Molecular modelling of Biomimetic Antifreeze Polymers

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Introduction

Ice crystal growth during storage of tissues presents a serious problem to the cryo-preservation of organs, food and crops in cold climates. In the UK alone the waiting list for organ transplantation currently exceeds 7000[1], and it is important that no organ is wasted. Inspired by antifreeze proteins and glycoproteins (AF(G)Ps), which permit the survival of organisms in sub-freezing climates, researchers are now seeking better and cheaper synthetic alternatives to prevent ice crystal growth or reduce them into manageable sizes.

Recently, our collaborators demonstrated the remarkable ability of low concentrations of poly(vinyl) alcohol (PVA) to preserve mammalian red blood cells after freeze-thawing. However, molecular mechanisms of antifreeze actions of PVA are not known.

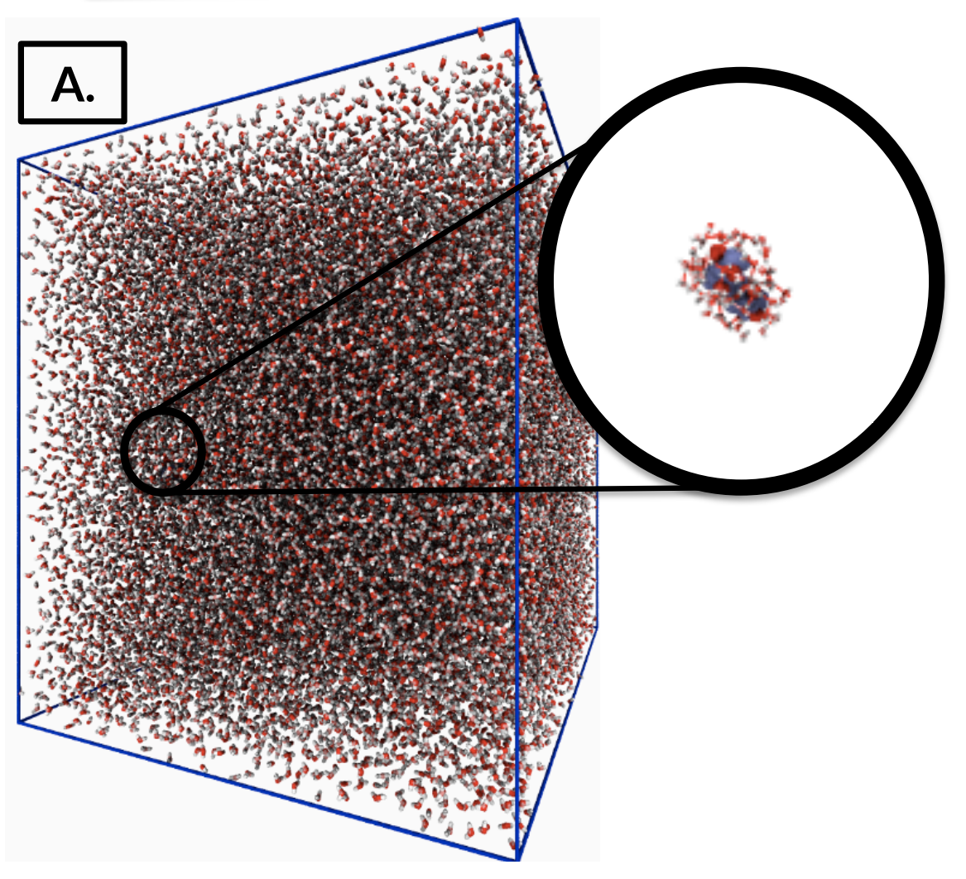
Here we employ molecular dynamics computer simulations to address this challenge. This understanding will enable us to correlate the structural properties of this polymer with different antifreeze characteristics e.g. thermal hysteresis, dynamic ice shaping or reduced ice recrystallisation, and facilitate the design of new, improved antifreeze polymers for specific control over crystal size and morphology. Our initial work has focused on PVA, and its antifreeze inactive isomer, poly(ethylene) glycol (PEG). We have simulated various chain lengths of PVA and PEG in solution and at the ice/water interface using the TIP4P/Ice water model and the OPLS-AA forcefield for the polymers.

