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RNA aptazyme-tethered large gold nanoparticles for on-the-spot sensing of the aptazyme ligand

Atsushi Ogawa *

Senior Research Fellow Center, Ehime University, 3 Bunkyo-cho, Matsuyama, Ehime 790-8577, Japan

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ABSTRACT

A single-step sensing system was developed to visually detect ligands of a cleavase-like RNA aptazyme at room temperature using aptazyme-tethered gold nanoparticles, the electrosteric stability of which was adjusted by increasing their diameter. In this system, the ligand induces self-cleavage of the aptazyme on gold nanoparticles to decrease the electrosteric stability of the gold nanoparticles, which causes them to visibly aggregate. In comparison to a previous multi-step system using aptazymes and gold nanoparticles separately, the present system requires only single handling and no special equipment, making it more suitable for on-the-spot sensing.

Visible sensing systems utilizing color changes of gold nanoparticles (AuNPs) with their state transitions (dispersion/aggregation) are very useful for on-the-spot sensing without any machine.[1] DNA-tethered AuNPs (DNA-Au) are especially applicable to the detection of a wide variety of molecules due to the functional abilities of tethered DNA: DNA hybridizes to its complementary strands; some DNAs (DNA aptamers) bind to their specific ligand molecule; and some other DNAs (cleavase-like DNA aptazymes) cleave themselves in the presence of their specific ligand. Many inventive sensing systems with these DNA-Au have been reported, with the target analyte (complementary DNA to DNA on AuNPs[2] or the ligand of DNA aptamers[3] or DNA aptazymes[4]) promoting or inhibiting DNA-Au aggregation via various mechanisms that can be detected by the naked eye. In contrast to many types of DNA-Au-based sensing systems, there have been a few systems using RNA-tethered AuNPs (RNA-Au),[5] despite their higher potential for binding (RNA aptamers) or allosteric activities (RNA aptazymes) due to the 2′-hydroxy group on the RNA. I report herein a novel sensing system to detect the ligand of a cleavase-like RNA aptazyme (cRaptz) with relatively large AuNPs tethering the cRaptz.

Recently, I and Maeda have reported a sensing system for detecting cRaptz activity using DNA-Au without conjugation between them (Fig. 1A).[6] However, this noncrosslinking AuNP aggregation-based system is not suitable for "on-the-spot" sensing because it requires multiple steps; in particular, an aptazyme reaction step is carried out separately from the AuNP-based detection step, and what is worse, a heating step is required for dissociation of the short cleaved RNA from the aptazyme. Although the heating step could be omitted by adjusting the strength of the stem I duplex, there remains another problem to be solved. Even if the aptazyme is hybridized to DNA on AuNPs to achieve a single-step system, it should never be cleaved with the ligand because it is hardly able to form an active structure (Fig. 1B). Therefore, this type of noncrosslinking AuNP aggregation is not available for constructing a single-step detection system, and I therefore decided to utilize another AuNP aggregation mechanism.

Li et al. have recently reported some inventive sensing systems using DNA-Au and other types of noncrosslinking AuNP aggregation, which is induced by a decrease in the electrosteric stability of AuNPs.[3d,e,4c] Inspired by these reports, I designed a system to detect cRaptz reactions utilizing this noncrosslinking AuNP aggregation mechanism (Fig. 1C). Because a cRaptz is a relatively long nucleic acid (~120 mer), it causes huge amounts of electrosteric repulsion among cRaptz-tethered AuNPs, making them stable under high salt concentrations. On the other hand, the cRaptz is extensively shortened (~30 mer) by self-cleavage with the ligand, which causes a decrease in electrosteric repulsion among the AuNPs and thus a decrease in their stability (i.e., salt resistance). Therefore, under tuned salt concentrations, aggregation should occur only in the presence of the ligand. However, so as to not decrease the cRaptz activity, the salt concentrations must be kept at optimal values for the aptazyme reaction to proceed (20 mM Mg^{2+}). Nonetheless, it has been reported that this salt concentration is not sufficient to aggregate normally prepared AuNPs tethering even short 12 mer DNA,[3d] so that normally prepared cRaptz-tethered AuNPs should keep dispersing at 20 mM Mg^{2+} after self-cleavage of the cRaptz on AuNPs. Thus, the stability of cRaptz-tethered AuNPs instead of salt concentrations must be tuned.

