

Supporting Information

Heat Shock Proteins as Key Biological Targets of the Marine Natural Cyclopeptide Perthamide C

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Tandem MS analysis of PRT C- 4,7,10-trioxa-1,13-tridecanediamine covalent adduct

To determine the reaction pathway between PRT C and $\text{NH}_2\text{-(PEG)}_2\text{-NH}_2$ spacer, the product formed after incubation and recorded at m/z 1226.54 was subjected to MSMS analysis. The MS/MS fragmentation spectra of this ion gave rise to two daughter ions at m/z of 1146.33 and 870.35, corresponding to the loss of SO_3 on the entire molecule and a fragment without PEG spacer, respectively. This fragmentation is in accordance with an adduct produced by the nucleophilic attack of the spacer terminal amino group onto the β -sulfated Asn carbonyl residue of PRT C, followed by urea loss.

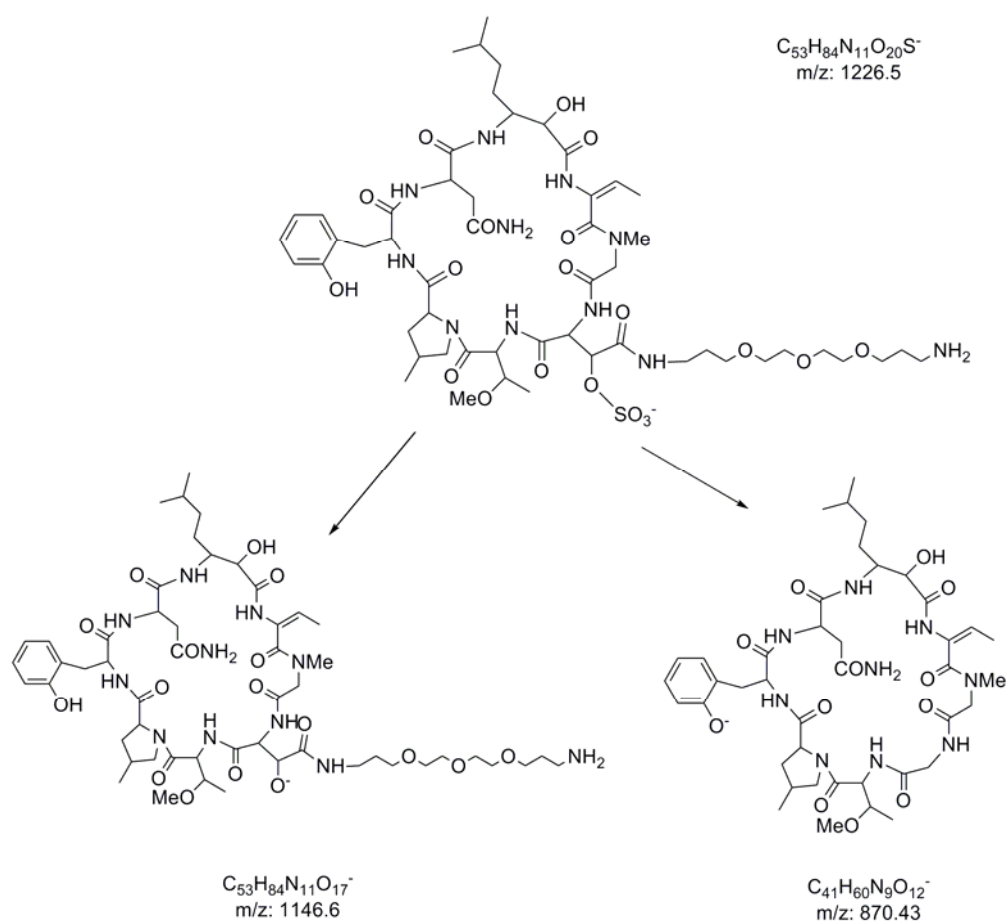


Fig. S1: Ion fragments obtained during tandem MS experiments on the parent ion at 1226.5

Mascot report for Endoplasmin and peak lists

Endoplasmin (Glucose Related Protein 94, GRP94) fitting mass values of three independent experiments are reported here. In addition, all the peak lists, which were sent to Mascot, are available in the pride database under the PRIDE experiment accession number 16675-16680 and the project title name “PRT C Based Chemical Proteomics Experiment”^[1].

