a fluorescently tagged protein provides half of the requisite donor/acceptor pair. If the second half of the FRET pair were a lipid soluble compound that could move freely through the bilayer, then the presence or absence of a FRET interaction could be controlled by the position of the lipid-soluble compound relative to the tagged protein. If the lipid-soluble compound is close to the outside surface of the cell, then FRET occurs. On the other hand, if the lipid-soluble compound is close to the inside surface of the cellular membrane, then no FRET interaction occurs as the distance between the donor and acceptor is too great. Thus, controlling the location of the lipid-soluble acceptor molecule is the key to successfully using FRET technology in this type of system. When the lipid-soluble acceptor is charged, alteration of the cell membrane potential will control the position of the acceptor molecule (Figure 4.17). Since membrane potentials are controlled by the action

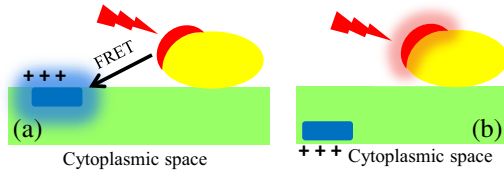

FIGURE 4.17 A cell surface protein (yellow) tagged with a FRET donor (red) can be coupled with a charged, lipid-soluble FRET acceptor (blue) that is sensitive to electrical charge. (a) When the exterior of the cell is positively charged, a negatively charged FRET acceptor will move to the exterior of the lipid layer, inducing FRET emission. (b) On the other hand, reversal of the membrane potential will cause the FRET acceptor to move toward the cytoplasmic space, preventing FRET emission.

of ion channels, this assay system can be used to monitor ion channel activity and screen for compounds that modulate ion channel function.[40]

The combination of FRET technology and fluorescent proteins provides a method for studying GPCR activity. In this case, the donor/acceptor pair is derived from genetically altered versions of the fluorescent proteins originally isolated from the jellyfish *Aequorea victoria*.[41] While the first fluorescent protein isolated produced a green color and was cleverly named green fluorescent protein (GFP), mutations in the protein sequence have provided access to fluorescent protein that provide a signal in different parts of the light spectrum, and pairs of overlapping absorbance/fluorescence spectra can be employed as FRET pairs. Importantly, in most cases, tagging of functional proteins with the jellyfish-derived fluorescent proteins has minimal impact on protein function, and this provides useful tools for monitoring protein function and location.[42] In the case of GPCRs, activation of the signaling pathway could be monitored by tagging the various subunits of the G-protein. For instance, tagging the Gα subunit with yellow fluorescent protein (YFP) and the Gγ subunit with a cyan fluorescent protein (CFP) would provide a donor/acceptor pair capable of undergoing a FRET interaction, provided they are