

## **Dataset Brief**

### **A Well-Characterised Peak Identification List of MALDI MS Profile Peaks for Human Blood Serum**

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**Non-standard abbreviations:** UKCTOCS, United Kingdom Collaborative Trial of Ovarian Cancer Screening; UKOPS, United Kingdom Ovarian Cancer Population Study.

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MALDI MS profiling, using easily available body fluids such as blood serum, has attracted considerable interest for its potential in clinical applications. Despite the numerous reports on MALDI MS profiling of human serum there is only scarce information on the identity of the species making up these profiles, particularly in the mass range of larger peptides. Here we provide a list of more than 90 entries of MALDI MS profile peak identities up to 10 kDa obtained from human blood serum. Various modifications such as phosphorylation were detected amongst the peptide identifications. The overlap with the few other MALDI MS peak lists published so far was found to be limited and hence our list significantly extends the number of identified peaks commonly found in MALDI MS profiling of human blood serum.

There has been great interest in exploiting simple MALDI MS profiling for the analysis of crude or little purified human serum or plasma samples for clinical diagnostics [1, 2]. However, it has become apparent that there are far more challenges related to its applicability to disease diagnosis than initially thought. The experimental bias and the sensitivity and diagnostic accuracy of MALDI MS profiling are of major concern [3, 4]. These are further aggravated by the wide range of abundances and the complexity of the endogenous molecular components in blood [2]. However, there have been now further reports substantiating the claim that MALDI MS profiling can indeed be a tool for disease (outcome) classification [5] and biomarker discovery [6].

The fact that little is known about most of these potential biomarkers presents a serious limitation for quantification and validation of potentially discriminatory peaks and restricts biomarker assay development, including immunoassays that might potentially overcome some of the above analytical limitations of MALDI MS profiling.

Unlike the in-depth analyses of human blood serum or plasma where many hundreds of peptides and proteins have been identified [7, 8], MALDI MS profiling focuses on the reproducible, rapid and inexpensive acquisition of MS peak patterns that can be used for discriminative analysis as needed in population screening or clinical triage.

Employing MALDI, peptide sequencing is more challenging and usually less sensitive and informative for identification purposes than ESI-LC-MS/MS which is mostly combined with extensive pre-fractionation but without any relation to MALDI MS profiles [9, 10]. Indeed, only a few identifications of MALDI MS profile peaks have been reported so far. The most comprehensive list of peptide identifications obtained from human blood serum was reported by Villanueva *et al.* [11], although it provides only peak identifications below 4 kDa. For blood plasma, Koomen *et al.* [12] reported a list of 249 sequenced plasma profile peptides with a maximum mass of 5.2 kDa.

In the present study, we used serum blood samples from healthy persons recruited as part of the United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKCTOCS) and United Kingdom Ovarian Cancer Population Study (UKOPS) (for details, see [13]). Polypeptides were enriched from 5  $\mu$ L of serum using a one-step protocol based on reversed phase pre-packed C18-ZipTips<sup>®</sup>. The eluates from this one-step preparation were used for this study. The acquired MS/MS data are available in the PRIDE database (Experiment Accession # 11692-11694). Details on the materials and methods employed can be found in Supplemental Data 1.

MALDI-TOF MS profiling of 60 serum samples revealed 108 aligned peaks within the combined UKCTOCS and UKOPS serum sample set, using an S/N ratio above 5 (Supplemental Data 2, A) and 156 peaks at S/N>3. Despite the high number of common peaks, the different sample collection and handling protocols for the UKOPS and UKCTOCS sample sets resulted in markedly different MALDI MS profiles and peak intensities (Figure 1). Thus, sera from both sample sets were employed to identify as many profile peaks as possible using the higher mass accuracy and sequencing capability of MALDI Q-TOF instrumentation.

Many of the peak identifications were obtained by direct switching from MALDI MS to MALDI CID MS/MS as described in Supplemental Data 1, analysing the precursor ions determined in the MALDI MS profile. This approach provided a total of 36 identities through searching the human subset of the NCBI nr protein database using MASCOT with an average mass accuracy of the precursor ion mass measurements of 13 ppm (Supplemental Data 2, B). The next approach was using the same samples for nanoESI MS/MS analysis, using the chip-based Nanomate system. Similarly to the MALDI analysis, MS profile spectra were first acquired and MS/MS analysis of the precursor ions of the MS profile provided their identity. In this approach, the MALDI and ESI MS profiles were compared to match peaks unambiguously and a total of 16 peaks were identified, of which 6 could not be identified by earlier MALDI MS/MS analysis.