Methods

We investigated 5 different polymer chain lengths of poly(vinyl) alcohol (antifreeze active) and pol(ethylene) glycol (antifreeze inactive). Models for each polymers were built, and observed in different environments.

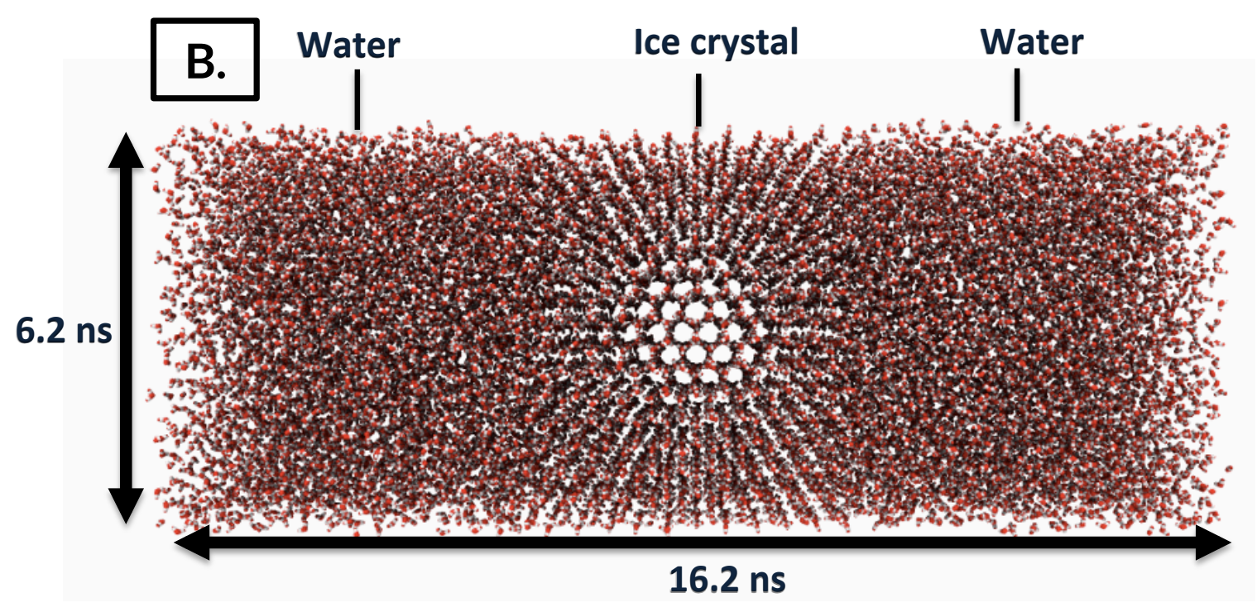
Firstly polymer simulations in pure TIP4P/Ice water were conducted with the dual purpose of:

- *Validation* – To confirm that the polymer models behave as expected in experimental solution.
- *Correlations* – To find predictive properties in solution to antifreeze activity.



