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for

Novel DNA Catalysts Based on G-Quadruplex Recognition

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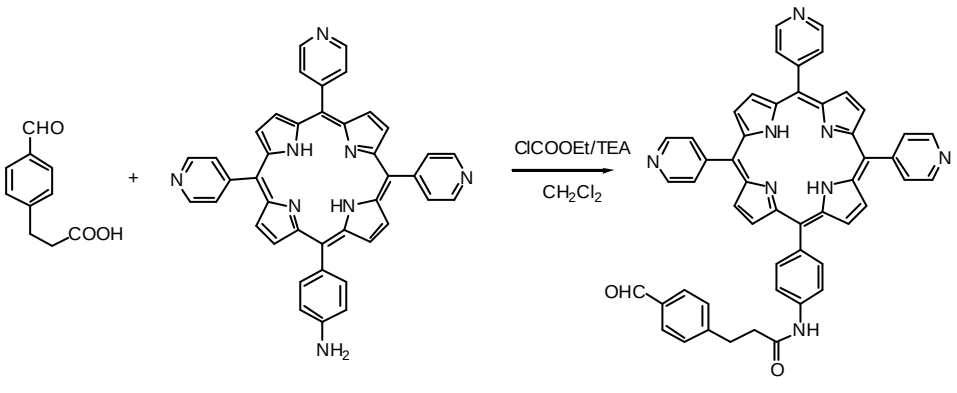
General: 3-(4-Formylphenyl)propanoic acid was purchased from Milestone Pham-Tech. Electrospray ionization ion trap (ESI-IT) mass spectra were recorded by using a Bruker Daltonics esquire 3000+ instrument in positive or negative mode. DNA oligonucleotide synthesis was carried out by using an Applied-Biosystems 392 DNA/RNA synthesizer using standard phosphoramidite protocols and purified by reverse phase HPLC with a TEAA/CH_3CN gradient. Reverse- phase HPLC was performed by using a Prominence HPLC (Shimadzu) system.

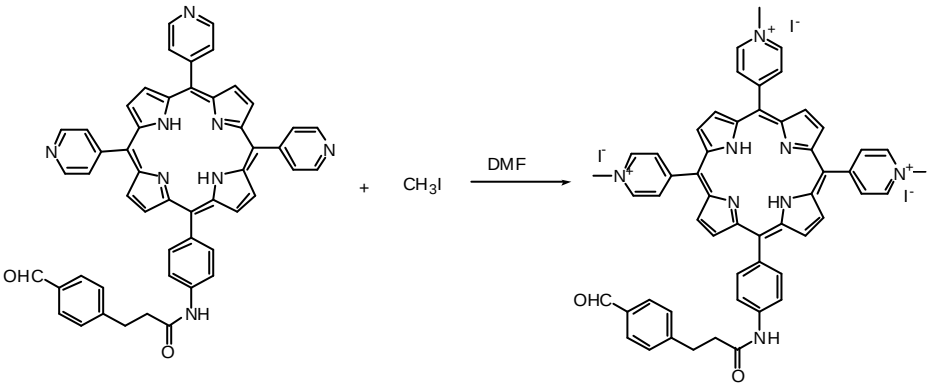
Synthesis of aldehyde 1a



1a

3-(4-formylphenyl)propanoic acid (0.16 mmol, 28 mg) was dissolved in dry THF (3 mL) at 0°C followed by the addition of triethylamine (0.2 mmol, 28 µL) and ethyl chloroformate (0.16 mmol, 15.3 µL). The reaction mixture was stirred for 30 min under N_2 atmosphere and evaporated to dryness under reduced pressure. The resulting solid was re-dissolved in dry DMF followed by the addition of triethylamine (0.2 mmol, 28 µL) and tris(4-pyridyl) -(4-aminophenyl) porphyrin[1] (0.08 mmol, 50 mg). After being stirred for 4 h at 50°C under N_2 atmosphere, the reaction mixture was evaporated under reduced pressure, then purified by preparative TLC (CH_2Cl_2/EtOH 5:1). Yield: 52%; ^{1}HNMR (250 MHz, $CDCl_3$) δ (ppm) -2.90 (s, 2H), 2.91 (t, *J*= 7.4Hz, 2H), 3.29 (t, *J*= 7.4Hz, 2H), 7.52 (d, *J*= 8.0Hz, 2H), 7.86 (d, *J*= 8.0Hz, 2H), 7.92 (d, *J*= 8.4Hz, 2H), 8.11-8.16(m, 8H), 8.79 (d, *J*= 4.9Hz, 2H), 8.83 (s, 4H), 8.93 (d, *J*= 4.9Hz, 2H), 9.01(bs, 6H), 9.99 (s, 1H); ESI- MS mass calculated for $(C_{51}H_{36}N_8O_2 + H)^+$ m/z 793.3, found m/z 793.2; UV/visible (DMF), ?/nm (e/10^3 $M^{-1}cm^{-1}$): 418 (298), 513 (16.3), 549 (6.6), 589 (2.06), 644 (2.94).

