Biotherapeutic Peptide Mapping

Information Independent SWATH® Acquisition 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 independent SWATH Acquisition method was employed to acquire high-resolution MS and MS/MS level data on the digested biologic protein product.

SWATH Acquisition utilizes either fixed or variable Q1 mass isolation window, transmitting all precursor ions in the defined Q1 window through to the collision cell. Transmitted ions are fragmented and analyzed at high-resolution. The Q1 isolation window is stepped across the entire mass range, with an LC compatible cycle time, resulting in the comprehensive acquisition of high-resolution MS/MS spectra for every precursor ion in a sample. This unbiased data acquisition approach ensures data completeness is maximized, and enables detection of low abundance peptides and modifications.

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.

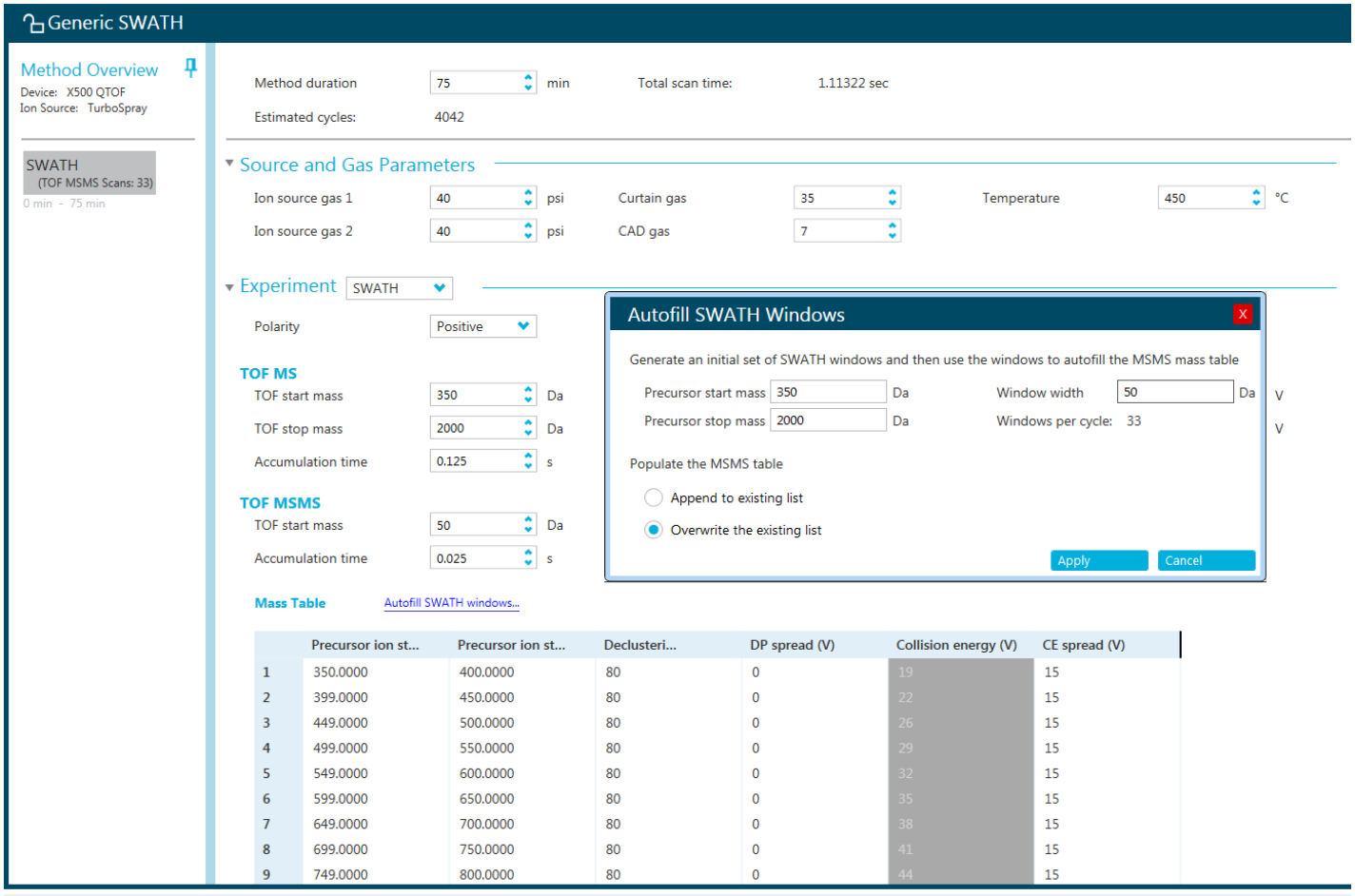
Step 7
- Reonstitute the digest into Mobile Phase A, to ~0.1 µg/µL just before the analysis.

