Electronic Supplementary Information

Hyperpolarized ^{29}Si Magnetic Resonance Spectroscopy of Selectively Radical-Embedded Silica Nanoparticles

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Materials

TEOS (98%) was purchased from ACROS Organics (Geel, Belgium). Anhydrous ethyl alcohol (EtOH, 99.9%) was obtained from Daejung Chemicals (Gyeonggi do, South Korea). Ammonium hydroxide (28–30%), 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO, ≥ 95%), 4-amino-2,2,6,6-tetramethylpiperidine-1-oxyl (≥ 95%) and deuterium oxide (D_2O-d, 99.9%) were supplied by Sigma–Aldrich (MO, USA). DMSO-d6 (99.9%) was purchased from Cambridge Isotope Laboratories (MA, USA). Distilled water (DI H_2O, 18.2 MΩ-cm) was obtained using an Arioso Power water purification system (Human Corporation, South Korea).

Preparation of 1-[3-(triethoxysilyl)propyl]-3-[4-(2,2,6,6-tetramethylpiperidine-1-oxyl)]urea (TEOS–TEMPO)

The TEOS-TEMPO was synthesized by following the method previously described in the literature.[1] Typically, a 10 mL round-bottom flask (RBF) was evacuated to dryness and filled with Ar. The flask was charged with a solution of 4-amino-2,2,6,6-tetramethylpiperidine-1-oxyl (100 mg, 0.58 mmol) in benzene (900 µL). A solution of 3-(triethoxysilyl)propyl isocyanate (150 µL, 0.60 mmol) in benzene (900 µL) was added dropwise to the mixture, which was stirred at room temperature for 9 h. The solvent was removed under reduced pressure conditions to afford TEMPO-(3-(triethoxysilyl)propyl)urea 1 (235 mg, quantitative) as a red liquid, which was used in the next step without further purification. The product was analyzed by TLC (n-Hexane/ethyl acetate (EtOAc) = 1:3, R_f = 0.25, UV and KMnO4 stain) and LRMS (ES-API) (((m/z): found 419.3, calc. 419.27 for $[C_{19}H_{41}N_3O_5Si]^+$ as $[M+H]^+$).

Synthesis of radical-embedded SiO2 NPs

The NORMAL sample was prepared according to a previous report.[2] A 100 mL RBF was charged with 50 mL of EtOH, 1 mL of DI H_2O, and 1.9 mL of NH4OH. The solution was vigorously stirred at 28 ℃ for 10 min. Afterward, 1 mL of TEOS was added dropwise to the solution, and the mixture was stirred at 28 ℃ for 6 h. The NORMAL sample was collected via high-speed centrifugation (Labogene, 2236R, South Korea) at 15,000 rpm for 30 min, and the nanoparticles were washed with ethanol three times (between centrifuging) and vacuum-dried at 60 ℃. Amount of the final product was approximately 230 mg. For radical-embedded silica samples, a mixture of TEOS and TEOS–TEMPO was used as an organically modified silica (ORMOSIL) precursor. The amounts of TEOS and TEOS–TEMPO were calculated to obtain a final radical concentration of 30 mM. The HOMO silica sample was synthesized using a process similar to that of the NORMAL sample with a specific amount of TEOS and TEOS–TEMPO mixture precursor. The CORE sample was prepared in two steps. First, the 60 nm TEMPO-embedded core was prepared using the same amount of EtOH, DI H_2O, and precursor as the HOMO sample; however, the volume of NH4OH was adjusted to 1.7 mL to achieve a small particle size. In the next step, 60 mg of the TEMPO-embedded core was dispersed in 25.2 mL DI H_2O and 96.0 mL anhydrous EtOH via sonication for 30 min. Subsequently, 3 mL NH4OH was added to this suspension and 0.2 mL of TEOS was then added dropwise to this mixture. The CORE sample was collected via high-speed centrifugation (Labogene, 2236R, South Korea) at 15,000 rpm for 30 min, and the nanoparticles were washed with ethanol five times (between centrifuging) and finally vacuum-dried at 60 ℃. Amount of the final product was approximately 90 mg.

The SHELL nanoparticles were synthesized in a manner similar to that of the CORE sample. The main difference in procedures is that a 60 nm normal core was prepared first, and then, TEOS and TEOS–TEMPO were added in the second step for shell formation.

