# Laplace Inversion for Kinetic Analysis of Hyperpolarized 13C data without a priori knowledge using a Hybrid Maximum Entropy Method (MEM)/ Non-Linear Least Square (NLS)

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**Purpose:** Hyperpolarized Magnetic Resonance[1] has been used to study metabolism in cancer, the heart, liver and the brain to study a variety of metabolic pathways *in vitro* in whole cells, *ex vivo* in perfused organs as well as *in vivo*. To obtain information on the underlying metabolic activities, hyperpolarized data are usually fit using kinetic models to derive rates. All kinetic models proposed so far assume a priori knowledge of the structure (e.g. number of compartments) of the metabolic pathway[2,3] and are challenged by the "multiple local minimum" problem. In this work, we show the feasibility of analysing hyperpolarized data without prior assumptions of a model and, at the same time, overcoming the "multiple local minimum" problem by applying a hybrid Maximum-Entropy/NonLinear-Least-Squares (MEM/NLS) method[4] to hyperpolarized $^{13}$C data both *in vitro* in whole blood and *ex vivo* in the isolated rat heart.

**Methods:** Pyruvic acid containing 15 mM trityl radical and 1 mM Gadolinium (Dotarem) was hyperpolarised in a HyperSense® (Oxford Instrument) DNP at 3.35 T and 1.4 K for ~ 1hr. In vitro $^{13}$C data: 100 µl of the hyperpolarized solution was mixed with 500 µl of whole blood (heparinised), previously collected from male Wistar rats (n=4, 225-250 g). Ex vivo data: 3ml of hyperpolarised [1-$^{13}$C] Pyruvate was injected in a Langendorff perfused rat heart (n=4) at constant flow (1ml/min). In both *in vitro* and *ex vivo* set up, a series of $^{13}$C-MRS spectra (Δt=2s) of hyperpolarised [1-$^{13}$C] pyruvate and its metabolites were detected with a Bruker 9.4 T at 310K and a small flip angle excitation (~10deg). Datasets were analysed using the hybrid MEM/NLS (MeMexp[4]). Using this approach, the number of components characterizing the experimental data is determined iteratively, using MEM fitting results as input parameters for NLS. Additionally, both *in vitro* and *ex vivo* experiments were fitted with NLS only (exponential fitting) and for *in vitro* data the rates obtained from the MEM/NLS hybrid fitting were also compared with those obtained using a traditional two-compartment model.

**Results:** A representative summed $^{13}$C spectrum is shown Fig. 2a and 2b for the *in vitro* blood and *ex vivo* heart experiments, respectively. An example of the MeMexp output for $^{13}$C hyperpolarized data is shown in Fig. (1a-b) (*in vitro*) and Fig. (4a-d) (*ex vivo*). *In vitro* the hybrid MEM/NLS method suggests a single component to characterize the pyruvate curve (Fig.1a), whereas two components were found for the lactate curve (Fig.1b). *Ex vivo,* two components were found for pyruvate and its metabolites bicarbonate, lactate and alanine (Fig.4a-c).

**Discussion:** Results from *in vitro* data show a good agreement between the number of components found with MEM and those obtained from MEM/NLS hybrid fitting (Fig.1a-b). Results from *ex vivo* data show a higher number of peaks from MEM than from MEM/NLS hybrid (Fig. 4a-d). The extra peaks shown in the MEM solution are likely to be artefacts due to the fact that the MEM biases the solution sought as well as the ill conditioned nature of Laplace Inversion[4]. Good agreement was found between the proposed hybrid approach and the two-compartment model for the rates derived in the *in vitro* data (Fig. 3).

**Conclusion:** In this work we showed a novel approach to analyse hyperpolarized data without *a priori* assumptions on the metabolic pathways by using a hybrid MEM/NLS method. Using the hybrid MEM/NLS avoids the "multiple local minimum" problem. The hybrid MEM/NLS will be useful in analysing hyperpolarized data that describe complex metabolic reactions or heterogeneous biological systems where simple kinetic models do not describe the actual biology and possibly fail because of a priori assumptions.

