SUPPORTING INFORMATION

Plasma Concentration Gradient Influences the Protein Corona Decoration on Nanoparticles

Mahdi Ghavami\textsuperscript{a}, Samaneh Saffar\textsuperscript{b}, Baharak AbdeEmamy\textsuperscript{a}, Afshin Peirovi\textsuperscript{b}, Mohammad A. Shokrgoza\textsuperscript{a}, Vahid Serpooshan\textsuperscript{c}, and Morteza Mahmoudi\textsuperscript{c,d,e}\textsuperscript{*}

\textsuperscript{a}National Cell Bank, Pasteur Institute of Iran, Tehran, Iran.

\textsuperscript{b}Core Facility Center, Pasteur Institute of Iran, Tehran, Iran.

\textsuperscript{c}Division of Pediatric Cardiology, Department of Pediatrics, Stanford University School of Medicine, Stanford, California 94305-5101, United States

\textsuperscript{d}Nanotechnology Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

\textsuperscript{e}Current Address: School of Chemical Sciences, University of Illinois at Urbana-Champaign, 600 South Mathews Avenue, Urbana, Illinois 61801

*Corresponding Author: Web: www.biospion.com; Email: Mahmoudi@illinoise.edu
Experimental Section

Materials

Nanoparticles (NPs). Carboxylated polystyrene NPs with the mean size of 100 nm were purchased from Invitrogen (USA). Plain silica NPs with 2 different sizes (100 and 200 nm) were purchased from Kisker-Biotech inc. (Germany).

Human plasma. Blood was withdrawn from 10 volunteers from the National Cell Bank staff (Pasteur Institute of Iran, Tehran, Iran), and collected into 10 ml K2EDTA coated tubes (BD Bioscience (UK)). Plasma was prepared following the HUPO BBB SOP guidelines.[1]

Hard corona preparation. Samples were incubated under non-gradient plasma (i.e. 10%, 30%, 50%, 70%, and 80%) and gradient-plasma (i.e. 10%-30%, 50%-70%, and 70%-80%) concentrations. Plasma solutions were diluted with PBS maintaining the final NP concentration constant and equal to 100 μg/ml. For the non-gradient plasma interactions, the NPs were incubated with the plasma solutions at 37°C for one hour. For the gradient plasma interactions, the NPs were incubated with the initial plasma solutions at 37°C for half an hour followed by an additional half an hour incubation with increased plasma (pure plasma was added to the incubated solution to increase the protein concentration). To obtain hard corona complexes, after the incubation in various types of plasmas, NPs were centrifuged to pellet the particle-protein complexes and separated from the supernatant plasma, as recognized as standard method[2]. The pellet was then re-suspended in 500 μl of PBS and centrifuged again for 3 minutes at 18 krcf at 4°C to pellet the particle-protein complexes. The standard procedure consisted of three washing steps before re-suspension of the final pellet to the desired concentration.

SDS PAGE. Upon completion of the last centrifugation step, the NP-protein corona pellet was re-suspended in protein loading buffer and boiled for 5 minutes at 100 °C. Subsequently, an equal sample volume was loaded in 15% and 20% polyacrylamide gels and gel
electrophoresis was performed at 120V, 400mA for about 60 minutes (for each sample), until the proteins neared the end of the gel. The gels were stained with silver staining method.

**Liquid chromatography mass spectrometry (LC-MS/MS) technique.** Associated proteins in the hard corona composition of the desired bands (Figure 3 b and d) were probed. The bands were cut and the proteins were collected. Samples were loaded onto a nanoAcquity UPLC system (Waters Corp., USA) equipped with a nanoAcquity Symmetry C_{18}, 5 µm trap (180 µm x 20 mm Waters) and a nanoAcquity BEH130 1.7 µm C_{18} capillary column (75 µm x 250 mm, Waters). The trap wash solvent was 0.1% (v/v) aqueous formic acid and the trapping flow rate was 10 µL/min. The trap was washed for 5 min before switching flow to the capillary column. The separation used a gradient elution of two solvents (solvent A: 0.1% (v/v) formic acid; solvent B: acetonitrile containing 0.1% (v/v) formic acid). The flow rate for the capillary column was 300 nL/min. Column temperature was set at 60°C and the gradient profile was as follows: initial conditions of 5% solvent B, followed by a linear gradient to 30% solvent B over 125 min, then a linear gradient to 50% solvent B over 5 min, followed by a wash with 95% solvent B for 10 min. The column was returned to initial conditions and re-equilibrated for 30 minutes before subsequent injections. The nanoLC system was interfaced with a maXis LC-MS/MS System (BrukerDaltonics) with a nano-electrospray source fitted with a steel emitter needle (180 µm O.D. x 30 µm I.D., Proxeon). Positive ESI- MS & MS/MS spectra were acquired using AutoMSMS mode. Instrument control, data acquisition and processing were performed using Compass 1.3 SR3 software (microTOF control, Hystar and DataAnalysis, BrukerDaltonics). Instrument settings were: ion spray voltage: 1,500 V, dry gas: 6 L/min, dry gas temperature 160 °C, ion acquisition range: m/z 50-2,200. AutoMSMS settings were: MS: 0.5 s (acquisition of survey spectrum), MS/MS (CID with N\textsubscript{2} as collision gas): ion acquisition range: m/z 300-1,500, 0.1 s
acquisition for precursor intensities above 100,000 counts, for signals of lower intensities down to 1,000 counts acquisition time increased linear to 1s, the collision energy and isolation width settings were automatically calculated using the AutoMSMS fragmentation table.; 5 precursor ions, absolute threshold 1,000 counts, preferred charge states: 2 – 4, singly charged ions excluded. 1 MS/MS spectrum was acquired for each precursor and former target ions were excluded for 30 s. Tandem mass spectral data were submitted to database searching using a locally-running copy of the Mascot program (Matrix Science Ltd., version 2.3), through the BrukerProteinScape interface (version 2.1). All spectra were searched against the Swiss-Prot database restricted to mammalia sequences only (63,676 sequences). Search parameters specified: Enzyme; Trypsin, Peptide Mass Tolerance; 10 ppm, Fragment Mass Tolerance; 0.1, Fixed Modifications; Methylthio (C), Variable Modification; Oxidation (M). All peptide identifications were filtered to only accept expect values of 0.05 or lower. Empirically derived estimates of peptide false discovery rate are calculated from searches against a Mascot generated decoy database.

