Optimising NMR Spectroscopy through Method and Software Development

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Chapter 4

NOAH

This final—and long—chapter describes my work on *NOAH* (NMR by Ordered Acquisition using ^{1}H detection) *supersequences*, pulse sequences which record multiple 2D datasets (*'modules'*) in the time required for one. This is an attractive NMR technique for several reasons: the time savings are clearly a key factor, but the flexibility of being able to combine almost any set of modules also makes NOAH supersequences applicable to a variety of contexts.

I begin by introducing the concepts underlying NOAH supersequences, as well as a general discussion of the time savings (and sensitivity per unit time) benefits thus realised. I then describe the GENESIS (GENEration of Supersequences In Silico) website, which allows users to generate Bruker pulse programmes for almost every imaginable NOAH supersequence. After this, my work on various aspects of the actual sequences themselves is described, with a special focus on newly developed and/or improved modules. Finally, the design of 'parallel' supersequences which use interleaved and/or time-shared modules is discussed.

This work was done in close collaboration with Ēriks Kupče (Bruker UK). However, all results and analysis shown in this chapter are mine. The work in this chapter forms the subject of several publications:

- Yong, J. R. J.; Hansen, A. L.; Kupče, Ē.; Claridge, T. D. W. Increasing sensitivity and versatility in NMR supersequences with new HSQC-based modules. *J. Magn. Reson.* **2021**, *329*, 107027, DOI: 10.1016/j.jmr.2021.107027

- Kupče, Ē.; Yong, J. R. J.; Widmalm, G.; Claridge, T. D. W. Parallel NMR Supersequences: Ten Spectra in a Single Measurement. *JACS Au* **2021**, *1*, 1892–1897, DOI: 10.1021/jacsau.1c00423

- Yong, J. R. J.; Kupče, Ē.; Claridge, T. D. W. Modular Pulse Program Generation for NMR Supersequences. *Anal. Chem.* **2022**, *94*, 2271–2278, DOI: 10.1021/acs.analchem.1c04

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- Yong, J. R. J.; Kupče, Ē.; Claridge, T. D. W. Uniting Low- and High-Sensitivity Experiments through Generalised NMR Supersequences. **2022**, manuscript in preparation

The material in the introductory sections also closely follow two reviews which I have contributed to:

- Kupče, Ē.; Frydman, L.; Webb, A. G.; Yong, J. R. J.; Claridge, T. D. W. Parallel nuclear magnetic resonance spectroscopy. *Nat. Rev. Methods Primers* **2021**, *1*, No. 27, DOI: 10.10 38/s43586-021-00024-3

- Yong, J. R. J.; Kupče, Ē.; Claridge, T. D. W. In *Fast 2D solution-state NMR: concepts and applications*, Giraudeau, P., Dumez, J.-N., Eds., forthcoming, 2022

4.1 Introduction

The characterisation of small molecules and biomolecules by NMR spectroscopy relies on a suite of standard 2D NMR experiments, which seek to detect heteronuclear scalar couplings (e.g. HSQC and HMBC), homonuclear scalar couplings (e.g. COSY and TOCSY), or through-space interactions (e.g. NOESY and ROESY). Although 2D experiments provide far superior resolution and information content compared to 1D spectra, they also require substantially longer experiment durations, as the indirect dimension must be constructed through the acquisition of many t_1 increments. This problem is further exacerbated by the fact that structural elucidation or verification often necessitates the acquisition of several different 2D experiments.

The acceleration of 2D NMR has thus proven to be a popular area of research. We may broadly categorise existing techniques into two classes: firstly, those which seek to directly speed up the acquisition of *individual* 2D spectra, and secondly, *multiple-FID* experiments which aim to collect two or more 2D spectra in the time required for one.[*] The former category includes methods such as non-uniform sampling (NUS),[7–10] fast pulsing (i.e. shortening of recovery delays),[11–14] ultrafast NMR,[5,15–19] Hadamard encoding,[20,21] and spectral aliasing;[22–24] whereas the latter encompasses time-shared NMR,[25,26] multiple-receiver NMR,[27–29] and—of course—NOAH supersequences.[5,30,31]

The scope of this introductory section will be limited to only NOAH supersequences. However, many of these techniques are closely related, and I will introduce concepts from elsewhere as needed. It should be noted that there are several other multiple-FID experiments which, while not explicitly advertised as such, are conceptually identical to NOAH experiments.[32–35] I do not discuss these here.

4.1.1 Time savings and sensitivity analyses

In a typical 2D NMR experiment, the majority of the experiment duration is taken up by the *recovery delay*—the time required for spins to return to their equilibrium polarisation, such that the next transient or t_1 increment can be recorded. The removal (or shortening) of recovery delays is thus a very effective way of speeding up 2D data acquisition. In NOAH supersequences, 2D experiments ('modules') can be directly concatenated without the addition of extra recovery delays between them: only one overall recovery delay is required for the entire supersequence. This means that, to a first approximation, a supersequence containing N modules ($N \geq 2$) can be acquired in the time needed for just one module. Figure 4.1 shows an example of a NOAH supersequence formed from two modules (HSQC and COSY): the various timings referred to in

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[*]These are by no means mutually exclusive: many of the techniques here can be combined to provide even greater efficiency.
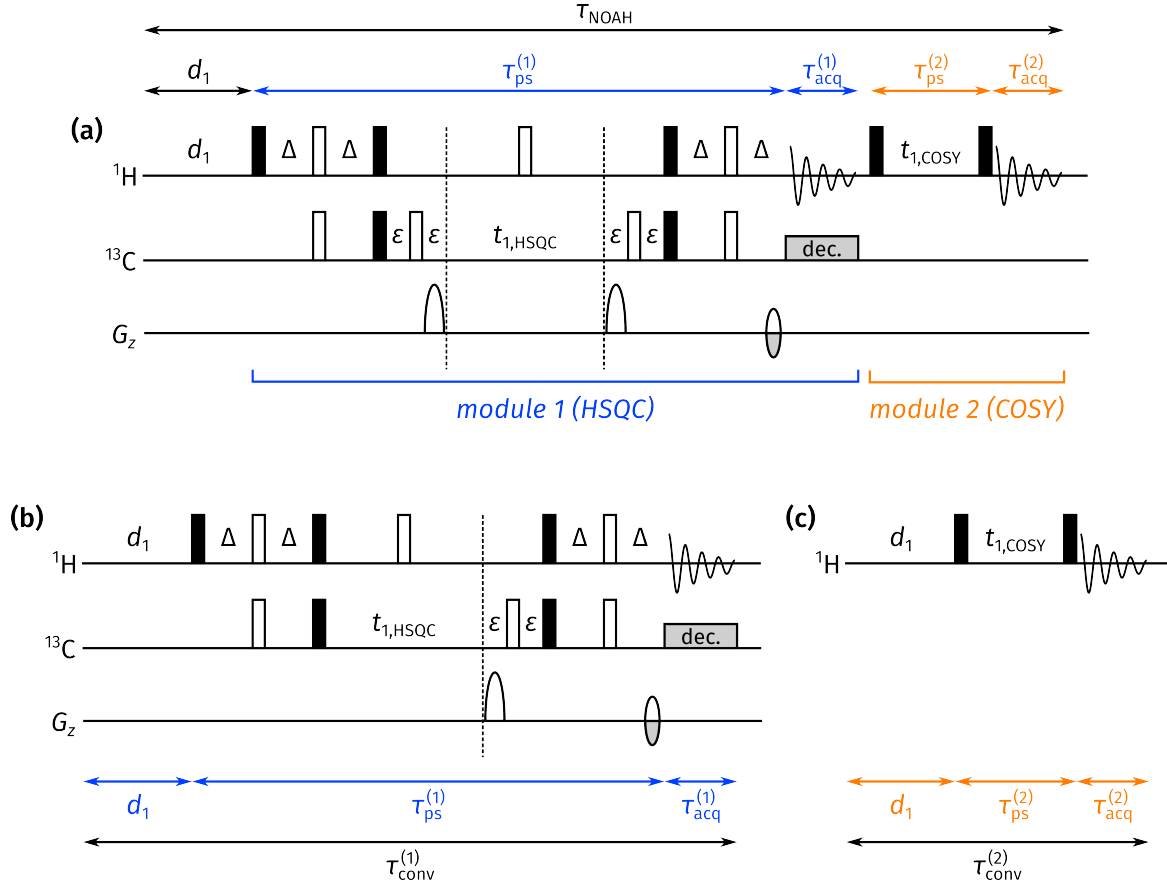
Figure 4.1: **(a)** NOAH-2 SC supersequence, comprising HSQC and COSY modules (see table 4.1 for an explanation of the single-letter module codes used). **(b)** 'Conventional' echo–antiecho HSQC (the same as in fig. 1.7). **(c)** 'Conventional' COSY. The timings referred to in the text are highlighted for all three experiments; I have assumed that d_1 for each experiment is the same. Note that the lengths are not to scale: d_1 is typically far longer than the τ_{ps} and τ_{acq}.

the text which follows are also marked on the diagram.

