Literature Report

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Article

Selective Mitochondrial Protein Labeling Enabled by Biocompatible Photocatalytic Reactions inside Live Cells

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2002年，北京大学，获得化学学士学位
2007年，普林斯顿大学，获得有机化学博士学位
2007年以后，哈佛大学和霍华德休斯医学研究所，博士后
2011年秋，上海有机化学研究所 生命有机化学国家重点实验室

陈以昀 研究员

2000-2006 北京理工大学 学士和硕士
2006-2010 德国慕尼黑-路德维希-马克西米利安大学 博士
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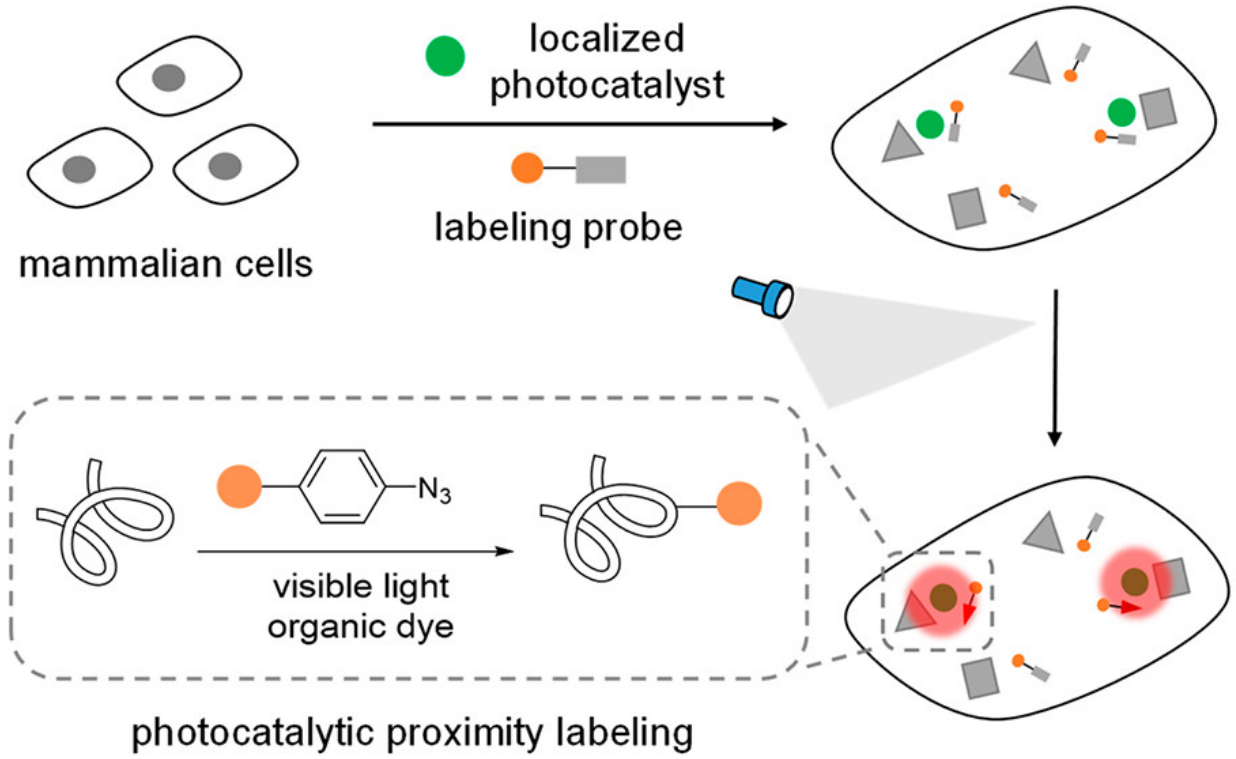
2013.9-至今 中国科学院生物与化学交叉研究中心

