Supporting Information

Breaking the T_1 Constraint for Quantitative Measurement in Magic Angle Spinning Solid-State NMR Spectroscopy

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Experimental Details

   Uniformly 99% ^{13}C, ^{15}N-labeled L-tyrosine (Tyr) that purchased from Sigma–Aldrich Inc., as well as commercially available natural abundant DL-alanine and AlPO$_4$ (aluminophosphate molecular sieves), were used in powder solid without further purification or recrystallization. All NMR experiments on L-Tyr, DL-Ala and AlPO$_4$ were carried out at room temperature on a Varian Infinityplus-400 solid-state NMR spectrometer with a magnetic field of 9.4 T. The resonance frequencies are 400.1, 100.6 and 162.0 MHz for ^{1}H, ^{13}C and ^{31}P, respectively. A double resonance magic angle spinning probe with an outer diameter of 4 mm rotor was used.

   The spinning speed was 14 kHz for NMR experiments of L-Tyr and DL-Ala, 7 kHz for NMR experiments of AlPO$_4$. The variation of the spinning rate was controlled to within ±2 Hz over the experimental time. The ^{1}H DARR irradiation strength was set to the corresponding sample spinning frequency for each quantitative experiment. For CP and QUCP experiments, a ramped rf field was applied on the I spin channel, and the matching conditions were optimized experimentally by varying the S rf field strength on the S spin channel. The recycle delays in the range from 1 s to 500 s were used, and the optimization was implemented for all quantitative single pulse experiments.

Table S1. The enhancement factors of uniformly labeled L-tyrosine calculated from ^{13}C spectra recorded with different experimental schemes. Conventional CP/MAS [1-4] and QUCP experiments with different recycle delays were recorded on ^{13}C isotope-enriched L-tyrosine spun at 14 kHz. The recycle delays of 1 s, 2 s, 5 s, 10 s and 15 s were used respectively. All the experimental parameters except the DARR [5-7] irradiation are identical for CP and QUCP experiments. With respect to the corresponding intensity of ^{13}C SP/MAS spectrum with optimized recycle delay of 500 s, as shown in Figure 2a, the enhancement factors are calculated for each experiment. By conventional CP scheme, the maximal enhancement difference ($\Delta\eta_{CP} = \eta_{CP}(C_\beta) - \eta_{CP}(C_o)$) is 0.35 in CP experiment with recycle delay of 1s, and is increased to 0.78 in CP experiment with recycle delay of 15 s, which is attributed to the recovery extent of proton magnetization. However, the ratio between enhancement factors keeps about 1.7 for different recycle delays, which demonstrates the non-uniform enhancement by CP scheme.

	Enhancement factor						
	C_o	C_4	$C_{2,6}$	C_1	$C_{3,5}$	C_α	C_β
CP(1s)	0.50	0.54	0.78	0.61	0.76	0.82	0.85
2s	0.69	0.74	1.07	0.84	1.05	1.13	1.17
5s	1.01	1.07	1.53	1.20	1.50	1.60	1.65
10s	1.14	1.21	1.75	1.37	1.72	1.83	1.90
15s	1.18	1.25	1.81	1.41	1.78	1.89	1.96
QUCP(1s)	0.67	0.67	0.67	0.66	0.66	0.65	0.68
2s	0.93	0.92	0.93	0.91	0.92	0.91	0.95
5s	1.31	1.29	1.31	1.28	1.30	1.28	1.32
10s	1.51	1.49	1.51	1.47	1.49	1.47	1.51
15s	1.55	1.54	1.54	1.53	1.56	1.53	1.57

Table S2. The enhancement factors of natural abundance DL-alanine calculated from ^{13}C QUCP spectra recorded with different recycle delays.

	Enhancement factor		
	C_o	C_α	C_β
QUCP(1 s)	2.02	1.99	1.99
2 s	2.05	2.02	2.05
5 s	2.08	2.09	2.07

Table S3. The enhancement factors of aluminophosphate molecular sieve AlPO$_4$-41 calculated from ^{31}P spectra recorded with different experimental schemes.

	Enhancement factor			
	P_1	P_2	P_3	P_4
CP(1 s)	1.36	1.14	1.02	0.96
5 s	1.86	1.57	1.40	1.32
10 s	1.91	1.59	1.43	1.34
QUCP(1 s)	1.06	1.06	1.06	1.05
5 s	1.47	1.45	1.44	1.44
10 s	1.48	1.47	1.47	1.46

