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Electronic Supplementary Information

Reversion of DNA strand displacement with functional nucleic acids as toeholds

Xiaowen Xu,[a,b] and Xiurong Yang*[a]

[a] State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, China. [b] University of Chinese Academy of Sciences, Beijing 100049, China. E-mail: xryang@ciac.jl.cn.

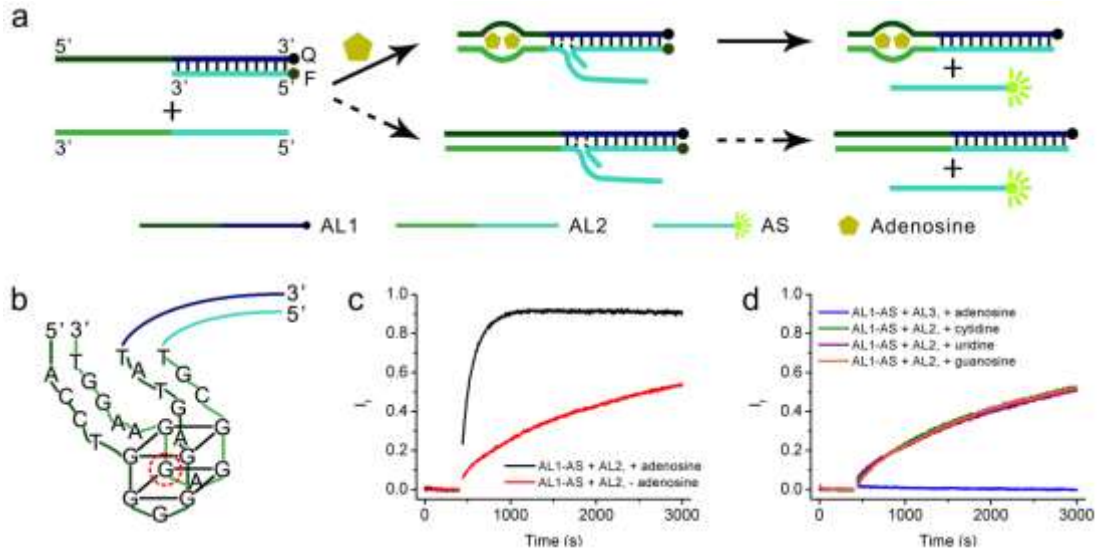

Fig. S1 Strand displacement between AL1-AS complex and AL2 with split aptamers as toeholds. (a) Principle of the strand displacement. The dark green and light green domains in AL1 and AL2 form ligand-stabilized structure in the presence of adenosine (upper path) or assembled heterodimer in the absence of adenosine (lower path), inducing toehold binding and subsequent branch migration to release AS. (b) The secondary structure of the assembled split adenosine-binding aptamer fragments. (c) Time-dependent fluorescence changes of the strand displacement between AL1-AS complex and AL2 in the presence (1 mM) and the absence of adenosine. (d) Time-dependent fluorescence changes of the strand displacement between AL1-AS complex and AL3 in the presence of adenosine (1 mM) and the strand displacement between AL1-AS complex and AL2 in the presence of cytidine, uridine and guanosine (1 mM each). The strand displacement proceeds in HEPES buffer (10 mM, pH 7.4) containing NaCl (300 mM).

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As outlined in Fig. S1a, AL1 contains one adenosine-binding aptamer fragment in the 5' portion while AL2 contains the other adenosine-binding aptamer fragment in the 3' portion. In the presence of adenosine (1 mM), the fluorescence intensifies promptly with time and levels off after about 1000 s (Fig. S1c), suggesting that adenosine accelerates the strand displacement. In this case, the split aptamer fragments embedded in AL1 and AL2 can reassemble to form adenosine-stabilized structures (schemed in Fig. S1b), thus AL2 can first bind to AL1 through the association of split aptamers in the exposed overhangs. Following the overhang binding, the strand displacement proceeds via branch migration and eventually releases AS, resulting in the detachment of fluorophore from quencher and the restoration of fluorescence (Fig. S1a, upper path). The toeholds utilized here are split aptamers and their association dependent on targets expedites the reaction. In the absence of adenosine, the fluorescence also intensifies with time, but is much retarded (Fig. S1c), indicating a slower strand displacement can also occur. The phenomenon is not difficult to understand since equilibrium exists between the disassociated parts and associated complex of the two split aptamers.[1] The two subunits of the aptamer keep the equilibrium that favors individual fragments over assembled heterodimer but shifts toward the latter only upon ligand binding. Therefore, strand displacement can still proceed due to the formation of assembled heterodimer under equilibrium but the absence of adenosine induces a less effective overhang binding and a slower displacement reaction (Fig. S1a, lower path). The presence of adenosine, therein, dramatically expedites the strand displacement reaction and acts as a stimulus to promote the reaction. If AL2 is mutated to AL3 with one base (indicated in red circle in Fig. S1b) in the split aptamer fragment domain altered, adenosine fails to accelerate the strand displacement (Fig. S1d), which emphasizes the role of sequence specificity in promoting the reaction. The base mutation inhibits the lower G-quartet plane formation and disrupts the folded aptamer structure, impeding the DNA binding to adenosine. Also, the analogues of adenosine (cytidine, uridine and guanosine) can not effectively enhance the strand displacement as adenosine can and only induce fluorescence changes similar to the case in the absence of adenosine, which

emphasizes the role of target specificity in promoting the reaction. All those illustrate that adenosine facilitates the strand displacement with its split aptamers as toeholds.

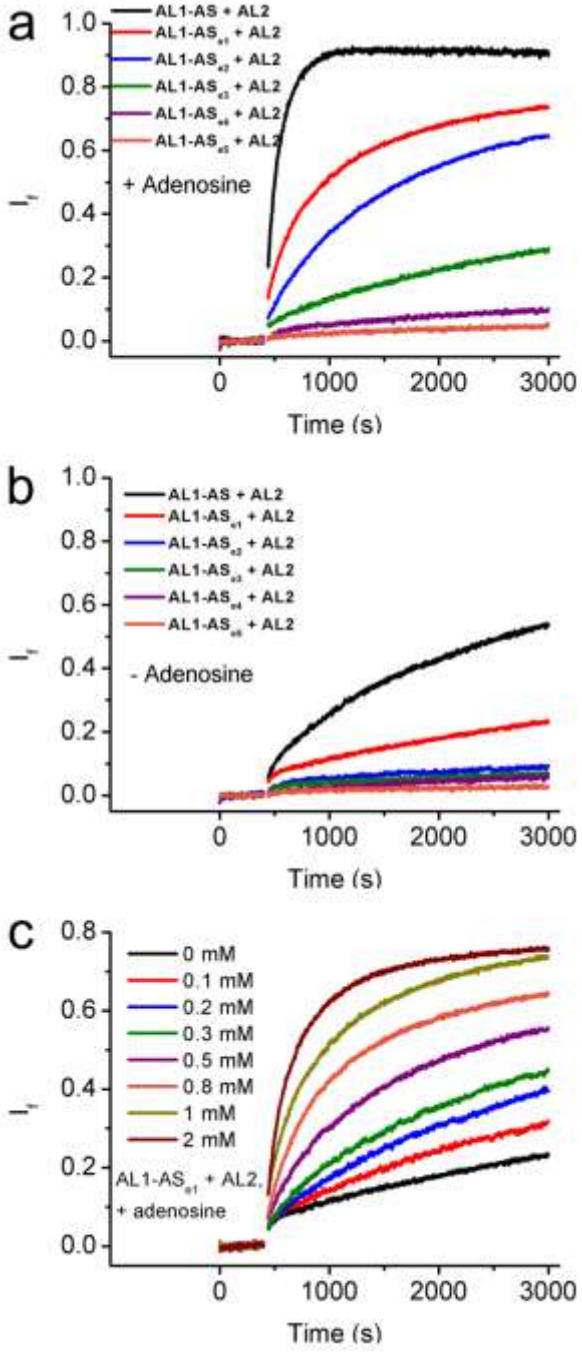

Fig. S2 Kinetics control of the strand displacement with split aptamers as toeholds. (a) Control of the reaction kinetics by extending the original shorter complementary sequence in the presence of adenosine (1 mM). (b) Control of the reaction kinetics by extending the original shorter complementary sequence in the absence of adenosine. (c) Control of the reaction kinetics by adjusting the concentration of adenosine. The strand displacement proceeds in HEPES buffer (10 mM, pH 7.4) containing NaCl (300 mM).

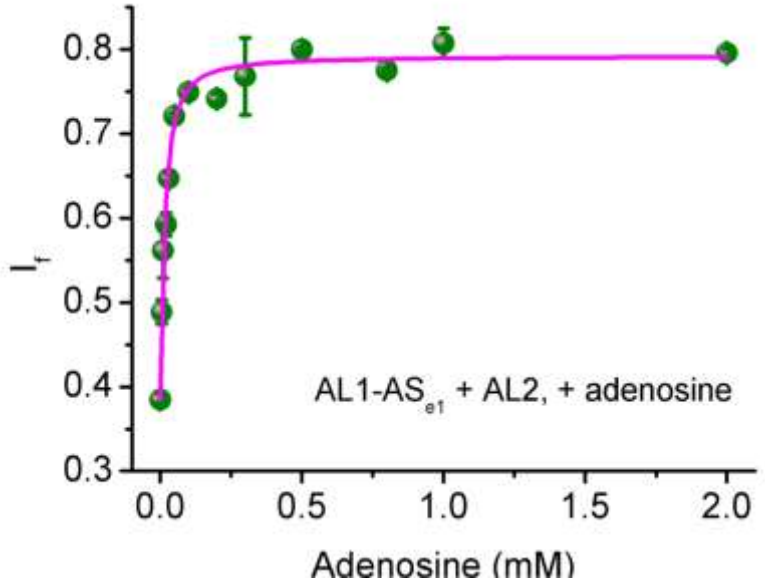

Fig. S3 Plot of normalized saturation fluorescence intensity versus adenosine concentration within the range from 0.005 mM to 2 mM for the strand displacement between AL1-AS_{e1} complex and AL2. The strand displacement proceeds in HEPES buffer (10 mM, pH 7.4) containing NaCl (300 mM).

