Post-Translational Modification Analysis

By Liquid Chromatography—Tandem Mass Spectrometry

Introduction

Proteins are subjected to a wide variety of covalent modifications after translation. Many of these post-translational modifications (PTMs) are critical to the protein's function. The common PTMs includes Phosphorylation, Ubiquitination, Methylation, Acetylation and Glycosylation. Our accumulated experiences enabled us to develop reliable workflows using available tools and technologies for the identification of PTMs.

Poochon's protein PTM analysis services are designed for identification of PTMs, including S/T/Y phosphorylation, R/K methylation, K/H acetylation, K ubiquitination, etc., from crude, partially purified, or purified protein samples by using a combination of liquid chromatography and tandem mass spectrometry (LC-MS/MS).

Specifications

- → **Method** – Protease digestion and LC-MS/MS
- → **Key Instruments** – Thermo Scientific™ Orbitrap Exploris™ 480 Mass Spectrometer, Thermo Scientific™ Vanquish™ Neo UHPLC System
- → **Detection Limit** – Target protein: ≥0.1 µg for PTM analysis; ≥1 ng for protein identification
- → **Acceptable Samples** – Purified protein in solution or dried (≥0.1 µg/sample), partially purified samples (≥1 µg/sample), tissue/cell lysate (≥100 µg/sample), cell pellet (>1 million cells/sample), solid tissue (≥50 mg/sample)
- → **Turnaround Time** – Typically, reports are available within 5 business days of sample receipt

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Schematic of Procedure Workflow

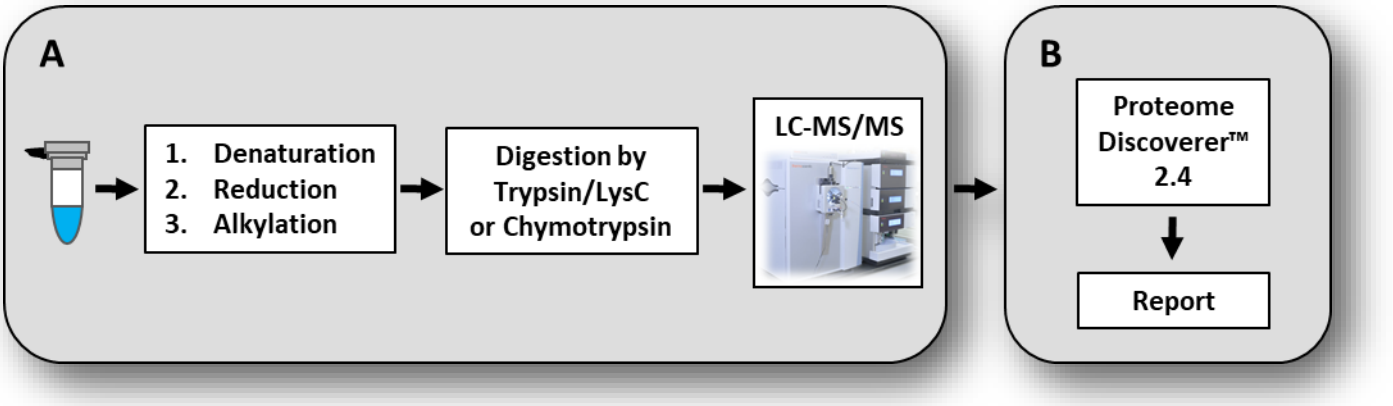

Figure 1:

A) Workflow for PTM and Protein ID analysis. *Note: LC-MS/MS = liquid chromatography and tandem mass spectrometry*

B) Bioinformatic analysis approach used for protein peptide and PTM identification of datasets.

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Example One

Identification of conversion of the proline (P) residue at amino acid 45 to glutamic acid (E) in human protein Peroxiredoxin-6

