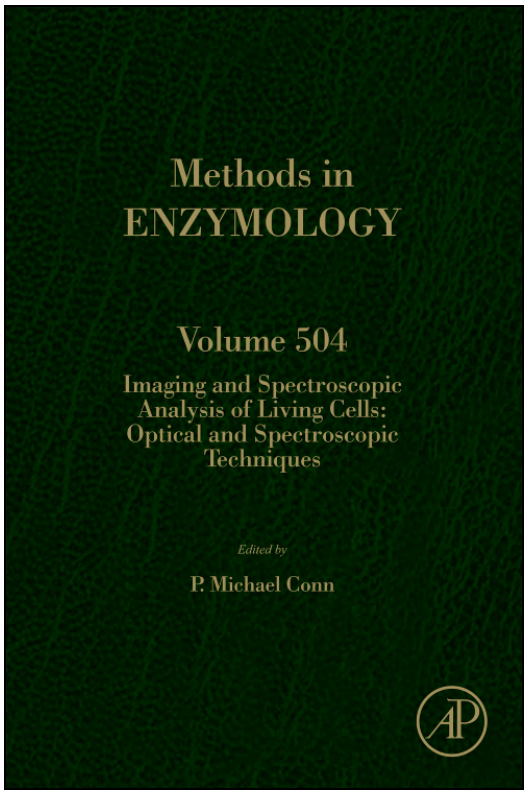

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CHAPTER EIGHTEEN

Coiled-Coil Tag–Probe Labeling Methods for Live-Cell Imaging of Membrane Receptors

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Contents

Abstract

Tag–probe labeling methods have advantages over conventional fusion with fluorescent proteins in terms of smaller labels, surface specificity, availability of pulse labeling, and ease of multicolor labeling. With this method, the gene of the target protein is fused with a short tag sequence, expressed in cells, and the protein is labeled with exogenous fluorescent probes that specifically bind to the tag. Various labeling principles, such as protein–ligand interaction, peptide–peptide interaction, peptide–metal interaction, and enzymatic reactions, have been applied to the tag–probe labeling of membrane receptors. We describe our coiled-coil tag–probe method in detail, including the design and synthesis of the tag and probe, labeling procedures, and observations by confocal microscopy. Applications to the analysis of receptor internalization and oligomerization are also introduced.

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1. Introduction

Fluorescence imaging of proteins in living cells has become a conventional technique to study the intracellular localization and dynamic behavior of the target proteins. The discovery and development of the green fluorescent proteins (GFPs) have drastically changed the procedures to obtain fluorophore-labeled proteins in living cells (Chudakov *et al.*, 2010; Giepmans *et al.*, 2006). The expression of genetic fusion constructs with fluorescent proteins into cultured cells (Fig. 18.1A) enables facile protein-specific labeling in cells

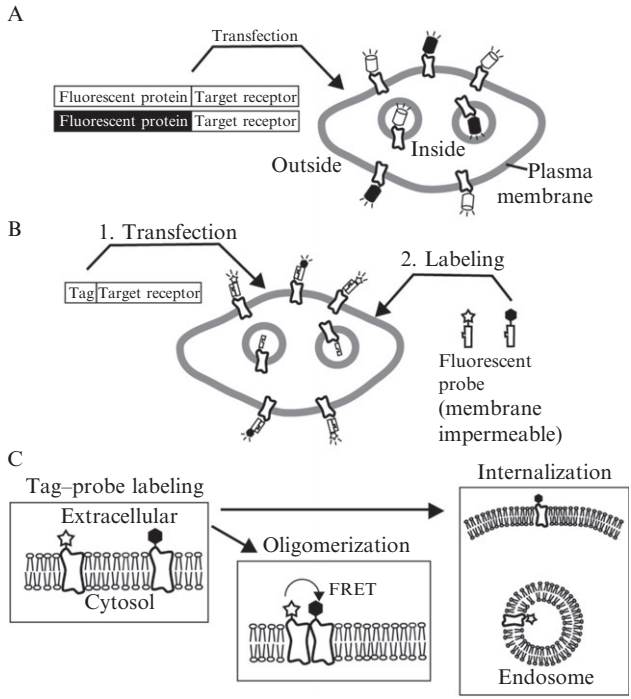

Figure 18.1 Principle of the tag–probe labeling method and its applications to the labeling of membrane proteins in living cells. (A) Labeling by genetic fusion with fluorescent proteins. The gene of the target protein fused with that of fluorescent protein is expressed in cells to obtain fluorophore-tagged target proteins. Multicolor labeling requires coexpression of multiple target genes fused with different tag proteins. Control of the labeling ratio is usually difficult. (B) Posttranslational labeling in the tag–probe method. The gene of the target protein is fused with a short tag sequence and expressed in cells. The expressed tag sequence is specifically labeled with an exogenous probe attached to a fluorophore. Surface labeling is achieved using a membrane-impermeable probe. Multicolor labeling is possible using probes labeled with different fluorophores. Precise control of the labeling ratio is possible. (C) Observation of internalization and oligomerization of membrane receptors by the tag–probe method. The surface specificity and easiness of multicolor labeling enable sensitive and accurate detection.

