

A Paper-Based Multicolor Colorimetric Aptasensor for the Visual Determination of Multiple Sulfonamides Based on Aptamer-Functionalized Magnetic Beads and NADH–Ascorbic Acid-Mediated Gold Nanobipyramids

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1. Chemicals and apparatus used in the experiments

Streptavidin-modified magnetic beads (~1.0 μm, 10 g/L) were purchased from ThermoFisher Tech, Inc. (USA). Trihydroxymethyl aminomethane (Tris), sodium ethylenediamine tetraacetate (EDTA) and bovine serum albumin (BSA) were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Tris-HCl solution (1 M, pH 7.35–7.45), NaCl, KCl, Tween-20, NaBH₄, HAuCl₄, cetyltrimethylammonium bromide (CTAB), hexadecyl trimethyl ammonium chloride (CTAC), silver nitrate (AgNO₃), L-ascorbic acid and 2-phosphate trisodium salt (AAP) were obtained from Sigma-Aldrich Inc. of Chian (Shanghai, China). Streptavidin-modified alkaline phosphatase (Streptavidin-ALP) was purchased from Shanghai Biyuntian Biotechnology Co., Ltd. (Shanghai, China). Reduced nicotinamide adenine dinucleotide I (NADH) and HCl were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Nylon membranes (average pore size 0.2 μm) were obtained from Jinteng Experiment Equipment Co., Ltd (Tianjin, China).

UV-visible extinction spectra of AuNBP s were measured using a Spark 10 M multifunctional microplate reader (Tecan, Switzerland). All photographs were recorded using an EOS 600D digital camera (Canon, Japan). Transmission electron microscope (TEM) images were obtained using an HT7700 transmission electron microscope (Hitachi, Japan).

Table S1: Detailed DNA sequences used in the experiment

DNA sequences	
Aptamer	5'- AGAACTTACGACACGGGGTCTTGGGGTGAGTCCTGCTGTGTCAG TGTGTCGTA-3'
cDNA-1	5'-CCGTGTCGTAAGTTCTGTTTTTTTT-biotin-3'
cDNA-2	5'-biotin-TTTTTTTTCACTGACACAGCAGG-3'

Table S2: Comparison of analytical performance among previous colorimetric aptasensors and our aptasensor for SA detection

Analytical methods	Detected SA kinds	Linear range	Visual LOD	Spectrometry LOQ	Spectrometry LOD	Color numbers	Ref.
Colorimetric aptasensor based on gold nanoparticles (AuNPs)	SDM	0.16–3.2 μM	N/A	0.16 μM	0.05 μM	single	1
A dichromatic aptasensor based on AuNPs and fluorescent dyeing	SDM	6.5–1.0 μM	N/A	6.5 μM	1.97 μM	single	2
Colorimetric aptasensor based on broad-specificity aptamer and dye displacement strategy	SQ, SMP, SMD, SCP and SPD	0.5–100 μM	2.0–5.0 μM	0.5 μM	0.15 μM	single	3
Colorimetric method based on ZIF-8 nanozyme	SMZ, ST and SDM	6.3–750 μM	N/A	6.3 μM	1.9 μM	single	4
Paper-based multicolor colorimetric aptasensor based on aptamer and gold nanobipyramids	SQ, SMP and SMD	0.3–1.0 or 5.0 μM	0.3–1.0 μM	0.3–0.5 μM	0.09–0.15 μM	multiple (> 8)	This work

References

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2. Chen, X.-X.; Lin, Z.-Z.; Hong, C.-Y.; Yao, Q.-H.; Huang, Z.-Y. A dichromatic label-free aptasensor for sulfadimethoxine detection in fish and water based on AuNPs color and fluorescent dyeing of double-stranded DNA with SYBR Green I. *Food Chem.* 2020, 309, 125712.
3. Xu, R.-Y.; Yang, C.; Huang, L.; Lv W.-C.; Yang, W.-J.; Wu, Y.-N.; Fu F.-F. Broad-specificity aptamer of sulfonamides: isolation and its application in simultaneous detection of multiple sulfonamides in fish sample. *J. Agric. Food Chem.* 2022, 70, 11804-11812.
4. Chen, G.-Y.; Zhou, X.; Tian, T.; Zhang, C.-Y.; Yin, S.-J.; Chen, H.; Xu, Y.; Yang, F.-Q. Nanozyme based on ZIF-8 for the colorimetric detection of sulfonamides in cow milk. *Analytical Biochemistry* 2022, 652, 114748.

