CHEMISTRY
A European Journal

Supporting Information

Functional Hallmarks of a Catalytic DNA that Makes Lariat RNA

Fatemeh Javadi-Zarnaghi[a, c] and Claudia Höbartner*[a, b]

chem_201503238_sm_miscellaneous_information.pdf

Functional Hallmarks of a Catalytic DNA that makes Lariat RNA

Fatemeh Javadi-Zarnaghi[a,b] and Claudia Höbartner[a]

[a] Research Group Nucleic Acid Chemistry, Max Planck Institute for Biophysical Chemistry, and Institute for Organic and Biomolecular Chemistry, Georg-August-University Göttingen, Germany

[b] Current address: Department of Biology, Faculty of Sciences, University of Isfahan, Isfahan, Iran.

General Information

Standard 5'-*O*-DMT-protected 3'-β-cyanoethyl phosphoramidites of N^6-benzoyl-2'-deoxyadenosine, N^4-acetyl-2'-deoxycytidine, N^2-(*N*,*N*-dimethylamino)methylene-2'-deoxyguanosine, and thymidine were from SAFC/Sigma-Aldrich. 5'-*O*-DMT-2'-*O*-TOM-protected 3'-β-cyanoethyl phosphoramidites of N^6-acetyladenosine, N^4-acetylcytidine, N^2-acetylguanosine, and uridine were from ChemGenes. Benzylthiotetrazole (BTT) was from Carbosynth (UK). Ribonucleotide triphosphates (NTPs) for in vitro transcription reactions were from Jena Bioscience, and α-^{32}P-dATP (3000 Ci/mmol) and γ-^{32}P-ATP (3000 Ci/mmol) was from Perkin Elmer. T4 RNA ligase, T4 polynucleotide kinase (PNK), and DNase I were from Fermentas, and Klenow fragment was from Jena Bioscience. The TMPyP4 (CAS 36951-72-1) was purchased from Calbiochem.

Table S1. Oligonucleotide sequences used in this study

a. RNA substrates and products

R1	*Acceptor RNA*	5′-AAGUCUCAUGUACUA**A**CAUCGAUUG-3′
R1i	*Inactive acceptor RNA, DMS probing*	5′-GUCUCAUGUACUA**A**-3′
R2	*Donor RNA, kinetic analyses*	5′-pppGUAUGUGCUAGCGG-3′
R2i	*Inactive Donor RNA, CD, DMS probing*	5′-GUAUGUGCUAGCGG-3′
R2s	*Short donor RNA, CoMA, dNAIM*	5′- pppGUAUGUGCUGG-3′
R3	*YLB059W*	5′-pppGUAUGCAUAGGCAAUAACUUCGGCCUCAUACUCAAAGAACACGUUUACUA**A**CAUAACUUAUUUACAUAG-3′
R4	*Branched RNA product*	(2′,5′-GUAUGUGCUAGCGG-3′) 5′- *AAGUCUCAUGUACUA***A** (3′,5′-*CAUCGAUUG*-3′)

Branch-site nucleotides in **R1,R3** and **R4** are bold and underlined. In branched RNA product **R4**, the RNA sequence that belonged to the acceptor RNA **R1** is shown Italic. The RNA sequence that belonged to the donor RNA **R2** is undelined.