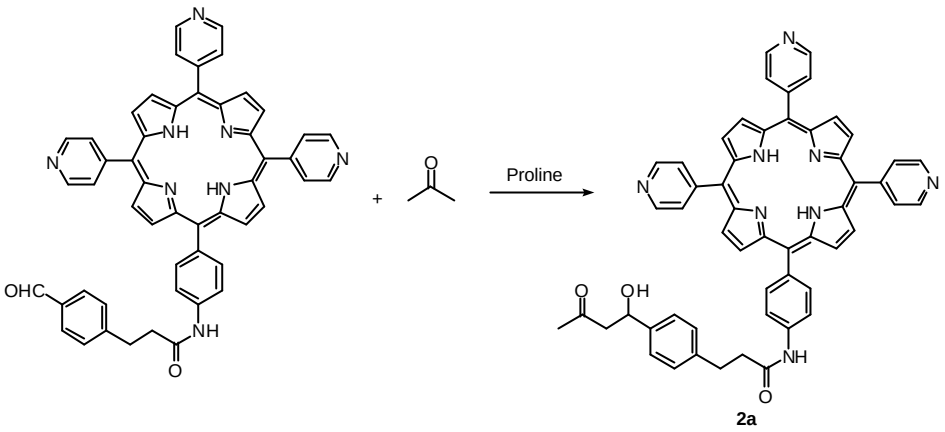
Synthesis of aldehyde 1



1a (10 mg) was dissolved in DMF (2 mL) and then methyl iodide (0.5 mL) was added. Then the solution was stirred at room temperature overnight. The reaction mixture was put into diethyl ether (10 mL) and kept at 4°C for 1 h. The methylated porphyrin **1** was obtained after filtering the solvent, washing the precipitate with diethyl ether and then drying the solid under vacuum. Yield: 90%; ^{1}H NMR (250 MHz, $CDCl_3$) δ (ppm) -2.88 (bs, 2H), 2.90 (t, *J*= 7.2Hz, 2H), 3.15 (t, *J*= 7.2Hz, 2H), 4.71 (s, 9H), 7.60 (d, *J*= 8.0Hz, 2H), 7.91 (d, *J*= 8.0Hz, 2H), 8.09 (d, *J*= 8.8Hz, 2H), 8.16 (d, *J*= 8.8Hz, 2H), 8.99-9.05(m, 10H), 9.17 (s, 4H), 9.46 (d, *J*= 6.6Hz, 6H), 10.00(s, 1H), 10.50 (s, 1H); ESI- MS mass calculated for $(C_{54}H_{45}N_8O_2)^{3+}$ [m-3I/3] 279.1, found *m/z* 279.3. UV/Vis

