Work Update - December

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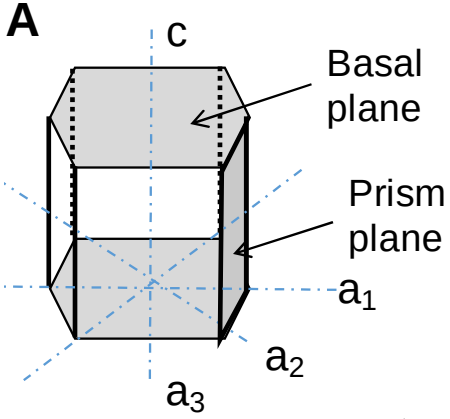
WARWICK

Introduction

- Ice formation and growth can be a serious problem
- Much research ongoing in many fields relating to ice
- Little understood about its mechanism
- This work investigates the ice growth process and mechanism of action of IRI active compounds; I have specifically been looking at dynamic ice shaping (modification of morphology of ice crystal) and ice recrystallisation inhibition
- As well as the dynamics of nuclei in frozen samples

A



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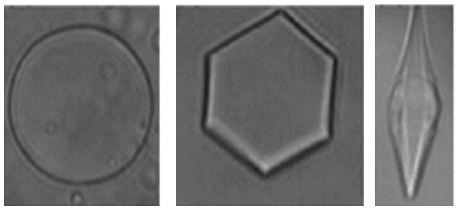

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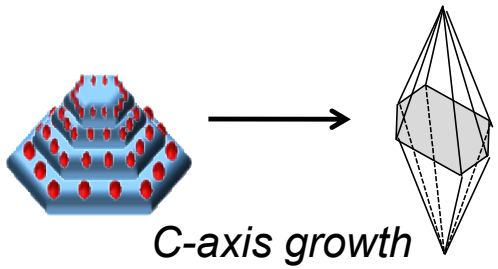


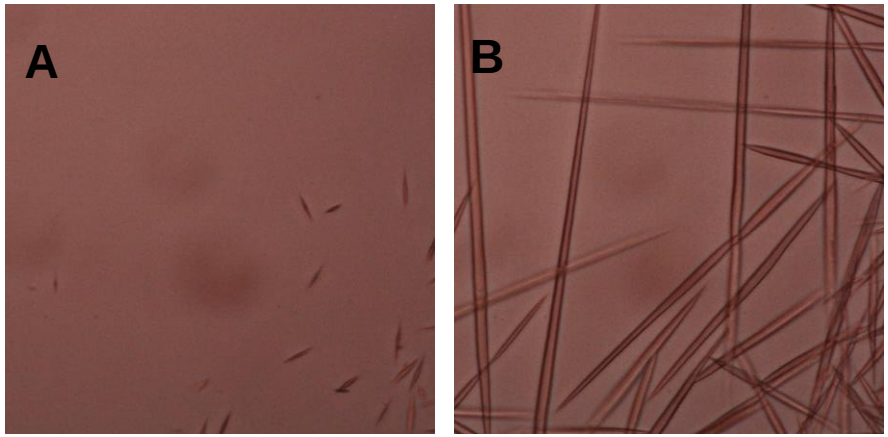
Projects & Aims

- Characterise the changes in ice structure upon addition of ice-active compounds (via microscopy, sucrose assays, X-ray scattering)
  - Antifreeze proteins
  - PVA
  - Safranin O
- Investigate what we can learn about the mobility of water and how it is affected by antifreezes using solid state NMR
  - Study relaxation rates
  - Compare motions in ice-active and inactive compounds, proteins
- Obtain further understanding of the mechanism of growth to improve techniques and designs for ice nucleation promoters and cryoprotectants.

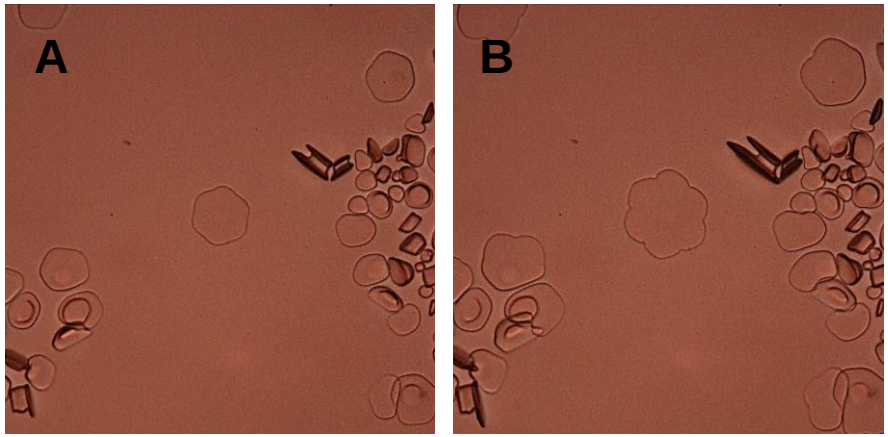
Sucrose Assay Results

- Used to study morphology of ice crystals
- Sample dissolved in 45 w% sucrose and PBS.
- Initial examples below for samples which ice shape and do not:
  - AFGP – shows the expected needles as the crystals grow.
  - For the copolymer so far it appears to not ice shape (hexagonal crystal)