Next, the serum sample eluates were fractionated by off-line C18 reversed-phase chromatography and the fractions were analysed by chip-based nanoESI as before. In contrast to many previous reports using major-protein depletion or multi-dimensional separation strategies where 200-400 peptides were identified [9, 10, 12], the objective of our fractionation strategy was to enhance the identification of peptides but to ensure that the obtained identities are the correct identities of the MALDI MS profile peaks. In all large-scale serum proteomic studies published so far this has not been achieved and can therefore result in a high false positive rate. Thus, we also monitored the change of the MALDI MS profile after fractionation by comparing the pre-fractionation MALDI MS profile with the MALDI MS (and ESI MS) profiles of each fraction. The fractions were then analysed by chip-based nanoESI MS/MS and MASCOT searching of the MS/MS data resulted in an additional 28 identities and another 9 confirmations of identities obtained by the two earlier approaches without LC fractionation.

As expected for peaks above  $m/z$  4,500, none of these approaches led to unambiguous peptide identification. The collected LC fractions were therefore digested with trypsin and analysed by chip-based nanoESI MS/MS. For each fraction, the protein hits from these MASCOT searches were then used to search peptide profile peaks from the undigested fractions for sequence mass matches within a mass tolerance of 50 ppm. Any putative matches were further interrogated by their isotopomer distribution, their known post-translational modifications and the likelihood of occurrence derived from their structural and functional information in protein databases. Where possible, fragment ion spectra from the previous methods were also used to substantiate putative identities. Overall, this approach led to an additional 11 peak identities.

In total, more than 80 polypeptides were identified with high confidence, representing peptides from 13 different but highly abundant proteins (see Supplemental Data 2, B). Among these there were three full length proteins, Apolipoprotein CII (Apo CII), Apolipoprotein CIII (Apo CIII) and Connective tissue-

activating peptide III (CTAP-III). The peaks at  $m/z$  1547 and 1616 were clearly identified by both MALDI MS/MS and ESI MS/MS without LC fractionation, and correspond to Fibrinopeptide A fragments phosphorylated at serine (see Supplemental Data 3, (a)-(d)). Interestingly, many of the Fibrinopeptide A/B peaks and others show a loss of 17 Da (ammonia) and sometimes of water. Some of the identified peptides/proteins were found to be oxidised and they were excluded from the list since many of them did not appear in the MALDI MS profiles and were most likely products obtained from the sample handling. This clearly shows that the MS peaks/profiles can substantially change with complex sample preparation methods, underlining the need for careful profile peak identification that is different to peptide identification of in-depth proteomic serum analyses.

A detailed comparison of our peak list with the peak lists reported by Villanueva *et al.* [11] and Peng *et al.* [14] shows that the lists are complementary rather than redundant (see Figure 2 and Table 1). It is worth noting that although the number of identified peaks from this study is comparable to the number reported by Villanueva *et al.* [11], only about 30% (25 peaks) are common between the two lists (Figure 2). Furthermore, the present study was able to identify nine polypeptides in the mass range of 4–10 kDa. However, within this mass range there were many more polypeptides for which the parent protein was identified after digestion but the data was inconclusive for assigning single identities to the MALDI MS profile peaks.

A comparison with a list of plasma peptide identifications reported by Koomen *et al.* [12] shows that again only about 25 peaks are common (about 10% of the 249 peptide identifications), reflecting the inherent difference between blood plasma and serum.

Combining this peak list with the lists from Villanueva *et al.* [11] and Peng *et al.* [14] increases the number of identified polypeptides of MALDI MS serum profiles to 139 (Table 1). This provides an extensive list of peptide identifications obtained from human blood serum that can be used for

provisional identification of MALDI MS profiling peaks below 10 kDa through simple comparison. Furthermore, this will allow, by quick comparison of MS profile and/or MS/MS data, the identification of many potential biomarkers commonly listed in reports without identification and thus facilitate biomarker validation.

The analysis of some of the MALDI MS profiling peaks resulted in MS/MS spectra different to those usually found for peptides (Supplemental Data 3, (e)-(f)). These fragmentation spectra were typical for phospholipids and their identification was carried out by comparing their accurate masses (within 10 ppm) to the calculated masses obtained from the LipidMaps database ([www.lipidmaps.org](http://www.lipidmaps.org)). The presence of ion fragments at  $m/z$  86.10, 104.11 and 184.07 in all MS/MS spectra is characteristic for choline, H<sub>2</sub>O-choline and phosphocholine, respectively (see Supplemental Data 3, (e)-(f), and Supplemental Data 2, C), and confirmed that these peaks are derived from phosphocholine lipids.

