Supporting Information

Coupling Green Fluorescent Protein Expression with Chemical Modification to Probe Functionally Relevant Riboswitch Conformations in Live Bacteria

Debapratim Dutta[a], Ivan A. Belashov[a] and Joseph E. Wedekind[a,*]

[a]*Department of Biochemistry & Biophysics and Center for RNA Biology, University of Rochester School of Medicine & Dentistry, Rochester NY 14642, USA.*

*Corresponding author: 585-273-4516 (J.E.W.); e-mail: joseph.wedekind@rochester.edu

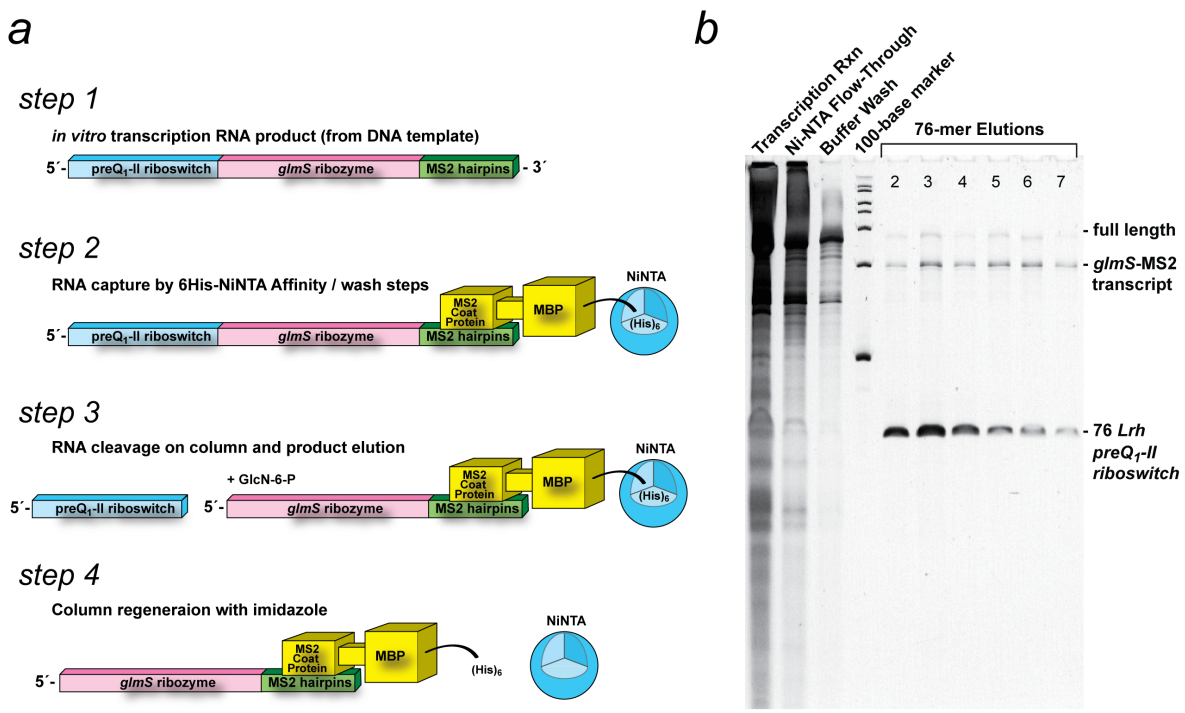

Figure S1 | Native purification scheme and representative results for the *Lrh* preQ$_1$-II riboswitch. (***a***) Graphical overview of the major *in vitro* steps used to generate the *Lrh* preQ$_1$-II riboswitch by co-transcriptional folding by T7 polymerase starting with PCR amplified DNA, as described in reference 26 of the main text. After capture on NiNTA resin and washing, glc-N-6-P (glucosamine-6-phosphate) is added to stimulate *glmS* ribozyme cleavage. This step produces uniform 3´-ends on the *Lrh* riboswitch and frees it from the NiNTA resin. A subtractive step using fresh NiNTA can be used to re-capture residual uncleaved transcript that co-elutes with the desired product (not shown). The resin can be regenerated by washing in high imidazole concentrations to release 6His-MBP-(MS2 Coat Protein); see reference 26 for details. (***b***) Results of the major steps for native purification in ***a*** were monitored by denaturing PAGE stained with SYBR Gold. The lanes are: T7 polymerase *in vitro* transcription (Transcription Rxn); Ni-NTA flow-through during loading; and