

Figure S1. Magnesium acetate gives higher plateau charging values for hmt tRNA^{Leu}_{UAA} transcript compared to magnesium chloride. Reactions were performed at 1 μ M LARS-2 in the presence of either magnesium chloride (red symbols and traces) (or) magnesium acetate (blue symbols and traces) at 37°C at concentrations of either 20 mM Mg²⁺ (open symbols) or 55 mM Mg²⁺ (closed symbols).

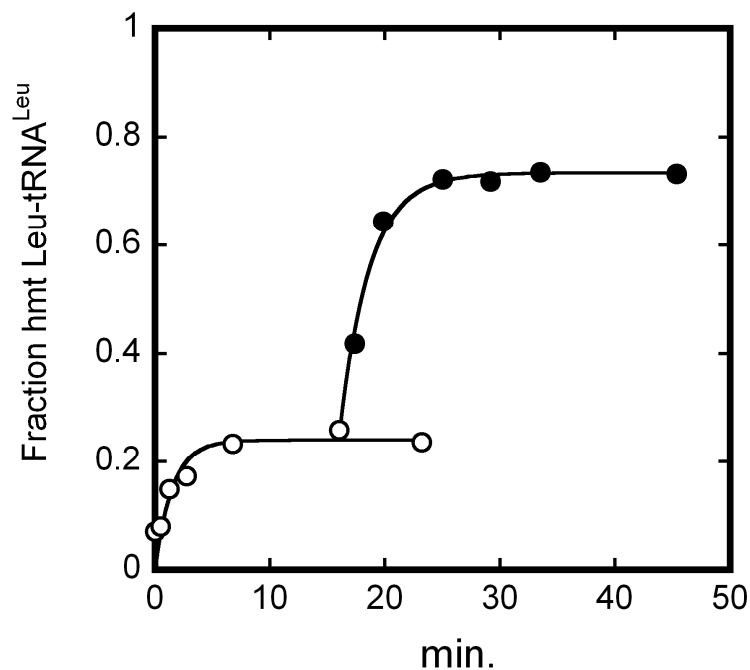


Figure S2. Aminoacylation plateau value is low at higher temperature ($>37^{\circ}\text{C}$) for hmt tRNA^{Leu} because of structural instability and not because of RNA being damaged at these temperatures. Leucylation of hmt tRNA^{Leu}_{UAA} was performed at 45°C and at 20 mM $\text{Mg}(\text{oAc})_2$, which resulted in a low plateau value for aminoacylation (0.25) (Open symbols and trace). A large aliquot was transferred to 21°C and aminoacylation monitored at 21°C (solid symbols and trace). Because the only difference that resulted in higher plateau value was a transfer to a lower temperature, we conclude that the tRNA was not damaged at 45°C but secondary and/or tertiary structure was severely perturbed at 45°C compared to 21°C .

