Supporting Information

Comparison of Solution and Crystal Structures of PreQ₁ Riboswitch Reveals Calcium-Induced Changes in Conformation and Dynamics

Qi Zhang,† Mijeong Kang,†‡ Robert D. Peterson,†‡ and Juli Feigon*,†‡
†Department of Chemistry and Biochemistry, and ‡the UCLA-DOE Institute for Genomics and Proteomics, University of California, Los Angeles, CA 90095-1569

Supporting Material and Methods

Sample preparation. Uniformly and base-specifically (U and C) ¹³C,¹⁵N-labeled preQ₁ riboswitch samples were prepared by in vitro transcription using T7 polymerase (P266L mutant) with synthetic DNA templates as described previously.† The RNA samples for NMR experiments were prepared in 50 mM KCl (pH 6.3-6.5) with two equivalents of preQ₁. The concentration of samples were 0.2 mM for titration experiments and ~1 mM for all other experiments. The concentration of CaCl₂ was 6mM for RDC and relaxation experiments. For RDC and titration experiments, H₂O samples were used, where 10% D₂O was added. For all relaxation experiments, D₂O samples were used, where the RNA solutions were repeatedly lyophilized and re-dissolved in the same volume of 99.996% D₂O (Sigma).

NMR spectroscopy and Data analysis.

All NMR experiments except the relaxation dispersion experiments were carried out on Bruker Avance 800 MHz spectrometer equipped with 5 mm triple-resonance cryogenic probe at 300K. Relaxation dispersion experiments were carried on Bruker DRX 600 MHz spectrometer equipped with 5 mm triple-resonance cryogenic probe at 300K.
**RDCs measurements and order tensor analysis.** One-bond C-H and N-H RDCs were measured on uniformly $^{13}$C,$^{15}$N-labeled samples in ~17-19 mg/ml Pf1 phage (ASLA Biotech, Ltd) using 2D $^1$H-$^{13}$C S$^3$CT-HSQC and standard $^1$H-$^{15}$N HSQC experiments as described previously. A total of 90 RDCs were measured for preQ$_1$ riboswitch in the absence and presence of Ca$^{2+}$, respectively (Table S1). For order tensor analysis, the lowest-energy structure from solution ensembles (PDB ID: 2L1V) and the crystal structure with higher resolution (PDB ID: 3K1V) were used as input coordinates for program RAMAH. Protons were added to the crystal structure with Insight II (Molecular Simulations, Inc.). Four RDCs from terminal residues (G1 and G2) were excluded from the analysis both in the absence and presence of Ca$^{2+}$ due to its high flexibility. RDCs from L2 residues except those from C19 were excluded in the order tensor analysis, since these residues are not visible in the crystal structure used in the analysis.

**$^{13}$C spin relaxation.** Longitudinal ($R_1$) and rotating-frame ($R_{1p}$) relaxation rates were measured for base carbons (C2, C6 and C8) using TROSY detected pulse sequences. A high power off-resonance spin lock was calibrated as previously described as $v_{SL} = 4208.4\pm 67.1$Hz. The carrier position was set to 143ppm and the offset of spin lock was 4000 Hz. Relaxation delays were 20, 400 (x2), 800 (x2) ms for $R_1$ experiments, and 2, 28 (x2), 56 (x2) ms for $R_{1p}$ experiments, where duplicated measurements were indicated as x2. Relaxation rates and errors were determined by fitting intensities to a mono-exponential decay, $I_t = I_0 e^{-R\times t}$, using NMRView and in-house software. The transverse relaxation rates ($R_2$) were obtained from $R_{1p}$ and $R_1$ rates according to $R_{1p} = R_1 \cos^2\theta + R_2 \sin^2\theta$, where $\theta = \arctan(v_{SL}/\Omega)$ is the effective tilt angle in the spinlock field, $v_{SL}$ is the spinlock field strength in Hz, and $\Omega$ is the resonance offset from the spinlock carrier frequency in Hz. The $R_1$ and $R_2$ values are summarized in Table S2.