buffer wash of the Ni-NTA column. Eluted fractions collected and analyzed after *glmS* ribozyme cleavage are labeled 2-7. The desired 76-mer riboswitch is labeled "76 *Lrh* preQ$_1$-II riboswitch". The sequence of the latter construct is shown in **Fig. 2*b***, except that a G76A change was made to promote *glmS* ribozyme cleavage.

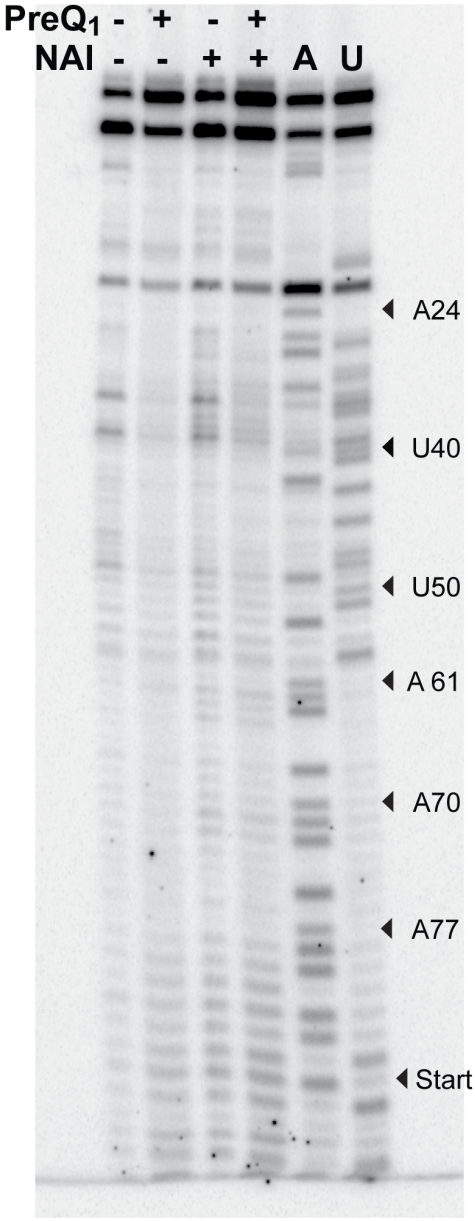

Figure S2 | Representative whole-gel PAGE analysis of icSHAPE cDNA obtained from primer extension. This uncropped image is the basis of the sequencing gel in **Fig. 4*a***. *Lane 1*: cells grown in no preQ$_1$ but treated with DMSO. *Lane 2*: cells grown in 2 μM preQ$_1$, treated with DMSO. *Lane 3*: cells grown without preQ$_1$, treated with NAI. *Lane 4*: cells grown in presence of 2 μM preQ$_1$, treated with NAI. *Lane 5*: cells grown without preQ$_1$, ddTTP added during primer

extension. *Lane 6*: cells grown without $preQ_1$, where ddATP was added during primer extension. Full-length cDNA transcripts are observed at the top with a major site of primer pausing just below. Innate primer pausing also occurred at nucleotide position 22. Analysis was curtailed beyond nucleotide 23 due to loss of resolution and high background from primer pausing. At the bottom of the gel, cDNA bands can be observed up to the second codon of the GFPuv gene; "A" of start codon is labeled "Start". There is some band-compression between U57 and A61 but the template DNA was sequence verified. Some degradation is observed in the control lanes past position 77 (*bottom*), but this region does not include the *Lrh* $preQ_1$-II riboswitch.

