# *Work Update - July*

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WARWICK

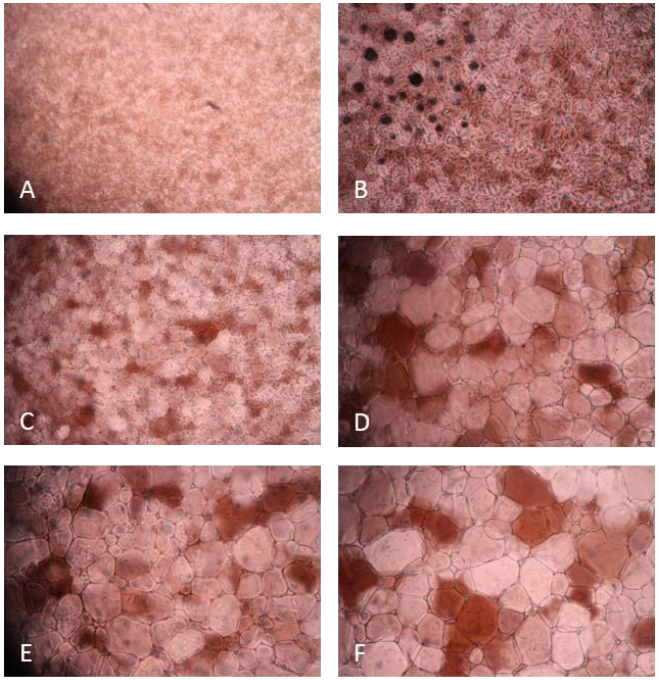
# *Introduction*

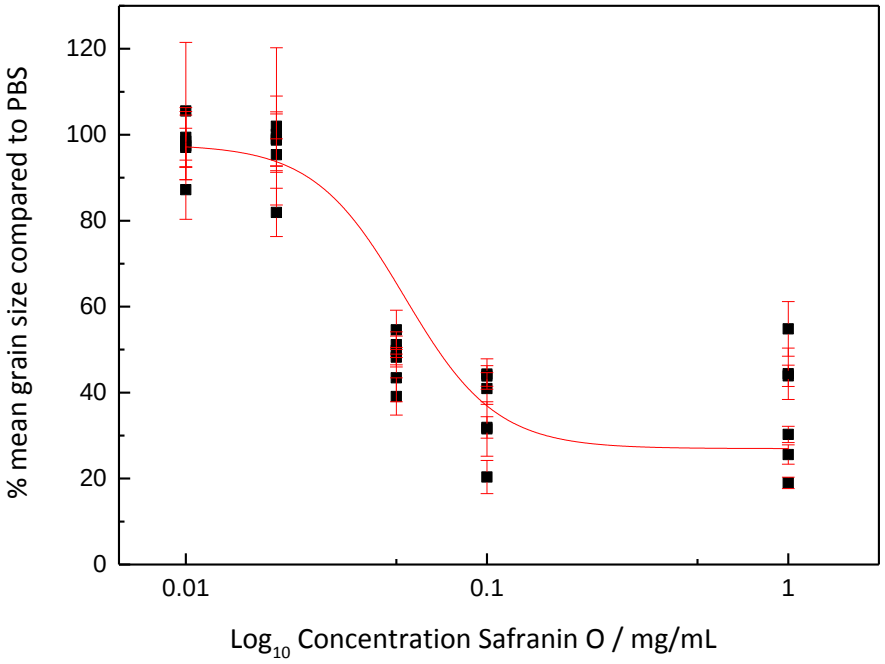
- Ice formation and growth can be a serious problem
- Little understood about its mechanism
- This work investigates the ice growth process and mechanism of action of IRI active compounds
- Goal to characterise ice structures and how they are affected by these compounds
- Using solid state NMR and XRD
- Increased understanding will help improvement of techniques for prevention of ice formation
- 3 key macroscopic effects associated with growth studied: DIS, IRI and TH

# *Aims so far*

- Characterise the changes in ice structure upon addition of antifreezes.
- Investigate what we can learn about the mobility of water and how it is affected by antifreezes using solid state NMR – studying relaxation rates.
- To assess structural changes upon water freezing via X-ray studies.
- Assay protective activity as well as toxicity of PEG/PVA etc on different proteins.
- Do the antifreezes have an optimum concentration/limit

