Supporting Information

for

A Novel Truncated DNAzyme Modified Paper Analytical Device for Point-of-Care Test of Copper Ions in Natural Waters

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List

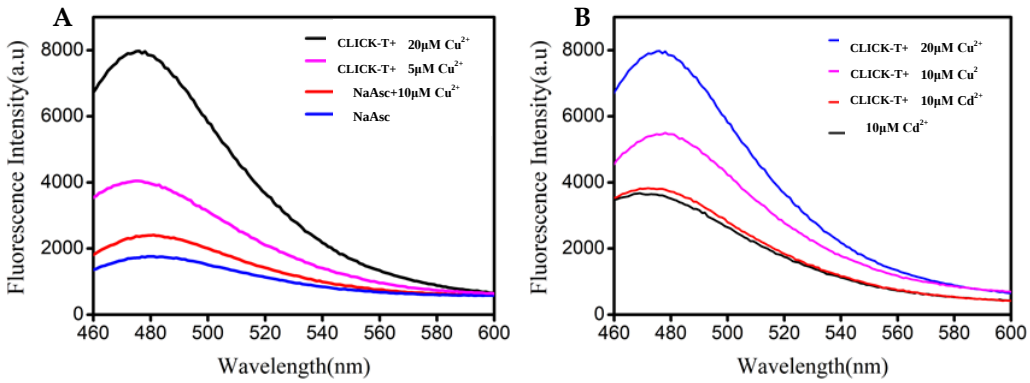

Figure S1. (**A**) The comparison of Cu^{2+} catalyzed azide-alkyne click reaction with 4μM CLICK T and conventional CuAAC based on 1mM NaAsc reducing Cu^{2+} to Cu^{+}. (**B**) The comparison of Cu^{2+} and Cd^{2+} catalyzed azide-alkyne click reaction with 4μM CLICK T. The concentrations of both AHC and BOL were 100 μM in pH 6.5 25mM HEPES buffer and reacted for 20min.

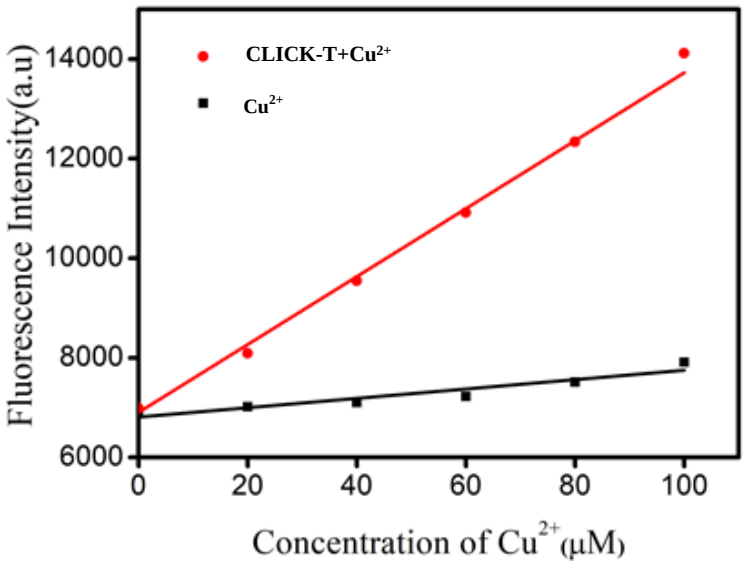

Figure S2. The comparison of Cu^{2+} catalyzed azide-alkyne click reaction with (red line) and without 4μM CLICK-T (black line). The concentrations of both AHC and BOL were 100 μM in pH 6.5 25mM HEPES buffer and reacted for 20min.