Interestingly, a comparison with a recently published list of endogenous serum peptides that were obtained by extensive prefractionation and ESI-MS/MS shows that more than 40% of the fibrinogen (P02671) peptides in Table 1 were not identified despite the much higher number of identified peptides (>1000) obtained in that study [15]. Other comparisons including lists of endogenous plasma peptides [16] and other proteins showed similar results. In addition, none of these lists include any post-translational modifications (PTMs), and only recently a few phosphorylated endogenous serum peptides were reported [17]. The presented list is based on MALDI MS profile peak identification and as such includes PTMs as well as other non-peptidic components. The focus on MALDI MS profile peak identification also provides an additional and decisive advantage. While other lists might provide many more endogenous peptides, these are not linked to MALDI MS profiling. As a result entries cannot be ranked according to their signal intensity in MALDI MS profiles and many of the entries will have the same mass value within a given mass tolerance. For instance, the above comparison (based

only on fibrinogen peptides) reveals that the profile peak at  $m/z$  1518.7 would have been wrongly assigned using the more extensive list of endogenous serum peptides published by Bakun et al. [15]. In the latter, the peptide at the corresponding mass was identified as NRGDSTFESKSYK (cf. Table 1). In the same list, there is no entry for the serine-phosphorylated peptide DSGEGDFLAEGGGV or for its associated peptide that has lost phosphoric acid, which has the same mass value as NRGDSTFESKSYK within a tolerance of 25ppm. In addition, the peptide entries for the profile peak at  $m/z$  1309.6 would have led to an ambiguous identification of either SSSYSKQFTSST or DSGEGDFLAEGGGV, again assuming a mass accuracy of around 25ppm or more. Even at 10ppm most fibrinogen peptides are not unique for their respective mass value. For instance, there are seven fibrinogen peptides with exactly the same  $m/z$  value of 1206.57. The above analysis clearly shows that any list of endogenous serum peptides can only be useful for MALDI MS profiling if the peak identifications can be directly linked to the MALDI MS profiles.

In general, a comparison with the published literature shows that this new list of MALDI serum profile peak identifications can be highly advantageous. It can be used for initial putative peak identification, particularly where similar strict sample handling procedures have been followed. Recently published MALDI serum profiling work [18] using FT-ICR-MS for the identification of acute leukemia biomarker candidate peaks at  $m/z$  1778 and 1865 revealed these peaks to be fragments of complement C3f as listed in Table 1. Other examples can be found for the average  $m/z$  1212, 1350, 1451, 1564, 1691, 1779, 1866 [19]; 3275 [20]; 1467 [21]; 5905 [22]; and 4283 [23]. Some of these profile peaks were even obtained from blood plasma. Nonetheless, one needs to be aware that the presented dataset is based on the analysis of C18-purified but otherwise non-fractionated serum and that its application to plasma or fractionated serum MS profile peaks may be limited. Some of the limitation with respect to sample handling can be seen in the comparison with the data from Villanueva et al. and Peng et al. (Table 1 and Figure 2). However, there are many reasons for the low degree of overlap between these



three studies. In our study we identified non-peptidic and larger peptide peaks and used exclusively healthy persons while the other two studies either focused on peptide identification of cancer-specific profile peaks or used SELDI chips for serum fractionation. Within our study we tried to extend the currently available and biased lists by employing a sample handling protocol that researchers in the field consider as best practice and performing peak identification that is not based on disease-specific biomarker candidates.

In conclusion, the presented dataset provides the identification of more than 90 serum MALDI MS profiling peaks with little overlap with already published datasets. The more than 80 identified polypeptides originate from only 13 proteins. Complementary to other published lists this dataset also provides the identification of very low molecular weight ( $< 1$  kDa) peaks often associated with phospholipids and peaks above 5 kDa. Together with previously published data the most comprehensive MALDI MS serum profiling list consisting of 148 peak identifications is now available (see Table 1).

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**Figure 1:**

Averaged MALDI-TOF mass spectra of human serum samples collected for UKCTOCS (upper panel) and UKOPS (lower panel) and obtained from purifications of 5 $\mu$ L-aliquots using pre-packed C18 ZipTips<sup>®</sup>. Averaged mass spectra were generated by ClinProTools software v2.1 after baseline subtraction, smoothing, normalisation and peak realignment.

**Figure 2:**

Venn diagram showing the number of overlapping and total number of peptides identified in this study and those of the studies of Villanueva *et al.* [11] and Peng *et al.* [14].

