

SUPPLEMENTARY INFORMATION

Novel Method for Formation of Monodisperse Superheated Perfluorocarbon Nanodroplets as Activatable Ultrasound Contrast Agents

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FIGURE S1. Specific components and operating parameters of the high-pressure homogenizer.

- a) Turn the pressure knob to the desired pressure (e.g., 13, 000 psi for nanodroplets homogenization)
- b) Detach the delivering syringe, fill it with the excipient solution (6 mL), remove all air bubbles, and reattach it to the curved tube.
- c) With the toggle switch still in the Jog position, hold the Retract button while gently pushing the plunger of the delivering syringe until the desired volume has been loaded or the plunger stops moving on its own.
- d) Turn the toggle switch to the Run position and wait until the green “Ready” light turns on.
- e) Press and hold the Extend button until the receiving syringe is no longer filling.
- f) Turn the toggle switch back to Jog and wait until you hear a difference in the sound made by the machine before continuing.
- g) Repeat Steps a-e three times.
- h) Perform multiple passes with the sample, switching the syringe positions and repeat Steps 4a-e
- i) To clean, repeat Steps a-e a minimum of three times (or until the solution in the receiving syringe is clear), alternating 6 mL milliQ water and 70 % isopropyl alcohol.

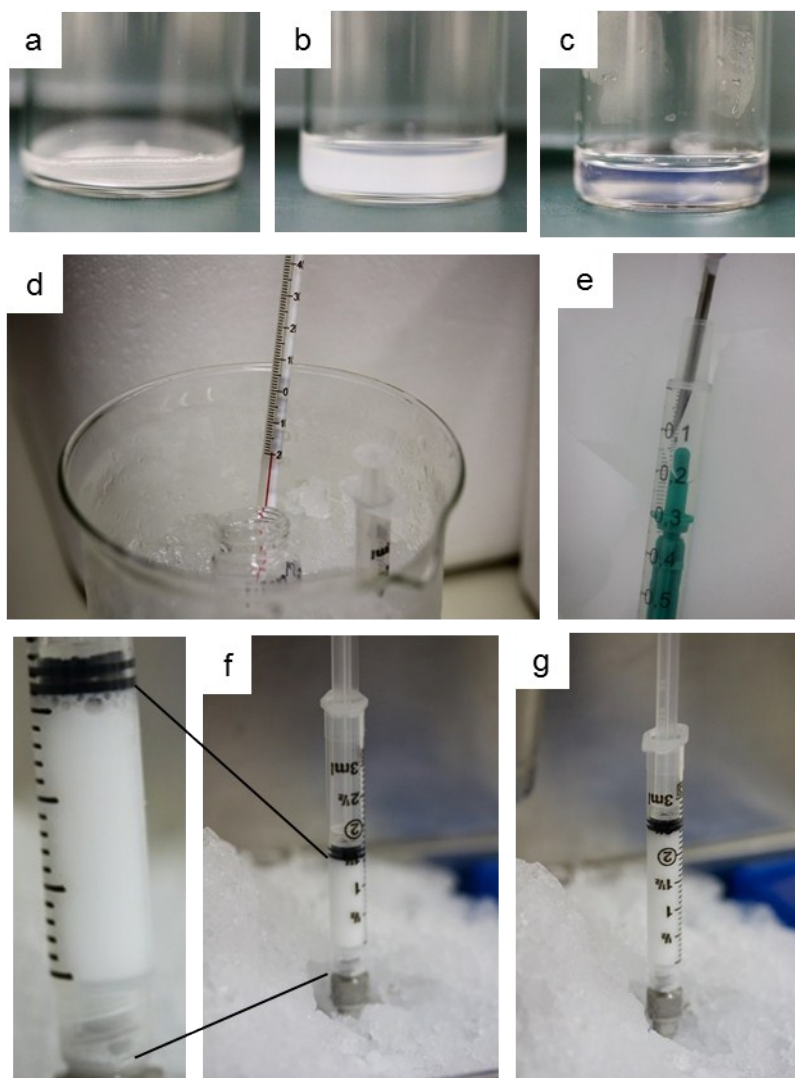


