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Identification of Post-translational Modifications of *Plasmodium yoelii* Glyceraldehyde-3-phosphate dehydrogenase by Mass Spectrometry

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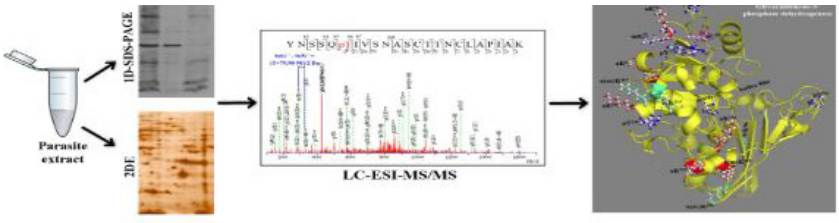
Abstract

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a glycolytic enzyme from *Plasmodium yoelii* exhibits diverse sub-cellular distribution with a multitude of electrophoretic variants. Recent studies have implicated this protein in multiple non-glycolytic functions such as vesicular transport and facilitating host cell invasion by merozoites and sporozoites. In the absence of any organelle specific signal sequence in GAPDH, PTMs that could enlarge molecular species of a protein with distinct functions are likely to form the structural basis for its diverse localization and functions. Such considerations have enthused our interest in chemically characterizing all species of this protein in the parasite. Here, an attempt was made for a comprehensive determination of the PTMs in PyGAPDH in blood stage parasites using LC-ESI-MS/MS of peptides obtained from in-gel digestion of appropriate protein bands. Twelve residues were identified that underwent modifications. These changes consisted of four phosphorylations (pS^{144}, pT^{146}, pS^{204} and pS^{213}), five ubiquitinations (uK^{73}, uK^{218}, uK^{222}, uK^{230} and uK^{336}), three acetylations (acK^{163}, acK^{230}, acK^{301}), two methylations (mK^{218} and mK^{230}), one dimethylation ($m2K^{230}$) and one nitrosylation (nC^{157}). It is hoped that such comprehensive analysis of PTMs in a single protein will pave the way to correlate structure with specific functions and provide the molecular basis for diverse intracellular distribution.



Graphical Abstract

Keywords: Glyceraldehyde-3-phosphate dehydrogenase; Mass Spectrometry; Moonlighting Functions; *Plasmodium*; Post-Translational Modifications; Protein Species

Abbreviations

GAPDH	:	Glyceraldehyde 3-phosphate dehydrogenase
PyGAPDH	:	*Plasmodium yoelii* GAPDH
rPfGAPDH	:	Recombinant *Plasmodium falciparum* GAPDH
G-3-P	:	D-Glyceraldehyde-3-phosphate
2-DE	:	2-Dimensional Electrophoresis
PTM	:	Post-Translational Modification
PBST	:	Phosphate Buffer Saline Tween
PVDF	:	Polyvinylidene fluoride

Introduction

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH, EC 1.2.1.12) has emerged as a multifunctional protein with several moonlighting functions. In mammalian cells, involvement of GAPDH in RNA transport, DNA replication, vesicular transport, cytoskeletal reorganization, membrane fusion, apoptosis etc. is well documented [1]. The structural basis for such functional diversity has been attributed to multiple species of GAPDH arising due to multiple PTMs [2]. A few chemical modifications have been correlated to specific functions [1d,3] while majority of GAPDH species are yet to be characterized. Since each chemical modification leads to a new protein speciation, multiple PTMs in different combinations could create a vast number of species leading to complexity in cellular functions without any expansion of genome. For a complete understanding of such systems, knowledge about the structure of each protein species, its function and spatio-temporal distribution inside the cell will be needed. Recent strategies of applying the advanced proteomics technologies for protein separation and sequencing, to multiple molecular species of a single gene product is providing robust structural data laying down the foundation for understanding the cellular physiology [4].

In *P. yoelii*, GAPDH is associated with multiple organelles viz. cytosol, nuclei, cell membranes, cytoskeletal elements etc. and has a distinct organelle specific electrophoretic variant profile. 2DE western blots of *P. yoelii* sub-cellular fractions showed ≥20-25 different species of GAPDH [5] arising due to post translational modifications. This structural diversity is forming the molecular basis for the involvement of the parasite GAPDH in multiple non-glycolytic functions such as vesicular transport and biogenesis of the apical complex [6], likely involvement in merozoite invasion of red blood cells [7] and invasion of liver cells by sporozoites [8] etc. Thus, the observed functional and localization diversity does correlate with the underlying structural heterogeneity. For understanding the molecular basis of various cellular functions of this protein, it is essential that we determine the chemical structure of each molecular species. Initial attempts to excise the relevant spots from a 2-Dimensional gel, digest the protein with trypsin and sequence the peptides for PTM determination did not succeed largely due to low resolution of spots in 2D-gel and inadequate sensitivity of our mass spectrometer. To tide over these limitations, we took an alternative approach where the whole cell extract was fractionated in soluble and particulate fractions and proteins were analyzed on a 1D-SDS-PAGE. PyGAPDH containing protein bands were digested with trypsin and subjected to MS and MS/MS analysis. Results showed that twelve residues underwent modifications. The changes consisted of four phosphorylations (pS^{144}, pT^{146}, pS^{204} and pS^{213}), five ubiquitinations (uK^{73}, uK^{218}, uK^{222}, uK^{230} and uK^{336}), three acetylations (acK^{163}, acK^{230}, acK^{301}), two methylations (mK^{218} and mK^{230}), one dimethylation ($m2K^{230}$) and one nitrosylation (nC^{157}).

Materials and Methods

P. yoelii culturing and whole cell extract preparation

The lethal strain of *P. yoelii* 17XL was grown in mice as described earlier [9]. The parasite pellet isolated from infected blood was suspended in lysis buffer (50 mM Tris-HCl, pH 7.4, 2 mM EDTA, 25 mM NaCl, 1 mM PMSF and 1x protease inhibitor cocktail) and subjected to 3-4 cycles of freeze-thaw in liquid nitrogen. This was labeled as the Whole Cell Extract (WCE). Animal experiments involving mice were approved by the Institutional Animal Ethics Committee (IAEC) of the Tata Institute of Fundamental Research, which is constituted by the 'Committee for the Purpose of Supervision and Experiments on Animals (CPCSEA)', Government of India (Project approval no: TIFR/IAEC/2010-4 and TIFR/IAEC/2012-5).

Generation of Anti-rPfGAPDH Serum and Immunoprecipitation

Anti-PfGAPDH sera were generated using purified recombinant PfGAPDH as described earlier [5]. For immuno-precipitation experiments, whole cell extract was centrifuged at 40,000xg and the supernatant was labeled as the 'Soluble fraction'. The pellet was dissolved in 1% NP-40 buffer and centrifuged at 40,000xg. The supernatant was collected and labeled as the 'Particulate fraction'. The protocol followed for immuno-precipitation was similar to that used earlier except that anti-rPfGAPDH IgGs were used in place of anti-rPfeno IgGs [9].

Electrophoresis and western blotting

Proteins were resolved on a 12% SDS-PAGE [10] and either stained with Coomassie Brilliant Blue R-250 or were transferred to a PVDF membrane as described earlier [5]. The blots were treated with mouse anti-rPfGAPDH serum (1:1000 dilution) followed by washing and incubation with HRP conjugated secondary antibody. The immunoblots were developed using di-anilinobenzene substrate.

In-Gel Tryptic Digestion and LC-ESI-MS/MS Analysis

Protein bands that corresponded to PyGAPDH positive in western blot were excised from a Coomassie stained gel and subjected to in-gel trypsin digestion as described earlier [9,11]. Extracted peptides were analyzed by LC-ESI-MS/MS using an Agilent 6520-Q-TOF. Details for the mass spectrometric analysis of peptides were as described earlier [9]. Briefly, the extracted peptides were re-suspended into 3 µL 0.1% formic acid (Solvent A) of which 2.8 µL was applied to Agilent HPLC chip (G4240-62002) (injected at a rate of 40 µL/min). Mobile phases (A): 0.1% formic acid, (B): 90% acetonitrile, 0.1% formic acid. After sample injection, the column was washed by a gradient 3-12% of phase B for 3 min and peptides were eluted with linear gradient of varying slopes viz. 12-60% B from 3 to 23 min, 60-95% of B from 23 to 27 min. Q-TOF MS conditions were: drying gas 4L/Min, 300°C; skimmer: 65 V; fragmentor: 175V; collision energy: slope 3.7 V, offset 2.5 V. The MS scan range was m/z =100-1700 and the scan rate was 5 spectra/sec. For MS/MS, scan range was m/z=100-1700 and scan rate was 3 spectra/sec. Active exclusion was set on for 0.5 min after 2 MS/MS spectra of a parent ion. For each MS, five most abundant precursor ions were sequenced. Preferred charge states were set to 2^+, 3^+ and 4^+.

Data Analysis Using Mascot

From all the MS data files, Mascot generic files (.mgf) were extracted using Agilent Mass hunter qualitative analysis software. All mass-spectrometric data were analyzed using a Matrix Science Mascot in house server [12]. MS/MS data were searched against the NCBInr database for *P. falciparum* and *P. yoelii.* Since a large fraction of *P. yoelii* than *P. falciparum* proteomes are unannotated, most proteins are marked as hypothetical. For obtaining as many proteins annotated as possible, databases for both species of *Plasmodium* were used for search. However, hits obtained against a species different from the one used in the experiments were analyzed cautiously for sequence differences before reporting the PTMs. Parameters used for the search were: peptide mass tolerance in MS was set to 10 ppm and for MS/MS to 0.6 Da; peptide charges were set to 2^+, 3^+ and 4^+; missed cleavage, 2; fixed modifications: carbamidomethyl (cysteine); variable modifications: oxidation of methionine and target modifications phosphorylation (Ser/Thr), phospho (Tyr), acetyl (Lys), methylation (Lys; mono, di and tri); nitrosylation on cysteine and Gly-Gly (Lys) for ubiquitination. All PTMs reported here were manually validated. This involved examining the ions detected in MS/MS spectra and if two consecutive ions differed in mass equivalent to the modified residue, it was treated as true positive. Although this approach is time consuming, it leads to greater confidence in identification and assignment of PTMs. Cases where a signature peak was missing, and if the site of modification could not be inferred from neighboring peaks, such PTMs were not reported.

The mass spectrometric data have been deposited at the ProteomeXchange Consortium [13] via the PRIDE partner repository [14] with the dataset identifier PXD002313 and 10.6019/PXD002313. The desired pride XML files were obtained from Mascot .dat files using the PRIDE converter 2 tool [15] and inspected using the PRIDE Inspector tool [16] before uploading them. These PRIDE XML files were deposited to the repository along with the raw data files (Agilent .d files), peak lists (Mascot .mgf files) and the search results files (Mascot .dat files).

Results

PyGAPDH Variants With MW ~51 kDa may Be Ubiquitinated

P. yoelii Whole Cell Extract (WCE) was subjected to centrifugation (40,000xg for 30 minutes). The supernatant was designated as the soluble fraction while the pellet containing nuclei, membrane vesicles and cytoskeletal elements was treated as the particulate fraction. All three fractions (WCE, Soluble and Particulate) were analyzed on a 12% SDS-PAGE and probed with anti-rPfGAPDH antisera in a western blot. As shown earlier [5], three major positive bands at ~27, 37 and 51 kDa were observed (Figure 1).

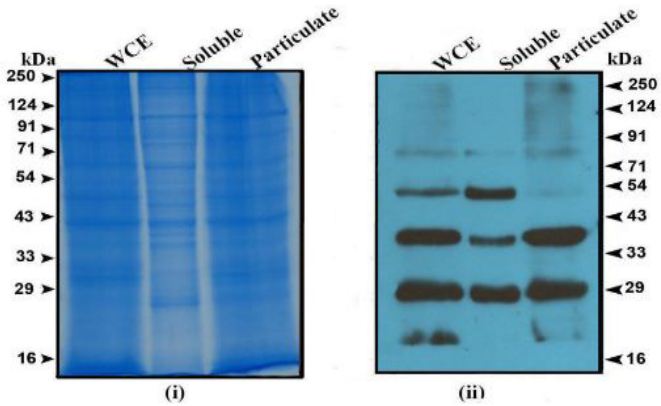


Figure 1: (i) Coomassie stained SDS-PAGE of *P. yoelii* whole cell extract (WCE) and the two fractions i.e. soluble and particulate. Fractionation was done by centrifugation at 40,000g for 30 minutes. (ii) Western blot of three fractions probed using anti-rPfGAPDH antibodies. Note the presence of PyGAPDH in three different sizes with MW ~27, 37 and 51 kDa. Absence of 51 kDa species in particulate fraction is quite evident.

Since molecular mass of PyGAPDH in its native state is 37 kDa, the lower mass band at ~27 kDa could arise as a result of controlled proteolysis. Observation of higher molecular mass species of PyGAPDH raised the possibility of post-translational modifications involving conjugation with multiple ubiquitin moieties or ubiquitin like modifiers (e.g. SUMO). To test the possibility of the higher molecular weight species of PyGAPDH in the soluble fraction of *P. yoelii* being ubiquitinated, an immuno-precipitation experiment was performed. Using purified fraction of IgGs derived from rPfGAPDH antisera, all variants of PyGAPDH present in the soluble and particulate (solubilized in 1% NP-40) fractions were pulled down (Figure 2A) and the proteins were run on a 12% SDS-PAGE. Blot of the gel (Western analysis) was probed using rabbit anti-ubiquitin antibody (Figure 2B(ii) & (iii)).

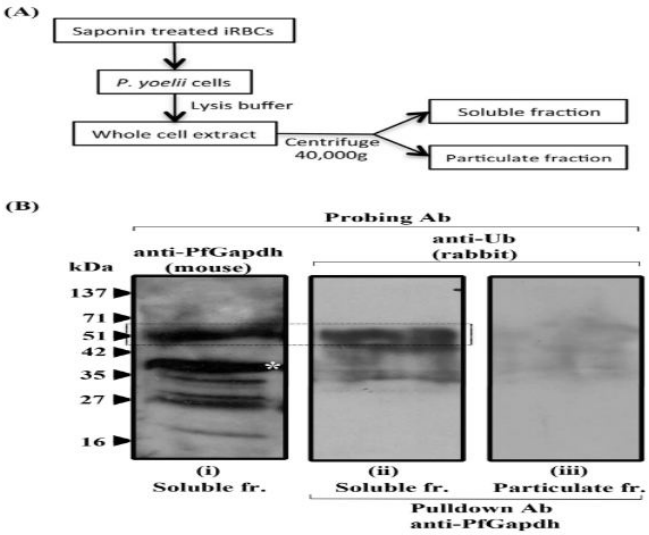


Figure 2: Antibody pull-down assay to determine ubiquitination of PyGAPDH. (A) Fractionation scheme for the preparation of soluble and particulate fractions. (B) (i) Soluble fractions showed three major species of PyGAPDH at MW ~27, 37 and 51 kDa. (ii) Probing a similar blot with anti-Ub antibody showed ~51 kDa band to be an ubiquitinated form of PyGAPDH (dotted box). *Represents the ~37 kDa form of PyGAPDH which showed a faint signal for ubiquitination. (iii) Blot showing absence of ubiquitination in PyGAPDH associated with particulate fraction of *P. yoelii* cell extract.

