Video Article

Genetically-encoded Molecular Probes to Study G Protein-coupled Receptors

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URL: <http://www.jove.com/video/50588>

DOI: [doi:10.3791/50588](https://doi.org/10.3791/50588)

Keywords: Genetics, Issue 79, Receptors, G-Protein-Coupled, Protein Engineering, Signal Transduction, Biochemistry, Unnatural amino acid, site-directed mutagenesis, G protein-coupled receptor, targeted photocrosslinking, bioorthogonal labeling, targeted epitope tagging

Date Published: 9/13/2013

Citation: Naganathan, S., Grunbeck, A., Tian, H., Huber, T., Sakmar, T.P. Genetically-encoded Molecular Probes to Study G Protein-coupled Receptors. *J. Vis. Exp.* (79), e50588, doi:10.3791/50588 (2013).

Abstract

To facilitate structural and dynamic studies of G protein-coupled receptor (GPCR) signaling complexes, new approaches are required to introduce informative probes or labels into expressed receptors that do not perturb receptor function. We used amber codon suppression technology to genetically-encode the unnatural amino acid, *p*-azido-L-phenylalanine (azF) at various targeted positions in GPCRs heterologously expressed in mammalian cells. The versatility of the azido group is illustrated here in different applications to study GPCRs in their native cellular environment or under detergent solubilized conditions. First, we demonstrate a cell-based targeted photocrosslinking technology to identify the residues in the ligand-binding pocket of GPCR where a tritium-labeled small-molecule ligand is crosslinked to a genetically-encoded azido amino acid. We then demonstrate site-specific modification of GPCRs by the bioorthogonal Staudinger-Bertozzi ligation reaction that targets the azido group using phosphine derivatives. We discuss a general strategy for targeted peptide-epitope tagging of expressed membrane proteins in culture and its detection using a whole-cell-based ELISA approach. Finally, we show that azF-GPCRs can be selectively tagged with fluorescent probes. The methodologies discussed are general, in that they can in principle be applied to any amino acid position in any expressed GPCR to interrogate active signaling complexes.

Video Link

The video component of this article can be found at <http://www.jove.com/video/50588/>

Introduction

Heptahelical G protein-coupled receptors (GPCRs) comprise a superfamily of highly dynamic membrane proteins that mediate important and diverse extracellular signals. In the classical paradigm, receptor activation is coupled with ligand-induced conformational changes.^{1,2} Recent advancements in structural biology of GPCRs have provided significant insight on the molecular mechanisms of transmembrane signaling.³⁻⁵ However, to understand with greater chemical precision the functional mechanism and structural dynamics of GPCR signaling, a toolkit of approaches is required to incorporate informative molecular and chemical probes to interrogate active signaling complexes.

To this end, we adapted a method to site-specifically introduce non- or minimally perturbing probes into expressed receptors based on unnatural amino acid (UAA) mutagenesis using amber codon suppression technology previously pioneered by Schultz and coworkers.⁶ We optimized the UAA mutagenesis methodology to achieve a high-yield expression and mutagenesis system for proteins such as GPCRs, which are difficult to express in most heterologous systems other than mammalian cells. Using an orthogonal engineered suppressor tRNA and evolved aminoacyl-tRNA synthetase pair for a specific UAA, we site-specifically introduced UAAs in expressed target GPCRs. The successful incorporation of UAAs, *p*-acetyl-L-phenylalanine (AcF), *p*-benzoyl-L-phenylalanine (BzF), and *p*-azido-L-phenylalanine (azF) has been demonstrated in our model GPCRs - rhodopsin and human C-C chemokine receptor, CCR5.^{7,8}

In principle, an UAA can be genetically encoded at any position within the protein sequence and this property is an invaluable biochemical tool as it enables single-codon scanning of a target GPCR. We focus here specifically on the versatility of the UAA, azF, which has a reactive azido moiety. In addition to serving as a unique infrared (IR) probe,^{8,9} azF can also serve as a photoactivatable cross-linker by reacting with neighboring primary amines or aliphatic hydrogens. Additionally, the biologically inert azido group can participate as a selective chemical handle in bioorthogonal labeling reactions. Here we present examples illustrating the useful applications of site-specific incorporation of azF into GPCRs, such as targeted photocrosslinking to trap a receptor-ligand complex, and modification of GPCRs by bioorthogonal epitope tagging and fluorescent labeling strategies.

