iTRAQ Quantitation with Libra

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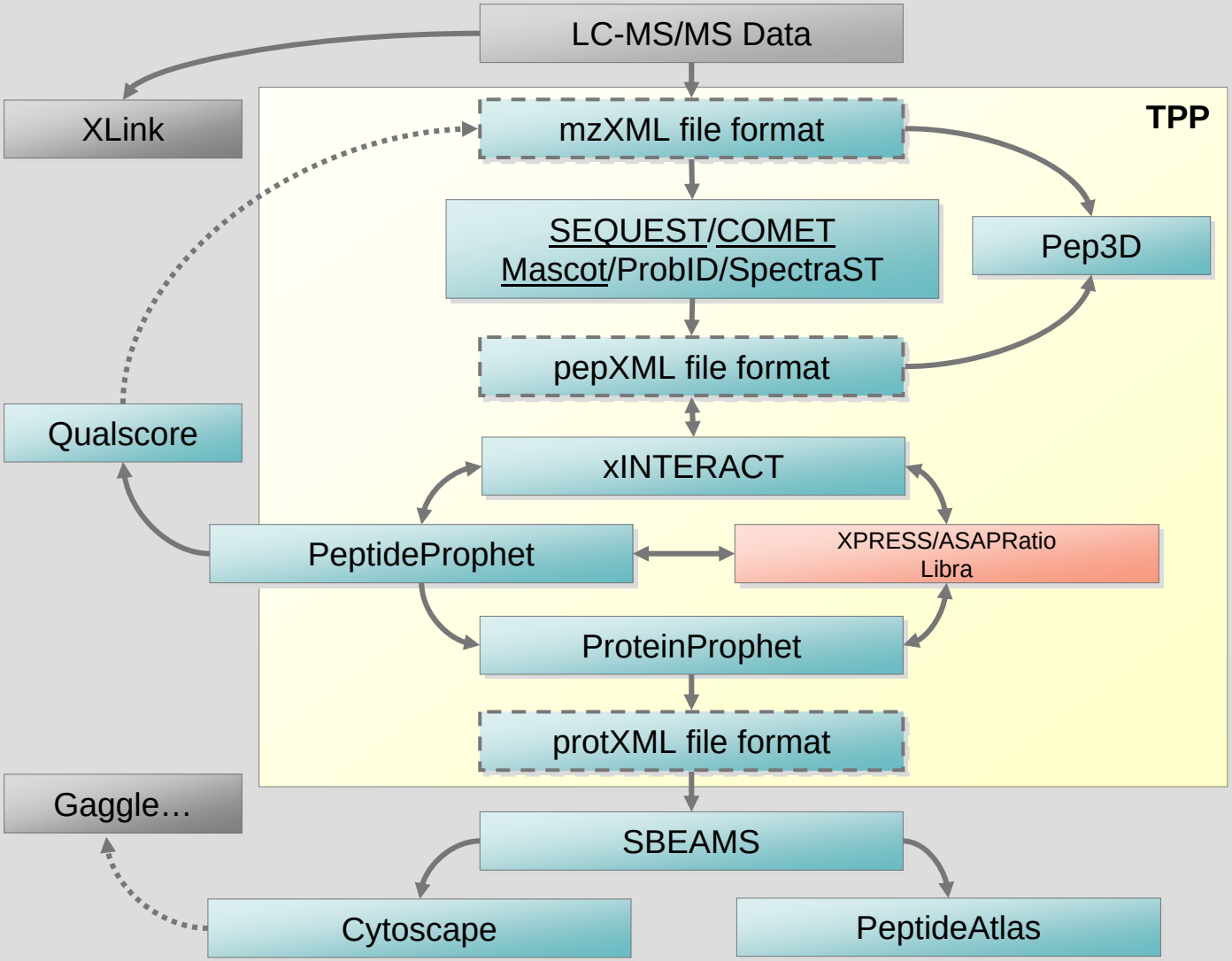
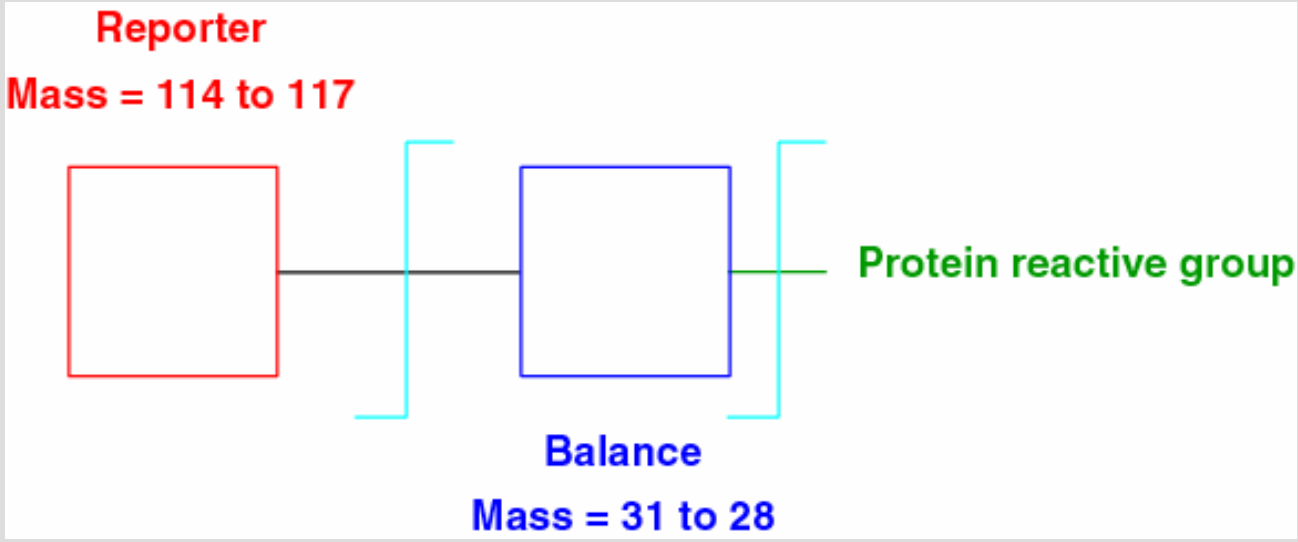
Peptide and Protein Quantitation