In a parallel experiment, the soluble fraction was also analyzed by Western using mouse anti-rPfGAPDH antibody. As expected, three major protein bands at MW ~27, 37 and 51 kDa were observed (Figure 2B (i)). Certain minor bands present are likely to arise due to proteolysis. Higher molecular wt. species (MW ~51 kDa) was present only in the soluble (cytosolic) fraction (Figure 2B). This is consistent with the earlier observations of electrophoretic variant profiles in 2DE [5]. In the anti-rPfGAPDH antibody pull down sample from the soluble (i.e. cytosolic) fraction, an intense band at ~51 kDa MW was observed that was positive for ubiquitin indicating it to be the ubiquitinated form of PyGAPDH. This sample also had ubiquitin positive band at ~37 kDa albeit of much lower intensity. Such a band could arise if the ~61kDa ubiquitinated form of PyGAPDH got proteolysed yielding a ~37 kDa form that still carried ubiquitin moieties (Figure 2B (ii)). The particulate fraction did not show any ubiquitinated form of PyGAPDH (Figure 2B (iii)). From the data presented here, we conclude that the higher molecular weight species (MW ~51 kDa) of PyGAPDH observed in the soluble fraction and in 2DE of cytosol [5] arose due to ubiquitination of native PyGAPDH. The ~51 kDa band in the soluble fraction that is visualized by both antibodies (anti-PfGAPDH and anti-Ub) has an addition of mass of ~15-17 kDa to the native PyGAPDH. Conjugation of two molecules of ubiquitin (MW ~8.5 kDa) to PyGAPDH can give rise to such species.

Detection and Sequence Coverage of PyGAPDH as Analyzed By LC-ESI-Q-TOF-MS

Identity of the three protein bands observed in Western blot analysis was further confirmed by the mass spectrometric analysis of peptides obtained from in-gel tryptic digestion of the three PyGAPDH species. Extracted peptides were separated on a reverse phase C-18 nano-chip and as the peptides eluted, MS and MS/MS spectra were acquired. The lists of matched m/z peptides for various fractions are presented in Table 1(A) to (D).

Table 1: Analysis of Post Translational Modifications in peptides derived from tryptic digests of GAPDH positive bands as shown in Figure 1. Three bands from the soluble fraction (with MW ~ 27, 37 and 51 kDa) and 37kDa band of particulate fraction were individually digested with trypsin and peptides were sequenced using MS/MS. All peptides that were derived from GAPDH and had post-translational modifications are listed below.

Calculated m/z	observed	Δ (ppm)	Peptide and observed PTMs (bold)	No. of hits
1345.7466	1345.7377	-7	^{2}AIT**K**VGINGFGR^{13}GG(K^{4})	1
818.4399	818.4384	-2	^{6}VGINGFGR^{13}	3
2687.388	2687.391	1	^{26}SDIEVVAINDPF**M**DINHLIYLLK^{48}(Oxi-M^{138})	6
1807.8485	1807.8454	-2	^{56}FPCEVTPTEGGIMVGSK^{72}	8
1823.8434	1823.854	6	^{56}FPCEVTPTEGGI**M**VGSK^{72}(Oxi-M^{68})	60
1998.0687	1998.0543	-7	^{73}KVVVYNERDPAQIPWGK^{89}	22
2112.1116	2112.0942	-8	^{73}KVVVYNERDPAQIPWGK^{89}GG(K^{73})	1
877.4658	877.4667	1	^{74}VVVYNER^{80}	2
1869.9737	1869.964	-5	^{74}VVVYNERDPAQIPWGK^{89}	9
1010.5185	1010.5164	-2	^{81}DPAQIPWGK^{89}	7
1774.8924	1774.8858	-4	^{90}HAIDVVCESTGVFLTK^{105}	9
1307.6833	1307.6922	7	^{106}ELSNAHI**K**GGA**K**^{117}ac(K^{113}K^{117})	1
841.4731	841.4731	0	^{119}VIMSAPPK^{126}	2
2470.2236	2470.2148	-4	^{119}VIMSAPPKDDTPIYVMGINHEK^{140}	9
1630.7661	1630.764	-1	^{127}DDTPIYV**M**GINHEK^{140}	5
1646.761	1646.7572	-2	^{127}DDTPIYVMGINHEK^{140}(Oxi-Met)	3
2499.1734	2499.1814	3	^{141}YNSSQTIVSNASCTTNCLAPIAK^{163}	6
2579.1397	2579.1187	-8	^{141}YNSSQ**T**IVSNASCTTNCLAPIAK^{163}p(T^{146})	2
2579.1397	2579.1187	-8	^{164}VIHENFGIVEGLMTTVHASTANQLVVDGPS^{194}	2
3278.6606	3278.6704	3	^{164}VIHENFGIVEGL**M**TTVHASTANQLVVDGPS^{194}(Oxi-M^{176})	4
1425.8191	1425.8109	-6	^{204}SALLNIIPASTGAAK^{218}	6
1505.7854	1505.7738	-8	^{204}**S**ALLNIIPASTGAAK^{218} p(S^{204})	2
1895.084	1895.0744	-5	^{204}SALLNIIPASTGAA**K**AVGK^{222}GG(K^{218})	1
1895.084	1895.0744	-5	^{204}SALLNIIPASTGAAKAVG**K**^{222}GG (K^{222})	1
868.5018	868.5002	-2	^{223}VLPELNGK^{230}	3
1654.9406	1654.9271	-8	^{223}VLPELNG**K**LTGVAFR^{237}ac(K^{230})	1
1726.973	1726.9629	-6	^{223}VLPELNGKLTGVAFR^{237}GG(K^{230})	2
762.4388	762.4381	-1	^{231}LTGVAFR^{237}	4
1512.8334	1512.8227	-7	^{238}VPIGTVSVVDLVCR^{251}	15
1070.5971	1070.5974	0	^{265}IKEASEGPLK^{274}	3
2265.055	2265.0621	3	^{275}GILGYTDEEVVSQDFVHDSR^{294}	9

808.4331	808.4311	-2	^{295}SSIFDLK^{301}	14
1208.619	1208.6122	-6	^{302}AGLALNDNFFK^{312}	9
1787.7903	1787.7889	-1	^{313}IVSWYDNEWGYSNR^{326}	4
1135.6965	1135.6951	-1	^{327}LLDLAIHITK^{336}	6
1249.7394	1249.7318	-6	^{327}LLDLAIHIT**K**^{336}GG(K^{336})	1
1272.7554	1272.7517	-3	^{327}LLDLAIHITKH^{337}	8
1386.7983	1386.7882	-7	^{327}LLDLAIHIT**K**H^{337}GG(K^{336})	6

Table 1A: MW ~51 kDa species from soluble fraction (data from 2 independent samples): Protein Score: 1246; Sequence coverage: 76%.

Calculated m/z	Observed m/z	Δ (ppm)	Peptide and observed PTMs (bold)	No of hits
818.4399	818.4432	4	^{6}VGINGFGR^{13}	2
3583.7422	3583.7541	3	^{26}SDIEVVAINDPF**M**DINHLI**Y**LL**K**HDSVHG**K**^{55} ac(K^{55}); m(K^{48}); Oxi (M^{38});p(Y^{45})	1
2687.388	2687.3895	1	^{26}SDIEVVAINDPF**M**DINHLIYLLK^{48}Oxi (M^{38})	11
1807.8485	1807.8427	-3	^{56}FPCEVTPTEGGI**M**VGSK^{72}Oxi (M^{68})	42
1998.0687	1998.0646	-2	^{73}KVVVYNERDPAQIPWGK^{89}	4
877.4658	877.4649	-1	^{74}VVVYNER^{80}	2
1869.9737	1869.9661	-4	^{74}VVVYNERDPAQIPWGK^{89}	11
1010.5185	1010.5217	3	^{81}DPAQIPWGK^{89}	4
1774.8924	1774.8968	2	^{90}HAIDVVCESTGVFLTK^{105}	10
2470.2236	2470.2051	-7	^{120}KVI**M**SAPPKDDTPIYV**M**GINHEK^{140}2Oxi (M^{123}& M^{136})	3
2486.2185	2486.2239	2	^{119}VI**M**SAPPKDDTPIYV**M**GINHEK^{140}Oxi (M^{121})	5
1646.761	1646.7663	3	^{127}DDTPIYV**M**GINHEK^{140}Oxi (M^{134})	2
2499.1734	2499.1679	-2	^{141}YNSSQTIVSNASCTTNCLAPIAK^{163}	33
3278.6606	3278.647	-4	^{164}VIHENFGIVEGL**M**TTVHASTANQLVVDGPSK^{194}Oxi (M^{176})	63
1425.8191	1425.8205	1	^{204}SALLNIIPASTGAAK^{218}	12
1505.7854	1505.7695	-11	^{204}**S**ALLNIIPASTGAAK^{218}p(S^{204})	2
1505.7854	1505.7815	-3	^{204}**S**ALLNIIPA**S**TGAAKAVGK$^{\mathbf{222}}$p(S^{204})	2
2853.5728	2853.6265	19	^{204}SALLNIIPA**S**TGAA**K**AVG**K**VLPELNG**K**^{230}GG(K^{230}); m(K^{218}& K^{222}); p(S^{213})	1
868.5018	868.506	5	^{223}VLPELNGK^{230}	2
1612.9301	1612.9243	-4	^{223}VLPELNGKLTGVAFR^{237}	2
762.4388	762.4433	6	^{231}LTGVAFR^{237}	2
1512.8393	1512.8334	4	^{238}VPIGTVSVVDLVCR^{251}	8
3026.611	3026.598	-4	^{238}VPIGTVSVVDLV**C**RLE**K**PA**K**YEDVA**K**^{263}ac(K); GG(K); m(K); n(C^{250})	1
1070.5971	1070.6003	3	^{265}IKEASEGPLK^{274}	1
2265.055	2265.052	-1	^{275}GILGYTDEEVVSQDFVHDSR^{294}	18
808.4331	808.4346	2	^{295}SSIFDLK^{301}	18
2041.052	2041.059	3	^{295}SSIFDL**K**AGLALNDNFFK^{312}ac(K^{301})	1
1208.619	1208.6233	4	^{302}AGLALNDNFFK^{312}	13
1787.7903	1787.793	2	^{313}IVSWYDNEWGYSNR^{326}	11
1272.7554	1272.7593	3	^{327}LLDLAIHITK^{336}	30

1386.7983	1386.7867	-8	^{327}LLDLAIHITKH^{337} GG(K^{301})	1
2159.1827	2159.1961	6	^{201}AGRSALLNIIPA**S**TGAAKAVGK^{222}	1
2513.1526	2513.1845	13	^{141}YNSSQTIVSNASCTTN**C**LAPIA**K**^{163}ac(K^{163}); n(C^{157})	1
2499.1734	2499.1759	1	^{141}YNSSQTIVSNASCTTNCLAPIAK^{163}	5
2579.1135	2579.1397	-10	^{141}YNS**S**QTIVSNASCTTNCLAPIAK^{163}p(S^{144})	1

Table 1B: MW ~37 kDa species from soluble fraction (data from 2 independent samples): Protein Score: 2116; Sequence coverage: 87%.

Calculated m/z	Observed m/z	Δ (ppm)	Peptide and observed PTMs (bold)	No of hits
818.4399	818.4413	2	^{6}VGINGFGR^{13}	2
2687.388	2687.3839	-2	^{26}SDIEVVAINDPF**M**DINHLIYLLK^{48}Oxi(M^{38})	5
1807.8485	1807.8507	1	^{56}FPCEVTPTEGGIMVGSK^{72}	5
1823.8434	1823.8386	-3	^{56}FPCEVTPTEGGI**M**VGSK^{72}Oxi(M^{68})	14
1998.0687	1998.0496	-10	^{73}KVVVYNERDPAQIPWGK^{89}	1
1869.9737	1869.9661	-4	^{74}VVVYNERDPAQIPWGK^{89}	3
1010.5185	1010.5174	-1	^{81}DPAQIPWGK^{89}	8
1774.8924	1774.8899	-1	^{90}HAIDVVCESTGVFLTK^{105}	7
1590.7953	1590.8005	3	^{114}GGA**K**KVIM**S**APP**K**^{126}GG (K^{117}& K^{126}); p(S^{122})	1
2486.2106	2486.2185	-3	^{119}VI**M**SAPPKDDTPIYV**M**GINHEK^{140}Oxi (M^{121}&M^{134})	2
1646.761	1646.761	0	^{127}DDTPIYV**M**GINHEK^{140}Oxi (M^{134})	3
2499.1734	2499.1799	3	^{141}YNSSQTIVSNASCTTNCLAPIAK^{163}	7
3278.6606	3278.6497	-3	^{164}VIHENFGIVEGL**M**TTVHASTANQLVVDGPSK^{194}Oxi (M^{176})	5
1425.8191	1425.8195	0	^{204}SALLNIIPASTGAAK^{218}	9
1265.7343	1265.7257	-7	^{219}AVGKVLPELNG**K**^{230}ac(K^{230})	1
868.5018	868.5046	3	^{223}VLPELNGK^{230}	6
1512.8334	1512.8344	1	^{238}VPIGTVSVVDLVCR^{251}	8
723.3439	723.3434	-1	^{258}YEDVAK^{263}	1
1070.5971	1070.5992	2	^{265}IKEASEGPLK^{274}	1
2265.055	2265.0405	-6	^{275}GILGYTDEEVVSQDFVHDSR^{294}	12
808.4331	808.4338	1	^{295}SSIFDLK^{301}	10
2041.052	2041.0647	6	^{295}SSIFDL**K**AGLALNDNFFK^{312} ac(K^{301})	2
1208.619	1208.6176	-1	^{302}AGLALNDNFFK^{312}	12
1787.7903	1787.7898	0	^{313}IVSWYDNEWGYSNR^{326}	8
1135.6965	1135.6921	-4	^{327}LLDLAIHITK^{336}	12
762.4388	762.4388	0	^{231}LTGVAFR^{237}	4

Table 1C: MW ~27 kDa species from soluble fraction (data from 2 independent samples): Protein Score: 918; Sequence coverage: 85%.

Calculated m/z	Observed m/z	Δ (ppm)	Peptide and observed PTMs (bold)	No of hits
1998.0687	1998.0501	-9	^{73}KVVVYNER DPAQIPWGK^{89}	1

2159.1827	2159.1961	6	^{201}AGRSALLNII PA**S**TGAA**K**AVGK^{222}m(K^{218}); p(ST^{213})	1
1425.8191	1425.8055	-10	^{204}SALLNII PASTGAAK^{218}	2
868.5018	868.5068	6	^{223}VLPELNGK^{230}	3
1512.8334	1512.8279	-4	^{238}VPIGTVSVVDLVCR^{251}	5
808.4331	808.4303	-3	^{295}SSIFDLK^{301}	1
1208.619	1208.6154	-3	^{302}AGLALNDNFFK^{312}	3
1135.6965	1135.6871	-8	^{327}LLDLAIHITK^{336}	1
1386.7983	1386.7867	-8	^{327}LLDL AIHIT**K**H^{337}GG(K^{336})	1

Table 1D: MW ~37 kDa species from particulate fraction: Protein Score: 149; Sequence coverage: 26%.

