Supporting information:

Analysis of metabolic pathways by ^{13}C-labelled molecular probes and HRMAS NMRspectroscopy: Isotopologues identification and quantification methods for medical applications.

H. Outilaft[1,2], C. Bund[1,2,3], M. Piotto[2,4] and I.J. Namer*[1,2,3]

[1] MNMS-Platform, Hôpital de Hautepierre, Hôpitaux Universitaires de Strasbourg, 1 avenue Molière, 67200 Strasbourg CEDEX, France
[2] ICube, Université de Strasbourg/CNRS, UMR 7357, 67091 Strasbourg CEDEX, France
[3] Service de Médecine Nucléaire et d'Imagerie Moléculaire, Institut de Cancérologie Strasbourg Europe, 67200 Strasbourg, France
[4] Bruker BioSpin, 34 rue de l'industrie, 67166 Wissembourg, France

Correspondence should be addressed to Izzie Jacques Namer (ij.namer@icans.eu)

NMR EXPERIMENTAL PARAMETERS

The experimental details of the eight pulse sequences used in this study are presented below:

a) 1D ^{1}H CPMG: This experiment was acquired with the following parameters: sweep width 14 ppm, number of points 32 k, relaxation delay 2 s and acquisition time 2.3 s. A total of 128 scans were acquired resulting in an acquisition time of 10 min. The inter-pulse delay between the 180° pulses of the CPMG pulse train was set to 285 µs and the number of loops was set to 328 giving the CPMG pulse train a total length of 93 ms. All spectra were recorded in such a manner that only a zero order phase correction was necessary to properly phase the spectrum.

b) 1D HSQC: Spectra were acquired with an acquisition time of 0.292 s in F2, a 14 ppm spectral width and a 2 s relaxation delay. 128 transients were averaged corresponding to a total acquisition time of 8 min. HSQC experiments using echo–antiecho gradient selection for phase-sensitive detection [19] were ac-quired with GARP ^{13}C decoupling. and a 2 s relaxation delay.

c) 1D ^{1}H POCE: Spectra were acquired with the following parameters: sweep width 14 ppm, number of points 5 k, relaxation delay 5 s and acquisition time 0.350 s. A total of 512 scans were acquired resulting in an acquisition time of 47 min. All spectra were processed using manual base line correction routines.

d) 2D Het-JRES: Spectra were acquired with an acquisition time of 2.33 s in F2 and 0.32 s in F1, a 14 ppm spectral width and a 2 s relaxation delay. Sixteen transients were averaged for each of the 128 increments during t1, corresponding to a total acquisition time of 2h36. Da-ta were zero filled to a 32k * 512 matrix and weighted with a shifted square sine bell function prior to Fourier transformation.

e) 2D HR-TOCSY: High resolution spectra were ac-quired with an acquisition time of 0.584 s in F2 and 0.073 s in F1, a 60 ms mixing time using an adiabatic mixing pulse [20], a 14 ppm

spectral width and a 2 s relaxation delay. Sixteen transients were averaged for each of the 1024 increments during t1, corresponding to a total acquisition time of 12h34min. Data were zero filled to a 4 k * 4 k matrix and weighted with a shifted square sine bell function prior to Fourier transformation.

f) 2D HSQC with high resolution in F1: Spectra were acquired with an acquisition time of 0.292 s in F2 and 0.049 s in F1, a 14 ppm spectral width in F2, a 165 ppm spectral with in F1 and a 2 s relaxation delay. Eight transients were averaged for each of the 2k increments during t1, corresponding to a total acquisition time of 10h 45 min. Data were zero filled to a 4 k * 4 k matrix and weighted with a shifted square sine bell function prior to Fourier transformation. HSQC experiments using echo–antiecho gradient selection for phase-sensitive detection [19] were acquired with GARP ^{13}C decoupling and a 2s relaxation delay.

g) 2D HR-HSQC with high resolution in F1 and F2, not decoupled in F2: Spectra were acquired with an acquisition time of 2.33 s in F2 and 0.049 s in F1, a 14 ppm spectral width in F2, a 165 ppm spectral with in F1 and a 1 s relaxation delay. Sixteen transients were averaged for each of the 2k increments during t1, corresponding to a total acquisition time of 30h. Data were zero filled to a 16 k * 4 k matrix and weighted with a shifted square sine bell function prior to Fourier transformation.

