Electronic Supplementary Information

Target-controlled formation of silver nanoclusters in abasic site-incorporated duplex DNA for label-free fluorescence detection of theophylline

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Table S1. DNA sequences employed in this study

Strand name	**DNA sequence (5'→ 3')**
13-mer abasic site-incorporated DNA[a]	TG GTG GXG GCA GC
13-mer complementary DNA (C)[b]	GC TGC CCC CAC CA
13-mer complementary DNA (A)[b]	GC TGC CAC CAC CA
13-mer complementary DNA (T)[b]	GC TGC CTC CAC CA
13-mer complementary DNA (G)[b]	GC TGC CGC CAC CA
FAM-labeled 13-mer complementary DNA (C)[b]	GC TGC CCC CAC CA-fluorescein
23-mer abasic site-incorporated DNA[a]	GA GAA TG GTG GXG GCA GC GAG AA
23-mer complementary DNA (C)[b]	TT CTC GC TGC CCC CAC CA TTC TC

[a]: X is an abasic site (Spacer C3, a propylene residue) in duplex DNA, which is in green color.

[b]: Receptor nucleobase opposite the abasic site in duplex DNA, which is in red color.

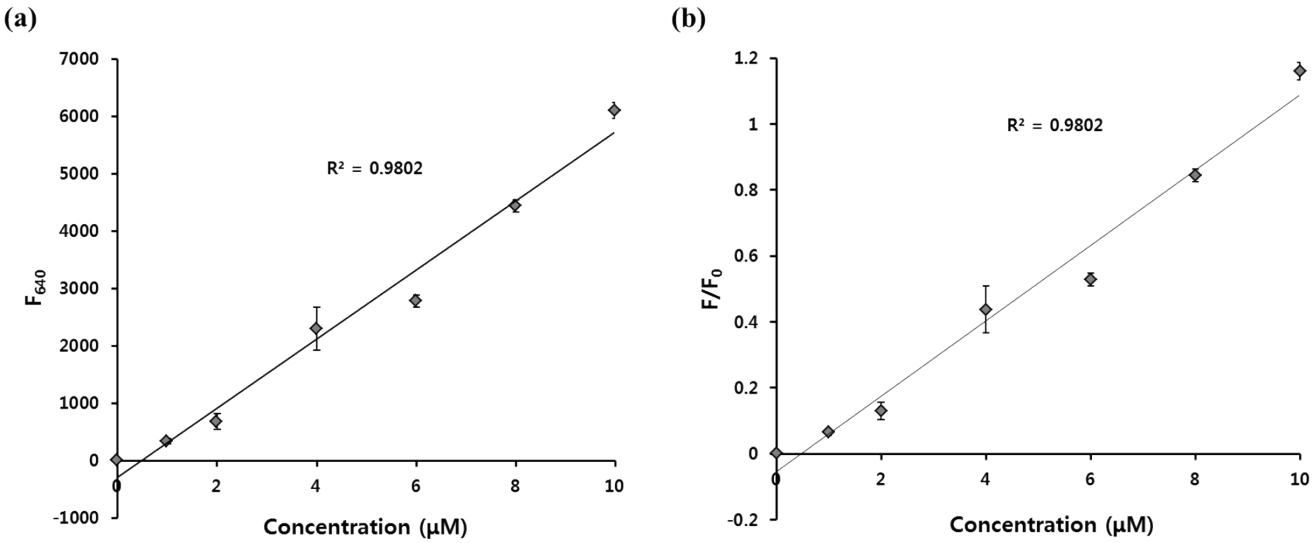

Fig. S1 (a) Fluorescence intensities of AgNCs generated from 13-mer duplex DNA at different concentrations. (b) Normalized fluorescence intensities is defined as F/F_0, where F and F_0 are the fluorescence intensities at 640 nm of AgNCs and 525 nm of FAM-labeled 13-mer duplex DNA at 50 nM, respectively.

Optimization for the conditions for analysis of theophylline using the new target-controlled formation of fluorescent AgNCs.