Sequence coverage for soluble fraction GAPDH bands was in the range of 76-87%. Since in each case two independent samples were analyzed, in final tally the sequence coverage was 313 out of 337 residues (92.9%). Several peptide m/z matched by inclusion of certain PTMs defined as fixed and variable. For insoluble fraction, only ~37 kDa band was analyzed (Table 1(D)). The sequence coverage obtained was ~26% that largely covered the C-terminal half of the molecule. Generally high sequence coverage is obtained for the soluble proteins as compared to the membrane bound forms [17]. However, our expectation was to obtain much greater sequence coverage similar to soluble fraction (87% coverage; Table 1(B)). Membrane association of PyGAPDH is likely to be mediated through post-translational modifications involving membrane anchoring groups such as prenyl, palmitoyl or Glycosyl Phosphatidyl Inositol (GPI) etc. or those that facilitate its binding with other membrane proteins. Lack of N-terminal peptides in ~37 kDa band from particulate fraction could arise because of post-translational modifications with membrane associating hydrophobic groups. Such regions may not be cleaved by trypsin or such peptides may not have eluted from C-18 chips that we used in our chromatographic separation. Recently, the possibility of N-terminal being palmitoylated to translocate GAPDH1 to cellular cortex in *Toxoplasma gondii* has been suggested [18]. Although the soluble fraction showed extensive coverage, certain stretches of sequence did not get covered. These consisted of ^{14}IGRLVFRSAQER^{23}, ^{195}GGKDWRAGR^{203} and ^{261}VAK^{263}. Trypsin digestion of these segments will generate peptides that are too small in size and could have been missed detection. Thus, MS data presented in Table 1 provided direct evidence that all the three different molecular mass species detected by anti-rPfGAPDH antibodies indeed contained GAPDH.

Identification of Post-Translational Modifications (PTMs)

Matched m/z in MS spectra led to identification of several peptides that have undergone modifications (marked in bold in Table 1). MS/MS spectra of all these peptides were manually verified and peptides that passed our acceptance criteria were selected. The peaks in MS/MS spectra were assigned to b and y ions and wherever possible, spectra for modified and unmodified forms of the peptide were compared to locate the modified residue and the PTMs. This approach is far superior and yields more reliable results as compared to most of the algorithms that automatically identify PTMs. Modified peptides identified with confidence in various fractions are listed in Table 2 along with the residue(s) (in bold) that have undergone the modification. The PTM search in PyGAPDH was set for phosphorylation of Ser, Thr and Tyr with the residue acquiring additional mass of 80 Da (Δm = 80 Da) or a neutral loss of 98 Da (Δm = -98 Da), acetylation (Δm = 42 Da), methylation (mono Δm = 14 Da; dimethylation Δm = 28 Da and trimethylation Δm = 43 Da) and ubiquitination (Δm =114 Da) of Lys and nitrosylation (Δm = 29 Da) of Cys. Addition of 80 Da in mass also occurs on sulfation of tyrosine [19].

Sr. No.	MW (kDa)	Peptide Sequence	PTM/ Residue Modified*	MWSE Score[!]
***P. yoelii* soluble fraction:**				
1.	~51	^{141}YNSSQ**T**IVSNASCTTNCLAPIAK^{163}	pT^{146}	35
2.	~51	^{223}VLPELNG**K**LTGVAFR^{237}	acK^{230}	23
3.	~51	^{73}**K**VVVYNERDPAQIPWK^{88}	uK^{73}	23

4.	~51	^{204}SALLNIIPASTGAA**K**AVGK^{222}	uK^{218}	32
5.	~51	^{204}SALLNIIPASTGAAKAVG**K**^{222}	uK^{222}	30
6.	~51	^{223}VLPELNG**K**LTGVAFR^{237}	uK^{230}	21
7.	~51	^{327}LLDLAIHIT**K**H^{337}	uK^{336}	24
8.	~37	^{141}YNS**S**QTIVSNASCTTNCLAPIAK^{163}	pS^{144}	37
9.	~37	^{204}SALLNIIPA**S**TGAA**K**AVG**K**VLPELNG**K**^{230}	pS^{213}, mK^{218}, uK^{222}, mK^{230}	30
10	~37	^{223}VLPELNG**K**LTGVAFR2^{37}	m2K^{230}	13
11.	~37	^{204}SALLNIIPASTGAAKAVG**K**^{222}	uK^{222}	30
12.	~37	^{141}YNSSQTIVSNASCTTN**C**LAPIAK^{163}	nC^{157}	19
13.	~37	^{204}**S**ALLNIIPASTGAAKAVGK^{222}	pS^{204}	20
14.	~37	^{204}**S**ALLNIIPASTGAAK^{218}	pS^{204}	29
15.	~37	^{295}SSIFDL**K**AGLALNDNFFK^{312}	acK^{301}	14
16.	~27	^{295}SSIFDL**K**AGLALNDNFFK^{312}	acK^{301}	39
17.	~37	^{141}YNSSQTIVSNASCTTN**C**LAPIA**K**^{163}	acK^{163}	15
***P. yoelii* particulate fraction:**				
18.	~37	^{201}AGRSALLNIIPA**S**TGAA**K**AVGK^{222}	pS^{213}, mK^{218}	9
19.	~37	^{327}LLDLAIHIT**K**H^{337}	uK^{336}	16
*p, phosphorylation; ac, acetylation; m, methylation; m2, dimethylation; n, nitrosylation; u, ubiquitination. [!]Modified residues that were detected in multiple samples, have been listed even if the score was low.				

Table 2: Post-translational modifications in PyGAPDH. List of validated PTMs with peptide sequence. Residues modified are marked in bold.

For making distinction between tyrosine phosphorylation or sulfation, more extensive experiments will be needed [20]. Here, we assumed phosphorylation as the modifying group. Figure 3 shows a few representative MS/MS spectra of the peptides in their native and modified forms.

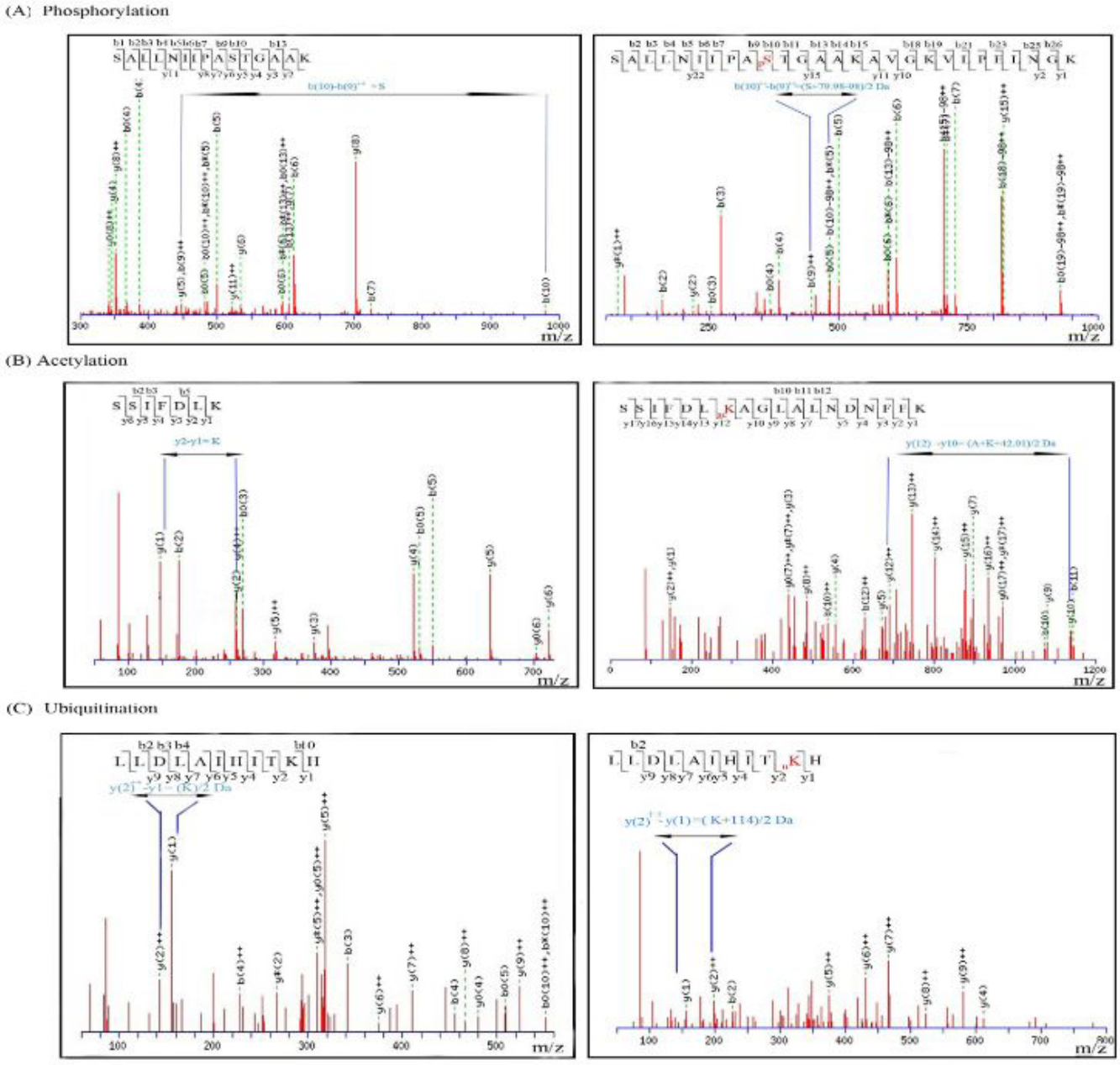


Figure 3: Some representative MS/MS spectra of peptides in their modified and unmodified forms. Parent ion m/z and Retention Times (RT) are stated. (A) phosphorylation- unmodified (parent ion m/z = 476.2814^{3+}; RT=22.6 minutes) and modified (parent ion m/z = 714.4139^{4+}; RT=22.71 minutes); (B) acetylation- unmodified (parent ion m/z = 405.2241^{2+}; RT=12.06 minutes) and modified (parent ion m/z = 681.3600^{3+}; RT=25.00 minutes) and (C) ubiquitination- unmodified (parent ion m/z = 319.1928^{4+}; RT=13.70 minutes) and modified (parent ion m/z = 347.7039^{4+}; RT=13.31 minutes). Insets (blue) mark the peaks that account for unmodified and modified residue masses.

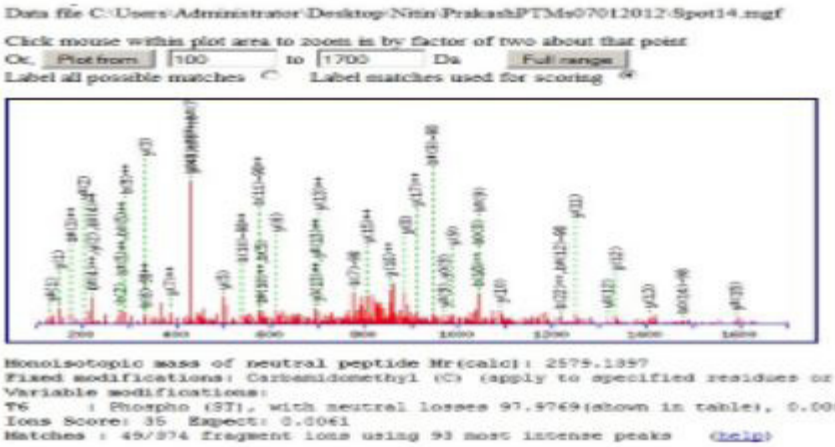
In all twelve residues were identified that underwent modifications. Different modifications included four phosphorylations (pS^{144}, pS^{204}, pS^{213} and pT^{146}), two methylations (mK^{218} and mK^{230}) and a dimethylation (m2K^{230}), three acetylations (acK^{163}, acK^{230} and acK^{301}), one nitrosylation (nC^{157}) and five ubiquitinations (uK^{73}, uK^{218}, uK^{222}, uK^{230}and uK^{336}) (Table 2). Some PTMs were detected in more than one band (Table 3).

Sr. No.	Residue	PTM*	MW of the species (kDa)	Fraction
1	K^{73}	u	~51	Soluble
2	S^{144}	p	~37	Soluble
3	T^{146}	p	~51	Soluble
4	C^{157}	n	~37	Soluble
5	K^{163}	ac	~37	Soluble
6	S^{204}	p	~37	Soluble
7	S^{213}	p	~37	Particulate
				Soluble
8	K^{218}	u	~51	Soluble
		m	~37	Soluble
				Particulate
9	K^{222}	u	~37	Soluble
			~51	Soluble
10	K^{230}	m	~37	Soluble
		m2	~37	Soluble
		ac	~51	Soluble
		u	~51	Soluble
11	K^{301}	ac	~27	Soluble
			~37	Soluble
12	K^{336}	u	~51	Soluble
			~37	Particulate

*p, phosphorylation; ac, acetylation; m, methylation; m2, dimethylation; n, nitrosylation; u, ubiquitination.
[!]Modified residues that were detected in multiple samples, have been listed even if the score was low.

Table 3: List of residues in PyGAPDH that undergo post-translational modifications (PTMs). Some residues showed multiple modifications. Data are from Table 2.

Figure S1 has all the data and corresponding MS/MS spectra for the peptides listed in Table 2. Examination of missed cleavage pattern among modified lysine residues indicated that trypsin could cut at C-terminal end of mono-methylated lysine (e.g. mK^{230}) but failed to cleave dimethylated residues. Trypsin cleavage at ubiquitinated lysines was also observed (e.g. SALLNIIPASTGAAKAVGu**K^{222}**). A non-tryptic peptide (-LLDLAIHIT**K**H-) that showed ubiquitination at K^{336} was present in the particulate fraction. This peptide is a product of the C-terminal end of the protein.



#	b	b^{++}	b^{*}	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y^{*}	y^{*++}	y^{0}	y^{0++}	#
1	164.0706	82.5389					Y							23
2	278.1135	139.5604	261.0870	131.0471			N	2319.1068	1160.0570	2302.0802	1151.5437	2301.0962	1151.0517	22
3	365.1456	183.0764	348.1190	174.5631	347.1350	174.0711	S	2205.0638	1103.0356	2188.0373	1094.5223	2187.0533	1094.0303	21
4	452.1776	226.5924	435.1510	218.0792	434.1670	217.5871	S	2118.0318	1059.5195	2101.0053	1051.0063	2100.0212	1050.5143	20
5	580.2362	**290.6217**	563.2096	282.1084	562.2256	281.6164	Q	2030.9998	1016.0035	2013.9732	1007.4903	2012.9892	1006.9982	19
6	663.2733	**332.1403**	646.2467	323.6270	645.2627	323.1350	T	1902.9412	951.9742	1885.9146	943.4610	1884.9306	942.9690	18
7	776.3573	388.6823	759.3308	380.1690	758.3468	379.6770	I	1819.9041	910.4557	1802.8775	901.9424	1801.8935	901.4504	17
8	875.4258	438.2165	858.3992	429.7032	857.4152	429.2112	V	1706.8200	853.9136	1689.7935	845.4004	1688.8095	844.9084	16
9	962.4578	481.7325	945.4312	473.2193	944.4472	472.7272	S	1607.7516	804.3794	1590.7251	795.8662	1589.7410	795.3742	15
10	1076.5007	**538.7540**	1059.4742	530.2407	1058.4901	529.7487	N	1520.7196	760.8634	1503.6930	752.3502	1502.7090	751.8581	14
11	1147.5378	**574.2726**	1130.5113	565.7593	1129.5273	565.2673	A	*1406.6766*	703.8420	1389.6501	695.3287	1388.6661	694.8367	13
12	1234.5699	617.7886	1217.5433	609.2753	1216.5593	608.7833	S	*1335.6395*	668.3234	1318.6130	659.8101	1317.6290	659.3181	12
13	1394.6005	697.8039	1377.5740	689.2906	1376.5899	688.7986	C	*1248.6075*	624.8074	1231.5810	616.2941	1230.5969	615.8021	11
14	1495.6482	748.3277	1478.6216	739.8145	1477.6376	739.3224	T	*1088.5769*	544.7921	1071.5503	536.2788	1070.5663	535.7868	10
15	1596.6959	798.8516	1579.6693	790.3383	1578.6853	789.8463	T	*987.5292*	494.2682	970.5026	485.7550	969.5186	485.2629	9
16	1710.7388	855.8730	1693.7122	847.3598	1692.7282	846.8678	N	*886.4815*	443.7444	869.4550	435.2311			8
17	1870.7694	935.8884	1853.7429	927.3751	1852.7589	926.8831	C	772.4386	386.7229	755.4120	378.2097			7
18	1983.8535	992.4304	1966.8270	983.9171	1965.8429	983.4251	L	*612.4079*	306.7076	595.3814	298.1943			6
19	2054.8906	1027.9489	2037.8641	1019.4357	2036.8801	1018.9437	A	*499.3239*	250.1656	482.2973	241.6523			5
20	2151.9434	1076.4753	2134.9168	1067.9621	2133.9328	1067.4700	P	*428.2867*	214.6470	411.2602	206.1337			4
21	2265.0274	1133.0174	2248.0009	1124.5041	2247.0169	1124.0121	I	*331.2340*	166.1206	314.2074	157.6074			3
22	2336.0646	1168.5359	2319.0380	1160.0226	2318.0540	1159.5306	A	*218.1499*	109.5786	201.1234	101.0653			2
23							K	*147.1128*	74.0600	130.0863	65.5468			1

