Supporting Information

A nucleic acid logic gate system that distinguish different sets of inputs from one miRNA collection with shared members

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S1. Additional Experiments

S1.1. Universal Reagents, Materials and Instruments

S1.1.1. DNA and RNA

All the DNA oligomers were synthesized by Sangon Biotech Co. Ltd. (Shanghai, China). And all the RNA sequences were synthesized by Genebase Co. Ltd. (Shanghai, China). Their sequences were shown in the corresponding sections of this document.

S1.1.2 Buffer solutions:

Imidazole-HCl solution was prepared by mixing 0.1 mol HCl with 0.1 M imidazole solution at appropriate ratio to reach a pH of 6.8.

TAE (Tris-acetate EDTA) buffer contains 40 mM tris(hydroxymethyl)aminomethane (tris), 2 mM EDTA and 20 mM acetic acid, and the pH was adjusted to 7.9.

PBS (phosphate buffered saline) buffer contains 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4 and 1.76 mM KH_2PO_4. The newly prepared PBS has a pH of 7.4; and the pH can be adjusted with HCl or NaOH if needed.

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Enzymes and relating buffers:

T4 DNA Ligase (EP0011) and the relating **buffer** were provided by Fermentas Inc. The buffer contains 400 mM Tris-HCl (pH 8.0 at 25°C), 100 mM $MgCl_2$, 100 mM DTT, and 5 mM ATP, and was diluted 10-fold when use.

Phi29 DNA Polymerase (EP0091) and the relating **Phi29 buffer (10×)** were provided by Fermentas Inc. The buffer contains 330 mM Tris-HCl (pH 7.9 at 25°C), 660 mM CH3COOK, 100 mM $(CH_3COO)_2$ Mg, 10 mM DTT, 1% Tween, and was diluted 10-fold when being used.

Nb.BbvCI (R0631) and the relating **NEBuffer2 (10×)** were provided by New England Labs. The buffer contains 100 mM Tris-HCl (pH 8.0 at 25°C), 50 mM $MgCl_2$ and 10 mM DTT, and was diluted 10-fold when use.

S1.2. Model Input miRNA collection

The input miRNA collection consisted of four miRNA members; their mature sequences were used in this study, according to the two online miRNA databases, miRNA Map [S1] and miRBase [S2].

Table S1. Sequences of input miRNAs

Name	Sequences
hsa-mir-92b (a)	5'- UAU UGC ACU CUC CCG GCC UCC -3'
hsa-miR-146a (b)	5'- UGA GAA CUG AAU UCC AUG GGU U -3'
hsa-mir-149 (c)	5'- UCU GGC UCC GUG UCU UCA CUC C -3'
hsa-mir-122a (d)	5'- UGG AGU GUG ACA AUG GUG UUU GU -3'
Mismatched hsa-mir-92b (a')	5'- UAU UGC ACU CGU CCG GCC UCC -3'
Mismatched hsa-mir-122a (d')	5'- UGA GAA CUU CAU UCC AUG GGU U -3'

S1.3. Initialization Stage

S1.3.1. Node

S1.3.1.1. The sequences of the DNA strands used in the node of the initial stage

Table S2. Sequences of the strands for initial stage

miRNA input	Strand role	Sequences
hsa-mir-92b (a)	Hairpin strand	5'-NH_2- ACA AGA CAC GCGA AGGC CCA GAA AGG C GGC ACT CGG GGA GGC CGG GAG AGT GCA ATA C CTC A▲GC CCG AGT GCC G -3'
	Messenger DNA	5'- GG CCT TTC TGG GCCT TCGC GTG TCT TGT GCTG TTT -3'
	Amp-primer	5'- C GGC ACT CGG -3'
hsa-miR-146a (b)	Hairpin strand	5'-NH_2- ATG ATC GTC GCCA ACCG ACC CTT TCC C GGC ACT CGG AAC CCA TGG AAT TCA GTT CTC A CTC A▲GC CCG AGT GCC G -3'
	Messenger DNA	5'- GG GGA AAG GGT CGGT TGGC GAC GAT CAT GCTG -3'
	Amp-primer	5'- C GGC ACT CGG -3'
hsa-mir-149 (c)	Hairpin strand	5'-NH_2- CTA TGA CAG CGGA ACGG GCA AGT AGC C GGC ACT CGG GGA GTG AAG ACA CGG AGC CAG A CTC A▲GC CCG AGT GCC G -3'
	Messenger DNA	5'- GG GCT ACT TGC CCGT TCCG CTG TCA TAG GCTG TTT - 3'
	Amp-primer	5'- C GGC ACT CGG -3'
hsa-mir-122a (d)	Hairpin strand	5'-NH_2- GTA GTT CGA CGCA AGCC CGT CAA GAG C GGC ACT CGG ACA AAC ACC ATT GTC ACA CTC CAC CTC A▲GC CCG AGT GCC G -3'
	Messenger DNA	5'- GG CTC TTG ACG GGCT TGCG TCG AAC TAC GCTG TTT - 3'
	Amp-primer	5'- C GGC ACT CGG -3'

The small triangle ▲ indicates the potential nicking point of Nb.BbvCI. A functional Nb.BbvCI nicking point would form on the complementary strand upon hybridization.

S 1.3.1.2. The initialization probes

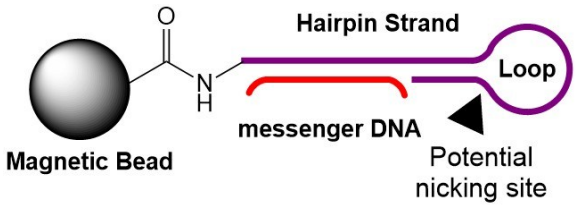
For each input miRNA, an initialization probe and an amplification primer (Amp-primer) were prepared for its transduction to messenger DNA and primary amplification; each initial probe was constructed with a hairpin strand and a messenger DNA bound on the hairpin; thus three DNA strands were needed for each input miRNA. The probes and primers were roughly based on our previous work[S3].

The design and construction of the initialization probe was based on one probe in our previous report[S3]. In this study, four instances of the probe (each for one input miRNA) were established under the same design (Scheme S1). They were all the hybridization product of one hairpin strand and one messenger DNA strand (corresponding to the according input miRNA in each instance). The hairpin strand hybridizes with the latter on its "stem" part, and the "loop" part of the hairpin was complementary to the according input miRNA; and a potential nicking site was placed on the loop part (indicated by the triangle). The entire probe is immobilized on the MBs at the 5'-end of the hairpin strand.

