# Targeting the Formation of $^{13}C$-$^{12}C$ Bonds *In-Vivo*: A Unique Approach to Probe Complex Processes

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## Introduction

NMR is unique in its ability to provide a wide range of metabolic information *in-vivo,* especially when combined with isotopic $^{13}C$ labelling.[1] *Daphnia magna* (water fleas) are one of the most commonly used organism for aquatic toxicity studies, see Figure 1. They are found in almost all freshwater bodies, have a well understood genome, and a relatively short lifestyle. Their lipid content is also solely provided from food.[2] This makes then an ideal candidate for *in-vivo* studies as they can be placed directly into the NMR using a 5 mm flow system, and provided food and oxygen keeping them alive indefinitely.[3]

2D $^{1}H$ - $^{13}C$ *in-vivo* datasets are complex, making it challenging to monitor specific processes of interest. Here, a new experiment is introduced that targets the formation of $^{12}C$ - $^{13}C$ - $^{1}H$ bonds.

**Figure 1.** Image of *Daphnia magna.*

## Sequence

A simplified version of the utilized pulse sequence can be seen in Figure 2, broken down into three blocks. The first block (A) is a reversed HSQC where the $CH_2$ are reflected arounds the carbon offset.[4] The result of block A is that $^{1}H$'s on $^{13}C$'s are selected. Block B allows the selected protons to correlate via TOCSY transfer to other protons in the spin system, but only to neighbouring atoms. Block C suppresses one bond $^{1}H$ -$^{13}C$ correlations leaving only the relays from a $^{1}H$ on a $^{12}C$ (not suppressed by the filter) correlating to the adjacent $^{13}C$ ($^{1}H$-$^{13}C$-$^{12}C$). In simple terms $^{12}C$-$^{13}C$ bonds are selectively detected. The data are collected in an interleaved fashion producing two data sets. One dataset contains all $^{1}H$-$^{13}C$ bonds while the other contains only units where a $^{13}C$ and $^{12}C$ are adjacent to each other.

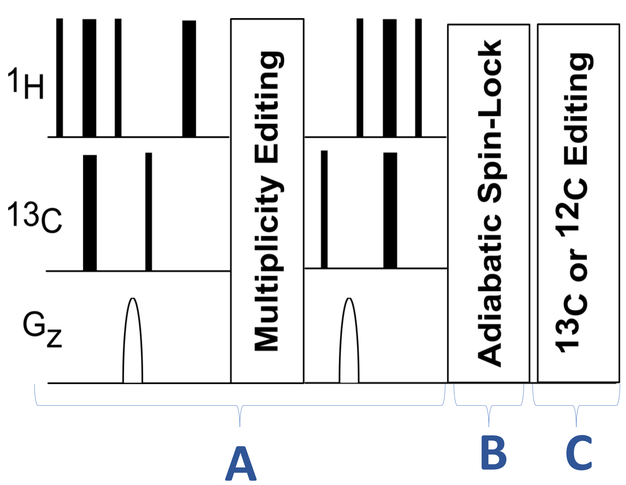


**Figure 2.** Schematic of the pulse sequence utilized to provide information on the $^{1}H$-$^{13}C$- $^{12}C$ bonds in samples of *D. magna.* A, B, and C are discussed in the text.

## Proof of Concept

As an example of utilizing this sequence, a spectrum of singly labelled glucose was examined, see Figure 3. (A) shows only the $^{1}H$-$^{13}C$ bonds where (B) shows the $^{1}H$-$^{13}C$-$^{12}C$ bonds.