# *Safranin O*




Nucleation and SPLAT assays

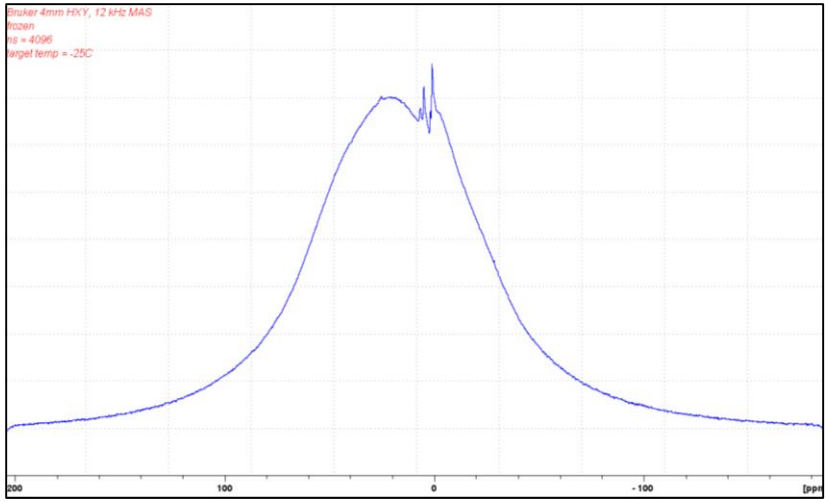
5 different concentrations compared to PBS standard.

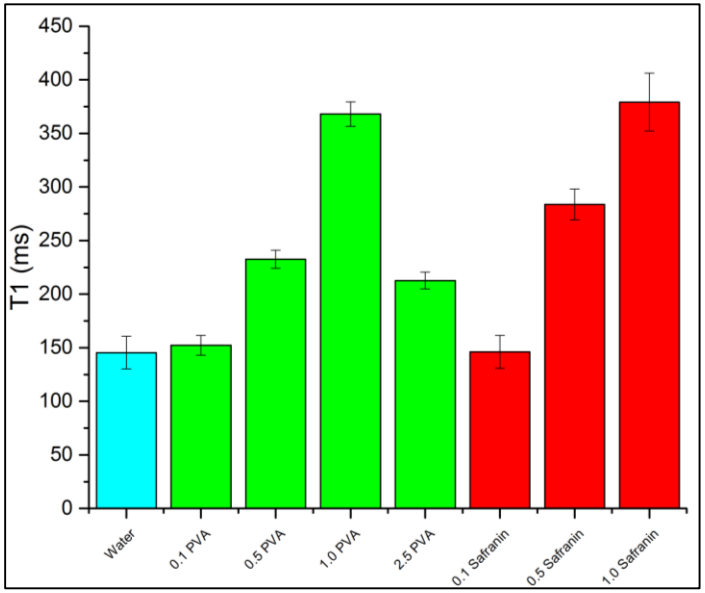
A) 1 mg/mL Safranin O, B) 0.1 mg/mL Safranin O, C) 0.05 mg/mL Safranin O, D) 0.02 mg/mL Safranin O, E) 0.01 mg/mL Safranin O F) PBS standard.

# *Solid State NMR*

Idea: Understand how various antifreezes interact with ice.

- Relaxation rates?
- Used TTMSS (reference) and methanol (internal thermometer).
- Initial studies of different antifreezes – tried AFP and PEG also but the peaks were difficult to analyse so data not trustworthy

