Supplementary Informations for

Nicking Enzyme-Mediated Signal Rollback Mechanism for Programmable Access Control of Molecular Systems

Wei Zhao [a, ‡], Xiaokang Zhang [b, ‡], Peijun Shi [b], Yao Yao [b], Lijun Sun [b], Yixin Xu [a], Huizi Man [a, *], Bin Wang [a].

This PDF file includes:

Supplementary Fig. S1 - Fig. S17

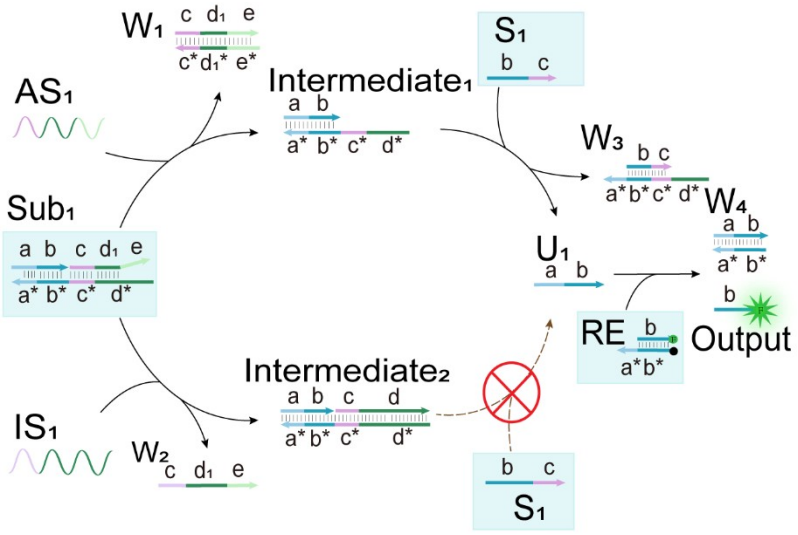

Fig. S1. Workflow of a traditional molecular sequential circuit.

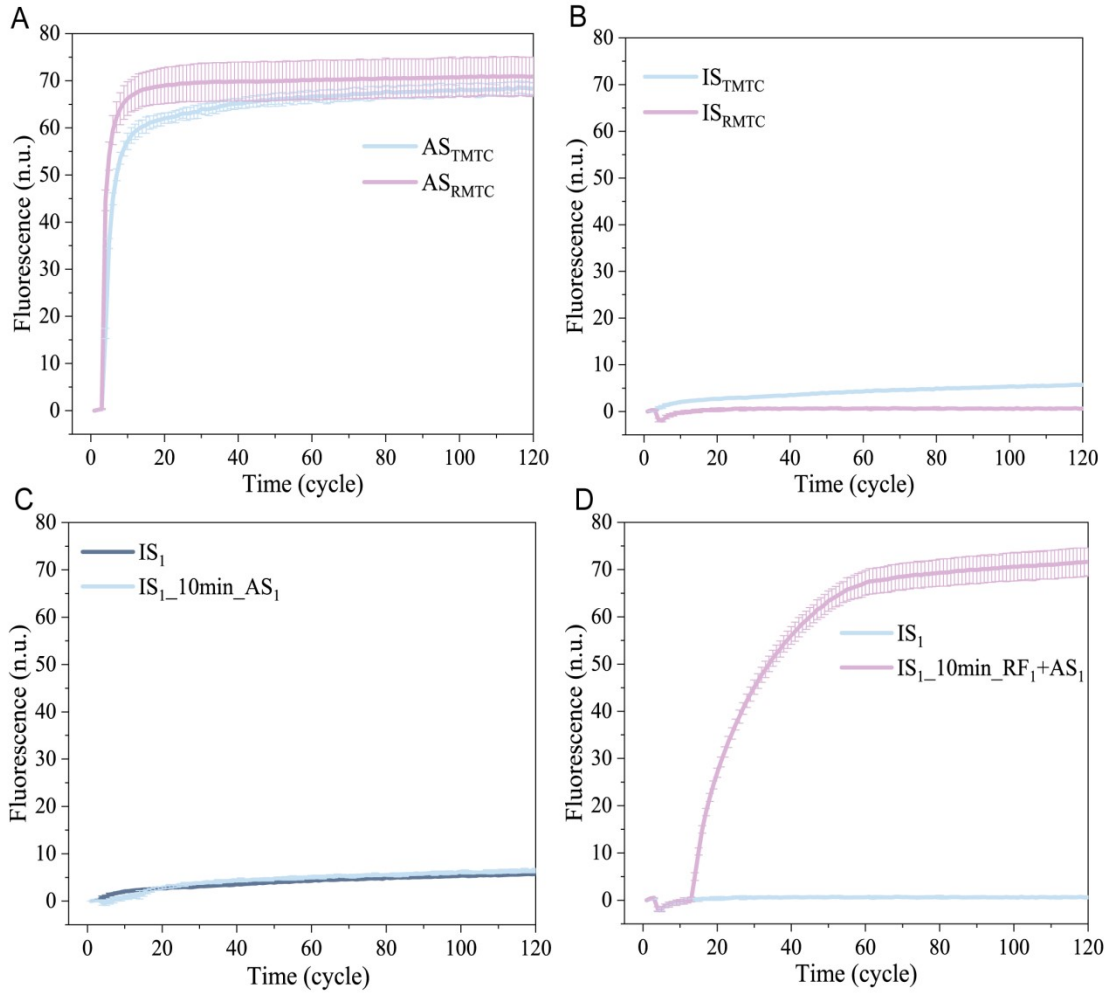

Fig. S2. Real-time fluorescence experimental analysis of traditional molecular timing circuits and signal rollback circuits. (A) Real-time fluorescence experimental results with activation signals added to each circuit ([AS_1] = 400 nM, [IS_1] = 400 nM). (B) Real-time fluorescence experimental results with suppression signals added to each circuit ([AS_1] = 400 nM, [IS_1] = 400 nM). (C) Real-time fluorescence experimental results of the traditional molecular timing circuit without rollback factor after suppression ([IS_1] = 200 nM, [AS_1] = 400 nM). (D) Real-time fluorescence experimental results of the signal rollback circuit with rollback factor added after suppression ([IS_1] = 200 nM, RF_1 = 2 units).

