

Figure S1. Size distribution of A12 and R20 in water and exposure medium.

Hydrodynamic diameter, as measured by dynamic light scattering (DLS), on A12 and R20 NPs dispersed in water (A12_water and R20_water) or dispersed in water and then diluted in cell culture medium (A12_medium and R20_medium).

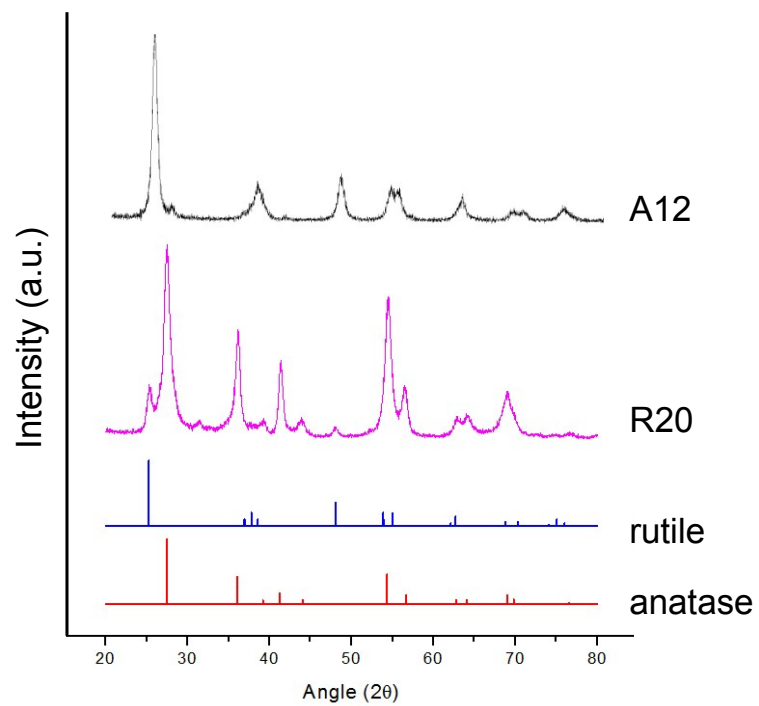


Figure S2. Experimental X-ray powder diffraction patterns of A12 and R20 nanoparticles.

Characteristic peaks of anatase and rutile are depicted in red and blue, respectively; patterns obtained on A12 and R20 are depicted in black and pink, respectively.

Table S1 (1/2). Composition of the protein corona that forms on A12^a

Protein name	Protein accession numbers	nr. unique pept.	nr. unique spectra	nr. total spectra	% sequ. cov.
Complement C3	sp Q2UVX4	48	64	69	35.6%
Serum albumin	sp P02769	26	36	48	41.7%
probable Complement component 4	tr E1BH06	24	30	30	19.7%
Apolipoprotein A-I	sp P15497	16	25	32	60.4%
Hemoglobin fetal subunit beta	sp P02081	11	17	23	84.8%
Alpha-2-HS-glycoprotein	sp P12763, tr B0JYN6	11	18	25	44.8%
Antithrombin-III	tr F1MSZ6	11	12	13	31.4%
Serotransferrin	sp Q29443, tr G3X6N3	10	11	11	17.5%
Complement component C9	sp Q3MHN2	9	9	10	18.8%
Thrombospondin-1	sp Q28178	7	7	7	7.18%
Alpha-fetoprotein	sp Q3SZ57	7	8	8	14.9%
Pigment epithelium-derived factor	sp Q95121	7	8	8	27.4%
Coagulation factor V	sp Q28107	5	5	5	2.67%
Hemoglobin subunit alpha	sp P01966	5	5	6	2.94%
Alpha-1-antiproteinase	sp P34955	5	5	5	16.1%
Collagen alpha-1(I) chain	sp P02453	4	5	6	2.94%
Prothrombin	sp P00735	4	4	4	8.00%
Apolipoprotein E	sp Q03247, tr Q0ZCB4	4	5	5	16.5%
Kininogen-1	sp P01044	4	4	4	8.53%
Complement Factor I	tr F1N4M7, tr Q32PI4	4	7	7	8.25%
Collagen alpha-2(I) chain	sp P02465	3	4	4	2.71%
Tetranectin	sp Q2KIS7	3	5	5	22.8%
probable Complement component 4	tr F1MVK1	3	3	3	11.1%
SERPING1 protein	tr E1BMJ0	3	3	3	11.1%
Complement C5a anaphylatoxin	tr F1MY85	3	3	3	2.44%