FIGURE S2. Formulation of DFB nanodroplets. a) Dry film composed of DSPC and DSPE-PEG2000. b) Dry film dispersion in 2mL PBS 1X/propylene glycol/glycerol in a 16:3:1 v/v/v ratio. c) Clear solution post sonication at 68 °C. d) Sample cooling for 2 min at -20 °C sodium chloride/ice bath prior to the addition of condensed DFB. e) Liquid DFB in the syringe kept on dry ice. f) Emulsion after first pass through the LV1. g) Emulsion after nine passes.



FIGURE S3. Formulation of OFP nanodroplets. a) OFP gas condensed by cooling at $-70\text{ }^{\circ}\text{C}$ using an ethanol-dry ice bath alongside a syringe containing clear lipid film dispersed in excipient

(propylene glycol/water 60:40). b) 100-150 μL of liquid OFP measured with a 1 mL syringe. c) Liquid OFP transfer into the sample-containing syringe. d) Emulsification using the LV1 microfluidizer cooled to $-30\text{ }^{\circ}\text{C}$ using a sodium chloride /ice/ and dry ice cooling bath. e) Milky suspension obtained after nine passes at 13,000 psi. f) Additional sample obtained by washing the system with 1.5 mL excipient.

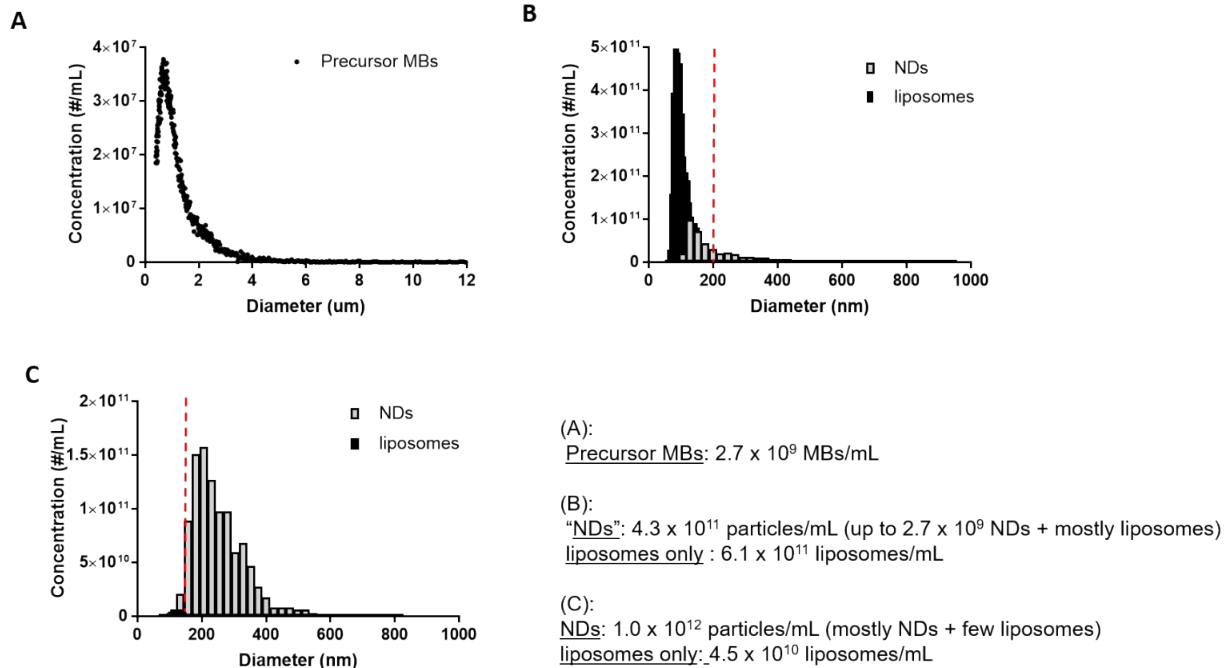


FIGURE S4. Representative size distribution of precursor microbubbles measured by Multisizer (A). Representative DFB ND emulsions and liposome size distributions measured by TRPS obtained by condensation (B) and our direct formulation (C). Particles with diameter on the left of the red bar are liposomes not NDs.