Libra Overview

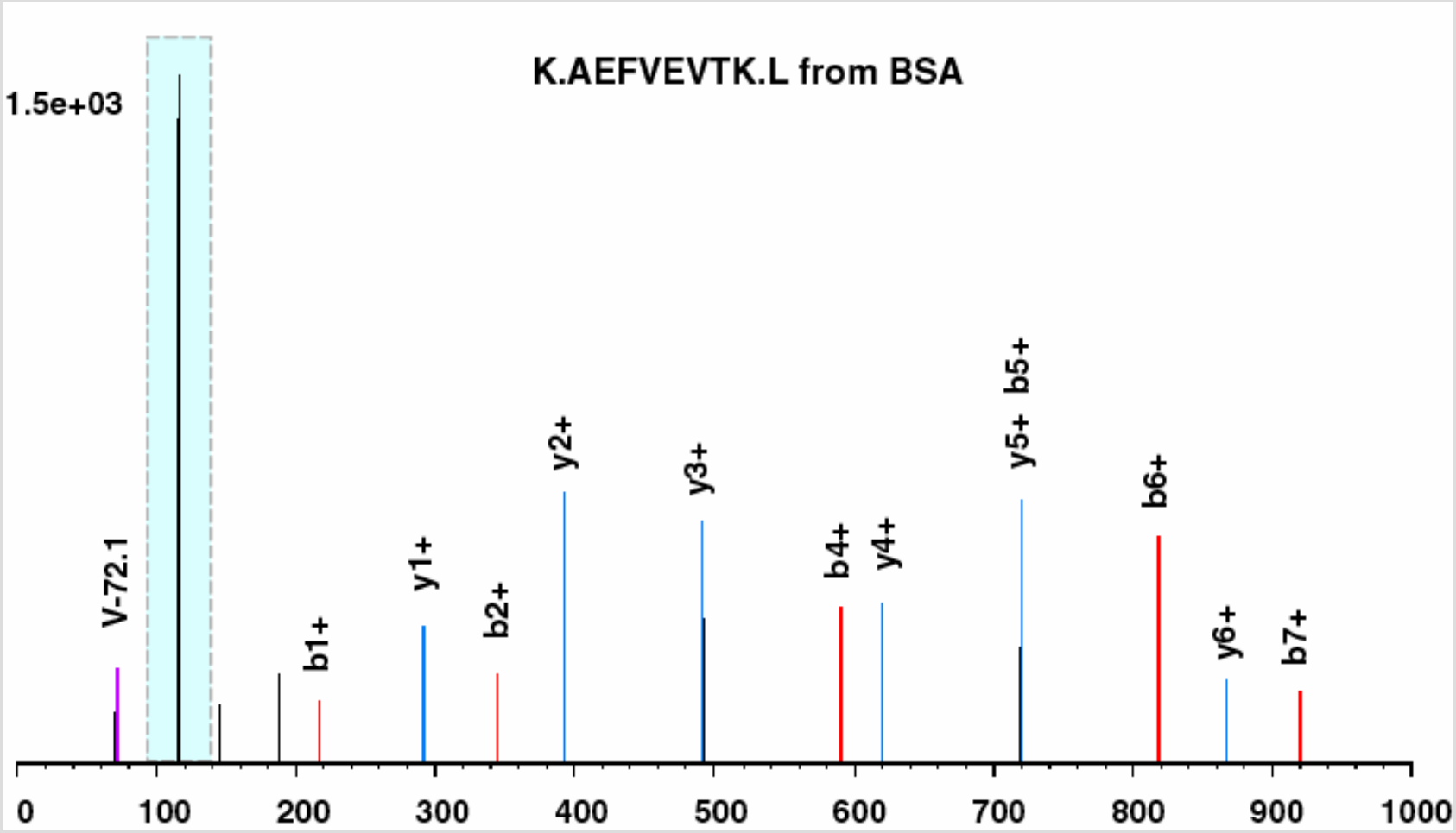
- Libra MS^2-level Quantitation
  - Not alternative to ASAPRatio or XPRESS
  - An alternative labeling technique
- Background on iTRAQ
- Peptide Quantitation
- Protein Quantitation
- Running Libra
- Looking at results

