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Probing long-range anisotropic interactions – A general and sign-sensitive strategy to measure ^1H - ^1H residual dipolar couplings as a key advance for organic structure determination

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Abstract

Residual dipolar couplings (RDCs) are amongst the most powerful NMR parameters for organic structure elucidation. In order to maximize their effectiveness in increasingly complex cases such as flexible compounds, a maximum of RDCs between nuclei sampling a large distribution of orientations is needed, including sign information. For this, the easily accessible one-bond ^1H - ^{13}C RDCs alone often fall short. Long-range ^1H - ^1H RDCs are both abundant and typically sample highly complementary orientations, but accessing them in a sign-sensitive way has been severely obstructed due to the overflow of ^1H - ^1H couplings. Here, we present a generally applicable strategy that allows the measurement of a large number of ^1H - ^1H RDCs, including the sign, that is based on a combination of an improved PSYCHEDELIC method and a new selective constant-time β -COSY experiment. The potential of ^1H - ^1H RDCs to better determine molecular alignment and to discriminate between enantiomers and diastereomers is demonstrated.

Main manuscript

Residual dipolar couplings (RDCs) have proven to be a powerful tool for conformational analysis, as well as configuration and constitutional elucidation for organic molecules or natural products.^{[1],[2]} In solution, RDCs (D_{ij}) only appear when a bias in molecular orientation relative to the magnetic field is present, in most cases achieved by introducing the molecule of interest into a dilute liquid crystalline phase or a stretched or compressed gel.^[1a-e] Like the scalar coupling (J_{ij}), the RDC between two nuclear spins i and j results in a splitting of the NMR signal. However, unlike the scalar coupling, which manifests as a through-bond interaction, the dipolar coupling is a through-space interaction, its value and sign determined by both the internuclear distance and the orientation of the internuclear vector relative to the magnetic field. It is the latter that makes the RDC such a powerful parameter for conformation and relative configuration determination, since each RDC reports on local molecular geometry relative to the same external reference, probing long-range structural order. However, especially in flexible molecules,^[2d,3] an effective analysis requires a large number of RDCs, importantly with internuclear vectors sampling a maximum of unique orientations. Thus, even if several one-bond ^1H - ^{13}C RDCs are measurable, if they all point in the same direction, e.g. as in β -glucose, alignment tensor determination by standard SVD fitting is precluded (although sometimes diastereomer identification may still be possible using RDC values directly^[2e,4]). Therefore, towards the application of RDCs in increasingly complex structure elucidation cases, there is a clear incentive to obtain RDCs between nuclei separated by multiple bonds, which typically sample orientations complementary to the more readily available one-bond RDCs. Unfortunately, such long-range RDCs are challenging to determine in a sign sensitive way. Indeed, most experiments yield the absolute value of the sum of both couplings $|T_{ij}| = |J_{ij} + 2D_{ij}|$ and, since for long-range couplings J_{ij} the magnitude is generally smaller or comparable to $2D_{ij}$, this results in sign ambiguity for D_{ij} . Although it is possible to perform structure optimization based on the magnitude of T_{ij} ,^[5] this inevitably amplifies restraint ambiguity and sacrifices much of the valuable information content. Until now, the pursuit of long-range RDCs was mostly aimed at heteronuclear (^1H - ^{13}C) couplings.^[5-6] These methods work well in particular cases, but the low sensitivity at ^{13}C natural abundance is a severe limitation, while obtaining the RDC sign remains challenging. ^1H - ^1H RDCs, instead, due to the high gyromagnetic ratio involved, would ensure both the highest sensitivity and a multitude of relatively large dipolar interactions. Therefore, a generally applicable technique for measuring both size and sign of ^1H - ^1H long-range RDCs is highly desirable.

Unfortunately, the abundance of long-range $^nD_{\text{HH}}$ couplings also results in multiplets that are severely broadened compared to isotropic samples (Fig. 1). This makes individual line splittings unresolvable, which is a key issue. Furthermore, this increases the likelihood of overlap between multiplets, exacerbating spectral crowding. Although in some applications fortunate spectral circumstances — often aided by ^1H - ^{13}C spectra — allows extraction of a limited number of ^1H - ^1H RDCs,^[4,6c,7] generally the amalgamation of all aforesaid complications have resulted in ^1H - ^1H RDCs remaining elusive for structural and conformational analysis of organic compounds, despite their high potential. Some limited precedence exists of methods specifically designed to measure T_{HH} magnitudes, but not signs, without the aid of heteronuclear couplings. These experiments are either doubly selective^[8] or specifically designed for oligomeric compounds.^[9] Although these work well in particular cases, they fail or become insensitive when coupled partners have close chemical

