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Electron Spin Density Matching for Cross-Effect Dynamic Nuclear Polarization†

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Abstract

A new design principle for a mixed broad (TEMPO) and narrow (Trityl) line radical to boost the dynamic nuclear polarization efficiency is *electron spin density matching*, suggesting a polarizing agent of one Trityl tethered to at least two TEMPO moieties.

Graphical Abstract
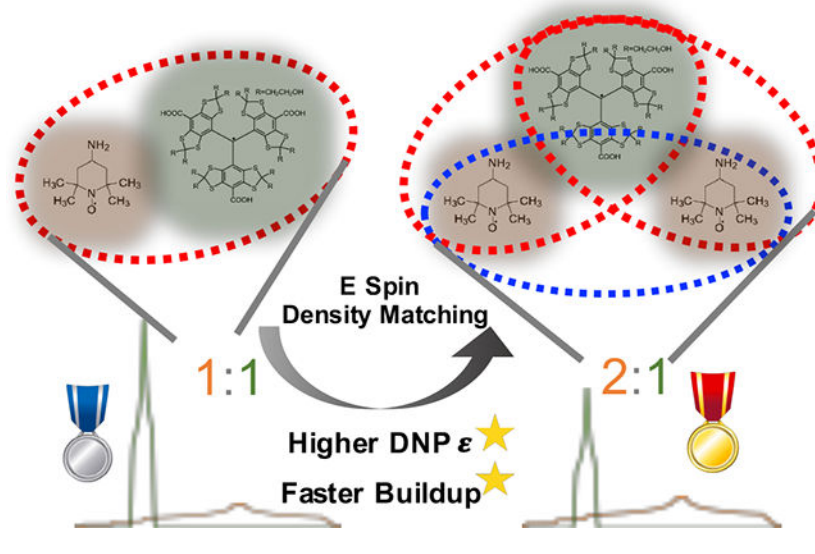
Dynamic Nuclear Polarization (DNP) is transforming the scope of solid-state nuclear magnetic resonance (NMR) by providing unprecedented access to the structural characterization of many challenging systems, such as amyloid fibrils, liposome embedded membrane proteins to inorganic surfaces and low-abundant NMR active nuclei such as ^{29}Si and ^{17}O.[1–9]

DNP normally requires exogenous polarizing agent (PA), to transfer polarization from its unpaired electron spins to coupled nearby nuclei, which is subsequently relayed to the bulk nuclei through the nuclear spin-diffusion mechanism. While many interesting applications of DNP-NMR have been demonstrated already, a prime research objective for DNP is to enhance its performance under the desired experimental conditions, including at high magnetic-fields (B_0), fast magic angle spinning (MAS) and/or at higher temperatures, while minimizing the microwave (μw) power requirement.

Among the different DNP mechanisms in insulating solids, the cross-effect (CE)[10] is the most promising mechanism at high B_0 and with the currently available state of the art μw instrumentation.[1] The CE relies on three-spin dynamics (two coupled electron spins, e, differently coupled to a nuclear spin, n), and is induced by two essential spin transitions: (i) the quantum mechanically allowed μw transition that depolarizes the ensemble of one of the two e spins involved in the CE transfer, thereby creating a large polarization difference between the two e spins relative to the n spin polarization, and (ii) the simultaneous flip-flop-flip of all three spins induced by second-order cross-terms between the e–e and e–n couplings. This is achieved when the CE resonance condition, $/\omega_{e1} - \omega_{e2}/ = /\omega_n/$, is satisfied. [11,12] Maximizing the CE transfer therefore requires maximizing the transition probabilities of these two processes.

