Native Top-Down Characterization of Protein Complexes with a Hybrid ECD-SID Device

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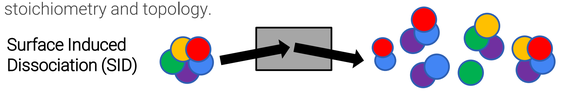
Introduction

- Dissociation of large biomolecules to reveal subunit stoichiometry, arrangement, sequence, and posttranslational modifications (PTMs) is a significant challenge for native mass spectrometry.
- Electron capture dissociation (ECD) fragments denatured proteins extensively. However, native protein noncovalent interactions necessitate supplemental activation to release ECD product ions.
- Surface induced dissociation (SID) is a valuable technique for dissociating large protein complexes to reveal higher order structure, including subunit stoichiometry and topology.



- Here, we present a hybrid ECD-SID device and the initial application to the characterization of native protein complexes.

Experimental

- The ExD cell for Thermo Scientific Q Exactive mass spectrometers[1] was redesigned to incorporate RF-only multipoles for improved transmission and focusing of ions into and out of the device.

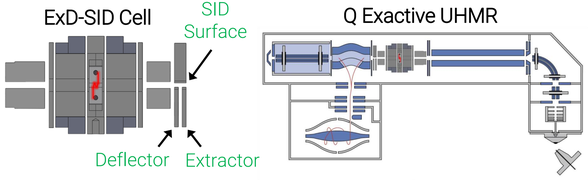


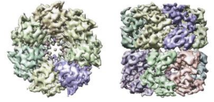
Figure 1. ExD cell for the Thermo Fisher Scientific Q Exactive platform incorporating RF-only quadrupoles, revised magnet and filament geometry, and lenses for SID.

- A Generation 3 SID device[2] (3 mm long surface) was incorporated at the exit of the ExD cell. The ExD-SID cell performance was characterized in modified Q Exactive and Q Exactive UHMR mass spectrometers.
- Data were processed using UniDec[3] for charge deconvolution of intact masses or ExD Viewer (e-MSion, Inc.) for high-resolution MS/MS data.

Results

Electron Capture Charge Reduction and SID of Protein Complexes

GroEL 14mer (801 kDa)



- Tunable charge reduction via electron capture without dissociation enables spectral decongestion for large native protein complexes (Figure 2).
- Electron capture charge reduction combined with SID yielded more native like dissociation of the GroEL 14mer and C-Reactive Protein 5mer compared to SID alone (Figure 3&4).

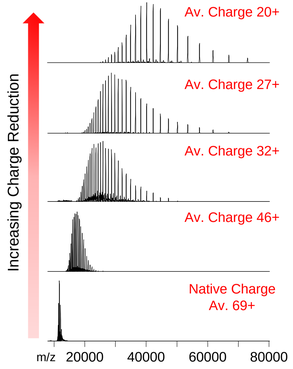


Figure 2. Tunable electron capture charge reduction of the GroEL 14mer.

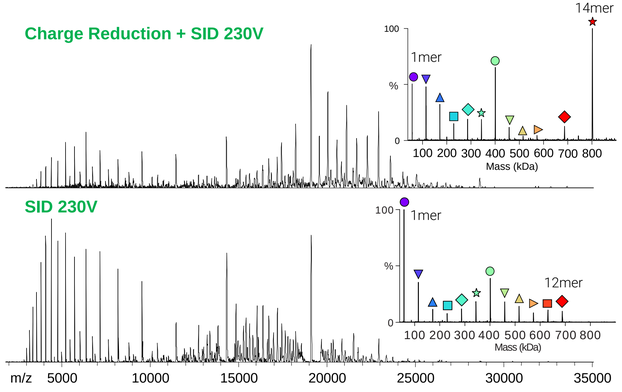
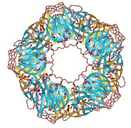
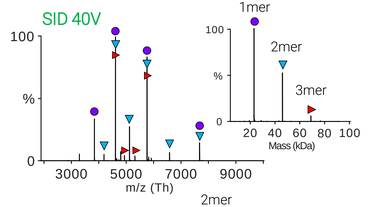


Figure 3. SID of charge reduced and native charge GroEL 14mer.

C-Reactive Protein Pentamer (115 kDa)





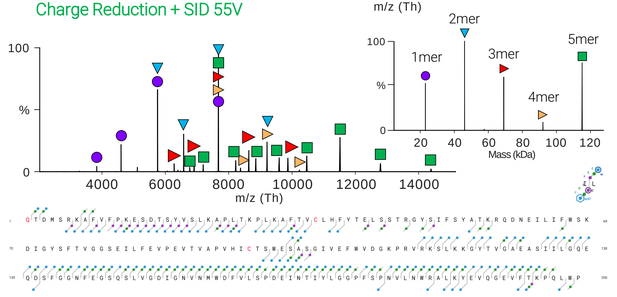


Figure 4. SID of native and electron capture charge reduced C-Reactive Protein 5mer and product ion map (64% coverage) for the 1mer ejected from the 5mer.

Native Top-Down ECD+SID Characterization of NIST mAb

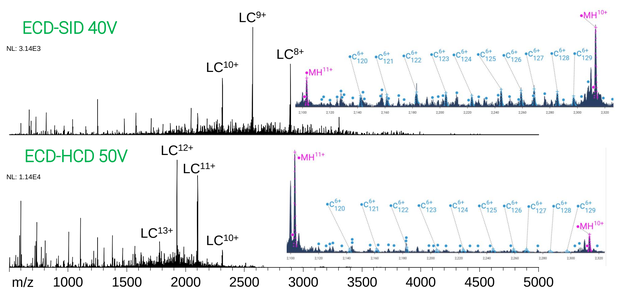


Figure 5. ECD-SID and ECD-HCD of native charge NIST mAb.

ECD-SID 40V: 66% Coverage

ECD-HCD 50V: 78% Coverage

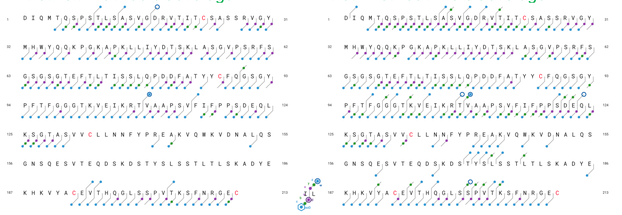


Figure 5. Light chain product ion maps for ECD-SID and ECD-HCD of native intact NIST mAb.

- ECD-SID and ECD-HCD experiments were performed using NIST mAb to evaluate sequencing of disulfide linked protein complexes.
- SID of ECD product ions shifted product ion distributions to higher m/z presumably due to more symmetric charge portioning between complementary product ions yielding spectral decongestion.
- Reduced sensitivity with SID is likely responsible for lower sequence coverage and will be addressed in future implementations

Conclusions

- A device combining ExD and SID was constructed to enhance Complex-Down and Native Top-Down characterization workflows.
- Electron capture alone yielded extensive charge reduction of large native protein complexes for spectral decongestion and enhanced SID.
- More symmetric charge partitioning between complementary product ions produced by ECD-SID yielded less congested MS/MS spectra for native top-down protein characterization.
- Future work will focus on improvements to SID efficiency and various combinations of ExD and SID (e.g., SID-ExD, SID-ExD-SID, etc.).

References

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2. Snyder, D. T.; Panczyk, E. M.; Somogyi, A.; Kaplan, D. A.; Wysocki, V. Anal. Chem. 2020, 92, 11195−11203.
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Acknowledgements

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