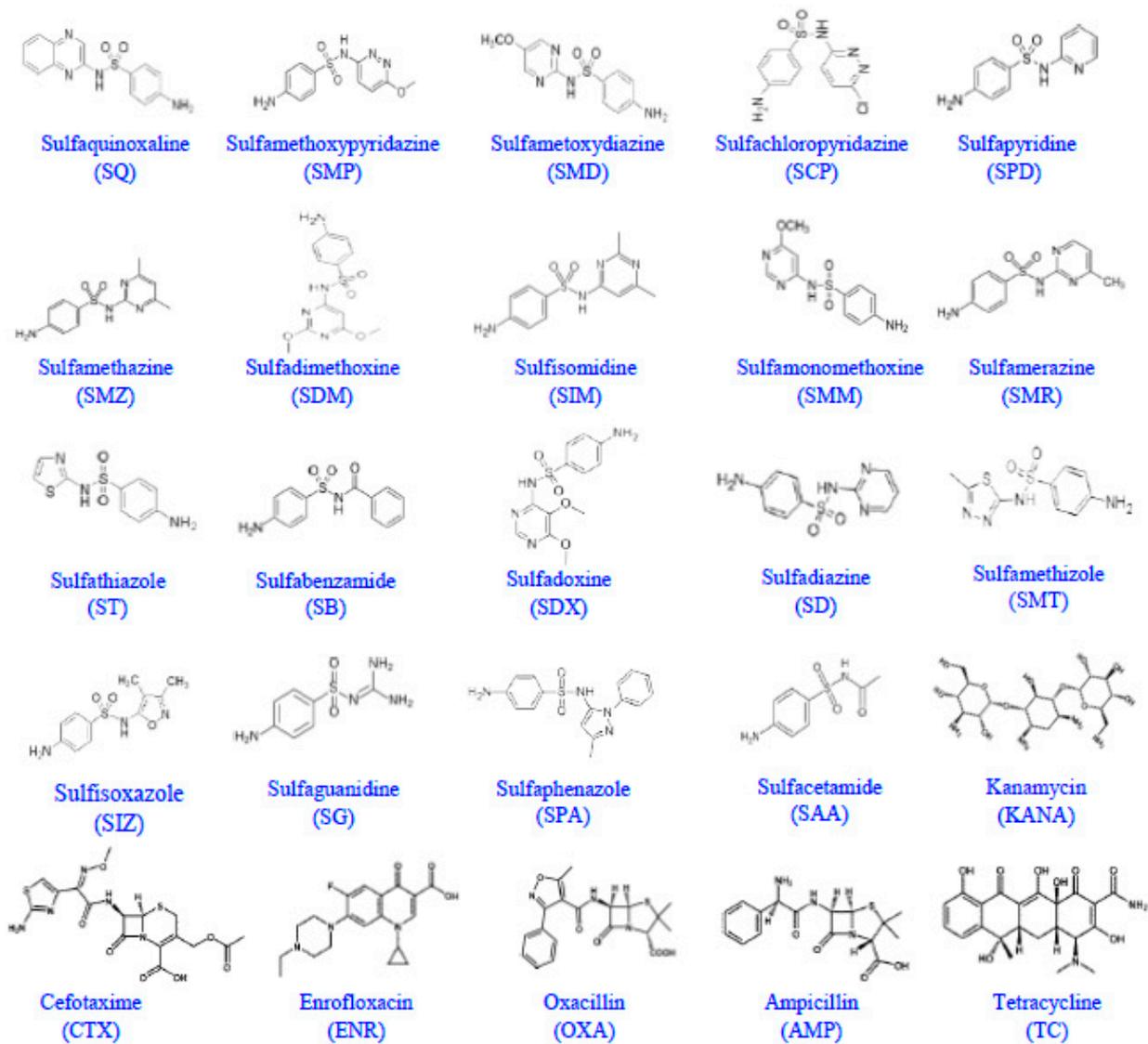


Figure S1: The full names, abbreviations and chemical structures of the 19 commonly used sulfonamides and 6 other antibiotics.