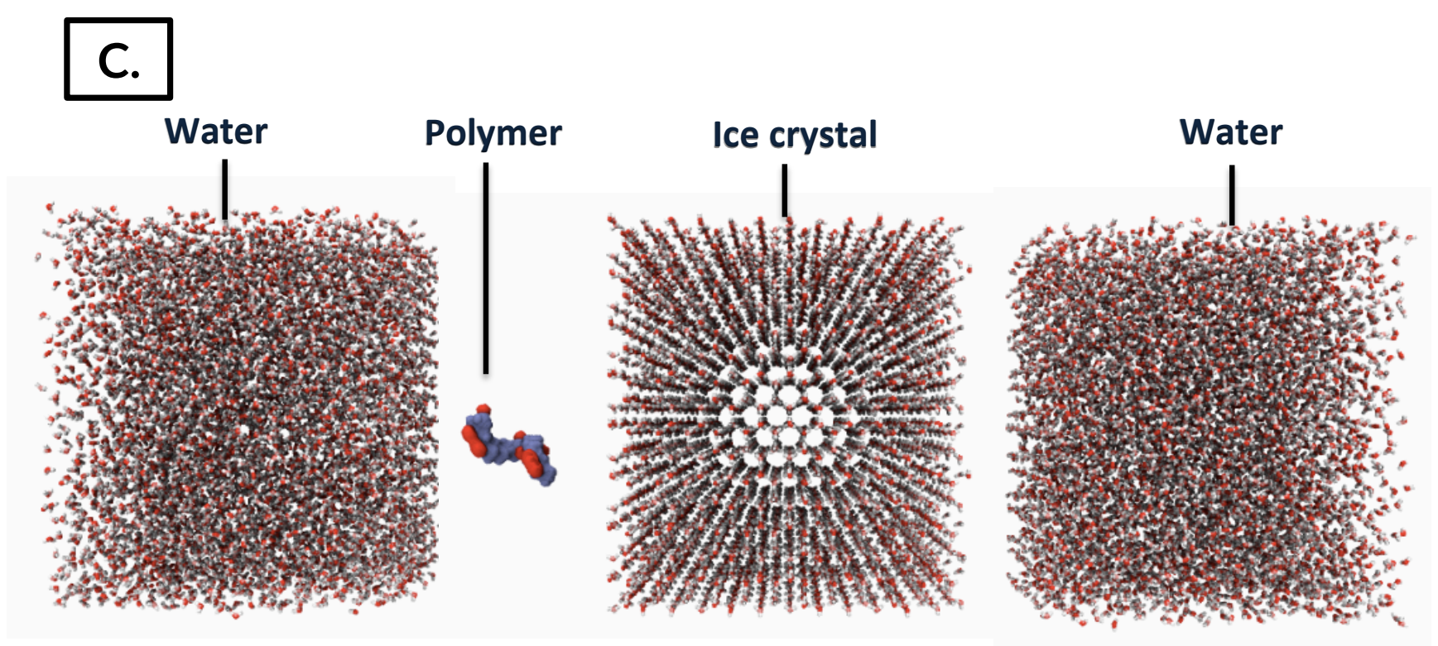
Polymer simulations

Next, ice/water simulations were conducted prior to adding polymers to the system. The starting structure (see left) was allowed to grow or melt at 10 different temperatures. This serves as a negative benchmark and provides a melting profile in the absence of additives.



Ice simulations

Finally the same ice system is allowed to grow or melt in the presence of a polymer of specific chain length. These simulation will help to visually, and numerically determine the atomistic mechanism of inhibition (and contribution from chain lengths). For example the new melting profiles will be useful in identifying and assigning thermal hysteresis values.



Polymer and Ice simulation

Fig. 1 Snapshots of the system set ups and dimensions for the 3 different simulations discussed in this poster. Top down: the first is for polymer simulations in water (A), the second for ice simulations in the absence of polymers (B) and finally with both systems combined.(C)

