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Supporting Information

Multi-Modal ^{19}F MRI Probe Using Perfluorinated Cubic Silsesquioxane-Coated Silica Nanoparticles for Monitoring Enzymatic Activity.

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Materials and Methods

General. ^{1}H NMR and ^{13}C NMR spectra were measured with JEOL EX-400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) spectrometer. ^{19}F and ^{29}Si NMR spectra were measured with JEOL JNM-A400 (370 MHz for ^{19}F and 80 MHz for ^{29}Si) spectrometer. Coupling constants (J value) are reported in hertz. The chemical shifts are expressed in ppm downfield from tetramethylsilane, using residual chloroform (δ = 7.24 in ^{1}H NMR, δ = 77.0 in ^{13}C NMR) as an internal standard and trifluoroacetic acid in $CDCl_3$ as an external standard. Masses were determined with a MALDI-TOF mass spectroscopy (acceleration voltage 21 kV, negative mode) with DHB (2,5-dihydroxybenzoic acid) as a matrix. Transmission electron microscopy was performed using a JEOL JEM-100SX operated at 100 kV. DLS measurements are executed using a FPAR-1000 particle analyzer with a He-Ne laser as a light source.

Octaammonium POSS (1). The synthesis of **1** was according to the reference 5 in the main text. (3-Aminopropyl)triethoxysilane (100 mL, 0.427 mol) and 35–37% HCl (135 mL) in MeOH (800 mL) produced **1** as a white precipitate after 2 days at room temperature. The crude product obtained after filtration, washing with cold MeOH, and drying. The product was spectroscopically pure in 30% yield (18.8 g). Recrystallization from hot MeOH afforded **1** (4.29 g, 3.66 mmol, 6.88%) as a white solid. ^{1}H NMR ($(CD_3)_2SO$, 25 °C): δ 8.23 (s, 24H), 2.76 (t, 16H), 1.71 (m, 16H), 0.72 (t, 16H). ^{13}C NMR ($(CD_3)_2SO$, 25 °C): δ 40.53, 20.13, and 7.96. ^{29}Si NMR($(CD_3)_2SO$, 25 °C): δ -66.4 (s).

F-POSS (2). To a suspension of POSS **1** (1 g, 0.852 mmol) and ethyl trifluoroacetate (406 μL, 3.41 mmol) in methanol (20 mL), triethylamine (2 mL, 14.4 mmol) was added, and the reaction mixture was stirred at room temperature for 3 h. The resulting mixture was evaporated, and the crude **2** was directly used in the next step. The analyzed sample as a clear oil was obtained after dialysis (895 mg, 83%); ^{1}H NMR (D_2O, 400 MHz) δ 3.26 (t, 8H, J = 7.0 Hz), 2.94 (t, 8H, J = 7.0 Hz), 1.70 (brs, 8H), 1.61 (brs, 8H), 0.64 (m, 16H): ^{13}C NMR (D_2O, 100 MHz) δ 155.9, 117.4, 41.52, 40.92, 21.76, 20.05, 8.77, 8.72: ^{29}Si NMR (D_2O, 80 MHz) δ –68.7, –68.4: ^{19}F NMR (D_2O, 373 MHz) δ –75.4 MALDI-TOF [$(M+H)^+$], [POSS-TFA_2] calcd. 1074.52, found 1073.29, [POSS-TFA_3] calcd. 1170.53, found 1170.00, [POSS-TFA_4] calcd. 1266.53, found 1266.16, [POSS-TFA_5] calcd. 1362.54, found 1361.76.

Preparation of F-POSS-coated silica nanoparticles. F-POSS obtained above (0.852 mmol) and $POCl_3$ (1 mL, 10.9 mmol) were mixed in 20 mL of chloroform containing 2 mL of triethylamine (14.4 mmol), and then 100 mg of 3-aminopropyltriethoxysilane-coated silica nanoparticles (150 nm and 280 nm, respectively) were added to the mixture. After 1 h stirring at room temperature, nanoparticles were centrifuged and washed with chloroform, water, and methanol. 101 mg of white powder was obtained after drying. The amount of F-POSS on the surface was estimated from the fitting of the signal intensity

of ^{19}F NMR on the standard curve using completely dissolved samples in 1 *N* NaOH*aq*.

