Electronic Supplementary Information

Self Assembly of Acetylcholinesterase on a Gold Nanoparticles-Graphene Nanosheet Hybrid for Organophosphate Pesticide Detection Using Polyelectrolyte as a Linker †

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Synthesis details of graphene oxide

Graphite powder (2 g, 325 meshes) was put into a mixture of 12 mL concentrated H_2SO_4, 3.0 g $K_2S_2O_8$ and 3.0 g P_2O_5. The solution was heated to 80 ^oC and kept stirring for 5 h by using oil-bath. Nextly, the mixture was cooled down to room temperature and diluted with deionized water (500 mL) overnight. Then, the product was obtained by filtering using 0.2 micron Nylon film and dried naturally. The pre-oxidized graphite was then re-oxidized by Hummers and Offeman method. Pretreated graphite powder was put into 0 °C concentrated H_2SO_4 (150 mL). Then, 25 g $KMnO_4$ was added gradually under stirring and the temperature of the mixture was kept to around 5 °C by using ice-bath. Successively, the mixture was stirred at 35 °C for 4 h, and then diluted with 250 mL dionized water by keeping the temperature under 50 ^oC. 1 L water was then injected into the mixture followed by adding 30 mL 30% H_2O_2 drop by drop. The mixture was filtered and washed with 1:10 HCl aqueous solution (1 L) to remove metal ions followed by 1L of dionized water to remove the acid. The resulting solid was dried in air and diluted to make graphite oxide dispersion (0.5% w/w). Finally, it was purified by dialysis for one week to remove the remaining metal species. Exfoliation was carried out by sonicating 0.1 mg mL^{-1} graphite oxide dispersion under ambient condition for 40 min. The resulting homogeneous yellow-brown dispersion was used for producing Au NPs/cr-Gs and chemically synthesized graphene.

Chemically reduced graphene oxide was prepared by adding 5 mL hydration hydrazine (80 %) into the 50 mL solution of 0.1 mg mL^{-1} graphene oxide and kept stirring for 24 h at 80^oC. Finally, black hydrophobic powder was obtained by filtration the production and drying in vacuum.

Principle of paraoxon detection based on acetylcholinesterase

To date, a host of various organophosphate pesticide (OPs) biosensors based on enzyme acetylcholinesterase (AChE) have been developed integrated with electrochemical or optical transducers. AChE is a most used biomarker for OPs monitoring because it could be inhibited easily after exposure to the poison. As shown in Scheme S1, paraoxon (a model compound for OP and nerve agent) can attack the serine residue of AChE and inhibit it readily by forming phosphorylated adducts.[1] By monitoring the electrochemical activity of catalysis product thiocholine (TCh) from its substrate acetylthiocholine (ATCh), AChE-based electrochemical biosensor has been considered as an efficient, direct and simple technique for OP detection (eq. S1).[2]

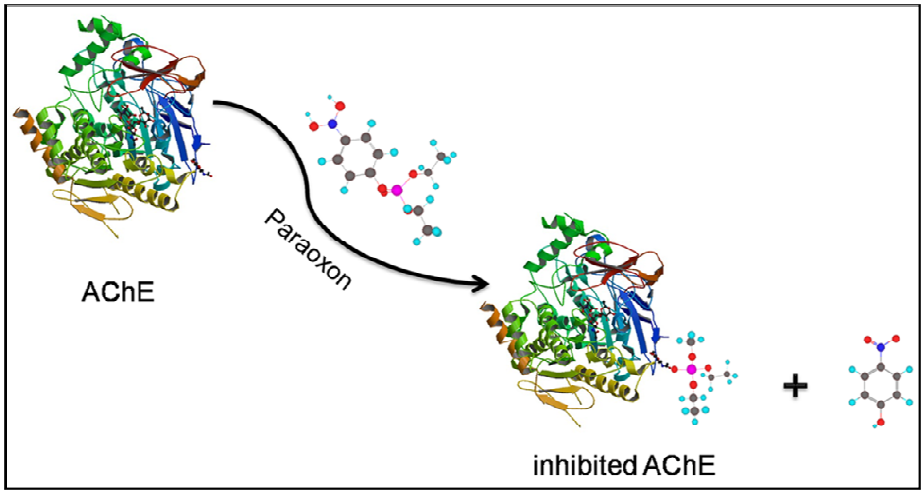
$$\mathrm{ATCh} + \mathrm{H_2O} \xrightarrow{\mathrm{AChE}} \mathrm{TCh} + \mathrm{acetate\ acid} \qquad \text{(S1)}$$

Reference

1. M. Pohanka, K. Musilek and K. Kuca, *Current Medicinal Chemistry*, 2009, **16**, 1790-1798.
2. J. Mukherjee and J. R. Kirchhoff, *Anal. Chem.*, 2009, **81**, 6996-7002.
3. M. Mascini, M. Sergi, D. Monti, M. Del Carlo and D. Compagnone, *Anal. Chem.*, 2008, **80**, 9150-9156.
4. P. Skladal, *Food Technology and Biotechnology*, 1996, **34**, 43-49.