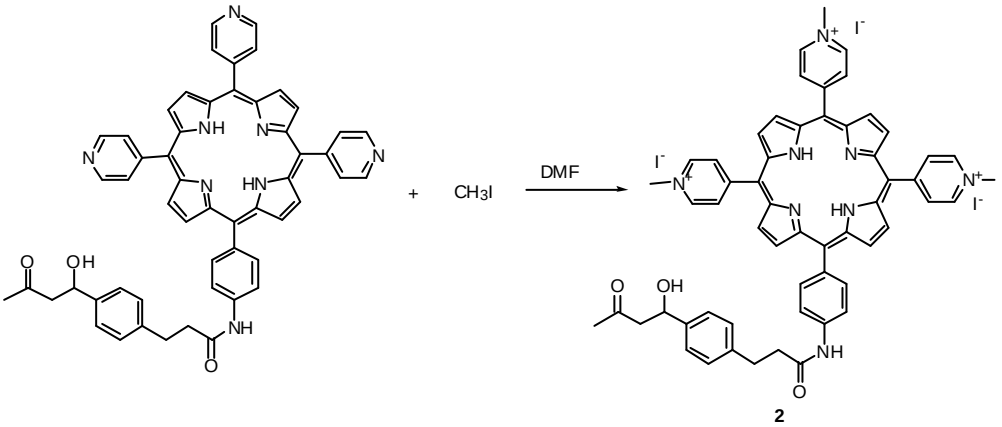
(DMSO), l/nm (e/10^3 M^{-1}cm^{-1}): 428 (254), 518 (17.8), 555 (7.9), 591 (6.6), 645 (2.9).

Synthesis of aldehyde 2a



To a mixture of anhydrous DMSO (500 µL) and acetone (100 µL) was added the corresponding aldehyde **1a** (0.02 mmol) followed by L-proline (20-30 mol %) and the re-sulting mixture was stirred at room temperature overnight. The reaction mixture was evaporated under reduced pressure, and then purified by preparative TLC (CH_2Cl_2/EtOH 5:1). Yield: 23%; ^{1}HNMR (250 MHz, $CDCl_3$) δ (ppm) -2.92 (s, 2H), 2.20 (s, 3H), 2.91-2.81 (m, 4H), 3.19 (t, J=7.4 Hz, 2H), 5.17(dd, J=4.0 Hz, J=8.2 Hz, 1H), 7.35 (d, J=8.6 Hz, 1H), 7.37 (d, J=8.6 Hz, 1H), 7.50 (s, 2H), 7.87 (d, J=8.9 Hz, 2H), 8.11-8.15 (m, 8H), 8.79 (d, J=4.9 Hz, 2H), 8.83 (s, 4H), 8.92 (d, J=4.9 Hz, 2H), 9.01(bs, 6H); ESI- MS mass calculated for $(C_{54}H_{43}N_8O_3 + H)^+$ m/z 851.3, found m/z 851.4; UV/Vis (DMF), l/nm (e/10^3 M^{-1}cm^{-1}): 418 (251), 514 (15.8), 549 (6.6), 590 (5.16), 644 (3.23).

Synthesis of aldehyde 2



The methylation protocol applied for the synthesis of **2** is the same as for **1**: Yield:

85%; ^{1}H NMR (250 MHz, $CDCl_3$) δ (ppm) -2.93 (bs, 2H), 2.13 (s, 3H), 2.72-2.88 (m, 4H), 3.09 (t, *J*=7.0 Hz, 2H), 4.71 (s, 9H), 4.99-5.04 (m, 1H), 7.33 (s, 4H), 8.10 (d, *J*= 8.6 Hz, 2H), 8.16 (d, *J*=8.6 Hz, 2H), 9.00 (d, *J*=6.9 Hz, 6H), 9.06 (br s, 4H), 9.16 (br s, 4H), 9.48 (d, *J*=6.4 Hz, 6H), 10.47 (s, 1H); ESI-MS mass calcd for $(C_{57}H_{51}N_8O_3)^{3+}$ [*M*-3I/3] 298.5, found *m/z* 298.4. UV/Vis (DMSO), λ/nm ($\varepsilon/10^3$ $M^{-1}cm^{-1}$): 428 (185), 518 (13.0), 556 (5.8), 589 (5.0), 645 (2.3).

Preparation of DNA catalyst:

Fmoc-proline NHS ester: *N*-hydroxysuccinimide (150 mg) was dissolved in anhydrous CH_2Cl_2 together with Fmoc-proline (1.0 equiv) and EDCI (2 equiv). The reaction was maintained at room temperature for 5 h. The crude reaction mixture was extracted with 2.5 % $NaHSO_4$ to remove the excess EDCI. The organic phase was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was dried and used directly for DNA conjugation.

