application by consumers in need of information on circulating levels of human chorionic gonadotropin. Increasing concentrations of this hormone can be detected via sandwich ELISA using properly designed urine test strips, and this forms the basis of the common in-home pregnancy test kits.[23]

There are many variations of ELISA techniques and their application has extended well beyond the field of drug discovery. *In vitro* diagnostic kits are common place in medical laboratories,[24] and the food industry employs ELISA methods to detect potential food allergens such as peanuts in food products.[25] Some types of illicit drugs can also be rapidly detected using ELISA systems.[26] It is also worth noting that the basic principles of ELISA technology (antibody/antigen complexes designed for detection) have been successfully employed in non-ELISA assays, making the original development of ELISA much more important to the drug discovery process.

FLUORESCENCE-BASED ASSAY SYSTEMS

Although the detection of signals created by the interaction of radiolabeled compounds and an appropriate scintillator can be an effective means of measuring biological function, the inherent limitations and the desire to avoid the generation of radioactive waste material led to the development of alternative detection systems. A number of modern assay systems take advantage of the fluorescent properties of selected classes of compounds. In general, fluorescence occurs when a substance absorbs light energy at one wavelength and then undergoes a near-immediate release of the energy at a lower wavelength (lower energy) in the visible spectrum due to non-radiative energy loss to the lowest vibrational energy level of the excited state. The difference in energy between the absorbed and emitted light is referred to as the Stokes shift (Figure 4.11).[27] The emission of

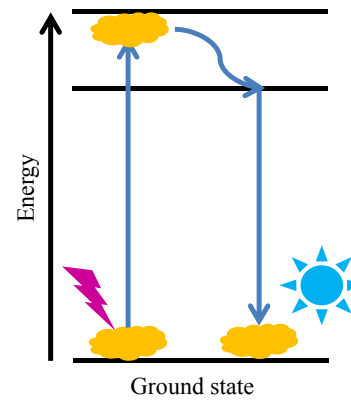

FIGURE 4.11 When a photon of energy from a light source is absorbed by a fluorophore (a compound that undergoes fluorescence, orange), an excited energy state is created. Relaxation of the excited state back to the ground state occurs with the emission of a photon at lower energy.