Supporting Information

Parallel DNA circuits by autocatalytic strand displacement and nanopore readout

Jinbo Zhu,[a,b] Jinglin Kong,[b] Ulrich F. Keyser[b] and Erkang Wang[a]**

[a] State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin 130022, P. R. China. E-mail: ekwang@ciac.ac.cn
[b] Cavendish Laboratory, University of Cambridge, JJ Thompson Avenue, Cambridge, CB3 0HE, UK. E-mail: jz421@cam.ac.uk

S1 Experiment details and data analysis

S1.1 Materials

DNA oligonucleotides were synthesized by Integrated DNA Technologies, Inc. (IDT). M13mp18 ssDNA was purchased from Guild BioSciences, Inc. BamHI-HF and EcoRI-HF were purchased from New England Biolabs. Other chemicals were of reagent grade and were used without further purification.

S1.2 Preparation of DNA flower (7-way junction)

Four DNA flowers (7-way junctions, 7WJa, 7WJb, 7WJc and 7WJd) were prepared separately for the four carriers. Taking 7WJa as an example, 4 μM DNA strand J1, J2, J3 and J4a were mixed together in TM buffer (10 mM Tris-HCl, 10 mM $MgCl_2$, pH 8.0) and heated at 90 ℃ for 5 min, then cooled down to 65 ℃ for 15 min, 45 ℃ for 15 min, 37 ℃ for 20 min, 25 ℃ for 20 min and finally 4 ℃ for 20 min. Strand J4a was substituted by J4b, J4c and J4d to prepared 7WJb, 7WJc and 7WJd, respectively.

S1.3 Native polyacrylamide gel electrophoresis (PAGE)

DNA substrates for each step of the ACSD circuit were separately annealed and then mixed to react for 3 hours at room temperature before gel analysis. 10 μL of DNA sample was mixed with 2 μL of 6 × loading buffer, and then analyzed by 15% native PAGE. Concentrations of DNA strands were 1 μM for *I*, *O4*, *I·W1*, *W3·O3* and *W4·O4·O5*, and 1.2 μM for *W1·O1* and *W2·O2*. 2 μM strand *I* was added into the mixture of lane 9 to trigger the reaction. Low molecular weight DNA ladder was mixed with 6 × loading buffer and added into the first lane. The electrophoresis was conducted in 1 × TBE buffer (pH 8.0) with 10 mM $MgCl_2$ at a constant voltage of 110V (10 V/cm) for 1.5 hour. The gel was stained by GelRed for 15 min before visualization on a UV transilluminator.

S1.4 Displacement efficiency (DE) and relative extent of reaction (RER)

We proposed DE to compare the fluorescence signal changes in ACSD and TMSD caused by initiator *I* and its mutant strands *M1*-*M5*. It is calculated by the following equation:

$$DE_X = \frac{I_X - I_B}{I_I - I_B}$$

where DE_X is the displacement efficiency of strand *X* (*X* = *I*, *M1*, *M2*, *M3*, *M4* or *M5*), I_X is the fluorescence intensity of the circuit triggered by strand *X*, I_B is the fluorescence intensity of the circuit without any initiator, and I_I is the fluorescence intensity of the circuit triggered by strand *I*.

RER was proposed to compare the reactions of different paths in the competition group in Figure 3. It is calculated by the following equation:

$$RER_X = \frac{I_X - I_B}{I_P - I_B}$$

where RER_X is the relative extent of reaction of path X (X = a or b), I_X is the fluorescence intensity of path X in the competition group at 600 min, I_B is the fluorescence intensity of the blank without initiator at 600 min, and I_P is the fluorescence intensity of the positive control (only path X is activated) at 600 min.

S1.5 Nanopore data analysis

We use home-made LabVIEW algorithms to collect and analyze the nanopore data, which were introduced in detail in our previous work.[1-3] The workflow can be divided into three main steps and they are briefly described as below.

Step 1: Event filter and search

The raw current data collected from the amplifier was firstly filtered by the home-made LabVIEW programs to remove the noise. Thresholds for minimum single standard deviation, current change and duration of translocation were set to conduct the first-round event search. Then event charge deficit (ECD) and nanopore dsDNA level were determined and used to exclude any fragments.

