Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Magnetite-Doped Polydimethylsiloxane (PDMS) for Phosphopeptide Enrichment

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Table S-1: *The sequence of β-Casein and predicted m/z values of its tryptic peptides.*

(a) The sequence of β-Casein (obtained from the UniProtKB database). **(b)** A list of predicted peptides with monoisotopic masses ($[M+H]^+$) in the 700-4,000 Da range resulting from a tryptic digest of β-Casein. The masses were obtained from Protein Prospector, assuming a maximum number of missed cleavages of 1 and taking into account known phosphorylation sites.

(a)

1	RELEELNVPG	EIVESLSSSE	ESITRINKKI	EKFQSEEQQQ	TEDELQDKIH
51	PFAQTQSLVY	PFPGPIPNSL	PQNIPPLTQT	PVVVPPFLQP	EVMGVSKVKE
101	AMAPKHKEMP	FPKYPVEPFT	ESQSLTLTDV	ENLHLPLPLL	QSWMHQPHQP
151	LPPTVMFPPQ	SVLSLSQSKV	LPVPQKAVPY	PQRDMPIQAF	LLYQEPVLGP
201	VRGPFPIIV				

(b)

m/z **(m_i)**	**Modifications**	**Start**	**End**	**Missed Cleavages**	**Sequence**
742.450		203	209	0	GPFPIIV
748.370		108	113	0	EMPFPK
780.498		170	176	0	VLPVPQK
830.452		177	183	0	AVPYPQR
873.486		98	105	1	VKEAMAPK
911.477		100	107	1	EAMAPKHK
1013.524		106	113	1	HKEMPFPK
1591.932		170	183	1	VLPVPQKAVPYPQR
1981.862		33	48	0	FQSEEQQQTEDELQDK
2061.829	1 Phospho	33	48	0	FQSEEQQQTEDELQDK
2186.168		184	202	0	DMPIQAFLLYQEPVLGPVR
2352.084		30	48	1	IEKFQSEEQQQTEDELQDK(I)
2432.050	1 Phospho	30	48	1	IEKFQSEEQQQTEDELQDK
2646.299		2	25	0	ELEELNVPGEIVESLSSSEESITR
2726.266	1 Phospho	2	25	0	ELEELNVPGEIVESLSSSEESITR
2802.400		1	25	1	RELEELNVPGEIVESLSSSEESITR
2806.232	2 Phospho	2	25	0	ELEELNVPGEIVESLSSSEESITR
2882.367	1 Phospho	1	25	1	RELEELNVPGEIVESLSSSEESITR
2886.198	3 Phospho	2	25	0	ELEELNVPGEIVESLSSSEESITR
2909.600		184	209	1	DMPIQAFLLYQEPVLGPVRGPFPIIV
2962.333	2 Phospho	1	25	1	RELEELNVPGEIVESLSSSEESITR
2966.165	4 Phospho	2	25	0	ELEELNVPGEIVESLSSSEESITR

2997.602		177	202	1	AVPYPQRDMPIQAFLLYQEPVLGPVR
3001.521		2	28	1	ELEELNVPGEIVESLSSSEESITRINK
3042.299	3 Phospho	1	25	1	RELEELNVPGEIVESLSSSEESITR
3081.488	1 Phospho	2	28	1	ELEELNVPGEIVESLSSSEESITRINK
3122.266	4 Phospho	1	25	1	RELEELNVPGEIVESLSSSEESITR
3161.454	2 Phospho	2	28	1	ELEELNVPGEIVESLSSSEESITRINK
3241.420	3 Phospho	2	28	1	ELEELNVPGEIVESLSSSEESITRINK
3321.387	4 Phospho	2	28	1	ELEELNVPGEIVESLSSSEESITRINK

Table S-2: *m/z values of potential LMW siloxane contaminants.*

Peak lists generated from spectra of eluted phosphopeptide fractions were searched for both cyclic and linear forms of potential PDMS oligomer contaminants (as there are reports of both in the literature). The dimethylsiloxane (C_2H_6SiO) monomer has a mass of 74.019 Da and example *m/z* values for siloxane oligomers with 5 repeating units are provided below. The lists were searched for any sequences of peaks with a 74.019 *m/z* difference for all repeating unit lengths with *m/z* values from 100 to 3,600, including searching for sodium (with values greater than those below by *m/z* 21.982) and ammonium (values greater by *m/z* 17.027) adducts. No corresponding sequences were found.