The duration of an NMR experiment, τ_{exp}, can be expressed as a sum of its parts:

$$\tau_{exp} = \tau_{ps} + \tau_{acq} + d_1, \tag{4.1} \quad \text{\{eq:exp\_duration\_2d\}}$$

where τ_{ps} is the time required for the pulse sequence itself (typically several milliseconds), τ_{acq} is the acquisition time (several hundred milliseconds), and d_1 is the recovery delay (one or more seconds). The *time-saving factor* ρ_t for a NOAH supersequence, as compared to a series of conventional standalone experiments, is then:

$$\rho_t = \frac{\sum_i \tau_{conv}^{(i)}}{\tau_{NOAH}} = \frac{\sum_i (\tau_{ps}^{(i)} + \tau_{acq}^{(i)} + d_1^{(i)})}{d_1 + \sum_i (\tau_{ps}^{(i)} + \tau_{acq}^{(i)})}, \tag{4.2} \quad \text{\{eq:rho\_t\}}$$

where τ_{NOAH} is the duration of the NOAH experiment, τ_{conv} is the duration of a conventional

experiment, and the superscript (i) represents the i-th module or conventional experiment being acquired. The sum runs from $i = 1$ to N, where N is the number of modules. If we assume that $d_1^{(i)} = d_1$ is the same for all N conventional experiments and the supersequence, then in the limit where

$$d_1 \gg \sum_i \tau_{\text{ps}}^{(i)} + \tau_{\text{acq}}^{(i)}, \tag{4.3}$$ {eq:d1_limit}

we have that $\rho_t \to N d_1 / d_1 = N$. This analysis makes plenty of assumptions, and is not entirely valid in practice. For example, *each* τ_{acq} is often around 5–10% of d_1, so is not entirely negligible, especially in longer supersequences. Furthermore, some modules require longer τ_{ps}: most notable is the NOESY module, which contains a mixing time of several hundred milliseconds. (HMBC, TOCSY, and ROESY spectra are also lesser offenders.) These factors serve to reduce ρ_t from its idealised value of N; generally, this deviation is larger as N increases, because eq. (4.3) becomes less and less valid. Nevertheless, the general point that time savings are approximately proportional to N stands.

For relatively concentrated samples, where sensitivity is not an issue, we can in fact end the discussion here. In this *sampling-limited regime*, the minimum 2D experiment duration is dictated purely by the number of t_1 increments needed to obtain sufficient resolution in the indirect dimension, as well as the minimum phase cycle required for artefact suppression.[*] NOAH supersequences are identical to conventional experiments in both aspects, but provide a time-saving factor of $\rho_t \sim N$.

The development of modern NMR instrumentation, including high-field magnets and cryogenic probes, means that the sampling-limited regime continues to be extended to ever lower concentrations. However, it is often not this simple: the opposite *sensitivity-limited regime* is still very commonly encountered, for example with naturally insensitive experiments (e.g. ADEQUATE), low-field benchtop NMR, or most simply, dilute samples.[†]

In such cases, it becomes mandatory to compare the SNRs of the NOAH modules and conventional experiments. To do so, we define for each module an *SNR factor* $A^{(i)}$, which is the SNR of the NOAH module divided by the SNR of a conventional experiment, acquired with the same parameters.[‡] In general, we have that $A \leq 1$, because NOAH modules frequently contain small modifications from conventional experiments (as will be explained in § 4.1.2). The *gain in*

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[*]With modern gradient-enhanced experiments, the minimum phase cycle may well not even be a 'cycle'; see also fig. 1.8.

[†]If the SNR factor $A^{(i)}$ as discussed below is *very small*, then it is possible that even concentrated samples may be shifted into the sensitivity-limited regime. This is never really the case in practice, though, as will be shown in § 4.1.3.

[‡]The relative SNR will likely vary from peak to peak in the spectrum, and $A^{(i)}$ should in theory be quoted either as an average over all peaks, or as a range. This is what I have done in this thesis. However, comparisons in the literature are not always as thorough.

sensitivity per unit time, $\varepsilon^{(i)}$, is then defined by

$$\varepsilon^{(i)} = A^{(i)}\sqrt{\rho_t},$$

(4.4)   {eq:varepsilon_i}

where the square root accounts for the fact that SNR scales only as the square root of the number of scans, or the number of times the experiment can be repeated in a given period. Of course, the exact values calculated for $A^{(i)}$ (and hence $\varepsilon^{(i)}$) will depend on the sample chosen for the comparison. These values should therefore be assumed to be valid only for similar samples.

If $\varepsilon^{(i)} > 1$, as is frequently the case, this means that the NOAH supersequence provides greater sensitivity per unit time in the i-th module compared to a standalone experiment. Equivalently, performing a NOAH experiment allows data of sufficient sensitivity to be obtained in less time. Naturally, this condition is most important for modules which are inherently insensitive, particularly heteronuclear correlation modules. For sensitive (typically homonuclear) modules, it is often perfectly tolerable to have $A < 1$ or even $\varepsilon < 1$, as even with this sensitivity penalty they are still more intense than the heteronuclear modules.

Another issue with NOAH supersequences is that each module is run with the same number of scans (phase cycle). Although this was touted as a benefit in the sampling-limited regime, this may in fact be undesirable in the sensitivity-limited regime, where insensitive experiments need to be run with more scans than sensitive ones. In this case, the *effective* time savings provided by NOAH experiments are smaller:

$$\rho_{t,\text{eff}} = \frac{\sum_i \tau_{\text{conv}}^{(i)}}{\tau_{\text{NOAH}}} = \frac{\sum_i S^{(i)}(\tau_{\text{ps}}^{(i)} + \tau_{\text{acq}}^{(i)} + d_1^{(i)})}{Sd_1 + S\sum_i(\tau_{\text{ps}}^{(i)} + \tau_{\text{acq}}^{(i)})},$$

(4.5)   {eq:rho_t_eff}

where each standalone experiment is acquired with $S^{(i)}$ scans and the NOAH experiment with S scans. Typically, S is simply the largest of the $S^{(i)}$. If $S^{(i)} = S$ for all i, then eq. (4.5) simply reduces to eq. (4.2); on the other hand, if the $S^{(i)}$'s are different, then we have that $\rho_{t,\text{eff}} < \rho_t$. In such a situation, it is probably more appropriate to describe a NOAH supersequence as 'measuring the most insensitive module and getting the others for free'. Indeed, if $S = S^{(i)} \gg S^{(j \neq i)}$, then 'the other' modules require almost no time to measure (relative to the least sensitive module), and $\rho_{t,\text{eff}}$ tends towards 1, meaning that even the time-saving utility of NOAH vanishes. A corollary of this is that NOAH supersequences are generally best constructed from modules which have similar intrinsic sensitivities and hence similar $S^{(i)}$'s.

As the reader can no doubt appreciate by now, the comparison of NOAH and conventional spectra is fraught with subtleties (which are sometimes glossed over in the literature, but invariably surface in real-life discussions). In fact, it is hardly even difficult to construct yet more edge cases. For example, one may not want to acquire all the individual spectra 'conventionally': for example,

NUS may be used for a HSQC experiment but not for others; or d_1 may be varied for different experiments. These will have an impact on both the durations of the experiments, as well as their sensitivities. To make any meaningful quantitative comparisons, it is therefore necessary to restrict the discussion to values of ρ_t, A, and ε, which can be objectively calculated. This is the approach I have taken in this thesis. These should of course be read with the qualitative understanding that depending on the context, these aforementioned factors may lead to *some*—but never a *complete*—decrease in the utility of NOAH experiments.

4.1.2 Magnetisation pools

subsec:noah__magpools

Having gotten this relatively dry material out of the way, I now turn to exactly how NOAH supersequences are constructed. Ordinarily, if the recovery delay is removed from an NMR experiment, its sensitivity will be greatly reduced because insufficient magnetisation will have recovered between repetitions; or in other words, $A^{(i)}$ will be very small. Such experiments would only really be useful well in the sampling-limited regime.

The key to avoiding this in NOAH supersequences is to make sure that *each module samples a different source of magnetisation.* For example, a HSQC module can be designed to only sample magnetisation of protons directly bonded to the 1.1%-natural abundance ^{13}C, and leave all other proton magnetisation untouched. Immediately following this, the remainder of the proton magnetisation can then be used to record (say) a COSY module, without needing a separate recovery delay. Using the notation of Orts and Gossert,[36] the magnetisation of ^{13}C–bound protons is denoted as $^1\text{H}^\text{C}$, and the magnetisation of protons *not* bonded to ^{13}C is denoted as $^1\text{H}^{!\text{C}}$. Protons not directly bonded to NMR-active heteronuclei are labelled $^1\text{H}^{!\text{X}}$, and will often be referred to as 'bulk' magnetisation, since (in natural-abundance samples) the majority of protons fall into this category.

