



## Reporting Transient Molecular Events by DNA Strand Displacement

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Complete List of Authors:	Mao, Chengde; University of Purdue, Department of Chemistry Liu, Zhiyu; Purdue University,

## COMMUNICATION

## Reporting Transient Molecular Events by DNA Strand Displacement

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Zhiyu Liu and Chengde Mao\*

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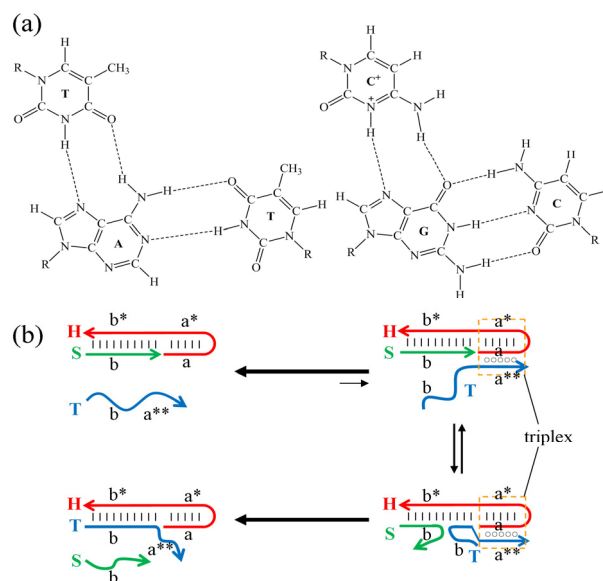
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**Transient formation of DNA triplex was reported by being coupled with a permanent strand displacement reaction.**

This manuscript reports a strategy to detect a rare and transient molecular event: formation of cytosine (C)-containing DNA triplex in neutral solution. Under neutral pH condition, C-containing DNA triplex is not stable and rarely forms; thus, very difficult to be detected. Here, we have coupled the triplex formation with a DNA strand displacement reaction. Though the triplex formation is transient, the DNA strand displacement results in permanent molecular changes, which reports the triplex formation. This strategy should be able to be adopted to monitor other rare and transient molecular events as well.

Many molecular events are rare and transient. Detection of those events represents a challenge. One approach is to couple the transient event of interest with a permanent (or long-life) chemical reaction.<sup>1</sup> In this work, we have applied this concept to detect C-containing DNA triplex formation in neutral solution. DNA triplexes can form in cells and are believed to be involved in some biological processes.<sup>2</sup> People have tried to use triplex formation for sequence-specific recognition of DNA duplexes to modulate specific gene expression.<sup>3</sup> Recently, DNA triplexes have been further introduced into DNA nanotechnology as sequence-specific targeting agents<sup>4</sup> or nanodevices.<sup>5</sup> Often a triplex consists of a homopurine (A/G) DNA strand and two homopyrimidine (T/C) DNA strands. The purine strand and one pyrimidine strand hybridize with each other to form a normal DNA duplex via Watson-Crick base-pairing. The third strand binds to the duplex in the major groove via Hoogsteen hydrogen bonds. While the T-A<sup>+</sup>T triad can readily form in aqueous solution, the C-G<sup>+</sup>C triad forms only when the C on the third strand is protonated at its N3 position as C<sup>+</sup>, which can form Hoogsteen hydrogen-bonds with guanosine (Figure 1a).<sup>6</sup> Thus, under an acidic condition, the C-containing triplex is stable. At neutral pH, however, C<sup>+</sup> will deprotonate into C and the triplex will mostly dissociate into a duplex and a homopyrimidine single strand. Consequently, the C-containing DNA triplex exists at low concentration with a short lifetime. Hence, its detection represents a great challenge. Here we have addressed this issue by applying the strategy of conditioned strand displacement, which couples the triplex formation with a DNA strand displacement reaction.

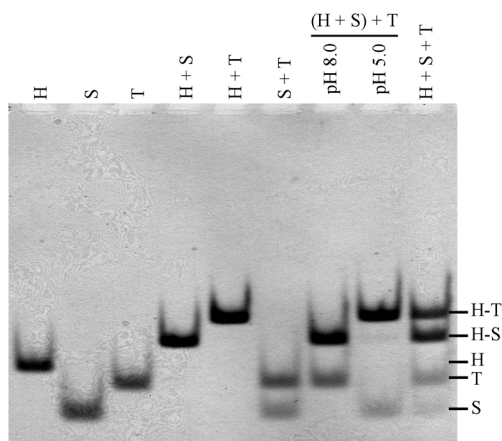


**Fig. 1.** Reporting transient triplex formation by proximity-induced strand displacement. (a) Structures of T-A<sup>+</sup>T and C-G<sup>+</sup>C<sup>+</sup> triads. Note that the protonated C (C<sup>+</sup>) forms Hoogsteen hydrogen bonds with G. (b) Mechanism of triplex-mediated strand displacement. Segments a/a\*, b/b\* are two complementary pairs. a is homopurine while a\* and a\*\* are homopyrimidines. When a\*\* binds to the hairpin (a-a\*) and form a triplex (a-a\*-a\*\*) domain, strand T is brought to proximity to strand H. Gradually, strand T will displace strand S. The first two steps are reversible, but the last step is essentially irreversible, thus drives the overall reaction to completeness.