b. Deoxyribozymes and other DNAs

D1	*6BX22 orig. for **R2***	5′-CAACGCTAGCACAT**CAGGGGGAGCGAGCACTAATACAAGCGGGTAGGAGGCCC**TAGTACATGAGAC-3′
D2	*6BX22 orig. for **R2s***	5′-CAACAGCACAT**CAGGGGGAGCGAGCACTAATACAAGCGGGTAGGAGGCCC**TAGTACATGAGACTT-3′
D3	*6BX22-min-1*	5′- CAACGCTAGCACAT**CAGGGGGAGCGGCGGGTAGGAGGCCC**TAGTACATGAGAC-3′
D4	*6BX22-min*	**5′-** CAACGCTAGCACAT**CAGGGGGAGCTTGCGGGTAGGAGGCCC**TAGTACATGAGAC-3′
D5	*6BX22-min catalytic core*	5′-**CAGGGGGAGCTTGCGGGTAGGAGGCCC**-3′
D6	*G-quadruplex DNA, human telomer*	5′-TAGGGTTAGGGT-3′
D7	*G-quadruplex complement*	5′-ACCCTAACCCTA-3′
D8	*Template for **R2***	5′-CCGCTAGCACATACTATAGTGAGTCGTATTACAGCGTGCGT-3′
D9	*Template for **R2s***	5′-CCAGCACATACTATAGTGAGTCGTATTACAGCGTGCGT-3′
D10	*Template for **R3***	5′-CTATGTAAATAAGTTATGTTAGTAAACGTGTTCTTTGAGTATGAGGCCGAAGTTATTGCCTATGCATAC TATAGTGAGTCGTATTACAGCGTGCGT-3′
D11	*T7 promoter*	5′-CTGTAATACGACTCACTATA-3′
D12	*Template DNA for 3′-^{32}P-labeling of 6BX22*	5′-TAGGGTTAGGGT-3′
D13	*6BX22 ori. for **R3***	5′-GTTATTGCCTATGCAT**CAGGGGGAGCGAGCACTAATACAAGCGGGTAGGAGGCCC**TAGTAAACGTGTTCTTTG-3′
D14	*6BX22 min for **R3***	5′-GTTATTGCCTATGCAT**CAGGGGGAGCTTGCGGGTAGGAGGCCC**TAGTAAACGTGTTCTTTG-3′

For deoxyribozymes, the bold nucleotides represent the catalytic domain. The non-bold nucleotides constitute the binding arms for the RNA substrates. For transcription templates, the underlined nucleotides are complementary with the T7 promoter and are not transcribed. In template for **R3**, the blue and red nucleotides correspond to sequences recognized by 5' and 3' binding arm of the catalytic DNAs, **D13** and **D14**.

Unmodified DNA oligonucleotides were purchased from Sigma Aldrich and purified by denaturing PAGE (20% or 15% polyacrylamide). The RNA donor strands were prepared by *in vitro* transcription using T7 RNA

polymerase and synthetic DNA templates. The RNA acceptor substrates and CoMA pools were prepared by solid-phase synthesis, following our previously described procedures.[1]

Synthesis of branched RNA (R4) by 6BX22-catalyzed RNA ligation

Synthesis of branched RNA (**R4**) was performed on 1 nmol scale using acceptor RNA (**R1**), deoxyribozyme 6BX22-min (**D4**) and donor RNA (**R2**) in equimolar ratio, at final a concentration of 10 µM each. Annealing was performed in 70 µl annealing buffer (5 mM HEPES pH 7.5, 15 mM NaCl, 0.1 mM EDTA) at 95°C for 2 min followed by incubation at room temperature for at least 15 min. To the annealed complex, 20 µl of 5X reaction buffer (250 mM HEPES pH 7.5, 750 mM NaCl and 10 mM KCl) was added. The sample was incubated for 10 min at 37°C, and the ligation reaction was initiated by the addition of 10 µl 200 mM $MnCl_2$. After incubation at 37°C for 3h, the reaction was quenched by addition of 50 µl stop solution (80% formamide, 1×TBE [89 mM each Tris and boric acid, pH 8.3], and 50 mM EDTA containing 0.025% bromophenol blue and xylene cyanol). Samples were loaded on 10% denaturing PAGE and electrophoresed for 45 min with 35W to separate branched product from deoxyribozyme and unligated RNAs. The bands were visualized by UV-shadowing. The desired bands were cut and oligonucleotides were extracted by crush and soak method, followed by ethanol precipitation. The branched RNA was isolated in a reliable yield of 50-55%.

