Ice nucleating properties of the sea ice diatom *Fragilariopsis cylindrus* and its exudates

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Supplemental Information

Determination of INP concentration

In the main paper, we defined f'_{ice} as the plateau region separating heterogeneous and homogenous freezing. Since f'_{ice} varies with the number of droplets containing at least one INP, an experimentally determined f'_{ice} value can be used to calculate the concentration of INPs for unknown samples using a variation of Eq. (7). Typically, a sample is investigated by means of a dilution series so that a different INP concentration is scanned in each experiment. If the INP concentration is too large, all droplets freeze heterogeneously, and if it is too low, no INP-induced heterogeneous nucleation occurs (apart from that induced by any impurity present) and, thus, all droplets freeze homogeneously. In both these cases, it is not possible to obtain the desired INP concentration. But if measurements are done in the Poisson relevant concentration range (see definition in the main paper), one can observe both heterogeneous as well as homogenous freezing of droplets, resulting in a plateau in the frozen fraction curve, as discussed above. With the frozen fraction value of this plateau, f'_{ice}, and the assumptions that, first, every INP induces heterogeneous freezing and that, secondly, all heterogeneously frozen droplets freeze before the first freezing of a homogenous frozen droplet, the following Equation can be obtained by rearranging Eq. (7):

$$c = -\frac{6\ln\left(1 - P_\lambda(k \geq 1)\right)}{\pi \cdot d^3} = -\frac{6\ln(1 - f'_{ice})}{\pi \cdot d^3} \qquad \text{(S1)}$$

A comparison with Fig. 3a in the original paper implies that $f_{ice}^{'}$ values of about 0.5 will lead to more accurate results than values close to the limits of the Poisson relevant range, because of the larger slope of the curve at $P_\lambda(k \geq 1)$ at intermediate $f_{ice}^{'}$ values.

In order to verify this method, we determine the concentrations c_{measured} of the investigated *P. syringae* samples by using the already determined values of $P_\lambda(k \geq 1)_{\text{measured}}$, from Table S3, for calculating $f_{ice}^{'}$. The resulting values for c_{measured} as well as the actually prepared concentrations of the samples c, are also listed in Table S4. The comparison shows that there are only minor differences between the prepared and measured concentrations, supporting the fact that this method provides a suitable and relatively accurate estimate of the INP concentration of an unknown sample. A similar treatment was performed for the *F. cylindrus* diatom samples and the related Fig. S4.

Figure S5 shows the cumulative number of ice-nucleating active sites n_N per *P. syringae* cell. As we will see below, the Poisson evaluation is required for this type of evaluation. The cumulative number of active ice-nucleating sites is given formally by Eq. (S2) (Budke and Koop, 2015):

$$n_N = \frac{-\ln(1 - f_{ice})}{c \cdot V} \tag{S2}$$

Here, f_{ice} is the frozen fraction, c is the INP concentration in absolute number of INPs per volume unit (i.e., the number density), and V is the volume of each individual droplet.

Figure S5 shows that for temperatures lower than about -35 °C, n_N obtains values that are larger than one per bacterium cell, implying that one bacterium initiates ice nucleation in more than one droplet, which is of course unreasonable. Instead, these high n_N values result from homogenous ice nucleation in droplets that do not contain any *P. syringae* bacteria, which normally is not considered in the classical n_N evaluation. By applying Eq. (7) on these measurements, the threshold value between droplets that do contain INPs and those that do not can be determined. This treatment results in maximum values for n_N of about one per bacterial cell, as indicated by the filled and open circles in Fig. S5.

Table S1: Salts used for the preparation of artificial seawater for the *F. cylindrus* ice nucleation experiments. The amounts of substances provided for each ion yield a mass of 500 g artificial seawater at a salinity of 34.5.

