Biotherapeutic Non-Reduced Peptide Mapping

Routine non-reduced peptide mapping of biotherapeutics on the X500B QTOF System

Method details for the routine non-reduced 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 MS and MS/MS level data on the digested biologic protein product for the purpose of localizing disulfide bonds. Either an information dependent acquisition (IDA) method or an information independent SWATH acquisition method can be employed for non-reduced peptide mapping analyses.

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, thus limiting the need to reanalyze a sample to obtain sufficient MS/MS spectra for disulfide bond location confirmation.

Sample Prep

A generic sample preparation strategy is shown for the digestion of an antibody biotherapeutic prior to a non-reduced peptide mapping LC-MS analysis.

Step 1
- Buffer exchange antibody sample (0.2mg) in Ammonium Bicarbonate (100mM) buffer and add 25 µL Trifluoroethanol (TFE) denaturation agent. 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 completely.

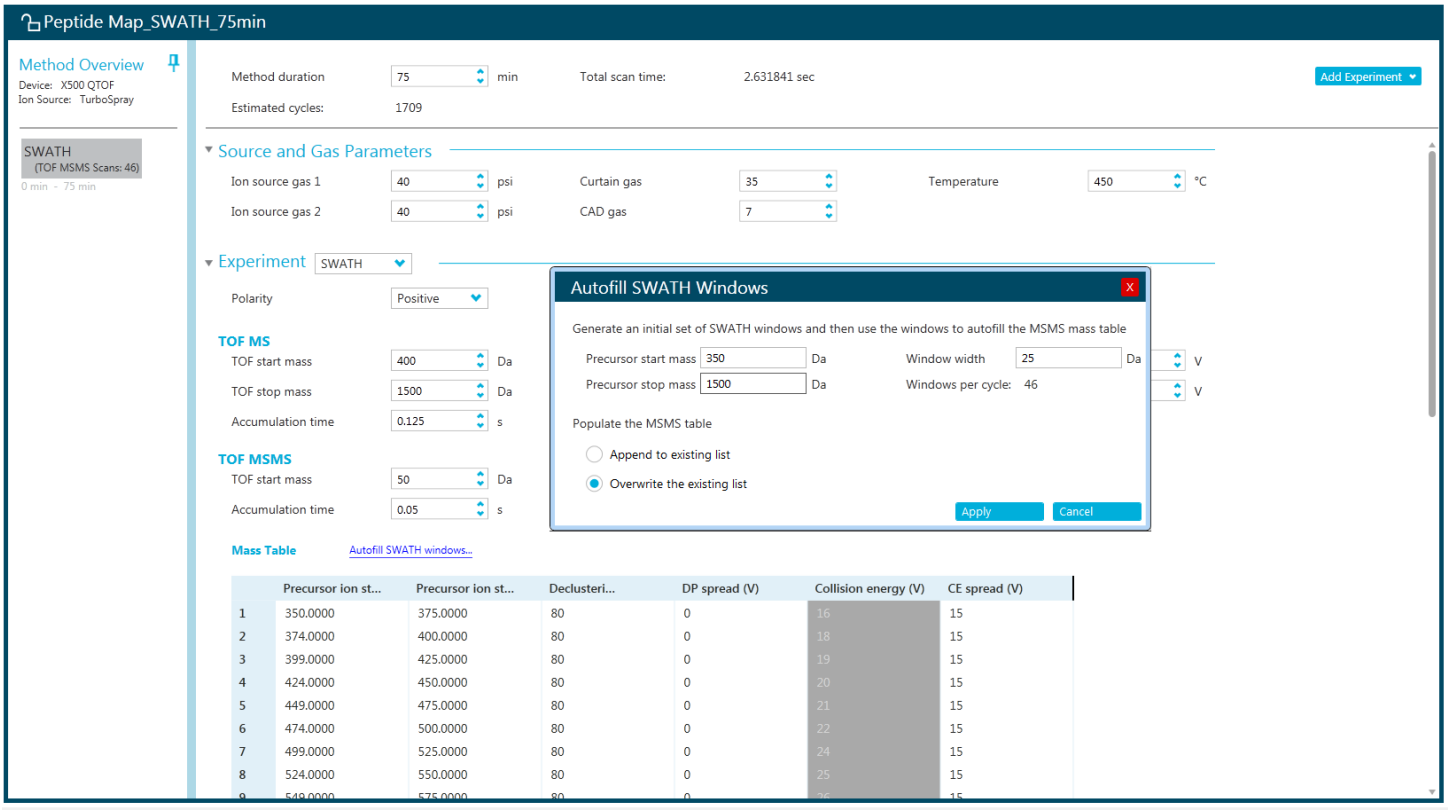
Step 7
- Reonstitute the digest into Mobile Phase A, to ~0.1 µg/µL just before the analysis.