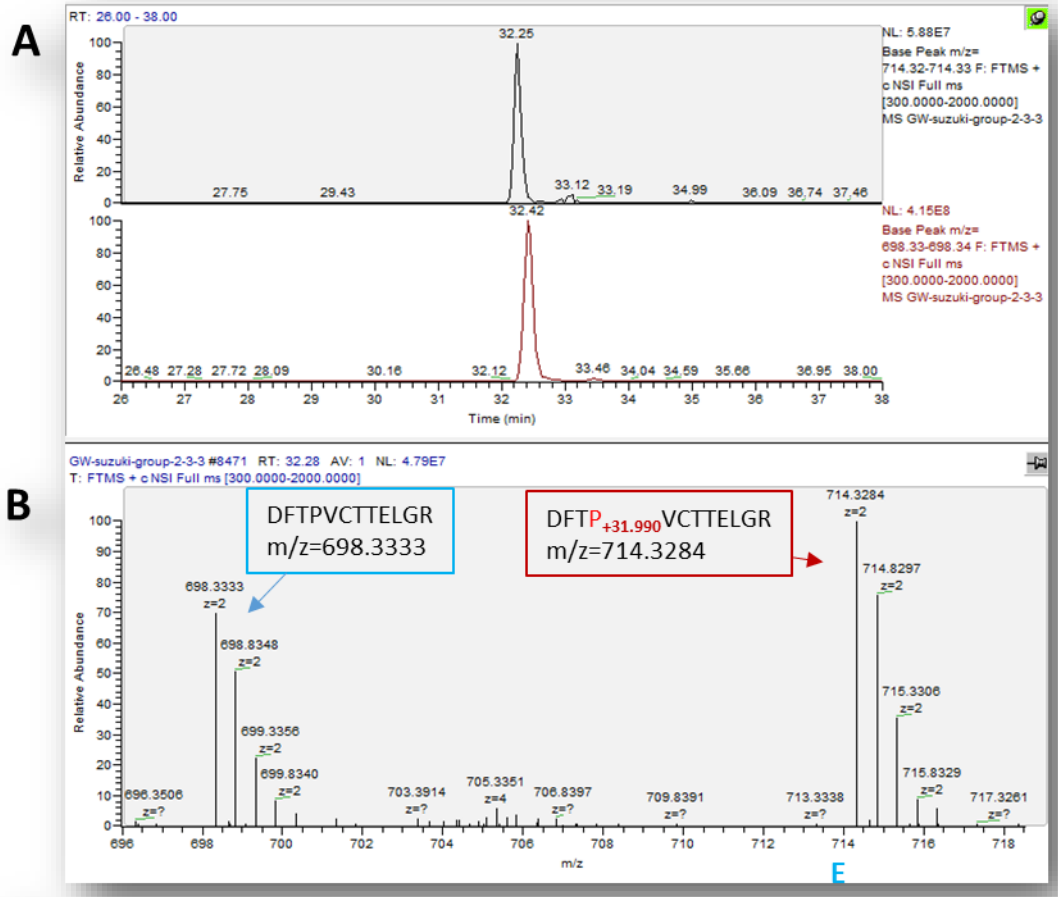

Figure 1: Nano LC-MS/MS verification of conversion of Pro 45 to Glu at PRDX6 (DFTP$_{+31.990}$VCTTELGR). **A)** Extracted ion chromatograms of PRDX6 peptide (DFTP$_{+31.990}$VCTTELGR, +2 charge, m/z=714.33) (top), and its non-conversion counterpart (DFTPVCTTELGR, +2 charge, m/z=698.33) (bottom). Both peptides were eluted at the same retention time and are from affinity-enriched cultured human cell extract using anti-PRDX6 antibody. **B)** High resolution MS spectra of co-elution of peptides (DFTP$_{+31.990}$VCTTELGR, +2 charge, m/z=714.33) (right), and its non-conversion counterpart (DFTPVCTTELGR, +2 charge, m/z=698.33) (left). **C)** Protein sequence