AFGP

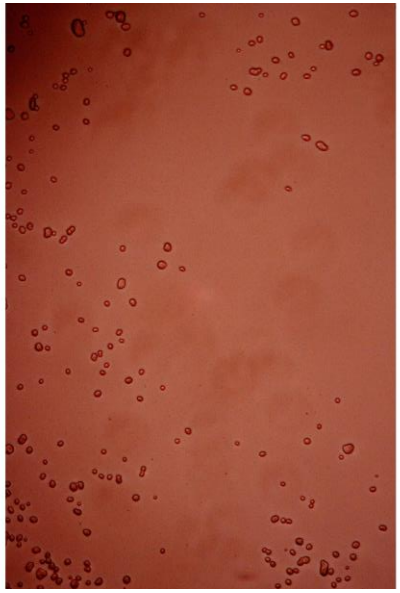


Ben G copolymer

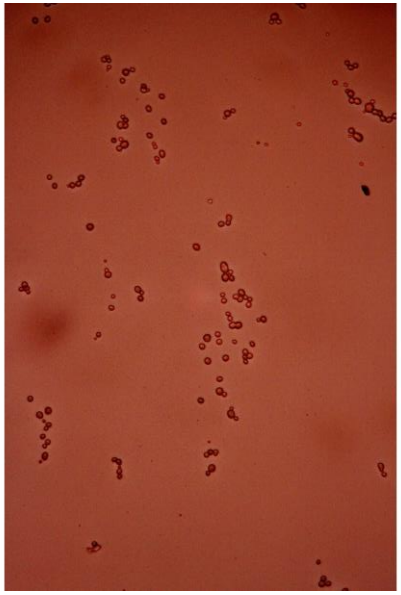


Comparisons of crystal growth morphology

- Study the morphologies of ice upon addition of PVA and AFPIII-SNAP and if they change when bound to gold nanoparticles

