Supplementary Information (SI) for Molecular Omics.

SUPPORTING INFORMATION

Selective Labeling of Tyrosine Residues in Proteins: Insights from PTAD Labeling and Tandem Mass Spectrometry Analysis

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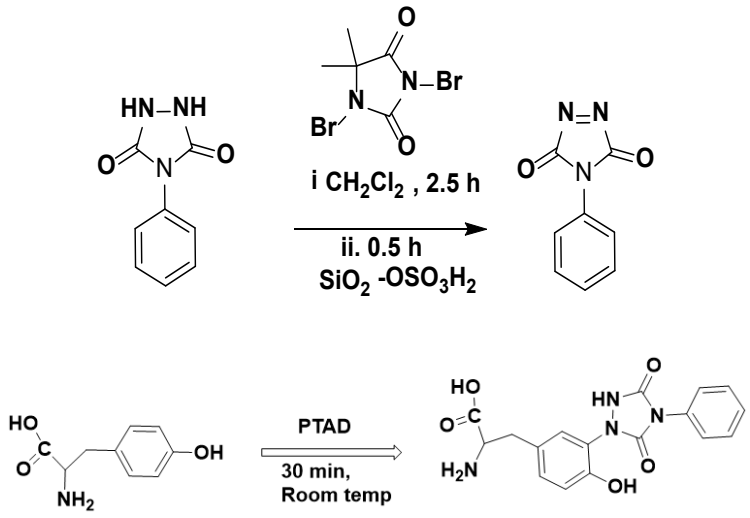
Scheme S1.



Synthesis scheme of PTAD (top) and PTAD labeling in a Tyrosine residue (bottom).

Synthesis of 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD)

PTAD was synthesized following a procedure available in literature. Briefly, a solution of 4-phenyl-1,2,4-triazolidine-3,5-dione (20mg, 0.11 mmol) in dichloromethane (5mL) was prepared. 1,3-dibromo-5,5-dimethylhydantoin (32 mg, 0.11 mmol) was added and the resulting solution was stirred for two hours. Then silica sulfuric acid (SiO_2-OSO_3H_2) (88mg, 0.55 mmol) was added and stirred at room temperature for thirty minutes. Silica sulfuric acid was removed by centrifugation and the volatile material was evaporated *in vacuo* to give the product as a red solid. ^{1}H NMR ($(CD_3)_2SO$): δ (ppm) 7.51-7.56 (2H, d), 7.36-7.48 (3H,m).

Neurotensin:

