Supplemental Information for:

Development and Characterization of a "Store and Create" Microfluidic Device to Determine the Heterogeneous Freezing Properties of Ice Nucleating Particles

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1. Microfluidic Device Fabrication

1.1 Wafer Fabrication

Prior to applying photoresist to the silicon wafer (Silicon Sense, INC.), the wafer was thoroughly cleaned with acetone (Fisher Scientific Education), isopropyl alcohol (Pharmco-Aaper), and rinsed off with de-ionized water (Milli-Q®). The wafer was then air dried with nitrogen gas and baked at 200 °C for 5 minutes to evaporate any residual water left on the surface during the cleaning process. Next, the silicon wafer was placed on a (Laurell) spin coater and a quarter sized droplet of SU-8 3050 (MicroChem) was placed on the center of the silicon wafer. The spin coater had a 2-step spread cycle. The first step was set to 500 RPM at a ramp of 85 RPM/second for 20 seconds. The second spin cycle was set to 1500 RPM at a ramp rate of 340 RPM/second for 30 seconds. The spread cycle described above should result in a SU-8 depth of approximately 100 μm. After the spin cycle, the SU-8 coated wafer was transferred to a hot plate for a two stage bake process. Stage one was set for 65 °C with a ramp rate of 300 °C/hour and held for 5 minutes, while stage two was set at 95 °C with a ramp rate of 300 °C/hour for 15 minutes.

After soft baking the SU-8 onto the silicon wafer, the wafer was removed from the heat and allowed to cool to room temperature (approximately 5-10-minute wait time). The cooled wafer is placed on top of a glass substrate and is used to sandwich a dark field transparency mask (Fig. 2b) between a UV wavelength filter glass substrate. The exposed regions on the transparency are exposed to the UV lamp for 90 seconds. The exposed wafer is carefully removed from the dark field transparency and is placed on a hot plate for another 2-stage heating. Stage 1 was set to 65 °C at a ramp rate of 300 °C/hour and held for 1 minute, while stage two was set to 95 °C at a ramp rate of 300 °C/hour and held for 5 minutes. When multiple wafers are on the same hot plate, the wafers were rotated every 30 seconds to ensure uniform baking. After the baking procedure the wafers are removed from the heat and allowed to cool back down to room temperature (approximately 5-10-minute wait time).

Lastly, the wafers were placed in a bath of SU-8 developer (MicroChem) and allowed to develop over the course of several hours. The wafers were inspected approximately every hour to evaluate the development of the wafers' surface features. At the inspection times, the wafer was removed from the developer and cleaned with isopropyl alcohol. If white surface features were still present after in areas that did not contain features, the wafer would be placed back into the development bathe and the process would be repeated.

1.2 PDMS Molding and Bonding Process

Polydimethylsiloxane (Sylgard^{TM}) and hardener were mixed at a 10:1 ratio of base to hardener. The mixture was placed in a Thinky AR-100 planetary mixer and experienced a two-stage mix and defoaming process. While the PDMS base and hardener were mixing in planetary mixer, a wafer with the desired surface features was placed in a 6-inch petri dish (VWR). Approximately 17 grams of the newly made mixture was poured on top of the wafer. An empty petri dish was filled with 8 grams of the mixture. When hardened the 8-gram mixture will serve as the base for the microfluidic device. These petri dishes would then be transferred to a degassing chamber to remove any air bubbles that may have been introduced during the pouring process. Approximately after 5 minutes, the devices are carefully removed from the chamber and placed in a 65 °C convection oven (Binder) for 3 hours. Lastly, the petri dishes were removed from the oven and allowed to cool down to room temperature.

