Electronic Supplementary Information

Multiple advanced logic gates made of DNA-Ag nanocluster and the application for intelligent detection of pathogenic bacterial genes

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Reagents and Materials

Silver nitrate was purchased from TianJin Tiangan chemical technology development Co., Ltd. Sodium borohydride was obtained from Alfa-Aesar chemicals Co., Ltd. SYBR@Gold Nucleic Acid Gel Stain (10,000×) was offered from Life Techonlogies. Acrylamide, ammonium persulfate, Boric acid, Tris (hydroxymethyl) aminomethane and N, N'-Methylenebisacrylamide were obtained from Aladdin Bio-chem technology Co., Ltd.. EDTA $Na_2 \cdot 2H_2O$ was purchased from GEN-VIEW Scientific Inc., N-Methyl Mesoporphyrin IX (NMM) was obtained from J&K Scientific Ltd.. All the Oligonucleotides (HPLC) are obtained from Sangon Biological Engineering Technology Co. Ltd., and all the detailed sequence information has been shown in Table S1. All chemicals used are of analytical reagent without further purification. All solutions are prepared with ultrapure water (18.2 MΩ·cm) is obtained from Milli-Q purification system.

Table S1. DNA sequences used in the investigation

DNA strand (P-DNA) for construction logic platform of DNA-AgNCs	CCCACCCACCCTCCC ATGCAAAATTTATGCAAGTCGTAT Segment for AgNCs Input-recognition segment
IN_{YES}	ATACGTGTGCTGTTGCTAATTTGCA GGGTGGGTGGGTGGGT
$IN1_{OR}$	ATACGACTTGCATAAATTTTGCA GGGTGGGGTGGGGTGGGG
$IN2_{OR}$	CTAAATACGACTTGCATAAATTTTGCA GGGTGGGGTGGGGTGGGG
$IN1_{AND}$	GGTTTTTTTTTTTTA GGGTGGGGTGGGGTGGGG
$IN2_{AND}$	ATACGACTTGCATAAATTTTGCAAATAAAAAAAAAAAACC
$IN2_{INH}$	CGACTTGGATAAATATTGCA GGGTGGGGTGGGGTGGGG
$IN1_{INH}$	ATACGACTTGCATAAATTTTGCATGGGATTTTTTTTTTTTT
$IN1_{XOR}$	ACCCATACGTGTGCTGTTGCTAATTTGCA GGGTGGGTGGGTGGGT
$IN2_{XOR}$	CCGCAACAGCACACGTATTAATGCGTTTAAATTTTGCAGGGTGGGT GGGT
$IN1_{HA}$	ATACGAGTTGCATAAATTTTGCA GGGTGGGTGGGT
$IN2_{HA}$	GGGTTGCAAAATTTATGCAACTCGATACGAGTTGCATAAATTTTCG AGGGTGGGTGGGT
$IN1_{HS}$	ATACGTGTGCTGTTGCTAATTTGCA GGGTGGGTGGGTGGGT
$IN2_{HS}$	CCCACCCGCAACAGCACACGTATTAATGCGTTTAAATTTTGCA GGGTGGGTGGGT
IN_{DMUX}	ATAGACGCAGTACGACATGGATAAATTTTGCAT GGGTGGGTGGGT
S_{DMUX}	GGGTATGCAAAATTTATCCATGTCGTACTGCGTCTA
$IN1_{MUX}$	ACTTGCATAAATTTTGCA GGGTGGGGTGGGGTGGGG
$IN2_{Mux}$	CTTTTATTTATATATTATTTAT GGGTGGGGTGGGGTGGGG
S_{MUX}	TTTTACGACTTGCATAAATTTTGCAATAAATAATATATAAATAAAA GTTAT
P1-AgNCs	CCCTTAATCCCCGAGATATTTCTCTACACCTTTATTAT
P2-AgNCs	CCCTTAATCCCCGAGACGGATGAGCGGCA
IN1 (*S. aureus*)	GGTGTAGAGAAATATGGTCCTGAAGCAAGTGCA
IN2 (*E. coli*)	TGCCGCTCATCCGCCACATATCCT
G-DNA	TGCACTTGCTTCAGGACCCTCT GGGTGGGGTGGGGTGGGG
Selector (S-DNA)	TGCACTTGCTTCAGGACCATATTTCTCTACATGGTTTTTTTTTTTC AGGATATGTGGCTCT GGGTGGGGTGGGGTGGGG
Comments	The black segment of the inputs was designed to hybridize with the input-recognition segment of the DNA-AgNCs.

Apparatus

Fluorescence spectra of silver nanocluster and NMM were recorded with a LUMINA Fluorescence Spectrometer (Thermo, USA). The gel images were obtained from the Gel Doc^{TM} XR+, Bio-RAD system (USA). The CD spectra of DNAs were collected by a Bio-Logic MOS450 (France). The background of the buffer solution was subtracted from the CD data.

