Intact biotherapeutic mass analysis

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

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

Sample Prep

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

Option 1

- Dilute stock of biotherapeutic into stock of Mobile Phase A to reach 0.1 mg/mL concentration

Option 2

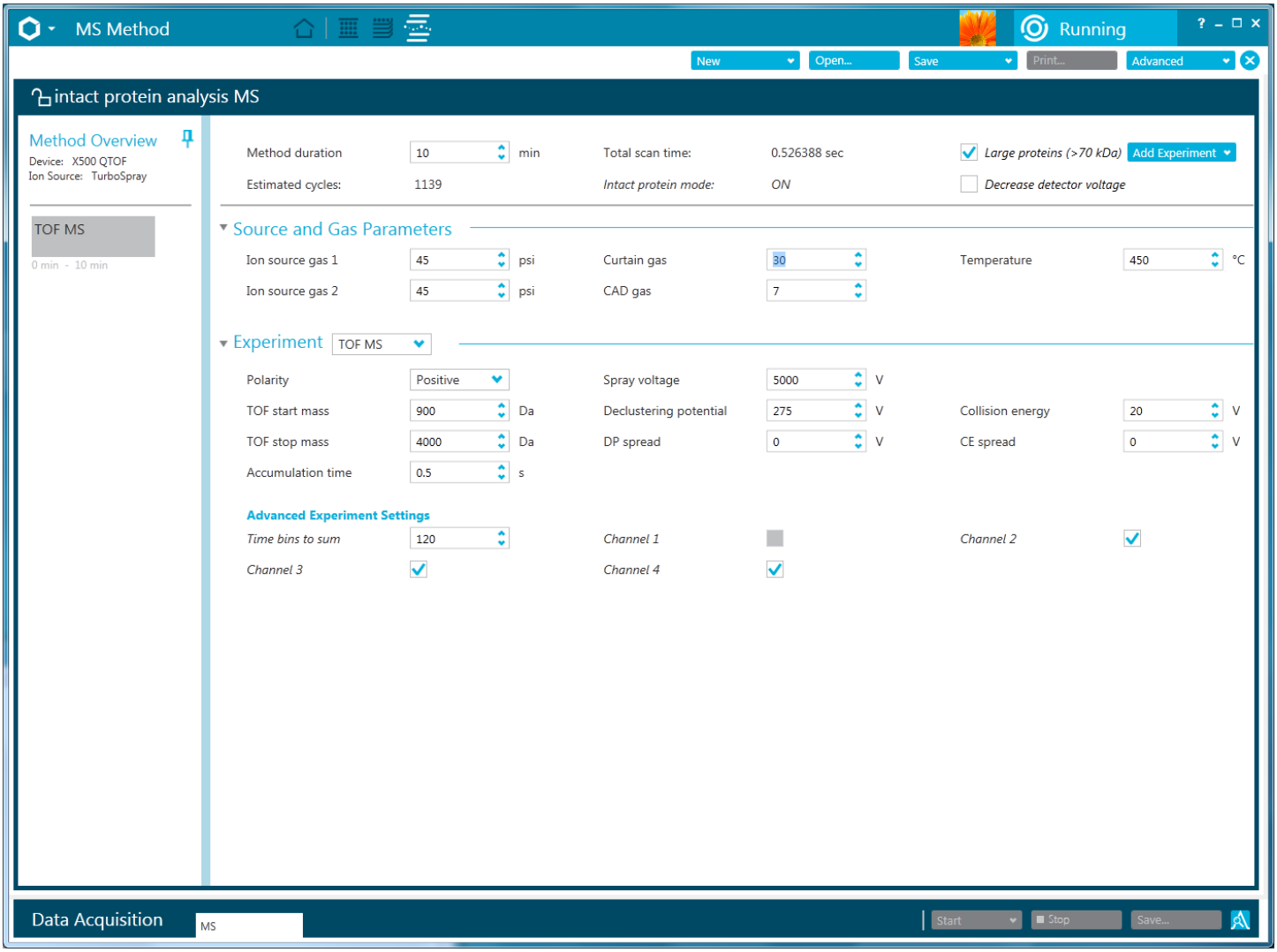
- -OR- Buffer exchange biotherapeutic sample using ultracentrifigal filter spin column (100K cutoff) using Mobile Phase A with 2-5 volumes of Mobile Phase. Reach a final biotherapeutic concentration of 0.1 mg/mL

