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Abstract

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BLAH

1 Introduction

One downside of the HSQC-TOCSY experiment discussed in the previous section is the (largely) indiscriminate transfer of magnetisation effected by the DIPSI mixing. It is difficult to determine how many transfer steps give rise to each peak, unless several HSQC-TOCSY spectra with different mixing times are recorded, which is a time-consuming process.[*]

For unambiguous spectral assignment, it can be preferable to have a mixing process which only effects coherence transfer across a single coupling: this can be described as an HSQC-COSY experiment. Similar experiments, including the H2BC,[1,2] 2BOB/H2OBC,[3] and HMQC-COSY[4] have previously been reported: the 2BOB experiment in particular has previously been incorporated in NOAH supersequences.[5] These often use constant-time techniques in order to remove $^nJ_{HH}$ splittings and minimise linewidths in the indirect dimension; however, the drawback of this approach is that peak *amplitudes* are modulated by $^nJ_{HH}$. Furthermore, it is not generally possible to obtain absorption-mode lineshapes for all peaks in the spectrum: typically the 'direct' responses are in-phase absorption, and 'indirect' responses antiphase dispersion. (Here, the terms in-phase and antiphase are used with respect to $^nJ_{HH}$; specifically, for the indirect peaks, this refers to the 'active' coupling over which the coherence was transferred).

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[*]Of course, as per the previous section, this could be done in a NOAH supersequence where each HSQC-TOCSY spectrum is assigned a fraction of the $^1H^C$ magnetisation. However, one must be wary of stretching the same magnetisation pool too thinly between many different modules: the resulting decrease in signal intensity may lead to a crossing into the sensitivity-limited regime.
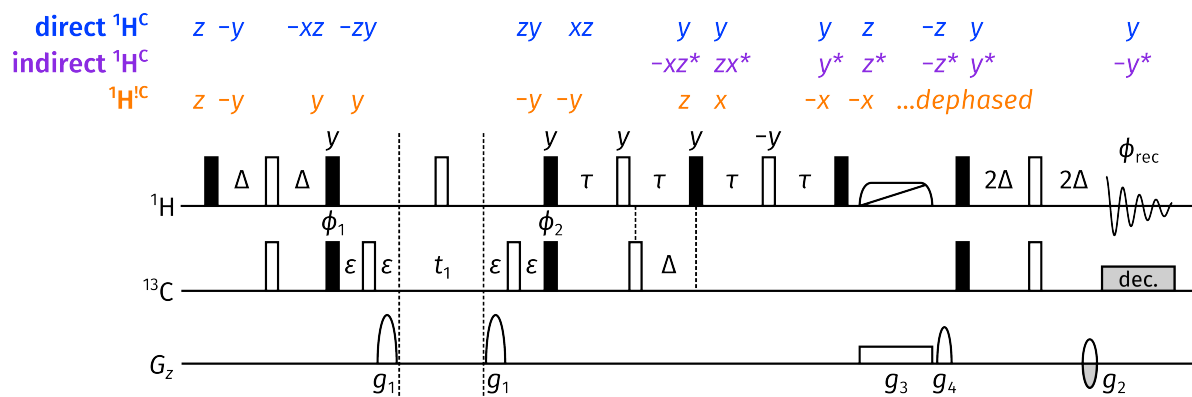
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2   HSQC-CLIP-COSY

Figure 1: HSQC-CLIP-COSY experiment with product operator analysis for the HSQC-COSY signal derived from the $^1\mathrm{H}^C$ magnetisation, as well as the bulk $^1\mathrm{H}^{!C}$ magnetisation. Both the 'direct', HSQC-type peaks, as well as the 'indirect' responses arising from coherence transfer in the perfect echo block, are analysed. The shorthand notation for product operators is expanded here to deal with a three-spin *IKS* system, where *I* and *S* are a mutually bonded $^1\mathrm{H}$–$^{13}\mathrm{C}$ pair as usual, and *K* is a 'remote' proton coupled to *I*. Terms with asterisks are on spin *K*; thus, for example, $zx*$ refers to an $I_z K_x$ term. The delay τ is chosen to be $1/(4 \cdot \sum {}^n\!J_{\mathrm{HH}})$; typically, this sum of couplings is set as 30 Hz, leading to a value of $\tau = 8.33$ ms. The ZQF gradient g_3 should be calibrated as per Thrippleton et al.;[6] g_4 is a purge gradient with arbitrary amplitude.

