

ARTICLE

Significance of surface charge and shell material of Superparamagnetic Iron Oxide Nanoparticles (SPIONs) based core/shell nanoparticles on the composition of the protein corona

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Electronic supplementary information

Materials

Gold (III) chloride trihydrate (HAuCl₄·3H₂O), tetraethylorthosilicate (TEOS, 99%), tetramethylammonium hydroxide pentahydrate (TMAOH, 25%wt solution), 3-aminopropyltriethoxysilane (APTES), sodium borohydride were purchased from Sigma-Aldrich Chemie GmbH, Buchs, Switzerland. Sodium citrate tribasic dehydrate, absolute ethanol and tetrakis (hydroxymethyl) phosphonium chloride (THPC, 80% in water) were from Fluka Chemie GmbH, Buchs, Switzerland. Titanium (IV) tetraethoxide (TEOT) was purchased from Merck (schweiz) AG, Zug, Switzerland. NH₄OH (25% aqueous solution) was purchased from BDH, VWR International AG, Dietikon, Switzerland. Phosphate buffer saline (PBS) and fetal bovine serum were purchased from Gibco BRL, Life Technologies, Basel, Switzerland. All chemicals were used as received. In all preparations, ultrapure deionized water (DI water) (Seralpur delta UV/UF setting, 0.055 µS/cm) was used.

Polyvinyl alcohol (PVA) coated SPION. All chemicals were of analytical reagent grade and were used without further purification. PVA (Mowiol® 3-85) with an average molecular weight (MW) of 14000 g/mol and a hydrolysis degree of 85%, and carboxylic acid functionalized polyvinyl alcohol (KL-506) i.e. a PVA with randomly distributed carboxylic acid groups, with an average molecular weight (MW) of 30000 – 50000 g/mol, a hydrolysis degree of 74 – 80% was supplied by were supplied by Kuraray Specialities Europe GmnH, Germany. Vinylalcohol/vinyl amine copolymer (M12), with an average MW of 80,000-140,000 g/mol was supplied by courtesy of ERKOL S.A, Tarragona Spain (now Sekisui Specialty Chemicals Europe).

POLYVINYL ALCOHOL COATED SPION.

Polymer solutions were prepared by dissolving the polymer powders in DI water followed by rapidly heating the solutions for 15 min (Mowiol® 3-85 and KL-506) to 4 h (M12) at 90°C. The polymer solutions were then filtered through a sterile filter (pore size: 200 nm). In order to obtain highly positively, positively, neutral and negatively charged PVA coated SPION, the different polymer solutions (i.e. 0.2% w/v M12, a mixture of Mowiol®3-85 and M12 at a mass ratio of 45, 10% w/v Mowiol®3-85 and 6% w/v KL-506 solutions) were mixed with 10 mg_{Fe}/ml SPION suspension at the v/v ratio of 1. The particle suspensions were stored at least one week and kept at 4°C until further use. The particle suspension was adjusted to pH 7.4 by 1M NaOH at least one day before used.

TEOS SILICA AND APTES-TEOS SILICA COATED SPION

Silica coated NPs were prepared according to Stöber sol-gel process¹. Briefly, stock SPION suspension was diluted with DI water to 0.1 mg_{Fe}/ml (total volume 10 ml). The obtained NP suspension was diluted in a solution of 200 µl of TEOS in 40 ml ethanol under vigorous magnetic stirring. Then 1.25 ml of aqueous ammonia (25% w/w) was added in one pot and the mixture was allowed to react for 1 h. Afterwards, the obtained NP suspension were centrifuged at 30000 g for 45 min. The obtained particles (SiO₂(TEOS)-SPION) were washed twice and dispersed in DI water (final concentration appx. 0.1mgFe/ml).

To produce positive charge silica coated SPION, after 1 h of reaction between SPION suspension and TEOS-ethanol solution, the silica particles were functionalized with amines to promote positively charged nanoparticle. 0.1 ml of APTES was

added to the suspension and kept under magnetic stirring overnight. The reaction mixture was further heat under refluxed at 80°C for 30 min to complete the reaction. The obtained particles (SiO₂(APTES)-SPION) were washed twice and dispersed in DI water (final concentration appx. 0.1mg_{Fe}/ml). The suspension pH was adjusted to pH 6 by HCl, in order to protonate the amine groups for better dispersibility. The nanoparticles were stored at 4°C.

GOLD COATED SPION

SiO₂(APTES)-SPION were produced as mentioned previously². A gold seed nanoparticles were produced by addition of 0.5 ml of 1 M NaOH to 45 ml of DI water, followed by 1 ml of a tetrakis (hydroxymethyl) phosphonium chloride (THPC) solution (12 µl of 80% THPC/ml of DI water). The mixture was stirred for 5 min before adding 2 ml of a fresh 1% w/w HAuCl₄ (aq) solution. After 15 min, the solution turned dark brown, indicating the formation of small (2-3 nm) gold seed particles. The gold hydroxide solution for shell deposition was prepared 1 day prior to shell deposition. Twenty-five milligrams of potassium carbonate was dissolved in 100 ml of DI water, followed by the addition of 1.5 ml of a 1.0% w/w HAuCl₄ solution. After stirring for 30 min, the solution appears colorless, indicating that hydrolysis of AuCl₄ has occurred. This gold hydroxide solution was then stored for 24 h in the dark and used immediately.

