Supplementary Information

New Cellulose-Silica Composite IMAC/C18 for Selective Enrichment of Phosphorylated Molecules and Improved Recovery of Hydrophilic Species

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Materials and Reagents

The chemicals and reagent used in this study are given in the Supporting information. Silica gel 60 (pore size 60 Å, 200-425 mesh, ≥98.5%), microgranular cellulose, (aminopropyl) trimethoxy silane (97%), iminodiacetic acid (IDA, 99%), octadecyl amine (ODA), potassium iodate (KIO_4), sodium acetate trihydrate ($CH_3COONa.3H_2O$), perchlorate ($HClO_4$), phospholipid mixture from soyabean, Bovine α-, β-casein, lysozyme, cytochrome c, myoglobin and BSA were purchased from Sigma-Aldrich. Ammonium hydroxide, acetic acid, toluene (99%), triethylamine (99.5%), dichloromethane, acetonitrile (ACN), were purchased from Fluka. Methanol (≥99.8%) was purchased from Merck. Ammonium hydrogen carbonate (NH_4HCO_3), and trypsin were obtained from Sigma (St. Luis, MO, USA). Cyano-4-hydroxycinnamic acid (HCCA) was from Bruker Daltonics (Madison, WI, USA). Tetrahydrofuran (THF) and thionyl chloride were purchased from domestic supplier. Purified water was used for all the experiments.

Synthesis of Aminopropyl Bonded Silica

One gram silica gel was suspended in 10 mL of anhydrous toluene; 1.5 mL silane reagent (trimethoxy-aminopropyl silane) was added into the suspension and mixture was refluxed for 10 h at 110 °C. The resulting product was washed with toluene, followed by dichloromethane and methanol-water (1:1). Derivatized silica was refluxed in methanol-water (1:1) for an hour at 70 °C to hydrolyze the un-reacted methoxy groups. Finally aminopropyl bonded silica (product A) was washed with methanol and dried under vacuum using rotary evaporator.

Selective Oxidation of Cellulose

Three gram cellulose was stirred with 0.01 M, 300 mL aqueous solution of potassium iodate (KIO_4) for 6 h at 25 °C, to form aldehyde group at C_2-C_3. The resulting product was filtered and mixed with 0.2 M, 300 mL aqueous solution of sodium chlorite ($NaClO_2$) which pH was

maintained at 6 using 0.1 M, 100 mL solution of CH_3COOH/CH_3COONa buffer in the ratio (1:18). The reaction mixture was stirred for 24 h at 25 °C. During this step chlorite (III) oxidized the aldehydes to carboxyl groups. Oxidized cellulose was refluxed in 10 mL dry thionyl chloride for 24 h at 76 °C. The unreacted thionyl chloride was vacuum distilled using rotary evaporator and the resulting brown solid (product B; cellulose acetyl chloride) was used, without air exposure, for further reaction.

Digestion of Standards and Biological Fluids

One milligram of standard protein (β-casein) was suspended in 1 mL of deionized water. For protein mixture, 1 mg of each protein (α-, β-casein, cytochrome c, lysozyme, myoglobin and BSA) was dissolved in 1 mg of deionized water. For non-fat milk and egg yolk, the lyophilized 1 mg powder was used and diluted with 1 mL of water. The resulting solution was aliquoted to 200 µL fractions. In case of serum, 20 µL was diluted with 1 M ammonium bicarbonate (NH_4HCO_3) to the final volume of 200 µL. 160 µL of 1 M aqueous solution of ammonium bicarbonate (NH_4HCO_3) and 50 µL of 45 mM aqueous solution of dithiothreitol (DTT) were added to aliquot. The aliquot was incubated using a thermomixer at 56 °C for 15 min. The resulting solution was cooled to room temperature followed by the addition of 50 µL of 100 mM aqueous solution of iodoacetamide. Subsequently, the solution was incubated for 15 min in dark at room temperature. 1400 µL of de-ionized water was added in the solution followed by adding 2 µg of trypsin (20 µL of 0.1 µg/µL). Protein solution was digested on thermomixer for 14 h at 37 °C. Finally the tryptic digestion was stopped, by acidifying solution with 10 µL of 1% trifluoroacetic acid (pH = 3). Protein digest was incubated for 5 min and stored at -20 °C.

Preparation of HeLa Cell Sample

HeLa cells were cultured in high glucose Dulbecco's modified Eagle's medium, supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 U/mL streptomycin. The cells were cultivated on 150 mm tissue culture plastic dishes at 37 °C in 5% CO_2 and 98% humidity. For total cell lysate preparation, cells were washed twice in ice cold PBS, harvested and centrifuged at 500xg for 4 min at 4 °C. Cells collected from 15 cm dish were re-suspended in 1 mL of lysis buffer (50 mM Tris-HCl, pH 8.0), 0.5% Triton X100, 150 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT, 10 mg/mL leupeptin, 10 mg/mL aprotinin and 1 mM PMSF. Lysis was performed for 30 min at 4 °C followed by the centrifugation at 16000xg for 10 min at 4°C. Protein concentration was determined using Coomassie Plus Protein Assay (Thermo Scientific).

For Selectivity/Sensitivity

β-casein (1 µL) was spiked in de-phosphorylated cell extract (0.1 mL, 0.5 mL, 1 mL, 1.5 mL and 2 mL) to measure the selectivity of cellulose-silica IMAC composite. For sensitivity, 5 dilutions of β-casein were prepared in concentrations of 100, 50, 25, 10 and 1 femtomole. The calculations were cross checked by solution dilution calculator provided by Sigma Aldrich.

Mass Spectrometric Analysis

The eluted content from RP was spotted on the MALDI target plate with 0.5 µL matrix solution (10 µg/µL α-cyano-4-hydroxy-cinnamic acid in 0.1% TFA: ACN, 1:1 v/v). For phosphopeptides, DHB buffer spiked with phosphoric acid was used. For phospholipids, gold nanoparticles with 0.1% TFA and carbon based LDI matrix strategy was applied. Ultraflex I (Bruker Daltonics) MALDI-TOF-MS in reflector mode was used to perform the mass spectrometric analysis. Mass spectra were recorded in the desired mass range. Validation of

all the data, including baseline subtraction, external calibration using Protein Standard I (Bruker Daltonics, Bremen, Germany) and all further data processing, were carried out by Flex analysis 3.3 software and the data acquisition by Flex control 3.0**.**

For serum analysis, mascot search engine was used with parameters as: taxonomy: Homo sapiens, Database: SwissProt, Enzyme: Trypsin, Allowed missed cleavage: 1, Fixed modification: Carboxymethyl (C), Variable modifications: Oxidation (M), Phospho (ST), Phospho (Y), monoisotopic, peptide mass tolerance: 150 ppm.

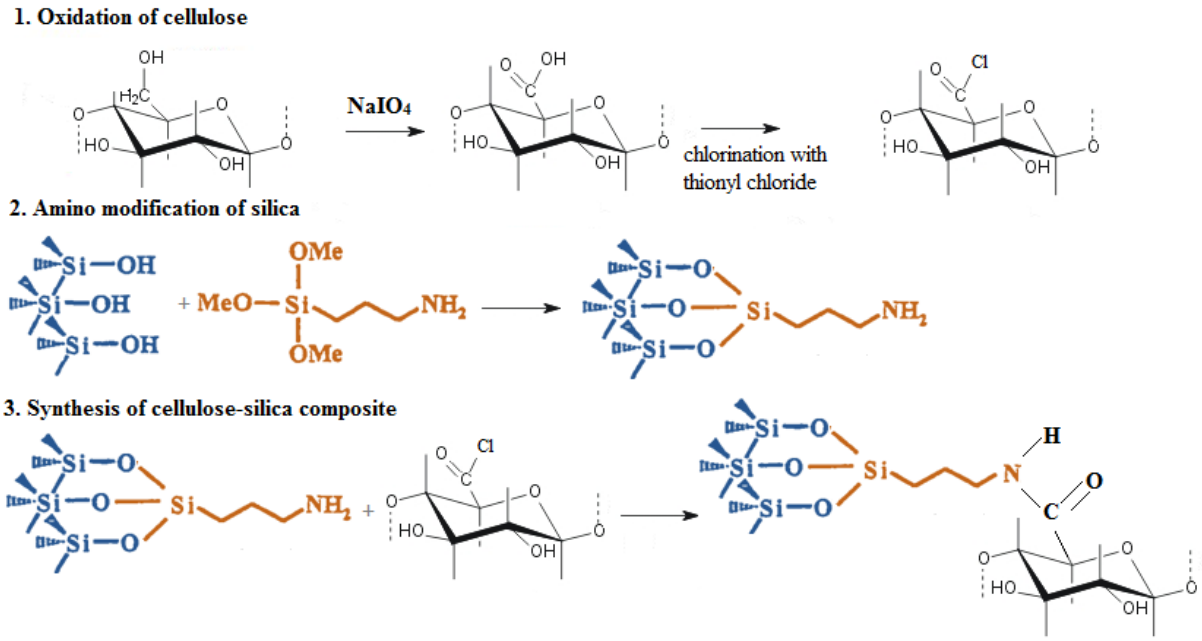

Figure S1: Schematic representation showing synthesis of cellulose-silica composite.