Once cooled, the PDMS structures and based were carefully cut from the mold to avoid ripping the features of interest. Both the base and the featured PDMS surface were coated in scotch tape to remove and limit the amount of debris that may deposit on the polymer surfaces. The tape was removed from the featured PDMS surface and inlets were drilled by using a 1 mm biopsy punch. The featured section of the PDMS slab were then coated in tape once again to remove debris. After removing the tape from both the base and featured PDMS slab, they were placed in a plasma cleaner (Harrick) and exposed to oxygen plasma for 1 minute. The PDMS slabs were removed and the exposed regions were placed in contact with each other. This process of bonding alters the PDMS's surface features from being hydrophobic to being hydrophilic. To promote further bonding between the base and the featured PDMS slabs and return of their hydrophobic surface features, they were placed in a 180 °C oven for 1 hour. After the 1 hour bake time, the devices were returned to room temperature and then placed in a vial filled with squalene to ensure the devices had a uniform coating of squalene in the microfluidic channels.

2. Freezing Assays and Temperature Calibrations

To perform a cooling assay, the droplet-filled microfluidic device is placed on the aluminum block of the cold plate and the temperature is held at 10 °C. A thin layer of squalene (VWR, ≥98%) coats the top and bottom of the microfluidic device to eliminate fogging of the device and ensure uniform thermal contact between the bottom of the microfluidic device and the cold plate surface. An acrylic lid and viewing window are placed on top of the aluminum base and are separated by an o-ring. The chamber is sealed, and a temperature ramp is applied in three stages. First, the temperature is ramped to 0 °C at a rate of 1 °C/min and then held at 0 °C for 10 minutes to perform a thermal soak that minimizes the thermal lag between the actual temperature of the droplets and the temperature reported by the thermistor. After the temperature hold, the temperature is ramped down to –40 °C at a rate of 1 °C/min, or until all the droplets in the microfluidic device have frozen.

The temperature at the locations of the droplets is calibrated by embedding a K-type thermocouple into six different batches of PDMS devices at a depth corresponding to that of the microfluidic channels. A device was removed from the squalene oil bath and a thin incision was cut at approximately the height of the microfluidic device. A K-type thermocouple was placed inside of the incision. The device was then placed inside the cold plate chamber and held down

with a thin sheet of scotch tape to ensure the thermocouple did not cause the device to lift off the chamber's floor. The cables of the K-type thermocouple were passed through the Swagelok inlet on the exterior of the chamber, as seen in Figure 2a. The cold plate was set to the normal temperature ramp, as described in section 2.2 of the main paper. The temperature of K-type thermocouple was recorded as a function of the cold plate temperature. The temperature ramp can be seen below in Figure S1.

Variation in the depth of the base layer did not contribute a noticeable difference in the thermocouple readings to within the reported uncertainty of the K-type thermocouple of ± 0.2 °C. As expected, the temperature at the channel height was offset from the cold plate temperature. To correct the temperature offset we used a linear fit of the PDMS temperature calibration as a function of the PID input temperature, where $T_{PDMS} = 0.933 \times T_{PID} + 1.410$. At 0 °C the channel temperature was ≈1.5 °C warmer that the setpoint, while at −40 °C the channel temperature was ≈4.1 °C warmer as shown in Figure S1. Each temperature ramp was repeatable to within the uncertainty of the K-type thermocouple. Freezes conducted during the winter and spring seasons did not require any external air flow over the chamber. For lab conditions with high relative humidity, external airflow may be required to eliminate condensation on the exterior of the chamber viewing window.

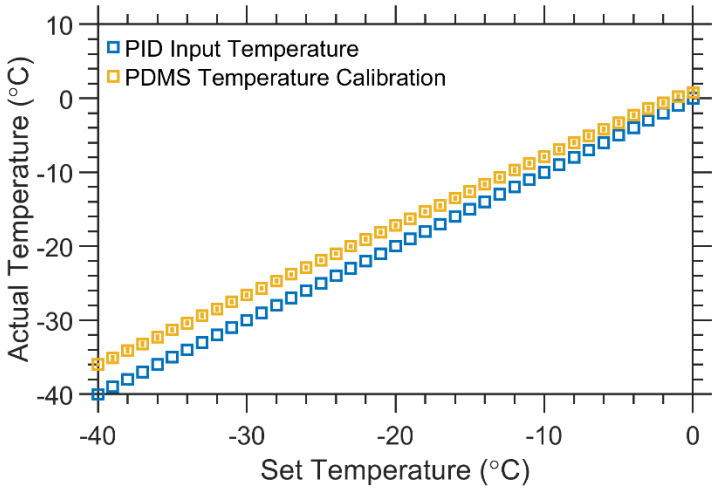

Figure S1: Temperature calibration results for our cold plate assembly with the set temperature plotted against the actual measured temperature. In yellow is the temperature of the K-type thermocouple and in blue is the temperature setpoint of the controller. The error bars on the K-type thermocouple curve are the standard deviation of 6 independent calibrations and are smaller than the symbol size.

