Supplementary Information

Fluorous Biphase Drug Delivery System Triggered by Low Frequency Ultrasound: Controlled Release from Perfluorous Discoidal Porous Silicon Particles

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SUPPLEMENTARY EXPERIMENTAL SECTION

1. Methods

Nuclear magnetic resonance (NMR). NMR spectra were recorded at 25 ^{o}C on a JEOL ECA-500 spectrometer. The chemical shifts were recorded in ppm relative to tetramethylsilane (TMS) and with the solvent resonance as the internal standard. ^{1}H, ^{13}C, and ^{19}F NMR data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, br = broad), coupling constants (*J*) were reported in hertz (Hz), integration.

Electrospray ionization liquid chromatography mass spectrometry (ESI-LC-MS). ESI-LC-MS analyses were performed on Thermo Finnigan LCQ Deca XP Plus LC/MS with a C18 column (Kinetex™ 5 μm XB-C18 100 Å, LC Column 50 × 4.6 mm, Phenomenex, USA) in gradient elution (10 – 95% Acetonitrile in H_2O, 12 min). The injection amount was 1 μL. Reactions requiring anhydrous conditions were performed under nitrogen or argon.

Transmission electron microscopy (TEM). An aliquot of diluted particle suspension was applied to a carbon holey film on a copper grid. TEM analysis was performed with JEOL 2000 FX and JEOL 2010 FEG microscopes equipped with energy dispersive spectrometers (EDS) at accelerating voltage of 200 kV. Each sample was analyzed by conventional and high resolution (HR) TEM, selective area electron diffraction (SAED) and energy dispersive spectroscopy (EDS).

Scanning electron microscopy (SEM). The scanning electron microscopy (SEM) images were taken by a SU8010 model instrument operated at an accelerating voltage of 10 kV. Samples for SEM imaging were prepared on a clean silicon wafer.

X-ray photoelectron spectroscopy (XPS). The unmodified or modified fluorous particles were deposited on a 200 μm × 200 μm silicon substrate. A PHI 5700 X-ray

photoelectron spectrometer equipped with a monochromatic Al Kα X-ray source (1486.7 eV) at a takeoff angle (TOA) of 45º was used to analyze the sample. The spectrometer was operated at both high and low resolutions with pass energy of 23.5 and 187.85 eV, respectively. Electron binding energies were calibrated with respect to the O1s at 532.9 eV.

Fourier transform infrared spectroscopy (FTIR). The unmodified or modified fluorous particles were directly transferred on the sample stage. FTIR spectra were acquired using a Nicolet™ iS™10 FT-IR Spectrometer. The spectra were collected over 16 scans at a resolution of 2 cm^{-1}.

UV-Vis spectroscopy. The UV-Vis absorption spectra were recorded UV-Vis spectrophotometer (Shimadzu UV-1800, Japan) in the range of 300 − 800 nm.

2. Synthesis of fluorous-tagged compounds

Synthesis of perfluorooctanyl fluorescein isothiocyanate (FITC-C_8F_{15}). FITC (15.6 mg, 0.04 mmol) was dissolved in ethanol (10 mL). 1H,1H-perfluorooctylamine (8 mg, 0.02 mmol) in ethanol (10 mL) was added dropwise into the FITC solution at room temperature under mild magnetic stirring and kept stirring for 24 hours. Afterwards, the solvent was evaporated and the residue was purified by flash column chromatography with 10% methanol in ethyl acetate as the eluent to give the product as a green powder, 22.0 mg, 93%. ^{1}H NMR (500 MHz, Acetone-d6) δ 9.05 (s, 2H), 7.98 – 7.89 (m, 2H), 7.26 (dd, *J* = 8.1, 7.0 Hz, 1H), 6.75 (d, *J* = 2.5 Hz, 2H), 6.72 (d, *J* = 4.0 Hz, 1H), 6.70 (d, *J* = 3.5 Hz, 1H), 6.65 – 6.64 (m, 1H), 6.63 (dt, *J* = 4.9, 1.8 Hz, 1H), 4.75 (dtd, *J* = 48.0, 16.4, 6.2 Hz, 2H), 4.47 (q, *J* = 7.2 Hz, 1H). ^{13}C NMR (126 MHz, Acetone-d6) δ 184.39, 169.11, 169.07, 160.30, 153.35, 153.32, 153.28, 149.98, 146.63, 141.81, 131.30, 130.17, 130.13, 130.09, 128.40, 125.88, 125.17, 125.12, 124.75, 119.22,

119.07, 117.36, 116.63, 113.30, 111.65, 111.58, 111.55, 103.33, 55.44. ^{19}F NMR (471 MHz, Acetone-d6) δ -117.45, -117.49, -117.51, -122.23, -122.46, -123.18, -123.75, -123.94, -126.59, -126.60, -126.62, -126.63, -126.65, -126.67. MS (ESI): [M+H] calcd for $C_{29}H_{18}F_{15}N_2O_5S$ = 791.06; found 791.10.

