Biotherapeutic Peptide Mapping
Information Dependent Acquisition (IDA) Method

Routine biotherapeutic accurate mass peptide mapping analysis on the X500B QTOF System

Method details for the routine peptide mapping of a biotherapeutic monoclonal antibody (mAb) protein by high-resolution accurate mass analysis on the X500B QTOF System, powered by SCIEX OS Software. An information dependent acquisition (IDA) method was employed to acquire high-resolution MS and MS/MS level data on the digested biologic protein product.

Sample Prep

A generic sample preparation strategy is shown for reduction and tryptic digestion of an antibody biotherapeutic prior to LC-MS analysis.

Step 1
- Buffer exchange antibody sample (0.2mg) in Ammonium Bicarbonate (100mM) buffer and add 25 µL Trifluoroethanol (TFE) denaturation agent. Add 2.5 µL DTT (200mM) solution. Vortex to mix. Heat to at least 60 ºC for 60min to denature (Alternatively, use 90 ºC for 20 min).

Step 2
- Add 10 µL IAM (200mM) solution. Vortex briefly. and allow to stand at room temperature for 1 hour in the dark.

Step 3
- Add 2.5 µL DTT (200mM) solution to destroy excess IAM. Allow to stand for 1 hour in the dark.

Step 4
- Add 300 µL water to dilute denaturant and add 100 µL ammonium bicarbonate (100mM) solution to raise pH. Trypsin will be denatured or less active if TFE level is greater than 5%. Typical pH value is 7.5-8.0; add more base if needed.

Step 5
- Add trypsin Gold stock solution (50mM acetic acid, 1ug/ul) at 1:25 enzyme:substrate. ratio. Vortex briefly. Incubate overnight at 37 ºC.

Step 6
- Stop the trypsin activity by adding 2 µL formic acid.Vortex briefly and vacuum dry the samples.

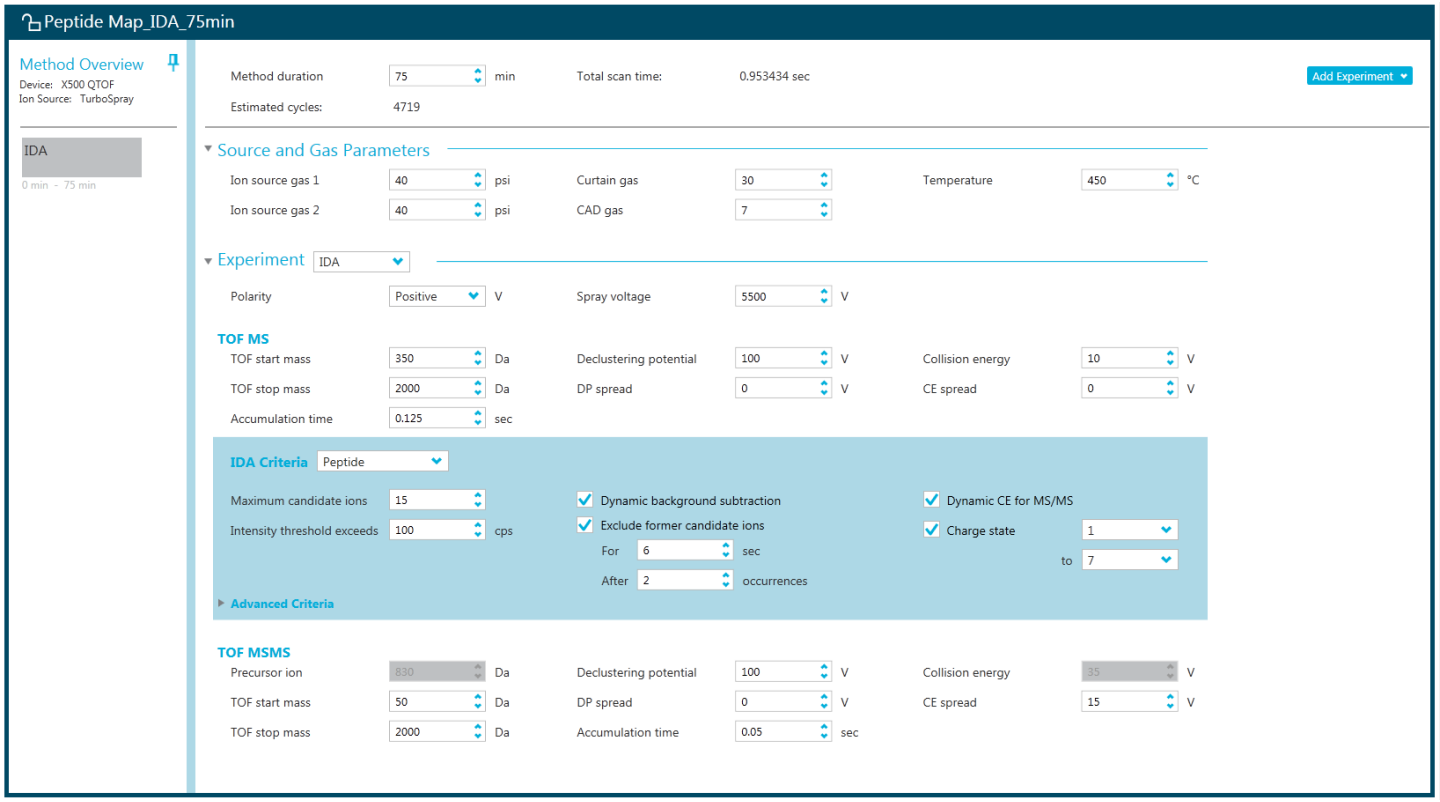
Step 7
- Reonstitute the digest into Mobile Phase A, to ~0.1 µg/µL just before the analysis.