Table S1 (2/2). Composition of the protein corona that forms on A12^a

Protein name	Protein accession nr.	nr. unique pept.	nr. unique spectra	nr. total spectra	% sequ. cov.
Insulin-like growth factor-binding protein	sp P13384	3	3	3	8.83%
Vitamin D-binding protein	sp Q3MHN5	3	3	3	6.96%
Adiponectin	sp Q3Y5Z3	3	5	5	15.4%
Alpha-enolase	sp Q9XSJ4, tr F1MB08	3	4	4	8.76%
ALB protein	tr B0JYQ0	3	6	6	41.7%
Complement Factor I	Q32PI4	3	6	6	6.15%
Coagulation factor XIII, B polypeptide	Q2TBQ1	3	3	3	7.11%
Coagulation factor XI	sp Q5NTB3, tr F1MUT4, tr Q6PZ62, tr Q6Q0I7	2	2	2	3.52%
putative tropomyosin alpha	tr A6QR15	2	3	3	3.87%
Histidine-rich glycoprotein	tr F1MKS5, tr Q9BGU1	2	3	3	5.22%
Collagen alpha-1(XI) chain	tr F1N0K0	2	2	2	2.37%
Actin, cytoplasmic 1	sp P60712, sp P63258, tr F1MRD0	2	2	2	6.93%
SERPIND1 protein	tr A6QPP2	2	2	2	4.44%
Coagulation factor X	sp P00743, tr Q3MHW2	2	2	2	4.07%
Apolipoprotein A-II	sp P81644	2	2	2	26.0%
Plasma kallikrein	sp Q2KJ63	2	2	2	3.62%
Elongation factor 1-alpha	Q862F6	2	2	2	5.19%

^aProtein corona was analyzed by nano LC/MS-MS, peptides were identified by database searching using MascotTM algorithm, using all proteins annotated for *Bos taurus* (Taxonomy ID 9913) in UniprotKB, their decoy equivalents and common contaminants (UniProtKB release of 2013-05-01, 63412 target+decoy entries). Nr.: number; pept.: peptide; sequ.: sequence; cov.: coverage.