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	40^0 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

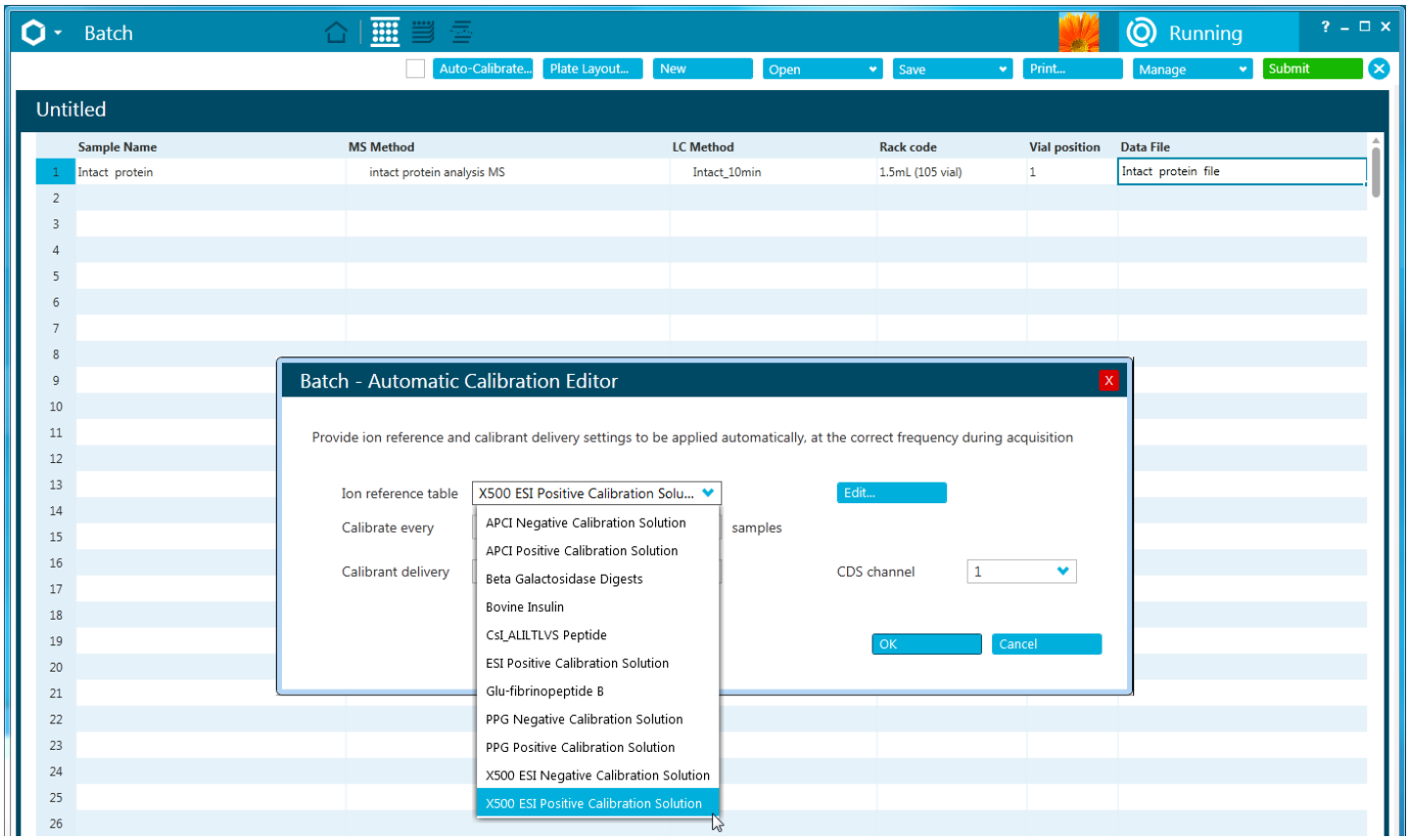
MS Method

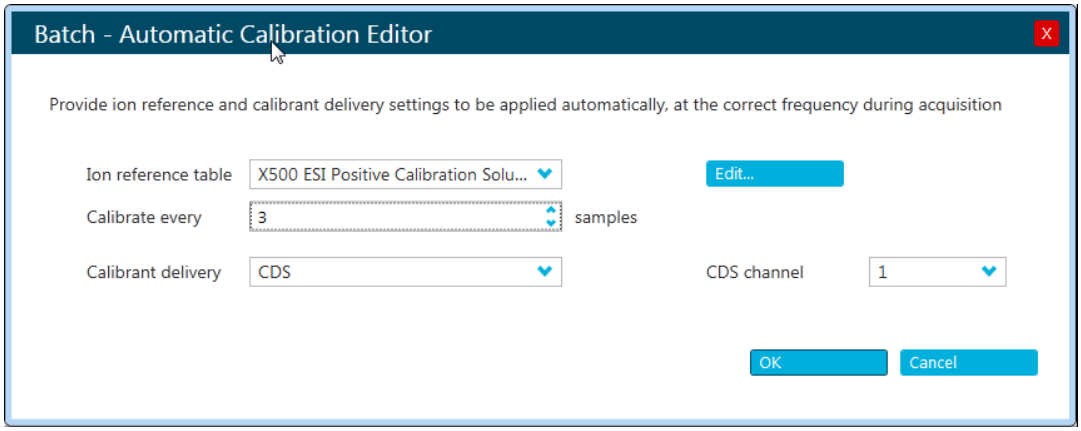
Suggested starting MS and MS/MS method parameters for routine SWATH based peptide mapping analysis as displayed in SCIEX OS user interface. The SWATH acquisition criteria are shown with a 50Da fixed SWATH window from 350-2000 m/z acquiring high-resolution MS/MS in each cycle. For best sequence coverage and sensitivity, the specific SWATH parameters should be optimized for the length of 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.

