

Review 1: Andrea Capozzi.

Where is the asymmetry coming from? [...]

We thank the reviewer for raising this interesting point. Given the complexity of these ^{13}C NMR lineshapes, we decided to limit the scope of this article to reporting the observed effect, quantifying it, and linking it to the absolute polarization measured by conventional means (by comparison with thermal equilibrium NMR signal). 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 $[2-^{13}\text{C}]$ sodium acetate has features which mainly originate from ^{13}C chemical shift anisotropy (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 $[2-^{13}\text{C}]$ sodium acetate.”

Usually, when introducing a new methodology (not yet established and broadly acknowledged), the new methodology has to be compared to the traditional one (i.e. measuring the enhancement from the ratio of the hyperpolarized proton signal to the thermal equilibrium one). In the methods part, you say to have a background free coil. Why don't you use it to show how much your new method is consistent and reliable?

Indeed, Figure 5 shows the asymmetry vs. the absolute polarization measured by comparison with the thermal equilibrium NMR signal. We thank the reviewer for pointing this out, and we will add a sentence in the corresponding Figure caption to make this point clearer.

Moreover, do you need a methyl group or a simple coupling with a ^1H nucleus can provide the same results?

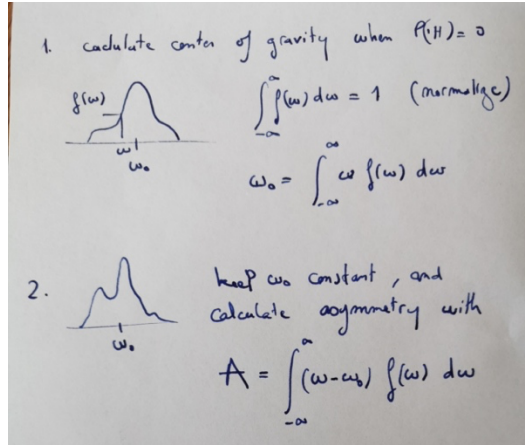
We believe that the strong HC dipolar coupling is involved in this phenomenon.

I would have expected at least one more probe molecule, in particular because in the discussion you mention sodium $[1-^{13}\text{C}]$ formate that has no methyl group.

We agree that it would be very interesting to investigate other molecules, and we intend to do so in the future. However, we have only seen such interesting lineshapes, i.e., ones with similarly pronounced features, for $[2-^{13}\text{C}]$ acetate thus far. Other ^{13}C -labelled methyl groups, such as gamma-picoline, and ^{13}C -labelled methylene groups might also exhibit exotic spectra under our experimental $d\text{DNP}$ conditions, and it is also possible that such effects could be observed in $[1-^{13}\text{C}]$ formate, if indeed one of the main requirements is the presence of a strong HC dipolar coupling. However, we have not yet investigated other suitable molecular candidates.

Last but not least, when you calculate the asymmetry you take into account the peaks intensity. These peaks are far from being resolved and the intensity of the first will most likely be influenced by the intensity of the second and vice versa. At the end of the discussion, you mention the correct data processing procedure to estimate the asymmetry (to deconvolute the two peaks by means of Voigt fits and evaluate the integral). I agree it is less straight forward than just measuring the intensity, but you are introducing a new methodology. You should at least prove that “the peak intensity method” provides, withing a decent error margin, the same result of the “the peak fitting method”. If you demonstrate that, then we will all use the intensity one, of course.