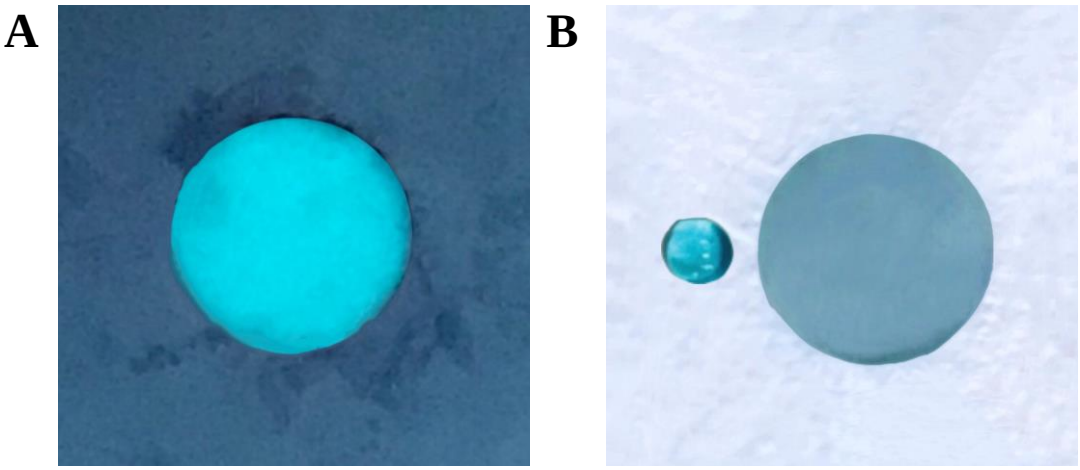

Figure S3. 10μL of 100 mM Cu^{2+} was dripped on CLICK-T modified PDAs (**A**) Under ultraviolet light (365 nm) or without (**B**) UV light.

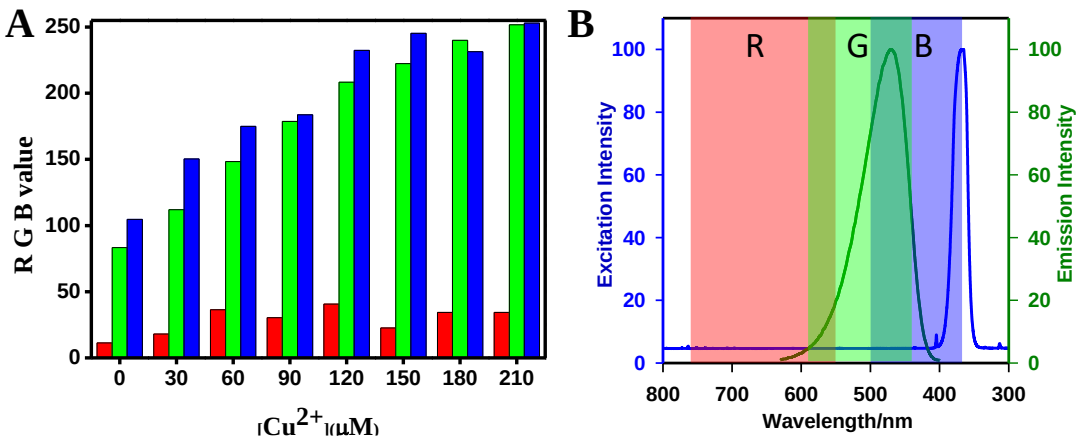

Figure S4. (**A**) PDA's RGB values obtained with the Pixolor App software app in the mobile phone versus the copper concentration. (**B**) The RGB distribution of the FL signal.

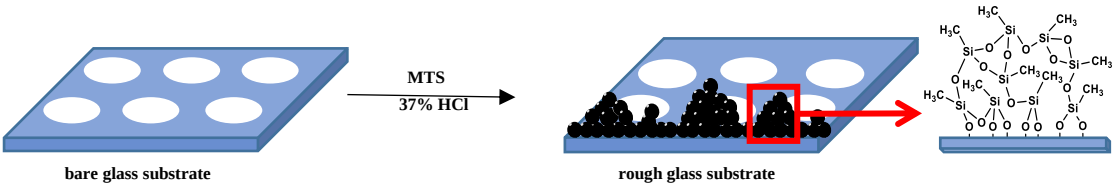

Figure S5. Schematic diagram of paper-based hydrophobization reaction.

