Polymer Self-Assembly Induced Enhancement of Ice Recrystallization Inhibition

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**ABSTRACT:** Ice binding proteins modulate ice nucleation/growth and have huge (bio)technological potential. There are few synthetic materials that reproduce their function, and rational design is challenging due to the outstanding questions about the mechanisms of ice binding, including whether ice binding is essential to reproduce all their macroscopic properties. Here we report that nanoparticles obtained by polymerization-induced self-assembly (PISA) inhibit ice recrystallization (IRI) despite their constituent polymers having no apparent activity. Poly(ethylene glycol), poly(dimethylacrylamide), and poly(vinylpyrrolidone) coronas were all IRI-active when assembled into nanoparticles. Different core-forming blocks were also screened, revealing the core chemistry had no effect. These observations show ice binding domains are not essential for macroscopic IRI activity and suggest that the size, and crowding, of polymers may increase the IRI activity of “non-active” polymers. It was also discovered that poly(vinylpyrrolidone) particles had ice crystal shaping activity, indicating this polymer can engage ice crystal surfaces, even though on its own it does not show any appreciable ice recrystallization inhibition. Larger (vesicle) nanoparticles are shown to have higher ice recrystallization inhibition activity compared to smaller (sphere) particles, whereas ice nucleation activity was not found for any material. This shows that assembly into larger structures can increase IRI activity and that increasing the “size” of an IRI does not always lead to ice nucleation. This nanoparticle approach offers a platform toward ice-controlling soft materials and insight into how IRI activity scales with molecular size of additives.

**INTRODUCTION**

Ice binding proteins (IBPs)1,2 (and also polysaccharides3,4) are a diverse class of biological macromolecules that achieve the molecular recognition of ice crystals in the presence of a large excess of water. INPs include those classed as antifreeze proteins (AFPs), which can depress the freezing point of water (and can also raise the melting point)5 and are ice recrystallization (growth) inhibitors (IRI). Ice nucleating proteins (INPs) that promote ice formation are another distinct class.6−8 There are a huge range of technological challenges where IBPs (or synthetic mimics) could have impact, spanning the cryopreservation of donor cells and tissues,9−13 protecting infrastructure against freeze/thaw damage14,15 and improving the texture of frozen foods.16,17 Inspired by IBPs, there has been significant interest in the development of synthetic materials that can modulate ice growth and/or materials that can enhance cellular cryopreservation.18−22 Synthetic materials have advantages in terms of scalability, cost, and tunability compared to biological ones, but there is real need to understand their function and to address questions around how the size and nanoscale dimensions may impact activity.23−26 Poly(vinyl alcohol) (PVA) is established as a uniquely potent polymeric IRI, even though its exact mechanism of action is still under study.27,28 Graphene oxide has been reported to interact with ice,29−31 and the self-assembly of saffranine leads to ice binding aggregates.32,33 Ben and co-workers have shown that short glycopeptides,34 or even some surfactants,35 can inhibit ice growth but do not lead to altered ice crystal morphologies. It has been discovered that thermal hysteresis activity (a macroscopic property which requires ice face binding) does not scale with IRI activity in several ice binding proteins,36,37 and hence there is increasing evidence that ice binding is not a prerequisite for the macroscopic effect of IRI. Removing the need for ice binding (which needs precision placement of the interacting groups) would simplify...
Figure 1. Synthesis of PEG-corona PISA-derived nanoparticles. (A) Synthesis of \( \text{PEG}_{45}\)-b-PDAAm\(_n\) diblock copolymer nanoparticles via aqueous RAFT-mediated PISA using a commercially available PEG\(_{45}\) macro-CTA (\([\text{solids}] = 10\% \text{ w/w}, [\text{NaCl}] = 0.05 \text{ M}\)). (B) Normalized SEC RI molecular weight distributions. \( M_n, \text{SEC}\) and \( \mathcal{D}\) values were calculated from PMMA standards with DMF + 5 mM NH\(_4\)BF\(_4\) as the eluent. (C) Evolution of \( M_n\) (black circles) and \( \mathcal{D}\) (red circles) values with increasing targeted DP\(_{\text{PDAAm}}\). (D) Zeta potential values for \( \text{PEG}_{45}\)-b-PDAAm\(_n\) diblock copolymer nanoparticles. (E) Representative dry-state images of \( \text{PEG}_{45}\)-b-PDAAm\(_n\) diblock copolymer nanoparticles, stained with 1 wt % uranyl acetate (UA) solution. (F) Intensity-weighted size distributions along with average \( D_h\) and PD values (the error shows the standard deviation from five repeat measurements).
the design process and enable a new range of IRI-active materials to emerge with potentially easier design rules.

