

Isolation of a DNA aptamer to develop the fluorescent aptasensor for pesticide thiamethoxam

Yu Luo^{1,2}, Ziyang Jin^{1,3}, Jine Wang¹, Pi Ding¹, Renjun Pei^{1,*}

¹ CAS Key Laboratory of Nano-Bio Interface, Suzhou Institute of Nano-Tech and Nano-Bionics, Chinese Academy of Sciences, Suzhou, 215123, China. E-mail: rjpei2011@sinano.ac.cn.

² Nano Science and Technology Institute, University of Science and Technology of China, Suzhou, 215123, China.

³Department of Biological Sciences, Xi'an Jiaotong-Liverpool University, Suzhou, 215123, China.

Table S1 Detailed sequences used in the SELEX procedure.

| Name | Sequence (5' to 3') |
|---------------|--|
| ssDNA library | GGAGGCTCTCGGGACGAC-(N)30- GTCCCGATGCTGCAATCGTAAGAAT |
| Bio-capture | GTCGTCCCGAGAGCCATA-biotin |
| P1 | GGAGGCTCTCGGGACGAC |
| P2 | ATTCTTACGATTGCAGCATCGGGAC |
| Bio-P2 | biotin-ATTCTTACGATTGCAGCATCGGGAC |

Table S2 SELEX round in details.

| Rounds | ssDNA (pmol) | Thiamethoxam (μM) | Incubation time (min) |
|--------|--------------|--------------------------------|-----------------------|
| 1 | 1000 | 200 | 10 \times 3 |
| 2 | 500 | 200 | 10 \times 3 |
| 3 | 500 | 200 | 10 \times 3 |
| 4 | 500 | 200 | 10 \times 3 |
| 5 | 200 | 200 | 10 \times 3 |
| 6 | 200 | 150 | 10 \times 3 |
| 7 | 200 | 150 | 10 \times 3 |
| 8 | 180 | 150 | 10 \times 3 |
| 9 | 180 | 150 | 8 \times 3 |
| 10 | 180 | 120 | 8 \times 3 |
| 11 | 180 | 120 | 8 \times 3 |
| 12 | 180 | 120 | 8 \times 3 |
| 13 | 180 | 120 | 8 \times 3 |

Table S3 Comparison of analytical parameters to detect thiamethoxam.

| Method | Material | LOD | Linear range | Reference |
|------------------------|---|-------------|------------------|--------------|
| Electrochemical method | Nanosilver/SDS | 100 nM | 0.1-9 μ M | [42] |
| Electrochemical method | Grapheneoxide | 8.3 μ M | 10-200 μ M | [43] |
| Electrochemical method | β -CD-rGO | 270 nM | 0.5-16 μ M | [44] |
| Electrochemical method | Graphene | 40 nM | 0.5-20 μ M | [45] |
| Electrochemical method | Co ₃ O ₄ @g-C ₃ N ₄ | 4.9 nM | 0.01-420 μ M | [46] |
| Flourescence sensor | Aptamer (ssDNA) | 1.23 nM | 0.01-1 μ M | Present work |