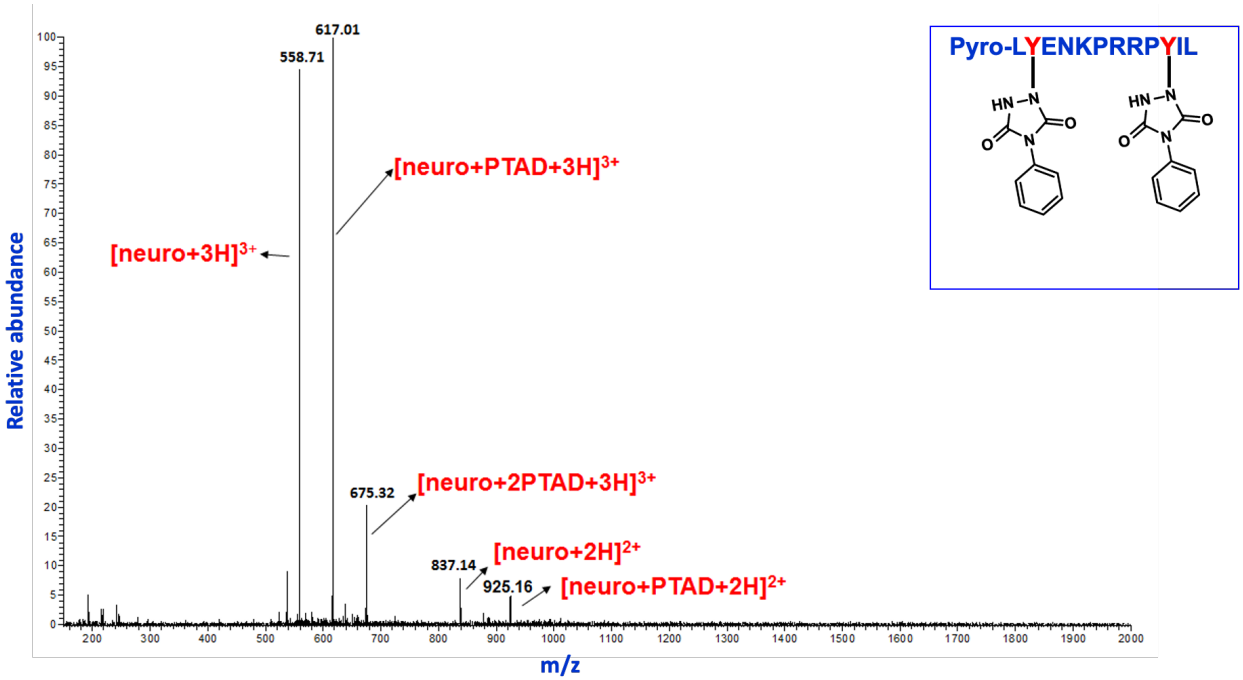


Figure S1: Full-MS spectrum of PTAD labeled neurotensin.

