Supplementary Material (ESI) for Chemical Communications

Supplementary information for

An i-motif-containing DNA Device that Breaks Certain Forms of Watson-Crick Interactions

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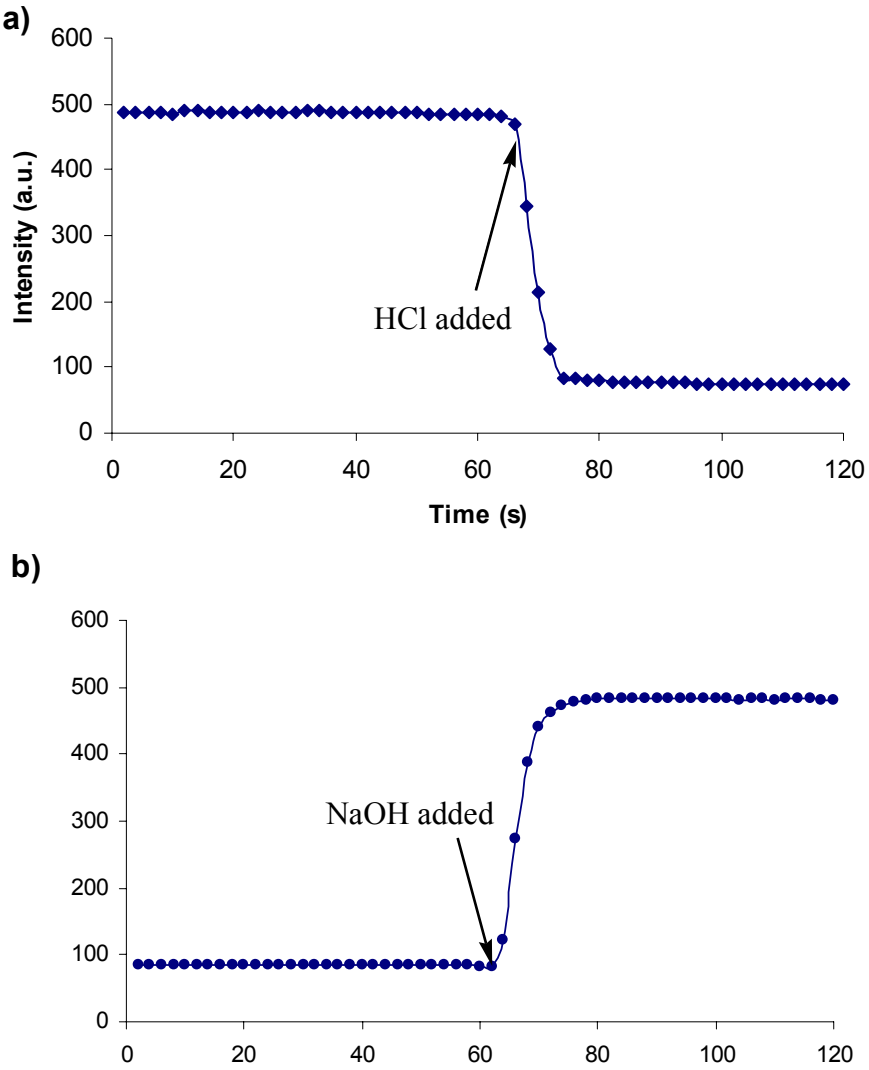

Fig. S1. Examination of operability of artificial DNA machines designed in the current studies using fluorescence spectroscopy.

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(a) Changes of emission intensity in the first half of the cycle as pH decreases from 7.5 to 5.5 (from State 1 to State 2 in Fig.1C). 1 M HCl was added to a solution containing 1 μM of Sequence 1, 1 μM of Sequence 2, 10 mM MES/HEPES (pH 7.5) and 1 M NaCl in a stepwise fashion until pH reached ~5.5. Emission intensity at 515 nm was recorded at 20 °C in every 2 s with excitation at 480 nm.

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(b) Changes of emission intensity in the second half of the cycle as pH increases from 5.5 to 7.5 (from State 1 to State 2 in Fig. 1C). 1 M NaOH was added to a solution containing 1 μM of Sequence 1, 1 μM of Sequence 2, 10 mM of MES/HEPES (pH 7.5) and 1 M NaCl in stepwise fashion until pH reached ~7.5. Emission intensity at 515 nm was recorded at 20 °C in every 2 s with excitation at 480 nm.

Detailed legend of Fig. 1

Fig, 1. Schematic representation of our approach for designing an i-motif-containing DNA machine capable of breaking down certain forms of Watson-Crick interactions.

(a) Formation and disintegration of i-motif structure as pH value of its environment varies. The two nucleotides at the termini of a 25-mer cytosine-rich oligonucleotide (Cytosine 1 and Cytosine 25) might not be able to move close to each other (***i***) under neutral condition and would be forced to align next to each other (***ii***) with the formation of i-motif under acidic condition.

