Electronic Supplementary Information

Exploiting the arginine distributions for selective and efficient depletion of arginine-rich plasma proteins

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Experimental section

Materials and reagents

Human plasma albumin (HSA, A9731), immunoglobulin G (IgG, I4506), transferring (Trf, T3309), conalbumin (Cona, C0880), lactoferrin (Lf, L9507), lysozyme (Lys, F62970), concanavalin A (ConA, L7647), myoglobin (Mb, M1882) are purchased from Sigma-Aldrich (St. Louis, MO, USA). The protein molecular weight marker (Broad, 3597A, Takara Biotechnology Co., Ltd., Dalian, China) is a mixture of nine purified proteins (myoglobulin, Mr 200 kDa; β-galactosidases, Mr 116 kDa; phosphorylase b, Mr 97.2 kDa; plasma albumin, Mr 66.4 kDa; ovalbumin, Mr 44.3 kDa; carbonic anhydrase, Mr 29.0 kDa; trypsin inhibitor, Mr 20.1 kDa; lysozyme, Mr 14.3 kDa; inhibitory peptide, 6.5 kDa). Amino propyltrethoxy silane (APTES, $H_2NCH_2CH_2Si(OC_2H_5)_3$), cetylpyridinium bromide ($C_{21}H_{38}BrN$, CPDB), cyclohexane (CH_4ON_2), n-butyl alcohol (C_6H_{12}), tetraethyl orthosilicate ($C_8H_{20}O_4Si$, TEOS), methanol (CH_3OH) and ethanol (CH_3CH_2OH) are obtained from Sinopharm Chemical Reagent Co., Ltd., (Shanghai, China). Ethylene glycol methacrylate phosphate ($C_{12}H_{19}O_8P$, EGMP) is purchased from J&K Scientific Co., Ltd., (Beijing, China). All chemicals used are at least of analytical reagent grade unless otherwise specified. Deionized water of 18 MΩ cm is used throughout.

Human blood samples are provided by the Hospital of Northeastern University from healthy volunteers. All experiments involving human samples are performed in compliance with the relevant laws and institutional guidelines and have been approved by the ethical committee of Northeastern University. Fresh blood is collected in an additive-free tube and anti-coagulated with potassium EDTA. After the removal of red blood cells via centrifugation at 5,000 rpm for 30 min, the plasma is collected and stored at -20℃.

The fabrication of EGMP@SiO_2 microspheres

Fibrous SiO_2 nanospheres are prepared according to a reported hydrothermal procedure [1]. Briefly, 1 g cetylpyridinium bromide, 1.8 g urea, 20 g cyclohexane and 1 g n-butyl alcohol are dissolved in 15 mL deionized water, and then 1.25 g TEOS is added under magnetic stirring. After stirring for 30 min at room temperature, and aged at 70℃ for 16 h, the product fibrous SiO_2 is collected after centrifugation, washed with acetone and deionized water. The residual cetylpyridinium bromide is removed by calcination at 550℃ for 6 h. Amino fibrous SiO_2 synthesized by coupling reaction. 100 mg fibrous SiO_2 is dispersed in 2 mL methanol, then 5 μL APTES is added under magnetic stirring. After stirring for 24 h at room temperature, the product is collected after centrifugation, washed with methanol, and the white powder amino fibrous SiO_2 is obtained after drying at 65°C overnight. EGMP@SiO_2 synthesized by addition reaction. 100 mg amino fibrous SiO_2 is dispersed in 2 mL ethanol, then 6 μL EGMP is added under magnetic stirring. After stirring for 24 h at room temperature, the product is collected after centrifugation, washed with ethanol, and the final product EGMP@SiO_2 microspheres are obtained after vacuum drying at 40°C overnight.

