Labeling and Imaging of Cell Surface Receptors Mediated by SNAP-tag®

Introduction

Many plasma membrane receptors, such as G-protein coupled receptors (GPCR), play a vital role in cell signaling and are often the therapeutic targets for drug discovery. Fluorescent imaging of the receptor pool on the plasma membrane of live cells is made possible by extracellular expression of a SNAP-tag fused to a receptor of interest and labeled with a cell membrane-impermeable substrate. The SNAP-tag enabled surface labeling technique provides a unique approach to monitoring localization, trafficking and turnover of a variety of cell membrane localized proteins (1-6).

The specific labeling and internalization of a GPCR protein, β2 Adrenergic Receptor (ADRβ2), is exemplified by using $SNAP_f$-tag as the fusion partner. To utilize this system, the coding region of ADRβ2 is first cloned in-frame to the C-terminus of the $pSNAP_f$ vector with a signal peptide coding sequence inserted upstream. The $SNAP_f$-ADRβ2 fusion protein is inserted in the plasma membrane with the $SNAP_f$-tag exposed to the extracellular side of the membrane. The following procedure is performed to visualize localization and internalization of $SNAP_f$-ADRβ2 using fluorescent microscopy after labeling with a SNAP-Surface fluorescent substrate. In addition, a comparative analysis of the $SNAP_f$-ADRβ2 and eGFP-ADRβ2 fusion constructs has been demonstrated.

General Protocol

1. Seed trypsinized cells in an 8-well Lab-Tek II Chambered Coverglass (Nalgene #155409).
2. Co-transfect the cells with 0.3 µg each of $SNAP_f$-ADRβ2 and eGFP-ADRβ2 fusion protein construct and incubate samples for 18-24 hours at 37°C, 5% CO_2.
3. Remove transfection complex media, wash cells twice with complete media, and label cells with 5 µM SNAP-Surface 549 (NEB #S9112) labeling media for 30 minutes at 37°C, 5% CO_2.
4. Remove the labeling media and add media containing 5 µM Hoechst 33342 to the cells for 2-3 minutes for nuclear counterstaining (optional).
5. Wash cells 3X with complete media and proceeded to imaging.

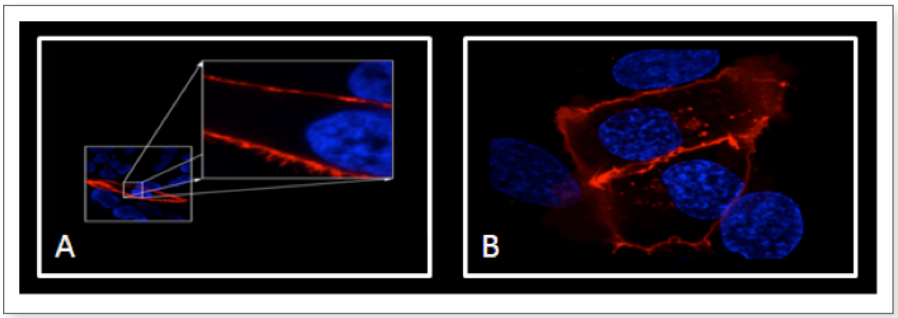

Figure 1. *Live cell imaging of surface localization and internalization of ADRβ2 receptor fused to $SNAP_f$-tag. A: Labeling of live U2OS cells transfected with $pSNAP_f$-ADRβ2 was carried out on ice for 15 minutes in the presence of 5 µM SNAP-Surface 549 (NEB #S9112) followed by washing and imaging by a confocal fluorescence scanning microscope. B: Internalization of ADRβ2 was visualized by confocal microscopy after labeling live HEK293 cells transiently expressing $SNAP_f$-ADRβ2 with 1.7 µM of SNAP-Surface 549 (NEB #S9112) for 15 minutes.*

Materials

- $pSNAP_f$-ADRβ2 (NEB #N9184)
- peGFP-ADRβ2
- SNAP-Surface® 549 (NEB #S9112)
- HEK293 or U2O cells
- DMEM medium
- Hoechst 33342
- Lab-Tek II Chambered Coverglass (Nalgene)

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Results

Localization and Internalization of Surface Receptors Fused to SNAP$_f$-tag

Live cell labeling of the SNAP$_f$-ADRβ2 pool on the plasma membrane was conducted using a cell membrane-impermeable substrate, SNAP-Surface 549 (NEB #S9112). The fluorescent signals of the surface localized receptor population were visualized by confocal fluorescence scanning microscope. As shown in Figure 1, the receptor endocytosis in cells transfected with SNAP$_f$-ADRβ2 can be monitored without the background from the intracellular pool of the fusion proteins. Figure 2 shows fluorescent images of HEK293 cells transiently co-expressing eGFP-ADRβ2 and SNAP$_f$-ADRβ2. When labeled with a cell-impermeable SNAP-Surface substrate, HEK293 cells give a distinct cell membrane fluorescent labeling pattern. In contrast, eGFP-ADRβ2 yields fluorescent signals from both the cell surface and intracellular compartments. A higher intracellular background of eGFP-ADRβ2 makes it more difficult to specifically monitor the internalization of tagged receptor. The data demonstrate a major advantage of the SNAP-tag labeling technique over the use of auto-fluorescent protein tags for the study of receptor trafficking.

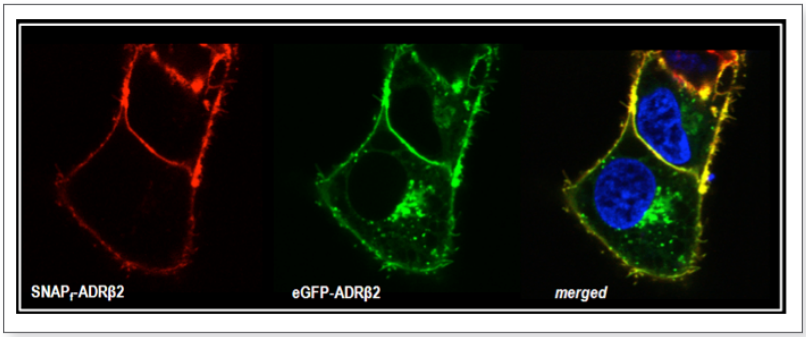

Figure 2. *Confocal microscopy of live HEK293 cells transiently co-expressing SNAP$_f$-ADRβ2 and eGFP-ADRβ2. Live cell labeling was carried out for 20 minutes on ice with 5 µM SNAP-Surface 549 (NEB #S9112S) and nuclei staining with Hoechst 33342.*

Summary

The use of SNAP-tag cell-impermeable substrates enables the fraction of a plasma membrane protein present on the surface of the cell to be visualized through specific labeling. This approach permits the discrimination of different populations of a cell surface protein: those properly translocated to the plasma membrane from those retained in the secretory pathway or already internalized, e.g., upon ligand binding. A wide range of selection of cell impermeable SNAP-Surface substrates offers excellent properties for the study of receptor localization, trafficking and protein-protein interactions. Our recent work together with the scientists at Yale University has further expanded the applications of the surface labeling approach by conducting live cell super-resolution microscopy of SNAP$_f$ tagged epidermal growth factor receptor and its ligand labeled with photostable organic dyes (5).

References:
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