

Supporting Information for Publication

Proteomic toolbox to standardize the separation of extracellular vesicles and lipoprotein particles

Tingting Wang^{‡#} and Illarion V. Turko^{‡.#,*}

[‡]Biomolecular Measurement Division, National Institute of Standards and Technology,
Gaithersburg, Maryland 20899, United States

[#]Institute for Bioscience and Biotechnology Research, Rockville, Maryland 20850,
United States

Table of Contents (Supporting Information)

Table S1. Transitions used for quantification.

Table S2. ¹⁵N-Labeling of LP1 and LP2 QconCATs.

Supporting Material 1. LP1 and LP2 QconCATs.

Figure S1. Scheme depicting separation protocols.

Table S1. Transitions used for quantification.

Protein	Peptide		Precursor (m/z)	Product Ions (m/z)		
				1	2	3
albumin	LVNEVTEFAK	L	573.1	937.5 (y8)	823.4 (y7)	694.4 (y6)
		H	581.3	947.4 (y8)	831.4 (y7)	701.4 (y6)
	VFDEFKPLVEEPQNLIK	L	682.4	900.0 (y15)	842.5 (y14)	777.9 (y13)
		H	689.3	909.5 (y15)	851.4 (y14)	786.4 (y13)
α -2-macroglobulin	LVHVEEPHTETVR	L	773.4	1097.52 (y9)	968.5 (y8)	839.4 (y7)
		H	783.4	1111.5 (y9)	981.4 (y8)	851.4 (y7)
	LPPNVVEESAR	L	605.8	1000.5 (y9)	789.4 (y7)	691.3 (y6)
		H	613.3	1013.5 (y9)	799.4 (y7)	699.3 (y6)
transferrin	DGAGDVAFVK	L	489.7	806.4 (y8)	678.4 (y6)	563.4 (y5)
		H	495.2	815.4 (y8)	685.4 (y6)	569.3 (y5)
apo A-I	DYVSQFEGSALGK	L	700.8	936.5 (y9)	808.4 (y8)	661.5 (y7)
		H	708.3	947.4 (y9)	817.4 (y8)	669.3 (y7)
	ATEHLSTLSEK	L	608.3	914.5 (y8)	777.4 (y7)	664.4 (y6)
		H	615.3	925.5 (y8)	785.4 (y7)	671.3 (y6)
apo A-II	EQLTPLIK	L	471.3	684.5 (y6)	571.3 (y5)	470.3 (y4)
