Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Highly specific enrichment of N-linked glycopeptides based on hydrazide functionalized soluble nanopolymers

*Lijuan Zhang, Hucong Jiang, Jun Yao, Yali Wang, Caiyun Fang, Pengyuan Yang and Haojie Lu**

Experiment details

Chemicals and reagents

Asialofetuin from fetal calf serum, myoglobin from equine heart, avidin from egg white, invertase form baker's yeast, dithiothreitol (DTT), iodoacetamide (IAA), sodium periodate ($NaIO_4$), sodium sulfite ($NaSO_3$), sodium acetate (CH_3COONa), ammonium bicarbonate (NH_4HCO_3), methyl acrylate, methanol, urea, α-cyano-4-hydroxycinnamic acid (CHCA), and L-1-tosylamido-2-phenylethylchloromethyl ketone (TPCK) treated trypsin from bovine pancreas, and PAMAM dendrimer (ethylenediamine core, generation 5.0 solution, provided as 5 wt. % in methanol), *p*-toluenesulfonyl hydrazide (polymer-bound, 100-200 mesh) were purchased from Sigma (St. Louis, MO, USA). BcMag™ Hydrazide-terminated magnetic beads (1 μm) was obtained from Bioclone (San Diego, CA, USA). Acetonitrile (ACN, 99.9%), trifluoroacetic acid (TFA) and formic acid (FA) were purchased from Merck (Darmstadt, Germany). Peptide-N-glycosidase (PNGase F) was obtained from New England Biolabs (Ipswich, MA, USA). Sep-Pak C18 columns were from Waters (Milford, MA, USA). Affi-Gel Hz hydrazide Gel was purchased from Bio-Rad (Hercules, CA, USA). Human serum sample was obtained from a healthy donor and stored at −80 °C before analysis. Pure water was prepared with a Milli-Q system (Millipore, Bedford, MA, USA). All other chemicals and reagents were of analytical grade and obtained from Shanghai Chemical Reagent.

Synthesis of hydrazide functionalized PAMAM

Methyl acrylate (60 μL) in 1.5 mL methanol was added dropwise to PAMAM (2 mL, 5 wt. % in methanol) over a period and then stirred at 0 ºC for 24 h. Both solvent and the residue methyl acrylate were removed with a rotatory evaporator. After drying in vacuum at 40 ºC, hydrazine hydrate (160 μL) in 2 mL methanol was added dropwise to the intermediate, PAMAM with methyl ester terminals, which was re-dissolved in 2 mL methanol and then refluxed and stirred at 120 ºC for 3 h. The solvent and residue hydrazine hydrate were removed with a rotary evaporator and dried in vacuum at 60 ºC for 24 h.

Characterization

^{1}H NMR measurements were carried out on Varian Mercury plus 400NMR spectrometer (400 MHz, 298 K) with dimethyl sulfoxide-d6 (DMSO-d6) as the solvent. Fourier-transform infrared (FT-IR) spectra were collected on a Nicolet Fourier spectrophotometer, using KBr pellets (USA).

Preparation of standard protein digests

Standard glycoprotein (asialofetuin, avidin, invertase) was prepared as 1 mg/mL solution in 25 mM ammonium bicarbonate (pH 8.5), and heated at 95 ºC for 5 min. After cooling to room temperature, the solution was treated with trypsin at 37 ºC (enzyme/protein ratio of 1:50, w/w) for 18 h. Digestion was stopped by heating the solution at 95 ºC for 5 min, and the obtained protein tryptic digests were stored at −20 ºC before use.

Enrichment of N-linked glycopeptides with hydrazide functionalized PAMAM

1 mg dried tryptic peptides were re-dissolved in 200 μL coupling buffer (100 mM sodium acetate, 150 mM NaCl, pH 5.5) using a 10 kDa MWCO filter (Vivacon® 500, Sartorius Stedium Biotech, Goettingen, Germany). To oxidize the cis-diol groups of carbohydrates to aldehydes, sodium periodate at 10 mM final concentration was introduced into the peptide solution, and the sample was incubated at room temperature for 1 h with continuous shaking. Then sodium sulfite was added to 20 mM final concentration and incubated for 10 min to deactivate the excess oxidant. After introducing hydrazide functionalized PAMAM (20 μL) into the quenched peptide solution, the coupling reaction was performed at 37 °C overnight with continuous shaking. After the coupling reaction, the excess unreacted reagents, the salts in the coupling buffer, and those non-bound peptides were removed into the filtrate collection through centrifugation at 4 ºC for 30 min. The glycopeptide-bound material was washed thoroughly and sequentially with 1.5 M NaCl, 30% MeOH, 0.1% TFA in 10% ACN, and 50 mM ABC and followed by a buffer exchange step to 50 mM ABC. Enzymatic cleavage of the N-linked peptides from the sugar moiety was carried out at 37 °C overnight by PNGase F at a concentration of 1 μL of PNGase F/1 mg of crude proteins. The supernatant, containing the released deglycosylated peptides, was collected into a new collection tube by centrifugation while the PAMAM material and the enzymes remained in the filter. The human serum sample was kindly provided by Fudan University Shanghai cancer center. The research followed the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of the Fudan University Shanghai cancer center.

