Supporting Information

Targeted Annotation of *S*-Sulfonylated Peptides by Selective Infrared Multiphoton Dissociation Mass Spectrometry

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Contents

Supporting Figures

Figure S1. CID Spectrum of Oxidized Laminin β.
CID MS/MS spectrum of doubly protonated, oxidized laminin β. * denotes product ions that contain the modification. All product ions are consistent with modification at the N-terminal Cysteine (ν denotes harmonics).

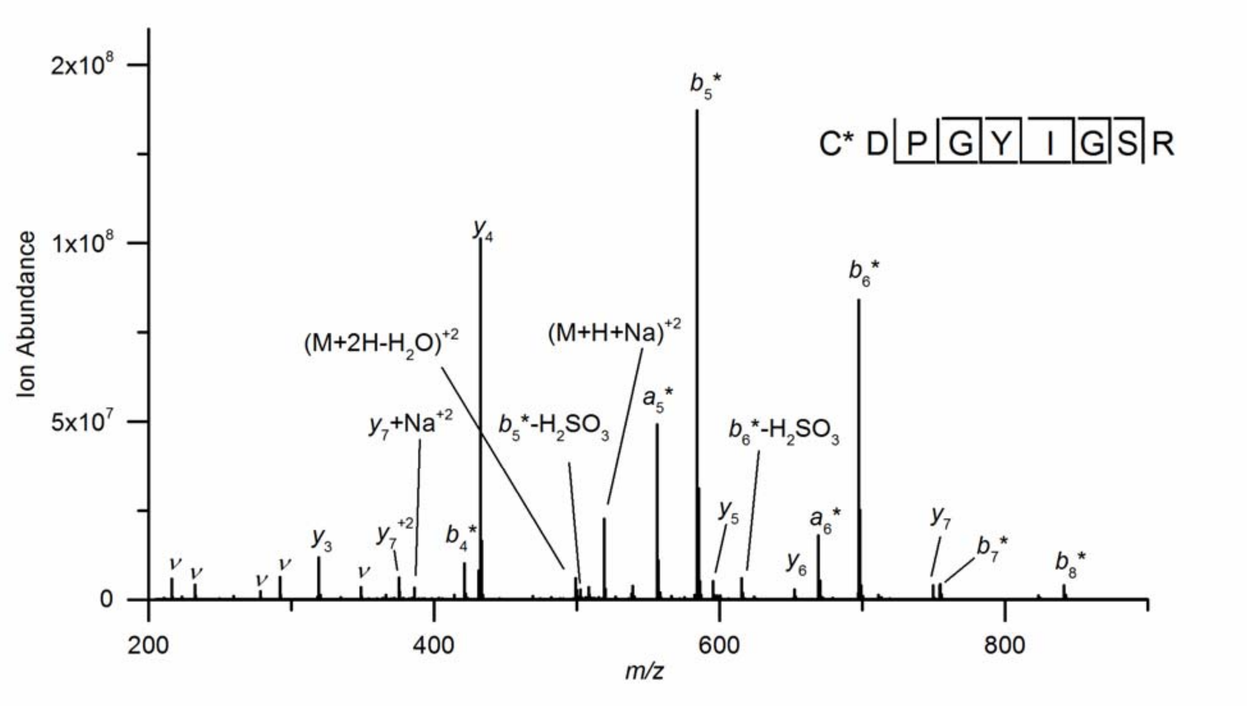


Figure S2. Unoxidized and Oxidized Laminin β Before and After IR Irradiation
Overlaid mass spectra of unoxidized and oxidized laminin β (CDPGYIGSR and C[SO_3]DPGYIGSR, respectively) isolated together using a wide window, before (black) and after (red) IR irradiation at 10.6 µm for 0.2 s at 23 W. Product ions that contain the sulfonic acid are designated with *.

