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It's Time to
See the Future
Differently





Analysis and quality control of biologics on SCIEX QqTOF platforms

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Overview

- Defining the goals for Biologics characterisation
- Routine Workflow Overview
  - Intact Mass
  - Subunit
  - Peptide Map
- Hardware and software that enable these workflows
- Summary





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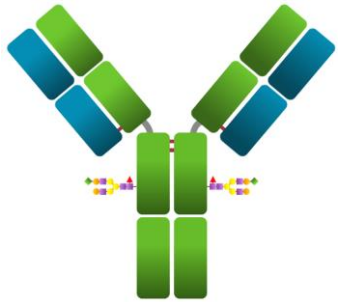
From small to LARGE

Trend towards BioPharmaceuticals

- The pharma industry is shifting towards larger molecules targeting diseases more specifically
- Biopharmaceuticals are produced using biological processes
  - Predominantly large peptides, recombinant proteins, antibodies, nucleic acids, and vaccines



Increasing Size of Therapeutic Molecules



Ibuprofen	Indolicidin	Insulin	Epogen	Herceptin
Small molecule	Peptide	rProtein	Glycosylated rProtein	monoclonal Antibody
MW ~ 200 Da	MW ~ 1,400 Da	MW ~ 6,000 Da	MW ~ 34,000 Da	MW ~ 147,000 Da

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TOP 12 Biologicals – Several Billion Dollars market

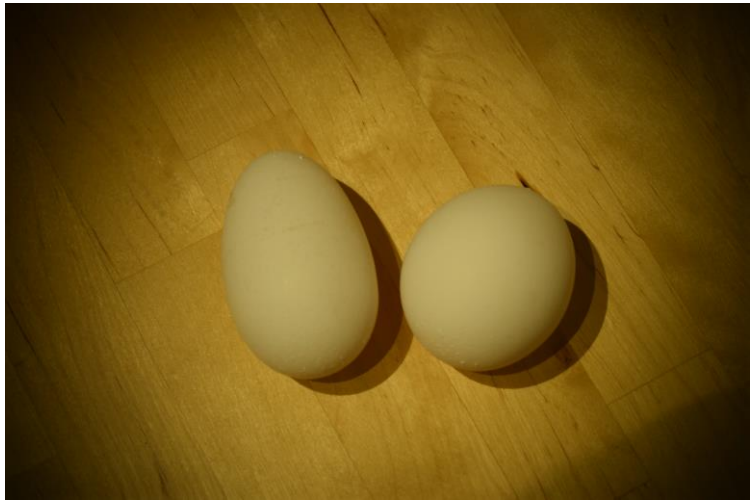
Protein	Biotherapeutic	Patent Expiry USA	Patent Expiry EU
Humanised mAb	Avastin (Bevacizumab)	Jul 2019	Jan 2022
	Herceptin (Trastuzumab)	Jun 2019	expired
	Synagis (Palivizumab)	expired	expired
Non-humanised Ab	Erbitux (Cetuximab)	expired	expired
	Enbrel (Etanercept)	expired	expired
	Humira (Adalimumab)	expired	April 2018
	Remicade (Infliximab)	Sep 2018	expired
	Rituxan (Rifluximab)	Sep 2018	expired
Non Ab product	Aranesp (Darbepoetin α)	May 2024	expired
	Epogen (Epoetin α)	expired	expired
	Neulasta (Pegfilgrastim)	expired	Aug 2017
	Neupogen (Filgrastim)	expired	expired

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Biosimilars are NOT Generics

Allowed differences because of production in living cells - unlike small molecules

- Approval of a biological product based on
  - High similarity to already approved biopharmaceutical (reference product)
  - No clinically meaningful differences (safety, efficacy, purity)



- Interchangeable product
  - Biosimilar which is expected to produce the same clinical results as the reference product in any given patient
  - Risks (safety and efficacy) of alternating or switching between products not greater than using the reference product only

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BioPharmaceuticals

No matter if originator or biosimilar, all biotherapeutics need to be fully characterized



Biologics Development



Biologics Bioanalysis

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What is needed?

- Hardware?



- Easy-to-use Software?



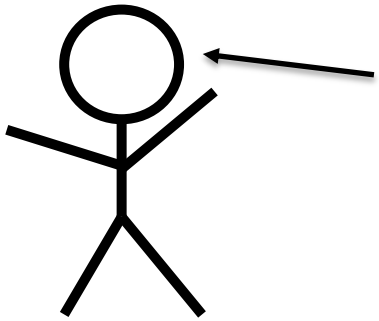
- “Plug and Play“ Methods?





- Training programs for staff?





Dedicated support to help you

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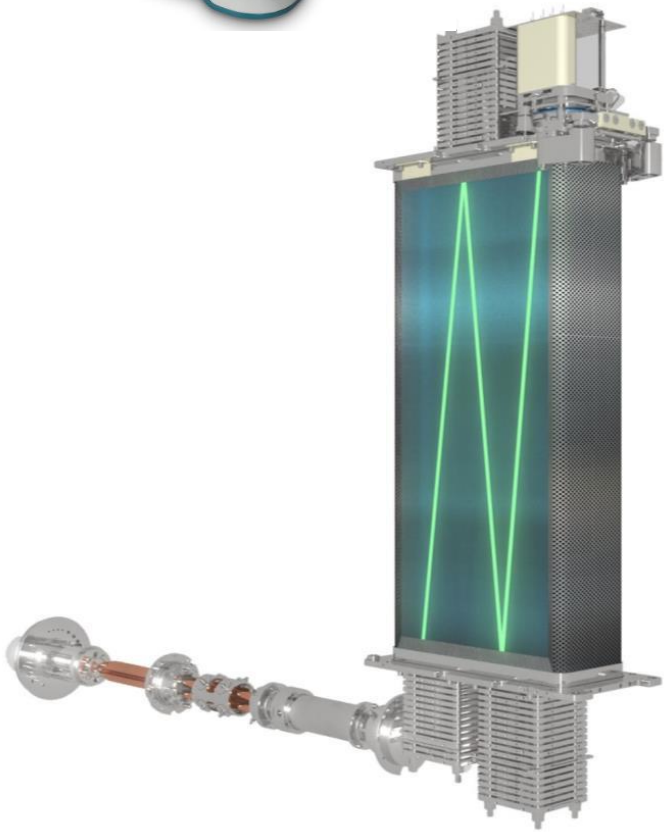
The SCIEX TripleTOF® and X500 Systems











BioPharmaView™ 2.0 Software for characterisation



Inviting you to process

- Fast – intact mass deconvolution and peptide mapping in minutes
- Designed for processing and reviewing batches
- Automated calculations for modification ratios
- Hundreds of built-in modifications, easy to add custom modifications
- Complete support for IDA and SWATH® data
- Key routine workflows supported:

Comparability

Intact Protein

- Subunits

Peptide Map

- Reduced
- Non-reduced
- Disulfide bond mapping

ADC DAR

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Example: Therapeutic Immunoglobulin Molecules

A lot to characterize on...

Changes in Sequence:

Sequence **V**ariant **A**nalysis

N-term blocking

(Gln/Glu → PyroGlu: -17/-18 Da)

Glycosylation Heterogeneity

(Fab & Fc: +1445, 1607, 1769 Da…)

Oxidation (Met, Trp: +16 Da)

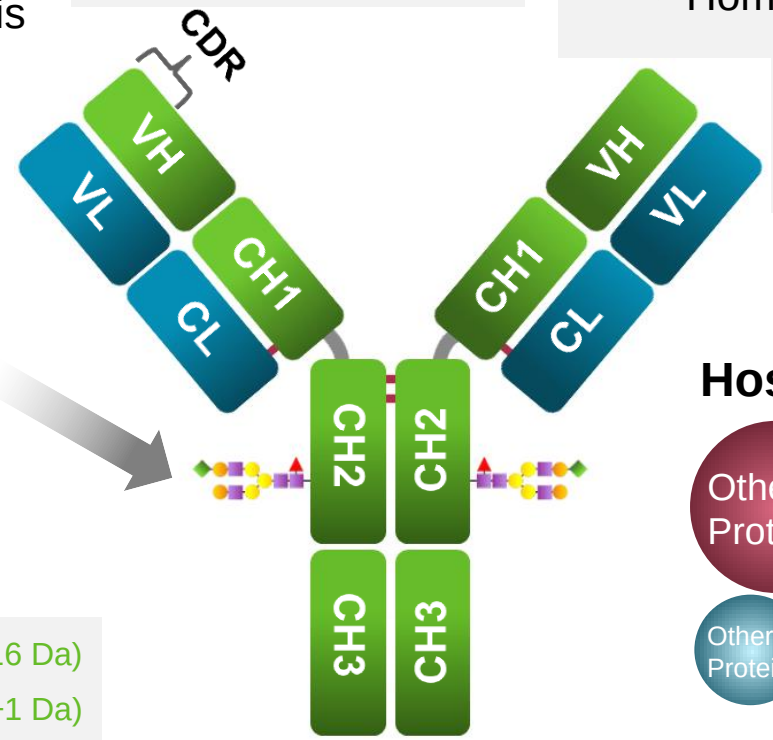
Deamidation (Asn: +1 Da)

Succinimide (Asn/Asp Succ: -17 Da)

Glycation (Lys-Glc: +162 Da)

Cysteinylation (+119 Da)

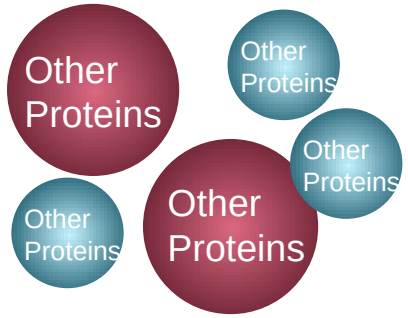
Thioether (-32 Da)



Intact IgG1: MW ~150 kDa

- Homodimer H2L2 (symmetrical)
  - 1300-1350 aa
  - 32 Cys - 16 disulfide bridges
    - 4 interchain + 12 intrachain

Host Cell Protein Analysis



C-term clipping (-Lys: -128 Da)

C-term amidation (OH → NH2: -1 Da)