Deglycosylation of N-linked glycopeptides by PNGase F

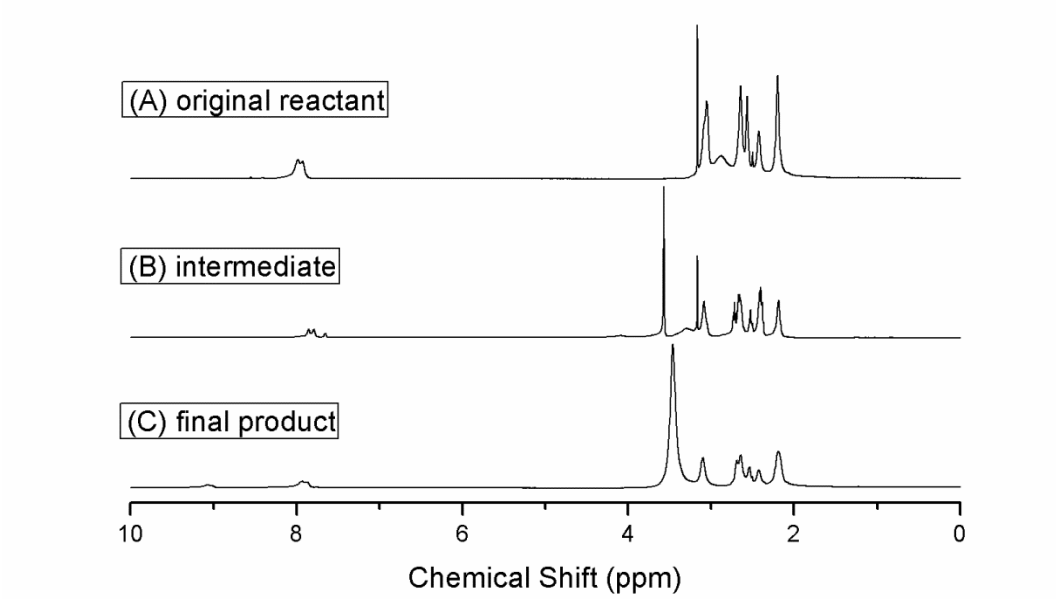
Tryptic digestion of the glycoprotein (100 μg) was dissolved in 100 μL 25 mM ammonium bicarbonate (pH 8.5), 0.1 μL PNGase F (500 units per μL) was added to the solution and incubated overnight at 37 ºC for N-glycan release. The reaction was stopped by heating to 95 ºC for 5 min, and then directly spotted on the MALDI target plate or analysis by nano-LC-MS/MS.

Database search and data analysis

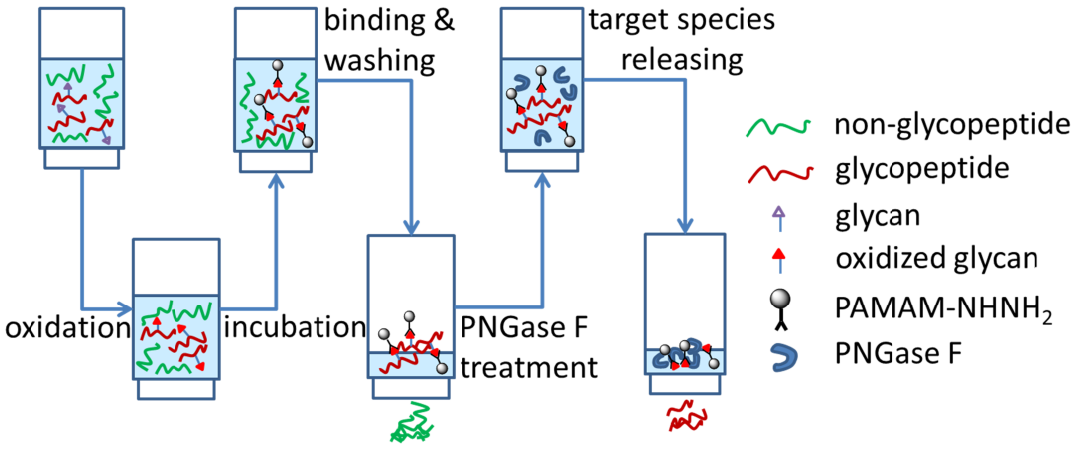
The raw data was initially converted into MGF format with MM File conversion software (Version 3.9). The acquired MS/MS spectra were searched against Swiss-Prot database using MASCOT software (version 2.3). The search criteria were set as follows: variable modifications of methionine oxidation (+16 Da), N-terminal acetylation, and deamidation (N) and fixed

modification of cysteine residues (+57 Da), at most two missed tryptic cleavage sites, 20 ppm error tolerance in MS and 1.00 Da error tolerance in MS/MS. The resulting data files were exported with the filtrations of significance threshold $p < 0.01$ and ion score ≥25. Since N-glycosylation occurs at a consensus N-X-S/T(X≠P) sequon, the remaining peptide sequences were additionally filtered to remove non-motif containing peptides.

Figure S1. ^{1}H NMR spectra of (A) the original reactant (G5 PAMAM), (B) the intermediate (PAMAM with methyl ester terminals), and (C) the final product (hydrazide functionalized PAMAM).



Scheme S1 Schematic illustration of hydrazide functionalized PAMAM based glycopeptide enrichment strategy using FASP mode.



