Abstract Submitted
for the MAR14 Meeting of
The American Physical Society

Structure and Dynamics of the tRNA-like Structure Domain of Brome Mosaic Virus MARIO VIEWEGER, University of Colorado, DAVID NESBITT, JILA - NIST/University of Colorado — Conformational switching is widely accepted as regulatory mechanism in gene expression in bacterial systems. More recently, similar regulation mechanisms are emerging for viral systems. One of the most abundant and best studied systems is the tRNA-like structure domain that is found in a number of plant viruses across eight genera. In this work, the folding dynamics of the tRNA-like structure domain of Brome Mosaic Virus are investigated using single-molecule Fluorescence Resonance Energy Transfer techniques. In particular, Burst fluorescence is applied to observe metal-ion induced folding in freely diffusing RNA constructs resembling the 3'-terminal 169nt of BMV RNA3. Histograms of E_{FRET} probabilities reveal a complex equilibrium of three distinct populations. A step-wise kinetic model for TLS folding is developed in accord with the evolution of conformational populations and structural information in the literature. In this mechanism, formation of functional TLS domains from unfolded RNAs requires two consecutive steps; 1) hybridization of a long-range stem interaction followed by 2) formation of a 3' pseudoknot. This three-state equilibrium is well described by step-wise dissociation constants K_1 *(328(30) μM)* and K_2 *(1092(183) μM)*for $[Mg^{2+}]$ and K_1 *(74(6) mM)* and K_2 *(243(52) mM)* for $[Na^{+}]$-induced folding. The kinetic model is validated by oligo competition with the STEM interaction. Implications of this conformational folding mechanism are discussed in regards to regulation of virus replication.

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Date submitted: 16 Nov 2013 Electronic form version 1.4