Supporting Information

Conquering 2-Aminopurine's Deficiencies: Highly Emissive Isomorphic Guanosine Surrogate Faithfully Monitors Guanosine Conformation and Dynamics in DNA

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1. Synthesis

Reagents were purchased from Sigma-Aldrich, Fluka, TCI, Acros and Synchem, Inc. (Elk Grove, IL), and were used without further purification unless otherwise specified. Solvents were purchased from Sigma-Aldrich and Fisher Scientific, and dried by standard techniques. NMR solvents were purchased from Cambridge Isotope Laboratories (Andover, MA). All reactions were monitored with analytical TLC (Merck Kieselgel 60 F254). All experiments involving air and/or moisture sensitive compounds were carried out under an argon atmosphere. Column chromatography was carried out with silica gel particle size 40-63 μm by CombiFlash® Rf 200 (Teledyne Isco). NMR spectra were obtained on Varian Mercury 400 MHz, Varian VX 500 MHz and Jeol ECA 500 spectrometers. Mass spectra were obtained on an Agilent 6230 HR-ESI-TOF MS at the Molecular Mass Spectrometry Facility at the

UCSD Chemistry and Biochemistry Department. Modified oligonucleotide (ODN) was quantified by Shimadzu UV 2450 at 70 °C. MALDI-TOF spectra were recorded on a PE Biosystems Voyager-DE STR MALDI-TOF spectrometer in negative-ion, delayed-extraction mode.

1.1. dthGphosphoramidite synthesis

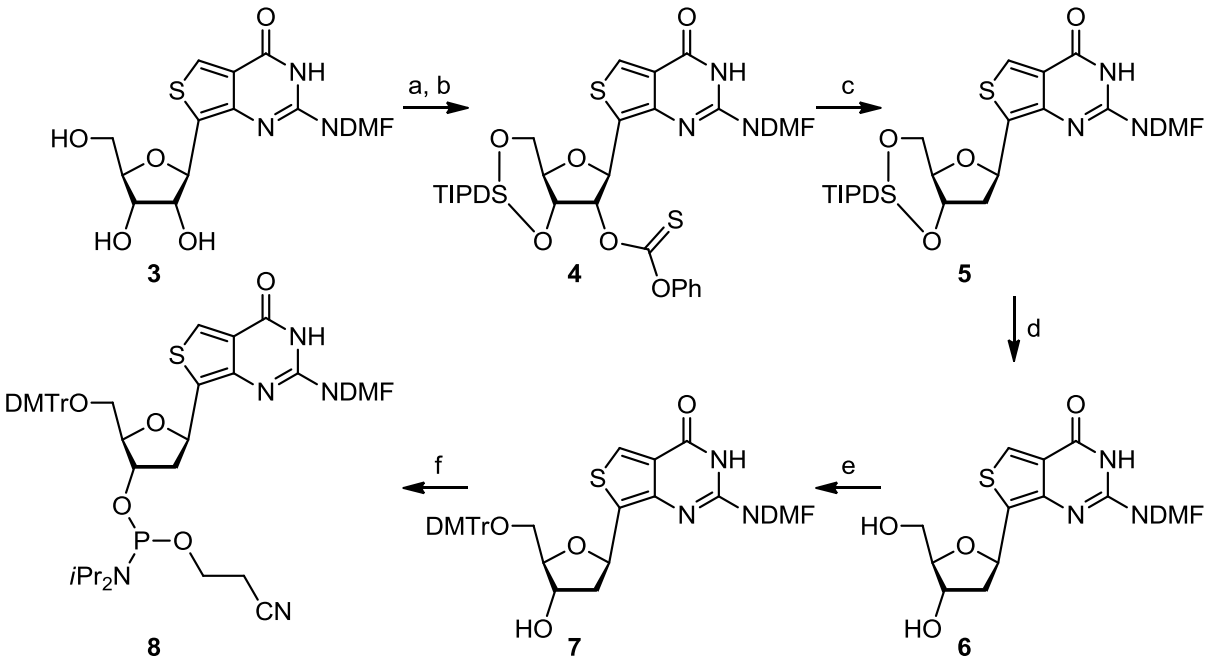

Figure S1. dthG-phosphoramidite synthesis[a]

[a] Reagents and conditions: (a) $TIPDSiCl_2$, Py, 89%; (b) PhOC(S)Cl, DMAP, Py, 78%; (c) Bu_3SnH, AIBN, toluene, 110 °C, 75%; (d) TEA•3HF, THF, 0 °C ~ RT, 94%; (e) DMTrCl, Py, 50 %; (f) 2-cyanoethyl *N,N*-diisopropylchlorophophoramidite, *i*Pr_2NEt, DCM, 0 °C ~ RT, 56 %.

