Supporting information

Interaction of nano-quantum dots (CdSe@ZnS) and extracellular proteins in activated sludge revealed by bio-nano science

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TEXT 1. Molecular weights characterization

Molecular weight (MW) was determined by a Waters liquid chromatography system that consisted of a Waters 2487 Dual l Absorbance Detector, Waters 1525 pump system. A Shodex KW 802.5 gel chromatography column (Shoko, Japan) was used for organic materials separation. The mobile phase buffered with 5 mM phosphate to pH 6.8, and 0.01 M NaCl, was filtered through a 0.22 mm membrane, and then degassed for 30 min by means of ultra-sonication before use. A 200 uL sample was injected at a flow rate of 0.8 mL/min, by the isocratic elution, at 25 ℃. Polystyrene sulfonate standards (Sigma-Aldrich, USA) of MWs 1.8−32 kDa were used for apparent molecular weight (AMW).

TEXT 2. Fourier transform infrared spectroscopy (FT-IR) and X-ray photoelectron spectroscopy (XPS) analyses

Fourier transform infrared spectrophotometer (Thermo Scientific Co., United States, Nicolet 6700) was performed to characterize the chemical groups of the samples (freeze-dried EPS, extracellular proteins and protein corona) mixed with KBr and ratios of 1:100. And the interaction of protein and nanoparticles was analyzed. The scanning parameters were as follows: a spectral range of 4000–400 cm^{-1}, 32 scans and a resolution of 4 cm^{-1}. X-ray photoelectron spectroscopy (XPS) measurements were used to determine the composition and functional groups of freeze-dried EPS, extracellular proteins and protein corona. XPS of the samples were performed using a XPS spectrometer (ESCALAB 250, Thermo Fisher Scientific Co., USA). The XPS measurements were conducted using Al Ka radiation.

TEXT 3. **Three-dimensional fluorescence spectrum (3D-EEM)**

The components of original EPS and purified protein type of were determined with a luminescence spectroscope (Hitachi F700, Japan), with 200−400 nm excitation

wavelength at intervals of 10 nm and 220−550 nm emission wavelength at intervals of 0.5 nm. The excitation and emission slit band widths were 10 nm for spectra, and were recorded at 12,000 nm min^{-1} scan rate. The excitation-emission fluorescence spectra (EEM) spectrum was delineated into five regions based on methods described in Chen et al. The 3D-EEM fluorescence spectra of extracellular proteins before and after binding with different concentrations of nanoparticles were obtained using 3D-EEM.

TEXT 4. Confocal laser scanning microscope (CLSM) and Transmission electron microscope (TEM)

Extracellular proteins, polysaccharides distributions in sludge and morphology features of protein corona were characterized with CLSM (Zeiss LSM 510). Prior to the observation, samples were preprocessed using fluorescein isothiocyanate (FITC) and Con A-Texas Red agent for protein and polysaccharides, respectively. The extracellular proteins could be dyed with FITC and showed in green with the scanning range at Ex = 543 nm, Em = 550−600 nm, while polysaccharides could be dyed with Con A +Texas Red and displayed in red with the scanning range at Ex = 488 nm, Em = 500−550 nm. Morphological characteristics of protein corona was obtained without any pretreatment and protein corona could be showed in green with the scanning range at Ex = 488 nm.

The morphology of EPS, extracellular proteins, CdSe@ZnS nano-quantum dots and protein corona were characterized by transmission electron microscopy (TEM) (Tecnai G220, FEI, Netherlands). The samples were diluted 100-fold in deionized water prior to recording the TEM images and protein corona was obtained about by mixing equal volumes of 10 nmol/L nanoparticle solutions with a suitable concentration of protein. And the above complex was incubated for 30 min.

TEXT 5. Zeta potentials and dynamic light scattering (DLS)

The zeta potential was measured by Zetasizer nano ZS90 (Malvern instrument company, UK). The effect of extracellular proteins on the surface charge of CdSe@ZnS nano-quantum dots was determined by measuring the ζ potential of the particles (10 nmol/L) in DI water in the presence of protein (0−7.5 mg/L). The size of nano-quantum dots and proteins corona were measured via a Malvern Zetasizer (Nano ZS, Malvern, UK) at 25 ± 1 °C. A monochromatic coherent He−Ne laser with a fixed wavelength of 633 nm was used as a light source, and the intensity of scattered light was measured by a detector at 173°. Each autocorrelation function was accumulated for 6 s, and a total of 10 autocorrelations were obtained for each measure.