without laborious processes such as microinjections of target proteins into cells after expression, purification, and chemical labeling with exogenous fluorophores (Adams *et al.*, 1991). Cloning and mutational studies have established numerous new fluorescent and luminescent proteins with unique characteristics, including photoswitchable probes, expanding their spectral ranges and applications (Chudakov *et al.*, 2010; Fernandez-Suarez and Ting, 2008).

In spite of their excellent specificity, fluorescent/luminescent protein tags have several disadvantages. Their large size (e.g., ~27 kDa for GFP) can disrupt the normal trafficking and function of target proteins (Hoffmann *et al.*, 2005; Lisenbee *et al.*, 2003). It is not easy to control the labeling ratio in multicolor labeling, which is crucial for the quantitative analysis of intermolecular fluorescence resonance energy transfer (FRET). Further, genetic fluorophores are not suitable for cell-surface-specific labeling of membrane proteins such as G protein-coupled receptors (GPCRs) because of uniform labeling of both cell surface and intracellular proteins.

To overcome these difficulties, a hybrid approach using a genetic tag and synthetic fluorescent (or other functional) probes that specifically bind to the tag has been actively studied (Lin and Wang, 2008; Yano and Matsuzaki, 2009; Fig. 18.1B). As shown in Table 18.1, tag–probe methods based on diverse interactions such as protein–ligand, peptide–peptide, and peptide–metal interactions have been reported. An alternative labeling principle is the use of enzymatic reactions. A more comprehensive list has been described elsewhere (Yano and Matsuzaki, 2009). By using tag–probe labeling, the size can be reduced to 2 kDa, although in many cases there is a trade-off between size and labeling specificity. Posttranslational labeling enables surface-specific labeling of membrane receptors using membrane-impermeable probes, pulse labeling with arbitrary timing, and easy control of the labeling ratio in multicolor labeling (Fig. 18.1B). Using these advantages, receptor internalization and oligomerization (Fig. 18.1C) have been quantitatively analyzed. In this chapter, we show examples of tag–probe methods useful for labeling membrane receptors, particularly focusing on procedures using the coiled-coil tag–probe method we developed.

2. Various Principles Used for Tag–Probe Labeling

2.1. Protein–ligand interactions

Labeling using interactions between a protein tag and a ligand probe can achieve high specificity without complicated labeling procedures, although the degree of miniaturization of the label is modest. Both noncovalent ligands and covalent ligands have been applied to the tag–probe labeling of proteins including membrane receptors. For example, labeling with

Table 18.1 Various tag–probe labeling methods

Labeling principle	Labeling system	Tag	Probe	Tag–probe size (approx.)	Cofactor/ enzyme	Affinity	Typical labeling conditions	Material availability/ note	Reference
Protein–ligand	SNAP-tag™	hAGT	Benzilguanine	20 kDa	No	Covalent	5 μM, 1 h	Commercial kit (NEB), intracellular labeling is also possible	Maurel *et al.* (2008)
Peptide–peptide	Coiled-coil	$(EIAALKE)_3$	$(KIAALEK)_3$ or $(KIAALEK)_4$	6 kDa	No	6 nM (K4), 64 nM (K3)	20 nM, 1 min	Commercial probe (Peptide Institute)	Yano *et al.* (2008)
Peptide–metal	Biarcenical-tetracycteine	FLNCCPG-CCMEP	Biarsenical fluorophores	2 kDa	Ethanedithiol	Covalent	0.5 μM, 1 h	Commercial probe (Invitrogen), intracellular labeling, washout is necessary	Hoffmann *et al.* (2010)
Enzymatic	ACP-tag™, MCP-tag™	Acyl carrier protein	Coenzyme A	9 kDa	PPTase	Covalent	5 μM, 40 min	Commercial kit (NEB)	Meyer *et al.* (2006)

