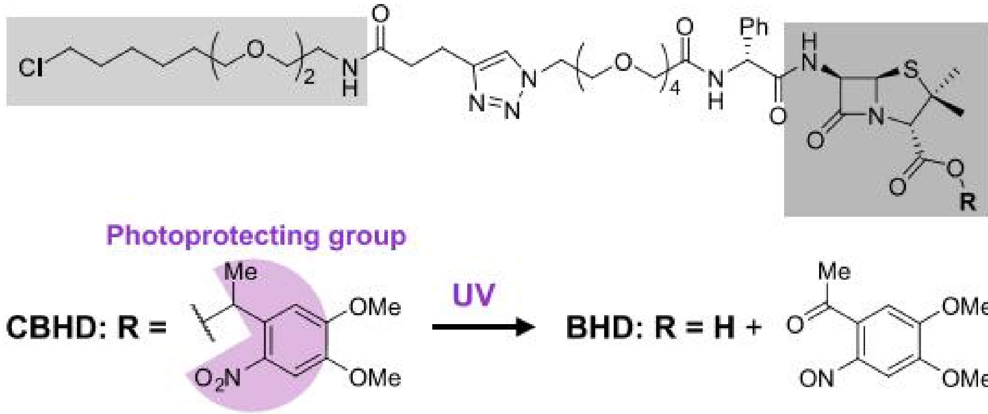
	R_1	R_2	*m*	*n*
CAP1	H		1	0
CAP2	H		2	0
FCAP2			2	0
FCAPO2			2	1
FCAPO2-DA			2	1

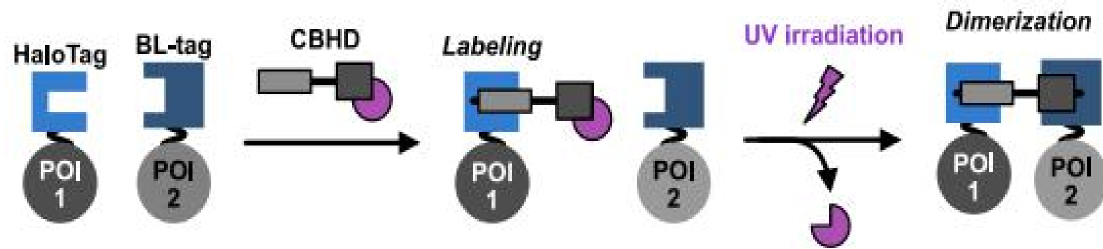
J. Am. Chem. Soc. 2012, 134, 3, 1623–1629

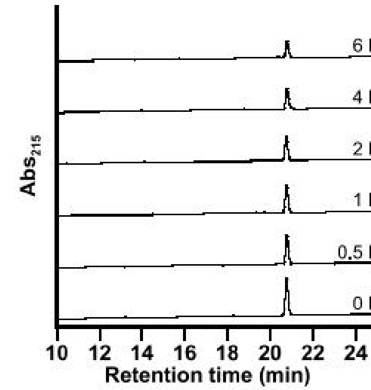
photoactivatable covalent protein-labeling system

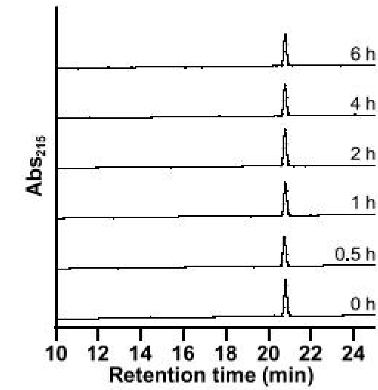
Figure 1. (a) Chemical structures of the synthesized caged BL-tag ligands (top), and schematic illustration of light-induced fluorescence labeling of the BL-tag-fused protein (bottom). (b) SDS-PAGE analysis of the light-induced labeling reaction of BL-tag with FPA-DMNPE. Coomassie Brilliant Blue (CBB)-stained and fluorescence gel images. [FPA-DMNPE] = 15 µM, [BL-tag] = 10 µM in 100 mM HEPES buffer (pH 7.4). Uncaging light: 365 ± 5 nm, 9.3 mW cm−2, for 5 min at rt. Excitation: 470 nm. Detection: 590 ± 40 nm. (c) Fluorescence intensities of the bands corresponding to the labeled BL-tags in the SDS-PAGE analysis shown in (b). The error bars represent s.d. (n = 4). (d) Confocal laser scanning microscopy images of BL-tag-fused EGFR (BL-EGFR) labeled with 100 nM FPA-DMNPE. For the condition of UV (+), FPA-DMNPE was uncaged by UV irradiation before probe addition to the live cells. Uncaging light: 365 ± 5 nm, 9.3 mW cm−2, for 5 min at rt. Scale bar: 40 µm.