In the absence of ice binding, there is emerging evidence that materials with hydrophobic patches can display moderate IRI (but less than AFPs)\(^3\) such as self-assembled metal-lobelhicles,\(^9\) ROMP-derived polymers,\(^40\) or nanocellulose.\(^41\) Balcerzak et al. synthesized a library of sequentially modified lysine derivatives showing increased hydrophobicity to lead to more IRI.\(^42\) In many cases, IRI activity increases with molecular weight, observed for proteins\(^43\) and polymers\(^27\) and in self-assembly.\(^32,44\) However, it is more complicated than “more polymers on a surface, or larger structures, are always better”; the incorporation of PVA (IRI active and ice binder) as the corona of micelles\(^45,46\) or grafted to gold nanoparticles\(^47\) led to no increase in activity, potentially due to the low surface density of the polymers. Similarly, dendrimer or gold nanoparticle conjugated AFPs showed no enhancement on a per-protein subunit basis but on a molar basis led to large enhancements in both IRI and thermal hysteresis activity.\(^48,49\)

Ice nucleating proteins (a distinct class of IBPs) have to be sufficiently large to match the minimum ice cluster size,\(^50,51\) and it is proposed that a key difference in activity between larger ice nucleating proteins and smaller antifreeze proteins is their size.\(^3,52,53\) Considering this evidence, it is clear that the impact of macromolecular size, but also crowding, plays a role in understanding IRI (but also other properties such as thermal hysteresis) but that there are size limits on what can be obtained by using linear polymers; increasing the size of proteins (e.g., linear assemblies of repeat units) is not practical and will not lead to identical folding or display of surfaces. Hence, there is a need to discover new platforms where nanoscale dimensions and composition can be tuned.

Polymerization-induced self-assembly (PISA) is a powerful approach for in situ development of block copolymer nanoparticles to generate multivalent nanomaterials at high concentrations of controlled morphology and size.\(^54–56\) During aqueous PISA, a water-soluble corona-forming block is chain-extended by using specific water-miscible (dispersion PISA) or water-immiscible (emulsion PISA) monomers that gradually form a second, insoluble core block (as the length of the core-forming block increases), resulting in nanoparticle formation. Higher-order morphologies such as worm-like micelles and polymersomes are typically being observed, resulting in higher packing density for the copolymer chains compared to spherical (micellar) particles.\(^57,58\) The versatility of this method has allowed the synthesis of nanoparticles for drug delivery,\(^59–62\) cell storage,\(^63–65\) and permeable nanoreactors,\(^60–69\) and has been extensively reviewed.\(^70–73\) PVA-graft-macroinitiators have been reported to lead to polymer nanoparticles with enhanced IRI (compared to the already very potent IRI activity of PVA), potentially due to the high local PVA density arising from PISA.\(^74\) However, the use of nanoparticle engineering to discover emergent AFP mimetic materials from components with no intrinsic IRI activity has not been explored.

Considering the above, we herein report the unexpected enhancement of IRI activity of polymerization-induced self-assembly derived nanoparticles from constituent components with no IRI activity of their own. A panel of nanoparticles with variation in size, corona-forming, and core-forming block chemistry/functionality were synthesized. In each case IRI activity was unexpectedly increased relative to the homopolymer, but only in the largest (vesicular) nanoparticles, providing a previously unreported mechanism to generate IRI-active materials. By use of complementary ice nucleation assays, it was found that the size increase did not substantially increase ice nucleation activity, showing that making IRI-active materials larger does not lead to ice nucleation in all cases. This demonstrates that self-assembled nanomaterials and multivalent presentation is a crucial tool to understand, and translate to application, ice growth modifiers.