LC Method

Column	Waters Acquity UPLC BEH C18 Column, 130 1.7 µm, 2.1 mm X 100 mm	
Mobile Phase A	Water, 0.1% Formic acid	
Mobile Phase B	Acetonitrile, 0.1% Formic acid	
Flow rate	200 µL/min	
Column temperature	40^{o} C	
Injection volume	10 µL, 1 µg total protein	
Gradient profile	**Time (min)**	**% B**
	8.0	2
	40.0	30
	60.0	50
	62.0	90
	66.0	90
	66.5	2
	75.0	2

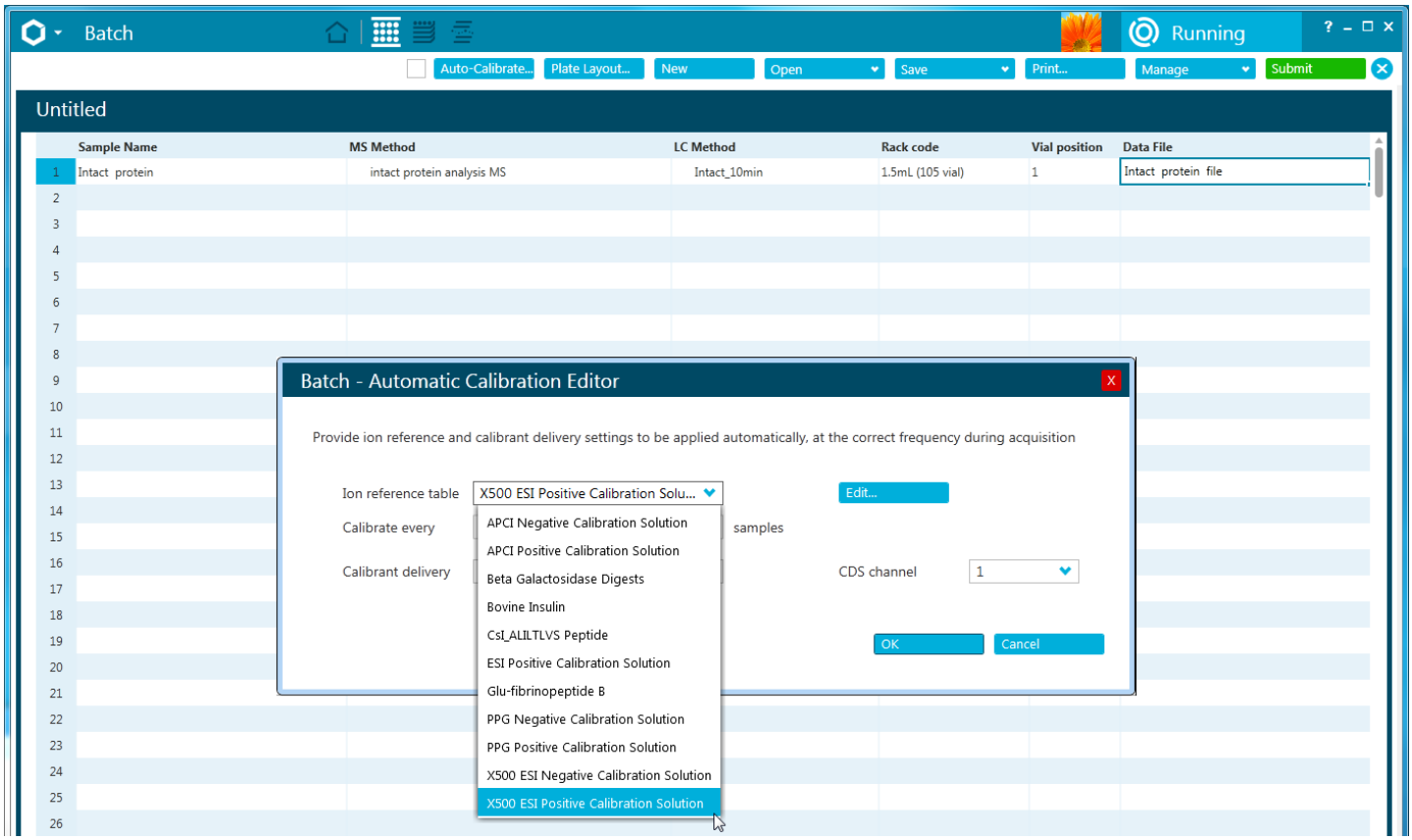
MS Method

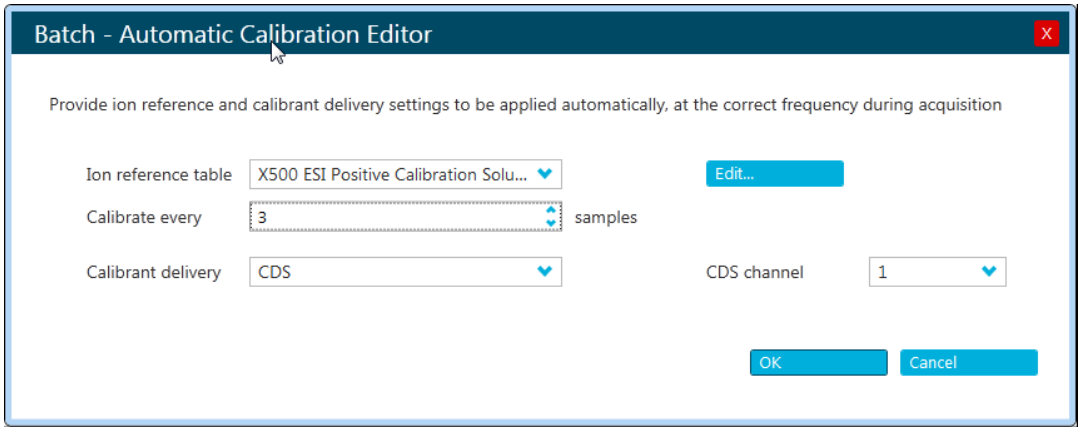
Suggested starting MS and MS/MS method parameters for routine peptide mapping analysis as displayed in SCIEX OS user interface. The information dependent acquisition (IDA) method criteria is shown for selecting the top 15 precursor ions for high-resolution MS/MS in each cycle. For best sequence coverage and sensitivity, the specific IDA criteria parameters should be optimized for each individual biotherapeutic and HPLC separation used.



Batch

In the 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.

