Supplementary Materials

Sensitive aptasensing of tobramycin through a rational design of catalytic hairpin assembly and hybridization chain reaction amplification monomers for CRISPR/Cas12a activation

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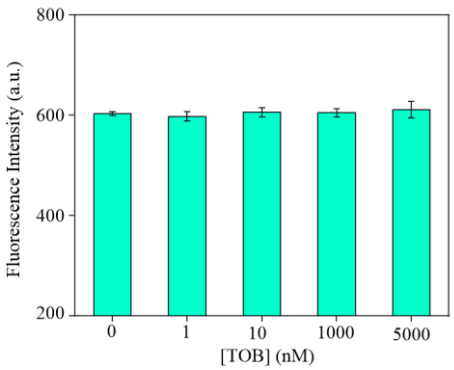

Figure S1 Dependence of the fluorescence intensity on the TOB concentration in the absence of AP.

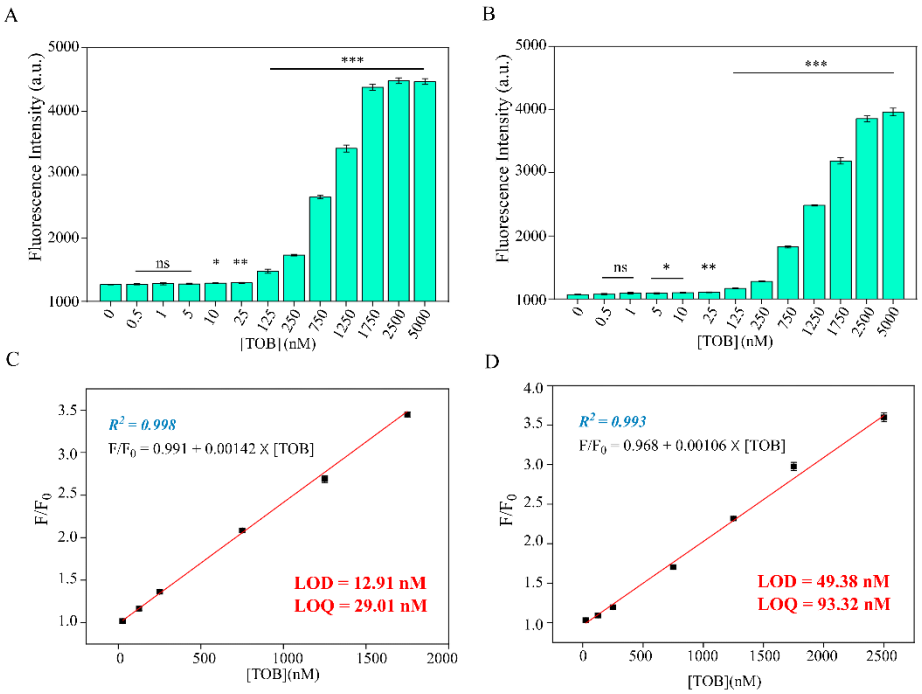

Figure S2 Analytical performance of the aptasensor for the detection of TOB in real samples. (A) Dependence of the fluorescence intensity on the TOB concentration in beef samples. (B) Dependence of the fluorescence intensity on the TOB concentration in milk samples. * $p \leq 0.05$, ** $p \leq 0.01$, $^{***}p \leq 0.001$, ns: no significant difference. (C) Linear relationship between the F/F_0 value and the TOB concentration in beef samples. (D) Linear relationship between the F/F_0 value and the TOB concentration in milk samples. The error bars represent the standard deviation of three repetitive measurements.