### RESULTS AND DISCUSSION

To investigate the hypothesis that aggregation and crowding of non-ice binding polymers in a nanoparticle format may induce IRI activity, a PISA strategy was followed to obtain polymer nanoparticles with tunable sizes and morphologies. Deionized water gives false positives in the ice growth assays used (below) as a eutectic phase where ice/water coexistence is essential, and hence saline solutes (or other solutes) are required.\(^38\) Therefore, to perform PISA, we first chose to use diacetone acrylamide (DAAm) as the core-forming monomer, which we have recently discovered to promote saline stability in PISA formulations (Figure 1A), unlike many other monomers.\(^74\) A series of aqueous dispersion polymerizations in the presence of [NaCl] = 0.05 M were performed for DAAm via thermally initiated RAFT polymerization using a PEG-based macromolecular chain transfer agent (macro-CTA, PEG\(_{45}\) mCTA, M\(_{n,SEC}\) = 4.1 kg mol\(^{-1}\), D\(_{w,m}\) = 1.1) as the corona-forming polymer. For these experiments the concentration of monomer was held constant at 10% w/w, the [macro-CTA]/[initiator] ratio was maintained at 1:0.1, and the [monomer]/[macro-CTA] ratio was varied to target different core-block degrees of polymerization. The polymerizations were carried out at 60 °C with 2,2’-azobis(2-methylpropionamide) dihydrochloride (V-50) as the water-soluble initiator. A gradual turbidity increase was noticed for polymerization solutions with increasing DP\(_{PDAAm}\) indicating the onset of particle formation typically observed in PISA. Quantitative monomer conversions (>95%) were achieved in all cases, as determined by \(^1\)H NMR spectroscopic analysis in methanol-\(d_4\), of the crude samples (Figure S5).

SEC analysis of PEG\(_{45}\)-b-PDAAm, diblock copolymers in DMF + 5 mM NH\(_4\)BF\(_4\) revealed the controlled character of the aqueous RAFT-PISA process (Figure 1B), with symmetric, monomodal molecular weight distributions shifting linearly toward higher molecular weight (M\(_n\)) values upon increasing the DP of PDAAm. A small low M\(_n\) shoulder of unconsumed macro-CTA was apparent, due to a small amount of unfunctionalized PEG. Calculated M\(_n\) values agreed well with theoretically expected values, while dispersity values remained relatively low for the polymerizations targeting DPs of 12, 25, 50, and 100 (D\(_w\) ≤ 1.6) but was higher for DP 150 (D\(_w\) = 2.1) throughout (Figure 1C and Table S1). The absence of charges on the outer surface of the obtained nanoparticles was confirmed by electrophoretic analysis at neutral pH (measured zeta potential ±0.5 mV) (Figure 1D).

DLS analysis of PEG\(_{45}\)-b-PDAAm formulations revealed the formation of particles with multiple populations and high polydispersity (PD) values for the lower DPs of PDAAm, consistent with worm-like micelles or nanoparticles with mixed morphologies.\(^34,75\) Single particle populations with low PD values were observed for DP\(_{PDAAm}\) ≥ 50, indicating the formation of uniform assemblies (Figure 1F). A single-exponential decay, smooth autocorrelation function with optimal signal-to-noise ratio was also recorded (Y-intercept
Supporting Information

Figure S6. Dry-state TEM analysis of IRI activity for PET-based nanoparticles. (A) IRI activity summary of PET-45-b-PDAAM100 nanoparticles corrected to [PEG]. Error bars are ± SD from a minimum of three repeats (key: S, spherical micelles; W, worm-like micelles; V, vesicles). The percent mean grain size (MGS) was reported relative to saline control ([NaCl] = 0.05 M). (B) Example cryomicrographs of ice wafers from the “splat” assay of PET-45-b-PDAAM100 nanoparticles, [PEG] = 10 mg mL−1.

Figure 2. Assessment of ice recrystallization inhibition activity for PET-based nanoparticles. (A) IRI activity summary of PET-45-b-PDAAM100 nanoparticles corrected to [PEG]. Error bars are ± SD from a minimum of three repeats (key: S, spherical micelles; W, worm-like micelles; V, vesicles). The percent mean grain size (MGS) was reported relative to saline control ([NaCl] = 0.05 M). (B) Example cryomicrographs of ice wafers from the “splat” assay of PET-45-b-PDAAM100 nanoparticles, [PEG] = 10 mg mL−1.