Synthesis of *O*$^{3',5'}$-TIPDS-*O*$^{2'}$-PTC-*N*^{2}-DMF-thG (4)

3 (0.22, 0.62 mmol), as prepared previously reported,[1] was co-evaporated with dry pyridine (2 × 3 mL) and dissolved in dry Py (4 mL). $TIPDSCl_2$ (0.20 mL, 0.62 mL) was added to the solution dropwise at 0 °C and stirred at RT for 16 hours. All volatiles were evaporated and the residue was partitioned between DCM (50 mL) and

saturated aq. $NaHCO_3$ (50 mL). The aq. layer was extracted with DCM (2 × 10 mL) and combined organic layer was dried over Na_2SO_4 then evaporated. The residue was purified by column chromatography with 0~10 % gradient MeOH in DCM to afford an off-white solid. Yield 0.32 g, 89 %. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (s, 1H), 8.15 (s, 1H), 8.12 (s, 1H), 5.60 (d, *J* = 3.3 Hz, 1H), 4.48 (t, *J* = 6.2 Hz, 1H), 4.27 – 4.20 (m, 1H), 4.12 – 3.96 (m, 3H), 3.17 (s, 3H), 3.07 (s, 3H), 1.14 – 0.98 (m, 28H); ESI-MS calculated for $C_{26}H_{45}N_4O_6SSi_2$ $[M+H]^+$597.26, found 597.19; ESI-HRMS calculated for $C_{26}H_{45}N_4O_6SSi_2$ $[M+H]^+$597.2593, found 597.2591.

The intermediate (0.22 g, 0.37 mmol) was co-evaporated with dry Py (2 × 4 mL) and dissolved in Py/ACN (2 mL/6 mL). DMAP (93 mg, 0.76 mmol) and O-phenyl chlorothionoformate (PTCCl, 76 µL, 0.56 mmol) were successively added to the solution and stirred for 16 hour at RT. All volatiles were evaporated, the residue was dissolved in DCM (20 mL), washed with saturated aq. $NaHCO_3$ (20 mL), dried over Na_2SO_4 then evaporated. The residue was purified by column chromatography with 0~1.5 % gradient MeOH in DCM to afford a yellow solid. Yield 0.21 g, 78 %. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.78 (s, 1H), 8.60 (s, 1H), 8.11 (s, 1H), 7.46 – 7.37 (m, 2H), 7.33 – 7.27 (m, 1H), 7.15 – 7.08 (m, 2H), 6.48 (d, *J* = 4.4 Hz, 1H), 4.58 (dd, *J* = 8.8, 4.7 Hz, 1H), 4.20 – 3.94 (m, 4H), 3.08 (s, 3H), 3.05 (s, 3H), 1.10 – 1.05 (m, 14H), 1.05 – 0.99 (m, 14H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 194.29, 158.45, 153.46, 129.60, 129.49, 127.84, 126.72, 125.67, 125.52, 121.89, 121.22, 86.95, 81.44, 78.10, 70.90, 61.00, 41.38, 35.19, 29.84, 17.61, 17.49, 17.45, 17.36, 17.34, 17.28, 17.25, 17.17, 13.53, 13.19, 12.99, 12.91; ESI-MS calculated for $C_{33}H_{49}N_4O_7S_2Si_2$ $[M+H]^+$733.26, found 733.22; ESI-HRMS calculated for $C_{33}H_{49}N_4O_7S_2Si_2$ $[M+H]^+$733.2576, found 733.2573

Synthesis of $O^{3',5'}$-TIPDS-2'-deoxy-N^2-DMF-thG (5)