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Intact Mass Analysis





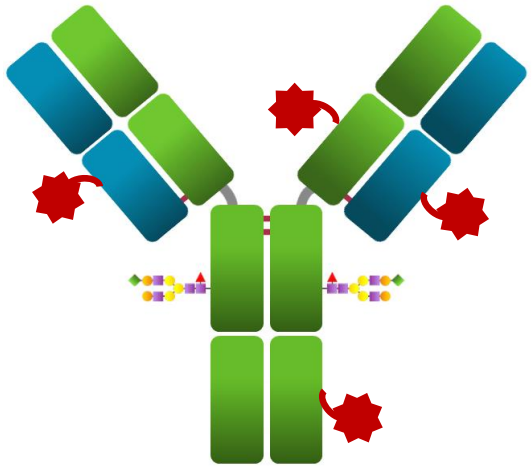
SCIEX

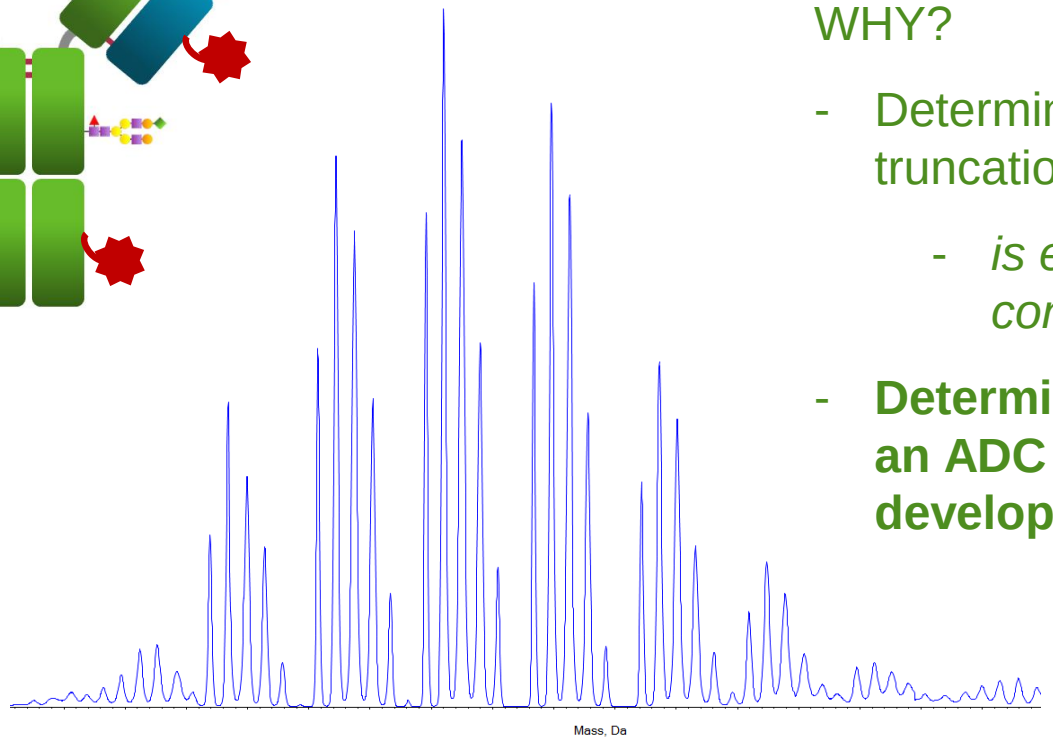
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Intact Protein ADC Analysis via MS

Main Workflows for Routine Characterisation











WHY?

- Determination of intact mass (observing truncations, and glycosylation)
  - *is everything alright before and after conjugation?*
- **Determination of drug-antibody-ratio (DAR) for an ADC – for each step of the way in ADC development**

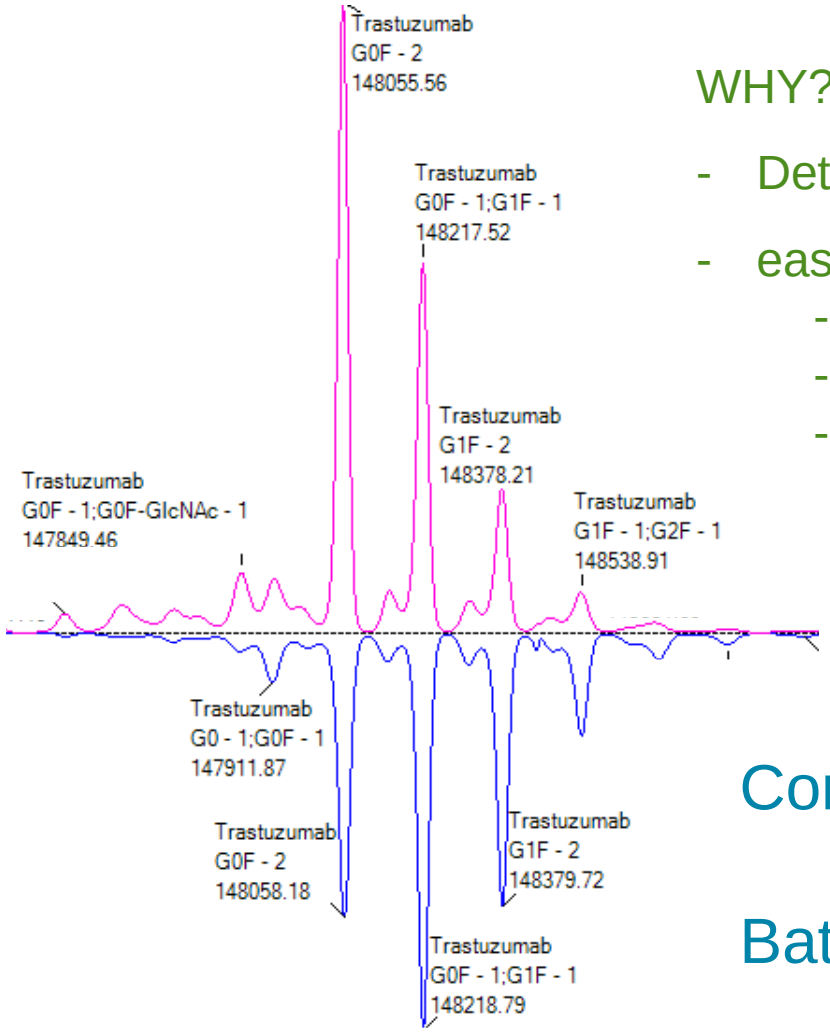
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Intact Protein Analysis via MS

Main Workflows for Routine Characterisation

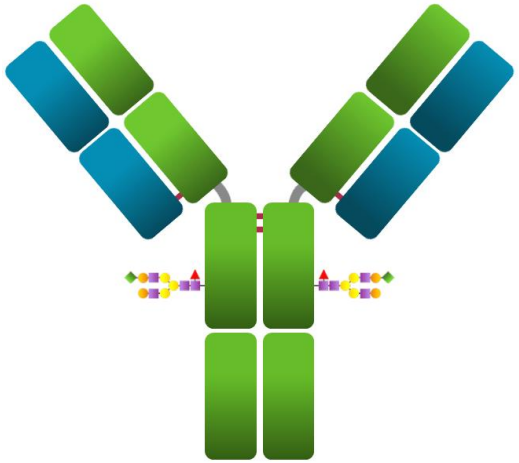


WHY?

- Determination of intact mass
- easily observe
  - Truncations (clipping)
  - Glycosylation pattern
  - Heterogeneity

Comparative analysis

Batch-to-batch assessments

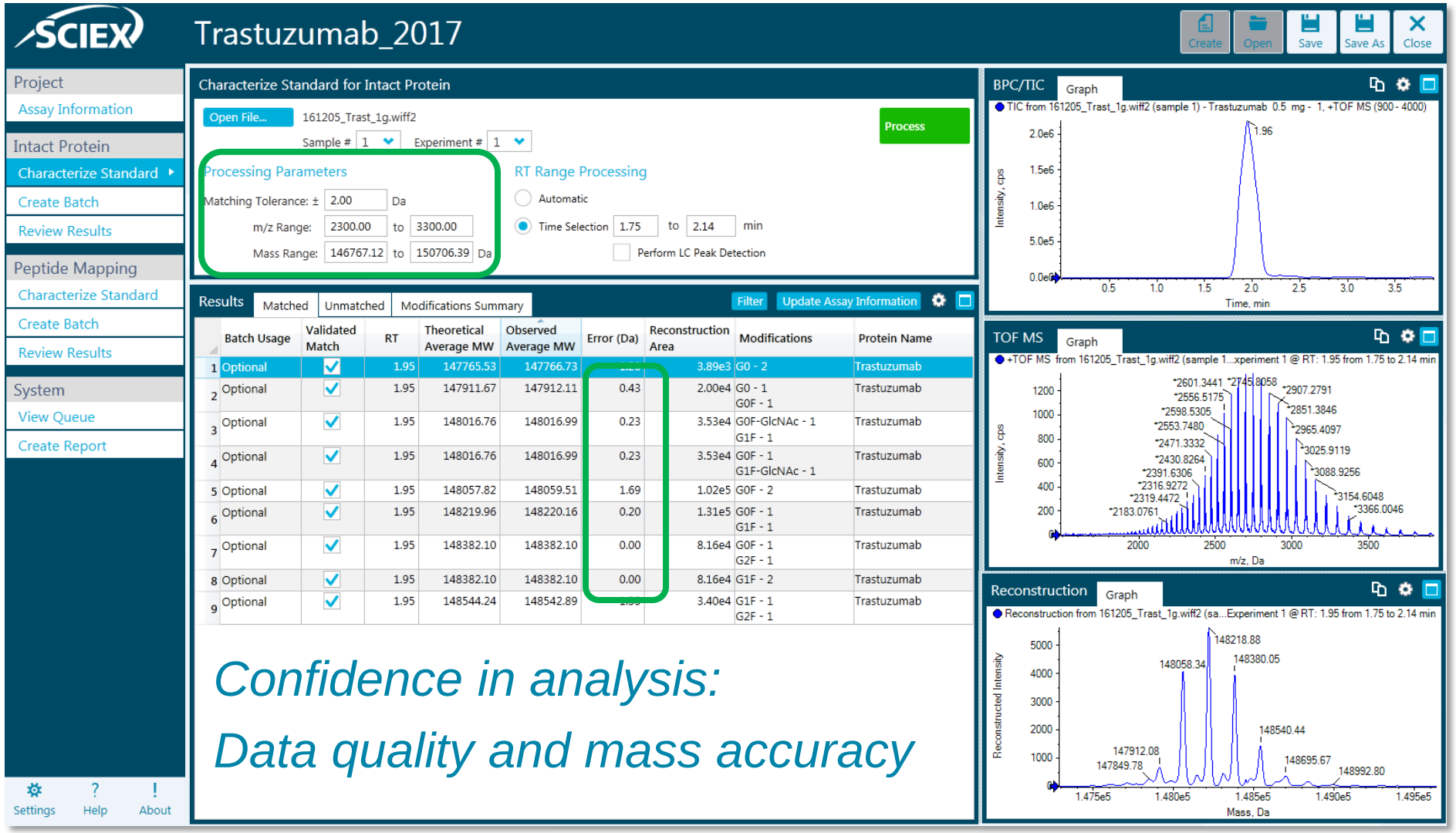


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Intact Protein Analysis – Characterise One…



Confidence in analysis:

Data quality and mass accuracy

Plenty of confidence in your product by a simple routine analysis

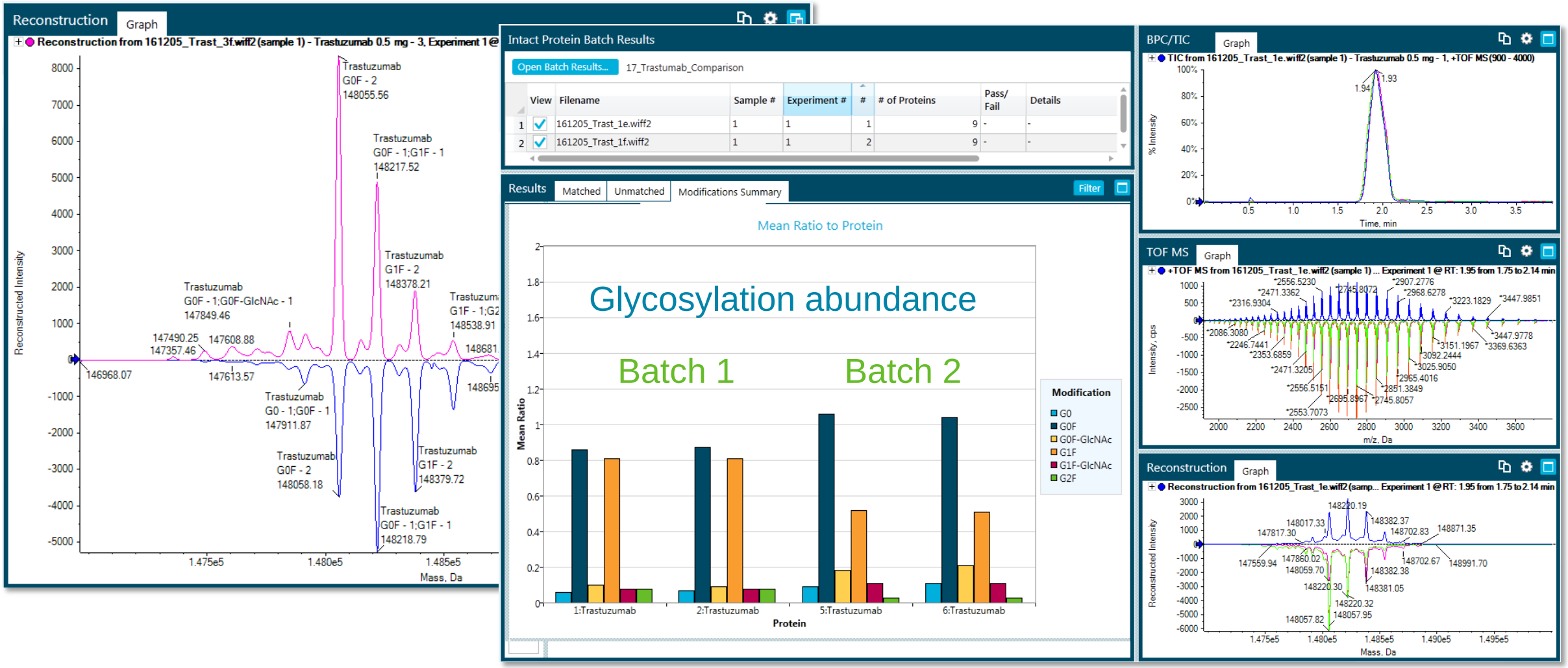
All the information available to you

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Intact Protein Analysis … Batch Analyse Many

Information - Comparability - Calculations



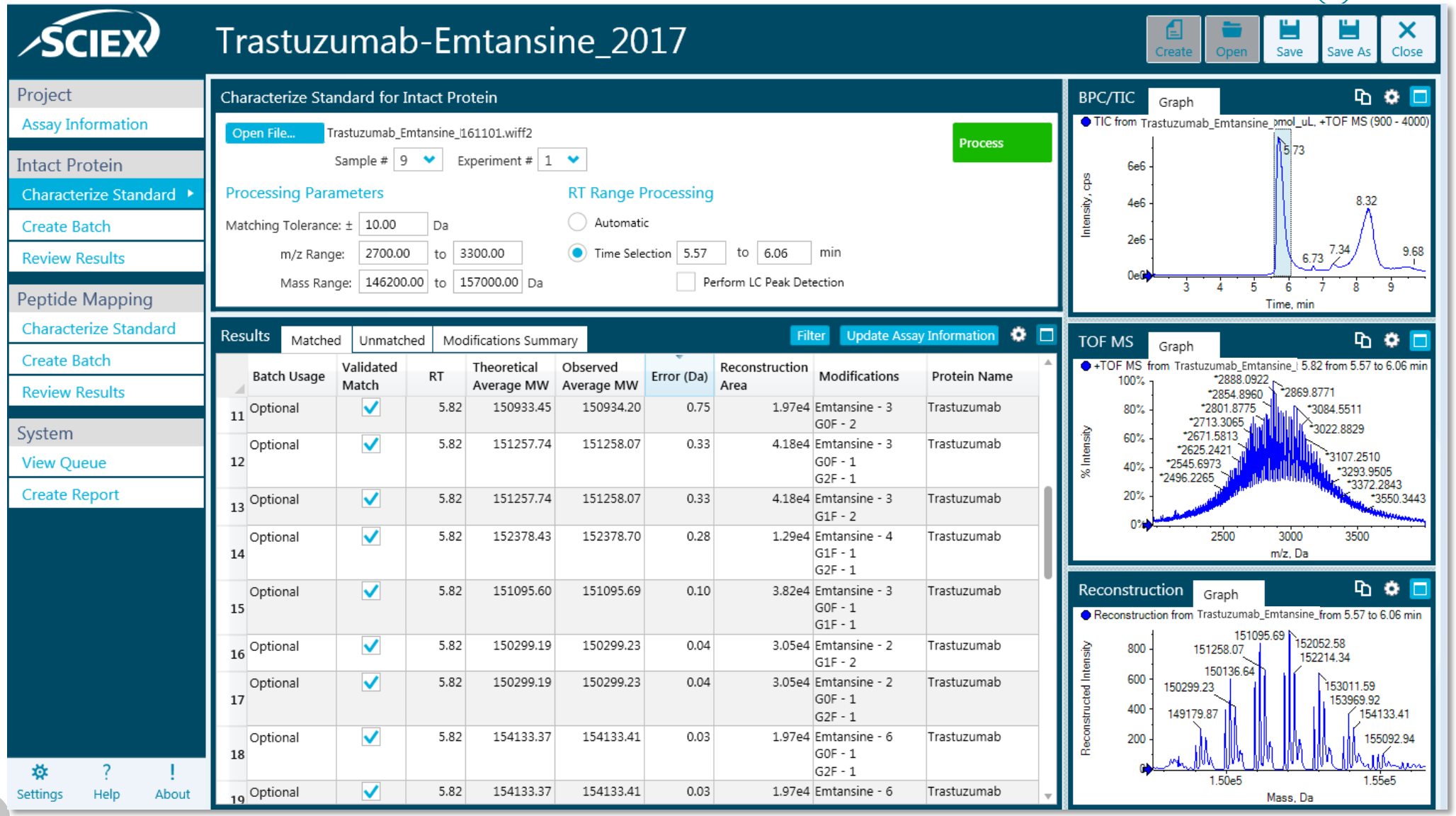
The example: two different batches of a mAb in duplicate injection

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Ado-trastuzumab emtansine (glycosylation on)

Example here a Lysine-linked non-deglycosylated ADC

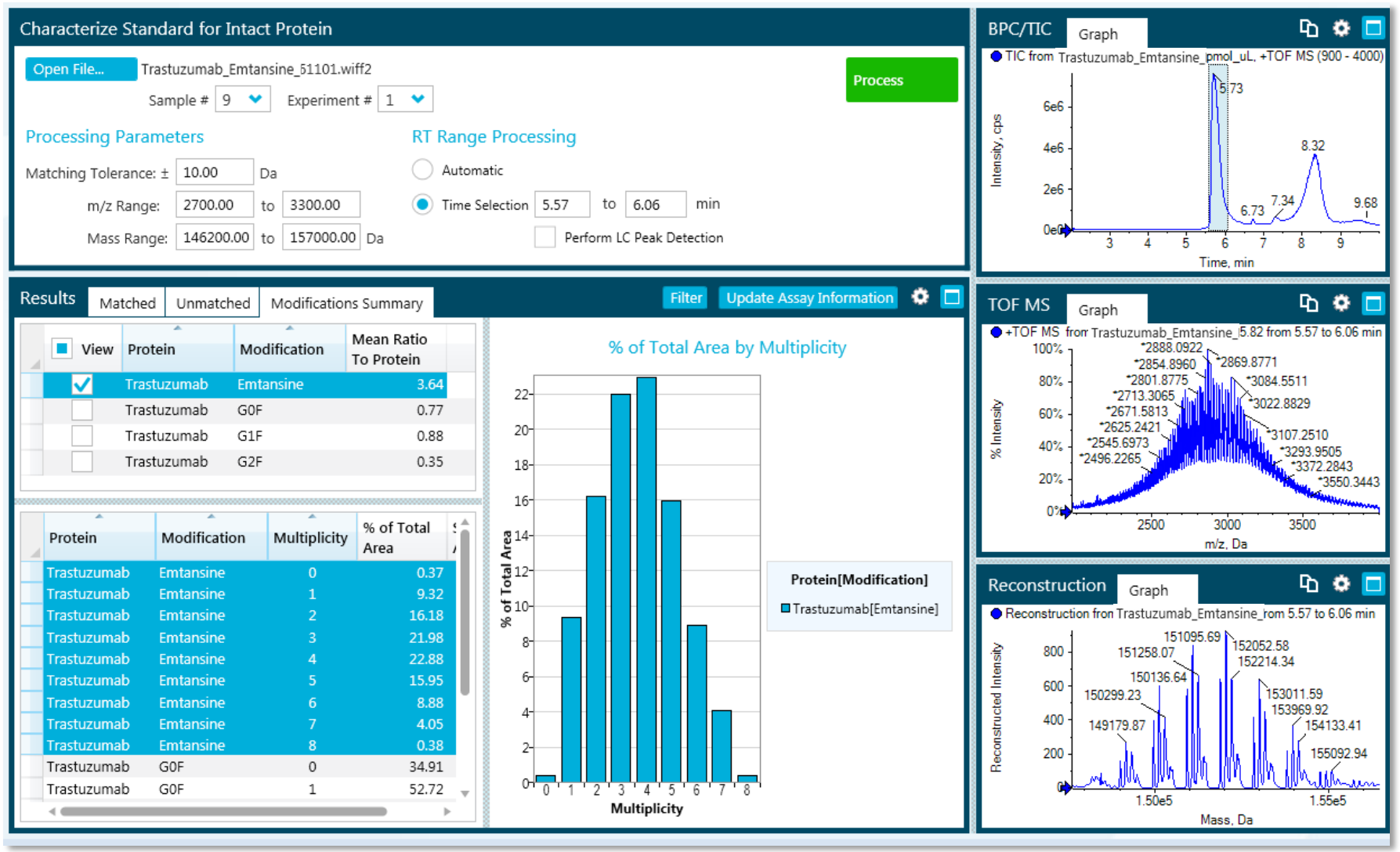


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Ado-trastuzumab emtansine (glycosylation on)