**On-resonance $^{13}$C relaxation dispersion.** The on-resonance relaxation dispersion profile for base carbon C6s of eight residues (including U8, U9, C10, U11, U15, C21, U22, and
U34) were measured using the recently developed 1D selective $R_{1p}$ experiment with individually U and C labeled samples.\(^8\) Each relaxation dispersion profile was composed of 12 points, two of which were duplicates for error analysis. The spin locks were calibrated and ranged from $\sim$500 Hz to $\sim$4000 Hz (Table S3). For each relaxation point, relaxation delays were 1, 5, 10, 15, 20 ms. Relaxation rates and errors were then determined by fitting intensities to a mono-exponential decay, $I_t = I_0e^{-R_xt}$, using NMRView and in-house software (Table S3). The measurement errors of relaxation rates were obtained from these fitting errors as well as deviations between two duplicates. During the analysis of dispersion data in the following, the input uncertainties for $R_2$ were set to be the larger values of either the measurement error or 2% of the relaxation rates. The relaxation dispersion profiles were fitted to a simple expression for fast chemical exchange,\(^9\)

$$R_{1p} = R_1 \cos^2 \theta + R_2 \sin^2 \theta + \sin^2 \theta \Phi_{ex} \frac{k_{ex}}{(k_{ex}^2 + \omega_c^2)}$$  \hfill (1)

where $\theta = \arctan(v_{SL}/\Omega)$ is the effective tilt angle, $v_{SL}$ is the spinlock field strength, and $\Omega$ is the resonance offset from the spinlock carrier, $\Phi_{ex} = \mu_0 n \mu_B \omega_c^2$ is the single fitting parameter, and $\omega_c = (v_{SL}^2 + \Omega^2)^{1/2}$ is the effective field in rotating frame. Since experiments were performed on resonance ($\Omega = 0$ and $\theta = 90^\circ$), the equation (1) can be simplified as

$$R_{2,obs} = R_2 + R_{ex} = R_2 + \Phi_{ex} \frac{k_{ex}}{(k_{ex}^2 + v_{SL}^2)}.$$  \hfill (2)

Each dispersion profile was analyzed either as a constant $R_2$ or as functions of $\Phi_{ex}$ and $k_{ex}$ according to equation (2). The choice of model was determined by F-statistics comparing these two models at the 95% confidence level. Data analysis and model comparison were conducted using Origin (Origin Lab Corporation).

**Ca\textsuperscript{2+} and Mg\textsuperscript{2+} titration experiments.** A series of 2D $^1$H–$^{13}$C HSQC spectra were recorded following incremental increases in the CaCl\textsubscript{2} and MgCl\textsubscript{2} concentrations as 0.05, 0.1, 0.2,
0.4, 0.8, 1.2, 1.6 and 2.0 mM. The observed chemical shift changes (Δδ_{obs}) were quantified using equation $\Delta\delta_{obs} = \sqrt{(\Delta\delta_H)^2 + (\Delta\delta_C/4)^2}$, where $\Delta\delta_H$ and $\Delta\delta_C$ are the chemical shift changes in the $^1$H and $^{13}$C dimensions. Resonances exhibiting chemical shift changes > 0.05 ppm at 2 equivalents of Ca$^{2+}$ were subjected to $K_d$ analysis. The apparent $K_d$s were obtained by fitting the chemical shift changes as function of metal ion concentration to the following equation,

$$\Delta\delta_{obs} = \frac{\Delta\delta_T}{2[RNA]_T} \left( [RNA]_T + [M] + K_d - \sqrt{([RNA]_T + [M] + K_d)^2 - 4[RNA]_T[M]} \right),$$

where $\Delta\delta_{obs}$ is the observed chemical shift changes, $\Delta\delta_T$ is the total chemical shift change between the free and fully bound states, $[RNA]_T$ is the total RNA concentration for the titration experiments (0.2 mM), and $[M]$ is the concentration of the added salt (CaCl$_2$ or MgCl$_2$). Data were analyzed using Origin (Origin Lab Corporation).

