UNIVERSITY OF NORTH DAKOTA





Ultrasensitive Hg^{2+} detection based on the T-Hg^{2+}-T base mismatch

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❖Mercury is a notoriously **toxic** element: accumulate in vital organs and tissues.

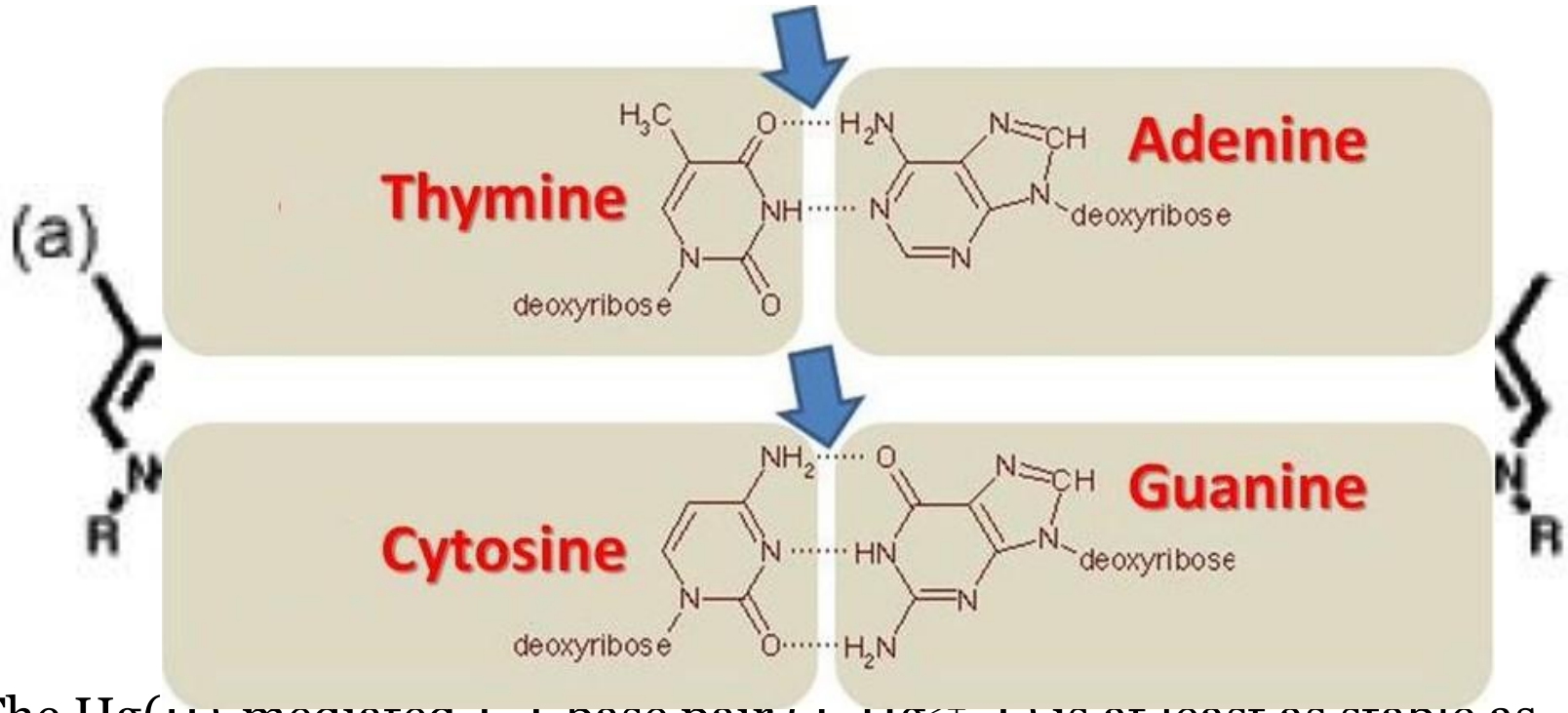
❖ Total mercury released into the environment reaches to **7500 tons** per year.

Since **1990**, the North Dakota Department of Health has obtained mercury data for many fish species found in the state's lakes and rivers.

- The maximum allowable level of mercury in drinking water is **10 nM**.
- Current applied instruments are **expensive and time-consuming**, such as AAS/AES, ICP-MS.
- It's extremely desirable to develop a **highly sensitive, selective, and practical sensor** to monitor mercury pollution.

