Chemical Approaches for Distinct Labelling of Proteins

Prof. Johannes Broichhagen

Leibniz-Forschungsinstitut für Molekulare Pharmakologie im Forschungsverbund Berlin e.V. (FMP)
10439 Berlin, Deutschland
broichhagen@fmp-berlin.de

The self-labelling enzyme HaloTag Protein (HTP) is a widely used platform for selective protein labeling in chemical biology. It is based on an engineered haloalkane dehalogenase that forms a rapid, irreversible covalent bond with synthetic chloroalkane ligands. When genetically fused to a protein of interest, HaloTag enables site-specific attachment of fluorophores, affinity tags, or functional probes in living cells. This modular system supports diverse applications, including live-cell imaging, pulse–chase analysis, and protein trafficking studies. With its key advantage over fluorescent proteins, such as GFP, is its temporal and chemical flexibility due to externally added HaloTag Ligands (HTLs) that carry a fluorophore for microscopic visualization. Recent advances have addressed limitations in selectively labeling cell-surface proteins. Many bright fluorophores are membrane permeable, leading to unwanted intracellular labeling. To overcome this, we developed the SHTL (sulfonated HaloTag ligand) approach. This strategy introduces a negatively charged sulfonate group into the HaloTag ligand, reducing membrane permeability while preserving efficient covalent binding and favorable photophysical properties. SHTL probes enable selective labeling of extracellular HaloTag fusion proteins in living cells, effectively distinguishing surface-expressed proteins from intracellular pools. This is particularly valuable for studying membrane receptors, where spatial resolution between surface and internalized populations is critical. The approach is modular and compatible with a variety of fluorophores, including those used in super-resolution imaging. Together with chemical chromophore and recombinant protein engineering, we show a bright palette of possibilities for imaging from the single molecule to the tissue level.