Electronic Supplementary Information

A universal molecular translator for non-nucleic acid targets that enables dynamic DNA assemblies and logic operations

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Text S1: Experimental Section

Materials and Reagents

All HPLC-purified DNA oligonucleotides were purchased from Sangon Inc. (Shanghai, China). The DNA sequences and modifications are listed in Table S1–S6. The DNA samples were dissolved in TE buffer (50 mM Tris(hydroxymethyl)metyl aminomethane (Tris), 1 mM ethylene diamine tetraacetic acid (EDTA); pH 8.0) and stored in the dark at 4 ^{o}C. Adenosine triphosphate (ATP), thymine triphosphate (TTP), cytosine triphosphate (CTP), and guanosine triphosphate (GTP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Bovine serum albumin (BSA), fibrinogen (FIB), human serum albumin (HSA), lysozyme (LZM), and streptavidin (SA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Thrombin (THR) was purchased from Enzyme Research (South Bend, IN, USA). Strontium chloride ($SrCl_2$) and all other chemicals employed were of analytical grade from Beijing Chemical Works (Beijing, China), and deionized water was used in all experiments.

Fluorescence Measurements

For preparation of **SO** duplex, strand **S** was mixed with strand **O** labeled by the 5'-TET and the 3'-TAMRA at 1:1 ratio in TNM buffer (50 mM Tris, 300 mM NaCl, and 5 mM $MgCl_2$; pH 7.5) with a final concentration of 1 μM for each strand. The resulting solutions were annealed by heating at 95 °C for 5 min, and then slowly cooled down to room

temperature over 2 hours.

Fluorescence measurements were performed by using an F-7000 fluorescence spectrometry (Hitachi, Japan) at 25 °C. Sample solutions were excited at 522 nm, and the emission signal was recorded with wavelength of 539 nm. For all time-dependent fluorescence tests, appropriate volumes of DNA stock solutions were added to TNM buffer to achieve 20 nM final concentration with a total volume of 1.0 mL in the cuvettes. Afterwards, corresponding concentration of ATP and strand $\mathbf{I_A}$, with 2 μL and 5 μL of volume, respectively, were added respectively and mixed quickly within 30 s. In all graphs, time $t = 0$ indicates the time of strand $\mathbf{I_A}$ being added to the solutions.

Fluorescence measurements for CHA were performed by using a DNA Engine Opticon 2 (MJ Research, USA) at 30 °C. The reaction mixture contained 50 nM $\mathbf{I_A}$, 20 nM $\mathbf{SO^*}$, 100 nM **H1**, 500 nM **H2**, 100 nM reporter **FQ**, and varying concentrations of ATP were prepared in TNM buffer. The time-dependent fluorescence of the reaction mixture was collected every 2 minutes by a multi-mode microplate reader with excitation/emission at 490/520 nm. One normalized unit (1 n.u.) of fluorescence corresponds to the fluorescence shift caused by the release of 1 nM fluorophore-labelled strand.

Native Polyacrylamide Gel Electrophoresis Experiments

In a typical experiment, the reaction mixture contained 1 μM **SO** duplex, 1 μM strand $\mathbf{I_A}$, and 2 mM ATP was incubated at room temperature for 30 min. 12% native polyacrylamide gel electrophoresis (PAGE) experiments were carried out at 110 V in 1×TBE buffer (90 mM Tris, 90 mM boric acid, 2 mM EDTA; pH 8.0) for 1 hours. After separation, PAGE gels containing DNA were stained using GelSafe Dye, and imaged by a Tanon 1600 imager (Tanon, China).

Agarose Gel Electrophoresis Experiments

20 μL samples with mixture of 2.5 μM $\mathbf{SO^*}$, 2.5 μM $\mathbf{I_A}$, 1 μM **H3**, 1 μM **H4**, and varying concentrations of ATP were prepared and incubated overnight at room temperature. The 1.5% agarose gels contained 0.05 μL SYBG I dye per milliliter of gel volume were prepared by using 1×TAE buffer. The gel was run at a constant voltage of 110 V for 40 min and scanned by a Tanon 1600 imager.

Surface Plasmon Resonance Measurements

All binding processes were monitored on-line by using a SPR-Navi 220A instrument (BioNavis, Tampere, Finland). Prior to modification, Au-sensor chip (BioNavis, Tampere, Finland) used was immersed in a boiling solution (30% H_2O_2, 28% ammonia, and deionized water in a volume ratio of 1:1:5) for 10 min. Then the cleaned sensor chip was rinsed thoroughly with deionized water, and dried by nitrogen gas prior to use. The SPR measurements were performed at 25 ^oC. A 0.1 μM **O**-SH solution was injected into the flow cell for on-line self-assembly on the gold surface for 20 min. Then, **S-SA** solution (the mixture of 100 nM streptavidin and 100 nM biotinlated **S**) was injected to hybridize with **O** for 25 min. At last, a premixed solution contained 1 μM $\mathbf{I_A}$ and 10 mM ATP was injected for 50 min.

Quartz Crystal Microbalance Measurements

All binding processes were monitored on-line by using a Q-Sense E4 QCM-D instrument (Q-Sense AB, Västra Frölunda, Sweden). Prior to modification, crystal chips (5 MHz, AT-cut) (Hrbio Co. Ltd, Beijing, China) used were immersed in a boiling solution (30% H_2O_2, 28% ammonia, and double distilled water in a volume ratio of 1:1:5) for 10 min. Then the cleaned chips were rinsed thoroughly with deionized water, and dried by nitrogen gas prior to use. The QCM-D measurements were performed at 20 ^oC with a flow rate of 10 μL min^{-1}. A 0.1 μM **O**-SH solution was injected into the QCM-D chamber for on-line self-assembly on the gold electrode of a crystal chip for 20 min. Then, **S-SA** solution (the mixture of 100 nM streptavidin and 100 nM biotinlated **S**) was injected to hybridize with **O** for 25 min. At last, a premixed solution contained 0.5 μM $\mathbf{I_A}$ and 10 mM ATP was injected for 50 min. The frequency changes were obtained at 5 overtones.