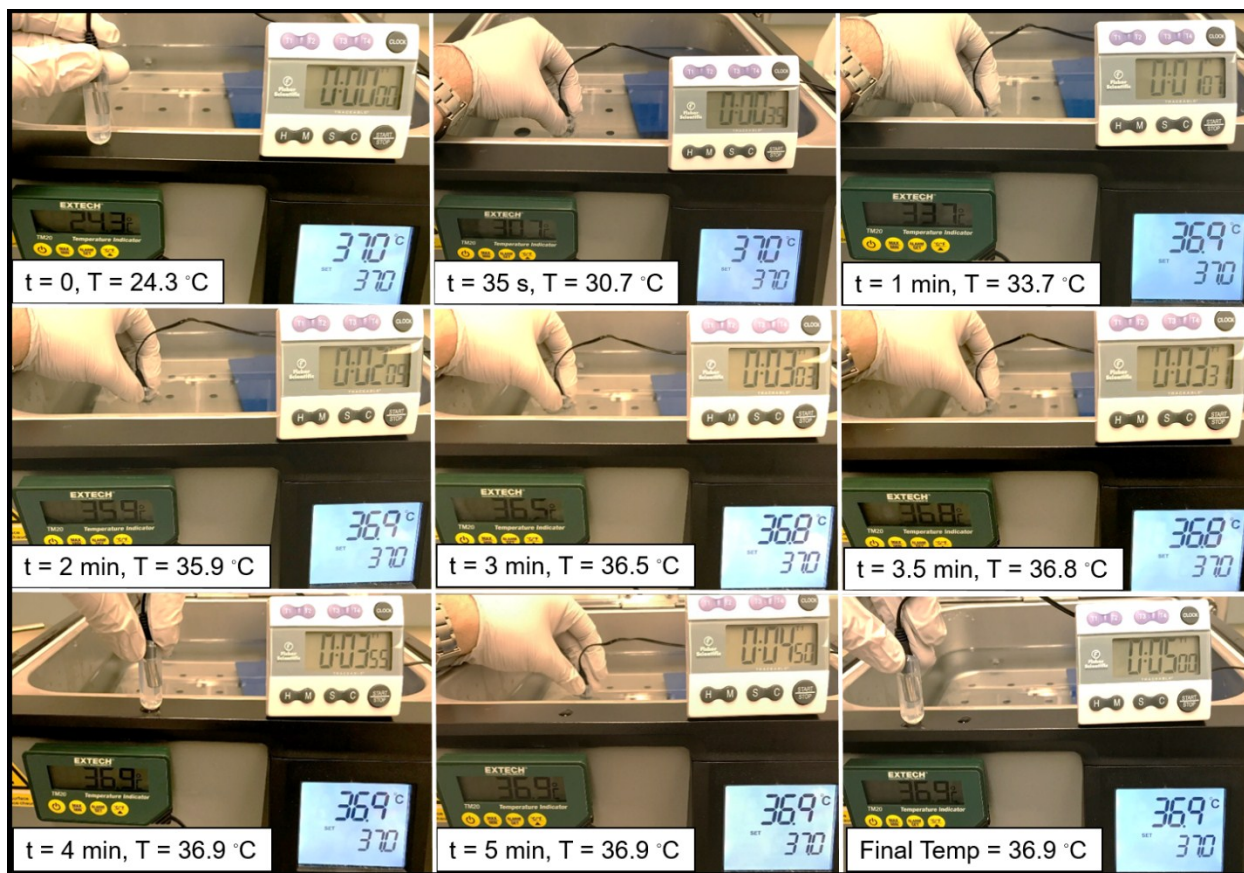


FIGURE S5. Temperature in bulb over time in heating bath. Bath temperature (37 ± 0.2 °C) is reached in the bulb after 3.5 min.

