N-terminal derivatization of peptides with 4'-formylbenzo-18-crown-6-ether for protein and species identification

Luka Ozdanovac (Luka.Ozdanovac@irb.hr),[1] Renata Biba (Renata.Biba@irb.hr),[1] Marijana Erk (Marijana.Erk@irb.hr),[1] Amela Hozić (Amela.Hozic@irb.hr),[1] Marta Zrno (eaamazr@students.eaaa.dk),[2] Mario Cindrić (Mario.Cindric@irb.hr)*

[1] Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb 10000, Croatia
[2] IT Technology, Business Academy Aarhus, Aarhus 8260, Denmark
*Corresponding author

Keywords: *de novo* sequencing, crown ether, *N*-terminal peptide derivatization, noise reduction, database matching, species identification.

Supplementary information

Table S1. The list of derivatized transferrin peptides with amino acid sequences, precursor ion masses and corresponding intensities derived from PLGS software analysis.

Number	Precursor MH+ (Da)	Sequence	Modifications	Intensity	Intensity sum
Derivatization of *N*-terminus					
1	5888.9507	(R)AIAANEADAVTLDAGLVYDAYLAPNN LKPVVAEFYGSKEDPQTFYYAVAVVK(K)	4-formylbenzo-18-crown 6-ether (1)	78672	4456899
2	1953.9696	(K)EDPQTFYYAVAVVK(K)	4-formylbenzo-18-crown 6-ether (1)	413992	
3	2082.0647	(K)EDPQTFYYAVAVVKK(D)	4-formylbenzo-18-crown 6-ether (1)	9102	
4	1302.6453	(K)DGAGDVAFVK(H)	4-formylbenzo-18-crown 6-ether (1)	343316	
5	1597.8087	(K)HSTIFENLANK(A)	4-formylbenzo-18-crown 6-ether (1)	169399	
6	2854.3901	(R)SMGGKEDLIWELLNQAQEHFGK(D)	4-formylbenzo-18-crown 6-ether (1)	104413	
7	2394.1833	(K)EDLIWELLNQAQEHFGK(D)	4-formylbenzo-18-crown 6-ether (1)	69496	
8	2637.3050	(K)EDLIWELLNQAQEHFGKDK(S)	4-formylbenzo-18-crown 6-ether (1)	28880	
9	1815.9140	(K)SKEFQLFSSPHGK(D)	4-formylbenzo-18-crown 6-ether (1)	25347	
10	1600.7897	(K)EFQLFSSPHGK(D)	4-formylbenzo-18-crown 6-ether (1)	33466	
11	1802.8892	(K)MYLGYEYVTAIR(N)	4-formylbenzo-18-crown 6-ether (1)	283273	
12	2483.1682	(K)IMNGEADAMSLDGGFVYIAGK(C)	4-formylbenzo-18-crown 6-ether (1)	539553	
13	1683.8461	(K)KSASDLTWDNLK(G)	4-formylbenzo-18-crown 6-ether (1)	4760	
14	1701.8584	(K)KSASDLTWDNLK(G)	4-formylbenzo-18-crown 6-ether (1)	100462	
15	1573.7616	(K)SASDLTWDNLK(G)	4-formylbenzo-18-crown 6-ether (1)	618734	
16	1607.7244	(K)EGYYGYTGAFR(C)	4-formylbenzo-18-crown 6-ether (1)	185836	
17	2299.1338	(K)HQTVPQNTGGKNPDPWAK(N)	4-formylbenzo-18-crown 6-ether (1)	129222	
18	1151.5632	(K)NPDPWAK(N)	4-formylbenzo-18-crown 6-ether (1)	854927	
19	2220.0679	(K)NLNEKDYELLCLDGTR(K)	4-formylbenzo-18-crown 6-ether (1)	49119	
20	1324.6538	(K)YLGEEYVK(A)	4-formylbenzo-18-crown 6-ether (1)	41629	
21	4278.1685	(R)AIAANEADAVTLDAGLVYDAYLAPNN LKPVVAEFYGSK(E)	4-formylbenzo-18-crown 6-ether (1)	362810	
22	2499.1630	(K)IMNGEADAMSLDGGFVYIAGK(C)	4-formylbenzo-18-crown 6-ether (1), Oxidation M (2)	10491	
Number	**Precursor MH+ (Da)**	**Sequence**	**Modifications**	**Intensity**	**Intensity sum**
Side chain derivatization					
23	6213.1120	(R)AIAANEADAVTLDAGLVYDAYLAPNN LKPVVAEFYGSKEDPQTFYYAVAVVK(K)	4-formylbenzo-18-crown 6-ether_2	1964	1626552
24	1953.9674	(K)EDPQTFYYAVAVVK(K)	4-formylbenzo-18-crown 6-ether_2	20786	
25	2438.2397	(R)SAGWNIPIGLLYCDLPEPR(K)	4-formylbenzo-18-crown 6-ether_2	14168	
26	1815.9140	(K)SKEFQLFSSPHGK(D)	4-formylbenzo-18-crown 6-ether_2	41134	
27	1490.7499	(K)HQTVPQNTGGK(N)	4-formylbenzo-18-crown 6-ether_2	1525708	

Table S1. The list of derivatized transferrin peptides with amino acid sequences, precursor ion masses and corresponding intensities derived from PLGS software analysis. (**continued**)

28	4602.3125	(R)AIAANEADAVTLDAGLVYDAYLAPNNLKPVVAEFYGSK(E)	4-formylbenzo-18-crown 6-ether_2	4052
29	5556.5700	(K)IECVSAETTEDCIAKIMNGEADAMSLDGGFVYIAGKCGLVPVLAENYNK(S)	4-formylbenzo-18-crown 6-ether_2, Oxidation M (17)	1449
30	2823.3157	(K)IMNGEADAMSLDGGFVYIAGK(C)	4-formylbenzo-18-crown 6-ether_2, Oxidation M (2)	6444
31	3437.6638	(R)SMGGKEDLIWELLNQAQEHFGKDK(S)	4-formylbenzo-18-crown 6-ether_2, Oxidation M (2)	10847

Note: The peptide sequences marked in red are doubly derivatized.