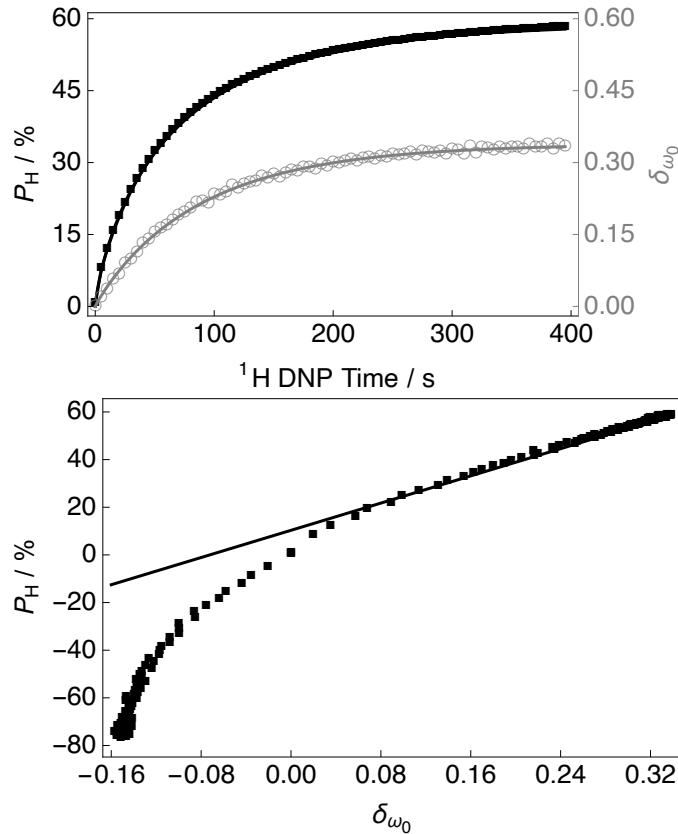
We agree that it would be interesting to compare our peak picking method with a fitting method. However, since the lineshape is more complicated than simply two lines, we propose an alternative here, alongside our original method, which is a simple calculation of an asymmetry parameter as described below, 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.

In the Methods you put a lot of emphasis on the necessity to have cross polarization to be able to use this method. Firstly, I think that with such a level of deuteration, at 6.7 T and with microwave modulation you should be able to achieve a decent SNR with direct ^{13}C DNP as well (see T. Cheng, PCCP 2013, 15 (48)). Secondly, if CP is required, 90% of the DNP users around the world could not take advantage of this method. Lastly, in the motivation you say that “in lack of ^1H rf coil” your method can be useful...How can you do CP without a proton coil???

We agree with the reviewer that CP is not necessary. We simply use it to offer more signal-to-noise on the ^{13}C side of our experiment. We will modify the Methods Section accordingly to stress the fact that CP is optional. Indeed, we measured the asymmetry without CP during with first data points of Figure 2.

IMHO the bare minimum to consider this paper for publication is to address these 3 points: give some theoretical insight about the spectral features; run new experiments to compare the new way of measuring ^1H polarization with the traditional way (preferably using also one more molecule); try without CP.

As suggested by the reviewer, we give more theoretical insights although this is not within the initial scope of this article. We indicate that we compare the measured polarizations with the traditional method, and we will also make it clear that CP is an optional tool, and that the asymmetry can be measured without CP just as well.

Line 11. “is emerging” for a technique invented in 2003 is not appropriate, rephrase like “dDNP allows to prepare proton polarization...”

We will use the suggested rephrasing of the reviewer at the appropriate point in our article.

Line 33. I think there are too many references. You are mentioning hyperpolarization methodology (dDNP, PHIP, SEOP, brute force). I suggest choosing one for each, there is no need to cite 5 or 6 reviews about dDNP. Moreover, the bullet DNP technique from Benno Meier is missing, please include it.

We will make the suggested changes at the appropriate point in our article and add the missing reference to Benno Meier's work.

Line 37. Here you can cite more recent papers. A lot has been achieved from 2013 to 2021 concerning human trials. Some suggestions: Kurhanewicz, J. et al. Hyperpolarized (^{13}C) MRI: Path to Clinical Translation in Oncology. *Neoplasia* **21**, 1–16 (2019); Chen, H.-Y. et al. Hyperpolarized ^{13}C -pyruvate MRI detects real-time metabolic flux in prostate cancer metastases to bone and liver: a clinical feasibility study. *Prostate Cancer Prostatic Dis.* (2019) doi:10.1038/s41391-019-0180-z; Gallagher, F. A. et al. Imaging breast cancer using hyperpolarized carbon- 13 MRI. *Proc. Natl. Acad. Sci.* **117**, 2092–2098 (2020).

We thank the reviewer for their comments on this and will include the above references.

Line 38. It is a super general statement. For sure Prof Jannin is a big player in the field, but I would cite Abragam, Goldman and or Borghini here.

We will make the suggested changes to the main text here.