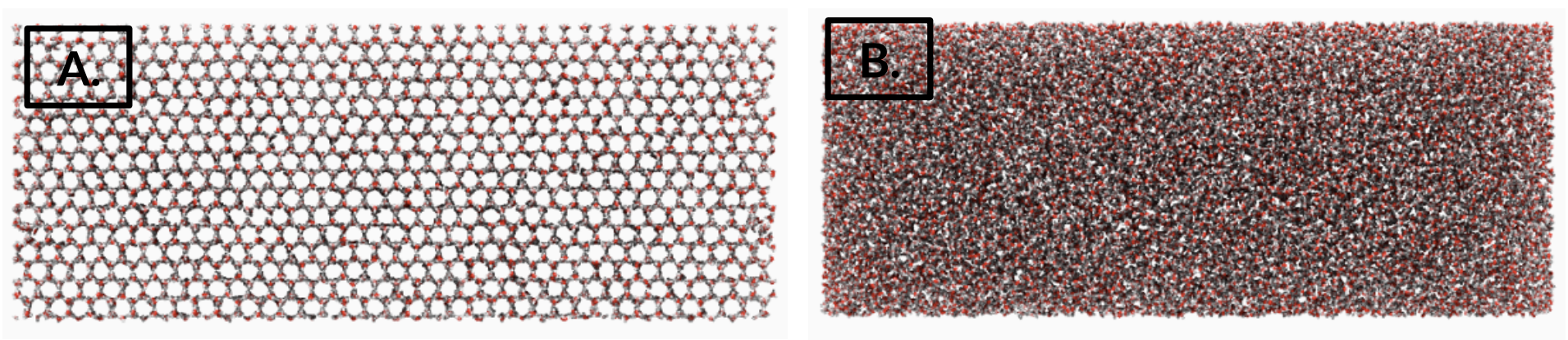


Fig. 2 Snapshots of the pure ice system completely molten at 310 K after 0.43 ns or completely frozen at 268K after 173 ns.