Characterization

FT-IR spectra are recorded on a Nicolet 6700 spectrometer (Thermo Fisher Scientific, USA) using a KBr disk from 400 to 4000 cm^{-1} with a resolution of 2.0 cm^{-1}. XRD patterns are taken on a Rigaku D/max-a X-ray diffractometer (Rigaku, Japan) with graphite-monochromatized Cu-Kα radiation (k=1.54056 Å), with a step size of 0.03°. The thermal stability of the product is evaluated by using a TG-DSC simultaneous thermal analyzer (Mettler Toledc, Switzerland) within a temperature range of 25-1000℃ in nitrogen atmosphere by heating at a rate of 10℃ min^{-1}. The nitrogen adsorption-desorption isotherms are obtained at -196℃ by using an ASAP 2020HD88 (Micromeritics Instrument, USA). SEM images are obtained on a ZEISS Ultra/Plus scanning electron microscope (ZEISS, Germany). Hydrodynamic diameter (Dh) and Zeta potential measurements are carried out by dynamic light scattering (DLS) with a Nano ZS90 (Malvern, U.K.). LC-MS/MS analysis is performed using an Easy nanoLC 1000 system (Thermo Fisher Scientific, Germany) interfaced with a Q Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific, Germany) for which the database employed is Swiss-Prot filtered for the Homo sapiens taxonomy. The quantitative detection of proteins is carried out by U-3900 UV-vis spectrophotometer (Hitachi, Japan). The pH values are measured by a PB-10 pH meter (Sartorius, Germany).

Capturing of peptides by EGMP@SiO_2 microspheres

The capturing behaviors of the synthesized 12 peptide chains on EGMP@SiO_2 microspheres are studied as following. Briefly, 1.0 mL 100 μg mL^{-1} peptide sample (BR, pH 5.0) is mixed with 1 mg EGMP@SiO_2 microspheres at room temperature and the mixture is shaken on an oscillator vigorously for 30 min to facilitate the capture of peptide species. After centrifugation at 6000 rpm for 5 min, the supernatant is collected to quantify the residual peptide content by monitoring the absorbance at 595 nm after binding with Coomassie brilliant blue (Bradford method).

The capturing efficiency of peptide (Q) is calculated by the following equation, where C_0 and C_1 represent the original and the residual peptide concentrations (mg L^{-1}), respectively. All tests are repeated three times.

$$Q= (C_0-C_1)/C_0\times100\%$$

Capturing of proteins by EGMP@SiO_2 microspheres

The performances of EGMP@ SiO_2 microspheres on protein capturing are investigated by using HSA, BSA, IgG, Trf, Cona, ConA, Lf, Mb and Lys as protein models. The selection of these 8 proteins are based on the different arginine number in their structure, and the numbers of arginine residues in these proteins are 37, 32, 26, 24, 13, 11, 6 and 2, respectively.

Briefly, 1.0 mL protein sample solution is mixed with 1 mg EGMP@SiO_2 microspheres at room temperature and the mixture is shaken on an oscillator vigorously for 30 min to facilitate the adsorption of protein species. After centrifugation at 6000 rpm for 5 min, the supernatant is collected to quantify the residual protein content by monitoring the absorbance at 595 nm after binding with Coomassie brilliant blue (Bradford method). The calculation of capturing efficiency of protein (Q) is same with that of peptides. All tests are repeated three times.

Applications

Depletion of proteins by EGMP@SiO_2 microspheres

For the depletion of arginine-rich proteins from human plasma by EGMP@SiO_2 microspheres, the human plasma sample is firstly diluted 500 times with BR buffer (pH 5.0), then 1.0 mL diluted plasma is mixed with 1 mg EGMP@SiO_2 microspheres under vigorous shaking for 30 min. After centrifugation at 6000 rpm for 5 min, the supernatant is collected for ensuing assay. Thereafter, EGMP@SiO_2 microspheres with captured proteins is prewashed with deionized H_2O, mixed with 1 mL of Tris-HCl (0.05 mol L^{-1}, pH 8.9) and shaken for 20 min to recover the captured proteins.

Validations of the protein depletion efficiency with LC-MS/MS

The effectiveness of arginine-rich protein consumption is assessed using LC-MS/MS. The plasma samples are trypsinized firstly, and then the obtained peptide sample is diluted with 0.1% TFA and injected into a loading/desalting column (C18 modified silica gel, 3 mm diameter, inner diameter 100 mm, length 20 mm) and eluted onto the analytical column (C18 modified silica gel, 1.9 mm bead size, inner diameter 150 mm, length 12 cm), flow rate 200 nL min^{-1}, unchanged in the following solvent gradient curves: 5-7% solvent B (0-2 minutes), 7-10% solvent B (3-10 minutes), 10-20% solvent B (11-50 minutes), 20-30% solvent B (51-70 minutes) and 90% solvent s (71-78 minutes). Solvent A is 0.1% TFA and solvent B is ACN / 0.1% TFA. The mass spectrometer has a scan range of 300-1400 ms^{-1} and the database used is a Swiss-Prot filter for Homo sapiens taxonomy.