Example here a Lysine-linked non-deglycosylated ADC



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RUO- MKT-11- 5502 - B

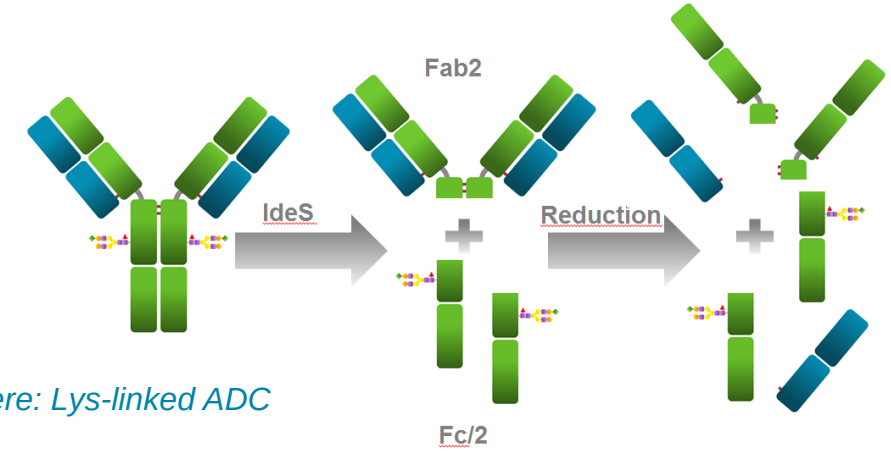
Subunit Analysis via MS

Main Workflows for Routine Characterisation



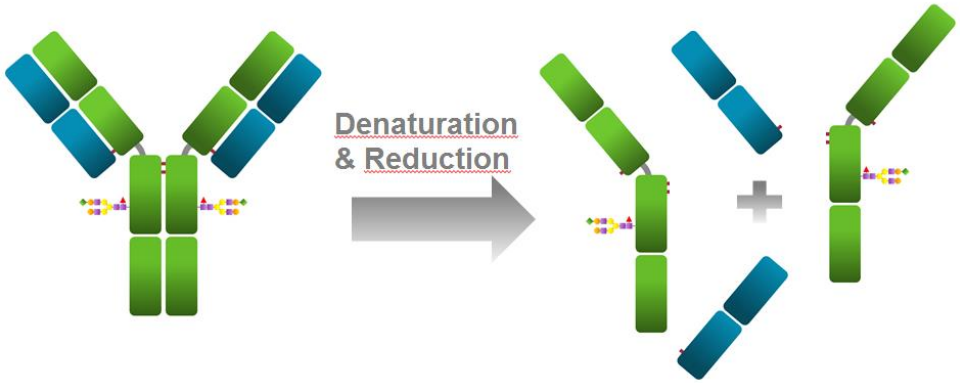
A) Enzymatic (IdeS/ FabRICATOR) digestion into large fragments

- Optional followed by: Denaturation and Reduction



Figures here: Lys-linked ADC

B) Denaturation & Reduction into heavy and light chains



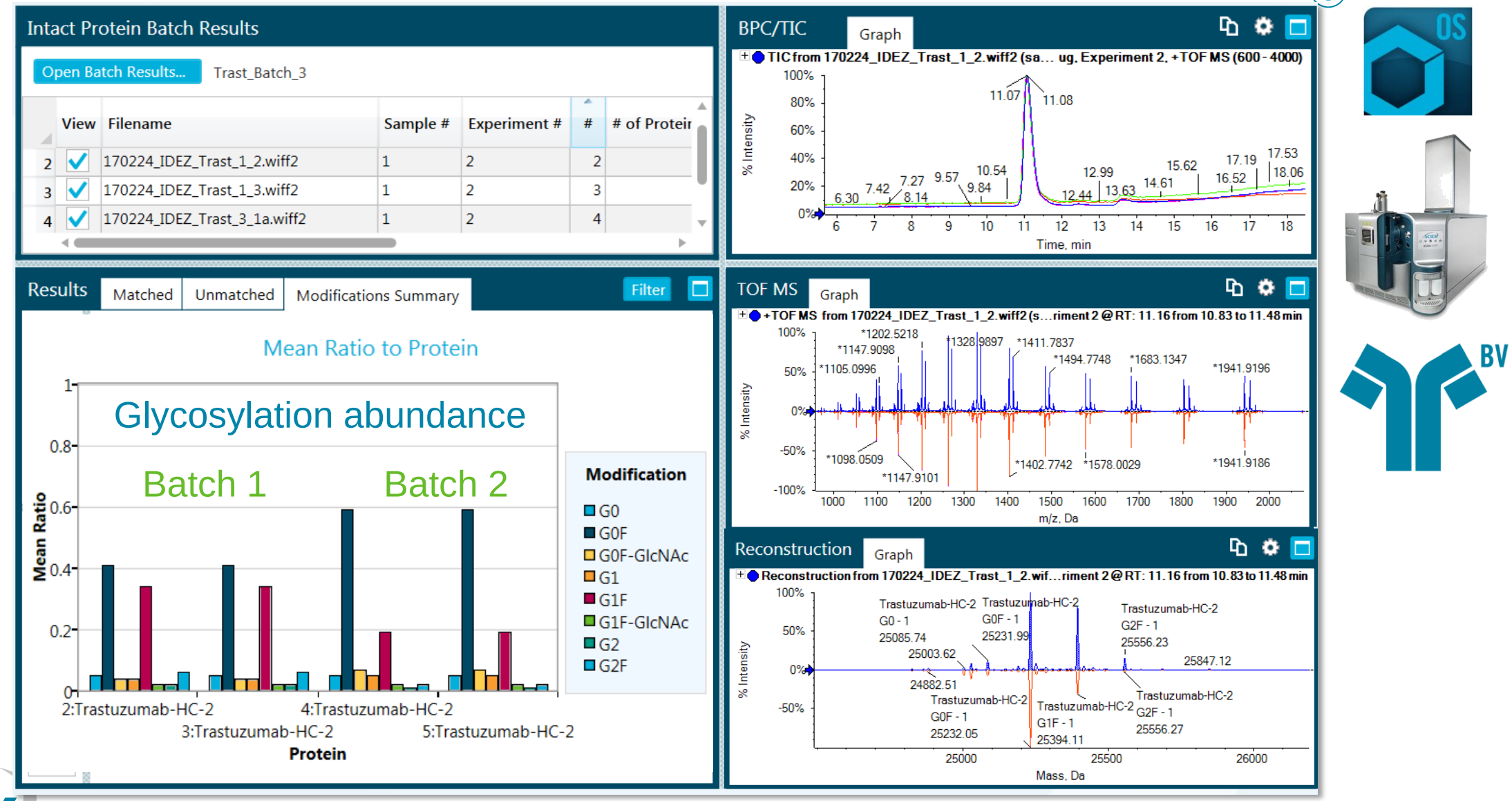
WHY sub-unit analysis?

- Deeper insight into
  - modifications (inc. Approximate Location)
  - Reducing complexity of the data

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Subunit Analysis – mAb IdeS FC Heavy Chain

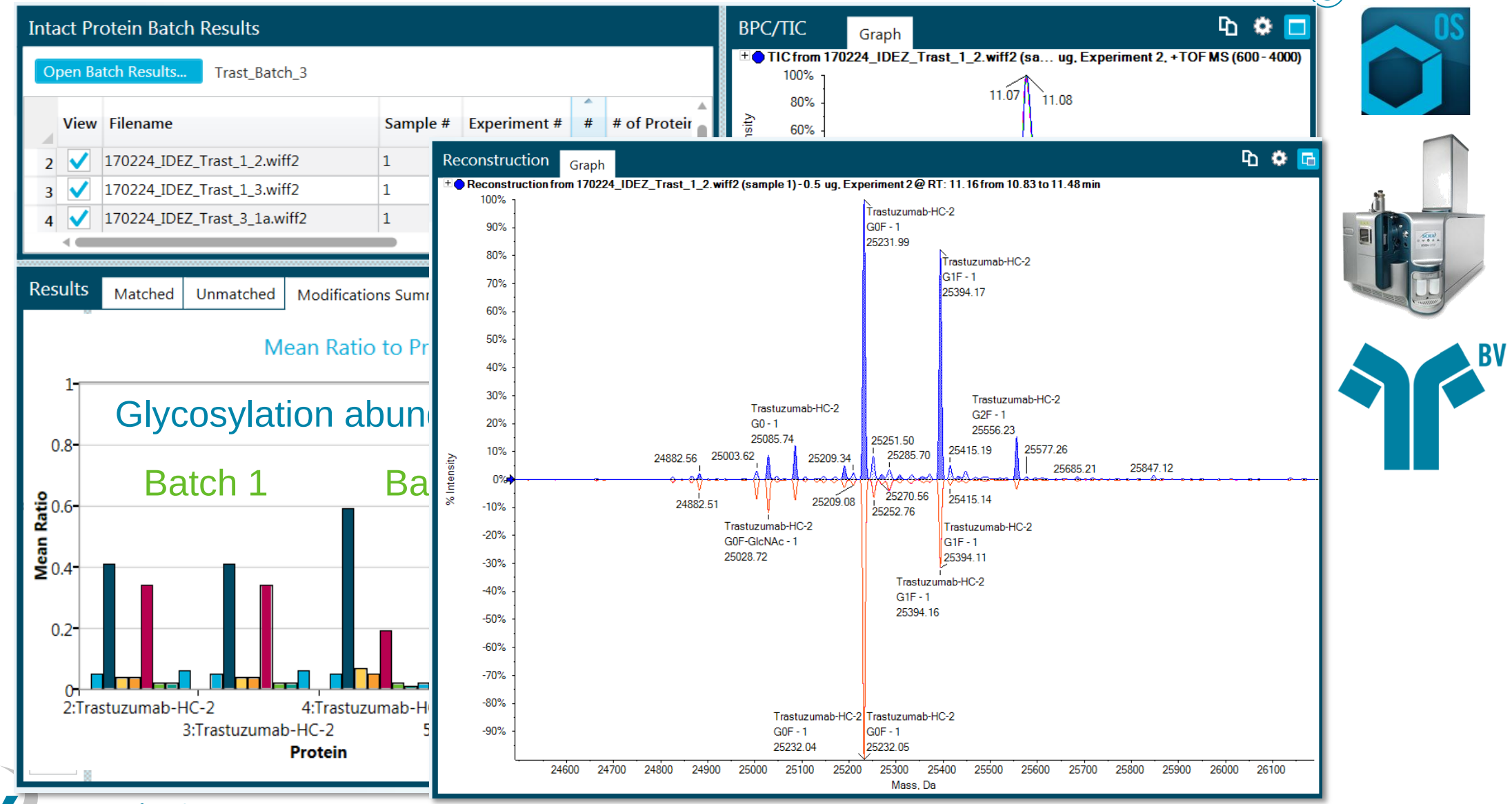
The example: two batches of an unconjugated, glycosylated mAb in duplicate injection



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Subunit Analysis – mAb IdeS FC Heavy Chain

The example: two batches of an unconjugated, glycosylated mAb in duplicate injection