Table S4. The recovery factors of ^{13}C, ^{15}N labeled alanine calculated from ^{13}C small flip-angle SP and QUSP spectra recorded with different recycle delays, the spectra were not shown. 16 FIDs were accumulated for each experiment. The optimized recycle delays were 50 s, 22 s and 30 s for 90°, 20° and 30° flip-angle single pulse quantitative experiments, respectively. For QUSP experiments, the mixing time of DARR irradiation was 1.0 s and the RF field intensity was 14 kHz. QUSP experiments with different recycle delays were performed. According to the quantitative single pulse spectrum, the calculated recovery factors were obtained. Although the quantitative NMR spectrum can be achieved by the small flip-angle single pulse experiments with a relatively short recycle delay, there is no obvious improvement with the efficiency gain. However, quite higher efficiency gains of 41.6 and 10.7 can be obtained in QUSP experiments with recycle delay of 0.5 s and 5 s, respectively, due to the NOE enhancement. For QUSP experiments with small flip-angle, the obtained efficiency gains are 14.4 and 3.6 for the recycle delay of 0.5 s and 5 s, respectively. In this case, the combination with small flip-angle technique cannot be used to achieve higher efficiency gain in QUSP experiment, because ^{13}C magnetizations mostly come from NOE transfer.

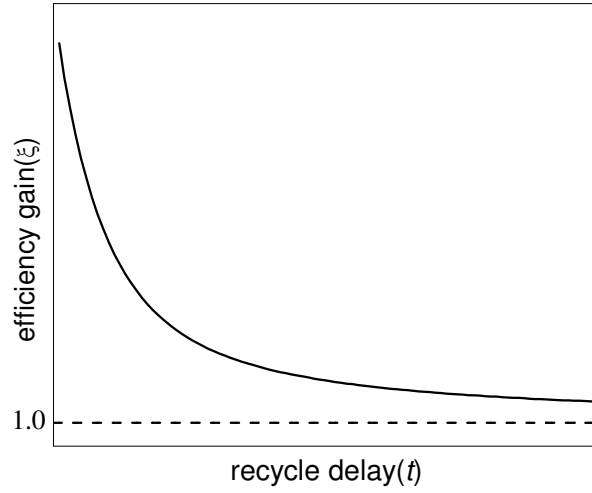| Exp (recycle delay, flip angle)	Recovery factor		
	C_o	C_α	C_β
Small flip-angle (22 s, 20°)	0.33	0.33	0.32
Small flip-angle (30 s, 30°)	0.50	0.50	0.48
QUSP (0.5 s, 90°)	1.25	1.25	1.26
QUSP (5 s, 90°)	1.28	1.27	1.29
QUSP (0.5 s, 20°)	0.43	0.45	0.44
QUSP (5 s, 20°)	0.44	0.43	0.42