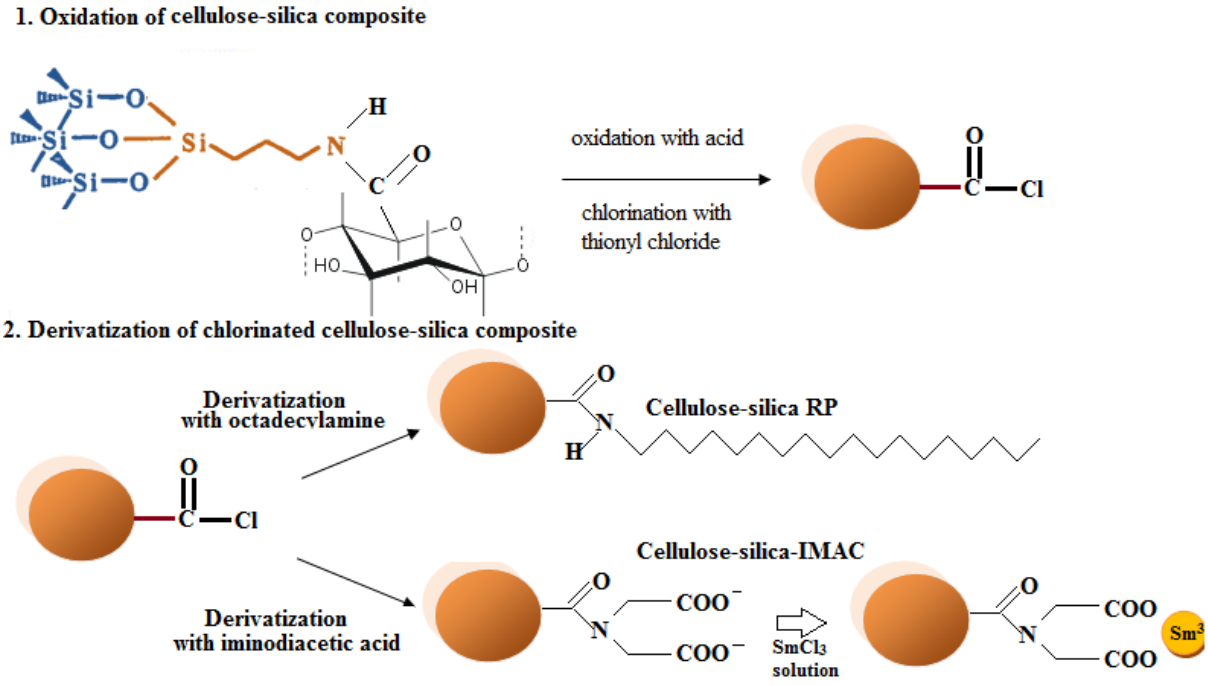

Figure S2: Schematic representation showing derivatization of cellulose-silica composite as IMAC and RP.

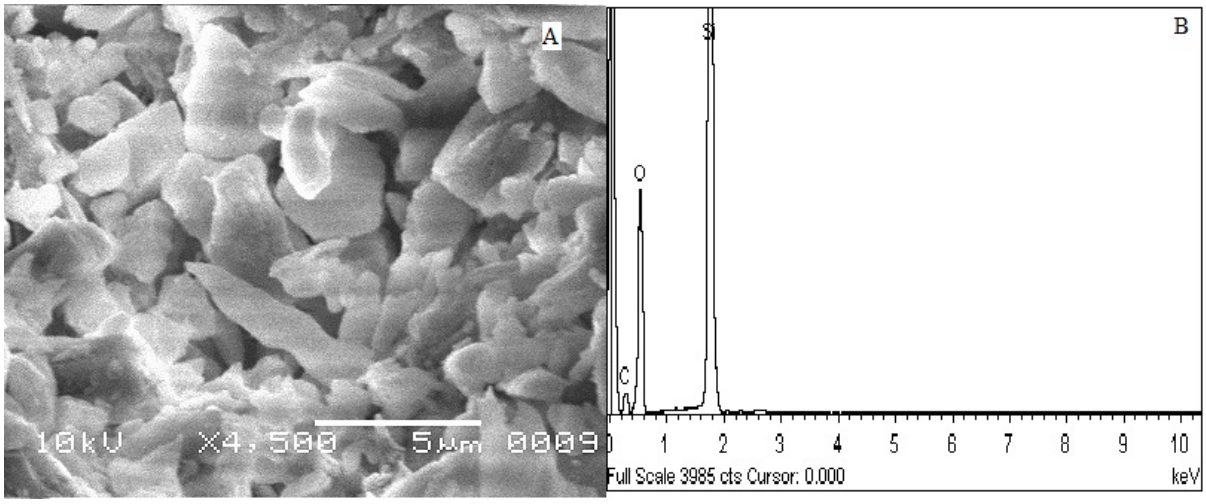

Figure S3: Characterisation of cellulose-silica composite; (A) morphology by SEM (b) elemental analysis by EDX.

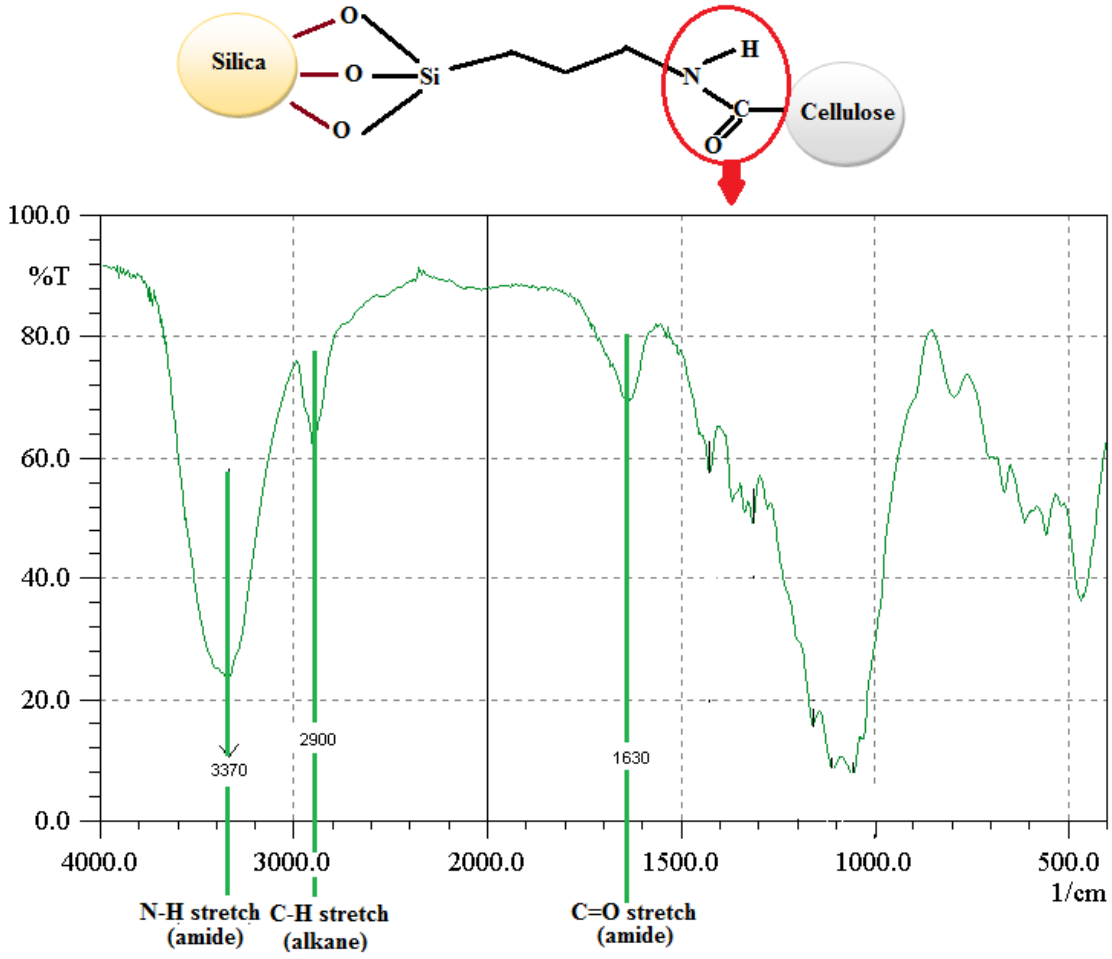

Figure S4: Synthesis of cellulose-silica composite through amide linkage. Pictorial illustration; FT-IR characterisation.