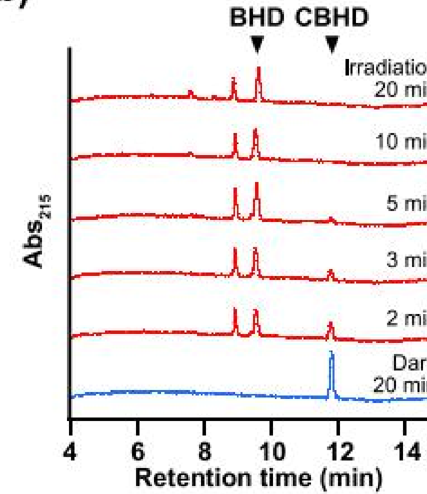
Design of the caged BL-tag–HaloTag dimerizer (CBHD)

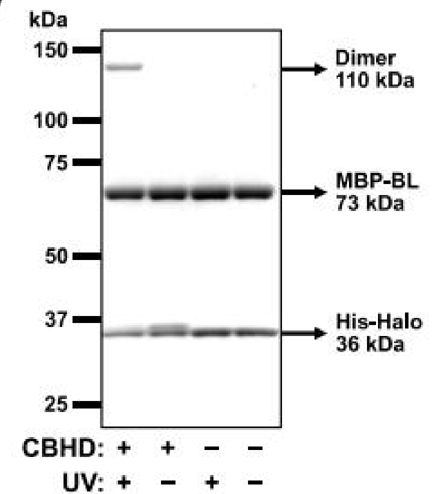






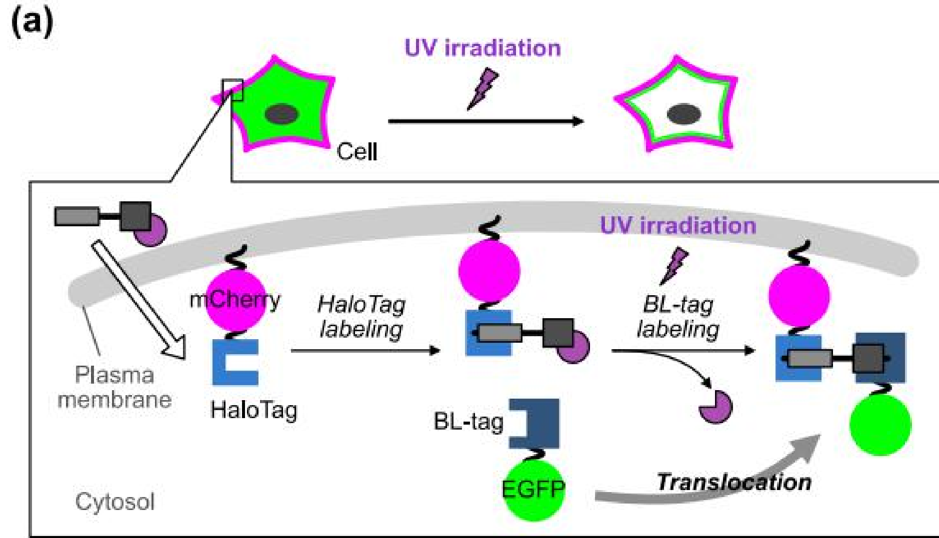