**SI References**

(1) Kang, M.; Peterson, R.; Feigon, J. *Mol. Cell* **2009**, 33, 784.
(2) Kim, N. K.; Zhang, Q.; Zhou, J.; Theimer, C. A.; Peterson, R. D.; Feigon, J. *J. Mol. Biol.* **2008**, 384, 1249.
(3) Losonczi, J. A.; Andrec, M.; Fischer, M. W. F.; Prestegard, J. H. *J. Magn. Reson.* **1999**, 138, 334.
(4) Bailor, M. H.; Musselman, C.; Hansen, A. L.; Gulati, K.; Patel, D. J.; Al-Hashimi, H. M. *Nat. Protoc.* **2007**, 2, 1536.
(5) Klein, D. J.; Edwards, T. E.; Ferre-D'Amare, A. R. *Nat. Struct. Mol. Biol.* **2009**, 16, 343.
(6) Hansen, A. L.; Al-Hashimi, H. M. *J. Magn. Reson.* **2006**, 179, 299.
(7) Hansen, A. L.; Al-Hashimi, H. M. *J. Am. Chem. Soc.* **2007**, 129, 16072.
(8) Hansen, A. L.; Nikolova, E. N.; Casiano-Negroni, A.; Al-Hashimi, H. M. *J. Am. Chem. Soc.* **2009**, 131, 3818.
(9) Palmer, A. G., 3rd; Kroenke, C. D.; Loria, J. P. *Methods Enzymol.* **2001**, 339, 204.
Table S1. RDCs measured for preQ1 riboswitch in the absence and presence of Ca$^{2+}$.

| Residue | No Ca$^{2+}$ | Ca$^{2+}$ | Residue | No Ca$^{2+}$ | Ca$^{2+}$ |
|---------|--------------|-----------|---------|--------------|-----------|
| 1 C8H8  | -0.9         | -0.8      | 18 C1'H1' | -20.8        | -19.6     |
| 1 C1'H1' | -3.4         | 5.7       | 19 C5H5  | -0.8         | 0.7       |
| 2 C8H8  | 0.9          | -7.9      | 19 C6H6  | 3.0          | 10.1      |
| 2 C1'H1' | -1.5         | -5.9      | 20 C5H5  | -0.4         | 0.9       |
| 3 C2H2  | -1.7         | -11.2     | 20 C6H6  | 10.1         | 7.4       |
| 3 C8H8  | -2.1         | -7.0      | 21 C1'H1' | -10.2        | 6.4       |
| 4 C8H8  | 3.4          | -1.1      | 21 C6H6  | 2.0          | 2.4       |
| 4 N1H1  | -4.3         | -1.4      | 21 C6H6  | 16.3         | 9.1       |
| 5 C2H2  | 5.3          | -1.4      | 22 C6H6  | 15.0         | 8.4       |
| 5 C8H8  | 12.8         | 6.8       | 22 C1'H1' | 3.6          | 15.3      |
| 5 C1'H1' | 2.3          | -6.9      | 22 C5H5  | 7.5          | 4.6       |
| 6 C8H8  | 16.9         | 9.3       | 22 C6H6  | 15.0         | 8.4       |
| 6 N1H1  | -6.4         | -2.9      | 22 N3H3  | -8.0         | -4.3      |
| 7 C8H8  | 14.5         | 9.4       | 23 C5H5  | 20.3         | 13.1      |
| 7 C1'H1' | 19.5         | 13.0      | 23 C6H6  | 7.8          | 2.5       |
| 7 N1H1  | -2.8         | -0.9      | 23 C1'H1' | 14.5         | 14.8      |
| 8 C5H5  | 12.4         | 2.8       | 24 C5H5  | 12.1         | 5.8       |
