Abstract
Freezing is initiated by generation of numerous embryo ice crystals in water, which grow and merge together to form their multicrystalline state, the ordinary ice block. Antifreeze protein (AFP) is capable of binding to these ice crystals to inhibit their growth, and disturbs such general ice formation. This mechanism is expected to solve many technical problems with regard to the frozen storage of water-containing materials, such as foods and tissues. The fish-derived AFP can also bind to the lipid bilayer to prolong the lifetime of a cell, which is applicable to the short-term hypothermic cell preservation. Natural fish AFP is a mixture of 2–13 isoforms that function together far more effectively than any single isoform. We have been therefore trying to develop mass preparation method of natural fish AFP (the mixture), and now AFP, AFPIII, and AFGP samples are available from NICHIREI CORPORATION, Japan (E-mail to s.tsuda@aist.go.jp or directly to N1000X016@nichirei.co.jp). Each AFP sample is highly purified (>95%) and contains neither cations nor buffer detergents. The samples are also sterilized using 0.22 μm syringe filter, so that directly applicable to any kind of experiment including medical tests. AFPI sample will also be released soon.

Functions
A single ice crystal consists of water molecules in hexagonal arrangement, while in solution it forms a disk-like shape (A-I). In AFP solutions, ice crystal growth is only allowed between the bound AFPs (A-II) according to the Gibbs-Thomson effect. The resultant convex ice front surrounded between the AFPs is terminated its growth to form a flat, AFP-accumulated surface (A-III). This process transfers the disk-shaped ice crystal into a hexagonal plate, and allows generation of a new disk on that plate through a mechanism called 2D-nucleation. Repeated AFP binding and a new disk generation causes successive stacking of smaller hexagonal ice plates in the direction of the c-axis (A-IV&V), forming a hexagonal bipyramid (A-V) onto which millions of AFPs are adsorbed. This unique ice crystal is further modified into its derivative forms, such as hexagonal trapezohedron (A-VII) and a lemon-like shape (A-VIII). The ice hexagonal plate remains unchanged and is not further modified into a bipyramid, when AFP concentration is below 40–50 μg/mL (depends on the condition), which we termed the “critical ice-shaping concentration” (CISC) (Mahatabuddin et al. 2016). The general disk-shape ice crystals (A-I) undergo ice recrystallization and forms multicrystalline state by freezing (C). This ice expands the volume and physically destroys inside texture of all of the frozen materials. AFP is capable of inhibiting growth of the ice crystals to form ice slurry (D). If we can minimize the size of each ice crystal ultimately small, the frozen state should become noncrystalline glass-like state (E). That is, AFP should be able to freeze and preserve the water-containing materials by filling their inside with numerous tiny ice crystals. This can be realized with a home freezer (-20 degC) without LN2 nor deep freezer.

Application 1
AFP exhibited a gel protection function, which made us to develop “gelation freezing method” to fabricate porous materials, for which AFP has a significant contribution (Fukushima et al. 2013). A solution of gelatin, ceramic powder, and AFP is initially cooled to form a gel and placed on a freezing plate to induce unidirectional freezing. Because AFP binds to the surface of the elongating ice crystals, extremely sharpened and uniformly aligned ice needles are created in the frozen gel. After sintering at 1,000°C, a ceramic containing numerous unidirectionally-aligned dendritic pores is created.

Application 2
Cell protection effect of AFP has been suggested in both cryo- and hypothermic conditions. The former used a very diluted solution (below CISC) so as not to create ice bipyramids. In nature, AFP functions with glucose, glycerol, lipids, ions, minerals, etc. The AFP protection will be maximized by optimal combinations of these substances, which should be different between the applications.

Summary
Highly purified natural fish AFP, III, and AFGP containing neither salts nor buffer detergents are now supplied from NICHIREI CORPORATION, Japan. Ref. Nishimiya et al. Synthesiology 2008, 1 (1) 4–17; Mahatabuddin et al. Cryobiology and Cryotechnology 2016, 62 (2) 95–103; Fukushima et al. 2013, J Am Ceram Soc 96, 1029–1031. This research was supported by JSPS (15K13760).

*AFP I is “BpAFP” introduced in Mahatabuddin et al. 2017 Concentration-dependent oligomerization of an alpha-helical antifreeze polypeptide makes it hyperactive, Scientific Reports 7, 42501.

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