Step 2: Remove folded events

We applied two more strict filter limits to remove the folded events, which are large current drops ($> I_{event}$ + 0.02 nA) at the beginning (first 5% of the translocation, folding at the entry) and deep current drops with less than 50% current points lying between $I_{event} \pm 0.05$ nA (folding in the middle). This algorithm was introduced in detail in our former work.[2] After that only the unfolded events with clear peaks were left.

Step 3: Barcode determination and occupied fraction calculation

Finally, we used a peak detection program to find the peaks in the unfolded events, readout the barcodes to classify them and calculate the occupied fraction at the sensing site. Threshold of 0.2 ($\Delta I/I_{event}$, Figure S4) was used to find the peaks of dumbbells and DNA flowers.

S2 Figures

W1•O1	–	–	+	–	–	–	–	+	+
W2•O2	–	–	–	+	–	–	–	+	+
W3•O3	–	–	–	–	+	–	–	+	+
W4•O4•O5	–	–	–	–	–	+	–	+	+
I	+	–	–	–	–	–	–	+	–
I•W1	–	+	–	–	–	–	–	–	–

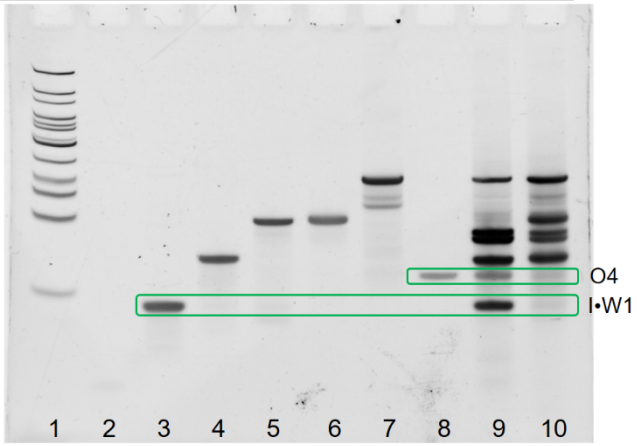

Figure S1. PAGE result of the ACSD circuit. DNA ladder is added in lane 1 and strand *O4* is added in lane 8 as a reference. The DNA strands added in each lane are given in the table above the gel. "+" in the table means the corresponding DNA is added and "-" means it is not added in the lane below the table. The DNA substrates, such as *W1·O1*, were annealed separately. In lane 9 and 10, the DNA substrates were mixed and incubated under room temperature for 3 hours before adding into the gel.

W1•O1	+	+	+	+	–
W2•O2	+	+	+	+	–
W3•O3	+	+	+	+	–
W4•O4•O5	–	–	+	+	–
W4•O4•O5s	+	+	–	–	–
I	–	+	–	+	–

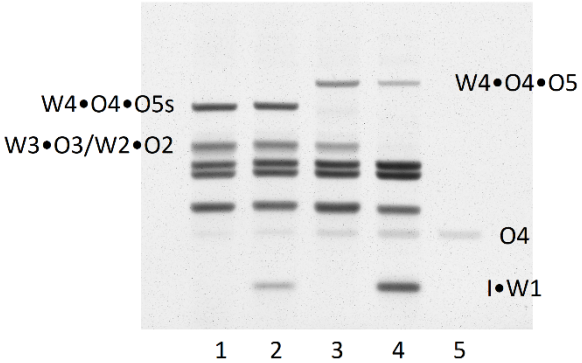

Figure S2. PAGE result of the ACSD circuits containing *O5* (lane 3 and 4) or *O5s* (lane 1 and 2). *O5s* is a control strand without domain *s1* compared to *O5*. Strand *O4* is added in lane 5 as a reference and the information of DNA strands added in other lanes is given in the table above the gel. In lane 1 and 2, there is no significant difference between the main bands after the addition of

strand *I* except the light band of *I·W1* formed in lane 2. In lane 4, the top band of *W4·O4·O5* gets light compared to the one in lane 3, the band of W3·O3/W2·O2 is missing, and a dark band of *I·W1* is formed. The band of *O4* in lane 4 is the most obvious in lane 1-4. The above result proves that a complete strand *O5* is essential for the ACSD circuit.