iTRAQ

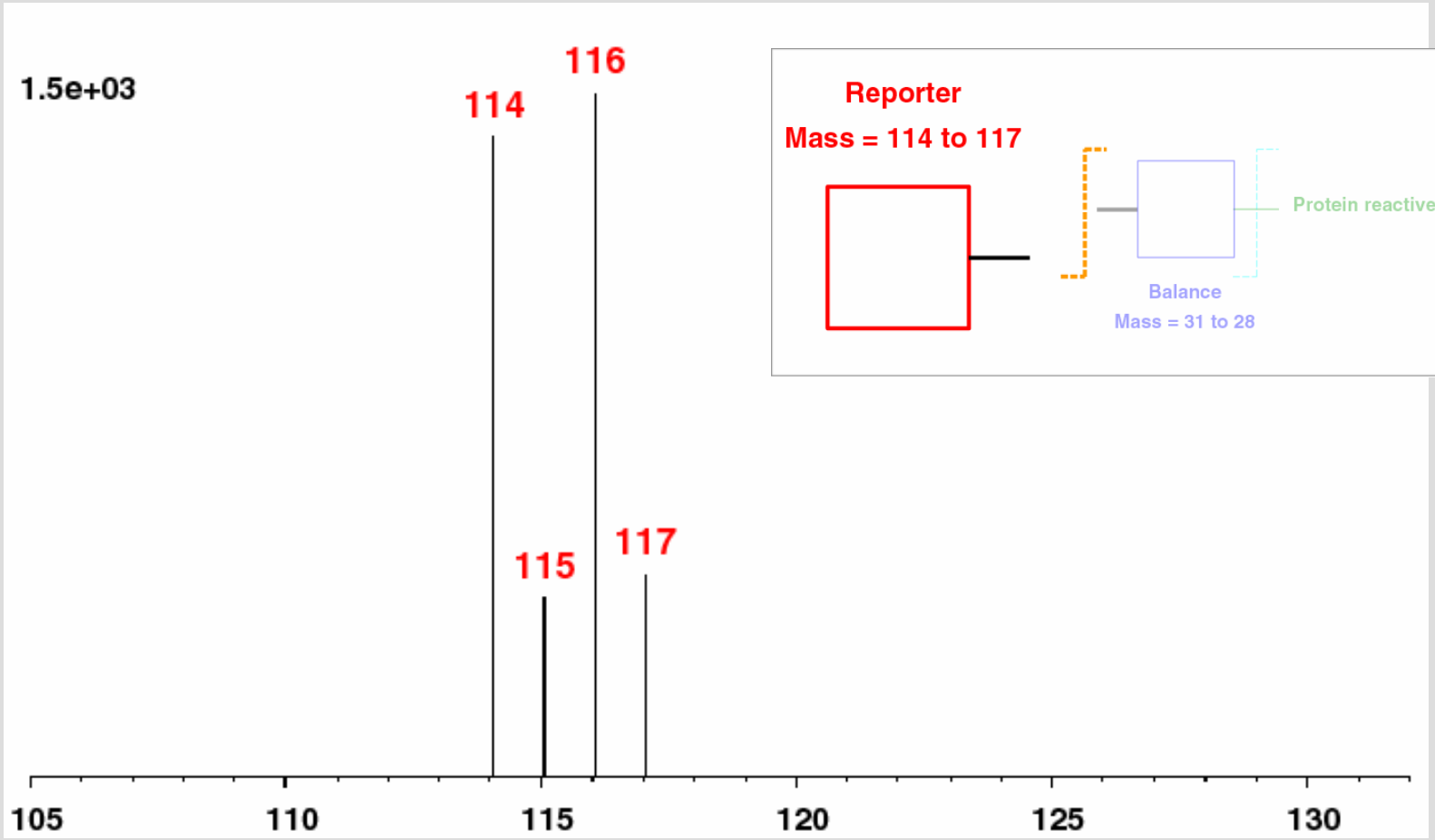


Four reagents with the same mass and same retention time on reverse phase chromatography, but different **reporter ions** upon fragmentation.

