Hydrogen/deuterium exchange during capillary electrophoresis allows proteome-specific characterization of protein higher-order structures

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Gold statement
Differential HDX was performed during electrophoretic separation of coexisting proteoforms followed by top-down MS analysis of sequentially eluted species.

Introduction
Characterization of structural differences between coexisting conformational states of protein is difficult with conventional biophysical techniques. Hydrogen/deuterium exchange (HDX) coupled with top-down mass spectrometry (MS) allows different conformers to be deuterated to different extents and distinguished through gas-phase separation based on molecular weight distributions prior to determination of deuteration levels at local sites for each isolated conformer. However, application of this strategy to complex systems is hampered by the interference from conformers with only minor differences in overall deuteration levels. In this work, we performed differential HDX while the different conformers were separated according to their differing charge to size ratios in capillary electrophoresis.

Body
We developed a differential HDX-MS approach to characterize conformational differences between coexisting protein states. Proteoforms with different conformations exhibited distinguishable charges or hydrodynamic sizes in solution, resulting in distinguishable electrophoretic mobility. The conformational features of these proteoforms were differentially labeled with 2H and were sequentially characterized by determination of deuteration level at local sites in real time using top-down MS.

Conclusion
The electrophoretic stage allows separation of proteoforms regardless of their overall deuteration level difference, thereby enabling integration of proteoform profiling and in-depth proteoform-specific characterization for a complex system. Modern CE instruments allow performance of HDX at low sample consumption in an automated manner, without the need for pre-equilibration in a flow system prior to each measurement. This strategy may open up additional possibilities for protein higher-order struct