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Electronic Supplementary Information (ESI):

Experimental separation steps influence the protein content of corona around mesoporous silica nanoparticles

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Experimental

Synthesis of magnetic mesoporous silica nanoparticles (M-MSNs)

Magnetic mesoporous silica nanoparticles (M-MSN) were synthesized according to an optimized procedure, previously described.[1] This two-step method allows the formation of monodisperse and homogeneous core@shell Fe_3O_4@MSN nanoparticles with a single magnetic iron oxide core per nanoparticle surrounded by a mesoporous silica shell. (Fig.1A)

Nanoparticle characterization

Transmission electron micrographs (TEM) with a JEOL 1200 EX II microscope (Fig.1B) was used for size measurements and verification of single magnetic iron oxide core presence per nanoparticle. Dynamic Light Scattering with a Zetasizer Nano ZS (Malvern Instruments Ltd, UK) was used for hydrodynamic diameter and zeta potential in physiological pH (n=3), according to the method used in Pisani *et al.*[2]

Exposure of M-MSNs to sera and purification

A stock solution of M-MSNs was made in PBS 1X (Invitrogen) at a concentration of 1mg/mL. After 2min of sonication in an ultrasonic bath (Elma Transsonic T780/H) at 4°C, the M-MSN suspension was supplemented with HS (human serum, Sigma-Aldrich). Exposure started at the time of serum addition. All experiments were performed in triplicate. After 30min of exposure, 1mL of the solution was sampled. Samples containing M-MSNs coated with proteins were washed three times with 1mL PBS 1X by gently mixing, followed by magnetization separation for 30s (1.4 Tesla magnets), or by centrifugation for 30s (5900 x g). Washing steps were necessary to eliminate non adsorbed proteins.

Sample preparation for Nano-LC Mass Spectrometry

M-MSNs exposed to serum were suspended in 20µL of PBS 1X and 10µL of LDS 1X (Lithium Dodecyl Sulfate, Invitrogen). LDS 1X working solution contained: 106mM Tris/HCl, 141mM Tris base, 2% lithium dodecyl sulfate, 10% glycerol, 0.51mM EDTA, 0.22mM G250 SERVA® Blue, 0.175mM Phenol Red, buffered at pH8.5 and was supplemented with 2.5% beta-mercaptoethanol. Samples were heated at 99°C during 5min and then loaded onto a 4-12% NuPAGE gel (Invitrogen) for a short denaturing electrophoresis (5min) at 200V in MES/SDS running 1X buffer. Gels were stained using Coomassie Blue Safe stain (Invitrogen).

The polyacrylamide bands containing all proteins were processed as previously described.[3] Briefly, after overnight destaining at 4°C with milliQ water, the protein content from each well was excised as a single polyacrylamide band with a scalpel. These bands were treated with 25mM DTT, with iodoacetamide and proteolyzed with 0.01% proteasMAX (Promega).

The resulting peptides (10µL) were analyzed with an ESI-Q Exactive HF mass spectrometer (ThermoFisher) incorporating an ultra-high-field Orbitrap analyzer and coupled to an Ultimate 3000 RSL Nano LC System (Dionex-LC Packings). For nano liquid chromatography, samples were loaded and desalted on line on a reverse phase Acclaim PepMap100 C18 micro precolumn (5µm, 100Å, 300µm internal diameter x 5mm, Thermofisher) and resolved on a Acclaim PepMap100 C18 nano column (3µm, 100Å, 75µm internal diameter x 50cm, Thermofisher) at a flow rate of 0.2µL/min, with a 4-25% gradient of solvent B (80% acetonitrile, 20% water, 0.1% formic acid) against solvent A (0.1% formic acid, 99.9% water) during 70min and then 25-40% during 20min, for a total gradient runtime of 90min. A Top20 data dependent method was used for MS/MS spectrum acquisition. Full scan mass spectra were measured from 350 to 1500 *m/z* with an Automatic Gain Control Target set at 3×10^6 ions and a resolution of 60,000. MS/MS scan was initiated at resolution of 15,000 for ions with potential charge of 2+ and 3+ and with a dynamic exclusion of 10s. MS/MS were recorded with an Automatic Gain Control Target set at 1×10^5 ions.