Photoactivatable reagents have been used to study biological systems since the 1960s.¹⁰ In this period, an abundance of receptor-ligand crosslinking experiments have been reported to study GPCR complexes, most of which involved the use of photoaffinity ligands.^{11,12} However, these applications are technically limited, as they require synthesis of ligands bearing a crosslinking group.¹³⁻¹⁵ Moreover, with the crosslinker moiety in the ligand, it is challenging to identify the location of the crosslink on the GPCR. Site-specifically introducing photocrosslinking groups as UAAs into proteins using the amber codon suppression technology is a valuable advancement.^{16,17} We developed a photocrosslinking

technique to identify the binding interface on a receptor that is involved in the formation of a receptor-ligand complex in live cells by introducing photolabile groups into GPCRs.^{18, 19} Here we describe the experimental protocol and method of data analysis for applying this targeted photocrosslinking technology to identify the binding site of a small-molecule ligand, tritiated maraviroc, on CCR5. This method capitalizes on the precise quantification of the radioactive handle on the ligand, in addition to retaining the native chemical structure of the ligand.

Fluorescence-based techniques support the precise understanding of the structural basis of receptor activation by directly probing the conformational state of the receptor.^{20, 21} However, techniques possessing the flexibility to introduce fluorescent labels into GPCRs site-specifically are limited. We are interested in employing bioorthogonal chemical modification strategies to facilitate single-molecule detection (SMD) of GPCR signaling complexes.²² The azido group can participate in bioorthogonal chemistries such as Staudinger-Bertozzi ligation,^{23, 24} copper-free strain-promoted azide-alkyne cycloaddition (SpAAC)²⁵ and copper-catalyzed azide-alkyne cycloaddition (CuAAC).²⁶ We focus here on the Staudinger-Bertozzi ligation reaction that involves the specific reaction between an azide and a phosphine. We demonstrate the use of two different phosphine derivatives, conjugated to a peptide epitope (FLAG peptide) or a fluorescent label (fluorescein) to achieve site-specific modification of GPCRs.

We previously optimized the conditions for site-specific labeling of rhodopsin azF variants using the X-ray crystal structure and dynamic simulations to choose target sites that are solvent exposed.^{27, 28} We also illustrated the feasibility to achieve background-free labeling by Staudinger-Bertozzi ligation.²⁹ We demonstrate here the general procedure employed to achieve fluorescent labeling of a detergent-solubilized receptor that is immobilized on an immunoaffinity matrix and subsequently visualized by in-gel fluorescence. Additionally, we demonstrate a useful extension on this labeling strategy to identify positions amenable to labeling on a receptor of unknown structure, CCR5. This is carried out using a targeted peptide-epitope tagging strategy that relies on bioorthogonal modification of azF-GPCR variants in a cell-based semi-high throughput format.³⁰ This method exploits the multi-step detection properties of a cell-surface ELISA to monitor labeling events.

The methodologies we discuss here are general and in principle can be applied to any GPCR incorporated with azF using the amber codon suppression technology. In the protocols presented here we detail the steps involved in mammalian cell expression of receptors incorporated with UAAs such as azF using the unnatural amino acid mutagenesis method and their subsequent applications to facilitate structural and dynamic studies of GPCRs.

