Rapid Probing of Glucose Influx into Cancer Cell Metabolism: Using Adjuvant and a pH-Dependent Collection of Central Metabolites to Improve In-Cell D-DNP NMR

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**Fig. S1** The pH invariance of $^{13}$C chemical shifts in metabolites containing neither carboxylic acid nor phosphoester group (such as carbohydrates, aldehydes, and alcohols; top), or in $\alpha$-ketoacids above pH 4.0 (such as $\alpha$-ketoglutaric acid, pyruvic acid, oxaloacetic acid; bottom). Changes relative to the $^{13}$C chemical shifts at pH 8.0 are plotted.
**Fig. S2** Distributions of changes in $^1$H and $^{13}$C chemical shift for CH groups in central metabolites upon acidification from pH 8.0 to 7.0, from 7.0 to 6.0, or from 6.0 to 5.0 (top). Distributions and correlations for the acidification from pH 8.0 to 4.0 are shown in the bottom. Redistribution in electron density elicits a weak anti-correlation between $^1$H and $^{13}$C chemical shift changes.
Fig. S3 The pH dependent changes to the $^1$H chemical shift (relative to the chemical shifts at pH 8.0) for some of the central metabolites exhibiting strongest pH dependence of both $^1$H and $^{13}$C chemical shifts, in comparison to pH invariant $^1$H chemical shifts in alcohols, polyols, and $\alpha$-ketoacids (bottom). Deviations up to +0.3 ppm for the $^1$H chemical shift can be expected for central metabolites upon acidification from pH 8.0 to 4.0.
Fig. S4 Alkali ion (K$^+$) dependent $^{13}$C chemical shifts at pH 7.0 for succinate indicate the absence of significant effects of alkali ions on chemical shifts in physiologically relevant regimes. Deviations by more than -3 ppm occur, in contrast, for both $^{13}$C chemical shift values upon acidification from $10^{-8}$ M to $10^{-4}$ M H$_3$O$^+$ concentration, to chemical shift values of 181.5 and 33.2 ppm.
Fig. S5 Alkali ion (K⁺) dependent ¹³C chemical shifts at pH 7.0 for citrate indicate the absence of significant effects of alkali ions in physiologically relevant regimes. Deviations by -0.6 to -3.6 ppm occur, in contrast, for ¹³C chemical shift values upon acidification from 10⁻⁸ M to 10⁻⁴ M H₃O⁺ concentration, to chemical shift values of 181.8, 178.4, 77.2, and 46.7 ppm. The observations of Fig. S5 and S6 indicate that hydronium ions have significantly stronger effects on chemical shifts than other monovalent Group I cations.
Fig. S6 Time series of 1D $^{13}$C NMR spectra recorded as a pseudo-2D spectrum after the injection of hyperpolarized D-[1-$^{13}$C, 1-$^2$H]glucose to PC3 cancer cells (40 mM phosphate buffer, pH 7.4) in the presence of 20 mM pyruvate. The presence of pyruvate renders influx of glucose into the pentose phosphate pathway visible through $^{13}$C signals for 6-phosphogluconate and CO$_2$/HCO$_3^-$ signal. Signals for the primary alcohol groups and phosphoesters in upper glycolysis and for pyruvate and lactate methyl groups are likewise highlighted.
**Fig. S7** Projection of 50 1D $^{13}$C NMR spectra acquired within 25 seconds after the injection of hyperpolarized D-[1-$^{13}$C,1-$^{2}$H]glucose to PC-3 cancer cells (40 mM phosphate buffer, pH 7.4) in the absence (blue) and in the presence of 20 mM pyruvate. Pyruvate and lactate signals are reporters of the cellular redox state, consistent with an enzymatic verification of increased NAD$^{+}$/NADH in the presence of exogeneous pyruvate relative to its absence.