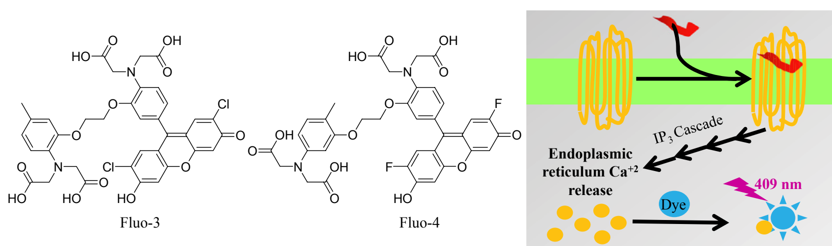

FIGURE 4.26 The presence of Ca^{+2} increases the fluorescent intensity produced upon irradiation of Flou-3 or Fluo-4. When a cell is preloaded with calcium-sensitive dyes, activation of a GPCR-IP_3 -mediated signaling event leads to downstream release of Ca^{+2} (orange circles) from the endoplasmic reticulum. The interaction between the Ca^{+2} and the dye produces a fluorescent signal upon irradiation with light. Changes in fluorescent signal intensity can be used as a means of identifying compounds that are functionally active at a target GPCR.

(Figure 4.26).[60] Both of these dyes fluoresce upon irradiation with a light source, such as an Argon laser (488 nm), but more importantly, the intensity of the fluorescent signal increases in the presence of increasing Ca^{+2} concentration. This phenomenon can be harnessed to monitor biological events that lead to changes in calcium concentration. GPCR signaling that proceeds through the IP_3 signaling cascade, for example, can be monitored, as activation of this signaling cascade by an agonist leads to the release of Ca^{+2} from cellular storage. In the presence of a fixed concentration of the aforementioned dyes, this increase in Ca^{+2} concentration leads to increased fluorescent signal intensity that can be monitored to determine the ability of test compounds to acts as functional agonists for the GPCR in question. In a similar manner, antagonists will block the ability of the GPCR's natural ligand to activate the signaling cascade, preventing calcium release from cellular stores. This also prevents the increase in fluorescent signal intensity that would have been observed with the natural ligand, providing a means for assessing a test compound's ability to behave as a functional antagonist for a particular GPCR.[61]

Of course, in order for these assays to function properly, the fluorescent dye must be capable of entering the cell and have little to no impact on other cellular functions. Also, like any other fluorescent system, interference from background fluorescence can be an issue. An alternative system, the aequorin-based assays, needs neither dyes nor outside irradiation in order to monitor calcium concentration changes related to cellular activity. These assays depend on the bioluminescence induced by the presence of Ca^{2+}, coelenterazine, and apoaequorin, the apo-enzyme of aequorin. This 22 kDa photoprotein was originally isolated from bioluminescent jellyfish, specifically the *Aequorea victoria* jellyfish, and is responsible for the blue light emitted by these creatures (Figure 4.27).[62] In the absence of Ca^{2+},