Protocol

1. Site-specific Genetic Incorporation of Unnatural Amino Acids into GPCRs

1. Maintain HEK293T cells in DMEM (4.5 g/L of glucose, 2 mM glutamine) supplemented with 10% fetal bovine serum (FBS) at 37 °C in a 5% CO₂ atmosphere.
2. Transfect the cells grown to 60-80% confluence in a 10-cm plate using Lipofectamine Plus reagent.
  1. To 750 µl DMEM, add 10 µl Plus reagent, 3.5 µg of GPCR cDNA (pMT4. Rho or pcDNA 3.1. CCR5) containing the amber stop codon at a desired position, 3.5 µg of suppressor tRNA cDNA (pSVB.Yam) and 0.35 µg of mutant amino-acyl tRNA synthetase cDNA for *p*-azido-L-phenylalanine (pcDNA.RS). Incubate at room temperature for 15 min. Also perform a similar transfection using the wt GPCR cDNA (not containing an amber stop codon) to serve as a control. Add this mixture to 750 µl of DMEM with 17 µl Lipofectamine. After equilibrating 15 min at room temperature, bring the total volume to 4 ml.
  2. Aspirate media on 10-cm plate, apply transfection mixture to cells, and return to 37 °C in 5% CO₂ atmosphere. After 4-6 hr, supplement cells with 4 ml DMEM containing 20% FBS and 1 mM azF.
3. The next day, replace the growth media with DMEM containing 10% FBS and 0.5 mM azF.
4. Harvest cells 48 hr post-transfection, to analyze expression or proceed to photocrosslinking or labeling procedures described in the following sections.
  1. Confirm expression of the GPCR amber mutant in the presence of the UAA in the growth media. We do this by Western immunoblot detection. Lyse the cell pellet in 1% (w/v) dodecyl-β-D-maltoside (DDM) in 20 mM Tris-HCl, pH 7.5, 100 mM NaCl for 1 hr at 4 °C. Centrifuge the lysate at 16,000 x g and resolve the supernatant under reducing conditions by SDS-PAGE. Transfer to a PVDF membrane and carry out immunoblot analysis to detect full-length receptor expression using the 1D4 mAb (National Cell Culture Center), which recognizes an engineered epitope at the C-terminus of the receptor.³¹

2. Mapping a Ligand Binding Site Using Targeted Photocrosslinking

1. 48 hr post-transfection, aspirate media from cells and replace with 4 ml 1x Phosphate Buffered Saline (PBS). Return to 37 °C for 15 min.
2. Resuspend the cells with 1x PBS and transfer to a Falcon tube. Centrifuge at 1,500 rpm for 2 min to pellet the cells and aspirate the supernatant.
3. Resuspend the cell pellet in 1x Hank's Buffered Salt Solution containing 20 mM HEPES, pH 7.5, 0.2% BSA and the tritiated ligand at saturating binding concentration for the required length of time and temperature to ensure receptor-ligand complex formation.
4. Transfer the cell suspension to a 24-well or 96-well plate for photoactivation of the azF at 365 nm (e.g. use a UV-A lamp) for 15 min at 4 °C. Maintain the plate on ice to avoid sample heating and cell lysis.
5. Transfer the cells to an Eppendorf tube, centrifuge to pellet the cells, remove the supernatant and proceed to analysis. The pellets can be stored at -20 °C for future use.
6. Resuspend the cell pellets in lysis buffer containing 1% (w/v) DDM, 20 mM Tris-HCl, pH 7.5 and protease inhibitors. After 1-hr incubation at 4 °C, centrifuge the cell lysate for 10 min at 16,000 x g, and transfer the supernatant to a new tube.

