Intact antibody drug conjugate (ADC) analysis

Routine high-resolution accurate mass analysis of intact ADCs on the X500B QTOF System

Method details for the routine characterization of an ADC biotherapeutic by high-resolution intact mass analysis using HPLC coupled with the X500B QTOF System, powered by the SCIEX OS Software.

Sample Prep

A generic sample preparation strategy is shown for deglycosylation and general clean-up of an intact ADC biotherapeutic prior to LC-MS analysis.

Step 1

- Glycosylated ADC (ado-trastuzumab emtansine) was diluted to a concentration between 50 to 500 µg in 45 µL of 50 mM Tris-HCl, pH 8.0

Step 2

- A 2 µL aliquot of N-Glycanase was added to the reaction mixture and incubated at 37°C for 2 hours

Step 4

- Reaction mixture was desalted and cleaned up using Zeba spin desalting column using buffer exchange with Mobile phase A.

Step 5

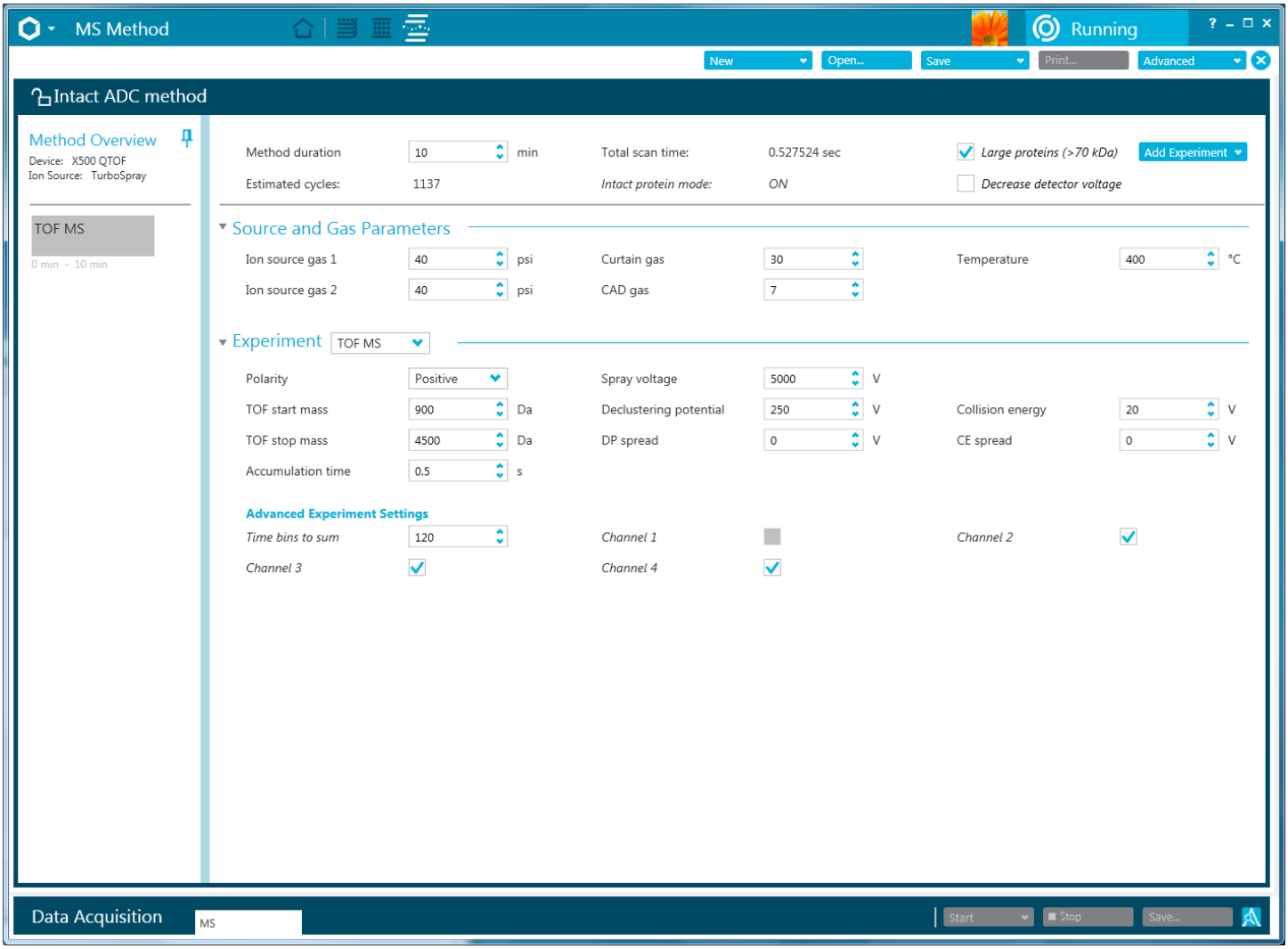
- The sample was diluted to 0.1 mg/mL.