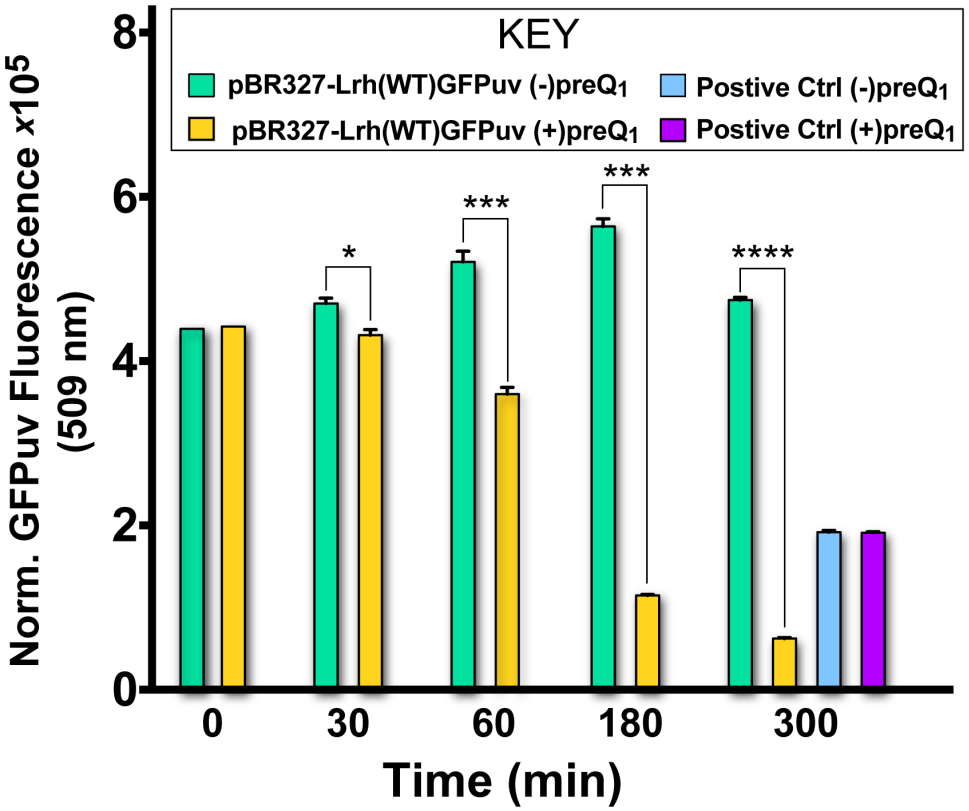

Figure S3 | Representative GFPuv fluorescence from effector-mediated regulation by the *Lrh* preQ$_1$-II riboswitch at various times during log-phase growth. To test changes in GFPuv fluorescence emission as a function of time, cells harboring the pBR327-Lrh(WT)GFPuv reporter were grown overnight in CSB media in the absence of preQ$_1$. These cells were used to inoculate fresh liquid cultures, giving an OD600 of 0.05 (time = 0 min); the fresh triplicate cultures were inoculated in the presence of 2 μM preQ$_1$ or in its absence. Positive and negative controls were also run; negative controls were used to subtract the background fluorescence levels. Values shown are normalized based on OD600 (see **Fig. 3** for details). At 30 min, a statistically significant difference in GFPuv fluorescence emission was noted when comparing (+)preQ$_1$ to (-)preQ$_1$ cultures. The reporter probability (*P*) equals 0.0284 based on an unpaired, two-tailed Welch's *t*-test (t = 5.4; df = 1.99); **P* < 0.05 is statistically significant. At 60 min, *P* equals 0.0002 (t = 18.2; df = 3.34). At 180 min, *P* equals 0.0001 (t = 81.3; df = 2.08). At 300 min, *P* equals <0.0001 (t =

208.1; df = 2.48). For 60, 180 and 600 min, ***$P < 0.001$ is statistically highly significant. The positive controls were not significantly different in response to $preQ_1$ as a function of time.