Cocrystalline matrices for hyperpolarization at room temperature using photoexcited electrons

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ABSTRACT: We propose using cocrystals as effective polarization matrices for triplet dynamic nuclear polarization (DNP) at room temperature. The polarization source can be uniformly doped into cocrystals formed through acid–acid, amide–amide, and acid–amide synthons. The dense-packing crystal structures, facilitated by multiple hydrogen bonding and π–π interactions, result in extended $T_1$ relaxation times, enabling efficient polarization diffusion within the crystals. Our study demonstrates the successful polarization of a DNP–magnetic resonance imaging molecular probe, such as urea, within a cocrystal matrix at room temperature using triplet-DNP.

INTRODUCTION

Nuclear magnetic resonance (NMR) is a potent technique for detecting molecules’ structural and dynamic attributes. Dynamic nuclear polarization (DNP) effectively enhances NMR signal intensities.1-2 Several DNP methodologies exist, encompassing cryogenic DNP utilizing radical molecules,3-4 parahydrogen-induced polarization,5-7 spin-exchange optical pumping,8-9 and photochemically induced DNP.10-11 Hyperpolarization of molecules for magnetic resonance imaging (MRI) probes, such as pyruvic acid, urea, and glutamine, has been harnessed as chemical sensors for biological analytes in living systems.12-13

Among various DNP techniques, triplet-DNP, utilizing photoexcited triplet electrons, can yield highly sensitive NMR spectra at room temperature and low magnetic fields.14-18 This capability arises from the fact that the polarization of optically polarized triplet electrons is independent of temperature and magnetic field. Consequently, triplet-DNP eliminates the need for cryogenic liquid (e.g., liquid N$_2$ and He) and bulky, expensive millimeter-wave generators (e.g., gyrotron). However, triplet-DNP application faces limitations due to two challenges: the low dopability of the polarization source and the short longitudinal relaxation time ($T_1$) at room temperature. A lengthy $T_1$ relaxation time, extending over several tens of seconds is crucial for the diffusion and enhancement of polarization generated from the source before relaxation occurs.19-21 Recently developed polarization sources for triplet-DNP,22 have enabled the polarization of functional materials such as metal–organic frameworks,23 drugs,20 and biomolecules,23 over 70–100 times enhancement at room temperature. Conversely, few reports exist on spin polarization matrices facilitating triplet-DNP of various molecules at room temperature. Relayed triplet-DNP from polarization domains to analyte domains in eutectic crystals has enabled the polarization of some biomolecules.21 However, synthesizing eutectics formed by microdomains (<100–200 nm) is challenging, limiting their applicability to specific molecules. The development of versatile spin polarization matrices encompassing analyte molecules and polarization sources while possessing a long $T_1$ relaxation time represents an attractive challenge.

In this study, cocrystals served as matrices for spin polarization via triplet-DNP. Cocrystals, defined as crystalline single-phase solids comprising two or more distinct molecules, were employed to polarize analyte molecules within the cocrystal structure, incorporating coformers and polarization sources. Within cocrystal engineering, supramolecular synthons such as acid–acid, amide–amide, acid–amidine, and acid–pyridine act as substructural units to form crystal structures.24-26 These supramolecular synthons play a vital role in enhancing solubility, bioavailability, and tableting of drugs.27 The dense-packing crystal structures formed by these synthons contribute to
extended $T_1$ time, as multiple intermolecular interactions reduce molecular motion, subsequently minimizing $T_1$ relaxation induced by fluctuations in the local magnetic field. Maintaining crystallinity is crucial for preserving the long $T_1$ relaxation time. Our focus lies on solid solutions of cocrystals, incorporating a minimal amount of polarization source to introduce it into cocrystals without compromising crystallinity. Pentacene, chosen for its highly polarized photoexcited triplet electrons and multiple phenyl rings fostering strong $\pi-\pi$ interactions with neighboring molecules, was selected as the polarization source. In the synthon motif, acid–acid, amide–amide, and acid–amide synthons collectively form a flat hexagonal structure. The flat structures and dimensions of benzoic acid and benzamide derivatives (coformers) closely resemble those of pentacene. During the solid-solution process, pentacene is strategically replaced into the position of the coformer (Figures 1c–d).

RESULTS AND DISCUSSION

Ascertainment of the position of pentacene. The specific position of pentacene in the solid solution of pentacene and organic crystals remains unclear. Density functional theory (DFT) calculations were conducted to ascertain whether pentacene could occupy the site of the coformer with acid–acid and amide–amide synthons (homosynthons). Interaction energies between coformers (without pentacene) and neighboring coformers and between pentacene and neighboring coformers (with pentacene) were computed for the structures of benzoic acid (BA) and picolinamide (PAm), respectively. Structural models for the DFT calculation were optimized using the Gaussian 16 package (Figure 2). The interaction energies without and with pentacene were $-2.57$ and $-2.75$ eV for BA and $-3.31$ and $-2.13$ eV for PAm, respectively. Negative interaction energies indicate that pentacene is stably located at the sites of the coformers. Triplet-DNP enhanced the $^1$H NMR spectrum of the solid solution of the coformers and pentacene supports the homogeneous distribution of pentacene in the solid solutions (Figure S1).