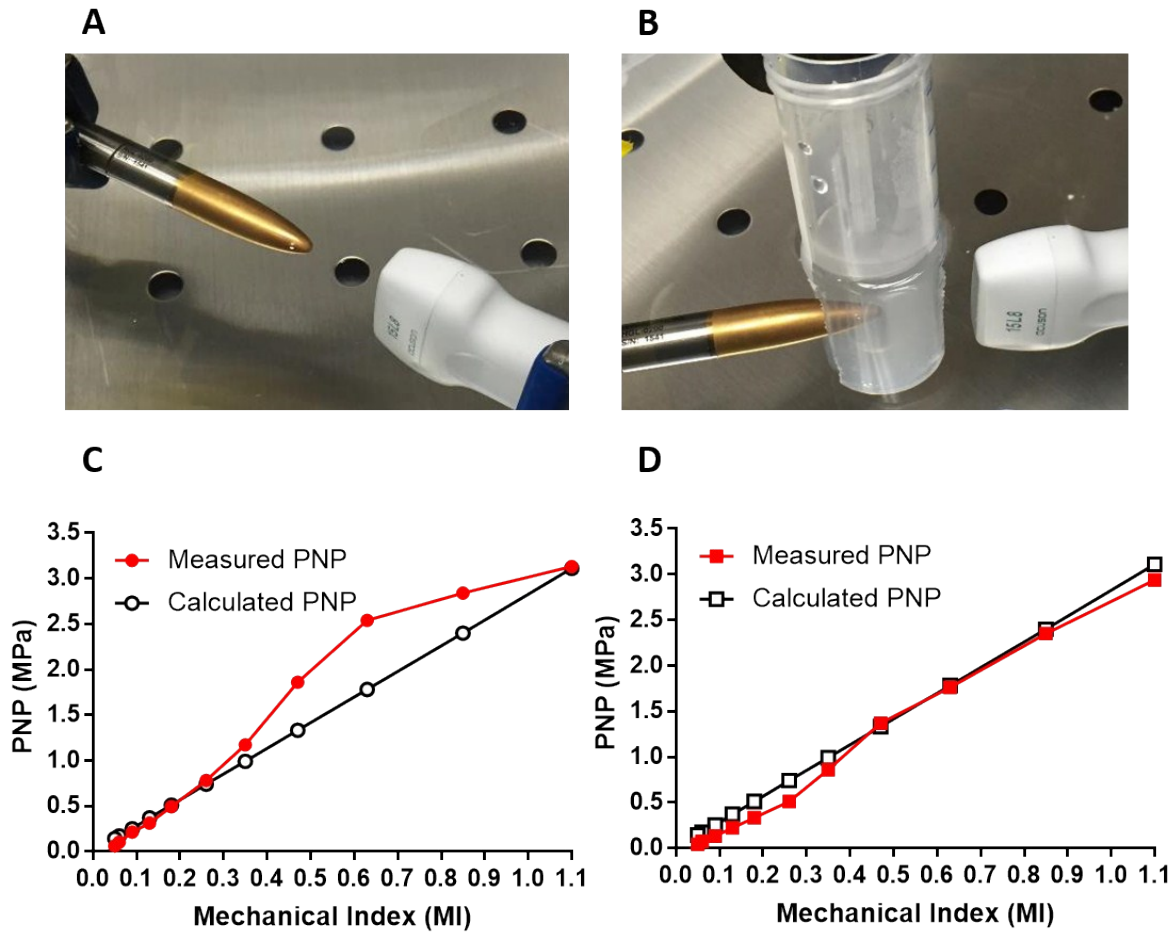


FIGURE S6. Calibration of peak negative pressure (PNP) output measurement by the clinical ultrasound machine used for vaporization experiments. (A) A broadband hydrophone (Onda HGL-200) was oriented towards the Siemens 15L8 transducer, with tip and aperture of the hydrophone held at the electronic focus of the transducer, 2 cm away from the transducer face. (B) To obtain recorded PNPs more accurately representing the experimental setup, agarose/cellulose and half of a pipette wall were placed in the path between the transducer and hydrophone. With settings identical to those used in the vaporization experiments, the maximal PNP amplitudes were recorded on an oscilloscope, with persistence recording mode used to find the largest amplitude peak at 10 different mechanical indices. PNPs recorded (C) using agarose/cellulose/pipette