7. Add 1D4 mAb-sepharose 2B resin to the supernatant and incubate overnight at 4 °C to immunopurify the GPCR using the engineered C-terminus 1D4 mAb epitope. Next day wash the beads several times with lysis buffer. Elute samples with 1% SDS at 37 °C for 1 hr with shaking.
8. Transfer a portion of the eluent to a 15 ml vial containing scintillation fluid and count on a beta scintillation counter to quantify the amount of specific binding of the tritiated ligand to the receptor.
9. Resolve the remaining 1D4 mAb purified eluent by standard gel electrophoresis. We use a 4-12% SDS-PAGE gel and then semi-dry transfer to a PVDF membrane for immunoblotting. We also include a lane with standard protein ladder to guide visual approximation of molecular weight.
  1. Block the PVDF membrane with 5% milk in TBS buffer containing 0.05% Tween-20. Probe the membrane to confirm full-length receptor expression with 1D4 mAb followed by HRP-conjugated anti-mouse IgG secondary antibody.
  2. Treat with enhanced chemiluminescence (ECL) reagent and expose membrane to autoradiography film to visualize bands.
10. Cut the membrane with a razor blade to separate each sample lane, followed by cutting at specific molecular weight markers. Transfer membrane segments to vials containing scintillation fluid. Quantify the amount of tritium in each membrane segment by counting on a beta-scintillation counter.
11. Identify the positive photocrosslink with the detection of tritium at the apparent molecular mass equal to the sum of that of the GPCR and the ligand.

3. Targeted Epitope Tagging and Cell Surface Enzyme-linked Immunosorbent Assay (ELISA)

1. 24 hr post-transfection, wash cells with 1 ml 1x PBS and trypsinize with 1 ml 0.25% trypsin for 3 min. Supplement with 9 ml DMEM containing 10% FBS and 0.5 mM azF. Resuspend cells and count the cell density. We use a standard hemocytometer.
2. Pre-treat a high-binding, clear-bottom 96-well plate with 100 µl of 0.01 mg/ml poly-D-lysine per well. Incubate for 30 min at room temperature, wash repeatedly with 1x PBS and air-dry.
3. Plate 200 µl of the transfected cells at a density of 6×10^4 - 8×10^4 cells/well to the 96-well plate and return to 37 °C in 5% CO₂ atmosphere.
4. Next day prepare cells for labeling. Gently wash three times with 100 µl/well 1x PBS to remove any azF containing growth media. Ensure cells remain adhered, for example, by using PBS containing Ca²⁺ and Mg²⁺.
5. Prepare 50-200 µM FLAG-triarylphosphine labeling reagent in 1x HBSS/PBS from a stock maintained at 5-20 mM in PBS. Apply 60 µl to each well (in triplicate for each amber mutant) and return to 37 °C for 30 min to 4 hr. Maintain a set of wells without label treatment in 1x HBSS/PBS. Also include a wt GPCR control to compare with azF-GPCR variants.
6. Wash cells 3 times with 100 µl/well of blocking buffer, BB (1x PBS, 0.5% BSA), to remove unreacted label completely.
7. Apply 100 µl/well of 4% paraformaldehyde (prepared from a 16% stock in 1x PBS) to the cells. Incubate for 20 min at room temperature. Wash three times with BB.
8. Perform standard cell surface ELISA protocol to detect labeled receptor and receptor expression. Incubate with primary antibody for 1.5 hr on ice followed by secondary antibody incubation at room temperature for 1 hr.
  1. Add 100 µl of anti-FLAG M2 antibody (e.g. 1:2,000 dilution of antibody made in BB) to detect FLAG peptide epitope of the FLAG-triarylphosphine labeling reagent. Also probe non-label treated wells as a negative control. Add anti-CCR5 2D7 (we use a 1:500 dilution) antibody to a separate set of wells to determine wt or azF-GPCR cell surface expression.
  2. Wash cells three times with BB, and incubate with 100 µl of HRP-conjugated anti-mouse IgG secondary antibody (1:2,000 dilution).
  3. Carefully wash cells five times with BB. Add 50 µl detection buffer: Amplex Red, 20 mM H₂O₂, 1x PBS (1:10:90 ratio) and incubate 15 min at room temperature. Collect spectral data on a fluorescence multi-well plate reader at λ_{ex} =530 and λ_{em} =590.

