Long-lived fluorescence of homopolymeric guanine–cytosine DNA duplexes

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Supporting Information

Synthesis and characterization of poly(dG)·poly(dC)

2'-deoxyribonucleoside 5'-triphosphates (dCMP and dGMP) were obtained from Sigma-Aldrich (USA), as well as other reagents and were used without further purification. Klenow fragment exonuclease minus of DNA Polymerase I from *Esherichia coli* lacking the 3'→5'exonuclease activity (Klenow exo$^-$) was purchased from Fermentas (Lithuania).

A reaction mixture containing: 60 mM phosphate buffer (K_2PO_4, $KHPO_4$) pH 7.4, 5 mM dithiothreitol, 4 mM $MgCl_2$ and 1.5 mM dCTP and 1.5 mM dGTP and was primed by 0.2-0.5 μM $(dG)_{10}$·$(dC)_{10}$. The synthesis was initiated by addition of the enzyme (Klenow exo$^-$) and was conducted for 2 hours at 37^{o}C. The synthesized poly(dG)·poly(dC) molecules were separated from the nucleotides and other components of the assay using size-exclusion HPLC. The separation was carried out with a TSK-gel G-DNA-PW HPLC column (7.8x300 mm) from TosoHaas (Japan) by isocratic elution with 20 mM Tris-Acetate, pH 7.4, at a flow rate of 0.5 ml/min, and was confirmed by electrophoresis and AFM (see Fig. SI-1, A and B respectively). The synthesized polymer moves as a single band on the agarose gel electrophoresis, showing that the molecules are uniform (Fig. SI-1 A). As clearly seen in AFM images (Fig. SI-1 B) the synthesized molecules are characterized by narrow length distribution. AFM imaging was performed on the molecules adsorbed onto muscovite mica surfaces. The DNA sample in 2 mM Tris-Acetate, pH 7.4, containing 2 mM $MgCl_2$, were incubated on freshly cleaved mica plates for 2-5 min, washed with distilled water, and dried with nitrogen gas. AFM images were obtained with a Solver PRO (NT-MDT, Russia). The measured average contour length of ~100 single molecules is equal to approximately 300 nm and corresponds to 1200 bp poly(dG)·poly(dC).[1]

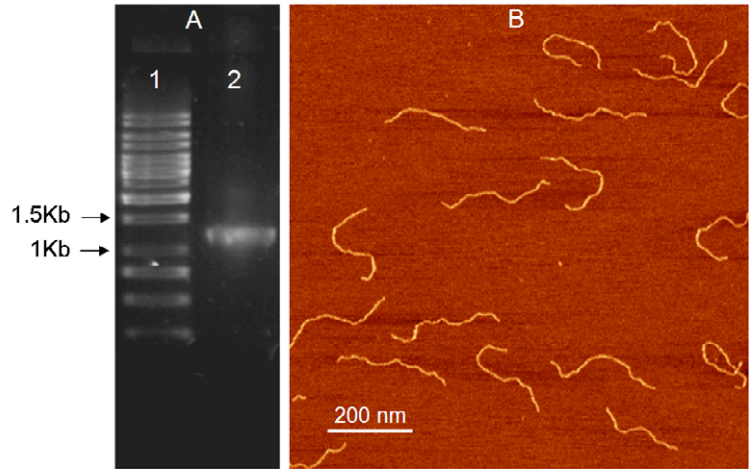

Figure SI-1. Electrophoresis and AFM of the synthesized DNA molecules. (A) Mobility of a DNA molecular weight ladder (lane 1) and the synthesized poly(dG)·poly(dC) molecules (lane 2) in 1.2% agarose gel. (B) AFM image of the poly(dG)·poly(dC) molecules. The molecules were electrophoresed and imaged on freshly cleaved mica and scanned in a semi-contact mode as described above.

Experimental setups and methods

Steady-state spectra were obtained with a Perkin-Elmer Lambda 900 spectrophotometer and a SPEX Fluorolog-3 according to a procedure described in detail elsewhere.[2] Fluorescence quantum yields were determined for dilute solutions (absorbance at 267 nm < 0.5 over 10 mm) using TMP as a reference.

