

Introduction to MS Database

Searching using MASCOT

G10

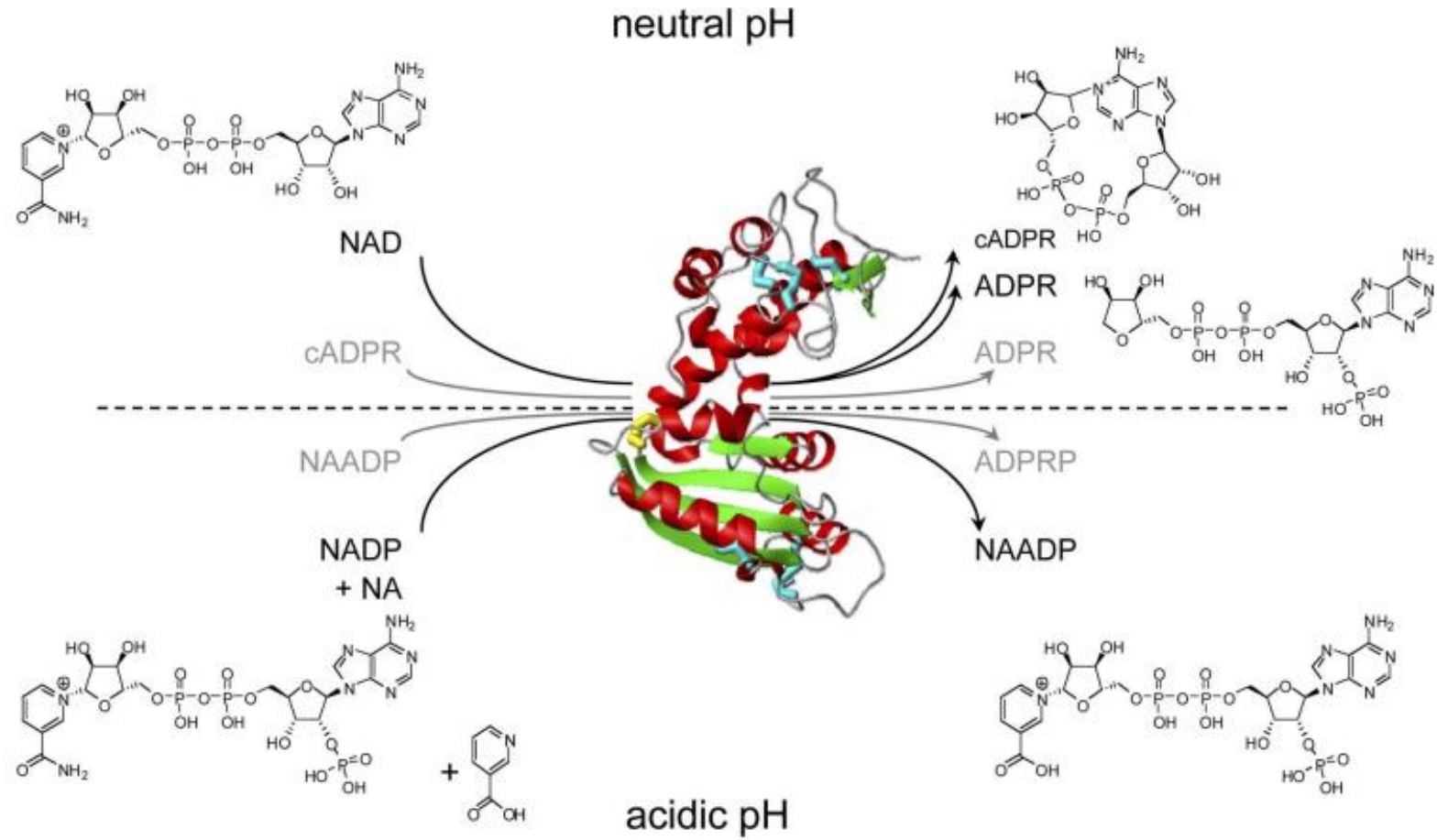
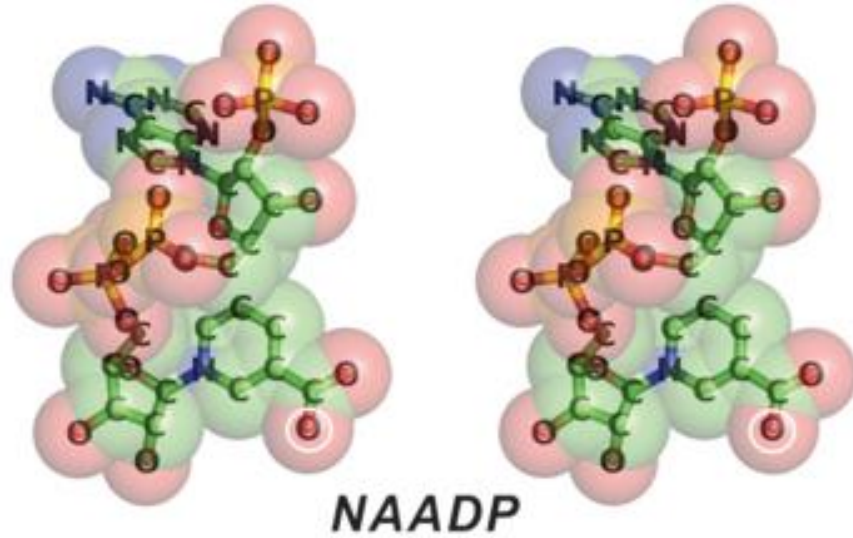
Yajie Chen