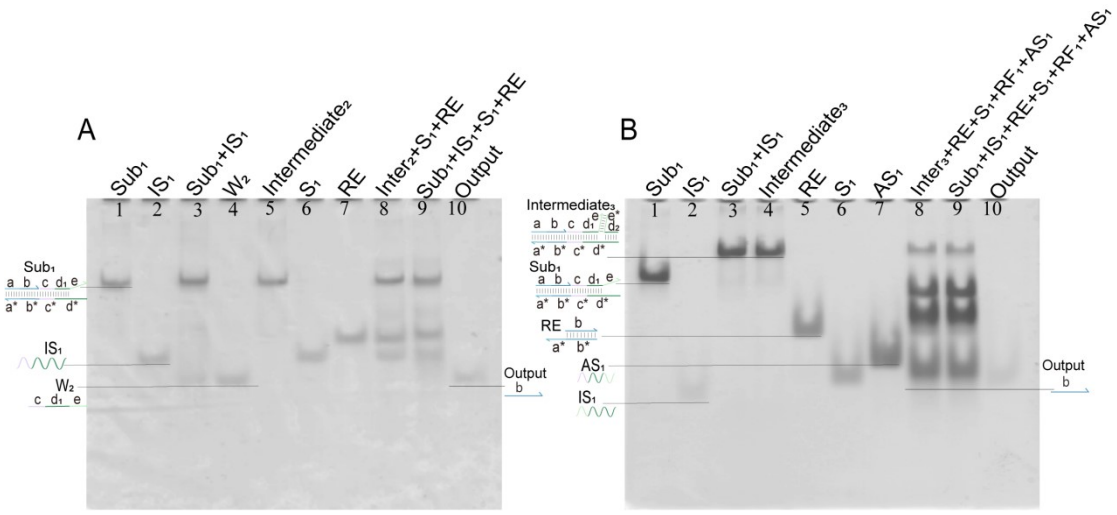

Fig. S3. Comparative analysis of the stepwise process of traditional molecular timing circuit and signal rollback circuit using polyacrylamide gel electrophoresis. (A) Experimental result of traditional molecular timing circuit analyzed by PAGE ([RE] = 0.8 μM, $[IS_1] = [S_1] = 1.6$ μM). (B) Experimental result of signal rollback circuit analyzed by PAGE ([RE] = $[IS_1]$ = 1 μM, $[S_1] = 2$ μM, $[AS_1] = 4$ μM, $RF_1 = 2$ units).

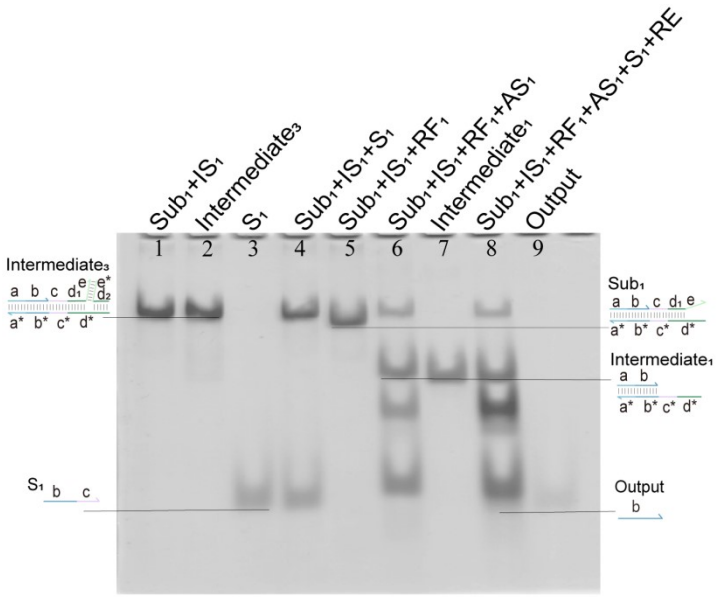

Fig. S4. Full flow analysis of the rollback path in the signal rollback circuit using polyacrylamide gel electrophoresis([RE] = [IS_1] = 1 μM, [S_1] = 2 μM, [AS_1] = 4 μM, RF_1 = 2 units).

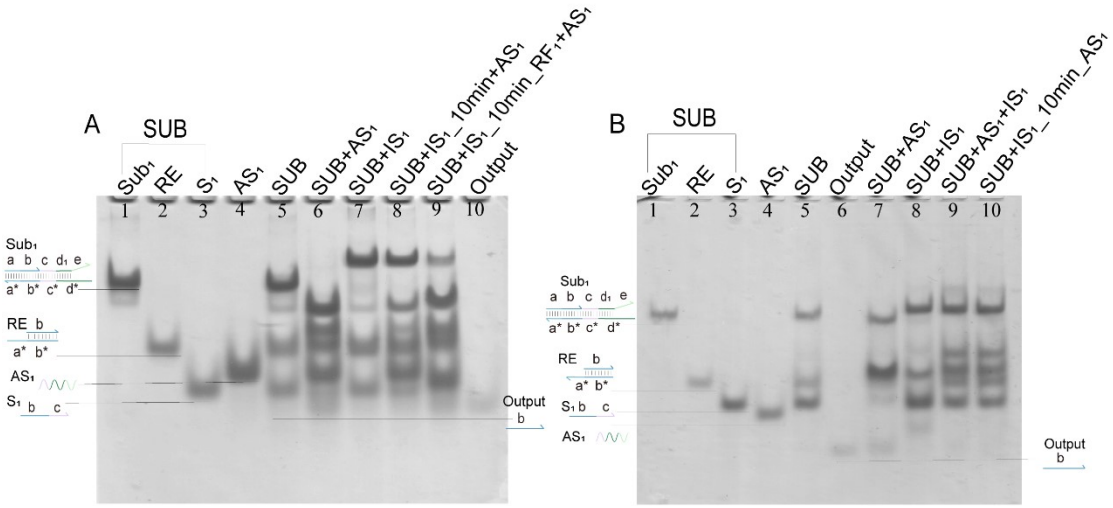

Fig. S5. Analysis of polyacrylamide gel electrophoresis experiments using signal rollback circuit and conventional molecular timing circuit. (A) Experimental results of signal rollback circuit analyzed by PAGE ([RE] = 0.8 uM, $[AS_1] = [IS_1] = [S_1]$ = 1.6 uM, RF_1 = 2 units). (B) Experimental results of conventional molecular timing circuit analyzed by PAGE ([RE] = 0.8 uM, $[AS_1] = [IS_1] = [S_1]$ = 1.6 uM).

