

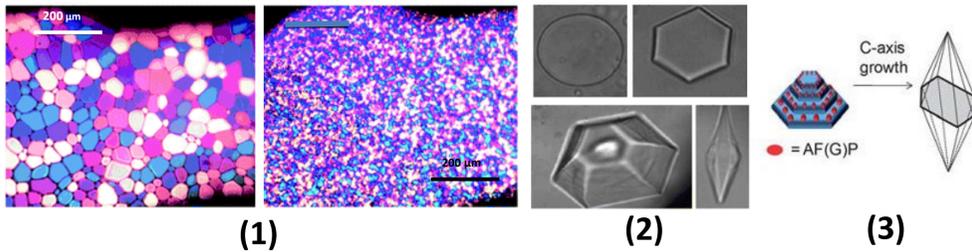
Developing Antifreeze Protein Mimics for Cryopreservation

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Control of Ice Crystal Size and Shape



Antifreeze proteins display strong ice recrystallisation inhibition (IRI) - slowing the rate of ice crystal growth (1), but causes damaging needle-like crystals to form, due to Dynamic Ice Shaping (2). This is due to adsorption of proteins onto the ice crystal surface (3), which also induces thermal hysteresis, lowering the freezing point of water.

Poly(vinyl alcohol) as an Antifreeze Agent

PVA has been shown to be the most potent synthetic recrystallisation inhibitor, but does **not** shape the ice or greatly affect the freezing point.

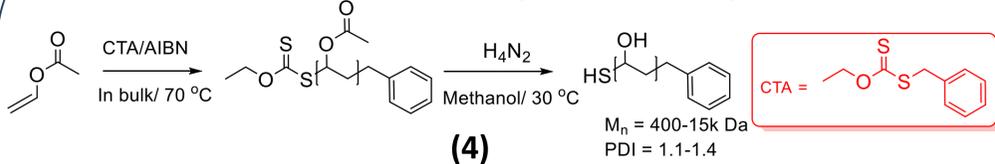


PVA is non-toxic and readily available, and has been recently shown to greatly enhance the cryopreservation of human red blood cells.

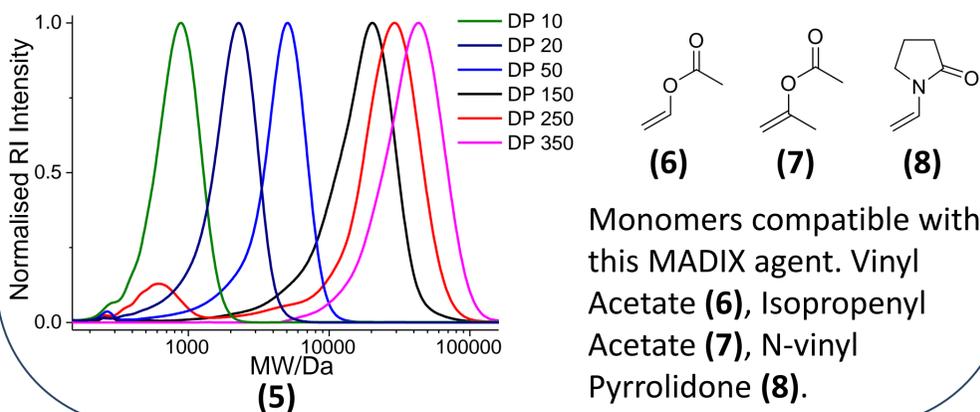
Deller, R.C.; M. & Gibson, M.I. *et al.* Nat. Comms., 2014, 5 (3244)

Deller, R.C.; Congdon, T.; Gibson, M.I., *et al* Biomat. Sci., 2013, 1, 478

Using Controlled Polymerisation to Probe Structure/Activity Relationships

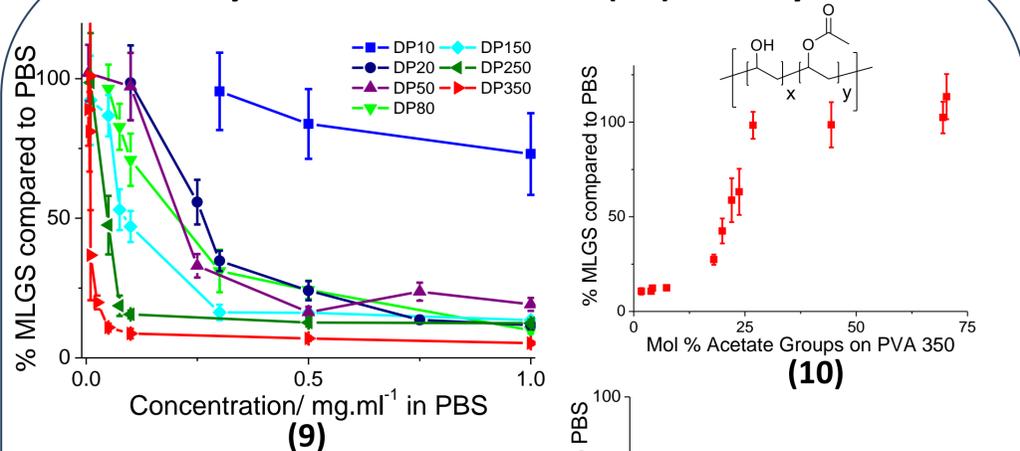


The specific properties/mechanisms that make PVA such an efficient ice recrystallisation inhibitor are currently unknown. In order to examine the effect of molecular weight, dispersity, and the effect of copolymers a MADIX methodology was employed (4).



Monomers compatible with this MADIX agent. Vinyl Acetate (6), Isopropenyl Acetate (7), N-vinyl Pyrrolidone (8).