张耀阳 研究员



陈以昀课题组

研究方向：主要发展新的生物相容的可见光化学方法用于分子科学及生命科学的研究。

研究内容：新型可见光反应发现及新型可见光化学生物学工具发展。

研究目标：通过光引发的生物相容反应（稳定的反应物及快速的反应动力学，多样化的成键断键反应类型，高时空分辨率的外源调控）实现光调控生物分子功能。

张耀阳课题组

研究方向：质谱和蛋白质组实验室利用生物质谱技术来研究蛋白质在生物体系中的多种特性。

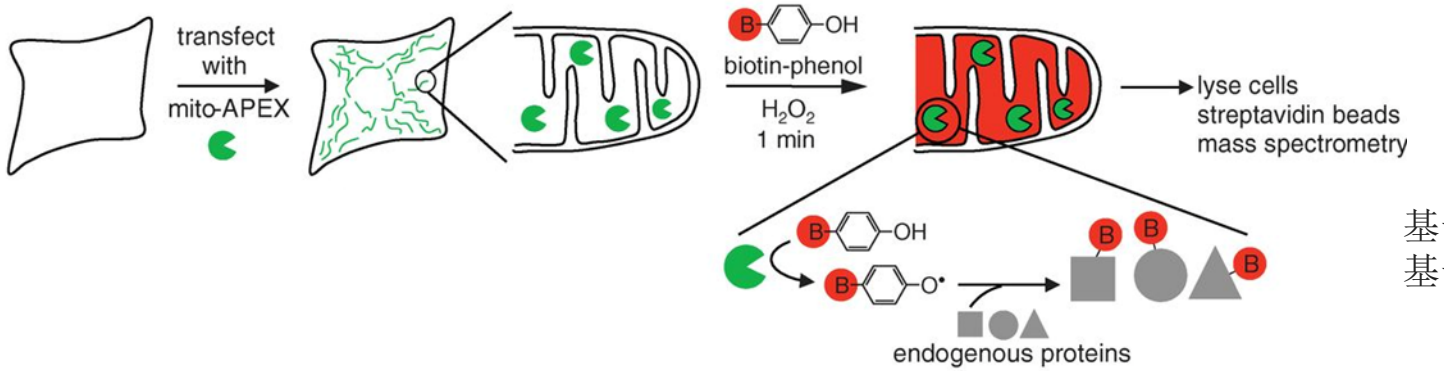
其研究方向主要包括：

1. 质谱蛋白质组学新技术和新方法的研究
2. 神经退行性疾病和衰老过程的相关蛋白质组学研究
3. 质谱蛋白质组学技术在生物学问题中的广泛应用

The development of subcellular protein labeling methods

The enzymatic proximity labeling methods

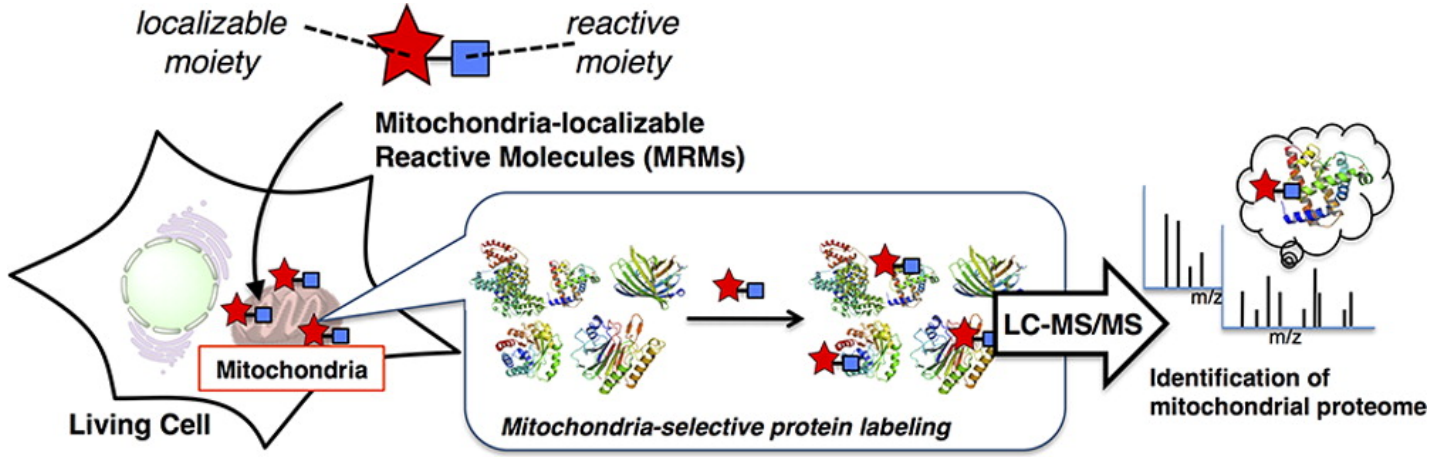
基本原理:将共价生物素标签引入活细胞中选定目标附近的蛋白质



基于生物素连接酶的邻近标记方法(BioID方法)
基于Horseradish Peroxidase的邻近标记方法(HRP方法)

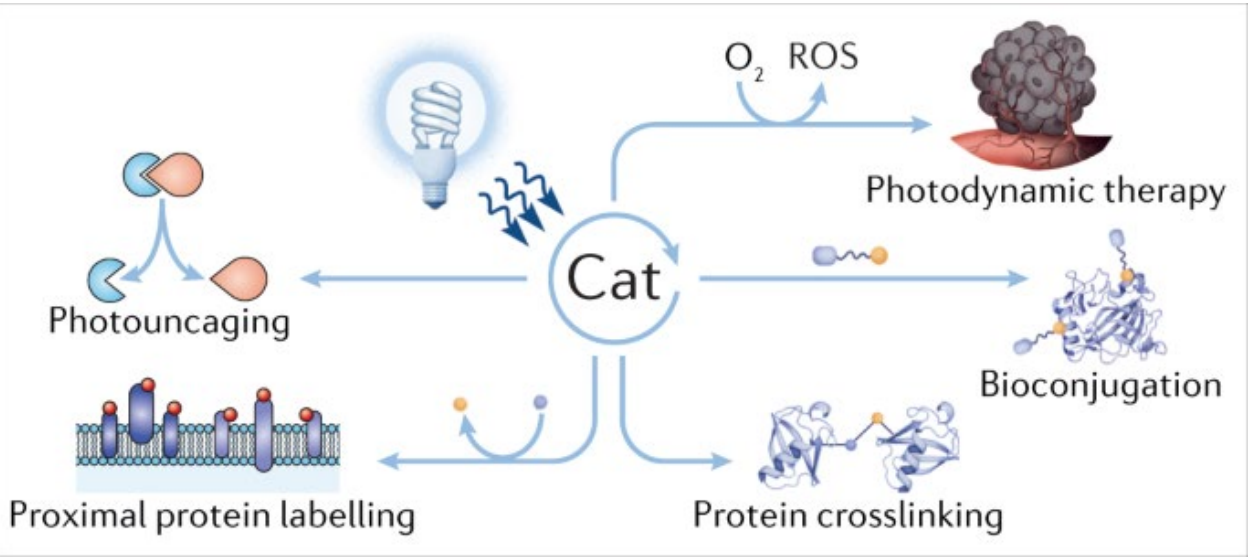
Science 2013, 339, 1328–1331.

