Electronic supplementary information

Female *versus* male biological identities of nanoparticles determine the interaction with the immune system in fish

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Supplemental description of experimental methods

Collection of zebrafish blood plasma

To obtain a large volume of blood from each fish, a centrifuge-based method reported by Babaei and colleagues[1] was employed with modifications to accommodate large-sized fish. Briefly, fish were first anesthetised with 0.02% tricaine (3-amino benzoic acid ethyl ester) and euthanised in ice-water. Prior to blood harvesting, a custom-made 1.5 ml microcentrifuge tube (Eppendorf) with its bottom excised was suspended in a 13 ml polypropylene tube (Sarstedt), kept on ice and rinsed with anticoagulation buffer prepared of PBS containing 10 mM EDTA (BioUltra, Sigma-Aldrich) and a mixture of 100 U/mL penicillin and 100 μg/mL streptomycin (Gibco). Each euthanised fish had its tail amputated, the wound dipped in the anticoagulation buffer and placed in the upper column of the double tube assembly, before being centrifuged at 40*g* for 5 min at 11°C. Centrifugation was repeated after the second excision as described,[1] and the blood collected was transferred to a Protein LoBind tube (Eppendorf) and spun at 13800*g* for 15 min at 8°C. If haemolysis was apparent, the tube was discarded. The clear supernatant (plasma) was pooled and stored at -80°C until use (within a month). Total protein concentration was quantified by the Bradford assay (Pierce Coomassie Plus, ThermoScientific) with a VersaMax ELISA Microplate Reader (Molecular Devices) following the manufacturer's instructions (absorbance at 595 nm). Normally 6 female or male fish were sacrificed in one session and each batch of pooled plasma had a typical protein yield of 100-200 μg per fish. The blood plasma thus obtained was named DrBP-F or DrBP-M (*Danio rerio* blood plasma - female or male, respectively). DrBP (Mix) is a 1:1 mixture of the two.

Incubation of SiO_2 nanoparticles to form nanoparticle-protein complexes

The protein concentrations of the DrBP series (DrBP-F, DrBP-M, DrBP-Mix) and FBS (Sigma) were adjusted to 1 mg/ml and 2 mg/ml, respectively, using the anticoagulant buffer. The protein solutions were centrifuged at 16000*g* for 3 min at room temperature (RT) to remove any insoluble protein aggregates, and the supernatants were then incubated with the SiO_2 nanoparticles (200 μg/ml) for 24 h in darkness at 26°C. To minimize non-specific binding of proteins to the tube wall, Protein Lobind tubes were used in all steps of incubation and centrifugal isolation of nanoparticle-protein complexes (described below). For the given mass concentration of 70 nm SiO_2 nanoparticles (200 μg/ml), nanoparticle number and outer surface area can be roughly calculated to be 6×10^{11}/ml and 86 cm^2/ml,

respectively. Based on these numbers we can then assume the minimal number of serum albumin molecules that are necessary to completely cover the nanoparticle surface.[2] To ensure a full surface coverage, an excess of proteins accounting for theoretical 5 layers has been recommended.[3] In this study, protein concentrations used for incubation correspond to approximately 2.5-5.0% of the original protein concentrations in whole blood/serum and were 1 mg/ml (the DrBP series) and 2 mg/ml (FBS). The selection of candidate concentrations was further short-listed by the dispersibility of the nanoparticle pellet after centrifugation, as analysed by dynamic light scattering (described below). At the chosen ratio of nanoparticles-to-proteins, theoretically, there is a 22 (the DrBP series) or 44 (FBS) times excess of proteins, enough to cover the nanoparticle surface.

Centrifugal isolation

The nanoparticle-protein complexes were isolated from unbound and loosely-bound proteins by a well-established centrifugation technique as described previously.[4] Briefly, after pelleting the nanoparticle-protein complexes by centrifugation at 16000*g* for 20 min at 21°C, the pellet was redispersed in PBS and centrifuged again. This washing process was repeated three times (the suspension was transferred to a new Protein Lobind tube after the second wash), before concentrated SDS-loading buffer (with 100 mM dithothreitol as a reducing agent; 5X Lane Marker Reducing Sample Buffer, ThermoScientific) was added to the nanoparticle pellet. The samples were heated at 98°C for 5 min to denature the proteins and strip off the hard protein corona from the nanoparticles, after which the nanoparticles were spun down at 16000*g* for 30 min at 4 °C and the supernatant was stored at -20°C until analysis. Nanoparticle-free blanks were separately prepared and we confirmed by SDS-PAGE that no protein aggregates were unintentionally spun down during the centrifugal isolation steps.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

The hard corona protein samples were diluted with PBS to adjust the SDS-loading buffer concentration, and for reference total protein samples were prepared by heating respective protein solutions (5 μg) in the presence of the SDS-loading buffer. The denatured proteins were separated by 4-20% gradient SDS-PAGE (Precise Protein Gels, ThermoScientific) along with a PageRuler Unstained Protein Ladder (ThermoScientific) as the molecular weight standard (10-200 kDa). Protein bands in the gels were detected by Imperial Protein Stain (Coomassie Brilliant Blue staining with sensitivity of 3 ng; ThermoScientific) following the manufacturer's instructions. The stained gels were scanned on an

Odyssey infrared imager (Li-Cor) and the images were processed with Fiji/ImageJ.[5, 6] For analysis, the plot profile tool was used to quantify the staining intensity and run length. The whole process starting from the formation of the nanoparticle-protein complexes to documentation of the stained gels was repeated three times to ensure reproducibility.

Mass spectrometry (MS)

In-gel digestion of selected proteins was performed essentially as described previously.[4] The tryptic peptides were micro-purified using C18 stage tips (Proxeon, ThermoScientific). Nano-electrospray ionization MS/MS (nanoESI-MS/MS) analyses were performed on an EASY-nLC II system (ThermoScientific) connected to a TripleTOF 5600+ mass spectrometer (AB SCIEX) equipped with a NanoSpray III source (AB SCIEX) operated under Analyst TF 1.6 control. The trypsin-digested samples were suspended in 0.1% formic acid, injected, trapped and desalted isocratically on a precolumn (ReproSil-Pur C18-AQ 3 μm resin, Dr. Maisch GmbH, Germany). The peptides were eluted and separated on a 15 cm analytical column (75 μm i.d.), pulled in-house (P2000 laser puller, Sutter Instrument), and packed with ReproSil-Pur C18-AQ 3 μm resin (Dr. Maisch GmbH, Germany). Peptides were eluted from the analytical column at a flow rate of 250 nL/min using a 30 min gradient from 5% to 35% of solution B (0.1% formic acid, 100% acetonitrile). The collected MS files were converted to Mascot generic format (MGF) using the AB SCIEX MS Data Converter beta 1.1 (AB SCIEX) and the "protein pilot MGF" parameters. The generated peak lists were searched using an in-house Mascot search engine (Matrix Science). Search parameters were allowing one missed trypsin cleavage site and propionamide as a fixed modification with peptide tolerance and MS/MS tolerance set to 20 ppm and 0.4 Da, respectively.