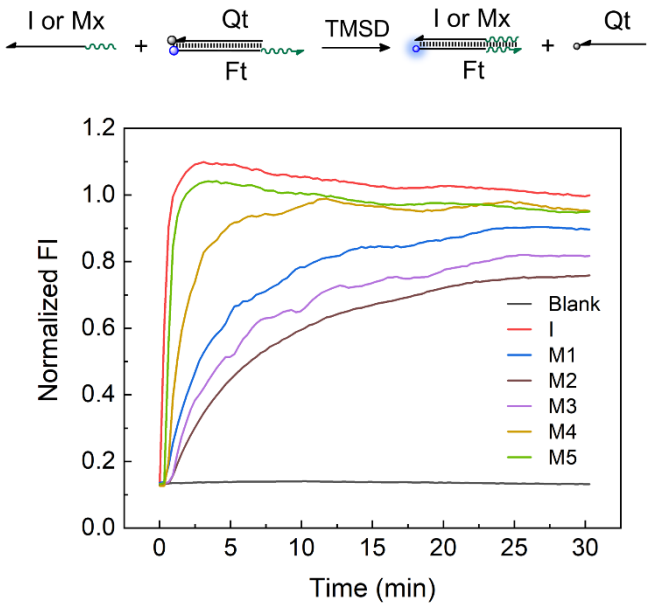

Figure S3. Scheme of the detection of target strand *I* and its mutants using the toehold-mediated strand displacement reaction (TMSD). Single base substitution is designed in the mutant strands M1-M5 at specific position. 25 nM target or mutant strand was mixed with 25 nM *Ft·Qt* complex in TM buffer at the beginning of the kinetics measurement. The sample was excited at 535 nm and the emission intensity was recorded at 556 nm.

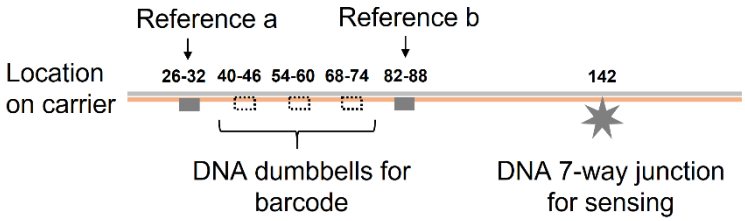

Figure S4. Design of carrier A-D for DNA output detection. There are 190 short staple strands (38 nt for each) hybridized with the linear M13 scaffold. The numbers above the DNA nanostructures indicate its location on the carrier.

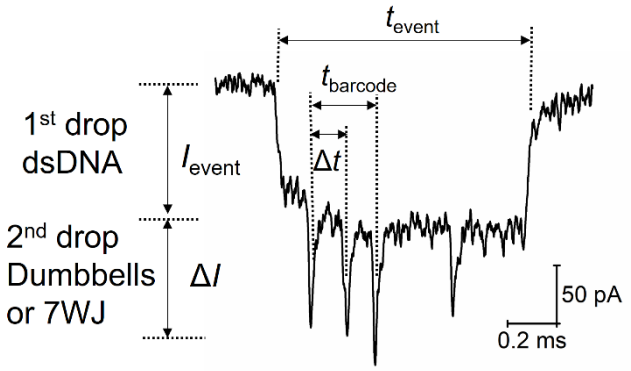

Figure S5. Analysis of a translocation event from the carrier B as an example. I_{event} is the first level current drop caused by the DNA carrier. ΔI is the second level current drop caused by DNA nanostructures on the carrier. Barcode can be determined by the relative presence time ($\Delta t/t_{barcode}$) of the downward peak between the two references. The single downward peak caused by DNA flower at the other side is used to detect the DNA target. Direction of the event can be easily judged by the asymmetric distribution of these two groups of secondary current drops.