| 8 C6H6  | 20.7         | 16.3      | 24 C6H6  | -2.8         | -5.1      |
| 8 C1'H1' | 8.8          | 4.7       | 24 C1'H1' | 14.4         | 8.6       |
| 8 N3H3  | -11.0        | -7.2      | 24 N3H3  | -1.0         | 1.8       |
| 9 C5H5  | NA           | 8.4       | 25 C2H2  | 10.9         | 7.4       |
| 9 C6H6  | 9.1          | 15.0      | 25 C8H8  | -3.2         | -11.9     |
| 9 C1'H1' | 17.1         | 14.4      | 25 C1'H1' | 13.7         | 12.6      |
| 10 C5H5 | 8.6          | 16.2      | 27 C2H2  | 13.6         | 3.6       |
| 10 C6H6 | -5.3         | -4.6      | 27 C8H8  | 21.2         | 16.3      |
| 11 C5H5 | 6.2          | -5.0      | 27 C1'H1' | -1.4         | NA        |
| 11 C6H6 | 4.1          | -1.5      | 28 C8H8  | 14.3         | 18.8      |
| 11 C1'H1' | -13.0       | -16.9     | 28 C1'H1' | 6.7          | NA        |
| 12 C2H2 | 0.0          | 0.4       | 29 C2H2  | 23.4         | 16.6      |
| 12 C8H8 | 10.8         | 8.8       | 29 C8H8  | 11.9         | 8.7       |
| 12 C1'H1' | -13.3       | -20.2     | 30 C8H8  | -1.1         | -0.7      |
| 13 C8H8 | 19.9         | 10.6      | 31 C2H2  | 16.3         | 17.9      |
| 13 C1'H1' | -7.9         | -16.0     | 32 C2H2  | -3.5         | 8.5       |
| 13 N1H1 | -8.8         | -4.1      | 33 C5H5  | -6.5         | 2.6       |
| 14 C5H5 | 3.4          | 1.6       | 33 C6H6  | 10.4         | 2.6       |
| 14 C6H6 | -0.1         | 3.8       | 33 C1'H1' | -21.1        | -7.2      |
| 14 C1'H1' | 3.3          | 2.0       | 34 C5H5  | 7.1          | 2.4       |
| 15 C6H6 | 3.2          | 2.4       | 34 C6H6  | 15.7         | 8.5       |
| 15 C1'H1' | -0.9         | -4.1      | 34 C1'H1' | -14.8        | -11.8     |
| 16 C2H2 | 15.0         | 7.2       | 34 N3H3  | NA           | -4.5      |
| 16 C8H8 | 13.9         | 5.7       | 35 C2H2  | 20.1         | 13.3      |
| 16 C1'H1' | 13.4         | 8.6       | 35 C8H8  | 8.3          | 6.5       |
| 17 C6H6 | -3.9         | -0.1      | 35 C1'H1' | -7.2         | -8.4      |
| 17 C1'H1' | 0.1          | 2.5       | 36 C2H2  | 7.8          | 2.4       |
| 18 C2H2 | 8.3          | 1.7       | 36 C8H8  | -1.8         | -4.0      |
| 18 C8H8 | -0.9         | 1.7       | 36 C1'H1' | -1.5         | -4.6      |
Table S2. $^{13}$C spin relaxation rates ($R_1$ and $R_2$) measured for preQ$_1$ riboswitch in the absence and presence of Ca$^{2+}$.

