Electronic Supplementary Material (ESI) for ChemComm.
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Supporting materials

Table of content

1. Oligonucleotides used in this study

Table 1: Oligonucleotides used in this study

Name	Sequence
MB1	5'-FAM-CGCGTTAACATACAATAGATCGCG-BHQ1-3'
YES1	5'-TATTGTGCGGGTTGTTCTCCGATC-3'
YES2	5'-CCCCAACAATGTTAACG-3'
I1m	5'-GGTCAACCCAACAGCGGGTTGTTCT-3'
I1	5'-Phos-CAGAACAACCCGCTGTTGGGGTTGACC
AND1	5'-TATGTCAGCGGGTTGTATTG-3'
AND2	5'-AACACCAATAACGCG-3'
AND3	5'-CGCGATCTTCTGACG-3'
Ia	5'-Phos-ACGTCGCGTCAGAACAACC-3'
Iam	5'-GGTTGTTCTGACGCGACGT-3'
Ib	5'-Phos-CGCTGTTGGTGTTGACCTG-3'
Ibm	5'-CAGGTCAACACCAACAGCG-3'

Underlined sections represent the toe-hold regions; 'Phos' is 5'-end phosphate group.

2. Figure S1. Fluorescent YES complex

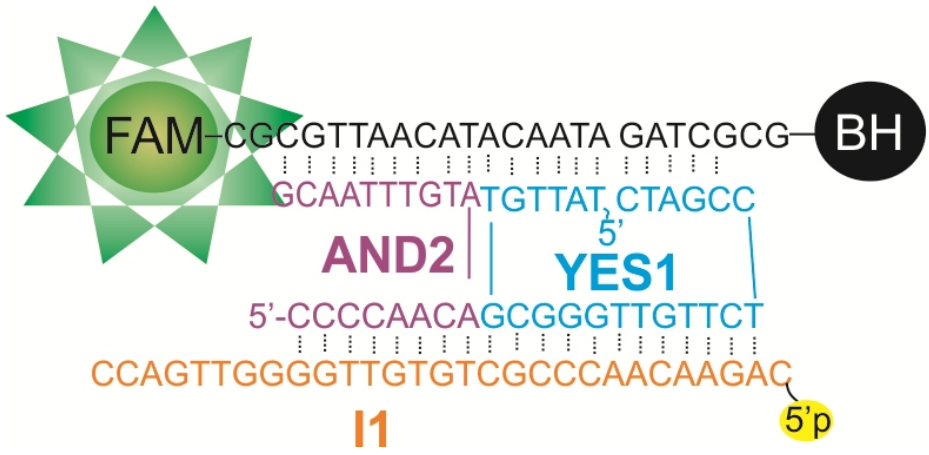

Figure S1. Predicted structure of fluorescent YES complex. The assembly of **MB1**, **YES1** (cyan), **YES 2** (magenta), and **I1** (orange) strands in a fluorescent complex. The 5'- and 3'- end of the oligonucleotides are indicated. 'FAM' is 5'-end conjugated fluorescein residue; BH, Black hole quencher 1; 5'p is a 5' phosphate group.

3. Figure S2. Fluorescent response of YES logic gate

The conditions for λ exo cleavage were thoroughly optimized for the resetting of the YES gates shown in Figure S1. The optimization included variations of temperature, concentrations of each DNA strand, concentration of $MgCl_2$ in order to increase the signal-to-background ratio (S/B) and reduce the time of operational cycle. The experiment under optimal conditions was reproduced three times (Figures 2a and S2).

