**Superposition of Orthogonal Mass Spectrometric Data Methods with Theoretical Intact Reconstruction from Bottom Up Data**

Yong Kil1, Marshall Bern1, Claire Bramwell1, St John Skilton1, Rose Lawler1, Michelle English1, Andrew Mahan2, Bo Zhai2, Hirsh Nanda2, Andrew Nichols1

Contact: anichols@proteinmetrics.com

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**Introduction**

A longstanding issue in the biopharmaceutical industry has been the comparison and evaluation of orthogonal methods to characterize - and potentially quantify - the covalent modification of critical quality attributes (CQAs) in proteins. This issue is particularly intensified in mass spectrometry due to the fecund and complex nature of the data it produces. Concretely, comparing bottom up methods such as peptide mapping to intact methods such as reduced and non-reduced LC-MS analysis, using human judgement, presents numerous challenges. However, by using bottom up data to build a theoretical reconstruction of the intact data and then juxtaposing it with the experimental intact data, differences and similarities between the two orthogonal methods can quickly be qualitatively determined. This paradigm can be used to inform scientists in many respects, such as the effectiveness of the acquisition and analysis parameters used, as well as whether modifications of CQAs are stochastic or non-stochastic in nature.

**Methods**

**Data Acquisition Graciously Performed by Janssen Pharmaceuticals:**

Tryptic Mapping: All samples are reduced with TCEP and alkylated with iodoacetamide. Mass spectrometric data is acquired by Thermo Orbitrap instruments. Intact LC-MS: When applicable, samples are reduced with TCEP and alkylated with iodoacetamide. Mass spectrometric data is acquired by Waters TOF instruments.

**Data Analysis:**

Tryptic Mapping Data Analysis: Performed using Byos-Peptide Analysis software. The PTM workflow was selected as a starting template in most cases. Intact Reconstruction: Performed using Byos-Intact Analysis software. The Reduced Reconstruction workflow was used for reduced reconstruction.

**Optimizing Data Analysis Parameters**

**Determining If Modifications are Stochastic**

Orthogonal Comparison of mAb Heavy Chain Glycosylation

Experimental reduced LC-MS mAb heavy chain juxtaposed with a theoretical reconstruction of a reduced mAb heavy chain using degradation quantification from tryptic mapping data show that estimation of glycan composition is most likely underestimated by tryptic map. The hypothesis being that larger glycans suppress ionization of the smaller tryptic peptide than they do a reduced heavy chain.

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**Conclusions**

- Intact reconstruction can be used to not only determine if modifications are stochastic or not, but perhaps also the nature of them. In the example shown, the authors posit that C-term cleavage of Trimer molecule is enzymatic rather than chemical in nature.
- Intact reconstruction can be used to quickly evaluate alignment of orthogonal methods vis-à-vis degradation quantification. Quantiﬁcation of glycans for instance by peptide map may not be the most accurate.
- Intact reconstruction may be used to shed light on the underlying modiﬁcations that contribute to intact LC-MS peak shape.
- Intact reconstruction can be used to quickly evaluate analysis parameters.