Experimental report

01/06/2020

Proposal: 9-12-588 **Council:** 10/2019

Title: Nanogel-Protein complex formation at the air/water interface

Research area: Chemistry

This proposal is a new proposal

Main proposer: Marina RESMINI

Experimental team: Pengfei LIU
Ali ZARBAKHSH

Local contacts: Armando MAESTRO

Samples: NIPAM

Instrument	Requested days	Allocated days	From	To
FIGARO	4	3	28/01/2020	31/01/2020

Abstract:

Nanogel-protein interaction determines the biological fates and functions of these gels when considering their bioavailability and tailored release as drug delivery vehicles. The cell ‘sees’ and interacts with the entire nanoparticle–protein corona complexes rather than with the ‘bare’ entity of nanoparticle because of the large concentration of proteins available in plasma. This proposal is focused for resolving the formation proteins/nanogels complexes and their conformations at air/water interface to develop a robust protocol, aiming to link the chemical structure of nanogels to the interfacial and complex formation behaviour.

1 PRINCIPAL INVESTIGATOR
Name and institution of the Principal Investigator
Prof. M Resmini Department of Chemistry Queen Mary University of London UNITED KINGDOM

2 EXPERIMENT DETAILS	
Experiment: 9-12-588	
Title: Nanogel-Protein complex formation at the air/water interface	
Instrument: FIGARO	
Dates scheduled: 28th January 2020 to 31st January 2020	No. Days allocated: 3
Date of experimental report: 30th May 2020	

3 EXPERIMENT OBJECTIVES

Nanogel-protein interaction determines the biological fates and functions of these gels when considering their bioavailability and tailored release as drug delivery vehicles. The cell 'sees' and interacts with the entire nanoparticle–protein corona complexes rather than with the 'bare' entity of nanoparticle because of the large concentration of proteins available in plasma. The aim was to resolve the formation proteins/nanogels complexes and their conformations at air/water interface to develop a robust protocol, aiming to link the chemical structure of nanogels to the interfacial and complex formation behaviour.

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.

This is the 2nd part of a back-to-back experiment. In the first part, we did a few screening experiments to explore the key parameters which governing interactions of nanogels with BSA. We found that both the charge and hydrophobicity played important roles on the nanogel-protein complex formation. In this experiment, we focused on the kinetics study of nanogels with and without BSA. Time-resolved measurements on nanogel-protein mixtures will allow us to resolve information about adsorption kinetics and structural progression of protein layers at the air/water interface in the absence and presence of nanogels in the bulk. This will help not only to elucidate the kinetics of protein adsorption at interfaces, but also contribute to identify the key structural features of the nanogels that facilitate interaction and permeation through membranes.

4 EXPERIMENT REPORT

Here an exemplary NR profiles of the kinetic study of positively charged nanogels MRFT130 with and without BSA in PBS buffer at room temperature. The chemical composition and scattering length density (SLD) of the nanogel are summarised in Table 1. The concentration of nanogel and BSA were 0.1 and 4.0 mg ml-1, respectively. Figure 1 shows the batch fitting of BSA on its own, nanogel on its own and the mixture of them. Parameters obtained from the fits were used to calculate to the volume fraction of water and layer thickness at the interface, which are presented in Figure 2. Both the nanogel and BSA were reasonably fitted with a single monolayer model. The layer thickness increased gradually in the first 20 minutes before reaching a plateau region. The thickness at equilibrium were 60 ± 5 and 70 ± 2 Å. The volume of water in the adsorbed BSA layer followed the same trend as described for the layer thickness. In contrast, it decreased slightly from 91% to 86% in the case of nanogels on its own, which suggests the

adsorbed nanogels layer became slight denser. Interestingly, a one-layer model was not adequate to fit the mixture of nanogels with BSA. This may indicate the complex formation of the nanogel-protein. We preliminarily fitted the data using a two-layer model while keeping the SLD of the 1st layer (2.0×10^{-6}Å^{-2}) and volume fraction of water of both layers fixed (72% for the 1st layer and 76% for the 2nd layer). The variations in SLD of the 2nd and layer thickness of both layers as a function of time are given in Figure 2c. Both layer thickness and SLD did not change significantly. Detailed data analysis is still in progress.

