Electronic Supporting Information for:

Formation of the Protein Corona on Nanodiamonds

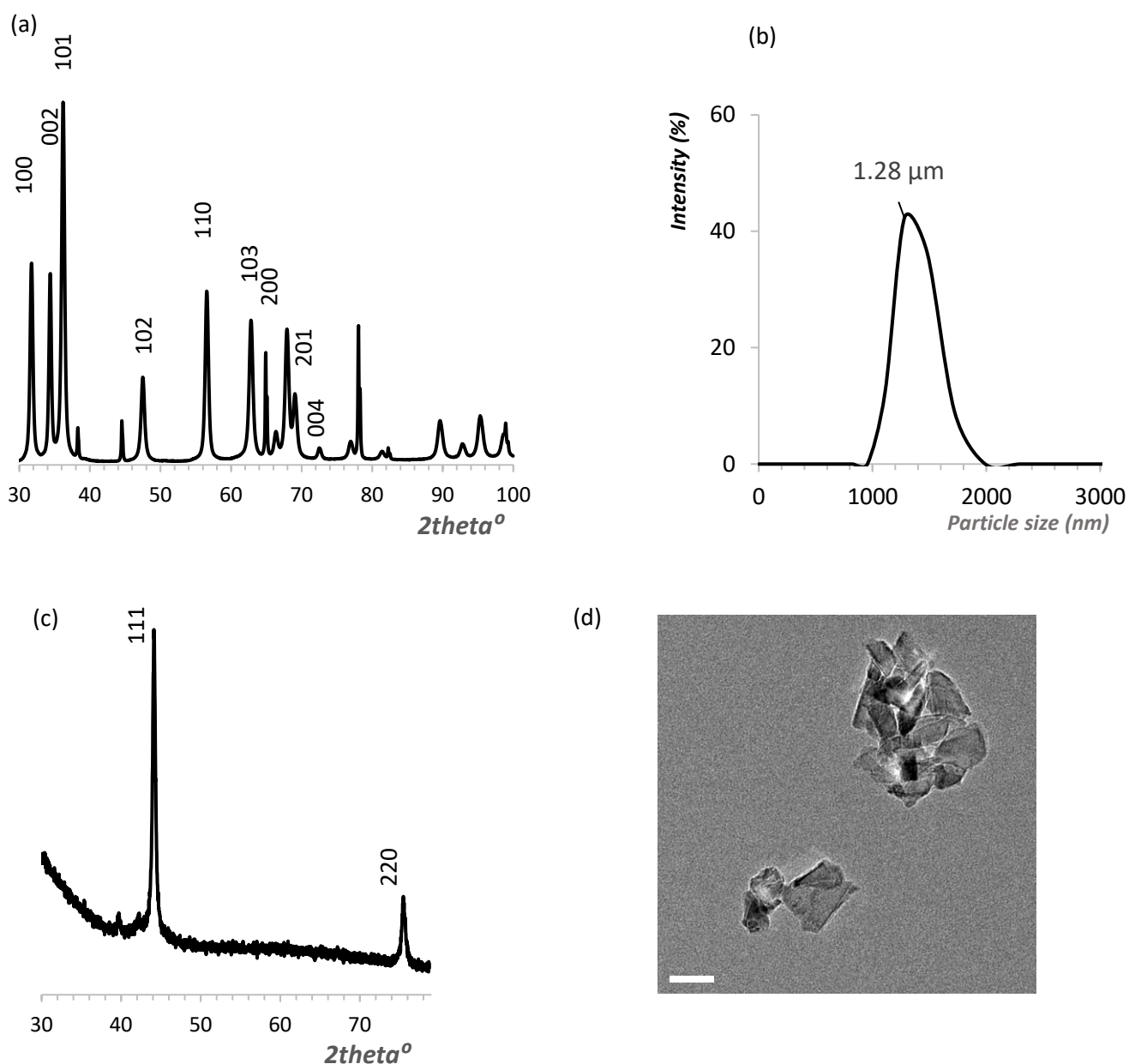*A. E. Garcia-Bennett, Arun E. Dass, Ishan Das Rastogi, Irene Moroni, Lindsay Parker, Nicolle Paker and Louise Brown*

SI-1. Materials Characterisation of zinc Oxide particles

SI-2. Zeta potential and Fourier Transform Infrared (FT-IR) spectra of as-received and purified NDs,