Instrumentation and characterization

The morphologies of these radical-embedded SiO_2 NPs were determined using SEM (Hitachi S4800, Japan) with an electron beam accelerated at 20 kV. For the SEM analysis, 2 mg of each sample was dispersed in 4 mL of EtOH using probe sonication for 1 min at 70% amplitude (Vibra–Cell, VCX 130, 130 W, Sonics and Materials Inc., USA). Thereafter, the suspension was dropped onto the Si chip substrates (SPI Supplies brand Si chip substrate 5 × 5 mm × 525 μm thick pack). The average size and standard deviation were estimated using the SEM images by averaging the diameters of more than 100 nanoparticles using ImageJ (NIH, USA).

For DLS measurements, a 0.5 mg/mL suspension of each sample was filtered through a PTFE membrane of 0.22 μm syringe filter. The residual suspension was placed in a quartz cell (10 × 10 mm, Hellma Analytics, Germany). This suspension was evaluated using a particle size analyzer (Anton Paar Litesizer 100, Austria) with a He–Ne laser (λ = 632.8 nm) at a backscattered angle of 173°. The concentration of the radicals was analyzed via electron spin resonance spectroscopy (ESR, JEOL, JES-X310) using an X-band waveguide (9.433 GHz, power: 0.998 mW) with a modulation frequency of 100.0 kHz and modulation width of 0.05 mT at 336.901 mT center field. The amplifications of radical-embedded SiO_2 NPs and the TEMPO sample are 8 and 0.1, respectively. The thin wall quartz EPR sample tube (710-SQ-250M, 5mm, Wilmad-Labglass) was filled with 20 mg of the radical-grafted silica samples, and 3 mg of TEMPO was selected as a standard sample for calculating the concentration of radical-embedded SiO_2 NPs based on the mass and concentration of TEMPO in DNP experiments (see equations 1–3). The g-factor and A-value were calculated using the 'pepper' fitting function employing the EASYSPIN program.[3] EASYSPIN was operated in MATLAB R2022a (MathWorks, CA, USA).

The integral of TEMPO (60 mg) was calculated using 3 mg of TEMPO and the difference in the amplification.

$$S_{TEMPO\_60mg} = \frac{S_{TEMPO\_3mg} \times 8 \times 60}{0.1 \times 3} \qquad (1)$$

The integral of the radical-embedded SiO_2 NPs (60 mg) was estimated by equation:

$$S_{radical\_60mg} = \frac{S_{radical\_20mg} \times 60}{20} \qquad (2)$$

The concentration of radical-embedded SiO_2 NPs is calculated using the equation:

$$C_{radical\_60mg} = \frac{60 \times S_{radical\_60mg}}{S_{TEMPO\_60mg} \times M_{TEMPO} \times V_{sample}} \qquad (3)$$

where $C_{radical\_60mg}$ is the concentration of radical-grafted SiO_2 NPs; M_{TEMPO} is the molecular weight of TEMPO (156.25 g/mol), S_{TEMPO_3mg} = 3711, and V_{sample} is the volume of all samples for DNP experiments (40×10^{-6} L).

Solid-state ^{29}Si NMR spectroscopy of the four samples was performed using a HPDEC pulse sequence on a Bruker 400 MHz NMR spectrometer equipped with a 4 mm MAS probe (Bruker Biospin, Billerica, MA) operated at a spinning rate of 10 kHz. Spectra were obtained using $\pi/2$ pulse excitations, and the pulse strength was $\gamma B_1/2\pi = 62.5$ kHz. A total of 1024 transients were obtained, and the time delay between each transient was 10 s. For each transient, 3020 data points were obtained for an acquisition time of 47.9 ms. During the acquisition, SPINAL64 ^{1}H decoupling was applied with a pulse strength of $\gamma B_1/2\pi = 104.2$ kHz. The raw data were zero-filled to 8192 complex data points. An exponential window function with 100 Hz line broadening was applied to the overall solid NMR spectra using TOPSPIN 3.7 prior to Fourier transformation. For calibration, the octakis(trimethylsiloxy)octa-silsesquioxane signal at 11.51 ppm was used as the reference for ^{29}Si chemical shifts.

