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Supporting Information

A simple thermometer based photothermometric assay for alkaline

phosphatase activity based on target-induced nanoprobe generation

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Fig. S1. (a) Photographs, (b) UV-vis absorption spectra and (c) temperature changes (Δ T) of the ALP-induced in situ PB NPs generation system with different components. The final concentrations of Fe³⁺, K₃[Fe(CN)₆], AAP, ALP and AA are 0.5 mM, 0.25 mM, 0.5 mM, 10 U/L, and 75 μ M, respectively (1: Fe³⁺, 2: K₃[Fe(CN)₆], 3: AAP, 4: ALP, 5: Fe³⁺+K₃[Fe(CN)₆], 6: Fe³⁺+AAP, 7: Fe³⁺+ALP, 8: K₃[Fe(CN)₆]+AAP, 9: K₃[Fe(CN)₆]+ALP, 10: AAP+ALP, 11: Fe³⁺+K₃[Fe(CN)₆]+AAP, 12: Fe³⁺+K₃[Fe(CN)₆]+ALP, 13: K₃[Fe(CN)₆]+AAP+ALP, 14: Fe³⁺+AAP+ALP, 15: Fe³⁺+K₃[Fe(CN)₆]+AAP+ALP, 16: Fe³⁺+K₃[Fe(CN)₆]+AA). Error bars indicate standard deviations (n=3).



Fig. S2. Optimization of the reaction condition for enzymatic incubation. (a) The incubation pH of enzyme. (b) The incubation time of enzyme. (c) Concentration of AAP. The final concentrations of Fe^{3+} , $K_3[Fe(CN)_6]$ and ALP are 0.5 mM, 0.25 mM, and 10 U/L, respectively.



Fig. S3. Optimization of the reaction condition. (a) Reaction pH. (b) Reaction time. (c) Fe^{3+} concentration. (d) $K_3[Fe(CN)_6]$ concentration. The final concentrations of AAP and ALP are 0.5 mM and 10 U/L, respectively.



Fig. S4. (a) The line chart of the relationship between Fe^{2+} concentrations (0.015-0.3mM) and the temperature increment (ΔT) of the colorimetric reaction system with different irradiation time (10-180 s). (b) Calibration plot of the temperature changes (ΔT) of the reaction solutions at 90 s vs the logarithm of the Fe²⁺ concentration.



Fig. S5. (a) UV-vis absorption spectra and (b) temperature changes of the reaction solution system with the addition of 0.25 U/L, 0.5 U/L and 1 U/L ALP. Inset: photographs of the corresponding solution.



Fig. S6. Temperature changes of the reaction solution system for eight sample solutions with the same ALP concentration (10 U/L).

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Fig. S7. The calculated result of the PNPP-based standard method. (The absorbance at 405 nm was linearly correlated with the logarithm of the ALP concentration with the square of the correlation coefficient of 0.995 (A=0.6530*Log(C_{ALP})-0.314). And then, the measured absorbance of the serum samples was brought into the regression equation. The serum sample concentrations were calculated as 47.3 U/L, 60.4 U/L, respectively.)