SNAP-tag, which is based on the irreversible transfer of an alkyl group from O^6-alkylguanine-DNA to human O^6-alkylguanine-DNA alkyltransferase (Keppler *et al.*, 2003), has been used to detect the internalization and oligomerization of GPCRs. Both membrane-permeable and impermeable probes are available from New England Biolabs (Ipswich, MA). The internalization of orexin and cannabinoid receptors following stimulation with their agonists was detected by labeling the receptors with a luminescent terbium chelate, resulting in an increase in detection sensitivity (Ward *et al.*, 2011). Taking advantage of the surface-specific labeling, time-resolved FRET from europium chelate to organic fluorophores has also been measured to investigate the oligomerization of GPCRs in HEK 293 cell membranes (Maurel *et al.*, 2008). The authors found that class C metabotropic glutamate receptors form strict dimers, whereas $GABA_B$ receptors can form dimer of dimers.

2.2. Peptide–metal interactions

Strong interactions between peptides and metals (or metalloids) are a fascinating tool for labeling. Organic fluorophores that contain metalloid atoms such as arsenic (Griffin *et al.*, 1998) and boron (Halo *et al.*, 2009) have been used to label tetracysteine and tetraserine motifs, respectively. The first tag–probe labeling system in living cells, reported in 1998, was based on a reversible covalent bond between an arsenic derivative of fluorescein (designated as FlAsh) and pairs of thiols (Griffin *et al.*, 1998). Thereafter, development of the tag sequence (Martin *et al.*, 2005), expansion of available fluorophores (Pomorski and Krezel, 2011), and refinement of labeling procedures (Hoffmann *et al.*, 2010) were reported. FlAsh labeling in combination with a genetic fluorescent protein is useful to detect conformational changes of GPCRs by intramolecular FRET (Hoffmann *et al.*, 2005).

The specific assembly of a chelator tag peptide and a chelator probe is possible through the coordination of metal cations, such as Ni^{2+} (Guignet *et al.*, 2004) and Zn^{2+} (Hauser and Tsien, 2007; Ojida *et al.*, 2006). An example is the membrane-impermeable HisZiFit probe that binds to a hexahistidine tag via Zn^{2+} coordination (Hauser and Tsien, 2007). Surface exposure of a membrane protein, stromal interaction molecule 1, from the endoplasmic reticulum in HEK293 cells was successfully detected using this method.

2.3. Enzymatic reactions

Enzymes that covalently attach a substrate to a specific site of a polypeptide have been applied to tag–probe labeling in living cells. A smaller size and tight labeling can be achieved with this approach, although a longer labeling

time in the presence of excess probes (=substrates) is usually required for efficient labeling. An example is the use of phosphopantetheinyl transferase which transfers part of a phosphopantetheinyl probe to an acyl carrier protein (ACP) tag consisting of ~80 amino acids (George *et al.*, 2004; Yin *et al.*, 2004; available from New England Biolabs). The lateral organization of neurokinin-1 (NK_1) receptors in HEK293 cell membranes was investigated by FRET from Cy3 to Cy5 attached to the receptors by ACP labeling (Meyer *et al.*, 2006). The authors analyzed FRET efficiency at various donor/acceptor ratios and expression levels, and concluded that NK_1 receptors are monomeric but locally concentrated in membrane microdomains to give FRET signals in HEK293 cell membranes. Consistent with the result, perturbation of the membrane microdomains by extraction of cholesterol with methyl-β-cyclodextrin decreased the FRET signal.

3. Coiled-Coil Tag–Probe Labeling

We have examined the utility of tag–probe labeling based on peptide–peptide interactions to achieve a reasonable balance between small size and labeling specificity. Relatively quick labeling will also be possible because of the simple principle involved (physicochemical binding). Coiled-coil tag–probe labeling is based on α-helical coiled-coil formation between negatively charged E_n peptides $(EIAALEK)_n$ and positively charged K_n peptides $(KIAAEKE)_n$ ($n = 3$ or 4), originally studied by Litowski and Hodges (2002). In addition to electrostatic attractions, leucine zipper-type hydrophobic interactions at the interface drive tight heterodimer formation (Fig. 18.2A). We found the E3 peptide to be suitable as the N-terminal extracellular tag of membrane proteins, which was specifically labeled with K3 and K4 peptide probes while retaining protein functions (Yano *et al.*, 2008). For example, E3-tagged β_2 adrenergic receptors (β_2ARs) transiently expressed in Chinese hamster ovary (CHO) cells were successfully labeled with K4 probes, as confirmed by costaining with fluorescent ligands (Fig. 18.2B). The labeling can be completed within 1 min, which is much faster than other tag–probe methods. Labeling of the E3 tag with the K3 probe ($K_d \sim 60$ n*M*) is reversible and the probe can be washed out whereas labeling with K4 is stronger ($K_d \sim 6$ n*M*) and therefore suitable for long-term observation. Because the charged peptides are membrane-impermeable, the label is surface-specific. Various fluorophores are available for the labeling (see Section 3.4), advantageous in multicolor labeling. Another peptide–peptide tag–probe pair based on heterotrimeric coiled-coil formation has also been reported (Tsutsumi *et al.*, 2009).