iTRAQ

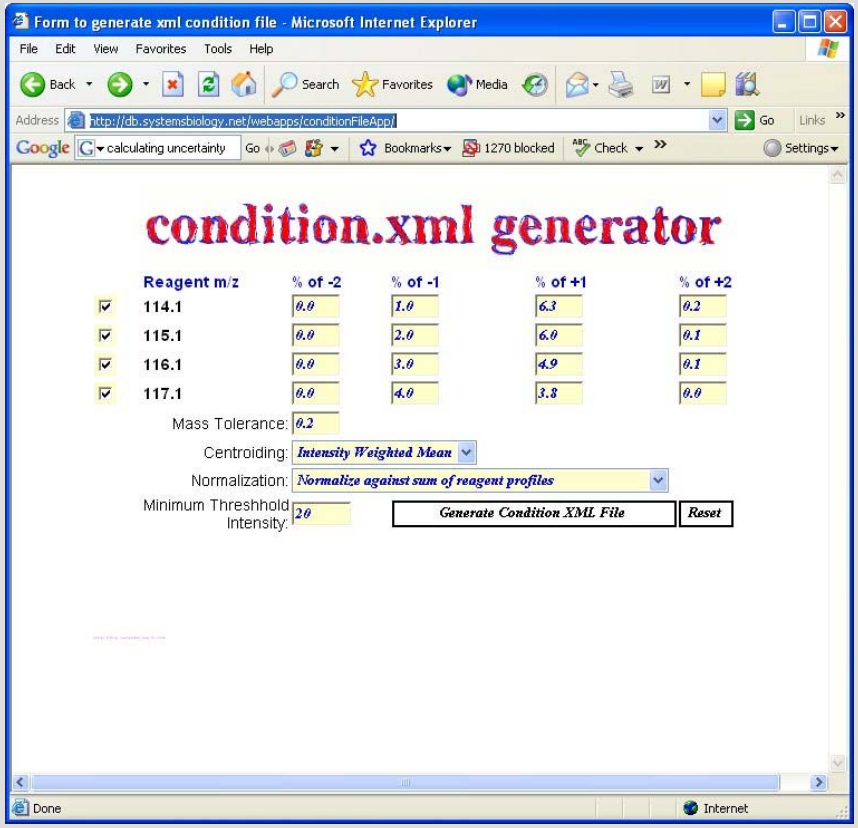

K.AEFVEVTK.L from BSA
1.5e+03
V-72.1
b1+
y1+
b2+
y2+
y3+
b4+
y4+
y5+
b5+
b6+
y6+
b7+
0
100
200
300
400
500
600
700
800
900
1000

iTRAQ


1.5e+03
114
116
115
117
105
110
115
120
125
130
Reporter
Mass = 114 to 117
Protein reactive group
Balance
Mass = 31 to 28

Libra

- Parses mzXML files to **extract intensities** for a defined list of m/z values
- Controlled by an **XML parameter file** (conditions.xml)

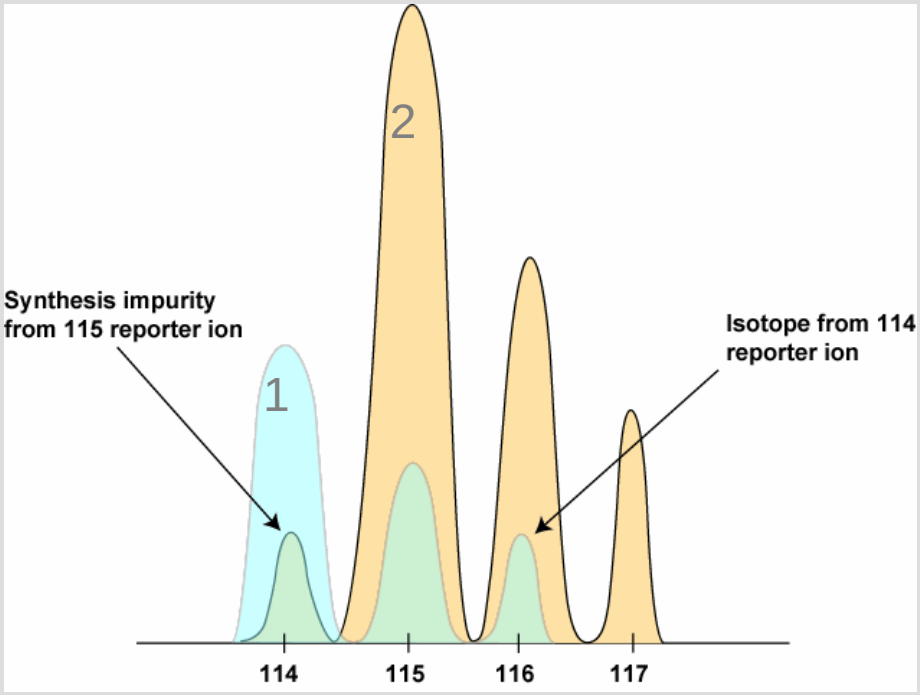


http://db.systemsbiology.net/webapps/conditionFileApp/

Intensity Correction

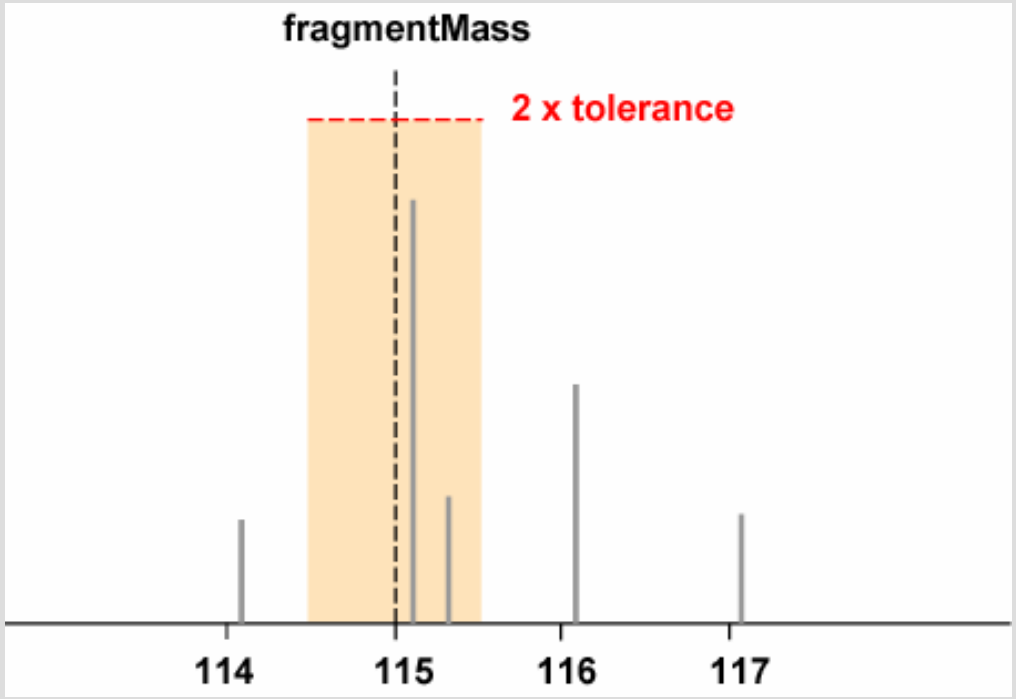
- Raw **intensities** must first be **adjusted**
  - corrects for isotopic and synthesis by-products

```
<isotopicContributions>
  <contributingMz value="1">

    <affected mz="2" correction="0.063" />
    <affected mz="3" correction="0.0020" />

  </contributingMz>
  <contributingMz value="2">

    <affected mz="1" correction="0.02" />
    <affected mz="3" correction="0.06" />
    <affected mz="4" correction="0.0010" />

  </contributingMz>
     .....
</isotopicContributions>
```