Prolinamide-conjugated oligonucleotides: Amino-modified oligonucleotides were synthesized by using standard automated solid-phase phosphoramidite coupling methods with corresponding amino-modifier (Link Technologies). Up to 200 µg of a 5'-amino DNA oligonucleotide was combined with 6 mg/mL Fmoc-proline NHS ester (final reaction = 10% DMSO) in 200 mM sodium phosphate (pH 7.2) at room temperature for 12 h. The reaction mixture was evaporated and washed with diethylether, then purified by reverse-phase HPLC and lyophilized. The Fmoc group was cleaved with 33% NH_3/H_2O at 55 °C for 1 h and evaporated.[2] The final product was purified by reversed-phase HPLC and characterized by mass spectrometry.

Figure S1. HPLC (260 nm) analysis of **1-Gq** and **13-Gq**. Retention time in minutes.

Aldol reaction catalyzed by DNA catalyst: DNA-templated catalytic reactions were performed by mixing DNA catalysts (60 pmol) and aldehyde (60 pmol) in a volume of 30 µL of 100 mM KCl and phosphate buffer (pH 7.2) at 25^{0}C. To this solution, acetone (6 µL) was added. After the defined time, the reaction mixture was lyophilized and analyzed by denaturing HPLC.

Denaturing HPLC: Chromatography was run using a Waters Xbridge column (4.6 x 50 mm) eluted at 50^{0}C at 1 mL/min with a linear gradient that increased the proportion of solvent B in solvent A from 5% to 50% in 20 min, where A: 20 mM triethylammonium trifluoroacetic acetate (pH 2.7) adjusted with concentrated acid, and B: CH_3CN.

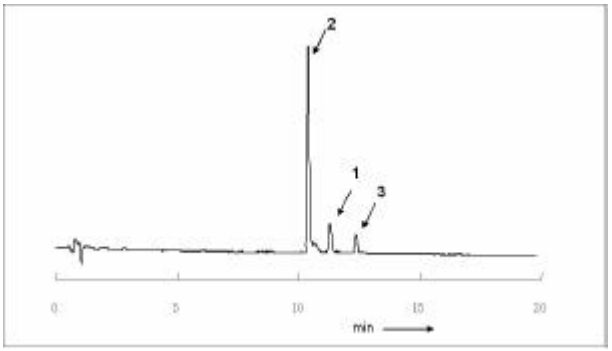


Figure S2. HPLC (420nm) analysis of the reaction between of **1** and acetone catalyzed by **13-Gq** in 100 mM KCl and phosphate buffer buffer (pH 7.2) at 25 °C. Retention time in minutes.

Determination of the initial rates: The reactions were initiated by the addition of acetone (36 µL) to a solution of DNA-tethered catalyst (300 pmol) and DNA-conjugated aldehyde **1** (300 pmol) in KCl (180 µL, 100 mM and phosphate buffer buffer (pH 7.2) at 25 °C. At several time intervals aliquots (36 µL) were removed, frozen and lyophilized. Then the samples were analyzed by denaturing HPLC. The values were obtained by fitting the data (time vs. product amount) obtained for the initial reaction until yields of 10 - 20% were reached.