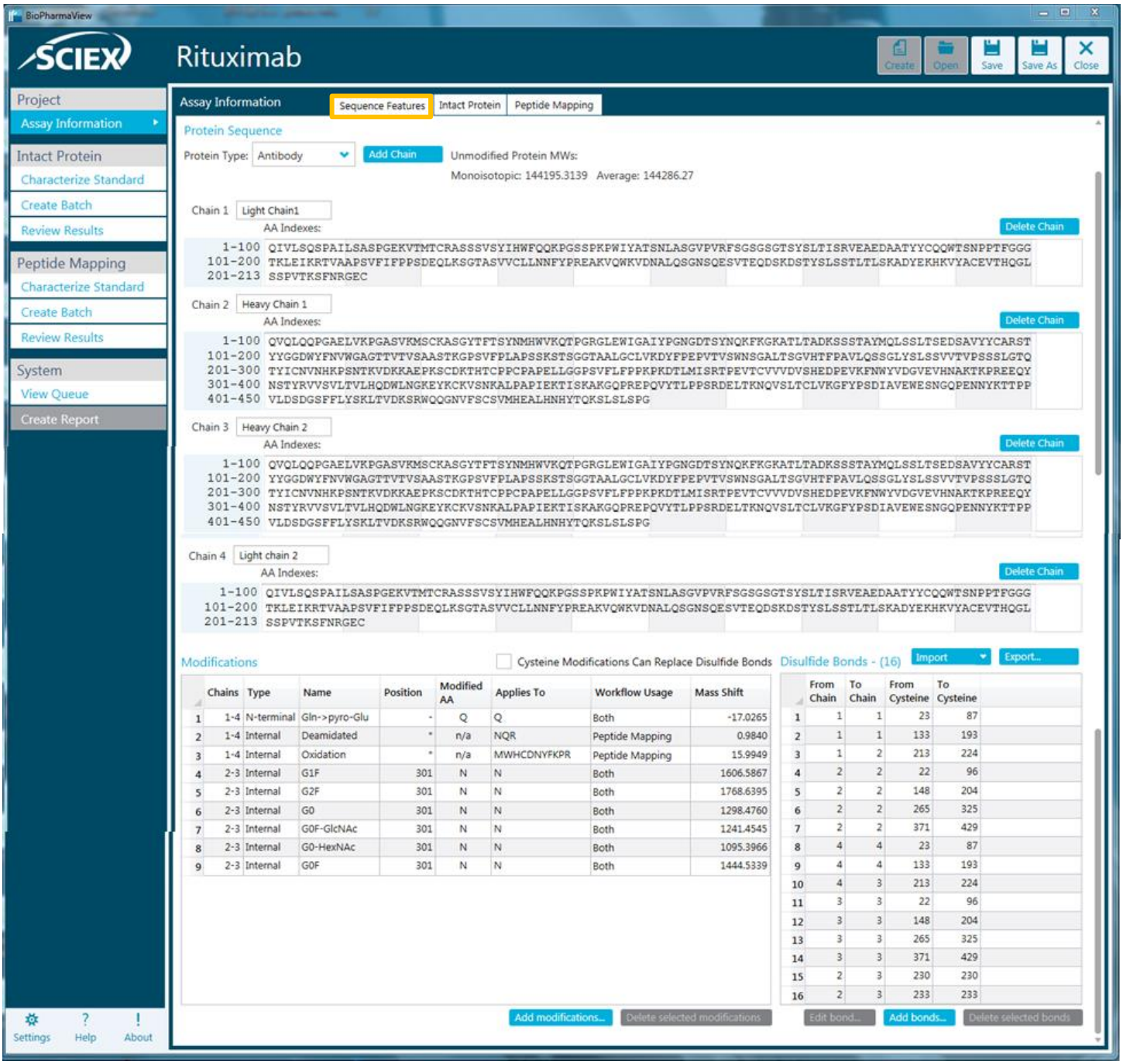




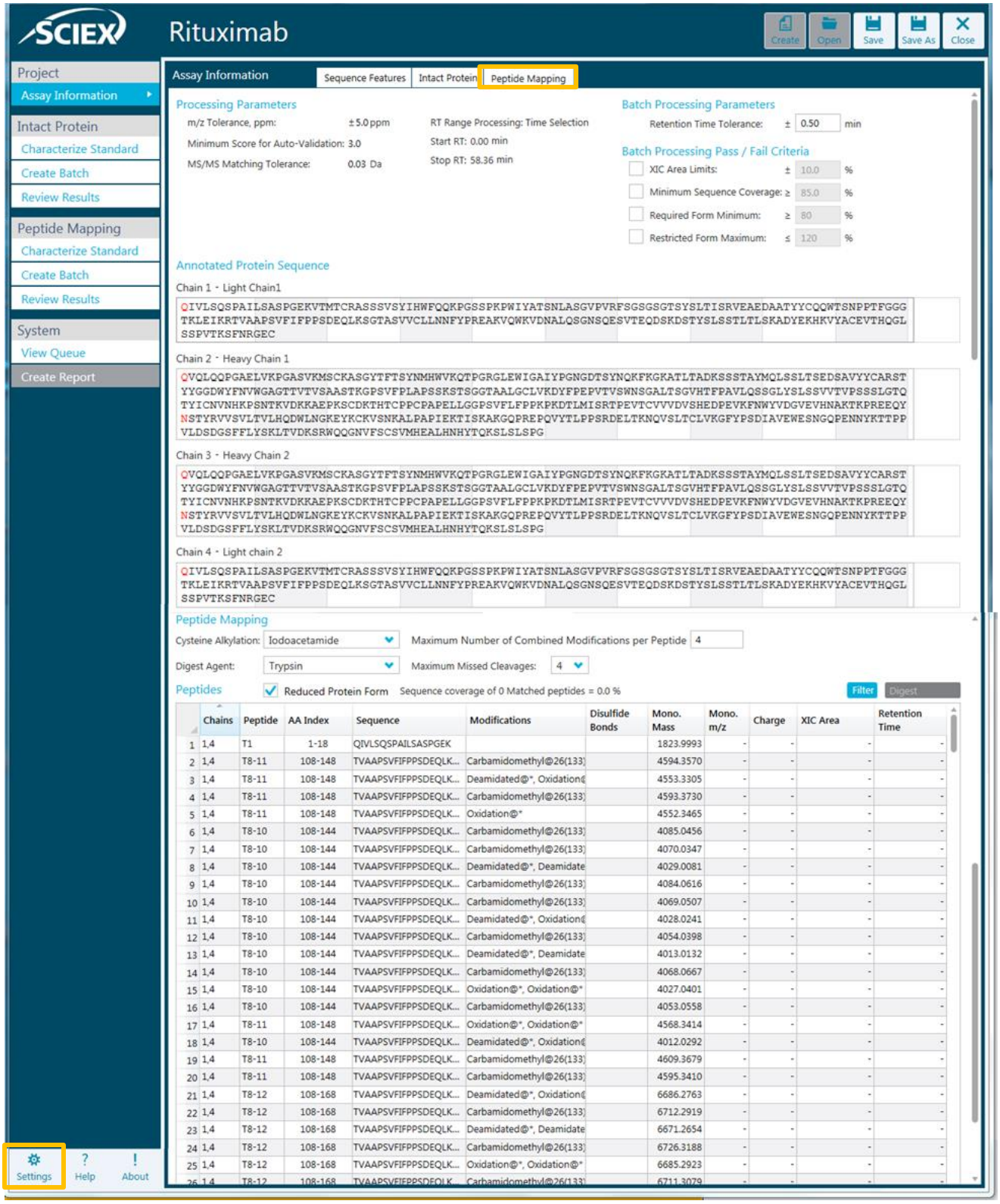
Data Processing

Process SWATH® biotherapeutic