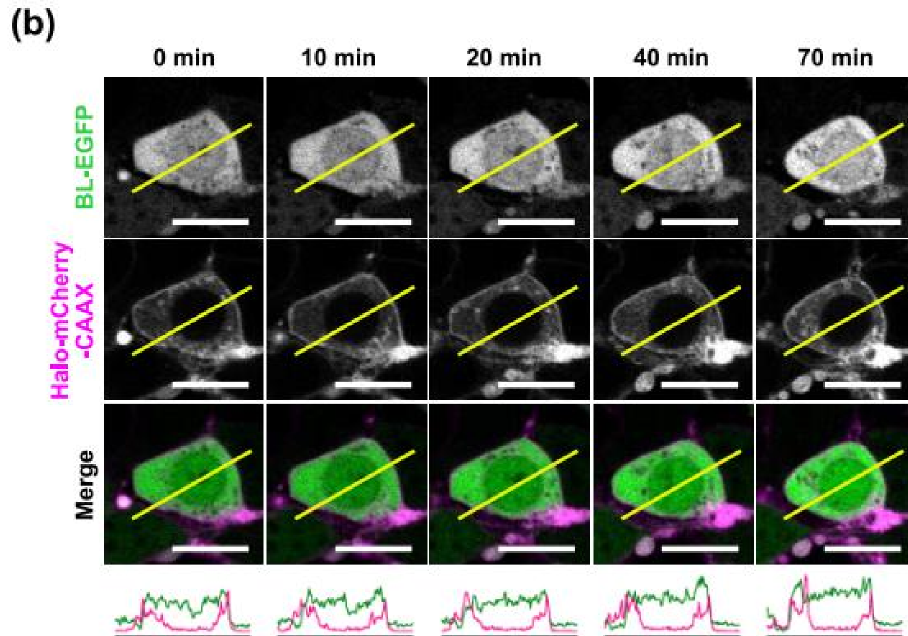


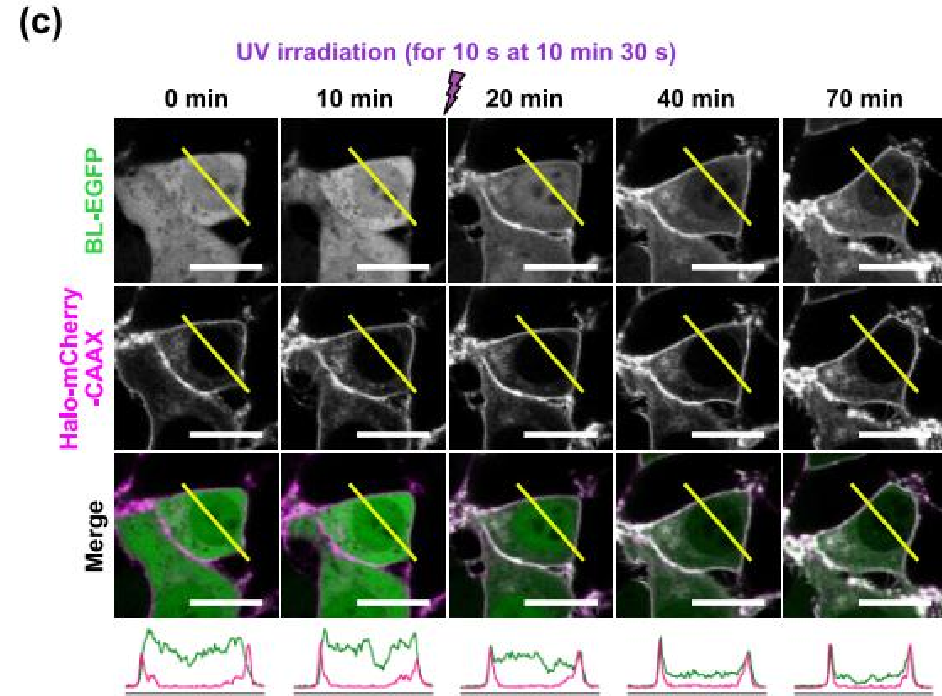


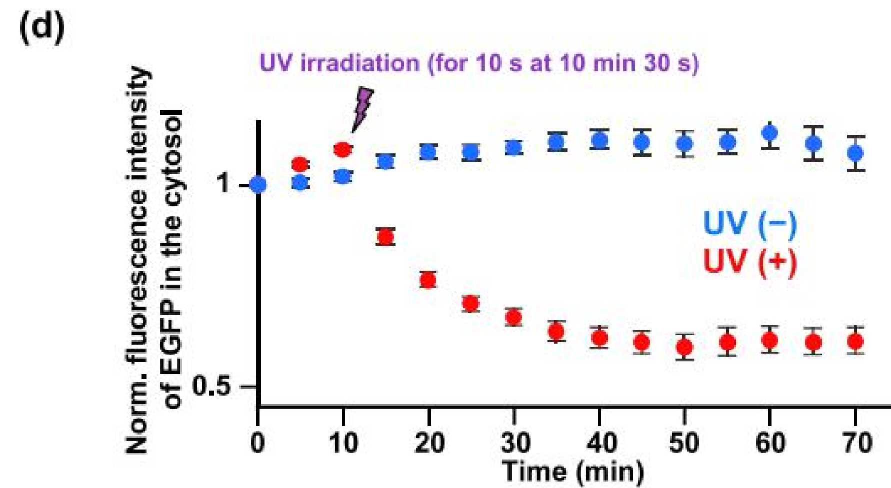
Intracellular protein translocation