3. Bacterial cell culture

Pseudomonas aeruginosa (PA) strain expressing green fluorescent protein and a carbenicillin resistance gene (PAO1-GFP) was purchased from ATCC. The bacteria were grown in LB media at 37 °C and harvested in the log growth phase. Cultures were centrifuged at 5000 rpm for 10 min to pellet cells.

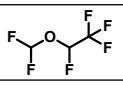
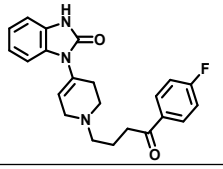
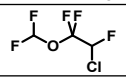
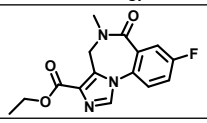
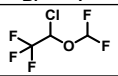
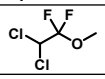
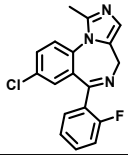
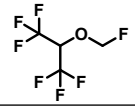
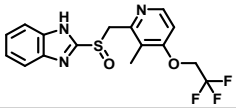
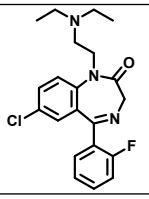
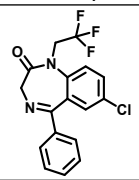
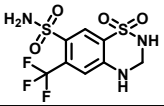
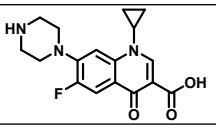
4. Plate counting assay

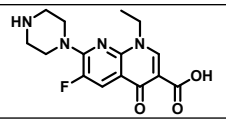
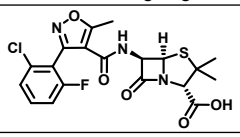
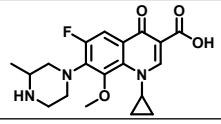
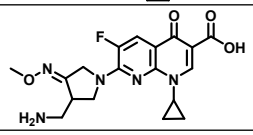
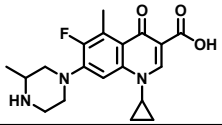
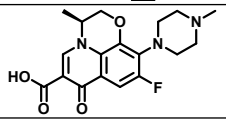
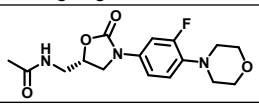
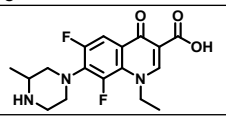
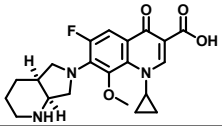
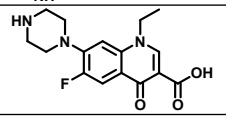
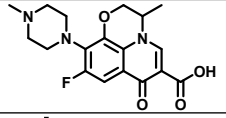
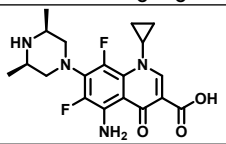
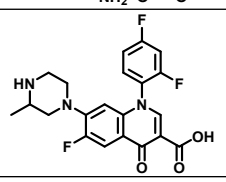
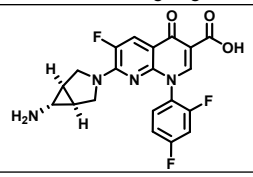
The biofilm formation on the bottom of the 48-well plate was examined by the plate counting assay. Briefly, after 24-hour static incubation, the culture solutions in the 48-well plate were gently removed. 1 mL of 0.01% SDS solution was added into each well and the bacteria were detached by sonication at 25 °C for 1 min. Afterwards, the bacterial suspensions were serially diluted (1, 10^{-2}, 10^{-4}, 10^{-6}) and 100 μL of each aliquot was immediately plated on LB agars. The colonies formed were counted after 24-hour static incubation at 37 °C.

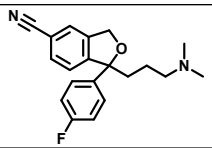
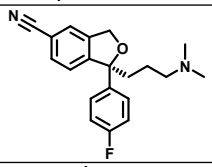
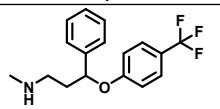
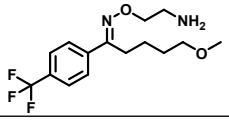
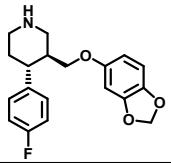
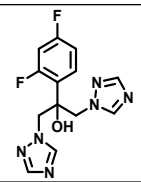
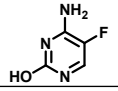
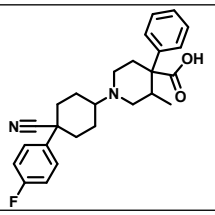
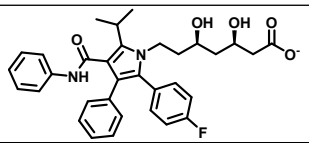
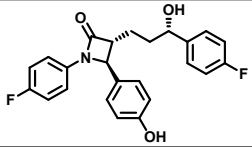
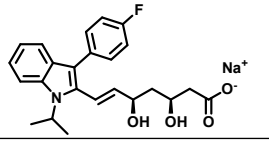
5. Statistical analysis

