Asymmetry of ^{13}C labeled 3-pyruvate affords improved site specific labeling of RNA for NMR spectroscopy

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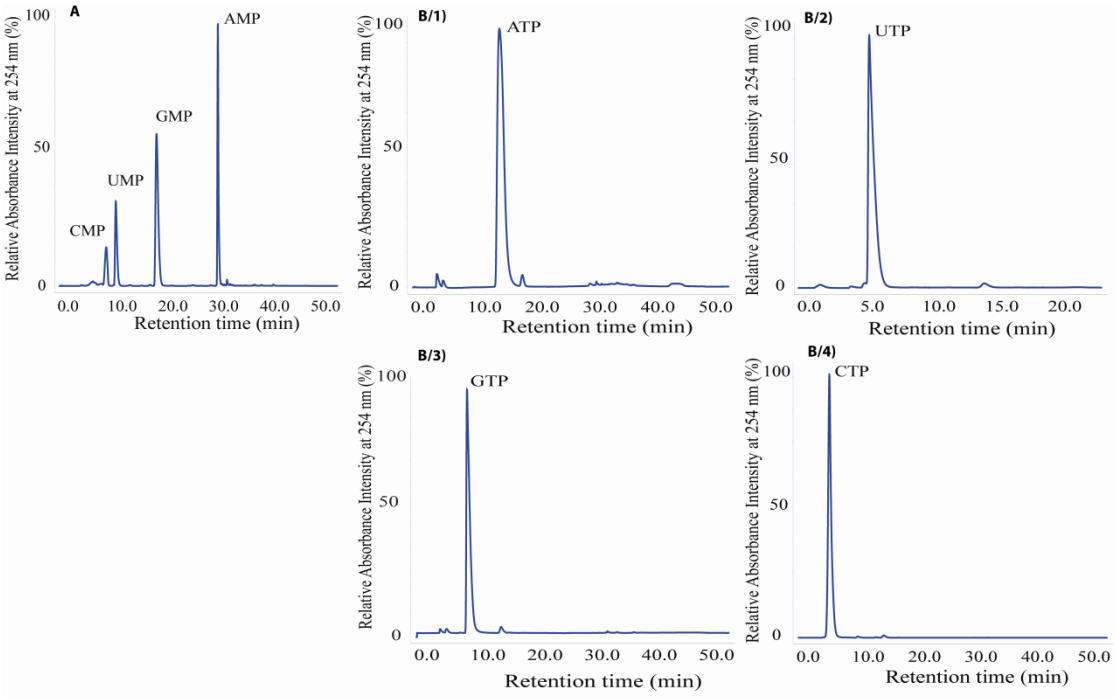

Figure S1. Reverse phase HPLC chromatograms of the separation of ribonucleoside monophosphates (NMPs) and enzymatic phosphorylation of labeled NMP to NTP. (**A**) NMPs mixture were separated on a Targa C18 reverse phase column (250 × 4.6 mm, 5 µm, Higgins Analytical, Inc.) at a flow-rate of 0.8 ml/min using solvent A (0.1 M KH_2PO_4) and B (20% v/v MeCN in 0.1 M KH_2PO_4) and detection at 254 nm. (**B**) Complete enzymatic phosphorylation of labeled NMP to NTP after 1 hr (same HPLC condition as (**A**).

