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RUO-

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The Simpler and Faster Solution for Routine Biopharmaceutical Characterisation

Alexandre Paccou, Sr Support Manager, EMEA

Overview

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

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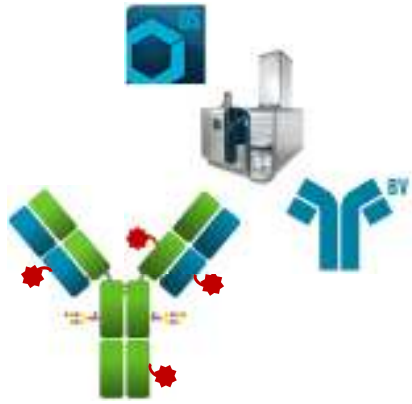
ADC Complexity – analysed routinely

- Assess and ensure the product
  - Quality in and after process of conjugation
  - Stability *in vitro* and *in vivo*
  - Conjugation sites – site occupancy and payload
  - Linker chemistry – stability

Need to know your mAb – for every batch

- Three Main Workflows for ADC Characterisation
  - Intact protein fingerprint of the whole product
  - Subunit analysis – insight on localisation – simpler data
  - Peptide mapping – drilling deeper on the site occupancy
- Easy to use – get the results – make the right conclusions
  - Inviting you to analyse – you can handle this



The need for information – a lot of it!

*Complexity is brought upon the antibody heterogeneity (glycosylation etc.) and the **multiple** possible sites of conjugation*

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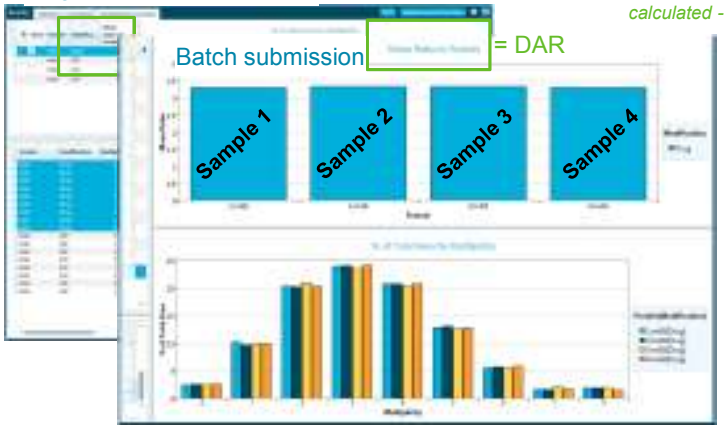
ADCs – DAR demystified

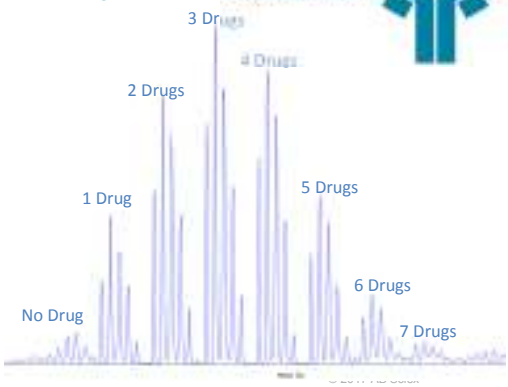
- Drug–to–Antibody Ratio
- Compound Complexity is not an issue

Sample here is non-deglycosylated Lys-linked Antibody-Drug-conjugate, from which we can get the DAR automatically calculated - for multiple samples

Single sample Calculations



Single sample reconstruction



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ADC characterisation by Mass Spectrometry made simple

SCIEX X500B QTOF System powered by SCIEX OS Software

X500B

SCIEX X500B QTOF system

SCIEX ExionLC™ AC system

SCIEX OS software

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SCIEX X500B QTOF System- Inviting you to analyse

The Beauty – in appearance and in the data quality

Engineered for simplicity
Optimal performance delivered through adjustment of only 2 voltage variables

TwinSpray
An independent calibrant delivery path for reliable auto-calibration

Service accessibility
Easy QJet® ion guide access for fast and efficient maintenance and single three stage split flow pump for increased system uptime

Minimized footprint
The benchtop stature (110 x 57 x 112 cm)* occupies less lab space than any other HRMS system on the market