Protein identification and quantification by proteomics

MS/MS spectra were analyzed with the SwissProt database (release SwissProt_2016). Searches for trypsic peptides were performed with the following parameters: full-trypsin specificity, a mass tolerance of 5ppm on the parent ion and 0.5Da on the MS/MS, carbamidomethylCys as static modification and oxidized Met as dynamic modification, and maximum number of missed cleavages set at 2. All peptide matched with a peptide score below a p-value of 0.05 were filtered. A protein was considered valid when at least two different peptides were detected in the same experiment. The false-positive rate for protein identification was estimated using the appropriate decoy database as below 1% at these parameters. The number of MS/MS spectra per protein (spectral counts, SC) was determined for each sample and compared using the TFold method of the PatternLab software v3.2.0.3 as previously described with a minimal SC cut-off set at 5. The normalized spectral abundance factor (NSAF) is a ratio calculated by normalizing spectral counts (SC) obtained for a protein by the respective molecular weight (MW) according to Pisani *et al.*[2]

Results

Nanoparticles characterization

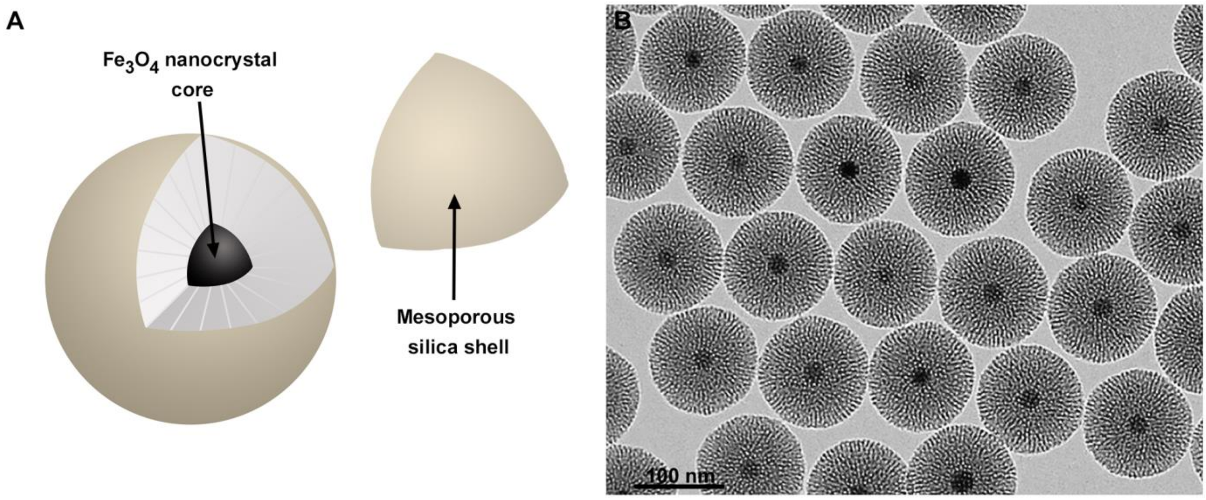

Fig.1. A) Representation of magnetic mesoporous silica nanoparticles (M-MSN). B) TEM image of single core M-MSN. Mean diameter: 116.6 ± 2.1 nm.

These nanoparticles were monodisperse (polydispersity index: 0.111 ± 0.024) and stable at physiological pH (Zeta potential: -39.1 ± 1.5 mV), with a hydrodynamic diameter of 143.5 ± 1.5 nm.

ESI Table 1. Relative proportions of human serum proteins within the corona after magnetization, obtained by NSAF ratio (%)