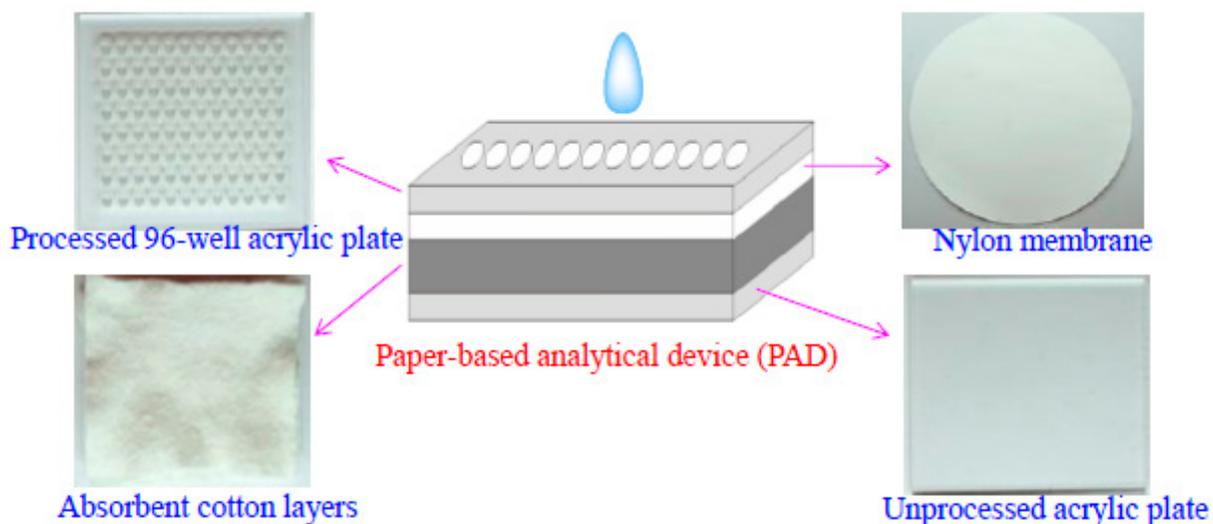


Figure S2: Structure of the paper-based analytical device (PAD).

