Experimental report

20/08/2015

Proposal: 9-13-540 **Council:** 4/2014

Title: The role of intrinsically disordered proteins under conditions of abiotic stress

Research area: Soft condensed matter

This proposal is a new proposal

Main proposer: Alexander ROUTH

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Local contacts: Robert BARKER

Samples: H-LEA protein synthesised from genetically modified e-coli
Citrate synthase from porcine heart
D-LEA protein synthesised from genetically modified e-coli

Instrument	Requested days	Allocated days	From	To
FIGARO Adsorption troughs	3	3	26/09/2014	29/09/2014

Abstract:

The ability of extremophile organisms to survive hostile conditions has caused us to reassess the requirements for life. Scientists and engineers strive to elucidate extremophile survival mechanisms that might allow the development of new technologies for preserving biological materials. LEA proteins are linked to the acquisition of cold and desiccation tolerance in plants and animals. As intrinsically disordered proteins, LEA proteins are inherently tolerant to stress-induced denaturation, and LEA proteins have been shown to protect globular proteins, such as pig citrate synthase and rabbit lactate dehydrogenase, and a human cell proteome from abiotic stresses. Our current results do not appear to align with the conventional models of interactions and, has led us to propose an additional protection mechanism: the preferential adsorption of LEA proteins at air/water interfaces, where the globular proteins are shielded from the irreversible damage at the interface. The aim of this proposal is to use neutron reflection to assess our new hypothesis by studying study the competitive adsorption an LEA protein at the air/water interface.

ILL Experimental Report

		Experiment Number:	9-13-540
Title of Experiment:	The role of intrinsically disordered proteins under conditions of abiotic stresses	**Local Contact:**	Robert Barker
Principal Proposer:	Dr Alex Routh	**Instrument:**	FIGARO
Affiliation:	University of Cambridge		
Experimental Team:	Fanny Yuen, Alex Routh, Matthew Watson	**Experiment Date:**	26-29/9/2014