Prior to shell growth, gold seed particles were adsorbed to SiO₂(APTES)-SPION which were mildly sonicated for 20 min to ensure uniform particle dispersion and then 2.5 ml of this dispersion (0.05 mg_{Fe}/ml) was added to a 15 ml centrifuge tube, followed immediately by the addition of 5 ml of undiluted gold seed particles. The pH was readjusted to 3 with a few drops of concentrated HCl, and the dispersion was gently shaken for 2 min. The mixture was stored in the dark at 40°C overnight to maximize the surface coverage of the gold nanoseeds. The resulting nanoparticle product was isolated by centrifugation at 2500g for 1 h. The supernatant was discarded, and the precipitate was redispersed and washed twice with sonication in 2.5 ml of DI water to remove any unattached gold remaining, as indicated by a colorless supernatant above the precipitate. Finally, the gold nanoshell was prepared by reduction of a gold solution with sodium borohydride in the presence of SiO₂(APTES)-SPION covered with gold seeds (gold seeds deposited NPs). A 4 ml of the as-prepared gold hydroxide reactant solution was added to a 20 ml glass vial and placed under magnetic stirring. After sonicating for 20min, gold seeds deposited NPs was then added to this reactant solution with amounts that depended on the desired gold shell thickness. The pH was adjusted from 6 to 8 by adding 1 to 2 µl of 25% ammonia to inhibit homogeneous Au particles nucleation. 1 ml of 6.6 mM NaBH₄ was added at 1 ml for every 10 ml of gold solution. The dispersion changes from colorless to blue over the course of about 10 min. The nanoshell product was isolated by centrifugation at 2500g for 1 h. After discarding the supernatant, the precipitate of gold nanoshells was redispersed in 4 ml of DI water with sonication.

SEMI-QUANTITATIVE ANALYSIS OF PROTEINS ASSOCIATED WITH NANOPARTICLES BY NLC-MS/MSM.

NPs were spun down by centrifugation at 20000 g for 10 min and pellet was resuspended in 25 µl of Sequencing Grade Trypsin (12.5 nanogram/microliter in 25 mM ammonium bicarbonate) and digested using a CEM Discover Microwave Digestor for 15 min at 55°C (70W). The digestion was stopped by addition of 200 µl of 50% acetonitrile + 5% formic acid, dried using a Thermo SpeedVac and resuspended in 13 µl of 5% acetonitrile containing 0.1% formic acid. Samples were analyzed by NanoLC-MS/MS on an Ultimate 3000 system (Dionex, Amsterdam, The Netherlands) interfaced on-line with a LTQ-Orbitrap-XL mass spectrometer (ThermoFisher Scientific, San Jose, CA). Re-dissolved peptides were loaded onto a 5 mm × 300 µm i.d. trapping micro column packed with C18 PepMAP100 5 µm particles (Dionex) in 0.1% FA at the flow rate of 20 µL/min. Upon loading and washing, peptides were back-flush eluted onto a 15 cm × 75 µm i.d. nano-column, packed with C18 PepMAP100 3 µm particles (Dionex). The following mobile phase gradient was delivered at the flow rate of 300 nL/min: in 5–50% of solvent B for 93 min; in 50–80% B for 5 min; in 80% B for 10 min, and back to 5% B for 5 min. Solvent A was 100:0 H₂O/acetonitrile (v/v) with 0.1% formic acid and solvent B was 10:90 H₂O/acetonitrile (v/v) with 0.1% formic acid. Peptides were infused into the mass spectrometer via dynamic Nanospray probe (ThermoElectron Corp.) with a stainless steel emitter (Proxeon, Odense, DK). Typical spray voltage was 1.6 kV with no sheath and auxiliary gas flow; ion transfer tube temperature was 200 °C. Mass spectrometer was operated in data-dependent mode. The automated gain control (AGC) was set to 5×10⁵ charges and 1×10⁴ charges for MS/MS at the linear ion trap analyzer. DDA cycle consisted of the survey scan within m/z 300–1300 at the Orbitrap analyzer with target mass resolution of 60,000 (FWHM, full width at half maximum at m/z 400) followed by MS/MS fragmentation of the five most intense precursor ions under the relative collision energy of 35% in the linear trap. Singly charged ions were excluded from MS/MS experiments, and m/z of fragmented precursor ions were dynamically excluded for further 90 s. Ion selection threshold for triggering MS/MS experiments set to 500 counts. An activation parameter q 0.25 and activation time of 30 ms were applied. PEAKS DB (version 5.3) was applied to spectra generated by LTQ-ORBITRAP-XL to screen the protein composition of corona on SPION shells. The false discovery rate was manually adjusted into zero. The normalized SpC amounts of each protein, identified in the LC-MS/MS study of smooth and jagged surfaces, were calculated by applying the following equation:

$$NpSpC_k = \left(\frac{(SpC/M_w)_k}{\sum_{i=1}^n (SpC/M_w)_i} \right) \times 100$$

where $NpSpCk$ is the normalized percentage of spectral count for protein k , SpC is the spectral count identified, and Mw is the molecular weight (in kDa) of the protein k . The MS analysis

including with $NpSpCk$ of tightly bound protein eluted from each type nanoparticle was represented in the supportive information (Table S1 to S9).

