Literature Report

Reporter: 许宁

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Article

Bioorthogonal Photocatalytic Decaging-Enabled Mitochondrial Proteomics

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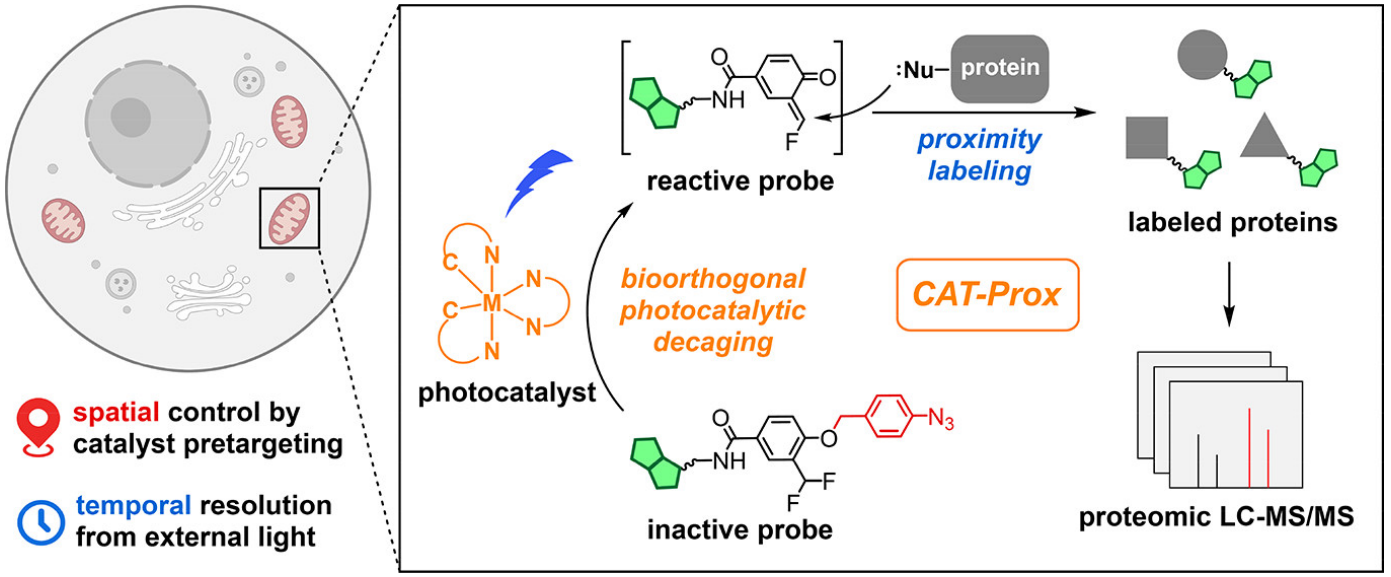


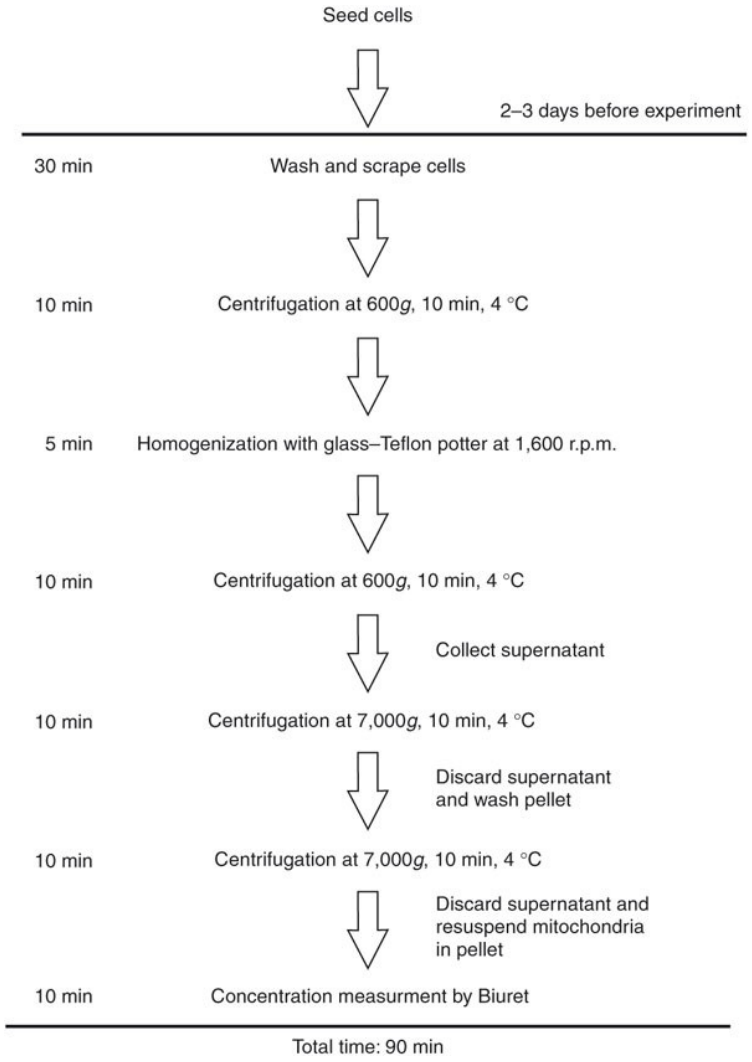
1998-2002 B.S. in Chemistry, Peking University
2002-2007 Ph.D in Chemistry University of Chicago
2007-2009, Postdoctoral Fellow, The Scripps Research Institute
2009/07-2014/07, Investigator, Department of Chemical Biology, Peking University
2011/05-2015/12, Investigator, Peking-Tsinghua Center for Life Sciences
2014/08-present, Professor, Department of Chemical Biology, Peking University
2015/01-present, Chairman, Department of Chemical Biology, Peking University
2016/01-present, Senior Investigator, Peking-Tsinghua Center for Life Science
2018/01-present, Cheung Kong Professor, Ministry of Education, P.R.China