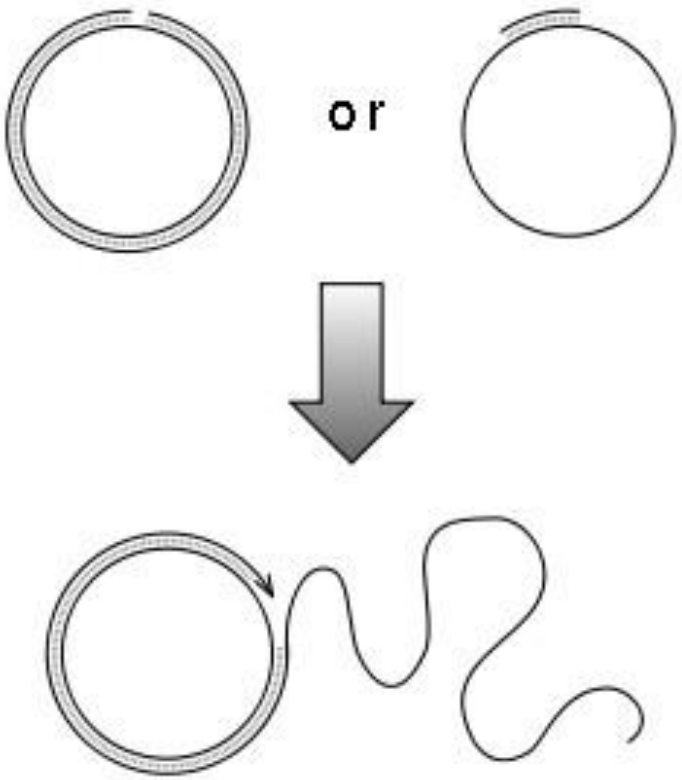
➡ Let DNA do the Hg^{2+} detection job!

The Formation of T-Hg^{2+}-T Mismatch



• The Hg(II)-mediated T-T base pair (T-Hg^{2+}-T) is at least as stable as normal Watson-Crick base pairs.

Rolling Circle Amplification (RCA)

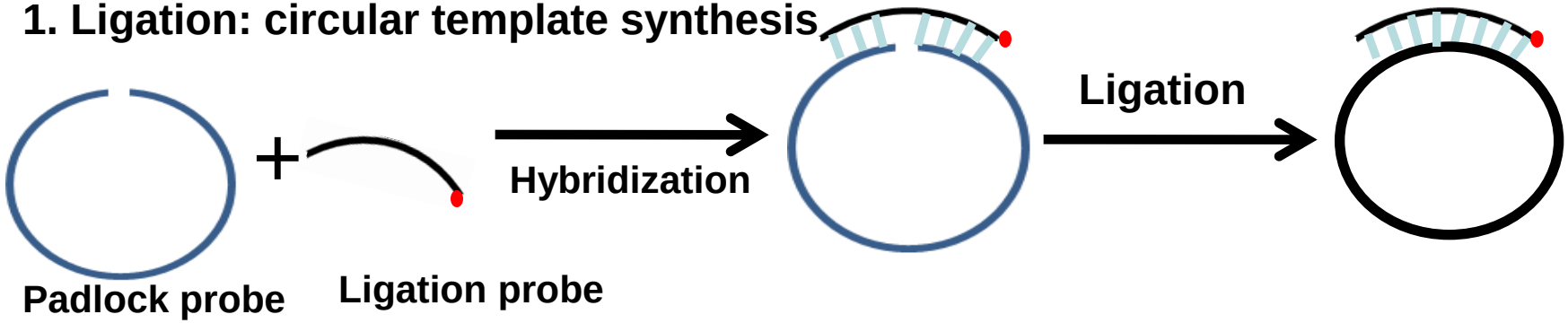


- RCA is a simple enzymatic process that can generate very long sing-strand DNA (ssDNA) with tandem repeats.