Figure 1 illustrates our strategy to report rare and transient formation of DNA triplex under neutral pH condition. The core idea is to exploit the strand proximity accompanying the formation of DNA triplex to induce an irreversible, DNA strand displacement. Since being developed, DNA strand displacement has been widely used in DNA nanotechnology to introduce conformational changes,<sup>7</sup> build nanomechanical devices,<sup>8</sup> perform molecular computations,<sup>9</sup> amplify analytical signals,<sup>10</sup> help nano-scale self-assembly,<sup>11</sup>

organize organic synthesis,<sup>12</sup> etc. The foremost requirement for the reaction to occur is the existence of a toehold region.<sup>13</sup> Traditionally, the toehold is a short single strand, which is complementary to the counterpart of the other strand. Through specific Watson-Crick base-pairing, hybridization of the toehold region brings relevant strands into proximity, which subsequently initiate branch migration. As predicted by thermodynamics, the extra base-pairings of the toehold region lead the equilibrium towards the total displacement of the corresponding strand.<sup>14</sup> Since DNA triplex formation can bring the relevant strands into proximity as well, we hypothesize that a triplex formation could facilitate strand displacement in a similar fashion as traditional, single-stranded toehold (Figure 1b). Such design ensures that the strand displacement takes place only when the following two prerequisites are satisfied: (1) the presence of the third complementary strand; (2) a triplex structure forms at the toehold region.

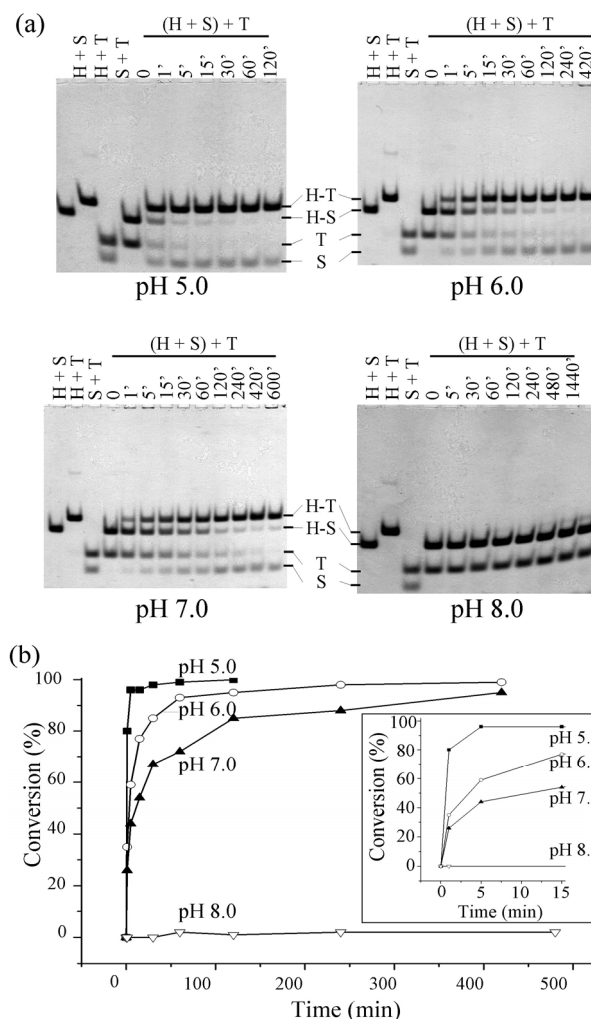
The experiment system (Figure 1b) contains three DNA strands: a hairpin-containing strand (H), a short strand (S), and a triplex-forming strand (T). Strands H and S associate with each other to form a continuous duplex. Part of the T strand can potentially bind to the major groove of the hairpin region of H strand to form a triplex domain. In the triplex domain, 60% of the triads are C-G<sup>+</sup>C<sup>+</sup> triads. Without triplex formation, strand T and complex H-S can not interact with each other. However, triplex formation (even for a very short time) will bring strand T and complex H-S together and initiate strand displacement. The output is the formation of a new complex H-T while strand S is set free. In the final complex H-T, the triplex may or may not be stable. A key molecular event is the triplex formation. It is reported by a proximity-induced strand displacement, which can be easily monitored by native polyacrylamide gel electrophoresis (PAGE) or fluorescence signals.<sup>15</sup> In the current work, we have used native PAGE for this study.