Most standard 2D experiments do not preserve unused magnetisation but instead dephase it through CTP gradient selection; thus, NOAH modules often require some modifications compared to standard experiments. For example, compared to the echo–antiecho HSQC (discussed in § 1.4.5), the NOAH HSQC module[30] adds an extra CTP gradient so that the bulk magnetisation is refocused and ultimately returned to the $+z$ equilibrium state (fig. 4.2). (This is largely identical to the 'symmetrised' ASAP-HSQC experiment.[37])

Sometimes, the modifications required are more extensive, as in the HMBC module. If this module is followed by a HSQC module (or any other module which draws on $^1\text{H}^\text{C}$ magnetisation), the initial 90° excitation pulse must be replaced with a *zz*-filter (fig. 4.3). This performs an *isotope-selective rotation* in that $^1\text{H}^\text{C}$ magnetisation is stored along the *z*-axis, but $^1\text{H}^{!\text{C}}$ magnetisation is excited (and subsequently detected). In general, sequences which are thus modified have lower
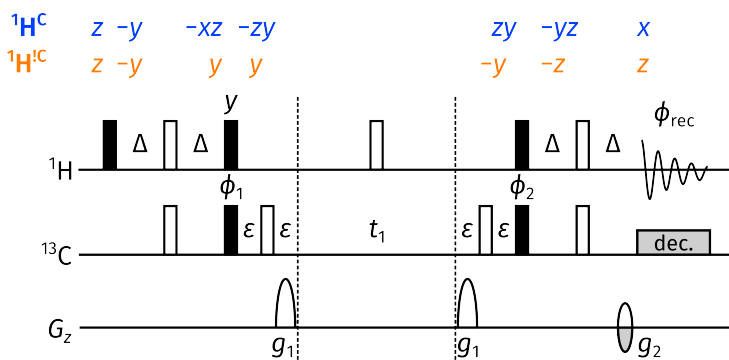
Figure 4.2: NOAH HSQC module. The delay Δ is set to $1/(4 \cdot {}^1J_{CH})$. Phase cycling is performed with $\phi_1 = (x, -x)$, $\phi_2 = (x, x, -x, -x)$, and $\phi_{rec} = (x, -x, -x, x)$. Gradient amplitudes are $(g_1, g_2) = (80\%, \pm40.2\%)$. The notation for product operator analysis is explained in the *Preface.*

sensitivities (i.e. $A < 1$) than the 'original' sequences from which they were derived.
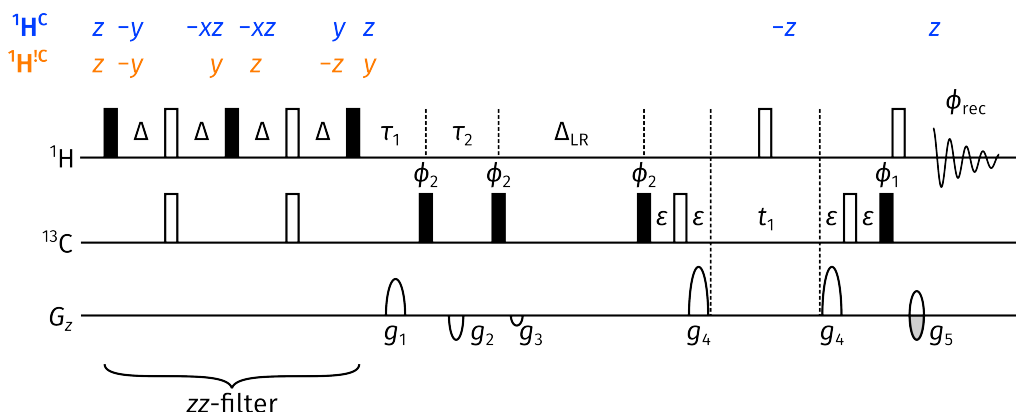
Figure 4.3: NOAH HMBC module. Delays are set as: $\Delta = 1/(2 \cdot {}^nJ_{CH})$; $\tau_1 = 1/(2 \cdot {}^1J_{CH,max})$; $\tau_2 = 1/(2 \cdot {}^1J_{CH,min})$ (see also § 3.4.7). Phase cycling is performed with $\phi_1 = (x, -x)$, $\phi_2 = (x, x, -x, -x)$, and $\phi_{rec} = (x, -x, -x, x)$. Gradient amplitudes are $(g_1, g_2, g_3, g_4, g_5) = (15\%, -10\%, -5\%, 80\%, \pm40.2\%)$.

In contrast, modules placed towards the *end* of a supersequence do not need to be modified, as they do not need to preserve any magnetisation. This includes virtually all homonuclear modules, which are allowed to simply consume any remaining magnetisation. Although this makes their implementation very straightforward, in general these modules will *also* suffer some losses in sensitivity, because the preceding modules do not perfectly retain all magnetisation.

Thus, in general, it is not possible for any module in a NOAH supersequence to have $A = 1$,

unless it is placed first in the supersequence *and* has not undergone any modifications.[*] Such cases are very rare, and it is thus necessary to accept some decreases in A, which are often fairly small (on the order of 10–20%). In the sampling-limited regime, sensitivity is not at a premium and this is often perfectly tolerable. In the sensitivity-limited regime, the full time savings ρ_t cannot be realised, but since ε is still typically larger than 1, there is still an overall boost in sensitivity per unit time.

4.1.3 Case studies

Using all that has been described in the previous sections, we now look at a few 'typical' supersequences to understand their construction. A quick note about the nomenclature of NOAH supersequences is warranted here. Supersequences are labelled by the number of modules N, plus a series of single-letter codes corresponding to the identity and ordering of the modules involved (table 4.1). Occasionally, superscripts or subscripts are used to qualify the modules involved.[†] Thus, a NOAH supersequence containing three modules—say ^{15}N HMQC, ^{13}C HSQC, and CLIP-COSY—would be referred to as a NOAH-3 $M_N SC^c$. Table 4.2 provides values of ρ_t and A for each module of several typical supersequences, which will be rationalised in the text which follows.

NOAH-2 SC: HSQC + COSY

We begin with perhaps the simplest example of a NOAH supersequence, one containing the HSQC and COSY modules: this is labelled as a NOAH-2 SC experiment (entry 1, table 4.2). As shown in fig. 4.2, the HSQC module only samples $^1H^C$ magnetisation, and leaves $^1H^{!C}$ magnetisation along the $+z$-axis Although the HSQC experiment has to be modified to preserve this $^1H^{!C}$ magnetisation, its sensitivity is practically unaffected as compared to a 'standard' HSQC ($A = 0.97$). Furthermore, the COSY module retains *most* of its sensitivity ($A = 0.90$). The small loss here is because the HSQC module does not *perfectly* preserve the $^1H^{!C}$ magnetisation: for example, evolution of J-couplings as well as relaxation occur during the HSQC pulse sequence, which are ignored in the product operator analysis in fig. 4.2.

The value of the time-saving factor, $\rho_t = 1.87$, is very close to the theoretical limit of $N = 2$. This reflects the fact that the pulse sequence itself, τ_{ps}, is fairly short for both the HSQC and COSY

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[*]Of course, this also depends on exactly *what* standalone experiment the NOAH supersequence is being compared against. Sometimes, in the literature, the NOAH experiment has been compared against its constituent modules acquired in a standalone fashion; in this case, the first module will always have $A = 1$. This tells us how much we gain through the act of concatenating modules, but is less meaningful in the 'real world' where one is interested in how useful NOAH is relative to 'typical' optimised 2D experiments. I therefore prefer to make comparisons against standard-library sequences.

[†]With the increasing number of modules, and the variety of modern NMR experiments which could be incorporated into NOAH supersequences, keeping these abbreviations short yet meaningful has been a challenge.

^{1}H–^{15}N modules		^{1}H–^{13}C modules		^{1}H–^{1}H modules	
Module	Code	Module	Code	Module	Code
HMQC	M_N	HSQC	S	COSY	C
HSQC	S_N	seHSQC	S^+	CLIP-COSY	C^c
seHSQC	S_N^+	HSQC-TOCSY	S^T	DQF-COSY	C
HMBC	B_N	HSQC-COSY	S^C	TOCSY	T
		2BOB	O	NOESY	N
		HMBC	B	ROESY	R
		ADEQUATE	A	PSYCHE	P
				TSE-PSYCHE	P^T
				PSYCHE 2DJ	J

tbl:noah_modules

Table 4.1: A (non-exhaustive) list of single-letter module codes for a selection of NOAH modules. Note that, in the literature, the ^{15}N HMQC module has been referred to simply by 'M', since the HSQC module is preferred for ^{1}H–^{13}C correlations. In this thesis, I include the subscript N throughout to avoid any ambiguity.