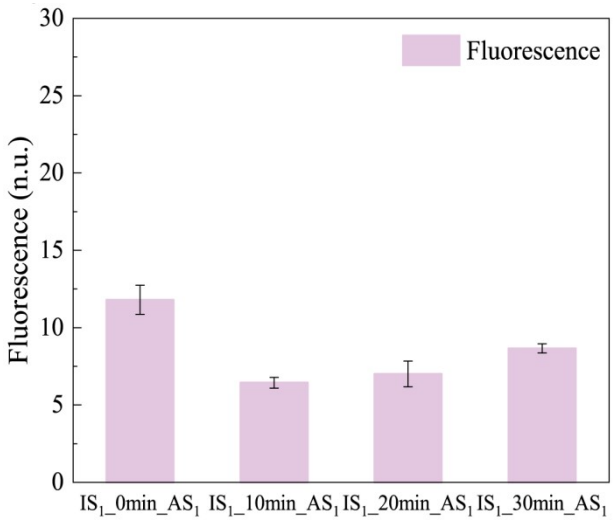

Fig. S6. Suppression effect of adding suppression signals at different time intervals in the rollback mechanism ([AS_1] = 600 nM, [IS_1] = 200 nM, [S_1] = 400 nM).

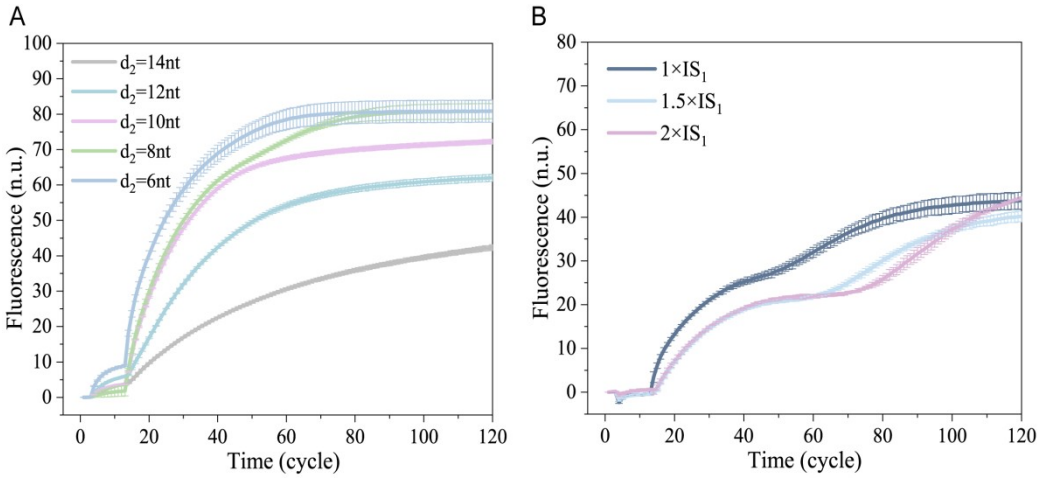

Fig. S7. Stability analysis of the binding of the inhibition signal to the substrate under different base lengths d_2. (A) Real-time fluorescence experiments with rollback at different d_2 base lengths ($[IS_1]$ = 200 nM, $[AS_1]$ = 600 nM, RF_1 = 2 units). (B) Real-time fluorescence experiments with rollback at different concentrations of inhibition signal when d_2=8nt ($[IS_1]$ = 1×, 1.5×, 2×, where 1× = 200 nM, $[AS_1]$ = 800 nM, $[S_1]$ = 400 nM).

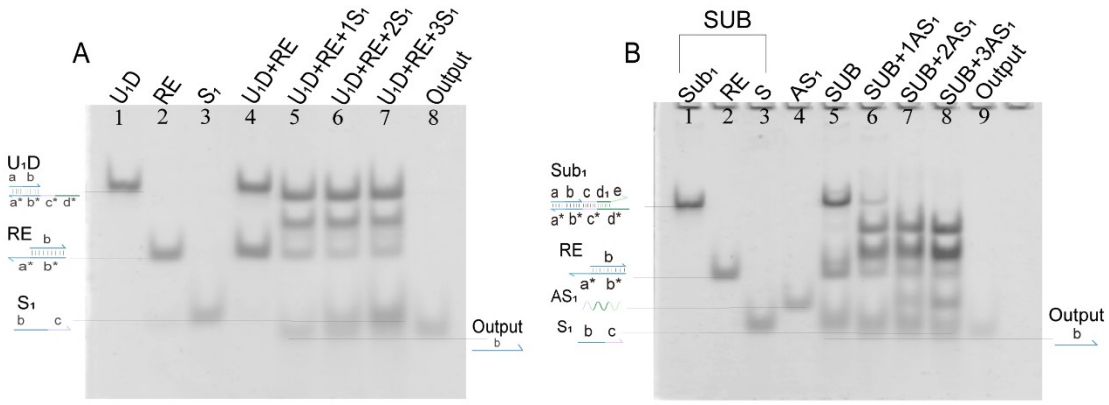

Fig. S8. Analysis of polyacrylamide gel electrophoresis experiments with different concentrations of relay and activation signals. (A) Experimental results of PAGE analysis of different concentrations of relay signal S_1 ([S_1] = 1×, 2×, 3×, where 1× = 0.8 μM). (B) Experimental results of PAGE analysis of different concentrations of activation signal AS_1 ([AS_1] = 1×, 2×, 3×, where 1× = 0.8 μM, [S_1] = 1.6 μM).

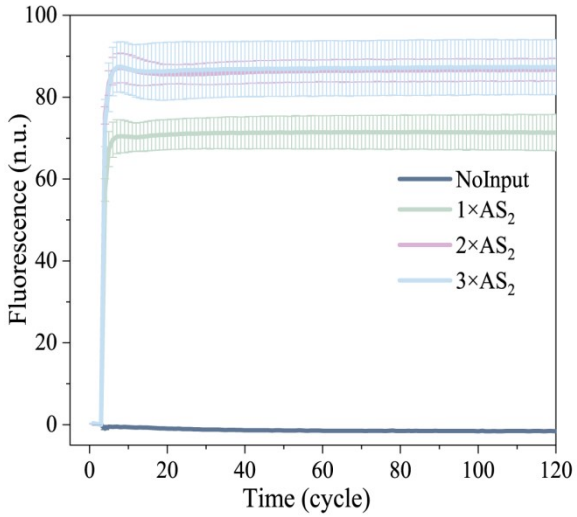

Fig. S9. Real-time fluorescence experimental results of activation signal AS_2 at different concentrations ([AS_2] = 0×, 1×, 2×, 3×, where 1× = 200 nM).

