Supporting Information

DNA-Modified Prussian Blue Nanozymes for Enhanced Electrochemical Biosensing

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Table S1. List of DNA sequences used in the study.

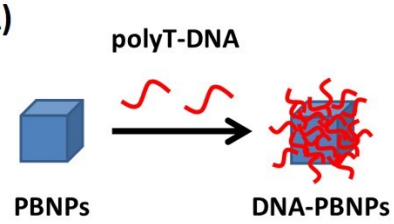
Name	Sequences (5'→3')
polyT-linker	5'-TTTTTTTTTTTTTTTTTTTTTTTTTGGTTTTTTTTTTTTTTTTTTTTTTT-3'
Cu^{2+}-DNAzyme	5'-GGTTCCTCAGCATCTCTTTCTAATACGACTCAGAATGAGTCTGGGCCTCT TTCTTTTAGAAAGAAC-3'
Product 1 (P_1)	5'-ACTCAGAATGAGTCTGGGCCTCTTTCTTTTAGAAAGAAC-3'
Product 2 (P_2)	5'-GGTTCCTCAGCATCTCTTTCTAATACG-3'
SH-Cu^{2+}-DNAzyme	5'-/5ThioMC6-D/ GGTTCCTCAGCATCTCTTTCTAATACGACTCAGAATGAG TCTGGGCCTCTTTCTTTTAGAAAGAAC-3'
Cu^{2+}-polyA-capture DNA	5'-AAAAAAAAAAAAAAAAAACGTATTAGAAAGAGA-3'
SH-VEGF aptamer (Aptamer1)	5'-/5ThioMC6-D/ATGACTAGGTGGGGGTGGACGGGCCGGGTAGA-3'
VEGF aptamer (Aptamer2)	5'-AAAAAAAAAAAAAAAAAAACAATTGGGCCCGTCCGTATGGTGGGT-3'

The sequences labeled in blue represent copper ion-dependent DNAzymes, and the triangles indicate the cut sites.

The sequences marked with an underline are complementary.

The sequences labeled in red are crucial for VEGF recognition.

(A)



(B)

	I	II	III
PBNPs (mg/mL)	0.1	0.05	0.03
polyT-DNA (μM)	10	10	10

(C)