* Tel.: +81 89 927 8450.
E-mail address: a-ogawa@ccr.ehime-u.ac.jp

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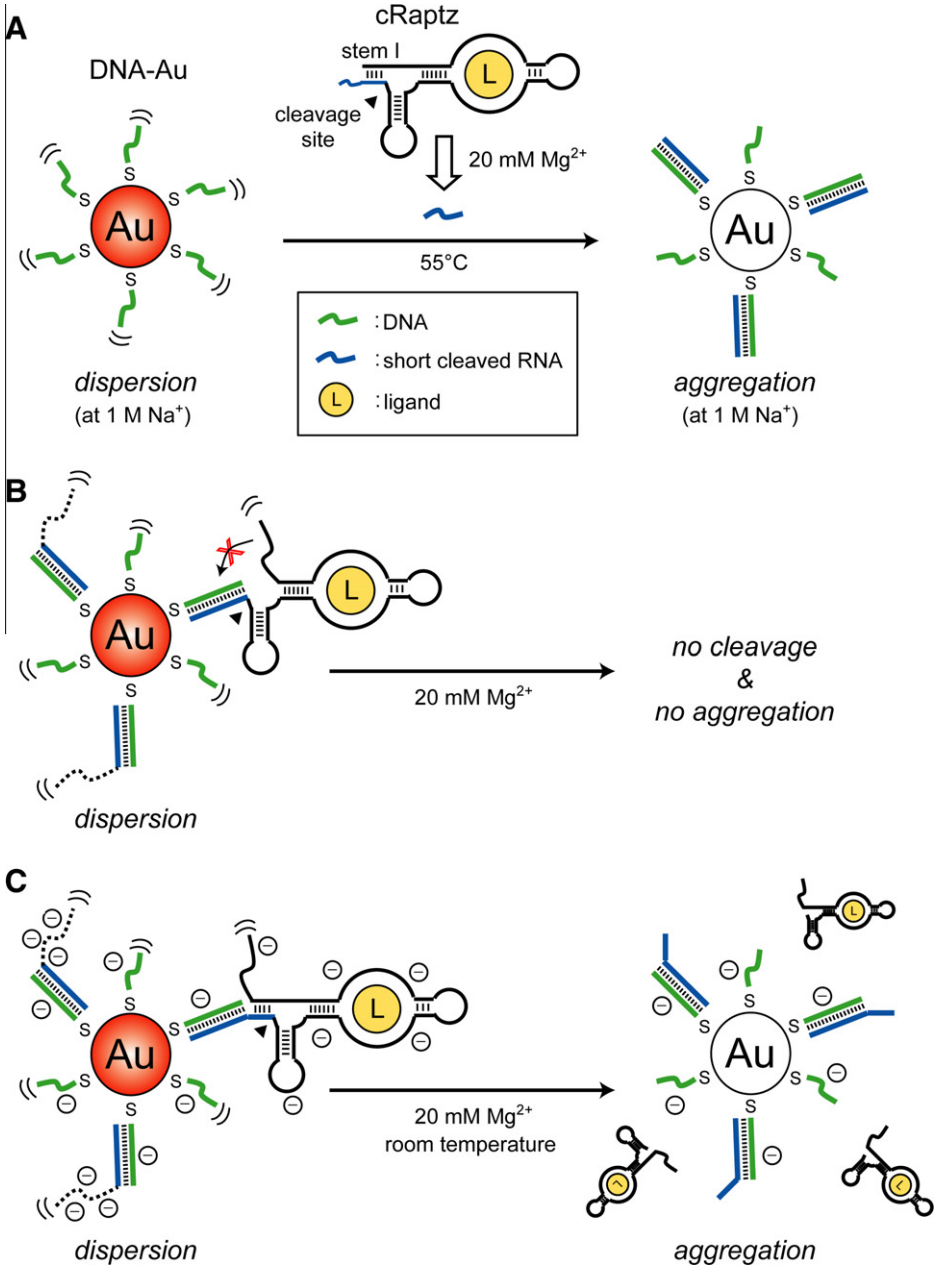

Figure 1. Visible sensing systems to detect a ligand of cRaptz using noncrosslinking AuNP aggregation. (A) A previously reported multi-step system.[6] Noncrosslinking AuNP aggregation occurs by blunt-end duplex formation between short cleaved RNA and DNA on AuNPs. (B) cRaptz-tethered AuNPs, in which a cleavage site of cRaptz is just opposite to the terminus of DNA on AuNPs. cRaptz is not cleaved due to the lack of stem I formation. (C) A single-step sensing system reported here. The cRaptz cleavage causes a decrease in the electrosteric stability of AuNPs to be aggregated at room temperature.