S1.3.1.3. Preparation of the probe

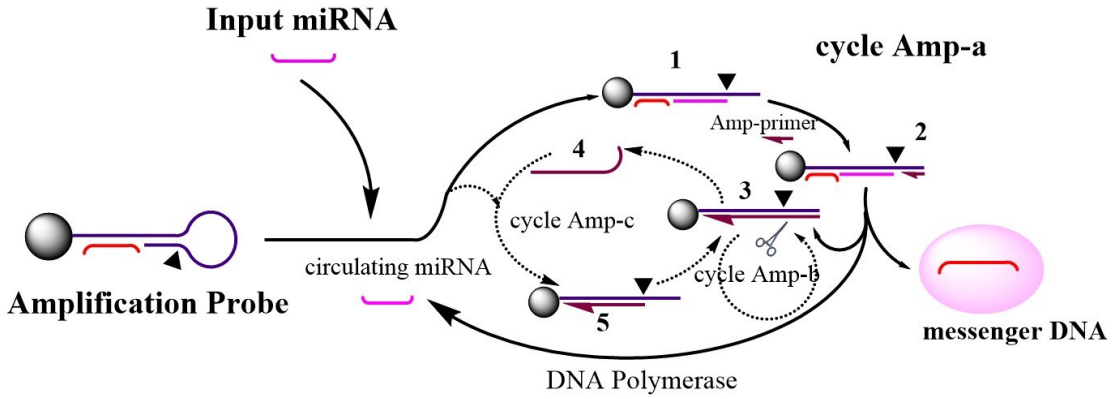
To prepare this probe, **the hairpin strand** was first attached to the MBs through the amido link. In detail, the magnetic beads were washed three times with 0.1 M imidazole-HCl solution (pH 6.8), and re-dispersed in TAE buffer to form a dispersion of 50 mg mL^{-1}. An aliquot of the dispersion (600 μL) was mixed with EDAC solution (1 mL, 0.1 M) and incubated for 60 min to activate the carboxyl groups on the magnetic beads. A portion (200 uL) aliquot of the solution of **the hairpin strand** (whose concentration varied in different experiments) was mixed with the imidazole-HCl solution described above (200 μL) for 30 min, and then the activated MB suspension was added. The linkage reaction was allowed to proceed for 12 h at 37 °C, after which the unattached DNA strands were removed by magnetic separation. The MB residues (with **the hairpin strand** attached) were then re-dispersed in TAE buffer to achieve a content of 50 mg mL^{-}

[1], and then mixed with **messenger DNA** (200 μL), and the mixture was then incubated for 60 min at 37 °C. The hybridized probes were then washed 3× with PBS, and magnetically separated. The residue was re-dispersed in TAE to form a suspension (500 μL) of a specific concentration (see below), which was maintained at a temperature < 4 °C.



Scheme S1. The detailed structure of the initialization probe

S1.3.2. Wire



Scheme S2. Diagram of the Initialization stage.

The detailed diagram of the Initialization Stage was shown in Scheme S2. Function: recognizing input miRNA (one miRNA in one instance) and transducing it into messenger DNA in amplified quantities; this is carried out in three designed reaction cycles; in practice, these cycles proceeds automatically.

Cycle INIT-a

Function: directly releasing the messenger sequence; providing first signal amplification

Triggering: interaction of the input miRNA with the initialization probe

Output: “messenger DNA” is displaced and released in a strand-displacement polymerization

Cyclic: input miRNA is displaced and released in the same strand-displacement polymerization

Cycle INIT-a is the main part of the initialization stage, which is supposed to transduce the miRNA to messenger DNA, and to provide initial amplification. Design of this cycle is inspired by the report of Guo et al. in 2009.[S4] It is a simple strand-displacement polymerization cycle that is based on the initialization probe and **the input miRNA**. This cycle is initialized by the interaction of the **input miRNA** and the loop part of the **hairpin strand** in **the initialization probe**, causing the **hairpin** to open. Amp-primer is thus allowed to attach to the newly formed overhang in the **opened initialziation probe** and start a strand-replacement polymerization. The extending new strand replaces the binding between **miRNA** and **the hairpin strand**, and the binding between **hairpin strand** and the **messenger DNA**. As a result, the **hairpin strand-based duplex** is formed on magnetic bead, while the **messenger DNA** and the **input miRNA** are released into the solution. The released **miRNA** can then bind to another **initialziation probe** to start another round of this cycle while the **messenger DNA** would be fed to the main logic stage in the supernatant after magnetic separation.

Cycle INIT-b and Cycle INIT-c

Triggering: nicking-polymerization cycle of the dsDNA based on the hairpin strand, producing ssDNA that interacts with the initialization probe through hybridization.

Output: messenger DNA is displaced and released in a strand-displacement polymerization

Cyclic: the dsDNA based on the hairpin strand is recovered in the strand-displacement polymerization

Cycle INIT-b: During the strand-displacement polymerization in cycle INIT-a, a functional Nb.BbvCI nicking site is formed in the **hairpin-based duplex**, initiating its self-cycle (cycle INIT-c). In this cycle, **hairpin-based duplex** is nicked, and the 3'-end of the nick acts as a starting point, from which a strand-replacement polymerization can be triggered in the presence of DNA polymerase. This process re-generates the **hairpin-based duplex**, while **hairpin-based ssDNA** is released; resulting in the self-cycle of **hairpin-based duplex** that repetitively releases **hairpin-based ssDNA**.

Cycle INIT-c: the **hairpin-based ssDNA** in cycle INIT-b is complementary to **the hairpin strand** in **initialization probe**. It binds to this probe to produce **hairpin-based partial duplex** with a single-strand "overhang", to which Amp-primer can bind. This process starts a strand-displacement polymerization that forms **hairpin-based duplex**, which can in turn enter cycle INIT-b to cause repetitive releases **hairpin-based ssDNA**, which then binds to another **initialization probe** to start over the next round of Cycle INIT-c. In this cycle, the **hairpin-based ssDNA** is complementary to **the hairpin strand in initialization probe**, so that it can hybridize with the latter to produce **hairpin-based partial duplex** with a single-strand "overhang", to which **the Amp-primer** can bind, and start a strand-displacement polymerization that forms **hairpin-based dsDNA**, which can in turn enter cycle INIT-b to cause repetitive releases of **hairpin-based ssDNA**, which then binds to another initialization probe to start next round of cycle, completing cycle INIT-c that operates in the similar mode as in cycle INIT-b.

S1.4. Logic Stage

S1.4.1. Node

S1.4.1.1. The sequences of the strands used in the node of the logic gate stage

Name	Sequences
Gate Template 1	5'- P-C ACA AGA CAC GCG AAG GC CCA GAA AGG CCTCA▲GC CTA TGA CAG CGGA ACGG GCA AGT AGC CCTCA▲GC ATG ATC GTC GCC AAC CG ACC CTT TCC CCTCA▲G -3'
Blocking strands for template 1	5'- TTT TCT TGT GC▲TGAGG GGA AAG TTT -3' 5'- TTT GAT CAT GC▲TGAGG GCT ACT TTT -3' 5'- TTT TCA TAG GC▲TGAGG CCT TTC TTT -3'
Gate Template 2	5'- P-C GTA GTT CGA CGC AAG CC CGT CAA GAG CCTCA▲GC CTA TGA CAG CGGA ACGG GCA AGT AGC CCTCA▲GC ATG ATC GTC GCC AAC CG ACC CTT TCC CCTCA▲G -3'
Blocking strands for template 2	5'- TTT AAC TAC GC▲TGAGG GGA AAG TTT -3' 5'- TTT GAT CAT GC▲TGAGG GCT ACT TTT -3' 5'- TTT TCA TAG GC▲TGAGG CTC TTG TTT -3'

Notes:

1) The small triangle ▲ indicates the potential nicking point of Nb.BbvCI. A functional Nb.BbvCI nicking point would form on the complementary strand upon hybridization.

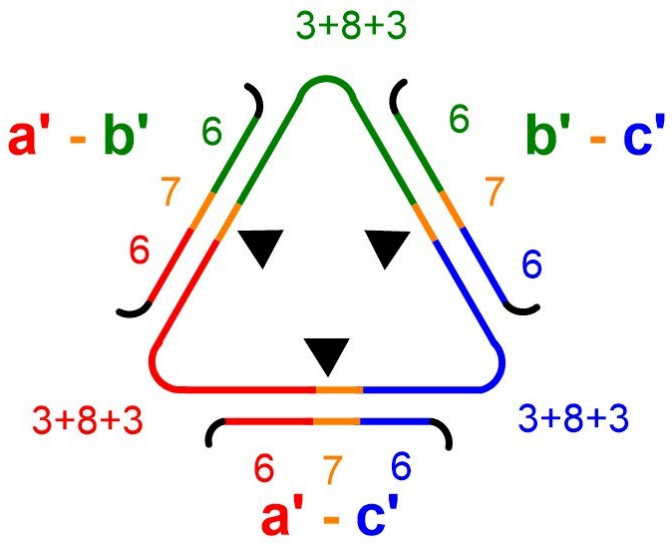
2) 5'-P- stands for the phosphate group.