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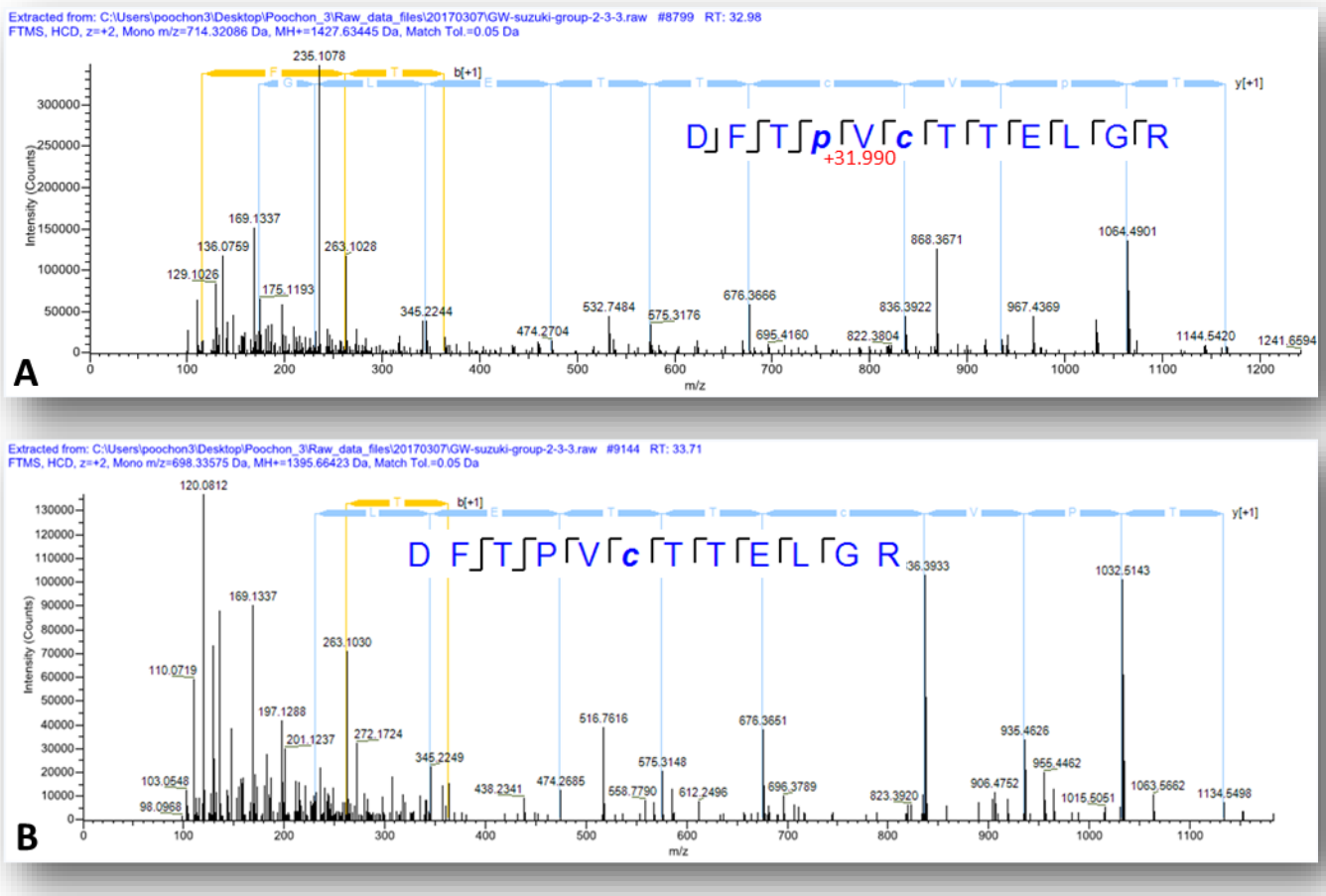

Figure 2: Nano LC-MS/MS verification of conversion of proline 45 to glutamic acid at PRDX6 (DFTP$_{+31.990}$VCTTELGR). **A)** High resolution MS/MS spectra of PRDX6 Glu conversion peptide (DFTP$_{+31.990}$VCTTELGR). **B)** High resolution MS/MS spectra of PRDX6 Pro 45 peptide (DFTPVCTTELGR).