According to the report by Li et al. the surface coverage of DNA on AuNPs has large effects on the stability of DNA-Au.[3d] In fact, the amount of DNA on AuNPs has been reduced to two thirds by adding 6-mercaptohexan-1-ol (MCH) to decrease the stability of DNA-Au. However, this method is attended with difficulty because the conditions of the MCH treatment must be optimized and firmly controlled to get reproducible results. On the other hand, Maeda et al. have reported that the surface coverage of DNA on large AuNPs is, due to the small curvature, usually lower than that on small AuNPs, wherein DNA is tethered on AuNPs with a standard Mirkin's method[2a] without MCH treatment.[7] Because the AuNP diameter that is used for DNA-Au in Li's systems described above is small (13 nm), I speculated that the MCH treatment should be omitted to prepare DNA-Au with low salt resistance (so as to tether cRaptz) by using AuNPs with a relatively large diameter.

I first compared the salt (Mg^{2+}) resistance of DNA-Au with AuNPs 15 nm and 40 nm in diameter, both of which were similarly prepared using 5′-thiolated 23 mer DNA (SH-DNA23) without MCH treatment (designated as DNA23-Au15 and DNA23-Au40, respectively).[8] As a result, while the smaller one (DNA23-Au15) was stable at 50 mM Mg^{2+} more than for several days (Fig. 2A), the larger one (DNA23-Au40) began to aggregate within 5 min at 20 mM Mg^{2+}, which is the optimal concentration in the cRaptz reaction (Fig. 2B). The average numbers of tethered DNA on each 15 and 40 nm AuNP were estimated to be 100 and 470, respectively, which corresponded to a surface coverage of 24 and 16 pmol/cm^2, respectively. Thus, DNA23 on 40 nm AuNPs has more open spaces near its root to be compacted on the AuNPs to cause their aggregation.[3e] The decrease ratio of the surface coverage on 40 nm AuNPs to that on 15 nm AuNPs is two-thirds, which is