The reaction kinetics can be regulated by two approaches; one is extending the original shorter complementary sequence and the other is adjusting the concentration of adenosine. In the first approach, the 3' domain of AS was extended with bases complementary to the split aptamer fragment in the 5' domain of AL1 to obtain strands AS_{en} (n represents the number of extended bases). AL2 was labeled with a fluorophore instead of AS_{en} to save cost. Although the proceeding of strand displacement leads to the proximity of the fluorophore to quencher, the signal change can be normalized into an increasing trend to allow an intuitive comparison. The kinetics of the strand displacement were recorded in the presence (1 mM) and the absence of adenosine when AS was extended with one to five bases. The extension of AS inhibits the adenosine-enhanced strand displacement, with longer extensions yielding slower reaction kinetics (Fig. S2a). On the other hand, the extension of AS with only one base greatly restrains the reaction in the absence of adenosine, and the extension with two to five bases further limits the reaction to a very low rate (Fig. S2b). So not only can the reaction kinetics for a given concentration of adenosine be tuned by the extended bases in AS, but the reaction in the absence of adenosine can be

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effectively restrained by such an extension to lower the background. The extended domain in AS impedes both the adenosine-induced association of split aptamers and the formation of assembled heterodimer in the absence of adenosine, since the hybridized bases in the split aptamer fragment of AL1 provide a barrier for the toehold binding. For the reaction using specific AS_{en}, the strand displacement always shows faster reaction kinetics in the presence of adenosine (1 mM) than that in the absence of adenosine, verifying the role of adenosine as a stimulus to promote the reaction. The rate constants are fitted using the 'fminunc' function in Matlab[2] and presented in Table S2; generally the extension of AS controls the displacement rates spanning 4 orders of magnitude in the presence of adenosine (1 mM) and 3 orders of magnitude in the absence of adenosine. The magnitude of rates control here exceeds or matches that of the rates control by introduction of remote toeholds in oligonucleotide-expedited displacement reaction.[3] The capability to control the releasing rate of the output strand provides possibility for the subsequent regulation of the nucleic acid output-triggered downstream reaction, toward the case in which the concentration of a stimulus is unalterable in the environment. For the sequences used here, the own unique domain in AS to trigger the downstream reaction is extended at the 5' end, and the extension at the 3' end to control the releasing rate does not bother its triggering character.

In the second approach, the strand displacement between AL1-AS_{e1} complex and AL2 was chosen in the investigation. Adenosine with different concentrations was introduced to the reaction and time-dependent fluorescence changes were recorded (Fig. S2c). The fluorescence intensifies more rapidly along with the increasing concentration of adenosine, indicating the reaction kinetics positively correlate with adenosine concentration. The presence of more adenosine drives the equilibrium of split aptamers toward the formation of ligand-stabilized structure to a greater extent, which promotes the overhang binding more effectively and induces a faster displacement reaction. The strand displacement is proved to be responsive to the quantity of the stimulus. The rate constants span 2 orders of magnitude (Table S3) and the reaction kinetics can be more precisely regulated. The apparent dissociation

constant (K_d)[4] is calculated to be 15.5 μM (Fig. S3) and is close to the known dissociation constant of the selected aptamer for adenosine (6 μM),[5] verifying the ligand concentration-dependent strand displacement. The splitting of aptamer, extension of AS and relatively weak target-aptamer interaction may all account for the higher calculated K_d value.

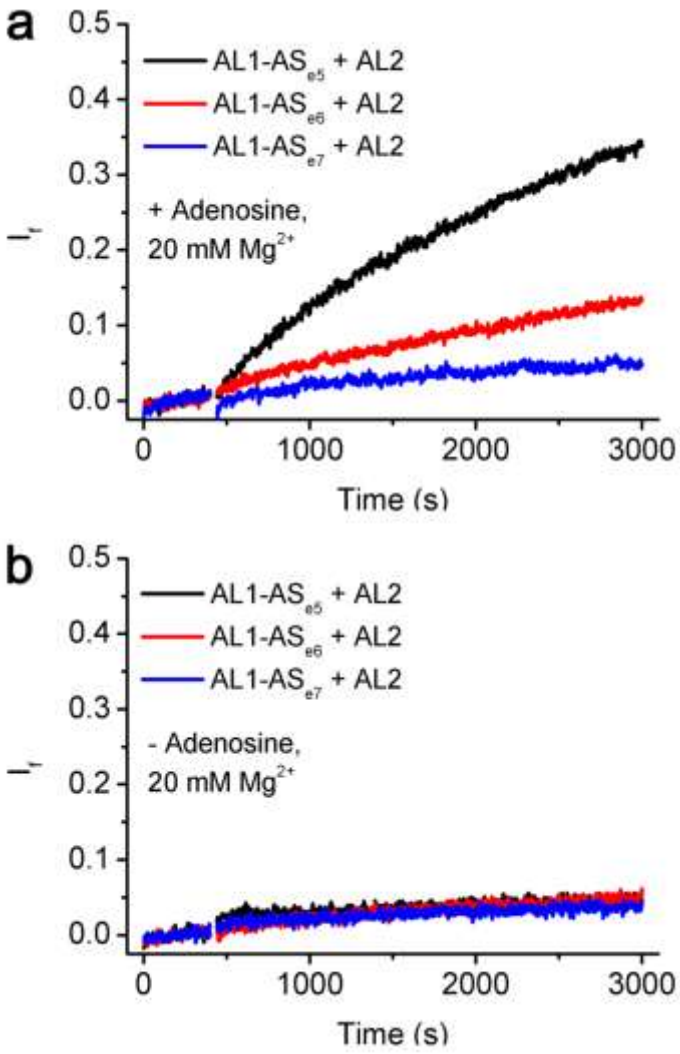

Fig. S4 Effect of Mg^{2+} (20 mM) on the strand displacement employing AS with relatively long extension. Time-dependent fluorescence changes of the strand displacement between AL1-AS_{e5} complex and AL2, AL1-AS_{e6} complex and AL2, and AL1-AS_{e7} complex and AL2 in the presence (1 mM) (a) and the absence (b) of adenosine in HEPES buffer (10 mM, pH 7.4) containing $MgCl_2$ (20 mM).

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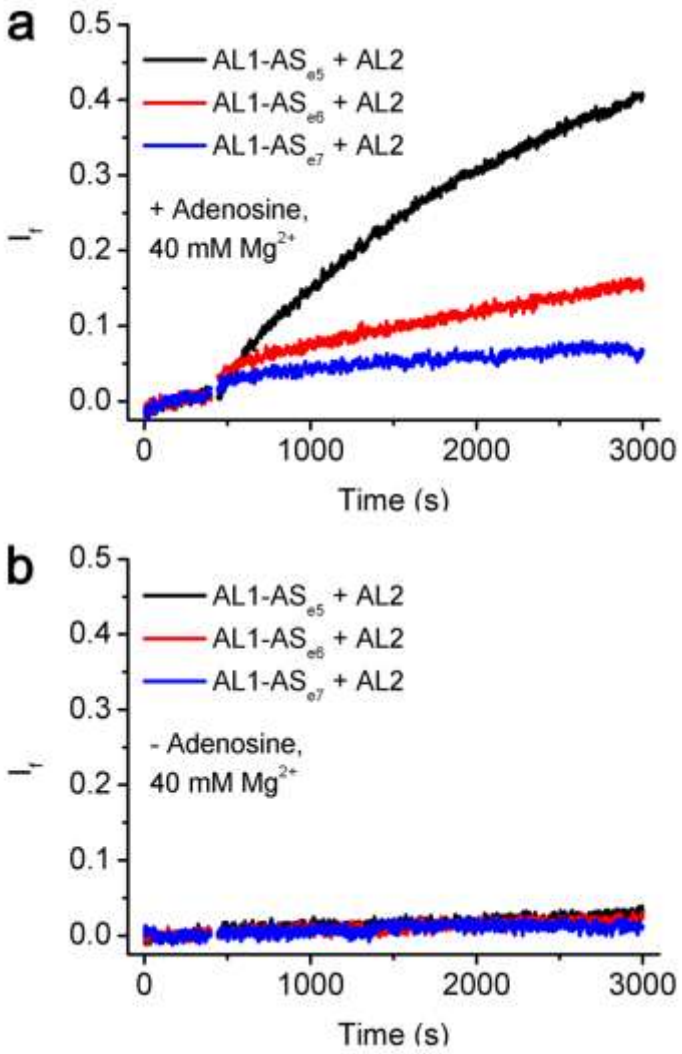

Fig. S5 Effect of Mg^{2+} (40 mM) on the strand displacement employing AS with relatively long extension. Time-dependent fluorescence changes of the strand displacement between AL1-AS_{e5} complex and AL2, AL1-AS_{e6} complex and AL2, and AL1-AS_{e7} complex and AL2 in the presence (1 mM) (a) and the absence (b) of adenosine in HEPES buffer (10 mM, pH 7.4) containing $MgCl_2$ (40 mM).

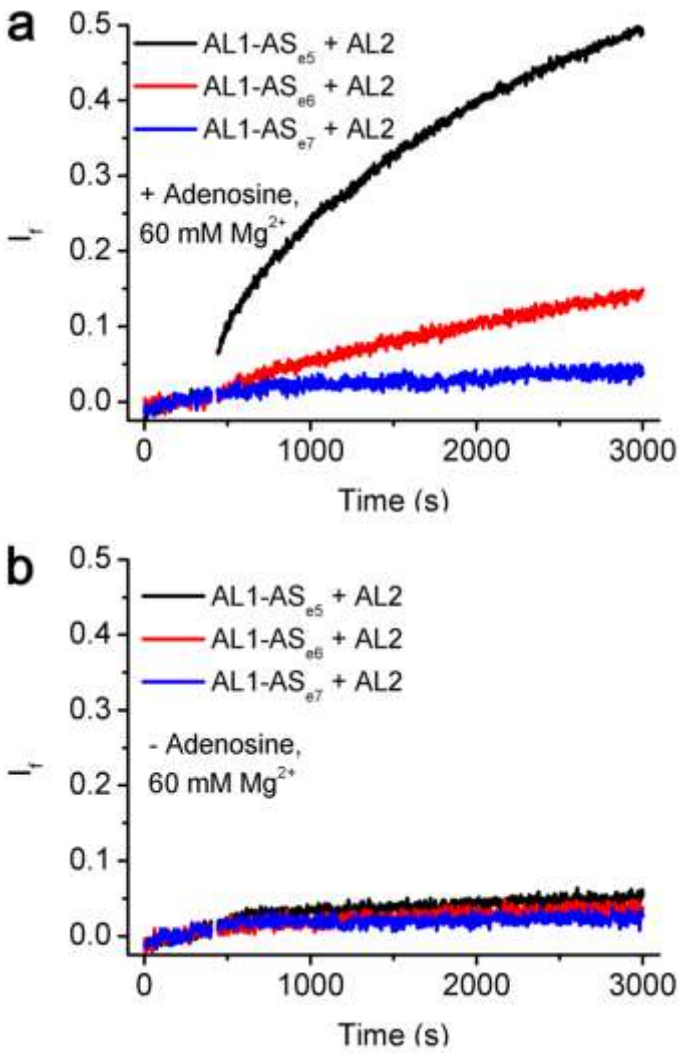

Fig. S6 Effect of Mg^{2+} (60 mM) on the strand displacement employing AS with relatively long extension. Time-dependent fluorescence changes of the strand displacement between AL1-AS_{e5} complex and AL2, AL1-AS_{e6} complex and AL2, and AL1-AS_{e7} complex and AL2 in the presence (1 mM) (a) and the absence (b) of adenosine in HEPES buffer (10 mM, pH 7.4) containing $MgCl_2$ (60 mM).

