P-1242 Diversifying NMR Supersequences with New HSQC-based Modules

Jonathan RJ Yong[1], Alexandar L Hansen[2], Ēriks Kupče[3], Tim DW Claridge[1]

[1]University of Oxford; [2]Campus Chemical Instrument Center; [3]Bruker UK

Summary

Slide 1: NOAH overview

- 2D NMR experiments ("modules") are combined into "supersequences" by removing relaxation delays, speeding data acquisition by up to 4×.
- Each module should only excite its share of magnetisation, e.g. ^{1}H–^{13}C HSQC should only excite ^{13}C–bound ^{1}H. "Standard" experiments must typically be modified to satisfy this.

Slide 2: Sensitivity-enhanced HSQC

- Adding a double spin echo ("ZIP element") at the start of the seHSQC lets it preserve ^{12}C– or ^{14}N–bound ^{1}H magnetisation for other modules.
- This new ^{1}H–^{13}C seHSQC module provides 1.2–1.8× S/N gain vs the original HSQC module.
- The ^{1}H–^{15}N seHSQC gives 2.0–4.5× gains vs the HMQC, partly via collapse of f_1 multiplet structure.

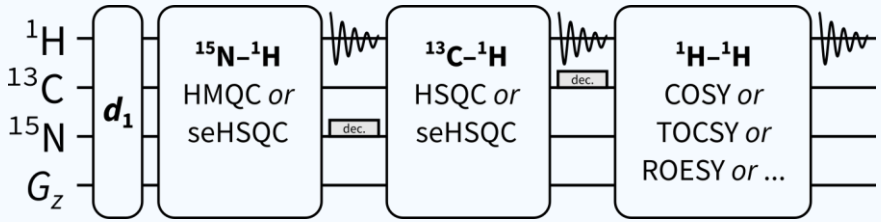
Slide 3: HSQC-TOCSY and HSQC-COSY

- These preserve ^{12}C–bound ^{1}H magnetisation, but also allow variable excitation of ^{13}C–bound ^{1}H.
- The unexcited ^{13}C–^{1}H magnetisation (plus any that recovers during FID) can then be used for a HSQC module, e.g. to extract multiplicities or $^{1}J_{CH}$.
- HSQC-COSY avoids "relay peaks" present in HSQC-TOCSY, even when short mixing times are used.

Slide 1: NOAH overview

Modules are combined into supersequences

Many typical 2D experiments e.g. HMBC, HSQC, COSY, NOESY can be acquired "in parallel" using the NOAH technique (**N**MR by **O**rdered **A**cquisition using ^{1}**H**-detection). Each constituent experiment is called a "module".



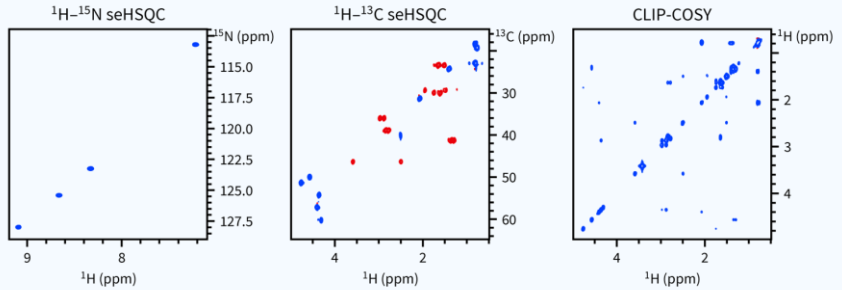
Only one recovery delay (d_1) needed

Because d_1 is the longest part of the pulse sequence, the elision of multiple recovery delays leads to substantial time savings (up to 4× depending on the modules employed).