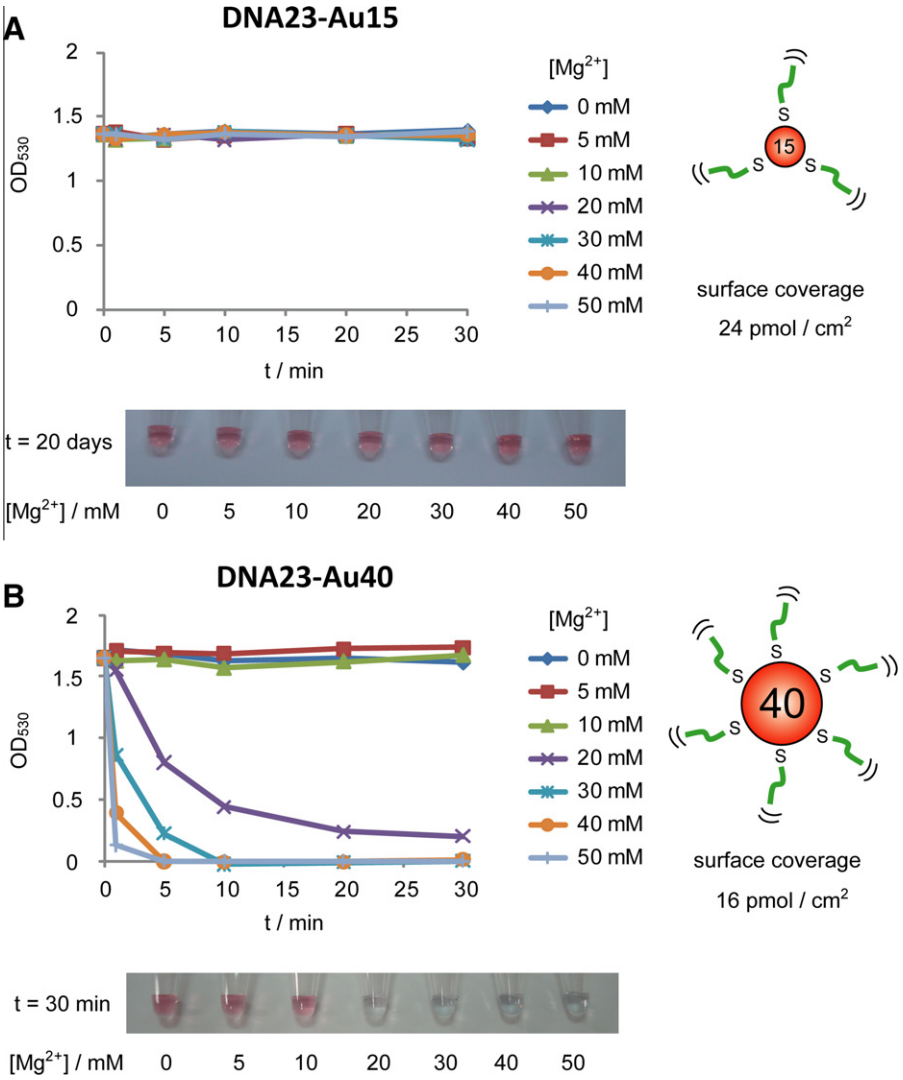

Figure 2. Mg^{2+} resistance of DNA23-Au15 (A) and DNA23-Au40 (B). The *X*-axes (t) are the time from the addition of Mg^{2+}. OD_{530} in *Y*-axes are relative values to OD_{800}. Photos of DNA23-Au15 in 20 days (A) and DNA23-Au40 in 30 min (B) are shown below each graph.

comparable to the previous study using MCH treatment.[3d] Therefore, the lower surface coverage of DNA23-Au40 is probably the main reason for its lower stability. In addition to the effect of surface coverage, a larger diameter results in stronger van der Walls forces between AuNPs.[7] This additional effect is also the reason why DNA23-Au40 has a greater tendency to aggregate at low salt concentrations.

I next designed a cRaptz sequence to be tethered on the DNA23-Au40 via DNA-RNA hybridization. A theophylline-dependent cRaptz[9] was chosen as the model cRaptz because it works with high sensitivity and selectivity in response to its ligand, theophylline (Fig. 3).[6,10] To spontaneously release the cleaved RNA from its mother cRaptz at room temperature after the self-cleavage (i.e., to avoid a heating step), the stem I was adjusted to be hybridized moderately. In addition, the cleaved RNA sequence to hybridize to DNA23 on AuNPs was designed so that three 3′-terminal bases of the DNA23 covered a part of the stem I. This is because a partial strand displacement of the stem I by the DNA23 was expected to facilitate ready release of the cleaved RNA from its mother.[11] Moreover, an extra sequence was fused to a 5′ terminus of the cRaptz to give it a larger electrosteric effect.

Before tethering the cRaptz on AuNPs, I also checked the Mg^{2+} resistance of AuNPs tethering a short cleaved RNA. DNA23-Au40 tethering the cleaved RNA was prepared by hybridization of the cleaved RNA to DNA23 on AuNPs and was found to aggregate at 20 mM Mg^{2+}, slightly more quickly than DNA23-Au40 (Supplementary Fig. S2). This quicker aggregation, which is advantageous to the present system, can be explained by the lower mobility of DNA/RNA duplex than that of single-stranded DNA, which leads to lower steric repulsion; in addition, Manning's theory[12] suggests that the double-stranded nucleic acids have slightly lower charges than the same lengths of single-stranded nucleic acids. Although five bases at a 5′ terminus of the cleaved RNA are dangling as single-stranded RNA, they are too short to overcome these effects caused by DNA/RNA duplex formation.

Finally, detection of the cRaptz ligand (theophylline) was performed at room temperature using theophylline-dependent cRaptz-tethered DNA23-Au40 (cRaptz-DNA23-Au40), which was prepared via hybridization between the cRaptz and DNA23 on AuNPs.[13] Figure 4A and B shows time-course analyses of AuNP aggregation at 20 mM Mg^{2+} in the presence of various concentrations of theophylline. In the absence of theophylline, cRaptz-DNA23-Au40 was stable at 20 mM Mg^{2+} for several hours due to the long, dangling single-stranded cRaptz, which have a large electrosteric effect that can overcome the DNA/RNA duplex effects described above. In contrast, 1 mM and 100 μM theophylline caused