Results: Polymer simulations

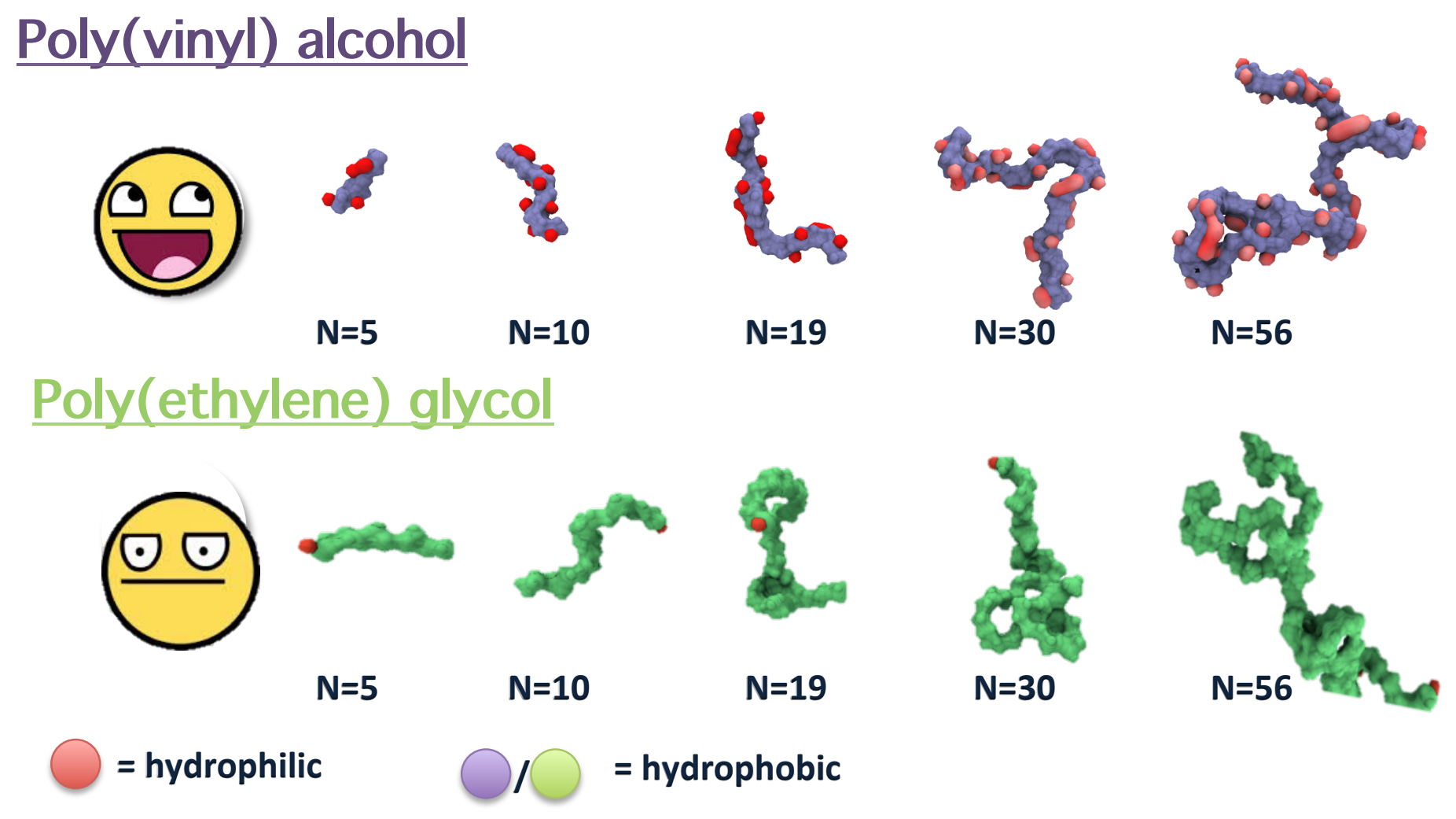


Fig. 3 snapshots of the solvent accessible surface area of two different polymers; the antifreeze active poly(vinyl) alcohol (purple) and antifreeze inactive poly(ethylene) glycol (green) at varying chain lengths (**N**).

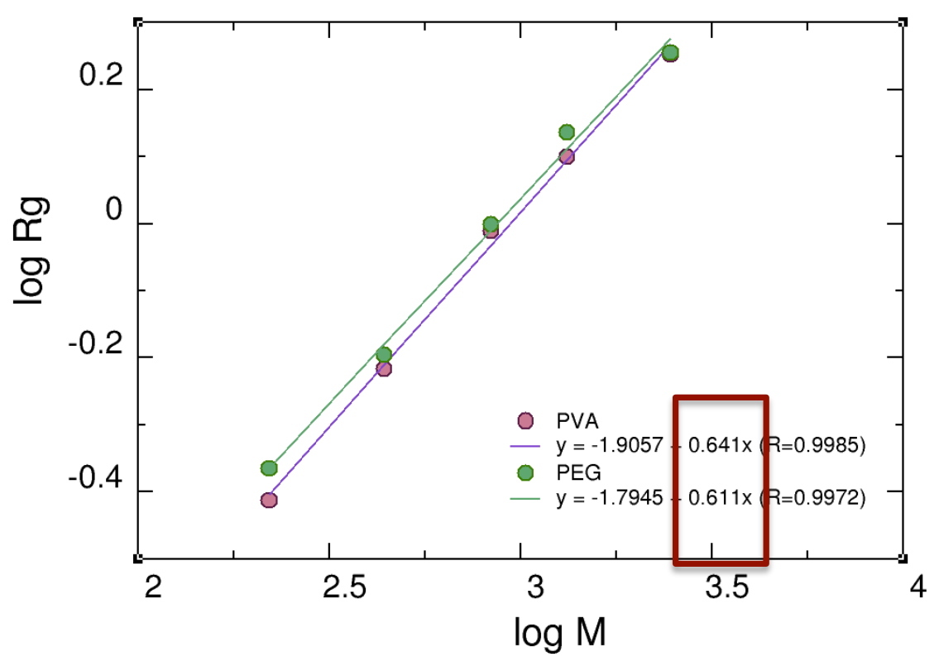


Fig. 4 Logarithmic plot of radius of gyration and molecular weight .

Polymer models were validated by plotting the linear correlation between the log radius of gyration (**Rg**) and log molecular weight (**M**). At approx. 0.6, our results are in agreement with experimental exponents of 0.588 and confirm their random coil form.

