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Triplet dynamic nuclear polarization of nanocrystals dispersed in water at room temperature

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While the dynamic nuclear polarization using photo-excited triplet electrons (triplet-DNP) can improve the sensitivity of nuclear magnetic resonance at room temperature, it has not been carried out in water. Here, we report the first example of triplet-DNP in water by downsizing the conventional bulk crystals to nanocrystals.

Nuclear magnetic resonance (NMR) and magnetic resonance imaging (MRI) are noninvasive methods to provide important structural information of molecules and spatial morphology. The major issue of NMR/MRI is the intrinsically poor sensitivity due to the low polarization of nuclear spins. For example, it is still difficult to analyze the structure and dynamics of low-concentration proteins in living cells.¹ To overcome this situation, a wealth of methods to improve the NMR/MRI sensitivity have been developed.²⁻⁴ Dynamic nuclear polarization (DNP) is one of the most powerful methods allowing various nuclear spins to be hyperpolarized. In the common DNP, organic free radicals are employed as polarizing agents, and their electron spin polarization is transferred to nuclear spin polarization of target compounds.⁵⁻¹⁸ The target molecules are hyperpolarized in glass matrix at low temperature around 1 K and rapidly dissolved to detect the enhanced signals. The method is powerful to hyperpolarize small molecules such as pyruvic acid.⁸ However, it is not suitable for large biomolecules such as proteins since the low-temperature condition tends to cause denaturation. As a complementary method to the radical-based dissolution-DNP, and towards the ultimate goal of nuclear polarization of biological substances in the living system, the development of a method to

increase nuclear polarization in water at room temperature is strongly desired.

Room-temperature DNP using photo-excited triplet electrons (triplet-DNP) has been achieved by utilizing the non-equilibrated polarization of triplet electron spins.¹⁹⁻²⁶ The typical triplet-DNP system consists of bulk organic crystals doped with polarizing agents (Fig. 1a). Photo-excitation of the polarizing agents and subsequent spin-selective intersystem crossing (ISC) result in the selective population of one specific sublevel among three sublevels of the triplet excited state. This triplet electron polarization is effectively transferred to nuclear spins by integrated solid effect (ISE) under microwave irradiation and magnetic field sweep, followed by

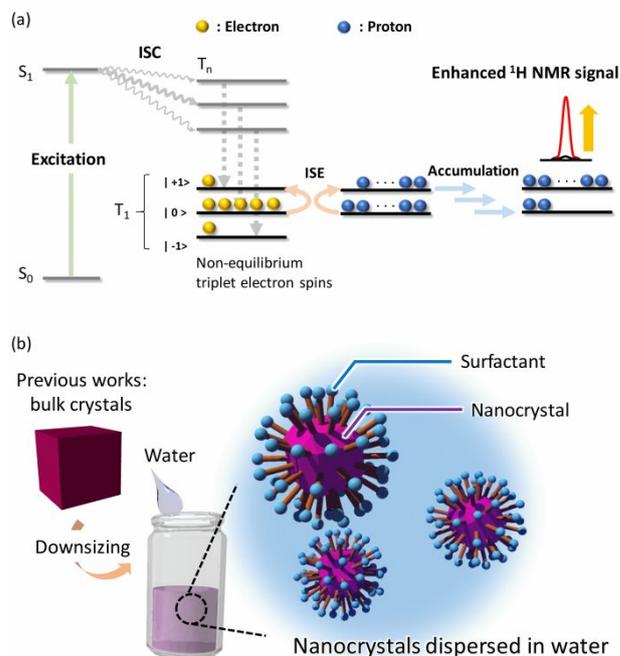


Fig. 1 (a) Typical scheme of triplet-DNP. Photo-excitation is followed by spin-selective intersystem crossing (ISC). The resulting triplet electron spin polarization is transferred to the nuclear spin through integrated solid effect (ISE) under microwave irradiation and magnetic field sweep. (b) Schematic representation of downsizing bulk crystals to nanocrystals for triplet-DNP in water.