For proof-of-principle experiments, the cis-diols of the glycans on glycopeptides were firstly oxidized to obtain the reactive aldehyde groups. After adding the home-made functionalized PAMAM into the coupling buffer, the hydrazide on the surface could react with these aldehyde groups efficiently undermild conditions. Both the oxidation and conjugation steps would be

carried out in a common ultrafiltration device with appropriate molecular weight cutoff (MWCO), and in our study we chose the 10 kDa MWCO filter. Aided by the filter device, the excess unreacted reagents, the salts in the coupling buffer, and the non-bound peptides were removed into the filtrate collection tube easily and quickly. After this, the glycopeptide-bound material was thoroughly washed by a series of washing buffers. These washing steps were also carried out in the same filter device, thus avoiding the possible sample loss during the transfer procedure and making all the captured glycopeptides remain on the filter. Finally, PNGase F was added to release the glycopeptides bound to the material into the solution. And this time the recovered glycopeptides were collected in a new collection tube while the PAMAM material and the enzymes remained on the filter. The schematic illustration of the whole procedure is shown in Scheme S1 (ESI†).

Figure S2 Plots graphically displaying the tendency between the S/N value of the formerly N-linked glycosylated peptide (VVHAVEVALATFNAESNGSYLQLVEISR) and the concentration of asialofetuin.

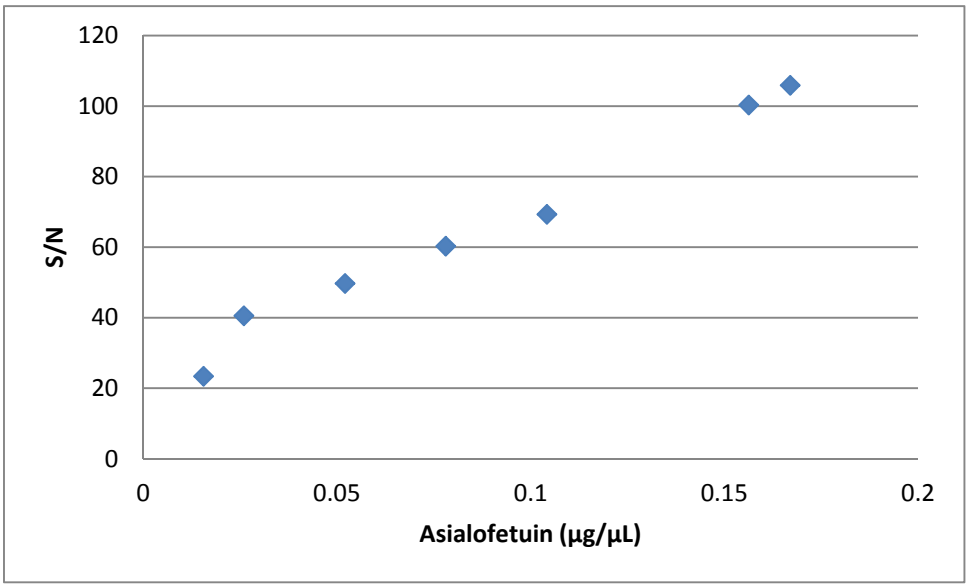


Figure S3 MALDI-TOF MS spectra of tryptic digest mixture of asialofetuin and myoglobin (with a mole ratio of asislofetuin : myoglobin = 1:10) by (A) direct analysis or (B) analysis after enrichment with hydrazide functionalized PAMAM and then deglycosylated by PNGase F. (The asterisk denotes the deglycosylated glycopeptide, the pound sign denotes the doubly charged species, and the circle denotes the unknown peak cluster.)

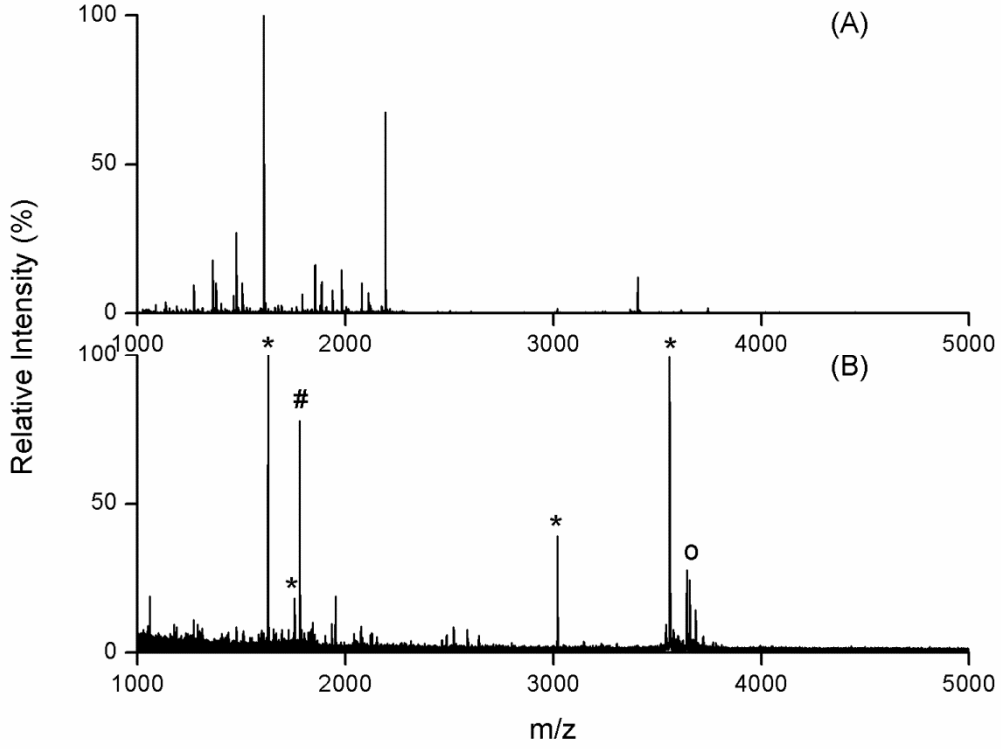


Table S1 Results of LTQ analysis of N-glycopeptides isolated from the three-glycoprotein mixture.