Integrated calibration
Maintains mass calibration through long runs without effect on sample flow

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SCIEX OS - inviting you to analyse

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SCIEX OS - Users

Basic - For Routine User

Advanced - For Method Developer / Lab Manager

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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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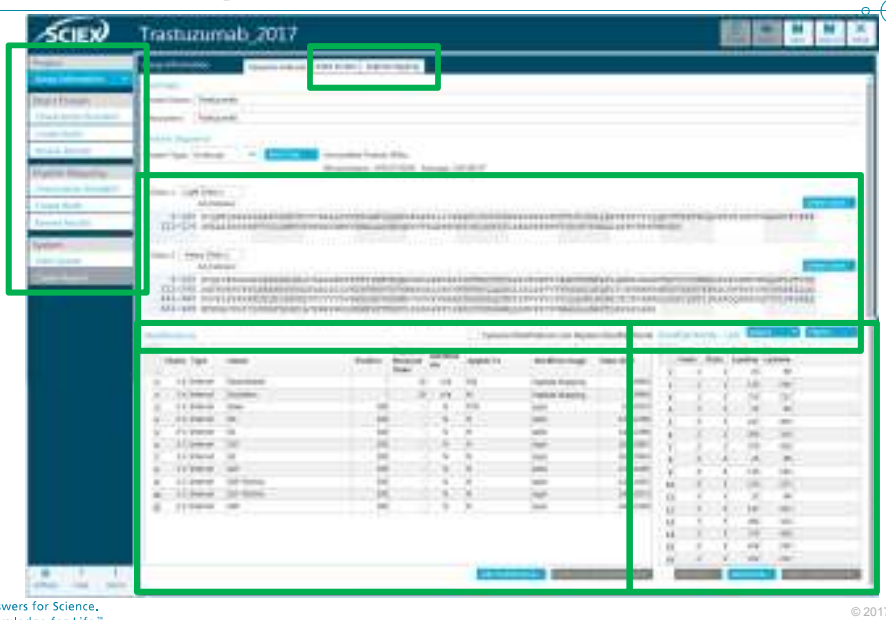
Easily Define Your Experiment – with ease



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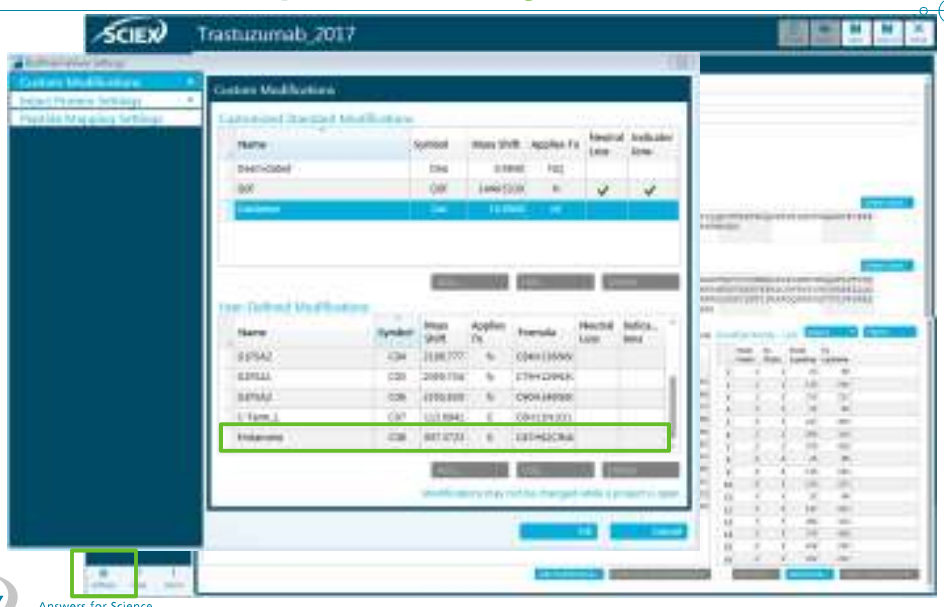
Easily Define Your Experiment for your ADC



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Intact Mass and Subunit Analysis

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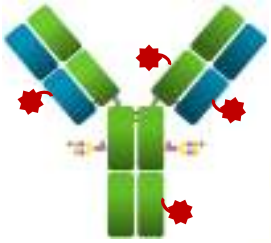
Intact Protein Analysis via MS

Main Workflows for Routine Characterisation



WHY?