PET-45 block copolymer vesicles were annealed for different time points (t = 30 and 60 min) showing complete inhibition even after this time (Figure S13). Colloidal stability for PET-45-b-PDAAM100 vesicles upon multiple freeze–thaw cycles was assessed by DLS analysis and showed the particles were recovered with no significant changes in their hydrodynamic diameter (Dh) (Figure S11). The IRI activity as a function of total solid (polymer) concentration is also plotted in Figure S12. This shows that even when the core-polymer mass is included (which is not in contact with ice/water), the nanoparticles are still more active than a PET (corona) alone on a mass basis. The larger particles were also still more active, and hence the activity is not an artifact of concentration calculations and the nanoparticle size is a key criterion. To further demonstrate the IRI activity, an alternative assay was conducted in 45 wt % sucrose (“sucrose sandwich”) which has a lower ice fraction. Figure S14 shows nucleated ice crystals, which after 30 min have grown significantly (recrystallized) in the presence of PET-45 macro-CTA, while Figure S15 shows that a solution containing 10 mg mL−1 of PET-45-b-PDAAM100
nanoparticles displays inhibition of ice recrystallization, demonstrating activity in a range of different formulations. To evaluate whether the observations made above were only due to the PEG corona (as the component with most solvent interactions), a higher $M_w$ PEG macro-CTA (PEG113 mCTA) was employed, and the composition of the core-forming block was separately examined. Few PISA core-forming monomers are reported to be saline-tolerant.\(^8\) We employed an acrylamide-based monomer that can undergo PISA, N-(isobutoxymethyl)acrylamide ($i$BuOMAm), which was selected based on \textit{in silico} computation/experimental study by O’Reilly and co-workers.\(^5\) $i$BuOMAm was polymerized via aqueous RAFT in the presence of $[\text{NaCl}] = 0.05 \text{ M}$ by using PEG45 mCTA at 10% w/w solids content targeting a DPP$i$BuOMAm of 100. The prepared PEG45-$b$-PiBuOMAm\(_{100}\) diblock copolymer possessed bimodal molecular weight distribution due to unconsumed PEG mCTA with relatively low dispersity value, as determined by SEC analysis ($M_{n,SEC} = 25.1 \text{ kg mol}^{-1}$, $D_M = 1.6$) (Figure S16). Dynamic light scattering (DLS) analysis revealed the formation of nanoparticles with a monomodal size distribution and mean hydrodynamic diameter ($D_h$) of 357.9 ± 1.3 nm and low polydispersity (PD = 0.24 ± 0.01) (Figure 3A). Dry-state transmission electron microscopy (TEM) imaging confirmed the development of uniform PEG45-$b$-PiBuOMAm\(_{100}\) porous spherical nanoparticles and uniform size (Figure S12D). Cryo-TEM imaging of PEG45-$b$-PiBuOMAm\(_{100}\) nanoparticles in solution confirmed the formation of perforated spherical nanoparticles (Figure S16E). A higher $M_w$ PEG macrorinitiator (PEG113 mCTA—to probe effect of coronal chain length) was subsequently used for the polymerization of DAAm to yield PEG113-$b$-PDAAm\(_{100}\) and PEG113-$b$-PDAAm\(_{400}\) (to enable comparison of the same morphology with PEG45-$b$-PDAAm\(_{100}\) vesicles) diblock copolymer nanoparticles. TEM and DLS analyses showed the formation of uniform spherical nanoparticles and unilamellar vesicles for DP$\text{PDAAm} = 100$ and 400, respectively (Figure 3B, Figures S17 and S18), with monomodal size distributions, mean hydrodynamic diameters ($D_h$) of 96.6 ± 1.8 nm (DP$\text{PDAAm} = 100$) and 280.8 ± 8.2 nm (DP$\text{PDAAm} = 400$), and low polydispersities (PD = 0.20 ± 0.04 and 0.08 ± 0.04). These particles were tested for IRI and were found to show nearly identical activity to those that had PDAAm cores (above). Additionally, PEG113-$b$-PDAAm\(_{400}\) vesicles showed higher IRI activities compared to PEG45-$b$-PDAAm\(_{100}\) vesicles. This experiment proves that the nanoparticle size and morphologies were crucial for IRI activity, but not the nature of the core-forming block (other than its role in ensuring saline stability), primarily because of its inaccessibility by the solvent molecules (Figure 3C).

