Xyloglucan endotransglycosylases (XET, EC 2.4.1.207) are unique enzymes that perform an endolytic cleavage of a xyloglucan chain with subsequent transfer of the newly created chain representing each protein into a single peak on a true molecular weight scale. Of these, 85% of the theoretical calculated masses of the glycoforms were sub 0.5 ppm. Two out of the three glycans were sub 3 ppm. Two out of the three glycans were sub 3 ppm. This is reflected in the poorer accuracy and precision. Another possibility is that there could be localised background interference affecting the fourth. The fourth column within Table 4 represents the accurately measured glycosylation mass compared with the theoretically calculated glycosylation masses.

The data provided in the table above are comparable to those obtained during the accurate mass analysis of compounds whose molecular weight is below 1000 Da. With such high-mass analytes, one can, therefore, determine average and total abundances, that may only differ in 1 ppm, such as heteroatom species. Thus, the presence of post-translational modifications, such as phosphorylation, glycosylation, oxidation and disulfide bonds, can be determined.

**REFERENCES**


**CONCLUSION**

Utilising orthogonal acceleration time-of-flight mass spectrometry and maximum entropy to obtain accurate molecular masses on a variety of glycoproteins: a study of sub 3 ppm mass accuracy on species above 10 kDa

**OVERVIEW**

When we determine accurate mass measurements of the molecules of a glycoprotein (~20 Da) using an external calibrator and Maximum Entropy (MEnt) deconvolution of the data obtained on an orthogonal acceleration time-of-flight (TOF) instrument.

**EXPERIMENTAL**

**Water/Micromass UCT Premier® Mass Spectrometer.**

Figure 1 shows a schematic diagram of Waters Micromass UCT Premier® and XeLIS mass spectrometers. Sample introduction is conducted into the instrument on the Vapi2™ high-performance capillary spray device (Waters Micromass). Samples were introduced into the instrument through a glass probe located within the sampling cone. A mixture of 5% acetic acid (v/v) and 0.1% formic acid (v/v) was used as the high voltage solvent. The gas flow through the glass probe and for the needle through the sampling cone was 100 µl/min. A mixture of 5% acetic acid (v/v) and 0.1% formic acid (v/v) was used as the high voltage solvent. The gas flow through the glass probe and for the needle through the sampling cone was 100 µl/min. The high voltage of the ion spray was 3.5 kV and the flow back towards the detector where the ions are recorded and sent to a computer display was +3.5 kV.

**RESULTS AND DISCUSSION**

Figure 2 shows a typical multiply charged glycosylation fragment obtained by adding a 10 µl solution of the mutant enzyme PttXET16A (E85A). Following deconvolution with Model 1 (2.4.1.207), neutral glycoforms were obtained (Figure 3). The measured mass of the most abundant species (15797.22 Da) was within experimental error of the average mass of the glycoprotein to give glycoform masses. This is reflected in the poorer accuracy and precision. Another possibility is that there could be localised background interference affecting the fourth. The fourth column within Table 4 represents the accurately measured glycosylation mass compared with the theoretically calculated glycosylation masses.

The data provided in the table above are comparable to those obtained during the accurate mass analysis of compounds whose molecular weight is below 1000 Da. With such high-mass analytes, one can, therefore, determine average and total abundances, that may only differ in 1 ppm, such as heteroatom species. Thus, the presence of post-translational modifications, such as phosphorylation, glycosylation, oxidation and disulfide bonds, can be determined.

**CONCLUSION**

Utilising the combination of electrospray ionisation, matrix and Maximum Entropy, one can accurately measure intact protein.

On 3 out of 4 glycosylation species, a standard deviation of ±1 Da (±3.2 ppm) was better obtained.

On 3 out of 4 glycosylation species, the values obtained, in comparison to the theoretically calculated mass measurements on the glycosylated species within ±0.3 Da. Two out of the three glycans were ±0.3 Da.

**REFERENCES**


**MAXIMUM ENTROPY-DECONVOLUTION**

These multiply charged glycosylation species belonging to the mutant enzyme PttXET16A (E85A) were chosen for deconvolution from the internally reallocated data. The mass output parameters ranged from 10,000 to 14,000 Da and were recorded under 0.2 Da/Channel. The Lowest Common Peak width and Half-Height for the Damage window was set at 0.4 Da. The Default Scale and S/B ratio value were set at 10. The left-right intensity values were set at 45, 45% respectively. A centroided mass was created from accurate average accurate molecular mass data. From this data set, mass error were extracted and standard devations were calculated to ±0.5 Da and ±0.01 ppm.

**MAGNITUDE ENHANCEMENT**

Figure 1 shows the typical multiply charged glycopeptide obtained by adding a 10 µl solution of the mutant enzyme PttXET16A (E85A) in the presence of horse heart myoglobin (final concentration of 100 µg/ml). Glycopeptide was not run under condition, with which a spectrum feature was not carried out. Upon spectral enhancement, Maximum Entropy 1 deconvolution can be used to generate an accurate mass change mass. Figure 4 for the protein under investigation.

**Figure 1. Instrument configuration of the Waters Micromass UCT Premier® Mass Spectrometer.**

**Figure 2. A typical Multiply-charged glycopeptide spectrum from PttXET16A (E85A) in the presence of 100 µg/ml horse heart myoglobin.**

**Figure 3. Background subtracted and smoothed electrospray spectrum from PttXET16A (E85A) in the presence of 100 µg/ml horse heart myoglobin.**

**Figure 4. A typical Multiply-charged glycopeptide spectrum obtained by adding a 10 µl solution of the mutant enzyme PttXET16A (E85A) in the presence of horse heart myoglobin (final concentration of 100 µg/ml).** Glycopeptide was not run under condition, with which a spectrum feature was not carried out. Upon spectral enhancement, Maximum Entropy 1 deconvolution can be used to generate an accurate mass change mass.

**Figure 5. A Typical Multiply-charged glycopeptide spectrum obtained by adding a 10 µl solution of the mutant enzyme PttXET16A (E85A) in the presence of horse heart myoglobin (final concentration of 100 µg/ml).** Glycopeptide was not run under condition, with which a spectrum feature was not carried out. Upon spectral enhancement, Maximum Entropy 1 deconvolution can be used to generate an accurate mass change mass.