h) 2D HR-HMBC with high resolution in F1 and F2, not decoupled in F2: Spectra were acquired with an acquisition time of 1.16 s in F2 and 0.037 s in F1, a 14 ppm spectral width in F2, a 220 ppm spectral with in F1 and a 1 s relaxation delay. Sixteen transients were averaged for each of the 2k increments during t1, corresponding to a total acquisition time of 21h. Data were zero filled to a 4 k * 4 k matrix and weighted with a shifted square sine bell function prior to Fourier transformation.

METABOLITES QUANTIFICATION

Metabolites quantification was performed on the 1D ^{1}H CPMG experiment using the Pulcon method (Wider, G. & Dreier, L. Measuring Protein Concentrations by NMR Spectroscopy. J. Am. Chem. Soc. 128, 2571–2576, 2006). The NMR spectrometer was calibrated with an external standard of Alanine in D_2O containing 68.9 nmoles of Alanine.

FIGURES SI

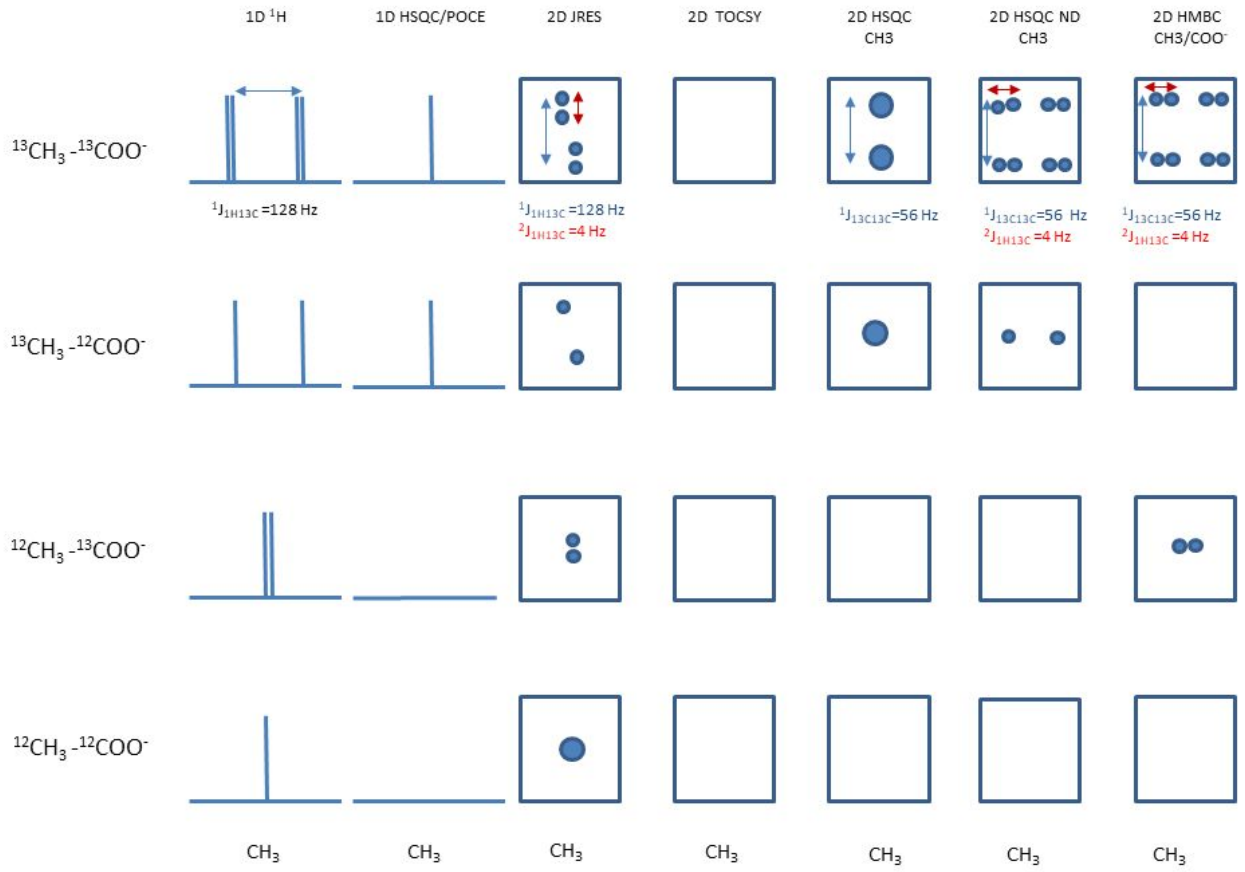

Figure S1: Theoretical 1D ^{1}H patterns for the methyl group of Acetate for the four possible isotopologues. $^{1}J_{1H13C}$ = 128 Hz, $^{2}J_{1H13C}$ = 4 Hz, $^{1}J_{13C13CO}$ = 56 Hz.

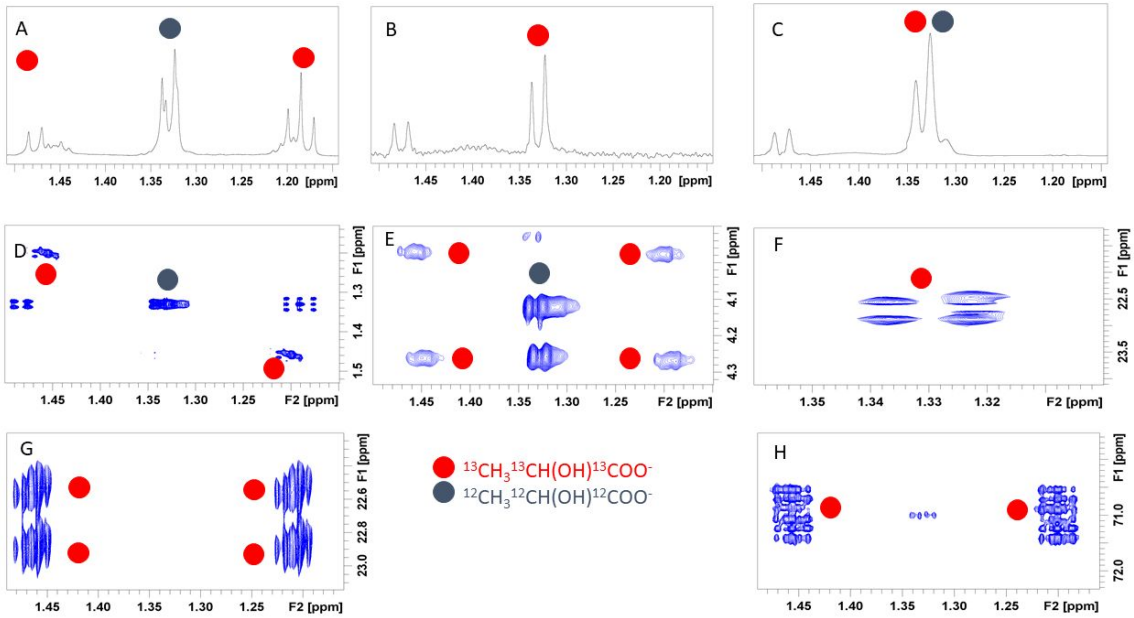

Figure S2: Osteosarcoma cell cultures, pattern observed for the CH_3 group for the set of NMR experiments used. (A) 1D ^{1}H CPMG (B) 1D ^{1}H HSQC (C) 1D ^{1}H POCE (D) 2D Het-JRES (E) 2D TOCSY (F) 2D HR-F1-HSQC (G) 2D HR-F1,F2-HSQC (H) 2D HR-F1-HMBC

