Characterization of a Fusion Protein Dimer by a UHR ESI - QTOF Mass Spectrometer

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Introduction:

Fusion proteins, the genetic combination of two or more originally separate proteins are highly successful biopharmaceuticals.
Here we illustrate the capabilities of the maXis II UHR ESI QTOF mass spectrometer (Bruker Daltonics, Billerica, MA) to characterize a fusion protein in denaturing and native conditions to determine the glycosylation pattern on the monomer and dimer. Additionally the high resolution of the maxis II enabled the detection of a sequence variant of – 8Da on a second fusion protein from two different clones. The dissect command was used to automatically find compounds on an LC-MS chromatogram trace; this algorithm is based on the principle that all ions that have the maximum intensity at the same time belong to the same compound thus making it possible to find compounds even if the peaks almost completely overlap.

Methods:

Two fusion protein samples were provided by Bristol Meyers Squibb, one for native spray analysis and the second for sequence variant determination. The workflow included the maXis II UHR-QTOF and Data Analysis software (Bruker Daltonics, Billerica, MA), and Ultimate™ 3000 RSLC (Thermo Scientific, San Jose, CA)

The first fusion protein sample was analyzed under denaturing LC-MS conditions with Acquity UPLC BEH C4, 2.1 x 100 mm column (Waters, Milford, MA). The buffers were water + 0.1 % formic acid +0.05 % TFA and acetonitrile + 0.1 % formic acid +0.05 % TFA with a 30 min gradient at a flow rate of 0.3 ml/min. The non-covalent dimer analysis was carried out under native conditions. After a buffer exchange to 10mM ammonium bicarbonate using a molecular weight cut off (30 kDa) spin filter, 15.6ug of the fusion protein was loaded on a Polyhydroxyethyl A 3um, 1 x 50mm column (PolyLC Inc., Columbia, MD) and eluted under isocratic conditions with 100mM ammonium at a flow rate of 15ul/min for 10 min.

A second fusion protein from a mixture of two different clones was compared to determine the presence of a sequence variant with an abundance of ~20% in one of the clones and corresponding to a mass shift of - 8 daltons.

