Supplementary Materials

A colorimetric/fluorescent dual-mode aptasensor of *Salmonella* based on aptamer magnetic separation and DNA nanotriangle programmed multivalent aptamer

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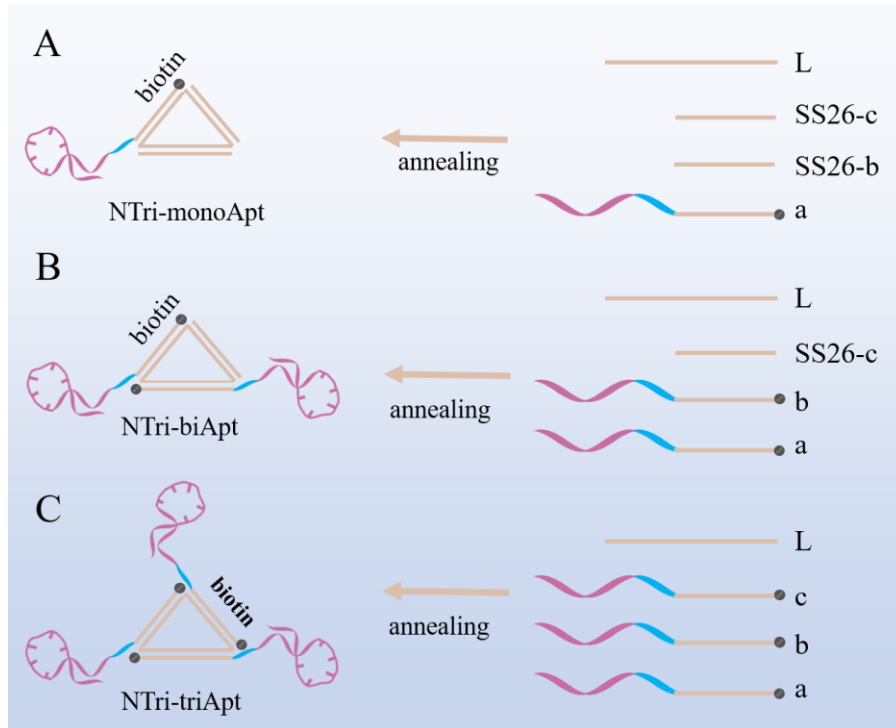
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Figure. S1 Illustration of the preparation processes of (A) NTri-monoApt, (B) NTri-biApt, (C) NTri-triApt.

Preparation of linear trivalent aptamers.

All DNA sequences used for linear trivalent aptamers were listed in Table S3. Briefly, eight short ssDNAs were prepared and classified into two categories: linker DNA (1-3 black lines) and spacer DNA (a-b red lines). After hybridization, the spacer DNAs can be sequentially connected through the linker DNA, leaving a gap of 26 bp between each spacer DNA, which just connects with the 26 bp extension fragment of the aptamer. In this way, linear trivalent aptamers were prepared, keeping the aptamer spacing consistent with planar NTri-Multi-Apt.
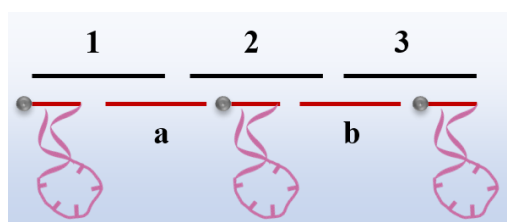