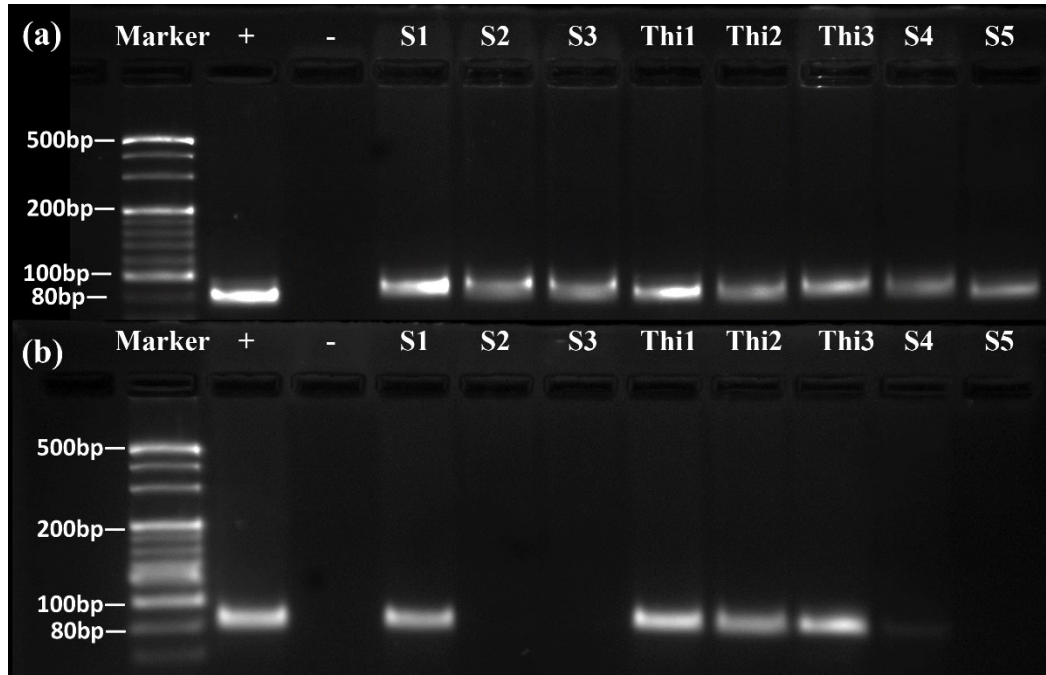


Fig. S1 The elution profile by 4% agarose gel electrophoresis for the first (a) round of selection and 13th (b) round of selection. The S bands (S1~S5) stand for the samples eluted by SELEX buffer. The Sar bands (Thi1~Thi3) stand for the samples eluted by thiamethoxam solution.

| No. | Sequence(5'-3') | Frequency |
|-----|----------------------------------|-----------|
| 18 | GGGAGGAGATGTAATTGCTCACTCTTGGC | 1 |
| 22 | GGTGGAGTAACGTGTTACTTCAGACACGA | 1 |
| 10 | GGACCGATACGTTGTAGTATGCGTCCACGA | 1 |
| 21 | GGAAAGGGAATGTTGGGGTACCCGAGCCGCC | 1 |
| 29 | GGAAATGTGGCGGTTTCGTTTCATTCGCC | 1 |
| 7 | GGCAGGAGCGTTAAGACACTACTACCGGAT | 1 |
| 8 | GGCAGTGCATCATGGATAAGAGTACCGGA | 1 |
| 6 | CAGGGATGAGATGGAGGTCCATCAAGGGA | 1 |
| 1 | CGGGCAAGAGCTCATGTCCCTTGTAAAGGTT | 1 |
| 9 | GGGAGATGTATAATCCCTCCCGGCAGGTCCCG | 3 |
| 2 | GGGGGGCAATGTTGAGACATGTCAACCATC | 1 |
| 20 | GGCAGTGAGGACATCATCCGAACTACCATC | 1 |
| 25 | GGCAGGGAAAGTGTAAAGTGCCTACTACCATC | 1 |
| 23 | ACGGTGCAGACATATTGCCCTACACCATGGA | 1 |
| 3 | CACAGCCGAGTATTAGGACTGGTAGGGGAC | 1 |
| 15 | ATACCCGCCAAATGTTAGTTGGCTATGGGTA | 1 |
| 4 | GGTGGGGGATAATGTAATGCCCTAACGATC | 1 |
| 24 | GGCCAAAGGCCATTCATTACCATGAACAGTC | 1 |
| 12 | GGCGAGTCATTGTACAATGGATAGCCCGGA | 3 |
| 13 | GGCAAGGTAACGTATGGGACCTTGGCACGA | 3 |
| 17 | AAAGGATGCCCGAAAAGACATCGTTCCGGTT | 1 |
| 5 | CACAGTCCGACGAAATGTATGAATGCCGGTGC | 1 |
| 26 | GGGTGTAGGGAGTAACTCTCCACACGGGA | 1 |
| 16 | GGGCGATACTTTTGGTGTGAGTCCGATC | 1 |

Fig. S2 Sequences alignment result by Clustalx 1.8.3.

