Homonuclear Decoupling in metabolomics

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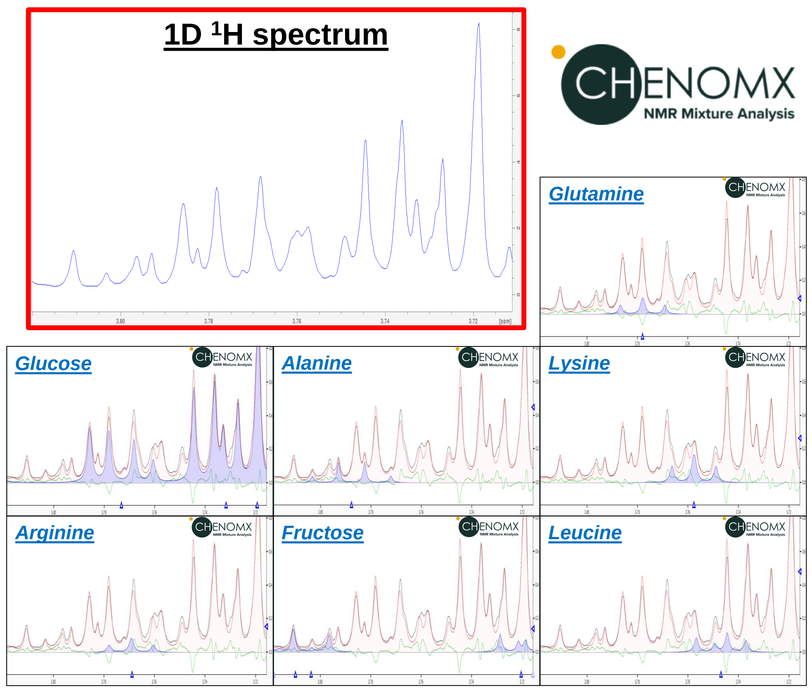
INTRODUCTION

1. Metabolomics: 1D NMR => signal overlap



2. Goal: improve resolution => 2D & HD

Resolution is a problem of ^{1}H 1D-NMR spectra. Techniques to increase resolution have been developed in the past decades, with multidimensional and "Pure-Shift" experiments. Pure-Shift, also known as Homonuclear Decoupling, consists in "removing" homonuclear coupling interactions causing the multiplicity.[1,2,3] (Fig 1)