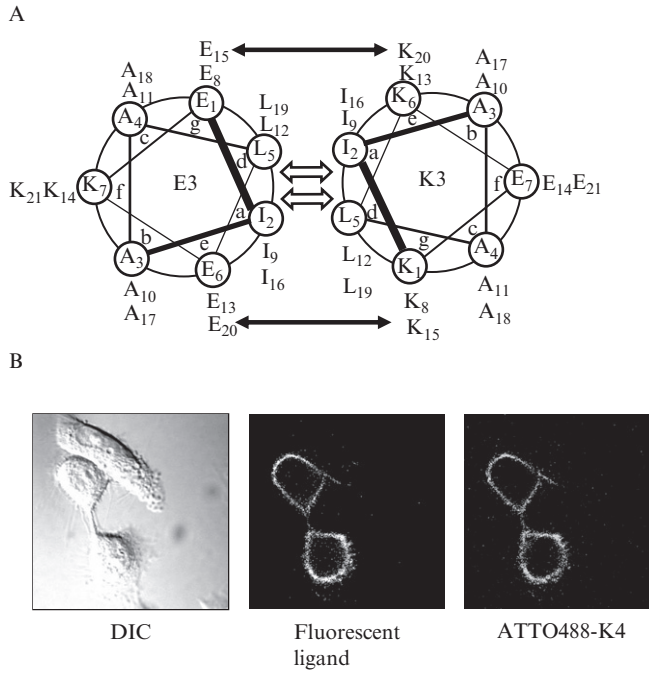

Figure 18.2 (A) Helical wheel representation of E3/K3 coiled-coil heterodimer. Hydrophobic and electrostatic interactions are indicated by white and black arrows, respectively. (B) Confocal imaging of E3-tagged β_2 adrenergic receptors (β_2ARs) transiently expressed in CHO cells. The receptors were colabeled with fluorescent ligands (CA 200689, 10 n*M*) and K4 probes (ATTO488-K4, 10 n*M*) for 10 min. Comparison with the differential interference contrast (DIC) image demonstrates specific labeling of cells that express the target receptors.

3.1. Design of tag-fused membrane proteins

In coiled-coil labeling, the gene of the target protein, which is cloned into mammalian expression vectors, is fused with the E3 sequence as an extracellular tag. Figure 18.3 shows an example of a plasmid map encoding the E3-tagged target protein (β_2AR) and the DNA sequence of the tag. The oligonucleotide encoding the E3 tag ($\sim$100 base pair) can be custom-made (e.g., Invitrogen, synthesis scale of 200 nmol with cartridge purification) and inserted at the N-terminal site immediately after the start codon by standard recombinant techniques. In the design of the tag sequence, a repetitive DNA sequence should be avoided to prevent undesired annealing. This is easily achieved by using different codons for the repeated amino acid sequence $(EIAALEK)_3$.