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	5
	6.0	90
	7.0	90
	8.1	5
	10	5

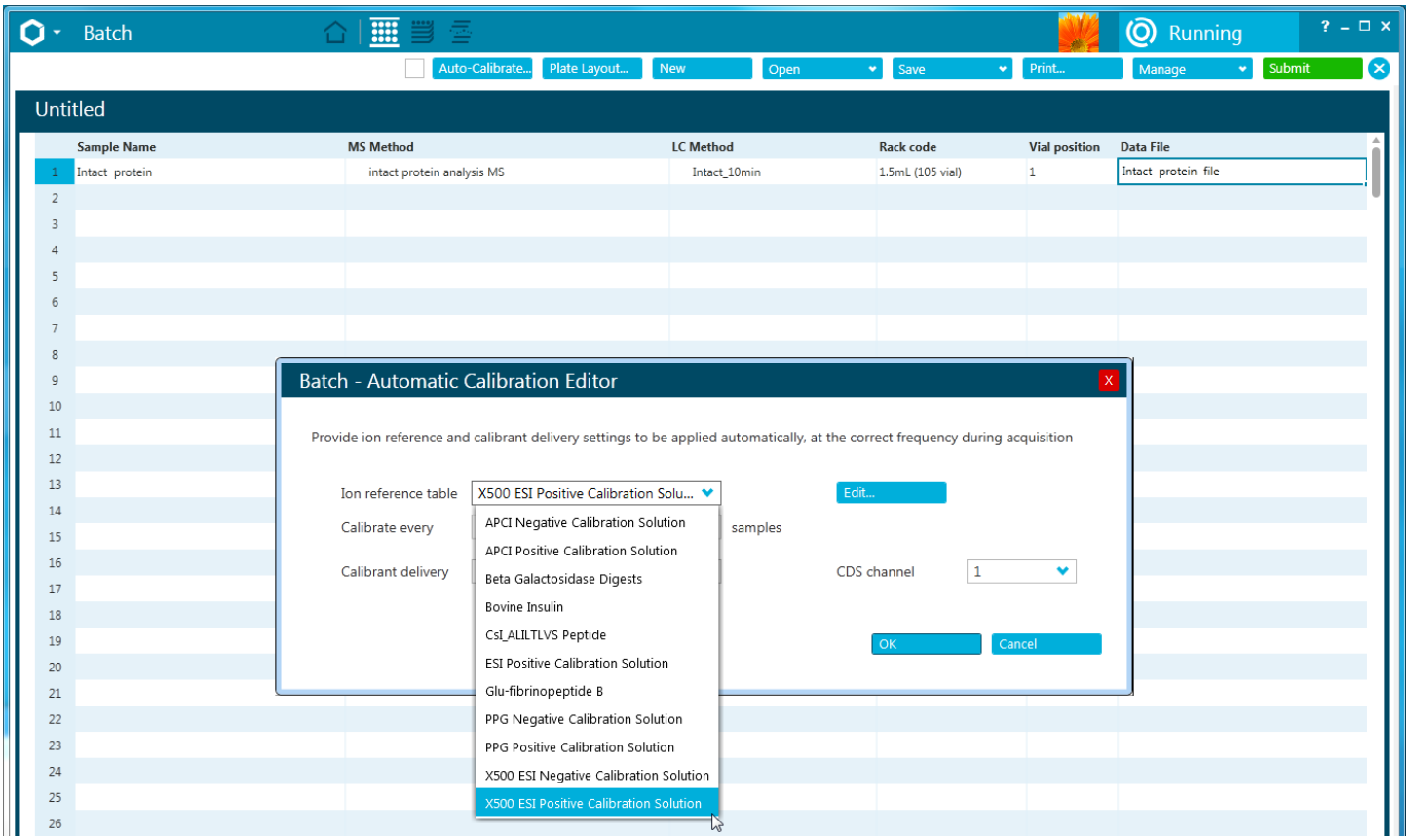
MS Method

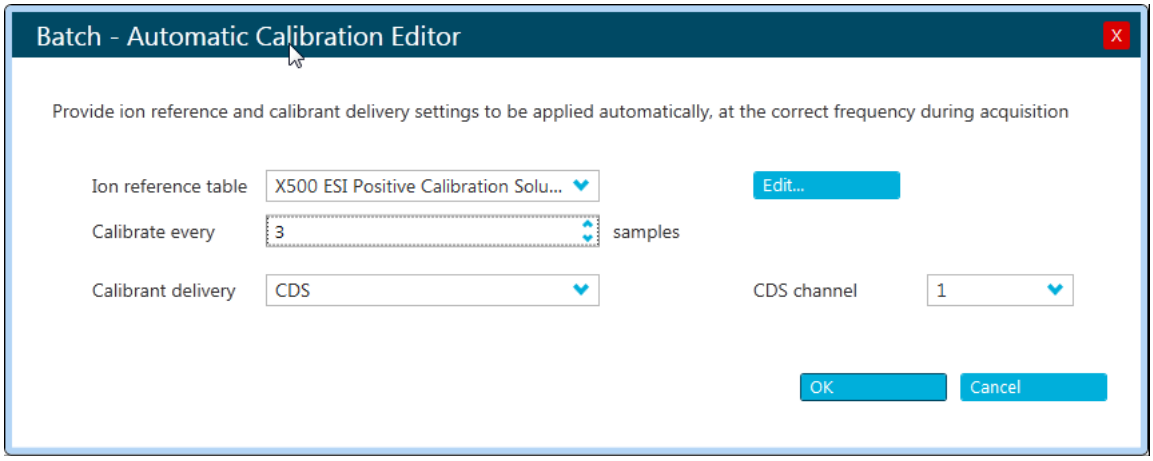
Suggested starting MS method parameters for routine intact mAb analysis as displayed in SCIEX OS. For best sensitivity and resolution, the declustering potential (DP) and collision energy (CE) parameters should be optimized for each individual biotherapeutic.



Batch

In 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.