Text S2: Fluorescence Data Processing

The 27 nt output DNA **O** is labeled with a TET fluorophore at 5' end and a TAMRA quencher at 3' end. When **O** hybridizes with **S** forming the **SO** duplex, the fluorophore and the quencher are separated by 26 base pair dsDNA (approximately 9 nm). When **O** is displaced from **SO**, a random coiling of the single-stranded DNA and hydrophobic interactions bring the TET and the TAMRA sufficiently close to each other that the fluorescence of TET is almost completely quenched.[1] The strand displacement kinetics can therefore be monitored by measuring the fluorescence intensity of **O** as a function of time. The fluorescence signals are normalized using the equation

$$Normalized\ Fluorescence = \frac{F_S - F_O}{F_{SO} - F_O},$$

where F_S is the fluorescence intensity of each sample, F_O is the fluorescence intensity of **O** lone, and F_{SO} is he fluorescence intensity of **SO**. F_O and F_{SO} are measured before the beginning of each run as the ingredients are successively added.

We presume that the proposed target-triggered strand displacement is the same as the standard toehold-based strand displacement as a simple bimolecular reaction.[1] When the initial concentration of invading DNA **I** is in large excess, we consider the target-triggered strand displacement as a pseudo-first-order reaction.[2] The Normalized Fluorescence-vs-time plots were approximately fitted into a single-exponential equation:

from which the k could be obtained.

Text S3: ATP Translator

Table S1 DNA oligonucleotide sequences used in ATP translation system.

Name	Sequence (from 5' to 3')
O	TET-AACTAATCCTCAGATCCAGCTAGTGTC-TAMRA
O-SH	SH-C6-AACTAATCCTCAGATCCAGCTAGTGTC
S	ATGATGTCGGATGT GACACTAGCTGGATCTGAGGATTAGTA
S-Biotin	Biotin-TEG-ATGATGTCGGATGT GACACTAGCTGGATCTGAGGATTAGTA
I_A(0,0,0)	ACTAATCCTCAGATCCAGCTAGTGTC ACCTGGGGGAGTATTGCGGAGGAAGGT ACATCCGA[a]
I_A(1,0,0)	ACTAATCCTCAGATCCAGCTAGTGTC ACCTGGGGGAGTATTGCGGAGGAAGGT A ACATCCGA
I_A(2,0,0) / I_A_8	ACTAATCCTCAGATCCAGCTAGTGTC ACCTGGGGGAGTATTGCGGAGGAAGGT AA ACATCCGA
I_A(2,0,1)	ACTAATCCTCAGATCCAGCTAGTGTC A ACCTGGGGGAGTATTGCGGAGGAAGGT TAA ACATCCGA
I_A(2,0,2)	ACTAATCCTCAGATCCAGCTAGTGTC AA ACCTGGGGGAGTATTGCGGAGGAAGGT TTAA ACATCCGA
I_A(3,0,0)	ACTAATCCTCAGATCCAGCTAGTGTC ACCTGGGGGAGTATTGCGGAGGAAGGT AAA ACATCCGA
I_A(4,0,0)	ACTAATCCTCAGATCCAGCTAGTGTC ACCTGGGGGAGTATTGCGGAGGAAGGT AAAA ACATCCGA
I_A(0,2,0)	ACTAATCCTCAGATCCAGCTAGTGTC AA ACCTGGGGGAGTATTGCGGAGGAAGGT ACATCCGA
I_A_6	ACTAATCCTCAGATCCAGCTAGTGTC ACCTGGGGGAGTATTGCGGAGGAAGGT AA ACATCC
I_A_7	ACTAATCCTCAGATCCAGCTAGTGTC ACCTGGGGGAGTATTGCGGAGGAAGGT AA ACATCCG
I_A_9	ACTAATCCTCAGATCCAGCTAGTGTC ACCTGGGGGAGTATTGCGGAGGAAGGT AA ACATCCGAC
I_A_10	ACTAATCCTCAGATCCAGCTAGTGTC ACCTGGGGGAGTATTGCGGAGGAAGGT AA ACATCCGACA

[a] The function domains are underlined.

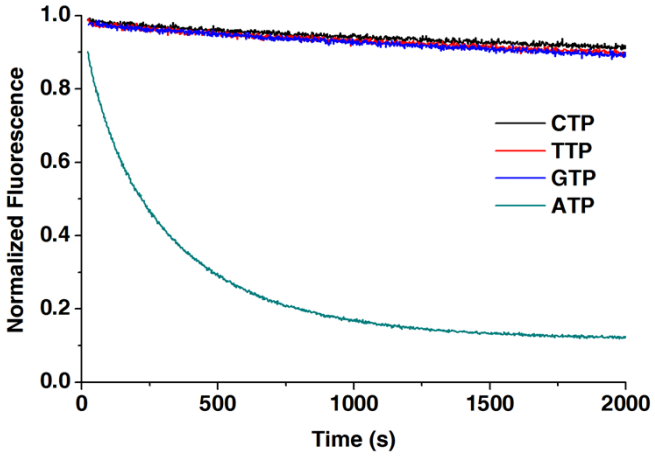

Fig. S1 Effect of ATP, TTP, CTP, and GTP on the translation kinetics of ATP translator. The reaction mixture contained 20 nM **SO** duplex, 100 nM strand $\mathbf{I_A}$, and 2 mM nucleoside triphosphate (ATP, TTP, CTP, or GTP).