The optimal reduction time needed to form fluorescent AgNCs was determined by measuring fluorescence intensities of AgNCs in the absence and presence of theophylline. Analysis of the plots, displayed in Fig. S2 (a), shows that the fluorescence signals of AgNCs from samples with and without theophylline increased with increasing reduction time and the apparent signal difference was observed after 1 h. As the time increased further, the overall fluorescence signal was enhanced, but the degree of signal reduction defined as $(F_0\text{-}F)/F_0$, where F_0 and F are the fluorescence intensities at 640 nm in the absence and presence of theophylline, respectively, was slightly decreased after a 1 h reduction time (Fig. S2 (b)). Thus, 1 h was chosen as the optimal reduction time to analyze the target theophylline. The optimal ratio between reducing agent ($NaBH_4$) and silver ions was also determined. The results show that higher fluorescent signals were obtained at the lower ratio of $NaBH_4$:Ag^+ (1.5:1) (Fig. S3 (a)). However, the degree of signal reduction induced by the presence of theophylline was higher when relatively high ratios of $NaBH_4$:Ag^+ (3:1 and 4.5:1) were employed (Fig. S3 (b)). Based on these results, 3:1 ratio between $NaBH_4$ and Ag^+ was employed throughout this study.

The effect of the length of duplex DNA on the detection of theophylline was also determined. For this purpose, 23-mer duplex DNA containing AP site paired with cytosine was prepared together with 13-mer duplex DNA and their capabilities to detect theophylline were compared. As illustrated in Fig. S4, the fluorescence intensity of AgNCs formed from 23-mer duplex DNA was lower than that of 13-mer duplex DNA. More importantly, no significant difference was observed in the degree of the fluorescence signal reduction induced by theophylline between the two duplex DNAs. This result is consistent with the previous report that the binding affinity of theophylline is not sensitive to the duplex DNA length.[1] Therefore, 13-mer duplex DNA showing higher fluorescence intensity was employed as a probe to detect theophylline in this study.

The effect of pH value on the detection of theophylline was also investigated. As illustrated in Fig. S5, AgNCs with the highest fluorescence signal were generated at pH 6, but this high fluorescence signal was not significantly diminished by the presence of theophylline.[1,2] In contrast, AgNCs formed at

pH 7 gave a lower fluorescence signal than that of pH 6, but the signal reduction caused by theophylline was the largest among the tested pH values. Therefore, pH 7 was employed to detect theophylline in this study.

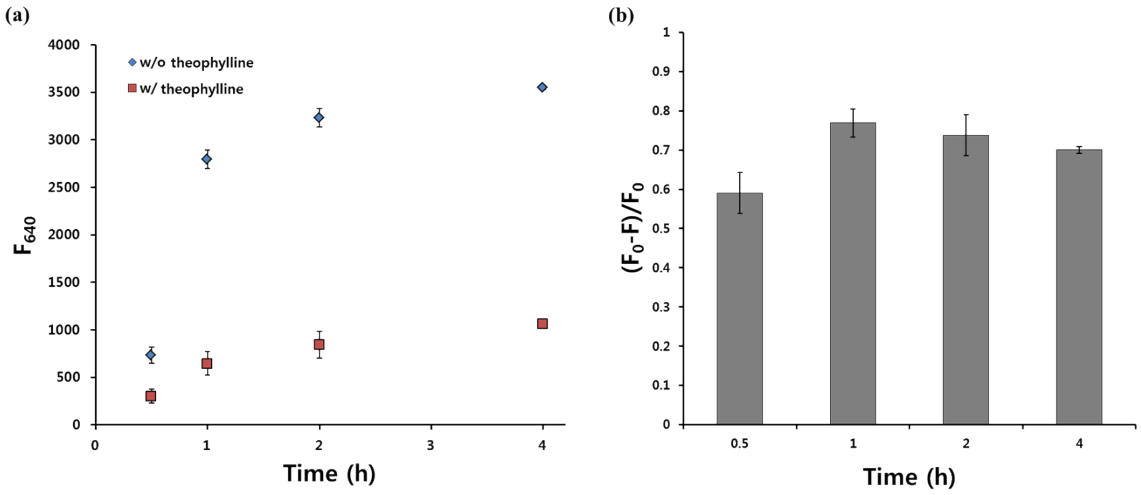

Fig. S2 Optimization of the reduction time for the formation of AgNCs in the absence and presence of theophylline (100 μM). (a) Fluorescence intensities and (b) degree of signal reduction in a bar form. The degree of signal reduction is defined as $(F_0\text{-}F)/F_0$, where F_0 and F are the fluorescence intensities at 640 nm in the absence and presence of theophylline, respectively.