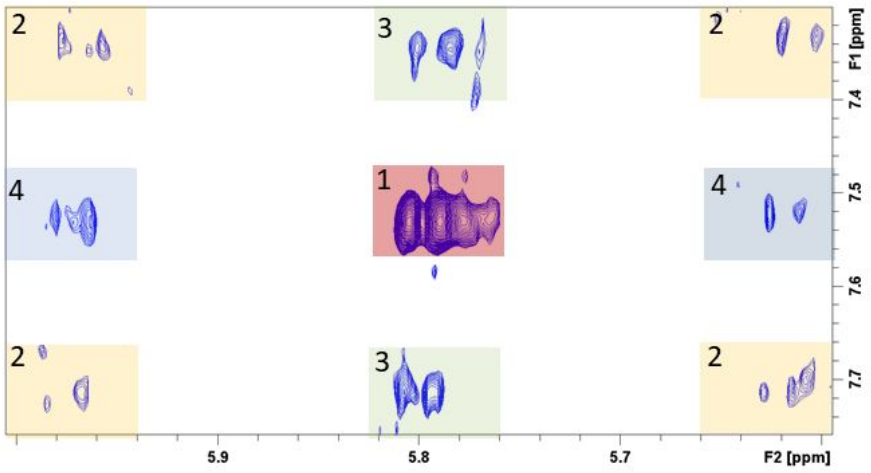

Figure S3: Osteosarcoma cells, pattern observed for the H5-H6 TOCSY cross peak in UXP. The four isotopomers can be detected in this spectrum: (1) ^{12}CH5-^{12}CH6, (2) ^{13}CH5-^{13}CH6, (3) ^{12}CH5-^{13}CH6 and (4) ^{13}CH5-^{12}CH6.

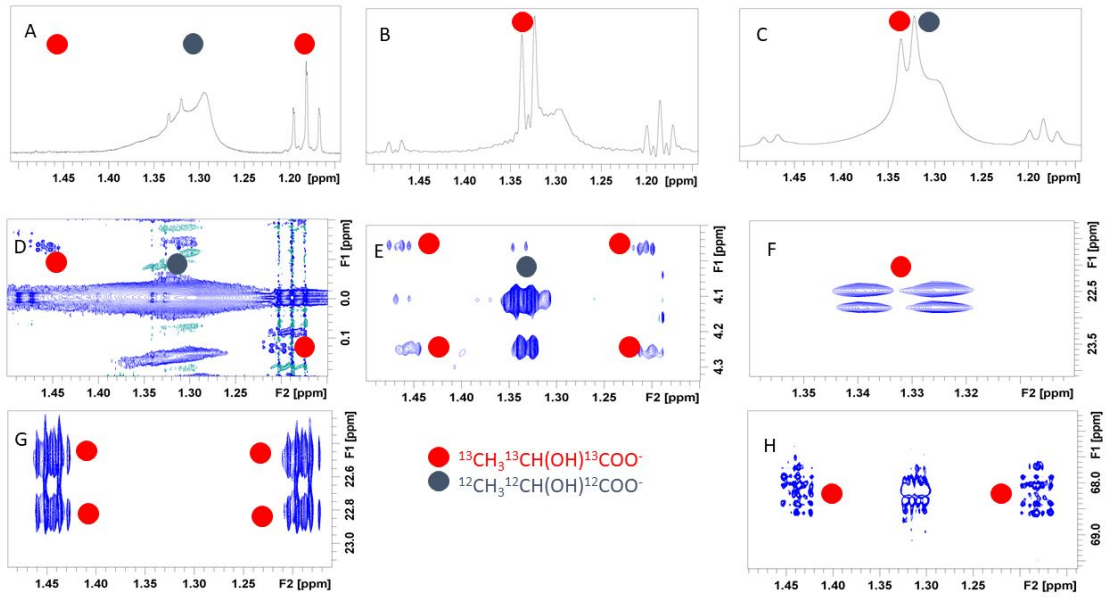

Figure S4: Xenografts biopsies, pattern observed for the CH_3 group for the set of NMR experiments used. (A) 1D ^{1}H CPMG (B) 1D ^{1}H HSQC (C) 1D ^{1}H POCE (D) 2D Het-JRES (E) 2D TOCSY (F) 2D HR-F1-HSQC (G) 2D HR-F1,F2-HSQC (H) 2D HR-F1-HMBC