Results and Discussion

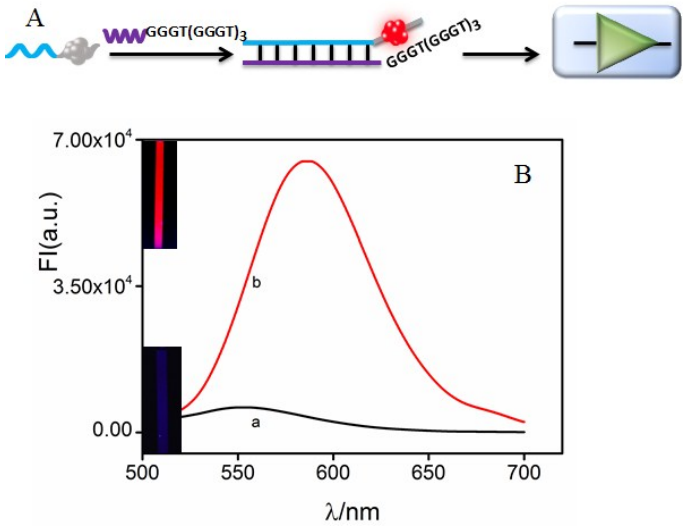
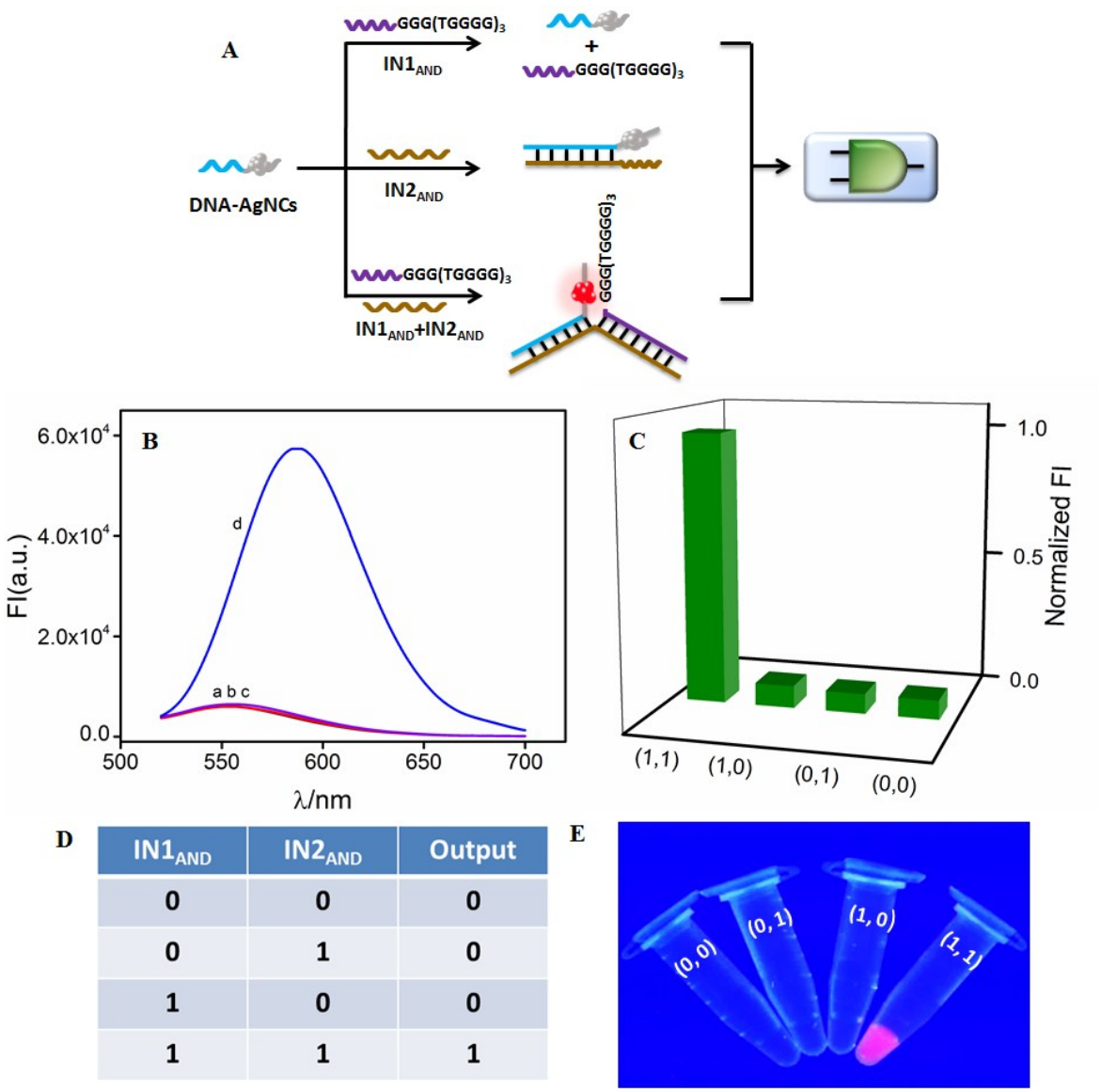


Fig. S1. (A) The principle scheme of the developed YES gate and the corresponding logic circuit. (B) YES logic gate results: The fluorescences of DNA-AgNCs before (a) and after hybridizing with G-rich DNA strands (b). The photographs of DNA-AgNCs before (bottom one) and after hybridized with G-rich DNA strands (upside one) under UV irridiation.

DNA strands containing G-rich segment approaches to the AgNCs through hybridization with DNA-AgNCs as shown in Fig. S1A, lighting up the fluorescence of AgNCs.[1] The dim fluorescence of AgNCs (Figure S1A (a)) was significantly enhanced, (Figure S1A (b)). This implemented the simplest YES logic function to generate a high output of "1" when the input exists as "1".



$IN1_{AND}$	$IN2_{AND}$	Output
0	0	0
0	1	0
1	0	0
1	1	1

Fig. S2. (A) The principle scheme of the developed AND gate and the corresponding logic circuit. (B) AND logic gate results: the fluorescent responses of DNA-AgNCs in the absence of inputs (a) and in the presence of $IN1_{AND}$ (b), $IN2_{AND}$ (c) and $IN1_{AND}$ +$IN2_{AND}$ (d). (C) The FI_{max} of DNA-AgNCs against various input combinations. The corresponding truth table (D) and photographs of the logic system under UV irradiation (E).

Fig. S2A depicts operation principle of the designed AND logic gate. For an AND logic gate, the system reported high output signal only when two inputs coexisted. The input 1 ($IN1_{AND}$) contained a G-rich segment while did not hybridize with the platform of DNA-AgNCs. Input 2 ($IN2_{AND}$) hybridized with the DNA-AgNCs, however, it did not contain a G-rich sequence. Thus, low output

signals were read in the above three cases, (0, 0), (0, 1), and (1, 0), as shown in Fig. S2B (a, b and c). Except for DNA-AgNCs, the $IN2_{AND}$ could also hybridize with $IN1_{AND}$ and acted as linkers, joining DNA-AgNCs and $IN1_{AND}$. The G-rich segment of $IN1_{AND}$ was close to the AgNCs to light up the fluorescence of the logic system (Fig. S2B, (d)). By plotting FI_{max} as a function of input combinations (Fig. S2C), the truth table clearly describes the operation of AND logic gate, (Fig. S2D), which could be conveniently seen from the photographs of the DNA-AgNCs against various input combinations under UV irradiation (Fig. S2E). The fluorescence of AgNCs could only be activated when both inputs were added together, meeting the feature of AND logic gate.