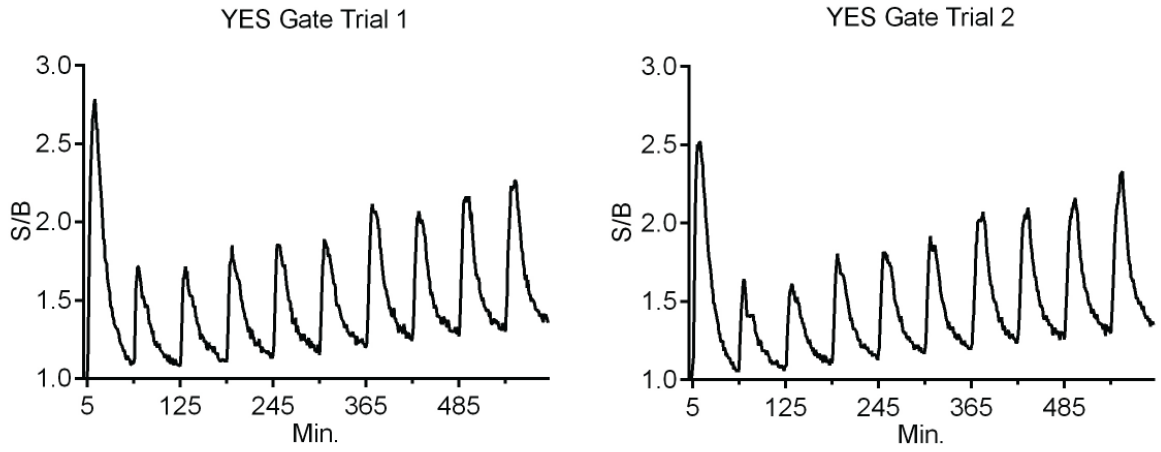

Figure S2. Fluorescent response of YES logic gate. Signal-to-background ratio (S/B) was calculated from the control sample lacking **I1**. DNA strands **MB1** probe, **YES1**, **YES2**, and **I1~~m~~** were incubated in the buffer (50 mM HEPES pH 7.4, 50 mM $MgCl_2$, 20 mM KCl, 120 mM NaCl,0.03% Triton X-100, 1% DMSO) containing 75 units of λ exo at 27.0°C. After a 5 min incubation, input oligonucleotide **I1** was added every 60 min **(5, 65, 125, 185, 245, 305, 365, 425, 485, and 545 min)**. The fluorescent response of the sample was continuously measured during 10 hrs.

We repeatedly observed greater S/B for the first operational round for both YES (Fig. S2 and for AND (Fig. S5) gates. We speculate that this is due to excess concentration of **I1** strand (plus the products of its partial degradation) over **YES1** and **YES2** after 2nd and subsequent operational rounds. Indeed, the total concentration of **I1** added in 2 after 2nd addition was 500 nM, while **YES1** and **YES2** were present at 375 nM. In excess of **I1** and its fragments**, YES1** and **YES2** can be bound to different **I1** or **I1** fragments, thus resulting in the complexes incapable to bind **MB1** probe and produce fluorescence. Indeed, **MB1** can be efficiently bound and elongated only if **YES1** and **YES2** cooperatively hybridize to form YES complex stabilized by a single **I1** (Fig S1). This hypothesis is supported by observation of bands B and C in a polyacrylamide gel, which might be the complexes of **YES1** or **YES2** with the fragment of **I1** obtained after its partial digestion.

The gradual background increase during the 10 hrs of operation (Fig. S2) is the result of incomplete dissociation of the fluorescent YES complex and accumulation of the products of incomplete input digestion which can still stabilize the association of **YES1** and **YES2** strands with **MB1** (see below Fig. S3, bands A, B and C in lanes 9-15).

4. **Figure S3. Polyacrylamide gel electrophoresis (PAGE) analysis of the reaction mixtures containing YES gate**