Enzymatic reaction with F-POSS-coated silica NPs. F-POSS-coated silica NPs (1.5 mg) were incubated in 500 μL of the reaction solutions containing AP (2.5 U) in 50 mM sodium phosphate, 25 mM Tris–HCl, and 0.05 mM EDTA (pH = 7.0) at 37 °C. The reaction was terminated by adding 50 μL of 10 mM EDTA to the reaction mixture, and then ^{19}F NMR spectra were taken with JEOL JNM-A400 (370 MHz).

Fluorescence measurement. The fluorescence from a solution containing 1.5 mg of F-POSS-coated Si NPs containing fluorescein carboxylic acid before (left) and after (right) 12 h incubation at 37 °C in 50 mM sodium phosphate, 25 mM Tris–HCl, and 0.05 mM EDTA (pH = 7.0) was measured using a transilluminator at 365 nm. Fluorescence imaging was performed using BioRad ChemiDoc XRS. The samples (1 μL) after enzymatic reaction were put onto the slide glass and illuminated with a 290-365 nm transilluminator. Image was taken through a 480-nm long pass emission filter.

Cell viability assay. Primary mouse hepatocytes were used to test the toxic effects of F-POSS as assessed in the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay (Figure S2). Cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum and incubated at 37 °C in humidified 5% CO_2. One day before F-POSS treatment, mouse primary hepatocytes were prepared by the method of collagenase perfusion (Seglen P. O. *Methods in Cell Biology* **1976**, *13*, 29–83) and were seeded at a 15,000 cells / 100 μl / well in 96 well microtiter plates. Three days after the cells were incubated with F-POSS, 10 μl of 5 mg / ml MTT in phosphate buffered saline was added to each well, and the plates were kept in a CO_2 incubator for additional 4 h. After MTT solution was removed, the cells were lysed by adding 100 μl of 10% SDS, 0.01 M NH_4Cl and were incubated overnight. The degree of MTT reduction (i.e., cell viability) in each sample was subsequently assessed by measuring absorption at 600 nm at 37 °C using a plate reader. The absorbances measured from the three wells were averaged, and the percentage MTT reduction was calculated by dividing this average by the absorbance measured from a control sample lacking F-POSS.

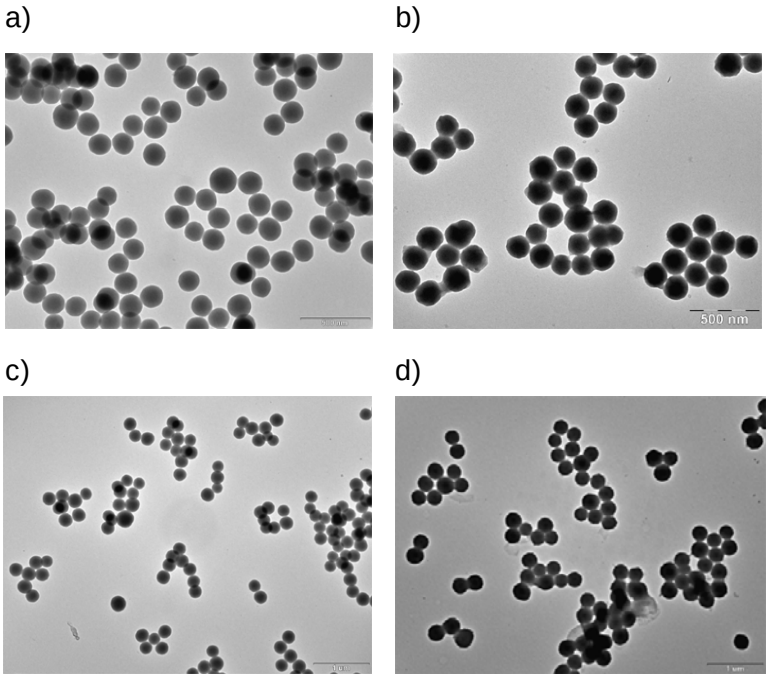

Figure S1. TEM images of the silica NPs (a) (150 nm diameter) (c) (280 nm diameter) before and (b) (150 nm diameter) (d) (280 nm diameter) after modification with F-POSS (scale bar: 500 nm in a and b, 1 μm in c and d).