shifts. Some sign-sensitive methods exist for proteins, but these require $^{13}\text{C}/^{15}\text{N}$ isotope enrichment and are not applicable to organic molecules.^[10] Ideally, a strategy to measure ^1H - ^1H RDCs at natural isotope abundance should report a maximum of RDCs while being minimally constrained by the overflow of couplings and spectral overlap, allows sign measurement, and is sensitive, *i.e.*, does not make use of low-natural abundance heteronuclei. Here we propose an effective, generally applicable two-step approach to measure both size and sign of ^1H - ^1H RDCs of molecules at natural abundance.

In a first step, the size of the couplings is measured, which involves the recently emerging pure shift methods.^[11] In an aligned sample, these methods dramatically increase resolution, even more so than in the isotropic case where only J-couplings are present (Fig. 1). Under aligned conditions, we generally find the PSYCHE method to be the most robust option.^[12] Whereas the effectiveness of bandselective, Zangger-Sterk and BIRD based methods^[11c, 13] are all contingent upon chemical shifts or homo- or heteronuclear coupling values, PSYCHE delivers high quality and sensitive spectra free of such constraints, which is key given the heightened intricacy of the coupling network in the spin system. The $^nT_{\text{HH}}$ coupling magnitudes can then be measured at pure shift resolution using the PSYCHEDELIC experiment (Fig. 2).^[14] PSYCHEDELIC provides near-identical spectral quality, sensitivity and spectral resolution as the pure shift PSYCHE experiment, but adds a 2D J-resolved (2DJ) dimension where couplings to a chosen proton are measured as simple doublets with purely absorptive lineshapes. This circumvents all complications arising from the excess of splittings in full multiplets. Figure 2 demonstrates that even small ^1H - ^1H total couplings of just a few Hz can be straightforwardly extracted from isopinocampheol ((+)-IPC) aligned in a lyotropic liquid crystalline phase of poly(γ -benzyl-D-glutamate (PBDG) in CD_2Cl_2 ,^[15] with some splittings purely resulting from dipolar couplings alone. Like this, RDCs between protons several bonds apart can be detected.

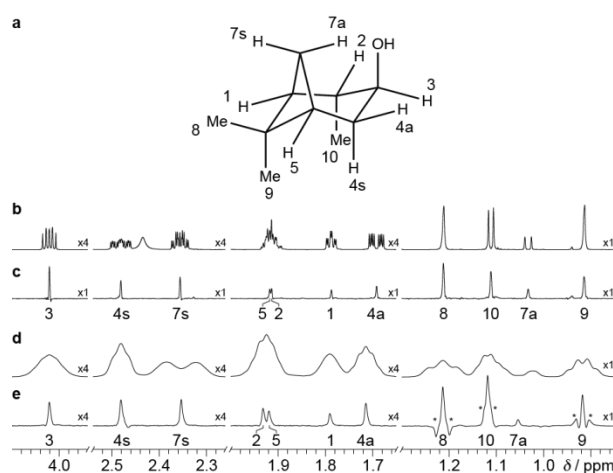


Figure 1: (a) Structure of (+)-IPC; (b) isotropic 1D ^1H spectrum in CD_2Cl_2 ; (c) TSE-PSYCHE^[12b] pure shift spectrum in CD_2Cl_2 (isotropic); (d) anisotropic 1D ^1H spectrum in PBDG/ CD_2Cl_2 ; (e) TSE-PSYCHE pure shift spectrum in PBDG/ CD_2Cl_2 (anisotropic). For each segment in the spectra, intensities were scaled as shown. The sensitivity of the TSE-PSYCHE spectra are about one order of magnitude lower than the 1D ^1H spectra. Assignments are shown below the pure shift spectra. Asterisks indicate artefacts due to the $^2D_{\text{HH}}$ coupling in methyl groups (see supporting information).

It should be noted that the $^2D_{\text{HH}}$ couplings within methyl groups lead to *D*-modulated doublet responses in PSYCHE and PSYCHEDELIC that are superimposed on the desired signals (Fig. 1e and Fig. 2), resulting from z-COSY-type transfer between the chemically equivalent protons within the methyl group (see supporting information). However, in most cases these

have no or minimal complications for long-range coupling measurement, and it should be remarked that other pure shift methods are fully ineffective at homodecoupling such equivalent protons. For this reason, PSYCHEDELIC greatly improves the applicability of ^1H - ^1H RDCs for enantiodiscrimination compared to previous methods,^[8d, 8e, 16] as it experiences no negative impact from $^2D_{\text{HH}}$ couplings in methyl groups and provides excellent results even in regions of the spectrum that are hard to decouple with selective pulses. The supporting information contains an example on ibuprofen with comparison to previous methods.

