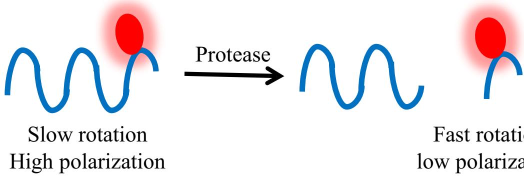

FIGURE 4.13 Polarized irradiation of a fluorescently tagged protein in solution will produce a fluorescent signal that maintains the polarization to a greater extent than fragments of the protein produced by the action of a suitable protease. The degree of depolarization in the presence of candidate compounds can be used to identify enzyme inhibitors.

will produce fluorescence, but the level of polarization will be decreased (Figure 4.13). Compounds that inhibit protease activity will decrease the rate of loss of polarization, and monitoring these changes allows quantification of the inhibitory activity of test compounds.[34]

Combinations of FP methods and antibody technology have provided assays capable of monitoring phosphorylation of substrates by various kinases (Figure 4.14). Substrate peptides labeled with a suitable

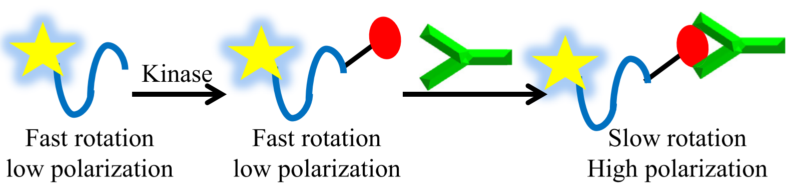

FIGURE 4.14 A fluorescently tagged peptide (blue) will rotate quickly in solution, leading to depolarization of emitted light as a result of irradiation with polarized light. Phosphorylation by a kinase in the presence of a suitable antibody (green), however, decreases rotation al speed. This preserves polarization upon emission.

fluorophore will emit a depolarized fluorescent signal upon irradiation with a polarized source. Kinase activity to produce the corresponding phosphorylated product in the presence of an antibody designed to bind with the phosphorylated product would produce an antibody/antigen complex that is significantly larger. The increased size decreases the rotational speed of the fluorophore in solution, leading to increased retention of polarization upon emission. In the presence of a kinase inhibitor, the rate of formation of the antibody/antigen complex is slowed, decreasing the rate of increase in the polarized fluorescent signal, providing insight into the potential utility of test compounds as kinase inhibitors.[35]