# Easy design of logic gates based on aptazymes and noncrosslinking gold nanoparticle aggregation†

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**We have developed an easy method for constructing aptazyme-based logic gates using noncrosslinking gold nanoparticle aggregation.**



Logic gates made of nano-molecules have high potential for computation at literally the "nano-size" level.[1] In particular, nano-sized DNA and RNA oligomers have recently become commonly used as components of nano-molecular logic gates due to their characteristic ability to hybridize with their complementary strands and/or to bind to specific target molecules.[2–6] In normal-nucleic-acid-based[2] or (deoxy)ribozyme-based logic gates,[3,4] input oligonucleotides for the ON state hybridize to the gate composed of nucleic acids by unwinding internal duplexes in the gate (*i.e.* hybridization switch) to produce some output signals such as fluorescence and ribozyme activity (output = "1"). Inversely, inputs for the OFF state prevent (or don't induce) the hybridization switch or induce a different one that doesn't lead to detectable output (output = "0"). Some aptamer-based logic gates have also been reported,[5,6] wherein the gate is constructed with a few aptamers that can bind tightly to their targets. In these aptamer-based logic gates, input targets for the ON state induce hybridization switches in the gate from internal duplexes into target-aptamer bindings to produce some output signals as in the normal-nucleic-acids-based gates. These aptamer-based logic gates have a great advantage in choosing input molecules because aptamers for any target are almost certain to be obtained through an *in vitro* selection method.[7] However, these aptamer-based logic gates have the disadvantage of requiring complex designs of hybridization switches for "each" aptamer (*i.e.* for each input molecule).

On the other hand, aptazymes, which are allosteric ribozymes activated by specific targets (called "cofactors" of aptazymes) and which can also be obtained *via in vitro* selection for any target as well as aptamers, have a switching mechanism in their own bodies.[8] Therefore, they have the same advantage as aptamers and additionally have the potential for omitting the difficult design of hybridization switches in constructing aptazyme-based logic gates. In fact, the cleavase-aptazyme-based riboswitches that we have reported recently were designed without taking hybridization switches into account.[9] Moreover, the molecule-dependency of these riboswitches can easily be altered just by changing the aptazyme part into an other-molecule-dependent aptazyme.[9] In this paper, we report the easy design of logic-gate elements (OR and AND) using cleavase-aptazymes.

In constructing cleavase-aptazyme-based logic gates, the first problem that must be addressed is how to convert the self-cleavage signal to an output signal. Although expression of a reporter gene using an aptazyme-based gene-regulation system is one possibility, aptazymes with extra sequences such as mRNA and tRNA have lower activity than non-fused aptazymes under translation conditions.[9,10] Nonetheless, classical analyses using gel electrophoresis for non-fused aptazymes require time and effort.[4,8] In contrast, we have recently developed a simple method of signal conversion from self-cleavage of an almost-non-fused cleavase-aptazyme to colorimetric change of a gold nanoparticle (AuNP) solution (Fig. 1).[11] In this method, a cleaved RNA from the aptazyme in the presence of the cofactor hybridizes to a probe-DNA on the AuNPs to induce "noncrosslinking" AuNP aggregation[12] that can be detected with the naked eye as a colorimetric change of the solution from red to almost colorless (Fig. 1B). The "noncrosslinking" AuNP aggregation occurs by the loss of flexibility of the AuNP surface terminus and stacking interactions between blunt ends of the duplex on individual AuNPs,[12] so that the aggregation depends strongly on the complementarity of the duplex terminus on AuNPs. Moreover, the aggregates are quick to glow and almost completely precipitate[13] with only a small amount of cleaved RNA.[11,14] This method therefore has some merits in constructing aptazyme-based logic gates: (1) aggregation can be easily controlled by (various combinations of) the sequences of the cleaved RNA; (2) ON ("1", aggregation) or OFF ("0", dispersion) is very clear due to the almost complete precipitation of aggregates in the ON state (Fig. 1C); (3) calculation results can be detected visibly (Fig. 1B).

