

## MATERIALS SCIENCE

## Peptide-DNA origami as a cryoprotectant for cell preservation

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Cryopreservation of cells is essential for the conservation and cold chain of bioproducts and cell-based medicines. Here, we demonstrate that self-assembled DNA origami nanostructures have a substantial ability to protect cells undergoing freeze-thaw cycles; thereby, they can be used as cryoprotectant agents, because their nanoscale morphology and ice-philicity are tailored. In particular, a single-layered DNA origami nanopatch functionalized with antifreezing threonine peptides enabled the viability of HSC-3 cells to reach 56% after 1 month of cryopreservation, surpassing dimethyl sulfoxide, which produced 38% viability. It also exhibited minimal dependence on the cryopreservation period and freezing conditions. We attribute this outcome to the fact that the peptide-functionalized DNA nanopatches exert multisite actions for the retardation of ice growth in both intra- and extracellular regions and the protection of cell membranes during cryopreservation. This discovery is expected to deepen our fundamental understanding of cell survival under freezing environment and affect current cryopreservation technologies.

## INTRODUCTION

Protection of cells from cryoinjuries has been a major challenge in cryopreservation. Dimethyl sulfoxide (DMSO) is one of the most common and widely used membrane-penetrating cryoprotective agents (CPAs) that hinder the formation and growth of ice crystals (1, 2). The optimal concentration of DMSO mixed with cell culture media is typically as high as 10% (v/v); however, the use of high concentrations of DMSO has been shown to produce cytotoxicity and induce adverse effects when thawed cells are infused into the patient (3–5). Therefore, DMSO is occasionally used together with other chemicals such as hydroxyethyl starch, sucrose polyvinyl pyrrolidone, dextran, and trehalose for reducing cytotoxicity (6). In addition, other polymers that have strong ice recrystallization inhibition (IRI) activity can be used with other CPAs; these polymers include synthetic polypeptides (7, 8), poly(vinyl alcohol) (PVA) (9, 10), and glycoconjugates (11, 12).

Another class of cell cryoprotectants is natural antifreeze (glyco-) proteins [AF(G)Ps] found in certain polar fish (13–15), water flounder (16, 17), and plants (18). They are effectively adsorbed onto specific ice planes, resulting in the thermal hysteresis of the solution and growth inhibition of ice seeds (19). Such binding characteristics of certain AF(G)Ps originate from periodically arranged peptides such as alanine (Ala) or threonine (Thr) (20, 21). The IRI ability of AF(G)Ps has led researchers to exploit them as cryoprotectants; when used in combination with DMSO or glycerol, the concentration of which was lower than that typically used for cryopreservation, certain AF(G)Ps showed similar effects on cryoprotective functions. However, the addition of AF(G)Ps was not necessarily successful in improving cell viability, possibly owing to the anisotropic ice-shaping behavior and poor cellular uptake efficiency (22). While the investigations

use ice-binding proteins (23, 24) and two-dimensional materials like graphene oxide (25, 26) with efficient antifreeze or ice nucleation properties, there have been no reports of an alternative CPA that can be used alone and that outperforms the cell recovery by 10% DMSO to date.

Here, we investigated an approach to achieve higher cell viability after long-term cryopreservation (up to 1 month) using self-assembled nanoscale DNA structures. Scaffolded DNA origami nanostructures, consisting of a long single-stranded DNA and multiple short strands that fold it into a designed geometry, have been widely used as intracellular delivery carriers or membrane-attachable structures (27–29). Their intracellular uptake ability without cytotoxicity (30, 31) and robust shape integrity after freeze-thaw cycles (32) prompted us to explore them as attractive candidates for CPA. We tested slender bundles and rectangular patch structures and found that the patch shape showed a noticeable cryoprotective performance. Inspired by the conjugation of antifreezing peptides onto gold nanoparticles (33), we functionalized DNA nanopatch (NP) with different types and numbers of antifreezing peptides using peptide nucleic acid (PNA)-based linkers (Fig. 1).

## RESULTS

## Design and self-assembly process

To investigate the cryoprotective performance of structured DNA assemblies, scaffolded DNA origami with lattice-packed geometry (34) was used to create DNA nanostructures with regular dimensions. Nanorod geometries consisting of 6 and 12 double-stranded DNA (dsDNA) helices (denoted as 6HB and 12HB, respectively) were designed with lengths of approximately 380 and 190 nm, respectively (Fig. 2A and fig. S1) (35). In addition, a single-layered rectangular nanopatch (90 nm by 70 nm) consisting of 24 dsDNA helices (denoted as NP) was prepared (Fig. 2A and fig. S1) (27). For the attachment of antifreezing peptides to the DNA nanopatch, 48 or 96 single-stranded overhangs were inserted at regular intervals, protruding at the single side of the nanopatch. We introduced a PNA-based linker consisting of two parts: (i) nucleic acid bases that are complementary to those of the overhangs and (ii) peptides of

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