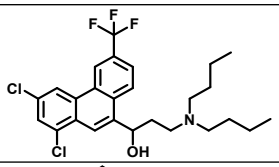
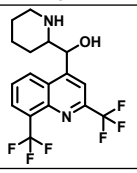
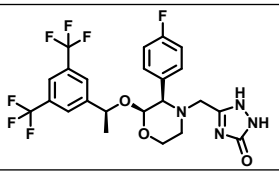
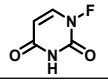
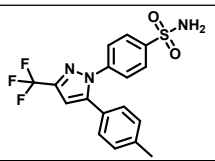
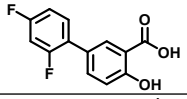
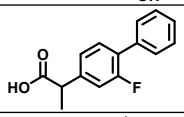
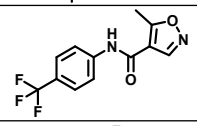
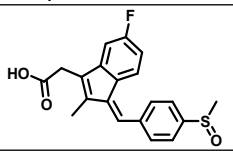
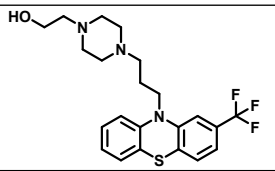
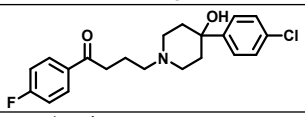
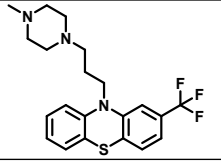
All data was obtained from at least three independent experiments and represented as mean ± standard deviation. The statistical significance of the data was determined by the Student's *t* test and the single factor one-way analysis of variance (ANOVA). A *P* value < 0.05 was considered to be statistically significant.

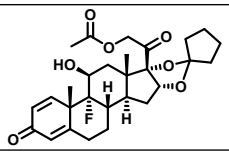
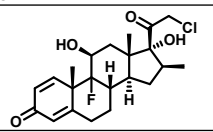
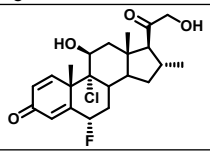
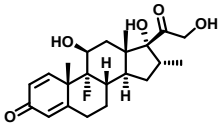
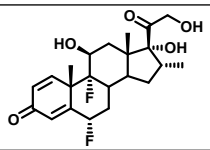
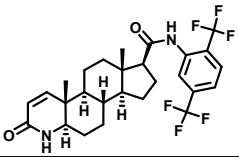
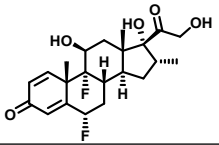
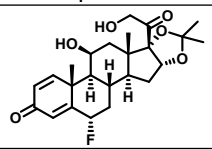
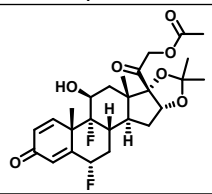
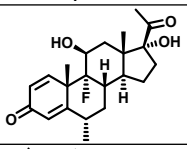
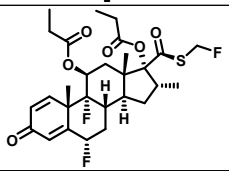
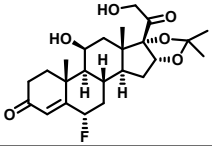
Table S1. List of FDA Approved Fluorinated Pharmaceuticals.

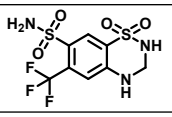
Uses	Drug Name	Brand Names	Chemical Structure
Anesthetics			
	Desflurane	Suprane	
	Droperidol	Inapsine	
	Enflurane	Ethrane, Compound 347	
	Flumazenil	Romazicon	
	Halothane	Fluothane	
	Isoflurane	Forane, Terrell	
	Methoxyflurane	Penthrane	
	Midazolam	Versed, Seizalam	
	Sevoflurane	Ultane, Sojourn	
Antacids			
	Lansoprazole	Prevacid	
Anti-anxiety			
	Flurazepam	Dalmane	
	Halazepam	Paxipam	
	Hydroflumethiazide	Saluron	
Antibiotics			
	Ciprofloxacin	Cipro	

