Supplementary information

Improving robustness of catalyzed hairpin assembly with three-arm nanostructure for nonenzymatic signal amplification

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Table S1. Sequences of oligonucleotides used in this work

Oligonucleotides[a,b]	5'-Sequences-3'[c]
Catalyst$_{_24}$ (C$_{_24}$)	GCAACA-CAG-AACTCG-TCG-ACTCCA
Catalyst$_{_30}$ (C$_{_30}$)	CTACAG-CAATTC-ACCATC-CTTAGC-ACTTCG
Catalyst_original (C$_{_O}$)	GCACTA-CTCCCT-AACATC-TCAAGC
H1$_{_24}$	TGGAGT–CGA–CGAGTT–CTG–TGTTGC–ACT–CCACCA–GCAACA–CAG–AACTCG-TCG
H2$_{_24}$	CGAGTT-CTG-TGTTGC-TGG-TGGAGT-AAC-TCGTCG-ACTCCA-CCA-GCAACA-CAG
H3$_{_24}$	TGTTGC-TGG-TGGAGT-CGA-CGAGTT-GCA-ACACAG-AACTCG-TCG-ACTCCA-CCA
H3_FAM$_{_24}$	/5IABkFQ/TGTTGC-TGG-TGGAGT-CGA-CGAGTT-GCA-ACACAG-AACTCG-TCG-ACTCCA-CCA/36-FAM/
H1$_{_30}$	CGAAGT-GCTAAG-GATGGT-GAATTG-GTGTAG-ACTTCG-GCTACA-CTACAC-CAATTC-ACCATC-CTTAGC
H2$_{_30}$	GATGGT-GAATTG-GTGTAG-TGTAGC-CGAAGT-ACCATC-CTTAGC-ACTTCG-GCTACA-CTACAC-CAATTC
H3$_{_30}$	GTGTAG-TGTAGC-CGAAGT-GCTAAG-GATGGT-CTACAC-CAATTC-ACCATC-CTTAGC-ACTTCG-GCTACA
H1$_{_O}$	GCTTGA-GATGTT-AGGGAG-TAGTGC-TCCAAT-CACAAC-GCACTA-CTCCCT-AACATC
H2$_{_O}$	AGGGAG-TAGTGC-GTTGTG-ATTGGA-AACATC-TCAAGC-TCCAAT-CACAAC-GCACTA
H3$_{_O}$	GTTGTG-ATTGGA-GCTTGA-GATGTT-GCACTA-CTCCCT-AACATC-TCAAGC-TCCAAT
Single-base substitution at toehold	GCAACA-CAG-AACTCG-TCG-ACCCCA
Single-base substitution at stem	GCAACA-CAG-AAATCG-TCG-ACTCCA
Single-base deletion	GCAACA-CAG-AA_TCG-TCG-ACTCCA

Single-base insertion	GCAACA-CAG-AAACTCG-TCG-ACTCCA

[a] Subscript $_O$: sequences of original 3-arm nanostructure[1], $_{24}$: circuit targeting 24 nt DNA, $_{30}$: circuit targeting 30 nt DNA.

[b] MTC: mismatched at toehold catalyst, MSC: mismatched at stem catalyst, DC: deleted catalyst, IC: inserted catalyst.

[c] The red portions represent the mutated bases in catalyst DNA., The underline represents deleted base.

Table S2. Probability of minimum free energy structure formation and free energy of secondary structure of selected sequences

Oligonucleotides	Free energy of secondary structure (kcal/mol)	Probability
$C_{\_24}$	0	0.126
$C_{\_30}$	0.8	0.385
$C_{\_O}$	0	0.387
$H1_{\_24}$	-27.48	0.947
$H2_{\_24}$	-26.72	0.521
$H3_{\_24}$	-26.41	0.277
$H1_{\_30}$	-33.88	0.705
$H2_{\_30}$	-34.16	0.524
$H3_{\_30}$	-34.64	0.424
$H1_{\_O}$	-24.13	0.845
$H2_{\_O}$	-24.75	0.415
$H3_{\_O}$	-23.59	0.473

All simulations were executed with 1 M Na^+ at 25 °C under default setting of NUPACK.

Table S3. The calculated reactivity (%) between catalyst and targeted hairpin and between two hairpins for 3-CHA-24

	H1_24	**H2_24**	**H3_24**
H1_24	0	0.18	0.60
H2_24	0.18	0	0.50
H3_24	0.60	0.50	0
C_24	92.3	-	-

All simulations were executed with 1 M Na^+ at 25 °C under default setting of NUPACK.