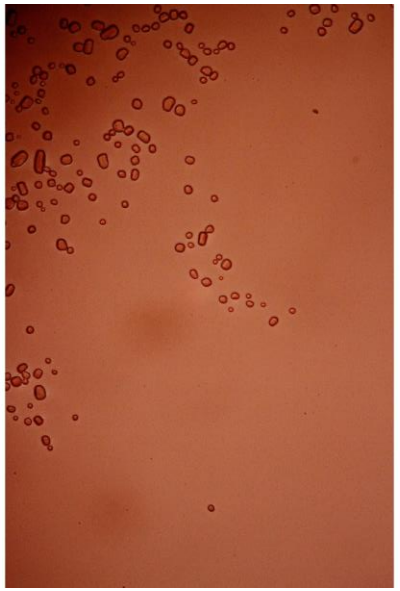


AFP-SNAP -Gold



AFP-SNAP

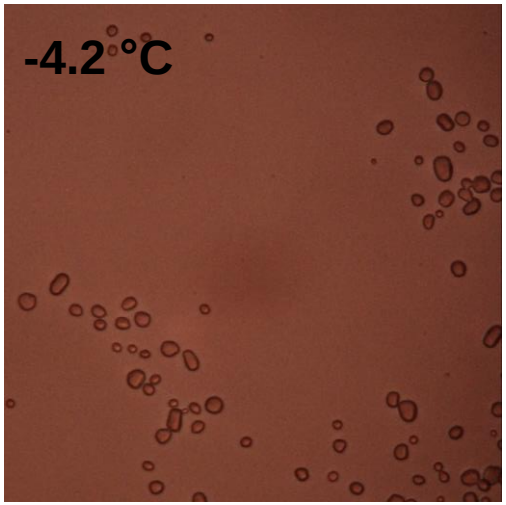
PVA-Gold

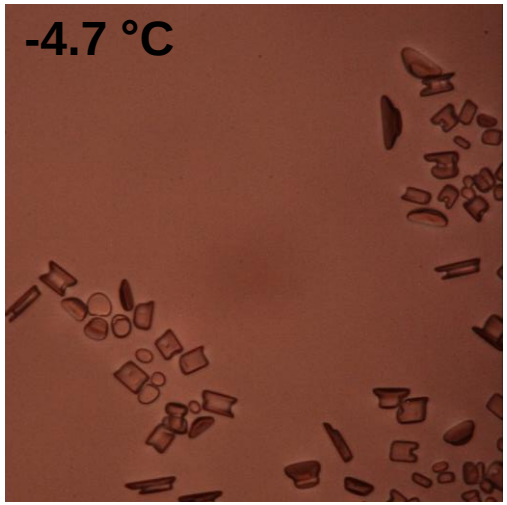


PVA

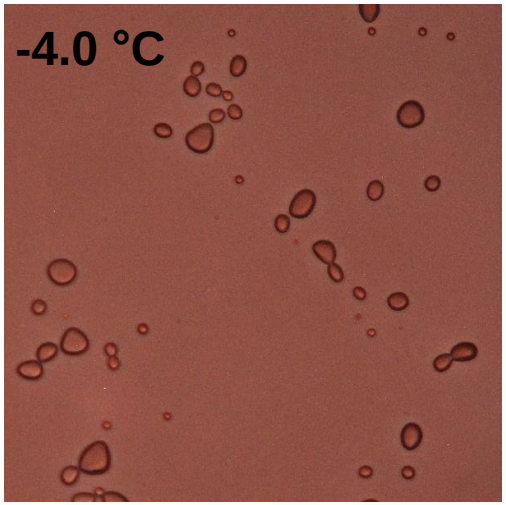
Close up on PVA comparison

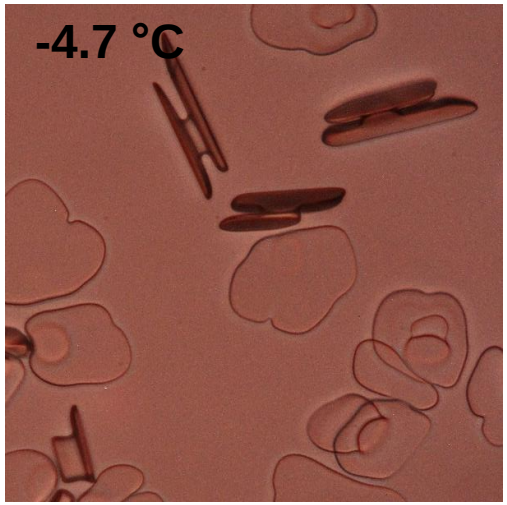
PVA
(0.1 mg.mL^{-1})



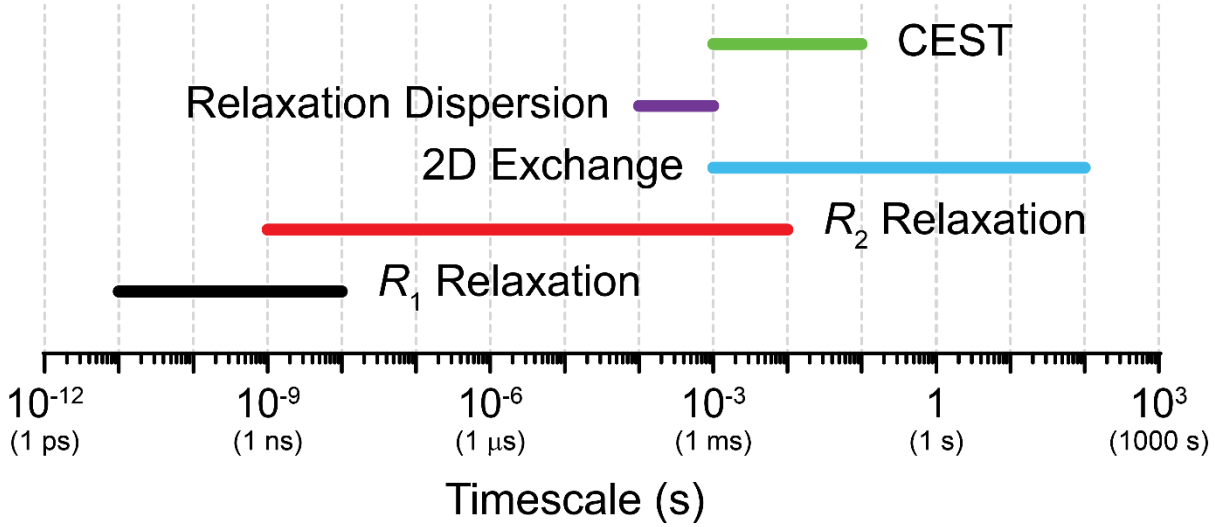


PVA-Gold





Solid State NMR



- Performed T1, T2 and T1rho experiments on water, CPAs and negative controls using a MAS frequency of 10 KHz
- Measured relaxation rates to study mobility of water by looking at dynamics of protons
- Tried multiple experiments at different timescales (shown in figure above), but we saw the most useful data from relaxation dispersion (purple) and variable temperature R_2 relaxation studies (red).