Table S2 (1/2). Composition of the protein corona that forms on R20^a

Protein name	Protein accession nr.	nr. unique pept.	nr. unique spectra	nr. total spectra	% sequ. cov.
Apolipoprotein A-I	sp P15497	13	17	20	54.3%
Complement C3	sp Q2UVX4	12	15	15	9.27%
Serum albumin	sp P02769	19	25	30	30.6%
probable Complement component 4	tr E1BH06	10	11	11	8.27%
BOVIN Hemoglobin fetal subunit beta	sp P02081	10	11	15	84.1%
Alpha-2-HS-glycoprotein	sp P12763, tr B0JYN6	10	13	15	39.8%
Antithrombin-III	tr F1MSZ6	6	8	8	13.3%
Serotransferrin	sp Q29443, tr G3X6N3	6	6	6	9.52%
Complement component C9	sp Q3MHN2	5	6	6	10.2%
Thrombospondin-1	sp Q28178, tr F1N3A1, tr Q28194	6	6	6	6.15%
Alpha-fetoprotein	sp Q3SZ57	3	4	4	6.56%
Coagulation factor V	sp Q28107	4	4	4	2.26%
Hemoglobin subunit alpha	sp P01966	5	9	11	47.2%
Alpha-1-antiproteinase	sp P34955	5	6	6	14.2%
Complement Factor I	tr F1N4M7, tr Q32PI4	3	3	3	6.15%
Collagen alpha-2(I) chain	sp P02465	2	2	2	1.54%
Tetranectin	sp Q2KIS7	4	6	6	27.2%
Vitamin D-binding protein	tr F1N5M2, tr I7CT57	3	5	5	7.38%
Adiponectin	sp Q3Y5Z3	3	4	4	15.4%
ALB protein	tr B0JYQ0	3	5	8	36.1%
Histidine-rich glycoprotein	tr F1MKS5, tr Q9BGU1	2	3	3	5.22%
Actin, cytoplasmic 1	sp P60712, sp P63258, tr F1MRD0	4	4	4	11.5%
Beta-2-glycoprotein 1	sp P17690	4	5	5	15.1%
Gelsolin	sp Q3SX14, tr F1MJH1, tr F1N1I6	3	3	3	4.92%
Alpha-enolase	sp Q9XSJ4, tr F1MB08	2	3	3	5.76%
Apolipoprotein E	Q0ZCB4	2	3	3	7.91%
Apolipoprotein A-II	sp P81644	2	2	2	26.0%
Pigment epithelium-derived factor	sp Q95121	2	2	2	10.1%
Fibrinogen alpha chain	sp P02672, tr A5PJE3	2	2	2	3.74%
Homogentisate 1,2-dioxygenase	tr B8YB76, tr F1MLF8, tr Q2KIH3	2	2	2	4.27%
Transthyretin	sp O46375	2	2	2	24.5%

Table S2 (2/2). Composition of the protein corona that forms on R20^a

Protein name	Protein accession nr.	nr. unique pept.	nr. unique spectra	nr. total spectra	% sequ. cov.
Plasma kallikrein	sp Q2KJ63	2	2	2	3.77%
Tropomyosin beta chain	sp Q5KR48, tr A6QR15	2	2	2	4.93%
Procollagen C-endopeptidase enhancer	tr Q2HJB6	2	2	2	6.21%
Kininogen-1	sp P01044, tr F1MNV5	2	2	2	3.90%

^aProtein corona was analyzed by nano LC/MS-MS, peptides were identified by database searching using MascotTM algorithm, using all proteins annotated for *Bos taurus* (Taxonomy ID 9913) in UniprotKB, their decoy equivalents and common contaminants (UniProtKB release of 2013-05-01, 63412 target+decoy entries). Nr.: number; pept.: peptide; sequ.: sequence; cov.: coverage.

Table S3. Proteins present in the corona formed on A12 but not on the corona formed on R20^a

Protein name	Protein accession nr.	nr. unique pept.	nr. unique spectra	nr. total spectra	% sequ. cov.
Collagen alpha-1(I) chain	sp P02453	4	5	6	2.94%
Prothrombin	sp P00735	4	4	4	8.00%
SERPING1 protein	tr E1BMJ0	3	3	3	11.1%
Complement C5a anaphylatoxin	tr F1MY85	3	3	3	2.44%
Insulin-like growth factor-binding protein	sp P13384	3	3	3	8.83%
Complement Factor I	Q32PI4	3	6	6	6.15%
Coagulation factor XIII, B polypeptide	Q2TBQ1	3	3	3	7.11%
Coagulation factor XI	sp Q5NTB3, tr F1MUT4, tr Q6PZ62, tr Q6Q017	2	2	2	3.52%
putative tropomyosin alpha	tr A6QR15	2	3	3	3.87%
Collagen alpha-1(XI) chain	tr F1N0K0	2	2	2	2.37%
SERPIND1 protein	tr A6QPP2	2	2	2	4.44%
Coagulation factor X	sp P00743, tr Q3MHW2	2	2	2	4.07%
Elongation factor 1-alpha	Q862F6	2	2	2	5.19%

^aProtein corona was analyzed by nano LC/MS-MS, peptides were identified by database searching using MascotTM algorithm, using all proteins annotated for *Bos taurus* (Taxonomy ID 9913) in UniprotKB, their decoy equivalents and common contaminants (UniProtKB release of 2013-05-01, 63412 target+decoy entries). Nr.: number; pept.: peptide; sequ.: sequence; cov.: coverage.