Table S4. Comparison of signal to background ratio with other approaches

Method[a]	Advantage	Limitation	F/F_0 ratio[b, c]	Ref
Enzyme-free nucleic acid amplified detection	Improved signal	Background noise	< 3.2 (500 nM C)	[2]
HSC	Real-time, minimized noise	Low sensitivity	6.1 (250 nM C)	[3]
CHA	High sensitivity and specificity	Complex sequence design	3 (100 pM C)	[4]
CHA cascade	Detailed kinetic analysis	Lack of practical data	8 (5 nM C)	[5]
ISDPR	High sensitivity, low background noise	Extra graphene oxide addition	< 3 (5 nM C)	[6]
Translator-mediated CHA	Sensitive colorimetric detection	Long reaction time	< 2.5 (500 nM C)	[7]
TB-CHA	Multisite fluorescence	MoS_2 addition	< 7.5 (150 nM C)	[8]
3-CHA	Simple, suppressed leakage, improve S/B ratio	Lower sensitivity	8.9 (100 nM C) 7.9 (500 nM C)	This work

[a]. HSC: hairpin stacking circuit; ISDPR: isothermal strand-displacement polymerase reaction; CHA: catalyzed hairpin assembly; TB-CHA: three-branched catalyzed hairpin assembly

[b]. F/F_0 ratio: signal-to-background ratio

[c]. C: catalyst

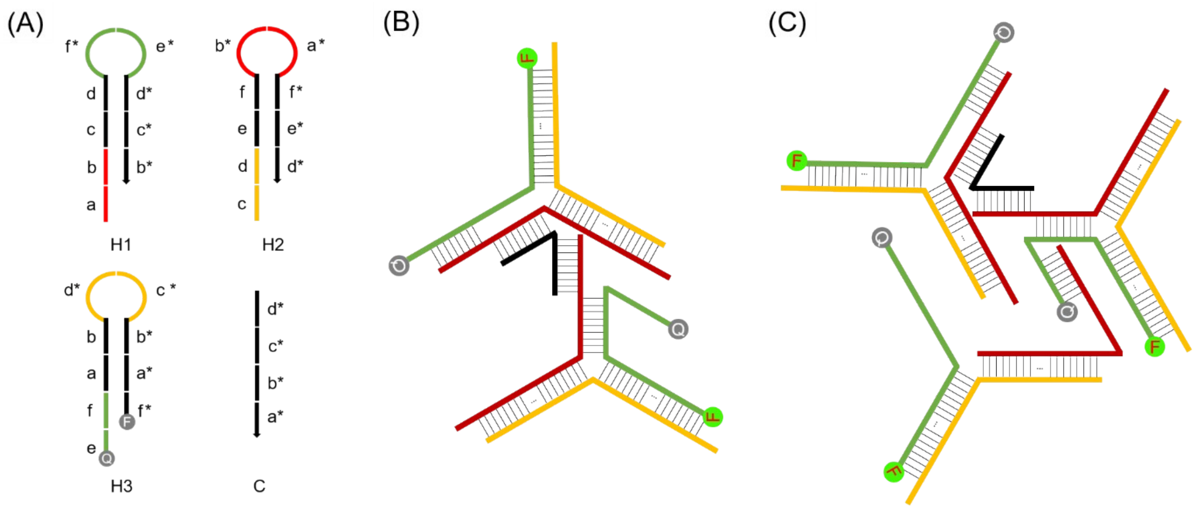

Figure S1. Domain organization of the original 3-arm nanostructure[1]. The color shows possible reaction sites due to disclosed ssDNA form which can induce spontaneous hybridization among hairpin DNAs. (B) (C) Possible multimeric forms of the amplified product, resulting the bands between 300 bp to 1000 bp in Figure 2 and Figure S2.