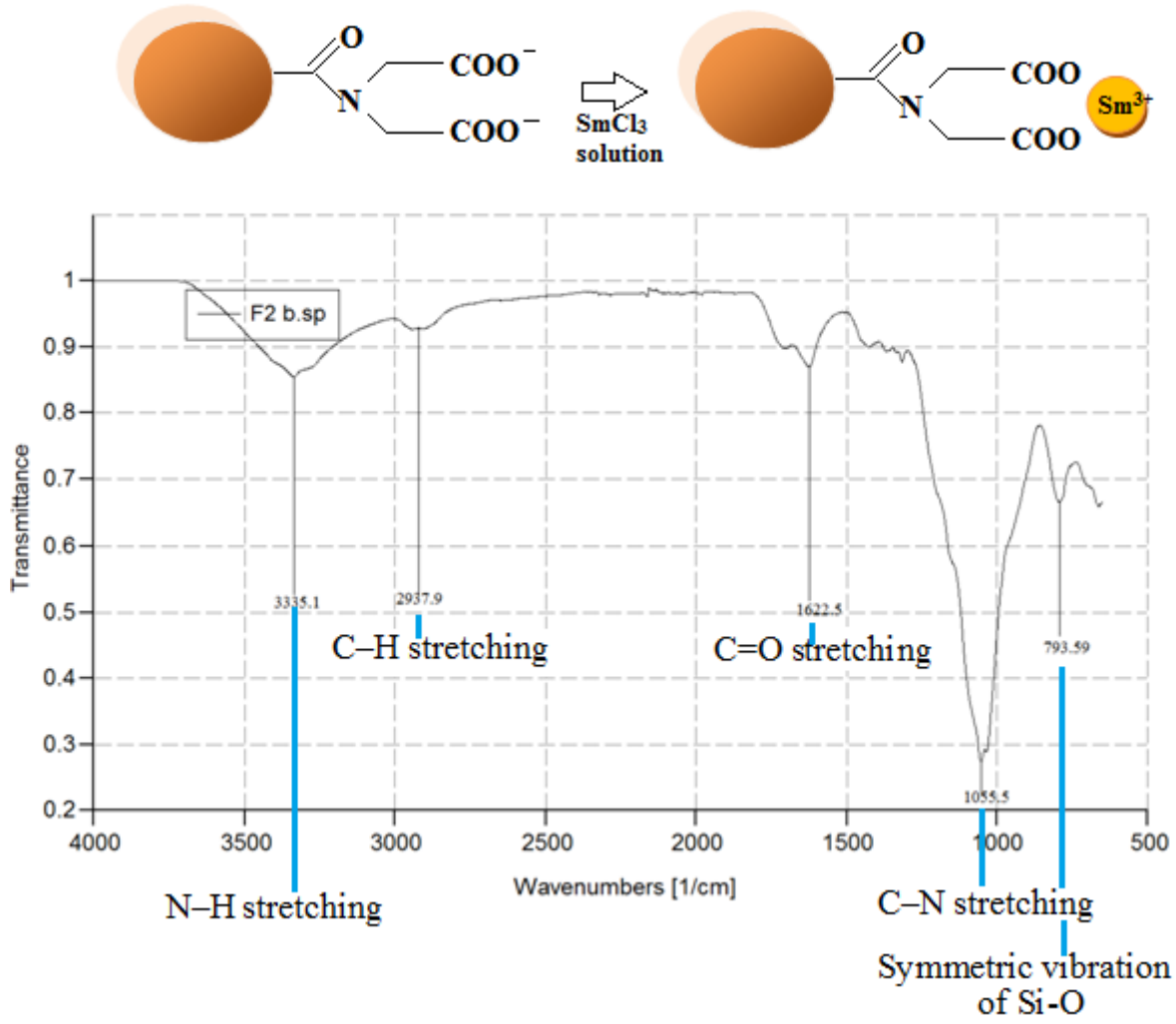

Figure S5: Functionalization of cellulose-silica composite as IMAC through iminodiacetic acid. Pictorial illustration; FT-IR characterisation.

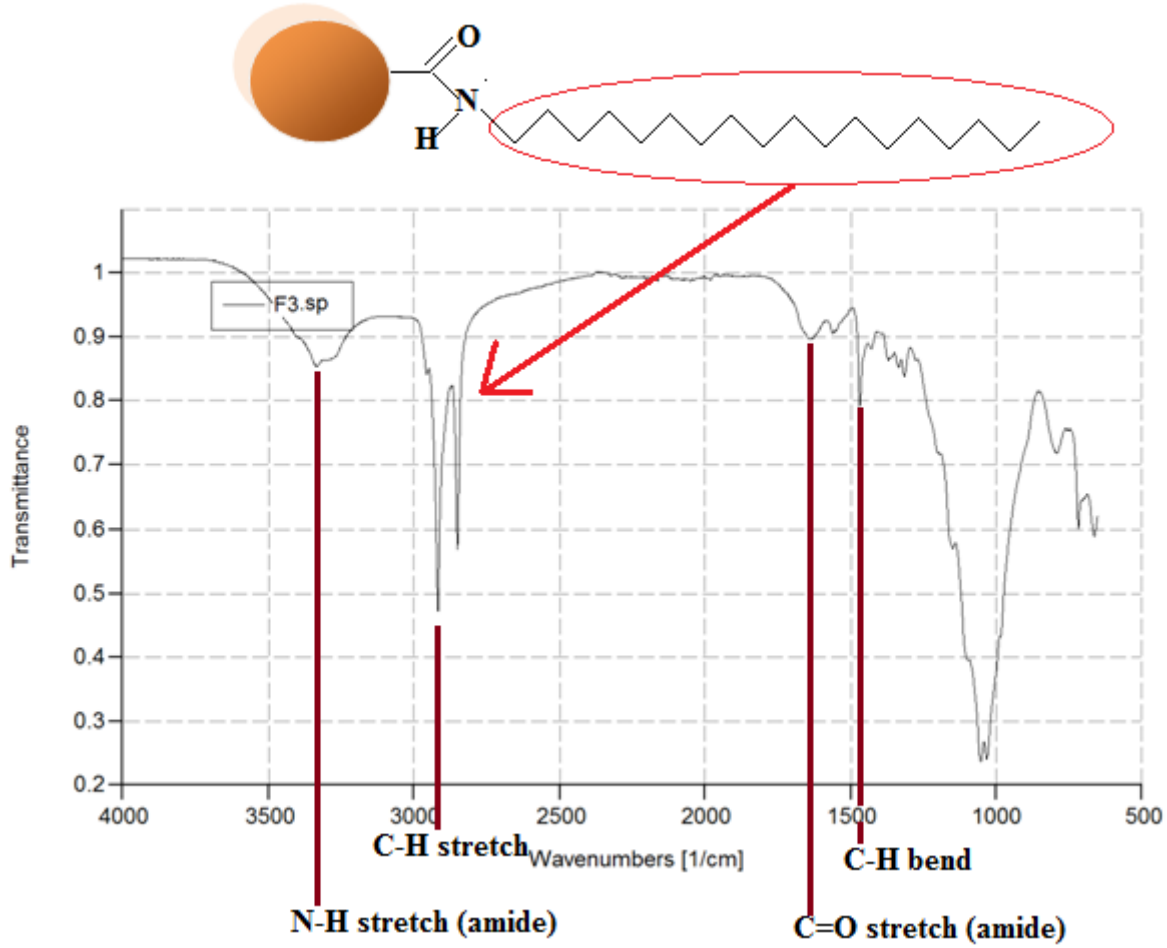

Figure S6: Cellulose-silica composite derivatized as reverse phase with octadecylamine: FT-IR characterisation.