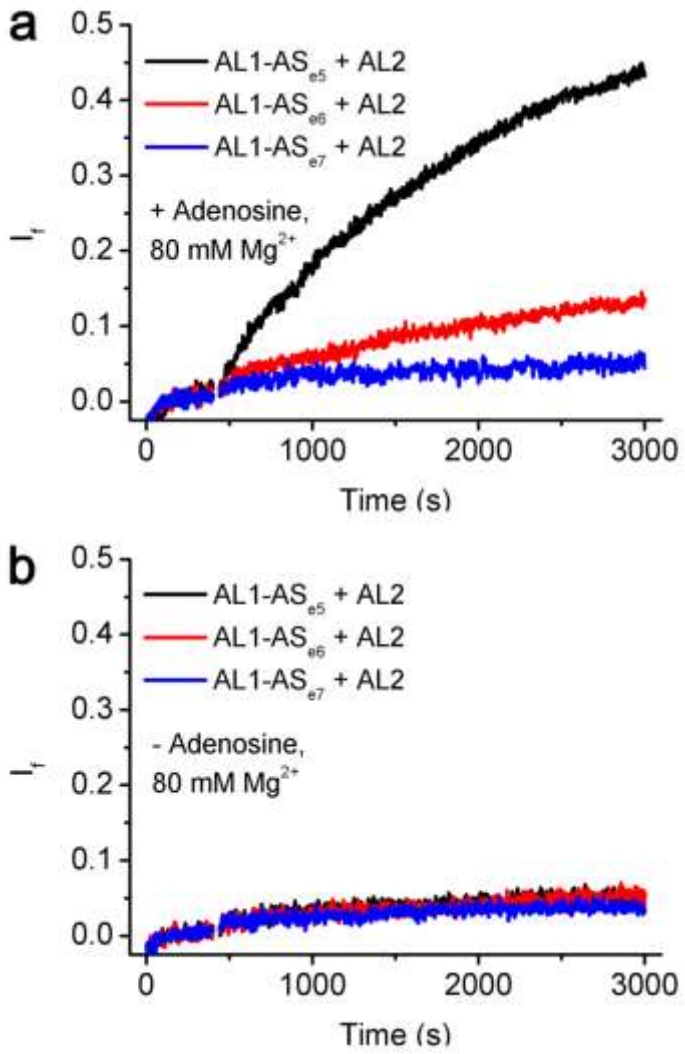

Fig. S7 Effect of Mg^{2+} (80 mM) on the strand displacement employing AS with relatively long extension. Time-dependent fluorescence changes of the strand displacement between AL1-AS_{e5} complex and AL2, AL1-AS_{e6} complex and AL2, and AL1-AS_{e7} complex and AL2 in the presence (1 mM) (a) and the absence (b) of adenosine in HEPES buffer (10 mM, pH 7.4) containing $MgCl_2$ (80 mM).

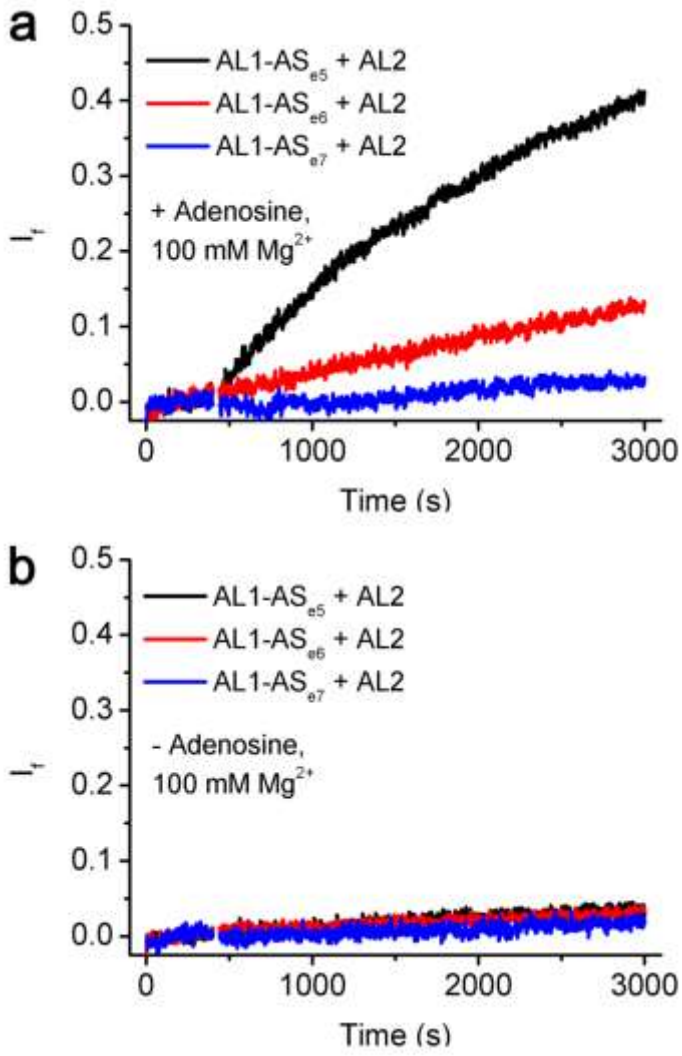

Fig. S8 Effect of Mg^{2+} (100 mM) on the strand displacement employing AS with relatively long extension. Time-dependent fluorescence changes of the strand displacement between AL1-AS_{e5} complex and AL2, AL1-AS_{e6} complex and AL2, and AL1-AS_{e7} complex and AL2 in the presence (1 mM) (a) and the absence (b) of adenosine in HEPES buffer (10 mM, pH 7.4) containing $MgCl_2$ (100 mM).

To achieve the reversion, the extension of AS should be relatively longer, which is mainly due to the following two reasons. First, the long extension of AS ensures a complete inhibition of the forward reaction in the absence of adenosine; otherwise the backward reaction contains the proceeding of forward reaction and is not thorough. Second, the long extension of AS can accelerate the backward reaction in the case excluding heating, because the rate of strand displacement expedited by oligonucleotides correlates significantly with the toehold length.[2] However, the

forward reaction in the presence of adenosine should also progress for such a long extension. Based on these considerations, further buffer conditions for the strand displacement between AL1-AS_{en} complex and AL2 were investigated by using AS_{en} with relatively longer extensions.

Experiments reveal that the introduction of magnesium ions (Mg^{2+}) to the system facilitates the forward reaction more efficiently than that of Na^{+}. Fluorescence kinetics spectra were recorded in the systems employing AS extended with five to seven bases in HEPES buffer (10 mM, pH 7.4) containing various concentrations of $MgCl_2$ (20 mM to 100 mM) (Figs S4 to S8). The greatly inhibited reaction between AL1-AS_{e5} complex and AL2 in the presence of adenosine in the buffer containing Na^{+} (Fig. S2a) can proceed unambiguously in the buffer containing Mg^{2+} (Figs S4a to S8a). Even for the case between AL1-AS_{e6} complex and AL2, the strand displacement can also occur in the presence of adenosine, albeit more slowly. The unique effect of Mg^{2+} is ascribed to two factors. First, Mg^{2+} enhances the adenosine-induced association of split aptamers since the selection buffer for adenosine-binding aptamer contains Mg^{2+}.[5] Second, previous studies reveal that the kinetics of a entropy-driven DNA catalytic reaction is much faster when using the TE buffer containing 12.5 mM Mg^{2+} than that containing 100 mM Na^{+},[6] and the rate of strand exchange is considerably higher in the presence of Mg^{2+} for the generation of two homoduplexes from two heteroduplex substrates via branch migration,[7] which both indicate that the presence of Mg^{2+} contributes to the proceeding of strand displacement. Further extension of AS to employ AS_{e7} suppresses the reaction, indicating that the reassembly of split aptamers in the presence of adenosine to compete off seven base-pairings is very difficult. In contrast, for all the three cases employing AS_{e5}, AS_{e6} and AS_{e7}, the strand displacement always proceeds slowly and is greatly restrained in the absence of adenosine (Figs S4b to S8b). This suggests that the only introduction of Mg^{2+} can not accelerate the strand displacement if adenosine is excluded, highlighting the role of adenosine as a stimulus. The adenosine-enhanced strand displacement yields the fastest kinetics upon introducing 60 mM Mg^{2+} under a series of concentrations investigated, thus 60 mM is used as the optimized Mg^{2+}

concentration.

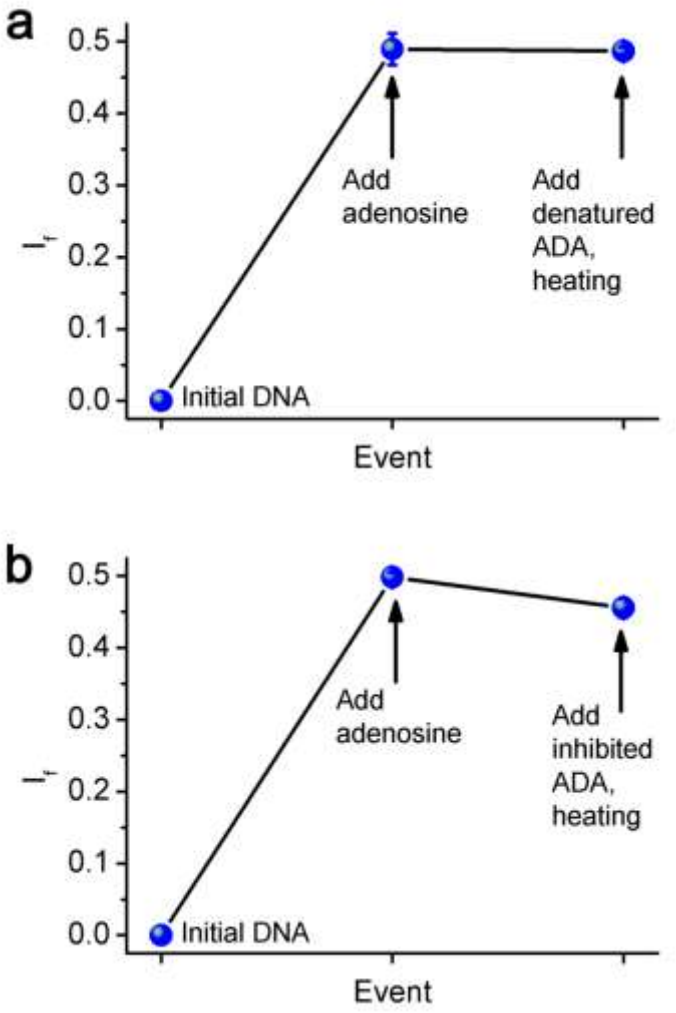

Fig. S9 Control experiments in the reversion by using inactive ADA enzyme. Normalized fluorescence intensities recorded in the following events: initial system with AL1-AS_{e5} complex and AL2; addition of adenosine (1 mM); and addition of denatured ADA (5 units) (a) or pre-incubated ADA (5 units) / EHNA (15 μM) mixture (b) with a later heating. The reaction proceeds in HEPES buffer (10 mM, pH 7.4) containing $MgCl_2$ (60 mM).