NOAH-3 MSC
18 min 26 sec

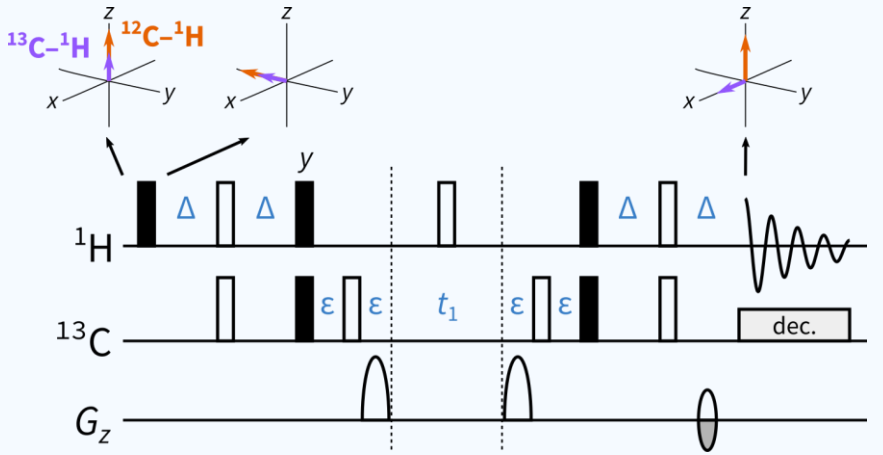
Conventional HMQC + HSQC + COSY
46 min 19 sec
(2.51× longer)

Kupče, Ē.; Claridge, T. D. W. *Angew. Chem. Int. Ed.* **2017,** *56* (39), 11779–11783.
Schulze-Sünninghausen, D.; Becker, J.; Luy, B. *J. Am. Chem. Soc.* **2014,** *136* (4), 1242–1245.



Resulting spectra are identical to standard 2Ds

Extra data processing consists merely of "splitting" the FIDs and is completely automated via AU programmes.



Modules excite ^{1}H magnetisation selectively

For example, the ^{13}C HSQC module above only excites protons directly bound to ^{13}C, leaving ^{12}C-bound protons untouched.

P-1242 Diversifying NMR Supersequences with New HSQC-based Modules

Jonathan RJ Yong[1], Alexandar L Hansen[2], Ēriks Kupče[3], Tim DW Claridge[1]

[1]University of Oxford; [2]Campus Chemical Instrument Center; [3]Bruker UK

Summary

Slide 1: NOAH overview

- 2D NMR experiments ("modules") are combined into "supersequences" by removing relaxation delays, speeding data acquisition by up to 4×.
- Each module should only excite its share of magnetisation, e.g. ^{1}H–^{13}C HSQC should only excite ^{13}C–bound ^{1}H. "Standard" experiments must typically be modified to satisfy this.

Slide 2: Sensitivity-enhanced HSQC

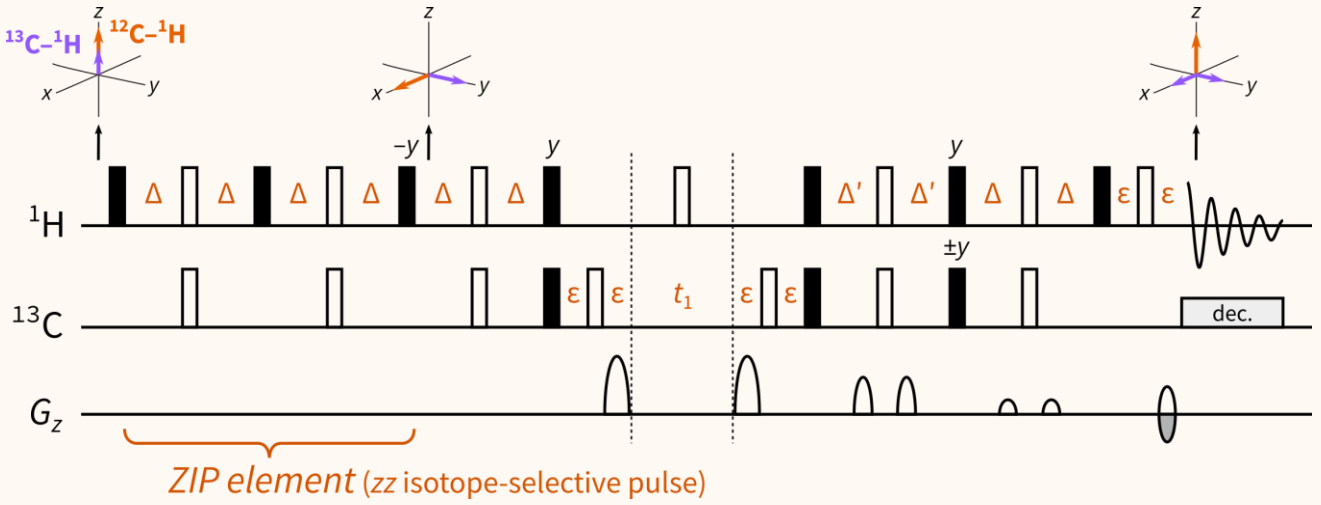
- Adding a double spin echo ("ZIP element") at the start of the seHSQC lets it preserve ^{12}C– or ^{14}N–bound ^{1}H magnetisation for other modules.
- This new ^{1}H–^{13}C seHSQC module provides 1.2–1.8× S/N gain vs the original HSQC module.
- The ^{1}H–^{15}N seHSQC gives 2.0–4.5× gains vs the HMQC, partly via collapse of f_1 multiplet structure.