Table S2. The results of *de novo* sequencing of unreduced and reduced datasets of BSA peptide ions: derivatized at *m*/*z* 1991.9664 and underivatized at *m*/*z* 1667.8094.

Correct peptide sequence: **MPCTEDYISIIINR**

Rank	***de novo* sequences (unreduced dataset)**	**Score**	**Rank**	***de novo* sequences (reduced dataset)**	**Score**
1	MAETEDYISIIINR	66.0	1	MAETEDYISIIINR	66.5
2	MPCTEFMLSLLLNR	64.7	2	MPCTEFMLSLLLNR	64.4
3	MEAETDYISIIINR	63.4	3	MAETEDYISIIIRN	61.6
4	MAEETDYISIIINR	62.6	4	NNCTEFMLSLLLNR	61.5
5	MPCTEFMLTVLLNR	62.5	5	MPCTEFLMSLLLNR	59.5
6	MPCTEFMLAELLNR	62.3	6	LDCTEFMLSLLLNR	57.8
7	NNCTEFMLSLLLNR	61.3	7	PMCTEFMLSLLLNR	57.8
8	MPCETFMLSLLLNR	61.3	8	DLCTEFMLSLLLNR	56.8
9	MPCTEFLMSLLLNR	59.9	9	VECTEFMLSLLLNR	56.8
10	MPCTEFFPSLLLNR	59.7	10	NNCTEFLMSLLLNR	56.6
time of analysis	1.08 s		time of analysis	0.44 s	

Matched peptide sequence: MAETEDYISIIINR

Permutations of matched peptide sequence: MAETEDYISIIINR, MAETEDYISIILNR, MAETEDYISILINR, MAETEDYISILLNR, MAETEDYISLIINR, MAETEDYISLILNR, MAETEDYISLLINR, MAETEDYISLLLNR, MAETEDYLSIIINR, MAETEDYLSIILNR, MAETEDYLSILINR, MAETEDYLSILLNR, MAETEDYLSLIINR, MAETEDYLSLILNR, MAETEDYLSLLINR, MAETEDYLSLLLNR

Note: The amino acid residues highlighted in green in the peptide sequences have been successfully identified at their corresponding indices. The same note applies to all subsequent supplementary tables.

Table S3. The results of *de novo* sequencing of unreduced and reduced datasets of BSA peptide ions: derivatized at *m*/*z* 1113.6301 and underivatized at *m*/*z* 789.4714.

Correct peptide sequence: **LVTDLTK**

Rank	***de novo* sequences (unreduced dataset)**	**Score**	**Rank**	***de novo* sequences (reduced dataset)**	**Score**
1	IVTDITK	62.4	1	IVTDITK	62.0
2	LVTPMTK	59.4	2	IVTPMTK	57.0
3	LVTDTLK	58.9	3	IVTEVTK	55.6
4	IVSEITK	56.7	4	IVTDTIK	54.8
5	LVTSELK	56.5	5	IVTDDVK	54.8
6	LVTPTMK	55.8	6	LVCLLTK	51.7
7	LVTDWR	55.2	7	KTLDTVL	51.5
8	LVTEVTK	55.2	8	VLCLLTK	51.3
9	LVTSLEK	55.2	9	IAEDITK	50.7
10	LVCLLTK	54.0	10	LSEVLTK	50.3
time of analysis	0.14 s		time of analysis	0.11 s	

Matched peptide sequence: IVTDITK

Permutations of matched peptide sequence: LVTDLTK, IVTDITK, IVTDLTK, LVTDITK

Table S4. The results of *de novo* sequencing of unreduced and reduced datasets of BSA peptide ions: derivatized at *m*/*z* 1629.8699 and underivatized at *m*/*z* 1305.7169.

Correct peptide sequence: **HLVDEPQNLIK**

Rank	***de novo* sequences (unreduced dataset)**	**Score**	**Rank**	***de novo* sequences (reduced dataset)**	**Score**
1	HIVDEPKNIIK	54.5	1	HIVDEPKNIIK	70.3
2	HIDVEPKNIIK	51.8	2	HIVDEKPNIIK	69.9
3	HLDDLPKNLLK	49.5	3	HIVDEKNPIIK	67.7
4	HLDEVPKNLLK	49.4	4	HIVDENKPIIK	67.3
5	HLVHKPFDGAGK	48.6	5	HIVDEKGGPIIK	65.9
6	HLVHKPVYGAGK	47.8	6	HMVDKPAGPLLK	46.7
7	HLVHKPFDGKK	45.3	7	HVMDKPAGPLLK	46.6
8	HLVHKPFDKGK	45.2	8	SSHLWPAGPLLK	41.6
9	HAGAAGEPKNIIK	44.1	9	SSLHWPAGPLLK	41.5
10	HLVHKPWFNK	42.3	10	AGPAADKPAGPLLK	41.3
time of analysis	0.42 s		time of analysis	0.30 s	

Matched peptide sequence: HIVDEPKNIIK

Permutations of matched peptide sequence: HIVDEPKNIIK, HIVDEPQNIIK, HIVDEPKNILK, HIVDEPQNILK, HIVDEPKNLIK, HIVDEPQNLIK, HIVDEPKNLLK, HIVDEPQNLLK, HLVDEPKNIIK, HLVDEPQNIIK, HLVDEPKNILK, HLVDEPQNILK, HLVDEPKNLIK, HLVDEPQNLIK, HLVDEPKNLLK, HLVDEPQNLLK

Table S5. The results of *de novo* sequencing of unreduced and reduced datasets of BSA peptide ions: derivatized at *m*/*z* 1991.9664 and underivatized at *m*/*z* 1667.8094.