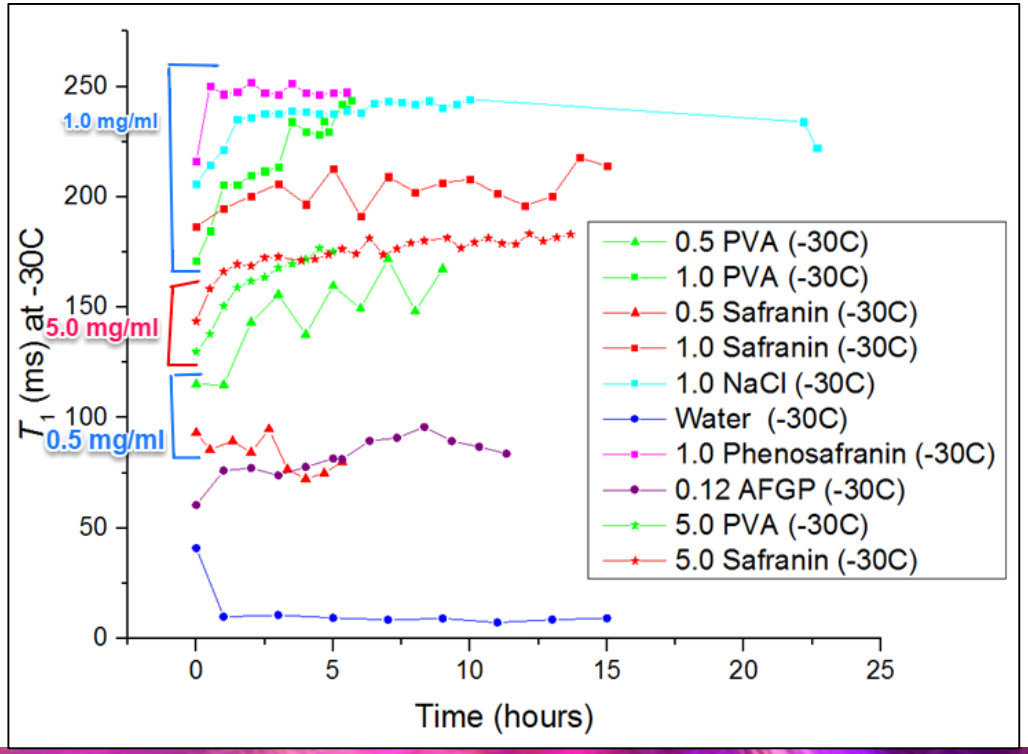


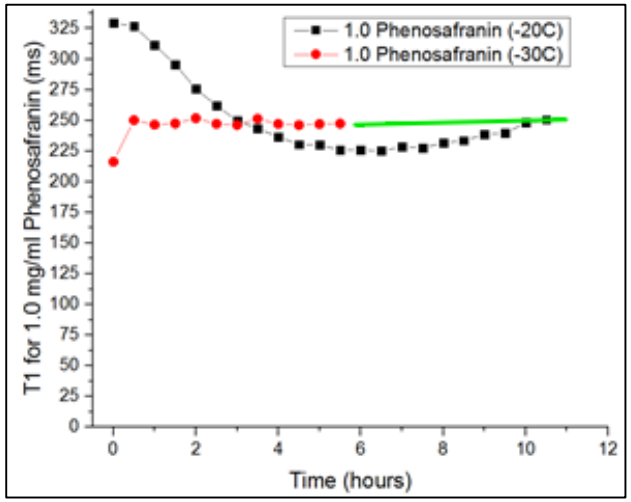



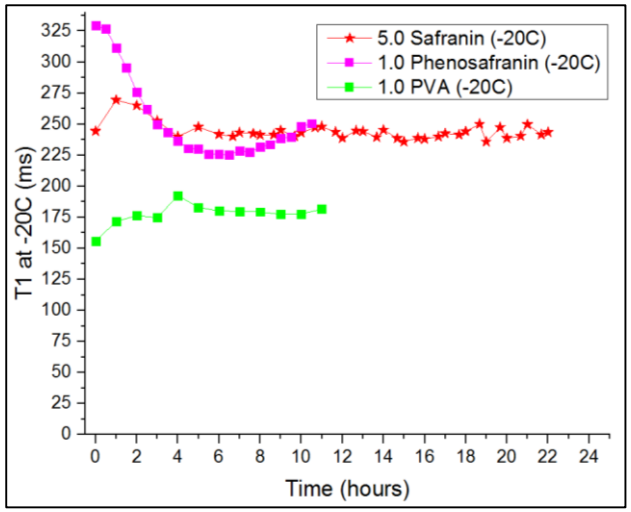
# *Solid State NMR*

Weren't sure of whether what we observed would change so decided on overnight studies