The chemical affinity labeling methods with organelle-targeting reactive probes

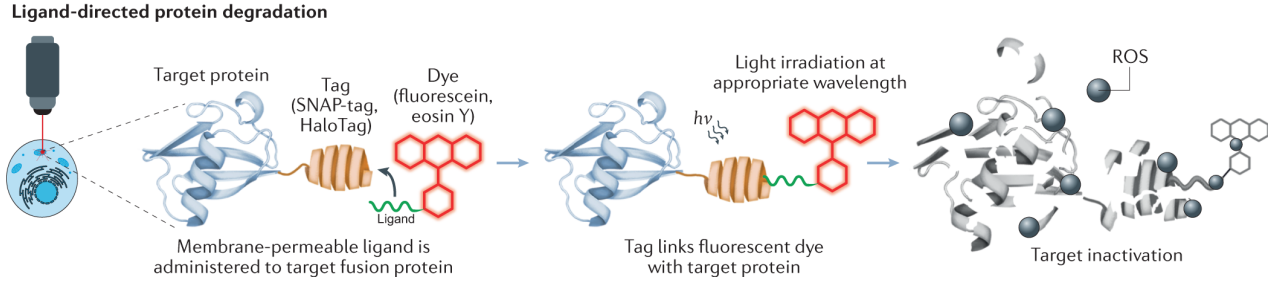


J. Am. Chem. Soc. 2016, 138, 24, 7592–7602

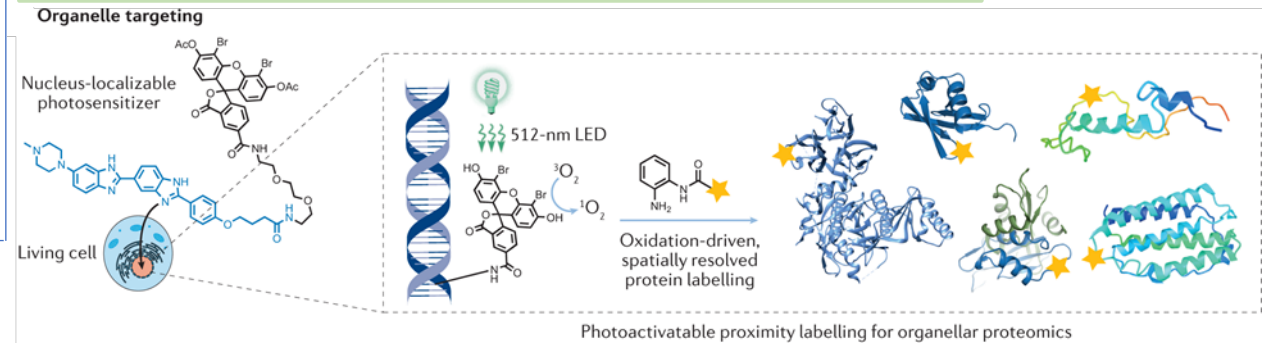
Interrogating biological systems using visible-light-powered catalysis



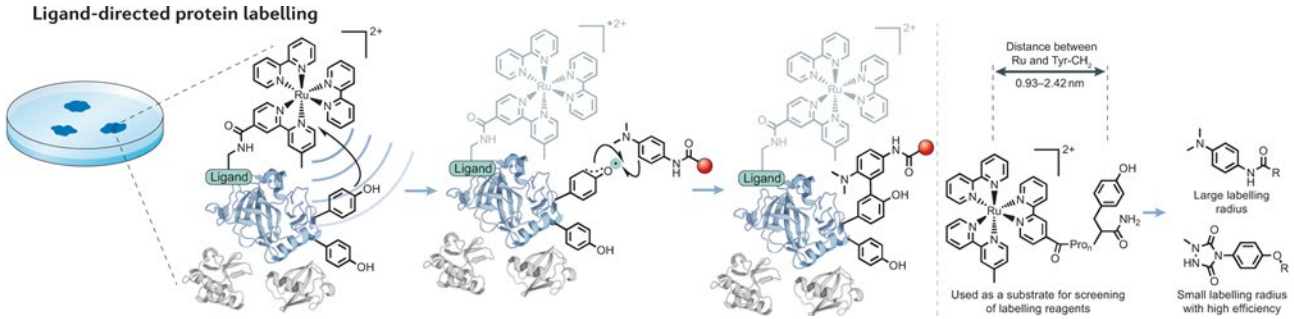
Ligand-targeted photoinactivation



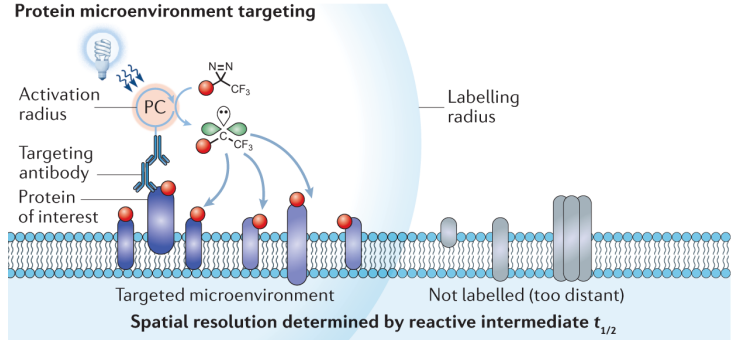
Organelle-specific photocatalytic protein labelling