There are two gates in the logic gate stage, each dealing with one input miRNA set (three members). Each gate was operated based on its own node, each consisting of one circular DNA strand and three blocking strands. According to the design of this study, the blocking strands would only be displaced by the full set of the corresponding messenger DNA strands.

S1.4.1.2. The Main Logic probes

The node of the logic stage centred on "main logic probe". There were two instances of the probe (each for one gate) under the same design (Scheme S3). The probe was constructed by a single-stranded closed DNA circular template and three "blocking" strands. The strands and the probe were designed as the following:

1) The circular template was able to bind the three blocking strands, as well as to bind the three corresponding messenger DNAs (Scheme S4). Their bindings have the following characteristics:
2) The binding section of the three messenger DNAs on the circular template surpasses the three blocking strands in length and GC ratio, allowing the former to replace the latter in the binding with the template through hybridization-displacement; the three messenger DNAs were equal in their lengths (32 nt each) and in their G-C ratios (20 nt each, GC% = 62.5%), as well as the three blocking strands (19 nt each, with 10 G/C each, 52.6%).
3) The blocking strands and the messenger DNAs bound to the circular template on staggered sections: the binding section of each blocking strand stretched across parts of those of the two neighboring messenger DNAs, and vice versa.
4) The circular probe contains three potential nicking sites (indicated by the black triangles in Scheme S3). The binding section of each blocking strand covers these sites to form functional nicking sites, in accordance in its designed function: it should be vulnerable to the nicking endonuclease when the blocking strands bind on (Scheme S4).
5) Due to the staggered binding described in 3), the binding section of each messenger DNA keeps away from the above-mentioned nicking sites, thus when the full set of messenger DNAs bind on the circular template, no nicking sites would be functional.



Scheme S3. The detailed structure of the main logic probe. The color codes are in accordance with those in Table S1; the numbers were the base numbers of each section; black triangles indicated the nicking sites.

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S1.4.1.3. Preparation of the circular template. The raw material of the circular template is an open-stranded sequence, with 5'-end modified by phosphate group for cyclization. To prepare the circular probe, the raw circular template and the according three blocking strands were mixed in equal concentration (typically, 2×10^{-8} M). And then, T4 DNA ligase and its buffer were added to complete cyclization, and the system was incubated at 25 °C for 2 h.

S1.4.2 Wire

Different reaction paths

Logic Stage (Scheme S4)

The wire of the logic stage had different reaction paths for the requirement of the production of on/off outputs; these paths were started by different binding statuses:

1.Various binding statuses of the circular template with the messenger DNAs or with the binding strands might occur according to the input; and different paths originated from different

binding statuses. While only two typical binding statuses were elucidated in the main text, an overall discussion will be given as the following:

a) Typical “Off” mode: The template remains bound with the three “blocking strands”, as in the initial status, or in the case that no messenger DNA (no input miRNA) was present;

b)Typical “On” mode: The template binds with the three messenger DNAs; this occurred when the whole set of characteristic miRNA inputs were present, and thus the blocking strands were removed through hybridization-displacement, due to their contrasts in the length and G-C contents.

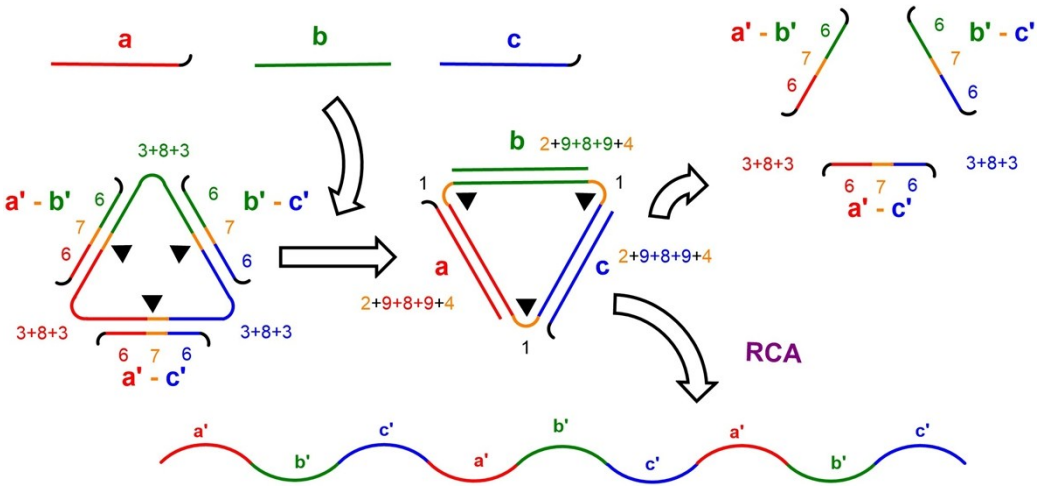
c) “Insufficient input” mode: Not the full set of messenger DNA, i.e. only one or two messenger DNAs in the set was inputted.

If only one member in the set was inputted, there would always be one “locking strand” that would not unaffected; simulation of hybridization with unpack.com[S6] shows that at least another blocking strand would remain bound on the circular template in prominent amounts;

When just two in the set was inputted, all the three locking strands would be affected, while simulation of hybridization with unpack.com shows that at least another blocking strand would remain bound on the circular template in prominent amounts;

In summary, in these cases, the complete displacement of the three blocking strands would not be guaranteed; portion of the blocking strands would still remain on the circular template, and un-neglectable amount of nicking sites would remain functional.

The following On/off nicking and the On/off rolling circle amplification (RCA) have been elucidated in the main text.



Scheme S4. Diagram for the Main Logic Stage

S1.5. Signal Stage

Node

Sequences

S1.1.4 The sequences for the signal production

For the production of the SERS signal, four strands were prepared. Two of them were designed for attaching the Raman signal tags on the RCA products of the two gates, respectively: one brought ROX (6-carboxy-x-rhodamine) on the product of gate 1, while the other attached MG (malachite green) on the product of gate 2 with the MG aptamer[S5]. Two other thiol-modified DNA oligomer strands were prepared for the construction of the matrix for the surface-enhancement of Raman scattering signals.

Table S3. Sequences of the strands used in the Logic Stage

Strand Name	**Sequence**
Raman tag 1 (ROX-tagged DNA)	5'- ROX - TTT TTT ACA AGA CAC GCG AAG GC CCA GAA AGG TT -3'
Raman tag 2 (MG -binding RNA)	5'- UUU UUU GUA GUU CGA CGC AAG CC CGU CAA GAG UU GGA UCC CGA CUG GCG AGA GCC AGG UAA CGA AUG GAU CC -3'
Matrix binding strand 1	5'- SH - TTT TTT GCG GCG GAG GAG GAG GCG GCG GCG GAG GTG GAG TTT TTT ATG ATC GTC GCC AAC CG ACC CTT TCC -3'
Matrix binding strand 2	5'- SH - TTT TTT CTC CAC CTC CGC CGC CGC CTC CTC CTC CGC CGC -3'

*** Notes:** ROX stands for 6-carboxy-x-rhodamine.

Gold nanoparticles (AuNPs) for the enhancement of the Raman scattering signals were prepared through the chemical reduction of chloroauric acid. 2.0% sodium citrate solution and chloroauric acid solution (0.01%) were filtered through filter membrane with average aperture of 0.2 μm, respectively. 1.0 mL sodium citrate solution was added dropwise into 100 mL boiling

chloroauric acid solution, and stirred for 10 min in persistent boiling. The prepared gold nanoparticles were stored under 4 °C before use. TEM examination indicated the prepared AuNPs were 15~20 nm in average diameter (Figure S1).

