**Supplementary Information**

Observation of preQ1-II riboswitch dynamics using single-molecule FRET

Chandani Warnasooriya, Clarence Ling, Ivan A. Belashov, Mohammad Salim, Joseph E. Wedekind and Dmitri N. Ermolenko

*Department of Biochemistry & Biophysics and Center for RNA Biology, School of Medicine and Dentistry, University of Rochester, Rochester, NY 14642*



**Supplementary Figure 1 | The *Lrh* preQ1-II riboswitch samples multiple conformations.** Representative smFRET traces show fluctuations of the riboswitch between the ~0.35 and ~0.8 FRET states (***a***), or ~0.5 and ~0.8 FRET states (***b***). The riboswitch was imaged in the presence of 0.5 mM Mg2+ and the absence of preQ1. Observed intensities of donor and acceptor fluorescence and the calculated apparent FRET efficiency are shown in blue, red, and black, respectively. Hidden Markov Model fit is shown in magenta.