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Twin-plate Ice Nucleation Assay (TINA) with infrared detection for high-throughput droplet freezing experiments with biological ice nuclei in laboratory and field samples

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For efficient analysis and characterization of biological ice nuclei under immersion freezing conditions, we developed a Twin-plate Ice Nucleation Assay (TINA) for high-throughput droplet freezing experiments, in which the temperature profile and freezing of each droplet is tracked by an infrared detector. In the fully automated setup, a couple of independently cooled aluminum blocks carrying two 96-well plates and two 384-well plates, respectively, are available to study ice nucleation and freezing events simultaneously in hundreds of microliter range droplets (0.1-40 μL). A cooling system with two refrigerant circulation loops is used for high-precision temperature control (uncertainty $<$ 0.2 K), enabling measurements over a wide range of temperatures ($\sim$272-233 K) at variable cooling rates (up to 10 K min^{-1}).

The TINA instrument was tested and characterized in experiments with bacterial and fungal ice nuclei (IN) from *Pseudomonas syringae* (Snomax®) and *Mortierella alpina*, exhibiting freezing curves in good agreement with literature data. Moreover, TINA was applied to investigate the influence of chemical processing on the activity of biological IN, in particular the effects of oxidation and nitration reactions. Upon exposure of Snomax® to O_3 and NO_2, the cumulative number of IN active at 270-266 K decreased by more than one order of magnitude. Furthermore, TINA was used to study aqueous extracts of atmospheric aerosols, simultaneously investigating a multitude of samples that were pre-treated in different ways to distinguish different kinds of IN. For example, heat treatment and filtration indicated that most biological IN were larger than 5 μm. The results confirm that TINA is suitable for high-throughput experiments and efficient analysis of biological IN in laboratory and field samples.