These problems are circumvented by the HSQC-CLIP-COSY experiment,[7,8] where the basic HSQC experiment is combined with clean in-phase (CLIP) coherence transfer using a perfect echo[9–11] and ZQF[6] (Figure 1). The use of an HSQC-type experiment means that ${}^n\!J_{\mathrm{HH}}$ does not evolve during t_1, and the CLIP transfer ensures that all peaks have in-phase absorption lineshapes. Much like the HSQC-TOCSY experiment before it, this experiment may be implemented with direct/indirect response editing: it is this version of the experiment which is shown in Figure 1.[†] If this editing is not desired, then the final 2Δ–180°(I, S)–2Δ spin echo can simply be reduced to a minimal gradient echo, i.e. ε–180°(I)–ε.

Unfortunately, the HSQC-CLIP-COSY experiment does not preserve $^1\mathrm{H}^{!C}$ magnetisation; thus, it cannot be directly used in a NOAH supersequence without sacrificing the sensitivity of terminal homonuclear modules. In particular, the ZQF used in the HSQC-CLIP-COSY experiment dephases all magnetisation that is not along z. If we wished to retain $^1\mathrm{H}^{!C}$ magnetisation, it would therefore have to (somehow) be placed along z during this period, and would have to be differentiated from the HSQC-COSY signal *after* the ZQF, which is not possible.[‡] On top of

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[†]Note that this direct/indirect editing is orthogonal to *multiplicity editing*, which labels both direct and indirect responses with a sign that depends on the multiplicity of the $^{13}\mathrm{C}$ nucleus detected in t_1. The addition of multiplicity editing leads to highly confusing spectra, so is ignored here—although the option to do so *is* provided in the GENESIS pulse programmes if the user so desires.

[‡]To be precise, although the $^1\!J_{\mathrm{CH}}$ Hamiltonian could be used to separate bulk magnetisation and the HSQC-type 'direct' responses, the 'indirect' responses cannot be differentiated.

that, the experiment also cannot be modified to provide partial excitation of $^1H^C$ magnetisation, which limits the ways in which it can be combined with other ^{13}C modules.
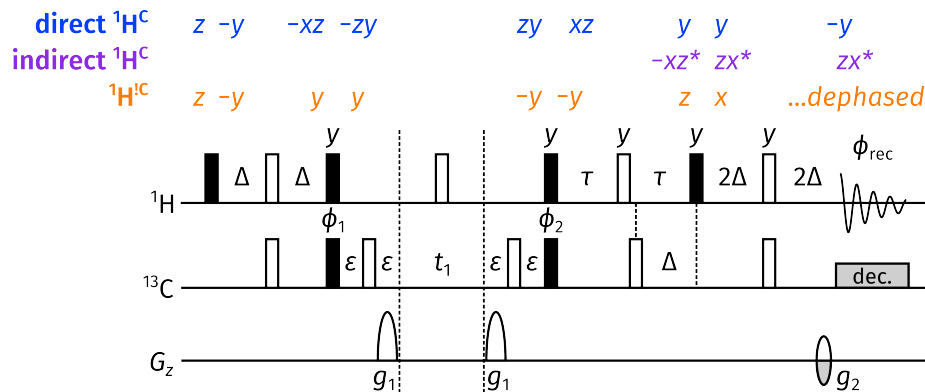
3   Double spin echo HSQC-COSY

Figure 2: 'Double spin echo' HSQC-COSY experiment; all symbols have the same meaning as in Figure 1.

