Supporting Information

Selective sensing of adenosine monophosphate (AMP) by a calix[6]triazolium-based colorimetric sensing ensemble

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Contents

1. ^{1}H NMR studies

A ^{1}H NMR study was also conducted to confirm the complexation of **BM** with calix[6]triazole composed only of triazole instead of triazolium (Fig. S1). Unlike **CT6**, when calix[6]triazole was gradually added to **BM**, the ^{1}H NMR spectrum of **BM** showed a slight downfield shift for all protons ($H_{a'}$–$H_{f'}$) of **BM** compared to that of calix[6]triazolium. All protons of **BM** ($H_{a'}$–$H_{f'}$) shifted downfield by 0.09 and 0.55 ppm on average in the presence of calix[6]triazole and **CT6**, respectively. This indirectly shows that the interaction with triazolium plays an important role in the formation of the **CT6**/**BM** complex.

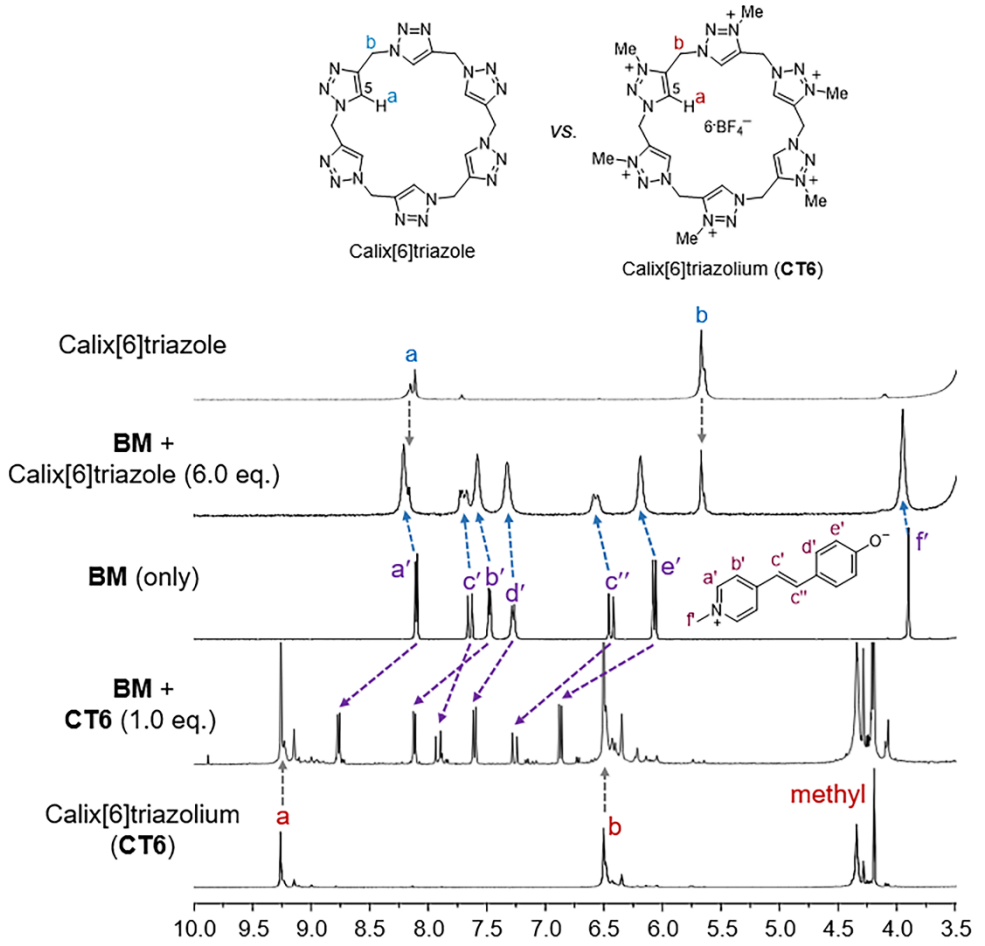

Fig. S1 ^{1}H NMR spectra of **BM** (1.5 mM) in DMSO-d_6 recorded in the presence of increasing concentrations of calix[6]triazole and **CT6**.

