Experimental report

01/06/2020

Proposal: 9-13-838 **Council:** 4/2019

Title: Structural studies of nanogels-proteins at air-water interface

Research area: Biology

This proposal is a new proposal

Main proposer: Ali ZARBAKHSH

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Samples: N-isopropylacrylamide
2-Acrylamido-2-methylpropane sulfonic acid

Instrument	Requested days	Allocated days	From	To
FIGARO	2	2	20/01/2020	22/01/2020

Abstract:

Proteins compete for the nanoparticle surface, leading to a protein corona that largely defines the biological identity of the particle. Thus, knowledge of rates, affinities, and stoichiometries of protein association with, and dissociation from, nanoparticles is important for understanding the nature of the particle surface seen by the functional machinery of cells. In this proposal, the interaction between nanogels with protein (lysozyme) at the air-water interface on a molecular scale will be investigated by using NR technique. This work will be carried out as a function of the nanogel structure in addition to the study of the kinetic of the adsorption process.

1 PRINCIPAL INVESTIGATOR

Name and institution of the Principal Investigator

Dr A Zarbakhsh
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2 EXPERIMENT DETAILS

Experiment: 9-13-838

Title: Structural studies of nanogels-proteins at air-water interface

Instrument: FIGARO

Dates scheduled: 20th January 2020 to 22nd January 2020	No. Days allocated: 2

Date of experimental report: 30th May 2020

3 EXPERIMENT OBJECTIVES

The use of nanomaterials for drug delivery offers important advantages, such as improved drug solubility and stability, increased bioavailability and tailored release. Although numerous studies have been conducted on the characterisation of the delivery vehicles and on maximising the drug loading, the reality is that the 'chemical entity' that is initially created in the laboratory, is significantly altered once introduced into the body. The large concentration of proteins available in plasma can interact with the nanoparticle, forming so called protein corona complexes. The cell 'sees' and interacts with the entire nanoparticle–protein corona complexes rather than with the 'bare' entity of nanoparticle. The biological fates and functions of these nanoparticles are determined by physiological responses toward these nanoparticle–protein complexes as the effective biological unit of nanoparticles. There is currently still limited understanding of the interfacial behaviour of these nanomaterial-protein corona complexes, especially with biological membranes, and its relationship with the chemical structure of the nanoparticles on the molecular level.

We have a track record in the development of polymeric nanogels for applications in catalysis, sensor and drug delivery vehicles. We have successfully synthesised a series of methylene-bis-acrylamide (MBA) crosslinked N-isopropylacrylamide (NIPAM) based nanogels via high dilution radical polymerisation and characterised them by neutron reflectivity (NR) measurements in combination with dynamic light scattering (DLS) and tensiometry to study the behaviour at the silicon/water and air/water interfaces as a function of concentration and temperature.

The focus of this experiment is to study and develop a robust protocol for resolving the formation proteins/nanogels complexes and their conformations at the air/water interface. The aim is to link the chemical structure of nanogels (hydrophobicity and charge) to the complex formation behaviour in the presence of protein. This information is important in the design of effective drug carriers.