Solid State NMR

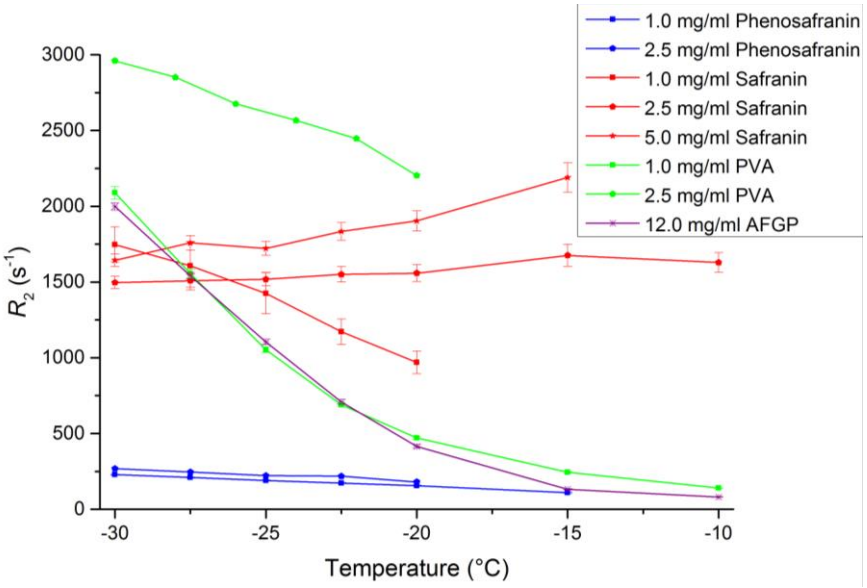


Fig 1. R_2 relaxation measurements between -30 and -10 ° C

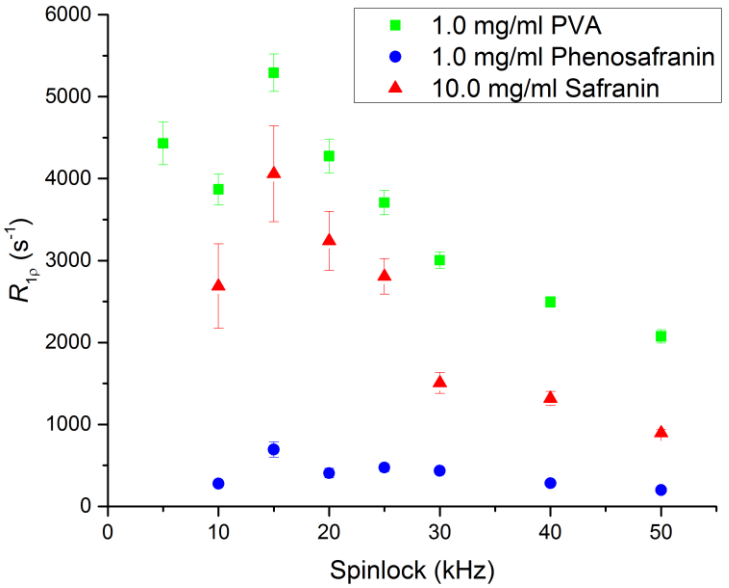


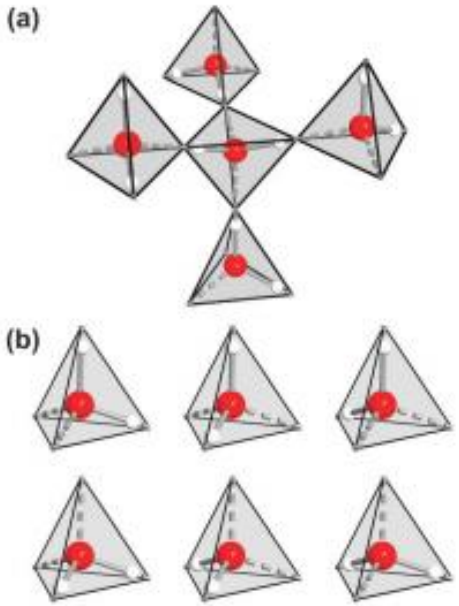
Fig 2. Relaxation dispersion data, R_1 against spinlock frequency