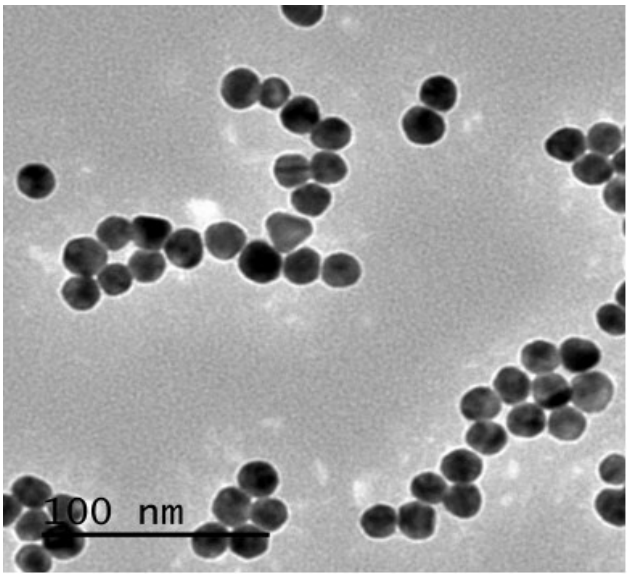


Figure S1. TEM image of the prepared AuNPs

Construction of the Enhancing Matrix

The matrix for the enhancement of the Raman scattering signal was the assembly of the gold thin film and gold nanoparticles. The gold nanoparticles were divided into two types, each were attached to one of the two "matrix binding strands" through the well-established Au-SH attaching protocol; and the gold thin film were attached with matrix binding strand 2. The two "matrix binding strands" can bind with each other through hybridization, thus the thin film and the two types of gold nanoparticles; thus the three components could be assembled into a matrix.

Matrix binding strand 1 also houses a sequence that binds with the RCA products (using a shared sequence on the two products of the two gates), enabling the constructed matrix to capture the products.

Procedures

The matrix binding strands were labeled on the AuNPs with the following procedures. AuNPs dispersion in the presence of TCEP (10 mM) in 1.5 mL Tris-acetate buffer (pH 5.2). After being stirred in the dark for 16 h, the DNA-AuNP conjugate was aged in 0.1 M NaCl solution containing 20 mM Tris-acetate (pH 7.0) for 24 h, and was separated through centrifugation at 15,000 rpm for 30 min. The red oily residue was washed with 100 μL solution containing 300 mM NaCl and 25

mM Tris-acetate (pH 8.2) and then re-centrifuged. The washing and centrifugating were repeated twice, and the residue was re-dispersed in 1 mL solution containing 100 mM NaCl and 25 mM tris-sodium acetate, and kept under 4 °C before use. UV-Vis spectra indicated the attaching strand have been bound with AuNPs (Figure S2). The thin gold film was attached with matrix attaching strand with similar procedures.

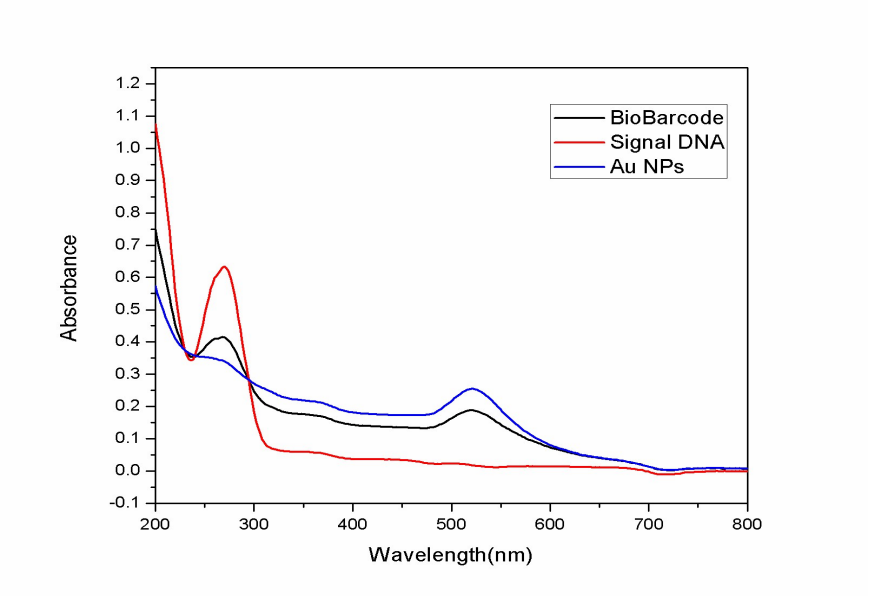


Figure S2. UV-Vis characterization of the binding between AuNPs and attaching strand.

a) AuNPs; b) attaching strand; c) attaching product

The matrix was assembled with the following procedures:

The two types of gold nanoparticles (attached with two matrix attaching strands respectively) were mixed in equal quantities, and drop on the thin film (after functionalized with matrix attaching strand 2). The complex was incubated at 37 °C for two hours. The prepared matrix was let stand to nearly drying, and used for Raman signal enhancement as soon as possible.

S1.6. Typical Assay Procedures

The four initial probes (8 μL each), Amp-primers (4 μL each), NEBuffer2 (×10, 16 μL), dNTP mixture (16 μL), Phi29 (4 μL), Nb.BbvCI (2 μL), and the miRNA sample solution (2 μL) were mixed and diluted to form a reaction mixture (100 μL), containing initial probes (2.0×10^{-7} M each), Amp-primers (5.0 μM each), NEBuffer2, dNTP mixture (0.25 mM), Phi29 polymerase (0.60 IU μL^{-1}), and Nb.BbvCI (0.40 IU μL^{-1}). The mixture was incubated at 37 °C for 2 h, magnetically separated, washed, and the supernatant were subjected to the logic stage.

The supernatant was briefly heated to 65 °C to deactivate the enzymes, and then further mixed with the two prepared logic gates, and was together incubated for. Then, further Nb.BbvCI and its buffer were added. The mixture was heated again. After another incubation for two hours, phi29 polymerase, its buffer and dNTP were added, and was further incubated at 37 °C for 2 h.

For the SERS measurements, ROX-tagged strand or MG-tagged aptamer of 1 μM were added into the resulting reaction mixture, according to the gate involved, and incubated at 37 °C for 1 h. Then, the mixture was sufficiently centrifuged in an EP tube, and washed with TAE buffer. Centrifugation and washing were repeated for three times. The residues were dispersed in water and then dropped on the enhancing matrix. After air-drying, the residue were subjected to SERS measurements.

All the SERS measurements were carried out on the Renishaw inVia Raman Microscope. For ROX (6-carboxy-x-rhodamine), the excitation wavelength was set to 633 nm; for MG (malachite Green), the excitation wavelength was set to 785 nm; these two tags produced Raman readouts under different excitation wavelengths, allowing the two gates to be measured respectively. For both cases, the Raman scattering spectra were recorded in the wavenumber range from 500 cm^{-1} to 1900 cm^{-1}.

S2. Additional Results

Verification of the process

S4. PAGE characterization of the network.

