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論文題目	Development of an optochemical method for spatially resolved proteome profiling *in vitro* and *in vivo*（光駆動近接ラベル化法の開発）		

In biological systems, proteins are organized, sometimes sequestered, into specific compartments to facilitate and economize the chemical reactions that sustain life. Often, the protein residents of these compartments are not only physically proximal to each other but also functionally interacting and intertwined in an elaborate network of protein-protein interactions (PPIs). Over the past decade, proximity labeling has emerged as a valuable tool for hypothesis-free protein interactome analyses in live cells and organisms. In proximity labeling, engineered enzymes (biotin ligases and peroxidases) are typically fused to a protein of interest to generate a short-lived reactive species that can promiscuously label proteins in the vicinity of the enzyme. Although this method is undoubtedly powerful, the fusion of an exogenous enzyme risks perturbing the native state of the protein of interest and may disrupt interactions that would otherwise occur. Moreover, existing proximity labeling methods suffer from a low temporal resolution when implemented in live animals. This thesis describes the development of an entirely chemical, nongenetic method for proximity labeling in live cells and organisms that aims to address these limitations. Named PhoxID (photooxidation-driven proximity labeling for protein identification), this method utilizes a small-molecule organic photosensitizer to locally generate $^1O_2$, which can oxidize proteins within tens of nanometers from the photosensitizer upon green light irradiation. The oxidized proteins are then tagged by a nucleophilic reagent bearing an enrichable handle for identification by mass spectrometry.

Chapter 1 describes the proof-of-concept of the PhoxID method *in vitro*. A DNA-binding photosensitizer and a cell-permeable labeling reagent were developed to label the nuclear proteome in live cell culture, and labeling was confirmed to occur in a light- and $^1O_2$-dependent fashion.

In **Chapter 2,** PhoxID was combined with ligand-directed chemistry to profile the interactomes of various neurotransmitter receptors *in vivo*. Specifically, the extracellular interactomes of the endogenous AMPA-type glutamate receptor (AMPAR), the $GABA_A$ receptor, and the δ2 glutamate receptor were successfully characterized in the live, genetically intact mouse brain with just minutes of in-brain photoirradiation. Furthermore, PhoxID's high temporal resolution was leveraged to capture 10-minute "snapshots" of the AMPAR neighborhood in the neonatal cerebellum, leading to the identification of IGSF3 and NECTIN3 as developmentally regulated AMPAR-proximal proteins.

Chapter 3 describes how an extracellularly bound photosensitizer can catalyze proximity labeling on the cytoplasmic side of a transmembrane protein. This strategy, dubbed "transmembrane PhoxID", could enable the mapping of elaborate multiprotein assemblies below the plasma membrane using traditionally cell-impermeable targeting modalities for the photosensitizer. As a proof-concept, intracellular and extracellular protein constituents of parallel fiber-Purkinje cell synapses were identified simultaneously using a photosensitizer conjugated to a nanobody for the δ2 glutamate receptor on isolated mouse brain tissues.