Supporting Information for

Establishment of a universal and rational gene detection strategy through three-way junction based remote transduction

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**Figure S1.** Scheme for a typical CHA pathway. The ssDNA C1 as a catalyst catalyzes the hairpin assembly.

**Figure S2.** Agarose gel electrophoresis shown the amplicons obtained by LAMP reaction using different concentrations of *ompA* gene as template. Line 1: no Target gene; Line 2: 200 copies *ompA* gene; Line 3: 2,000 copies *ompA* gene; Line 4: 20,000 copies *ompA* gene.
**Figure S3.** End-point detection of LAMP amplicons for *ompA* gene using 3W-CHA detector. The TP (TP2\textsubscript{ompA}TT) and CHA components (CHA2-H1\textsubscript{ompA}, CHA2-H2, CHA2-F and CHA2-Q) were added after LAMP reactions finished. Note: Reaction rate could not be used as a quantitative standard due to for different LAMP reactions, side reactions could happen randomly and thus affect the yields of correct amplicons.

**Figure S4.** One-tube one-channel detection of T1, T2 and T3. Kinetic curves (left) and respective initial rate (right) of the CHA reactions catalyzed by different targets with different concentrations of H1.
**Figure S5.** The self-fabricated mini portable reactor (in a dimension of 14.5 cm × 8 cm × 5.5 cm) could provide colorimetric fluorescence reading. The reactor has a two two-way buttons. Right one is for temperature setting. II side could be set to any temperature ranging from room temperature to 99 °C. We usually set it to 95 °C for lysis or 60 °C for LAMP reaction. For the left button, II side is to light up blue LED source to excite FAM emission. I side is to open a fan to avoid overheating. The photograph was taken by one of the authors of this work.
Comparison between the transduction strategies based on 3W-associative trigger, 4W-associative trigger, and hairpin transduction

Systematic comparison between the three kinds of transduction strategies should be much more complicated than that we could show in Figure 9. Ideally, each method has its own advantages and disadvantages, which could finally meet different practical applications. However, particular in this work, we thought some potential problems of 4W-associative trigger and hairpin transduction may make them relatively harder to use and design to be as efficient as the 3W-associative trigger.

The ideal pathway of transduction based on 4W-associative trigger is shown in Figure 9B1. While in detecting real nucleic acid targets (not limited to LAMP amplicons), two non-expected situations may happen. The first potential problem is formation of side products as shown in Figure 9B2. It shows a possibility that transducer-TH and transducer-BM bind with two of targets (e.g. LAMP loop amplicons), producing side products that will not induce OSD or CHA signal. In other words, certain amount of target sequences could only bind one of transducer, either TH or BM. This problem would become more serious when the target amount is comparable with or even higher than that of any one of transducer. As shown in Figure S6B, when the T\textsubscript{ompA} concentration is over than 20 nM (equal to concentration of TH), the catalytic rate of CHA reaction, induced by 4W-associative trigger, starts to decrease sharply. As a matter of fact, during detecting isothermal reactions such as LAMP, it would be really hard to predict how many amplicons will be produced and how many loop amplicons can be efficiently probed. So it will be relatively hard to determine the concentrations of TH and BM to be used. The transduction strategies based on 3W-associative trigger and hairpin transducer will not have this problem (Figure S6A and S6C). The second potential problem is inefficient hybridization. In previous study\textsuperscript{1}, we have concluded that formation of efficient 4W-associative trigger relies on stable hybridization of both m-m* and n-n*. The target (m-n) has to be long enough to stabilize both m-m* and n-n* hybridization, especially at high temperature around 55 °C in this work. To make efficient LAMP reaction, the single stranded loop amplicon should be no more than \textasciitilde 35-mer. Therefore, sometimes the binding energy between loop amplicon and TH (or/and BM) is too low to enable complete amplicon/TH/BM hybridization (Figure 9B2), thus showing less CHA efficiency under the same environments as 3W-associative trigger (As compared in Figure 9D and Figure S7). As predicted, if we artificially extend two bases to the end of both m and n domains, more 4W-associative trigger would be formed under the same condition, thus producing higher CHA signals (Comparison of Figure S7B and Figure S8B).
Figure S6. Comparison between three types of universal transduction strategies in presence of different concentrations of T_ompA. All the comparison was done under the same buffer situation and same concentration of TP2_ompA TT, hairpin transducer, and transducer-TH (20 nM) at 55 °C.

Figure S7. Fitting using Software of Nupack^2. It indicates that in presence of 20 nM T_ompA, 20 nM TH, and 30 nM BM, only about 7.2 nM efficient 4W-associative trigger could be formed. While in presence of 20 nM T_ompA and 20 nM TP2_ompA TT, about 19 nM efficient 3W-associative trigger would be formed.
Figure S8. Demonstration that detection based on 4W-associative trigger could be improved via increasing the binding energy of m-m* and n-n* hybridization. (A) Fitting using Software of Nupack when we artificially extend two bases at both ends of target T_{ompA} (forming new mimic target T*_{ompA}). It indicates that in presence of 20 nM T*_{ompA}, 20 nM TH, and 30 nM BM, about 15 nM efficient 4W-associative trigger could be formed. It is more than the one with T_{ompA} target (Figure S7A). (B) Detection of different concentrations of T*_{ompA}. It shows CHA reaction rate in presence of T*_{ompA} is faster than that of T_{ompA} under the same concentration (Figure S6B).