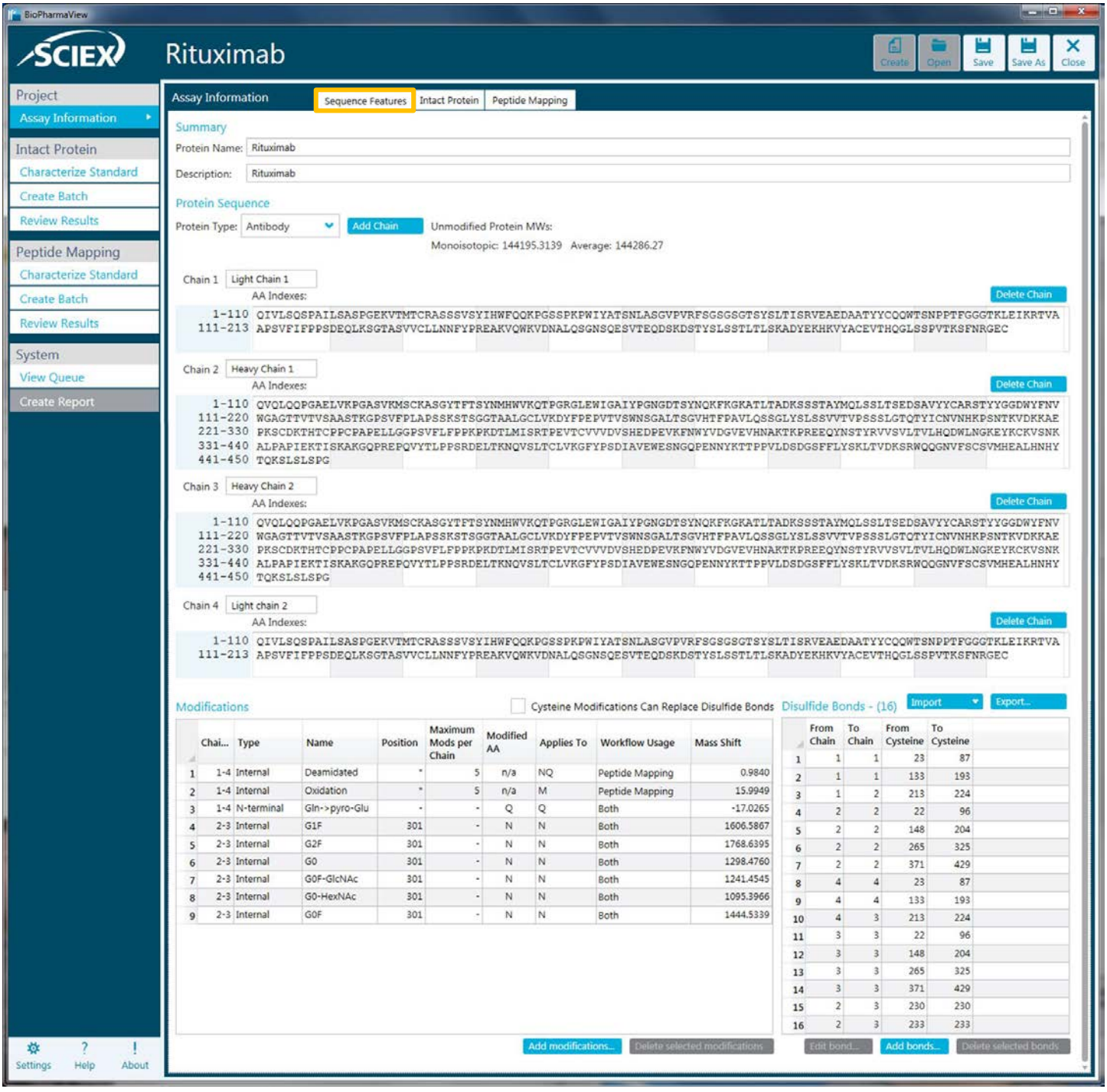




Data Processing

Process intact biotherapeutic data in BioPharmaView™ Software 2.0.