- Protein bioorthogonal chemistry
- Protein therapeutic engineering
- Chemical Immunology

2003-2010 B.S & M.S., Lanzhou University
2010-2014 PhD, Institute of Chemical Research of Catalonia,
2015-2017, Postdoctoral Fellow, Peking-Tsinghua Center for Life Sciences, Peking University
2017-2019, Associate Professor, Nanjing Tech University
2019-present, Research Associate Professor, College of Chemistry and Molecular Engineering, Peking University

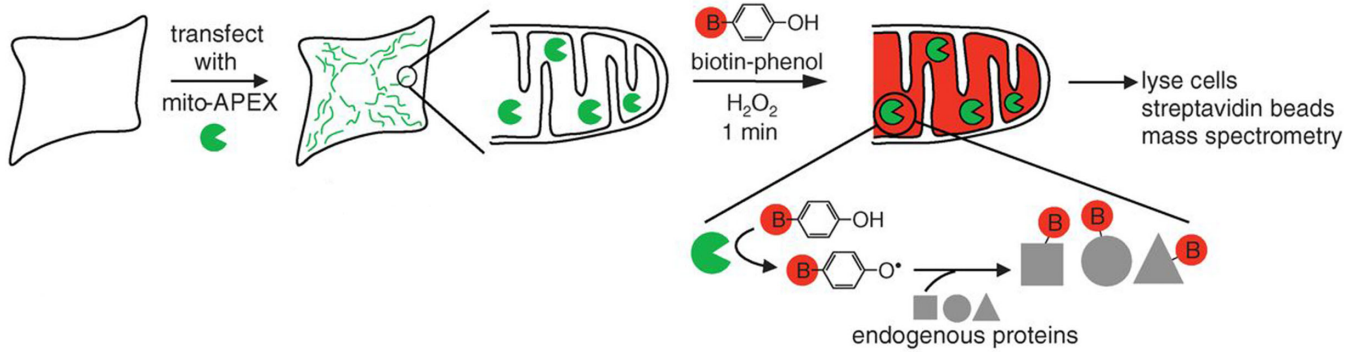
Profiling methods of the mitochondria proteome

Organelle isolation: functional mitochondria from mouse liver, muscle and cultured filroblasts



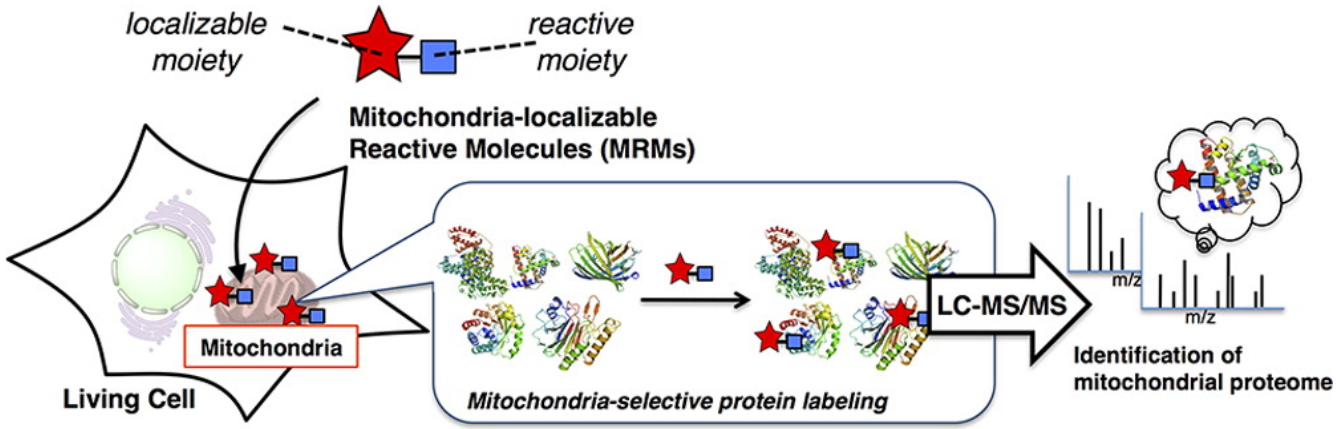
Nat. Protoc. **2007**, 2, 287– 295.

Proteomic Mapping of Mitochondria in Living Cells via Spatially Restricted Enzymatic Tagging



Science **2013**, 339, 1328–1331.