Correct peptide sequence: **DAFLGSFLYEYSR**

Rank	***de novo* sequences (unreduced dataset)**	**Score**	**Rank**	***de novo* sequences (reduced dataset)**	**Score**
1	DAFIGSFIYHGANR	81.8	1	DAFIGSFIYEYSR	80.7
2	WFLGSYPYEYSR	80.2	2	SVFIGSFIYEYSR	77.6
3	WFLGSYPYEYDK	79.4	3	SGGSSAGSFIYEYSR	70.7
4	FSVLGSYPYEYSR	78.9	4	SGGSTGGSFIYEYSR	69.9
5	FSVLGSYPYEYDK	78.4	5	SGGSASGSFIYEYSR	69.8
6	SFVLGSYPYEYSR	77.6	6	SGGSGTGSFIYEYSR	69.8
7	SFVLGSYPYEYDK	77.0	7	SGGSASGSFIYYGFAP	59.6
8	WFLGSYNFEYNK	73.8	8	SGGSGTGSFIYYGFAP	59.5
9	WFLGSYPYAMSFV	72.5	9	DAFEPHGPYHGANR	37.1
10	WFLGSYPYAMSVF	72.5	10	DAFPEHGPYHGANR	36.3
time of analysis	0.91 s		time of analysis	0.45 s	

Matched peptide sequence: DAFIGSFIY

Permutations of matched peptide sequence: DAFIGSFIY, DAFIGSFLY, DAFLGSFIY, DAFLGSFLY

Table S6. The results of *de novo* sequencing of unreduced and reduced datasets of Elongation factor Tu peptide ions: derivatized at *m*/*z* 1161.6515 and underivatized at *m*/*z* 837.4926.

Correct peptide sequence: **EHILLGR**					
Rank	***de novo* sequences (unreduced dataset)**	**Score**	**Rank**	***de novo* sequences (reduced dataset)**	**Score**
1	EHILIGR	52.8	1	EHIIIGR	52.6
2	HEILIGR	52.1	2	(111.08)HMIIGR	50.6
3	(111.08)HMLIGR	51.8	3	TFMLLGR	43.7
4	EHILTPK	48.9	4	FTMLLGR	42.7
5	HEILTPK	48.2	5	EHIIIRG	40.6
6	(111.08)HMLTPK	47.1	6	(111.08)HIMIGR	39.4
7	(111.08)HIMIGR	45.2	7	EYSIIGR	39.4
8	EHILTKP	44.5	8	(177.13)DSIIGR	37.5
9	HELLTKP	43.8	9	(177.13)TTIIGR	37.1
10	EHILIRG	43.3	10	SSGIIIFT	33.3
time of analysis	0.06 s		time of analysis	0.06 s	
Matched peptide sequence: EHILIGR					
Permutations of matched peptide sequence: EHIIIGR, EHIILGR, EHILIGR, EHILLGR, EHLIIGR, EHLILGR, EHLLIGR, EHLLLGR					

Table S7. The results of *de novo* sequencing of unreduced and reduced datasets of Elongation factor Tu peptide ions: derivatized at *m*/*z* 1538.7867 and underivatized at *m*/*z* 1214.6315.

Correct peptide sequence: **FESEVYILSK**					
Rank	***de novo* sequences (unreduced dataset)**	**Score**	**Rank**	***de novo* sequences (reduced dataset)**	**Score**
1	FESEVYIISK	74.1	1	FESEVYLLNT	43.1
2	FEDTVYIISK	72.3	2	EFSEVYLLNT	42.0
3	FESEVYIINT	66.1	3	FEDTVYLLNT	41.7
4	EFSEVYLLNT	65.6	4	YLDTVYLLNT	40.9
5	LYSEVYLLNT	65.3	5	LYVESYLLNT	40.8
6	FEDTVYIINT	64.9	6	YLSEVYLLNT	40.8
7	LYDTVYLLNT	63.5	7	EFDTVYLLNT	40.6
8	LYSEVLYLNT	63.1	8	LYSEVYLLNT	40.6
9	EFSEVLYLNT	62.8	9	LYDTVYLLNT	40.3
10	FESEVLYLNT	62.5	10	FEVESYLLNT	39.9
time of analysis	0.81 s		time of analysis	0.39 s	
Matched peptide sequence: FESEVYLL					
Permutations of matched peptide sequence: FESEVYII, FESEVYIL, FESEVYLI, FESEVYLL					

Table S8. The results of *de novo* sequencing of unreduced and reduced datasets of Elongation factor Tu peptide ions: derivatized at *m/z* 2120.1155 and underivatized at *m/z* 1795.9569.

Correct peptide sequence: **TKPHVNVGTIGHVDHGK**

Rank	***de novo* sequences (unreduced dataset)**	**Score**	**Rank**	***de novo* sequences (reduced dataset)**	**Score**
1	KTPHVNVGTIGHVDNHA	51.7	1	TKPHVNVSAIGHVDHKG	63.5
2	KTPHVAAAGTIGHVDNHA	51.3	2	TKPHVVNSAIGHVDHKG	61.2
3	TKPHVNVGTIGHVDNHA	48.9	3	KTPHVNTRMPSPVFGK	56.8
4	KTPHVNTRLDPKGVMC	48.2	4	TKPHVNTRMAPPCKLC	56.5
5	KTPHVNTRLDPKVGMC	47.6	5	KTPHVNTRMAPPCKLC	56.5
6	KTPEVHGLRTTGAGGVLC	47.4	6	HGVGNGVAGISANVVHPPN	56.5
7	KTPEVHGLRTTGANVLC	47.1	7	TKPHVNTRMDPRKLC	56.4
8	KTPEVHGLRTTAGGGVLC	47.1	8	KTPHVNTRMDPRKLC	56.4
9	KTPHVNTRTSAGHVGSF	47.0	9	KTPHVNTRMDPPAGKF	56.3
10	KTPHVNTRLGHVEPNP	46.9	10	KTPHVNTRMPSVPFGK	56.1
time of analysis	2.78 s		time of analysis	0.41 s	

Matched peptide sequence: PHVNVSAIGHVD

Permutations of matched peptide sequence: PHVNVSAIGHVD, PHVNVSALGHVD

Table S9. The results of *de novo* sequencing of unreduced and reduced datasets of Elongation factor Tu peptide ions: derivatized at *m/z* 2128.0427 and underivatized at *m/z* 1803.8895.