Figure S1. Theoretical simulation of the recycle delay dependence of the efficiency gain by QUCP experimental scheme. For a quantitative NMR spectrum with truthful intensities, the experimental efficiency gain of QUCP experiment over the conventional quantitative single pulse experiment can be expressed as, $\xi_{exp} = \dfrac{\tau_{rd}^{SP}\eta_{QUCP}}{\tau_{rd}^{QUCP}+\tau_{DARR}}$. Where τ_{rd}^{sp} denotes the recycle delay of single pulse experiment. τ_{rd}^{QUCP} and τ_{DARR} denote the recycle delay and the mixing time in QUCP experiment, respectively. The enhancement factor of QUCP is denoted as η_{QUCP}. Here made an approximation that the contact time and the finite pulse width are not taken into account due to their tininess compared to the recycle delays. According to the above expression, the efficiency gains can be obtained easily. Considering the quantitative NMR measurement of ^{13}C isotope enriched L-tyrosine, the optimized relaxation delays were found to be 15 s and 500 s for CP and SP experiments, respectively. The calculated efficiency gains are 167.5, 155.0, 108.3, 67.7 and 48.4 for the QUCP experiment with relaxation delays of 1s, 2 s, 5 s, 10 s and 15 s, respectively. Theoretically, the efficiency gain of QUCP experiment is dependent on spin-lattice relaxation (T_1^H, T_1^C) and the enhancement factor (η_{QUCP}), as given by, $\xi_{theo} = \eta_{QUCP}\dfrac{1-e^{-t/T_1^H}}{1-e^{-t/T_1^C}}$. where t denotes the recycle delay of QUCP experiment, τ_{rd}^{QUCP}. The plot shows that ξ attenuates with the increasing recycle delay, approaching to 1.0 for infinitely long recycle delay, which is consistent with the result of QUCP experiment.
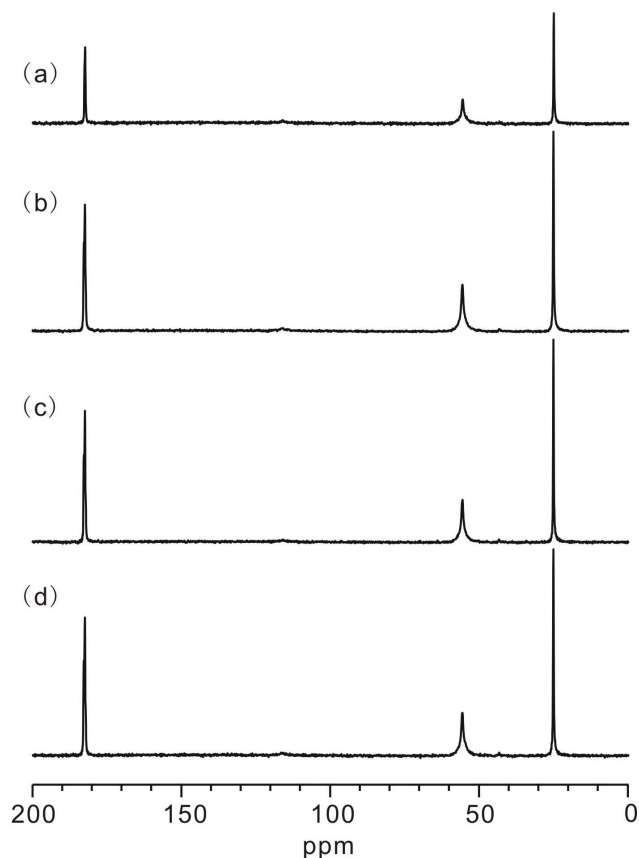
Figure S2. ^{13}C SP (a) and QUCP (b~d) NMR spectra of natural abundance DL-alanine spun at 14 kHz. The spectra are plotted on the same amplitude scale, and 200 FIDs were accumulated for each experiment. The relaxation delays were 200 s, 1 s, 2 s and 5 s (a) ~ (d), respectively. For QUCP experiments, the contact time for cross polarization was 0.6 ms and the time of DARR irradiation on proton was 10.0 s. RF field intensity of 14 kHz was used for DARR irradiation. Generally, the duty cycle of a pulse sequence should be less than 0.2 for the present NMR instruments. This requirement is certainly guaranteed in our method because the RF field strength for the DARR irradiation pulse is only about 1/5 of the maximal value which converts to a duty cycle of about 4%, much lower than the allowed duty cycle. Because the DARR field strength is set to the spinning speed which is normally from 10 to 20 kHz, the requirement of a duty cycle less than 0.2 can always be satisfied by properly setting recycle according to the DARR irradiation strength (higher MAS speed and longer DARR time requires longer recycle delay). For instance, for a DARR time of 20 s with spinning speed of 20 kHz, assuming the maximal RF field of 80 kHz, with a recycle delay of 5 seconds, the duty cycle would be 0.13. In addition, even the NMR hardware has lower specifications and cannot stand such long irradiation, windowed DARR may be used. For the QUCP experiments on rare nuclei with natural abundance, the polarization transfer rate during the mixing time is reduced seriously due to very weak *S-S* homonuclear dipolar couplings and low paired spin percentage (PSP) [8,9]. This results in that a mixing time of several seconds or longer is required for all spins to reach quasiequilibrium and achieve uniform enhancement. Compared to the quantitative single pulse spectrum shown in Figure S2a, the enhancement factors of carbonyl, methenyl and methyl carbons for QUCP experiment with different relaxation delays can be obtained, as shown in Table S2. It can be seen that the enhancement factors of three magnetically nonequivalent ^{13}C sites are almost similar, with a deviation of ±0.02, whatever the corresponding recycle delay is. Taking the optimized recycle delay for single pulse experiment and the mixing time for DARR irradiation into account, the calculated efficiency gains are 36.2, 33.5 and 27.6 for the QUCP experiment with recycle delays of 1s, 2 s and 5 s, respectively. The QUCP efficiency gain decreases with the increasing recycle delay, which is consistent well with the theoretical prediction. Higher efficiency gain can be obtained with a shorter recycle delay in the QUCP experiment.
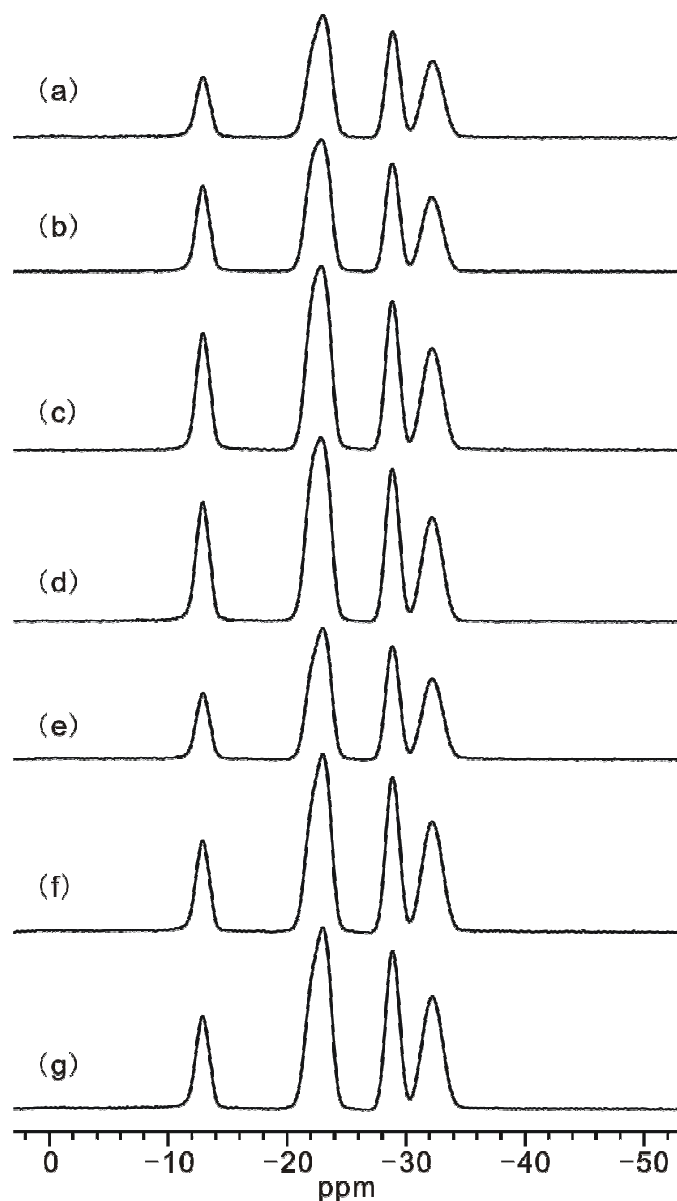
Figure S3. ^{31}P SP (a), CP (b~d) and QUCP (e~f) MAS NMR spectra of calcined rehydrated $AlPO_4$-41. The spectra are plotted on the same amplitude scale. A MAS frequency of 7 kHz was used. 8 scans were accumulated for each experiment. The recycle delays were 500 s, 1 s, 5 s, 10 s, 1 s, 5 s, and 10 s, respectively. The same CP matching condition was used for both CP and QUCP experiments. The mixing time with DARR irradiation was 1.0 s and RF field intensity was 7.0 kHz. The obtained enhancement factors of nonequivalent ^{31}P sites for different experiments are shown in Table S3. ^{31}P resonant sites from low field to high field are labeled as P_1~P_4, respectively. It is shown obviously that the uniform enhancement of QUCP experiment can be achieved easily whatever the recycle delay is, while CP experimental scheme cannot. Moreover, in CP experiments, the maximum difference of non-uniform enhancement factors increases with the relaxation delay due to the effect of spin-lattice relaxation. The calculated efficiency gains are 262.5, 120.8 and 66.8 for the QUCP experiment with the relaxation delay of 1s, 5 s and 10 s, respectively.
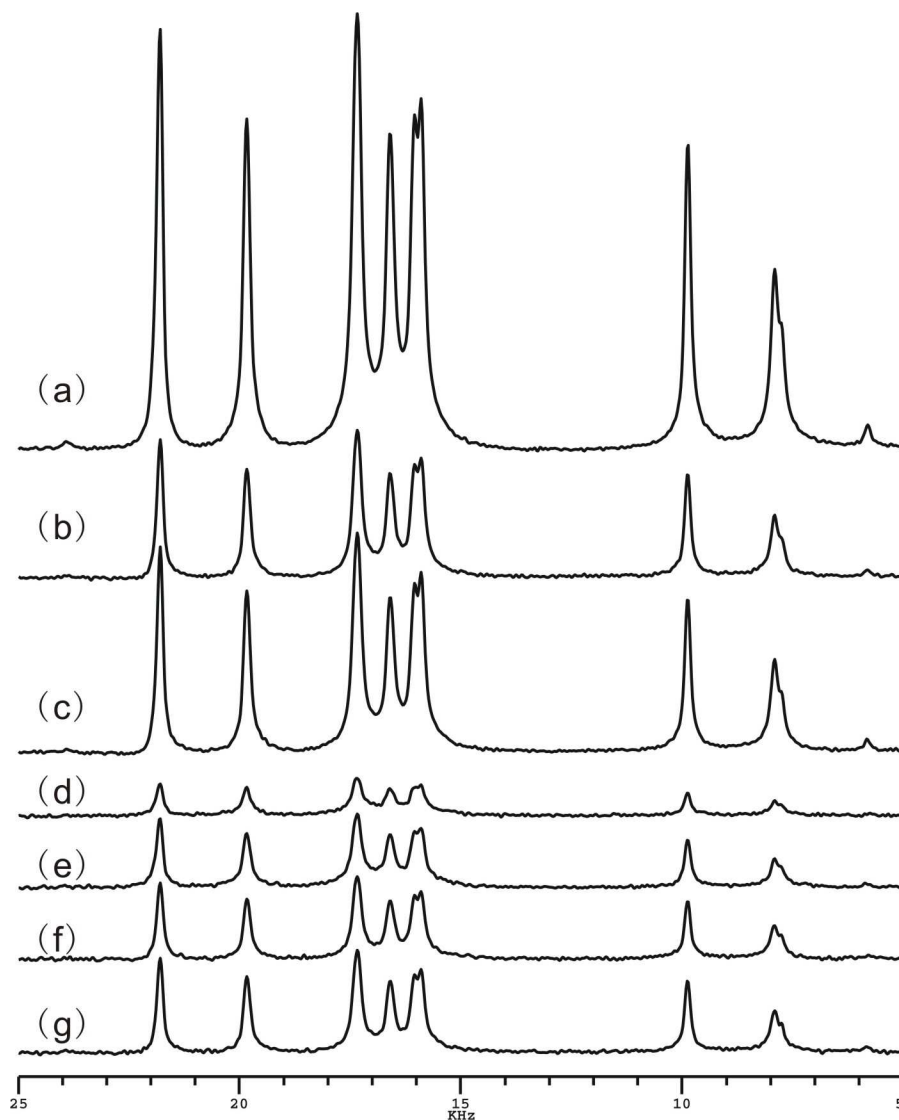
Figure S4. ^{13}C conventional SP (a), small flip-angle SP (b, c) and QUSP (d~g) NMR spectra of ^{13}C, ^{15}N-labeled L-tyrosine spun at 14 kHz. 16 FIDs were accumulated for each spectrum. The optimized recycle delays were 500 s, 220 s and 300 s for (a), (b) and (c) respectively. 20° and 30° single pulse was used for the small flip-angle experiments (b) and (c), respectively. For QUSP experiments, the mixing time of DARR irradiation was 1.0 s and the RF field intensity was 14 kHz. The recycle delay was 5 s for (d) and (f), 15 s for (e) and (g). 20° small flip-angle was used for (f) and (g). By the conventional single pulse scheme, the ^{13}C spin magnetizations cannot be recovered completely in a relatively short recycle delay (less than $5T_1^C$), which results in non-quantitative ^{13}C spectra. Although the small flip-angle technique can be used to achieve quantitative NMR spectrum with a relatively short recycle delay, the required recycle delay depends on the size of small flip-angle and still needs to be optimized for the quantitative experiments. For the QUSP experiment, however, the MAS averaged ^{13}C-^{13}C dipolar couplings are reintroduced by the DARR irradiation on proton, which drives polarization transfer among the non-uniformly recovered ^{13}C magnetizations. The quantitative ^{13}C NMR MAS spectra can be obtained so long as the mixing time is sufficiently long to reach a quasi-equilibrium state, where the T_1 constraint is not required, even with a small flip-angle as shown in (f) and (g). Similar to the mechanism of the QUCP, the present QUSP is another quantitative NMR method breaking the T_1 constraint. In this case, the non-uniform initial magnetization in the mixing period is not caused by CP, but by the magnetization recovery of the spin-lattice relaxation. Compared to the quantitative single pulse MAS spectrum of L-tyrosine as shown in Figure (a), the recovery factor of non-equivalent ^{13}C sites in QUSP spectra with different recycle delays can be obtained easily. The calculated recovery factors are 0.080,

