Supplemental Information

A simple label-free electrochemical method for the detection of polynucleotide kinase activity by peroxidase mimetics: TiO_2 nanotube array

Baojuan Wang*[a], Yunfang Xiong[b], Lin Lin[b], Xiaojun Zhang[b], Guangfeng Wang[bc]*

[a] College of Life and Science, Anhui Normal University,Wuhu,241000, P R China

[b] College of Chemistry and Materials Science, Anhui Normal University, Wuhu, 241000, P R China

[c] State Key Laboratory of Chemo/Biosensing and Chemometrics, Hunan University, Changsha, 410082, P R China

Corresponding author. Fax: +86-553-3869303; Tel: +86-553-3869303

E–mail: wangyuz@mail.ahnu.edu.cn

Optimization of assay conditions

In order to achieve an optimal experimental result, the effects of several experimental conditions were investigated as shown in Fig. S1. In the process of phosphorylation, a number of experimental conditions played important roles for the detection of T4 PNK activity based on the TiO_2 Nanotube Array fabricated biosensor, for instance, the effect of pH value, phosphorylation time, and reaction temperature, shown as Fig. S1. In brief, the effect of pH on the biosensor based on TiO_2 NTA was performed over the pH range 5.0-10.0. As shown in Fig. S1A, when the pH value increased in the range of 5.0-7.5, the result of electrocatalytic current relative to that of TiO_2 NTA electrode (recorded as Δi) decreased gradually, which was attributed to the protonation of nucleic acid reduced its interaction with T4 PNK. Then the relative current increased a little when the pH was higher than 7.5. Considering the physiological conditions, 0.05 M Tris-HCl (pH 7.4) was used in the subsequent studies. As shown in Fig. S1B, the resulting relative response increased obviously with reaction temperature of phosphorylation ranging from 25 to 34 °C. While the phosphorylation temperature increasing from 34 to 40 °C, the resulting change decreased rapidly. Thus the optimized phosphorylation temperature was chosen at 34°C. In this experiment, the time of phosphorylation also had important effect. As shown in Fig. S1C, the relative response of the resulting electrocatalytic signal was intensified with the time of phosphorylation. The change increased very slightly when the time of phosphorylation was higher than 120 min. In the process of phosphorylation, ATP is significant because of that T4 PNK

cannot phosphorylate DNA without ATP. The effect of the concentration of ATP on the response was studied as shown in Fig. S1D. The relative response of the resulting electrocatalytic signal increased with the increase of the concentration of ATP. However, a slight change was observed when the concentration of ATP was more than 6 mM, indicating that higher concentrations of ATP may hinder the reaction between DNA and ATP. Thus the appropriate concentration of ATP was 6 mM in the following experiments.

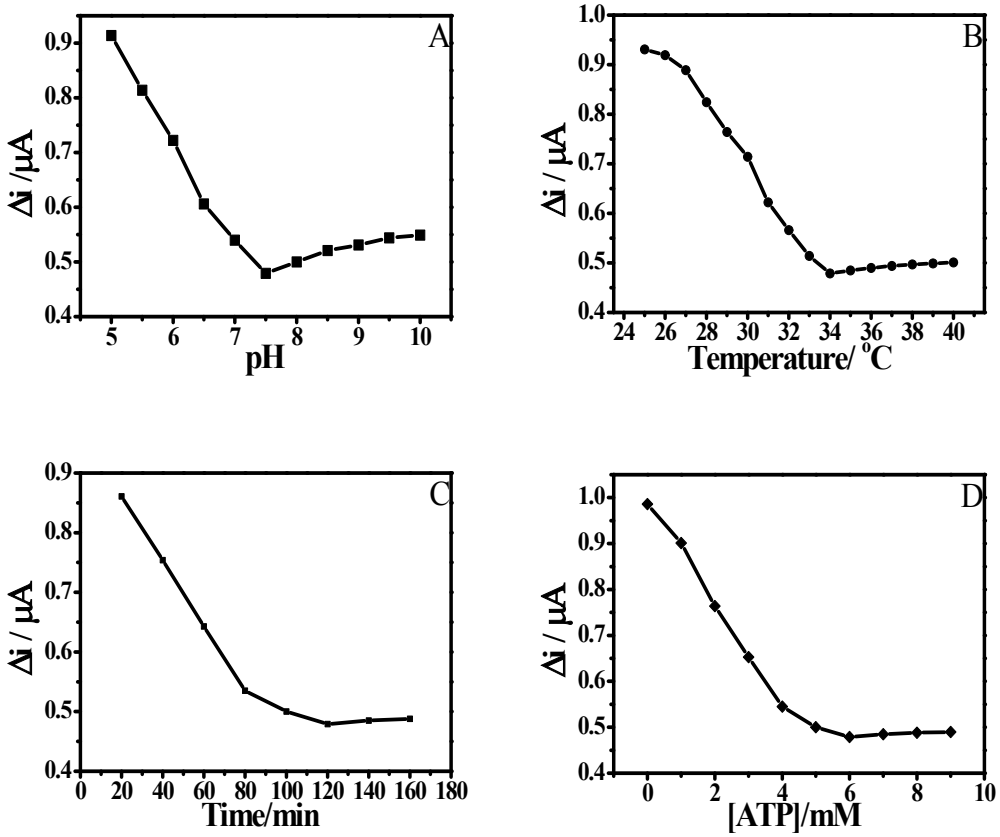

Fig. S1 Optimization of experimental conditions: (A) pH value, (B) phosphorylation time, (C) reaction temperature, (D) different concentration of ATP in the process of phosphorylation. All tested TiO2 NTA electrodes were incubated in the Tris-HCl buffer containing 100 μM H_2O_2.