interaction is one of the strongest non-covalent interactions known, with a dissociation constant in the femtomolar range, making this bonding interaction virtually inert under conditions generally used for biological screening. Binding of a protein to the surface of a 96-well plate can be accomplished by taking advantage of the strength of this bond and the presence of four biotin binding sites on streptavidin (Figure 4.6). In this instance, the plastic surface

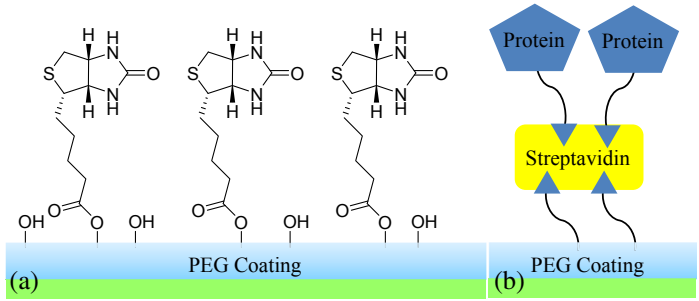

FIGURE 4.6 (a) Polyethylene glycol coating of the surface of a microtiter plate provides open hydroxyl groups that can be capped with biotin. (b) Streptavidin binds to the bicyclic portion of biotin (blue triangle) linked to the surface of the microtiter plate. A second biotin-tagged molecule will also bind to the streptavidin, creating a labeled surface suitable for use in various assay platforms.

of a 96-well plate could be covalently linked to a biotin with an ester or amide bond. Addition of streptavidin to the 96-well plate would lead to binding of streptavidin to biotin on the surface of the 96-well plate. A second protein suitably functionalized with a biotin molecule on the external surface of the protein, away from the active site, could be added to the 96-well plate and would bind to the streptavidin coating. The strength of the streptavidin/biotin interaction creates a surface on the 96-well plate that is effectively coated with the protein of interest that will stay attached to the surface of the plate under the vast majority of biological screening conditions. Alternatively, a streptavidin-labeled protein in solution could be brought together with a biotin-tagged molecule also in solution in order to generate an assay signal that can be used as a biological readout to determine the biological activity of test compounds (e.g., FRET, TRFRET, SPA assays systems).

BIOCHEMICAL VERSUS CELLULAR ASSAYS

The vast majority of modern screening assays can be divided into two basic categories, biochemical and cellular assays. Each has advantages and disadvantages and the choice of which is appropriate for a given study is largely dependent on the goal of the program. Typically, drug discovery