Experimental report

24/01/2024

Proposal: 8-04-941 **Council:** 10/2022

Title: Dynamical effects of protein-nanoplastic interactions

Research area: Biology

This proposal is a new proposal

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Experimental team: Stefano DA VELA

Local contacts: Tilo SEYDEL

Samples: BSA
Silica nanoparticles 150 nm
PS100 nanoparticles

Instrument	Requested days	Allocated days	From	To
IN16B Si 111 BATS	4	2	03/07/2023	05/07/2023

Abstract:

Proteins can interact with the surface of nanometer-scale plastic particles (nanoplastics) to form a so-called protein corona. This process requires adhesion of the protein, not necessarily followed by unfolding, to the particle surface. We wish to investigate the global and internal short-time dynamics of a model blood serum protein (BSA) upon corona formation, which are expected to be dramatically affected by this process. Nanoplastics are object of current studies as an environmentally relevant pollutants, and a better understanding of the dynamical consequence of their interactions with proteins is desirable.

Experimental Report Experiment 8-04-941 IN16b

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Introduction and aims

In order to gain a better understanding of nanoplastics as pollutants and of their effect on biological macromolecules, we focused in these experiments on the changes in the dynamics of the model protein Bovine Serum Albumin (BSA) upon interaction and adhesion on polystyrene nanoparticles in a saline buffer. In this and similar buffers the formation of a so-called "protein corona" around the particles has been detected [1,2]. The main aim of this first series of measurements was to verify whether changes in protein dynamics due to the presence of the nanoplastics are detectable at all, with a secondary goal to obtain data which can be used to model the details of the diffusive and internal dynamics of both adsorbed and free protein.

Materials and sample preparation

Polystyrene particles, 100 nm diameter (Sigma-Aldrich, provided as H_2O dispersion) were exchanged to D_2O using 30 kDa MWCO centrifugal concentrators, obtaining a stock at 10% particles in D_2O. BSA stock solutions at 100, 200, 400 mg/mL were prepared by dissolving an appropriate mass of BSA powder (Sigma-Aldrich) in a D_2O-buffer at twice the final desired salts concentration for the samples (PBS2x, containing 308 mM NaCl, 11.1 mM Na_2HPO_4 and 4.2 mM KH_2PO_4 adjusted to pD 8). BSA comparison solutions at 50 and 200 mg/mL were prepared by dissolving the appropriate mass of BSA powder in (D_2O-)PBS1x (154 mM NaCl, 5.6 mM Na_2HPO_4 and 2.1 mM KH_2PO_4 adjusted to pD 8). Samples containing both polystyrene and BSA in PBS1x were prepared by mixing equal volumes of polystyrene particles stock solution and BSA stock solution at twice the final desired concentrations by pipetting and incubated ~1h at room temperature before measurements.

Measurements

The following solutions were measured on IN16b in BATS configuration using cylindrical aluminium sample containers:

- Polystyrene particles 5% in D2O
- Polystyrene particles 5% + BSA 50 mg/mL in PBS 1x
- Polystyrene particles 5% + BSA 100 mg/mL in PBS 1x
- Polystyrene particles 5% + BSA 200 mg/mL in PBS 1x
- BSA 200 mg/mL in PBS 1x
- BSA 50 mg/mL in PBS 1x
- PBS1x

All solutions were measured at 293 K. Two additional measurements were repeated at 310K for the samples with 5% polystyrene particles and 100 and 200 mg/mL BSA to check for temperature effects.

Preliminary evaluation of the results

While as expected polystyrene particles alone show only a residual QENS signal due to their larger size and slow diffusion, we can induce a small but appreciable change in the QENS spectra of BSA solutions in the presence of the particles (Fig.1 showing the effect for 50 mg/mL BSA, zoom in the inset). This is still detectable, albeit weaker probably due to obfuscation from

free BSA, at higher protein concentrations (Fig.2). Additionally, the effect seems to be not too sensitive to the temperature in the range probed (not shown).