	Enoxacin	Penetrex	
	Flucloxacillin	Floxapen	
	Gatifloxacin	Zymaxid, Zymar, Tequin	
	Gemifloxacin mesylate	Factive	
	Grepafloxacin	Raxar	
	Levofloxacin	Levaquin, Quixin, Iquix	
	Linezolid	Zyvox	
	Lomefloxacin	Maxaquin	
	Moxifloxacin	Avelox	
	Norfloxacin	Noroxin	
	Ofloxacin	Floxin	
	Sparfloxacin	Zagam	
	Temafloxacin	Omniflox	
	Trovafloxacin mesylate	Trovan	
Antidepressants			

	Citalopram	Celexa	
	Escitalopram	Lexapro	
	Fluoxetine	Prozac, Sarafem, R apiflux	
	Fluvoxamine maleate	Luvox	
	Paroxetine	Pexeva, Paxil, Brisdelle	
Anti-fungal antibiotics			
	Fluconazole	Diflucan	
	Flucytosine	Ancobon	
	Voriconazole	Vfend	
Antihistamines			
	Levocabastine	Livostin	
Antilipemics			
	Atorvastatin	Lipitor	
	Ezetimibe	Zetia	
	Fluvastatin sodium	Lescol	

Anti-malarial			
	Halofantrine	Halfan	
	Mefloquine	Lariam	
Antimetabolites			
	Aprepitant	Emend	
	Fluorouracil	Adrucil	
Arthritis			
	Celecoxib	Celebrex	
	Diflunisal	Dolobid	
	Flurbiprofen	Ansaid, Ocufen	
	Leflunomide	Arava	
	Sulindac	Clinoril	
Psychotropic			
	Fluphenazine	Prolixin	
	Haloperidol	Haldol	
	Trifluoperazine	Stelazine	
Steroids			

	Amcinonide	Cyclocort	
	Clobetasol	Temovate, Clobex, Dermovate, Impoyz	
	Clocortolone	Cloderm	
	Dexamethasone	Baycadron, Decadron, Dexamethasone Intensol, DexPak, TaperDex, Zema-Pak, ZoDex, Zonacort	
	Diflorasone	Psorcon, ApexiCon E, Apexicon, Maxiflor	
	Dutasteride	Avodart	
	Flumethasone	Flovent	
	Flunisolide	Aerospan, Aerobid	
	Fluocinonide	Lidex, Vanos	
	Fluorometholone	FML, Flarex	
	Fluticasone propionate	Cutivate, Flonase, Flonase Allergy Relief	
	Flurandrenolide	Cordran, Nolix	

	Hydroflumethiazide	Saluron	

SUPPLEMENTARY DATA

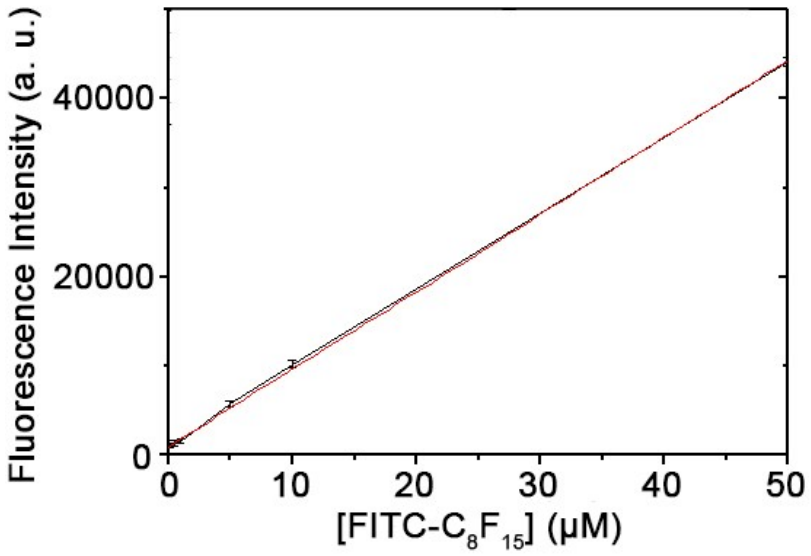


Fig. S1 Calibration curve of the fluorescence intensity of emitted at 535 nm in function of the FITC-C_8F_{15} concentration. Fluorescence Intensity = [FITC-C_8F_{15}] × 865850.7 + 985.6 (R^2 = 0.9994, red line is the corresponding linear fitting curve).