Mass Tolerance

- The m/z **tolerance** for matching a **target ion**
- If multiple ions are in the tolerance interval only the most intense one will be used

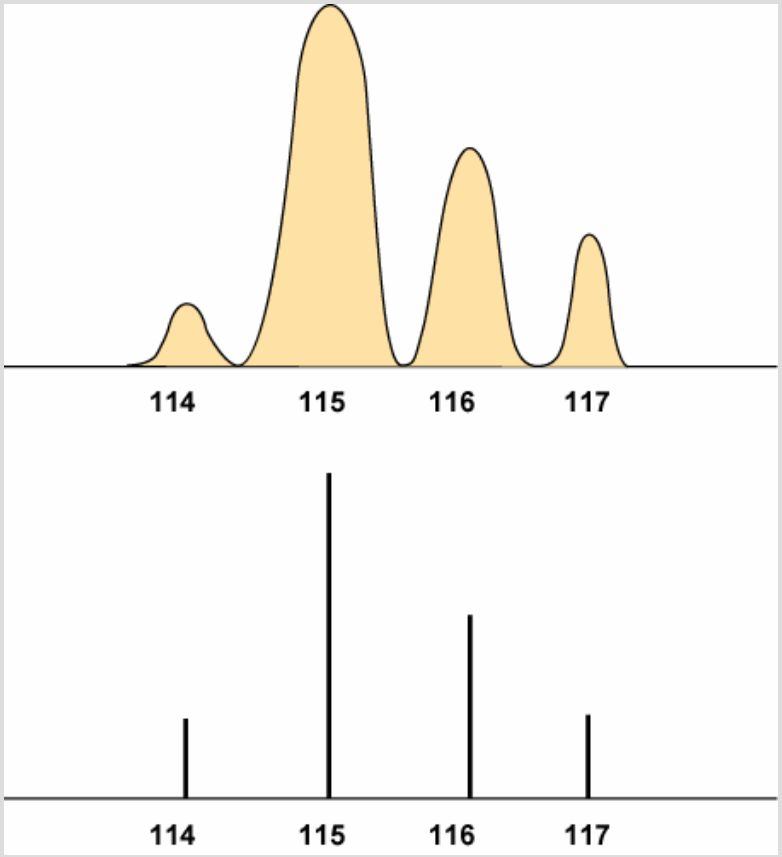


Centroiding

Controls conversion of **profile** data into **centroid** mode.

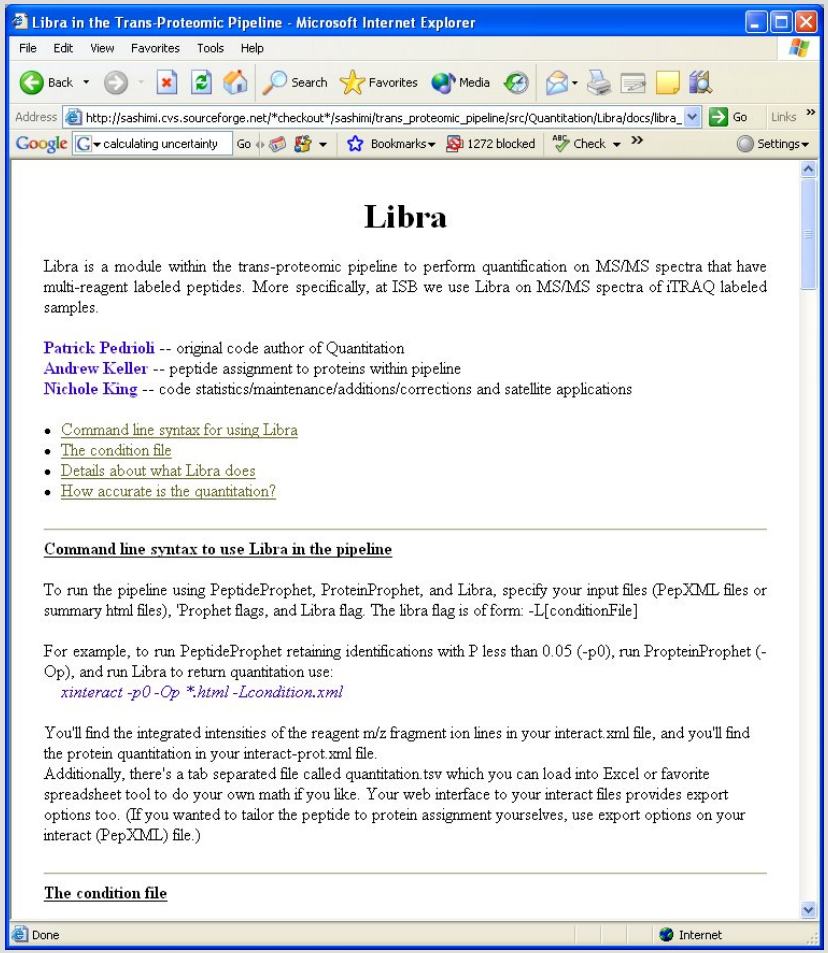
Types:

0. None
1. Mathematical
2. Intensity weighted



Other Libra Parameters

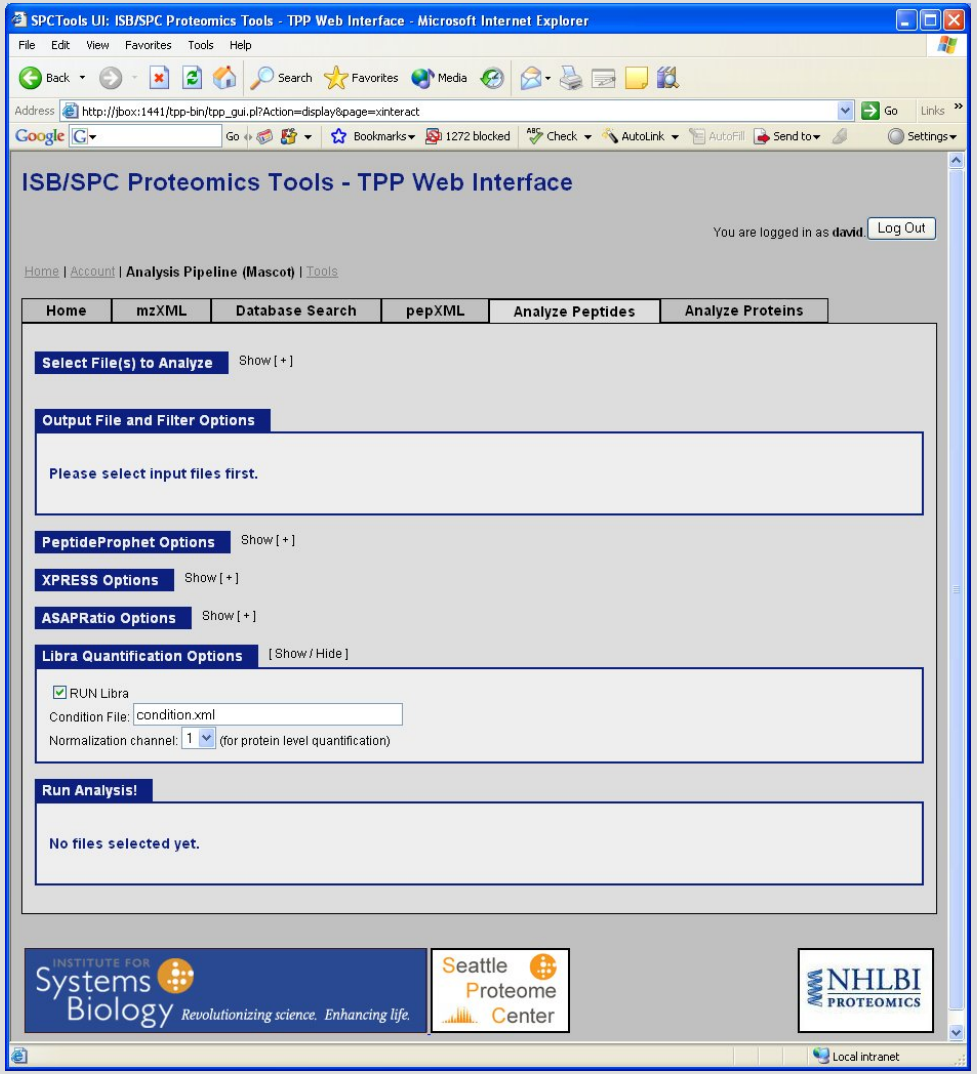
- Normalization
- Target MS level
  - default 2
- Output Options



