Electronic Supplementary Material (ESI) for Molecular BioSystems.


## ELECTRONIC SUPPORTING INFORMATION

# Dual Input DNA-Based Molecular Switch

**Irina V. Nesterova,*  Siddieg O. Elsiddieg,  Evgueni E. Nesterov**

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## Materials

All the chemicals of "Reagent" grade or above were obtained from established commercial suppliers (e.g. Sigma-Aldrich (St. Louis, MO), VWR/Alfa Aesar (West Chester, PA), and others) and were used as received unless otherwise noted. Nuclease-free water (Promega, Madison, WI) was used for all sample preparation involving DNA. In-house DI water was used for preparation of buffers for PAGE experiments. Sodium phosphate (20 mM)/potassium chloride (100mM) buffers for pH-dependent CD measurements were prepared in house. Bst large fragment polymerase, dNTPs, BSA, 50-bp DNA ladder, NEBuffer 1 (NEB 1, 10 mM Bis-Tris-Propane-HCl, 10 mM $MgCl_2$, 1 mM Dithiothreitol, pH 7.0) and NEBuffer 2 (NEB 2, 50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM Dithiothreitol, pH 7.9) were obtained from New England Biolabs (Ipswich, MA). Acrylamide/Bis 29:1 (30%) was purchased from BioRad. Bromphenol blue gel loading buffer was obtained from Sigma-Aldrich (St. Louis, MO). Single stranded DNAs (sequences included in Table S1) were obtained from IDT (Coralville, IA) and reconstituted with nuclease-free water. SYBR Green I and SYBR Gold were obtained from Invitrogen (Carlsbad, CA).

## Experimental procedures

### pH denaturation studies via Circular Dichroism (CD)

CD experiments were conducted using a J-815 Circular Dichroism Spectrometer (Jasco, Easton, MD) equipped with a Peltier-based thermostatted sample compartment.

Samples containing $\mathbf{I_i}$ (at ~ $3\times10^{-6}$ M) were prepared in appropriate phosphate buffers, heated at 95°C for 10 minutes, slowly (overnight) cooled to room temperature. Three scans for each sample were recorded using a 0.1 cm optical path cuvette, averaged, and corrected for a corresponding buffer blank. The i-motif formation was monitored at 290 nm where C-quadruplexes are responsible for a characteristic positive band, while single stranded fragments are characterized by a positive band at 260-280 nm.[1]

### pH Denaturation studies via UV spectroscopy

pH Denaturation studies via UV spectroscopy were performed using a Cary 5000 UV-Vis-NIR spectrophotometer (Agilent Technologies, Santa Clara, CA). Typically, measurements were performed on a 500-µL aliquot of a 0.5-1.0 µM sample (prepared as described above for CD-based measurements) using a 10 mm optical path cuvette. The i-motif transformations were monitored at 295 nm where C-quadruplex unfolding is accompanied by the hypochromic effect.[2] The measurements were taken at room temperature and corrected against corresponding blank buffers.

**Thermal denaturation studies via UV spectroscopy**

The thermal denaturation studies were conducted as described by us before.[3] Briefly, the samples containing approx. 500 nM of $\mathbf{I_i}$ were equilibrated at 80°C for 30 minutes before beginning a cooling cycle. Upon completion of the cooling cycle, the samples were stored overnight at 5°C (refrigerated) and, on the next morning, equilibrated to the lowest temperature of the temperature range evaluated (12-15°C) in a sample compartment of a spectrometer for 30 min before heating cycle began. Temperature gradients of 0.2°C $min^{-1}$ were used. Concentrations used for the denaturation studies (~500 nM) were below the range in which monomolecular folded hairpin (1 μM-1 mM)[4] or i-motif (2-10 μM)[5] have been demonstrated. Cary 5000 UV-Vis-NIR spectrophotometer (Agilent Technologies, Santa Clara, CA) equipped with a Cary Dual Cell Peltier Accessory (Varian, Palo Alto, CA) was utilized. The measurements were taken every 1°C. The differential spectra (obtained by subtraction of sample spectrum obtained at the lowest temperature (15°C) from the spectrum obtained at the highest temperature (80°C)) were evaluated to derive the best wavelength for a conformation change monitoring. Changes at 295 nm are exclusively indicative of i-motif opening. Those observations agree well with literature.[6]