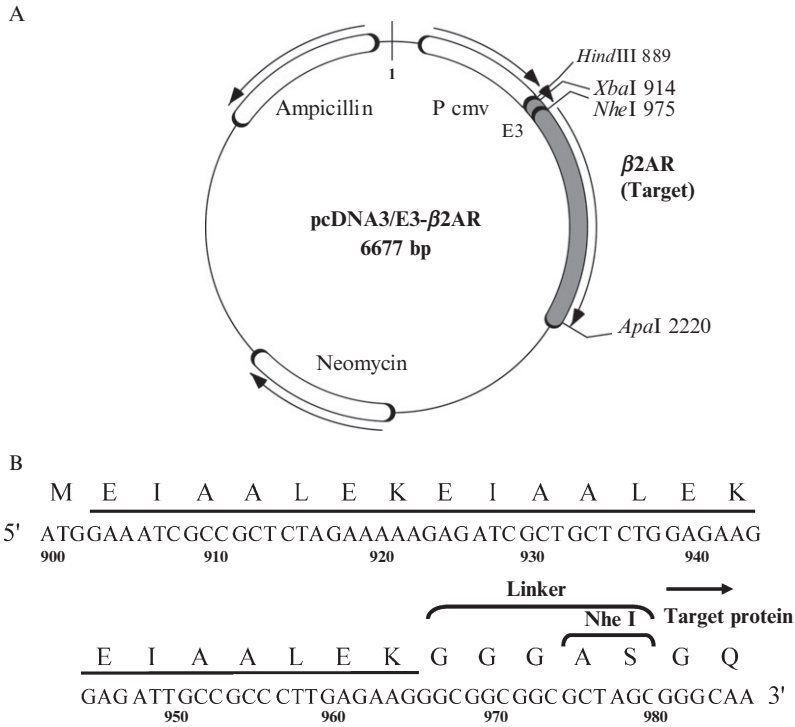

Figure 18.3 **An example of design of the tag-fused receptor. (A) Plasmid map for E3-tagged human β2 adrenergic receptor (β_2AR). Representative restriction sites are shown. (B) DNA sequence for the E3 tag and a linker between the tag and target receptor.**

A spacer (e.g., Gly-Gly-Gly-Ala-Ser) was inserted between the tag and the target protein. When the E3 tag is fused at the N-terminus of a protein that has a signal sequence (e.g., EGF receptors), the tag sequence should be inserted after the signal sequence instead of the start codon.

3.2. Expression of tagged membrane proteins in living cells

A variety of strategies are available to introduce genes into eukaryotic cells. We have used biochemical transfection reagents (Lipofectamine) according to the manufacturer's instructions. Both transient and stable expressions are available by using standard expression vectors. In transient expression, the cells are typically observed 24–48 h after transfection. The transfection reagents should be washed out after transfection (e.g., 5 h for Lipofectamine) and the cells incubated for 1 day to reduce the damage to cell membranes and nonspecific binding of probes.

- *Host cells*: It is important to select appropriate host cells for successful labeling using the coiled-coil method, because the positively charged K probes may nonspecifically bind to the surface of highly negatively charged cells due to electrostatic adsorption. In our experience, CHO cells have negligible nonspecific labeling and are suitable for coiled-coil labeling compared with other cell lines such as HEK-293, COS-7, and PC-12. The K4 probes labeled with negatively charged fluorophores (Alexa 568 and Alexa 647) can decrease nonspecific labeling for HEK 293 cells (K. Kawano *et al.*, unpublished observation).
- *Glass bottom dish*: For fluorescence imaging, the cells are seeded on a ϕ35-mm glass bottom dish 1 day before transient transfection or 1 day before confocal observation of cells stably expressing the target gene. A glass bottom dish coated with Advanced TC™ polymer (#627965 advanced glass bottom; Greiner Bio-One, Frickenhausen, Germany) significantly suppresses nonspecific absorption of the probes on the glass. Other coating reagents (poly-L-lysine and collagen) did not improve the nonspecific binding compared with normal dishes, although normal glass bottom dishes (e.g., IWAKI #3911-035 and Matsunami #D110400) are also available for confocal imaging with coiled-coil labeling.

3.3. Preparation of fluorophore-labeled probe peptides

The K probe peptides are synthesized by a standard 9-fluorenylmethyloxycarbonyl (Fmoc)-based solid phase method (Amblard *et al.*, 2006). After elongation of the peptide sequence from the C-terminus on the resin, the N-terminal amino group can be used as a specific labeling site with amino-reactive fluorophores, followed by detachment of the peptide from the resin and deprotection of side chains by treatment with trifluoroacetic acid (TFA). Procedures for N-terminal labeling with fluorophores are given below.

Dried K4-attached resin ($\sim$1 μmol of peptide, which typically corresponds to 5–10 mg of resin) is placed in a regular 1.5-ml polypropylene tube, and swelled with 500 μl of *N*, *N*-dimethylformamide (DMF) overnight on a shaker. After precipitation of the resin by standing, the DMF is replaced, the resin washed by shaking, and as much of the supernatant removed as possible. The *N*-hydroxysuccinimide ester of fluorophores (1–1.5 μmol or 1 mg) is dissolved in a minimum amount of DMF ($<$100 μl) and added to the resin, followed by gentle stirring with a small magnetic bar for 48 h in the dark. Addition of a base (e.g., 5% *N*, *N*-diisopropylethylamine) may be required for efficient coupling, particularly when the fluorophores are provided as a salt form with acids such as HCl. After the coupling, wash the resin by repeated replacement and shaking for 5 min with organic solvents until the supernatant becomes colorless (typically, DMF 3 $\times$ followed by methanol 5 $\times$). Vacuum-dry the methanol-washed resin for deprotection.