2. Complexation studies between CT6 and BM

(1) UV-Vis titration of **BM** with **CT6**: Stock solutions of **CT6** (10 mM) and **BM** (100 μM) in DMSO:-H_2O (5:1) solution were prepared separately. Three milliliters of **BM** solution was transferred to a cuvette, and an initial spectrum was taken. Aliquots of the **CT6** solution (0–15 μL) were added to the cuvette, and spectra were recorded after each addition. The binding constant was analyzed by BindFit, plotting UV-Vis absorption values at 260 nm against equivalents of the **CT6** added. The graph of the UV-Vis titration of **BM** using **CT6** is shown in Fig. 2 in the manuscript.

(2) Job plot for the binding stoichiometric ratio between **CT6** and **BM**: Stock solutions of equal concentrations of **CT6** (100 μM) and **BM** (100 μM) in DMSO:H_2O (5:1) solution were prepared separately. Ten vials were each filled with 10 mL of **CT6** and **BM** solution in the following ratios (**CT6**:**BM**): 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8 and 1:9. A Job plot was constructed by plotting the change in UV-Vis absorption of **BM** at 260 nm against the molar fraction of the host.

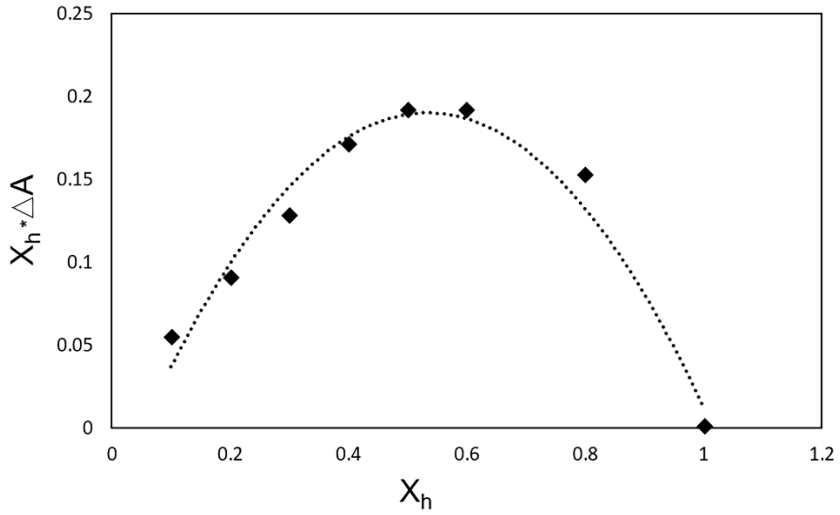

Fig. S2 Job plot generated from the UV-Vis titration data of **BM** with **CT6** in DMSO:H_2O (5:1) solution.

(3) Association constant calculated by BindFit

Fig. S3 Screenshot of the summary window of http://app.supramolecular.org/bindfit/. This screenshot shows the raw data for the UV-Vis titration of **BM** with **CT6** following the UV-Vis absorption values at 260 nm vs. the data fitted to the 1:1 UV binding model, the corresponding residual plot and the association constants with the calculated asymptotic standard errors.