Correct peptide sequence: **GITINTSHVEYDTPTR**

Rank	***de novo* sequences (unreduced dataset)**	**Score**	**Rank**	***de novo* sequences (reduced dataset)**	**Score**
1	GITINTSHVEYDTPTR	76.2	1	LGTINTSHVEYDTPTR	78.3
2	VATINTSHVEYDTPTR	76.2	2	TLGINTSHVEYDTPTR	71.8
3	GITINTSHVEYDTTPR	75.9	3	RDINTSHVEYDTPTR	68.2
4	VATINTSHVEYDTTPR	75.9	4	RDINTSHVEYDTTPR	67.8
5	VATIASGSHVEYDTPTR	71.6	5	RDGGITSHVEYDTPTR	67.8
6	VATGGITSHVEYDTPTR	69.7	6	RDGGITSHVEYDTTPR	67.5
7	GLTLNTSHVEYDTPSVA	67.7	7	RDINTSHVEYDTTGVP	60.4
8	GLTLNTSHVEYDTPSAV	67.6	8	RDINTSHVEYDTGTVP	59.3
9	GLTLNTSHVEYDTPSGL	67.6	9	RDGGITSHVEYDTGTVP	57.0
10	GLTLNTSHVEYDTPSLG	67.6	10	AAENLTSHGVHSTNLPR	52.8
time of analysis	2.06 s		time of analysis	1.05 s	

Matched peptide sequence: TINTSHVEYDTPTR

Permutations of matched peptide sequence: TINTSHVEYDTPTR, TLNTSHVEYDTPTR

Table S10. The results of *de novo* sequencing of unreduced and reduced datasets of Elongation factor Tu peptide ions: derivatized at *m*/*z* 2441.3184 and underivatized at *m*/*z* 2117.1638.

Correct peptide sequence: **AIDKPFLLPIEDVFSISGR**

Rank	***de novo* sequences (unreduced dataset)**	**Score**	**Rank**	***de novo* sequences (reduced dataset)**	**Score**
1	TAALEDLRRKTPVFSLSGR	42.9	1	ALDKPFLLPLEDVFSLSGR	85.1
2	LPEDATSRRKTPVFSLSGR	42.8	2	LADKPFLLPLEDVFSLSGR	84.9
3	TAALELDRRKTPVFSSLGR	41.9	3	SPDKPFLLPLEDVFSLSGR	84.8
4	LPEDATSRRKTPVFSSLGR	41.9	4	PSDKPFLLPLEDVFSLSGR	84.8
5	TAALEDLRRKTPVFSSLGR	41.8	5	KNGKPFIIPIEDVFSISGR	84.2
6	WGNKDLRRKTPVFSLSGR	41.4	6	ALDKLMLLPLEDVFSLSGR	83.6
7	WGLNRSRRKTPVFSSLGR	41.2	7	LADKLMLLPLEDVFSLSGR	83.6
8	WGNLRSRRKTPVFSSLGR	40.9	8	SPDKLMLLPLEDVFSLSGR	83.6
9	WLGNRSRRKTPVFSSLGR	40.8	9	PSDKLMLLPLEDVFSLSGR	83.6
10	WGNKDLRRKTPVFSSLGR	40.1	10	AGNGKPFIIPIEDVFSISGR	83.6
time of analysis	3.45 s		time of analysis	0.72 s	

Matched peptide sequence: VFSLSGR

Permutations of matched peptide sequence: VFSISGR, VFSLSGR

Table S11. The results of *de novo* sequencing of unreduced and reduced datasets of Elongation factor Tu peptide ions: derivatized at *m*/*z* 2286.1755 and underivatized at *m*/*z* 1962.0199.

Correct peptide sequence: **ILELAGFLDSYIPEPER**

Rank	***de novo* sequences (unreduced dataset)**	**Score**	**Rank**	***de novo* sequences (reduced dataset)**	**Score**
1	IIEIAGFLDSYLPESHSA	52.5	1	LLELAGFMPSYLPESVPV	73.3
2	IIEIAGFLDSYLPESVVP	52.4	2	LLELAGFMPSYLPEWVP	73.2
3	LLNKASMVDSYLPESVVP	47.0	3	LLELAGFMPSYLPESVVP	73.1
4	LLNKASMVDSYLPESVPV	46.9	4	LLELAGFMPSYLPEWPV	73.1
5	LLNKASMVDSYLSLALHS	46.5	5	KNLLAGFMPSYLPESVPV	73.1
6	LLNKASMVDSYLSLALSH	46.5	6	LLSSEPLYSPMFGALLSH	72.9
7	LLNKASMVDSYLSALLSH	46.5	7	LLSSEPLYSPMFGALLHS	72.9
8	LLNKASMVDSYLSALLHS	46.4	8	NKLLAGFMPSYLPESVPV	72.9
9	LLELASMVDSYLPESVVP	46.2	9	KNLLAGFMPSYLPESVVP	72.9
10	LLELASMVDSYLPESVPV	46.1	10	NKLLAGFMPSYLPESVVP	72.7
time of analysis	1.99 s		time of analysis	0.88 s	

Matched peptide sequence: IIEIAGFMPSYLPE

Permutations of matched peptide sequence: IIEIAGFMPSYIPE, IIEIAGFMPSYLPE, IIELAGFMPSYIPE, IIELAGFMPSYLPE, ILEIAGFMPSYIPE, ILEIAGFMPSYLPE, ILELAGFMPSYIPE, ILELAGFMPSYLPE

Table S12. The results of *de novo* sequencing of unreduced and reduced datasets of HSA peptide ions: derivatized at *m*/*z* 1550.7594 and underivatized at *m*/*z* 1226.6095.

