Biotherapeutic subunit mass analysis

Routine high-resolution accurate mass analysis of biotherapeutic subunits on the X500B QTOF System

Method details for the routine characterization of trastuzumab biotherapeutic protein following an IdeS subunit digestion strategy. Analysis performed by high-resolution accurate mass detection using HPLC coupled with the X500B QTOF System, powered by SCIEX OS Software.

Sample Prep

A generic sample preparation strategy is shown for IdeS subunit digestion and clean-up of an intact biotherapeutic prior to LC-MS analysis.

Step 1

- Dilute the antibody sample into a cleavage buffer- a neutral buffer such Tris-HCl bufered to pH 7 - to a concentration of about 1 mg/ml of the antibody

Step 2

- Digestion with IdeS protease (50 units of IdeS per 1 ug of antibody) 37ºC for 30-60 minutes

Step 3

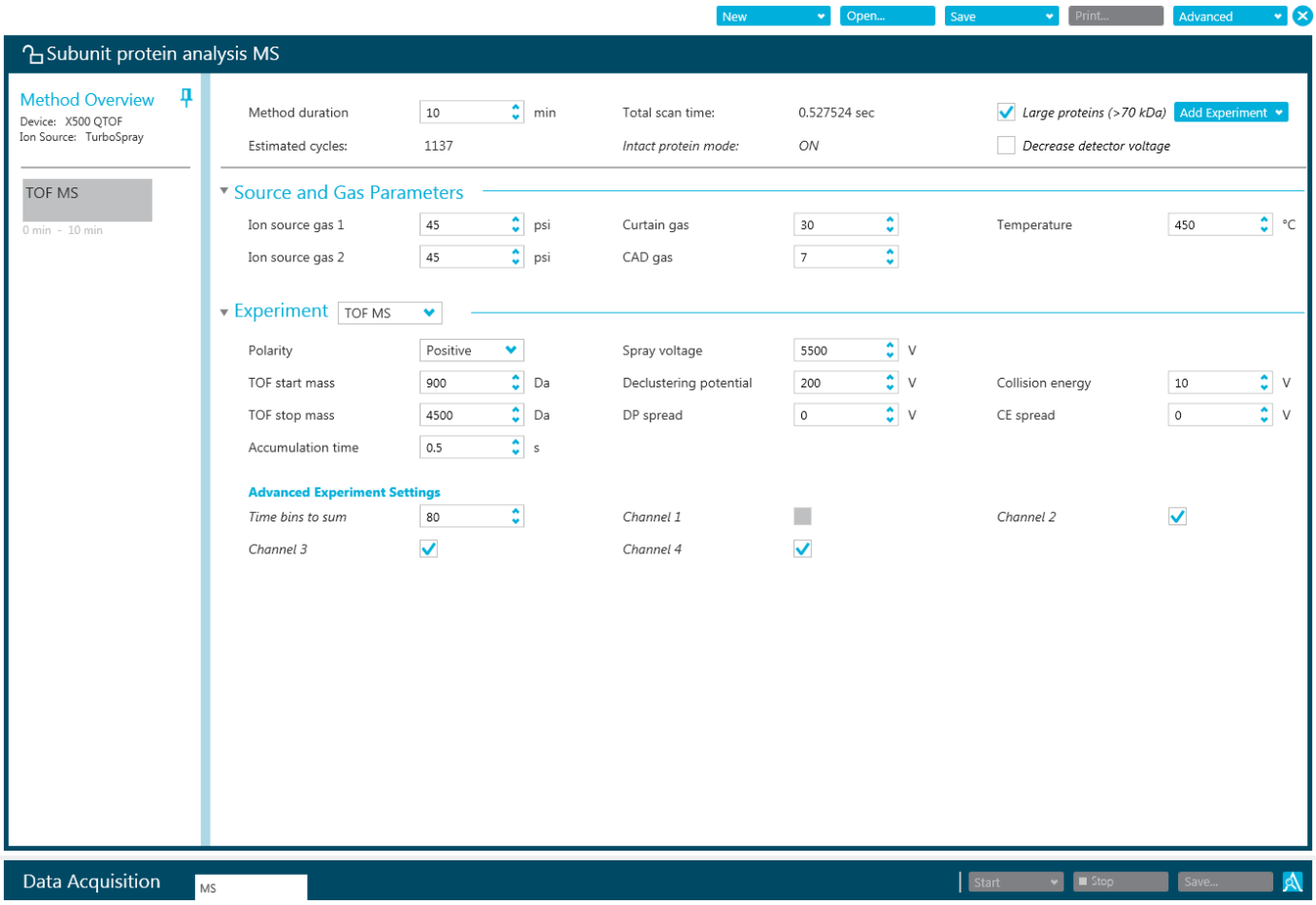
- Dilution into Mobile Phase A, to ~0.1 µg/µL