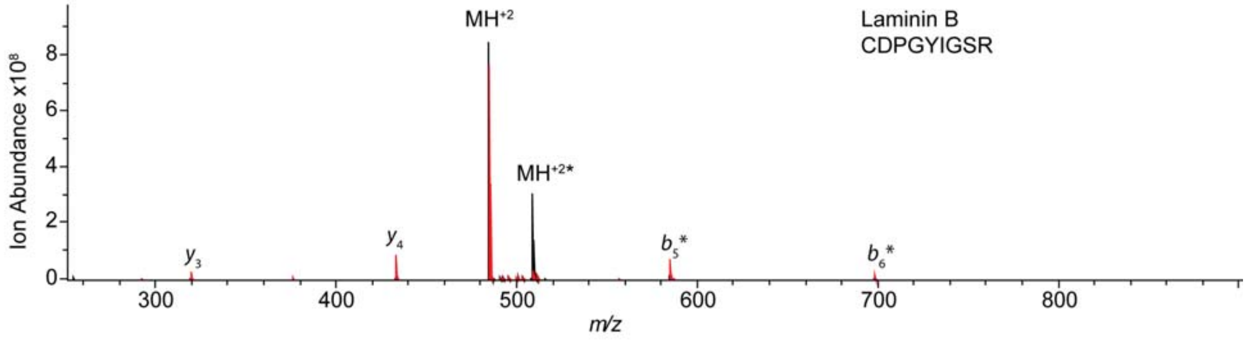


Figure S3. Unoxidized, Oxidized, and Alkylated Laminin β Before and After IR Irradiation
Overlaid mass spectra of unoxidized, oxidized, and alkylated laminin β (CDPGYIGSR, C[SO_3]DPGYIGSR, and C[SC_2H_3NO]DPGYIGSR) isolated together using a wide window, before (black) and after (red) IR irradiation at 10.6 μm for 0.2 s at 23 W. Alkylated and oxidized species are indicated using # and *, respectively.

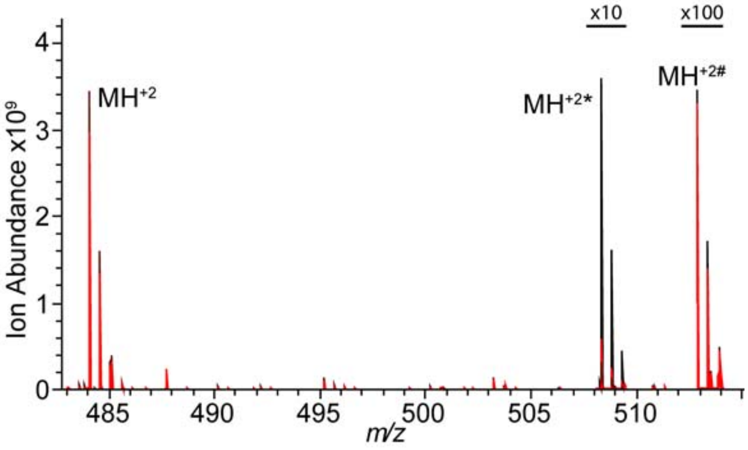


Figure S4. Mass spectra of LC separated standard peptides before and after IR irradiation.

Representative mass spectra for oxidized Laminin β (Figure 2, peak 1), oxidized ACTH 1-10 (Figure 2, peak 2), ACTH 1-10 (Figure 2, peak 3), and oxidized Insulin chain B (Figure 2, peak 6) before (black) and after (red) IR irradiation (15 W for 0.15 s). Laminin β inlay included to more clearly illustrate the preferential fragmentation of the doubly charged peptide (*m/z* = 508.2153).