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polarized nuclear spin diffusion to the bulk crystals.^{27,28} Since its first report in 1990,¹⁹ many efforts have been paid to increase the enhancement factor of the room-temperature triplet-DNP, and it has been applied to neutron spin filter and dissolution-DNP.^{23, 25, 26, 29} However, the nuclear polarization process using the photo-excited triplets has been limited to the solid state. This is mainly because the effective ISE and the accumulation of spin polarization through spin diffusion require the solid crystals with long spin-lattice relaxation time T_1 . In addition, paramagnetic oxygen molecules severely quench the photo-excited triplet state. Therefore, it has not been clear whether it is possible to achieve triplet-DNP in water containing dissolved oxygen molecules.

Here, we show the first demonstration of triplet-DNP in water by downsizing the conventional bulk crystals to nanocrystals (Fig. 1b). This is also the first example of triplet-DNP of nanosized crystals. As a proof-of-concept, we employ pentacene and *p*-terphenyl as benchmark polarizing agent and crystalline matrix, respectively (Fig. 2a). A stable aqueous dispersion of pentacene-doped *p*-terphenyl nanocrystals is obtained by simple ball-milling of the bulk crystals in a surfactant solution. The photo-excited triplet state of pentacene is protected against oxygen quenching in dense *p*-terphenyl nanocrystals. The triplet-DNP process of the nanocrystals in water achieves a high polarization enhancement over 360 times.

Bulk *p*-terphenyl crystals doped with 0.5 mol% pentacene were obtained by melting and cooling of a mixture of pentacene and *p*-terphenyl. The bulk crystals (1.0 wt%) in an aqueous solution of cetyltrimethylammonium bromide (CTAB, 1.4 mM) were ball-milled for 3 hours at 280 rpm. Large micrometer-sized particles were removed by slow centrifugation (6000 rpm, 5 min). Nanoparticles were then concentrated by faster centrifugation (12000 rpm, 30 min), giving a purple milky suspension (Fig. 2a, particle concentration: 5.3 wt%). The particle size was evaluated by scanning electron microscopy (SEM) and dynamic light scattering (DLS). The SEM image showed corner-round nanoparticles with an average size of 92 ± 22 nm (Fig. 2b, S2, ESI). The DLS analysis of the aqueous dispersion showed an average diameter of 151 ± 26 nm, agreeing with the SEM result (Fig. 2c). The positive and large zeta potential of $+43.5 \pm 8.9$ mV was observed for the aqueous dispersion, confirming the surface coverage of nanoparticles with cationic CTAB molecules and the

good colloidal stability. Indeed, the nanoparticles were stably dispersed over 24 hours thanks to the highly charged surfaces.

The crystallinity of *p*-terphenyl and the electronic structure of pentacene were found to be well maintained after the ball-milling. Powder X-ray diffraction (PXRD) patterns were similar for the bulk crystals and nanocrystals (Fig. 3a). The maintenance of good crystallinity was confirmed by the unchanged PXRD peak width. The good dispersibility of polarizing agent is important for achieving the high nuclear polarization because the aggregation of polarizing agent induces the electron spin relaxation.³⁰ The good dispersibility of pentacene in the *p*-terphenyl nanocrystals was confirmed by absorption, fluorescence lifetime and transient absorption measurements. It has been reported that pentacene molecules can be doped in bulk *p*-terphenyl crystals without aggregation.³¹ The absorption spectrum of pentacene in bulk *p*-terphenyl crystals exhibited π - π^* absorption bands at 507, 543 and 590 nm (Fig. S3, ESI). On the other hand, the absorption spectrum of neat pentacene powder showed red shifts to around 660 nm and peak broadening due to intermolecular interactions among aggregated pentacene molecules. The absorption spectrum of the pentacene-doped *p*-terphenyl nanocrystals in water did not show any peak shift nor broadening compared with the pentacene-doped bulk *p*-terphenyl crystals, indicating the molecularly-dispersed state of pentacene in the nanocrystals.³² The good dispersibility was confirmed by similar fluorescence lifetimes of pentacene in bulk *p*-terphenyl crystals (7.8 ns) and nanocrystals (6.7 ns) (Fig. S4, ESI).