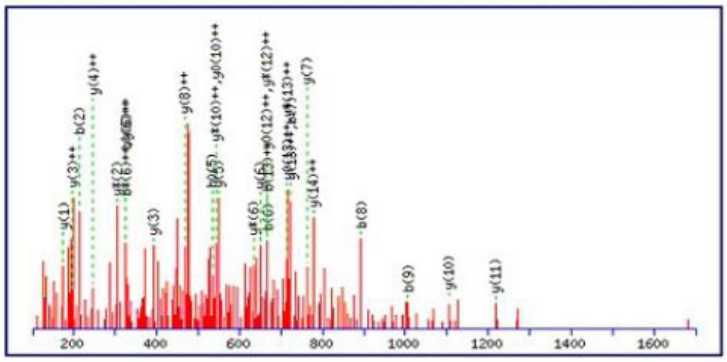
Peptide 1

Data file C:\Users\Administrator\Desktop\Nitin\PrakashPTMs07012012\Spot14.mgf

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

Or, Plot from 100 to 1700 Da Full range

Label all possible matches ○ Label matches used for scoring ◉



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1	100.0757	50.5415					V							15
2	213.1598	107.0835					L	1556.8795	778.9434	1539.8530	770.4301	1538.8689	769.9381	14
3	310.2125	155.6099					P	1443.7954	722.4014	1426.7689	713.8881	1425.7849	713.3961	13
4	439.2551	220.1312			421.2445	211.1259	E	1346.7427	673.8750	1329.7161	665.3617	1328.7321	664.8697	12
5	552.3392	276.6732			534.3286	267.6679	L	*1217.7001*	609.3537	1200.6735	600.8404	1199.6895	600.3484	11
6	666.3821	333.6947	649.3556	325.1814	648.3715	324.6894	N	*1104.6160*	552.8116	1087.5895	544.2984	1086.6055	543.8064	10
7	723.4036	362.2054	706.3770	353.6921	705.3930	353.2001	G	990.5731	495.7902	973.5465	487.2769	972.5625	486.7849	9
8	893.5091	447.2582	876.4825	438.7449	875.4985	438.2529	K	933.5516	467.2795	916.5251	458.7662	915.5411	458.2742	8
9	1006.5932	503.8002	989.5666	495.2869	988.5826	494.7949	L	*763.4461*	382.2267	746.4196	373.7134	745.4355	373.2214	7
10	1107.6408	554.3241	1090.6143	545.8108	1089.6303	545.3188	T	*650.3620*	325.6847	633.3355	317.1714	632.3515	316.6794	6
11	1164.6623	582.8348	1147.6358	574.3215	1146.6517	573.8295	G	*549.3144*	275.1608	532.2878	266.6475			5
12	1263.7307	632.3690	1246.7042	623.8557	1245.7202	623.3637	V	492.2929	246.6501	475.2663	238.1368			4
13	1334.7678	667.8876	1317.7413	659.3743	1316.7573	658.8823	A	*393.2245*	197.1159	376.1979	188.6026			3
14	1481.8362	741.4218	1464.8097	732.9085	1463.8257	732.4165	F	322.1874	161.5973	305.1608	153.0840			2
15							R	*175.1190*	88.0631	158.0924	79.5498			1

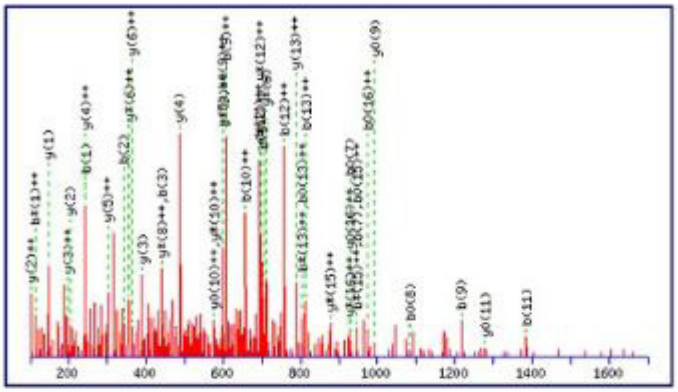
Peptide 2

Data file C:\Users\Administrator\Desktop\Nitin\PrakashPTMs07012012\Spot14.mgf

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

Or, Plot from 100 to 1700 Da Full range

Label all possible matches ○ Label matches used for scoring ◉



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1	*243.1452*	122.0762	226.1186	113.5629			K							17
2	*342.2136*	171.6104	325.1870	163.0972			V	1870.9810	935.9941	1853.9545	927.4809	1852.9704	926.9889	16
3	*441.2820*	221.1446	424.2554	212.6314			V	1771.9126	886.4599	1754.8860	877.9467	1753.9020	877.4547	15
4	540.3504	270.6788	523.3239	262.1656			V	1672.8442	836.9257	1655.8176	828.4125	1654.8336	827.9204	14
5	*703.4137*	352.2105	686.3872	343.6972			Y	1573.7758	787.3915	1556.7492	778.8782	1555.7652	778.3862	13
6	817.4567	409.2320	800.4301	400.7187			N	1410.7124	705.8599	1393.6859	697.3466	1392.7019	696.8546	12
7	*946.4993*	473.7533	929.4727	465.2400	928.4887	464.7480	E	1296.6695	648.8384	1279.6430	640.3251	1278.6589	639.8331	11
8	1102.6004	551.8038	1085.5738	543.2905	1084.5898	542.7985	R	1167.6269	584.3171	1150.6004	575.8038	1149.6164	575.3118	10
9	*1217.6273*	609.3173	1200.6008	600.8040	1199.6167	600.3120	D	1011.5258	506.2665	994.4993	497.7533	993.5152	497.2613	9
10	1314.6801	657.8437	1297.6535	649.3304	1296.6695	648.8384	P	896.4989	448.7531	879.4723	440.2398			8
11	*1385.7172*	693.3622	1368.6906	684.8490	1367.7066	684.3570	A	799.4461	400.2267	782.4196	391.7134			7
12	1513.7758	757.3915	1496.7492	748.8782	1495.7652	748.3862	Q	728.4090	364.7081	711.3824	356.1949			6
13	1626.8598	813.9336	1609.8333	805.4203	1608.8493	804.9283	I	600.3504	300.6788	583.3239	292.1656			5
14	1723.9126	862.4599	1706.8860	853.9467	1705.9020	853.4547	P	487.2663	244.1368	470.2398	235.6235			4
15	1909.9919	955.4996	1892.9654	946.9863	1891.9813	946.4943	W	390.2136	195.6104	373.1870	187.0972			3
16	1967.0134	984.0103	1949.9868	975.4970	1949.0028	975.0050	G	204.1343	102.5708	187.1077	94.0575			2
17							K	147.1128	74.0600	130.0863	65.5468			1

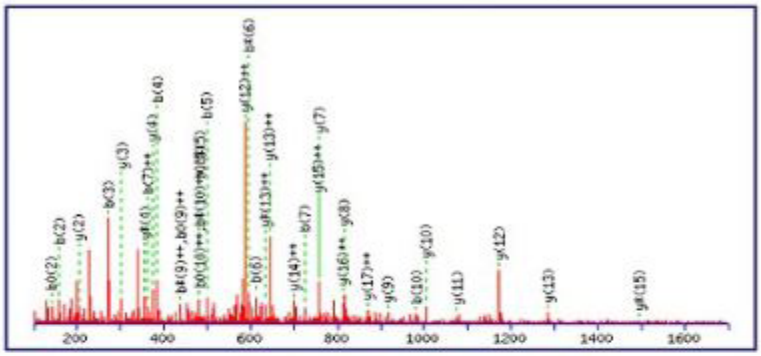
Peptide 3

Data file C:\Users\Administrator\Desktop\Nitin\PrakashPTMs07012012\Spot14.mgf

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

Or, Plot from 100 to 1700 Da Full range

Label all possible matches ○ Label matches used for scoring ◉



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Variable modifications:
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Ions Score: 32 Expect: 0.012
Matches : 35/192 fragment ions using 112 most intense peaks (help)

#	b	b^{++}	b^*	b^{*++}	b^0	b^{0++}	Seq.	y	y^{++}	y^*	y^{*++}	y^0	y^{0++}	#
1	88.0393	44.5233			70.0287	35.5180	S							19
2	*159.0764*	80.0418			141.0659	71.0366	A	1809.0593	905.0333	1792.0327	896.5200	1791.0487	896.0280	18
3	*272.1605*	136.5839			254.1499	127.5786	L	1738.0221	869.5147	1720.9956	861.0014	1720.0116	860.5094	17
4	*385.2445*	193.1259			367.2340	184.1206	L	1624.9381	812.9727	1607.9115	804.4594	1606.9275	803.9674	16
5	*499.2875*	250.1474	482.2609	241.6341	481.2769	241.1421	N	1511.8540	756.4306	1494.8275	747.9174	1493.8435	747.4254	15
6	*612.3715*	306.6894	595.3450	298.1761	594.3610	297.6841	I	1397.8111	699.4092	1380.7845	690.8959	1379.8005	690.4039	14
7	*725.4556*	363.2314	708.4291	354.7182	707.4450	354.2262	I	*1284.7270*	642.8672	1267.7005	634.3539	1266.7165	633.8619	13
8	822.5084	411.7578	805.4818	403.2445	804.4978	402.7525	P	*1171.6430*	586.3251	1154.6164	577.8118	1153.6324	577.3198	12
9	893.5455	447.2764	876.5189	438.7631	875.5349	438.2711	A	*1074.5902*	537.7987	1057.5636	529.2855	1056.5796	528.7935	11
10	*980.5775*	490.7924	963.5510	482.2791	962.5669	481.7871	S	*1003.5531*	502.2802	986.5265	493.7669	985.5425	493.2749	10
11	1081.6252	541.3162	1064.5986	532.8030	1063.6146	532.3109	T	*916.5211*	458.7642	899.4945	450.2509	898.5105	449.7589	9
12	1138.6466	569.8270	1121.6201	561.3137	1120.6361	560.8217	G	*815.4734*	408.2403	798.4468	399.7271			8
13	1209.6838	605.3455	1192.6572	596.8322	1191.6732	596.3402	A	*758.4519*	379.7296	741.4254	371.2163			7
14	1280.7209	640.8641	1263.6943	632.3508	1262.7103	631.8588	A	687.4148	344.2110	670.3883	335.6978			6
15	1522.8588	761.9330	1505.8322	753.4197	1504.8482	752.9277	K	616.3777	308.6925	599.3511	300.1792			5
16	1593.8959	797.4516	1576.8693	788.9383	1575.8853	788.4463	A	*374.2398*	187.6235	357.2132	179.1103			4
17	1692.9643	846.9858	1675.9377	838.4725	1674.9537	837.9805	V	*303.2027*	152.1050	286.1761	143.5917			3
18	1749.9858	875.4965	1732.9592	866.9832	1731.9752	866.4912	G	*204.1343*	102.5708	187.1077	94.0575			2
19							K	147.1128	74.0600	130.0863	65.5468			1

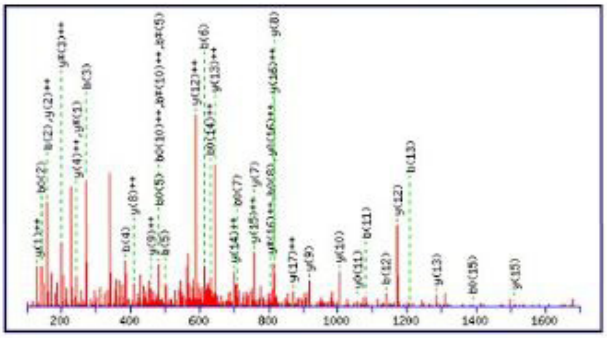
Peptide 4

Data file C:\Users\Administrator\Desktop\Nitin\PrakashPTMs07012012\Spot14.mgf

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

Or, Plot from 100 to 1700 Da Full range

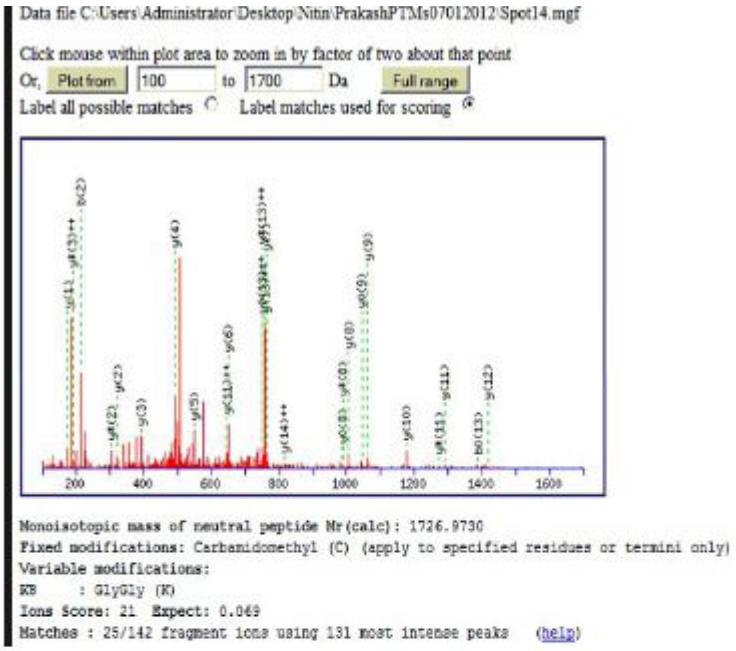
Label all possible matches ○ Label matches used for scoring ●



Monoisotopic mass of neutral peptide Mr(calc): 1895.0840
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Variable modifications:
K19 : GlyGly (K)
Ions Score: 30 Expect: 0.022
Matches : 40/192 fragment ions using 124 most intense peaks (help)