Figure 1. Schematic representation of cocrystals as matrices for spin polarization using triplet-DNP. (a) Depicts the analyte, coformer of benzoic acid and benzamide derivatives, and pentacene as the polarization source. (b) Illustrates that most analyte molecules form crystal structures without pentacene doping. Solid solutions are shown for (c) coformer and pentacene and (d) analyte, coformer, and pentacene. (e–f) Describes the generation of photoexcited electrons through intersystem crossing (ISC) in pentacene. The electron polarization in the triplet state is transferred to the proton spin near pentacene through the integrated solid effect (ISE). Proton polarization then diffuses throughout the entire cocrystal via spin diffusion. The figure includes orange and blue spheres with arrows representing electron and proton spins, respectively.
Figure 2. DFT models for calculating interaction energy in coformer-neighboring coformers and pentacene-neighboring coformers. (a, b) represent BA clusters, and (c, d) represent PAm clusters.

**Triplet-DNP measurements of cocrystals.** Three cocrystals—salicylic acid–benzamide (SA–BAm), salicylic acid–picolinamide (SA–PAm), and 3-nitrobenzoic acid–benzamide (NBA–BAm)—were chosen for illustrating triplet-DNP in solid solutions of pentacene and coformers with acid–amide synthons (heterosynthons) (Figure 1c). BA derivatives (SA or NBA) connect BAm derivatives (PAm or BAm) through acid–amide synthons (Figures 3a–c). Stacked coformers with π–π interactions are densely packed with intermolecular forces like CH-π (SA–BAm and SA–PAm) and CH-NO2 (NBA–BAm) (Figure S2). Solid solutions of pentacene at 0.04 mol% with SA–BAm (A1), SA–PAm (A2), and NBA–BAm (A3) were prepared using the melt-quenching method. UV-Vis spectra of the solid solution show peaks at 500–600 nm attributed to pentacene, reflecting its distribution in the solid solutions (Figure S5). Interaction energies in pentacene-doped cluster models were −2.42, −2.25, and −2.24 eV for SA–BAm, SA–PAm, and NBA–BAm, respectively. The negative interaction energies support the replacement of pentacene into the position of the coformers (Figure S6).

Triplet-DNP measurements at 298 K for the solid solutions with an ISE repetition rate of 50 Hz revealed polarization ratios (P) and enhancement factors (ε) of 0.10% and 7.5 × 102 times for A1, 0.079% and 5.9 × 102 times for A2 in a 600 s triplet-DNP process, and 0.019% and 1.4 × 102 s for A3 in a 420 s triplet-DNP process, respectively (Figures 3d and e). The buildup time constant (τ) for polarization in the DNP process and T1 of the polarized magnetization were 2.7 × 102 and 7.3 × 102 s for A1, 1.2 × 102 and 1.8 × 102 s for A2, and 81 and 41 s for A3, respectively (Figure S7). The extended τ of A1 and A2 due to the prolonged T1 relaxation time, allows for high polarization.

Figure 3. Crystal structures of (a) SA–BAm, (b) SA–PAm, and (c) NBA–BAm. Hydrogen bonds between derivatives are depicted as black dotted lines. (d) 1H NMR spectra of A1, A2, and A3 under triplet-DNP and thermal conditions at 0.39 T and 298 K. (e) 1H polarization buildup curves at 298 K. Blue, red, and green represent A1, A2, and A3, respectively.

**Triplet-DNP measurements of biomolecules using cocrystal matrices.** Biomolecules, such as urea and succinic acid, were chosen as analytes. Urea serves as a DNP-MRI molecules probe for perfusion monitoring, while succinic acid plays a role in the citric acid cycle. Cocrystals of urea–nicotinamide (U–NAm) and succinic acid–nicotinamide (SuA–NAm) were selected as matrices for biomolecular polarization. Analytes form bonds with coformers, stacked through π–π interactions, and hydrogen bonds (O–NH2 in U–NAm and COOH–N in SuA–NAm), respectively (Figures 4a–c). A solid solution of 0.04 mol% pentacene with U–NAm (B1) and SuA–NAm (B2) was synthesized through the melt-quenching method. Sharp x-ray diffraction peaks in B1 and B2 indicate high solid-solution crystallinity (Figure S3). UV–Vis spectra and negative
interaction energies from DFT calculations (−2.20 and −1.20 eV for pentacene-doped U–NAm and SuA–NAm) indicate solid solutions with pentacene doped in the coformer’s site (Figures S5 and S6). In triplet-DNP experiments at 50 Hz ISE repetition rate and 298 K, B1 exhibited 0.0064%, 48 times, and 35 s for $P$, $\varepsilon$, and $\tau$, while B2 showed 0.0055%, 41 times, and 30 s for $P$, $\varepsilon$, and $\tau$, respectively (Figures 4 d and e). $T_1$ of the polarized magnetization was 18 and 38 s for B1 and B2 (Figure S7).