Entry	Sequence	τ_{NOAH}	ρ_t	A				
				HMBC	seHSQC	HSQC	COSY	TOCSY
1	SC	15 min 0 s	1.87			0.97	0.90	
2	SCT	16 min 25 s	2.60			1.01	0.99	0.79
3	BS	15 min 40 s	1.82	0.93		0.87		
4	SB	15 min 35 s	1.83	0.99		0.96		
5	BSCT	17 min 48 s	3.22	0.95		0.90	0.36	0.28
6	BS_N^+SCT	18 min 57 s	3.74	0.95	0.71	0.66	0.38	0.30
7	S_N^+BSCT	18 min 56 s	3.75	0.76	0.79	0.74	0.33	0.26

tbl:noah_sensitivities

Table 4.2: Sensitivity and time-saving analyses of several typical NOAH supersequences. All experiments were acquired with 2 scans per increment, 256 t_1 increments, an acquisition time of 67 ms, and a recovery delay of 1.5 s. The following Bruker library sequences were used as the 'conventional' experiments: `hmbcetgpl2nd`, `hsqcetf3gpsi2`, `hsqcetgpsp.2`, `cosygpqf`, and `dipsi2gpphzs`. *Data code:* 7Z-220224.

modules; the deviation therefore chiefly arises from the acquisition time, τ_{acq}. In all respects, this is therefore an example of an 'ideal' NOAH supersequence, where the combination of two modules provides time savings without compromising on sensitivity.

It is worth pointing out that the order of the modules cannot be reversed: the COSY module cannot be (easily) modified to preserve ^{1}H^C magnetisation. In a hypothetical NOAH-2 CS supersequence, the later HSQC module would only be able to use magnetisation recovered during the COSY FID, leading to substantial sensitivity drops.

A final point to consider would be whether the NOAH data has comparable spectral quality in
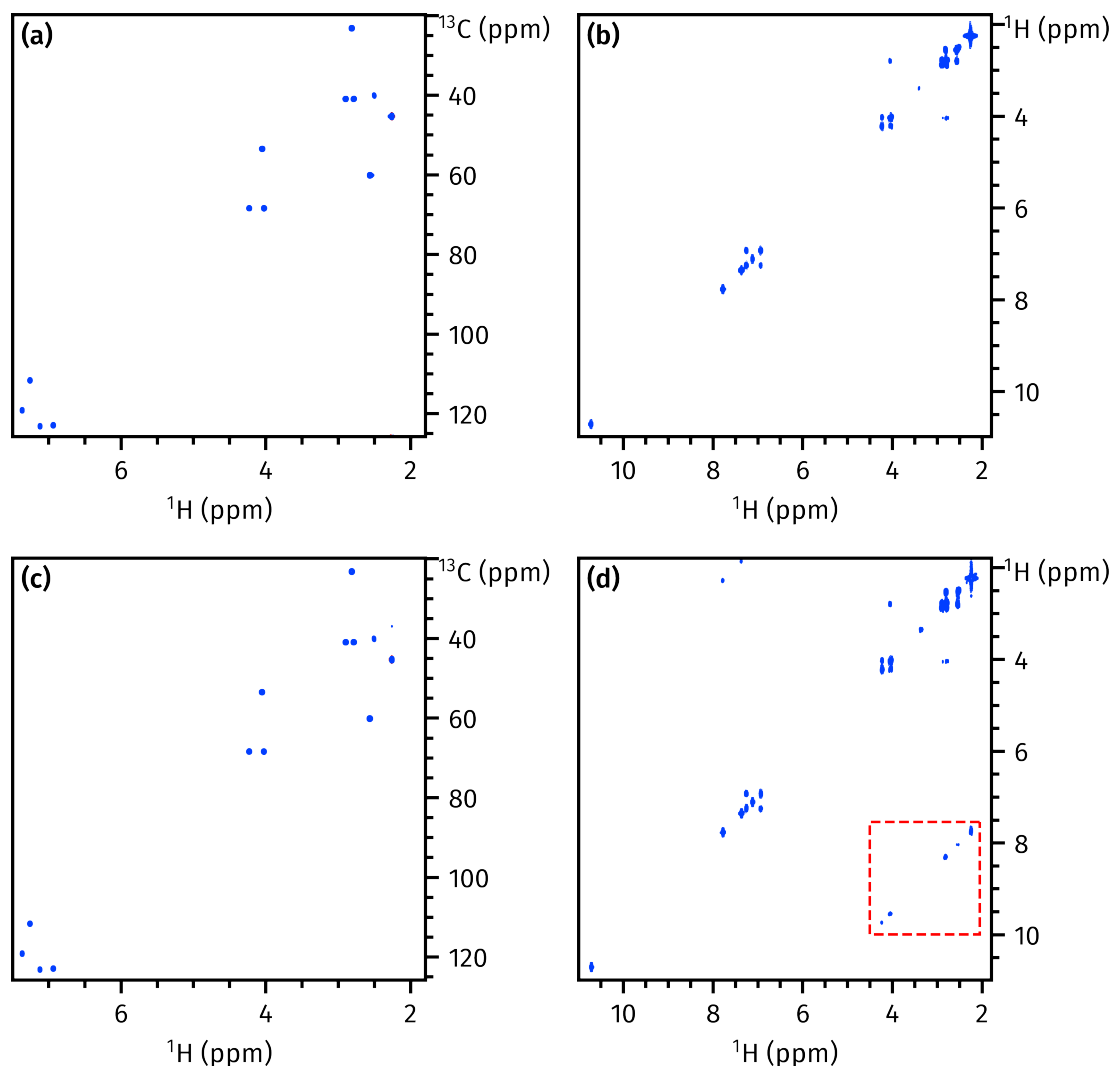
Figure 4.4: **(a)** HSQC from NOAH-2 SC supersequence. **(b)** COSY from NOAH-2 SC supersequence. **(c)** Standalone HSQC. **(d)** Standalone COSY; off-diagonal artefacts are highlighted in the red box. *Data code:* 7Z-220224.

terms of (for example) artefacts. In this case, the answer is yes: the NOAH HSQC spectrum is virtually identical to the standalone (figs. 4.4a and 4.4c; both spectra have low-level artefacts of different kinds, which do not seriously impede the interpretation and are not shown). On the other hand, the NOAH COSY spectrum seems to actually *improve* on the standalone COSY, in that it better suppresses off-diagonal artefacts (figs. 4.4b and 4.4d). These artefacts likely arise in the standalone COSY because of accidental refocusing of magnetisation which has not completely relaxed between t_1 increments.[38] In contrast, the NOAH COSY module has an extra set of HSQC gradients between every repetition of the COSY, so accidental refocusing is less likely. (Similar artefacts have been noted before in the DQF-COSY experiment,[39,40] and have also shown to be attenuated in the corresponding NOAH module.[41]) That said, such improvements are not always guaranteed: there are sometimes artefacts which arise uniquely in NOAH experiments,

some of which are discussed in the following sections.

NOAH-3 SCT: HSQC + COSY + TOCSY

Evidently, the fact that the HSQC preserves almost all $^1H^{!C}$ magnetisation means that *any* homonuclear module—or a combination thereof—can be placed after it. In general, since homonuclear modules tend to consume any remaining bulk magnetisation, it is very difficult to create combinations of homonuclear modules which do not lead to significant reductions in sensitivity. The only real exceptions are COSY/X combinations, where X can be NOESY, ROESY, or TOCSY: instead of concatenating the COSY and X modules, the COSY pulse sequence can instead be nested *within* the X module, as was first demonstrated with X = NOESY.[42,43] Here, we use the COSY/TOCSY combination as an example.[44] The COSY, TOCSY, and combined COSY/TOCSY modules are shown in fig. 4.5.
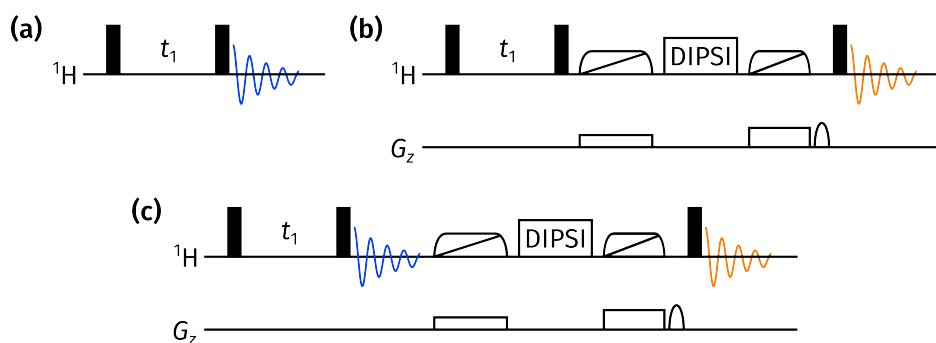
Figure 4.5: **(a)** COSY module. **(b)** TOCSY module; zero-quantum suppression is employed before and after the isotropic mixing period. **(c)** Combined COSY/TOCSY module, where the COSY FID is acquired immediately before the TOCSY mixing.