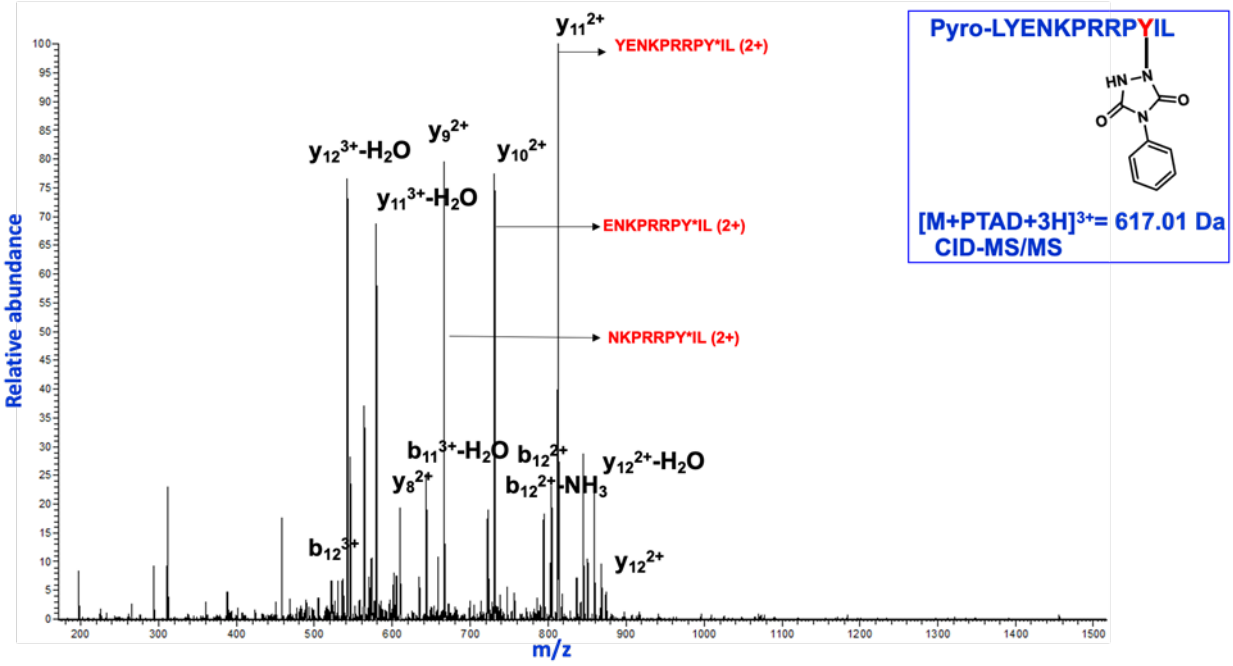
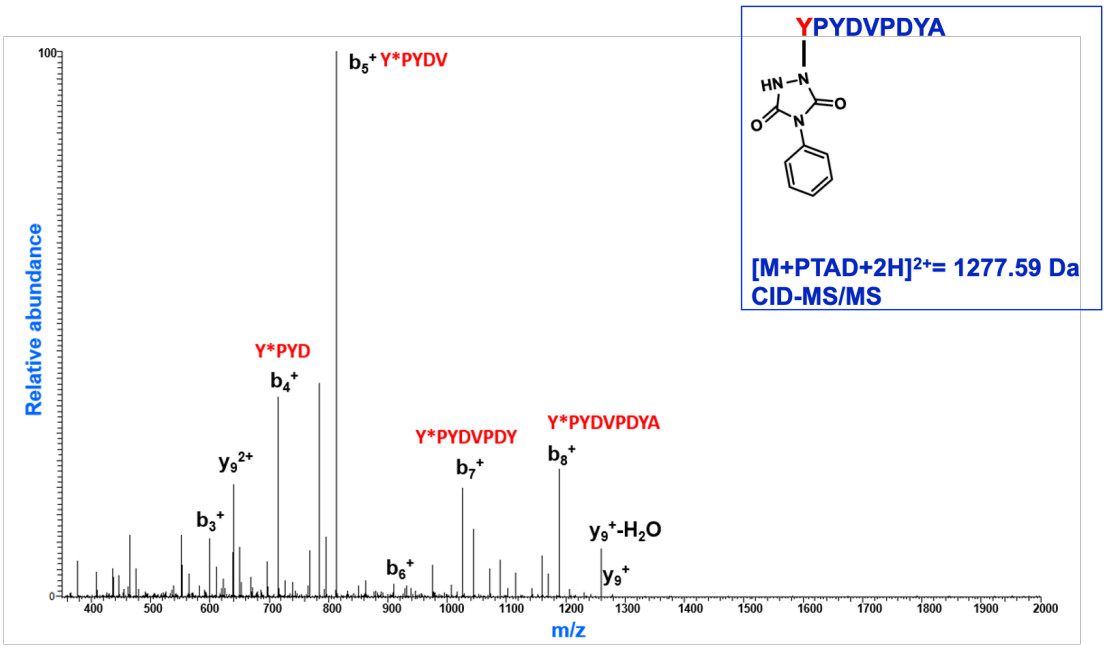


Figure S2: CID-MS/MS of a singly tagged neurotensin precursor m/z 617.07 Da (3+).

HA Peptide:

S3 A)



S3 B)

