physical changes that alter the noncovalent secondary bonds responsible for the 3D folding and stabilization of a biopharmaceutical). These additional physicochemical changes, along with the sequence of amino acids, constitute a biopharmaceutical's final *total* structure. However, the presence of these additional physicochemical structural changes does not necessarily occur in every molecule of a biopharmaceutical. In fact, each structural change is typically distributed independently among the biopharmaceutical molecules, such that the percentage of biopharmaceutical molecules with each of these additional changes can vary greatly, ranging from every biopharmaceutical molecule having all of the physicochemical changes observed in a biopharmaceutical sample to some biopharmaceutical molecules having only a partial fraction of the total observed changes and others having none of the observed changes.

In addition, a number of chemical structure modifications observed on biopharmaceuticals can occur at multiple sites on a given biopharmaceutical molecule, offering another route for distributing these structural alterations. Coupling all of this with a certain level of variability in these distributions of structural changes in biopharmaceutical molecules on a lot-to-lot basis, and with the multitude of different types of changes that can occur to a biopharmaceutical, gives rise to many different subpopulations or "drug-product variants" (as simply illustrated in Figure 2.4 A through C). The end result is that a biopharmaceutical can be considered to be a heterogeneous mixture of protein molecules (or proteoforms; Smith and Kelleher, 2013) in comparison to a pharmaceutical, as shown experimentally in Figure 2.4D using a modern separation technology called capillary electrophoresis (CE). In many cases, this heterogeneity of biopharmaceuticals is frequently referred to as microheterogeneity owing to the relatively small size of these additional chemical changes or the area of a biopharmaceutical that undergoes a physicochemical change, especially in comparison to the entire size of the biopharmaceutical molecule.

This heterogeneity or wide collection of drug-product variant forms of a biopharmaceutical can be broken into two general major classes called *drug-product-related substances* (which are comparable to the main form of the drug in terms of potency and safety) and *drug-product-related impurities* (which are not comparable to the main form of the drug in terms of potency and safety). It is thus this additional fine-detail structural complexity and its variability on a lot-to-lot basis (which gives rise to the structural heterogeneity of an innovator's biopharmaceutical) that a biosimilar manufacturer must *uncover*, and then *duplicate* and *control*, to successfully develop a biosimilar.

2.4 POSTTRANSLATIONAL MODIFICATIONS

In the previous section, the discussion focused on the additional structural changes that can occur to the polypeptide chain(s) of a biopharmaceutical during its production. Most of these changes are due to covalent (chemical or primary structural) modifications that occur *in vivo* (inside the cell) after the polypeptide chain is synthesized and released from the ribosome, and they are referred to as posttranslational modifications (PTMs). It should be noted, however, that some of these covalent modifications can actually occur while the polypeptide chain is still attached to the