Experiment 1										
ENPL_MOUSE Mass: 92703 Score: 41 Matches: 3(1) Sequences: 3(1) emPAI: 0.10										
Endoplasmin OS=Mus musculus GN=Hsp90b1 PE=1 SV=2										
Check to include this hit in error tolerant search										
Query	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Score	Expect	Rank	Unique	Peptide
13	638.3018	1274.5890	1274.6354	-36.35	0	25	0.27	1	U	R.ELISNASDALDK.I
15	645.2770	1288.5394	1288.5935	-41.96	0	4	29	2	U	K.DISTNYASQK.K
58	743.3461	1484.6776	1484.7471	-46.78	0	37	0.017	1	U	K.GVVSDDLLPLNVS.R.E
Experiment 2										
ENPL_MOUSE Mass: 92703 Score: 107 Matches: 9(3) Sequences: 7(2) emPAI: 0.22										
Endoplasmin OS=Mus musculus GN=Hsp90b1 PE=1 SV=2										
Check to include this hit in error tolerant search										
Query	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Score	Expect	Rank	Unique	Peptide
14	638.2910	1274.5674	1274.6354	-53.29	0	38	0.013	1		R.ELISNASDALDK.I
15	638.3164	1274.6182	1274.6354	-13.44	0	(36)	0.023	1		R.ELISNASDALDK.I
63	765.3560	1528.6974	1528.7668	-45.36	0	9	13	2	U	K.NLLHVTDGVMTR.E + Oxidation (M)
68	772.8810	1543.7474	1543.8205	-47.35	1	(18)	1.7	1		R.ELISNASDALDKIR.L
69	772.8945	1543.7744	1543.8205	-29.86	1	19	1.2	1		R.ELISNASDALDKIR.L
129	1015.4975	2028.9804	2029.0579	-38.16	0	13	5.4	1	U	R.LISLTDENALAGNEELTVK.I
145	750.6515	2248.9327	2249.0349	-45.45	0	64	4.4e-05	1	U	R.FQSSHSTDITSLDQYVER.M
188	910.0768	2727.2086	2727.3311	-44.93	1	3	61	2	U	R.TDEVVQREEEAIQLDGLNASQIR.E
195	917.7828	2750.3266	2750.4048	-28.44	2	31	0.094	1	U	R.IKEDEDDKTVMDLAVVLFETATLR.S
Experiment 3										
ENPL_MOUSE Mass: 92703 Score: 105 Matches: 7(3) Sequences: 6(3) emPAI: 0.16										
Endoplasmin OS=Mus musculus GN=Hsp90b1 PE=1 SV=2										
Check to include this hit in error tolerant search										
Query	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Score	Expect	Rank	Unique	Peptide
10	638.3296	1274.6446	1274.6354	7.27	0	30	0.063	1	U	R.ELISNASDALDK.I
11	638.3450	1274.6754	1274.6354	31.4	0	(19)	0.62	1	U	R.ELISNASDALDK.I
15	645.3071	1288.5996	1288.5935	4.75	0	22	0.51	1	U	K.DISTNYASQK.K
51	743.3839	1484.7532	1484.7471	4.14	0	64	2.4e-05	1	U	K.GVVSDDLLPLNVS.R.E
60	763.3728	1524.7310	1524.7195	7.58	0	47	0.0017	1	U	K.EEASDYLELDTIK.N
100	750.6837	2249.0293	2249.0349	-2.50	0	12	4.4	1	U	R.FQSSHSTDITSLDQYVER.M
116	910.1188	2727.3346	2727.3311	1.27	1	2	40	2	U	R.TDEVVQREEEAIQLDGLNASQIR.E

Figure S2. Mascot report of all matched peptides of GRP94 in three independent experiment

MSMS spectra of GRP94

MATRIX *SCIENCE* Mascot Search Results

Peptide View

MS/MS Fragmentation of **ELISNASDALDK**

Found in **ENPL_MOUSE**, Endoplasmic OS=Mus musculus GN=Hsp90b1 PE=1 SV=2

Match to Query 14: 1274.567448 from(638.291000,2+) intensity(7925.0684) index(19)

