Electronic Supplementary Information (ESI)

A fluorescent ELISA based on the enzyme-triggered synthesis of poly(thymine)-templated copper nanoparticles

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EXPERIMENTAL SECTION

Chemicals and Apparatus. The oligonucleotides poly(thymine) (T30) (5′-TTT TTT TTT TTT TTT TTT TTT TTT TTT-3′) was synthesized and purified by SangonBiotech Company, Ltd. (Shanghai, China). Sodium L-ascorbate, L-ascorbic acid 2-phosphate trisodium salt, magnesium chloride, hydroxylammonium chloride, sodium hydroxide, hydrazine monohydrate were purchased from Aladdin Industrial Corporation (Shanghai, China). Alkaline phosphatase (ALP) from bovine intestinal mucosa, acetylcholinesterase, bovine serum albumin, choline oxidase, glucose oxidase, lysozyme, IgG (from human serum), peroxidase from horseradish, trypsin, sodium borohydride, tetrakis(hydroxymethyl)phosphonium chloride, sodium citrate tribasic dihydrate, 3-(N-morpholino)propanesulfonic acid (MOPS) and copper(II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Rabbit anti-human IgG antibody was purchased from Santa Cruz (CA, USA), Alpha-fetoprotein (AFP) was bought from ProSpec (Ness Ziona, Israel). Rabbit anti-AFP antibody was purchased from Proteintech (Wuhan, China). ALP-conjugated secondary antibody (goat anti-rabbit IgG labeled with ALP) was purchased from Abcam (Cambridge, MA, USA). Both the wash buffer and antibody diluent buffer for ELISA was purchased from Boster (Wuhan, China). All reagents were analytical grade and used as received without any further purification. The ultrapure water from a Millipore system was used in all aqueous solution.

The fluorescence emission spectra of all samples were recorded in a Hitachi F-4600 spectrofluorometer (Tokyo, Japan), using a 0.2 cm path length quartz cell at room temperature. Fluorescence photographs were taken by a common digital camera under illumination of a hand-held ultraviolet-lamp with a wavelength of 365 nm.

Sensing ALP activity based on the synthesis of fluorescent polyT-CuNPs. A fluorescent ALP activity assay was performed using the following procedures. Volumes of 10 μL of AA2P (20 mM), 20 μL of $MgCl_2$ (5 mM) and 20 μL of water were injected into a 1.5 mL microcentrifugal tube. The 50 μL of freshly prepared ALP aqueous solutions with different activities ranging from 0 to 600 mU/mL were then added into the mixtures, respectively. These ALP-treated AA2P solutions were incubated at 37 °C for

60 min, and mixed with the solution containing 20 μL of MOPS buffer (100 mM MOPS, 1.5 M NaCl, pH 7.4), 20 μL of $CuSO_4$ (2 mM) and 60 μL of polyT (T30, 10 μM). The fluorescence spectra of the above mixture solutions were recorded after a brief mixing (30 second). The selectivity of this sensing system for ALP activity was assessed by using other routine proteins/enzymes instead of ALP.

Fluorescent ELISA for the Model Protein. Prior to the fluorescent ELISA, the 96-well microplates were modified and functionalized as follows: first, 100 μL of human IgG standards with various concentrations was added into wells and incubated at 4 °C overnight (Note: After each following step until the enzyme reaction, the wells were rinsing three times with the wash buffer). Second, the wells were blocked with 5 % bovine serum albumin (BSA) at 37 °C for 1 h. Third, 100 μL of diluted rabbit anti-IgG (1:500) in antibody diluent buffer was added and finally we added 100 μL of (goat antirabbit IgG labeled with ALP (1:500), and the wells were incubated at 37 °C for 1 h.

Subsequently, 10 μL of AA2P (20 mM), 20 μL of $MgCl_2$ (5 mM), and 70 μL of water were added into the wells for an enzyme reaction at 37 °C for 1 h. And then the solution containing 20 μL of MOPS buffer, 20 μL of $CuSO_4$ (2 mM) and 60 μL of polyT (10 μM) was mixed with the above solution in the wells and reacted at room temperature for 30 second before the fluorescence spectra measurements. The detailed procedures for sensing another model protein, AFP, are based on that for the human IgG by just changing the addition of IgG standards and rabbit anti-IgG to AFP standards and rabbit anti-AFP, respectively.