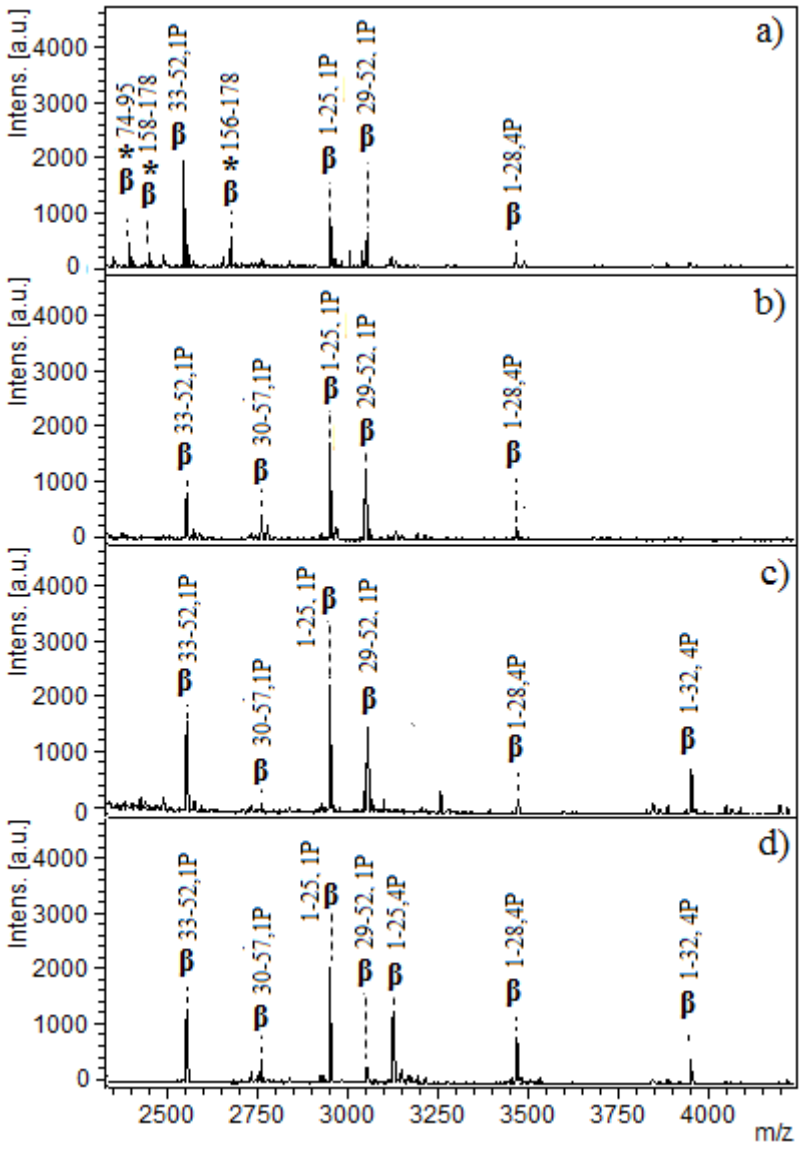

Figure S7: MALDI-MS spectra of tryptic β-casein digest using cellulose-silica-IMAC with immobilization of different metal ions; (a) eluted fraction of phosphopeptides from cellulose-silica-IMAC-Fe^{3+} (b) eluted fraction of phosphopeptides from cellulose-silica-IMAC-Zr^{4+} (c) eluted fraction of phosphopeptides from cellulose-silica-IMAC-La^{3+} (d) eluted fraction of phosphopeptides from cellulose-silica-IMAC-Sm^{3+}. The symbols **β** for phosphopeptides and **β*** for non-phosphopeptides are derived from β-casein. Amino acid position and number of phosphate groups are also given with each m/z value.

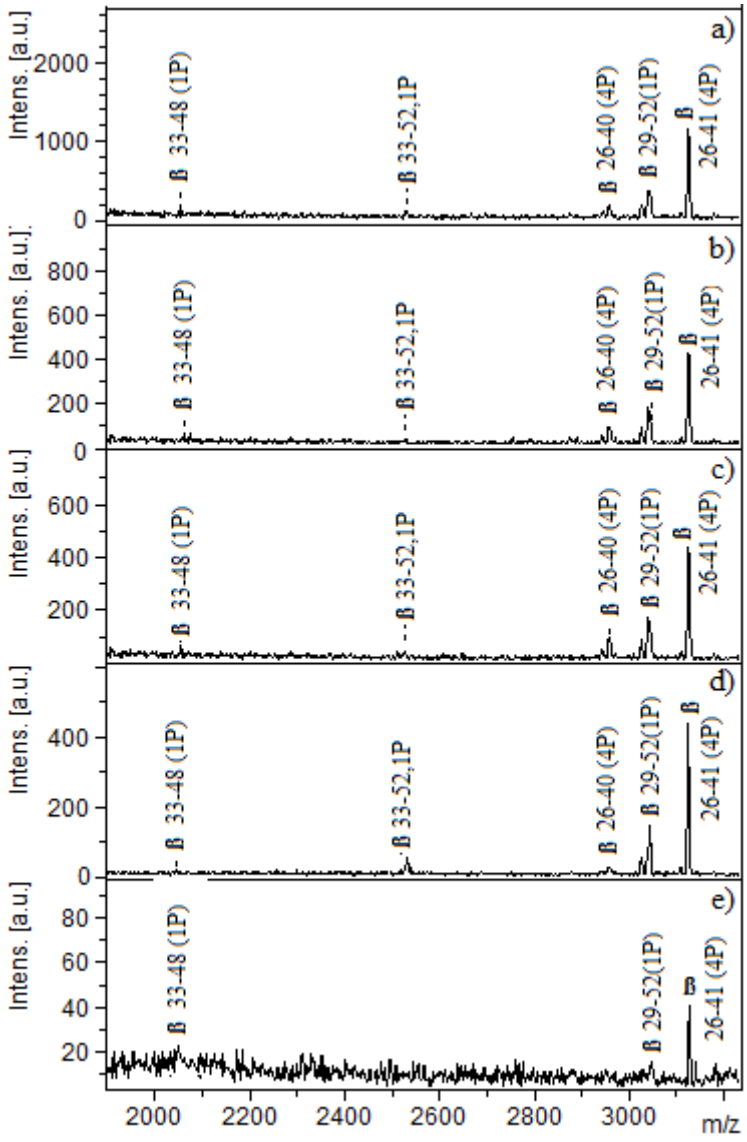

Figure S8: Sensitivity measurements for cellulose-silica-IMAC-Sm^{3+} using dilutions of β-casein digest in concentrations as (a) 100 femtomole (b) 50 femtomole (c) 25 femtomole (d) 10 femtomole and (e) 1 femtomole. The symbols β is used to label phosphopeptide with position and number of phosphorylated serine.

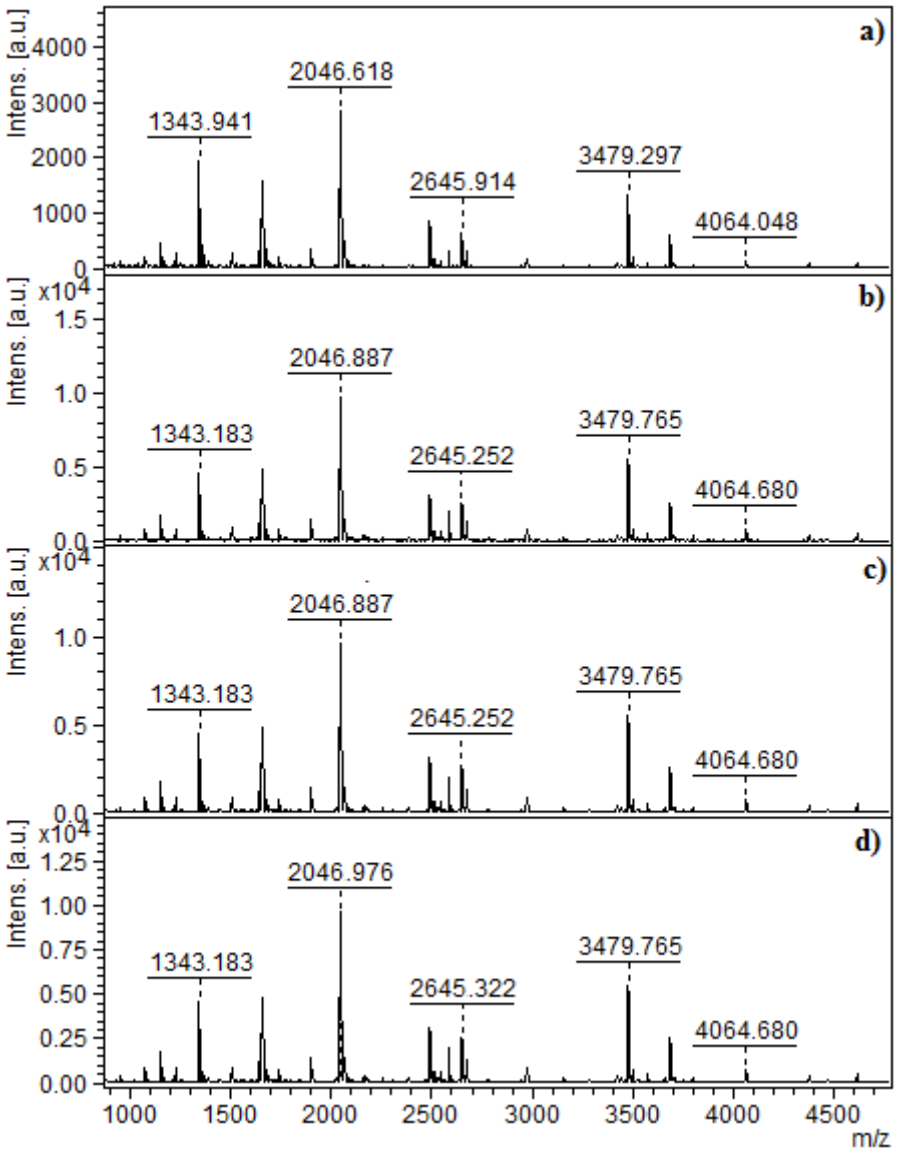

Figure S9: Method and material reproducibility using serum sample on cellulose-silica-IMAC-Sm^{3+}.