Correct peptide sequence: **FKDLGEENFK**					
Rank	***de novo* sequences (unreduced dataset)**	**Score**	**Rank**	***de novo* sequences (reduced dataset)**	**Score**
1	KFDLGEENKF	47.9	1	KFDLGEEPYE	70.7
2	KFDLGSGNNKF	46.8	2	RHNEEGIDKE	70.4
3	KFDLGSGMPKF	45.7	3	RHKDEGIDKE	69.2
4	KDFGAFGMPKE	45.3	4	HRNEEGLDKE	69.2
5	KFDLGSMGPKF	45.0	5	RHNEWLDKE	68.3
6	KDFGAFGLDKE	44.7	6	RHDRTGLDKE	68.0
7	KFDGAFGLDKE	44.5	7	HRKDEGLDKE	67.9
8	KFPMGSGMPKF	44.4	8	RPGPEEGIDKE	67.8
9	KDFMGSGLDKE	43.9	9	RHPSSTGLDKE	67.4
10	KDFGMSGLDKE	43.9	10	RHPCATGLDKE	67.3
time of analysis	0.41 s		time of analysis	0.16 s	
Matched peptide sequence: DLGEE					
Permutations of matched peptide sequence: DIGEE, DLGEE					

Table S13. The results of *de novo* sequencing of unreduced and reduced datasets of HSA peptide ions: derivatized at *m*/*z* 1964.0887 and underivatized at *m*/*z* 1639.9382.

Correct peptide sequence: **KVPQVSTPTLVEVSR**					
Rank	***de novo* sequences (unreduced dataset)**	**Score**	**Rank**	***de novo* sequences (reduced dataset)**	**Score**
1	AGVPKVSTPTIVEDAR	65.3	1	RAPKVMGPTIVEVSR	84.7
2	KVPKVSTPTIVEVSR	61.2	2	KVPKVMGPTIVEVSR	83.9
3	AGVPKVSTPTIVEVDK	60.5	3	RAPKVMGPTINNVSR	82.4
4	AGVPKVSTPTIVEVSR	59.9	4	ARPKVMGPTIVEVSR	81.8
5	AGPVKVSTPTLVEDAR	58.9	5	PLNKVMGPTLVEVSR	80.4
6	KVNEVLGPTLVEVSR	57.1	6	LPNKVMGPTLVEVSR	80.4
7	KVNEVLGPTLVEDAR	56.9	7	PLNKVMGPTLVEWR	79.7
8	NLNEVLGPTLVEVSR	56.4	8	LPNKVMGPTLVEWR	79.7
9	AGVNEVLGPTLVEVSR	55.9	9	PLNKVMGPTLVLDSR	79.1
10	KVNEVLGPTLVEWR	55.7	10	LPNKVMGPTLVLDSR	79.1
time of analysis	1.00 s		time of analysis	0.22 s	
Matched peptide sequence: KVPKVMGPTIVEVSR					
Permutations of matched peptide sequence: KVPKVMGPTIVEVSR, KVPQVMGPTIVEVSR, QVPKVMGPTIVEVSR, QVPQVMGPTIVEVSR, KVPKVMGPTLVEVSR, KVPQVMGPTLVEVSR, QVPKVMGPTLVEVSR, QVPQVMGPTLVEVSR					

Table S14. The results of *de novo* sequencing of unreduced and reduced datasets of Ile-RS peptide ions: derivatized at *m*/*z* 1584.7349 and underivatized at *m*/*z* 1260.5763.

Correct peptide sequence: **EHGSNVWFER**

Rank	***de novo* sequences (unreduced dataset)**	**Score**	**Rank**	***de novo* sequences (reduced dataset)**	**Score**
1	EHGSNVGEFER	61.5	1	EHGSNVVSFER	63.5
2	EHGSNVWFER	61.2	2	CYGSNVWFER	62.3
3	EHGSNVVSFER	61.0	3	EHGSNVWFER	62.1
4	EHEEVGEFER	57.1	4	HEGSNVWFER	59.7
5	CYGSNVGEFER	57.0	5	YCGSNVWFER	58.2
6	EHEEVWFER	56.8	6	EHGSNVFDAER	54.3
7	CYGSNVWFER	56.7	7	EHGSNVFVSER	54.3
8	EHGSNVGFEER	54.4	8	EHGSNVFPSMR	52.6
9	EHGSNVGEFRE	53.3	9	EHGSNVFPSGET	49.5
10	EHEVLGANSCTT	51.7	10	EHEEVWFER	49.0
time of analysis	0.58 s		time of analysis	0.22 s	

Matched peptide sequence: EHGSNVWFER

Permutations of matched peptide sequence: EHGSNVWFER

Table S15. The results of *de novo* sequencing of unreduced and reduced datasets of Ile-RS peptide ions: derivatized at *m*/*z* 1738.8632 and underivatized at *m*/*z* 1414.7085.