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

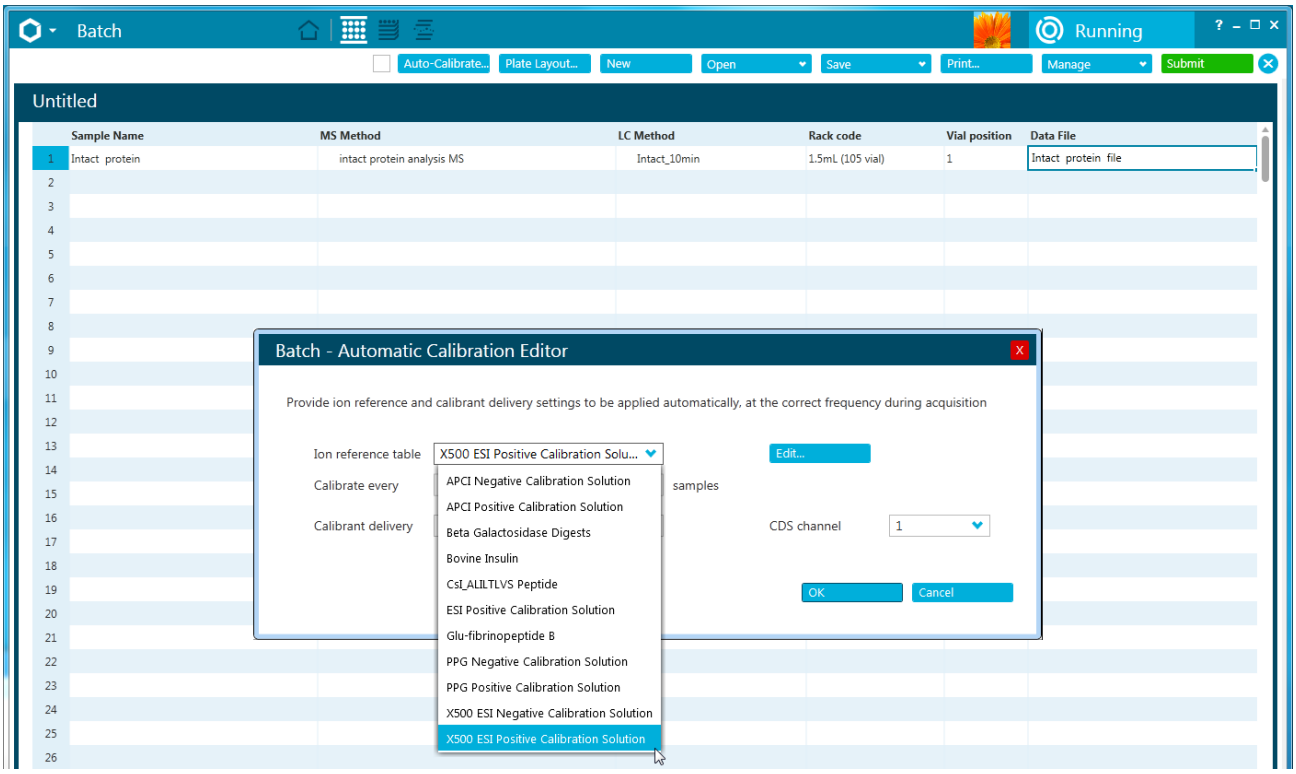
MS Method

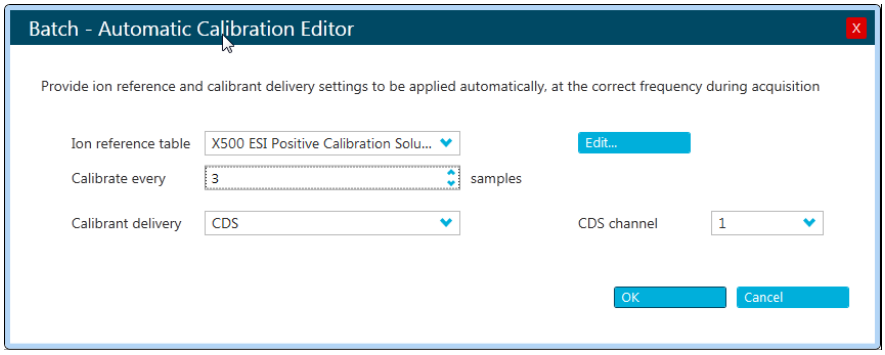
Suggested starting MS and MS/MS method parameters for routine SWATH based peptide mapping analysis as displayed in SCIEX OS user interface. The SWATH acquisition criteria are shown with a 25Da fixed SWATH window from 350-1500 m/z acquiring high-resolution MS/MS in each cycle. For best sequence coverage 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.