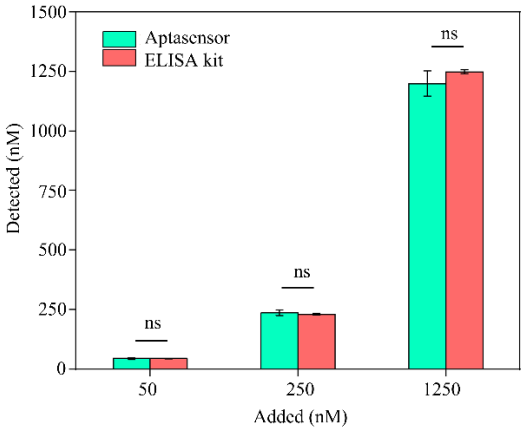

Figure S3 Comparison of the proposed aptasensor with a commercially available ELISA kit for detecting TOB in milk samples. ns: no significant difference. Data are presented as means and error bars represent the standard deviations of three repetitive measurements.

Table S1. Sequence of the oligonucleotides used in APF-CRISPR for ATP detection.

Name	Sequence (5'-3')
$H1_{SL\text{-}HCR}$	CACACCGACTCCAGGTTTCGTATAGTACAGCGAAACCTGGAGTC
$H2_{SL\text{-}HCR}$	ACAGCGAAACCTGGAGTCGGTGTGGACTCCAGGTTTCGCTGTACTATA
$H1_{SL\text{-}CHA}$	CACACCGACTCCAGGTTTCGTATAGTACAGCGAAACCTGGAGTC
$H2_{SL\text{-}CHA}$	CAGGTTTCGCTGTACTATACGAAACCTGGAGTCTTTCGTATAGTACAGC
$H1_{PF\text{-}HCR}$	GAGGAACAGGTTTCGTATAGTACAGCGTGATTTGGCTGTACTATACGAAACCTG
$H2_{PF\text{-}HCR}$	GCTGTACTATACGAAACCTGTTCCTCCAGGTTTCGTATAGTACAGCCAAATCAC
$H1_{PF\text{-}CHA}$	CACACCGACTCCAGGTTTCGTTTCGCTGTACTATACGAAACCTGCGAAACCTGGAGTC
$H2_{PF\text{-}CHA}$	GTTTCGCAGGTTTCGTATAGTACAGCGAAACGAAACCTGGAGTCGCTGTACTATACGAAACCTG
Initiator $_{SL\text{-}HCR}$	CGAAACCTGGAGTCGGTGTG
Initiator $_{SL\text{-}CHA}$	CGAAACCTGGAGTCGGTGTG
Initiator $_{PF\text{-}HCR}$	GCTGTACTATACGAAACCTGTTCCTC
Initiator $_{PF\text{-}CHA}$	CGAAACCTGGAGTCGGTGTG
AP (ID: 1 bp, AD: 7 bp)	GACTAGGCACTAGTCCCGAAACCTGGAGTCGGTGTGGACTAGT
AP (ID: 2 bp, AD: 7 bp)	GACTAGGCACTAGTCCACGAAACCTGGAGTCGGTGTGGACTAGT
AP (ID: 3 bp, AD: 7 bp)	GACTAGGCACTAGTCCACCGAAACCTGGAGTCGGTGTGGACTAGT
AP (ID: 4 bp, AD: 7 bp)	GACTAGGCACTAGTCCACACGAAACCTGGAGTCGGTGTGGACTAGT
AP (ID: 5 bp, AD: 7 bp)	GACTAGGCACTAGTCCACACCGAAACCTGGAGTCGGTGTGGACTAGT
AP (ID: 6 bp, AD: 7 bp)	GACTAGGCACTAGTCCACACACGAAACCTGGAGTCGGTGTGGACTAGT
AP (ID: 3 bp, AD: 6 bp)	GACTAGGCACTAGTCCACCGAAACCTGGAGTCGGTGTGGACTAG
AP (ID: 3 bp, AD: 7 bp)	GACTAGGCACTAGTCCACCGAAACCTGGAGTCGGTGTGGACTAGT
AP (ID: 3 bp, AD: 8 bp)	GACTAGGCACTAGTCCACCGAAACCTGGAGTCGGTGTGGACTAGTG
AP (ID: 3 bp, AD: 9 bp)	GACTAGGCACTAGTCCACCGAAACCTGGAGTCGGTGTGGACTAGTGC
AP (ID: 3 bp, AD: 10 bp)	GACTAGGCACTAGTCCACCGAAACCTGGAGTCGGTGTGGACTAGTGCC
AP (ID: 3 bp, AD: 11 bp)	GACTAGGCACTAGTCCACCGAAACCTGGAGTCGGTGTGGACTAGTGCCT
TS	CAGGTTTCGTATAGTACAGCCAAATCAC
NTS	GTGATTTGGCTGTACTATACGAAACCTG
crRNA	UAAUUUCUACUAAGUGUAGAUGCUGUACUAUACGAAACCUG
FQ probe	FAM - TTTATT - BHQ1