Supporting figure

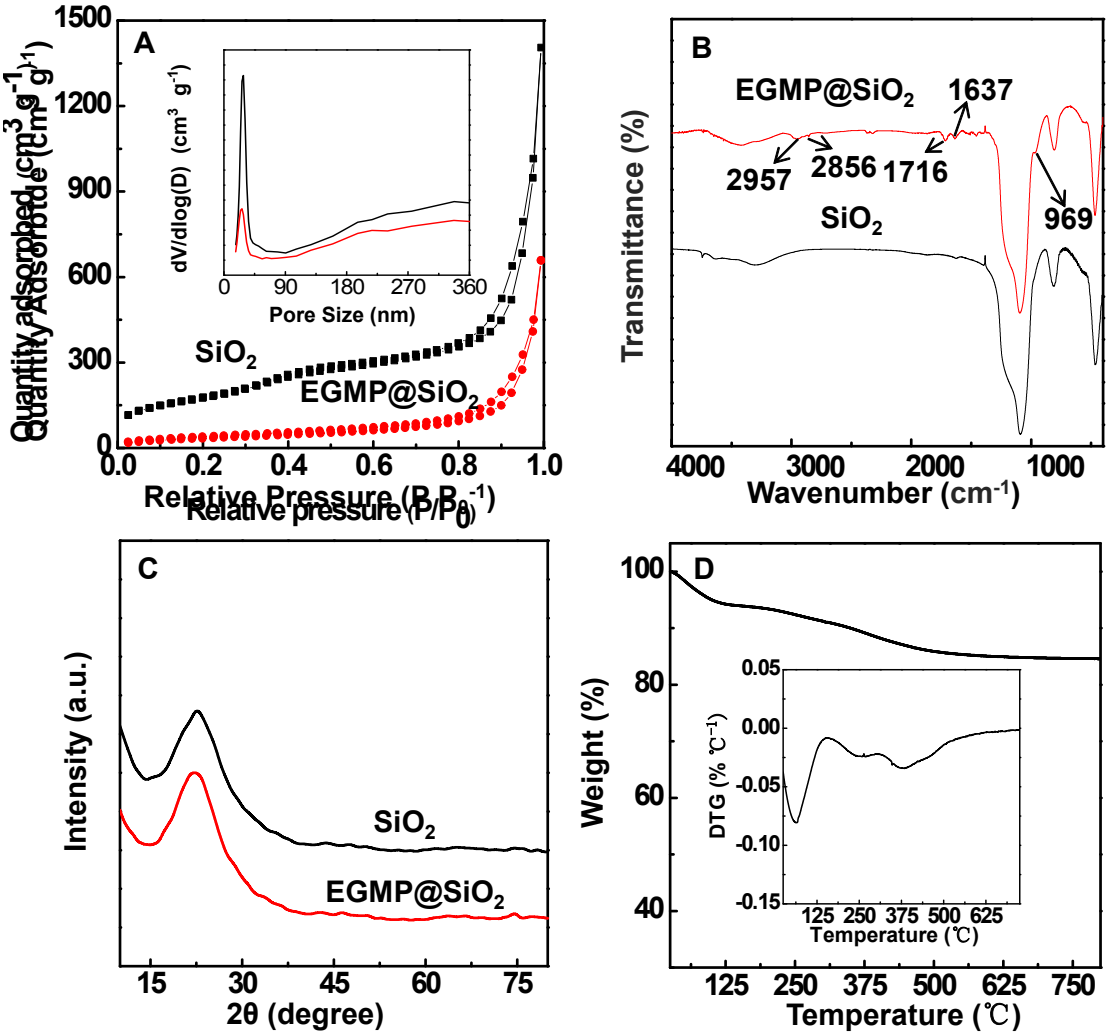

Figure S1. Nitrogen adsorption-desorption isotherms of SiO_2 and EGMP@SiO_2 (A); Pore size distribution of SiO_2 and EGMP@SiO_2 microspheres (A inset). FT-IR spectra of fibrous SiO_2 and EGMP@SiO_2 microspheres (B); XRD patterns of fibrous SiO_2 and EGMP@SiO_2 microspheres (C); TGA curves of SiO_2 and EGMP@SiO_2 microspheres (D); DTG curves of SiO_2 and EGMP@SiO_2 microspheres (D inset).