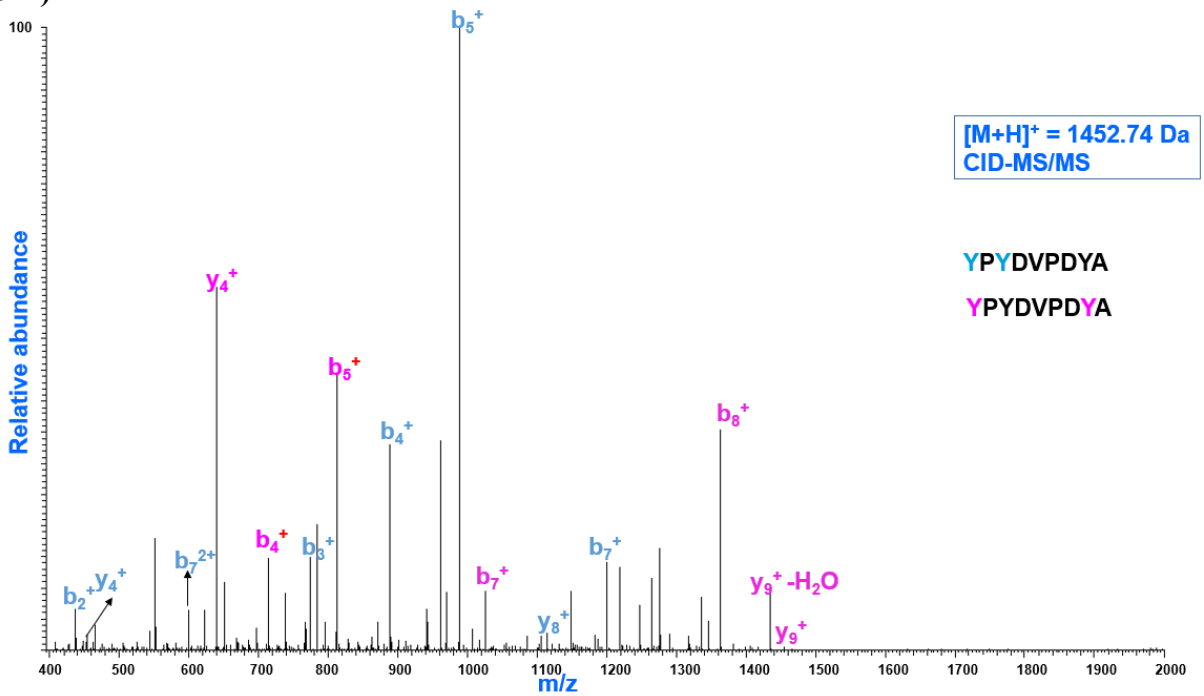


Figure S3: A) CID-MS/MS of m/z 1277.59 Da PTAD labeled HA peptide with one PTAD-tagged tyrosine residue. B) CID-MS/MS of m/z 1452.73 Da PTAD labeled HA peptide, two types of double labeled HA peptide are observed. MS/MS of the mixtures of the same m/z precursors produced mixtures of b/y ions.