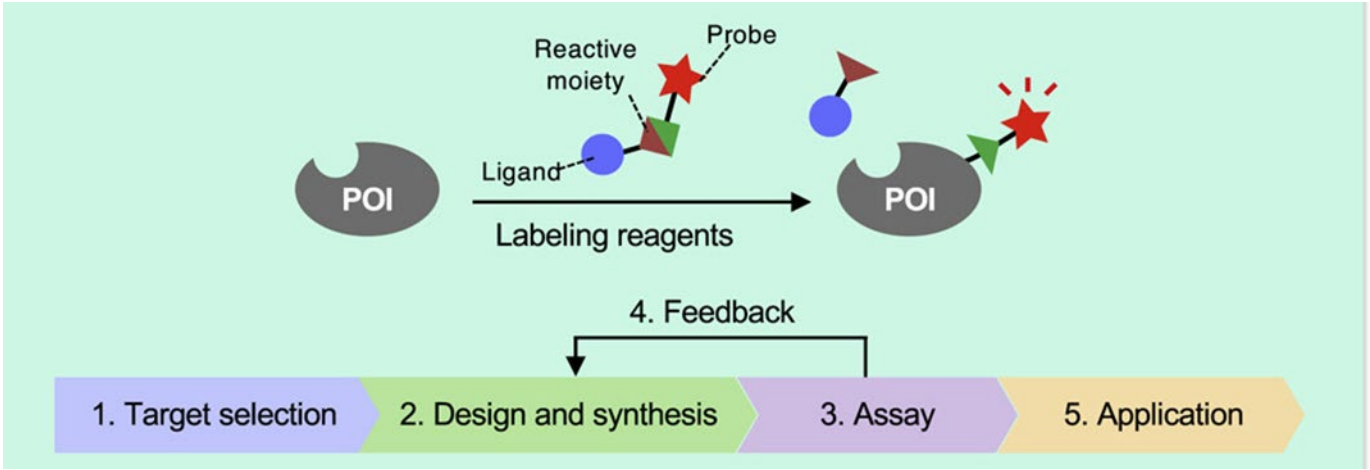
Strategy of Mitochondria-Localizable Reactive Molecules (MRMs)



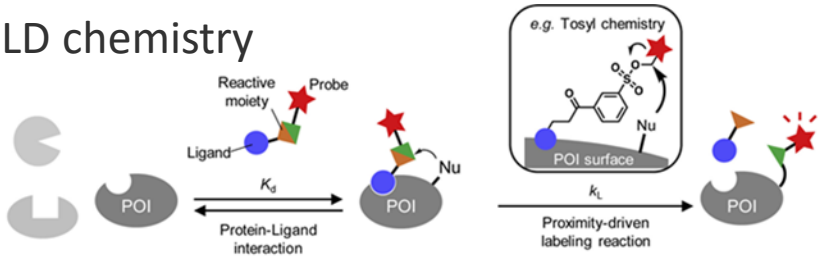
J. Am. Chem. Soc. **2016**, 138, 24, 7592–7602.

Chemical Tools for Endogenous Protein Labeling and Profiling

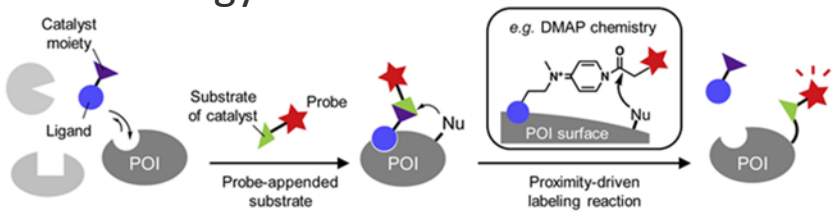
1. Selective Chemical Labeling of Endogenous Proteins



