**1st reviewer**

Before I will comment further on the paper I have to say that this manuscript is close to being unreadable without an intense recourse to the first paper on the subject published by the same author in Angew. Chemie.

Reply: As stated in the manuscript, the focus of the current work is on "extending the applicability of the P3D simulation model". The P3D simulation has already been published in Angew Chem and key features of the method were summarized in the current manuscript. We are convinced that this procedure is fully in agreement with scientific practice.

Furthermore, the manuscript does not include any Supporting Information, which additionally compromises the comprehension of its scientific content.

Reply: We included all results in the tables and figures of the manuscript.

This means that this manuscript cannot stand on its own which is a very unpleasant fact for the reader (and the reviewer!).

Reply: Scientific findings/publications are generally based on previous studies and the corresponding information is provided through references. In addition, we summarized the key aspects of the P3D simulation, which was previously published in Angew Chem, in the section "Methods" of the manuscript.

Moreover, the introduction should be rewritten in order to give credit to the people doing pioneering work in the field. Ad Bax may have coined the term RDCs, but this doesn't mean that he invented the technique or that the paper of Bax and Tjandra was a seminal one. Courtieu, Prestegard, Lesot and of course Emsley to name just a few, should be mentioned here. [1-4] In general the citation policy of the manuscript is far from being of good scientific practice (the LLC-phase literature, [5-7] the structure of PBLG – its helicity is known since 1954[8] not 2009 and so on. . .). But apart from these formal deficiencies, the scientific value of the paper is more than questionable.

Reply: We added the suggested references.

P3D simulations are based on MD derived PBLG snapshot geometries, around which a cubic grid is placed. The molecular geometries of the analytes under investigation (strychnine, IPC, and sucrose) are placed on this grid, and Boltzmann-averaged RDC sampling is carried out by evaluating the averages over all grid points and all analyte orientations. The interaction between the analytes and the alignment polymer PBLG are evaluated purely on the basis of static (pre-computed grids) interactions that include steric (excluded volume) and electrostatic (based atomic charges) terms. This type of simulation represents a fast, but surely very crude model to simulate the analyte-polymer interactions as well as the alignment process. Not only are vdW interactions completely ignored, but also all dynamic and entropic contributions to the alignment process are neglected. The rather coarse-

grained grid used in the simulations (grid spacing 0.4A) and the rough sampling of molecular orientations (1800 per grid point) must necessarily lead to large uncertainties. It is not even clear, whether a cubic grid superimposed to a rod-shaped, cylindrical polymer may introduce systematic errors. Certainly, the excluded-volume simulations are apt to introduce large degrees of order even at large polymer-analyte distances when first contacts become possible. The Boltzmann-averaging is highly sensitive towards energies used, and simple electrostatic interactions using static molecular models are with some certainty crude oversimplifications.

Reply: In fact, in our previous publication, one of the most exciting conclusions was that just the steric and electrostatic factors are able to discriminate the correct diastereomer and we showed that with 6 different small molecules. The grid spacing and the number of molecular orientations were selected as described in our Angew Chem paper, i.e. smaller grid spacings or increased numbers of orientations did not significantly change the predicted RDCs.

The RDCs obtained from the P3D-PBLG simulation are then compared to experimental data obtained from diverse alignment media (Figure 2), though obviously these media do have vastly differing alignment properties (see Figure 2, RDCs across different alignment media also differ vastly in their magnitude). It is unclear how the P3D derived RDCs were scaled to account for different degrees of order

Reply: Please note that the different magnitude is irrelevant when the Pearson correlation coefficient (R) is used. In addition, when calculating the Q factor, the RDCs were normalized by the slope of the linear fitting (as stated on line 90: "*the RDC quality factor Q = rms($D^{exp}$-$D^{P3D}$)/rms($D^{exp}$) scaled by the slope of the $D^{exp}$ vs $D^{P3D}$ fitting*")

And how a PBLG simulation should compare to chemically different alignment media such as PELG, PMMA, PS, PA, etc.