description	accession	pI	mass (Da)	Relative proportion (NSAF%)	Standard deviation
Serum albumin	P02768	5.9	69322	33.407	2.309
Ig kappa chain C region	P01834	5.6	11602	5.902	1.198
Ig gamma-1 chain C region	P01857	8.5	36083	5.182	0.741
Apolipoprotein A-I	P02647	5.6	30759	3.652	0.389
Serotransferrin	P02787	6.8	77014	3.122	0.178
Ig lambda-2 chain C regions	P0CG05	6.9	11287	2.688	1.056
Vitamin D-binding protein	P02774	5.4	52929	2.222	0.313
Complement C3	P01024	6.0	187030	2.212	0.133
Alpha-2-HS-glycoprotein	P02765	5.4	39300	2.190	0.099
Hemopexin	P02790	6.6	51643	2.144	0.136
Alpha-1-antitrypsin	P01009	5.4	46707	1.967	0.077
Beta-2-glycoprotein 1	P02749	8.3	38273	1.929	0.233
Ig gamma-2 chain C region	P01859	7.7	35878	1.202	0.083
Apolipoprotein A-IV	P06727	5.3	45372	1.060	0.121
Complement factor B	P00751	6.7	85479	1.041	0.099
Alpha-1-antichymotrypsin	P01011	5.3	47621	0.969	0.022
Apolipoprotein A-II	P02652	6.3	11168	0.856	0.150
Ig alpha-1 chain C region	P01876	6.1	37631	0.855	0.195
Retinol-binding protein 4	P02753	5.8	22995	0.852	0.075
Ig gamma-3 chain C region	P01860	8.2	41260	0.843	0.240
Gelsolin	P06396	5.9	85644	0.825	0.084
Plasminogen	P00747	7.0	90510	0.825	0.197
Complement C4-B	P0C0L5	6.9	192631	0.767	0.060
Apolipoprotein E	P02649	5.7	36132	0.761	0.169
Histidine-rich glycoprotein	P04196	7.1	59541	0.759	0.042
Kininogen-1	P01042	6.3	71912	0.758	0.036
Inter-alpha-trypsin inhibitor heavy chain H4	Q14624	6.5	103293	0.737	0.021
Ig kappa chain V-III region SIE	P01620	8.7	11768	0.687	0.036
Haptoglobin	P00738	6.1	45177	0.651	0.081
Transthyretin	P02766	5.5	15877	0.649	0.193
Pigment epithelium-derived factor	P36955	6.0	46283	0.596	0.070
Ceruloplasmin	P00450	5.4	122128	0.588	0.011
Antithrombin-III	P01008	6.3	52569	0.588	0.122
Apolipoprotein D	P05090	5.1	21262	0.533	0.233
Heparin cofactor 2	P05546	6.4	57034	0.527	0.081
Complement component C9	P02748	5.4	63133	0.509	0.036
Vitronectin	P04004	5.6	54271	0.487	0.052
Plasma protease C1 inhibitor	P05155	6.1	55119	0.464	0.113
Serum paraoxonase/arylesterase 1	P27169	5.1	39706	0.455	0.055
Prothrombin	P00734	5.6	69992	0.444	0.024
Complement factor H	P08603	6.2	139005	0.425	0.079
Clusterin	P10909	5.9	52461	0.423	0.076
Apolipoprotein C-III	P02656	5.2	10846	0.410	0.117
Complement component C8 beta chain	P07358	8.5	67004	0.376	0.034
Alpha-2-antiplasmin	P08697	5.9	54531	0.376	0.021
N-acetylmuramoyl-L-alanine amidase	Q96PD5	7.3	62178	0.361	0.025
Serum amyloid P-component	P02743	6.1	25371	0.357	0.143
Alpha-1-acid glycoprotein 2	P19652	5.0	23588	0.339	0.127
Lumican	P51884	6.2	38405	0.338	0.069
Alpha-1B-glycoprotein	P04217	5.6	54220	0.322	0.094
Complement component C8 gamma chain	P07360	8.5	22264	0.320	0.084
Angiotensinogen	P01019	5.9	53121	0.311	0.006
Ig heavy chain V-III region BRO	P01766	6.4	13218	0.311	0.095
Inter-alpha-trypsin inhibitor heavy chain H1	P19827	6.3	101326	0.304	0.033
Alpha-1-acid glycoprotein 1	P02763	4.9	23497	0.296	0.045
Inter-alpha-trypsin inhibitor heavy chain H2	P19823	6.4	106397	0.293	0.036
Coagulation factor XII	P00748	8.0	67748	0.292	0.041
Kallistatin	P29622	7.3	48511	0.287	0.086
Protein AMBP	P02760	6.0	38974	0.286	0.126
Tetranectin	P05452	5.5	22522	0.285	0.053
Ig kappa chain V-I region AG	P01593	5.7	11985	0.268	0.233
Afamin	P43652	5.6	69024	0.255	0.017
Ig lambda chain V-III region SH	P01714	6.0	11386	0.236	0.037
Complement component C7	P10643	6.1	93457	0.223	0.039
Ig heavy chain V-III region TRO	P01762	9.7	13464	0.221	0.049
Complement component C6	P13671	6.4	104718	0.212	0.004
Insulin-like growth factor-binding protein complex acid labile subunit	P35858	6.3	65994	0.206	0.039
Complement factor I	P05156	7.7	65707	0.206	0.041
Ig kappa chain V-II region TEW	P01617	5.7	12308	0.201	0.114