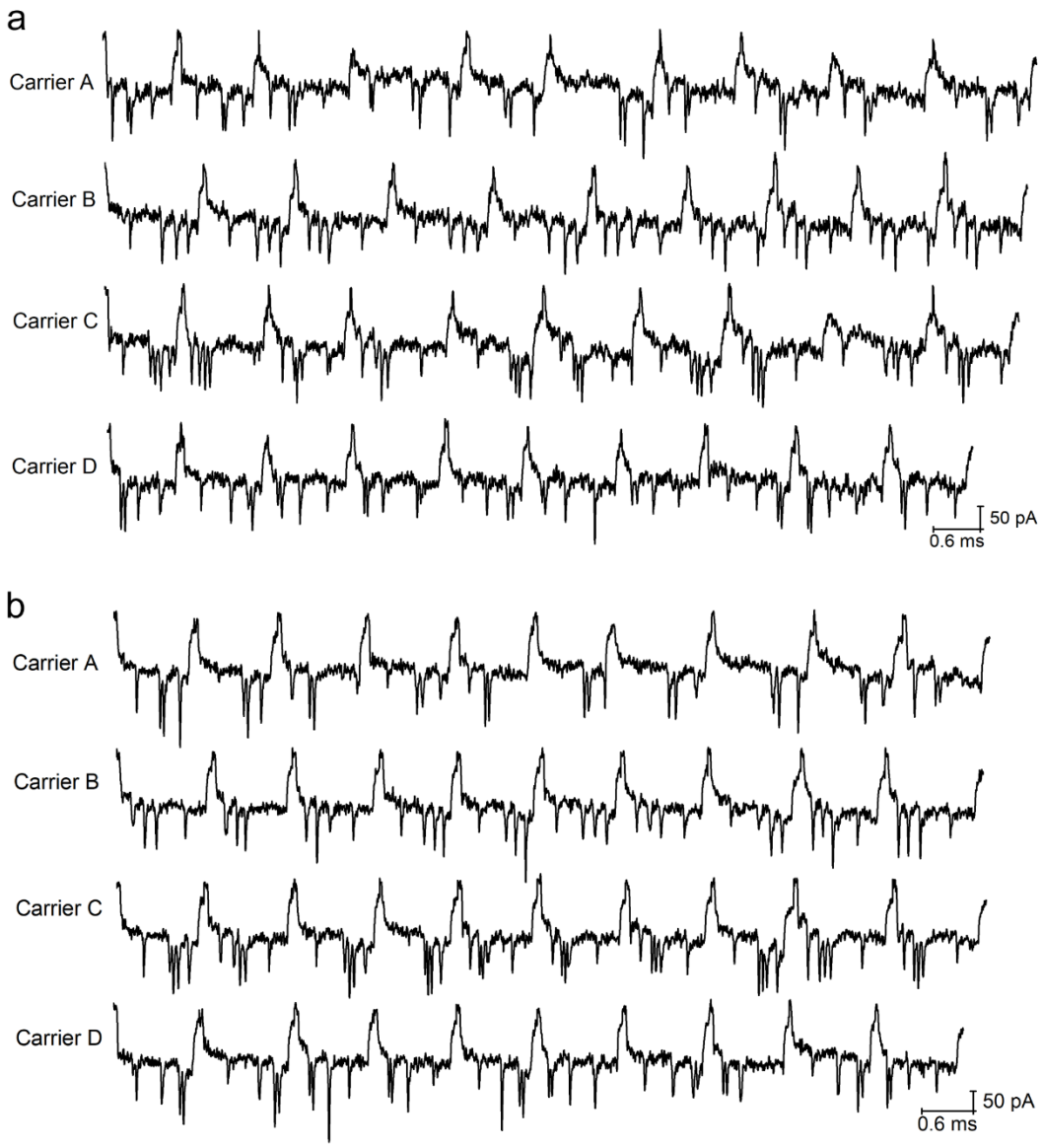

Figure S6. First ten unfolded translocation events of each carrier before (a) and after (b) the addition of strand *Oa4*.

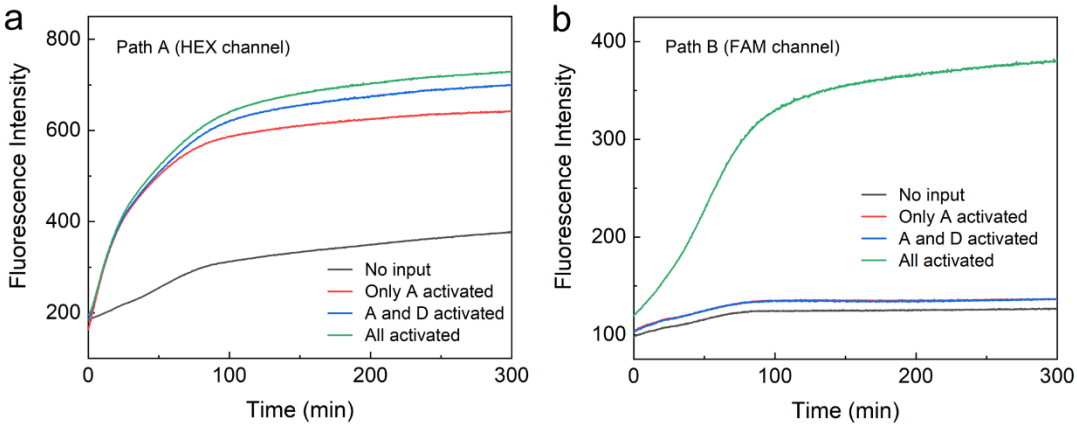

Figure S7. Fluorescence kinetics measurements of the 4 parallel paths selection system for (a) path