**Analyzing the LC-MS/MS results.** In order to assess the alterations in the amount of various proteins (due to the interaction of particles with gradient plasma), a semi-quantitative assessment of the protein quantity was conducted by utilizing the spectral counting method (SpC). The normalized SpC amounts of each protein, as previously described in the MS study of smooth and jagged surfaces[^3^], were calculated using the following equation:

\[
NpSpC_k = \left( \frac{SpC}{(M_w)_k} \right) \times 100
\]

\[
\sum_{i=1}^{n} \left( \frac{SpC}{(M_w)_i} \right) = \sum_{i=1}^{n} \left( \frac{SpC}{(M_w)_i} \right) \times 100
\]

[^3^]: [3]
where $N_pS_pC_k$ is the normalized percentage of spectral count for protein $k$, $SpC$ is the spectral count identified, and $Mw$ is the molecular weight (in kDa) of the protein $k$. Using equation 1, the protein size can be calculated and the actual contribution of each protein to the hard corona composition can be evaluated.[4] In order to determine the change in the protein amount, the equation below was used:

$$
\frac{(N_pS_pC_{k(non-gradient)} - N_pS_pC_{k(gradient)})}{N_pS_pC_{k(non-gradient)}}(2)
$$

The values below 0.6 (60%) were considered as significant decrease in protein amount and presented in Table 1.
**Differential Centrifugal Sedimentation (DCS).**

DCS experiments were performed using a CPS Disc Centrifuge DC24000. The Disc Centrifuge device is a particle size analyzer for measuring particles in the range of 0.01µm to 40µm. The analyzer measures particle size distributions using centrifugal sedimentation within an optically clear spinning disc that is filled with fluid. Sedimentation is stabilized by a density gradient within the fluid and the accuracy of the measured size is insured through the use of a known size calibration standard run before each measurement. The use of a biological sample with a large amount of proteins requires a new sucrose gradient to be prepared for each measurement. Concentration of the particles at each size is determined by continuously measuring the turbidity of the fluid near the outside edge of the rotating disc. The turbidity measurements are converted to a weight distribution by Mie theory light scattering calculations. The choice of the experimental parameters such as fluid gradient and the speed of rotation is based on the type of arterial being analyzed and the range of sizes being measured. The primary information from the analytical disk centrifuge is the time taken for the particles to travel from the centre of the disk through a defined viscous sucrose gradient to a detector placed at the outer rim of the disk under a strong centrifugal force. For materials with homogenous density and simple shape (e.g. spherical particles) this time can be directly related to the particle size. When objects are inhomogeneous, or irregular in shape, the different arrival times still allow to distinguish between the particles. In the simplest approximation, it is possible to approximate them to an equivalent uniform sphere. This was applied to the entire figures presented in this work, by citing the sizes on the x-axis; hence, in the case of aggregated particles (e.g. dimers and trimers), the cited size should be considered as the ‘apparent’ size for particles. Moreover, for the sake of clarity in the comparisons made between different samples, we chose to present the data as relative weight particle size distribution. The tallest peak (highest weight value) in the distribution is called the ‘base’ peak (has a value of 100%) and all other particle size
(multimer) peaks are then normalized against this base peak to give a relative weight distribution. It is noteworthy that the conversion from absorption raw data to molecular weight data is correct as long as the optical parameters and the density for particles and fluid are correct and the particles are spherical. In this regard, the information we obtain for the large NP-protein clusters at about 500 nm are merely indicative of their existence but do not provide insight to their actual size or their absolute amount. It is notable that different sucrose gradient (1.018g/ml and 1.064g/ml) were employed for polystyrene and silica particles, respectively.
**Figure S1:** (a) 15% and (b) 20% SDS-PAGE gel of human plasma proteins obtained from silica(200 nm in size)-protein complexes free from excess plasma following incubation with human plasma at various concentrations (both non-gradient and gradient situations); from left to right, the lanes stand for protein ladder, 10%, 30%, 10%-30%, 50%, and 30%-50%. (c) 15% and (d) 20% SDS-PAGE gel similar to (a) and (b) with different plasma concentration from left to right, the lanes stand for protein ladder, 50%, 70%, 50%-70%, 80%, and 70%-80%.
**Figure S2:** DCS results for silica (200 nm) nanoparticles interacted with human plasma proteins at various non-gradient and gradient circumstances *in situ*; right graph is the enlargement of the main peak areas of the DCS graphs to enhance the shift to smaller size after interaction with plasma proteins.
References


