Experimental report

23/05/2016

Proposal: 8-02-710 **Council:** 10/2014

Title: Life in extreme environments: The role of intrinsically disordered proteins at ice-liquid water interfaces

Research area: Soft condensed matter

This proposal is a new proposal

Main proposer: Alexander ROUTH

Experimental team: Fanny YUEN
Matthew WATSON
Alexander ROUTH

Local contacts: Robert BARKER

Samples: LEA protein in water

Instrument	Requested days	Allocated days	From	To
D17	4	3	23/06/2015	26/06/2015

Abstract:

The ability of extremophile organisms to survive hostile conditions has intrigued scientists as they develop new technologies to preserve biological materials. Intrinsically disordered proteins (IDPs), such as LEA proteins, have been found to be upregulated in some plants and animals as these organisms gain cold and desiccation tolerance. LEA proteins have been shown to protect globular proteins, such as pig citrate synthase (CS) and rabbit lactate dehydrogenase, and a human cell proteome, from abiotic stress. The mechanism behind these protective abilities is still unclear, but leading hypotheses involve chaperone or shield interactions. However, our preliminary experiments suggest that these interactions are insufficient to explain the protection observed. This has led us to propose that LEA proteins preferentially adsorb onto surfaces generated in the freeze-thaw process, thereby excluding folded proteins from interfaces where they would undergo irreversible aggregation. The aim of this study is to investigate the adsorption and structural conformation of an LEA protein and CS at an ice/water interface analogue in order to better understand how IDPs protect globular proteins.

ILL Experimental Report

		Experiment Number:	8-02-710
Title of Experiment:	Life in extreme environments: The role of intrinsically disordered proteins at ice-liquid water interfaces	**Local Contact:**	Robert Barker
Principal Proposer:	Dr Alex Routh	**Instrument:**	D17
Affiliation:	University of Cambridge		
Experimental Team:	Fanny Yuen, Alex Routh, Matthew Watson	**Experiment Date:**	23-26/6/2015

