

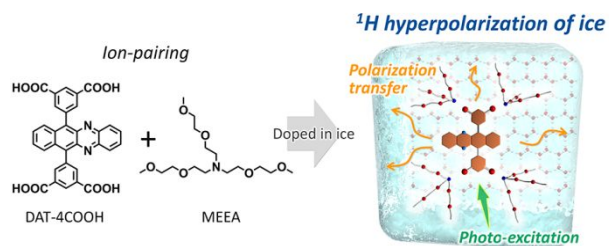


ChemComm

**Triplet dynamic nuclear polarization of crystalline ice using
water-soluble polarizing agents**

Journal:	<i>ChemComm</i>
Manuscript ID	CC-COM-02-2020-000836.R1
Article Type:	Communication

SCHOLARONE™
Manuscripts



The first example of triplet dynamic nuclear polarization of crystalline ice is demonstrated by developing a water-soluble triplet polarizing agent.

COMMUNICATION

Triplet dynamic nuclear polarization of crystalline ice using water-soluble polarizing agents

Received 00th January 20xx,
Accepted 00th January 20xx

Hironori Kouno,^a Kana Orihashi,^a Koki Nishimura,^a Yusuke Kawashima,^a Kenichiro Tateishi,^{*b}
Tomohiro Uesaka,^b Nobuo Kimizuka^{*a} and Nobuhiro Yanai^{i*a,c}

DOI: 10.1039/x0xx00000x

Triplet dynamic nuclear polarization (triplet-DNP) allows the hyperpolarization at a moderate temperature. While the triplet-DNP of water is strongly desired, water-soluble triplet polarizing agents have not been reported. Herein, the first example of triplet-DNP of crystalline ice is demonstrated by molecularly dispersing a novel water-soluble polarizing agent into ice.

Dynamic nuclear polarization (DNP) is a promising technique to overcome the sensitivity limitation of nuclear magnetic resonance (NMR) and magnetic resonance imaging (MRI).¹⁻⁹ Water works as a contrast agent in magnetic resonance angiography (MRA) for diagnosing various medical conditions such as emboli, stenosis, and aneurysms.^{10, 11} Furthermore, water is a ubiquitous polarization source for biological substances such as metabolites, peptides, and proteins. The hyperpolarization of water has been attained by using polarized electron spins of free radicals at around 1.5 K.¹²⁻¹⁴ The mixture of water and glycerol/DMSO, so-called DNP juice, was hyperpolarized at the cryogenic temperature, and then the DNP juice was dissolved and injected into the NMR tube containing biomolecules. Through proton exchange, the water hyperpolarization was transferred to biomolecules such as amino acids, peptides, and proteins.¹²⁻¹⁴ While significant polarization enhancements of biological substances have been achieved by using this dissolution-DNP method, the use of cryogenic temperatures around 1.5 K inevitably increases the cost of the instruments.¹⁵

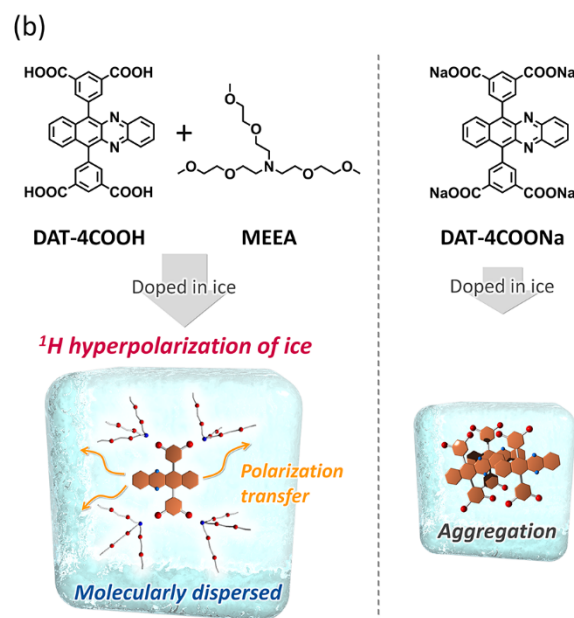
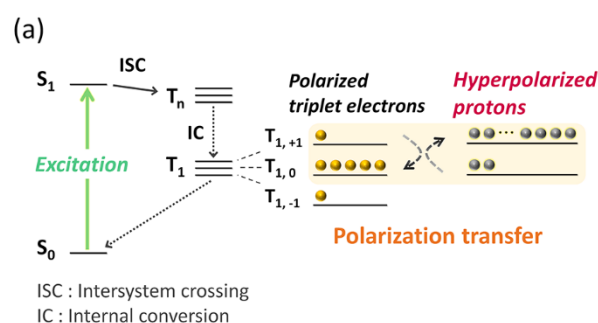


