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A new s-adenosylhomocysteine hydrolase-linked method for adenosine detection based on DNA-templated fluorescent Cu/Ag nanoclusters

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ABSTRACT

We herein describe a novel fluorescent method for the rapid and selective detection of adenosine by utilizing DNA-templated Cu/Ag nanoclusters (NCs) and employing s-adenosylhomocysteine hydrolase (SAHH). SAHH is allowed to promote hydrolysis reaction of s-adenosylhomocysteine (SAH) and consequently produces homocysteine, which would quench the fluorescence signal from DNA-templated Cu/Ag nanoclusters employed as a signaling probe in this study. On the other hand, adenosine significantly inhibits the hydrolysis reaction and prevent the formation of homocysteine. Consequently, highly enhanced fluorescence signal from DNA-Cu/Ag NCs is retained, which could be used to identify the presence of adenosine. By employing this design principle, adenosine was sensitively detected down to 19 nM with high specificity over other adenosine analogs such as AMP, ADP, ATP, cAMP, guanosine, cytidine, and urine. Finally, the diagnostic capability of this method was successfully verified by reliably detecting adenosine present in a real human serum sample.

1. Introduction

Recently, metal nanoclusters (NCs) have been extensively employed as a new alternative of organic fluorophores to construct fluorescent systems to detect various chemical or biological analyte (Park et al., 2014; Park and Park, 2015; Zhang et al., 2014). Since DNA-templated metal NCs exhibit several attractive inherent properties including high stability, biocompatibility, lower toxicity, and a strong fluorescence emission compared with organic fluorophores or quantum dots (Díez et al., 2012; Enkin et al., 2014; Park and Park, 2014), various applications such as optical sensing (Murphy, 2002), biological imaging (Michalet et al., 2005), and optoelectronic devices (Katz and Willner, 2004) have been developed based on DNA-templated metal NCs.

The representative examples are found in colorimetric biosensors based on DNA-templated metal NCs (Li et al., 2012; Park and Park, 2015; Qian et al., 2014; Yeh et al., 2010, 2011). The systems take advantage of the fact that non-emissive DNA-templated metal NCs are converted to bright emitters when placed in the proximity of a guanine-rich DNA sequence. Other systems rely on the quenching phenomena of fluorescent DNA-templated metal NCs induced by their strong interaction with sulfide atom, which led to the development of novel methods to detect sulfide ion and thiol-containing chemicals by utilizing this unique feature (Chen et al., 2011; Su et al., 2010).

In recent years, adenosine has received great attention due to its significant biological functions as an endogenous modulator which regulates various physiological processes in diverse tissues and organs (Spychala, 2000; Yan et al., 2009). In addition, adenosine plays a key role as a fundamental component for cell energy generation and protein metabolism, and many researches have demonstrated the possibility of adenosine as a biomarker for cancer detection (Gessi et al., 2004; Stagg and Smyth, 2010; Yang et al., 2002). Therefore, various methods have been developed for adenosine detection, which include radioimmunoassay (Siragy and Linden, 1996), high-performance liquid chromatography (HPLC) (Sottofattori et al., 2001), capillary electrophoresis (CE) (Tzeng et al., 2006) and aptamer-based methods (Chen et al., 2008; Xiang et al., 2009; Zhu et al., 2011). However, these methods inevitably require complicated sample preparation procedures or modified fluorescent probes.

Therefore, there is a great incentive existing for the development of much more convenient and accurate method for the detection of adenosine. Along this line, we herein designed a new system to detect target adenosine by utilizing DNA-templated Cu/Ag NCs. The fluorescence signal from DNA-templated Cu/Ag NCs is quenched by Hcy produced through SAHH-promoted hydrolysis reaction of SAH. However, target adenosine inhibits the hydrolysis

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reaction and prevents the quenching process, consequently leading to highly enhanced fluorescence signal. The diagnostic capability of this strategy was successfully verified by reliably detecting adenosine with high specificity.

2. Materials and methods

2.1. Materials

The DNA oligonucleotide (5′-CCCTTAATCCCC-3′) used in the study was purchased and purified from Bioneer® (Daejeon, Korea). Cysteine, sodium borohydride, human serum, adenosine, adenosine monophosphate (AMP), adenosine diphosphate (ADP), adenosine triphosphate (ATP), cyclic adenosine monophosphate (cAMP), cytidine, guanosine, urine, s-adenosylhomocysteine, L-homocysteine, and s-adenosylhomocysteine hydrolase were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of analytical grade and used without further purification (Ahn et al., 2015). Aqueous solutions were prepared using ultrapure DNase/RNase-free distilled water purchased from Bioneer®.