- Are there T1 and T2 differences over time?
- Effect of temperature
- *Do these antifreezes have an optimum concentration?*



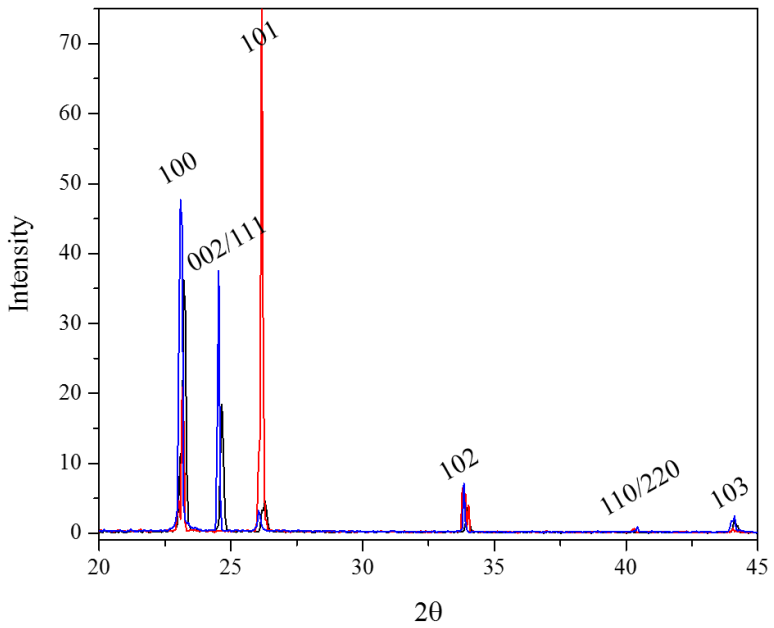
# *Solid State NMR explanations & more ideas*

- Difficult to study
- Still working on negative controls as PEG has too extreme peaks and NaCl gives bizarre results – trying phenosafranin
- We have had problems spinning the liquid samples (unsure why)
- There is definitely an effect
- Would like to do 2D experiments on antifreezes - How does the motion change when at room temp and when frozen?
- Differences between -20 and -30 experiments.

# *WAXS so far*

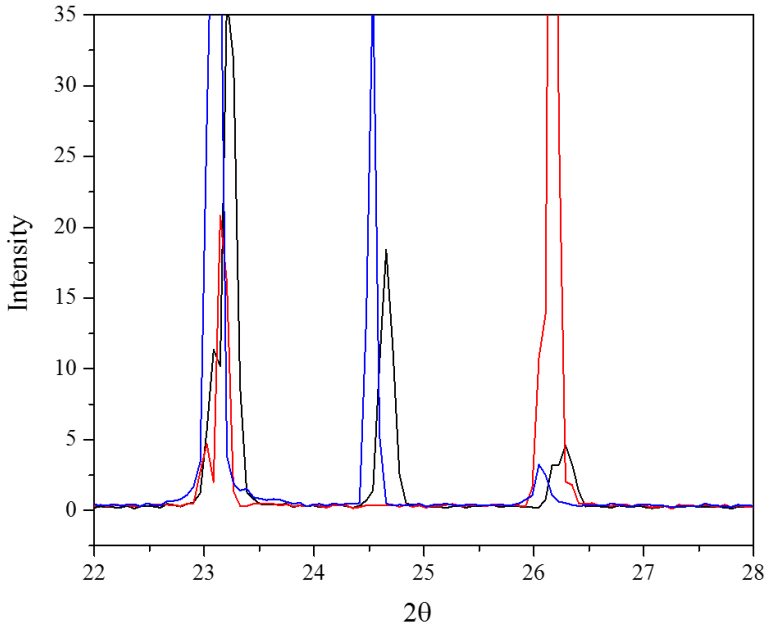
- WAXS analysis of water diffraction patterns from -10 to 10 degrees and the effect of PVA, PEG
- Cubic ice → Hexagonal ice observed (not shown)

Cooling comparisons (-30 ºC)