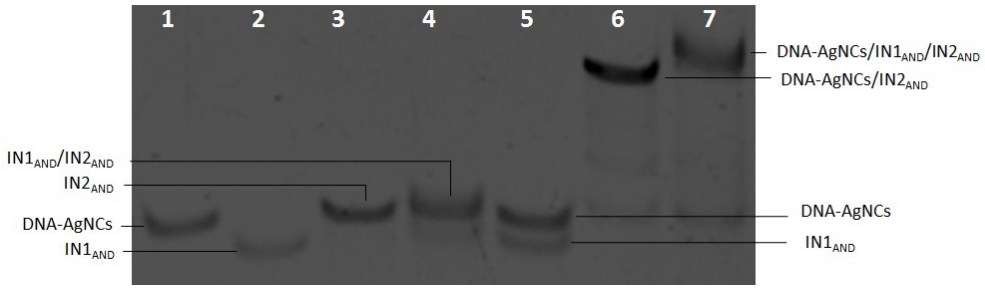
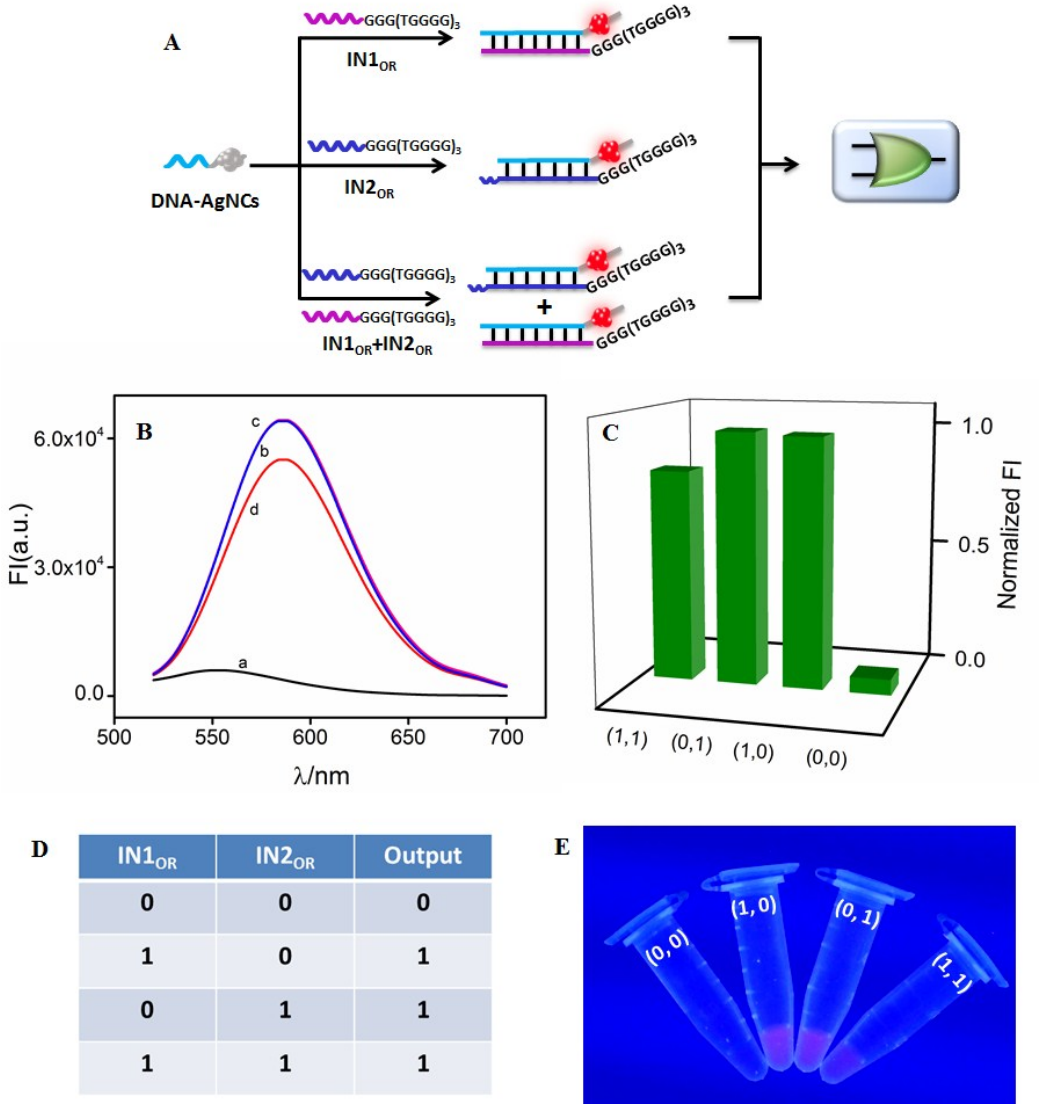


Fig. S3. Native polyacrylamide gel (20%) analysis of the interaction among DNA-AgNCs, $IN1_{AND}$ and $IN2_{AND}$ used in the developed AND logic gate. Lane 1: DNA-AgNCs, Lane 2: $IN1_{AND}$, Lane 3: $IN2_{AND}$, Lane 4: $IN1_{AND}$ and $IN2_{AND}$, Lane 5: DNA-AgNCs + $IN1_{AND}$, Lane 6: DNA-AgNCs + $IN2_{AND}$, Lane 7: DNA-AgNCs + $IN1_{AND}$ + $IN2_{AND}$.

