# Rapid & comprehensive comparison of five versions of bevacizumab: Avastin versus its biosimilars

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## Introduction

As the pace of biosimilar development and commercialization increases, there is a clear need for LCMS analytical methods to support or disprove similarity to an originator product. Analytical scientists rely on techniques ranging from peptide mapping, to intact mass analysis, to detailed MS/MS analysis of PTMs and sequence variants to show similarity with originator products, as well as lot to lot similarity.

As the speed and sensitivity of mass spectrometers continue to improve, there is a concomitant need for software tools to provide rapid yet detailed and robust analysis of complex MS datasets. Here we present a study showing comprehensive analysis of five different productions of a therapeutic antibody – bevacizumab (Avastin) -- using the Protein Metrics suite of analysis software.

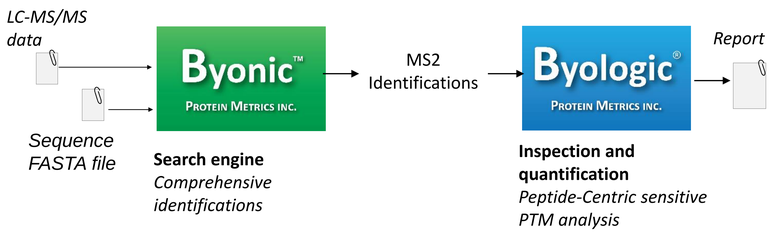
## Methods

Five samples of bevacizumab were sourced: two commercial versions of Avastin (from U.S. and E.U. production facilities), and three different test lots of the biosimilar bevacizumab. All five samples were analyzed for similarity using an Orbitrap Fusion or Q-Exactive mass spectrometer with an EASYnLC HPLC (Thermo Fisher). Raw MS/MS data were analyzed with Byonic and Byologic software packages (Protein Metrics). Samples were quantitatively compared, and reports exported automatically.

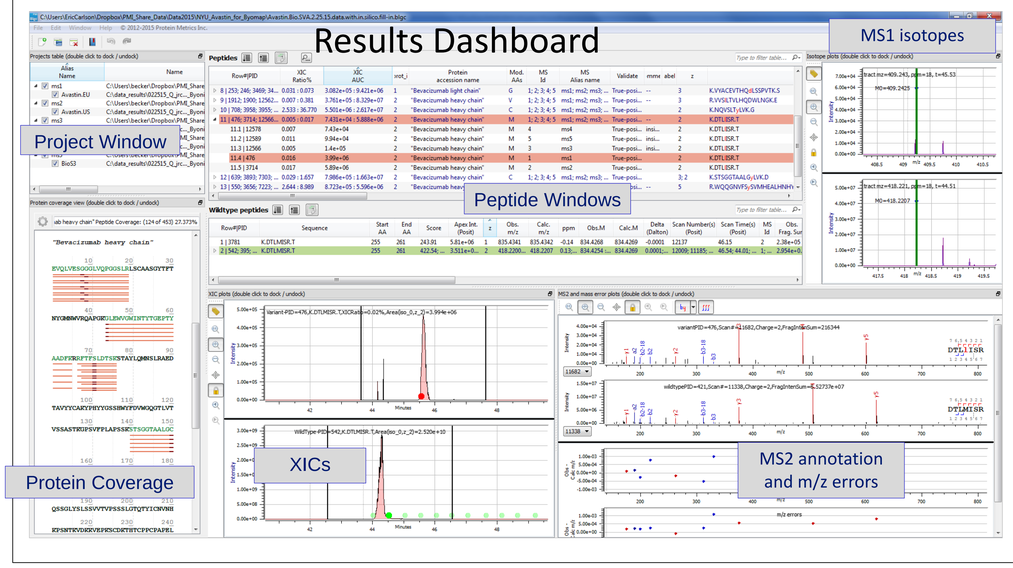
Raw LC-MS/MS data was searched with the Byonic search engine and the search results, MS1 and MS2 data were imported into Byologic for subsequent analysis. Comprehensive analysis included automatic annotation of peptide maps (not shown in this poster) and detailed study of oxidation, deamidation, glycosylation, glycation and sequence variants.