First, we constructed an easier OR gate by combining two aptazymes[8] for theophylline and cGMP, both of which had the same sequence after the cleavage site (*i.e.* future cleaved RNA) with the corresponding probe-DNA-tethered AuNPs (Fig. 2A). Because both of the input molecules (*i.e.* theophylline and cGMP) make each dependent aptazyme yield the same cleaved RNA that can hybridize to probe-DNA on AuNPs with a blunt end, AuNP aggregation should occur in the presence of either, or of course both, of them (Fig. 2A, except far left).[15] Fig. 2B and 2C show the calculation results with this OR gate, which are in correspondence with the ideal OR gate responses. It should be noted that the precipitation of AuNP

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† Electronic supplementary information (ESI) available: Materials and methods, absorbance spectra, design strategy and calculation results for the NOT gate. See DOI: 10.1039/b910288d

 

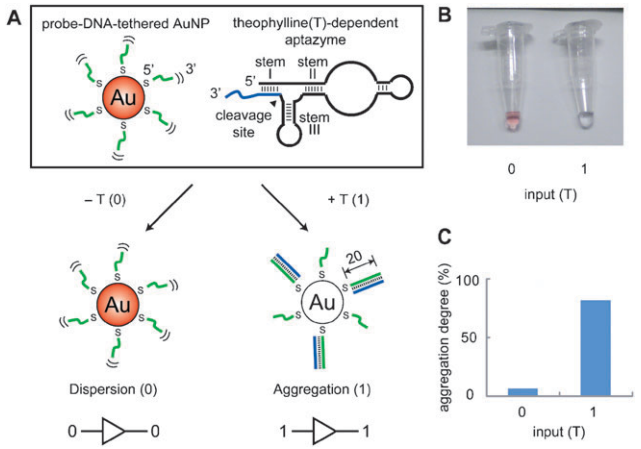


**Fig. 1** Aptazyme-based noncrosslinking AuNP aggregation (YES gate).[11] (A) Illustration of aptazyme-based AuNP aggregation. In the absence of an input target (theophylline (T) here) (input = 0), AuNPs remain dispersed (output = 0) (left). In the presence of the input target (input = 1), the aptazyme is self-cleaved and the cleaved RNA (blue strand) hybridizes to a probe-DNA (green strand) on AuNPs to induce AuNP aggregation (output = 1) (right). The number beside the double-headed arrows indicates the length of the probe-DNA or the cleaved RNA. (B, C) Calculation results of the YES gate in the absence (input = 0) or presence (input = 1, 1 mM) of theophylline with 5-min reaction. Photograph of AuNP solutions (B) and their aggregation degree (C). The aggregation degree was calculated as follows: Aggregation degree (%) = $[(A_{529}^{ref} - A_{529}^{obs})/A_{529}^{ref}] \times 100$, wherein $A_{529}^{ref}$ and $A_{529}^{obs}$ are the absorbances at 529 nm of only AuNP and observed AuNP solution after calculation, respectively.