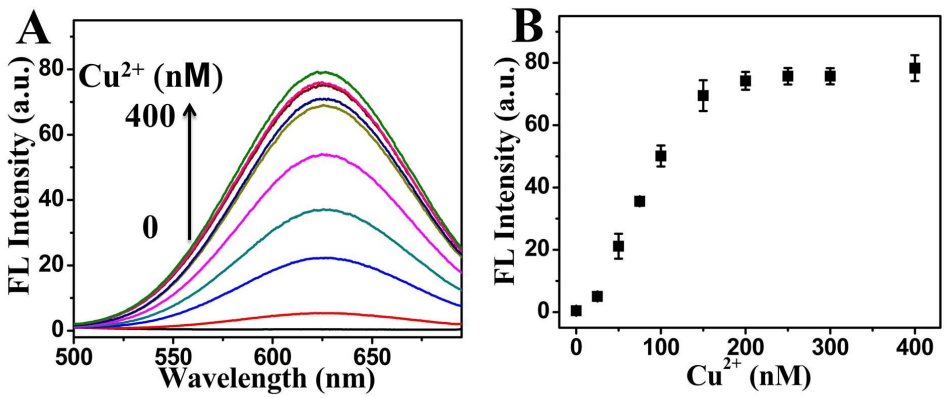

Fig. S1 Fluorescence emission spectra (A) and intensities at 625 nm (B) of solutions containing constant sodium ascorbate (1 mM) and polyT (3 μM) in MOPS buffer (10 mM MOPS, 150 mM NaCl, pH 7.4) toward Cu^{2+} ions with various concentrations.

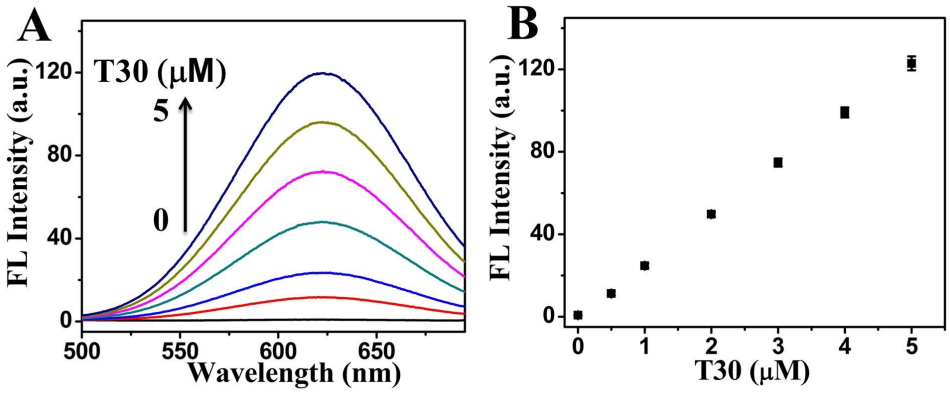

Fig. S2 Fluorescence emission spectra (A) and intensities at 625 nm (B) of solutions containing constant sodium ascorbate (1 mM) and Cu^{2+} ions (200 μM) in MOPS buffer (10 mM MOPS, 150 mM NaCl, pH 7.4) toward polyT with various concentrations.

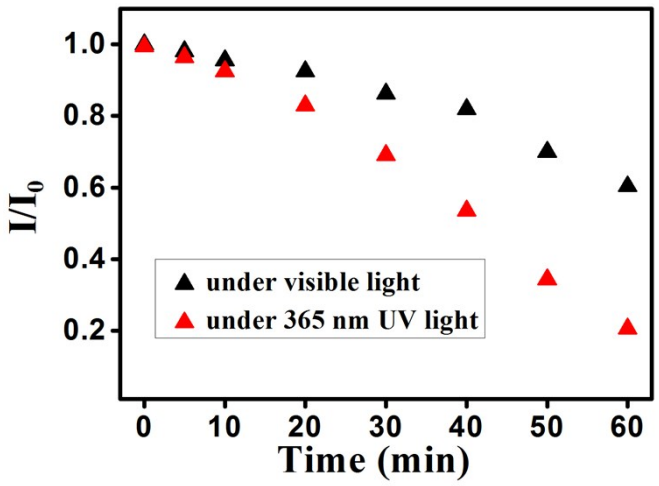

Fig. S3 Stablility of the fluorescent polyT-CuNPs system measured with the relative fluorescence intensity at 625 nm of the as-prepared polyT-CuNPs (the sodium ascorbate and polyT in MOPS buffer was mixed with Cu^{2+} ions and reacted for 5 min) as a function of irradiation time under the visible and 365 nm ultraviolet light.