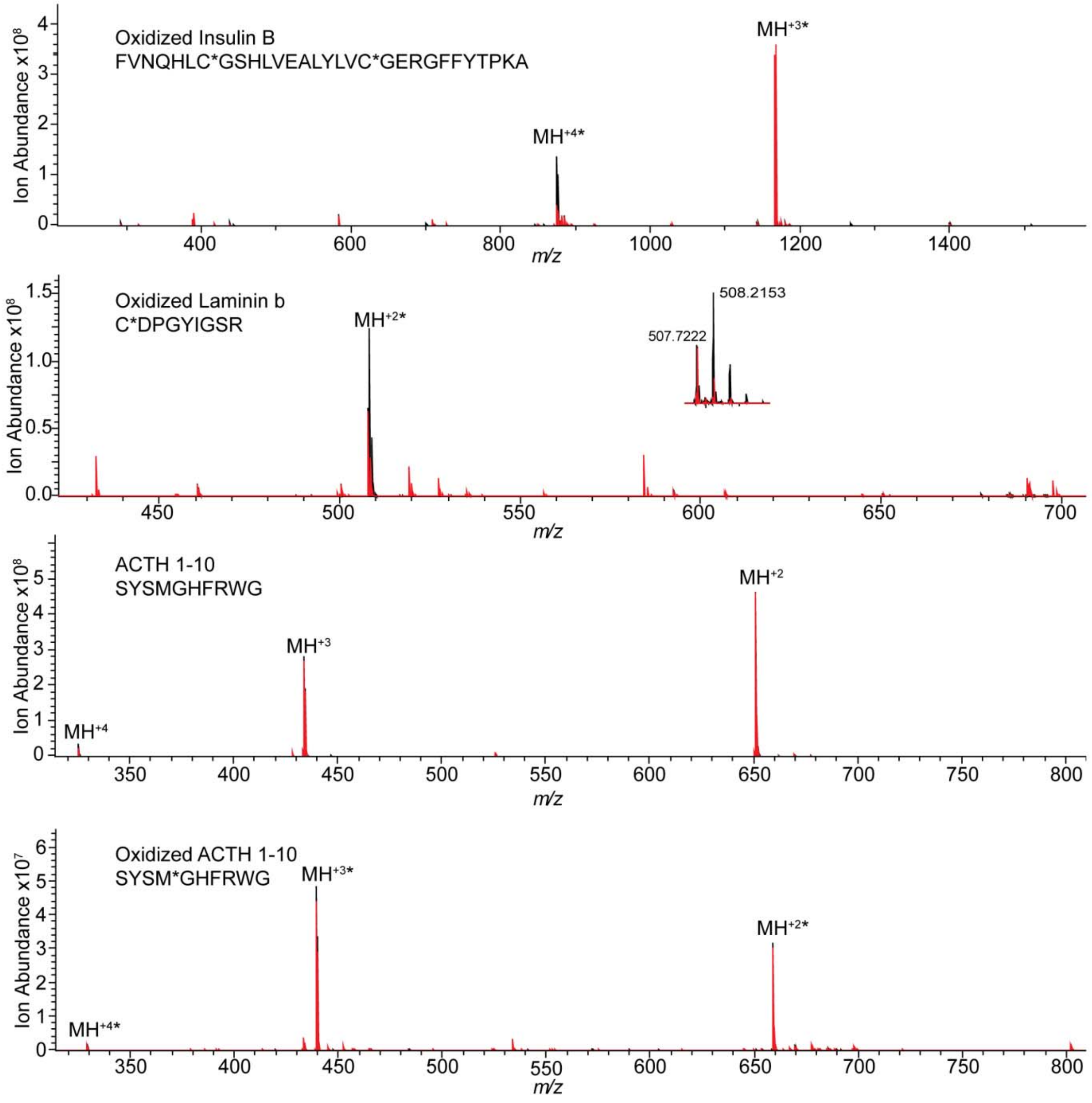


Figure S5. Sample DJ-1 peptides before and after IR irradiation
Mass spectra for A) DJ-1 peptide 13-27 (Figure 3, peak 1) and B) DJ-1 peptide 64-89 (Figure 3, peak 3) before (black) and after (red) IR irradiation (15 W for 0.15 s).

