Electronic Supplementary Information

Novel multifunctionalized perylenetetracarboxylic/amines supramolecules for electrochemical assay

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1 Experimental

1.1 Reagents and apparatus

Affimag PSC Magnetic Bead (MB) was purchased from Tianjin baseline ChromTech Research Centre (Tianjing, China). Trishydroxymethylaminomethane hydroch-loride (tris) was obtained from Roche (Switzerland). Thrombin (TB), hemoglobin (Hb), and bovine serum albumin (BSA) were acquired from Sigma Chemical Co. (St. Louis, MO, USA). Hexanethiol (96%, HT), gold chloride (1%, $HAuCl_4$), ethylenediamine (EDA), diethylamide (DEA) and triethylamine (TEA) were obtained from Shanghai Chemical Reagent Co. (Shanghai, China). 3,4,9,10-Perylenetetracarboxylic dianhydride ($C_{24}H_8O_6$, PTCDA) was received from Lian Gang Dyestuff Chemical Industry Co. Ltd. (Liaoning, China). Human immunoglobulin G (IgG) and L-cysteine (L-cys) were obtained from Chengdu kelong chemical Industry (Chendu, China). The oligonucleotide was synthesized by Sangon Biotech. Co. Ltd. (Shanghai, China) with the sequence of the oligonucleotide as followed:

Thrombin binding aptamer (TBA): 5'-NH_2-$(CH_2)_6$-GGTTGGTGTGGTTGG-3'

Phosphate buffered solution (PBS) (pH 7.0) was prepared using 10 mM Na_2HPO_4, 10 mM KH_2PO_4 and 2 mM $MgCl_2$ as working buffer. 20 mM Tris-HCl buffer (pH 7.4) containing 140 mM NaCl, 5 mM KCl and 1 mM $MgCl_2$ was used to prepare aptamer and thrombin (TB) solutions. The human serum samples were obtained from the Ninth people's Hospital of Chongqing, China. All other reagents were of analytical reagents or guaranteed reagents and used as received without further purification. Ultrapure water obtained from a Millipore water purification system (⩾18 MΩ, Milli-Q, Millipore) was used throughout this study.

Electrochemical Cyclic voltammetry (CV) was performed on a CHI 660D electrochemical workstation (Shanghai Chenhua Instrument, China). A conventional three-electrode system was used for all electrochemical measurements: a platinum wire as auxiliary electrode, the modified glassy carbon electrode (GCE, Φ = 4 mm) as working electrode, and a saturated calomel electrode (SCE) as reference electrode. CVs of the electrode fabrication were performed in PBS (pH 7.0) with a scanning potential from -0.8 to 0.1 V at a scan rate of 100 mV/s. The scanning electron micrographs were taken with scanning electron microscope (SEM, S-4800, Hitachi, Japan). And the transmission electron microscopy (TEM) was performed by transmission electron microscope (Tecnai G2 F20 S-TWIN, FEI, USA). All calculations were implemented with the Gaussian 09 program. The molecular structures of PTCA/EDA, PTCA/DEA and PTCA/TEA were fully and geometrically optimised using the B3LYP method to locate all of the stationary points with 6-

311++G (d, p) basis set for C, H, N, and O atoms.[1-3] Meanwhile, the stability of the density function theory (DFT) wave function was tested with repeating appropriately to reduce the wave function of the reoptimizations.[4,5] The relationship between bond energy E_{HB} and potential energy density $V(r)$ at corresponding the bond critical point (BCP) could be approximately described as $E_{HB} = V(r_{bcp})/2$ for the hydrogen bonding [X-H···O(X=C, N, O)].[6] We evaluated the energies of hydrogen bonding in our system by this formula with the Multiwfn Software.