Standard glycoprotein	Sequences of identified glycopeptides [a]	Theoretical glycosylation sites	Identified glycosylation sites
Asialofetuin	K.LCPDCPLLAPLNDSR.V R.KLCPDCPLLAPLNDSR.V R.VVHAVEVALATFNAESNGSYLQLVEISR.A R.RPTGEVYDIEIDTLETTCHVLDPTPLANCSVR.Q	3	3
Chicken avidin	K.WTNDLGSNMTIGAVNSR.G	1	1
Invertase	R.FATNTTLTK.A K.NPVLAANSTQFR.D K.AEPILNISNAGPWSR.F K.NPVLAANSTQFRDPK.V K.RNDSGAFSGSMVVDYNNTSGFFNDTIDPR.Q K.ANSYNVDLSNSTGTLEFELVYAVNTTQTISK.S K.FSLNTEYQANPETELINLKAEPILNISNAGPWSR.F	13	8

a N denotes the N-linked glycosylation site.

Table S2. List of identified glycoproteins from 5 μL human plasma captured by hybrid hydrazide functionalized PAMAM. N denotes the N-linked glycosylation site.

Protein accession number	Protein name/Protein group	Peptide sequence
AFAM_HUMAN	Afamin OS=Homo sapiens GN=AFM PE=1 SV=1	DIENFNSTQK
AFAM_HUMAN	Afamin OS=Homo sapiens GN=AFM PE=1 SV=1	YAEDKFNETTEK
A1AG1_HUMAN	Alpha-1-acid glycoprotein 1 OS=Homo sapiens GN=ORM1 PE=1 SV=1	ENGTISR
A1AG1_HUMAN	Alpha-1-acid glycoprotein 1 OS=Homo sapiens GN=ORM1 PE=1 SV=1	QDQCIYNTTYLNVQR
A1AG1_HUMAN	Alpha-1-acid glycoprotein 1 OS=Homo sapiens GN=ORM1 PE=1 SV=1	QDQCIYNTTYLNVQRENGTISR
A1AG2_HUMAN	Alpha-1-acid glycoprotein 2 OS=Homo sapiens GN=ORM2 PE=1 SV=2	NEEYNK
A1AG2_HUMAN	Alpha-1-acid glycoprotein 2 OS=Homo sapiens GN=ORM2 PE=1 SV=2	NEEYNKSVQEIQATFFYFTPNKTEDTIFLR
A1AG2_HUMAN	Alpha-1-acid glycoprotein 2 OS=Homo sapiens GN=ORM2 PE=1 SV=2	QNQCFYNSSYLNVQR

A1AG2_HUMAN	Alpha-1-acid glycoprotein 2 OS=Homo sapiens GN=ORM2 PE=1 SV=2	QNQCFYNSSYLNVQRENGTVSR
AACT_HUMAN	Alpha-1-antichymotrypsin OS=Homo sapiens GN=SERPINA3 PE=1 SV=2	APDKNVIFSPLSISTALAFLSLGAHNTTLTEILK
AACT_HUMAN	Alpha-1-antichymotrypsin OS=Homo sapiens GN=SERPINA3 PE=1 SV=2	FNLTETSEAEIHQSFQHLLR
AACT_HUMAN	Alpha-1-antichymotrypsin OS=Homo sapiens GN=SERPINA3 PE=1 SV=2	GLKFNLTETSEAEIHQSFQHLLR
AACT_HUMAN	Alpha-1-antichymotrypsin OS=Homo sapiens GN=SERPINA3 PE=1 SV=2	KLINDYVKNGTR
AACT_HUMAN	Alpha-1-antichymotrypsin OS=Homo sapiens GN=SERPINA3 PE=1 SV=2	LINDYVKNGTR
AACT_HUMAN	Alpha-1-antichymotrypsin OS=Homo sapiens GN=SERPINA3 PE=1 SV=2	YTGNASALFILPDQDK
AACT_HUMAN	Alpha-1-antichymotrypsin OS=Homo sapiens GN=SERPINA3 PE=1 SV=2	YTGNASALFILPDQDKMEEVEAMLLPETLK
AACT_HUMAN	Alpha-1-antichymotrypsin OS=Homo sapiens GN=SERPINA3 PE=1 SV=2	YTGNASALFILPDQDKMEEVEAMLLPETLKR
A1AT_HUMAN	Alpha-1-antitrypsin OS=Homo sapiens GN=SERPINA1 PE=1 SV=3	ADTHDEILEGLNFNLTEIPEAQIHEGFQELLR
A1AT_HUMAN	Alpha-1-antitrypsin OS=Homo sapiens GN=SERPINA1 PE=1 SV=3	QLAHQSNSTNIFFSPVSIATAFAMLSLGTK
A1AT_HUMAN	Alpha-1-antitrypsin OS=Homo sapiens GN=SERPINA1 PE=1 SV=3	YLGNATAIFFLPDEGK
A1AT_HUMAN	Alpha-1-antitrypsin OS=Homo sapiens GN=SERPINA1 PE=1 SV=3	YLGNATAIFFLPDEGKLQHLENELTHDIITK
A1BG_HUMAN	Alpha-1B-glycoprotein OS=Homo sapiens GN=A1BG PE=1 SV=3	EGDHEFLEVPEAQEDVEATFPVHQPGNYSCSYR
FETUA_HUMAN	Alpha-2-HS-glycoprotein OS=Homo sapiens GN=AHSG PE=1 SV=1	AALAAFNAQNNGSNFQLEEISR
A2MG_HUMAN	Alpha-2-macroglobulin OS=Homo sapiens GN=A2M PE=1 SV=2	GCVLLSYLNETVTVSASLESVR
A2MG_HUMAN	Alpha-2-macroglobulin OS=Homo sapiens GN=A2M PE=1 SV=2	GNEANYYSNATTDEHGLVQFSINTTNVMGTSLTVR
A2MG_HUMAN	Alpha-2-macroglobulin OS=Homo sapiens GN=A2M PE=1 SV=2	VSNQTLSLFFTVLQDVPVR
ANGT_HUMAN	Angiotensinogen OS=Homo sapiens GN=AGT PE=1 SV=1	VYIHPFHLVIHNESTCEQLAK
ANT3_HUMAN	Antithrombin-III OS=Homo sapiens GN=SERPINC1 PE=1 SV=1	AAINKWVSNKTEGR
ANT3_HUMAN	Antithrombin-III OS=Homo sapiens GN=SERPINC1 PE=1 SV=1	LFGDKSLTFNETYQDISELVYGAK
ANT3_HUMAN	Antithrombin-III OS=Homo sapiens GN=SERPINC1 PE=1 SV=1	LGACNDTLQQLMEVFK

