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Electronic supplementary information

Ring-shaped corona proteins influence the toxicity of engineered nanoparticles to yeast

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Experimental supplementary information

Synthesis and characterization of CdS QDs

X-ray diffraction (XRD) analysis was performed using a Thermo ARL X'tra diffractometer (Cu Kα source, Θ–Θ Bragg-Brentano geometry, 10^{-4} degree accuracy) and the XRD pattern was reported in Fig. S1A. All peaks have been indexed according to JCPDS no. 80-0006 and no impurity phases have been detected. Grain size was estimated by Scherrer calculation on FWHM of the main peak. XRD analysis showed that CdS QDs exhibited hexagonal shape (Fig. S1A).

For scanning transmission electron microscopy (STEM) analysis, CdS QDs have been dispersed onto Cu/lacey-carbon TEM grids and characterized using a field emission high resolution JEM-2200 FS TEM (JEOL; Scherzer resolution of ~0.19 nm) working at 200 kV. STEM image reported in Fig. S1B confirms the CdS QDs size deduced from Scherrer formula and show that these NPs had uniform morphology.

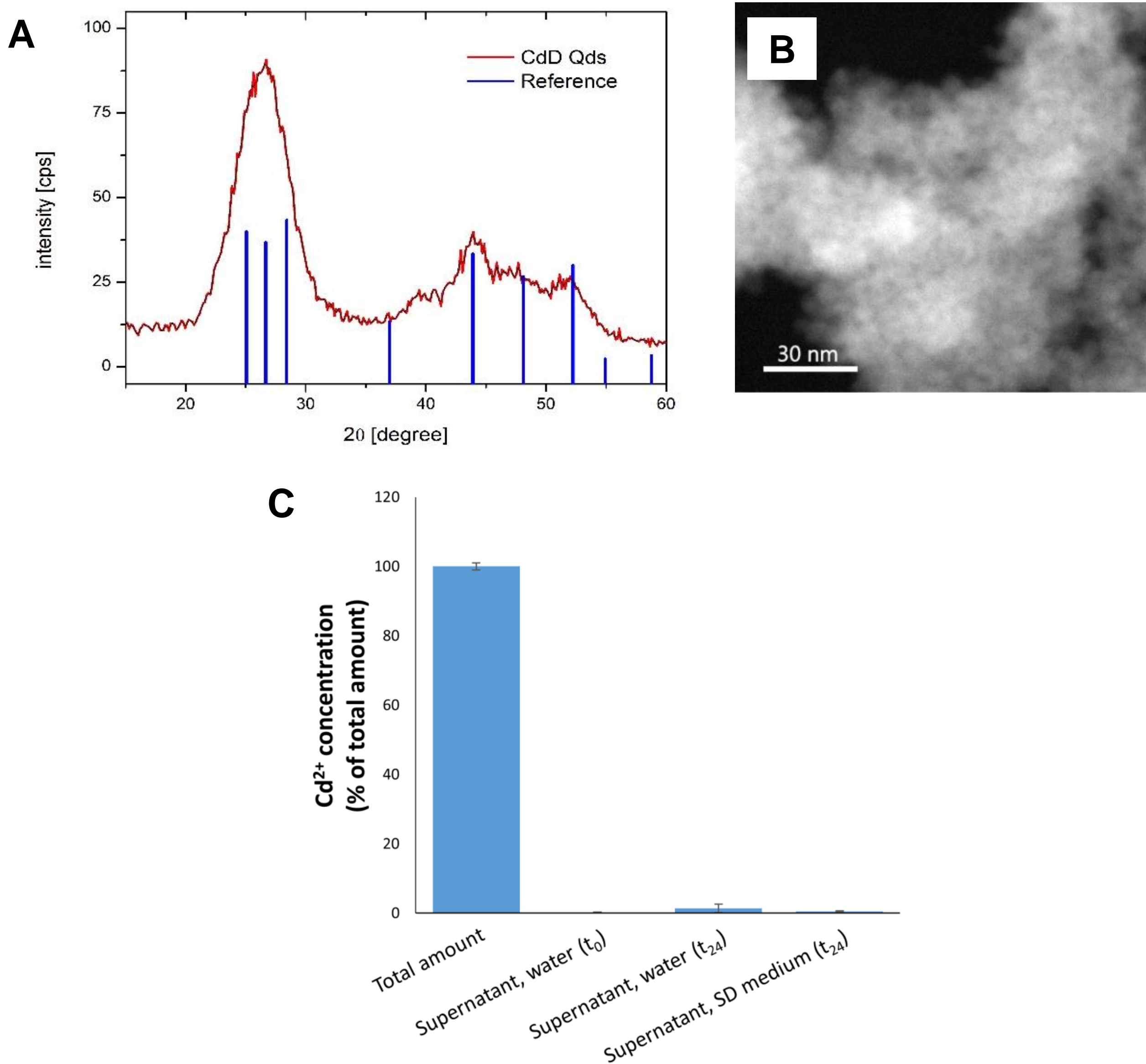

Fig. S1. Physico-chemical characterization of CdS QDs. (A) XRD pattern of CdS QDs. Blue lines refer to hexagonal CdS (greenockite) as reported in JCPDS database (card no. 80-0006). (B) STEM image of CdS QDs. (C) Cd^{2+} ion concentration was measured using AAS analysis (see "Experimental" for details). "Total amount" (100%) represents the Cd^{2+} ion concentration determined by AAS analysis at the maximal dose used in the biological experiments (250 mg/L). Only negligible amounts of Cd^{2+} ions (less than 0.1% of the total amount) were found in the supernatants of aqueous CdS QD solutions (250 mg/L) obtained by centrifugation for 5' at 21000 *g* [*supernatant, water* (t_0)]. Very low amounts of Cd^{2+} ions were also found in the centrifuged supernatants of CdS QD solutions (250 mg/L) prepared in water [*supernatant, water* (t_{24})] or in yeast culture medium [synthetic medium supplemented with

glucose (SD); *supernatant, SD medium (t_{24})*]. These QD solutions are incubated at 28°C for 24h with gently shaking, prior to centrifugation (for 5' at 21000 *g*) and AAS analysis. These results show a low rate of dissolution of CdS QDs in these experimental conditions.

