Spatiotemporally resolved subcellular proteomic profiling with photocatalytic proximity labeling

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Mapping the subcellular organization of proteins is crucial for understanding their biological functions. Herein, we report a reactive oxygen species induced protein labeling and identification (RinID) method for profiling subcellular proteome in the context of living cells. Our method capitalizes on a genetically encoded photocatalyst, miniSOG, to locally generate singlet oxygen that reacts with proximal proteins. Labeled proteins are conjugated *in situ* with an exogenously supplied nucleophilic probe, which serves as a functional handle for subsequent affinity enrichment and mass spectrometry-based protein identification. From a panel of nucleophilic compounds, we identify biotin-conjugated aniline and propargyl amine as highly reactive probes. As a demonstration of the spatial specificity and depth of coverage in mammalian cells, we apply RinID in the mitochondrial matrix, capturing 477 mitochondrial proteins with 94% specificity. We further demonstrate the broad applicability of RinID in various subcellular compartments, including the nucleus and the endoplasmic reticulum (ER). The temporal control of RinID enables pulse-chase labeling of ER proteome in Hela cells, which reveals substantially higher clearance rate for secreted proteins than ER resident proteins.

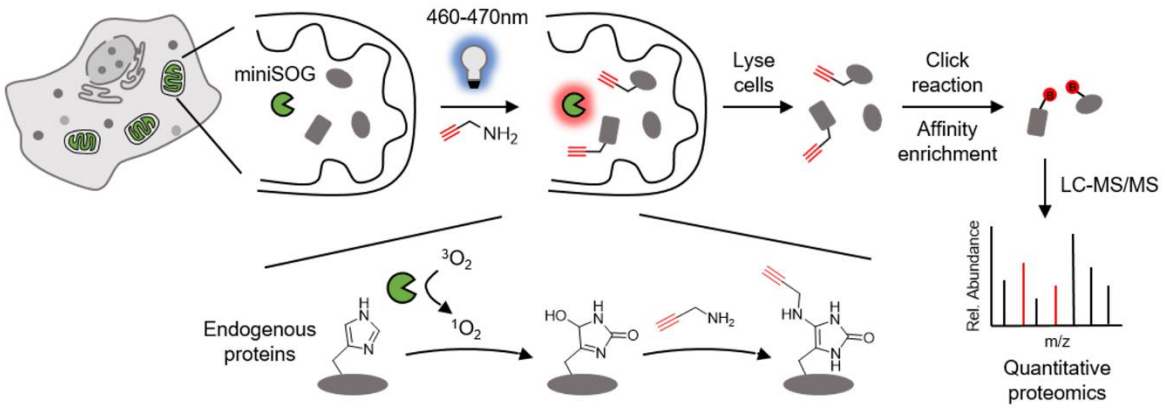

Figure 1. Spatially resolved mapping of subcellular proteome with genetically encoded photocatalytic proximity labeling.

References

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