Fig. 1 (a) Typical scheme of triplet-DNP. Photoexcitation of a polarizing agent is followed by spin-selective intersystem crossing (ISC). The resulting large electron spin polarization is transferred to the nuclear spin polarization through the integrated solid effect (ISE). (b) Schematic illustration of aggregation-free dispersion of the ion pair between DAT-4COOH and MEEA for triplet-DNP in crystalline ice. DAT-4COONa aggregates in crystalline ice.

^a Department of Chemistry and Biochemistry, Graduate School of Engineering, Center for Molecular Systems (CMS), Kyushu University, 744 Moto-oka, Nishi-ku, Fukuoka 819-0395, Japan. E-mail: yanai@mail.cstm.kyushu-u.ac.jp, n-kimi@mail.cstm.kyushu-u.ac.jp

^b Cluster for Pioneering Research, RIKEN, RIKEN Nishina Center for Accelerator-Based Science, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan. E-mail: kenichiro.tateishi@riken.jp

^c PRESTO, JST, Honcho 4-1-8, Kawaguchi, Saitama 332-0012, Japan

† Electronic Supplementary Information (ESI) available: Experimental details, synthetic schemes of DAT-4COOH and DAT-2COOH, dynamic light scattering (DLS), absorption spectra, excitation spectra, photoluminescence spectra, triplet polarization parameters. See DOI: 10.1039/x0xx00000x

To achieve hyperpolarization at a much higher temperature, another DNP method using polarized electron spins of photo-excited triplet state (triplet-DNP) has attracted much attention (Fig. 1a).¹⁶⁻²⁵ In triplet-DNP, polarized triplet electrons are produced by spin-selective intersystem crossing (ISC) from photo-excited singlet state (S_1), which is followed by polarization transfer from triplet electron spins to nuclear spins through the integrated solid effect (ISE). Despite its potential, the applications of triplet-DNP have been limited to organic bulk crystals,¹⁶⁻²¹ nanocrystals,²² metal-organic frameworks (MOFs),²³ or molecular glass.^{24, 25} The key to achieving the water hyperpolarization at a higher temperature is to utilize the long spin-lattice relaxation time (T_1) of ^1H in crystalline ice over 5 minutes. However, there have been no reports on water-soluble polarizing agents. Therefore, to dissolve hydrophobic polarizing agents, it has been required to use the glass matrix of ethanol- d_6 : water = 80 : 20 (w/w) mixture, whose T_1 was only ~ 10 s.²⁵ There is a clear need to develop water-soluble triplet polarizing agents for the hyperpolarization of crystalline ice.

Here, we report the first example of a water-soluble triplet polarizing agent as well as the first demonstration of triplet-DNP of crystalline ice (Fig. 1b). Conventionally, pentacene has been the only and best option as the triplet polarizing agent, but pentacene is immediately decomposed in solution by oxidation.^{26, 27} To solve this stability issue, our group has reported that 5,12-diazatetracene (DAT) shows significant air stability as well as the high polarizing ability comparable to pentacene.²¹ Based on this previous work, we design a novel water-soluble polarizing agent DAT-4COOH by modifying DAT with four carboxylic acid groups (Fig. 1b). While its sodium salt DAT-4COONa aggregated in ice, an ion-pair composed of DAT-4COOH and bulky hydrophilic tris[2-(2-methoxyethoxy)ethyl]amine (MEEA) is successfully dispersed in ice without aggregation. We also found that the number of carboxylic acid groups is important to be dispersed in ice since DAT with two carboxylic acid moieties could not be dispersed. The polarization transfer from DAT-4COOH/MEEA triplet to water ^1H is realized with a significantly long build-up time over tens of minutes, reflecting the long ^1H T_1 of crystalline ice.