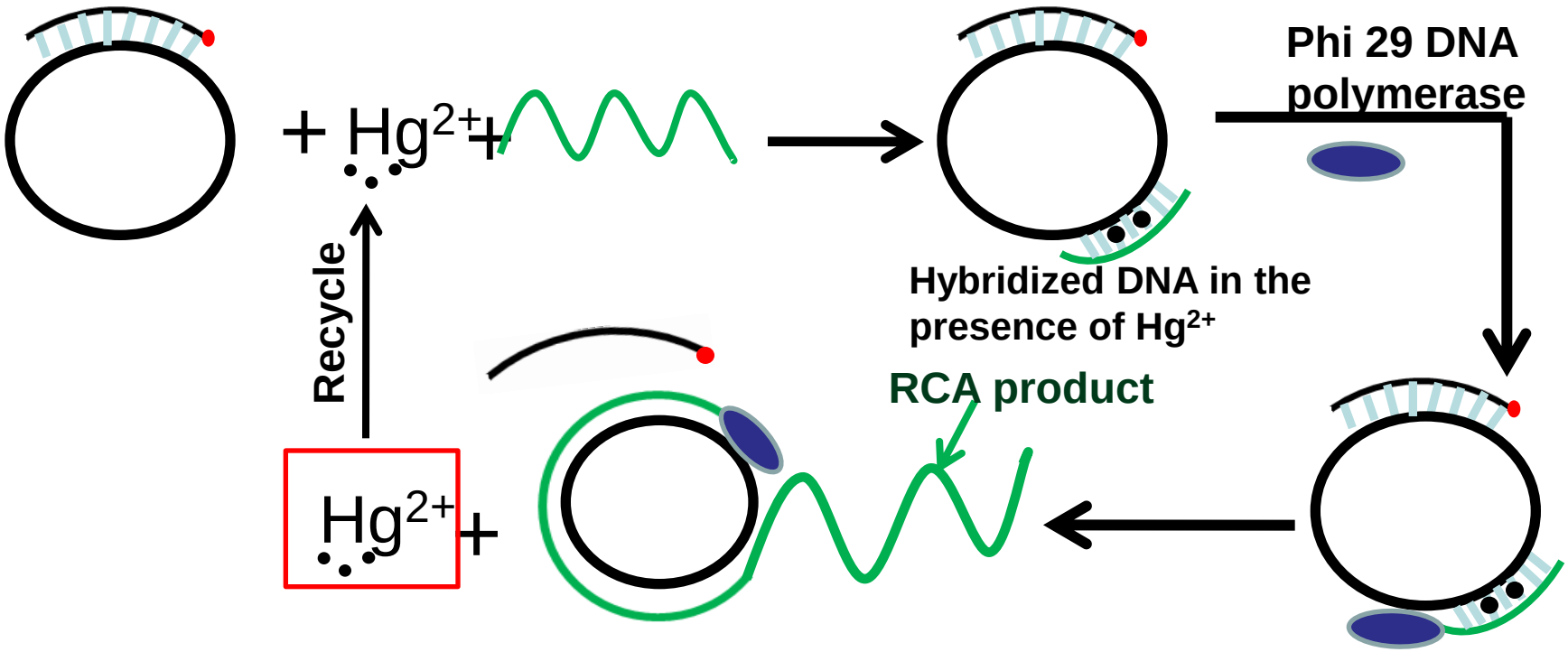
- A primer DNA first anneals to a circular DNA template.

- The added DNA polymerase extends the primer continuously around the circular DNA generating a long DNA product that consists of many repeated copies of the circle.

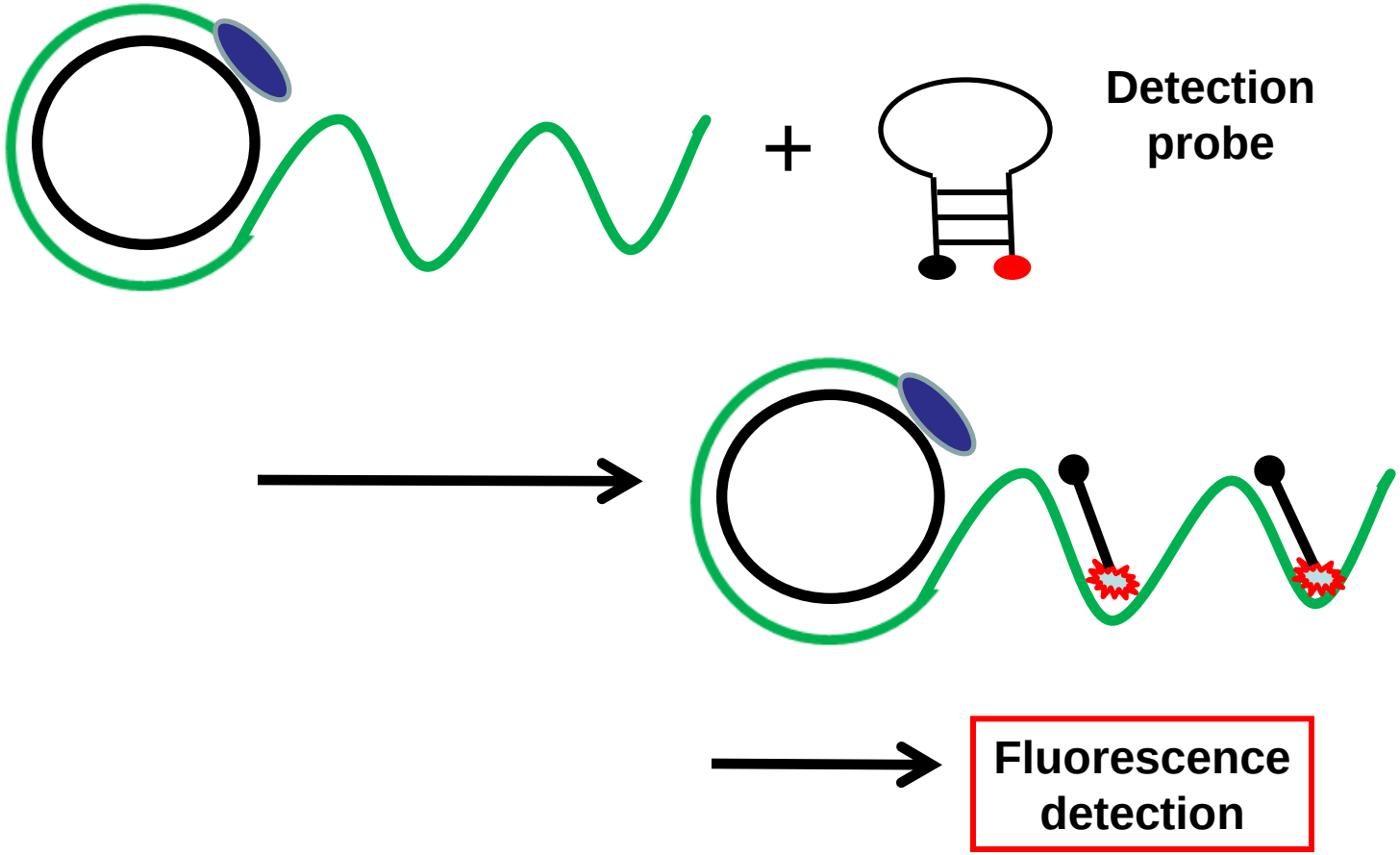
1. Ligation: circular template synthesis