Transmission electron microscopy (TEM)

The primary particle size distribution of the SiO_2 nanoparticles was examined under a Phillips CM20 transmission electron microscope operating at 200 keV. To establish a size distribution ($n \geq 1000$) from several TEM images across the grid, the scanning probe image software SPIP (Image Metrology, Denmark) was used. For imaging of the SiO_2 nanoparticle-protein complexes, the complexes were prepared as described above except that they were washed with MilliQ water (18.2 MΩ) instead of PBS for desalting purposes. A drop of the colloids was directly deposited onto an oxygen plasma-treated copper grid with a formvar/carbon membrane (Ted Pella, CA) and left for 1 minute. The excess liquid

was wipe-dried and a drop of freshly prepared uranyl acetate aqueous solution (1% w/v) was applied to stain the specimen. After 1 minute, the excess contrast agent solution was dried-off in a similar manner.

Nanoparticle tracking analysis (NTA)

The hydrodynamic size distribution of SiO_2 nanoparticle-protein complexes was assessed using a NanoSight LM10-HS (Malvern Instruments, UK) instrument with a laser wavelength of 405 nm. Prior to analysis each sample was diluted in sterile-filtered PBS (or MilliQ water for the pristine sample) in order to have a working concentration of ca. 10^8 nanoparticles/ml. Three videos of 90 seconds each were recorded and processed individually (NanoSight NTA 2.3 build 025). To avoid cross-contamination, the measurement cell was flushed with the diluent after each measurement. Signal-to-noise ratio was high enough to confidently differentiate light scattering of SiO_2 nanoparticles from that of proteins and, if any, protein aggregates.

Dynamic light scattering (DLS) and zeta potential analysis

Light scattering analysis of the SiO_2 nanoparticle-protein complexes was performed on a Malvern ZetasizerNano (Malvern Instruments, UK) with the laser wavelength of 633 nm. For DLS, a detection angle at 173° was used and the correlation function obtained was fitted to a multiple exponential model (CONTIN algorithm) using the Zetasizer Software 7.11 (Malvern Instruments, UK). In comparison to NTA, DLS allows sizing of SiO_2 nanoparticles at concentrations relevant for *in vitro* cell assays (50-200 μg/ml) in the presence of proteins provided that contribution of the proteins to light scattering is negligible. The SiO_2 nanoparticle-protein complexes were thus further studied following redispersion and 2 h additional incubation in complete cell culture medium (cCCM; details described below). For zeta potential measurements, the SiO_2 nanoparticle-protein complexes with or without the 2 h additional incubation in cCCM were desalted (pelleted and redispersed in MilliQ water) and the electrophoretic mobility was immediately analysed. For the calculation of zeta potentials the Henry equation was applied with the Smoluchowski approximation ($f(\kappa a) = 1.5$) using the Malvern's software.

Intact image of the gel used in Figure 1 (Fig. S1)

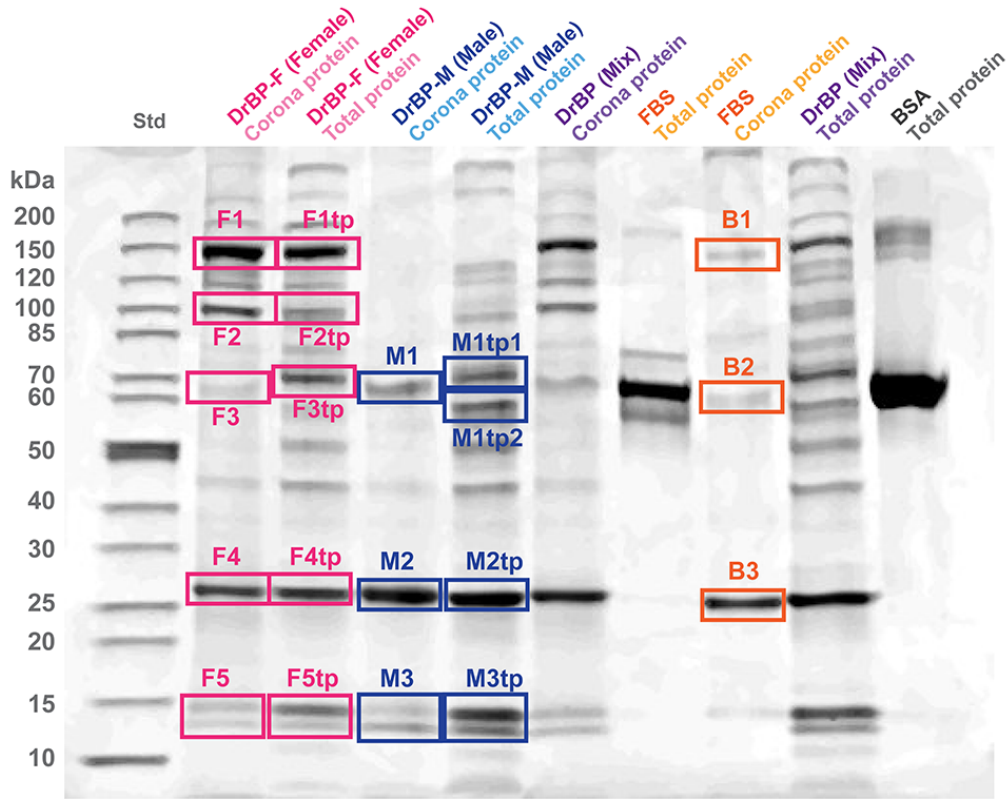

Figure S1. The intact image of the Coomassie Brilliant Blue-stained gel shown in Figure 1. Boxes denote the positions of each band excised for tandem mass spectrometry analysis. A unique band ID was assigned to each box. See Tables S1 & S2 for proteins identified for each band ID (Supplementary Tables).