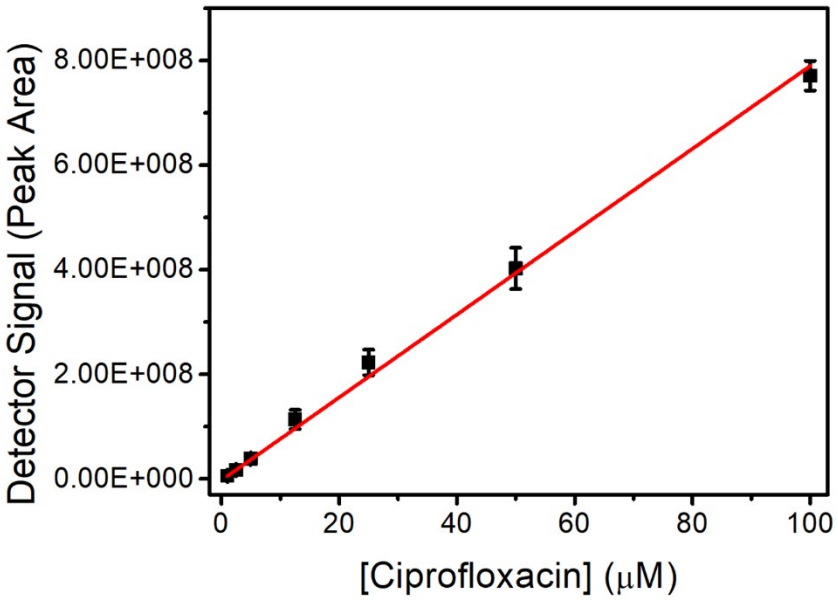


Fig. S2 Calibration curve for ciprofloxacin concentration *vs.* peak area. Each data point is the mean of four replicates and the error bar represents the standard deviation.

Calculation of the load rate of ciprofloxacin (Cip) on the fluorous particles:

(1) The Cip concentration in the solution was obtained from the mean detector signal (MDS) with reference to the standard curve in Fig. S2 using formula 1:

$$[Cip] = \frac{MDS + 2.76 \times 10^6}{7.93 \times 10^6} \quad (1)$$

(2) The load rate of Cip on the fluorous particles was calculated and the results were summarized in the Table S2.

Table S2. Calculation of the load rate of ciprofloxacin (Cip) on the fluorous particles.

Sample ID	MDS	Concentration (μM)	Load Rate
Sample 1	5273883		
Sample 2	5040741		
Sample 3	5370629		
Avg	5228418	1.01	58%
STD	169578	0.13	

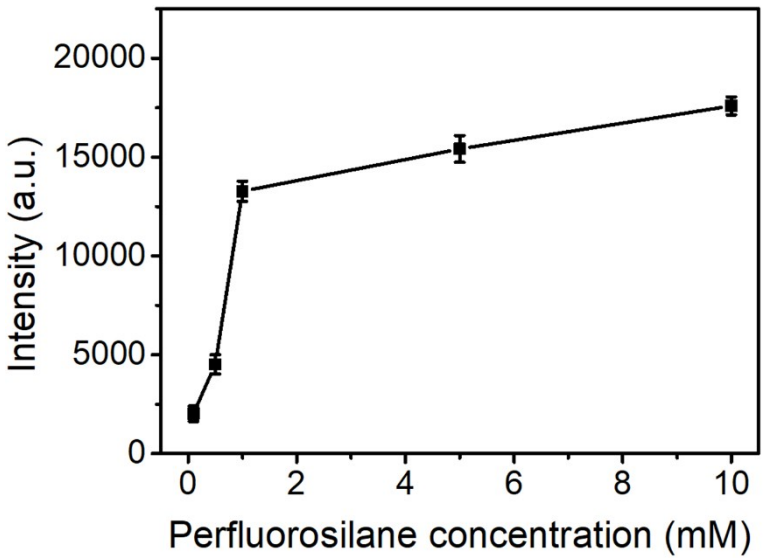


Fig. S3 High resolution F1s peak intensity of FAS17 functionalized porous silicon particles on a silicon wafer using perfluorosilane solution (0.1 – 10 mM).