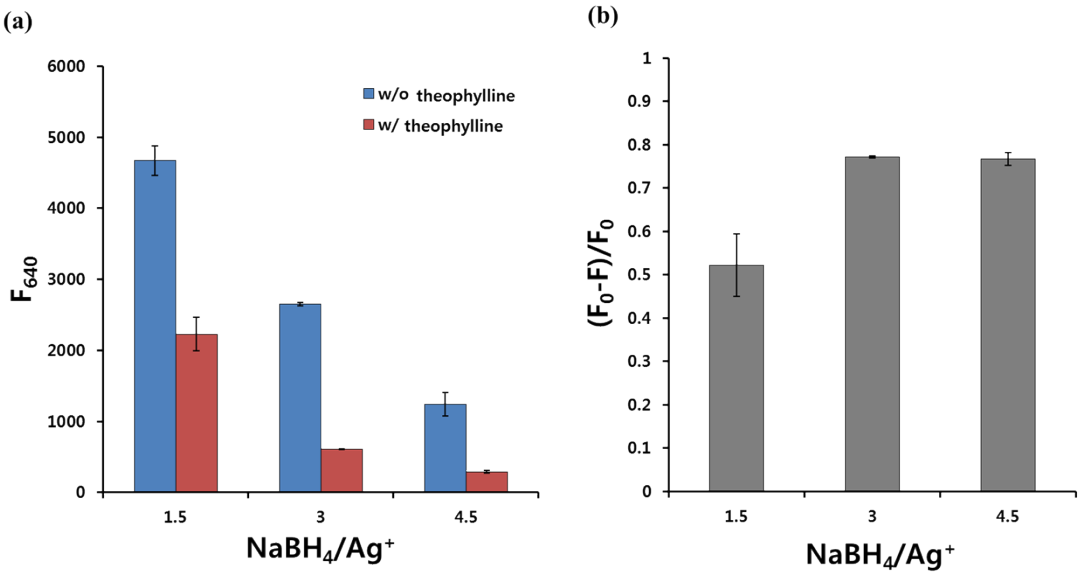

Fig. S3 Optimization of the ratio between $NaBH_4$ and Ag^+ ions for the formation of AgNCs in the absence and presence of theophylline (100 μM). (a) Fluorescence intensities and (b) degree of signal reduction in a bar form. The degree of signal reduction is defined as $(F_0\text{-}F)/F_0$, where F_0 and F are the fluorescence intensities at 640 nm in the absence and presence of theophylline, respectively.