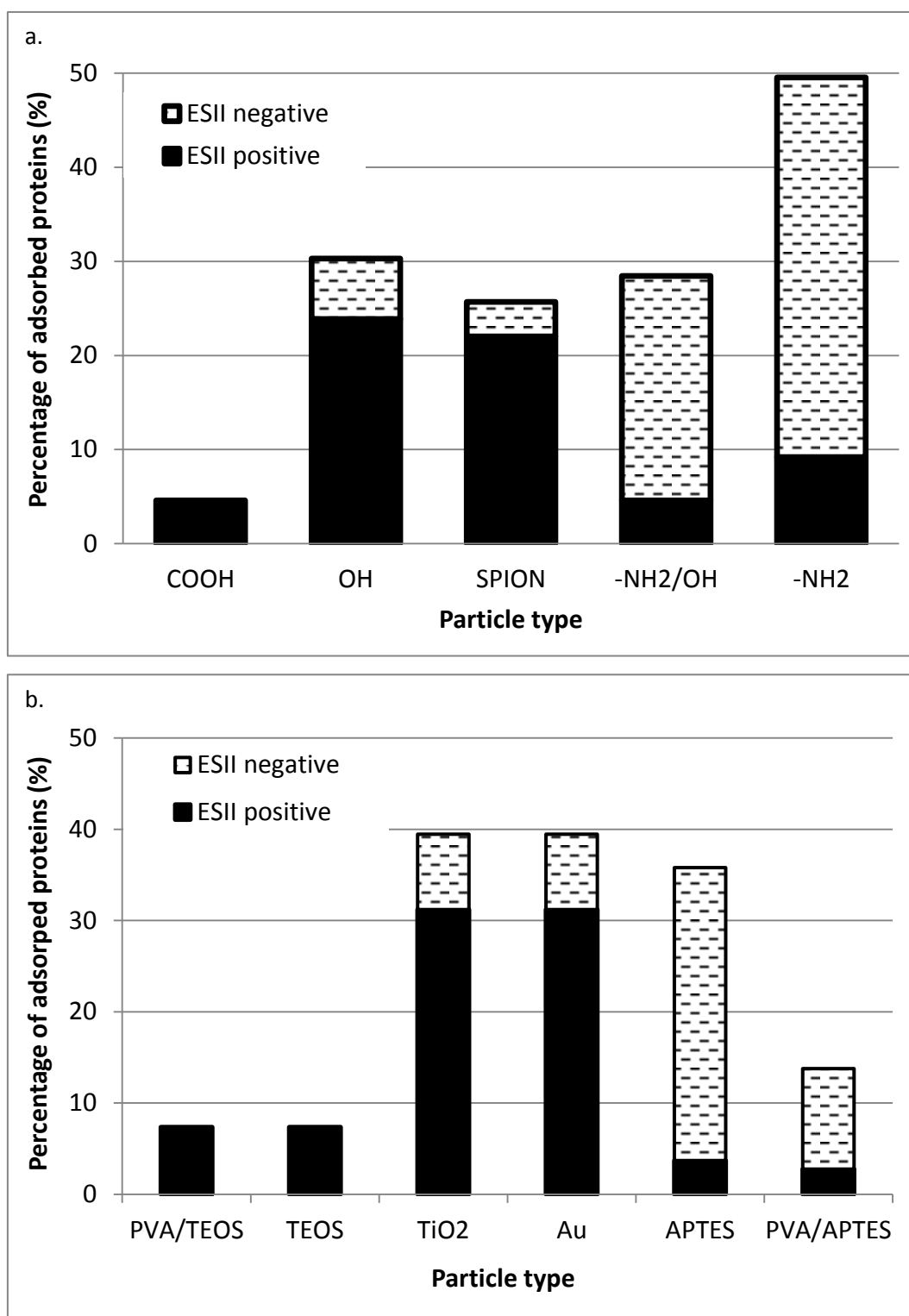


Figure S1. Influence of the particle type and coating on the adsorption of proteins from with a positive ESII (repulsive interaction) and a negative ESII (attraction)

Table S1: Protein identification of tightly bound protein eluted from naked SPION (total: 24 identified proteins).

Accession	Mw (Da)	Description	NpSpCk
P02070 HBB_BOVIN	15954	Hemoglobin subunit beta	9.63
P34955 A1AT_BOVIN	46104	Alpha-1-antitrypsin	8.33
P02081 HBBF_BOVIN	15859	Hemoglobin fetal subunit beta	8.07
P15497 APOA1_BOVIN	30276	Apolipoprotein A-I	7.61
P12763 FETUA_BOVIN	38419	Alpha-2-HS-glycoprotein	6.00
P81187 CFAB_BOVIN	85366	Complement factor B	4.20
Q03247 APOE_BOVIN	35979	Apolipoprotein E	3.56
P02769 ALBU_BOVIN	69294	Serum albumin	2.22
Q95121 PEDF_BOVIN	46229	Pigment epithelium-derived factor	2.21
P00741 FA9_BOVIN	46785	Coagulation factor IX (Fragment)	2.19
P28800 A2AP_BOVIN	54711	Alpha-2-antiplasmin	1.87
P01045 KNG2_BOVIN	68710	Kininogen-2	1.86
P01044 KNG1_BOVIN	68890	Kininogen-1	1.86
P60712 ACTB_BOVIN	41737	Actin, cytoplasmic 1	1.84
P63258 ACTG_BOVIN	41793	Actin, cytoplasmic 2	1.84
Q2UVX4 CO3_BOVIN	187252	Complement C3	1.64
P56652 ITIH3_BOVIN	99551	Inter-alpha-trypsin inhibitor heavy chain H3	1.54
P19120 HSP7C_BOVIN	71241	Heat shock cognate 71 kDa protein	1.44
Q28178 TSP1_BOVIN	129534	Thrombospondin-1	1.38
Q7SIH1 A2MG_BOVIN	167575	Alpha-2-macroglobulin	1.37
Q0VCX2 GRP78_BOVIN	72400	78 kDa glucose-regulated protein	1.06
P02672 FIBA_BOVIN	67012	Fibrinogen alpha chain	0.76
P00735 THRB_BOVIN	70506	Prothrombin	0.73
P01030 CO4_BOVIN	3101551	Complement C4 (Fragments)	0.03

Table S2: Protein identification of tightly bound protein eluted from highly positively charged PVA coated SPION (PVA_(NH₂)-SPION) (total: 55 identified proteins).