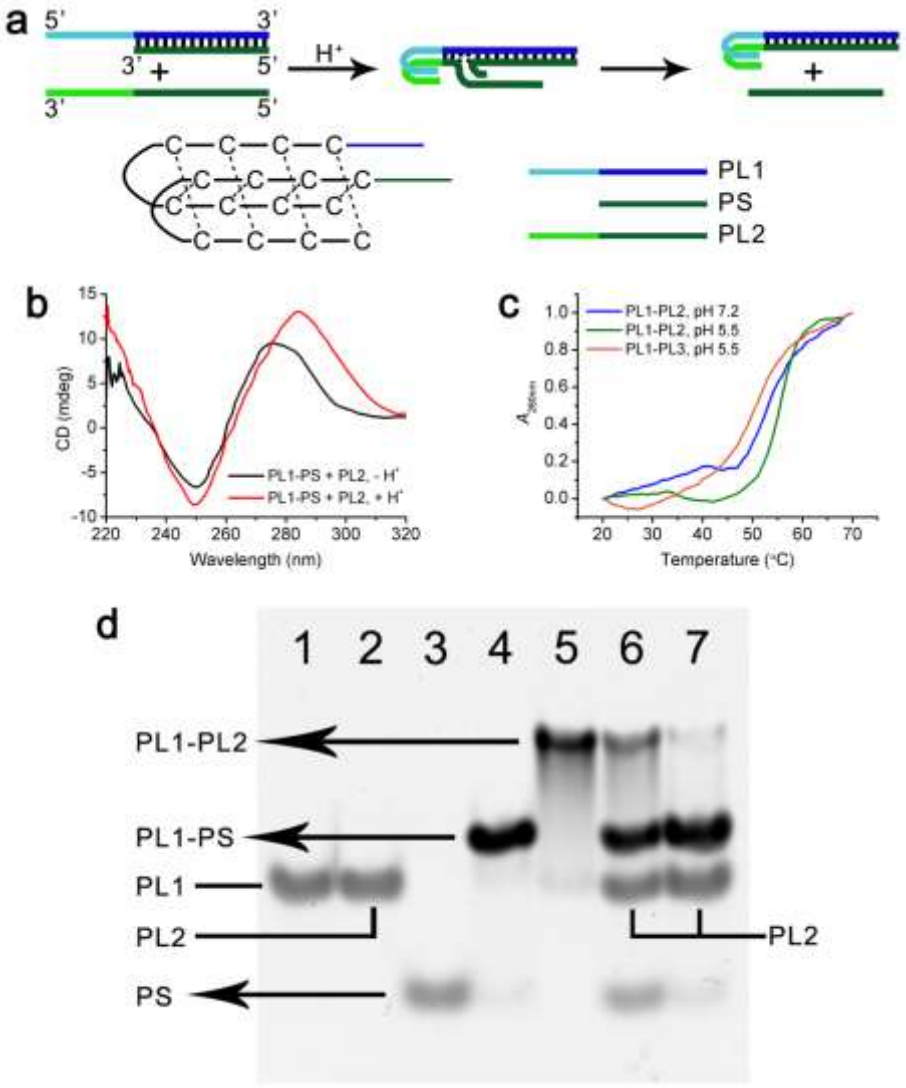

Fig. S10 Strand displacement between PL1-PS complex and PL2 with cytosine-rich sequences as toeholds. (a) Mechanism of the DNA strand displacement responsive to proton. The light blue domain in strand PL1 and the light green domain in strand PL2 form the intermolecular i-motif structure upon proton introduction, and the overhang binding promotes the branch migration to release strand PS. (b) CD spectra of PL1-PS complex and PL2 in 50 mM MES / 300 mM NaCl buffer at pH 7.2 and at pH 5.5. (c) Normalized melting curves at 260 nm UV absorbance of PL1-PL2 complex in 50 mM MES / 300 mM NaCl buffer at pH 7.2 and at pH 5.5 and PL1-PL3 complex in 50 mM MES / 300 mM NaCl buffer at pH 5.5. (d) Native PAGE characterization. Lane 1: strand PL1, lane 2: strand PL2, lane 3: strand PS, lane 4: PL1-PS complex, lane 5: PL1-PL2 complex, lane 6: PL1-PS complex, PL2 and HCl, lane 7: PL1-PS complex and PL2. Typically, HCl (37 mM) was introduced to corresponding DNA in MES buffer (50 mM, pH 7.2) containing NaCl (300 mM) to lower the pH to 5.5, with measurements performed after 1 h.

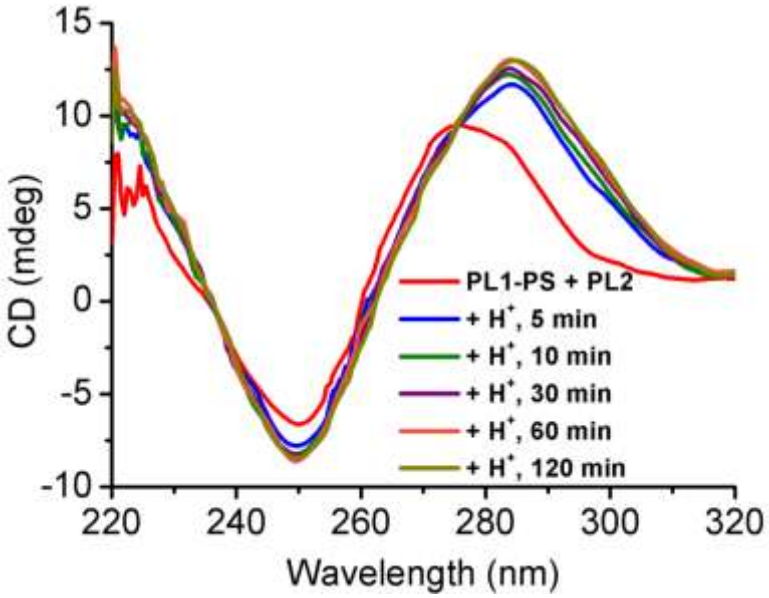

Fig. S11 Time-dependent CD spectra changes of PL1-PS complex and PL2 in MES buffer (50 mM, pH 7.2) containing NaCl (300 mM) upon HCl (37 mM) introduction. The introduction of acid lowers the buffer pH to 5.5 and induces the formation of intermolecular i-motif structure between PL1 and PL2.

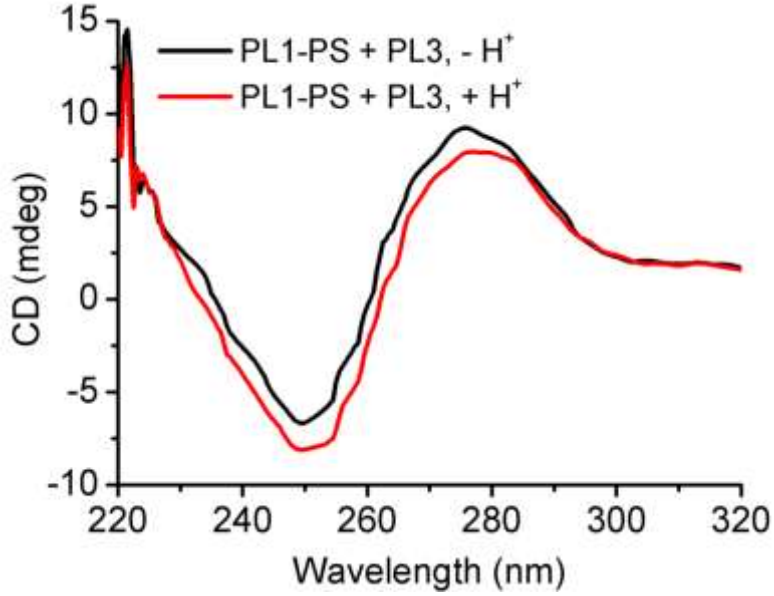

Fig. S12 CD spectra of PL1-PS complex and PL3 in MES buffer (50 mM, pH 7.2) containing NaCl (300 mM) buffer before and after the addition of HCl (37 mM). The introduction of acid lowers the buffer pH to 5.5 but does not induce the formation of intermolecular i-motif structure between PL1 and PL3.

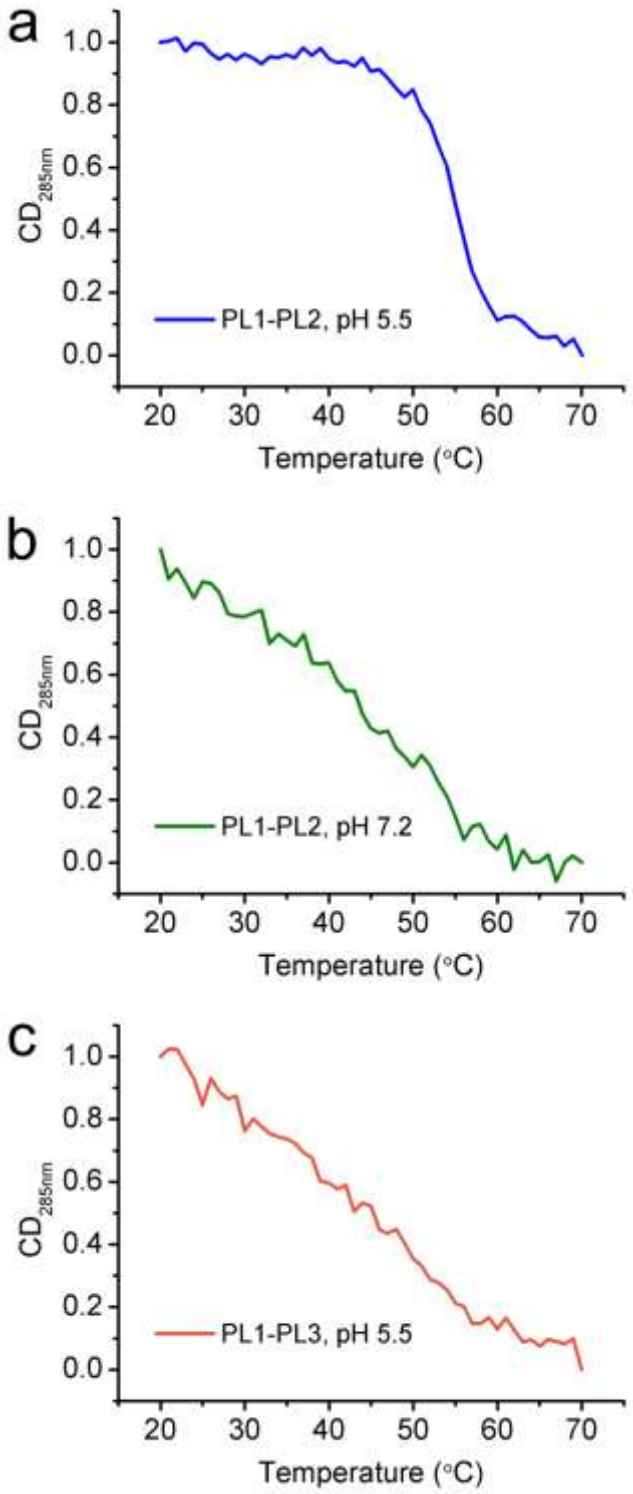

Fig. S13 Melting transition of CD positive peak at 285 nm. (a) Normalized melting curve at 285 nm CD of PL1-PL2 complex in MES buffer (50 mM, pH 5.5) containing NaCl (300 mM). (b) Normalized melting curve at 285 nm CD of PL1-PL2 complex in MES buffer (50 mM, pH 7.2) containing NaCl (300 mM). (c) Normalized melting curve at 285 nm CD of PL1-PL3 complex in MES buffer (50 mM, pH 5.5) containing NaCl (300 mM).