Before tackling these problems directly, I discuss a simpler version of the HSQC-COSY experiment, which uses a simple spin echo for $^nJ_{HH}$ evolution instead of the perfect echo of the CLIP version. Together with the final 4Δ spin echo, this forms a 'double spin echo' (DSE) version of the HSQC-COSY experiment (Figure 2); the 'double' refers only to the mixing section after t_1. The removal of the CLIP coherence transfer element leads to mixed lineshapes in this experiment, where the direct responses are (mostly) in-phase absorption, and the indirect responses (mostly) antiphase dispersion.§

Like the CLIP version, this DSE experiment does not preserve bulk magnetisation; it is also incompatible with partial $^1H^C$ excitation. However, it does provide more raw sensitivity than the CLIP version: in the CLIP version, any antiphase signal at the end of the perfect echo is destroyed by the ZQF. All of this available signal is sampled in the DSE version, but this comes at the cost of not having pure absorption lineshapes.

It should be mentioned that prepending this DSE HSQC-COSY with the ZIP element would in fact return the bulk $^1H^{!C}$ magnetisation to $+z$ at the end of the sequence. However, I did not manage to test this experimentally, as my focus was on the development of the *triple* spin echo HSQC-COSY below.

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§The qualifier *mostly* is required because evolution of $^nJ_{HH}$ during the final spin echo leads to mixtures of in-phase absorption and antiphase dispersion. This evolution is generally small, though, because 2Δ is smaller than τ.
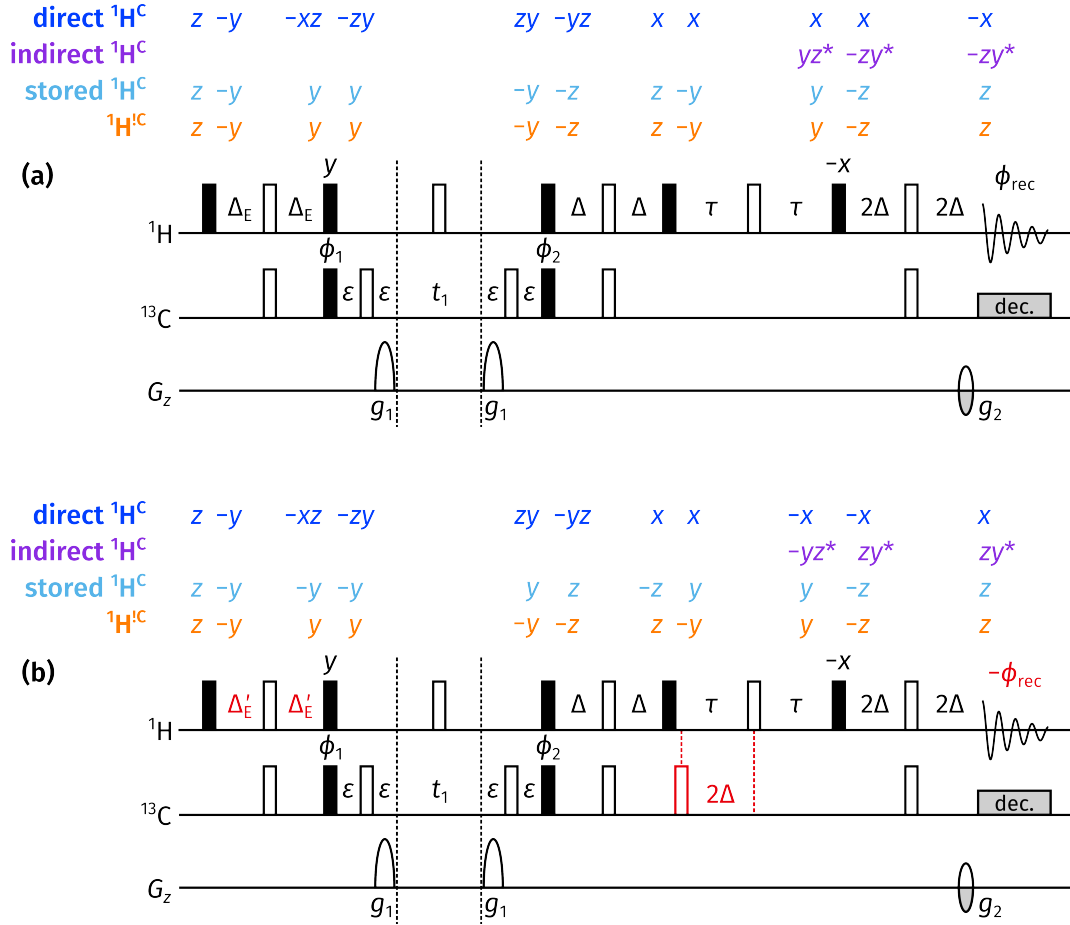
4 Triple spin echo HSQC-COSY