SI-3. Proteomics data: abundancy versus protein number and *Heat* map for the full protein data of NDs
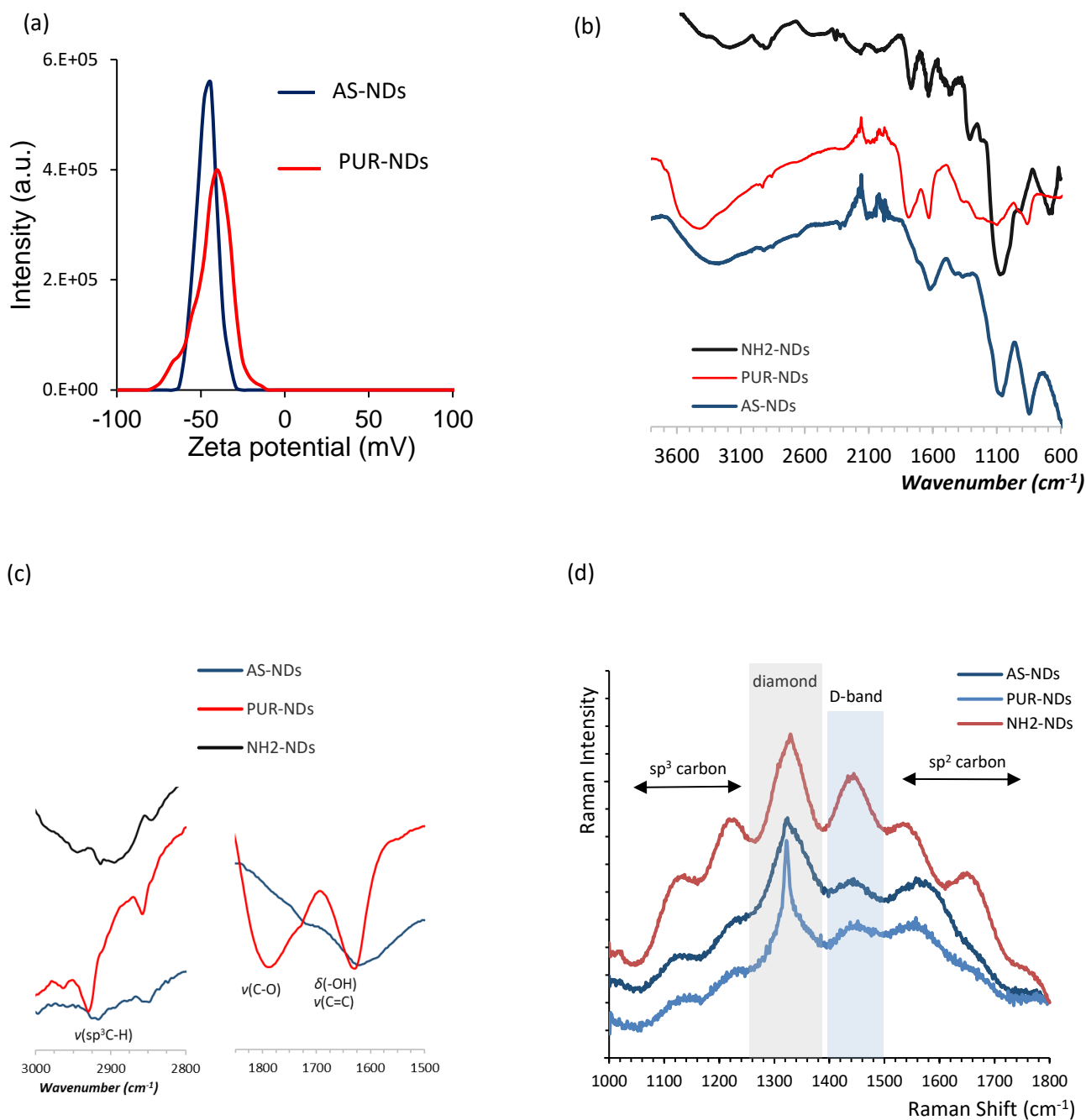
SI-4-7 Additional Proteomics data and PRIDE access details