		H	476.3	691.4 (y6)	577.4 (y5)	475.3 (y4)
apo A-IV	LLPHANEVSQK	L	618.4	1009.5 (y9)	912.5 (y8)	775.4 (y7)
		H	626.3	1023.5 (y9)	925.4 (y8)	785.4 (y7)
	IDQNVEELK	L	544.3	859.5 (y7)	731.4 (y6)	617.4 (y5)
		H	550.3	869.4 (y7)	739.4 (y6)	623.3 (y5)
	SELTQQLNALFQDK	L	545.6	948.5 (y8)	835.4 (y7)	721.4 (y6)
		H	551.9	959.5 (y8)	845.4 (y7)	729.4 (y6)
	LGEVNTYAGDLQK	L	704.4	1009.5 (y9)	895.5 (y8)	794.4 (y7)
		H	712.3	1021.5 (y9)	905.4 (y8)	803.4 (y7)
apo A-V	VEQIHQQK	L	505.3	781.4 (y6)	653.4 (y5)	540.3 (y4)
		H	512.3	793.4 (y6)	663.3 (y5)	549.3 (y4)
	VQELQEQLR	L	571.8	915.5 (y7)	786.4 (y6)	673.4 (y5)
		H	579.3	927.5 (y7)	797.4 (y6)	683.3 (y5)
	SVAPHAPASPAR	L	580.8	903.5 (y9)	806.4 (y8)	669.4 (y7)
		H	589.3	917.4 (y9)	819.4 (y8)	679.3 (y7)
	IQQNLDQLR	L	564.3	886.5 (y7)	758.4 (y6)	644.4 (y5)
		H	572.3	899.4 (y7)	769.4 (y6)	653.3 (y5)
apo C-I	EFGNTLEDK	L	526.7	776.3 (y7)	605.3 (y5)	504.3 (y4)
		H	532.2	785.4 (y7)	611.3 (y5)	509.3 (y4)
apo C-II	TYLPAVDEK	L	518.3	771.4 (y7)	658.3 (y6)	561.3 (y5)
		H	523.3	779.4 (y7)	665.3 (y6)	567.3 (y5)
apo C-III	DALSSVQESQVAQQAR	L	573.0	1016.5 (y9)	800.4 (y7)	672.4 (y6)
		H	580.6	1031.5 (y9)	813.4 (y7)	683.3 (y6)
apo C-IV	ELLETVVNR	L	536.8	830.5 (y7)	717.4 (y6)	588.3 (y5)
		H	543.2	841.4 (y7)	727.4 (y6)	597.3 (y5)
apo J	ASSIIDELFQDR	L	697.4	1035.5 (y8)	922.4 (y7)	678.4 (y5)
		H	705.3	1047.5 (y8)	933.4 (y7)	687.3 (y5)
	ELDESLQVAER	L	644.8	802.4 (y7)	715.4 (y6)	602.3 (y5)
		H	652.3	813.4 (y7)	725.4 (y6)	611.3 (y5)
apo B-100	FPEVDVLTK	L	524.3	803.5 (y7)	674.4 (y6)	575.3 (y5)