Sequence coverage maps for vitellogenin 1 (Fig. S2)

F1 (120–150 kDa)

MRAVVLALTVALVACQQFNLVPEFAHDKTYVYK**YEALLLGGLPQEGLAR**AGIKVSSK**VLISATTENTYLMKLMDPLLYEYAGTWPKDPFVPATKLTSALAAQLQIPIKFEYANGVVGKVFAPAGVSPTVMNLHRGILNILQLNLK**
KTQNIYEMQEAGAQGVCR**THYVINEDPKANHIIVTK**SKDLSHCQER**IMKDVGLAYTER**CAECTERVKSLIETATYNYIMKPADNGALIAEATVEEVYQFSPFNEIHGAAMMEAK**QTLAFVEIEKTPVVPIKADYMPR**GSLQYEFA
TEILQTPIQLMK**ISDAPAQIVEVLKHLVSNNKDMVHDDAPFKFVQLVQLLR**VASLEK**IEAIWSQFK**DKPVYRR**WLLDALPAVGTPVIIK**FIKEKFLAGEFTTPEFIQTLVIALQMVTADPETIK**MTASLATHEKFATIPALREVV**
MLGYGSLIAKYCVAVPTCPAELLRPIHEIATEAISK**NDIPEITLALKVMGNAGHPSSLKPIMK**LLPGLR**TAANALPIRVQVDAILALR**NIAKKEPK**LVQPVALQLVLDRALHPEVR**MVACIVLFEAEPSVALVSSLAGALR**IEPN**
MHVASFAYSHIKSLTR**ITAPDMASVAGAANVAIK**LMSRKLDRLNYRYSRAFQMDYYYTPLMIGAAGSAYMINDAATILPRAVVAKAR**AYLAGAAADVIEFGVRTGGIHEALLKSPAADESADR**ITKIKRTLRALTNWK**ALPTDKP**
LASAYLKVFGQEVAYVNFDKTIIEEAIPMATGPKPRALLKEALKALQEGVAFQYAKPLLAAEVRRILPTAVGVPMEFSWYTAAVAAASVNVQATITPALPEK**LESMTYEQLKK**TDVQFQAEARPSVALQTFAVMGVNTAFIQAAV
MARGKIRTIAPGKVAARADILKGNYKVEALPVELPEHIASASFETYAVVR**NIEDHSAER**SVPLVPELSLQNSQASYAGDLSSEMSSVASVR**APAPFDR**TLCYAVPYIEIKGCVEVHSHNAAFIRNSTLFYIIGHHSVRAAVAR**AE**
GPAVERLEFEVQVGPRAAERLVK**QINIIDDDTPEGQAFLLKLREILDTEAK**NAPVSSESSSSRNSRSSSSRSTSTSTSSSSSSSSSSSSSSSSSMSSSRMSK**TATIIEPFRK**FHKDRYLAHHSATKDTSSGSAAASFEQMQKQNR
FLGNDIPPVFAIIARAVRADQK**LLGYQLAAYFDKPTARVQLIVSSIAENDNMK**ICADGALLSKHKVTGKFSWGAECK**QYAVFAKAEAGVLGEFPAARLEVEWERLPIIVTTYAK**KLGK**HILTAAYDTGFR**FER**ATNSEKEIELTA**
ALPSQRSLNIIARIPEITMSKRDIYLPVAVPINPDGTFSIETYEDFLAWIQKYIKEE

F2+F4 (overlaps in purple) (F1 sequence coverage map underlined)

MRAVVLALTVALVACQQFNLVPEFAHDKTYVYK**YEALLLGGLPQEGLAR**AGIKVSSK**VLISATTENTYLMKLMDPLLYEYAGTWPKDPFVPATKLTSALAAQLQIPIKFEYANGVVGKVFAPAGVSPTVMNLHRGILNILQLNLK**
KTQNIYEMQEAGAQGVCR**THYVINEDPKANHIIVTK**SKDLSHCQER**IMKDVGLAYTER**CAECTERVKSLIETATYNYIMKPADNGALIAEATVEEVYQFSPFNEIHGAAMMEAK**QTLAFVEIEKTPVVPIKADYMPR**GSLQYEFA
TEILQTPIQLMK**ISDAPAQIVEVLKHLVSNNKDMVHDDAPFKFVQLVQLLR**VASLEK**IEAIWSQFK**DKPVYRR**WLLDALPAVGTPVIIK**FIKEKFLAGEFTTPEFIQTLVIALQMVTADPETIK**MTASLATHEKFATIPALREVV**
MLGYGSLIAKYCVAVPTCPAELLRPIHEIATEAISK**NDIPEITLALKVMGNAGHPSSLKPIMK**LLPGLR**TAANALPIRVQVDAILALR**NIAKKEPK**LVQPVALQLVLDRALHPEVR**MVACIVLFEAEPSVALVSSLAGALR**IEPN**
MHVASFAYSHIKSLTR**ITAPDMASVAGAANVAIK**LMSRKLDRLNYRYSRAFQMDYYYTPLMIGAAGSAYMINDAATILPRAVVAKAR**AYLAGAAADVIEFGVRTGGIHEALLKSPAADESADR**ITKIKRTLRALTNWK**ALPTDKP**
LASAYLKVFGQEVAYVNFDKTIIEEAIPMATGPKPRALLKEALK**ALQEGVAFQYAKPLLAAEVR**RILPTAVGVPMEFSWYTAAVAAASVNVQATITPALPEK**LESMTYEQLKK**TDVQFQAEARPSVALQTFAVMGVNTAFIQAAV
MARGKIRTIAPGKVAARADILKGNYKVEALPVELPEHIASASFETYAVVR**NIEDHSAER**SVPLVPELSLQNSQASYAGDLSSEMSSVASVR**APAPFDR**TLCYAVPYIEIKGCVEVHSHNAAFIRNSTLFYIIGHHSVRAAVAR**AE**
GPAVERLEFEVQVGPRAAERLVKQINIIDDDTPEGQAFLLKLREILDTEAKNAPVSSESSSSRNSRSSSSRSTSTSTSSSSSSSSSSSSSSSSSMSSSRMSKTATIIEPFRKFHKDRYLAHHSATK**DTSSGSAAASFEQMQK**QNR
FLGNDIPPVFAIIARAVRADQK**LLGYQLAAYFDKPTARVQLIVSSIAENDNMK**ICADGALLSKHKVTGKFSWGAECK**QYAVFAKAEAGVLGEFPAARLEVEWERLPIIVTTYAK**KLGK**HILTAAYDTGFR**FER**ATNSEKEIELTA**
ALPSQRSLNIIARIPEITMSKRDIYLPVAVPINPDGTFSIETYEDFLAWIQKYIKEE

F2 (85–100 kDa)