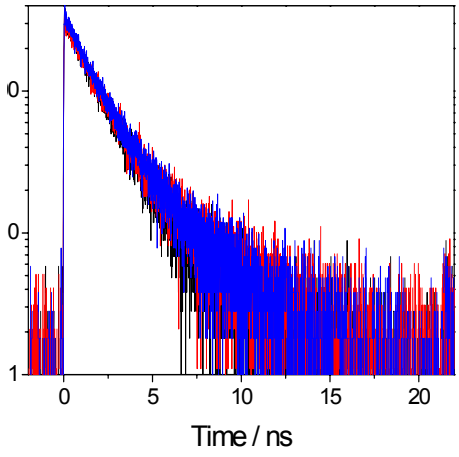

Figure SI-2. Successive fluorescence decays obtained for poly(dG).poly(dC) in phosphate buffer solution (0.25 M NaCl) at 310 nm by TCSPC.

The TCSPC setup used the third harmonic of a titanium-sapphire laser at a repetition rate of 4.75MHz. The average laser power (0.3 mW) was measured with a Melles Griot broadband powermeter. The irradiated area on the surface of the cell was ca. 0.2 cm^2. Solutions (typically 3.5 ml with an absorbance of 0.5 at 267 nm) were contained in a 10 mm x 10 mm quartz cell

and continuously stirred. Successive measurements gave identical decays (Fig. SI-2), which were eventually merged to decrease the signal-to-noise ratio. The polarization on the excitation side was controlled by a half-wave plate whereas that of the emission side was fixed vertically by a Glan-Thomson prism set at the magic angle. The instrumental response function (50 ps, fwhm) was given by the Raman line of water.

Fluorescence decays for each wavelength were analysed by a non-linear fitting procedure using a three-exponential model function: $F(t) = \sum \alpha_i \exp(-t/\tau_i)$ (i = 1 to 3). This model function was convoluted with an apparatus function G(t) obtained by measuring the Raman signal in neat water at 295 nm. As an example, in Figure SI-3 we show the fluorescence decay of poly(dG).poly(dC) in phosphate buffer solution (0.25 M NaCl) at 310 nm together with the best fit.

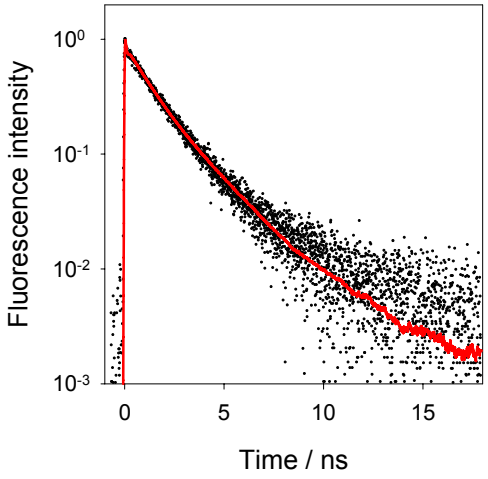

Figure SI-3. Fluorescence decay of poly(dG).poly(dC) in phosphate buffer solution (0.25 M NaCl) at 310 nm together with the best fit.

Table SI-1. Parameters derived from the fits of the decays with three-exponential functions: $\Sigma a_i exp(-t/\tau_i)$; $p_i = \alpha_i\tau_i / (\alpha_1\tau_1 + \alpha_2\tau_2 + \alpha_3\tau_3)$ represents the weight of each time constant τ_i (in ns).

decay	τ_1	α_1	p_1	τ_2	α_2	p_2	τ_3	α_3	p_3
H, 310 nm, 0.25 M NaCl	0.03	0.56	0.02	1.3	0.32	0.53	2.9	0.12	0.45
H, 340 nm 0.25 M NaCl	0.03	0.68	0.03	1.3	0.23	0.43	4.2	0.09	0.54
H, 310 nm 0.1 M NaCl	0.01[a]	0.92	0.09	0.2	0.01	0.03	1.4	0.06	0.88
A, 310 nm 0.25 M NaCl	0.0002[b]	0.77	0.005	1.4	0.21	0.78	4.7	0.02	0.22

[a] Fixed to 10 ps, which corresponds to the time resolution of our setup after deconvolution.

[b] Fixed to 0.2 ps, as determined by fluorescence upconversion experiments.[4]

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