Figure 3: 'Triple spin echo' (TSE) HSQC-COSY experiment. **(a)** The first part of the experiment; this can be used on its own, but leads to spurious 'relayed' peaks arising via coherence transfer over two scalar couplings. **(b)** The second part of the experiment; co-adding this dataset with the first part leads to suppression of the relayed peaks. All symbols have the same meaning as in Figure 1.

In the DSE HSQC-COSY, the first of the two spin echoes after t_1 serves a dual purpose: $^1J_{\mathrm{CH}}$ is allowed to evolve for a duration of $\tau - (\tau - \Delta) + \Delta = 2\Delta$ (thus generating peaks which are in-phase with respect to $^1J_{\mathrm{CH}}$), and $^nJ_{\mathrm{HH}}$ evolves for the total duration of 2τ (allowing coherence transfer to remote spins). The triple spin echo (TSE) HSQC-COSY is derived from the DSE version by separating the first spin echo into two distinct parts: one for $^1J_{\mathrm{CH}}$ refocusing, and one for $^nJ_{\mathrm{HH}}$ evolution. As shown by the product operator analysis in Figure 3a, this not only preserves the bulk $^1\mathrm{H}^{!C}$ magnetisation, but is also compatible with partial $^1\mathrm{H}^C$ excitation.

The problem with the TSE version is the spectral quality: consider an even larger spin system of the form $\mathrm{S-I-K-L}$, where $\{I, K, L\}$ are $^1\mathrm{H}$ and S is $^{13}\mathrm{C}$. At the end of t_1, single-quantum magnetisation on spin I is present. Any evolution of J_{IK} in the first spin echo (of duration 2Δ) leads to terms of the form $2I_yK_z$, and can then be transferred onto spin K by the subsequent 90°_x pulse. This magnetisation can then further evolve under J_{KL} during the 2τ spin echo, and

then be transferred a *second* time by the 90°_{-x} pulse to spin L. Since the intensity of this transfer pathway is proportional to $\sin(2\pi J_{IK}\Delta)$, these 'relay' artefacts are especially prominent for large J_{IK}.

In order to suppress these, the experiment must be run a second time but with an additional 180° ^{13}C pulse inserted during the second spin echo (Figure 3b).¶ Any magnetisation still on spin I will therefore evolve under $^1J_{CH}$ for a total period of 4Δ, leading to net inversion. However, magnetisation which has already been transferred to spin K at this point will not be inverted: it is this magnetisation which is responsible for the relay artefacts. Therefore, by subtracting the two datasets (or equivalently, inverting the receiver phase and adding the two datasets), the desired peaks will be added up and the artefacts cancelled through subtraction.

The insertion of this 180° pulse also causes any unexcited $^1\mathrm{H}^C$ magnetisation to be inverted. In order to ensure that this is returned to $+z$ at the end of the sequence (and not $-z$), the initial INEPT delay must be *lengthened* instead of shortened, as per:

$$\Delta'_E = \frac{2\Delta(\pi - \arcsin f)}{\pi},\tag{1}$$

where, as before, f is the proportion of magnetisation to be excited.

5 Supersequences

The spectral quality, and sensitivity, of all of these modules is captured in Figures 4 and 5. The CLIP version (Figures 4a and 5a) has the best lineshapes, but its sensitivity is slightly lower. The DSE version (Figures 4b and 5b) provides greater sensitivity, but at the cost of impure lineshapes.