As outlined in Fig. S10a, the overhangs of PL1 and PL2 are both single-stranded cytosine-rich stretches and DNA oligonucleotides were dissolved in MES buffer (50 mM, pH 7.2) containing NaCl (300 mM). After introduction of specified amount of proton, the solution pH is lowered to 5.5. At acid pH cytosine is partially protonated and the noncanonical C-CH$^+$ base pair forms,[8] leading to the formation of intermolecular i-motif structure[9] and the connection of two cytosine-rich stretches in PL1 and PL2. The overhang binding can then initiate the subsequent branch migration to progress the strand displacement between PL1-PS complex and PL2 to release PS. Therefore, the proton-driven strand displacement is achieved.

Circular dichroism (CD) spectroscopy for oligonucleotides system containing PL1-PS complex and PL2 is recorded and shown in Fig. S10b. At pH 7.2, CD spectrum shows a positive band at 275 nm, revealing a B-form duplex DNA structure;[10] a negative band at 250 nm originates from the unstructured single-stranded PL2.[11] When pH is reduced to 5.5, a strong positive band at 285 nm assigned to the distinct characteristic of i-motif structure[12] appears, confirming the formation of the i-motif structure and the overhang binding between PL1 and PL2. Time-dependent CD spectra changes demonstrate that the intermolecular i-motif structure forms very quickly once the acid is introduced, with the characteristic band appearing after 5 min (Fig. S11). The positive peak near 285 nm gradually increases along with time and the CD spectrum in the whole wavelength range reaches a plateau after 1 h, indicating the fast response and accomplishment of the strand displacement stimulated by proton (also suggested in Fig. S14). If PL2 is mutated into PL3 with four cytosine residues in the cytosine-rich domain changed, the strong positive band near 285 nm is not observed in the CD spectrum after acid introduction (Fig. S12). The intermolecular i-motif structure fails to form due to the insufficient number of C-CH$^+$ base pairs and the overhang binding is impeded.

The melting curve at a wavelength of 260 nm of PL1-PL2 complex is measured and shown in Fig. S10c. The melting temperature is obtained at the maximum of the first derivative of the melting transition. At pH 7.2, PL1-PL2 complex melts at 53 °C. The introduction of acid to lower pH to 5.5 raises the melting temperature of PL1-PL2

complex to 56 °C, indicating a higher thermal stability of PL1-PL2 complex at acid pH. The increased stability is attributed to the formation of the intermolecular i-motif structure between PL1 and PL2. This is confirmed by recording the melting curve of CD positive peak at 285 nm. For PL1-PL2 complex at acid pH, the 285 nm CD shows a sharp transition as temperature increases and the i-motif structure is disrupted at 56 °C (Fig. S13a), coinciding with the melting temperature of PL1-PL2 complex. By contrast, at acid pH, PL1-PL3 complex melts at only 50 °C (Fig. S10c). The intermolecular i-motif structure can not form due to the mutated bases in PL3 and the acid condition somewhat decreases the duplex stability.[13] Since no i-motif structure forms for PL1-PL2 complex at pH 7.2 and PL1-PL3 complex at pH 5.5, their CD melting curves at 285 nm do not show sharp transitions and no melting temperature is obtained (Figs S13b and S13c).

The proceeding of the strand displacement is characterized by native polyacrylamide gel electrophoresis (PAGE) (Fig. S10d). The introduction of proton accelerates the strand displacement between PL1-PS complex and PL2, with the original shorter complementary strand PS released and the new complex PL1-PL2 generated (lane 6). By contrast, the displacement reaction can not be expedited if proton is excluded (lane 7). A very small number of PL1-PL2 complexes are produced at pH 7.2, which is attributed to that very few i-motif structures might fold at physiological or neutral pH.[14,15] In spite of this, the quantity of released PS at slightly alkaline pH is much less than that at acid pH and their ability to trigger the downstream DNA cascading reaction differs greatly. Due to consumption of the reactants PL1-PS complex and PL2 upon proton introduction, the corresponding bands in the gel become narrowed in comparison with the case excluding proton.

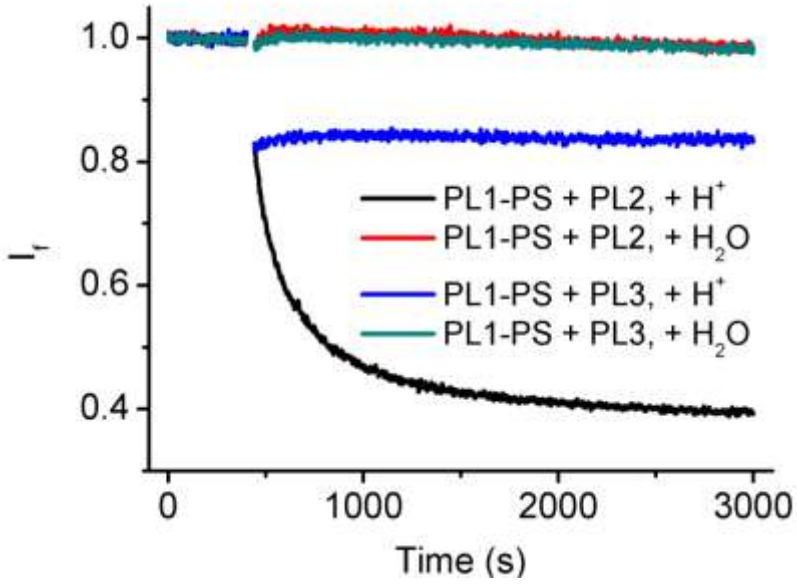

Fig. S14 Time-dependent fluorescence changes of the strand displacement between PL1-PS complex and PL2 upon addition of HCl or water and the strand displacement between PL1-PS complex and PL3 upon addition of HCl or water.

When PL1 is labeled with a quencher (BHQ-1) at its 3' end and PL2 is labeled with a fluorophore (rhodamine green) at its 5' end, the proceeding of proton-stimulated strand displacement between PL1-PS complex and PL2 will lead to the approach of fluorophore to quencher and the quenching of the fluorescence. For the strand displacement between PL1-PS complex and PL2, the fluorescence decreases fast and then gradually levels off with time upon proton introduction (black curve), verifying the proton-expedited displacement reaction. Control experiment reveals that the strand displacement between PL1-PS complex and PL2 can not be accelerated if proton is excluded (red curve). If PL1 is mutated to PL3, the strand displacement can not proceed upon proton introduction (blue curve), which results from the failing formation of intermolecular i-motif structure in the overhang and the failure of toehold binding. The decrease of fluorescence, however, is due to the fluorescence change of rhodamine green influenced by pH. This is validated by the control reaction between PL1-PS complex and PL3 excluding proton (cyan curve) and is in accordance with the previous report,[16] although the insensitivity of rhodamine green to pH between 4 and 9 is declared.[17]

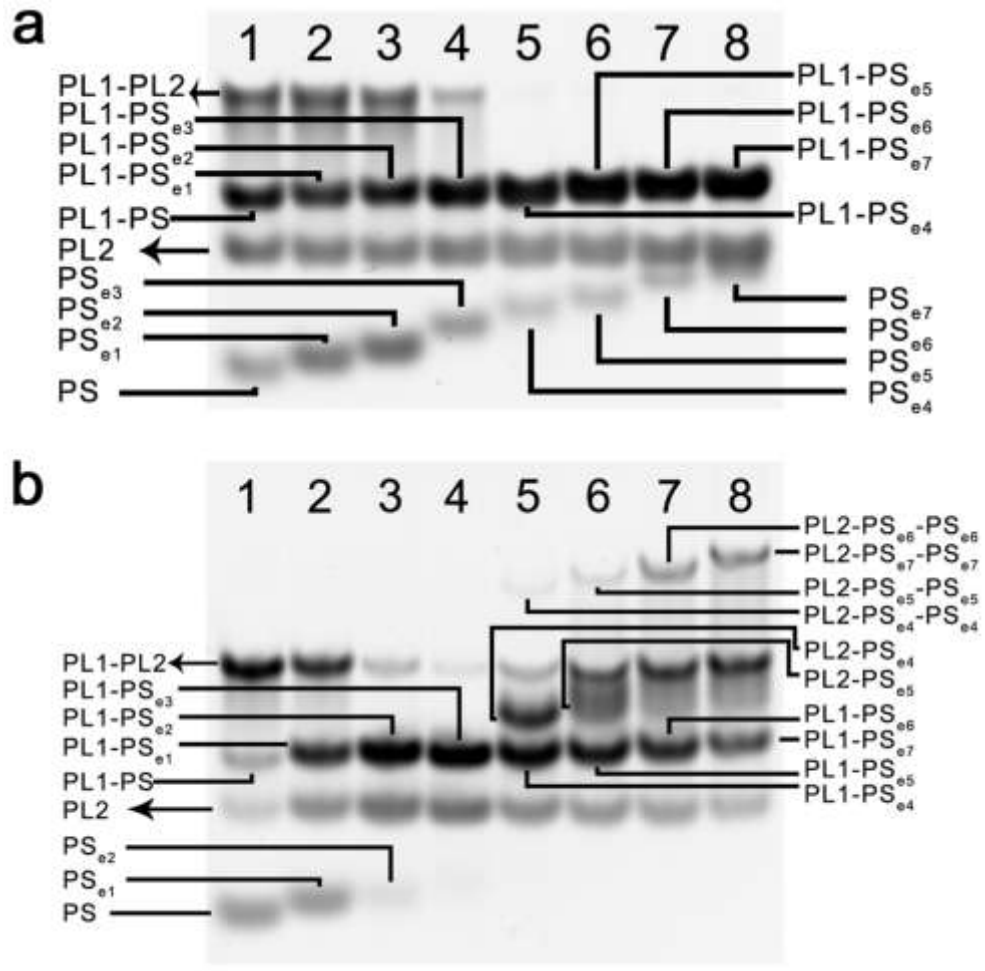

Fig. S15 (a) Strand displacement between PL1-PS$_{en}$ complex and PL2 expedited by proton with cytosine-rich stretches as toeholds. Lane 1: PL1-PS complex, PL2 and HCl, lane 2: PL1-PS$_{e1}$ complex, PL2 and HCl, lane 3: PL1-PS$_{e2}$ complex, PL2 and HCl, lane 4: PL1-PS$_{e3}$ complex, PL2 and HCl, lane 5: PL1-PS$_{e4}$ complex, PL2 and HCl, lane 6: PL1-PS$_{e5}$ complex, PL2 and HCl, lane 7: PL1-PS$_{e6}$ complex, PL2 and HCl, lane 8: PL1-PS$_{e7}$ complex, PL2 and HCl. (b) Strand displacement between PL1-PL2 complex and PS$_{en}$ at slightly alkaline pH. Lane 1: PL1-PL2 complex and PS, lane 2: PL1-PL2 complex and PS$_{e1}$, lane 3: PL1-PL2 complex and PS$_{e2}$, lane 4: PL1-PL2 complex and PS$_{e3}$, lane 5: PL1-PL2 complex and PS$_{e4}$, lane 6: PL1-PL2 complex and PS$_{e5}$, lane 7: PL1-PL2 complex and PS$_{e6}$, lane 8: PL1-PL2 complex and PS$_{e7}$.