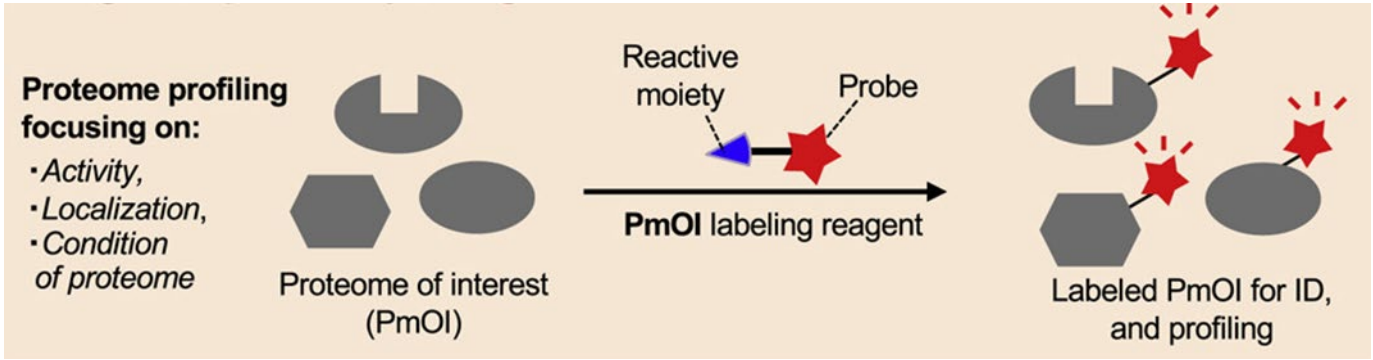
LD chemistry

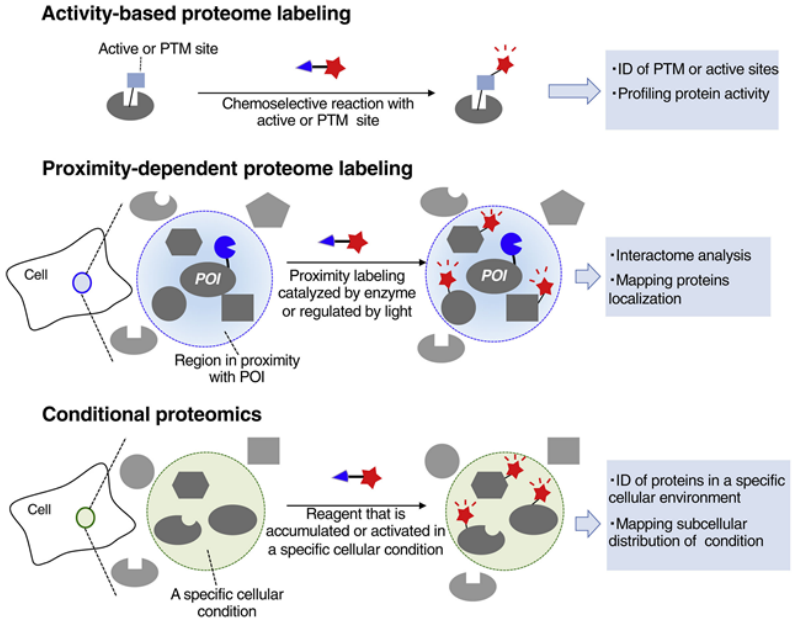


AGC strategy



2. Proteome-Directed Chemical Modification



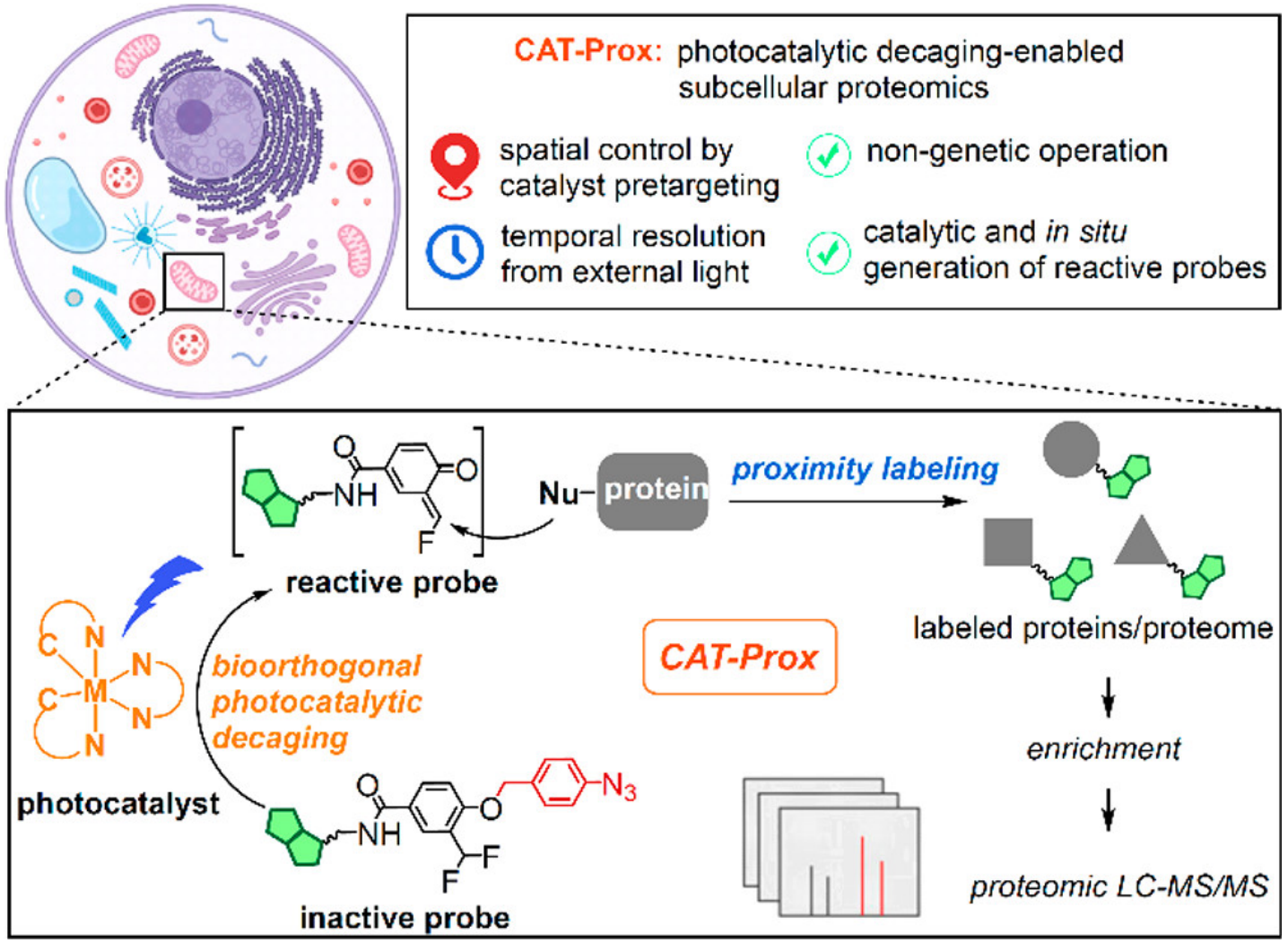


Cell Chem. Biol. **2020**, *27*, 970– 985.

Schematic Illustration of the CAT-Prox Strategy and the Workflow of Subcellular Proteomics

Areas for improvement:

1. Low temporal resolution
2. Lack of spatial control
3. Need the gene transfection procedures
4. The direct usage of reactive probes might cause off- labeling



Bioorthogonal and photocatalytic decaging-enabled proximity labeling strategys (Cat-Prox)