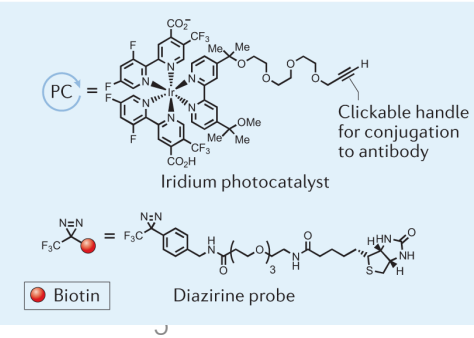


Ligand-directed photocatalytic protein labelling

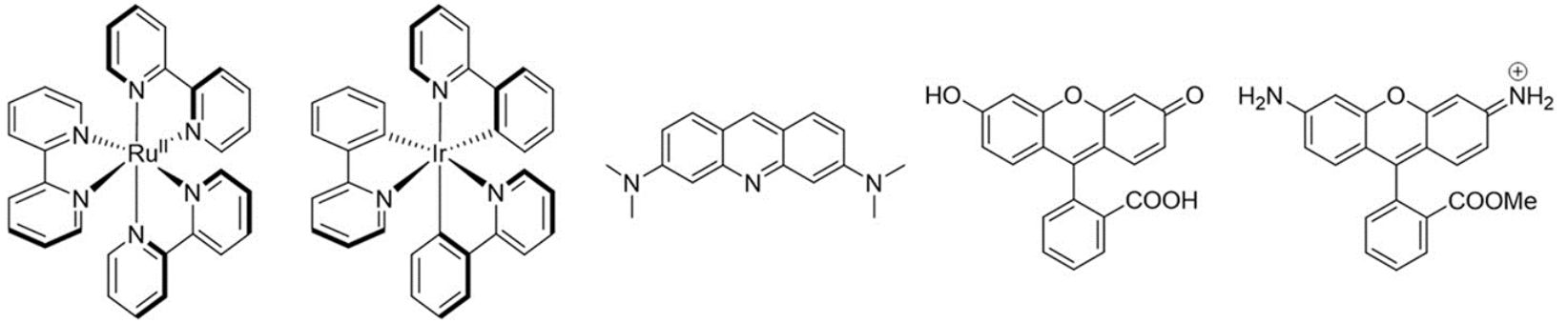


Cellular microenvironment mapping via photoproximity labelling

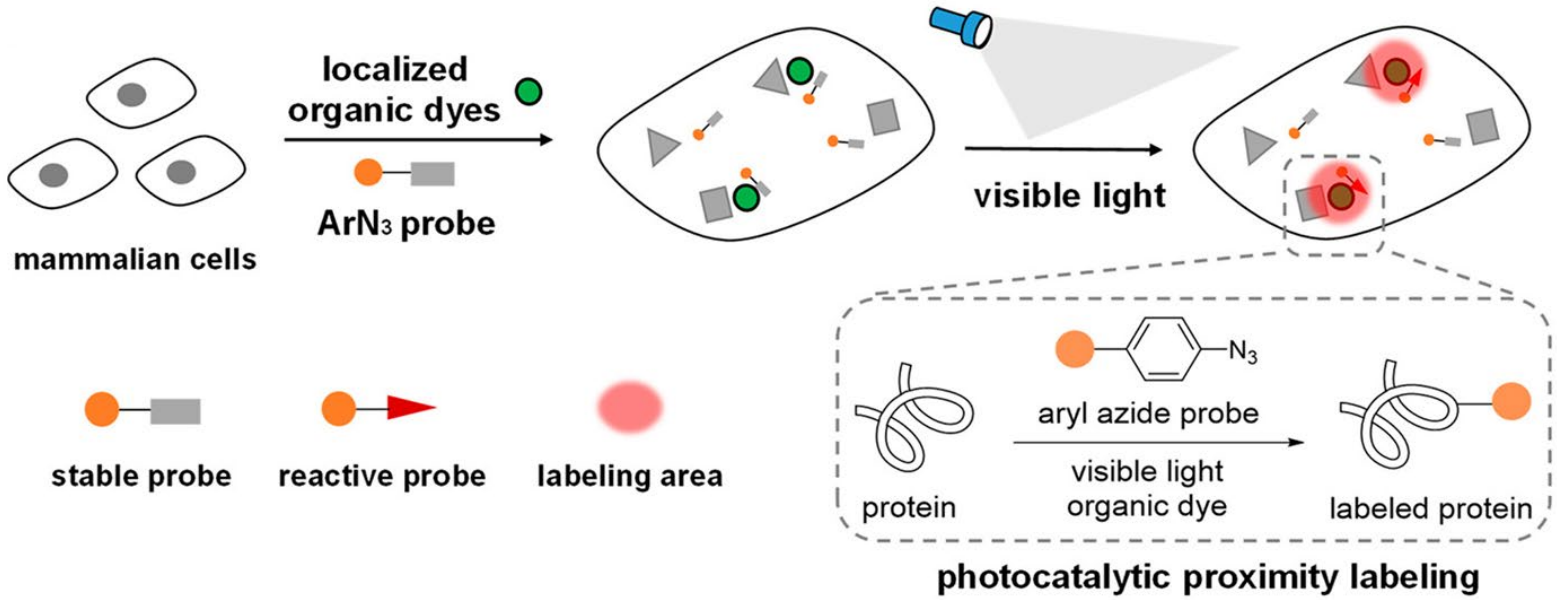




Photocatalytic proximity labeling with localized organic dyes and aryl azide



Representative transition-metal photocatalysts based on heavy metals ruthenium and iridium and organic photocatalysts acridine orange (AO), fluorescein (Fluo), and rhodamine 123 (Rh 123).



Stable aryl azide probes were activated to the reactive probes only in the proximity of the localized organic dyes for spatial protein labeling reactions under visible light irradiation.

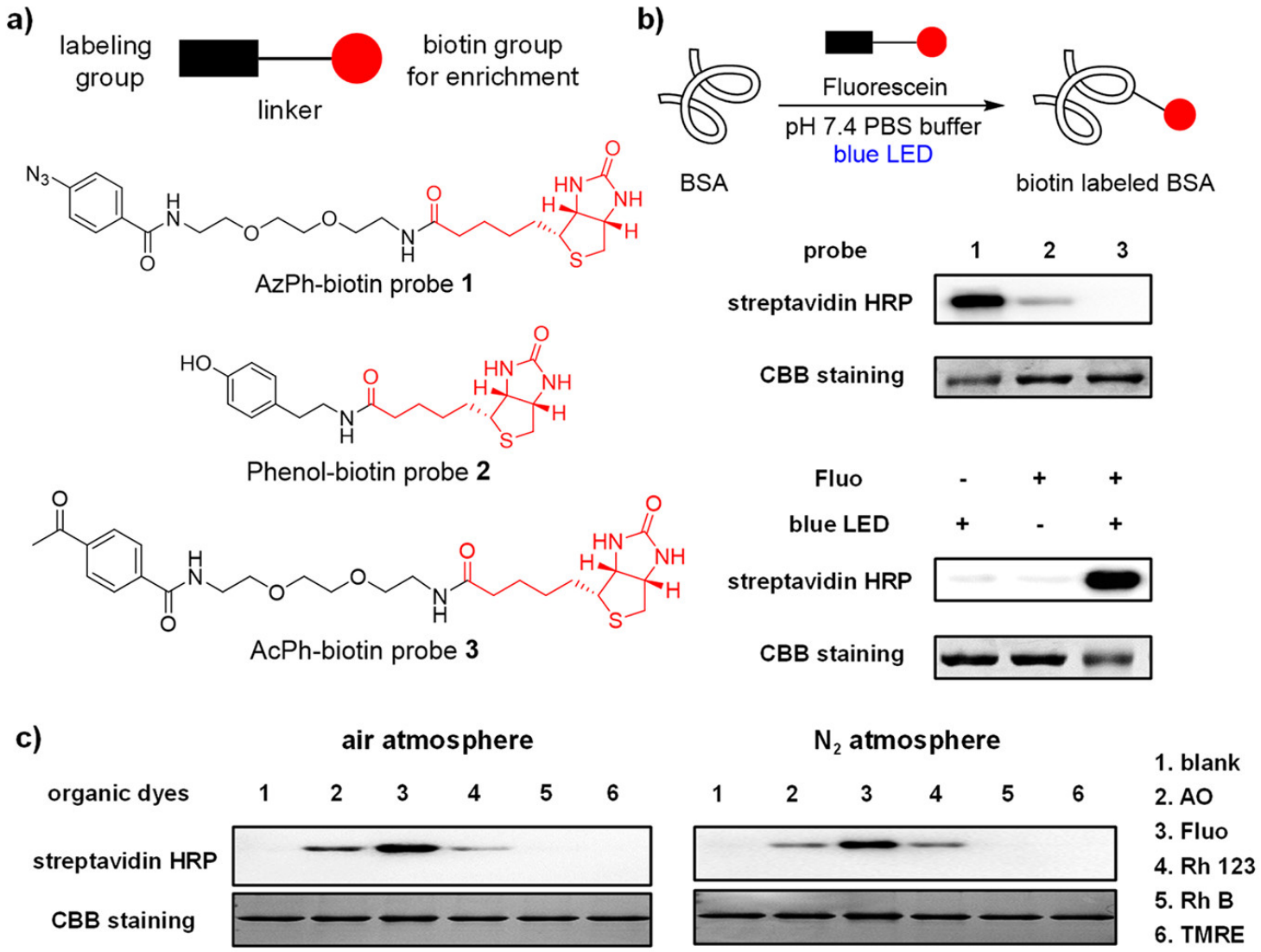
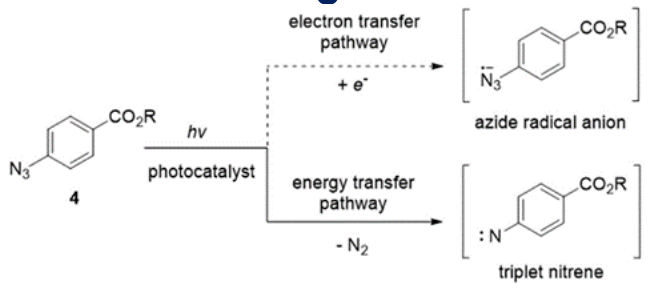