Fig. S1 (a) X-ray diffraction (XRD) patterns of highly crystalline zinc oxide nanoparticles showing characteristic peaks for the wurtzite structure with some peaks assigned to undetermined impurities. (b) Dynamic Light Scattering particle size distribution of zinc oxide particles. (c) XRD pattern of amine functionalised nanodiamonds (NH$_2$-NDs) and characteristic TEM image of NH$_2$-NDs showing the agglomerated particles.
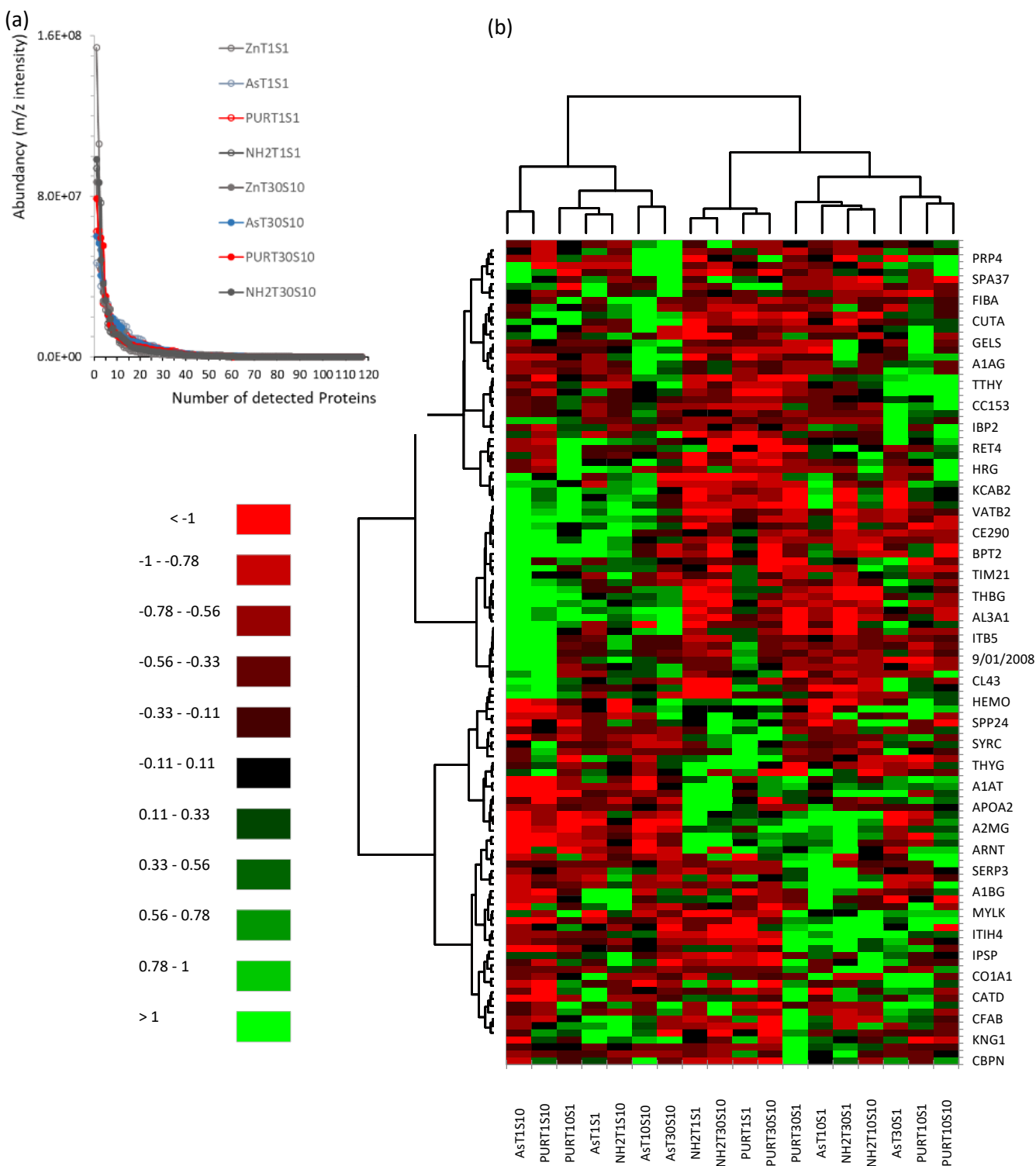
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Fig. S2 (a) Zeta potential measurements for As-NDs and PUR-NDs conducted in water media. (b) FT-IR spectra of NDs and (c) enlarged selected areas of FT-IR spectra of NDs (omitted y-axis represents intensity in arbitrary units). (d) Raman spectra of the powdered ND samples recorded after excitation at 632.8 nm, showing the following features: G and D bands (1400-1500 cm^{-1} and 1550-1700 cm^{-1}), main diamond peak centred around 1320 cm^{-1} and the amorphous carbon region (<1300 cm^{-1}). Note a considerable sharpening of the diamond peak as a result of the purification process indicative of more exposed diamond particle core in comparison to AS-NDs.