Results

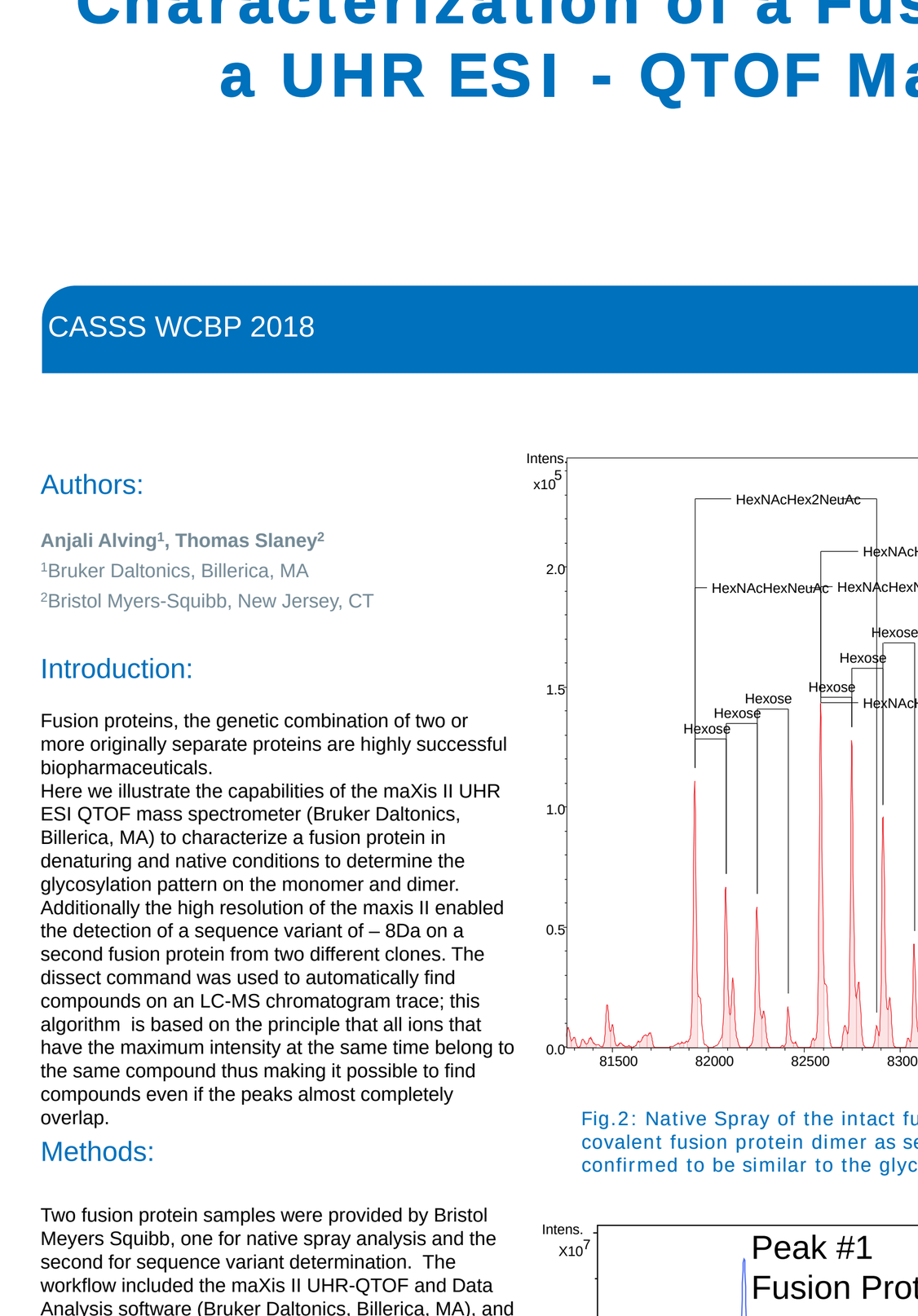

Fig.1: Fusion protein monomer acquired under denaturing conditions. The high resolving power on the maXis II enables isotopic resolution of the fusion protein as well as maintaining true isotopic pattern™ (TIP) of isotope spacing and intensity under fast HPLC conditions. The SNAP II peak picking algorithm was used to determine the monoisotopic mass of the fusion protein cluster as well as the glycosylation pattern with high mass accuracy.

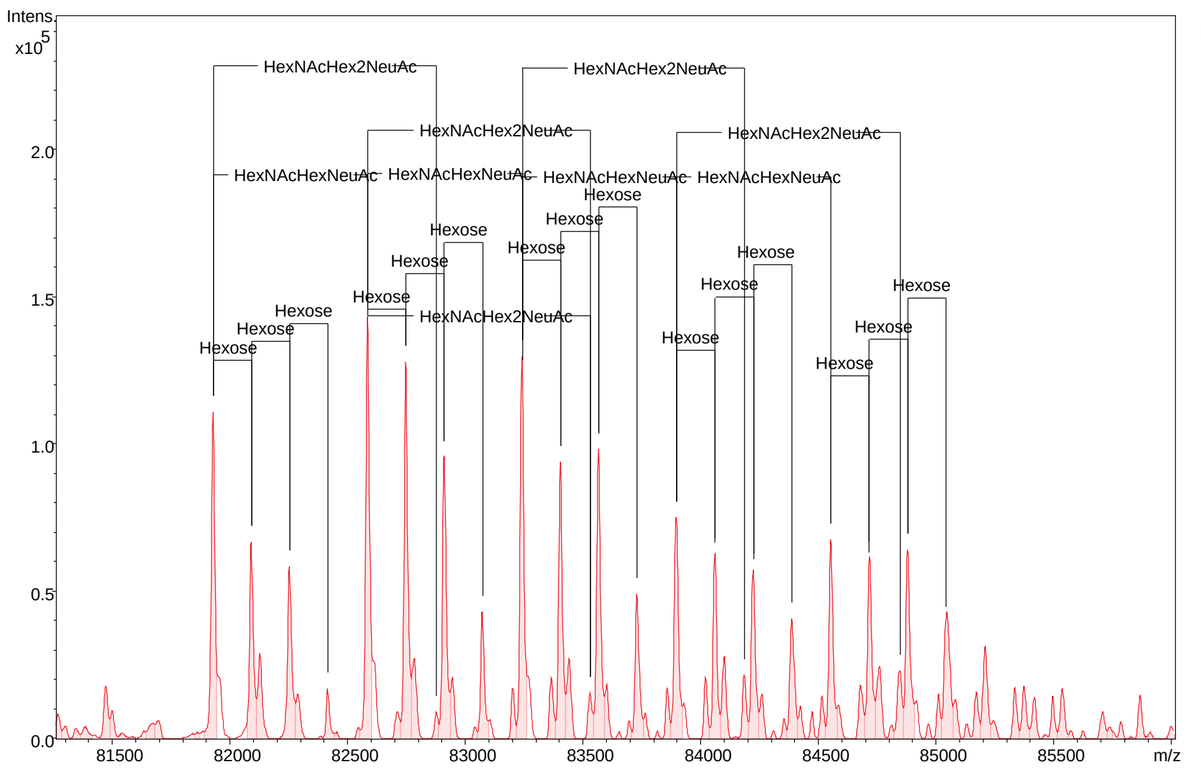

Fig.2: Native Spray of the intact fusion protein allowed the visualization of the non-covalent fusion protein dimer as seen in Fig.2. The complex glycosylation pattern was confirmed to be similar to the glycosylation pattern on the monomer of the fusion protein.