光激活蛋白从细胞质移动到细胞质膜



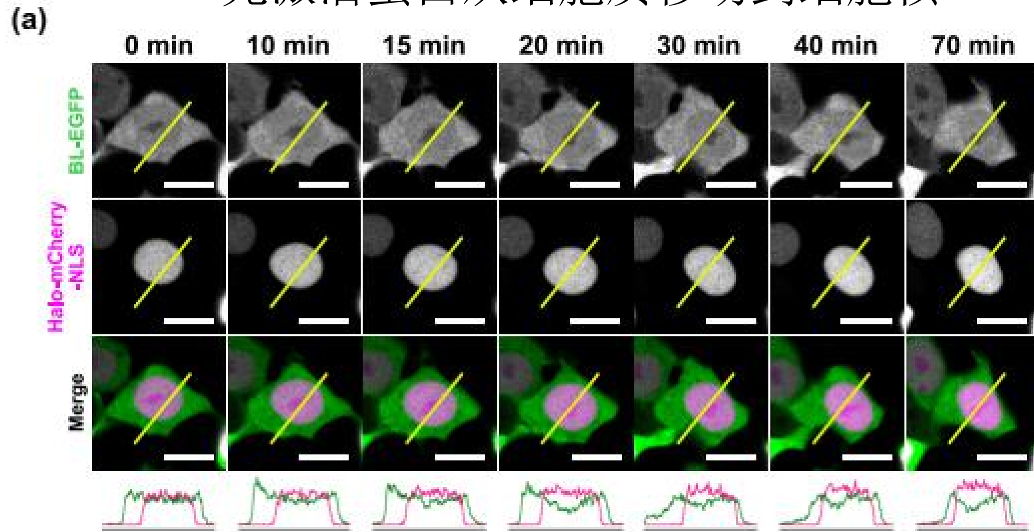


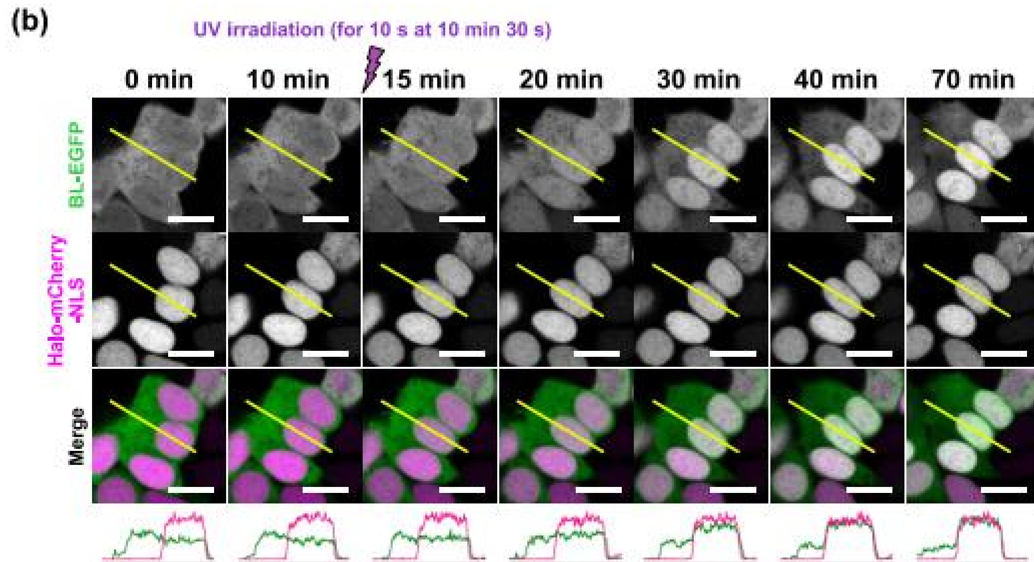


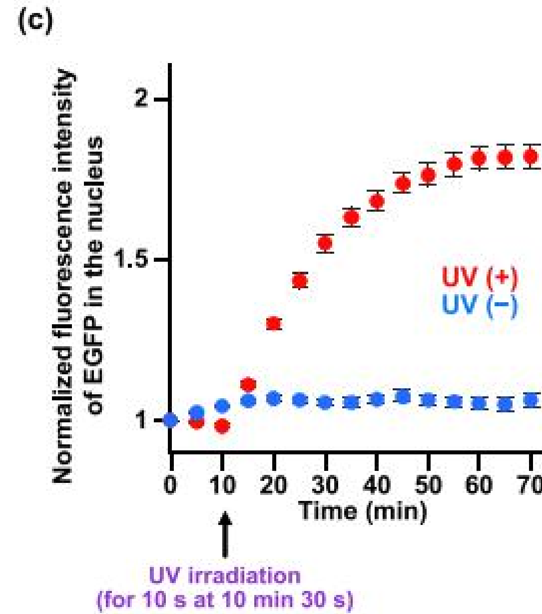