LC Method

Column	Waters Acquity UPLC BEH C4, 2.1mm x 50mm, 300A, 1.7 um	
Mobile Phase A	Water, 0.1% Formic acid	
Mobile Phase B	Acetonitrile, 0.1% Formic acid	
Flow rate	200 µL/min	
Column temperature	80^{0} C	
Injection volume	10 µL, 1 µg total protein	
Gradient profile	**Time (min)**	**% B**
	2.0	10
	6.0	90
	7.0	90
	7.1	10
	10	10

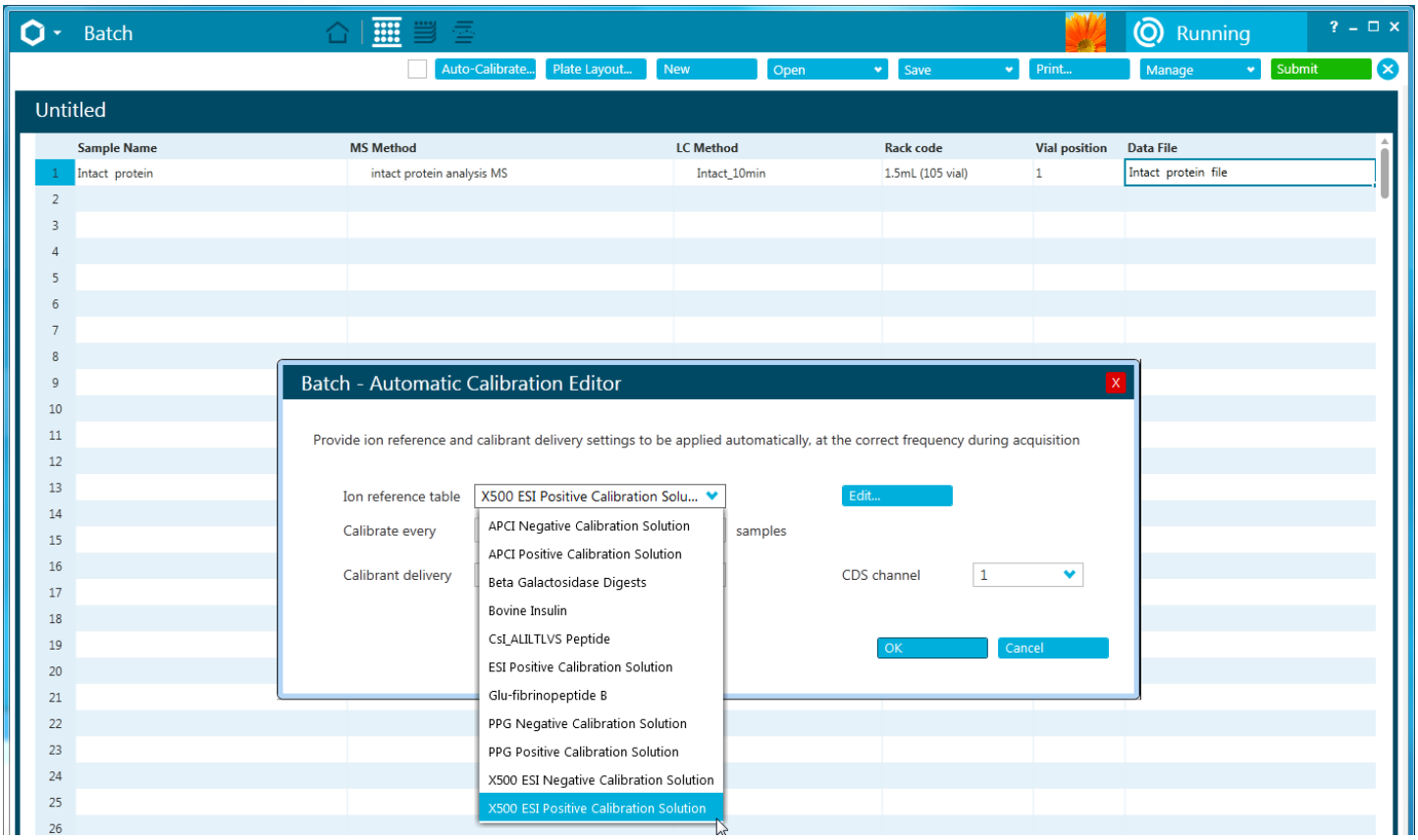
MS Method

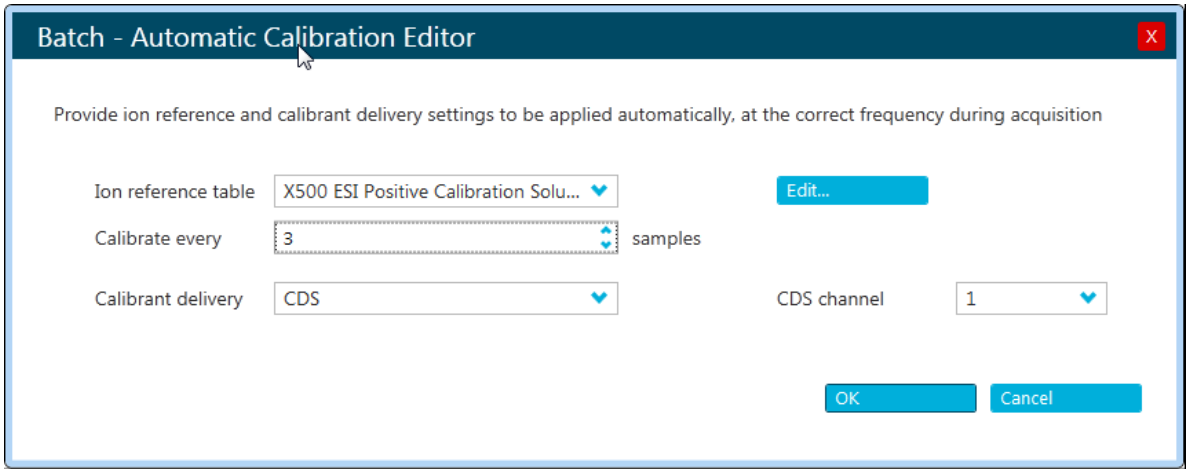
Suggested starting MS method parameters for routine intact ADC analysis as displayed in SCIEX OS. For best sensitivity and resolution, the declustering potential (DP) and collision energy (CE) parameters should be optimized for each individual biotherapeutic.



Batch

In Batch setup, open the ‘Automated Calibration Editor’ window in order to select the use of the autocalibration function. Designate use of the ‘X500 ESI Positive Calibration Solution’, and then determine how often you would like the system to perform a fast, automated calibration. These short calibrations will be added automatically to your queue once you have submitted a sample batch.