The labeling of fluorophores that degrade in TFA may be difficult. In that case, a Cys residue for specific labeling should be introduced after deprotection of the peptide. Purify the crude peptide by HPLC using common reversed-phase octadecylsilyl ODS columns. Phenyl-based columns (e.g., PLRP-S series; Agilent Technologies) are also useful to separate fluorophore-labeled peptides from unlabeled peptides. After lyophilization of the purified peptide, dissolve it in *distilled water* to obtain a stock solution (CAUTION: do not use buffers for the stock solution). Depending on the attached fluorophore, the probe may not dissolve in water (e.g., the fluorescein-K4 probe aggregates in water). We found that 0.01 *M* NaOH could stably solubilize the fluorescein-K4 probe. Avoid exposure of the probes to light as much as possible. Determine the concentration of the probe by absorbance based on the extinction coefficients of the fluorophores. To avoid significant loss of the probes by adsorption on the tube, the concentration of stock solution should be above 10 μ*M*. To prevent the degradation of the peptide, store small aliquots of the stock solution below −20 °C in the dark. In the case of multicolor labeling, preparation of a premixed probe solution is recommended. In time-lapse imaging, addition of quenchers for reactive oxygen species, such as ascorbic acid and crocetin (Tsien *et al.*, 2006), might be required to suppress the phototoxicity of the probes.

3.4. Labeling of E3-tagged proteins with K probes

Thaw the probe stock solutions and keep them on ice or in a refrigerator. Avoid exposure of the probes to light as much as possible. The thawed solutions should be used within a few days. Take out the glass bottom dish from a CO_2 incubator and replace the culture medium (αMEM or F-12) with 1 ml of PBS (+). When the labeling is performed in medium, the solution should be buffered (e.g., by adding 10 m*M* HEPES). Dilute the stock solution with PBS (+) to obtain a fresh labeling solution (final concentration: typically 20 n*M* K4), which should be prepared *immediately before* application to the cells. Replace PBS (+) with 1 ml of the labeling solution and incubate, for example, for 1–2 min with 20 n*M* probe or for 10–15 min with 2 n*M* probe before starting confocal imaging. If necessary, wash out the probes by replacing the solution with PBS (+) before observation, although background fluorescence from free probes can be negligible compared with that on cell membranes in many cases.

- *Confocal microscopy*: We usually use a 60× Plan Apochromat water-immersion objective lens to obtain confocal images of the target receptors on cell membranes, focusing 2–3 μm above the glass surface. The excitation laser power and sensitivity of the detector should be optimized to

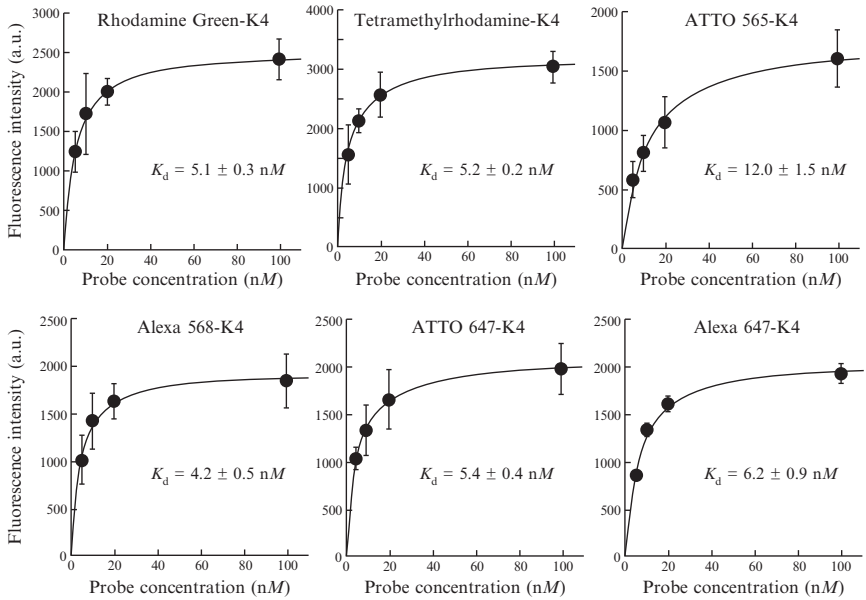

Figure 18.4 **Determination of affinity between the tag and probe. The K4 probes labeled with various fluorophores were added to CHO cells stably expressing E3-β_2ARs at probe concentrations [P] of 5, 10, 20, and 100 nM. Averaged fluorescence intensity (F) at each probe concentration was determined from the confocal images by defining regions of interest at cell membranes ($n = 10$). The dissociation constant (K_d) was obtained from the fitting, $F = F_{max}$*[P]/([P] + K_d), where F_{max} indicates maximal fluorescence intensity.**