Accession	Mw (Da)	Description	NpSpCk
P01966 HBA_BOVIN	15184	Hemoglobin subunit alpha	6.69
P02081 HBBF_BOVIN	15859	Hemoglobin fetal subunit beta	5.12
P15497 APOA1_BOVIN	30276	Apolipoprotein A-I	3.69
P02070 HBB_BOVIN	15954	Hemoglobin subunit beta	3.18
P34955 A1AT_BOVIN	46104	Alpha-1-antitrypsin	3.08
P02769 ALBU_BOVIN	69294	Serum albumin	2.78
P12763 FETUA_BOVIN	38419	Alpha-2-HS-glycoprotein	2.64
Q2UVX4 CO3_BOVIN	187252	Complement C3	2.49
P81947 TBA1B_BOVIN	50152	Tubulin alpha-1B chain	2.43
Q03247 APOE_BOVIN	35979	Apolipoprotein E	2.26
P81948 TBA4A_BOVIN	49924	Tubulin alpha-4A chain	2.24
P02584 PROF1_BOVIN	15057	Profilin-1	2.02
Q76LV2 HS90A_BOVIN	84731	Heat shock protein HSP 90-alpha	1.92
P60712 ACTB_BOVIN	41737	Actin, cytoplasmic 1	1.70
P63258 ACTG_BOVIN	41793	Actin, cytoplasmic 2	1.70
Q5E9B5 ACTH_BOVIN	41877	Actin, gamma-enteric smooth muscle	1.70
P62739 ACTA_BOVIN	42009	Actin, aortic smooth muscle	1.69
Q3ZC07 ACTC_BOVIN	42019	Actin, alpha cardiac muscle 1	1.69
P68138 ACTS_BOVIN	42051	Actin, alpha skeletal muscle	1.69
Q0VCG9 PTX3_BOVIN	42021	Pentraxin-related protein PTX3	1.45
Q2KJD0 TBB5_BOVIN	49671	Tubulin beta-5 chain	1.43
P52556 BLVRB_BOVIN	22132	Flavin reductase (NADPH)	1.38
Q76LV1 HS90B_BOVIN	83253	Heat shock protein HSP 90-beta	1.34
O46375 TTHY_BOVIN	15727	Transthyretin	1.29
Q7SIH1 A2MG_BOVIN	167575	Alpha-2-macroglobulin	1.27
P17697 CLUS_BOVIN	51114	Clusterin	1.19
P81187 CFAB_BOVIN	85366	Complement factor B	1.19
Q95121 PEDF_BOVIN	46229	Pigment epithelium-derived factor	1.10
P63103 1433Z_BOVIN	27745	14-3-3 protein zeta/delta	1.10
Q3SWY2 ILK_BOVIN	51447	Integrin-linked protein kinase	0.99
P61223 RAP1B_BOVIN	20825	Ras-related protein Rap-1b	0.98
P02676 FIBB_BOVIN	53339	Fibrinogen beta chain	0.95
P56652 ITIH3_BOVIN	99551	Inter-alpha-trypsin inhibitor heavy chain H3	0.92
Q5E9B1 LDHB_BOVIN	36724	L-lactate dehydrogenase B chain	0.83
P68103 EF1A1_BOVIN	50141	Elongation factor 1-alpha 1	0.81
Q05443 LUM_BOVIN	38756	Lumican	0.79
Q3SZV7 HEMO_BOVIN	52209	Hemopexin	0.78
Q3MHN5 VTDB_BOVIN	53342	Vitamin D-binding protein	0.76
P19120 HSP7C_BOVIN	71241	Heat shock cognate 71 kDa protein	0.71
Q3T052 ITIH4_BOVIN	101513	Inter-alpha-trypsin inhibitor heavy chain H4	0.70
P06868 PLMN_BOVIN	91216	Plasminogen	0.67
P52193 CALR_BOVIN	48039	Calreticulin	0.63
Q2KJ83 CBPN_BOVIN	52669	Carboxypeptidase N catalytic chain	0.58
Q27965 HS71B_BOVIN	70229	Heat shock 70 kDa protein 1B	0.58

Q27975 HS71A_BOVIN	70259	Heat shock 70 kDa protein 1A	0.58
Q2KJH9 AL9A1_BOVIN	53977	4-trimethylaminobutyraldehyde dehydrogenase	0.56
P00978 AMBP_BOVIN	39235	Protein AMBP	0.52
Q3SZ57 FETA_BOVIN	68588	Alpha-fetoprotein	0.44
Q27991 MYH10_BOVIN	229097	Myosin-10	0.44
P31836 NCAM1_BOVIN	93894	Neural cell adhesion molecule 1	0.43
P00735 THRB_BOVIN	70506	Prothrombin	0.43
Q28107 FA5_BOVIN	248981	Coagulation factor V	0.41
P12799 FIBG_BOVIN	50244	Fibrinogen gamma-B chain	0.40
P28800 A2AP_BOVIN	54711	Alpha-2-antiplasmin	0.37
P81282 CSPG2_BOVIN	369991	Versican core protein	0.19

Table S3: Protein identification of tightly bound protein eluted from positively charged PVA coated SPION (PVA_(NH₂/OH)-SPION) (total: 31 identified proteins).