Figure 2. Photocatalytic labeling of bovine serum albumin (BSA) in vitro. (a) Structures of probes **1**–**3**. The chemical labeling groups and linkers are shown in black, and the biotin groups for enrichment are shown in red. (b) Photocatalytic BSA labeling with biotin probes **1**–**3** using fluorescein (Fluo) under the blue LED irradiation (468 nm, 5.8 mW/cm^2) at 25 °C for 60 min. Control experiments with AzPh-biotin probe **1** showed the fluorescein and the light irradiation were critical for the reaction. The biotinylated-BSA was analyzed by HRP-conjugated streptavidin (streptavidin-HRP), and the BSA proteins were detected by Coomassie brilliant blue staining (CBB staining). BSA (2 μM in pH 7.4 PBS buffer), fluorescein (100 μM), and probes **1**–**3** (100 μM). (c) Photocatalytic BSA labeling with AzPh-biotin probe **1** (100 μM) by different organic dyes (100 μM) in the air atmosphere or the nitrogen atmosphere under a Xe lamp source at 25 °C for 1 min (15 cm from Xe lamp, 19.8 mW/cm^2 with 500 nm band-pass filter for lanes 1–4, 550 nm band pass filter for lanes 5 and 6).

Mechanism Investigation of the Photocatalytic Azide-Promoted Protein Labeling

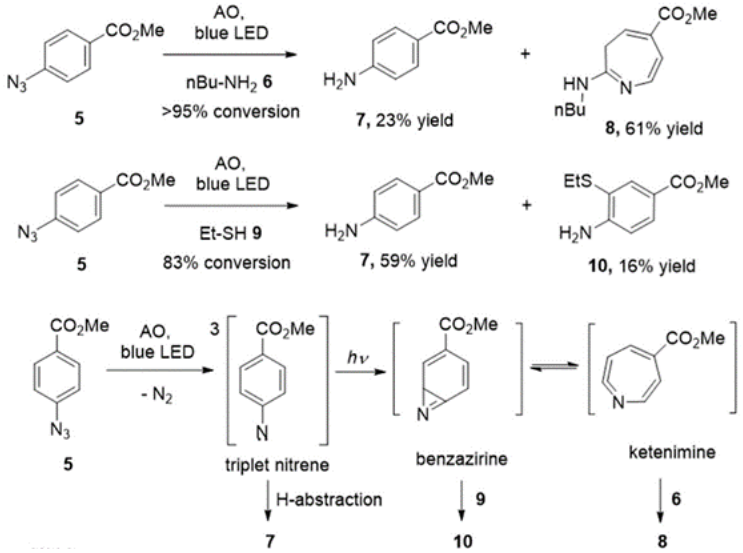
a)



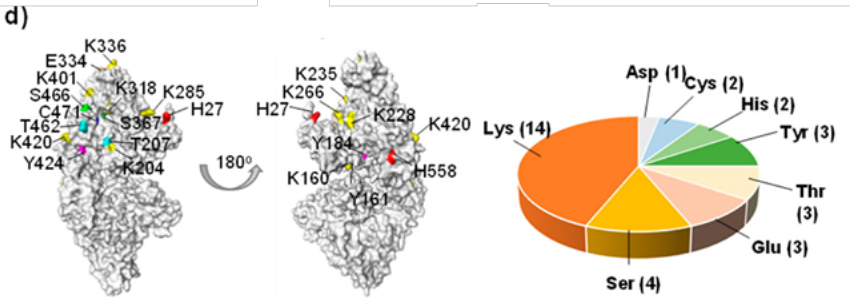
b)

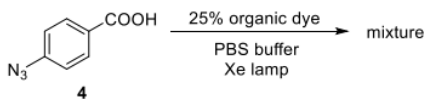
organic dyes	AO	Fluo	Rh 123	Rh B	TMRE
conv. of **4** (air)	40%	28%	54%	<5%	<5%
conv. of **4** (N_2)	70%	33%	50%	<5%	6%
E_T (eV)	2.13	1.94	1.90	1.80	1.80

c)



d)



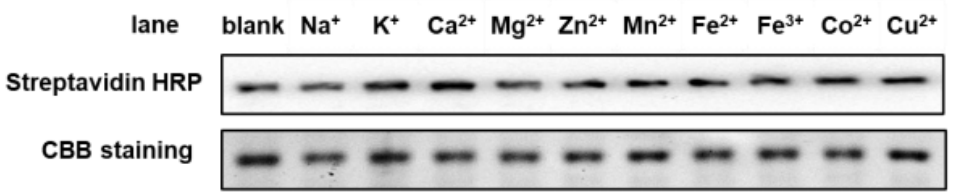


entry	organic dye	light	λ_{max}	E_T (eV)	$E_{1/2}$(red)	conversion (in air)	conversion (in N_2)
1	blank	500 nm				<5%	<5%
2	Acridine Orange	500 nm	495 nm	2.13	-0.92 V	40%	70%
3	Fluorescein	500 nm	491 nm	1.94	-1.22 V	28%	33%
4	Rhodamine 123	500 nm	505 nm	1.90	-0.89 V	54%	50%
5	Rhodamine B	550 nm	550 nm	1.80	-0.96 V	<5%	<5%
6	TMRE	550 nm	550 nm	~1.80	~ -0.96 V	<5%	6%

