difference between two very large and highly similar signals that also contain signal noise. In such cases, the resulting small differences will likely be buried in the noise of the two biophysical measurements unless one can find ways to improve the signal-to-noise ratio in these measurements (for a graphical illustration of this problem, see Figure 3.1 in Houde and Berkowitz, 2014b). Nevertheless, the collective nature of the information obtained from low-resolution biophysical techniques does offer, as a pooled dataset, some underpinning of a fingerprint of the HOS of the RP and biosimilar; it's just that in most cases the ability of this fingerprint to reveal a small difference in HOS is going to be significantly limited.

One approach that might help improve the capability of some of these low-resolution biophysical methods to better detect the presence of small differences in the HOS between biopharmaceutical samples is to apply identical amounts of a physical or/and chemical stress to the samples being compared. The premise here is that if an inherent small difference in the HOS actually exists and makes that biopharmaceutical slightly less stable, applying an appropriate amount of stress over time may make that biopharmaceutical preferentially undergo some further structural change and/or a structural change at a different rate relative to the unaltered form of the same biopharmaceutical, as illustrated in Figure 2.11. Hence, for the sample having the slightly altered HOS, the introduction of stress might now provide a significantly large enough signal output in response to this stress, so that the actual difference present between both samples can be detected. Consequently, if one can introduce in an identical manner the same form and amount of an appropriate stress to biopharmaceutical samples being compared, this approach could be effective in assessing the biophysical biosimilarity using these low-resolution biophysical tools. Indeed, it is exactly this type of an approach that is employed in differential scanning calorimetry (DSC) that tends to make this particular biophysical tool fairly useful in detecting HOS differences between highly similar biopharmaceuticals (Demarest and Frasca, 2014). In this case, the controlled reproducible application of an increasing amount of heat (stress, which eventually denatures the biopharmaceutical) is used to reveal differences in the HOS of biopharmaceutical samples.

This general concept of applying stress to help facilitate the detection of underlying structural difference(s) between two or more samples is the basis for revealing the underlying stability issues when developing any biopharmaceutical or pharmaceutical via the use of accelerated stability studies. However, in these stability studies, the level of stress is greatly reduced, resulting in the amount of time to potentially reveal a structural problem that is very long (months). Consequently, the application of stress being called for here to potentially help low-resolution biophysical tools to be more useful in revealing possible structural differences in biopharmaceutical samples is significantly higher in order to carry out these measurements in a much shorter time scale (minutes).

Overall low-resolution biophysical tools provide only a coarse footing for assessing biosimilarity from a biophysical perspective. To improve upon this situation, one will need to use much higher resolution biophysical tools to generate more sensitive assessments of consistency and biosimilarity in terms of HOS. As a result, as mentioned at the end of Section 2.6.2, a growing interest in finding and developing such analytical tools for the biopharmaceutical area has attracted a fair amount of interest in recent years (Berkowitz et al., 2012; Marino et al., 2015).