**References: 1.**Ardenkjaer-Larsen JH, *Proc. Natl. Acad. Sci. U. S. A.* Sep 2 2003;100(18):10158-10163**. 2.** Zierhut ML, *Journal of Magnetic Resonance.* Jan 2010;202(1):85-92. **3.** Day SE, et al. Nat. Med. Nov 2007;13(11):1382-1387. **4.**Steinbach PJ, *Biophys. J.* Apr 2002;82(4):2244-2255.

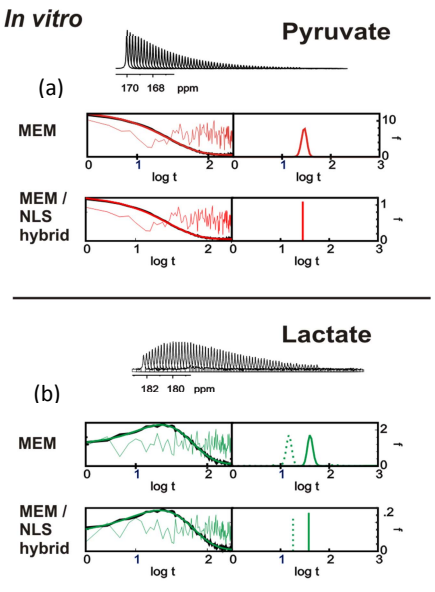


**Figure1**: Example of MeMexp output for *in vitro* data. One component was found to characterize the pyruvate curve (Fig.1a), whereas two components were suggested for the lactate curve (Fig.1b). Solid peaks are the positive rates describing the build up of the curves, whereas dashed peaks are the negative rates describing the decay of the curves.

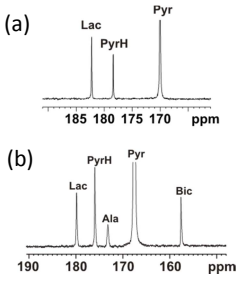


**Figure2:** Representative $^{13}$C hyperpolarized spectrum obtained by summing the entire time-series for *in vitro* (Fig.2a) and *ex vivo* experiments (Fig.2b).

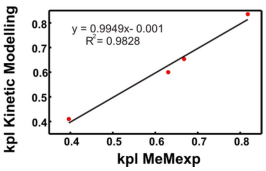


**Figure (3):** Comparison between the rates obtained from the hybrid MEM/NLS method with those obtained by from traditional two-compartment model.

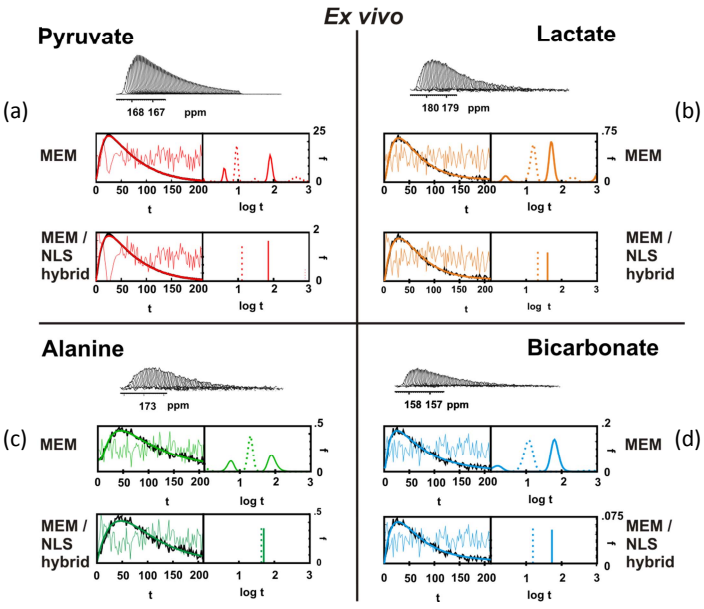


**Figure (4):** Example of MeMexp output for *ex vivo* data. Two components are found for pyruvate and its metabolites (Fig.4a-c). Solid peaks are the positive rates describing the build up of the curves, whereas dashed peaks are the negative rates describing the decay of the curves.