light-induced protein translocation to different subcellular regions

光激活蛋白从细胞质移动到细胞核

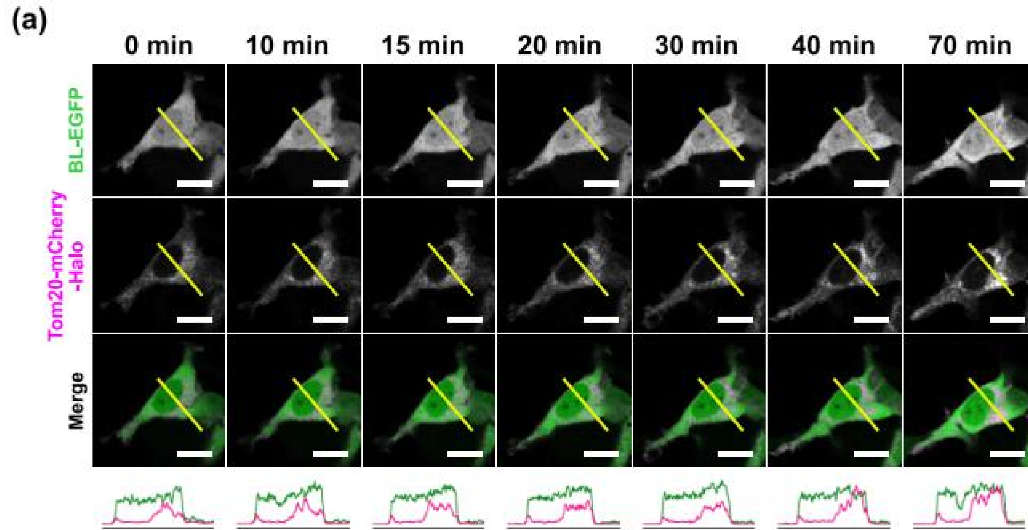


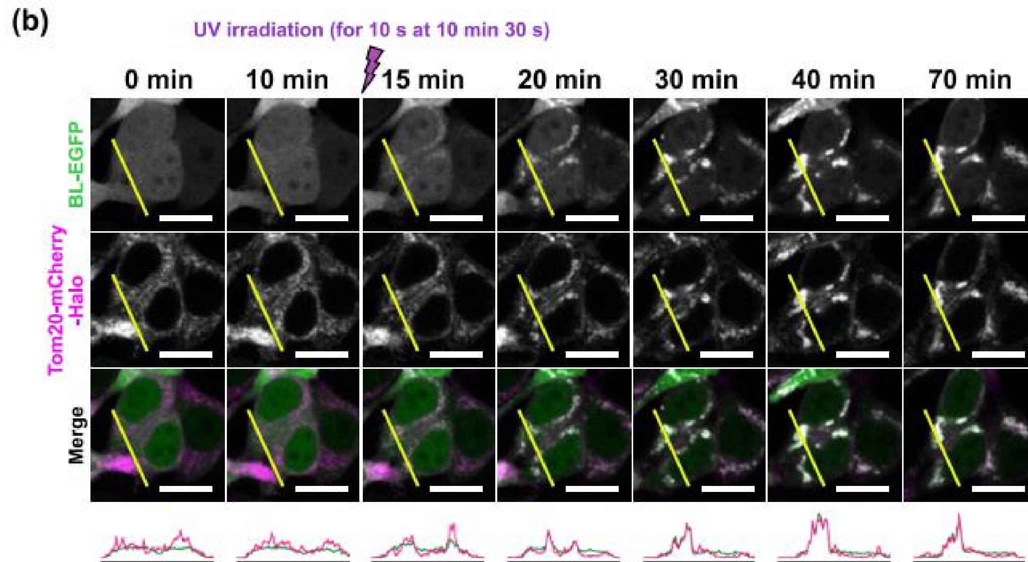


