Size-Tunable Micro-/Nanofluidic Channels Fabricated by Freezing Aqueous Sucrose

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ABSTRACT: Upon freezing aqueous sucrose at temperatures higher than the eutectic point (−14 °C in this case), two phases, that is, ice and freeze concentrated solution (FCS), are spontaneously separated. FCS forms through-pore fluidic channels when thin ice septum is prepared from aqueous sucrose. Total FCS volume depends on temperature but is independent of the initial sucrose concentration. This allows us to control the size of the FCS channels simply by changing the initial sucrose concentration as long as temperature is kept constant. In this paper, we show that the size of the channel, which has a layered structure, can be controlled in a range from 50 nm to 3 μm. Thus, the FCS channel is suitable for size-sorting of micro- and nanoparticles. We discuss the size-sorting efficiency of the channel and demonstrate the separation of particles with different sizes.

INTRODUCTION

Size-selective separation of particles is an important task in various fields, including micro/nano-science/technology, life science, environmental chemistry, and industrial applications. Particle separation has been conducted using field flow fractionation (FFF), hydrodynamic chromatography, electrophoresis, dielectrophoresis, and so forth. In these separation methods, physical fields are utilized to induce different distributions of particles according to their physical properties, such as sizes and charges. For example, an electric field has been used in a number of methods, such as FFF, electrophoresis, and dielectrophoresis. Also, flow field plays an important role in FFF and hydrodynamic chromatography to differentiate the elution of particles. Other physical fields employed for particle separation include magnetic, acoustic, and thermal fields. The recent development of micro/nanofluidic devices has put complicated on-line procedures at our disposal, including mixing and separating streamlines in the micrometer range and incorporating physical interactions of particles with structures fabricated in a channel. However, new principles and concepts for particle separation are still required because of the limited separation efficiency and versatility of the methods currently available.

We have reported various separation and reaction systems using frozen aqueous solutions. When an aqueous solution is frozen, solutes are expelled from ice and concentrated in a freeze concentrated solution (FCS). The FCS coexits with ice at temperatures higher than the eutectic point, and its volume is predictable from the equilibrium freezing point depression curve of the system. Thus, we can control the volume of the FCS by changing the temperature and solute concentration. Another critical point in the utilization of an FCS for analytical purposes is the morphological control of the FCS. As noted above, although the total volume of the FCS is thermodynamically known, thermal equilibrium does not predict its morphology, which depends on various factors, such as the freezing rate, solute distribution, and the physicochemical nature of the FCS. In our previous work, the dendritic growth of ice was confirmed when an aqueous sucrose solution was frozen. Straight channel-like grooves are formed between ice dendrites and are filled with the FCS. This structure is highly reproducible if the same freezing conditions are employed.

The grooves formed on the ice surface act as fluidic channels suitable for size-sorting of nano/microparticles. The most remarkable property of this channel is that the groove width can be controlled in a range from 200 nm to 4 μm with high reproducibility, simply by changing the working temperature. When the groove width becomes smaller than the size of a particle introduced therein, the migration of particles is severely restricted because the channel is not accessed from the ice walls of the groove. This concept was extended to the evaluation of the interaction of antifreeze proteins with the ice surface. In this method, particles were spontaneously introduced in the grooves by a freezing aqueous particle suspension because they are expelled from the ice phase. Although this approach is effective for evaluating the size of a particle and its interaction with the ice walls, particle separation or fractionation is severely restricted because the channel is not accessed from outer solutions; it is impossible to move particles in and out of the channel. In this paper, we extend this concept to the size-selective separation of micro/nanoparticles, which facilitates the application of freeze-control of channel dimensions in the micro- to nanometer range.
RESULTS AND DISCUSSION