The first break-through in CE radical design was accomplished by Hu *et al.* who introduced bis-nitroxide radicals.[13] Thereafter, efforts have been devoted to optimize the electron spin relaxation rates, linker size, molecular geometry, and solubility.[13–16] However, bis-nitroxide radicals loses efficacy at high B_0 field and under fast MAS, conditions under which the relevant adiabatic CE transition probabilities decrease.[12]

A promising solution to this dilemma emerged from the discovery of Hu *et al.*,[17] showing that mixed Trityl (narrow line) and TEMPO (broad line) radicals display favorable g-tensor difference that can effectively increase the CE e_1–e_2 spin pairs for H DNP. This mixture improved the CE-DNP enhancement by a factor of 3 compared to an equivalent amount of TEMPO at 90 K, 5 T, 5 kHz MAS, when using high-power (10 W) μw irradiation. Taking advantage of this favorable condition, significant efforts have been invested into synthesizing a PA archetype of a broad nitroxide radical tethered with a narrow Trityl or a BDPA radical, while preserving their anisotropic g-tensor features[18,19] for efficient DNP, as first successfully demonstrated by Mathies *et al.* for TEMPO-Trityl,[20] and recently by Wisser *et al.* for TEMPO-BDPA.[19]

In this study, we add a new perspective for further enhancing the CE DNP of mixed radical-based PAs by matching the electron spin density for the broad and narrow line radicals. The conceptual underpinning for testing ratios other than 1:1 (conventional) between the broad and narrow radical is that the two radicals have very different EPR line-widths and relaxation properties.[21] This raises an obvious question- Is 1:1 ratio of the radicals the optimum composition? The question we address is whether a significant boost in the DNP enhancement can be achieved under static and MAS conditions by matching the electron spin density for cross-effect DNP. The outcome of this study will offer a key design principle for the type and composition of mixed-radicals that maximize their DNP performance.

An ideal testing system would be a narrow line radical tethered to more than one broad line radicals. However, the synthesis of such radical system, and searching for the adequate length and geometry of the chemical linker is synthetically challenging, especially when a Trityl or a BDPA radical is involved. Before proposing with the design and synthesis of such systems, the viability of this concept must be tested first. To this end, we carried out the initial test by increasing the relative portion of the broad line radical in the physical mixture of Trityl and TEMPO. In this study we chose not to focus on the physical mixture of TEMPO and BDPA, another commercially available narrow line radical, given the low solubility of BDPA in toluene which significantly impairs the CE probability between the two radicals. The here reported DNP experiments were performed under static, as well as MAS conditions, at 6.9 T powered by a tunable solid-state microwave source.[22–24] Two factors will be closely investigated: the change in DNP enhancement factor and the DNP buildup rate, as these factors constitute the DNP enhancement per unit time.

Mixtures with 1:1 and 2:1 TEMPO:Trityl ratios, respectively, with a total concentration of 30 mM in DNP-juice (60/30/10 vol% d_8-glycerol/D_2O/H_2O) were chosen to commence the study. The simulated EPR spectra of the two mixtures are shown in Fig. 1(a). The Trityl and TEMPO center frequencies are separated by roughly the ^{1}H Larmor frequency, while the electron spin density is concentrated at the Trityl resonance. However, whether or not the DNP enhancement (ε) will be boosted by electron spin density matching of the mixed radicals cannot be inferred from the EPR spectra, given the role of electron spin relaxation, electron-electron coupling and electron spectral diffusion (eSD) in modulating DNP. Here, we empirically examined the effect of electron spin density matching using our DNP setup.