**Polyacrylamide Gel Electrophoresis**

A 50 μL sample containing one of the oligonucleotides **p**, $\mathbf{I_h}$, $\mathbf{I_i}$ or $\mathbf{I_s}$ (28 nM) in NEB 1 or NEB 2 was prepared by heating the solution at 95°C for 10 minutes followed by slow (overnight) cooling to room temperature. After addition of BSA (to yield 100 μg/mL), dNTP mixture (to yield 400 μM each), and DNA template **II** (final concentration of 28 nM) the resulting solution was incubated at 37°C for 600 seconds in a VWR dry block heater (West Chester, PA). Following the incubation, 0.4 unit of Bst large fragment polymerase was added to the sample and reaction

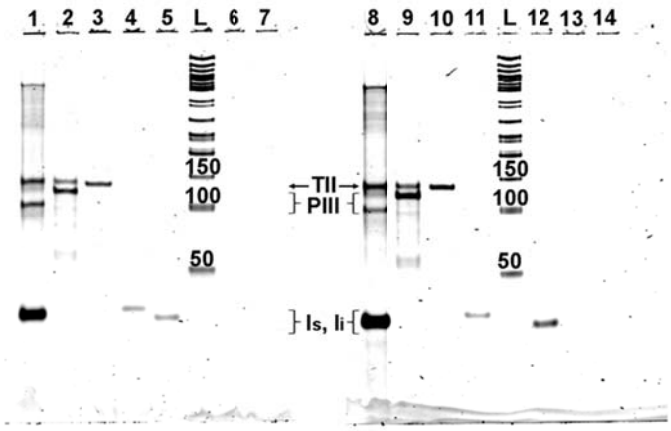


**Figure S1.** Raw image of a non-denaturing polyacrylamide gel demonstrating: (*i*) separation between single-stranded template **II** (**TII**) and double-stranded product **III** (**PIII**) (Lines 1, 2, 8, 9) and (*ii*) no interference from potential self-primed extension of **p** (lines 7 and 14), $\mathbf{I_h}$ (lines 6 and 13), $\mathbf{I_s}$ (lines 5 and 12), $\mathbf{I_i}$ (lines 4 and 11) and **TII** (lines 3 and 10). Non-denaturing PAGE (12 %) separations of reactions conducted in NEB 1 (Lines 1-7) and NEB 2 (lines 8-14) were performed using conditions described in the SI text. A 50-bp DNA ladder was analyzed in line L. Polymerase extension reactions containing $\mathbf{I_s}$ and **TII** (lines 1 and 8) and primer **p** and **TII** (lines 2 and 9) were included to provide markers for corresponding extension products. Note, that PAGE conditions necessary to separate **TII** and **PIII** do not allow detecting short strands $\mathbf{I_h}$ and **p** (lines 6, 7, 13, 14) on the same gel, however, no interferences at the area of expected **PIII** migration and no self-primed extension are intended goals of analyzing the samples. All the sequences are included in Table S1.

was allowed to proceed for 1200 seconds at 37°C. After the appropriate time, the reaction was quenched by adding 8 μL of gel loading buffer and immediate cooling to 0°C (on ice). The samples were kept refrigerated until ready for electrophoresis. A 12 μL aliquot of the reaction mixture was injected on the gel.

12% non-denaturing polyacrylamide gel electrophoresis (PAGE) experiments were carried out using Vertical Gel Electrophoresis System (VWR, West Chester, PA). The separation was performed in 0.5×TBE at room temperature at ~ 15 V $cm^{-1}$ for 2 hours. The 12 % gels (0.5×TBE) were prepared in house with Acrylamide/Bis 29:1, Ammonium Persulfate and TEMED using standard protocols and were stained post-separation with SYBR Gold for 30 minutes. Stained gels were imaged on a Typhoon 9410 scanner (GE, Fairfield, CT) using a 488 nm laser for excitation and a 555±20 nm filter for signal collection.