The DNA interaction involved in AND logic operation was validated by conducting PAGE experiment, Fig. S3. From Lane 1 to Lane 3, the belts exhibit the individual DNA strands of DNA-AgNCs, $IN1_{AND}$ and $IN2_{AND}$ in sequence. The coexistence of $IN1_{AND}$ and $IN2_{AND}$ generates a new belt, Lane 4, indicating the formation of duplex of $IN1_{AND}/IN2_{AND}$. However, two individual belts still could be observed in the coexistence of DNA-AgNCs and $IN1_{AND}$, Lane 5, suggesting that no interaction happens between them. In the coexistence of DNA-AgNCs and $IN2_{AND}$, a new belt is monitored at position which is different from that of DNA-AgNCs or $IN2_{AND}$, Lane 6. The result indicates the formation of duplex of DNA-AgNCs/$IN2_{AND}$. The coexistence of DNA-AgNCs, $IN1_{AND}$ and $IN2_{AND}$

results in a new belt over all other belts (Lane 7) due to the hybridization among of the three DNA strands.



D

$IN1_{OR}$	$IN2_{OR}$	Output
0	0	0
1	0	1
0	1	1
1	1	1

Fig. S4. (A) The principle scheme of the developed OR gate and the corresponding logic circuit. (B) OR logic gate results: the fluorescent responses of DNA-AgNCs in the absence of inputs (a) and in the presence of $IN1_{OR}$ (b), $IN2_{OR}$ (c) and $IN1_{OR}$ +$IN2_{OR}$ (d). (C) The FI_{max} of DNA-AgNCs against various input combinations. The corresponding truth table (D) and photographs of the logic system under UV irradiation (E).

An OR logic gate reports high output signal in the presence of any input. The operation principle of the developed OR logic gate is illustrated in Fig. S4A. Here, both of the DNA inputs, $IN1_{OR}$ and $IN2_{OR}$, contained G-rich segment and hybridized with DNA-AgNCs via the input-recognition segment to form

duplexes of DNA-AgNCs/$IN1_{OR}$ and DNA-AgNCs/$IN2_{OR}$, respectively. Such formation of the duplexes resulted in the proximity of G-rich segment of the inputs to the AgNCs. The dim fluorescence of DNA-AgNCs (Fig. S4B, (a)) was then significantly enhanced (Fig. S4B, (b and c)). In the coexistence of $IN1_{OR}$ and $IN2_{OR}$, the system still reported high output signals caused by both DNA-AgNCs/$IN1_{OR}$ and DNA-AgNCs/$IN2_{OR}$ (Fig. S4B, (d)). By plotting the output signal against various input combinations (Fig. S4C), a truth table was produced (see Fig. S4D), which fits the feature of an OR logic gate. The logic operation could be instantly visualized from photographs of the DNA-AgNCs against various input combinations obtained under irradiation with UV (Fig. S4E). Logic output values of "0" and "1" were related to dark and light fluorescence of AgNCs, respectively. Obviously, the DNA-AgNCs could be lighted up by any one of the inputs or by both of them.

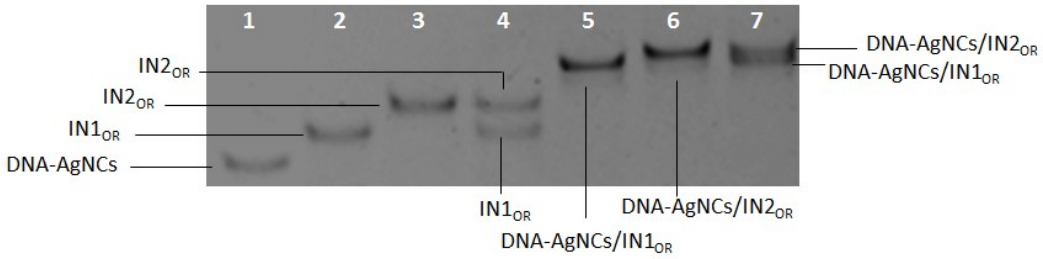
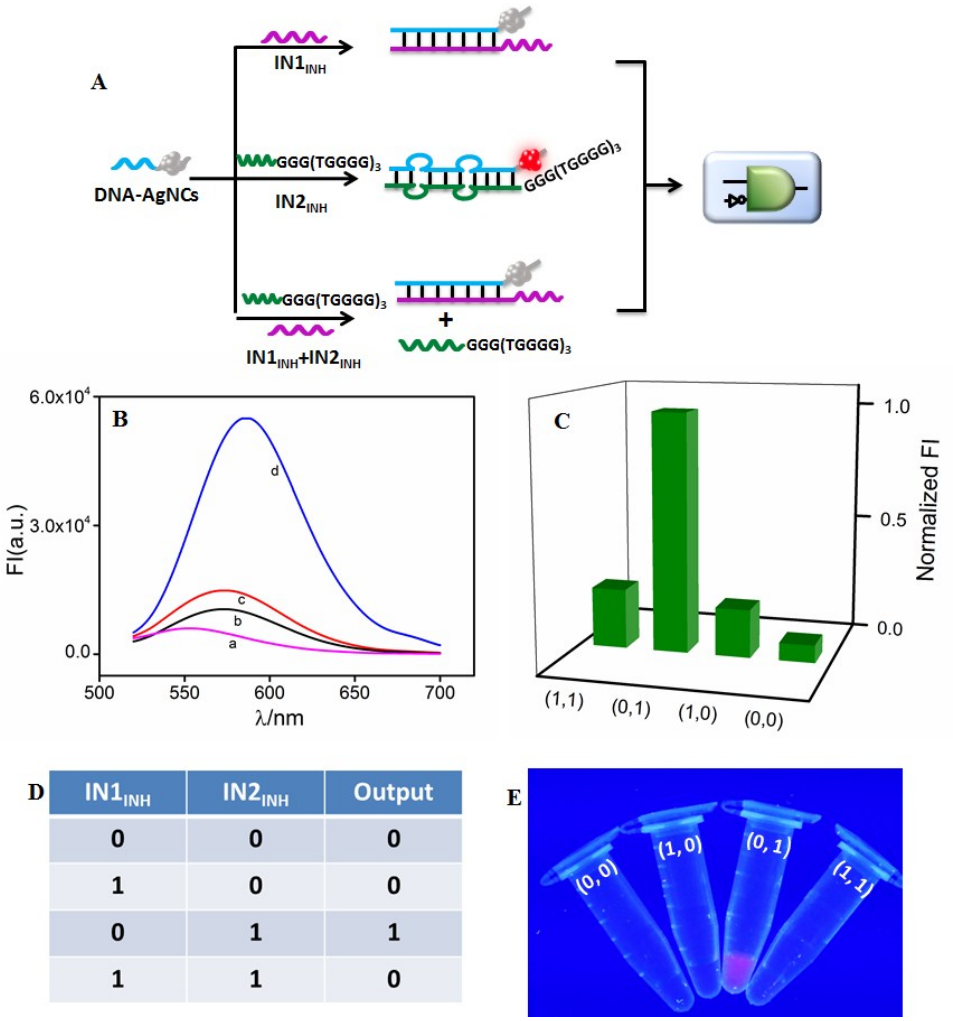