A solution of **4** (0.20 g, 0.27 mmol), Bu_3SnH (0.22 mL, 0.81 mmol) and AIBN (22 mg, 0.14 mmol) in freshly distilled toluene over sodium (5 mL) was degased with argon bubbling for 20 min at RT, and then the mixture was heated at 110 °C for 2 hours. Solvent was evaporated and residue was dissolved in DCM (20 mL), washed with saturated aq. $NaHCO_3$ (20 mL), dried over Na_2SO_4 then evaporated. The residue was purified by column chromatography with 0~2 % gradient MeOH in DCM to afford a yellow foam. Yield 0.12 g, 75 %. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.70 (s, 1H), 8.50 (s, 1H), 8.10 (s, 1H), 5.87 (s, 1H), 4.64 – 4.53 (m, 1H), 4.13 – 4.05 (m, 1H), 3.92 – 3.82 (m, 3H), 3.19 (s, 3H), 3.08 (s, 3H), 2.49 (s, 1H), 2.43 – 2.23 (m, 1H), 1.69 – 1.59 (m, 1H), 1.18 – 0.98 (m, 28H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.41, 157.98, 153.97, 131.34, 129.50, 125.45, 121.25, 86.09, 76.59, 73.61, 72.11, 63.66, 43.00, 41.55, 17.71, 17.67, 17.61, 17.57, 17.44, 17.28, 17.21, 17.13, 13.59, 13.48, 13.09, 12.69; ESI-HRMS calculated for $C_{26}H_{45}N_4O_5SSi_2$ $[M+H]^+$581.2644, found 581.2645.

Synthesis of 2'-deoxy-N^2-DMF-thG (6)

To a solution of **5** (0.11 g, 0.19 mmol) in dry THF (2 mL) was added TEA•3HF (0.14 mL, 0.86 mmol) dropwise at 0 °C then was stirred for 7 hours at RT. All volatiles were evaporated and the residue was purified by column chromatography with 0~10 % gradient MeOH in DCM to afford an off-white foam. Yield 60 mg, 94 %. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.82 (s, 1H), 8.48 (s, 1H), 8.05 (s, 1H), 5.56 (dd, *J* = 11.1, 5.4 Hz, 1H), 4.85 (s, 1H), 4.59 (d, *J* = 5.1 Hz, 1H), 4.13 (d, *J* = 1.0 Hz, 1H), 3.86 (dd, *J* = 12.0, 2.7 Hz, 1H), 3.71 (d, *J* = 11.7 Hz, 1H), 3.14 (s, 3H), 3.03 (s, 3H), 2.70 (td, *J* = 13.1, 5.2 Hz, 1H), 2.57 (s, 1H), 2.21 (dd, *J* = 13.2, 5.5 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.52, 158.16, 154.63, 146.20, 127.78, 126.30, 124.50, 88.31, 75.57, 75.27, 63.80, 43.52, 41.30, 35.04; ESI-MS calculated for $C_{14}H_{19}N_4O_4S$ $[M+H]^+$339.11,

found 399.19; ESI-HRMS calculated for $C_{14}H_{19}N_4O_4S$ $[M+H]^+$339.1122, found 339.1125.

Synthesis of *O*$^{5'}$-DMT-2'-deoxy-*N*^{2}-DMF-thG (7)

6 (59 mg, 0.17 mmol) was coevaporated with dry Py (2 × 2 mL) and was dissolved in dry Py (1 mL). DMTrCl (69 mg, 0.20 mmol) was added to the solution at RT, and was stirred for 16 hours. The reaction was quenched by addition of MeOH (1 mL) and evaporated. The residue was purified by column chromatography with 0~2 % gradient MeOH in DCM with 1 % Py to afford an off-white foam. Yield 54 mg, 50 %. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.84 (s, 1H), 8.57 (s, 1H), 8.03 (s, 1H), 7.45 – 7.37 (m, 2H), 7.37 – 7.24 (m, 4H), 7.24 – 7.04 (m, 3H), 6.73 (d, *J* = 8.4 Hz, 4H), 5.91 (dd, *J* = 9.9, 5.2 Hz, 1H), 4.42 (d, *J* = 5.4 Hz, 1H), 4.01 (dd, *J* = 6.6, 4.5 Hz, 1H), 3.69 (s, 3H), 3.69 (s, 3H), 3.20 (qd, *J* = 9.9, 4.7 Hz, 2H), 3.02 (s, 3H), 2.93 (s, 3H), 2.52 (s, 1H), 2.33 (dd, *J* = 13.1, 5.2 Hz, 1H), 2.18 (ddd, *J* = 13.1, 10.5, 5.8 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.82, 158.49, 158.48, 157.92, 154.00, 146.49, 144.93, 136.14, 136.06, 130.85, 130.20, 130.18, 129.10, 128.55, 128.29, 127.92, 126.85, 125.33, 125.23, 113.19, 86.35, 86.24, 74.81, 73.14, 64.48, 55.32, 43.97, 41.35, 35.12; ESI-MS calculated for $C_{35}H_{37}N_4O_6S$ $[M+H]^+$641.24, found 640.86; ESI-HRMS calculated for $C_{35}H_{37}N_4O_6S$ $[M+H]^+$641.2428, found 641.2426