As shown in table 4.2 (entry 2), this nesting of the COSY module does not materially affect the TOCSY sensitivity. A small loss of approximately 20% is observed, which is partly due to the imperfect magnetisation preservation by the HSQC, and perhaps also due to relaxation during the COSY acquisition period. As for the time-saving factor, a slightly larger deviation ($\rho_t = 2.60$) is observed from the ideal value of 3. This reflects the addition of a TOCSY mixing period, which contributes to τ_{ps}.

NOAH-2 BS: HMBC + HSQC

As mentioned previously, the HMBC module shown in fig. 4.3 is designed to retain $^1H^C$ magnetisation through the addition of the *zz*-filter. This can be used in a subsequent HSQC module in a NOAH-2 BS supersequence. Entry 3 of table 4.2 shows that the addition of the *zz*-filter to eh HMBC causes a relatively small 7% decrease in sensitivity; on the other hand, the HSQC loses 13% of its sensitivity because of incomplete magnetisation preservation.

Generally, it has been recommended that less sensitive modules are placed earlier in the supersequence so that they can access a larger proportion of the equilibrium magnetisation. Since the HMBC is less sensitive of the two modules, this rule of thumb suggests that the BS supersequence would be better than the alternative SB supersequence. In fact, the opposite is true, as entry 4 of table 4.2 shows. The HSQC module has a boost in sensitivity because it is placed first in the supersequence, and no longer needs to rely on the $^1\mathrm{H}^\mathrm{C}$ magnetisation preserved by the HMBC; and the HMBC also benefits because the *zz*-filter modification is no longer needed. Arguably, the ordering of modules in a supersequence should be considered on a case-by-case basis.

NOAH-4 BSCT: HMBC + HSQC + COSY + TOCSY

We now move on to a longer supersequence containing four modules, with a correspondingly larger ρ_t value of 3.22. The sensitivity of the HSQC module is practically the same as in the NOAH-2 BS supersequence just described: however, the COSY and TOCSY modules expose one weakness of the HMBC module which has so far been overlooked. In principle, the HMBC module should only excite magnetisation of protons which are long-range coupled to $^{13}\mathrm{C}$ (which we could, for example, denote as $^1\mathrm{H}^{\mathrm{C(LR)}}$). This magnetisation pool should be separate from both the directly coupled protons ($^1\mathrm{H}^\mathrm{C}$), as well as protons which are not coupled to any $^{13}\mathrm{C}$ at all ($^1\mathrm{H}^{!\mathrm{C}}$). Unfortunately, this is not the case: it is not actually possible to separate the $^1\mathrm{H}^{\mathrm{C(LR)}}$ and $^1\mathrm{H}^{!\mathrm{C}}$ magnetisation pools. The HMBC excites both of these magnetisation sources, dephases the latter using CTP gradients, and detects the signal arising from the former.

What this means, of course, is that the COSY/TOCSY module which rely on $^1\mathrm{H}^{!\mathrm{C}}$ magnetisation will have substantially lower sensitivities. The signal detected in these two modules derives only from whatever has recovered during the previous two acquisition periods, as shown in entry 5 of table 4.2: *A* for COSY and TOCSY is 0.36 and 0.28 respectively. That said, this is in fact not likely to be an issue *even* for sensitivity-limited samples. Because the intrinsic sensitivity of the HMBC is orders of magnitude lower than the COSY and TOCSY, even with these large losses in sensitivity, the COSY and TOCSY spectra still have greater intensities than the HMBC. Thus, as long as the entire supersequence is acquired with enough scans to make the HMBC SNR sufficient, the SNR in the COSY and TOCSY will *also* be acceptable. This is illustrated in fig. 4.6.

A rather more insidious problem is that different signals relax at different rates: thus, the COSY and TOCSY spectra (or indeed, any homonuclear module) will have uneven intensities and are frequently asymmetric. This can be seen in the COSY spectrum, where a pair of asymmetric crosspeaks are highlighted. Adding a period of isotropic mixing before the COSY module[45] can help to ameliorate this to some extent (this was not performed when acquiring the data in fig. 4.6).
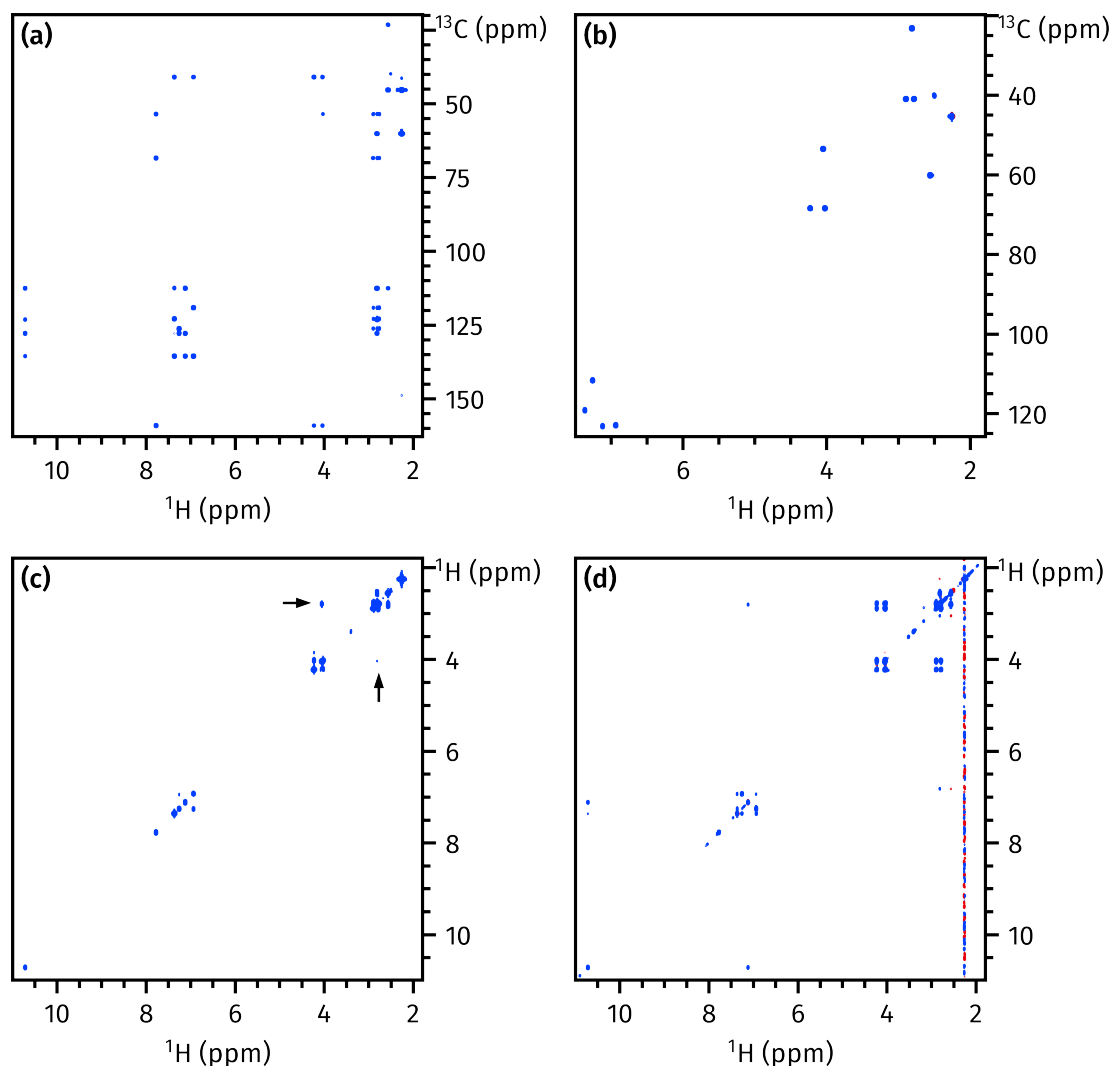
Figure 4.6: Spectra obtained from a NOAH-4 BSCT supersequence. **(a)** HMBC. **(b)** HSQC. **(c)** COSY; a pair of asymmetric crosspeaks are highlighted with red arrows. **(d)** TOCSY (60 ms DIPSI-2 mixing). Despite the COSY and TOCSY having only ∼ 30% sensitivity compared to standalone experiments, the intensity of the spectra obtained is still perfectly acceptable (the contour levels chosen are 1–2 orders of magnitude larger than for the HMBC). *Data code:* `7Z-220224`.

NOAH-5 BS$_N^+$SCT: HMBC + ^{15}N seHSQC + HSQC + COSY + TOCSY

As the final example, we add a further magnetisation pool to the mix, namely protons directly coupled to ^{15}N (i.e. ^{1}H^N). As of the time of writing, the implementation of multiple-FID experiments on Bruker spectrometers limits *N* to a maximum of 5, so a supersequence such as the present NOAH-5 BS$_N^+$SCT is the current limit. (However, there is no *scientific* argument forbidding $N > 5$, and it is likely that in future versions of TopSpin this restriction will be lifted.)