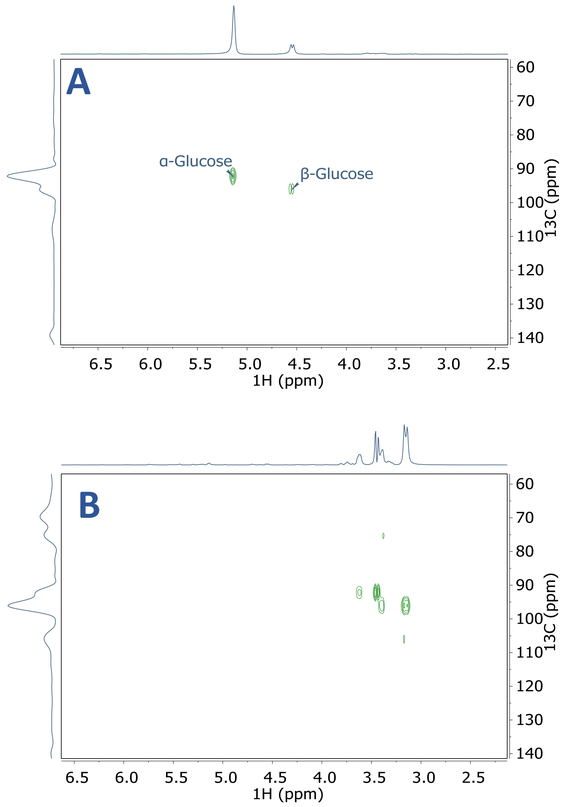


**Figure 3.** $^{13}C$-1-Glucose measured with the new sequence. A) only the $^{13}C$ and B) the $^{13}C$-$^{12}C$ bonds.

## Monitoring a Reaction

The next step is to follow a simple process. For this purpose the $^{13}C$-1-Glucose and bakers yeast (*Saccharomyces cerevisiae*) were used to examine the formation of ethanol over 24 hours, see Figure 4. The initial spectrum matches the two above, the resulting spectra contain the ethanol product, which can only be seen on the $^{13}C$ spectrum as the OH peak is directly bonded to carbon 1. βα

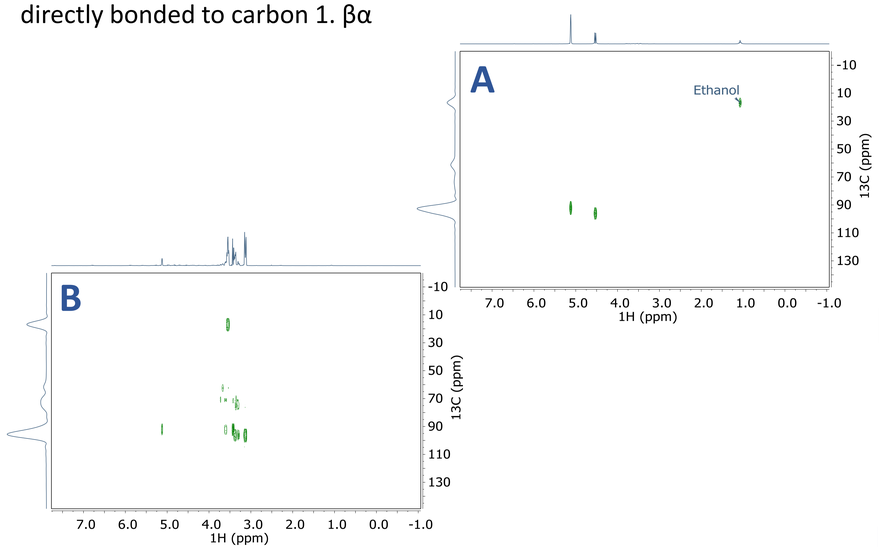


**Figure 4.** $^{13}C$-1-Glucose and bakers yeast used to examine the formation of labelled ethanol after 48 hours A) the $^{1}H$-$^{13}C$ spectrum with and B) examining the $^{12}C$-$^{13}C$ bonds.

## *In-Vivo* Applications

To demonstrate the concept, fully $^{13}C$ enriched *D. magna* were fed $^{12}C$ *Chlamydomonas reinhardtii* for 96 hours, see Figure 5. (A) shows only the $^{13}C$ bonds after feeding of non-enriched algae for 96 hours and (B) shows only the $^{12}C$-$^{13}C$ bonds. In Figure 6, the horizontal projections of the feeding process, as seen from this, there exists the presence of energy molecules early on that are rapidly consumed to allow the formation of sugars and lipids over time.