Salt	Supplier	m [g]	Na^+ [mmol]	K^+ [mmol]	Mg^{2+} [mmol]	Ca^{2+} [mmol]	Cl^- [mmol]	SO_4^{2-} [mmol]	H_2O [mmol]
NaCl	VWR Chemicals	11.8446	202.68				202.68		
KCl	VWR Chemicals	0.3758		5.04			5.04		
$MgCl_2 \cdot 6H_2O$	ITW Reagents	5.3280			26.21		52.42		157.25
$Na_2SO_4 \cdot 10H_2O$	Acros Organics	4.4902	27.87					13.94	139.36
$CaCl_2 \cdot 2H_2O$	ITW Reagents	0.7460				5.07	10.15		10.15
H_2O	double distilled water	477.23							26490.26
artificial seawater		**500.01**	**230.55**	**5.04**	**26.21**	**5.07**	**270.28**	**13.94**	**26797.02**

Table S2: Temperature parameters used in the microfluidic freezing experiments. The first number in each triplet is the final temperature of the respective step in °C, the second number indicates the rate of cooling or heating in °C per min, and the third number indicates the holding time at the final temperature in min. Reference samples were always investigated with the same parameters as those given for each sample.

Step	*F. cylindrus*	*F. cylindrus* (filtered)	*F. cylindrus* (pure cells)	*F. cylindrus* (Medium)	*fc*IBP11	*P. syringae*
1	-20/-5/2	-20/-5/2	-20/-5/2	-20/-5/2	-20/-5/2	-5/-5/2
2	-45/-1/0	-45/-1/0	-45/-1/0	-45/-1/0	-45/-1/0	-40/-1/0
3	-10/5/2	-10/5/2	-10/5/2	-10/5/2	-10/5/2	-10/5/2
4	5/1/0	5/1/0	5/1/0	5/1/0	5/1/0	5/1/0

Table S3: As prepared concentrations $\boldsymbol{c}$ of the *P. syringae* samples, calculated fractions of droplets with at least one bacterium $\boldsymbol{P_\lambda(k \geq 1)}_{\text{calculated}}$, as well as measured fractions $\boldsymbol{P_\lambda(k \geq 1)}_{\text{measured}}$ and experimentally determined concentrations $\boldsymbol{c}_{\textbf{measured}}$ based on the approach outlined above using Eq. (S1).

$\boldsymbol{c}$ / mL^{-1}	$\boldsymbol{P_\lambda(k \geq 1)}$ calculated	$\boldsymbol{P_\lambda(k \geq 1)}$ measured	$\boldsymbol{c}$ measured / mL^{-1}
1.4x10^7	$1.00^{+0.00}_{-0.01}$	0.99	1.2x10^7
2.8x10^6	$0.66^{+0.06}_{-0.06}$	0.61	2.5x10^6
1.4x10^6	$0.41^{+0.05}_{-0.05}$	0.39	1.3x10^6

Table S4: Shifts in ice nucleation temperature relative to the ΔT_{50} of artificial seawater for the untreated *F. cylindrus* samples, as well as for the samples filtered with a 0.22 µm syringe filter.

c	unfiltered ΔT_{50}	filtered ΔT_{50}
$5x10^7$ mL^{-1}	7.2 °C	6.4 °C
$1x10^7$ mL^{-1}	6.0 °C	5.2 °C
$2x10^6$ mL^{-1}	5.4 °C	3.1 °C
$1x10^6$ mL^{-1}	4.8 °C	2.6 °C
$5x10^5$ mL^{-1}	2.8 °C	0.0 °C