(b) Formation and disintegration of i-motif structure that is embedded in a 36-mer circular oligonucleotides. Sequence 1 (***iii***) is a 36-mer circular oligonucleotide constituted by a cytosine-rich stretch (25 nucleotides in length) and a non-cytosine segment (11 nucleotides in length) (***iii***) respectively. As pH varies from neutral to acidic, a structural entity of i-motif structure will form from the cytosine-rich tract, which will force Cytosine 1 and Cytosine 25 to line align adjacent to each other (***iv***). This relative movement of Cytosine 1 and Cytosine 25 (from ***iii*** to ***iv***) will cause a certain degree of backbone bending on the 11-mer non-cytosine segment. As pH changes from acidic to neutral, the backbone bending will be released due to disintegration of i-motif (from ***iv*** to ***iii***).

(c) Breakdown of the Watson-Crick interaction that upholds 11-mer T- and A-rich duplex segments. Under neutral condition, Sequence 1 and Sequence 2 will form an 11-mer duplex structure via their complementary segments, an entity sustained by hydrogen bonding (State 1). As pH decreases (from State 1 to State 2), a certain degree of curvature on the non-cytosine segment will be forced to occur by the formation of structural entity of i-motif. Owing to the absence of extra force to drive Sequence 2 to curve with its complementary segment, the corresponding hydrogen bonds between the 11-mer duplex will be broken down.

Detailed legend of Fig. 2

Fig. 2. Polyacrylamide gel electrophoretic analysis of oligonucleotides as components of the artificial DNA machines designed in the present studies.

(a) Preparation of Sequence 1 from its linear precursor on a 15-mer template catalyzed by T4 DNA ligase. Reaction mixtures containing 1 μM Sequence 3 (*p-TAAAAACCCCTTTCCCCTTTCCCCTTTCCCCAAAAT), 3 μM Sequence 4 (GGTTTTTAATTTTGG, as Template), 1 x T4 DNA ligase buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP, 25 μg/ml BSA, pH 7.8), 30 mM NaCl and 100 U T_4 DNA ligase were incubated at 20 °C for different periods of time. Lane 1: Sequence 3 alone, lane 2 to lane 5: T_4 DNA ligase-catalyzed ligation reactions lasted for 5, 10, 30 and 60 min respectively, lane 6: 66-mer as a molecular weight marker.

(b) Confirmation of circularity of Sequence 1 in its backbone. A solution containing 1 x exonuclease I buffer (67 mM Glycine-KOH, 6.7 mM $MgCl_2$, 10 mM 2-mercaptoethanol, pH 9.5), oligonucleotides (Sequence 1 or Sequence 3) and 80 U exonuclease I was incubated at 37 °C for 60 min. Lane 1: Sequence 3 in the absence of exonuclease I (as control), lane 2: Sequence 3 in the presence of

exonuclease I, lane 3: Sequence 1 in the absence of exonuclease I (as control), lane 4: Sequence 1 in the presence of exonuclease I.

(c) Confirmation of presence of duplex structure between Sequence 1 and Sequence 2 (State 1 in Figure 1c) at pH > 6.2. A solution containing 1 μM Sequence 2, 1 μM ^{32}P-labled Sequence 1, 1 x Mse I buffer (10 mM Tris-HCl, 50 mM NaCl, 10 mM MgCl2 1 mM Dithiothreitol, pH 7.9), 100 mM NaCl and 60 U Mse I was incubated at 20 °C for different time periods. Lane 1: Sequence 3 alone, lane 2: Sequence 1 alone, lane 3 to lane 6: Mse I-catalyzed hydrolysis reactions lasted for 10 min, 60 min, 120 min and 240 min respectively.

Detailed legend of Fig. 3

Fig. *3.* Fluorescence Spectroscopic analysis of formation and disintegration of duplex structure associated with the artificial machines designed in the current studies.

a) Emission spectra of Sequence 1 and Sequence 2 as pH increases. Fluorescence spectra of a sample containing 1 μM Sequence 1, 1 μM Sequence 2, 10 mM MES/HEPES (pH 7.5) and 1 M NaCl was recorded at 20 °C with excitation at 480 nm. Variation of pH was carried out by addition of 1 M NaOH to the system in a stepwise fashion.

b) Emission spectra of Sequence 1 and Sequence 2 as pH decreases [from State 1 to State 2 in Fig. 1c). Fluorescence spectra of a sample containing 1 μM Sequence 1, 1 μM Sequence 2, 10 mM MES/HEPES (pH 5.5) and 1 M NaCl was recorded at 20 °C with excitation at 480 nm. Variation of pH was carried out by addition of 1 M HCl to the system in a stepwise fashion.

Detailed legend of Fig. 5

Fig. *5.* Examination of operability of artificial DNA machines designed in the current studies using fluorescence spectroscopy. Changes of emission intensity as the designed DNA machines were operated for five cycles. For the first half of the cycle, 1 M NaOH was added to a solution containing 1 μM Sequence 1, 1 μM Sequence 2, 10 mM of MES/HEPES (pH 7.5) and 1 M NaCl in stepwise fashion until pH reached ~5.5. 1 M HCl was added next to the above solution until pH reached ~ 7.5 to complete the second half of the cycle. Emission intensity at 515 nm was recorded at 20 °C in every 2 s with excitation at 480 nm.