Experimental report

24/07/2021

Proposal: EASY-662 **Council:** 4/2020

Title: Protein corona formation on surface-functionalized polystyrene nanoparticles

Research area: Chemistry

This proposal is a new proposal

Main proposer: Maria MARTINEZ NEGRO

Experimental team:

Local contacts: Sylvain PREVOST

Samples: Polystyrene nanoparticles/ serum albumin/fibrinogen

Instrument	Requested days	Allocated days	From	To
D11	12	12	29/08/2020	30/08/2020

Abstract:

Blood proteins can actively interact with nanoparticles introduced into the body, giving rise to the formation of the “protein corona”. As a consequence, nanostructures can lose their chemical properties, leading to dramatic changes of their predesigned functionality. The magnitude of this effect depends on the nature and concentration of proteins forming the corona. Although great efforts have been made in recent years to understand the protein corona formation process, there are still significant issues that need to be addressed to design more biocompatible nanoparticles. For this reason, we aim at investigating the changes in nature and conformation of common plasma proteins interacting with polymeric nanoparticles. Also we want to evaluate the influence of different surface functionalization such as different chain length poly(ethylene glycol) (PEG) and SDS to unravel their role in the protein corona formation

Investigation of protein corona formation on surface-functionalized polystyrene nanoparticles

Maria Martinez Negro (Main Proposer), Sylvain Prevost (Local Contact)

Scientific Background. Nanomedicine's aim is the development of nano sized materials with unique properties that can be used for improved methods of diagnosis and therapy. However, blood proteins can actively interact with nanoparticles introduced into the body, giving rise to the formation of the "protein corona". As a consequence, nanostructures can lose their chemical properties, leading to dramatic changes of their predesigned functionality. The magnitude of this effect depends on the nature and concentration of proteins forming the corona. Parameters such as the physicochemical stability, blood circulation time, cellular uptake, transportation, or accumulation of the nanoparticles are strongly determined by the nature of the adsorbed proteins. In this sense, certain proteins are involved in the immunological response to pathogens or promotion of their clearance from the bloodstream, whereas other proteins can act as a receptor of cancer cells or avoid the recognition by the reticuloendothelial system. The understanding of the protein corona formation requires investigations on the interplay between the nanoparticles composition and surface functionalization with the distinct proteins found in the blood stream. For example, most nanoparticles require the use of different ligands or surfactants to provide them colloidal stability via electrostatic repulsions or steric hindrance, which play a pivotal role in the protein corona formation. Among the available surfactants, those containing polyethylene glycol (PEG) chains enable enhanced colloidal stability and low toxicity.

Experimental Part.

Proteins such as the human serum albumin (HSA, the most abundant protein), fibrinogen (involved in coagulation processes) and Myoglobin were studied studied by SANS using D11 beamline at the Institut Laue-Langevin (ILL, Grenoble, France). The interaction of these proteins with deuterated polystyrene nanoparticles functionalized with either a negatively charged surfactant (SDS) or a neutral one (Lutensol AT50) will be characterized.

1. Characterization of naked NPs and pure surfactants (Lutensols and SDS) and pure proteins, HSA, Myoglobin and fibrinogen.
2. Study of NPs stabilized with Lutensol and SDS as well as their interaction to the proteins after incubation for 1h at 37C

The data analysis was performed using SASview software which enables for obtaining the radius of nanoparticles, thickness shell of the surfactante and the the thickness shell of the protein around the nanoparticle. Information about the polydispersity can be also obtained from the analysis of SANS results. Furthermore, information about the scattering length density profiles of the supramolecular architectures can be obtained. In the following graphs it is shown the scattering profiles obtained for the NPs and the protein HSA as an examples. Similar results were obtained for the Myoglobin protein. In the case of fibrinogen, a lot of aggregation was found. For the modelling in case of NPs+ HSA a model considering a core shell sphere and fractal shell sphere were used.

Results. Figure 1 shows the SANS profiles obtained over the whole accessible Q-range for naked NPs presenting different average size and the corresponding fitting curves obtained using a model that considers the geometrical parameters of a fractal shell and core shell.

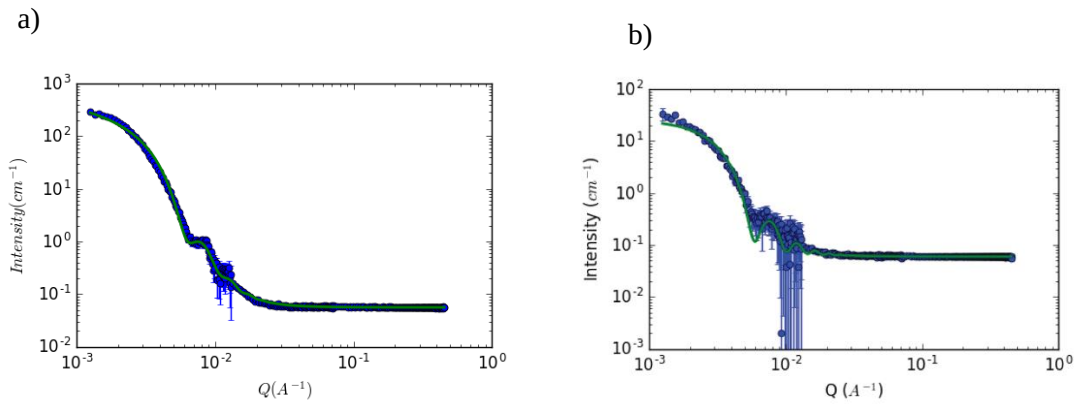

Figure 1. SANS profiles for polystyrene nanoparticles stabilized with SDS (a) and Lutensol (b). Corresponding fitting curves obtained using a Fractal core shell model (a) and a shell model (b)