As to the PAGE results of proton-stimulated strand displacements between PL1-PS$_{en}$ complex and PL2 (Fig. S15a), bands of PL1-PL2 complex in lane 2 and lane 3 illustrate that the displacement reaction can still progress with the extent of reaction unreduced when PS is extended with one or two bases. The weakened band of PL1-PL2 complex in lane 4 suggests that the displacement reaction is suppressed

when the bases extension reaches three. No band of PL1-PL2 complex can be observed from lane 5 to lane 8 thus further extending PS with four to seven bases completely inhibits the strand displacement. Very weak bands of strands PS_{e4} to PS_{e7} can be observed in the gel; this is attributed to the non-specific adsorption of oligonucleotides onto microcentrifuge tubes and pipette tips[2,6] leading to the slightly more amounts of shorter complementary DNA in the annealing process. As to the PAGE results of strand displacements between PL1-PL2 complex and PS_{en} at slightly alkaline pH (Fig. S15b), the gradually diminished band of PL1-PL2 complex and gradually strengthened band of PL1-PS_{en} complex from lane 1 to lane 4 indicate an enhanced displacing ability as PS extends till three bases; other evidences are the diminishing of band PS_{en} owing to the consumption and the strengthening of band PL2 owing to the release. The phenomenon that displacement reaction by the displacement domain sequence PS can proceed to a small extent (lane 1) is consistent with the previous report.[18] The strengthening of band PL1-PL2 complex and weakening of band PL1-PS_{en} complex from lane 5 to lane 8 suggest that further extension of PS with four to seven bases suppresses the displacement reaction. Dimers (Fig. S16) and trimers (Fig. S17) formed between the released PL2 and the free PS_{en} mainly poison the displacement reaction when PS is extended with four / five bases and six / seven bases respectively. The lower migration rate of PL2-PS_{en} complex than PL1-PS_{en} complex (n = 4, 5) is attributed to its more outstretched structure retarded by the sieving effect of the gel (lanes 5 and 6),[19] and the consumption of a large amount of PS_{en} (n = 6, 7) to form trimers leaves reasonable quantity of PL1-PL2 complex unreacted (lanes 7 and 8).

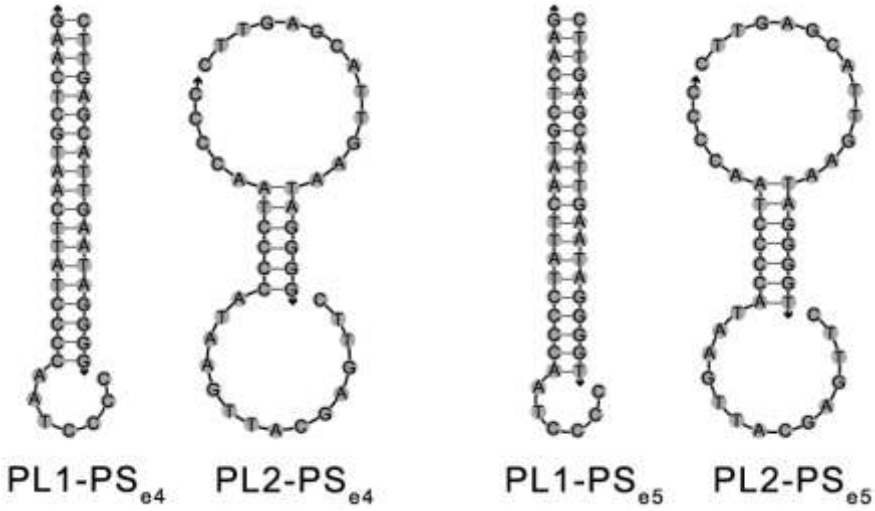

Fig. S16 Secondary structures of dimers PL1-PS$_{e4}$ complex, PL2-PS$_{e4}$ complex, PL1-PS$_{e5}$ complex and PL2-PS$_{e5}$ complex predicted by NUPACK. Detailed parameters under the experimental condition are 6 μM DNA monomers, 0.3 M Na^+ and a reaction temperature of 15 °C.

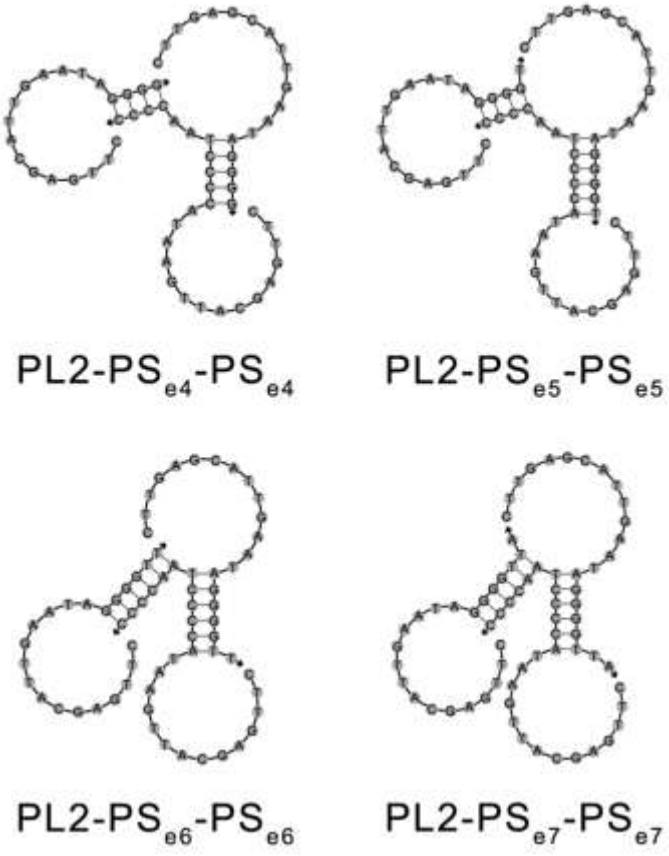

Fig. S17 Secondary structures of trimers PL2-PS$_{e4}$-PS$_{e4}$ complex, PL2-PS$_{e5}$-PS$_{e5}$ complex, PL2-PS$_{e6}$-PS$_{e6}$ complex and PL2-PS$_{e7}$-PS$_{e7}$ complex predicted by NUPACK. Detailed parameters under the experimental condition are 6 μM DNA monomers, 0.3 M Na^+ and a reaction temperature of 15 °C.

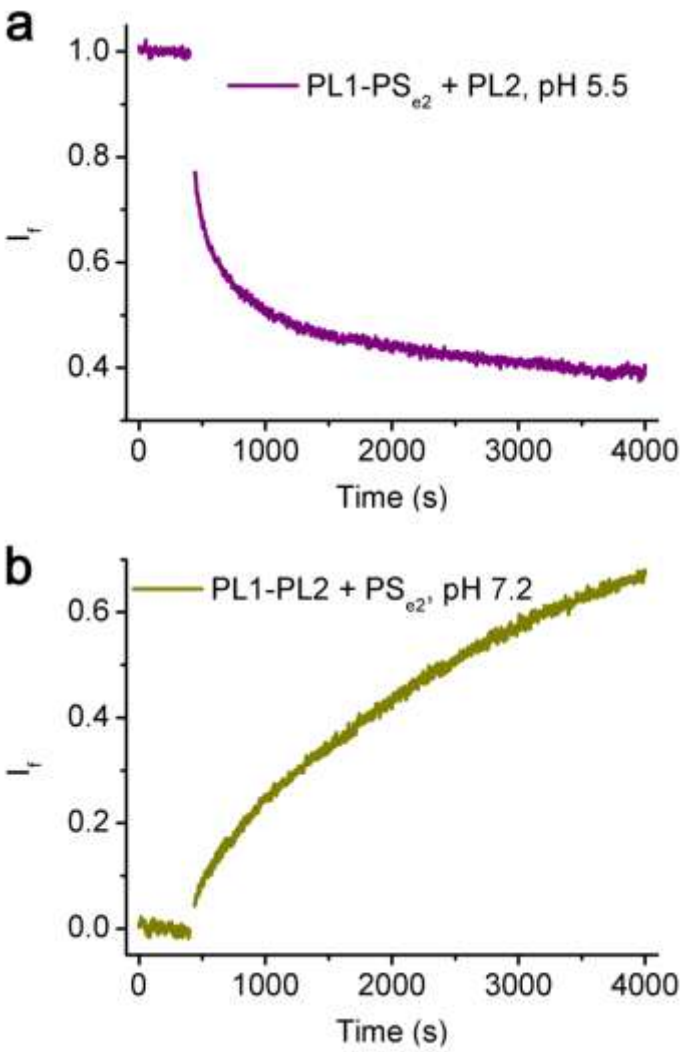

Fig. S18 Time-dependent fluorescence changes of the strand displacement between PL1-PS_{e2} complex and PL2 under pH 5.5 (a) and the strand displacement between PL1-PL2 complex and PS_{e2} under pH 7.2 (b).

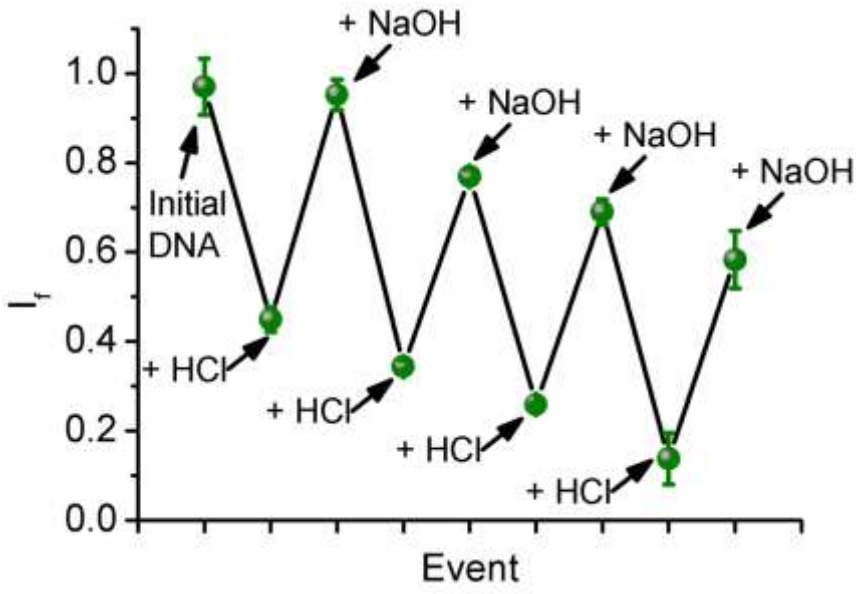

Fig. S19 Fluorescence characterization of the reversible strand displacement between PL1-PS_{e2} complex and PL2 regulated by HCl and NaOH.