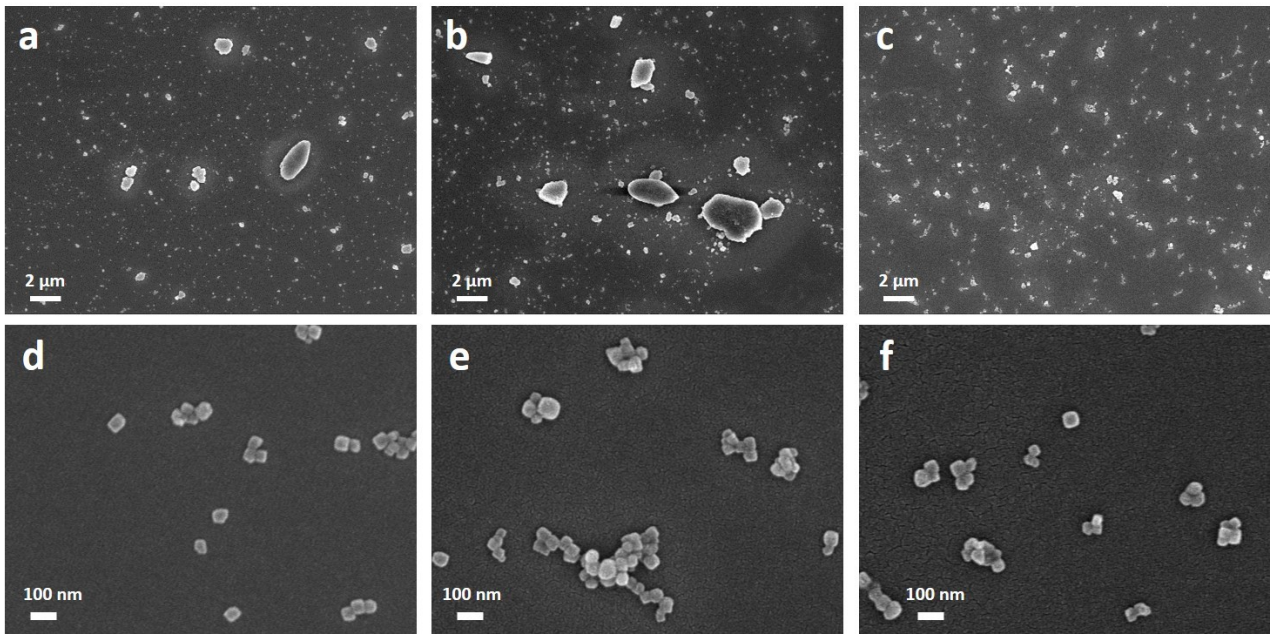


Figure S1. SEM images of DNA-PBNPs synthesized with varying ratios of PBNPs to polyT-DNA. (A) Scheme of DNA-modified PBNPs by DNA adsorption on the porous surface of PBNPs. (B) Table of three concentration ratios of PBNPs to polyT-DNA used in DNA-PBNP synthesis. (C) SEM images of DNA-PBNPs synthesized at different PBNP to polyT-DNA ratios: (a, c) Condition I: 0.1 mg/mL PBNPs and 10 μM poyT-DNA; (b, e) Condition II: 0.05 mg/mL PBNPs and 10 μM poyT-DNA; (c, f) Condition III: 0.03 mg/mL PBNPs and 10 μM poyT-DNA. Scale bar: (a-c) 2 μm and (d-f) 100 nm.

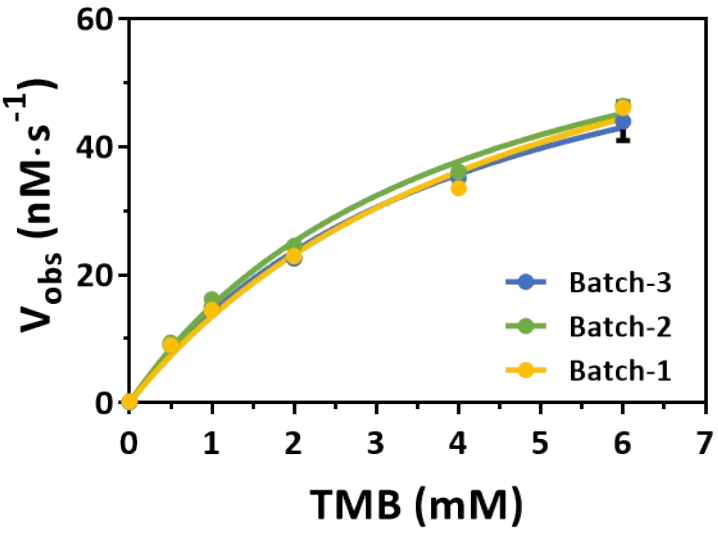


Figure S2. Catalytic activity of the DNA-PBNPs with different synthesized batches.

(A) Rates of oxidation of different concentrations of TMB_{red} to TMB_{ox} by different batch of DNA-PBNPs in the presence of H_2O_2. In all experiments, [H_2O_2] = 2 mM, [PBNP] or [DNA-PBNPs] = 10 μg mL^{-1}.

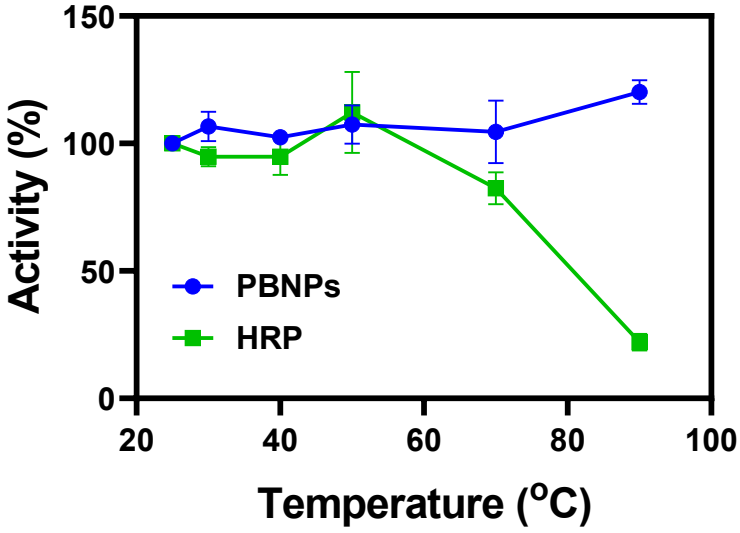


Figure S3. Comparative analysis of catalytic activity between PBNPs and horseradish peroxidase (HRP) at various temperatures. After PBNPs and HRP were maintained for 15 minutes at different temperatures, their catalytic activity was tested using K-Blue reagent as the substrate. [PBNP] and [HRP] = 10 μg mL^{-1}.