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Peptide Mapping





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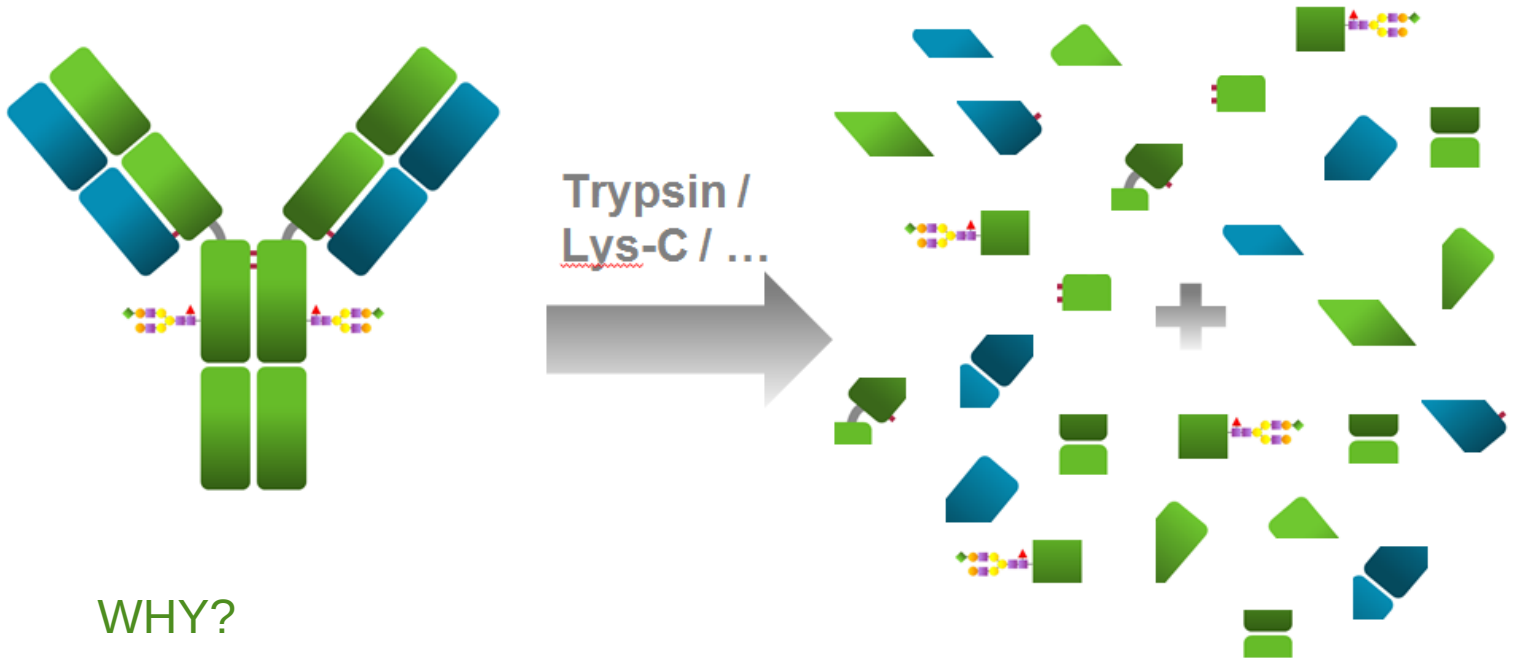
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Peptide Mapping via MS

Main Workflows for Routine Characterisation

- Denaturation and optional Reduction & Alkylation
- Enzymatic digestion into peptides





WHY?

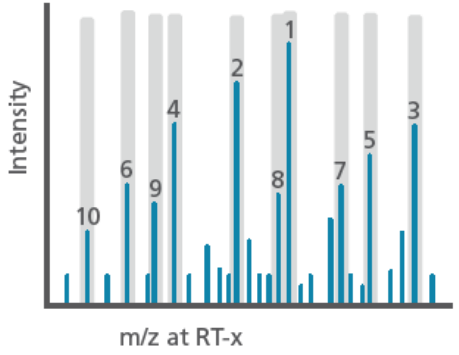
- Determination of modifications: quality attributes (QA) which have an impact on safety on efficacy
- Confirmation of disulfide bonds

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Peptide Mapping Acquisition Modes

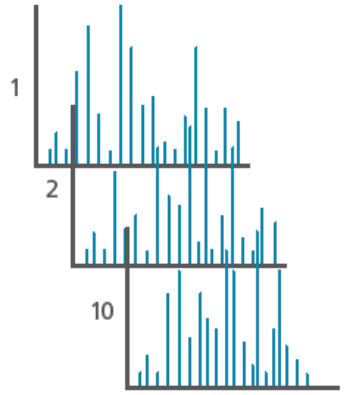
IDA Workflow

Information Dependent Triggering



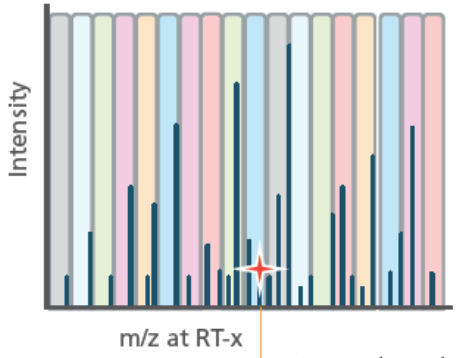
IDA Top 10-15

High-Resolution MS/MS spectra of **Selected** Precursors

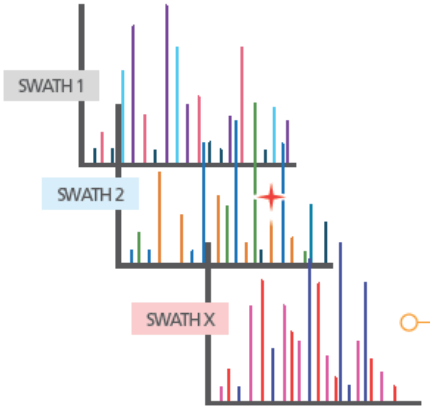


SWATH® Acquisition

Information Independent Triggering



High-Resolution MS/MS spectra of **ALL** Precursors



Low abundance peptide with PTM acquired by SWATH, but missed by IDA

Simple, Automated Processing

100% sequence coverage from IDA



100% sequence coverage from SWATH Acquisition



Gain additional insights into low abundance PTMs







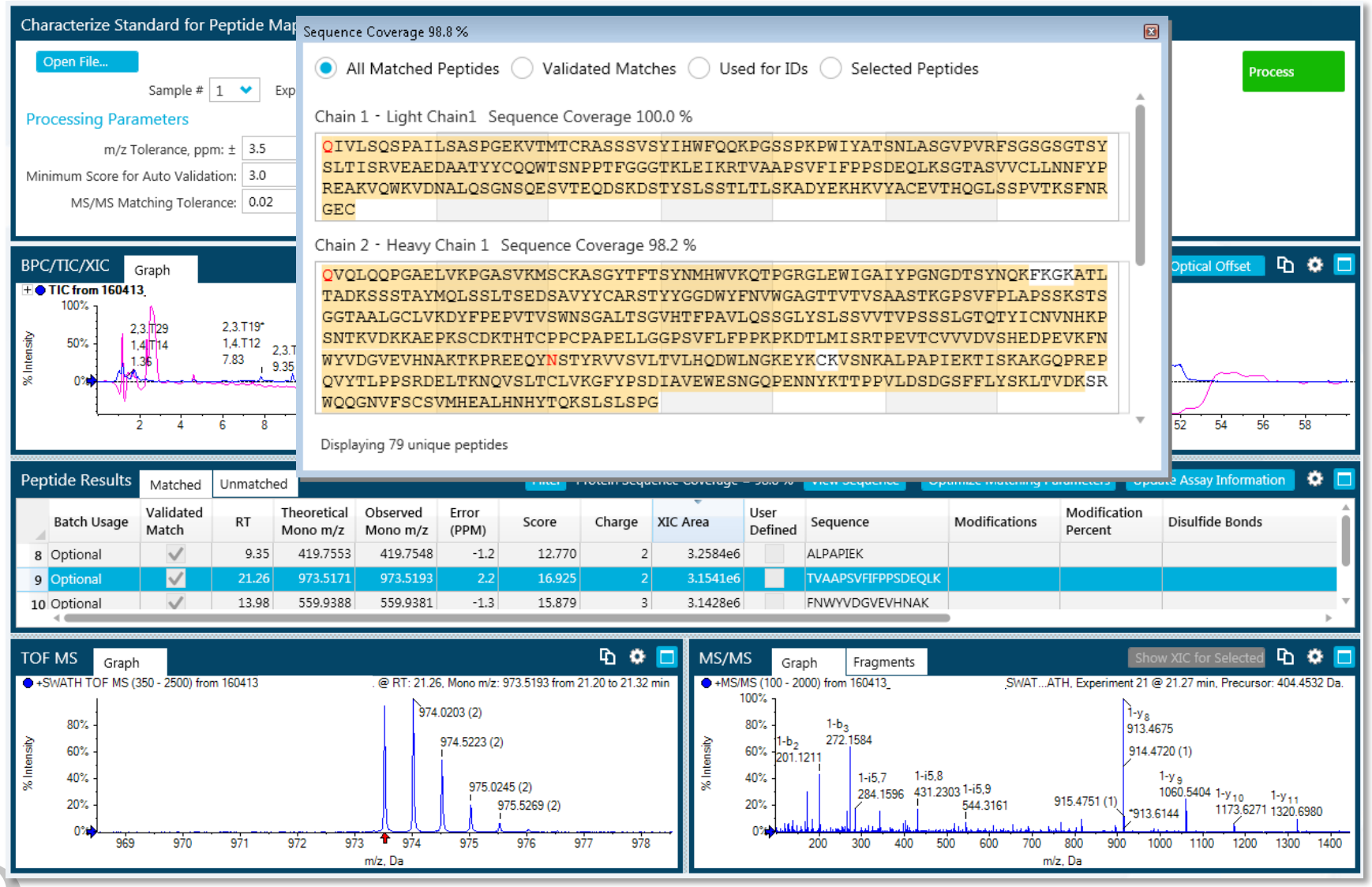


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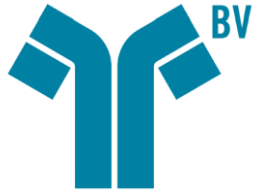
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Peptide maps in a flash