ANT3_HUMAN	Antithrombin-III OS=Homo sapiens GN=SERPINC1 PE=1 SV=1	LGACNDTLQQLMEVFKFDTISEK
ANT3_HUMAN	Antithrombin-III OS=Homo sapiens GN=SERPINC1 PE=1 SV=1	LVSANRLFGDKSLTFNETYQDISELVYGAK
ANT3_HUMAN	Antithrombin-III OS=Homo sapiens GN=SERPINC1 PE=1 SV=1	SLTFNETYQDISELVYGAK
APOB_HUMAN	Apolipoprotein B-100 OS=Homo sapiens GN=APOB PE=1 SV=1	FEVDSPVYNATWSASLK
APOB_HUMAN	Apolipoprotein B-100 OS=Homo sapiens GN=APOB PE=1 SV=1	FNSSYLQGTNQITGR
APOB_HUMAN	Apolipoprotein B-100 OS=Homo sapiens GN=APOB PE=1 SV=1	FVEGSHNSTVSLTTK
APOB_HUMAN	Apolipoprotein B-100 OS=Homo sapiens GN=APOB PE=1 SV=1	LATALSLSNKFVEGSHNSTVSLTTK
APOB_HUMAN	Apolipoprotein B-100 OS=Homo sapiens GN=APOB PE=1 SV=1	QVLFLDTVYGNCSTHFTVK
APOB_HUMAN	Apolipoprotein B-100 OS=Homo sapiens GN=APOB PE=1 SV=1	VNQNLVYESGSLNFSK
APOB_HUMAN	Apolipoprotein B-100 OS=Homo sapiens GN=APOB PE=1 SV=1	VNQNLVYESGSLNFSKLEIQSQVDSQHVGHSVLTAK
APOB_HUMAN	Apolipoprotein B-100 OS=Homo sapiens GN=APOB PE=1 SV=1	YDFNSSMLYSTAK
APOC4_HUMAN	Apolipoprotein C-IV OS=Homo sapiens GN=APOC4 PE=1 SV=1	ELLETVVNR
APOC4_HUMAN	Apolipoprotein C-IV OS=Homo sapiens GN=APOC4 PE=1 SV=1	MKELLETVVNR
APOD_HUMAN	Apolipoprotein D OS=Homo sapiens GN=APOD PE=1 SV=1	ADGTVNQIEGEATPVNLTEPAK
APOD_HUMAN	Apolipoprotein D OS=Homo sapiens GN=APOD PE=1 SV=1	ADGTVNQIEGEATPVNLTEPAKLEVK
APOD_HUMAN	Apolipoprotein D OS=Homo sapiens GN=APOD PE=1 SV=1	CIQANYSLMENGK
ATRN_HUMAN	Attractin OS=Homo sapiens GN=ATRN PE=1 SV=2	DLDMFINASK
ATRN_HUMAN	Attractin OS=Homo sapiens GN=ATRN PE=1 SV=2	IDSTGNVTNELR
ATRN_HUMAN	Attractin OS=Homo sapiens GN=ATRN PE=1 SV=2	VFHIHNESWVLLTPK
APOH_HUMAN	Beta-2-glycoprotein 1 OS=Homo sapiens GN=APOH PE=1 SV=3	LGNWSAMPSCK
APOH_HUMAN	Beta-2-glycoprotein 1 OS=Homo sapiens GN=APOH PE=1 SV=3	VYKPSAGNNSLYR
BTD_HUMAN	Biotinidase OS=Homo sapiens GN=BTD PE=1 SV=2	DVQIIVFPEDGIHGFNFTR