Channel Size and Its Control. Figure 1 compares a fluorescence microscopic image of the ice septum ($c_{\text{suc}} = 3.38$ wt %) formed immediately after its preparation by freezing at $-14.0 \, ^\circ\text{C}$ with that after annealing at $-5.0 \, ^\circ\text{C}$ (Figure 1B) and $-3.0 \, ^\circ\text{C}$ (Figure 1C). Fluorescein was added to visualize the FCS. While no clear FCS structures are seen in the septum immediately after freezing, channel-like veins filled with the FCS are developed after annealing. The development of channel structures can be explained by the growth of dendritic ice.26,27 This leads to the formation of long dendrite twigs for $c_{\text{suc}} = 3.38$ wt %, particles with $d = 3 \, \mu\text{m}$, and $y_{\text{f}}$ values, which are filled by the FCS, as found on the ice surface in our previous work. Bogdan et al.28 indicated that a continuous ice framework rather than isolated ice crystals is formed when an aqueous sucrose is frozen. Their microscopic observation showed that the FCS is present between ice twigs. A similar phenomenon occurs inside the frozen aqueous sucrose and allows the formation of long through-channels in the ice septum. It is obvious that the long channel formation is related to the FCS viscosity because through-channel formation was rarely confirmed in frozen aqueous glycerol, although the results are not shown. The FCS viscosity for aqueous glycerol at $-4.0 \, ^\circ\text{C}$ is $4.25 \, \text{mPa} \cdot \text{s}$,20 which is one-third of that in frozen sucrose. Thus, the FCS viscosity is a critical factor for through-channel formation in frozen aqueous solution.

The total volume of the FCS is a function of the initial concentration of the main solute (sucrose in the present case, $c_{\text{suc}}^{\text{ini}}$) and temperature. In contrast, the concentration of the FCS ($c_{\text{suc}}^{\text{fcs}}$) is a function of temperature but independent of $c_{\text{suc}}^{\text{ini}}$. Bogdan et al. showed that the ice framework is immersed in FCS, and more concentrated FCS is present between ice dendrite twigs for $c_{\text{suc}}^{\text{ini}} = 3.38$ wt %.28 Because the present $c_{\text{suc}}^{\text{ini}}$ was lower than 3.38 wt % ($=100 \, \text{mM}$), the FCS is present between ice twigs and no sucrose concentration gradient exists. The freezing depression curve for the sucrose/water system is shown in Figure S1.30 At temperatures above the eutectic point ($-14 \, ^\circ\text{C}$), the channels are filled with the FCS with the concentration of $c_{\text{suc}}^{\text{fcs}}$, which can be read from the freeze depression curve. In this work, the working temperature was kept constant at $-4.0 \, ^\circ\text{C}$, while $c_{\text{suc}}^{\text{ini}}$ was varied in the range of $0.034 \text{–} 3.38 \text{ wt }\%$. Thus, the FCS volume can be controlled by changing $c_{\text{suc}}^{\text{ini}}$ because $c_{\text{suc}}^{\text{fcs}}$ is constant at $1.3 \, \text{M}$.

The shape of the FCS-channel cross section was evaluated by confocal fluorescence microscopy. Figure 2 summarizes micrographs of the cross-sections of the channels fabricated using $c_{\text{suc}}^{\text{ini}} = 0.68$ wt %. Fluorescein was contained in the FCS for visualization. Although fluorescence imaging does not provide the proper morphologies of the channels because of light penetration into ice and scattering at the ice/FCS interface, all of the channel cross-sections have large aspect ratios, that is, rectangular or oval. This can be understood by considering the origin of the FCS channel formation in the frozen aqueous solution. Figure S2 schematically shows the FCS channel development between two ice dendrites. The FCS is sandwiched between two facets of ice dendrites facing each other and forms a thin layer. The ice crystal size after ripening is typically in the range of $100 \, \mu\text{m}$.29,31,32 Thus, the typical length and width of the FCS channel are in this dimensional range. As shown later, particles often pass through other particles in the same channel, suggesting that the channel has a large width orthogonal to the direction of particle migration. Also, our electrochemical measurements indicated that the FCS forms a thin layer on the electrode surface.33 Thus, the cross-section with a high aspect ratio is reasonably explained.

