Experimental report

14/09/2023

Proposal: 8-03-1058 **Council:** 4/2021

Title: The role of plant prion-like domains in temperature sensing and response through liquid-liquid phase separation

Research area: Biology

This proposal is a new proposal

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Samples: EARLY FLOWERING 3 (ELF3)

Instrument	Requested days	Allocated days	From	To
D22	1	1	20/09/2021	21/09/2021

Abstract:

The increased average temperatures and prolonged periods of extreme heat due to global warming have already altered plant phenology for both wild and domesticated species, presenting a critical challenge for food security in the coming decades. Plants are able to perceive temperature and subsequently reprogram their growth and development for optimal reproduction and survival. However, the underlying mechanisms plants use to sense changes temperature remain poorly understood. Recent studies suggest that one important mechanism allowing a fast response to temperature stimuli is the formation of dynamic liquid-liquid phase separated states.
liquid-liquid phase separation (LLPS) has become recognised as an important player with many biological functions. Intrinsically disordered proteins (IDPs) often act as drivers of phase separation in vitro and in vivo. Phase separation of IDPs depends on factors such as protein concentration, protein binding partners, ionic strength of the solution, pH and temperature. However, many studies of phase separation are merely descriptive of the process. We propose to use SANS to structurally probie IDPs in the soluble monomeric or condensed phase.

Report on the proposal: 8-03-1058

The role of plant prion-like domains in temperature sensing and response through liquid-liquid phase separation

Our team prepared four constructs of the protein, early flowering 3 (ELF3) for SANS measurements. WE had shown that ELF3 go throughs liquid-liquid phase separation (LLPS) with changes in temperature, pH, concentration and NaCl concentration. SAXS experiments showed that with increasing temperature the proteins would form a distinct peak at ~0.0385 Å and increased in intensity with increasing temperature up to 22 °C. When the temperature was decreased to 5 °C the peak dissipated showing the LLPS is reversible up to 22 °C.

We aimed to investigate the formation of the peak further using SANS to mitigate the radiation damage that we saw with SAXS.

We carried out temperature ramps over several hours with ELF3 constructs on D22 and saw that we were able to get a good signal to noise but unfortunately, we did not see the formation of LLPS (fig1). This is thought to be because ELF3 is very sensitive to pH and when regulating the pD this caused the protein to move out of its narrow range of LLPS formation.

We also tested the deuterated forms of ELF3 and showed that these behaved the same as the hydrogenated forms.

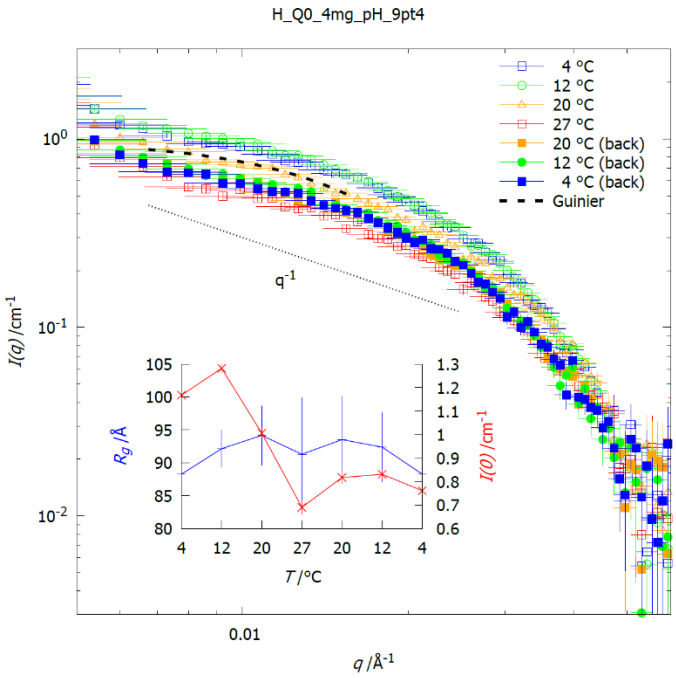

fig 1. SANS profiles of a Hydrogenated ELF3 Q0 temperature ramp. 7 temperatures were recorded going from 4 to 27 °C and then reversed back to 4 °C

Concluding remarks.

Although we were not able to definitively see the formation of lamella stacking in the ELF3 protein with increasing temperature. We believe that SANS is a viable technique that can explore the structure factor formation during LLPS. This will require further testing of phase changes with pD when compared to pH but this can be carried out prior to any experiments.

We can now also form an ELF3 hydrogel, this will also be valuable to study with SANS.