LC Method

Column	Agilent Zorbax SB300 C-8 1mm X 75mm	
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	25
	6.0	60
	7.0	60
	7.1	80
	9.0	80
	9.5	25
	10.5	25

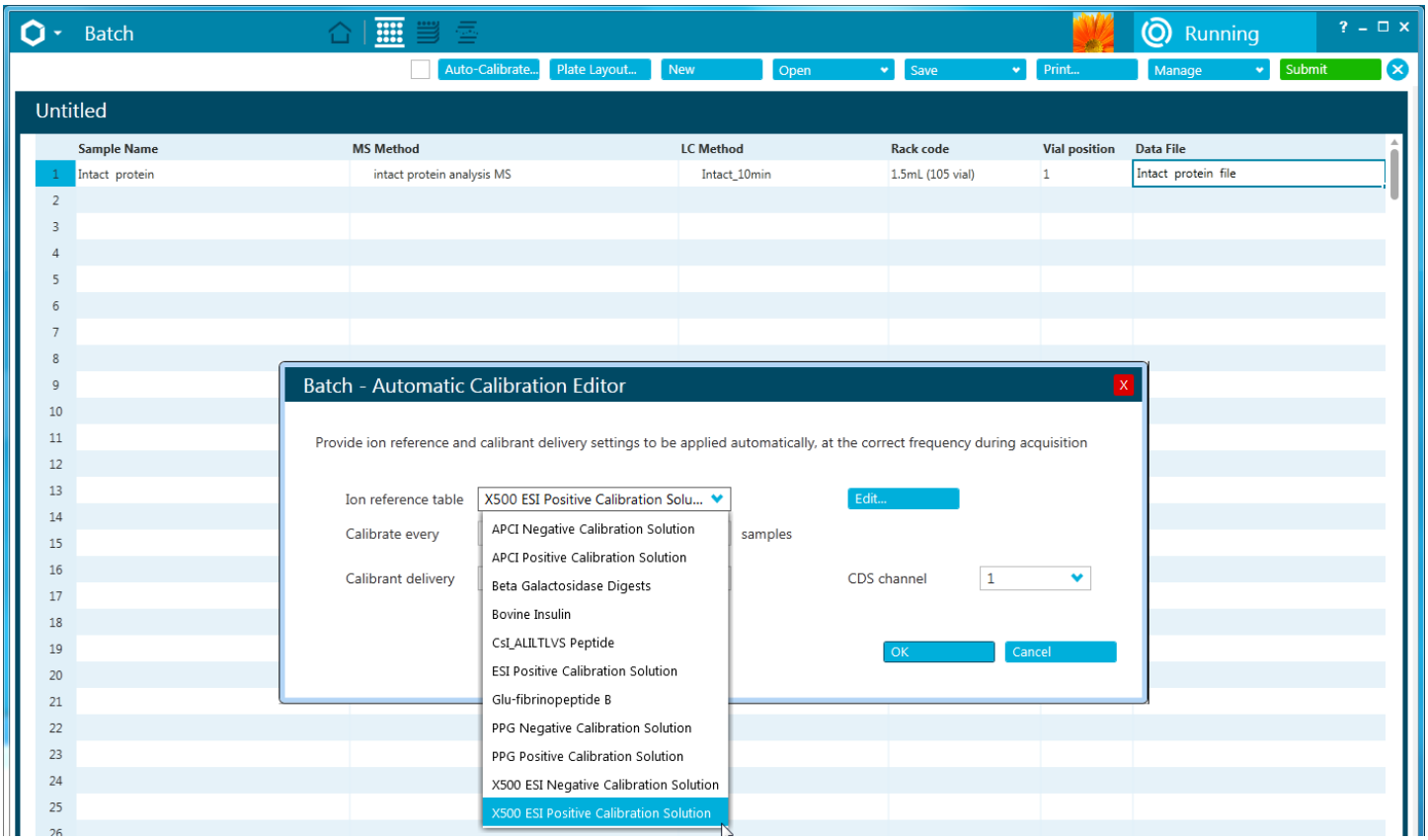
MS Method

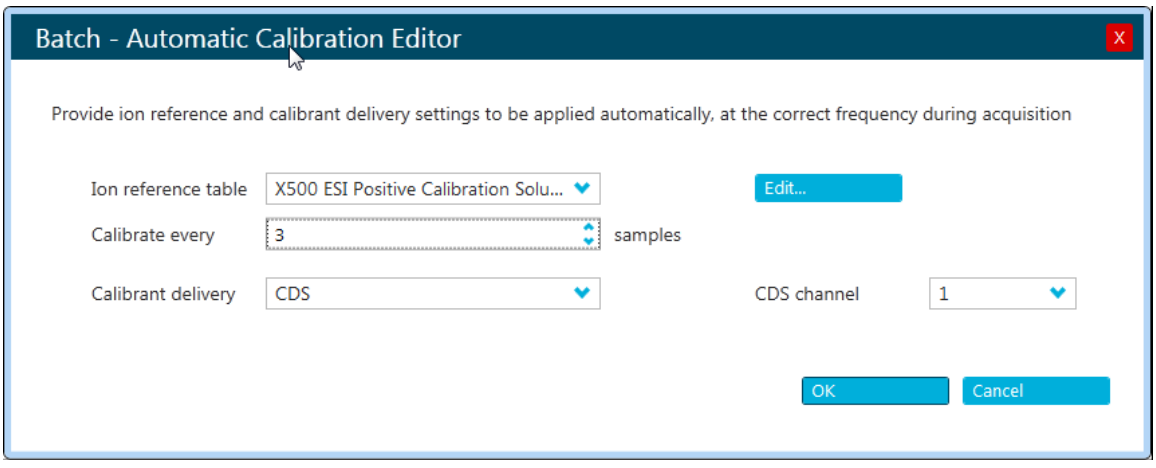
Suggested starting MS method parameters for routine mAb subunit analysis as displayed in SCIEX OS. For best sensitivity and resolution, the declustering potential (DP) and collision energy (CE) parameters should be optimized for each individual biotherapeutic.



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.