Stable stacked coformers binding analytes through hydrogen bonds suppress molecular motion, enabling a sufficiently long $T_1$ for polarization diffusion in solids. Triplet-DNP experiments with 100 and 200 Hz ISE repetition rates were conducted to achieve higher polarization. B1 displayed 0.0094% and 70 times for $P$ and $\varepsilon$ at 100 Hz for 90 s, and 0.014% and 102 times for $P$ and $\varepsilon$ at 200 Hz for 60 s. B2 exhibited 0.010% and 77 for $P$ and $\varepsilon$ at 100 Hz for 90 s (Figure S8). Applying a higher ISE repetition rate and longer buildup time led to sample melting due to laser irradiation heating. This experiment marks the first demonstration of triplet-DNP for an MRI molecular probe at 298 K.

Figure 4. Crystal structures of U–NAm (a) along the $c$–axis and (b) 1D chain of urea and neighboring NAm. (c) Crystal structure of SuA–NAm along the $a$–axis. Black, pink, and green dotted lines indicate hydrogen bonds in the coformer, analytes, and analyte–coformer. (d) $^1$H NMR spectra of B1 and B2 under triplet-DNP and thermal conditions at 0.39 T and 298 K. (e) $^1$H polarization buildup curves of the cocrystals at 298 K. Red and black represent B1 and B2, respectively.

Dissolution triplet-DNP measurements of cocrystals. One advantage of cocrystals is improving solubility without altering the molecular structure. The obtained cocrystals were soluble in aqueous solution and alcohol. (Table S1). Their solubility indicates suitability for dissolution triplet-DNP applications as a spin polarization matrix. We conducted dissolution triplet-DNP for A1 and A2, displaying sufficient polarization. First, after polarizing the cocrystals with 50 Hz ISE repetition rates for 420 s at 0.39 T, they were transferred to a superconducting magnet at 11.7 T (Figure S9). Second, the cocrystals dissolved in methanol at 298 K within the magnet. Finally, solution NMR experiments for the cocrystal were conducted (Figure 5). $P$ and $\varepsilon$ for solution NMR were 0.072% and 17 times for A1, and 0.034% and 8.5 times for A2, respectively. $P$ decreased by about half compared to solids (0.15% for A1 and 0.098% for A2) due to relaxation during dissolution (approximately 1–2 s). We also attempted dissolution triplet-DNP for a 0.5M Na$_2$CO$_3$ solution of A1 and A2. However, the dissolution NMR spectra were not enhanced as polarizations relaxed during dissolution over 10 s, longer than $T_1$ of SA (4.1 s), BAm (5.2 s), and PAm (6.2 s) in the 0.5M Na$_2$CO$_3$ solution. Optimizing the aqueous solution and dissolution process poses a further challenge.

Figure 5. Solution $^1$H NMR spectra of DNP-enhanced and thermal (a) A1 and (b) A2 at 11.7 T, respectively. The NMR spectra under thermal equilibrium conditions were accumulated 16 times.

Prospects for the future. Optimizing combinations of analytes and conformers could enhance the polarization ratio and broaden the range of applicable molecules. Crystal engineering and extensive crystal structural databases like the Cambridge Crystallographic Data Center facilitate this optimization. Physicochemical properties of cocrystals, such as solubility, hydration, tableting, and bioavailability, prove valuable for dissolution triplet-DNP and MRI. Conversely, in sample preparation using the melt-quenching method, all compounds should be miscible in the hot liquid state without sublimation or molecular structural changes. Analyte molecules should exhibit heat tolerance and affinity for BA and BAm derivatives. Alternative synthetic methods, including crystallization in solvents containing soluble polarization sources like 5,12-diazatetracene derivative and 6,13-diphenylpentacene, would be beneficial in obtaining cocrystals without the hot melting process.
CONCLUSIONS

In summary, we have demonstrated cocryystals as matrices for room temperature triplet-DNP. Coformers, composed of BA and BAm derivatives, act as pentacene sites in the solid solution. Multi-intermolecular interactions stabilize molecular mobility, ensuring long enough T1 relaxation times for effective polarization diffusion in solids. Our study successfully polarized biomolecules at room temperature using cocryystal matrices, making them applicable to various small molecules with hydrogen bond-capable functional groups. The high solubility of cocryystals adds value to dissolution triplet-DNP. This research contributes to developing highly sensitive MRI studies and dissolution DNP-NMR for NMR-based drug discovery screenings 40.

ASSOCIATED CONTENT

Supporting Information. Additional experimental details, synthesis, DFT calculations, measurement conditions, powder XRD data, DSC profiles, UV–Vis spectra, 1H polarization buildup curves, T1 of polarized magnetization, and solubility.

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ABBREVIATIONS

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