Text S4: Thrombin, Sr^{2+}, and H^+ Translators

Table S2 DNA oligonucleotide sequences used in thrombin translation system.

Name	Sequence (from 5' to 3')
I_T(0,0,0)	ACTAATCCTCAGATCCAGCTAGTGTC AGTCCGTGGTAGGGCAGGTTGGGGTGACT ACATCCGACA
I_T(0,1,0)	ACTAATCCTCAGATCCAGCTAGTGTC A AGTCCGTGGTAGGGCAGGTTGGGGTGACT ACATCCGACA
I_T(1,0,0)	ACTAATCCTCAGATCCAGCTAGTGTC AGTCCGTGGTAGGGCAGGTTGGGGTGACT A ACATCCGACA
I_T(0,2,0)	ACTAATCCTCAGATCCAGCTAGTGTC AA AGTCCGTGGTAGGGCAGGTTGGGGTGACT ACATCCGACA
I_T(2,0,0) / I_T_10	ACTAATCCTCAGATCCAGCTAGTGTC AGTCCGTGGTAGGGCAGGTTGGGGTGACT AA ACATCCGACA
I_T(3,0,0)	ACTAATCCTCAGATCCAGCTAGTGTC AGTCCGTGGTAGGGCAGGTTGGGGTGACT AAA ACATCCGACA
I_T_9	ACTAATCCTCAGATCCAGCTAGTGTC AGTCCGTGGTAGGGCAGGTTGGGGTGACT AA ACATCCGAC
I_T_11	ACTAATCCTCAGATCCAGCTAGTGTC AGTCCGTGGTAGGGCAGGTTGGGGTGACT AA ACATCCGACAT

Table S3 DNA oligonucleotide sequences used in Sr^{2+} translation system.

Name	Sequence (from 5' to 3')
I_S(0,0,4)	ACTAATCCTCAGATCCAGCTAGTGTC AAAA GGTTGGTGTGGTTGG TTTT ACATCCGACA
I_S(0,0,5)	ACTAATCCTCAGATCCAGCTAGTGTC AAAAA GGTTGGTGTGGTTGG TTTTT ACATCCGACA
I_S(0,0,6)	ACTAATCCTCAGATCCAGCTAGTGTC AAAAAA GGTTGGTGTGGTTGG TTTTTT ACATCCGACA
I_S(0,0,7)	ACTAATCCTCAGATCCAGCTAGTGTC AAAAAAA GGTTGGTGTGGTTGG TTTTTTT ACATCCGACA
I_S(0,1,6)	ACTAATCCTCAGATCCAGCTAGTGTC A AAAAAA GGTTGGTGTGGTTGG TTTTTT ACATCCGACA
I_S(1,0,6)	ACTAATCCTCAGATCCAGCTAGTGTC AAAAAA GGTTGGTGTGGTTGG TTTTTT A ACATCCGACA
I_S(0,2,6)	ACTAATCCTCAGATCCAGCTAGTGTC AA AAAAAA GGTTGGTGTGGTTGG TTTTTT ACATCCGACA
I_S(2,0,6) / I_S_10	ACTAATCCTCAGATCCAGCTAGTGTC AAAAAA GGTTGGTGTGGTTGG TTTTTT AA ACATCCGACA
I_S_9	ACTAATCCTCAGATCCAGCTAGTGTC AAAAAA GGTTGGTGTGGTTGG TTTTTT AA ACATCCGAC
I_S_11	ACTAATCCTCAGATCCAGCTAGTGTC AAAAAA GGTTGGTGTGGTTGG TTTTTT AA ACATCCGACAT

Table S4 DNA oligonucleotide sequences used in H^+ translation system.

Name	Sequence (from 5' to 3')
$I_H(0,0,0)$	ACTAATCCTCAGATCCAGCTAGTGTC CCCTAACCCTAACCCTAACCC ACATCCGACA
$I_H(0,0,1)$	ACTAATCCTCAGATCCAGCTAGTGTC A CCCTAACCCTAACCCTAACCC T ACATCCGACA
$I_H(0,0,2)$	ACTAATCCTCAGATCCAGCTAGTGTC AA CCCTAACCCTAACCCTAACCC TT ACATCCGACA
$I_H(0,0,3)$	ACTAATCCTCAGATCCAGCTAGTGTC AAA CCCTAACCCTAACCCTAACCC TTT ACATCCGACA
$I_H(0,1,1)$ / I_H_10	ACTAATCCTCAGATCCAGCTAGTGTC AA CCCTAACCCTAACCCTAACCC T ACATCCGACA
$I_H(0,2,1)$	ACTAATCCTCAGATCCAGCTAGTGTC AAA CCCTAACCCTAACCCTAACCC T ACATCCGACA
$I_H(1,0,1)$	ACTAATCCTCAGATCCAGCTAGTGTC A CCCTAACCCTAACCCTAACCC TA ACATCCGACA
$I_H(2,0,1)$	ACTAATCCTCAGATCCAGCTAGTGTC A CCCTAACCCTAACCCTAACCC TAA ACATCCGACA
$I_H(0,1,2)$	ACTAATCCTCAGATCCAGCTAGTGTC AAA CCCTAACCCTAACCCTAACCC TT ACATCCGACA
$I_H(0,2,2)$	ACTAATCCTCAGATCCAGCTAGTGTC AAAA CCCTAACCCTAACCCTAACCC TT ACATCCGACA
$I_H(1,0,2)$	ACTAATCCTCAGATCCAGCTAGTGTC AA CCCTAACCCTAACCCTAACCC TTA ACATCCGACA
$I_H(2,0,2)$	ACTAATCCTCAGATCCAGCTAGTGTC AA CCCTAACCCTAACCCTAACCC TTAA ACATCCGACA
I_H_11	ACTAATCCTCAGATCCAGCTAGTGTC AA CCCTAACCCTAACCCTAACCC T ACATCCGACAT
I_H_12	ACTAATCCTCAGATCCAGCTAGTGTC AA CCCTAACCCTAACCCTAACCC T ACATCCGACATC
I_T_13	ACTAATCCTCAGATCCAGCTAGTGTC AA CCCTAACCCTAACCCTAACCC T ACATCCGACATCA