Figure. S2. Schematic design of linear trivalent aptamer.
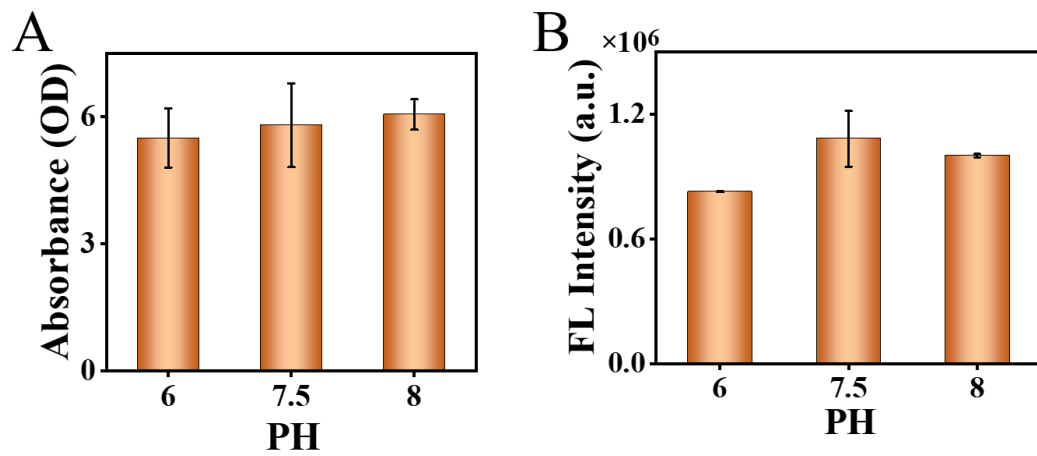
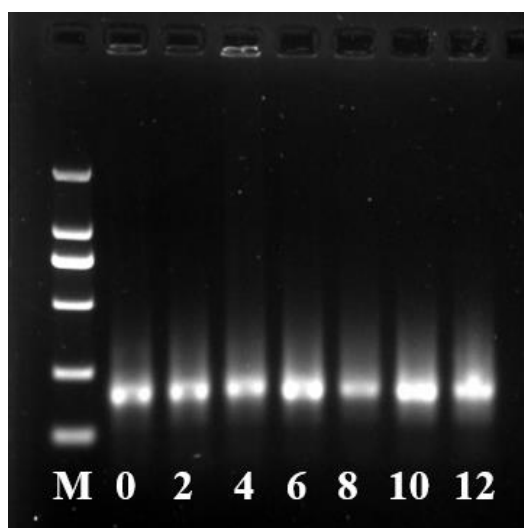**Figure. S3.** The effect of pH on the colorimetric (A) and fluorescent (B) detection.

Figure. S4. Agarose gel electrophoresis analysis of NTri-Multi-Apt under different storage durations (0, 2, 4, 6, 8, and 12 days) at 4°C.

Table S1. Sequences information for the nucleic acids of NTri-Multi-Apts in this study.

Name	Sequences (5' to 3')
L	GATGACTCGGCTTTCAGTCTAACGCTGACGTAGGCCCTTCGTAGGACCTTGCCATGATAGGCCCTTGCTCAGTAGACACCC
a	Biotin-TCTAGGGCCTACGTCAGCGTTAGACTGAACAAAACTTGGGCGGTTGGTGTGATGGGCTTTTTTCGTTGGGCCGG
b	Biotin-TATGCCGAGTCATCGGGTGTCTACTGAGCCAAAACTTGGGCGGTTGGTGTGATGGGCTTTTTTCGTTGGGCCGG
c	Biotin-TGTAGGGCCTATCATGGCAAGGTCCTACGCAAAACTTGGGCGGTTGGTGTGATGGGCTTTTTTCGTTGGGCCGG
S26-a	FAM-TCTAGGGCCTACGTCAGCGTTAGACTGAACAAAACTTGGGCGGTTGGTGTGATGGGCTTTTTTCGTTGGGCCGG
S26-b	FAM-TATGCCGAGTCATCGGGTGTCTACTGAGCCAAAACTTGGGCGGTTGGTGTGATGGGCTTTTTTCGTTGGGCCGG
S26-c	FAM-TGTAGGGCCTATCATGGCAAGGTCCTACGCAAAACTTGGGCGGTTGGTGTGATGGGCTTTTTTCGTTGGGCCGG
SS26-b	GCCGAGTCATCGGGTGTCTACTGAGC
SS26-c	AGGGCCTATCATGGCAAGGTCCTACG
Biotin-Apt	biotin-TTTTTTTTTTTTTTTCTTGGGCGGTTGGTGTGATGGGCTTTTTTCGTTGGGCCGG

Table S2. Sequence information for the nucleic acids of NTri-Multi-Apt with different interval lengths in this study.

