Electronic Supplementary Material (ESI) for Soft Matter.

Electric Supplementary Information for

Photoinduced adsorption of oligonucleotides on polyvinyl chloride films containing malachite green derivative

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Fluorescence intensity of film after rinsing procedure

The polyvinyl chloride film was exposed to UV light, and a Fluo-dT_{24} solution (6 μL, 537 μM) was placed on the film to cover the irradiated area. After 10 min, the Fluo-dT_{24} solution was removed using a tissue paper. A fresh buffer solution (6 μL) was added to the irradiated area and subsequently removed using tissue paper to rinse the film. The film was repeatedly rinsed using this procedure, and the effect of rinsing on the fluorescence intensity of the film was examined. Figure S1 shows the dependence of fluorescence intensity of the green signal on the number of rinses. When rinsing was insufficient (n=1), the fluorescence intensity was as high as that without rinsing. The fluorescence intensity decreased with increasing number of rinses, and was almost unchanged when the rinsing was repeated more than 2 times. Therefore, the non-adsorbed Fluo-dT_{24} was removed after rinsing the film for the fourth time.

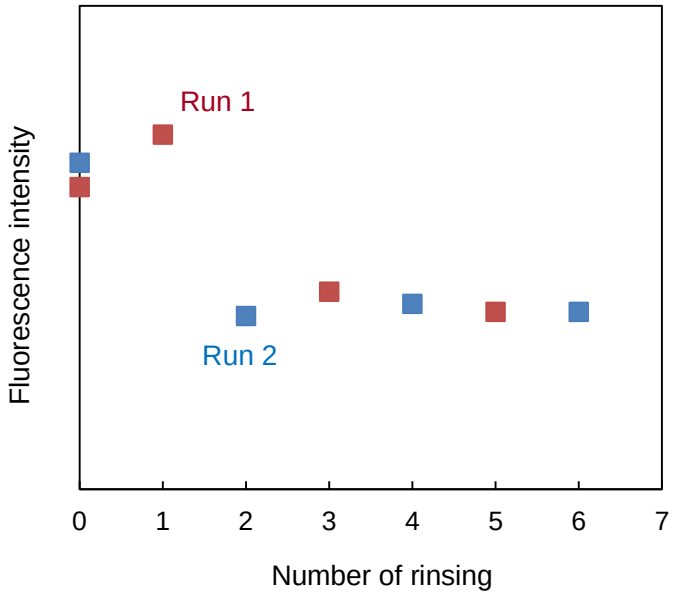

Figure S1. Dependence of the fluorescence intensity of Fluo-dT_{24} on the number of rinses. Two different films were tested.

Damage of UV light exposure to oligonucleotide

To check that the oligonucleotide remained undamaged by UV light (0.22 mW/cm^2 at 254 nm), a TE buffer solution containing oligonucleotide (1 mg/L, 200 μL) was exposed to UV light for 10 min, and the concentration of the undamaged oligonucleotide was subsequently determined with the Quant-iT ssDNA assay kit using a Qubit fluorometer (Invitrogen). The concentrations of oligonucleotides used in this work were 1 ± 0.05 mg/L after irradiation, and we found that the oligonucleotide remained undamaged under these exposure conditions.

Film thickness

Part of the film mounted on the microscope slide was wiped off using a paper towel dampened with THF, and the film was observed using a confocal microscope (OPTELICS HYBRID, Lasertec, Japan). A typical cross-sectional profile of the film is shown in Figure S2. The film thickness can be measured by the difference between the thickness of the film mounted on the slide and that of the bare slide, as indicated by an arrow in Figure S2. The average thickness was found to be 2.2±0.3 μm for 15 measurements at different positions.