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
Protein (BSA)	NRW	D2O	CM2.5 (CMbsa)
SLD of BSA (10^{-6} Å^{-2})	2.0	3.3	2.5

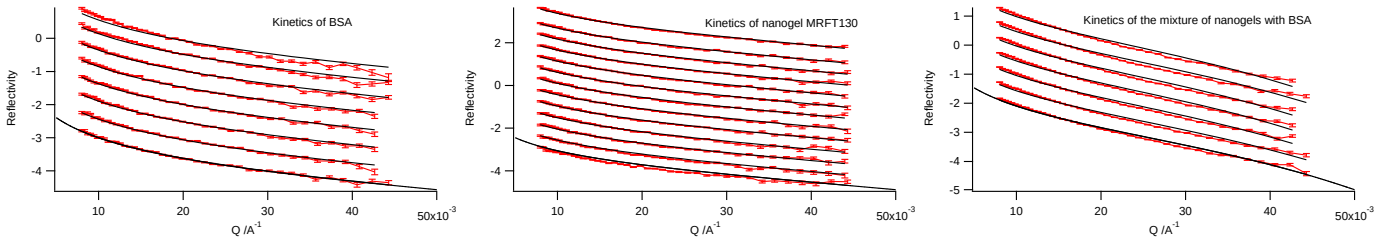

Fig. 1. Batch fitting of kinetics NR profiles of MRFT130 nanogels with and without BSA at the air/water interface at 25°C. The concentration of nanogel and lysozyme are 0.1 and 4 mg ml^{-1}, respectively.

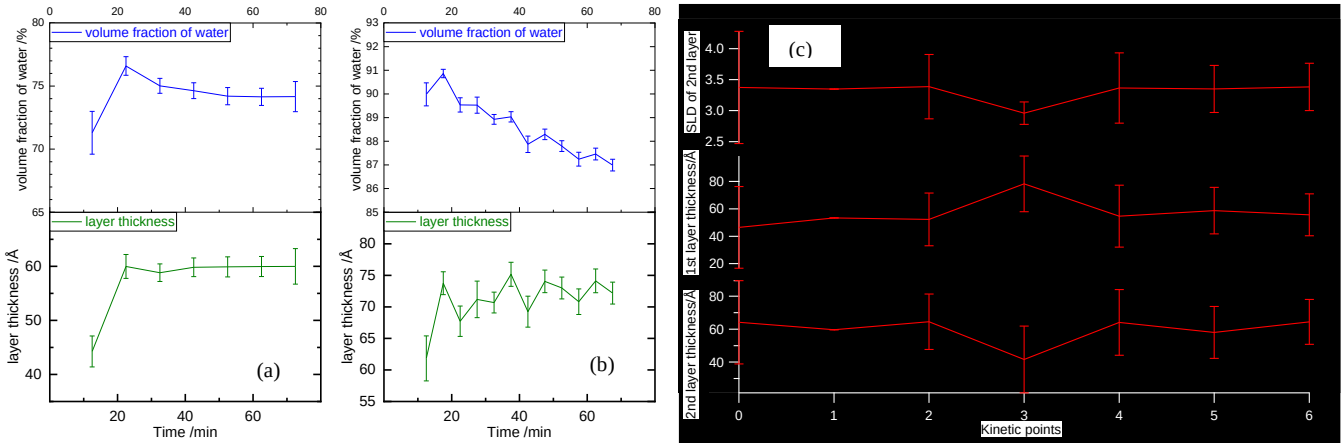

Fig. 2. Calculated volume fraction of water, layer thick and scattering length density of adsorbed layers at the air-water interface of (a) BSA on its own, (b) nanogel on its own and (c) mixture of nanogels with BSA. The concentration of nanogel and BSA are 0.1 and 4 mg ml^{-1}, respectively.

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