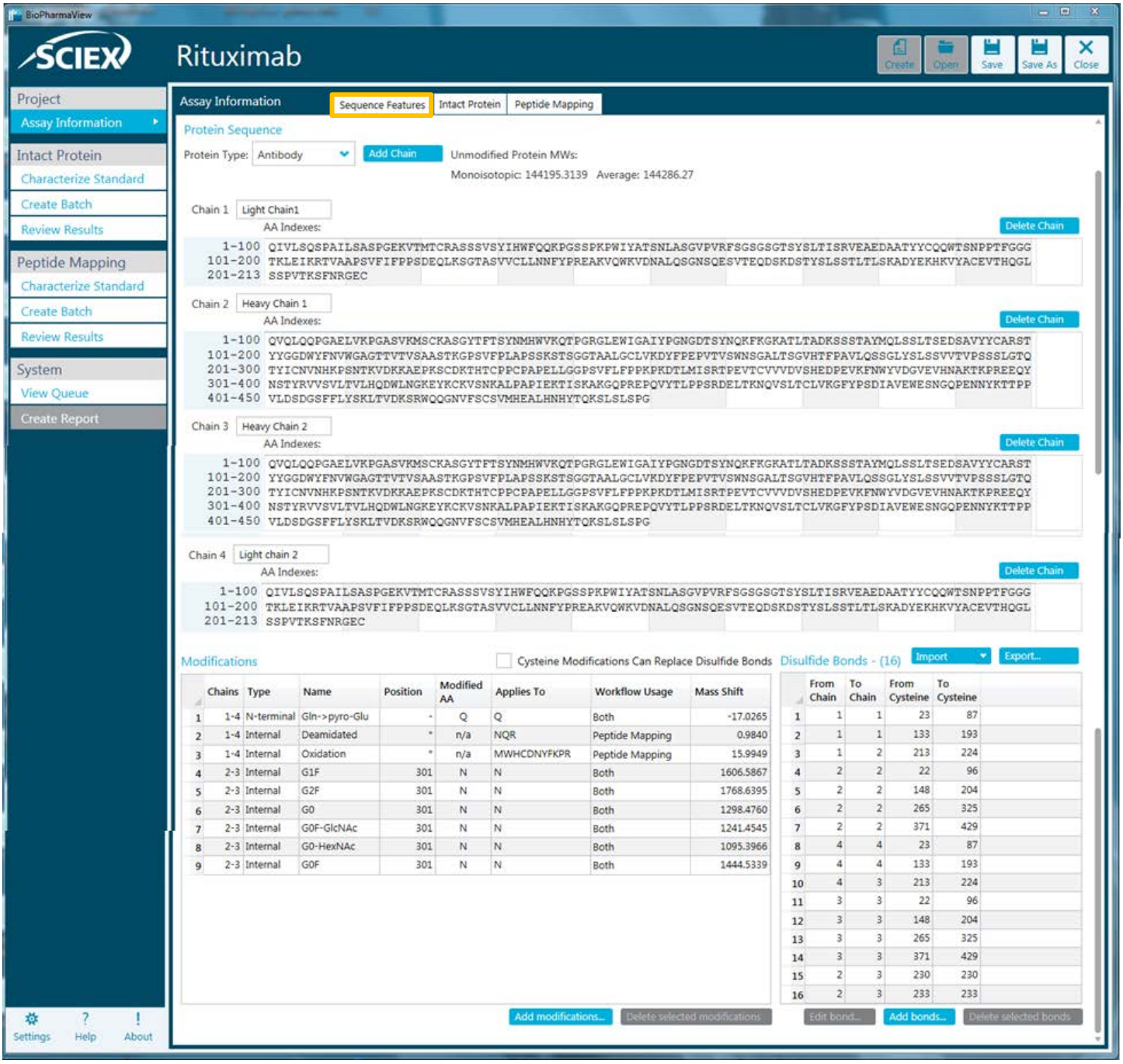




Data Processing

Process biotherapeutic peptide mapping data in BioPharmaView™ Software 2.0.