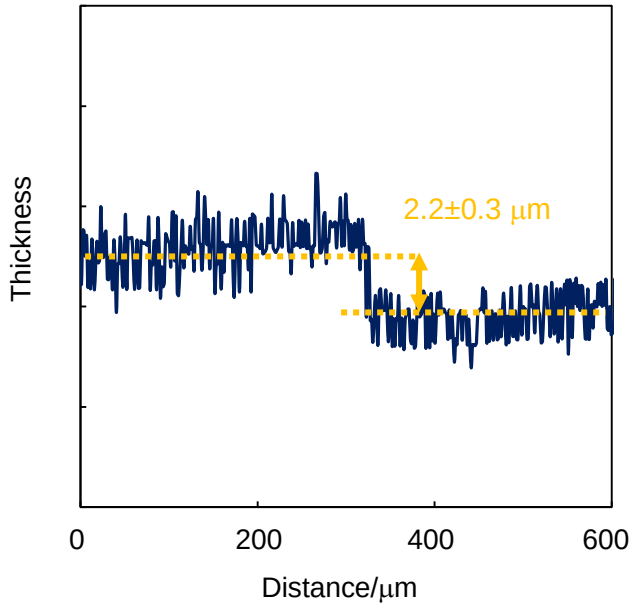

Figure S2. Typical cross-sectional profile of the film used in this study.

Absorption spectra of the film

The microscope slide, on which the film prepared in this study was mounted, was attached to the sample holder of a spectrophotometer (UV-2400, Shimadzu, Japan). The changes in the absorption pattern of the films are shown in Figure S3. The film was UV-irradiated for 10 min (0.22 mM/cm^2 at 254 nm) without a photomask. UV irradiation decreased the peak at λ_{max} = 270 nm, which was assigned to the neutral forms of MGL. Peaks around 620 nm and 430 nm which were absent under dark conditions, appeared upon irradiation and were assigned to the malachite green cation.

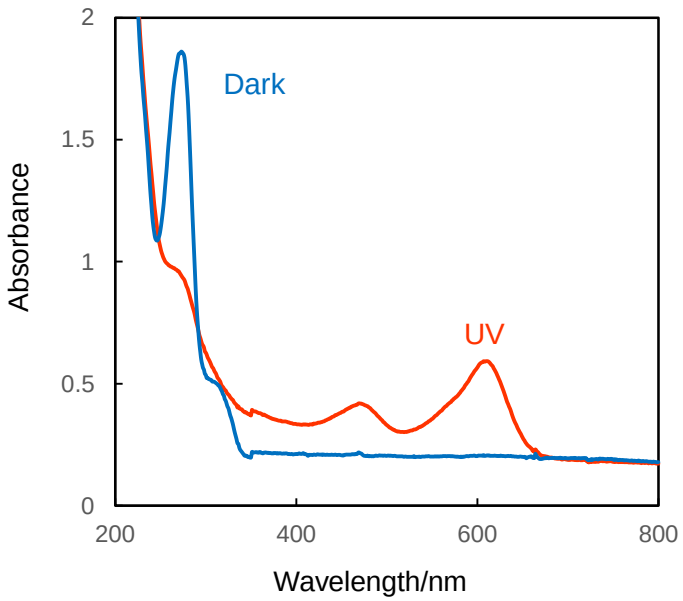

Figure S3. Changes in the absorption spectra of the films in this study. Irradiation; 10 min.

Image of photomask

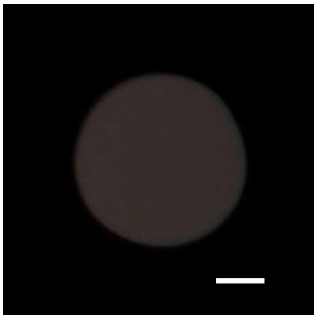

Figure S4. Microscopic image of photomask pattern used for Fluo-dT_{24} adsorption. Bar = 25 μm.

Fluorescence spectrum of malachite green oxalate

Figure S5 shows the fluorescence emission spectrum of malachite green oxalate (cationic form of malachite green) in a viscous solution of glycerol. A peak appeared at 660 nm. This result agreed with the reports that demonstrate the enhancing role of triphenylmethane dye cations such as malachite green and crystal violet on the fluorescence spectra in viscous and cold environments.[1,2]

