Mapping a glutamate receptor interactome in living mice by photoactivated proximity labeling

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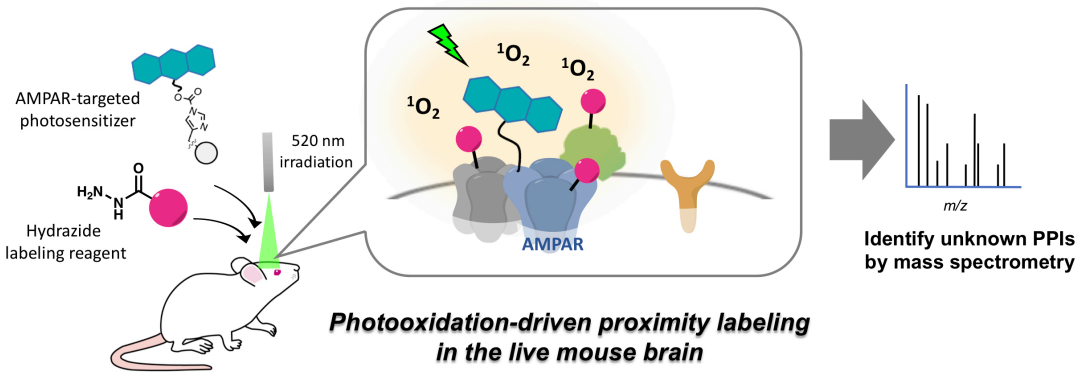
Nearly all aspects of cellular activity are regulated by an intricate network of protein-protein interactions (PPIs), and the discovery of unknown PPIs is essential to deciphering biological processes at the molecular level. In the past decade, enzymatic proximity labeling has emerged as a powerful tool for detecting potential PPIs in the native environment of the cell, and has been particularly effective for identifying transient and low-affinity associations.[1] However, when applied to live animal studies, existing methods suffer either from toxicity or a low temporal resolution on the order of hours to days, as well as the need for a potentially disruptive genetic modification of the organism under study.[2]

To address this challenge, we have developed **PhoxID** (photooxidation-driven proximity labeling for protein identification),[3] an optochemical proximity labeling method for profiling protein interactions that can be applied to live, genetically intact animals. In this strategy, an organic small-molecule photosensitizer is tethered to a protein of interest and irradiated with visible light to locally generate singlet oxygen ($^1O_2$). The diffusion radius of $^1O_2$ in biological environments is estimated to be several tens of nanometers[4] – thus, only proteins that are physically proximal to the protein of interest are oxidized by $^1O_2$ and tagged by a nucleophilic labeling reagent for identification by mass spectrometry. We demonstrated the tissue and *in vivo* compatibility of PhoxID by identifying multiple members of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-type glutamate receptor (AMPAR)-interactome in the brains of live mice with just minutes of photoirradiation.



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