Solid State NMR

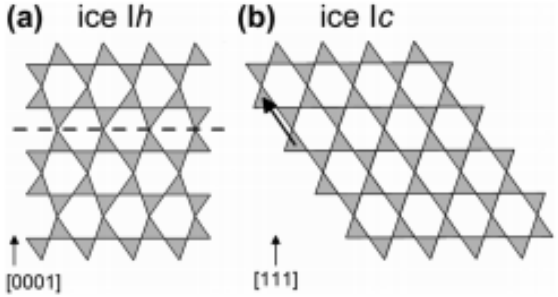
- Initially difficult to study ice
- We are hoping to compare macroscopic effects (eg. DIS, IRI) with the motions at different timescales.
- We so far have seen there is definitely a difference, just need to look at more samples eg AFGP.
- Perform viscosity measurements to compare to R2

Ice structures

1)



2)



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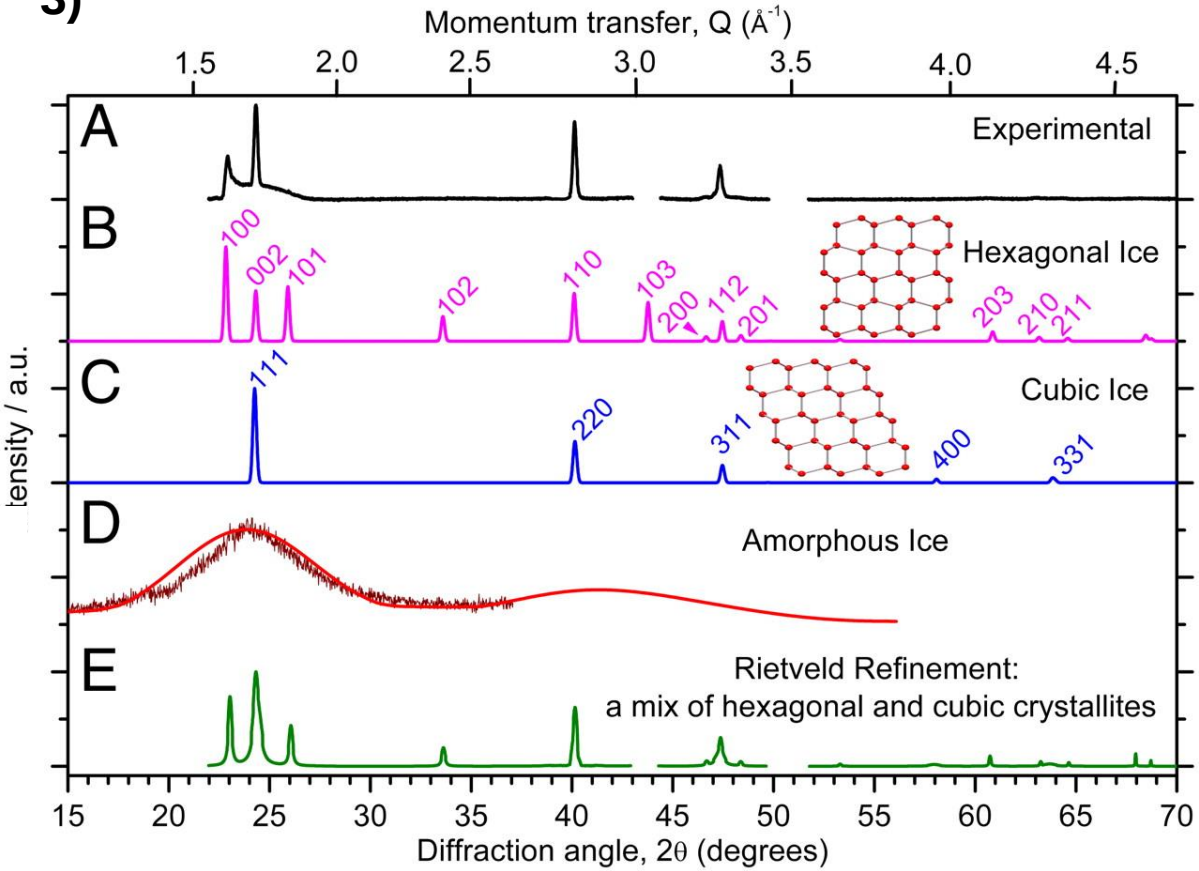

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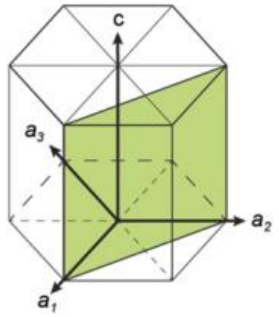


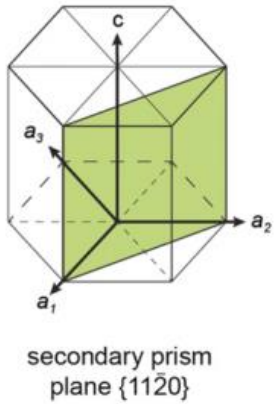
Freezing points

Sample	Freezing point (°C)	Melting point	$\bar{x}$ T_n (microscopy)
PBS:AFP III	-17.5	2.83	?
H_2O:Safranin	-23.66	4.33	
PBS	-24.03	3.39	-34.09
MilliQ	Ca. -26	Ca. 4	Ca. -40
H_2O:PEG	Ca. -24	Ca. 2.5	
H_2O:PVA	Ca. -22	Ca. 4	-37.38
PBS:Polyampholyte	-22.35	1.99	
PBS:AFP III-Snap	-21.5		-20.70