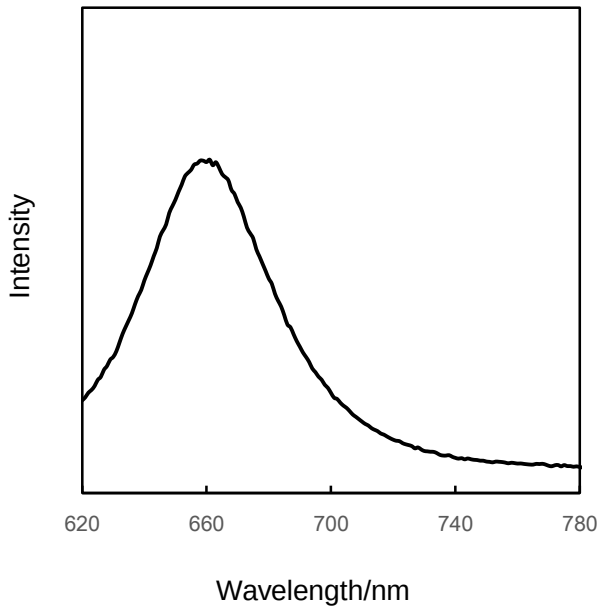

Figure S5. Fluorescence spectrum of malachite green oxalate (20 μM) in 90 wt% aqueous glycerol solution. λ_{ex} = 590 nm.

1. M. S. Baptista, G. L. Indig, *J. Phys. Chem. B*, 1998, **102**, 4678.
2. L. A. Brey, G. B. Schuster and H. G. Drickamer, *J. Chem. Phys.*, 1977, **67**, 2648.

Fluorescence spectra of Fluo-dT$_{24}$

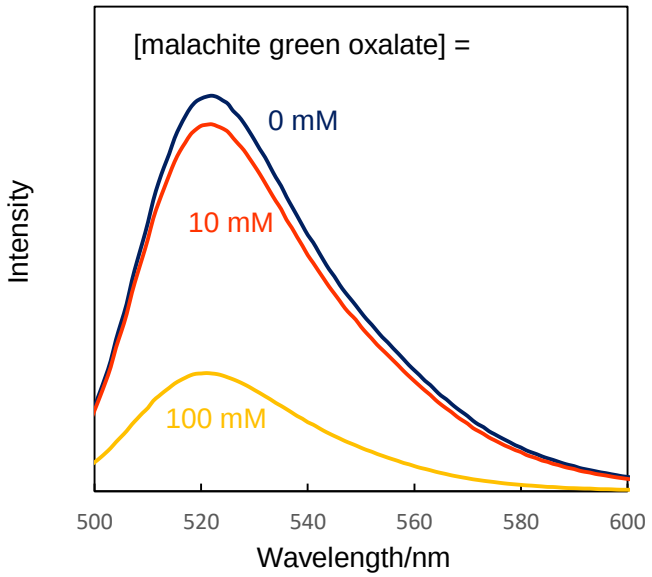

Figure S6. Fluorescence spectra of Fluo-dT$_{24}$ (10 μM) in the TE buffer solution containing malachite green oxalate at various concentrations. λ_{ex} = 494 nm.

The fluorescence intensity of Fluo-dT$_{24}$ at 520 nm decreased by the addition of malachite green oxalate. This may have occurred because ionized malachite green has an absorption peak in the visible-light region (Figure S3). Because the λ_{em} of the fluorescence peak was almost unchanged in the presence of malachite green oxalate, the interactions between malachite green and fluorescein via π stacking were negligible.

Estimation of the oligonucleotide concentration adsorbed on the film

The fluorescence intensity of the solution containing Qubit® ssDNA reagent and oligonucleotide was plotted against the oligonucleotide concentration. Typical plots are shown in Figure S7. The intensity increased with increasing oligonucleotide concentration, and the calibration curves were obtained from the fluorescence intensity in the linearly increasing range. Because the fluorescence was slightly different for each film, the concentration of the oligonucleotide adsorbed on the films were calculated from the calibration curve obtained from the same films. Six irradiated spots were observed on the films. Out of them, one was for oligonucleotide adsorption, while the others were used for the calibration curve.