The passage of particles through the ice septum was observed using the fluorescence microscope. The passage of particles of various sizes (diameter, $d = 50 \, \text{nm}$ to $3 \, \mu\text{m}$) through the ice septum prepared from the sucrose solution of $c_{\text{suc}}^{\text{ini}} = 0.034 \text{–} 3.38 \text{ wt }\%$ was studied. The ice septum was prepared with the same $c_{\text{suc}}^{\text{ini}}$ at least three times, and the passage of more than ten particles through each septum was tested. The migration and passage of relatively large particles ($d \geq 0.5 \, \mu\text{m}$) were directly observed using a microscope. The passage of smaller particles through the ice septum was confirmed by measuring the fluorescence intensity at the outlets of the channels. The results are summarized in Table 1 and Figure 3. Table 1 summarizes the confirmation of the passage for particles with $d = 50 \, \text{nm} \text{–} 3 \, \mu\text{m}$. For example, the ice septum prepared with $c_{\text{suc}}^{\text{ini}} = 3.38 \text{ wt }\%$ allowed all of the particles tested to pass through the septum. In contrast, when $c_{\text{suc}}^{\text{ini}}$ decreased to $1.70 \text{ wt }\%$, particles with $d = 3 \, \mu\text{m}$ became impassable, whereas smaller ones remained passable. Similarly, the threshold particle size became smaller as $c_{\text{suc}}^{\text{ini}}$ decreased.

Based on the above observations, the channel thickness ($t$) was estimated that determines the size selectivity of the FCS channel. When a particle passes through a channel, the channel thickness, $t$, is larger than the particle diameter. In contrast, when the particle passage is not confirmed, the channel
migration rate decreased with a decrease in linearly with much less sensitive to the decrease in the ice volume. Thus, a change in the ice volume is, example, an increase in the size of ice becomes smaller. However, the shrinkage of ice thickness should be smaller than the particle diameter. Figure 3 shows that there is a linear relationship between log t and log $c_{\text{suc ini}}$. The slope is almost unity, suggesting that t is proportional to $c_{\text{suc ini}}$. As discussed above, the major channels are formed between two ice crystal facets. When $c_{\text{suc ini}}$ increases, the FCS grows and its volume linearly increases with $c_{\text{suc ini}}$. In contract, the size of ice becomes smaller. However, the shrinkage of ice is much smaller than the development of the FCS. For example, an increase in $c_{\text{suc ini}}$ from 0.034 to 0.34 wt % results in a ten-fold increase in the FCS volume but a 0.7% decrease in the ice volume. Similarly, an increase in $c_{\text{suc ini}}$ from 0.34 to 3.38 wt % results in a ten-fold increase in the FCS volume but only a 7% decrease in the ice volume. Thus, a change in the ice volume is much less sensitive to the $c_{\text{suc ini}}$ change than that in the FCS. Because the width and length of the channel are determined by the size of ice, as shown in Figure S2, these dimensions can be regarded as almost constant during a change in $c_{\text{suc ini}}$. Therefore, a change in $c_{\text{suc ini}}$ has an impact only on t, which increases linearly with $c_{\text{suc ini}}$ as a result. Thus, the critical channel size, that is, thickness t, which determines size selectivity, is proportional to $c_{\text{suc ini}}$. This allows us to control the channel dimension in a simple way, that is, by adjusting $c_{\text{suc ini}}$.

### Electric Field in the Channel

Figure 4 shows the dependence of the migration rate of a $3\mu$m particle in the FCS channel on $c_{\text{suc ini}}$. The voltage for these measurements was kept at 40 V; the average electric field was $8.0 \times 10^3$ V m$^{-1}$. The migration rate decreased with a decrease in $c_{\text{suc ini}}$ and became zero between $c_{\text{suc ini}} = 2.38$ and 2.71 wt %. Although Table 1 indicates that the threshold $c_{\text{suc ini}}$ is in the range of 1.7–3.38 wt % for a $3\mu$m particle, Figure 4 gives a more specific threshold $c_{\text{suc ini}}$ value.

### Table 1. Particle Passage through Ice Septum Prepared with Various $c_{\text{suc ini}}$

| $d/\mu$m | $c_{\text{suc ini}}$/wt % |
|---------|-------------------------|
| 3       | 3.38                    |
| 1       | 1.70                    |
| 0.5     | 0.68                    |
| 0.3     | 0.34                    |
| 0.1     | 0.034                   |