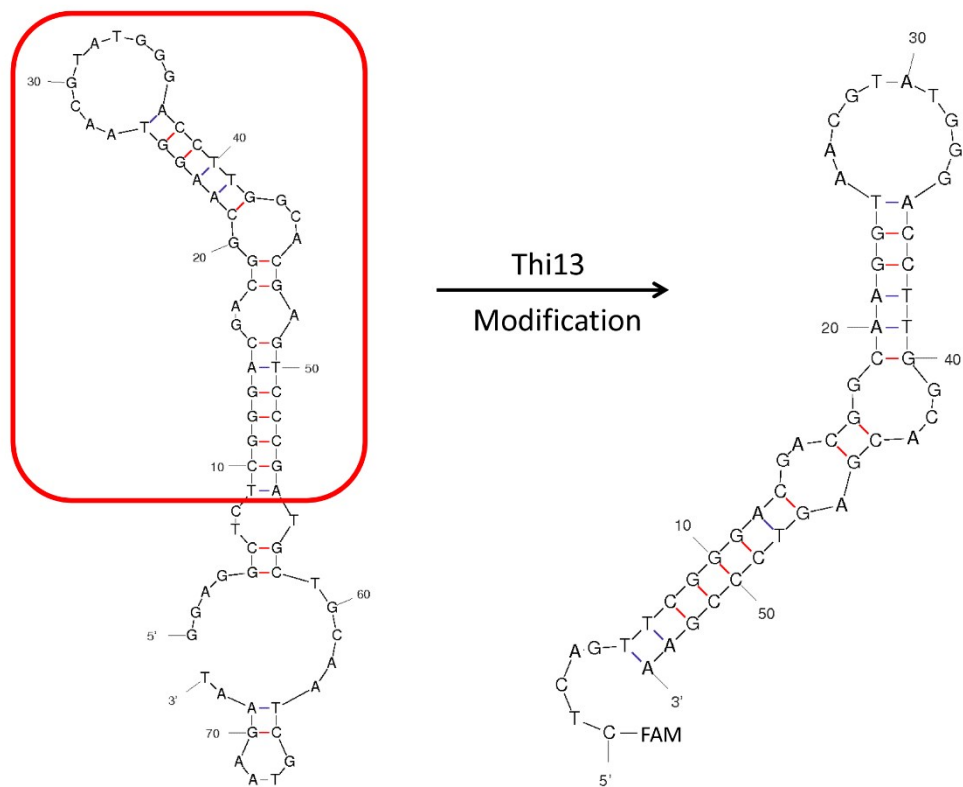


Fig. S3 The secondary structures of Thi13 and FAM-Thi13.

