Multiple-oocyte in vivo localized MR spectroscopy

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Introduction: *Xenopus laevis* oocytes are used as model, for example, in developmental biology or in pharmacokinetic studies. MR spectroscopy is a potential tool for the investigation of oocytes, because it allows for observation of cellular metabolites. PRESS localization to small voxels within single cells has been reported to afford separation of MR spectra from the nucleus and the cytoplasm [1]. This approach required micro-coil equipment and implied very long measurement times. Here, we introduce a novel approach, which combines solvent-localized (SOLO) NMR [2] with the use of chemical shift agents, and makes localization in samples with multiple oocytes possible.

Theoretical background: SOLO NMR spectroscopy allows separating MR spectra of solutes in different solvents, based on selective excitation of the solvent. The method is based on the effect of the distant dipolar field (DDF) that originates from the longitudinal magnetization of the sample. The 2D pulse sequence (Fig. 1) was derived from the HOMOGENIZED experiment [3] and creates a DDF with a frequency selective second pulse [4]. The DDF locally refocuses transverse magnetization leading to detectable signal [4, 5]. The local reach of the action of the DDF can be adjusted by a correlation gradient (CG), of the strength G and duration T, which spatially modulates the magnetization. When the second pulse is applied frequency selectively, the effective DDF is created only in a volume where solvent spins are excited. Thus, signal is only detected from regions inside the probe that contains the excited solvent.

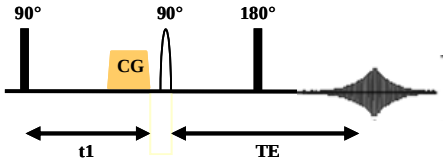

Fig. 1: 2D pulse sequence used for multiple-oocyte localization with SOLO

Experiments: Measurements were performed on a Bruker Avance 750 WB spectrometer. PRESS spectra of single oocytes were acquired with TR/TE=1000/20 ms using a 1.6 mm microcoil. SOLO spectra were acquired on a sample of about 100 oocytes in a 5 mm NMR tube using a 5 mm birdcage coil. 64 t_1 increments were acquired with a relaxation delay of 3 s and a +/- phase cycle on the first and third pulse. TE was 100 ms and 1024 points were acquired. Further SOLO experiments were performed on a 700 MHz high-resolution NMR system equipped with a cryo-probe. The required difference in chemical shift (2ppm) between intra and extracellular water was reached by doping the medium with 10 mM Tm-DOTP, which remained in the extracellular space. For metabolite/drug uptake studies the oocytes were incubated in Barth medium containing either 125 mM choline (Cho) or 10 mM tetracycline.

Results: After incubation with Cho, PRESS spectra from cubic 250-micron voxels in different intracellular regions (Fig. 2) showed different concentrations of Cho. While in the vegetal hemisphere no Cho was found, high Cho concentrations were observed in animal hemisphere and nucleus. The intracellular Cho concentration was estimated from relative peak intensities and relaxation time measurements to 60 mM. From a sample of 100 oocytes, SOLO allowed separating intracellular from extracellular spectra. Upon selective excitation of either intracellular or extracellular water, only intracellular or extracellular Cho was observed, respectively (Fig. 3). SOLO spectra were intrinsically averaged over all oocytes in the sample, compensating for the low sensitivity of the method. Comparing SNR-efficiency of SOLO and PRESS (Table 1), SOLO afforded a more than 30-fold gain in sensitivity. The high sensitivity enabled observation of drug uptake. After incubation with 10 mM tetracycline, the $N(CH_3)_2$ peak became observable in intracellular PRESS spectra (500 µm voxel) after 68 min averaging. SOLO spectra acquired in 3.3 hours showed an intracellular tetracycline $N(CH_3)_2$ peak with an SNR of 69. This sensitivity renders SOLO a promising tool for studying pharmacokinetics of cellular uptake of drug molecules. SOLO, furthermore, averages over 100 oocytes and, thus, provides intrinsic statistics, which can not be reached with single cell investigations. Another great advantage of SOLO is that it requires only a single-axis gradient. Implementation of the method on a high-resolution 700 MHz system with cryo-probe afforded creation of an intracellular fingerprint of the oocytes.

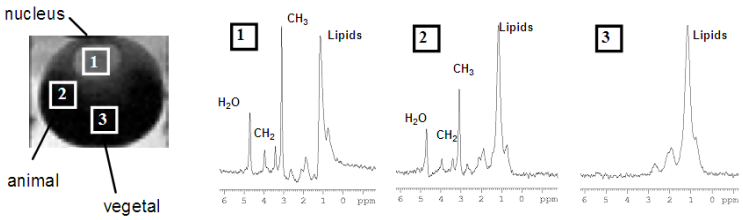

Fig. 2: Choline uptake into cellular compartments. Localized PRESS spectra from a voxel size of $(250\ \mu m)^3$ of nucleus (1), and animal (2) and vegetal (3) cytoplasm.

	Localization	SNR/√min
$(250\ m)^3$ (n=6)	Nucleus	0.73±0.13
$(500\ \mu m)^3$ (n=6)	Whole cell	1.36±0.26
SOLO (n=4)	Global 100 oocytes	44.4±2.1

Table 1: SNR-efficiency of PRESS and SOLO

Conclusion: Combination of SOLO and doping the extracellular medium with chemical shift agents allows separating extra from intracellular spectra of oocytes. Compared to PRESS SOLO affords a more than 30-fold sensitivity gain. Because only a single-axis gradient is required, SOLO can further benefit from the sensitivity of high-resolution cryo-probe NMR systems and is readily applicable on most NMR spectrometers.

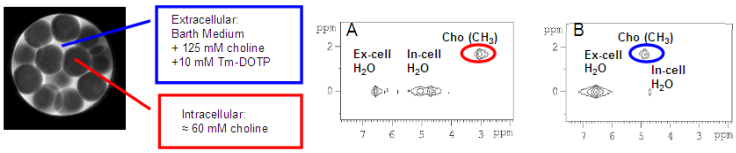

Fig. 3: Left: 2D GE image across 5 mm sample tube with 100 oocytes. Right: SOLO spectra averaged over 100 oocytes with selective excitation of intracellular (A) or extracellular (B) water. Only intracellular (A) or extracellular (B) choline was observed.

References:
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