Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Information

Complementary Mass Spectrometric Techniques for the Quantification of the Protein Corona: A Case Study on Gold Nanoparticles and Human Serum Proteins

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Table S1: Experimental conditions for the coupling SEC-ICP-MS

ICP-MS	
Instrument	Double-focusing sector field ICP-MS (Element 2, Thermo Fisher Scientific, Bremen, Germany)
RF power	1300 W
Auxiliary gas flow	0.87 L min^{-1}
Coolant gas flow	15.5 L min^{-1}
Nebulizer gas flow	0.95 L min^{-1}
Sampler cone	Nickel
Skimmer cone	Nickel
Isotopes monitored	$^{32}S^{+}$ and $^{34}S^{+}$
Chromatography	
Instrument	Agilent 1100 Series Liquid Chromatograph
Chromatographic column	Superdex-75 10/300 GL, 13 µm average particle size, 10×300 mm
Flow rate	0.5 mL·min^{-1} (room temperature)
Injection volume	50 µL
Mobile phase	50 mmol·L^{-1} ammonium acetate, pH = 6.8

Table S2: ICP-MS conditions for the flow-injection analysis of total S and Au concentrations

ICP-MS	
Instrument	Double-focusing sector field ICP-MS (Element 2, Thermo Fisher Scientific, Bremen, Germany)
RF power	1300 W
Auxiliary gas flow	0.87 L min^{-1}
Coolant gas flow	15.5 L min^{-1}
Nebulizer gas flow	0.95 L min^{-1}
Sampler cone	Nickel
Skimmer cone	Nickel
Isotopes monitored	$^{32}S^{+}$, $^{34}S^{+}$ and $^{103}Rh^{+}$ as internal standard (R = 4,000) $^{197}Au^{+}$ and $^{103}Rh^{+}$ (R = 300)
Flow rate	1 mL min^{-1}
Injection volume	50 µL

Incubations with bovine serum albumin (BSA) as model protein

The incubations of the GNPs with BSA were carried out following method 2 as described for the serum incubations (Chapter: Instrumentation and methods). The particle number concentration of the GNPs was set to approx. $4 \cdot 10^9$/mL in 50 mg/mL BSA.

The results were as follows.

Table S3: Results of incubations with BSA as model protein

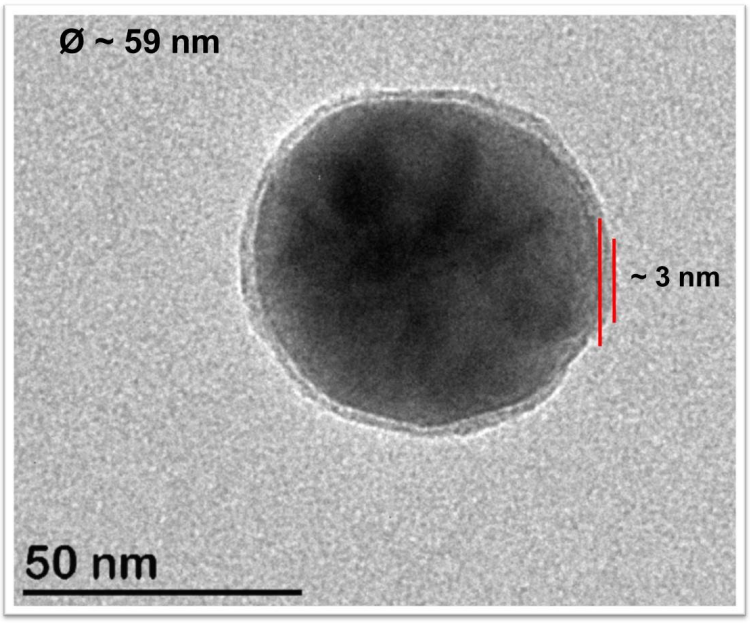
Incubated GNP	**Number of proteins per GNP (N = 3)**
10 nm	2725 ± 280
30 nm	2460 ± 406
60 nm	1510 ± 213

Transmission Electron Microscopy (TEM)

