

Review 2: Anonymous.

The appearance of the spectra in Figures 3a) and 3d) are not “single peaks” but rather spectra with multiple shoulders (spanning several 100 ppm). What is the origin of those? I would be curious to know if the appearance of the spectra is identical at shorter ^1H DNP times in the sequence – does this line(shape) build homogeneously?

We see at least five peaks in the ^{13}C NMR spectrum of our sample. The peaks do not appear equally spaced, and so are not a multiplicity effect. We therefore believe that this corresponds to different chemical environments, being polarized on different time scales, which could be associated with complicated factors such as inhomogeneous sample freezing. There is a chemical environment at approximately -300ppm, which is very deshielded for a ^{13}C nuclear spin and is perhaps an effect of hyperfine couplings with the radical spins. The ^{13}C NMR spectra in fact change most rapidly at shorter ^1H DNP times when the rate of change of the ^1H polarization level is fastest. The ^{13}C NMR lineshape does indeed also change homogeneously, as shown by the ^{13}C NMR spectra presented in the supplement. Furthermore, looking at the ^{13}C NMR spectra acquired at the CP contacts in our experiment, at which point the ^1H polarization has reached a constant value whilst the ^{13}C NMR signal continues to grow, it is observed that there is minimal distortion to the ^{13}C lineshape, indicating that it is the ^1H polarization is mostly responsible for this phenomenon

What is the role of CSA or possible dipole-dipole interactions, and how are those manifest under both positive and negative microwave irradiation? What is the preferred energy state for coupling to $P(^1\text{H}) = +$ versus $P(^1\text{H}) = -$?

We agree with the reviewer that more information should be given about the origin of this asymmetry, we will therefore add a paragraph listing the different interactions involved:

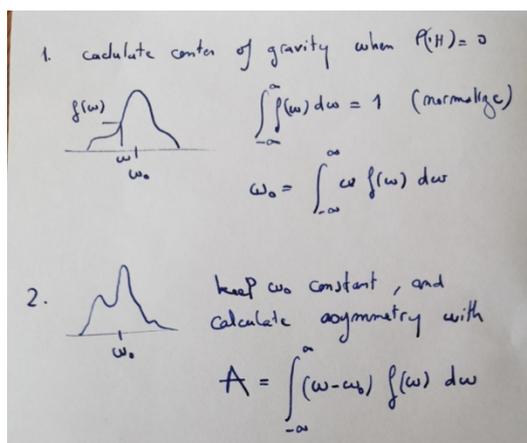
“The ^{13}C NMR lineshape of [2- ^{13}C]sodium acetate has features which mainly originate from ^{13}C CSA (typ. ~ 1.5 kHz at our magnetic field of 7.05 T) and ^1H - ^{13}C dipolar couplings (typ. -22.7 kHz) that are affected by possible methyl group rotation. Since the ^{13}C CSA is negligible with respect to the ^1H - ^{13}C dipolar couplings, it is assumed that the ^1H - ^{13}C dipolar couplings play the key role in the ^{13}C NMR lineshape of [2- ^{13}C]sodium acetate.”

Presumably the glycerol carbon and the quaternary carbon of the formate both contribute to the spectrum. Where are those, and how are those influenced by both cross-polarization and microwave irradiation?

The main ^{13}C peak includes contributions from the ^{13}C Glycerol-d8 peaks (these peaks are typically within ca. 30ppm of the [2- ^{13}C]sodium acetate peak). Although Glycerol-d8 is deuterated it can still be polarized by ^1H - ^{13}C cross-polarization (CP), but such deuterated systems typically require much longer CP contact times for efficient polarization transfer. We therefore hypothesize that the influence of the Glycerol-d8 ^{13}C nuclear spins has a reduced impact on the hyperpolarized spectrum of [2- ^{13}C]sodium acetate than at lower levels of ^{13}C polarization. Under microwave irradiation, the natural abundance ^{13}C spins of Glycerol-d8 will be polarized by microwave irradiation with their own build-up rate and maximum polarization, although this is anticipated to be slower and lower than those of [2- ^{13}C]sodium acetate. We did not trial formate in this study.