Synthesis of *O*$^{3'}$-(2-Cyanoethyldiisopropylphosphoramidite)-*O*$^{5'}$-DMT-2'-deoxy-*N*^{2}-DMF-thG (8)

7 (49 mg, 76 µmol) was coevaporated with dry Py (2 × 1 mL) and dried under high vacuum overnight then dissolved in dry DCM (1 mL). DIPEA (53 µL, 0.30 mmol) and 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (25 µL, 0.11 mmol) were

successively added to the solution at 0 °C and the mixture was stirred at RT for 2 hours. All volatiles were evaporated and the residue was purified by column chromatography with 0~2 % gradient MeOH in DCM with 1 % Py to afford a white foam. Yield 36 mg, 56 %. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.70 (s, 1H), 8.69 (s, 2H), 8.17 – 8.15 (m, 2H), 8.11 (s, 1H), 8.11 (s, 1H), 7.52 – 7.45 (m, 4H), 7.41 – 7.33 (m, 8H), 7.32 – 7.23 (m, 4H), 7.23 – 7.16 (m, 2H), 6.85 – 6.75 (m, 8H), 5.99 – 5.91 (m, 2H), 4.64 – 4.53 (m, 2H), 4.24 – 4.17 (m, 2H), 4.17 – 4.09 (m, 1H), 3.91 – 3.82 (m, 1H), 3.82 – 3.76 (m, 18H), 3.76 – 3.67 (m, 2H), 3.67 – 3.43 (m, 5H), 3.40 – 3.30 (m, 2H), 3.27 – 3.12 (m, 7H), 3.08 (s, 6H), 2.81 – 2.72 (m, 2H), 2.62 (t, *J* = 6.5 Hz, 2H), 2.56 (dd, *J* = 13.3, 5.1 Hz, 1H), 2.46 (dd, *J* = 12.1, 5.7 Hz, 3H), 2.37 – 2.20 (m, 2H), 1.30 – 1.23 (m, 9H), 1.19 – 1.14 (m, 9H), 1.05 (d, *J* = 6.8 Hz, 6H); ^{31}P NMR (202 MHz, $CDCl_3$) δ 148.58, 148.29; ESI-MS calculated for $C_{44}H_{54}N_6O_7PS$ $[M+H]^+$841.35, found 840.86; ESI-HRMS calculated for $C_{44}H_{54}N_6O_7PS$ $[M+H]^+$841.3507, found 841.3503.

1.2. Synthesis of non-labeled and dthG/d2Ap-labeled ODNs

Non-modified (–)PBS, (+)PBS, T12(+)PBS and d2Ap7(–)PBS were synthesized and purified by IBA GmbH Nucleic Acids Product Supply (Germany). For dthG7(–)PBS, solid-phase ODN synthesis was performed on an Expedite 8909 synthesizer using commercially available reagents and phosphoramidites (Glen Research). The modified phosphoramidite was chemically synthesized as described above and incorporated into ODN with coupling efficiency comparable to the commercially available phosphoramidites. The solution of the modified phosphoramidite was dried for 16 hours over molecular sieve 3A (dried for 2 days at 300 °C under high vacuum) and was filtered using syringe filter right before use. ODNs were synthesized (with trityl-off) on a 500 Å CPG solid support column (1 μmol scale). Cleavage from the