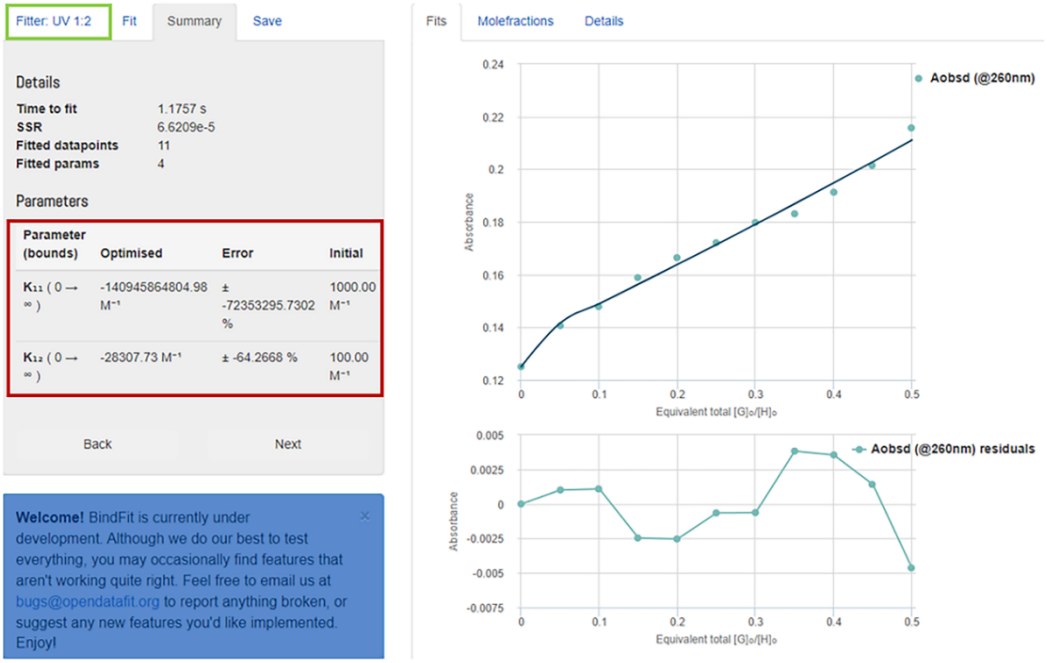

Fig. S4 Screenshot of the summary window of http://app.supramolecular.org/bindfit/. This screenshot shows the raw data for the UV-Vis titration of **BM** with **CT6** following the UV-Vis absorption values at 260 nm vs. the data fitted to the 1:2 UV binding model, the corresponding residual plot and the association constants with the calculated asymptotic standard errors.

Fig. S5 Screenshot of the summary window of http://app.supramolecular.org/bindfit/. This screenshot shows the raw data for the UV-Vis titration of **BM** with **CT6** following the UV-Vis absorption values at 260 nm vs. the data fitted to the 2:1 UV binding model, the corresponding residual plot and the association constants with the calculated asymptotic standard errors.

Table S1 Summary of association constants between **CT6** and **BM** according to different binding models.[a]

Binding models		
1:1	1:2	2:1
40402.24 (±12.99%)	K_{11} -140945864804.98 (±-72353295.73%) K_{12} -28307.73 (±-64.27%)	K_{11} 17.05 (±136.67%) K_{21} 31224538.44 (±312.77%)

[a]BindFit software from *supramolecular.org* was used for data analysis.

3. Indicator displacement of the CT6/BM complex with AMP

(1) UV-Vis titration spectra of **CT6/BM** complex upon addition of AMP

UV-Vis titration of the **CT6**/**BM** complex with AMP: Stock solutions of **CT6** (10 mM), **BM** (100 μM) and AMP (10 mM) in DMSO:H_2O (5:1) solution were prepared separately. An aliquot of 3 mL of **BM** solution was transferred to a cuvette to take an initial spectrum, and the minimum amount of **CT6** solution was added for complete color change of **BM**. Aliquots of each AMP solution (0–70 μL) were added to the cuvette, and spectra was recorded after each addition. The binding constant was analyzed by BindFit, plotting UV-Vis absorption values at 260 nm against equivalents of the AMP added. The graph of the indicator displacement of **CT6/BM** complex with AMP is shown in Fig. 5 in the manuscript.

(2) Association constant calculated by BindFit

Fig. S6 Screenshot of the summary window of http://app.supramolecular.org/bindfit/. This screenshot shows the raw data for the UV-Vis titration of the **CT6/BM** complex with AMP following the UV-Vis absorption values at 260 nm vs. the data fitted to the 1:1 UV binding model, the corresponding residual plot and the association constants with the calculated asymptotic standard errors.