Name	Sequences (5' to 3')
L21	GATGACTCGTTTCAGTCTAACGCTGACGTAGTCGTAGGACCTTGCCATGATAGTGCTCAGTAGACA
S21-a	Biotin-TCTCTACGTCAGCGTTAGACTGAACAAAACTTGGGCGGTTGGTGTGATGGGCTTTTTTCGTTGGGCCGG
S21-b	Biotin-TATCGAGTCATCTGTCTACTGAGCCAAAACTTGGGCGGTTGGTGTGATGGGCTTTTTTCGTTGGGCCGG
S21-c	Biotin-TGTCTATCATGGCAAGGTCCTACGCAAAACTTGGGCGGTTGGTGTGATGGGCTTTTTTCGTTGGGCCGG
L26	GATGACTCGGCTTTCAGTCTAACGCTGACGTAGGCCCTTCGTAGGACCTTGCCATGATAGGCCCTTGCTCAGTAGACACCC
S26-a	Biotin-TCTAGGGCCTACGTCAGCGTTAGACTGAACAAAACTTGGGCGGTTGGTGTGATGGGCTTTTTTCGTTGGGCCGG
S26-b	Biotin-TATGCCGAGTCATCGGGTGTCTACTGAGCCAAAACTTGGGCGGTTGGTGTGATGGGCTTTTTTCGTTGGGCCGG
S26-c	Biotin-TGTAGGGCCTATCATGGCAAGGTCCTACGCAAAACTTGGGCGGTTGGTGTGATGGGCTTTTTTCGTTGGGCCGG
L32	GATGACTCGGCCAATTTCAGTCTAACGCTGACGTAGGCCCTACCAAATCGTAGGACCTTGCCATGATAGGCCCTACCAAATGCTCAGTAGACACCCATA
S32-a	Biotin-TCTTTTGGTAGGGCCTACGTCAGCGTTAGACTGAACAAAACTTGGGCGGTTGGTGTGATGGGCTTTTTTCGTTGGGCCGG
S32-b	Biotin-TATTTGGCCGAGTCATCTATGGGTGTCTACTGAGCCAAAACTTGGGCGGTTGGTGTGATGGGCTTTTTTCGTTGGGCCGG
S32-c	Biotin-TGTTTTGGTAGGGCCTATCATGGCAAGGTCCTACGCAAAACTTGGGCGGTTGGTGTGATGGGCTTTTTTCGTTGGGCCGG

Table S3. Sequence information for the nucleic acids of linear trivalent aptamer in this study.

Name	Sequences (5' to 3')
1	GCGTCCAGCTCAGGCTACGTACACTTC
2	ATGATGAGGGAGCAGTAGGCGTCGGT
3	CAGGTACGGACAGCGTC
Apt1	Biotin-GAAGTGTACGTAGCCTGATTCTTGGGCGGTTGGTGTGATGGGCTTTTTTCGTTGGGCCGG
Apt2	Biotin-TACTGCTCTTCTTGGGCGGTTGGTGTGATGGGCTTTTTTCGTTGGGCCGG
Apt3	Biotin-CGTACCTGTTCTTGGGCGGTTGGTGTGATGGGCTTTTTTCGTTGGGCCGG
A	GCTGGACGCACCGACGCC
B	CCTCATCATGACGCTGTC

Table S4. Sequence information for the nucleic acids of S1, S2, S3 and the corresponding DNA sequences that make up the NTri-Multi-Apt.