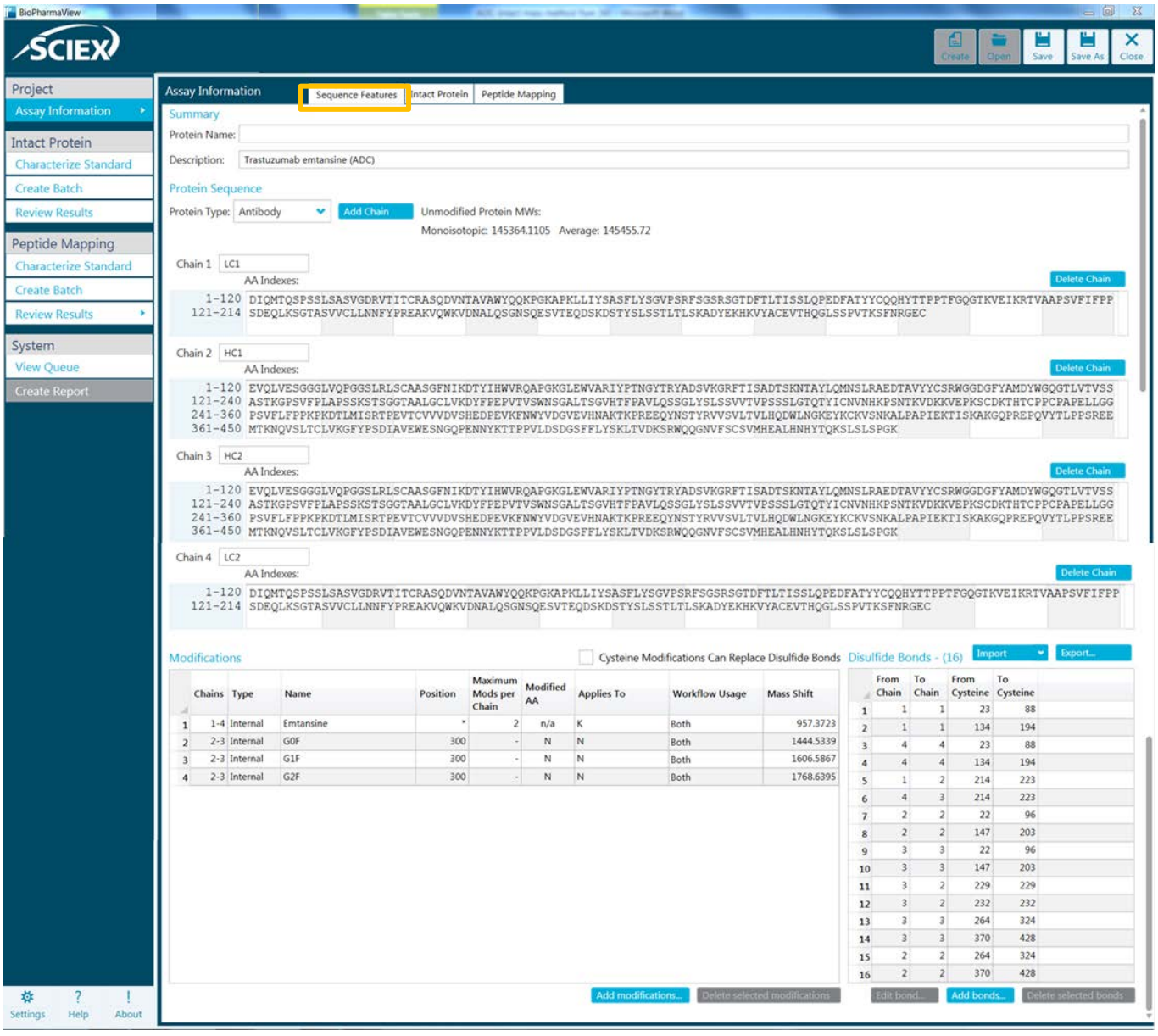




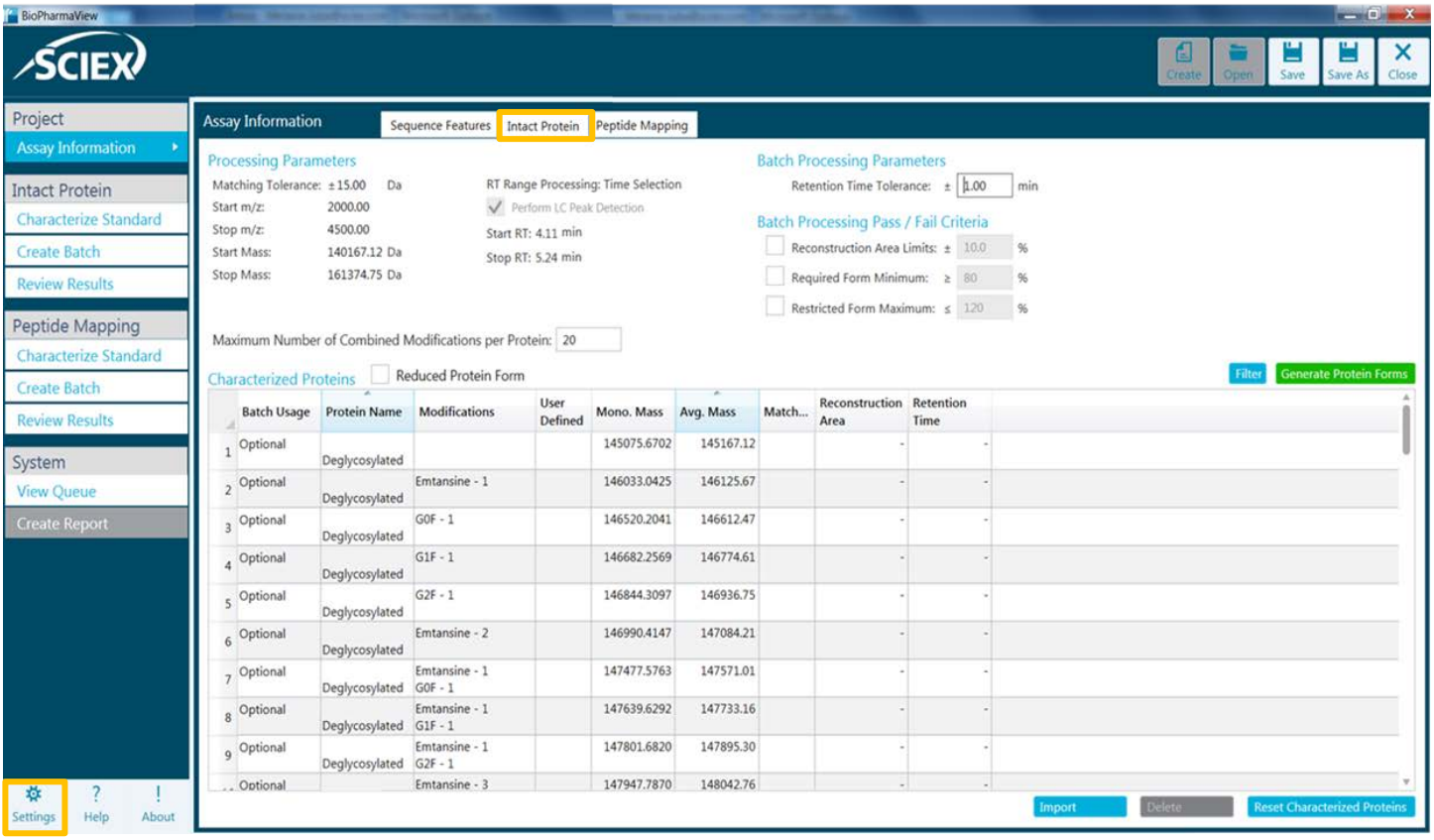
Data Processing

Process intact biotherapeutic data in BioPharmaView™ Software 2.0.

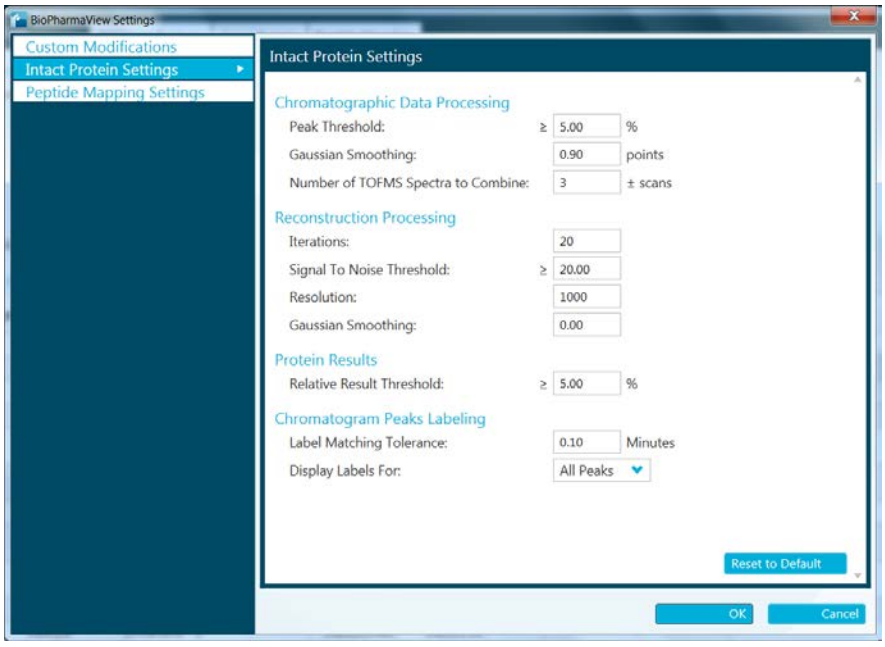
Input the protein sequence, and assign potential modifications in the ‘Assay Information’ window. Click on the ‘Add Modifications’ tab to insert the drug conjugate mass information, as well as expected location. In this case the DM1/Emtansine drug conjugate and linker is conjugated to lysine residues within the protein. The DM1/ Emtansine has an average mass of 957.37 Da.



Navigate to the ‘Intact Protein’ tab complete processing parameters and to generate the protein forms for matching.

