Single particle tracking: the HaloTag® system

What is a HaloTag®?

Spatial visualization and real-time investigation of single-particle dynamics is of great research interest and can provide valuable mechanistic understanding. To achieve the highest resolution, fixed-cell imaging has traditionally been superior to that of live-cell, due to wider availability of labeling strategies. Here is a brief introduction to how HaloTag® labeling can be used to overcome some of the challenges of live-cell imaging and single-particle tracking (SPT).

Genetically encoded proteins, such as GFP and other variants have revolutionized the field of biology, as their labeling permits live-cell imaging and removes the need for harsh permeabilization and fixation steps. Whilst fluorescent proteins are relatively small and label with high specificity, they can interfere in protein folding and substrate binding dynamics, emit far fewer photons and are less stable than organic dyes (Dempsey et al., 2011). Consequently, the superior brightness of organic dyes coupled with their emission properties make them advantageous to that of traditional fluorescent proteins for live-cell imaging.

The HaloTag® system is an increasingly popular labeling alternative, and works by combining the advantages of genetically encoded fluorescent proteins with the brightness and photostability of organic dyes. This is particularly suitable for single-molecule imaging techniques such as PALM (Photo-Activated Localization Microscopy) that uses photoactivatable and photoconvertible fluorophores to ensure only a subset of molecules fluoresce simultaneously. SPT-PALM using a HaloTag® provides researchers the ability to measure single-molecule dynamics in real time, where proteins exist in dense populations (Figure 1). Other similar genetically encoded tags have been developed to permeate live cells include SNAP®-, FLAG™- and CLIP®-tags, which are also gaining interest and becoming widely available.

Benefits of HaloTag® over conventional fluorescent proteins

Advantages	Disadvantages
Higher photon counts	Requirement to genetically tag protein of interest with HaloTag®
Better photostability	Laborious, and may interfere with folding, cell treatments and time-course experiments
Control of labeling density	Removal of unbound ligands requires extensive washing
Photoactivatable dyes enable SPT-PALM and multi-colour tracking	Restricted to limited number of commercially available ligands
Permeable and non-permeable ligands available	Can be expensive

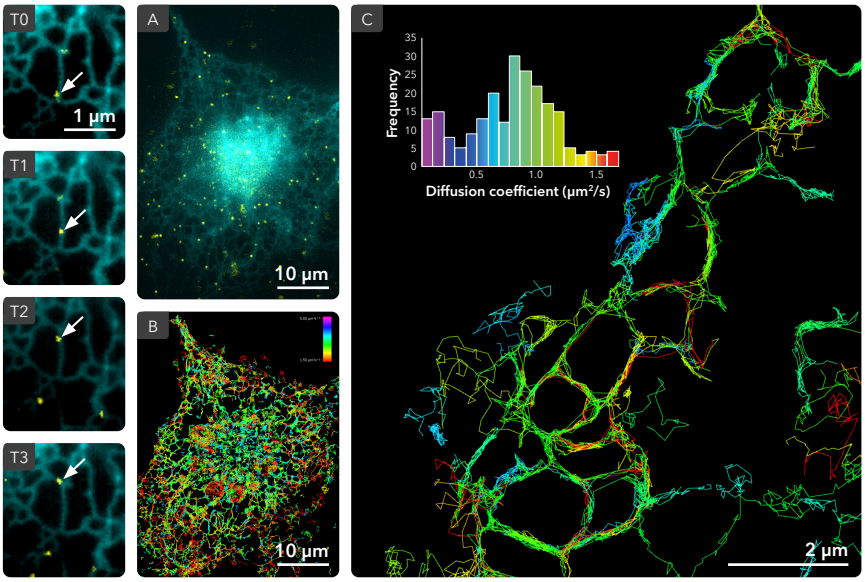

Figure 1 | Atlastin1 protein tagged with Halo tag-PAJF647 walking along the endoplasmic reticulum in fibroblast cells. *Dr. Christopher Obara, Jennifer Lippincott-Schwartz lab, USA.*

A) Halotag-Atlastin1 (yellow) has been labeled with a PA-JF647 ligand, and the ER using KDEL-GFP (blue).

B) Atlastin1 tracked walking along the ER. (T0-3) Timelapses of Atlastin1 walking along ER, at different time points.