2015.6.27

Outline

- A grail of NAADP signal pathway
- The use of Mascot

NAADP is a potent Ca^{2+} -releasing second messenger



An debate about the intracellular stores targeted by NAADP and the molecular identity of the NAADP receptors

nature

Vol 459 | 28 May 2009 | doi:10.1038/nature08030

Cell

LET

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Photoaffinity Labeling of Nicotinic Acid Adenine Dinucleotide Phosphate (NAADP) Targets in Mammalian Cells*[†]

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Robert Hooper^{§2}, Eugen Brailoiu^{**}, Sandip Patel[§], and Jonathan S. Marchant^{‡3}
& Michael

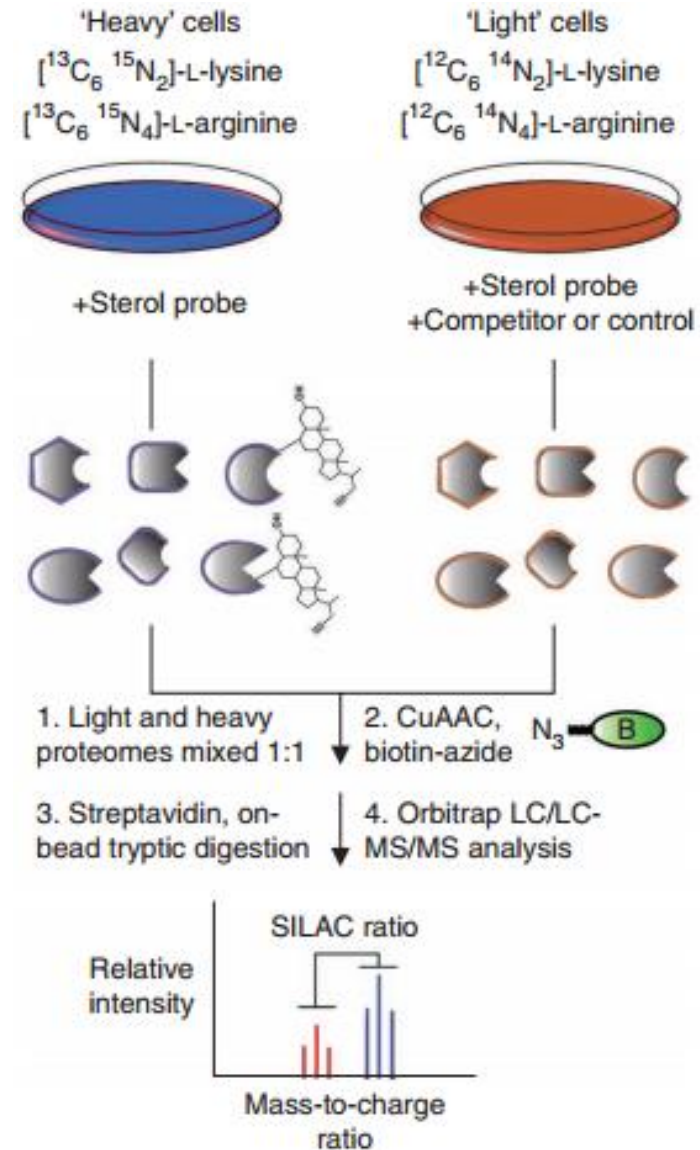
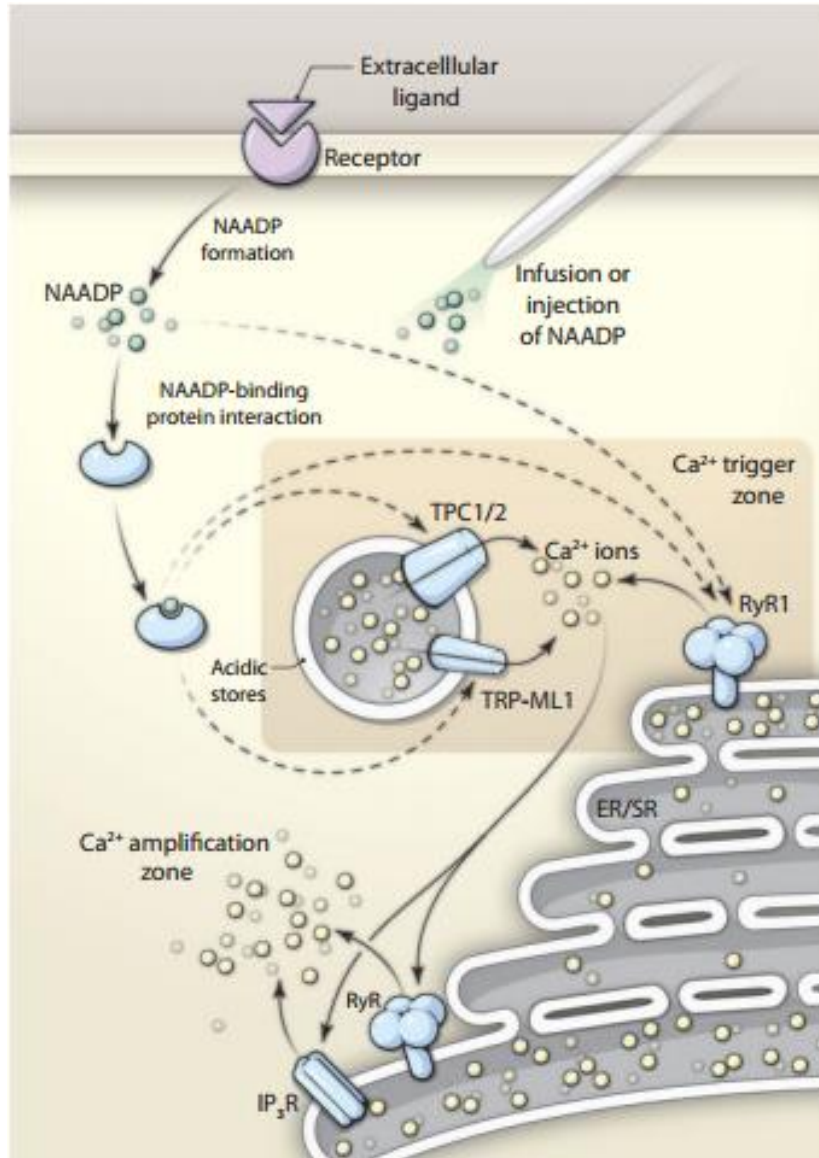
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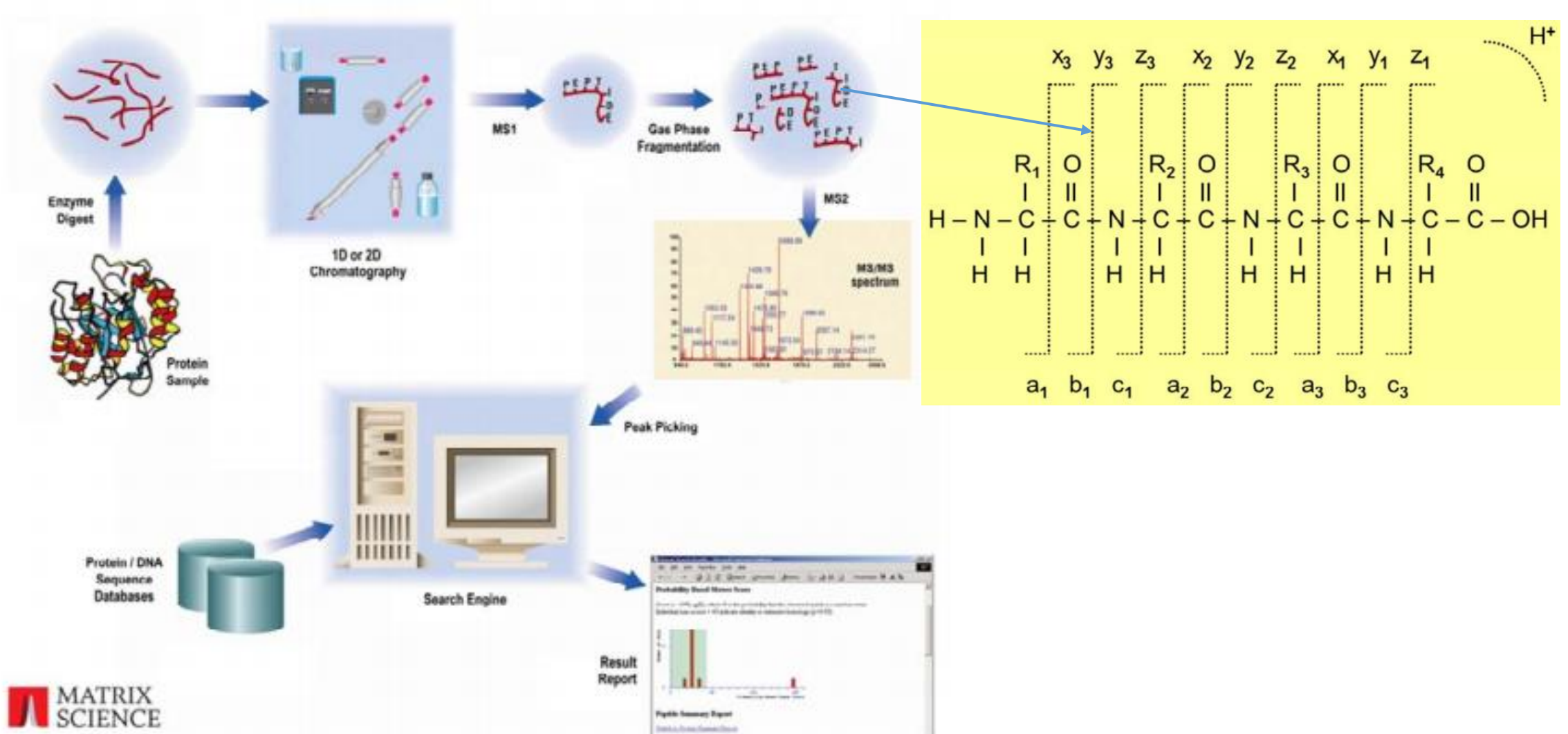
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<http://dx.doi.org/10.1016/j.cell.2012.08.036>