For the TSE version, we first look at the importance of the relay artefact suppression procedure described above. The extra relay artefacts are clearly visible in Figure 4c, acquired using the 'basic' sequence in Figure 3a only. (As previously mentioned, these arise from large $^nJ_{HH}$ which evolve during the 2Δ spin echo; for this specific compound, the offending couplings are ^3J between two axial protons in a six-membered ring, and ^2J in diastereotopic methylenes). The presence of these peaks largely defeats the purpose of using an HSQC-COSY experiment; in fact, this unoptimised TSE HSQC-COSY is qualitatively very similar to an HSQC-TOCSY acquired with a short mixing time of 10 ms (Figure 4d).‖ However, these artefacts can be efficiently removed using the suppression technique described in the text above; the result in Figure 4e is qualitatively similar to the two other HSQC-COSY experiments. Using this suppression technique, the

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¶A very similar strategy is used in the Bruker seHSQC pulse sequence `hsqcedetgpsisp2.4` to suppress the COSY-type artefacts. However, to the best of my knowledge, this sequence has not been published anywhere.

‖The HSQC-TOCSY even gives better peak shapes, since the DIPSI mixing transfers in-phase magnetisation to in-phase magnetisation.
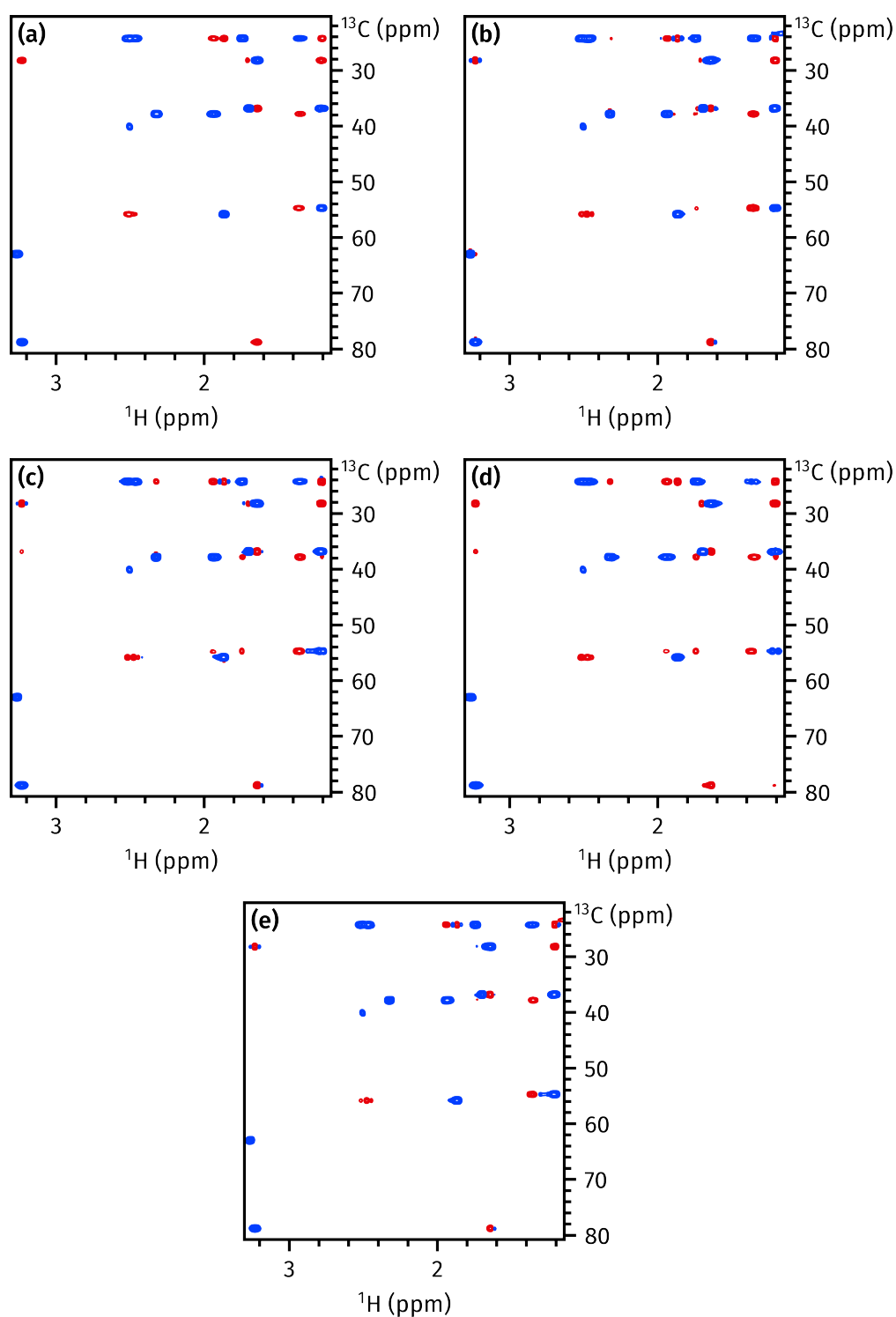
Figure 4: HSQC-COSY and HSQC-TOCSY spectra, taken from (respectively) NOAH-3 $S^C SC^c$ and NOAH-3 $S^T SC^c$ experiments. **(a)** HSQC-CLIP-COSY. **(b)** DSE HSQC-COSY. **(c)** TSE HSQC-COSY without suppression of relay artefacts (described in the text). **(d)** HSQC-TOCSY with 10 ms mixing time. **(e)** TSE HSQC-COSY with suppression of relay artefacts. 7A-210303
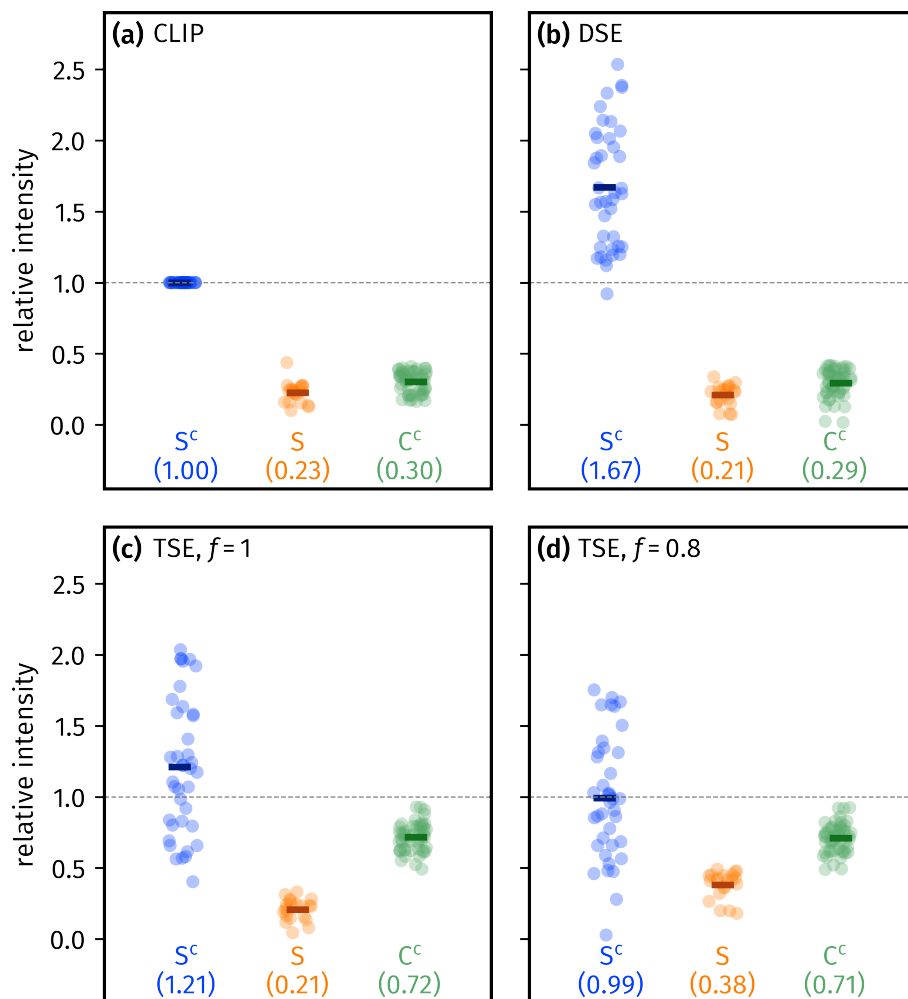
Figure 5: Sensitivity comparisons for all three modules in NOAH-3 $S^C SC^c$ supersequences. Peak intensities are relative to the HSQC-CLIP-COSY module itself (the leftmost column in (a)), as well as HSQC and CLIP-COSY spectra from a NOAH-2 SC^c experiment. Numbers in parentheses indicate averages over all peaks. **(a)** HSQC-CLIP-COSY. **(b)** DSE HSQC-COSY. **(c)** TSE HSQC-COSY, acquired with $f = 1$. **(d)** TSE HSQC-COSY, acquired with $f = 0.8$. 7A-210723

sensitivity of the TSE HSQC-COSY (when acquired with $f = 1$) falls between that of the CLIP and DSE versions.