solid support and deprotection were accomplished with AMA (ammonium hydroxide/methylamine in water = 1/1) at 65 °C for 30 min. The oligonucleotides were purified by 20 % preparative polyacrylamide gel electrophoresis (PAGE) using the crush and soak method; the desired band was cut out, pulverized, extracted with 50 mM TEAA (pH 7.0) for 12 hours (while shaking) and decanted. The buffer containing the purified ODN was lyophilized and the residue was taken up in 0.2 M TEAB (pH 7.0) buffer and desalted on a Sep-pak C18 (Waters). The ODNs were eluted with 40 % acetonitrile in water. The $d^{th}G$ containing (–)PBS DNA was > 98 % pure as determined by analytical high resolution PAGE. The purified ODN was quantified by UV absorbance at 260 nm at 70 °C with the following extinction coefficients ($M^{-1}cm^{-1}$): dCMP, 7050; dTMP, 8840; dGMP, 12010; dAMP, 15200; and $d^{th}G$, 5500, and confirmed by MALDI-TOF mass spectrometry: calculated M 5483.52, found 5482.70 $[M-H]^+$.

2. UV/visible absorption and steady-state fluorescence measurements. Spectroscopic grade solvents were used for absorption and fluorescence spectroscopy. To determine the concentrations, extinction coefficients of 4150 $M^{-1}cm^{-1}$ at 321 nm and 157280 $M^{-1}cm^{-1}$ at 260 nm for $d^{th}G$ and $d^{th}G7$(–)PBS, respectively and 6800 $M^{-1}cm^{-1}$ at 303 nm and 143550 $M^{-1}cm^{-1}$ at 260 nm for d2Ap and d2Ap7(–)PBS, respectively, were used. All experiments were performed in 25 mM TRIS-HCl (pH = 7.5), 30 mM NaCl, 0.2 mM $MgCl_2$ at 20 °C.

Absorption spectra were recorded on a Cary 4000 UV-visible spectrophotometer (Varian). Fluorescence spectra were recorded on a FluoroMax 4 spectrofluorimeter (JobinYvon) equipped with a thermostated cell compartment at 20 ± 0.5 °C. Fluorescence spectra were corrected for Raman scattering, lamp fluctuations and instrumental wavelength-dependent bias. QY of $d^{th}G$- and d2Ap-labeled ODNs were

calculated using quinine sulfate in 0.5 M sulfuric acid (QY = 0.546),[2] and free 2Ap deoxyriboside (QY = 0.68)[3] as references, respectively. Excitation wavelength was 380 nm for $d^{th}G$ and 315 nm for d2Ap. Melting temperatures were determined by measuring absorbance changes at 260 nm as a function of the temperature using a Varian Cary 400 spectrophotometer equipped with a Peltier temperature controller. Absorbance was recorded in the forward and backward directions from 20 to 80 °C at a rate of 0.5 °C/min. Prior to the melting experiment, (+)PBS and (–)PBS samples were denatured at 90 °C for 3 min and then slowly cooled down to allow their annealing. For melting experiments, the complementary ODNs were at 1 µM in 25 mM TRIS (pH = 7.5), 30 mM NaCl and 0.2 mM $MgCl_2$. Melting temperatures were determined from the first derivative of thermal denaturation curves.

3. Time-resolved fluorescence intensity decays

Time-resolved fluorescence measurements were performed with the time-correlated single-photon counting technique. Excitation pulses at 315 nm with a repetition rate of 4 MHz were generated by a pulse-picked frequency-tripled Ti-sapphire laser (Tsunami, Spectra Physics) pumped by a Millenia X laser (Spectra Physics).[4] The fluorescence emission was collected at 500 nm through a polarizer set at magic angle and a 16 mm band-pass monochromator (Jobin Yvon). The single-photon events were detected with a micro-channel plate photomultiplier (Hamamatsu) coupled to a pulse pre-amplifier HFAC (Becker-Hickl GmbH) and recorded on a time correlated single photon counting board SPC-130 (Becker-Hickl GmbH). The instrumental response function (IRF) recorded with a polished aluminum reflector was characterized by a ≈ 50 ps full-width at half-maximum. The mean lifetime $<\tau>$ was calculated from the individual fluorescence lifetimes (τ_i) and their relative

amplitudes (α_i) according to $<\tau> = \sum \alpha_i \times \tau_i$. The population of dark species (α_0) was calculated by:

$\alpha_0 = 1 - \tau_{free}/(\tau_{ODN} \times R_m)$ (1),

where τ_{free} is the lifetime of the free nucleoside, τ_{ODN} is the measured mean lifetime of the probe within the ODN and R_m is the ratio of their corresponding QYs. The amplitudes of the fluorescent populations α_{ic} were recalculated according to $\alpha_{ic} = \alpha_i \times (1 - \alpha_0)$. Time-resolved intensity data were fitted using the maximum entropy method (Pulse 5 software).[5] In all cases, the χ^2 values were close to 1, indicating an optimal fit.