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Fig. S3 (a) Abundancy versus number of proteins detected from protein corona samples prepared for all materials at low serum concentration (S_1) and short incubation times (1 minute) and high serum concentrations (S_{10}) and long times (30 minutes). The plot demonstrates that the top 30 proteins dominate the corona. **(b)** Heat map for the full protein data set for all ND samples showing four major protein clusters (rows), and a large variability in the abundancy of proteins as a function of type of functional group of the nanodiamond surface. A full list of proteins found is included at the end of this document.

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Table S4 Composition of the Top 10 proteins for coronas of as received A-NDs, purified P-NDs, NH_2-NDs and zinc oxide particles (Zn-) as a function of serum concertation (S-) and incubation time (T-). Values are expressed as a percentage of the total abundancy.

AsT_1S_1	%	$AsT_{10}S_1$	%	$AsT_{30}S_1$	%
α-1-antiproteinase	9.35	Cullin	15.30	α-1-antiproteinase	15.70
Hemoglobin-α	9.02	α-1-antiproteinase	12.11	Hemoglobin-α	15.40
Spleen trypsin inhibitor	7.03	Hemoglobin-α	8.65	Prosalusin	9.27
Vitamin K-depend. protein C	6.66	α-2-macroglobulin	7.66	Vitamin K-depend. protein C	8.47
Protein CutA	5.64	Protein CutA	6.14	Protein CutA	5.57
Prosalusin	5.28	Spleen trypsin inhibitor	5.24	Cullin	3.44
Cathepsin D	3.76	Prosalusin	3.29	α-2-macroglobulin	3.41
Clusterin	3.74	SCO-spondin	2.98	SCO-spondin	3.30
α-2-macroglobulin	3.71	Complement factor D	2.82	60S ribosomal protein	2.87
Aldehyde dehydrogenase	3.47	Clusterin	2.46	Prefoldin subunit 4	2.37
AsT_1S_{10}	%	$AsT_{10}S_{10}$	%	$AsT_{30}S_{10}$	%
Vitamin K-depend. protein C	10.20	Protein CutA	14.17	α-1-antiproteinase	14.17
Protein CutA	9.69	Hemoglobin-α	6.79	Protein CutA	6.79
Spleen trypsin inhibitor	8.05	Prosalusin	6.38	Hemoglobin-α	6.38
SCO-spondin	6.47	α-1-antiproteinase	6.04	Prosalusin	6.04
Peptidase inhibitor 16	5.34	Vitamin K-depend. protein C	5.23	Vitamin K-depend. protein C	5.23
Clusterin	4.26	Cullin	3.93	60S ribosomal protein L19	3.93
Aldehyde dehydrogenase	4.19	Prefoldin subunit 4	3.78	Prefoldin subunit 4	3.78
Prefoldin subunit 4	3.96	Spleen trypsin inhibitor	3.25	Cullin	3.25
60S ribosomal protein L19	3.93	SCO-spondin	3.23	Aldehyde dehydrogenase	3.23
Pantetheinase	3.08	60S ribosomal protein L19	3.11	SCO-spondin	3.11