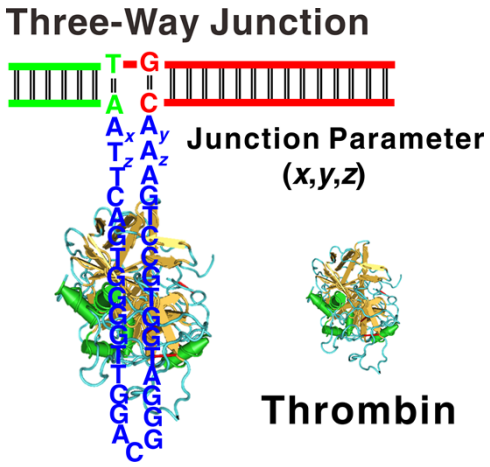

Fig. S2 Molecular translator for thrombin. Schematic of the TWJ with the designed junction parameters.

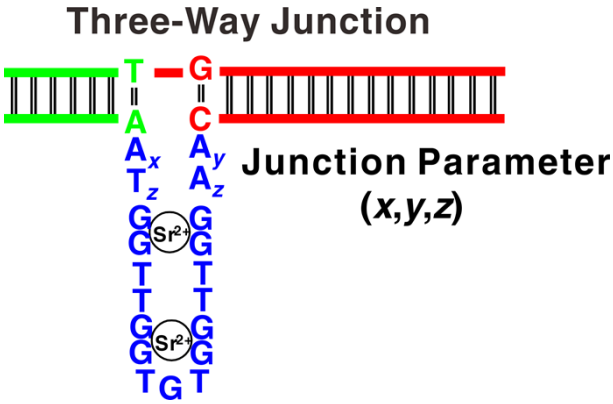

Fig. S3 Molecular translator for Sr^{2+}. Schematic of the TWJ with the designed junction parameters.

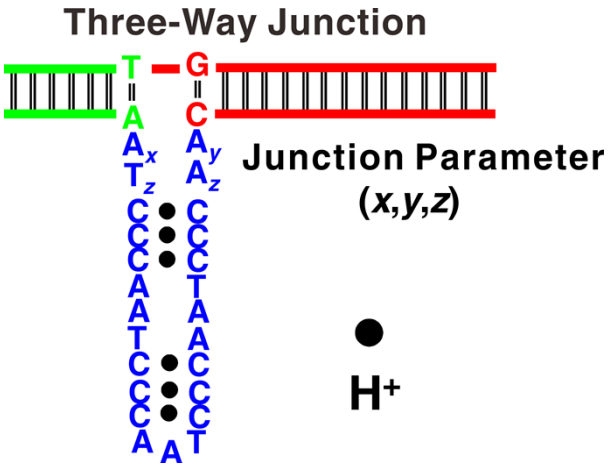

Fig. S4 Molecular translator for H^{+}. Schematic of the TWJ with the designed junction parameters.

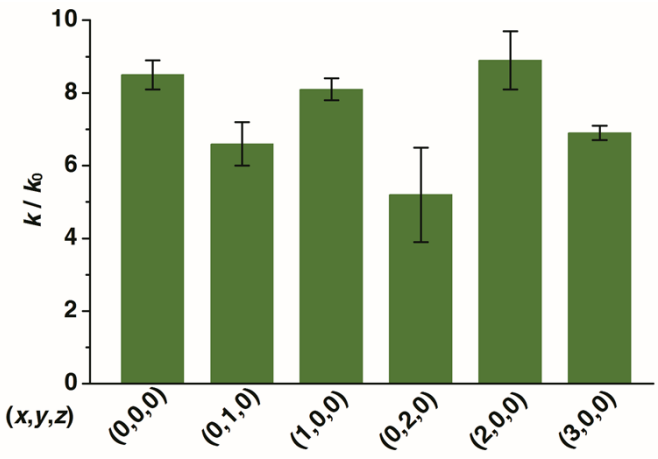

Fig. S5 Effect of the junction parameters for thrombin translator on the ratio of reaction rate constants (k/k_0). Initial concentrations: 20 nM **SO**, 100 nM $\mathbf{I_T}$, and 100 nM thrombin. Error bars represent the standard deviation of three measurements.

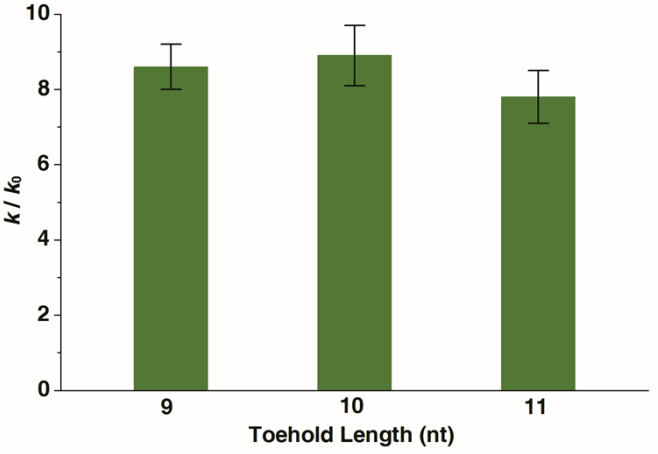

Fig. S6 Effect of the toehold lengths for thrombin translator on the ratio of reaction rate constants (k/k_0). Initial concentrations: 20 nM **SO**, 100 nM $\mathbf{I_T}$, and 100 nM thrombin. Error bars represent the standard deviation of three measurements.