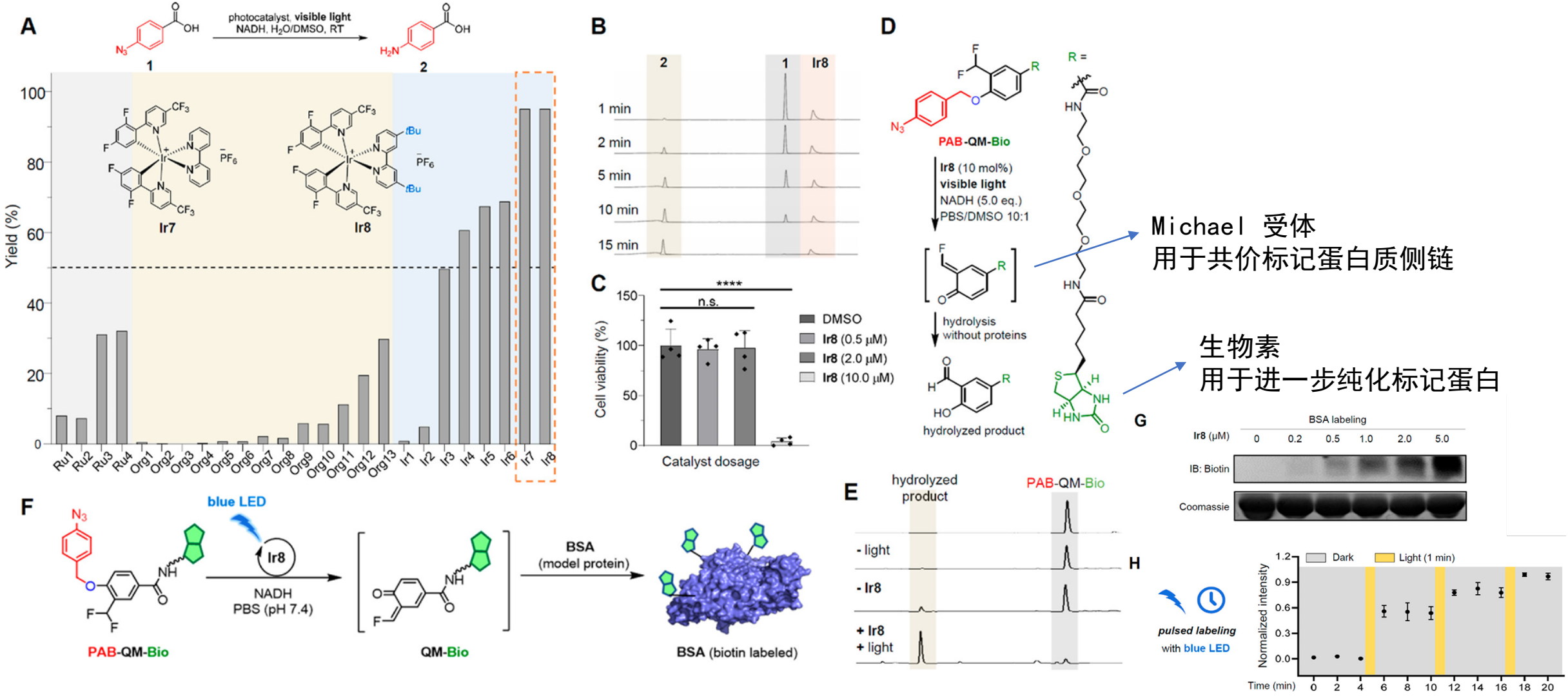
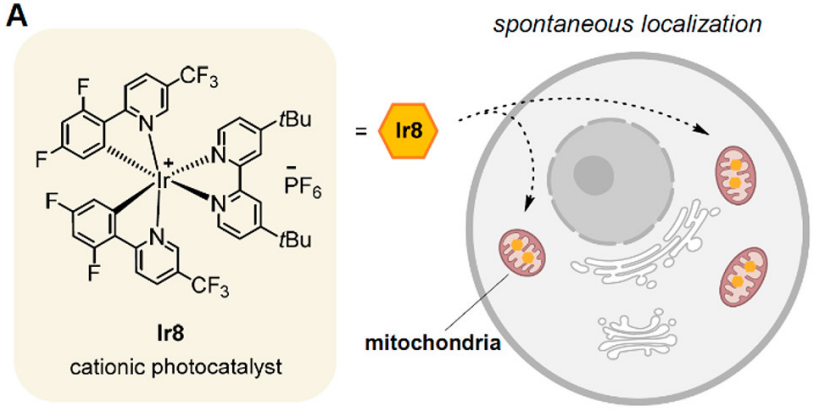
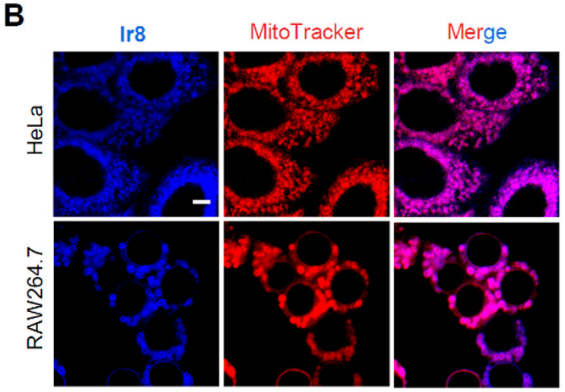
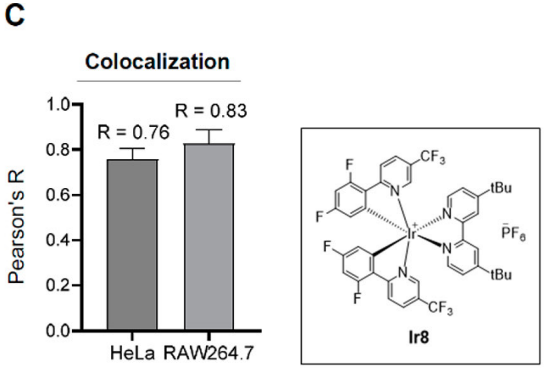