Importantly, transient absorption measurements showed an identical pentacene triplet lifetime (83 μ s) in the bulk crystals and the nanocrystals (Fig. 3b). These results indicate that pentacene is molecularly dispersed in *p*-terphenyl nanocrystals, and the triplet state of pentacene is well protected from the paramagnetic relaxation by dissolved oxygen molecules even in the nano-sized crystals thanks to the dense *p*-terphenyl crystal structure that remained intact after the ball-milling.

Electron spin properties of pentacene were estimated by time-resolved electron spin resonance (ESR). The bulk *p*-terphenyl crystals doped with pentacene showed a high polarization of 71% and a long electron spin polarization lifetime of 21 μ s (Fig. S5, Table S1, S2, ESI), which agrees with the previous report.²¹ Unfortunately, ESR signal of the nanocrystal dispersion in water cannot be observed due to the microwave (9.1 GHz) absorption of water at the low microwave intensity (ca. 1 μ W). Instead, we measured dried nanocrystals and observed a similar high polarization and long polarization lifetime (Fig. S6, Table S1, S2, ESI).

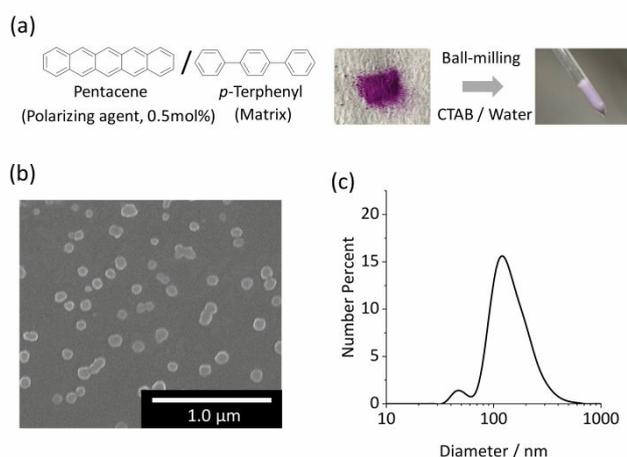


Fig. 2 (a) Chemical structures of pentacene (polarizing agent) and *p*-terphenyl (crystalline matrix). Bulk crystals of *p*-terphenyl doped with 0.5 mol% pentacene were ball-milled in an aqueous solution of CTAB (1.4 mM), resulting in an aqueous dispersion of nanocrystals. (b) SEM image of the nanocrystals. (c) DLS profile of the aqueous dispersion of nanocrystals.

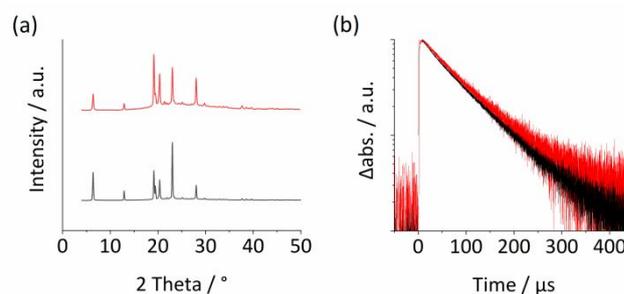


Fig. 3 (a) PXRD patterns of bulk *p*-terphenyl crystals doped with 0.5 mol% pentacene (black) and *p*-terphenyl nanocrystals doped with 0.5 mol% pentacene (red). (b) Transient absorption intensity ($\lambda_{ex} = 590$ nm, $\lambda_{det} = 540$ nm) of bulk *p*-terphenyl crystals doped with 0.5 mol% pentacene (black) and the aqueous dispersion of *p*-terphenyl nanocrystals doped with 0.5 mol% pentacene (red).