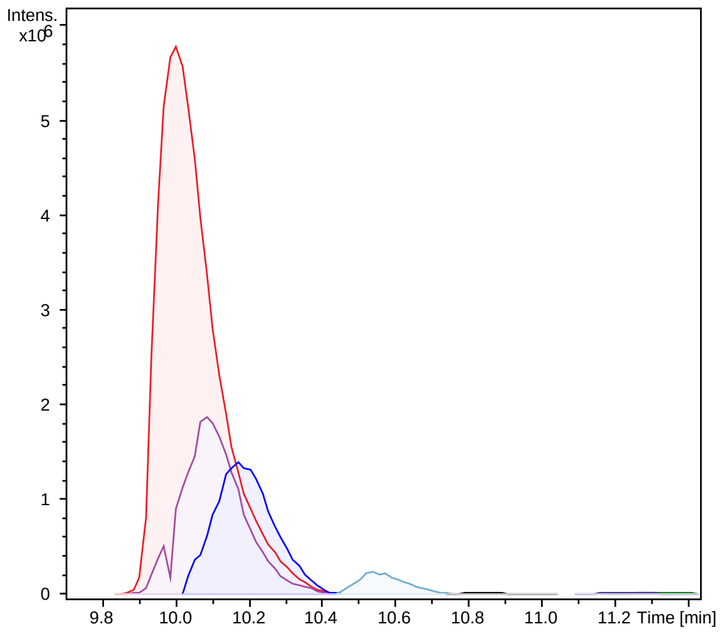

Fig. 5 illustrates the dissect algorithm that is used for compound detection in an LC-MS chromatogram even if the peaks completely overlap. 4 compounds were picked by the dissect software and their corresponding mass spectra seen in Fig. 6 shows distinctly different species.

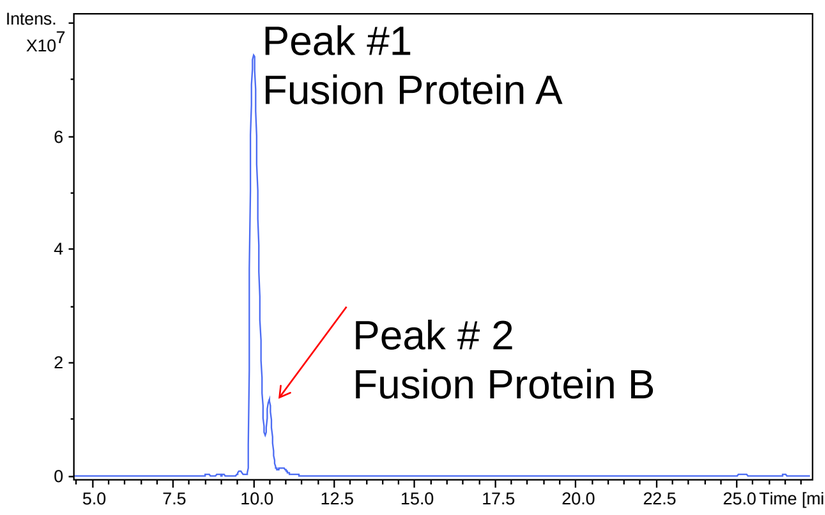

Fig. 3 shows the chromatographic separation of a fusion protein from two different clones(A and B). The mutant clone has a sequence variant and is seen separated as the small peak # 2 in fusion protein B.