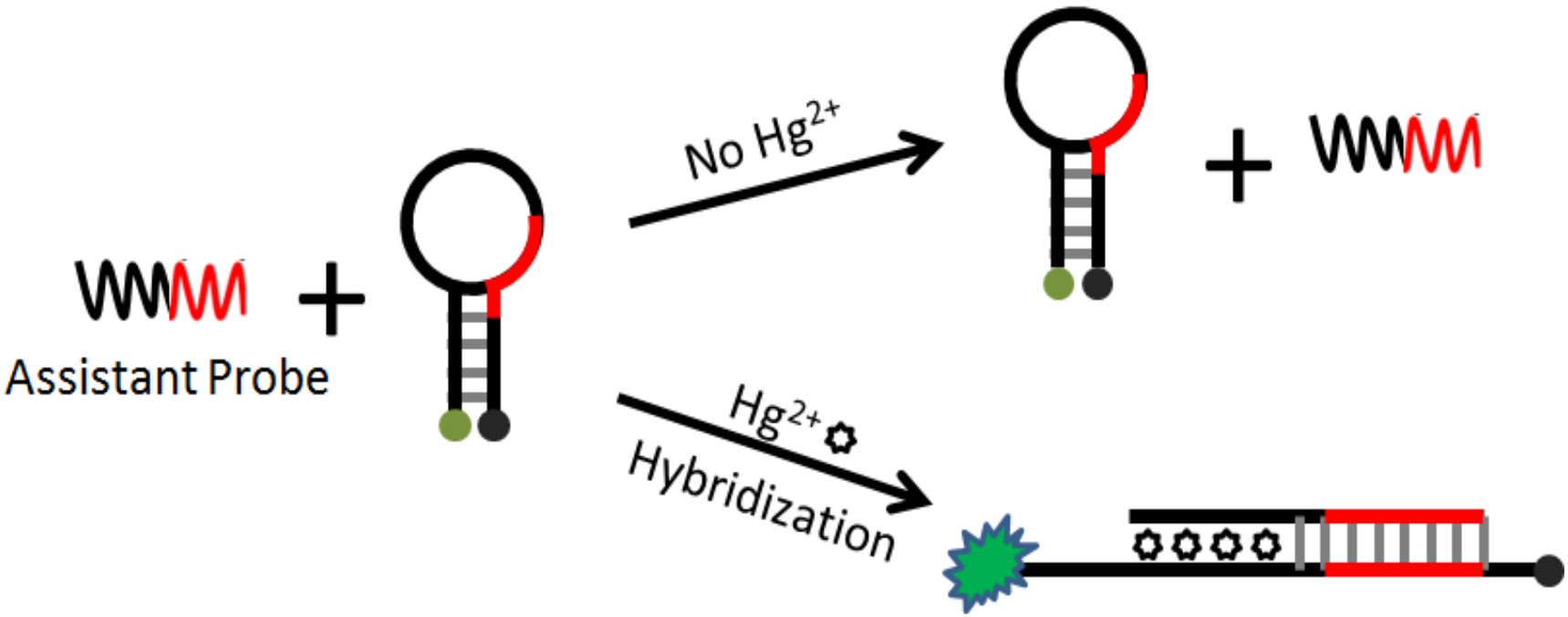
2. Rolling circle amplification



3. Signal detection



Proof of Concept Experiment



Sensing strategy of the Hg^{2+} detection using the molecular beacon.