We examined the impact of glutathione (GSH) on the self-cleavage process of Cu^{2+}-DNAzyme using DNA electrophoresis (Figure S3). Gel electrophoresis of the self-cleavage assay showed that the DNA enzyme remained intact in the presence of only GSH or only Cu^{2+}. In contrast, when GSH and Cu^{2+} coexist, the gel displays cleavage products (P1 and P2). These results revealed that both copper ions and glutathione (GSH) are indispensable for activating the catalytic capability of DNAzyme.

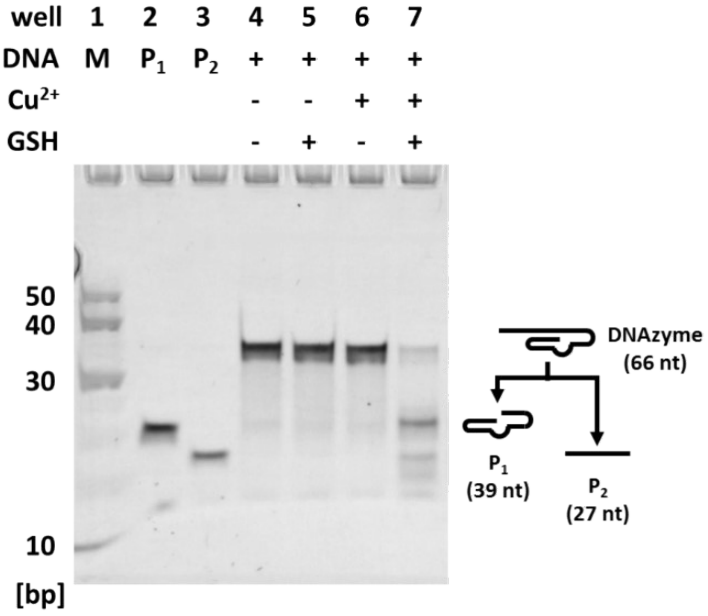


Figure S4. Influence of glutathione on the self-cleavage reaction of Cu^{2+}-DNAzyme.

M is the DNA ladder; P_1 and P_2 are the product of the self-cleavage reaction of Cu^{2+}-DNAzyme. The catalytic assay of Cu^{2+}-DNAzyme was performed in a 20 μL reaction mixture containing 0.5 μM Cu^{2+}-DNAzyme, 50 μM Cu^{2+}, 50 μM GSH, 0.3 M NaCl, and 50 mM Tris-HCl (pH 7.0). The reaction mixture incubated at room temperature for 1 hour. The experiment was conducted on 12% acrylamide (19:1) denature gel with 1× TBE running buffer under 120 V, 50 minutes.

The metal ion selectivity of Cu^{2+}-DNAzyme was investigated by different metal ions (Cu^{2+}, Mg^{2+}, K^{+}, Ca^{2+}, Fe^{3+}, Ni^{2+}, and Zn^{2+}) in the presence of GSH. Only Cu^{2+} efficiently triggered self-cleavage of DNAzyme, resulting the production of cleavage products (P_1 and P_2). According to the result, this self-cleavage reaction of the Cu^{2+}-DNAzyme is specific for Cu^{2+}.

