Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supplementary Information

A different protein corona cloaks "true-to-life" nanoplastics with respect to synthetic polystyrene nanobeads

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Details for Human blood collection and plasma preparation

Peripheral blood was obtained from healthy subjects (Geriatrics Unit, Spedali Civili of Brescia, Italy). The study was approved by the ethics committee of Civil Hospitals of Brescia on 8th June 2016 (Informed consent was obtained from all the subjects enrolled in the study). EDTA was used as anticoagulant. Blood samples were processed within 2 h from the withdrawal and kept at room temperature. Plasma-EDTA (plasma) was centrifuged at 800 *g* for 10 min (5804R Eppendorf Centrifuge, A-4-44 rotor, 15 ml tubes), 2,500 *g* for 15 min and then centrifuged a second time at 2,500 *g* for 15 min. All centrifugation steps were made at room temperature with low acceleration and avoiding the application of the centrifuge brake. After each centrifugation plasma was collected in a fresh plastic tube, leaving 1 cm of plasma above the buffy layer to not disturb it. Plasma was finally transferred into cryo-vials in 1 ml aliquots and stored at -80˚C.[41]

Water pre-treatment

The proper pre-treatment of Milli-Q water is a crucial step to avoid external and unknown contamination in the concentrated T2LNP pellet. Figures S1 a-c show AFM topography images of untreated Milli-Q water following the same centrifugation steps of separation and concentration set for the T2LNPs production. It is possible to note AFM fields rich in interfering nanoparticles. Figures S1 d-f show properly treated Milli-Q water (see Materials and Methods section), confirming neglecting contaminations.