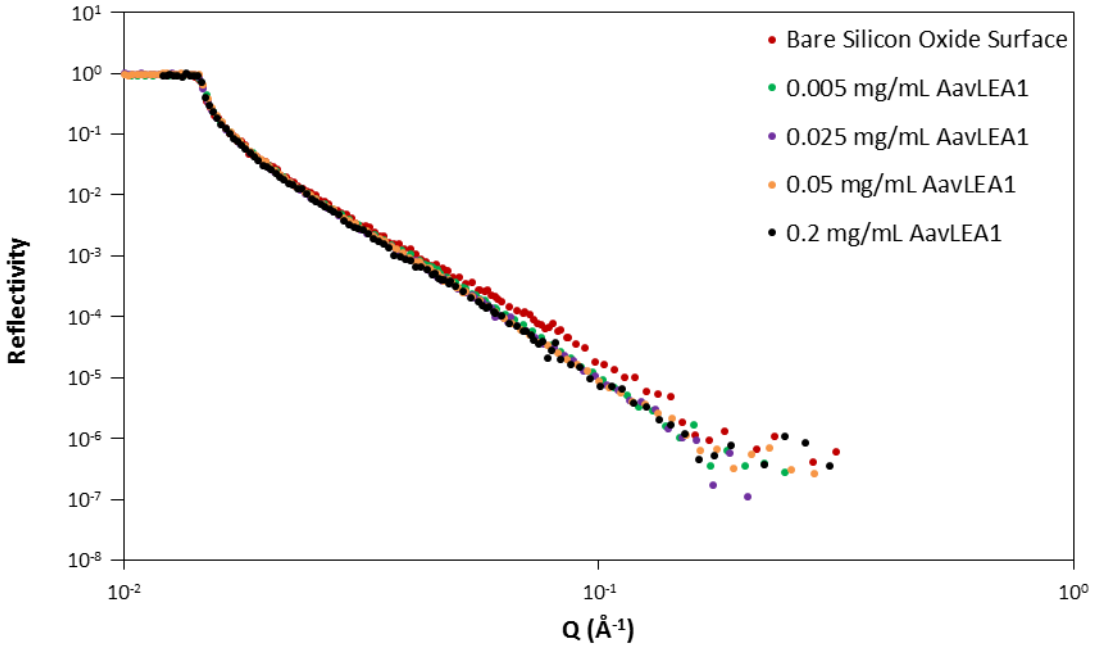

Figure 1: Silicon oxide-water neutron reflectivity profiles for AavLEA1 in D_2O at various concentrations. The error bars have been omitted for clarity.

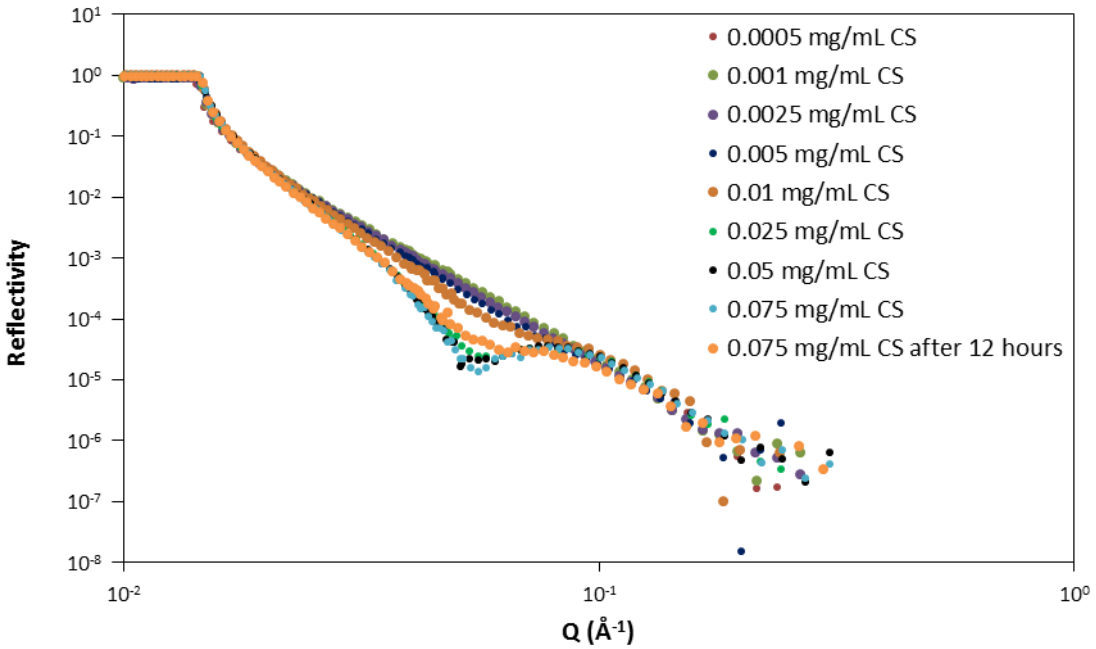

Figure 2: Silicon oxide-water neutron reflectivity profiles for CS in D_2O at various concentrations. The error bars have been omitted for clarity