MRAVVLALTVALVACQQFNLVPEFAHDKTYVYK**YEALLLGGLPQEGLAR**AGIKVSSK**VLISATTENTYLMKLMDPLLYEYAGTWPKDPFVPATKLTSALAAQLQIPIKFEYANGVVGKVFAPAGVSPTVMNLHRGILNILQLNLK**
KTQNIYEMQEAGAQGVCR**THYVINEDPKANHIIVTK**SKDLSHCQER**IMKDVGLAYTER**CAECTERVKSLIETATYNYIMKPADNGALIAEATVEEVYQFSPFNEIHGAAMMEAK**QTLAFVEIEKTPVVPIKADYMPR**GSLQYEFA
TEILQTPIQLMK**ISDAPAQIVEVLKHLVSNNKDMVHDDAPFKFVQLVQLLR**VASLEK**IEAIWSQFK**DKPVYRR**WLLDALPAVGTPVIIK**FIKEKFLAGEFTTPEFIQTLVIALQMVTADPETIK**MTASLATHEKFATIPALREVV**
MLGYGSLIAKYCVAVPTCPAELLRPIHEIATEAISK**NDIPEITLALKVMGNAGHPSSLKPIMK**LLPGLR**TAANALPIRVQVDAILALR**NIAKKEPK**LVQPVALQLVLDRALHPEVR**MVACIVLFEAEPSVALVSSLAGALR**IEPN**
MHVASFAYSHIKSLTR**ITAPDMASVAGAANVAIK**LMSRKLDRLNYRYSRAFQMDYYYTPLMIGAAGSAYMINDAATILPRAVVAKAR**AYLAGAAADVIEFGVRTGGIHEALLKSPAADESADR**ITKIKRTLRALTNWK**ALPTDKP**
LASAYLKVFGQEVAYVNFDKTIIEEAIPMATGPKPRALLKEALK**ALQEGVAFQYAKPLLAAEVR**RILPTAVGVPMEFSWYTAAVAAASVNVQATITPALPEK**LESMTYEQLKK**TDVQFQAEARPSVALQTFAVMGVNTAFIQAAV
MARGKIRTIAPGKVAARADILKGNYKVEALPVELPEHIASASFETYAVVR**NIEDHSAER**SVPLVPELSLQNSQASYAGDLSSEMSSVASVR**APAPFDR**TLCYAVPYIEIKGCVEVHSHNAAFIRNSTLFYIIGHHSVRAAVAR**AE**
GPAVERLEFEVQVGPRAAERLVKQINIIDDDTPEGQAFLLKLREILDTEAKNAPVSSESSSSRNSRSSSSRSTSTSTSSSSSSSSSSSSSSSSSMSSSRMSKTATIIEPFRKFHKDRYLAHHSATKDTSSGSAAASFEQMQKQNR
FLGNDIPPVFAIIARAVRADQK**LLGYQLAAYFDKPTAR**VQLIVSSIAENDNMKICADGALLSKHKVTGKFSWGAECKQYAVFAKAEAGVLGEFPAAR**LEVEWERLPIIVTTYAK**KLGKHILTAAYDTGFRFERATNSEKEIELTA
ALPSQRSLNIIAR**IPEITMSK**RDIYLPVAVPINPDGTFSIETYEDFLAWIQKYIKEE

F4 (25–30 kDa)

MRAVVLALTVALVACQQFNLVPEFAHDKTYVYKYEALLLGGLPQEGLARAGIKVSSKVLISATTENTYLMKLMDPLLYEYAGTWPKDPFVPATKLTSALAAQLQIPIKFEYANGVVGKVFAPAGVSPTVMNLHRGILNILQLNLK
KTQNIYEMQEAGAQGVCR**THYVINEDPK**ANHIIVTKSKDLSHCQERIMKDVGLAYTERCAECTERVKSLIETATYNYIMKPADNGALIAEATVEEVYQFSPFNEIHGAAMMEAKQTLAFVEIEK**TPVVPIK**ADYMPRGSLQYEFA
TEILQTPIQLMKISDAPAQIVEVLKHLVSNNKDMVHDDAPFKFVQLVQLLRVASLEKIEAIWSQFKDKPVYRRWLLDALPAVGTPVIIKFIKEKFLAGEFTTPEFIQTLVIALQMVTADPETIKMTASLATHEKFATIPALREVV
MLGYGSLIAKYCVAVPTCPAELLRPIHEIATEAISKNDIPEITLALK**VMGNAGHPSSLKPIMK**LLPGLR**TAANALPIRVQVDAILALR**NIAKKEPKLVQPVALQLVLDRALHPEVRMVACIVLFEAEPSVALVSSLAGALRIEPN
MHVASFAYSHIKSLTRITAPDMASVAGAANVAIKLMSRKLDRLNYRYSRAFQMDYYYTPLMIGAAGSAYMINDAATILPRAVVAKARAYLAGAAADVIEFGVRTGGIHEALLKSPAADESADRITKIKRTLRALTNWKALPTDKP
LASAYLKVFGQEVAYVNFDKTIIEEAIPMATGPKPRALLKEALKALQEGVAFQYAKPLLAAEVRRILPTAVGVPMEFSWYTAAVAAASVNVQATITPALPEK**LESMTYEQLKK**TDVQFQAEARPSVALQTFAVMGVNTAFIQAAV
MARGKIRTIAPGKVAARADILKGNYKVEALPVELPEHIASASFETYAVVRNIEDHSAERSVPLVPELSLQNSQASYAGDLSSEMSSVASVR**APAPFDR**TLCYAVPYIEIKGCVEVHSHNAAFIRNSTLFYIIGHHSVRAAVARAE
GPAVERLEFEVQVGPRAAERLVKQINIIDDDTPEGQAFLLKLREILDTEAKNAPVSSESSSSRNSRSSSSRSTSTSTSSSSSSSSSSSSSSSSSMSSSRMSKTATIIEPFRKFHKDRYLAHHSATK**DTSSGSAAASFEQMQK**QNR
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ALPSQRSLNIIARIPEITMSKRDIYLPVAVPINPDGTFSIETYEDFLAWIQKYIKEE

Figure S2. Sequence coverage maps for vitellogenin 1 (Q1LWN2) identified by tandem mass spectrometry. See Figure S1 for the band positions of F1, F2 and F4. The F1 band corresponds to the theoretical molecular weight of full-length vitellogenin 1 (148 kDa), F2 the cleaved product lipovitellin heavy chain (114 kDa), and F4 another cleaved product lipovitellin light chain (26 kDa). See the review by Finn (2007)[7] for details of the molecular mass prediction for vitellogenins.