The DNP frequency profiles (^{1}Hε vs. $n_{\mu w}$) of the radical mixtures with 1:1 and 2:1 ratio for TEMPO:Trityl, with a total e spin concentration (C_{total}) fixed to 30 mM, were recorded at 4 K from the ratio of the integrated solid-echo ^{1}H NMR signal at μw-on and μw-off, as displayed in Fig. 1(d). The positive maximum enhancement, ε^+, is obtained at $n_{\mu w} = 193.6$ GHz for both mixtures, and the negative maximum enhancement, ε^-, at 193.9 and 194 GHz for 1:1 and 2:1, respectively. The absolute value for ε^- is significantly lower than ε^+ in both cases. The overlaid profiles show that the 2:1 mixture yields 72% greater enhancements, with $\varepsilon^+ = 224$ for the 2:1 mixture compared to $\varepsilon^+ = 130$ for the 1:1 mixture with 120 mW μw power ($P_{\mu w}$) irradiation for 60 s. To investigate the $P_{\mu w}$ dependence on DNP, ^{1}Hε^+ values were obtained as a function of $P_{\mu w}$ at the optimum frequency of $v_{\mu w} = 193.6$ GHz. The power profiles showed (Fig. 1(e)) an increase, followed by a decrease in ε with increasing $P_{\mu w}$ beyond an optimum threshold value. This is attributed to *oversaturation*, an effect originating from beyond optimum e spin depolarization caused by eSD.[25] Notice that under the applied DNP conditions, we readily reach the maximum ε with a low $P_{\mu w}$ of ~30 mW, making the mixed-radical system particularly beneficial for DNP under μw power-limited conditions. At the optimum $v_{\mu w}$ and $P_{\mu w}$, the 2:1 mixture yields greater ε of 347, which is 95% larger than that of the 1:1 mixture. The electron spin dynamics, probed using T_{1e} and electron-electron double resonance (ELDOR) measurement at the DNP conditions, suggests that the benefit of the 2:1 mixture mainly comes from a higher number density of CE-fulfilling spins overall, as properties of spin dynamics and saturation appear rather comparable for the two ratios under static conditions. EPR measurement also revealed that the T_{1e} at the Trityl resonance is shorter compared to that at the TEMPO resonance, perhaps

due to the clustering of Trityl in DNP-juice. This shows that the increased DNP efficiency of mixed radical radical can not attributed to slow relaxation of Trityl, but rather to its narrower line. See SI for details.

The vital question is whether the benefit of the 2:1 over the 1:1 mixture will be maintained under MAS, where the CE mechanism is principally different from static conditions due to energy oscillations of the relevant spin states.[11,12] The energy oscillations, mainly originating from the large g-anisotropy, typically increase the combinations of spin pairs fulfilling the CE conditions. Since the g-anisotropy of Trityl is very small compared to TEMPO, the effective number of CE resonances per rotor period (τ_r) is not limited by Trityl but by TEMPO, and depends heavily on TEMPO's g-tensor orientation. Thus, the multi-spin effect under MAS is considerably more complex, and optimizing the mixed-radical composition is critical to maximize its CE-DNP.

The investigation of mixed-radicals under MAS were carried out at 6.9 T, using a custom MAS probe (Revolution NMR, LLC.)[26] operational at 25 K and powered by a 350 mW μw source. The DNP frequency profiles, as obtained by ^{1}H-^{13}C Cross Polarization (CP) under 3 kHz MAS measured with 2-^{13}C glycine (to avoid background signal from the MAS system), are shown in Fig. 2(a). The $\varepsilon_{on/off}$ enhancements are determined from the ratio of the integrated μw-on and μw-off NMR signal. Overall, the frequency profiles under MAS were found to be similar in appearance to those obtained under static condition. The MAS DNP profile of AMUPOL in DNP-juice, acquired under the same conditions, is overlaid in grey, for comparison. At the optimum $\nu_{\mu w}$, the 2:1 mixture leads to a 28% greater $e_{on/off}$ than the 1:1 mixture at the same $C_{total} = 40$ mM. The $\varepsilon^+_{on/off}$ observed for 1:1 and 2:1 are 109 and 140, respectively. This is a phenomenal result, considering the relatively low $P_{\mu w} = 350$ mW at the source output available for our DNP setup. We also tested the 3:1 mixture with $C_{total} = 53.2$ mM. This resulted in a decreased $\varepsilon^+_{on/off} = 82$, which may be due to faster T_{1e} relaxation with increased spin concentration. Impressively, $\varepsilon^+_{on/off}$ of the 1:1, 2:1 and 3:1 mixtures were all higher than of AMUPOL at an optimum $C_{total} = 20$ mM ($\varepsilon^+_{on/off} = 55$).[16,20] The lower $\varepsilon^+_{on/off}$ observed with AMUPOL is due to high μw power requirement for bis-nitroxides.[20,27] The mixed TEMPO-Trityl radical has also been shown to exhibit minimal ^{1}H depolarization[28,29] under MAS in contrast to AMUPOL.[26] Hence, the absolute ^{1}H enhancement for AMUPOL will be even lower if its large depolarization factor at MAS exceeding 1.5 kHz is considered, as reported in the literature.[26] See SI for further discussion.