BTD_HUMAN	Biotinidase OS=Homo sapiens GN=BTD PE=1 SV=2	NPVGLIGAENATGETDPSHSK
CBPB2_HUMAN	Carboxypeptidase B2 OS=Homo sapiens GN=CPB2 PE=1 SV=1	KQVHFFVNASDVDNVK
CBPB2_HUMAN	Carboxypeptidase B2 OS=Homo sapiens GN=CPB2 PE=1 SV=1	QVHFFVNASDVDNVK
CPN2_HUMAN	Carboxypeptidase N subunit 2 OS=Homo sapiens GN=CPN2 PE=1 SV=2	AFGSNPNLTK
CERU_HUMAN	Ceruloplasmin OS=Homo sapiens GN=CP PE=1 SV=1	AGLQAFFQVQECNK
CERU_HUMAN	Ceruloplasmin OS=Homo sapiens GN=CP PE=1 SV=1	AGLQAFFQVQECNKSSSK
CERU_HUMAN	Ceruloplasmin OS=Homo sapiens GN=CP PE=1 SV=1	AGLQAFFQVQECNKSSSKDNIR
CERU_HUMAN	Ceruloplasmin OS=Homo sapiens GN=CP PE=1 SV=1	EHEGAIYPDNTTDFQR
CERU_HUMAN	Ceruloplasmin OS=Homo sapiens GN=CP PE=1 SV=1	ELHHLQEQNVSNAFLDK
CERU_HUMAN	Ceruloplasmin OS=Homo sapiens GN=CP PE=1 SV=1	ELHHLQEQNVSNAFLDKGEFYIGSK
CERU_HUMAN	Ceruloplasmin OS=Homo sapiens GN=CP PE=1 SV=1	ENLTAPGSDSAVFFEQGTTR
CERU_HUMAN	Ceruloplasmin OS=Homo sapiens GN=CP PE=1 SV=1	NLASRPYTFHSHGITYYKEHEGAIYPDNTTDFQR
CLUS_HUMAN	Clusterin OS=Homo sapiens GN=CLU PE=1 SV=1	EDALNETR
CLUS_HUMAN	Clusterin OS=Homo sapiens GN=CLU PE=1 SV=1	KKEDALNETR
CLUS_HUMAN	Clusterin OS=Homo sapiens GN=CLU PE=1 SV=1	LANLTQGEDQYYLR
CLUS_HUMAN	Clusterin OS=Homo sapiens GN=CLU PE=1 SV=1	MLNTSSLLEQLNEQFNWVSR
C1QA_HUMAN	Complement C1q subcomponent subunit A OS=Homo sapiens GN=C1QA PE=1 SV=2	NPPMGGNVVIFDTVITNQEEPYQNHSGR
C1QA_HUMAN	Complement C1q subcomponent subunit A OS=Homo sapiens GN=C1QA PE=1 SV=2	RNPPMGGNVVIFDTVITNQEEPYQNHSGR
C1R_HUMAN	Complement C1r subcomponent OS=Homo sapiens GN=C1R PE=1 SV=2	EHEAQSNASLDVFLGHTNVEELMK
CO2_HUMAN	Complement C2 OS=Homo sapiens GN=C2 PE=1 SV=2	QSVPAHFVALNGSK
CO4A_HUMAN	Complement C4-A OS=Homo sapiens GN=C4A PE=1 SV=1	FSDGLESNSSTQFEVK
CO4A_HUMAN	Complement C4-A OS=Homo sapiens GN=C4A PE=1 SV=1	FSDGLESNSSTQFEVKK

CO4A_HUMAN	Complement C4-A OS=Homo sapiens GN=C4A PE=1 SV=1	GLNVTLSSTGR
CO4A_HUMAN	Complement C4-A OS=Homo sapiens GN=C4A PE=1 SV=1	GLNVTLSSTGRNGFK
CO6_HUMAN	Complement component C6 OS=Homo sapiens GN=C6 PE=1 SV=3	LSSNSTKK
CO6_HUMAN	Complement component C6 OS=Homo sapiens GN=C6 PE=1 SV=3	VLNFTTK
CO8A_HUMAN	Complement component C8 alpha chain OS=Homo sapiens GN=C8A PE=1 SV=2	GGSSGWSGGLAQNR
CO9_HUMAN	Complement component C9 OS=Homo sapiens GN=C9 PE=1 SV=2	AVNITSENLIDDVVSLIR
CFAB_HUMAN	Complement factor B OS=Homo sapiens GN=CFB PE=1 SV=2	IVLDPSGSMNIYLVLDGSDSIGASNFTGAK
CFAB_HUMAN	Complement factor B OS=Homo sapiens GN=CFB PE=1 SV=2	IVLDPSGSMNIYLVLDGSDSIGASNFTGAKK
CFAB_HUMAN	Complement factor B OS=Homo sapiens GN=CFB PE=1 SV=2	KIVLDPSGSMNIYLVLDGSDSIGASNFTGAK
CFAB_HUMAN	Complement factor B OS=Homo sapiens GN=CFB PE=1 SV=2	KIVLDPSGSMNIYLVLDGSDSIGASNFTGAKK
CFAH_HUMAN	Complement factor H OS=Homo sapiens GN=CFH PE=1 SV=4	IPCSQPPQIEHGTINSSR
CFAH_HUMAN	Complement factor H OS=Homo sapiens GN=CFH PE=1 SV=4	MDGASNVTCINSR
CBG_HUMAN	Corticosteroid-binding globulin OS=Homo sapiens GN=SERPINA6 PE=1 SV=1	AQLLQGLGFNLTER
CBG_HUMAN	Corticosteroid-binding globulin OS=Homo sapiens GN=SERPINA6 PE=1 SV=1	AVLQLNEEGVDTAGSTGVTLNLTSKPIILR
FINC_HUMAN	Fibronectin OS=Homo sapiens GN=FN1 PE=1 SV=3	DQCIVDDITYNVNDTFHK
FINC_HUMAN	Fibronectin OS=Homo sapiens GN=FN1 PE=1 SV=3	LDAPTNLQFVNETDSTVLVR
LG3BP_HUMAN	Galectin-3-binding protein OS=Homo sapiens GN=LGALS3BP PE=1 SV=1	ALGFENATQALGR
LG3BP_HUMAN	Galectin-3-binding protein OS=Homo sapiens GN=LGALS3BP PE=1 SV=1	GLNLTEDTYKPR
LG3BP_HUMAN	Galectin-3-binding protein OS=Homo sapiens GN=LGALS3BP PE=1 SV=1	YKGLNLTEDTYKPR
HPT_HUMAN	Haptoglobin OS=Homo sapiens GN=HP PE=1 SV=1	MVSHHNLTTGATLINEQWLLTTAK
HPT_HUMAN	Haptoglobin OS=Homo sapiens GN=HP PE=1 SV=1	NLFLNHSENATAK
HPT_HUMAN	Haptoglobin OS=Homo sapiens GN=HP PE=1 SV=1	VVLHPNYSQVDIGLIK