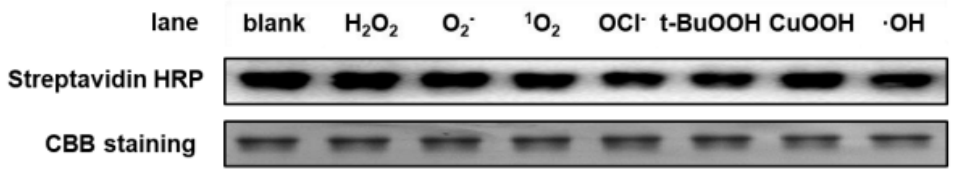
Figure 3. Mechanistic studies of the photocatalytic azide-promoted protein labeling reaction. (a) Plausible photocatalysis reaction pathways (single electron transfer or energy transfer) to generate the reactive intermediates from aryl azides. (b) Conversion of 4-azidobenzoic acid 4 (R = H) (200 μM) after visible light irradiation at 25 °C for 1 h in PBS buffer (10 mM, pH 7.4) (xenon lamp equipped with band-pass filters, 19.8 mW cm–2) by organic dyes (50 μM) with different triplet energies. c) The formation of aniline 7, azepine 8, and thiolether-substituted aniline 10 supported the triplet nitrene, ketenimine, and benzazirine intermediates. The solution of 5 (0.10 mmol), Acridine Orange (0.020 mmol) and amine or thiols (5 mmol) in methanol were under blue LED (10.4 mW/cm2) irradiation at 25 °C for 40–48 h. (d) LC-MS/MS analysis of the AzPh-biotin probe 1 labeled nucleophilic amino acid residues on BSA protein.

Selective Mitochondrial Proteins Labeling Inside Live Cells

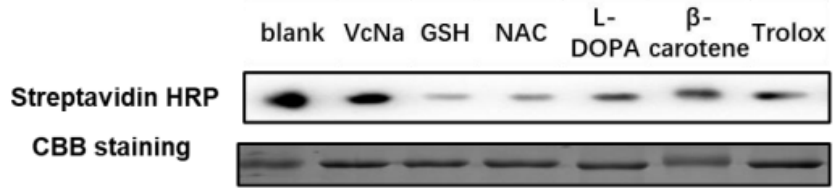
Effects of the metal ions on the photocatalytic BSA labeling