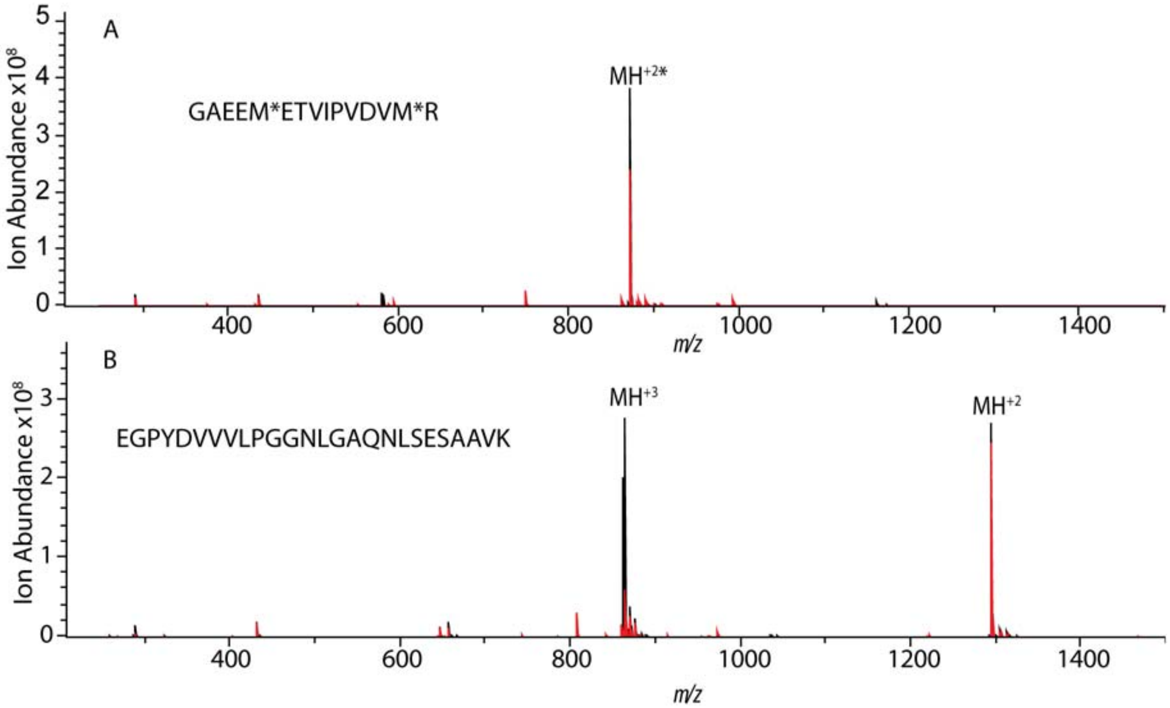
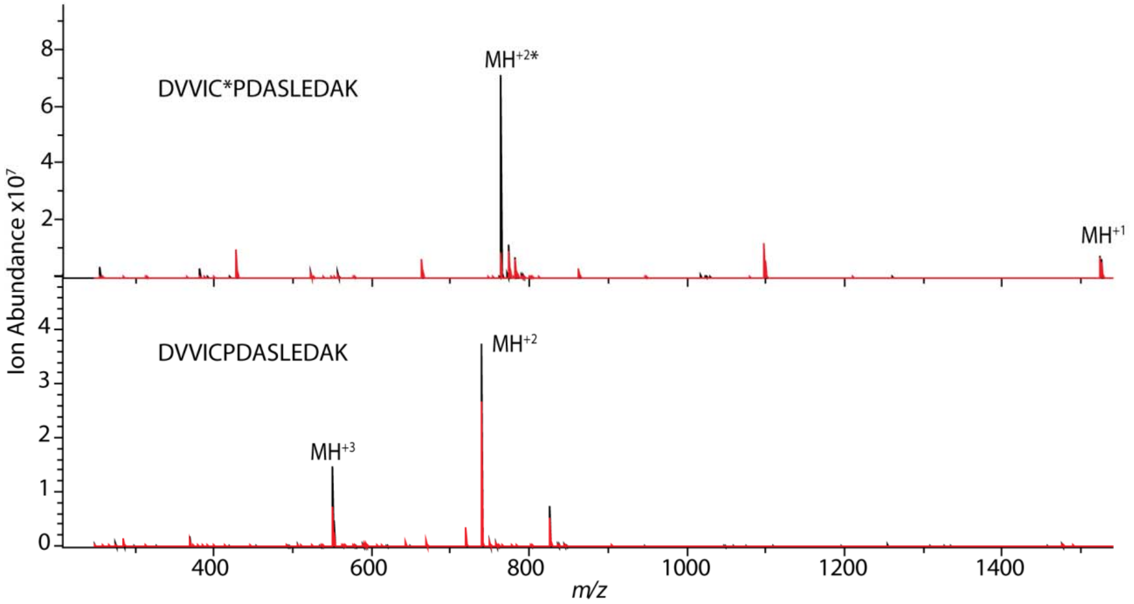


Figure S6. IR irradiation of DJ-1 peptide DVVICPDASLEDAK before and after oxidation.
Mass Spectra of oxidized (top) and unoxidized (bottom) DJ-1 peptide 49-62. The spectra obtained following IR irradiation (red) are overlaid on the spectra obtained without irradiation (black).