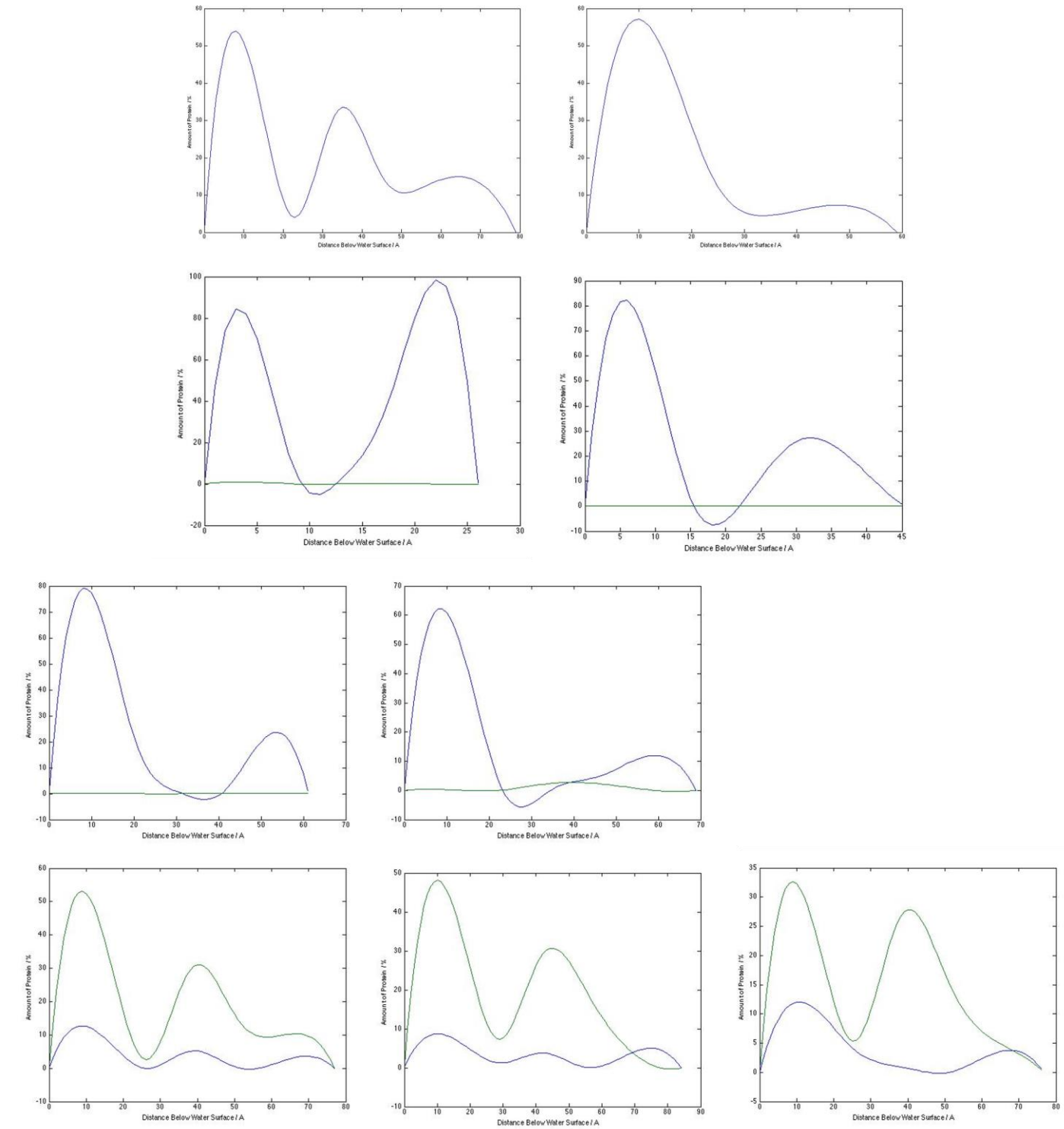

Figure 1: Neutron reflection protein density profiles for (a) 0.6 mg/mL CS at 2 h, (b) 0.6 mg/mL LEA protein at 2 h, (c) 0.6 mg/mL CS + 0.6 mg/mL LEA at 30 s, (d) 0.6 mg/mL CS + 0.6 mg/mL LEA at 1 min, (e) 0.6 mg/mL CS + 0.6 mg/mL LEA at 5 min, (f) 0.6 mg/mL CS + 0.6 mg/mL LEA at 15 min, (g) 0.6 mg/mL CS + 0.05 mg/mL LEA at 2 h, (h) 0.6 mg/mL CS + 0.15 mg/mL LEA at 2 h, (i) 0.6 mg/mL CS + 0.6 mg/mL LEA at 2 h.

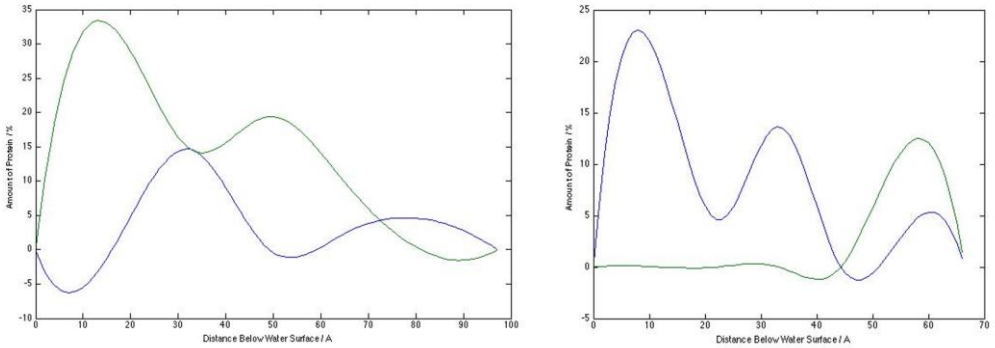

Figure 2: Neutron reflection protein density profiles for the sequential binding of (a) 0.6 mg/mL CS then 0.6 mg/mL LEA (a) 0.6 mg/mL LEA then 0.6 mg/mL CS.

Disordered LEA proteins are found to be upregulated as extremophiles gain cold and desiccation tolerance. It was shown by previous surface tension experiments that LEA proteins are surface active and due to its unstructured nature, are hypothesised to move more quickly to interfaces in comparison to globular proteins. The aim of this experiment is to study the competitive adsorption and structure conformations of LEA protein at the air/water interface.

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

The standard layer approaches customarily used to fit neutron reflection data were found to be insufficient to accurately describe our system. Therefore, a new custom layer model approach was developed by Dr. Robert Barker to analyse this system.