3. Droplet Freezing Detection Algorithm

With the images acquired from the CMOS camera, we counted the number of droplets in the microfluidic device using an image processing subroutine written in MATLAB. To maximize the number of droplets detected in the microfluidic array, we preprocess the final image in which all the droplets are frozen. The frozen droplets appear white and have a higher contrast with the background than the liquid droplets, which appear clear. The image was converted to grey scale and the built-in MATLAB edge detection subroutine searches for circles within a designated region of the image. The program records the location and radius of detected droplets in pixel units, where one pixel is approximately equivalent to 8 µm. Droplets that are deformed or outside

the size selection criterion of 18 to 20 pixels are excluded from the analysis. Droplets with a radius larger than 20 pixels, or 160 µm, are not physically possible since the traps have a physical radius of approximately 150 µm. The radius range of 19 ± 1 pixels (144 to 160 µm) was chosen to detect droplets that may have otherwise been lost due to picture quality. A visual inspection of the filled chip image is used to eliminate droplets that are falsely detected or have irregular contact lines with the surface of the PDMS, indicating droplet pinning to the PDMS surface. Pinning of a droplet to the PDMS surface is a stochastic process observed in channels that have irregular surface roughness or other features, embedded debris, or improper plasma surface treatments.

For each individually detected droplet found by the subroutine, we tracked and saved the total interior pixel intensity. As the droplets freeze, they transition from clear to white, resulting in large changes in their total greyscale pixel value. To track freezing events, we compared the initial total pixel intensity to the subsequent total pixel intensity for each frame. When a droplet freezes, there is a large change in the slope of total pixel greyscale value versus temperature, on the order of 10^3-10^4 per °C as shown in Figure S2. For a droplet with a pixel intensity slope greater than the slope of the typical background signal for pure water droplets, the maximum slope of the total grey scale value of the droplet can be used to identify the frame in which a droplet froze. Since each frame is timestamped, we can extract the freezing temperature of the droplets by cross-checking with the temperature data logger on the thermistor. This analysis can be further extrapolated to droplet-on-substrate techniques, where droplets transition from white to black, by taking the absolute value of the slope.

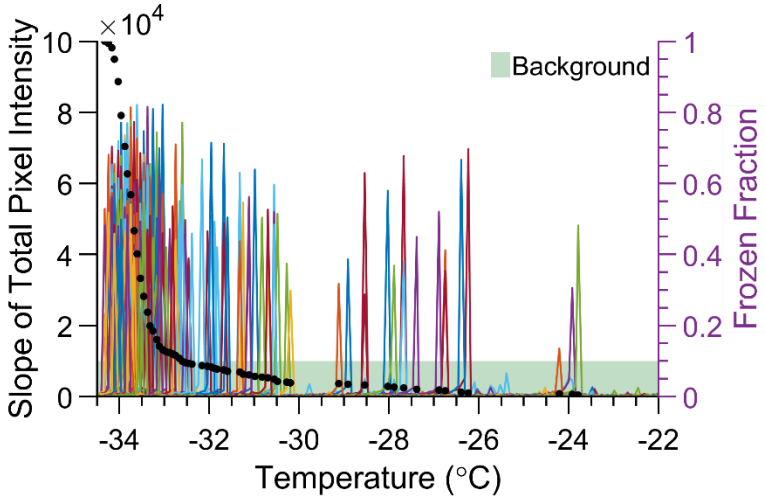

Figure S2. Example thresholding for detecting the freezing of pure water droplets on a single microfluidic chip using reflectance microscopy. The slope of the total pixel intensity per droplet is shown on the y-axis, as temperature is decreased in an assay from right to left. Each of the peaks above the baseline are associated with a droplet freezing event. The frozen fraction is shown on the right y-axis for this data set, plotted as black filled circles. The slope for each droplet was found by tracking the interior pixel intensity of each droplet.

