Contribution ID: **73** Type: **Poster**

Protein Characterization with CIU and MSn eXd on the timsOmni platform

Protein characterization is a very wide-reaching discipline which can encompass a broad range of techniques and approaches. Mass spectrometry is making an increasingly significant contribution to this field as the technology matures and top-down workflows are improved upon with new hardware and software innovations. The new timsOmniTM platform combines trapped ion mobility spectrometry (TIMS) with the Omnitrap® multidimensional MSn ion processor, providing the necessary analytical flexibility required for the increasingly complex studies aimed at sequencing and structural characterisation of biopharmaceuticals.
The configuration of the timsOmni instrument contributes greatly to the experimental options that are available, including a variety of ion activation methods which can be applied in multiple different locations along the ion path. Firstly, the protein desolvation unit (PDU) is positioned upstream of the TIMS cell and can be used for desolvation or unfolding of protein ions. Here, we demonstrate collision induced unfolding (CIU) of proteins in the PDU prior to mobility analysis in the TIMS analyzer. This was then combined with electron capture dissociation (ECD), a soft fragmentation technique, which was applied to proteins at differing levels of unfolding in the Omnitrap section Q5 to reveal which sequence regions become more flexible as the protein unfolds.
In-source collision induced dissociation (isCID) can be applied directly downstream of the TIMS analyzer and is typically used for desolvation or all-ion CID. For MS3, fragment ions produced by isCID can be isolated in the quadrupole ion filter, accumulated in the Omnitrap section Q2 and fragmented again by electron activated dissociation (eXd) in Q5. For pseudo MS4 experiments, eXd products can be isolated and accumulated prior to fragmentation by CID in the collision cell. The MSn eXd workflow is demonstrated for NISTmAb, producing up to 88% sequence coverage for N-terminus light and heavy chain fragments produced by isCID.

User consent

yes

Author: PENGELLEY, Stuart (Bruker Daltonics GmbH & Co. KG, Bremen, Germany)

Co-authors: APALATEGUI, Amalia (Bruker Daltonics GmbH & Co. KG, Bremen, Germany); GRIGORIADIS, Anastasios (Fasmatech Science & Technology, Chalandri, Greece); SMYRNAKIS, Athanasios (Fasmatech Science & Technology, Chalandri, Greece); GEBHARDT, Christoph (Bruker Daltonics GmbH & Co. KG, Bremen, Germany); PAPANASTASIOU, Dimitrios (Fasmatech Science & Technology, Chalandri, Greece); CARRASCOSA, Eduardo (Bruker Daltonics GmbH & Co. KG, Bremen, Germany); BUSCH, Florian (Bruker Switzerland AG, Fällanden, Switzerland); KOSMOPOULOU, Maria-Aggeliki (Fasmatech Science & Technology, Chalandri, Greece)

Presenter: PENGELLEY, Stuart (Bruker Daltonics GmbH & Co. KG, Bremen, Germany)

Session Classification: Poster Session 1