Supporting Information for

Direct measurement of the rates and barriers on forward and reverse diffusions of intramolecular collision in overhang oligonucleotides

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1. The duplex melting temperature of the overhang oligonucleotides.

To make sure all oligonucleotide molecules labeled by TMR were hybridized, the concentration of the complementary oligonucleotides without TMR was kept in 4-fold excess in all the three types of overhang sequences. Fig.S1 shows the examples of the fluorescence measurement on the duplex melting curves of the overhang oligonucleotides. The concentration of the strands with TMR was 10 nM. It is seen that the fluorescence of the control dsDNA (Oh30T) decreases with temperature due to the thermal quenching of the fluorescence. The melting temperature did not show up in Oh30T, because the melting did not change the status of TMR fluorescence as expected. The fluorescence from both Oh2GG26 (end-to-interior type) and Oh2GG (end-to-end type) showed sudden recovery around the melting temperature (T_m) of the duplex, 80 ^oC, because TMR was no longer quenched by G bases after the duplex was dissociated. Our experimental temperatures of 10 - 30 ^oC were well below the melting temperature, so that all the TMR labeled DNA molecules were in their duplex forms.

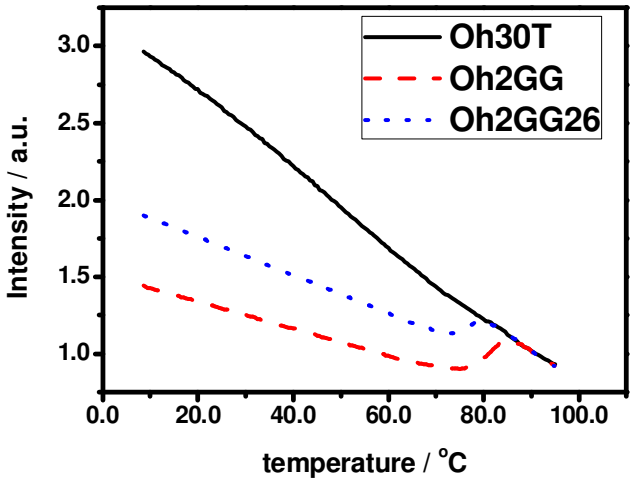

Figure S1. Fluorescence of Oh30T, Oh2GG, and Oh2GG26 against temperature in 1×TE buffer, pH 7.5, containing 500 mM NaCl.

2. The addition of a second G does not vary the dynamics of intrachain motion in the overhang oligonucleotides.

To improve the signal to noise ratio, we put a double guanosines site in the sequences instead of one. Here we show that the dynamic property of double dGs remains the same as that of one dG in the overhang oligonucleotides for both end-to-interior and end-to-end collision. Tables S1 shows the sequences we compared, in which Oh2GG26 and Oh2G27 for end-interior collision, Oh2G and Oh2GG for end-to-end collision, sharing the same separation length between TMR and dG but different number of dG. As shown in Figure S2, double dGs increased the quenching efficiency evidently.

Fitting the FCS curves through

$$G(\tau) = \frac{A}{[1+(\tau/\tau_D)]}(1 + B\exp[-(\frac{\tau}{\tau_r})]) \qquad \text{(S1)}$$

the characteristic relaxation time was obtained and summarized in Table S1. In respective collision type, the overhang oligonucleotides with one dG and two dGs presents identical relaxation time, suggesting that the addition of a second dG does not influence the FCS dynamic property of the intrachain diffusion.

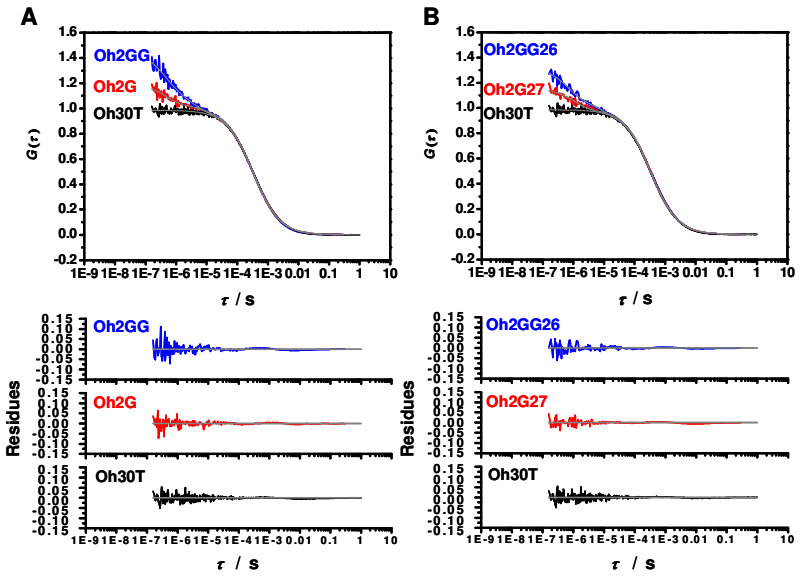

Figure S2. FCS curves of Oh2G27, Oh2GG26, Oh2G, and Oh2GG with Oh30T as a reference in 1×TE buffer, pH 7.5, containing 500 mM NaCl at 19 °C. The gray lines are the fitting and the residues of the fitting are shown at the bottom. The respective characteristic times of the intrachain diffusion were derived from the fit according to eq. (S1) and are summarized in Table S1.