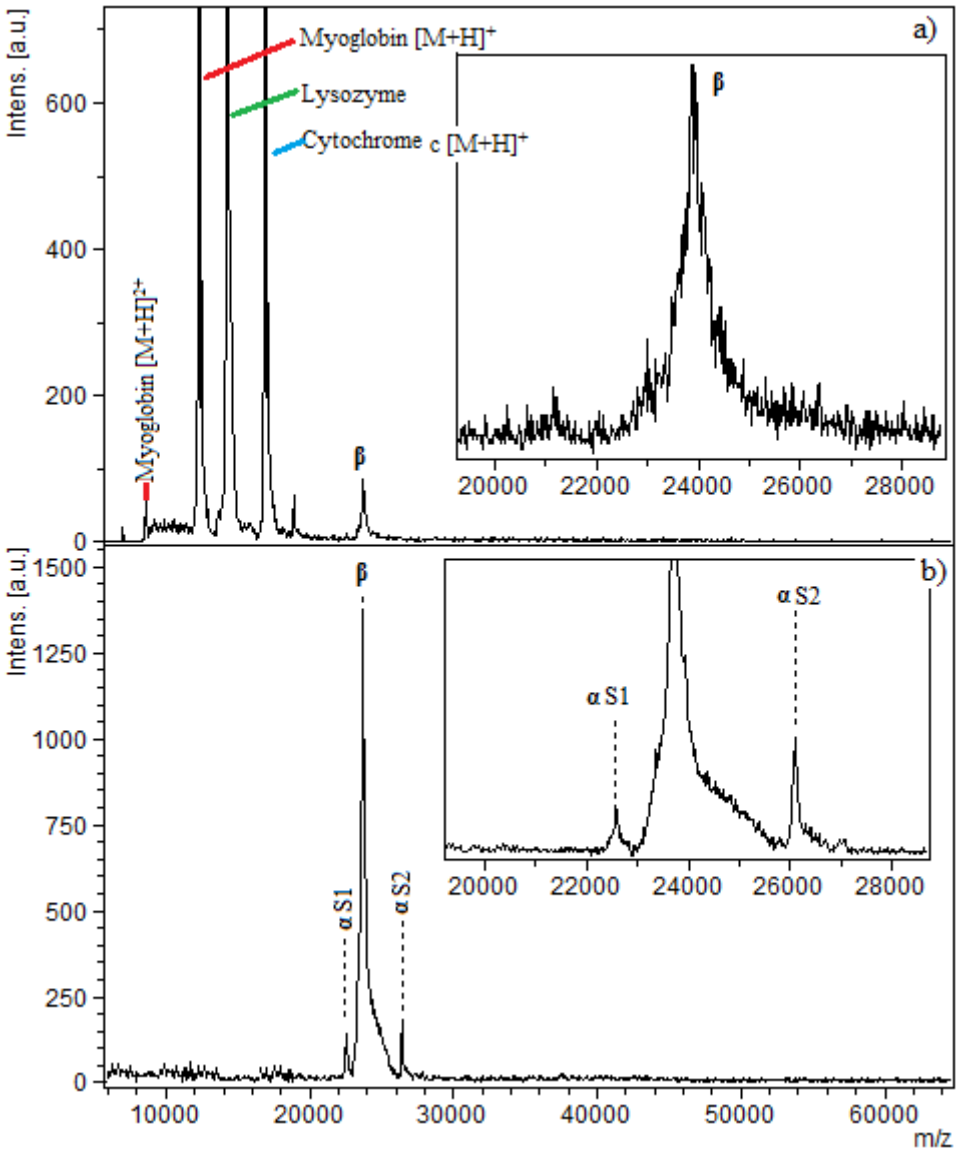

Figure S10: MALDI-MS spectra of enriched phosphoproteins (α-casein, αS1 at 24 kDa; αS2 at 26 kDa and β-casein at 25 kDa) from protein mixture (containing lysozyme, cytochrome c, myoglobin and BSA) (a) before enrichment and (b) after enrichment on cellulose-silica-IMAC-Sm^{3+}.

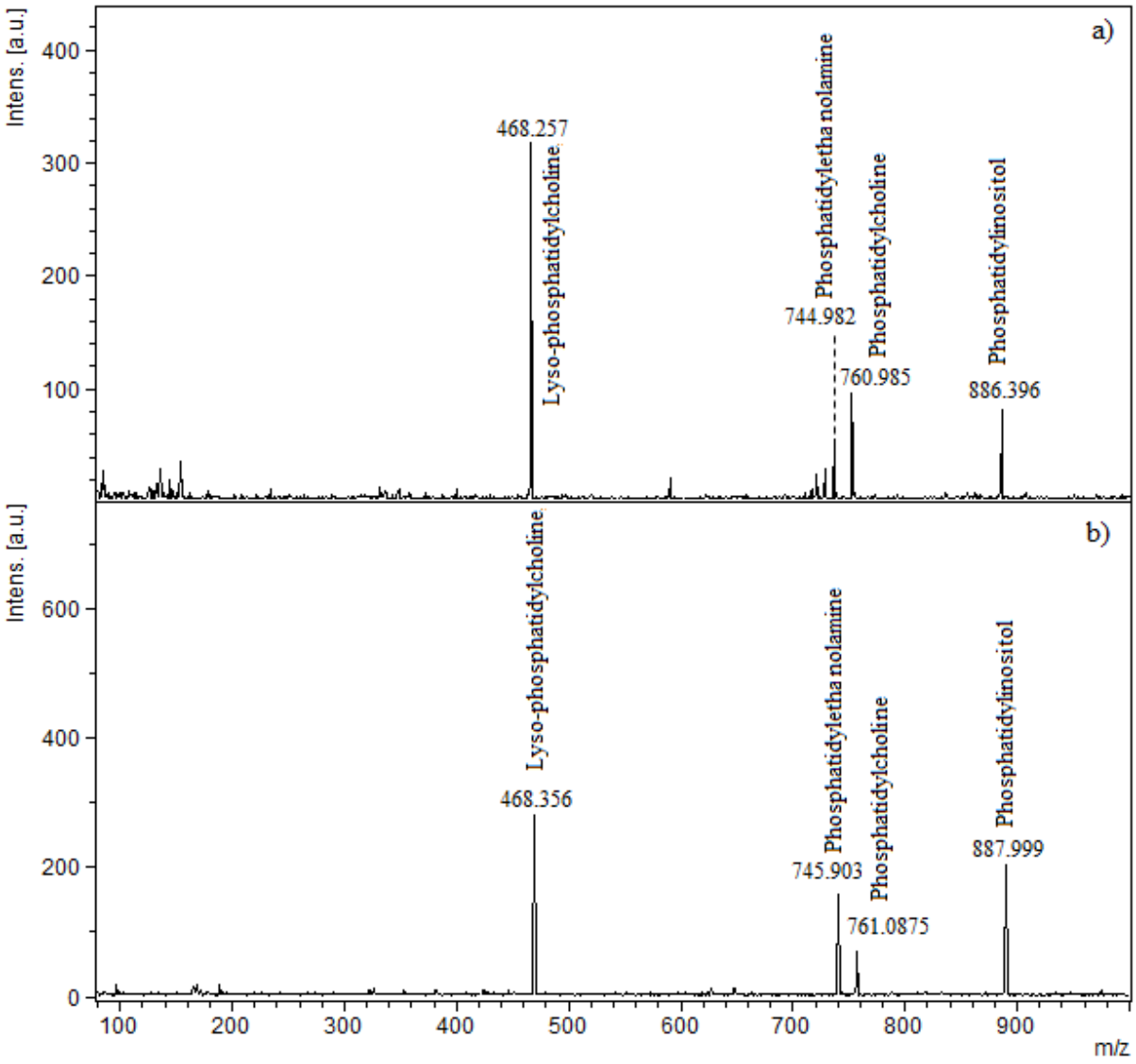

Figure S11: MALDI-MS spectra of phospholipid mixture (Sigma Aldrich) after enrichment on cellulose-silica-IMAC-Sm^{3+} using (a) gold nanoparticles in 0.1% TFA as LDI matrix (b) carbon based LDI matrix.