Table S4. Proteins present in the corona formed on R20 but not on the corona formed on A12^a

Protein name	Protein accession nr.	nr. unique pept.	nr. unique spectra	nr. total spectra	% sequ. cov.
Beta-2-glycoprotein 1	sp P17690	4	5	5	15.1%
Gelsolin	sp Q3SX14, tr F1MJH1, tr F1N1I6	3	3	3	4.92%
Fibrinogen alpha chain	sp P02672, tr A5PJE3	2	2	2	3.74%
Homogentisate 1,2-dioxygenase	tr B8YB76, tr F1MLF8, tr Q2KIH3	2	2	2	4.27%
Transthyretin	sp O46375	2	2	2	24.5%
Tropomyosin beta chain	sp Q5KR48, tr A6QR15	2	2	2	4.93%
Procollagen C-endopeptidase enhancer	tr Q2HJB6	2	2	2	6.21%

^aProtein corona was analyzed by nano LC/MS-MS, peptides were identified by database searching using MascotTM algorithm, using all proteins annotated for *Bos taurus* (Taxonomy ID 9913) in UniprotKB, their decoy equivalents and common contaminants (UniProtKB release of 2013-05-01, 63412 target+decoy entries). Nr.: number; pept.: peptide; sequ.: sequence; cov.: coverage.

Method for protein corona identification

Protein corona identification

Protein elution and electrophoretic in gel concentration

One microgram of TiO₂-NP water-suspension was incubated for 1 h at 37°C in 2 mL of DMEM+10% FBS, then washed three times for 5 min at 4°C with 1 mL of 10 mM HEPES buffer, on a rotating wheel, followed by centrifugation at 15,000g for 20 minutes. This washed TiO₂-NP pellet containing the adsorbed proteins was incubated for one hour on a rotating wheel with 125 µl of elution buffer (8M urea, 50mM DTT, 100mM Tris-HCl, pH 7.5) in order to desorb the proteins adsorbed on the surface of NPs. The suspension was then centrifuged at 15,000g for 20 minutes, the supernatant was stored and its protein concentration determined with a Bradford assay. SDS (2% final concentration) and tracking dyes (bromophenol blue and pyronin Y) were then added. Ten micrograms of proteins were loaded for each sample on a SDS gel (10 % acrylamide, 15x15x0.15 cm) on which a 5 cm long stacking gel was cast (5 % acrylamide in Tris HCl 125 mM pH 7.5). The samples were loaded on alternate lanes to avoid cross contamination, and the empty lanes were loaded with an equivalent amount of sample buffer, but using bromocresol green as the tracking dye. The gels were migrated at 50 V for 30 minutes and then at 100 V until they reached the middle of the stacking gel. The protein bands (blue band) were then excised, leaving as much as possible of free SDS (pink band) as possible.

In-gel digestions

After in-gel reduction and alkylation using the MassPrep Station (Waters, Milford, MA, USA), proteins were in-gel digested with trypsin at 37°C overnight. After digestion, peptides were extracted with a 60% acetonitrile, 0.1% formic acid solution prior to mass spectrometry analyses.

