

then bind to a TRFRET-labeled antibody that targets the phosphorylated species. Addition of a streptavidin-tagged acceptor molecule, such as the protein Allophycocyanin, a fluorescent protein, provides the necessary donor/acceptor pairing, which will undergo a long-lived FRET interaction upon irradiation. Test compounds that inhibit kinase activity will block phosphorylation, leading to a decrease in the formation of the FRET donor/acceptor complex, decreasing the intensity of the assay signal in a quantifiable manner.⁵³

Downstream cellular activity can also be monitored with TRFRET technology (Figure 4.22). As mentioned in Chapter 3, activation of a GPCR signaling pathway can lead to a number of downstream cellular events

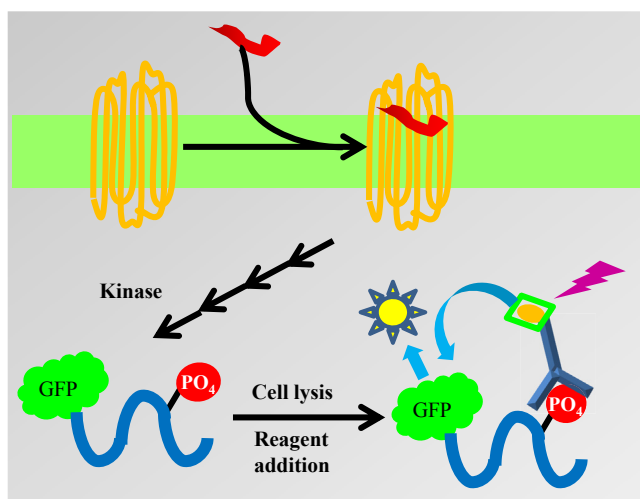


FIGURE 4.22 GPCR activation by the natural ligand or suitable agonist induces downstream events culminating in the phosphorylation of a GFP-labeled substrate. Cell lysis followed by the addition of an antibody tagged with a TRFRET fluorophore leads to close association of the donor/acceptor pair and FRET signaling. Antagonist will block induction of the downstream events leading to FRET emission by preventing GPCR activation by the natural ligand.

including the activation of various kinases. If a substrate is known to be phosphorylated by a kinase that is activated as a downstream event as a consequence of GPCR activation, then tagging the substrate with a fluorescent label, such as green fluorescent protein (GFP) provides an opportunity to establish a TRFRET assay to measure GPCR activation in cellular system. This requires stable cell lines that produce the substrate with a GFP tag in a way that does not interfere with normal kinase activity. Activation of the GPCR signaling pathway with the natural ligand or a synthetic agonist will produce the phosphorylated substrate. Cell lysis and addition of a lanthanide-tagged antibody TRFRET reagent and irradiation with the