To verify whether the reaction system worked as planned as described above (or, as in Scheme 1), non-denaturing polyacrylamide gel electrophoresis (PAGE) was carried out to testify the DNA reaction part. The details of operation of the pathways are elucidated below, and the results are shown in Figure S3. Non-denaturing polyacrylamide gel electrophoresis (PAGE) was carried out on a DYCZ-24DN cell with Liuyi DYY-6C power supply (Beijing Liuyi Instruments, Beijing, China), and the PAGE patterns were imaged on a WD-9413B gel imaging system (same supplier as above). Typically, the PAGE was run at 120 V for 2 h.

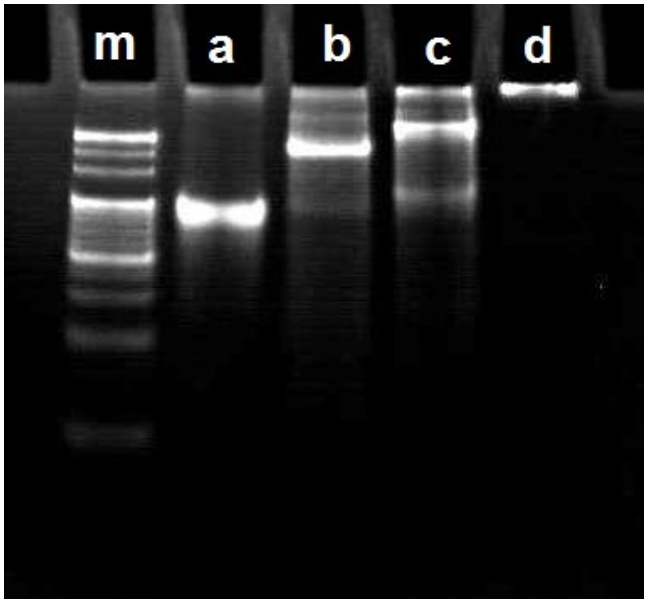


Figure S3. Polyacrylamide gel electrophoresis (PAGE) pattern. *m* : Marker ; *a* : Circular probe for the logic stage; *b* : circular probe with three assisstant strands; *c* : circular probe, three assisstant strands, and three messenger DNAs; *d* : RCA product.

Through the PAGE investigations, the process of the logic gate stage was examined.

Lane (a): the circular probe;

Lane (b): the circular probe plus three assistant strands.

Lane (c): the circular probe plus three assistant strands and three messenger strands.

Lane (d): the RCA product.

Comparison of Lane (a) and Lane (b) indicated that the three assistant strands were attached on the circular probe;

Comparison of Lane (b) and Lane (c) indicated that the three messenger strands attached on the circular probe, and displaced the three assistant probes;

Lane (d) indicated the appearance of the RCA product.

Performance of the Assay

S2.1. Optimization of the conditions of the assay

Various factors affected nicking-polymerization, and thus affected the total amplification efficiency of the network. For these factors, the temperature and pH were predetermined by the user manual for enzymes used in this study, *Phi29* DNA Polymerase ("Phi29" for short), and the nicking endonuclease, Nb.BbvCI. Fortunately, the enzymes share the same optimal temperature (37°C) and the same optimal pH (8.0). Thus, the variable conditions, i.e. the time duration and the content of the enzymes, were examined as described below.

Common reaction conditions: transduction template, 2×10^{-7} M each; logic gate circular template, 1×10^{-8} M each; assistant strands, 1.2×10^{-8} M each; input miRNAs, 1×10^{-10} M each; NEBuffer2; dNTP mixture (0.25 mM each)

To investigate the influence of reaction time, the reaction system was carried out under the following variable conditions: Phi29 (1.0 IU μL^{-1}), Nb.BbvCI (0.6 IU μL^{-1}). The mixture was incubated at 37 °C for different reaction times ranging from 1 h to 5 h. The SERS response reached a platform after a reaction time of 3 h. This timespan was then chosen as the optimal time (Figure S4A).

The effects of the enzymes on the output of the network were also examined.

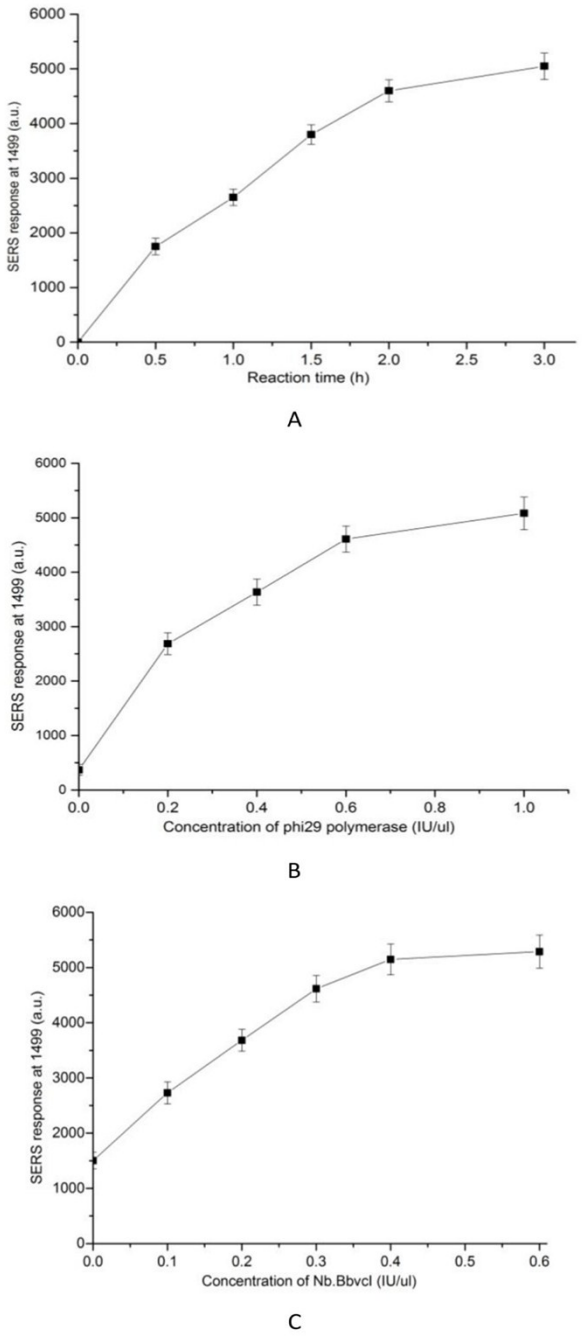


Figure S4. Optimization of reaction conditions. (A) Effect of reaction time; (B) Effect of Phi29 DNA Polymerase. (C) Effect of Nb.BbvCI content; Common reaction conditions: circular probe (10^{-8} M), NEBuffer2, Phi29 (1.0 IU μL^{-1}), Nb.BbvCI (0.6 IU μL^{-1}), dNTP mixture (0.25 mM each), incubated at 37 °C for 3 h.

To investigate the influence of Nb.BbvCI concentration, the reaction system was carried out under the following variable conditions: Phi29 (1.0 IU μL^{-1}), Nb.BbvCI (various concentration from 0 to 0.6 IU μL^{-1}). The mixture was incubated at 37 °C for 3 h. The SERS response reached a

platform after the Nb.BbvCI concentration of 0.4UμL^{-1}. This concentration was then chosen as the optimal (Figure S4B).