TEXT 6. Supplementary Methods.

(1) Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

1mL proteins solution was transferred into ultrafiltration device (10Kd) and concentrated to 80 μL by centrifugation (5000g, 4°C). After that, the concentrated solution was transferred into 1.5mL centrifuge tube for mixed with the addition of 80μL lysis buffer SDT（4% SDS，100 mM Tris-HCl，100 mM DTT，pH 8.0） and treated by at 100 °C for 5 min. Subsequently, 10 μL of the above solution was heat for 5 min by adding 1μL 6×Loading buffer and then conducted by SDS polyacrylamide gel electrophoresis analysis. Finally, the polyacrylamide gel was analyzed by fast silver-staining.

(2) Proteins of enzymolysis

Proteins sample (100 μL) was enzymolysised by filter-aided sample preparation (FASP) [1] at 100 °C for 5 min. After cool to room temperature, the sediment was collected by centrifugation (14000 g, 30 min), after the above solution was transferred in to ultrafiltration device (30 Kd) with addition of 100μL UA buffer (8M Urea, 150 mM Tris-HCl pH 8.5). Then, repeat as the same operation three times. Next, the

sediment was added in sequence with 100 μL iodoacetamide (IAA) (50 mM IAA in UA), 100 μL UA buffers and 100 μL 25 mM/L trimethylammonium bicarbonate (ABC) buffer. Every time the samples were collected by centrifugation (14000 g, 30 min) after addition of IAA, UA and ABC buffer. And the same operation repeats 3 ~ 4 times. Next, the sediment was put in eppendorf thermomixer comfort (300 rpm, 18h, 37°C) after with addition of 44μL Trypsin buffer (2μg Trypsin in 40μL 100 mM/L ABC buffer), and filtrate was collected by centrifugation (14000 g, 30 min). Finally, the filtrate was collected by centrifugation (14000 g, 30 min) after the addition of 40 μL 25 mM/L ABC buffer in a new collecting pipe. And the filtrate was analyzed by OD280.

(3) LC-MS/MS

Enzymatic hydrolysate (peptides) were subjected to analysis using a Thermo Scientific UHPLC system equipped with an Orbitrap Fusion system (Thermo Finnigan, San Jose, CA) for identification, incorporating a C18 column (Thermo Scientific analytical column (75μm×25 cm, 5μm, 100 Å, C18). The mobile phase comprised a mixture of water with 0.1% FA (formic acid) as gradient solvent A, the gradient solvent B, (0.1% FA in 98% ACN) was increased from 5% to 28% for 40 min, 28% to 90% for 2 min, and then held at 90% for the last 18 min. After desalination and separation, the enzymatic hydrolysate was conducted by mass spectrometric analysis with Orbitrap Fusion (Thermo Finnigan, San Jose, CA). Mass spectra were acquired by using data-dependent acquisition with a full mass scan (375-1800 m/z) followed by 10 MS/MS scans. And the analysis of time was maintained at 60 min. The mass charge ratio of polypeptides and polypeptide fragments was collected by the following methods: each first-order mass spectral resolution first-order scan (MS1) was followed by second-order mass spectral resolution (MS2) and the detail parameters can be seen in table S-6.1.

Table S-6.1 Parameters of mass spectrometry

Items	Parameters
Resolution ratio of MS1	120,000
Scanning range of MS1	Start from m/z 200
Resolution ratio of MS2	50,000
Scanning range of MS2	Start from m/z 200
AGC target of MS1	4e5
AGC target of MS2	1e5
Maximum IT of MS1	50 ms
Maximum IT of MS2	105 ms