A and (b) path B under different conditions. The same DNA substrates were used as Figure 4d except the reporters. Nanopore reporter carrier C and D were absent, and carrier A and B were replaced by fluorescent reporters used in Figure 2d. Concentrations of the DNA substrates were 75 nM for reporter, 50 nM for Substrate 3 and 4, 60 nM for Substrate 1 and 2. 250 nM strand *I* was added to initiate the reaction.

S3 Tables

Table S1. Sequences of DNA strands used in this work.

Type	Strand Name	Sequence
Reporter	F (Fa)	HEX- TGAGGATGTGTAGGTTGAGG AGTGATG
	Q (Qa)	CCTCAACCTACACATCCTCA-IBFQ
	Ft	HEX-TGGATGATGATG AGTGAG
	Qt	CATCATCATCCA-IBFQ
	Fb	FAM - CGAGTGCTGTATGAGAGGTG AGTGATG
	Qb	CACCTCTCATACAGCACTCG – IBFQ
	RA	GATGGTTTAATTTCAACTTTAATCATTGTGAATTACCT TT
		TGAGGATGTGTAGGTTGAGG AGTGATG
	RB	GATGGTTTAATTTCAACTTTAATCATTGTGAATTACCT TT
		CGAGTGCTGTATGAGAGGTG AGTGATG
	RC	GATGGTTTAATTTCAACTTTAATCATTGTGAATTACCT TT
		TGAGAGATTGGATTGTGGTG AGTGATG
	RD	GATGGTTTAATTTCAACTTTAATCATTGTGAATTACCT TT
		AGAGAGTAGGTAGGTGAAGG AGTGATG
Initiator	I1	CTCACT CATCATCATCCA
	M1	CTAACT CATCATCATCCA
	M2	CTCACT AATCATCATCCA
	M3	CTCACT CATAATCATCCA
	M4	CTCACT CATCATAATCCA
	M5	CTCACT CATCATCAACCA
ACSD	O1 (Oa1)	ACCAACTACATC CTCACT CATCATCATCCA
circuit (Path	O1s (Oa1s)	TCC ACCAACTACATC CTCACT CATCATCATCCA
A)	W1 (Wa1)	GAAGAG TGGATGATGATG AGTGAG
	O2 (Oa2)	TACATTCACCAG TCAGCATCACTC CTATCC ACCAACTACATC
	W2 (Wa2)	AGTGAG GATGTAGTTGGT GGATAG
	W2s (Wa2s)	GAG GATGTAGTTGGT GGATAG
	W2p1	AGTGAG GATGTA TAAGAGACGAAG
	W2p2	GGCTATTGGAGA GTTGGT GGATAG
	Linker	CTTCGTCTCTTA TCTCCAATAGCC
	O3 (Oa3)	CATCTACATTCACCAG GTCTTTAC TCAGCATCACTC
	W3 (Wa3)	GGATAG GAGTGATGCTGA CTGGTGAATGTA
	O4 (Oa4)	GTCTTTACTCAG CATCACTC CTCAACCT ACACATCC
	O5 (Oa5)	CATCATCATCCA CTCTTC CATCTACATTCA
	O5s	CTCTTC CATCTACATTCA
	W4 (Wa4)	GAGTGATG CTGAGTAAAGAC CTGG TGAATGTAGATG GAAGAG

