Supplementary Information

AI-assisted Hyperbranched RCA-based Colorimetric Biosensing Platform for Ultrasensitive Detection of BPA

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Synthesis of Gold Nanoparticles (AuNPs)

All glassware used for synthesis was pre-treated by soaking in freshly prepared aqua regia (a 1:3 volume ratio of nitric acid to hydrochloric acid) for at least 1 hour, followed by thorough rinsing with ultrapure water and drying. Gold nanoparticles were synthesized using the classical Turkevich method[1]. Briefly, 5 mL of trisodium citrate solution (38.8 mM) was rapidly added to 50 mL of boiling $HAuCl_4$ $4H_2O$ (1 mM) under vigorous stirring. Upon addition, the solution color changed from pale yellow to wine red, indicating the formation of AuNPs. The reaction mixture was continuously stirred until it cooled to room temperature. The resulting colloidal solution was stored at 4 °C in the dark for further use.

Preparation of AuNPs-DP

Hairpin DNA modified with a thiol group at the 5′ end (200 μM) was incubated with 0.1 M TCEP at room temperature for 1 h to reduce disulfide bonds. The activated DNA was then added to 5 mL of AuNPs solution and incubated on a shaker at room temperature for 16 h. Subsequently, 500 μL of 0.1% SDS was added to stabilize the conjugates for 1 h, followed by the slow addition of 1 mL of 3 M NaCl. The mixture was further incubated on a shaker for 16 h. The resulting AuNPs-DP conjugates were purified by centrifugation twice, and the pellet was resuspended in 5 mL of PBS (pH 7.4). The functionalized AuNPs-DP were stored at 4 °C in the dark until use.

Characterization of AuNPs-DP

The colorimetric detection illustrated in Figure 1 was based on the aggregation behavior of AuNPs. To achieve specific recognition of the complementary sequence of the HRCA product, thiol-modified DNA strands were immobilized onto the surface of AuNPs through gold–thiol interactions, forming functionalized AuNPs probes. The successful modification of DNA onto the AuNPs was confirmed by UV–vis spectrophotometry. As shown in Figure S2, the UV–vis spectrum of the functionalized AuNPs displayed a surface plasmon resonance (SPR) peak at 525 nm, red-shifted by approximately 5 nm relative to that of the bare AuNPs. This red shift indicates the successful conjugation of thiol-modified DNA onto the AuNPs surface[2].

Table S1. The DNA sequence used in this work for BPA detection.

Name	Sequence (5'-3')
Padlock Probe	P'-AAGGAGTGAAGCGTGCGTAATAGTGTCAAGGAATTCAATCAAAGCTGAAGAAGCT
Ligation template (LT)	CTCCTTAGCTTCTTCAGCT
Capture Probe (CP)	TGGGTGGTCAGGTGGGATAGCGTTCCGCGTATGGCCCATTTTTAGCTGAAGAAGCTCCCACCTGACCACCCA
H1	GCGAGCTTCTTTGATTGAATTCCTAGCTGAAGAAGCT
AuNPs-DNA Probe	SH-ATCGATGGTGAAGCGTGCGTAATACCATCGAT

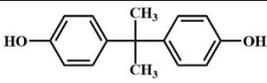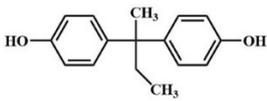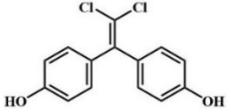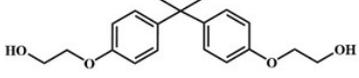**Table S2.** Chemical properties of bisphenol A and its analogs.

Name	IUPAC name	Abbreviation	Formula	Molecular weight (g/mole^{-1})	2D structure
Bisphenol A	4-[2-(4-hydroxyphenyl)propan-2-yl]phenol	BPA	$C_{15}H_{16}O_2$	228.29	
Bisphenol B	4-[2-(4-hydroxyphenyl)butan-2-yl]phenol	BPB	$C_{16}H_{18}O_2$	242.31	
Bisphenol C	4-[2,2-dichloro-1-(4-hydroxyphenyl)ethenyl]phenol	BPC	$C_{14}H_{10}Cl_2O_2$	281.13	
Ethoxylated bisphenol A	2,2'-((propane-2,2-diylbis(4,1-phenylene))bis(oxy))bis(ethan-1-ol)	BPE	$C_{19}H_{24}O_4$	316.39	
Bisphenol F	4,4'-methylenediphenol	BPF	$C_{13}H_{12}O_2$	200.24	
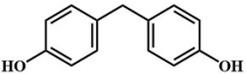
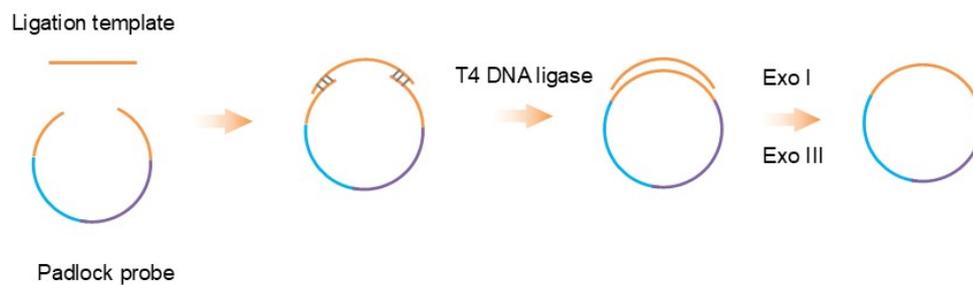**Figure S1** Schematic diagram of the preparation of CT used for HRCA.