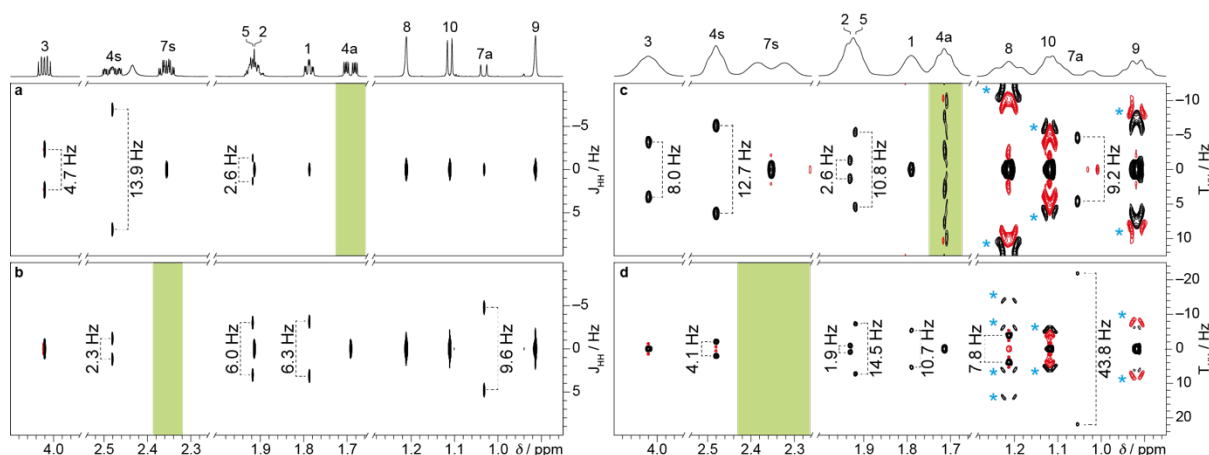


Figure 2: TSE-PSYCHEDELIC spectra of (+)-IPC in CD_2Cl_2 (a, b) and in PBDG/ CD_2Cl_2 (c, d), selecting protons 4a (a, c) and 7s (b, d), respectively. Green rectangles indicate the region covered by the bandselective pulse. Asterisks indicate artefacts due to the $^2D_{\text{HH}}$ coupling in methyl groups (see supporting information).

The possibility of large RDCs and the increased coupling network compared to isotropic conditions make additional spectral responses resulting from strongly coupled protons more likely than under isotropic conditions. Therefore, we developed an improved triple spin-echo (TSE) PSYCHEDELIC sequence, where the original four hard 180° pulses are replaced by two frequency-swept pulses applied during gradients (Fig. 3).^[12b, 17] Such pulses minimize the ‘mixing effect’ between strongly coupled protons, and attenuate interfering responses. Furthermore, the new experiment does not require shearing the $2D_J$ spectrum to separate chemical shifts from couplings, thereby avoiding lineshape distortions.^[18]

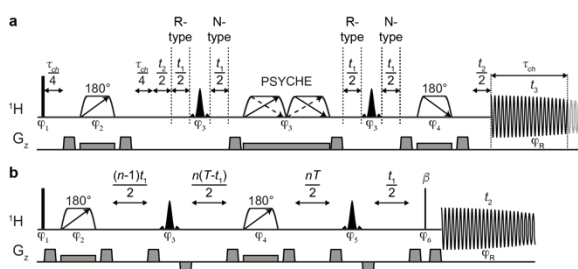


Figure 3: (a) TSE-PSYCHEDELIC and (b) CT- β -COSY pulse sequences. Wide rectangles indicate 90° RF pulses, the narrow rectangle is an RF pulse with flip angle β , typically 20 - 30° . Trapezoids with single arrow indicate frequency-swept (chirp) 180° pulses. PSYCHE is represented by two trapezoids with double arrows, indicating low-power chirp pulses of net flip angle β (typically 10 - 25°), sweeping frequencies in opposite directions simultaneously.^[12a] Black-filled shaped pulses are selective 180° pulses applied to the selected proton. Pulsed field gradients are shown on the line G_z . In (a), both Normal (N) and Reverse (R) type spectra are recorded separately, and differ in the location of the t_1 delays as indicated.^[19] The highlighted part of the FID of duration τ_{ch} in (a) shows the chunk of data acquired for each increment in t_2 (interferogram acquisition).^[11c] In (b), T is the maximum duration of t_1 , and n is a factor by which the selected ^1H - ^1H coupling can be upscaled in F_1 . Phase cycles are given in the supporting information.