Accession	Mw (Da)	Description	NpSpCk
P15497 APOA1_BOVIN	30276	Apolipoprotein A-I	7.16
P01966 HBA_BOVIN	15184	Hemoglobin subunit alpha	6.12
P34955 A1AT_BOVIN	46104	Alpha-1-antitrypsin	6.05
P02081 HBBF_BOVIN	15859	Hemoglobin fetal subunit beta	5.86
P12763 FETUA_BOVIN	38419	Alpha-2-HS-glycoprotein	4.43
Q03247 APOE_BOVIN	35979	Apolipoprotein E	3.87
P02769 ALBU_BOVIN	69294	Serum albumin	3.80
P02070 HBB_BOVIN	15954	Hemoglobin subunit beta	2.91
P20959 IBP3_BOVIN	31570	Insulin-like growth factor-binding protein 3	1.96
P60712 ACTB_BOVIN	41737	Actin, cytoplasmic 1	1.85
P63258 ACTG_BOVIN	41793	Actin, cytoplasmic 2	1.85
P02672 FIBA_BOVIN	67012	Fibrinogen alpha chain	1.85
Q2UVX4 CO3_BOVIN	187252	Complement C3	1.65
P01045 KNG2_BOVIN	68710	Kininogen-2	1.58
P01044 KNG1_BOVIN	68890	Kininogen-1	1.57
P02754 LACB_BOVIN	19883	Beta-lactoglobulin	1.56
Q7SIH1 A2MG_BOVIN	167575	Alpha-2-macroglobulin	1.39
Q27967 SPP24_BOVIN	23134	Secreted phosphoprotein 24	1.34
P02676 FIBB_BOVIN	53339	Fibrinogen beta chain	1.16
Q95121 PEDF_BOVIN	46229	Pigment epithelium-derived factor	1.00
P56652 ITIH3_BOVIN	99551	Inter-alpha-trypsin inhibitor heavy chain H3	0.93
P81948 TBA4A_BOVIN	49924	Tubulin alpha-4A chain	0.93
P17697 CLUS_BOVIN	51114	Clusterin	0.91
P28800 A2AP_BOVIN	54711	Alpha-2-antiplasmin	0.85
Q32PJ2 APOA4_BOVIN	43018	Apolipoprotein A-IV	0.72
Q3T052 ITIH4_BOVIN	101513	Inter-alpha-trypsin inhibitor heavy chain H4	0.61
P07589 FNC_BOVIN	272151	Fibronectin	0.57
P00743 FA10_BOVIN	54510	Coagulation factor X	0.57
P00735 THRB_BOVIN	70506	Prothrombin	0.44
P06868 PLMN_BOVIN	91216	Plasminogen	0.34
Q27991 MYH10_BOVIN	229097	Myosin-10	0.20

Table S4: Protein identification of tightly bound protein eluted from neutral PVA_(OH)-SPION (total: 34 identified proteins).

Accession	Mw (Da)	Description	NpSpCk
P01966 HBA_BOVIN	15184	Hemoglobin subunit alpha	9.49
P02081 HBBF_BOVIN	15859	Hemoglobin fetal subunit beta	7.27
P15497 APOA1_BOVIN	30276	Apolipoprotein A-I	6.19
P34955 A1AT_BOVIN	46104	Alpha-1-antitrypsin	3.75
Q03247 APOE_BOVIN	35979	Apolipoprotein E	3.20
P20959 IBP3_BOVIN	31570	Insulin-like growth factor-binding protein 3	3.20
Q27967 SPP24_BOVIN	23134	Secreted phosphoprotein 24	3.11
P12763 FETUA_BOVIN	38419	Alpha-2-HS-glycoprotein	3.00
P02253 H12_BOVIN	10365	Histone H1.2 (Fragment)	2.78
P02769 ALBU_BOVIN	69293	Serum albumin	2.50
P01045 KNG2_BOVIN	68710	Kininogen-2	2.31
P01044 KNG1_BOVIN	68890	Kininogen-1	2.30
P31096 OSTP_BOVIN	30904	Osteopontin	1.87
P31098 OSTK_BOVIN	30969	Osteopontin-K	1.86
P60712 ACTB_BOVIN	41737	Actin, cytoplasmic 1	1.38
P63258 ACTG_BOVIN	41793	Actin, cytoplasmic 2	1.38
Q5E9B5 ACTH_BOVIN	41877	Actin, gamma-enteric smooth muscle	1.38
P62739 ACTA_BOVIN	42009	Actin, aortic smooth muscle	1.37
Q3ZC07 ACTC_BOVIN	42019	Actin, alpha cardiac muscle 1	1.37
P68138 ACTS_BOVIN	42051	Actin, alpha skeletal muscle	1.37
Q2KIS7 TETN_BOVIN	22144	Tetranectin	1.30
A7MAZ5 H13_BOVIN	22154	Histone H1.3	1.30
Q95121 PEDF_BOVIN	46229	Pigment epithelium-derived factor	1.25
Q5E9B1 LDHB_BOVIN	36724	L-lactate dehydrogenase B chain	1.18
Q2UVX4 CO3_BOVIN	187252	Complement C3	1.15
P02672 FIBA_BOVIN	67012	Fibrinogen alpha chain	1.08
Q7SIH1 A2MG_BOVIN	167575	Alpha-2-macroglobulin	0.86
P17697 CLUS_BOVIN	51114	Clusterin	0.85
Q95117 SFRP3_BOVIN	36234	Secreted frizzled-related protein 3	0.80
Q32PJ2 APOA4_BOVIN	43018	Apolipoprotein A-IV	0.67
Q27991 MYH10_BOVIN	229097	Myosin-10	0.44
P81187 CFAB_BOVIN	85366	Complement factor B	0.34
P06868 PLMN_BOVIN	91216	Plasminogen	0.32
Q28178 TSP1_BOVIN	129534	Thrombospondin-1	0.22

