## Supporting information

## A Semi-Dry Chemistry Hydrogel-Based Smart Biosensing Platform for On-Site Detection of Metal Ions

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Figure S1Schematic illustration of the preparation process of DNA incorporated<br/>agarosehydrogelandhydrogel-basedanalysis.



Figure S2 Manual analysis process by ImageJ software.



**Figure S3** Feasibility of the fluorescent detection of K<sup>+</sup>. (A) Fluorescence spectra of 100 mM Tris-HCl buffer solutions containing different reagents: NMM, NMM + DNA probe, and NMM + DNA probe + K<sup>+</sup>, respectively (left part). And corresponding photographic images under UV light (right part). The concentration of NMM, DNA probe, and K<sup>+</sup> were 50  $\mu$ M, 1.5  $\mu$ M, and 0.25 mM, respectively. (B) Selectivity verification of K<sup>+</sup> detection. (C) Fluorescence spectra in the detection of different concentrations of K<sup>+</sup>. (D) Linear relationship of the fluorescence intensity at 620 nm and the concentration of K<sup>+</sup>. The inset shows the fluorescence intensity in the presence of different concentrations of K<sup>+</sup>.



**Figure S4** The stability of fluorescence signal of the reaction solution. 5 mM K<sup>+</sup>, 1.5  $\mu$ M DNA probe, and 50  $\mu$ M NMM were reacted in 100 mM Tri-HCl (pH = 7.4) at 25 °C for 120 min.



Figure S5 Effect of the concentration of agar on the fluorescence intensity. 5 mM K<sup>+</sup>, 2  $\mu$ M DNA probe, and 50  $\mu$ M NMM were reacted in 100 mM Tri-HCl at 25 °C for 120 min.



**Figure S6** Effect of the concentration of buffer solution on the fluorescence intensity. 5 mM K<sup>+</sup>, 2  $\mu$ M DNA probe, and 50  $\mu$ M NMM were reacted at 25 °C for 90 min.



Figure S7 Effect of the pH values of buffer solution on the fluorescence intensity. 5 mM K<sup>+</sup>, 2  $\mu$ M DNA probe, and 50  $\mu$ M NMM were reacted in 200 mM Tri-HCl at 25 °C for 90 min.



**Figure S8** Effect of the concentration of DNA probe on the fluorescence intensity. 5 mM K<sup>+</sup> and 50  $\mu$ M NMM were reacted in 200 mM Tri-HCl (pH = 7.4) at 25 °C for 90 min.



Figure S9 Linear relationship between the fluorescence intensity and the concentration of  $K^+$ . Data was acquired by manual analysis based on ImageJ software.



**Figure S10** Comparison of the fluorescent detection of Na<sup>+</sup> and K<sup>+</sup> in solution. (A) Fluorescence spectra of 100 mM Tris-HCl buffer solutions containing different reagents: NMM, NMM + DNA probe, NMM + DNA probe + Na<sup>+</sup>, and NMM + DNA probe + K<sup>+</sup>, respectively (left part), and corresponding photographic images under UV light (right part). The concentration of NMM, DNA probe, Na<sup>+</sup> and K<sup>+</sup> were 50  $\mu$ M, 1.5  $\mu$ M, 0.25 mM, and 0.25 mM, respectively.



**Figure S11** Feasibility of the fluorescent detection of  $Hg^{2+}$  based on  $Hg^{2+}$ -sensitive DNA probe. (A) Photographic images of the detection of different concentrations of  $Hg^{2+}$  under UV light. (B) Fluorescence spectra in the detection of different concentrations of  $Hg^{2+}$ . (C) Linear relationship of the fluorescence intensity at 520 nm and the concentration of  $Hg^{2+}$ . The inset shows the allosteric switch of the DNA probe in the presence of  $Hg^{2+}$ .



Figure S12 Linear relationship between the fluorescence intensity and the concentration of  $Hg^{2+}$ . Data was acquired by manual analysis based on ImageJ software.

Method	Limit of detection	Linear range	Reference
Pyrene-labeled G-quadruplex oligonucleotide	1 mM	$2 \text{ mM} \sim 10 \text{ mM}$	[1]
DNA/aptamer-based optical biosensors	0.4 mM	$0.6 \text{ mM} \sim 20 \text{ mM}$	[2]
G-quadruplex-specific fluorescent probe	0.5 mM	$2 \text{ mM} \sim 20 \text{ mM}$	[3]
DNA hydrogel-based plate	0.34 mM	$1 \text{ mM} \sim 40 \text{ mM}$	This work

Table S1 The comparison of detection performances of our method and other methods in the detection of  $K^+$ .

Table S2 The comparison of detection performances of our method and other methods in the detection of  $\rm Hg^{2+}.$ 

Method	Limit of detection	Linear range	Reference
Carbon quantum dots/3- aminophenylboronic acid Hybrid	38.1 nM	$0.1~\mu M \sim 6.0~\mu M$	[4]
Cellulose nanofiber substrate- supported luminescent gold nanoparticles	1 nM	1 nM ~ 1 mM	[5]
Hollow AuAg nanocages	10 nM	$30~nM\sim35~\mu M$	[6]
Mercury-stimulated Ag <sub>3</sub> PO <sub>4</sub> microcubes	20 nM	$0.1~\mu M \sim 7~\mu M$	[7]
DNA hydrogel-based plate	5.6 nM	$10~nM\sim 2.5~\mu M$	This work

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