**Table 1.** List of MALDI MS profile peaks

Calculated Monoisot. [M+H] <sup>+</sup>	Calculated Average [M+H] <sup>+</sup>	Protein/Peptide or Lipid		Peptide Sequences Identified	Study		
		UniProt Entry	Name (Fragment)		Tiss et al.	Villanueva et al.	Peng et al.
445.2518	445.4944	P02671	FPA (12-16)	E . GGGVR	√		
496.3426		NA	PC(16:0/0:0)	NA	√		
518.3236		NA	PC(18:3/0:0)	NA	√		
520.3393		NA	PC(18:2/0:0)	NA	√		
522.3590		NA	PC(18:1/0:0)	NA	√		
524.3742		NA	PC(18:0/0:0)	NA	√		
574.2944	574.6087	P02671	FPA (11-16)	A . EGGGVR	√		
645.3315	645.6867	P02671	FPA (10-16)	L . AEGGGVR	√		
740.2846	740.7172	P02675	FPB (1-7) – NH <sub>3</sub> – H <sub>2</sub> O	Q <sub>(-NH<sub>3</sub>)</sub> GVND <sub>(-H<sub>2</sub>O)</sub> NE . E	√		
758.4155	758.8522	P02671	FPA (9-16)	F . LAEGGGVR	√		√
758.5701		NA	PC(16:0/18:2 or 18:0/16:2)	NA	√		
760.5915		NA	PC(16:0/18:1 or 18:0/16:1)	NA	√		
822.4297	822.9731	P10909	Clusterin precursor (217-222)	P . HFFFPK . S			√
842.3944	842.9214	Q14624	ITIH4 (681-687)	D . HAAYHPF . R			√
864.4097	864.9299	P02671	FPA (7-15)	D . DFLAEGGGV . R	√		
869.3272	869.8334	P02675	FPB (1-8) – NH <sub>3</sub> – H <sub>2</sub> O	Q <sub>(-NH<sub>3</sub>)</sub> GVND <sub>(-H<sub>2</sub>O)</sub> NEE . G	√		
904.4676	905.0324	P01042	Bradykinin (1-8)	RPPGFSPF . R			√
905.4839	906.0288	P02671	FPA (8-16)	D . FLAEGGGVR	√		√
920.4624	921.0385	P01042	Bradykinin (1-8) [Pro_Hydroxyl]	RPPGFSP <sub>(Hydroxyl)</sub> F . R	√		√
926.3486	926.8856	P02675	FPB (1-9) – NH <sub>3</sub> – H <sub>2</sub> O	Q <sub>(-NH<sub>3</sub>)</sub> GVND <sub>(-H<sub>2</sub>O)</sub> NEEG . F	√		
942.4680	943.0358	P01024	Complement C3f fragment (9-16)	I . HWESASLL . R			√
944.3592	944.8817	P02675	FPB (1-9) – NH <sub>3</sub>	Q <sub>(-NH<sub>3</sub>)</sub> GVNDNEEG . F	√		
991.6807		NA	PC(16:0/0:0) dimer	NA	√		
998.4955	999.1075	Q14624	ITIH4 (681-688)	D . HAAYHPFR .			√
1015.6831		NA	PC(16:0/0:0) + PC(18:2/0:0) dimer	NA	√		
1020.5108	1021.1174	P02671	FPA (7-16)	G . DFLAEGGGVR	√		√
1055.5520	1056.1937	P01024	Complement C3f fragment (8-16)	R . IHWESASLL . R			√
1060.5787	1061.2185	P01042	Bradykinin	RPPGFSPFR			√
1073.4171	1074.0634	P02675	FPB (1-10) – NH <sub>3</sub> – H <sub>2</sub> O	Q <sub>(-NH<sub>3</sub>)</sub> GVND <sub>(-H<sub>2</sub>O)</sub> NEEGF . F	√		
1076.5736	1077.2179	P01042	Bradykinin [Pro_Hydroxyl]	RPPGFSP <sub>(Hydroxyl)</sub> FR			√
1077.5323	1078.1693	P02671	FPA (6-16)	E . GDFLAEGGGVR	√		√
1091.4277	1092.0559	P02675	FPB (1-10) – NH <sub>3</sub>	Q <sub>(-NH<sub>3</sub>)</sub> GVNDNEEGF . F	√		
1108.4542	1109.1005	P02675	FPB (1-10)	QGVNDNEEGF . F	√		
1194.5273	1195.2274	P02671	FPA (3-15)	D . SGEGDFLAEGGGV . R	√		
1206.5749	1207.2848	P02671	FPA (5-16)	G . EGDFLAEGGGVR	√		√
1211.6531	1212.3798	P01024	Complement C3f fragment (7-16)	H . RIHWESASLL . R			√
1220.4855	1221.2413	P02675	FPB (1-11) – NH <sub>3</sub> – H <sub>2</sub> O	Q <sub>(-NH<sub>3</sub>)</sub> GVND <sub>(-H<sub>2</sub>O)</sub> NEEGFF . S	√		
1238.4961	1239.2519	P02675	FPB (1-11) – NH <sub>3</sub>	Q <sub>(-NH<sub>3</sub>)</sub> GVNDNEEGFF . S	√		
1263.5963	1264.3367	P02671	FPA (4-16)	S . GEGDFLAEGGGVR	√		√