2.2. Preparation and characterization of DNA-Cu/Ag NCs

DNA-templated Cu/Ag NCs were synthesized according to a previously reported procedure with slight modification (Su et al., 2010). Briefly, 14 μL of a DNA solution (100 μM) was added to 41 μL of a phosphate buffer (50 mM, pH 7). Then, 4 μL of a 1 mM $Cu(NO_3)_2$ solution and 11 μL of a 1 mM $AgNO_3$ solution were added and the resulting mixture was incubated in ice bath for 15 min and reduced by adding 30 μL of 1 mM $NaBH_4$ under vigorous shaking. The reduced DNA-templated Cu/Ag NCs solution was stored in the dark for 60 min at room temperature. The prepared DNA-Cu/Ag NCs show good fluorescence stability for at least 2 weeks when stored at 4 °C in the dark (Fig S1). The concentration of the as-prepared DNA-Cu/Ag NCs is denoted as "1X" (Park et al., 2013). Dynamic light scattering (DLS) measurements were performed on a Malvern particle size analyzer (Malvern, UK). In the experiment, 1 mL of 1X DNA-Cu/Ag NCs solution was added in a polystyrene cuvette and the sample was scanned for 9 min (three runs) to obtain one set of raw data. The average size of the prepared NCs is estimated to be about 18 nm based on the DLS analysis (Fig. S2). Energy dispersive analysis of x-ray (EDAX) was performed by using field emission transmission electron microscopy (Tecnai, FEI, Netherlands) with accelerating voltages up to 200 kV.

2.3. SAHH-induced fluorescence quenching of DNA-Cu/Ag NCs

20 μL of phosphate buffer (100 mM, pH 7) containing 1 mM SAH is added to the 60 μL of the 1X as-prepared NCs solution. 20 μL of 26.4 unit/L SAHH is added to this solution (0.75 × NCs solution) and the resulting solution is incubated at 37 °C for 30 min. Fluorescence intensities were measured using a Tecan Infinite M200 pro microplate reader (Mnnedorf, Switzerland) and 384-well Greiner Bio-One microplates (Ref. 781077, Courtaboeuf, France) at the excitation wavelength of 480 nm. The CD spectra were recorded on a Jasco-815 spectropolarimeter (Tokyo, Japan) in the range of 200–300 nm in 0.5 mm path-length cuvettes, using a scanning speed of 50 nm/min, a response time of 1 s, a bandwidth of 1 nm, and an accumulation of 3 scans.

2.4. Adenosine detection procedure

Sample solutions (10 μL) containing adenosine at different concentrations or 1 μM of AMP, ADP, and ATP are added to 80 μL of the 0.75 × NCs. 10 μL of 52.8 unit/L SAHH is added to this solution and the resulting solution is incubated at 37 °C for 30 min. Fluorescence intensities were measured following the same procedure.

2.5. Human blood serum test

A human serum sample was first spiked by adenosine at various concentrations followed by 20 times dilution with the phosphate buffer (20 mM, pH 7). The resulting serum sample (10 μL) was then analyzed by following the adenosine detection procedure. To determine the amount of adenosine, the calibration curve was first constructed with a set of standards containing a known amount of adenosine in human serum. The fluorescence intensities from the unknown samples were measured and interpolated to determine the concentration of adenosine present in the sample based on the calibration curve.

2.6. Data analysis

All data points represent measurements carried out in triplicates (n=3) and each experiment was repeated three to four times. The data were statistically analyzed using the OriginPro software of OriginLab® and statistical errors are shown as standard deviations (SD).

3. Results and discussion

3.1. The overall scheme

The overall procedure to detect adenosine is illustrated in Fig. 1. In the procedure, an unknown sample is first mixed with a solution containing SAH and DNA-Cu/Ag NCs followed by the application of SAHH, which is then allowed to promote the hydrolysis reaction of SAH. As a result of the reaction, Hcy is produced and significantly reduces the fluorescence from DNA-Cu/Ag NCs by quenching through strong interaction with the NCs. In the presence of adenosine, however, it binds to SAHH enzyme with high affinity and inhibits the SAHH-catalyzed hydrolysis reaction of SAH. The inhibition leads to the reduced production of the Hcy, which accordingly reduces the Hcy-induced quenching process and retains the highly enhanced fluorescence signal from the NCs. Therefore, the fluorescence signal could be used to identify adenosine present in the sample.