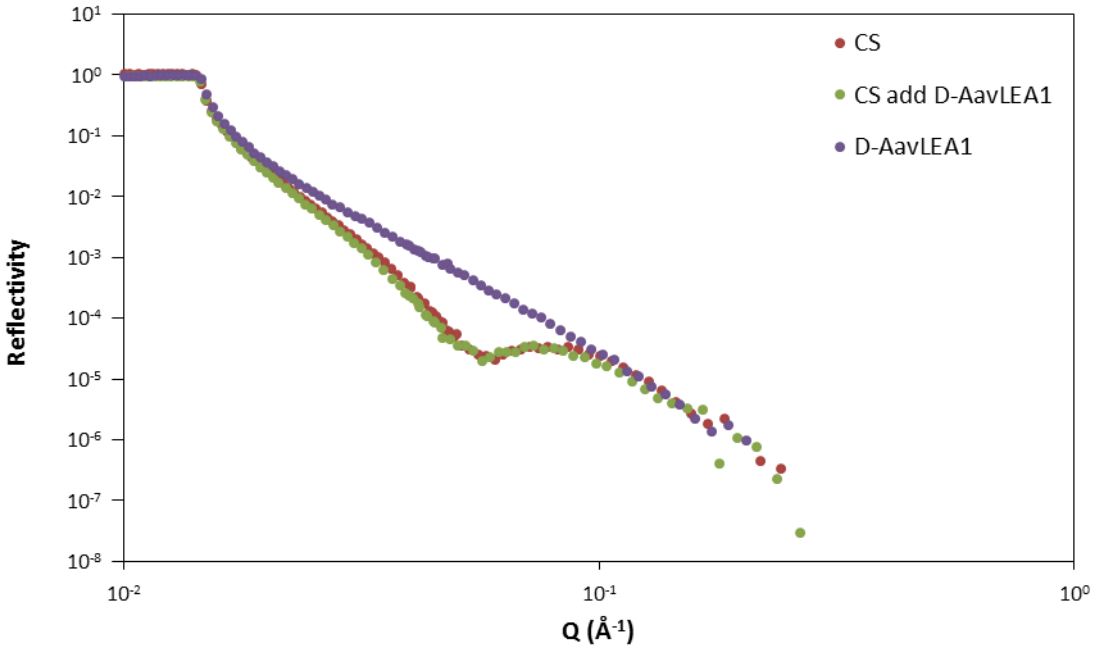

Figure 3: Silicon oxide-water neutron reflectivity profiles for the sequential adsorption of 0.025 mg/mL CS then 0.025 mg/mL AavLEA1 in D_2O with 0.025 mg/mL CS and 0.025 mg/mL D-AavLEA1 for comparison. The error bars have been omitted for clarity.