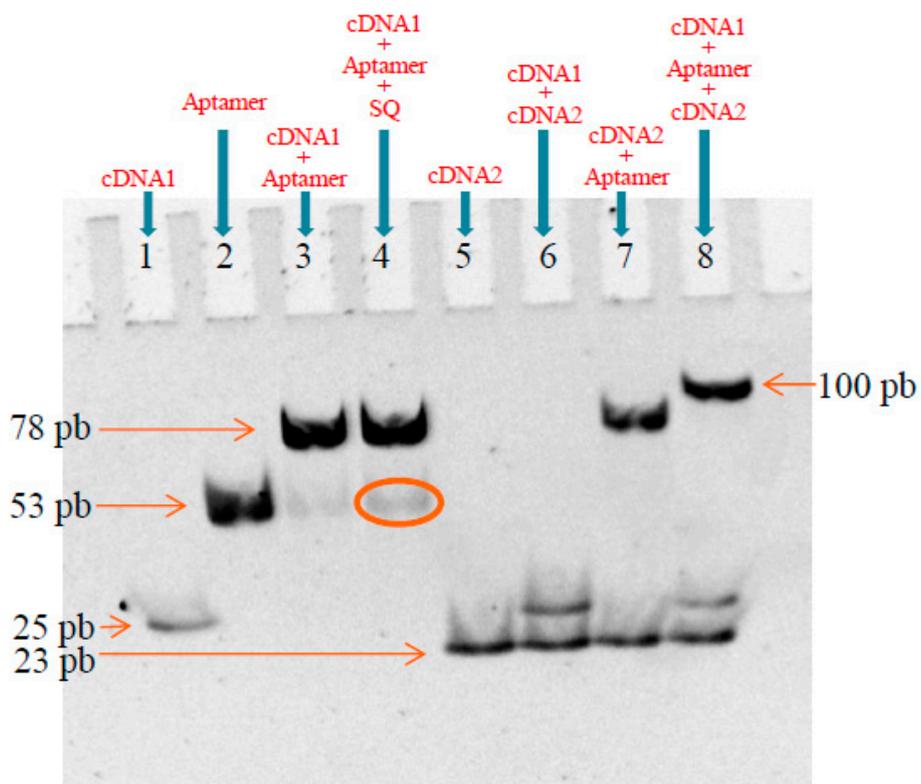


Figure S3: Polyacrylamide gel electrophoresis analysis of different DNA complexes. The concentrations of aptamer, cDNA1, cDNA 2 are all 500 nM, and the concentration of SQ is 2.0 μ M.

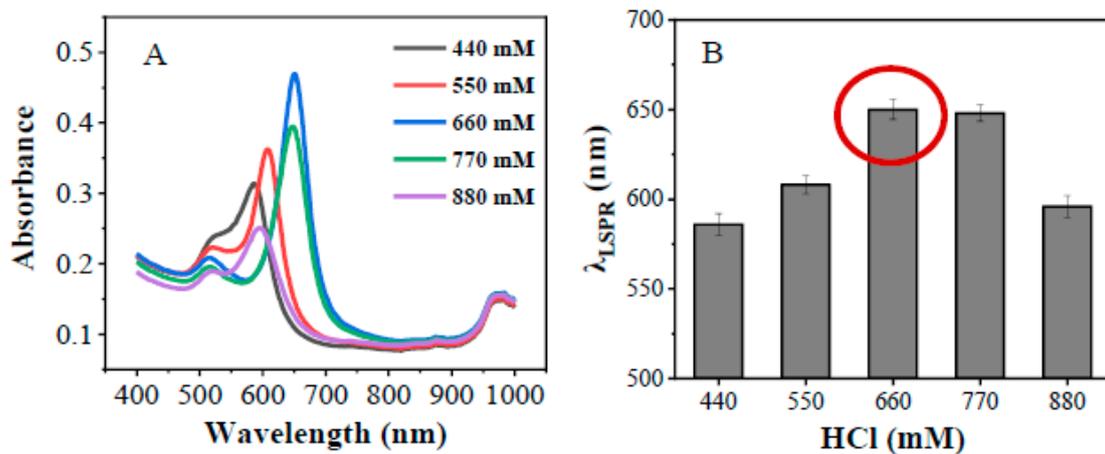


Figure S4: (A) Effect of the HCl concentration on NADH-AA-mediated AuNBPs growth system on the extinction spectra, (B) the wavelength of longitudinal LSPR peaks (λ_{LSPR}) of the generated AuNBPs.