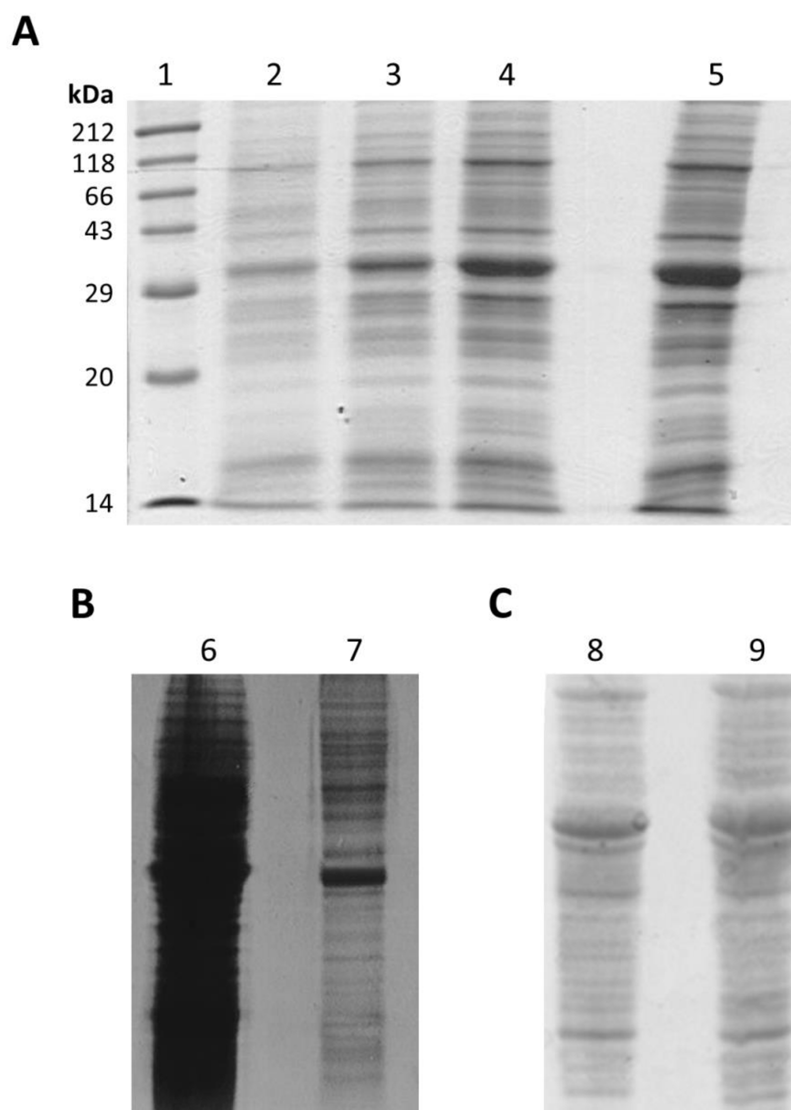

Figure S2. Proteomic analysis of the yeast proteins adsorbed onto CdS QD surface. (A) Increasing the titer of protein extract presented for binding (*lane 2*: 0.1 mg, *lane 3*: 0.3 mg, *lane 4*: 0.6 mg, *lane 5*: 1 mg) enhanced the amount of yeast proteins absorbed onto the CdS QDs. (B) Decreasing the time available for binding (*lane 6*: 24 h, *lane 7*: 1 h) reduced the amount of corona proteins which became bound. (C) There was no significant differences in electrophoretic profiles of proteins absorbed on the CdS QD surface at 4°C (*lane* 8) or 37°C (*lane 9*). In each case, the CdS QDs were rinsed only three times in a salt-free buffer (see “Experimental” for additional details). *Lane 1*: protein molecular weight ladder (weights in kDa as shown).

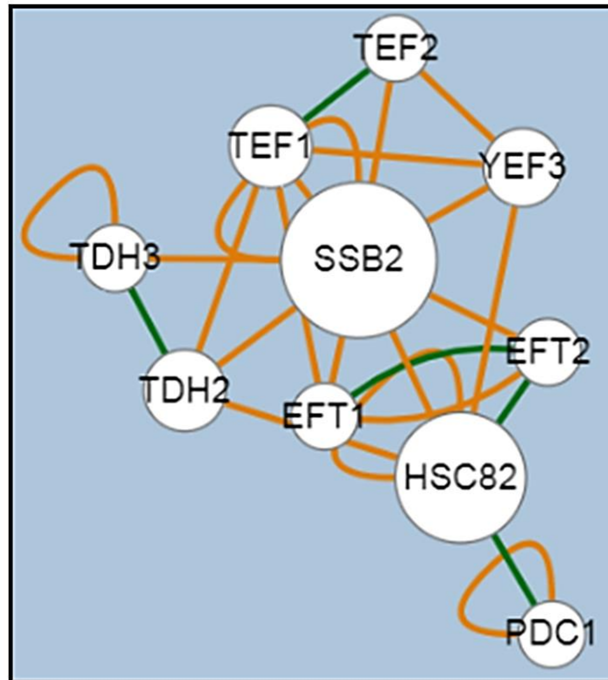

Figure S3. The hard corona is an complex set of yeast proteins. Well documented interactions (Biogrid database, https://thebiogrid.org/) between the corona proteins were observed. The presence of a highly connected node (Ssb2) in this small protein–protein interaction network is expected because Ssb2 is a molecular chaperone. Genetic and physical interactions between the various hard corona proteins are indicated by, respectively, *green* and *orange* lines. Biological network was built with the online tool esyN (http://www.esyn.org).