attenuation medium and (D) without attenuation medium, showing that attenuation medium resulted in recorded PNPVs very similar to those calculated from on screen mechanical indices.

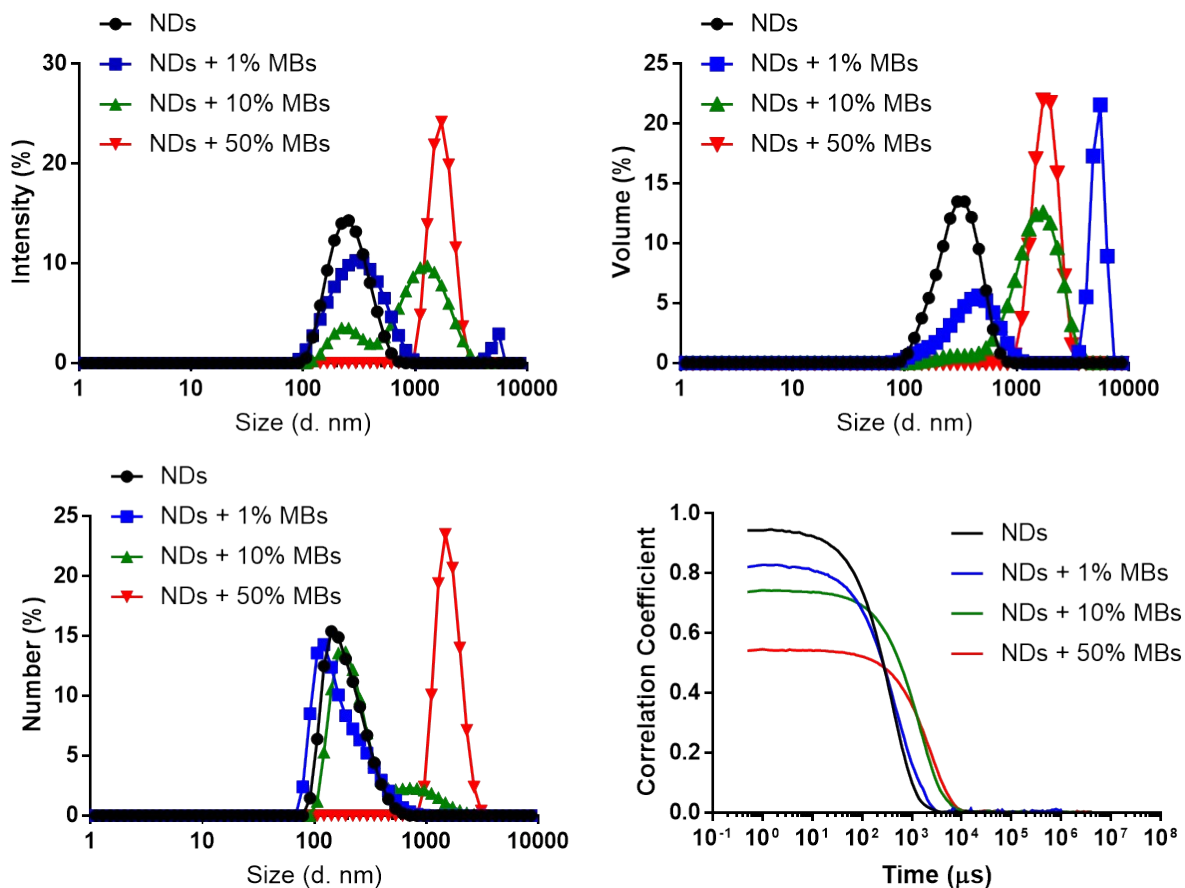


FIGURE S7. Size distributions (weighted by intensity, volume and number), and correlation functions of DFB NDs when in coexistence with Definity MBs (1, 10 and 50%).