Scheme 1

Paraoxon can attack on the serine residue of AChE and inhibit it readily by forming phosphorylated adducts. For molecular structures, gray circles represent carbon atoms; pink circles represent phosphate atoms; red circles represent oxygen atoms; blue circles represent nitrogen atoms and green circles represent hydrogen atoms.



Figures

Figure S1. Cyclic voltammograms of Au NPs/cr-Gs immobilized GC electrode in 0.01 M phosphate buffer (pH 7.4) and 0.2 M H_2SO_4. Scan rate, 100 mV/s.

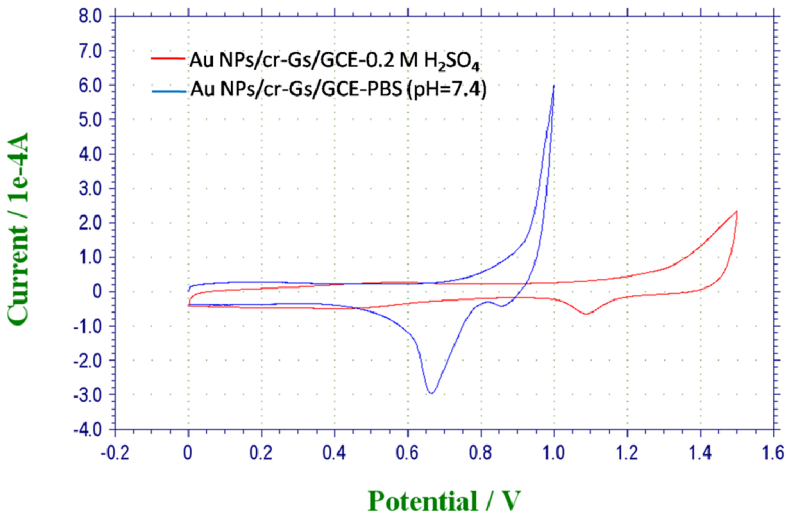


Figure S2. Cyclic voltammograms of Au NPs/cr-Gs and AChE/ Au NPs/cr-Gs on GC electrode in 0.01 M phosphate buffer (pH 7.4). Scan rate, 100 mV/s.

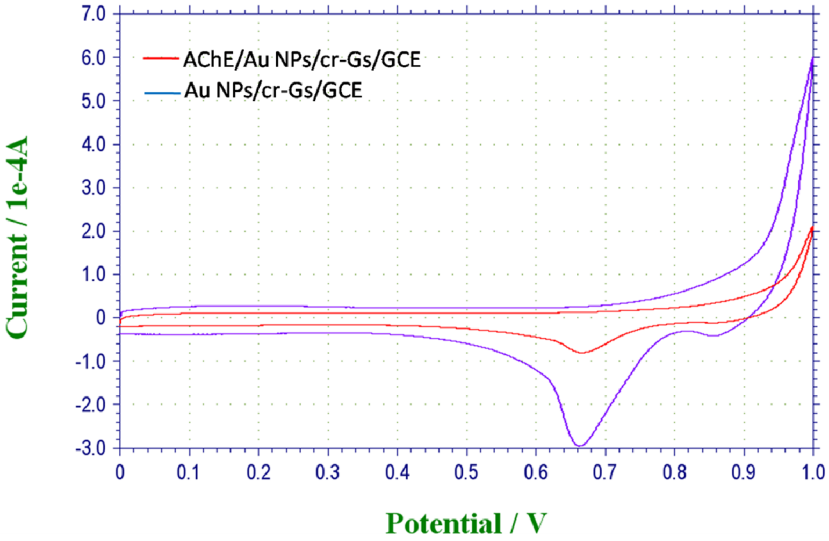


Figure S3. Effect of concentration of AChE (X axis, M), concentration of Au NPs/cr-Gs aqueous solution (Y axis, mg/mL) on current intensities of 2 mM ATCh on AChE/Au NPs/cr-Gs/GC electrode.