

# Silicon Nanoparticles as Hyperpolarized Magnetic Resonance Imaging Agents

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The use of nanoparticles for biomedical applications has benefited from rapid progress in nanoscale synthesis of materials with specific optical<sup>1–3</sup> and magnetic properties,<sup>4</sup> as well as biofunctionalization of surfaces, allowing targeting,<sup>5–7</sup> *in vivo* tracking,<sup>1,7,8</sup> and therapeutic action.<sup>3,9</sup> Porous silicon nanostructured materials are of interest for molecular and cell-based biosensing, drug delivery, and tissue engineering applications.<sup>10,11</sup> For magnetic resonance imaging (MRI), superparamagnetic nanoparticles have extended susceptibility-based contrast agents toward targeted imaging,<sup>4</sup> though achieving high spatial resolution with high contrast remains challenging, especially in regions with natural magnetic susceptibility gradients. An alternative approach is direct MRI of hyperpolarized materials with little or no background signal. Hyperpolarized noble gases<sup>12–14</sup> and <sup>13</sup>C-enhanced biomolecules<sup>15,16</sup> have demonstrated impressive image contrast, but are limited by short *in vivo* enhancement times (~10 s for noble gases,<sup>12</sup> ~30 s for <sup>13</sup>C biomolecules<sup>15,16</sup>).

Nuclear magnetic resonance (NMR) in silicon has been widely investigated for half a century<sup>17</sup> and with renewed interest recently in the context of quantum computation.<sup>19</sup> It is known that bulk silicon can exhibit multihour nuclear spin relaxation ( $T_1$ ) times at room temperature<sup>17</sup> and can be hyperpolarized *via* dynamic nuclear polarization (DNP).<sup>18</sup> The low natural abundance of spin- $1/2$  <sup>29</sup>Si nuclei (4.7%) embedded in a lattice of zero-spin <sup>28</sup>Si nuclei isolates the active nuclear spins from one another and

**ABSTRACT** Magnetic resonance imaging of hyperpolarized nuclei provides high image contrast with little or no background signal. To date, *in vivo* applications of prehyperpolarized materials have been limited by relatively short nuclear spin relaxation times. Here, we investigate silicon nanoparticles as a new type of hyperpolarized magnetic resonance imaging agent. Nuclear spin relaxation times for a variety of Si nanoparticles are found to be remarkably long, ranging from many minutes to hours at room temperature, allowing hyperpolarized nanoparticles to be transported, administered, and imaged on practical time scales. Additionally, we demonstrate that Si nanoparticles can be surface functionalized using techniques common to other biologically targeted nanoparticle systems. These results suggest that Si nanoparticles can be used as a targetable, hyperpolarized magnetic resonance imaging agent with a large range of potential applications.

**KEYWORDS:** silicon nanoparticle · contrast agent · hyperpolarized · molecular imaging · functionalized nanoparticle · magnetic resonance imaging (MRI) · nuclear magnetic resonance · nuclear spin relaxation

from the environment, leading not only to long  $T_1$  times, but also decoherence ( $T_2$ ) times of up to tens of seconds.<sup>19</sup> Moreover, the weak dipole–dipole coupling of the sparse <sup>29</sup>Si atoms, together with the isotropic crystal structure and the absence of nuclear electric quadrupole moment conspire to keep any induced nuclear polarization aligned with even very weak external fields as the nanoparticle tumbles in space, which occurs, for instance, in fluid suspensions.

