## **Supplementary materials**

## Enzymatically-generated long polyT Templated-Copper

## Nanoparticles for Versatile Biosensing Assay of DNA-related

**Enzymes Activity** 

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**Figure S1**. Fluorescence responses at 625 nm of DNA-CuNPs templated by  $1\mu$ M T<sub>40</sub> (1) or (1) with TdT buffer (2), 1 mM dTTP (3), or 400 U/mL TdT (4).



**Figure S2.** Detection of repeatability of DNA-CuNPs as biosensor involving ten samples in the pressence of 400 U/mL TdT.



Figure S3. Influence of polymerization time on the formation of fluorescent CuNPs in the presence of 1  $\mu$ M DNA-P records at  $\lambda_{em} = 625$  nm.



Figure S4. Influence of dTTP concentration on the formation of fluorescent CuNPs in the presence of 1  $\mu$ M DNA-P records at  $\lambda_{em} = 625$  nm.



Figure S5. Influence of  $Cu^{2+}$  concentration on the formation of fluorescent CuNPs in the presence of 1  $\mu$ M DNA-P records at  $\lambda_{em} = 625$  nm.



Figure S6. Fluorescence spectra of DNA-CuNPs synthesized by  $T_{40}$  of 0  $\mu$ M (1), 0.1  $\mu$ M (2), 1  $\mu$ M (3), 2  $\mu$ M (4), 4  $\mu$ M (5), 6  $\mu$ M (6) and by 0.1  $\mu$ M TdT-generated DNA-P-polyT (7).



Figure S7. (A) TEM characterization of DNA-CuNPs templated by  $T_{40}$ . And (B) DNA-CuNPs diameters supported by  $T_{40.}$ 

Number	Added/U	Found/U	Recovery/%	RSD/%
1	1	0.92	92.11	3.49
2	1.5	1.41	93.56	1.78
3	2	1.81	90.67	2.34
4	3	2.79	93.31	3.25
5	4	3.75	93.08	1.76

 Table S1. Measurement results of TdT in complex biological medium.



**Figure S8.** Assay of the inhibition of tetrasodium pyrophosphate (PP) on TdT. The inhibitor analysis was conducted by incubation of 400 U/mL TdT with varying concentrations of PP in the presence of 1  $\mu$ M DNA-P and 1 mM dTTP. *IC*<sub>50</sub> = 1.85 mM.



**Figure S9**. The fluorescence responses of the proposed biosensing system based on TdT-generated DNA-CuNPs to BamHI (25 U/mL) and other non-target enzymes. The concentration of non-target enzyme was 120 U/mL.



**Figure S10.** The fluorescence responses of the proposed biosensing system based on TdT-generated DNA-CuNPs to ALP (1 U/mL) and other non-target enzymes. The concentration of non-target enzyme was 120 U/mL.