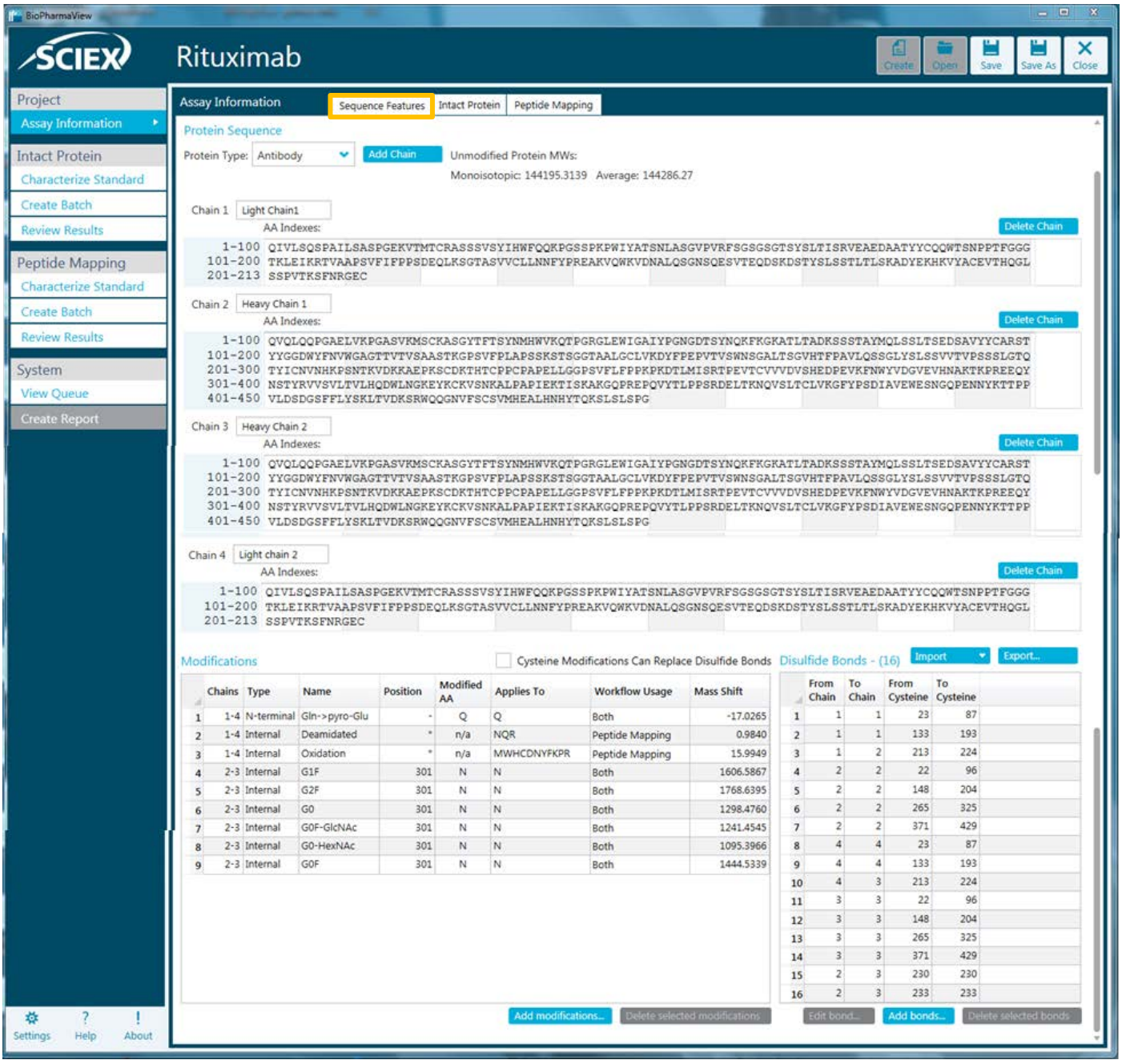




Data Processing

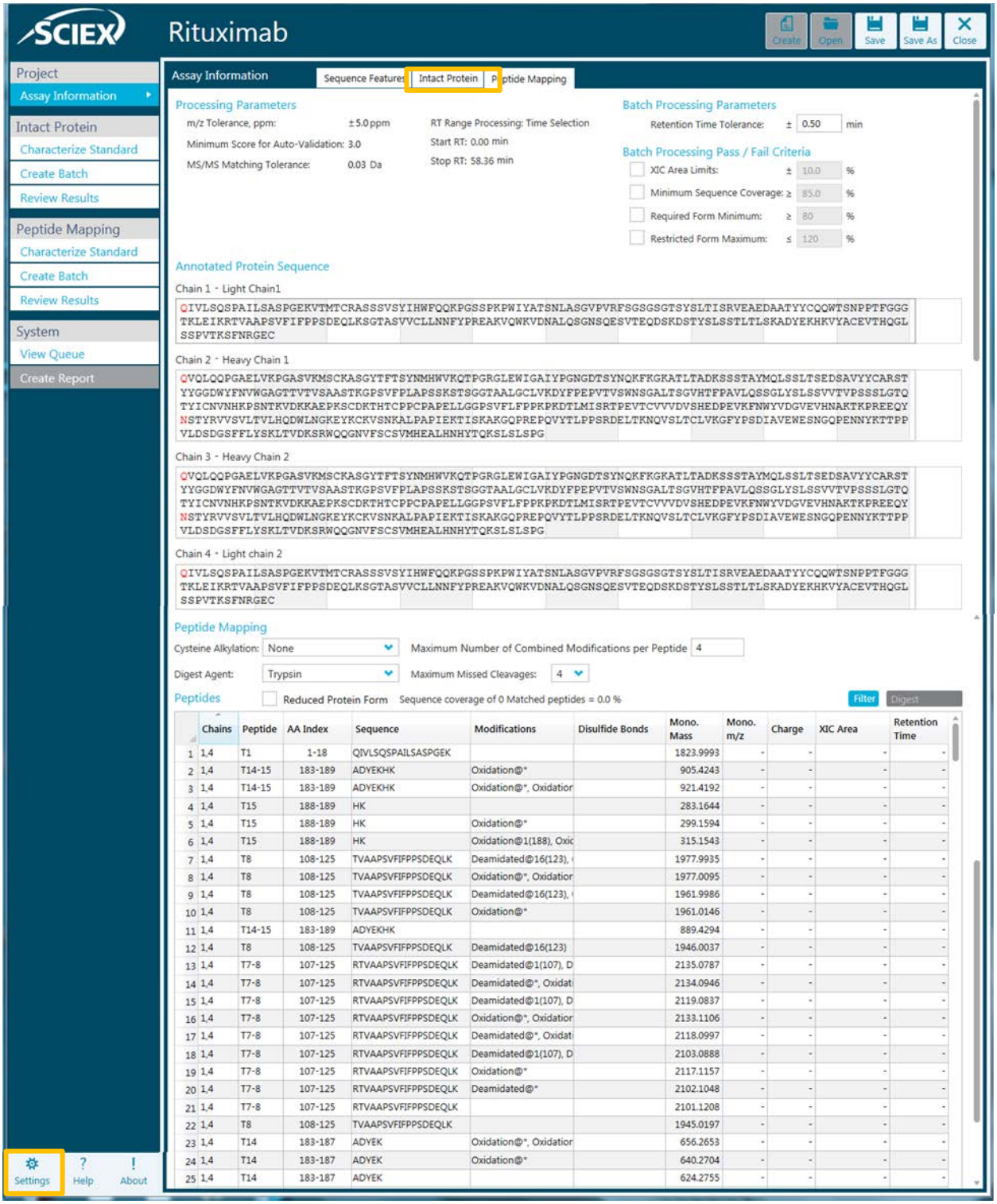
Process SWATH® biotherapeutic peptide mapping data in BioPharmaView™ Software 2.0.