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

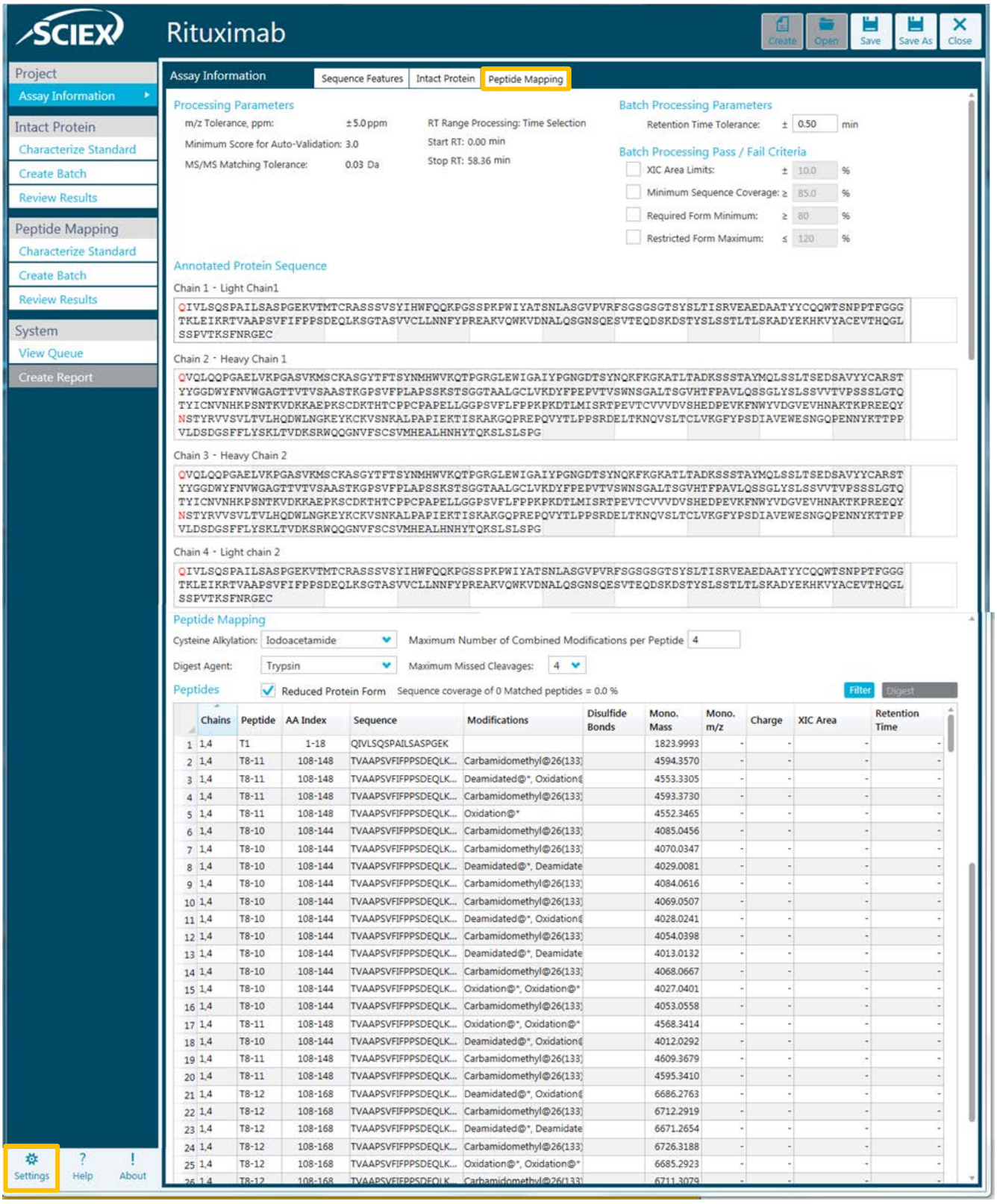


	Chains	Type	Name	Position	Modified AA	Applies To	Workflow Usage	Mass Shift
1	1-4	N-terminal	Gln->pyro-Glu	-	Q	Q	Both	-17.0265
2	1-4	Internal	Deamidated	*	n/a	NQR	Peptide Mapping	0.9840
3	1-4	Internal	Oxidation	*	n/a	MWHCDNYFKPR	Peptide Mapping	15.9949
4	2-3	Internal	G1F	301	N	N	Both	1606.5867
5	2-3	Internal	G2F	301	N	N	Both	1768.6395
6	2-3	Internal	G0	301	N	N	Both	1298.4760
7	2-3	Internal	G0F-GlcNAc	301	N	N	Both	1241.4545
8	2-3	Internal	G0-HexNAc	301	N	N	Both	1095.3966
9	2-3	Internal	G0F	301	N	N	Both	1444.5339