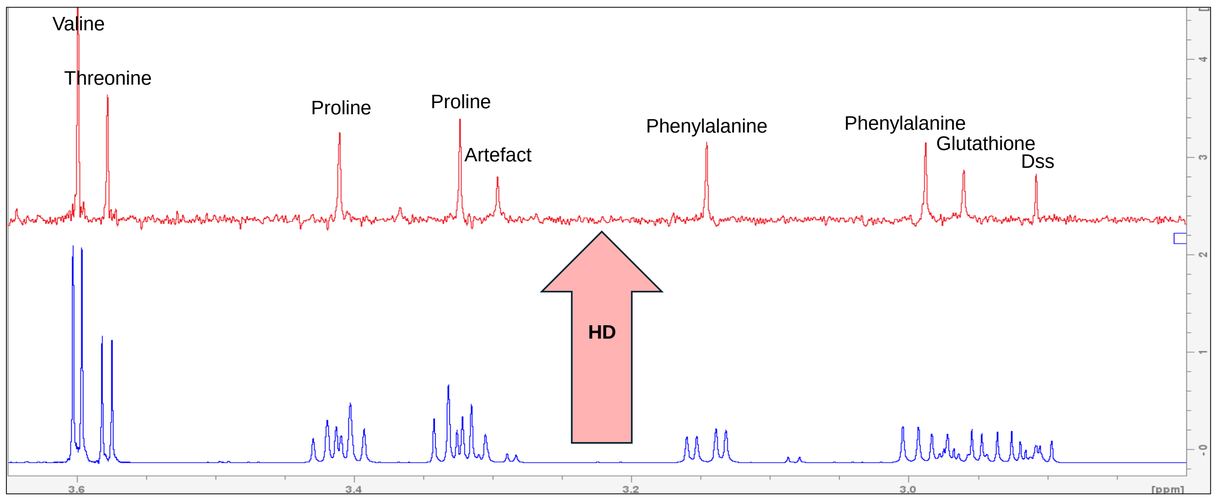
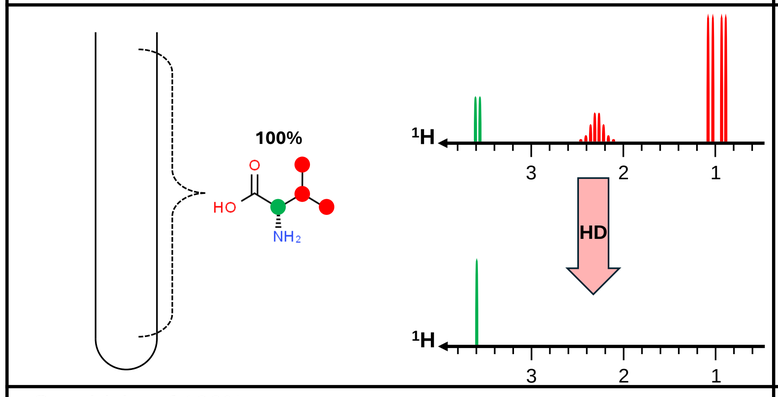
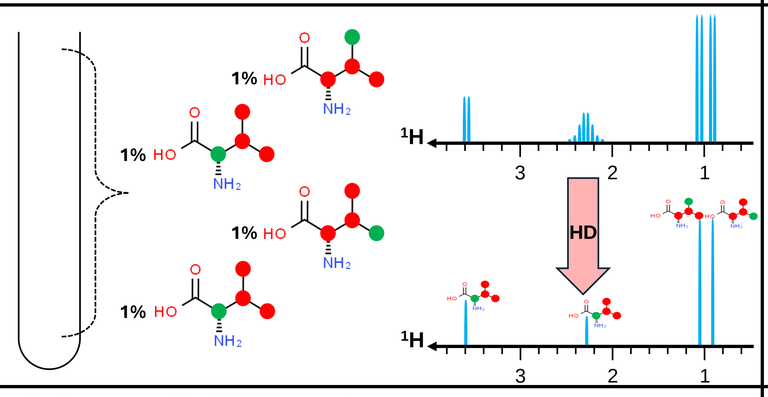
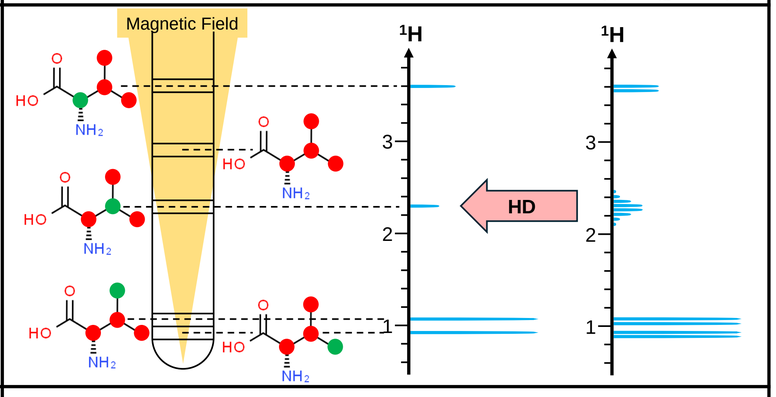


Figure 1 – ^{1}H NMR spectra acquired convetionnally (blue) and with homonuclear decoupling (red) on a D_2O sample containing 6 metabolites and DSS. Spectra have been normalised on the the DSS-singlet at 0.00 ppm. A resolution increase is notable on both spectra as signals are thiner.

METHOD

The general idea is to separate spins in two groups: the active ones which are observed, and the passive ones which are not. Based on this separation, spins are individually manipulated: 360°-rotation for active spins and 180°-rotation for passive spins. In the end, this results in suppressing the interactions of passive spins on the active ones. The key point is this selection, which can be obtained through three different ways.[4] These are displayed here-below with the example of the molecule of valine.
The active protons are displayed in green and the passive protons in red. The decoupled ^{1}H-spectrum is displayed beneath the theoretical ^{1}H-spectrum, both in light blue.

HOBS – **HO**modecoupled **B**and **S**elective	BIRD – **BI**linear **R**otation **D**ecoupling	ZS / PSYCHE – **Z**angger-**S**terk / **P**ure **S**hift **Y**ielded by **Ch**irp **E**xcitation
Selection based on a **selective chemical shift excitation**. Active spins: protons in the excited frequency band (*green*) Passive spins: protons outside the excited frequency band (*red*)	Selection based on **isotopic discrimination**. Active spins: protons attached to ^{13}C (*green*) Passive spins: protons attached to ^{12}C (*red*)	Selection based on a **spatial discrimination** obtained by magnetic field gradients and chirp pulses.[5] Each small volume ("slice") has a different active spins (*green*)
		
+ Sensitivity of 100% + Easy - Only selective chemical shift window - If coupled spins both excited => no decoupling	+ Perfect use with HSQC experiments + Broadband decoupling - Sensitivity of 1% (^{13}C abundance) - Geminal protons not decoupled	+ Most sensitive broadband decoupling - Sensitivity of 10-20% (1% for ZS) - Strong couplings can generate artefacts

RESULTS - DISCUSSION

Discussion

HOBS: As we are focussed on broadband decoupling, we did not explore the HOBS technique. It is henceforth not considered in this discussion.