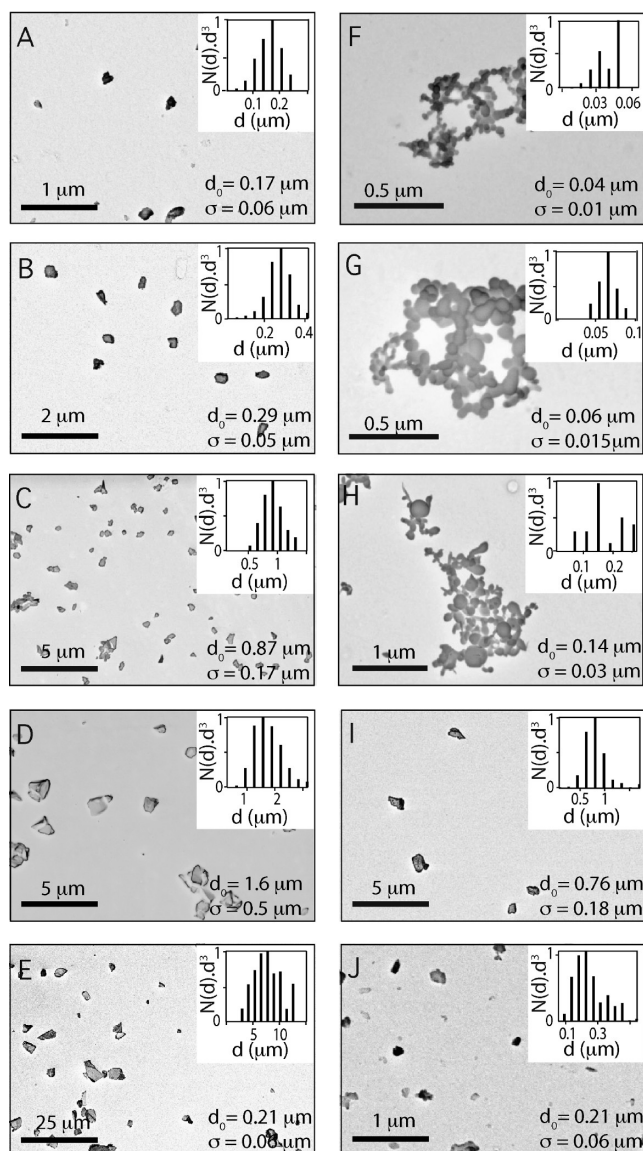
This paper investigates in detail two critical properties of Si nanoparticles for their use as targetable hyperpolarized MRI imaging agents. First, we demonstrate for the first time that Si nanoparticles retain long  $T_1$  times at room temperature into the submicrometer regime, and investigate how  $T_1$  depends on size for a variety of commercial and ball-milled Si nanoparticles. This dependence is compared to a model of nuclear spin

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**Figure 1.** Sizes and shapes of silicon particles. Electron micrographs of Si nanoparticles (A–E) ball milling high-resistivity silicon wafer, (F–G) wet synthesis (Meliorum), (H) plasma synthesis (MTI), (I) electrical explosion (NanoAmor), and (J) ball milling low-resistivity wafer. Insets: Volume-weighted histograms of diameters following size segregation along with averages  $d_0$  and standard deviations  $\sigma$  based on Gaussian fits to distributions.

diffusion,<sup>18</sup> yielding reasonable consistency between theory and experiment. Second, we demonstrate that long- $T_1$  Si nanoparticles can be surface functionalized by methods similar to those used to prepare other targeted nanoparticle systems.<sup>20,21</sup>

## RESULTS

**Particle Characterization.** Particle size determines regimes of application to biomedicine<sup>22</sup> as well as NMR properties.<sup>18</sup> We investigated room-temperature NMR properties of Si particles spanning four orders of magnitude in mean diameter, from 40 nm to 1 mm. Particles were made by various methods, including ball-milling of nominally undoped (high-resistivity 30–100 k $\Omega$ -cm) and highly doped (low-resistivity 0.01–0.02  $\Omega$ -cm)

commercial silicon wafers, followed by segregation by size (see Methods). We also investigated chemically synthesized Si nanoparticles with mean diameters 40 nm (wet synthesis, 99.99% elemental purity, Meliorum Corp.), 60 nm (wet synthesis, 99.99% elemental purity, Meliorum Corp.), 140 nm (plasma synthesis, 99% elemental purity, MTI Corp.), and 600 nm (electrical explosion synthesis, 98% elemental purity, Nanostructured & Amorphous Materials, Inc.), obtained commercially. Figure 1 shows representative scanning electron microscope (SEM) images of all measured particles, along with volume-weighted size distributions obtained by SEM image analysis.