4. Bioorthogonal Fluorescent Labeling of a GPCR

1. Lyse 10^7 harvested cells expressing wt or azF-Rhodopsin variants in 1 ml solubilization buffer containing 1% (w/v) DM, 50 mM HEPES or Tris-HCl, pH 6.8, 100 mM NaCl, 1 mM CaCl₂ and protease inhibitors for 1 hr at 4 °C. Centrifuge the cell lysate at 15,000 x g for 10 min and collect the supernatant fraction.
2. Add 100 µl 1D4-mAb sepharose 2B resin to capture receptor using the engineered C terminus 1D4 epitope. Incubate for 12 hr at 4 °C. Wash the resin three times with 0.1% (w/v) DDM in 0.1 M sodium phosphate buffer, pH 7.3.
3. Add 0.1 mM Fluorescein-phosphine in a total volume of 0.3 - 0.5 ml to label receptor immobilized on the immunoaffinity matrix (1D4-mAb sepharose 2B). Incubate for 12 hr at room temperature. Centrifuge and remove supernatant. Wash the resin to remove unreacted label.
4. Elute the labeled receptor with 100 µl elution buffer containing 0.1% (w/v) DDM and 0.33 mg/ml nonapeptide (C9 peptide against the 1D4 epitope) in 2 mM phosphate buffer, pH 6.0 by incubation on ice for 1 hr.
5. Resolve labeled samples by SDS-PAGE under reducing conditions. Wash gels briefly in PBS and then visualize labeling of azF-GPCR by in-gel fluorescence. For example, use a confocal fluorescence scanner with a 488-nm wavelength laser to detect receptor modified with fluorescein.

Representative Results

We employed the unnatural amino acid mutagenesis methodology to site-specifically introduce molecular probes into a GPCR using the amber codon suppression technology. The scheme in **Figure 1** outlines the salient steps of the methodology and the various applications of incorporating the versatile UAA, *p*-azido-L-phenylalanine (azF), into GPCRs. Expression of azF-GPCRs in mammalian cells enables targeted photocrosslinking to ligands, and targeted peptide-epitope tagging or fluorescent modifications of a GPCR via bioorthogonal labeling chemistries.

The photocrosslinking technique can be used to investigate the structural aspects of GPCR-ligand complexes in live cells (**Figure 1a**).

Representative data obtained from one such experiment is shown in **Figure 2**.¹⁹ Here we used a radioactive labeled small-molecule ligand, tritiated maraviroc, to identify its binding site on CCR5. In this example, UV irradiation on cells expressing azF-CCR5 variants pre-incubated with tritiated maraviroc results in covalently crosslinked complexes that can be identified using the sensitive radioactive tag on the ligand. Amongst the several positions on CCR5 tested, I28azF and W86azF were able to photocrosslink to tritiated maraviroc as depicted with significantly higher scintillation counts (red bars, bottom panel, **Figure 2**). The wt receptor showed no crosslinking to the radioactive ligand.

Bioorthogonal labeling chemistries can be used to site-specifically modify azF-GPCR variants. We employed the Staudinger-Bertozzi ligation chemistry to label two GPCRs: CCR5 and rhodopsin using phosphine derivatives (**Scheme 1**). One strategy involves the targeted epitope tagging of a GPCR in live cells using a peptide-conjugated label (**Figure 1b**). We demonstrate one such experiment, in which a FLAG peptide-triarylphosphine labeling reagent is used to site-specifically and selectively modify azF-CCR5 variants. In **Figure 3** we display representative data from a sensitive and multi-step ELISA detection strategy used to identify positions that are tagged with the FLAG peptide.³⁰ Amongst the positions on CCR5 investigated, all azF-CCR5 variants were expressed at the cell surface (red bars, **Figure 3**) and approximately half of them successfully underwent targeted epitope tagging (blue bars, **Figure 3**). wt CCR5 served as a negative control for epitope tagging, exhibited by the lack of anti-FLAG signal. In an alternative strategy, we labeled detergent-solubilized azF-GPCRs immobilized on an immunoaffinity matrix, such as anti-1D4 mAb conjugated to sepharose 2B resin, with a fluorophore-conjugated label (**Figure 1c**). The in-gel fluorescence image shown in **Figure 4** demonstrates the successful fluorescent labeling of the GPCR, rhodopsin, at three different positions incorporated with azF by the Staudinger-Bertozzi ligation using a phosphine derivative of fluorescein.²⁹ As expected, the wt rhodopsin showed no background labeling as seen by the absence of a fluorescent band at the expected molecular weight of the receptor.