***m/z* Values for Oligomers with Five Repeating Units**	
$+ H^+$	371.102
$+CH_3 +Si(CH_3)_3 +H^+$	459.173
$+CH_3 +Si(CH_3)_2OH +H^+$	461.152
$+OH +Si(CH_3)_2OH +H^+$	463.131

Table S-3: *Casein and HeLa Phosphopeptides used to generate XIC in figures*

a) Casein peptides and phosphopeptides used to generate figure 4.

m/z	Charge	Retention time (min)	Sequence	Protein	Label in figure
797.877	2+	58	K.TVDMESTEVFTKK.T + Phospho (ST)	α-S2-casein	P1
733.828	2+	71	K.TVDMESTEVFTK.K + Phospho (ST)	α-S2-casein	P2
651.335	3+	79	K.YKVPQLEIVPNSAEER.L + Phospho (ST)	α-S1-casein	P3
830.92	3+	80	K.VPQLEIVPNSAEER.L + Phospho (ST)	α-S1-casein	P4
373.74	2+	46	K.VIPYVR.Y	α-S2-casein	N1
579.764	2+	48	K.ENLCSTFCK.E	α-S2-casein	N2
598.357	2+	56	R.NAVPITPTLNR.E	α-S2-casein	N3
446.576	2+	68	K.HIQKEDVPSER.Y	α-S1-casein	N4

b) Hea cell phosphopeptides used to generate figure 5.

m/z	Charge	Retention time (min)	Sequence	Protein source	Label in figure
785.8333	2+	50	R.LTPVSPESSSTEEK.S + Phospho (ST)	Sequestosome-1	P1
503.5609	2+	53	K.AEEDEILNRSPR.N + Phospho (ST)	Calnexin	P2
701.307	2+	56	R.LRLSPSPTSQR.S + 2 Phospho (ST)	Prelamin-A/C	P3
709.3091	3+	67	K.EYIPGQPPLSQSSDSSPTR.N + Phospho (ST)	Bifunctional glutamate/proline--tRNA ligase	P4
735.6183	3+	74	K.GNAEGSSDEEGKLVIDEPAK.E + 2 Phospho (ST)	Hepatoma-derived growth factor	P5
865.8424	4+	80	K.CGSGPVHISGQHLVAVEEDAESEDEEEEDVK.L + Phospho (ST)	Nucleophosmin	P6
915.1088	3+	80	K.KPATPAEDDEDDDIDLFGSDNEEEDKEAAQLR.E + Phospho (ST)	Elongation factor 1-delta	P7
985.9095	4+	91	K.VQGEAVSNIQENTQTPTVQEESEEEEVDETGVEVK.D + Phospho (ST)	Nascent polypeptide-associated complex subunit alpha	P8
742.3148	3+	109	K.DELHIVEAEAMNYEGSPIK.V + Phospho (ST)	Nucleophosmin	P9
982.9263	2+	109	K.SSSPAPADIAQTVQEDLR.T + Phospho (ST)	Ras GTPase-activating protein-binding protein 1	P10

Figure S-1: *Typical spectra demonstrating phosphopeptide enrichment of a β-Casein digest using a magnetite-PDMS microwell.*

(a) MALDI-TOF spectra obtained from an unenriched β-Casein sample (lower spectrum) and from an enriched fraction eluted from a magnetite-PDMS well (upper spectrum). (b&c) Expanded views of the monophosphate (b) and tetraphosphate (c) regions, with the upper spectra being from the phosphopeptide enriched fraction and the lower spectra from the unenriched digest. These spectra were obtained using a 50 ng sample of trypsin digested β-Casein and an incubation time of 5 min in the magnetite-PDMS well. The numbered peaks correspond to the β-Casein tryptic peptides listed in the top right box, with *m/z* 3122.3 being the tetraphosphate, 3042.3 and 2962.3 being the tetraphosphate with the loss of one (-80 *m/z*) or two (-160*m/z*) phosphate groups respectively, and 2061.8 being the monophosphate. Other significant peaks that can be seen in the upper panel of (c) include those corresponding to dephosphorylation by β-elimination (-98 *m/z*) and metastable decay of the ions.