Synthesis of control penta-nucleotide for CoMA

Before the first step of CoMA (synthesis of DNA mutant libraries) analytical pentamers with the sequence of dTdT(rN:dN)dTdT were synthesized with all 16 rN:dN mixtures. After synthesis and basic deprotection with $MeNH_2/NH_4OH$, the analytical samples were injected on anion exchange HPLC (Dionex DNAPac PA200 column). Pentamers which contained rN (dTdTrNdTdT) had the 2'-*O*-TOM group and therefore their retention time was increased compared to the pentamers which contained the parent dN (dTdTdNdTdT). The integrated area under UV^{260} peaks for dTdTrNdTdT and dTdTdNdTdT were extracted and the efficiency of rN incorporation was calculated. The results are summarized in Table S2.

Table S2. Efficiency of rN incorporation in a mixture of rN:dN phosphoramidites 10:90. Data were obtained experimentally for dTdT(rN:dN)dTdT penta-nucleotides .

Parent (dN)	rA (%)	rC (%)	rG (%)	rU (%)
dA	7.7	4.3	7.7	5.5
dC	8.8	5.9	7.1	9.1
dG	8.1	6.5	8.4	6.7
dT	8.5	6.3	6.6	6.9

Combinatorial kinetic analysis of 6BX22 rA library

For rA mutant library of 6BX22, separations of active mutants were performed with various ligation times in presence of 20 mM Mn^{2+}. The hydrolysis patterns for 0.5, 2, 3 and 5h were similar except for the band A31 which only appears after 2 h. Presence of the 2'-OH chemical tag in position A31 caused an interference value above 2.0 when 6BX22 reaction was stopped at 30 min but the observed interference value was reduced at longer time points.

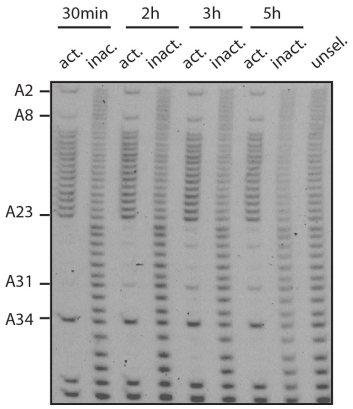

Figure S1. Combinatorial kinetic analysis of 6BX22 rA mutant library.

Combinatorial mutation interference analysis of 6BX22 for all libraries

All mutant libraries of 6BX22, were subjected to separations of active mutants in presence of 20 mM Mn^{2+} for 3h, followed by alkaline hydrolysis. The lanes of this gel corresponding to the active fractions are shown in **Figure 2b**.

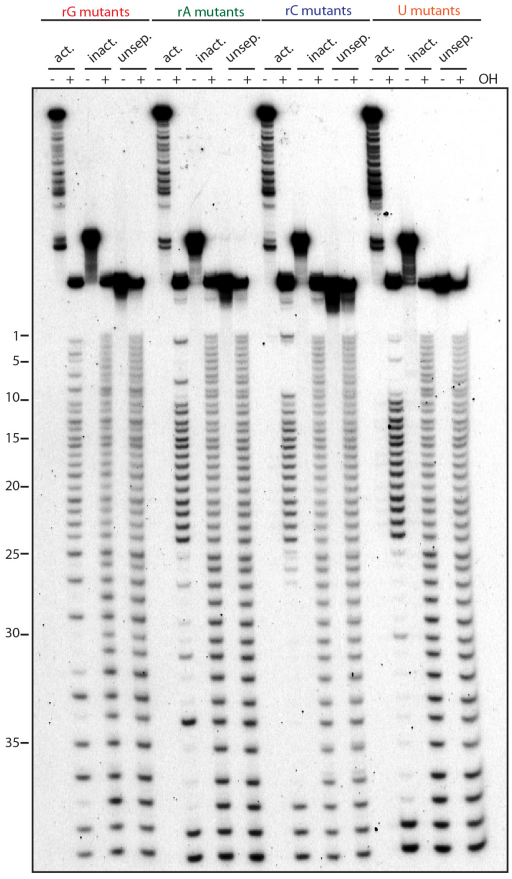

Figure S2. Full data set of 6BX22 CoMA in presence of 20 mM Mn^{2+}. For each mutant library, the active and inactive fractions and also the unselected library were subjected to hydrolysis and the products were resolved on denaturing sequencing gel. In all cases, hydrolysis was performed with 10 mM freshly made NaOH by heating at 95°C for 10 min. (A water control was prepared for each hydrolysis sample and was run aside the sample shown in the gel, labeled "–").The band intensities were quantified using ImageQuant 5.2 manually as described earlier[1]. The interference values were calculated as: Interference value $^{pos.\ n}$ = intensity $^{pos.\ n}{}_{unseparated\ library}$/ intensity $^{pos.\ n}{}_{active\ fraction}$.