Finally, the ^1H - ^1H RDC sign needs to be determined in a second experiment, as the TSE-PSYCHEDELIC experiment only measures coupling magnitudes. Sign information of ^1H - ^1H couplings is ideally obtained using COSY-type experiments that retain ^1H spin-states of passive protons in the polarization transfer step, by using low flip angles (P.E.COSY, z-COSY)^[20] or spin-state selective procedures (E.COSY, soft-COSY).^[21] In such spectra, the cross-peak between two multiplets displays a tilted 2D pattern depending on the signs of couplings to other ‘passive’ protons, thus reporting on the relative signs (*i.e.*, equal or opposite) between these couplings. Unfortunately, just as in 1D ^1H spectra, the extensive ^1H - ^1H long-range RDC network results in complicated and broad cross-peaks, and tilted patterns from particular pairs of couplings are obscured (Fig. 4b-d). We therefore designed a new experiment, CT- β -COSY, that in F_1 only features couplings to a chosen passive proton using a selective pulse, and thus has similar selection constraints as the PSYCHEDELIC experiment. As illustrated in Fig. 4f-h, this makes assessment of the relative ^1H - ^1H coupling signs straightforward. The new experiment is based on a simple gradient N-type COSY, with the final pulse given at a low flip angle β (Fig. 3b). Pure shift and selected coupling evolution occurs via a combination of constant-time (CT) t_1 incrementation and the selective pulses. Similar to the TSE-PSYCHEDELIC sequence, frequency-swept 180° pulses applied during magnetic field gradients are needed to suppress additional responses from strongly coupled protons, which are known to be severe in constant-time experiments.^[17, 22] The experiment cannot be designed to feature pure absorption mode lineshapes (see supporting information), implying the coupling magnitudes cannot be as accurately measured as in TSE-PSYCHEDELIC. Both experiments thus provide complementary information.