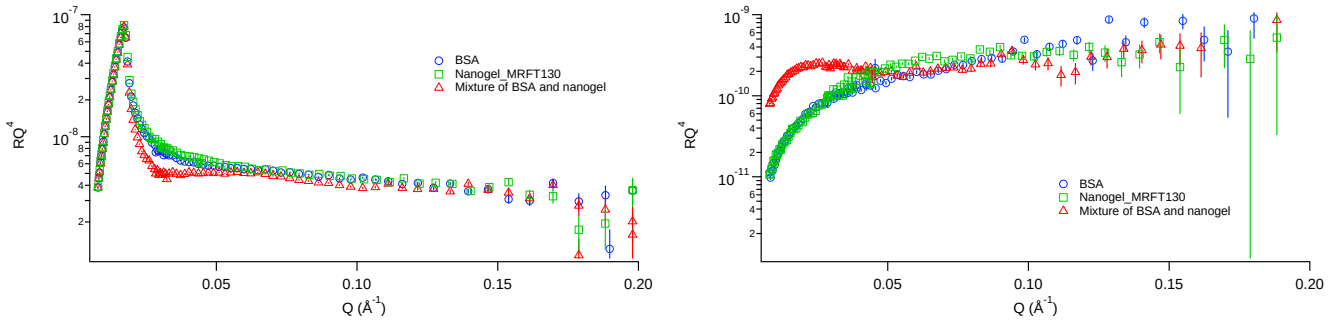
4 EXPERIMENT REPORT

We studied the interactions of 4 positively changed nanogels with BSA in PBS buffer at room temperature. The chemical composition and scattering length density (SLD) are summarised in table 1. The concentration of nanogel and BSA were 0.1 and 4.0 mg ml-1, respectively. Several contrasts including D2O, CMAir. CMBSA and CMNano were used in order to resolve the interfacial behaviour of nanogels with/without BSA in details. Exemplary NR profiles of MRFT130/MRFT132 nanogels with and without BSA are presented (Fig 1 and Fig 2). In both D2O and CMAir contrasts, the original NR profiles are significantly different from both the nanogels and BSA on its own, which evidently suggests the very dissimilar interfacial behaviour of the nanogels at the presence of BSA. This may indicate the complexion of nanogels with BSA at the air/water interface. Detailed structural parameters from the

fitting of these profiles (which is still on progress) can provide evidence distinguish the nanogel-protein interaction from simply co-adsorption of nanogels with BSA.

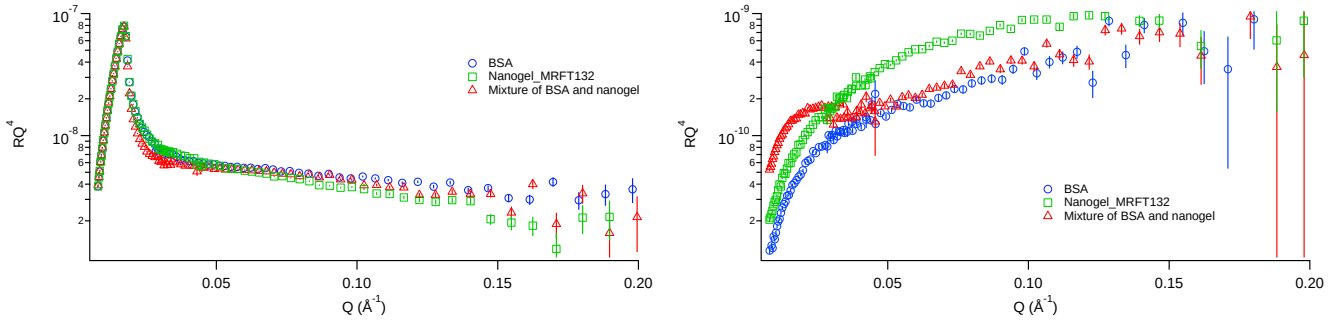
Table 1. Nanogels and the protein used in this experiment

Nanogels	Chemical composition	SLD	surface active?
MRFT130	60%AAm-d3 + 20%GUA + 20%MBA	3.4	YES
MRFT131	60%AAm + 20%GUA + 20%MBA	1.8	YES
MRFT132	60%NIPAM-d7 + 20%GUA + 20%MBA	4.2	YES
MRFT133	60%NIPAM + 20%GUA + 20%MBA	1.4	YES
Protein (BSA)	NRW	D2O	CM2.5 (CMbsa)
SLD of BSA (10^{-6} Å^{-2})	2.0	3.3	2.5



(a) (b)

Fig. 1. NR profiles for MRFT130 nanogels with and without BSA in (a) D2O and (b) CMAir contrast at the air/water interface at 25°C. The concentration of nanogel and lysozyme are 0.1 and 4 mg ml^{-1}, respectively.



(a) (b)

Fig. 1. NR profiles for MRFT132 nanogels with and without BSA in (a) D2O and (b) CMAir contrast at the air/water interface at 25°C. The concentration of nanogel and lysozyme are 0.1 and 4 mg ml-1, respectively.

5 LIKELY OUTCOMEs FROM EXPERIMENT

Please indicate what the experiment is likely to lead to by putting an 'x' next to one or more of the possible outcomes below.

Likely outcome	
Journal publication	*x*
Data for thesis	*x*
Follow-up experiment at ILL	-
Follow-up experiment at another facility	*x*
Other	*x*
No outcome anticipated	-

6 SUGGESTIONS FOR IMPROVEMENTS TO YOUR EXPERIMENT, EQUIPMENT OR THE FACILITY

NA