Rapid Identification and Quantitation of Disulfide Bonds in Infliximab (Remicade vs Remsima)

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Introduction

Disulfide bonds influence protein higher order structure and thus have a direct effect on a protein's biological function and activity. Unpaired cysteines or variant disulfide bonding (shuffling) can adversely affect monoclonal antibodies (mAbs) or other proteins, and hence there is great interest in mapping and quantifying disulfide variants down to low concentrations. This problem is also important for antibody-drug conjugates (ADCs) or for bi-specific antibodies. One technique for conjugating a drug replacing disulfide bonds with drug-containing linkers in order to attach a linker and drug to cysteine.

We present here an automated method for rapidly characterizing and quantifying disulfide bonds, including their shuffling.

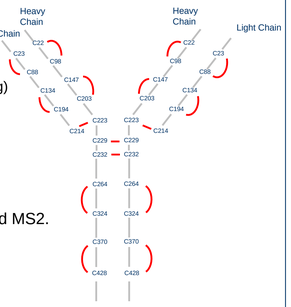
Application is made to innovator (Remicade) and biosimilar (Remsima) versions of infliximab.

Methods

Infliximab, an IgG1 [$C_{6428}H_{9912}N_{1694}O_{1987}S_{46}$ = 144,193 Da]

Originator
Remicade (RC)
Janssen Biotech Inc
Approved for multiple indications

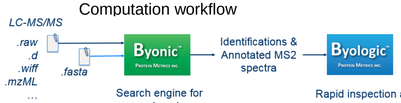
Biosimilar
Remsima (RS)
Celltrion Inc.
EU , CA, and S Korea approvals (US pending)



Sample Preparation.
- Unreduced protein, trypsin digestion

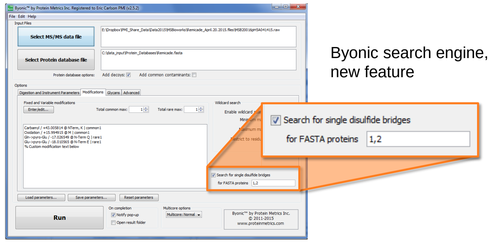
LC-MS. Mass spectral analysis by Thermo Q-Exactive, high resolution MS1 and MS2.

Computation workflow



Search engine for comprehensive identifications (from tandem MS data)

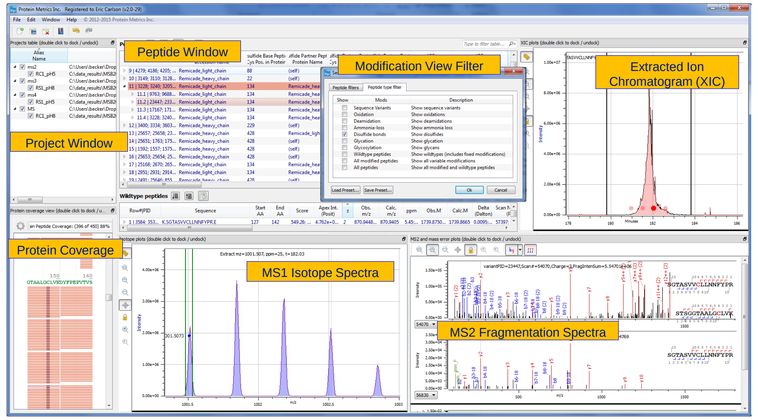
Rapid inspection and quantification of low-to-moderate concentration species



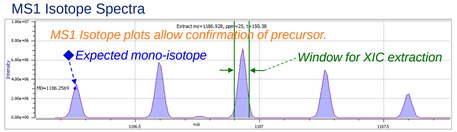
Byonic search engine, new feature

Byologic® Software: Results and Inspection Dashboard

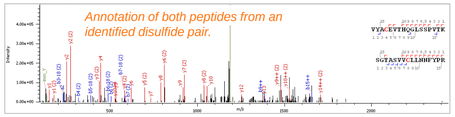
Interactive windows allow an analyst to review details of identified disulfide pairs.



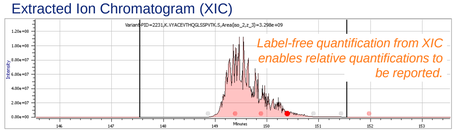
MS1 Isotope Spectra



MS2 Fragmentation Spectra



Extracted Ion Chromatogram (XIC)



Results of Comparison: Remicade vs Remsima

Expected Disulfide Pairs: Total XIC (Normalized to Injection Amount)

Chain 1	Cys 1	Chain 2	Cys 2	Remicade	Remsima
LC	88	LC	23	1.8E+04	2.6E+04
LC	134	LC	194	9.4E+04	1.0E+05
LC	214	HC	223	1.7E+01	1.4E+00
HC	22	HC	98	1.5E+04	2.2E+04
HC	203	HC	147	3.5E+04	2.7E+04
HC	229	HC	229	1.9E+04	1.7E+04
HC	264	HC	324	7.1E+04	5.8E+04
HC	428	HC	370	4.5E+04	5.8E+04

