If excitation is achieved through the use of polarized light, the nature of the ensuing fluorescent emission will depend upon the molecular motion available to the fluorophore. If the fluorophore is immobilized, then the fluorescent emission will remain polarized and occur in the same plane as the original excitation source. On the other hand, if the fluorophores are in solution, the level of polarization of the emitted light will decrease in a predictable manner based on the size of the fluorophore. When the fluorophore is small, such as a potential drug molecule, molecular motion in solution is fast relative to the time interval between excitation and emission, leading to randomization/depolarization of the fluorescent signal. If, however, the fluorophore is part of a larger complex, such as a ligand/protein complex, molecular motion is substantially slower than the ligand alone. In this scenario, there is less opportunity for randomization/depolarization of the emission signal, leading to an increase in the amount of polarized emission relative to the free ligand emission. Thus, quantification of the ratio of polarized versus depolarized emission can be used to measure binding interaction and monitor biological processes (Figure 4.12).[33]

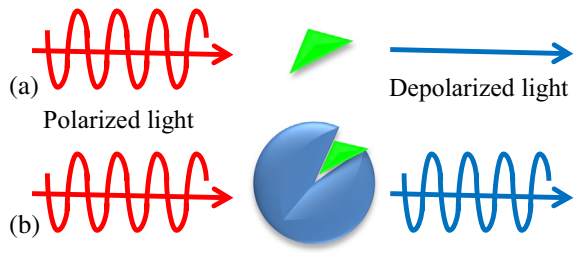

FIGURE 4.12 (a) Irradiation of an unbound small molecule fluorophore (green) in solution with polarized light produces depolarized emission. (b) Interaction of the same small molecule with a biomolecule (blue) will preserve polarization upon emission.

In practice, fluorescence polarization assay techniques have been applied to a wide range of biological interactions. Monitoring the enzymatic reactions, for example, can be accomplished by monitoring either increases or decreases in polarization depending on the nature of the enzyme and assay design. Screening for protease inhibitor can be accomplished with a fluorescently tagged protease substrate. In the absence of the protease, a large protein tagged with a fluorophore will rotate relatively slowly in solution. Upon excitation with polarized light, polarization will be maintained upon emission, leading to a higher signal. Protease activity will cleave the protein into smaller fragments that rotate more quickly in solution. Emission induced by excitation with polarized light