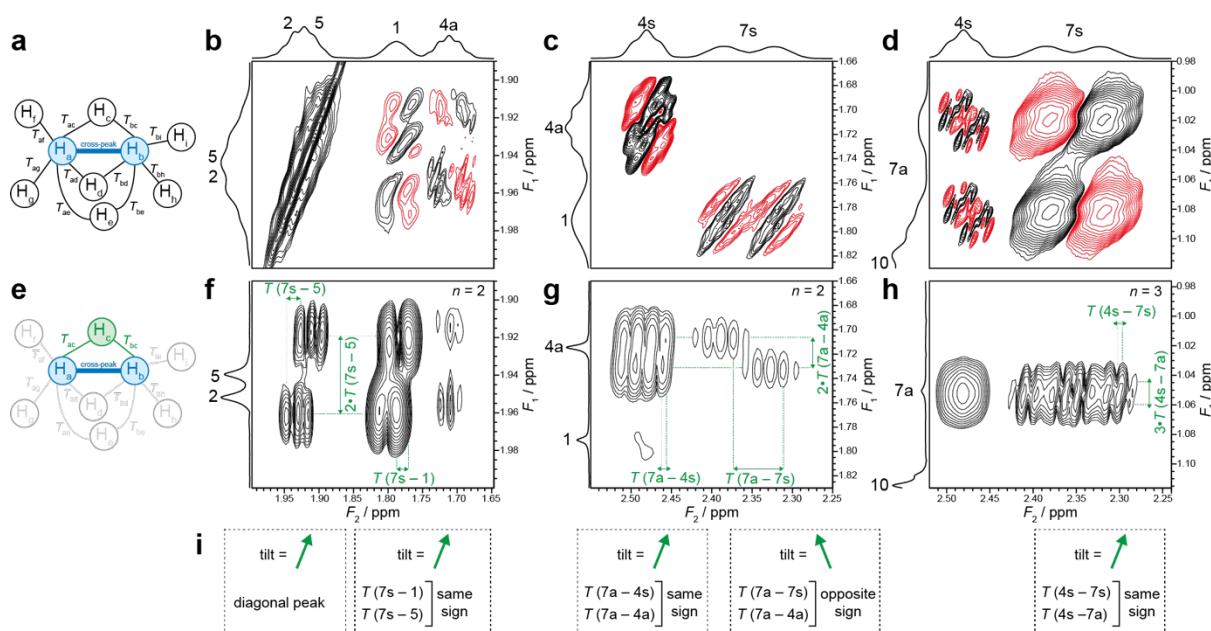


Figure 4: (a) Illustration showing that a z-COSY cross-peak between H_a and H_b is complex due to many coupling partners, and shows several tilted 2D patterns due to multiple mutual coupling partners. (b-d) Extracts from z-COSY 2D spectra^[20c] of (+)-IPC in PBDG/ CD_2Cl_2 . (e) The CT- β -COSY experiment selects a single coupling partner H_c , so that a single tilted 2D pattern resulting from the T_{ac} and T_{bc} couplings are visible. (f-h) Extracts from CT- β -COSY spectra that selected protons H7s (f), H7a (g) or H4s (h), and with upscale factor n in F_1 as shown. (i) The sense of the tilted 2D patterns either follows or opposes the diagonal, revealing relative coupling sign information.

From several CT- β -COSY experiments, each selecting a different passive proton, most relative ^1H - ^1H coupling signs can be deduced. However, for absolute sign determination, one

or more coupling signs must be known. For non-flexible molecules, this, to a certain extent, can be assessed through trial-and-error data fitting of the alignment tensor. More elegantly, alternative experiments such as P.E.HSQC, P.E.HSQMBC, JHH-TOCSY or signed COSY^[7b, 23] can be used that provide the ^1H - ^1H coupling sign relative to the large one-bond $^1T_{\text{CH}}$ coupling, which in practice may always be assumed positive (see supporting information). However, the aforementioned experiments have much lower sensitivity due to the ^{13}C natural abundance, are mostly restricted to specific types of molecular fragments, and do not feature pure shift resolution. Ideally, if sensitivity allows, a limited number of ^1H - ^1H coupling signs are determined using such experiments, and this is subsequently greatly extended using CT- β -COSY.

Just as for isotropic conditions, the only cases where our two-step approach will fail to unambiguously extract individual coupling information is when both coupling partners cannot be selectively irradiated without a third mutual coupling partner, or when protons are very strongly coupled. The latter presents a fundamental limit imposed by spin dynamics, and can be due to either chemical shift degeneracy or a too strong molecular alignment. The approach thus works best under conditions where the degree of alignment strikes a balance between inducing sufficiently strong dipolar interactions and avoiding the breakdown of the weak coupling approximation.

Using this strategy, 32 $^nT_{\text{HH}}$ couplings with sign could be experimentally determined for (+)-IPC in PBDG/ CD_2Cl_2 , including RDCs between protons separated by 5 or 6 bonds. This large ^1H - ^1H RDC dataset not only complements the one-bond ^1H - ^{13}C RDCs for alignment tensor calculation – both datasets showing high consistency – it could also be used to independently calculate the alignment tensor with excellent reproducibility and allowed discrimination between different diastereomer structure proposals (supporting information). To the best of our knowledge, this is the first time this is achieved using ^1H - ^1H RDCs alone for small molecules. Similarly, 28 ^1H - ^1H RDCs, 18 with sign, were experimentally determined in (+)- β -pinene in a recently developed thermoresponsive alignment-medium co-PBLA-PPLA/TCE.^[24] β -Pinene is a case in which the determination of the alignment tensor orientation is notoriously ill-posed due to undersampling of unique orientations by $^1D_{\text{CH}}$ alone. Thus comparisons of tensor orientations – as is done frequently when assessing the enantiodiscriminating abilities of new alignment media – are error prone. By adding the $^nD_{\text{HH}}$ couplings in the analysis, the precision of the alignment tensor calculation improved significantly (supporting information). This demonstrates the potential of long range ^1H - ^1H RDCs towards improving data fitting statistics, and thus discriminating enantiomers and/or diastereomers.

In conclusion, we have developed a general and sensitive approach to measure ^1H - ^1H RDCs with a minimum of spectral constraints and with a means to experimentally establish their sign. We expect it to find wide application within, for instance, natural product configuration determination, enantiomeric excess determination, or conformational analysis of organic compounds. Experimental sign determination especially opens the door for use of ^1H - ^1H RDCs in flexible molecules,^[3b] where signs cannot simply be back-calculated from one-bond RDCs. Ideally one alignment tensor per chiral element can be determined, such that local tensors, which allow relating these stereogenic elements, become accessible routinely.^[25] This is only conceivable if $^nT_{\text{HH}}$ are used. Future demonstrations of the application of ^1H - ^1H RDCs are in progress and will be reported in due course.

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