Electronic Supplementary Information

Instrument-free quantitative gold nanoparticle-based liquid-phase colorimetric assays for use in resource-poor environments

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Experimental Section

Reagents and Apparatus

Streptavidin (from *Streptomyces avidinii*, >17 U/mg), hydrogen tetrachloroaurate (III) ($HAuCl_4 \cdot 3H_2O$), sodium citrate, glutaraldehyde, cysteamine, formamide, dimethyl formamide and ethanol were purchased from Sigma-Aldrich. Adenosine, cytidine, uridine, guanosine, lysine and polyethylene glycol (molecular weight range 7000 ~ 9000) are the products of Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China). Human serum samples were collected from healthy volunteers. Amine-coated superparamagnetic microparticles (SMPs, ca. 0.5 μm in diameter) and epoxide-modified poly(glycidyl methacrylate) nanoparticles (PNPs, ca. 0.1 μm in diameter) were obtained from Tianjin BaseLine Chrom Tech Research Centre (Tianjin, China). All other chemicals were of analytical grade and were used as received without further purification. The buffer includes 10 mM phosphate buffer (PBS, pH 7) prepared from Na_2HPO_4 and KH_2PO_4. Unless stated otherwise, all stock and buffer solutions were prepared with deionized water (with a specific resistivity ≥ 18.2 MΩ·cm).

Gold nanoparticles (GNPs) with an average diameter of ca. 15 nm were synthesized with a domestic microwave oven. Optical characterization of GNPs was performed on a UV-Vis spectrometer (Cary 50, Varian, USA). The characterization of their morphology was carried out using a high resolution transmission electron microscope (HRTEM, JEM-2100F, JEOL, Japan). Images of all solutions and real-time processes of the cysteamine-induced aggregation of GNPs were recorded using a smart phone (Apple iPhone 5s).

General Method for Oligonucleotide Synthesis and Purification

The thermodynamic parameters of the used oigonucleotides were calculated using bioinformatics software (http://www.bioin-fo.rpi.edu/applications/).[1] The sequences of the oligonucleotides (from 5' to 3') were as follows: capture DNA strand, biotin-CCC AGG TCA GTG GAG-$(CH_2)_6$-NH_2; aptamer strand, CAC TGA CCT GGG GGA GTA TTG CGG AGG AAG GT (the sequence for adenosine binding is underlined).[2,3]

Standard DNA phosphoramidites, solid supports, and additional reagents were purchased from Link Technologies Ltd and Applied Biosystems Ltd. All oligonucleotides were synthesized on an Applied Biosystems 394 automated DNA/RNA synthesizer using a standard 1.0 μM phosphoramidite cycle of acid-catalyzed detritylation, coupling, capping, and iodine oxidation. Stepwise coupling efficiencies and overall yields were determined by the automated trityl cation conductivity monitoring facility and in all cases were >98.0%. All β-cyanoethyl phosphoramidite monomers were dissolved in anhydrous acetonitrile to a concentration of 0.1 M immediately prior to use. The coupling time for normal A, G, C, and T monomers was 60 s, and the coupling time for the modified phosphoramidite monomers was extended to 600 s. Cleavage of the oligonucleotides from the solid support and deprotection was achieved by exposure to concentrated aqueous ammonia solution for 60 min at room temperature followed by heating in a sealed tube for 5 h at 55 °C. Purification of oligonucleotides was carried out by reversed-phase HPLC on a Gilson system using a Brownlee Aquapore column (C8, 8 mm × 250 mm, 300Å pore) with a