Input the protein sequence, and assign potential modifications as well as expected localization of disulfide bonds in the ‘Assay Information’ window.



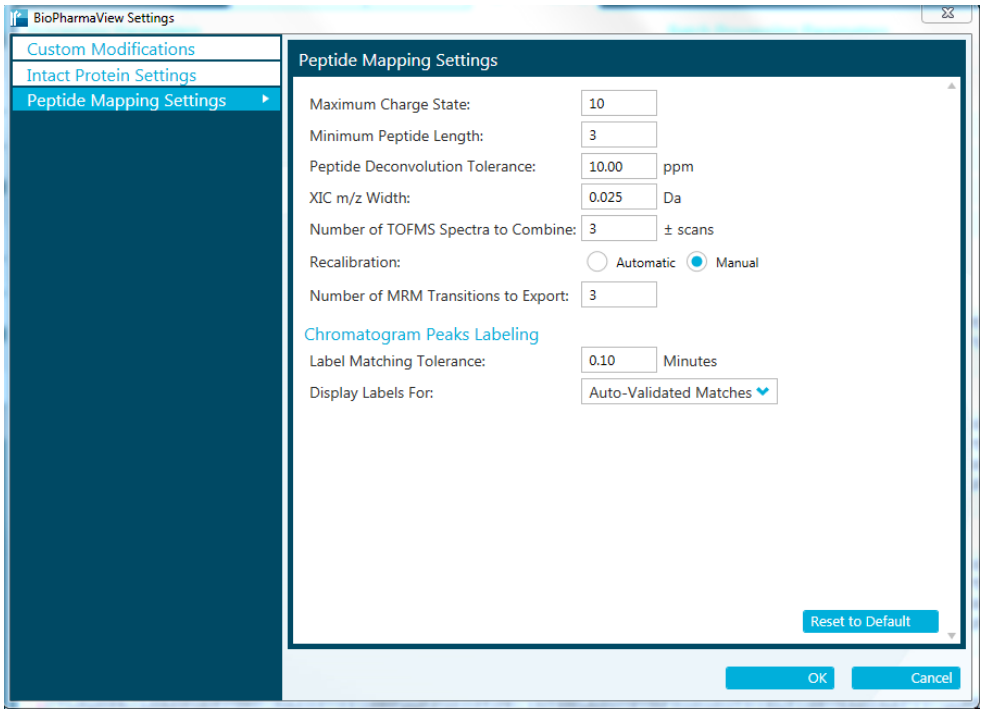
Pharma and Biopharma

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



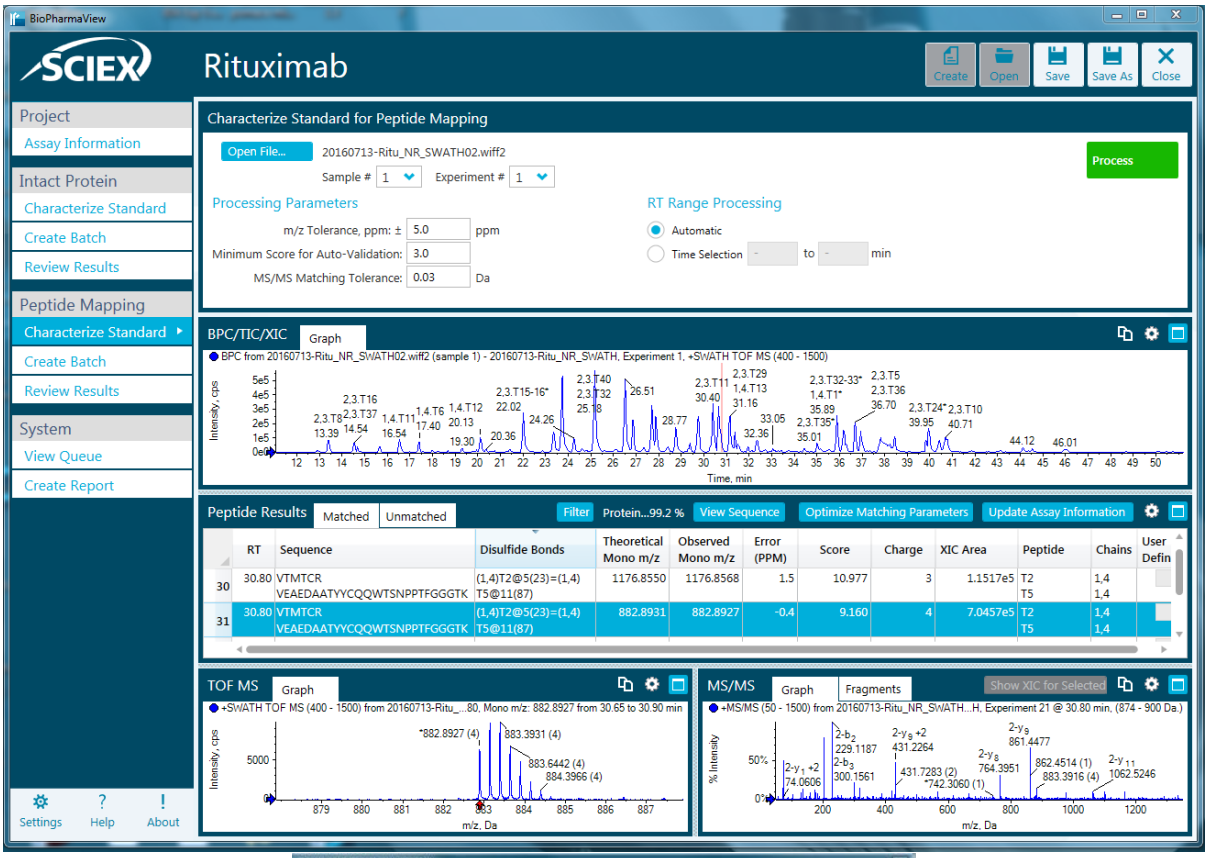
	Chains	Peptide	AA Index	Sequence	Modifications	Disulfide Bonds	Mono. Mass	Mono. m/z	Charge	XIC Area	Retention Time
1	1,4	T1	1-18	QIVLSQSPAILSASPGEK			1823.9993	-	-	-	-
2	1,4	T14-15	183-189	ADYEKHK	Oxidation@*		905.4243	-	-	-	-
3	1,4	T14-15	183-189	ADYEKHK	Oxidation@*, Oxidatior		921.4192	-	-	-	-
4	1,4	T15	188-189	HK			283.1644	-	-	-	-
5	1,4	T15	188-189	HK	Oxidation@*		299.1594	-	-	-	-
6	1,4	T15	188-189	HK	Oxidation@1(188), Oxic		315.1543	-	-	-	-
7	1,4	T8	108-125	TVAAPSVFIFPPSDEQLK	Deamidated@16(123), (		1977.9935	-	-	-	-
8	1,4	T8	108-125	TVAAPSVFIFPPSDEQLK	Oxidation@*, Oxidatior		1977.0095	-	-	-	-
9	1,4	T8	108-125	TVAAPSVFIFPPSDEQLK	Deamidated@16(123), (		1961.9986	-	-	-	-
10	1,4	T8	108-125	TVAAPSVFIFPPSDEQLK	Oxidation@*		1961.0146	-	-	-	-