description	accession	pI	mass (Da)	Relative proportion (NSAF%)	Standard deviation
Complement component C8 alpha chain	P07357	6.1	65121	0.196	0.096
Plasma serine protease inhibitor	P05154	9.3	45646	0.189	0.033
Ig mu chain C region	P01871	6.4	49276	0.178	0.063
Zinc-alpha-2-glycoprotein	P25311	5.7	34237	0.169	0.083
Ig gamma-4 chain C region	P01861	7.2	35918	0.169	0.071
Apolipoprotein C-I	P02654	8.0	9326	0.162	0.281
Lipopolysaccharide-binding protein	P18428	6.2	53350	0.154	0.033
Ig lambda-1 chain C regions	P0CG04	7.9	11341	0.147	0.254
Ig heavy chain V-III region GAL	P01781	8.7	12722	0.138	0.076
Complement C5	P01031	6.1	188186	0.138	0.021
Apolipoprotein C-II	P02655	4.7	11277	0.133	0.123
Ig kappa chain V-I region Ni	P01613	5.3	12238	0.128	0.056
Complement factor H-related protein 1	Q03591	7.4	37626	0.127	0.067
Selenoprotein P	P49908	8.1	43147	0.126	0.043
Alpha-2-macroglobulin	P01023	6.0	163188	0.117	0.041
Fetuin-B	Q9UGM5	6.5	42028	0.115	0.026
Plasma kallikrein	P03952	8.6	71323	0.102	0.045
Carboxypeptidase B2	Q96IY4	7.6	48393	0.101	0.027
Serum amyloid A-4 protein	P35542	9.2	14737	0.100	0.130
Haptoglobin-related protein	P00739	6.6	39005	0.090	0.043
Apolipoprotein M	O95445	5.7	21239	0.090	0.057
Ig kappa chain V-I region Lay	P01605	8.0	11827	0.089	0.155
Coagulation factor XI	P03951	8.5	70064	0.089	0.074
Complement C2	P06681	7.2	83214	0.088	0.057
Actin, cytoplasmic 1	P60709	5.3	41710	0.085	0.018
Hyaluronan-binding protein 2	Q14520	6.1	62630	0.084	0.065
Monocyte differentiation antigen CD14	P08571	5.8	40051	0.084	0.028
Glutathione peroxidase 3	P22352	8.3	25536	0.075	0.047
Complement C1q subcomponent subunit C	P02747	8.6	25757	0.075	0.104
Ig heavy chain V-I region 2	P23083	9.2	13076	0.069	0.120
Ig heavy chain V-II region ARH-77	P06331	8.5	16218	0.065	0.113
C4b-binding protein alpha chain	P04003	7.2	66989	0.060	0.048
Leucine-rich alpha-2-glycoprotein	P02750	6.5	38154	0.053	0.016
Complement factor H-related protein 2	P36980	6.0	30631	0.052	0.025
Protein Z-dependent protease inhibitor	Q9UK55	8.3	50674	0.048	0.032
Inter-alpha-trypsin inhibitor heavy chain H3	Q06033	5.5	99787	0.046	0.036
Ig delta chain C region	P01880	8.1	42227	0.038	0.018
Coagulation factor XIII B chain	P05160	6.0	75461	0.038	0.036
Vitamin K-dependent protein S	P07225	5.5	75074	0.036	0.006
Hepatocyte growth factor activator	Q04756	7.0	70636	0.034	0.003
Hepatocyte growth factor-like protein	P26927	8.0	80268	0.031	0.017
Fibrinogen alpha chain	P02671	5.7	94914	0.030	0.025
Coagulation factor X	P00742	5.7	54697	0.029	0.013
Procollagen C-endopeptidase enhancer 1	Q15113	7.4	47942	0.028	0.049
Complement C1s subcomponent	P09871	4.9	76635	0.027	0.019
Phosphatidylinositol-glycan-specific phospholipase D	P80108	5.9	92278	0.027	0.024
Sulfhydryl oxidase 1	O00391	9.1	82526	0.024	0.012
Coagulation factor V	P12259	5.7	251546	0.019	0.020
Complement C4-A	P0C0L4	6.7	192664	0.018	0.005
Fibulin-1	P23142	5.1	77162	0.017	0.015
EGF-containing fibulin-like extracellular matrix protein 1	Q12805	5.0	54604	0.014	0.024
Apolipoprotein B-100	P04114	6.6	515283	0.014	0.009