Figure 5 also shows the relative sensitivities of the later modules in NOAH-3 $S^C SC^c$ supersequences. In all of the first three cases (CLIP, DSE, and TSE HSQC-COSY with $f = 1$, Figures 5a to 5c), the HSQC sensitivity is low (ca. 20%) because no $^1H^C$ magnetisation is retained for it to use. Thus, the signal derives only from $^1H^C$ magnetisation which has recovered during the HSQC-COSY FID. However, when the TSE version is used, partial $^1H^C$ excitation can be used to control this sensitivity: for example, when $f = 0.8$ (Figure 5d), the HSQC-COSY sensitivity is decreased (on average equalling that of the HSQC-CLIP-COSY), but the sensitivity of the HSQC module is almost doubled. Generally, choosing a value of $f < 1$ allows for the HSQC-COSY and HSQC sensitivities to be better balanced.

Finally, the CLIP-COSY module suffers when the HSQC-CLIP-COSY or the DSE HSQC-COSY are used, because both of these dephase $^1\text{H}^{!C}$ magnetisation; however, the TSE version successfully preserves around 70% of this magnetisation for it, regardless of the value of f. (This value is slightly lower than the approximately 90% magnetisation preserved by the HSQC module, because the bulk magnetisation is placed in the transverse plane during the 2τ spin echo (Figure 3a) and experiences losses due to $^n J_{\text{HH}}$ evolution.)

6 HSQC-COSY in context
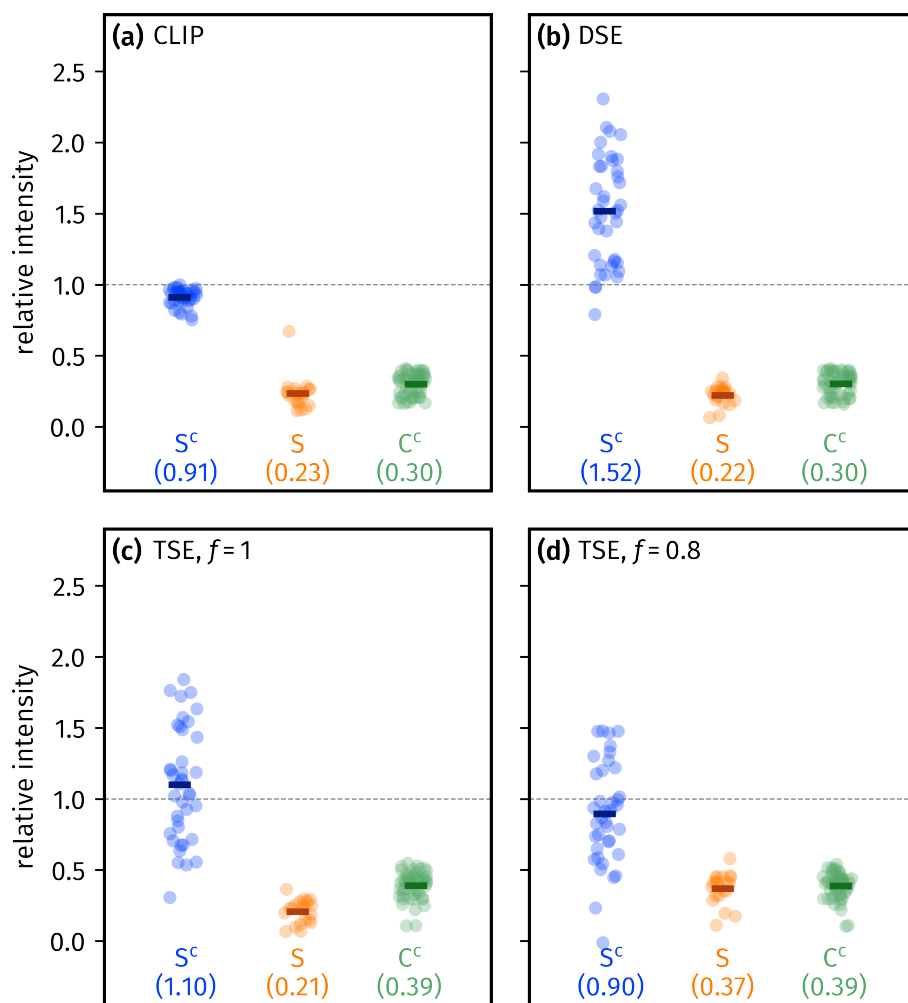
Figure 6: Sensitivity comparisons for the three last modules in NOAH-4 BS^{C}SC^c supersequences. Peak intensities are relative to the HSQC-CLIP-COSY module from a NOAH-3 S^{C}SC^c supersequence, and HSQC and CLIP-COSY spectra from a NOAH-2 SC^c experiment (these are the same reference spectra as used in Figure 5). Numbers in parentheses indicate averages over all peaks. **(a)** HSQC-CLIP-COSY. **(b)** DSE HSQC-COSY. **(c)** TSE HSQC-COSY, acquired with $f = 1$. **(d)** TSE HSQC-COSY, acquired with $f = 0.8$. 7A-210723