A unifying hypothesis for NAADP signaling



The experimental workflow for MS/MS



A wide choice of search engines for performing searches of uninterpreted MS/MS data

MS/MS Ions Search Servers on the Web

Inspect / MS-GFDB	http://proteomics.ucsd.edu/ProteoSAFe/
Mascot	http://www.matrixscience.com/search_form_select.html
MassMatrix	http://www.massmatrix.net/mm-cgi/search_form.py
MS-Tag (Protein Prospector)	http://prospector.ucsf.edu/prospector/mshome.htm
PepFrag (Prowl)	http://prowl.rockefeller.edu/prowl/pepfrag.html
RAId_DbS	http://www.ncbi.nlm.nih.gov/CBBResearch/qmbp/RAId_DbS/index.html
Sonar (Knexus)	http://hs2.proteome.ca/prowl/knexus.html
X!Tandem (The GPM)	http://thegpm.org/TANDEM/index.html
Not on-line	Byonic, Comet, Crux, greylag, Morpheus, Myrimatch, Paragon, Peaks DB, PepSplice, pFind, Phenyx, ProBID, ProLuCID, ProteinLynx GS, Sequest, SIMS, SpectrumMill, etc.

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Access Mascot Server

You are welcome to submit searches to this free Mascot Server. Searches of MS/MS data are limited to 1200 spectra and some functions, such as no enzyme searches, are unavailable. Automated searching of batches of files is not permitted. If you want to automate search submission, perform large searches, search additional sequence databases, or customise the modifications, quantitation methods, etc., you'll need to [license your own, in-house copy of Mascot Server](#).

H. Sequence Queries	PDF	PDF	PDF
I. Mascot Daemon	PDF	PDF	PDF
J. Quantitation	PDF	PDF	PDF
K. Sequence Database Administration	PDF	PDF	PDF
L. Administration and Configuration	PDF	PDF	PDF

More info

- > [Mascot overview](#)
- > [Search parameter reference](#)
- > [Data file format](#)
- > [Results report overview](#)

Mascot database search > Access Mascot Server > MS/MS Ions Search

MASCOT MS/MS Ions Search

<u>Your name</u>	<input type="text"/>	<u>Email</u>	<input type="text"/>
<u>Search title</u>	<input type="text"/>		
<u>Database(s)</u>	<div style="border: 1px solid black; padding: 2px;"><ul style="list-style-type: none">Invertebrates_ESTHuman_ESTFungi_ESTEnvironmental_ESTSwissProt</div>	<u>Enzyme</u>	Trypsin <input type="button" value="v"/>
<u>Taxonomy</u> Homo sapiens (human) <input type="button" value="v"/>		
<u>Fixed modifications</u>	<div style="border: 1px solid black; padding: 2px;">--- none selected --- <input type="button" value="v"/></div>	<input type="button" value="v"/> <input type="button" value="v"/>	<div style="border: 1px solid black; padding: 2px;"><ul style="list-style-type: none">Acetyl (K)Acetyl (N-term)Acetyl (Protein N-term)Amidated (C-term)Amidated (Protein C-term)Ammonia-loss (N-term C)Biotin (K)Biotin (N-term)Carbamidomethyl (C)Carbamyl (K)Carbamyl (N-term)</div>
	Display all modifications <input type="checkbox"/>		
<u>Variable modifications</u>	<div style="border: 1px solid black; padding: 2px;">--- none selected --- <input type="button" value="v"/></div>	<input type="button" value="v"/> <input type="button" value="v"/>	
<u>Peptide tol. ±</u>	1.2 <input type="button" value="Da v"/>	<u># 13C</u>	0 <input type="button" value="v"/>
<u>MS/MS tol. ±</u>	0.6 <input type="button" value="Da v"/>		
<u>Peptide charge</u>	2+ <input type="button" value="v"/>	<u>Monoisotopic</u>	<input checked="" type="radio"/> Average <input type="radio"/>
<u>Data file</u>	<input type="button" value="选择文件"/> 未选择文件	<u>Precursor</u>	<input type="text"/> m/z
<u>Data format</u>	Mascot generic <input type="button" value="v"/>	<u>Error tolerant</u>	<input type="checkbox"/>
<u>Instrument</u>	Default <input type="button" value="v"/>	<u>Report top</u>	AUTO <input type="button" value="v"/> hits
<u>Decoy</u>	<input type="checkbox"/>	<input type="button" value="Start Search ..."/>	<input type="button" value="Reset Form"/>

