

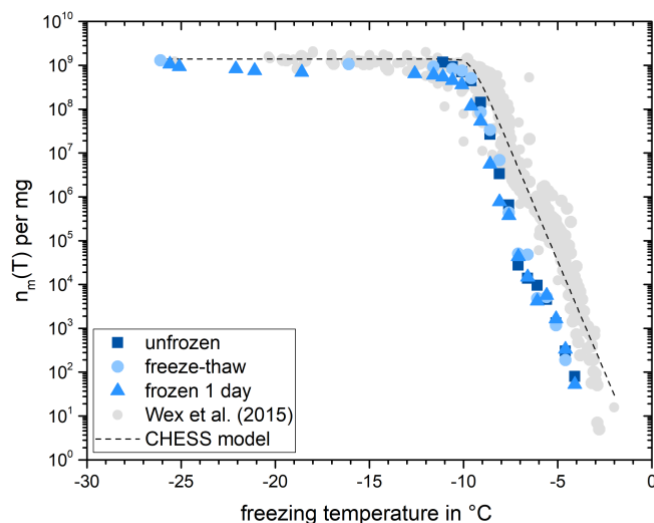
Referee comments #1

Major comments:

1. The authors recommend storing Snomax suspensions in aliquots. While I agree that the ice nucleation activity (INA) of Snomax suspensions with such a treatment shows improved reproducibility among repeating experiments. It's noted that its INA has a lower value compared to the freshly prepared samples (Figure 5). This indicates that the sample experiences changes during storage. Therefore, the statement that aliquot storage is a better treatment for preserving Snomax samples requires further justification.

Thank you for this comment. It is correct that the INA activity of the frozen Snomax is starting at lower temperatures compared to the curve shown in Figure 5(a). However, we attribute this to the variability between the suspensions. We have done an additional experiment to evaluate the impact of freezing and thawing on the Snomax suspension and found that the activity is not reduced by freezing. We have added Figure S5 to the Supplementary and the following sentence in the manuscript:

“The lower onset freezing temperature in Fig. 5b is attributed to the large variability between freshly prepared Snomax suspensions and not due to a decrease in activity upon freezing. We evaluated the impact of freezing and thawing to the suspension and found that the variations are within the measurement uncertainty (Supplementary S5).”



2. The authors claim that the INAs of illite NX obtained in the present studies are comparable to those reported by previous studies. However, a difference in INAs obtained between the two datasets can reach up to 3 orders of magnitude (Figure 6). This conclusion on “The micro-PINGUIN instrument was validated using the well-studied substances Snomax and Illite NX and the results obtained in this study are consistent with already existing instruments.” needs justification.

We agree that the measurements presented in this study are up to 3 orders of magnitude lower than the previous studies shown as a grey background. However, also

between these previous studies we find that the measurements differ by up to 3 orders of magnitude. Within the temperature range from -5°C to -10°C , so far only one instrument showed activity (Harrison et al., 2018), therefore the spread for higher temperatures is not yet defined. Thus, we argue that the results are consistent with what was found in previous studies. Further, we have corrected the temperatures by half of the vertical gradient to account for the temperature deviation within the well. This was not done by studies using other instruments that employ larger volumes, which would have shifted the presented freezing curves to lower temperatures (Table A1).

3. The temperature bias introduced by the inherent uncertainties from Pt 100 is not indicated in the present work.

In the Appendix A2 and A3 we discuss the measurement uncertainties of the Pt100 with regard to the long-term drift, the uncertainty and repeatability of the PT100 and the uncertainty of the calibration device. We have rewritten the section A3 to clarify the Pt100 repeatability measurements.

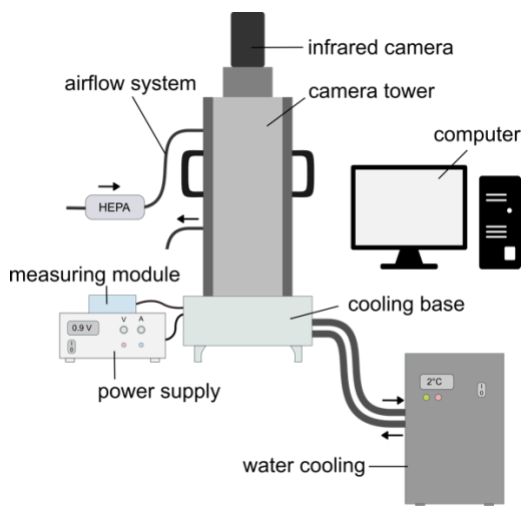
“After the calibration, the stability of the Pt100 temperature probe reading was examined by recording the temperature at steady state conditions at 0°C for 3 minutes. The standard deviation of the temperature reading was calculated as 0.0016°C and is used to derive the temperature probe repeatability contribution δT_{TR} .”

4. Specific details are absent in the schematic of the experimental setup, as outlined below.

Minor comments:

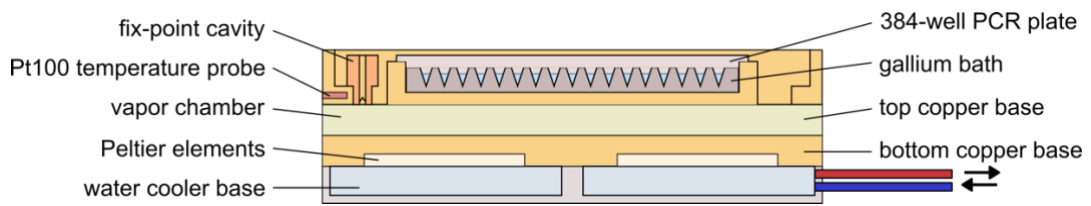
1. Comments on figures: (1) For all figures: The name of each component can be indicated in the figure for clarity reasons. (2) Figure 1: Components E and D are not connected to any other units, can you make it more specific? If B is “water cooling”, how come the temperature of B goes to -2°C ?

