## Electronic supplementary information

# Homogeneous DNA-Only Keypad Locks Enable One-Pot Assay of Multi-Inputs

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# S1. Materials and Methods

## S1.1. Materials

DNA oligonucleotides and 4S GelRed used in this study were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). The 6-FAM/Dabcyl-modified oligonucleotides were purified through high-performance liquid chromatography (HPLC), while ULTRAPAGE for non-modified oligonucleotides. All oligonucleotides were dissolved in 1×TAE/$Mg^{2+}$ buffer (40 mM Tris-Acetate, 1 mM EDTA, and 12.5 mM $Mg^{2+}$) and quantified by measuring the absorbance at 260 nm using NanoDrop 2000 Spectrophotometer (Thermo Fisher, USA) before use, then stored at -20 ℃. Other chemicals were purchased from Sinopham Chemical Reagent Co., Ltd. (China).

## S1.2. Preparation and Purification of the DNA Substrates

The multi-stranded junction substrates used in this study were prepared according to our previous report,[1] through assembling multiple purified double-stranded DNA motifs in a nominally equal molar ratio at 25 ℃ for 2 h. These double-stranded DNA motifs were annealed in a nominally equal ratio, heating up to 95 ℃ for 5 min and then slowly cooling down to 25 ℃ at a rate of 0.1 ℃/s.

Purification of the double-stranded DNA motifs and eliminators were performed using 12% non-denaturing PAGE (polyacrylamide gel electrophoresis), which was made by mixing 21 mL 40% acrylamide/bis (19 : 1), 1.4 mL 50XTAE, 420 uL APS, 42 ul TEMED in 47 mL ultrapure water and continuously stirring for 7 min. After loading the sample pre-mixed with 33% glycerol per lane, gel was run at 240 V for 6 hours using Hoefer standard vertical electrophoresis unit (Hoefer, Inc., San Francisco, CA, USA) with a PS300-B power supply at the freezer to prevent the temperature from elevating. Stained by GelRed dye, the desired substrate bands were able to be seen under UV light and cut from the gel into pieces, then soaked in buffer at 4 ℃ for two days to ensure sufficient extraction of the DNA. Finally, the supernatants were separated from gel pieces and re-quantified using NanoDrop 2000 Spectrophotometer. The purified DNA substrates were redissolved in 1×TAE/$Mg^{2+}$ buffer and stored at -20 ℃.

## S1.3. Fluorescence measurements

All fluorescence experiments were measured by Hitachi F-7000 fluorescence spectrophotometer (Hitachi High-Technologies Corporation, Tokyo, Japan) in 1×TAE/$Mg^{2+}$ buffer at 25 ℃. To confirm the performance of multi-input keypad lock security system, every reaction with mixture of 15 nM junction substrate and 30 nM each eliminator was carried out by sequentially adding 30 nM inputs in different permutations every three hours. With the use of 6-FAM fluorophore, the excitation/emission were set at 492/520 nm for kinetic characterization.

## S2. Supplementary Table

**Table S1.** Strand sequences used in the optimization of the junction substrate.

| Strand | Sequence |
|---|---|
| b1-F | **6-FAM-**TTGTGTGGTAGTATGAGTTGAGATTGGTTGTTTAGTTTG |
| t1-Q ($p_5$-15nt) | GTGTTGATAGGTAGGATCTCAACTCATACTACCACACAA-**Dabcyl** |
| t1-Q ($p_5$-5nt) | GTAGGATCTCAACTCATACTACCACACAA**-Dabcyl** |
| b2 | CAAACTAAACAACCATTGATAAGTTGGTATGAAGTAAATGTAAGTAGATGG |
| t2 ($p_5$-0nt) | TTACATTTACTTCATACCAACTTTCA |
| t2 ($p_5$-5nt) | TTACATTTACTTCATACCAACTTTCACCTAC |
| t2 ($p_5$-8nt) | TTACATTTACTTCATACCAACTTTCACCTACCTA |
| t2 ($p_5$-12nt) | TTACATTTACTTCATACCAACTTTCACCTACCTATCAA |
| t2 ($p_6$-3nt) | TTACATTTACTTCATACCAACTTTCACCTAC |
| t2 ($p_6$-4nt) | TTACATTTACTTCATACCAACTATCACCTAC |
| t2 ($p_6$-5nt) | TTACATTTACTTCATACCAACCATCACCTAC |
| t2 ($p_6$-6nt) | TTACATTTACTTCATACCAAACATCACCTAC |
| t2 ($p_6$-7nt) | TTACATTTACTTCATACCACACATCACCTAC |
| t2 ($p_6$-8nt) | TTACATTTACTTCATACCTCACATCACCTAC |
| A | CCATCTACTTACATTTACTTCATACC |
| B (8/5) | AACTTATCATCTCAACTCATACTACCA |
| B (8/4) | AACTTATCATCTCAACTCATACTACCAC |
| B (8/3) | AACTTATCATCTCAACTCATACTACCACA |
| B (8/2) | AACTTATCATCTCAACTCATACTACCACAC |
| B (8/1) | AACTTATCATCTCAACTCATACTACCACACA |
| B (8/0) | AACTTATCATCTCAACTCATACTACCACACAA |