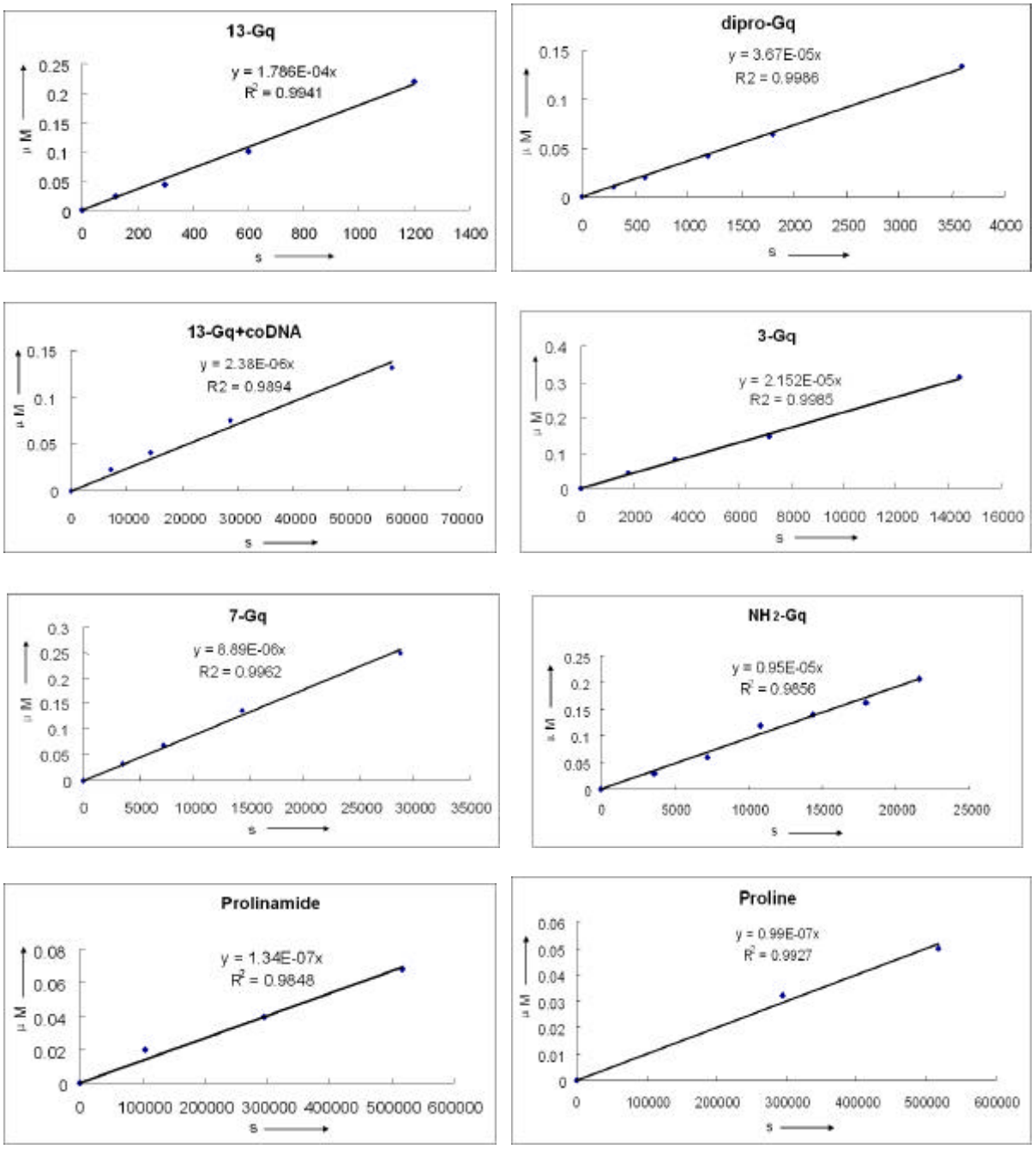
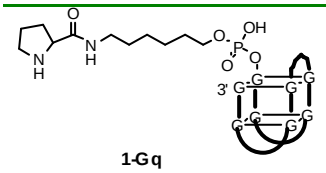
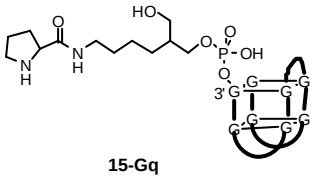
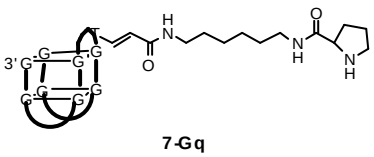


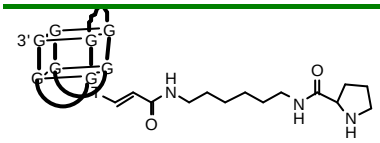
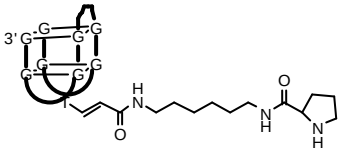
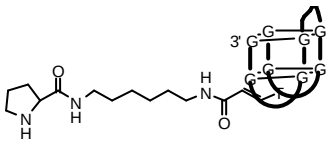
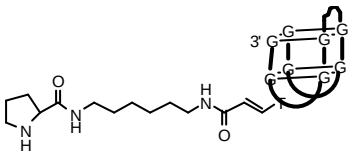
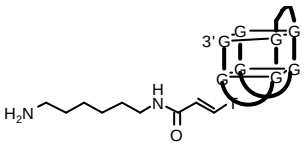
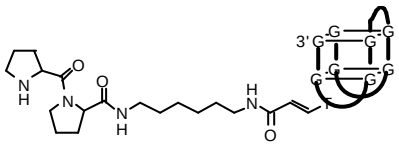
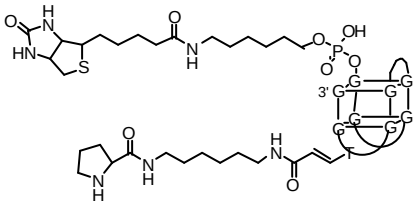
Figure S3. Initial product formation promoted by the indicated catalysts.