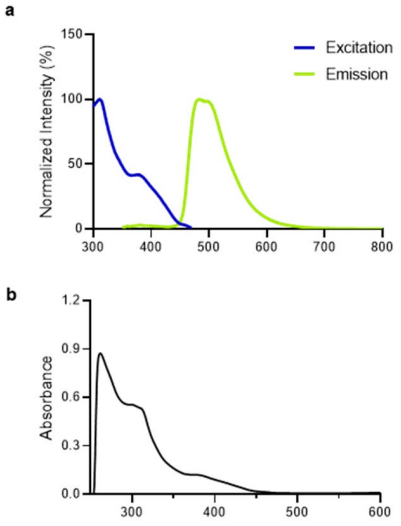
Figure 1. Development of the bioorthogonal and photocatalytic decaging-enabled proximity labeling strategy (Cat-Prox). (A) Catalyst screening for the aryl azide reduction reaction. The reaction was carried out with **1** (0.1 mmol) and NADH (0.2 mmol) in 0.5 mL mixture of water and DMSO (1/1) with photocatalyst (10 mol %, 0.01 mmol) at room temperature under white LED irradiation for 30 min. (B) HPLC traces of photocatalytic reduction of **1** under blue LED at given time points. Conditions: 0.1 mmol **1**, 0.2 mmol NADH, 10 mol % **Ir8**, in 0.5 mL water/DMSO (1/1), irradiated by mild blue LED (4 mW/cm^2). (C) Cytotoxicity evaluation of **Ir8**. (D) Design of quinone methide-based labeling probe (PAB-QM-Bio) and photocatalytic decaging of PAB-QM-Bio in aqueous media. (E) HPLC traces of the photocatalytic decaging of PAB-QM-Bio probe. Conditions: 0.1 mM PAB-QM-Bio, 0.5 mM NADH, 10 mol % **Ir8**, in PBS/DMSO (10/1) buffer, irradiated by blue LED (4 mW/cm^2) for 15 min. (F) *In vitro* evaluation of CAT-Prox using BSA as model protein. (G) Photocatalyst concentration-dependent labeling of BSA. Biotinylated BSA and total BSA were analyzed by immunoblotting and Coomassie blue staining, respectively. (H) Time-resolved BSA labeling by switching of the photostimuli.