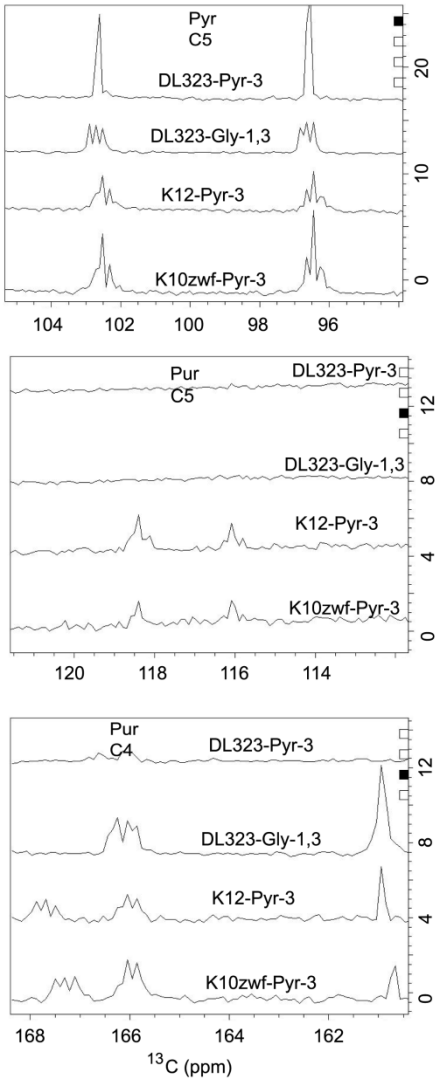

Figure S2. Labeling pattern of a mixture of four rNMPs isolated from K10*zwf,* K12, and DL323 E. coli grown in a [3-^{13}C]-Pyruvate background, and DL323 *E. coli* grown in a [1, 3-^{13}C]-glycerol background. The direct carbon detection 1D spectrum shows all the labeled carbon positions. A long recycle delay of 5 s was used to allow for sufficient magnetization recovery and proton decoupling was limited to the acquisition period only. The residual ^{13}C-^{13}C coupling observed in a [1, 3-^{13}C]-glycerol is absent with the [3-^{13}C]-Pyruvate only in DL323; K10 and K12 grown in [3-^{13}C]-Pyruvate still retain the residual ^{13}C-^{13}C coupling.

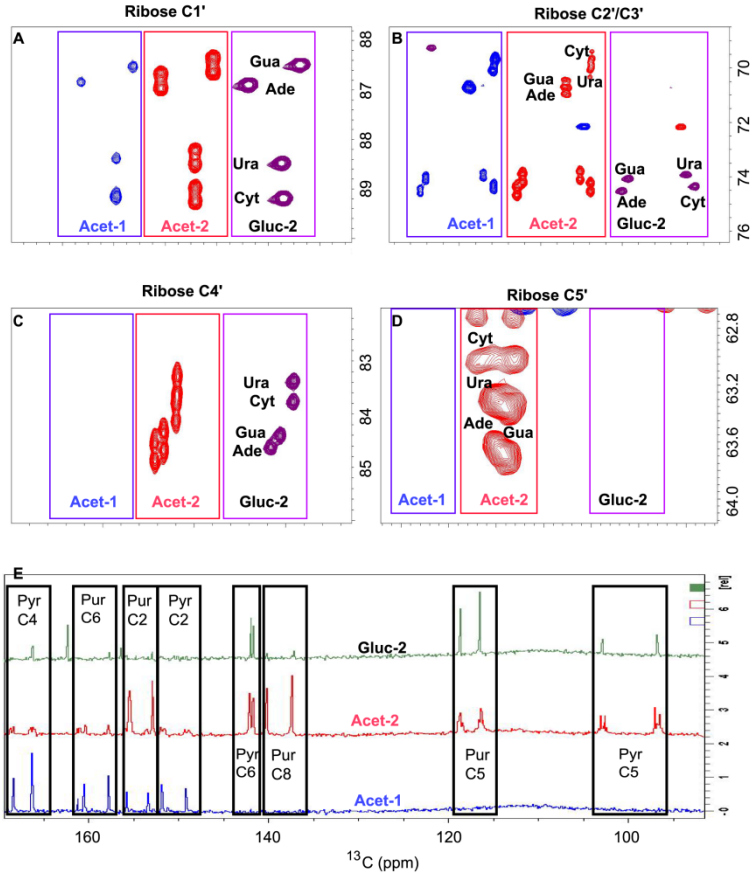

Figure S3.