| Residue | Carbon | No Calcium | With Calcium |
|---------|--------|------------|--------------|
|         |        | $R_1$ (Hz) | $R_2$ (Hz) | $R_1$ (Hz) | $R_2$ (Hz) |
| A3      | C2     | 1.301±0.013 | 39.45±0.53 | 1.310±0.014 | 40.83±0.54 |
| A3      | C8     | 1.171±0.007 | 34.20±0.68 | 1.236±0.012 | 41.12±0.88 |
| G4      | C8     | 1.151±0.019 | 44.63±1.31 | 1.183±0.028 | 43.24±1.40 |
| A5      | C2     | 1.341±0.014 | 40.64±0.56 | 1.302±0.013 | 42.95±0.58 |
| A5      | C8     | 1.215±0.010 | 35.04±0.79 | 1.198±0.011 | 37.57±0.84 |
| G6      | C8     | 1.233±0.027 | 34.85±1.29 | 1.220±0.056 | 38.03±2.24 |
| G7      | C8     | 1.193±0.020 | 34.31±1.05 | 1.183±0.028 | 35.72±1.30 |
| U8      | C6     | 1.796±0.054 | 46.38±1.82 | 1.683±0.054 | 53.77±2.04 |
| U9      | C6     | 1.705±0.081 | 52.40±2.79 | 1.680±0.070 | 58.54±3.00 |
| C10     | C6     | 1.726±0.045 | 51.14±1.72 | 1.702±0.020 | 45.50±0.96 |
| U11     | C6     | NA         | NA          | 1.679±0.101 | 62.11±3.99 |
| A12     | C2     | 1.282±0.051 | 39.71±1.64 | 1.293±0.015 | 43.28±0.66 |
| A12     | C8     | 1.283±0.032 | 38.41±1.25 | 1.205±0.050 | 45.51±2.15 |
| G13     | C8     | 1.145±0.091 | 34.47±3.51 | 1.275±0.049 | 36.37±2.10 |
| U14     | C6     | 2.366±0.014 | 22.57±0.42 | 2.228±0.009 | 25.90±0.44 |
| U15     | C6     | 2.376±0.015 | 24.81±0.44 | 2.287±0.010 | 27.10±0.45 |
| A16     | C2     | 1.429±0.017 | 38.39±0.60 | 1.453±0.013 | 37.81±0.46 |
| A16     | C8     | 1.288±0.011 | 30.58±0.64 | 1.305±0.007 | 31.60±0.60 |
| U17     | C6     | 2.172±0.013 | 24.78±0.45 | 2.174±0.009 | 26.46±0.45 |
| A18     | C2     | 1.348±0.014 | 39.23±0.53 | 1.312±0.012 | 40.00±0.47 |
| A18     | C8     | 1.211±0.010 | 35.79±0.77 | 1.178±0.008 | 36.15±0.76 |
| C19     | C6     | 1.704±0.030 | 50.57±1.42 | 1.706±0.016 | 51.74±1.13 |
| C20     | C6     | 1.687±0.018 | 48.29±1.06 | 1.693±0.016 | 51.63±1.07 |
| C21     | C6     | 1.748±0.023 | 49.05±1.14 | 1.710±0.019 | 52.00±1.09 |
| U22     | C6     | 1.752±0.024 | 49.19±1.18 | 1.726±0.028 | 50.62±1.25 |
| C23     | C6     | 1.693±0.023 | 50.14±1.14 | 1.654±0.050 | 53.26±1.79 |
| U24     | C6     | 1.652±0.043 | 49.83±1.66 | 1.655±0.047 | 49.70±1.71 |
| A25     | C2     | 1.292±0.014 | 37.77±0.51 | 1.300±0.016 | 39.41±0.57 |
| A25     | C8     | 1.138±0.009 | 33.60±0.64 | 1.138±0.014 | 34.97±0.74 |
| A27     | C2     | 1.337±0.013 | 38.88±0.52 | 1.311±0.022 | 41.83±0.76 |
| A27     | C8     | 1.146±0.015 | 32.90±0.78 | 1.115±0.025 | 34.86±1.05 |
| A28     | C2     | NA         | NA          | 1.937±0.080 | 37.44±1.70 |
| A28     | C8     | 1.121±0.026 | 34.08±1.09 | 1.094±0.038 | 36.69±1.43 |
| A29     | C2     | 1.337±0.013 | 39.23±0.55 | 1.274±0.015 | 42.35±0.65 |
| A29     | C8     | 1.177±0.016 | 34.48±0.85 | 1.160±0.018 | 36.65±0.93 |
| A30     | C8     | 1.205±0.013 | 35.42±0.85 | 1.191±0.012 | 38.00±0.88 |
| A31     | C2     | 1.372±0.019 | 38.95±0.64 | 1.332±0.015 | 41.87±0.58 |
| A32     | C2     | NA         | NA          | 1.290±0.020 | 42.83±0.73 |
| C33     | C6     | 1.688±0.037 | 50.16±1.61 | 1.705±0.020 | 52.17±1.25 |
| U34     | C6     | 1.740±0.036 | 47.81±1.42 | 1.745±0.021 | 51.37±1.12 |
| A35     | C2     | 1.391±0.049 | 38.17±1.34 | 1.338±0.017 | 40.40±0.62 |
| A35     | C8     | 1.268±0.019 | 34.33±0.93 | 1.189±0.014 | 36.31±0.84 |
| A36     | C2     | 1.497±0.030 | 31.48±0.70 | 1.499±0.017 | 32.49±0.45 |
| A36     | C8     | 1.405±0.050 | 28.34±1.52 | 1.366±0.020 | 28.95±0.75 |
Table S3. $^{13}$C relaxation profiles of base carbon C6s measured for preQ$_1$ riboswitch in the absence and presence of Ca$^{2+}$.