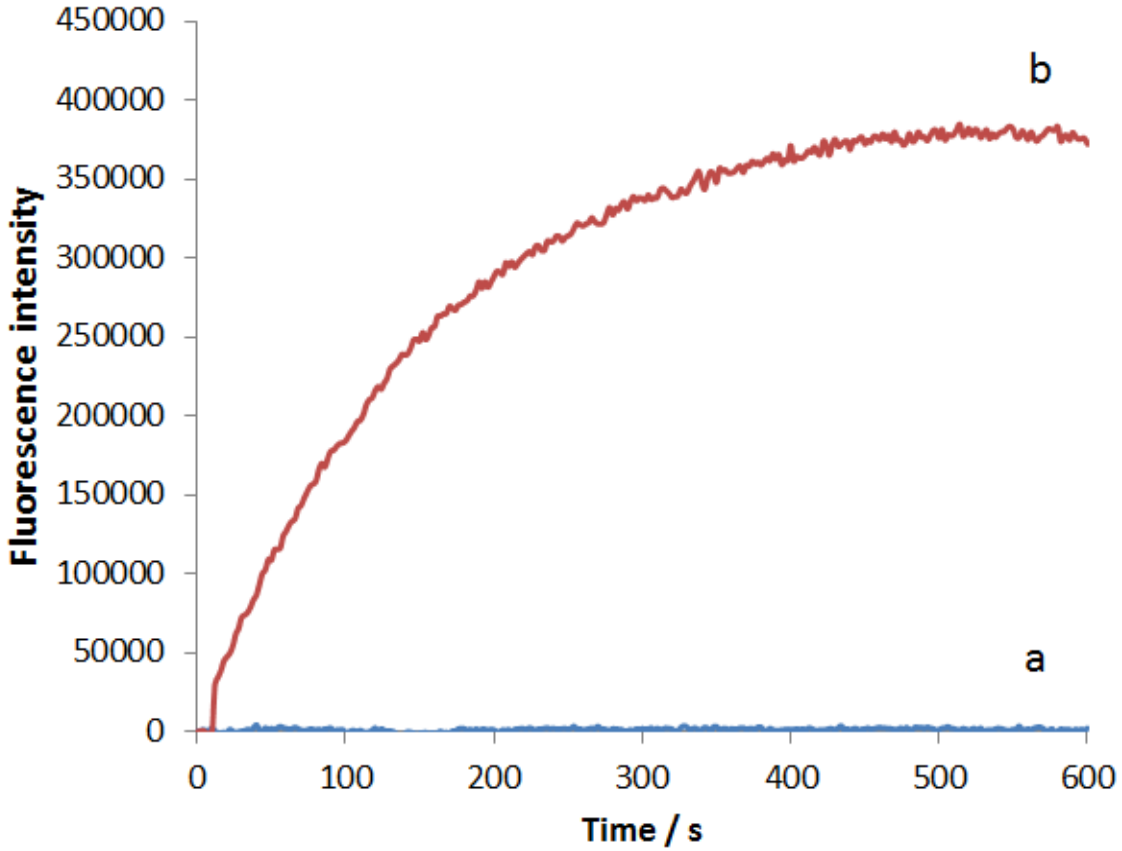


Figure 1. Fluorescence intensity of the sensor response to Hg^{2+} with the time. (a) the solution containing 10 nM MB and 200 nM assistant probe; (b) the addition of 300 nM Hg^{2+} into (a). Excitation: 480 nm, Emission:518 nm.

