Low field biological *J*-coupling spectroscopy

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Introduction: Low-field NMR spectroscopy offers advantages for studying cells metabolism in their native environment. This is especially relevant for in-vitro studies, where low-field NMR could be used to analyze biological samples with reduced equipment size and cost, potentially benchtop research. However, the study of cellular metabolism at low, milli-Tesla, magnetic fields remains an unsolved problem. In this work, we tackle this problem by combining J-coupling spectroscopy [1] and parahydrogen-induced polarization (PHIP) [2] with our multinuclear scanner built in-house.

Methods: We developed and optimized the following steps: **1)** The PHIP protocol for providing biologically compatible [2-^{13}C] pyruvate. **2)** The design and in-house construction of a multinuclear low-field NMR spectrometer with B_0 = 0.066 T, a triple-nuclear RF coil for ^{1}H, ^{13}C and ^{23}Na channels, and five shimming. **3)** The fingerprinting matching method for quantifying the pyruvate and lactate concentration. **4)** The validation of the methodology by measuring synthetic enzymatic pyruvate-to-lactate conversion. Finally, we measure metabolism in PanC02 cancer cells for three different pyruvate and lactate concentrations.

Results and discussion: Fig. 1 shows a summary of the main results obtained from our work. This includes the implementation of strong J-couplings for [2-^{13}C] lactate that allow the differentiation with pyruvate at low magnetic fields, the optimization of a PHIP protocol that provides biocompatible [2-^{13}C] pyruvate with signal enhancements of 20,000 that facilitates its detection at low magnetic fields, and the multinuclear low-field NMR spectrometer developed in-house (top section of Fig. 2). Finally, the right box of Fig. 1 shows a pyruvate-to-lactate conversion measured at 0.066 T for PanC02 cancer cells. The pyruvate + lactate peak can be differentiated from the lactate peak, allowing for concentration quantification. Thus, we implemented a fingerprinting matching method to determine the pyruvate concentration and magnetic field homogeneity. The estimated ΔB_0 = 35 Hz describes the observed line broadening. The correlation of 0.9682 shows a good agreement between the simulated and acquired spectra.

Conclusion: Our results represent the first time that a J-coupling spectroscopy is implemented in biological samples at low magnetic fields. The differentiation of the pyruvate and lactate demonstrated that is possible to study metabolism in milli-Tesla fields.

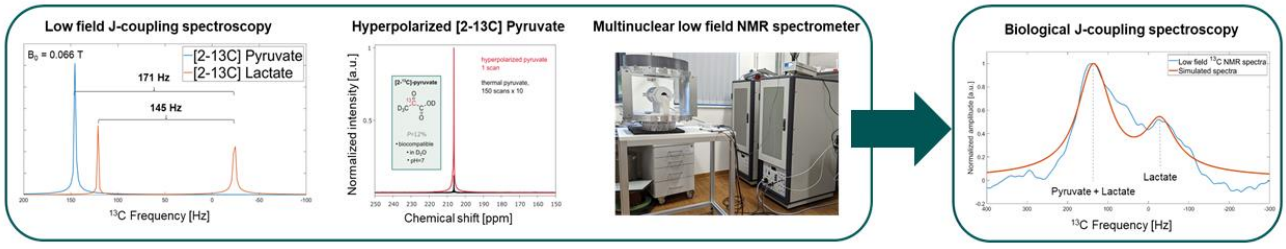

Fig. 1: The simulated pyruvate and lactate spectra at 0.066 T and ΔB_0 = 0 Hz are shown in the left. This was combined with a PHIP protocol optimized to provide a biocompatible solution with 20,000-fold signal enhancement middle. Additionally, we developed a multinuclear low-field NMR spectrometer in-house to be able to acquire the data. This includes the main magnet, shimming and RF coils (right image). Finally, we measured the pyruvate and lactate spectra in PanC02 cancer cells and calculated the pyruvate concentration and field inhomogeneity through fingerprinting matching (right box).

References: [1] Appelt, Pys. Rev. A (2010). [2] Hovener, Angew. Chem. Int. Ed. Engl. (2018).