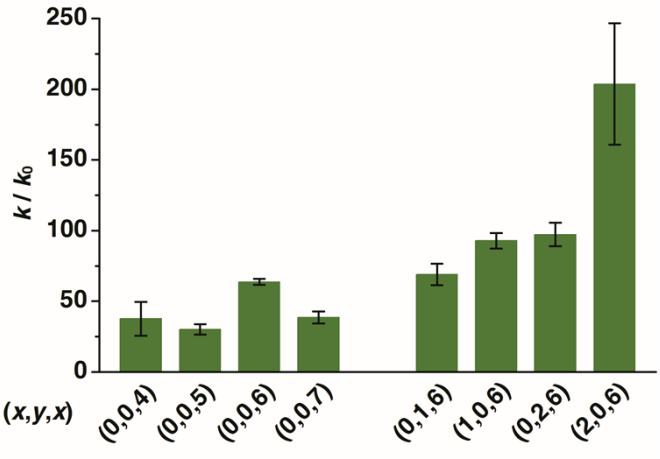

Fig. S7 Effect of the junction parameters for Sr^{2+} translator on the ratio of reaction rate constants (k/k_0). Initial concentrations: 20 nM **SO**, 100 nM $\mathbf{I_S}$, and 20 mM Sr^{2+}. Error bars represent the standard deviation of three measurements.

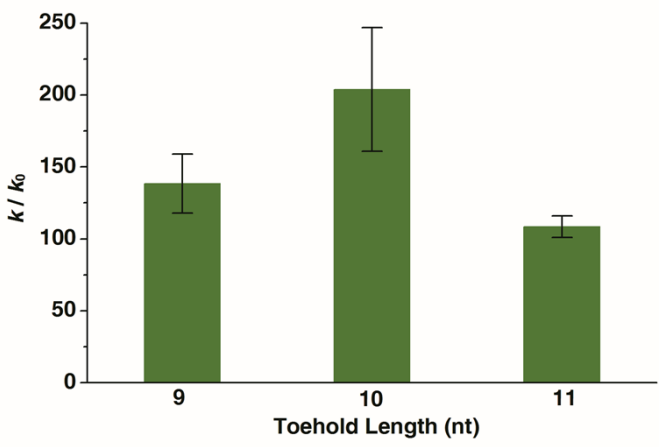

Fig. S8 Effect of the toehold lengths for Sr^{2+} translator on the ratio of reaction rate constants (k/k_0). Initial concentrations: 20 nM **SO**, 100 nM $\mathbf{I_S}$, and 20 mM Sr^{2+}. Error bars represent the standard deviation of three measurements.

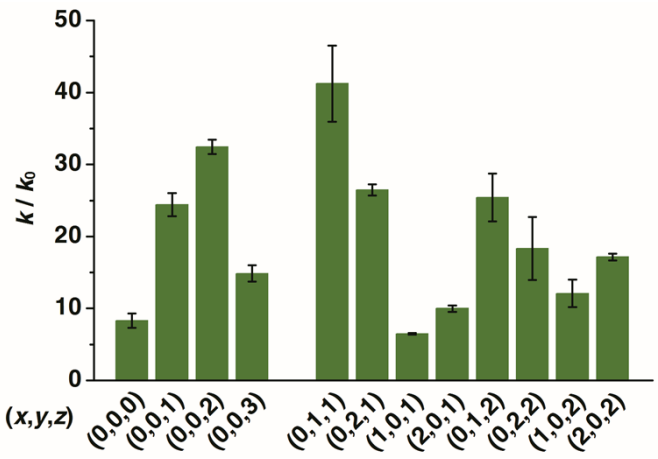

Fig. S9 Effect of the junction parameters for H^+ translator on the ratio of reaction rate constants (k/k_0). Initial concentrations: 20 nM **SO**, 100 nM $\mathbf{I_H}$, in pH 5.4 or 7.5. Error bars represent the standard deviation of three measurements.

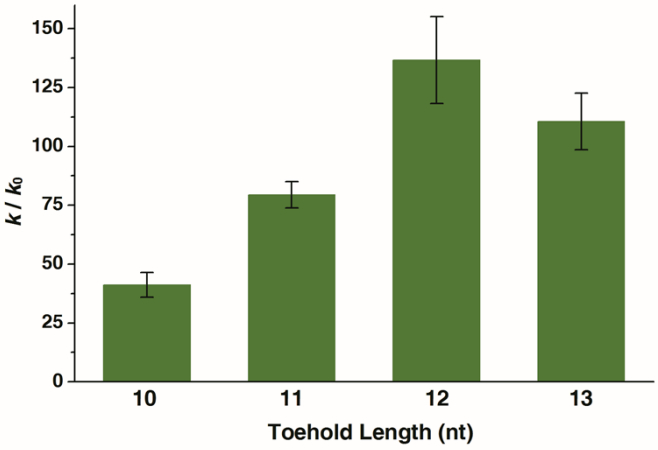

Fig. S10 Effect of the toehold lengths for H^+ translator on the ratio of the reaction rate constants (k/k_0). Initial concentrations: 20 nM **SO**, 100 nM $\mathbf{I_H}$, in pH 5.4 or 7.5. Error bars represent the standard deviation of three measurements.