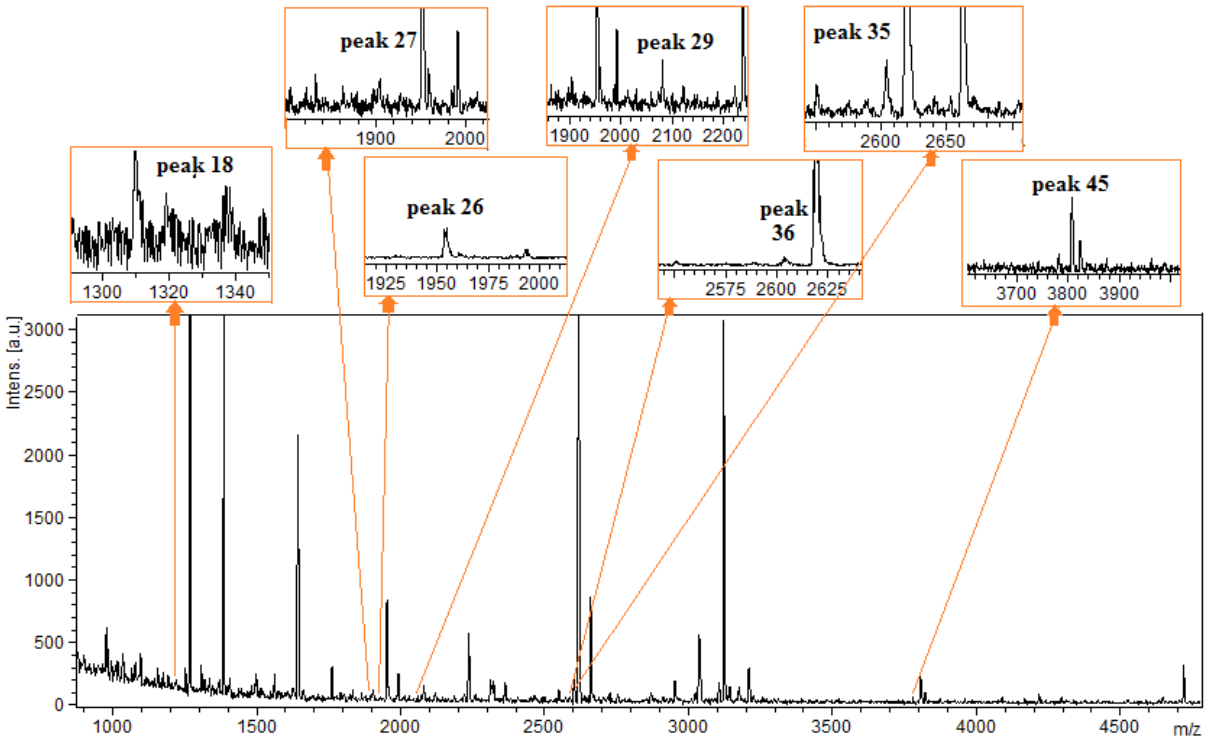

Figure S12: MALDI-MS analysis of serum phosphopeptides enriched by cellulose-silica-IMAC-Sm^{3+}. Phosphopeptides related to prostate cancer are shown in inset.

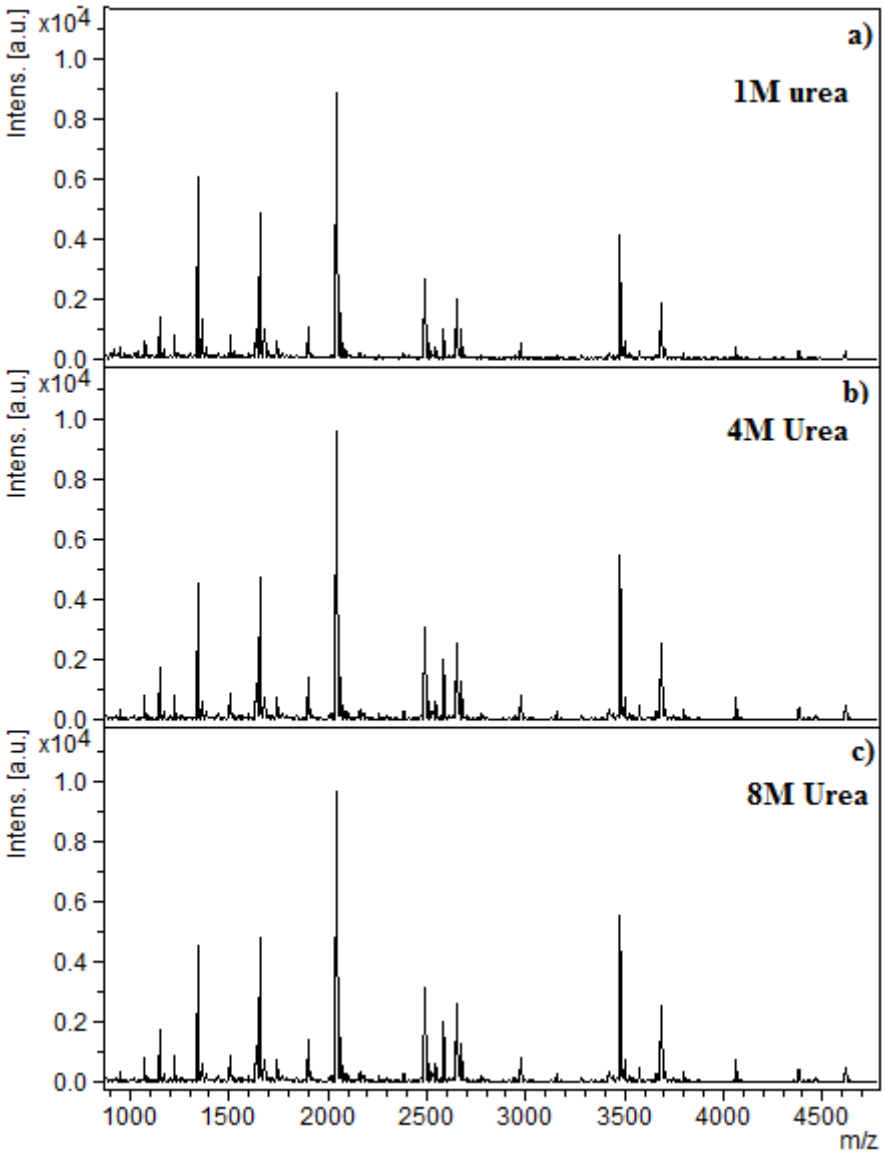

Figure S13: Desalting efficiency of cellulose-silica C18 for peptide mixture obtained as a result of tryptic digestion of α-, β-casein in different salt concentrations; (a) 1 M urea (b) 4 M urea and (c) 8 M urea.

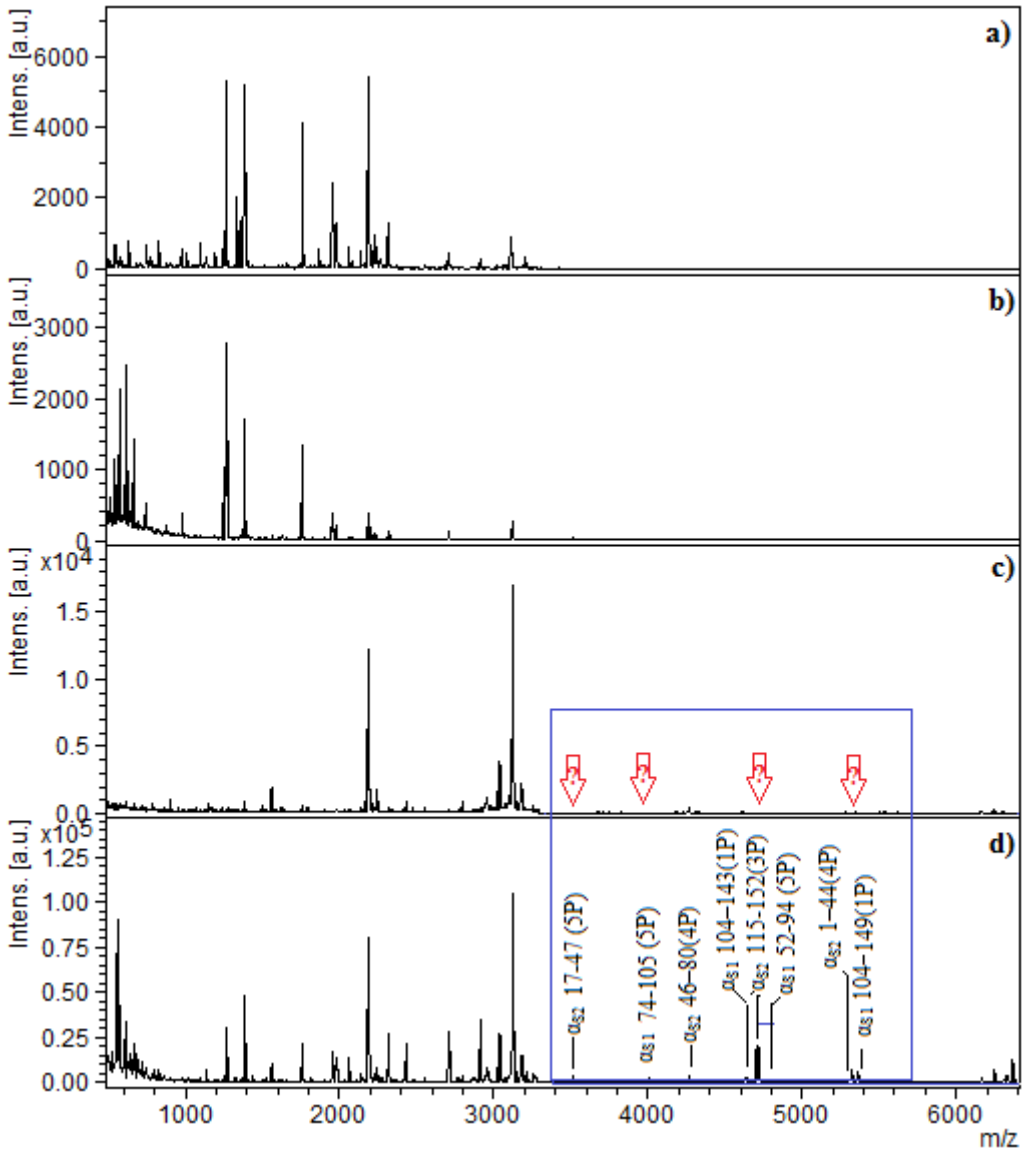

Figure S14: MALDI-MS spectra to show comparison of commercial desalting materials in relevance to recovery of hydrophilic species from α-casein digest after desalting; (a) SDB Tip (GL Sciences), (b) ZipTip C18 (Millipore), (c) Graphite tip (GL Sciences), (d) Cellulose-silica C18.