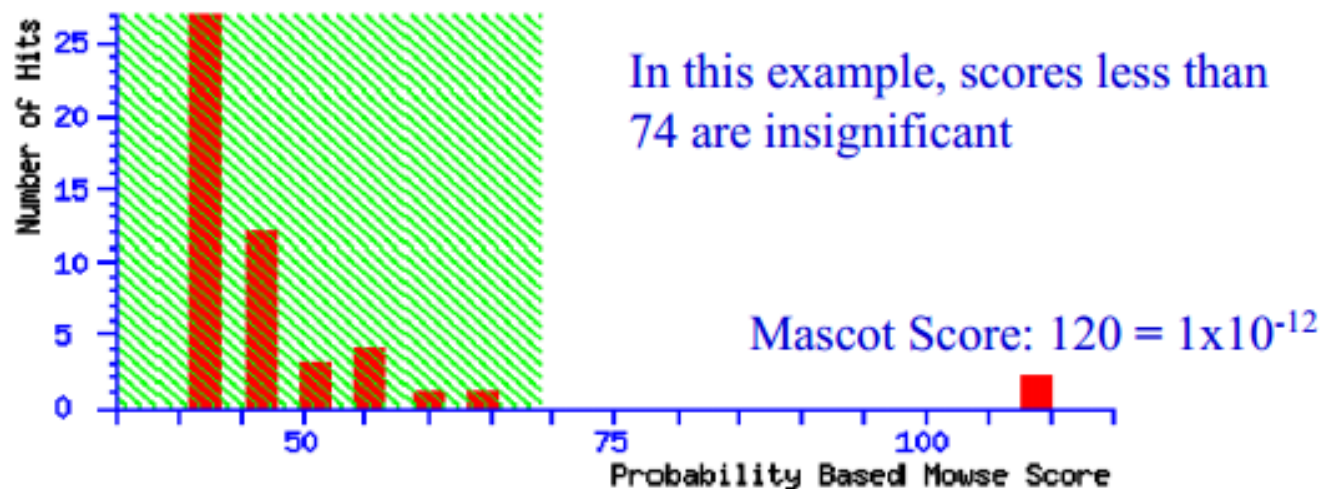
Mascot Search Results

User :
Email :
Search title : **MS/MS Example**
Database : **SwissProt 2014_04 (544996 sequences; 193815432 residues)**
Timestamp : **20 May 2014 at 11:00:46 GMT**
Protein hits : [CH60 HUMAH](#) 60 kDa heat shock protein, mitochondrial OS=Homo sapiens GN=MSPD1 PE=1 SV=2
[CH60 DROME](#) 60 kDa heat shock protein, mitochondrial OS=Drosophila melanogaster GN=Hsp60 PE=1 SV=3
[CH60 CAEEL](#) Chaperonin homolog Hsp-60, mitochondrial OS=Caenorhabditis elegans GN=hsp-60 PE=1 SV=2
[CH60 STRM5](#) 60 kDa chaperonin OS=Stenotrophomonas maltophilia (strain R551-3) GN=groL PE=3 SV=1

匹配到的蛋白

Mascot Score Histogram

Ions score is $-10 \cdot \log(P)$, where P is the probability that the observed match is a random event.
Individual ions scores > 41 indicate identity or extensive homology ($p < 0.05$). 本次搜索中, 大于41分意味着成功匹配。
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.