peptide mapping data in BioPharmaView™ Software 2.0.

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

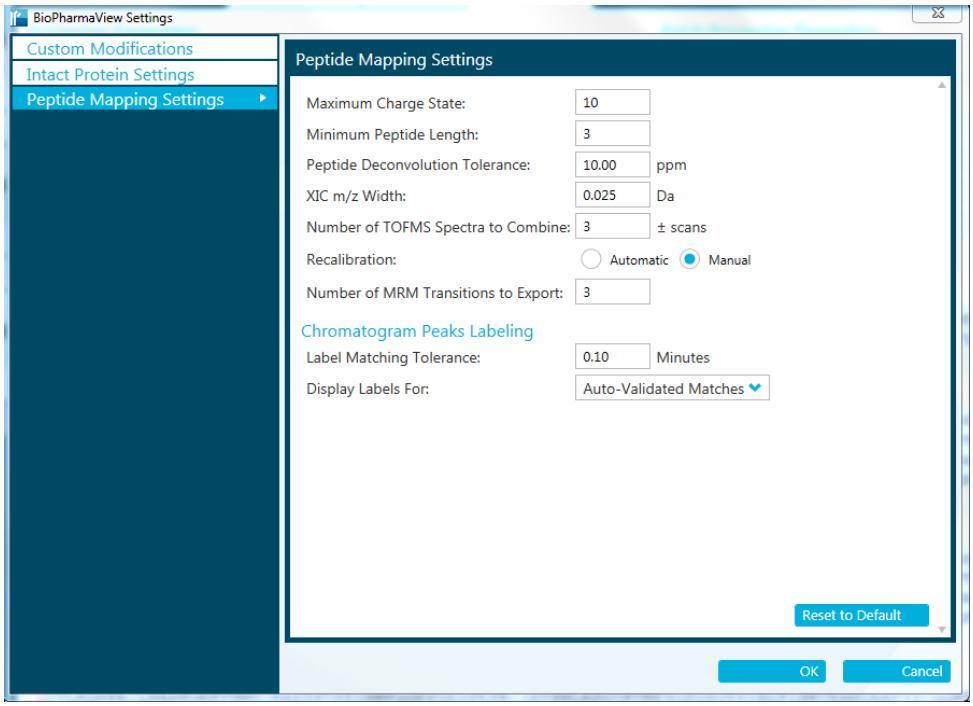


Navigate to the ‘Peptide Mapping’ tab complete processing parameters and to generate all peptide forms for matching.