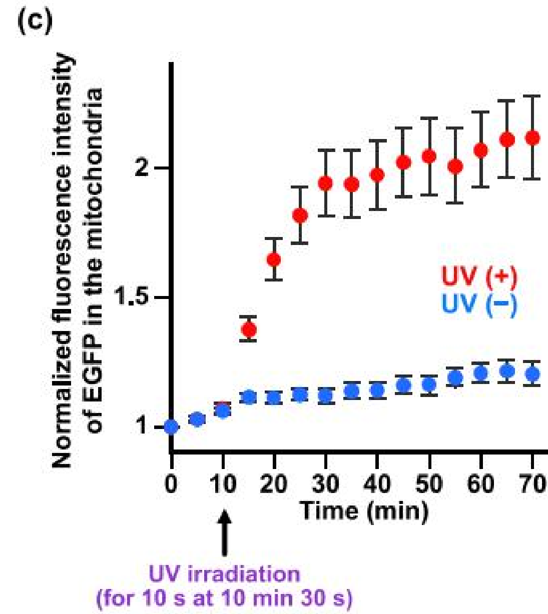


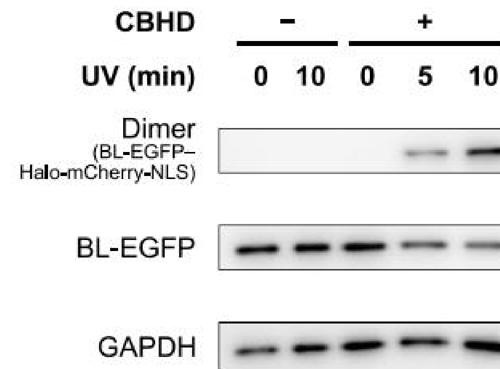
light-induced protein translocation to different subcellular regions