1277.7153	1278.5258	P10909	Clusterin precursor (217-226)	P . HFFFPKSRIV . R			√
1309.5542	1310.3160	P02671	FPA (2-15)	A . DSGEGDFLAEGGGV . R	√		
1325.5281	1326.3076	P02675	FPB (1-12) – NH <sub>3</sub>	Q <sub>(-NH<sub>3</sub>)</sub> GVNDNEEGFFS . A	√		
1348.7120	1349.5194	P01024	Complement C3f fragment (6-16)	T . HRIHWESASLL . R			√
1350.6284	1351.4149	P02671	FPA (3-16)	D . SGE GDFLAEGGGVR	√		√
1396.5652	1397.4098	P02675	FPB (1-13) – NH <sub>3</sub>	Q <sub>(-NH<sub>3</sub>)</sub> GVNDNEEGFFSA . R	√		
1447.6447	1448.4929	P02671	FPA (2-16) – H <sub>2</sub> O	A . DS <sub>(-H<sub>2</sub>O)</sub> GEGDFLAEGGGVR	√		
1449.7597	1450.6235	P01024	Complement C3f fragment (5-16)	I . THRIHWESASLL . R			√
1465.6554	1466.5035	P02671	FPA (2-16)	A . DSGEGDFLAEGGGVR	√		√
1498.7873	1499.6562	P0C0L4	Complement C4-A (1337-1349)	R . NGFKSHALQLNNR . Q			√
1518.6818	1519.5717	P02671	FPA – H <sub>2</sub> O	ADS <sub>(-H<sub>2</sub>O)</sub> GEGDFLAEGGGVR	√		
1530.8692	1531.8273	Q59FS1	IGIH4	R . RPHFFFPKSRIV . R			√
1536.6924	1537.5823	P02671	FPA	ADSGEGDFLAEGGGVR	√		√
1542.5939	1543.4566	P02671	FGA chain (605-619)	A . DEAGSEADHEGTHST . K	√		
1545.6000	1546.4482	P02671	FPA (2-16) + P	A . DS <sub>p</sub> GEGDFLAEGGGVR	√		
1562.8438	1563.7813	P01024	Complement C3f fragment (4-16)	K . ITHRIHWESASLL . R			√
1616.6594	1617.5493	P02671	FPA + P	ADS <sub>p</sub> GEGDFLAEGGGVR	√		
1626.8459	1627.7857	P0C0L4	Complement C4-A (1337-1350)	R . NGFKSHALQLNNRQ . I			√
1690.9387	1691.9540	P01024	Complement C3f fragment (3-16)	S . KITHRIHWESASLL . R			√
1739.9299	1740.9619	P0C0L4	Complement C4-A (1337-1351)	R . NGFKSHALQLNNRQI . R	√		√
1751.9187	1752.9510	P01024	Complement C3f fragment (1-15)	. SSKITHRIHWESASL . L			√
1762.9222	1763.9653	P0C0L4	Complement C4-A (1353-1368)	R . GLEELQFSLGSKINV . K			√
1768.8236	1769.8406	P01024	Complement C3 (1322-1337)	S . EETKENEGFTVTAEGK . G	√		
1771.8497	1772.8893	P06727	Apolipoprotein A-IV (288-303)	K . SLAELGGHLDQQVEEF . R			√
1777.9708	1779.0314	P01024	Complement C3f fragment (2-16)	S . SKITHRIHWESASLL . R			√
1786.8547	1787.9504	Q14624	ITIH4 (671-687)	L . GLPGPPDVPDHAAYHPF . R	√		√
1807.9297	1808.9884	P02647	Apolipoprotein A-I (149-163)	A . ELQEGARQKLHELQE . K	√		√
1847.8379	1848.8859	P02671	FGA chain (607-624)	E . AGSEADHEGTHSTKRGA . K	√		
1855.8555	1856.9375	P01024	Complement C3 (1321-1337)	R . SEETKENEGFTVTAEGK . G	√		
1865.0028	1866.1295	P01024	Complement C3f fragment (1-16)	SSKITHRIHWESASLL . R	√		√
1883.8114	1884.8701	P02671	FGA chain (605-622)	A . DEAGSEADHEGTHSTKRG . H	√		
1884.9899	1886.0639	P02671	FGA chain (606-622)	D . HEGTHSTKRGHAKSRPV . R			√
1896.0311	1897.1494	P0C0L4	Complement C4-A (1336-1351)	G . RNGFKSHALQLNNRQI . R	√		√
1927.9508	1929.0755	P06727	Apolipoprotein A-IV (288-304)	K . SLAELGGHLDQQVEEFR . R			√
1943.9080	1945.0261	P01042	HMW Kininogen (440-456)	H . NLGHGKHERDQGHGHQ . R	√		√
1971.0433	1972.2228	P02647	Apolipoprotein A-I (251-267)	K . VSFLSALEEYTKKLNTQ			√
2008.0788	2009.3518	Q0P5N8	TMSB4X (31-47)	K . PDMAEIEKFDKSKLKT . E	√		
2010.9708	2012.2352	Q14624	ITIH4 (669-687) – NH <sub>3</sub>	R . Q <sub>(-NH<sub>3</sub>)</sub> LGLPGPPDVPDHAAYHPF . R	√		
2021.1039	2022.2950	P01024	Complement C3f fragment	SSKITHRIHWESASLLR			√
2027.9974	2029.2378	Q14624	ITIH4 (669-687)	R . QLGLPGPPDVPDHAAYHPF . R			√
2045.9172	2047.1100	P01042	HMW Kininogen (480-497)	L . DDDLEHQGGHVLHDHGKH . K	√		
2053.0812	2054.2822	P02647	Apolipoprotein A-I (220-238)	K . ATEHLSTLSEKAKPALEDL . R			√
2067.1921	2068.4016	P02775	Platelet Basic Protein precursor (110-127)	D . APRIKKIVQKLAGDESAD			√