Correct peptide sequence: **GVLSHGFALDGEGR**

Rank	***de novo* sequences (unreduced dataset)**	**Score**	**Rank**	***de novo* sequences (reduced dataset)**	**Score**
1	GVLSHGFAIDGEGR	71.8	1	RLSHGFALDWGR	40.4
2	RLSHGFAIDDAGR	71.4	2	RLSHGFALDWRG	35.6
3	RLSHGFAIDGEGR	71.2	3	RLSHGFALDWVN	35.5
4	RLSHGFAIDGDAR	67.5	4	VGISHGFAIDDARG	35.5
5	RLSHGFANGGGEGR	67.5	5	RISHGFAIDDARG	35.4
6	RLSHGFAIDGADR	66.8	6	RLSHGFALDWNV	35.3
7	RLSHGFANNGEGR	66.6	7	RISHGFAIDPGNM	35.1
8	RLSHGFAIDGERG	61.7	8	VGISHGFAIDNGMP	34.3
9	RLSHGFAIDPGSGT	58.3	9	RISHGFAIDNGMP	34.2
10	RLSHGFAIDGPSGT	58.1	10	RISHGFAIDNMGP	34.0
time of analysis	0.84 s		time of analysis	0.06 s	

Matched peptide sequence: LSHGFAID

Permutations of matched peptide sequence: ISHGFAID, ISHGFALD, LSHGFAID, LSHGFALD

Table S16. The results of *de novo* sequencing of unreduced and reduced datasets of Ile-RS peptide ions: derivatized at *m*/*z* 1759.9598 and underivatized at *m*/*z* 1435.8032.

Correct peptide sequence: **YVVATELIETVAK**					
Rank	***de novo* sequences (unreduced dataset)**	**Score**	**Rank**	***de novo* sequences (reduced dataset)**	**Score**
1	YVVATEIIETVAK	71.2	1	YVVATEIIETVAQ	47.1
2	YVVATEIIEEAAK	71.1	2	YVVATEIIETVKA	47.1
3	YVVATNKIETVAK	68.0	3	YVVTAEIIETVAQ	44.6
4	YVVDGEIIETVAK	63.1	4	YVVTAEIIETVKA	44.6
5	YVVANLLEHLHK	63.0	5	YVVATELLNAFVP	43.6
6	YVVATELLNAFPV	62.8	6	YVVATELLNAFPV	43.6
7	YVVATELLNAFVP	62.8	7	YVVPGFIIETVAQ	43.5
8	YVVANLLETAVPF	61.7	8	YVVTSIIIETVAQ	43.5
9	YVVATELLNAVFP	61.7	9	YVVATELLNAVML	43.2
10	YVVATELLNAVML	61.7	10	YVVANLLETAVML	43.1
time of analysis	0.73 s		time of analysis	0.47 s	
Matched peptide sequence: YVVATEIIETVAK					
Permutations of matched peptide sequence: YVVATEIIETVAK, YVVATEILETVAK, YVVATELIETVAK, YVVATELLETVAK					

Table S17. The results of *de novo* sequencing of unreduced and reduced datasets of Ile-RS peptide ions: derivatized at *m*/*z* 2237.0098 and underivatized at *m*/*z* 1912.8541.

Correct peptide sequence: **GHMTNEAPGFEGLFYDK**					
Rank	***de novo* sequences (unreduced dataset)**	**Score**	**Rank**	***de novo* sequences (reduced dataset)**	**Score**
1	GHMTGGEAPGFEGIFYDK	71.3	1	HGMTGGEAPGFEGIFYDK	76.5
2	GHMTNEAPGFEGIFYDK	70.7	2	HGMGSAEAPGFEGIFYDK	75.0
3	GHMGTGEAPGFEGIFYDK	70.6	3	HGMTNEAPGFEGIFYDK	74.7
4	GPNGTNEAPGFEGIFYDK	63.3	4	HGMTGGEAPGFDAIFYDK	71.8
5	HGMTGGEAPGSCSAGKTTYT	52.0	5	PGNGTGGEAPGFEGIFYDK	70.2
6	HGMTNEAPGSCSAGKTTYT	51.8	6	NGPGTGGEAPGFEGIFYDK	70.2
7	HGMTGGEAPGSCSAFVLEY	51.6	7	NGPGTNEAPGFEGIFYDK	70.2
8	HGMTNEAPGSCSAFVLEY	51.5	8	PGNGTNEAPGFEGIFYDK	70.1
9	HGMTNEAPGSCSASLHAGW	47.8	9	PGAGSGGGEAPGFEGIFYDK	67.3
10	HGMTNEASCSGPASLHADK	46.9	10	GHMTGGEAPGMKDRWPW	59.6
time of analysis	2.08 s		time of analysis	0.50 s	
Matched peptide sequence: MTNEAPGFEGIFYDK					
Permutations of matched peptide sequence: MTNEAPGFEGIFYDK, MTNEAPGFEGLFYDK					

Table S18. The results of *de novo* sequencing of unreduced and reduced datasets of Transferrin peptide ions: derivatized at *m*/*z* 1302.6453 and underivatized at *m*/*z* 978.4911.

Correct peptide sequence: **DGAGDVAFVK**					
Rank	***de novo* sequences (unreduced dataset)**	**Score**	**Rank**	***de novo* sequences (reduced dataset)**	**Score**
1	DGAGDVAFVK	68.2	1	DKGDVAFVK	62.9
2	DGAGITAFVK	67.4	2	DKGDVAFAR	60.8
3	ATAGDVAFVK	66.4	3	DAGGDVAFVK	56.7
4	DKGDVAFVK	63.7	4	DGAGDVAFVK	56.7
5	DAGGDVAFVK	63.6	5	DKGDAVFVK	55.4
6	DAGGLTAFVK	63.5	6	DKGDAVFAR	54.8
7	DGATAVAFVK	62.5	7	DAGGDVAFAR	54.6
8	SRGDVAFVK	62.3	8	DGAGDVAFAR	54.6
9	SGVGDVAFVK	61.6	9	DGKDVAFVK	54.2
10	DAGTAVAFVK	60.6	10	ADGGDVAFVK	53.1
time of analysis	0.28 s		time of analysis	0.14 s	
Matched peptide sequence: DGAGDVAFVK					
Permutations of matched peptide sequence: DGAGDVAFVK					

Table S19. The results of *de novo* sequencing of unreduced and reduced datasets of Transferrin peptide ions: derivatized at *m*/*z* 1573.7616 and underivatized at *m*/*z* 1249.6104.