The new polarizing agent DAT-4COOH was synthesized and characterized by ^1H NMR and elemental analysis (Scheme S1, ESI[†]). Since the aggregation of triplet polarizing agents has been known to largely decrease the effect of triplet-DNP, we attempted to increase the solubility of DAT-4COOH by the complexation with MEEA that works as hydrophilic and bulky cations.²⁸ When the DAT-4COOH/MEEA mixed solution was frozen by being soaked in liquid nitrogen, the sample color and fluorescence were close to those of molecularly-dispersed DAT-4COOH/MEEA in frozen DMSO (Fig. 2a). Indeed, excitation and fluorescence peaks of DAT-4COOH/MEEA in water at 140 K (557 nm and 596 nm, respectively) were close to those of DAT-4COOH/MEEA molecularly-dispersed in DMSO at the same temperature (560 nm and 609 nm, respectively) (Fig. 2b, S1, S2, S3, ESI[†]). On the other hand, an aqueous solution of DAT-4COONa, obtained by neutralization of DAT-4COOH with NaOH, showed a different color (dark purple) and very weak fluorescence (Fig. 2a). Compared with DAT-4COOH/MEEA in ice,

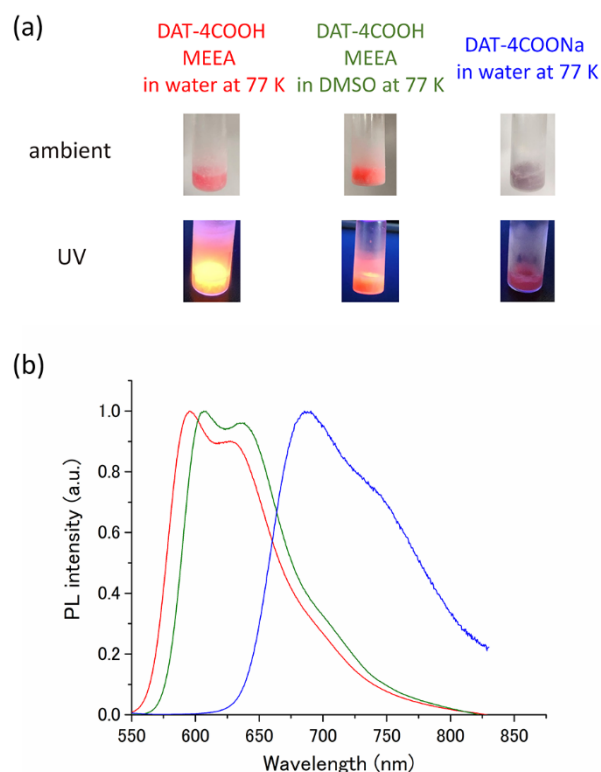


Fig. 2 (a) Photographs of DAT-4COOH (1 mM)/MEEA (4 mM) mixture in water at 77 K, DAT-4COOH (1 mM)/MEEA (4 mM) mixture in DMSO at 77 K, and 1 mM DAT-4COONa in water at 77 K under ambient light and UV light. (b) Photoluminescence (PL) spectra ($\lambda_{\text{ex}} = 532$ nm) of DAT-4COOH (1 mM)/MEEA (4 mM) mixture in water at 140 K (red), DAT-4COOH (1 mM)/MEEA (4 mM) mixture in DMSO at 140 K (green), and 1 mM DAT-4COONa in water at 140 K (blue).

excitation and fluorescence peaks of DAT-4COONa in frozen ice showed a redshift to 590 nm and 690 nm, respectively (Fig. 2b, S3, ESI[†]). Significantly, the ion-pairing with MEEA improved the hydrophilicity of the polarizing agent to be able to be molecularly dispersed in ice.