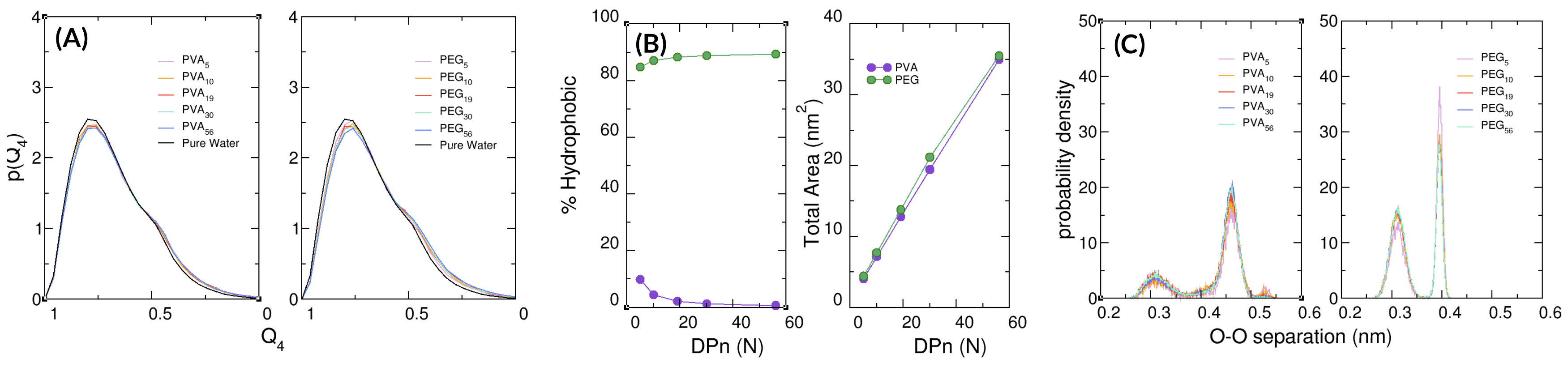


Fig. 5 The Q4 order parameter, where 1 is ice like and 0 is random gas arrangement of water molecules. The solvent accessible surface areas of polymers as a function of chain length (N). And the distribution oxygen-oxygen separation distance.

One hypothesis is that the antifreeze molecules structure liquid water either in an ice-like configuration, which could promote binding to a growing ice crystal, or in a disordered configuration that could inhibit ice crystal growth. Our simulations indicate that PVA does not have significant ordering or disordering effect on surrounding water molecules in solution. On the other hand the O-O separation of PVA in solution is 0.46 nm (compared to 0.31 nm and 0.39 nm in PEG), which corresponds to the O-O separation distance on the prism plane of ice, suggesting that direct binding to ice may play an important role in the mechanism. A delicate balance of hydrophobicity, (approx. 2 %) may responsible or correlated with the adopted conformations. E.g. induced collapse or for folding.

Results: Simulation of polymers and ice

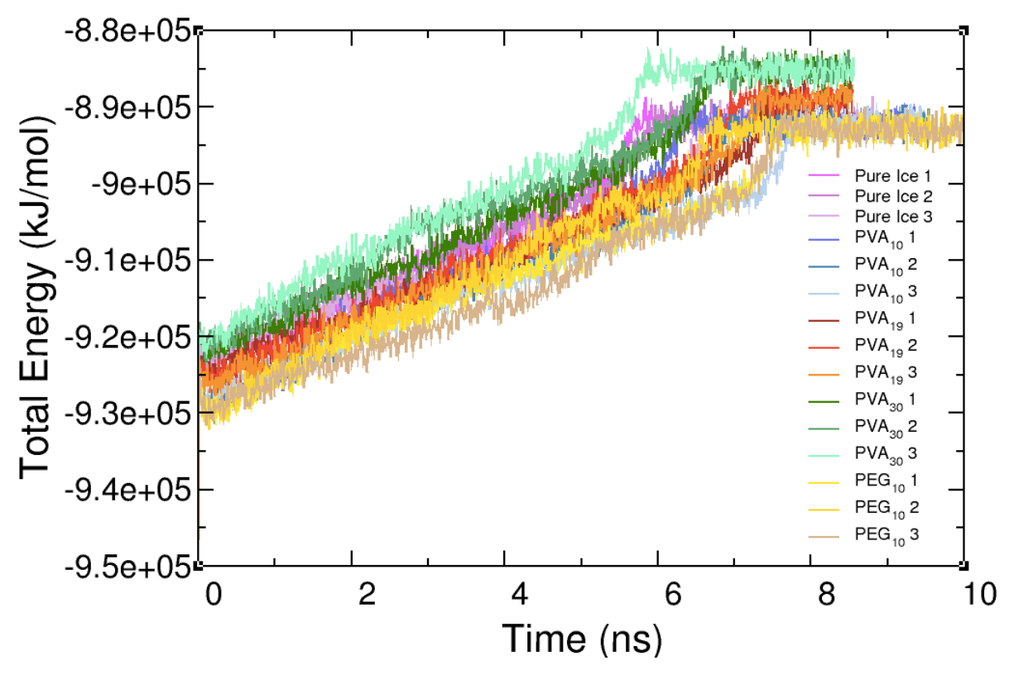


Fig. 6 The total energy against time in the presence and absence of polymers at 280 K.

