ribosome and is being synthesized (or translated). In this latter case, the chemical modifications are referred to as cotranslational modifications (Fedorov and Baldwin, 1997). However, for the purpose of this chapter, we will simply consider all types of chemical changes to a biopharmaceutical's polypeptide chain(s) inside the cell (whether they occur during the synthesis or after the complete synthesis of that polypeptide) and even outside the cell as PTMs.

Most of the *in vivo* covalent PTMs found on a biopharmaceutical result from enzymatic reactions that usually add a simple chemical group to different amino acid side chains in the polypeptide chain (e.g., phosphate, sulfate; see Figure 2.5A). Since the diversity of different simple chemical groups that can be added is rather large (Walsh, 2006a; Walsh and Jefferis, 2006; Walsh et al., 2005), this is a significant source for generating heterogeneous biopharmaceuticals. In some cases, however, the type of chemical group that is added constitutes a basic collection of somewhat similar building blocks (e.g., monosaccharides) that can be covalently strung together in different ways to generate their own unique source of complexity. This type of PTM is exemplified by a process called glycosylation where a wide range of uniquely linked monosaccharides can give rise to structures called oligosaccharides (which corresponds to a collection of typically 3–9 monosaccharides chemical linked in varying complex configurations) that can be found attached to a given site(s) on the polypeptide chain(s) of a biopharmaceutical during its production inside a cell (Kyte, 1995; see Figure 2.5B and Section 2.6.1.2 for further discussion on this topic). In other cases, covalent PTMs can involve the cleavage of the polypeptide (Walsh, 2006b; see Figure 2.5C) or the formation and cleavage of intrachain disulfide bonds involving two cysteine amino acids within the same polypeptide chain (see Figure 2.5D) or interchain disulfide bonds also involving two cysteine amino acids located in a different polypeptide chain of the biopharmaceutical. In the case of forming/cleaving disulfide bonds, more complex situations can arise that involve a process called disulfide scrambling (Lu and May, 2012; Wang et al., 2011), as illustrated in Figure 2.5D where the disulfide scrambling between two intrachain disulfide bonds in a biopharmaceutical form two different intrachain disulfide bonds (see Section 2.6.1.3 for further discussion of this topic).

A critical feature of these covalent PTMs is that they can be accompanied by changes in the HOS and/or surface properties of the biopharmaceutical. Such changes can serve important functional roles in controlling the biological activity and therefore the therapeutic activity of the biopharmaceutical via interactions that the biopharmaceutical will have with other biological molecules when injected into a patient (see Chapter 7 in this book). In other cases, however, PTMs simply constitute a form of degradation (especially those PTMs that occur outside the cell). In this latter form of PTMs, the resulting changes in the HOS and/or surface properties of the biopharmaceutical serve no useful function. More importantly such PTMs could cause adverse effects (e.g., aggregation), which can induce life-threatening immunogenicity issues (Filipe et al., 2010; Rosenberg, 2006) or alter cellular function that can lead to fatal disease states (Bucciantini et al., 2002; Dobson, 2001) (also see Section 2.9).

Once a biopharmaceutical makes its way outside the cell, it is still susceptible to chemical (covalent) and physical (noncovalent) modifications. These extracellular or *in vitro* PTMs correspond to a range of degradation processes that are predominately