Name	Sequences (5' to 3')
S1	CTTGGGCGGTTGGTGTGATGGGCTTTTTTCGTTGGGCCGG
S2	TATGGCGGCGTCACCCGACGGGGACTTGACATTATGACAG
S3	GAGTTAATCAATACAAGGCGGGAACATCCTTGGCGGTGC
L-d	GATGACTCGGCTTTCAGTCTAACGCTGACGTAGGCCCTTCGTAGGACCTTGCCATGATAGGCCCTTGCTCAGTAGACACCC
S26-1-a	Biotin-TCTAGGGCCTACGTCAGCGTTAGACTGAACAAAACTTGGGCGGTTGGTGTGATGGGCTTTTTTCGTTGGGCCGG
S26-1-b	Biotin-TATGCCGAGTCATCGGGTGTCTACTGAGCCAAAACTTGGGCGGTTGGTGTGATGGGCTTTTTTCGTTGGGCCGG
S26-1-c	Biotin-TGTAGGGCCTATCATGGCAAGGTCCTACGCAAAACTTGGGCGGTTGGTGTGATGGGCTTTTTTCGTTGGGCCGG
S26-2-a	Biotin-TCTAGGGCCTACGTCAGCGTTAGACTGAACAAAATATGGCGGCGTCACCCGACGGGGACTTGACATTATGACAG
S26-2-b	Biotin-TATGCCGAGTCATCGGGTGTCTACTGAGCCAAAATATGGCGGCGTCACCCGACGGGGACTTGACATTATGACAG
S26-2-c	Biotin-TGTAGGGCCTATCATGGCAAGGTCCTACGCAAAATATGGCGGCGTCACCCGACGGGGACTTGACATTATGACAG
S26-3-a	Biotin-TCTAGGGCCTACGTCAGCGTTAGACTGAACAAAAGAGTTAATCAATACAAGGCGGGAACATCCTTGGCGGTGC
S26-3-b	Biotin-TATGCCGAGTCATCGGGTGTCTACTGAGCCAAAAGAGTTAATCAATACAAGGCGGGAACATCCTTGGCGGTGC
S26-3-c	Biotin-TGTAGGGCCTATCATGGCAAGGTCCTACGCAAAAGAGTTAATCAATACAAGGCGGGAACATCCTTGGCGGTGC

Table S5. Recovery of the proposed colorimetric and fluorescent method in *Salmonella*-spiked samples.

Sample	*Salmonella* Spiked (CFU/mL)	Colorimetric			Fluorescent		
		Detected (CFU/mL)	Recovery (%)	CV (%)	Detected (CFU/mL)	Recovery (%)	CV (%)
Milk	1×10^2	98	98.10	6.72	113	112.83	8.54
	1×10^3	1164	116.44	5.74	999	99.89	9.53
	1×10^4	11549	115.49	4.25	10566	105.66	5.42
Egg white	1×10^2	92	92.16	9.71	103	103.25	3.78
	1×10^3	1072	107.18	8.51	1068	106.8	7.8
	1×10^4	10322	103.21	9.36	11085	110.85	9.49
Chicken meat	1×10^2	108	108.63	8.66	94	93.9	7.85
	1×10^3	1097	109.70	4.84	1015	101.45	5.56
	1×10^4	9584	95.84	9.03	10387	103.87	6.57

Table S6. Comparison with different methods of recently developed for *Salmonella* detection.

Detection method	Linear range (CFU/mL)	LOD (CFU/mL)	Reference
fluorescent	7×10^3 - 3×10^8	1×10^3	[1]
fluorescent	1503 - 96938	733 and 464	[2]
fluorescent	4.9×10^3 - 4.9×10^7	4.9×10^3	[3]
fluorescent	10^3 - 10^7	10^3	[4]
fluorescent	4.6×10^2 - 4.6×10^7	82	[5]
Colorimetric	1×10^3 - 1×10^8	10^3	[6]
Colorimetric	10^5 - 10^8	10^5	[7]
Colorimetric	8×10^1 - 8×10^4	62	[8]
Colorimetric	$10 - 10^7$	100	[9]
Colorimetric/fluorescent	$1.88 \times 10^4 - 1.88 \times 10^7$	$1.88 \times 10^4 / 3.75 \times 10^3$	[10]
Colorimetric/fluorescent	10^2 - 10^7	316/60	This work

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