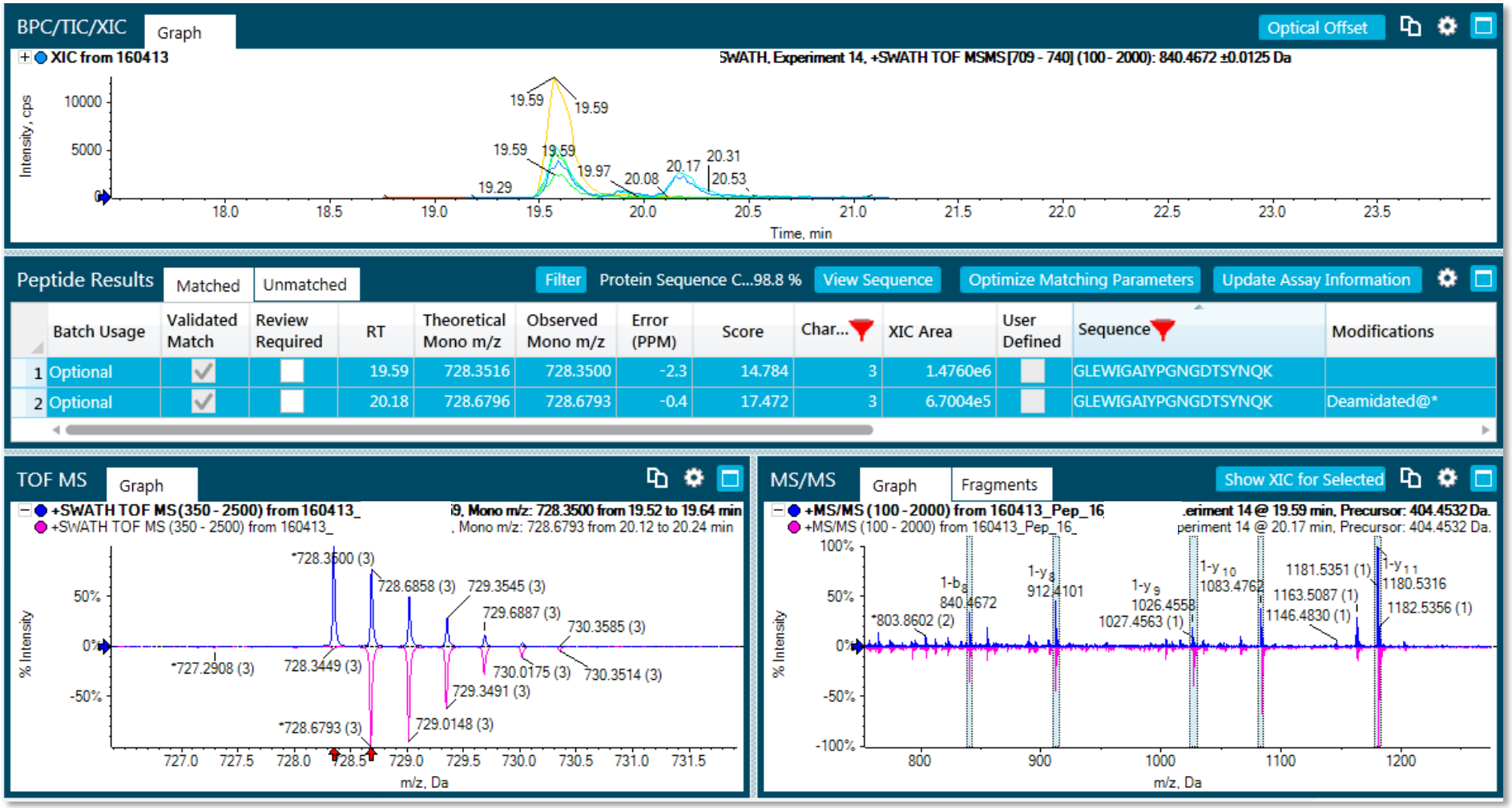




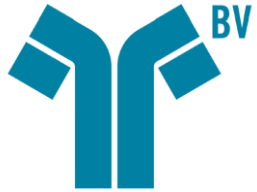
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SWATH Acquisition and Processing for Ultimate Confidence



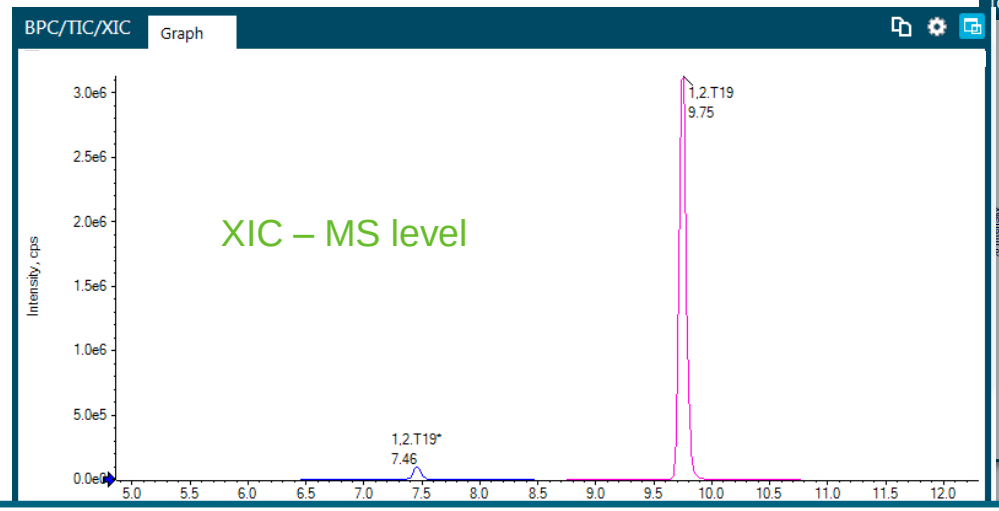


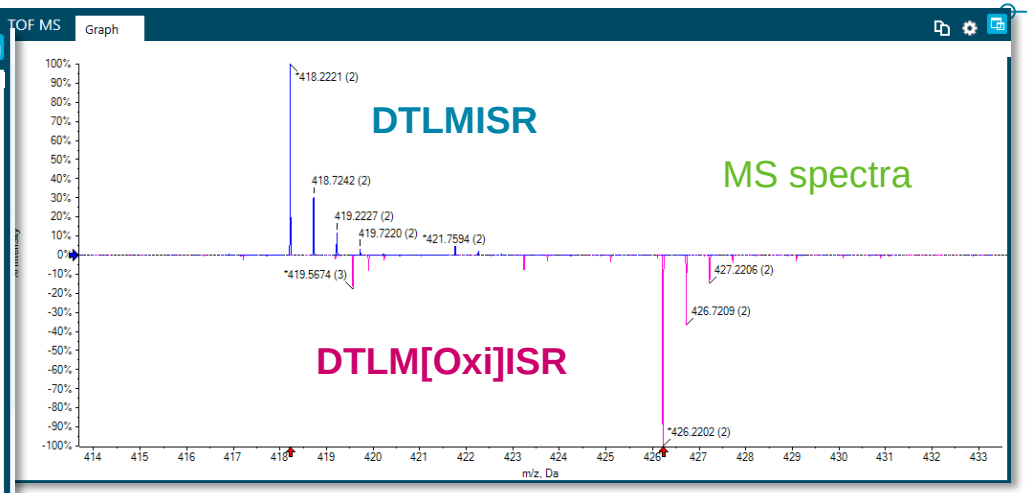


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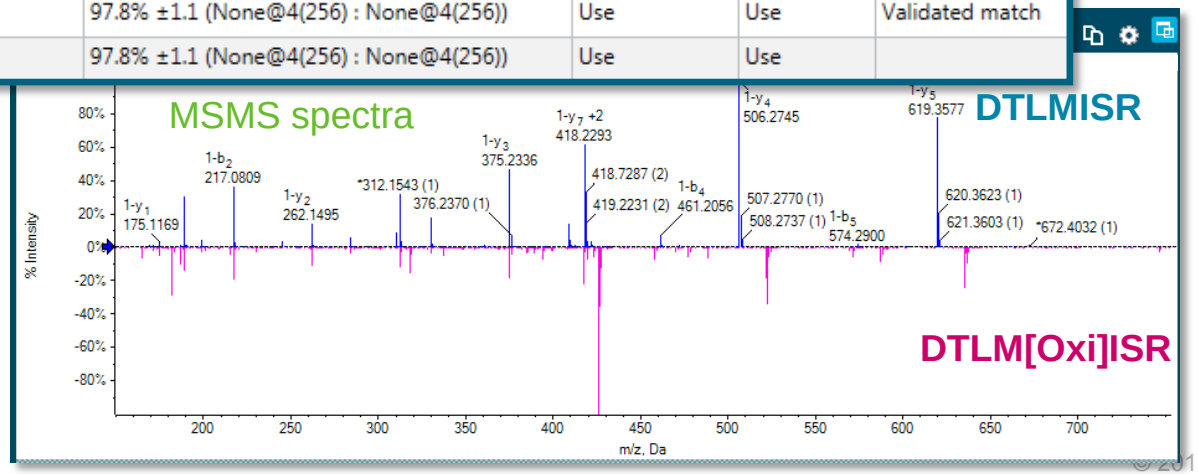
Modification ratios





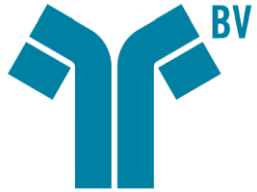
Peptide Details

	Validated Match	RT	Observed Mono m/z	Charge	XIC Area	Sequence	Modifications	Modification Percent	Use for Quant	Use for ID	Notes
1		7.45	851.4295	1	6.7285e4	DTLMISR	Oxidation@4(256)	2.2% ±1.1 (Oxidation@4(256) : None@4(256))	Use	Use	
2	✓	7.46	426.2202	2	4.7631e5	DTLMISR	Oxidation@4(256)	2.2% ±1.1 (Oxidation@4(256) : None@4(256))	Use	Use	Validated match
3	✓	9.75	418.2221	2	1.3938e7	DTLMISR		97.8% ±1.1 (None@4(256) : None@4(256))	Use	Use	Validated match
4		9.75	835.4351	1	5.9211e6	DTLMISR		97.8% ±1.1 (None@4(256) : None@4(256))	Use	Use	









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Click. Compare. Report.









Intact Protein Batch Report

Project	RituximabPDB
Batch Name	160407_BatchOfThree_2

Matched Proteins Applied Filters	
Unmatched Proteins Applied Filters	

Assay Information Settings

Cysteine Modifications Can Replace Disulfide Bonds	Off
Reduced Protein Form	Off

Processing Settings

Matching Tolerance	15.00 Da
Start m/z	2500.00
Stop m/z	3500.00
Start Mass	145185.89 Da
Stop Mass	149793.28 Da
RT Range Processing	Time Selection
Start Time (min)	5.86
Stop Time (min)	6.10
Perform LC Peak Detection	Off

Chromatographic Data Processing

Peak Threshold	≥ 5.00%
Gaussian Smoothing	0.90 points
Number of TOFMS Spectra to Combine	± 3 scans

Protein Results

Relative Result Threshold	≥ 5.00%
Maximum Combined Modifications for Any Protein	20

Chromatogram Peak Labeling

Label Matching Tolerance:	0.10 Minutes
Display Labels For:	All Peaks

Batch Processing Pass/Fail Criteria

	RT Tolerance	1.00
On	Reconstruct Area Tolerance	40.0%
Off	Required Protein Tolerance	-%
Off	Restricted Protein Tolerance	-%

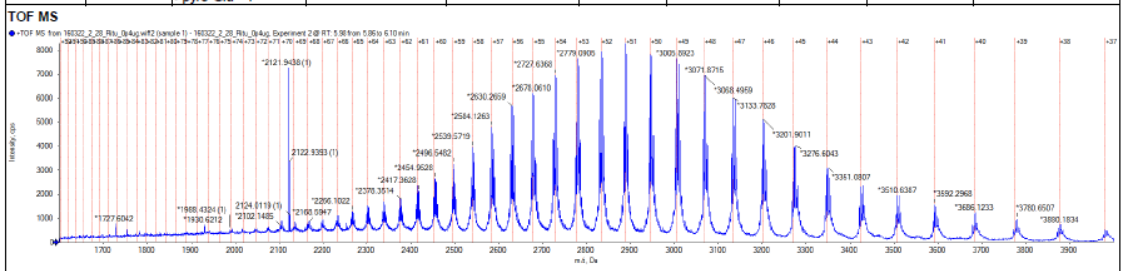
Summary

File Name	Sample #	Experiment #	#	# of Proteins	Pass/Fail	Details
160322_2_10_Ritu_1p0ug.wiff2	1	2	1	8	Fail	Reconstruc for one or r
160322_2_28_Ritu_0p4ug.wiff2	1	2	2	8	Pass	-
160322_2_29_Ritu_0p4ug.wiff2	1	2	3	9	Pass	-