The generation of polarized triplet electrons in ice was confirmed by time-resolved electron paramagnetic resonance (EPR) measurements with a home-built setup.²¹ Under pulsed excitation at 532 nm, EPR spectra of DAT-4COOH/MEEA in water at 140 K showed the typical shape for photo-excited triplet state of DAT (Fig. 3a). The EPR spectrum was simulated by using the EasySpin toolbox in MATLAB.²⁹ The zero-field splitting parameters ($|D| = 1484$ MHz and $|E| = 133$ MHz) and relative zero-field populations ($P_x : P_y : P_z = 0.56 : 0.21 : 0.23$) of DAT-4COOH/MEEA were close to the values of DAT (Table S1, ESI[†]).²¹ The hydrophilic modification to DAT skeleton has no significant effect on triplet polarization properties. A lifetime of EPR signal decay of DAT-4COOH/MEEA in water at 140 K was 37 μs (Fig. 3b, Table S2, ESI[†]), being long enough for triplet-to-nuclei polarization transfer. We also checked the effect of MEEA concentration (4, 6, 8, 20, 40 mM) in 1 mM DAT-4COOH aqueous dispersion. The EPR signal intensity at 140 K was not

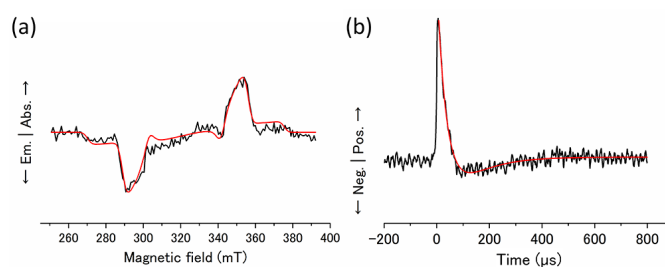


Fig. 3 (a) Time-resolved EPR spectrum (black) and simulated spectrum (red) of DAT-4COOH/MEEA in water at 140 K just after pulsed photoexcitation at 532 nm ([DAT-4COOH] = 1 mM, [MEEA] = 4 mM). (b) The decay of the EPR peak at 354 mT under pulsed photoexcitation at 532 nm of DAT-4COOH/MEEA in water at 140 K (black), and its fitting result according to the following equation, $A \exp(-t/\tau_A) + B \exp(-t/\tau_B) + C$ (red).

largely changed by the increased MEEA concentration (Fig. S4, ESI[†]), and thus four equivalents of MEEA are sufficient to disperse DAT-4COOH in ice.

The triplet-DNP process was conducted for water doped with DAT-4COOH/MEEA at 140 K by the integrated solid effect (ISE) sequence. The triplet polarizing agent was photo-excited by 400 Hz pulsed 532 nm laser, followed by a 17.7 GHz microwave irradiation for 30 μ s under a magnetic field sweep (\pm 30 mT). After repeating the triplet-DNP sequence to accumulate the spin polarization, the polarized sample was shuttled to the coil above the resonator within 3 s and ^1H NMR signal was measured. Typically, the spin-lattice relaxation time (T_1) of crystalline ice is longer than 5 min at 200K, and that of glassy water is much shorter around 5 s.^{30, 31} The buildup curve was fitted with the following equation, $A[1-\exp(-t/T_B)]$, and T_B was estimated as 11.6 minutes (Fig. 4). This long buildup time clearly indicates that the triplet electron polarization is transferred to crystalline water ^1H with long T_1 . An enhancement factor (ϵ) of 23 at 140 K was obtained for ^1H of water molecules. The enhancement factor was calculated by comparing the thermal equilibrium signal of methanol at 140 K. When the NMR signal intensity is compared with the room-temperature signal, ϵ was 49. While the further improvement of enhancement factor is necessary towards actual NMR and MRI applications, the first example of triplet-DNP of crystalline water is achieved by molecularly dispersing the water-soluble polarizing agent with the help of bulky hydrophilic counter cation.