DNP experiments

The home-built DNP system was configured in a manner similar to that in a previous report.[4] SiO_2 NP samples (60 mg) were prepared inside 5 mm NMR tubes, which were shortened by $\sim$15 mm. Next, 40 μL of 30 mM TEMPO or 40 μL of DMSO/D_2O (1:1 v/v) solution was added to the NMR tube and mixed thoroughly. An NMR tube (loaded with a given sample) was affixed to the bottom of a carbon pipe before loading in the microwave-active region of the DNP instrument. During polarization, the temperature was maintained at 3.3 K, and an optimal microwave frequency of 140 GHz was used for irradiation at a maximum power of 500 mW. The enhanced ^{29}Si MR signal was monitored with a small flip-angle (10°) pulse for 66 min with a 6 min delay between pulses. Afterward, the microwave was turned off, and the decay experiments were conducted with 30° pulses for 66 min, using a 6 min delay between the pulses. Thermal polarization was measured using the same setup as used for DNP experiments. The temperature was kept at 3.3 K, and the samples were incubated for 66 min to reach the thermal equilibrium before applying a 90° hard pulse. All DNP data were analyzed using the TNMR program (Tecmag Inc., TX, USA) and the OriginPro 2016 program (OriginLab Corp., MA, USA).

Density of radical in each radical grafted SiO_2 NPs

The density of radical in each sample was calculated based on the equation below.

$$\text{density of radical} = \frac{\text{\# radical molecules}}{V_{\text{embedding partial}}} \sim \frac{1}{V_{\text{embedding partial}}} \tag{4}$$