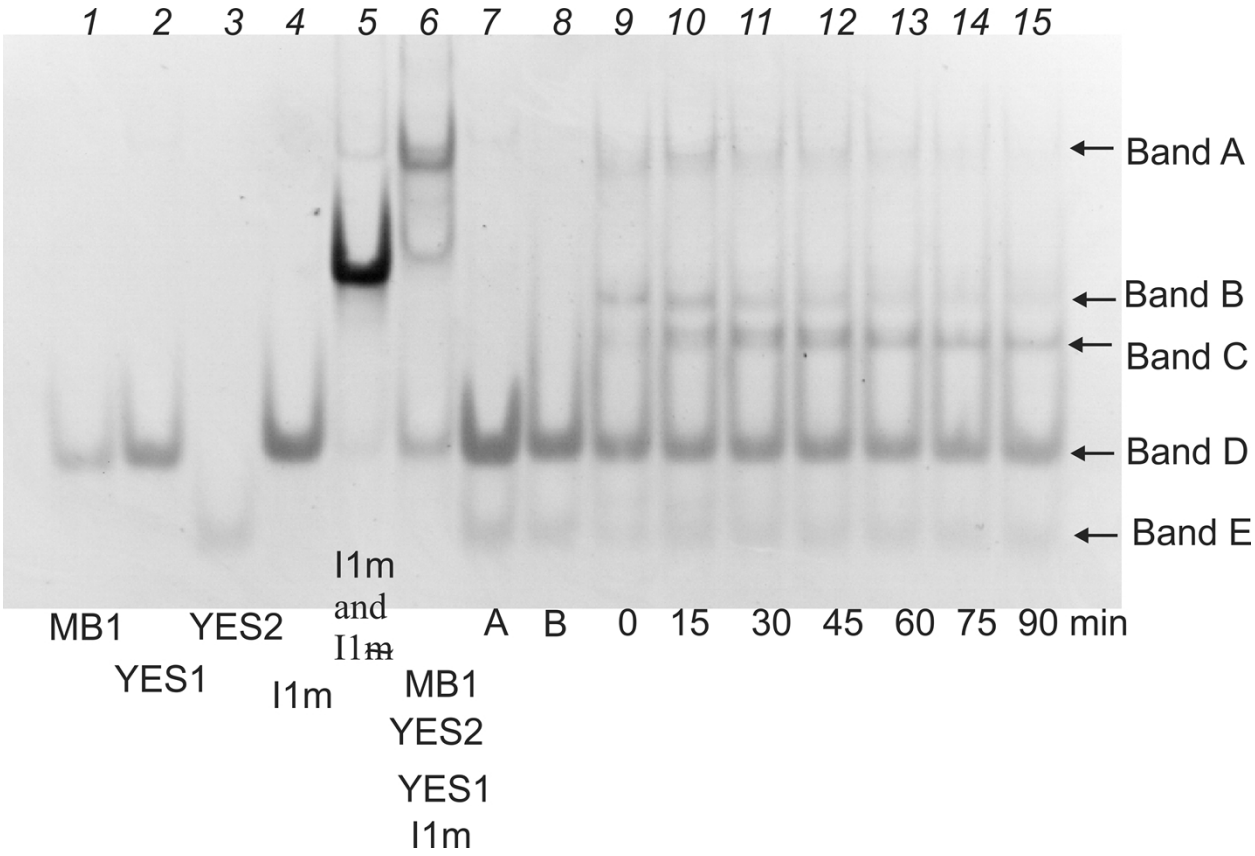

Figure S3. Native PAGE analysis of YES gate. The complex of the YES gate was analyzed in native 15% polyacrylamide gel containing 1xTBE buffer (89 mM Tris-base, 89 mM Boric acid, 2 mM EDTA) and 10 mM $MgCl_2$. The gel was run at 150 V for 4 hrs in a Thermo Owl electrophoresis apparatus with the temperature maintained at 3 ℃. The gel was stained with Gel Red (Biotium) for 7 min. Lanes 1-4 contained individual strands as indicated. Lane 5 contained input-complement duplex **(I1- I1m)**, formed by the toe-mediated strand displacement. Lane 6 contained strands **YES 1**, **YES 2**, **MB1**, and **I1**. Lanes 7-15 contain samples taken from the reaction mixture for YES gate at double concentration to ensure visualization. Lane 7 (A) is the reaction mixture before addition of λ exo. Lane 8 (B) is reaction mixture post addition of λ exo. Lanes 9-15 contain active reaction mixture (Fig.2) taken sequentially in 15 min intervals. Each sample was frozen by submersion in Dry-Ice Acetone bath (approximately -80℃) for 5 min and stored in -80℃ freezer until completion of the trial.