**Table S2.** Strand sequences used in the two-, three-, and four-input keypad lock systems.

| circuit | Strand | Sequence |
|---|---|---|
| Two-input | b1-F | **6-FAM-**TTGTGTGGTAGTATGAGTTGAGATTGGTTGTTTAGTTTG |
| | t1-Q | GTAGGATCTCAACTCATACTACCACACAA**-Dabcyl** |
| | b2 | CAAACTAAACAACCATTGATAAGTTGGTATGAAGTAAATGTA |

| | | |
|---|---|---|
| | | AGTAGATGG |
| | t2 | TTACATTTACTTCATACCACACATCACCTAC |
| | $E_B$-b | TGGTAGTATGAGTTGAGATGATAA |
| | $E_B$-t | ATCTCAACTCATACTACCA |
| | $E_A$-b | TATGAAGTAAATGTAAGTAGA |
| | $E_A$-t | TTACATTTACTTCATA |
| | A | CCATCTACTTACATTTACTTCATACC |
| | B | AACTTATCATCTCAACTCATACTACCACACA |
| Three-input | b1-F | **6-FAM-**TTGTGTGGTAGTATGAGTTGAGATTGGTTGTTTAGTTTG |
| | t1-Q | GTAGGATCTCAACTCATACTACCACACAA**-Dabcyl** |
| | b2 | CAAACTAAACAACCATTGATAAGTTGGTATGAAGTAAATGTAATGGAGGTAATGAATG |
| | t2 | GATAGTTACATTTACTTCATACCACACATCACCTAC |
| | b3 | CATTCATTACCTCCATTGTAGATGGGAGGTGTTGTTGAAAGGTGTGTTTGA |
| | t3 | ACCTTTCAACAACACCTCCACCAACACTATC |
| | $E_C$-b | TGGTAGTATGAGTTGAGATGATAA |
| | $E_C$-t | ATCTCAACTCATACTACCA |
| | $E_B$-b | TATGAAGTAAATGTAAGTAGA |
| | $E_B$-t | TTACATTTACTTCATA |
| | $E_A$-b | GGTGTTGTTGAAAGGTGTGTT |
| | $E_A$-t | ACCTTTCAACAACACC |
| | A | TCAAACACACCTTTCAACAACACCTC |
| | B | CCATCTACTTACATTTACTTCATACC |
| | C | AACTTATCATCTCAACTCATACTACCACACA |
| Four-input | b1-F | 6-FAM-TTGTGTGGTAGTATGAGTTGAGATTGGTTGTTTAGTTTG |
| | t1-Q | GTAGGATCTCAACTCATACTACCACACAA-Dabcyl |
| | b2 | CAAACTAAACAACCATTGATAAGTTGGTATGAAGTAAATGTAATGGAGGTAATGAATG |
| | t2 | GATAGTTACATTTACTTCATACCACACATCACCTAC |
| | b3 | CATTCATTACCTCCATTGTAGATGGGAGGTGTTGTTGAAAGGTTGTTTGAAGTTAGTG |
| | t3 | GTGTTACCTTTCAACAACACCTCCACCAACACTATC |

| | | |
|---|---|---|
| | b4 | CACTAACTTCAAACATTGTGTTTGAGAGTGGTAGTAATAGGTG TATGAGGT |
| | t4 | CACCTATTACTACCACTCTACCCACAAACAC |
| | $E_D$-b | TGGTAGTATGAGTTGAGATGATAA |
| | $E_D$-t | ATCTCAACTCATACTACCA |
| | $E_C$-b | TATGAAGTAAATGTAAGTAGA |
| | $E_C$-t | TTACATTTACTTCATA |
| | $E_B$-b | GGTGTTGTTGAAAGGTGTGTT |
| | $E_B$-t | ACCTTTCAACAACACC |
| | $E_A$-b | GTGGTAGTAATAGGTGTATGA |
| | $E_A$-t | CACCTATTACTACCAC |
| | A | ACCTCATACACCTATTACTACCACTC |
| | B | TCAAACACACCTTTCAACAACACCTC |
| | C | CCATCTACTTACATTTACTTCATACC |
| | D | AACTTATCATCTCAACTCATACTACCACACA |