	LDFSSQADLR	H	529.3	811.4 (y7)	681.4 (y6)	581.3 (y5)
		L	576.3	923.5 (y8)	776.4 (y7)	689.3 (y6)
	NIILPVYDK	H	583.3	935.4 (y8)	787.4 (y7)	699.3 (y6)
		L	537.8	847.5 (y7)	734.4 (y6)	621.3 (y5)
		H	543.3	855.5 (y7)	741.4 (y6)	627.3 (y5)
apo B100/B48	SVSLPSLDPASAK	L	636.3	885.4 (y9)	788.4 (y8)	473.2 (y5)
		H	643.3	895.4 (y9)	797.4 (y8)	479.3 (y5)
		L	653.9	902.5 (y8)	803.5 (y7)	706.4 (y6)
	EFQVPTFTIPK	H	660.3	911.5 (y8)	811.4 (y7)	713.4 (y6)
		L	654.8	975.6 (y9)	878.5 (y8)	664.4 (y6)
	GFEPTLEALFGK	H	661.3	985.5 (y9)	887.5 (y8)	671.3 (y6)
apo D	AATVGSLAGQPLQER	L	499.9	827.4 (y7)	642.4 (y5)	545.3 (y4)
		H	506.6	839.4 (y7)	651.3 (y5)	553.3 (y4)
apo E	ELQAAQAR	L	443.7	644.3 (y6)	516.3 (y5)	445.3 (y4)
		H	450.2	655.3 (y6)	525.3 (y5)	453.2 (y4)
	EQVAEVR	L	415.7	573.3 (y5)	474.3 (y4)	403.2 (y3)
		H	421.2	581.3 (y5)	481.2 (y4)	409.2 (y3)
	LGPLVEQGR	L	484.8	701.4 (y6)	588.3 (y5)	489.2 (y4)
		H	491.3	711.4 (y6)	597.3 (y5)	497.2 (y4)
	LEEQAQQIR	L	557.8	872.5 (y7)	615.4 (y5)	544.3 (y4)
		H	565.3	885.4 (y7)	625.3 (y5)	553.3 (y4)
	SELEEQLTPVAEETR	L	577.6	902.5 (y8)	801.4 (y7)	704.4 (y6)
		H	583.9	913.4 (y8)	811.4 (y7)	713.3 (y6)
apo M	WIYHLTEGSTDLR	L	795.9	991.5 (y9)	878.4 (y8)	777.4 (y7)
		H	805.4	1003.5 (y9)	889.4 (y8)	787.3 (y7)
	AFLTPR	L	409.3	599.4 (y5)	486.3 (y4)	373.2 (y3)
		H	414.2	607.4 (y5)	493.3 (y4)	379.2 (y3)
apo H	ATVVYQGER	L	511.8	850.4 (y7)	751.4 (y6)	652.3 (y5)
		H	518.2	861.4 (y7)	761.3 (y6)	661.3 (y5)
	EHSSLAFWK	L	552.8	838.4 (y7)	751.4 (y6)	664.4 (y5)
		H	559.3	847.4 (y7)	759.4 (y6)	671.4 (y5)
HSPA8	FEELNADLFR	L	627.3	735.3 (y6)	848.5 (y7)	977.5 (y8)
		H	634.3	745.3 (y6)	859.4 (y7)	989.5 (y8)
moesin	IGFPWSEIR	L	552.8	690.4 (y5)	787.4 (y6)	991.5 (y8)
		H	559.3	699.3 (y5)	797.4 (y6)	1003.5 (y8)
HSP90AA1	ADLINNLGTIAK	L	621.9	716.4 (y7)	830.5 (y8)	943.6 (y9)
		H	629.3	725.4 (y7)	841.4 (y8)	955.5 (y9)
flotillin-1	VTGEVLDILTR	L	608.4	617.4 (y5)	730.4 (y6)	829.5 (y7)
		H	615.3	625.3 (y5)	739.4 (y6)	839.5 (y7)
HSP90AB1	YESLTDPSK	L	520.3	547.3 (y5)	660.4 (y6)	747.4 (y7)
		H	525.2	553.3 (y5)	667.3 (y6)	755.4 (y7)
TSG101	ASLISAVSDK	L	495.8	519.3 (y5)	606.3 (y6)	719.4 (y7)
		H	501.3	525.3 (y5)	613.3 (y6)	727.4 (y7)
L1CAM	DATQITQGPR	L	543.8	558.3 (y5)	671.4 (y6)	799.4 (y7)
		H	551.3	567.3 (y5)	681.4 (y6)	811.4 (y7)

