Electronic Supplementary Information

Enzymatic Polymerization of Poly (Thymine) for Copper Nanoparticles Synthesis with Tunable Size and Its Application in Enzyme Sensing

Tai Ye[a], Chunying Li[b], Chen Su[a], Xinghu Ji[a], Jiao Zheng[a], Philip Tinnefeld[c] and Zhike He[a]*

[a] *Key Laboratory of Analytical Chemistry for Biology and Medicine (Ministry of Education), Wuhan University, 430072, P. R. China Email: zhkhe@whu.edu.cn*

[b] *Department of Chemistry, Xinxiang Medical University, Xinxiang, 453003, P. R. China*

[c] *NanoBioSciences Group, Institute for Physical and Theoretical Chemistry and Braunschweig Integrated Centre of Systems Biology (BRICS), and Laboratory for Emerging Nanometrology (LENA), Braunschweig University of Technology, Braunschweig, 38106, Germany.*

Experiment sections

Materials

All oligonucleotide with different sequences were synthesized and HPLC purified by Sangon Biotechnology Co., Ltd (Shanghai, China). The sequences of the oligonucleotide used in this work are as follows:

Primer DNA: 5'-CCCTTAATCCCC-3'

T4 PNK Probe: 5'-P-CCCTTAATCCCC-P-3'

Poly-adeine: 5'-AAAAAAAAAAAA-3'

Copper sulfate ($CuSO_4 \cdot 5H_2O$) and ascorbic acid were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 3-(N-morpholino) propanesulfonic acid (MOPS) and adenosine diphosphate (ADP) were purchased from Amersco (USA). T4 DNA ligase, DNase, exonuclease III, and Deoxythymidine 5'-triphosphate (dTTP, 100 mM) were obtained from Takara (Dalian, China), while T4 polynucleotide kinase was purchased from New England Biolabs (NEB). Terminal deoxynucleotidyl transferase (TdT, 20 U/μL), and the 5× reaction buffer (1 M potassium cacodylate, 0.125 M Tris, 0.05 % (v/v) Triton X-100, 5 mM $CoCl_2$, pH 7.2 at 25 °C) were purchased from Fermentas (St. Leon-Rot, Germany). All chemical reagents were of analytical grade and used without further purification. All solutions were prepared with ultra-pure water (18.25 MΩ·cm) from a Millipore system.

Instruments

Fluorimetric spectra were obtained with a RF-5301PC spectrophotometer (Shimadzu, Japan)

equipped with a 150 W xenon lamp (Ushio Inc, Japan). All optical measurements were performed at room temperature under ambient conditions, and the fluorescence emission intensity was measured at 600 nm with excitation wavelength at 340 nm. Gels were imaged by a ChemiDoc XRD system (Bio-Rad). Morphology observation of Cu NPs was carried out on high-resolution transmission electron microscopy machine (JEM-2010 FEF) at an acceleration voltage of 200 kV. Time-resolved fluorescence decay curves were performed using a FLS920 single-photon counting fluorometer (Edinburgh Analytical Instruments, UK) with a pulsed microsecond nitrogen lamp as excitation source.

Polymerization of poly-T DNA template by TdT:

The polymerization mixture consisted of 10 μM DNA primer, 10 mM dTTP, and various concentration of TdT in 20 μL of 1× TdT reaction buffer. The mixture was incubated at 37 ºC for 5 h, and then heated to 75 ºC for 10 min to inactivate the enzyme. The polymerization mixture was subsequently used for the synthesis of Cu NPs.

Argrose gel Analysis of poly (thymine) yielded by TdT:

3 % agarose gel was prepared using 1× TBE buffer. The loading samples were prepared by mixing 10 μL of the extended product with 20 μM poly-adenine in PBS buffer (10 mM PB, 150 mM NaCl, pH 7.6). The mixture were then stained by SYBR Green I. DNA gel electrophoresis was carried out on a horizontal electrophoresis system at 100 V for 40 min in 1× TAE buffer.

Synthesis of Cu NPs:

Firstly, the products of enzymatic polymerization were diluted to desired concentration with the MOPS buffer (10 mM MOPS, 150 mM NaCl, pH 7.6), Then, 1 mM ascorbic acid was added into the DNA solutions. After blending completely, corresponding concentration of Cu^{2+} was added and incubated at room temperature to form fluorescent Cu NPs.

Detection of T4 PNK concentration:

Briefly, a total volume of 20 μL of solution containing varying concentrations of T4 PNK, T4 PNK Probe (1 μM), and dTTP (10 mM) was incubated at 37 ºC for 30 min. Then, 10 U TdT and 6 μL 5× TdT reaction buffer were added to give a final volume of 33 μL. After incubation for 5h, the enzymatic products were diluted to form Cu NPs according to the above-mention procedure.

