alteration that does occur to the HOS of the biopharmaceutical samples when they are in mass spectrometer's gas phase, the resulting alterations to these samples should be the same. Consequently, the resulting charge state distribution (and gas-phase separation profile in the case of IM-MS) of the samples being compared should be highly similar if the HOS of the biopharmaceutical samples entering the mass spectrometer were highly similar to each other to begin with. Any difference that is discerned between the MS outputs from the different samples being compared would likely be indicative of a difference in the starting HOS of these samples (again assuming that all samples are in the same matrix, are processed under identical conditions and that MS experimental conditions used in these assessments are also the same). Consequently, the act of conducting these types of comparisons in a gas-phase environment of the mass spectrometer effectively implies the application of an approach similar to that discussed in Section 2.6.2.1 and illustrated in Figure 2.11, where a stress is applied to help facilitate a comparison. Since the temperature and voltage of the native MS can be controlled, additional variables for applying different levels of stress can readily be changed to fine tune this approach in challenging the samples being compared to help reveal their potential underlying HOS differences.

2.6.2.2.3 Antibody Array Footprinting to Assess HOS

In Section 2.6.2.2.1, the discussion on footprinting techniques called H/DX-MS and covalent labeling MS made use of MS to monitor the labeling process and assess what parts of the biopharmaceutical's HOS were potentially different. Recently, an interesting alternate form of protein footprinting has been introduced that avoids the need of MS. This relatively new footprinting technique relies on using specifically designed collections (kits) of antibodies, referred to as antibody arrays (Wang et al., 2013) or protein conformational arrays (Davies et al., 2015), as a labeling reagent. Each kit of antibodies is unique to a specific biopharmaceutical. This form of footprinting offers an interesting and simple approach for allowing anyone to conduct high-resolution footprinting without the complexity and high cost of acquiring a mass spectrometer or other sophisticated instrumentation and the use of special fragmentation/cleavage procedures to conduct HOS comparisons on a biopharmaceutical.

What is involved in antibody array footprinting is the synthesis of a set of short overlapping peptides (about 30) that collectively correspond to the entire amino acid sequence of a particular target biopharmaceutical. These peptides are then used to develop a set of unique polyclonal antibodies (anti-peptide antibodies) that only bind to their respective targeted peptide (which corresponds predominantly to only a small specific linear amino acid sequence region in the polypeptide chain(s) of the targeted biopharmaceutical called a linear epitope). Since many of these peptides will normally be buried within the interior of the biopharmaceutical, they will not be accessible to bind to their probing antibodies. Hence, when each of these antipeptide antibodies is separately added to a different sample of the same biopharmaceutical (using specially designed 96-well plates), it generates a unique binding profile of the antipeptide antibodies that bind to that biopharmaceutical using the sensitive and common detection capability of enzyme-linked immunosorbent assay (ELISA) technology. If another sample of the same biopharmaceutical has incurred a change in its HOS, it may trigger a change(s) in the profile of the peptides that are exposed on the