Scheme 1. The Staudinger-Bertozzi ligation reaction between a triarylphosphine and an azide to form a stable amide-linked adduct.

Figure 1. Scheme illustrating the site-specific incorporation of an UAA (azF) and its applications to study GPCRs. Site-specific UAA mutagenesis is achieved by co-transfection of three separate plasmids into mammalian cells that carry: an evolved orthogonal amino acyl-tRNA synthetase specific for the UAA, an orthogonal suppressor tRNA that recognizes an amber stop codon (UAG), and the gene encoding the GPCR containing a TAG mutation at a desired position. Cells grown in the presence of the UAA express a full-length GPCR with a site-specifically introduced azF. These cells can then be (a) incubated with ligand and activated with UV-light to identify crosslinks between a GPCR and its ligand, (b) used to site-specifically epitope-tag a GPCR in culture with a peptide-conjugated chemical label, or (c) solubilized to purify GPCRs, which are then bioorthogonally modified with a fluorescent label under conditions where the receptor is immobilized on an antibody-conjugated sepharose resin. [Click here to view larger image](#)

Figure 2. Targeted photocrosslinking of a GPCR to a tritiated ligand. Displayed here are the representative results from a crosslinking experiment between azF-CCR5 variants and a tritium-labeled small molecule ligand, maraviroc. Top panel: The Western immunoblot generated after a typical experiment, showing the expression of the tested azF-CCR5 variants. The colored segments highlight where the PVDF membrane was cut for scintillation counting and correspond to the detected scintillation counts plotted in the bar graph in the bottom panel. The red segment represents the expected molecular mass (around 40 kDa) of the covalent crosslinked complex between the receptor and ligand. Therefore, we would expect to detect tritium in those segments only when azF is introduced at a position in the receptor that is in close proximity to the ligand-binding pocket and can form a covalent crosslink with the ligand. Reprinted (adapted) with permission from Grunbeck, A., *et al.* Genetically encoded photo-cross-linkers map the binding site of an allosteric drug on a G protein-coupled receptor. *ACS Chemical Biology* **7**, 967-972 (2012). Copyright (2012) American Chemical Society. [Click here to view larger image](#)

Figure 3. ELISA detection of targeted epitope-tagging of a GPCR. Top panel: Cell surface expression of HEK 293T cells expressing azF-CCR5 variants and wt and mock transfected controls probed with anti-CCR5 mAb 2D7 using the whole cell-based ELISA (red bars). Bottom panel: Cells from the same transfection were treated with 0.05 mM FLAG-triarylphosphine for 1 hr in live cells and then quantified by subsequent ELISA using anti-FLAG M2 mAb to determine the extent of peptide-epitope tagging at each position (blue bars). Error bars represent the standard error of the mean for two or more replicate data sets. Reprinted (adapted) with permission from Naganathan, S., *et al.* Site-specific epitope tagging of GPCRs by bioorthogonal modification of a genetically encoded unnatural amino acid. *Biochemistry* DOI: 10.1021/bi301292h (2013). Copyright (2013) American Chemical Society. [Click here to view larger image](#)

Figure 4. Fluorescent labeling of rhodopsin azF incorporated at various positions. Wt and azF-rhodopsin mutants immobilized on an immunoaffinity matrix labeled with fluorescein-phosphine. Purified samples were separated by 4-12% SDS-PAGE and the in-gel fluorescence image shown was taken with a confocal 488-nm laser fluorescence scanner using an emission filter set optimized for fluorescein detection. Figure adapted from Huber, T., *et al.* Bioorthogonal labeling of functional G protein-coupled receptors at genetically encoded azido groups. submitted (2013). [Click here to view larger image](#)

