Portable "dual-signal-on" colorimetric and photothermal biosensor for accurate detection of Hg^{2+} based on split Cas12a-mediated cascade strand displacement and phosphorothioate-modified G-quadruplex DNAzyme

Yi Tian [a, b], Jiaxuan Chen [a, b], Fengzheng Chen [a, b], Yu Ling [a], Shengyue Zheng [a], Daizhen Jian [a], Qiong Tang [a*], Song Wang [c*], Kai Shi [a, b*]

[a]College of New Energy Materials and Chemistry, Leshan Normal University, Leshan, Sichuan 614000, P. R. China

[b]Sichuan Province Key Laboratory of Natural Products and Small Molecule Synthesis, Leshan, Sichuan 614000, P. R. China

[c]Yangtze Laboratory, Wuhan 430205, P. R. China.

* Corresponding authors.

E-mail address: tangqiong75@163.com, Aden_wong@yangtzelabs.ac.cn, shikai9901@163.com

1. UV-vis spectroscopy and fluorescence measurements

For fluorescence measurement, 5 μL of the sRNA/SS (5 μmol/L) was added to a 45 μL of NEB2.1 buffer containing 55.6 nmol/L Cas12a/hRNA complex and 3 μmol/L FQ-reporter, and react at 37℃ for 30 min. The fluorescence signals of all samples were measured within the range of 500 nm to 675 nm using an edge light F98 fluorescence spectrophotometer (Shanghai, China). 485 nm was used as the excitation wavelength, and the fluorescence emission intensity at 520 nm was detected.

For UV-vis spectroscopy measurement, 1 μL of Hg^{2+} with different concentrations (100 fM-10 nM), 1.0 μmol/L PS/ SS and 1.0 μmol/L sRNA probe were reacted at 37°C for 90 min in 10 mmol/L Tris-HCl buffer (12.5 mmol/L $MgCl_2$, pH 8.0) to produce sRNA/SS. Then, 1 μL of the above solution was added to a NEB2.1 buffer containing 50 nmol/L Cas12a/hRNA complex and 200 nmol/L psHG4 probe, and react at 37℃ for 30 min. Subsequently, adding 58 μL of 20 mmol/L Tris HCl buffer (pH 7.0, Triton X-100 (0.01%), 150 mmol/L NaCl, DMSO (1%), 20 mmol/L KCl) and 1 μL hemin at 25℃ to generate a psG4/hemin complex. After 30 min, TMB, H_2O_2, and phosphate buffer (0.2 mol/L, pH=5.4) were added to the above mixture and reacted at 25℃ for 5 min. All absorbance data were adopted on a TU-1901 UV–vis spectrophotometer (PERSEE, Beijing, China).

2. Supplemental Table

Table S1 Comparisons between different methods for Hg^{2+} detection.

Methods	Strategy	Linear range	Sensitivity	Portable	Ref
Fluorescence	Cas12a	0.05-200 nM	23 pM	No	29
Glucometer	CSDR and Cas12a	100 fM-10 nM	40 fM	Yes	3
Fluorescence	PCR and Cas12a	-	10 pM	No	30
Colorimetric	Pyomelanin	4.9-1250 nM	1.2 nM	Yes	31
Colorimetric	Indigoidine	-	0.008 μM	Yes	32
Colorimetric	Deoxyviolacein	0.183-750 nM	0.183 nM	Yes	33
Colorimetric and photothermal	CSDR and SCas12a	1 pM-100 nM and 100 fM-10 nM	0.5 pM and 60 fM	Yes	This work

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3. Supplemental Figures

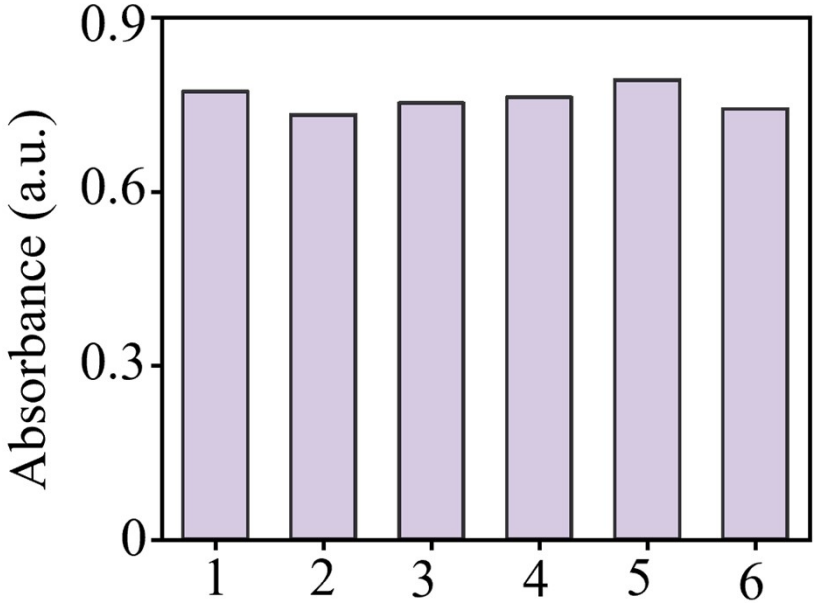

Fig. S1. Reproducibility of the SCas12a/CSDR-psHG4 system for Hg^{2+} detection.