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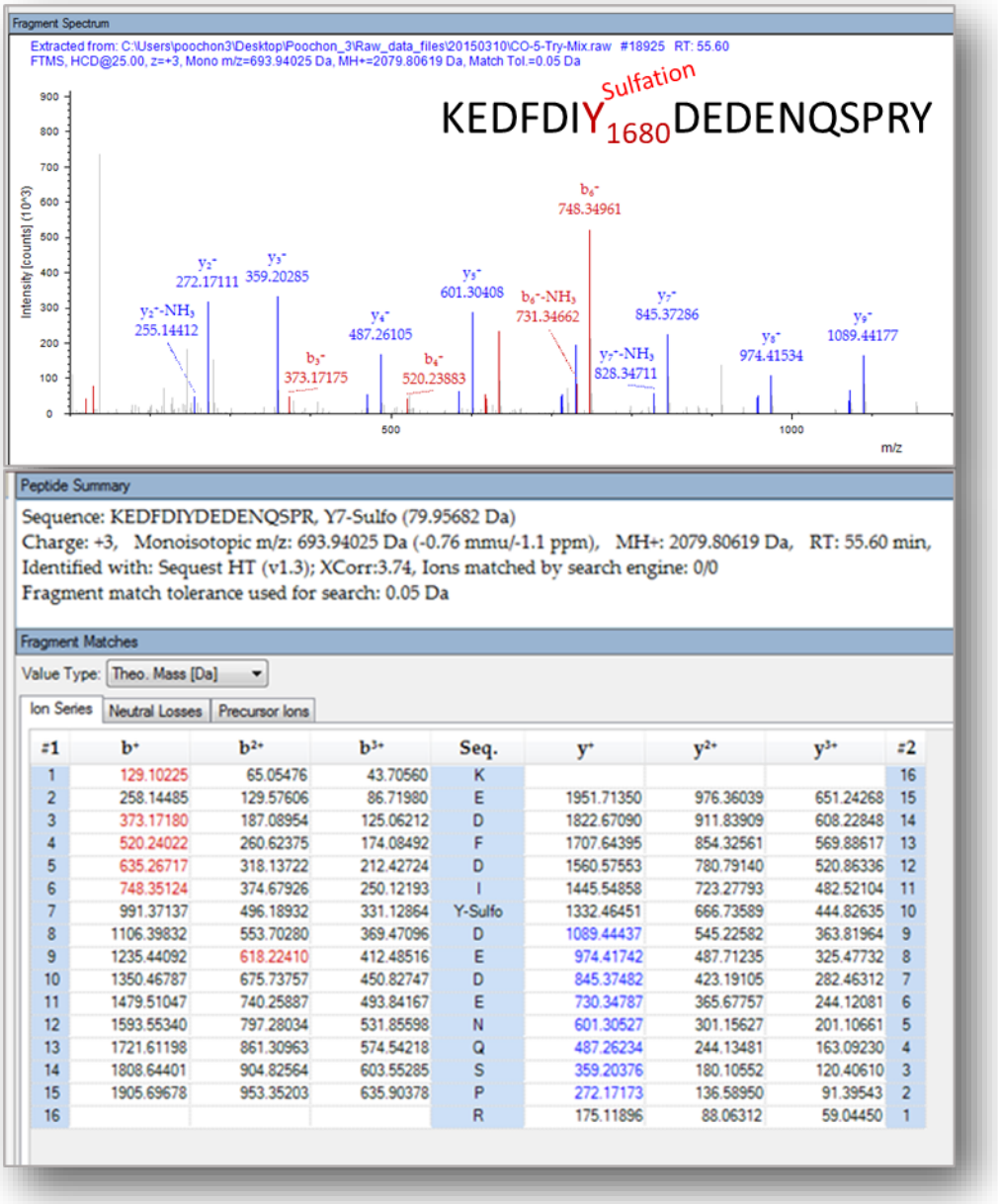
Example Two

PTM Analysis of Factor VIII



#1	b+	b2+	b3+	Seq.	y+	y2+	y3+	#2
1	129.10225	65.05476	43.70560	K				16
2	258.14485	129.57606	86.71980	E	1951.71350	976.36039	651.24268	15
3	373.17180	187.08954	125.06212	D	1822.67090	911.83909	608.22848	14
4	520.24022	260.62375	174.08492	F	1707.64395	854.32561	569.88617	13
5	635.26717	318.13722	212.42724	D	1560.57553	780.79140	520.86336	12
6	748.35124	374.67926	250.12193	I	1445.54858	723.27793	482.52104	11
7	991.37137	496.18932	331.12864	Y-Sulfo	1332.46451	666.73589	444.82635	10
8	1106.39832	553.70280	369.47096	D	1089.44437	545.22582	363.81964	9
9	1235.44092	618.22410	412.48516	E	974.41742	487.71235	325.47732	8
10	1350.46787	675.73757	450.82747	D	845.37482	423.19105	282.46312	7
11	1479.51047	740.25887	493.84167	E	730.34787	365.67757	244.12081	6
12	1593.55340	797.28034	531.85598	N	601.30527	301.15627	201.10661	5
13	1721.61198	861.30963	574.54218	Q	487.26234	244.13481	163.09230	4
14	1808.64401	904.82564	603.55285	S	359.20376	180.10552	120.40610	3
15	1905.69678	953.35203	635.90378	P	272.17173	136.58950	91.39543	2
16				R	175.11896	88.06312	59.04450	1

Figure 3: Analysis of tyrosine sulfation of Factor VIII by LC-MS/MS. High resolution MS/MS spectra of one peptide (KEDFDIY$_{1680}$DEDENQSPRY) with sulfation on Y1680.