Correct peptide sequence: **SASDLTWDNLK**					
Rank	***de novo* sequences (unreduced dataset)**	**Score**	**Rank**	***de novo* sequences (reduced dataset)**	**Score**
1	SASDLTWDNLK	60.3	1	SASDLTWDNLK	65.2
2	SASDITADDNLK	59.9	2	SASDITDADNLK	64.4
3	SASDITDADNLK	59.8	3	ASSDLTWDKVK	62.9
4	SASDLTWDKVK	56.4	4	SASDLTWDKVK	62.2
5	SASPMTWDNLK	54.9	5	TGSDLTWDKVK	61.9
6	SASDKWTLDVK	53.5	6	GTSDLTWDKVK	61.9
7	SASDLRMDNLK	53.2	7	ASSDLTWDKAR	61.7
8	SASKDWTLDVK	53.1	8	SASDLTWDKAR	58.8
9	SASDLTWKDVK	52.8	9	ASSDSARTLDVK	58.5
10	SASDKWTMPVK	52.0	10	TGSDLTWDKAR	58.5
time of analysis	0.55 s		time of analysis	0.38 s	
Matched peptide sequence: SASDLTWDNLK					
Permutations of matched peptide sequence: SASDLTWDNLK					

Table S20. The results of *de novo* sequencing of unreduced and reduced datasets of Transferrin peptide ions: derivatized at *m*/*z* 1953.9696 and underivatized at *m*/*z* 1629.8147.

Correct peptide sequence: **EDPQTFYYAVAVVK**

Rank	***de novo* sequences (unreduced dataset)**	**Score**	**Rank**	***de novo* sequences (reduced dataset)**	**Score**
1	EDPKTFYYAVAVVK	65.0	1	EDPKTFYYAVAVVK	66.4
2	EDPKTFYYAVGIVK	64.7	2	EDPKTFYYAVGIVK	63.8
3	EDPKTFYYAVVAVK	64.3	3	EDPKTFYYAVAVRA	59.4
4	EDPKTFYAYVAVVK	63.6	4	EDPKTFYYAVGIRA	57.0
5	EDPKTFYAYVAVVK	59.1	5	EDNDPFYYAVAVVK	56.7
6	EDPKTEGGTEAVAVVK	50.6	6	EDPKTFYPHVAVVK	46.2
7	EDPKTEGTGEAVAVVK	49.8	7	EDPKTTKAVHAAAYK	40.8
8	EDPNPEPELEYAVK	30.9	8	EDPKTTKAVHYNVK	39.4
9	EDNETDFTYVAVVK	30.5	9	EDPKTTKAVHYVNK	39.2
10	EDNETVYTYVAVVK	30.2	10	EDPKTTKAVHACCVK	37.7
time of analysis	1.98 s		time of analysis	0.67 s	

Matched peptide sequence: EDPKTFYYAVAVVK

Permutations of matched peptide sequence: EDPKTFYYAVAVVK, EDPQTFYYAVAVVK

Table S21. The results of *de novo* sequencing of unreduced and reduced datasets of Transferrin peptide ions: derivatized at *m*/*z* 2483.1682 and underivatized at *m*/*z* 2159.0125.

Correct peptide sequence: **IMNGEADAMSLDGGFVYIAGK**

Rank	***de novo* sequences (unreduced dataset)**	**Score**	**Rank**	***de novo* sequences (reduced dataset)**	**Score**
1	ADAMSLDGGFVYLAGK	67.2	1	RTEASADAMSNNGGFVYIAGK	64.8
2	LMNWADAMSLDGGFVYLAGK	67.1	2	RTEASADAMSNNNFVYIAGK	64.4
3	LMNGEADAMSLDNFVYLAGK	67.0	3	RTEASADAMVSEGGFVYIAGK	63.2
4	LMNWADAMSLDNFVYLAGK	67.0	4	RTEATGDAMSNNGGFVYIAGK	62.0
5	LMNGEWAMSLDGGFVYLAGK	64.6	5	ADAEASADAMSNNGGFVYIAGK	61.6
6	LMNGEAWMSLDGGFVYLAGK	64.2	6	ATGPMSADAMSNNGGFVYIAGK	61.6
7	LMNGEWAMSLDNFVYLAGK	64.1	7	VSAEASADAMSNNGGFVYIAGK	61.4
8	LMNWWAMSLDGGFVYLAGK	64.0	8	ADAEASADAMSNNNFVYIAGK	61.2
9	LMNWAWMSLDGGFVYLAGK	63.8	9	ADNMIADAMSNNGGFVYIAGK	61.1
10	LMNGEAWMSLDNFVYLAGK	63.5	10	RTEASADAMSNNGGFVYIAKG	57.7
time of analysis	2.89 s		time of analysis	0.53 s	

Matched peptide sequence: ADAMSLDGGFVYLAGK

Permutations of matched peptide sequence: ADAMSLDGGFVYLAGK, ADAMSLDGGFVYIAGK

Table S22. The results of *de novo* sequencing of unreduced and reduced datasets of Val-RS peptide ions: derivatized at *m*/*z* 1399.6724 and underivatized at *m*/*z* 1075.5183.

