Supporting Information for

Detecting Intramolecular Conformational Dynamics of Single Molecules in Short Distance Range with Sub-Nanometer Sensitivity

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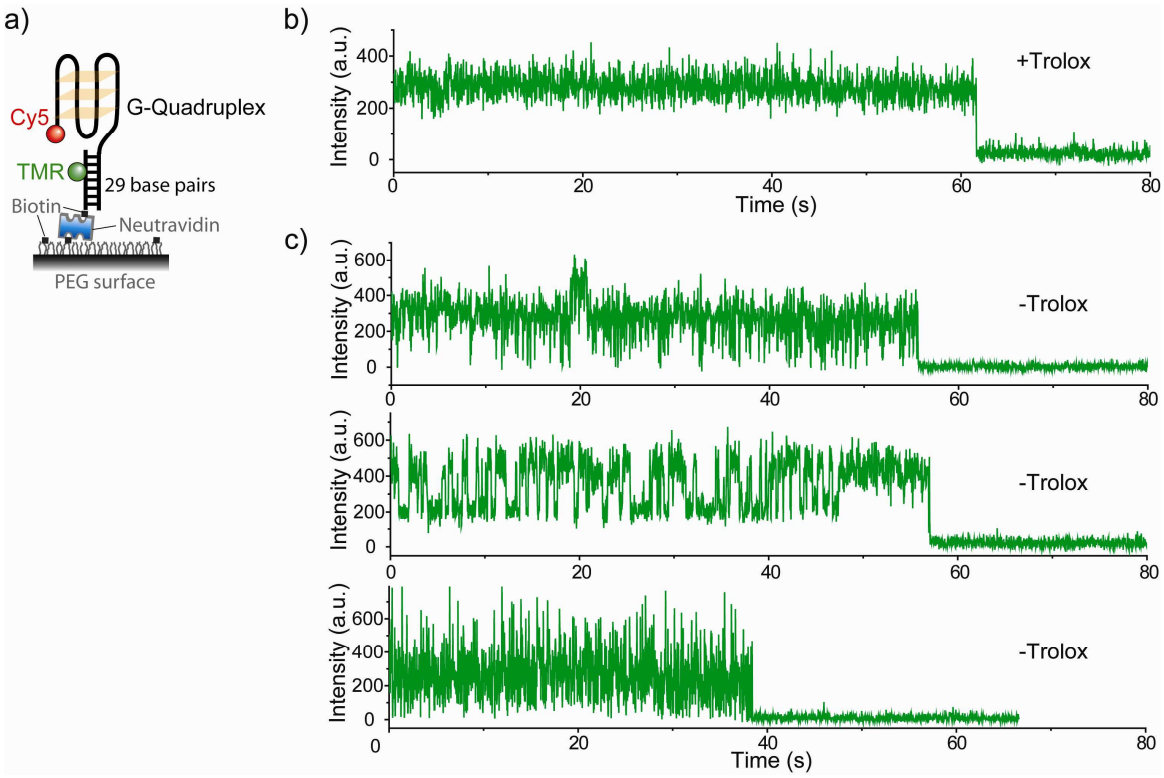

Figure. S1 A TMR-labeled DNA construct previously used for G-Quadruplex study [1, 2] was used to test whether oxygen removal and trolox addition in the imaging buffer sufficiently suppress the blinking of TMRs and increase the photostability of TMRs. a) The experimental scheme. The TMR-labeled DNA molecules were immobilized on PEG surface via biotin-nertravidin interactions. The sample preparation is as described previously and the same single molecule TIR setup was used for the data acquisition as we used for the ParM experiments[1, 2]. The Cy5 fluorophore on the DNA constructs was photobleached before data acquisition by directly exciting Cy5 using a 633 nm laser for the surface-immobilized DNA constructs. b) A representative fluorescence-intensity time trace of a single TMR-labeled DNA molecule that shows one-step photobleaching in the buffer that we used for the ParM experiments (30 mM Tris-HCl (pH 7.5), 25 mM KCl, 3 mM $MgCl_2$, 4 mM Trolox, 0.1 mg/ml BSA, 1 mg/ml glucose oxidase, 0.4% (w/v) D-glucose, 0.04 mg/ml catalase) . c) Representative fluorescence-intensity time traces of a single TMR-labeled DNA molecule that show one-step photobleaching in the buffer without trolox addition (30 mM Tris-HCl (pH 7.5), 25 mM KCl, 3 mM $MgCl_2$, 0.1 mg/ml BSA, 1 mg/ml glucose oxidase, 0.4% (w/v) D-glucose, 0.04 mg/ml catalase). Our data suggest the addition of trolox indeed suppressed the blinking of TMRs and hence