gradient of acetonitrile in triethylammonium bicarbonate (TEAB) increasing from 0% to 50% buffer B over 30 min with a flow rate of 4 mL/min (buffer A: 0.1 M triethylammonium bicarbonate, pH 7.0, buffer B: 0.1 M triethylammonium bicarbonate, pH 7.0 with 50% acetonitrile). Elution of oligonucleotides was monitored by ultraviolet absorption at 295 or 300 nm. After HPLC purification, oligonucleotides were freeze dried then dissolved in water without the need for desalting. For long oligonucleotides, polyacrylamide gel electrophoresis was used for purification. Oligonucleotide bands were then visualized using a UV lamp and the desired bands excised, crushed and soaked in water overnight at 37 °C. After evaporation, samples were desalted using NAP-25 followed by NAP-10 columns (G.E. Healthcare Life Sciences). All oligonucleotides were characterised by electrospray mass spectrometry using a Bruker micrOTOF II focus ESI-TOF MS instrument in ESI^- mode. Data were processed using MaxEnt.

Synthesis of GNPs

The GNPs were synthesized by a slightly modified microwave-assisted sodium citrate reduction method.[4] Briefly, 1 mL of a 1 wt% (w/v) $HAuCl_4 \cdot 3H_2O$ solution and 2 mL of a 1 wt% (w/v) sodium citrate solution were rapidly mixed with 97 mL of water, followed by heating in the microwave oven at high power (800 W) for 8 min. After the resulting red GNP solution was allowed to cool to room temperature (ca. 25°C, over 1 h), it was diluted with water to the original volume (100 mL) and then stored at 4 °C for further use.

The freshly synthesized GNPs, which were coated with citrate ions and were negatively charged, were used directly to design the red-blue 2D GNP-LPCA for detection of cysteamine in PBS buffer. Before their use in the blue-red 2D GNP-LPCA cysteamine and adenosine assays, the GNPs were first isolated via centrifugal separation and then dispersed in formamide. The resultant GNP solution also appeared red because this organic solvent does not allow the particles to aggregate. It was stored at 4 °C until used.

Preparation of Aptamer-SMP Bioconjugates

For the preparation of aptamer-SMP bioconjugates, aptamer and amine-terminated capture DNA oligonucleotides (1 μM each in PBS buffer) were mixed in equimolar amounts. The mixture was heated to 70 °C, incubated for 10 min, and then allowed to slowly cool to room temperature (over 2h) where upon DNA duplexes were formed via hybridization. 1 mL of a 0.5% (w/v) SMP suspension was mixed with 5 mL of a 5% (w/v) glutaraldehyde solution and the mixture was incubated for 3 h at room temperature. The SMPs were then isolated magnetically and washed three times with PBS buffer to remove unreacted glutaraldehyde molecules. The aldehyde-activated SMPs were dispersed in 1 mL of the duplex DNA solution (1 μM) and incubated under continuous gentle stirring at 4 °C overnight. Next, 1 mL of 1 mM lysine was further used to block the remaining aldehyde groups on the sub-microparticles. After magnetic separation and washings, the resultant aptamer-SMP bioconjugates were re-suspended

in 1 mL of PBS buffer (containing 1.5% (w/v) polyethylene glycol) and stored at 4 °C for further use.

Preparation of Streptavidin-PNP Bioconjugates

The conjugation process was performed as follows: 1.5 mL of a 0.25% (w/v) PNP suspension was mixed with 1 mL of a 1 mg mL^{-1} streptavidin solution in PBS buffer for incubation at 4 °C overnight. The streptavidin was covalently conjugated with the PNPs via ring-opening reactions of the epoxide groups on the particle surface with the amine moieties of the protein.[5] Then, 0.5 mL of a 100 mM cysteamine solution was used to block the remaining epoxide groups on the nanoparticles. After centrifugal separation and washing, the resultant streptavidin-PNP bioconjugates were re-suspended in 1 mL of PBS buffer (containing 1.5% (w/v) polyethylene glycol) and stored at 4 °C until used.