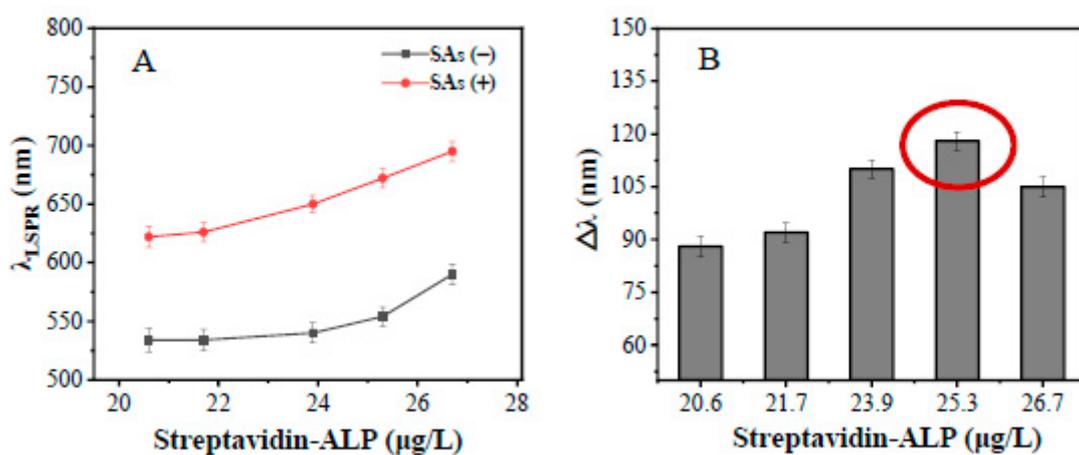


Figure S5: Effect of the streptavidin-ALP concentration in the competitive aptamer-binding system (A) on the λ_{LSPR} of the generated AuNBPs and (B) the $\Delta\lambda_{LSPR}$ of the multicolor colorimetric aptasensor for detecting SQ.

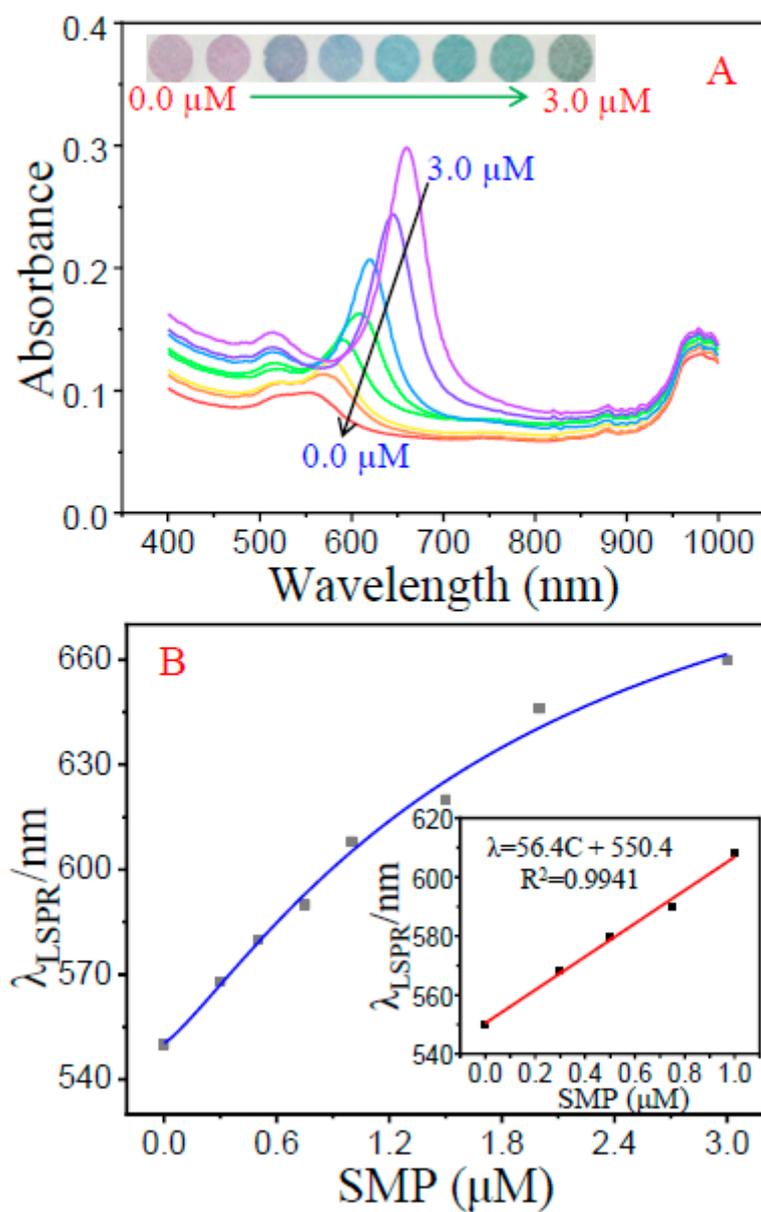


Figure S6: The photographs and UV-vis extinction spectra (A) of multicolor colorimetric aptasensor for detecting different concentrations of SMP (0.0, 0.3, 0.5, 0.75, 1.0, 1.5, 2.0 and 3.0 μM), and (B) the variation of the λ_{LSPR} of AuNBPs versus SMP concentrations. The insert in (B) is the linear relationship between the λ_{LSPR} of AuNBPs and SMP concentrations in the range of 0.0–1.0 μM .