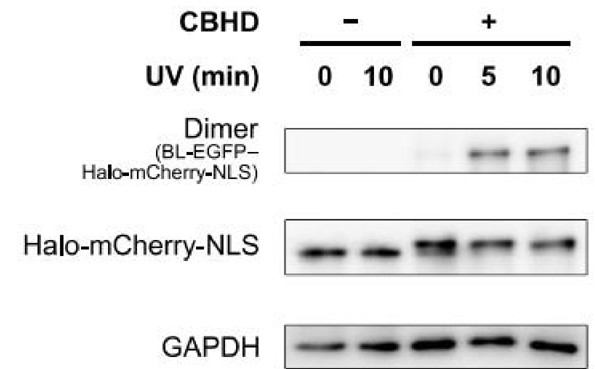
光激活蛋白从细胞质移动到线粒体外膜





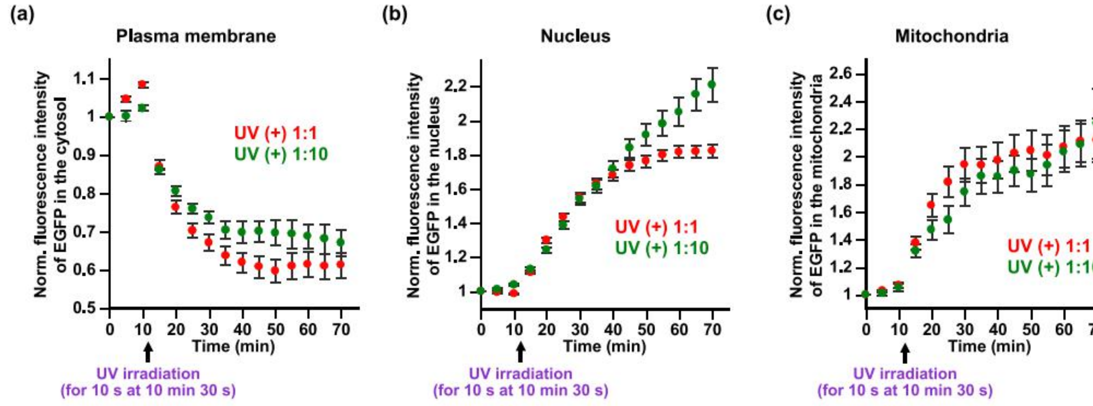


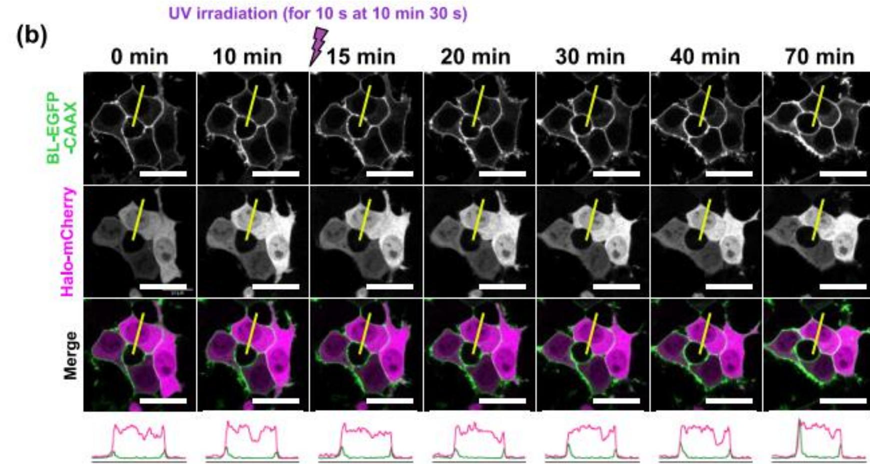


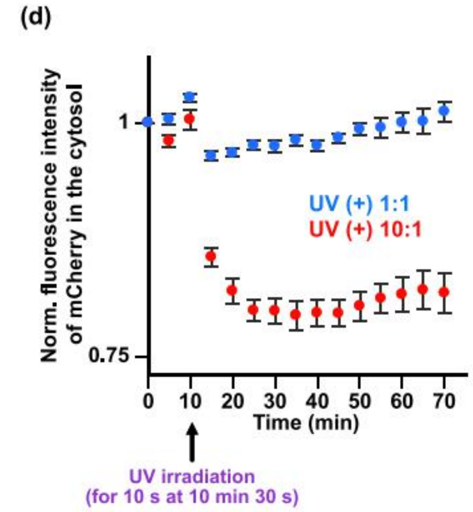