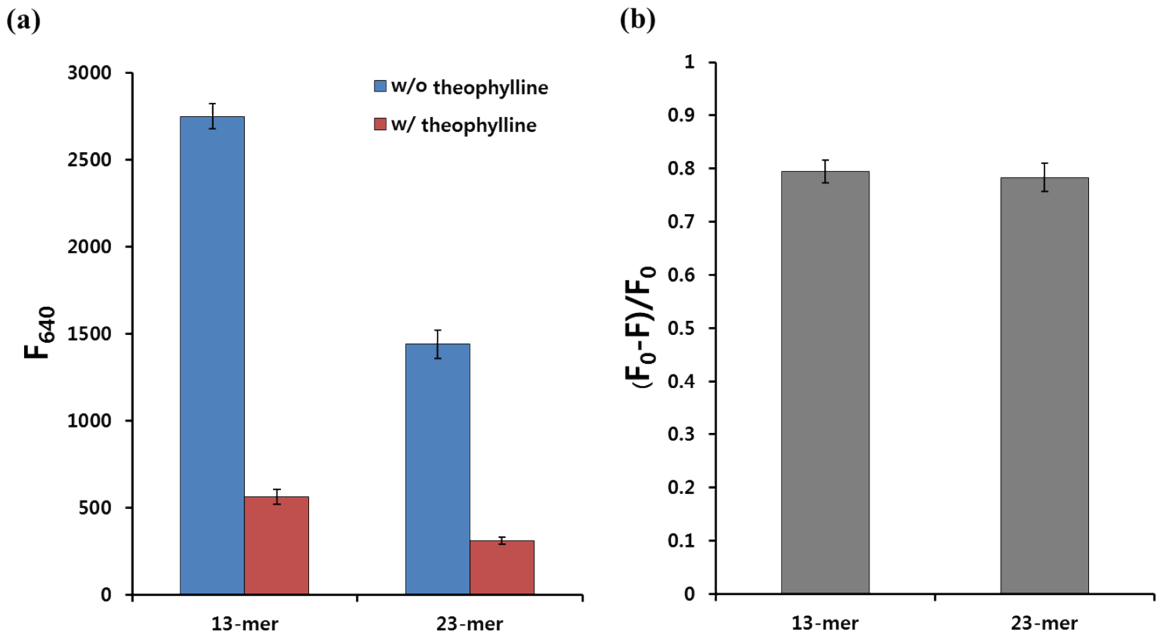

Fig. S4 (a) Fluorescence intensities of AgNCs generated from 13-mer or 23-mer duplex DNA having AP site paired with a cytosine nucleobase in the absence and presence of theophylline (100 μM) and (b) degree of signal reduction in a bar form. The degree of signal reduction is defined as $(F_0-F)/F_0$, where F_0 and F are the fluorescence intensities at 640 nm in the absence and presence of theophylline, respectively.

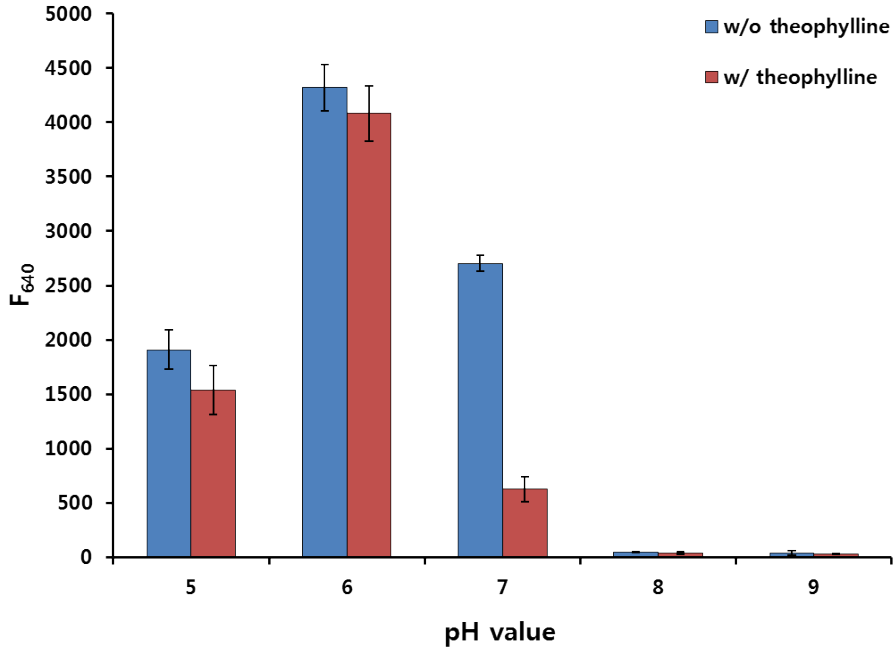

Fig. S5 Fluorescence intensities of AgNCs generated from 13-mer duplex DNA having AP site paired with a cytosine nucleobase at different pH values in the absence and presence of theophylline (100 μM).

Characterization of the synthesized AgNCs

The synthesized AgNCs were characterized using transmission electron microscopy (TEM), which indicates that they are spherical in shape and have a diameter of *ca.* 2 nm (Fig. S6). This value of diameter is consistent with the previous report for AgNCs.[3] Besides, the DNA-AgNCs were quite stable for 12 hours emitting the high fluorescence signal without any significant reduction (Fig. S7).

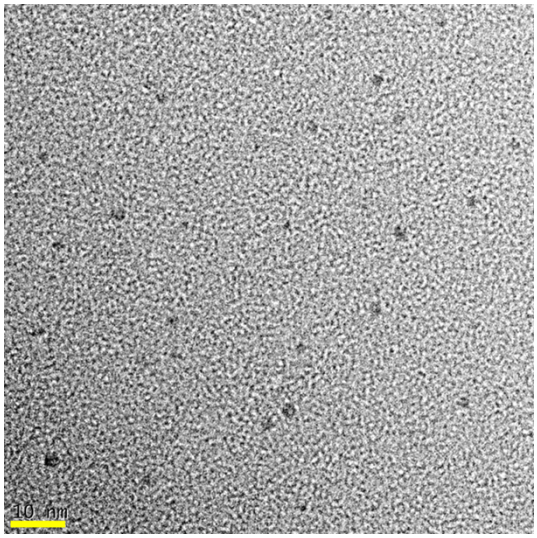

Fig. S6 A representative TEM image of DNA-AuNCs (Scale bar: 10 nm).