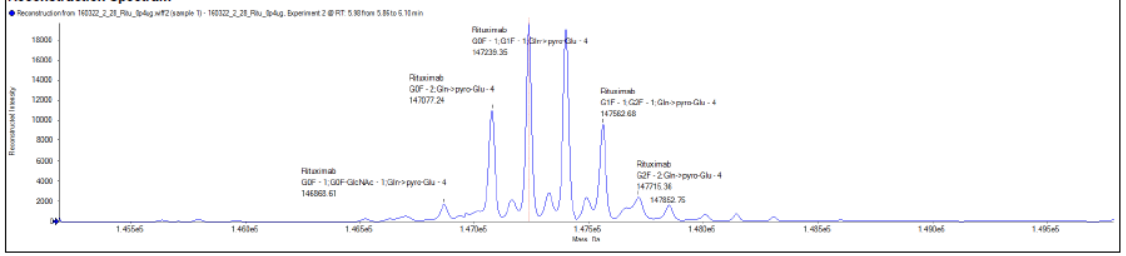
Detailed Results: 160322_2_28_Ritu_0p4ug.wiff2 - Rituximab

Batch Usage	Validated Match	Modifications	Observed Average MW	Theoretical Average MW	Error (Da)	Reconstruction Area	Abundance Ratio	RT
Optional	True	G0F - 1;G1F - 1;Gln->pyro-Glu - 4	147239.35	147238.73	0.62	6.00e+05	1.000	5.98

TOF MS



Reconstruction Spectrum

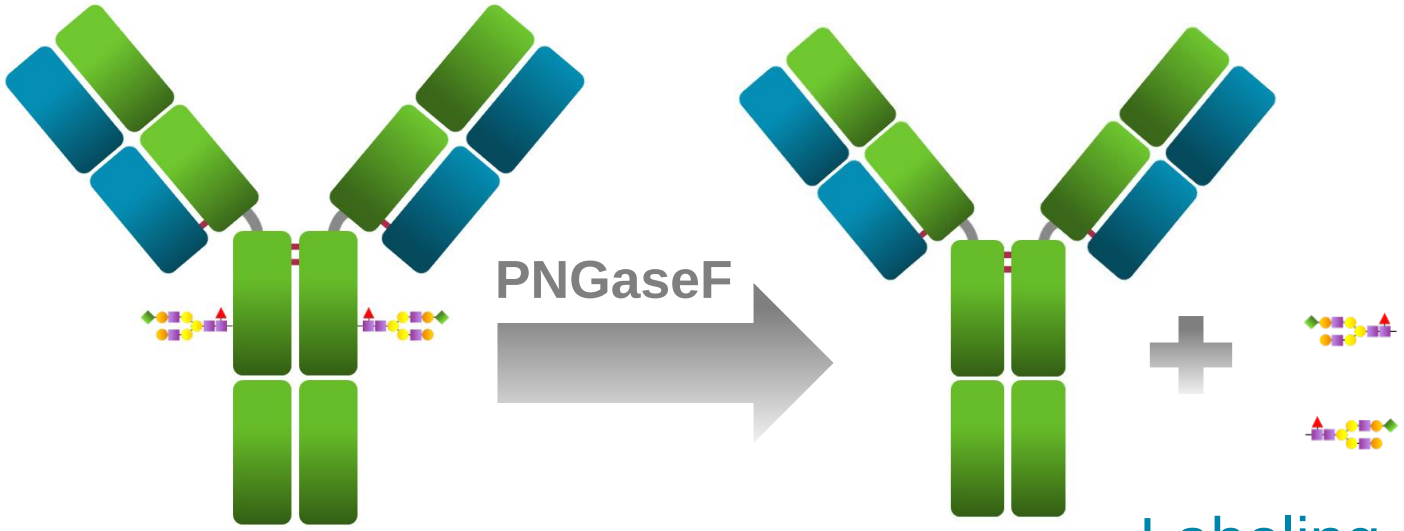


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Main Workflows for Characterization IV

Released Glycans Analysis via MS



WHY?

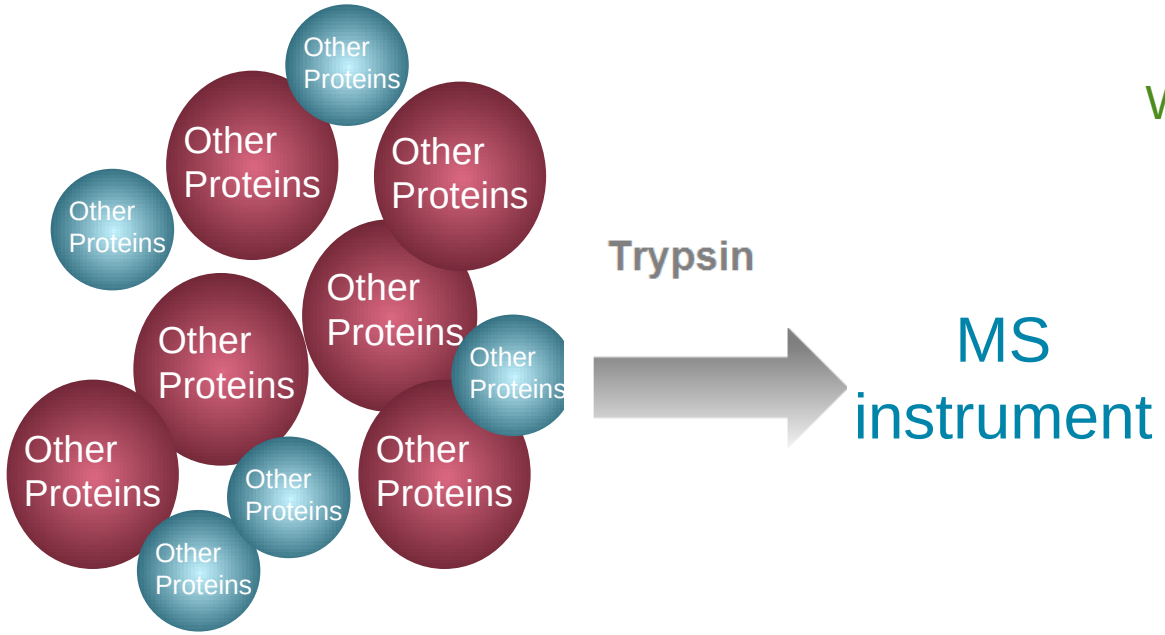
- Identification and Characterization of N-linked Glycans
- Quantitation of Glycans

Labeling of Glycans with e.g. 2-AB

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Additional Workflows for Characterization

Host Cell Protein (HCP) Analysis via MS



WHY?

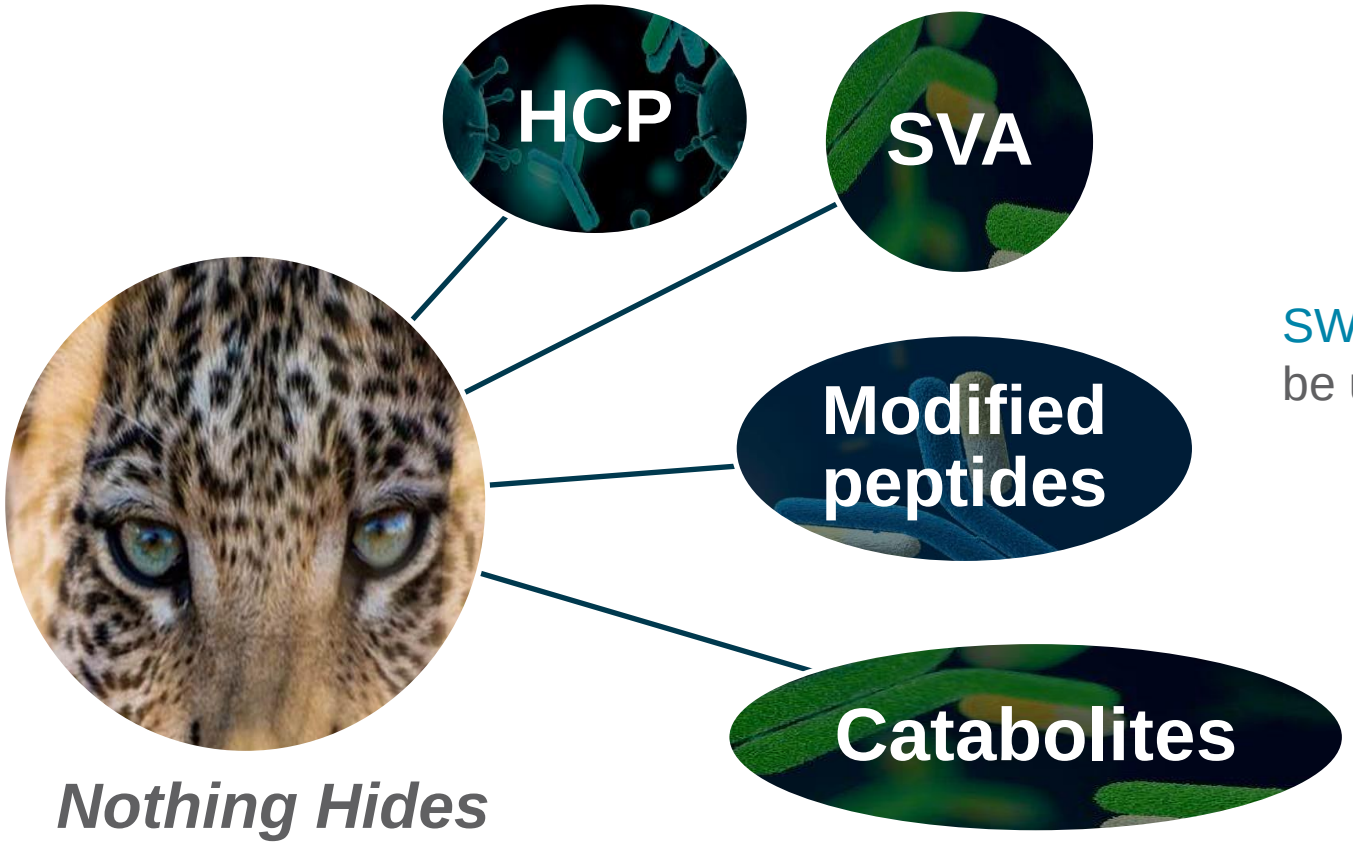
- Proteins derived from the Host Cell are not removed completely during Purification Processes
- HCP could harm the Patient when provoking Immunogenic Responses (CQA!)

→ Optimizing Purification and ensuring Safety of a Biopharmaceutical Product

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What is SWATH® and why is it useful for BioPharmaceutical Characterisation?

Nowhere to run, nowhere to hide...