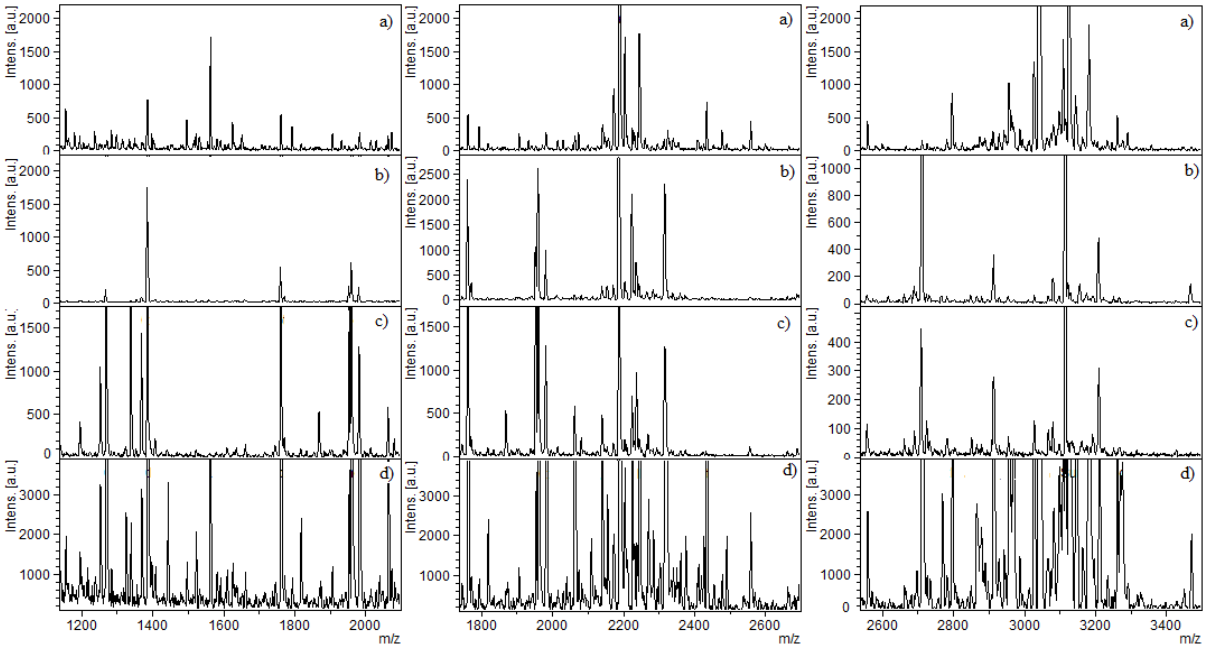

Figure S15: Comparison of different desalting tips with cellulose-silica C18 for tryptic milk digest: (a) SDB Tip (styrene-divinyl benzene, GL Sciences); (b) ZipTip C18 (Millipore); (c) Graphite Tip (GL Sciences) and (d) Cellulose-silica C18. The comparison of sequence coverage for milk proteins (casein, lactoferrin and lactoglobulin) is given in Table S2.

Table S1: Overview of synthesis and application of composites fabricated using cellulose and silica.

Composite	Synthesis	Application	Reference
NH_2-functionalized CA/silica composite nano-fibrous membranes	Cellulose acetate modified with silane	Water treatment	1
Cellulose-silica composite fibre	cellulose derivatized with silane reagent in ethanol/water	Affect of silica concentration on crystallinity of cellulose	2
Cellulose–Silica Nanocomposite Aerogels*	Silane reagent is used with cellulose, calcination	Nano-porous materials synthesis	3
Cellulose/silica hybrid (CSH) composites**	silane reagent for treatment of cellulose	Comparison to commercial insulation foams	4

*fabrillar morphology of composite inherited from the cellulose network

**silica network in cellulosic materials increased their dimensional stability, hydrophobicity, thermal stability and bending strength

Table S2: Method and material reproducibility of selected independent measurements using cellulose-silica-IMAC-Sm^{3+} for serum digest.

	Spectra A	Spectra B	Spectra C	Spectra D	SD
$[M+H]^+$	1343.941	1343.183	1343.183	1343.183	0.379
	2046.618	2046.887	2046.887	2046.976	0.150
	2645.914	2645.252	2645.252	2645.332	0.319
	3479.297	3479.765	3479.765	3479.765	0.234
	4064.048	4064.680	4064.680	4064.680	0.316

Table S3: Detail of phosphopeptides identified for cellulose-silica IMAC-Sm^{3+} from serum digest. Phosphopeptides associated to prostate cancer are highlighted.

Tree Hierarchy	Meas. Mass	Calc. MH^+	Int.	Dev (Da)	M	Sequence
39S ribosomal protein L54, mitochondrial OS=Homo sapiens GN=MRPL54 PE=1 SV=1 RM54_HUMAN						
peak 17	1268.42	1268.54	3777.37	-0.122	0	TLEELDPESR 1: Phospho (ST)
peak 37	2661.03	2661.09	523.408	-0.06	0	DPDVCTDPVQLTTYAMGVNIYK 14: Phospho (Y) 21: Phospho (Y) 5: Carboxymethyl (C)
Coiled-coil domain-containing protein 124 OS=Homo sapiens GN=CCDC124 PE=1 SV=1 CC124_HUMAN						
peak 26	1953.13	1952.92	785.957	0.212	0	TIEDAIAVLSVAEEAADR 1: Phospho (ST)
Receptor-transporting protein 2 OS=Homo sapiens GN=RTP2 PE=2 SV=1 RTP2_HUMAN						
peak 22	1495.56	1495.58	89.741	-0.025	1	CTSLTTCEWKK 2: Phospho (ST) 1: Carboxymethyl (C) 7: Carboxymethyl (C)
peak 24	1642.81	1642.62	2213.66	0.185	1	MCTSLTTCEWKK 1: Oxidation (M) 3: Phospho (ST) 2: Carboxymethyl (C) 8: Carboxymethyl (C)
Twist-related protein 1 OS=Homo sapiens GN=TWIST1 PE=1 SV=1 TWST1_HUMAN						
peak 20	1385.51	1385.39	3317.65	0.117	0	MEGAWSMSASH 1: Oxidation (M) 6: Phospho (ST) 7: Oxidation (M) 8: Phospho (ST)
peak 21	1407.47	1407.51	97.862	-0.04	0	MASCSYVAHER 1: Oxidation (M) 6: Phospho (Y) 4: Carboxymethyl (C)
peak 43	3145.08	3145.04	33.158	0.042	0	MMQDVSSSPVSPADDSLSNSEEEPDR 1: Oxidation (M) 6: Phospho (ST) 7: Phospho (ST) 8: Phospho (ST) 11: Phospho (ST)
peak 44	3209.47	3209.01	138.327	0.456	0	MMQDVSSSPVSPADDSLSNSEEEPDR 6: Phospho (ST) 7: Phospho (ST) 8: Phospho (ST) 11: Phospho (ST) 16: Phospho (ST)
cAMP-specific 3',5'-cyclic phosphodiesterase 4C OS=Homo sapiens GN=PDE4C PE=1 SV=2 PDE4C_HUMAN						
peak 36	2618.02	2618.2	2861.58	-0.179	1	ALDPQSSPGLGRIMQAPVPHSQR 6: Phospho (ST) 7: Phospho (ST) 14: Oxidation (M)
SAP30-binding protein OS=Homo sapiens GN=SAP30BP PE=1 SV=1 S30BP_HUMAN						
peak 47	4719.3	4719.47	66.828	-0.175	0	WDSAIPVTTIAQPTILTTTATLPAVVTVTTSASGSK 3: Phospho (ST) 8: Phospho (ST) 9: Phospho (ST) 14: Phospho (ST) 17: Phospho (ST) 18: Phospho

