Supporting Information

Specific Labeling of Threonine Methyl Groups for NMR Studies of Protein-Nucleic Acid Complexes.

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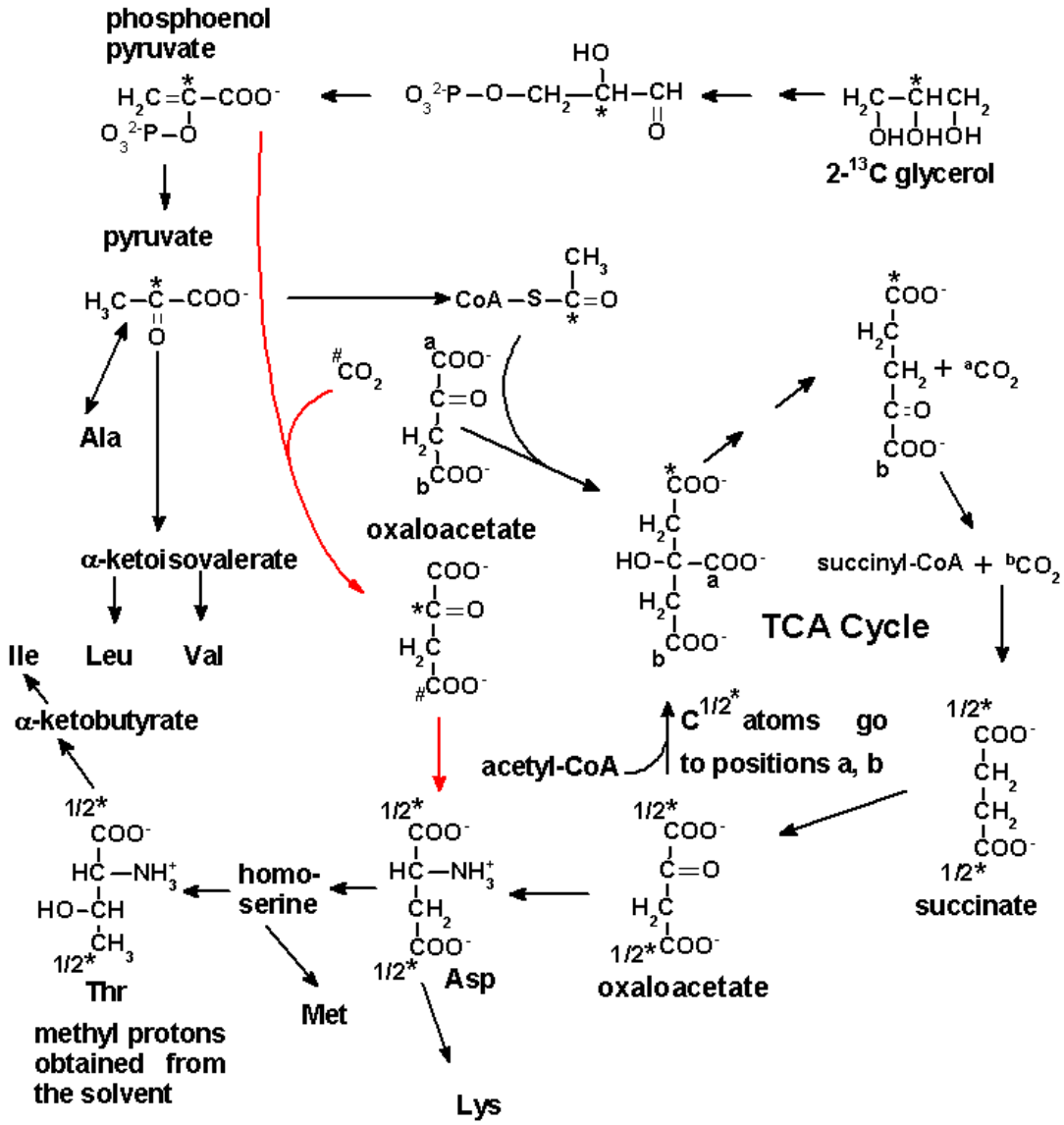

Figure S1. Schematic representation of the important pathways leading to Thr biosynthesis from glycerol. The fate of the 2-^{13}C atom of glycerol is traced through the pathway (*) for one round of the TCA cycle. If the oxaloacetate re-enters the TCA cycle its labeled carbons (indicated by a and b) are lost as CO_2. The red arrows indicate carboxylation of phosphoenolpyruvate to regenerate oxaloacetate that is lost due to biosynthetic processes; a # indicates the ^{13}C from CO_2. This carbon would become the methyl group of Thr. Also shown are the important reactions that lead to the dilution of the ^{13}C label.