NanoLC-MS/MS analyses

Digestion peptides were analyzed on a nanoACQUITY UltraPerformance LC[®] (UPLC[®]) system coupled to a MaXis 4G Q-ToF (Bruker Daltonics, Bremen, Germany) equipped with a nano-spray ion source. The system was fully controlled by HyStar[™] 3.2 (BrukerDaltonics). The peptides were first trapped on a nanoAcquity[™] UPLC[™] pre-column (Symmetry C18 Trap, 5 µm, 180 µm x 20 mm, Waters) and then separated on a nanoAcquity[™] UPLC[™] column (BEH C18, 1.7 µm, 75 µm x 250 mm, Waters). The solvent system consisted of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). Peptides were trapped during 1 min at 15 µL/min with 99 % A and 1 % B. Elution was performed at a flow rate of 450 nL.min⁻¹, using a linear gradient of 6-35% B over 55 min at 45°C. The mass spectrometer was operating in positive mode, with the following settings: source temperature was set to 160°C while dry gas flow was at 5 liters per minute. The nanoelectrospray voltage was optimized to -5000 V. External mass calibration of the TOF was achieved using Tuning Mix (Agilent Technologies, Paolo Alto, USA) in the mass range of 300-2700 m/z. Mass correction was achieved by recalibration of acquired spectra to the applied lock masses (methylstearate ([M+H]⁺ 299.2945 m/z) and hexakis(2,2,3,3,-tetrafluoropropoxy)phosphazine ([M+H]⁺ 922.0098 m/z)). For tandem MS experiments, the system was operated

with automatic switching between MS and MS/MS modes in the range of 100-2500 m/z (MS acquisition time of 0.4 s, MS/MS acquisition time between 0.05 s (intensity > 250000) and 1.25 s (intensity <5000)). The 6 most abundant ions (absolute intensity threshold of 1500), were selected from each MS spectrum for further isolation and CID fragmentation using nitrogen as collision gas. Peak lists in mascot generic format (.mgf) were generated using DataAnalysis™ software (version 4.0; Bruker Daltonics, Bremen, Germany).

MS/MS data interpretation

Data from nanoLC-MS/MS analyses were interpreted by database searching using the Mascot™ algorithm installed on a local server (version 2.4.1, Matrix Science, London, UK). Spectra were searched against a database containing all proteins annotated for *Bos taurus* (Taxonomy ID 9913) in UniprotKB, their decoy equivalents and common contaminants (UniProtKB release of 2013-05-01, 63412 target+decoy entries). The database was built with our in-house developed software pipeline MSDA (Mass Spectrometry Data Analysis, <https://msda.unistra.fr>). Searches were performed with a mass tolerance of 10 ppm in MS and of 0.05 Da in MS/MS mode, allowing one missed cleavage. Carbamidomethylation of cysteine residues was set as fixed modification and oxidation of methionine residues and acetylation on protein N-termini were set as variable modifications. Following the guidelines for proteomic data publication [1,2] filtering criteria based on probability-based scoring of the identified peptides have been taken into account for high confidence identifications and false discovery rates in protein identification were controlled thanks to the decoy hits. The Scaffold™ software (v.3.6.5, Proteome software Inc., Portland, OR, USA) was used to validate identifications using the following criteria:

proteins were validated when identified with a minimum of 2 unique peptides and MS/MS spectra showing ion scores higher than Mascot's threshold score of identity (95% confidence level) and ion scores higher than 25.

References

1. Carr S, Aebersold R, Baldwin M, Burlingame A, Clauser K, Nesvizhskii A: **The need for guidelines in publication of peptide and protein identification data : working group on publication guidelines for peptide and protein identification data.** *Mol Cell Proteomics* 2004, **3(6)**: 531-3.
2. Wilkins MR, Appel RD, Van Eyk JE, Chung MCM, Görg A, Hecker M, Huber LA, Langen H, Link AJ, Paik YK, Patterson SD, Pennington SR, Rabilloud T, Simpson RJ, Weiss W, Dunn MJ: **Guidelines for the next 10 years of proteomics.** *Proteomics* 2006, **6**: 4-8.