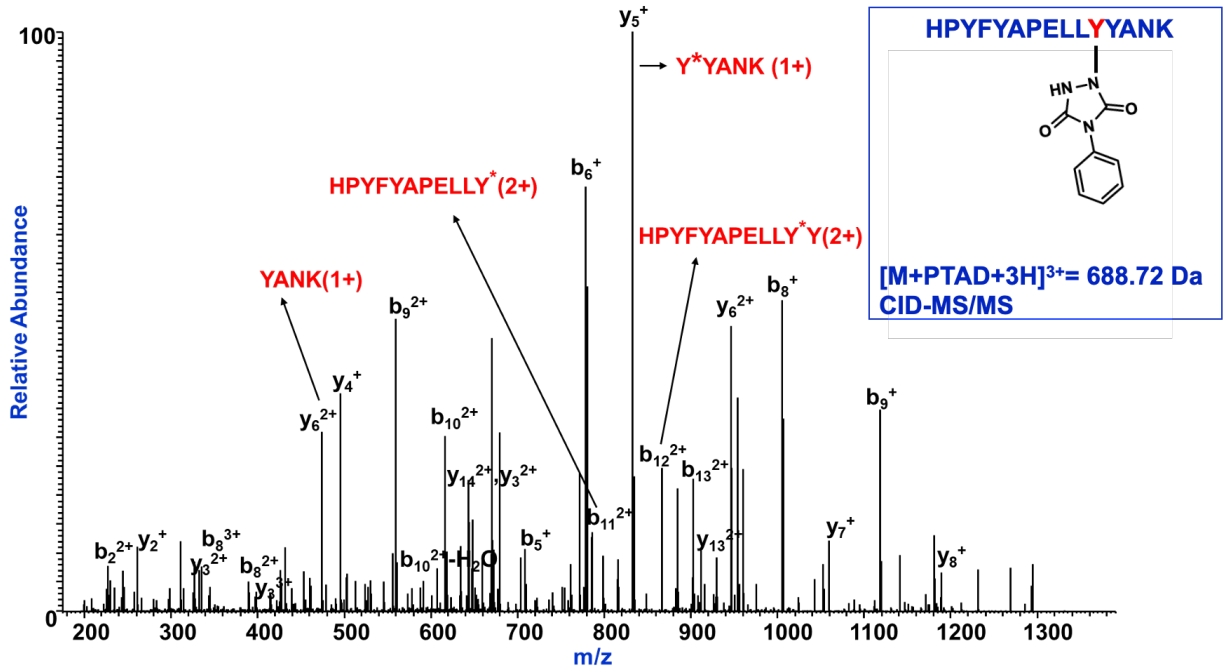


Figure S4: PTAD-tagged BSA peptide (Y179).

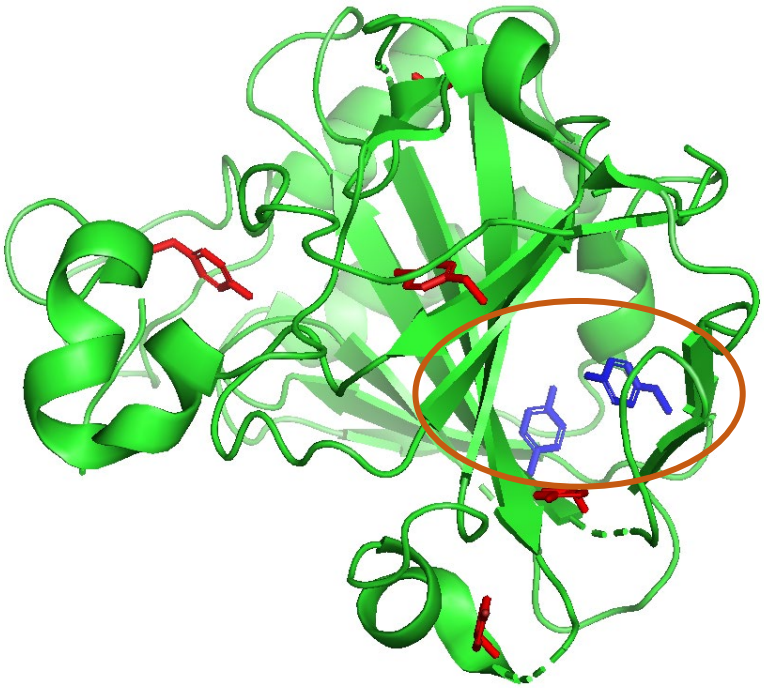


Figure S5: PTAD-tagged Carbonic Anhydrase (pdb 1G67). Blue-labeled sites, Red-Unlabeled sites.