avoid rapid photobleaching and saturation of signals. The linearity between the concentration of the probe and detector response can be checked by titrating free probes in water.

Figure 18.4 shows the concentration-dependence of fluorescence intensity on cell membranes for the K4 probes labeled with various fluorophores, quantified from confocal images for E3-β_2ARs stably expressed on CHO cells. The dissociation constants (K_d) for the tag–probe pairs were estimated from the fitting. Most fluorophores (tetramethylrhodamine (TMR), Rhodamine green (RG), ATTO 647, Alexa 568, and Alexa 467) attached to K4 gave dissociation constants of around 5 nM, whereas the attachment of ATTO 565 slightly decreased the affinity ($K_d \sim 12$ nM). Unexpectedly, the negatively charged fluorophore Alexa 647 (−3), which may partially neutralize positive charges of the K4 probe, did not reduce the formation of the coiled-coil. These results indicate that diverse fluorophores are available for the labeling.

4. Applications

4.1. Receptor internalization

Upon the stimulation of cell-surface receptors with ligands, sequestration to endosomes is often observed, which is a mechanism of receptor desensitization (Wolfe and Trejo, 2007). Because receptor internalization is a ubiquitous process irrespective of downstream signaling pathways, it is applicable to the monitoring of activities of a wide variety of membrane receptors including orphan receptors. Fluorescence imaging of receptors fused with fluorescent proteins has been used to visualize the internalization of target receptors (McLean and Milligan, 2000). However, fluorescent proteins inevitably label receptors even in intracellular compartments, which can partially obscure observations of internalization. On the other hand, the coiled-coil method has an advantage for the observation of internalization because of cell-surface labeling. Further, the quickness and reversibility of coiled-coil labeling enable pulse-chase labeling (Fig. 18.5). A shorter K3 probe ($K_d \sim 60$ n*M*) is useful for reversible labeling. After stimulation of the TMR-K3-labeled E3-β_2AR with an agonist, the receptors on the cell (receptors that had not been internalized and newly externalized receptors) could be labeled by the second probe FL-K4 after the washout of TMR-K3 (PBS, 10 times). The absence of TMR fluorescence on the cell surface indicates the reversibility of the labeling (Yano *et al.*, 2008).

4.2. Receptor oligomerization

Protein–protein interactions in membrane environments drive the formation of noncovalent oligomeric structures for membrane proteins that are necessary for function. For example, potassium channels function as tightly assembled homotetramers (MacKinnon, 2003). Although rhodopsin (Bayburt *et al.*, 2007) and β_2AR (Whorton *et al.*, 2007) can function as monomers, many GPCRs are believed to form homo-oligomers and hetero-oligomers with other receptors. Experimental evidence of receptor oligomerization includes the cointernalization and coexpression of receptors (Sartania *et al.*, 2007; Uberti *et al.*, 2005). Also, recent single molecule studies using fluorescent ligands for the M1 muscarinic receptor (Hern *et al.*, 2010) and the *N*-formyl peptide receptor (Kasai *et al.*, 2011) revealed that the receptors diffuse on cell membranes accompanied by transient colocalization with each other, consistent with reversible receptor oligomerization. However, it is not clear from these results whether the receptors come directly into contact with each other or are locally concentrated into membrane domains (Jacquier *et al.*, 2006; Meyer *et al.*, 2006). Further, the relationship between