***Table S1**: Kinetic Data*

Catalyst	Initial rate (M/s)	Relative ratio
3-Gq-DNA	2.15×10^{-11}	217
7-Gq-DNA	8.89×10^{-12}	89
13-Gq-DNA	1.78×10^{-10}	1797
13-Gq-DNA+coDNA	2.38×10^{-12}	24
13-dipro-Gq-DNA	3.67×10^{-11}	370
13-NH_2-Gq-DNA	0.95×10^{-11}	96
Prolinamide	1.34×10^{-13}	1.4
Proline	0.99×10^{-13}	1

ESI mass spectrometry: The purified oligonucleotide templates (50–100 pmol) were dissolved in a solution of 2-propanol (20%) containing NEt_3 (1%). The mass measurements were carried out by using an Esquire 3000+ (Bruker) instrument and nitrogen was used as the nebulizing gas (12 psi) at 300 ^{0}C. The samples were injected into the system with the aid of a syringe pump (180 µLh^{-1}).

Oligonucleotide	Expected mass	Observed mass
 **1-Gq**	5002.3	5000.4
 **15-Gq**	5032.2	5031.9
 **7-Gq**	4977.3	4977.3

 **4-Gq**	4977.3	4974.8
 **12-Gq**	4977.3	4976.2
 **3-G**	4977.3	4976.8
 **13-Gq**	4977.3	4977.0
 **NH_2-Gq**	4880.2	4879.2
 **dipro-Gq**	5074.6	5073.2
	5382.4	5382.7

Circular dichroism (CD) spectroscopy: CD spectra were recorded on a Jasco-715 spectropolarimeter (Jasco) by using a quartz cell (1 mm optical path length) and an instrument scanning speed of 20 nm/min with a response time of 1 s, over a wavelength range of 220–450 nm. CD spectra were determined with 10 µM **Gq** with different amounts of phophyrin tethered aldehyde **1** in MilliQ water.