HELA Cell lysate

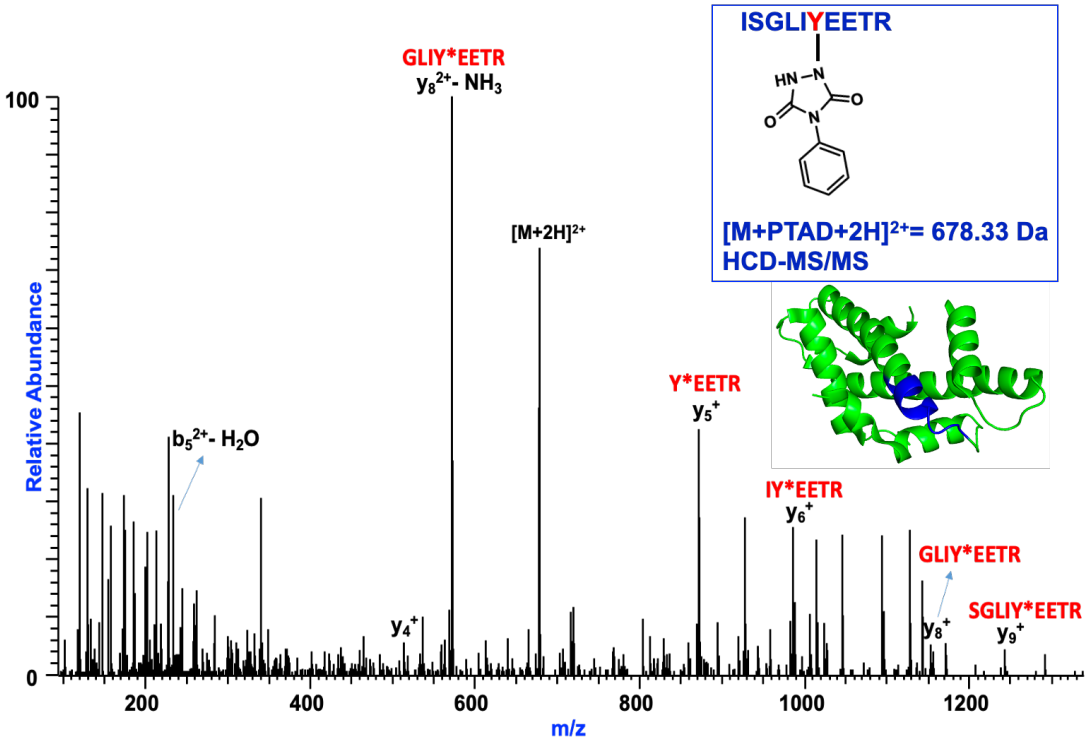


Figure S6 An HCD-MS/MS spectrum of m/z 678.33 Da (2+) of PTAD-tagged Histone H-4 protein from HeLa cell lysate. Location of the PTAD-tagged site is shown in the crystal structure (3nqj)

Table 1: List of PTAD-labeled high-confidence proteins obtained from HeLa cell lysate. The labeled peptide sequences have been listed with their associated protein names, accession numbers, XCorr values, and unique peptides.

Protein name	Annotated Sequence	Modifications	# Proteins	Master Protein Accessions	XCorr	Unique peptides
Histone H1.3	ALAAAGyDVEK	Y7(PTAD)	5	ENST00000244534	2.53	4
Protein disulfide-isomerase A3	DGEEAGAyDGPR; mDATANDVPSPyEVR	[Y8(PTAD)]; [M1(Oxidation); Y12(PTAD)]	1	ENST00000300289	2.47; 3.39	14
Heterogenous nuclear ribonucleoprotein H3	DGmDNQGGyGSVGR	M3(Oxidation); Y9(PTAD)	2	ENST00000265866	1.94	5