Figure 3. Synthesis, characterization, and ice recrystallization inhibition activity of PEG-coronal nanoparticles using PiBuOMAm- and PDAAm-based cores. (A) PEG45-$b$-PiBuOMAm\(_{100}\) (B) PEG113-$b$-PDAAm\(_{100}\) (i), and PEG113-$b$-PDAAm\(_{400}\) vesicles (ii) synthesized at [solids] = 10% w/w via aqueous RAFT-mediated PISA in the presence of $[\text{NaCl}] = 0.05 \text{ M}$ with commercially available PEG45 and PEG113 macro-RAFT agents. Insets show intensity-weighted size distributions along with average $D_h$ and PD values (the error shows the standard deviation from five repeat measurements) and representative dry-state TEM images stained with 1 wt % uranyl acetate (UA) solution. (C) IRI activity summary corrected to [PEG].
With the above observation of PEG activity and confirmation that the nature of core was not crucial, we envisioned that introducing a different corona composition, as it is the component that is in direct contact with the aqueous/ice
phases, would result in different IRI activities. PEG is not known to present IRI, so as it was important to compare it to other PISA-derived particles with a linear non-ionic hydrophilic corona-forming polymer, with similar repeat unit dimensions (to avoid comb-type PEG-(meth)acrylates which might affect packing density). Here, poly(vinylpyrrolidone) (PVP) was employed for the first time as a steric stabilizing block for performing PISA by using DAAm as the core-forming monomer (Figure 4). Initially, N-vinylpyrrolidone (NVP) was polymerized by using xanthate chain transfer of 2-(ethoxycarbonothioyl)sulfanyl propanoate (EXEP) (see Supporting Information for full synthetic procedure, Figures S3 and S4) via photoinitiated RAFT/MADIX to ensure maximum end-group fidelity (PVP₄₀, Mₙ,SEC,RI = 4600 g mol⁻¹, D_M = 1.3) (Figure S19).⁸⁴,⁸⁵ Then, a series of aqueous dispersion polymerizations in the presence of [NaCl] = 0.05 M were performed for DAAm via RAFT by using the synthesized PVP₄₀ macromolecular chain transfer agent. The concentration of monomer was held constant at 20% w/w, and the [monomer]/[macro-CTA] ratio was varied to target DP_DAAm = 25, 50, 100, and 200. A gradual turbidity increase was noticed for polymerization solutions with increasing DPPDAAm, indicating the onset of particle formation. Quantitative monomer conversions (>97%) were achieved in all cases after 6 h, as determined by ¹H NMR spectroscopic analysis in methanol-_, of the crude samples (Figure S20 and Table S2).

SEC analysis of PVP₄₀-b-PDAAmₙ diblock copolymers revealed a rather uncontrolled character of chain extensions of PVP₄₀ macro-CTA with high dispersity values (D_M > 2.0) as would be expected for this macroinitiator (Figure S21).⁸⁶ PVP is known to undergo undesirable side reactions in aqueous media, leading to poor RAFT control and incomplete monomer conversions,⁸⁷,⁸⁸ and it can only be readily copolymerized with a limited set of other lesser activated monomers (LAM), such as vinyl acetate or acrylics,⁸⁹,⁹⁰ but no LAM dispersion PISA monomer is known, necessitating this approach. It is crucial to note that a relatively high proportion of PVP stabilizer chains remained unreacted at the end of the polymerization, leading to bimodal molecular weight distributions and is discussed in terms of the IRI activity below. Peak deconvolution was also performed on SEC curves for resulting block copolymer nanoparticles (Figures S23−S26), and the ratios of the areas between chain extended and unconsumed PVP macro-CTA are summarized in Table S3.