Fig. S5. Native polyacrylamide gel (20%) analysis of the interaction among DNA-AgNCs, $IN1_{OR}$ and $IN2_{OR}$ used in the developed OR logic gate. Lane 1: DNA-AgNCs, Lane 2: $IN1_{OR}$, Lane 3: $IN2_{OR}$, Lane 4: $IN1_{OR}$ and $IN2_{OR}$, Lane 5: DNA-AgNCs+$IN1_{OR}$, Lane 6: DNA-AgNCs+$IN2_{OR}$, Lane 7: DNA-AgNCs+$IN1_{OR}$+$IN2_{OR}$.

PAGE experiment was conducted to validate the DNA interaction of OR logic system, Fig. S5. From Lane 1 to Lane 3, the belts exhibit the individual DNA strands of DNA-AgNCs, $IN1_{OR}$ and $IN2_{OR}$ in sequence. Two separated belts appear in the coexistence of $IN1_{OR}$ and $IN2_{OR}$ (Lane 4), indicating that no hybridization occurs. In the presence of (DNA-AgNCs and $IN1_{OR}$) or (DNA-AgNCs and $IN2_{OR}$), new belts are observed from Lane 5 and Lane 6, respectively. The results indicate the formation of duplexes of DNA-AgNCs/$IN1_{OR}$ and DNA-AgNCs/$IN2_{OR}$. The coexistence of DNA-AgNCs, $IN2_{OR}$

and $IN1_{OR}$ generates belts (Lane 7) which appear at the similar position as those in Lane 5 and Lane 6, suggesting the coexistence of DNA-AgNCs/$IN1_{OR}$ and DNA-AgNCs/$IN2_{OR}$. The PAGE results validate the DNA reaction of OR logic gate occurs as expected, which are consistent with the fluorescence results, Fig. S4.



$IN1_{INH}$	$IN2_{INH}$	Output
0	0	0
1	0	0
0	1	1
1	1	0

Fig. S6. (A) The operation principle of the developed INH gate and the corresponding logic circuit. (B) Results of INH logic gate: the fluorescent response of DNA-AgNCs in the absence of inputs (a) and in the presence of $IN1_{INH}$ (b), $IN2_{INH}$ (c) and $IN1_{INH}$+$IN2_{INH}$ (d). (C) The FI_{max} of DNA-AgNCs against various input combinations. The corresponding truth table (D) and photographs of the logic system under UV irradiation (E).

A two-input INH logic gate generates a high output signal only in the presence of one of the two inputs, (0, 1), and low outputs in other cases, (0, 0), (1, 0) and (1, 1). As illustrated in Fig. S6A, the

input 1 ($IN1_{INH}$) formed a duplex with DNA-AgNCs, which reports a low fluorescence response (Fig. S6B, (b)) similar as that of DNA-AgNCs at the initial state (Fig. S6B, (a)). The hybridization of DNA-AgNCs with the input 2, $IN2_{INH}$, approached the G-rich segment of $IN2_{INH}$ to the AgNCs. A high fluorescence output of "1" was then monitored (Fig. S6B, (c)). In the coexistence of $IN1_{INH}$ and $IN2_{INH}$, the DNA-AgNCs preferred to react with $IN1_{INH}$, since the affinity of DNA-AgNCs/$IN1_{INH}$ was stronger than that of DNA-AgNCs/$IN2_{INH}$. In this case, the logic system still reported a low output signal (Fig. S6B, (d)). The FI_{max} against input combinations with the corresponding truth table were depicted in Fig. S6C and Fig. S6D, respectively. The photograph under UV excitation (Fig. S6E) directly exhibited the INH operation that the fluorescence of AgNCs can be activated by $IN2_{INH}$ alone.