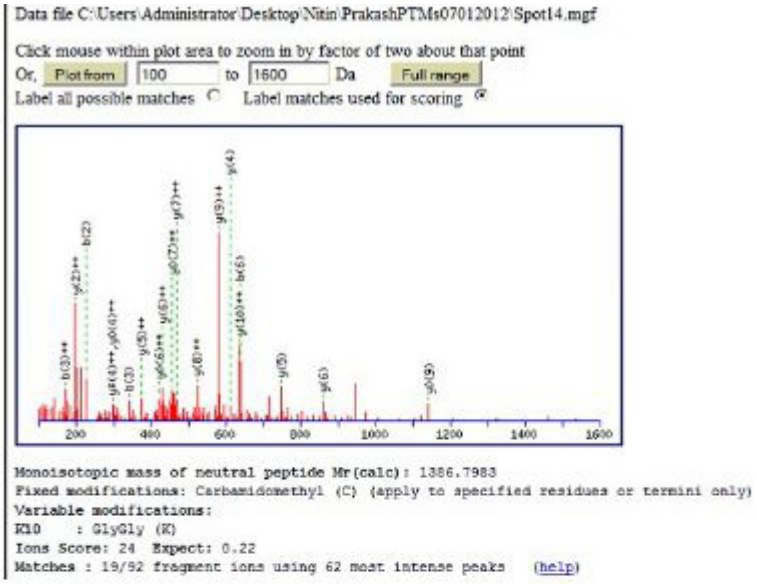
#	b	b^{++}	b^*	b^{*++}	b^0	b^{0++}	Seq.	y	y^{++}	y^*	y^{*++}	y^0	y^{0++}	#
1	88.0393	44.5233			70.0287	35.5180	S							19
2	159.0764	80.0418			141.0659	71.0366	A	1809.0593	905.0333	1792.0327	896.5200	1791.0487	896.0280	18
3	272.1605	136.5839			254.1499	127.5786	L	1738.0221	869.5147	1720.9956	861.0014	1720.0116	860.5094	17
4	385.2445	193.1259			367.2340	184.1206	L	1624.9381	812.9727	1607.9115	804.4594	1606.9275	803.9674	16
5	499.2875	250.1474	482.2609	241.6341	481.2769	241.1421	N	1511.8540	756.4306	1494.8275	747.9174	1493.8435	747.4254	15
6	612.3715	306.6894	595.3450	298.1761	594.3610	297.6841	I	1397.8111	699.4092	1380.7845	690.8959	1379.8005	690.4039	14
7	725.4556	363.2314	708.4291	354.7182	707.4450	354.2262	I	1284.7270	642.8672	1267.7005	634.3539	1266.7165	633.8619	13
8	822.5084	411.7578	805.4818	403.2445	804.4978	402.7525	P	1171.6430	586.3251	1154.6164	577.8118	1153.6324	577.3198	12
9	893.5455	447.2764	876.5189	438.7631	875.5349	438.2711	A	1074.5902	537.7987	1057.5636	529.2855	1056.5796	528.7935	11
10	980.5775	490.7924	963.5510	482.2791	962.5669	481.7871	S	1003.5531	502.2802	986.5265	493.7669	985.5425	493.2749	10
11	1081.6252	541.3162	1064.5986	532.8030	1063.6146	532.3109	T	916.5211	458.7642	899.4945	450.2509	898.5105	449.7589	9
12	1138.6466	569.8270	1121.6201	561.3137	1120.6361	560.8217	G	815.4734	408.2403	798.4468	399.7271			8
13	1209.6838	605.3455	1192.6572	596.8322	1191.6732	596.3402	A	758.4519	379.7296	741.4254	371.2163			7
14	1280.7209	640.8641	1263.6943	632.3508	1262.7103	631.8588	A	687.4148	344.2110	670.3883	335.6978			6
15	1408.8158	704.9116	1391.7893	696.3983	1390.8053	695.9063	K	616.3777	308.6925	599.3511	300.1792			5
16	1479.8530	740.4301	1462.8264	731.9168	1461.8424	731.4248	A	488.2827	244.6450	471.2562	236.1317			4
17	1578.9214	789.9643	1561.8948	781.4510	1560.9108	780.9590	V	417.2456	209.1264	400.2191	200.6132			3
18	1635.9428	818.4751	1618.9163	809.9618	1617.9323	809.4698	G	318.1772	159.5922	301.1506	151.0790			2
19							K	261.1557	131.0815	244.1292	122.5682			1

Peptide 5



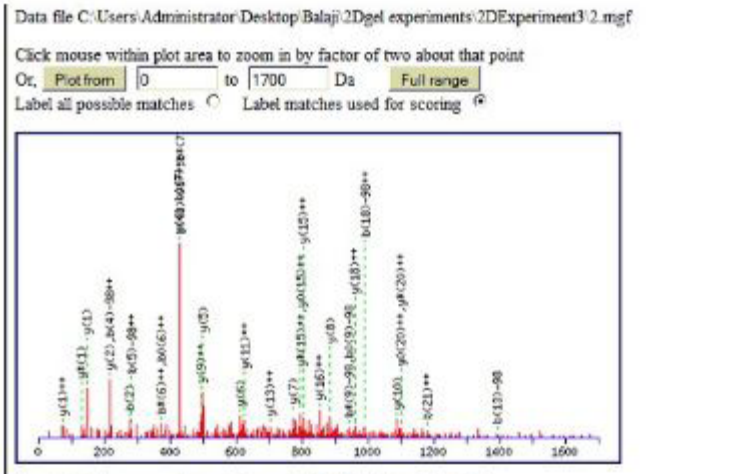
#	b	b^{++}	b^{*}	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y^{*}	y^{*++}	y^{0}	y^{0++}	#
1	100.0757	50.5415					V							15
2	213.1598	107.0835					L	1628.9119	814.9596	1611.8853	806.4463	1610.9013	805.9543	14
3	310.2125	155.6099					P	1515.8278	758.4175	1498.8013	749.9043	1497.8172	749.4123	13
4	439.2551	220.1312			421.2445	211.1259	E	*1418.7750*	709.8912	1401.7485	701.3779	1400.7645	700.8859	12
5	552.3392	276.6732			534.3286	267.6679	L	*1289.7324*	645.3699	1272.7059	636.8566	1271.7219	636.3646	11
6	666.3821	333.6947	649.3556	325.1814	648.3715	324.6894	N	*1176.6484*	588.8278	1159.6218	580.3146	1158.6378	579.8225	10
7	723.4036	362.2054	706.3770	353.6921	705.3930	353.2001	G	*1062.6055*	531.8064	1045.5789	523.2931	1044.5949	522.8011	9
8	965.5415	483.2744	948.5149	474.7611	947.5309	474.2691	K	*1005.5840*	503.2956	988.5574	494.7824	987.5734	494.2904	8
9	1078.6255	539.8164	1061.5990	531.3031	1060.6150	530.8111	L	*763.4461*	382.2267	746.4196	373.7134	745.4355	373.2214	7
10	1179.6732	590.3402	1162.6467	581.8270	1161.6626	581.3350	T	*650.3620*	325.6847	633.3355	317.1714	632.3515	316.6794	6
11	1236.6947	618.8510	1219.6681	610.3377	1218.6841	609.8457	G	*549.3144*	275.1608	532.2878	266.6475			5
12	1335.7631	668.3852	1318.7365	659.8719	1317.7525	659.3799	V	*492.2929*	246.6501	475.2663	238.1368			4
13	1406.8002	703.9037	1389.7736	695.3905	1388.7896	694.8985	A	*393.2245*	197.1159	376.1979	188.6026			3
14	1553.8686	777.4379	1536.8421	768.9247	1535.8580	768.4327	F	*322.1874*	161.5973	305.1608	153.0840			2
15							R	*175.1190*	88.0631	158.0924	79.5498			1

Peptide 6



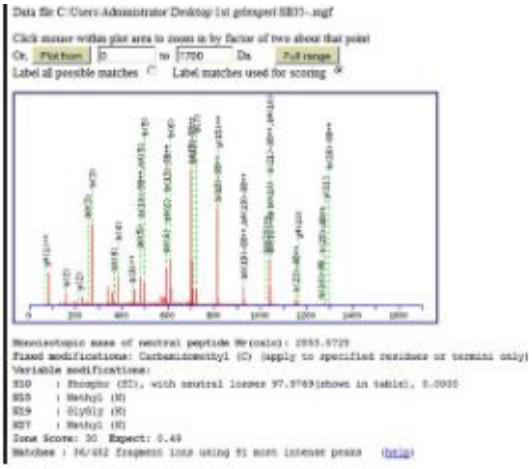
#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	114.0913	57.5493					L							11
2	227.1754	114.0913					L	1274.7215	637.8644	1257.6950	629.3511	1256.7110	628.8591	10
3	342.2023	171.6048			324.1918	162.5995	D	1161.6375	581.3224	1144.6109	572.8091	1143.6269	572.3171	9
4	455.2864	228.1468			437.2758	219.1416	L	1046.6105	523.8089	1029.5840	515.2956	1028.6000	514.8036	8
5	526.3235	263.6654			508.3130	254.6601	A	933.5265	467.2669	916.4999	458.7536	915.5159	458.2616	7
6	639.4076	320.2074			621.3970	311.2022	I	862.4894	431.7483	845.4628	423.2350	844.4788	422.7430	6
7	776.4665	388.7369			758.4559	379.7316	H	749.4053	375.2063	732.3787	366.6930	731.3947	366.2010	5
8	889.5506	445.2789			871.5400	436.2736	I	612.3464	306.6768	595.3198	298.1636	594.3358	297.6715	4
9	990.5982	495.8028			972.5877	486.7975	T	499.2623	250.1348	482.2358	241.6215	481.2518	241.1295	3
10	1232.7361	616.8717	1215.7096	608.3584	1214.7256	607.8664	K	398.2146	199.6110	381.1881	191.0977			2
11							H	156.0768	78.5420					1

Peptide 7



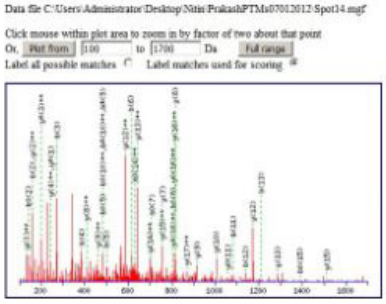
#	b	b^{++}	b^*	b^{*++}	b^0	b^{0++}	Seq.	y	y^{++}	y^*	y^{*++}	y^0	y^{0++}	#
1	164.0706	82.5389					Y							23
2	278.1135	139.5604	261.0870	131.0471			N	2417.0837	1209.0455	2400.0571	1200.5322	2399.0731	1200.0402	22
3	365.1456	183.0764	348.1190	174.5631	347.1350	174.0711	S	2303.0407	1152.0240	2286.0142	1143.5107	2285.0302	1143.0187	21
4	552.1439	276.5758	535.1174	268.0623	534.1334	267.5703	S	2216.0087	1108.5080	2198.9821	1099.9947	2197.9981	1099.5027	20
5	680.2025	340.6049	663.1759	332.0916	662.1919	331.5996	Q	2029.0103	1015.0088	2011.9838	1006.4955	2010.9998	1006.0035	19
6	781.2502	391.1287	764.2236	382.6155	763.2396	382.1234	T	1900.9518	950.9795	1883.9252	942.4662	1882.9412	941.9742	18
7	874.3342	437.6708	857.3077	429.1575	856.3237	428.6655	I	1819.9041	910.4557	1802.8775	901.9424	1801.8935	901.4504	17
8	973.4027	487.2050	956.3761	478.6917	955.3921	478.1997	Y	1706.8200	853.9136	1689.7935	845.4004	1688.8095	844.9084	16
9	1060.4347	530.7210	1043.4081	522.2077	1042.4241	521.7157	S	1607.7516	804.3794	1590.7251	795.8662	1589.7410	795.3742	15
10	1174.4776	587.7424	1157.4511	579.2292	1156.4670	578.7372	N	1520.7196	760.8634	1503.6930	752.3502	1502.7090	751.8581	14
11	1245.5147	623.2610	1228.4882	614.7477	1227.5042	614.2557	A	1406.6766	703.8420	1389.6501	695.3287	1388.6661	694.8367	13
12	1332.5468	666.7770	1315.5202	658.2637	1314.5362	657.7717	S	1335.6395	668.3234	1318.6130	659.8101	1317.6290	659.3181	12
13	1492.5774	746.7923	1475.5509	738.2791	1474.5668	737.7871	C	1248.6075	624.8074	1231.5810	616.2941	1230.5969	615.8021	11
14	1593.6251	797.3162	1576.5985	788.8029	1575.6145	788.3109	T	1088.5769	544.7921	1071.5503	536.2788	1070.5663	535.7868	10
15	1694.6728	847.8400	1677.6462	839.3267	1676.6622	838.8347	T	987.5292	494.2682	970.5026	485.7550	969.5186	485.2629	9
16	1808.7157	904.8615	1791.6891	896.3482	1790.7051	895.8562	N	886.4815	443.7444	869.4550	435.2311			8
17	1968.7463	984.8768	1951.7198	976.3635	1950.7358	975.8715	C	772.4386	386.7229	755.4120	378.2097			7
18	2081.8304	1041.4188	2064.8038	1032.9056	2063.8198	1032.4136	L	612.4079	306.7076	595.3814	298.1943			6
19	2152.8675	1076.9374	2135.8410	1068.4241	2134.8569	1067.9321	A	499.3239	250.1656	482.2973	241.6523			5
20	2249.9203	1125.4638	2232.8937	1116.9505	2231.9097	1116.4585	P	428.2867	214.6470	411.2602	206.1337			4
21	2363.0043	1182.0058	2345.9778	1173.4925	2344.9938	1173.0005	T	331.2340	166.1206	314.2074	157.6074			3
22	2434.0415	1217.5244	2417.0149	1209.0111	2416.0309	1208.5191	A	218.1499	109.5786	201.1234	101.0653			2
23							K	147.1128	74.0600	130.0863	65.5468			1

Peptide 8



#	b	b^{++}	b^*	b^{*++}	b^0	b^{0++}	Seq.	y	y^{++}	y^*	y^{*++}	y^0	y^{0++}	#
1	88.0393	44.5233			70.0287	35.5180	S							27
2	*159.0764*	80.0418			141.0659	71.0366	A	2669.5712	1335.2893	2652.5447	1326.7760	2651.5607	1326.2840	26
3	*272.1605*	136.5839			254.1499	127.5786	L	2598.5341	1299.7707	2581.5076	1291.2574	2580.5236	1290.7654	25
4	*385.2445*	193.1259			367.2340	184.1206	L	2485.4501	1243.2287	2468.4235	1234.7154	2467.4395	1234.2234	24
5	*499.2875*	250.1474	482.2609	241.6341	481.2769	241.1421	N	2372.3660	1186.6866	2355.3395	1178.1734	2354.3554	1177.6814	23
6	*612.3715*	306.6894	595.3450	298.1761	594.3610	297.6841	I	2258.3231	1129.6652	2241.2965	1121.1519	2240.3125	1120.6599	22
7	*725.4556*	363.2314	708.4291	354.7182	707.4450	354.2262	I	2145.2390	1073.1231	2128.2125	1064.6099	2127.2284	1064.1179	21
8	822.5084	411.7578	805.4818	403.2445	804.4978	402.7525	P	2032.1550	1016.5811	2015.1284	1008.0678	2014.1444	1007.5758	20
9	893.5455	*447.2764*	876.5189	438.7631	875.5349	438.2711	A	1935.1022	968.0547	1918.0756	959.5415	1917.0916	959.0494	19
10	962.5669	*481.7871*	945.5404	473.2738	944.5564	472.7818	S	1864.0651	932.5362	1847.0385	924.0229	1846.0545	923.5309	18
11	1063.6146	532.3109	1046.5881	523.7977	1045.6041	523.3057	T	1795.0436	898.0254	1778.0171	889.5122	1777.0330	889.0202	17
12	1120.6361	560.8217	1103.6095	552.3084	1102.6255	551.8164	G	1693.9959	847.5016	1676.9694	838.9883	1675.9854	838.4963	16
13	1191.6732	*596.3402*	1174.6467	587.8270	1173.6626	587.3350	A	1636.9745	818.9909	1619.9479	810.4776	1618.9639	809.9856	15
14	*1262.7103*	631.8588	1245.6838	623.3455	1244.6997	622.8535	A	1565.9374	783.4723	1548.9108	774.9590	1547.9268	774.4670	14
15	1404.8209	*702.9141*	1387.7944	694.4008	1386.8104	693.9088	K	1494.9002	747.9538	1477.8737	739.4405	1476.8897	738.9485	13
16	1475.8580	738.4327	1458.8315	729.9194	1457.8475	729.4274	A	1352.7896	676.8985	1335.7631	668.3852	1334.7791	667.8932	12
17	1574.9265	787.9669	1557.8999	779.4536	1556.9159	778.9616	V	1281.7525	641.3799	1264.7260	632.8666	1263.7419	632.3746	11
18	1631.9479	*816.4776*	1614.9214	807.9643	1613.9374	807.4723	G	1182.6841	591.8457	1165.6575	583.3324	1164.6735	582.8404	10
19	1874.0858	937.5466	1857.0593	929.0333	1856.0752	928.5413	K	1125.6626	563.3350	1108.6361	554.8217	1107.6521	554.3297	9
20	1973.1542	987.0807	1956.1277	978.5675	1955.1437	978.0755	V	883.5247	442.2660	866.4982	433.7527	865.5142	433.2607	8
21	2086.2383	*1043.6228*	2069.2117	1035.1095	2068.2277	1034.6175	L	784.4563	392.7318	767.4298	384.2185	766.4458	383.7265	7
22	2183.2910	1092.1492	2166.2645	1083.6359	2165.2805	1083.1439	P	671.3723	336.1898	654.3457	327.6765	653.3617	327.1845	6
23	2312.3336	*1156.6705*	2295.3071	1148.1572	2294.3231	1147.6652	E	574.3195	287.6634	557.2930	279.1501	556.3089	278.6581	5
24	2425.4177	1213.2125	2408.3912	1204.6992	2407.4071	1204.2072	L	445.2769	223.1421	428.2504	214.6288			4
25	2539.4606	*1270.2340*	2522.4341	1261.7207	2521.4501	1261.2287	N	332.1928	166.6001	315.1663	158.0868			3
26	2596.4821	*1298.7447*	2579.4555	1290.2314	2578.4715	1289.7394	G	218.1489	109.5786	201.1234	101.0653			2
27							K	161.1285	81.0679	144.1019	72.5546			1