In NOAH-3 S^{C}SC^c-type supersequences, using the TSE HSQC-COSY module here appears to be a sensible option as it is capable of preserving some $^1\text{H}^C$ magnetisation for the HSQC, as well

as all $^1\text{H}^{!C}$ magnetisation for the CLIP-COSY. However, this may not necessarily be so important in the context of a larger supersequence—particularly one which begins with the HMBC module, which *already* dephases $^1\text{H}^{!C}$ magnetisation (meaning that there is not much of it to preserve).

Figure 6 provides the same sensitivity comparisons as in Figure 5, but in the context of a NOAH-4 BS^{C}SC^c supersequence instead. The HSQC-COSY and HSQC modules largely follow the same pattern as before, but with an approximate 10% loss across the board: this reflects the imperfect preservation of $^1\text{H}^C$ magnetisation by the zz-HMBC module. The CLIP-COSY module, however, has a substantially lower sensitivity regardless of which HSQC-COSY module is chosen. When the CLIP or DSE HSQC-COSY modules are used, the CLIP-COSY retains only roughly 30% of its original intensity: this is the same as in Figure 5. With the TSE HSQC-COSY, this is boosted to around 40% because there is one extra FID in which the $^1\text{H}^{!C}$ polarisation can be recovered. However, the use of the HMBC module at the beginning effectively places an upper limit on the amount of signal available to this module.

For virtually all homonuclear modules (including the CLIP-COSY), this small difference in sensitivity will not make a real difference in the interpretability of the spectrum. This is especially so considering that the HMBC module—which has a far lower sensitivity—is also present in the supersequence: a CLIP-COSY with 30% of its original sensitivity is still more intense than the HMBC experiment. This argument was used in justifying the NOAH-3 BSC^c experiment, and logically, should be equally applicable to the NOAH-4 BS^{C}SC^c experiment. In this case, the only compelling reason to use the TSE HSQC-COSY would be to preserve a portion of $^1\text{H}^C$ magnetisation for a later ^{13}C module. Thus, in this context, the decision of which HSQC-COSY module to use is slightly more nuanced: the cleaner lineshapes provided by the CLIP version, or the sensitivity of the DSE version, may be more preferable.

Acknowledgements

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Supporting Information

for

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Contents

S1 Software and raw data

All processing was carried out using TopSpin 3 or 4. Plots are generated in Python 3, using the `numpy`, `scipy`, and `penguins` libraries. The raw data used for this paper, as well as all scripts required for regenerating the plots, are available on GitHub: `https://github.com/yongrenjie/hsqc-cosy-paper`.