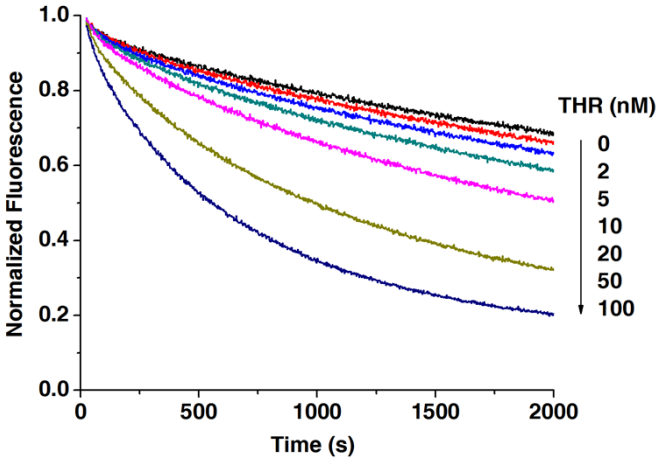

Fig. S11 Effect of the concentrations of thrombin on the translation kinetics. In a typical experiment, the **SO** duplex (1 mL, 20 nM) was placed in a cuvette, and then both $\mathbf{I_T}$ (5 μL, 20 μM) and thrombin (THR) (2 μL at the proper concentration) were added and mixed quickly within 30 s to initiate the reaction.

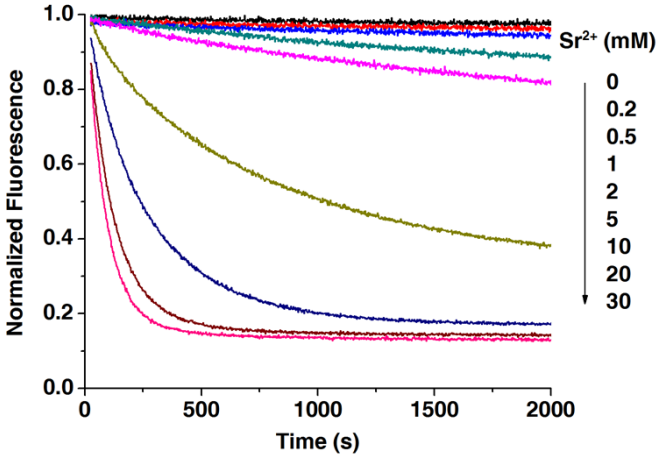

Fig. S12 Effect of the concentrations of Sr^{2+} on the translation kinetics. In a typical experiment, the **SO** duplex (1 mL, 20 nM) was placed in a cuvette, and then both $\mathbf{I_S}$ (5 μL, 20 μM) and Sr^{2+} (2 μL at the proper concentration) were added and mixed quickly within 30 s to initiate the reaction.

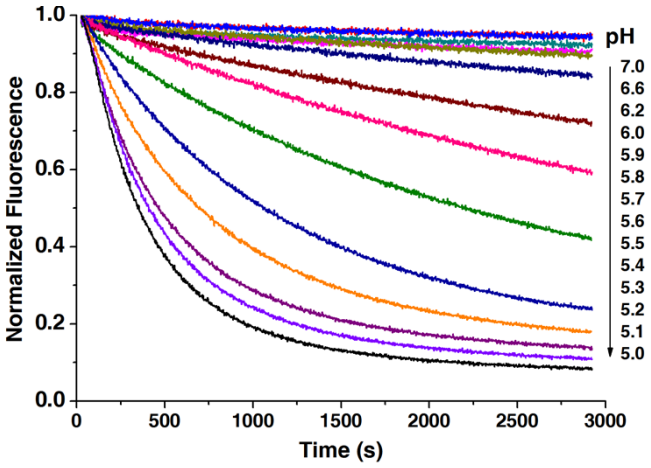

Fig. S13 Effect of the concentrations of H^+ on the translation kinetics. In a typical experiment, the **SO** duplex (1 mL, 20 nM) was placed in a cuvette with various pH solutions, and then $\mathbf{I_H}$ (5 μL, 20 μM) was added and mixed quickly within 30 s to initiate the reaction.

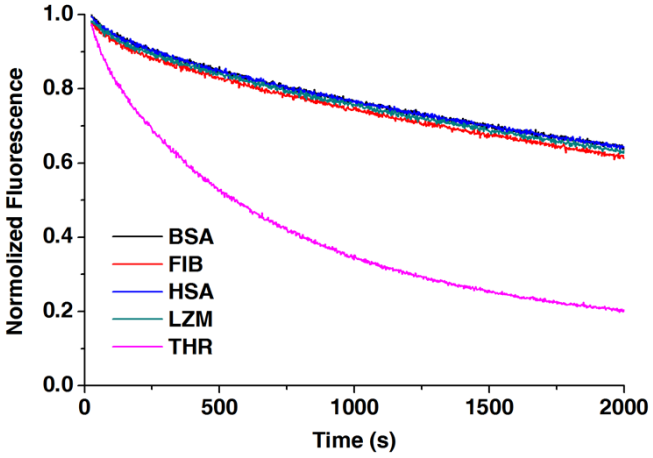

Fig. S14 Effect of BSA, FIB, HSA, LZM, and THR on the translation kinetics of THR translator. The reaction mixture contained 20 nM **SO** duplex, 100 nM strand $\mathbf{I_T}$, and 100 nM protein.