Data file Experiment2_PRT_band2 (2).pkl

Click mouse within plot area to zoom in by factor of two about that point

Or, to Da

Label all possible matches Label matches used for scoring

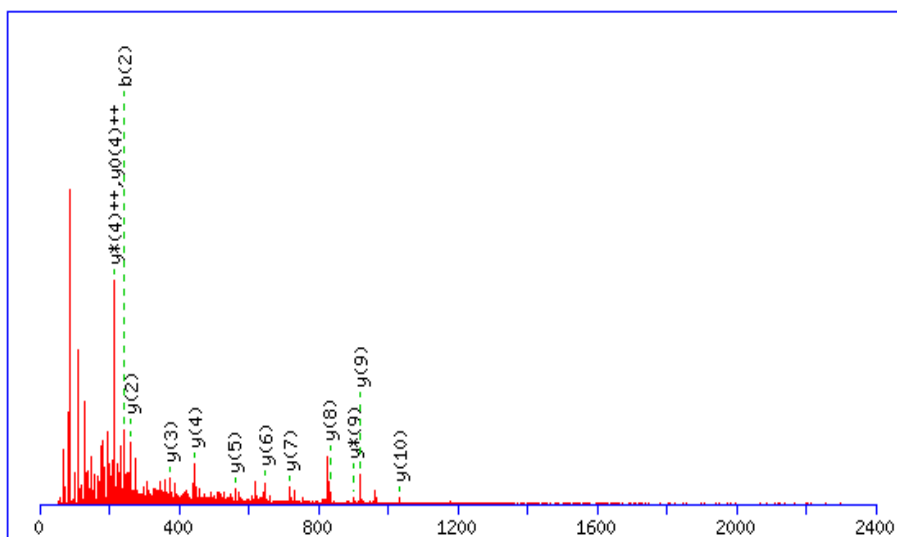


Figure S3. Tandem mass spectrum of the Endoplasmic peptide (ELISNASDALDK)

{MATRIX} **{SCIENCE}** Mascot Search Results

Peptide View

MS/MS Fragmentation of **EEASDYLELDTIK**

Found in **ENPL_MOUSE**, Endoplasmin OS=Mus musculus GN=Hsp90b1 PE=1 SV=2

Match to Query 60: 1524.731048 from(763.372800,2+) intensity(574.5045) index(116)

Data file Experiment3_PRT_band2.pkl

Click mouse within plot area to zoom in by factor of two about that point

Or, to Da

Label all possible matches Label matches used for scoring

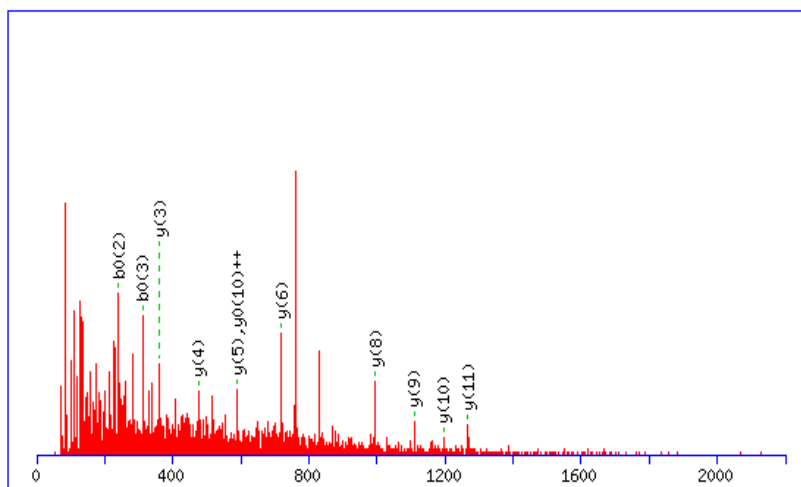


Figure S4. Tandem mass spectrum of the Endoplasmin peptide (EEASDYLELDTIK)

Comparison between canine and murine isoform of GRP94

All the SPR experiments were performed using a canine isoform of GRP94 that was commercially available. To make sure that the homology between the murine and canine isoform was very high, we aligned all sequences using a web software named Clustal W (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>). As reported in **Figure S5**, the sequence alignment reveals that the two proteins have almost the same amino acid sequence. Thus, we have enough confidence that the canine protein is a good model to perform SPR experiments.

CLUSTAL 2.0.12 multiple sequence alignment		
sp P08113 ENPL_MOUSE	MRVLWVLGLCCVLLTFGFSVRADDEVVDGTVVEEDLGKSREGSRTDDEVVQREEEAIQLDG	60
sp P41148 ENPL_CANFA	MRALWVLGLCCVLLTFGFSVRADDEVVDGTVVEEDLGKSREGSRTDDEVVQREEEAIQLDG	60
**.....*		
sp P08113 ENPL_MOUSE	LNASQIRELREKSEKFAFQAEVNRMMKLIINSLYKNKEIFLRELISNASDALDKIRLISL	120
sp P41148 ENPL_CANFA	LNASQIRELREKSEKFAFQAEVNRMMKLIINSLYKNKEIFLRELISNASDALDKIRLISL	120