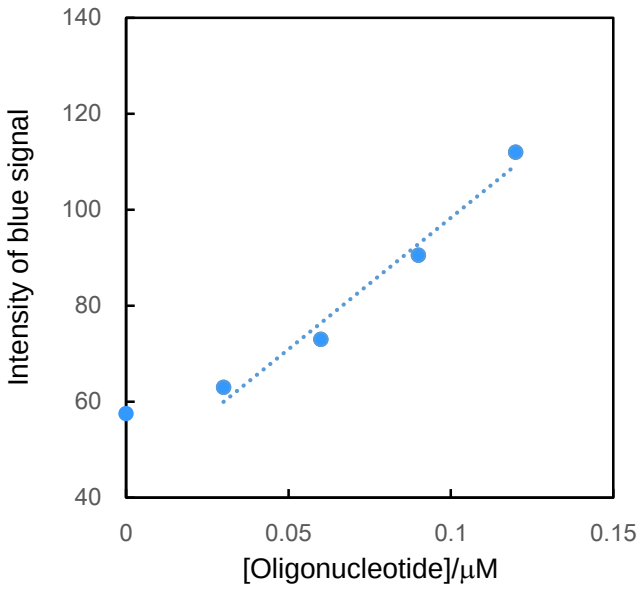

Figure S7. Typical calibration curve for estimating the oligonucleotide concentration in the irradiated film.

Dependence of oligonucleotide adsorption on irradiation time

Oligonucleotide adsorption was examined several times by UV irradiation. Figure S8 shows the adsorption of oligonucleotides with a random sequence of 5'-ACCGGTGCTTTACCGGTTCTCGATCGGTACGTACCGTGCCTCAATGCCGT–3'.

Longer irradiation leads to a higher concentration of adsorbed oligonucleotides. The absorption spectra of the film provided information about the ionization of MGL (Figure S3). In Figure S9, the absorption difference at 620 nm before and after UV irradiation ($\Delta A_{UV\text{-}dark}$) is plotted against the irradiation time. Longer irradiation yields a higher concentration of ionized MGL (Figure S9). The dependance of $\Delta A_{UV\text{-}dark}$ on irradiation time at 620 nm corresponds to the dependence of the adsorbed oligonucleotide concentration on irradiation time. Based on these results, it can be stated that the amount of ionized MGL determines the amount of oligonucleotides adsorbed on the film.

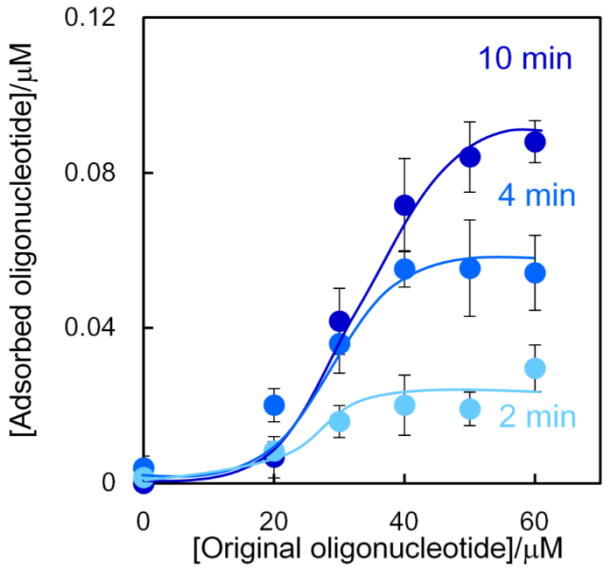

Figure S8. Effect of irradiation time on adsorbed oligonucleotide concentration. Data has been presented as the average ± standard deviation of three different films.