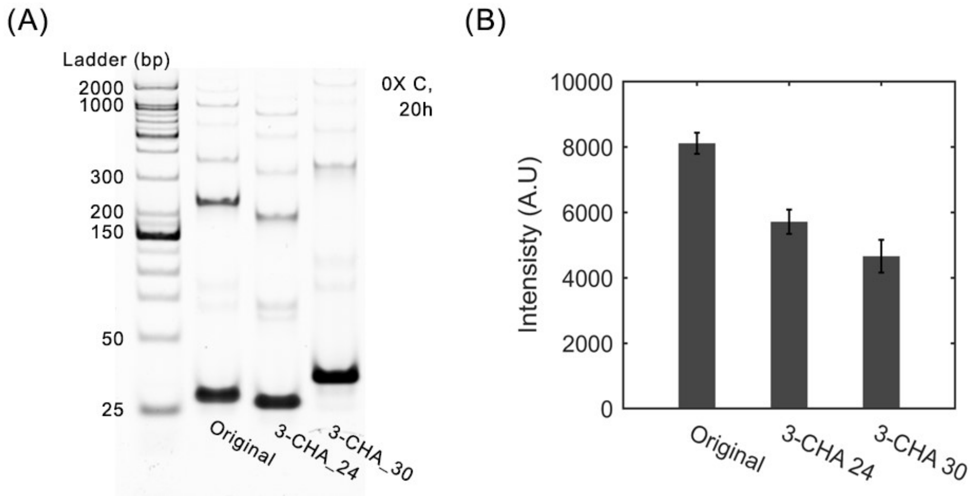

Figure S2. Assembly reaction in absence of catalyst of different 3-CHA circuits after 20 h incubation. (A) Comparison of different circuits by gel electrophoresis. (B) False-positive or leakage reaction.

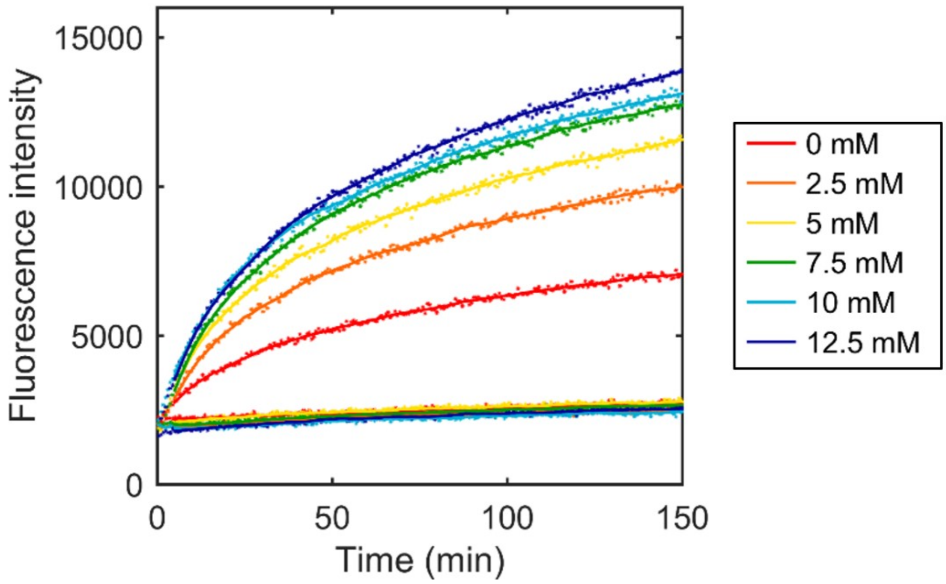

Figure S3. Time-coursed fluorescence depending on different concentration of Mg^{2+} in reaction buffer. Dots and lines represent collected data and digitally processed data, respectively.

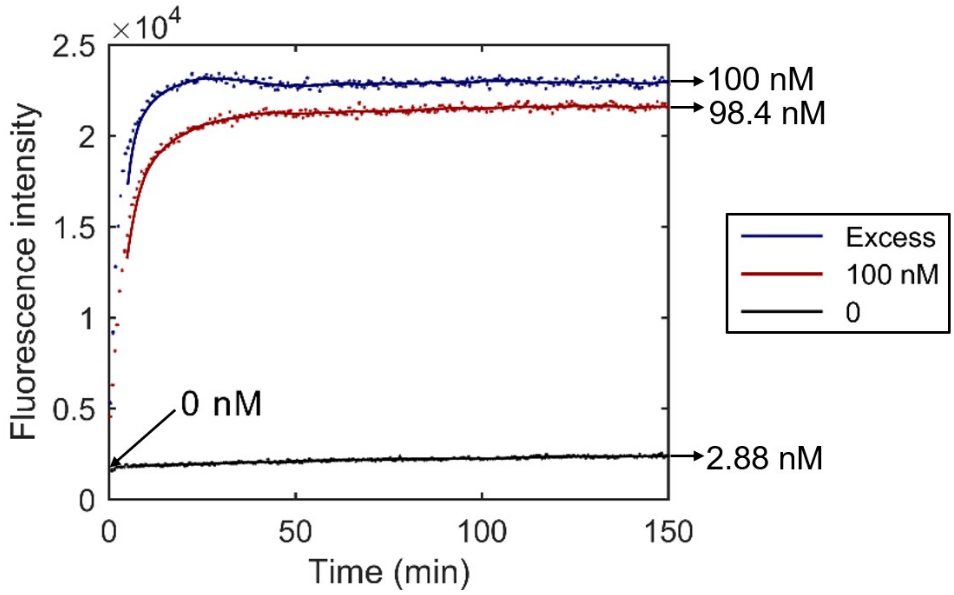

Figure S4. Control experiment for converting fluorescence intensity to concentration of product. Excess: 250 nM H1 + 250 nM H2 + 100 nM H3 + 100 nM C, 100 nM: 100 nM H1 + 100 nM H2 + 100 nM H3 + 100 nM C, 0: 100 nM H1 + 100 nM H2 + 100 nM H3 + 0 C, Dots and lines represent collected data and digitally processed data, respectively.

