First HR-MAS MRS Slice-Localized Spectroscopy (S.L.S.) and (frequency/space) C.S.I. of living drosophila.

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- Target audience: People interested in: genetics animal models, neurodegenerative animal models, MRS methodology.

- Purpose: For ten years, in addition to the models developed in monkeys and rodents, many human diseases have been successfully modeled in an insect, *Drosophila melanogaster*. This organism has specific advantages for genetic studies, and discoveries made on these models are often found to have a significant impact on the understanding of the disease. Up to now, few MRS studies have been done, and only in a non-localized manner (1). We wanted to show that it is possible to get localized metabolite information.

- Methods: NMR spectrometer Bruker (17.6T, 8cm bore vertical magnet equipped with a HR-MAS probe). Pulse sequences: custom made "Semi selective slice localized spectroscopy" (SLS) and (frequency/space) CSI. Drosophila is placed in an HRMAS rotor (3.2 mm inner diameter) and spun up to 2.6 kHz. Anesthesia was provided by decreasing the temperature around 3°C. Drosophila : For demonstration of feasibility, we used two groups, up^{101} mutant and wild type (wt). The *up* mutant cannot take flight and shows a wings-up phenotype because of indirect flight muscles (IFM) degeneration.

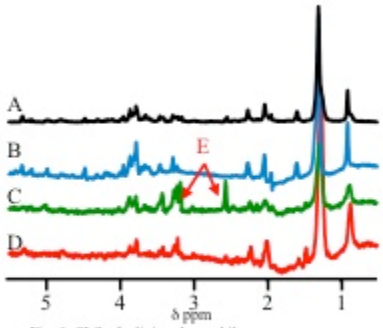

Fig. 1. SLS of a living drosophila
A: Whole fly; B: head; C: thorax; D: abdomen
E: β-alanine

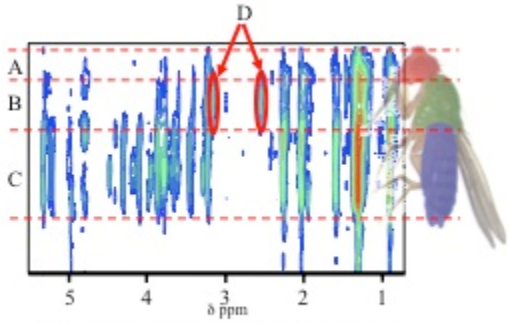

Fig. 2. CSI of a living drosophila (chemical shift/space)
A: head; B: thorax; C: abdomen; D: β-alanine

- Results:. To obtain a meaningful information, it is not necessary to make a "voxel" localization of the measurement, a "virtual slice localization" is sufficient. SLS allows to make a semi-localization perpendicular to the rotation/gradient axis: head, thorax, abdomen (fig 1). The same type of information can be obtained in only one experiment with the (frequency/space) CSI (fig 2).

As a demonstration of an efficient localization: a metabolite present in muscles, β-alanine is only identified in thorax (fig 1 and 2). β-alanine signals are highly reduced in SLS and CSI spectra of the thorax of mutant up^{101}.

- Discussion: The efficiency of the semi-localized acquisition of ^{1}H HRMAS spectra has been proved by 2 different techniques, SLS and frequency/space CSI. These results open numerous possibilities of study of metabolism in genetically modified drosophila. The semi-localization (head/thorax/abdomen) (SLS) (fig. 1) gives metabolome information in 3 experiments. CSI brings up information on the spatial relations of metabolites in one experiment. These techniques are complementary, depending of what we want to know.

- Conclusion:

We have shown that it is possible to obtain semi-localized MRS spectra (head, thorax, abdomen) of alive drosophila by using HR-MAS technique associated with rotating magnetic field gradients and very high magnetic field (17.6T). Applications to the study of mutants up^{101} will be shown. Studies on neurodegenerative flies models is ongoing.

- References:

1) Righi, V. *et al. International Journal of Molecular Medicine* 26, 175-184 (2010).