Transitions are listed for both unlabeled, light (L) and fully ¹⁵N-labeled, heavy (H) peptides. All precursors were +2 charge and product ions were +1 charge. Additionally, y-ion information is included for reference.

Table S2. ¹⁵N-labeling of LP1 and LP2 QconCATs

QconCAT	LP1			LP2		
Peptide	VQPYLDDFQK	ASSIIDELFQDR	TYLPAVDEK	ELQAAQAR	SEYQADYESLR	FPEVDVLTK
¹⁵ N-labeling efficiency, %	98.69	99.39	97.27	99.39	97.40	99.00
Average*	98.45 ± 0.62			98.60 ± 0.61		

* Final labeling efficiency was determined by averaging 3 representative peptides in each QconCAT

Supporting Material 1. LP1 and LP2 QconCATs

LP1 - Number of amino acids: 592; Molecular weight: 68284.16; Theoretical pI: 8.22; Grand average of hydropathicity (GRAVY): -0.784.

MATKTAKDALSSVQESQVAQQARGWVTDGFSSLKDYWSTVRGRIKELLETVVNRTD
GWQPWDRVKDLATVYVDVLKDSGRDYVSQFEGSALGKQLNLKLLIEKVKSPELQAEA
KSYFEKSKAEQLTPLIKKAGTELSAKIREWFSETFQKVKEKLKALHARIQQNLDQLREELS
RAEYHAKATEHLSTLSEKAKPALEEVKAKVQPYLDDFQKKWQEEIFLTQVKESLSSYW
ESAKTAAQNLYEKTYLPAVDEKLRDLVSGDKGRVEQIHQQKIAREPALDKLKEFGNTLE
DKARELISHVDALRTHLAPYSDELRRQLAARLSNNAKEAVEHLQKSELTQQNLALFQD
KLGEVNTYAGDLQKKLVPFATELHERLAKDSEELRARLLPHANEVSQKIGDNLRELQQ
RLEPYADQLRTQVNTQAEQLRRQLTPYDELKAKIDQNVEELKGRLTPYADEFKVKIDQ
TEQVALRVQELQEQLRVVGEDTVQELHRSVAPHAPASPARLSRAVQDHFSSRASSIIDE
LFQDRFFFTREPQAKLRRELDSESLQVAERLTRKYNSKYVNKEIQNAVNGVKQIKTLIKLA
AALEHHHHHH

Peptides (marked red) with 6 a.a. residues flanking sequences on both sides (marked black) from 9 proteins listed below were randomly concatenated in LP1 sequence. 1 Cys was replaced with Ala (marked green) and 5 Met were replaced with Ile (marked blue) in the flanking sequences. 6 x His tag and linker sequence (marked orange) was from expression vector.

Apo A-I

1. PWDRVKDLATVYVDVLKDSGRDYVSQFEGSALGKQLNLKL (1234.68 and 1399.66)
2. EEVKAKVQPYLDDFQKKWQEEI (1251.61)
3. HVDALRTHLAPYSDELRRQLAAR (1300.64)
4. AEYHAKATEHLSTLSEKAKPALE (1214.61)

Apo A-II

1. LIEKVKSPELQAEAKSYFEKSKAEQLTPLIKKAGTEL (971.49 and 940.56)

Apo A-IV

1. LSNNAKEAVEHLQKSELTQQNLALFQDKLGEVNTYAGDLQKKLVPFATELHERLAKDSE (952.50, 1633.83, 1406.70 and 1310.70)
2. EELRARLLPHANEVSQKIGDNLRELQQRLPYADQLRTQVNTQAEQLRRQLTPY (1234.67, 1103.56 and 1286.66)
3. DELKAKIDQNVEELKGRLTPYADEFKVKIDQT (1086.56 and 1082.53)

Apo A-V

1. SGD KGRVEQIHQQKIAREPA (1008.53)
2. EQVALRVQELQEQLRVVGEDT (1141.61)
3. VQELHRSVAPHAPASPARLSRAVQ (1159.61)
4. KALHARIQQNLDQLREELSRA (1126.61)

Apo C-I

1. ALDKLKEFGNTLEDKARELIS (1051.48)
2. LSAKIREWFSETFQKVKEKLK (1200.55)

Apo C-II

1. FLTQVK**ESLSSYWESAKTAAQNLYEKTYLPAVDEK**LRDLYS (1285.58, 1036.52 and 1034.53)

Apo C-III

1. ATKTA**KDALSSVQESQVAQQARGWTDGFSSLK**DYWSTV (1715.84 and 1195.59)

Apo C-IV

1. VRGR**IKELLETVVNR**TRDGWQ (1071.59)

Apo J (clusterin)

1. SKYVN**KEIQNAVNGVK**QIKTLI (1070.57)

2. QDHFSR**ASSIIDELFQDR**FFTREP (1392.69)

3. QAKLRR**ELDESLQVAER**LTRKYN (1287.63)

LP2 - Number of amino acids: 595; Molecular weight: 67492.11; Theoretical pI: 6.08; Grand average of hydropathicity (GRAVY): -0.587