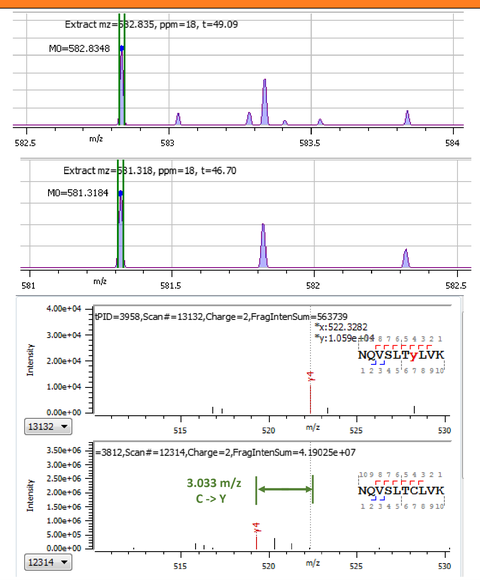
Label-free quantification was performed using extracted ion chromatograms (XIC's), with comparison of the modified peptide forms to those of the unmodified ("wildtype") peptides. Results were exported in formatted reports.

Data analysis workflow:



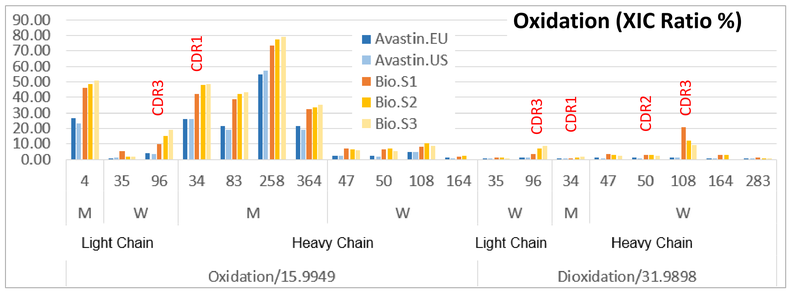

## Data Analysis

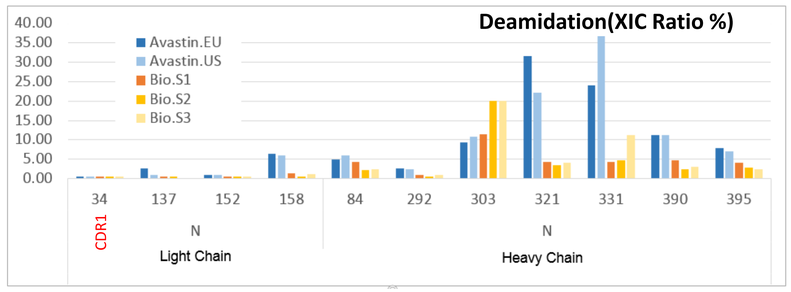


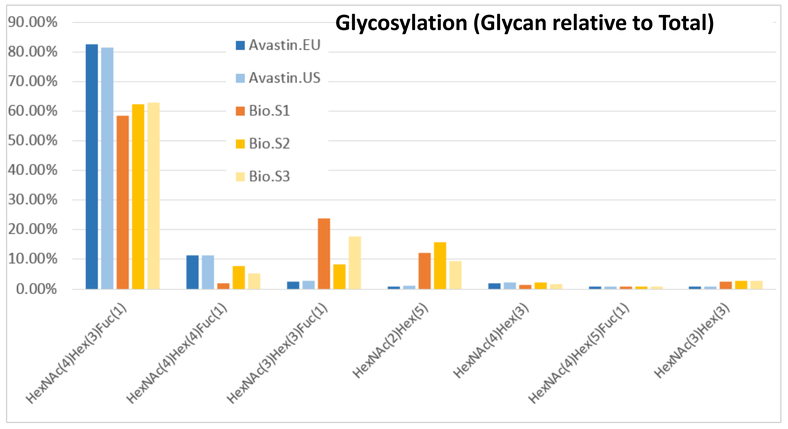



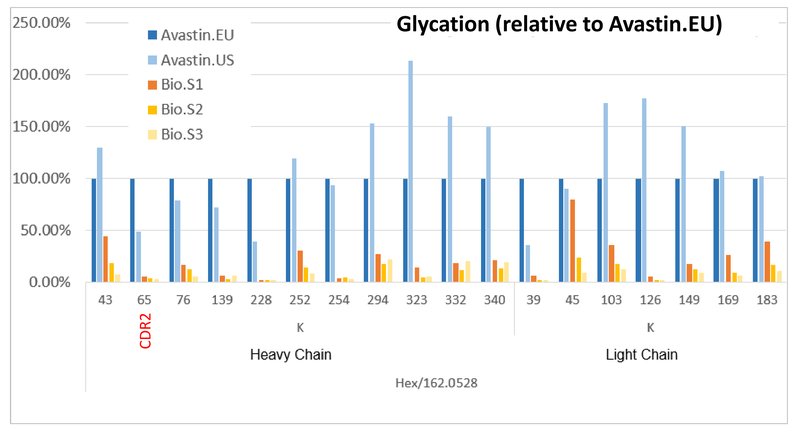

Selected Inspection Tools
Variant peptide shown in panel above unmodified (wildtype) peptide

## Post-translational Modifications: Selected Sites




## Selected Sequence Variants - percentages

| Modification | mAb Chain | AA Residue | AA Position | Region | Avastin.EU | Avastin.US | BioS1 | BioS2 | BioS3 |
|---|---|---|---|---|---|---|---|---|---|
| Cys->Tyr/3.0327 | HC | C | 150 | Fab | 0.03 | 0.04 | 1.49 | 0.53 | 0.43 |
| | | → | 373 | Fc | 3.03 | 2.53 | 36.77 | 11.65 | 14.18 |
| | | → | 431 | Fc | 3.71 | 2.64 | 8.99 | 4.29 | 4.82 |
| Gly->Asp/58.0055 | LC | G | 200 | Fab | 0.070 | 0.073 | 0.051 | 0.031 | 0.049 |
| | HC | G | 8 | Fab | 0.07 | 0.09 | 0.08 | 0.06 | 0.12 |
| | | | 9 | Fab | 0.031 | 0.047 | 0.053 | 0.038 | 0.091 |
| | | | 44 | Fab | 0.024 | 0.022 | 0.022 | 0.005 | |
| Adjacent to Lys cut site | | | 322 | Fc | 1.58 | 2.69 | | 0.02 | 0.01 |
| Val->Leu/14.0157 | HC | V | 311 | Fc | 0.06 | 0.02 | 0.38 | 0.01 | 0.01 |
| Ser->Asn/27.0109 | LC | S | 202 | Fc | 0.016 | 0.021 | | | 0.000 |
| | HC | S | 71 | Fab | 0.022 | 0.028 | 0.001 | 0.002 | 0.001 |
| | | | 310 | Fc | 0.019 | 0.015 | 0.024 | 0.039 | 0.043 |
| Met->Leu/-17.9564 | HC | M | 258 | Fc | 0.016 | 0.017 | 0.005 | 0.007 | 0.011 |