- Determination of intact mass (observing truncations, and glycosylation)
  - *is everything alright before conjugation?*
- Determination of drug-antibody-ratio (DAR) for an ADC

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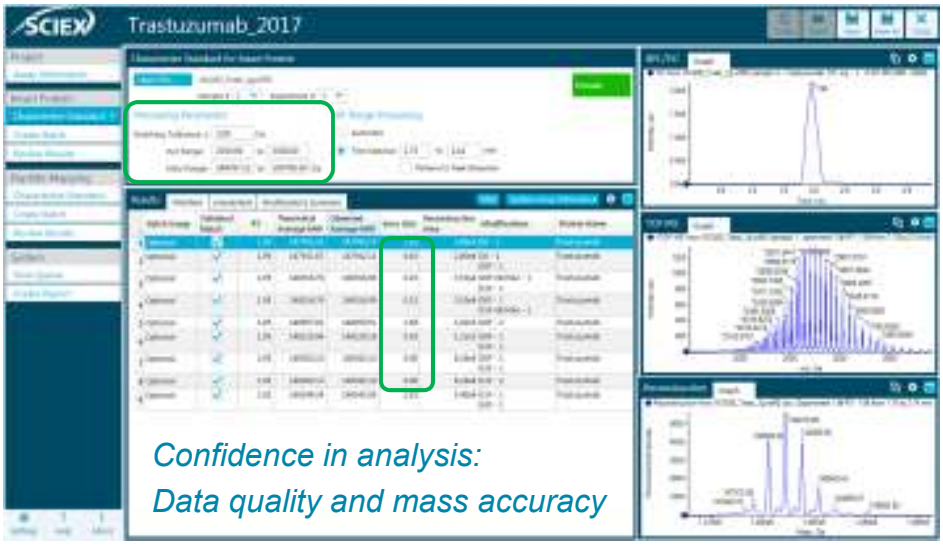
Intact Protein Analysis – Characterise One…

The example: an unconjugated, glycosylated mAb



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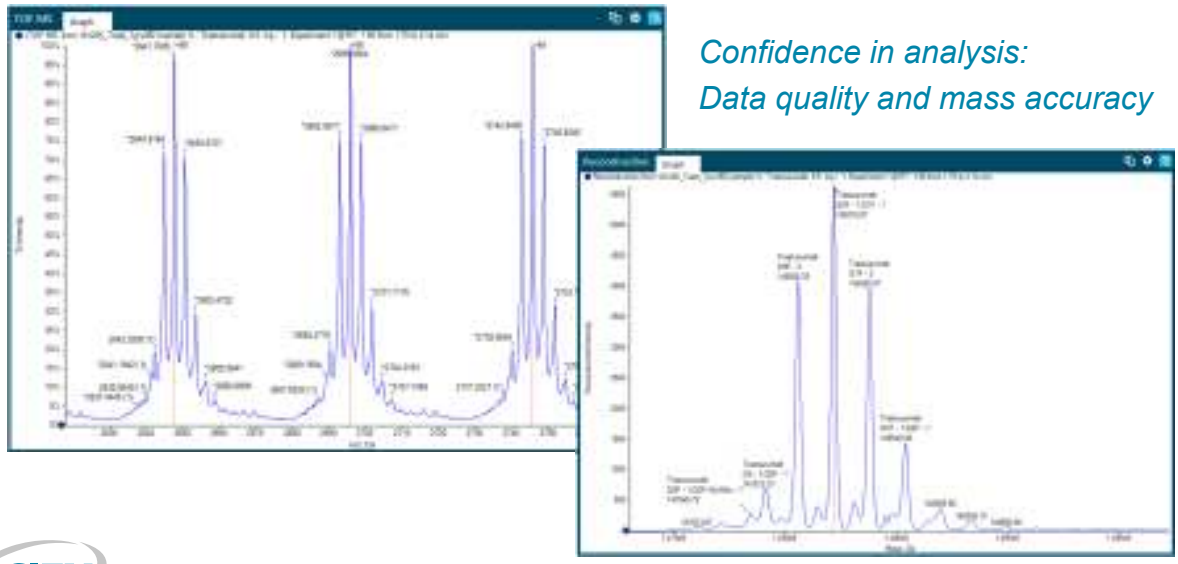