The values of *A* for each module are given in entry 6 of table 4.2. If the HMBC module is placed at

the beginning of the supersequence, then in order to preserve *both* $^1H^N$ and $^1H^C$ magnetisation, the *zz*-filter must be extended to include ^{15}N pulses.[46] As before, the ^{15}N seHSQC and ^{13}C HSQC modules both suffer drops in sensitivity. For the ^{15}N seHSQC, this is partly because of imperfect preservation of $^1H^N$ magnetisation by the HMBC, but also stems from the addition of the *zz* isotope-selective pulse (ZIP) element to the seHSQC pulse sequence; this is described further in § 4.3.2. On the other hand, for the ^{13}C HSQC, the sensitivity loss stems purely from imperfect retention of $^1H^C$ magnetisation. Finally, because the HMBC dephases $^1H^{IX}$ magnetisation, the COSY and TOCSY at the end have lower sensitivities: however, as discussed above, this is not an issue in practice.

It is also possible to move the ^{15}N seHSQC module to the front: this gives it a slightly greater sensitivity, at the cost of the HMBC (entry 7, table 4.2). In general, these two modules tend to have comparable sensitivity, and which of these two arrangements is better depends on which module the sensitivity needs to be optimised for.

Lastly, the value of ρ_t given here of 3.74 represents an effective upper limit on the time-saving factor. Although ρ_t increases with N, the extent to which it deviates from the ideal value of N also increases: it is very difficult to obtain $\rho_t > 4$, even with five modules in the supersequence. Of course, it is possible to increase ρ_t further by lengthening the recovery delay d_1 used for the experiments: for example, if d_1 is increased to 2 s from its present value of 1.5 s, ρ_t increases to 3.94. Obviously, this can only be pushed so far before it becomes meaningless.

4.2   GENESIS: automated pulse programme creation

In this section, I discuss the development of the GENESIS (GENEration of Supersequences In Silico) website (fig. 4.7): as the name suggests, it automatically generates pulse programmes for arbitrary NOAH supersequences. The website also provides extensive instructions on acquiring and processing NOAH data. Although this may at first glance appear slightly out of chronological order, in that the paper[3] was published later than much of the other work in this chapter, an early version of the GENESIS tool was in fact created much earlier (by July 2020).

The present version of GENESIS is available at https://nmr-genesis.co.uk; the source code can also be obtained from https://github.com/yongrenjie/genesis.

4.2.1   Motivation

From the preceding discussion in § 4.1, it is clear that modules which use different magnetisation pools can be combined almost at will. It does not matter *what* modules they are, merely what magnetisation pools they consume (and preserve). Thus, for example, the NOAH-2 SC super-

fig:genesis_frontpage

Figure 4.7: The front page of the GENESIS website (<https://nmr-genesis.co.uk>), as of 10 September 2022.

sequence can in fact be generalised to any $^1\mathrm{H}^\mathrm{C}$ module plus any $^1\mathrm{H}^{!\mathrm{C}}$ module. Very broadly speaking, we may define a generic supersequence as having any or all of the following:

- a HMBC module, which actually uses $^1\mathrm{H}^{!\mathrm{X}}$ magnetisation but can be placed at the front as discussed in the NOAH-3 BSC example above;

- a $^1\mathrm{H}^\mathrm{N}$ module;

- one or more $^1\mathrm{H}^\mathrm{C}$ modules (it is possible to partition the $^1\mathrm{H}^\mathrm{C}$ magnetisation pool between two modules, as will be discussed in § 4.3.4);

- one or more $^1\mathrm{H}^{!\mathrm{X}}$ modules which consume bulk magnetisation.

In the first NOAH paper in 2017,[30] a total of 285 'viable' supersequences were already listed. If we further take into account some of the new modules which were developed over the course of my DPhil (§ 4.3), the generic formula above provides for over 4000 viable supersequences.* ('Non-viable' sequences would be those which have undesirable drawbacks: for example, wrongly ordered modules like in a NOAH-2 CS supersequence.)

In spite of this, *only around 45 pulse programmes* had been made available (either via the supplementary information of NOAH papers, or the Bruker User Library). Traditionally, pulse

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*This is also ignoring the 'parallel' supersequences, which are discussed in § 4.5. The support for parallel supersequences in GENESIS is not complete: integrating these fully would require substantial changes to the user interface, which I have not had time to do.

programmes must be written by hand, which is a laborious and fairly error-prone process made worse by the sheer length of NOAH experiments. Doing this for thousands of supersequence is clearly impractical; furthermore, each time a new module is developed, or an old module is improved, updating every relevant supersequence would itself already be a mammoth task. This explains why—although the NOAH concept provides a clear blueprint for how supersequences may be constructed—there is still a huge gap between theory and practice.

To bridge this gap, I turned towards the *programmatic* generation of pulse programmes.* This not only allows for existing supersequences to be generated at will, but also provides an easy way for updates to be rapidly disseminated to the NMR community. Furthermore, a website can serve as a 'one-stop' shop where—after downloading pulse programmes—users may download the associated NOAH processing scripts and also access instructions on how to run NOAH experiments. This information did already exist, but was scattered across several different websites and/or journal supplementary information documents, and would have been needlessly confusing to a new user (not to mention the different versions of scripts available in different publications).

4.2.2 Implementation details

genesis_implementation

I will now describe a few features of GENESIS pulse programmes, as well as how these are implemented. The GENESIS code is written in TypeScript; during deployment, this is compiled to JavaScript, which can then be directly executed in the client's web browser. No server-side code is required, meaning that the GENESIS web page is actually a static site (it is currently hosted using GitHub Pages).

Overall structure

The algorithm used for pulse programme construction can loosely be separated into three parts, which are shown in listing 4.1:

1. the *preamble*, which consists of everything up until the beginning of the actual pulse sequence (the ze command). This includes header comments as well as definitions of parameters, such as delays and pulse widths;

2. the *main section*, which contains the actual pulse sequence;

3. the *epilogue*, which contains phase cycle information as well as footer comments describing

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*This is actually a bit of a lie: GENESIS was initially created for my own convenience. Throughout this chapter, I have had to perform many comparisons of different supersequences, and this tool spared me from having to write everything by hand (and—more often than not—subsequently discover mistakes which invalidated the results). Of course, it soon became apparent that it could find much wider use.

```
/* PREAMBLE */
; gns_noah2-SCqf
; 13C HSQC
; 1H magnitude-mode COSY
"d4          = 0.25s/cnst2"  ; 13C INEPT
"in0         = inf1/2"       ; 13C HSQC increment
"in11        = 2*dw"         ; COSY increment
; ...
define delay DC_HSQCa
"DC_HSQCa    = d4-p14/2"
"l0          = td1/2"        ; TD1/NBL

/* MAIN SECTION */
1 ze
4 d1 st0
  ; MODULE 1 - HSQC
  (p1 ph0):f1
  ; ...
  goscnp ph30 cpd2:f2
  50u do:f2
  2m st
  ; MODULE 2 - COSY
  (p1 ph12):f1
  ; ...
  go=2 ph26
  1m iu1          ; TD1/NBL counter
  1m igrad EA     ; echo-antiecho gradients
  1m id11         ; COSY t1
  30m wr #0 if #0 zd
if "l1 % 2 == 0" {
  1m id0          ; HSQC t1
}
  lo to 4 times l0
exit

/* EPILOGUE */
ph0=0
;gpnam4: SMSQ10.100
;gpz4: 70% (13C CTP)
;cpd2:wvm:wudec: cawurst_d-20(220 ppm, 1.4 ms; L2H)
;d4: 1/4J(CH)
;auprog: noah_hsqc:noah_cosy QF
;module identifiers: C_HSQC H_COSY_QF
;pulse programme created by genesis-v2.2.2, https://nmr-genesis.co.uk
;Sun Sep 11 2022 16:04:54 GMT+0800 (Malaysia Time)
```

Listing 4.1: Abridged GENESIS pulse programme for the NOAH-2 SC supersequence shown in fig. 4.1a.

lst:genesis_sc

each parameter. Instructions for generating shaped pulses using Bruker's WaveMaker software are also included here, as well as instructions for automatic processing (§ 4.2.3), and comments indicating how the pulse programme was generated (for reproducibility purposes).

The construction of the preamble and main section is largely accomplished through the collation of module-specific information, the most important of which are:

- information about the module itself, which go into the header comments;

- parameter definitions, which are collated to form the preamble. Duplicates must be removed here to avoid errors; and

- the pulse programmes themselves, which are directly concatenated to form the main section.

These, as well as other smaller bits of information (e.g. relevant citations, appropriate processing scripts), are stored within `NOAHModule` objects (an example is provided in listing 4.2). Each distinct module corresponds to one such object. Therefore, if one wants to add a new module to GENESIS, most of the work can be completed by simply defining a new `NOAHModule` object: no changes to the algorithm itself are needed.

