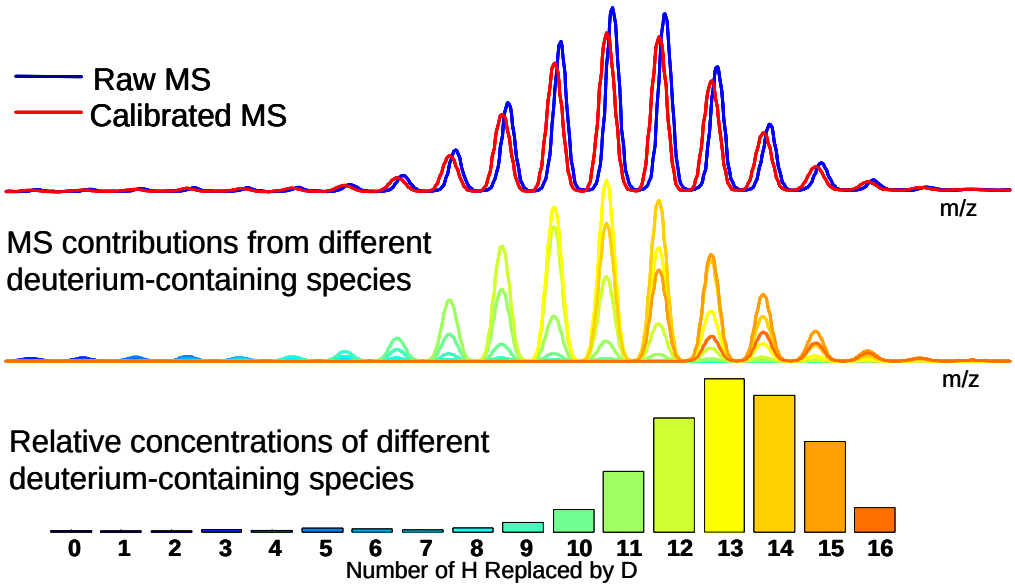
Cerno Application Note

Extending the Limits of Mass Spectrometry

Exact MS Mixture Analysis for Hydrogen-Deuterium Exchange

The biological activity of proteins is largely determined by their high order structure or conformation in aqueous solutions, which can be probed by hydrogen-deuterium exchange mass spectrometry (HDX-MS). Since the mass spectral responses from various ion species of different numbers of deuterium atoms are very similar to each other (especially with multiple charges) and these components are typically not easily separated by chromatography, the degree of deuterium exchange is conventionally determined by the mass shift calculation using MS data from a higher resolution system such as TOF [1] or through a matrix calculation based on MS centroids [2]. None of these methods provides a mathematically exact solution to yield detailed information on the kinetics or distributions of various deuterium-exchanged ion species.

With the unique MassWorks™ calibration that corrects not only the m/z but more importantly the MS peak shape [3], a mathematically exact solution could be found by using raw profile mode MS data to arrive at the relative concentrations of all ion species involved. With the mixture search capability now expanded in Version 4.0 MassWorks software (either CLIPS™ or sCLIPS™ [4]) to more than three components, accurate quantitation of 16 different HDX-MS species can be accomplished, resulting in a relative concentration distribution of all these species for each mass spectrum acquired, which may come from any given time point during an HDX kinetics study. Below graphs shows typical results with data kindly provided by Prof. Weis [1]. Interestingly, this may also be accomplished on a single quadrupole LC/MS or GC/MS system.



References

[1] Weis, D. D. et al, *J. Am. Soc. Mass Spectrom.* **2006**, 17, 1700-1703.

[2] Chik, J. K. et al, *Anal. Chem.* **2006**, 78, 207-214.

[3] Wang, Y.; Gu, M. *Anal. Chem.* **2010**, *82*, 7055-7062.

[4] Erve, J. C. L. et al, *J. Am. Soc. Mass Spectrom.* **2009**, 20, 2318-2333.

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40 Richards Avenue, 3rd Fl• Norwalk, CT 06854, USA • Tel 203-312-1150
Email: info@cernobioscience.com • Web: www.cernobioscience.com