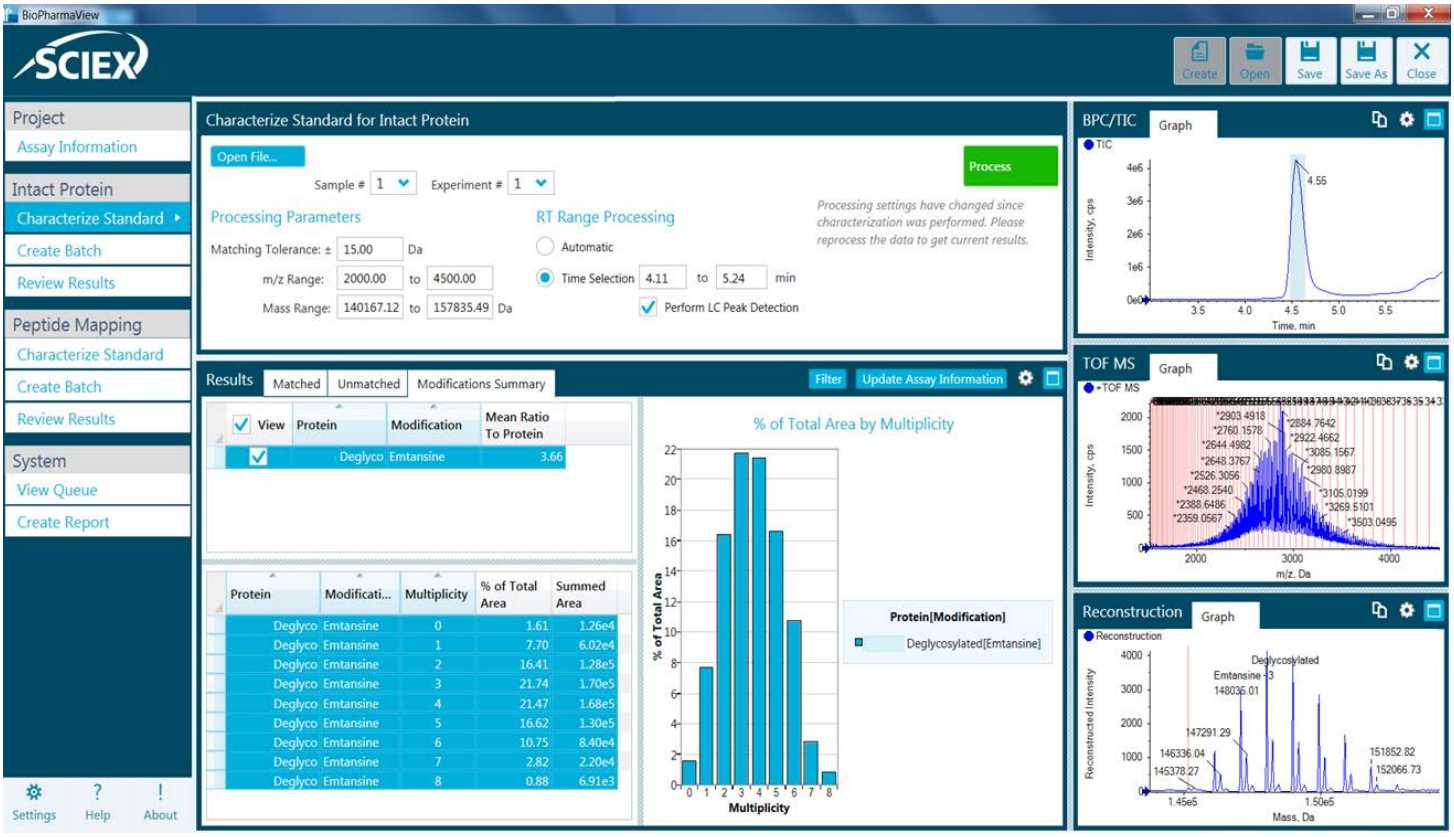


Navigate to the ‘Settings’ icon and review your global ‘Intact Protein Settings’



Pharma and Biopharma

Intact protein deconvolution can be performed in seconds, on either a single datafile, or on multiple samples using the batch processing function. Review your intact ADC protein deconvolution results in the BioPharmaView Software window. Annotated reconstruction mass graph (bottom right) hyperlinks to the raw spectra (middle right) to confirm peak identity and show fidelity between raw and deconvoluted data. Detailed information on drug load can be found on the bottom left, with automated calculation of the drug to antibody ratio (DAR) presented clearly in the window.



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