As to PAGE characterization of the reversible strand displacement between PL1-PS_{e2} complex and PL2 shown in Fig. 2 in the main text, one may argue that pH of the buffer for electrophoresis (pH 8.3) will influence the stability of starting materials. The molecular proof of the reversible strand displacement is, however, indeed observed through PAGE. Although running buffer will greatly change pH of the sample with acid introduced (samples in lanes 2, 4, 6 and 8), the proceeding of backward strand displacement needs time (Fig. S18b). Samples for PAGE characterization were injected at the same time and electrophoresis was started immediately (see Experimental Section for details). The application of electric potential will separate the invading strand from DNA complex, and the displacement reaction can be greatly inhibited once the oligonucleotides penetrate into gel which is an inhomogenous, non-solution phase. Therefore, in spite of the pH change of starting materials influenced by running buffer, we believe PAGE here is a proper means to provide molecular information for the DNA reactants and products in displacement reactions and to make a clear comparison in one gel to validate the reversibility. Fluorescence characterization also confirms the reversible strand displacement (Fig. S19). The addition of HCl will initiate the forward reaction expedited by proton,

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inducing the approach of labeled fluorophore on PL2 to labeled quencher on PL1 and the decrease of fluorescence; while the addition of NaOH will initiate the backward reaction, inducing the separation of fluorophore on PL2 from quencher on PL1 and the increase of fluorescence. The gradual fall of fluorescence with cycles results from the dilution of solution when acid and alkali are added.

Table S1 Sequences of DNA oligonucleotides used in DNA strand displacement with split aptamers as toeholds.

Name	Sequence (5' to 3')	Length (nt)
AL1	**ACCTGGGGGAGTAT***CTATCTTCAAAC*-BHQ-1	26
AL2 (unlabeled)	*GTTTGAAGATAG***TGCGGAGGAAGGT**	25
AL2 (labeled)	6-FAM-*GTTTGAAGATAG***TGCGGAGGAAGGT**	25
AL3	*GTTTGAAGATAG*TGCGGA**T**GAAGGT	25
AS (AS_{e0})	6-FAM-*GTTTGAAGATAG*	12
AS_{e1}	*GTTTGAAGATAG*A	13
AS_{e2}	*GTTTGAAGATAG*AT	14
AS_{e3}	*GTTTGAAGATAG*ATA	15
AS_{e4}	*GTTTGAAGATAG*ATAC	16
AS_{e5}	*GTTTGAAGATAG*ATACT	17
AS_{e6}	*GTTTGAAGATAG*ATACTC	18
AS_{e7}	*GTTTGAAGATAG*ATACTCC	19

The bold letters in above sequences represent the split adenosine-binding aptamer fragment domains. The italic letters represent the complementary domains with the underlined domain showing complementarity to that without the underline. AL3 is a mutated sequence from AL2 with one base (marked in bold and underlined) in the split aptamer fragment domain altered. AS_{e1} to AS_{e7} extend AS by adding bases complementary to the split aptamer fragment domain of AL1 at their 3' ends.

Table S2 Rate constant (*k*) regulation of the strand displacement with split aptamers as toeholds by extending the original shorter complementary sequence. The reaction proceeds in HEPES buffer (10 mM, pH 7.4) containing NaCl (300 mM).

Stimulus / *k* / System	In the presence of 1 mM adenosine	In the absence of adenosine
AL1-AS + AL2	5.006×10^4 M^{-1} S^{-1}	1.977×10^3 M^{-1} S^{-1}
AL1-AS_{e1} + AL2	5.885×10^3 M^{-1} S^{-1}	4.389×10^2 M^{-1} S^{-1}
AL1-AS_{e2} + AL2	3.128×10^3 M^{-1} S^{-1}	2.016×10^2 M^{-1} S^{-1}
AL1-AS_{e3} + AL2	5.832×10^2 M^{-1} S^{-1}	1.611×10^2 M^{-1} S^{-1}
AL1-AS_{e4} + AL2	1.941×10^2 M^{-1} S^{-1}	92.72 M^{-1} S^{-1}
AL1-AS_{e5} + AL2	83.11 M^{-1} S^{-1}	51.41 M^{-1} S^{-1}

Table S3 Rate constant (*k*) regulation of the strand displacement with split aptamers as toeholds by adjusting the concentration of adenosine. The reaction proceeds in HEPES buffer (10 mM, pH 7.4) containing NaCl (300 mM).

System / *k* / c (Adenosine)	AL1-AS_{e1} + AL2
0 mM	4.389×10^2 M^{-1} S^{-1}
0.1 mM	7.247×10^2 M^{-1} S^{-1}
0.2 mM	1.076×10^3 M^{-1} S^{-1}
0.3 mM	1.406×10^3 M^{-1} S^{-1}
0.5 mM	2.317×10^3 M^{-1} S^{-1}
0.8 mM	3.946×10^3 M^{-1} S^{-1}
1 mM	5.885×10^3 M^{-1} S^{-1}
2 mM	9.710×10^3 M^{-1} S^{-1}

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For the system composed of the same DNA reactants, the rate constants in the presence of adenosine are always larger than that in the absence of adenosine (Table S2), numerically supporting the remark of adenosine as a stimulus to accelerate the strand displacement. The rate constant shows a big increase (over 1 order of magnitude) upon treatment with adenosine when AS is employed or the extension of AS is relatively short (n = 1, 2), and shows a small increase upon treatment with adenosine when the extension of AS is relatively long (n = 3, 4 and 5). This can be understandable as the long extension of AS hinders the adenosine-induced association of split aptamers while that association is less influenced by the short extension. For a fixed displacement system, the rate constant increases along with the increasing concentration of adenosine (Table S3). The span of the rate constant controlled by adjusting the concentration of adenosine is narrower than that controlled by extending of the original shorter complementary strand. This result can be anticipated since the variation of the rate constant tuned by adenosine concentration is supposed to be confined within the value range of rate constant in certain specified system in Table S2.

Table S4 Sequences of DNA oligonucleotides used in DNA strand displacement with cytosine-rich sequences as toeholds.

Name	Sequence (5' to 3')	Length (nt)
PL1	**CCCCTAACCCC***TATTCAATGCTCAAG*	26
PL2	*CTTGAGCATTGAATA***CCCCTAACCCC**	26
PL3	*CTTGAGCATTGAATA*C**TA**CTAAC**TA**C	26
PS (PS_{e0})	*CTTGAGCATTGAATA*	15
PS_{e1}	*CTTGAGCATTGAATA*G	16
PS_{e2}	*CTTGAGCATTGAATA*GG	17
PS_{e3}	*CTTGAGCATTGAATA*GGG	18
PS_{e4}	*CTTGAGCATTGAATA*GGGG	19
PS_{e5}	*CTTGAGCATTGAATA*GGGGT	20
PS_{e6}	*CTTGAGCATTGAATA*GGGGTT	21
PS_{e7}	*CTTGAGCATTGAATA*GGGGTTA	22
PL1 (labeled)	**CCCCTAACCCC***TATTCAATGCTCAAG*-BHQ-1	26
PL2 (labeled)	Rhodamine Green-*CTTGAGCATTGAATA***CCCCTAACCCC**	26
PL3 (labeled)	Rhodamine Green-*CTTGAGCATTGAATA*C**TA**CTAAC**TA**C	26

The bold letters in above sequences represent the cytosine-rich stretches. The italic letters represent the complementary domains with the underlined domain showing complementarity to that without the underline. The mutated bases in PL3 are underlined and marked in bold. PS_{e1} to PS_{e7} extend PS by adding bases complementary to the cytosine-rich stretch of PL1 at their 3' ends.

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Supporting Matlab Code. Sample Matlab code for fitting rate constants (adapted from reference 2).

The data from 1 s to 440 s are deleted before loading each sample since the fluorescence related to kinetics is recorded from 441 s. The normalized fluorescence value multiplies the concentration of produced AS (240 nM) in F_{max} definition or AL1-AL2 complex (240 nM) in F_{min} definition to obtain the concentration of products. Assuming the linearity of reaction in the initial stage, the variable K_0 is calculated through dividing the concentration of products by the reaction time and then by the initial concentration of DNA substrates.[20] The variable iiiii is the assumed time in which the amount of products increases linearly, and can be optimized through the reported 'fval' value and the simulated curve. For example, in the following code to load sample 'Ka12.txt' iiiii is 150 s. All fitted rate constants multiply 10^9 to convert the unit from $nM^{-1}\ S^{-1}$ to $M^{-1}\ S^{-1}$.

The main program is as follows.

```
data.data = load('./Ka12.txt');

iiiii = 150;

k0 = (data.data(iiiii,2) - data.data(1,2))/(data.data(iiiii,1) -
data.data(1,1)) / 240^2;

options = optimset( 'Display', 'iter',     ...
                    'DiffMaxChange',1e-5, ...
                    'DiffMinChange',1e-8, ...
                    'MaxFunEvals',  100000 ,  ...
                    'MaxIter',   10000 ,     ...
                    'TolFun',   1e-12 ,      ...
                    'TolPCG',    0.1 ,       ...
                    'TolX',     1e-12      );

data.y0 = [240-data.data(1,2)  240-data.data(1,2)  data.data(1,2)
data.data(1,2)];

[k, fval] = fminunc(@(k) errfunction(k,data), k0);

load('temp.mat')
```

```
figure

hold on

plot(data.data(:,1),data.data(:,2))
plot(redata(:,1),redata(:,2),'r')

grid on
axis([0 3500 0 250])

title(['k = ' num2str(k)])
```

The error function is as follows; the Matlab function *ode23s* is used to simulate the reactions defined in the function *react*.

```
function err = errfunction(k,data)

t = data.data(:,1);
y0 = [data.y0 k];

%options = odeset('RelTol', 1e-4, 'AbsTol', 1e-30);

[t,y] = ode23s(@react, t, y0);

%save('y.mat','y')

err = sum( (y(2:end,4) - data.data(2:end,2)).^2 ./ y(2:end,4) )

redata = [t'; y(:,4)'];

save('temp.mat','redata')
```

The *react* function is defined as follows.

```
function dy = react(t,y)

dy = zeros(5,1);

dy(1) = - y(5) * y(1) * y(2) ;
dy(2) = - y(5) * y(1) * y(2) ;
dy(3) =   y(5) * y(1) * y(2) ;
dy(4) =   y(5) * y(1) * y(2) ;
```

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Experimental section

Materials. DNA oligonucleotides were synthesized and purified by HPLC in Sangon Biotech (Shanghai) Co., Ltd., and Rhodamine Green-labeled oligonucleotides were synthesized and purified by HPLC in Takara Biotechnology (Dalian) Co. Ltd. Sequences of DNA oligonucleotides (see Tables S1 and S4) were designed by aid of NUPACK and confirmed by NUPACK (http://www.nupack.org/) to have minimal secondary structures. Concentrations of DNA were determined according to UV absorbance at 260 nm and the extinction coefficient of each sequence. 4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES), 2-morpholinoethanesulphonic acid (MES) monohydrate, adenosine, adenosine deaminase (ADA) and erythro-9-(2-hydroxy-3-nonyl)adenine hydrochloride (EHNA) were purchased from Sigma. Cytidine, uridine and guanosine were bought from Bio Basic Inc.

