Supporting Information

Plasmonic AuNP/g-C_3N_4 Nanohybrid-Based Photoelectrochemical Sensing Platform for Ultrasensitive Monitoring of Polynucleotide Kinase Activity Accompanying DNAzyme-Catalyzed Precipitation Amplification

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Fabrication of g-C_3N_4-Based PNK Sensing Platform. For the immobilization of HP1 on g-C_3N_4 surface, the as-prepared g-C_3N_4-modified electrode was placed in 5% glutaraldehyde (GA) in 0.01 M PBS (pH 7.4) for 4 h. Subsequently, the electrode was incubated in 10 mM Tris-HCl buffer (pH 7.4) containing 1.0 μM of HP1 (with a 3′-terminal amino group). In order to neutralize the unreacted glutaraldehyde, the resulted electrode was left overnight in 10^{-5} M aqueous solution of propylamine. Finally, the electrode was blocked using BSA and stored in Tris-HCl buffer according to the above-mentioned method.

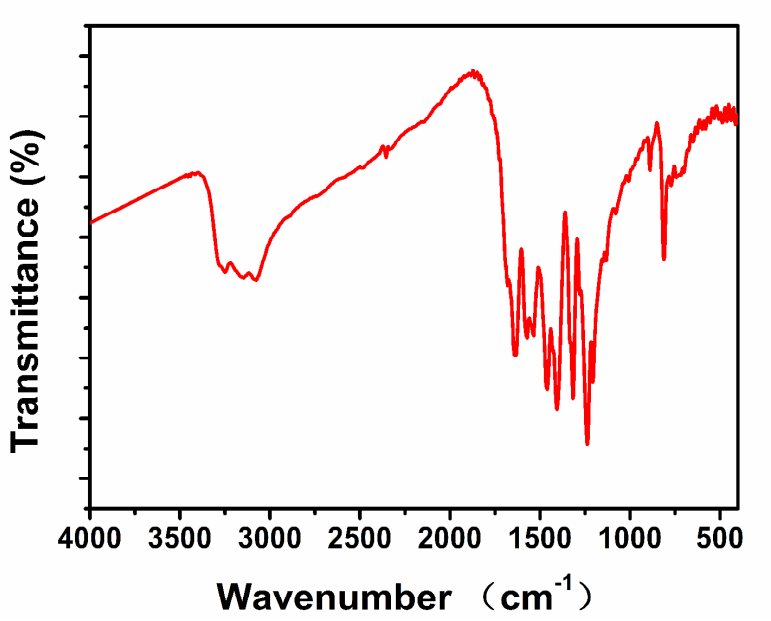

Figure S1. FTIR spectra of the as-synthesized g-C_3N_4 nanosheets.

As shown from Figure S1, a broad band at approximately 3200-3500 cm^{-1} was observed at the newly synthesized g-C_3N_4 nanosheets, which attributed to NH stretching vibration modes and the surface-adsorbed hydroxyl groups. The presence of NH stretching vibration modes indicated that some amine functional groups existed in the carbon nitride layer. In addition, several strong bands in the range of 1200-1650 cm^{-1} dominated the spectrum with peaks at approximately 1241, 1320, 1405, and 1640 cm^{-1}, which correspond to the typical stretching mode of CN heterocycles. The bands at about 3290-3335 cm^{-1} were consistent the N-H or C-H stretching vibration. The absorption band at 2161-2209 cm^{-1} was attributed to C-N stretching vibration. The peaks observed at 1570-1634 cm^{-1} was correspond to the C=N bond. The band observed at approximately 1570-1634

cm^{-1} was typical for C-N that contained CN heterocycles and associated with skeletal stretching vibration of this aromatic ring. The absorption at 810-880 cm^{-1} was characteristic of the out-of-plane bending modes of CN heterocycles of triazine units.

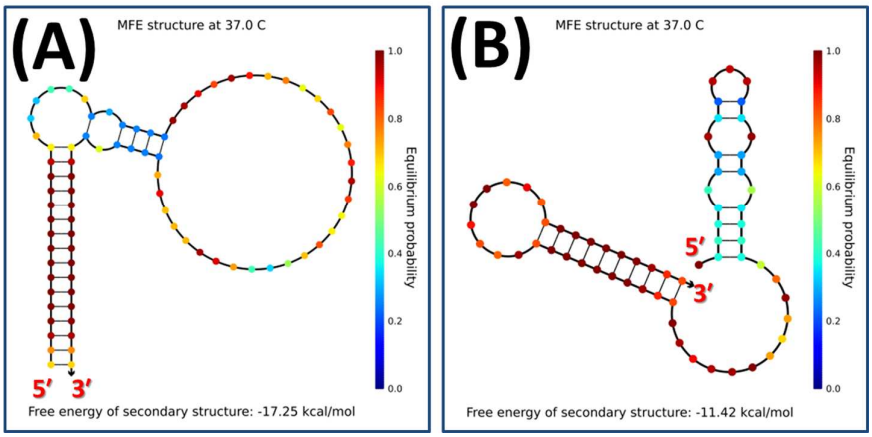

Figure S2. The secondary structure of hairpin DNA_2 (HP_2) before (A) and after (B) partial hydrolysis by λ-exo.