0.082, 0.082, 0.083, 0.083, 0.078 and 0.081 for C_o, C_4, $C_{2,6}$, C_1, $C_{3,5}$, C_α and C_β sites, respectively, in QUSP experiment with a recycle delay of 5 s. For the QUSP experiment with a recycle delay of 15 s, the recovery factors are 0.164, 0.169, 0.167, 0.169, 0.168, 0.165 and 0.166, respectively. It can be seen that the uniform recovery factors in the range of ±0.003 can be obtained by QUSP scheme whatever the recycle delay is. Similarly, the calculated uniform recovery factors are 0.187±0.002 and 0.235±0.003 for QUSP experiments (f) and (g) with a small flip-angle of 20°, respectively. Taken the mixing time τ_{DARR} into account, the efficiency gain of QUSP experiment can be calculated, which is 6.7 and 5.2 for the experiment with recycle delay of 5 s and 15 s, respectively. However, the efficiency gains are 15.6 and 7.33 for the small flip-angle QUSP with recycle delay of 5 s and 15 s, respectively. It shows that higher efficiency gain can be achieved in QUSP experiments when combined with the small flip-angle technique.
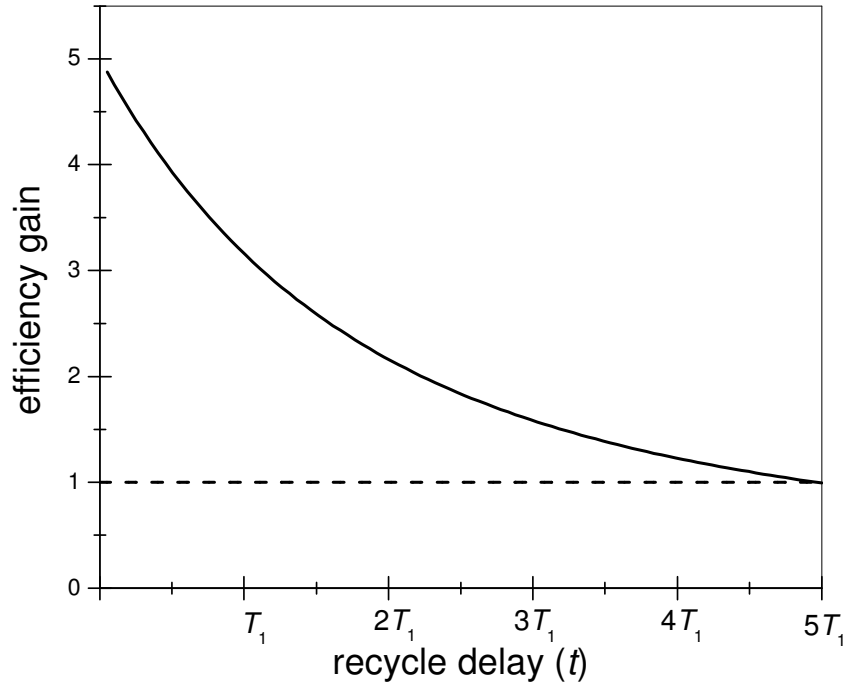