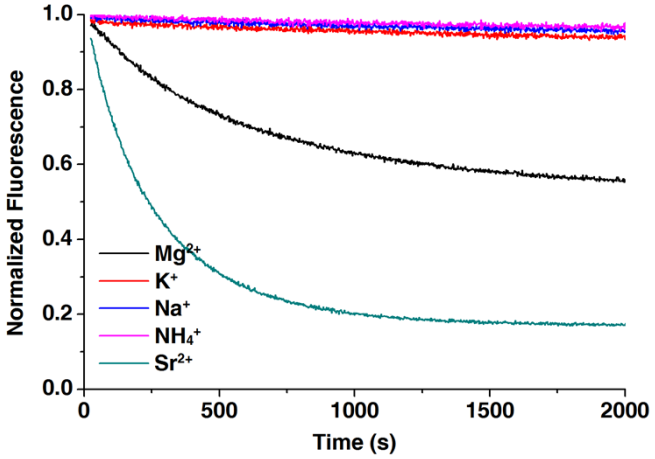

Fig. S15 Effect of Mg^{2+}, K^+, Na^+, NH_4^+, and Sr^{2+} on the translation kinetics of Sr^{2+} translator. The reaction mixture contained 20 nM **SO** duplex, 100 nM strand $\mathbf{I_S}$, and 10 mM ion.

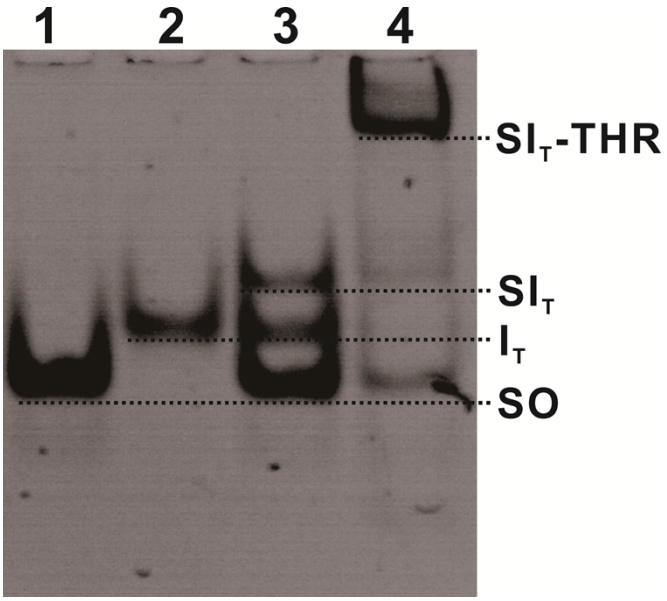

Fig. S16 Native PAGE (12%) analysis. Lane 1: 1 μM **SO**, lane 2: 1 μM $\mathbf{I_T}$, lane 3: 1 μM **SO** and 1 μM $\mathbf{I_T}$, lane 4: 1 μM **SO**, 1 μM $\mathbf{I_T}$, and 100 nM thrombin.

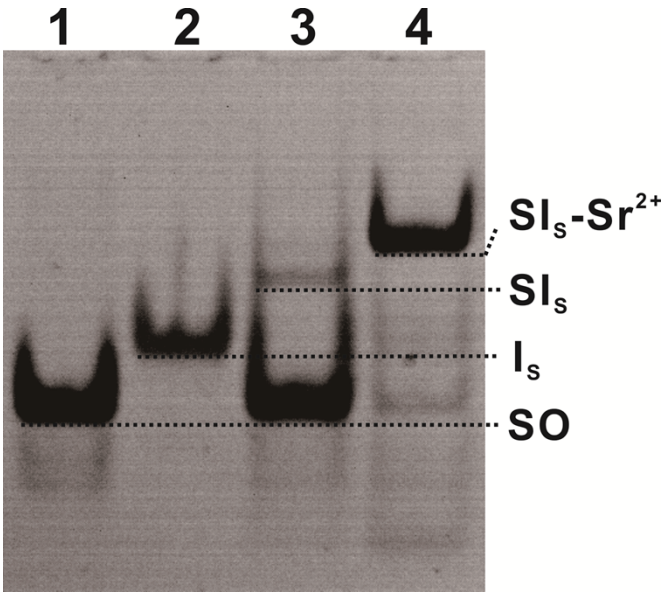

Fig. S17 Native PAGE (12%) analysis. Lane 1: 1 μM **SO**, lane 2: 1 μM $\mathbf{I_S}$, lane 3: 1 μM **SO** and 1 μM $\mathbf{I_S}$, lane 4: 1 μM **SO**, 1 μM $\mathbf{I_S}$, and 20 mM Sr^{2+}.

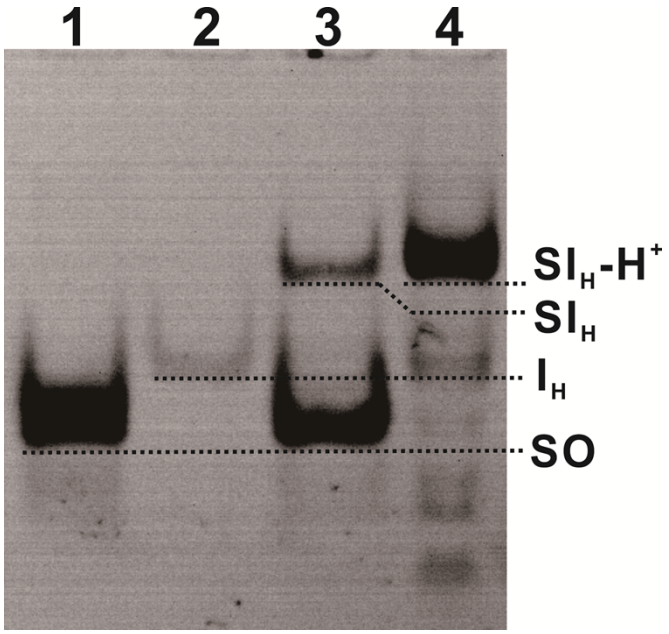

Fig. S18 Native PAGE (12%) analysis. Lane 1: 1 μM **SO**, lane 2: 1 μM $\mathbf{I_H}$, lane 3: 1 μM **SO** and 1 μM $\mathbf{I_H}$ at pH 7.5, lane 4: 1 μM **SO**, 1 μM $\mathbf{I_H}$ at pH 5.4.