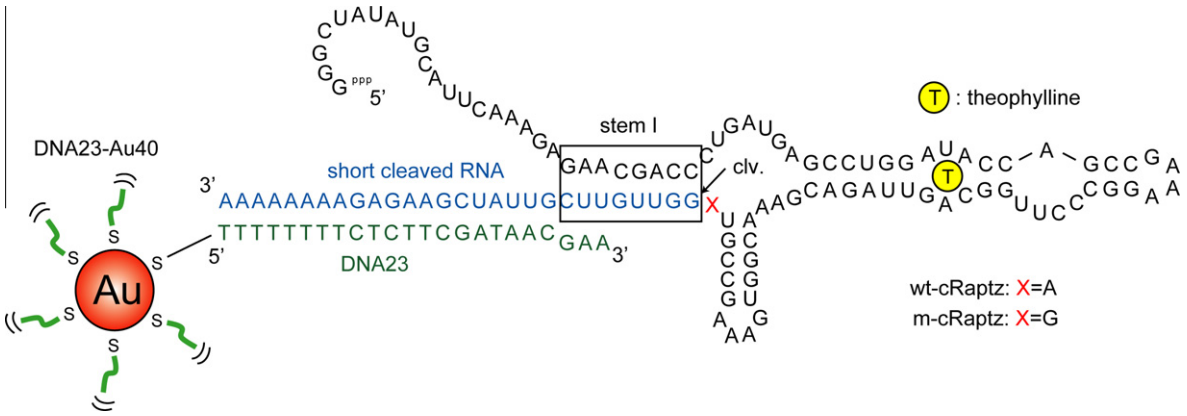

Figure 3. Nucleotide sequences of theophylline-dependent cRaptz[9] and DNA23. The stem I is boxed. The cleavage site is shown by an arrow.

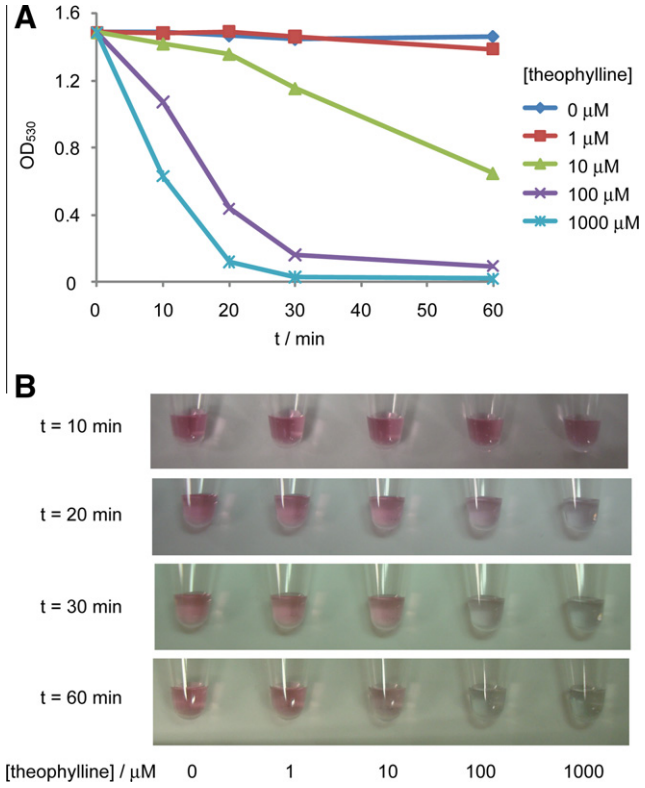

Figure 4. Detection of theophylline using cRaptz-DNA23-Au40. Time course of AuNP aggregation at 20 mM Mg^{2+} with various concentrations of theophylline. (A) Changes of OD_{530} relative to OD_{800}. The *X*-axis (t) is the time from the addition of theophylline. (B) Photos of AuNPs solutions 10, 20, 30, and 60 min after the addition of theophylline.

AuNPs to begin to aggregate in a few minutes and to almost completely precipitate in 30 min, when AuNPs with 10 μM theophylline also began to aggregate. With the naked eye, 1 mM, 100 μM, and 10 μM theophylline were detected within 10, 20, and 60 min, respectively (Fig. 4B). On the other hand, 1 mM caffeine, which has a similar structure to that of theophylline, did not induce AuNP aggregation (Supplementary Fig. S3A). Moreover, AuNPs tethering an inactive A90G-mutated cRaptz (m-cRaptz, Fig. 3 and Supplementary Fig. S1) did not aggregate for several hours, even with 1 mM theophylline (Supplementary Fig. S3B). These results clearly indicate that ligand-dependent self-cleavage of the cRaptz on AuNPs resulted in AuNP aggregation. In fact, a reasonable amount of larger cleaved cRaptz was detected only in the supernatant of AuNPs aggregated by theophylline (Supplementary Fig. S4).