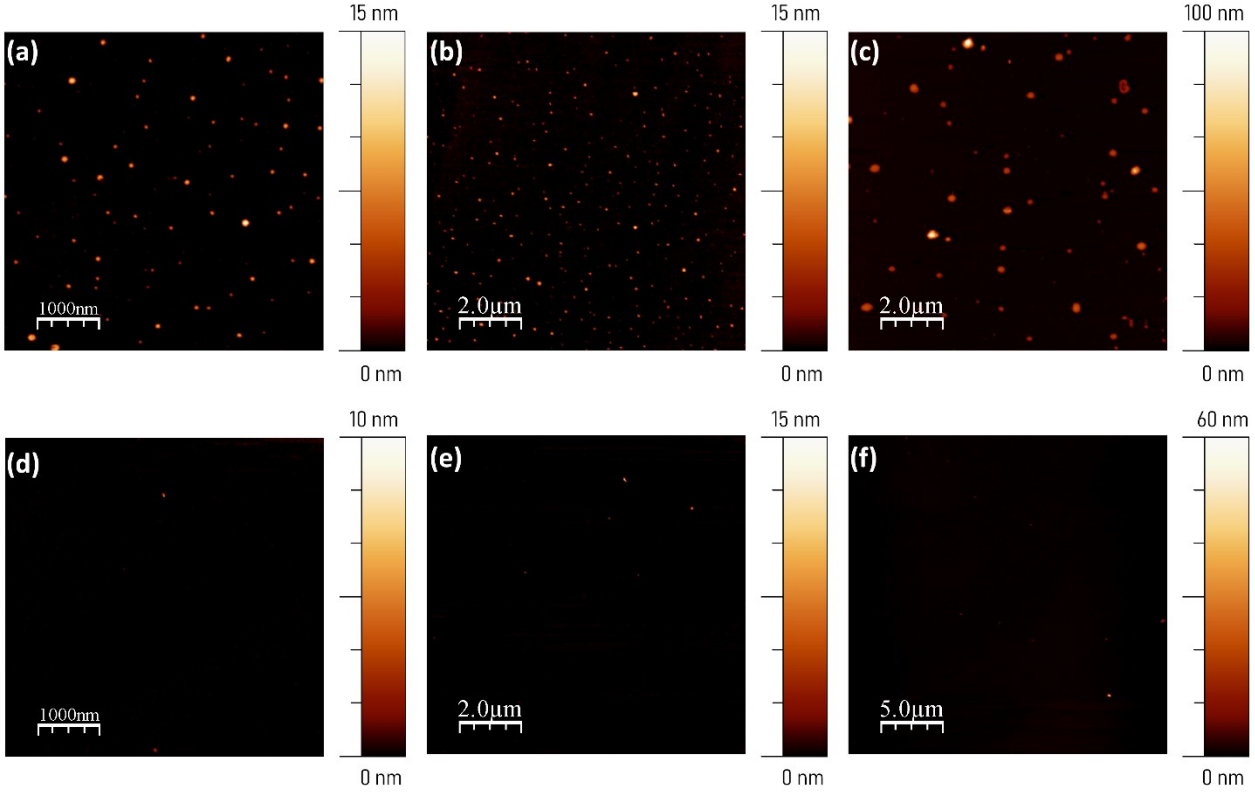

Figure S1: Water check before centrifugation (a-c) and after centrifugation (d-f).

FT-IR measurements of thermal degradation of T2LNPs

In the proposed protocol, we set a preliminary embrittlement phase in liquid nitrogen of 30 minutes, followed by the milling step under a constant flow of liquid nitrogen. The correct coupling of mechanical fragmentation and the use of liquid nitrogen ensures the protection of the polymer from thermal degradation induced by the local heat generated from the mechanical forces acting during the process. Figure S2 shows a FT-IR spectrum of T2LNPs without a correct time of embrittlement and a continuous flow of nitrogen during the fragmentation, in comparison of parental polystyrene macro piece. FT-IR spectrum shows the loss of characteristic bands associated with polystyrene, suggesting the chemical degradation of nanoplastics.

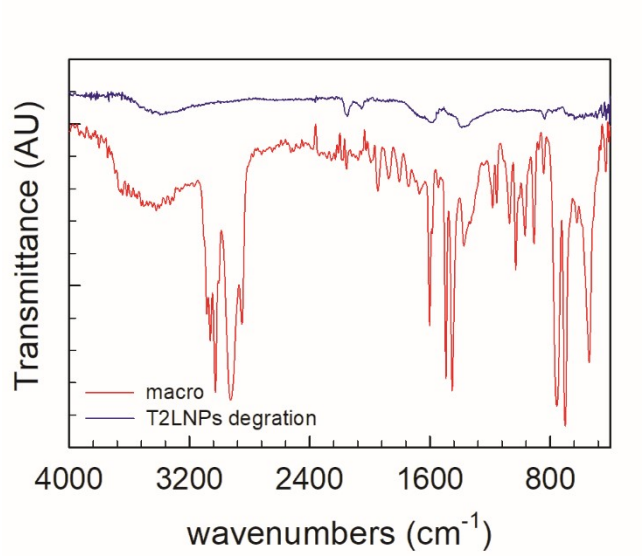

Figure S2. FT-IR spectra of polystyrene macro pieces and T2LNPs without a proper time of embrittlement.

AFM on nanobeads

Polystyrene nanobeads have been characterized using the same techniques employed for T2LNPs, specifically: FT-IR spectroscopy for chemical characterization (see Figure 2 in the manuscript, the green spectrum corresponds to the nanobeads) and AFM for the morphological characterization: in Figure S3, a representative AFM image is reported, showing that nanobeads have a monodisperse size distribution of 165 nm.