The experimental curves show similar q-dependences for the scattered intensity for both nanoparticles. Some models were applied to find the best one. A fractal-core shell model satisfies the curve (a) at low q and the parameters present similar values to those previously obtained with a simple core-shell model. On the other side, the PS-NPs-Lut was adjusted to a core-shell model (b) with a polydispersity on the core radius that perfectly fit the curve to the model. The fractal core-shell model does not improve the fitting. The fact that a core-shell model perfectly adjusts the PS-NPs-Lut curve and together to a lower polydispersity needed to enhance the fit implies that the PS-NPs-Lut are more monodisperse than PS-NPs-SDS.

After incubation with the protein HSA and myoglobin, the scattering profiles were adjusted to different model. Figure 2 shows the PS-NPs-SDS + HSA (a) and PS-NPs-Lut + HAS (b)

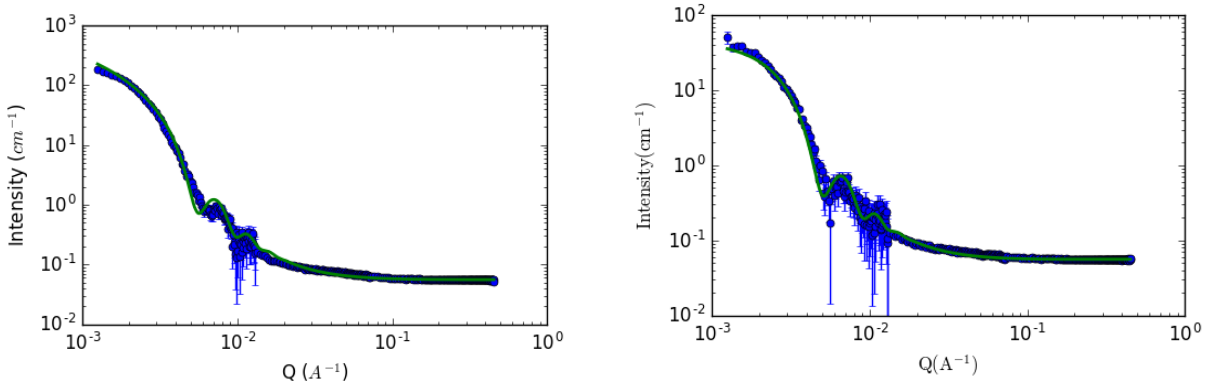

Figure 2. SANS profiles for polystyrene nanoparticles stabilized with SDS (a) and Lutensol (b) + HAS corresponding fitting curves obtained using a Fractal core shell model (a) and a shell model (b)

The same models than the naked NPs were applied for both NPs, such as the fractal shell model for PS-NP-SDS and a core-shell model for PS-NPs-Lut. The SLD of the shell was imposed from the HSA, whereas it was kept from the polystyrene for the core-shell.

Table 1 summarize all the data from the analysis. The radius for PS-NPs- SDS is 650 Å, whereas for PS-NPs-Lut is 683 Å. These values depend entirely on the amount of detergent added at the beginning of the synthesis procedure. Since the surfactants are different and it is not possible to precisely tune the outcoming size on a single nanometer scale, the variation in the radius values between NPs is entirely expected. Furthermore, a shell thickness of 2 Å for PS-NP-SDS matches with the presence of SDS molecules, whereas 3Å shell thickness for PS-NPs-Lut is slightly thicker. This value may suit Lutensol molecules spread out on the nanoparticle surface, sticking out from the surface into the solution with a brush or mushroom conformation. In this case, a low Lutensol density is expected, which implies likely a mushroom conformation than the brush.

NPs	RADIOUS (Å)	SHELL THICKNESS (Å)
PS-NPs-SDS	650	2
PS-NPs-Lut	693	3
PS-NPs-SDS + HSA	650	14
PS-NPs-Lut + HSA	693	18

Table 1. Data from the radious and shell thickness are summarized for all the samples.

Conclusions. An ellipsoidal model fits for the native HSA was an ellipsoidal model with an equatorial radius of 40 Å and 20 Å for the polar one. After the HSA incubation with the NPs an increase of the shell thickness is expected due to the protein adsorption. The shell thickness increases to 14 Å for PS-NPs- SDS and raised to 18 Å PS-NPs- Lut. Thus, both shell thickness values are significantly lower than for native HSA, which may agree with a completely denatured HSA on the surfaces. A total protein denaturation on PS-NPs- SDS may justify a shell thickness on average slightly thinner than for PS-NPs- Lut where it can reveal partially native HSA. The results obtained in this proposal are intended to be published in amanuscript that is at the present time in preparation