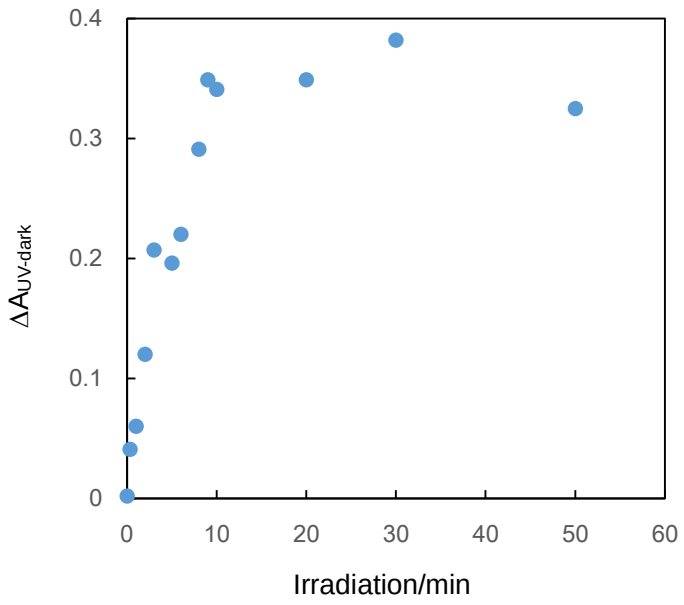

Figure S9. Dependence of $\Delta A_{UV\text{-}dark}$ at 620 nm on irradiation time.

Oligonucleotide adsorption induced by cationic substrate

To investigate the adsorption induced by electrostatic interactions, cationic cetyltrimethylammonium chloride (CTAC) was used instead of MGL. The CTAC film was prepared using PVC (50 mg), DOS (100 mg), and CTAC at various concentrations, and the amount of DNA adsorbed was investigated in the same manner as that for the MGL film; however, the CTAC film was not irradiated with UV light. Figure S10 shows the dependence of the adsorbed dT_{50} on the weight percentage of CTAC present in the film. The adsorbed dT_{50} concentration increased with increasing CTAC concentration, indicating that electrostatic interactions between nucleotides and the cationic film play an important role in oligonucleotide adsorption. Although the concentration of adsorbed dT_{50} on the CTAC film (Figure S10) was higher than that on the MGL film (Figure 4), it cannot be stated that MGL was less effective than CTAC. The concentration of ionized MGL in the film is difficult to evaluate and therefore cannot be compared with the corresponding CTAC concentration.

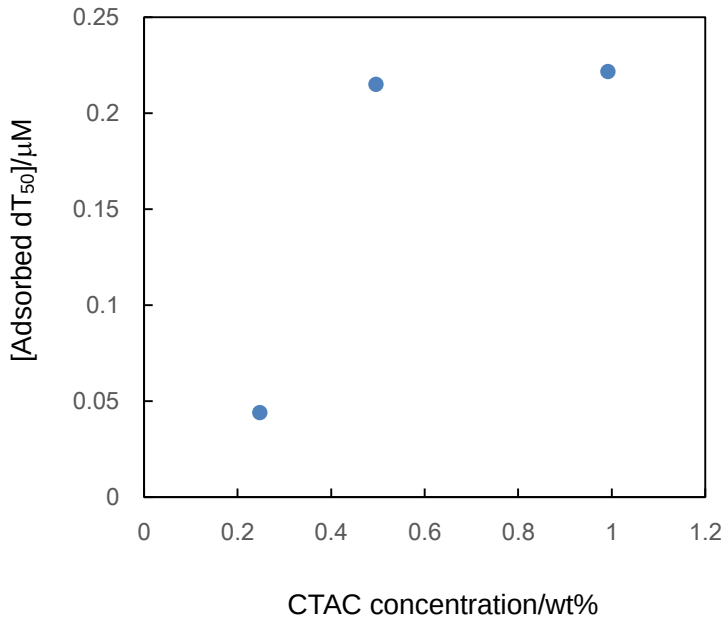

Figure S10. Concentration of adsorbed dT_{24} on the CTAC film.