| SL Field (Hz) | U8 $R_2$ (Hz) | U9 $R_2$ (Hz) | C10 $R_2$ (Hz) | U11 $R_2$ (Hz) | U15 $R_2$ (Hz) | C21 $R_2$ (Hz) | U22 $R_2$ (Hz) | U34 $R_2$ (Hz) |
|---------------|---------------|---------------|----------------|----------------|----------------|----------------|----------------|---------------|
| **No Calcium**|               |               |                |                |                |                |                |               |
| 485.7         | 40.48±0.60    | 55.14±0.59    | 51.21±0.77     | 87.68±0.66     | 20.92±0.53     | 43.10±0.61     | 38.88±0.87     | 44.24±0.94    |
| 582.8         | 42.26±0.60    | 53.19±0.58    | 48.95±0.73     | 86.74±0.63     | 21.30±0.53     | 42.37±0.65     | 38.49±0.84     | 44.06±0.95    |
| 582.8         | 40.25±0.66    | 54.25±0.63    | 49.24±0.81     | 86.58±0.69     | 21.22±0.57     | 42.52±0.75     | 40.21±0.92     | 43.45±1.01    |
| 777.1         | 41.58±0.61    | 52.38±0.59    | 49.53±0.75     | 83.56±0.60     | 20.47±0.54     | 41.44±0.69     | 40.56±0.88     | 41.86±0.95    |
| 971.4         | 40.90±0.59    | 52.59±0.56    | 46.50±0.71     | 79.64±0.58     | 21.70±0.53     | 42.86±0.69     | 41.47±0.84     | 41.26±0.92    |
| 1457.0        | 40.54±0.62    | 50.82±0.59    | 48.06±0.77     | 70.56±0.54     | 21.06±0.57     | 44.99±0.75     | 40.66±0.90     | 41.58±0.98    |
| 1942.7        | 41.07±0.60    | 47.59±0.56    | 45.48±0.73     | 62.60±0.50     | 20.23±0.53     | 43.91±0.71     | 38.83±0.86     | 41.58±0.96    |
| 2428.3        | 42.16±0.57    | 47.42±0.51    | 45.75±0.67     | 58.34±0.44     | 21.01±0.52     | 42.39±0.66     | 39.55±0.82     | 44.19±0.91    |
| 2914.1        | 41.08±0.57    | 45.34±0.51    | 43.43±0.65     | 56.61±0.42     | 20.66±0.52     | 41.92±0.64     | 40.46±0.82     | 41.82±0.90    |
| 2914.1        | 39.95±0.62    | 45.88±0.55    | 44.19±0.71     | 56.38±0.46     | 20.65±0.55     | 40.61±0.72     | 40.18±0.90     | 43.16±0.97    |
| 3399.8        | 41.65±0.59    | 45.97±0.53    | 43.62±0.68     | 54.66±0.44     | 20.36±0.53     | 43.22±0.66     | 39.47±0.85     | 41.81±0.95    |
| 3883.7        | 41.14±0.62    | 43.00±0.55    | 42.49±0.72     | 53.48±0.45     | 20.27±0.56     | 40.38±0.62     | 41.02±0.91     | 43.33±0.98    |
| **With Calcium**|               |               |                |                |                |                |                |               |
| 485.7         | 45.84±1.21    | 53.53±0.65    | 38.13±0.39     | 77.84±1.13     | 23.74±0.44     | 40.98±0.72     | 42.11±1.54     | 41.10±0.96    |
| 582.8         | 46.63±1.21    | 53.45±0.63    | 38.95±0.39     | 74.95±1.07     | 23.66±0.43     | 42.05±0.72     | 43.13±1.51     | 41.84±0.95    |
| 582.8         | 47.01±1.30    | 52.90±0.69    | 39.57±0.42     | 73.71±1.18     | 23.59±0.46     | 41.99±0.78     | 42.37±1.63     | 42.66±1.06    |
| 777.1         | 48.80±1.25    | 54.64±0.64    | 38.59±0.39     | 69.32±1.03     | 23.64±0.44     | 43.27±0.73     | 46.42±1.53     | 41.17±0.97    |
| 971.4         | 47.23±1.21    | 52.11±0.62    | 40.22±0.39     | 66.42±0.99     | 24.00±0.43     | 44.21±0.72     | 39.91±1.48     | 42.35±0.96    |
| 1457.0        | 47.94±1.27    | 50.01±0.65    | 39.47±0.42     | 62.94±0.99     | 24.58±0.46     | 42.86±0.77     | 43.64±1.64     | 43.94±1.06    |
| 1942.7        | 45.12±1.20    | 49.76±0.62    | 39.21±0.40     | 57.92±0.93     | 23.22±0.44     | 41.93±0.73     | 45.15±1.59     | 41.73±0.99    |
| 2428.3        | 45.41±1.15    | 48.72±0.57    | 39.14±0.38     | 58.43±0.87     | 24.32±0.42     | 41.97±0.69     | 42.67±1.49     | 42.89±0.95    |
| 2914.1        | 42.98±1.12    | 48.07±0.57    | 38.56±0.37     | 57.62±0.86     | 23.53±0.43     | 42.07±0.70     | 44.81±1.46     | 42.92±0.95    |
| 2914.1        | 45.08±1.25    | 48.72±0.62    | 38.96±0.40     | 57.78±0.92     | 23.99±0.45     | 41.36±0.75     | 43.72±1.54     | 42.52±1.02    |
| 3399.8        | 44.74±1.20    | 46.66±0.59    | 39.27±0.40     | 56.98±0.89     | 23.56±0.44     | 43.07±0.74     | 39.36±1.50     | 44.93±1.00    |
| 3883.7        | 45.81±1.26    | 45.59±0.62    | 38.75±0.41     | 55.08±0.94     | 22.96±0.46     | 41.36±0.77     | 42.74±1.63     | 42.72±1.04    |
### Table S4. Chemical exchange parameters of U9, C10 and U11.