Analytical Procedures Using Red-Blue 2D GNP-LPCA for Detection of Cysteamine

In a typical assay, 15 μL of a cysteamine sample in PBS was firstly mixed with 15 μL of formamide. Then, this mixture was dropped into 100 μL of a red GNP solution in a graduated test tube at room temperature. After the dropwise addition, a top-bottom red-blue reaction mixture was formed, as the cysteamine mediated the aggregation of the GNPs mainly at the bottom part of the solution. The length of the blue column was proportional to the cysteamine level in the sample. Counting the blue length via the

marked bars of the graduated test tube by eye enabled quantitative detection of the target.

Analytical Procedures Using Blue-Red 2D GNP-LPCA for Detection of Cysteamine and Adenosine in Buffer Samples

In the cysteamine assay, 30 μL of a cysteamine sample in the buffer was dropped into 100 μL of a red GNP solution in formamide in a graduated test tube at room temperature. The dropwise addition led to the production of a top-bottom blue-red reaction mixture, since most of the cysteamine-mediated GNP aggregation took place in the upper phase. Visual quantitative detection of the analyte was realized by simply counting the red length-related marked bars of the graduated test tubes.

For the aptamer-based adenosine assay, 10 μL of an adenosine sample in PBS was mixed with 10 μL of aptamer-SMP bioconjugate and 10 μL of streptavidin-PNP bioconjugate. Incubation was carried out for 40 min at 37 °C to allow aptamer-adenosine binding which exposed the biotin moieties on the capture DNAs to further capture the PNPs onto the SMP surfaces via the biotin-streptavidin interaction. The mixture was magnetically separated and washed with the PBS buffer. The sediments were then resuspended in 40 μL of a freshly prepared GNP solution in water and incubated at 37 °C for 50 min. GNPs were bound onto the PNP surfaces via the thiol-gold chemistry. After the resulting SMP-PNP-GNP complexes were magnetically isolated and washed, they were next dispersed in 40 μL of 10 μM cysteamine in buffer. After a 40 min incubation at 37 °C and magnetic separation, 30 μL of the supernatant

was dropped into 100 μL of a red GNP solution in formamide in a graduated tube at room temperature, resulting in the formation of a top-bottom blue-red mixture. The red length-related marked bars were inversely proportional to the concentration of the adenosine target in the sample. It took totally about 2.5 h to complete the whole adenosine assay. Control experiments were performed in the same manner above but using PBS buffer, cytidine, uridine or guanosine instead of adenosine.

Analytical Procedures Using Blue-Red 2D GNP-LPCA for Detection of Adenosine in Human Serum Samples

To assess the practicability of the aptamer-based 2D GNP-LPCA, the detection of adenosine in undiluted human serum samples was carried out according to the above general procedures. The "found" concentrations of the adenosine in the serum samples were calculated from the corresponding number of the red bars on the test tubes and the regression equation: y (N_{bar}) = 1.1386x (Log[adenosine]) + 3.5200 (R = 0.9948).

Next, different levels of standard solutions of adenosine were added into the human serum. Then the analyte-spiked serum samples were assayed according to the above general procedures. The "total" concentrations of the adenosine in the spiked serum samples were also calculated from the corresponding number of the red bars on the test tubes and the above regression equation. The recovery was defined as the ratio of the estimated adenosine concentration and the added value in the human serum.

References

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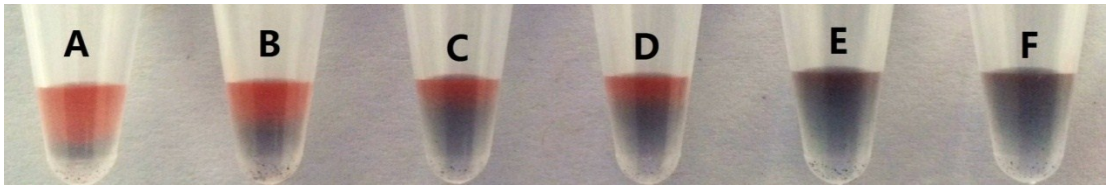

Fig. S1 Volume optimization for cysteamine solution and formamide (v/v = 1/1). Each colorimetric result was obtained by dropwise addition of different volumes of the mixture consisting of 40 μM cysteamine and formamide (v/v = 1/1) into 100 μL of a red GNP solution: (A) 15, (B) 20, (C) 25, (D) 30, (E) 40, and (F) 50 μL. 30 μL was chosen as the optimal volume as it gave the clearest colorimetric result.