Circular dichromic (CD) spectra of homo oligomers

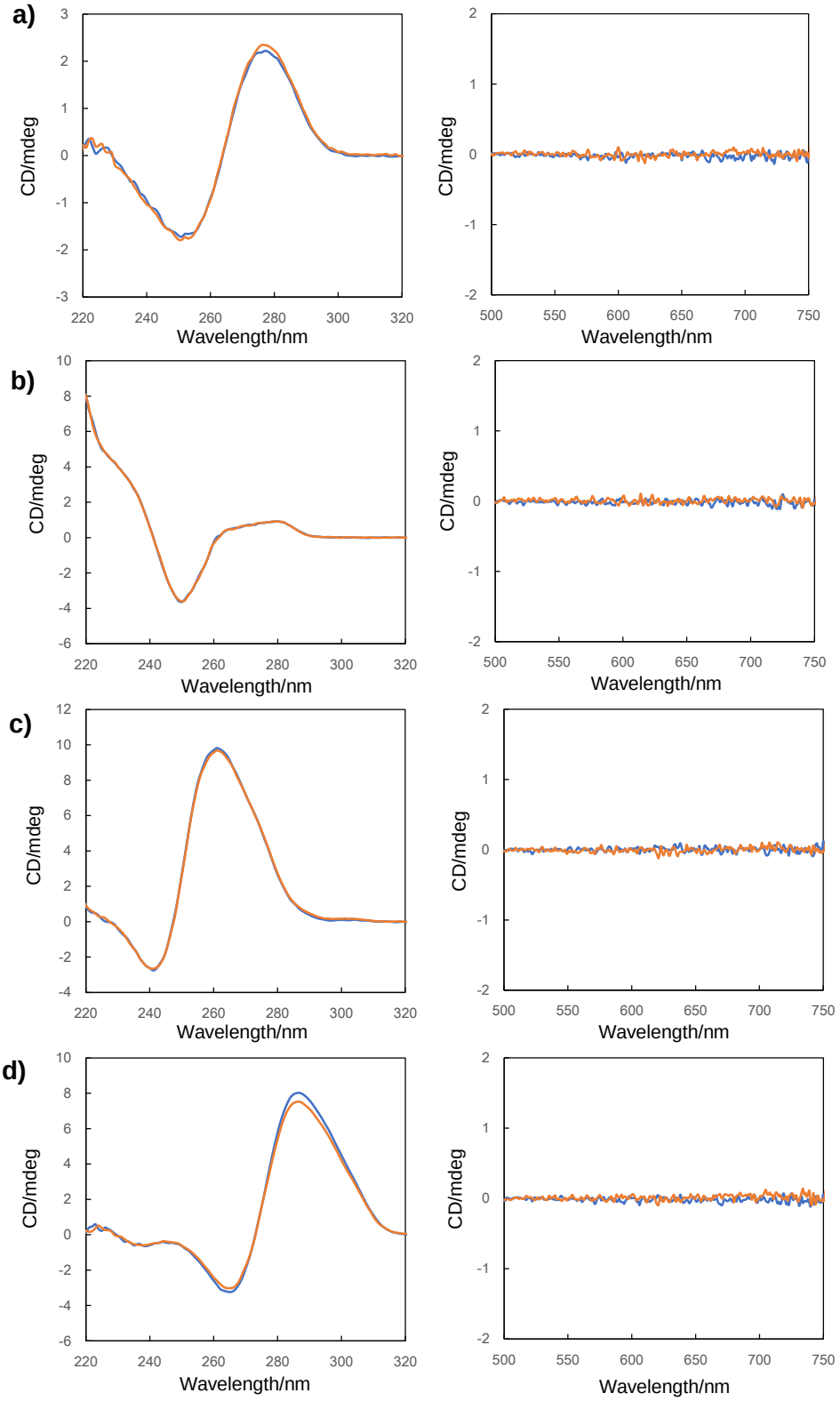

Figure S11. CD spectra of dT_{24} a), dA_{24} b), dG_{24} c), and dC_{24} d) in the TE buffer solution excluding (blue) and including (red) MG oxalate. [oligonucleotide] = 5 μM. [MG oxalate] = 5 μM.

Because cationic dyes such as telomestatin and thioflavin T are known to induce structural changes in oligonucleotides,[3-5] circular dichromic (CD) spectra of the homo oligomers were measured in the presence of MG oxalate (Figure S11). The CD spectra in the wavelength range 220–320 nm were almost unchanged by the addition of MG oxalate. Therefore, we conclude that the unfolded structure of the oligonucleotides was intact upon interaction with MG oxalate.

If malachite green binds to the G-quadruplex, an induced CD signal is observed at approximately 620 nm.[6] Because no significant peak was observed in the wavelength range of 500–750 nm, it can be stated that the homo oligomers hardly form G-quadruplexes.