Results: Our results concur with the Literature.
If the resolution enhancement is promising for both 1D and 2D spectra (*see results on the right*), the signal-to-noise ratio loss stands as a major hinder to a wider application. Nevertheless, it is noteworthy to mention two points: there is no sensitivity loss for BIRD-HSQC as the isotopic selection is intrinsic to HSQC experiments, and singlets are relatively more intense than multiplets. This means that some experiments can show little SNR loss, or even higher SNR (*see results 2D*)
Another drawback of HD-experiments are un-perfect decoupling (geminal protons for BIRD and strong-couplings for PSYCHE), which can false the signal identification.

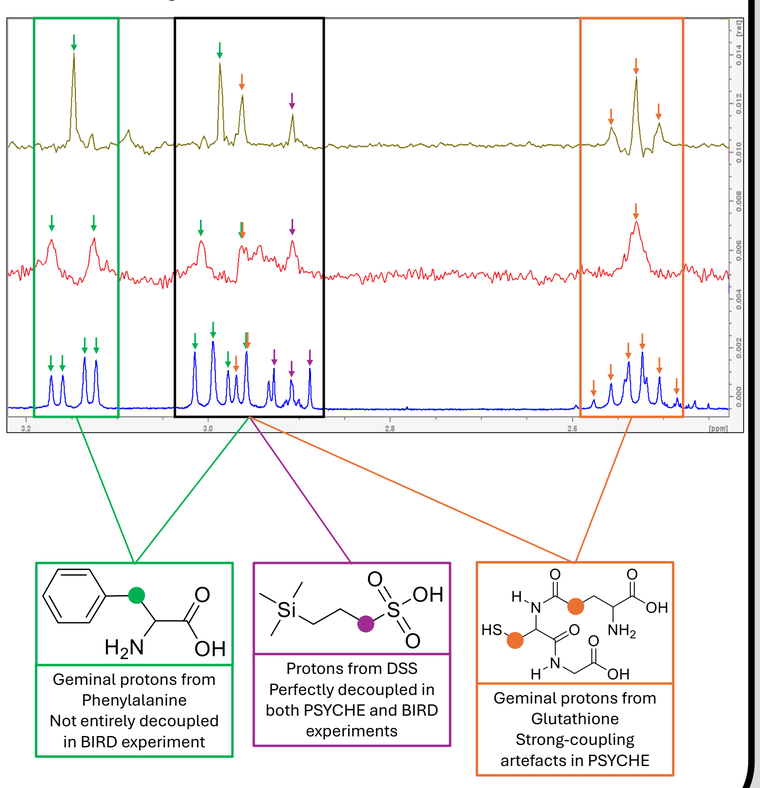
Discussion: In metabolomics, considering all processing and database protocols are based on coupled signals and the sensitivity loss of HD experiments, we are sceptic to the application of HD experiments for 1D spectra. However, the results obtained on 2D-spectra show resolution enhancement without major sensitivity loss (even increase in some points). These results and the current lack of established processing protocols for 2D spectra convince us of a future use of HD experiments for 2D spectra in metabolomics.

References

1. Giraudeau, P., *Analyst*. 2020, **145**, 2457-2472.
2. Dumez, J. N. *Chem. Commun.* 2022, **58**, 13855-13872.
3. Chen, X., Bertho. G., Caradeuc, C., Giraud, N., and Lucas-Torres, C., *Magn. Reason. Chem.* 2023, **61**, 654-673.
4. Bowyer, B., and Crouch, R., preprint at www.jeolusa.com (general)
5. Foroozandeh, M., *et al.* 2014, **53**, 6990-6992 (invention psyche)
6. Pulse sequences coming from Gareth Morris laboratory (Manchester): https://nmr.chemistry.manchester.ac.uk/?q=node/255

Results - 1D spectra

1D ^{1}H spectra were acquired with homonuclear decoupling (psyche in *green* and bird in *red*) and without homonuclear decoupling (*blue*) on a sample containing 6 metabolites and DSS in D_2O. The spectra intensity has been adjusted to allow a convenient comparison
(blue: 16 scans * factor 0.0125; red: 128 scans * factor 2; green: 2 scans * factor 4)
Arrows show the attribution of signals to metabolites (Phenylalanine in *green*, DSS in *purple*, and Glutathione in *orange*). Boxes show the specific protons attributed to the signals.



Results - 2D spectra[6]

HSQC and TOCSY spectra were acquired with (*red*) and without (*blue*) homonuclear decoupling on a sample containing 6 metabolites and DSS in D_2O. Boxes display perfectly decoupled signals (*green*), problematic signals (*purple*) and sensitivity improvement (*light blue*).