$PURT_1S_1$	%	$PURT_{10}S_1$	%	$PURT_{30}S_1$	%
α-1-antiproteinase	12.49	A-1-antiproteinase	14.07	α-1-antiproteinase	16.87
Cullin	12.38	Prosalusin	8.14	Cullin	15.40
Hemoglobin-α	8.70	Protein CutA	7.43	α-2-macroglobulin	11.02
Prosalusin	5.32	α -2-macroglobulin	6.26	Hemoglobin-α	10.51
α-2-macroglobulin	5.28	Hemoglobin-α	6.20	Prosalusin	5.27
SCO-spondin	5.05	Cullin	6.11	Protein CutA	3.23
Vitamin K-depend. protein C	4.10	Spleen trypsin inhibitor	4.82	Spleen trypsin inhibitor	2.91
Spleen trypsin inhibitor	3.69	Glycolipid transfer protein	3.61	Cathepsin D	2.86
Protein CutA	3.48	SU4/U6 small nuclear ribonucleoprotein Prp4	2.70	SCO-spondin	2.59
Prefoldin subunit 4	2.65	Cathepsin D	2.67	Vitamin K-depend. protein C	2.45
$PURT_1S_{10}$	%	$PURT_{10}S_{10}$	%	$PURT_{30}S_{10}$	%
SCO-spondin	9.08	α-1-antiproteinase	15.86	α-1-antiproteinase	15.75
Vitamin K-depend. protein C	6.94	Cullin	12.20	Hemoglobin-α	12.53
Spleen trypsin inhibitor	6.76	Hemoglobin-α	10.76	α-2-macroglobulin	11.83
Protein CutA	6.19	Protein CutA	7.37	Cullin	11.09
Cullin	5.54	Prosalusin	7.21	Prosalusin	6.01
60S ribosomal protein L19	4.27	α-2-macroglobulin	6.47	Protein CutA	4.21
α-1-antiproteinase	3.99	SU4/U6 small nuclear ribonucleoprotein Prp4	3.91	SU4/U6 small nuclear ribonucleoprotein Prp4	3.19
Clusterin	3.85	SCO-spondin	2.95	SCO-spondin	2.91
Septin-8	3.67	Vitamin K-depend. protein C	2.14	Keratin	2.49
Peptidase inhibitor 16	3.31	Vitamin D-binding protein	1.98	Prefoldin subunit 4	2.11

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NH$_2$T$_1$S$_1$	%	NH$_2$T$_{10}$S$_1$	%	NH$_2$T30S$_1$	%
A-1-antiproteinase	18.77	Protein CutA	13.02	A-1-antiproteinase	15.64
Cullin	15.63	A-1-antiproteinase	9.48	Cullin	15.23
Hemoglobin-α	15.33	Spleen trypsin inhibitor	7.00	Hemoglobin-α	14.91
A-2-macroglobulin	6.45	Vitamin K-depend. protein C	5.73	A-2-macroglobulin	10.14
Prosalusin	5.33	Hemoglobin-α	4.80	Prosalusin	4.77
Keratin	3.32	Prefoldin subunit 4	4.17	Cathepsin D	2.62
SCO-spondin	2.53	Peptidase inhibitor 16	3.90	Protein CutA	2.60
Protein CutA	2.32	Cullin	3.89	Keratin	2.60
Cathepsin D	2.31	Prosalusin	3.84	SCO-spondin	2.52
Spleen trypsin inhibitor	2.28	Complement factor D	3.81	Apolipoprotein A-I	2.13
NH$_2$T$_1$S$_{10}$	**%**	**NH$_2$T$_{10}$S$_{10}$**	**%**	**NH$_2$T30S$_{10}$**	**%**
Cullin	9.29	Hemoglobin-α	15.74	α-1-antiproteinase	19.65
α-1-antiproteinase	9.14	α -1-antiproteinase	13.39	Hemoglobin-α	17.29
Hemoglobin-α	7.19	Cullin	11.63	Cullin	9.66
Spleen trypsin inhibitor	6.13	Prosalusin	7.65	A-2-macroglobulin	7.27
SCO-spondin	5.18	A-2-macroglobulin	6.87	Prosalusin	5.65
Protein CutA	4.81	Protein CutA	5.19	Protein CutA	4.43
Vitamin K-depend. protein C	4.12	SCO-spondin	2.83	Keratin	3.80
A-2-macroglobulin	3.98	Pantetheinase	2.48	SCO-spondin	2.83
Prefoldin subunit 4	3.07	Complement factor D	2.36	Vitamin D-binding	2.25
Prosalusin	3.02	SU4/U6 small nuclear ribonucleoprotein Prp4	2.35	Cathepsin D	2.24