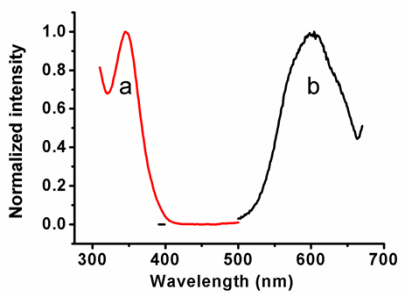

Figure S1 Excitation (a) and emission (b) spectra for the bottom-up synthesis of Cu NPs.

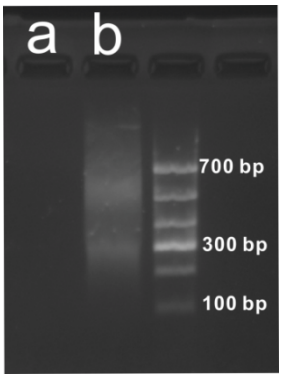

Figure S2 Gel electrophoresis image of products of the bottom-up strategy in the absence (a) and presence (b) of TdT. The concentration of Poly-adenine and TdT were 20 μM and 20 U, respectively. Incubation times: 5h.

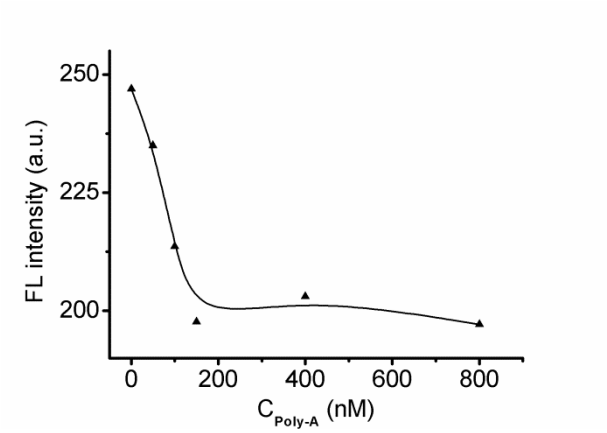

Figure S3 The fluorescence intensity of the Cu NPs after hybridization with various concentrations of Poly-adenine.

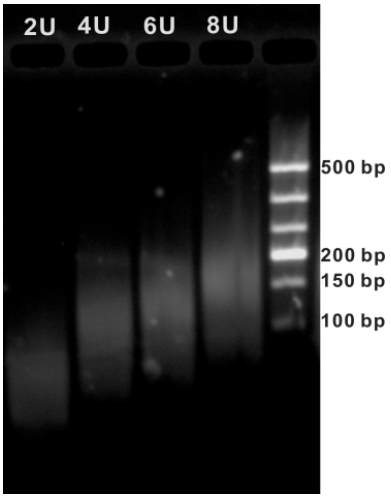

Figure S4 Agarose gel electrophoresis results showing enzymatically products with four different concentrations of TdT: 2 U, 4 U, 6 U, and 8 U (From the left to the right). The concentration of Poly-A sequence was 20 μM. Incubation times: 5h.

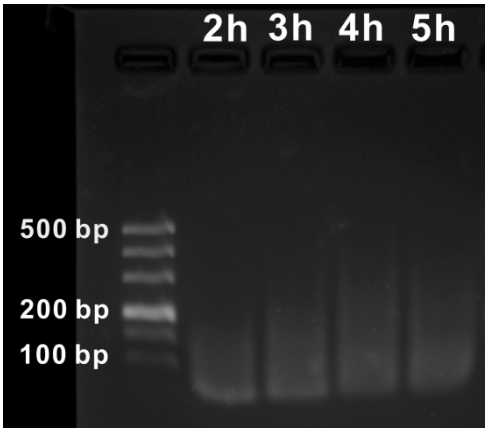

Figure S5 Agarose gel electrophoresis results showing enzymatically products with different incubation times. (From the left to the right: 2 h, 3 h, 4 h, and 5 h). The concentration of Poly-adenine sequence and TdT were 20 μM and 4U, respectively.

