Inverse or Direct Detect Experiments and Probes: Which are "best" for *In-vivo* Research of ^{13}C Enriched Organisms?

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In *in-vivo* research spectral overlap observed in 1D ^{1}H NMR experiments makes identification of metabolites difficult. This can be overcome by the additional spectral dispersion afforded by 2D experiments, for example, the peak capacity of ^{1}H NMR is reported as 3000 while ^{1}H-^{13}C 2D NMR approaches 2,000,000.[1] Owing to the sensitivity of ^{1}H, inverse detected multidimensional NMR experiments are often ideal, as such many modern NMR probes are optimized for this purpose. Despite its sensitivity, ^{1}H detected NMR experiments can suffer from multiple drawbacks compared to ^{13}C, such as reduced spectral width, faster relaxation, and complications from water requiring suppression, especially for *in-vivo* NMR samples. The increase in sensitivity of NMR cryoprobe technology has greatly improved the sensitivity of ^{13}C detection experiments. This has led to a renewed interest in experiments based on ^{13}C detection.[2] Therefore, novel ^{13}C experiments show great potential for metabolomic studies. Further, they offer a very interesting alternative for *in-vivo* research especially as organisms are commonly ^{13}C enriched for *in-vivo* analysis.[3,4]

In this study we compare inverse and observe experiments (HSQC vs HETCOR) on inverse and observe prodigy cryoprobes (TCI vs BBO) for collecting metabolic data on ^{13}C enriched *Daphnia Magna*. The key questions being:

1) Which experiment identifies the most metabolites? Or do HSQC and HETCOR produce complementary information and both experiments should be run?
2) In *in-vivo* research, data is continuously collected, as such cryoprobes cannot be changed during a study. Assuming both HSQC and HETCOR need to be run, is it best to run them on an inverse probe (optimal for ^{1}H detected HSQC, and use the decoupling channel for ^{13}C detected HETCOR) or BBO (optimal for HETCOR but compromised for HSQC)?

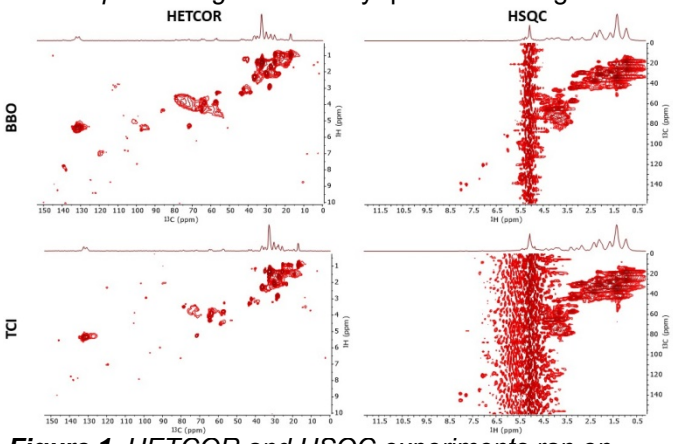

***Figure 1**. HETCOR and HSQC experiments ran on BBO and TCI cryoprobes for 15 minutes.*

Figure 1 shows example data with each experiment taking 15 minutes to collect. Signals in HSQC are more intense while HETCOR is water free. As expected, the HETCOR on the BBO contains stronger signals. Interestingly, while the signals in HSQC are stronger on the TCI, the water is also detected more effectively and the wide water base leads to a much wider region of the spectrum being unusable. However, with longer experiment times (24 hrs) (see figure 2), the signal from the sample becomes larger making the water contributions less problematic in HSQC. At the same time the signal intensity in the HETCOR also improves, with both spectra permitting the identification of over 50 metabolites *in-vivo*. The presentation will compare in detail the various experiments and probes over different experimental timeframes in order to attempt to identify the most efficient probe/ experiment combinations for metabolic screening *in-vivo*.

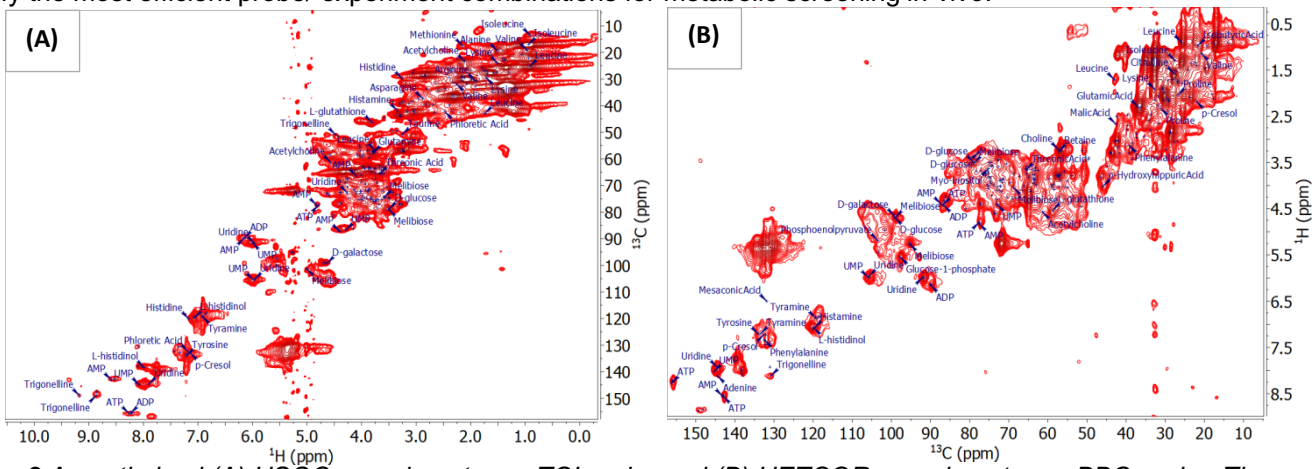

***Figure 2** An optimized (A) HSQC experiment on a TCI probe and (B) HETCOR experiment on a BBO probe. The spectra have over 50 metabolites identified and assigned.*

References: 1. Hertkorn, N. *et al. Anal. Bioanal. Chem.* 389**,** 1311–1327 (2007). 2. Bermel, W., *et al. Concepts Magn. Reson. Part A* 32A**,** 183–200 (2008). 3. Soong, R. *et al. MRC* 53, 774–779 (2015). 4. Mobarhan, Y. L. *et al. Chem. Sci.* 7, 4856–4866 (2016).