

# Some Recent Enhancements to Protein Prospector

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## Multi-Sample Data

The ABI 4700 TOFTOF instrument allows each spot on a sample plate to contain a single digest sample with both MS and MSMS data available. As the MSMS data isn't acquired for every peak the MS data can be used to identify post-translation modifications, peptides formed by non-specific enzymatic cleavages or to distinguish proteins with similar sequences.

The MS data can either be searched against the proteins identified by MSMS or the whole database.

The MSMS hits can be used to calibrate the MS scan.

# Display of Multi-Sample Results

The Batch-Tag and Search Compare programs can produce separate protein and peptide reports for each spot on a sample plate. Both the MS and MSMS matches for the proteins identified can be displayed.

1 MPSQEVSVNR **VIVHPLVLLS VVDHFNRR** IGMKRRVGVV LIGCVRSKGV LDVSNSEAVP FDEDDKDSV WFLDHDYLEN  
 81 NYGHFQVNA REPVVGMHT GPKLQNDIA ENLIVRYCP NSVLVIDAK PMLGLPTA YISVEEVDD GSPTSSTEN  
 161 **VPSIGAREE REVGVEHLR** DEDITVGLS SQKITNQLMG LKGLNAQLD IKQYLQVGD SKMPINHOIV YQLQDIFMLL  
 241 PDITNDQFTG THYVKTNDQ LVVYLASHVR SIALLNHLIN NKLANDARE GSDSREAKE KMDSDSDEN KETDQDKGK  
 321 ABEKADKGD EGGKSRK

Protein Score 1	m/z	Error (ppm)	Peptide	F	S	R	#	Score	Score Diff	Disc Score
342.2	2677.4878	66	TFEHVPSIGAREEAEVGVVEHLR	1	23	7	3	109.7	73.5	4.67
	1534.8872	35	LHONDIANELVR	1	23	3	2	120.2	71.6	4.61
	1957.2274	45	VIVHPLVLLSVVDHFNRR	1	23	3	4	66.8	31.5	3.41
	1033.5969	24	ITNQLMGLK	1	23	6	4	45.5	7.3	2.68

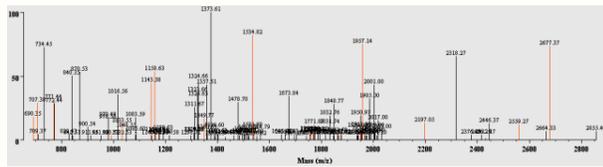
## MSMS Coverage

1 MPSQEVSVNR **VIVHPLVLLS VVDHFNRR** IGMKRRVGVV LIGCVRSKGV LDVSNSEAVP FDEDDKDSV WFLDHDYLEN  
 81 NYGHFQVNA REPVVGMHT GPKLQNDIA ENLIVRYCP NSVLVIDAK PMLGLPTA YISVEEVDD GSPTSSTEN  
 161 **VPSIGAREE REVGVEHLR** DEDITVGLS SQKITNQLMG LKGLNAQLD IKQYLQVGD SKMPINHOIV YQLQDIFMLL  
 241 PDITNDQFTG THYVKTNDQ LVVYLASHVR SIALLNHLIN NKLANDARE GSDSREAKE KMDSDSDEN KETDQDKGK  
 321 ABEKADKGD EGGKSRK

