Electronic Supplementary Information

Phase-transition nanocapsule contrast agent triggered by low-intensity ultrasound

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1. Materials

1,1,1,3,3-Pentafluorobutane (PFB; 99%, Apollo), and 2,2,3,3,4,4,4-heptafluoro-1-butanol (HFB; 98%, Matrix Scientific) were purchased from J&K Scientific Ltd. (Beijing, China). Perfluorohexane (PFH; 98+%), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC; 99%), and N-hydroxysuccinimide (NHS; 99%) were purchased from Adamas Reagent Ltd. (Shanghai, China). α-Methoxy-ω-hydroxyl poly(ethylene glycol) [mPEG-OH; molecular weight (M.W.): 2000 g/mol], and tin(II) 2-ethylhexanoate [$Sn(Oct)_2$; CR] were provided by Sigma-Aldrich Co. LLC. (Milwaukee, USA). α-Methoxy-ω-amino poly(ethylene glycol) (mPEG-NH_2; M.W.: 2000 g/mol) was prepared from mPEG-OH following reported method (M. D. Bentley et al., US Patent 2010/0063328 A1). ε-Caprolactone (ε-CL; 99+%, Shenzhen Esun Industrial Co., Ltd., Shenzhen, China) was purified by vacuum distillation over calcium hydride (CaH_2) prior to use. Dialysis tube (M.W. cut-off: 14 kDa; Shanghai Green Bird Technology Development Co., Ltd., China) was boiled in pure water for 10 minutes and stored in 1 mM ethylenediamine tetraacetic acid (EDTA) aqueous solution prior to use. All other regents are of analytical grade, and dried or redistilled before use.

2 Synthesis of Poly(ε-caprolactone) Homopolymer and Block Copolymer

The polymers in this work were synthesized according to the route presented in Figure S1.

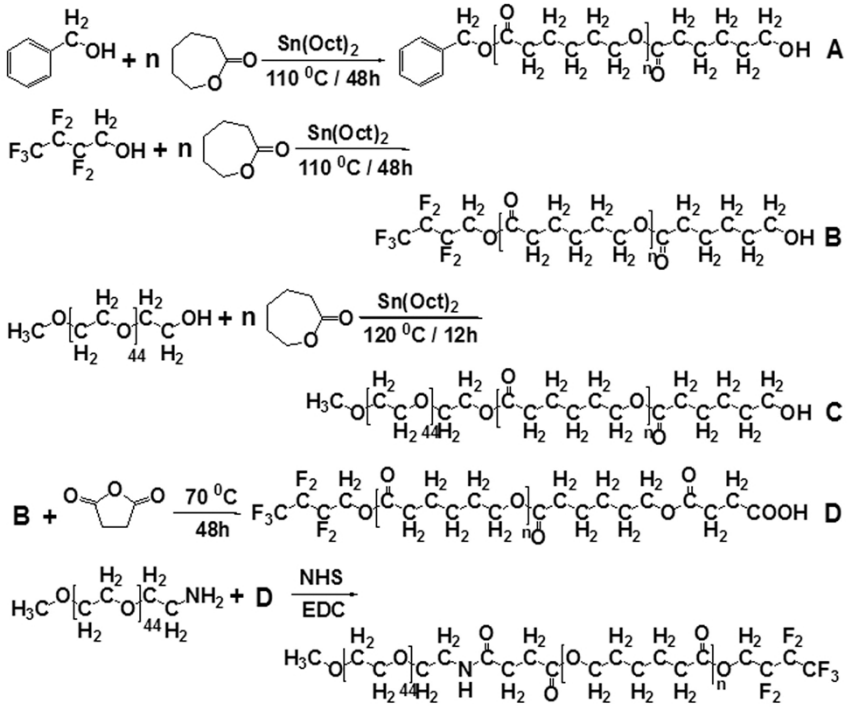


Figure S1. Synthesis of Bz-PCL (A), HFB-PCL (B), PEG-PCL (C) and PEG-PCL-HFB (E).