ESI Table 2. Relative proportions of human serum proteins within the corona after centrifugation, obtained by NSAF ratio (%)

description	accession	pI	mass	Relative proportion (%)	Standard deviation
Apolipoprotein A-I	P02647	5.6	30759	7.547	2.313
Serum albumin	P02768	5.9	69322	5.990	1.844
Complement C3	P01024	6.0	187030	4.481	0.243
Alpha-2-HS-glycoprotein	P02765	5.4	39300	3.639	0.496
Apolipoprotein E	P02649	5.7	36132	3.095	0.925
Ig kappa chain C region	P01834	5.6	11602	2.897	0.753
Apolipoprotein A-IV	P06727	5.3	45372	2.613	0.707
Ig lambda-2 chain C regions	P0CG05	6.9	11287	2.310	0.587
Ig gamma-3 chain C region	P01860	8.2	41260	2.242	0.941
Beta-2-glycoprotein 1	P02749	8.3	38273	2.210	0.922
Apolipoprotein D	P05090	5.1	21262	2.035	0.408
Complement factor H	P08603	6.2	139005	1.798	0.388
Kininogen-1	P01042	6.3	71912	1.701	0.138
Gelsolin	P06396	5.9	85644	1.652	0.369
Apolipoprotein B-100	P04114	6.6	515283	1.635	0.187
C4b-binding protein alpha chain	P04003	7.2	66989	1.578	0.149
Clusterin	P10909	5.9	52461	1.576	0.397
Serum amyloid A-4 protein	P35542	9.2	14737	1.535	0.398
Antithrombin-III	P01008	6.3	52569	1.482	0.541
Histidine-rich glycoprotein	P04196	7.1	59541	1.419	1.033
Vitronectin	P04004	5.6	54271	1.382	0.286
Alpha-1-antitrypsin	P01009	5.4	46707	1.334	0.227
Serotransferrin	P02787	6.8	77014	1.308	0.447
Hemopexin	P02790	6.6	51643	1.305	0.861
Inter-alpha-trypsin inhibitor heavy chain H4	Q14624	6.5	103293	1.271	0.223
Complement C4-B	P0C0L5	6.9	192631	1.251	0.072
Ig gamma-1 chain C region	P01857	8.5	36083	1.162	0.592
Plasminogen	P00747	7.0	90510	1.142	0.157
Prothrombin	P00734	5.6	69992	1.108	0.227
Vitamin D-binding protein	P02774	5.4	52929	1.107	0.372
Ig mu chain C region	P01871	6.4	49276	0.982	0.296
Coagulation factor XII	P00748	8.0	67748	0.884	0.244
Heparin cofactor 2	P05546	6.4	57034	0.839	0.407
Ig alpha-1 chain C region	P01876	6.1	37631	0.808	0.121
Complement factor B	P00751	6.7	85479	0.759	0.358
Serum paraoxonase/arylesterase 1	P27169	5.1	39706	0.742	0.151
Apolipoprotein A-II	P02652	6.3	11168	0.731	0.104
Tetranectin	P05452	5.5	22522	0.725	0.338
Apolipoprotein M	O95445	5.7	21239	0.711	0.117
Plasma kallikrein	P03952	8.6	71323	0.677	0.151
Haptoglobin	P00738	6.1	45177	0.666	0.054
Alpha-2-antiplasmin	P08697	5.9	54531	0.659	0.132
Plasma protease C1 inhibitor	P05155	6.1	55119	0.639	0.176
Complement factor H-related protein 1	Q03591	7.4	37626	0.562	0.087
Transthyretin	P02766	5.5	15877	0.544	0.169
Actin, cytoplasmic 1	P60709	5.3	41710	0.540	0.038
Retinol-binding protein 4	P02753	5.8	22995	0.532	0.373
Complement C1r subcomponent	P00736	5.8	80067	0.518	0.140
Immunoglobulin lambda-like polypeptide 5	B9A064	9.1	23049	0.516	0.386
Complement C1q subcomponent subunit B	P02746	8.8	26705	0.498	0.060
Apolipoprotein C-III	P02656	5.2	10846	0.494	0.117
Complement C1s subcomponent	P09871	4.9	76635	0.487	0.098
Ig gamma-2 chain C region	P01859	7.7	35878	0.451	0.085
Insulin-like growth factor-binding protein complex acid labile subunit	P35858	6.3	65994	0.451	0.055
Ig kappa chain V-III region WOL	P01623	9.1	11739	0.447	0.193
Plasma serine protease inhibitor	P05154	9.3	45646	0.439	0.031
Complement C1q subcomponent subunit C	P02747	8.6	25757	0.420	0.062
Protein AMBP	P02760	6.0	38974	0.407	0.152
Haptoglobin-related protein	P00739	6.6	39005	0.405	0.095
Alpha-2-macroglobulin	P01023	6.0	163188	0.403	0.119
Inter-alpha-trypsin inhibitor heavy chain H2	P19823	6.4	106397	0.390	0.058
Complement component C6	P13671	6.4	104718	0.373	0.057
Apolipoprotein L1	O14791	5.6	43947	0.372	0.140
Vitamin K-dependent protein S	P07225	5.5	75074	0.372	0.039