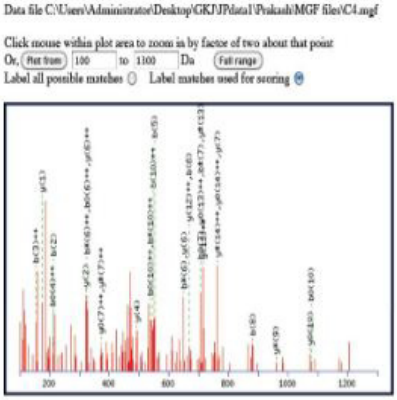
Peptide 9



Monoisotopic mass of neutral peptide Mr(calc): 1895.0840
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Variable modifications:
K19 : GlyGly (K)
Ions Score: 30 Expect: 0.022
Matches : 40/192 fragment ions using 124 most intense peaks (help)

#	b	b^{++}	b^*	b^{*++}	b^0	b^{0++}	Seq.	y	y^{++}	y^*	y^{*++}	y^0	y^{0++}	#
1	88.0393	44.5233			70.0287	35.5180	S							19
2	159.0764	80.0418			141.0659	71.0366	A	1809.0593	905.0333	1792.0327	896.5200	1791.0487	896.0280	18
3	272.1605	136.5839			254.1499	127.5786	L	1738.0221	869.5147	1720.9956	861.0014	1720.0116	860.5094	17
4	385.2445	193.1259			367.2340	184.1206	L	1624.9581	812.9727	1607.9315	804.4594	1606.9275	803.9674	16
5	499.2875	250.1474	482.2609	241.6341	481.2769	241.1421	N	1511.8540	756.4306	1494.8275	747.9174	1493.8435	747.4254	15
6	612.3715	306.6894	595.3450	298.1761	594.3610	297.6841	I	1397.8111	699.4092	1380.7845	690.8959	1379.8005	690.4039	14
7	725.4556	363.2314	708.4291	354.7182	707.4450	354.2262	I	1284.7270	642.8672	1267.7005	634.3539	1266.7165	633.8619	13
8	822.5084	411.7578	805.4818	403.2445	804.4978	402.7525	P	1171.6430	586.3251	1154.6164	577.8118	1153.6324	577.3198	12
9	893.5455	447.2764	876.5189	438.7631	875.5349	438.2711	A	1074.5902	537.7987	1057.5636	529.2855	1056.5796	528.7935	11
10	980.5775	490.7924	963.5510	482.2791	962.5669	481.7871	S	1003.5531	502.2802	986.5265	493.7669	985.5425	493.2749	10
11	1081.6252	541.3162	1064.5986	532.8030	1063.6146	532.3109	T	916.5211	458.7642	899.4945	450.2509	898.5105	449.7589	9
12	1138.6466	569.8270	1121.6201	561.3137	1120.6361	560.8217	G	815.4734	408.2403	798.4468	399.7271			8
13	1209.6838	605.3455	1192.6572	596.8322	1191.6732	596.3402	A	758.4519	379.7296	741.4254	371.2163			7
14	1280.7209	640.8641	1263.6943	632.3508	1262.7103	631.8588	A	687.4148	344.2110	670.3883	335.6978			6
15	1408.8158	704.9116	1391.7893	696.3983	1390.8053	695.9063	K	616.3777	308.6925	599.3511	300.1792			5
16	1479.8530	740.4301	1462.8264	731.9168	1461.8424	731.4248	A	488.2827	244.6450	471.2562	236.1317			4
17	1578.9214	789.9643	1561.8948	781.4510	1560.9108	780.9590	V	417.2456	209.1264	400.2191	200.6132			3
18	1635.9428	818.4751	1618.9163	809.9618	1617.9323	809.4698	G	318.1772	159.5922	301.1506	151.0790			2
19							K	261.1557	131.0815	244.1292	122.5682			1

Peptide 10

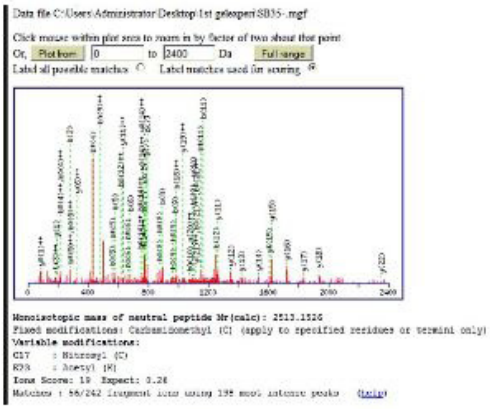


Monoisotopic mass of neutral peptide Mr(calc): 1641.9454
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Variable modifications:
N6 : Deamidated (NQ)
K8 : Dimethyl (K)
Ions Score: 13 Expect: 2.3
Matches : 31/142 fragment ions using 67 most intense peaks (help)

#	b	b^{++}	b^*	b^{*++}	b^0	b^{0++}	Seq.	y	y^{++}	y^*	y^{*++}	y^0	y^{0++}	#
1	100.0757	50.5415					V							15
2	213.1598	107.0835					L	1543.8843	772.4458	1526.8577	763.9325	1525.8737	763.4405	14
3	330.2125	165.6099					P	1430.8002	715.9037	1413.7736	707.3905	1412.7896	706.8985	13
4	439.2551	220.1312			421.2445	211.1259	E	1333.7474	667.3774	1316.7209	658.8641	1315.7369	658.3721	12
5	552.3392	276.6732			534.3286	267.6679	L	1204.7048	602.8561	1187.6783	594.3428	1186.6943	593.8508	11
6	667.3661	334.1867	650.3396	325.6734	649.3556	325.1814	N	1091.6208	546.3140	1074.5942	537.8007	1073.6102	537.3087	10
7	724.3876	362.6974	707.3610	354.1842	706.3770	353.6921	G	976.5938	488.8006	959.5673	480.2873	958.5833	479.7953	9
8	880.5138	440.7606	863.4873	432.2473	862.5033	431.7553	K	919.5724	460.2898	902.5458	451.7765	901.5618	451.2845	8
9	993.5979	497.3026	976.5714	488.7893	975.5873	488.2973	L	763.4461	382.2267	746.4196	373.7134	745.4355	373.2214	7
10	1094.6456	547.8264	1077.6190	539.3132	1076.6350	538.8211	T	650.3620	325.6847	633.3355	317.1714	632.3515	316.6794	6
11	1151.6671	576.3372	1134.6405	567.8239	1133.6565	567.3319	G	549.3144	275.1608	532.2878	266.6475			5
12	1250.7355	625.8714	1233.7089	617.3581	1232.7249	616.8661	V	492.2929	246.6501	475.2663	238.1368			4
13	1321.7726	661.3899	1304.7460	652.8767	1303.7620	652.3846	A	393.2245	197.1159	376.1979	188.6026			3
14	1468.8410	734.9241	1451.8144	726.4109	1450.8304	725.9189	F	322.1874	161.5973	305.1608	153.0840			2
15							R	175.1190	88.0631	158.0924	79.5498			1

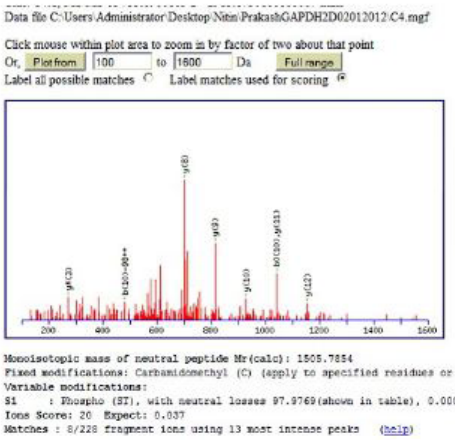
Peptide 11

Citation: Jindal N, Balaji C, Sangolgi PB, Dutta S, Jarori GK (2017) Identification of Post-translational Modifications of *Plasmodium yoelii*Glyceraldehyde-3-phosphate dehydrogenase by Mass Spectrometry. Adv Proteomics Bioinform: APBI -103. DOI: 10.29011/APBI -103. 100003



#	b	b^{++}	b^*	b^{*++}	b^0	b^{0++}	Seq.	y	y^{++}	y^*	y^{*++}	y^0	y^{0++}	#
1	104.0706	82.5389					V							23
2	278.1135	139.5604	261.0870	131.0471			N	2351.0966	1176.0519	2334.0700	1167.5387	2333.0860	1167.0466	22
3	365.1456	183.0764	348.1190	174.5631	347.1350	174.0711	S	2287.0837	1119.0505	2270.0571	1110.5372	2219.0431	1110.0252	21
4	452.1776	226.5924	435.1510	218.0792	434.1670	217.5871	S	2150.0216	1051.5145	2132.9951	1067.0012	2132.0111	1066.5092	20
5	580.2362	290.6217	563.2096	282.1084	562.2256	281.6164	Q	2062.9896	1031.9984	2045.9631	1023.4852	2044.9790	1022.9932	19
6	681.2838	341.1456	664.2573	332.6323	663.2733	332.1403	T	1934.9310	967.9692	1917.9045	959.4559	1916.9205	958.9639	18
7	794.3679	397.6876	777.3414	389.1743	776.3573	388.6825	I	1833.8833	917.4453	1816.8568	908.9320	1815.8728	908.4400	17
8	893.4363	447.2218	876.4098	438.7085	875.4258	438.2165	V	1720.7993	860.9033	1703.7727	852.3900	1702.7887	851.8980	16
9	980.4684	490.7378	963.4418	482.2245	962.4578	481.7325	S	1621.7309	811.3691	1604.7043	802.8558	1603.7203	802.3638	15
10	1094.5125	547.7599	1077.4847	539.2460	1076.5007	538.7540	N	1534.6988	767.8531	1517.6723	759.3398	1516.6883	758.8478	14
11	1165.5496	583.2785	1148.5230	574.7646	1147.5390	574.2726	A	1420.6559	710.8316	1403.6294	702.3183	1402.6453	701.8263	13
12	1252.5816	626.7938	1235.5539	618.2806	1234.5699	617.7886	S	1349.6188	675.3130	1332.5923	666.7998	1331.6082	666.3078	12
13	1412.6311	706.8092	1395.5843	698.2959	1394.6003	697.8039	C	1262.5768	631.7920	1245.5502	623.2837	1244.5762	622.7917	11
14	1513.6587	757.3330	1496.6322	748.8197	1495.6482	748.3277	T	1102.5361	551.7717	1085.5296	543.2684	1084.5456	542.7764	10
15	1614.7064	807.8569	1597.6799	799.3436	1596.6959	798.8516	T	1001.5084	501.2579	984.4819	492.7446	983.4979	492.2526	9
16	1728.7494	864.8783	1711.7228	856.3650	1710.7388	855.8730	N	900.4608	450.7340	883.4342	442.2207			8
17	1860.7487	930.8780	1843.7222	922.3647	1842.7381	921.8727	C	786.4178	393.7126	769.3913	385.1993			7
18	1973.8328	987.4200	1956.8062	978.9067	1955.8222	978.4147	L	654.4185	327.7129	637.3919	319.1996			6
19	2044.8699	1022.9386	2027.8433	1014.4253	2026.8593	1013.9333	A	541.3344	271.1709	524.3079	262.6576			5
20	2141.9226	1071.4650	2124.8961	1062.9517	2123.9121	1062.4597	P	470.2973	235.6523	453.2708	227.1390			4
21	2255.0667	1128.0370	2237.9402	1119.4737	2236.9561	1118.9817	I	373.2445	187.1259	356.2180	178.6126			3
22	2326.0458	1163.5265	2309.0173	1155.0123	2308.0333	1154.5203	A	260.1605	130.5839	243.1339	122.0706			2
23							K	189.1234	95.0653	172.0968	86.5520			1

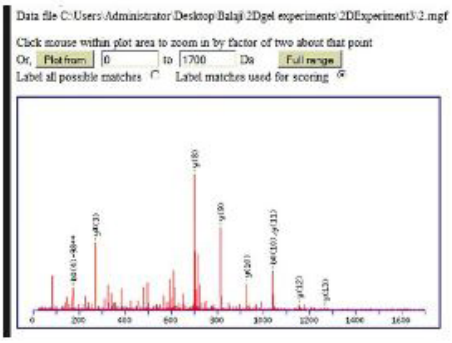
Peptide 12



#	b	b^{++}	b^*	b^{*++}	b^0	b^{0++}	Seq.	y	y^{++}	y^*	y^{*++}	y^0	y^{0++}	#
1	70.0287	35.5180			52.0182	26.5127	S							15
2	141.0659	71.0366			123.0553	62.0313	A	1339.7044	670.4008	1322.7678	661.8876	1321.7838	661.3955	14
3	254.1499	127.5786			236.1394	118.5733	L	1268.7573	634.8823	1251.7307	626.3690	1250.7467	625.8770	13
4	367.2340	184.1206			349.2234	175.1153	L	1155.6732	578.3402	1138.6467	569.8270	1137.6626	569.3350	12
5	481.2769	241.1421	464.2504	232.6288	463.2663	232.1368	N	1042.5891	521.7982	1025.5626	513.2849	1024.5786	512.7929	11
6	594.3610	297.6841	577.3344	289.1708	576.3504	288.6788	I	928.5462	464.7767	911.5197	456.2635	910.5356	455.7715	10
7	707.4450	354.2262	690.4185	345.7129	689.4345	345.2209	I	815.4621	408.2347	798.4356	399.7214	797.4516	399.2294	9
8	804.4978	402.7525	787.4713	394.2393	786.4872	393.7473	P	702.3781	351.6927	685.3515	343.1794	684.3675	342.6874	8
9	875.5349	438.2711	858.5084	429.7578	857.5243	429.2658	A	605.3253	303.1663	588.2988	294.6530	587.3148	294.1610	7
10	962.5669	481.7871	945.5404	473.2738	944.5564	472.7818	S	534.2882	267.6477	517.2617	259.1345	516.2776	258.6425	6
11	1063.6146	532.3109	1046.5881	523.7977	1045.6041	523.3057	T	447.2552	224.1317	430.2296	215.6185	429.2456	215.1264	5
12	1120.6361	560.8217	1103.6095	552.3084	1102.6255	551.8164	G	346.2085	173.6079	329.1819	165.0946			4
13	1191.6732	596.3402	1174.6467	587.8270	1173.6626	587.3350	A	289.1870	145.0972	272.1605	136.5839			3
14	1262.7103	631.8588	1245.6838	623.3455	1244.6997	622.8535	A	218.1499	109.5786	201.1234	101.0653			2
15							K	147.1128	74.0600	130.0863	65.5468			1