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Example Two

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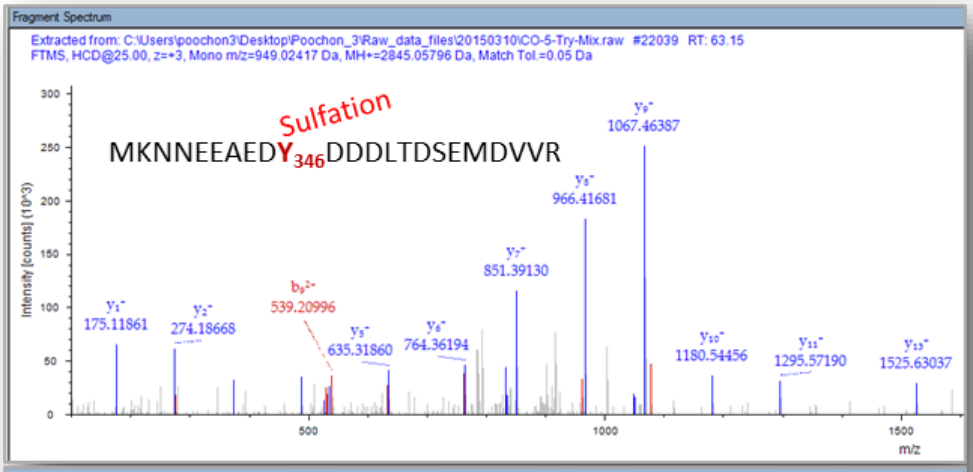

Peptide Summary

Sequence: MKNNEEAEDYDDDLTDSEMDVVR, M1-Oxidation (15.99492 Da), Y10-Sulfo (79.95682 Da), M19-Ox
Charge: +3, Monoisotopic m/z: 949.02417 Da (-0.88 mmu/-0.93 ppm), MH+: 2845.05796 Da, RT: 63.15 min,
Identified with: Sequest HT (v1.3); XCorr:6.17, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.05 Da

Fragment Matches

Value Type: Theo. Mass [Da]

Ion Series | Neutral Losses | Precursor Ions

#1	b+	b2+	b3+	Seq.	y+	y2+	y3+	#2
1	148.04269	74.52498	50.01908	M-Oxidation				23
2	276.13766	138.57247	92.71740	K	2698.02518	1349.51623	900.01324	22
3	390.18059	195.59393	130.73171	N	2569.93021	1285.46874	857.31492	21
4	504.22352	252.61540	168.74602	N	2455.88728	1228.44728	819.30061	20
5	633.26612	317.13670	211.76022	E	2341.84435	1171.42581	781.28630	19
6	762.30872	381.65800	254.77442	E	2212.80175	1106.90451	738.27210	18
7	833.34584	417.17656	278.45346	A	2083.75915	1042.38321	695.25790	17
8	962.38844	481.69786	321.46766	E	2012.72203	1006.86465	671.57886	16
9	1077.41539	539.21133	359.80998	D	1883.67943	942.34335	628.56466	15
10	1320.43553	660.72140	440.81669	Y-Sulfo	1768.65248	884.82988	590.22234	14
11	1435.46248	718.23488	479.15901	D	1525.63235	763.31981	509.21563	13
12	1550.48943	775.74835	517.50133	D	1410.60540	705.80634	470.87332	12
13	1665.51638	833.26183	555.84364	D	1295.57845	648.29286	432.53100	11
14	1778.60045	889.80386	593.53833	L	1180.55150	590.77939	394.18868	10
15	1879.64813	940.32770	627.22089	T	1067.46743	534.23735	356.49399	9
16	1994.67508	997.84118	665.56321	D	966.41975	483.71351	322.81143	8
17	2081.70711	1041.35719	694.57389	S	851.39280	426.20004	284.46912	7
18	2210.74971	1105.87849	737.58809	E	764.36077	382.68402	255.45844	6
19	2357.78512	1179.39620	786.59989	M-Oxidation	635.31817	318.16272	212.44424	5

Figure 4: Analysis of tyrosine sulfation of Factor VIII by LC-MS/MS. High resolution MS/MS spectra of one peptide (MKNNEEAEDY$_{346}$DDDLTDSEMDVVR) with sulfation on Y346.