3.2. SAHH-induced fluorescence quenching of DNA-Cu/Ag NCs

We first synthesized DNA-Cu/Ag NCs and examined their fluorescence spectra. As shown in Fig. S3. maximum fluorescent intensity was observed at 576 nm. Only when both silver and copper ions are simultaneously applied for the formation the DNA-NCs, high fluorescence signals are observed from the resulting NCs, which consist of silver and copper as evidenced by the EDAX data (Fig. S4). Optimal conditions for fluorescence quenching of NCs were next determined by examining the intensities of the fluorescence signals reduced by SAHH-catalyzed SAH hydrolysis reaction. The results of experiments, in which SAH concentrations and incubation time were varied, indicate that 50 μM and 30 min incubation time are ideal for optimal sensing (Fig. S5). A set of experiments performed under the optimized conditions shows that the reduction of fluorescence from NCs happens only when the solution containing SAH and NCs is treated with SAHH while DNA-Cu/Ag NCs freely emit strong fluorescence at 576 nm in the absence of SAHH (Fig. 2). In addition, circular dichroism (CD) spectra were also recorded to examine the conformational changes of the DNA-NCs after treatment with Hcy or product of SAHH-catalyzed SAH hydrolysis reaction, which supports the fluorescent spectral

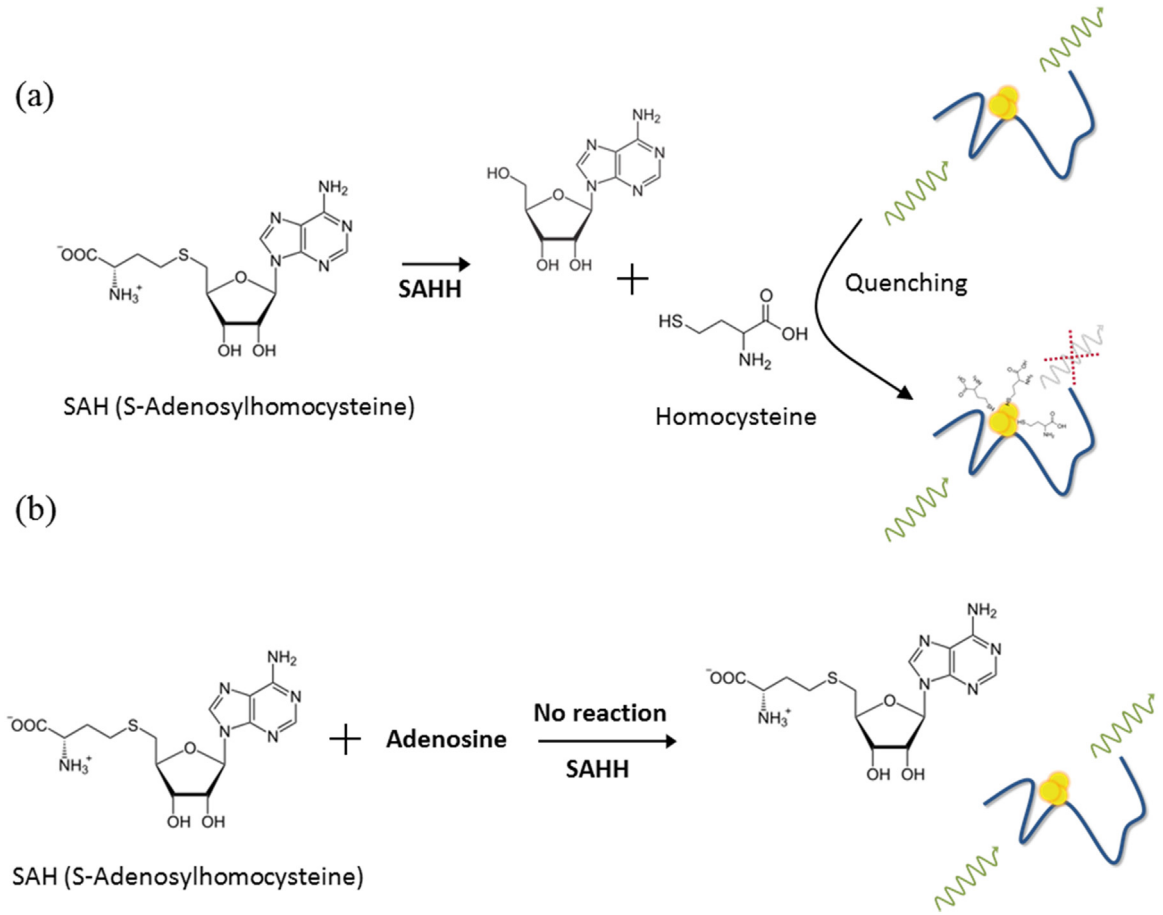

Fig. 1. Schematic illustration of adenosine detection utilizing fluorescent DNA-templated Cu/Ag NCs. (a) Fluorescence signal quenched by homocysteine produced through SAHH-promoted hydrolysis reaction of SAH. (b) Restored fluorescence signal in the presence of adenosine.

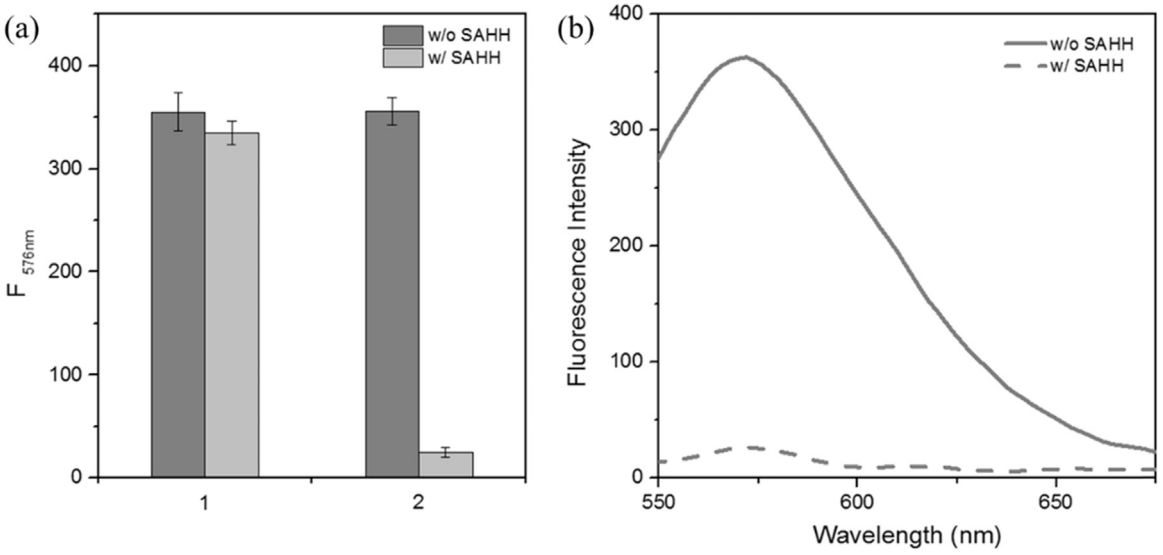

Fig. 2. SAHH-induced fluorescence quenching of DNA-Cu/Ag NCs. (a) fluorescence intensities of DNA-Cu/Ag NCs in the absence and presence of SAHH (52.8 Units/L) (1: without SAH, 2: with SAH). (b) fluorescence emission spectra generated from Cu/Ag NCs in the absence and presence of SAHH (52.8 Units/L).

data (Fig. S6). All these observations clearly demonstrate that Hcy, which is produced from SAH hydrolysis reaction catalyzed by SAHH, effectively reduces the fluorescence intensities from DNA-Cu/Ag NCs.