Note: The underlined sequences represent the complementary region. The sequences of PAM and its complimentary sequences are in purple font. The sequences in green font are the target sequence (TS) of CRISPR/Cas12a. In the AP sequence, the aptamer of TOB is in red font and HCR initiator is in blue font. ID means the duplex region in the initiator domain, and AD means the duplex region in the aptamer domain. The optimized AP sequence is shown with a yellow background.

Table S2. Comparison of the detection performances of the aptasensor for TOB determination

Signal	Amplification Strategy	Linear range	Limit of detection (LOD)	Samples	References
Fluorescence	-	80 nM – 2 μM	21.86 nM	Human serum	[1]
Fluorescence	-	0.1 – 6 μM	0.063 μM	Human serum and Milk	[2]
Fluorescence	-	50 – 500 nM	15.3 nM	Milk	[3]★
Fluorescence	-	1000 – 8125 pM	860 pM	Milk	[4]★
Colorimetric	-	0.1 – 100 nM	70 pM	Milk	[5]★
Colorimetric	-	40 – 200 nM	23.3 nM	Milk and egg	[6]★
PEC	-	5 – 50 nM	4.28 nM	Milk	[7]★
PEC	-	0.01 – 50 ng/ml	4.27 pg/ml	Milk and water	[8]★
ECL	-	1 pM – 1 μM	18 pM	Human serum	[9]★
SERS	-	6.67 – 300 nM	1.26 nM	Beef and mutton	[10]★
LFA	-	0.1 – 4 nM	0.02 nM	Milk and honey	[11]★
Electrochemical	HCR	5 – 1000 nM	3.51 nM	Milk	[12]★
Fluorescence	HCR	0.3 – 50 μM	17.37 nM	Milk	[13]△★
Fluorescence	SDA	10 – 300 pM	3.719 pM	Milk and water	[14]
Colorimetric	SDA	20 – 800 nM	12.24 nM	Milk and water	[15]
Electrochemical	SDA	10 – 200 nM	5.13 nM	Milk	[16]★
Fluorescence	SDA&HCR	0.5 – 30 nM	0.06 nM	Milk and water	[17]★
Fluorescence	HCR	0.125 – 25 nM 25 – 2500 nM	92.87 pM	Milk and beef	This work

△Magnetic separation

★Nanomaterials labeling

Photoelectrochemical (PEC)

Electrochemiluminescence (ECL)

Surface-enhanced Raman Scattering (SERS)

Lateral Flow Assay (LFA)

Hybridization Chain Reaction (HCR)

Strand Displacement Amplification (SDA)

References:

[1] Z. Khajavian, M. Esmaelpourfarkhani, M. Ramezani, M. Alibolandi, K. Abnous, S.M. Taghdisi, A highly sensitive, simple and label-free fluorescent aptasensor for tobramycin sensing based on PicoGreen intercalation into DNA duplex regions of three-way junction origami, Microchemical Journal, 160 (2021).

[2] S. Naeeminejad, K. Abnous, S.M. Taghdisi, A simple and label-free fluorescent aptasensor for detection of tobramycin: Appropriate for on-site antibiotic monitoring, Microchemical Journal, 165 (2021).

[3] X. Wang, R. Xu, Y. Tian, X. Gao, W. Zhang, Z. Sun, Y. Mou, X. Sun, Y. Guo, F. Li, A fluorescent aptasensor based on ZIF-8 @PdNCs and DNA-AgNCs for tobramycin detection in milk, Sensors and Actuators B: Chemical, 388 (2023).