At extended ^1H DNP times, there are additional intriguing details – the claim that these are now two separate resonances doesn't quite fit with the initial picture (of a “single [peak]”). Defining Eq 1 based on the fractional intensities of these two “peaks” feels somewhat arbitrary. Without knowing what these I_h and I_l features represent, it's somewhat difficult to tell this is arising from the ^1H polarization or from some other effect.

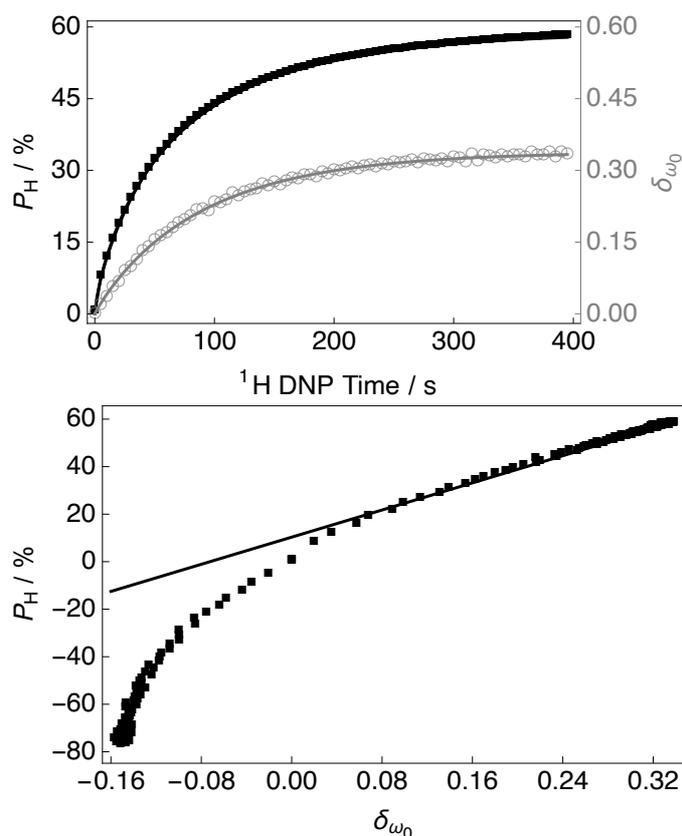
We propose an alternative here, alongside our original method, which is a simple calculation of an asymmetry parameter as described below, which can be easily applied and generalized to any lineshape:



This procedure should work very easily and give a very general way to calculate asymmetry on any lineshape. In this way, any laboratory/group can adopt the procedure and reproduce the result. We furthermore build on the procedure given above and define the following quantity:

$$\delta_{\omega_0} = \frac{\int_{-\infty}^{\infty} (\omega - \omega_0(P_H = 0\%)) f(\omega) d\omega}{\sqrt{\int_{-\infty}^{\infty} (\omega(P_H = 0\%) - \omega_0(P_H = 0\%))^2 f(\omega(P_H = 0\%)) d\omega}}$$

which is normalized by the linewidth (at FWHM at $P_H = 0\%$) to yield a dimensionless quantity. The procedure above produces the results of the kind show below, with δ_{ω_0} being the normalized shift from ω_0 at $P_H = 0\%$:



A line of best fit had been added in the linear regime of the curve as an additional tool for the reader. This methodology is also robust with respect to inhomogeneous magnetic fields. A discussion of the above kind will be added to the manuscript, alongside the discussion of our original approach.

The different slopes for Figure 5 are explained empirically (lines 36-37) but is there a physical reason why the ^1H polarization (or the asymmetry of the carbon resonances) would be more sensitive to negative microwave irradiation?

The reviewer is indeed correct and we thank them for this comment. We believe that radiation damping is responsible for the difference between the two curves. In the case of negative DNP, radiation damping leads to an overestimation of the ^1H polarization, particularly at high ^1H polarizations, and hence a change in the slope of the calibration curve.

Minor point: the term “crusher” is unfamiliar to me. Do you mean “saturation” sequence or “saturating comb”?

This text, and Figure 1, will be changed from “crusher” to “saturating”.