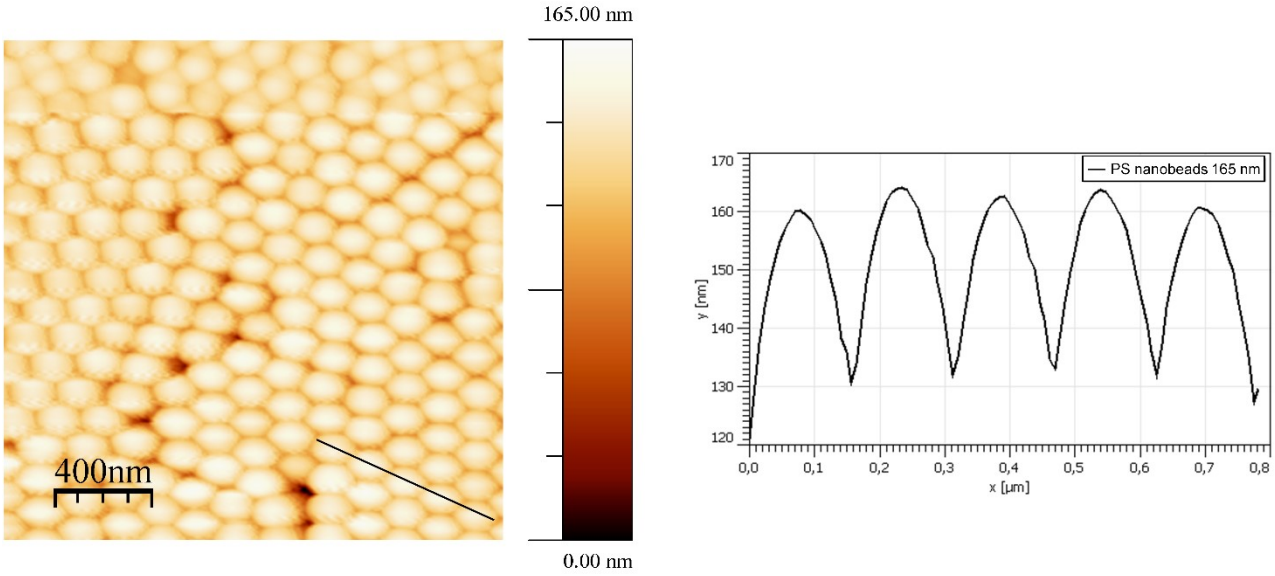

Figure S3: AFM image of nanobeads (a) and profile analysis (b).

Inter-sample size distribution analysis of two T2LNPs samples from AFM images

Two different T2LNP samples independently prepared were analyzed by AFM to provide a strong and statistically representative size distribution, as reported in the manuscript. An inter-samples analysis between the two different T2LNPs samples was also performed, to verify the production repeatability of the proposed mechanical fragmentation protocol.

In Figure S4, size distribution analysis is reported, highlighting the overlap between the two T2LNPs sample distributions. In Table S1, statistical details provide median values of z-dimension of 6.8 nm for the first sample and 8.0 nm for the second one and median values of diameter of 22.9 nm and 27.6 nm for the two samples, respectively. Mean values of z-dimension and diameter are (14.2 ± 0.2) nm and (41.3 ± 0.5) nm, respectively, for the first sample, and (12.9 ± 0.3) nm and (44.7 ± 0.8) nm, respectively, for the second sample.