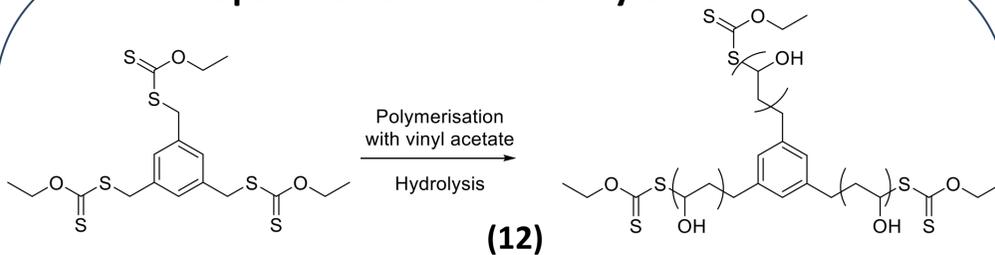
Ice Recrystallisation Inhibition (IRI) Activity of PVA



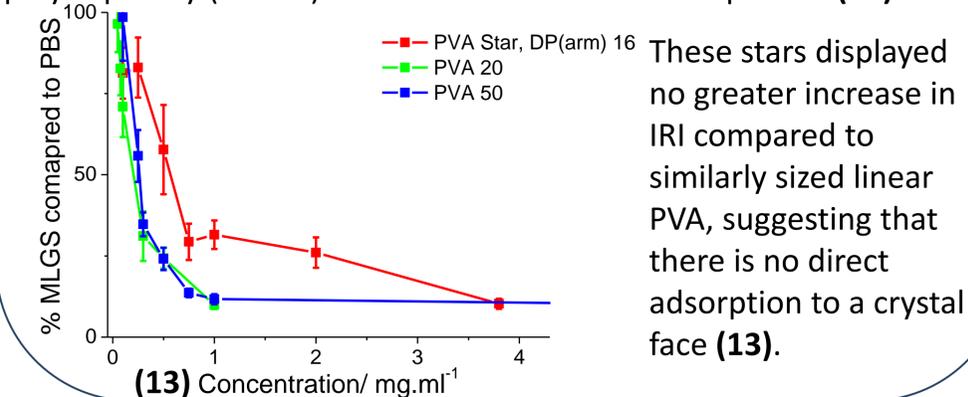
PVA oligomers display activity similar to longer chain PVA, with activity 'switching on' between 10 and 20 repeat units (9). The inclusion of acetate groups stops IRI activity above 25 mol% (10), whereas pyrrolidone groups lessen activity (11).

Congdon, T.C.; Notman, R., Gibson, M.I., *Biomac*, 2013, 14, (1578)

Preparation and IRI Activity of PVA Stars



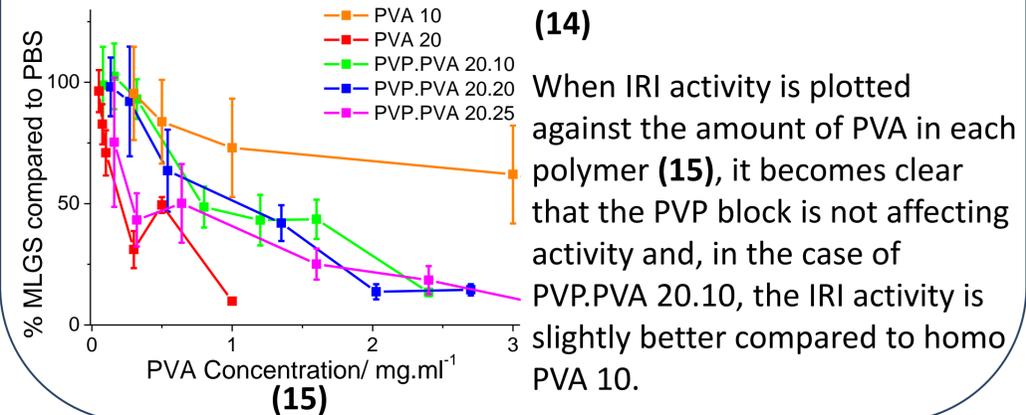
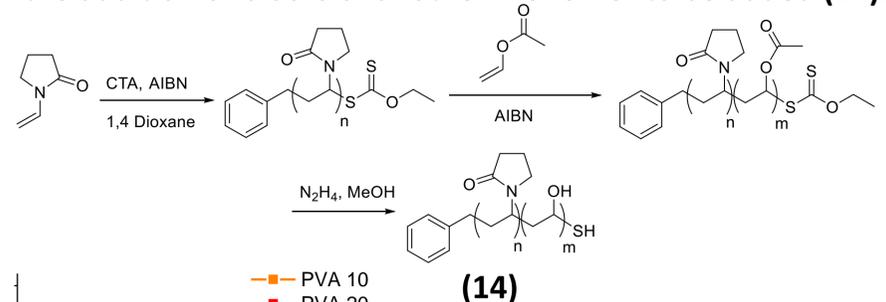
Three-arm stars were prepared using a novel trifunctional MADIX agent, which afforded stars that even at high conversion had a low polydispersity (\bar{D} 1.17) and no trace of a linear component (12).



These stars displayed no greater increase in IRI compared to similarly sized linear PVA, suggesting that there is no direct adsorption to a crystal face (13).

IRI Activity of PVA(b)PVP Block Copolymers

The 'living' polymerization of vinyl pyrrolidone using MADIX allows for the addition of blocks of another monomer to be added (14).



When IRI activity is plotted against the amount of PVA in each polymer (15), it becomes clear that the PVP block is not affecting activity and, in the case of PVP.PVA 20.10, the IRI activity is slightly better compared to homo PVA 10.

Background References

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O'Reilly, R.K., *et al.*, *Macromol.*, 2011, 4, 886
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