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

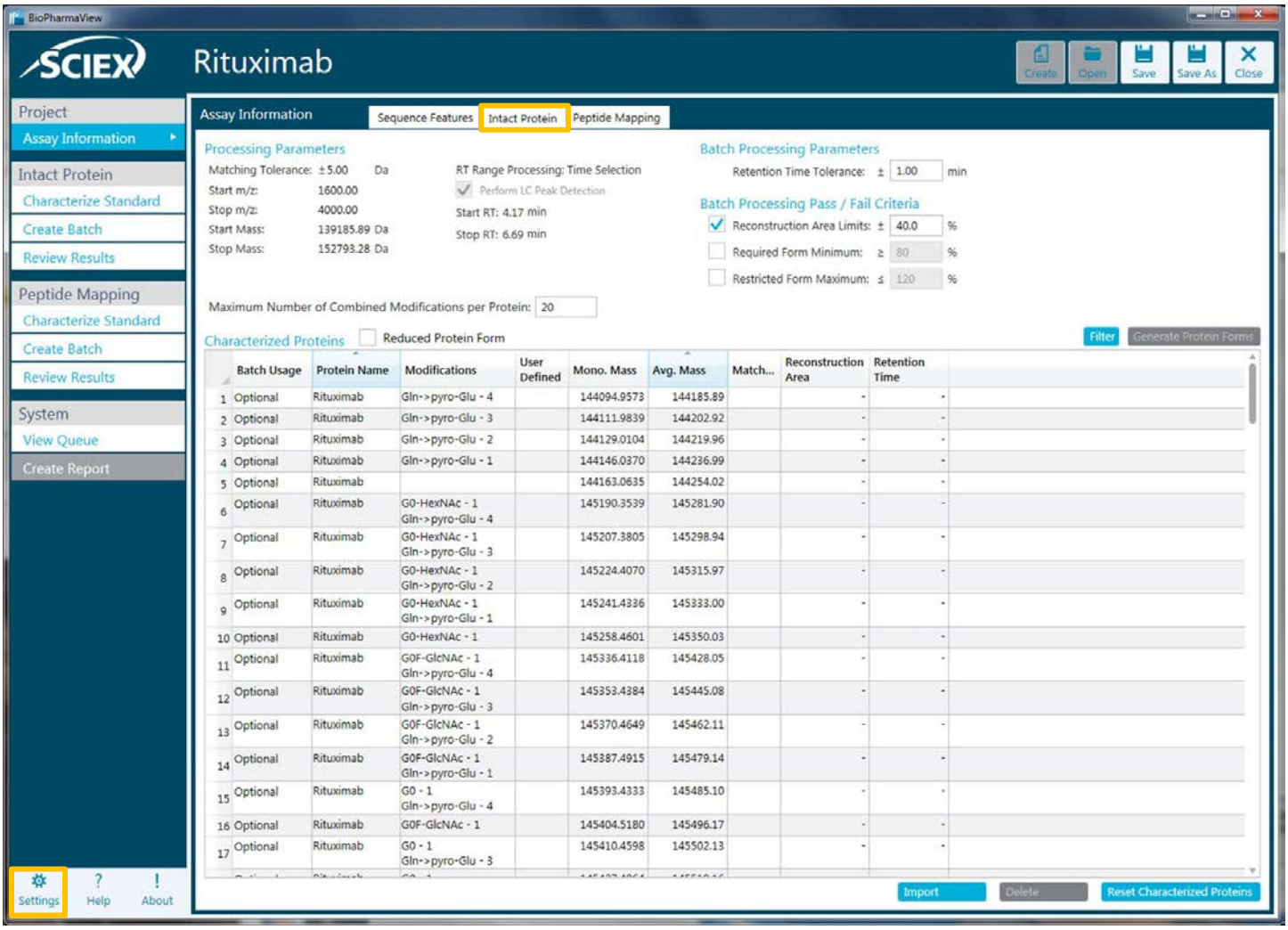


	Chai...	Type	Name	Position	Maximum Mods per Chain	Modified AA	Applies To	Workflow Usage	Mass Shift
1	1-4	Internal	Deamidated	*	5	n/a	NQ	Peptide Mapping	0.9840
2	1-4	Internal	Oxidation	*	5	n/a	M	Peptide Mapping	15.9949
3	1-4	N-terminal	Gln->pyro-Glu	-	-	Q	Q	Both	-17.0265
4	2-3	Internal	G1F	301	-	N	N	Both	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

