Abstract

The plasma membrane is an integral part of the cell transmitting signals from the extracellular environment to the inside of the cell. Proteins are crucial components of the plasma membrane acting as receptors, adhesion molecules, transporters, and enzymes and are uniquely positioned to interact with the extracellular environment and with other proteins on the cell. Proximity-dependent biotinylation approaches, including BioID combined with mass spectrometry, have begun illuminating the landscape of proximal protein interactions within intracellular compartments, however their application at the cell surface is limited. In my thesis work, I present extracellular TurboID (ecTurboID), an adapted BioID approach designed to profile extracellular proximal interactions at the cell surface in short timescales, utilizing the fast-labeling enzyme TurboID.

I started by optimizing the approach using a simple construct designed to allow trafficking of TurboID to the plasma membrane and anchoring it to the cell surface by fusing it with a transmembrane domain (TMD). Using ecTurboID-TMD, I optimized the labeling conditions and times required for sufficient extracellular labeling, while minimizing intracellular labeling resulting from biotin entry into the cell. Using fluorescence microscopy, protein blots and mass spectrometry, I show that ecTurboID fused to TMD or a lipid anchor is expressed at the cell surface and induces biotinylation of cell surface proteins.

I then explored the applicability of ecTurboID at the cell surface by profiling the extracellular interactomes of five type I membrane proteins including the epidermal growth factor receptor (EGFR). Upon stimulation with the ligand EGF, there was a change in the extracellular interactome of EGFR, showing low-density lipoprotein receptor (LDLR), among other proteins,

as novel ligand-dependent associations. This association is mediated by an EGF-induced change in LDLR localization and association with proteins that regulate EGFR signaling.

Collectively, this thesis presents ecTurboID as a versatile, easy-to-use approach capable of profiling dynamic extracellular proximal interactions.