Optimization of sensor conditions

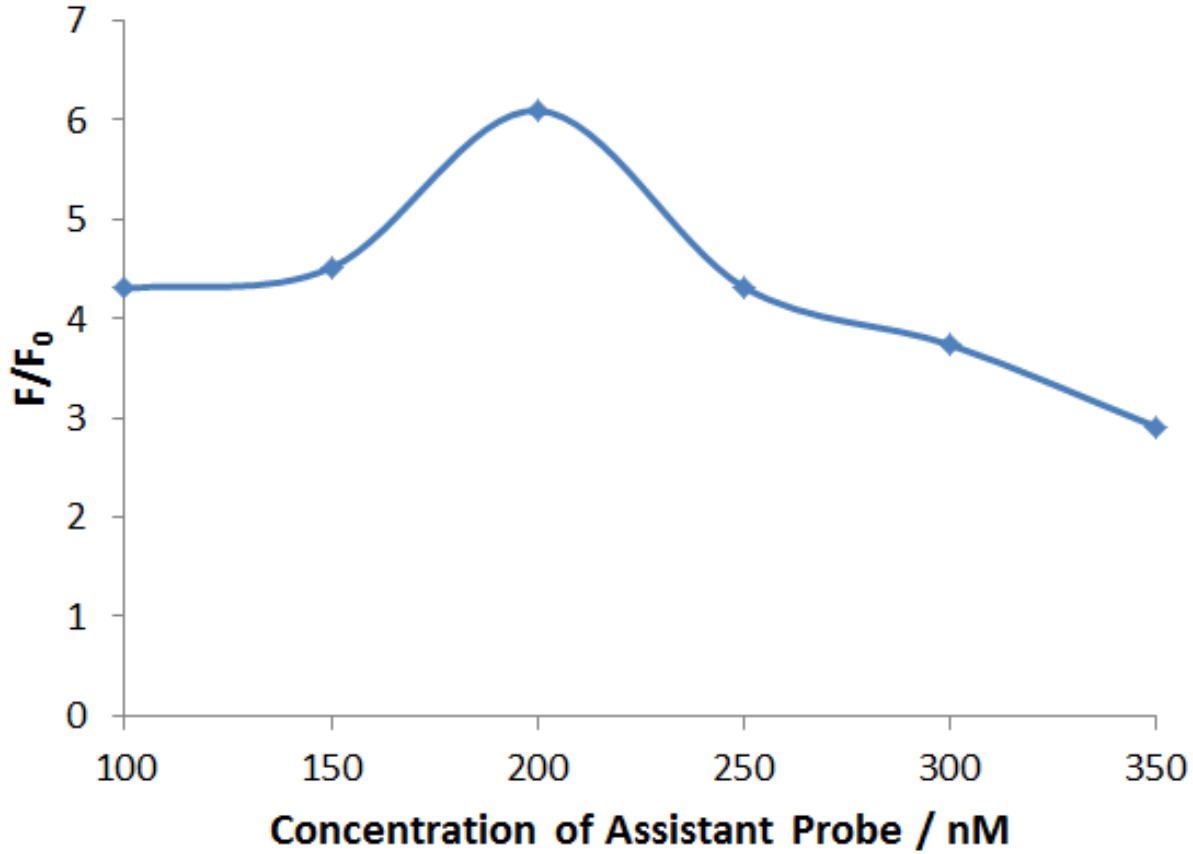


Figure 2. Fluorescence intensity of the sensor at different concentration of assistant probe. F: fluorescence intensity of the sensor in the presence of the 300 nM Hg^{2+}; F_0: fluorescence intensity of the sensor in the absence of the Hg^{2+}. MB: 10 nM.