| Residues | $R_2$ (Hz) | $k_{ex}$ (sec$^{-1}$) | $\Phi$ (sec$^{-2}$) |
|----------|------------|---------------------|---------------------|
| No Calcium |            |                     |                     |
| U9       | 40.5±1.8  | 13.7±2.8x10$^3$    | 1.96±0.58x10$^5$    |
| C10      | 40.5±2.2  | 13.8±5.0x10$^3$    | 1.32±0.71x10$^5$    |
| U11      | 47.8±0.7  | 8.8±0.4x10$^3$     | 4.04±0.19x10$^5$    |
| Global Fit |          |                     |                     |
| U9       | 42.9±0.6  | 1.22±0.12x10$^5$   |                     |
| C10      | 42.2±0.5  | 9.4±0.7x10$^3$     | 0.81±0.10x10$^5$    |
| U11      | 46.9±1.3  |                     | 4.29±0.34x10$^5$    |
| With Calcium |       |                     |                     |
| U9       | 42.8±2.4  | 16.1±4.6x10$^3$    | 1.80±0.84x10$^5$    |
| U11      | 55.3±0.6  | 4.5±0.6x10$^3$     | 1.44±0.08x10$^5$    |

### Table S5. Order tensor analysis of P1/L3 RDCs measured in the absence and presence of Ca$^{2+}$ using the lowest-energy solution structure (2L1V)$^1$ with program RAMAH.$^6$

|               | No Ca$^{2+}$ | With Ca$^{2+}$ |
|---------------|--------------|----------------|
| N             | 41           | 38             |
| RMSD (Hz)     | 1.6          | 1.7            |
| Q (%)         | 14           | 16             |
| CN            | 2.3          | 2.3            |
| $\Theta$ (x10$^{-3}$) | 0.57±0.02   | 0.54±0.02     |
| $\eta$       | 0.93±0.05    | 0.69±0.06      |
| $\alpha$ ($^\circ$) | -124.9       | 173.2          |
| $\beta$ ($^\circ$)  | -0.4         | -21.2          |
| $\gamma$ ($^\circ$) | 125.7        | -147.3         |
| $\alpha+\gamma$ ($^\circ$) | 0.8          | 26.0           |

Note: The input structure was pre-rotated into the principle axis system (PAS) frame of the best-fit order tensor computed with the P1/L3 RDCs measured in the absence of Ca$^{2+}$.