Black = MilliQ
Red = PEG
Blue = PVA

Cooling comparisons (-30 ºC) Magnification




Black = MilliQ
Red = PEG
Blue = PVA

Labels for peaks based on Salzmann 2012

# *WAXS explanations (?)*

Hypothetical mechanisms of action

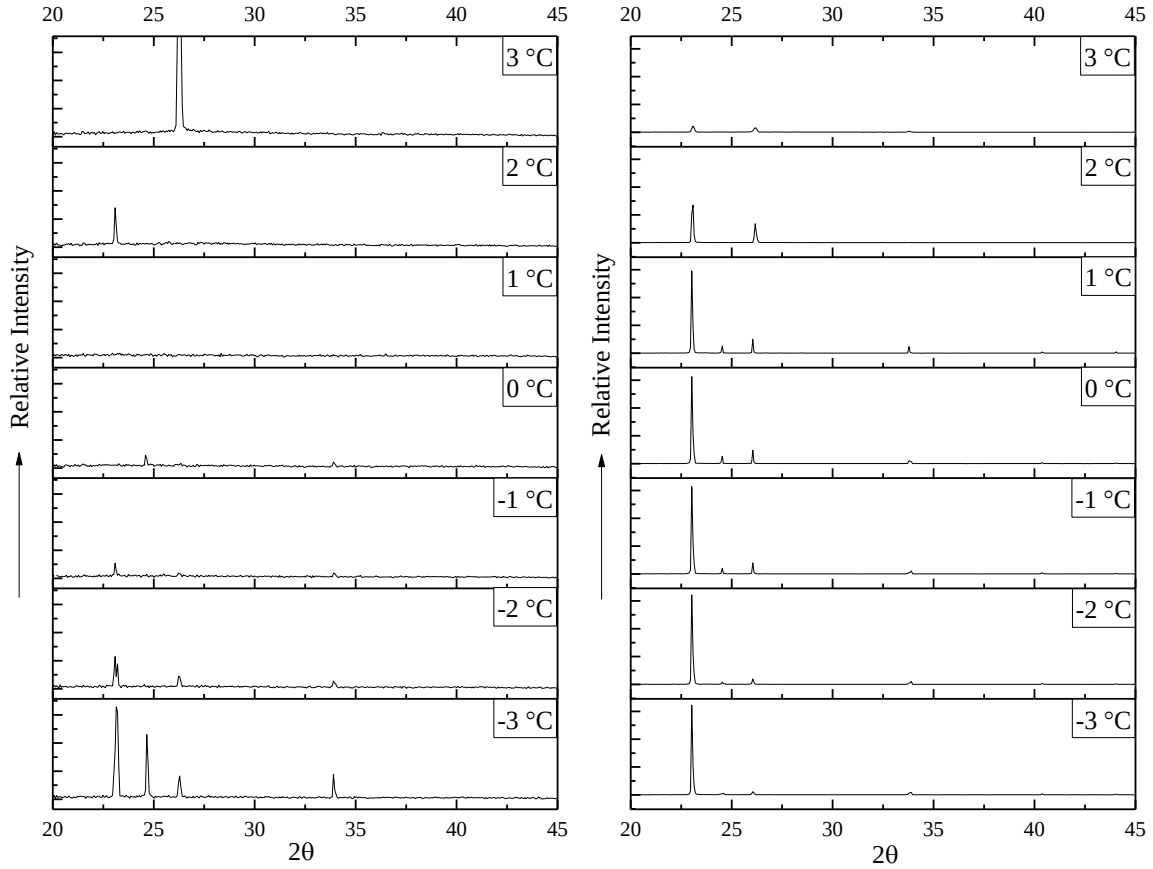
- Direct ice/antifreeze interaction
- Antifreeze partitions into the liquid layer

Possible reasons for peak splitting:

- Deformation of hexagonal ice by surface active components
- Potentially artifacts form
- Crystallisation of a solute – but do the peaks not correspond to the known crystalline forms of solutes in systems studied
- Another ice polymorph – ice IV/III?
- Ih structure changes due to pressure build-up due to volume expansion during water-ice transition.