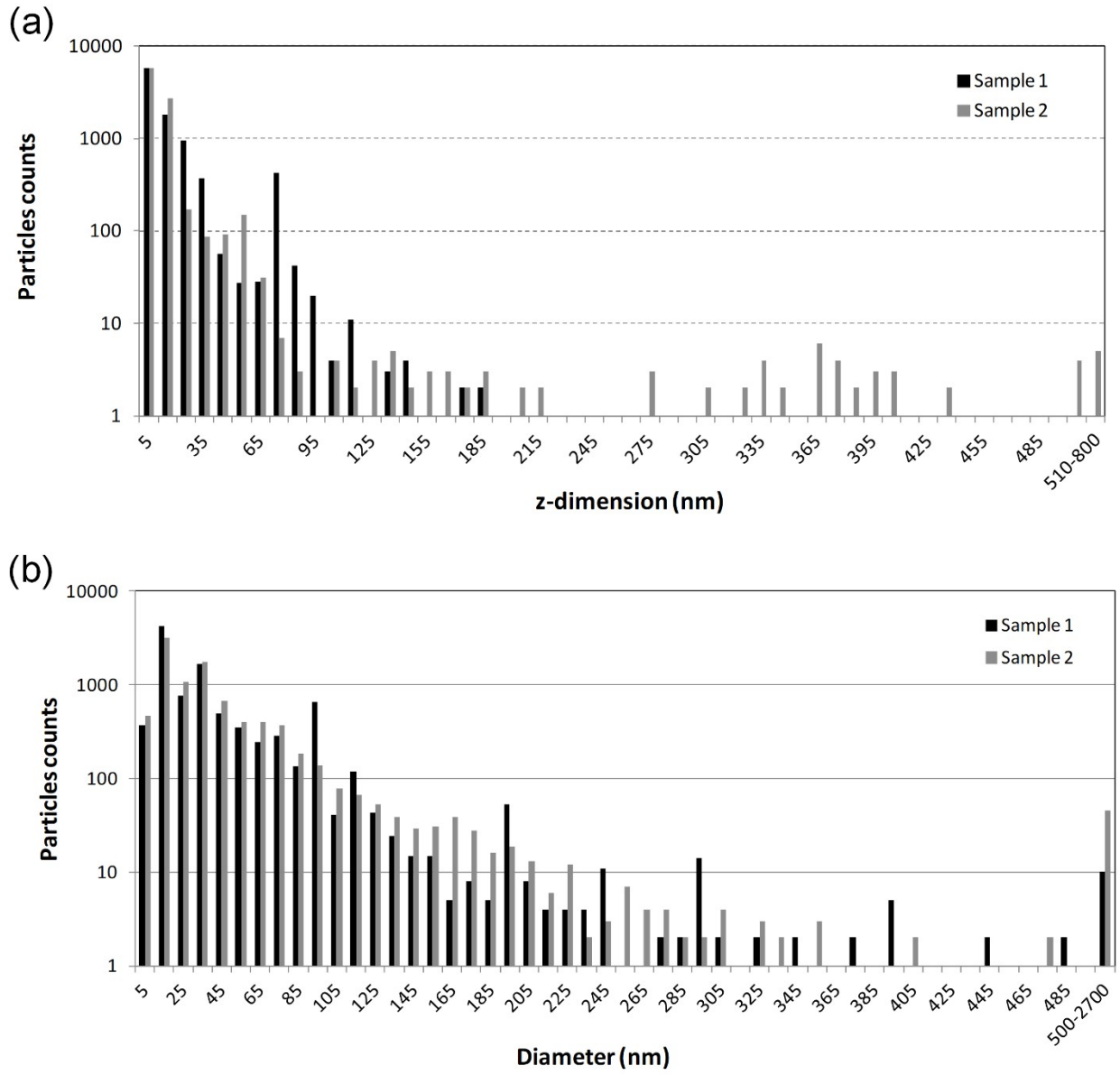

Figure S4. Inter-sample size distribution analysis of T2LNPs samples from AFM images: (a) z-dimension and (b) diameter of two independent samples of T2LNPs.

Z-dimension: Statistics

	Sample 1	Sample 2
Total number of values	9557	9121
Number of excluded values	0	0
Number of binned values	9557	9121
Minimum	1,2	1,3
25% Percentile	3,8	5,2
Median	6,8	8,0
75% Percentile	15,8	12,1
Maximum	275	798
Mean	14,2	12,9
Std. Deviation	19	32
Std. Error of Mean	0,2	0,3
Lower 95% CI of mean	14	12
Upper 95% CI of mean	15	14

Diameter: Statistics

	Sample 1	Sample 2
Total number of values	9557	9121
Number of excluded values	0	0
Number of binned values	9557	9121
Minimum	7,8	7,8
25% Percentile	19,5	19,5
Median	22,9	27,6
75% Percentile	45,7	45,8
Maximum	1120	2690
Mean	41,3	44,7
Std. Deviation	45	77
Std. Error of Mean	0,5	0,8
Lower 95% CI of mean	40,4	43,1
Upper 95% CI of mean	42,2	46,3

Table S1. Statistics of size distribution analysis from AFM images for two independent T2LNPs samples.