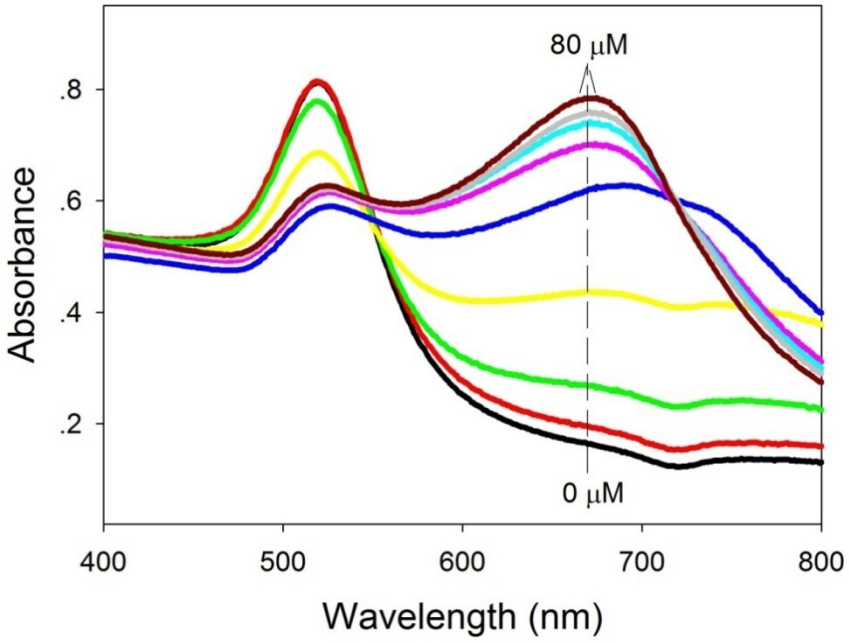

Fig. S2 UV-Vis spectra obtained from the detection of cysteamine samples in PBS with concentrations ranging from 0 to 80 μM by using the 1D GNP-LPCA method.