2091.9074	2093.0900	P02671	FGA chain (605-624)	A . DEAGSEADHEGTHSTKRGHA . K	√		
2113.0771	2114.3166	Q0P5N8	TMSB4X (52-70)	E . KNPLPSKETIEQEKQAGES	√		
2115.0512	2116.3462	Q14624	(ITI4) (347-367)	R . NVHSAGAAGSRMNFPRGVLSS . R			√
2122.8618	2124.1563	P02671	FGA chain (600-619)	K . SYKMADEAGSEADHEGTHST . K	√		
2127.0128	2128.2565	P01042	HMW Kininogen (458-477)	R . GHGLGHGHEQQHGLGHGKHF . K			√
2162.9445	2164.1688	P02671	FGA chain (604-624)	M . ADEAGSEADHEGTHSTKRGHA . K	√		
2184.0985	2185.4238	Q14624	ITI4 (669-688)	R . QLGLPGPPDVPDHAAYHPF . R			√
2209.0619	2210.3192	P01042	HMW Kininogen (438-456)	R . KHNLGHGKHERDQGHGHQ . R			√
2267.1891	2268.5127	P02649	Apolipoprotein E (212-232)	A . TVGSLAGQPLQERAQAWGERL . R			√
2271.1305	2272.5274	Q14624	ITI4 (667-687)	S . SRQLGLPGPPDVPDHAAYHPF . R	√		√
2279.2718	2280.6046	P02775	Platelet Basic Protein precursor (108-127)	D . PDAPRIKKIVQKKLAGDESAD			√
2305.2034	2306.5524	P0C0L4	Complement C4-A (1353-1374)	R . GLEEEELQFSLGSKINVKVGNS . K			√
2353.1518	2354.5363	Q0P5N8	TMSB4X (50-70) – NH <sub>3</sub>	T . QEKN <sub>(-NH3)</sub> PLPSKETIEQEKQAGES	√		
2358.1626	2359.5787	Q14624	ITI4 (666-687)	S . SSRQLGLPGPPDVPDHAAYHPF . R			√
2371.1624	2372.5469	Q0P5N8	TMSB4X (50-70)	T . QEKN <sub>(deam)</sub> PLPSKETIEQEKQAGES	√		
2378.2098	2379.6342	P0C0L4	Complement C4-A (1429-1449)	K . DDPDAPLQPVTPPLQFEGRRN . R	√		
2379.0371	2380.4173	P02671	FGA (577-597)	S . SSSYSKQFTSSTSYNRGDSTFE . S			√
2409.2633	2410.6688	P02649	Apolipoprotein E (210-232)	R . AATVGSLAGQPLQERAQAWGERL . R			√
2451.2051	2452.6608	P02766	Transthyretin precursor (101-123)	K . ALGISPFHEHAEVVFTANDSGPR . R			√
2453.9610	2455.4140	Q0P5N8	TMSB4X (49-70) – NH <sub>3</sub>	E . TQ <sub>(-NH3)</sub> EKNPLPSKETIEQEKQAGES	√		
2464.0793	2465.5698	P02671	FGA chain (600-622)	K . SYKMADEAGSEADHEGTHSTKRG . H	√		
2471.2260	2472.6679	Q0P5N8	TMSB4X (49-70)	E . TQEKPLPSKETIEQEKQAGES	√		