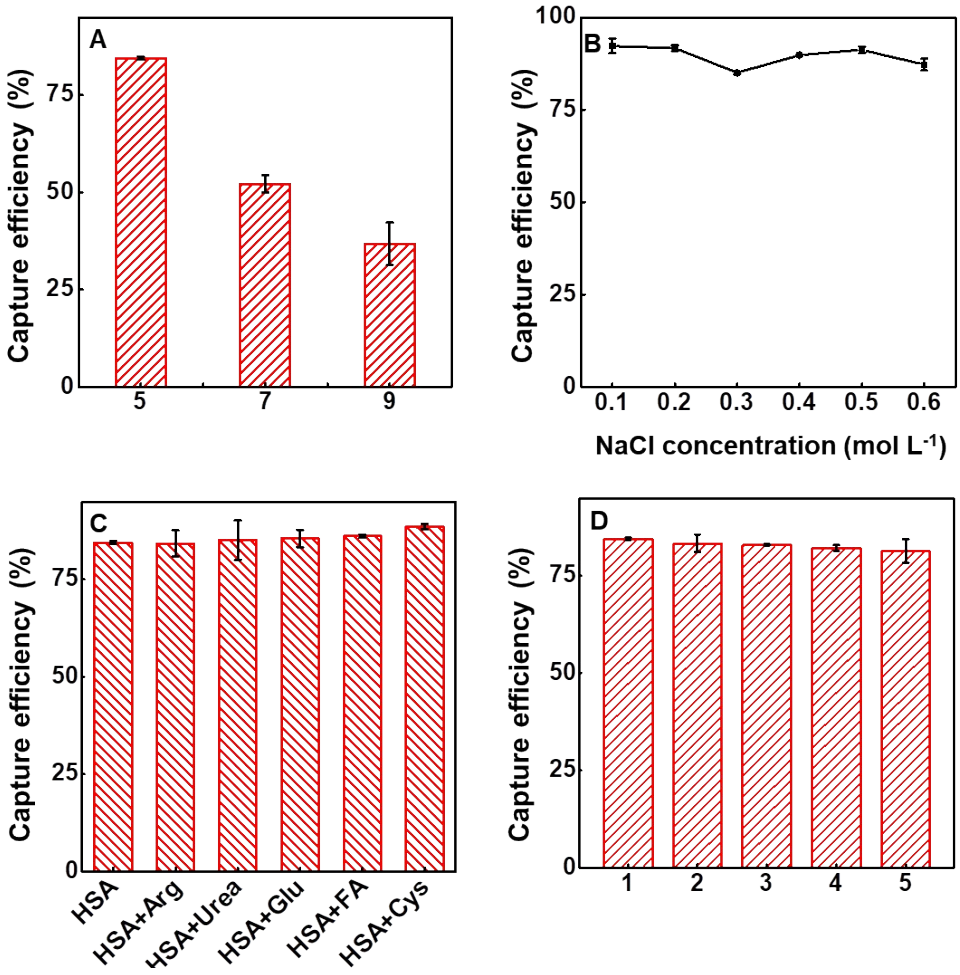

Figure S2. The effect of ionic strength on the adsorption of HSA by EGMP@SiO_2 microspheres (A). The effect of pH on the adsorption of HSA by EGMP@SiO_2 microspheres (B); The anti-interference ability of EGMP@SiO_2 microspheres against small molecules in plasma (C); The reusability of EGMP@SiO_2 microspheres for HSA adsorption/desorption (D); Protein solution: 100 mg L^{-1}, 1.0 mL, EGMP@SiO_2 microspheres: 1.0 mg; adsorption time: 30 min; pH 5.0. Arginine (Arg): 80 μmol L^{-1}; Urea, Glucose (Glu) and Cysteine (Cys) concentration: 10mmol L^{-1}; Folic acid (FA) concentration: 10 μmol L^{-1}.

Supporting table

Table S1. Amino acid sequence of the designed peptide chains.