2 Results and discussion

2.1 Preparation of PTCA/triethylamine (PTCA/TEA) multifunctionalized supramolecule nanomaterials

PTCA/TEA multifunctionalized supramolecule nanomaterials were prepared by a simple hydrogen bonding approach. In a typical experiment, PTCA was synthesized by hydrolyzed PTCDA according to Caruso method.[7] First, 500 μL TEA was added in the prepared PTCA aqueous solution with magnetic stirring for 24 h at the room temperature. After centrifugated at 12 000 rpm for 20 min to remove surplus TEA, the sediment of the resulting PTCA/TEA nanomaterials were resuspended in ultrapure water and stored at 4 °C for further use. PTCA/ethylenediamine (PTCA/EDA) and PTCA/diethylamide (PTCA/DEA) were prepared with the same method.

2.2 The electrochemical characterization of the stepwise modified electrode

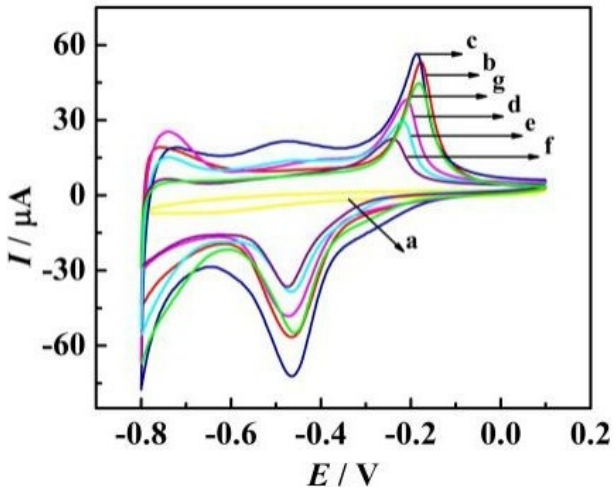

Fig. S1. The CV of the stepwise modified electrodes: CV of bare GCE (a); GCE-PTCA/TEA (b); GCE-PTCA/TEA-nano-Au (c); GCE-PTCA/TEA-nano-Au-TBA (d); GCE-PTCA/TEA-nano-Au-TBA-HT (e); GCE-PTCA/TEA-nano-Au-TBA-HT-TB (f); GCE-PTCA/TEA-nano-Au-TBA-HT-TB-TBA/AuNPs/MB (g).

To further confirm the successfully assemble process of the aptasensor with PTCA/TEA supermolecule nanomaterials as redox carrier, CV was employed to characterize the modified electrodes. CVs of different modified electrodes in the presence of 0.1 M PBS (pH 7.0) were acquired (Fig. 9). No obvious redox peak was observed (curve a) due to lack of redox carrier. However, after formation of a lay of PTCA/TEA, a well-defined redox peaks appeared (curve b), which ascribed to the fact that PTCA/TEA could act as an excellent redox carriers. Owing to the conductivity of nano-Au, an increase of signal was obtained after the electrochemical deposition of nano-Au (curve c). However, after the consecutive assembling of negative TBA, inert HT and biological macromolecule TB, the signals decreased with the stepwise decrease of electron transfer (curve d, e, f, respectively). Once the complex of the TBA/AuNPs/MB was incubated in the modified electrode due to the formation of G-quadruplex between TBA and TB,[8] the signal of CV in the PBS increased obviously (curve g), which contributed that the MB presented well catalytic activity to the novel

supermolecule nanomaterials PTCA/TEA, leading to the increase of the electrochemical signal. These results indicated that the proposed aptasensor was constructed successfully.