## S3. Supplementary Figure

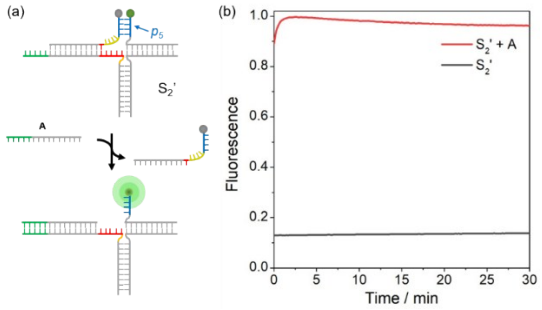


**Fig. S1** The double-stranded $P_5$ of $S_2$ was labelled with FAM and Dabcyl on the adjacent terminals to test its initial stability and disassociation efficiency upon the displacement reaction between input A and $S_2$'. In these experiments, [$S_2$'] = 15 nM, [A] = 30 nM. Fluorescence signals were normalized to 1.0, which corresponds to 15 nM of released FAM-labelled strand. Experiments were run at 25 °C in Tris-acetate-EDTA bu□er containing 12.5 mM $Mg^{2+}$ (1× TAE/$Mg^{2+}$).

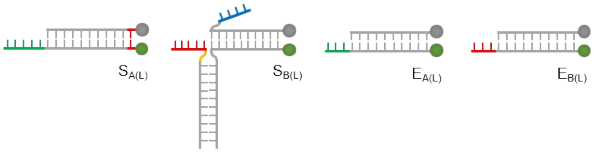


**Fig. S2** FAM- and Dabcyl-labelled $S_{A(L)}$, $S_{B(L)}$, $E_{A(L)}$, and $E_{B(L)}$.

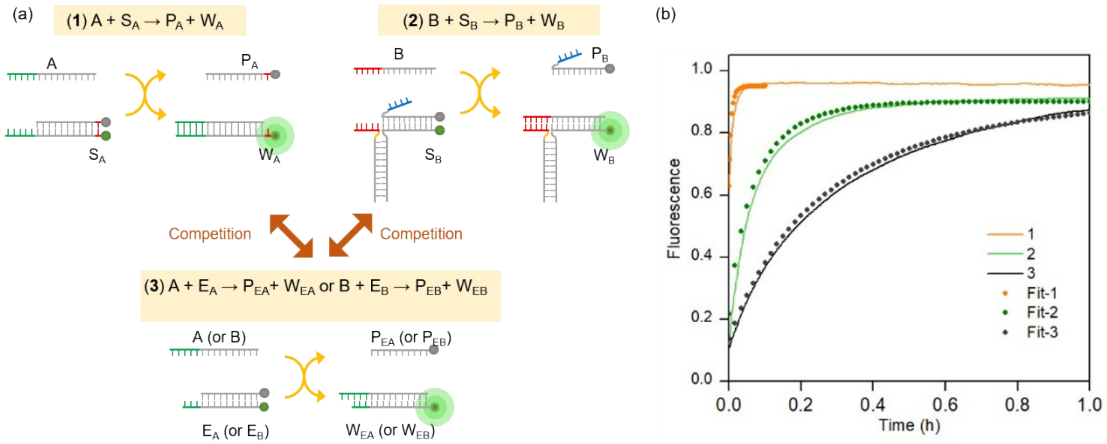


**Fig. S3** Kinetic characterization of the competitive reactions. (a) Schematics of the reactions. (b) Experimental and simulation results of the reactions. The solid curves show the experimental results, while the corresponding dotted lines denote simulations of a second-order displacement reaction with the corresponding experimental best-fit rate constants ($k_1$ = 1.85 × $10^6$ $M^{-1}$ $s^{-1}$, $k_2$ = 1.53 × $10^5$ $M^{-1}$ $s^{-1}$, and $k_3$ = 3.86 × $10^4$ $M^{-1}$ $s^{-1}$). In the experiment, [$S_A$] = [$S_B$] = 15 nM, [$E_A$] = 30 nM, [A] = [B] = 30 nM. The substrates were initially in solution at the indicated concentration, and the inputs were added at 0 h.

# References

1 X. B. Sun, B. Wei, Y. J. Guo, S. Y. Xiao, X. Li, D. B. Yao, X. Yin, S. Y. Liu, H. J. Liang, *J. Am. Chem. Soc.*, 2018, **140**, 9979-9985.