Slide 3: HSQC-TOCSY and HSQC-COSY

- These preserve ^{12}C–bound ^{1}H magnetisation, but also allow variable excitation of ^{13}C–bound ^{1}H.
- The unexcited ^{13}C–^{1}H magnetisation (plus any that recovers during FID) can then be used for a HSQC module, e.g. to extract multiplicities or $^{1}J_{CH}$.
- HSQC-COSY avoids "relay peaks" present in HSQC-TOCSY, even when short mixing times are used.

Slide 2: Sensitivity-enhanced HSQC

Aim: Boost SNR for the least sensitive heteronuclear modules.



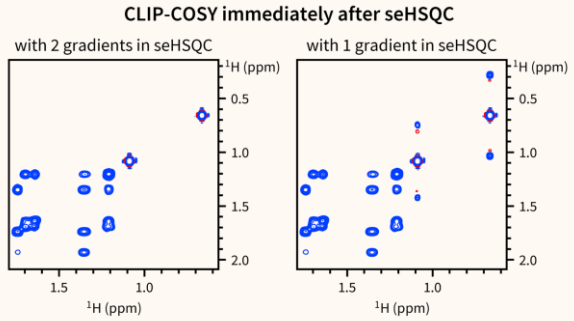
ZIP element distinguishes ^{13}C- and ^{12}C-bound ^{1}H

This ensures that the ^{12}C-bound proton magnetisation is ultimately returned to +z, meaning that it can be used for other modules.

The concept is readily extended to ^{15}N and ^{14}N-bound protons, too.

Gradient pair in t_1 prevents chemical shift evolution of ^{12}C-bound ^{1}H

Unsuppressed evolution of δ in multiple t_1 periods leads to "wing artefacts" in later NOAH modules.



Palmer, A. G.; Cavanagh, J.; Wright, P. E.; Rance, M. *J. Magn. Reson.* **1991,** *93* (1), 151–170.
Hansen, A. L., Brüschweiler, R. *et al.,* submitted for publication, **2021**.
Yong, J. R. J.; Hansen, A. L.; Kupče, Ē.; Claridge, T. D. W., submitted for publication, **2021**.

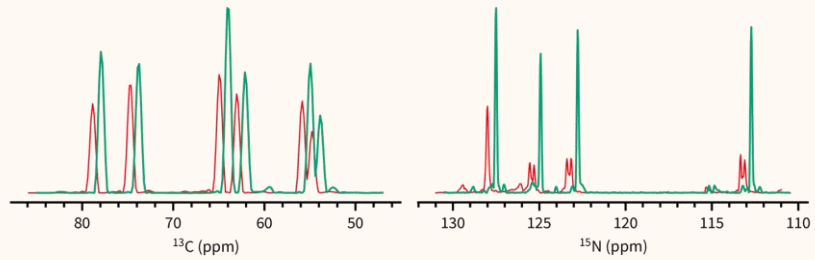
The seHSQC provides substantial SNR gains versus existing NOAH modules

^{13}C seHSQC vs ^{13}C HSQC

1.2–1.8× gains

^{15}N seHSQC vs ^{15}N HMQC

2.0–4.5× gains



P-1242 Diversifying NMR Supersequences with New HSQC-based Modules

Jonathan RJ Yong[1], Alexandar L Hansen[2], Ēriks Kupče[3], Tim DW Claridge[1]

[1]University of Oxford; [2]Campus Chemical Instrument Center; [3]Bruker UK

Summary

Slide 1: NOAH overview

- 2D NMR experiments ("modules") are combined into "supersequences" by removing relaxation delays, speeding data acquisition by up to 4×.
- Each module should only excite its share of magnetisation, e.g. ^{1}H–^{13}C HSQC should only excite ^{13}C–bound ^{1}H. "Standard" experiments must typically be modified to satisfy this.