This moderate polarization enhancement is partly due to the quantum yield of triplet formation through ISC (Φ_{ISC}). 0.05 mol% DAT doped in *p*-terphenyl crystalline powder showed a very low fluorescence quantum yield $\Phi_F < 1\%$ at 77 K, implying the efficient ISC. On the other hand, Φ_F of DAT-4COOH/MEEA in ice at 77 K was 77%, indicating the maximum Φ_{ISC} of 23%. Another possible reason is the disorder of ice crystals around the DAT-4COOH/MEEA ion pair. When dilute aqueous solutions are frozen, the coexistence of the water-rich crystalline part and the solute-rich glassy part is widely observed.³² The glassy region around the polarizing agents might induce a faster T_1

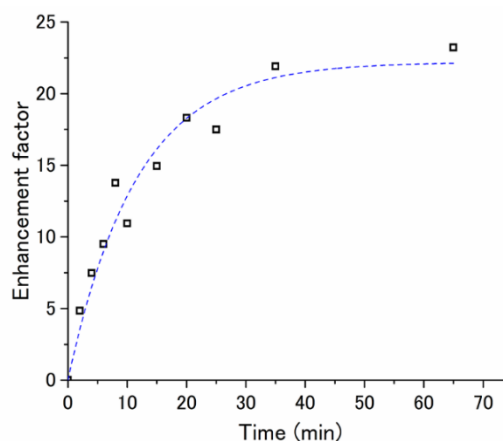


Fig. 4 Buildup curve of the ^1H NMR signal of water doped with DAT-4COOH/MEEA at 140 K and 664 mT. The broken line is a fitting curve with the following equation, $A[1-\exp(-t/T_B)]$.

relaxation.³³⁻³⁵ Indirect information about the size of the glassy region is provided by the characterizations of DAT-4COOH/MEEA in water at 300 K. Compared with the molecularly-dispersed state of DAT-4COOH/MEEA in ice, DAT-4COOH/MEEA in water at 300 K showed a red-shift and broadening in excitation and fluorescence spectra (Fig. S5, ESI[†]). These results indicate the aggregation of DAT-4COOH/MEEA in water at room temperature, which was also supported by the red-shift and broadening in absorption and fluorescence spectra compared with the molecularly-dispersed DAT-4COOH/MEEA in DMSO at 300 K (Fig. S6, ESI[†]). Dynamic light scattering (DLS) measurements of DAT-4COOH/MEEA in water at room temperature confirmed the presence of nanoscale aggregates with an average size of 64 nm (Fig. S7, ESI[†]). Therefore, interestingly, the nanoscale aggregates of DAT-4COOH/MEEA disassemble during the freezing of water. Taking into account that DAT-4COONa aggregates in ice, MEEA should play an important role to form amorphous water structure around the polarizing agents, which enables the aggregation-free dispersion of the polarizing agents.³⁶ It would be reasonable to consider that the solute (DAT-4COOH/MEEA)-rich, glassy nanodomains are formed within the crystalline ice, which results in the coexistence of two solid parts with different ^1H T_1 values.

To get more insight into the molecular design, DAT modified with a fewer number (two) of carboxylic acid moieties (DAT-2COOH) was also synthesized (Scheme S2, ESI[†]). An ion-pair of DAT-2COOH/MEEA ([DAT-2COOH] = 1 mM, [MEEA] = 2 mM) in water showed a weak fluorescence peak around 670 nm at 300 K and the peak was blue-shifted to 642 nm at 140 K (Fig. S8, ESI[†]). However, the fluorescence peak of DAT-2COOH/MEEA at 140 K was considerably red-shifted (642 nm) compared with that of DAT-4COOH/MEEA (596 nm). Importantly, DAT-2COOH/MEEA ([DAT-2COOH] = 1 mM, [MEEA] = 2 mM) in ice at 140 K didn't show any EPR signals, indicating that the electron spin polarization was not generated due to the aggregated DAT moieties. These results suggest that the hydrophilicity of the dicarboxylic acid derivative DAT-2COOH complexed with two

MEEA molecules was not sufficient to be molecularly dispersed in frozen water. It is important to introduce enough number of ion-pairs to prevent the chromophore aggregation and to achieve the triplet-to-nuclei polarization transfer in crystalline ice.

