Supporting Information

Ultrasensitive Therapeutic Drug Monitoring of Methotrexate by Structure-Switching Aptamer with Cascade Primer Exchange Reaction

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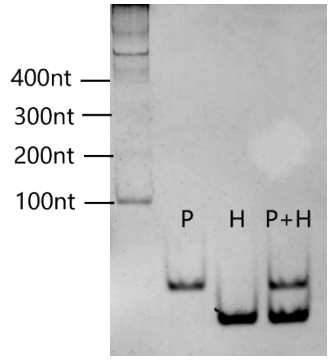

Fig. S1. Native PAGE analysis of Apt-P (P), PER catalytic hairpin (H) and Apt-P plus PER catalytic hairpin (means 0 h of PER reaction) (P + H).

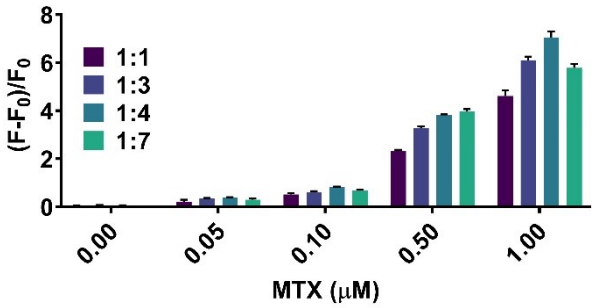

Fig. S2. Signal of Apt-PER assay corresponding to different concentration of MTX in buffer with different ratio of Apt-PC to cDNA. Error bars are means and SDs from three independent repeats.

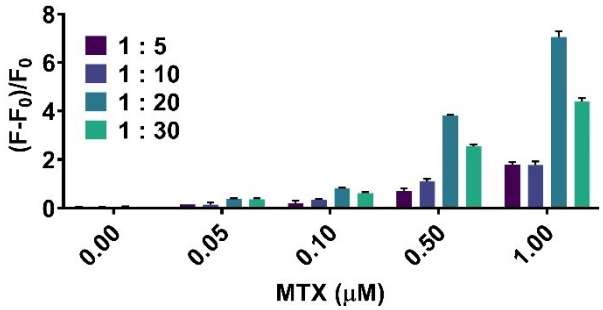

Fig. S3. Signal of Apt-PER assay corresponding to different concentration of MTX in buffer with different ratio of Apt-PC to Detector. Error bars are means and SDs from three independent repeats.

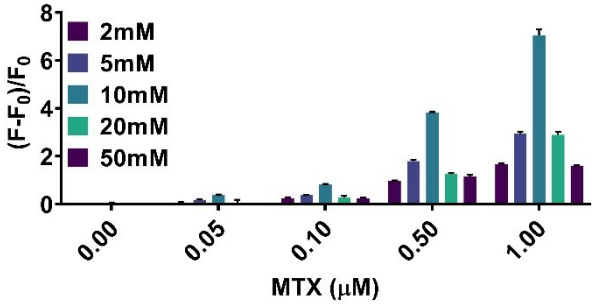

Fig. S4. Signal of Apt-PER assay corresponding to different concentration of MTX in buffer with different Mg^{2+} concentration. Error bars are means and SDs from three independent repeats.

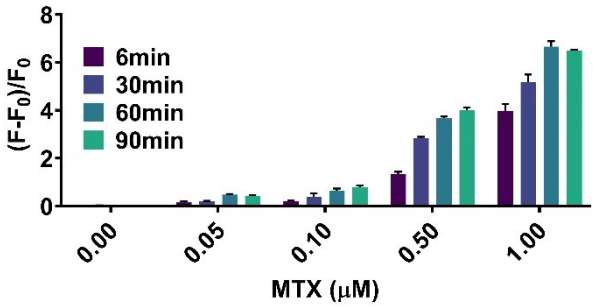

Fig. S5. Signal of Apt-PER assay corresponding to different concentration of MTX in buffer with different MTX incubation time. Error bars are means and SDs from three independent repeats.

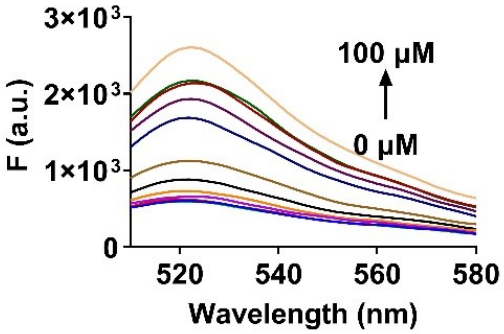

Fig. S6. Fluorescence spectra of the Apt-One sensor corresponding to different concentration of MTX (0, 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100 μM) in buffer.