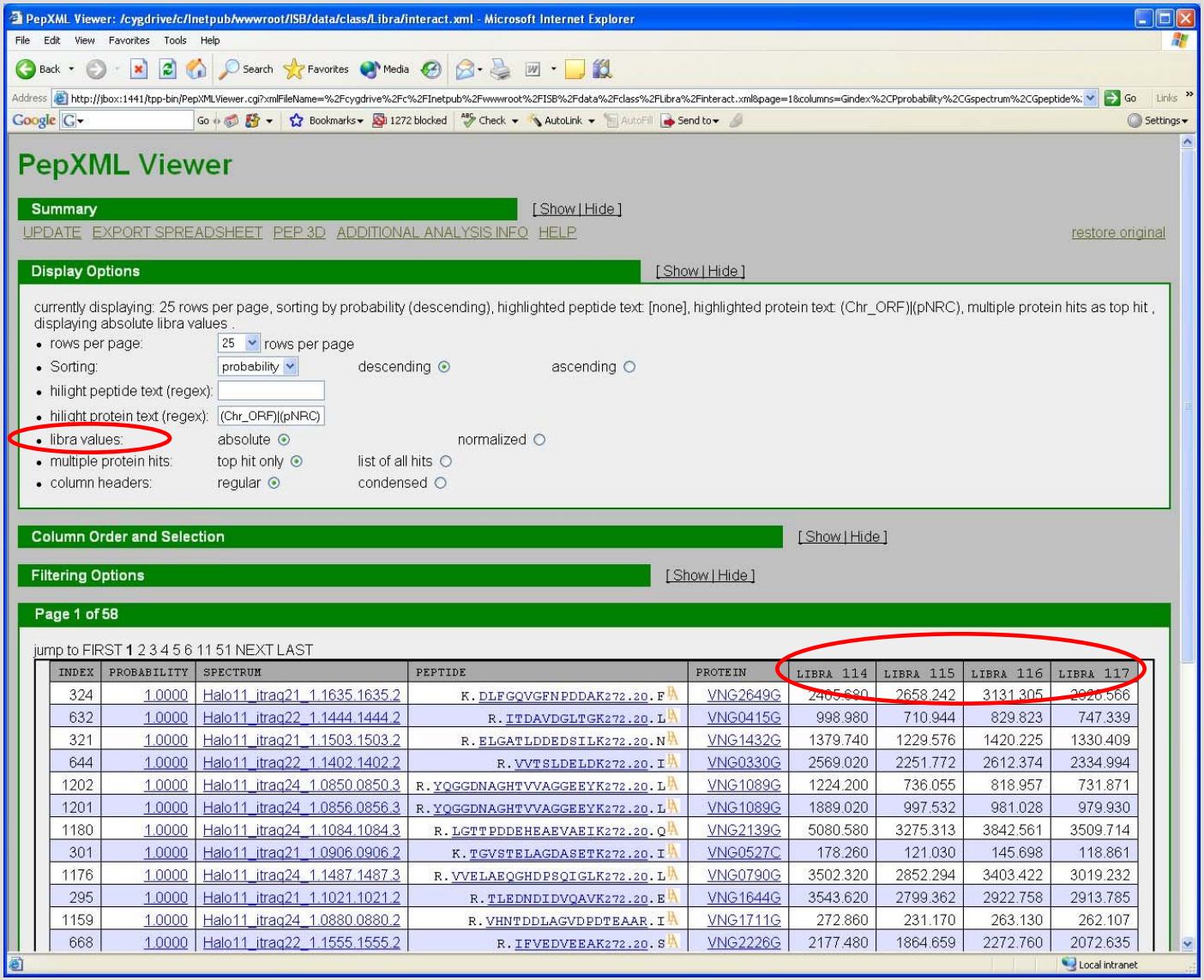
http://tools.proteomecenter.org/Libra.php

Running Libra

1. Generate a condition.xml file
2. Copy the condition file to data directory
3. Add input files and set Prophet options
4. Check “RUN Libra” and specify condition file by name