The steady state DNP buildup time ($t_{buildup}$) shows a marked decrease as the TEMPO:Trityl ratio is increased in the mixture (Fig. 2(b)). In fact, $t_{buildup}$ (5 s) for untethered 3:1 mixture is slightly shorter than that of AMUPOL (8 s), where the two e spins are strongly coupled within the tethered biradical. It is anticipated that chemically tethering two or three nitroxide moieties to Trityl will significantly boost ε and shorten the $t_{buildup}$ time.

However, unlike tethered mixed radical systems, the physical mixtures cannot maintain a radical distribution where one Trityl radical is strongly coupled to one/two tethered TEMPO radicals. To further examine if increasing the TEMPO composition as well as tethering can be beneficial, quantum mechanical simulations of DNP-NMR were performed using the Spin Evolution package.[30] We simulated CE DNP at 6.9 T, under 10 kHz MAS and a μw B_1

of 0.2 MHz for a 1:1 ($e_1 - e_2 - {}^1$H) and 2:1 ($e_1 - e_2 - e_3 - {}^1$H) type radical system, with e_1 representing a Trityl and e_2 and e_3 representing TEMPO moieties. In both cases, we fixed the e_1-e_2 and e_1-^{1}H dipolar couplings to 6 and 2 MHz, respectively. Only the e_1-e_3 dipolar couplings were varied, while keeping its relative orientations fixed. All the other dipolar couplings were turned off for simplicity, including the e_2-e_3 dipolar coupling from TEMPO-TEMPO interaction. Additional relevant details of the simulation are provided in the SI.

The aim of the simulation was to ask: (i) can the CE between Trityl and TEMPO radicals be increased by increasing the relative number density of the TEMPO, and (ii) will increased e-e coupling by chemical tethering further improve CE for the 2:1 mixture? The simulated ^{1}He as a function of μw irradiation time (t$_{buildup}$) with $v_{\mu w}$ fixed to the Trityl center frequency is shown in Fig. 3(a). Remarkably, the net ^{1}He increased by ~26% by merely changing the radical type from 1:1 (blue line, ^{1}H$e = 173$) to 2:1 (red line, ^{1}H$e = 217$) with t$_{buildup} = 3$ s (steady state), corroborating the experimental observations of the physical mixture. The ^{1}He and the buildup rates could be further augmented by increasing the coupling strength, as observed when e_1-e_3 coupling is increased from 6 MHz (20Å) in red to 12 MHz (16Å) in black. This observation confirms that the increased proportionality of TEMPO with respect to Trityl will be even more advantageous when these radicals are chemically tethered. This is because optimally increasing the e-e coupling strength increases the CE transition probability, while also maintaining a large steady state polarization difference between the e spins as a result of adiabatic e-e polarization exchange, resulting in higher CE enhancement. Here, we want to assert that the design principle introduced here may not work for oligomeric nitroxide-radical due to its more stringent orientation dependence or selectivity. For instance, for the same simulation parameters, replacing the narrower e_1 (Trityl) with a broad line (TEMPO) radical abates the DNP enhancement for $e_1 - e_2 - e_3 - {}^1$H compared to $e_1 - e_2 - {}^1$H.