Table headings: Number of RDCs (N), RMSD between measured and back-calculated RDCs, quality factor (Q), condition number (CN), generalized degree of order ($\Theta$), asymmetry parameter ($\eta$), and Euler angles ($\alpha$, $\beta$, $\gamma$) which rotate PDB from the input molecular frame to the PAS frame of the computed best-fit order tensor.
Figure S1. Order tensor analysis of RDCs measured in the absence of Ca$^{2+}$. Shown are structures and correlation plots between measured and back-calculated RDCs using (A, C) the lowest-energy solution structure (PDB ID 2L1V) and (B, D) the crystal structure (PDB ID 3K1V). The RDCs (filled symbols) were fitted independently into either the P1/L3 domain (red) or the P2/L1 domain (blue). The correlation plots between measured and back-calculated RDCs that were not used in determining order tensors are shown in open symbols.
Figure S2. Relaxation dispersion profiles measured for C6 carbons in the (A) absence and (B) presence of Ca$^{2+}$. Solid lines represent the best fits of relaxation rates using the fast exchange equation. The exchange rates ($k_{ex}$) shown were obtained from individual fitting of dispersion profiles.
Figure S3. Chemical shift mapping of Ca\(^{2+}\) binding. (A) Plot of chemical shift differences between the absence and presence of 2 equivalents of Ca\(^{2+}\) vs C1’H1’, C2H2, C5H5, C6H6 and C8H8 resonances. Dashed line corresponds to Δδ\(_{\text{obs}}\) of 0.05 ppm. (B) Overlay of 2D \(^1\text{H}-^{13}\text{C}\) HSQC spectra (C1’H1’, C2-H2, C5-H5, C6-H6 and C8-H8) in the absence (black) and presence of 2 equivalents (green) and 10 equivalents (blue) Ca\(^{2+}\).
Figure S4. (A-C) Titration curves as a function of Ca\(^{2+}\) concentration for resonances with chemical shift changes > 0.05 ppm at 2 equivalents of Ca\(^{2+}\). (D) Plot of apparent K\(_d\)s vs. residues obtained from fitting each titration curve. (E) Titration curves for the residues at the weaker binding site as function of Ca2+ concentration, yielding an apparent K\(_d\) of 361 \(\mu\)M from a global fit.
Figure S5. Chemical shift mapping of Mg\(^{2+}\) binding. (A) Plot of chemical shift differences between the absence and presence of 2 equivalents of Mg\(^{2+}\) vs C1’H1’, C2H2, C5H5, C6H6 and C8H8 resonances. Dashed line corresponds to \(\Delta\delta_{\text{obs}}\) of 0.05 ppm. (B) Overlay of 2D \(^1\text{H}-^{13}\text{C}\) HSQC spectra (C1’H1’, C2-H2, C5-H5, C6-H6 and C8-H8) in the absence (black) and presence of 2 equivalents (green) and 10 equivalents (blue) of Mg\(^{2+}\).
Figure S6. (A-B) Plots of RDCs measured in the absence and presence of calcium vs residue. (C) Correlation plots between RDCs measured in the absence and presence of calcium. Symbols are colored as the secondary structure.
Figure S7. Order tensor analysis of RDCs measured in the presence of Ca$^{2+}$. Shown are structures and correlation plots between measured and back-calculated RDCs using (A) the lowest-energy solution structure (PDB ID 2L1V) and (B) the crystal structure (PDB ID 3K1V). The RDCs (filled symbols) were fitted independently into either the P1/L3 domain (red) or the P2/L1 domain (blue). The correlation plots between measured and back-calculated RDCs that were not used in determining order tensors are shown in open symbols.
Figure S8. Pico- to nanosecond timescale dynamics of preQ₁ riboswitch in the presence of calcium. (A) Plot of $2R_2-R_1$ value vs C2, C6, and C8 derived from $R_1$ and $R_{10}$ measurements. Dash lines are the average values excluding loop L2 residues. (B) Correlation plot between the $2R_2-R_1$ values in the absence and presence of calcium. The solid line is the best-fit line.