We changed the figures accordingly as shown below.



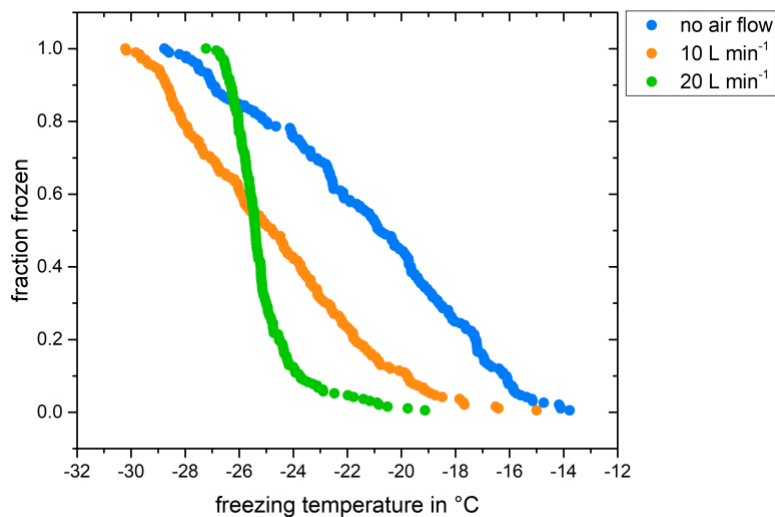
(3) Figure 2: What are the red and blue bars, current or circulating cooling fluid?

We updated the figure legend to clarify that the bars are showing the circulation of cooling liquid. **Figure 1:** Schematic drawing of the cooling base. The red and blue tubes connected to the water cooler base indicate the circulation of cooled water which removes the heat generated by the Peltier elements."



(4) Figure 3: Can you also specify the flow rate in the legend?

We have updated the figure legend.



(5) Figure 7: clarify the meaning of horizontal error bars – whether they represent standard deviations or the previously mentioned temperature uncertainties.

We have updated the figure legend **Figure 2:** (a) Fraction frozen for Snomax suspensions with a concentration between 10⁻² mg ml⁻¹ and 10⁻⁷ mg ml⁻¹. The data for 10⁻⁵ mg ml⁻¹ and 10⁻⁶ mg ml⁻¹ are not shown for illustrative purposes. Data points represent

the mean values of three measurements and the horizontal error bars indicate the standard deviation between these three measurements. (b) Standard deviations for the different concentrations are shown as box plots with 25th and 75th percentiles.”

2. L170: What do you mean by “dry air”? What are the components of the dry air and how was it produced? Why the largely deviated freezing curve was not observed in other studies, most of which are used under room temperature and relative humidity conditions? Do you have any films to isolate the suspensions and air?

The dry air is air with a humidity <10%RH that is provided from a centralized compressor supplying the entire building. Due to the use of an infrared camera, we cannot cover the droplets as done in some other studies. Previous studies describe a similar approach: for example, Schiebel et al. (2017) and Budke and Koop (2015) who apply a flow of dry air or N₂ to the instrument to avoid frost formation.

We modified the sentence in line 161: “Tests with a flow of compressed air with a low humidity (<10 % RH) passing through the camera tower showed that condensation was avoided during the experiment and that the freezing temperatures decreased with increasing air flow until T₅₀ temperatures, corresponding to the temperature where 50 % of the droplets are frozen, of around -25 °C were reached (Fig. 3).”

3. L259: Do you mean the thermal conductivity between gallium and PCR trays?

We have modified the sentence to clarify this point “The vertical gradient was not dependent on the cooling rate in the range between 0.3 °C min⁻¹ to 3 °C min⁻¹ (Supplementary S3) and thus, we conclude that the gradient is not attributed to a poor thermal conductivity between the individual parts of the instrument but rather to the warm air above the sample surface. “

4. L125: Ensure consistency in referring to the "temperature probe" by specifying if it corresponds to the "pt100 probe" throughout the main text.

We have exchanged “temperature probe” by “Pt100 temperature probe” throughout the text.

5. Figure 3: Will higher flow rates >20 L/min introduce any changes in the freezing curve? I wonder if 20 L/min represents the optimal condition for your measurement.

In section 2.2 we describe the airflow system. The 20 L/min flow shown in Figure 3 is only used to evaluate the impact of a dry air flow. The conditions during the experiment are described in line 170: “Before each run, the camera tower is flushed with a high flow of dry air (20 l min⁻¹). The flow is reduced to 10 l min⁻¹ during the measurement to minimize disturbance of the samples and the introduction of warm air.”

We added the following sentence to clarify this point: “We measured the relative humidity in the camera tower for this procedure and found a flow of 10 l min⁻¹ is sufficient to maintain a low relative humidity.”

6. L352: Define "type A and type C INPs" for better comprehension. Provide an explanation for these terms.

We added the following explanation in line 351:

“Bacterial ice nucleation proteins show distinct freezing behaviour depending on the size of the proteins and can be divided into different classes (Turner et al., 1990; Yankofsky et al., 1981; Hartmann et al., 2013; Budke and Koop, 2015). Budke and Koop (2015) identified two classes of INPs for Snomax, the high active but less abundant class A INPs nucleate ice at around $-3.5\text{ }^{\circ}\text{C}$ while class C INPs are frequently observed but nucleate at lower temperature of $-8.5\text{ }^{\circ}\text{C}$.”