Fig. S7 Screenshot of the summary window of http://app.supramolecular.org/bindfit/. This screenshot shows the raw data for the UV-Vis titration of the **CT6/BM** complex with AMP following the UV-Vis absorption values at 260 nm vs. the data fitted to the 1:2 UV binding model, the corresponding residual plot and the association constants with the calculated asymptotic standard errors.

Fig. S8 Screenshot of the summary window of http://app.supramolecular.org/bindfit/. This screenshot shows the raw data for the UV-Vis titration of the **CT6/BM** complex with AMP following the UV-Vis absorption values at 260 nm vs. the data fitted to the 2:1 UV binding model, the corresponding residual plot and the association constants with the calculated asymptotic standard errors.

Table S2 Summary of association constants between the **CT6/BM** complex and AMP according to different binding models.[a]

Binding models		
1:1	1:2	2:1
2808.94 (±1.93%)	K_{11} -1913.67 (±-0.71%) K_{12} -541.39 (±-1.18%)	K_{11} -1178.97 (±-1.00%) K_{21} -201.43 (±-0.34%)

[a]BindFit software from *supramolecular.org* was used for data analysis.

4. Detection limit measurements

The detection limit of AMP by the **CT6**/**BM** complex (100 μM) in DMSO:H_2O (5:1) solution was determined using the equation LOD = 3σ/K, where σ denotes the standard deviation of blank solution (without analyte) and K is the slope between UV absorbance intensity and AMP concentration. σ was obtained from the results of 10 separate measurements.

Receptor	Slope, K	R^2	Standard deviation, σ	Limit of detection (LOD) (μM)
CT6/**BM** complex	0.01	0.9236	0.0000963	0.03

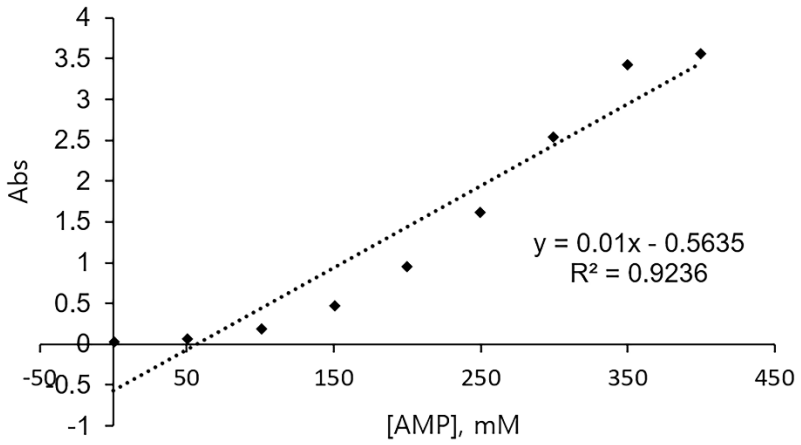

Fig. S9 Calibration curve for the **CT6**/**BM** complex (100 μM) in DMSO:H_2O (5:1) solution. The UV absorbance intensities of the **CT6**/**BM** complex were recorded at 530 nm.