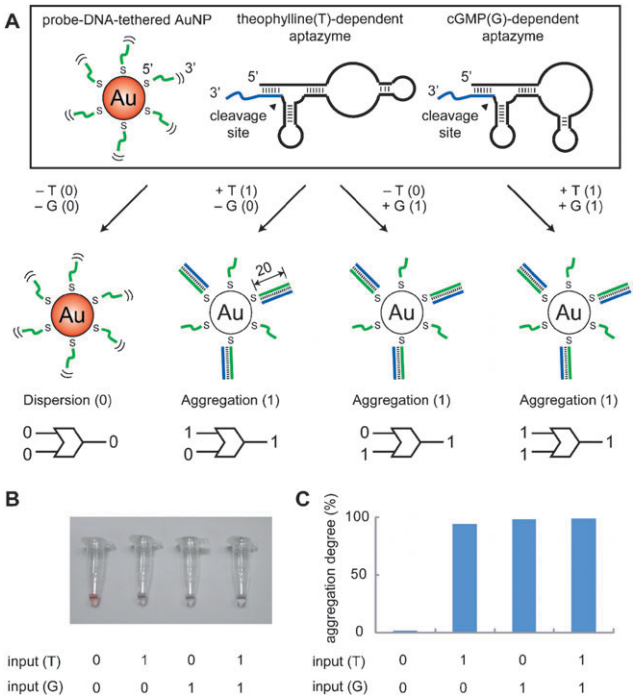


**Fig. 2** The OR gate. (A) Illustration of the design strategy of the OR gate. In the absence of inputs (input T/G = 0/0), AuNPs remain dispersed (output = 0) (far left). In the presence of either or both inputs (input T/G = 1/0, 0/1, 1/1), the aptazyme is self-cleaved and the cleaved RNA (blue strand) hybridizes to the probe-DNA (green strand) on AuNPs to induce AuNP aggregation (output = 1) (second left, second right, far right, respectively). The number beside double-headed arrows indicates the length of the probe-DNA or the cleaved RNA. (B, C) Calculation results of the OR gate in the absence (input = 0) or presence (input = 1, 1 mM) of input molecules (theophylline and/or cGMP) with 5-min reaction. Photograph of AuNP solutions (B) and their aggregation degree (C).

aggregates in the presence of either input was as complete as with both inputs. This was due to the amount of cleaved RNA produced by each input being sufficient to induce complete AuNP precipitation. It is therefore not surprising that the phenomenon remains unchanged with twice as much cleaved RNA (in the presence of both inputs). As above, it is easy to construct an OR gate by designing only one hybridization scheme between the cleaved RNA and its complementary probe-DNA on AuNPs.

We next constructed an AND gate, the design of which also was not difficult (Fig. 3A). The gate is designed to be composed of two kinds of aptazymes and one kind of AuNP having exactly the same probe-DNA as in the OR gate. The sequences of the cleaved RNA from two aptazymes are different from each other (a longer one and a shorter one), unlike in the OR gate.[16] The longer cleaved RNA (30-mer) from theophylline-dependent aptazyme partially hybridizes to the probe-DNA on AuNPs with its 3′-terminal (14-mer), so that its 5′-terminal end (16-mer) is dangling after hybridization (Fig. 3A, second left).[17] In contrast, the shorter one (16-mer) from cGMP-aptazyme is able to hybridize not to the probe-DNA (Fig. 3A, second right) but to the dangling 16-mer of the longer cleaved RNA from theophylline-aptazyme with a blunt end (Fig. 3A, far right). Therefore, in this gate both inputs are necessary for AuNP aggregation to occur. Fig. 3B and 3C show the calculation results with this AND gate; these results are also consistent with ideal responses. It should be noted that AuNPs with either of the two inputs were dispersed almost as completely as without any inputs, whereas aggregation took place for AuNPs with both inputs. This result occurred because hybridization "with a blunt end" on AuNPs is absolutely required for noncrosslinking aggregation. In constructing an AND gate using aptazymes and AuNPs, all we have to do is design two hybridization schemes: the first occurs between a part of the longer cleaved RNA and the probe-DNA, and the second between the dangling part of the longer cleaved RNA and the shorter cleaved RNA.

In summary, we easily constructed logic gate elements (OR and AND), wherein the inputs are small molecules (theophylline and cGMP in this paper) and outputs are colorimetric changes in AuNP solutions, utilizing some kinds of molecule-dependent cleavase-aptazymes and one kind of AuNP having probe-DNA. Although "aptamer"-based logic gates using "crosslinking" AuNP aggregation as outputs have been reported previously,[6] the "aptazyme"-based logic gates using "noncrosslinking" AuNP aggregation used in the present study can be designed more easily, only requiring consideration of one or two hybridization schemes without consideration of hybridization switches. In addition, calculation results, with the output being either "0" or "1", can be determined much more clearly by the naked eye due to almost complete