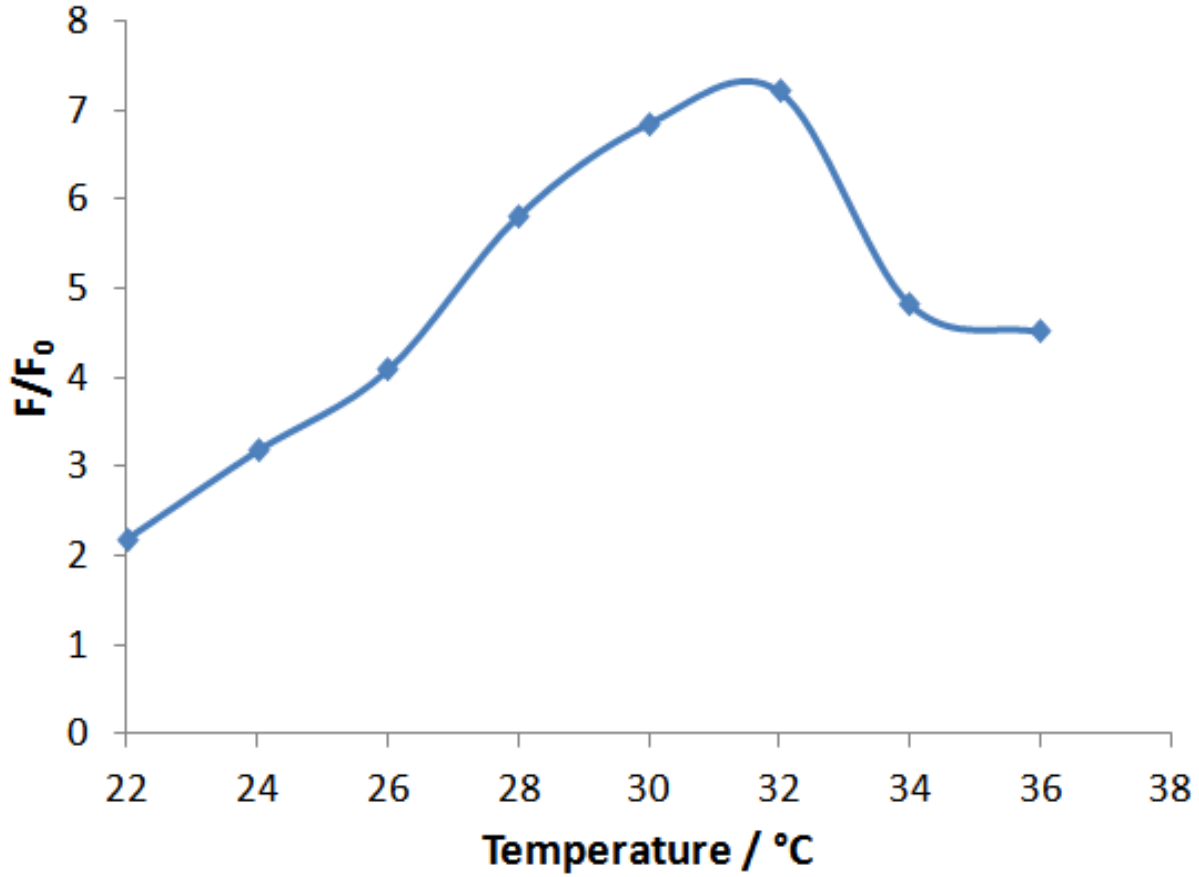


Figure 3. Fluorescence intensity of the sensor at different temperature. F: fluorescence intensity of the sensor in the presence of the 300 nM Hg^{2+}; F_0: fluorescence intensity of the sensor in the absence of the Hg^{2+}. MB: 10 nM; assistant probe: 200 nM.

Sensitivity investigation

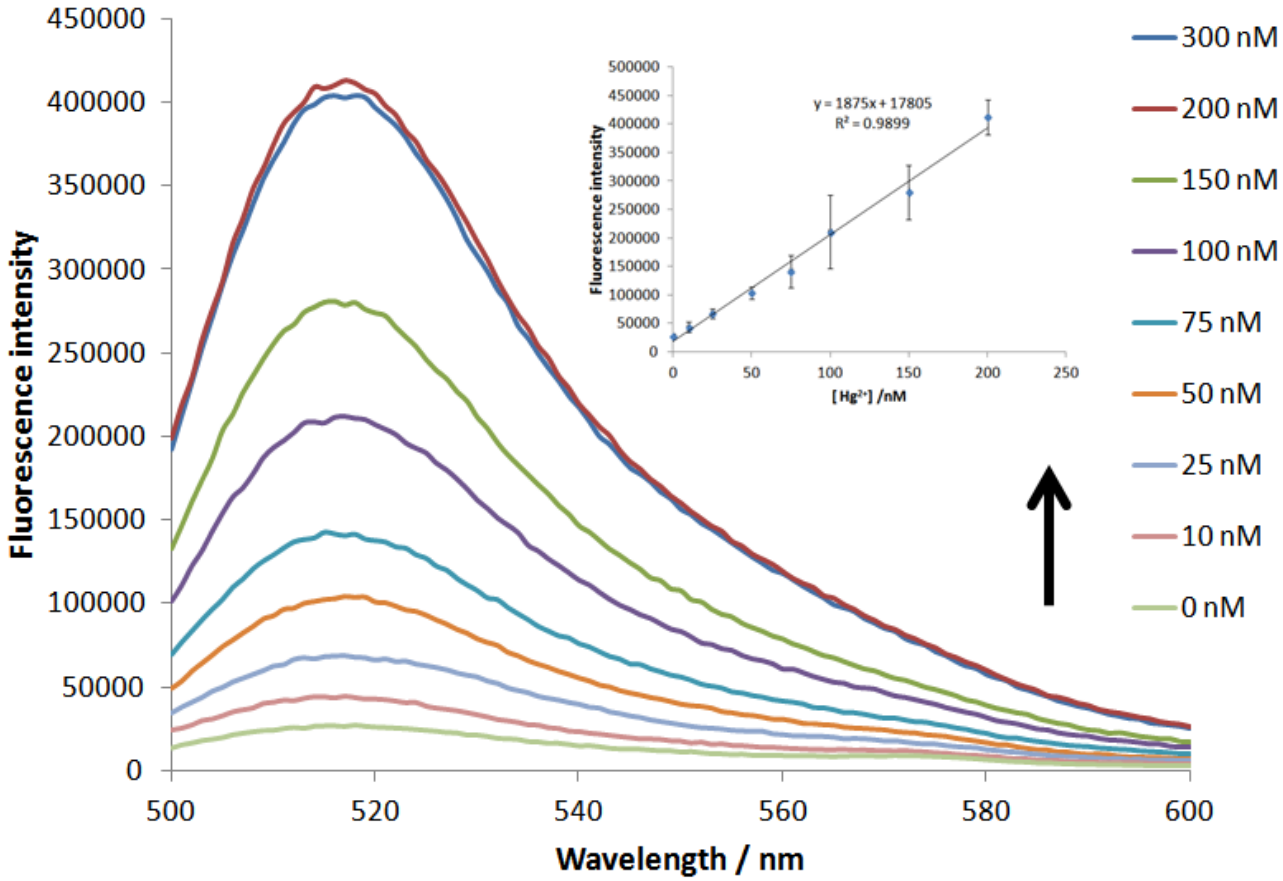


Figure 4. Changes of the fluorescence spectra of the sensor system with different concentrations of Hg^{2+}. The inset is the calibration curve of the sensor system to the detection of Hg^{2+}.