3.3. Adenosine detection by utilizing DNA-Cu/Ag NCs

We next applied the SAHH-induced fluorescence quenching of DNA-Cu/Ag NCs to construct a new method to detect adenosine by focusing on that adenosine inhibits the SAHH-promoted hydrolysis

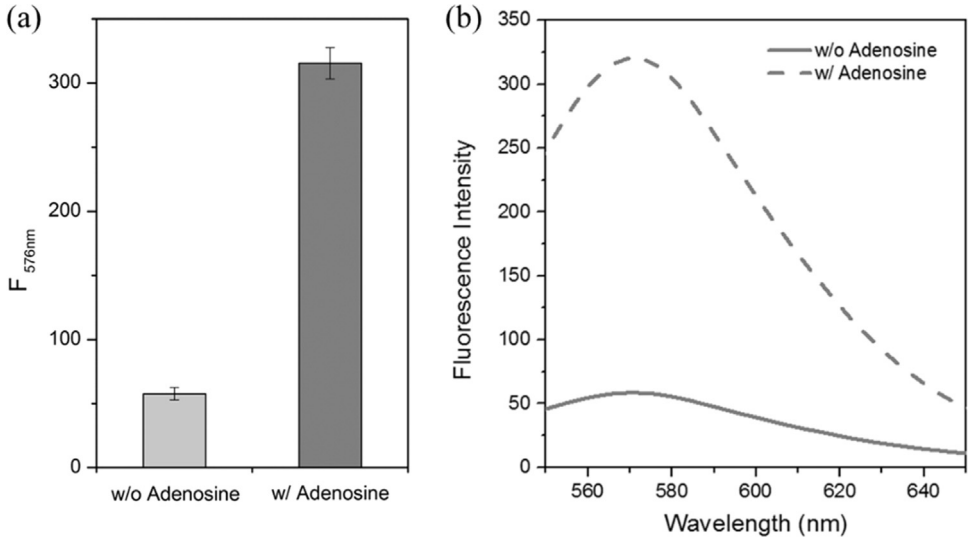

Fig. 3. Adenosine-mediated restoration of the quenched fluorescence of DNA-Cu/Ag NCs. (a) Fluorescence intensities and (b) fluorescence emission spectra generated from DNA-Cu/Ag NCs in the absence and presence of adenosine (1 μM).

of SAH. As envisioned, the quenching phenomena caused by hydrolysis of SAH was quite significantly prevented by the presence of adenosine, retaining the highly enhanced fluorescence signal of NCs (Fig. 3). The result proves the feasibility of our new system for adenosine detection.

The selectivity of the new detection system toward target adenosine was investigated. For this purpose, adenosine analogs such as AMP, ADP, ATP, cAMP, guanosine, cytidine, and urine were examined for their capabilities to inhibit SAHH-promoted hydrolysis of SAH and compared with that of adenosine. As presented in Fig. 4, a highly retained fluorescence is observed only when there is target adenosine present in the sample while the other adenosine analogs resulted in significantly reduced fluorescence indicating that they were not able to inhibit SAHH activity to catalyze the hydrolysis reaction of SAH. This result confirms high selectivity of our detection strategy for target adenosine.

The detection sensitivity of our adenosine detection system was also determined by measuring fluorescence intensities at 576 nm as a function of adenosine concentrations. The results

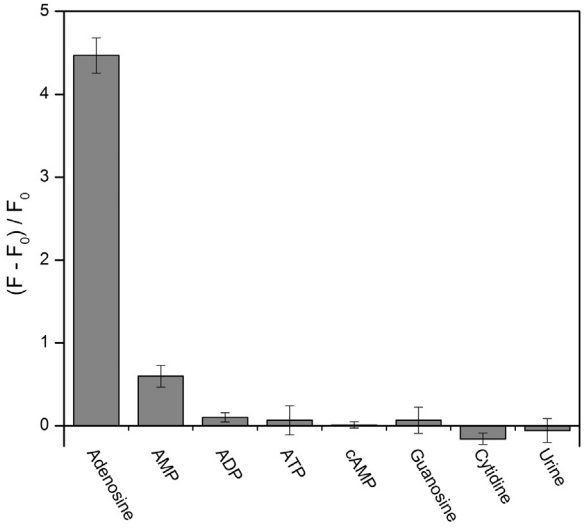

Fig. 4. Selectivity of the adenosine detection system. The degree of signal increase is defined as $(F-F_0)/F_0$, where F_0 and F are the fluorescence intensity at 576 nm in the absence and presence of adenosine, AMP, ADP, ATP, cAMP, guanosine, cytidine, and urine at 1 μM.

show that fluorescence intensities increase with increasing concentrations of adenosine up to 1 μM, and then plateau at concentrations over 1 μM (Fig. 5). An excellent linear relationship ($R^2=0.996$) exists in the range of 0–1 μM of the plot and the limit of detection (LOD) (3σ/slope) is ca. 19 nM, which is comparable with those of previously reported methods. (Chen et al., 2008; Fu et al., 2013; Liu et al., 2011; Shahdost-fard et al., 2014; Zhu et al., 2011).