Neutron reflectivity of CS at the air-water interface was measured in three contrasts (D_2O, 42% D_2O, and 8% D_2O). The profiles were simultaneously fitted to yield a protein density profile (Figure 1a). The protein density profile of CS alone after 2 hr showed a double layer configuration with a total thickness of around 8 nm. The layer closest to the air-water interface was estimated to be around 2.5 nm and the second bimodal layer around 6 nm. The two layers are separated by a thin layer of water molecules. The R_g of CS is 3 nm, as determined previously by neutron scattering. Therefore, it follows that the first CS layer does not consist of native CS protein, but, instead, contains denatured CS. In contrast, the second layer is more diffuse and likely contains native CS.

Neutron reflectivity of LEA after 2 hr at the air-water interface was measured in six contrasts (D_2O, 42% D_2O, and 8% D_2O for both H-LEA and D-LEA). The protein density profile showed a concentration of LEA protein at the interface with a diffuse layer as one moves away from the interface totalling around 6 nm (Figure 1b).

These results show that both proteins have the propensity to adsorb to the air-water interface.

Competitive adsorption experiments of CS and LEA were conducted at a single angle measuring for only 30 s. These measurements were performed in five contrasts (CS + H-LEA in D_2O and 8% D_2O, and CS + D-LEA in D_2O, 42% D_2O, and 8% D_2O) and are able to show us what is occurring at the interface as shorter time frames to allow for direct comparison with the our surface tension data. The protein density profiles for 30 s, 1 min, 5 min and 15 min are shown in Figure 1c, 1d, 1e, and 1f. In all four profiles, the surface is clearly dominated by LEA. No citrate synthase was detected on the surface until 15 min profile where a small amount of CS starts to appear. This data provides further evidence that LEA has the ability to out compete CS to the interface at short time-frames.

Competitive adsorption experiments of CS and LEA were undertaken at three LEA concentrations (0.05 mg/mL, 0.15 mg/mL and 0.6 mg/mL) and at five contrasts (CS + H-LEA in D_2O and 8% D_2O, and CS + D-LEA in D_2O, 42% D_2O, and 8% D_2O) after 2 h of equilibration (Figure 1g, 1h, and 1i). Both proteins were mixed together in solution and the mixture was loaded and left for two hours to allow equilibration.

For all LEA concentrations, the both components were shown to be present at the surface, but there is considerably more CS than LEA. CS continued to

appear in a two-layer conformation similar to that of its native state. The LEA concentration at the interface remained consistent with increasing bulk LEA concentration. However, the concentration of CS at the interface was considerably decreased with increased bulk LEA concentration.

Although at short times, LEA out competes CS to adsorb to the air-water surface, at longer time frames, it appears that CS does eventually reach the surface, displacing some LEA, and forming a layer containing both proteins. This aligns well with the previous ST data. As noted previously, the ST data of CS revealed that the can progressively depress the surface tension with time. We had proposed that this may be due to denaturation of CS once reaching the interface.

Sequential adsorption experiments were conducted by first loading the troughs with one protein, allowing two hours for equilibration and then carefully pipetting the second protein below the surface at the bottom of the trough (Figure 2). The system was then allowed to equilibrate for an additional two hours. Each system was measured in three contrasts (D_2O, 42% D_2O, and 8% D_2O).

When CS was loaded first, the resulting protein density profile showed CS continued to appear in a two-layer conformation similar to that of its native state. No LEA was seen at the interface until around 2 nm below the surface where LEA protein was seen to be present between the two CS layers. Therefore, it appears that when CS was first loaded onto the interface, it formed one protein layer at the interface that was not displaced by the addition of LEA protein. Previously we conjectured that this top layer was likely to contain denatured CS. A denatured CS layer is likely to bind tightly at the interface and it is completely reasonable that the LEA cannot penetrate this layer. When D-LEA was loaded first, the LEA continued to be seen at the interface and no major contributions from CS appear until 4.5 nm from the interface.

This study has provided corroborating evidence to our previous surface tension data and have helped definitively prove that LEA is surface active and its ability to preferentially adsorb to an air-water interface in comparison to CS. The results of this study have been instrumental in understanding the surface conformations resulting from the competition of a disordered and a globular protein mixture.