A representative gel image demonstrating *(1)* separation of reaction product **III** (**PIII**) and template **II** (**TII**), and *(2)* non-interference from potential self-primed extension products of template **II**, **p**, $\mathbf{I_i}$, $\mathbf{I_s}$ and $\mathbf{I_h}$ is included in Figure S1.

**Fluorescence experiments**

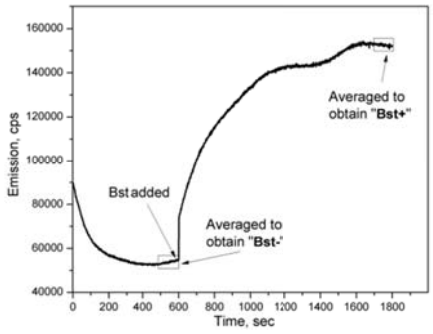


**Figure S2**. Emission (525 nm) of SYBR Green I in a sample containing oligonucleotide **p**, BSA, dNTPs, DNA template **II** in NEB 1 before and after addition of Bst polymerase (at 600 sec). Increase in emission after addition of Bst is caused by the enhanced sensitivity of SYBR Green I towards double stranded polymerase extension product formed.

Time-based fluorescence measurements were carried out with a QuantaMaster4/2006SE spectrofluorimeter (PTI, Birmingham, NJ) equipped with a temperature-controlled cuvette holder. A 60 μL sample containing either **p**, $\mathbf{I_h}$, $\mathbf{I_i}$ or $\mathbf{I_s}$ (28 nM) in NEB 1 or NEB 2 was prepared by heating the solution at 95°C for 10 minutes followed by slow (overnight) cooling down to room temperature. After addition of BSA (to yield 100 μg/mL), dNTP mixture (to yield 400 μM each), DNA template **II** (final concentration of 28 nM) and SYBR Green I (to yield 0.05×), a 50 μL aliquot of the resulting mixture was transferred into an appropriate fluorescence cuvette and equilibrated for 600 seconds at 37°C in the thermo-controlled cuvette holder of the spectrofluorimeter. Emission at 525 nm (excitation at 497 nm) was monitored during the time to ensure a stable baseline. The last 120 seconds of the stabilized baseline were averaged to obtain (after normalization) the **"Bst–"** value (Figure 3 in the main text). Post equilibration, 0.4 unit of Bst large fragment polymerase was added to the cuvette and a 525-nm emission monitoring was continued for 1200 seconds. The last 120 seconds

of the measurements were averaged to obtain the **"Bst+"** value (Figure 3 in the main text). An example of emission monitoring graph is presented in Figure S2.

## SI References


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**Table S1. DNA sequences**

| Designation | Sequence 5′→3′ [b] |
|---|---|
| $\mathbf{I_i}$ | **CTG CAG AAA ACC CCC TTT CCC CCT TTC CCC CTT TCC CCC CAT GCC TGC AG**<br>1* 3 2 1a 1 |
| **p** | **CAT GCC TGC AG**<br>1a 1 |
| $\mathbf{I_h}$ | **CTG CAG AAA ACA TGC CTG CAG**<br>1* 3 1a 1 |
| $\mathbf{I_s}$ | **CTG CAG AAA ACA TTG ACC TTG GAA CTG ATA GAA AAG AAC CAT GCC TGC AG**<br>1* 3 2a 1a 1 |
| Template **II** **(TII)** | **GAA ACA GCT ATG ACC ATG ATT ACG AAT TCG AGC TCG GTA CCC GGG GAT CCT CTA GAG TCG ACC TGC AGG CAT GCA AGC TTG GCA CTG GCC GTC GTT TTA C**<br>1* 1a* |