2.1 Synthesis of poly(ε-caprolactone) homopolymer (PCL)

Poly(ε-caprolactone) homopolymers with different end groups were synthesized *via* ring-opening polymerization (ROP) of ε-CL using benzyl alcohol (BA; related to Bz-PCL) or HFB (related to HFB-PCL) as the initiator and $Sn(Oct)_2$ as a catalyst. Typically, catalytic amount of $Sn(Oct)_2$ was dried under vacuum in a round bottom flask for 30 min., and then 10 g of ε-CL, 10 mL of anhydrous toluene, and 104 μL of BA or 330 μL of HFB were added to the flask under dry argon atmosphere, a condenser was equipped to the flask during the polymerization. After 48 hours stirring at 110 °C, the crude products were purified by precipitation in excessive diethyl ether for two times and dried in vacuum. Both the M.W. of Bz-PCL and HFB-PCL were ~10,000 g/mol as determined by the relative peak integral of phenyl protons (C_6H_5-) in Bz (~7.19 ppm), methylene protons (-CH_2-) in HFB (~4.52 ppm), and ε-CH_2- in repeating units of PCL (~4.04 ppm) in ^{1}H-NMR spectrum (Avance III, Bruker, Germany).

2.2 Synthesis of methoxy-poly(ethylene glycol)-*b*-poly(ε-caprolactone) diblock

copolymer (mPEG-PCL)

mPEG-PCL was synthesized through mPEG-OH-initiated ROP of ε-CL in the presence of $Sn(Oct)_2$ as catalyst. In detail, 2.0 g of mPEG-OH were dried under vacuum at 60 °C for 2 hours and cooled to room temperature. Subsequently, 10 g of ε-CL, catalytic amount of $Sn(Oct)_2$, and anhydrous toluene were added to mPEG-OH under dry argon atmosphere, a condenser was equipped to the flask during the polymerization. After 12 hours' stirring at 120 °C, the product were isolated after two-time precipitation in excessive diethyl ether and vacuum dried. The M.W. of PCL block in mPEG-PCL were ~10,000 g/mol as determined by the relative peak integral of $-CH_2-$ in repeating units of PEG block (~3.56 ppm, see peak "a" in Figure S2A) and $\varepsilon-CH_2-$ in repeating units of PCL block (~4.04 ppm, see peak "e" in Figure S2A) in ^{1}H-NMR spectrum.

2.3 Synthesis of HFB-terminated mPEG-PCL copolymer (mPEG-PCL-HFB)

10 g of HFB-PCL (M.W. ~10,000 g/mol) and 0.3002 g of succinic anhydride were dissolved in 15 mL of anhydrous chloroform ($CHCl_3$), and refluxed at 70 °C for 48 hours. The product was isolated by precipitation in cold ethanol and dried in vacuum to obtain HOOC-PCL-HFB. 6.3 g of HOOC-PCL-HFB, 85.3 mg of EDC, and 63.3 mg of NHS were mixed in anhydrous $CHCl_3$ and stirred for 2 hours, then a determined volume of $CHCl_3$ solution containing 1 g of mPEG-NH_2 and catalytic amount of triethylamine were added. After stirring at 35 °C for additional 12 hours, the resulting solution was concentrated and then added drop wise into deionized water with high-speed stirring to form polymeric micelles, followed by rotary evaporation to remove $CHCl_3$. The purified products (mPEG-PCL-HFB) were obtained by dialysis in deionized water for 3 days and lyophilization.