Name	Amino acid sequence
Peps1	MKWAGATFDSGAKDAHDKSW*
Peps2	MKWAGATFDSRAKDAHDKSW
Peps3	MKWAGATFDRRAKDAHDKSW
Peps4	MKWAGATFRRRRKDAHDKSW
Peps5	MKWAGARRRRRRRRRAHDKSW
Peps6	MKWVRRTFISLLKIAHDK
Peps7	MRKWVTFRISLLRIAHRD
Peps8	MKWVRRTFISLLIRAHDR
Peps9	MKWVRRTFISLLRRIAHD
Peps10	RKWRGARFDRGARDARSWRR
Peps11	RRWAGATRRSGAKDRRRRSW
Peps12	MRRAGARRDSGRRDAHRRSW

*M: methionine; K: lysine; W: tryptophan; A: alanine; G: glycine; T: threonine; F: phenylalanine; D: aspartic acid; S: serine; H: histidine; V: valine; I: isoleucine; L: leucine; R: arginine.

Table S2. Amino acid sequences of the selected proteins

Protein	Amino acid sequences	Data Sources
Lactoferrin (Lf)	APRKNVRWCTISQPEWFKCRWQWR MKKLGAPSITCVRRAFALECIRAEKK AVTLDGGMVFEAGRDPYKRPVAAEI YGTKESPQTHYYAVAVVKKGSNFQL QGRKSCHTGLGRSAGWI[IPMGILRPY LSWTESLEPLQGAVAKFFSASCVPCID RQAYPNLCQLCKGEGENQCACSSREP YFGRSGAFKCLQDGAGDVAFVKETT VFENLPEKADRDQYELLCLNNSRAPV DAFKECHLAQVPSHAVVARSVDGKE DLIWKLLSKAQEKFGKNKSRSFQLFG SPPGQRDLLFKDSALGFLRIPSKVDSA LYLGSRYLTTLKNLRETAEEVKARYT RVVWCACGPEEQKKCQQWSQQSGQ NVTCATASTTDDCIVLVLKGEADALN LDGGTIYTAGKCGLVPVLAENRKSSK HSSLDCVLRPTEGYLAVAVVKKANE GLTWNSLKDKKSCHTAVDRTAGWNI PMGLIVNQTGSCAFDEFFSQSCAPGA DPKSRLCALCAGDDQGLDKCVPNSK EKYYGYTGAFRCLAEDVGDVAFVKN DTVWENTNGESTADWAKNLNREDFR LLCLDGTRKPVTEAQSCHLAVAPNHA VVSRSDRAAHVKQVLLHQQALFGKN GKNCPDKFCLFKSETKNLLFNDNTEC LAKLGGRPTYEEYLGTEVVTAIANLK KCSTSPLLEACAFLTR	RCSB Protein Data Bank
Conalbumin (Cona)	APPKSVIRWCTISSPEEKKCNNLRDLT QQERISLTCVQKATYLDCIKAIANNEA DAISLDGGQAFEAGLAPRKLKPIAAE VYEHTEGSTTSYYAVAVVKKGTEFTV NDLQGKTSCHTGLGRSAGWNIPIGTL LHRGAIEWEGIESGSVEQAVAKFFSAS CVPGATIEQKLCRQCKGDPKTKCARN APYSGYSGAFHCLKDGKGDVAFVKH TTVNENAPDQKDEYELLCLDGSRQPV DNYKTCNWARVAAHAVVARDDNKV EDIWSFLSKAQSDFGVDTKSDFHLFGP PGKKDPVLKDLLFKDSAIMLKRVPSL	RCSB Protein Data Bank