Mascot Search Results

31个肽段归属于这个蛋白，其中有27个肽段的可信度单独超过阈值分数这里是大于41分。

1. **CH60_HUMAN** Mass: 61016 Score: 1225 Matches: 31(27) Sequences: 19(17)
60 kDa heat shock protein, mitochondrial OS=Homo sapiens GN=HSPD1 PE=1 SV=2
 Check to include this hit in error tolerant search

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Unique	Peptide
<input checked="" type="checkbox"/> 11	417.1822	832.3498	832.3828	-0.0329	0	45	0.03	1		K.APGFGDNR.K
<input checked="" type="checkbox"/> 12	422.7433	843.4720	843.5066	-0.0346	0	46	0.035	1	U	K.VGEVIVTK.D
<input checked="" type="checkbox"/> 13	430.7328	859.4510	859.4837	-0.0327	0	36	0.31	1	U	K.IPAMTIAK.N + Oxidation (M)
<input checked="" type="checkbox"/> 15	451.2499	900.4853	900.5280	-0.0428	0	52	0.008	1	U	K.LSDGVAVLK.V
<input checked="" type="checkbox"/> 16	456.7806	911.5467	911.5804	-0.0337	0	59	0.0011	1	U	K.VGLQWAVK.A
<input checked="" type="checkbox"/> 21	480.7447	959.4748	959.5036	-0.0288	0	45	0.035	1	U	R.VTDALNATR.A
<input checked="" type="checkbox"/> 24	595.7855	1189.5565	1189.6012	-0.0447	0	(57)	0.0022	1	U	K.EIGNIISDAMK.K
<input checked="" type="checkbox"/> 25	603.7720	1205.5294	1205.5962	-0.0668	0	60	0.001	1	U	K.EIGNIISDAMK.K + Oxidation (M)
<input checked="" type="checkbox"/> 26	608.3099	1214.6052	1214.6507	-0.0455	0	73	4.6e-05	1	U	K.NAGVEGSLIVEK.I
<input checked="" type="checkbox"/> 27	617.2857	1232.5569	1232.5885	-0.0316	0	81	8.5e-06	1	U	K.VGGTSDVEVNEK.K
<input checked="" type="checkbox"/> 31	672.8375	1343.6605	1343.7085	-0.0480	0	64	0.00033	1	U	R.TVIEQSWGSPK.V
<input checked="" type="checkbox"/> 34	714.8884	1427.7623	1427.8058	-0.0435	0	(65)	0.00029	1	U	R.GVMLAVDAVIAELK.K
<input checked="" type="checkbox"/> 35	714.8938	1427.7730	1427.8058	-0.0327	0	(73)	4.4e-05	1	U	R.GVMLAVDAVIAELK.K
<input checked="" type="checkbox"/> 36	722.8849	1443.7552	1443.8007	-0.0455	0	75	2.5e-05	1	U	R.GVMLAVDAVIAELK.K + Oxidation (M)
<input checked="" type="checkbox"/> 37	722.8934	1443.7722	1443.8007	-0.0285	0	(73)	4.5e-05	1	U	R.GVMLAVDAVIAELK.K + Oxidation (M)
