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Kinetic Studies of Lysine Riboswitch Folding Using Single-Molecule FRET LARRY FIEGLAND, JILA, NIST and University of Colorado, Boulder, CO, ANDREW GARST, University of Colorado, Boulder, CO, J. FIORE, JILA, NIST and University of Colorado, Boulder, CO, ROBERT BATEY, University of Colorado, Boulder, CO, DAVID NESBITT, JILA, NIST and University of Colorado, Boulder, CO — Riboswitches regulate gene expression through conformational changes induced by metabolite binding. This regulation of gene expression depends on the kinetics of metabolite binding and structural changes. Therefore, an understanding of these dynamics is crucial to developing a compete knowledge of riboswitch functionality. To probe the binding of a metabolite and subsequent folding, a metabolite-binding domain of the *Bacillus subtilis* lysine riboswitch was transcribed and hybridized to a fluorescent-labeled RNA strand, which allows FRET monitoring of ligand-induced conformational changes. The RNA construct was studied using single-molecule FRET methods that allowed for characterization of the folding dynamics. In the presence of lysine, we observed two states, of which the relative populations are perturbed by lysine concentration. We measured the folding and unfolding rates of the inter-conversion between these states. We also observe that $[Mg^{2+}]$ affects the lysine-free conformation and the lysine sensitivity of the riboswitch.

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