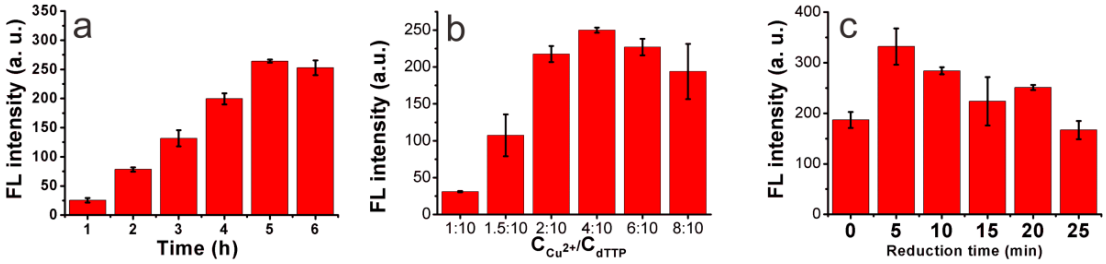

Figure S6 The fluorescence intensity of Cu NPs upon different incubation time of the elongation reaction (a), the addition of different concentrations ratios between Cu^{2+} and dTTP (b), and the reduction time of Cu NPs synthesis (c). Experimental conditions: primer DNA, 10 μM; dTTP, 10 mM; ascorbic acid, 1 mM.

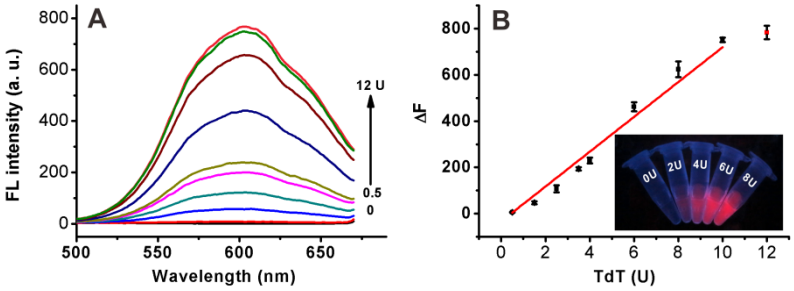

Figure S7 (A) Fluorescence spectra of the assay system in the presence of different concentrations of TdT. (B) Linear relationship between the fluorescence intensity and TdT concentrations. Inset: photographs of the Cu NPs solutions form at different concentations of the TdT under the UV beam of 365 nm.

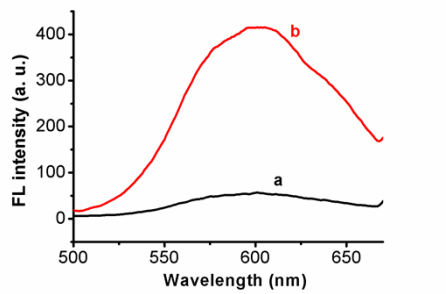

Figure S8 Fluorescence emission spectra of the sensing system in the presence of different concentrations of T4 PNK (a: 0 and b: 1.25 U mL^{-1}). Experimental conditions: TdT, 10 U; T4 PNK probe, 1 μM; dTTP, 10 mM.

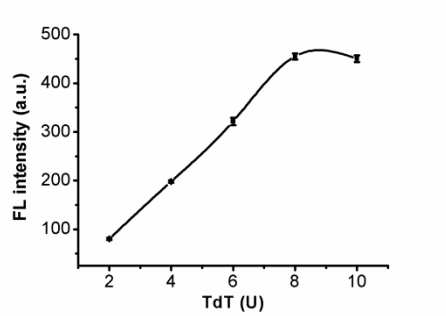

Figure S9 Effects of the concentration of TdT on the fluorescent intensity of the T4 PNK sensing system.

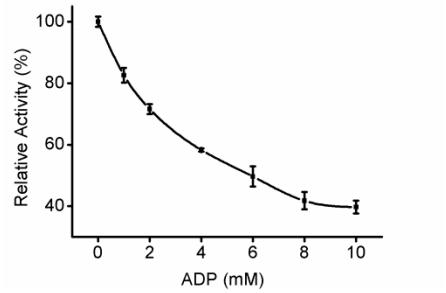

Figure S10 Inhibition effects of ADP on the concentration of T4 PNK. The concentration of T4 PNK was 1.25 U mL^{-1}. The error bar indicates the standard deviation from three independent experiments.

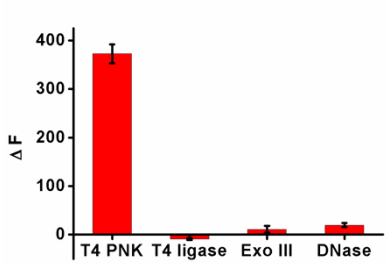

Figure S11. The selectivity of the proposed strategy for T4 PNK assay. The concentration of TdT was 1.25 U mL^{-1}, and the concentration of the other species were 12.5 U mL^{-1} Error bars represent standard deviations (n =3)