

Since the energy input wavelength, donor fluorescence, and acceptor fluorescence all occur at different wavelengths, it is possible to measure each and monitor the course of events in a properly arranged biological system. Early efforts to identify HIV protease inhibitors, for example, benefited from the application of FRET technology. Scientists at Abbot Laboratories identified a small peptide substrate of HIV protease, Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln, that is cleaved at the Tyr-Pro bond. The peptide chain was converted to a FRET donor/acceptor system by attaching 5-(2'-aminoethyl)aminonaphthalene sulfonic acid (EDANS) to the carboxy terminus (Gln) of the peptide and 4'-(Dimethylamino)-4-azobenzenecarboxylic acid (DABCYL) to the amino-terminus (Ser) of the peptide. In isolation, excitation of EDANS by irradiation at 340 nm results in a fluorescent response at 490 nm. However, within the context of the aforementioned modified peptide, the normal fluorescent response by EDANS is quenched by a resonance energy transfer to DABCYL. In the presence of HIV protease, however, the modified peptide is cleaved, separating the donor/acceptor complex, allowing EDANS to fluoresce at 490 nm. Thus, the enzymatic activity of HIV protease can be quantified by monitoring the intensity of EDANS fluorescence over time. HIV protease inhibitors will prevent cleavage of the donor/acceptor complex, preventing EDANS fluorescence in a quantifiable manner (Figure 4.16).³⁹

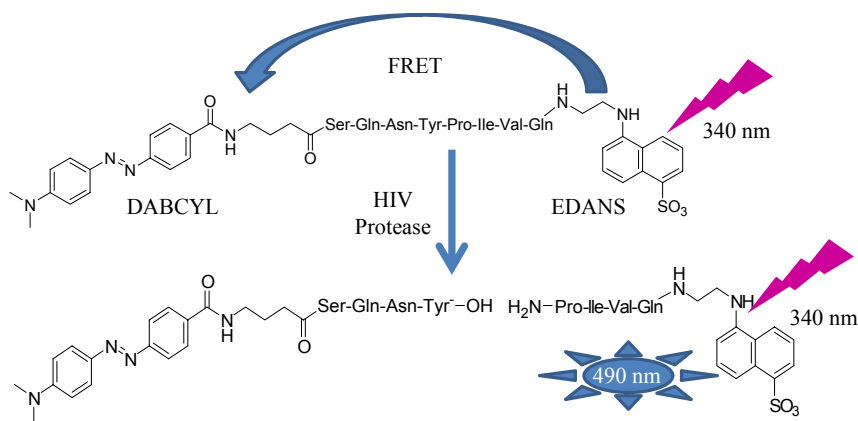


FIGURE 4.16 The seven amino acid HIV protease substrate maintains the EDANS/DABCYL donor/acceptor pair within the minimum distance required for energy transfer, which quenches the fluorescent emission of EDANS (490 nm). Peptide cleavage by HIV protease separates the pair, allowing EDANS fluorescence. HIV protease inhibitors can be identified and compared by monitoring changes in EDANS fluorescence intensity.

FRET technology has also been employed in the study of membrane potentials and ion channels. Labeling of the outside of a cell membrane with