In fig 6, the plots of the total energy against time highlights the conversion process. Complete melting is observed at 280 K, and is reflected by a rise in total energy. The reverse is true for freezing. Results indicate that the times taken for melting at 280 K is ordinarily an average of 6.33 (±0.55) ns without any additives. In the presence of PVA_{10}, PVA_{19} and PVA_{30} this changes to 7.24 (±0.87), 7.26 (±0.16) and 6.48 (±0.54) respectively. For PEG_{10} the value 7.35 (±0.61). Snapshots of the completed systems are shown in fig 2A and 2B.

Fig 7A and B display the times taken for completion of melting at different temperatures with and without polymers. As the leading, proposed adsorption mechanism suggests, it would be expected that the polymers would slow down the rate of freezing as well as encourage superheating. In fact we have found that all additives increased the time taken for melting to occur, including PEG which is antifreeze inactive and typically takes longer. This is the case for 310 K, 290 K, 280 K and 275 K. It suggests that the mechanism may not be the applicable in the case of the reduced ice recrystallization properties of PVA. Experimentally it has been shown that antifreeze activity "switches on" from 10 units of PVA to 20. Additional chain lengths are required to draw better correlations in chain length between the polymers however at high temperatures, larger polymers appear to disrupt the melting time to a lesser extent.