4. Time-resolved fluorescence anisotropy decays

Time-resolved fluorescence anisotropy was obtained from the fluorescence decay curves recorded in directions parallel $I_\parallel$ and perpendicular $I_\perp$ alternatively, to the excitation beam polarization and was analyzed by:

$r(t) = \frac{I_\parallel(t) - G \times I_\perp(t)}{I_\parallel(t) + 2G \times I_\perp(t)} = r_0 \sum_i \beta_i \times \exp\left(-\frac{t}{\theta_i}\right)$ (2),

where β_i are the amplitudes of the rotational correlation times θ_i, r_0 is the initial anisotropy, and G is the geometry factor at the emission wavelength, determined in an independent experiment. Time-resolved anisotropy data were fitted using the maximum entropy method (Pulse 5 software) or according to a non-linear least-square analysis using an iterative reconvolution method (software provided by G. Krishnamoorthy). The r_0 values were found to be 0.32-0.33 for the 2Ap-containing sequences, while those (r_0) for the d^{th}G-containing sequences were 0.23-0.25. In all cases, the χ^2 values were close to 1, indicating an optimal fit.

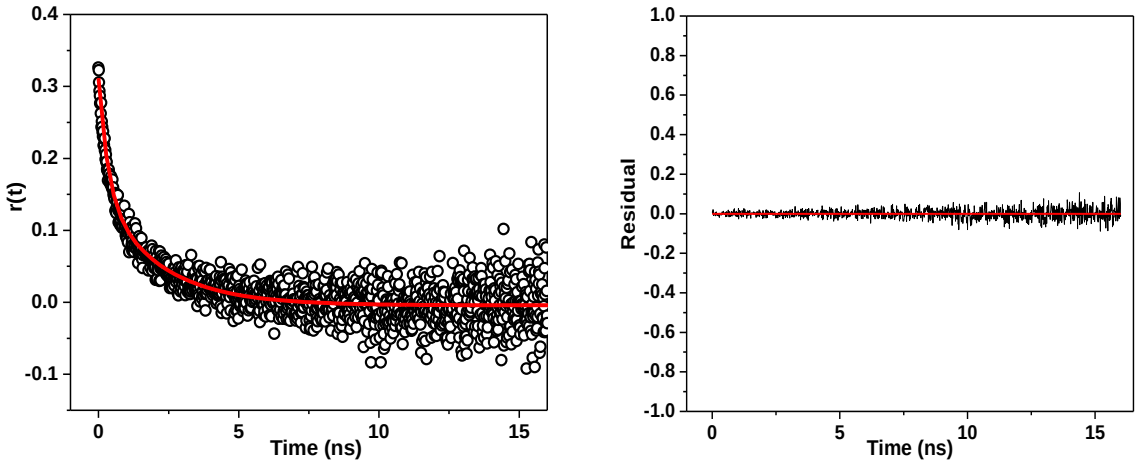

Figure S2. Anisotropy decay and corresponding residual plot for d2Ap7(–)PBS.The continuous line in the left panel corresponds to the fit of the data with the parameters in Table 2. Excitation wavelength was at 315 nm.