(4) Data analysis

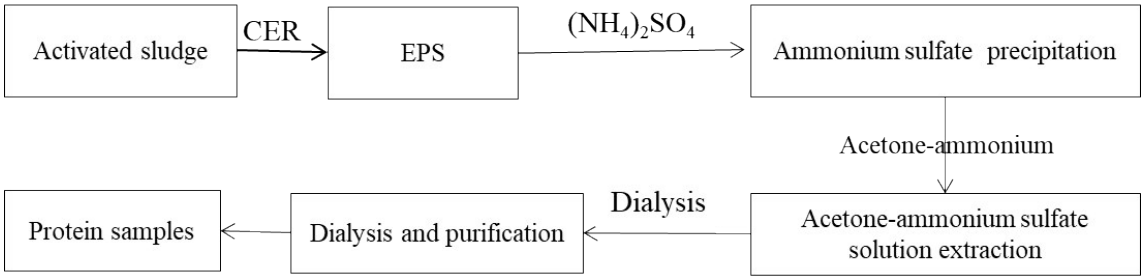
The original data of mass spectrometry named RAW, and be analyzed by MaxQuant1.5.1.7 for the identification and quantitative analysis [2]. Database Mock_Comm_RefDB_V3. fasta. fasta [3] was adopted to treat the data and the lookup parameters are shown in the following table S-6.2.

Table S-6.2 Parameters of Check the library

Title	Parameters
Variable modifications	Oxidation (M) Acetyl (Protein N-term)
Fixed modification	Carbamidomethylating (C)
Digestion mode	Trypsin
Max. missed cleavages	2
Label-free quantification	LFQ iBAQ
Main search ppm	6
Database	Database Mock_Comm_RefDB_V3.fasta.fasta
Decoy mode	Revert
PSM FDR	0.01
Protein FDR	0.01
Min. peptide length	2
Peptides for quantification	Unique+Razor peptide
FTMS MS/MS tolerance	20 ppm
FTMS top peaks per 100 Da	12

Table S1 Characteristics of waste activated sludge

Factor	Moisture content (%)	pH	VSS/TSS	TSS	Zeta potential
Value	96.7%	7.48	0.71	2.65g/100 mL	-28.1



Scheme S1 Procedure of extracellular proteins extraction and purification

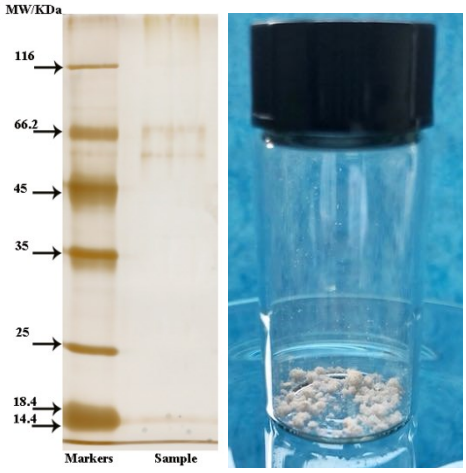


Fig. S1 Separation and detection of extracellular proteins by SDS-PAGE (a) and photograph of the obtained protein sample (b)