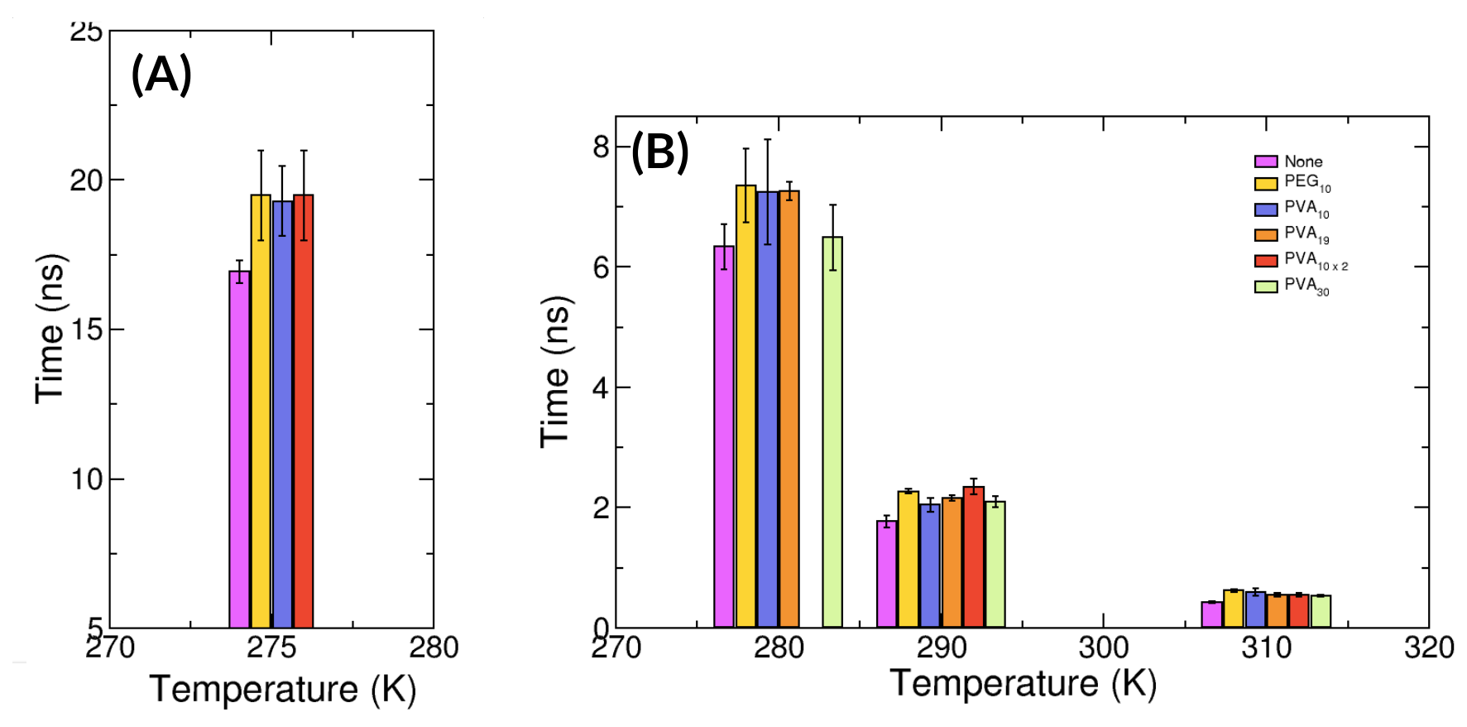


Fig. 7 Time taken for the completion of melting at 4 different temperatures with and without the polymers. Note that only 5 and 4 polymers are shown for 280 K and 275 K respectively.

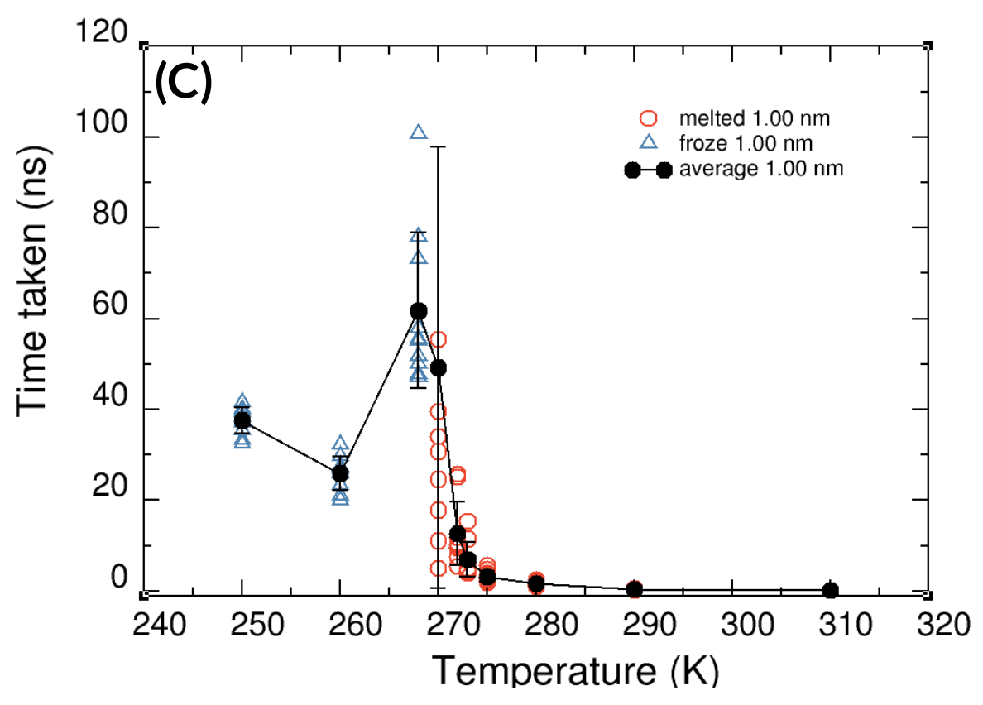


Fig. 8 Example of a melting profile. The time taken for melting and freezing of a smaller ice system size. Across different temperatures

Further work

Lower and higher temperatures will give greater insight into the thermal hysteresis behavior to govern the structure/property relationships that govern the reduced ice recrystallisation activity of PVA, and the lack of it in PEG.

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[1] NHS Organ Donation Annual Statistics
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