Table S1. Sequences and their characteristic relaxation times from FCS at 20 °C.

Abbreviation	Sequence	τ_r / μs
Oh2G27	5'-TTGTTGGTGATGTGGTTGTA$(T)_2$**G**$(T)_{27}$-3'	1.1±0.1
	3'-AACAACCACTACACCAACAT-TMR-5	
Oh2GG26	5'-TTGTTGGTGATGTGGTTGTA$(T)_2$**GG**$(T)_{26}$-3'	1.2±0.1
	3'-AACAACCACTACACCAACAT-TMR-5'	
Oh2G	5'-TTGTTGGTGATGTGGTTGTA$(T)_2$**G**-3'	1.1±0.1
	3'-AACAACCACTACACCAACAT-TMR-5'	
Oh2GG	5'-TTGTTGGTGATGTGGTTGTA$(T)_4$**GG**-3'	1.0±0.1
	3'-AACAACCACTACACCAACAT-TMR-5'	

3. Our rate constants were consistent with previous measurements.

We noticed that our rates of intrachain diffusion in the overhang dsDNA were generally slower than those for single stranded DNA reported by Kim *et al.*[1] and Uzawa *et al.*[2] To make sure that this slowness is intrinsic for dsDNA, we compared the rate constant of Oh2GG26 with that of 5'-TMR-TTTTTG (5TG), and the results are shown in Fig S3. Fitting the FCS curves at 19 °C we obtained $k_{d+} = 3.0\times10^5$ s^{-1} and $k_{d-} = 9.3\times10^5$ s^{-1} for Oh2GG26 and $k_{d+} = 1.0\times10^6$ s^{-1} and $k_{d-} = 2.8\times10^6$ s^{-1} for 5TG. Therefore, it is an experimental fact that the respective rates of overhang on dsDNA are slower than those of ssDNA.

Kim *et al.*[1] report that when 5 dTs are placed between MR121 and dG the end-to-end collision rate is 1.73×10^6 s^{-1} at 20 °C. From the figure provided by Uzawa *et al.*[2] the end-to-end collision rate is 2.8×10^6 s^{-1} at 22 °C when the chain length is 6. Our end-to-end collision rate of 1.0×10^6 s^{-1} on 5TG is consistent with their results. The minor difference among different authors may come from different experimental conditions, such as different probes, different temperatures, and different salt concentrations.

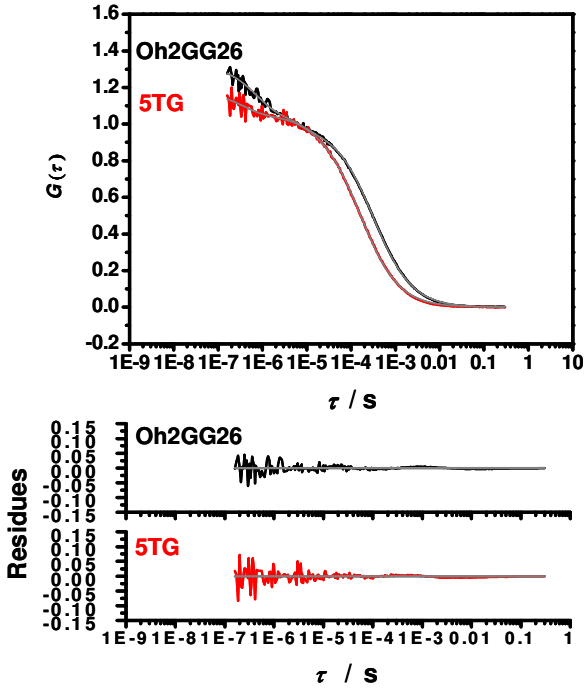

Figure S3. FCS curves of Oh2GG26 and 5TG in 1×TE buffer, pH 7.5, containing 500 mM NaCl at 19 °C. The gray lines are the fitting and the residues of the fitting are shown at the bottom.

References

(1) Kim, J.; Doose, S.; Neuweiler, H.; Sauer, M. *Nucleic Acids Res.* **2006**, *34*, 2516-2527.

(2) Uzawa, T.; Cheng, R. R.; Cash, K. J.; Makarov, D. E.; Plaxco, K. W. *Biophys. J.* **2009**, *97*, 205-210.