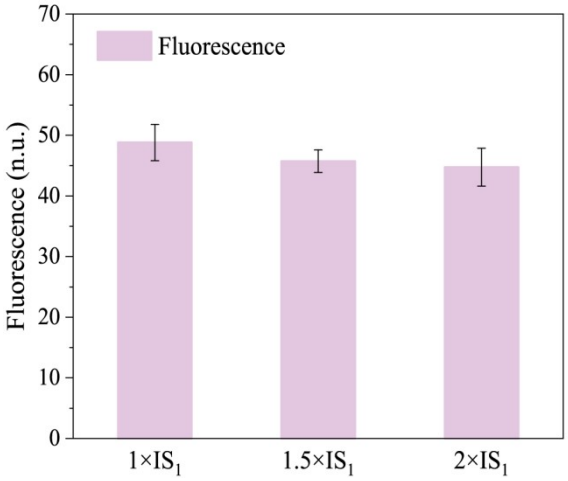

Fig. S10. Fluorescence output results of inhibition signal IS_1 at different concentrations ($[IS_1]$ = 1×, 1.5×, 2×, where 1× = 200 nM, $[AS_1]$ = 800 nM, $[S_1]$ = 400 nM).

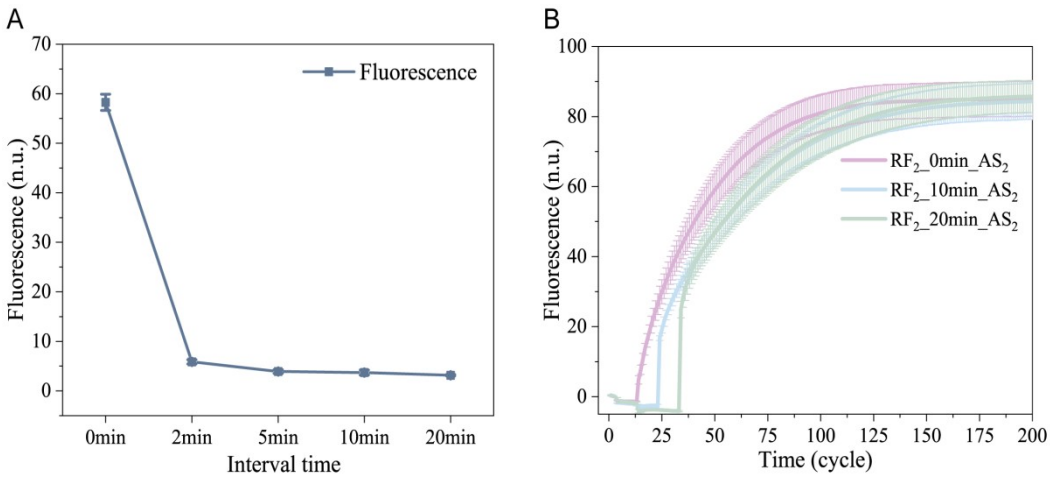

Fig. S11. Verification of the universality and feasibility of introducing another restriction endonucleases (Nb.BtsI) into the rollback mechanism. (A) Inhibitory effect of adding inhibitory signals at different times ([S_2] = 400 nM, [IS_2] = 200 nM, [AS_2] = 800 nM). (B) Real-time fluorescence experimental results of adding rollback factors and activation signals at different time intervals to perform signal rollback ([S_2] = 400 nM, [IS_2] = 200 nM, [AS_2] = 800 nM, RF_2 = 2 units).

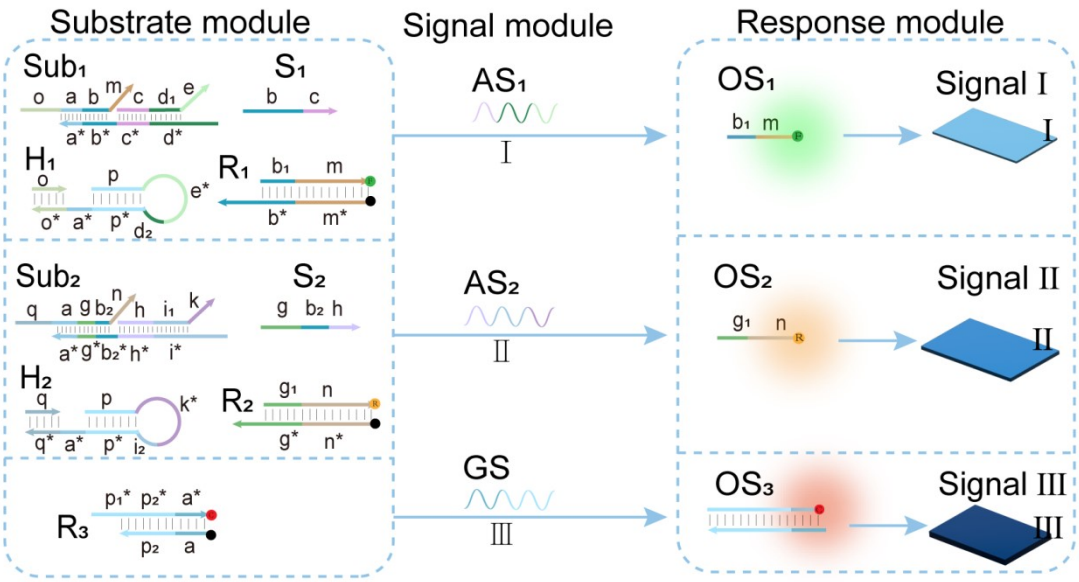

Fig. S12. Three core components of a molecular safety system based on a signal rollback mechanism.