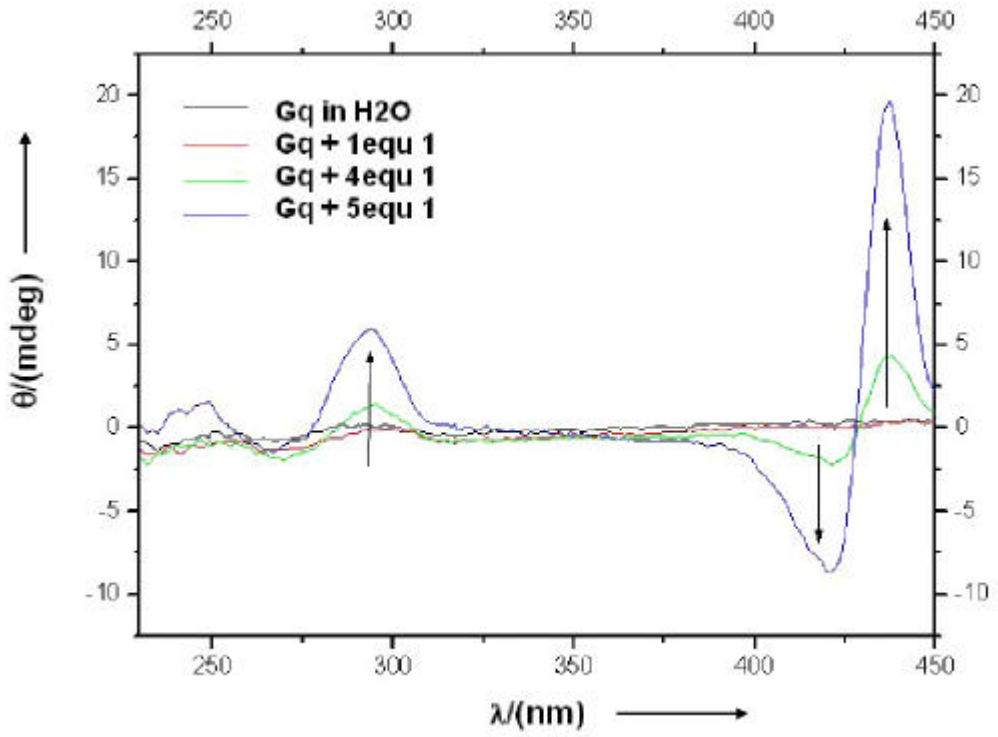


Figure S4. CD spectra of complexes of **Gq** DNA and varied amounts of **1**.

Surface Plasmon Resonance (SPR): All SPR experiments were carried out in KCl (100 mM) and phosphate buffer buffer (pH 7.2) using a streptavidin functionalized chip (Biacore Series S Sensor Chip SA) on a Biacore T100 system (Biacore Control and Evaluation Software Version 1.1). All experiments were carried out at 25^{o}C with ten data points taken per second. In a typical experiment, *biotin*-**13-Gq** was immobilized on the chip to a final immobilization level of about 200 RU on flow cell 2, leaving flow cell 1 as a blank to control for bulk refractive index changes and signal drift. Porphyrins were injected for a contact time of five minutes followed by a dissociation time of 5 min at a flow rate of 30 µL/min. Subsequently, the flow cells were regenerated by washing with "Regeneration Buffer" (50 mM NaOH, 1 M NaCl) three times for 30 s at a flow rate of 30 µL/min. Increasing concentrations of porphyrins were used as indicated below.

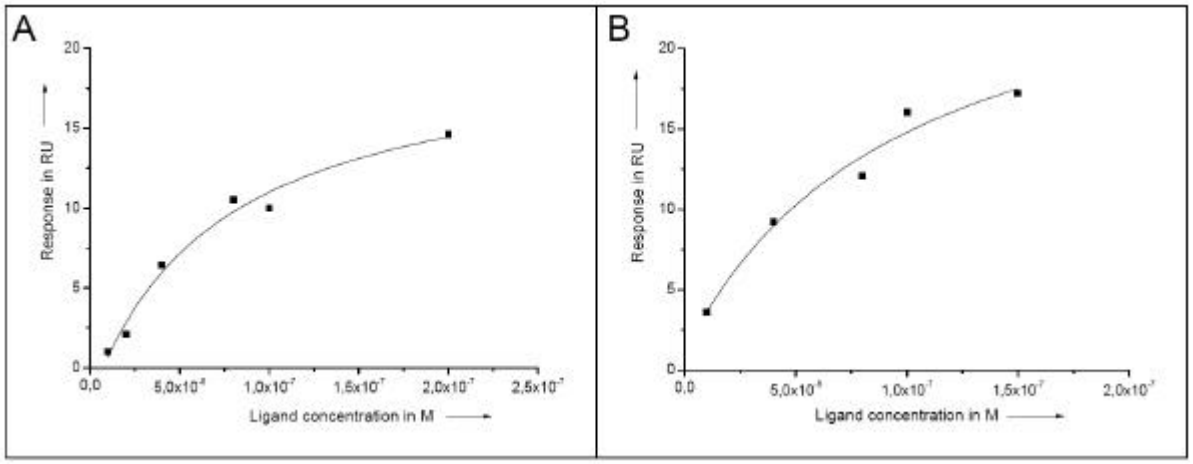


Figure S5. Surface plasmon resonance studies of binding of A) porphyrinic substrate (aldehyde) and B) aldol product to immobilized 13-Gq DNA.

Reference:

[1] H. Li, O. S. Fedorova , A. N. Grachev, W. R. Trumble, G. A. Bohach , L. Czuchajowski, *Biochim. Biophys. Acta,* **1354** ,1997, 252.

[2] Z. Tang, A. Marx, *Angew. Chem. Int. Ed.* **2007**, *49*, 7297.