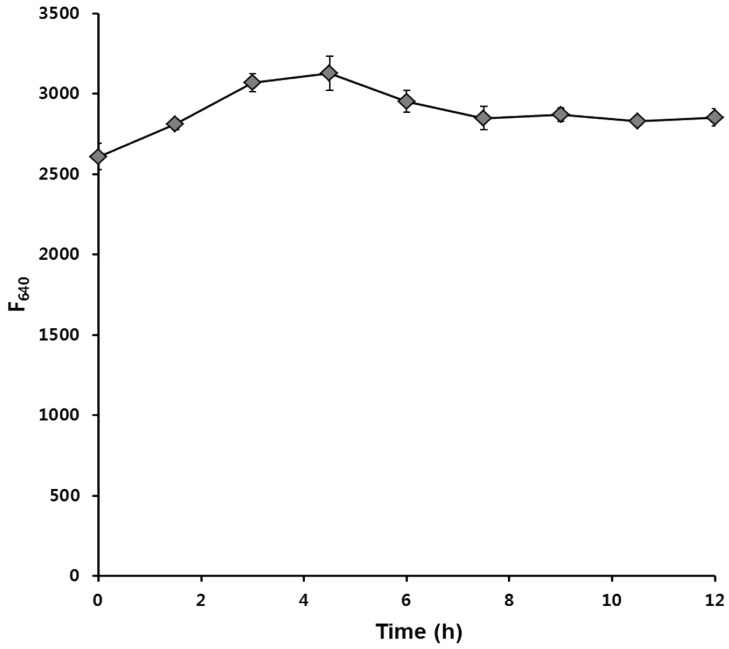

Fig. S7 Stability of the synthesized DNA-AgNCs.

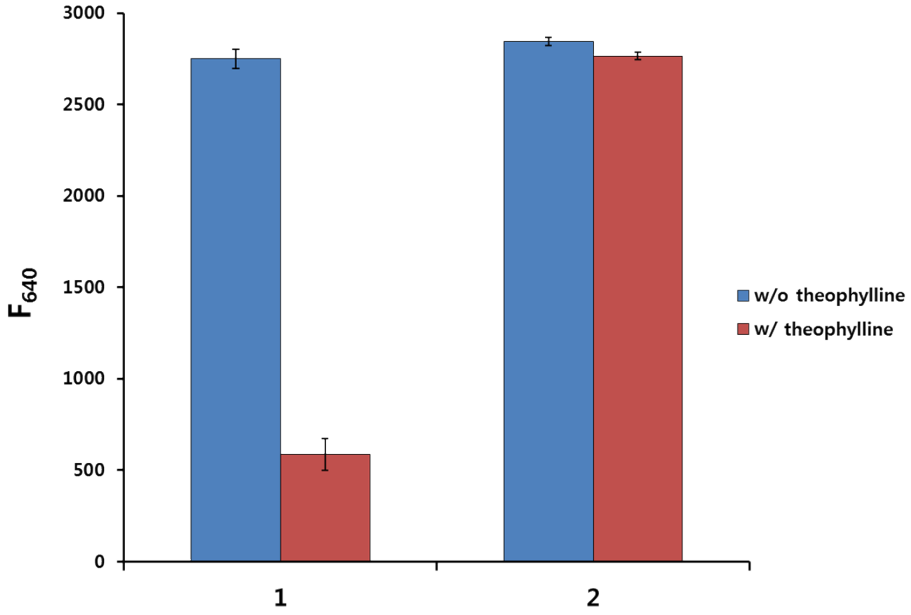

Fig. S8 Fluorescence intensities of AgNCs generated from 13-mer duplex DNA having AP site paired with a cytosine nucleobase in the abasence and presence of theophylline. Theophylline (100 μM) is applied before (1) and after (2) the formation of AgNCs.