In Fig. 3(b), we show steady state ^{1}He for 50 different orientations in powder for the same parameters as used in Fig. 3(a). We observe that ^{1}He increased statistically for most orientations at 2:1 ratio (red and black) and with stronger coupling (black). In other words, for the majority of e spin pairs, the CE between e_1-e_2 and e_1-e_3 constructively adds up, shifting the net enhancement and buildup rate to much higher values. This result makes sense as the CE resonance conditions between the broad and narrow radical are determined/limited by the orientation of the broad line radical. Therefore, increasing the broad line proportionality increases the net effective CE frequency per τ_r (see SI for histogram distribution of e). We can conclude that the gain in both ^{1}He and buildup rates are due to increased probability for CE between Trityl and the TEMPO radicals. Note that in the simulation, the mechanistic prerequisites for TEMPO-TEMPO CE are turned off (no e_2-e_3 coupling). In other words, for low-power μw source, the benefit from the 2:1 ratio is not primarily due to indirect effects coming from increased TEMPO-TEMPO couplings, while such effects are certainly present additionally under experimental conditions. The SE mechanism is also eliminated since ^{1}H is hyperfine coupled only to the narrow line e_1 spin and the μw irradiation is on-resonance with e_1. Turning on more couplings, such as between e_2-e_3, e_2-^{1}H and e_3-^{1}H can further increase the net DNP enhancement.

Taken together, we demonstrated that tuning the e spin density by simply altering the ratio of mixed broad and narrow line radical dramatically enhanced the CE-DNP performance, under both static and MAS conditions. Surprisingly, even a simple physical mixture between TEMPO and Trityl, when the e spin density is matched without chemical tethering, can outperform the broadly used AMUPOL biradical when relying on low-power μw solid-state diode sources that is becoming increasingly popular owing to its low cost and μw versatility, as well as pulsing capability.[23,24] A very recent joint venture by Bruker Biospin and Virginia diodes has shown that a 250 mW solid-state source with optimized μw transmittance can lead to an impressive enhancement of ~61 at 9.4 T, 100 K and 8 kHz spinning.[31]

In summary, this study reveals a new design principle for next generation PAs targeting greater CE-DNP enhancement factors and DNP buildup rates. Quantum mechanical simulations elucidated that a single narrow line radical can constructively perform CE polarization transfer with multiple coupled broad line radical partners under MAS. While our present study was carried out with a physical mixture, we expect tethered TEMPO-Trityl systems with a higher than 1:1 TEMPO:Trityl ratio to further and dramatically enhance CE-DNP under static and MAS conditions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