Line 56. This is conclusion not introduction.

We will move this statement to the conclusion.

Line 65. You use one sample, call it just “the sample”, no need to call it **I**.

We will remove all instances of “**I**” in the main text, and simply refer to the sample as suggested by the reviewer.

Line 77. Add brand and model of microwave source.

This information will be added to the manuscript at this point.

Line 91. How did you calculate 11 ms? Is there a particular reason (demanding spectrometer duty cycle)? In a solid-state saturation sequence the important thing is that the inter-pulse delay is $> 3T_2^*$. I guess your proton line is 40 – 50 kHz broad. Therefore, the T_2^* is 20 μs . With an inter-pulse delay of 100 μs the magnetization in the x-

y plane is completely dephased before the next pulse comes and you do not risk flipping back magnetization on z.

In our experimental setup, the above delay combined with the phase cycling given in Figure 1 of the manuscript is sufficient to remove all magnetization before commencing each experiment. Even if this procedure does not work perfectly, the amount of magnetization maintained is very small compared to what is built-up by 1H DNP or CP.

Line 95. Is the acquisition time of the FID perturbing the signal? Consider rephrasing

A suitable rephrasing will be given at the point in the manuscript.

Line 129. The mw gating paragraph is not Methods, it is discussion how it is written here.

Thank you to the reviewer for pointing this out. It should indeed be included in the Methods Section. We will make suitable amendments to the manuscript to move this information to the Methods section.

Line 151. This paragraph is Discussion not Results. Moreover, the explanation is not clear. Consider rephrasing like: “The microwaves are ON and the ^{13}C nuclear ensemble relaxes towards the spin temperature value it would have achieved in the case of direct DNP (no cross polarization)”. Moreover, there is a 3rd factor to consider. The radical concentration and temperature are in the good range for thermal mixing (Guarin et al, JPCL 2017, 8 (22)); the ^{13}C are polarized, the ^1H are saturated. The two nuclear pools most likely exchange energy via the electron non-Zeeman reservoir. This also affects the time evolution of the ^{13}C until ^1H achieves the same spin temperature.

This text will be moved to the Discussion Section and will also be made much clearer as per the suggestion of the reviewer. We also acknowledge the important contribution from the reviewer regarding thermal mixing. We will add a discussion of this kind at the appropriate place of the main text to address this comment.

Line 173. Are not mirror images of each other with respect to the x-axis or the y-axis?

With respect to a reflection about the y-axis (after a 180° phase correction). This issue will be made clear at this point of the main text.

Line 196. If you use a stretched exponential, technically, you are not using “a sole” build-up time constant, but a linear combination of many (infinite) build up time constants. Moreover, in the discussion I would like to understand why you observe a stretched buildup. Is beta close to 1 or to 0.5? Please provide the value. Could it be that having polarized ^{13}C and depolarized ^1H forces a stretched exponential buildup of protons?

This is a “typo” and will be corrected in the revised version of the manuscript. Beta is close to 0.77 (+ve DNP) and 0.87 (-ve DNP). We originally decided to remove a discussion of this behaviour from the manuscript, since it is a different and complicated issue. It is possible that having highly polarized ^{13}C nuclear spins yields a stretched exponential behaviour of the ^1H polarization build-up or that the methyl group protons polarize rapidly (possibly due to enhanced nuclear spin relaxation from the adjacent ^{13}C spin labelled site) whilst the bulk of the sample polarizes more slowly (likely attributable to ^1H - ^1H spin diffusion processes).

Line 236. I don’t understand this sentence. Can you justify why your calibration curve (pol vs asymmetry) changes slope between positive and negative DNP?

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.

Line 239. This is discussion.

This text will be moved to the Discussion Section.

Line 256. This remains an open question until you don’t measure the polarization in the traditional way.

Figure 5 shows the asymmetry vs. the absolute polarization measured by comparison with the thermal equilibrium NMR signal. We will add a sentence in the caption of Figure 5 to make this point clearer.

Line 87. “DNP equilibrium” not “DNP equilibria”.

This “typo” will be corrected.

Line 87. I have never heard the term “crusher” rf pulses. Consider using “saturating” rf-pulses.

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