Supporting Tables

Table S1. Relative ion abundance of peptide standards after IR irradiation.
Relative abundance of irradiated peptide standards post IR irradiation.

Peptide	Sequence	Modification	Relative Abundance*
Insulin Chain A	GIVEQCCASVCSLYQLENYCN	Sulfonic Acid	0.4%
Tyrosine Phosphopeptide	TSTEPQYQPGEN	Phosphorylated	4.2%
Neuropeptide F	KRSYEEHIP	Phosphorylated	4.5%
Laminin β-1 Chain (925-933)	CDPGYIGSR	Sulfonic Acid	9.9%
Insulin Chain B	FVNQHLCGSHLVEALYLVCGERGFFYTPLA	Sulfonic Acid	49.0%
Substance P (5-11)	pyro-QQFFGLM-NH$_3$	Oxidized	83.4%
Substance P (5-11)	pyro-QQFFGLM-NH$_3$	n/a	84.2%
Laminin β-1 Chain (925-933)	CDPGYIGSR	n/a	90.8%
Angiotensin I	DRVYIHPFHL	n/a	92.8%
Substance P	RPKPQQFFGLM-NH$_3$	n/a	92.9%
ACTH 1-10	SYSMEHFRWG	n/a	94.1%
Substance P	RPKPQQFFGLM-NH3	Oxidized	98.0%
Leucine Enkephalin	YGGFL	n/a	99.8%
ACTH 1-10	SYSMEHFRWG	Oxidized	107.2%

*Relative ion abundance was calculated by dividing the average remaining ion abundance after IR irradiation by the average ion abundance prior to irradiation

Table S2. Relative ion abundance of DJ-1 and AhpC tryptic peptides after IR irradiation.
Relative abundance of irradiated DJ-1 and AhpC peptides post IR irradiation