FT-IR measurements after protein corona formation

The formation of the protein corona was also checked by performing FT-IR measurements after the incubation of nanobeads and T2LNPs in human plasma. It is possible to note the presence of typical absorption bands of proteins in the region around 1650 and 1540 cm^{-1}, ascribed to amide I and amide II. In particular, they are primarily due to the carbonyl stretching vibration and N–H bending vibrations, respectively. The lower in intensity in some regions could be attributed to the three washing steps after protein corona formation and the consequent material loss.

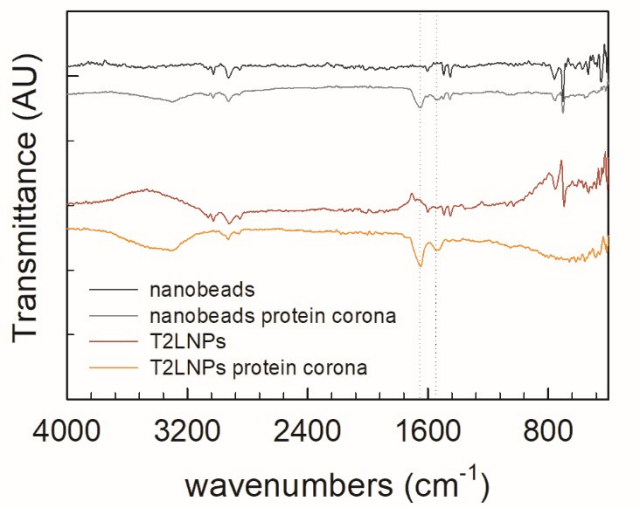

Figure S5. FT-IR measurements before and after protein corona formation on nanobeads and T2LNPs.