Peptide 13

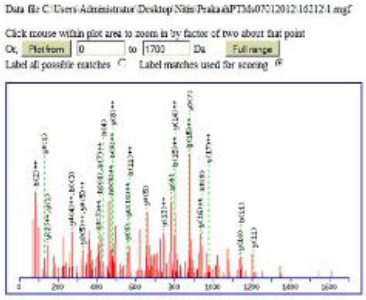
Citation: Jindal N, Balaji C, Sangolgi PB, Dutta S, Jarori GK (2017) Identification of Post-translational Modifications of *Plasmodium yoelii*Glyceraldehyde-3-phosphate dehydrogenase by Mass Spectrometry. Adv Proteomics Bioinform: APBI -103. DOI: 10.29011/APBI -103. 100003



Monoisotopic mass of neutral peptide Mr(calc): 1809.7854
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Variable modifications:
S1 : Phospho (ST), with neutral losses 97.9768(shown in table), 0.0010
Ions Score: 29 Expect: 0.16
Matches : 9/228 fragment ions using 15 most intense peaks (help)

#	b	b^{++}	b^*	b^{*++}	b^0	b^{0++}	Seq.	y	y^{++}	y^*	y^{*++}	y^0	y^{0++}	#
1	70.0287	35.5180			52.0182	26.5127	S							15
2	141.0659	71.0366			123.0553	62.0313	A	1339.7044	670.4058	1322.7678	661.8876	1321.7838	661.3955	14
3	254.1499	127.5786			236.1394	118.5733	L	*1268.7575*	634.8824	1251.7307	626.3690	1250.7467	625.8770	13
4	367.2340	184.1206			349.2234	175.1153	L	*1155.6732*	578.3402	1138.6467	569.8270	1137.6626	569.3350	12
5	481.2769	241.1421	464.2504	232.6288	463.2663	232.1368	N	*1042.5891*	521.7982	1025.5626	513.2849	1024.5786	512.7929	11
6	594.3610	297.6841	577.3344	289.1708	576.3504	288.6788	I	*928.5462*	464.7767	911.5197	456.2635	910.5356	455.7715	10
7	707.4450	354.2262	690.4185	345.7129	689.4345	345.2209	I	*815.4621*	408.2347	798.4356	399.7214	797.4516	399.2294	9
8	804.4978	402.7525	787.4713	394.2393	786.4872	393.7473	P	*702.3781*	351.6927	685.3515	343.1794	684.3675	342.6874	8
9	875.5349	438.2711	858.5084	429.7578	857.5243	429.2658	A	585.3253	293.1663	568.2988	284.6530	567.3148	284.1610	7
10	962.5669	481.7871	945.5404	473.2738	944.5564	472.7818	S	534.2882	267.6477	517.2617	259.1345	516.2776	258.6425	6
11	1063.6146	532.3109	1046.5881	523.7977	1045.6041	523.3057	T	447.2562	224.1317	430.2296	215.6185	429.2456	215.1264	5
12	1120.6361	560.8217	1103.6095	552.3084	1102.6255	551.8164	G	346.2085	173.6079	329.1819	165.0946			4
13	1191.6732	596.3402	1174.6467	587.8270	1173.6626	587.3350	A	289.1870	145.0972	*272.1605*	136.5839			3
14	1262.7103	631.8588	1245.6838	623.3455	1244.6997	622.8535	A	218.1499	109.5786	201.1234	101.0653			2
15							K	147.1128	74.0600	130.0863	65.5468			1

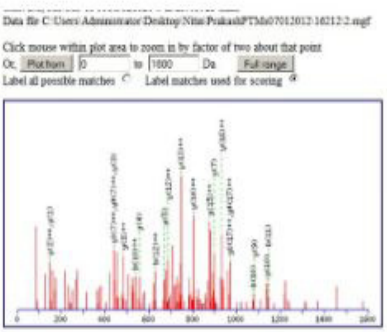
Peptide 14



Monoisotopic mass of neutral peptide Mr(calc): 2041.3520
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Variable modifications:
K7 : Acetyl (K)
Ions Score: 14 Expect: 0.097
Matches : 33/184 fragment ions using 139 most intense peaks (help)

#	b	b^{++}	b^*	b^{*++}	b^0	b^{0++}	Seq.	y	y^{++}	y^*	y^{*++}	y^0	y^{0++}	#
1	*88.0393*	44.5233			70.0287	35.5180	S							18
2	175.0713	*88.0393*			157.0608	79.0340	S	1955.0273	978.0173	1938.0007	969.5040	1937.0167	969.0120	17
3	288.1554	144.5813			270.1448	135.5761	I	1867.9953	934.5013	1850.9687	925.9880	1849.9847	925.4960	16
4	435.2238	218.1155			417.2132	209.1103	F	1754.9112	877.9592	1737.8846	869.4460	1736.9006	868.9540	15
5	550.2508	275.6290			532.2402	266.6237	D	1607.8428	804.4250	1590.8162	795.9118	1589.8322	795.4197	14
6	663.3348	332.1710			645.3243	323.1658	L	1492.8158	*746.9116*	1475.7893	738.3983	1474.8053	737.9063	13
7	833.4403	*417.2238*	816.4138	408.7105	815.4298	408.2185	K	1379.7318	690.3695	1362.7052	681.8563	1361.7212	681.3642	12
8	904.4775	452.7424	887.4509	444.2291	886.4669	443.7371	A	*1209.6262*	605.3168	1192.5997	596.8035	1191.6157	596.3115	11
9	961.4989	*481.2531*	944.4724	472.7398	943.4884	*472.2478*	G	*1138.5891*	569.7982	1121.5626	561.2849	1120.5786	*560.7929*	10
10	1074.5830	537.7951	1057.5564	529.2819	1056.5724	528.7898	L	1081.5677	541.2875	1064.5411	532.7742	1063.5571	532.2822	9
11	*1145.6201*	*573.3137*	1128.5936	564.8004	1127.6095	564.3084	A	968.4836	*484.7454*	951.4571	476.2322	950.4730	475.7402	8
12	1258.7042	629.8557	1241.6776	621.3424	1240.6936	620.8504	L	897.4465	449.2269	880.4199	440.7136	*879.4359*	440.2216	7
13	1372.7471	686.8772	1355.7205	678.3639	1354.7365	677.8719	N	*784.3624*	392.6849	767.3359	384.1716	766.3519	383.6796	6
14	1487.7740	744.3907	1470.7475	735.8774	1469.7635	735.3854	D	670.3195	335.6634	*653.2930*	*327.1501*	652.3089	*326.6581*	5
15	1601.8170	*801.4121*	1584.7904	792.8988	1583.8064	792.4068	N	*555.2926*	278.1499	538.2660	*269.6366*			4
16	1748.8854	*874.9463*	1731.8588	866.4331	1730.8748	865.9410	F	441.2496	221.1285	424.2231	212.6152			3
17	1895.9538	948.4805	1878.9272	939.9673	1877.9432	939.4753	F	294.1812	*147.5942*	277.1547	139.0810			2
18							K	*147.1128*	74.0600	*130.0863*	65.5468			1

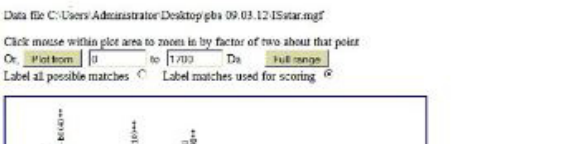
Peptide 15



Monoisotopic mass of neutral peptide Mr(calc): 2041.0520
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Variable modifications:
ST : Acetyl (N)
Ions Score: 39 Expect: 0.0019
Matches : 22/184 fragment ions using 41 most intense peaks (help)

#	b	b^{++}	b^*	b^{*++}	b^0	b^{0++}	Seq.	y	y^{++}	y^*	y^{*++}	y^0	y^{0++}	#
1	88.0393	44.5233			70.0287	35.5180	S							18
2	175.0713	88.0393			157.0608	79.0340	S	1955.0273	978.0173	1938.0007	969.5040	1937.0167	969.0120	17
3	288.1554	144.5813			270.1448	135.5761	I	1867.9953	934.5013	1850.9687	925.9880	1849.9847	925.4960	16
4	435.2238	218.1155			417.2132	209.1103	F	1754.9112	877.9592	1737.8846	869.4460	1736.9006	868.9540	15
5	550.2508	275.6290			532.2402	266.6237	D	1607.8428	804.4250	1590.8162	795.9118	1589.8322	795.4197	14
6	663.3348	332.1710			645.3243	323.1658	L	1402.8158	746.9116	1475.7893	738.3983	1474.8053	737.9063	13
7	833.4403	417.2238	816.4138	408.7105	815.4298	408.2185	K	1379.7318	690.3695	1362.7052	681.8563	1361.7212	681.3642	12
8	904.4775	452.7424	887.4509	444.2291	886.4669	443.7371	A	1209.6262	605.3168	1192.5997	596.8035	1191.6157	596.3115	11
9	961.4989	481.2531	944.4724	472.7398	943.4884	472.2478	G	1138.5891	569.7982	1121.5626	561.2849	1120.5786	560.7929	10
10	1074.5830	537.7951	1057.5564	529.2819	1056.5724	528.7898	L	1081.5677	541.2875	1064.5411	532.7742	1063.5571	532.2822	9
11	1145.6201	573.3137	1128.5936	564.8004	1127.6095	564.3084	A	968.4836	484.7454	951.4571	476.2322	950.4730	475.7402	8
12	1258.7042	629.8557	1241.6776	621.3424	1240.6936	620.8504	L	897.4465	449.2269	880.4199	440.7136	879.4359	440.2218	7
13	1372.7471	686.8772	1355.7205	678.3639	1354.7365	677.8719	N	784.3624	392.6849	767.3359	384.1716	766.3519	383.6798	6
14	1487.7740	744.3907	1470.7475	735.8774	1469.7635	735.3854	D	670.3195	335.6634	653.2930	327.1501	652.3089	326.6581	5
15	1601.8170	801.4121	1584.7904	792.8988	1583.8064	792.4068	N	555.2926	278.1499	538.2660	269.6366			4
16	1748.8854	874.9463	1731.8588	866.4331	1730.8748	865.9410	F	441.2496	221.1285	424.2231	212.6152			3
17	1895.9538	948.4805	1878.9272	939.9673	1877.9432	939.4753	F	294.1812	147.5942	277.1547	139.0810			2
18							K	147.1128	74.0600	130.0863	65.5468			1

Peptide 16



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S13 : Phospho (ST), with neutral losses 97.9769(shown in table), 0.0000
K18 : Methyl (K)
Ions Score: 9 Expect: 0.96
Matches : 28/352 fragment ions using 78 most intense peaks (help)

#	b	b^{++}	b^*	b^{*++}	b^0	b^{0++}	Seq.	y	y^{++}	y^*	y^{*++}	y^0	y^{0++}	#
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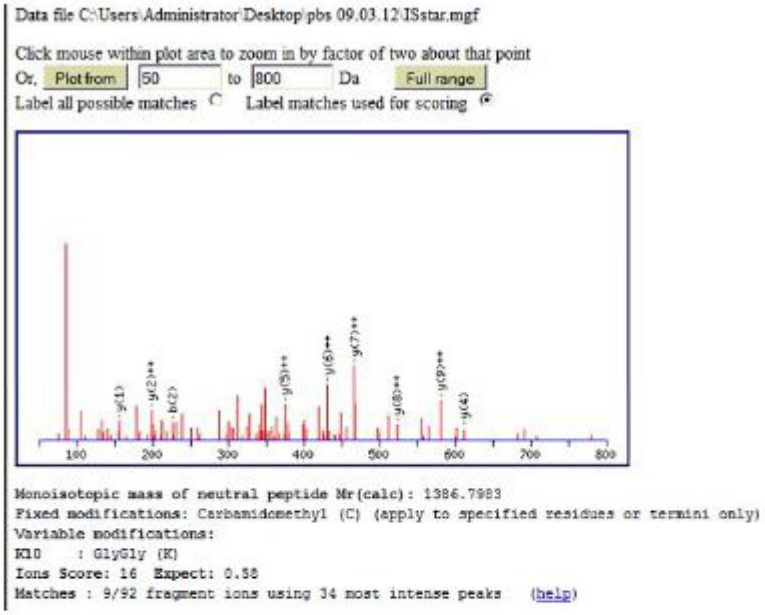
2	129.0659	65.0366					G	1991.1780	996.0926	1974.1495	987.5784	1973.1655	987.0864	21
3	285.1670	143.0871	268.1404	134.5738			R	1934.1546	967.5809	1917.1280	959.0676	1916.1440	958.5756	20
4	372.1990	186.6031	355.1724	178.0899	354.1884	177.5979	S	1778.0534	889.5304	1761.0269	881.0171	1760.0429	880.5251	19
5	443.2361	222.1217	426.2096	213.6084	425.2255	213.1164	A	1691.0214	846.0143	1673.9949	837.5011	1673.0108	837.0091	18
6	556.3202	278.6637	539.2936	270.1504	538.3096	269.6584	L	1619.9843	810.4958	1602.9578	801.9825	1601.9737	801.4905	17
7	669.4042	335.2058	652.3777	326.6925	651.3937	326.2005	L	1506.9002	753.9538	1489.8737	745.4405	1488.8897	744.9485	16
8	783.4472	392.2272	766.4206	383.7139	765.4366	383.2219	N	1393.8162	697.4117	1376.7896	688.8983	1375.8056	688.4064	15
9	896.5312	448.7693	879.5047	440.2560	878.5207	439.7640	I	1279.7732	640.3903	1262.7467	631.8770	1261.7627	631.3850	14
10	1009.6153	505.3113	992.5887	496.7980	991.6047	496.3060	I	1166.6892	583.8482	1149.6626	575.3350	1148.6786	574.8429	13
11	1106.6681	553.8377	1089.6415	545.3244	1088.6575	544.8324	P	1053.6051	527.3062	1036.5786	518.7929	1035.5946	518.3009	12
12	1177.7052	589.3562	1160.6786	580.8429	1159.6946	580.3509	A	956.5524	478.7798	939.5258	470.2665	938.5418	469.7745	11
13	1246.7286	623.8679	1229.7021	615.3547	1228.7181	614.8627	S	885.5152	443.2613	868.4887	434.7480	867.5047	434.2560	10
14	1347.7743	674.3908	1330.7478	665.8775	1329.7637	665.3855	T	816.4938	408.7505	799.4672	400.2373	798.4832	399.7452	9
15	1404.7958	702.9015	1387.7692	694.3883	1386.7852	693.8962	G	715.4465	358.2269	698.4199	349.7136			8
16	1475.8329	738.4201	1458.8063	729.9068	1457.8223	729.4148	A	658.4246	329.7160	641.3981	321.2027			7
17	1546.8700	773.9386	1529.8433	765.4254	1528.8594	764.9334	A	587.3875	294.1974	570.3610	285.6841			6
18	1688.9806	844.9939	1671.9541	836.4807	1670.9701	835.9887	K	516.3504	258.6788	499.3239	250.1656			5
19	1760.0177	880.5125	1742.9912	871.9992	1742.0072	871.5072	A	374.2308	187.6191	357.2132	178.1103			4
20	1859.0861	930.0467	1842.0596	921.5334	1841.0756	921.0414	V	303.2677	152.1375	286.1701	143.5917			3
21	1916.1076	958.5574	1899.0811	950.0442	1898.0970	949.5522	G	204.1343	102.5708	187.1077	94.0575			2
22							K	147.1128	74.0600	130.0863	65.5468			1

Peptide 17



#	b	b^{++}	b^{*}	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y^{*}	y^{*++}	y^{0}	y^{0++}	#
1	114.0913	57.5493					L							11
2	227.1754	114.0913					L	1274.7215	637.8644	1257.6950	629.3511	1256.7110	628.8591	10
3	342.2023	171.6048			324.1918	162.5995	D	1161.6375	581.3224	1144.6109	572.8091	1143.6269	572.3171	9
4	455.2864	228.1468			437.2758	219.1416	L	1046.6105	523.8089	1029.5840	515.2956	1028.6000	514.8036	8
5	526.3235	263.6654			508.3130	254.6601	A	933.5265	467.2669	916.4999	458.7536	915.5159	458.2616	7
6	639.4076	320.2074			621.3970	311.2022	I	862.4894	431.7483	845.4628	423.2350	844.4788	422.7430	6
7	776.4665	388.7369			758.4559	379.7316	H	749.4053	375.2063	732.3787	366.6930	731.3947	366.2010	5
8	889.5506	445.2789			871.5400	436.2736	I	612.3464	306.6768	595.3198	298.1636	594.3358	297.6715	4
9	990.5982	495.8028			972.5877	486.7975	T	499.2623	250.1348	482.2358	241.6215	481.2518	241.1295	3
10	1232.7361	616.8717	1215.7096	608.3584	1214.7256	607.8664	K	398.2146	199.6110	381.1881	191.0977			2
11							H	156.0768	78.5420					1

Peptide 18

Figure S1: MS/MS spectra and fragment ion masses for all the peptides listed in Table 2.