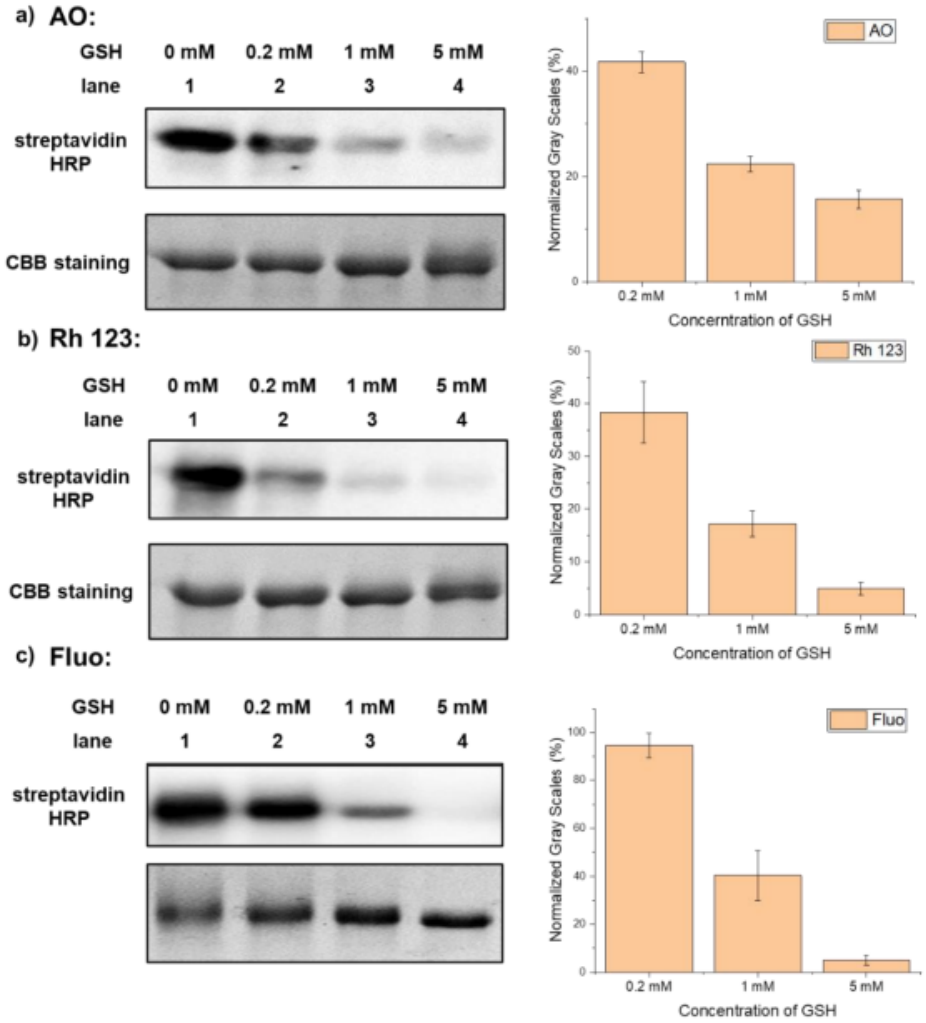


Effects of the reactive oxygen species on the photocatalytic BSA labeling

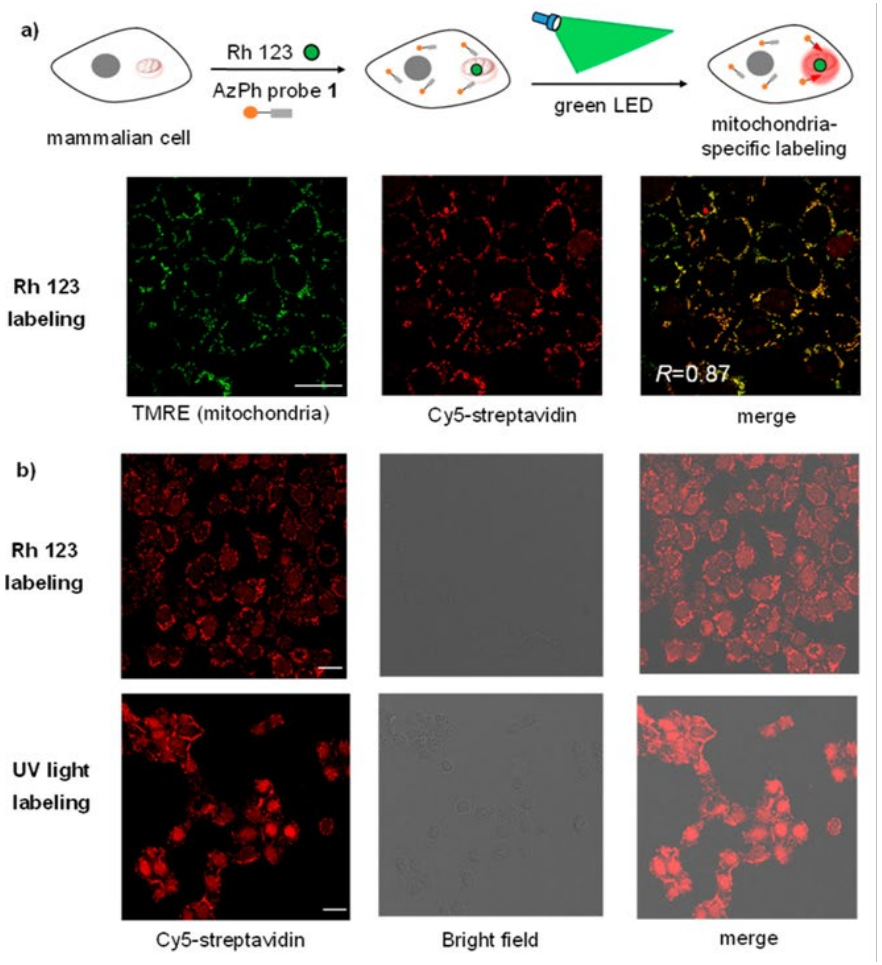


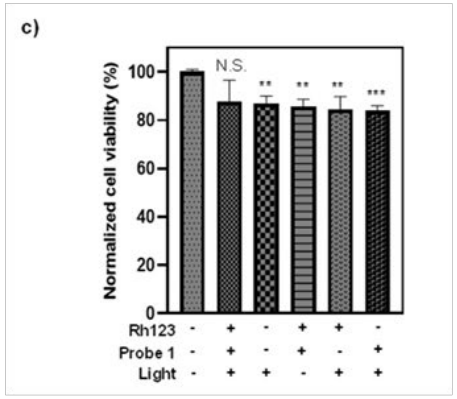
Effects of the radical quenchers or reductants on the photocatalytic BSA labeling

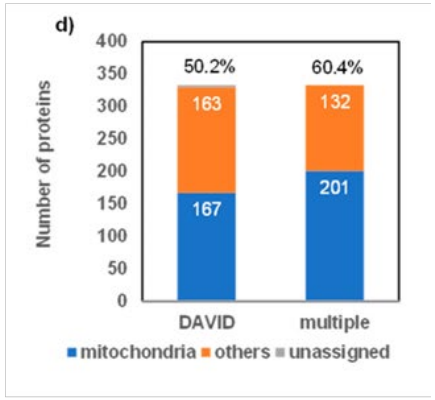




Selective Mitochondrial Proteins Labeling Inside Live Cells







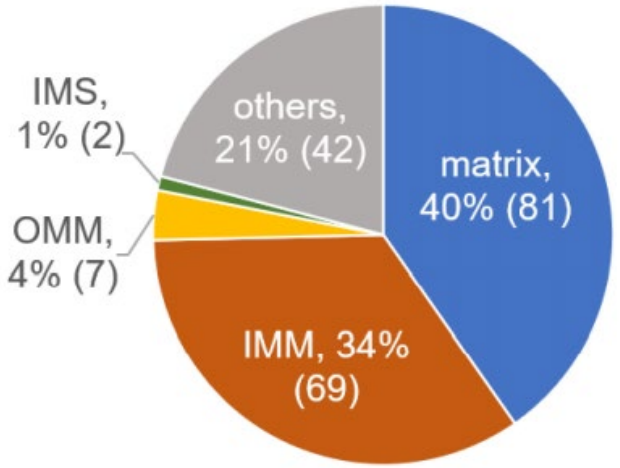
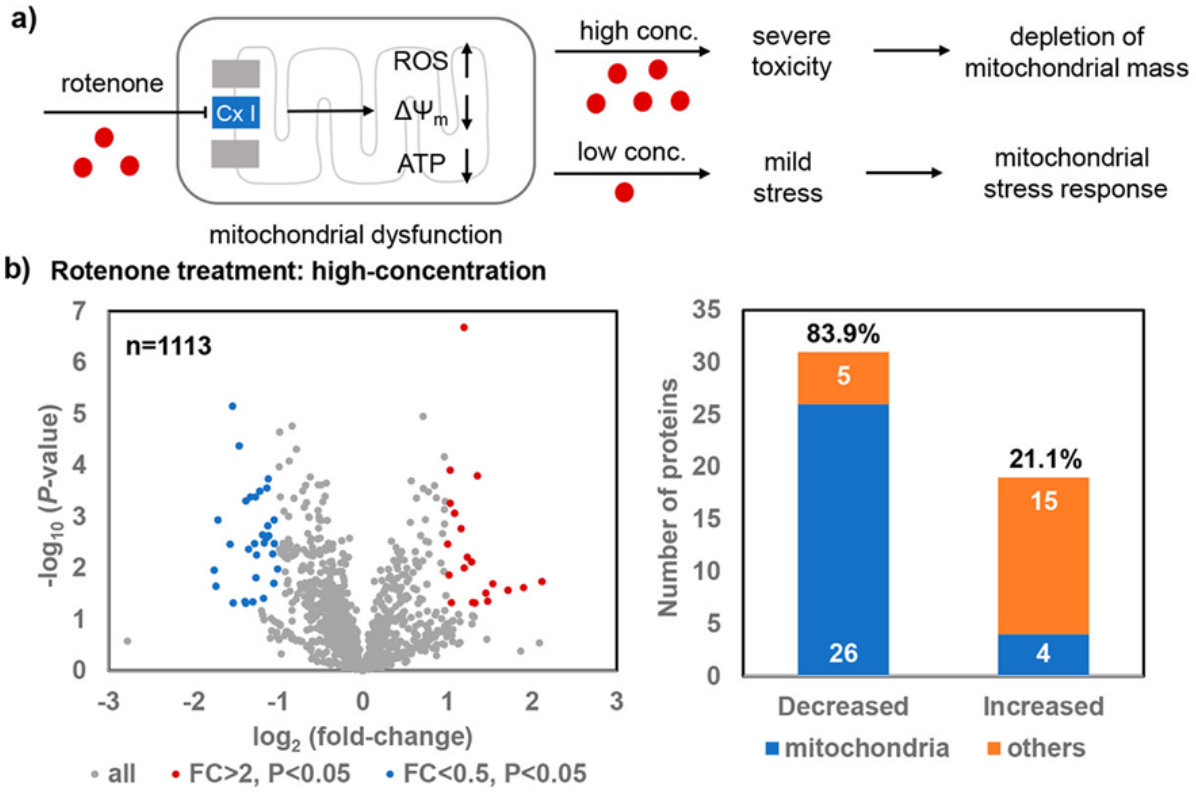
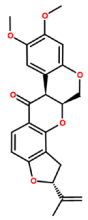