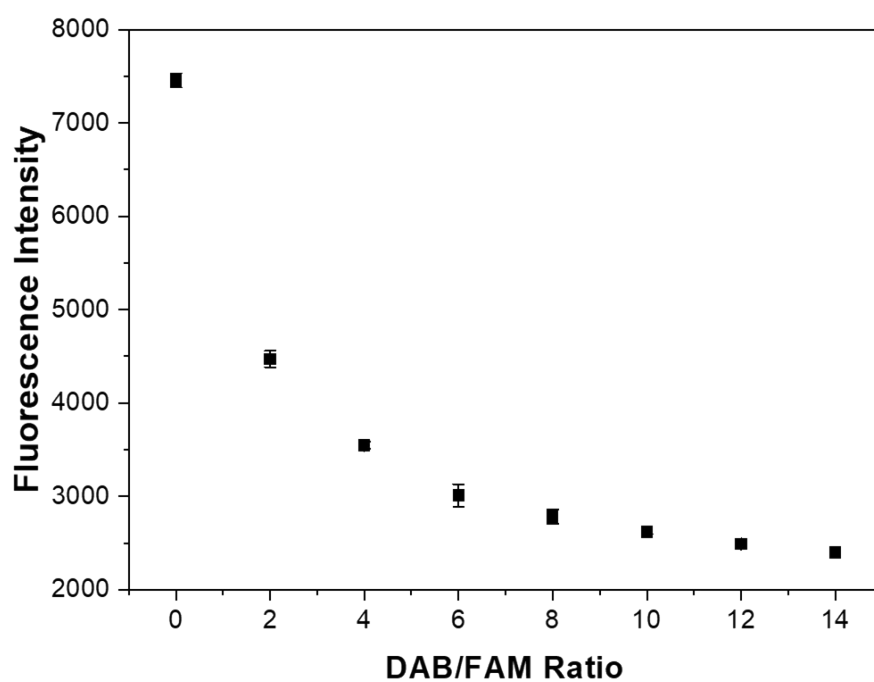


Fig. S4 Fluorescence quenching efficiency of different concentration ratios of quenching strand with FAM-Thi13. The concentration of FAM- Thi13 is 0.1 μ M. DAB represents the quenching strand, and FAM represents FAM-Thi13.

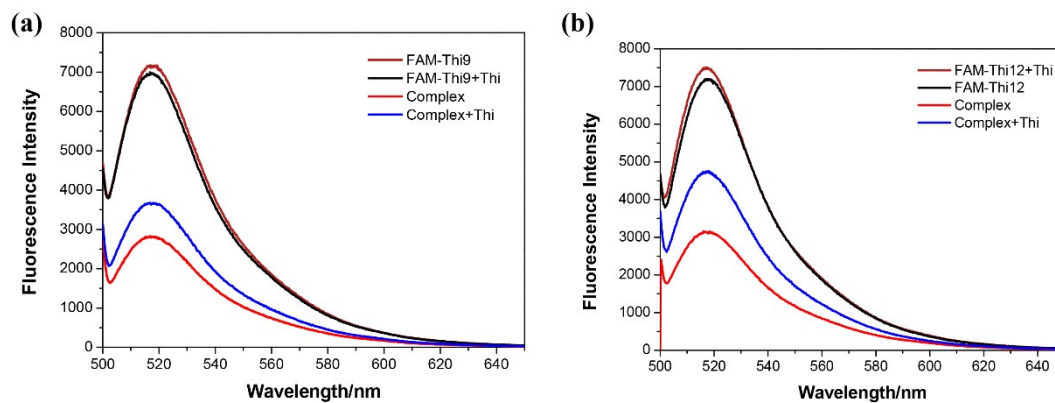


Fig. S5 Fluorescence spectra of FAM-aptamer (FAM-Thi9 (a) and FAM-Thi12 (b)) and FAM-aptamer-quenching strand complexes alone and in the presence of thiamethoxam. The concentration of FAM-aptamer is 0.1 μM and the ratio of FAM-aptamer and quenching strand is 1:6.

