Supplementary Material (ESI) for Chemical Communications


# Single Molecule Conformational Analysis of the Biologically Relevant DNA G-Quadruplex in the Promoter of the Proto-Oncogene *c-MYC*

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## I. Materials

The fluorophore-labeled and unlabeled DNA oligonucleotides used in this study were purchased from IBA (Gottingen, Germany).

## II. Single-Molecule Experiments

A home-built dual-channel confocal fluorescence microscope was used to detect freely diffusing single molecules. For the details of this apparatus, refer to ref. 1. The donor, Cy3, was excited by an argon ion laser (model 35LAP321–230, Melles Griot, Carlsbad, CA) with 150 μW at 514.5 nm. Donor and acceptor fluorescence were collected through an oil-immersion objective (Nikon Plan Apo x 60, numerical aperture 1.45) and detected separately by two photon-counting modules (SPCMAQR14, Perkin–Elmer). The outputs of the two detectors were recorded by two computer-implemented multichannel scalar cards

(MCS-PCI, ORTEC, Canada). Sample solutions of 50 pM were used to achieve single-molecule detection. All the samples contained 200 μM sodium ascorbate and 0.01% Tween 20 to reduce photobleaching and adsorption of DNA molecules onto the glass surface, respectively. A threshold of 30 counts per ms bin for the sum of the donor and acceptor fluorescence signals was used to differentiate single molecule bursts from the background. A background of between 2 and 3 counts per ms, obtained from independent measurements of buffer solutions without labeled samples, was subtracted from each burst. Apparent FRET efficiencies, $E_{app}$, of each burst were calculated according to $E_{app} = \eta_A/(\eta_A + \gamma\eta_D)$, where $\eta_A$ and $\eta_D$ are the acceptor and donor counts, respectively. The percentage of crosstalk from the donor signal to the acceptor signal was measured and corrected in $\eta_A$. $\gamma = (\phi_A\eta_A)/(\phi_D\eta_D)$ is a factor accounting for the difference in the quantum yields, $\phi_A$ and $\phi_D$, and detection efficiencies, $\eta_A$ and $\eta_D$, for the acceptor and donor channels, respectively. This factor has been previously measured to be close to 1 for our setup.[1]

**III. Hybridization Kinetics**

The hybridization of excess (200 nM, 4000-fold) complementary oligonucleotide **III** to **I:II** interrogates the unfolding of *c-MYC* quadruplexes by trapping the duplex. The change in the populations in solution was monitored by recording a burst trajectory over about 300 min, and from this a series of FRET efficiency histograms was created. These histograms were used to follow the evolution of the various subpopulations within the solution. Removal of the zero peaks was in this case carried out by subtraction of the zero peak of the first histogram from all histograms in the series. In this way the disappearance of high FRET and medium FRET, could be followed. Figure S1 show few last histograms where the medium

FRET population still exist at about 300 min. The fall of the high FRET population (quadruplex) was fitted with single exponential (Figure S2).

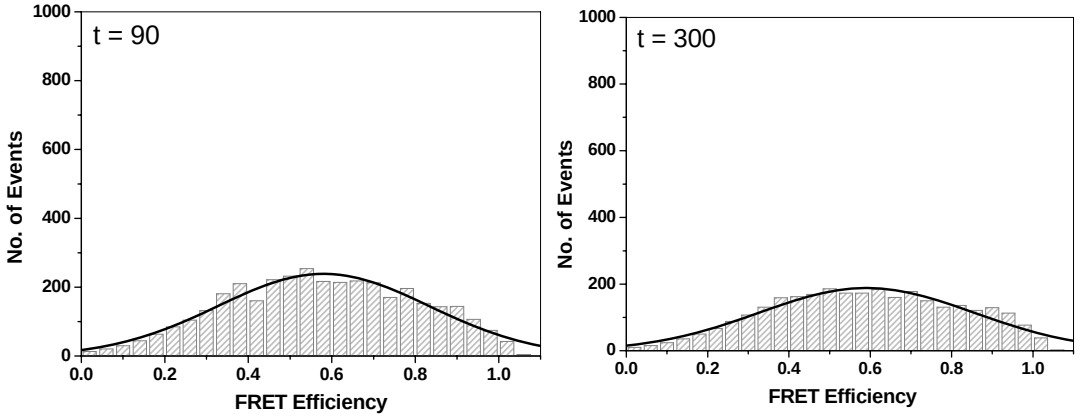


**Figure S1.** Single-molecule histograms of FRET efficiencies for DNA quadruplex opening kinetics (**I:II:III**). All are in 100 mM KCl and 10 mM sodium cacodylate (pH 7.4) at 20°C. Solid curves are the best fit to the Gaussian functions. 't' is in minutes.

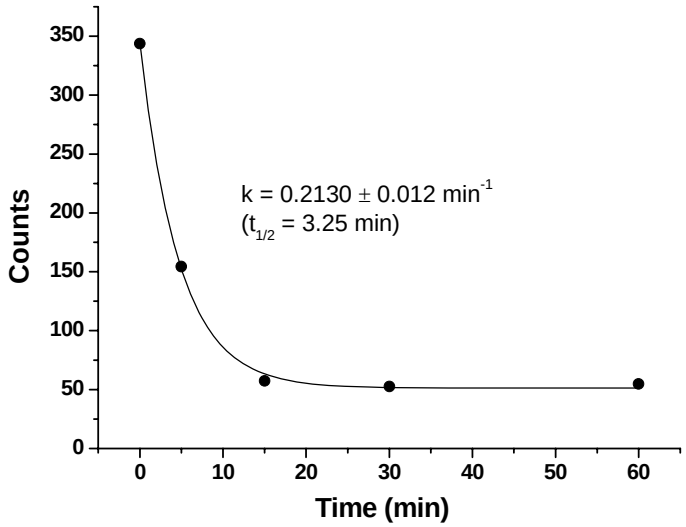


**Figure S2.** Traces showing the decrease in populations of the high FRET quadruplex species as function of time after addition of 200 nM **III** to 50 pM **I:II** in 100 mM KCl and 10 mM Sodium Cacodylate pH 7.4 with 200 µM sodium ascorbate and 0.01% Tween 20 at 20°C. Single exponential fit to the data (line) is also shown.

[1] L. M. Ying, M. I. Wallace, S. Balasubramanian, D. Klenerman, *J. Phys. Chem. B*, **2000**, *104*, 5171.