Table S5: Protein identification of tightly bound protein eluted from negatively charged PVA coated SPION (PVA_(COOH)-SPION) (total: 5 identified proteins).

Accession	Mw (Da)	Description	NpSpCk
P12763 FETUA_BOVIN	38419	Alpha-2-HS-glycoprotein	7.31
P34955 A1AT_BOVIN	46104	Alpha-1-antitrypsin	6.09
P02769 ALBU_BOVIN	69294	Serum albumin	5.07
Q76LV2 HS90A_BOVIN	84731	Heat shock protein HSP 90-alpha	3.31
Q2UVX4 CO3_BOVIN	187252	Complement C3	2.62

Table S6: Protein identification of tightly bound protein eluted from negatively charged SiO₂ coated SPION (TEOS-SPION) (total: 19 identified proteins).

Accession	Mw (Da)	Description	NpSpCk
P15497 APOA1_BOVIN	30276	Apolipoprotein A-I	11.18
P01966 HBA_BOVIN	15184	Hemoglobin subunit alpha	8.36
P60712 ACTB_BOVIN	41737	Actin, cytoplasmic 1	5.07
P63258 ACTG_BOVIN	41793	Actin, cytoplasmic 2	4.05
Q5E9B5 ACTH_BOVIN	41877	Actin, gamma-enteric smooth muscle	4.04
P62739 ACTA_BOVIN	42009	Actin, aortic smooth muscle	4.03
Q3ZC07 ACTC_BOVIN	42019	Actin, alpha cardiac muscle 1	4.03
P68138 ACTS_BOVIN	42051	Actin, alpha skeletal muscle	4.02
Q3SX14 GELS_BOVIN	80731	Gelsolin	2.62
P01045 KNG2_BOVIN	68710	Kininogen-2	2.46
P01044 KNG1_BOVIN	68890	Kininogen-1	2.46
Q03247 APOE_BOVIN	35979	Apolipoprotein E	2.35
P12763 FETUA_BOVIN	38419	Alpha-2-HS-glycoprotein	2.20
Q2UVX4 CO3_BOVIN	187252	Complement C3	2.03
P81187 CFAB_BOVIN	85366	Complement factor B	1.98
P02672 FIBA_BOVIN	67012	Fibrinogen alpha chain	1.89
P28800 A2AP_BOVIN	54711	Alpha-2-antiplasmin	1.55
P02453 CO1A1_BOVIN	138939	Collagen alpha-1(I) chain	1.52
Q28178 TSP1_BOVIN	129534	Thrombospondin-1	0.98

Table S7: Protein identification of tightly bound protein eluted from positively charged SiO₂ coated SPION (APTES-SPION) (total: 40 identified proteins).

Accession	Mw (Da)	Description	NpSpCk
P15497 APOA1_BOVIN	30276	Apolipoprotein A-I	18.25
P81644 APOA2_BOVIN	1122	Apolipoprotein A-II	5.58
P01966 HBA_BOVIN	15184	Hemoglobin subunit alpha	3.43
P02081 HBBF_BOVIN	15859	Hemoglobin fetal subunit beta	3.29
P02070 HBB_BOVIN	15954	Hemoglobin subunit beta	3.27
P34955 A1AT_BOVIN	46104	Alpha-1-antitrypsin	3.17
Q03247 APOE_BOVIN	35979	Apolipoprotein E	2.32
Q32PJ2 APOA4_BOVIN	43018	Apolipoprotein A-IV	1.94
Q5KR49 TPM1_BOVIN	32695	Tropomyosin alpha-1 chain	1.91
Q5KR47 TPM3_BOVIN	32819	Tropomyosin alpha-3 chain	1.91
Q2UVX4 CO3_BOVIN	187252	Complement C3	1.73
P02769 ALBU_BOVIN	69293	Serum albumin	1.66
Q76LV2 HS90A_BOVIN	84731	Heat shock protein HSP 90-alpha	1.60
Q95121 PEDF_BOVIN	46229	Pigment epithelium-derived factor	1.58
P63103 1433Z_BOVIN	27745	14-3-3 protein zeta/delta	1.50
P68250 1433B_BOVIN	28081	14-3-3 protein beta/alpha	1.49
P05059 CMGA_BOVIN	50015	Chromogranin-A	1.46
P12763 FETUA_BOVIN	38419	Alpha-2-HS-glycoprotein	1.36
P60712 ACTB_BOVIN	41737	Actin, cytoplasmic 1	1.25
P63258 ACTG_BOVIN	41793	Actin, cytoplasmic 2	1.25
P81187 CFAB_BOVIN	85366	Complement factor B	1.22
Q27965 HS71B_BOVIN	70229	Heat shock 70 kDa protein 1B	1.04
Q27975 HS71A_BOVIN	70259	Heat shock 70 kDa protein 1A	1.04
Q76LV1 HS90B_BOVIN	83253	Heat shock protein HSP 90-beta	1.00
Q5E9B5 ACTH_BOVIN	41877	Actin, gamma-enteric smooth muscle	1.00
P62739 ACTA_BOVIN	42009	Actin, aortic smooth muscle	0.99
Q3ZC07 ACTC_BOVIN	42019	Actin, alpha cardiac muscle 1	0.99
P68138 ACTS_BOVIN	42051	Actin, alpha skeletal muscle	0.99
Q7SIH1 A2MG_BOVIN	167575	Alpha-2-macroglobulin	0.87
P00743 FA10_BOVIN	54510	Coagulation factor X	0.77
Q28034 GLU2B_BOVIN	60151	Glucosidase 2 subunit beta	0.69
Q3SX14 GELS_BOVIN	80731	Gelsolin	0.65
P02672 FIBA_BOVIN	67012	Fibrinogen alpha chain	0.62
P17697 CLUS_BOVIN	51114	Clusterin	0.61
P00735 THRB_BOVIN	70506	Prothrombin	0.44
Q0VCX2 GRP78_BOVIN	72400	78 kDa glucose-regulated protein	0.43
Q27991 MYH10_BOVIN	229097	Myosin-10	0.32
P56652 ITIH3_BOVIN	99551	Inter-alpha-trypsin inhibitor heavy chain H3	0.31
Q3T052 ITIH4_BOVIN	101513	Inter-alpha-trypsin inhibitor heavy chain H4	0.31
Q28107 FA5_BOVIN	248981	Coagulation factor V	0.29