TEM image of pristine SiO_2 nanoparticles (Fig. S3)

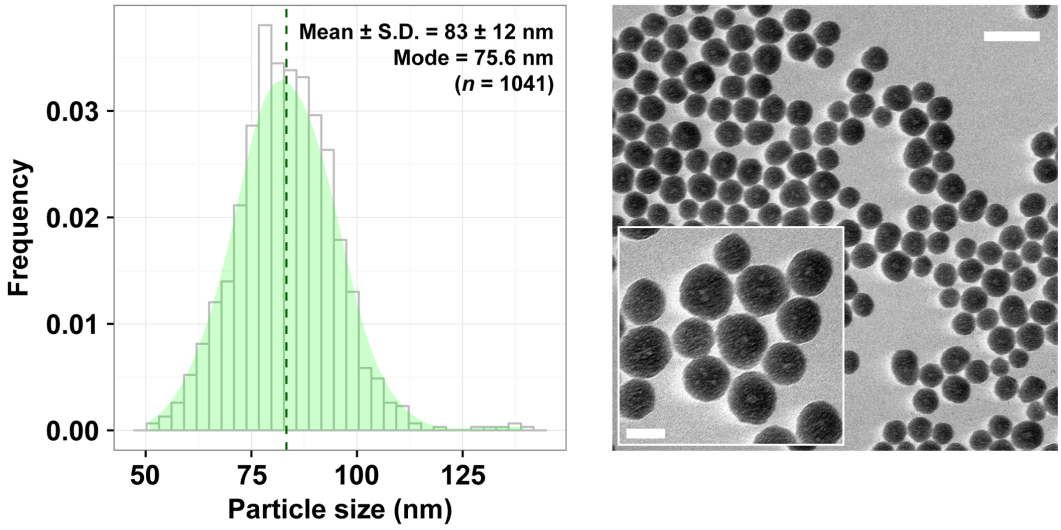

Figure S3. Electron micrographs of SiO_2 nanoparticles. A TEM image of pristine nanoparticles with associated size statistics. Scale bars = 200 nm and 50 nm (inset).

Colloidal stability under the exposure condition (Fig. S4)

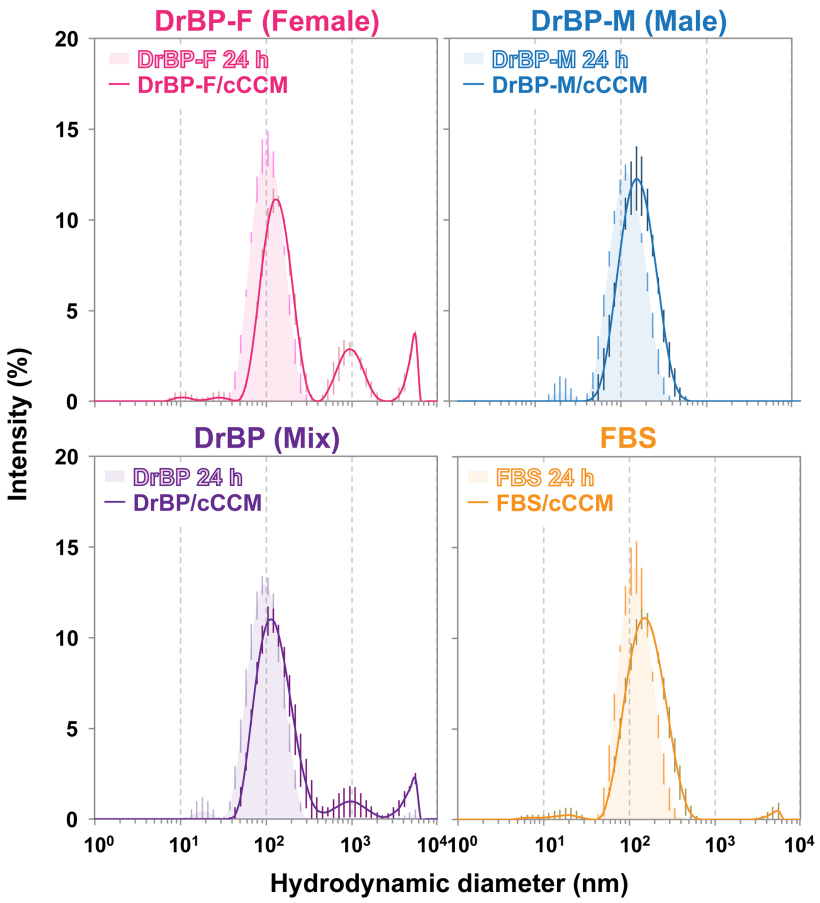

Figure S4. Colloidal stability of SiO_2 nanoparticle-protein complexes under the exposure condition (2 h incubation at 26°C in cCCM). The filled histograms and solid lines show the size distribution of the complexes before and after the 2 h incubation, respectively. Note that shown are light scatter intensity-based size distributions; the minor peaks in the μm range are negligible in volume or number. Mean values of three measurements are plotted, and the error bars represent standard deviations. cCCM, complete cell culture medium (containing 5% FBS).

Protein corona profiles under the exposure condition (Fig. S5)