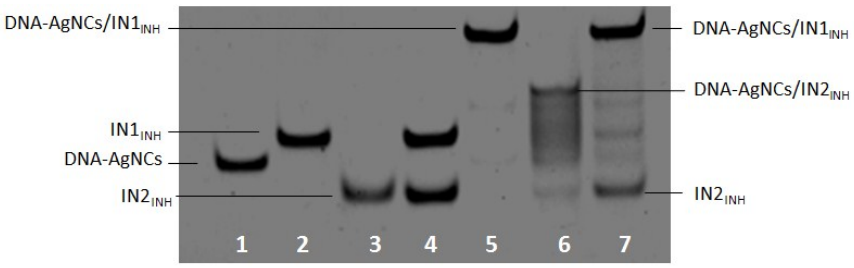
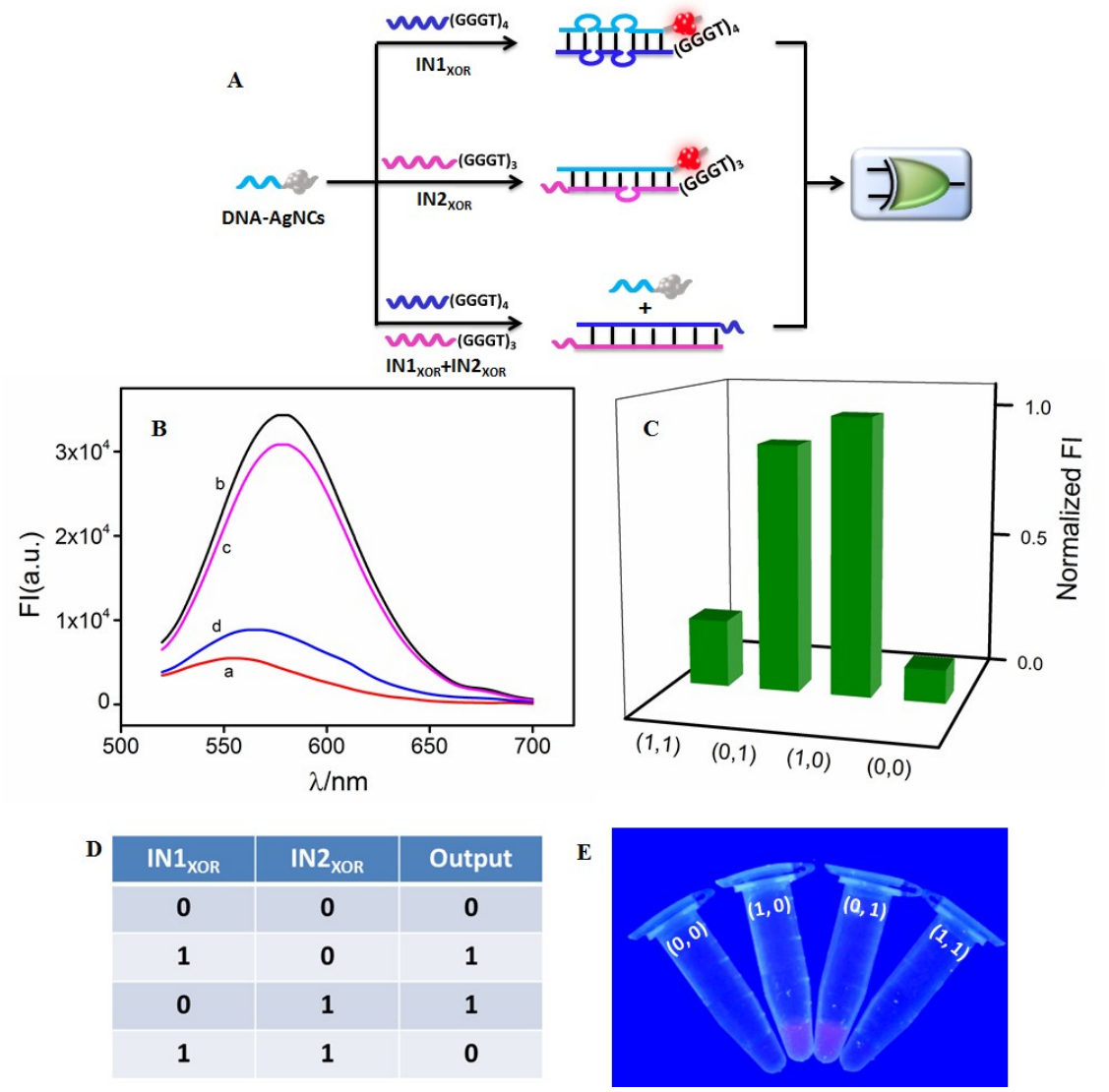


Fig. S7. Native polyacrylamide gel (20%) analysis of the interaction among DNA-AgNCs, $IN1_{INH}$ and $IN2_{INH}$ used in the developed INH logic gate. Lane 1: DNA-AgNCs, Lane 2: $IN1_{INH}$, Lane 3: $IN2_{INH}$, Lane 4: $IN1_{INH}$ and $IN2_{INH}$, Lane 5: DNA-AgNCs+$IN1_{INH}$, Lane 6: DNA-AgNCs+$IN2_{INH}$, Lane 7: DNA-AgNCs+$IN1_{INH}$+$IN2_{INH}$.

Fig. S7 validates the DNA interactions of the developed INH logic gate. From Lane 1 to Lane 3, the belts exhibit the individual DNA strands of DNA-AgNCs, $IN1_{INH}$ and $IN2_{INH}$ in sequence. No hybridization between $IN1_{INH}$ and $IN2_{INH}$ results in two separated belts, Lane 4. The belts in Lane 5 and Lane 6 indicate the formation of duplexes of DNA-AgNCs/$IN1_{INH}$ and DNA-AgNCs/$IN1_{INH}$, respectively. In the coexistence of DNA-AgNCs, $IN1_{INH}$ and $IN2_{INH}$ (Lane 7), a belt is detected on the same position of DNA-AgNCs/$IN1_{INH}$, suggesting that the hybridization occurs between DNA-AgNCs

and $IN1_{INH}$. The PAGE results validate the DNA reaction of INH logic gate occurs as expected and are consistent with the fluorescence results, Fig. S6.



$IN1_{XOR}$	$IN2_{XOR}$	Output
0	0	0
1	0	1
0	1	1
1	1	0

Fig. S8. (A) The principle scheme of the developed XOR gate and the corresponding logic circuit. (B) Results of the XOR logic gate: the fluorescent responses of DNA-AgNCs in the absence of inputs (a) and in the presence of $IN1_{XOR}$ (b), $IN2_{XOR}$ (c), and $IN1_{XOR}$+$IN2_{XOR}$ (d). (C) The FI_{max} of DNA-AgNCs against various input combinations. The corresponding truth table (D) and photographs of the logic system under UV irradiation (E).