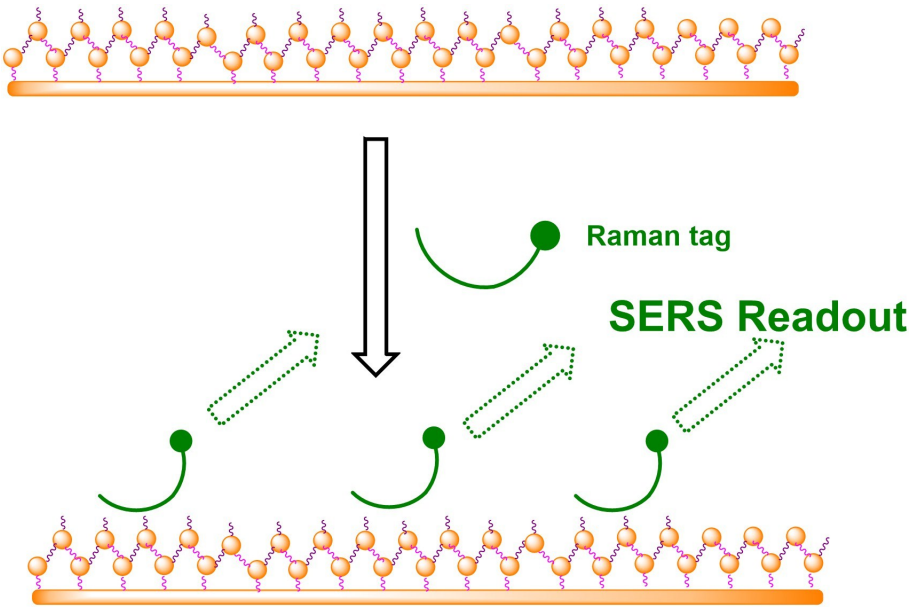
To investigate the influence of Phi29 DNA polymerase concentration, the reaction system was carried out under the following variable conditions: Phi29 (various concentration from 0 to 1.0 μL^{-1}), Nb.BbvCI (0.6 IU μL^{-1}). The mixture was incubated at 37 °C for 3 h. The SERS response reached platform after the Phi29 concentration of 0.6 μL^{-1}. This concentration was then chosen as the optimal (Figure S4C).

Performance of the Assay

S2.2. Signal Amplification Performance

Mode "No amplification" (Basepoint for amplification comparison)

To provide a basepoint for the amplification, the SERS responses of ROX-tagged strands of known concentrations were measured, i.e. in the case no amplification was applied. The diagram of this mode is shown in Scheme S5. To carry out this measurement, ROX-tagged strands were drip on the prepared SERS matrix. After air-drying, the residue were subjected to SERS measurements. The SERS responses were shown in Figure S5.



Scheme S5. Diagram of the mode "no amplification"

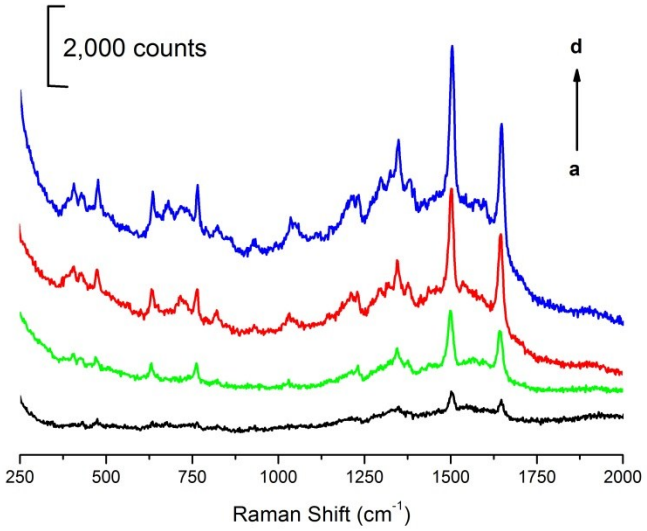


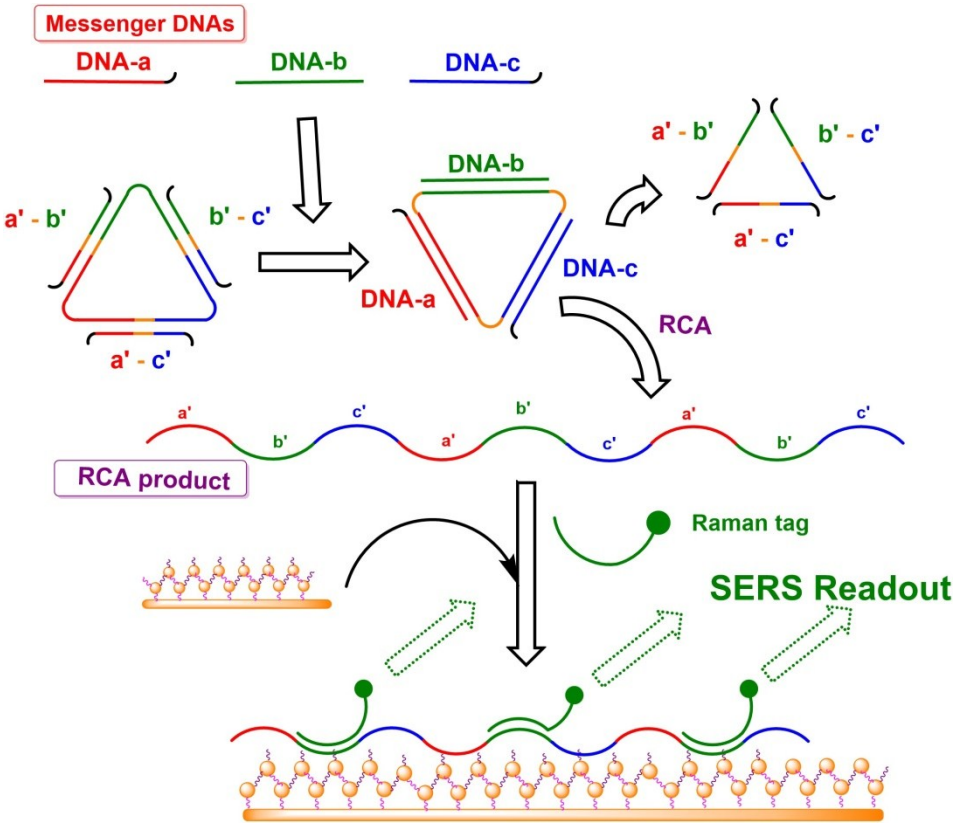
Figure S5. SERS responses in the "no amplification" mode.

Simulated transcripts concentration: a) 10^{-8} M; b) 10^{-7} M; c) 10^{-6} M; d) 10^{-5} M..

Mode "RCA only"

The diagram of the mode "RCA only" is shown in Scheme S6. In this mode, only the logic stage and the signal producing stage were carried out, i.e. the transduction stage was omitted.

The prepared messenger DNAs were directly input, instead of being generated in the transduction stage (tested from 10^{-13} M to 10^{-10} M in the mixture). The mixture was incubated at 37 °C for 30 min to allow a thorough hybridization. Then, the mixture was incubated at 37 °C for 3 h. The SERS responses were listed in Figure S6, and the calibration curve was shown in Figure 2 in the main text.



Scheme S6. Diagram of the mode “RCA only”

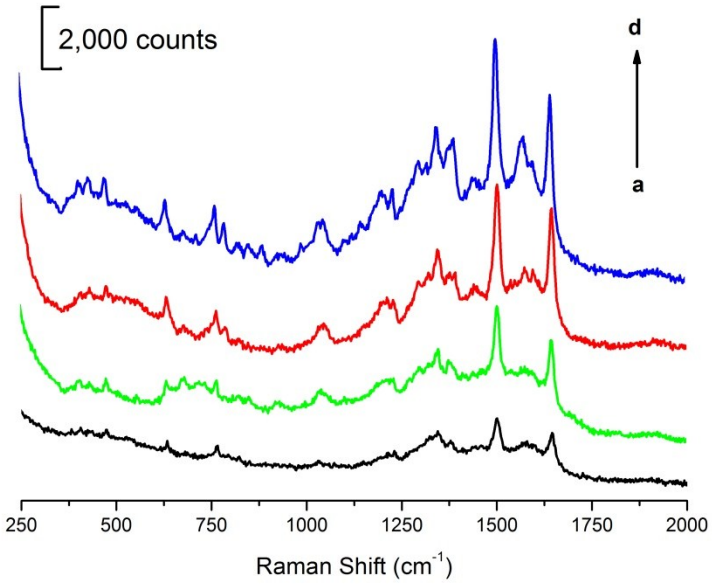


Figure S6. SERS responses in the “RCA only” mode.

a) 1.0×10^{-14} M, *b)* 1.0×10^{-12} M, *c)* 1.0×10^{-11} M, *d)* 1.0×10^{-10} M.