Development and validation of CAT-Prox for mitochondria proteomics

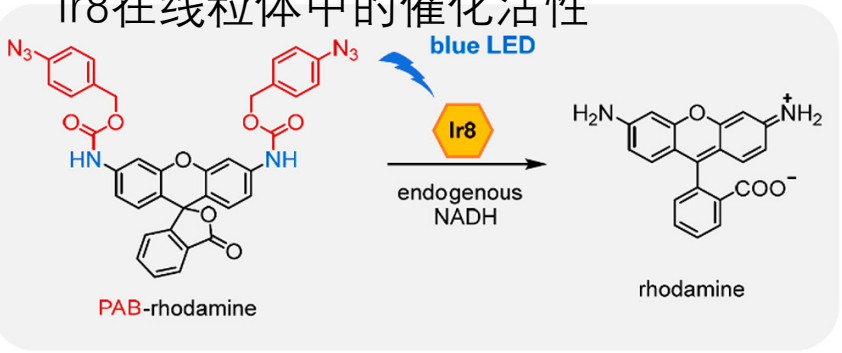


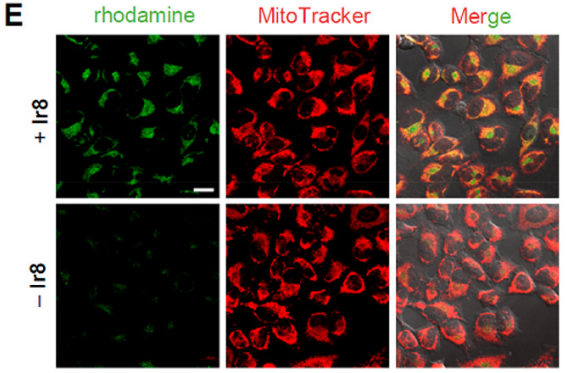


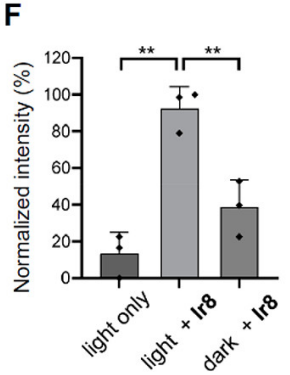


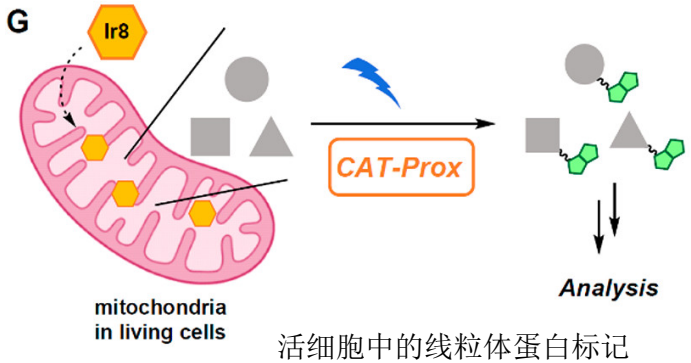


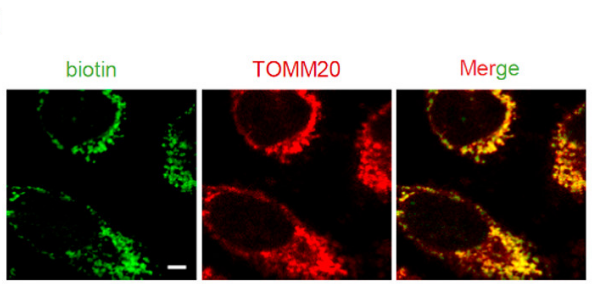
D Ir8在线粒体中的催化活性

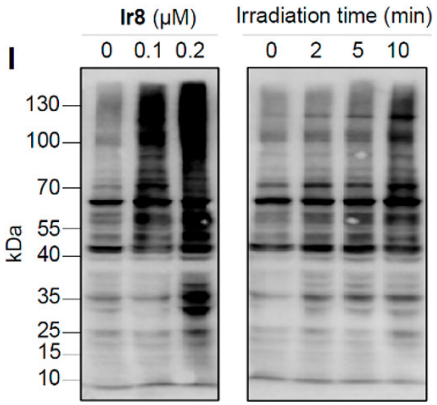






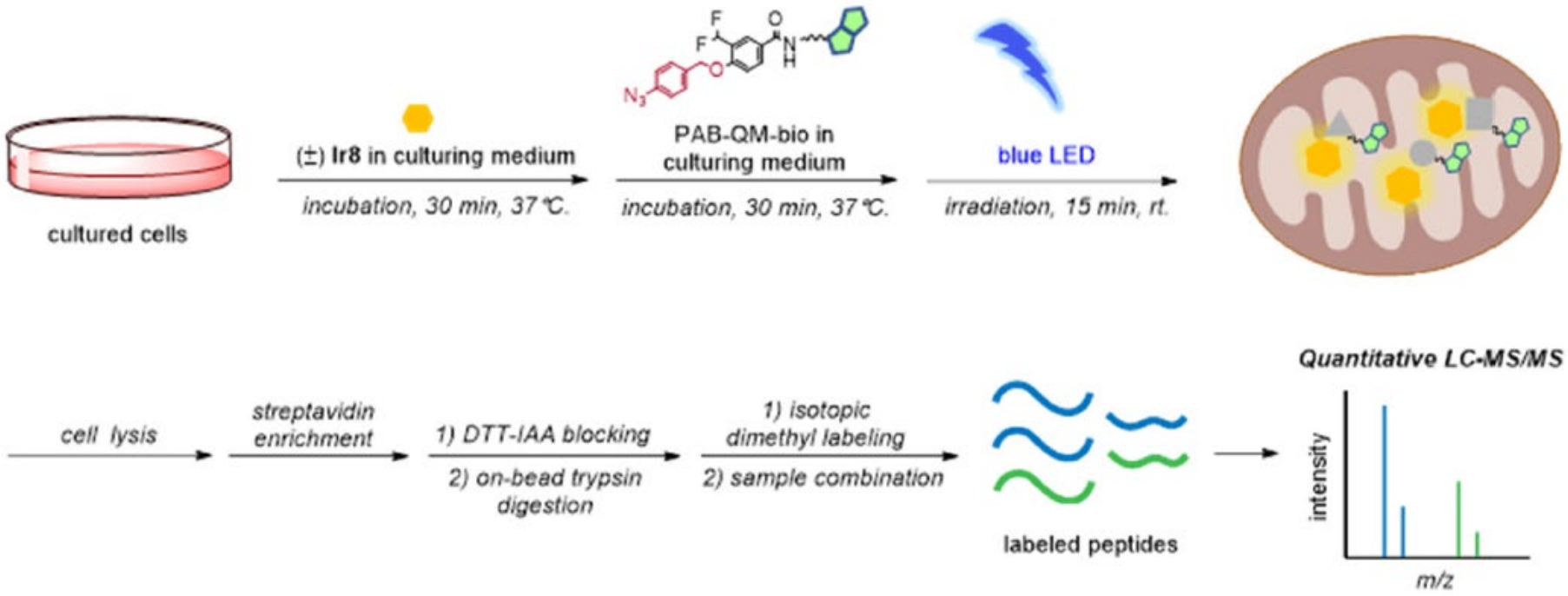




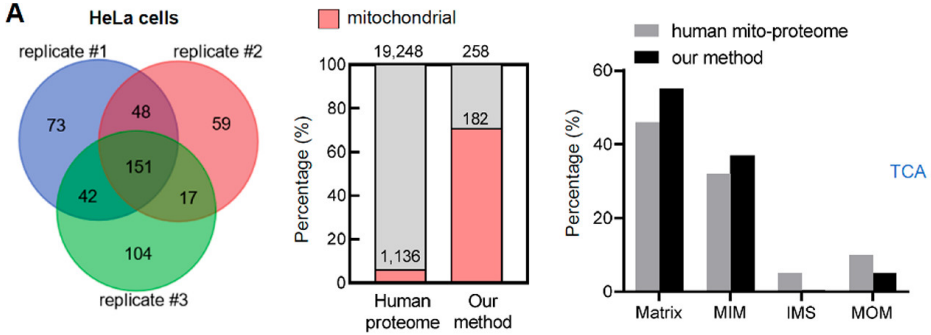


活细胞中的线粒体蛋白标记

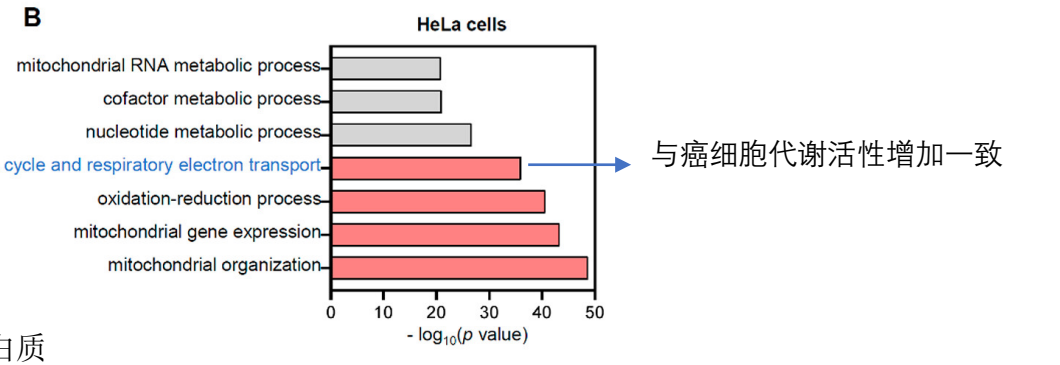
Technical details for photocatalytic proximity labeling in mitochondria