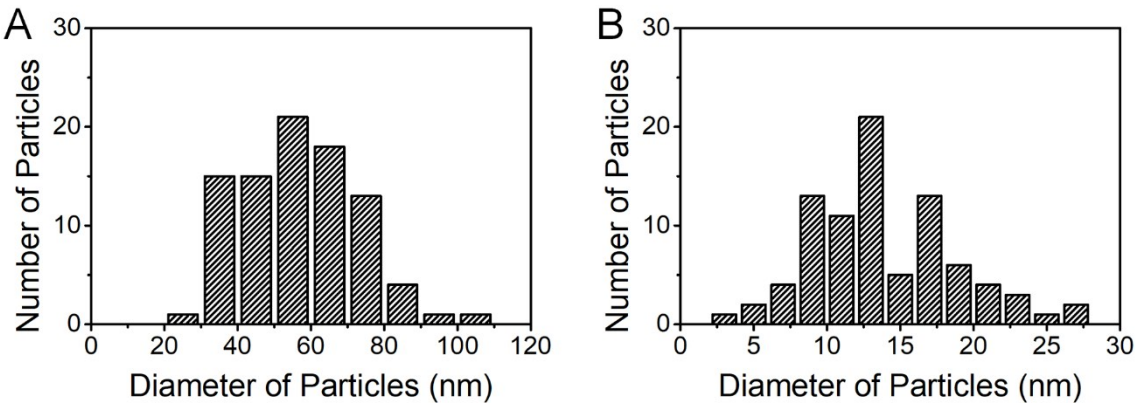


Fig. S4 The pore size distribution of the release layer (A) and the device layer (B).

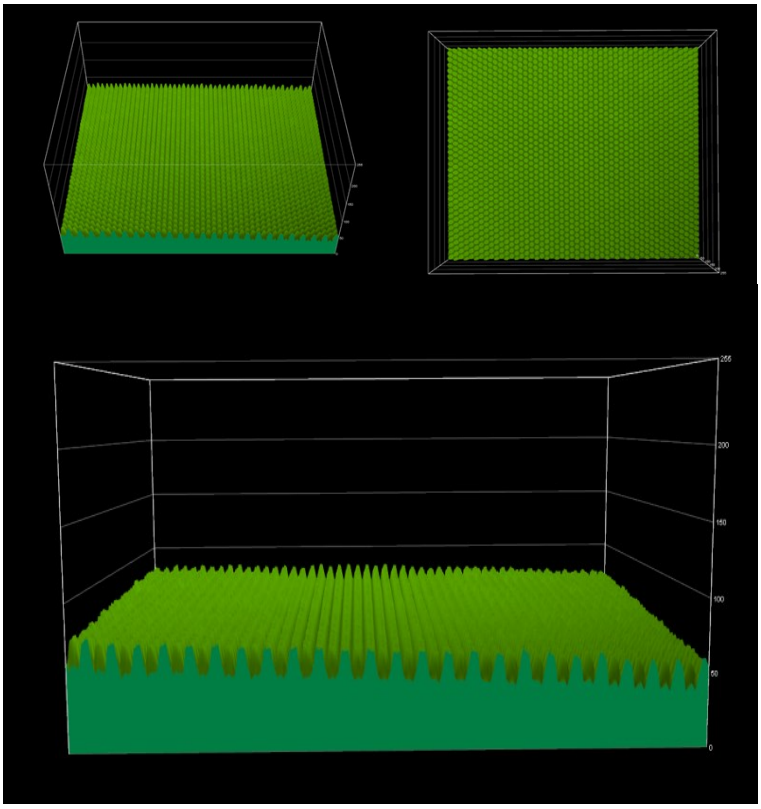


Fig. S5 Surface plot images at three different angles for the perfluorinated porous silicon particles after fluorinated FITC incorporation.

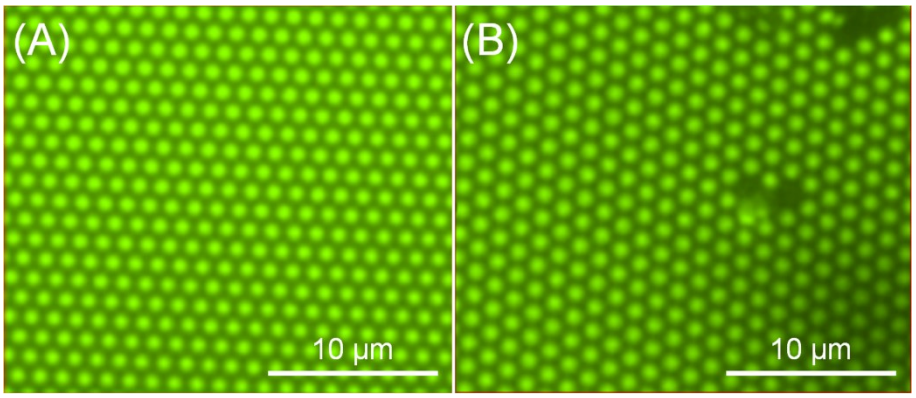


Fig. S6 Fluorescence microscopy images of the fluorinated FITC molecules incorporated fluorous particles before (A) and after (B) LFUS application without C_5F_{12} incorporation. The mean fluorescent intensity (MFI) was extracted from these images (A = 642 µm^2).