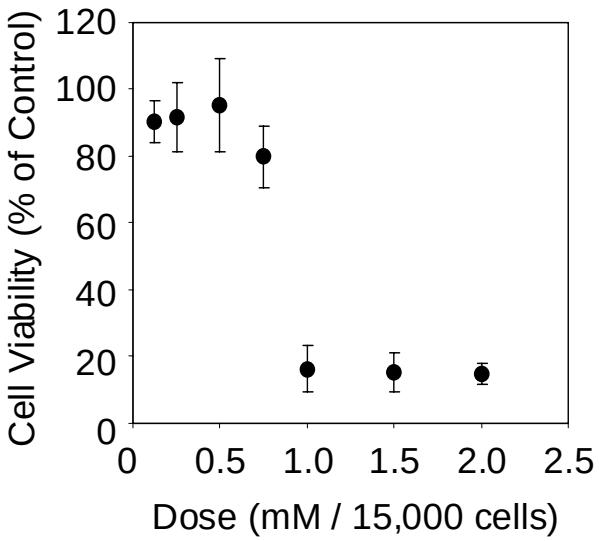

Figure S2. Effect of F-POSS on the viability of primary hepatocytes. Cells were incubated with various concentrations of F-POSS for 72 hours. Results are expressed as viability (% viable cells in comparison with the control) versus complex concentration. Each experiment was performed in triplicate and the error bars represent SD values.

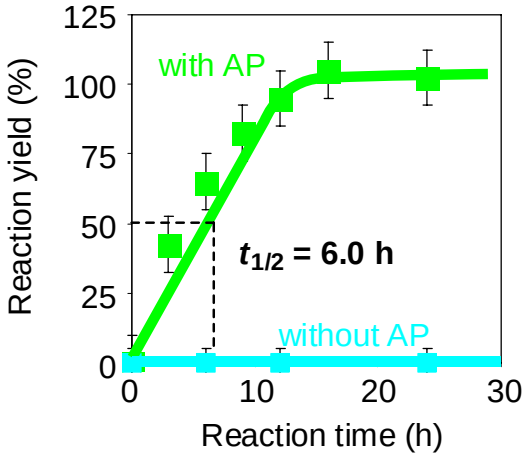

Figure S3. Time-course of enzymatic hydrolysis of the silica NPs in diameter of 280 nm. F-POSS-coated silica NPs (3 mg) were incubated in 500 μL of the reaction solutions containing AP (2.5 U) in 50 mM sodium phosphate, 25 mM Tris–HCl, and 0.05 mM EDTA (pH = 7.0) at 37 °C. The reaction yields were monitored with ^{19}F NMR and calculated by the fitting on the standard curve.

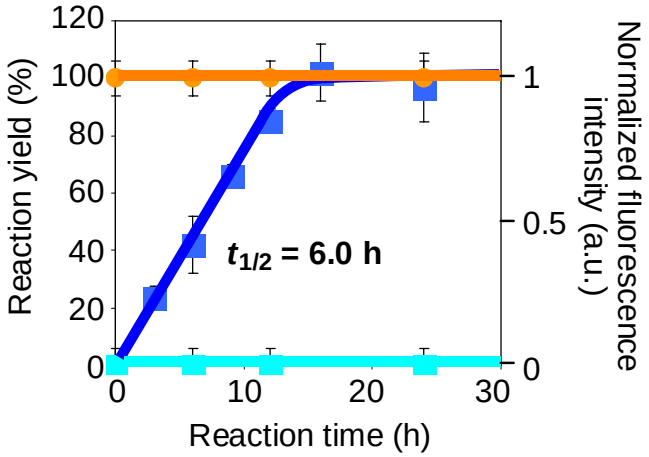

Figure S4. Time-course of the enzymatic reaction. Fluorophore-containing F-POSS-coated silica NPs (3 mg) were incubated in 500 μL of the reaction solutions in the absence (light blue line) and presence (blue line) of AP (2.5 U) in 50 mM sodium phosphate, 25 mM Tris–HCl, and 0.05 mM EDTA (pH = 7.0) at 37 °C. The reaction yields were monitored with ^{19}F NMR and calculated by the fitting on the standard curve. The fluorescence intensity of the reaction mixture was monitored (orange line) with the excitation at 490 nm.