The complete reaction system.

The SERS responses at different concentrations of input miRNA sets, illustrated using were shown in Figure S7.

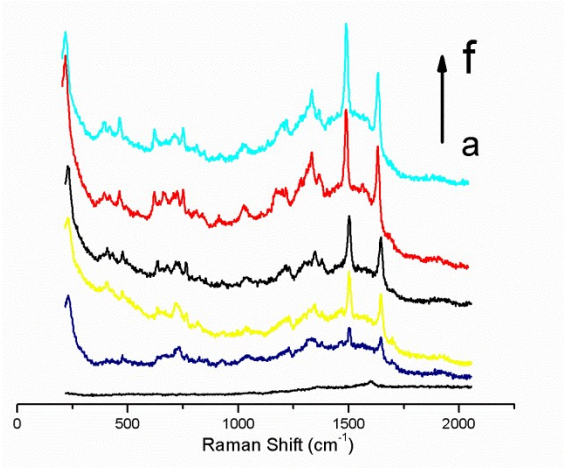


Figure S7. SERS responses for complete reaction system.

Target miRNA concentrations: *a)* blank, *b)* 1.0×10^{-16} M, *c)* 5.0×10^{-16} M, *d)* 1.0×10^{-15} M, *e)* 5.0×10^{-15} M, *f)* 1.0×10^{-14} M.

Performance of the Assay

S2.3. Logic Behaviors

Common Reaction Conditions (The Optimal Conditions):

Initialization template, 2×10^{-7} M each; logic gate circular template, 1×10^{-8} M each; assistant strands, 1.2×10^{-8} M each; input miRNAs, 1×10^{-10} M each; NEBuffer2; dNTP mixture (0.25 mM each); Phi29 (1.0 IU μL^{-1}), Nb.BbvCI (0.6 IU μL^{-1}). The mixture was incubated at 37 °C for 3h.

Original SERS reaction curves with the histograms

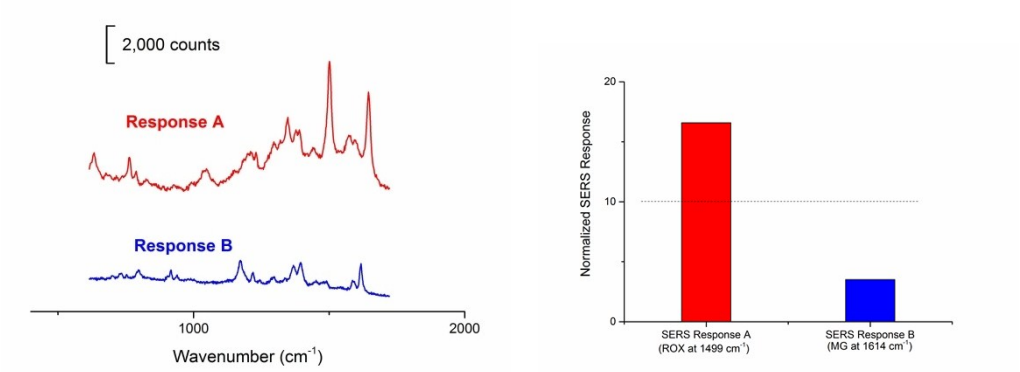


Figure S8. The responses of the linear threshold gate in the presence of miRNA a (hsa-mir-92b), b (hsa-miR-146a) and c (hsa-mir-149). Their concentrations were all 10^{-13} M.
（A）Original Raman curves（B）Histogram of the Raman intensities at the characteristic peaks

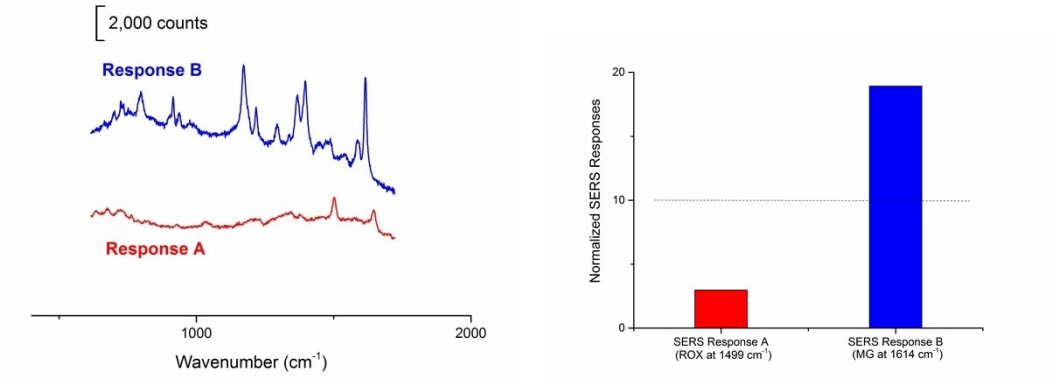


Figure S9. The responses of the linear threshold gate in the presence of miRNA b (hsa-miR-146a), c (hsa-mir-149) and d (hsa-mir-122a). Their concentrations were all 10^{-13} M.
（A）Original Raman curves（B）Histogram of the Raman intensities at the characteristic peaks

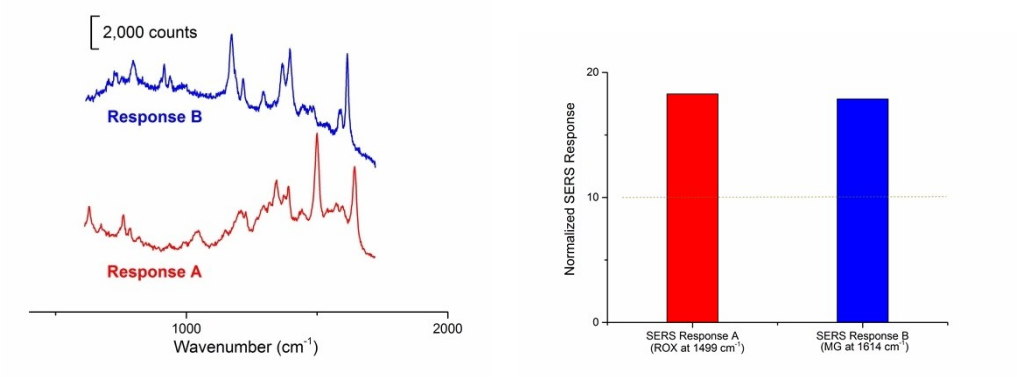


Figure S10. The response of the linear threshold gate in the presence of miRNA a (hsa-mir-92b), miRNA b (hsa-miR-146a), c (hsa-mir-149) and d (hsa-mir-122a). Their concentrations were all 10^{-13} M.