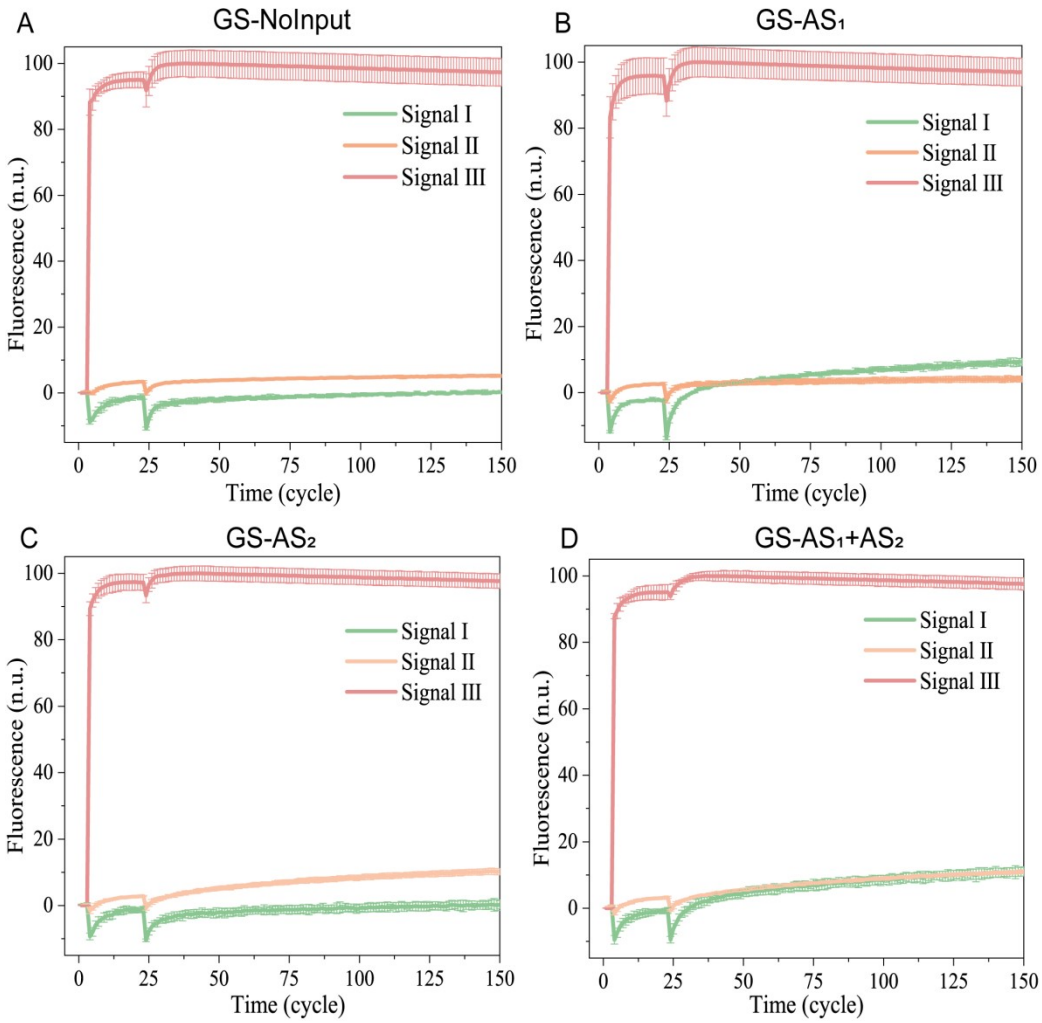

Fig. S13. Real-time fluorescence experimental results with different interface inputs in the pre-closed state of the system. (A) Real-time fluorescence experimental results with only the general signal added. (B) Real-time fluorescence experimental results with AS_1 interface input after adding the general signal GS. (C) Real-time fluorescence experimental results with AS_2 interface input after adding the general signal GS. (D) Real-time fluorescence experimental results with both AS_1 and AS_2 interfaces input simultaneously after adding the general signal GS ([GS] = [AS_1] = [AS_2] = 600 nM).

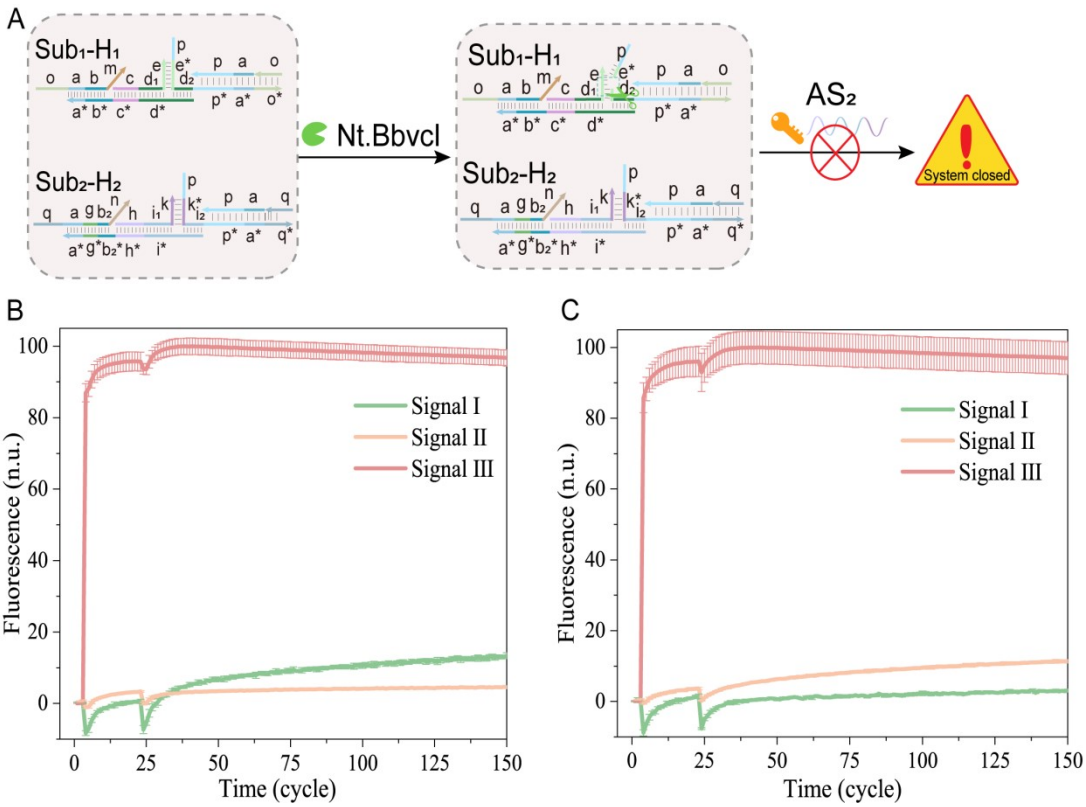

Fig. S14. Results of unauthorized user execution of information backtracking. (A) Schematic diagram of access performed with rollback factor RF_1 and activation signal AS_2. (B) Real-time fluorescence experimental results of access performed with rollback combination RF_1 and AS_2 ([GS] = [AS_2] = 600 nM, RF_1 = 2 units). (C) Real-time fluorescence experimental results of access performed with rollback combination RF_2 and AS_1 ([GS] = [AS_1] = 600 nM, RF_2 = 2 units).