Confidence in analysis:
Data quality and mass accuracy

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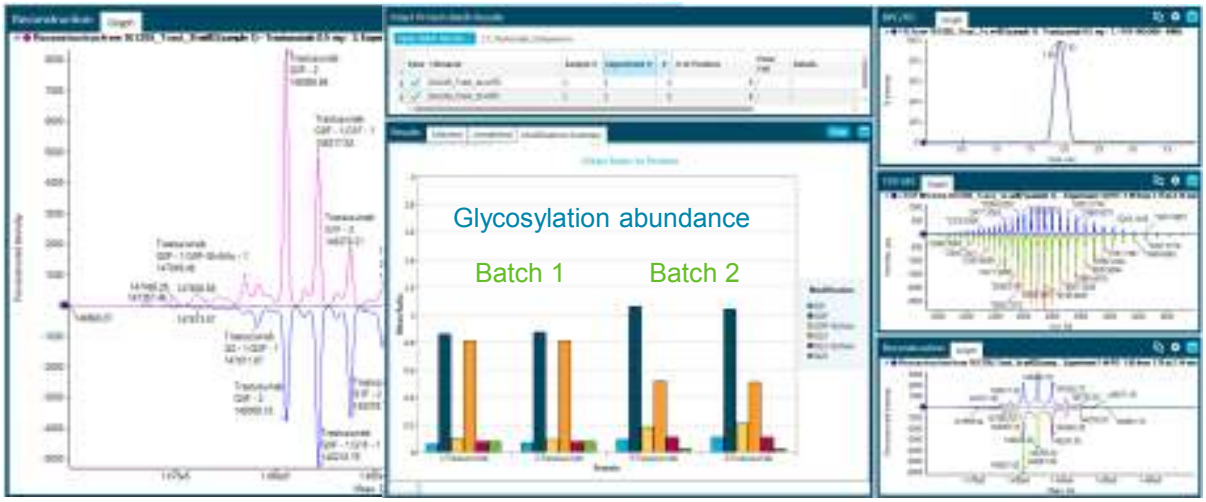
Intact Protein Analysis … Batch Analyse Many

Information - Comparability - Calculations



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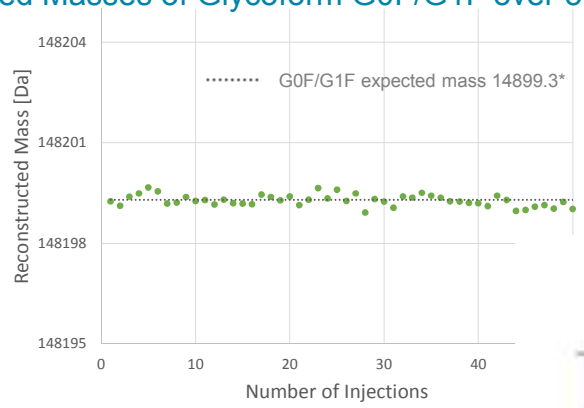
The example: two batches of an unconjugated, glycosylated mAb in duplicate injection