To put the epilogue together, the pulse programme constructed so far is scanned for pulse phases, shaped pulses, and all other parameters. Using predefined lookup tables, GENESIS then outputs pulse phase definitions, WaveMaker directives (where appropriate), and comments containing textual descriptions of each parameter. These comments are mostly cosmetic, but are very useful to the user as they are displayed in the `ased` screen when setting up an experiment. Finally, instructions for automatic processing of the NOAH data (explained in § 4.2.1) are added to the bottom, together with the version number and a timestamp (for reproducibility purposes).

Phase/delay incrementation and looping

Since NOAH experiments are 2D experiments, there is one additional complication: the pulse programme must contain appropriate looping statements, together with pulse phase and delay incrementation, in order to correctly generate the indirect dimension. In many existing NOAH pulse programmes, looping in 2D experiments was written using the equivalent of `for` loops (listing 4.3, *left*). Although this suffices for the vast majority of supersequences, whenever anything must be incremented in a different way (e.g. for parallel supersequences, or the PSYCHE module which uses a different number of t_1 increments), the nested loop structure must be modified. I therefore opted to change the structure to use only one loop, and to control the phase and delay incrementation using modular arithmetic (listing 4.3, *right*). The outcome is entirely equivalent,

```
let shortDescription = `; 13C HSQC`

let preamble = `
"d4      = 0.25s/cnst2"                 ; 13C INEPT
; ...
"D[ID]a  = d4-p14/2"     // [ID] is later replaced with the module code
`


let pulprog = `
  ; 13C-1H HSQC

  ; INEPT
  (p1 ph0):f1
  ; ...
  goscnp ph30 cpd2:f2
  50u do:f2
`


const mod = new NOAHModule(
    "C_HSQC",            // internal module code
    "c13",              // module category
    "S",                // single-letter code, Table 4.1
    [],                 // relevant citations (if any)
    "noah_hsqc",        // AU programme for processing
    shortDescription,   // short description
    [AF_EDIT],          // available acquisition flags
    preamble,           // preamble text
    pulprog,            // pulse programme text
    1,                  // number of FIDs
    false               // flag for 'parallel' modules
);
export default mod;
```

Listing 4.2: An excerpt of the `NOAHModule` object for the HSQC module (internal code C_HSQC.

lst:module_c_hsqc

but edge cases can be implemented simply by adding another check on the loop counter L1.

Parameter standardisation

Each NOAH module contains a number of parameters, including pulse widths, delays, gradient amplitudes, shaped pulse waveforms. Since different modules are stored separately (as differ-ent objects), directly concatenating their pulse programmes may lead to conflicting parameter definitions. GENESIS avoids this by maintaining a global table of parameter definitions which are applied to all modules: when new modules are added, they must be checked against this to

```
"l0 = td1/4"                        "l0 = td1/2"
                                    "l1 = 0"


1 ze                                1 ze
3 1m                                4 d1 st0
4 d1 st0

  ; ... (pulse sequence goes here)    ; ... (pulse sequence goes here)

  ; in inner loop                     ; on every pass
  1m igrad EA   ; HSQC gradients       1m iu1          ; loop counter
  1m id11       ; COSY t1              1m igrad EA   ; HSQC gradients
  30m wr #0 if #0 zd                   1m id11         ; COSY t1
  lo to 4 times 2                      30m wr #0 if #0 zd


  ; in outer loop                     ; on every second pass
  1m ip5*2      ; HSQC 13C 90          if "l1 % 2 == 0" {
  1m ip30*2     ; HSQC receiver          1m ip5*2        ; HSQC 13C 90
  1m id0        ; HSQC t1                1m ip30*2       ; HSQC receiver
  lo to 3 times l0                       1m id0          ; HSQC t1
                                      }
                                      lo to 4 times l0


end                                 end
```

Listing 4.3: Implementation of phase/delay incrementation and looping in previous NOAH sequences (*left*, using nested loops) and in GENESIS (*right*, using modular arithmetic).

lst:genesis_looping

ensure that there are no inconsistencies.

In general, where possible, these parameters are chosen to be consistent with pulse programmes in the Bruker standard library: thus, for example, P1 is the ^{1}H 90° pulse width, and CNST2 is the $^1J_{CH}$ value used for calculating INEPT delays. This makes it easy to read in parameters either from the *prosol* relation tables in TopSpin, or from other existing parameter sets. Some delays are module-specific and do not need to be reused, and in standard library sequences, are often labelled as DELTA1, DELTA2, and so on. To avoid conflicting definitions and also to improve readability, I rename these such that they include the name of the module: thus, in a ^{13}C HSQC these may be labelled DC_HSQC_1. Here, C_HSQC is the name associated with the NOAHModule object.

If combined with some caution when adding new modules, these measures ensure that *within* a supersequence there are no parameter clashes: we may view this as a *local uniqueness* of parameters. However, the impact of this design choice is even more far-reaching: since parameters

are stored globally, they will always have the same value in *all* possible supersequences (or in other words, the parameters are *globally unique*). This makes it exceptionally easy to set up multiple different supersequences in TopSpin: virtually all of the parameter values may simply be copied from a previous NOAH dataset.

One potential issue with this strategy is that TopSpin provides a finite number of named pulse widths (for example). Thus, there are only so many different parameters which can be stored in a global table before running into inevitable conflicts. A workaround would be to sacrifice the global uniqueness of each parameter, and only have it be unique within a given supersequence. Fortunately, this situation has not (yet) surfaced.*

Parameter descriptions

At the epilogue of the pulse programme, extra comments are added for every parameter present in the pulse programme. Most of these are purely textual, and appear in the TopSpin `ased` parameter setup screen; this naturally helps to make the pulse programmes as easy to use as possible. However, some of these comments have especial meaning: gradient amplitudes and shapes, for example, are specified in a way which allows them to be automatically populated using the `gppp` Python script in TopSpin. Furthermore, WaveMaker directives are also specified for some shaped pulses, allowing them to be created in an on-the-fly manner using the `wvm` command. This means that the user (generally) need not separately download and install a set of shaped pulses.

Module choice

One final issue which must be overcome is the fact that some NOAH modules may be implemented differently depending on the supersequence which it is being used in. The HMBC module described in § 4.1.3 is one such example: the form of the *zz*-filter depends on whether the HMBC module must retain $^1\text{H}^\text{C}$ and/or $^1\text{H}^\text{N}$ magnetisation for subsequent modules. Thus, in the NOAH-2 BS supersequence the *zz*-HMBC must be used, but in the NOAH-2 SB supersequence the 'original' HMBC without a *zz*-filter is preferable. Since they have different pulse programmes, each of these 'versions' of the HMBC are described by *separate* `NOAHModule` objects.

However, it is unlikely that the majority of users would want to manually configure the supersequence in such detail. Thus, the GENESIS web interface actually hides these different

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*The number of *pulse phases* in particular, though, is dangerously close to the maximum number of 32. In fact, the global uniqueness criterion is not really important for pulse phases, because—unlike, say, delays—pulse phases are hardcoded in the pulse programme, and cannot be copied from one dataset to another. So, if necessary, I could dispense with the global uniqueness for pulse phases, at the cost of some increased code complexity. I did briefly contemplate this possibility, but since I am at the end of my DPhil and am unlikely to add any new modules soon, this will remain a hypothetical—for now.

versions from the user, only showing one button labelled 'HMBC'. Under the hood, the correct version of the HMBC is automatically chosen based on what other modules the user has chosen (fig. 4.8).
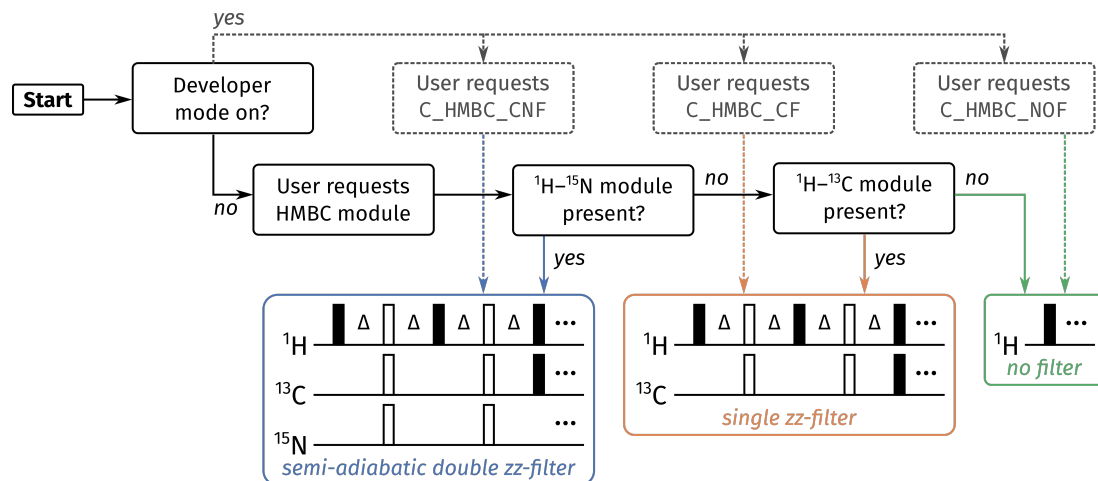

Figure 4.8: Flowchart showing how the correct 'version' of the HMBC module is determined when constructing a supersequence using GENESIS. When developer mode is on, the user directly chooses module codes corresponding to each version of the HMBC. When it is off, the appropriate version is chosen based on which other modules are present in the supersequence.