Figure 4. Photocatalytic labeling of the mitochondrial proteome by AzPh-biotin probe 1 in MCF-7 cells. (a) Photocatalytic mitochondrial proteome labeling scheme and confocal fluorescence microscopy analysis of AzPh-biotin probe 1 with Rh 123 in MCF-7 cells. The green dots represent the organic dye Rh 123, and the red circles represent the mitochondrial proteome labeled in the proximity of Rh 123. Cells were irradiated by the green LED with Rh 123 at 37 °C for 1 h (515 nm, 2.9 mW/cm2). TMRE (green) stains the mitochondria, and Cy5-streptavidin (red) detects the biotinylated proteins. The merged image shows good colocalization (R = 0.87, colocalization was quantified using Pearson's R-value calculated with Coloc 2 in ImageJ). Scale bar: 20 μm. Rh 123 (20 μM), AzPh-biotin probe 1 (200 μM). (b) Proteome labeling in MCF-7 cells with AzPh-biotin probe 1 by green LED irradiation with Rh 123 for 1 h (515 nm, 2.9 mW/cm2) or UV light irradiation for 20 min (365 nm, 11.8 mW/cm2) at 37 °C. (c) MTT cell viability assay for the cytotoxicity of Rh 123 labeling using AzPh-biotin probe 1 under green LED irradiation at 37 °C for 1 h (515 nm, 2.9 mW/cm2). (d) Counts of the photocatalytic labeled MCF-7 mitochondrial proteins by the LC-MS/MS analysis with Rh 123 and the AzPh-biotin probe **1**. The annotations were performed by the DAVID (167/333, 50.2%, DAVID) or by the combined databases including DAVID, Uniprot, and MitoCarta 2.0 (201/333, 60.4%, multiple).

Mitochondrial Proteins Mapping for Stress-Response Proteins Identification





Rotenone：一种广泛应用于研究细胞凋亡和神经退行性疾病的线粒体复合物I抑制剂，可导致线粒体的膜电位降低(ΔΨm）、ATP耗竭和ROS的生成。

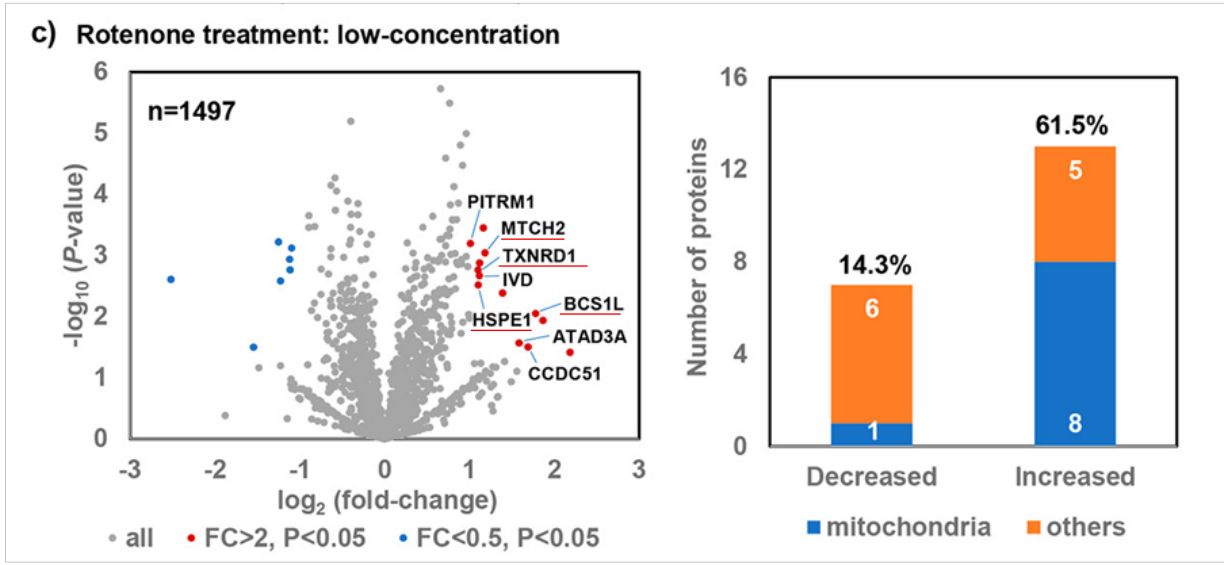


Figure 5. Photocatalytic proteome mapping of the mitochondria for the rotenone-induced stress response. (a) HeLa cells underwent mitochondrial stress response with the mild stress induced by the low-concentration of rotenone, while the depletion of mitochondrial mass was detected with the severe mitochondrial toxicity induced by the high-concentration of rotenone. (b) Volcano plot of the identified proteins by the high concentration (2 μM) of rotenone treatment and the changed protein counts. A significant portion of the decreased protein measurement was observed in mitochondria (26/31, 83.9%). (c) Volcano plot of the identified proteins by the low concentration (0.02 μM) of rotenone treatment and the changed protein counts. A significant portion of the increased protein measurement was observed in mitochondria (8/13, 61.5%). The decreased protein measurement is colored in blue, and the increased protein measurement is colored in red. FC: fold-change.