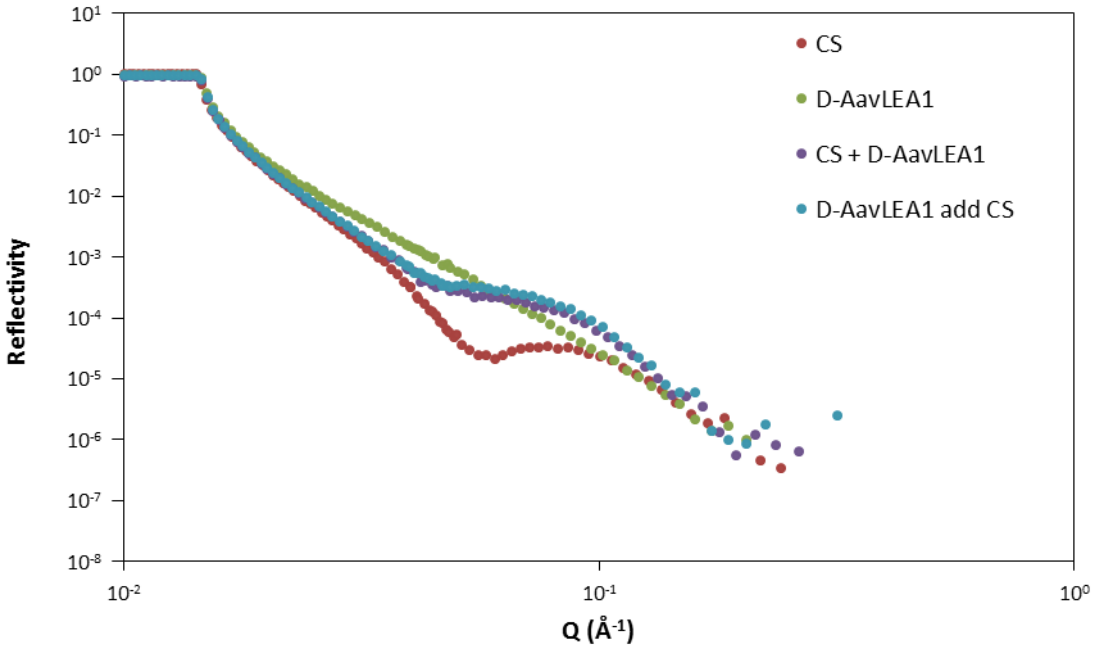

Figure 4: Silicon oxide-water neutron reflectivity profiles for the competitive adsorption of 0.025 mg/mL CS + 0.025 mg/mL D-AavLEA1 in D_2O , with the sequential adsorption of 0.025 mg/mL D-AavLEA1 then 0.025 mg/mL CS, 0.025 mg/mL CS and 0.025 mg/mL D-AavLEA1, for comparison. The error bars have been omitted for clarity.

The ability of extremophile organisms to survive hostile conditions has intrigued scientists and engineers as they develop new technologies to preserve biological materials. Intrinsically disordered proteins, such as LEA proteins, have been found to be upregulated in some plants and animals as these organisms gain cold and desiccation tolerance. LEA proteins have been shown to protect globular proteins, such as pig heart citrate synthase (CS) and rabbit muscle lactate dehydrogenase, and a human cell proteome, from abiotic stress. The mechanism behind these protective abilities is still unclear. We propose that LEA proteins preferentially adsorb onto surfaces generated in the freeze-thaw process, thereby excluding folded proteins from interfaces where they would otherwise undergo irreversible aggregation. The aim of this study is to investigate the adsorption and structural conformation of an LEA protein and CS at an ice/water interface analogue in order to better understand how IDPs protect globular proteins.

The interfacial properties of the CS + LEA protein system were investigated using the D17 instrument to study the self-assembled interfacial layers. Deuterated LEA protein (D-LEA) was synthesized by growing genetically modified bacteria in deuterated media.

Conducting neutron reflection experiments on an ice-water interface is currently not possible. However, previously, researchers studying anti-freeze proteins have used hydrophilic silicon oxide as an ice substitute. Therefore, this neutron reflection experiments was also performed using silicon oxide as a model substrate.

The reflectivity curves for AavLEA1 were different from those of the bare silicon oxide (Figure 1). This provided evidence that the protein alone adsorbed to the silicon oxide interface. The thickness of the adsorbed layer increased with increasing CS concentration (Figure 2). At concentrations above 0.025 mg/mL CS, the reflectivity profile showed a striking fringe, which indicated a thick CS layer at the surface. Measuring the sample after 12 h showed a minor decrease in this fringe, which may indicate some dissolution of the protein back into solution. The critical concentration for both CS and AavLEA1 was determined to be 0.025 mg/mL because higher concentrations did not yield marked changes to the reflection profile, indicating that the surface was saturated.

Sequential binding experiments where CS was first loaded onto the surface revealed that the addition of D-AavLEA1 did not result in any change in the reflectivity profile (Figure 3). On the other hand, loading the D-AavLEA1 first showed a profile different from that of either the D-AavLEA1 or CS alone (Figure 4). It is clear that the D-AavLEA1 at the interface changes the ability of CS to adsorb to the surface.

Most interestingly, when CS and D-AavLEA1 were loaded together at the same time, the resulting profile was nearly identical to that of the sequential binding experiments where D-AavLEA1 was loaded onto the interface first (Figure 4). We believe that these results reveal that AavLEA1 adsorbed to the silicon oxide interface before the CS.

The results of this study have provided crucial information for understanding the competitive adsorption process for a disordered and a globular protein mixture at solid-water interface.