## MSMS Hits

m/z	MH*	Intensity	Delta ppm	Modifications	Start	End	Missed Cleavages	Sequence
690.35	690.34	69.3	13		328	334	1	(K)IKDRDQK (G)
690.35	690.36	69.3	-9.0	pyroGha	213	217	0	(K)QYLQR(V)
690.35	690.38	69.3	-4.0		315	320	2	(K)IKGQK (A)
707.38	707.38	109.5	-10		213	217	0	(K)QYLQR (V)
771.44	771.45	119.7	-13		203	209	0	(K)ILNAQLR (D)
990.53	990.50	12.6	32		328	337	2	(K)IKDRDQKSR (K)
1033.55	1033.57	49.0	-18	1Met-ox	194	202	0	(K)ITNQLMGLK(G)
1143.38	1143.60	169.5	-13		94	103	0	(R)YGVVYHTQPK (L)
1158.63	1158.65	204.4	-10		37	46	0	(R)YGVVYHTQPK (S)
1176.38	1176.60	15.0	-17	pyroGha	213	222	1	(K)QYLQRVGDGSK(M)
1349.77	1349.79	64.2	-13		271	282	0	(K)IHALNHLNKK (L)
1534.82	1534.83	311.7	-7.9		104	116	0	(K)LHONDIANELVR (R)
1755.87	1755.88	24.0	-5.7	1Met-ox	256	270	0	(K)TNDQMLVYVYLAQMVR(S)
1771.87	1771.87	46.5	-2.0	2Met-ox	256	270	0	(K)TNDQMLVYVYLAQMVR(S)
1873.02	1873.04	15.5	-6.6		117	132	1	(R)YVPSVIVDQKPK (D)
1953.99	1953.91	37.1	40		49	66	0	(K)YVLSNSEAVVDFDQK (D)
1957.14	1957.14	285.2	2.8		11	27	0	(K)VIVHPLVLLSVVDHFNRR (M)
2197.05	2197.03	52.0	10		49	68	1	(K)YVLSNSEAVVDFDQK (S)
2559.27	2559.21	45.7	24		133	156	0	(K)ILOLPTAIVSVEVDDQSETSK (T)
2677.37	2677.31	280.2	24		157	180	0	(K)TFEHVPSIGAREEAEVGVVEHLR (D)

## MS Coverage

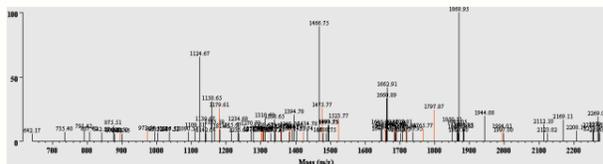


## MS Spectrum

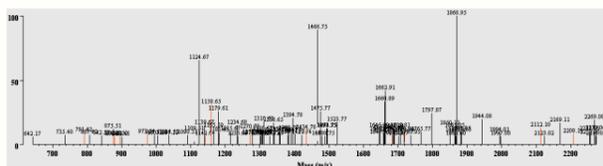
## MS Hits

# Better Matches Using MS-Homology

## Swiss Prot Matches

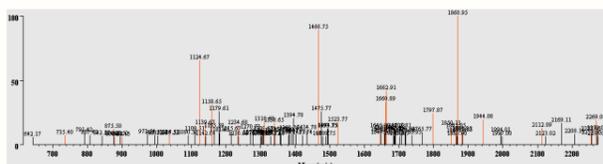


HUMAN Keratin, type II cytoskeletal 1



DICDI 26S protease subunit 6B homolog

## NCBI nr Match identified by MS-Homology

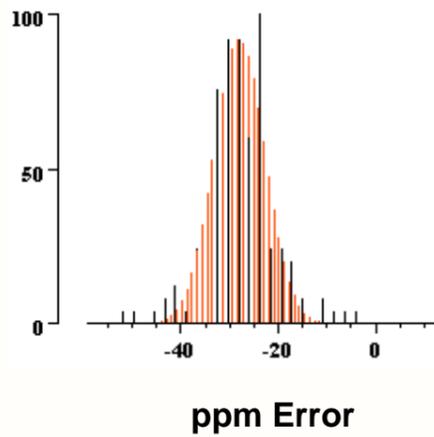


DROME 26S proteasome regulatory complex subunit p50

Identified by using the MSMS hit sequences in an MS-Homology search.

MSMS Hits: VD[I|L][I|L]DPA[I|L][I|L]R, GV[I|L][I|L]YGPPGTGK, YEE[I|L][K|Q][I|L]TAGR

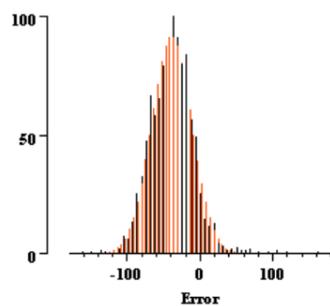
## Recalibration of Data Based on Results



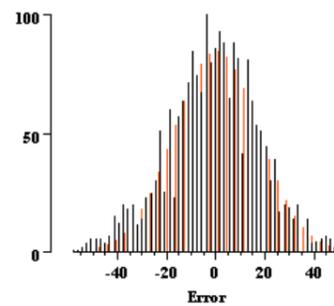
A histogram of the MSMS parent errors of the correctly identified peptides shows a normal distribution with a systematic error of  $-27\text{ppm}$ . The data can be resubmitted with an error tolerance of  $35\text{ ppm}$  rather than the original  $100\text{ ppm}$ .