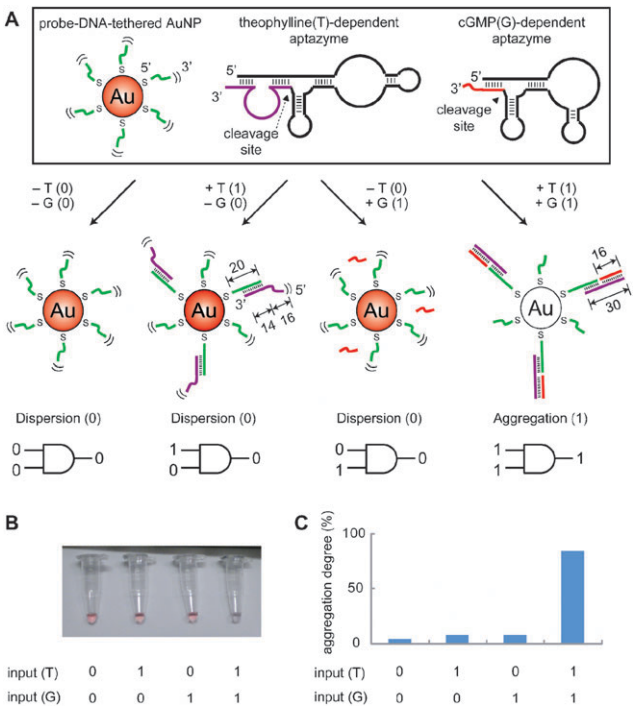


**Fig. 3** The AND gate. (A) Illustration of the design strategy of the AND gate. In the absence of inputs (input T/G = 0/0) or in the presence of either input (input T/G = 1/0, 0/1), AuNPs remain dispersed (output = 0) (far left, second left, second right, respectively). In the presence of both inputs (input T/G = 1/1), two kinds of cleaved RNAs (purple and red strand) and the probe-DNA (green strand) form duplexes with a blunt end to induce AuNP aggregation (output = 1) (far right). The numbers beside double-headed arrows indicate the lengths of the probe-DNA or the cleaved RNA. (B, C) Calculation results of the AND gate in the absence (input = 0) or presence (input = 1, 1 mM) of input molecules (theophylline and/or cGMP) with 30-min reaction.[16] Photograph of AuNP solutions (B) and their aggregation degree (C).

precipitation of AuNP aggregates. More importantly, the sequences of the cleaved RNA used in this study can be shared with other aptazymes,[18] so that re-design is not required if the input molecules are changed.[19] Moreover, these logic gates require only one kind of DNA-tethered AuNP unlike methods using "crosslinking" AuNP aggregation.[6,20] In these aptazyme-based logic gates, the key to their functionality is two consecutive information transductions: "from the existence of small molecules into the sequences of the cleaved RNA *via* aptazymes" and "from the sequences of the cleaved RNA into AuNP aggregation (*i.e.* colorimetric change) *via* duplex formation with a blunt end at the surface terminal of the AuNPs". It is therefore expected that the present method can be applied to the construction of more complex logic gates such as combinatorial ones and used for simultaneous detection of various small molecules utilizing a device "at the level of hybridization of nucleic acids". Further work along these lines is now underway.