UV-vis absorption spectra of the DNA-AgNCs

As can be seen from Fig. S9, there was the absorption band around 430 nm observed in both cases with and without theophylline, which is ascribed to the non-fluorescent larger silver nanoparticles, but even t he solution in the absence of theophylline did not show the characteristic visible absorption band with a maximum at 580 nm corresponding to the fluorescence excitation wavelength of DNA-AgNCs.[4] This ob servation may be due to the fact that the concentration of the AgNCs generated in the absence of theoph ylline is not high enough to be detected in UV-vis absorption spectroscopy.[5]

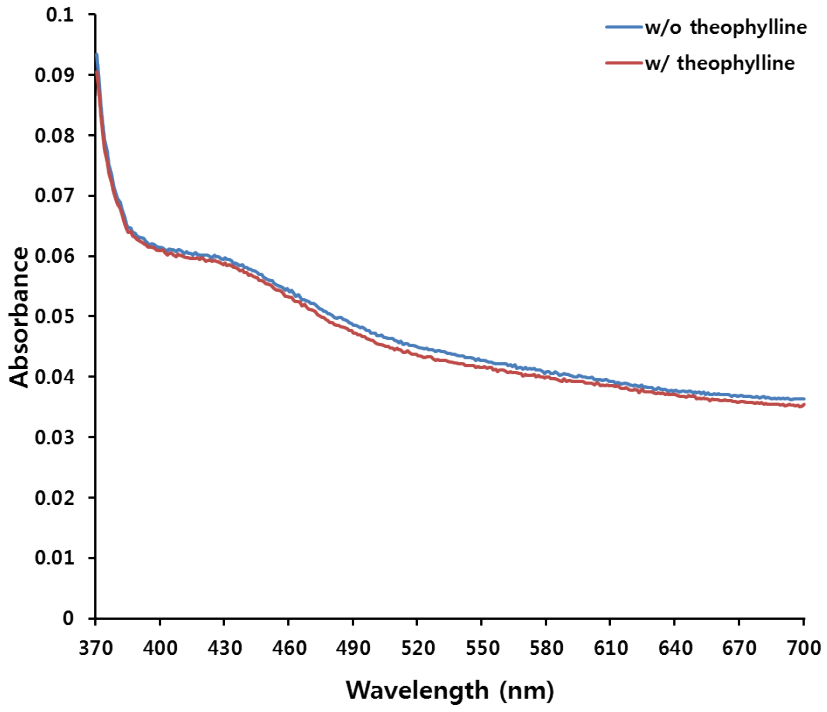

Fig. S9 UV-vis absorption spectra of DNA-AgNCs generated from 13-mer duplex DNA having AP site paired with a cytosine nucleobase in the absence and presence of theophylline (100 μM).

Copper ions interfering with the direct measurement of theophylline in blood serum

It is known that copper ions alter the fluorescence property of AgNCs.[3b, 6] In real situation, the direct measurement of theophylline in blood serum can be disturbed by copper ions. To solve this problem, ion selective-exchange resins which have the high selectivity and high uptake capacity for copper ions can be used in the pretreatment step, which consequently leads to the successful analysis of theophylline in blood serum.[7] In an alternative way, without the pretreatment step, 13-mer duplex DNA having AP site paired with a cytosine nucleobase is first incubated with the theophylline in blood serum and then separated using the centrifugal filter device whose molecular weight cut off is below the molecular weight of the 13-mer duplex DNA probe. This filtration allows the duplex DNA that is already complexed with theophylline to be separated from copper ions contained in blood serum. Using these suggested ideas, the problems caused by copper ions in blood serum could be overcome.