WAXS

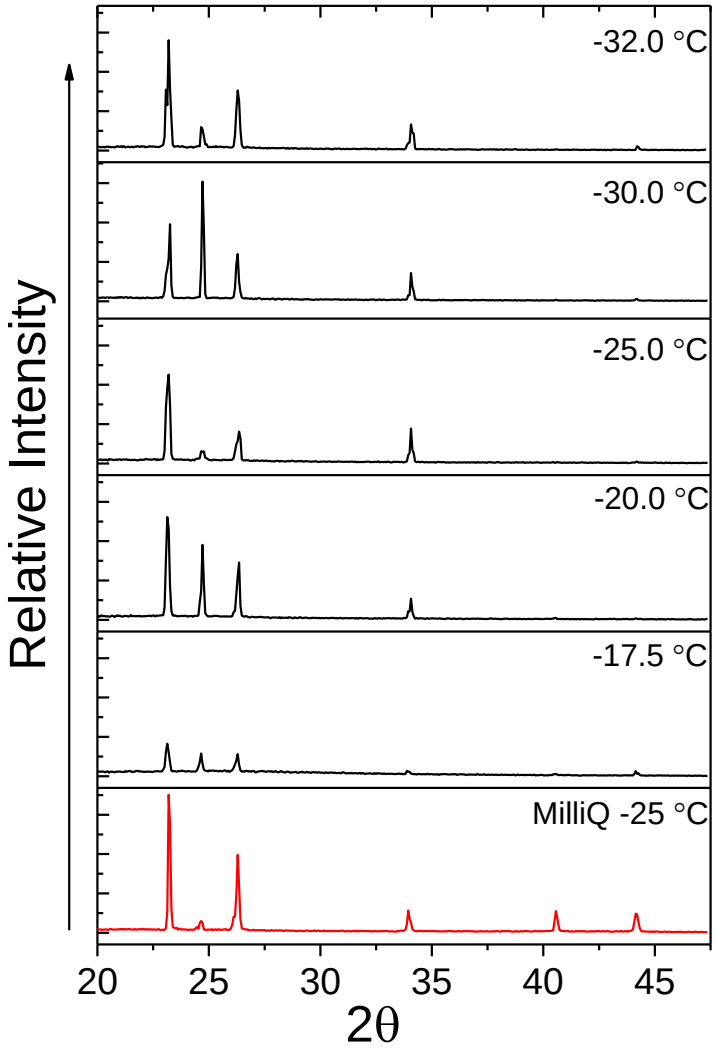
Samples run:

1. PEG/PVA
2. AFPIII
3. Safranin O
4. PBS
5. MilliQ
6. PEG
7. PVA
8. Polyampholyte
9. AFP-SNAP
10. AFGP



secondary prism plane {11$\bar{2}$0}

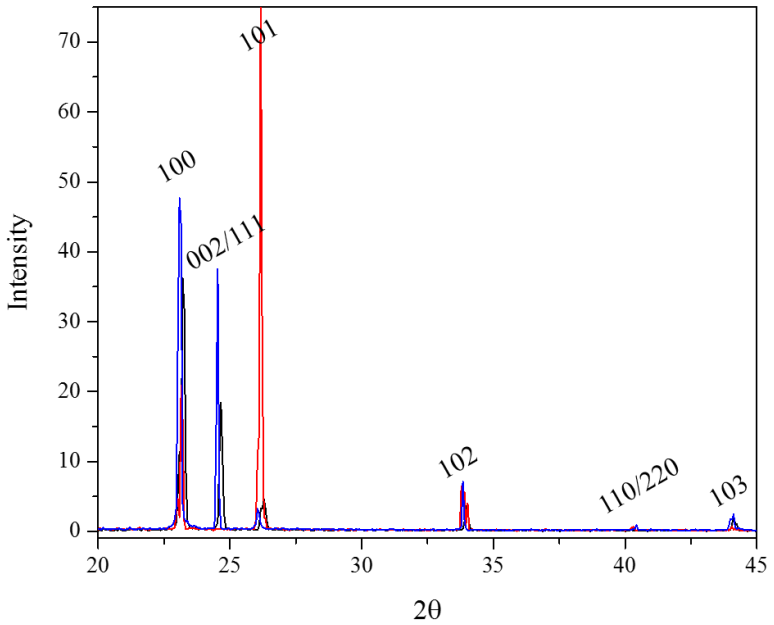
Peak at 40 is the Secondary (110) prism face of hexagonal ice, (1120) secondary prism plane