**<sup>29</sup>Si NMR Measurements.** Nuclear  $T_1$  times of dry Si nanoparticles were measured at room temperature at a magnetic field of 2.9 T using a saturation-recovery NMR pulse sequence with repeated spin–echoes for signal enhancement (see Methods). Values for  $T_1$  are extracted from exponential fits,  $A \propto 1 - \exp(-\tau_{\text{pol}}/T_1)$ , to the Fourier amplitude,  $A$ , of the free induction decay (FID) and echoes as a function of polarization time,  $\tau_{\text{pol}}$  (see Figure 2, inset). Figure 2 shows  $T_1$  as a function of volume-weighted average particle diameter for the various samples, as well as a shell–core nuclear spin diffusion model,<sup>18</sup> which has no free parameters. The model assumes  $T_1$  is determined by nuclear spin diffusion to the particle surface, where nuclear spin is quickly relaxed. Undoped ball-milled samples follow a roughly linear dependence on size,  $T_1 \propto d_0$ , for  $d_0 < \sim 10$   $\mu\text{m}$ , saturating at  $T_1 \sim 5$  h for larger particles. The trend of increasing  $T_1$  in larger particles is qualitatively consistent with the shell–core model, and suggests that  $T_1$  is governed by surface relaxation. Electron spin resonance (ESR) measurements (see Supporting Information S1) show a single peak corresponding to a  $g$ -factor of  $g = 2.006$ , characteristic of  $P_b$ -type defect centers at the Si–SiO<sub>2</sub> interface.<sup>23</sup> The shift toward lower  $T_1$  compared to the core–shell model presumably reflects relaxation within the core, which can be attributed to defects and strain induced either by ball milling<sup>24</sup> or noncrystallinity, depending on the method of synthesis. The highly doped ball-milled particles have  $T_1 \approx 200$  s, independent of size. Here  $T_1$  is shortened due to relaxation by free carriers. Smaller commercial particles formed by wet synthesis (>99.99% elemental purity, Meliorum) and plasma synthesis (>99% elemental purity, MTI) have  $T_1$  times as long as 700 s, exceeding the predictions of the core–shell model. Larger commercial particles formed by electrical explosion (>98% elemental purity, NanoAmor) have shorter  $T_1$  than the comparably sized high-resistivity ball-milled particles.

We have also measured the inhomogeneous dephasing times,  $T_2^*$ , as a function of mean particle diameter for undoped ball-milled samples at 4.7 T (using a Bruker DMX-200 NMR console).  $T_2^*$  ranges from 0.3 ms for  $d_0 \sim 0.2$   $\mu\text{m}$  to 1.8 ms for  $d_0 \sim 1000$   $\mu\text{m}$ . We note that while  $T_1$  changes by two orders of magni-

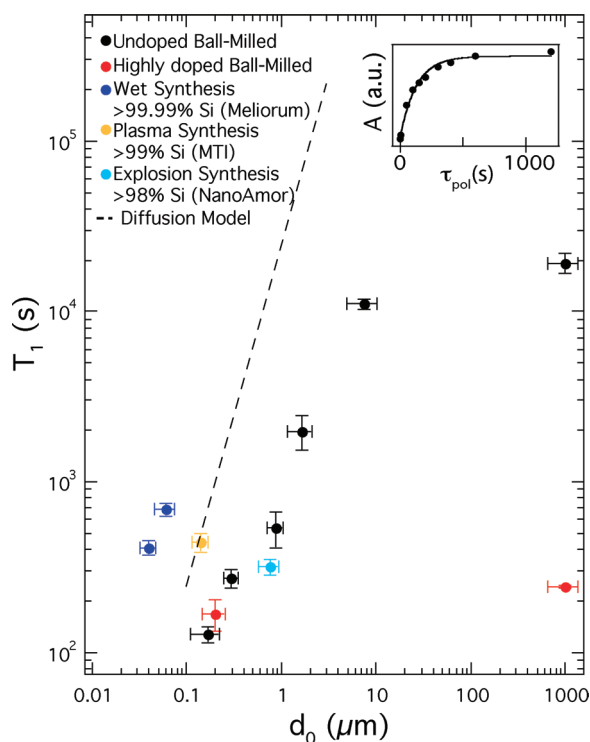
tude over the range of measured particle sizes,  $T_2^*$  changes only by a factor of  $\sim 6$ .