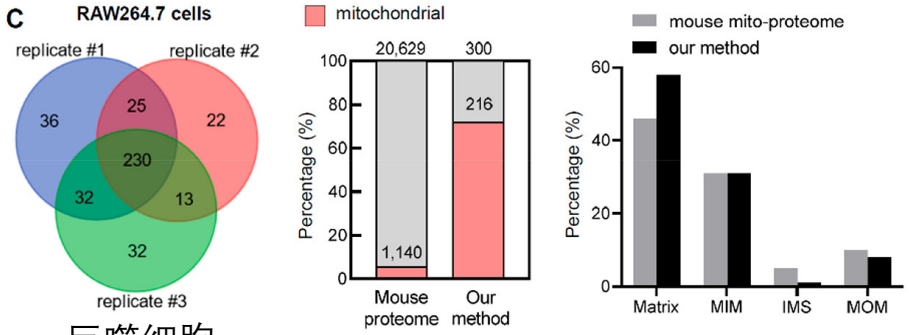
CAT-Prox-enabled profiling of mitochondria proteome and dynamics



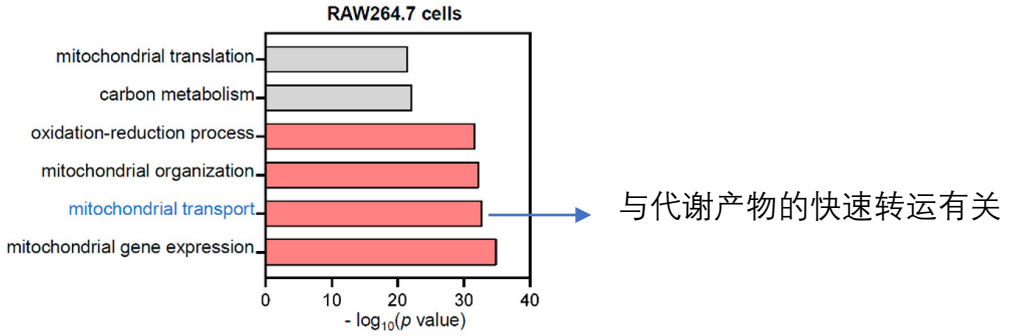
识别线粒体不同亚区的蛋白质



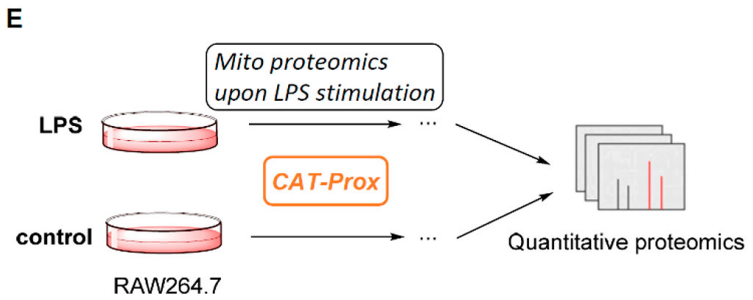
与癌细胞代谢活性增加一致



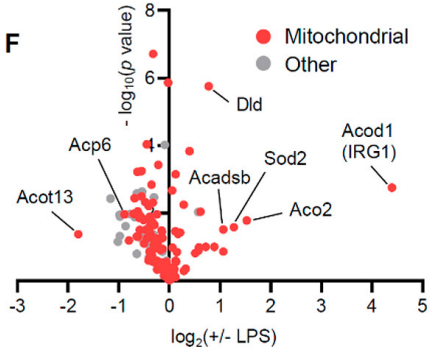
巨噬细胞

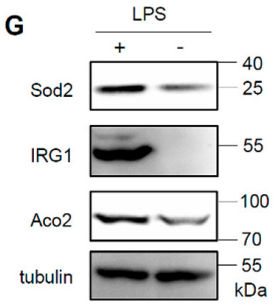


与代谢产物的快速转运有关



线粒体蛋白质组动力学对巨噬细胞炎症反应的影响





Aco2和IRG1：负责产生itaconate
Acadsb：与反应性亲电物种的增加有关
Sod2：与LPS诱导的氧化应激有关

Figure 3. CAT-Prox-enabled profiling of mitochondria proteome and dynamics in living cells. (A) Coupling CAT-Prox with mass spectrometry to identify the labeled subcellular proteome of HeLa cells; 182 mitochondrial proteins were identified from 258 enriched proteins according to the MitoCarta3.0 database (70.5% specificity). Proteins identified as mitochondrial proteins were classified into 'matrix', 'mitochondrial inner membrane (MIM)', 'intermembrane space (IMS)', and 'mithochondrial outer membrane (MOM)'. (B) Gene ontology analysis (Biological Processes) for identified protein from HeLa cells. (C) Protein identification in RAW264.7 cells; 216 mitochondrial proteins from 300 enriched proteins were identified (72% specificity). (D) Gene ontology analysis of the identified proteins from RAW264.7 cells. (E) Combination of CAT-Prox with quantitative protein mass spectrometry analysis based on isotopic dimethyl labeling to dissect the mitochondria proteome dynamics during the inflammatory process of LPS stimulated RAW264.7 cells. (F) Volcano blot showing the regulation of mitochondrial proteins in the inflammatory process of RAW264.7 cells. (G) Immunoblotting analysis of the representative proteins identified above.