Unexpected (Shuffled) Disulfide Pairs: Total XIC (Normalized to Injection Amount)

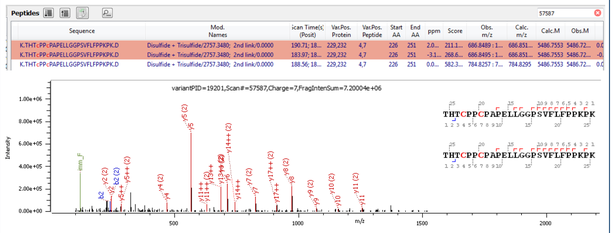
Cys-1	Cys-2	Remicade	Remsima
LC-023	LC-088	3.6E+01	3.0E+02
	LC-134	1.6E+02	1.5E+02
	LC-194	3.7E+01	3.8E+01
	HC-223		1.8E+02
	HC-264	1.1E+02	5.9E+01
	HC-428	5.5E+01	2.6E+01
LC-134	LC-088		6.7E+01
	LC-134	8.8E+01	1.5E+02
	HC-223	1.0E+02	9.5E+01
	HC-264	1.2E+02	1.5E+02
	HC-428		3.8E+01
LC-194	LC-088	4.5E+01	1.0E+02
	LC-194	6.8E+01	4.3E+01
	HC-022	5.2E+01	8.7E+01
	HC-232	2.2E+01	6.0E+00
	HC-264	1.3E+02	7.8E+01
	HC-428	5.5E+01	7.7E+01
LC-214	LC-134	3.8E+02	4.4E+02
	LC-194	1.5E+02	1.2E+02
	HC-022		1.7E+01
	HC-264	2.4E+02	1.6E+02
	HC-428		1.4E+01
HC-022	HC-264	4.9E+01	6.4E+01
HC-098	LC-134	1.1E+02	1.1E+02
	HC-264	5.9E+01	5.1E+01
intra	HC-229, 232	2.8E+02	5.2E+02

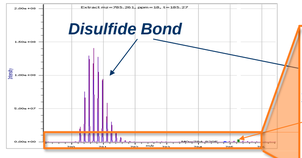
Cys-1	Cys-2	Remicade	Remsima
HC-147	LC-134	4.5E+02	5.1E+02
	HC-022		7.4E+01
	HC-264	9.4E+01	1.2E+02
HC-223	LC-134	1.5E+01	5.6E+01
	HC-022		2.2E+01
	HC-264	1.4E+02	6.6E+01
	HC-428		7.4E+01
HC-264	LC-134	1.2E+02	1.5E+02
	HC-022	8.3E+01	1.2E+02
	HC-264	3.1E+02	3.0E+02
HC-324	HC-147	2.3E+01	1.7E+01
	LC-134	8.3E+01	4.2E+01
	LC-194	3.1E+01	3.3E+01
	HC-022	2.1E+01	3.3E+01
	HC-098		2.7E+00
	HC-428	3.9E+01	4.1E+01
HC-370	LC-134	2.2E+02	3.1E+02
	HC-022		3.0E+01
	HC-264		3.1E+01
HC-428	LC-134	1.5E+02	1.9E+02
	HC-229		3.7E+01
	HC-022	1.6E+01	4.9E+01
	HC-264	2.2E+02	2.0E+02
	HC-428	1.7E+01	4.5E+01

Observations

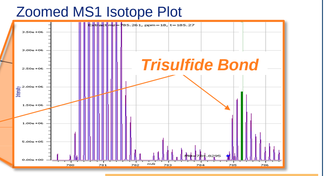
- Analyze both expected and shuffled disulfide bonds
- Most prevalent shuffled disulfide bonds
  Remicade → LC.134—LC.214, LC.134-HC.147
  Remsima → LC.134—LC.214, LC.134-HC.147, HC.229-232

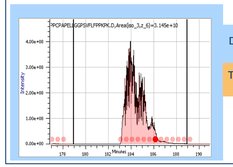
Identify and Quantify Tri-sulfide Bonds





Zoomed MS1 Isotope Plot

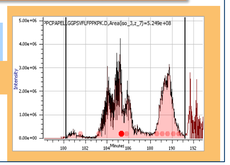




Di-sulfide XIC Integrated Area = 3.2×10^{10}

Tri-sulfide XIC Integrated Area = 5.3×10^{8}

Disulfide / Tri-sulfide Ratio ≈ 100



Discussion and Conclusions

- In both Originator (Remicade) and Biosimilar (Remsima), unexpected (shuffled) disulfide peaks are, in aggregate, ~ 100x less prevalent than the expected disulfide bonds.
- Remsima samples show slightly more disulfide shuffling than Remicade samples.
- Byonic→Byologic offers a platform and workflow for the efficient identification, inspection, and relative quantification of disulfide bonds pairs (both expected and unexpected)
- Byonic→Byologic can be used to identify and quantify tri-sulfide bonds.

Acknowledgements

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