In the case of transduction based on hairpin transducer, efficient design is even harder. Relatively high non-target induced background leaking is almost non-avoidable because the whole CHA catalyst ($\alpha$-2-3) always exists in the solution, nothing but $\alpha$ domain is blocked by the hairpin stem. During the stem breathing, partial of $\alpha$ might be released and finally generates non-target induced CHA leakage (as shown in Figure 9C2 and 9D, blue curve). In order to block $\alpha$ sequence better, the stem of hairpin transducer has to be equal or longer than $\alpha$. Therefore there is also possibility to form self-dimer or self-trimmer products once the hairpin is opened by the target (as shown in Figure 9C2 and S9). In these side products, the domain $\alpha$ of hairpin transducer are re-trapped, decreasing the ability to trigger CHA reaction. Particular in detecting LAMP reactions, the transduction may also subject to head-to-head hybridization with the hairpin transducer, which will further limit the opening efficiency.
Figure S9. Fitting using Software of Nupack. It indicates that if 20 nM transducer was opened by the target, about 4.7 nM of them would self-bind to each other due to α-α* re-hybridization.
Name | Sequences (from 5ʹ to 3ʹ) | Label | Note
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OSD-Acceptor | TACCATTTTTTC_GTAAATGCACCGC_CCATCTCCCTACC_CTCCGA_CTGTAACCGG | 4_3* 2* α* | Probe triplex used in 3W-one step detector
OSD-F | GGGTGCCATTTCAAGAAAATGGTA | 4* | 3’ Inverted dT
OSD-Q | GTGTCGTGTAGTA_GCTTCATGTCGAG | 3’FAM

CHA1-H1_ompA | ACCACGGAGCAGG_CATCATCCAGAG_AAGATTATCGAG_CCACCGTCCCTACC_CTCCGA_TAAATCTCCTGATGTAGG_CCTTGTCAC_TACGCAGACAC | a* 2* 3* 4_3 2_5_6 | Sequences used in CHA1
CHA1-H1_multi | CATGCCCCTTCTC_CATCATCCAGAG_AAGATTATCGAG_CCACCGTCCCTACC_CTCCGA_TAAATCTCCTGGATGTAGG_CCTTGTCAC_TACGCAGACAC | a* 2* 3* 4_3 2_5_6
CHA1-H2 | AGGATTATCGAG_GGTTAGGGAGATGG_CCTGATATACTCTCCTGAGTATA_CCATC_CCTTGTCAC_TACGCAGACAC | 3_4* 3_2 2_4
CHA1-F | CGA_GTGCTGCGTA_GTGACAGGGG_CATCATCCAGAG | 5’FAM
CHA1-Q | CTTTGTCAC_TACGCAGACAC_TCG | 3’BHQ1

CHA2-H1_ompA | ACCACGGAGCAGG_GATCACACCCG_CAGACGTTGA_CCACGCTGCTAGCA_TCAACGTCTG | a* 2_3* 4_3 2_5_6 | Sequences used in CHA2
CHA2-H1_multi | CATGCCCCTTCTC_GATCACACCG_CAGACGTTGA_CCACGCTGCTAGCA_TCAACGTCTG | a* 2_3* 4_3 2_5_6
CHA2-H1 | GATCACACCG_TCTACACATGG_CGACATCT_AACCTAGC_CCTTGTCATA_AGAGCAC | 3*_4*_3_2_4
CHA2-F | CGA_GTGGTTGCTG_CAATCGTAGG_GGATGGG_TCAACGTCTG | 5’FAM
CHA2-Q | C_CCTTGTCATA_CCACGACAC_TCG | 3’BHQ1

H1-T1 | AGTATGAGTC_GCTAGGTT_AGATGTCG_CCATGTGTAGA_CGACATC_TAACTAGC_CCTTGTCAT_AGACGAC | a* 2_3* 4_3 2_5_6 | Sequences used in CHA3
H1-T2 | TAGAGATGAC_TGCTAGGTT_AGATGTCG_CCATGTGTAGA_CGACATC_TAACTAGC_CCTTGTCAT_AGACGAC | a* 2_3* 4_3 2_5_6
H1-T3 | AGGTTCCTCAC_GCTAGGTT_AGATGTCG_CCATGTGTAGA_CGACATCT_AACCTAGC_CCTTGTCAT_AGACGAC | a* 2_3* 4_3 2_5_6
H2 | AGATGTCG_TCTACATCGG_GGACATCT_AACCTAGC_CCAGTGGTAGA | 5’FAM
F | CGA_GTGCTGCT_ATGACAGGG_GCTAGGTT | 3_4* 3_2_4
Q | C_CCTTGTCAT_AGAGCAC_TCG | 3’BHQ1

T*ompA | AGCCAGCTGGGCAGCCAGG_CGCCTCCCTCCGTGTGGT | β_α
T*ompA | AAAGCCAGTGGGCAGCCAGG_CGCCTCCCTCCGTGTGGT | β_α
T*ompA-OSD (target used for 3W-OSD detection in Figure 2A) | AAGCCCGTTTACAGTGTG_GCCCTTATACGCCATGCGCCC | α_β

MisT_ompA1 | AGCCAGCTGGGCAGCCAGG_CGCCTCCCTCCGTGTGGT | β_α
MisT_ompA2 | AGCCAGCTGGGCAGCCAGG_CGCCTCCCTCCGTGTGGT | β_α
MisT_ompA3 | AGCCAGCTGGGCAGCCAGG_CGCCTCCCTCCGTGTGGT | β_α

Targets detected in this paper
Table S1. Sequence of oligonucleotides used in this work. Domains are separated by underscores.
Notes and references