	MDSQLYLGFEYYSAIQSMRKDQLTPS PRENRIQWCAVGKDEKSKCDRWSVV SNGDVECTVVDETKDCIIKIMKGEAD AVALDGGLVYTAGVCGLVPVMAER YDDESQCSKTDERPASYFAVAVARK DSNVNWNNLKGKKSCHTAVGRTAG WVIPMGLIHNRTGTCNFDERFSEGCA PGSPPNSRLCQLCQGSGGIPPEKCVAS SHEKYFGYTGALRCLVEKGDVAFIQH STVEENTGGKNKADWAKNLQMDDF ELLCTDGRRANVMDYRECNLAEVPT HAVVVRPEKANKIRDLLERQEKRFG VNGSEKSKFMMFESQNKDLLFKDLT KCLFKVREGTTYKKEFLGDKFYTVIS SLKTCNPSDILQMCSFLEGK	
Transferring (Trf)	KTVRWCAVSEHEATKCQSFRDHMKS VIPSDGPSVACVKKASYLDCIRAIAAN EADAVTLDAGLVYDAYLAPNNLKPV VAEFYGSKEDPQTFYYAVAVVKKDS GFQMNQLRGKKSCHTGLGRSAGWNI PIGLLYCDLPEPRKPLEKAVANFFSGS CAPCADGTDFPQLCQLCPGCGCSTLN QYFGYSGAFKCLKDGAGDVAFVKHS TIFENLANNKAKRDQYELLCLDNTRK PVDEYKDCHLAQVPSHTVVARSMGG KEDLIWELLNQAQEHFGKDKSKEFQL FSSPHGKDLLFKDSAHGFLKVPPRMD AKMYLGYEYVTAIRNLREGTCPEAPT DECKPVKWCALSHHERLKCDEWSVN SVGKIECVSAETTEDCIAKIMNGEADA MSLDGGFVYIAGKCGLVPVLAENYN KSDNCEDTPEAGYFAVAVVKKSASD LTWDNLKGKKSCHTAVGRTAGWNIP MGLLYNKIMHCRFDEFFSEGCAPGSK KDSSLCKLCMGSGLNLCEPNNKEGY YGYTGAFRCLVEKGDVAFVKHQTVP QNTGGKNPDPWAKNLNEKDYELLCL DGTRKPVEEYANCHLARAPNHAVVT RKDKEACVHKILRQQQHKFGSNVTD CSGNFCLFRSETKDLLFRDDTVCLAK LHDRNTYEKYLGEEYVKAVGNLRKC STSSLLEACTFRRP	RCSB Protein Data Bank

Human plasma albumin (HSA)	DAHKSEVAHRFKDLGEENFKALVLIA FAQYLQQCPFEDHVKIVNEVIEFAKTC VADESAENCDKSLHTLFGDKLCTVAT LRETYGEMADCCAKQEPERNECFLQ HKDDNPNLPNLPRLVRPEVDVMCTA FHDNEETFLKKYEIARRHPYFYAPELL FFAKRYKAAFTECCQAADKAACLLP KLDELRDEGKASSAKQRLKCASLQKF GERAFKAWAVARLSQRFPKAEFAEV SKLVTDLTKVHTECCHGDLLECADD RADLAKYICENQKSISSKLKECCEKPL LEKSHCIAEVENDEMPADLPSLAADF VESKDVCKNYAEAKDVFLGMFLYEY ARRHPDYSVVLLLRLAKTYETTLEKC CAAADPHECYAKVFDEFKPLVEEPQN LIKQNCELFEQLGEYKFQNALLVRYT KKVPQVSTPTLVEVSRNLGKVGSKCC KHPEAKRMPCAEKYLSVVLNQLCVL HEKTPVSDRVTKCCTESLVNRRPCFS ALEVDETYVPKEFNAETFTFHADICTL SEKERQIKKQTALVELVKHKPKATKE QLKAVMDDFAAFVEKCCKADDKETC FAEEGKKLVAASQAALGL	RCSB Protein Data Bank
Immunoglobulin G (IgG)	MDWTWRFLFVVAAATGVQSQMQVV QSGAEVKKPGSSVTVSCKASGGTFSN YAISWVRQAPGQGLEWMGGIIPLFGT PTYSQNFQGRVTITADKSTSTAHMELI SLRSEDTAVYYCATDRYRQANFDRA RVGWFDPWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFP EPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKKVEPKSCDKTHTCPPCP APELLGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKLPAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQ VSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	NCBI

Lysozyme (Lys)	KVFGRCELAAAMKRHGLDNYRGYSL GNWVCAAKFESNFNTQATNRNTDGS TDYGIILQINSRWWCNDGRTPGSRNL CNIPCSALLSSDITASVNCAKKIVSDG NGMNAWVAWRNRCKGTDVQAWIR GCRL	RCSB Protein Data Bank
Concanavalin A (ConA)	ADTIVAVELDTHPNTDIGDPSYPHIGI DIKSVRSKKTAKWNMQNGKVGTAHI IYNSVDKRLSAVVSYPNADSATVSHD VDLDNVLPEWVRVGLSASTGLYKET NTILSWSFTSKLKSNSTHETNALHFMF NQFSKDQKDLILQGDATTGTDGNLEL TRVSSNGSPQGSSVGRALFYAPVHIW ESSAVVASFEATFTFLIKSPDSHPADGI AFFISNIDSSIPSGSTGRLLGLFPDAN	RCSB Protein Data Bank
Myoglobin (Mb)	GLSDGEWQQVLNVWGKVEADIAGH GQEVLIRLFTGHPETLEKFDKFHLKTE AEMKASEDLKKHGTVVLTALGGILK KKGHHEAELKPLAQSHATKHKIPIKY LEFISDAIIHVLHSKHPFGADAQGAMT KALELFRNDIAAKYKELGFQG	RCSB Protein Data Bank

Table S3. Low abundance proteins in liquid supernatant after treating with EGMP@SiO_2 microspheres.

