

Insulin Binding Aptamer Conjugated Graphene Oxide for Insulin Detection

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Supporting Information

1. Aptamer sequences used in this work.

Insulin binding aptamer (IBA) : 5'-GGT GGT GGG GGG GGT TGG TAG GGT GTC
TTC-FAM-3'

2. Preparation of graphene oxide.

Graphene oxide(GO) was synthesized from natural graphite powder following literature methods^{1,2}. Generally, graphite powder (2 g, 325 mesh) was put into an 80°C mixture of 12 mL concentrated H₂SO₄, 3.0 g K₂S₂O₈ and 3.0 g P₂O₅. The mixture was kept at 80°C for 4.5 hr using a hotplate with stirring. Afterwards, the mixture was cooled to room temperature and diluted with 0.5 L DI water and left overnight. Then the mixture was centrifuged and washed several times to remove the residual acid. The product was dried overnight under ambient conditions. This pre-oxidized graphite was then put into 0°C concentrated H₂SO₄ 120 mL, and 15g KMnO₄ was added gradually with stirring in an ice bath to keep the temperature below 20°C. The mixture was stirred at 35°C for 2h and then diluted with 250 mL DI water. During the dilution, an ice bath was used to keep the temperature below 50°C. The mixture was stirred for 2hr, and another 0.7 L DI water was added. Shortly after, 20 mL 30% H₂O₂ was added to the mixture, and the color of the mixture changed into brilliant yellow along with bubbling. The mixture was centrifuged and washed with 1 L 1:10 HCl aqueous solution to remove metal ions followed by 1 L DI water to remove the acid. The resulting solid was dried in air and diluted to make a GO 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 GO (2 mg/mL) dispersion under ambient conditions for 5 hours. The resulting sample was centrifuged at 12000 rpm for 10 min, and the upper solution was collected for future experiments.

3. Fluorescence response of 1:1 aptamer-based assay towards Insulin.

FAM-labeled insulin aptamer was diluted to 100nM in insulin buffer (50mM Tris-HCl, 10mM KCl, 100mM NaCl). An aliquot of the GO suspension (about 0.5 mg/mL) was added to the working solution (ratio of DNA and GO was 1nmol DNA to 1mg GO) and allowed to incubate for 30 min at room temperature to quench the fluorescence. Afterwards, 4-(1-pyrenyl) butyric acid (PBA) (3 μ L, 100 μ M) was added to the mixture solution as a surface-blocking agent. Then, different amounts of insulin were added into the GO-aptamer solution and incubated for 2 hours. The final concentration of insulin ranged from 5nM to 50 μ M. Finally the fluorescence intensities of the GO-aptamer complex were detected.

4. Fluorescence response of amplified aptamer-based assay towards Insulin.

FAM-labeled insulin aptamer was diluted to 100nM in insulin buffer (50mM Tris-HCl, 10mM KCl, 100mM NaCl). An aliquot of the GO suspension (about 0.5 mg/mL) was added to the working solution and allowed to incubate for 30 min at room temperature to quench the fluorescence. Afterwards, 4-(1-pyrenyl) butyric acid (PBA) (3 μ L, 100 μ M) was added to the mixture solution as a surface-blocking agent. Then, DNase I (25U) and different amounts of insulin were added to the GO-aptamer solution and incubated for 2 hours. The final concentration of insulin ranged from 5nM to 50 μ M. Finally the fluorescence intensities of the GO-aptamer complex were detected.

References

1. C. H. Lu, J. Li, M. H. Lin, Y. W. Wang, H. H. Yang, X. Chen and G. N. Chen, *Angew Chem Int Ed Engl*, **49**, 8454-8457.
2. Y. X. Xu, H. Bai, G. W. Lu, C. Li and G. Q. Shi, *Journal of the American Chemical Society*, 2008, **130**, 5856-5857.