Discussion

It is increasingly being realized that post-translational modifications and combinations thereof play an important role in determining sub-cellular distribution and functions of a protein [21]. Mass spectrometry has become the tool of choice to obtain precise chemical structures of various protein species at single protein level [9,21,22]. The ultimate objective of defining precise chemical structure is to correlate it to cellular function [4a,4b,4d]. Work presented in this manuscript has used single protein analysis approach to obtain solid chemical data about the PTMs in PyGAPDH.

Western blot analysis using anti-rPfGAPDH antibodies showed the presence of PyGAPDH in three different sizes in the parasite cell extracts. These were further confirmed by MS and MS/MS analysis of peptides obtained by trypsin digestion of the proteins. Origin of ~51 kDa form present in cytosol was found to be due to ubiquitination of the native 37 kDa species of PyGAPDH. Extensive MS/MS sequencing of peptides derived from the three bands led to identification of several PTMs in PyGAPDH. At least twelve different residues in PyGAPDH were modified with five different kinds of chemical modifications. Most modifications mapped to the C-terminal domain of the protein. There were eleven modifications in the C-terminal half while N-terminal half had only five (Table 3). Residues 201-237 had most modifications. Functionally, N-terminal domain has the NAD+ binding site and the C-terminal domain forms a glyceraldehyde 3-phosphate binding site. A flexible S-loop (extending from residue 180 to 210) is believed to be the region that transmits

structural changes induced by substrate binding to neighboring subunits (allosteric regulation) [18,23]. Examination of the pattern of PTMs in various regions of the molecule could provide some insightful information about the regulation of underlying physiological processes (Figure 4).

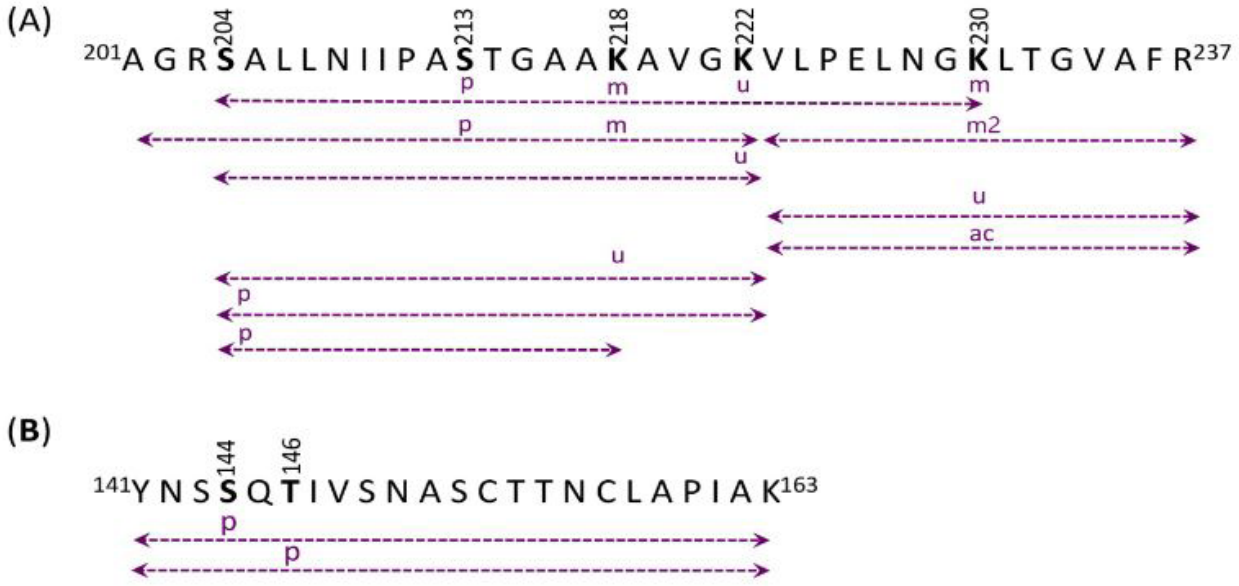


Figure 4: Schematic representation of modifications in various peptides. (A) Peptide containing residues from 201-237 and (B) peptide containing residues 141-163 showing the PTMs that occur in combinations or in exclusion of each other in PyGAPDH.

There are two serine residues in the peptide 201-237. Both underwent phosphorylation. However, these phosphorylations were exclusive of each other i.e. in a given molecule only one of the two was phosphorylated. Further, a peptide containing pS^{204}- did not show any modifications at K^{218} or K^{222}. However, when S^{213} had phosphorylation (pS^{213}), one or more modifications at the two lysines were observed. Further, we observed that K^{218} could either be methylated or ubiquitinated, but these modifications probably required phosphorylation of S^{213}. A serine residue in *Toxoplasma gondii* (S^{203}) that is homologous to S^{204} of *P. yoelii* also undergoes phosphorylation [24]. This modification has been implicated in the regulation enzyme activity presumably by interfering with oligomerization and allosteric activation [18]. *P. yoelii* as well as *T. gondii* have two neighboring residues (S^{213} and T^{214}) that can be phosphorylated. Interestingly S^{213} is phosphorylated in *P. yoelii* while T^{214} is phosphorylated in *T. gondii* [24]. Thus, there appears to be a high degree of conservation in PTMs among the related organisms. K^{230} is rather unusual in undergoing four different types of modifications i.e. mono or dimethylation / acetylation / ubiquitination. Although all three lysines in peptide 201-237 could undergo ubiquitination, modification occurred only at one of these lysines. In another peptide covering residues 141-163, phosphorylations of S^{144} and T^{146} were observed. These were also exclusive of each other. It is likely that phosphorylation of either of these two residues could mediate the similar physiological function(s). Two mono-methylations at $K^{218}$and K^{230} were detected. These could occur in the same molecule or in different molecules individually. Both these lysines are conserved in all four-species compared here (Figure S2).

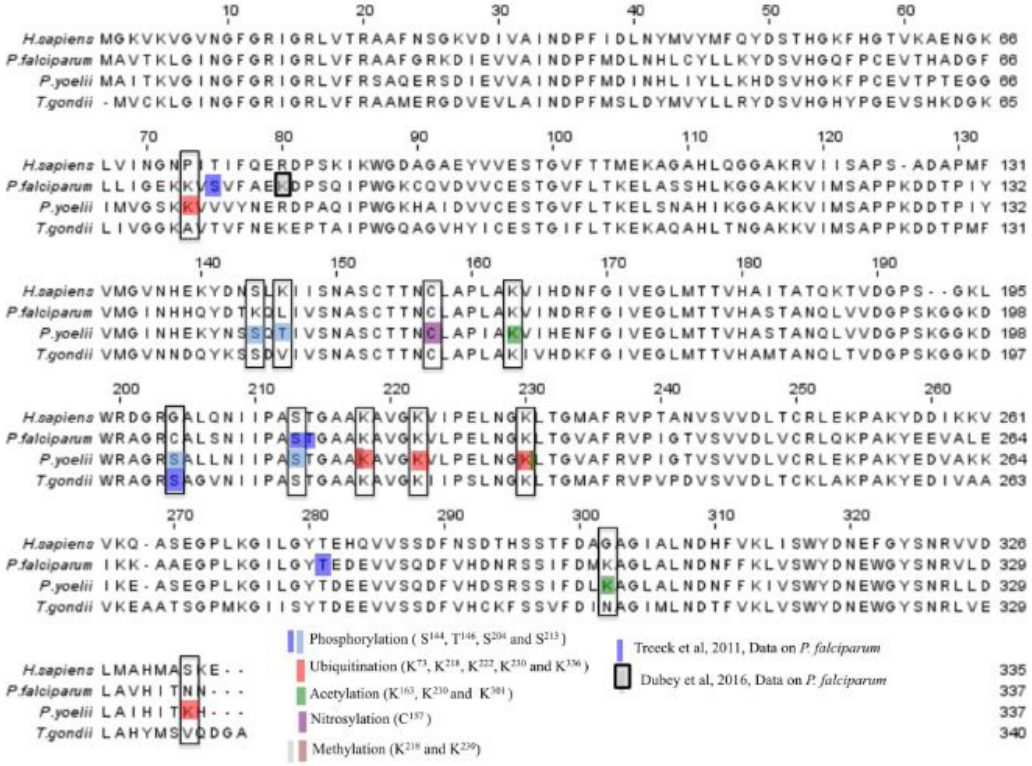


Figure S2: Sequence homology among *P. falciparum, P. yoelii* and human GAPDH. Analysis of phosphoproteome of *P. falciparum* led to identification of four sites. Modified residues detected in PyGAPDH are marked. S^{144}, S^{204}, S^{213} and T^{146} that undergo phosphorylation in PyGAPDH, only S^{213} is conserved in *P. falciparum*.

Dimethylation of K^{230} was also detected. In a recent study, methylated lysine proteome of blood stage *P. falciparum* was analyzed. However, in this proteome-wide lysine methylation analysis, trimethylation of K^{80} (^{74}VSVFAE**K**DPSQIPGW^{88}) [25] was the only modification reported. This residue in *P. yoelii* GAPDH is replaced by R^{80}. All methylated residues detected in *P. yoelii* are conserved in all four species of *Plasmodia* (Figure S2) suggesting that such methylations are likely to be present in *Plasmodium falciparum* GAPDH too.

For understanding the functional significance of PTMs, it is essential to determine combinations of various PTMs that occur together and the spatio-temporal distribution of each distinct chemical species inside the cell. In the absence of such information, functional implications of PTMs will be difficult to establish. PTMs such as phosphorylations are one important mechanism by which the parasite controls the process of invasion and modification of the host cells [26]. Phosphorylation of four different residues viz. pS^{144}, pT^{146}, pS^{204} and pS^{213} were observed in *P. yoelii* 17XL GAPDH. In *P. falciparum* 3D7 GAPDH, four phosphorylation sites (pS^{75}, pS^{213}, pT^{214} and pT^{280}) have been identified [26]. Although three of these four residues (S^{213}, T^{214} and T^{280}) are conserved in both species, only phosphorylation of S^{213} is observed in both species. Residues that are phosphorylated only in *P. yoelii* (pS^{144}, pT^{146} and pS^{204}) are not conserved in *P. falciparum* (K^{144}, $L^{146}$and C^{204}). Such variation may imply species-specific physiological roles for different modifications.

There are two Cys residues in the active site of PyGAPDH viz. C^{153} and C^{157}. One of these was found to be nitrosylated. Nitrosylation of GAPDH has been reported in macrophages. In mammalian cells, cysteine residue at the catalytic site (C^{152}) undergoes nitrosylation that triggers the binding of GAPDH to Siah-1 (an E3 ubiquitin ligase) followed by nuclear translocation and apoptosis[1d]. This cascade of S-nitrosylated-GAPDH and Siah-1 may represent an important molecular mechanism of apoptotic cell death [1d,1e]. The catalytic Cys ($C^{153}$and C^{157}) are not only conserved in PyGAPDH, but one of these also undergoes nitrosylation (nC^{157}). This raises the possibility that nitrosylation of C^{157}may play a role in nuclear localization of PyGAPDH.

Observations of modified lysine residues at C-terminus of tryptic peptides (e.g. mK^{230} and uK^{222}) indicate that trypsin does cut at modified lysines. This was in contrast to earlier belief that such modified residues were not cleaved by trypsin. Cleavage at ubiquitinated lysines was also observed in the analysis of ubiquitome

of MCF-7 breast cancer cells [27]. In all, five residues (K^{73}, K^{218}, K^{222}, K^{230} and K^{336}) were detected that were ubiquitinated of which K^{222} and K^{336} were present in ~37 kDa as well as ~51 kDa species (Table 3) while $K^{218}$and K^{230} were restricted to ~51 kDa species only. 51 kDa species could arise either by conjugation with a diubiquitin moiety or by bi-ubiquitination of the native 37 kDa form. Since tagging a protein for proteasomal degradation requires a K^{48} or K^{11} linked chain of >4-5 Ub subunits attached to a protein [28], it is unlikely that ubiquitinations observed here served as a signal for protein degradation. Di (or Bi) ubiquitinations of PyGAPDH are likely to have some other regulatory function(s) essential for maintaining the cellular homeostasis. Monoubiquitination has been shown to play a role in endocytic pathways and in some cases, single monoubiquitination was sufficient for internalization of the membrane proteins [29]. Our observation of ubiquitination in low MW forms of PyGAPDH (ubiquitination at K^{222} and K^{336}) would suggest their origin from 51 kDa form by limited proteolysis. There have been a few reports about ubiquitination of parasite proteins that are likely to be important in functions other than tagging for proteasomal degradation, e.g. actin [30], histone H2B [31] and enolase [9]. A recent study on *P. falciparum* ubiquitome from erythrocytic stages led to identification of 73 different proteins [32] that included the three proteins mentioned above.

Conclusions

Results presented here provide evidence for multiple structural modifications in *Plasmodium* spp. GAPDH that could easily account for several moonlighting functions that this protein may have [33]. The main objective for the identification of PTMs was to define precise chemical structure of each species and understand their functions. This task of exact correlation between structural variant and its function and/or sub-cellular localization remains yet to be accomplished.

Authors declare no conflict of interest

Author contribution

GKJ conceived and designed the experiments. NJ, CB and SD performed the experiments and collected data. NJ, CB, SD and GKJ analyzed and interpreted the data. GKJ wrote the manuscript. All the authors have read the manuscript and agreed with its content.

Funding

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