NO.	Protein Name	UniProt ID	Abundance %
1	HUMAN Keratin, type I cytoskeletal 10	P13645	12.405484
2	HUMAN Keratin, type II cytoskeletal 2 epidermal	P35908	2.816092
3	HUMAN Keratin, type I cytoskeletal 14	P02533	1.062239
4	HUMAN Keratin, type II cytoskeletal 5	P13647	0.972775
5	HUMAN Hornerin	Q86YZ3	0.188619
6	HUMAN Keratin, type I cytoskeletal 16	P08779	0.173143
7	HUMAN Rootletin	Q5TZA2	0.126253
8	HUMAN Histone H3.3C	Q6NXT2	0.116485
9	HUMAN Desmoplakin	P15924	0.115231
10	HUMAN Junction plakoglobin	P14923	0.10671
11	HUMAN Keratin, type II cytoskeletal 6A	P02538	0.102469
12	HUMAN Calmodulin-like protein 5	Q9NZT	0.09502
13	HUMAN Immunoglobulin kappa variable 3D-7	A0A0C4DH55	0.09242
14	HUMAN Dermcidin	P81605	0.082729
15	HUMAN Cystatin-A	P01040	0.064079
16	HUMAN Inhibitor of growth protein 5	Q8WYH8	0.054758
17	HUMAN RAC-gamma serine/threonine-protein kinase	Q9Y243	0.052587
18	HUMAN Filaggrin-2	Q5D862	0.040375
19	HUMAN Keratin, type II cuticular Hb5	P78386	0.035821
20	HUMAN Keratinocyte proline-rich protein	Q5T749	0.032965
21	HUMAN Pachytene checkpoint protein 2 homolog	Q15645	0.032284

22	HUMAN Caspase-14	P31944	0.030493
23	HUMAN Collagen alpha-5(VI) chain	A8TX70	0.026635
24	HUMAN Uncharacterized protein ZSWIM9	Q86XI8	0.026369
25	HUMAN Prelamin-A/C	P02545	0.026334
26	HUMAN Ankyrin-3	Q12955	0.025991
27	HUMAN Histone H1.2	P16403	0.023372
28	HUMAN Protein POF1B	Q8WVV4	0.023281
29	HUMAN Lysozyme C	P61626	0.022193
30	HUMAN Annexin A2	P07355	0.021192
31	HUMAN Lactotransferrin	P02788	0.019943
32	HUMAN Immunoglobulin heavy variable 4-28	A0A0C4DH34	0.017412
33	HUMAN Speriolin	Q76KD6	0.016848
34	HUMAN Desmocollin-1	Q08554	0.01665
35	HUMAN Keratin, type I cytoskeletal 17	Q04695	0.016528
36	HUMAN Immunoglobulin heavy variable 3-73	A0A0B4J1V6	0.014866
37	HUMAN Immunoglobulin heavy variable 6-1	A0A0B4J1U7	0.014248
38	HUMAN Protein S100-A7	P31151	0.013838
39	HUMAN Immunoglobulin heavy variable 3-15	A0A0B4J1V0	0.012791
40	HUMAN FACT complex subunit SSRP1	Q08945	0.012438
41	HUMAN Keratin, type II cytoskeletal 74	Q7RTS7	0.012156
42	HUMAN Cornifin-A	P35321	0.011594
43	HUMAN Small proline-rich protein 2A	P35326	0.011419
44	HUMAN Ubiquitin-60S ribosomal protein L40	P62987	0.010993