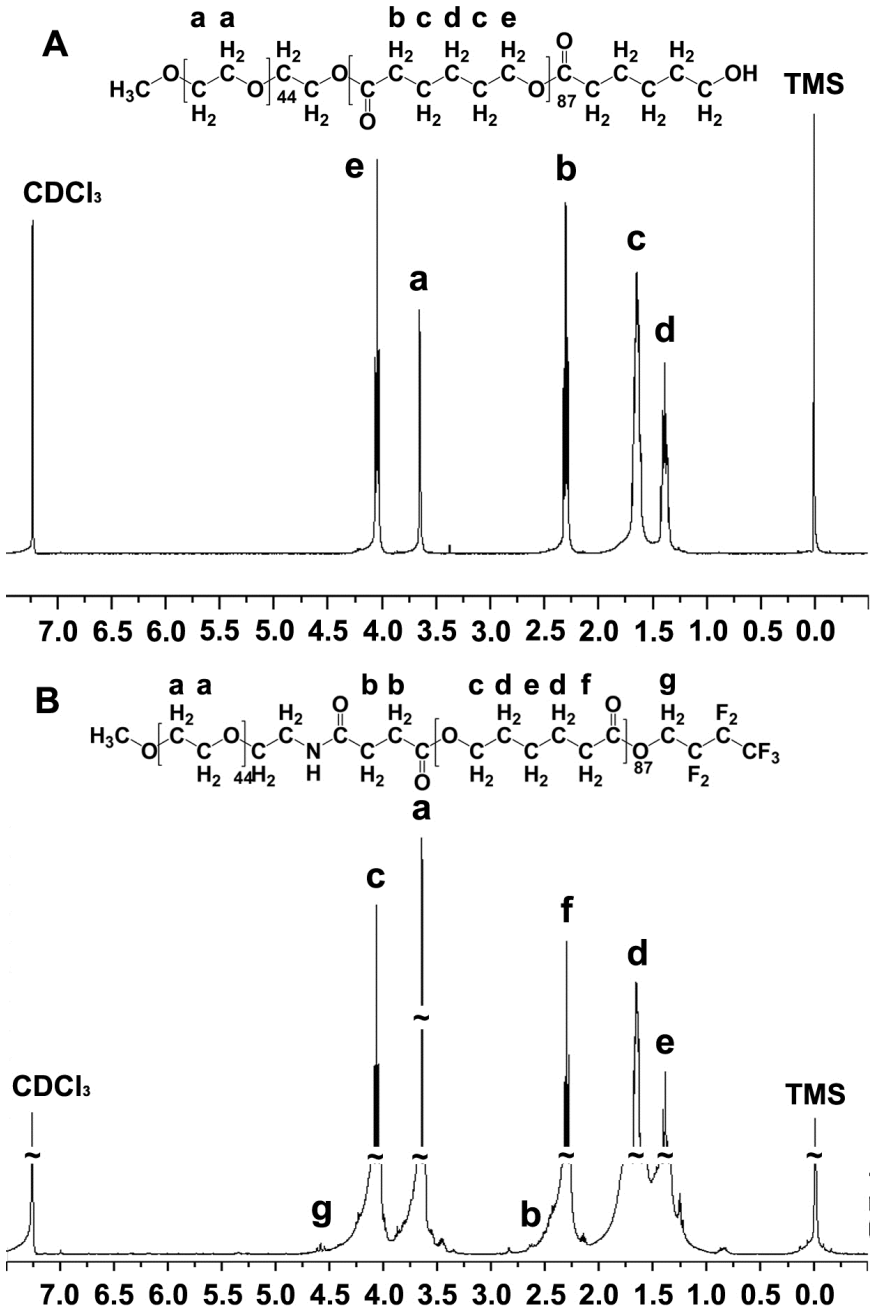


Figure S2 ^{1}H-NMR spectra of mPEG-PCL (A) and mPEG-PCL-HFB (B).

3 Preparation of PEG-PCL nanocapsules using ultrasonic triple- emulsification

100 μL of PFH or PFB was firstly dispersed in 0.5 mL of dichloromethane (DCM) by ultrasonic emulsification (S-4000, Misonix, U.S.A.), and then mixed with 0.5 mL of DCM solution containing 10 mg of mPEG-PCL or mPEG-PCL-HFB under sonication. Subsequently, the mixed solution was added drop wise into 10 mL of ultrapure water under sonication. Finally, redundant DCM in the resulting emulsion was removed by

rotary evaporation to form fluorocarbon-encapsulated nanocapsule solution, followed by particle sizing on a Nano ZS90 Zetasizer (Marlvern, U.K.).

4 Characterization of PEG-PCL nanocapsules

4.1 Transmission electron microscopy (TEM)

A drop of aqueous solution containing PFH-loaded mPEG-PCL-HFB nanocapsules was floated on 400 mesh carbon-coated copper grid, followed by staining with 0.02% (w/w) phosphotungstic acid solution. Nanocapsules was imaged on a JEM-1400 transmission electron microscope (JEOL, Japan) at 120 kV and 6,000 magnification.

4.2 Contact angle

The static water contact angle (WCA) of Bz-PCL, HFB-PCL, mPEG-PCL and mPEG-PCL-HFB were measured by contact angle meter (SL200B, KINO, U.S.A) at ambient temperature. According to the sessile drop method, 0.1 g of melted polymer was filmed on cover glass, and then a droplet of ultrapure water (about 2 μL) from a hydrophobized needle of a micro-syringe was dropped onto the polymer surface. The resulting images were captured with a video camera and their WCA values were determined by an image analysis software. An average of at least three measurements was taken for each sample.