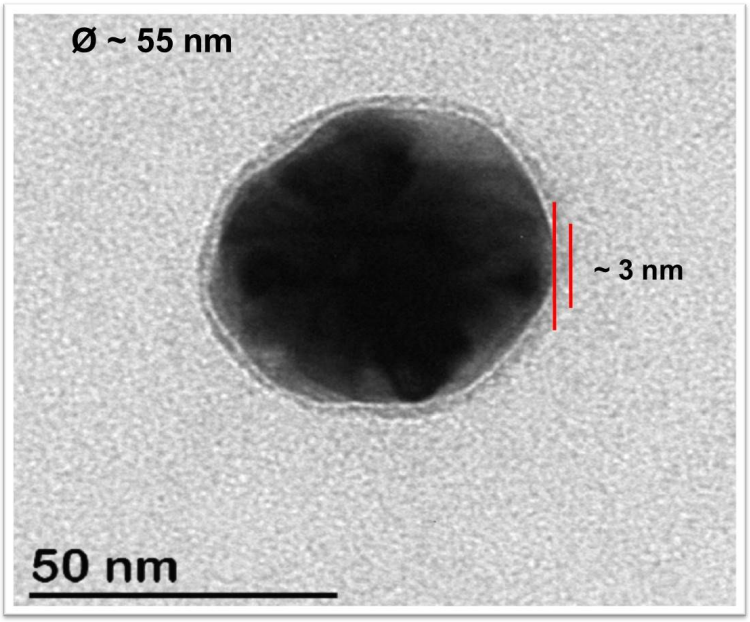
In order to obtain a deeper insight into the protein-nanoparticle bioconjugates, the biological layer around GNPs after the incubation and purification steps was visualized by TEM. This study was performed on a high-resolution transmission electron microscope, JEOL JEM-2100 (JEOL Ltd. Tokyo, Japan) operating at an accelerating voltage of 200 kV, by using Gatan 636 liquid N2 cooling holder (100 K). A 10 µL aliquot of the sample solution was diluted with distilled water, loaded on a carbon-coated copper grid, and then negatively stained with an aqueous solution of phosphotungstic acid (PTA, 2%). TEM images revealed the formation of protein corona around the spherical GNPs.

Figure S1: TEM images of purified and isolated GNPs after incubation (a) with BSA (60 nm nominal size), (b) with human serum (60 nm nominal size), (c) with BSA (30 nm nominal size), and (d) with human serum (30 nm nominal size).

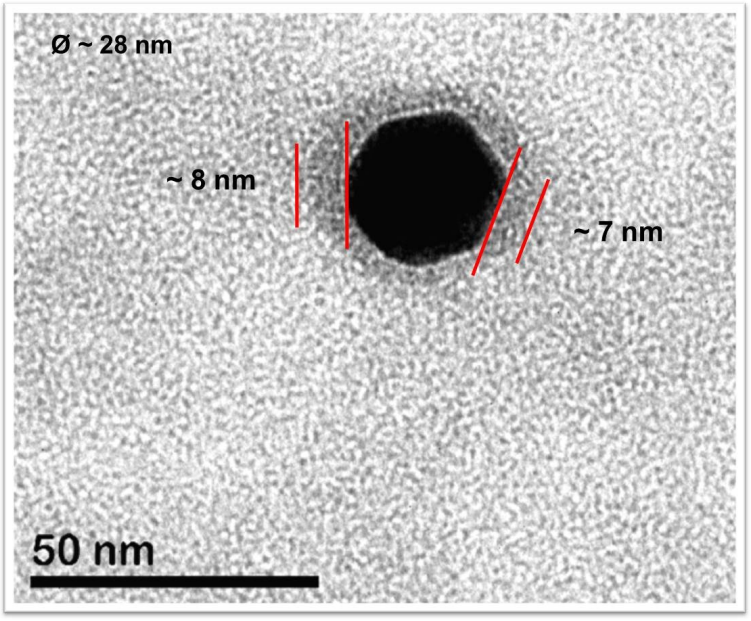
(Incubations of GNPs in both, BSA and human serum, resulted in the formation of protein layer with a thickness of approx. 3 nm in the case of the 60 nm GNPs. This indicated the formation of a mono-layered protein corona. Incubations with 30 nm GNPs showed the formation of a thicker protein layer (~ 7 - 8 nm) indicating the formation of a multilayer.)

(a) 

(b)



(c)



(d)