Nothing Hides from SWATH® Acquisition

SWATH® Acquisition can be used for multiple tasks

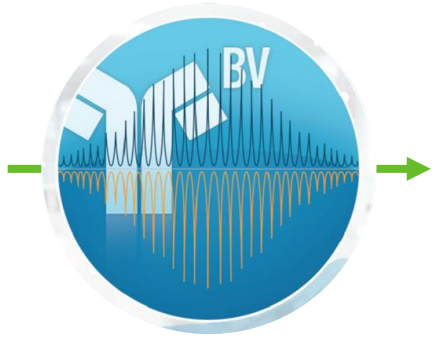
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Confidence for Your Characterization on all Levels

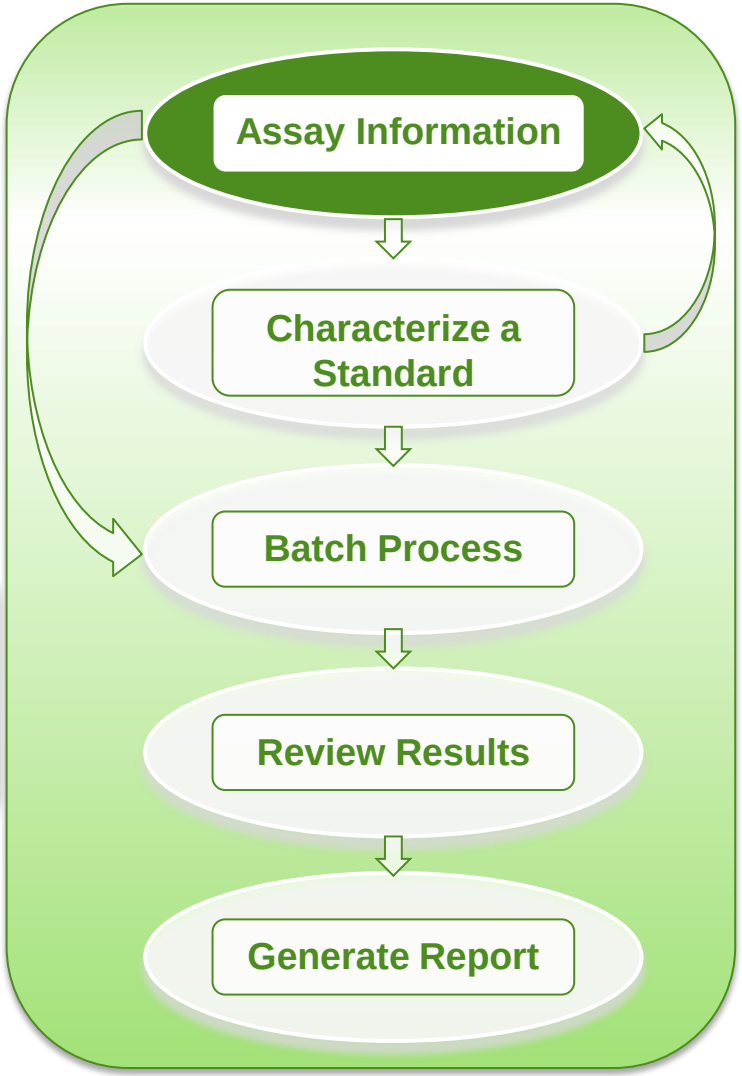
With BioPharmaView™ 2.0 Software



Data Acquisition



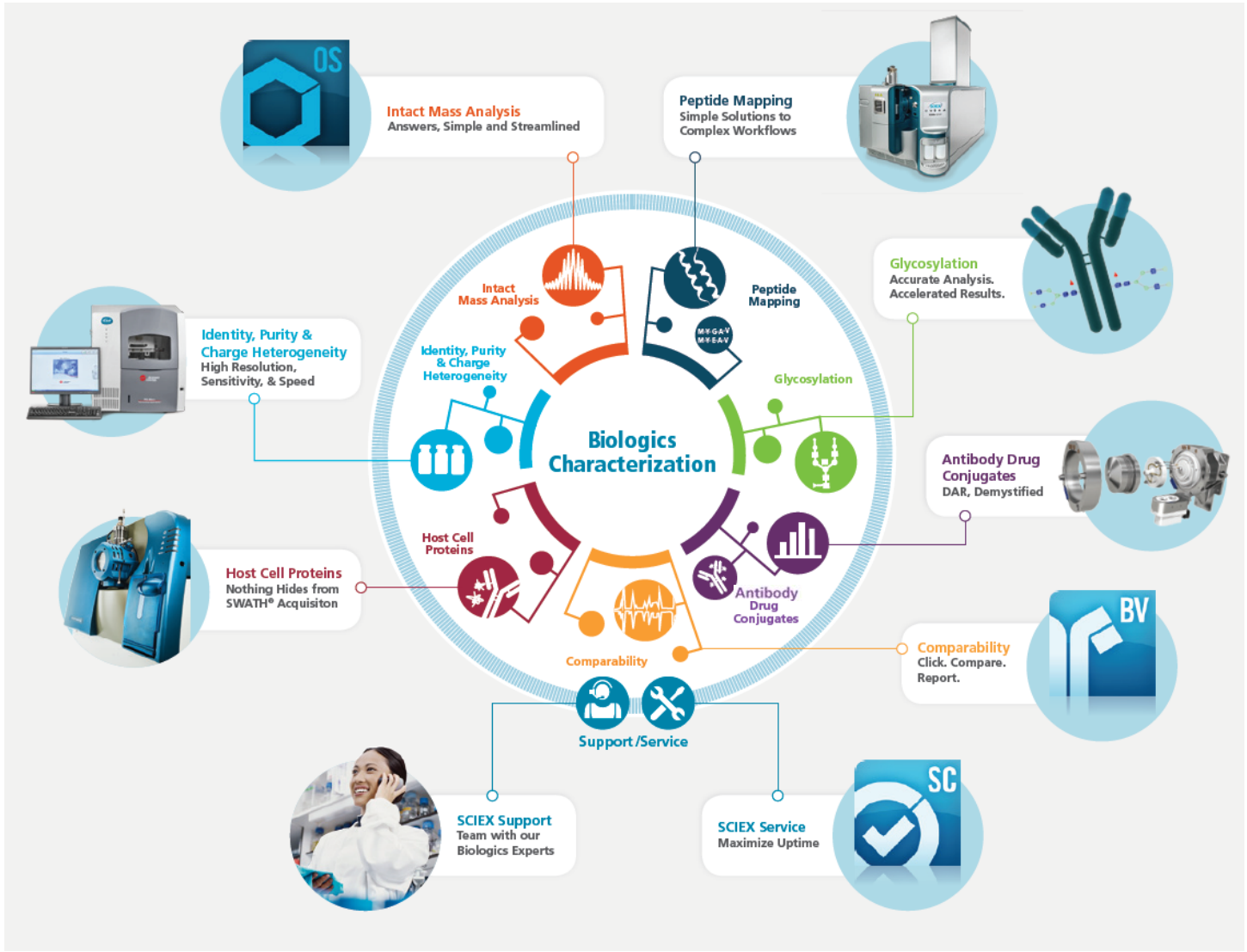
Powerful new solution for automation of core biologics development workflows



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Unique Solutions to Meet Biologics Characterization Challenges





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감사

Благодаря!

Tesekkurler

Kiitos

ขอบคุณ

متشكرم

Tak

Danke

Dziękuję

Thanks

Děkuji

Niżżik ħajr

Salamat po

Gracias

ありがとう

Спасибо

cảm ơn

Paldies

дєкую

धन्यवाद

Thank you for your attention

谢谢

ধন্যবাদ

Гялайлаа

Tapadh leibh

Hvala

Obrigado

Ačiu

תודה רבה

Köszönöm

Merci

(بهت) شکریه

Mulţumesc

감사

Ευχαριστω

Þakka þér

Dank

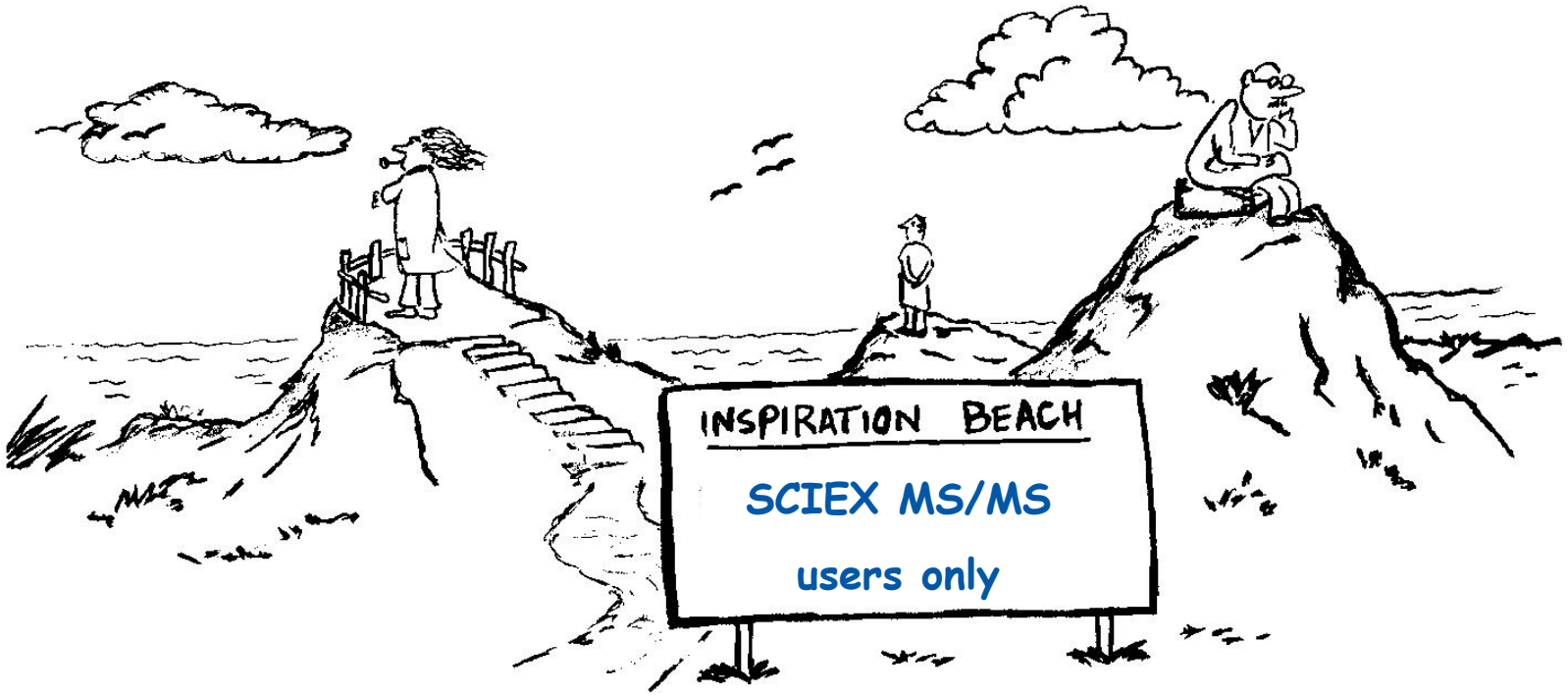
Tack

Děkuji

Grazie

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Thank you for listening !



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