Excellent Mass Accuracy for Highly Reproducible Results

Reconstructed Masses of Glycoform G0F/G1F over 50 Injections



*NIST Monoclonal Antibody Reference Material 8671

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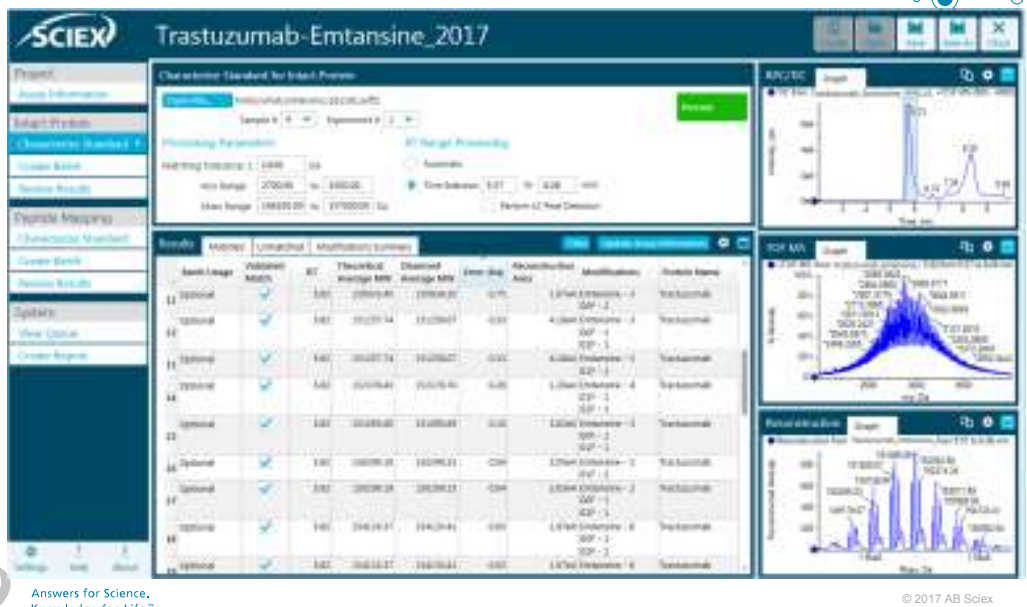
Ado-trastuzumab emtansine (glycosylation on)

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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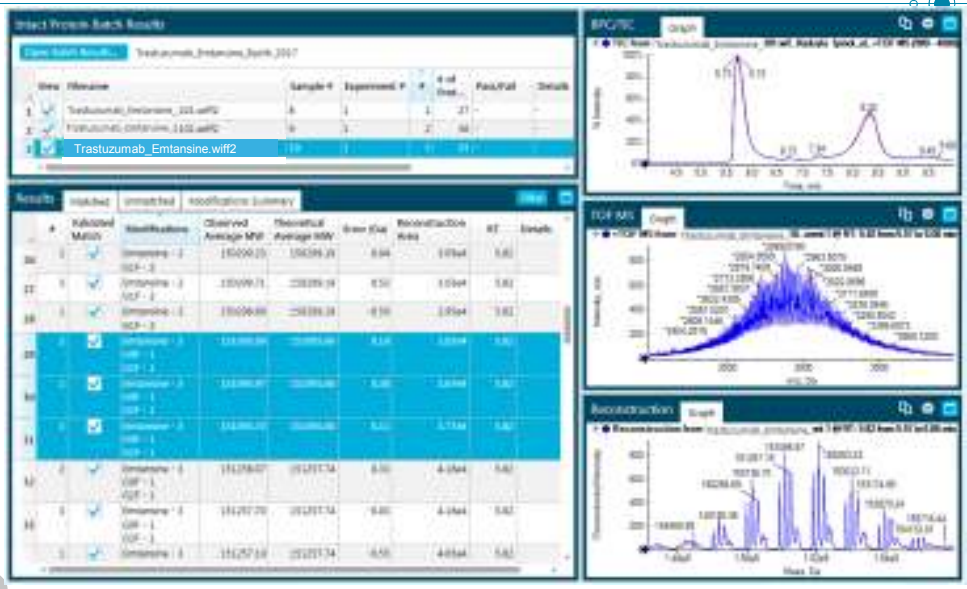
Ado-trastuzumab emtansine – Batch processing

Example here multiple injections of a Lysine-linked non-deglycosylated ADC



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Ado-trastuzumab emtansine – Batch processing

Example here multiple injections of a Lysine-linked non-deglycosylated ADC

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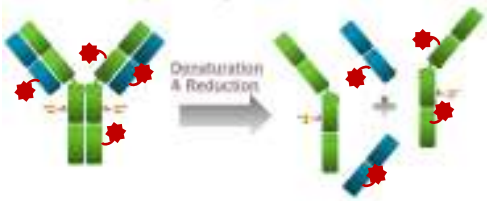
Subunit Analysis via MS

Main Workflows for Routine Characterisation

A) Enzymatic (IdeS/ FabRICATOR) digestion into large junks
- 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. Location)
- In the case Cys-Linked: RP can be used, as the non-covalently bound complex no-longer needs to be held together (better sensivities)

