Experimental report

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Proposal: **8-02-877** **Council:** 4/2019

Title: Kinetics of UBX Self-Assembly at the Air/Water Interface

Research area: Biology

This proposal is a new proposal

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Samples: Ultrabithorax protein

Instrument	Requested days	Allocated days	From	To
FIGARO	2	2	14/01/2020	16/01/2020

Abstract:

Control of mechanical properties and chemical functionality are key drivers in a range of implanted structures for biomedical applications. This proposal focusses on a new material with the ability to control both of these properties. Ultrabithorax (UBX) protein has been shown to spontaneously self-assemble at the air-water interface to form elastomeric films that can be drawn into fibres or deposited onto a substrate. However, preliminary investigations using laboratory-based techniques have uncovered considerable complexity in the film formation process. In particular, there appears to be a consistent non-monotonic development in the film with time. It appears that the key changes correspond to either submicron lateral structural developments, or structural changes normal to the interface. It is the kinetics of these changes that we would like to probe with neutron reflectivity.

Kinetics of UBX Self-Assembly at the Air/Water Interface

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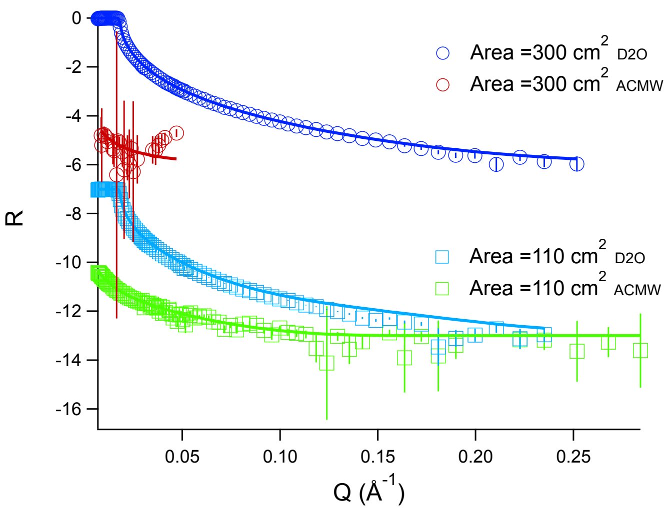
Ultrabithorax (UBX) protein is a transcription factor, derived from the fruit fly which plays a key role in the regulation of information transfer between RNA and DNA. Monomeric Ubx has been shown to spontaneously self-assemble at the air-water interface to form a film. This film can be pulled in fibres or wrapped in films and can be used in different applications, such as implant coatings for enhanced biocompatibility and environmental sensing.

At the state of art, the early chemical and physical mechanisms involved in film-formation are unknown. This lack of information is crucial for future industrial applications, since it prevents us to totally control the phenomenon and to tune the material properties depending on the specific application.

After preliminary investigations using laboratory-based technique, we investigated with neutron reflectivity the film properties of EGFP-Ubx, a fusion between Ubx and Enhanced Green Fluorescent Protein (EGFP). The main goal of the experiments was to investigate the time evolution of nanostructural details of the air-water interface in the presence of Ubx. We performed the experiments on FIGARO, which was equipped with a Langmuir Blodgett trough (LB trough) where the sample was kept in the horizontal position. The reflectivity profile was measured as a function of time to follow the evolution of the nanostructure of the UBX aggregates at the air-water interface. We used 50 µl of 10µM solution of EGFP-Ubx, in 140 mL of buffer, Air Contrast Matched Water (ACMW, 8.13 % v/v D_2O + 91.87% v/v H_2O) and pure D_2O. The total area (barriers fully opened) of the LB trough was 300 cm^2 and the smallest area (barriers fully closed) 110 cm^2. In order to study better the compression effect on film structure we measured the reflectivity at two additional halfway areas 200 cm^2 and 150 cm^2.

In the following paragraph we will present the results obtained during the experiments. Note that one day of the two assigned was lost due to technical problem on the instrument.

In the figure below the reflectivity curves for D2O and ACMW are shown at two different areas (300 cm^2 and 110 cm^2). The data at two areas are shifted for clarity. The first visible result is the difference between the reflectivity for ACMW at A = 300 cm^2 and 110 cm^2. When the barriers are fully open at 300 cm^2, the signal is overall noisy and not measurable at Q > 0.05 $Å^{-1}$. After the film compression the resulting signal is stronger and measurable over the whole Q range. This result is coherent with surface-pressure (π-A) values: at the beginning, the Ubx-monomers interactions are minimal, and the monomers start to aggregate forming small islands. As the barriers move closer, the interactions become stronger because of the decrease of area per molecule. This results in an increase of π-A values and consequently in a more compact film structure. A preliminary analysis of the reflectivity curves was done by modelling the protein layer with a simple slab. The surface excess Γ can be expressed as $\Gamma = \frac{\rho d\varphi}{bN_A}$, where ρ is the scattering length density of the protein contained in the slab, d is the thickness of the slab, b the scattering length of the protein. In terms of surface excess, the preliminary fitting analysis resulted in a Γ= 0.79 and 0.13mg/m^2 for A=110 cm^2 and 300 cm^2,



respectively. This experiment show that valuable information can be obtained on Figaro equipped with a LB trough for this particular protein at the solid liquid interface.