Figure S5. Theoretical simulation of the recycle delay dependence of the efficiency gain by QUSP experimental scheme. Assuming a single pulse NMR spectrum with the relaxation delay of $5T_1$ to be quantitative, the efficiency gain of QUSP experiment can be theoretically expressed as, $\xi_{QUSP}^{theo} = \dfrac{5T_1}{t + \tau_{DARR}}(1 - e^{-t/T_1})$, where t denotes the recycle delay, and τ_{DARR} denotes the mixing time in QUCP experiment. It can be seen that the maximal efficiency gain by QUSP scheme is about 5, which diminishes gradually with the recycle delay. Minimal efficiency gain can be obtained when the recycle delay is $5T_1$. Generally, for $\tau_{rd} << T_1$, an efficiency gain of more than 4 can be achieved, which is definitely a significant reduction of the experimental time. This kind of efficiency gain is different from that achieved by general small flip-angle single pulse scheme. Although the short recycle delay might be reasonable for obtaining quantitative determination by small flip-angle SP experiments, the signal intensity is reduced by a factor $\sin\theta$ (θ denotes the flip angle) simultaneously. Theoretically, the required recycle delay to achieve quantitative SP spectrum depends on the size of the flip angle greatly, and it can be expressed as the expression (a SP spectrum with a recycle delay of $5T_1$ is supposed as quantitative), $\tau_{required} = 5T_1 + T_1 \ln(1 - \cos\theta)$. Only when the flip angle θ is close to or smaller than 6.5°, can an