Selectivity investigation

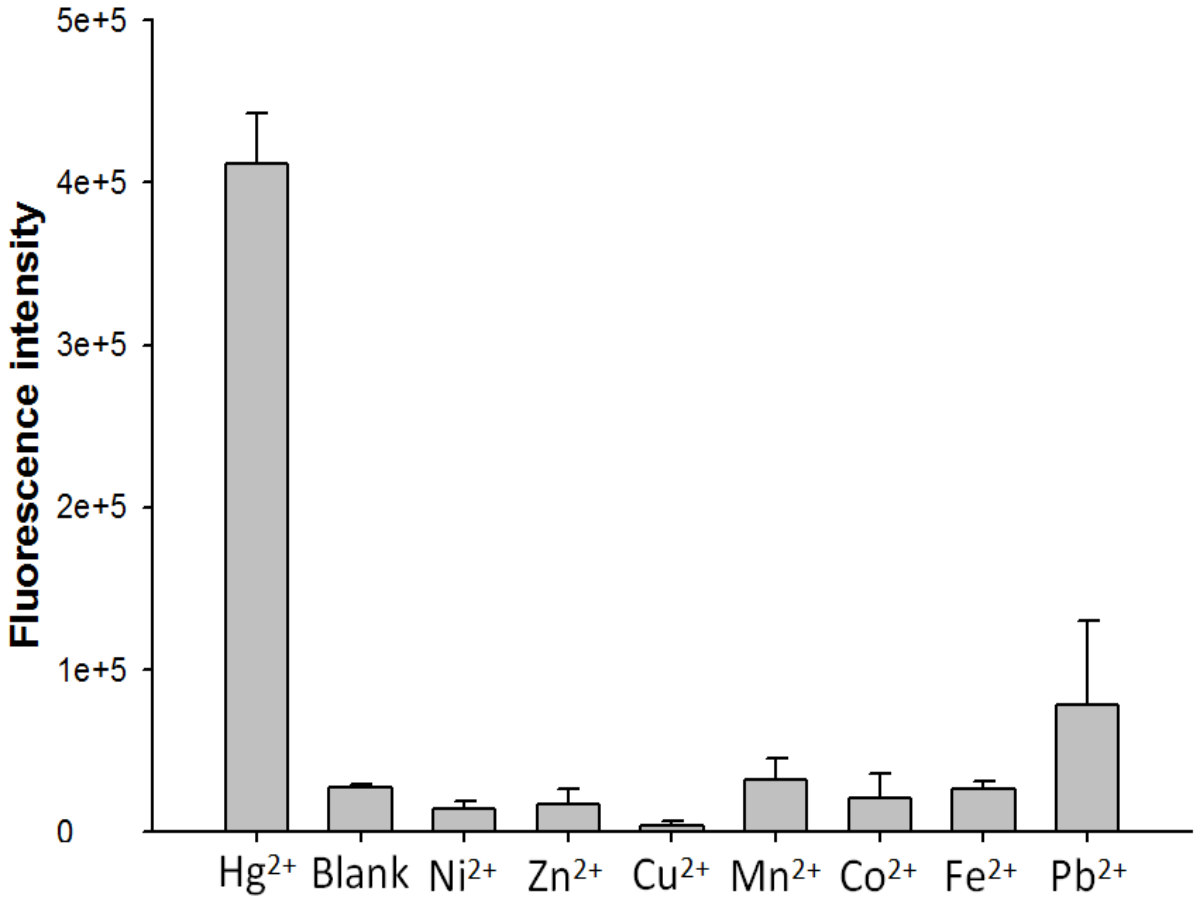


Figure 5. The fluorescence intensity of the sensor system with addition of other metal ions. Concentration of Hg^{2+}: 200 nM; concentration of other metal ions: 1000 nM.

Conclusions

• Molecular beacon/T-Hg^{2+}-T based Hg^{2+} sensor shows high selectivity and sensitivity (LOD = 8.3 nM).

• Detection limit can go down as low as 8.3 nM even without any signal magnification process.

• By applying the enzymatic process of RCA, a sensor with much lower LOD is expected.

Thank you!
Questions?