Reply: One of the aims of the study is to analyze how the small molecules align in different alignment media, that's why we compare the alignment in chemically different alignment media using the simulated PBLG as reference.

Even for the simulated and experimental data of PBLG, there are huge discrepancies between the individual RDCs of up to 115 Hz (Figure 2, strychnine, CH3 RDC)!!!

Reply: This data is the same as in our previous publication. Of course, errors in the simulation can affect more some of the RDCs than others. This is a consequence of the fact that we didn't optimize the parameters independently for each of the simulations in order to avoid a bias. While we do not claim that our simulations are perfect, the correlation between experimental and P3D-predicted RDCs are consistently of high quality (as demonstrated by high Pearson's correlation coefficients).

The invalid comparisons are continued in Figure 3, were matrices of Pearson correlations are given in color-coded form. Many of these correlations are negative (marked by dots in Figure 3), and thus raise additional doubts on the assumptions made by the P3D simulations

Reply: We described in the manuscript the reason of negative correlations for different alignment media (line 236):
"*The negative slope indicates that the major alignment axis in PMMA is oriented orthogonal to the field, while PBLG aligns with its helix axis parallel to the magnetic field. Indeed, the PMMA gel was compressed, while the stretched PS gel displayed a positive correlation with the P3D-calculated RDCs. In other words, strychnine has in PMMA a highly similar alignment tensor as in PBLG/PS but with an opposite sign of the axial component of the alignment (Da)*."

By the way: the color-code used in Figure 3 is also highly misleading, as “green” color obviously indicate bad correlations.

Reply: We used red as hot color to indicate a good correlation. In the revised version of the manuscript, we changed green to cyan in Fig. 3.

The obviously invalid cross-alignment media/P3D – PBLG comparisons are then continued in Figure 4 for different diastereomers of strychnine, yet it remains unclear how such a crude alignment simulation that neglects almost all relevant interactions (including all dynamic alignment polymer properties) can differentiate the molecular configurations.

Reply: Please, read our Angew Chem paper, in which we demonstrate with 6 different molecules & several different tests that the P3D simulation indeed works.

The investigations are then extended to the more flexible structure of sucrose, were only eight out of 23 RDCs that have been reported in the literature have been used. It is open to speculation why only this small subset of experimental data is used – may be the rest of the data doesn’t fit well?

Reply: With due respect, this is not open to speculation; we explain why in the manuscript (lines 322-324): "*Following the same rationale as before (Ibáñez de Opakua et al., 2020), we selected the one-bond CH RDCs (Fig. 5b) because they are the largest RDCs in small molecules, i.e. can be measured with high accuracy, and there is less ambiguity in the assignment*."
Please also note that the 23 anisotropic NMR parameters, which were reported by Ndukwe and colleagues, are not only RDCs, but include 12 RCSAs. This is also stated on lines 316-318 of our manuscript: “*On the basis of 11 RDCs and 12 RCSAs, the conformational ensemble of sucrose in … (Ndukwe et al., 2019)*.“
From these 11 RDCs, 3 belong to averaged RDCs from both geminal protons of a CH2 group that are not independently assigned. Thus, no cherry picking was done.

Three different sucrose conformers are evaluated, the geometries of which were taken from the literature. Figure 5 details the results for the three individual sucrose conformers, where large deviations of the experimental and calculated RDCs are observed indicated by significant deviations of the correlations from the diagonal of the plots given in Figure 5c. The relative contributions of the sucrose conformers are then optimized by maximizing the RQ parameter, and an "an almost perfect fit (R=0.996; QS=0.076)" was finally obtained (Figure 6). However, given the sparsity of the NMR data used, and the number of conformers evaluated, it is clear that a multi-conformer fit represents sort of an over-fitting scenario, which is not supported by an adequate amount of experimental data.