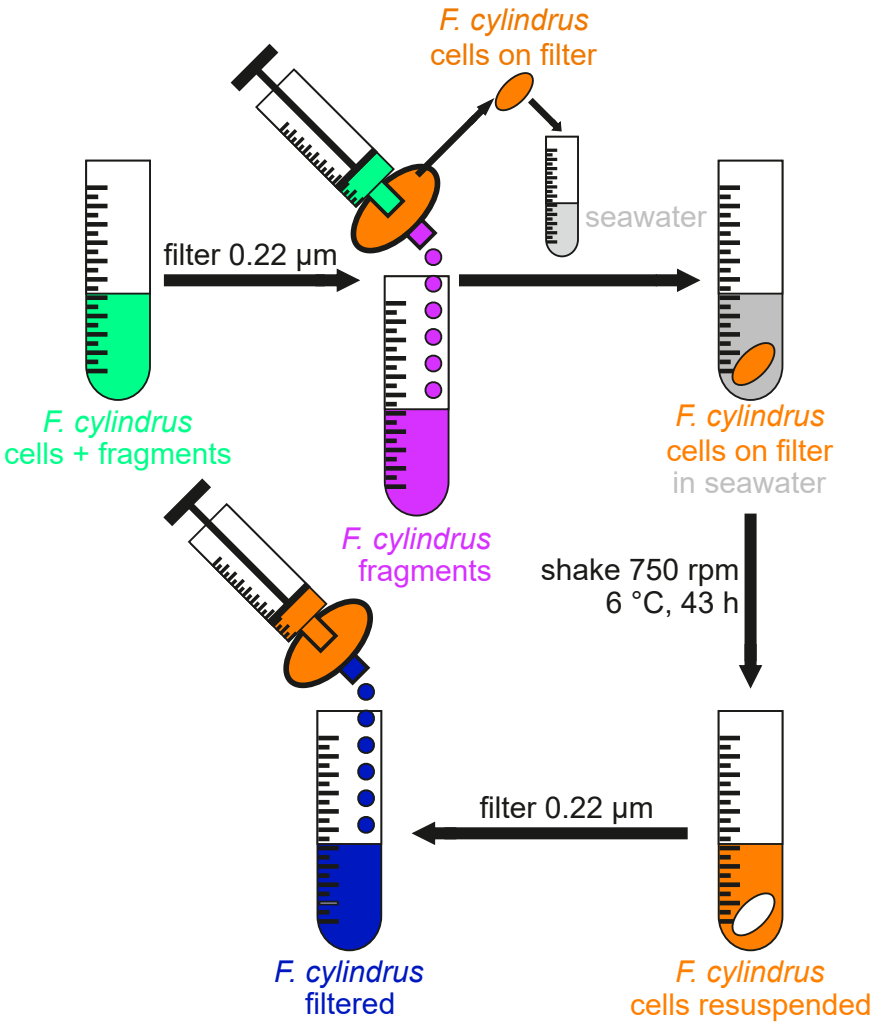

Figure S1: Extraction of the pure *F. cylindrus* cells by filtration of the stock solution (green). After filtration, the filtrate (purple) should only contain smaller cell fragments and soluble molecules such as *fc*IBP, while whole cells and larger fragments remain on the filter (orange filter). By shaking the filter in artificial seawater (grey), the cells were resuspended (orange solution). As a finally test, filtration of this suspension (blue) should not show any ice nucleation results different from those of pure artificial seawater.