Instrumentation. Time-dependent fluorescence changes were recorded on a luminescence spectrometer (PerkinElmer LS55) with a data interval of 1 s. When recording adenosine-accelerated strand displacement, the excitation and emission wavelengths were set at 486 nm and 520 nm respectively and the excitation and emission slits were set as 5 nm and 10 nm respectively. When recording proton-regulated strand displacement, the excitation and emission wavelengths were set at 502 nm and 530 nm respectively. UV-vis absorption spectra were recorded on a UV-Visible spectrophotometer (Varian Cary 50). CD spectra and melting curves were recorded on a spectropolarimeter (JASCO J-820) equipped with a programmable temperature-control unit. CD spectra were collected from 220 nm to 320 nm with a scan speed of 100 nm/min. Melting transitions were obtained by monitoring the UV absorbance at 260 nm or CD at 285 nm as a function of temperature at a rate of 1 °C/min. Native PAGE (20%) experiments were run at 40 V for 6 h in 1 × TBE buffer consisting of Tris (89 mM), boric acid (89 mM) and EDTA (2 mM). After electrophoresis, the gels were stained with SYBR Gold nucleic acid gel stain (Invitrogen) for 1 h and photographed with a fluorescence imaging system (Vilber Infinity 3000). The annealing processes were performed with a PCR thermal cycler

(Applied Biosystems 2700) to obtain DNA complexes. Stoichiometric amounts of oligonucleotides were mixed and heated to 90 °C for 2 min and cooled to 15 °C at a constant rate over a course of 75 min.

Strand displacement with split aptamers as toeholds. For the strand displacement between AL1-AS complex and AL2, AL1-AS complex (240 nM) was mixed with adenosine (1 mM) in HEPES buffer (10 mM, pH 7.4) containing NaCl (300 mM), followed by the fluorescence recording. AL2 (240 nM) was added at 400 s and the solution in the cuvette was mixed through rapid pipetting within 40 s. The fluorescence changes were continuously recorded till 3000 s and the background signals during the addition of AL2 were omitted.[21] To test the sequence specificity in promoting the reaction, AL3 was used instead of AL2 to process the strand displacement. To test the target specificity in promoting the reaction, cytidine, uridine and guanosine were introduced instead of adenosine to process the strand displacement.

For controlling reaction kinetics by the extension of AS, 6-FAM-labeled AL2 (240 nM) was mixed with adenosine (1 mM) in HEPES buffer (10 mM, pH 7.4) containing NaCl (300 mM), followed by fluorescence recording. AL1-AS_{en} complex (240 nM) was added at 400 s and the solution was mixed and continuously measured as mentioned above. For controlling reaction kinetics by the concentration of adenosine, 6-FAM-labeled AL2 (240 nM) was mixed with different concentrations of adenosine within the range from 0.1 mM to 2 mM in HEPES buffer (10 mM, pH 7.4) containing NaCl (300 mM), followed by fluorescence recording. AL1-AS_{e1} complex (240 nM) was added at 400 s and the solution was mixed and continuously measured. As to K_d calculation, 6-FAM-labeled AL2 (240 nM) was mixed with different concentrations of adenosine within the range from 0.005 mM to 2 mM, and the average fluorescence recorded in 400 s was taken as the initial intensity. AL1-AS_{e1} complex (240 nM) was added and the mixture was incubated for 12 h to render the reaction to equilibrium, and the average fluorescence again recorded in 400 s was taken as the saturation intensity. The normalized saturation intensity was plotted against the adenosine concentration and K_d was taken as the target concentration that induced half-maximal

fluorescence intensity change.[4]

For the investigation of the effect of magnesium ions on the reaction, 6-FAM-labeled AL2 (240 nM) was mixed with adenosine (1 mM) in HEPES buffer (10 mM, pH 7.4) containing $MgCl_2$ (20 mM, 40 mM, 60 mM, 80 mM, and 100 mM), followed by fluorescence recording. AL1-AS_{en} (n = 5, 6 and 7) complex (240 nM) was added at 400 s and the solution was mixed and continuously measured. Stock solution of oligonucleotides was prepared in HEPES buffer (10 mM, pH 7.4) and was diluted to the desired concentration with HEPES buffer containing corresponding $MgCl_2$ (100 mM, 200 mM, 300 mM, 400 mM, and 500 mM) at a 4:1 ratio directly preceding experiments.[2]

For the reversion of the forward reaction, 6-FAM-labeled AL2 (240 nM) was mixed with AL1-AS_{e5} complex (240 nM) in HEPES buffer (10 mM, pH 7.4) containing $MgCl_2$ (60 mM). Adenosine (1 mM) was added to initiate the forward reaction and the strand displacement was allowed to proceed for 1 h. To reverse the reaction, ADA (5 units) was added and the mixture was incubated at 25 °C for 2 h, followed by heating to 90 °C and cooling to room temperature in 65 min. ADA was at the same time denatured in the heating. Fluorescence changes were recorded for 400 s at the end of each event to obtain the average intensity. Heat-denatured or EHNA-inhibited ADA was used in the control experiment. For the backward strand displacement, adenosine (1 mM) was added to the mixture of 6-FAM-labeled AL2 (240 nM), AL1-AS_{e5} complex (240 nM) and ADA (5 units), and the process was recorded by time-dependent fluorescence changes.

Strand displacement with cytosine-rich sequences as toeholds. PL1-PS complex (1 μM) and PL2 (1 μM) were mixed in MES buffer (50 mM, pH 7.2) containing NaCl (300 mM) and HCl (37 mM) was added to trigger the reaction, with CD spectra recorded after 1 h; PL3 was used as a control sequence. PL1-PL2 complex (1 μM) or PL1-PL3 (1 μM) complex in MES buffer was mixed with HCl (37 mM) to perform melting analyses. HCl (37 mM) was introduced to PL1-PS complex (6 μM) and PL2 (6 μM) in MES buffer (50 mM, pH 7.2) containing NaCl (300 mM), and the displacement reaction was allowed to proceed for 1 h with samples characterized by

PAGE. HCl (37 mM) was added to the mixture of PL1-PS_{en} complex (6 μM) and PL2 (6 μM) in MES buffer with an incubation time of 1 h, and PL1-PL2 complex (6 μM) and PS_{en} (6 μM) were mixed in MES buffer with an incubation time of 1 h, followed by PAGE characterization respectively. For reversion of the strand displacement, HCl (37 mM) and NaOH (37 mM) were alternately added to the mixture of PL1-PS_{e2} complex (6 μM) and PL2 (6 μM) in MES buffer, with the displacement reaction allowed to proceed for 1 h. To inject samples for electrophoresis at the same time, the sample in lane 9 (shown in Fig. 2) was prepared first, because it needed 8 times of acid/alkali introduction and took the longest time. Then parallel samples from lane 8 to lane 1 were prepared in order, according to the shortened preparing time they took. Finally all the samples were injected to the lanes simultaneously and the electrophoresis was started immediately. Cyclic introduction of HCl (37 mM) and NaOH (37 mM) to MES buffer (50 mM, pH 7.2) containing NaCl (300 mM) switched the solution pH between 5.5 and 7.2.

For fluorescence characterization of the proton-stimulated strand displacement between PL1-PS complex and PL2, PL1-PS complex (300 nM) and PL2 (300 nM) were mixed in MES buffer (50 mM, pH 7.2) containing NaCl (300 mM), followed by fluorescence recording. HCl (37 mM) was added at 400 s and the solution in the cuvette was mixed through rapid pipetting within 40 s. The fluorescence changes were continuously recorded till 3000 s and the background signals during the addition of HCl were omitted. To test the sequence specificity in responding to proton, PL3 was used instead of PL2 to process the strand displacement. The addition of water instead of HCl was conducted as a control. For the strand displacement between PL1-PS_{e2} complex and PL2 expedited by proton, PL1-PS_{e2} complex (300 nM) and PL2 (300 nM) were mixed in MES buffer (50 mM, pH 7.2) containing NaCl (300 mM), followed by fluorescence recording. HCl (37 mM) was added at 400 s and the solution was mixed and continuously measured. For the strand displacement between PL1-PL2 complex and PS_{e2}, fluorescence recording was applied to PL1-PL2 complex (300 nM) in MES buffer (50 mM, pH 7.2) containing NaCl (300 mM). PS_{e2} (300 nM) was added at 400 s and the solution was mixed and continuously measured. For the

reversible strand displacement, HCl (37 mM) and NaOH (37 mM) were alternately added to the mixture of PL1-PS_{e2} complex (300 nM) and PL2 (300 nM) in MES buffer (50 mM, pH 7.2) containing NaCl (300 mM). The forward reaction was allowed to proceed for 1 h while the backward reaction was allowed to proceed for 2 h, followed by fluorescence recording.

Fluorescence normalization. For the strand displacement using BHQ-1-labeled AL1, 6-FAM-labeled AS and AL2, the fluorescence intensity is normalized as:[22]

$$I_f = (F - F_0)/(F_{max} - F_0) \qquad \text{Eq. S1}$$

In equation S1, F represents the fluorescence intensity recorded at each time interval and F_0 represents the average fluorescence intensity recorded in initial 400 s. AL2 (240 nM) and AL1-AS complex (240 nM) are annealed in HEPES buffer (10 mM, pH 7.4) containing NaCl (300 mM) in the presence of adenosine (1 mM), and the average fluorescence intensity recorded in 400 s after cooling obtains F_{max}.

For the strand displacement using BHQ-1-labeled AL1, AS_{en} (n = 1 ~ 7) and 6-FAM-labeled AL2, the fluorescence intensity is normalized as:

$$I_f = (F_0 - F)/(F_0 - F_{min}) \qquad \text{Eq. S2}$$

As to the kinetics curves, F and F_0 in equation S2 have the same meaning as in equation S1. As to the point-line graph, F represents the average fluorescence intensity recorded in 400 s at the end of each event and the average intensity of the first event obtains F_0. AL1 (240 nM) and AL2 (240 nM) are annealed in HEPES buffer (10 mM, pH 7.4) containing corresponding salt in the presence of adenosine (1 mM), and the average fluorescence intensity recorded in 400 s after cooling obtains F_{min}.

For testing the strand displacement upon proton introduction using BHQ-1-labeled PL1 and Rhodamine Green-labeled PL2 or Rhodamine Green-labeled PL3, the fluorescence intensity is normalized as:

$$I_f = (F-F_{min})/(F_0-F_{min}) \qquad \text{Eq. S3}$$

In equation S3, F and F_0 have the same meaning as in equation S1. PL1 (300 nM) and PL2 (or PL3, 300 nM) are annealed in MES buffer (50 mM, pH 5.5) containing NaCl (300 mM), and the average fluorescence intensity recorded in 400 s after

cooling obtains F_{min}.

For the reversible strand displacement regulated by acid and alkali, the fluorescence intensity is normalized as:

$I_f = (F-F_{min})/(F_{max}-F_{min})$ Eq. S4

In equation S4, F represents the average fluorescence intensity recorded in 400 s at the end of each event and F_{min} has the same meaning as in equation S3. PL1-PL2 complex (300 nM) and PS_{e2} (300 nM) are annealed in MES buffer (50 mM, pH 7.2) containing NaCl (300 mM), and the average fluorescence intensity recorded in 400 s after cooling obtains F_{max}.

For the strand displacement between PL1-PL2 complex and PS_{e2} in MES buffer (50 mM, pH 7.2) containing NaCl (300 mM), the fluorescence intensity is normalized according to equation S1, in which F_{max} has the same meaning as in equation S4.

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