The sample of YES gates were analyzed at different stages of a single operational cycle by polyacrylamide gel electrophoresis (PAGE), which corresponds to the fluorescent results. Lanes 9-15 demonstrate the degradation of the full complex (Band A) with substantial reduction in intensity noted at 1 hr (Lane 13). The full complex is shown as a control in lane 6. Residual intensity of band A in lanes 14 and 15 is thought to represent background fluorescence. A small shift is noted for the **I1- I1m** duplex (band B) with the expected degradation from λ exo cleavage occurring in lanes 9-12. A band not present in controls (band C) is located beneath the expected

Il- I1m duplex in lanes 9-15. This band displays an inverse relationship to the **I1- I1m** duplex and is thought to represent a partial digestion product from the premature release by λ exo, forming a 3'-overhang. The partial digestion product increases in concentration until the full **I1- I1m** duplex is exhausted. The intensity of band C's intensity increases to maximum in lane 12, which corresponds to the disappearance of the **I1- I1m** (band B), with noted reduction by lane 15. The specificity of λ exo is demonstrated by the consistent intensity of component strands, bands D and E, in lanes 9-15. Unspecific digestion of component strands would negatively impact further logic operations.

Optimization Notes:

1. Component Strand Concentration: Significant optimization is necessary to achieve proper logic function. High concentrations of component strands are necessary to favor the formation of the fluorescent complex. Additionally, incubation 15 min necessary for the YES gate and 30 min for the AND gate. This establishes equilibrium between component strands, which is disturbed by addition of the input, pushing the reaction toward complex formation.
2. Temperature: 27℃ represents the optimal balance between high enzyme activity and YES complex stability. Higher temperatures result in more efficient λ exo activity but lower the maximum signal. This issue could be circumvented by altering strand sequences or lengthening the complex in order to improve its stability at higher temperatures.

5. Figure S4. Fluorescent AND complex

Figure S4. Predicted structure the fluorescent AND complex. The assembly of **MB1**, **AND1** (orange), **AND2** (red), **AND3** (cyan), **Ia** (magenta) and **Ib** (green) strand in a signal producing complex. The 5'- and 3'- ends of the oligonucleotides are indicated. 'FAM' is 5'-end conjugated fluorescein residue; BH, Black hole quencher 1; 5'p is a 5' phosphate group.

6. Figure S5. Fluorescent response of AND logic gate

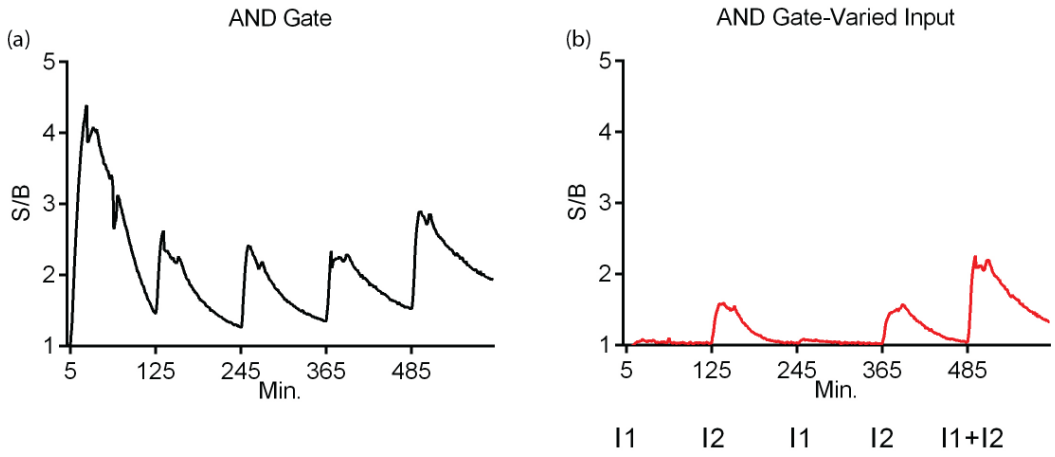

Figure S5. Fluorescent response of AND logic gate. (a) Both **Ia** and **Ib** inputs were added every 120 min after an initial 5 min pre-incubation (5, 125, 245, 365, 485 min). Signal to background (S/B) calculated from the control sample lacking the inputs. (b) Either **Ia** or **Ib** was added for first four cycles. At the final cycle (485 min) both **Ia** and **Ib** were added.