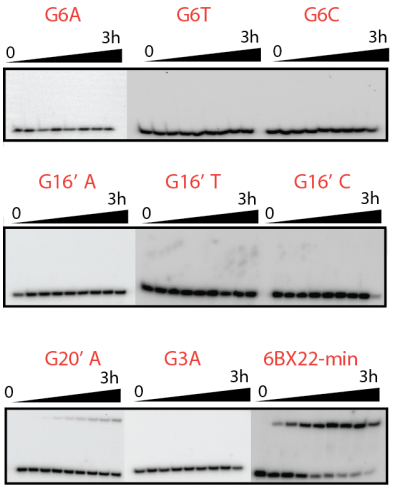

Figure S3. Kinetic analysis of point mutants of 6BX22-min, to examine positions at which the 2'-OH was detrimental. Time points were 0, 5, 10, 20, 40, 60, 90, 120, 180 min. All the mutants were inactive except G20'A which showed 20% yield at 3h. The nucleotides which have different numbers after minimization are marked with primed numbers.

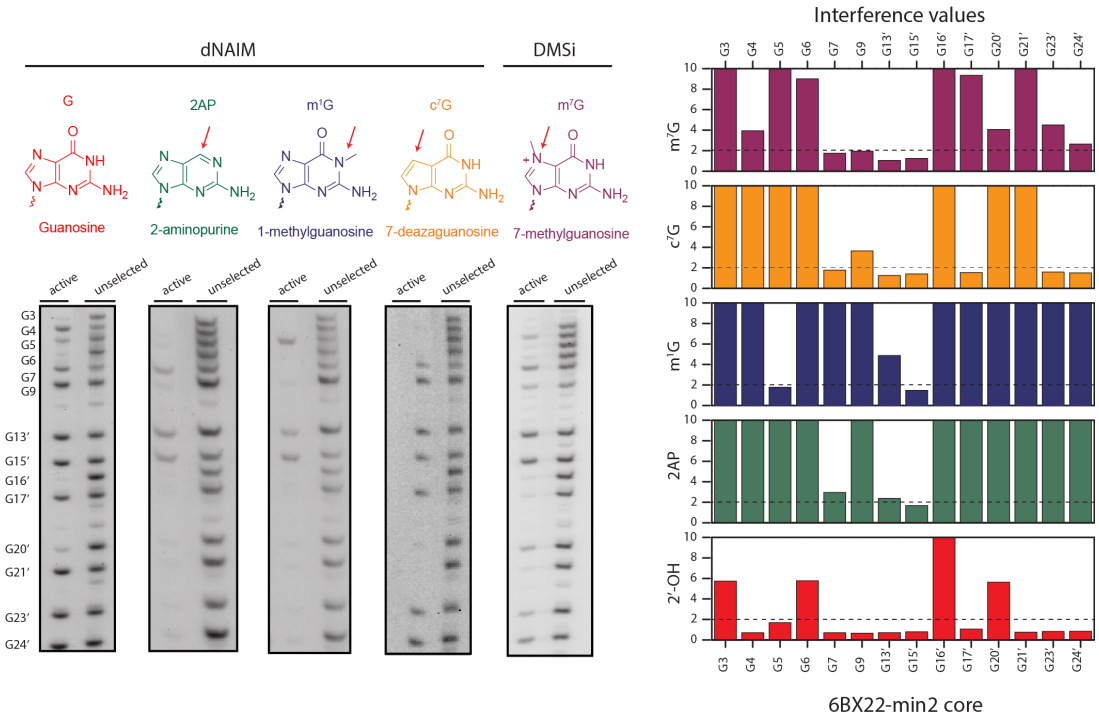

Figure S4. Nucleotide analogue interference mapping of DNA (dNAIM) and DMS-interference (DMSi) of guanines of the 6BX22-min. Modifications of dNAIM libraries were incorporated to the 6BX22 sequence during solid phase synthesis. Modification of the DMSi library was post synthetic using 0.1% DMS. 6BX22 reactions for separation of active modified DNAs were performed in presence of 20 mM Mn^{2+} at 37°C for 3h. Interference values were defined as in CoMA by the ratio of a band in the unselected library to the corresponding band in the active fraction.

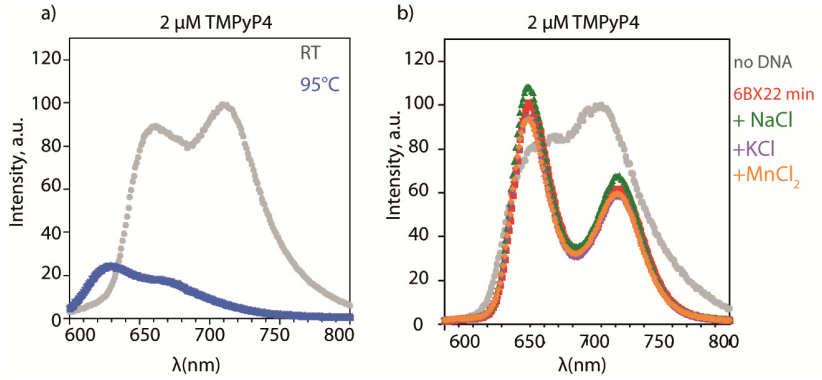