**C → Y some very high concentrations; disulfide bond disruption**
observed mass change 3.0327 = difference between tyrosine and carbamidomethylated cysteine

## Discussion and Conclusions

Extensive primary sequence and PTM analysis was performed on 5 lots of bevacizumab: commercial Avastin from the U.S., commercial Avastin from the E.U., and 3 experimental biosimilar lots. Byonic and Byologic software tools were employed to perform this analysis and produce automated reports.

PTMs reported here included: (1) oxidation, (2) deamidation, (3) glycosylation, and (4) glycation.

Oxidation and deamidation percentages were sometimes very high. It is known that oxidation and deamidation can be sensitive to sample handling and preparation conditions; further work will be necessary to determine to what extent these are *in vitro* artifacts; these are not intentionally stressed samples.

Significant differences were seen in glycosylation, and relative degree of glycation (considerably higher in Avastin). Absolute quantification of glycation will require a follow-on study with a digestion enzyme that does not cut at lysine because glycation strongly affects trypsin digestion efficiency.

Major surprises were observed for sequence variants, notably the substitution of cysteine by tyrosine (C to Y). This will disrupt disulfide bonding and three-dimensional structure. Although some very high amounts were found in the experimental biosimilar material, as much as 3% variant was found in Avastin.

Our integrated software workflow allows exhaustive analysis of these proteins.

## Acknowledgment

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