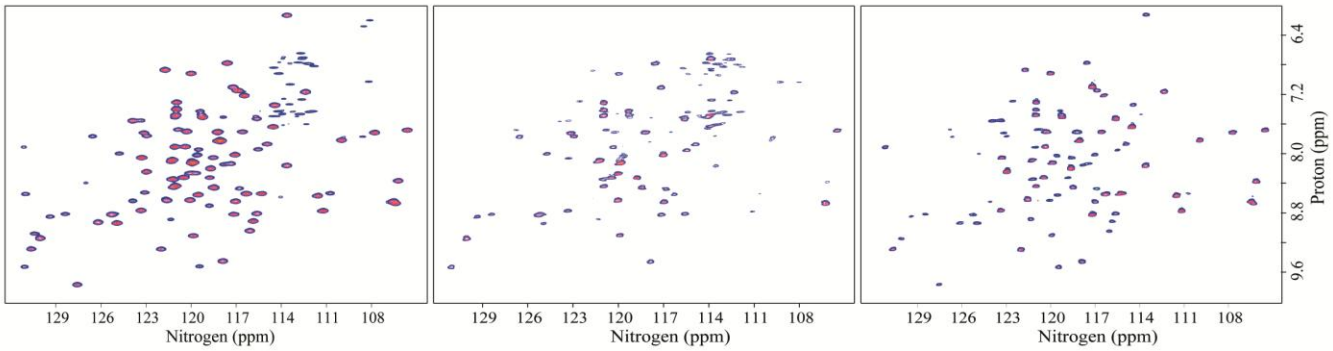

Figure S2. 2D ^{1}H-^{15}N spectra of rho130. A) HSQC showing all the peaks B) carbonyl edited (2D HNCO) to detect peaks with ^{13}C at (i-1) carbonyl position and C) C_α filtered (2D HNCA) to detect NH with ^{13}C at either i or (i-1) C_α.

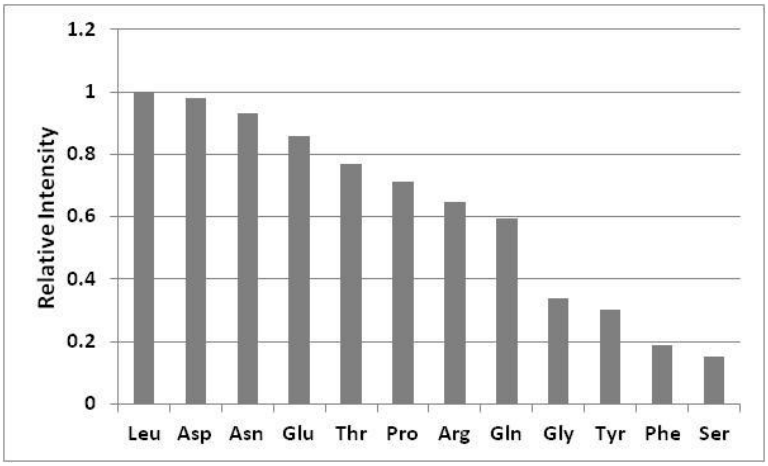

Figure S3. Histogram showing level of carbonyl labeling for residues in rho130. The intensities of the peaks in the carbonyl edited spectrum were normalized to the intensities in the non-edited HSQC. The residue type of the carbonyl labeled residue was obtained from the known NH resonance assignments.

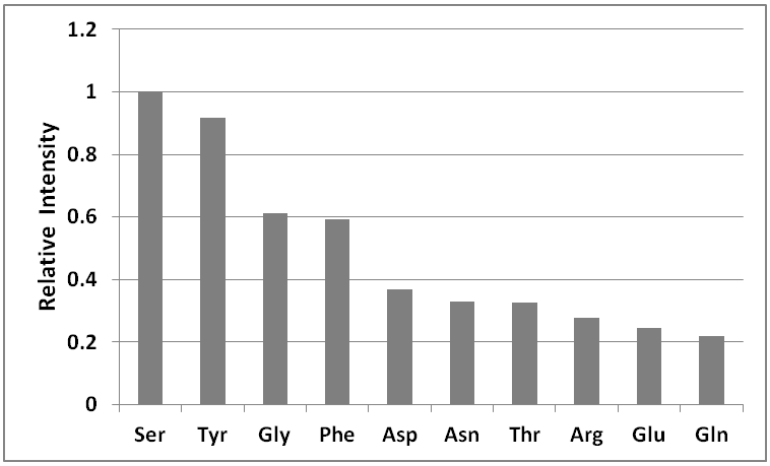

Figure S4. Histogram showing level of C_α labeling for residues in rho130. The intensities of the peaks in the C_α edited spectrum were normalized to the intensities in the non-edited HSQC. The residue type of the labeled residue was obtained from the known resonance assignments.