<input checked="" type="checkbox"/> 39	752.8643	1503.7141	1503.7490	-0.0349	0	90	8.9e-07	1	U	K.TLNDELEIEGK.F
<input checked="" type="checkbox"/> 40	760.8461	1519.6777	1519.7439	-0.0662	0	(89)	1e-06	1	U	K.TLNDELEIEGK.F + Oxidation (M)
<input checked="" type="checkbox"/> 45	640.3281	1917.9625	1918.0636	-0.1010	0	102	4.3e-08	1	U	K.ISSIQSIVPALEIANAHR.K
<input checked="" type="checkbox"/> 46	960.0327	1918.0509	1918.0636	-0.0127	0	(87)	1.1e-06	1	U	K.ISSIOSIVPALEIANAHR.K
<input checked="" type="checkbox"/> 48	1019.5106	2037.0067	2037.0153	-0.0087	0	52	0.0031	1	U	R.IQEIIQLDVTSEYEK.E
<input checked="" type="checkbox"/> 51	1057.0537	2112.0929	2112.1323	-0.0394	0	116	1.4e-09	1	U	R.ALMLQGVLLADAVAVTMGPK.G
<input checked="" type="checkbox"/> 52	1065.0399	2128.0653	2128.1272	-0.0619	0	(72)	3.7e-05	1	U	R.ALMLQGVLLADAVAVTMGPK.G + Oxidation (M)
<input checked="" type="checkbox"/> 53	1065.0623	2128.1100	2128.1272	-0.0172	0	(26)	1.3	1	U	R.ALMLQGVLLADAVAVTMGPK.G + Oxidation (M)
<input checked="" type="checkbox"/> 54	1073.0477	2144.0809	2144.1221	-0.0412	0	(93)	2.8e-07	1	U	R.ALMLQGVLLADAVAVTMGPK.G + 2 Oxidation (M)
<input checked="" type="checkbox"/> 58	789.1062	2364.2968	2364.3264	-0.0296	0	(56)	0.0011	1	U	R.KPLVIAEDVDGEALSTLVLR.L
<input checked="" type="checkbox"/> 59	1183.1570	2364.2994	2364.3264	-0.0270	0	(65)	0.00011	1	U	R.KPLVIAEDVDGEALSTLVLR.L
<input checked="" type="checkbox"/> 60	789.1094	2364.3063	2364.3264	-0.0201	0	95	1.3e-07	1	U	R.KPLVIAEDVDGEALSTLVLR.L
<input checked="" type="checkbox"/> 61	828.1238	2481.3495	2481.3942	-0.0447	0	(26)	0.89	1	U	R.TALLDAAGVASLLTTAEVWVTEIPK.E
<input checked="" type="checkbox"/> 62	828.1322	2481.3748	2481.3942	-0.0194	0	48	0.0064	1	U	R.TALLDAAGVASLLTTAEVWVTEIPK.E
<input checked="" type="checkbox"/> 64	854.0588	2559.1545	2559.2413	-0.0868	0	75	1.1e-05	1		K.LVQDVANNTNEEAGDPTTATVLAR.S
<input checked="" type="checkbox"/> 65	1038.5031	3112.4873	3112.5023	-0.0150	0	13	14	1	U	K.DMAIATGGAVFGEGLTLNLEDVQPHDLGK.V + Oxidation (M)