（A）Original Raman curves（B）Histogram of the Raman intensities at the characteristic peaks

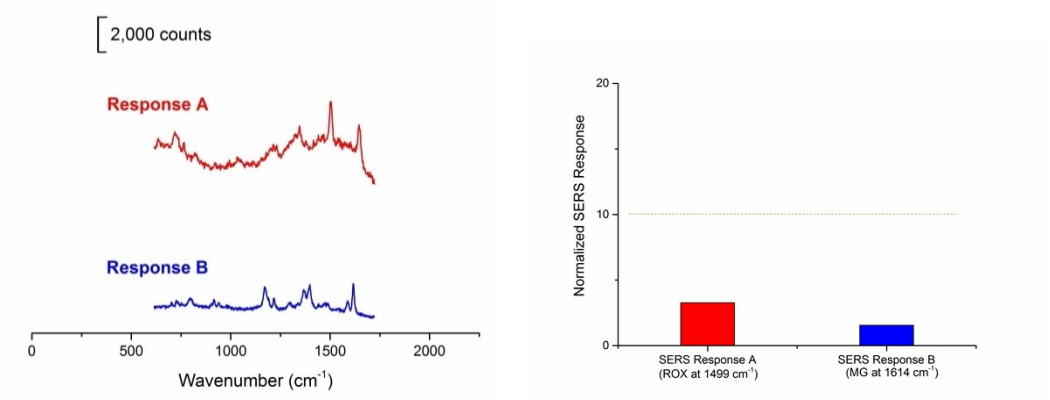


Figure S11. The response of the linear threshold gate in the presence of miRNA b (hsa-miR-146a) and c (hsa-mir-149). Their concentrations were all 10^{-13} M.

（A）Original Raman curves（B）Histogram of the Raman intensities at the characteristic peaks

S2.4. Interference test of the logic gates

Common Reaction Conditions (The Optimal Conditions):

Initialization template, 2×10^{-7} M each; logic gate circular template, 1×10^{-8} M each; assistant strands, 1.2×10^{-8} M each; input miRNAs, 1×10^{-10} M each; NEBuffer2; dNTP mixture (0.25 mM each); Phi29 (1.0 IU μL^{-1}), Nb.BbvCI (0.6 IU μL^{-1}). The mixture was incubated at 37 °C for 3h.

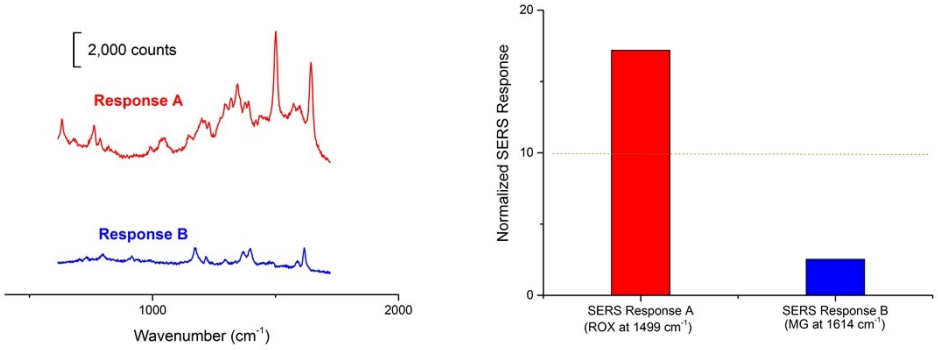


Figure S12. The response of the linear threshold gate in the presence of miRNA a (hsa-mir-92b), miRNA b (hsa-miR-146a), c (hsa-mir-149) and d' (mismatched hsa-mir-122a). Their concentrations were all 10^{-13} M.

（A）Original Raman curves（B）Histogram of the Raman intensities at the characteristic peaks

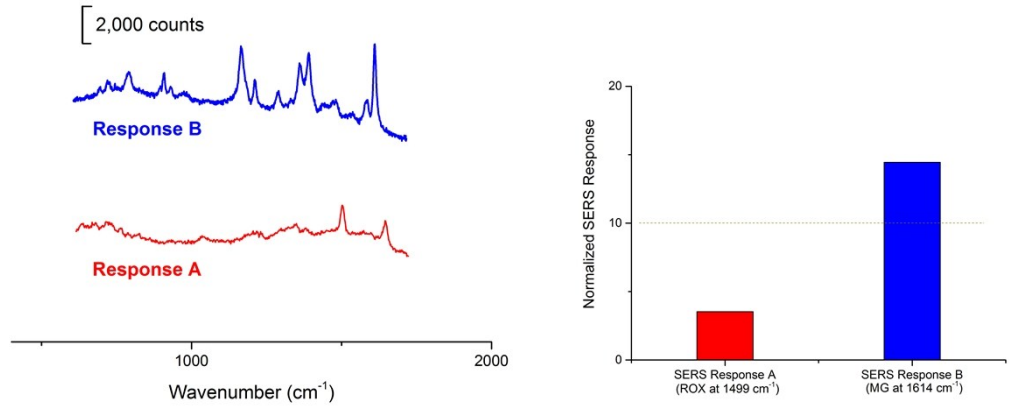


Figure S13. The response of the linear threshold gate in the presence of miRNA a' (mismatched hsa-mir-92b), miRNA b (hsa-miR-146a), c (hsa-mir-149) and d' (hsa-mir-122a). Their concentrations were all 10^{-13} M.

（A）Original Raman curves（B）Histogram of the Raman intensities at the characteristic peaks

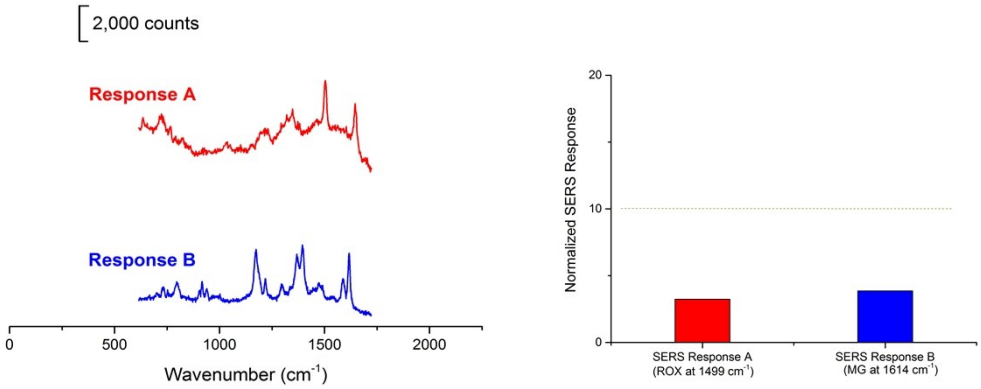


Figure S14. The response of the linear threshold gate in the presence of miRNA a' (mismatched hsa-mir-92b), miRNA b (hsa-miR-146a), c (hsa-mir-149) and d' (mismatched hsa-mir-122a). Their concentrations were all 10^{-13} M.

（A）Original Raman curves（B）Histogram of the Raman intensities at the characteristic peaks

S3. Supplementary References

[S1] miRNA Map: http://mirnamap.mbc.nctu.edu.tw/

[S2] miRBase: http://www.mirbase.org/

[S3] Rui Ren, Zhaotao Yu, Yuanming Zou, Shusheng Zhang. Enhancing the Sensitivity of Aptameric Detection of Lysozyme with a "Feed-forward" Reaction Network of Cycling DNA-Related Reactions. *Chem.– Eur. J.* 2012, 18, 14201-14209

[S4] Q. Guo, X. Yang, K. Wang, W. Tan, W. Li, H. Tang, H. Li, Sensitive fluorescence detection of nucleic acids based on isothermal circular strand-displacement polymerization reaction, Nucleic Acids Res. 2009, 37, e20

[S5] CalTech: http://www.nupack.org/

[S6] M. N. Stojanovic, D. M. Kolpashchikov, Modular Aptameric Sensors, J. Am. Chem. Soc. 2004, 126, 9266-9270