

# Ice Nucleation Promotion Impact on Ice Recrystallization Inhibition Activity of Polyols

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## ABSTRACT

Heterogenous ice nucleation occurs vis-à-vis nucleating agents already present in solution yet can occur within a rather broad range of temperature (0 to circa -30 °C). Controlling this temperature and the growth of resulting ice crystals is crucial for the survival of biological organisms (certain insects, fish, plants that endure sub-zero temperatures), as well as in the context of medical cryopreservation and food science. In these environments, uncontrolled crystal shape and size can rupture the cell membrane causing irreversible and catastrophic damage. Antifreeze (AF) proteins and synthetic AF analogs address this role to restrict crystal growth and to shape ice crystals. Although several synthetic AF approaches for the exploitation of these proteins have been investigated, challenges remain in the design of biomimetic polymers. Similar to biological AF proteins, poly(vinyl alcohol) (PVA) has potent IRI activity, however by comparison PVA has very little THA. We have systematically explored structural variations to polyol-based polymers to contrast with PVA as a control and we have identified several key structural elements for potent performance in IRI, THA, as well as in ice nucleation inhibition (INI). However, if the nucleation temperature is not controlled, and where diffusion of AF macromolecules becomes more restricted the lower the temperature, nascent ice crystals will have grown to a significantly larger size before the AF agents can be active on their surface to halt or slow the Ostwald ripening process during recrystallization. While antifreeze proteins, the inspiration for these synthetic analogs, are always applied in a salt buffer aqueous environment (most typically PBS buffer), the ions and other external impurities present lead to a stochastic nucleation event. Silver iodide (AgI), however is a highly effective ice nucleation promoter as its crystal lattice structure is a 98% lattice match to the basal plane of hexagonal ice (Ih) crystals acting as a template for water molecule orientation and decreasing the interfacial free energy. Here, we expose the advantage of purposely seeding such nascent ice crystals with AgI at a defined and high temperature (-7 °C), and in ultrapure water (UPW), resulting in the most potent synthetic IRI observed to date (at concentrations as low as 0.001 mg.ml<sup>-1</sup>).