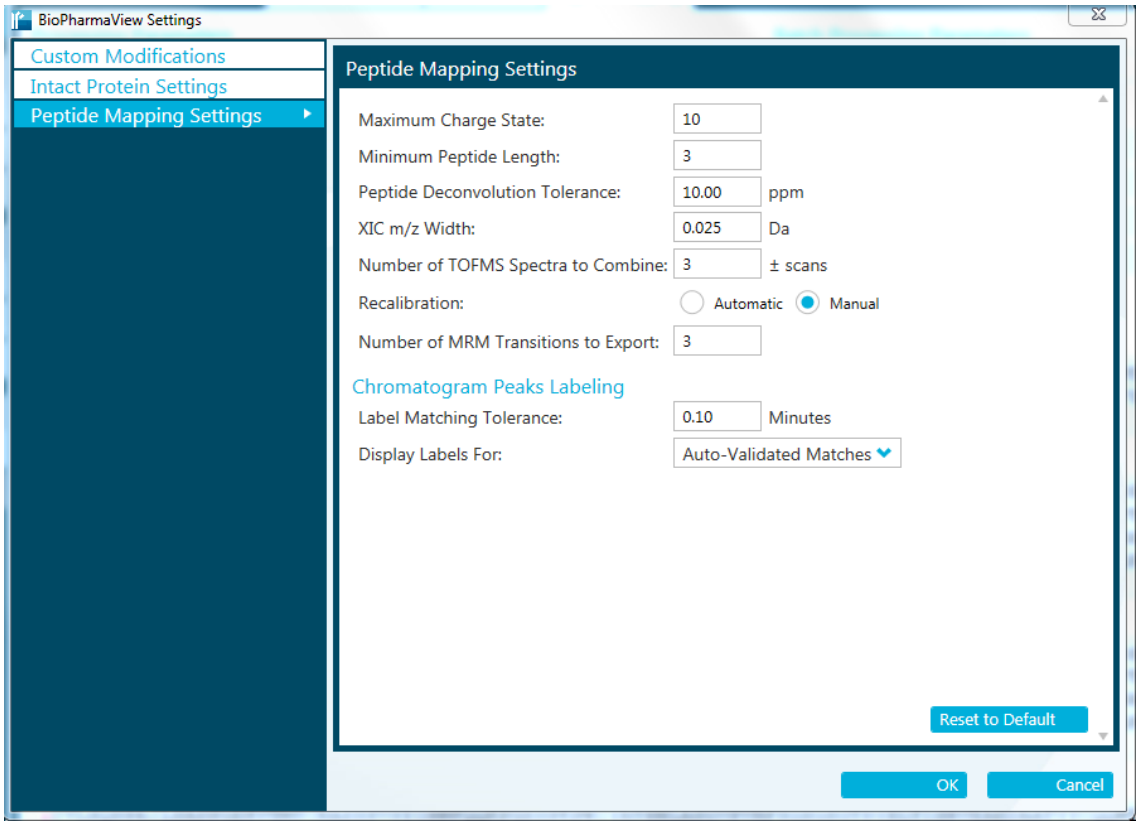
	From Chain	To Chain	From Cysteine	To Cysteine
1	1	1	23	87
2	1	1	133	193
3	1	2	213	224
4	2	2	22	96
5	2	2	148	204
6	2	2	265	325
7	2	2	371	429
8	4	4	23	87
9	4	4	133	193
10	4	3	213	224
11	3	3	22	96
12	3	3	148	204
13	3	3	265	325
14	3	3	371	429
15	2	3	230	230
16	2	3	233	233

Pharma and Biopharma

Navigate to the ‘Peptide Mapping’ tab complete processing parameters and to generate all peptide forms for matching.