Fig. S8 outlines the operation principle of the XOR logic gate. Both input 1 ($IN1_{XOR}$) and input 2 ($IN2_{XOR}$) contained G-rich segments. The low fluorescence of DNA-AgNCs (Fig. S8B, (a)) could be significantly amplified in the presence of either $IN1_{XOR}$ (Fig. S8B, (b)) or $IN2_{XOR}$ (Fig. S8B, (c)) so that

the formation of duplexes of DNA-AgNCs/$IN1_{XOR}$ or DNA-AgNCs/$IN2_{XOR}$ brings G-rich segments in the inputs close to the AgNCs. In the addition of the two inputs, duplex of $IN1_{XOR}$/$IN2_{XOR}$ rather than DNA-AgNCs/$IN1_{XOR}$ or DNA-AgNCs/$IN2_{XOR}$ was produced due to the higher affinity of $IN1_{XOR}$/$IN2_{XOR}$. The DNA-AgNCs was left and reported low output signals (Fig. S8B, (d)). The FI_{max} against input combinations (Fig. S8C) generated the corresponding truth table depicted in Fig. S8D. The system reported high output of "1" if logic values of the two inputs were different, i.e. (1, 0) or (0, 1). A low output of "0" was reported if the two inputs were identical, i.e. (0, 0) or (1, 1). The logic operation could be instantly monitored under ultraviolet rays (UV) irradiation (Fig. S8E). The fluorescence of AgNCs could be lightened only when the logic values of the two inputs differed, which was consistent with the fluorescence result and meets the feature of XOR logic gate.

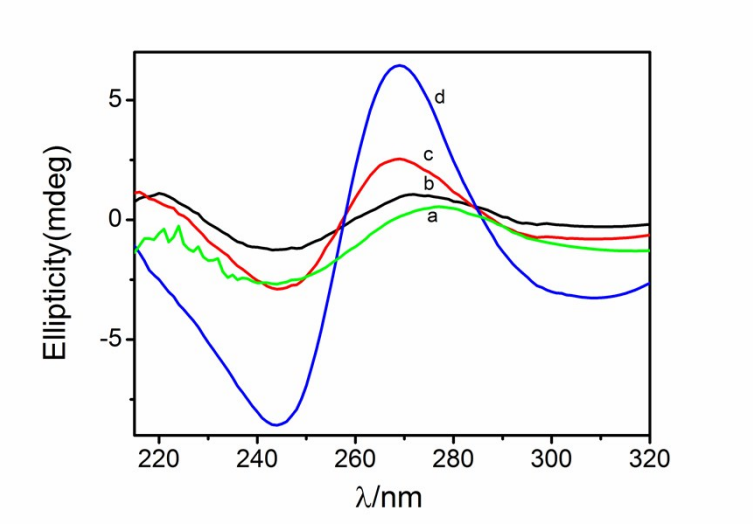


Fig. S9. CD spectra of DNAs used in the developed HA: platform DNA (P-DNA) used as template of AgNCs (a), P-DNA+$IN1_{HA}$ (b), P-DNA+$IN2_{HA}$ (c), $IN1_{HA}$+$IN2_{HA}$ (d). All the concentration of DNA was 4 μM.

The circular dichroism (CD) spectrum of platform DNA (P-DNA) used as template of AgNCs is of relatively low amplitude (Fig. S9, (a))，indicating a random strand structure. The addition of $IN1_{HA}$ or $IN2_{HA}$ causes slight enhance of amplitude due to the formation of duplex of P-DNA/ $IN1_{HA}$ (Fig. S9,

(b)) or P-DNA/ $IN2_{HA}$ (Fig. S9, (c)). Different from that, the amplitudes of negative peak near 242 nm and positive peak near 268 nm are significantly enhanced in the coexistence of $IN1_{HA}$ and $IN2_{HA}$ (Fig. S9, (d)), suggesting formation of stabilized G-quadruplex (G4) with a parallel configuration.[2] The results were consistence with the fluorescence results of Fig. 1 as shown in the main text.

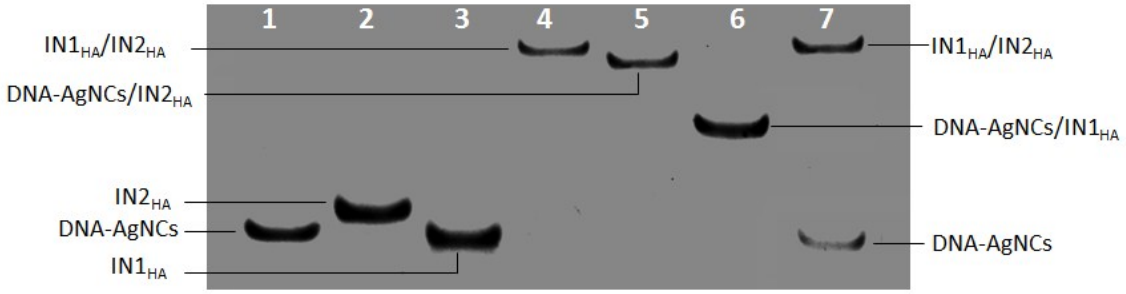


Fig. S10. Native polyacrylamide gel (20%) analysis of the interaction among DNA-AgNCs, $IN1_{HA}$ and $IN2_{HA}$ used in the developed HA logic gate. Lane 1: DNA-AgNCs, Lane 2: $IN2_{HA}$, Lane 3: $IN1_{HA}$, Lane 4: $IN1_{HA}$ and $IN2_{HA}$, Lane 5: DNA-AgNCs+$IN2_{HA}$, Lane 6: DNA-AgNCs+$IN1_{HA}$, Lane 7: DNA-AgNCs+$IN1_{HA}$+$IN2_{HA}$.