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## Notes and references

1 U. Pischel, *Angew. Chem., Int. Ed.*, 2007, **46**, 4206.
2 B. M. Frezza, S. L. Cockroft and M. R. Ghadiri, *J. Am. Chem. Soc.*, 2007, **129**, 14875; D. Miyoshi, M. Inoue and N. Sugimoto, *Angew. Chem., Int. Ed.*, 2006, **45**, 7716.
3 M. N. Stojanovic, T. E. Mitchell and D. Stefanovic, *J. Am. Chem. Soc.*, 2002, **124**, 3555; M. N. Stojanovic, S. Semova, D. Kolpashchikov, J. Macdonald, C. Morgan and D. Stefanovic, *J. Am. Chem. Soc.*, 2005, **127**, 6914; H. Lederman, J. Macdonald, D. Stefanovic and M. N. Stojanovic, *Biochemistry*, 2006, **45**, 1194.
4 R. Penchovsky and R. R. Breaker, *Nat. Biotechnol.*, 2005, **23**, 1424.
5 D. M. Kolpashchikov and M. N. Stojanovic, *J. Am. Chem. Soc.*, 2005, **127**, 11348; W. Yoshida and Y. Yokobayashi, *Chem. Commun.*, 2007, 195; J. Elbaz, B. Shlyahovsky, D. Li and I. Willner, *ChemBioChem*, 2008, **9**, 232; V. Sharma, Y. Nomura and Y. Yokobayashi, *J. Am. Chem. Soc.*, 2008, **130**, 16310.
6 J. Liu and Y. Lu, *Adv. Mater.*, 2006, **18**, 1667.
7 A. D. Ellington and J. W. Szostak, *Nature*, 1990, **346**, 818; C. Tuerk and L. Gold, *Science*, 1990, **249**, 505.
8 G. A. Soukup and R. R. Breaker, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**, 3584; G. A. Soukup, G. A. M. Emilsson and R. R. Breaker, *J. Mol. Biol.*, 2000, **298**, 623; M. Koizumi, G. A. Soukup, J. N. Q. Kerr and R. R. Breaker, *Nat. Struct. Biol.*, 1999, **6**, 1062; G. A. Soukup, E. C. DeRose, M. Koizumi and R. R. Breaker, *RNA*, 2001, **7**, 524.
9 A. Ogawa and M. Maeda, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 3156; A. Ogawa and M. Maeda, *ChemBioChem*, 2008, **9**, 206; A. Ogawa and M. Maeda, *ChemBioChem*, 2008, **9**, 2204.
10 M. N. Win and C. D. Smolke, *Science*, 2008, **322**, 456.
11 A. Ogawa and M. Maeda, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 6517.
12 K. Sato, K. Hosokawa and M. Maeda, *J. Am. Chem. Soc.*, 2003, **125**, 8102; K. Sato, K. Hosokawa and M. Maeda, *Nucleic Acids Res.*, 2005, **33**, e4; K. Sato, M. Onoguchi, Y. Sato, K. Hosokawa and M. Maeda, *Anal. Biochem.*, 2006, **350**, 162.
13 In the case that oligonucleotides hybridized to the probe-DNA are not DNA but RNA, the color of the AuNP solution after aggregation is more colorless than purple.[11] See the Supplementary Information for absorbance spectra†.
14 Aggregation can be induced by a small amount of the cleaved RNA that is equivalent to only 7.5% amounts of the probe-DNA on AuNPs.[11]
15 Aggregation effects in each pair (the theophylline-dependent aptazyme and the AuNP pair or the cGMP-dependent aptazyme and the AuNP pair) have previously been verified.[11] We also verified that these two pairs are orthogonal (theophylline didn't cleave the cGMP-aptazyme and cGMP didn't cleave the theophylline-aptazyme).
16 The sequences of the stem I in these two aptazymes were altered from the *in vitro*-selected sequences. However, it should be noted that the sequence of this stem I is less important for the switching efficiency of the aptazyme if it only forms a duplex. Although interactions between stem I and stem II in the hammer-head ribozyme are important to the ribozyme activity (*i.e.* reaction rate), the increase (decrease) in the ribozyme activity induced by their alteration leads to an increase (decrease) in the activities of the aptazymes both in the ON and OFF state.[21] Therefore, although activities of the aptazymein the ON state for the AND gate decreased somewhat, we compensated for this decrease by slightly extending the reaction time (see the Supplementary Information†).
17 The 3′-terminal end of the theophylline-dependent aptazyme was designed to partially hybridize to its 5′-terminal so as to prevent hybridization to the probe-DNA on AuNPs before the self-cleavage.
18 In fact, a FMN-dependent aptazyme having the same cleaved RNA sequence has high activity.[11]
19 Of course, the corresponding aptazymes must be obtained through *in vitro* selection.[7,8]
20 A NOT gate, another logic element, can also be constructed using the same probe-DNA-tethered AuNPs (see the Supplementary Information†).
21 K. H. Link, L. Guo, T. D. Ames, L. Yen, R. C. Mulligan and R. R. Breaker, *Biol. Chem.*, 2007, **388**, 779.

 