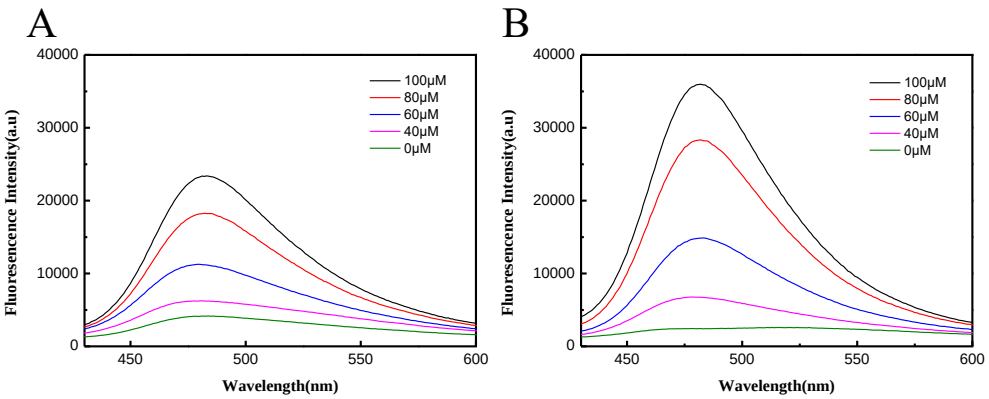

Figure S6. (**A**) Cu^{+} catalyzed the click reaction of 3-azide-7-hydroxycoumarin-hexynol. (**B**) Cu^{+} catalyzed the click reaction of 3-azide-7-hydroxycoumarin-butynol. The concentrations of AHC was 100 μM in pH 6.5 25 mM HEPES buffer and reacted for 20 min.

Table S1. The base sequence of CLICK-17 and other DNA with random sequence.

Name	Base sequence
CLICK-17	5′-GGA TCG TCA GTG CAT TGA GAT TA TTA TGC AAC TCTAT GGG TCC ACT CTG TGA ATG TGA CGG TGG TAT CCG CAA CGG GTA-3′
Other DNA	5′-TAA TCC AGA TAG GGA GCA AAT CGT ACT CCC ATC TATA AAA CCC TCT AGA CCT TTA ATA GGC AGT AAG TCA ATT TAA AAA ACC-3′

It has been reported that the shorter the ssDNA, the stronger its tissue penetration ability; moreover, an appropriate truncation of the DNAzyme could remove additional bases that might hinder target binding [1]. The CLICK-17 that was obtained through the SELEX had 79 bases (Table S1). Its affinity constant to Cu(II) was shown in Table S2. Because the preparation cost of CLICK-17 was relatively high. Hence, it is necessary to optimize the original CLICK-17 DNAzyme by removing the primers at both ends of CLICK-17.

CLICK-T1 (Table S1) was a sequence consisting of 40 bases obtained by removing the primers at both ends of CLICK-17. The Kd value showed it had good affinity for the target. Thus, we furthermore removed 3 base at both ends of structure of CLICK-T1, thereby yielded sequence CLICK-T2. It also had a slightly higher affinity for the target than CLICK-T1 (Table S1). Subsequently,3 bases were removed simultaneously from the 5′ and 3′ ends of the CLICK-T2 sequence, generating the desirable sequence CLICK-T which was 18 bases, and reducing the Kd value of the sequence to 28.12 ± 2.77 nM. Next, both ends of the CLICK-T were simultaneously and successively truncated, and sequences CLICK-T3 and CLICK-T4 were obtained. However, the affinity of these two sequences was not ideal, and their Kd values increased by an order of magnitude. Therefore, we employed CLICK-T as our final truncated DNAzyme for Cu(II)AAC. All the explanation and Table S2 was added in the latest supplementary file.

Table S2. Sequence and dissociation constants (K_d) for the studied *DNAzyme.*

Name	Sequence（5′to 3′）	K_d[nM]
CLICK-17	GGA TCG TCA GTG CAT TGA GAT TA TTA TGC AAC TCTAT GGG TCC ACT CTG TGA ATG TGA CGG TGG TAT CCG CAA CGG GTA	52.32±3.43
CLICK-T1	CAT TGA GA TTA TTA TGC AAC TCTAT GGG TCC ACT CTG TGA	37.25±3.34
CLICK-T2	TGA GA TTA TTA TGC AAC TCTAT GGG TCC ACT	33.2 ± 1.2
CLICK-T	TTA TTA TGC AAC TCTAT G	28.12 ± 2.77
CLICK-T3	TTA TGC AAC TCTAT GGG	55.61±7.34
CLICK-T4	TTA TTA TGC AAC TCTA	78.65±8.12

Table S3. The pH, dissolved oxygen, ion concentrations of the water samples.

Sample	pH	Dissolved Oxygen (mg/L)	Ion Concentrations (mg/L)
Fenghua river	6.5	7.6	320
Yuyao river-1	6.5	8.3	400
Yong river-2	7.2	9.5	620
Ningbo university	7.4	8.4	420
Baixi reservoir	7.1	10.2	200

Reference

[1] Sun, Y., Duan, N., Ma, P., Liang, Y., Zhu, X., Wang, Z. Colorimetric aptasensor based on truncated aptamer and trivalent DNAzyme for Vibrio parahemolyticus determination. Journal of agricultural and food chemistry, 2019, 67(8), 2313–2320.