efficiency gain larger than 1.0 ~~can~~ be obtained by the small flip-angle SP scheme. Otherwise, the efficiency gain will be less than the conventional single pulse experiment. Here one drawback should be noted that the accumulated signal would get smaller than $\sin\theta$ with the scans being increased due to the incomplete recovery in small flip-angle experiment, which does not happen with QUSP scheme. However, in practical examples, usually a single pulse experiment with a recycle delay longer than $5T_1$ is performed to obtain a quantitative reference, which results in overestimated efficiency factor, as seen in QUSP experiment of L-tyrosine. In addition, when the system containing fast motion groups such as methyl, it is expected to obtain higher efficiency gain due to NOE enhancement effect, which is presented in QUSP experiments of natural abundance DL-alanine.
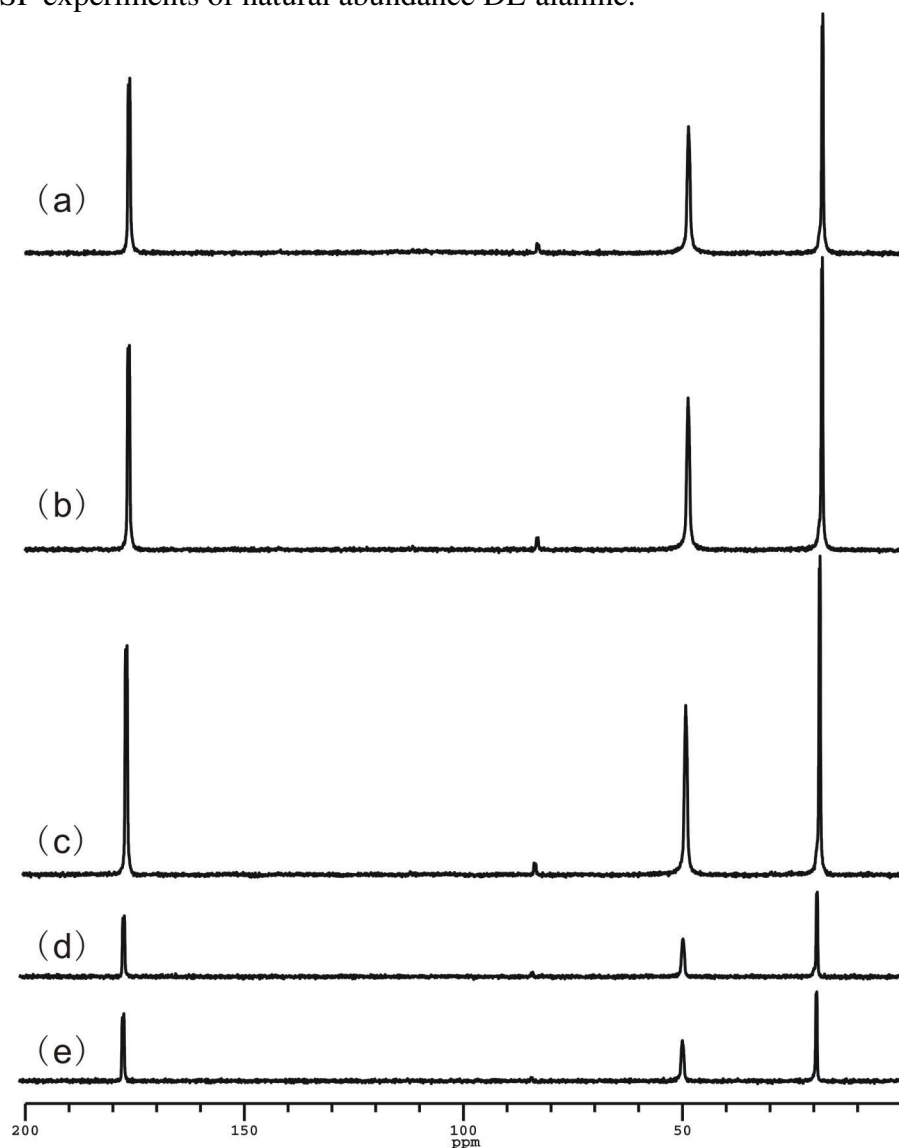