description	accession	pI	mass	Relative proportion (%)	Standard deviation
Apolipoprotein C-IV	P55056	9.2	14543	0.367	0.188
Complement factor I	P05156	7.7	65707	0.366	0.193
Complement component C8 beta chain	P07358	8.5	67004	0.365	0.182
Pigment epithelium-derived factor	P36955	6.0	46283	0.359	0.239
Complement component C8 gamma chain	P07360	8.5	22264	0.349	0.150
Complement component C8 alpha chain	P07357	6.1	65121	0.349	0.167
Inter-alpha-trypsin inhibitor heavy chain H1	P19827	6.3	101326	0.348	0.067
Coagulation factor XI	P03951	8.5	70064	0.341	0.148
Glutathione peroxidase 3	P22352	8.3	25536	0.339	0.090
Complement factor H-related protein 5	Q9BXR6	6.8	64377	0.329	0.117
Complement component C7	P10643	6.1	93457	0.306	0.100
Selenoprotein P	P49908	8.1	43147	0.300	0.101
Lipopolysaccharide-binding protein	P18428	6.2	53350	0.297	0.036
Ig heavy chain V-III region BRO	P01766	6.4	13218	0.295	0.044
Complement component C9	P02748	5.4	63133	0.294	0.146
Coagulation factor XIII B chain	P05160	6.0	75461	0.278	0.119
Insulin-like growth factor-binding protein 3	P17936	9.0	31654	0.272	0.092
CD5 antigen-like	O43866	5.3	38063	0.269	0.084
EGF-containing fibulin-like extracellular matrix protein 1	Q12805	5.0	54604	0.262	0.022
Hepatocyte growth factor-like protein	P26927	8.0	80268	0.254	0.030
Adipocyte plasma membrane-associated protein	Q9HDC9	5.8	46451	0.240	0.057
Cystatin-C	P01034	9.0	15789	0.237	0.099
Complement factor D	P00746	7.7	27016	0.226	0.058
N-acetylmuramoyl-L-alanine amidase	Q96PD5	7.3	62178	0.214	0.118
Extracellular matrix protein 1	Q16610	6.3	60635	0.213	0.073
Ig lambda chain V-III	P01714	6.0	11386	0.212	0.109
Fibulin-1	P23142	5.1	77162	0.208	0.029
Carboxypeptidase B2	Q96IY4	7.6	48393	0.201	0.029
Kallistatin	P29622	7.3	48511	0.201	0.130
Ig kappa chain V-I region Ni	P01613	5.3	12238	0.197	0.101
Cholesteryl ester transfer protein	P11597	5.7	54721	0.195	0.003
Ig heavy chain V-III region TRO	P01762	9.7	13464	0.193	0.039
Complement factor H-related protein 2	P36980	6.0	30631	0.192	0.027
Complement C1q subcomponent subunit A	P02745	9.3	26000	0.192	0.049
Sulfhydryl oxidase 1	O00391	9.1	82526	0.190	0.043
Angiotensinogen	P01019	5.9	53121	0.187	0.024
Hyaluronan-binding protein 2	Q14520	6.1	62630	0.186	0.045
Protein Z-dependent protease inhibitor	Q9UK55	8.3	50674	0.183	0.005
Afamin	P43652	5.6	69024	0.174	0.084
Alpha-1-antichymotrypsin	P01011	5.3	47621	0.173	0.050
Apolipoprotein(a)	P08519	5.6	500995	0.172	0.015
Serum amyloid P-component	P02743	6.1	25371	0.161	0.048
Cartilage oligomeric matrix protein	P49747	4.4	82808	0.156	0.027
Fetuin-B	Q9UGM5	6.5	42028	0.148	0.113