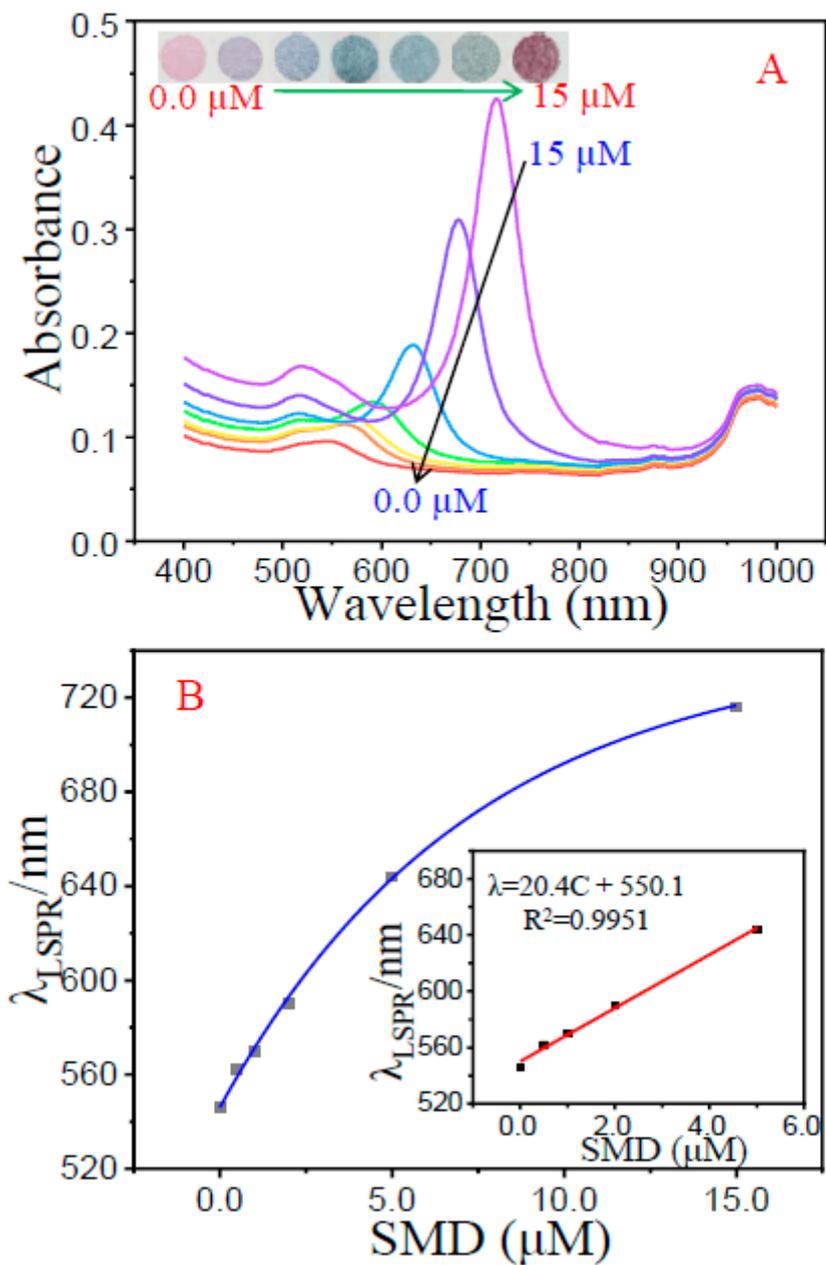


Figure S7: The photographs and UV-vis extinction spectra (A) of multicolor colorimetric aptasensor for detecting different concentrations of SMD (0.0,0.5, 1.0, 2.0, 5.0, 8.0 and 15 μM), and (B) the variation of the λ_{LSPR} of AuNBPs versus SMD concentrations. The insert in (B) is the linear relationship between the λ_{LSPR} of AuNBPs and SMD concentrations in the range of 0.0–5.0 μM .