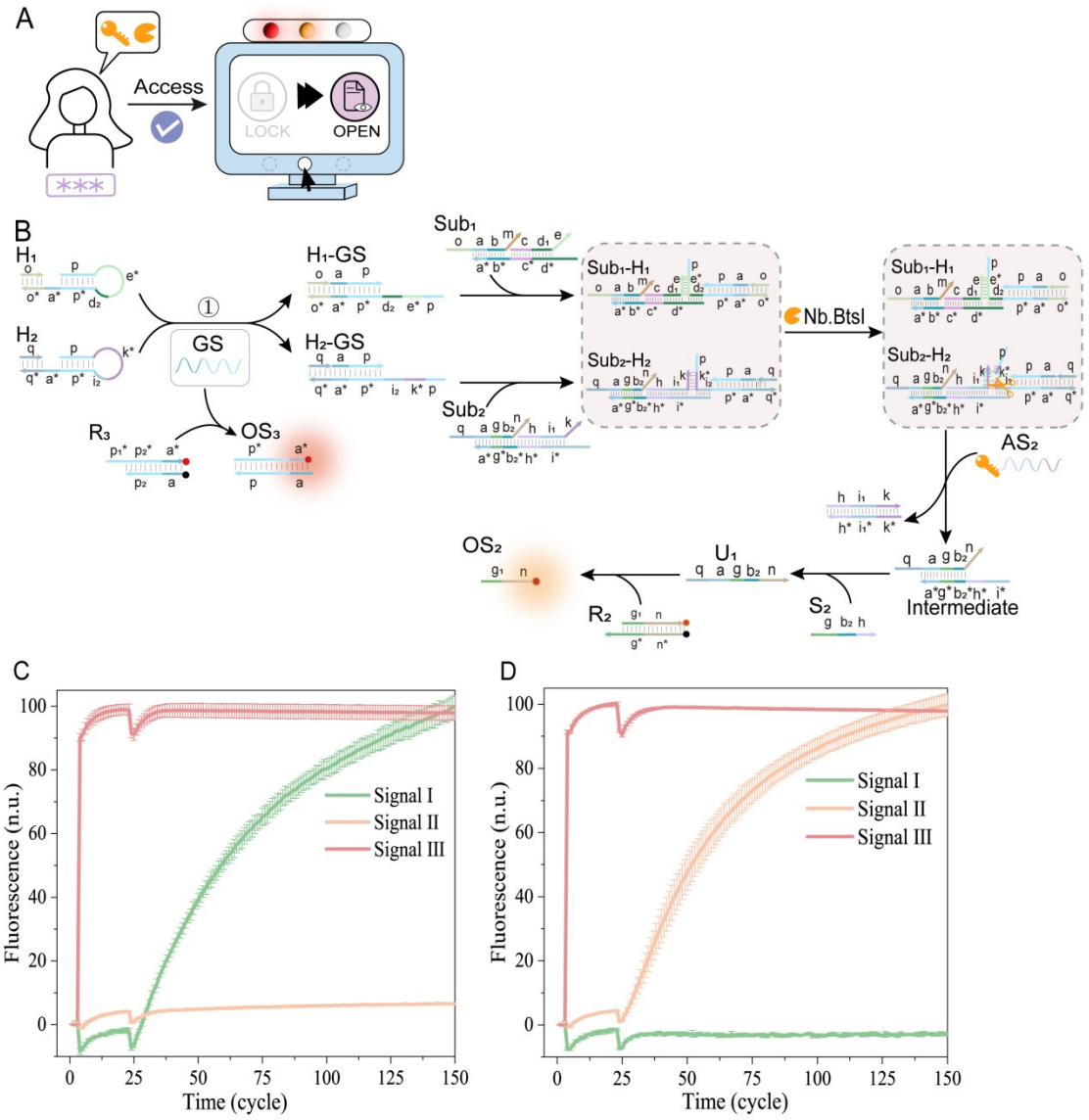

Fig. S15. Results of authorized user execution of information backtracking. (A) Schematic diagram of authorized user inputting RF_2 and AS_2 to execute information backtracking. (B) Workflow of authorized user inputting RF_2 and AS_2 to execute information backtracking. (C) Real-time fluorescence experimental results of authorized user inputting RF_1 and AS_1 backtracking information III and I ([GS] = $[AS_1]$ = 600 nM, RF_1 = 2 units). (D) Real-time fluorescence experimental results of authorized user inputting RF_2 and AS_2 backtracking information III and II ([GS]=$[AS_2]$ = 600 nM, RF_2 = 2 units).