Discussion

We describe here a robust methodology for site-specific incorporation of a reactive probe, azF, into GPCRs and demonstrate three useful applications of this tool to study the structure and dynamics of GPCRs. Our method to site-specifically incorporate UAAs circumvents a fundamental problem with an alternative strategy based on chemically labeling^{32, 33} or attaching photocrosslinkers³⁴ to a single accessible cysteine mutant. Although, chemistries that target cysteine thiol groups have been used to attach fluorescent probes, such a strategy necessitates the generation of a cysteine-free protein background. This can be limiting since all GPCRs possess more than one cysteine, many of which may be essential to maintain proper receptor function. Therefore, the feasibility to introduce a UAA that is amenable to chemical modification or can be activated by UV light is extremely attractive.

The covalent reaction of a photoactivatable crosslinker with a target molecule is dependent on distance and neighboring side chains. Therefore, these probes can be utilized in identifying interactions that are within a specific distance from the crosslinker, for example, benzophenone-based crosslinkers are suggested to have a distance dependency of 3 Å.³⁵ Previously, we introduced both azF and BzF, a UAA containing a

benzophenone reactive group, to identify residues in a GPCR that are within a defined distance from a fluorescein or tritium labeled ligand.¹⁸

¹⁹ The general applicability of this technology depends only on the availability of such labeled ligands and the generation of amber mutants of a target GPCR. Additionally, in theory this targeted photocrosslinking technique can be applied to identify interactions between GPCRs and their other binding partners, such as a G protein or β -arrestin. The data gained from these crosslinking studies can be used to create more accurate molecular models of GPCR signaling complexes.

Several bioorthogonal chemical reactions have been described in the literature that allow for the site-specific modification of UAA residues. The UAA, AcF, for example, possesses a keto group that can partake in hydrazone or oxime ligation reactions.^{36, 37} We previously compared the modification of keto groups and azido groups with biotinylated and fluorescein conjugated reagents.²⁹ Our observations indicated that the azido group of azF is truly more bioorthogonal than its keto counterpart. Hence, we subsequently focused on the use of the Staudinger-Bertozzi ligation to modify two GPCRs with a peptide epitope tag or a fluorescent label. It is noteworthy, however, that the Staudinger-Bertozzi ligation exhibits sub-stoichiometric³⁸ and poor kinetic properties of labeling. Moreover, phosphines are highly susceptible to air oxidation.²⁵ We have observed that the Staudinger-Bertozzi ligation only achieves 30% labeling after incubation for 12 hr at room temperature.²⁹ For these reasons, we are currently evaluating alternative labeling chemistries such as CuAAC and SpAAC to site-specifically label azF-GPCRs.

Albeit the drawbacks of Staudinger-Bertozzi ligation reactions, we demonstrate here the use of a live-cell peptide-epitope tagging strategy to identify positions on a target GPCR that are accessible for future fluorescent labeling. Additionally, this epitope tagging strategy has numerous other useful applications, such as, the introduction of an epitope tag at a single modified residue in contrast to inserting the entire epitope by replacing native sequences. This feature is of specific importance with GPCRs since modifying the length and sequence of highly conserved loop segments could dramatically alter function. Finally, we also illustrated here the proof-of-concept experiments to label GPCRs with fluorophores, making them suitable for single-molecule detection experiments.

In conclusion, azF is a versatile tool for the study of the structure and function of GPCRs. We have shown that azF is site-specifically incorporated into GPCRs expressed in mammalian cells using the amber codon suppression system. This allows for the modification of a GPCR at only one single position. The structure and function of the labeled receptor can then be evaluated either in the native plasma membrane environment or in a detergent solution. We are now poised to investigate GPCR signaling complexes with the flexibility of the azF probe.

Disclosures

Authors have nothing to disclose.

Acknowledgements

We thank the generous support of several foundations and philanthropic donors (see SakmarLab.org).

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