sp P08113 ENPL_MOUSE	TDENALAGNEELTVKIKCDKEKNLLHVTDGTVGMTREELVKNLGTIAKSGTSEFLNKMTE	180
sp P41148 ENPL_CANFA	TDENALAGNEELTVKIKCDKEKNLLHVTDGTVGMTREELVKNLGTIAKSGTSEFLNKMTE	180

sp P08113 ENPL_MOUSE	AQEDGQSTSELIGQFGVGFYS AFLVADKVVITSKHNNDTQHIWESDSNEFSVIADPRGNT	240
sp P41148 ENPL_CANFA	AQEDGQSTSELIGQFGVGFYS AFLVADKVVITSKHNNDTQHIWESDSNEFSVIADPRGNT	240

sp P08113 ENPL_MOUSE	LGRGTTITLVLKEEASDYLELDTIKNLVRKYSQFINFPIYVWSSKTETVEEPLDEEAAK	300
sp P41148 ENPL_CANFA	LGRGTTITLVLKEEASDYLELDTIKNLVRKYSQFINFPIYVWSSKTETVEEPMEEEAAK	300

sp P08113 ENPL_MOUSE	EEKESDDEAAVEEEEEKPKTKKVEKTVWDWELMNDIKPIWQRPSEVEDEYKAFYK	360
sp P41148 ENPL_CANFA	EEKESDDEAAVEEEEEKPKTKKVEKTVWDWELMNDIKPIWQRPSEVEDEYKAFYK	360

sp P08113 ENPL_MOUSE	SFSKESDDPMAYIHFTAEGEVTFKSILFVPTSAPRGLFDEYGSKKSQDIKLYVRRVFTID	420
sp P41148 ENPL_CANFA	SFSKESDDPMAYIHFTAEGEVTFKSILFVPTSAPRGLFDEYGSKKSQDIKLYVRRVFTID	420

sp P08113 ENPL_MOUSE	DFHDMMPKYLNFVKGVDSDDLPLNVSRETLQQHKLLKVIKRLVTKLDMIKKIADKEY	480
sp P41148 ENPL_CANFA	DFHDMMPKYLNFVKGVDSDDLPLNVSRETLQQHKLLKVIKRLVTKLDMIKKIADKEY	480

sp P08113 ENPL_MOUSE	NDTFWKEFGNIKLVGIEDHSNRTRLAKLLRFQSSHHSTDITSLDQYVERMKEKQDKIYF	540
sp P41148 ENPL_CANFA	NDTFWKEFGNIKLVGIEDHSNRTRLAKLLRFQSSHHSTDITSLDQYVERMKEKQDKIYF	540

sp P08113 ENPL_MOUSE	MAGSSRKEAESSPFVERLLKKGYEVIYLTEPVDEYCIQALPEFDGKRFQNVAKEGVKFE	600
sp P41148 ENPL_CANFA	MAGSSRKEAESSPFVERLLKKGYEVIYLTEPVDEYCIQALPEFDGKRFQNVAKEGVKFE	600

sp P08113 ENPL_MOUSE	SEKTKESREAEI EKEFEP LLNWMKDKALKDKIEKAVVSQRLTESPCALVASQYWGSGNMER	660
sp P41148 ENPL_CANFA	SEKTKESREAEI EKEFEP LLNWMKDKALKDKIEKAVVSQRLTESPCALVASQYWGSGNMER	660

sp P08113 ENPL_MOUSE	IMKAQAYQTGKDISTNYASQKKTFEINRHP LIRDLRRIKEDDDKTVM DLAVVLFET	720
sp P41148 ENPL_CANFA	IMKAQAYQTGKDISTNYASQKKTFEINRHP LIRDLRRIKEDDDKTVM DLAVVLFET	720

sp P08113 ENPL_MOUSE	ATLRSGYLLPDTKAYGDRIERMLRSLNIDPEAQVEEPEEPEPTSEDAEDSEQDEGEE	780
sp P41148 ENPL_CANFA	ATLRSGYLLPDTKAYGDRIERMLRSLNIDPEAQVEEPEEPEETTEDTTEQDDEE	780

sp P08113 ENPL_MOUSE	MDAGTEEEETEKESTEKDEL--	802
sp P41148 ENPL_CANFA	EMDAGTDDEEQETVKKSTA EKDEL	804
. :*: : : :		

Fig. S5. Sequence alignment of murine and canine GRP 94

Reference List

- [1] J.A. Vizcaíno, R. Côté, F. Reisinger, J.M. Foster, M. Mueller, J. Rameseder, H. Hermjakob, L. Martens, *Proteomics* **2009**, *18*, 4276-4283.