Table S8: Protein identification of tightly bound protein eluted from TiO₂ coated SPION (TiO₂-SPION) (total: 53 identified proteins).

Accession	Mw (Da)	Description	NpSpCk
P01966 HBA_BOVIN	15184	Hemoglobin subunit alpha	8.78
P81644 APOA2_BOVIN	11202	Apolipoprotein A-II	7.93
P15497 APOA1_BOVIN	30276	Apolipoprotein A-I	7.63
P62326 TYB4_BOVIN	5053	Thymosin beta-4	7.04
P02081 HBBF_BOVIN	15859	Hemoglobin fetal subunit beta	4.48
P02070 HBB_BOVIN	15954	Hemoglobin subunit beta	4.46
P19035 APOC3_BOVIN	10692	Apolipoprotein C-III	4.16
P21752 TYB10_BOVIN	4805	Thymosin beta-10	3.70
P02672 FIBA_BOVIN	67012	Fibrinogen alpha chain	3.32
P34955 A1AT_BOVIN	46104	Alpha-1-antitrypsin	2.89
Q3SX14 GELS_BOVIN	80730	Gelsolin	2.75
P01888 B2MG_BOVIN	13677	Beta-2-microglobulin	2.60
O46375 TTHY_BOVIN	15727	Transthyretin	2.26
P80425 FABPL_BOVIN	14227	Fatty acid-binding protein, liver	1.87
P12763 FETUA_BOVIN	38419	Alpha-2-HS-glycoprotein	1.85
P19034 APOC2_BOVIN	11061	Apolipoprotein C-II	1.61
P81187 CFAB_BOVIN	85366	Complement factor B	1.35
Q03247 APOE_BOVIN	35979	Apolipoprotein E	1.24
P80724 BASP1_BOVIN	23011	Brain acid soluble protein 1	1.16
Q3SZK8 NHRF1_BOVIN	39603	Na(+)/H(+) exchange regulatory cofactor NHE-RF1	1.12
P28800 A2AP_BOVIN	54711	Alpha-2-antiplasmin	0.97
Q95121 PEDF_BOVIN	46229	Pigment epithelium-derived factor	0.96
P60712 ACTB_BOVIN	41737	Actin, cytoplasmic 1	0.85
P63258 ACTG_BOVIN	41793	Actin, cytoplasmic 2	0.85
Q32PJ2 APOA4_BOVIN	43018	Apolipoprotein A-IV	0.83
Q2KIS7 TETN_BOVIN	22144	Tetranectin	0.80
Q9TS87 TAGL_BOVIN	22599	Transgelin	0.79
Q9N212 IPSP_BOVIN	45297	Plasma serine protease inhibitor	0.78
P01045 KNG2_BOVIN	68710	Kininogen-2	0.78
Q6URK6 CADH5_BOVIN	87467	Cadherin-5	0.71
P02769 ALBU_BOVIN	69293	Serum albumin	0.64
Q5E9B5 ACTH_BOVIN	41877	Actin, gamma-enteric smooth muscle	0.64
P62739 ACTA_BOVIN	42009	Actin, aortic smooth muscle	0.63
Q3ZC07 ACTC_BOVIN	42019	Actin, alpha cardiac muscle 1	0.63
P68138 ACTS_BOVIN	42051	Actin, alpha skeletal muscle	0.63
P31096 OSTP_BOVIN	30904	Osteopontin	0.58
P31098 OSTK_BOVIN	30969	Osteopontin-K	0.57
P05059 CMGA_BOVIN	50015	Chromogranin-A	0.53
P13384 IBP2_BOVIN	34015	Insulin-like growth factor-binding protein 2	0.52
Q2UVX4 CO3_BOVIN	187252	Complement C3	0.52
Q0VCX2 GRP78_BOVIN	72400	78 kDa glucose-regulated protein	0.49
Q2HJ49 MOES_BOVIN	67975	Moesin	0.39
P0CB32 HS71L_BOVIN	70389	Heat shock 70 kDa protein 1-like	0.38
P00735 THRB_BOVIN	70506	Prothrombin	0.38
P17697 CLUS_BOVIN	51114	Clusterin	0.35