Copies of ^{1}H, ^{13}C, and ^{19}F NMR spectra

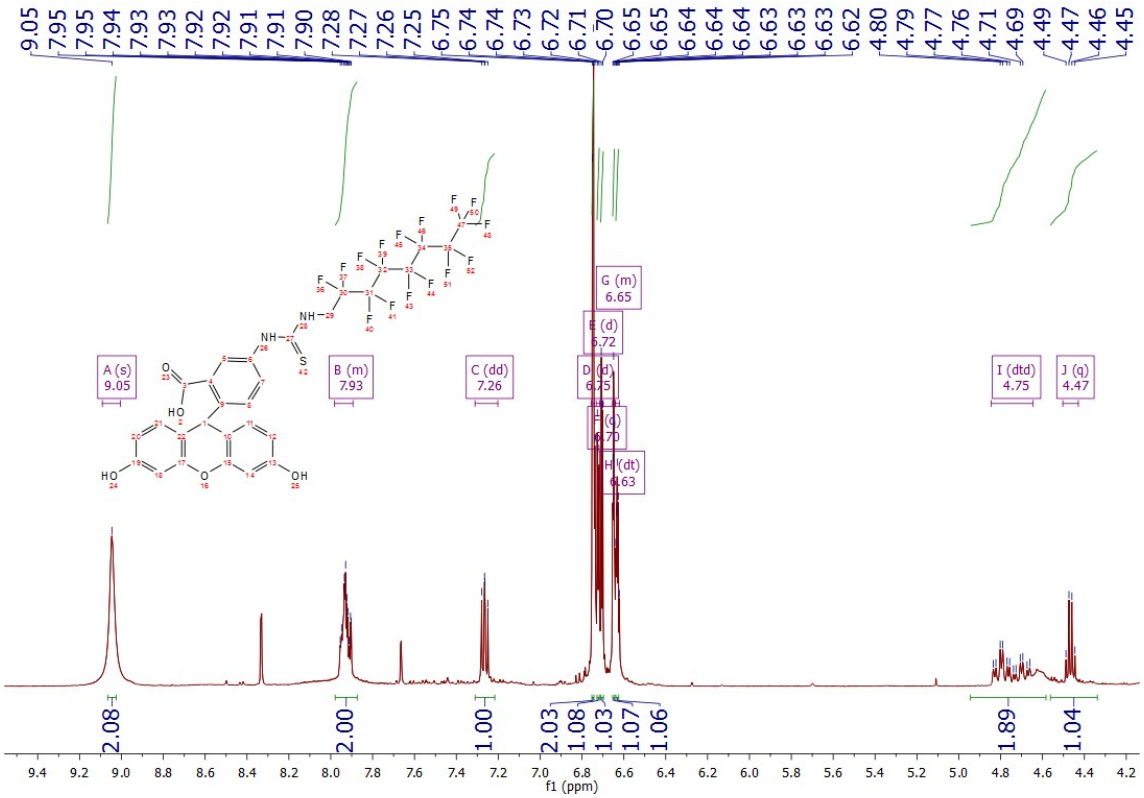


Fig. S7 Copy of ^{1}H NMR spectrum of perfluorooctanyl fluorescein isothiocyanate (FITC-C_8F_{15}).