[4] Y. Yin, Y. Tian, Y. Mou, L. Han, X. Sun, Y. Guo, F. Li, Dual-emissive DNA-stabilized fluorescent silver nanoclusters for the simultaneous detection of two antibiotics in milk, Food Control, 168 (2025).

[5] R. Mahjub, O.H. Shayesteh, K. Derakhshandeh, A. Ranjbar, F. Mehri, A. Heshmati, A novel label-free colorimetric polyA aptasensing approach based on cationic polymer and silver nanoparticles for detection of tobramycin in milk, Food Chemistry, 382 (2022).

[6] Q. Ma, Y. Wang, J. Jia, Y. Xiang, Colorimetric aptasensors for determination of tobramycin in milk and chicken eggs based on DNA and gold nanoparticles, Food Chemistry, 249 (2018) 98-103.

[7] X. Liu, Y. Jiang, J. Luo, X. Guo, Y. Ying, Y. Wen, H. Yang, Y. Wu, A SnO2/Bi2S3-based photoelectrochemical aptasensor for sensitive detection of tobramycin in milk, Food Chemistry, 344 (2021).

[8] Y. Zhang, Y. Zhu, T. Zeng, L. Qiao, M. Zhang, K. Song, N. Yin, Y. Tao, Y. Zhao, C. Zhang, Y. Zhang, Self-powered photoelectrochemical aptasensor based on hollow tubular g-C3N4/Bi/BiVO4 for tobramycin detection, Analytica Chimica Acta, 1250 (2023).

[9] F. Nasri, M. Hosseini, S.M. Taghdisi, M.R. Ganjali, M. Ramezani, Design and application of an ultrasensitive and selective tobromycin electrochemiluminescence aptasensor using MXene /Ni/Sm-LDH-based nanocomposite, Microchimica Acta, 191 (2024).

[10] S. Bi, Y. Yuan, F. Zhang, Y. Wang, J. Liu, B. Yang, D. Song, A sensitive surface-enhanced Raman spectroscopy detection for gentamicin and tobramycin using γ-Al2O3-modified silver nanoparticles coated with bovine serum albumin as substrate, Talanta, 260 (2023).

[11] X. Jin, L. Chen, Y. Zhang, X. Wang, N. Zhou, A lateral flow strip for on-site detection of tobramycin based on dual-functional platinum-decorated gold nanoparticles, The Analyst, 146 (2021) 3608-3616.

[12] Y. Zhao, Q. Chen, Y. Liu, B. Jiang, R. Yuan, Y. Xiang, A sensitive tobramycin electrochemical aptasensor based on multiple signal amplification cascades, Bioelectrochemistry, 160 (2024).

[13] J. Wang, H. Li, C. Du, Y. Li, X. Ma, C. Yang, W. Xu, C. Sun, Structure-switching aptamer triggering signal amplification strategy for tobramycin detection based on hybridization chain reaction and fluorescence synergism, Talanta, 243 (2022).

[14] D. Li, S. Ling, H. Wu, Z. Yang, B. Lv, CRISPR/Cas12a-based biosensors for ultrasensitive tobramycin detection with single- and double-stranded DNA activators, Sensors and Actuators B: Chemical, 355 (2022).

[15] Y. Ou, X. Jin, J. Fang, Y. Tian, N. Zhou, Multi-cycle signal-amplified colorimetric detection of tobramycin based on dual-strand displacement and three-way DNA junction, Microchemical Journal, 156 (2020).

[16] J. Nie, L. Yuan, K. Jin, X. Han, Y. Tian, N. Zhou, Electrochemical detection of tobramycin based on enzymes-assisted dual signal amplification by using a novel truncated aptamer with high affinity, Biosensors and Bioelectronics, 122 (2018) 254-262.

[17] D. Li, S. Ling, D. Meng, B. Zhou, P. Liang, B. Lv, Sensitive fluorescent aptasensing of tobramycin on graphene oxide coupling strand displacement amplification and hybridization chain reaction, International Journal of Biological Macromolecules, 220 (2022) 1287-1293.