*Not examined.*

If the electrophoretic force ($F_{\text{el}}$) is balanced by the Stokes resistance ($F_{\text{St}}$), the following relationship can be derived

$$F_{\text{el}} = QE = F_{\text{St}} = 6\pi\eta r v$$

where $E$ is the electric field, $\eta$ is the viscosity of the medium (=12.7 mPa s in the present case), $r$ is the radius of a particle, and v is the electrophoretic velocity. When a $3\mu$m particle electrophoretically migrates at $v = 1.6 \times 10^{-5}$ m s$^{-1}$ ($c_{\text{suc ini}} = 3.38$ wt %), $F_{\text{el}}$ acting on it is estimated to be 5.8 pN. The electrophoretic force decreased to $9.8 \times 10^{-6}$ and $8.3 \times 10^{-6}$ m s$^{-1}$ at $c_{\text{suc ini}} = 3.05$ and 2.71 wt %, respectively, and then to zero at $c_{\text{suc ini}} = 2.38$ wt %. Therefore, the interaction force with the ice wall increases by 2.3, 2.8, and 5.8 pN as $c_{\text{suc ini}}$ decreases from 3.38 to 3.05, 2.71, and 2.38 wt %, respectively.

Microchip electrophoresis of a $3\mu$m particle indicated that its mobility is $4.79 \times 10^{-9}$ m s$^{-2}$ V$^{-1}$ in 38.1 wt % aqueous sucrose at $-4.0$ °C. Thus, v under an electric field of $8.0 \times 10^3$ V m$^{-1}$ is $3.83 \times 10^{-5}$ m s$^{-1}$ in free solutions, in which no physical interference from the channel walls is imposed on the particle. However, according to Figure 4, v at $c_{\text{suc ini}} = 3.38$ wt % is $1.5 \times 10^{-5}$ m s$^{-1}$, which is smaller than the corresponding v in the free solution. There are three possibilities for the origin of this discrepancy: (1) the particle migration is hindered even at $c_{\text{suc ini}}$, which determines size selectivity, is proportional to $c_{\text{suc ini}}$. This allows us to control the channel dimension in a simple way, that is, by adjusting $c_{\text{suc ini}}$.

As shown in Figure 1, channels fabricated in the ice septum were not straight but rather tortuous. The electric path length is thus longer than the interval between two electrodes, and, in addition, the local electric field strength is different for different channels because the area of the channel cross-section (wt % in Figure S2) also varies from one channel to the other; although t can be controlled by $c_{\text{suc ini}}$ and temperature, as discussed above, v cannot be controlled by these parameters. Thus, the local electric field cannot be known from the apparent migration rate of the particle, and, also the migration rates of particles measured in different channels cannot be compared directly.

The migration ratios of two different particles in the same channel were studied to cancel the local electric field effect. Figure 5 shows snapshots of the passage of particles with different sizes through the same channel. We can confirm that a $1\mu$m particle passes through a $3\mu$m particle and then migrates ahead. In this figure, the migration of relatively large particles is shown to demonstrate the observation of
simultaneous passage of the particles in the same channel. Because the separation of nanoparticles would be of more significance in nanotech applications, the migration ratio of 100 and 300 nm particles was studied in the FCS channel prepared with \( c_{\text{ini}} = 0.68-3.38 \) wt % in detail.

When the contribution from the electro-osmotic flow is taken into account, the ratio of the apparent migration rates is given by

\[
\frac{v_{\text{app}}^{100}}{v_{\text{app}}^{300}} = \frac{v_{\text{ep}}^{100} + v_{\text{eo}}^{100}}{v_{\text{ep}}^{300} + v_{\text{eo}}^{300}} = \frac{\mu_{\text{ep}}^{100} E + \mu_{\text{eo}}^{100} E}{\mu_{\text{ep}}^{300} E + \mu_{\text{eo}}^{300} E} = \frac{\mu_{\text{ep}}^{100} + \mu_{\text{eo}}^{100}}{\mu_{\text{ep}}^{300} + \mu_{\text{eo}}^{300}}
\]

where subscripts denote the size of the particle and the superscripts ep and eo represent electrophoresis and electro-osmosis, respectively. Because both electrophoretic and electro-osmotic velocities are proportional to the electric field strength, using the ratio of apparent electrophoretic migration rates allows the cancellation of \( E \). The migration of particles was measured in the ice septum, which was prepared with \( c_{\text{ini}} = 0.68, 1.70, \) and 3.38 wt %. Septum preparation was repeated several times for each \( c_{\text{ini}} \) and the migration rates of the particles in the same FCS channel were determined. The migration data are summarized in Table S1, where the average values of migration rates for 0.1 and 0.3 \( \mu \)m particles (\( v_{\text{app}}^{100} \) and \( v_{\text{app}}^{300} \)) are listed with standard deviations. The ratios (\( v_{\text{app}}^{100}/v_{\text{app}}^{300} \)) are plotted in Figure 6. The ratio of the electrophoretic mobility of 100 and 300 nm particles (\( \mu_{\text{ep}}^{100}/\mu_{\text{ep}}^{300} \)), which were determined in bulk 1.3 M sucrose solution at \(-4.0 \) °C, is also shown by the horizontal line for comparison.