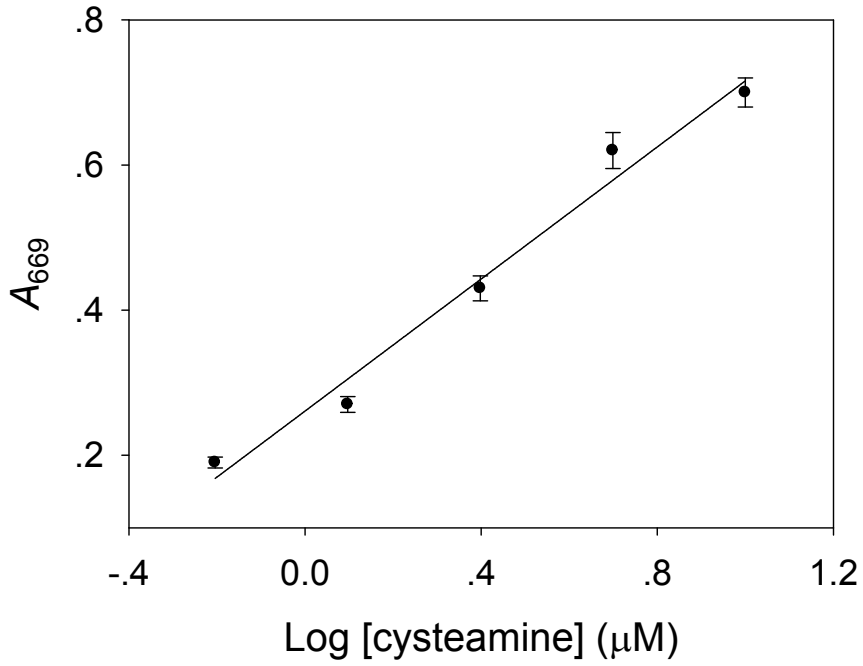

Fig. S3 The calibration curve for the 1D GNP-LPCA shows the linear relationship between the UV-Vis absorbance at 669 nm (A_{669}) of the GNP solutions obtained from Figure S2 as a function of (Log[cysteamine]). Each error bar represents a standard deviation across three replicate experiments.

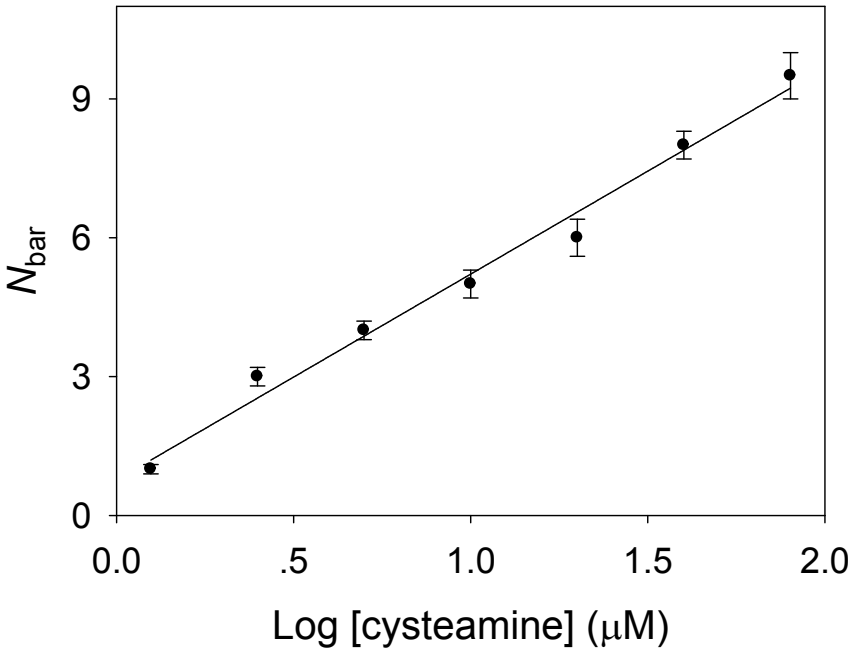

Fig. S4 The calibration curve for the 2D GNP-LPCA shows the linear relationship between the number of blue bars (N_{bar}) on the graduated test tubes shown in Figure 2D (top) in the main text as a function of (Log[cysteamine]). Each error bar represents a standard deviation across three replicate experiments.