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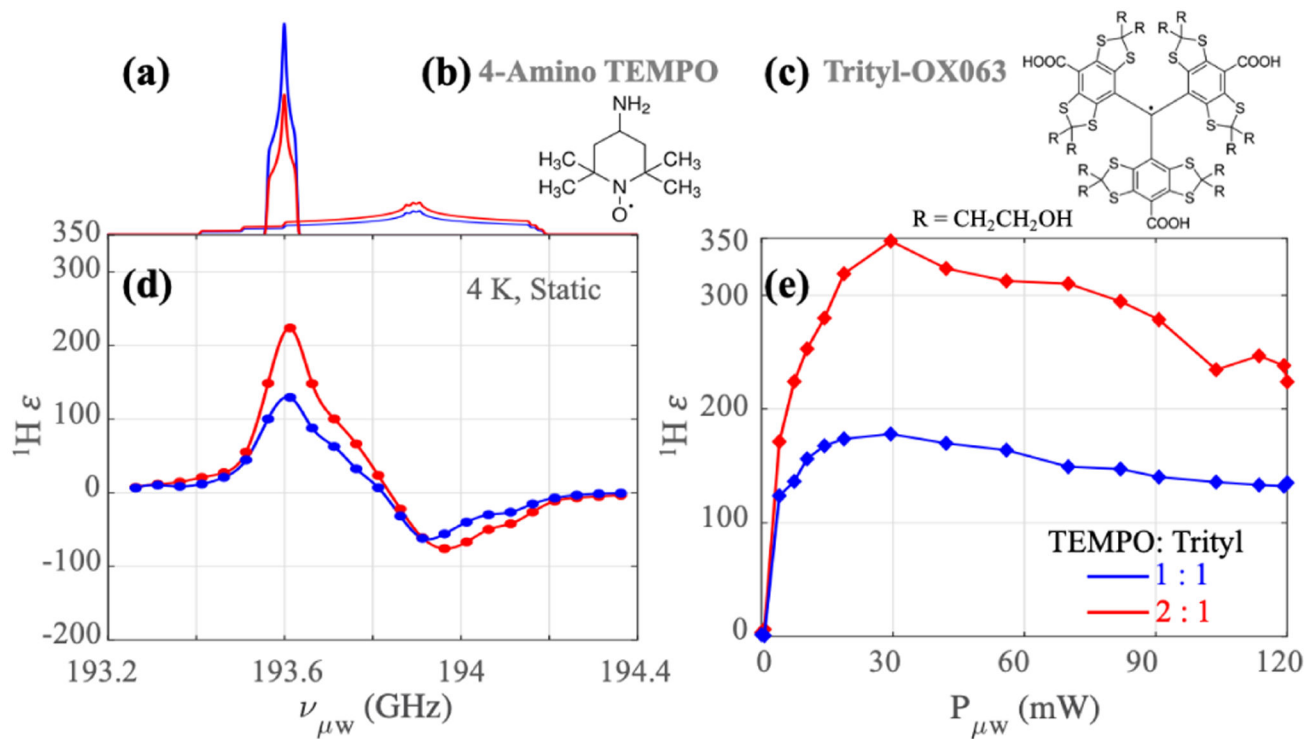
Fig. 1.
Mixed-radical with a total concentration C_{total} = 30 mM, TEMPO:Trityl = 1:1 (blue) and 2:1 (red). (a) Simulated EPR spectra at 6.9 T. Chemical structures of (b) 4-Amino TEMPO and (c) Trityl-OX063.(d) Experimental ^{1}Hε vs. $\nu_{\mu w}$ at static condition, 4 K measured with solid-echo detected ^{1}H signal in DNP-juice and $P_{\mu w}$ = 120 mW, t$_{buildup}$ = 60 s. (e) ^{1}Hε vs $P_{\mu w}$ obtained at optimum $\nu_{\mu w}$ = 193.6 GHz.
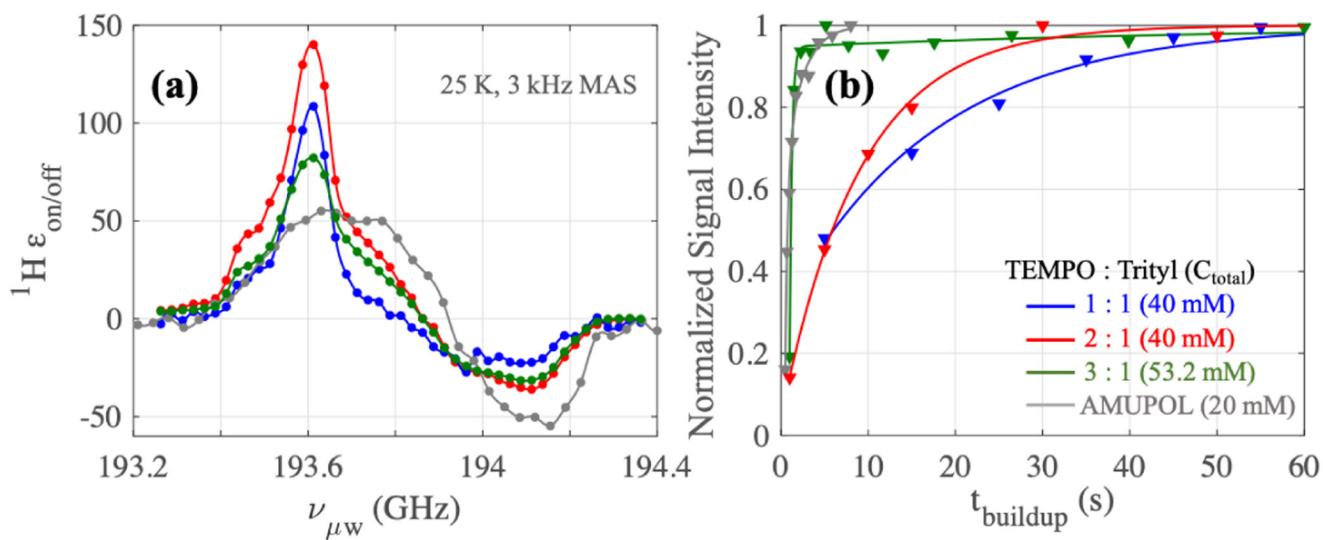
Fig. 2.
(a) Experimental ^{1}Hε vs. $\nu_{\mu w}$ at 3 kHz MAS, 25 K for TEMPO:Trityl = 1:1 (C_{total} = 40 mM, blue), 2:1 (C_{total} = 40 mM, red), and 3:1 (C_{total} = 53.2 mM, green), and C_{total} = 20 mM AMUPOL in DNP-juice, measured using 2-^{13}C glycine through ^{1}H-^{13}C CP experiment. $P_{\mu w}$ = 350 mW and t$_{buildup}$ = 60 s. (b) DNP buildup at optimum $\nu_{\mu w}$.