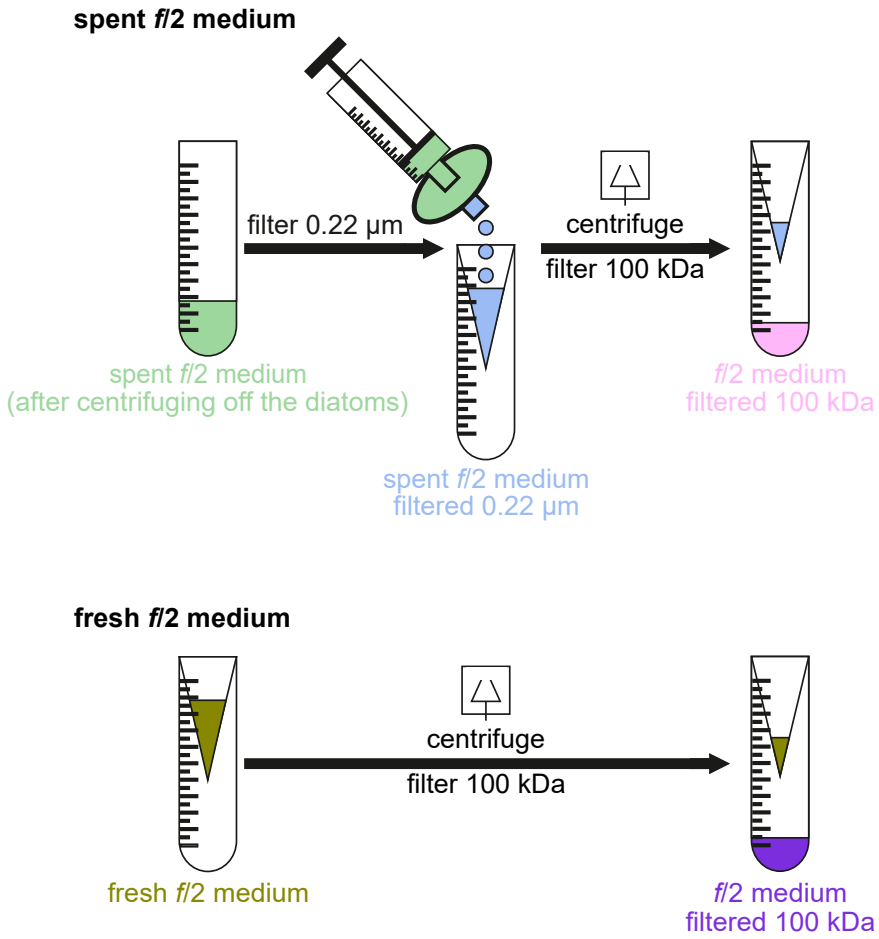

Figure S2: Sample preparation for the ice nucleation experiments with the *f*/2 medium. The spent medium should only contain a few diatoms, because the diatoms were separated from the medium by centrifugation before (green vial). By filtration with a syringe-filter, we removed the remaining cells and retained smaller *F. cylindrus* fragments and the *fc*IBP in the filtrate (blue solution). The solution was filtered by centrifugation filtration and the resulting filtrate should only contain soluble macromolecules smaller than 100 kDa, e.g. *fc*IBP (pink vial). The fresh *f*/2 medium (olive solution) does not contain any cells, fragments or *fc*IBP and was also filtered by centrifugation filtration as a reference (purple vial).

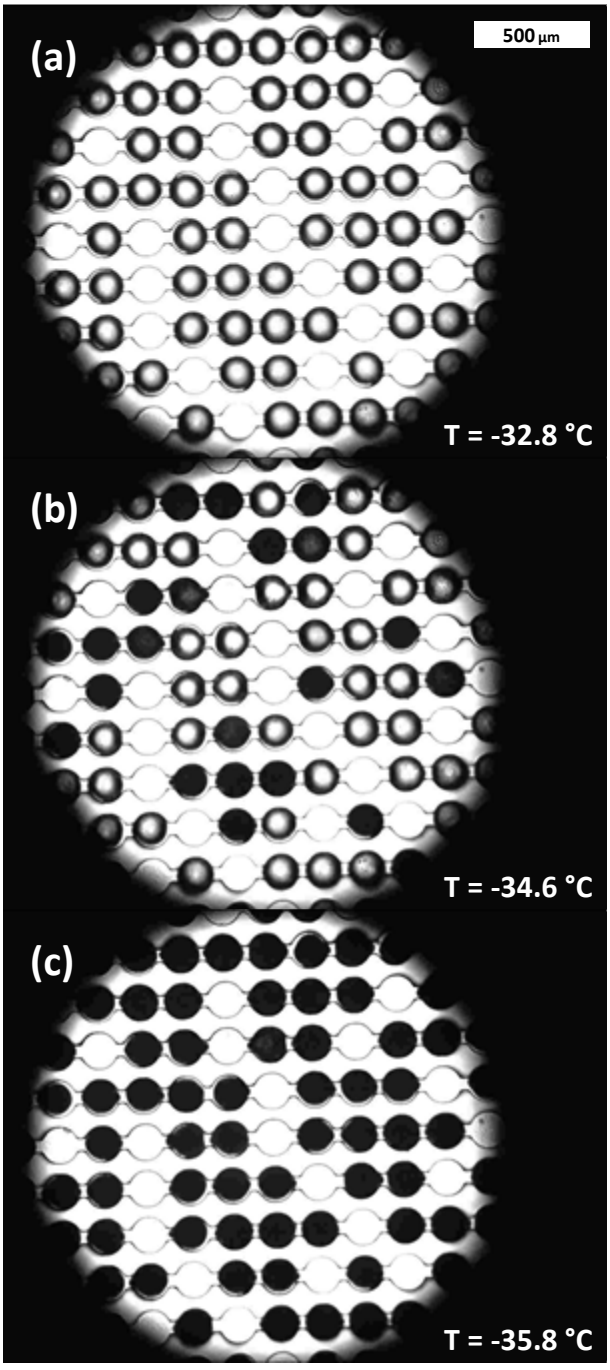

Figure S3: Optical photomicrographs of the freezing of microfluidic droplets during one of three freezing experiments with unfiltered *F. cylindrus* suspensions in artificial seawater (concentration of 2×10^6 cells per mL). The white scale bar in the top left indicates a length of 500 µm and is the same for all three images. The droplets' diameter is about (90 ±5) µm. **a:** At a temperature of -32.8 °C all droplets are still liquid. This is the last picture before the freezing of the first droplet during this experiment. **b:** At a temperature of -34.6 °C some droplets are already frozen (black), while other droplets are still liquid (white). **c:** At a temperature of -35.8 °C all droplets are frozen. This is the first picture after the freezing of the last droplet in this experiment.

