already been fully activated.[11] It is also worth noting that if two different tissues express the same receptor, but with different receptor reserve levels, then the impact of a set concentration of an agonist may have different physiological outcomes. Consider, for example, the case in which one cell type has a no receptor reserve (the number of receptors is equal to the level of signal transduction capacity) and a second cell type has a 100/1 ratio of receptor to signal transduction capacity. If there are no other differences, then full response requires 100% occupancy for the first cell type, but only 1% occupancy for the second cell type. In other words, an agonist will appear to be more potent in cell with higher receptor reserve. This can be an advantage if target cells have high receptor reserve as compared to other cells containing the same receptor, or a disadvantage if the opposite is true. Also, it is important to consider that receptor overexpression in artificial cell lines can lead to higher receptor reserve than seen in physiologically relevant systems, potentially leading to overstatement of the potency of test compounds. If, for example, an overexpressing cell line used for an assay has a 100/1 ratio of receptor to signal transduction capacity, while the naturally occurring cell is closer to 10/1, then compounds screened could appear to be nearly 10 fold more potent in the artificial cell line.

STREPTAVIDIN AND BIOTIN

In many cases, it is necessary to create a linkage point between materials that are useful in the elucidation of biochemical process without significant chemical modifications. Binding proteins to the surface of a 96 plate, for example, can simplify high throughput screening procedures, but chemical reaction required for attachment of the protein to the surface of a plate are not always conducive to retaining activity of the protein. The exceptionally strong interaction between biotin and the protein streptavidin (Figure 4.5[14]) is often employed in biotechnology in order to create stable, strong, non-covalent interactions.[15] The streptavidin/biotin binding

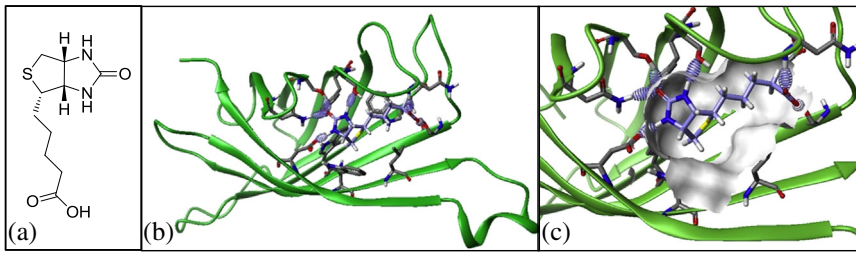

FIGURE 4.5 (a) Biotin (b) Crystal structure of Streptavidin monomer bound to biotin. (c) Close up image of biotin binding site. Hydrogen bonds between biotin and the protein backbone are indicated by blue ellipses and the surface of the binding site is designated by the gray surface (RCSB file 3RY2).