Slide 2: Sensitivity-enhanced HSQC

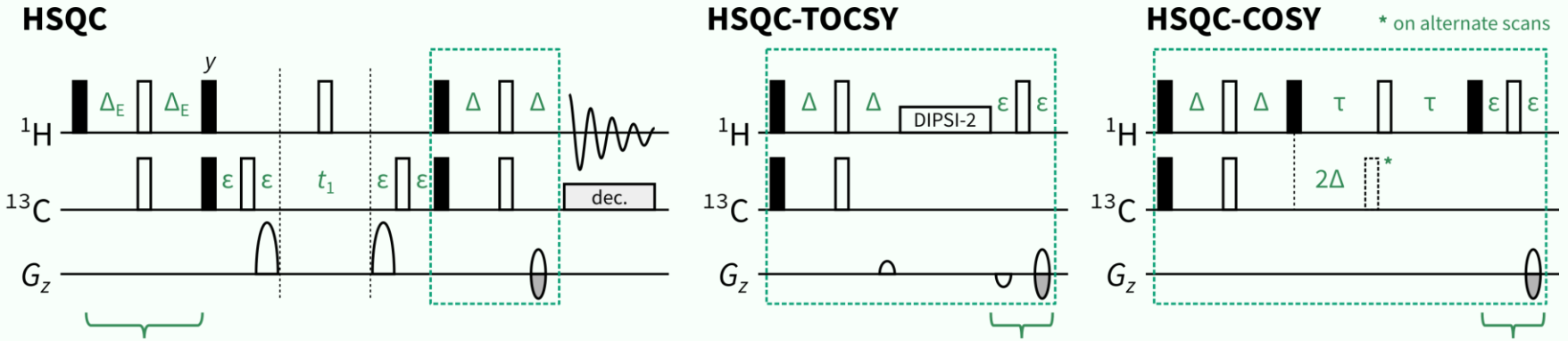
- Adding a double spin echo ("ZIP element") at the start of the seHSQC lets it preserve ^{12}C– or ^{14}N–bound ^{1}H magnetisation for other modules.
- This new ^{1}H–^{13}C seHSQC module provides 1.2–1.8× S/N gain vs the original HSQC module.
- The ^{1}H–^{15}N seHSQC gives 2.0–4.5× gains vs the HMQC, partly via collapse of f_1 multiplet structure.

Slide 3: HSQC-TOCSY and HSQC-COSY

- These preserve ^{12}C–bound ^{1}H magnetisation, but also allow variable excitation of ^{13}C–bound ^{1}H.
- The unexcited ^{13}C–^{1}H magnetisation (plus any that recovers during FID) can then be used for a HSQC module, e.g. to extract multiplicities or $^{1}J_{CH}$.
- HSQC-COSY avoids "relay peaks" present in HSQC-TOCSY, even when short mixing times are used.

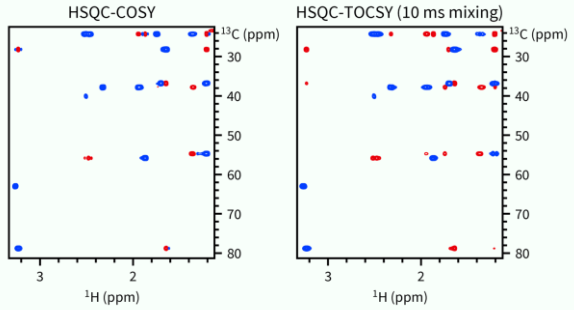
Slide 3: HSQC-TOCSY and HSQC-COSY

Aim: Increase variety of ^{13}C–^{1}H correlations available via NOAH.



HSQC-COSY suppresses relayed peaks present in HSQC-TOCSY

This is accomplished using a ^{13}C 180° pulse applied on alternate scans which inverts relayed peaks.



Becker, J.; Luy, B. *et al. J. Magn. Reson.* **2019,** *300,* 76–83.
Gyöngyösi, T.; Timári, I.; Kövér, K. E. *et al. Anal. Chem.* **2021,** *93* (6), 3096–3102.
Yong, J. R. J.; Hansen, A. L.; Kupče, Ē.; Claridge, T. D. W., submitted for publication, **2021**.

Modified INEPT delay Δ_E enables variable ^{13}C–^{1}H excitation (as in ASAP-HSQC)

Magnetisation can be partitioned between multiple HSQC-based modules in the same supersequence. Relaxation during FID acquisition, or isotropic mixing between modules, can also increases the available signal.