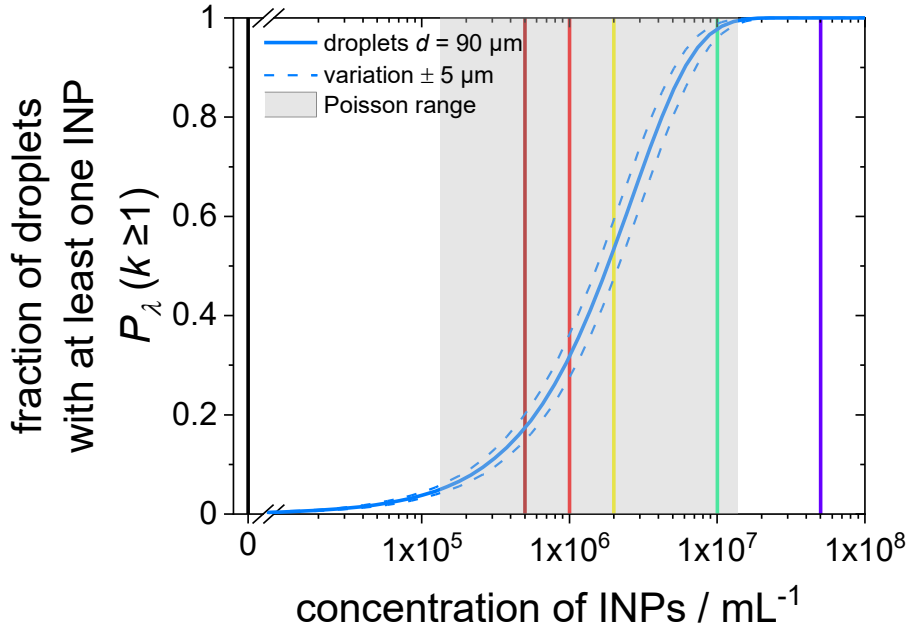

Figure S4: Fraction of droplets with at least one INP, $\boldsymbol{P_\lambda(k \geq 1)}$, as a function of INP concentration in the investigated samples. The solid blue curve represents the values of $\boldsymbol{P_\lambda(k \geq 1)}$ for the droplets in the microfluidic experiment with a diameter of 90 µm. The dashed curves indicate the values for a variation of ±5 µm in droplet diameter, i.e. 85-95 µm. The calculations of these curves are based on Eq. (7). The grey shaded area shows the Poisson relevant range, see main text for definition, with the lower and the upper limits at the INP concentrations corresponding to $\boldsymbol{P_\lambda(k \geq 1)}$ = 0.050 and $\boldsymbol{P_\lambda(k \geq 1)}$ = 0.995. The vertical bars mark the concentration of the *F. cylindrus* diatom suspensions used in the experiments: 5×10^5 mL^{-1} (dark red), 1×10^6 mL^{-1} (bright red), 2×10^6 mL^{-1} (yellow), 1×10^7 mL^{-1} (green) and 5×10^7 mL^{-1} (purple) and pure seawater (black).