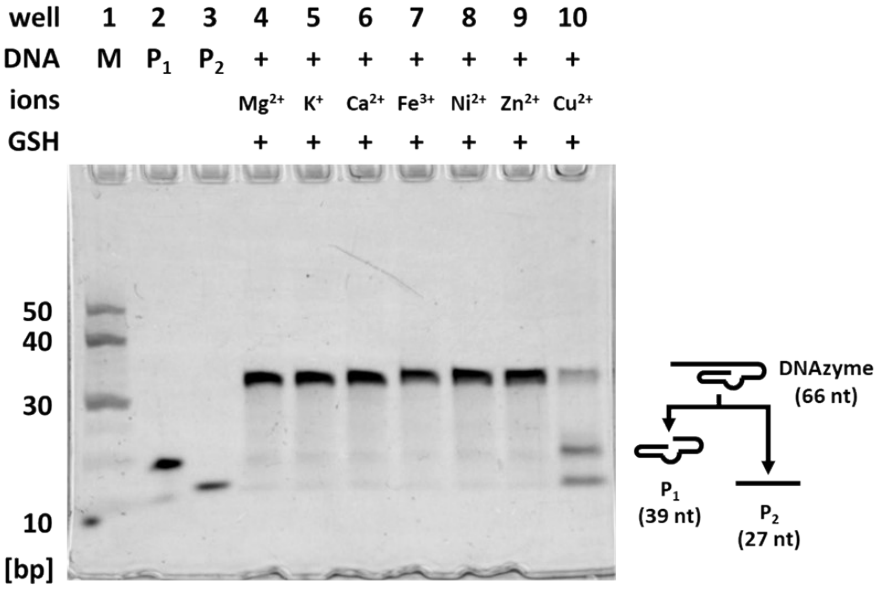


Figure S5. Influence of metal ions on the self-cleavage reaction of Cu^{2+}-DNAzyme.

M is the DNA ladder; P_1 and P_2 are the product of the self-cleavage reaction of Cu^{2+}-DNAzyme. The catalytic assay of Cu^{2+}-DNAzyme was performed in a 20 μL reaction mixture containing 0.5 μM Cu^{2+}-DNAzyme, 50 μM different metal ions (Mg^{2+}, K^{+}, Ca^{2+}, Fe^{3+}, Ni^{2+}, Zn^{2+}, and Cu^{2+}, respectively), 50 μM GSH, 0.3 M NaCl, and 50 mM Tris-HCl (pH 7.0). The reaction mixture was incubated at room temperature for 1 hour. The experiment was conducted on 12% acrylamide (19:1) denature gel with 1ᘔ TBE running buffer under 120 V, 55 minutes.

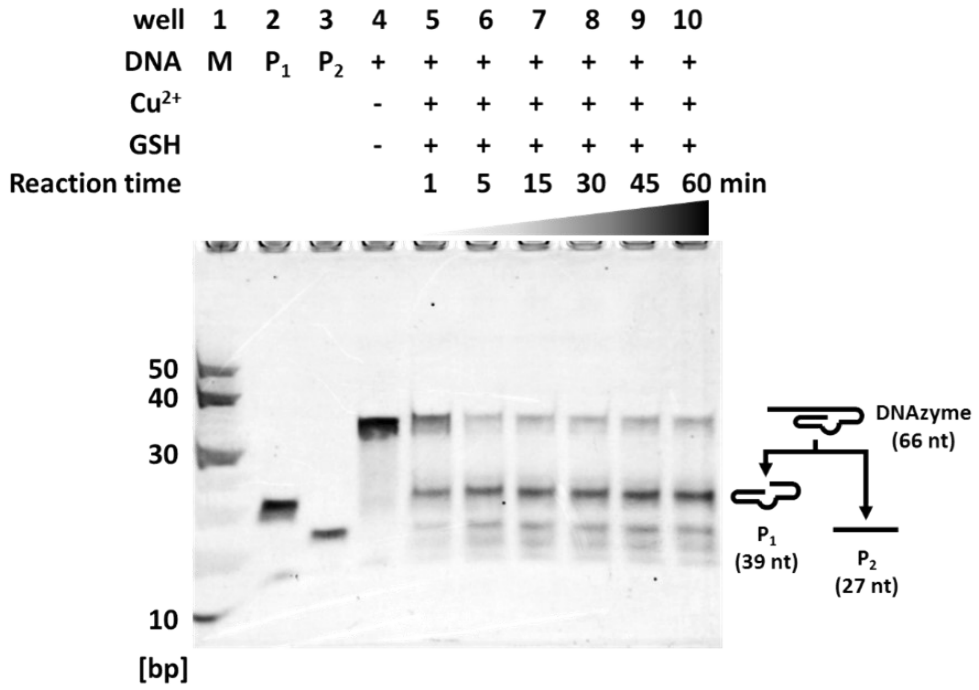


Figure S6. Influence of reaction time on self-cleavage the reaction of Cu^{2+}-DNAzyme.