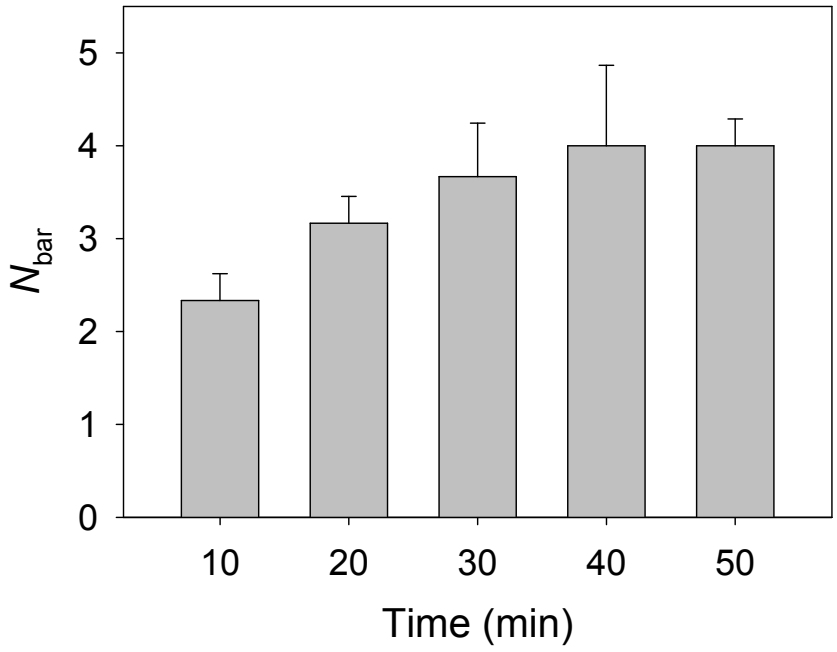

Fig. S5 Time optimization for the incubation of the adenosine sample and the aptamer-SMP bioconjugate probes and streptavidin-PNP bioconjugate probes. 40 min was chosen as the optimal incubation time. Each error bar represents a standard deviation across three replicate experiments.

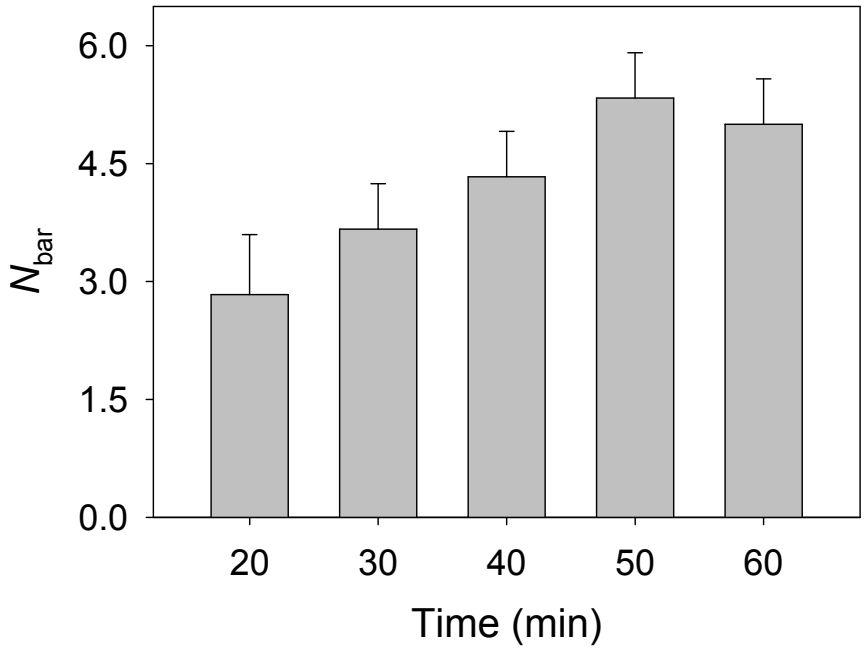

Fig. S6 Time optimization for the incubation of the SMP-PNP complexes and the GNPs. 50 min was chosen as the optimal incubation time as it gave the highest signal. Each error bar represents a standard deviation across three replicate experiments.