2.3 Specificity, reproducibility and stability of the aptasensor

The specificity of the aptasensor played an important role in analyzing biological samples. As shown in Fig. S2, no obvious responses were observed upon the same addition of interfering agents (L-cys, IgG and Hb). However, for the contrast test, as demonstrated in Fig. S2, obvious signals increase were obtained for TB (10 nM) and the mixture of TB (10 nM), Hb (100 nM), L-cys (100 nM) and IgG (100 nM), demonstrating that the aptasensor possessed high selectivity for determination of TB. The reproducibility of the present aptasensor toward TB determination was investigated, five freshly prepared electrodes were incubated with TB (10 nM) respectively and a relative standard deviation (RSD) of 4.7% was acquired. Whereas the aptasensor with one electrode was repeated for five measurements with 10 nM TB under the same condition, a RSD of 6.4% was obtained, revealing the high reproducibility of the present aptasensor. To check the stability, the proposed aptasensor was evaluated every 5 days for long-term storage at 4 °C. The aptasensor retained 95.1% of its initial current after 5 days storage and 91.6% after 25 days storage. The above experimental data effectively suggested the aptasensor with good stability.

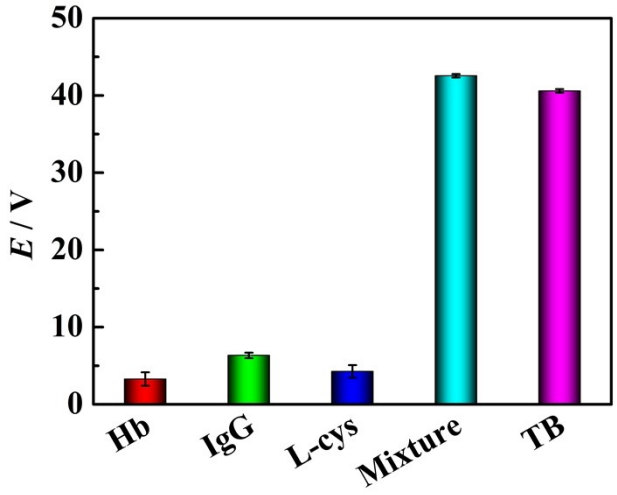

Fig. S2. Selectivity investigation compared with different targets: Mixture (10 nM TB + 100 nM Hb + 100 nM L-cys + 100 nM IgG), TB (10 nM), L-cys (100 nM), IgG (100 nM), Hb (100 nM).

Table S1. Determination of TB added in human blood serum (n=3) with the proposed aptasensor.

Sample number	Amount of TB added to the serum/nM	Amount of TB detected by the sensor/nM	Recovery/%	RSD/%
1	0.1	0.1025	102.5	3.5
2	1.0	0.9139	91.39	3.9
3	10.0	10.82	108.2	5.6
4	20.0	19.97	99.85	6.9
5	30.0	30.14	100.5	4.7

References

1 J. T. Miao, C. Fan, R. Sun, Y. J. Xu and J. F. Ge, *J. Mater. Chem. B*, 2014, **2**, 7065-7072.
2 M. W. Wong, *Phys. Lett.*, 1996, **256**, 391-399.
3 Z. Y. Bin, L. Duan and Y. Qiu, ACS Appl. Mater. Interfaces 2015, **7**, 6444-6450.
4 K. Tsurui, M. Murai, S. Y. Ku, C. J. Hawker and M. Robb, *J. Adv. Funct. Mater.*, 2014, **24**, 7338-7347.
5 M. J. McQuaid, T. Nokami, T. Matsuo, Y. Inatomi, N. Hojo, T. Tsukagoshi, H. Yoshizawa, A. Shimizu, H. Kuramoto, K. Komae, H. Tsuyama and J. Yoshida, *J. Am. Chem. Soc.*, 2012, **134**, 19694-19700.
6 E. Espinosa, E. Molins and C. Lecomte, *Chem. Phys. Lett.*, 1998, **285**, 170-173.
7 L. Y. Cao, Y. L. Liu, B. H. Zhang and L. H. Lu, *ACS Appl. Mater. Interfaces*, 2010, **2**, 2339-2346.
8 M. Wang, Z. Mao, T. Kang, C. Wong, J. Mergny, C. Leung and D. Ma, *Chem. Sci.*, 2016, **7**, 2516-2523.