Dry-state TEM analysis showed an evolution in morphology upon increasing the DP of the core-forming block from spheres (DP_DAAm = 25 and 50) to mixed morphologies of spheres and vesicles (DP_DAAm = 100) to pure single-phase vesicles of uniform size (Figure 4B). For DP_DAAm = 100, the appearance...
of mixed spheres and vesicles can be attributed to the high dispersity values with a large proportion of PVP stabilizer chains being chain-extended to different degrees. Cryo-TEM analysis further confirmed the resulting morphologies (Figure S27). DLS analysis supported TEM findings and revealed the stable formation of particles with single populations and low polydispersity (PD) values (Figure 4C and Figure S28). The absence of charges on the outer surface of the obtained nanoparticles was also confirmed by electrophoretic analysis at neutral pH (measured zeta potential \( < \pm 5 \) mV) (Figure 4D). A stabilizing block of poly(\( N \)-dimethylacrylamide) (PDMAC) was also employed to perform PISA by using DAAm as the core-forming monomer (Figure 4E). DMAC was initially polymerized in dioxane at 70 °C using \(-(((\text{butylthio})\text{carbonothiolyl})\text{thio})\)propanoic acid as the chain transfer agent (see Supporting Information for full synthetic procedure, Figures S5 and S6). The resulting homopolymer was analyzed by \(^1\)H NMR and SEC (PDMAC\(_{40}\) \( M_{n,\text{SEC,R1}} = 4800 \) g mol\(^{-1}\), \( D_M = 1.1 \)) (Figure S30). This water-soluble polymer precursor was chain-extended with DAAm via RAFT aqueous dispersion polymerization at 60 °C and 20% w/w solids in the presence of [NaCl] = 0.05 M, targeting a final DP\(_{\text{PDAAm}}\) of 200. This system has been extensively studied in the past, and previous synthetic procedures were followed to achieve vesicular morphologies.\(^79,80,91−93\) Quantitative monomer conversion (>98%) was achieved after 6 h, as determined by \(^1\)H NMR spectroscopic analysis in methanol-\( d_4 \) of the crude sample (Figure S31). The prepared PDMAC\(_{40}\)-b-PDAAm\(_{200}\) diblock copolymer possessed monomodal molecular weight with relatively low dispersity value, as determined by SEC analysis in DMF + 5 mM NH\(_4\)BF\(_4\) (\( M_{n,\text{SEC}} = 31600 \) g mol\(^{-1}\), \( D_M = 1.5 \)) (Figure S32). Dynamic light scattering (DLS) analysis revealed the formation of nanoparticles with a monomodal size distribution and mean hydrodynamic diameter (\( D_h \)) of 308.8 ± 5.5 nm and low polydispersity (PD = 0.12 ± 0.02) (Figure 4Fiii). Dry-state and cryo-TEM imaging finally confirmed the development of single-phase unilamellar vesicles (Figure 4Fi).