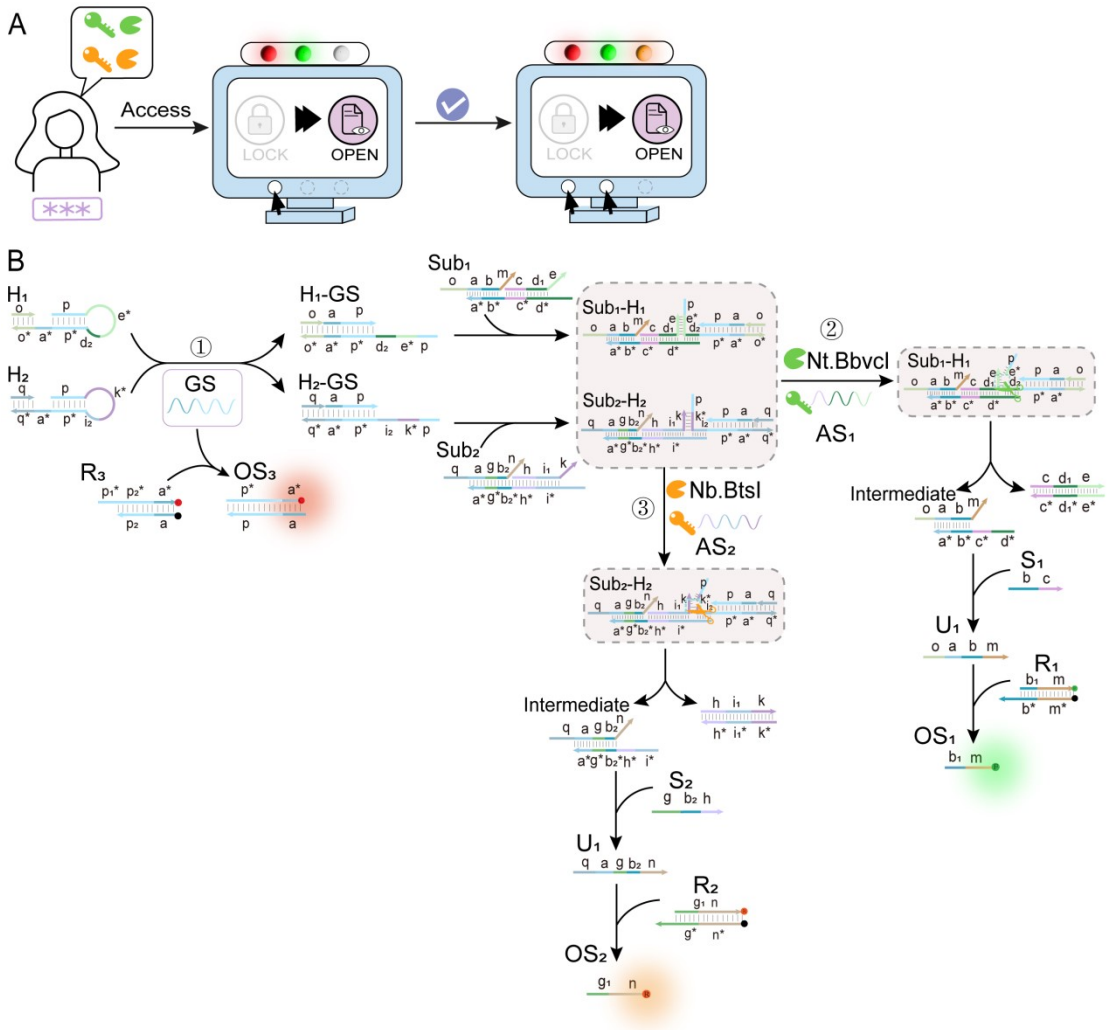

Fig. S16. Schematic diagram of authorized user performing complete information backtracking from III to I to II. (A) Schematic diagram of authorized user first inputting RF_1 and AS_1, then inputting RF_2 and AS_2 to perform complete information backtracking. (B) Response flow of authorized user first inputting RF_1 and AS_1, then inputting RF_2 and AS_2 to perform complete information backtracking.