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Example Three

S1943 Phosphorylation Analysis of MYH9

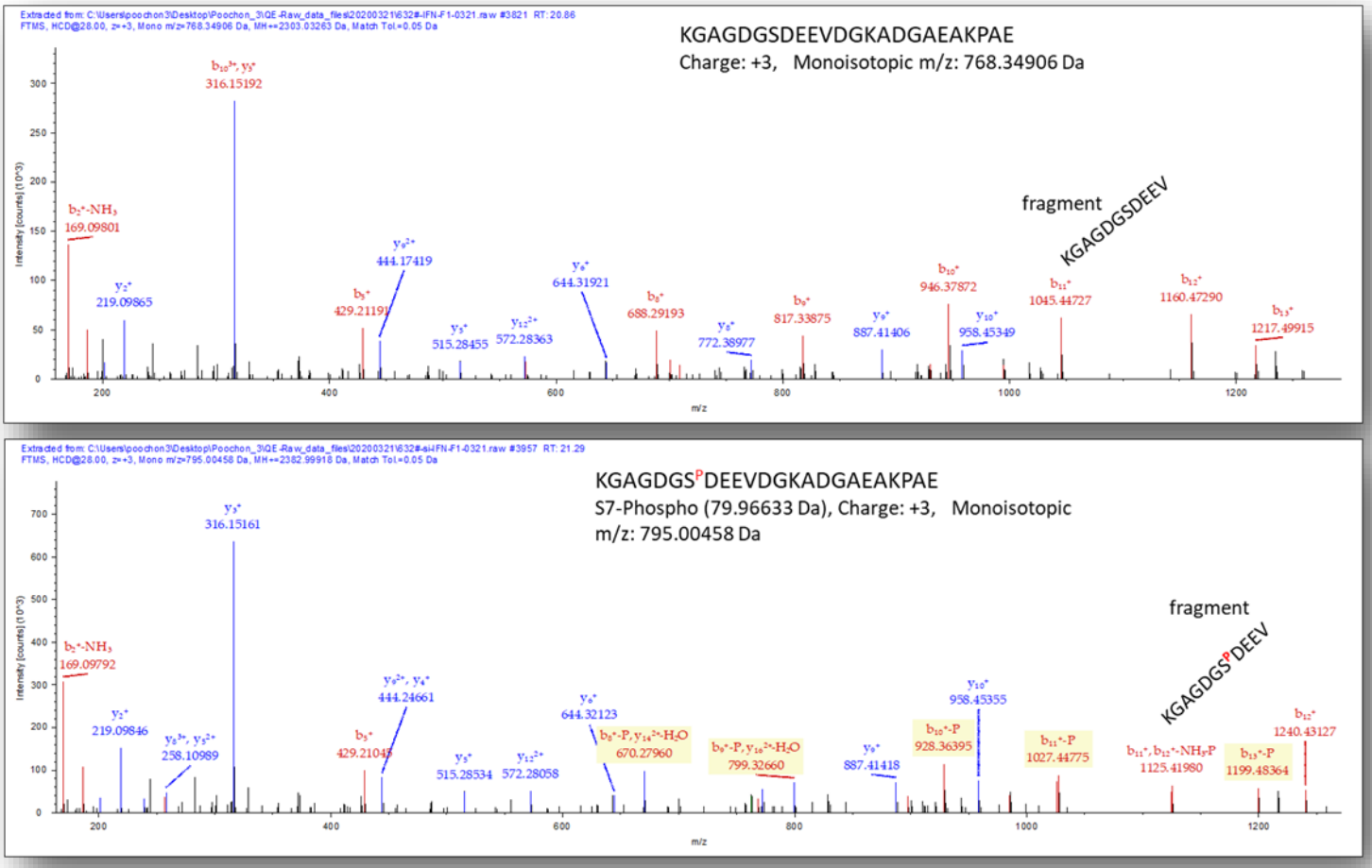

Figure 5: Nano LC-MS/MS verification of MYH9 S1943 phosphorylation. **A)** High resolution MS/MS spectra of MYH9 peptide (KGAGDGSDEEVDGKADGAEAKPAE). **B)** High resolution MS/MS spectra of MYH9 peptide (KGAGDGS[P]DEEVDGKADGAEAKPAE, S7-Phospho (79.96633 Da).

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