5. ^{1}H NMR titration and 2D NOESY NMR studies

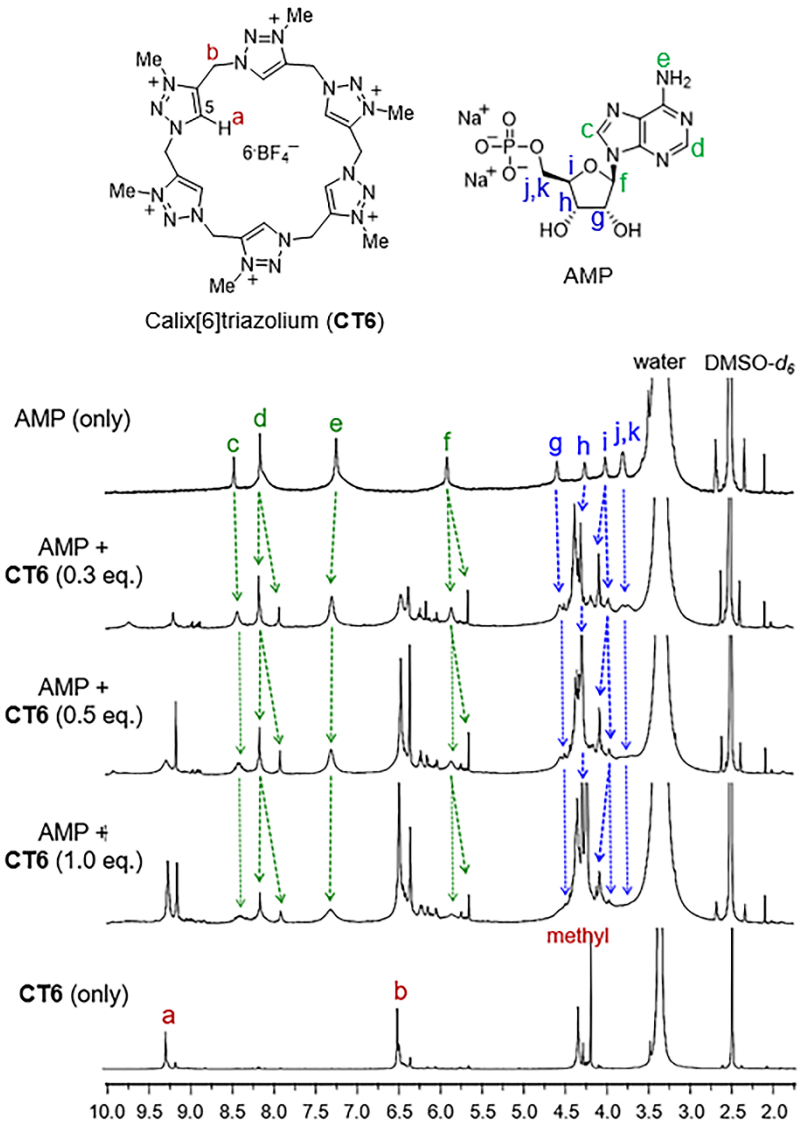

Fig. S10 ^{1}H NMR spectra of AMP (4 mM) in DMSO-d_6 recorded in the presence of increasing concentrations of **CT6**.