HEMO_HUMAN	Hemopexin OS=Homo sapiens GN=HPX PE=1 SV=2	ALPQPQNVTSLLGCTH
HEMO_HUMAN	Hemopexin OS=Homo sapiens GN=HPX PE=1 SV=2	GHGHRNGTGHGNSTHHGPEYMR
HEMO_HUMAN	Hemopexin OS=Homo sapiens GN=HPX PE=1 SV=2	NGTGHGNSTHHGPEYMR
HGFA_HUMAN	Hepatocyte growth factor activator OS=Homo sapiens GN=HGFAC PE=1 SV=1	DSVSVVLGQHFFNR
HRG_HUMAN	Histidine-rich glycoprotein OS=Homo sapiens GN=HRG PE=1 SV=1	IADAHLDRVENTTVYYLVLDVQESDCSVLSR
HRG_HUMAN	Histidine-rich glycoprotein OS=Homo sapiens GN=HRG PE=1 SV=1	VIDFNCTTSSVSSALANTK
IGHA1_HUMAN	Ig alpha-1 chain C region OS=Homo sapiens GN=IGHA1 PE=1 SV=2	LAGKPTHVNVSVVMAEVDGTCY
IGHA1_HUMAN	Ig alpha-1 chain C region OS=Homo sapiens GN=IGHA1 PE=1 SV=2	LSLHRPALEDLLLGSEANLTCTLTGLR
IGHG1_HUMAN	Ig gamma-1 chain C region OS=Homo sapiens GN=IGHG1 PE=1 SV=1	EEQYNSTYR
IGHG1_HUMAN	Ig gamma-1 chain C region OS=Homo sapiens GN=IGHG1 PE=1 SV=1	EEQYNSTYRVVSVLTVLHQDWLNGKEYK
IGHG2_HUMAN	Ig gamma-2 chain C region OS=Homo sapiens GN=IGHG2 PE=1 SV=2	EEQFNSTFR
IGHG3_HUMAN	Ig gamma-3 chain C region OS=Homo sapiens GN=IGHG3 PE=1 SV=2	EEQYNSTFR
IGHM_HUMAN	Ig mu chain C region OS=Homo sapiens GN=IGHM PE=1 SV=3	GLTFQQNASSMCVPDQDTAIR
IGHM_HUMAN	Ig mu chain C region OS=Homo sapiens GN=IGHM PE=1 SV=3	YKNNSDISSTR
IGJ_HUMAN	Immunoglobulin J chain OS=Homo sapiens GN=IGJ PE=1 SV=4	ENISDPTSPLR
IGJ_HUMAN	Immunoglobulin J chain OS=Homo sapiens GN=IGJ PE=1 SV=4	IIVPLNNRENISDPTSPLR
ITIH1_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H1 OS=Homo sapiens GN=ITIH1 PE=1 SV=3	ANLSSQALQMSLDYGFVTPLTSMSIR
ITIH1_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H1 OS=Homo sapiens GN=ITIH1 PE=1 SV=3	DKICDLLVANNHFAHFFAPQNLTNMNK
ITIH2_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H2 OS=Homo sapiens GN=ITIH2 PE=1 SV=2	GAFISNFSMTVDGK
ITIH4_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H4 OS=Homo sapiens GN=ITIH4 PE=1 SV=4	AFITNFSMIIDGMTYPGIIK
ITIH4_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H4 OS=Homo sapiens GN=ITIH4 PE=1 SV=4	AFITNFSMIIDGMTYPGIIKEK
ITIH4_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H4 OS=Homo sapiens GN=ITIH4 PE=1 SV=4	GPDVLTATVSGKLPTQNITFQTESSVAEQEAEFQSPK