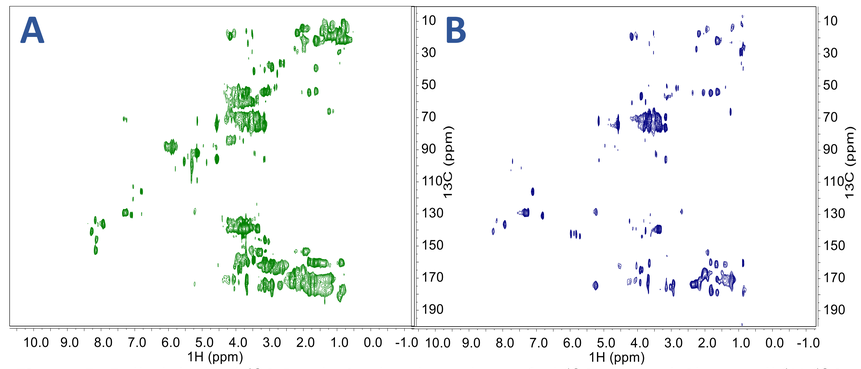


**Figure 5.** Fully labelled $^{13}C$ Daphnia that have been fed $^{12}C$ algae a) Shows all $^{1}H$-$^{13}C$ bonds in the sample. b) shows ) the $^{13}C$-$^{12}C$ bonds.

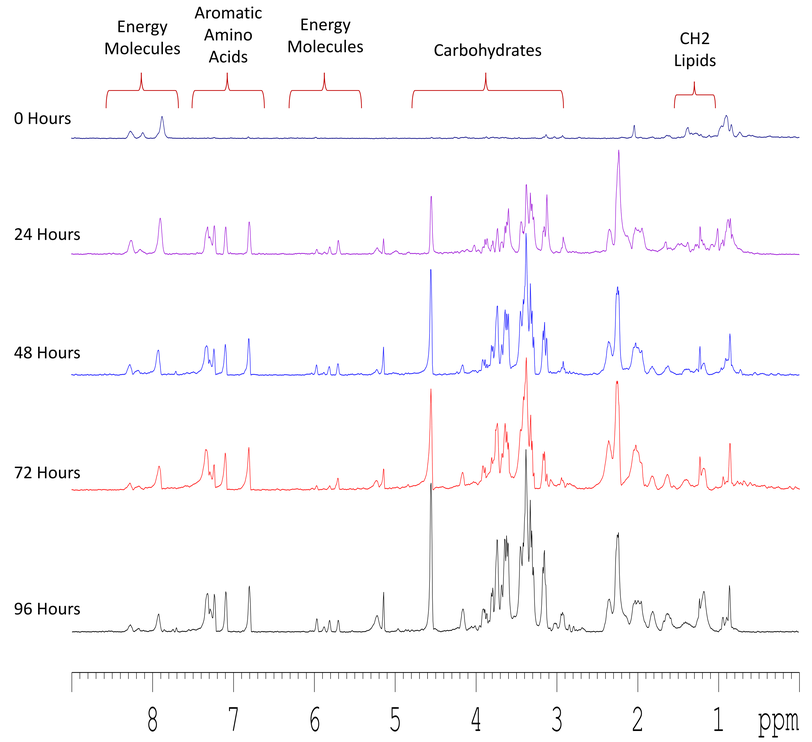


**Figure 6.** Horizontal Projections of the $^{12}C$-$^{13}C$ bonds over time in fully $^{13}C$ enriched *Daphnia* fed *C. reinhardtii* for 96 hours.

## Discussion

This approach can easily be extended to follow the fate of drugs, contaminants, or target nutrients *in-vivo*. Future work will examine how fully labelled compounds can be used as probe molecules to examine how non-enriched organisms use and convert specific substances in their systems.

## References

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