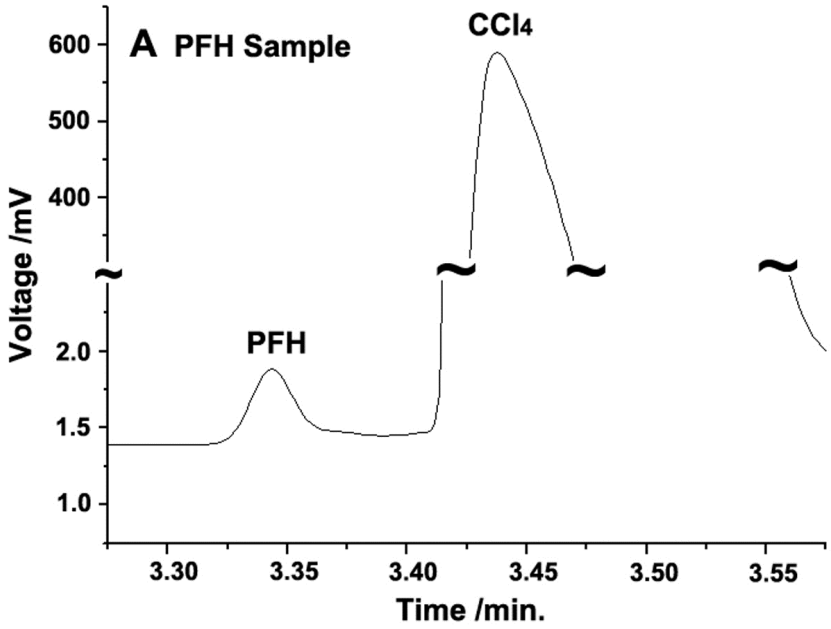
4.3 Differential scanning calorimetry (DSC)

Both blank nanomicelles and "fluorocarbon-loaded nanocapsules" were subjected to DSC analysis on a TA Q20 instrument (U.S.A) under nitrogen gas (flow rate: 10 mL/min); It should be noted that those "fluorocarbon-loaded nanocapsules" were actually empty nancapsules since the fluorocarbons would be removed during the lyphilization process. Typically, the samples were heated or cooled within temperature range of 15 to 70 °C at a rate of 1 °C/min. The cold-crystallization temperature (Tcc) was estimated as the peak value of the crystallization exothermic peak in the second cooling run, and the melting temperature (Tm) was estimated as the peak value of the melting endothermic peak in the first heating run, respectively.

4.4 Gas chromatography (GC)

9 mL of aqueous solution of nanocapusle loaded with PFH or PFB was concentrated to 2mL by centrifugal ultrafiltration using 15 mL Amicon Ultra-15 filter (Millipore,

Germany; MW cut-off: 100 kDa), and then mixed with 2 mL of tetrahydrofuran (THF) to disrupt the nanocapsule. Afterwards, the PFH or PFB was extracted three times from the mixed solution with an appropriate good solvent [carbon tetrachloride (CCl_4) for PFH; n-propyl bromide ($CH_3CH_2CH_2Br$) for PFB]. Analysis of the sample extracts were performed on a SP-6890 GC instrument (Rainbow Chemical Instrument Co., Ltd. Shandong Lunan, China). 0.2 μL of extract was injected on a split mode (split ratio: 30:1) at 40 °C. The separation was achieved on a HP-INNOWAX capillary column (30 m×0.32 mm i.d. ×0.25 μm film thickness, 19091N-113, Agilent Technologies, USA) using N_2/H_2 mixed gas (volume ratio: 10:1) as carrier gas at constant flow rate of 30 mL/min. Both the vaporizing chamber and electrical conductivity detector (ECD) were set at 110 °C. Two representative chromatograms of resulting sample extracts were shown in Figure S3. The contents of fluorocarbon were calculated according to the linear equation between the integral area of fluorocarbon elution peak and the fluorocarbon concentration of sample solution. Finally, both the loading efficiency (L_{FC}) and the encapsulation efficiency of liquid fluorocarbon (E_{FC}) in the polymeric nanocapsules were obtained. (see Table 1)