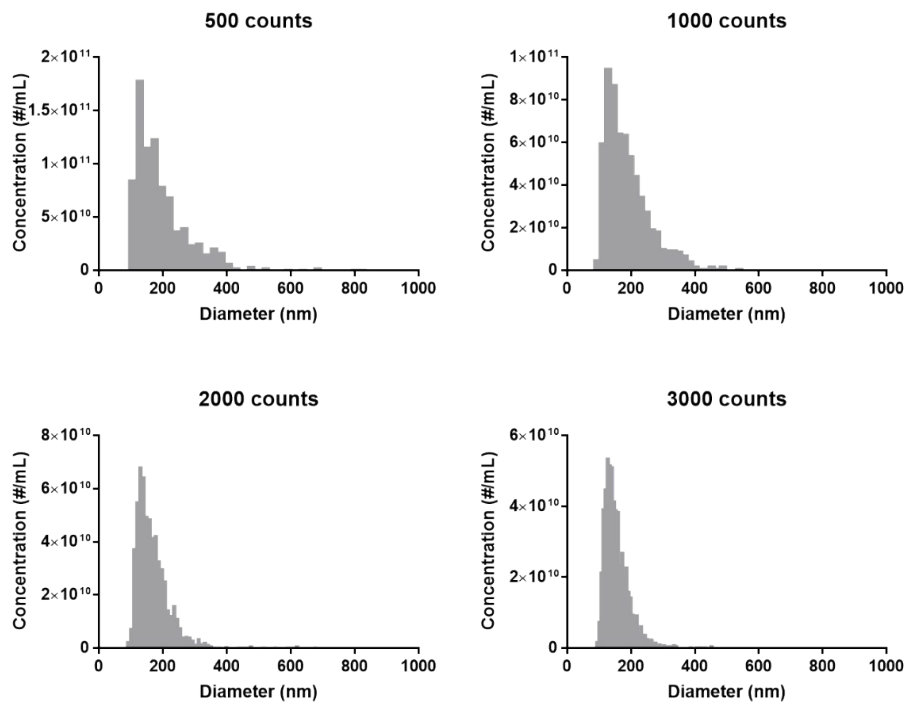


FIGURE S8. Representative DFB ND emulsion size distributions measured by TRPS (concentration in NDs/mL) as a function of counting events (500, 1000, 2000, and 3000).

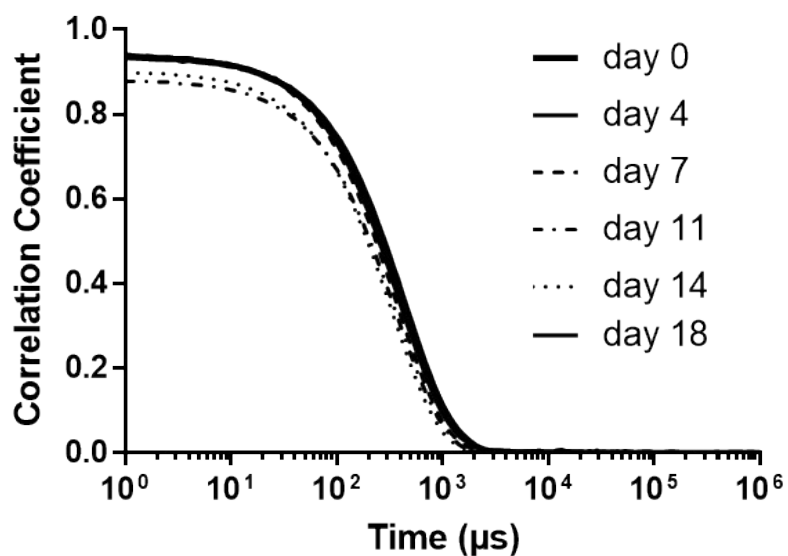


FIGURE S9. Representative correlation functions of DFB NDs over an 18-day observation period. A smooth, single exponential decay functions confirm the presence of mono-size particle dispersions.