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Subunit Analysis – mAb Heavy Chain

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

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Peptide Mapping

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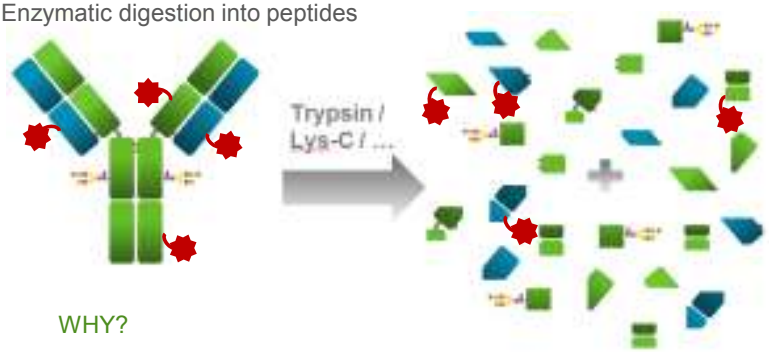
Peptide Mapping via MS

Main Workflows for Routine Characterisation

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



WHY?

- Determination of modifications: conjugated drug location and quality attributes (QA) which have an impact on safety on efficacy
- Determination of remaining disulfide bonds for Cys –linked ADCs

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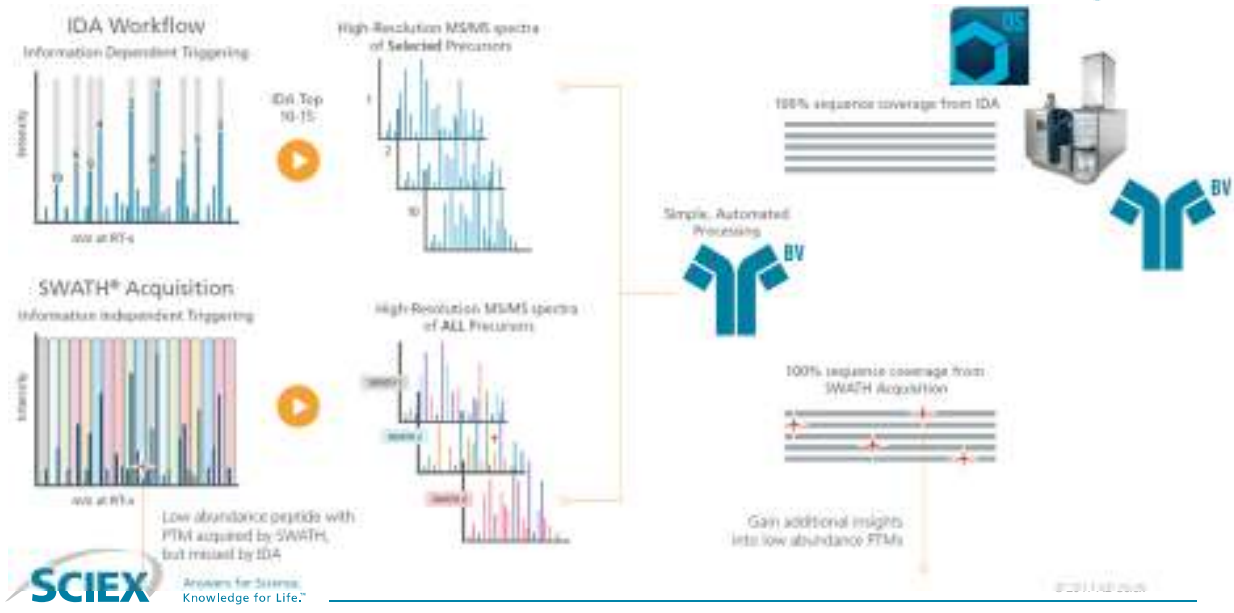
Peptide Mapping Acquisition Modes