## Recalibration of Multi-Fraction Data

Each fraction in a multi-fraction data set can have a separate calibration.



Before Calibration

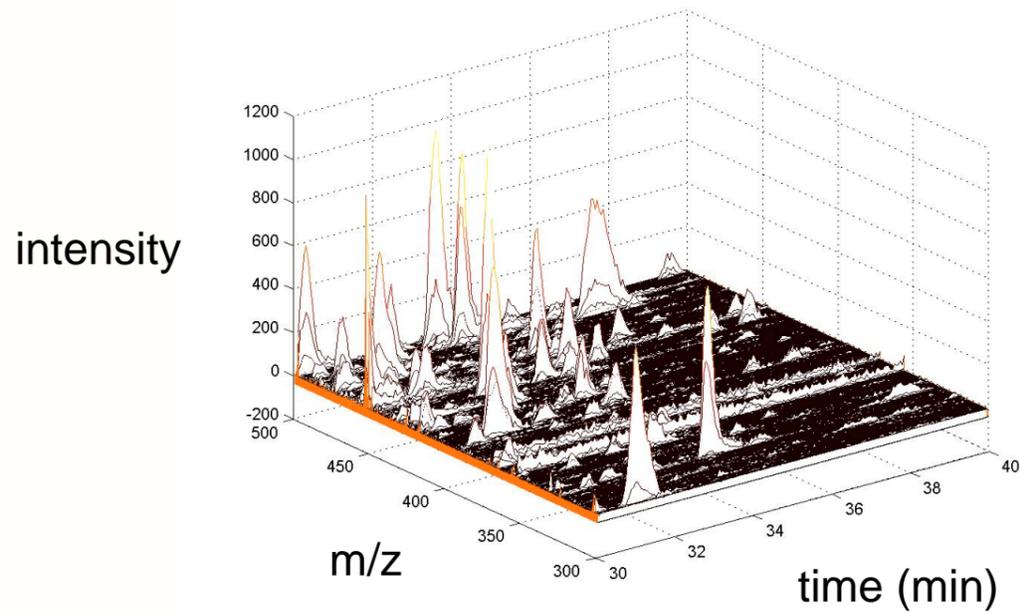


After Calibration

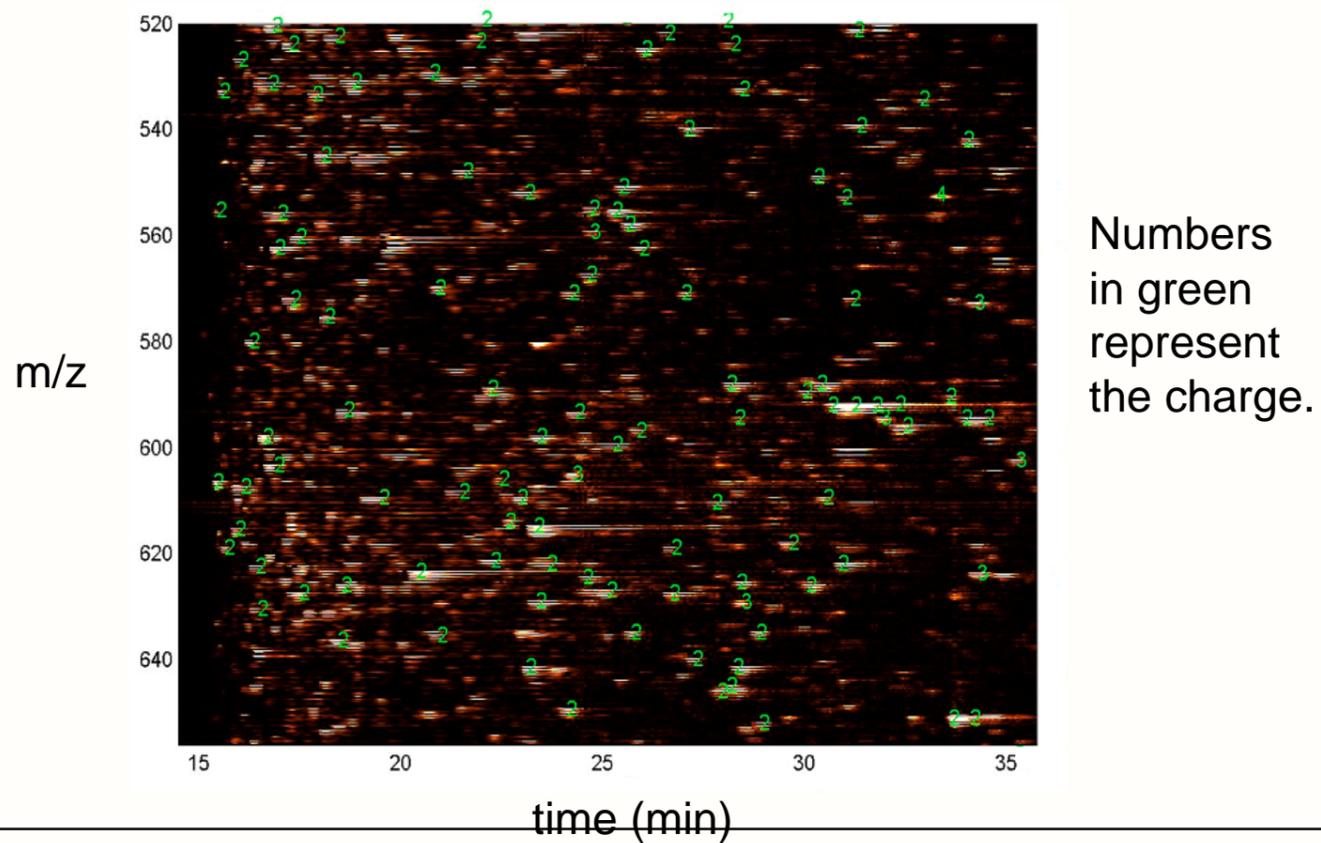
### Distribution of Errors in a 6 Fraction Data Set

The calibration enabled the data to be re-searched with a tolerance of  $\pm 