Labeling Protocol: The labeling scheme was tested on the 130 residue RNA binding domain of the transcriptional termination factor rho (rho130). C3013 cells (New England Biolabs), a standard T7 expression host, was used for protein expression. A modified Studier's PG medium (Studier, F. W. (2005), *Protein expression and purification* **41**, 207-234) was used: glucose was replaced by [2-^{13}C]-glycerol (5g/L) and $(^{15}NH_4)_2SO_4$ was used as the nitrogen source.

The cells were grown to an A_{550} of 0.6 at 37°C. At this point, deuterated α-ketoisovalerate (200mg/L), deuterated Ala (800mg/L, 8.9 mM), deuterated Ile (80mg/L, 0.6 mM), Met (150mg/L, 1.0 mM) and Lys (200mg/L, 1.4 mM) were added to

the media. ^{13}C labeled sodium bicarbonate (if used) was also added at this point. The cells were allowed to grow for 60 minutes and isopropylthiogalactoside (IPTG) (1mM) was added to induce protein expression. The cells were harvested 3 hours after induction.

The amounts of α-ketoisovalerate, deuterated Ala and deuterated Ile were based on the Ala-labeling method described by Ayala et al. (Ayala, I., Sounier, R., Use, N., Gans, P., and Boisbouvier, J. (2009), *J. Biomol. NMR* **43**, 111-119).

Methionine levels were chosen because the addition of 1mM Met to the growth media suppresses Met synthesis by 90-100% (Wijesundera, S., and Woods, D.D. (1960) *J. Gen. Microbiol.*, **22**, 229-241). The degree of suppression is somewhat dependent on the bacterial strain used, typically greater than 90%, but as low as 63% for one strain (Rowbury, R.J., and Woods, D.D. (1961) *J. Gen. Microbiol.*, **24**, 129-144). Consequently, we directly determined the level of ^{13}C-Met incorporation into rho130 using an internal standard (^{13}C alanine) and found that the level of incorporation was greater than 97%. Under the conditions used here, the exogenously supplied Met accounts for essentially all of the Met used in protein synthesis.

Lysine concentrations were chosen to reduce the flux of carbon from oxaloacetate to Lys, without inhibiting Thr biosynthesis. The biosynthesis of both lysine and threonine begin with the conversion of Asp to Asp-P by the action of aspartate kinase. Of the three isozymes of aspartate kinase in E. coli, one of the isozymes is subject to inhibition by Lys with a K_I = 0.39 mM. Lysine also inhibits homoserine kinase, an enzyme in the threonine synthesis pathway, with a K_I of 9 mM (Chassagnole, C., Rais, B., Quentin, E., Fell, D.A., and Mazat, J.-P. (2001) *Biochem J.* **356**, 415-423). Consequently, the levels of lysine used here partially repress flux through one of the aspartate kinase isozymes, while not affecting threonine kinase activity. This level of Lys suppresses endogenous synthesis because the activity of the last enzyme in lysine synthesis, diaminopimelate decarboxylase, is inhibited by 60-80% when cells are grown in media supplemented with 1.0 mM lysine (White, P.J., Kelly, B., Suffling, A., and Work, E. (1964), *Biochem. J.* **91**, 600-610).

Modifications to the protocol for growth in 100% D_2O: 100% D_2O media was nominally 99% D_2O and made using anhydrous salts. When using 100% D_2O media, the cells were adapted to D_2O by growth in media of increasing D_2O concentrations (50%, 75%, 90%) before inoculating the 100% D_2O culture. At all times during this adaptation period the cell density was kept above 0.1 A_{550}. After adding the amino acids and ketovalerate at A_{550} of 0.6, the cells were allowed to grow for 90 minutes before IPTG (1mM) was added to induce protein expression. The cells were harvested 10 hours after induction.

Cost of Labeling: We estimate the cost per liter of the labeling scheme in H_2O to be approximately $1500. Currently, deuterated alpha-keto-isovalerate is available only as a component of the Ala methyl labeling kit (Cambridge Isotopes), thus it is difficult to estimate its cost if supplied separately. Although not investigated here, the overall cost could be reduced somewhat by using lower amounts (e.g. 100mg/L) of α-ketoisovalerate to suppress labeling of Leu and Val methyl groups (Goto, N.K., Gardner, K.H., Mueller, G.A., Willis, R.C., L.E. Kay (1999) *J. Biomol. NMR* **13**, 369-374). Presently, there is no cost effective process of synthesizing Thr specifically labeled at the methyl position (personal communication with the vendor).