Protein	Start	End	Sequence	Modifications	Relative Abundance*
DJ-1	64	89	(K)EGPYDVVVLPGGNLGAQNLSESAAVK(E)	n/a	1.2%
DJ-1	-21	4	(-)AHHHHHHVGTGSNDDDDKSPDPMASK(R)	Oxidized	1.9%
DJ-1	100	122	(K)GLIAAICAGPTALLAHEIGFGSK(V)	Sulfonic Acid	3.4%
DJ-1	49	63	(R)DVVICPDASLEDAKK(E)	Sulfonic Acid	7.2%
DJ-1	100	115	(K)GLIAAICAGPTALLAH(E)	Sulfonic Acid	7.4%
DJ-1	49	62	(R)DVVICPDASLEDAK(K)	Sulfonic Acid	12.5%
DJ-1	33	48	(K)VTVAGLAGKDPVQCSR(D)	Sulfonic Acid	17.5%
AhpC	21	50	(E)FIEITEKDTEGRWSVFFFYPADFTFVCPTE(L)	3 oxidations Sulfonic Acid	18.2%
AhpC	124	143	(F)VVDPQGIIQAIEVTAEGIGR(D)	n/a	19.7%
DJ-1	64	81	(K)EGPYDVVVLPGGNLGAQN(L)	n/a	27.7%
DJ-1	64	89	(K)EGPYDVVVLPGGNLGAQNLSESAAVK(E)	Oxidized	28.8%
AhpC	21	50	(E)FIEITEKDTEGRWSVFFFYPADFTFVCPTE(L)	2 Oxidations Sulfonic Acid	31.5%
DJ-1	51	75	(V)VICPDASLEDAKKEGPYDVVVLPGG(N)	Sulfonic Acid	53.4%
AhpC	172	187	(K)EGEATLAPSLDLVGKI(-)	n/a	58.8%
AhpC	179	186	(A)PSLDLVGK(I)	n/a	63.8%
DJ-1	13	27	(K)GAEEMETVIPVDVMR(R)	Oxidized	64.7%
AhpC	181	186	(S)LDLVGK/(I)-(L)DLVGKI	n/a	66.0%
DJ-1	49	63	(R)DVVICPDASLEDAKK(E)	n/a	66.8%
AhpC	113	120	(R)EDEGLADR(A)	n/a	68.7%
AhpC	172	187	(K)EGEATLAPSLDLVGKI(-)	Oxidized	69.1%
DJ-1	49	62	(R)DVVICPDASLEDAK(K)	n/a	69.1%
DJ-1	90	93	(K)EILK(E)	n/a	70.2%
AhpC	107	120	(R)NFDNMREDEGLADR(A)	Oxidized	72.2%
AhpC	147	150	(S)DLLR(K)	n/a	73.0%
DJ-1	63	89	(K)KEGPYDVVVLPGGNLGAQNLSESAAVK(E)	n/a	73.5%
AhpC	92	106	(K)IKYAMIGDPTGALTR(N)	n/a	74.9%
AhpC	146	150	(A)SDLLR(K)	n/a	76.4%
DJ-1	123	130	(K)VTTHPLAK(D)	n/a	78.7%
DJ-1	13	27	(K)GAEEMETVIPVDVMR(R)	n/a	79.8%
AhpC	18	27	(K)NGEFIEITEK(D)	n/a	84.1%
DJ-1	78	90	(N)LGAQNLSESAAVK(E)	n/a	85.1%
DJ-1	183	189	(K)APLVLKD(-)	n/a	85.1%
DJ-1	7	12	(A)LVILAK(G)	n/a	91.7%
DJ-1	33	41	(K)VTVAGLAGK(D)	n/a	92.1%
DJ-1	152	156	(L)ILTSR(G)	n/a	94.4%
DJ-1	149	156	(K)DGLILTSR(G)	n/a	96.1%
DJ-1	165	175	(F)ALAIVEALNGK/(E)	n/a	96.5%
DJ-1	34	41	(V)TVAGLAGK(D)	n/a	96.5%
DJ-1	118	122	(I)GFGSK(V)	n/a	96.9%
AhpC	144	150	(R)DASDLLR(K)	n/a	100.2%
DJ-1	184	188	(A)PLVLK(D)	n/a	100.7%
DJ-1	183	188	(K)APLVLK(D)	Oxidized	101.0%
DJ-1	7	11	(A)LVILA(K)	n/a	101.2%

Protein	Start	End	Sequence	Modifications	Relative Abundance*
AhpC	172	182	(K)EGEATLAPSLD(L)	n/a	101.3%
AhpC	170	186	(K)WKEGEATLAPSLDLVGK(I)	n/a	103.2%
DJ-1	157	164	(R)GPGTSFEF(A)	n/a	103.2%
DJ-1	9	12	(V)ILAK(G)	n/a	106.3%
DJ-1	35	41	(T)VAGLAGK(D)	n/a	107.1%
DJ-1	153	156	(I)LTSR(G)	n/a	107.7%
DJ-1	8	12	(L)VILAK(G)	n/a	108.0%
DJ-1	36	41	(V)AGLAGK(D)	n/a	110.8%
DJ-1	133	145	(K)MMNGGHYTYSENR(V)	Oxidized	110.9%
AhpC	107	120	(R)NFDNMREDEGLADR(A)	n/a	110.9%
DJ-1	185	188	(P)LVLK(D)	n/a	113.7%
AhpC	109	120	(F)DNMREDEGLADR/(A)	n/a	115.9%
DJ-1	6	12	(R)ALVILAK(G)	n/a	117.1%
DJ-1	183	188	(K)APLVLK(D)	n/a	118.8%
AhpC	172	186	(K)EGEATLAPSLDLVGK(I)	n/a	122.5%
AhpC	94	106	(K)YAMIGDPTGALTR(N)	n/a	123.7%
AhpC	170	187	(K)WKEGEATLAPSLDLVGKI(-)	n/a	124.1%
DJ-1	176	182	(K)EVAAQVK(A)	n/a	131.1%
AhpC	64	80	(K)LGVDVYAVSTDTHFTHK(A)	n/a	131.9%

*Relative ion abundance was calculated by dividing the average remaining ion abundance after IR irradiation by the average ion abundance prior to irradiation