Mascot Search Results

Protein View: CH60_HUMAN

60 kDa heat shock protein, mitochondrial OS=Homo sapiens GN=HSPD1 PE=1 SV=2

Database: SwissProt
Score: 1225
Nominal mass (M_r): 61016
Calculated pI: 5.70
Taxonomy: [Homo sapiens](#)

Sequence similarity is available as [an NCBI BLAST search of CH60_HUMAN against nr.](#)

Search parameters

Enzyme: Trypsin: cuts C-term side of KR unless next residue is P.
Variable modifications: [Oxidation \(M\)](#)

Protein sequence coverage: 49%

Matched peptides shown in **bold red**.

```
1 MLRLPTVFRQ MRPVSRVLAP HLTRAYAKDV KFGADARALM LQVDLLADA
51 VAVTMGPKGR TVIIEQSWGS PKVTKDGVTV AKSIDLKDKY KNIGAKLVQD
101 VANNTNEEAG DGTTTATVLA RSIAKEGF EK ISKGANPVEI RRGVMLAVDA
151 VIAELKKQSK PVTTP EEIAQ VATISANGDK EIGNIISDAM KKVGRKGVIT
201 VKDGTKLNDE LEIIEGMKFD RGYISPYFIN TSKGQKCEFQ DAYVLLSEKK
251 ISSIQSIVPA LEIANAHRKP LVIIAEDVDG EALSTLVLNR LKVGLQWAV
301 KAPGFGDNRK NQLKDMAIAT GGAVFGEEGL TLNLEDVQPH DLGKVGEVIT
351 TKDDAMLLKG KGDKAQIEKR IQEIIEQLDV TTSEYEKEKL NERLAKLSDG
401 VAVLKVGGTS DVEVNEKKDR VTDALNATRA AVEEGIVLGG GCALLRCIPA
451 LDSLTPANED QKIGIEIIKR TLKIPAMTIA KNAGVEGSLI VEKIMQSSSE
501 VGYDAMAGDF VNMVEKGIID PTKVVRTALL DAAGVASLLT TAEVVTEIP
551 KEEKDPGMGA MGGMGGGMGG GMF
```

Take home message

- MS/MS is a powerful way to identify the protein primary structure.
- <http://www.matrixscience.com>

Thank You