Should the user indeed desire to control the exact module used, the website also offers a 'developer mode' switch: turning this on allows the user to directly choose the desired module code. (Enabling developer mode also reveals a handful of extra modules which were used exclusively for my DPhil and are not generally of interest.)

Tests

One risk inherent in any NMR experiment is the possibility of causing spectrometer damage when executing malformed pulse programmes. To minimise the chances of this, each version of GENESIS is checked against a series of automatic tests before being released. The most important tests ensure that each module containing decoupling statements also turn off decoupling immediately after acquisition. On top of this, there are also a series of regression tests where (Some) supersequences are checked against those generated by previous versions: any differences in these are automatically flagged for review.

None of this can actually *stop* wrong pulse programmes from being written. For example, if a module are incorrectly programmed (which is still very easy), then any supersequence containing that module will not be fully functional. However, the inclusion of tests minimises the chance of creating faulty pulse programmes.

It should be mentioned that this is not a *new* problem which GENESIS introduces: errors in pulse

programmes can arise equally easily (if not *even more easily*) when they are handwritten. There is no way to really circumvent this, except through careful inspection of the pulse programme prior to execution. The real difference with GENESIS is that users may be more likely to blindly use the pulse programmes it creates, without applying an equal amount of caution. The tests are therefore not meant to ensure perfection, but rather to avoid some of the worst consequences which may surface. Of course, it is also not possible for GENESIS to prevent users from setting up parameters incorrectly (e.g. overly long acquisition times).

Reproducibility

The final section of a GENESIS pulse programme consists of comments intended solely for reproducibility purposes. These comments indicate exactly which `NOAHModule` objects were used to create the pulse programme (these can be manually entered via developer mode), as well as a GENESIS *version number*. Since GENESIS has been updated with some regularity during my DPhil, each release is assigned a version number, which broadly follows the principles of *semantic versioning*. Old versions of GENESIS may be accessed by navigating to the specific URL `https://nmr-genesis.co.uk/X/Y/Z` for version `X.Y.Z`. Together, these two pieces of information allow all pulse programmes to be regenerated whenever necessary: this is important for ensuring that the spectra thus acquired are reproducible (as far as possible).

How smart is GENESIS?

Having written several pages of text about the *features* present in GENESIS, it is tempting to think that it is 'intelligent' in its design of pulse programmes. At various points in time, extensions to the concept have even been proposed: in GENESIS, the building blocks used for pulse programme construction are NOAH modules, but one could envision breaking up these into the smallest possible units of pulses, delays, gradients, and so on, and using something like GENESIS to construct *arbitrary* pulse programmes. This was even speculatively mentioned in the paper.

However, in truth, GENESIS is a long way from being able to do such things. In particular, it does not have any actual understanding of the Bruker pulse programming syntax: *almost* all of the pulse programme instructions are hardcoded as strings.[*] Thus, the creation of pulse programmes is not *truly* being done from the ground up: instead of 'combining pulse sequence elements', a more accurate description is that the *text* corresponding to different pulse sequence elements is being concatenated. In a way, there is a lot of brute force involved.

Of course, it is hardly a trivial task to write something more sophisticated, and I do not think I can do it without rather more knowledge in computer science (especially a course on compilers

---

[*]There are several exceptions where some parameters are dynamically generated, mainly to avoid clashes between, for example, DIPSI mixing in different parts of the supersequence.

and/or programming language theory). However, this was one of the things which I would have really liked to do had I had more time!

4.2.3   Processing improvements

ah__genesis_processing

Finally, I touch on a few small improvements in processing NOAH data: these are not strictly made possible by the GENESIS website, but having a unified source for pulse programmes and processing scripts does make it substantially easier to propagate these developments.

AU script processing

When processing NOAH experiments, the first step is to split up the FIDs of different modules into different files: this is done by the `splitx_au` AU programme. However, after that is done, `splitx_au` *also* calls on auxiliary AU programmes to process each of the resulting datasets. Each module is associated with a specific auxiliary AU programme: for example, HSQC data is processed with `noah_hsqc`, COSY data with `noah_cosy`, and so on.

Previously, these auxiliary AU programmes had to be specified manually using the `USERP1` through `USERP5` processing parameters, which respectively apply to the first through fifth modules in a supersequence. Although this was a perfectly serviceable setup, I realised that it was a source of annoyance when running multiple different supersequences, as modules would often be processed incorrectly if the processing parameters were inadvertently copied. Even when just running a single supersequence, this was still an extra cognitive load, especially for users who may not have been familiar with NOAH experiments.

Since the pulse programme specifies which modules are run, and the 'correct' processing parameters also depend on the modules, it stands to reason that the processing parameters can be encoded in the pulse programme itself. Thus, all GENESIS pulse programmes contain a line near the bottom specifying which AU programmes are to be used for each of the modules present. I also modified the `splitx_au` AU programme to parse the pulse programme for this extra information. Thus, the `USERP1`, …parameters no longer need to be specified, as long as a GENESIS pulse programme and a recent version of `splitx_au` are used. These parameters still serve a purpose, though; if they are non-empty, they will override the 'default' processing instructions found in the pulse programme. It is therefore possible for advanced users to customise the processing being carried out. This also maintains backwards compatibility with older versions of pulse programmes and `splitx_au`.

Non-uniform sampling

Another small improvement relates to the implementation of non-uniform sampling (NUS) in NOAH supersequences. Because the indirect dimension in NOAH pulse programmes is generated through explicit looping (listing 4.3), instead of the `mc` macro used in most 2D experiments, the standard NUS implementation in TopSpin cannot be used. The original solution[41] relied on explicitly defining a list of t_1 increments to sample (stored as the `VCLIST` parameter), and modifying the pulse programme to only use t_1 values from this list using a Python script (`noah_nus.py`). This required storing two copies of the same pulse programme, one with NUS and one without. Furthermore, enabling NUS was a (relatively) irreversible process: NUS could not be disabled without manually resetting the pulse programme and other parameters.

There is no way to circumvent the requirement for the `VCLIST` parameter, but in GENESIS pulse programmes, I opted to no longer create a new pulse programme: instead, NUS is implemented as an acquisition flag which can be toggled. This solves both of the above problems: there is no need to store two versions of the same pulse programme, and it is possible to seamlessly switch between non-uniform and uniform sampling as desired.

Unfortunately, this change is slightly more problematic than the `splitx_au` change: this new NUS implementation is not backwards-compatible, so requires a new NUS setup script which I have called `noah_nus2.py`. This script cannot be used with old pulse programmes, and the old script cannot be used with GENESIS pulse programmes.[*] Nevertheless, it does represent a real improvement over the previous implementation: the GENESIS website also helps to make the changeover as smooth as possible.

4.3 Discussion of individual modules

4.3.1 ^{13}C sensitivity-enhanced HSQC

^{13}C seHSQC

4.3.2 ^{15}N sensitivity-enhanced HSQC

^{15}N seHSQC

4.3.3 HMQC

Suppression of wing artefacts (GENESIS paper)

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[*]Also, there were still some bugs in the new NUS implementation right up until August 2022, so this change was not as smooth-sailing as I had hoped for.

4.3.4 HSQC-TOCSY

`subsec:noah__hsqctocsy`

HSQC + DIPSI + HSQC combos

Extension to HSQC-TOCSY

Cite ASAP work (Luy)

4.3.5 HSQC-COSY

`subsec:noah__hsqccosy`

Comparison of several versions of HSQC-COSY (JACS Au SI)

4.3.6 2DJ and PSYCHE

`subsec:noah__2djpsyche`

cnst37 scaling

SAPPHIRE

4.3.7 HMBC

`subsec:noah__hmbc`

Suppression of $^1J_{\text{CH}}$ artefacts (GENESIS paper)

Investigation of gradient schemes (no difference was really observed, but that's fine)

Also ^{15}N HMBC

4.3.8 ADEQUATE

`subsec:noah__adequate`

Recent stuff.

4.4 Solvent suppression in NOAH

`sec:noah__solvsupp`

GENESIS paper.

4.5 Parallel and generalised NOAH supersequences

`sec:noah__parallel`

Blah.

4.6 References

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