MSAIRER**LGPLVEQGRVRAATVGS**LAGQPLQ**ERAQAWGELKAYKSELEEQLTPVAEE**
TRARLSKELQAAQARLGAD**IERLDEVKEQVAEVR**AK**LEEQAQQIRLQAEAFQAR**LKSW
 FEKVPVK**KATVVYQGERVKIQEKL**NIPK**LDFSSQADLR**NEIKTL**AVPRKWIYHLTEGSTD**
LRTEGRPDDLQKRLAVYQAGAREGAERGTFAHR**DFSAEYEEDGKYEGLQEWEGKAH**
 LNIKDNVPK**ATGVLYDYVNKYHWEHTLPEVKFPEVDVLT**KYSQPEDVRFQ**KAA**SGTTG
TYQEWKDKAQNLQNALLR**SEYQADYESLRFFS**LLS**QATLKEVYGFNPEGK**ALLK**KT**SN
 DQEK**AHLDIAGSLEGH**LRFLK**NIILPVYDKSLWDFL**NYQLYK**SVSLPSLDPASAKIEGNLI**
 GLEGK**GFEPTLEALFGKQGFFPD**FHLPSR**EFQVPTFTIPKLYQLQVPKAFKEHSS**LAF
WKTDASDVNAIEGKSNTVASLHTEKNTLELSALDSKAFLLT**PRNQEAELADTVAKVQ**
GVEFSHRLNTDIAWILARN**PNLPPETVDSLKNILTSNWYEIEKIPTTFENGRAIQANYKLA**
AALEHHHHHH

Peptides (marked red) with 6 a.a. residues flanking sequences on both sides (marked black) from 6 proteins listed below were randomly concatenated in LP2 sequence. 6 Cys were replaced with Ala (marked green) and 1 Met was replaced with Ile (marked blue) in the flanking sequences. 6 x His tag and linker sequence (marked orange) was from expression vector.

Apo B-100/B-48

1. SQ**ATLKEVYGFNPEGK**ALLKKT (1138.53)

2. NYQLYK**SVSLPSLDPASAKIEGNLI** (1270.68)

3. IGLEGK**GFEPTLEALFGKQGFFPD** (1307.68)

4. FHLPSR**EFQVPTFTIPKLYQLQV** (1305.70)

5. QNALLR**SEYQADYESLRFFS**LLS (1359.59)

6. ADTVAK**VQGVESHRLNTDIA** (1057.53)

Apo B-100 only

1. NAIEGK**SNTVASLHTEKNTLELS** (1185.60)

2. KLNIPK**LDFSSQADLR**NEIKTL (1150.56)

3. SNDQEK**AHLDIAGSLEGH**LRFLK**NIILPVYDKSLWDFL** (1487.78 and 1073.61)

4. TLPEVK**FPEVDVLT**KYSQPED (1046.56)

5. GTFahr**DFSAEYEEDGKYEGLQEWEGK**AHLNik (1288.51 and 1237.56)
6. KDNVPK**ATGVLYDYVNKYHWEHT** (1241.63)
7. DVRFQK**AASGTTGTyQEWK**DKAQNL (1398.64)

Apo D

1. WYEIEK**IPtTFENGR**AIQANY (1033.52)
2. AWILAR**NPnLPPETVDSL**KNILTSN (1422.74)

Apo E

1. ELKAYK**SELEEQLTPVAEETR**ARLSK**ELQAAQAR**LGAD**I**E (1729.84 and 885.47)
2. DDLQKR**LAVYQAGAREGAERG** (947.52)
3. SAIRER**LGPLVEQGRVRAATVGS**LAG**QPLQER**AQAWGE (967.55 and 1496.79)
4. RLDEVK**EQVAEVRAL**EE**QAQQIRLQAEAFQAR**LKSWFE (829.43, 1113.58 and 1032.54)

Apo H (beta-2-glycoprotein 1)

1. KVPVKK**ATVVYQGER**VKIQEK (1021.52)
2. VPK**AFKEHSSLAFWK**TDASDV (1103.54)

Apo M

1. L**AVPRKWIYHLTEG**STD**LR**TEGRPD (1589.78)
2. S**ALDSKAFL**L**TPRNQEA**EL (816.49)

Figure S1. Scheme depicting separation protocols.