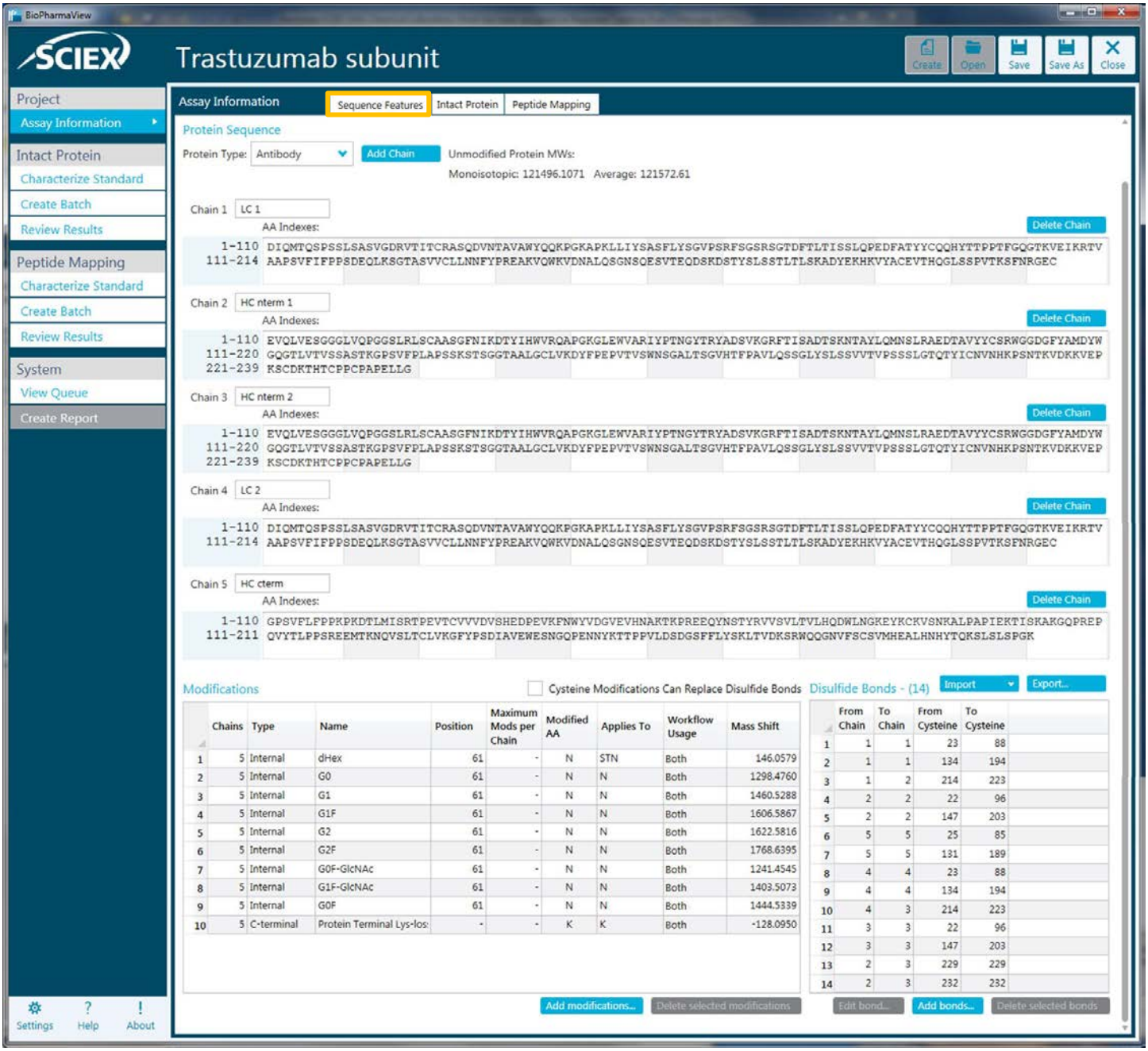




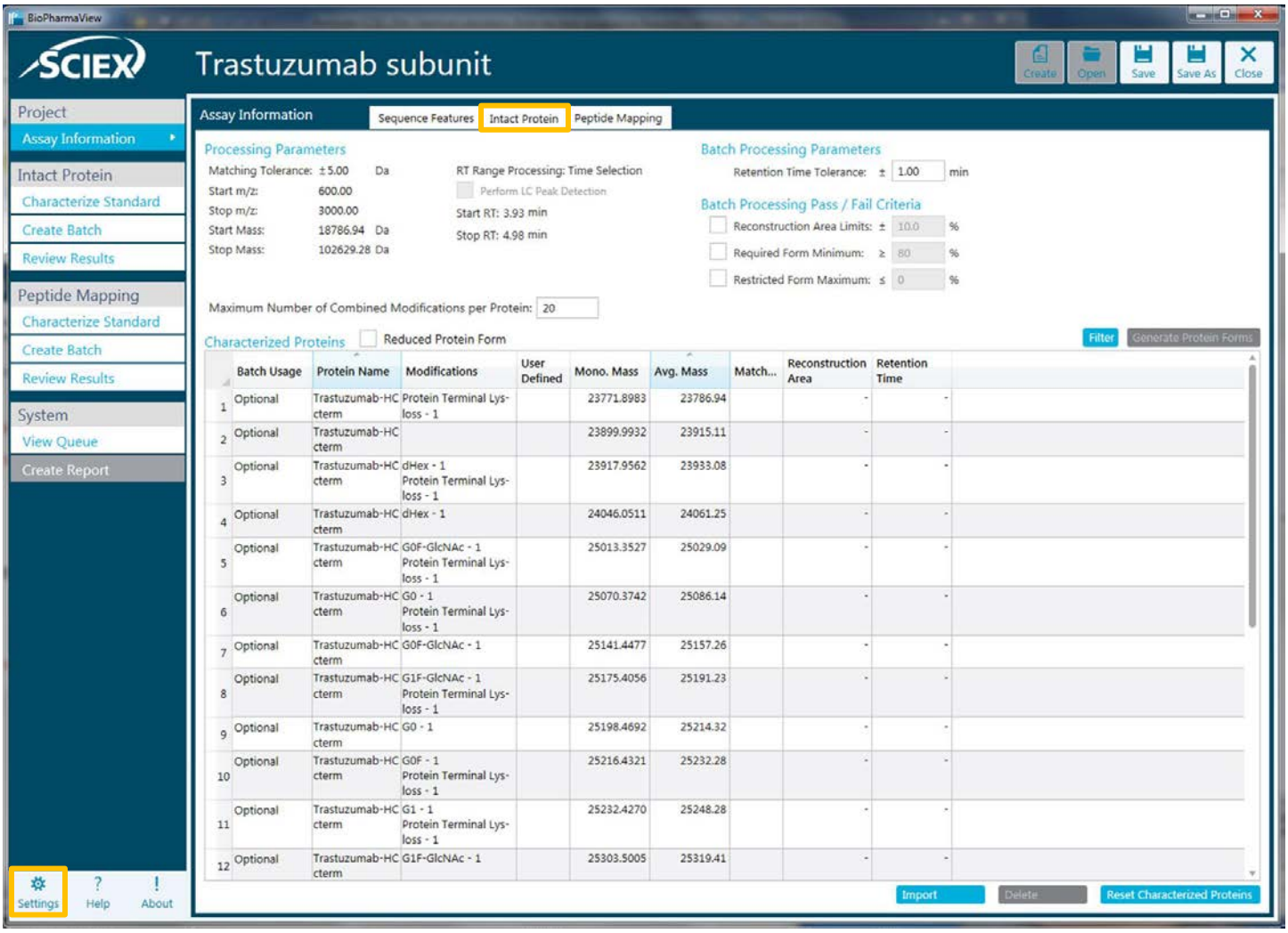
Data Processing

Process intact biotherapeutic data in BioPharmaView™ Software 2.0.

Input the protein sequence, and assign potential modifications in the ‘Assay Information’ window.