50\text{ ppm}$  rather than the original  $\pm 120\text{ ppm}$ .

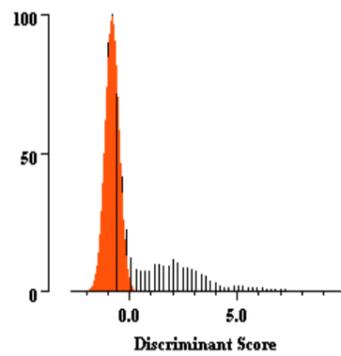
## LC MSMS Waterfall Plots



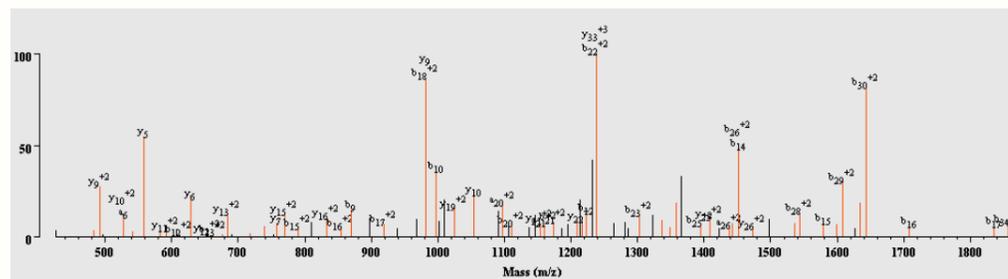
## Visualization of Peaks Chosen for MSMS



## Support for LTQ Data

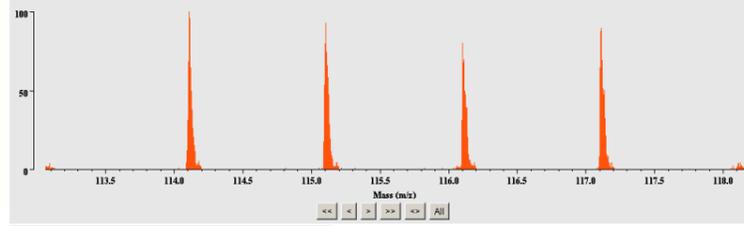


Discriminant score graph from data set with 116075 spectra from 82 fractions.