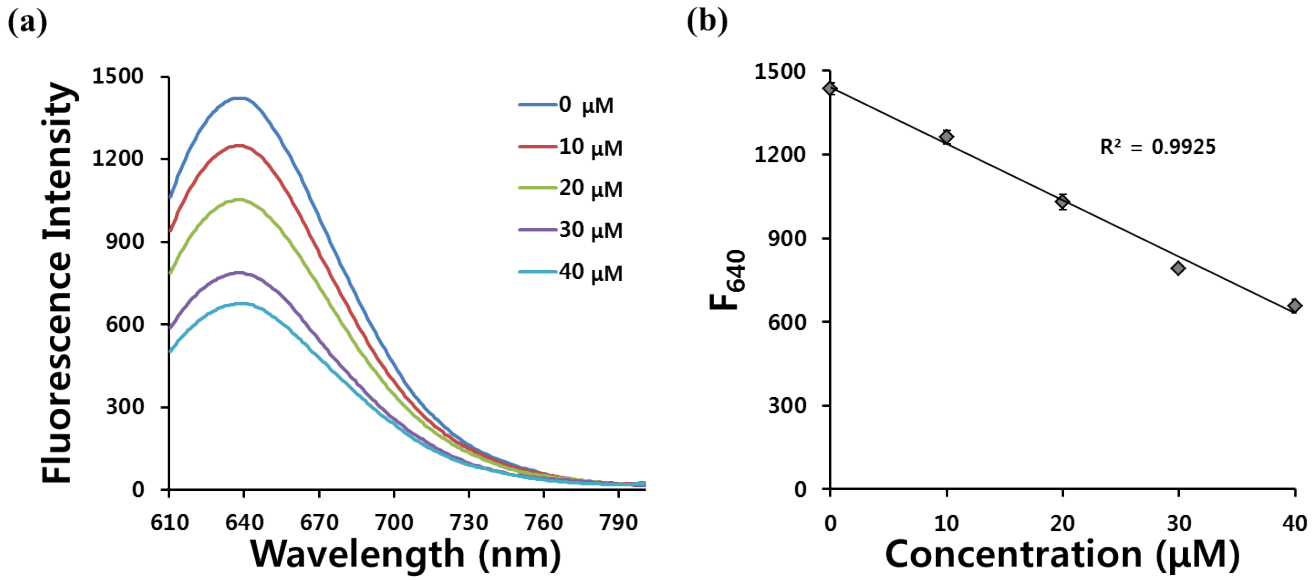

Fig. S10 (a) Fluorescence spectra of AgNCs generated from 13-mer duplex DNA having AP site paired with a cytosine nucleobase in the presence of diluted human serum spiked with various concentrations of theophylline. (b) Theophylline concentration-dependent change of fluorescence intensity (λ_{max} = 640 nm).

The effect of blood serum on the formation of AgNCs

As presented in Fig. S11, the fluorescence signal of AgNCs was decreased in the presence of diluted blood serum (1 %). This result indicates that formation of AgNCs was disturbed by proteins and other contaminants contained in serum.[8] It seems that the silver ions, precursor for the formation of fluorescent AgNCs, are consumed by biological thiols and other biological molecules that have the affinity to silver ions.[9] However, the theophylline-induced signal change was high enough to be discriminated from the background (Fig. S10), and the calibration curve in the serum samples was similar to that in the buffer solution (Fig. 5), demonstrating that the method has great potential for real clinical and diagnostic applications.

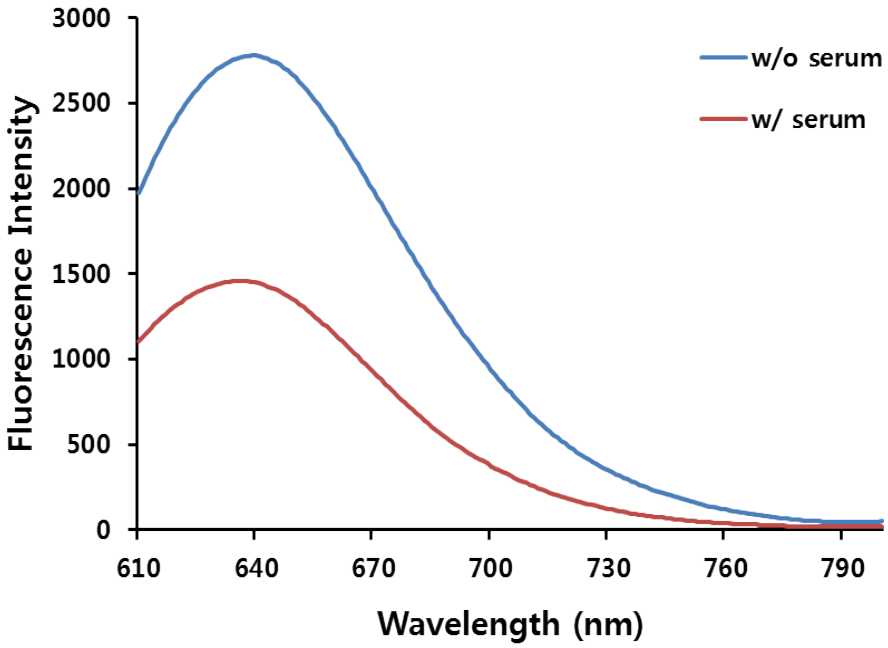

Fig. S11 Fluorescence spectra of AgNCs generated from 13-mer duplex DNA having AP site paired with a cytosine nucleobase in the absence and presence of diluted human serum (1 %).

References

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