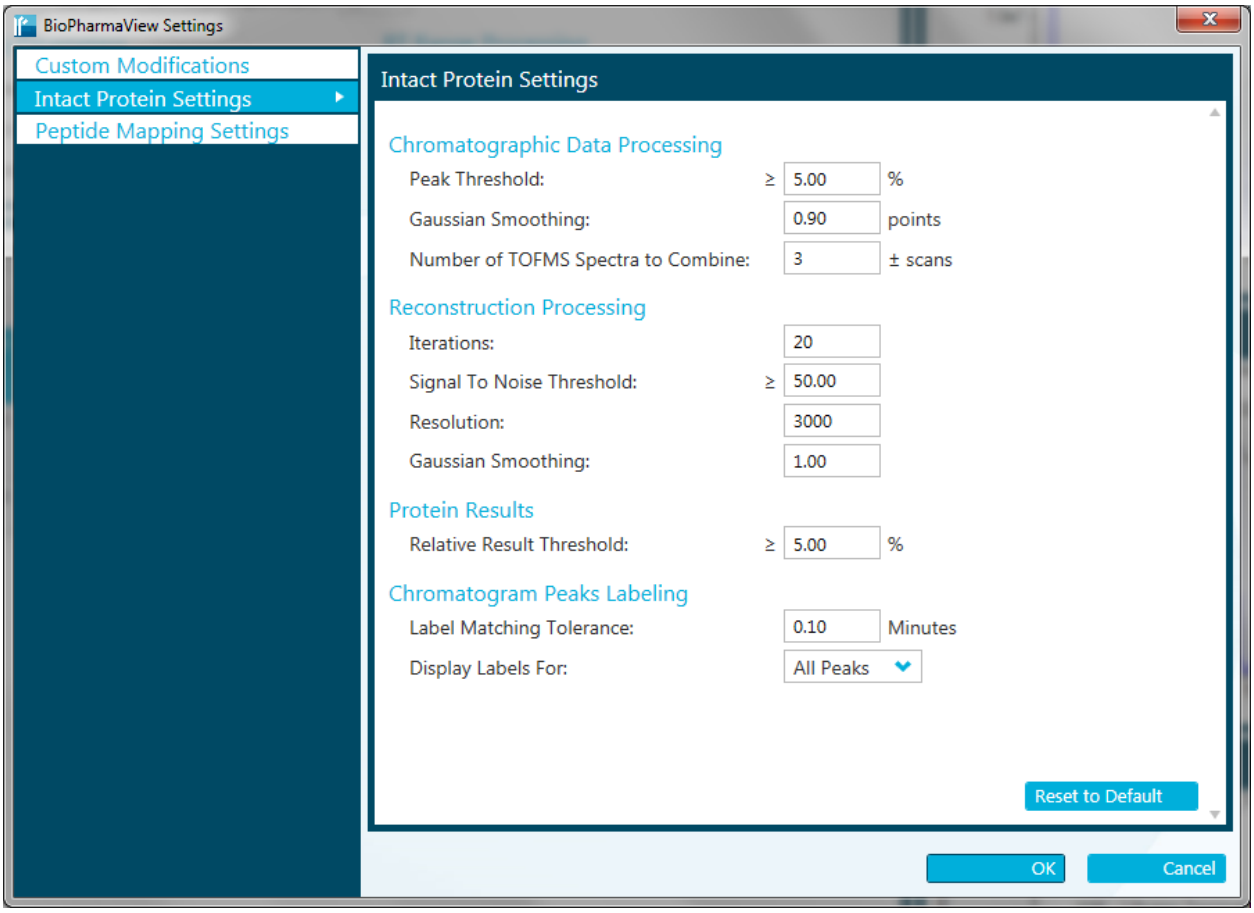


Navigate to the ‘Intact Protein’ tab complete processing parameters and to generate the protein forms for matching.