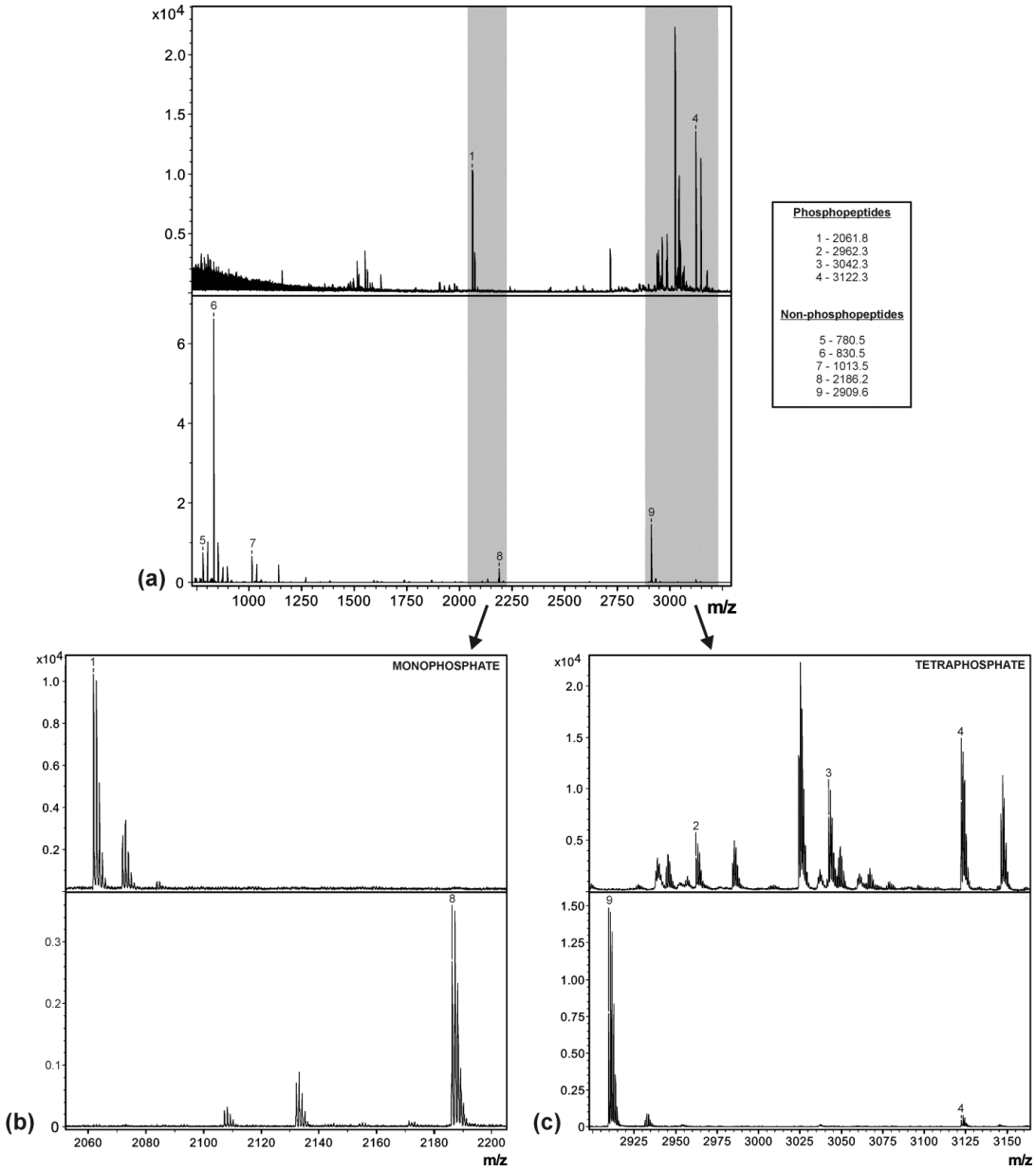

Figure S-2: *The effect of acetonitrile (*ACN*) in the elution buffer on the spectra obtained from fractions enriched for phosphopeptides.*

Four traces, highlighting a region containing the tetraphosphate peptide peak (*m/z*, +1, 3122.35) and other related peaks resulting from dephosphorylation and metastable decay of ions, are shown for spectra obtained with different sample loading and elution conditions. The upper two panels show spectra acquired from fractions eluted using 10% ACN in the 0.1 M ammonium hydroxide eluent, whilst the lower two spectra correspond to the use of 0.1 M ammonium hydroxide alone. The first and third traces were acquired from 20 ng β-casein samples enriched for phosphopeptides, the second and the fourth from 50 ng samples. In order to detect any peaks with the 10% ACN eluent, the laser power had to be increased from that used for the ACN -free eluent samples. It can be seen that the resolution of the resulting spectra is noticeably poorer, with strongly overlapping isotopic peaks.