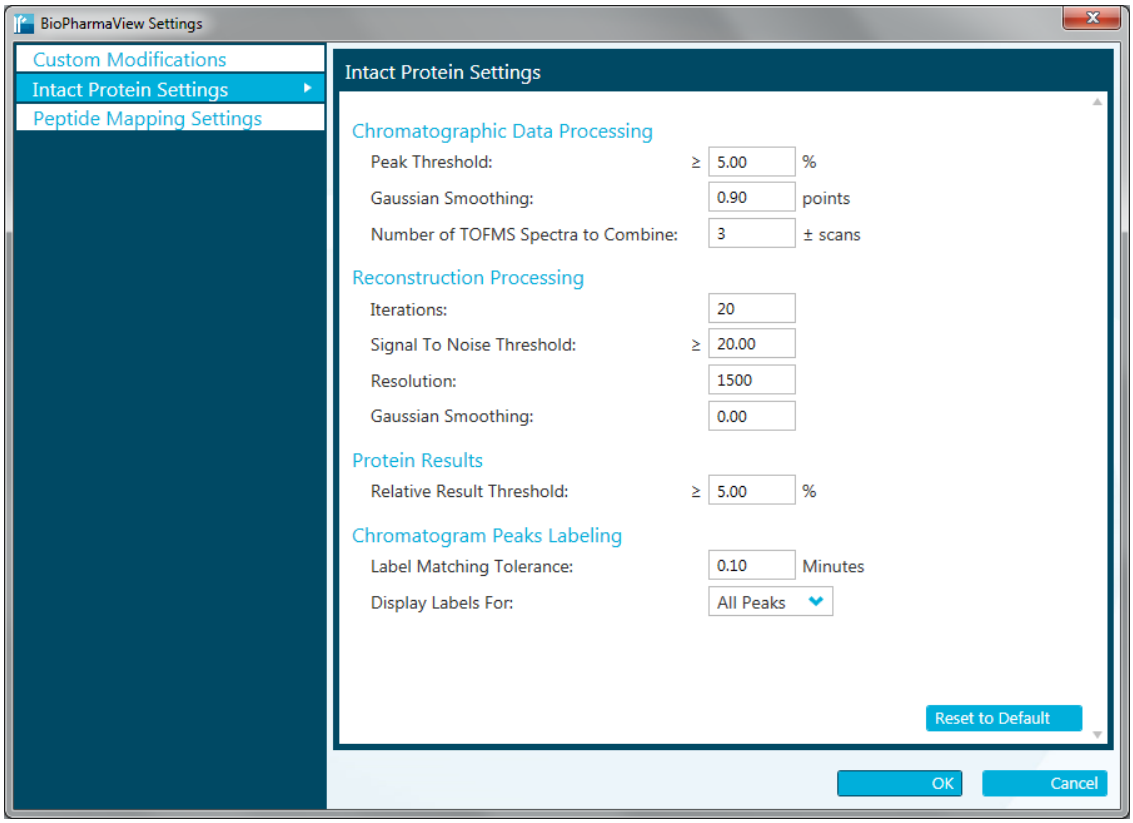
Pharma and Biopharma

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



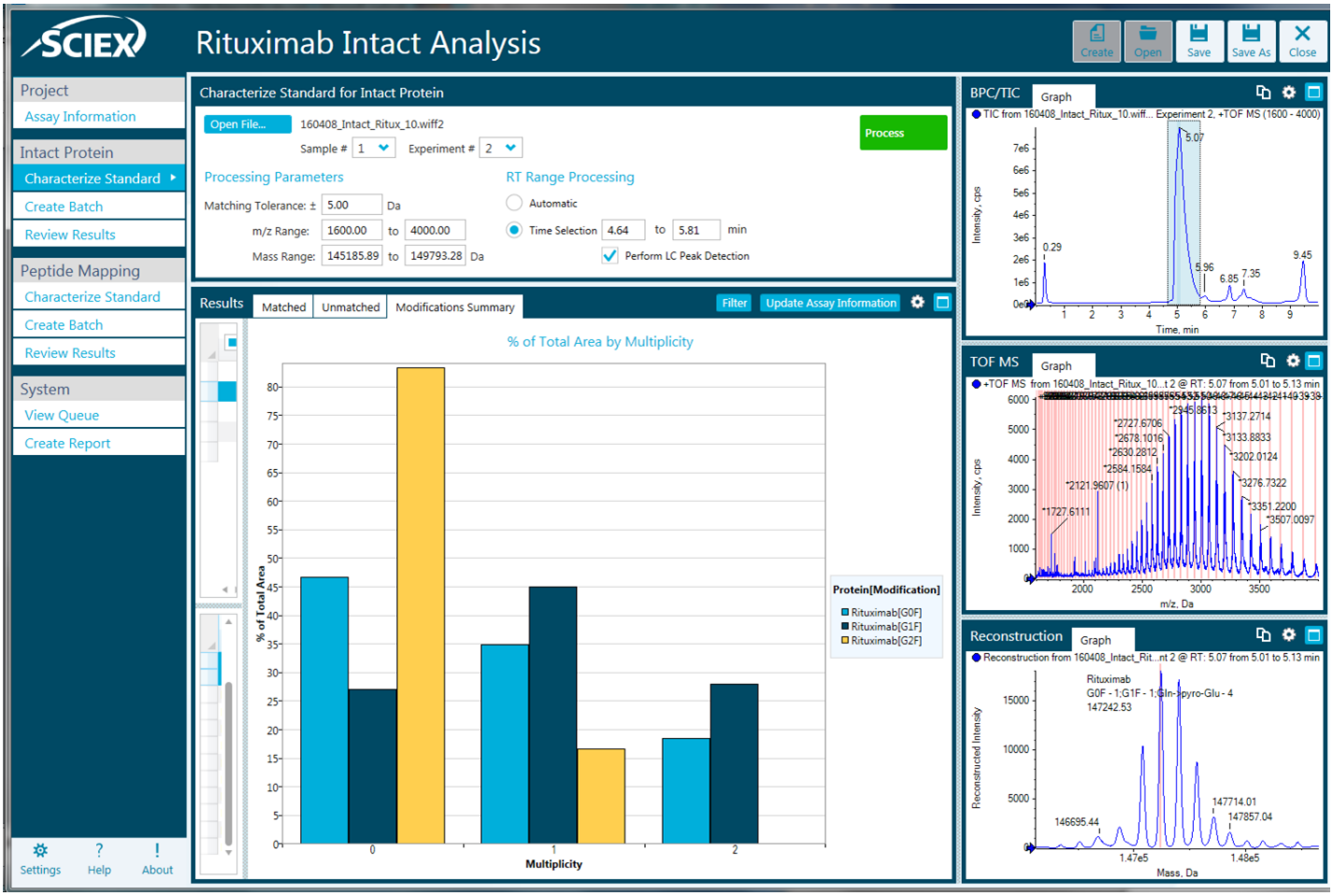
	Batch Usage	Protein Name	Modifications	User Defined	Mono. Mass	Avg. Mass	Match...	Reconstruction Area	Retention Time
1	Optional	Rituximab	Gln->pyro-Glu - 4		144094.9573	144185.89		-	-
2	Optional	Rituximab	Gln->pyro-Glu - 3		144111.9839	144202.92		-	-
3	Optional	Rituximab	Gln->pyro-Glu - 2		144129.0104	144219.96		-	-
4	Optional	Rituximab	Gln->pyro-Glu - 1		144146.0370	144236.99		-	-
5	Optional	Rituximab			144163.0635	144254.02		-	-
6	Optional	Rituximab	G0-HexNAc - 1 Gln->pyro-Glu - 4		145190.3539	145281.90		-	-
7	Optional	Rituximab	G0-HexNAc - 1 Gln->pyro-Glu - 3		145207.3805	145298.94		-	-
8	Optional	Rituximab	G0-HexNAc - 1 Gln->pyro-Glu - 2		145224.4070	145315.97		-	-
9	Optional	Rituximab	G0-HexNAc - 1 Gln->pyro-Glu - 1		145241.4336	145333.00		-	-
10	Optional	Rituximab	G0-HexNAc - 1		145258.4601	145350.03		-	-
11	Optional	Rituximab	G0F-GlcNAc - 1 Gln->pyro-Glu - 4		145336.4118	145428.05		-	-
12	Optional	Rituximab	G0F-GlcNAc - 1 Gln->pyro-Glu - 3		145353.4384	145445.08		-	-
13	Optional	Rituximab	G0F-GlcNAc - 1 Gln->pyro-Glu - 2		145370.4649	145462.11		-	-
14	Optional	Rituximab	G0F-GlcNAc - 1 Gln->pyro-Glu - 1		145387.4915	145479.14		-	-
15	Optional	Rituximab	G0 - 1 Gln->pyro-Glu - 4		145393.4333	145485.10		-	-
16	Optional	Rituximab	G0F-GlcNAc - 1		145404.5180	145496.17		-	-
17	Optional	Rituximab	G0 - 1 Gln->pyro-Glu - 3		145410.4598	145502.13		-	-

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 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 modifications, such as glycosylation, can be found in the 'Modification Summary' window (bottom left).



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Document number: RUO-MKT-02-4521-A

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