4. Biomass-burning aerosol collection

Biomass-burning aerosol (BBA) was created and collected in the combustion facility located in

the Center for Atmospheric Particle Studies at Carnegie Mellon University. Cutgrass and sawgrass collected from Savannah Coastal Refuges Complex (Georgia and South Carolina, U.S.A.), was burned under mostly flaming-phase conditions in an open pan. The resulting smoke was injected into a 12 m^3 Teflon smog chamber using a Dekati eductor diluter.[1] Before collection onto the nuclepore filters the particles passed through an activated carbon and carbonate-coated annular denuders to remove gas-phase components in the aerosol suspension. The chamber aerosol was collected onto a nuclepore polycarbonate filter (Whatman, 111103) in an in-line filter sampler. Nuclepore filters have smooth surfaces that facilitate particle extraction into water. To maximize the concentration of BBA particles collected from the filter for the ice nucleating experiments, we rinsed particles off the filter using 3 mL of filtered water. Droplet arrays were generated in the device shortly after particle suspensions were prepared or the BBA was extracted off the filter.

5. High concentration of illite comparison with prior results of Beydoun *et al.* (2017)

The high concentrations of illite NX discussed in Section 3.2.4 in the main text made the droplets optically opaque. The droplet freezing analysis algorithm described above was able to discern minute changes in the pixel intensity of the droplet and record individual freezing events. Normally we avoid such high particle mass concentrations as physical artifacts such as particle coagulation and settling become prominent (Beydoun et al. 2016). In this case high concentrations were used to match the total illite particle mass in each 6 nL droplet with that used in the 0.1 µL droplets studied by Beydoun *et al.* (2017). Since the droplets were optically opaque, the remainder of the droplets could not be analyzed since they were below the greyscale pixel background noise level. Therefore, the spectrum is not complete with 100% of droplets freezing (blue data series in Fig. S3).

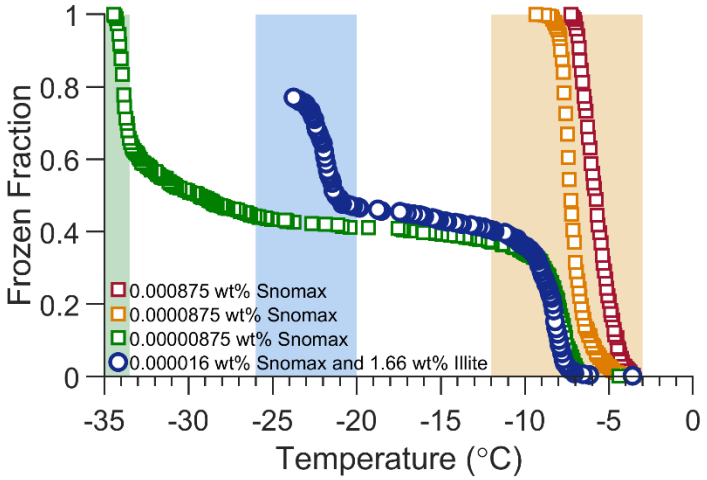

Figure S3: Individual droplet freezing temperature spectra of 6 nL microfluidic droplets containing different concentrations of Snomax bacterial particles and illite NX mineral particles. Orange and red symbols represent suspensions of Snomax. Blue symbols represent a mixture of highly concentrated illite NX combined with a dilute suspension of Snomax. Green symbols represent a highly dilute suspension of Snomax in pure water. Orange shading represents the freezing temperature regime of Snomax ice nucleants, blue the regime of illite NX, and green the regime where background freezing of filtered water is prominent for 6 nL microfluidic droplets.

References

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