where # radical molecules are number of radical molecules in 1 nanoparticle, $V_{\text{embedding partial}}$ is partial volume containing the radicals in 1 nanoparticle. For example, $V_{\text{HOMO}} = 268,082$ nm^3, $V_{\text{SHELL}} = 154,985$ nm^3, and $V_{\text{CORE}} = 113,097$ nm^3.
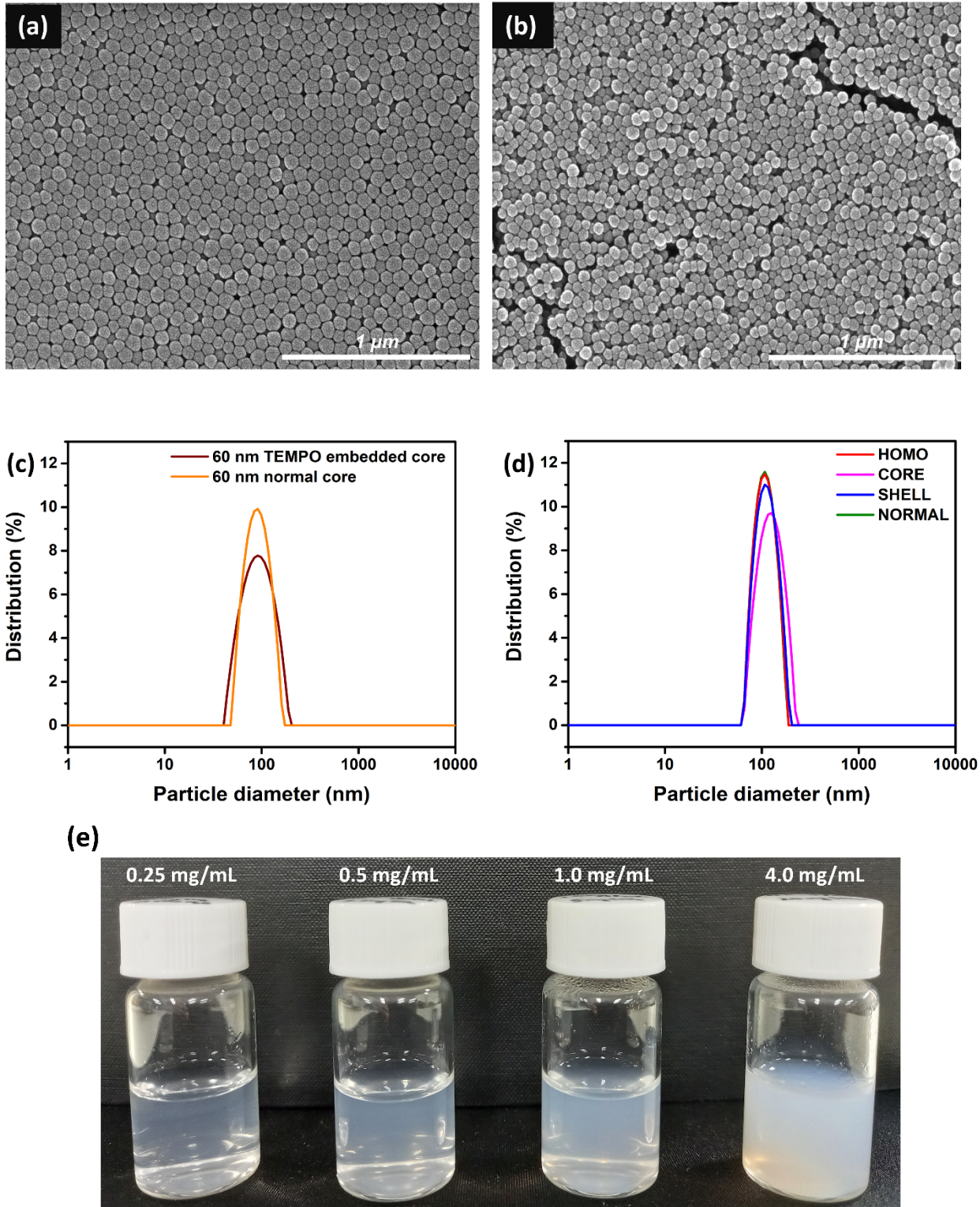
Fig. S1 SEM image of a) 60 nm normal core and b) 60 nm TEMPO-embedded core. DLS spectra of c) 60 nm normal core and 60 nm TEMPO-embedded core and d) 80 nm radical-grafted silica NPs and e) The colloidal solutions of the NORMAL sample with four different particle concentrations (0.25, 0.5, 1.0, and 4.0 mg/mL from left to right, respectively).
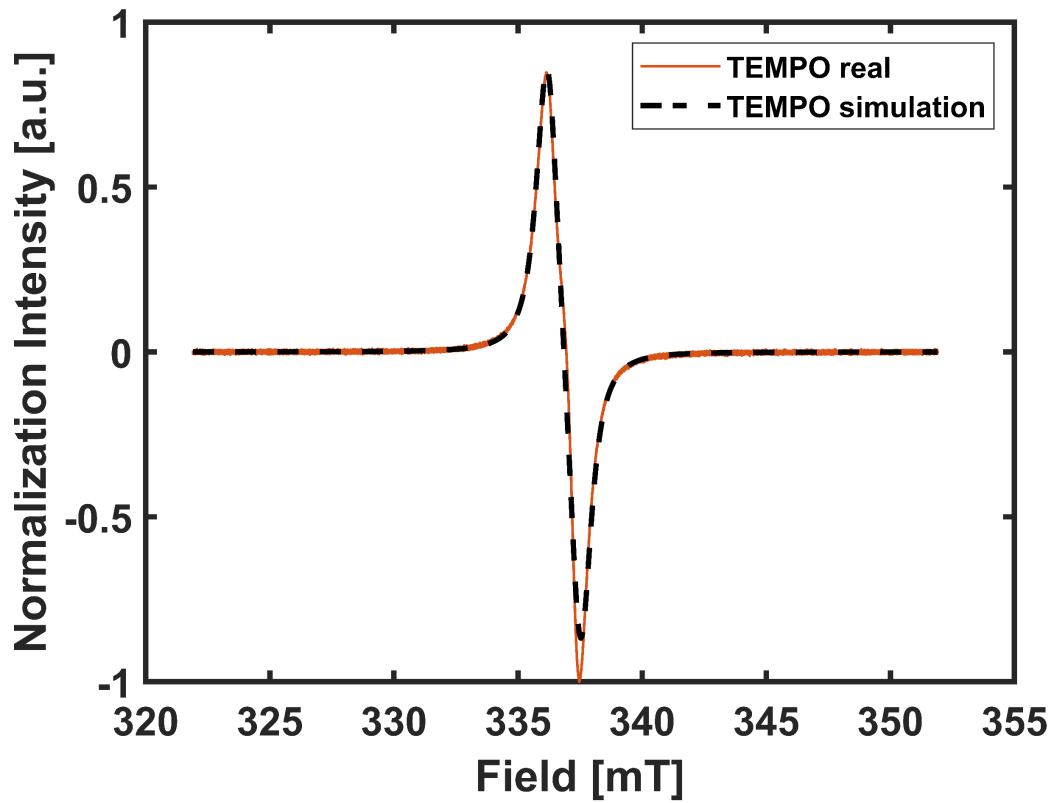
Fig. S2 EPR spectrum of TEMPO radical powder (solid line). Simulated spectrum of the sample is displayed with the dashed line.
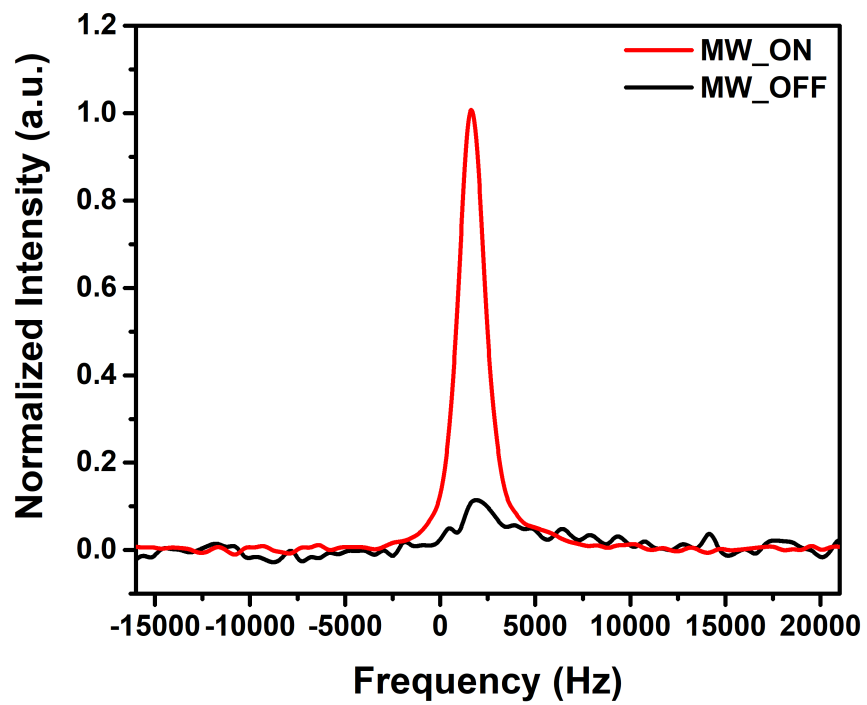
Fig. S3 Hyperpolarized spectra of NORMAL sample measured with microwave on and off at 3 K. Signal intensity in the spectrum with the microwave off was scaled up 2 times.