11	1,4	T14-15	183-189	ADYEKHK			889.4294	-	-	-	-
12	1,4	T8	108-125	TVAAPSVFIFPPSDEQLK	Deamidated@16(123)		1946.0037	-	-	-	-
13	1,4	T7-8	107-125	RTVAAPSVFIFPPSDEQLK	Deamidated@1(107), D		2135.0787	-	-	-	-
14	1,4	T7-8	107-125	RTVAAPSVFIFPPSDEQLK	Deamidated@*, Oxidat		2134.0946	-	-	-	-
15	1,4	T7-8	107-125	RTVAAPSVFIFPPSDEQLK	Deamidated@1(107), D		2119.0837	-	-	-	-
16	1,4	T7-8	107-125	RTVAAPSVFIFPPSDEQLK	Oxidation@*, Oxidatior		2133.1106	-	-	-	-
17	1,4	T7-8	107-125	RTVAAPSVFIFPPSDEQLK	Deamidated@*, Oxidat		2118.0997	-	-	-	-
18	1,4	T7-8	107-125	RTVAAPSVFIFPPSDEQLK	Deamidated@1(107), D		2103.0888	-	-	-	-
19	1,4	T7-8	107-125	RTVAAPSVFIFPPSDEQLK	Oxidation@*		2117.1157	-	-	-	-
20	1,4	T7-8	107-125	RTVAAPSVFIFPPSDEQLK	Deamidated@*		2102.1048	-	-	-	-
21	1,4	T7-8	107-125	RTVAAPSVFIFPPSDEQLK			2101.1208	-	-	-	-
22	1,4	T8	108-125	TVAAPSVFIFPPSDEQLK			1945.0197	-	-	-	-
23	1,4	T14	183-187	ADYEK	Oxidation@*, Oxidatior		656.2653	-	-	-	-
24	1,4	T14	183-187	ADYEK	Oxidation@*		640.2704	-	-	-	-
25	1,4	T14	183-187	ADYEK			624.2755	-	-	-	-