C) Zoomed region of tracking localization map. Tracks are colored by diffusion coefficient and represented on histogram.

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How does HaloTag® work?

The HaloTag® labeling technology is based on the formation of a highly specific, rapid and irreversible covalent bond with a synthetic HaloTag® ligand. The system is interchangeable, with several ligands commercially available that contain different functional groups. This allows for a multitude of experimental approaches including visualization, immobilization/purification and pulldown assays to capture binding partners. The modular visualization HaloTag® ligands that can be used for SPT include Alexa Fluor® 488, Oregon Green®, diAcFAM and TMR (Tetramethyl Rhodamine).

Following genetic fusion with a protein of interest, a specific HaloTag® ligand can be added and imaged (Figure 2). The rapid covalent binding of the HaloTag® fusion protein with the ligand occurs via the hydrolysis of the chloroalkane substrate halogen that works as a linker. The resulting alkyl-enzyme intermediate has been engineered to form an irreversible bond that persists in a stable state, even in harsh environments, permitting high stability and coupling reliability.

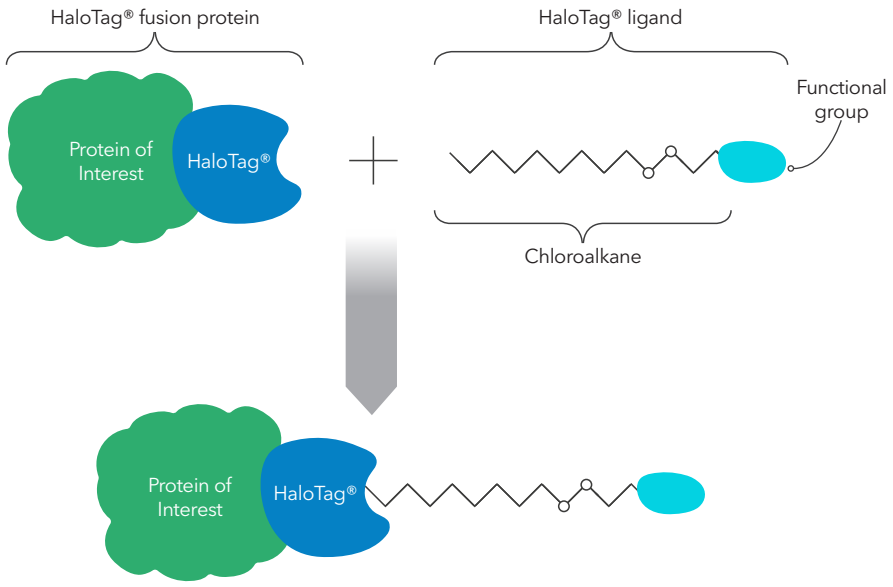

Figure 2 | Schematic representation of HaloTag® ligand binding.

Available fluorescent dyes for HaloTag®

Ligand	Excitation max (nm)	Emission max (nm)	Comments
Cell-Permeant Ligands (for intracellular labeling)			
Oregon Green®	492	520	Useful for applications that require quantitation of fluorescence or the use of high excitation energies with a green ligand
diAcFAM	492	521	Readily crosses the cell membrane to label intracellular proteins
TMR (Tetramethyl Rhodamine)	552	578	Popular choice, TMR conjugates (TMR-star) can also be used for SNAP-tag®
Janelia Fluor® 549	549	571	Twice as bright as TMR with excellent cell permeability
Janelia Flour® 646	646	664	
PA-Janelia Fluor® 549	549	571	Photoactivatable versions: good for high-speed and dual-colour live-cell imaging
PA-Janelia Flour® 646	646	664	
Cell-Impermeant Ligands (for cell-surface labeling)			
Alexa Fluor® 488	494	517	Similar spectra to Oregon Green®
Alexa Fluor® 660	663	690	Suitable far-red dye that is water soluble and pH-insensitive between pH 4 - pH 10

References | *G. T. Dempsey, J. C. Vaughan, K. H. Chen, M. Bates and X. W. Zhuang. Evaluation of fluorophores for optimal performance in localization-based super-resolution imaging. Nature Methods. 2011, 8, 1027-1035.*

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