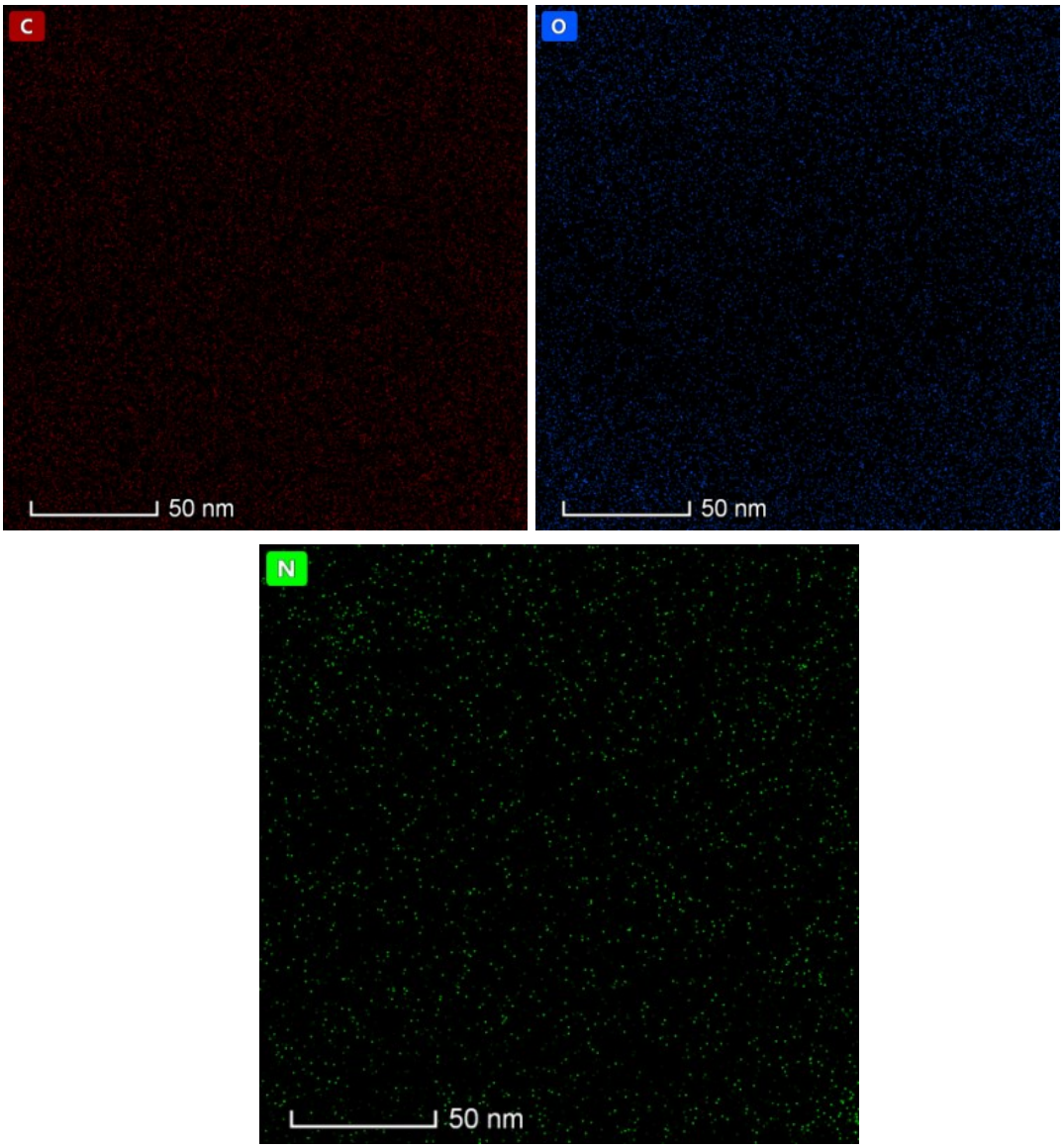


Fig. S2 TEM-mapping images of protein

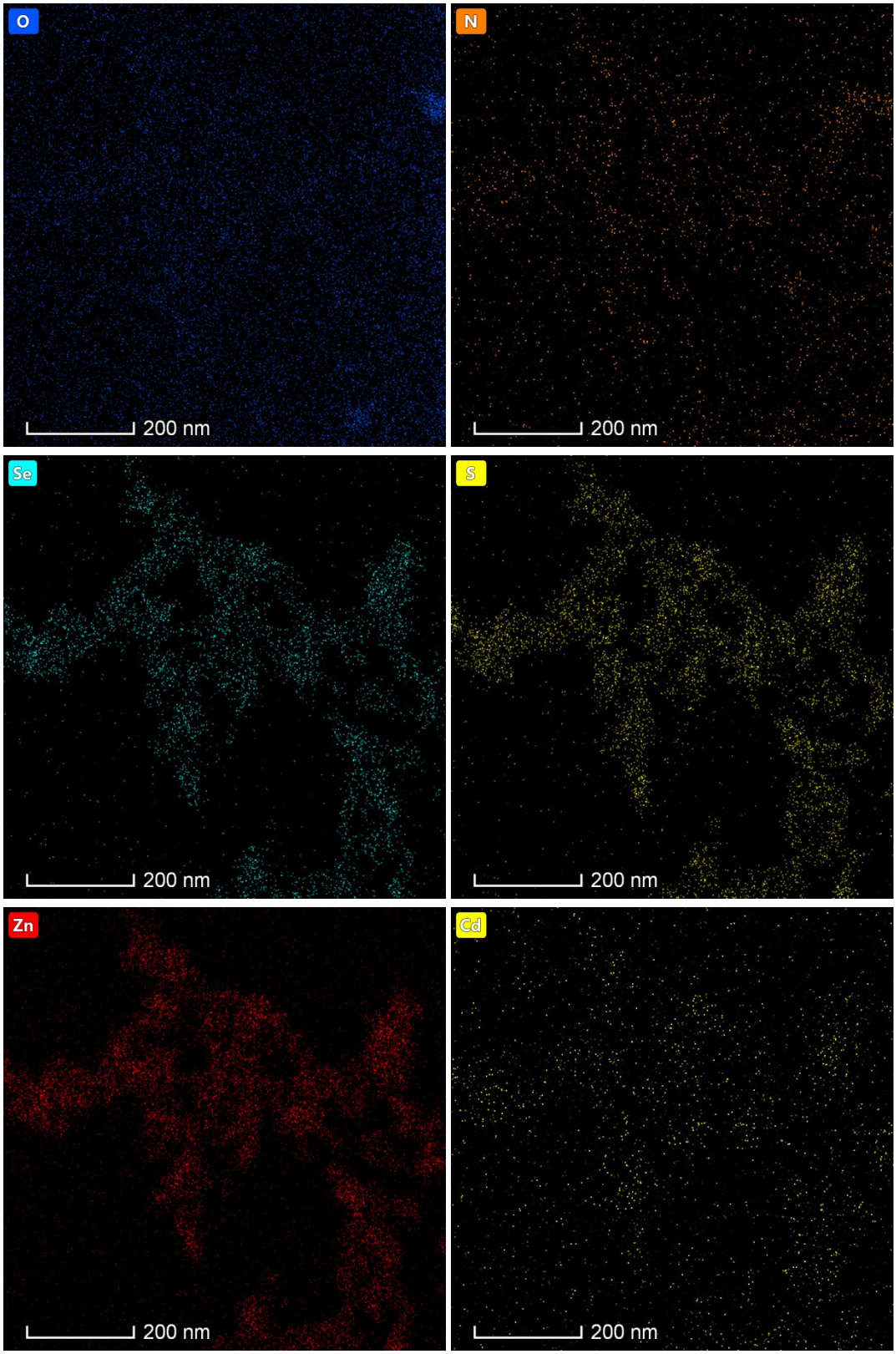


Fig. S3 TEM-mapping images of protein corona

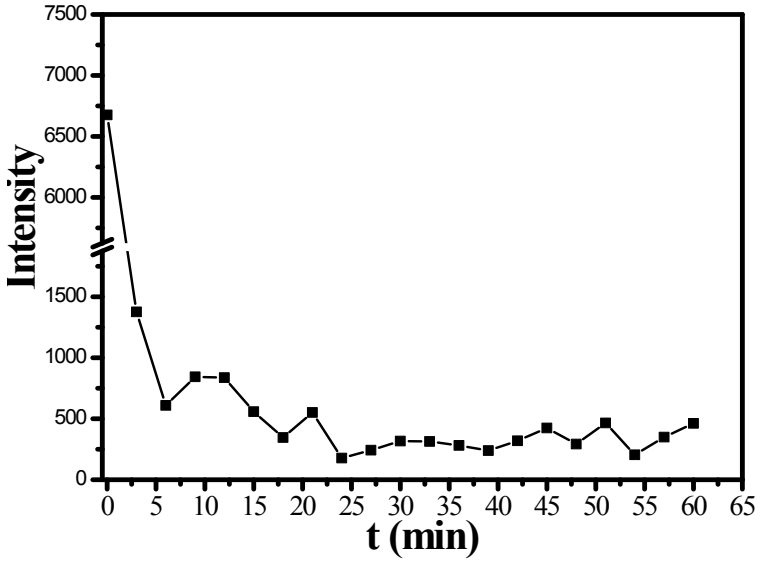


Fig. S4 Change of fluorescence intensity as reaction time

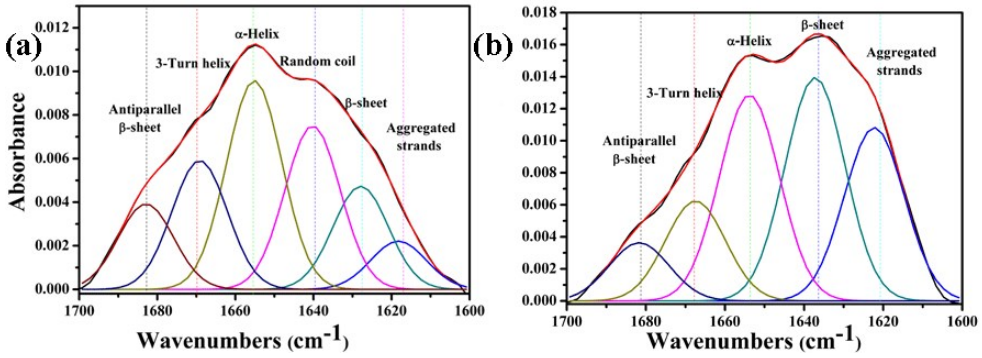