2508.3529	2509.7985	P06727	Apolipoprotein A-IV (256-278)	R . ISASAEELRQLAPLAEDVRGNL . K			√
2544.2297	2545.6650	P02671	FGA chain (606-629)	D . EAGSEADHEGTHSTKRGHAKSRPV . R			√
2551.1769	2552.7529	P0C0L4	Complement C4-A (957-979)	R . TLEIPGNSDPNMLPDGDFNSYVR . V			√
2553.1012	2554.5991	P02671	FGA chain (576-598)	K . SSSYSKQFTSSTSYNRGDSTFES . K	√		√
2565.3644	2566.8549	P02649	Apolipoprotein E (210-233)	R . AATVGSLAGQPLQERAQAWGERLR . A			√
2567.3729	2568.9088	P02768	Albumin precursor (27-48)	A . HKSEVAHRFKDLGEENFKALVL . I	√		
2582.3402	2583.9185	Q14624	ITI4 (617-639)	R . NVHSGSTFFKYLLQGAKIPKPEA . S	√		
2599.2635	2600.7734	P02647	Apolipoprotein A-IV (280-303)	K . GNTEGLQKSLAELGGHLDQQVEEF . R			√
2602.3107	2603.8204	P00488	Factor XIIIa (14-38)	R . AVPPNNSNAEEDDLPTVELQGVVPR . G			√
2627.3400	2628.9194	Q14624	ITI4 (663-687)	P . GVLSSRQLGLPGPPDVPDHAAYHPF . R			√
2659.2567	2660.7791	P02671	FGA chain (605-629)	A . DEAGSEADHEGTHSTKRGHAKSRPV . R	√		√
2704.4516	2706.0385	P0C0L4	Complement C4-A (1353-1378)	R . GLEEEELQFSLGSKINVKVGNSKGTL . K			√
2724.3893	2726.0348	Q14624	ITI4 (662-687)	R . PGVLSRQLGLPGPPDVPDHAAYHPF . R			√
2753.4369	2755.1053	P02768	Albumin precursor (25-48)	R . DAHSEVAHRFKDLGEENFKALVL . I	√		
2755.3646	2756.9595	P06727	Apolipoprotein A-IV (280-304)	K . GNTEGLQKSLAELGGHLDQQVEEFR .			√
2768.2282	2769.8514	P02671	FGA chain (576-600)	K . SSSYSKQFTSSTSYNRGDSTFESKS . Y	√		√
2778.4520	2780.0741	P02654	Apolipoprotein C-I (29-53)	P . DVSSALDKLKEFGNTLEDKARELIS . R			√
2816.3234	2817.9581	P02671	FGA (548-574)	R . GSESGIFTNTKESSSHHPGIAEFP SRG . K			√
2829.4112	2831.0626	Q0P5N8	TMSB4X (46-70)	K . KTETQEKPLPSKETIEQEKQAGES	√		
2861.3343	2863.6505	P02671	FGA chain (603-629)	K . MADEAGSEADHEGTHSTKRGHAKSRPV . R	√		√
2931.2915	2933.0274	P02671	FGA chain precursor (576-601)	K . SSSYSKQFTSSTSYNRGDSTFESKSY . K	√		√