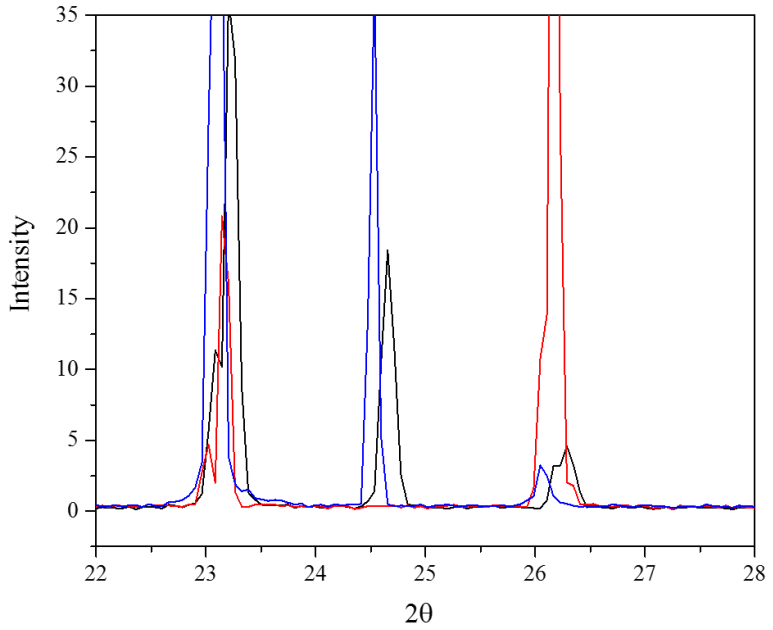
Diffraction patterns AFP III (black) compared to MilliQ (red) as the temperature decreases

WAXS

- WAXS analysis of water diffraction patterns from -10 to 10 degrees and the effect of PVA, PEG
- Peak splitting, slight shifts and changes in intensity observed



Black = MilliQ
Red = PEG
Blue = PVA



Black = MilliQ
Red = PEG
Blue = PVA

Labels for peaks based on Salzmann 2012

WAXS ideas

Possible reasons for peak splitting:

- Deformation of hexagonal ice by surface active components
- Potentially artefacts 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.

Other results:

- Safranin O works differently to AFPIII, PVA and PEG.
- Support for PVA/PEG solutions working synergistically

To do:

- Need to analyse the latest data sets on AFGP
- Perform splat assay in x-ray

Protein Work & Cryopreservation

- AFPIII expression and purification – to compare to PEG/PVA
- Cryopreservation of different bacteria strains