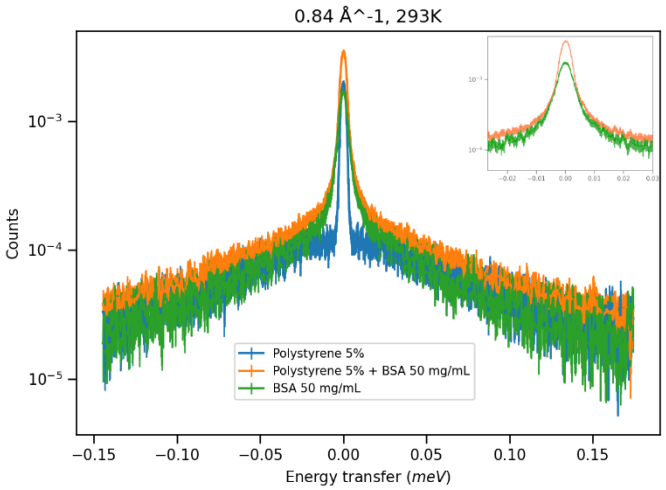

Figure 1

As working hypothesis, we assume that the self-diffusion on or in the vicinity of the surface of the polystyrene surface (in/near the protein corona) is altered due to increased chances of interaction between proteins or to confinement effects.

Fig.3 shows an example of a first preliminary fit of QENS spectra for Polystyrene 5% in D_2O (light blue squares) and for Polystyrene 5% + BSA 50 mg/mL in PBS (dark blue circles, data for q=0.6 $Å^{-1}$, T=293 K). The PS contribution gives rise to an elastic line (magenta solid line), and the solvent to a very broad Lorentzian. Additional Lorentzians are used to describe the BSA signal. The analysis of the data is ongoing and further experiments will be greatly beneficial to allow a clearer modelling of this phenomenon.

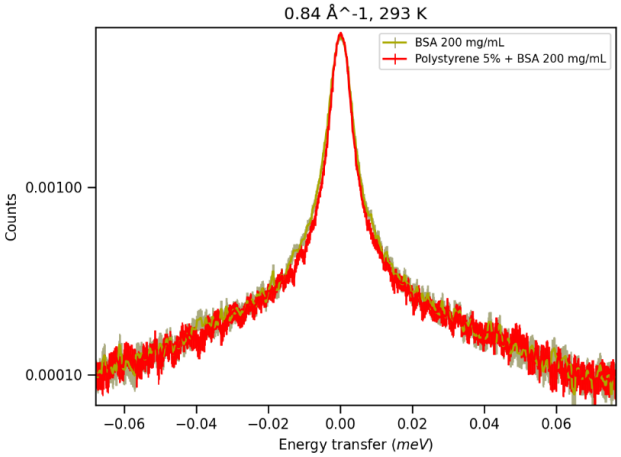

Figure 2

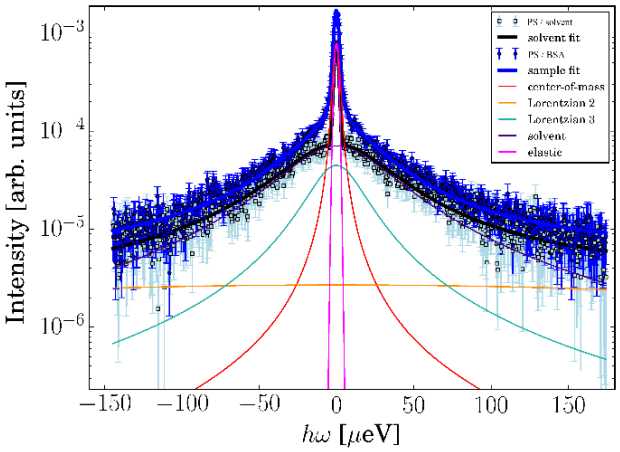

Figure 3

References

[1] Gräwert, M. et al. *in preparation*

[2] Kihara, S. et al. *Bioconjugate Chemistry* 30.4 (2019): 1067-1076