3.4. Adenosine detection in a serum sample

To verify the practical utility of the system, we finally employed our system to detect adenosine present in human blood serum containing various biological components which may interfere the detection reliability of the strategy. As presented in Fig. S7, fluorescence signal increases with increasing adenosine in 5% human serum, showing almost same pattern with the sample containing only adenosine. The linear relationship ($R^2=0.983$) is also observed in the same range of 0–1 μM. For the samples spiked by an unknown amount of adenosine, our method exhibited excellent reproducibility and precision yielding a coefficient of variation (CV) less than 0.1% and a recovery ratio within $100 \pm 5\%$ (Table 1), ensuring the reliability of our strategy.

4. Conclusions

In the study described above, we developed a novel strategy for the reliable detection of adenosine. Our strategy employs DNA-Cu/Ag NCs as a signaling unit whose fluorescence signal is designed to be recovered from the quenching state in a response to adenosine. Based on this design principle, adenosine was very successfully identified with high sensitivity and specificity. Importantly, the experimental procedure can be conducted in a quite simple manner because it does not need any complicated preparation steps which are generally inevitable in conventional methods (Tzeng et al., 2006). Finally, to the best of our knowledge, this is the first study to apply DNA-Cu/Ag NCs to achieve biomolecule detection. The observations presented in this study may initiate a new platform for the convenient and cost-effective biomolecular detection by utilizing DNA-templated fluorescent NCs.

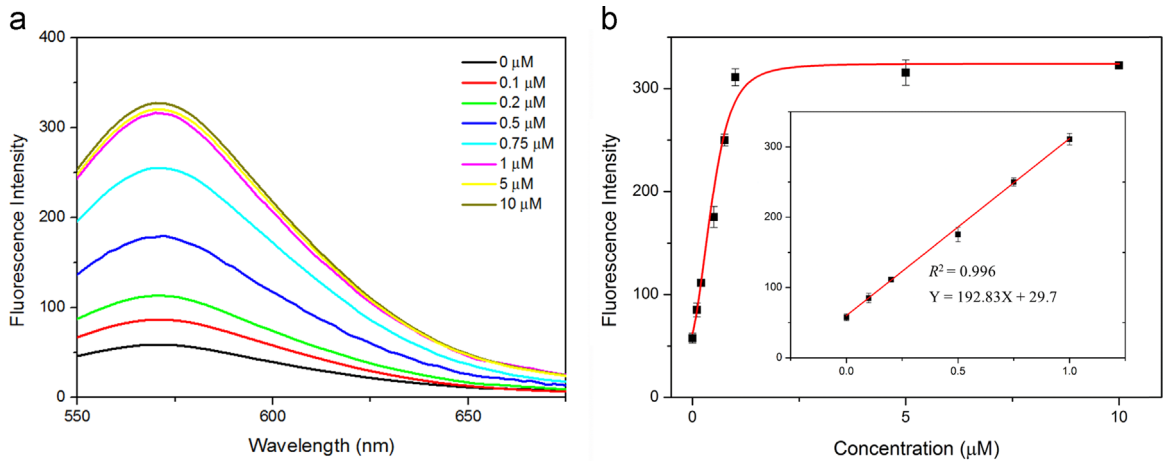

Fig. 5. Sensitivity of adenosine detection system. (a) Fluorescence emission spectra of DNA-Cu/Ag NCs in the presence of adenosine at varying concentrations. (b) Adenosine concentration-dependent changes of fluorescence intensity at 576 nm. Inset: Linear between $F_{576\ nm}$ and the adenosine concentration (0–1 μM).

Table 1
Determination of adenosine in human serum.

Added adenosine (μM)	Measured adenosine (μM)[a]	SD[b]	CV[c] (%)	Recovery[d] (%)
0.2	0.207	0.016	0.079	103.7
0.6	0.571	0.031	0.056	95.1
0.9	0.910	0.044	0.048	101.2

[a] Mean of three measurements.
[b] Standard deviation of three measurements.
[c] Coefficient of variation = SD/mean × 100.
[d] Measured value/added value × 100.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2016.08.058.

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