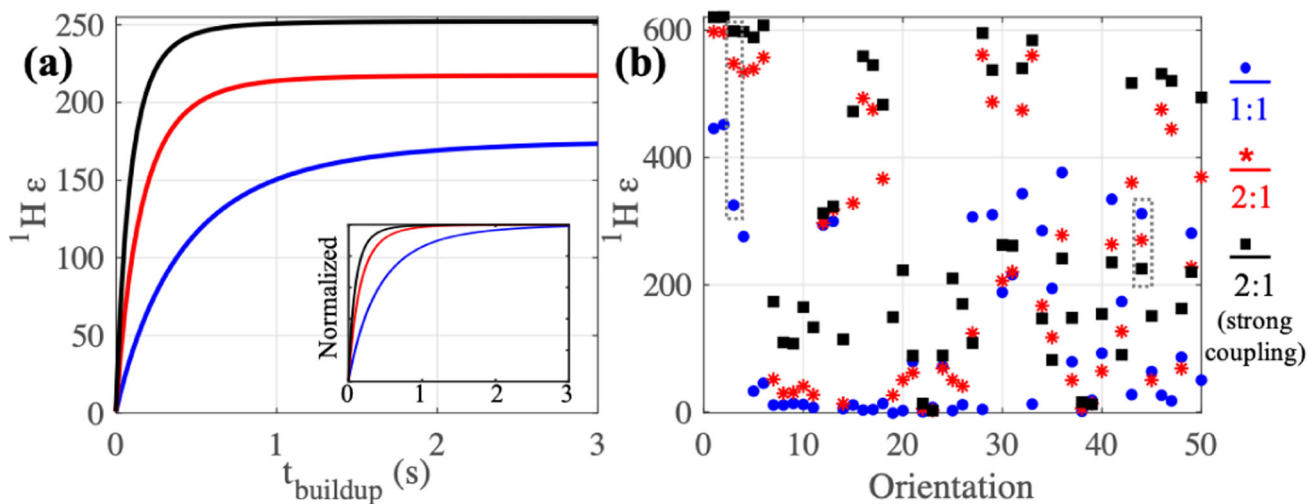**Fig. 3.**
(a)Simulated ^{1}He for e_1-e_2-H (blue) and $e_1 - e_2 - e_3 - {}^1$H (red and black) spin systems for 232 powder orientations, at 6.9 T, 10 kHz MAS and 0.2 MHz $P_{\mu w}$. e_1 mimics Trityl, and e_2 and e_3 mimic TEMPO. e_1 - e_3 dipolar coupling is set to 6 and 12 MHz, in red and black curve, respectively. T_{1H} and T_{1e} were set to 4 s and 3 ms, respectively. Inset shows normalized ^{1}He. (b) ^{1}He mapped for 50 different orientations.