	Chains	Peptide	AA Index	Sequence	Modifications	Disulfide Bonds	Mono. Mass	Mono. m/z	Charge	XIC Area	Retention Time
1	1,4	T1	1-18	QIVLSQSPAILSASPGEK			1823.9993	-	-	-	-
2	1,4	T8-11	108-148	TVAAPSVFIFPPSDEQLK...	Carbamidomethyl@26(133)		4594.3570	-	-	-	-
3	1,4	T8-11	108-148	TVAAPSVFIFPPSDEQLK...	Deamidated@*, Oxidation@		4553.3305	-	-	-	-
4	1,4	T8-11	108-148	TVAAPSVFIFPPSDEQLK...	Carbamidomethyl@26(133)		4593.3730	-	-	-	-
5	1,4	T8-11	108-148	TVAAPSVFIFPPSDEQLK...	Oxidation@*		4552.3465	-	-	-	-
6	1,4	T8-10	108-144	TVAAPSVFIFPPSDEQLK...	Carbamidomethyl@26(133)		4085.0456	-	-	-	-
7	1,4	T8-10	108-144	TVAAPSVFIFPPSDEQLK...	Carbamidomethyl@26(133)		4070.0347	-	-	-	-
8	1,4	T8-10	108-144	TVAAPSVFIFPPSDEQLK...	Deamidated@*, Deamidate		4029.0081	-	-	-	-
9	1,4	T8-10	108-144	TVAAPSVFIFPPSDEQLK...	Carbamidomethyl@26(133)		4084.0616	-	-	-	-
10	1,4	T8-10	108-144	TVAAPSVFIFPPSDEQLK...	Carbamidomethyl@26(133)		4069.0507	-	-	-	-
11	1,4	T8-10	108-144	TVAAPSVFIFPPSDEQLK...	Deamidated@*, Oxidation@		4028.0241	-	-	-	-
12	1,4	T8-10	108-144	TVAAPSVFIFPPSDEQLK...	Carbamidomethyl@26(133)		4054.0398	-	-	-	-
13	1,4	T8-10	108-144	TVAAPSVFIFPPSDEQLK...	Deamidated@*, Deamidate		4013.0132	-	-	-	-
14	1,4	T8-10	108-144	TVAAPSVFIFPPSDEQLK...	Carbamidomethyl@26(133)		4068.0667	-	-	-	-
15	1,4	T8-10	108-144	TVAAPSVFIFPPSDEQLK...	Oxidation@*, Oxidation@*		4027.0401	-	-	-	-
16	1,4	T8-10	108-144	TVAAPSVFIFPPSDEQLK...	Carbamidomethyl@26(133)		4053.0558	-	-	-	-
17	1,4	T8-11	108-148	TVAAPSVFIFPPSDEQLK...	Oxidation@*, Oxidation@*		4568.3414	-	-	-	-
18	1,4	T8-10	108-144	TVAAPSVFIFPPSDEQLK...	Deamidated@*, Oxidation@		4012.0292	-	-	-	-
19	1,4	T8-11	108-148	TVAAPSVFIFPPSDEQLK...	Carbamidomethyl@26(133)		4609.3679	-	-	-	-
20	1,4	T8-11	108-148	TVAAPSVFIFPPSDEQLK...	Carbamidomethyl@26(133)		4595.3410	-	-	-	-
21	1,4	T8-12	108-168	TVAAPSVFIFPPSDEQLK...	Deamidated@*, Oxidation@		6686.2763	-	-	-	-
22	1,4	T8-12	108-168	TVAAPSVFIFPPSDEQLK...	Carbamidomethyl@26(133)		6712.2919	-	-	-	-
23	1,4	T8-12	108-168	TVAAPSVFIFPPSDEQLK...	Deamidated@*, Deamidate		6671.2654	-	-	-	-
24	1,4	T8-12	108-168	TVAAPSVFIFPPSDEQLK...	Carbamidomethyl@26(133)		6726.3188	-	-	-	-
25	1,4	T8-12	108-168	TVAAPSVFIFPPSDEQLK...	Oxidation@*, Oxidation@*		6685.2923	-	-	-	-
26	1,4	T8-12	108-168	TVAAPSVFIFPPSDEQLK...	Carbamidomethyl@26(133)		6711.3079	-	-	-	-

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

AB Sciex is doing business as SCIEX.

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