Fig. S5 Second derivative resolution-enhanced curve-fitted amide I region (1700-1600 cm^{-1}) for protein (a) and protein corona (b)

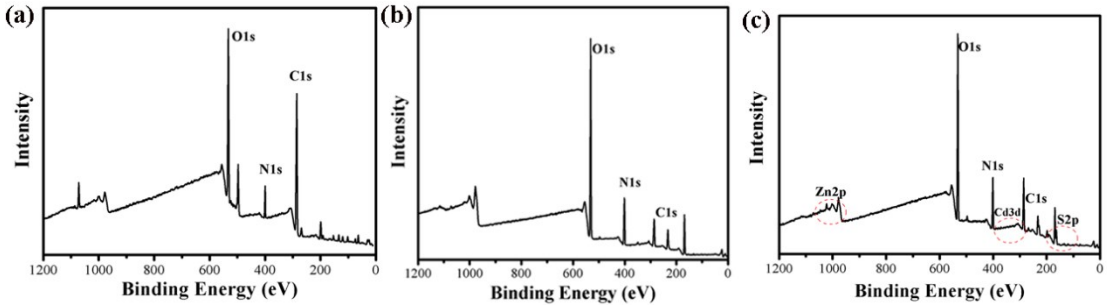


Fig. S6 XPS survey scan spectra of EPS (a), extracellular proteins (b) and protein corona (c)

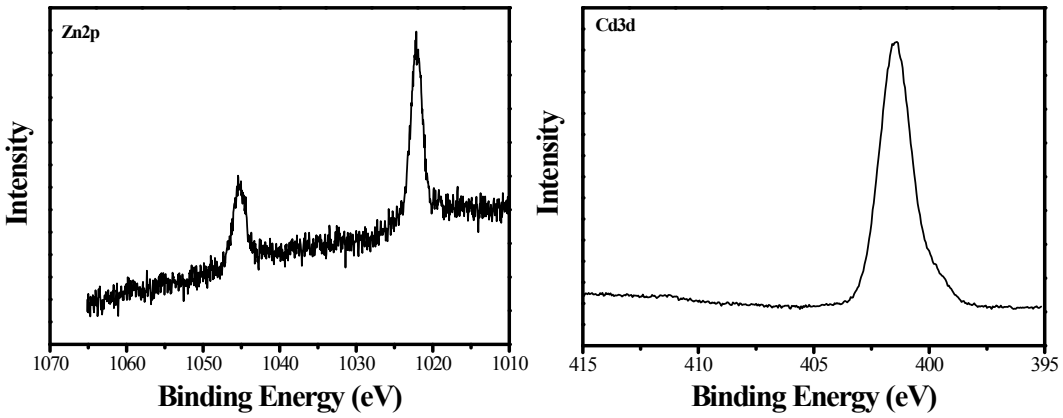


Fig. S7 High-resolution Zn2p and Cd3d spectra of protein corona

References

[1] J. R. Wieniawski, A. Zeugma, N. Nagaraj, M. Mann. Universal sample preparation method for proteome analysis, Nat. Methods, 2009 (6) 359–362.

[2] S. Tyanova, T. Temu, J. Cox. The MaxQuant computational platform for mass-spectrometry-based shotgun proteomics, Nat. Protoc., 2016 (11) 2301–2319.

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