P50448 F12AI_BOVIN	51723	Factor XIIa inhibitor	0.34
Q28178 TSP1_BOVIN	129534	Thrombospondin-1	0.34
Q28107 FA5_BOVIN	248981	Coagulation factor V	0.32
Q7SIH1 A2MG_BOVIN	167575	Alpha-2-macroglobulin	0.32
Q29RQ1 CO7_BOVIN	93090	Complement component C7	0.29
Q3T052 ITIH4_BOVIN	101512	Inter-alpha-trypsin inhibitor heavy chain H4	0.26
Q28085 CFAH_BOVIN	140374	Complement factor H	0.25
Q2KJ63 KLKB1_BOVIN	70994	Plasma kallikrein	0.25

Table S9: Protein identification of tightly bound protein eluted from gold coated SPION (Au-SPION) (total: 50 identified proteins).

Accession	Mw (Da)	Description	NpSpCk
P01966 HBA_BOVIN	15184	Hemoglobin subunit alpha	7.69
P02081 HBBF_BOVIN	15859	Hemoglobin fetal subunit beta	7.36
P15497 APOA1_BOVIN	30276	Apolipoprotein A-I	7.19
P02070 HBB_BOVIN	15954	Hemoglobin subunit beta	4.39
P34955 A1AT_BOVIN	46104	Alpha-1-antitrypsin	3.88
Q3SX14 GELS_BOVIN	80730	Gelsolin	2.99
P19035 APOC3_BOVIN	10692	Apolipoprotein C-III	2.91
P02672 FIBA_BOVIN	67012	Fibrinogen alpha chain	2.79
P02769 ALBU_BOVIN	69293	Serum albumin	2.47
Q2UVX4 CO3_BOVIN	187252	Complement C3	2.20
P81644 APOA2_BOVIN	11202	Apolipoprotein A-II	2.08
Q03247 APOE_BOVIN	35979	Apolipoprotein E	1.95
Q9N212 IPSP_BOVIN	45297	Plasma serine protease inhibitor	1.89
P12763 FETUA_BOVIN	38419	Alpha-2-HS-glycoprotein	1.82
Q3T052 ITIH4_BOVIN	101513	Inter-alpha-trypsin inhibitor heavy chain H4	1.76
Q2KIS7 TETN_BOVIN	22144	Tetranectin	1.76
P81187 CFAB_BOVIN	85366	Complement factor B	1.73
P01888 B2MG_BOVIN	13677	Beta-2-microglobulin	1.71
P01045 KNG2_BOVIN	68710	Kininogen-2	1.70
Q95121 PEDF_BOVIN	46229	Pigment epithelium-derived factor	1.68
P60712 ACTB_BOVIN	41737	Actin, cytoplasmic 1	1.49
P63258 ACTG_BOVIN	41793	Actin, cytoplasmic 2	1.49
Q3T0A3 CFAD_BOVIN	27878	Complement factor D	1.40
P13384 IBP2_BOVIN	34015	Insulin-like growth factor-binding protein 2	1.14
Q3SZ57 FETA_BOVIN	68588	Alpha-fetoprotein	1.13
Q5E9F5 TAGL2_BOVIN	22426	Transgelin-2	1.04
P41361 ANT3_BOVIN	52347	Antithrombin-III	1.04
P06868 PLMN_BOVIN	91216	Plasminogen	1.02
P50448 F12AI_BOVIN	51723	Factor XIIa inhibitor	0.90
P28800 A2AP_BOVIN	54711	Alpha-2-antiplasmin	0.85
P17697 CLUS_BOVIN	51114	Clusterin	0.76
Q58D62 FETUB_BOVIN	42663	Fetuin-B	0.73
Q28085 CFAH_BOVIN	140374	Complement factor H	0.67
P17690 APOH_BOVIN	38252	Beta-2-glycoprotein 1	0.61
Q29443 TRFE_BOVIN	77753	Serotransferrin	0.60
Q3SZV7 HEMO_BOVIN	52209	Hemopexin	0.60
Q3MHN5 VTDB_BOVIN	53342	Vitamin D-binding protein	0.58
Q7SIH1 A2MG_BOVIN	167575	Alpha-2-macroglobulin	0.51
Q29RQ1 CO7_BOVIN	93090	Complement component C7	0.50
Q28178 TSP1_BOVIN	129534	Thrombospondin-1	0.48
P56652 ITIH3_BOVIN	99551	Inter-alpha-trypsin inhibitor heavy chain H3	0.47
P35445 COMP_BOVIN	82362	Cartilage oligomeric matrix protein	0.38
Q3SWW8 TSP4_BOVIN	105974	Thrombospondin-4	0.37
P02465 CO1A2_BOVIN	129064	Collagen alpha-2(I) chain	0.36
P02453 CO1A1_BOVIN	138939	Collagen alpha-1(I) chain	0.34

Q0VCM5 ITI1_BOVIN	101237	Inter-alpha-trypsin inhibitor heavy chain H1	0.31
P00735 THRB_BOVIN	70506	Prothrombin	0.22
Q2KJ63 KLKB1_BOVIN	70994	Plasma kallikrein	0.22
P07589 FNC_BOVIN	272151	Fibronectin	0.14
P01030 CO4_BOVIN	3101551	Complement C4 (Fragments)	0.02

Notes and references

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