# iTRAQ Quantitation

Link to raw  
quantitation data



Acc. #: [P16858](#) Species: MOUSE Name: Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)  
pI of Protein: 8.4  
Protein.MW: 35810.3

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1      11      21      31      41      51      61      71
MVKVGVNCGF RIGRLVTRAA ICSGKVEIVA INDPFDLNY HVYMFQYDST HGKFNQTVKA ENGLKLVNGK PIIIFQERDP
81      91     101     111     121     131     141     151
TNIKWGEAGA EYVVESTGVF TTEKAGAGHL RCGAKRVIIS APSADAPHEV HGVNHEKYDN SLKIVSNASC TTNCLAPLAK
161     171     181     191     201     211     221     231
VIHDFGIVE GLMTTVHAIT ATQKTVDGFS GKLRWDGRGA DENLIPASTG ARKAVGRVIP ELNGKLTGMR FRVPTPNVSV
241     251     261     271     281     291     301     311
VDLTCRLKPK AKYDDIKGVV RQASEGPLK ILCVTFDQVV SCDPNSNSHS STFDAGACIA LNDNFVKLLS WVDNEYGYSN
321     331
RVVDLMAYMGR SKE
    
```

Int Pk 1	Int Pk 2	Int Pk 3	Int Pk 4	Area Pk 1	Area Pk 2	Area Pk 3	Area Pk 4
1840	1710	1480	1650	1182	1167	9850	1143

Num Unique	m/z	Error (ppm)	Peptide	F	S	R	#	Int Pk 1	Int Pk 2	Int Pk 3	Int Pk 4	Score	Score Diff	Disc Score
13	<a href="#">860.4192</a>	-6.3	<a href="#">WGEAGAEYVVESTGVFTTMEK</a>	4	79.26	2	1	1840	1710	1480	1650	52.3	38.0	5.53
	<a href="#">865.7486</a>	-8.9	<a href="#">WGEAGAEYVVESTGVFTTmEK</a>	3	74.67	1	1	2390	2390	2000	2320	49.5	33.2	5.00
	<a href="#">862.4451</a>	-9.1	<a href="#">LSWVDNEYGYSNR</a>	2	64.91	2	1	841.0	752.0	573.0	705.0	48.4	31.6	4.82
	<a href="#">829.4695</a>	-10	<a href="#">GAAQNIPASTGAAK</a>	14	46.72	2	1	636.0	555.0	473.0	544.0	45.5	31.5	4.81
	<a href="#">758.4183</a>	8.5	<a href="#">VVDLMAYMASK</a>	11	76.19	2	1	482.0	443.0	338.0	374.0	44.6	30.2	4.67
	<a href="#">703.3749</a>	0.14	<a href="#">IVSNASCTTNCLAPLAK</a>	11	57.05	2	1	497.0	527.0	443.0	510.0	38.7	26.3	4.24
	<a href="#">822.9295</a>	-4.4	<a href="#">VVDLMAYMASKE</a>	9	79.28	2	1	649.0	615.0	498.0	638.0	39.6	24.8	4.08
	<a href="#">470.2616</a>	-5.9	<a href="#">LTGMAFR</a>	9	51.68	2	1	789.0	729.0	625.0	701.0	36.6	18.6	3.39
	<a href="#">559.6275</a>	3	<a href="#">VVDLMAYmASKE</a>	8	51.32	1	1	518.0	345.0	285.0	379.0	25.3	12.7	2.75
	<a href="#">516.6098</a>	5.3	<a href="#">VVDLMAYmASK</a>	12	54.58	2	1	576.0	572.0	524.0	513.0	23.0	12.3	2.70
	<a href="#">554.2877</a>	-3.6	<a href="#">VVDLMAYmASKE</a>	9	61.59	1	1	817.0	737.0	650.0	691.0	25.3	5.2	1.92
	<a href="#">511.2686</a>	-13	<a href="#">VVDLMAYmASK</a>	9	59.86	2	1	724.0	636.0	550.0	605.0	27.2	5.1	1.91

## Acknowledgements

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