The triplet-DNP process was carried out for the aqueous dispersion of nanocrystals by using optimized ISE sequence based on the previous reports (see ESI for details).²¹ All experiments were conducted in the ambient condition. After a pulsed laser excitation at 532 nm, the electron spin polarization was transferred to ^1H spins by tuning the electron sublevels with microwave irradiation (17.7 GHz, <300 W) under the magnetic field sweep (676 mT \pm 30 mT). After repeating this triplet-DNP process for a certain duration, ^1H -NMR spectra were measured. Fig. 4a shows ^1H NMR spectra of the nanocrystal dispersion at thermal equilibrium and after the triplet-DNP process for different durations. Without the triplet-DNP process, only a sharp peak from water was observed due to the weak and broad signal of nanocrystals. Significantly, the intensity of the broad peak from nanocrystals gradually increased by elongating the triplet-DNP duration. The ^1H NMR spectra after triplet-DNP can be fitted with two Gaussian functions (Fig. 4b). The sharp and broad peaks are assignable to water and nanocrystals, respectively. The enhancement factor is defined as the signal intensity ratio between a thermal spectrum of the pentacene-doped bulk *p*-terphenyl

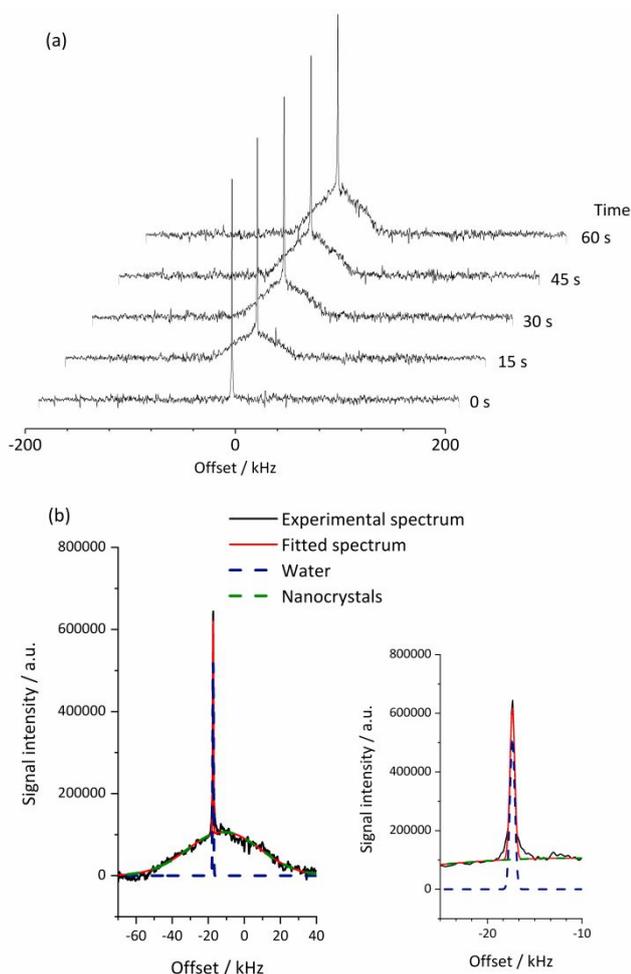


Fig. 4 (a) ^1H NMR spectra (0.676 T) of the aqueous dispersion of *p*-terphenyl nanocrystals doped with 0.5 mol% pentacene at the thermal equilibrium and after triplet-DNP process for different duration at room temperature in air. (b) The experimental NMR spectrum of the nanocrystal dispersion after 60 s triplet-DNP process (black) and fitted spectrum (red). The spectrum was separated into two components, water (blue) and the nanocrystals (green). An enlarged spectrum is also shown (right). The simulated spectrum is fitting curves with the following equation, $A \exp[-(x-B)^2/C] + D \exp[-(x-E)^2/F]$.