The \( v_{\text{app}}^{100}/v_{\text{app}}^{300} \) ratios obtained for ice channels prepared from \( c_{\text{ini}} = 1.70 \) and 3.38 wt % agreed with \( \mu_{\text{ep}}^{100}/\mu_{\text{ep}}^{300} \). Our previous work indicated that the surface of ice is negatively charged under most aqueous conditions.25 This predicts the electro-osmotic flow from the anode to the cathode, that is, the electroosmotic flow direction is opposite to that of the electrophoresis of the particles. However, the agreement between \( v_{\text{app}}^{100}/v_{\text{app}}^{300} \) and \( \mu_{\text{ep}}^{100}/\mu_{\text{ep}}^{300} \) at \( c_{\text{ini}} = 1.70 \) and 3.38 wt % indicates that the electroosmotic flow in the present case is so small that the apparent electrophoretic migration ratio can be considered equivalent to the electrophoretic mobility ratio. Also, the agreement between the ratios suggests that particle migration was not affected by the physical interference from the ice channel under these conditions because the FCS channel size is sufficiently large. In contrast, the \( v_{\text{app}}^{100}/v_{\text{app}}^{300} \) ratio for \( c_{\text{ini}} = 0.68 \) wt % was significantly smaller than \( \mu_{\text{ep}}^{100}/\mu_{\text{ep}}^{300} \). This indicates that 0.3 \( \mu \)m particles undergo greater physical interference from the ice wall than do 100 nm particles at \( c_{\text{ini}} = 0.68 \) wt %. Although both 100 and 300 nm particles can pass through the channel under this condition, the migration of the 300 nm particle is hindered by the wall interaction to a larger extent. This makes it easy to differentiate 100 and 300 nm particles, which have similar intrinsic electrophoretic mobility.

Figure 5. Simultaneous migration of different particles (1 and 3 \( \mu \)m) in the FCS channel prepared with \( c_{\text{ini}} = 3.38 \) wt %. Arrows show the time lapse. Orange and red circles indicate the positions of 3 and 1 \( \mu \)m particles, respectively. The FCS channel runs along the thick blue curve.

Figure 6. Relative apparent migration ratios (\( v_{\text{app}}^{100}/v_{\text{app}}^{300} \)) in the FCS channels prepared with \( c_{\text{ini}} = 0.68, 1.70, \) and 3.38 wt %. Measurements were repeated on the ice platforms independently prepared; four, two, and five platforms for \( c_{\text{ini}} = 0.68, 1.70, \) and 3.38 wt %, respectively. Error bars represent the standard deviation of measurements on a given platform (the number of measurements is provided in Table S2). The purple broken line shows the migration ratio obtained in free solution (1.3 M aqueous sucrose) at \(-4.0 \) °C.

Figure 7B shows the time change in the fluorescence intensity near the ice septum was measured at \( \lambda > 570 \) nm. Figure 7B shows the time change in the fluorescence intensity near the FCS/ice interface in the receiver phase. The intensity increased almost linearly with time and continued to increase 10 min after the voltage application. Although the migration of the 3 \( \mu \)m particles toward the ice septum was confirmed, they could not enter the channels and were repelled from the septum in the source phase because the channel size is smaller than 3 \( \mu \)m.

Similarly, the separation of YO-dyed 500- and YG-dyed 100 nm particles was studied using an ice septum prepared with \( c_{\text{ini}} = 1.70 \) wt %.

The intensity of YG increased with time >30 min, whereas that for YO was almost constant. Thus, these particles were successfully...
separated by the ice septum, in which the FCS channel dimension was controlled by $c_{\text{sucini}}$.