3. M.-Y. Kim, M. Gleason-Guzman, E. Izbicka, D. Nishioka, L. H. Hurley, *Cancer Res.*, 2003, **63**, 3247.
4. X. Tan, Y. Wang, B. A. Armitage, M. P. Bruchez, *Anal. Chem.* 2014, **86**, 10864.
5. A. Renaud de la Faverie, A. Guédin, A. Bedrat, L. A. Yatsunyk, J.-L. Mergny, *Nucleic Acids Res.* 2014, **42**, e65.
6. R. M. Uda, T. Matsui, M. Takei, *Supramol. Chem.*, 2017, **29**, 553.

Circular dichromic (CD) spectra of d(T_2AG_3)$_4$

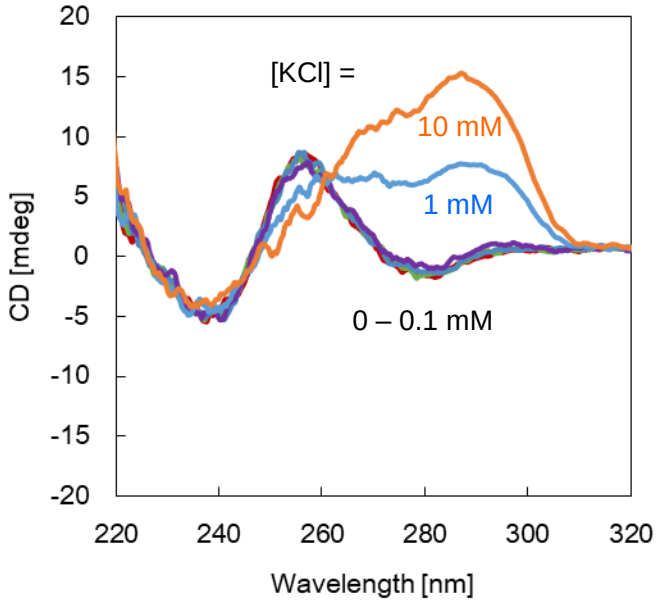

Figure S12. CD spectra of d(T_2AG_3)$_4$ in the TE buffer solution containing KCl at various concentrations. [d(T_2AG_3)$_4$] = 4 μM.

The CD spectra of d(T_2AG_3)$_4$ are shown in Figure S12. It is known that the repeated sequence of TTAGGG in telomeric DNA forms a G-quadruplex.[7] In the presence of KCl, the hybrid-type topology of the G-quadruplex was evaluated by a positive peak at approximately 290 nm, a shoulder peak at approximately 265 nm, and a negative CD peak at approximately 240 nm.[8] When the concentration of KCl was below 0.1 mM, the peaks assigned to the reported structure were not observed.

The results obtained from Figures S11 and S12 show that the oligonucleotides used in this study hardly form G-quadruplexes to which MG oxalate can bind.

7. S. Neidle, G. N Parkinson, *Curr. Opin. Struct. Biol.*, 2003, **13**, 275.

8. E. M. Rezler, J. Seenisamy, S. Bashyam, M.-Y. Kim, E. White, W. D. Wilson, L. H. Hurley, *J. Am. Chem. Soc.*, 2005, **127**, 9439.

Emission spectra of 1-anilino-8-naphthalene sulfonate (ANS)

Figure S13a shows ANS emission spectra. In the TE buffer solution, a small emission peak was observed around 520 nm, which shifted to 500 nm in the TE buffer solution containing 10 wt% PVA. Since the wavelength of the ANS emission peak is dependent on solvent polarity,[9] the blue-shift of ANS emission indicated that PVA provided a less polar environment to the TE buffer solution. The ANS emission spectrum of the films is shown in Figure S13b. The emission peak was observed at approximately 460 nm, and the environment around the film was considerably less polar than that in the TE buffer containing PVA.

