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Combining advanced fragmentation techniques and spectral simplification for deep proteoform interrogation

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The "proteoform hypothesis"postulates that the proteome-to-phenotype connection is better explained through the characterization of the actual molecules present in a cell or tissue, or proteoforms, than by cataloguing "protein groups"that represent undistinguished molecule ensembles. The top-down (TD) approach to proteomics (i.e., the direct analysis of proteoforms) can theoretically ensure the access to the proteoform landscape of cells and tissues. However, TD is particularly challenging to properly implement, as both intact and fragmentation mass spectra of proteoforms suffer from problems such as signal dilution and ion signal overlap. Additionally, to remain true to its declared mission of molecularly defining proteoforms, top-down mass spectrometry should in principle analyze also typically neglected post-translational modifications such as Cys-linked ones, which remain present on proteins only if disulfide bond reduction is avoided.

We find that the use of ion-ion and photon-ion reactions in the gas-phase leads to extensive sequence coverage of a variety of proteoforms, natural or artificial. Case studies include 66 kDa human serum albumin, which comprises 13 disulfide bonds, and chemically modified proteoforms of antibody-drug conjugates.

We demonstrate that the use of advanced fragmentation methods such as activated ion electron transfer dissociation (AI-ETD), where low-energy IR photons are used to denature protein cations while ETD takes place, are beneficial for both increasing the number of identified backbone cleavages and sequencing disulfide-protected regions that remain otherwise uncharacterized. We also show how incorporating collisional activation of product ions post AI-ETD (referred to as AI-EThcD) further increases proteoform sequence coverage.

Finally, we show that reducing signal overlap of product ions via ion-ion reactions, specifically proton transfer charge reduction (PTCR), does not only give access to additional sequence information generated by these fragmentation methods, but it also dramatically facilitates the interpretation of complex mass spectra, substantially reducing the number of false positive matches.

User consent

yes

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