All DNA interactions for HA operation were further validated by conducting native polyacrylamide gel electrophoresis (PAGE) experiment (Fig. S10). From Lane 1 to Lane 3, the belts exhibit the individual DNA strands of DNA-AgNCs, $IN2_{HA}$, and $IN1_{HA}$ in sequence. Hybridization would occur to form duplexes in the presence of any two DNA strands, which were validated by the new belts in coexistence of $IN1_{HA}$ and $IN2_{HA}$ (Lane 4), DNA-AgNCs and $IN2_{HA}$ (Lane 5), and DNA-AgNCs and $IN1_{HA}$ (Lane 6). In the coexistence of DNA-AgNCs, $IN1_{HA}$ and $IN2_{HA}$, one belt appeared at position of $IN1_{HA}$/$IN2_{HA}$. A further belt appeared at the position of DNA-AgNCs, confirming that $IN1_{HA}$ and $IN2_{HA}$ would hybridize together rather than with DNA-AgNCs. The PAGE results validate the DNA reactions of HA logic gate occurred as expected.

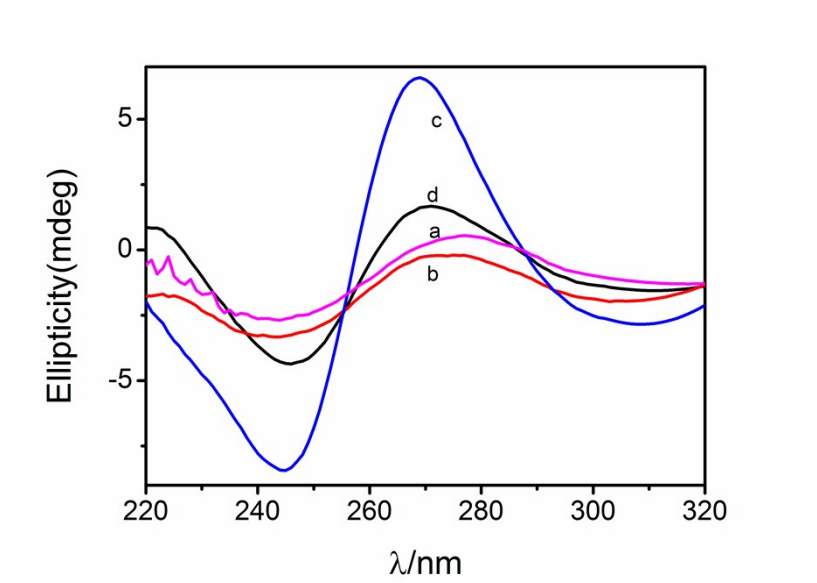


Fig. S11. CD spectra of DNAs used in the developed HS: P-DNA (a), P-DNA+$IN2_{HS}$ (b), P-DNA+$IN1_{HS}$ (c), $IN1_{HS}$ + $IN2_{HS}$ (d). All the concentration of DNA was 4 μM.

As illustrated in Fig. S11, the circular dichroism (CD) spectrum of $IN2_{HS}$ and P-DNA is of relatively low amplitude, indicating a random strand structure, Fig. S11 (a, b). The CD spectrum of $IN1_{HS}$ presents distinguished negative peak near 242 nm and positive peak near 268 nm, suggesting the formation of G-quadruplex with a parallel configuration, Fig. S11 (c). In the coexistence of $IN1_{HS}$ and $IN2_{HS}$, the amplitude of the CD spectrum is significantly reduced due to the formation of $IN1_{HS}$/$IN2_{HS}$ duplexer, Fig. S11 (d), which inhibits the generation of G-quadruplex. The results are consistence with the fluorescence results of Fig. 2 as shown in the main text.

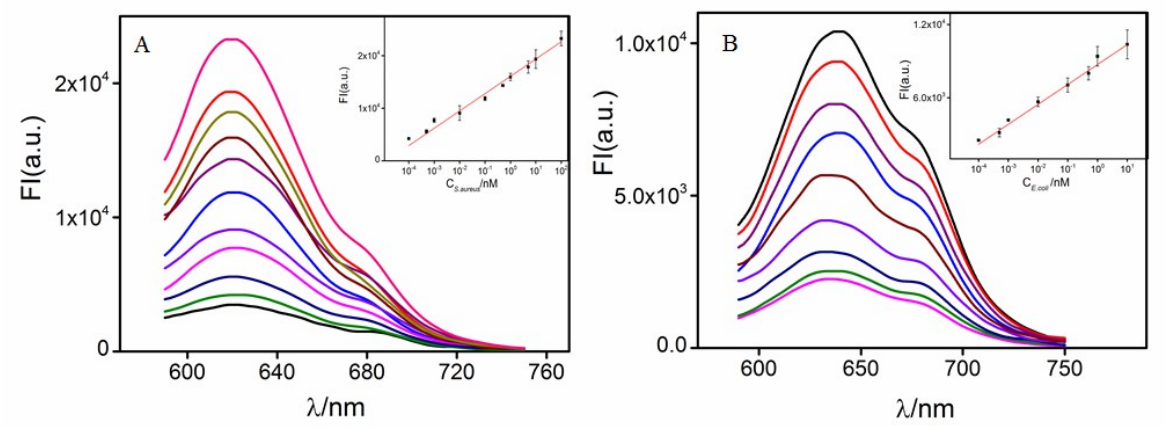


Fig. S12. (A) The fluorescence responses of the multiplexer on *S.aureus* gene with different concentration in the absence of selector. The concentrations of *S.aureus* were 0 fM, 100 fM, 500 fM, 1pM, 10 pM, 100 pM, 500 pM, 1 nM, 5 nM, 10 nM and 100 nM from bottom to up. (B) The fluorescence responses of the multiplexer on *E.coli* gene with different concentration in the presence of selector. The concentrations of *E.coli* were 0 fM, 100 fM, 500 fM, 1 pM, 10 pM, 100 pM, 500 pM, 1 nM and 10 nM from bottom to up.

References

1. W. W. Guo, J. P. Yuan, Q. Z. Dong and E. K. Wang, *J. Am. Chem. Soc.,* 2010, **132**, 932-934.

2. Y. Q. Liu, J. T. Ren, Y. A. Qin, J. Li, J. Y. Liu and E. K. Wang, *Chem. Commun.,* 2012, **48**, 802-804.