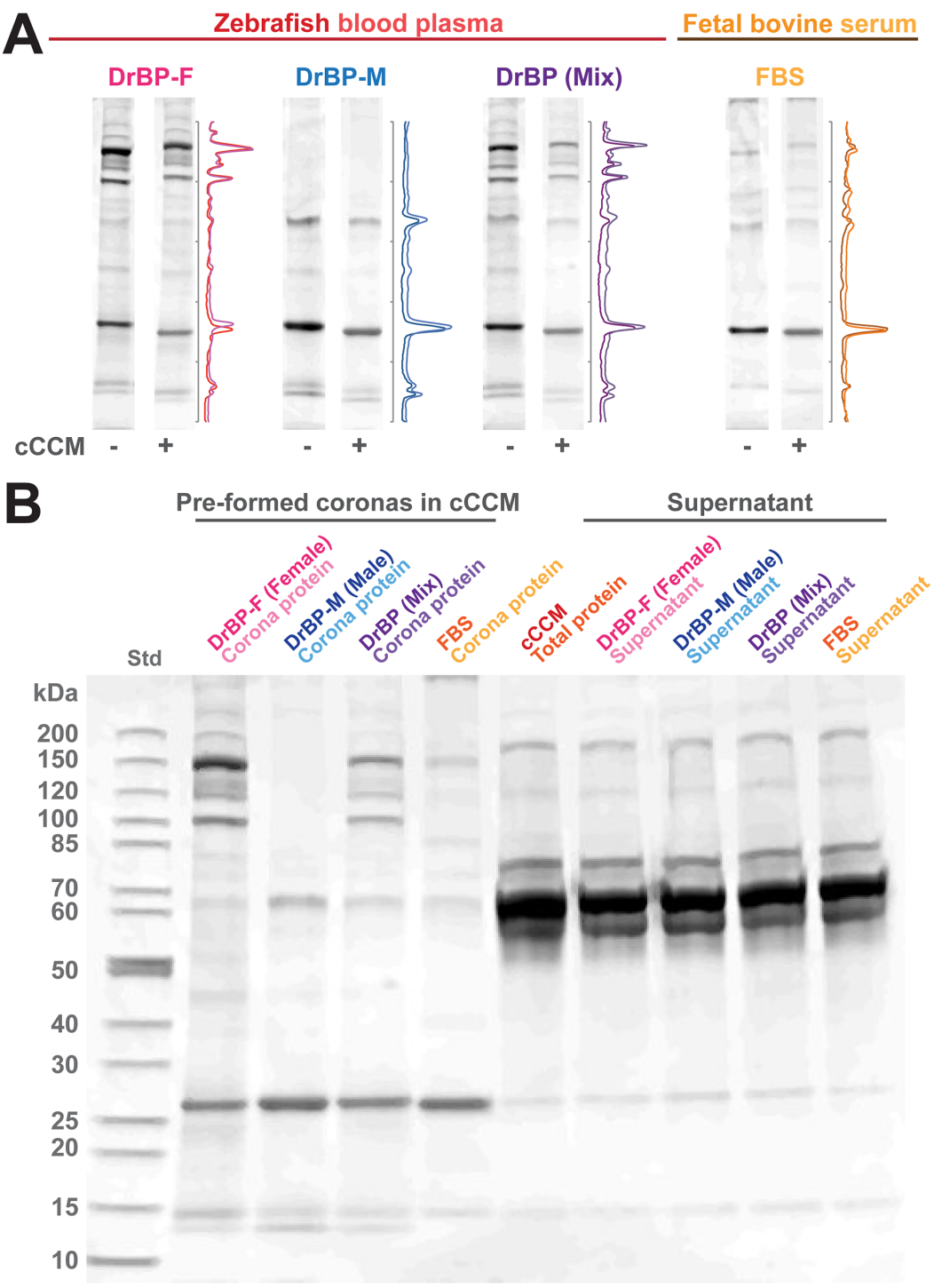

Figure S5. Protein corona profiles under the exposure condition (2 h incubation at 26°C in cCCM). (A) Side-by-side comparisons of the protein patterns before (left) and after (right) the 2 h incubation of the SiO_2

nanoparticle-protein complexes in cCCM. Note that each set of the two lanes derives from two representative gels. The associated spectra in solid lines are intensity profiles normalized to the molecular weight standard in each gel, allowing direct comparisons of the run length and intensity. Intact gel images can be found in Figure S1 (left lanes) and the panel B of this figure. (B) The intact image of the gel, some lanes of which appeared in (A). The remaining lanes show the profiles of free proteins in the supernatant. A representative gel of three independent experiments is shown.

FITC intensity distribution profile (Fig. S6)

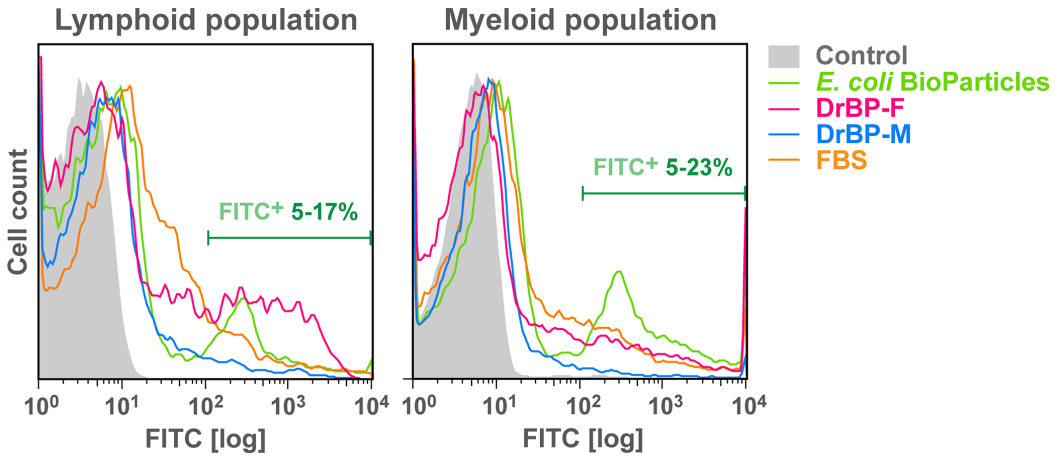

Figure S6. Representative profiles of the FITC fluorescence intensity distribution analysed by flow cytometry. Filled histograms and solid lines represent the control and the treatments (50 μg/ml), respectively. The increases of the cell count in the right tail of the peaks indicate that, during the 2 h exposure, only a minor fraction of the WKM cells accumulated BioParticles or SiO_2 nanoparticle-protein complexes to a remarkable extent. Some cells in the myeloid population, in particular in the DrBP-F treatment, had fluorescence intensity higher than the upper threshold; those cell counts were stacked at the end of the tail at the arbitrary fluorescence unit of 10^4.

Cellular accumulation of SiO_2 nanoparticles, non-pooled (Fig. S7)