Table S1. Size information of all silica NP samples determined by SEM and DLS analysis.

Sample	Size_SEM (nm)	RSD (%)	Size_DLS (nm)	PDI
NORMAL	81.5 ± 6.5	8.0	111.0 ± 2.9	0.074
HOMO	82.8 ± 3.9	4.7	111.7 ± 1.6	0.094
60 nm TEMPO-embedded core	58.0 ± 4.9	8.4	95.5 ± 4.5	0.184
CORE	78.5 ± 6.9	8.8	126.4 ± 0.9	0.133
60 nm normal core	62.5 ± 4.6	7.4	93.6 ± 1.3	0.106
SHELL	80.9 ± 5.6	6.9	114.4 ± 3.0	0.078

Table S2. Summary of solid-state NMR spectra of the Q_3 and Q_4 band values in all silica NP samples.

Sample	Chemical shift, δ (ppm)		FWHM (Hz)		Q_4/Q_3 intensity ratio
	Q_3	Q_4	Q_3	Q_4	
NORMAL	-99.7	-108.4	724.7	631.9	1.1
HOMO	-99.3	-108.0	941.8	747.1	1.1
CORE	-99.6	-108.1	735.1	709.4	1.4
SHELL	-99.5	-108.6	743.1	629.7	1.2

Table S3. EPR analysis of all silica NP samples.

Sample	g_x	g_y	g_z	A_x (G)	A_y (G)	A_z (G)
HOMO	2.0028	2.0012	1.9977	6.2	11.5	37.0
CORE	2.0034	2.0014	1.9978	3.0	12.5	36.9
SHELL	2.0026	2.0016	1.9982	2.8	14.1	37.1
TEMPO	2.0010	2.0010	2.0010	-	-	-

Table S4. Estimated radical concentrations and number of radicals embedded in silica NPs.

Sample	Area	Radical concentration (mM)	Number of radicals
HOMO	7491	32.95	7.94×10^{17}
CORE	7533	33.14	7.98×10^{17}
SHELL	6787	29.86	7.19×10^{17}

Table S5. Summary of DNP parameters of all silica NP samples.

Sample	$t_{build-up}$	T_1 (min)	ε	Relative ε
NORMAL	83	65	19.9	1.0
HOMO	22	14	49.4	2.5
CORE	16	8	14.1	0.7
SHELL	36	31	17.0	0.8

References

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