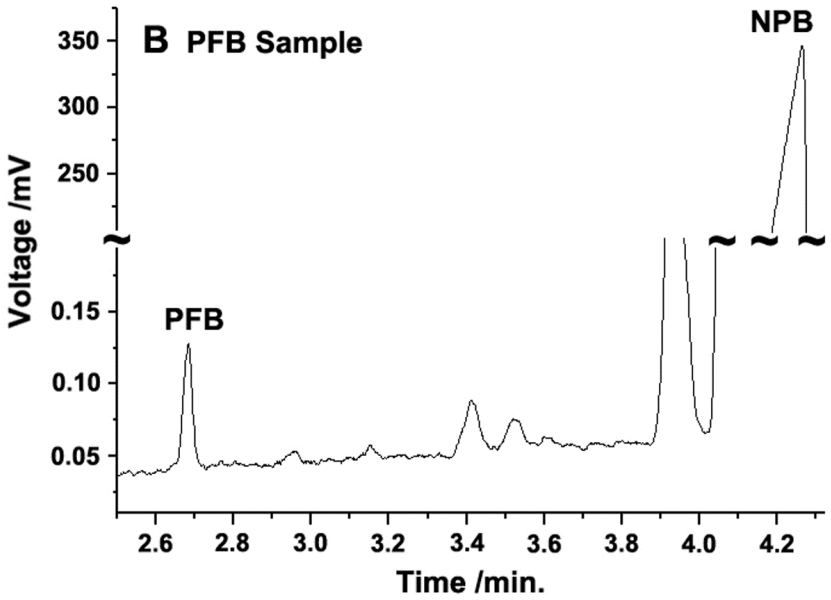


Figure S3 Representative chromatograms of PFH (A) and PFB (B) extracts.

Typically, L_{FC} is expressed as:

$$L_{FC} = \frac{m_{FC}}{m} \times 100\% = \frac{m_{FC}}{m_{FC} + m_P} \times 100\% \quad \textbf{Equation 1},$$

where m is the total mass of the liquid fluorocarbon-loaded polymeric nanocapsules, and m_{FC} and m_P are the mass of fluorocarbon and the copolymer, respectively.

Similarly, E_{FC} is expressed as:

$$E_{FC} = \frac{m_{FC}}{m_{FC}^*} \times 100\% \quad \textbf{Equation 2},$$

where m_{FC}* is the feed of liquid fluorocarbon.

4.5 Microscopic observation

Nanocapsules loaded with PFH or PFB was diluted 3 times with ultrapure water, 20 μL of the aliquot was transferred in between a slide and coverslip that were mounted on a heating stage under a DM2500p microscope (Leica, Germany), images were photographed at 1,000X magnification every 0.5 s. The heating stage for sample was programmed as follows: the temperature of PFH-loaded nanocapsules was increased from 35 °C to 37 °C at a rate of 1 °C/min and was held for 1 min, and subsequently increased to 55°C at a rate of 20 °C/min and held for 1 min (no photos), afterwards increased to 61 °C at a rate of °C/min and held for 1 min, and finally increased to 65 °C at a rate of 1 °C/min and held for 1 min; for PFB-loaded

nanocapsules, the temperature was increased from 35 °C to 37 °C at a rate of 1°C/min and held for 1 min, subsequently increased to 41°C at a rate of 1 °C/min and held for 1 min, and finally increased to 45 °C at a rate of 1 °C/min and held for 1 min. All the visible micron-sized bubbles in the field were counted for quantitative analysis.

4.6 *In vitro* ultrasound imaging

Agarose model (depth: 80 mm; inner diameter: 9 mm; wall thickness: 3 mm): 3 g of agarose was dissolved in 100 mL of ultrapure water at 90 °C, and then degassed for 30 min at 60 °C to remove redundant bubbles using a SG3200HBT ultrasonic cleaner (59 kHz, 100% power) (Shanghai Gutel Ultrasonic Instrument Co., Ltd., China). Afterwards, a glass rod with diameter of 9 mm was inserted into the degassed agarose solution inside a 15 mL centrifuge tube. The tube was cooled to 25 °C under sonication. Finally, an agarose model without any bubbles was obtained after the removal of inserted glass rod.

In vitro ultrasound imaging: 2 mL of solution of nanocapsules loaded with PFH or PFB was manually injected with a syringe into 2 mL of 2 w.t.% poly(vinyl alcohol) (PVA, 2,000 g/mol, Acros, Thermo Fisher Scientific Inc.) aqueous solution inside agarose model. Hereinto, the agarose model was immersed at 43 °C in a water bath, and the inner PVA aqueous solution was kept constant at 40 °C. *In vitro* ultrasound imaging was performed and recorded using a Siemens Sequoia 512 clinical imaging system with a 15L8W-S probe in B-mode [frequency: 10 MHz; mechanical index (MI): 0.11; focus depth: 1.5 cm]. During the entire imaging process, screenshots of recorded videos every 1 second were analyzed to determine the gray values within the region of agarose model using Image J software. Similarly, ultrasound images with 5, 10 and 40 times-diluted nanocaupsule solutions were analyzed respectively according to the same protocol. The PVA solution by itself was used as blank control.