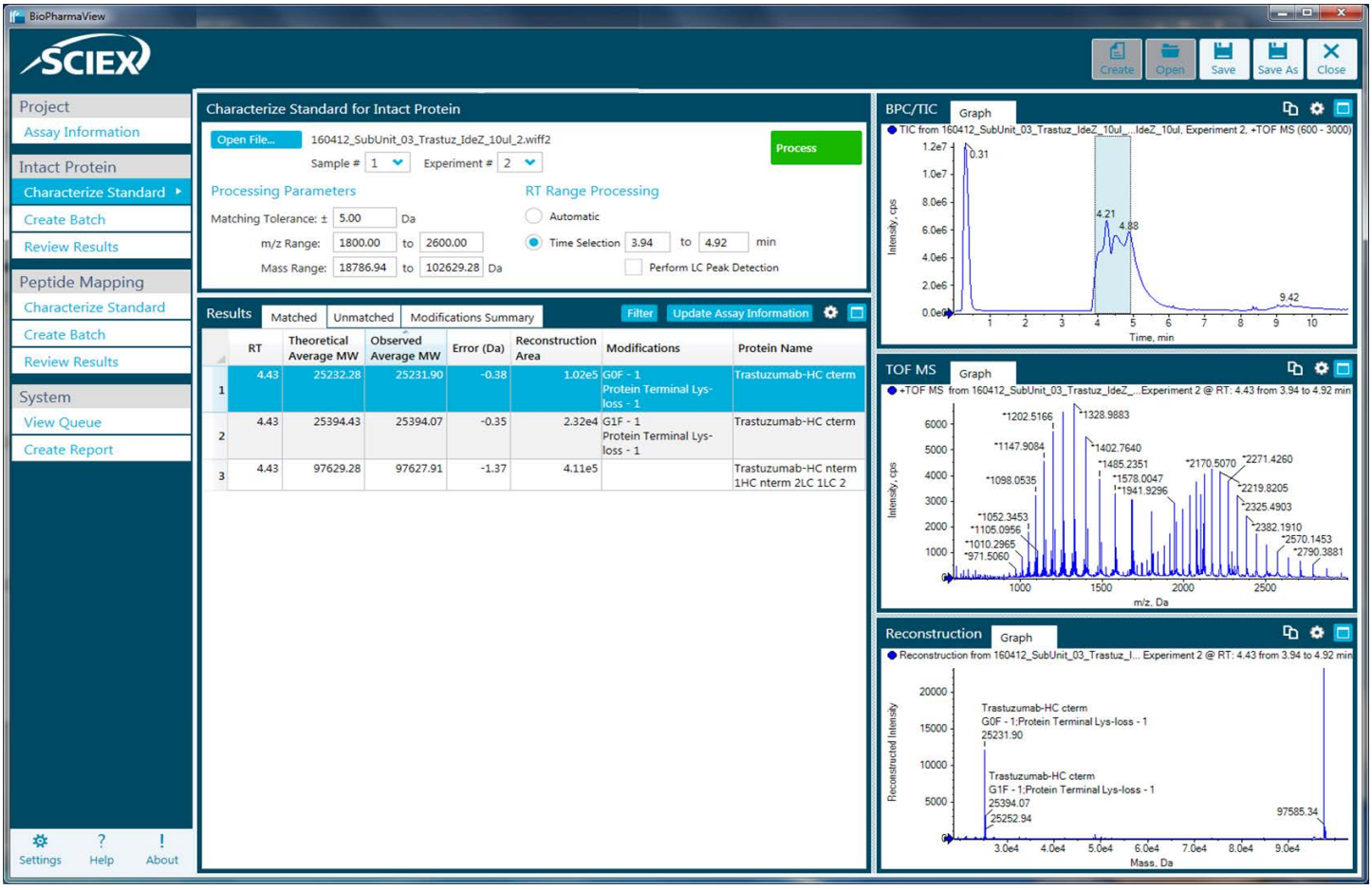
	Batch Usage	Protein Name	Modifications	User Defined	Mono. Mass	Avg. Mass	Match...	Reconstruction Area	Retention Time
1	Optional	Trastuzumab-HC cterm	Protein Terminal Lys-loss - 1		23771.8983	23786.94		-	-
2	Optional	Trastuzumab-HC cterm			23899.9932	23915.11		-	-
3	Optional	Trastuzumab-HC cterm	dHex - 1 Protein Terminal Lys-loss - 1		23917.9562	23933.08		-	-
4	Optional	Trastuzumab-HC cterm	dHex - 1		24046.0511	24061.25		-	-
5	Optional	Trastuzumab-HC cterm	G0F-GlcNAc - 1 Protein Terminal Lys-loss - 1		25013.3527	25029.09		-	-
6	Optional	Trastuzumab-HC cterm	G0 - 1 Protein Terminal Lys-loss - 1		25070.3742	25086.14		-	-
7	Optional	Trastuzumab-HC cterm	G0F-GlcNAc - 1		25141.4477	25157.26		-	-
8	Optional	Trastuzumab-HC cterm	G1F-GlcNAc - 1 Protein Terminal Lys-loss - 1		25175.4056	25191.23		-	-
9	Optional	Trastuzumab-HC cterm	G0 - 1		25198.4692	25214.32		-	-
10	Optional	Trastuzumab-HC cterm	G0F - 1 Protein Terminal Lys-loss - 1		25216.4321	25232.28		-	-
11	Optional	Trastuzumab-HC cterm	G1 - 1 Protein Terminal Lys-loss - 1		25232.4270	25248.28		-	-
12	Optional	Trastuzumab-HC cterm	G1F-GlcNAc - 1		25303.5005	25319.41		-	-

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



Pharma and Biopharma

Protein deconvolution of each subunit can be performed in seconds, on either a single datafile, or on multiple samples using the batch processing function. Below is shown the deconvolution results for the Fc domain of the biotherapeutic, as well as the Fab2 domain of the biotherapeutic (non-reduced).



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Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
Phone 508-383-7700
sciex.com

International Sales
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