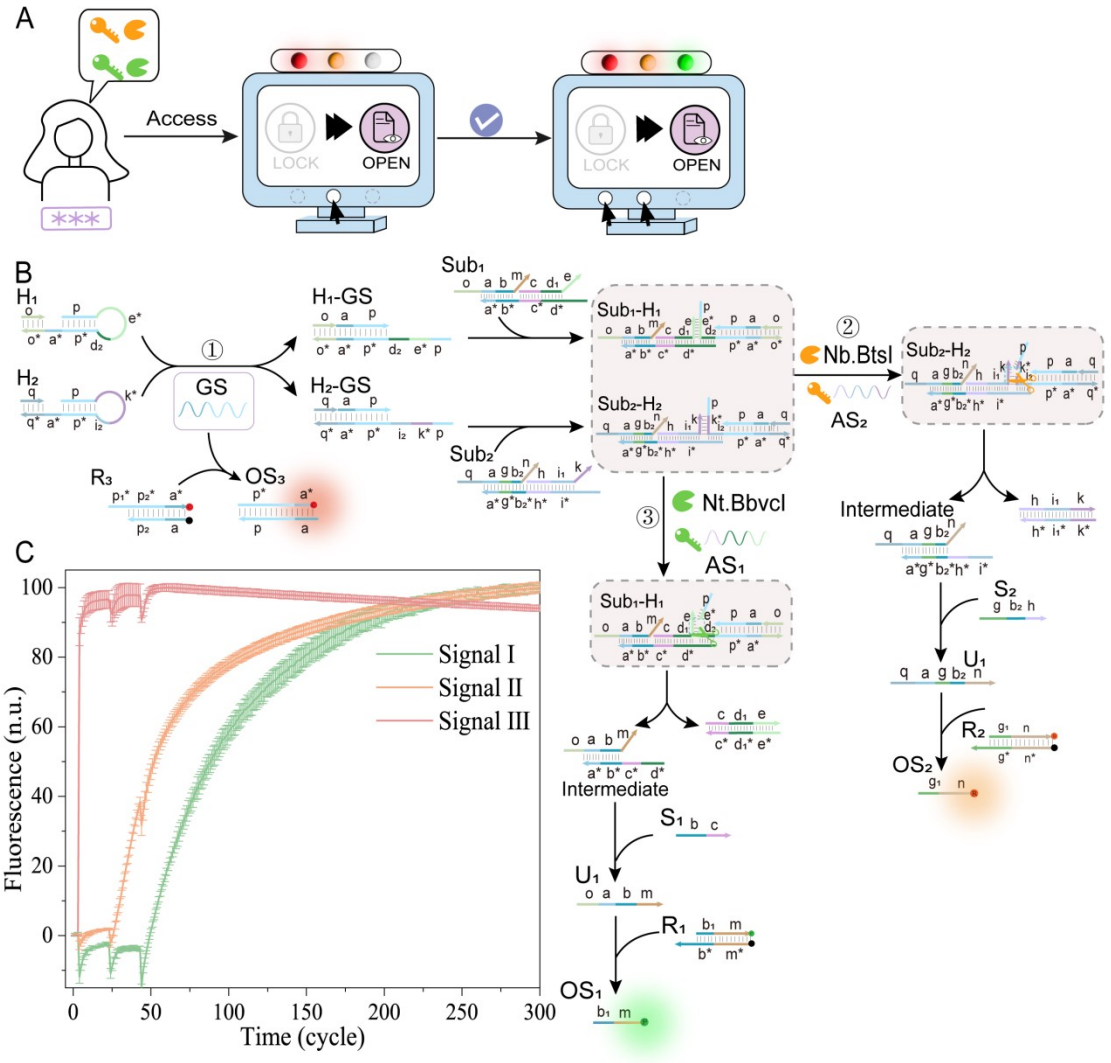

Fig. S17. Schematic diagram of authorized user performing complete information backtracking from III to II to I. (A) Schematic diagram of authorized user first inputting RF_2 and AS_2, then inputting RF_1 and AS_1 to perform complete information backtracking. (B) Workflow diagram of authorized user first inputting RF_2 and AS_2, then inputting RF_1 and AS_1 to perform complete information backtracking. (C) Real-time fluorescence experimental results of authorized user performing complete information backtracking from III to II to I ([GS] = [AS_1] = [AS_2] = 600 nM, RF_1 = RF_2 = 2 units).

Supplementary Table S1- Table S4

Table S1. Sequence of signal rollback mechanism

Strand		Domain	Sequence (5'->3')	Purity
AS_1		e* d_1* c*	CTAACCTCAGCAACCTCCACTACCACTATCTCTGAC	PAGE-purified
IS_1		e* d_2	CTAACCTCAGCAACTTACTAGACACA	PAGE-purified
S_1		b c	TACATCCAGAACTCACACTTGACCGTCAGA	PAGE-purified
Sub_1	Sub_1-U_1	a b	CAGCATTACATCCAGAACTCACACTTGACC	PAGE-purified
	Sub_1-U_2	c d_1 e	GTCAGAGATAGTGGTAGTGGAGGTTGCTGAGGTTAG	PAGE-purified
	Sub_1-D	d*c*b*a*	TGTGTCTAGTCTCCACTACCACTATCTCTGACGGTCAA GTGTGAGTTCTGGATGTAATGCTG	PAGE-purified
RE	RE-Reu	b	TACATCCAGAACTCACACTTGACCA-FAM	>95% (HPLC)
	RE-Red	b* a*	BHQ1-TGGTCAAGTGTGAGTTCTGGATGTAATGCTG	>95% (HPLC)

Table S2. Sequence of conditional optimization of signal rollback mechanism

Strand		Domain	Sequence (5'->3')	Purity
R	R-Reu	c d_1 d_2	ROX-GTCAGAGATAGTGGTAGTGGAGACTAGA	>95% (HPLC)
	R-Red	d_1* c*	CTCCACTACCACTATCTCTGAC-BHQ2	>95% (HPLC)

Table S3. Sequence of specify rollback module

Strand		Domain	Sequence (5'->3')	Purity
AS_2		k* i_1* h*	CTGAATCACTGCTACCTCTTCTACACCATTCCAACAC	PAGE-purified
IS_2		k* i_2	CTGAATCACTGCTACTTACTACGAGAG	PAGE-purified
S_2		g h	GAGAAGTGAGTGGTAAGATGTTACGTGTTG	PAGE-purified
Sub_2	Sub_2-U_1	f g	GATCAGGAGAAGTGAGTGGTAAGATGTTAC	PAGE-purified
	Sub_2-U_2	h i_1 k	GTGTTGGAATGGTGTAGAAGAGGTAGCAGTGATTCAG	PAGE-purified
	Sub_2-D	i*h*g*f*	CTCTCGTAGTCTCTTCTACACCATTCCAACACGTAACA TCTTACCACTCACTTCTCCTGATC	PAGE-purified
RE_2	RE_2-Reu	g	GAGAAGTGAGTGGTAAGATGTTAG-ROX	>95% (HPLC)
	RE_2-Red	g* f*	BHQ2-CTAACATCTTACCACTCACTTCTCCTGATC	>95% (HPLC)

Table S4. Sequence of molecular safety systems based on signal rollback mechanism

Strand		Domain	Sequence (5'->3')	Purity
H_1-o		o	CACTGACATCACGTACATCA	PAGE-purified
H_1-rest		pe*d_2p* a*o*	CTCCACTACCACTATCTCCTAACCTCAGCAACACTAGACAGGA GATAGTGGTAGTGGAGGAGTTCTGATGTACGTGATGTCAGTG	PAGE-purified
H_2-q		q	CAGTCACTCAACTCACTCTG	PAGE-purified
H_2-rest		pk*i_2p* a*q*	CTCCACTACCACTATCTCCTGAATCACTGCTACACTACGAGGA GATAGTGGTAGTGGAGGAGTTCCAGAGTGAGTTGAGTGACTG	PAGE-purified
GS		a p	GAACTCCTCCACTACCACTATCTCAGCTAC	PAGE-purified
Sub_1	Sub_1-U_1	o a b m	CACTGACATCACGTACATCAGAACTCTACATCCAGAACTCAC ACTTGACCTCCACACATGATCCAC	PAGE-purified
	Sub_1-D	d*c*b* a*	TGTGTCTAGTCTCCACTACCACTATCTCTGACGGTCAAGTGTG AGTTCTGGATGTAGAGTTC	PAGE-purified
Sub_2	Sub_2- U_1	q a g b_2 n	CAGTCACTCAACTCACTCTGGAACTCGAGAAGTGGAACTCAA GATGTTACAATGCTATCTACGATT	PAGE-purified
	Sub_2-D	i*h*b_2* g*a*	CTCTCGTAGTCTCTTCTACACCATTCCAACACGTAACATCTTG AGTTCCACTTCTCGAGTTC	PAGE-purified
R_1	R_1-Reu	b_1 m	GACCTCCACACATGATCCAC-FAM	>95% (HPLC)
	R_1-Red	m* b*	BHQ1-GTGGATCATGTGTGGAGGTCAAGTGT	>95% (HPLC)
R_2	R_2-Reu	g_1 n	TTACAATGCTATCTACGATT-ROX	>95% (HPLC)
	R_2-Red	n* g*	BHQ2-AATCGTAGATAGCATTGTAACATCTT	>95% (HPLC)
R_3	R_3-Reu	p_1* p_2* a*	GTAGCTGAGATAGTGGTAGTGGAGGAGTTC-Cy5	>95% (HPLC)
	R_3-Red	a p_2	BHQ3-GAACTCCTCCACTACCACTATCTC	>95% (HPLC)