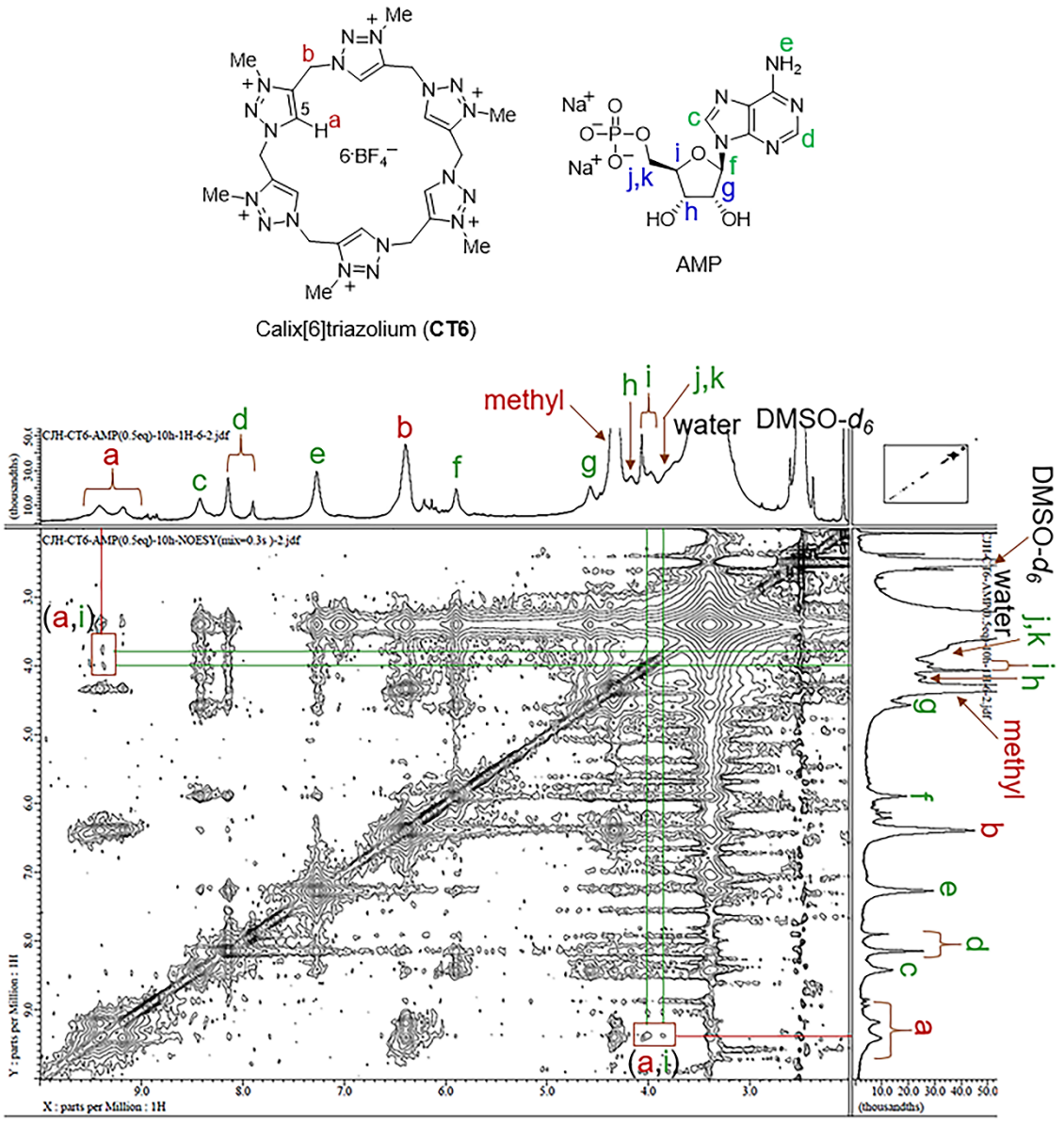

Fig. S11 2D NOESY NMR (600 MHz, DMSO-d_6, 298 K, mixing time = 300 ms) spectrum of AMP (4 mM) with 0.5 equiv. of **CT6**.