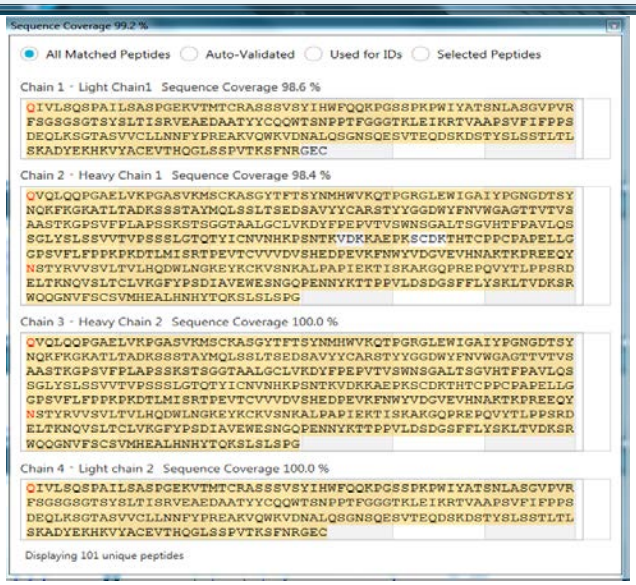
Navigate to the ‘Settings’ icon and review your global ‘Peptide Mapping Settings’



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. Disulfide bond locations are automatically identified and high-resolution MS/MS spectra for disulfide bond containing peptides are annotated to allow for fast review and bond confirmation.

Pharma and Biopharma





For more information, please visit sciex.com/X500B

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