Background: Dynamic Nuclear Polarization (DNP) provides enhancement of NMR sensitivity by factors (~10^4) sufficient to allow the monitoring of the conversion of ^{13}C labeled substrates in live cells over time periods of many 10s of seconds. Cell preparations are described in separate SOPs. The general procedure is to separately polarize a substrate, inject the substrate into an NMR tube containing cells to be examined, and collect data in a series of time points spanning periods from a few tens of seconds to a minute. The length depends on the polarization storage (spin relaxation) properties of the substrate. Non-protonated ^{13}C sites in isotope enriched substrates prove to have suitable spin relaxation properties.

Materials:

Hyperpolarizer: Hypesense from Oxford instruments (3.35T, 94 GHz)
NMR spectrometer: Varian INOVA 500 MHz equipped with an 8mm broad band observation probe
Sample: ^{13}C1 – pyruvic acid (Cambridge Isotopes)
Free radical: trityl oxo63 (GE Healthcare)
Solvent: D_2O, glycerol-d8, (Cambridge Isotopes)

Procedure:

Part 1: Substrate preparation

1. Determine the final substrate concentration needed according to the experimental goal. For pyruvate, a typical concentration is 5 mM.
2. Dissolve the desired amount of substrate in polarization juice. For a 3 mL dissolution of pyruvic acid: 1.2 μL ^{13}C1-pyruvic acid, 1.2 μL glycerol-d8, 0.3 μL 150 mM oxo63 (free radical).
3. Place substrate and free radical solution in a polarization capsule (max volume 100uL)

Part 2: Substrate polarization

1. About 3 hr before data acquisition, turn on the polarizer and allow the system to cool down to a temperature of ~1.4K at a pressure of ~3 mbar (usually takes 10-25 minutes) and then load the polarization cup containing substrate an into the polarizer (HyperSense, Oxford Instruments).
2. Irradiate with microwaves (~ 100 mW at 94 GHz) for 1-2 hours depending upon polarizing properties of the substrate.
3. Build up can be monitored via a ^{13}C detect coil built into the cryostat as seen in Figure 1. Microwave irradiation frequency can be optimized by scanning the frequency and selecting a frequency giving maximum positive enhancement as illustrated in Figure 2.

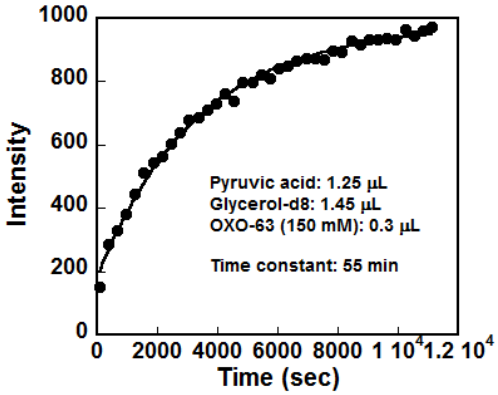

Figure 1. The polarization build-up curve of 1.25 µL pyruvic acid.

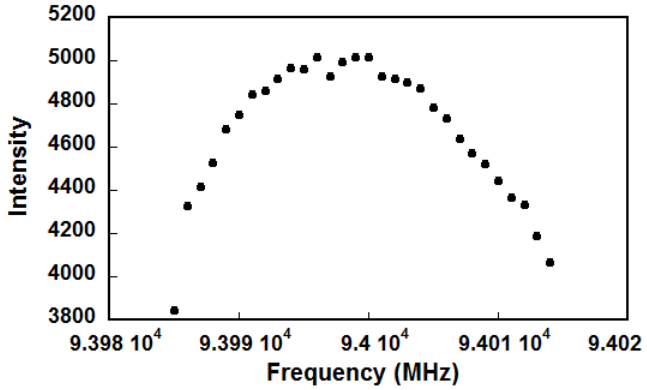

Figure 2. Determination of the optimal irradiation frequency by the frequency sweep.

Part 3: Data acquisition

1. About 30 min before the experiment, transfer about 2 mL of a cell prep to an 8 mm NMR tube equipped for substrate injection or cell perfusion (Figure 3). See SOP such as: "Observing Metabolic Flux by DNP NMR: Adherent Cell Sample Preparation". Allow the cell preparation to settle for a few minutes before placing in the NMR spectrometer and remove extra solution on the top.

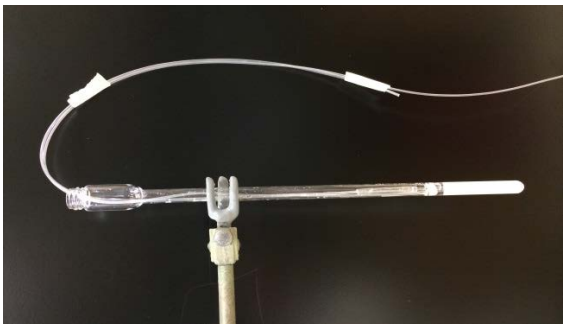

Figure 3. The 8 mm NMR tube with the cell retention module and microcarrier beads.

2. Insert the cell retention module into the NMR tube, including sample injection line, filters, and overflow line. The cells may be washed with PBS in the case of immediate manual injection, or continuous perfusion with medium may be established in the case of a programmed injection.
3. Increase the NMR probe temperature to 37 C, let it equilibrate (5 min), insert the NMR tube in the magnet, tune and match the coil, and then maximize the magnet homogeneity by shimming on the water peak (< 35 Hz is OK).
4. Load the acquisition program into the spectrometer (For a VARIAN spectrometer, type "s2pul" in the command line to load the standard single pulse sequence). The following commands and settings then implement data collection with a one pulse sequence with no proton decoupling and no NOE (dm='nnn'; delay time, d1=0.7 sec; acquisition time, at=0.3 sec; pulse width with an approximate 10° flip angle, pw=3.5 μsec at tpwr=54). Set repetition for 120 times by arraying d1 with zero increment for 120 steps. The entire acquisition time will be about 2 min.
4. As soon as the substrate in the polarizer is ready for injection into NMR tube, load the dissolution solvent in the polarizer injection bomb. A typical dissolution solvent is: 40 mM HEPES, 125 mM NaCl, pH 7.6, 3.5 mL. The final pH after dissolution of polarized pyruvic acid: 7.4.
5. Dissolution and data collection can be initiated by typing "go" at the spectrometer command prompt when a signal cable is connected between the spectrometer and polarizer. Dissolution can also be triggered manually and captured substrate solution injected manually by syringe with acquisition initiated after a few seconds settling time.

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