Figure S5. TMPyP4 spectral change upon heat or addition of metal ions. a) TMPyP4 emission spectra before and after 1 min incubation at 95°C. Measurement conditions: TMPyP4: 2 µM, HEPES pH 7.5: 50 mM and metal ions: not present. Volume: 120 µl. Excitation wavelength: 433 nm. b) Stepwise addition of concentrated small volumes (1-6 µl) of 6BX22 min DNA, mono- and divalent metal ions to TMPyP4. Initial condition: TMPyP4: 2 µM, HEPES pH 7.5: 50 mM. Final concentrations: DNA: 4 µM, NaCl: 150 mM, KCl: 2 mM, $MnCl_2$: 20 mM. Excitation wavelength: 433 nm. The spectrum was corrected for the volume change upon addition of DNA and metal ions.

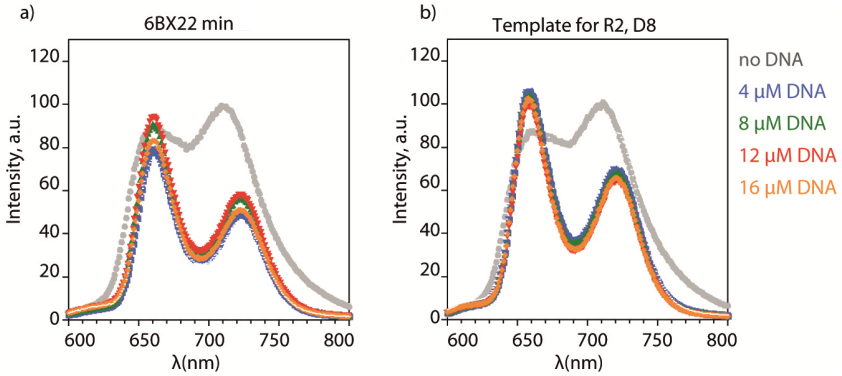

Figure S6. DNA titrations and TMPyP4 spectral change. Emission spectra of 2 µM TMPyP4 with increasing concentrations of 6BX22-min DNA enzyme (D4) or non G-quadruplex forming DNAs, D12 and D13 as negative controls. Measurement conditions: HEPES pH 7.5: 50 mM, NaCl: 150 mM, KCl: 2 mM, $MnCl_2$: 20 mM. The 660 nm and 720 nm peak intensities of TMPyP4 in presence of 6BX22-min was in the same range as for negative controls.