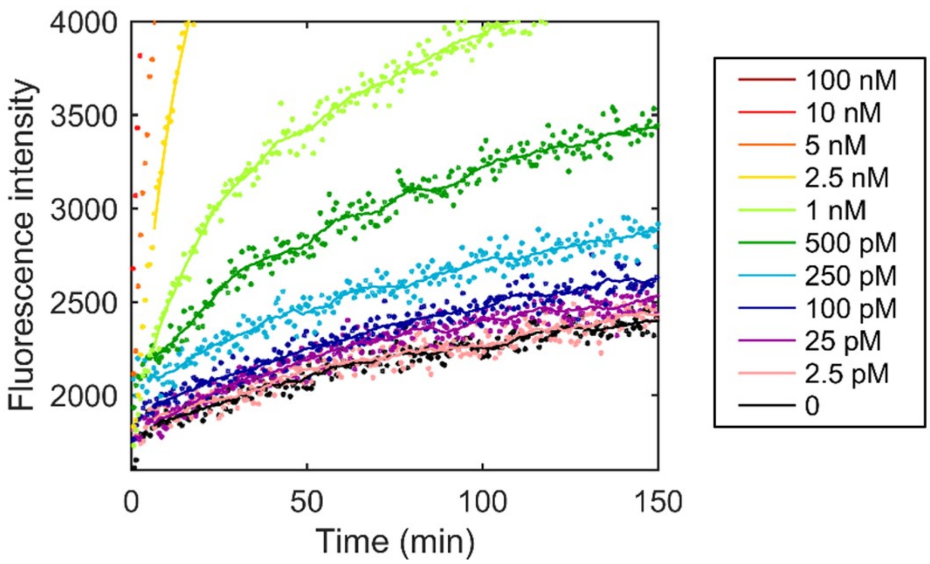

Figure S5. Time-coursed fluorescence of 3-CHA_24 depending on different concentration C, especially for low concentration. Dots and lines represent collected data and digitally processed data, respectively.

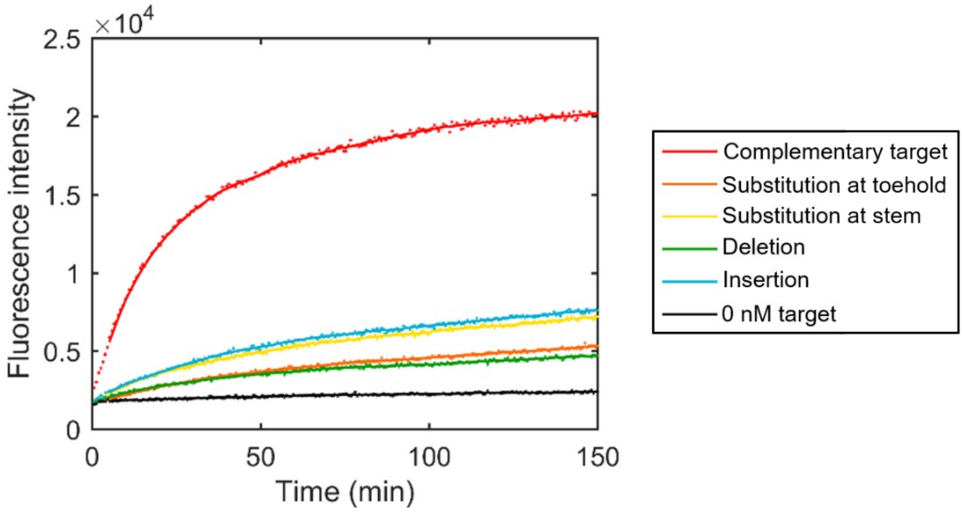

Figure S6. Signal amplification of 3-CHA_24 with perfectly complementary catalyst and SNPs. Catalytic reaction rate was significantly reduced with SNPs, showing selectivity of the 3-CHA. Dots and lines represent collected data and digitally processed data, respectively.

Reference

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