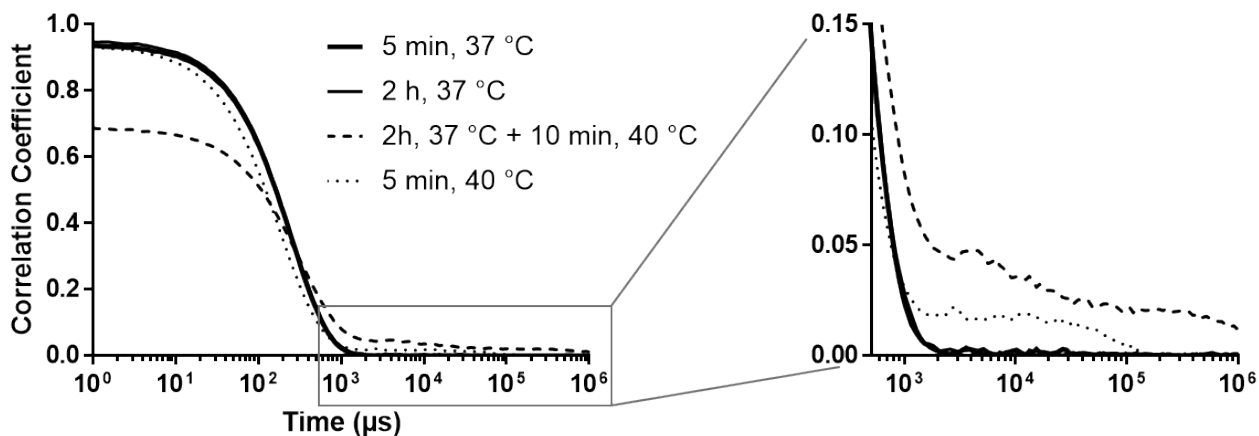


FIGURE S10. Correlation function curves of DFB NDs after a variety of temperature exposures as shown in the legend. Full 10^6 μs curve on the left and an expanded view from 0.5×10^3 to 10^6 μs on the right.

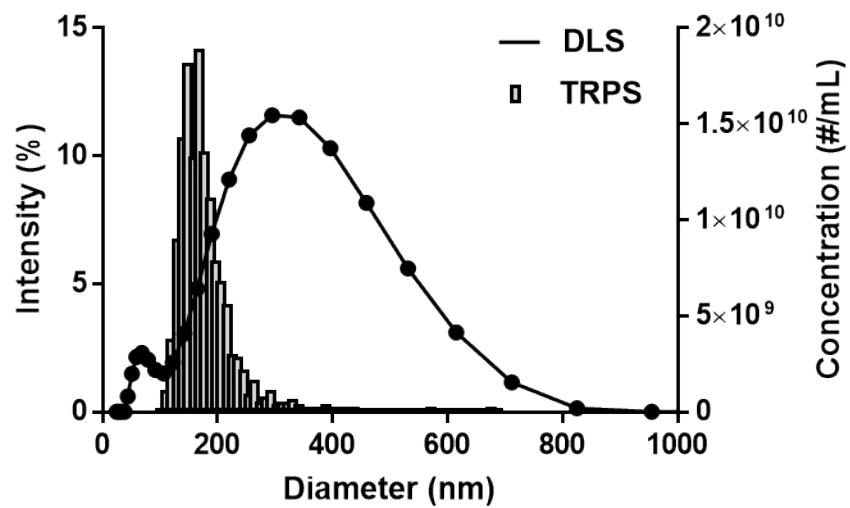


FIGURE S11. Representative OFP NDs size distributions measured by TRPS (histograms, concentration in NDs/mL) and DLS (solid lines, intensity in %).