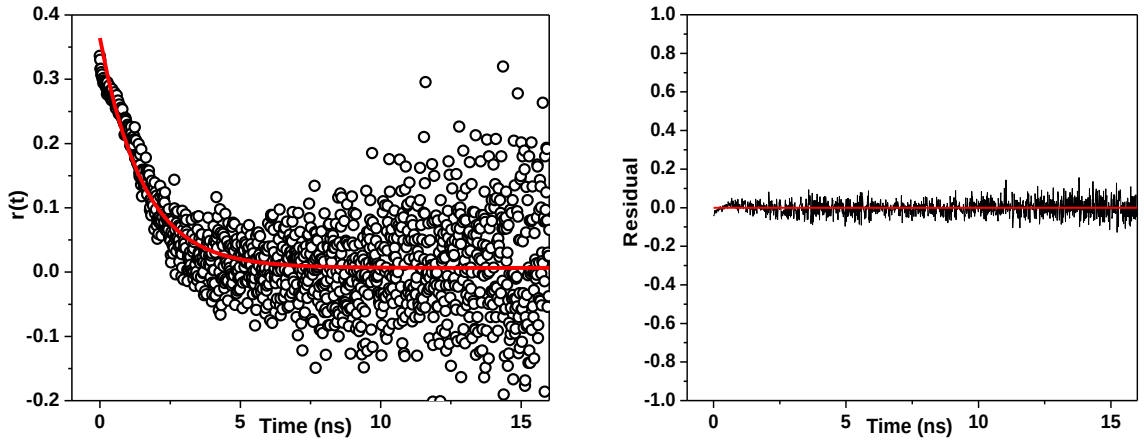

Figure S3. Anisotropy decay and corresponding residual plot for d2Ap7(–)/T12(+)PBS.The continuous line in the left panel corresponds to the fit of the data with the parameters in Table 2. Excitation wavelength was at 315 nm.

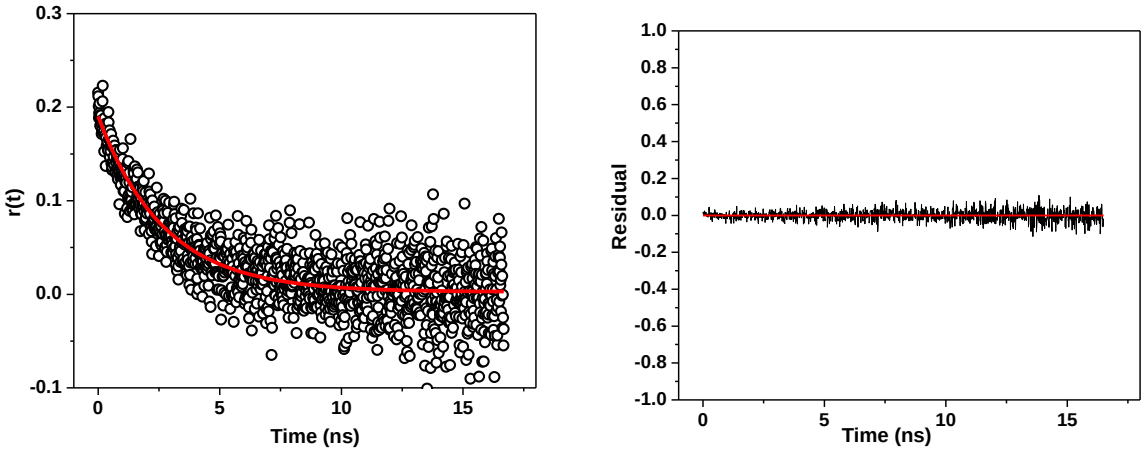

Figure S4. Anisotropy decay and corresponding residual plot for d^{th}G7(–)PBS.The continuous line in the left panel corresponds to the fit of the data with the parameters in Table 2. Excitation wavelength was at 315 nm.