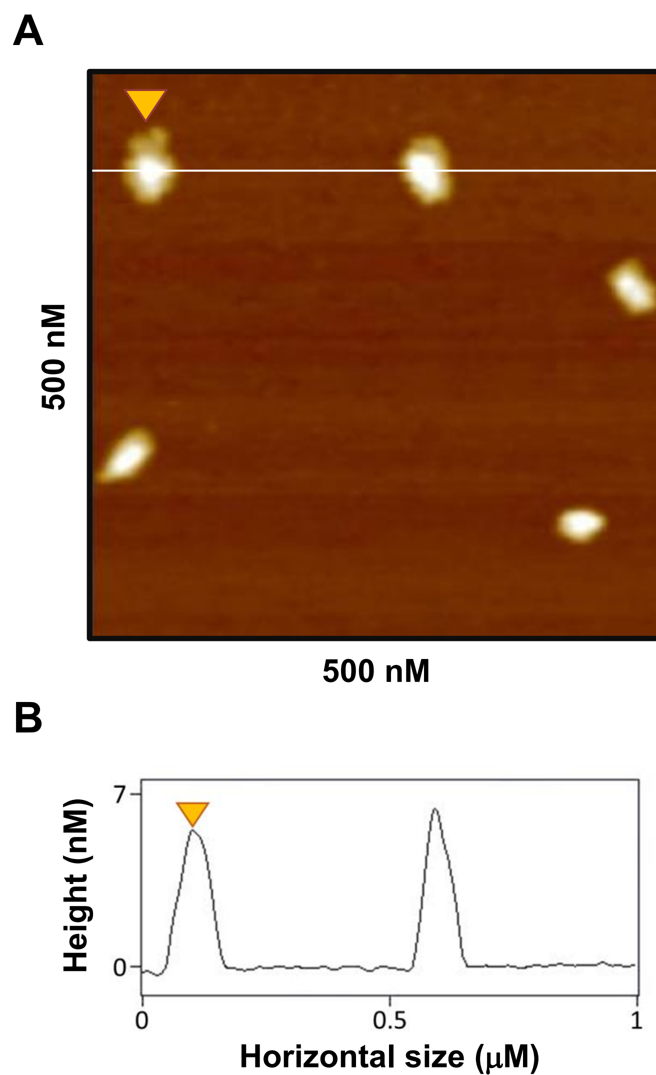

Figure S4. AFM analysis of the CdS QD-protein corona deposited onto freshly cleaved mica. (A) The AFM image showed that yeast protein corona was characterized by round-shaped structures. (B) Height distribution of the QD-protein corona structures along the white line shown in A.

Table S1. Average hydrodynamic diameters of CdS QD solutions prepared in water and yeast culture medium.

Sample[a]	**Average hydrodynamic diameter (nm)**
CdS QDs (water)	163.7 ± 4.3
CdS QDs (yeast medium)[b]	160.4 ± 10.7

[a]Samples were prepared at the maximal dose used in the biological experiments (250 mg/L) and incubated at 28°C for 24 h with gently shaking.

[b]Synthetic medium supplemented with glucose (SD).

Table S2. Primer sequences used in real-time PCR analysis.

Gene name	Amplicon length	Primer sequence (5'-3')	Primer concentration (final)
ACT1	67 bp	FW: GAGGTTGCTGCTTTGGTTATTGA RE: CGTCGTCACCGGCAAAA	50 nM
CDC19	104 bp	FW: ATCTTCACCACCGATGACAAGT RE: TAGATGATTCTACCAGCGGAGA	50 nM
EFT2	111 bp	FW: ATGCTAAGAAATTCGGTGTCGAC RE: CTTCAGCATCAGTGTCCTTGTTG	75 nM
HSC82	105 bp	FW: CTCTATCTGCTGGTGCCGA RE: CATTGTTCTTGGAAATAACTTGA	50 nM
PDC1	114 bp	FW: ATGCTGAATCCGAAAAGGAAGTC RE: TCAGCCTTGACGTCGTGTCTG	250 nM
SSB2	114 bp	FW: ATGTTTCCTTGTTGCACATTGCTG RE: CAGCCTTGAAGTGTTCCAACAAG	100 nM
TDH3	107 bp	FW: TCATGAGAATTGCTTTGTCTAGAC RE: TAAGTAGCAATCTTCTTACCATCG	250 nM
TEF1	106 bp	FW: ATGGTCAAACCAGAGAACACGC RE: AATCTGGATTCGTCCCATTTGAC	200 nM
YEF3	99 bp	FW: ATGCCAGAATTGATTCCAGTC RE: AGTTTCGGTAGCCTTGGTCATG	40 nM

Table S3. Amino acid frequencies in the corona protein sequences.

