## Supplementary material:

## Single VS ribozyme molecules reveal dynamic and hierarchical folding toward catalysis

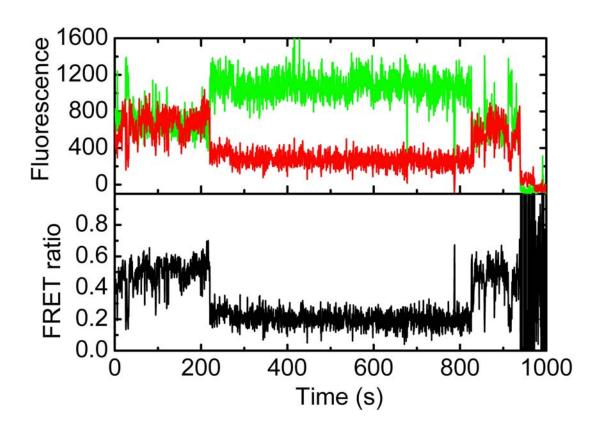
Miguel J. B. Pereira<sup>1</sup>, Evgenia N. Nikolova<sup>1,2</sup>, Shawna L. Hiley<sup>3</sup>, Dominic Jaikaran<sup>3</sup>, Richard A. Collins<sup>3</sup>, and Nils G. Walter<sup>1,\*</sup>

<sup>1</sup>Department of Chemistry, Single Molecule Analysis Group, 930 N. University Ave., University of Michigan, Ann Arbor, MI 48109-1055, USA

<sup>2</sup>Chemical Biology Doctoral Program, 930 N. University Ave., University of Michigan, Ann Arbor, MI 48109-1055, USA

<sup>3</sup>Department of Molecular and Medical Genetics, University of Toronto, Toronto, ON M5S 1A8, Canada

<sup>\*</sup>Corresponding author. Department of Chemistry, Single Molecule Analysis Group, 930 N. University Ave., University of Michigan, Ann Arbor, MI 48109-1055, USA. E-mail address: nwalter@umich.edu.



**Figure S1:** Raw fluorescence and FRET signals of a misfolded wild-type VS ribozyme molecule; the catalytically competent H state of FRET  $\approx 0.76$  is never occupied. 10% of all WT time traces show this behavior, at least partially explaining the typically  $\sim 30\%$  of inactive ribozyme in our ensemble cleavage assays (Fig. 1c).