**MRI of Hyperpolarized Si Nanoparticles.** A first demonstration of imaging hyperpolarized Si nanoparticles is shown in Figure 3. A phantom in the shape of the letter H was filled with undoped ball-milled particles ( $d_0 = 1.6 \mu\text{m}$ ) and allowed to equilibrate at low temperature (4.2 K) and high field (5 T) for 60 h,<sup>15</sup> which enhanced the nuclear spin polarization a factor of  $\sim 16$  compared to room-temperature polarization at that field. The sample was then removed and imaged at room temperature at 4.7 T (using a Bruker DMX-200 spectrometer with a microimaging gradient set). The transfer from the low temperature environment to the imager required  $\sim 60$  s, much shorter than the  $T_1$  of the nanoparticles. The phantom was imaged using a small-tip-angle gradient-echo sequence<sup>25</sup> with the following parameters: tip angle  $\theta = 9^\circ$ , echo time  $\tau = 1.2$  ms, field of view = 15 mm, sample thickness = 2.5 cm, single pass (no averaging), acquisition time = 11 s. The resulting image is shown in Figure 3B. MRI of the same sample equilibrated in the field of the imager at room temperature yielded no detectable image.

**Surface Functionalization.** To examine the applicability of Si nanoparticles to targeted MRI, we prepared the Si nanoparticle surface for attachment to biological-targeting ligands. Nanoparticles were aminated using either (3-aminopropyl)triethoxysilane (APTES) or a 1:2 mixture by volume of APTES with bis-(triethoxysilyl)ethane (BTEOSE) or (3-triethoxysilyl)propyl methylphosphonate (THPMP) (see Figure 4A and Methods).<sup>26</sup> Results are shown for ball-milled high resistivity nanoparticles ( $d_0 = 200$  nm). Successful amination was assessed using fluorescence spectroscopy (Figure 4B). The high level of fluorescence observed for aminated particles results from the covalent bonding of surface amino groups with fluorescamine, showing these functional groups were accessible for further reaction.

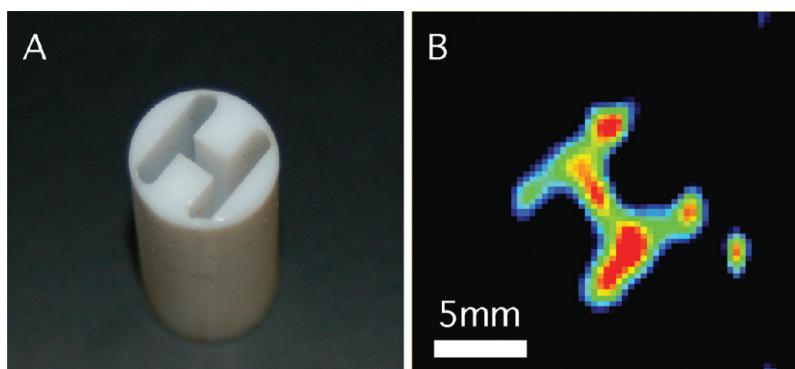
In addition to chemical assays, the accumulation of amines was indirectly monitored by measuring the surface charge of the particles in solution, or zeta potential ( $\zeta$ )<sup>27</sup> (Figure 4C). The surface of the unmodified silicon nanoparticles is composed of hydroxyl groups from the silicon dioxide and thus shows a negative zeta potential. Particles treated with APTES have surfaces coated with propylamines, which become protonated and positively charged in acidic solutions and show a positive zeta potential.<sup>27</sup>

Aminated particles were coated with polyethylene glycol (PEG) polymers to confer stability and biocompatibility. PEG coating of silica and iron-oxide nanoparticles has been shown to be nontoxic<sup>28</sup> and to reduce the rate of clearance by organs such as the liver