According the secondary structure predicted by *Nucleic Acid Package* (NUPACK, provided by NUPACK webserver), the original structure of probe HP_2 consisted of a 16 bp stem as designed and an uncertain small stem (Figure S2-A). However, the equilibrium probability of the uncertain small stem was very low that it might not exist in real situation. Thus, the HP_2 was likely to possess a 16 bp stem and 49 loop as designed. Similarly, the HP_2 after partial hydrolysis by λ exo possessed a 10 bp stem as designed and some ignorable small stems with low equilibrium probability (Figure S2-B). Importantly, the free energies of probe HP_2 before and after partial hydrolysis were -17.25 and -11.42 kcal mol^{-1} respectively, indicating that the unit I of original HP_2 might tend to become part of the 16 bp stem instead of hybridization with unit II for the higher stability.

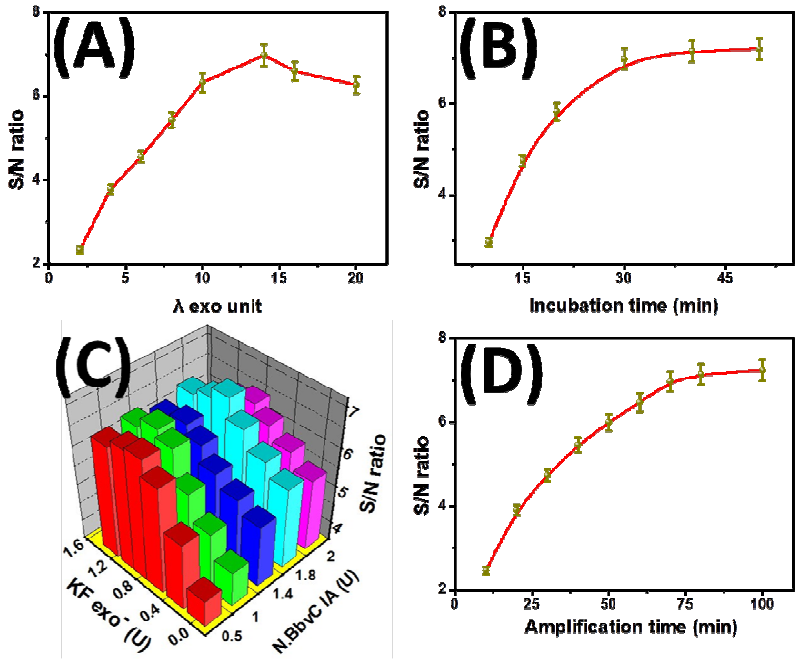

Figure S3. Effects of (A) λ-exo$^-$ concentration, (B) the time for HP_2 phosphorylation and hydrolysis by PNK/λ exo couple, (C) concentrations of KF exo$^-$ and N.BbvC IA, and (D) the isothermal amplification time on the signal-to-noise (S/N) ratio of the AuNP/g-C_3N_4-based PEC detection system.

To achieve an optimal analytical performance for the AuNP/g-C_3N_4-based PEC detection system, some experimental conditions including the concentration of enzymes (including λ exo$^-$, KF exo$^-$ and N.BbvC IA), the time required for HP_2 phosphorylation and hydrolysis by PNK/λ exo couple and reaction time for the isotherm amplification, were investigated and evaluated by using 50 mU mL^{-1} PNK as an example. To synthetically evaluate the effect of these parameters on noise and response signal, the signal-to-noise (S/N) ratio was employed as a judgment. As shown in Figure S3-A, the S/N ratio increased with the increasing amount of λ exo and reached a maximum value at the λ exo amount of 14 units, the further increase of λ exo amount was observed to decrease the S/N ratio. The reason might be attributed to the fact that excessive could cause the hydrolysis of HP_2 even without phosphorylation by PNK, leading to a high noise signal. Next, the time required for HP_2 phosphorylation and hydrolysis by PNK/λ exo couple was investigated. As shown in Figure S3-B, the S/N ratio increased gradually with the increase of reaction time and tended to level off at

30 min, suggesting the complete phosphorylation and cleavage process. Thus, 30 min was chosen for HP_2 phosphorylation and hydrolysis.

To ensure the smooth progress of HP_2 mediated signal amplification assisted by polymerase/nicking enzyme, the concentrations of KF exo^- and N.BbvC IA should be optimized. As shown in Figure S3-C, a maximum S/N ratio could be achieved when 1.0 U KF exo^- and 1.8 U N.BbvC IA were employed. Hence, 1.0 U KF exo^- and 1.8 U N.BbvC IA were used for the HP_2-mediated signal amplification step. In addition, the time required for this signal amplification reaction was also evaluated. As shown in Figure S3-D, the S/N ratio was observed to increase with the increase of amplification time and reached its maximum value at 70 min. So, 70 min was chosen for the amplification reaction and synthesis of trigger DNA fragments.