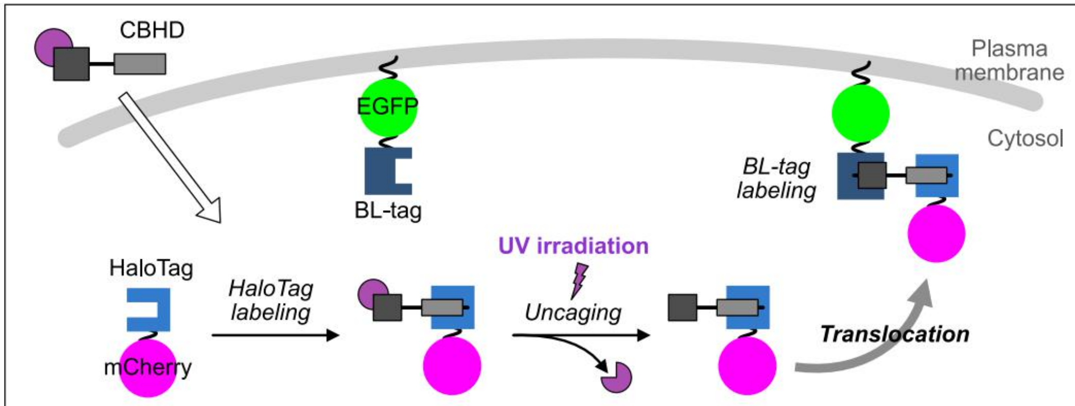
Intracellular protein translocation

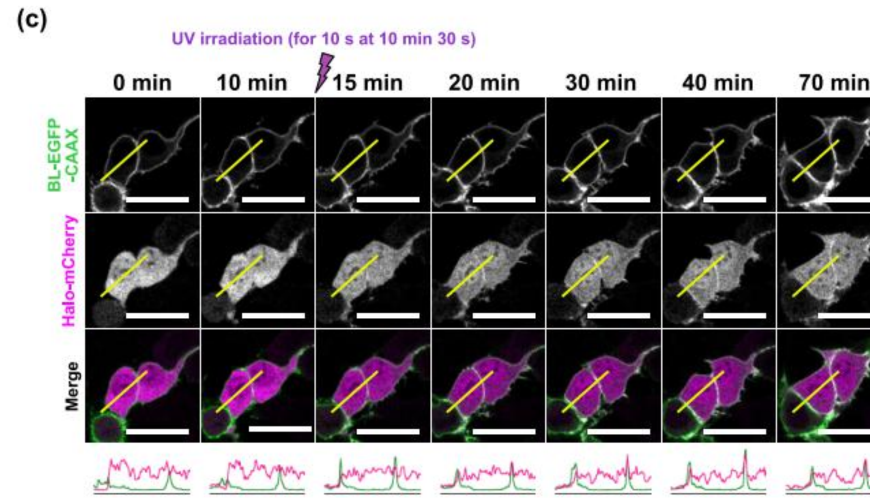
光激活的蛋白易位的动力学和效率











Intracellular protein translocation

蛋白快速易位到一个光照射的微区