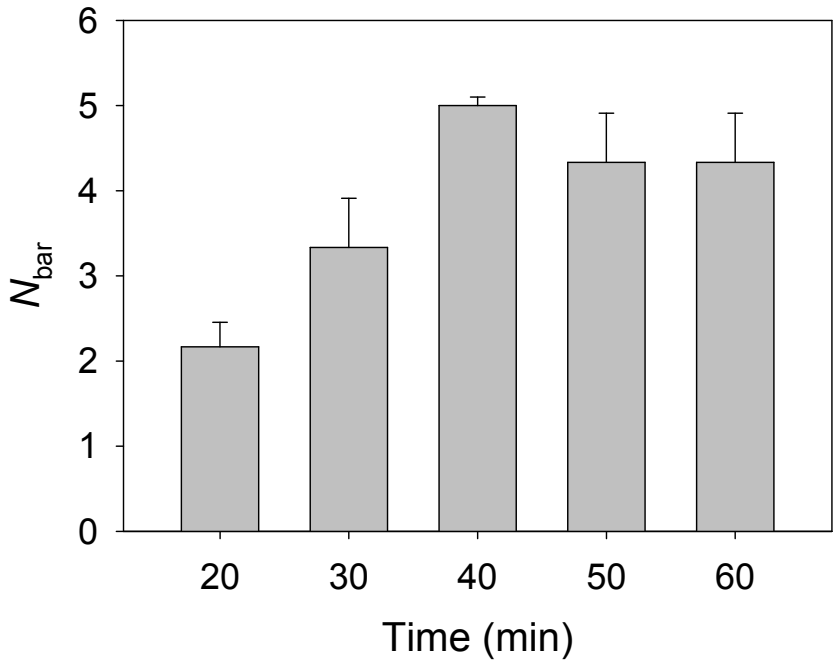

Fig. S7 Time optimization for the incubation of the SMP-PNP-GNP complexes and the cysteamine solution. 40 min was chosen as the optimal incubation time as it gave the highest signal. Each error bar represents a standard deviation across three replicate experiments.

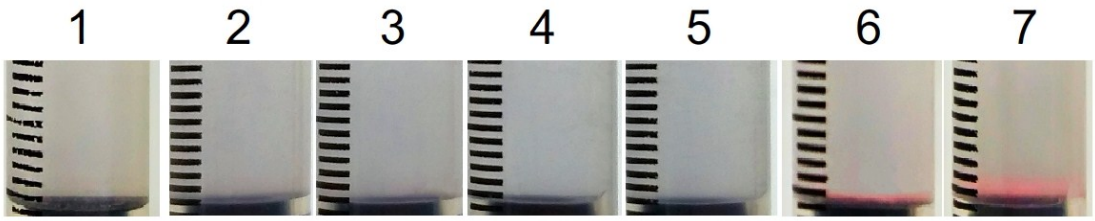

Fig. S8 Colorimetric results obtained from different adenosine samples in PBS buffer using the aptamer-based blue-red 2D GNP-LPCA method: 1) 4.8, 2) 9.3, 3) 9.4, 4) 9.5, 5) 9.6, 6) 9.7, and 7) 39 nM. The lowest level that was capable of producing a top-bottom blue-red reaction mixture (image 6), i.e., 9.7 nM, was estimated to be the visual limit of detection for the adenosine.

Table S1 Comparison of the new aptamer-based adenosine assay and several existing quantitative aptamer-based adenosine assays

quantitative strategy	limit of detection (nM)	portable	cost	Ref.
Raman scattering	1	no	very high	1
Raman scattering	1.5	no	very high	2
fluorescence	200	no	very high	3
fluorescence	101	no	very high	4
fluorescence	1000	no	very high	5
fluorescence	9.3	no	very high	6
fluorescence	0.75	no	very high	7
electrochemistry	2.5	no	high	8
electrochemistry	0.75	no	high	9
equipment-free	9.7	yes	very low	This work

References:

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Table S2 Recvery of adenosine in human serum samples

sample	found[a] (μM)	added (μM)	total[a] (μM)	recovery (%)	RSD[b] (%)
1	0.041	0.5	0.57	105.4	8.8
2	0.045	2.0	1.88	91.9	7.4
3	0.043	8.0	7.30	90.8	11.1

[a]The "found" and "total" concentrations were calculated from the corresponding number of the red bars on the test tubes measured before and after the additions of adenosine solutions at fixed concentrations in the human serum samples and the regression equation: $y(N_{bar}) = 1.1386x(\text{Log[adenosine]}) + 3.5200$ ($R = 0.9948$); [b]The relative standard deviations (RSDs) were obtained from six repetitive experiments.