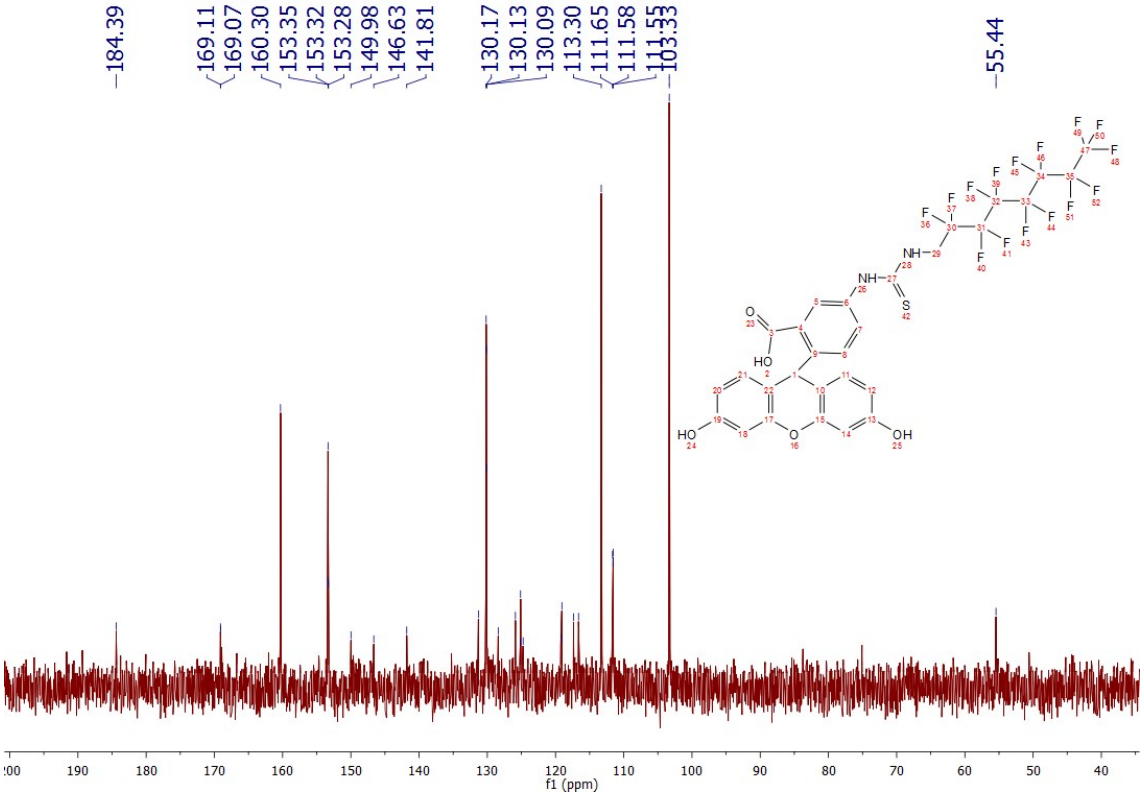


Fig. S8 Copy of ^{13}C NMR spectrum of perfluorooctanyl fluorescein isothiocyanate (FITC-C_8F_{15}).

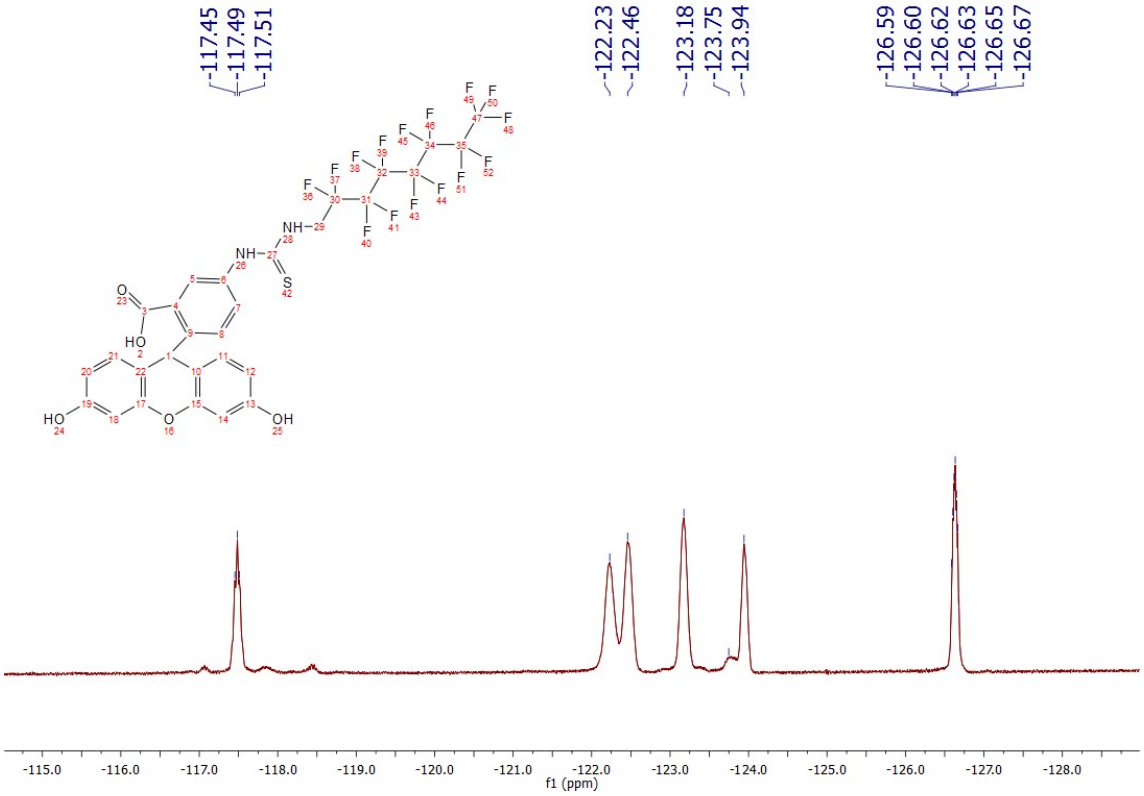


Fig. S9 Copy of ^{19}F NMR spectrum of perfluorooctanyl fluorescein isothiocyanate (FITC-C_8F_{15}).