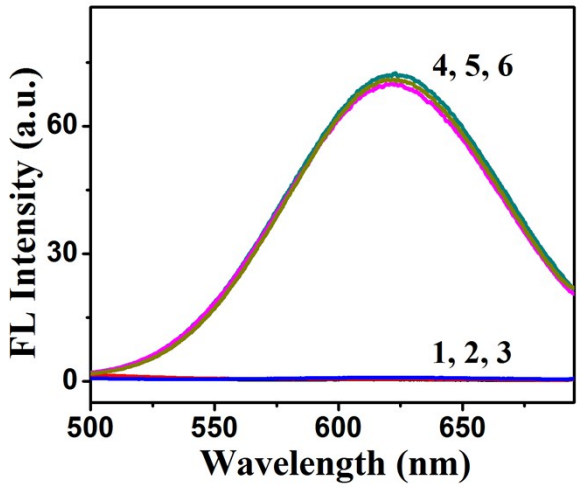

Fig. S4 Fluorescence emission spectra of the solutions containing polyT and Cu^{2+} ions mixed without (1−3) and with (4−6) sodium ascorbate in the presence of no additional agent (1, 4), rabbit antihuman IgG (2, 5), and goat antirabbit IgG labeled with ALP (3, 6), respectively, in MOPS buffer (pH 7.4).

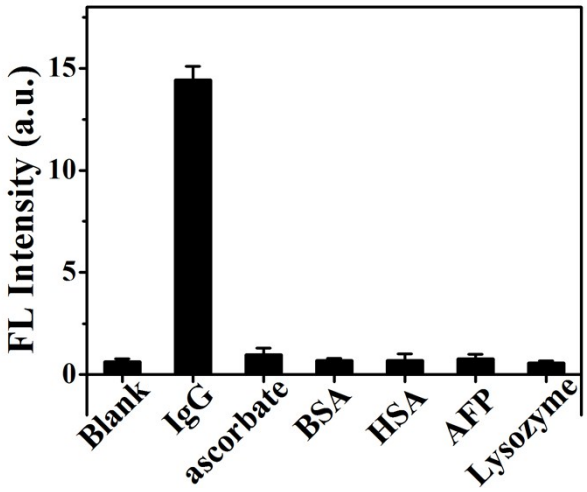

Fig. S5 Fluorescence responses of the developed fluorescent ELISA against the human IgG and potential interferences (ascorbate and proteins).

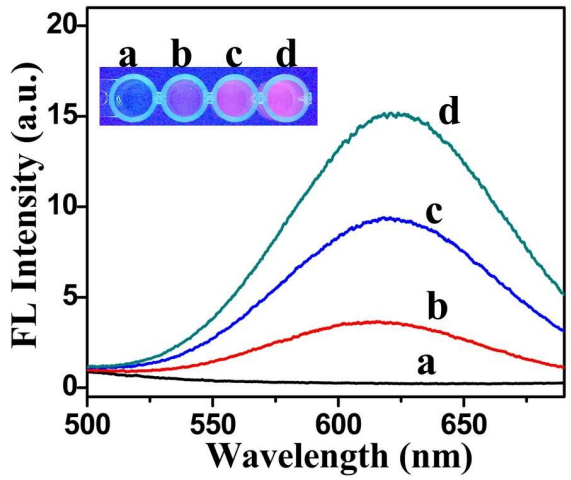

Fig. S6 Fluorescence emission spectra and photographs under 365 nm light (inset) of the ELISA system toward various concentrations of human IgG in fetal bovine serum (0 for a, 50 ng/mL for b, 100 ng/mL for c, 200 ng/mL for d).

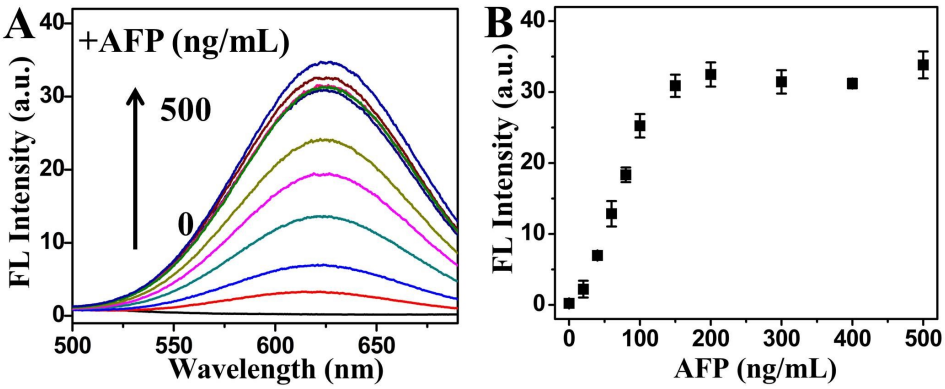

Fig. S7 The fluorescence emission spectra (A) and fluorescence intensities at 625 nm (B) of the ELISA toward AFP standards with various concentrations.