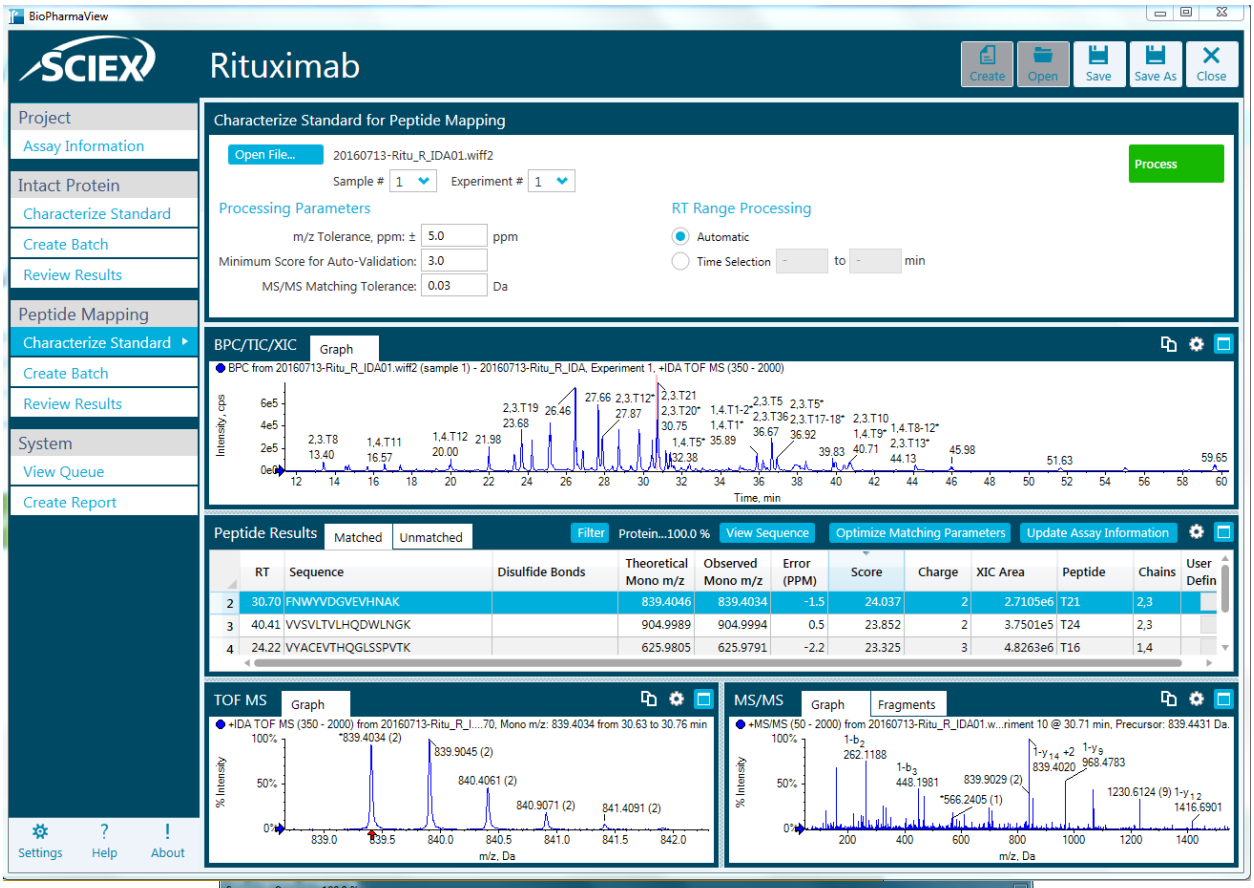


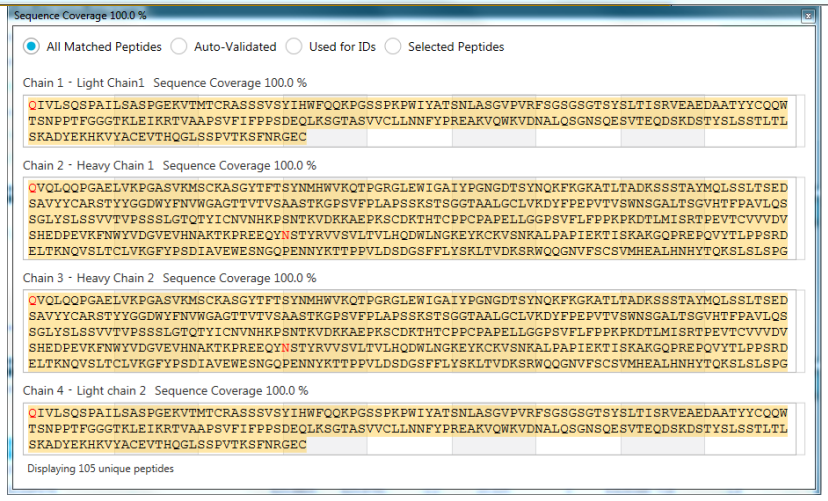
Navigate to the ‘Settings’ icon and review your global ‘Peptide Mapping Settings’



Data extraction, including peptide matching can be performed in minutes, on either a single datafile, or on multiple samples using the batch processing function. Review your peptide mapping results in the BioPharmaView Software window. Full sequence coverage of matched peptides can be viewed by clicking ‘View Sequence’. Peptide matches can be reviewed in the ‘Peptide Results’ window. For each selected peptide, corresponding TOF-MS raw spectrum (lower left) and high-resolution, annotated MS/MS spectrum (lower right) are shown for easy confirmation.

Pharma and Biopharma





For more information, please visit sciex.com/X500B

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