Figure S6. [13]C conventional SP (a) and QUCP (b~e) NMR spectra of ^{13}C, ^{15}N-labeled alanine spun at 14 kHz. 16 FIDs were accumulated for each spectrum. The optimized recycle delay was 50 s for single pulse spectrum. For QUCP experiments, DARR and RFDR homonuclear recoupling techniques were used during the mixing time, respectively. For DARR irradiation (b, c), the mixing time was 1.0 s and the RF field intensity was 14 kHz. For RFDR irradiation (d, e), XY-8 phase cycles were used and the mixing time was 8 ms. RFDR pulse block is shown in Figure S8(a). The recycle delay was 0.5 s for (b) and (d), 5 s for (c) and (e). According to the quantitative single pulse spectrum, the uniform enhancement in the range of 1.20±0.02, 1.33±0.01, 0.34±0.02 and 0.38±0.02 were calculated for (b) ~ (d), respectively. It can be demonstrated that quantitative CP/MAS spectra can be achieved with various

broadband homonuclear recoupling sequences. Compared with DARR irradiation, RFDR results in a faster transfer rate and shorter time to reach quasi-equilibrium state, even in a mixing time of 8.0 ms, however, partial signal loss comes with RFDR irradiation. The efficiency gain by RFDR irradiation was about three tenth of that by DARR irradiation.
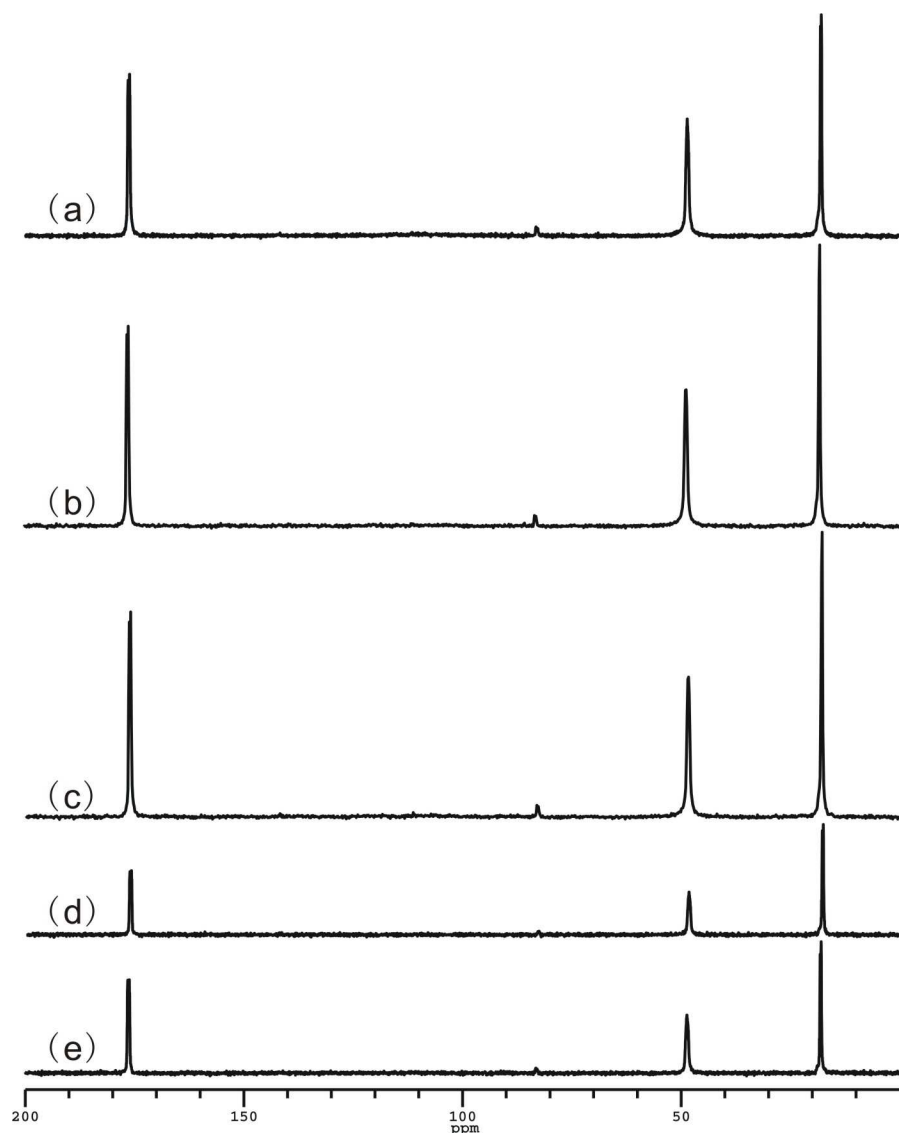