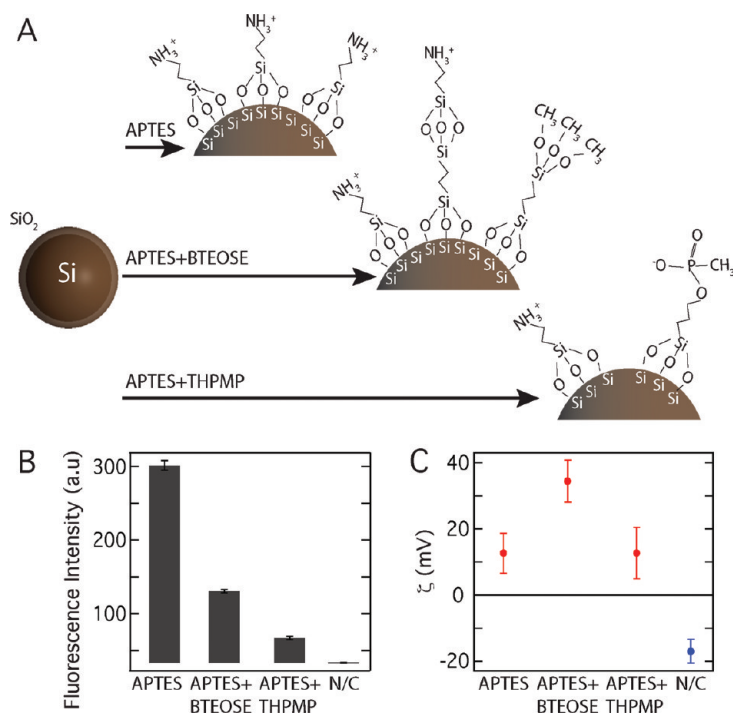


**Figure 2.** NMR Properties of Silicon Particles. Nuclear spin relaxation ( $T_1$ ) times at 2.9 T as a function of particle diameter  $d_0$  for various Si particles. Vertical error bars are from exponential fits to relaxation data; horizontal error bars are  $\sigma$  of size distributions (see Figure 1). Inset: Fourier-transform NMR peak amplitude,  $A$ , as a function of polarization time  $\tau_{\text{pol}}$  for the ball-milled high-resistivity particles with  $d_0 = 0.17 \mu\text{m}$ .  $T_1$  values were measured using a saturation recovery spin echo pulse sequence described in the text.

or kidneys, thus increasing the particle's circulation time *in vivo*.<sup>28</sup> Pegylation was performed with either  $\alpha$ -methyl-PEG-succinimidyl  $\alpha$ -methylbutanoate (mPEG-SMB) (Nektar) or maleimide-PEG-*N*-hydroxysuccinimide (MAL-PEG-NHS) (Nektar) (see Methods section). Both SMB and NHS are reactive with amines on the particle surface. The stability of nanoparticles in solution was assessed using both dynamic light scattering (DLS) (Nano ZS90, Malvern) as a measure of the particles' hydrody-



**Figure 3.**  $^{29}\text{Si}$  Magnetic resonance imaging of hyperpolarized Si nanoparticles. (A) An H-shaped phantom filled with high-resistivity Si particles ( $d_0 = 1.6 \mu\text{m}$ ) prepolarized at low temperature ( $T = 4.2$  K) and high magnetic field ( $B = 5$  T) for 60 h and warmed and transferred to a 4.7 T imager. (B) Single  $^{29}\text{Si}$  image of phantom in panel A. See text for imaging details. No  $^{29}\text{Si}$  image could be obtained without hyperpolarization using the same sequence.



**Figure 4.** Biological surface modification of silicon nanoparticles. (A) Silicon particles ( $d_0 = 0.2 \mu\text{m}$ ) were aminated using either (3-aminopropyl)triethoxysilane (APTES) alone or as a 1:2 mixture by volume of APTES with bis-(triethoxysilyl)ethane (BTEOSE) or (3-trihydroxysilyl)propyl methylphosphonate (THPMP in  $\text{H}_2\text{O}$ ). (B) Fluorescence spectroscopy confirmed the success of the amination reaction. No fluorescence was evident with the negative control (N/C). (C) A change in the sign of the surface charge, or zeta potential of the particles was evident after amination with the three amine groups (red) when compared to the negative control (blue).

nanic radius, and visual determination of flocculation and sedimentation. The particles treated with mPEG-SMB and NHS-PEG-MAL were both stable in phosphate-buffered saline (PBS) for a period of two days with no significant change in the hydrodynamic radius (see Supporting Information, S2). As a control, mPEG-amine polymer, which does not contain amine-reactive groups, was used. The aminated particles treated with mPEG-amine aggregated after centrifugation and resuspension in PBS. These results are consistent with

## METHODS

**Nanoparticle Preparation and Size Separation.** Nominally undoped float-zone grown Si wafers (Silicon Quest International) were (111) oriented, with residual p-dopants and nominal resistivity 30–100  $\text{k}\Omega\text{-cm}$ , depending on batch. Highly doped wafers (Virginia Semiconductor) were Czochralski grown, (100) oriented, boron-doped (p-type), with nominal resistivity 0.01–0.02  $\Omega\text{-cm}$ .