Correct peptide sequence: **LGNSVDWER**

Rank	***de novo* sequences (unreduced dataset)**	**Score**	**Rank**	***de novo* sequences (reduced dataset)**	**Score**
1	LGGGSVDWER	72.0	1	IGNSVDVSER	65.9
2	LGNSVDWER	70.4	2	LGNSVDWER	65.4
3	LGGGSVDAMRL	65.2	3	LGNWDWER	61.6
4	LGGGSVDVCRL	65.1	4	RFWDVCHL	56.4
5	LGGGSVDMARL	65.1	5	LGNSTLWER	56.4
6	LNGSVDWER	64.9	6	NTRVDWER	55.9
7	LGNSVDAMRL	63.7	7	NGLSVDWER	55.9
8	LGNSVDMARL	63.6	8	ASGRVDWER	55.0
9	LGNSVDVCRL	63.6	9	TGGRVDWER	54.8
10	LGGGSVDSDRL	61.8	10	NGPWMWER	54.6
time of analysis	0.30 s		time of analysis	0.17 s	

Matched peptide sequence: LGNSVDWER

Permutations of matched peptide sequence: IGNSVDEWR, LGNSVDWER

Table S23. The results of *de novo* sequencing of unreduced and reduced datasets of Val-RS peptide ions: derivatized at *m*/*z* 1536.8185 and underivatized at *m*/*z* 1212.6606.

Correct peptide sequence: **LYKEDLIYR**

Rank	***de novo* sequences (unreduced dataset)**	**Score**	**Rank**	***de novo* sequences (reduced dataset)**	**Score**
1	LYKEDIIYR	52.2	1	LYKEDLLYR	88.8
2	(205.08)AKEDIIYR	50.7	2	LYKEDLLRY	80.8
3	LYKEDLYLR	49.7	3	IYKEDII(319.16)	77.8
4	LYKEPMIYR	46.6	4	IYKEDIAA(290.13)	76.3
5	LYKEPMYLR	45.6	5	IYKEDIPHTP	75.9
6	LYKEVELYR	44.6	6	LYSGLDLLYR	70.3
7	LHGPEDIIYR	44.3	7	LYGSLDLLYR	69.9
8	LYKEPLMYR	43.6	8	LYSGLDLLRY	63.9
9	LYKELPMYR	42.9	9	LYGSLDLLRY	63.6
10	(135.07)IREDIIYR	42.2	10	ISHIIDERTE	59.3
time of analysis	0.22 s		time of analysis	0.05 s	

Matched peptide sequence: LYKEDIIYR

Permutations of matched peptide sequence: IYKEDIIYR, IYQEDIIYR, IYKEDILYR, IYQEDILYR, IYKEDLIYR, IYQEDLIYR, IYKEDLLYR, IYQEDLLYR, LYKEDIIYR, LYQEDIIYR, LYKEDILYR, LYQEDILYR, LYKEDLIYR, LYQEDLIYR, LYKEDLLYR, LYQEDLLYR

Table S24. The results of *de novo* sequencing of unreduced and reduced datasets of Val-RS peptide ions: derivatized at *m*/*z* 2962.5647 and underivatized at *m*/*z* 2638.4082.

Correct peptide sequence: **SKGNVIDPLDMVDGISLPELLEKR**					
Rank	***de novo* sequences (unreduced dataset)**	**Score**	**Rank**	***de novo* sequences (reduced dataset)**	**Score**
1	KSGNVLDPLDMVDGLSLPELLEKR	84.1	1	KSVNGDIPIDVMDGLSIPEILEKR	71.7
2	SKGNVLDPLDMVDGLSLPELLEKR	83.1	2	SKVNGDIPIDVMDGLSIPEILEKR	71.0
3	NTGNVLDPLDMVDGLSLPELLEKR	81.5	3	AGSVNGDIPIDVMDGLSIPEILEKR	70.4
4	AGSGNVIDPIDMVDGISIPELLEKR	81.4	4	KSVNGEVPIVDMDGLSIPEILEKR	69.4
5	TNGNVLDPLDMVDGLSLPELLEKR	81.1	5	AGSVNGDIPIVDMDGLSIPEILEKR	69.0
6	KSGNVDLPLDMVDGLSLPELLEKR	80.9	6	AGSVNGEVPIVDMDGLSIPEILEKR	68.9
7	KSGNVLDPMPMVDGLSLPELLEKR	80.8	7	PTSTVLDPLDMVDGLSLDKSLTLAH	66.5
8	KSGNVLDPDLMVDGLSLPELLEKR	80.8	8	TPSTVLDPLDMVDGLSLDKSLTLAH	66.5
9	KSGPGTADPLDMVDGLSLPELLEKR	80.8	9	VVSTVLDPLDMVDGLSLDKSLTLAH	66.5
10	SKGNVDLPLDMVDGLSLPELLEKR	79.9	10	VVSEALDPLDMVDGLSLDKSLTLAH	66.3
time of analysis	2.67 s		time of analysis	0.78 s	
Matched peptide sequence: SKVNGDIPIDVMDGLSIPEILEKR					
Permutations of matched peptide sequence: SKGNVIDPLDMVDGISLPELLEKR, SKGNVIDPLDMVDGISLPELLEQR, SQGNVIDPLDMVDGISLPELLEKR, SQGNVIDPLDMVDGISLPELLEQR, SKGNVIDPLDMVDGLSLPELLEKR. SKGNVIDPLDMVDGLSLPELLEQR, SQGNVIDPLDMVDGLSLPELLEKR, SQGNVIDPLDMVDGLSLPELLEQR, SKGNVLDPLDMVDGISLPELLEKR, SKGNVLDPLDMVDGISLPELLEQR, SQGNVLDPLDMVDGISLPELLEKR, SQGNVLDPLDMVDGISLPELLEQR, SKGNVLDPLDMVDGLSLPELLEKR, SKGNVLDPLDMVDGLSLPELLEQR, SQGNVLDPLDMVDGLSLPELLEKR, SQGNVLDPLDMVDGLSLPELLEQR					