2989.4292	2991.2246	P02671	FGA chain (602-629)	Y . KMADEAGSEADHEGTHSTKRGHAKSRPV . R	✓		
3156.6265	3158.5491	Q14624	ITIH4 (617-644)	R . NVHSGSTFFKYLLQGAKIPKPEASFSPR . R	✓	✓	✓
3182.7347	3184.6627	P02647	Apolipoprotein A-I (240-267)	R . QGLLPVLESFKVSFLSALEEYTKKLNTO		✓	
3190.4270	3192.3659	P02671	FGA (576-603)	K . SSSYSKQFTSSTSYNRGDSTFESKSYKM . A		✓	✓
3200.8001	3202.6864	P0C0L4	Complement C4-A (1353-1382)	R . GLEELQFSLGSKINVKVGNSKGTLLKVLRL . T		✓	
3206.4219	3208.3653	P02671	FGA (576-603) [Met-ox]	K . SSSYSKQFTSSTSYNRGDSTFESKSYKM <sub>ox</sub> . A		✓	
3239.5246	3241.4788	P02671	FGA chain (600-629)	K . SYKMADEAGSEADHEGTHSTKRGHAKSRPV . R	✓	✓	✓
3261.4641	3263.4440	P02671	FGA chain (576-604)	K . SSSYSKQFTSSTSYNRGDSTFESKSYKMA . D	✓	✓	✓
3272.6422	3274.6954	Q14624	ITIH4 (658-687)	R . MNFRPGVLSRQLGLPGPPDVPDHAAYHPP . R	✓	✓	
3277.4590	3279.4434	P02671	FGA (576-604) [Met-ox]	K . SSSYSKQFTSSTSYNRGDSTFESKSYKM <sub>ox</sub> . A . D		✓	
3377.7230	3379.7435	P02647	Apolipoprotein A-I (148-176)	R . AELQEGARQKLHELQEKLSPLGEEM <sub>ox</sub> RDRA . R		✓	
3675.9075	3678.0877	Q0P5N8	TMSB4X (39-70)	K . FDKSKLKKTTETQEKNLPLPSKETIEQEKQAGES	✓		
3953.9098	3956.4034	Q14624	ITIH4 (645-681)	R . RGWNRQAGAAGSRMNFPRPGVLSRQLGLPGPPDVPDHA . A	✓		
3970.9882	3973.4255	Q14624	ITIH4 (650-687)	R . QAGAAGSRMNFPRPGVLSRQLGLPGPPDVPDHAAYHPP . R		✓	
4280.1319	4282.7883	Q59FS1	IGIH4 (602-642)	R . NVHSAGAAGSRMNFPRPGVLSRQLGLPGPPDVPDHAAYHPP . R	✓		✓
4961.3111	4964.3899	P02671	FGA chain (529-574) or (513-558)	T . FPGFFSPMLGEFVSETESRGSSEGIF <sup>+</sup> TNTKESSSHHPGIAEFPSRG . K or R . HPDEAAFFDTASTGKTFPGFFSPMLGEFVSETESRGSSEGIF <sup>+</sup> TNTK . E	✓		
5334.3536	5337.5402	P02671	FGA chain (577-624)	K . SSSYSKQFTSSTSYNRGDSTFESKSYKMADEAGSEADHEGTHSTKRGH . A . K	✓		✓
5901.7029	5905.2293	P02671	FGA chain (577-629)	K . SSSYSKQFTSSTSYNRGDSTFESKSYKMADEAGSEADHEGTHSTKRGHAKSRPV . R	✓		✓
6627.3167	6631.4463	P02655	Apolipoprotein C-II (32-90)	M . PSPTFLTQVKESLSSYWEAKTAAQNLYEKTYLPAVDEKLRDLYSKSTAAMSTYTGIFT . D	✓		
8200.0508	8205.1815	P02655	Apolipoprotein C-II (29-101)	Q . DEMPSTFLTQVKESLSSYWEAKTAAQNLYEKTYLPAVDEKLRDLYSKSTAAMSTYTGIFTDQVLSVLKGE	✓		
8760.2248	8765.6732	P02656	Apolipoprotein C-III	SEAEDASLLSFMQGYMKHATKAKDALSSVQESQVAQQARGWVTDGFSLLKDYWSTVVKDKFSE FWDLDPEVRPTSAVAA	✓		
8910.3855	8915.9261	P02655	Apolipoprotein C-II (Chain A)	TQQPQQDEMPSTFLTQVKESLSSYWEAKTAAQNLYEKTYLPAVDEKLRDLYSKSTAAMSTY TGIFTDQVLSVLKGE	✓		
9282.8208	9288.7182	P02775	CTAP-III	NLAKGKEESLSDLYAELRCMCIKTTSGIHPKNIQSLEVIGKGTNCQVEVIATLKDGRKICL DPDAPRIKKIVQKLAGDESAD	✓		

\* *In the MS/MS spectra of these peaks, Choline, H<sub>2</sub>O-Choline and PhosphoCholine peaks were dominant but no corresponding phospholipids were found in the LipidMaps database.*

*CTAP-III: Connective tissue-activating peptide III; FGA: Fibrinogen alpha; FPA: Fibrinopeptide A; FPB: Fibrinopeptide B; HMW Kininogen: High molecular weight kininogen; IGIH4: Inter-alpha (Globulin) inhibitor H4 (Plasma Kallikrein-sensitive glycoprotein) variant; ITIH4: Inter-alpha-trypsin inhibitor heavy chain H4; NA: Not applicable; PC: Phosphatidylcholine; TMSB4X: Thymosin, beta 4. [M+H]<sup>+</sup> values were calculated using IsotopePattern Software (Bruker Daltonics).*

## Synopsis

Despite the numerous reports on MALDI MS profiling of human serum there is only scarce information on the profile peaks' identities. Here, we provide a well-characterised list of peak identities obtained from human blood serum that can be used for provisional identification of these peaks and show that in-depth proteomic analysis of many thousands of peptides by (multidimensional) LC-ESI-MS/MS cannot provide the necessary information for identifying MALDI-generated profile peaks.