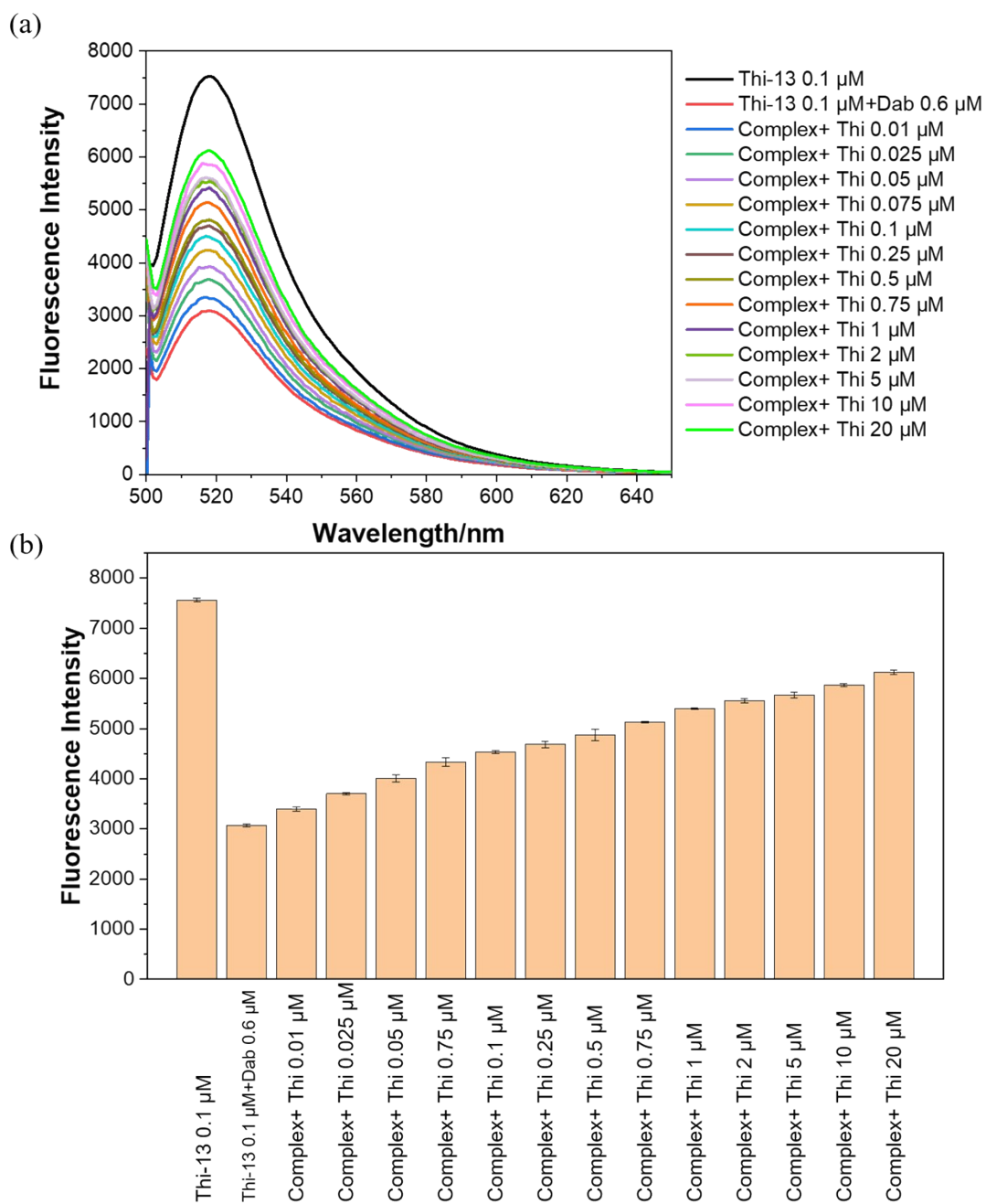


Fig. S6 Raw fluorescence spectra (a) and the fluorescence intensity at $\lambda_{ex}=520$ nm (b). The fluorescence intensity of the FAM-aptamer-quenching strand complex after adding various concentrations (0.01-20 μ M) of thiamethoxam, in which the ratio of aptamer to quencher is 1:6 and the concentration of aptamer is 0.1 μ M.