AFM images after protein corona formation on T2LNPs

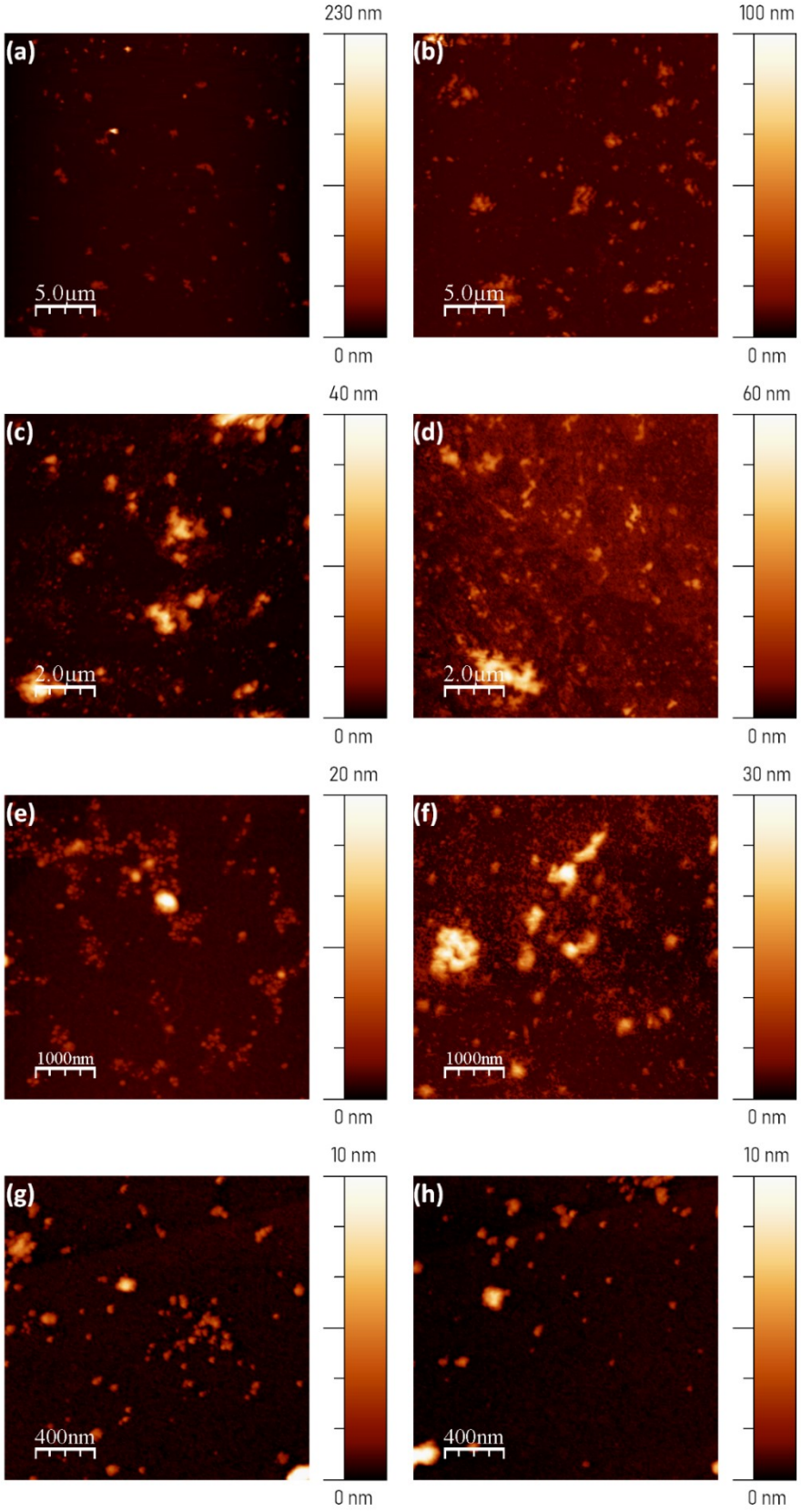

Figure S6. AFM on T2LNP samples after incubation with human plasma and protein corona formation.

Influence of nanobeads concentration of protein corona formation

Three nanobeads samples of different concentration (10 µg/ml, 25 µg/ml and 50 µg/ml) were incubated in human plasma and analyzed for protein corona formation, to assess the effect of nanoparticles dose on corona formation.

This analysis clearly demonstrates that changes in the nanobead concentration do not affect the type of proteins that interact with nanobead surface, but only intensity of signals (i.e., the number of proteins), that increases as the nanobead concentration increases.

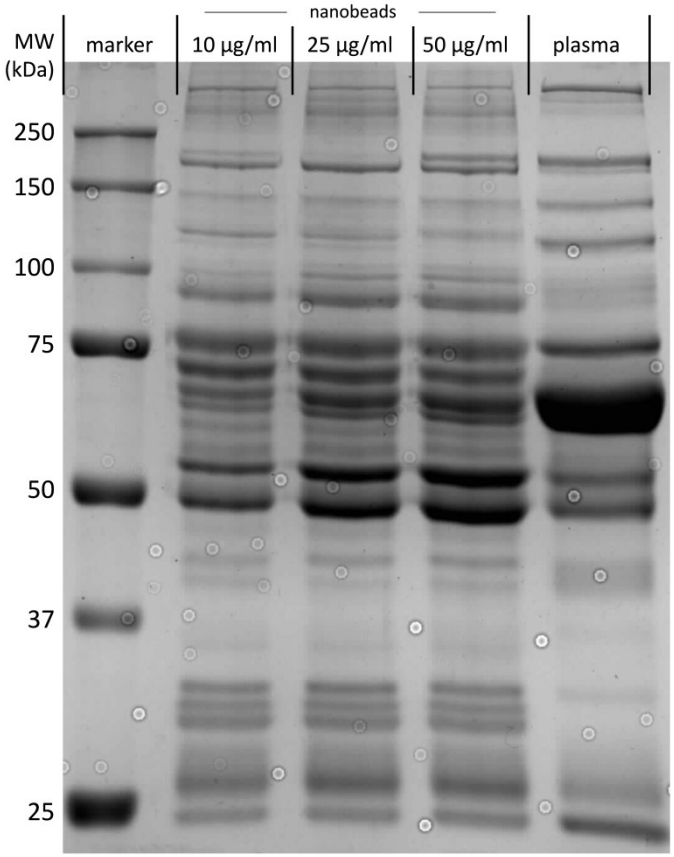

Figure S7. SDS-PAGE analysis of protein corona formed on nanobead samples of increasing concentrations.