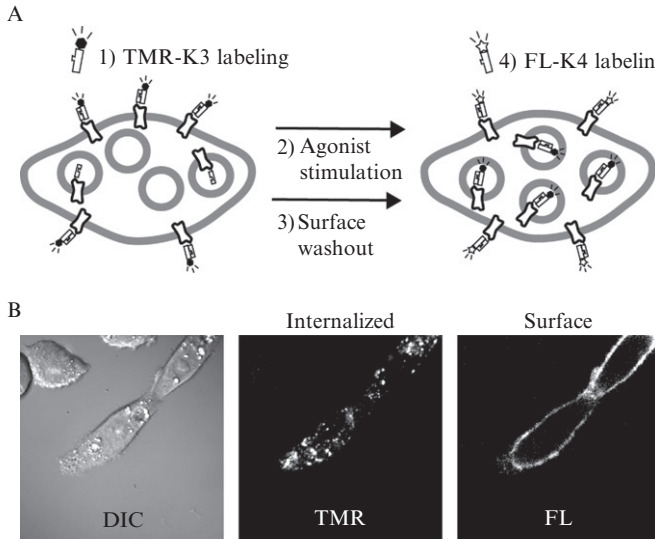

Figure 18.5 Selective labeling of internalized receptors and surface-remaining receptors. (A) The labeling scheme. CHO cells expressing E3-β_2AR were labeled with TMR-K3 (60 n*M*) for 2 min, and then stimulated with the agonist isoproterenol (10 μ*M*) for 5 min. After the cells were washed with PBS, fluorescein-K4 (20 n*M*) was added and the cells were observed by confocal microscopy. (B) Images for DIC, TMR, and FL channels.

oligomerization of receptors and biological function is unknown, although pharmacological studies indicate the presence of allosteric regulatory mechanisms for GPCRs (Han *et al.*, 2009). Detection of oligomerization by resonance energy transfer is direct evidence of close contact between receptors within ca. 5 nm. FRET and bioluminescent resonance energy transfer studies using GPCR fused with fluorescent/luminescent proteins have concluded that most receptors form oligomers. However, quantitative analysis of the oligomerization is not easy, as exemplified in controversial results for self-association of β_2AR reported from different research groups (Bouvier *et al.*, 2007; James *et al.*, 2006). Development of an improved method of analysis for receptor oligomerization is important.

Taking advantage of the ease of multicolor labeling of the coiled-coil method, we measured the self-association of metabotropic glutamate receptors (mGluRs), a class C GPCR that forms dimers (Maurel *et al.*, 2008), by FRET from RG to TMR (critical transfer distance: ~55 Å). To clarify fluorescence from the donor and acceptor, spectral imaging was performed using a Nikon C1Si confocal microscope. Figure 18.6A indicates fluorescence spectra for mGluR-expressing CHO cell membranes doubly labeled with RG-K4 and TMR-K4 excited at 488 nm, at which the donor RG is selectively excited. In addition to the donor RG fluorescence (~530 nm),

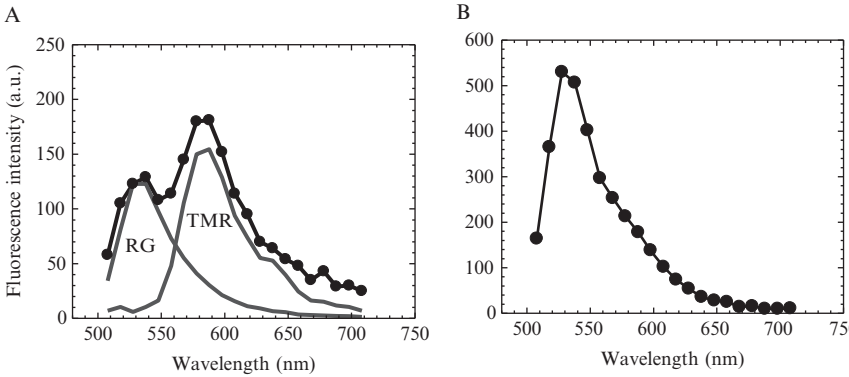

Figure 18.6 **Detection of receptor oligomerization by FRET using fluorescence emission spectra in CHO cells expressing (A) E3-tagged metabotropic glutamate receptors (E3-mGluRs) and (B) E3-tagged glycophorin A G83I mutant. The target proteins were doubly labeled with a 1/1 mixture of RG-K4 and TMR-K4 (25 n*M* each), the donor RG was excited at 488 nm, and spectral images in the membranes were obtained. The fluorescence spectrum originating from direct excitation of TMR has been subtracted for clarity. In the spectrum for mGluR (A), contributions from RG and TMR fluorescence are also shown, which were determined by a least square fitting using the reference spectra.**

sensitized emission from the acceptor TMR (~580 nm) was clearly observed, indicating strong FRET by oligomerization of the receptors. On the other hand, a monomeric control protein (Glycophorin A G83I mutant) did not show any sensitized emission (Fig. 18.6B). These results demonstrate that correct evaluation of receptor oligomerization is possible using coiled-coil labeling.

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