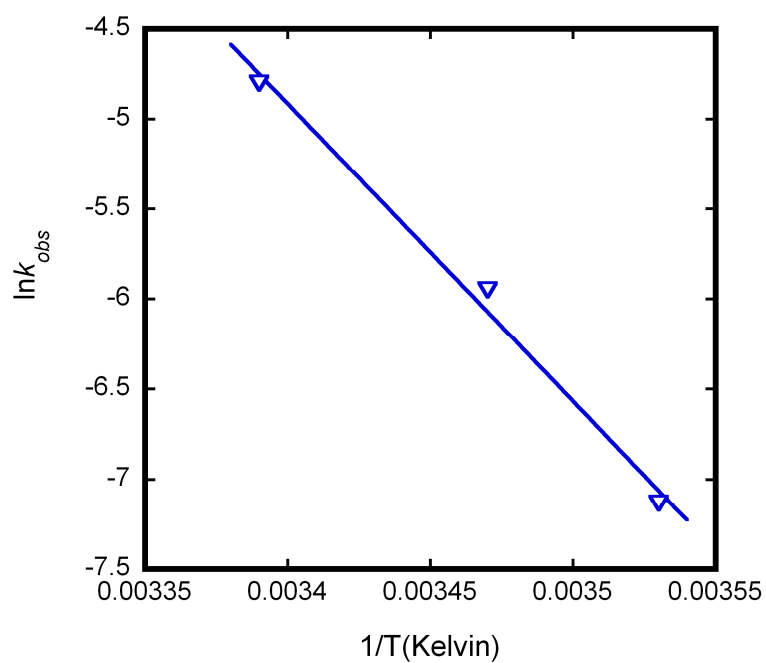


Figure S3. Log-linear plot of the rate constant against $1/T$ (Kelvin) for folding of the hmt tRNA^{Leu}_{UAA} transcript (see Figure 4). The slope is equal to $-E_a/R$, where E_a is the activation energy and R is the gas constant ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$). The fit gives E_a of $13.7 \times 10^4 \text{ J mol}^{-1}$ ($32.8 \text{ kcal mol}^{-1}$).

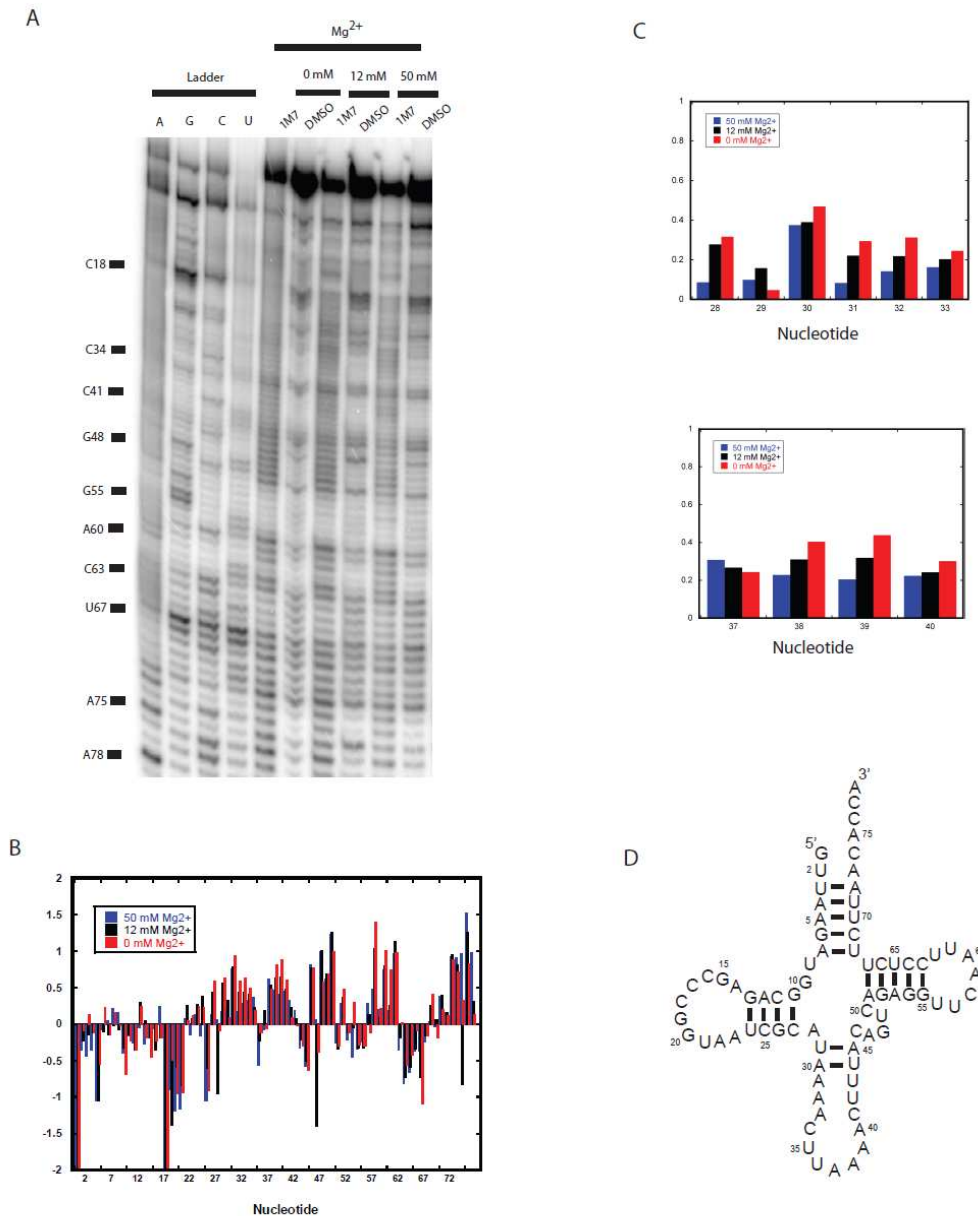
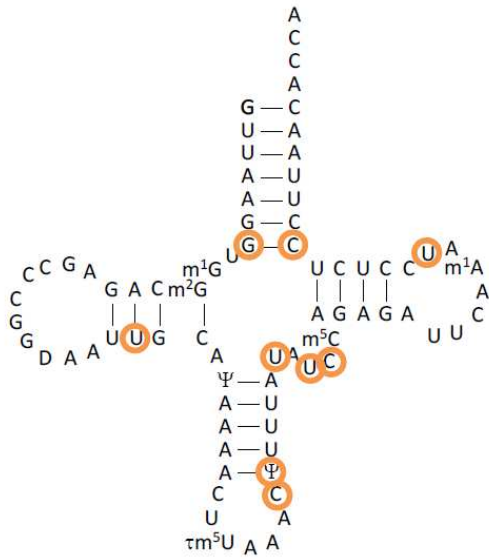