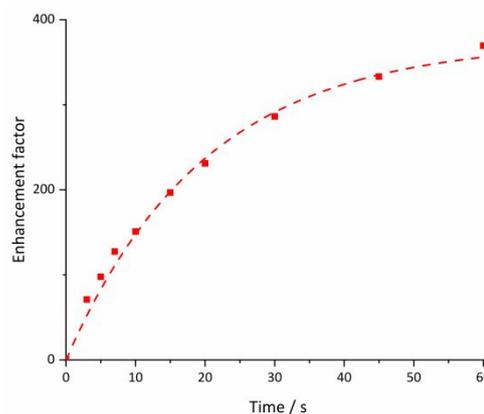


Fig. 5 ^1H polarization buildup curve of aqueous dispersion of *p*-terphenyl nanocrystals doped with 0.5 mol% pentacene at 0.676 T and room temperature. The broken line is a fitting curve with the following equation, $A[1-\exp(-t/T_B)]$.

crystals (Fig. S7, ESI) and the spectra (broad component) of aqueous nanocrystal dispersion after triplet-DNP (see ESI for details). By increasing the triplet-DNP duration to 60 s, the enhancement factor reached over 360 times (0.083%, Fig. 5). This is the first example of nuclear hyperpolarization through triplet-DNP in water at the ambient condition. While it has not been clear whether the triplet-DNP is possible in water partly due to the microwave absorption by water, we found that the moderate microwave irradiation of < 300 W is enough for the polarization transfer. The increase of sample temperature was suggested by the partial evaporation of water after the triplet-DNP process. Future optimization of microwave irradiation condition and introduction of temperature-control system would be necessary to suppress the temperature increment.³³

We also estimated the enhancement factor for the pentacene-doped bulk *p*-terphenyl crystals (Fig. S8, S9, ESI). When the duration of the triplet-DNP process is short (< 50 s), the enhancement factor of the bulk crystals is almost comparable to the nanocrystals, indicating that the polarization transfer is not almost impaired by water. After the longer triplet-DNP process (> 600 s), the bulk crystals showed a larger enhancement factor of about 1300 times (0.30%) than the nanocrystal aqueous dispersion. The buildup curves are fitted with an equation $A[1-\exp(-t/T_B)]$ (T_B : buildup time constant). The nanocrystal aqueous dispersion showed a shorter T_B of 20 s than that of bulk crystals (145 s). To understand this difference, we measured the spin-lattice relaxation time T_1 of ^1H that determines the accumulative duration of polarization. The measurements were carried out by the saturation recovery method with magic angle spinning (MAS) at the ambient condition (Fig. S10, ESI). A shorter T_1 was observed for the dried nanocrystals (22 s) than the bulk crystals (510 s). Therefore, the difference in enhancement factor and T_B between bulk crystals and nanocrystals would be due to the different T_1 values. Possible reasons for the prompted spin relaxation in the nanocrystal dispersion include the spin fluctuation caused by the flexible alkyl chains of the surfactant CTAB³⁴ and the partial structural disorder near nanocrystal surface.

In conclusion, we demonstrated the first example of triplet-DNP in water by downsizing the conventional bulk crystals to nanocrystals and also the first example of triplet-DNP of nanosized crystals. The simple ball-milling process produced the stable aqueous dispersion of pentacene-doped *p*-terphenyl nanocrystals. The photo-excited

triplet state of pentacene is well protected against oxygen quenching in the dense *p*-terphenyl nanocrystals. The significantly large polarization enhancement over 360 times was obtained for the aqueous dispersion of nanocrystals. The polarization enhancement of water ^1H was not observed in the current system. The polarization transfer from nano-diamonds to water has been reported although the resulting polarization enhancement remains low.^{35,36} Therefore, the future optimization of the nanocrystal surface structure would enable the polarization transfer from nanocrystals to surrounding water molecules, leading to selective hyperpolarization of solvent-exposed residues of proteins through proton exchange at the ambient condition.^{37,38} This work provides the important first step towards this goal.

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Conflicts of interest

There are no conflicts to declare.

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Room-temperature triplet dynamic nuclear polarization (triplet-DNP) of nanocrystals dispersed in water is achieved for the first time.