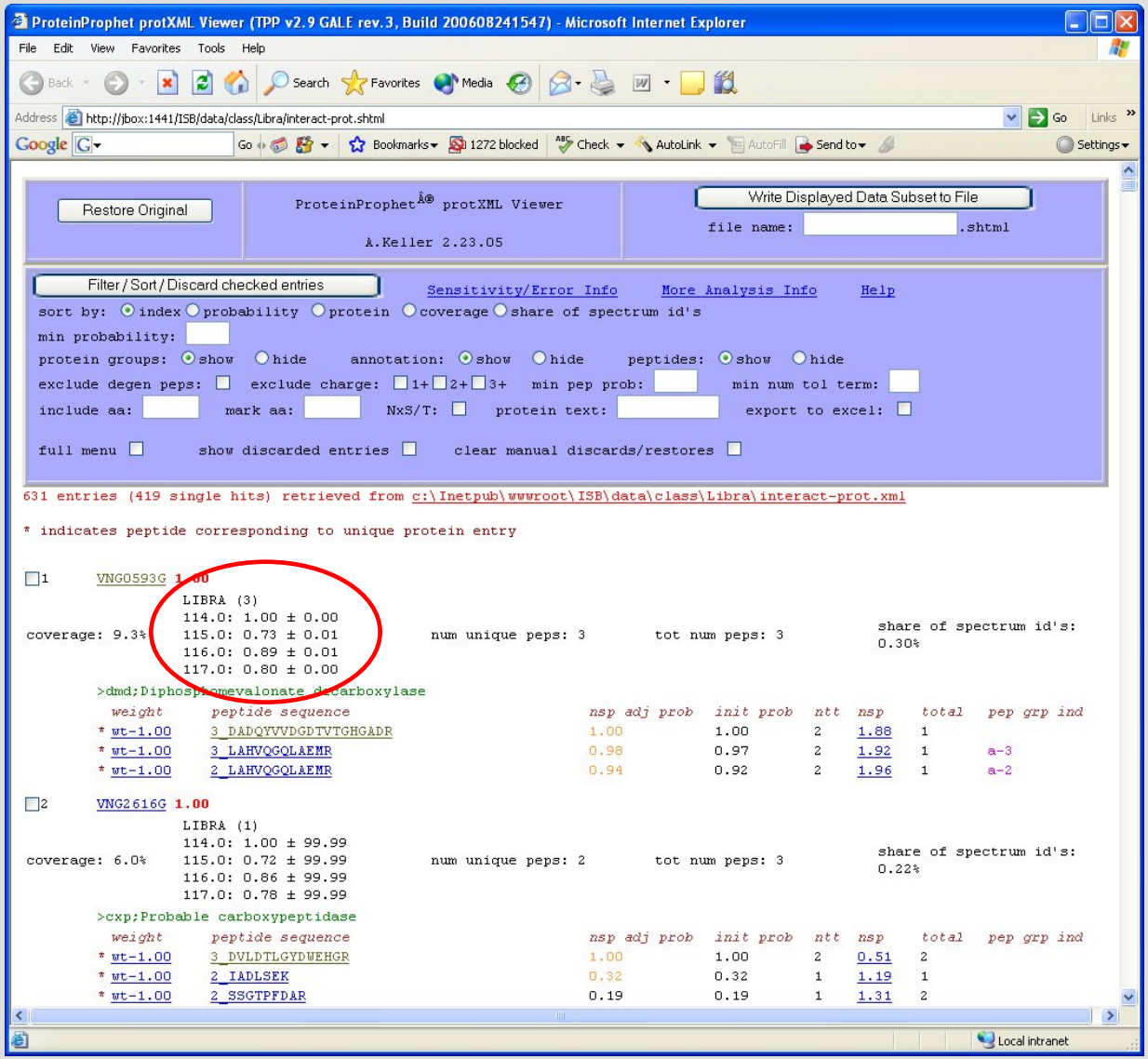


Libra PeptideProphet Results



INDEX	PROBABILITY	SPECTRUM	PEPTIDE	PROTEIN	LIBRA 114	LIBRA 115	LIBRA 116	LIBRA 117
324	1.0000	Halo11_itraq21_1.1635.1635.2	K.DLFGQVGFNPDDAK272.20.F	VNG2649G	2465.680	2658.242	3131.305	2926.566
632	1.0000	Halo11_itraq22_1.1444.1444.2	R.ITDAVDGLTGK272.20.L	VNG0415G	998.980	710.944	829.823	747.339
321	1.0000	Halo11_itraq21_1.1503.1503.2	R.ELGATLDDEDSILK272.20.N	VNG1432G	1379.740	1229.576	1420.225	1330.409
644	1.0000	Halo11_itraq22_1.1402.1402.2	R.VVTSLDELDK272.20.I	VNG0330G	2569.020	2251.772	2612.374	2334.994
1202	1.0000	Halo11_itraq24_1.0850.0850.3	R.YQGGDNAGHTVVAGGEEYK272.20.L	VNG1089G	1224.200	736.055	818.957	731.871
1201	1.0000	Halo11_itraq24_1.0856.0856.3	R.YQGGDNAGHTVVAGGEEYK272.20.L	VNG1089G	1889.020	997.532	981.028	979.930
1180	1.0000	Halo11_itraq24_1.1084.1084.3	R.LGTTPDDEHEAEVAEIK272.20.Q	VNG2139G	5080.580	3275.313	3842.561	3509.714
301	1.0000	Halo11_itraq21_1.0906.0906.2	K.TGVSTELAGDASETK272.20.I	VNG0527C	178.260	121.030	145.698	118.861
1176	1.0000	Halo11_itraq24_1.1487.1487.3	R.VVELAEQGHDPSQIGLK272.20.L	VNG0790G	3502.320	2852.294	3403.422	3019.232
295	1.0000	Halo11_itraq21_1.1021.1021.2	R.TLEDNDIDVQAVK272.20.E	VNG1644G	3543.620	2799.362	2922.758	2913.785
1159	1.0000	Halo11_itraq24_1.0880.0880.2	R.VHNTDDLAGVDPDTEAAR.I	VNG1711G	272.860	231.170	263.130	262.107
668	1.0000	Halo11_itraq22_1.1555.1555.2	R.IFVEDVEEAK272.20.S	VNG2226G	2177.480	1864.659	2272.760	2072.635

Libra ProteinProphet Results



weight	peptide sequence	nsp adj prob	init prob	ntt	nsp	total	pep grp ind
* wt-1.00	3_DADQYVVDGDTVTGHGADR	1.00	1.00	2	1.88	1	
* wt-1.00	3_LAHVQGQLAEMR	0.98	0.97	2	1.92	1	a-3
* wt-1.00	2_LAHVQGQLAEMR	0.94	0.92	2	1.96	1	a-2

weight	peptide sequence	nsp adj prob	init prob	ntt	nsp	total	pep grp ind
* wt-1.00	3_DVLDTLGYDWEHGR	1.00	1.00	2	0.51	2	
* wt-1.00	2_IADLSEK	0.32	0.32	1	1.19	1	
* wt-1.00	2_SSGTPFDAR	0.19	0.19	1	1.31	2	