Text S5: Molecular Translator-Directed CHA and HCR

Table S5 DNA oligonucleotide sequences used in CHA and HCR system.

Name	Sequence (from 5' to 3')
O*	CACCTCACTCCACTCTACTAATCC TCAGATCCAGCTAGTGTC
H1	GGATTAGTAGAGTGGAGTGAGGTGACCCGCACTTCCACCTCAC TCCACTCTCACCCTCATTTCACTC
H2	GTGAGGTGGAAGTGCGGGTCACCTCACTCCACTCTACCCGCACTTC
H3	GGATTAGTAGAGTGGAGTGAGGTGCCTACCTTCACCTCACTCCACTCT
H4	CACCTCACTCCACTCTACTAATCCAGAGTGGAGTGAGGTGAAGGTAGG
F	FAM-GAGTGAAATGAGGGTGAGAGTGGA
Q	CACCCTCATTTCACTC-Dabcyl

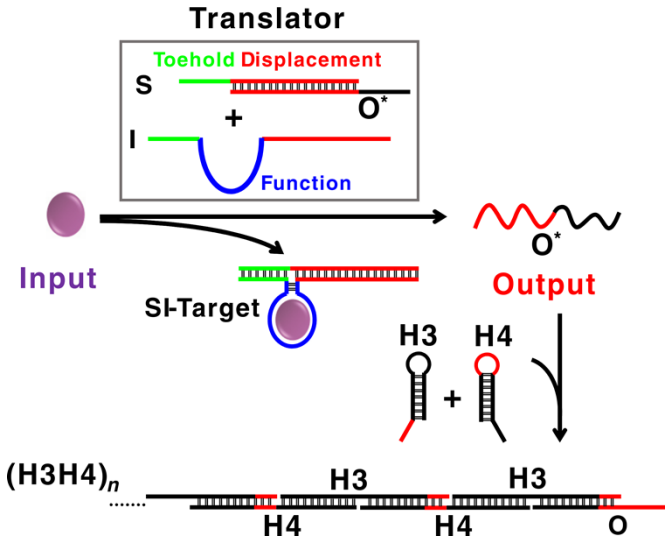

Fig. S19 Principle of the translator-directed HCR.

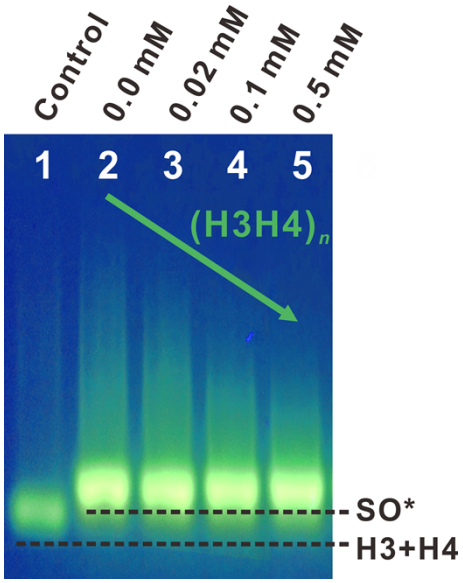

Fig. S20 Agarose gel electrophoresis (1.5%) analysis of the translator-directed HCR. The test solutions contained 2.5 μM **SO***, 2.5 μM $\mathbf{I_A}$, 1 μM **H3**, 1 μM **H4**, and varying ATP concentrations were incubated at room temperature overnight, respectively. Control test solution contained 1 μM **H3** and 1 μM **H4**.

Text S6: Logic Gates

Table S6 DNA oligonucleotide sequences used in logic gate systems.

Name	Sequence (from 5' to 3')
O	TET-AACTAATCCTCAGATCCAGCTAGTGTC-TAMRA
S_x	CCGGTTTCACATTACTTTTGCTGCCTTACGAGTCTTC
S_y	ACATCCGACTAAACCTAAACATAACCGAAGACTCGTAAGGCAGCAAAAGTAA
S_z	GGTTATGTTTAGGTTTAGTCGGATGTGACACTAGCTGGATCTGAGGATTAGT
I_x	GAAGACTCGTAAGGCAGCAAAAGTAAAGTCCGTGGTAGGGCAGGTTGGGGTGACTAATGTGAAACCGG
I_y	TACGAGTCTTCAAAAAAGGTTGGTGTGGTTGGTTTTTTAAGGTTATGTTTAGGTTTAGTCGGATGT
I_z	ACTAATCCTCAGATCCAGCTAGTGTCACCTGGGGGAGTATTGCGGAGGAAGGTTTACATCCGAC
S_{OR}	ATGATGTCGGATGTGACACTAGCTGGATCTGAGGATTAGTA
I_T	ACTAATCCTCAGATCCAGCTAGTGTCAGTCCGTGGTAGGGCAGGTTGGGGTGACTAAACATCCGACA
I_S	ACTAATCCTCAGATCCAGCTAGTGTCAAAAAAGGTTGGTGTGGTTGGTTTTTTAAACATCCGACA
I_A	ACTAATCCTCAGATCCAGCTAGTGTCACCTGGGGGAGTATTGCGGAGGAAGGTTTACATCCGA

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