Figure S7. ^{13}C conventional SP (a) and QUSP (b~e) NMR spectra of ^{13}C, ^{15}N-labeled alanine spun at 14 kHz. 16 FIDs were accumulated for each spectrum. The optimized recycle delay was 50 s for single pulse quantitative NMR spectrum. During the mixing time, DARR and RFDR homonuclear recoupling techniques were used for QUSP experiments (b, c) and (d, e), respectively. The experimental parameters were same as Figure S6. According to the quantitative single pulse spectrum (a), the uniform recovery factors in the range of 1.26±0.01, 1.28±0.01, 0.44±0.01 and 0.43±0.01 were calculated for (b) ~ (d), respectively. It can be seen that quantitative SP/MAS spectra can be achieved by performing various broadband homonuclear recoupling sequences during the mixing time. Similar to QUCP experiments, the efficiency gain by QUSP with RFDR irradiation was about three tenth of that by DARR irradiation.
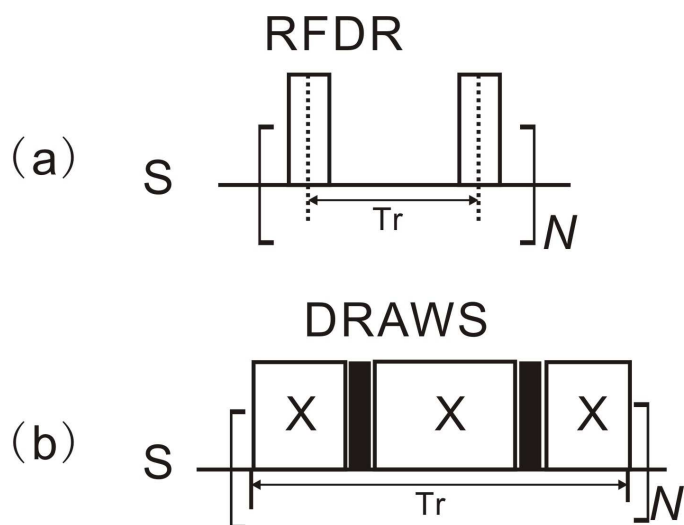
Figure S8. Alternative homonuclear recoupling pulse sequences used in the mixing time for QUCP/QUSP experiments. The blank and solid bars denote π and $\pi/2$ pulses, respectively. Tr denotes the rotational period. *N* is the cycle number of the elements. In pulse scheme (b), continuous wave RF field irradiation is applied during the intervals between $\pi/2$ pulses. RFDR [10] (a) and DRAWS [11] (b) broadband homonuclear recoupling schemes are performed to reintroduce S-S dipolar coupling which drive the polarization transfer among S spins. Both of them are applied on the observation nuclei, which is suitable for the system without abundant spin *I* or with invalid cross polarization. QUCP and QUSP with DARR irradiation presented in the manuscript are suitable for the quantitative measurement of rare nuclei system with abundant spins. During the mixing period, RF field irradiation is applied on the proton channel, hetero- and homo- nuclear dipolar couplings are reintroduced, which effectively drive broadband polarization transfer among rare nuclei. However, the absence of abundant nuclei in inorganic systems like dehydrated aluminophosphate molecular sieve results in that proton dipolar assisted recoupling (i.e. DARR) is disabled. It is reasonable to introduce other homonuclear recoupling techniques (such as RFDR, DRAWS) during the mixing period. Although great efficiency gain like QUCP experiments cannot be obtained within such systems, an efficiency gain close to 5 is expected to achieve with relatively short recycle delays.

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