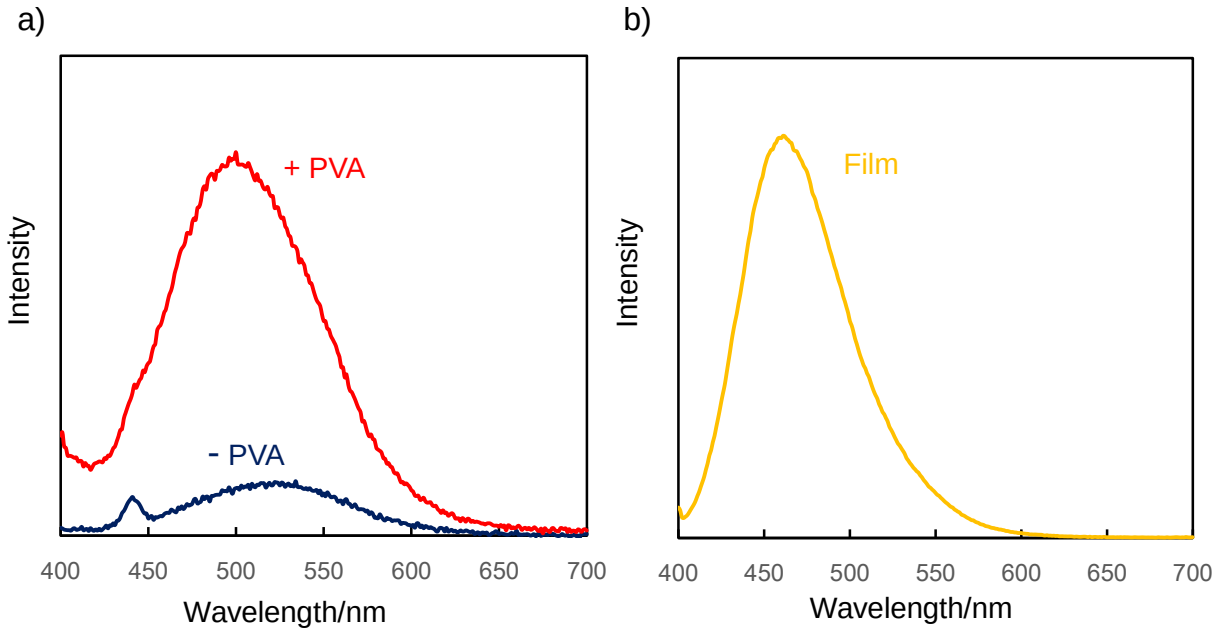

Figure S13. ANS emission in TE buffer solution with and without PVA a) and in film b). λ_{ex} = 380 nm. The ANS concentration in the TE buffer solution was 10 μM. The films were composed of PVC (3.75 mg), DOS (7.5 mg), and ANS (0.15 μmol). The film was prepared in the same manner as for the oligonucleotide adsorption experiment.

9. L. Stryer, *J. Mol. Biol.*, 1965, **13**, 482.

Viscosity of TE buffer solution containing PVA

Figure S14 shows the dependence of viscosity on the PVA concentration. The viscosity increased on increasing the PVA concentration. Although the viscosity of the TE buffer, excluding PVA, was not determined, it was less than 1.5 mPa·s.

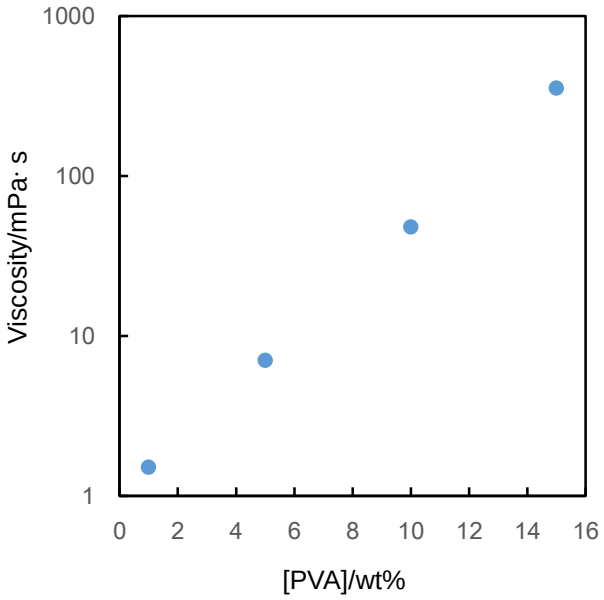

Figure S14. Viscosity of the TE buffer solutions containing PVA. The measurements were performed at 25 °C using an RE-85 viscometer (Toki Sangyo, Japan).