increased the photostability of TMRs. This result is similar as what we observed for Cyanine dyes [3].

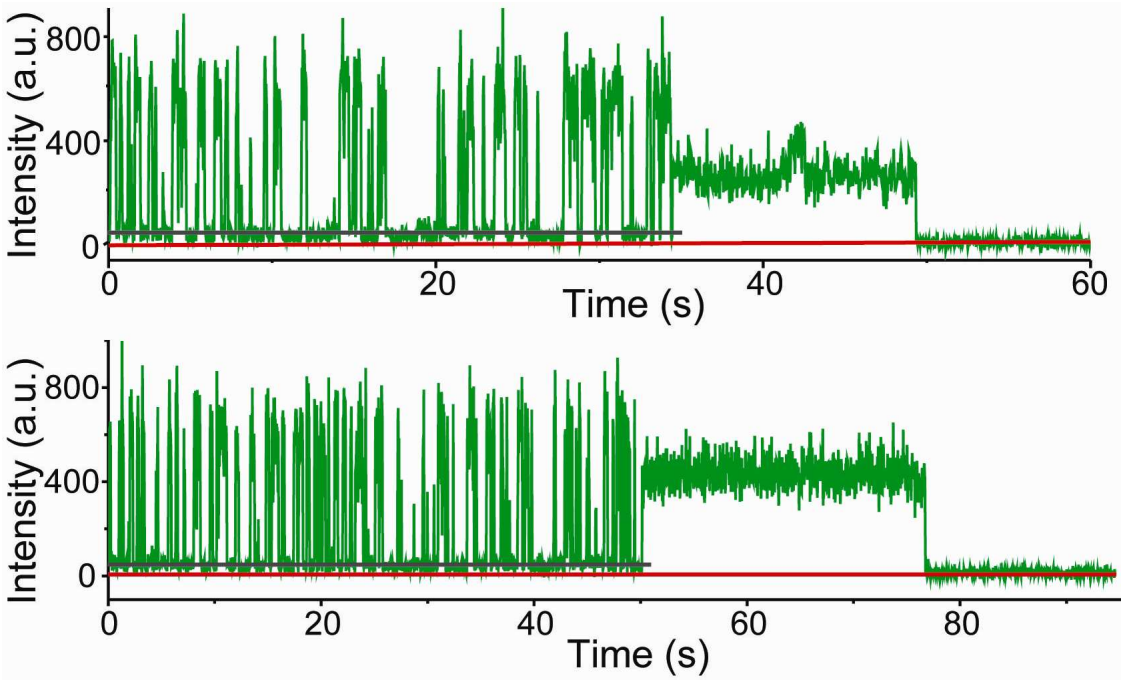

Figure.S2 Representative fluorescence-intensity time traces of a single ParM-based ADP sensor obtained at 20 μM ADP. After one of the two TMRs on the protein is photobleached, the fluorescence emission of the protein goes into an intermediate fluorescence level. Later after the second TMR is photobleached, the fluorescence emission goes down to a background value which has been corrected to zero. The red solid line shows the zero background value. The average fluorescence emission in the quenched (or apo) state (gray solid lines) typically shows a non-zero value (above the red line).

References

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2. Jena, P. V.; Shirude, P. S.; Okumus, B.; Laxmi-Reddy, K.; Godde, F.; Huc, I.; Balasubramanian, S.; Ha, T. *Journal of the American Chemical Society* **2009,** 131, (35), 12522-12523.
3. Rasnik, I.; McKinney, S. A.; Ha, T. *Nature Methods* **2006,** 3, (11), 891-893.