In conclusion, we showed the first example of the water-soluble triplet-DNP polarizing agent and demonstrated the first example of ^1H NMR signal enhancement of crystalline ice by triplet-DNP. The ion-pairing of the triplet polarizing agent with the bulky and hydrophilic amine allows the good dispersibility of the polarizing agent in the crystalline ice matrix. The polarized photo-excited triplet state is produced in water at 140 K, and the electron polarization is successfully transferred to ^1H nuclei of water molecules in crystalline ice. This work provides an important initial step toward the hyperpolarization of various biomolecules through proton exchange with water and the contrast enhancement in MRA to diagnose various organs.

This work was partly supported by JST-PRESTO program on "Creation of Life Science Basis by Using Quantum Technology" (grant number JPMJPR18GB), JSPS KAKENHI (grant number JP17H04799, JP16H06513, JP17J04506), Nakatani Foundation, The Shinnihon Foundation of Advanced Medical Treatment Research, RIKEN Cluster for Science, Technology and Innovation Hub (RCSTI), and the RIKEN Pioneering Project "Dynamic Structural Biology".

Conflicts of interest

There are no conflicts to declare.

References

1. A. W. Overhauser, *Phys. Rev.*, 1953, **92**, 411-415.
2. T. R. Carver and C. P. Slichter, *Phys. Rev.*, 1953, **92**, 212-213.
3. D. A. Hall, D. C. Maus, G. J. Gerfen, S. J. Inati, L. R. Becerra, F. W. Dahlquist and R. G. Griffin, *Science*, 1997, **276**, 930-932.
4. F. A. Gallagher, M. I. Kettunen, S. E. Day, D. E. Hu, J. H. Ardenkjær-Larsen, R. Zandt, P. R. Jensen, M. Karlsson, K. Golman, M. H. Lerche and K. M. Brindle, *Nature*, 2008, **453**, 940-943.
5. A. J. Rossini, A. Zagdoun, M. Lelli, A. Lesage, C. Copéret and L. Emsley, *Acc. Chem. Res.*, 2013, **46**, 1942-1951.
6. Q. Chappuis, J. Milani, B. Vuichoud, A. Bornet, A. D. Gossert, G. Bodenhausen and S. Jannin, *J. Phys. Chem. Lett.*, 2015, **6**, 1674-1678.
7. Y. Su, L. Andreas and R. G. Griffin, *Annu. Rev. Biochem.*, 2015, **84**, 465-497.
8. D. Kurzbach, E. Canet, A. G. Flamm, A. Jhajharia, E. M. M. Weber, R. Konrat and G. Bodenhausen, *Angew. Chem. Int. Ed.*, 2017, **56**, 389-392.
9. A. Ajoy, K. Liu, R. Nazaryan, X. Lv, P. R. Zangara, B. Safvati, G. Wang, D. Arnold, G. Li, A. Lin, P. Raghavan, E. Druga, S. Dhomkar, D. Pagliero, J. A. Reimer, D. Suter, C. A. Meriles and A. Pines, *Sci. Adv.*, 2018, **4**, eaar5492.
10. M. D. Lingwood, T. A. Siaw, N. Sailasuta, O. A. Abulseoud, H. R. Chan, B. D. Ross, P. Bhattacharya and S. Han, *Radiology*, 2012, **265**, 418-425.
11. K. W. Lipsø, E. S. S. Hansen, R. S. Tougaard, C. Laustsen and J. H. Ardenkjær-Larsen, *Magn. Reson. Med.*, 2018, **80**, 1165-1169.