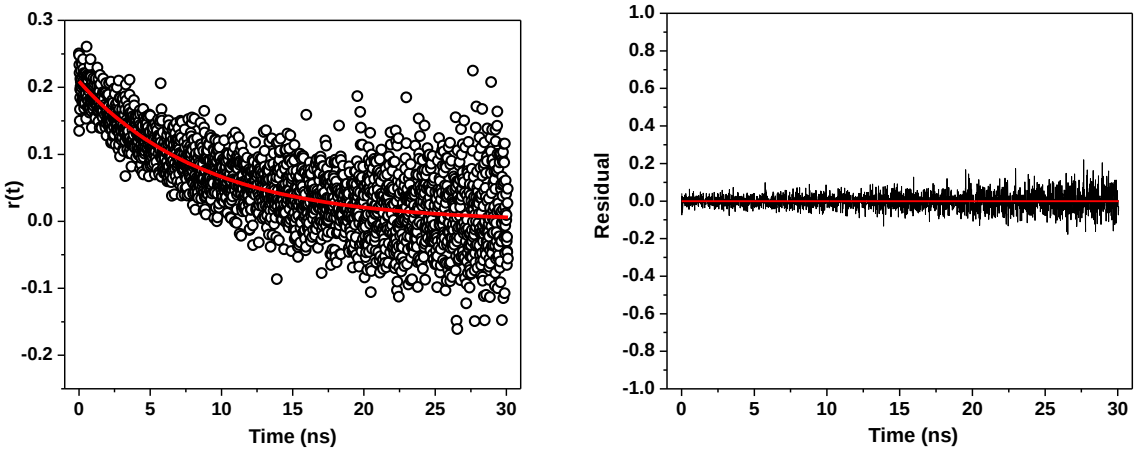

Figure S5. Anisotropy decay and corresponding residual plot for d^{th}G7(–)/(+)PBS.The continuous line in the left panel corresponds to the fit of the data with the parameters in Table 2. Excitation wavelength was at 315 nm.

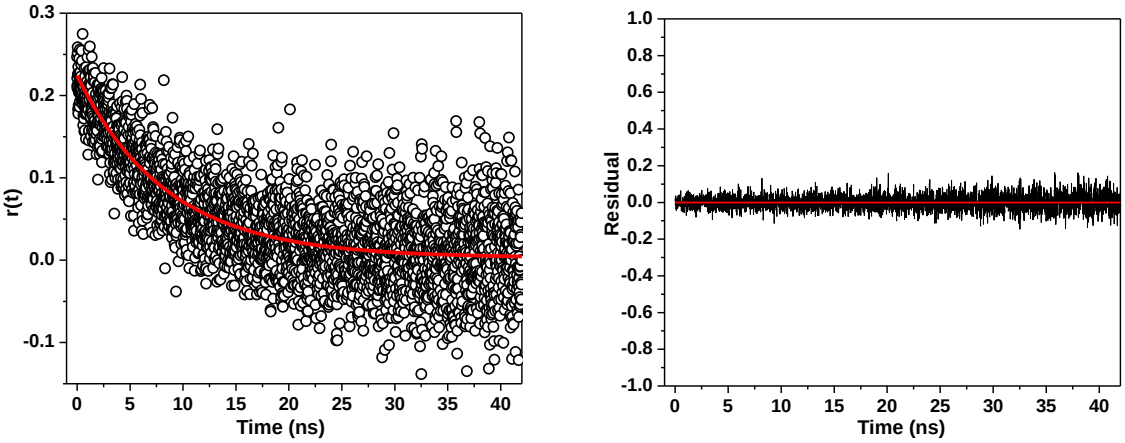

Figure S6. Anisotropy decay and corresponding residual plot for d^{th}G7(–)/T12(+)PBS.The continuous line in the left panel corresponds to the fit of the data with the parameters in Table 2. Excitation wavelength was at 315 nm.

5. Quenching measurements

Fluorescence quenching by potassium iodide (KI) was carried out by adding aliquots of a concentrated aqueous stock of KI to the labeled ODNs. $Na_2S_2O_3$ was added to the KI stock solution to prevent its oxidation. Fluorescence intensity was corrected for dilution. The change in fluorescence intensity as a function of quencher concentration was fitted by the Stern-Volmer equation:

$$\frac{F_0}{F} = 1 + k_q \tau_0 [Q] \quad (3),$$

where F and F_0 are the intensities in the presence and absence of quencher, respectively, k_q is the diffusion-controlled quenching rate, and τ_0 is the lifetime in the absence of the quencher.

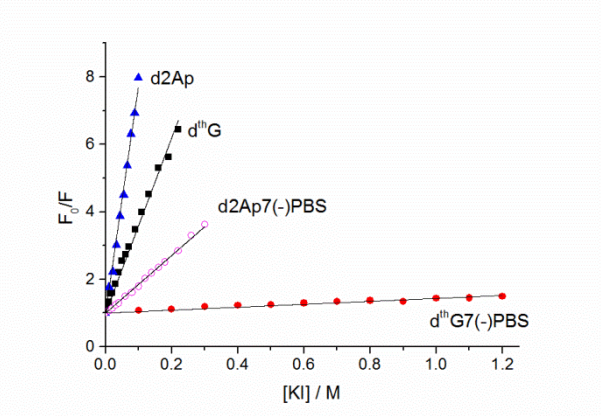

Figure S7. Stern-Volmer plots for KI quenching of dthG (black squares), dthG7(–)PBS (red disks), d2Ap (blue triangles), and d2Ap7(–)PBS (magenta circles). Black lines represent their linear fits.

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