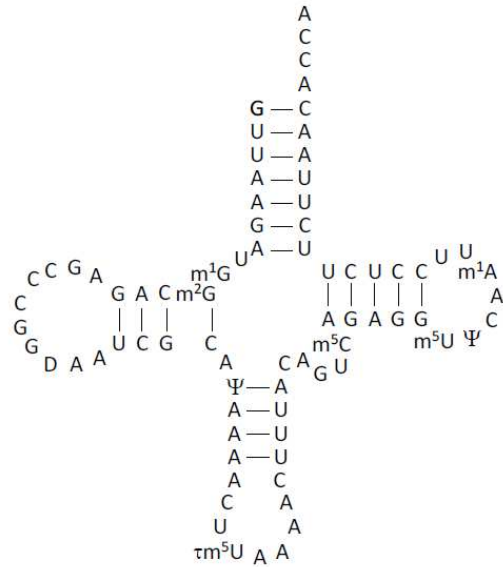
Figure S4. 1M7 SHAPE analysis at different Mg²⁺ concentrations. (A) SHAPE footprinting gel showing DMSO and 1M7 intensities at 0, 12 and 50 mM MgCl₂. (B) Absolute SHAPE reactivities at 0, 12 and 50 mM MgCl₂. (C) Absolute SHAPE reactivities of a subset of nucleotides at 0, 12 and 50 mM MgCl₂. Nucleotides 28, 31, 32, 33, 38, 39 and 40 show clear protections at increasing MgCl₂ up to 50 mM.

Figure S5. Predicted optimal (upper left) and sub-optimal secondary structures of hmt tRNA^{Leu}_{UAA} transcript tRNA. Structures were predicted using minimal energy criteria using MC-fold.

Bos taurus



Homo sapiens



○ Differences in sequence from Homo sapiens

Figure S6. Predicted native secondary structures (based on comparative sequence analysis) of human and bovine transcript tRNA^{Leu}_{UAA}. Differences in primary sequence are circled in the *Bos taurus* tRNA.