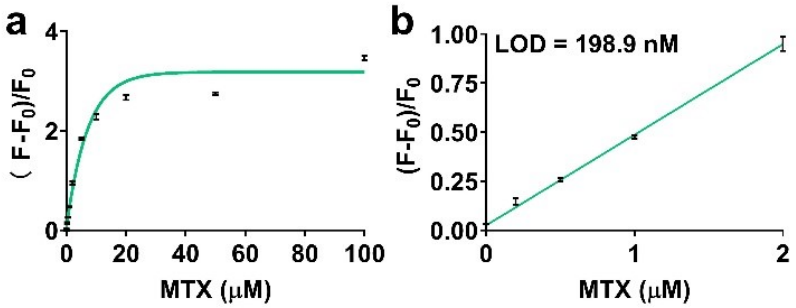

Fig. S7. (a) Detection signal of Apt-One assays response to various concentrations of MTX in buffer. (b) Linear relationship between signal of Apt-One assay and the corresponding MTX concentration in buffer ($Y = 0.4616 *X + 0.02480$, $R^2 = 0.9937$).

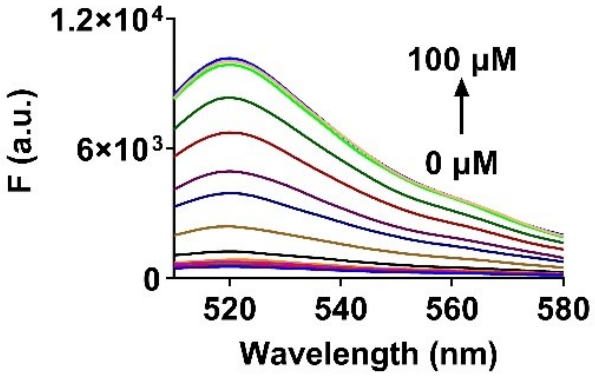

Fig. S8. Fluorescence spectra of the Apt-PER sensor corresponding to different concentration of MTX (0, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100 μM) in buffer.

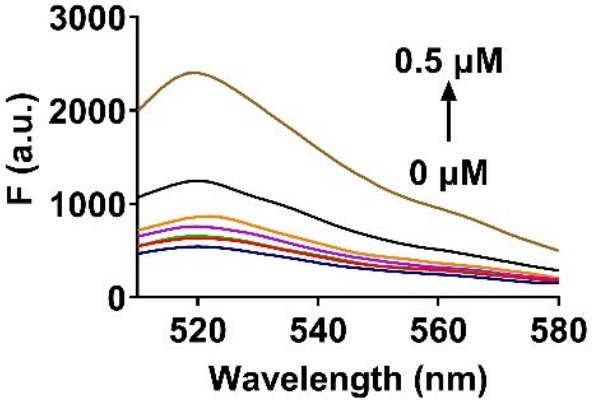

Fig. S9. Fluorescence spectra of the Apt-PER sensor corresponding to low concentration of MTX (0, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5 μM) in buffer.

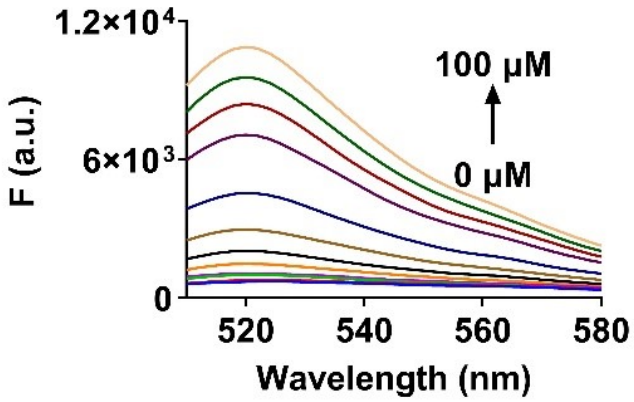

Fig. S10. Fluorescence spectra of the Apt-PER sensor corresponding to different concentration of MTX (0, 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100 μM) in 10 % FBS.

Table S1. Sequences used in this work.

Name	Sequence
Apt-P	*CTCTC**CGAACG***C**GGGATGTTTGGGGGACCCACGTTCG**aC TTTTTTTC
PER Catalytic Hairpin	ACTTTTTTTCGGGCCTTTTGGCCCGAAAAAAAGTGAAAAA AAG-Inverted dT
Apt-One	*CTCTC**CGAACG***C**GGGATGTTTGGGGGACCCACGTTCG**aC TTTTTTTCACTTTTTTTC
cDNA	CGTTCGGAGAG-PEG Biotin
Detector	FAM-ttCTTTTTTTCACTTTTTTTC

Table S2. Comparison of previously reported methods for MTX detection.

Method	Time	Sample	Cost	LOD	Linear range	Cited Reference
HPLC	+++	Serum	+++	0.003 μM	0.025 - 5.00 μM	1
FPIA	+++	Serum	+++	/	20 - 1000 nM	2
LC/MS-MS	+++	Serum	+++	3 nM	10 - 1000 nM	3
EMIT	+++	Serum	++	0.05 μM	0.05 - 1.00 μM	4
SPR	+	Serum	+	28 nM	28 - 500 nM	5
Aptamer	+	Serum	+	0.18 μM	0.5 - 10μM	6
This work	+	Serum / Blood	+	12.4 nM / 63.73 nM	0.05 - 2 μM / 0.2 - 5 μM	

The label "+++" means time-consuming or expeisive, "++" means acceptable, "+" means time-saving or cheap.

References:

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2. E. den Boer, B. C. Koch, R. Huisman and R. de Jonge, *Ther Drug Monit*, 2014, **36**, 819-823.
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