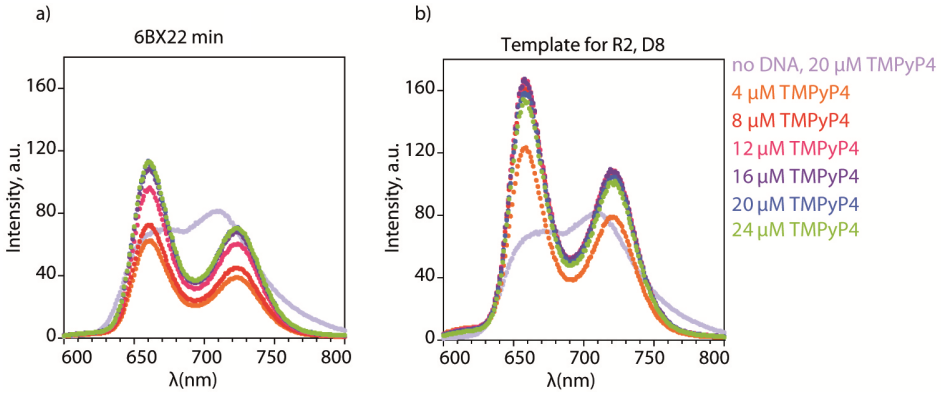

Figure S7. TMPyP4 titrations and spectral change. Emission spectra of TMPyP4 in presence of 20 µM DNA (D4 and D12). Increase in TMPyP4 concentration resulted in higher intensities. The effect is even stronger for non G-quadruplex forming DNA, D12. Additionally, emission spectrum of 20 µM TMPyP4 in the absence of any DNA is plotted on the graphs for comparison. The instrument setting adjusted for higher intensities of TMPyP4.

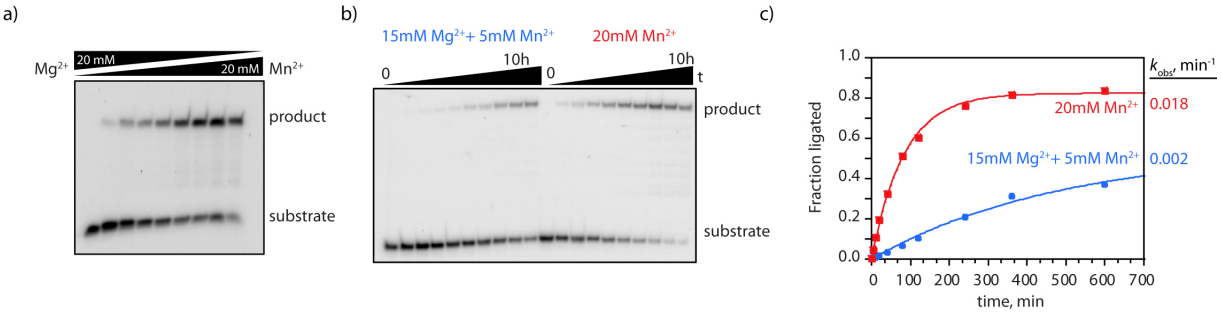

Figure S8. Kinetics of 6BX22 enzyme in presence of reduced concentrations of Mn^{2+}, compensated with Mg^{2+} to keep constant divalent metal ion concentration. a) Ligated fraction after 3 h incubation at different combinations of Mg^{2+} and Mn^{2+}, total concentration of 20 mM M^{2+}. In all samples ionic strength were kept constant by addition of 150 mM NaCl, 2 mM KCl and appropriate concentration of $MgCl_2$ to reach the final concentration of 20 mM divalent metal ion for each sample. b and c) Kinetic activity of 6BX22-min in presence of 50 mM HEPES 7.5, 150 mM NaCl, 2 mM KCl and 20 mM $MnCl_2$ or 5mM $MnCl_2$+15 mM $MgCl_2$. Time points: 0, 5, 10, 20, 40, 80 min, 2, 4, 6 and 10 h.

