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Reply on RC2

Felix Torres et al.

Author comment on "Exploration of the close chemical space of tryptophan and tyrosine reveals importance of hydrophobicity in CW-photo-CIDNP performances" by Felix Torres et al., Magn. Reson. Discuss., https://doi.org/10.5194/mr-2021-1-AC2, 2021

Dear Reviewer,

Thank you for the careful reading and the interesting comments. Your statement in short is that the use of constant-wave (CW) photo-CIDNP is irrelevant while we are absolutely convinced that the approach we have is relevant in the purpose of our ultimate goal in bringing CW photo CIDNP into the field of biomedical research. The different views we attribute to the point of perspective. From a physical point of view (which is yours) we fully agree with you that the elucidation of the mechanism of photo-CIDNP which is a highly complex interplay between two molecules can only be elucidated with time resolved photo-CIDNP (which we recently have done in collaboration with Alexandra Yurkovskaya for the specific molecule HOPI). However, when exploring the chemical space towards the elucidation of (many) highly active photo-CIDNP active compounds at concentration interesting for biomedical research requiring evtl. CW-photo-CIDNP, a screening approach is an alternative allowing for the establishment of a qualitative correlation between properties and polarization observed (our approach).

Please allow us to provide more detailed explanations for our perspective. We sincerely hope this will change your mind on the global significance of this work.

"his qualitative study of hydrophobicity could only be relevant, if the authors had applied time resolved detection and analyzed the CIDNP signals in the products of geminate recombination, often called geminate CIDNP. Under continuous illumination, however, the resulting CIDNP effects are formed as a result of a complex interplay of spin and molecular dynamics of free radicals, but it is wrong to predict CIDNP intensities based alone on the properties of the reacting diamagnetic molecules as it was done in the manuscript. As is known, some properties of free radicals can be very different from the properties of molecules in the diamagnetic state. For example, the pKa values of radicals (tryptophan and tyrosine) and the same molecules in the diamagnetic state differ by several units. Spin is the magnetic moment of nuclei and electrons; therefore, the "key players" in the formation of geminate CIDNP are the magnetic properties of radicals, as shown by R. Kaptein, namely the hyperfine coupling (HFC) constants, the difference in the g-factor of radicals in the spin-correlated pair, and the magnetic field strength. Polarization in an F-pair, as is also well known, depends on the competition between the rate of paramagnetic nuclear relaxation and the bimolecular rate of radical recombination. They can be different too for the set of chosen compounds. In addition, paramagnetic nuclear relaxation in radicals is determined by the anisotropy of the HFC. None of these

parameters is mentioned in the manuscript for the ten compounds studied. I'd like to stress, that Kaptein's work in the field of CIDNP goes much deeper than the simple sign rules"

The reviewer points out with clarity the theoretical frame of photo-CIDNP. He demonstrates the difficulty to correlate the intensity of the spin sorting happening during to geminate polarization and the final intensity when light is irradiated for several seconds with as part free radical encountering (F-pair). Therefore, the reviewer rejects the interpretation of CW-photo-CIDNP results as they may not correspond to what would be observed from TR-photo-CIDNP experiments. However, the authors have no such intention of performing TR-photo-CIDNP experiments to explore fine radical-pair mechanism. On contrary, the goal of the authors is to explore the possibility of implementing CW-photo-CIDNP as a simple method to obtain hyperpolarization in solution state biomedical NMR. The reason for our choice in using solely CW-photo-CIDNP are the following:

1) We want to promote photo-CIDNP as a readily implementable technique for solution state biomedical NMR polarization. CW-lasers prices are relatively attractive (2000 USD) and extremely simple of use. Because of this, CW-photo-CIDNP impact could be important for the biomedical NMR community contrasting the very expensive DNP approaches.

2) The concentrations used are significantly lower than what is typically used in TR-photo-CIDNP. As an example, while we use 0.1 mM of molecule and 0.02 mM of photosensitizer, Saprygina et al. Use 1.1 to 40 mM of molecule and 2 mM of photosensitizer (Figure 4 of *Saprygina et al. J. Phys. Chem. A 2014, 118, 339-349*). In biomedical research the use of molecule concentration in the range of a few microM is essential and was achieved by other studies such as Okuno et al. J Phys Chem B, 2016, 715-723, who demonstrated the use of CW-photo-CIDNP to detect low micro-molar concentration of tryptophan with fluorescein.For this reason, we perform CW-photo-CIDNP, to reach the maximal polarization achievable to measure the molecules and not to understand the mechanism of polarization. It is about applying photo-CIDNP. We are happy to share the polarization build-ups with increasing irradiation time to support our statement in a revised version of the manuscript.