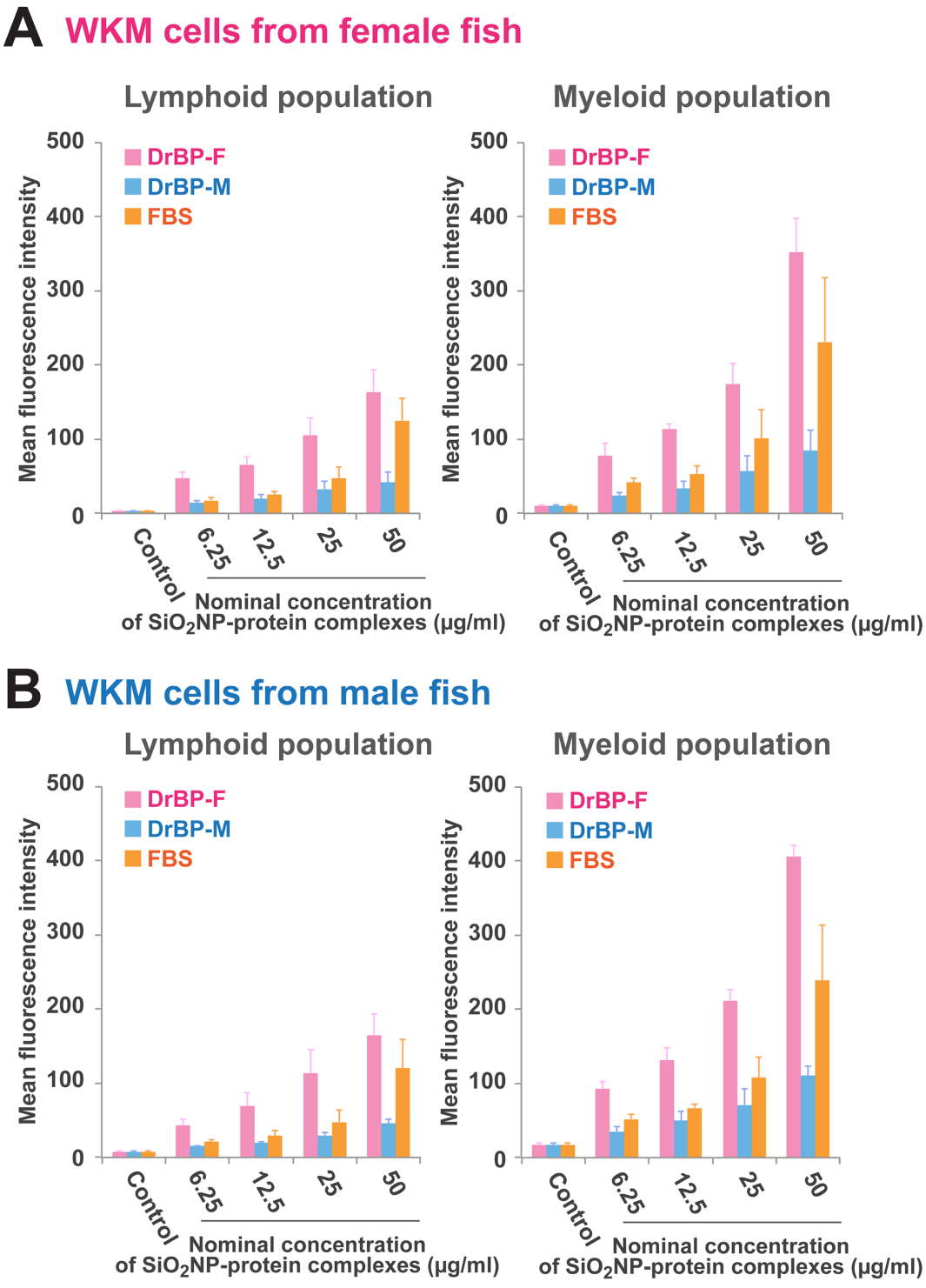

Figure S7. Cellular accumulation of SiO_2 nanoparticle-protein complexes pre-formed with three

different protein corona types (a non-pooled version of Figure 4). Results for WKM cells harvested from (A) female fish and (B) male fish are shown. Values are mean ± SE of three independent assays. No significant effect of the interaction of sex and the treatments (at 50 μg/ml) was observed for both of lymphoid and myeloid populations (Two-way ANOVA, $p = 0.795$ for lymphoid and $p = 0.870$ for myeloid).

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Table S1. List of *Danio rerio* blood plasma proteins identified in the hard corona around 70 nm SiO_2 nanoparticles.

Protein source: DrBP-F (*Danio rerio* blood plasma, female)

band ID	Mw range (kDa)	gene/Protein name	accession #	Mw[a] (kDa)	pI[a]	GRAVY score[a]	Mascot score	
							corona protein	total protein
F1 (tp)	120 – 150	vitellogenin 1	Q1LWN2	149	8.74	0.019	3530	7338
		vitellogenin 4	F1Q7L0	149	8.92	-0.009	3429	6247
		vitellogenin 6	F1QV15	150	8.83	-0.016	3183	6332
		vitellogenin 5	F1R2S5	149	8.84	-0.012	2890	6257
		vitellogenin 7	A3KMS4	149	8.76	0.009	2524	5258
F2 (tp)	85 – 100	vitellogenin 1	Q1LWN2	149	8.74	0.019	1025	3654
		vitellogenin 6	F1QV15	150	8.83	-0.016	867	3067
		vitellogenin 4	E9QFD8	149	8.86	0.014	813	3048
		vitellogenin 5	F1R2S5	149	8.84	-0.012	787	2741
		vitellogenin 7	A3KMS4	149	8.76	0.009	658	1943
		vitellogenin 2	Q1MTC4	180	8.70	-0.060	435	1618
F3 (tp)	60 – 70	vitellogenin 1	Q90YN8	150	8.68	0.029	347	1371
		alpha-2-HS-glycoprotein 1 (fetuin)	Q5U3D8	51	7.13	-1.116	220	-
F4 (tp)	25 – 30	apolipoprotein A-I b	E7FES0	30	6.04	-0.647	2636	3384
		apolipoprotein A-I a	O42363	30	5.06	-0.615	1915	3427
		vitellogenin 1	Q1LWN2	149	8.74	0.019	706	506
F5 (tp)	12 – 15	Haemoglobin subunit beta-2	Q90485	16	7.70	0.056	844	1161
		Haemoglobin subunit beta-1	Q90486	16	7.70	0.048	815	1110
		apolipoprotein A-II	B3DFP9	16	6.59	0.070	803	722
		Novel protein similar to zebrafish haemoglobin alpha-adult 1	Q6ZM17	16	8.81	0.131	569	-
		novel beta globin*	Q6DGK4	16	8.92	0.046	478	228
		haemoglobin, alpha adult 1	Q803Z5	16	7.97	0.170	464	1074
		haemoglobin, alpha adult 2	Q7SZV9	16	8.81	0.148	458	1001
		vitellogenin 5	F1R2S5	149	8.84	-0.012	328	-
		haemoglobin subunit alpha*	Q5BJC7	15	9.16	0.172	327	336
		vitellogenin 1	Q1LWN2	149	8.74	0.019	322	-
		apolipoprotein A-I b	E7FES0	30	6.04	-0.647	218	-