ITIH4_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H4 OS=Homo sapiens GN=ITIH4 PE=1 SV=4	KAFITNFSMIIDGMTYPGIIK
ITIH4_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H4 OS=Homo sapiens GN=ITIH4 PE=1 SV=4	LPTQNITFQTESSVAEQEAEFQSPK
ITIH4_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H4 OS=Homo sapiens GN=ITIH4 PE=1 SV=4	LQDRGPDVLTATVSGKLPTQNITFQTESSVAEQEAEFQSPK
ITIH4_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H4 OS=Homo sapiens GN=ITIH4 PE=1 SV=4	NQALNLSLAYSFVTPLTSMVVTKPDDQEQSQVAEKPMEGESR
KAIN_HUMAN	Kallistatin OS=Homo sapiens GN=SERPINA4 PE=1 SV=3	DFYVDENTTVR
KAIN_HUMAN	Kallistatin OS=Homo sapiens GN=SERPINA4 PE=1 SV=3	FLNDTMAVYEAK
KNG1_HUMAN	Kininogen-1 OS=Homo sapiens GN=KNG1 PE=1 SV=2	ITYSIVQTNCSK
KNG1_HUMAN	Kininogen-1 OS=Homo sapiens GN=KNG1 PE=1 SV=2	KYNSQNQSNNQFVLYR
KNG1_HUMAN	Kininogen-1 OS=Homo sapiens GN=KNG1 PE=1 SV=2	LNAENNATFYFK
KNG1_HUMAN	Kininogen-1 OS=Homo sapiens GN=KNG1 PE=1 SV=2	YNSQNQSNNQFVLYR
A2GL_HUMAN	Leucine-rich alpha-2-glycoprotein OS=Homo sapiens GN=LRG1 PE=1 SV=2	KLPPGLLANFTLLR
A2GL_HUMAN	Leucine-rich alpha-2-glycoprotein OS=Homo sapiens GN=LRG1 PE=1 SV=2	LPPGLLANFTLLR
A2GL_HUMAN	Leucine-rich alpha-2-glycoprotein OS=Homo sapiens GN=LRG1 PE=1 SV=2	MFSQNDTR
LUM_HUMAN	Lumican OS=Homo sapiens GN=LUM PE=1 SV=2	AFENVTDLQWLILDHNLLENSK
LUM_HUMAN	Lumican OS=Homo sapiens GN=LUM PE=1 SV=2	KLHINHNNLTESVGPLPK
LUM_HUMAN	Lumican OS=Homo sapiens GN=LUM PE=1 SV=2	LGSFEGLVNLTFIHLQHNR
LUM_HUMAN	Lumican OS=Homo sapiens GN=LUM PE=1 SV=2	LHINHNNLTESVGPLPK
LUM_HUMAN	Lumican OS=Homo sapiens GN=LUM PE=1 SV=2	LSHNELADSGIPGNSFNVSSLVELDLSYNK
PHLD_HUMAN	Phosphatidylinositol-glycan-specific phospholipase D OS=Homo sapiens GN=GPLD1 PE=1 SV=3	LGTSLSSGHVLMNGTLK
PHLD_HUMAN	Phosphatidylinositol-glycan-specific phospholipase D OS=Homo sapiens GN=GPLD1 PE=1 SV=3	LNVEAANWTVR

PLTP_HUMAN	Phospholipid transfer protein OS=Homo sapiens GN=PLTP PE=1 SV=1	IYSNHSALESLALIPLQAPLK
KLKB1_HUMAN	Plasma kallikrein OS=Homo sapiens GN=KLKB1 PE=1 SV=1	GVNFNVSK
KLKB1_HUMAN	Plasma kallikrein OS=Homo sapiens GN=KLKB1 PE=1 SV=1	IYPGVDFGGEELNVTFVK
KLKB1_HUMAN	Plasma kallikrein OS=Homo sapiens GN=KLKB1 PE=1 SV=1	IYSGILNLSDITK
KLKB1_HUMAN	Plasma kallikrein OS=Homo sapiens GN=KLKB1 PE=1 SV=1	IYSGILNLSDITKDTPFSQIK
IC1_HUMAN	Plasma protease C1 inhibitor OS=Homo sapiens GN=SERPING1 PE=1 SV=2	DTFVNASR
IC1_HUMAN	Plasma protease C1 inhibitor OS=Homo sapiens GN=SERPING1 PE=1 SV=2	GVTSVSQIFHSPDLAIRDTFVNASR
IC1_HUMAN	Plasma protease C1 inhibitor OS=Homo sapiens GN=SERPING1 PE=1 SV=2	VGQLQLSHNLSLVILVPQNLK
IC1_HUMAN	Plasma protease C1 inhibitor OS=Homo sapiens GN=SERPING1 PE=1 SV=2	VLSNNSDANLELINTWVAK
ZPI_HUMAN	Protein Z-dependent protease inhibitor OS=Homo sapiens GN=SERPINA10 PE=1 SV=1	ETFFNLSK
ZPI_HUMAN	Protein Z-dependent protease inhibitor OS=Homo sapiens GN=SERPINA10 PE=1 SV=1	LPYQGNATMLVVLMEK
THRB_HUMAN	Prothrombin OS=Homo sapiens GN=F2 PE=1 SV=2	GHVNITR
TRFE_HUMAN	Serotransferrin OS=Homo sapiens GN=TF PE=1 SV=2	CGLVPVLAENYNK
PON1_HUMAN	Serum paraoxonase/arylesterase 1 OS=Homo sapiens GN=PON1 PE=1 SV=2	HANWTLTPLK
PON1_HUMAN	Serum paraoxonase/arylesterase 1 OS=Homo sapiens GN=PON1 PE=1 SV=2	VTQVYAENGTVLQGSTVASVYK
PON1_HUMAN	Serum paraoxonase/arylesterase 1 OS=Homo sapiens GN=PON1 PE=1 SV=2	VTQVYAENGTVLQGSTVASVYKGK
TSP1_HUMAN	Thrombospondin-1 OS=Homo sapiens GN=THBS1 PE=1 SV=2	VVNSTTGPGEHLR
TITIN_HUMAN-R	TITIN_HUMAN-R	KNLSPGIR
ZA2G_HUMAN	Zinc-alpha-2-glycoprotein OS=Homo sapiens GN=AZGP1 PE=1 SV=1	AREDIFMETLKDIVEYYNDSNGSHVLQGR
ZA2G_HUMAN	Zinc-alpha-2-glycoprotein OS=Homo sapiens GN=AZGP1 PE=1 SV=1	DIVEYYNDSNGSHVLQGR