associated with the surface plasmon resonance that can be quantified to measure binding affinities. In practice, this is accomplished by exposing the tagged-side of the thin metal film to a continuous flow of a solution containing potential binding partners in a flow cell. The application microfluidics technology provides a means for miniaturization of this technology to create "labs on a chip." The most common drug discovery application of surface plasmon resonance is the Biacore assay system. In this particular variation, a gold surface is coated with a layer of carboxymethylated dextran, creating a hydrophilic environment that preserves the non-denatured state of attached biomolecules. Although the throughput of these systems is not as high as other methods, the high degree of sensitivity has led to wide adoption of Biacore assays in the drug discovery process (Figure 4.38).[85]

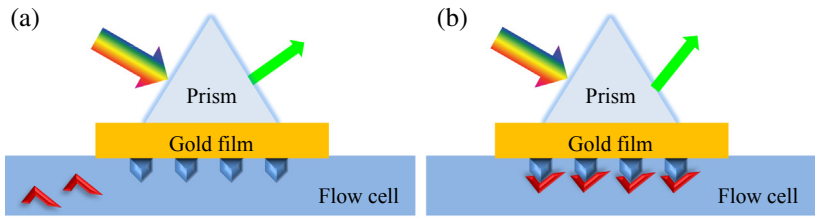

FIGURE 4.38 (a) In the absence of a binding partner, a tagged gold biosensor will undergo a surface plasmon resonance at a specific angle. (b) Addition of a binding partner via a flow cell will change the angle of reflected light associated with the surface plasmon resonance in a concentration-dependent manner.

ELECTROPHYSIOLOGICAL PATCH CLAMP

As discussed in Chapter 3, the flow of electrical currents and creation of voltage gradients across cellular membranes are critical to a wide range of cellular functions. Ion channels are an important aspect of these processes, and as such, monitoring their activity can provide a great deal of insight into the biochemical processes for both normal and pathological conditions. Earlier sections of this chapter have described fluorescent-based systems that are capable of providing indirect evidence regarding ion channel activity (e.g., calcium-sensitive dyes, charged lipid-soluble FRET acceptors), but none of the methods discussed thus far are capable of providing a direct measurement of channel activity, as each uses a surrogate marker (a fluorescent signal). To date, electrophysiological patch clamp remains the gold standard for assessing ion channel activity, as it is the only available method capable of providing direct measurement of ion flow through cellular