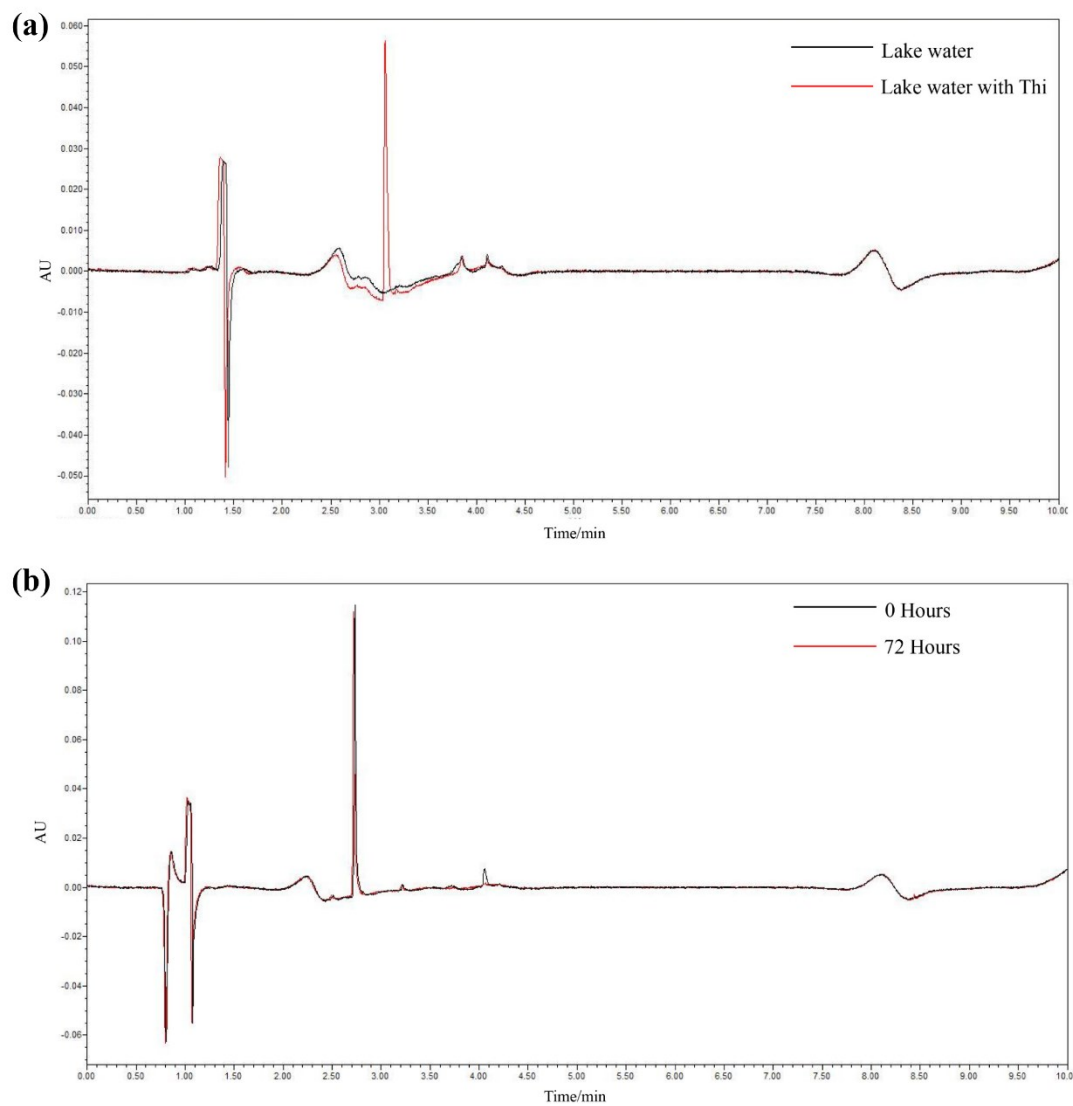


Fig. S7 (a) The original concentration of thiamethoxam in Dushu Lake. (b) The stability of thiamethoxam in binding buffer.