3) The scope of the study is to answer the question: what is the impact of chemical modification on the polarization performance of CW-photo-CIDNP? We provide here a view on the effect of side chain modification on the performances. We provide here a result which is the trend of the performance according to hydrophobicity in CW-photo-CIDNP and with this approach we were successful in finding the compound 3-(2-(piperazin)ethyl)-indole to be polarizable by a factor of 100 at 600 MHz on 1H just by screening.

4) As there is no simple correlation between TR-photo-CIDNP and CW-photo-CIDNP results, and we are interested in the performance of CW-photo-CIDNP. It would be irrelevant to interpret results from TR-photo-CIDNP to predict what would happen in the CW regime. For this reason, we opted for an empirical approach consisting in exploring the chemical space in the conditions of the final application which is CW-photo-CIDNP.

We regret that we may have not make this perspective clear enough in our manuscript. We are happy to correct that if necessary.

"The CIDNP method in its continuous mode should be applied with great care when used for any quantitative analysis. The concentration of radical pairs in the reaction of a triplet dye with a quencher essentially depends on the quenching rate constant. In the cited work of Saprygina et al. J. Phys. Chem. A 2014, 118, 339-349, the quenching rate constants were obtained from optical studies, the conditions for the time resolved

experiment were carefully adjusted, and only geminate CIDNP was considered for quantitative analysis of the pH dependence. This was not done in the paper under review."

This study, conducted under TR-photo-CIDNP, is focusing on the quenching rates in conditions relatively far than the conditions we have been using during this study. The concentrations are significantly higher (see point 2) and the type of dye is different. Indeed, the TCBP which is used, possessed 4 negatively charged carboxylates, the dyes that we have been using are significantly less charged. The pH dependencies could be provided, if required by the reviewer.

"There is a well-documented study where the terms "hydrophobicity" and "hydrophobic collapse" were shown to be misleading for explanation of the absence of CIDNP signals of tryptophan residues in the unfolded HEWL protein under cw-illumination. This work is cited as reference 8 in the reviewed manuscript. In ref 8, the time-resolved CIDNP detection revealed CIDNP tryptophan signals of similar strength at the geminate stage for the unfolded and native state of HEWL. It means, that hydrophobicity was not a relevant parameter for the description of CIDNP in the native and unfolded state of HEWL. Instead, the reaction of intramolecular electron transfer from tyrosine to the tryptophan radical on a microsecond time scale in the unfolded protein was found to be the main cause of a decrease in the Trp signal and an increase in the tyrosine signal."

This interesting case study demonstrates the presence of intramolecular electron transfer and its effect on photo-CIDNP anomalous lines intensities. However, the phenomenon described here does not apply to our study, as we do not have intramolecular electron transfer. Moreover, we took care of comparing only molecules sharing the same aromatic system in order to minimize the difference in the magnetic parameters. This idea is already present in the paper from Saprygina et al., when the effect of charge is compared by modifying the side chain.

"A direct comparison of the CIDNP data obtained under continuous illumination without measuring the quenching rate constant is inappropriate, since different concentrations of the quencher and different rate constants lead to different concentrations of the formed pair of geminate radicals."

As stated before, this is why we focus our work on chemical exploration and we take an empirical approach. A bottom-up approach (from ns to second irradiation) will find potentially many ambushes in the context of chemical space exploration due to the complexity of the mechanism.

More importantly, the quenching rates are not measured in the present manuscript.

"I highly recommend reading the following articles by Robert Kaptein: Kaptein, R.; Den Hollander, J. A. Chemically induced dynamic nuclear polarization. X. On the magnetic field dependence. J. Am. Chem. Soc. 1972, 94 (18), 6269-80. Kaptein R. Chemically induced dynamic nuclear polarization. VIII. Spin dynamics and diffusion of radical pairs. J. Am. Chem. Soc. 1972, 94 (18), 6251-62. Stob, S.; Kaptein R. Photo-CIDNP of the amino acids. Photochem. Photobiol. 1989, 49 (5), 565-577."

We would like to thank the reviewer for the relevant suggested reading. Field dependency, with the two different dyes would be interesting to demonstrate the equivalence of the magnetic parameters, however we do not have a spectrometer adapted to this kind of study.

"The over-interpretation of the small data set as presented here is misleading. Also, the historical overview of Kaptein's contribution to CIDNP theory is rather superficial, because it does not go deeper than just application his simple rule for the polarization sign.

With my regret, I recommend to reject the manuscript in its present form."

As of the data set, we are happy to operate a statistical test to demonstrate the significance of the trend line.

We are sorry to see that the illustration of the Kaptein's rules were not appreciated. To our knowledge, the HOPI and the dH-TRP are the only biological molecules for which the polarization sign alternates when the dye is switched.

We hope that the careful reading of our response to the reviewer's comments will change his (your) opinion on our work. We are happy to modify parts of the manuscript to enlighten better the purpose of our scientific approach, as it may have not been clear enough.

Sincerely yours

The authors