**Fig. 2.** Proof of concept for triplex-induced strand displacement by native polyacrylamide gel electrophoresis (PAGE) at pH 8.0. The composition in each lane and the chemical identity of each band are indicated above and beside the gel image, respectively. All samples are prepared at pH 8.0 except the sample [(H + S) + T (pH5.0)], which was prepared at pH 5.0.

We first tested the idea whether strand displacement can be initiated by triplex-generated proximity (Figure 2). Strands H and S were hybridized to form an H-S complex (sample H+S). When strand T was added at neutral pH [sample (H+S)+T, pH 8.0], triplex could not form and no strand displacement happened. In the solution, only H-S complex and free strand T existed. We also prepared a sample that had the same composition, but was exposed to pH 5.0 for one hour [sample (H+S)+T, pH 5.0]. It was well known that

triplexes could readily form and were stable at pH 5.0.<sup>16</sup> No surprise that strand displacement effectively carried out and resulted in complex H-T and free strand S. Though the triplex primarily dissociate due to the decreased stability when the sample pH was brought back to pH 8.0, the complex H-T was stable. Note that the overall process was kinetically, instead of thermodynamically, controlled. Complexes H-T and H-S had roughly the same stability. When the three strands (H, S, and T) were directly hybridized together, roughly the same amount of complexes H-S and H-T were produced.



**Figure 3.** Kinetics study of triplex-induced strand displacement. (a) Reaction time course monitored by native PAGE. The composition in each lane and the chemical identity of each band are indicated above and beside the gel image, respectively. Samples are prepared in solution at indicated pH and incubated for the indicated time. (b) Plot of the kinetics summarized from PAGE. Inset is a close-up view of the first 15 min.

We then apply this strategy to detect transient DNA triplex formation under different solution pH values. A C-containing DNA triplex readily forms at acidic condition (pH 5.0). As the solution acidity decreases (pH increases), the DNA triplex becomes unstable and its equilibrium concentration decreases. Consequently, the probability of the triplex-induced strand displacement will drop and the overall kinetics of strand displacement will slow down as well. This hypothesis has been proven by time course experiment in solutions with varied pH values (Figure 3). At pH 5.0, triplexes

readily form and the overall strand displacement goes quite fast and reaches 80% completeness within 1 min. But at pH 6.0 and 7.0, it takes ~ 20 and 100 min, respectively, to reach the same completeness. At pH 8.0, triplexes hardly form and only very slight sign shows reaction happens even after 24 hours. This result is consistent with previous NMR study. A triplex signal can be clearly seen under acidic environment.<sup>17</sup> It becomes weaker as the solution increases and eventually disappears at pH 7.4.

In conclusion, we have developed a strategy to report the transient triplex formation by DNA strand displacement, a commonly used method in structural DNA nanotechnology. The DNA strand proximity is brought by triplex formation between the DNA complex and the displacing strand, instead of the duplex formation at a traditional single-stranded toehold. This study raised three possibilities: (1) We may use strand displacement to monitor other transient molecular event if it can bring a DNA displacing strand into proximity to a DNA complex. (2) This study expands our tool box for structural DNA nanotechnology by introducing a new way to regulate DNA strand displacement. It is conceivable that strand displacement can be initiated by other molecular events that can bring different DNA strands into proximity.<sup>18</sup> (3) The current work has added another layer of regulation, pH-dependence, to strand displacement in addition to being sequence dependent. Such double regulation increases our control on the operation of designed experimental system and is valuable for construction of DNA nanomachines.<sup>19</sup>

## Notes and references

Department of Chemistry, Purdue University, West Lafayette, IN, USA.

E-mail: mao@purdue.edu

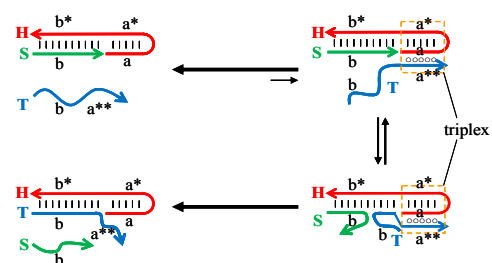
† Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/c000000x/

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## TOC



Coupling transient molecular event with a permanent chemical reaction.