Fibrinogen alpha chain	P02671	5.7	94914	0.145	0.065
Complement C5	P01031	6.1	188186	0.145	0.017
Fibronectin	P02751	5.5	262460	0.144	0.056
Ceruloplasmin	P00450	5.4	122128	0.143	0.024
Alpha-1B-glycoprotein	P04217	5.6	54220	0.141	0.016
Complement C2	P06681	7.2	83214	0.140	0.021
ig heavy chain V-III region 23	P01764	8.5	12574	0.139	0.149
Ig heavy chain V-III region GAL	P01781	8.7	12722	0.135	0.083
Monocyte differentiation antigen CD14	P08571	5.8	40051	0.135	0.095
Hepatocyte growth factor activator	Q04756	7.0	70636	0.132	0.054
Prenylcysteine oxidase 1	Q9UHG3	5.8	56604	0.132	0.016
Phosphatidylinositol-glycan-specific phospholipase D	P80108	5.9	92278	0.131	0.030
Ig kappa chain V-I region Hau	P01600	8.7	11664	0.126	0.217
Procollagen C-endopeptidase enhancer 1	Q15113	7.4	47942	0.122	0.084
Phospholipid transfer protein	P55058	6.5	54705	0.114	0.020
Proteoglycan 4	Q92954	9.5	150984	0.112	0.073
Properdin	P27918	8.3	51242	0.100	0.032
Mannosyl-oligosaccharide 1,2-alpha-mannosidase IA	P33908	6.0	72922	0.094	0.020
Thrombospondin-1	P07996	4.7	129300	0.092	0.015
Coagulation factor IX	P00740	5.3	51745	0.092	0.057
Carboxypeptidase N catalytic chain	P15169	6.9	52253	0.091	0.045
Inter-alpha-trypsin inhibitor heavy chain H3	Q06033	5.5	99787	0.089	0.021
Cartilage acidic protein 1	Q9NQ79	5.0	71376	0.083	0.014
C4b-binding protein beta chain	P20851	5.1	28338	0.078	0.031
Coagulation factor V	P12259	5.7	251546	0.078	0.012
Ig delta chain C region	P01880	8.1	42227	0.070	0.041
Lumican	P51884	6.2	38405	0.066	0.037
Fibrinogen gamma chain	P02679	5.4	51479	0.065	0.016
Platelet-activating factor acetylhydrolase	Q13093	7.2	50045	0.065	0.006
Coagulation factor X	P00742	5.7	54697	0.065	0.058
Ficolin-3	O75636	6.2	32882	0.063	0.034

description	accession	pI	mass	Relative proportion (%)	Standard deviation
Ig gamma-4 chain C region	P01861	7.2	35918	0.061	0.018
Transforming growth factor-beta-induced protein ig-h3	Q15582	7.6	74634	0.057	0.013
Beta-Ala-His dipeptidase	Q96KN2	5.1	56670	0.051	0.028
Vitamin K-dependent protein C	P04070	5.9	52038	0.049	0.030
Fibrinogen beta chain	P02675	8.5	55892	0.043	0.016
Sex hormone-binding globulin	P04278	6.2	43752	0.036	0.019
Plastin-2	P13796	5.3	70244	0.035	0.025
Moesin	P26038	6.1	67778	0.032	0.010
78 kDa glucose-regulated protein	P11021	5.1	72288	0.020	0.018
Complement C4-A	P0C0L4	6.7	192664	0.018	0.003
Basement membrane-specific heparan sulfate proteoglycan core protein	P98160	6.1	468532	0.018	0.016
Collagen alpha-3(VI) chain	P12111	6.3	343457	0.014	0.001

References of Experimental section

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