						(ST) 19: Phospho (ST) 21: Phospho (ST) 27: Phospho (ST) 29: Phospho (ST) 30: Phospho (ST) 31: Phospho (ST) 33: Phospho (ST) 35: Phospho (ST)
Zinc finger protein 439 OS=Homo sapiens GN=ZNF439 PE=2 SV=1 ZN439_HUMAN						
peak 23	1499.31	1499.51	144.093	-0.199	1	SHMGEKAYQCK 1: Phospho (ST) 8: Phospho (Y) 10: Carboxymethyl (C)
peak 42	3122.21	3122.22	1656.36	-0.012	1	ASPEVKSCDSFVCEVGLGNSSSNMNIR 2: Phospho (ST) 7: Phospho (ST) 24: Oxidation (M) 8: Carboxymethyl (C) 13: Carboxymethyl (C)
Regulator of telomere elongation helicase 1 OS=Homo sapiens GN=RTEL1 PE=1 SV=2 RTEL1_HUMAN						
peak 34	2365.67	2365.95	87.348	-0.289	0	VLSYWCFSPGHSMHELVR 3: Phospho (ST) 4: Phospho (Y) 6: Carboxymethyl (C)
Leukocyte receptor cluster member 8 OS=Homo sapiens GN=LENG8 PE=1 SV=2 LENG8_HUMAN						
peak 33	2323.6	2323.83	79.42	-0.236	1	NSGDITTELAYLTRELK 2: Phospho (ST) 6: Phospho (ST) 7: Phospho (ST) 11: Phospho (Y) 13: Phospho (ST)
Synaptonemal complex central element protein 3 OS=Homo sapiens GN=SYCE3 PE=3 SV=1 SYCE3_HUMAN						
peak 27	1960.18	1959.91	76.499	0.265	1	EEMEKNWQELLHETK 3: Oxidation (M)
Mitochondrial brown fat uncoupling protein 1 OS=Homo sapiens GN=UCP1 PE=1 SV=3 UCP1_HUMAN						
peak 18	1310.19	1310.32	204.061	-0.129	1	SRQTMDCAT 1: Phospho (ST) 4: Phospho (ST) 9: Phospho (ST) 7: Carboxymethyl (C)
peak 45	3806.25	3806.56	68.099	-0.315	0	NNILADDVPCHLVSALIAGFCATAMSSPVDVVK 14: Phospho (ST) 23: Phospho (ST) 26: Phospho (ST) 27: Phospho (ST) 10: Carboxymethyl (C) 21: Carboxymethyl (C)
Butyrophilin subfamily 1 member A1 OS=Homo sapiens GN=BTN1A1 PE=1 SV=3 BT1A1_HUMAN						
peak 46	4218.63	4218.25	14.566	0.382	1	LTPWIVAVAVILMVLGLLTIGSIFFTWRLYNERPR 2: Phospho (ST) 30: Phospho (Y)
Aminopeptidase N OS=Homo sapiens GN=ANPEP PE=1 SV=4 AMPN_HUMAN						
peak 29	2082.29	2082	65.92	0.293	1	GASVLRMLSSFLSEDVFK 3: Phospho (ST) 7: Oxidation (M)
peak 39	3037.43	3037.3	294.364	0.122	0	NANSSPVASTTPSASATTNPASATTLDQSK 4: Phospho (ST) 5: Phospho (ST)
[Pyruvate dehydrogenase (acetyl-transferring)] kinase isozyme 1, mitochondrial OS=Homo sapiens GN=PDK1 PE=1 SV=1 PDK1_HUMAN						
peak 27	1960.18	1960.36	76.499	-0.18	0	SFSSDSGSSPASER 1: Phospho (ST) 3: Phospho (ST) 4: Phospho (ST) 6: Phospho (ST) 8: Phospho (ST) 9: Phospho (ST) 12: Phospho (ST)
peak 35	2603.56	2603.92	52.543	-0.36	1	LFNYMYSTAPRPRVETSR 4: Phospho (Y) 5: Oxidation (M) 6: Phospho

						(Y) 7: Phospho (ST) 8: Phospho (ST) 16: Phospho (ST)
Complement C1q subcomponent subunit A OS=Homo sapiens GN=C1QA PE=1 SV=2 C1QA_HUMAN						
peak 26	1953.13	1952.94	785.957	0.194	1	VGYPGPSGPLGARGIPGIK 3: Phospho (Y) 7: Phospho (ST)
peak 41	3107.72	3107.41	37.211	0.316	1	EGPRGWLVLCVLAISLASMVTEDLCR 15: Phospho (ST) 18: Phospho (ST) 10: Carboxymethyl (C) 25: Carboxymethyl (C)
Zinc finger protein 726 OS=Homo sapiens GN=ZNF726 PE=2 SV=3 ZN726_HUMAN						
peak 25	1760.99	1760.72	260.125	0.263	1	NLYRNVMLENYR 3: Phospho (Y) 7: Oxidation (M) 11: Phospho (Y)
peak 36	2618.02	2618.2	2861.58	-0.188	1	AFNQSSNYTTHKVTHTGEKPYK 8: Phospho (Y)
Homeobox protein Hox-C10 OS=Homo sapiens GN=HOXC10 PE=1 SV=2 HXC10_HUMAN						
peak 40	3042.96	3043.1	33.664	-0.138	1	AAYRLEQPVGRPLSSCSYPPSVK 3: Phospho (Y) 14: Phospho (ST) 15: Phospho (ST) 17: Phospho (ST) 18: Phospho (Y) 21: Phospho (ST) 16: Carboxymethyl (C)
Putative uncharacterized protein FLJ45831 OS=Homo sapiens PE=5 SV=1 YQ050_HUMAN						
peak 19	1336.34	1336.37	114.268	-0.023	0	EHSSGVSSVK 3: Phospho (ST) 4: Phospho (ST) 7: Phospho (ST) 8: Phospho (ST)
peak 32	2317.48	2317.23	66.872	0.246	1	GVDNSKISVLILLGCELEQTK 15: Carboxymethyl (C)
Protein phosphatase 1 regulatory subunit 3D OS=Homo sapiens GN=PPP1R3D PE=1 SV=1						
peak 28	1992.33	1991.32	1991.74	-0.4152	1	MSRGPSSAVLPSALGSRK: 4 Phospho (ST)
peak 11	1077.14	1076.13	1075.36	0.7772	0	RYTFSGWRS: 2 Phospho (ST)
peak 14	1157.22	1156.22	1155.32	0.8928	0	RYTFSGWRS: Phospho (Y); 2 Phospho (ST)
peak 5	995.161	994.154	993.363	0.791	0	RDYSLTCRN: Phospho (ST)
Protein FAM3D OS=Homo sapiens GN=FAM3D PE=1 SV=1						
peak 8	1013.14	1012.14	1012.49	-0.3522	1	MRVSGVLRL: Oxidation (M); Phospho (ST)
peak 12	1077.14	1076.13	1075.38	0.7582	0	R.SYMSFSMKT: Oxidation (M); Phospho (Y)
peak 16	1253.33	1252.32	1251.3	1.0156	0	R.SYMSFSMKT: 2 Oxidation (M); 2 Phospho (ST); Phospho (Y)
peak 30	2236.51	2235.5	2235.97	-0.4634	1	KYKCGLIKPCPANYFAFKI: 2 Phospho (Y)
peak 4	885.069	884.062	884.39	-0.3284	0	R.MIMSPVKN: Phospho (ST)
peak 38	2953.57	2952.56	2953.13	-0.5709	1	KEIPGGALVLVASYDDPGTKMNDESRK: 3 Phospho (ST); Phospho (Y)

Fibrinogen gamma chain OS=Homo sapiens GN=FGG PE=1 SV=3						
peak 9	2313.56	2312.55	2311.98	0.5667	1	RVELEDWNGRTSTADYAMFKV: Phospho (Y)
peak 31	1019.12	1018.11	1017.34	0.7715	1	RWYSMKKT: Oxidation (M); Phospho (ST); Phospho (Y)
Angiopoietin-related protein 3 OS=Homo sapiens GN=ANGPTL3 PE=1 SV=1						
peak 15	1195.11	1194.1	1194.44	-0.3345	0	KNMSLELNSKL : 2 Phospho (ST)
peak 2	881.187	880.18	881.37	-1.1903	0	KIYSIVKQ: Phospho (ST); Phospho (Y)
peak 13	1099.18	1098.17	1098.48	-0.3086	1	RLYSIKSTKM: Phospho (ST); Phospho (Y)
Zinc finger protein 474 OS=Homo sapiens GN=ZNF474 PE=2 SV=1						
peak 1	880.133	879.126	879.314	-0.1883	1	KTDTQKKR: 2 Phospho (ST)
peak 6	980.079	979.072	980.233	-1.1613	0	KEYTNSKQ: 2 Phospho (ST); Phospho (Y)
Synaptonemal complex protein 3 OS=Homo sapiens GN=SYCP3 PE=1 SV=1						
peak 10	1036.08	1035.07	1035.45	-0.3723	1	KRLEMYTKA: Oxidation (M); Phospho (Y)

Table S4: Comparison of sequence coverage for milk tryptic digest containing peptides from casein (α-casein, β-casein and κ-casein), lactoglobulin and lactoferrin on GL SDB tip, graphite spin column (GL sciences), ZipTip C18 (Millipore) and cellulose-silica C18.

Milk Protein	Variants with Swiss Prot Accession No.	Sequence Coverage			
		SDB tip (GL Sciences)	ZipTip C18 (Millipore)	Graphite (GL Sciences)	Cellulose-silica C18
Casein	α	16.0%	18.0%	25.8%	34.8%
	β	18.7%	12.98%	30.2%	58.4%
	κ	12.3%	27.4%	20.7%	32.9%
Lactoglobulin	α (P00711)	23.7%	34.4%	43.2%	64.9%
	β (P02754)	20.2%	28.9%	33.5%	72.9%
Lactoferrin		19.1%	17.3%	36.4%	54.8%

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