2D non-constant time HSQC spectra and 1D direct carbon detection spectra of all four labeled nucleotides extracted from K12 grown on [1-^{13}C]-acetate (blue) or [2-^{13}C]-acetate (red) or [2-^{13}C]-glucose (purple or green). (**A**) Ribose C1′, (**B**) Ribose C2′/C3′, (**C**) Ribose C4′ and (**D**) Ribose C5′. The resonances from each of the four nucleotides are annotated for adenine (Ade), cytosine (Cyt), guanine (Gua), and uracil (Ura). Slight differences in pH and salt conditions between samples leads to a noticeable shift in the chemical shift positions such that some of the singlets are not exactly centered relative to the doublets in the carbon dimension. The direct carbon detection 1D spectrum shows all the labeled carbon positions. A long recycle delay of 5 s was used to allow for sufficient magnetization recovery and proton decoupling was limited to the acquisition period only. (**E**) The spectra are shown for K12 grown on the following: [1-^{13}C]-acetate (blue), [2-^{13}C]-acetate (red), and [2-^{13}C]-glucose (green).

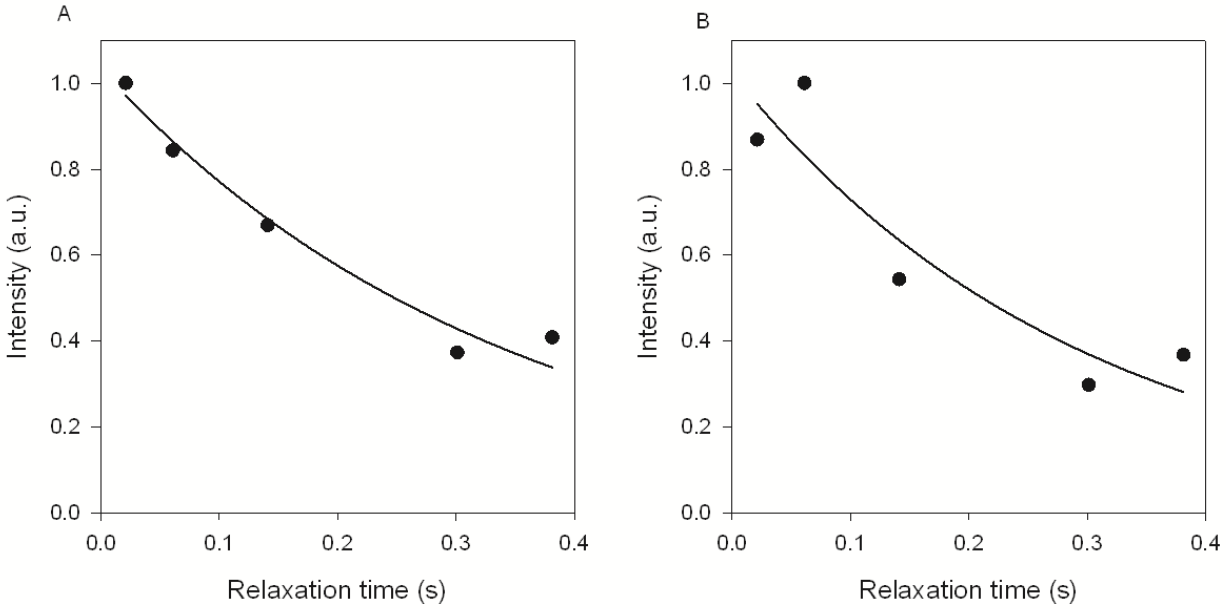

Figure S4.

Representative longitudinal R_1 relaxation decay curves showing marked deviation from monoexponential decay for uniformly labeled samples. Base C5 R_1 relaxation measurements at 25°C for the A-site RNA labeled with (**A**) site selectively-labeled CTP and (**B**) uniformly $^{13}C/^{15}N$-labeled CTP.