Path B	Ob1	TTCAGATACATC CTCACT CATCATCATCCA
	Wb1	GTGTAG TGGATGATGATG AGTGAG
	Ob2	CATTACTCTCCCTCAG CATCACTC GTTACC TTCAGATACATC
	Wb2	AGTGAG GATGTATCTGAA GGTAAC
	Ob3	CAACCATTACTCTCCC GTCTTTAC TCAGCATCACTC
	Wb3	GGTAAC GAGTGATGCTGA GGGAGAGTAATG
	Ob4	GTCTTTACTCAG CATCACTC ACCTCTCA TACAGCAC
	Ob5	CATCATCATCCA CTACAC CAACCATTACTC
	W4	GAGTGATG CTGAGTAAAGAC GGGA GAGTAATGGTTG GTGTAG
Path C	Oc1	CTCTACTCAACT CTCACT CATCATCATCCA
	Wc1	GAGTTG TGGATGATGATG AGTGAG
	Oc2	TCCACATC CTCG CTTC CATCACTC CACTCT CTCTACTCAACT
	Wc2	AGTGAG AGTTGAGTAGAG AGAGTG
	Oc3	ACAATCCACATC CTCG TCTCTCAT CTTC CATCACTC
	Wc3	AGAGTG GAGTGATG GAAG CGAG GATGTGGA
	Oc4	TCTCTCATCTTC CATCACTC ACCACAAT CCAATCTC
	Oc5	CATCATCATCCA CAACTC ACAATCCACATC
	Oc5s	CAACTC ACAATCCACATC
	Wc4	GAGTGATG GAAGATGAGAGA CGAG GATGTGGATTGT GAGTTG
Path D	Od1	CAATCACCTTCA CTCACT CATCATCATCCA
	Wd1	AGTGAG TGGATGATGATG AGTGAG
	Od2	ACTATCAC ACCG TCAGCATCACTC CTTCAC CAATCACCTTCA
	Wd2	AGTGAG TGAAGGTGATTG GTGAAG
	Od3	TCCT ACTATCAC ACCG GTCTTTAC TCAG CATCACTC
	Wd3	GTGAAG GAGTGATGCTGA CGGT GTGATAGT
	Od4	GTCTTTACTCAG CATCACTC CTTCACCT ACCTACTC
	Od5	CATCATCATCCA CTCACT TCCTACTATCAC
	Wd4	GAGTGATG CTGAGTAAAGAC CGGT GTGATAGTAGGA AGTGAG
DNA flower	J1	GGATCAGAGCTGGACG ACAATGACGTAGGTCC TTTT GGACCTACGTCATTGT ACTATGGCACACATCC
	J2	GCAAGACTCGTGCTCA CCGAATGCCACCACGC TTTT GCGTGGTGGCATTCGG CGTCCAGCTCTGATCC
	J3	GGTTCAGCCGCAATCC TCGCCTGCACTCTACC TTTT GGTAGAGTGCAGGCGA TGAGCACGAGTCTTGC
	J4a	GGATGTGTGCCATAGT GGATTGCGGCTGAACC TT CCTCAACCTACACATCCTCA
	J4b	GGATGTGTGCCATAGT GGATTGCGGCTGAACC TT CACCTCTCATACAGCACTCG
	J4c	GGATGTGTGCCATAGT GGATTGCGGCTGAACC TT CACTCCTACTCCAACTCACT
	J4d	GGATGTGTGCCATAGT GGATTGCGGCTGAACC TT CCTTCACCTACCTACTCTCT
Dumbbells for barcodes	Staple 26-32 Substitutes	
	CTGAAAGCGTAAGAATACGTGGCACAGACAATATTTTTGAATGGCT	
	ACATCACTTGTCCTCTTTTGAGGAACAAGTTTTCTTGTCCTGAGTAGA	
	AGAACTCAAATCCTCTTTTGAGGAACAAGTTTTCTTGTCTATCGGCCT	
	TGCTGGTAATTCCTCTTTTGAGGAACAAGTTTTCTTGTATCCAGAACA	
	ATATTACCGCTCCTCTTTTGAGGAACAAGTTTTCTTGTCAGCCATTGC	