Figure 5 shows the IRI activity of the non-PEG-based corona formulations. For PVP, the same particle size dependence on activity as seen for PEG was observed with larger particles (vesicles) being more active than smaller ones.
which may indicate ice binding (as opposed to a non-specific interaction), which leads to segregated ice crystals whose morphology can be tuned to nucleate ice at a given temperature. The ice critical cluster size increases rapidly as temperature approaches the melting point. For example, the critical ice cluster has a diameter of about 20 nm at −5 °C. This means large aggregates of (for example) bacterial ice nucleating proteins are required to induce ice nucleation close to 0 °C. There is theoretical evidence that molecular size is a key criteria for ice nucleation, and it has been observed experimentally that some AFPs can also nucleate ice, with the size of the AFP being an essential descriptor. Given that some of the polymer particles synthesized bind ice, all show IRI and are large compared to ice critical nuclei; it might be thought that they would be capable of nucleating ice at warm temperatures as seen for higher molecular weight antifreeze proteins. To test this, microliter ice nucleation assays were undertaken by using a bespoke apparatus described in the Supporting Information. This instrument freezes around 50 droplets per experiment to establish the spread of likely nucleation temperatures for a given heterogeneous nucleator. The ice nucleation temperatures of 1 mg mL−1 nanoparticle and polymer solution are reported in Figure 6D,E. It was found that the PEG- and PDMAC-based formulations nucleated ice at average temperatures of −25.8 and −26.8 °C, respectively, only slightly warmer than the average freezing temperature of pure water on the instrument, −28.1 °C. PVP and the various PVP-based nanoparticles tested nucleated ice at average temperatures between −23.6 and −24.5 °C. Notably, the fraction frozen curves for PVP and PVP40-b-PDAAm100 were very similar. While the PEG- and PDMAC-derived nanoparticles do not nucleate ice at slightly warmer temperatures than their precursor polymers, the difference is not large. Figure 6E shows a slight increase in nucleation temperature with increasing particle size for PVP-derived nanoparticles; however, the enhancement is not large. The nucleation data show that aggregation of polymers into particles does not substantially enhance ice nucleation effectiveness. This supports the theory that correct long- and short-range molecular mechanisms are required for a nucleant to be highly effective. These combined observations support emerging evidence that the observation that ice binding proteins are not always caused by the same underlying molecular mechanisms, and that a particular property can be dialled into a material. There is an opportunity to achieve desirable levels of ice growth inhibition (for a specific application) by deploying nanoparticle formulations such as those presented here, even if their overall activity is substantially weaker than IBPs, and indeed their mechanism is non-specific.

**CONCLUSIONS**

We report that biomimetic ice recrystallization inhibition activity can be introduced into polymer nanoparticles from components which themselves have no ice binding or associated activity. Saline-tolerant polymerization induced self-assembly (PISA) was exploited to obtain nanoparticles of controlled size and morphology by using a library of different corona-forming polymers [poly(ethylene glycol), poly(vinylpyrrolidone) (for the first time via dispersion PISA), and...
poly(methylacrylamide)] and core-forming blocks [poly-(diacetone acrylamide) and poly((isobutoxymethyl)-acrylamide)]. In all cases, ice recrystallization inhibition was observed despite the constituent homopolymers (i.e., core-forming blocks) having no activity themselves, suggesting that size and confinement of the coronal blocks can enhance this macroscopic property. Larger particles (vesicles) were more active than smaller ones (micelles/spheres and intermediate morphologies), confirming that the size and nanoarchitecture of the particles were crucial for inducing activity. The activity enhancement relative to linear polymers was seen both in terms of the total coronal mass (as the solvent-contacting component) or as the total mass (including the core), showing this was not simply due to the increased mass applied in a nanoparticle formulation. The core-forming blocks were also varied, and this had minimal or no impact on the observable ice recrystallization inhibition activity, confirming this was a general effect. In the case of poly(vinylpyrrolidone) coronal particles, there was observation of ice shaping, suggesting these particles may engage specific ice faces, which was not seen with the polymers alone. This additional binding did not lead to significantly higher IRI activity though, suggesting IRI was dominated by their size and packing density for each morphology. Ice nucleation was also studied, but no activity was seen. This was a crucial observation as it has been reported that larger assemblies of antifreeze proteins can lead to ice nucleation activity but that the assembly of these polymers does not lead to ice nucleation. This may suggest a unique mechanism, but the huge structural differences between the particles and AFPs make a real comparison difficult. The data here also supports emerging evidence that the magnitude of each macroscopic property associated with ice binding proteins mimics does not scale equally between all materials. The exact molecular mechanism for this increased activity is not clear and may be simply related to the overall size and molecular weight of the particles, which exceeds what is accessible with linear polymers. However, the PVP data supports that increased ice binding can occur in these assemblies. These observations show that coronal confinement of polymers can induce the macroscopic effect of ice recrystallization inhibition but that the same is not true for ice-nucleation activity, providing evidence that simply enlarging IRI-active components does not guarantee ice nucleation. While the overall magnitude of activity here is less than ice binding proteins, there is huge technological potential in the development of freeze-stable colloids, exploiting this size-driven enhancement to translate to applications, as well as a tool to study the fundamentals of ice-binding/modifying materials.

■ ASSOCIATED CONTENT

+ Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.1c01963.

Full synthetic details and characterization of polymers and nanomaterials (PDF)

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Notes
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