Ball-milled particles were processed as follows. Whole wafers were shattered using a mortar and pestle. Batches of 8.5 g wafer shards were dry ground for 10 min at 400 rpm in a planetary ball mill (Retsch PM100) using 10 1-cm diameter zirconia balls. The resulting powder was mixed with 20 mL of ethanol and milled under similar conditions for another 3.8 h. For a final milling, also at 400 rpm, 50 3-mm diameter zirconia balls were used. The slurry was milled for times ranging from 1 to 26 h, to give an approximately uniform size distribution between 100 nm and 1  $\mu\text{m}$ . The ball-milled silicon nanoparticles in ethanol were sepa-

other reports of the successful pegylation of  $\text{SiO}_2$  nanoparticles.<sup>29,30</sup>

## DISCUSSION

We have demonstrated several key features of Si nanoparticles that establish their potential as a hyperpolarized imaging agent for MRI, including long nuclear relaxation times and receptivity to surface modification with biologically compatible ligands. Room temperature nuclear relaxation ( $T_1$ ) times for all measured particles were found to be considerably longer than those of previously reported hyperpolarized MRI imaging agents,<sup>12,14–16</sup> in the range of tens of minutes to hours. Moreover,  $T_1$  in the Si system can be tuned by size and doping, allowing optimization for specific applications in biomedical imaging. We examined  $T_1$  as a function of diameter for particles made by ball-milling undoped silicon wafers as well as chemically synthesized nanoparticles. Preliminary measurements on other surface-functionalized silicon nanoparticles<sup>31</sup> indicate that the functionalization process does not reduce the nuclear  $T_1$  of the particles. MRI of Si nanoparticles was demonstrated at modestly enhanced polarization using low-temperature equilibration. While these polarizations are presumably too small for practical use, the results demonstrate that nanoparticles can be successfully transported through large magnetic and temperature gradients without a significant loss of an enhanced polarization. Significantly higher nuclear polarizations (exceeding  $10^4$  times room-temperature equilibrium polarization at 3T) are expected using DNP, with corresponding improvements in image resolution and contrast.<sup>12–16</sup> Optimizing DNP to achieve high polarization will be the subject of future work. The demonstrated coatings with APTES and PEG are important steps for further surface functionalization and, ultimately, biological targeting. In conclusion, the data presented here are necessary for establishing the utility of Si nanoparticles as a flexible platform for imaging agents in MRI.

rated by size using a centrifugational sedimentation process. Parameters were calculated using the Stokes equation.<sup>34</sup> From repeated sonication and centrifugal separation, a number of discrete particle size groups could be obtained.

**Scanning Electron Microscopy and Size Characterization.** Scanning electron microscopy and particle-measuring software (Gatan Digital Micrograph) were used to determine the size distributions of the nanoparticles. Dilute suspensions of silicon nanoparticles in ethanol were sonicated for 10 mins before being pipetted onto a vitreous carbon planchett which was mounted on a standard specimen holder with conducting carbon tape. An acceleration voltage of 2 kV was used. For each sample, >1000 particles were analyzed, sourced from ~50 images. Particle agglomeration seen in dry Meliorum and MTI samples has been reported in similarly sized silica nanoparticles,<sup>29</sup> but is not expected to occur after pegylation. In these cases (Meliorum, MTI), individual measurement of the particle diameter from SEM images was used instead of software analysis.