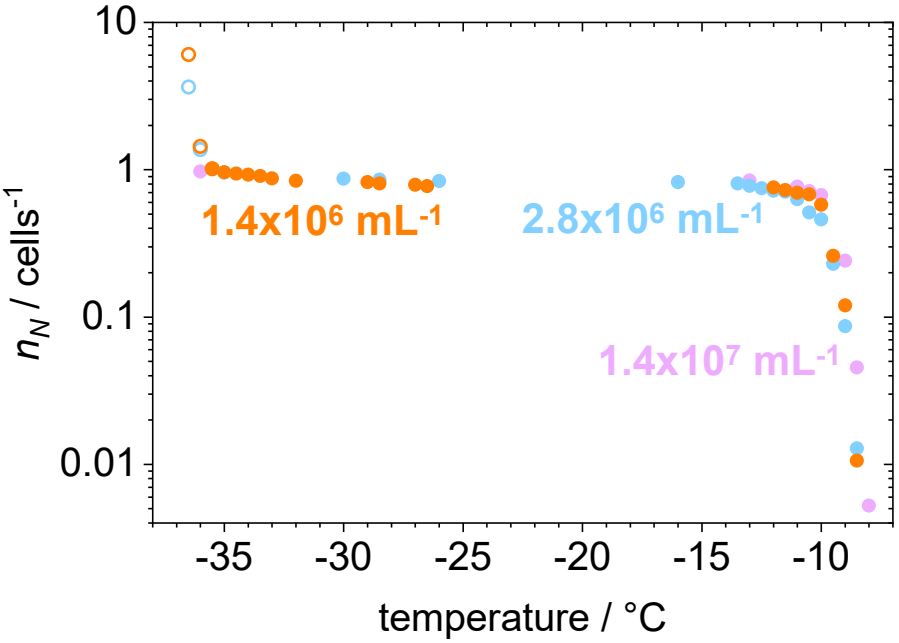

Figure S5: Cumulative number of ice nucleating active sites, $\boldsymbol{n_N}$, for three different *P. syringae* bacteria suspensions with colours indicating the respective concentration. Filled circles represent droplets containing bacteria, as calculated from Eq. (7), while open circles represent droplets devoid of INP.

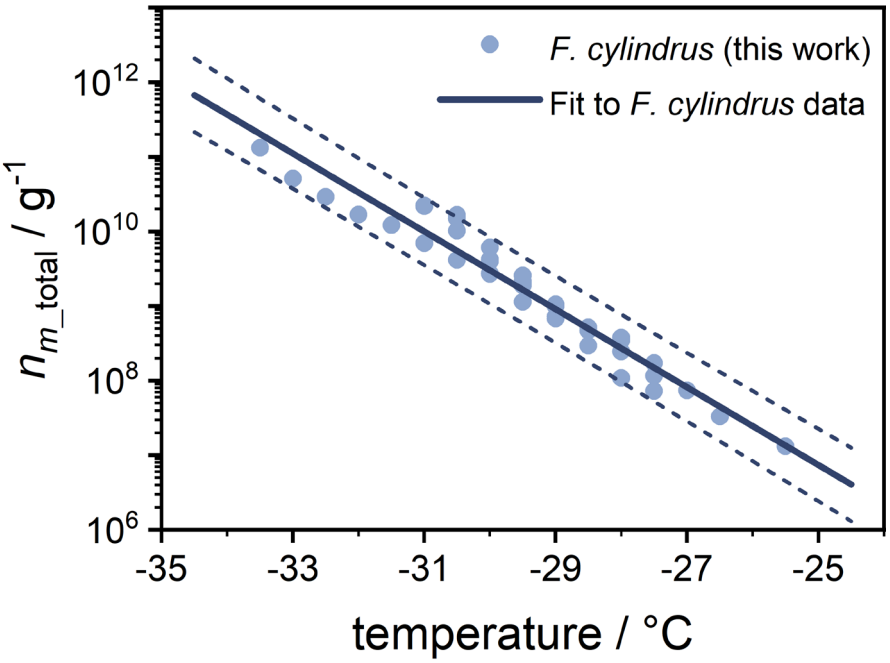

Figure S6: Measured values for $\boldsymbol{n_{m\_total}}$ of *F. cylindrus* diatoms. The solid line represents a fit of the experimental $\boldsymbol{n_{m\_total}}$ values (blue symbols) for the *F. cylindrus* diatoms. The parameterization of the fit is given in Eq. (S3). The dotted lines indicate to the upper and lower 2σ prediction bands of this fit. The temperatures are corrected for the freezing point depression of artificial seawater and, thus, represent ice nucleation temperatures in pure water.

References

Budke, C. and Koop, T.: BINARY: an optical freezing array for assessing temperature and time dependence of heterogeneous ice nucleation, Atmos. Meas. Tech., 8, 689–703, doi:10.5194/amt-8-689-2015, 2015.