AACAGGAAAATCCTCTTTTGAGGAACAAGTTTTCTTGTACGCTCATGG

AAATACCTACTCCTCTTTTGAGGAACAAGTTTTCTTGTATTTTGACGC

TCAATCGTCTTCCTCTTTTGAGGAACAAGTTTTCTTGTGAAATGGATT

ATTTACATTGTCCTCTTTTGAGGAACAAGTTTTCTTGTGCAGATTCAC

CAGTCACACGTCCTCTTTTGAGGAACAAGTTTTCTTGTACCAGTAATA

AAAGGGACATTCCTCTTTTGAGGAACAAGTTTTCTTGTTCTGGCCAAC

AGAGATAGAATCCTCTTTTGAGGAACAAGTTTTCTTGTCCCTTCTGAC

Staple 40-46 Substitutes

AATATAATCCTGATTGTTTGGATTATACTTCTGAATAATGGAAGGG

CACTAACAACTCCTCTTTTGAGGAACAAGTTTTCTTGTTAATAGATTA

GAGCCGTCAATCCTCTTTTGAGGAACAAGTTTTCTTGTTAGATAATAC

ATTTGAGGATTCCTCTTTTGAGGAACAAGTTTTCTTGTTTAGAAGTAT

TAGACTTTACTCCTCTTTTGAGGAACAAGTTTTCTTGTAAACAATTCG

ACAACTCGTATCCTCTTTTGAGGAACAAGTTTTCTTGTTTAAATCCTT

TGCCCGAACGTCCTCTTTTGAGGAACAAGTTTTCTTGTTTATTAATTT

TAAAAGTTTGTCCTCTTTTGAGGAACAAGTTTTCTTGTAGTAACATTA

TCATTTTGCGTCCTCTTTTGAGGAACAAGTTTTCTTGTGAACAAAGAA

ACCACCAGAATCCTCTTTTGAGGAACAAGTTTTCTTGTGGAGCGGAAT

TATCATCATATCCTCTTTTGAGGAACAAGTTTTCTTGTTTCCTGATTA

TCAGATGATGTCCTCTTTTGAGGAACAAGTTTTCTTGTGCAATTCATC

Staple 54-60 Substitutes

TGTAAATGCTGATGCAAATCCAATCGCAAGACAAAGAACGCGAGAA

TACCTTTTTTTCCTCTTTTGAGGAACAAGTTTTCTTGTAATGGAAACA

GTACATAAATTCCTCTTTTGAGGAACAAGTTTTCTTGTCAATATATGT

GAGTGAATAATCCTCTTTTGAGGAACAAGTTTTCTTGTCCTTGCTTCT

GTAAATCGTCTCCTCTTTTGAGGAACAAGTTTTCTTGTGCTATTAATT

AATTTTCCCTTCCTCTTTTGAGGAACAAGTTTTCTTGTTAGAATCCTT

GAAAACATAGTCCTCTTTTGAGGAACAAGTTTTCTTGTCGATAGCTTA

GATTAAGACGTCCTCTTTTGAGGAACAAGTTTTCTTGTCTGAGAAGAG

TCAATAGTGATCCTCTTTTGAGGAACAAGTTTTCTTGTATTTATCAAA

ATCATAGGTCTCCTCTTTTGAGGAACAAGTTTTCTTGTTGAGAGACTA

CCTTTTTAACTCCTCTTTTGAGGAACAAGTTTTCTTGTCTCCGGCTTA

GGTTGGGTTATCCTCTTTTGAGGAACAAGTTTTCTTGTTATAACTATA

Staple 68-74 Substitutes

TCATCGAGAACAAGCAAGCCGTTTTTATTTTCATCGTAGGAATCAT

AGAATATAAATCCTCTTTTGAGGAACAAGTTTTCTTGTGTACCGACAA

AAGGTAAAGTTCCTCTTTTGAGGAACAAGTTTTCTTGTAATTCTGTCC

AGACGACGACTCCTCTTTTGAGGAACAAGTTTTCTTGTAATAAACAAC

ATGTTCAGCTTCCTCTTTTGAGGAACAAGTTTTCTTGTAATGCAGAAC

GCGCCTGTTTTCCTCTTTTGAGGAACAAGTTTTCTTGTATCAACAATA

GATAAGTCCTTCCTCTTTTGAGGAACAAGTTTTCTTGTGAACAAGAAA

AATAATATCCTCCTCTTTTGAGGAACAAGTTTTCTTGTCATCCTAATT

TACGAGCATGTCCTCTTTTGAGGAACAAGTTTTCTTGTTAGAAACCAA

TCAATAATCGTCCTCTTTTGAGGAACAAGTTTTCTTGTGCTGTCTTTC

CTTATCATTCTCCTCTTTTGAGGAACAAGTTTTCTTGTCAAGAACGGG
TATTAAACCATCCTCTTTTGAGGAACAAGTTTTCTTGTAGTACCGCAC
Staple 82-88 Substitutes
AGATAGCCGAACAAAGTTACCAGAAGGAAACCGAGGAAACGCAATA
AAAAATGAAATCCTCTTTTGAGGAACAAGTTTTCTTGTATAGCAGCCT