# *Recrystallisation?*

- MilliQ: intensity disappears from 0 to 1 ºC, then reappears (2 ºC).
- PVA: intensity reduces as temperature increases but no disappearance.
- Need to identify the peaks as the spectra for MilliQ at 2 and 3 ºC doesn't look like the normal $I_h$ or $I_c$ spectra.

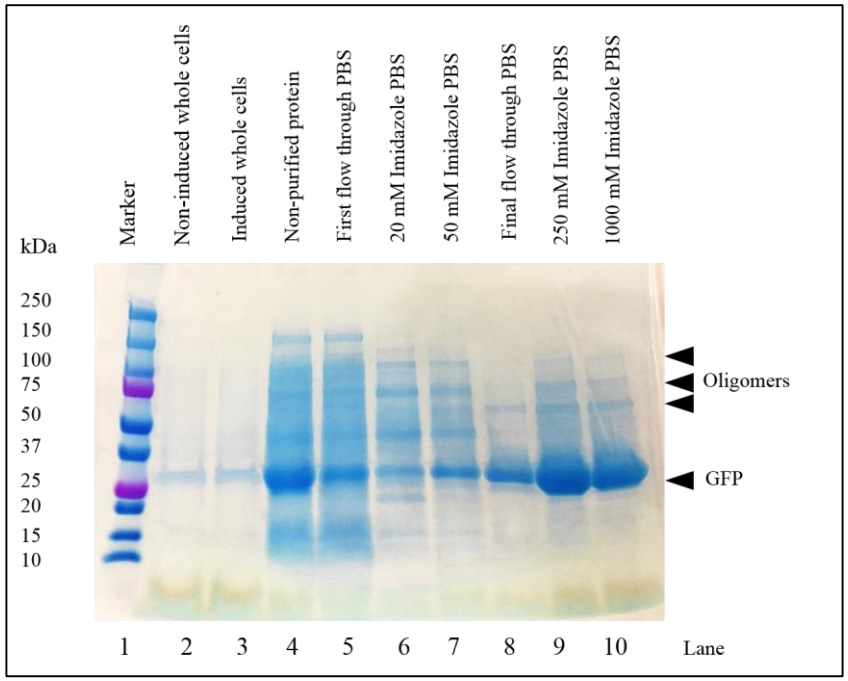


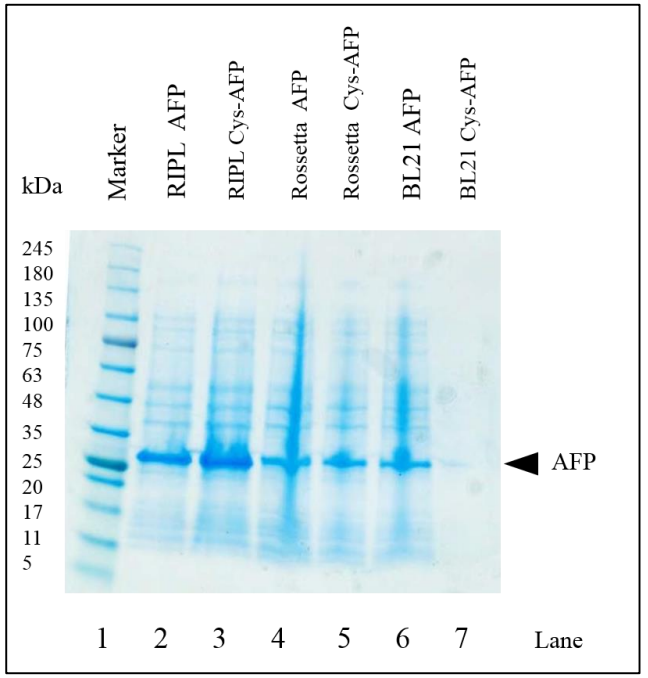

MilliQ

PVA

# *Protein expression - Gels*

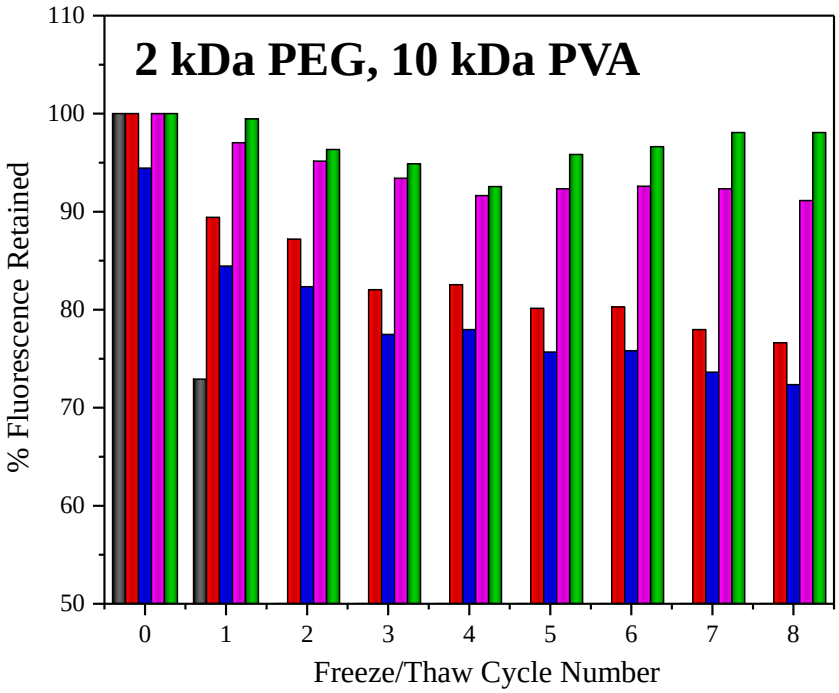
- Successful expression generally
- GFP – For freeze thaw studies
- AFP (I and III) – to compare to PEG/PVA etc
- Optimisation of AFP expression (difficult to get good yields)