45	HUMAN Fatty acid-binding protein 5	Q01469	0.009956
46	HUMAN Signal transducer and activator of transcription 5B	P51692	0.009483
47	HUMAN Pre-mRNA-processing factor 19	Q9UMS4	0.007965
48	HUMAN Splicing factor, proline- and glutamine-rich	P23246	0.007455
49	HUMAN Putative bifunctional UDP-N-acetylglucosamine transferase and deubiquitinase ALG13	Q9NP73	0.00726
50	HUMAN Elongation factor 2	P13639	0.006395
51	HUMAN Ankyrin repeat and SAM domain-containing protein 1A	Q92625	0.006353
52	HUMAN RNA-binding protein 8A	Q9Y5S9	0.005219
53	HUMAN Nucleosome-remodeling factor subunit BPTF	Q12830	0.005193
54	HUMAN Desmoglein-1	Q02413	0.004937
55	HUMAN Histone deacetylase 1	Q13547	0.00484
56	HUMAN Signal transducer and activator of transcription 3	P40763	0.003867
57	HUMAN Keratin, type I cuticular Ha1	Q15323	0.003702
58	HUMAN Suppressor of tumorigenicity 7 protein	Q9NRC1	0.003656
59	HUMAN Filamin-B	O75369	0.003604
60	HUMAN Gamma-glutamylcyclotransferase	O75223	0.003208
61	HUMAN Prolactin-inducible protein	P12273	0.002853
62	HUMAN Antileukoproteinase	P03973	0.002758
63	HUMAN Myosin-9	P35579	0.002746
64	HUMAN Suprabasin	Q6UWP8	0.002745
65	HUMAN Filaggrin	P20930	0.00265
66	HUMAN Plakophilin-1	Q13835	0.002429

67	HUMAN Heterogeneous nuclear ribonucleoproteins A2/B1	P22626	0.00238
68	HUMAN FACT complex subunit SPT16	Q9Y5B9	0.002346
68	HUMAN Pre-mRNA-splicing factor ATP-dependent RNA helicase DHX15	O43143	0.0022
70	HUMAN Nucleophosmin	P06748	0.001935
71	HUMAN Keratin, type II cytoskeletal 1b	Q7Z794	0.001883
72	HUMAN Dipeptidyl peptidase 4	P27487	0.001747
73	HUMAN Clathrin heavy chain 1	Q00610	0.001719
74	HUMAN X-ray repair cross-complementing protein 6	P12956	0.001658
75	HUMAN Probable ATP-dependent RNA helicase DDX5	P17844	0.001537
76	HUMAN Prostaglandin E synthase 3	Q15185	0.001426
77	HUMAN Heterogeneous nuclear ribonucleoprotein F	P52597	0.00123
78	HUMAN Keratin, type II cytoskeletal 78	Q8N1N4	0.001092
79	HUMAN Cathepsin D	P07339	0.000979
80	HUMAN ATP-citrate synthase	P53396	0.000955
81	HUMAN SUMO-activating enzyme subunit 2	Q9UBT2	0.000926
82	HUMAN Polypyrimidine tract-binding protein 1	P26599	0.000818
83	HUMAN DNA replication licensing factor MCM4	P33991	0.000739
84	HUMAN Protein kinase C theta type	Q04759	0.000702
85	HUMAN Twinfilin-1	Q12792	0.000655
86	HUMAN Transketolase	P29401	0.000591
87	HUMAN Annexin A1	P04083	0.000585
88	HUMAN GTP-binding nuclear protein Ran	P62826	0.000568
89	HUMAN Heat shock cognate 71 kDa protein	P11142	0.000544

90	HUMAN MOB kinase activator 2	Q70IA6	0.00052
91	HUMAN WAP four-disulfide core domain protein 2	Q14508	0.000271
92	HUMAN E3 ubiquitin-protein ligase RBX1	P62877	0.000261
93	HUMAN Protein S100-A9	P06702	0.000255
94	HUMAN Transmembrane protein 87A	Q8NBN3	0.000231
95	HUMAN Serine/threonine-protein kinase RIO2	Q9BVS4	0.000185
96	HUMAN Protein argonaute-1	Q9UL18	0.000167
97	HUMAN Synaptopodin-2	Q9UMS6	0.000159
98	HUMAN Kinesin-like protein KIF26B	Q2KJY2	0.000132
99	HUMAN Protein TASOR 2	Q5VWN6	0.000121
100	HUMAN Keratin, type II cytoskeletal 3	P12035	0.000105

Notes and references

1 A. E. Visser, R. P. Swatloski, W. M. Reichert, R. Mayton, S. Sheff, A. Wierzbicki, J. H. Davis and R. D. Rogers, Chem Commun, 2001, 10, 135.