Heat shock protein HSP 90-alpha	DNSTmGymAAK; NPDDITNEEyGEFYK; yIDQEELNK	[M5(Oxidation), Y7(PTAD), M8(Oxidation)]; [Y10(PTAD)];[Y 1(PTAD)]	2	ENST00000334701	2.27; 2.79; 2.14	9
14-3-3 protein epsilon	EAAENSLVAyK	Y10(PTAD)	2	ENST00000264335	2.77	5
Small ribosomal subunit protein uS3	ELAEDGySGVEVR	Y7(PTAD)	3	ENST00000278572	2.36	6
Transgelin-2	GASQAGmTGyGmPR	M7(Oxidation); Y10(PTAD); M12(Oxidation)	3	ENST00000368096	2.61	4
Heat shock 70 kDa protein 1A	IINEPTAAAIAyGLDR	Y12(PTAD)	3	ENST00000400040	3.18	15
Histone H4	ISGLIyEETR	Y6(PTAD)	1	ENST00000340756	2.25	5
Tubulin beta chain	ISVYyNEATGGK	Y5(PTAD)	1	ENST00000383564	2.41	5
Endoplasmic reticulum chaperone BiP	ITPSyVAFTPEGER	Y5(PTAD)	1	ENST00000324460	3.05	23
14-3-3 protein beta/alpha	LAEQAERyDDmAAA mK	Y8(PTAD); M11(Oxidation); M15(Oxidation)	3	ENST00000353703	2.55	3
Large ribosomal subunit protein uL23	LAPDyDALDVANK	Y5(PTAD)	1	ENST00000422514	2.65	3
Protein disulfide-isomerase A3	LAPEyEAAATR	Y5(PTAD)	1	ENST00000300289	2.01	14
Transferrin receptor protein 1	LLNENSyVPR	Y7(PTAD)	1	ENST00000360110	2.11	8
Heterogeneous nuclear ribonucleoprotein A1	NQGGYGGSSSSSSy GSGR	Y14(PTAD)	3	ENST00000340913	3.1	9
Annexin A2	RAEDGSVIDyELIDQD AR; TPAQyDASELK	Y10(PTAD); Y5(PTAD)	2	ENST00000332680	3.32; 3.14	18
Vimentin	SLyASSPGGVYATR	Y3(PTAD)	1	ENST00000224237	2	27
Nascent polypeptide-associated complex	SPASDTyIVFGEAK	Y7(PTAD)	1	ENST00000393891	2.17	4

subunit alpha						
Heterogeneous nuclear ribonucleoprotein A1	SSGPYGGGGQyFAKPR	Y11(PTAD)	3	ENST00000340913	2.75	9
Beta-actin	SyELPDGQVITIGNER	Y2(PTAD)	11	ENST00000331789	4.36	16
Heterogeneous nuclear ribonucleoprotein K	TDyNASVSVPDSSGPER	Y3(PTAD)	2	ENST00000376281	3.21	11
Heterogeneous nuclear ribonucleoprotein R	TGyTLDVTTGQR	Y3(PTAD)	4	ENST00000374616	2.2	2
Heat shock cognate 71 kDa protein	TTPSyVAFTDTER	Y5(PTAD)	8	ENST00000227378	2.54	17
Protein disulfide-isomerase	VDATEESDLAQQyGVR	Y13(PTAD)	1	ENST00000331483	3.65	13
Endoplasmic reticulum chaperone BiP	VTHAVVTVPAyFNDAQR	Y11(PTAD)	1	ENST00000324460	3.12	23
14-3-3 protein zeta/delta	yLAEVAAGDDKK	Y1(PTAD)	2	ENST00000395956	2	4
Large ribosomal subunit protein P2	yVASYLLAALGGNSSPSAK	Y1(PTAD)	1	ENST00000321153	2.29	8

Table 2: List of PTAD-labeled peptides observed in different proteins. (*) depicts the labeled tyrosine residues.

Protein	Peptide	Precursor	Theoretical mass (Da)	Observed mass (Da)
Neurotensin+PTAD	Pyro-LYENKPRRPY*IL	[M+PTAD+2H]2+	617.03	617.07
Neurotensin+2PTAD	Pyro-LY*ENKPRRPY*IL	[M+2PTAD+2H]2+	1012.62	1012.62
Neurotensin+2PTAD	Pyro-LY*ENKPRRPY*IL	[M+2PTAD+3H]3+	675.42	675.32
BSA	RHPEY*AVSVLLR	[M+PTAD+3H]3+	539.28	538.98
BSA	HPYFYAPELLY*YANK	[M+PTAD+3H]3+	688.70	688.72
Carbonic anhydrase	MVNNGHSFNVEY*DDSQDK	[M+PTAD+3H]3+	759.11	758.67
β - casein	DM(ox)PIQAFLLY*QEPVLGPVR	[M+PTAD+2H]2+	1189.87	1189.43
Histone H4-Hela	ISGLIY*EETR	[M+PTAD+2H]2+	678.3890	678.33
Beta actin-Hela	SY*ELPDGQVITIGNER	[M+PTAD+2H]2+	983.5245	984.39