**$T_1$  Measurements.** Nuclear  $T_1$  times of the Si nanoparticles, segregated by size and packed dry in Teflon NMR tubes, were measured at room temperature at a magnetic field of 2.9 T using a spin-echo Fourier transform method with a saturation recovery sequence. Following a train of 16 hard  $\pi/2$  pulses to null any initial polarization, the sample was left at field to polarize for a time  $\tau_{\text{pol}}$ , followed by a Carr-Purcell-Meiboom-Gill (CPMG) sequence  $(\pi/2)_x - [\tau - (\pi)_y - \tau - \text{echo}]^n$  with  $\tau = 0.5$  ms and  $n = 200$ . In Si and other nuclear-dipole-coupled materials echo sequences can yield anomalously long decay tails.<sup>33</sup> However, the Fourier amplitude of the echo train still provides a signal proportional to initial polarization.<sup>33</sup> Values for  $T_1$  are extracted from exponential fits,  $A \propto 1 - \exp(-\tau_{\text{pol}}/T_1)$ , to the amplitude,  $A$ , of Fourier transform of the echo train for 200 echoes as a function of polarization time (see Figure 2a, inset for an example).

**Amination.** Amination was performed using either (3-aminopropyl)triethoxysilane (APTES, Sigma, 99%) alone or as a 1:2 mixture by volume of APTES with bis-(triethoxysilyl)ethane (BTEOSE, Aldrich, 96%) or (3-triethoxysilyl)propyl methylphosphonate (THPMP, Aldrich, 42 wt % in H<sub>2</sub>O). The surface oxide was first etched with a dilute solution of hydrofluoric acid (8% in ethanol) followed by resuspension of the particles in ethanol. Approximately 100 mg of silicon nanoparticles were added to 45 mL of acidified 70% ethanol (0.04% v/v, adjusted to pH 3.5 with HCl) or methanol buffer (0.1 mM NaHCO<sub>3</sub> in methanol), and the solution was placed in an ultrasonic bath for 5 mins. Saline (0.10–0.15 M) was then added and the solution was shaken for 18–24 h. Silanes were removed from the nanoparticle solution by washing and resuspending three times in methanol buffer, with the final resuspension performed with 10 mL of ethanol or methanol buffer.

**Fluorescamine Assay.** The concentrations of all of the particles were equalized by adjusting their absorption at 420 nm using a spectrophotometer (SpectraMax Plus, Molecular Devices). The fluorescamine reagent was prepared by dissolving 3.5 mg of fluorescamine (Sigma) in 1 mL of dimethyl sulfoxide (DMSO). Within a 96-well standard opaque tray, 10  $\mu\text{L}$  of the fluorescamine solution were added simultaneously to each well containing 40  $\mu\text{L}$  of nanoparticles and mixed thoroughly for 1 min. Fluorescence was measured using an excitation at 390 nm and emission at 465 nm (SpectraMax Gemini XPS, Molecular Devices).

**Pegylation.** A 10 mg portion of PEG was mixed in 500  $\mu\text{L}$  of methanol buffer and heated briefly at 50 °C to dissolve. Approximately 0.1 mg of aminated particles (100  $\mu\text{L}$  in solution) were added to this solution and it was placed in an ultrasonic bath for 1–3 h. To remove the unreacted PEG, samples were centrifuged and resuspended twice in methanol and finally in a phosphate-buffered saline solution (PBS, 0.1 M Na<sub>2</sub>HPO<sub>4</sub>, 0.015 M NaCl buffer).

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**Supporting Information Available:** Electron spin resonance measurements, particle stability following PEGylation. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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# Supporting Information - Silicon Nanoparticles as Hyperpolarized Magnetic Resonance Imaging Agents

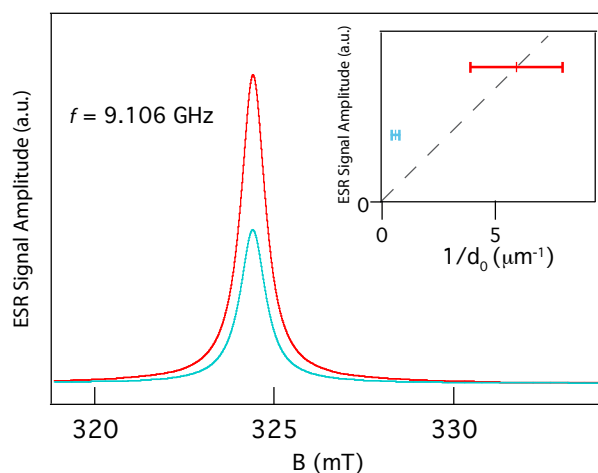
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## S1. Electron Spin Resonance Measurements