Peptide maps in a flash

The example: an unconjugated, glycosylated mAb

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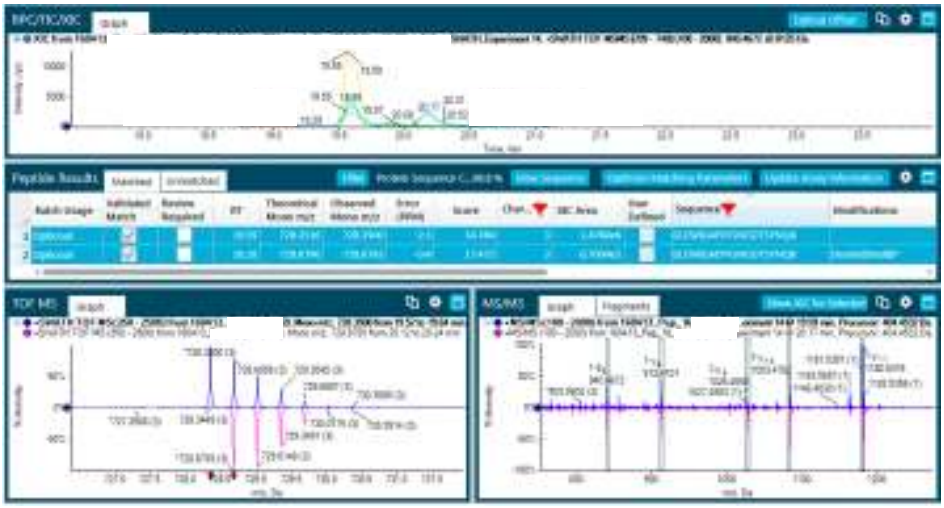
SWATH Acquisition and Processing for Ultimate Confidence



The example: an unconjugated, glycosylated mAb

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Modification ratios

The example: an [illegible]cosylated mAb

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Modification ratios

The example: ted, glycosylated mAb

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Click. Compare. Report.

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ADC Complexity – analysed routinely

- Assess and ensure the product
  - Quality in and after process of conjugation
  - Stability *in vitro* and *in vivo*
  - Conjugation sites – site occupancy and payload
  - Linker chemistry – stability

Need to know your mAb – for every batch

- Three Main Workflows for ADC Characterisation
- Easy to use – *Inviting you to acquire and process*
- Fits to your lab – a Benchtop accurate mass instrument

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Unique Solutions to Meet Biologics Characterization Challenges

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X500 – Next Generation Mass Spectrometer

- Next generation platform Architecture. Most complex project since the API 300 (1995)
- 70% of HW R&D worked on the project, 60% of SW R&D on SCIEX OS
- Used predictive modeling and testing for enhanced reliability
- Significant investment in industrial design and usability testing with customers

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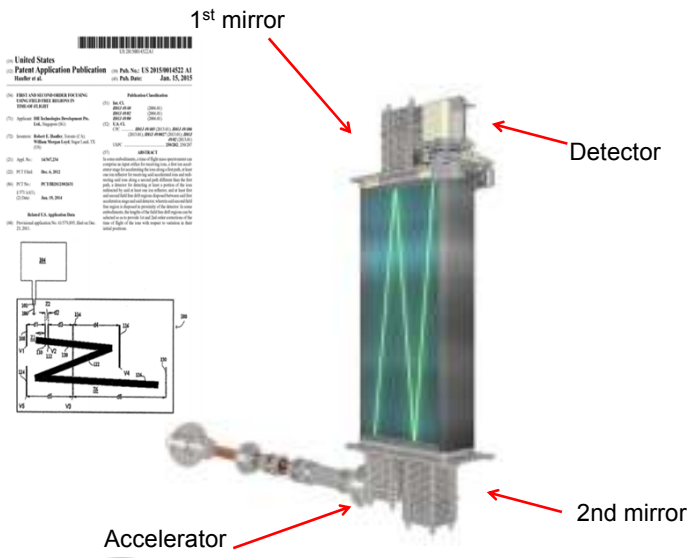
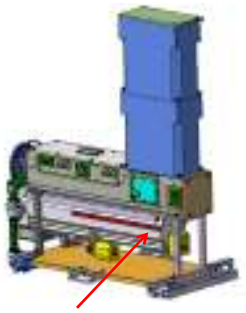
Novel Optics and Vacuum Technology





Pfeiffer triple Inlet
Turbo pump

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SCIEX OS

Single Software Platform for MS Control, Data Quantitation and Reporting

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