12. T. Harris, O. Szekely and L. Frydman, *J. Phys. Chem. B*, 2014, **118**, 3281-3290.
13. J. Kim, R. Mandal and C. Hilty, *J. Phys. Chem. Lett.*, 2019, **10**, 5463-5467.
14. A. Sadet, C. Stavarache, M. Bacalum, M. Radu, G. Bodenhausen, D. Kurzbach and P. R. Vasos, *J. Am. Chem. Soc.*, 2019, **141**, 12448-12452.
15. S. Jannin, J. N. Dumez, P. Giraudeau and D. Kurzbach, *J. Magn. Reson.*, 2019, **305**, 41-50.
16. A. Henstra, T.-S. Lin, J. Schmidt and W. Th. Wenckebach, *Chem. Phys. Lett.*, 1990, **165**, 6-10.
17. M. Iinuma, Y. Takahashi, I. Shaké, M. Oda, A. Masaike, T. Yabuzaki and H. M. Shimizu, *J. Magn. Reson.*, 2005, **175**, 235-241.
18. K. Takeda, *Triplet State Dynamic Nuclear Polarization*, VDM Verlag Dr. Müller, Saarbrücken, Germany, 2009.
19. K. Tateishi, M. Negoro, S. Nishida, A. Kagawa, Y. Morita and M. Kitagawa, *Proc. Natl. Acad. Sci. USA*, 2014, **111**, 7527-7530.
20. M. Negoro, A. Kagawa, K. Tateishi, Y. Tanaka, T. Yuasa, K. Takahashi and M. Kitagawa, *J. Phys. Chem. A*, 2018, **122**, 4294-4297.
21. H. Kouno, Y. Kawashima, K. Tateishi, T. Uesaka, N. Kimizuka and N. Yanai, *J. Phys. Chem. Lett.*, 2019, **10**, 2208-2213.
22. K. Nishimura, H. Kouno, K. Tateishi, T. Uesaka, K. Ideta, N. Kimizuka and N. Yanai, *Phys. Chem. Chem. Phys.*, 2019, **21**, 16408-16412.
23. S. Fujiwara, M. Hosoyamada, K. Tateishi, T. Uesaka, K. Ideta, N. Kimizuka and N. Yanai, *J. Am. Chem. Soc.*, 2018, **140**, 15606-15610.
24. K. Tateishi, M. Negoro, A. Kagawa and M. Kitagawa, *Angew. Chem. Int. Ed.*, 2013, **52**, 13307-13310.
25. K. Tateishi, M. Negoro, H. Nonaka, A. Kagawa, S. Sando, S. Wada, M. Kitagawa and T. Uesaka, *Phys. Chem. Chem. Phys.*, 2019, **21**, 19737-19741.
26. M. Yamada, I. Ikemoto and H. Kuroda, *Bull. Chem. Soc. Jpn.*, 1988, **61**, 1057-1062.
27. A. Maliakal, K. Raghavachari, H. Katz, E. Chandross and T. Siegrist, *Chem. Mater.*, 2004, **16**, 4980-4986.
28. K. Ishiba, T. Noguchi, H. Iguchi, M. A. Morikawa, K. Kaneko and N. Kimizuka, *Angew. Chem. Int. Ed.*, 2017, **56**, 2974-2978.
29. S. Stoll and A. Schweiger, *J. Magn. Reson.*, 2006, **178**, 42-55.
30. D. E. Barnaal and I. J. Lowe, *J. Chem. Phys.*, 1968, **48**, 4614-4618.
31. E. Koivula, M. Punkkinen, W. H. Tanttila and E. E. Ylinen, *Phys. Rev. B*, 1985, **32**, 4556-4564.
32. Y. Suzuki, *J. Chem. Phys.*, 2019, **150**, 224508.
33. X. Ji, A. Bornet, B. Vuichoud, J. Milani, D. Gajan, A. J. Rossini, L. Emsley, G. Bodenhausen and S. Jannin, *Nat. Commun.*, 2017, **8**, 13975.
34. C. G. Salzmann, *J. Chem. Phys.*, 2019, **150**, 060901.
35. Q. Wang, X. Huang, W. Guo and Z. Cao, *Phys. Chem. Chem. Phys.*, 2019, **21**, 10293-10299.
36. M. Gemmei-Ide, T. Motonaga, R. Kasai and H. Kitano, *J. Phys. Chem. B*, 2013, **117**, 2188-2194.