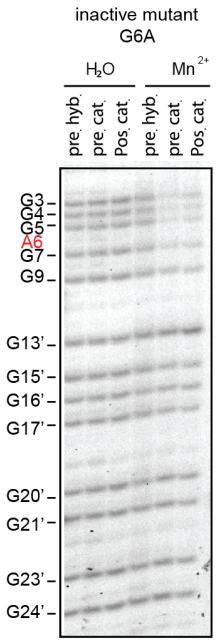

Figure S9. DMS probing of an inactive mutant of 6BX22-min (G6A). Three oligonucleotide complexes were analyzed in presence or absence of buffer and metal ions. Unlike in the active 6BX22 (compare **Figure 6c**), the protrusion of G20' is not observed in the inactive mutant.

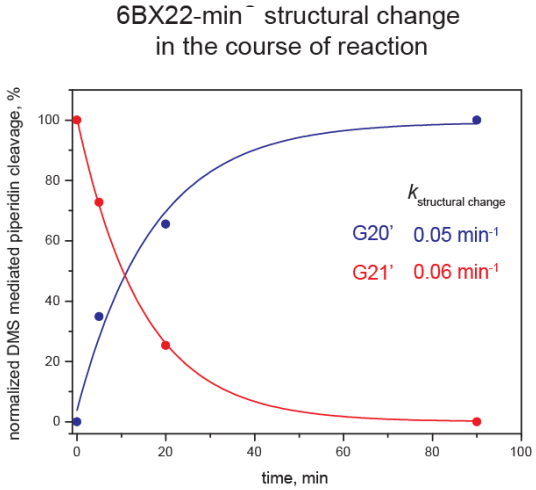

Figure S10. Timecourse of DMS probing. Band intensities for G20' and G21' of gel image in **Figure 6d** were quantified by area analysis, normalized, plotted against reaction time, and fitted to pseudo-first order kinetics.

Table S3. Output of QGRS mapper for 27 nt 6BX22-min core (5'-CAGGGGGAGCTTGCGGGTAGGAGGCCC) (http://bioinformatics.ramapo.edu/QGRS).[2]

Position	Length	QGRS	G-Score
3	19	GGGGGAGCTTGCGGGTAGG	13
3	19	GGGGGAGCTTGCGGGTAGG	12
3	19	GGGGGAGCTTGCGGGTAGG	15
3	19	GGGGGAGCTTGCGGGTAGG	14
3	22	GGGGGAGCTTGCGGGTAGGAGG	13
3	22	GGGGGAGCTTGCGGGTAGGAGG	12
3	22	GGGGGAGCTTGCGGGGTAGGAGG	8
3	22	GGGGGAGCTTGCGGGTAGGAGG	15
3	22	GGGGGAGCTTGCGGGTAGGAGG	14
3	22	GGGGGAGCTTGCGGGGTAGGAGG	10
3	22	GGGGGAGCTTGCGGGTAGGAGG	12
3	22	GGGGGAGCTTGCGGGTAGGAGG	11
4	18	GGGGAGCTTGCGGGTAGG	14
4	18	GGGGAGCTTGCGGGTAGG	13
4	21	GGGGAGCTTGCGGGTAGGAGG	14
4	21	GGGGAGCTTGCGGGTAGGAGG	13
4	21	GGGGAGCTTGCGGGGTAGGAGG	9
4	21	GGGGAGCTTGCGGGTAGGAGG	13
4	21	GGGGAGCTTGCGGGTAGGAGG	12
5	20	GGGAGCTTGCGGGTAGGAGG	14
5	20	GGGAGCTTGCGGGTAGGAGG	13
6	19	GGAGCTTGCGGGTAGGAGG	15
6	19	GGAGCTTGCGGGTAGGAGG	14

References for supporting information

[1] F. Wachowius, F. Javadi-Zarnaghi, C. Höbartner, *Angew. Chem. Int. Ed.* **2010**, *49*, 8504-8508.
[2] O. Kikin, L. D'Antonio, P. S. Bagga, *Nucleic Acids Res.* **2006**, *34*, W676-682.