M is the DNA ladder; P_1 and P_2 are the product of the self-cleavage reaction of Cu^{2+}-DNAzyme. The catalytic assay of Cu^{2+}-DNAzyme was performed in a 20 μL reaction mixture containing 0.5 μM Cu^{2+}-DNAzyme, 50 μM Cu^{2+}, 50 μM GSH, 0.3 M NaCl, and 50 mM Tris-HCl (pH 7.0). The reaction mixture was incubated at room temperature for 1, 5, 15, 30, 45, and 60 minutes, respectively. The reaction mixture was incubated at room temperature for 1 hour. The experiment was conducted on 12% acrylamide (19:1) denature gel with 1× TBE running buffer under 120 V, 55 minutes

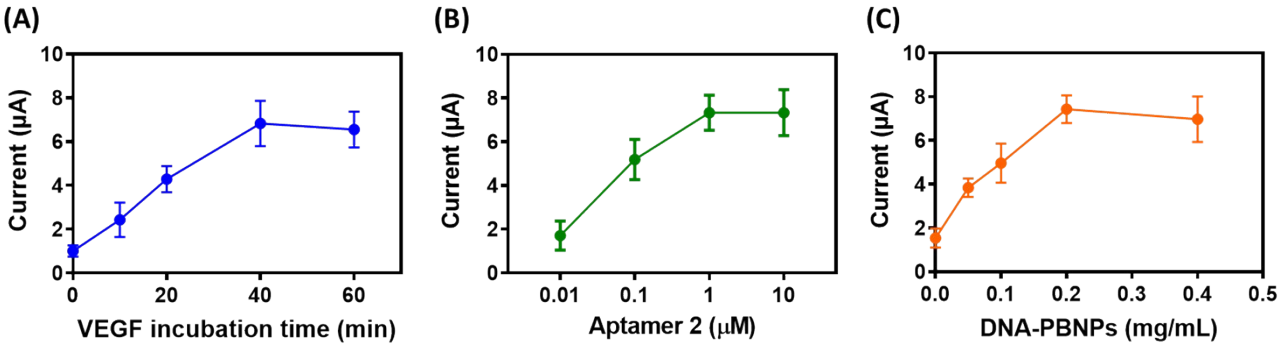


Figure S7. Optimization of DNA-PBNPs-based biosensor for VEGF detection.

The effect on the output signal due to varying factors is as follows: (A) the duration of VEGF incubation (ranging from 0 to 60 minutes), (B) the concentrations of Aptamer 2 (ranging from 0.01 to 2 μM), and (C) the concentrations of DNA-PBNPs (ranging from 0 to 0.4 mg/mL). N=3, mean ± SD.

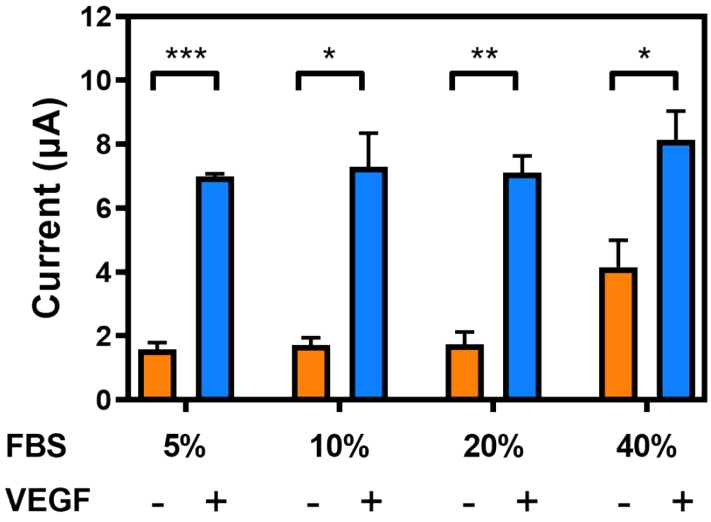


Figure S8. Matrix effect of DNA-PBNPs-based biosensor for VEGF detection.

The output signals of the biosensor under various percentages of fetal bovine serum (FBS) in the absence and presence of VEGF (10 ng/mL). (*: $P \leq 0.05$, **: $P \leq 0.01$, ***: $P \leq 0.001$)