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Single-Molecule Spectroscopic Investigations of RNA Structural Dynamics JULIE L. FIORE, DAVID J. NESBITT, JILA, National Institute of Standards and Technology and University of Colorado — To function properly, catalytic RNAs (ribozymes) fold into specific three-dimensional shapes stabilized by multiple tertiary interactions. However, only limited information is available on the contributions of individual tertiary contacts to RNA conformational dynamics. The Tetrahymena ribozymes's P4–P6 domain forms a hinged, "candy-cane" structure with parallel helices clamped by two motifs, the GAAA tetraloop-tetraloop receptor and adenosine (A)-rich bulge–P4 helix interactions. Previously, we characterized RNA folding due to a tetraloop-receptor interaction. In this study, we employ time-resolved single-molecule FRET methods to probe A-rich bulge induced structural dynamics. Specifically, fluorescently labeled RNA constructs excited by a pulsed 532 nm laser are detected in the confocal region of an inverted microscope, with each photon sorted by arrival time, color and polarization. We resolve the kinetic dependence of A-rich bulge-P4 helix docking/undocking on cationic environment (e.g. Na^+ and Mg^{2+} concentration.) At saturating [Mg^{2+}], the docked structure appears only weakly stabilized, while only 50% of the molecules exhibit efficient folding.

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