Continuous wave electron spin resonance (cw-ESR) measurements were taken on bulk samples of particles using a JEOL **FE-3XG** X-Band spectrometer at a frequency of 9.106 GHz. The a.c. field (amplitude 0.01mT,  $f_{mod} = 100$  kHz) was swept from 315 mT to 335 mT over a period of 30 s. For each sample, a single peak at  $B = 324$  mT, corresponding to a g-factor of 2.006 was recorded. This is consistent with the reported g-factor of  $P_b$  defects at the silicon-silicon dioxide interface.<sup>2</sup> ESR spectra of ball milled silicon particles with sizes  $0.17 \mu\text{m}$  and  $1.6 \mu\text{m}$ . are shown in Fig. S1. Curves are scaled vertically by sample weight, giving a measure of density of electron spins. Smaller particles have greater defect density, scaling roughly as the inverse diameter (inset, Fig. S1), suggesting that the defects are on the surface of the nanoparticle.<sup>1</sup>



**Figure S1 - Electron spin resonance measurements of silicon particles.** Weight adjusted ESR spectra of ball milled silicon particles with sizes  $0.17 \mu\text{m}$  and  $1.6 \mu\text{m}$ . Inset: ESR peak area vs inverse particle diameter.

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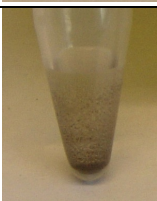


## S2. Evidence of Peylation via Stability of Particles

The aminated particles in this experiment were pegylated with either mPEG-SMB or NHS-PEG-MAL. Both SMB and NHS are reactive with amines on the particle surface. As a negative control, mPEG-Amine polymer was used because it does not contain amine-reactive groups and therefore should not conjugate to the nanoparticle surface. The stability of nanoparticles in solution was assessed using both dynamic light scattering (DLS) and visual determination of flocculation and sedimentation. The DLS-based size measurements of aminated and pegylated particles are shown in Table S1. As expected, the aminated particles treated with mPEG-Amine aggregated after centrifugation and resuspension in phosphate-buffered saline (PBS). However, the particles treated with mPEG-SMB and NHS-PEG-MAL were both stable in PBS.

Table 1: DLS size measurements showing size before and after pegylation.

Silane	PEG <sup>1</sup>	Size after pegylation (nm)		
		Measured in MeOH	Measured in PBS	After two days in PBS
APTES only	None	220 ± 88	-	-
	Amine	Aggregated	Aggregated	Aggregated
	SMB	360 ± 127	271 ± 84	260 ± 70
	NPM	240 ± 95	396 ± 126	371 ± 140
APTES & BTEOSE	None	235 ± 100	-	-
	Amine	Aggregated	Aggregated	Aggregated
	SMB	300 ± 151	314 ± 165	520 ± 200
	NPM	255 ± 100	Aggregated	326 ± 117
APTES & THPMP	None	235 ± 100	-	-
	Amine	Aggregated	Aggregated	Aggregated
	SMB	490 ± 200	295 ± 200	360 ± 200
	NPM	295 ± 126	295 ± 139	295 ± 200

Particle stability was also assessed visually, as shown in Fig. S2. These particles were pegylated in methanol, washed, and re-suspended in PBS. The particles treated with mPEG-Amine could not be re-suspended, as they had formed a large aggregate at the bottom of the tube. After two days in solution, some of the particles pegylated with mPEG-SMB and NHS-PEG-MAL had settled but immediately re-dispersed after gentle flicking.

Silane	mPEG-Amine	mPEG-SMB	NHS-PEG-MAL
(a) APTES only			
(b) APTES & BTEOSE			
(c) APTES & THPMP			

**Figure S2 - Stability of pegylated silicon nanoparticles.** Stability of pegylated particles after two days in PBS and gentle flicking, post amination with (a) APTES, (b) APTES and BTEOSE, and (c) APTES and THPMP.

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