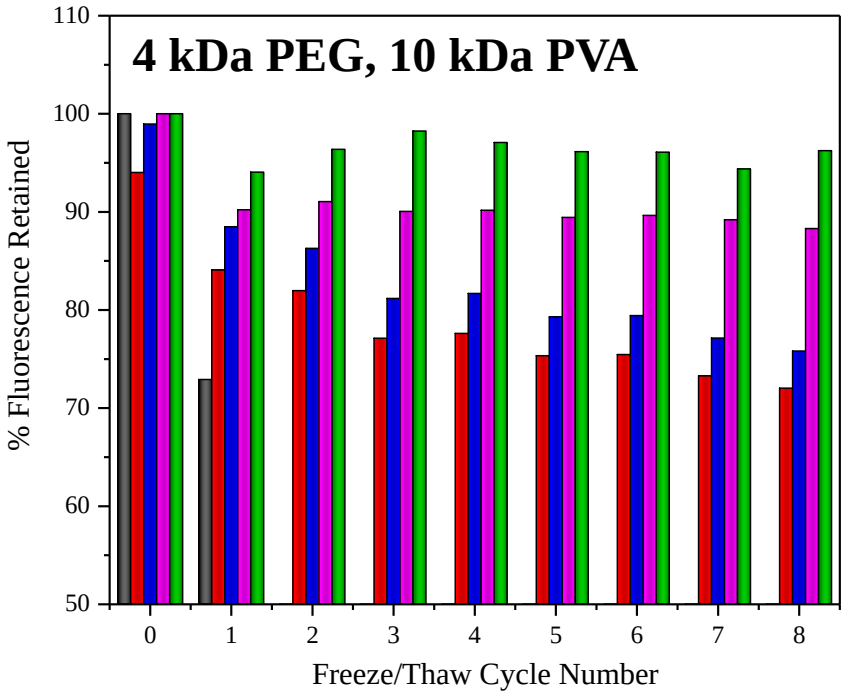


# *Freeze Thaw Assay Results*

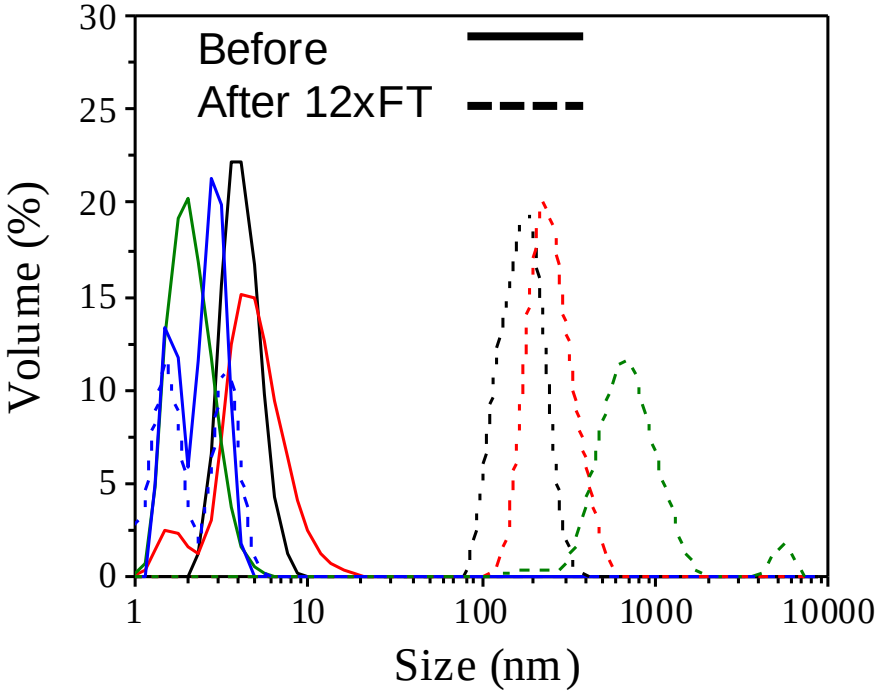
GFP Fluorescence (8 FT cycles)

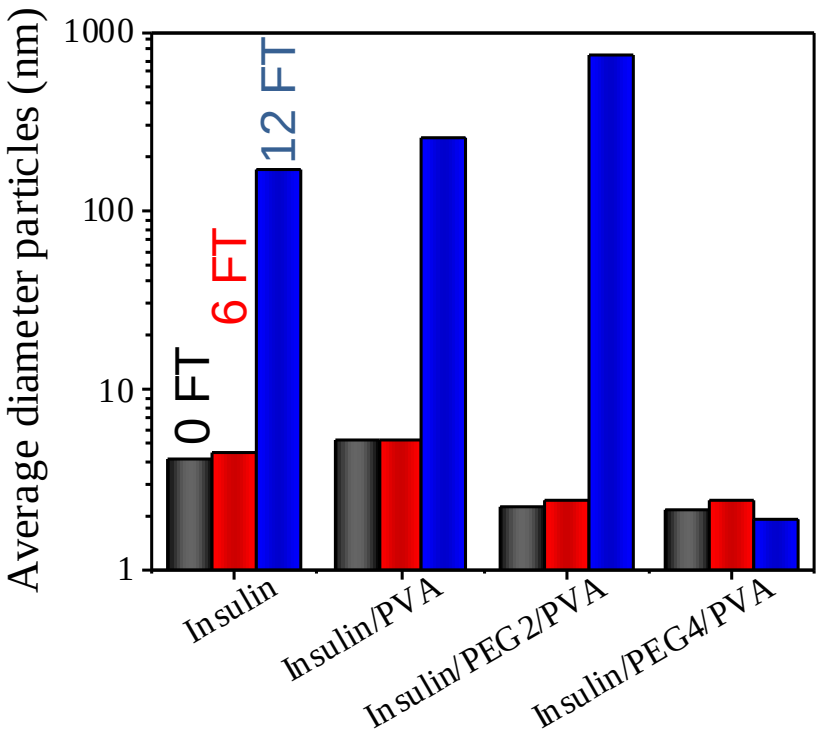


# *Freeze Thaw Assay Results*

Insulin aggregation (12 FT cycles)



# *What to do next*

- Continue with NMR
- Continue WAXS and then move on to SAXS using PBS to compare results directly with what we see from SPLAT assays
- Analyse all the data to see what it means
- Look at ice nucleating compounds (Microarrays)
- AFP expression optimisation

Potentials:

- Raman
- Viscosity studies
- Micro CT Imaging
- Phospholipids

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