In summary, I have developed a visible sensing system to detect a ligand of cRaptz using cRaptz-tethered large AuNPs with moderate salt resistance. In this system, the ligand can be detected at room temperature with visible AuNP aggregation induced by a loss of electrosteric stability (i.e., salt resistance) of the AuNPs, which is caused by ligand-dependent self-cleavage of the cRaptz on the AuNPs. In comparison to the previous multi-step method, which included a heating step (Fig. 1A),[6] this system requires only single handling and no thermostatic bath, making it more suited to on-the-spot sensing. In addition, the oligonucleotide-tethered AuNPs with moderate stability required for this system were easily prepared by using AuNPs with large diameter without any cumbersome treatment such as MCH treatment. This preparation method with the large AuNPs is available for other systems utilizing the electrosteric stability of DNA-tethered AuNPs.[3d,e,4c,14]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.11.048.

References and notes

1. Zhao, W.; Brook, M. A.; Li, Y. *ChemBioChem* **2008**, *9*, 2363.
2. (a) Elghanian, R.; Storhoff, J. J.; Mucic, R. C.; Letsinger, R. L.; Mirkin, C. A. *Science* **1997**, *277*, 1078; (b) Sato, K.; Hosokawa, K.; Maeda, M. *J. Am. Chem. Soc.* **2003**, *125*, 8102.
3. (a) Liu, J.; Lu, Y. *Angew. Chem., Int. Ed.* **2006**, *45*, 90; (b) Huang, C.-C.; Huang, Y.-F.; Cao, Z.; Tan, W.; Chang, H.-T. *Anal. Chem.* **2005**, *77*, 5735; (c) Xue, X.; Wang, F.; Liu, X. *J. Am. Chem. Soc.* **2008**, *130*, 3244; (d) Zhao, W.; Chiuman, W.; Brook, M. A.; Li, Y. *ChemBioChem* **2007**, *8*, 727; (e) Zhao, W.; Chiuman, W.; Lam, J. C. F.; McManus, S. A.; Chen, W.; Cui, Y.; Pelton, R.; Brook, M. A.; Li, Y. *J. Am. Chem. Soc.* **2008**, *130*, 3610.
4. (a) Liu, J.; Lu, Y. *J. Am. Chem. Soc.* **2003**, *125*, 6642; (b) Liu, J.; Lu, Y. *Anal. Chem.* **2004**, *76*, 1627; (c) Zhao, W.; Lam, J. C. F.; Chiuman, W.; Brook, M. A.; Li, Y. *Small* **2008**, *6*, 810.
5. For example of RNA-Au, see: Javier, D. J.; Nitin, N.; Levy, M.; Ellington, A.; Richards-Kortum, R. *Bioconjugate Chem.* **2008**, *19*, 1309.
6. (a) Ogawa, A.; Maeda, M. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6517; (b) Ogawa, A.; Maeda, M. *Chem. Lett.* **2009**, *38*, 848; (c) Ogawa, A.; Maeda, M. *Chem. Commun.* **2009**, 4666.
7. Sato, K.; Onoguchi, M.; Sato, Y.; Hosokawa, K.; Maeda, M. *Anal. Biochem.* **2006**, *350*, 162.

8. Zhao, W.; Lin, L.; Hsing, I.-M. *Bioconjugate Chem.* **2009**, *20*, 1218.
9. (a) Soukup, G. A.; Emilsson, G. A. M.; Breaker, R. R. *J. Mol. Biol.* **2000**, *298*, 623; (b) Roth, A.; Breaker, R. R. *Methods Mol. Biol.* **2004**, *252*, 145.
10. (a) Ogawa, A.; Maeda, M. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3156; (b) Ogawa, A.; Maeda, M. *ChemBioChem* **2008**, *9*, 206; (c) Ogawa, A.; Maeda, M. *ChemBioChem* **2008**, *9*, 2204; (d) Ogawa, A. *ChemBioChem* **2009**, *10*, 2465.
11. The theophylline-dependent cRaptz was efficiently cleaved with theophylline even in the presence of the DNA23, while it was almost not cleaved in the presence of DNA28 (Supplementary Fig. S1), which hybridizes to the cRaptz to prevent the stem I from hybridizing, as shown in Figure 1B.
12. Manning, G. S. *Q. Rev. Biophys.* **1978**, *11*, 179.
13. The hybridization efficiency of cRaptz to DNA23 on AuNPs was estimated to be ~20%, which is smaller than that of the cleaved RNA (~70%), because cRaptz has larger charges.
14. Liu, T.; Zhao, J.; Zhang, D.; Li, G. *Anal. Chem.* **2010**, *82*, 229.