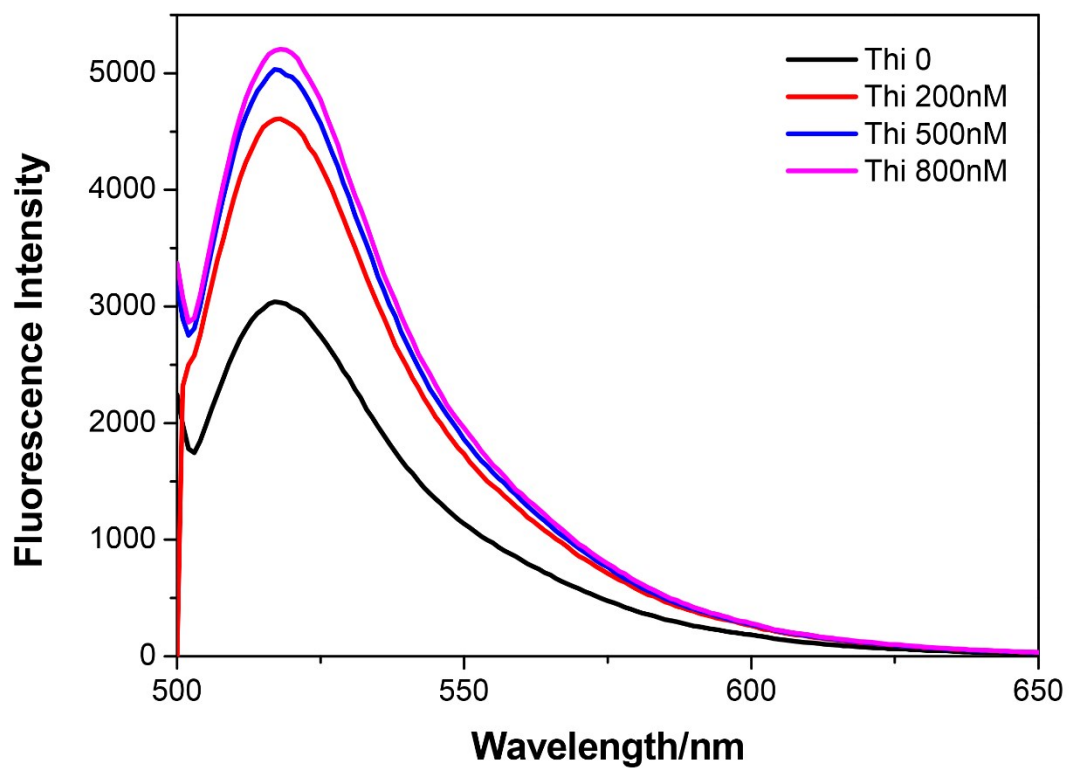


Fig. S8 Fluorescence spectra of the recovery experiment. Sample Thi 0 means to the FAM-aptamer-quencher strand complex without adding thiamethoxam. The concentration of aptamer is 0.1 μM and ratio of aptamer to quencher is 1:6.

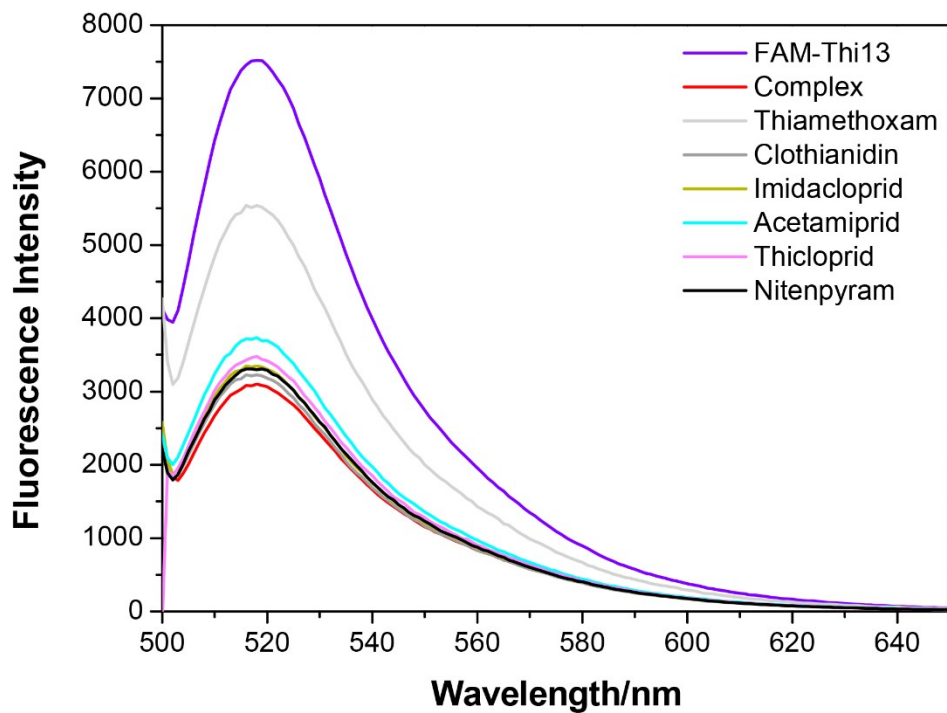


Fig. S9 Fluorescent spectra for the selectivity experiment. The fluorescence intensity of the FAM-aptamer-quenching strand complex after adding thiamethoxam and its analogues, in which the ratio of aptamer to quencher is 1:6 and the concentration of aptamer is 0.1 μM . The concentration of thiamethoxam is 2 μM , while that of its analogues are 20 μM .