the target window of biosimilarity and tell a biosimilar manufacturer what physicochemical attributes need to be changed and how close or far away those attributes are from that target window (thus guiding the biosimilar manufacturer in its attempts to find and produce a biosimilar with specific physicochemical attributes that will be highly similar to its RP). Unfortunately, however, analytics cannot tell us how to make those changes or what exactly needs to be changed in the manufacturing process so that the resulting biosimilar produced will be highly similar to the RP. That is a totally different problem and challenge.

2.6.1 Analytics at the Biochemical Level: Primary Structure

In dealing with the daunting challenges of characterizing the biochemical heterogeneity of a biopharmaceutical at the level of its primary structure, today's biopharmaceutical scientists are heavily dependent on separation technologies that are combined with various types of physicochemical detectors. The most prominent of these separation techniques include liquid chromatography (LC) (Fekete and Guillarme, 2014; Fekete et al., 2012, 2015; Haverick et al., 2014; Reusch et al., 2015a; Sandra et al., 2014) (which can separate biopharmaceuticals based on their chemical composition and spatial arrangement of those chemical groups on the biopharmaceutical's exposed surface in combination with the biopharmaceutical's overall size and shape), electrophoresis (Anderson et al., 2012; Berkowitz et al., 2005; Fekete et al., 2013; Moritz et al., 2015; Rustandi et al., 2008; Tamizi and Jouyban, 2015) (which can separate biopharmaceuticals on the basis of their net charge, given their physicochemical environment, in combination with the biopharmaceutical's overall size and shape), and MS (Kaltashov et al., 2012; Leurs et al., 2015; Zhang et al., 2009) (which separates biopharmaceuticals simply, in most cases, on the basis of their mass-to-charge ratio, *m*/*z*). Using these separation modes independently or in appropriate combinations with various detectors offers significant capabilities in revealing the underlying fingerprint heterogeneity in the physicochemical structure and properties of biopharmaceuticals. Such a situation is shown in Figure 2.4D where capillary zone electrophoresis (CZE, a mode of CE) in combination with simple UV detection was used to fractionate an intact biopharmaceutical (IFNβ) under conditions capable of maintaining the native-like structure of this biopharmaceutical to reveal its underlying microheterogeneity. The complex electropherogram shown in Figure 2.4D is due predominantly to the primary structural differences (in terms of charge) present and distributed among the biopharmaceutical molecules; however, additional separation is achieved through the contributing factors arising from a biopharmaceutical's HOS, for example, size and shape (or hydrodynamic properties).

In the case of MS, the separation and analysis capability that it can provide are tightly linked. This unique feature of MS allows it to stand on its own as an especially powerful independent analytical separation-analysis tool. However, when MS is coupled to LC or CE to add multidimensional separation capability to enhance its ability to fractionate complex mixtures, some of the most powerful separation-analysis tools feasible for characterizing and analyzing biopharmaceuticals are created (see Figure 2.8). Such a multidimensional arrangement of hyphenated separations and analysis capabilities now dominates the landscape of biopharmaceutical