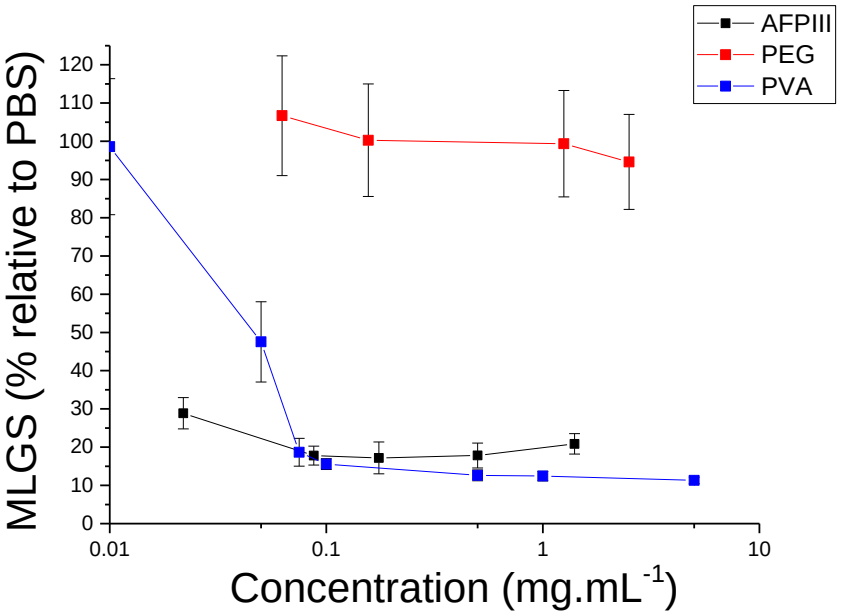


Fig 1. concentration dependence of IRI activity

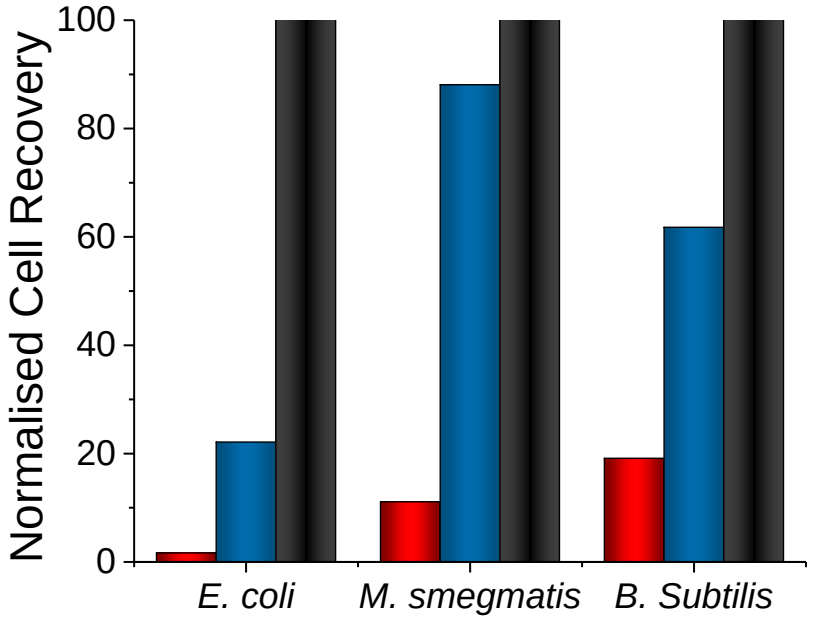
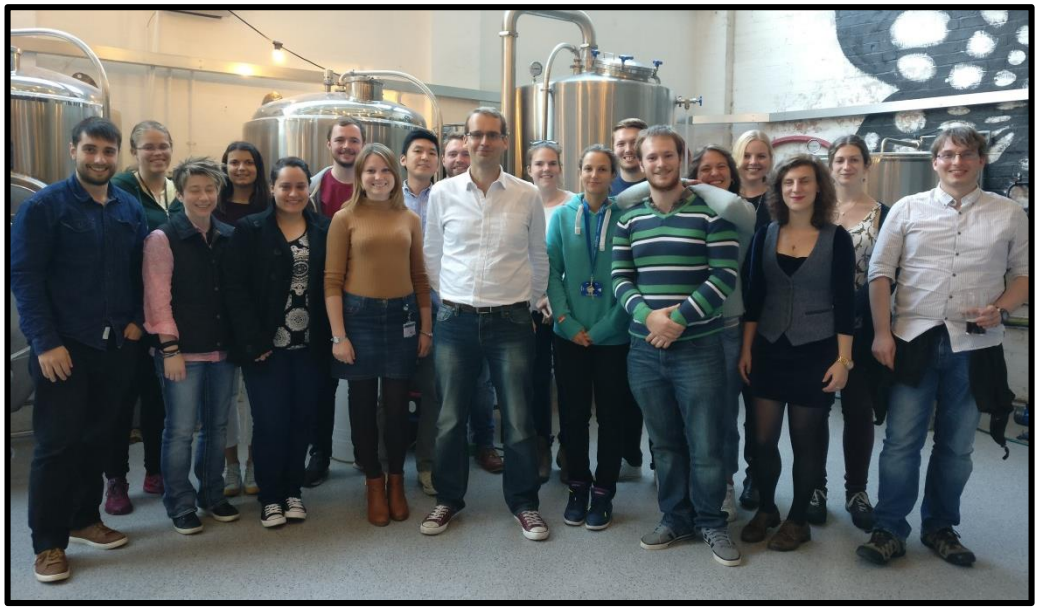


Fig 2. Cell recovery after 7 FT with no CPA (red), glycerol (blue) and PEG/PVA (black)

What to do next

- Continue WAXS and then move on to SAXS to compare results directly with what we see from SPLAT assays
- Can also look at the gold nanoparticles in SAXS to first observe scattering of nanoparticles and then to see if they are coated by the proteins/how scattering is affected
- Analyse all the data to see what it means
- NMR of different DP PVAs
- Viscosity studies

Acknowledgements – GibsonGroup 2017



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- Dr Collette Guy
- Dr Lucienne Otten
- Dr Muhammed Hasan
- Dr Antonio Laezza

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- Segun Wahab

Phd Students
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- Lewis Blackman
- Benjamin Martyn
- Joseph Healey
- Chris Stubbs
- Ben Graham
- Trisha Bailey
- Laura Wilkins
- Marie Grypioti
- Julia Lipecki
- Vinko Varas
- Gabriel Erni Cassola
- Robyn Wright
- Alice Fayter
- Alex Baker
- Ruben Tomas
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