ZnT$_1$S$_1$	%	ZnT$_{10}$S$_1$	%	ZnT30S$_1$	%
Spleen trypsin inhibitor I	30.74	Cullin	17.08	α-1-antiproteinase	19.65
Clusterin (Glycoprotein III)	21.18	α-1-antiproteinase	16.88	Prosalusin	19.19
α-1-antiproteinase	10.65	Prosalusin	12.64	Cullin	18.89
Hemoglobin-α	5.51	Hemoglobin-α	8.86	Hemoglobin-α	11.74
Protein CutA	4.68	SCO-spondin	6.41	α-2-macroglobulin	5.69
α-2-macroglobulin	2.96	Protein CutA	5.48	SCO-spondin	3.44
Prosalusin	2.48	α-2-macroglobulin	4.14	60S ribosomal protein	2.35
Centrosomal protein	2.43	60S ribosomal protein L19	3.63	Protein CutA	1.42
Cullin	2.33	U4/U6 small nuclear ribonucleoprotein Prp4	1.87	Pantetheinase	1.25
Apolipoprotein	2.12	Spleen trypsin inhibitor I	1.65	Complement C9	1.06
ZnT$_1$S$_{10}$	**%**	**ZnT$_{10}$S$_{10}$**	**%**	**ZnT30S$_{10}$**	**%**
α-1-antiproteinase	14.17	Prosalusin	16.22	Serum albumin	17.39
Prosalusin	11.70	α-1-antiproteinase	14.98	α-1-antiproteinase	11.78
Cullin	11.61	Cullin	14.01	α -2-HS-glycoprotein	8.73
Protein CutA	9.65	Hemoglobin-α	10.92	Keratin	5.54
SCO-spondin	8.31	SCO-spondin	5.37	Serotransferrin	5.33
Hemoglobin-α	7.95	Protein CutA	5.33	Hemoglobin-α	4.87
60S ribosomal protein L19	3.98	60S ribosomal protein L19	4.43	α-fetoprotein	4.71
U4/U6 small nuclear ribonucleoprotein Prp4	3.03	α-2-macroglobulin	3.97	Apolipoprotein A-I	3.16
Septin-8	2.46	U4/U6 small nuclear ribonucleoprotein Prp4	3.01	α-2-macroglobulin	3.02
Spleen trypsin inhibitor I	2.40	Septin-8	2.40	Keratin	2.92

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Fig. S5 Molecular Weight Distribution of the top 30 proteins measured in Zinc Oxide particles incubated at different time points at serum concentrations of 1% (ZnS1) and 10% (ZnS10).
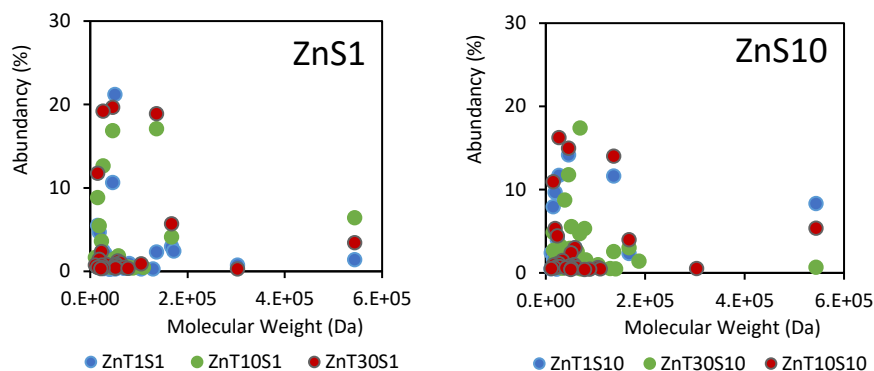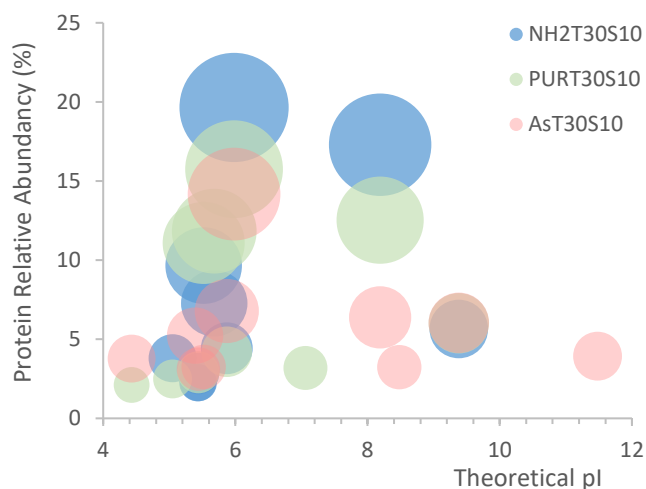

Fig. S6 Isoelectric point (pI) of the top 10 proteins for the protein corona of selected NDs. Theoretical pI obtained from the ExPaSy online freeware package (SIB Swiss Institute of Bioinformatics, https://web.expasy.org/compute_pi/)

S7. Raw mass spectra data files and SWATH library in PRIDE which can be accessed free of charge, with the details below:

Project accession: PXD013437

Username: reviewer47244@ebi.ac.uk

Password: hHRXIxKc