**CONCLUSIONS**

In this paper, we reported the fabrication of size-tunable micro/nano-channels using frozen aqueous sucrose. The channel thickness, which determines the size selectivity, can be controlled in the range of 50 nm to 3 $\mu$m. Although we did not attempt larger dimensions, it should be possible using higher sucrose concentrations. In contrast, it was not possible to fabricate channels with thickness smaller than 50 nm because the FCS formed discrete pools rather than through-channels. This may arise because the volume of the FCS is too small. As stated in the main text, the formation of a long channel is related to the viscosity of the FCS because it causes interfacial fluctuation. Also, the interfacial tension between the FCS and ice surface is an important factor to determine channel dimensions because the FCS of high ice-wettability can be penetrated between ice phases to allow the formation of thin fluidic channels. Under the condition where a highly viscous FCS wets the ice surface better than the sucrose FCS, thinner FCS channels may possibly be formed in an ice septum. Also, it was indicated that two different FCSs are formed in frozen aqueous sucrose by Bogdan et al.,

The FCS channel dimension was controlled by $c_{\text{sucini}}$. However, the temperature control of the channel dimension should be more useful because channel sizes can be varied simply by changing the working temperature on the same ice platform. However, it is difficult to realize this operation in the present system because $c_{\text{FCS}}$ is a function of temperature, and the sucrose concentration in the source and receiver phases will vary with the temperature. Nevertheless, temperature control should be possible by fabricating the ice septum in a tunnel opened in bulk ice. In such a system, if the temperature is changed, $c_{\text{FCS}}$ in the solution phase is automatically adjusted by melting of the tunnel wall or freezing of the FCS.

Hence, there remain a number of tasks to be overcome to enhance the usefulness of the present approach. However, in this study, we have successfully demonstrated the potential of a frozen solution for fabricating micro-/nano-fluidic channel structures. The present concept may inspire researchers to attempt various frozen solutions for nano-fabrication. This highly versatile approach is expected to bring about a breakthrough in nanoscience/technology.

**EXPERIMENTAL SECTION**

The instrumental setup is schematically given in Figure 8. A homemade Cu cell was placed on a Peltier array, which was driven by a Peltier controller (Cell System, Yokohama Japan). The cell had a solvent reservoir (16 mm diameter, 3 mm depth), which was filled with 1:1 water/ethylene glycol. A capillary (450 $\mu$m i.d., 980 $\mu$m o.d.) was inserted through holes drilled on the side walls of the cell and was immersed in water/ethylene glycol.
ethylene glycol. The depth of the capillary from the surface of water/ethylene glycol was approximately 1 mm. An ice septum was fabricated in a capillary kept at −14.0 °C. A small portion of aqueous sucrose (sucrose concentration \(c_{suc} \)) was placed at the center of the capillary using a microsyringe. The thickness of the septum ranged from 300 to 500 μm depending on its preparation. After the septum was annealed at −3.0 °C for 15 min, the temperature was decreased to −4.0 °C, at which measurements were performed. Thus, the inside of the capillary was separated into two compartments by the ice septum. Both compartments were filled with unfrozen sucrose solution, the composition of which was determined from the phase diagram of the sucrose/water system (Figure S1); the sucrose concentration at −4.0 °C was 38.1 wt % (±1.3 M). Fluorescent particles were added in one compartment, which acted as the source phase; the other one acted as the receiver phase. Two Ag/AgCl electrodes were inserted in both compartments. The interval between the electrodes was kept at 5.0 mm. A voltage was applied to the electrodes with a power supply (PAK60-12A, Kikusui). The current was measured using a 2000 Multimeter (Keithley).

Fluorescent particles were added in one compartment, which served as the light source. Two mirror units were used: U-MGFPHQ, laser wavelength 480 nm, dichroic mirror 485 nm; and U-MGFPHQ, laser wavelength 540 nm, dichroic mirror 485 nm. Images were acquired using a CCD camera, model EM-CCD (Hamamatsu Photonics), and processed using the software MetaMorph (Molecular Devices). In some experiments, a laser confocal microscope (FV1200, Olympus) was used to observe particle behavior and channel developments.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.9b01966.

Apparent migration velocities of 300 and 100 nm particles in the FCS channel; freezing depression curve for the water/sucrose system; and schematic of the FCS channel formed in the continuous ice framework (PDF)

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### Notes

The authors declare no competing financial interest.

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