6. Competition study

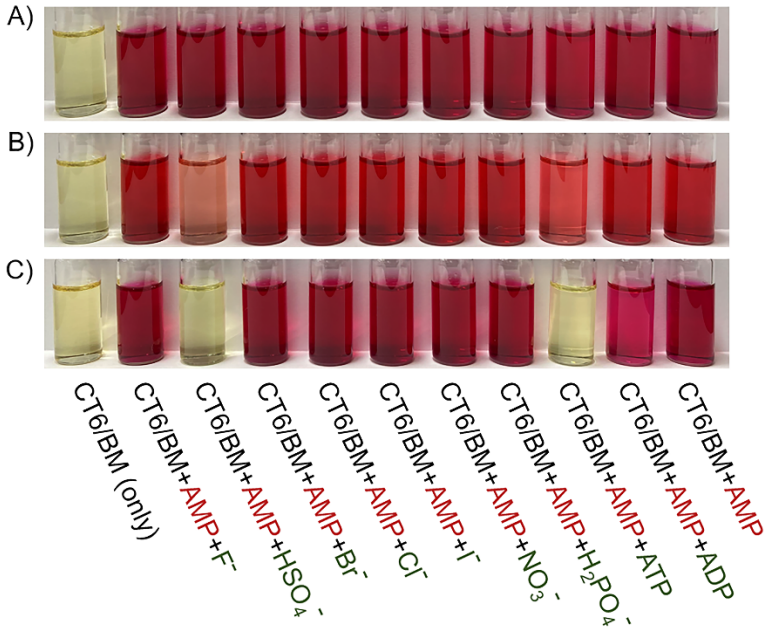

Fig. S12 Changes in the color of the **CT6/BM** complex (1 mM) in the presence of varying concentrations ((a) 10, (b) 20 and (c) 30 equiv.) of interfering anions with and without AMP (30 equiv.) in DMSO:H_2O (5:1) solution.

7. Paper-based colorimetric assay

(1) Fabrication of the paper-based device

Test papers were prepared by immersing ADVANTEC® paper discs into a DMSO:H_2O (5:1) solution of a mixture of **CT6** (50 μM) and **BM** (100 μM) for approximately 5 minutes. After removal from the **CT6**/**BM** complex solution and drying under vacuum, the paper discs were directly applied for the determination of AMP.

(2) Smartphone-based colorimetric analysis

Images of the colors from the paper disc were taken with a smartphone (iPhone 11) under laboratory lights. The acquired images were imported into ImageJ software (US National Institutes of Health, http://imagej.nih.gov/ij/) for quantitative analysis. ImageJ software cannot directly measure magenta color intensity values. Therefore, magenta color intensity values were quantified from an inverted green channel image based on the fact that magenta and green colors are complementary to each other.[S1] A calibration curve (Fig. 7d) was constructed using the quantified magenta color intensity values. Based on this, the AMP concentration used in the mixed sample was determined (Table S3).

(3) Determination of AMP in artificially mixed sample

Table S3 Determination of AMP in artificially mixed sample

Sample	AMP added (mM)	Measured color intensity[a]	SD[b]
Artificial sample[c]	2.7	60.696	10.69

[a]The images of paper discs acquired by the smartphone (iPhone 11) were processed using ImageJ software. [b]Standard deviation of three independent measurements. [c]Prepared by DMSO:H_2O (5:1) solution, 2.7 mM: F^-, Cl^-, Br^-, I^- and AMP.

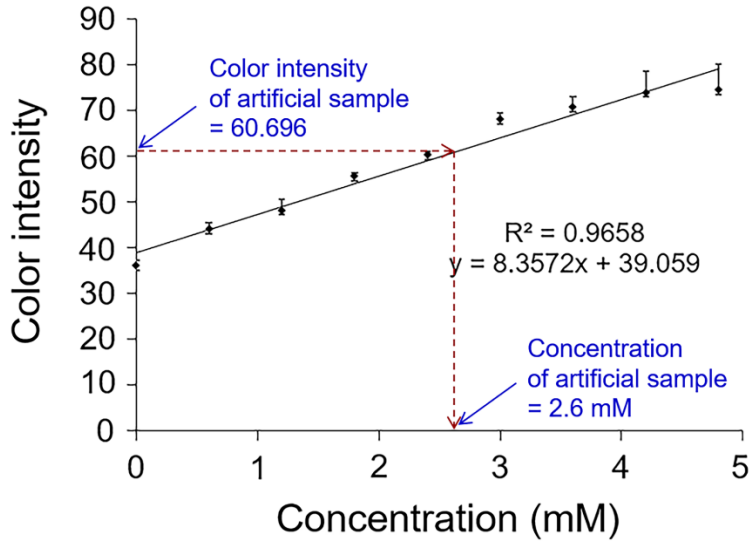

Fig. S13 Calibration curve for the determination of AMP concentration in artificially mixed sample.

8. References

S1 K. Yamada, D. Citterio and C. S. Henry, “Dip-and-read” paper-based analytical devices using distance-based detection with color screening, *Lab Chip*, 2018, **18**, 1485–1493.