Protein source: DrBP-M (*Danio rerio* blood plasma, male)

band ID	Mw range (kDa)	gene/Protein name	accession #	Mw[a] (kDa)	pI[a]	GRAVY score[a]	Mascot score	
							corona protein	total protein
M1	60 – 70	alpha-2-HS-glycoprotein 1 (fetuin)	Q5U3D8	51	7.13	-1.116	753	-
		alpha-2-HS-glycoprotein 1 (fetuin)	E7FF41	51	7.01	-1.131	509	-
		Haemoglobin subunit beta-2	Q90485	16	7.70	0.056	333	-
M2 (tp)	25 – 30	apolipoprotein A-I b	E7FES0	30	6.04	-0.647	5157	4518
		apolipoprotein A-I a	O42363	30	5.06	-0.615	2161	3556
M3 (tp)	12 – 15	apolipoprotein A-II	B3DFP9	16	6.59	0.070	1052	1708
		Haemoglobin subunit beta-2	Q90485	16	7.70	0.056	617	1956
		Haemoglobin subunit alpha	Q90487	16	7.97	0.172	491	1326
		haemoglobin, alpha adult 2	Q7SZV9	16	8.81	0.148	392	1486
		novel beta globin*	Q6DGK4	16	8.92	0.046	370	494
		haemoglobin subunit alpha*	Q5BJC7	15	9.16	0.172	274	785
		histone h2b*	R4GE02	27	10.17	-0.594	211	-

Protein source: FBS (Fetal bovine serum)

band ID	Mw range (kDa)	gene/Protein name	accession #	Mw[a] (kDa)	pI[a]	GRAVY score[a]	Mascot score	
							corona protein	total protein
B1	120 – 150	Thrombospondin-1	F1N3A1	130	4.72	-0.717	844	n.a.
		Complement factor H	Q28085	140	6.43	-0.651	473	n.a.
B2	60 – 70	Serum albumin	B0JYQ0	69	5.95	-0.432	1499	n.a.
		Serum albumin	P02769	69	5.82	-0.429	1414	n.a.
		Complement component 3	A0A0F6QNP7	187	6.46	-0.349	948	n.a.
		Alpha-1-antiproteinase	P34955	46	6.05	-0.056	766	n.a.
		Apolipoprotein A-I	P15497	30	5.71	-0.619	547	n.a.
B3	25 – 30	Apolipoprotein A-I	P15497	30	5.71	-0.619	5450	n.a.

GRAVY, Grand average of hydropathy.
* Annotated based on the BLAST (blastp) hits.
[a] Before post-translational modification, computed using SIB Bioinformatics Resource Portal (ExPASy) from the complete amino acid sequence.

Table S2. List of *Danio rerio* blood plasma proteins identified in the protein source but not in the hard corona.

Protein source: DrBP- F (*Danio rerio* blood plasma, female)

band ID	Mw range (kDa)	gene/Protein name	accession #	Mw[a] (kDa)	pI[a]	GRAVY score[a]	Mascot score	
							corona protein	total protein
F2tp	85 – 100	alpha 2-macroglobulin*	F1QQY9	128	5.36	-0.097	-	1610
		alpha 2-macroglobulin*	X1WC44	125	5.28	-0.100	-	1567
		alpha 2-macroglobulin-like*	F1R8N2	160	5.38	-0.063	-	1466
		alpha 2-macroglobulin-like	A0JMP8	160	5.30	-0.068	-	1423
		alpha 2-macroglobulin-like*	X1WBT0	160	5.94	-0.130	-	1179
F3tp	60 – 70	Serotransferrin	F1R858	74	6.61	-0.344	-	4635
		complement component c3a, duplicate 2	F1QV29	185	6.42	-0.333	-	1944
		complement component c3a, duplicate 1	B8JKW4	183	6.17	-0.320	-	1657
		vitellogenin 5	F1R2S5	149	8.84	-0.012	-	1229
		complement component c3a, duplicate 3	Q3MU73	185	6.48	-0.312	-	1076
		complement component c3a, duplicate 3	F1QX13	185	6.49	-0.322	-	970
		complement component 5	F1R0S4	118	7.95	-0.177	-	967
		vitellogenin 7	A3KMS4	149	8.76	0.009	-	934
F4tp	25 – 30	apolipoprotein A-IV b, tandem duplicate 1	F1QHR0	30	4.77	-0.625	-	1859
		apolipoprotein A-IV b, tandem duplicate 2	B3DHC5	29	4.78	-0.630	-	1732
		apolipoprotein A-IV b, tandem duplicate 3	F1QJD1	29	4.76	-0.589	-	1526
F5tp	10 – 15	Actin, cytoplasmic 1	Q7ZVI7	42	5.30	-0.213	-	1024
		actin, alpha 1a, skeletal muscle	F1QUN8	42	5.22	-0.227		690
		Histone H4	Q0D294	11	11.36	-0.574	-	289

Protein source: DrBP- M (*Danio rerio* blood plasma, male)

band ID	Mw range (kDa)	gene/Protein name	accession #	Mw[a] (kDa)	pI[a]	GRAVY score[a]	Mascot score	
							corona protein	total protein
M1tp1	60 – 70	Serotransferrin	F1R858	74	6.61	-0.344	-	4973
		complement component c3a, duplicate 2	F1QV29	185	6.42	-0.333	-	2492
		complement component c3a, duplicate 1	B8JKW4	183	6.17	-0.320	-	1838
		complement component c3a, duplicate 3	Q3MU73	185	6.48	-0.312	-	1540
		Carboxylic ester hydrolase	F1R9X5	60	5.49	-0.056	-	1289
		Carboxylic ester hydrolase	Q1LYL6	61	5.43	-0.041	-	1076
		complement component 5	F1R0S4	118	7.95	-0.177	-	1037
M1tp2	60 – 70	Hemopexin	Q6PHG2	51	6.14	-0.522	-	4245
		Serpin peptidase inhibitor, clade A, member 7	Q5XJ64	43	5.16	-0.340	-	1489
		Fibrinogen, B beta polypeptide	Q6NYE1	54	8.07	-0.679	-	1101
		apoliporotein Ba	E7FBD3	496	5.44	-0.216	-	1062
		serpin peptidase inhibitor, clade A, member 7	A8E5C1	43	5.16	-0.351	-	933
M2tp	25 – 30	apolipoprotein A-IV b, tandem duplicate 1	F1QHR0	30	4.77	-0.625	-	1888
		apolipoprotein A-IV b, tandem duplicate 2	B3DHC5	29	4.78	-0.630	-	1696
		apolipoprotein A-IV b, tandem duplicate 3	F1QJD1	29	4.76	-0.589	-	1515
M3tp	10 – 15	Haemoglobin subunit beta-1	Q90486	16	7.70	0.048	-	1905
		Myoglobin	Q6VN46	16	6.96	0.086	-	387

GRAVY, Grand average of hydropathy.
***** Annotated based on the BLAST (blastp) hits.
[a] Before post-translational modification, computed using SIB Bioinformatics Resource Portal (ExPASy) from the complete amino acid sequence.