	Yeast proteome (mean)[a]	Corona proteins[b]								
		Cdc19	Pdc1	Tdh2	Tdh3	EF-1α	eEF-2	Yef3	Hsc82	Ssb2
Hydrophobic amino acids										
Ala	5.7	8.6 (*)	9.6 (*)	9.9 (*)	9.6 (*)	8.1 (*)	8.1 (*)	8.7 (*)	6.2	10.0 (*)
Gly	5.2	6.8 (*)	7.5 (*)	7.5 (*)	7.8 (*)	9.2 (*)	7.0 (*)	6.0 (*)	3.5 (#)	6.9 (*)
Val	5.8	9.6 (*)	7.1 (*)	10.8 (*)	11.1 (*)	10.0 (*)	9.6 (*)	6.8 (*)	6	8.5 (*)
Pro	4.3	5.0 (*)	4.6	3.3 (#)	3.6 (#)	5.0 (*)	4.8	4.1	3.8	3.3 (#)
Ile	6.5	7.4	6.6	6	5.7	6.6	5.9	7.5	6.7	5.9
Leu	9.6	7.0 (#)	9.6	6.3 (#)	6.3 (#)	5.2 (#)	8.2 (#)	8.3 (#)	9.6	8.3 (#)
Met	2.2	2.2	2.3	2.4	2.1	1.7 (#)	2.7 (*)	2.4	1.8 (#)	1.5 (#)
Phe	4.7	3.0 (#)	4.1	3.3 (#)	3.0 (#)	3.7 (#)	4.2	3.4 (#)	4.8	4.2
Trp	1.1	0.2	1.2	0.9 (#)	0.9 (#)	1.3 (*)	1	1.1	0.7 (#)	0.2 (#)
Tyr	3.4	3	3	3	3.3	1.7 (#)	2.4 (#)	1.9 (#)	2.7 (#)	1.5 (#)
Acidic amino acids (negatively charged)										
Asp	5.5	6.4 (*)	5.2	7.2 (*)	7.2 (*)	5.2	6.9 (*)	5.6	6.5 (*)	6.2
Glu	6.3	5.6	5.3 (#)	4.5 (#)	4.5 (#)	6.8	6.5	8.8 (*)	13.6 (*)	8.0 (*)
Basic aminoacids (positively charged)										
Arg	4.7	4.8	2.7 (#)	3.3 (#)	3.3 (#)	3.9 (#)	4.9	4.3	3.8 (#)	4.7
His	2.2	1.4 (#)	2.1	2.4	2.4	2.4	1.9	2.3	0.6 (#)	0.8 (#)
Lys	7.4	7.4	6.2 (#)	7.8	7.8	10.7 (*)	7.2	8	10.4 (*)	7.7
Polar amino acids with uncharged groups										
Cys	1.5	1.4	0.7 (#)	0.6 (#)	0.6 (#)	1.5	1.0 (#)	1.4	0 (#)	0.3 (#)
Ser	8.7	5.4 (#)	5.3 (#)	7.5 (#)	7.8	4.6 (#)	5.2 (#)	5.7 (#)	6.4 (#)	7.2 (#)
Thr	5.8	7.6 (*)	7.8 (*)	6.9 (*)	7.2 (*)	6.1	5.7	6	5.7	7.3 (*)
Asn	5.7	5.2	5.2	4.2	3.9 (#)	3.5 (#)	3.1 (#)	4.7 (#)	4 (#)	3.6 (#)
Gln	3.9	2.0 (#)	3.9	1.8 (#)	1.5 (#)	2.6 (#)	3.8	2.8 (#)	3.1 (#)	4.1

[a]The average frequency of each amino acid residue in the yeast proteome (http://www.yeastgenome.org/).

[b]Amino acid residue frequencies in the corona proteins. Those which showed a higher (*) or a lower (#) abundance of a given residue relative to their abundance in the proteome as a whole are indicated (see “Experimental” for details).

Table S4. Yeast protein structures available in PDB database.

Proteins	PDB ID	Resolution (Å)	Cofactor[a]	Multimeric state
Cdc19	1A3W	3	ATP	homotetramer
Pdc1	2W93	1.6	TPP	homotetramer
Tdh3	3PYM	2	NAD^+	homotetramer
EF-1α	1F60	1.67	GTP	heterodimer (with EFB1)
eEF-2	1N0V	2.85	GTP	monomeric
Yef3	2IW3	2.4	ATP	homodimer
Hsc82	*not available*		ATP	
Ssb2	*not available*		ATP	

[a]TPP, thiamine pyrophosphate.