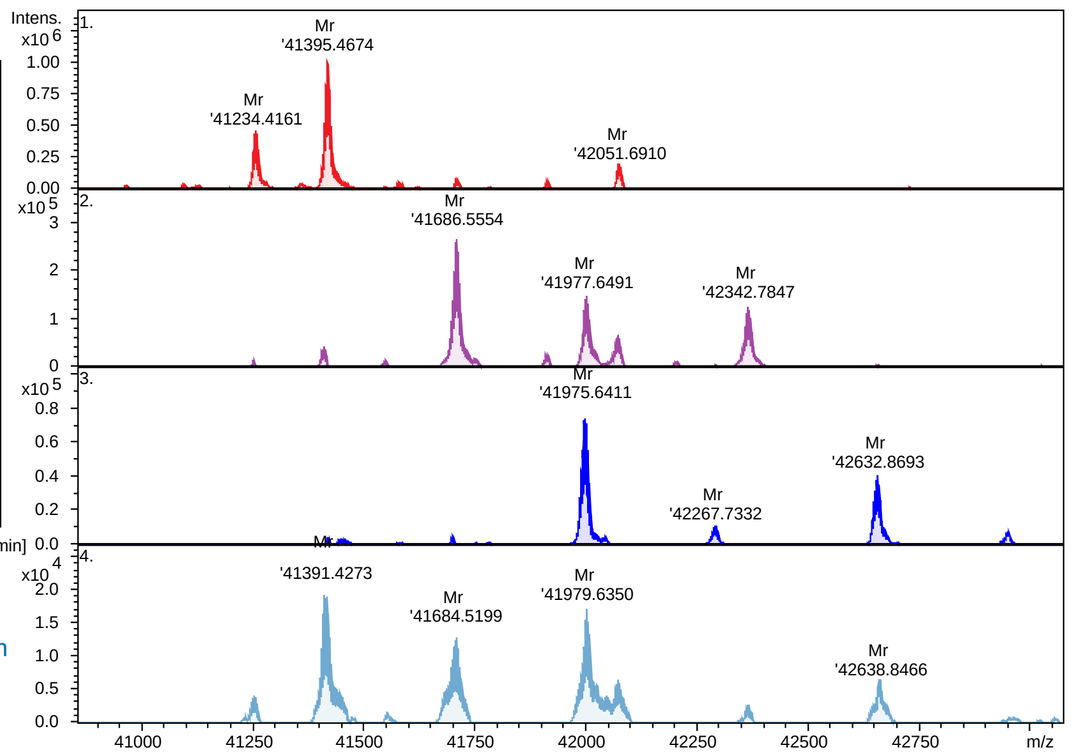

Fig.6 displays the spectra under the main peak and lower intensity heterogeneities eluting under the overlapping compounds picked by dissect peak picking software.

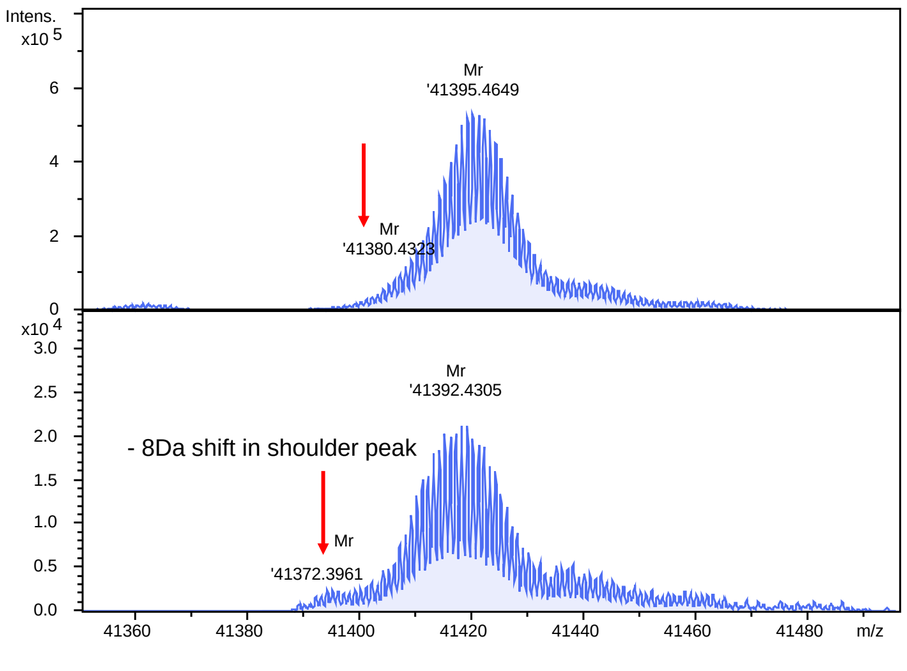

Fig.4: An examination of the mass spectra corresponding to the two chromatographic peaks revealed a -8 daltons mass shift in the small peak # 2.

Conclusions:

- The maXis II UHR ESI QTOF mass spectrometer was used to obtain isotopic resolution on the 40kDa monomer of a fusion protein and the SNAP II peak picking algorithm calculated the monoisotopic molecular weight from isotopically resolved peaks with high isotopic fidelity (True Isotopic Patten ™)
- Fusion protein dimer analyzed under native conditions confirmed the expected glycosylation pattern
- The maXis II UHR ESI QTOF mass spectrometer was also able to detect a sequence variant corresponding to a -8 Da mass shift in a mutant that was ~20% abundant.

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