Reply: A better agreement between experimental and predicted RDCs is of course reached as one increases the degrees of freedom. But the main aim here was to reproduce - with sparse data - the results obtained using SVD (by Ndukwe and colleagues). In contrast to SVD, the P3D-based approach does not rely on the assumption that the 3 conformers have an identical alignment tensor.

Given the wealth of conformational data available for a common compound such as sucrose, a more thorough evaluation against the literature data available is mandatory. The most highly populated structure of sucrose seems to be close to its solid-state conformation, but this must not necessarily be the correct description for the flexibility of sucrose as the solution conformation may differ significantly therefrom. The claim stated in the conclusion of this paper that "molecular alignment simulations might – with further improvements – become crucial for the determination of the absolute configuration" is, based on these results, an unjustified expectation as these simulations supposedly must treat molecular interactions on a much finer and much more detailed level, which even may be out-of-reach altogether at least in the (near) future.

Reply: Thanks for the suggestion. To better stress the need for further improvements, we state in the revised version of the manuscript: *"To determine the absolute configuration, atomistic descriptions are required that link the NMR anisotropic parameters obtained from chiral alignment media with the correct enantiomer. A next step towards this goal could be the inclusion of specific interactions between the solute and the alignment medium, for example salt bridges, into molecular alignment simulations.*"

In view of the roughness of the model, the complete neglect of dynamic effects, the over-simplifications of the molecular interactions, and the invalidity of the cross-alignment comparisons employed here I cannot see how these P3D simulations may be used to elucidate even the relative configuration of slightly more complex natural products of unknown configuration beyond reasonable doubt. For these reasons publication in Magnetic Resonance is not recommended.

Reply: With due respect, we do not agree. While certain interactions are currently not used in the P3D simulations, P3D simulations do take into account the molecular structure of the alignment medium and the solute, as well as steric and electrostatic interactions. In the Angew Chem paper we also performed several tests, which demonstrated that the simulations are robust against dynamic changes in the structure of the alignment medium

and the solute. In addition, we already showed in the Angew Chem paper that the relative configuration of small molecules can be determined using P3D. Therefore this is not the focus of the current manuscript.

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**2nd reviewer**

There are some discrepancies in the sucrose data that should be clarified. In figure 5 the numbering of the sucrose structure (a) and the notations in the table (b) don't agree. CH vectors with numbers 1,2,3 and 4 in the structure displayed are nearly parallel and should have very similar RDCs. The data appear to come from the Ndukwe reference, which does show this trend. In the manuscript table RDCs with the Ndukwe values are numbered 5,9, 10 and 11.

Reply: Thanks for detecting this mistake. We corrected the numbers in the revised version of the manuscript.

Also, it is not clear where the populations are coming from. These appear to be fitted parameters? The text seems to suggest that the populations are consistent with free ender estimates on line 315. They are not. Also, some comment might be made in comparison to water MD simulations (Case) where only M1 and M2 are highly populated with a difference of only 0.3kcal, something more in line with populations.

Reply: The populations of Fig. 5 come from the Ndukwe reference and the populations of Fig. 6 are fitted. We added a comment to the figure legend to avoid this confusion. The populations are based on the three conformations present in the M1 (called S3-i, S3-iii and S3-iv in that paper). The structures were taken from table S14. The description of the populations is just a short summary of the work from Ndukwe et al. We are not trying to suggest that the free energy from DFT explains the calculated populations. The information is just descriptive.

There are a few places that the text could be improved for clarity: Line 31 – not clearly worded. Maybe: "alignment requires a minimum concentration of lyotropic medium and then often aligns strongly at this concentration, resulting in .."

Reply: Thanks, we changed it to: "*Alignment requires a minimum concentration of lyotropic medium and often aligns strongly at this concentration, which limits the tunability of the alignment strength*."

line 87: RQ might be defined here as opposed to much later.

Reply: Changed.