It is also possible to design FP assays capable of detecting changes in protein/protein interactions and DNA/protein interactions (Figure 4.15). Labeling one of the two partners in an interaction of macromolecules with

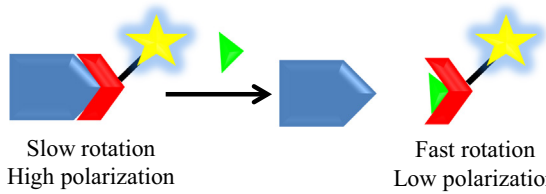

FIGURE 4.15 Compounds that disrupt the interaction between two macromolecules, one of which is fluorescently labeled, will cause a loss of polarized emission in a fluorescence polarization assay. Disruption of the macromolecular complex decreases the size of the tagged material, increasing its rotational speed and loss of polarization upon emission.

a fluorescent tag provides the necessary signal source, while the inherent change in molecular weight associated with formation and disassociation of the macromolecular complex of interest provides the change in size required to measure a difference in polarization of a fluorescent signal. Compounds that interfere with the formation of the macromolecular complex will produce changes in the observed FP in a quantifiable manner that can be used to determine binding constants of test compounds.[36]

Fluorescence Resonance Energy Transfer (FRET)

A number of biological assay have been designed to take advantage of the Theodor Förster's discovery of fluorescence resonance energy transfer.[37] In this process, a fluorescent donor molecule absorbs electromagnetic energy, and this energy is transferred to a nearby fluorescent acceptor molecule, which then fluoresces at a lower wavelength than the donor molecule. The exchange of energy between a donor and an acceptor pair occurs without the generation of thermal energy, requires no molecular collision, but is distance-dependent. Much like scintillation proximity assays, the amount of energy transferred, and thus the level of fluorescents, decreases as the distance between the donor and acceptor molecules increases. The distance requirements are larger than interatomic distance, usually on the order of 10–100 Å, depending on the nature of the donor/acceptor pair. In addition, there must be an overlap between the donor molecule's fluorescent spectrum and the acceptor molecule's absorbance spectrum in order for resonance energy transfer to occur. In effect, the fluorescent signal that would normally be emitted by the donor upon irradiation with a lamp or laser is decreased at the expense of the acceptor's fluorescent signal.[38]