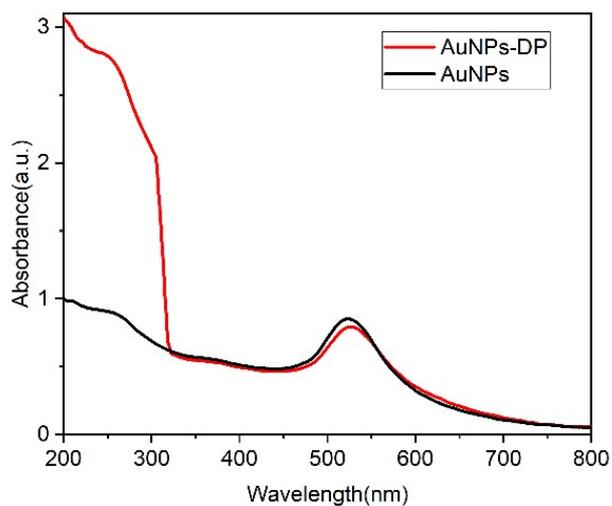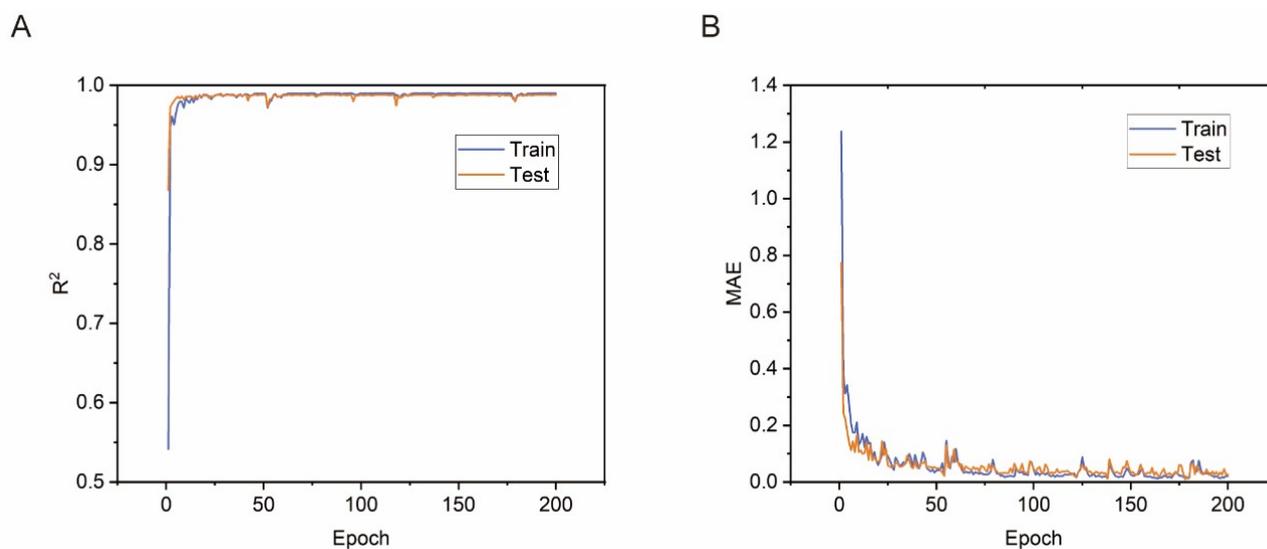**Figure S2** UV-visible spectra analysis of AuNPs and AuNPs-DP.

Figure S3 The coefficient of determination (R^2) values and mean absolute error (MAE) values for the training and testing datasets.

A

B

1. Turkevich, J., P.C. Stevenson, and J.J.D.o.t.F.S. Hillier, *A study of the nucleation and growth processes in the synthesis of colloidal gold.* 1951. **11**.

2. Dharanivasan, G., et al., *DNA templated self-assembly of gold nanoparticle clusters in the colorimetric detection of plant viral DNA using a gold nanoparticle conjugated bifunctional oligonucleotide probe.* RSC Advances, 2016. **6**(14): p. 11773-11785.