TTACAGAGAGTCCTCTTTTGAGGAACAAGTTTTCTTGTAATAACATAA
AAACAGGGAATCCTCTTTTGAGGAACAAGTTTTCTTGTGCGCATTAGA
CGGGAGAATTTCCTCTTTTGAGGAACAAGTTTTCTTGTAACTGAACAC
CCTGAACAAATCCTCTTTTGAGGAACAAGTTTTCTTGTGTCAGAGGGT
AATTGAGCGCTCCTCTTTTGAGGAACAAGTTTTCTTGTTAATATCAGA
GAGATAACCCTCCTCTTTTGAGGAACAAGTTTTCTTGTACAAGAATTG
AGTTAAGCCCTCCTCTTTTGAGGAACAAGTTTTCTTGTAATAATAAGA
GCAAGAAACATCCTCTTTTGAGGAACAAGTTTTCTTGTATGAAATAGC
AATAGCTATCTCCTCTTTTGAGGAACAAGTTTTCTTGTTTACCGAAGC
CCTTTTTAAGTCCTCTTTTGAGGAACAAGTTTTCTTGTAAAAGTAAGC

Table S2. Statistic of translocation events of carrier A-D under different conditions.

Samples	I_{event} (nA)[b]	OF of carrier A	OF of carrier B	OF of carrier C	OF of carrier D
Only 4 carriers	0.140	66/76=86.8%	47/52=90.4%	49/55=89.1%	62/74=83.8%
4 carriers + *Oa4*	0.123	45/121=37.2%	64/95=67.4%	72/96=75.0%	72/109=66.1%
No initiator *I*[a] (4 carriers + circuits)	0.127	110/157=70.1%	77/112=68.8%	79/107=73.8%	98/147=66.7%
	0.148	61/84=72.6%	49/64=76.6%	36/43=83.7%	50/61=82.0%
	0.122	57/74=77.0%	31/43=72.1%	34/55=61.8%	54/73=74.0%
Only Path A activated[a]	0.111	57/146=39.0%	93/137=67.9%	99/151=65.6%	115/194=59.3%
	0.124	19/59=32.2%	23/34=67.6%	24/46=52.2%	41/64=64.1%
	0.106	32/81=39.5%	34/48=70.8%	31/54=57.4%	54/83=65.1%
Path A and D activated[a]	0.125	33/93=35.5%	55/97=56.7%	57/98=58.2%	46/138=33.3%
	0.152	20/69=29.0%	42/70=60.0%	39/74=52.7%	39/126=31.0%
	0.125	55/136=40.4%	79/108=73.1%	39/61=63.9%	22/66=33.3%
All paths activated[a]	0.131	18/108=16.7%	18/70=25.7%	26/84=31.0%	36/135=26.7%
	0.141	23/101=22.8%	16/72=22.2%	35/106=33.0%	19/72=26.4%
	0.133	22/90=24.4%	22/72=30.6%	32/110=29.1%	33/115=28.7%

[a] The same sample was measured by three different nanopores and the average data was given in Figure 4d.

[b] I_{event} was determined by the nanopore size.

Table S3. Standard errors of occupied fractions in Figure 4d based on three repeated measurements.

	Path A	Path B	Path C	Path D
No initiator I	± 2.26%	± 2.03%	± 6.33%	± 4.42%
A activated	± 2.36%	± 1.03%	± 3.90%	± 1.78%
AD activated	± 3.32%	± 5.02%	± 3.24%	± 0.794%
All activated	± 2.36%	± 2.42%	± 1.13%	± 0.727%

Reference

[1] N. A. W. Bell, U. F. Keyser, *Nat. Nanotechnol.* **2016**, 11, 645.

[2] K. Chen, M. Juhasz, F. Gularek, E. Weinhold, Y. Tian, U. F. Keyser, N. A. W. Bell, *Nano Lett.* **2017**, *17*, 5199-5205.

[3] J. Zhu, N. Ermann, K. Chen, U. F. Keyser, Small **2021**, 17, 2100711.