Supporting Information

Target triggered ultrasensitive electrochemical Polychlorinated biphenyls aptasensor based on DNA microcapsules and nonlinear hybridization chain reaction

*Ting Han, Shaozhen Wang, Feifan Sheng, Sicheng Wang, Tianyue Dai, Xiaojun Zhang, Guangfeng Wang**

Key Laboratory of Chem-Biosensing, Anhui province; Key Laboratory of Functional Molecular Solids, Anhui province; College of Chemistry and Materials Science, Center for Nano Science and Technology, Anhui Normal University, Wuhu 241000, PR China

Corresponding Author: Guangfeng Wang

E-mail: wangyuz@mail.ahnu.edu.cn

Fax: +86-553-3869303; Tel: +86-553-3869302

Contents

1. Experimental Procedures

Chemicals and Materials. MB, 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid sodium salt (HEPES base), 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES acid) and tris(hydroxymethyl)aminomethane (Tris) were purchased from Sangon Biotech. Co., Ltd. (Shanghai, China). Poly (allylamine hydrochloride) (PAH, 58 kDa MW) was purchased from Sigma. Sodium chloride, magnesium chloride, ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA), were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 2,2',5,5'-Tetrachlorobenzidene and 3,3'-dichlorobenzidine, 2,4,4'-Trichlorobiphenyl (PCB-28), 2,2',5,5'-tetrachlorobiphenyl (PCB-52), 2,3',5,5'-tetrachlorobiphenyl (PCB-72), 2,2',4,5,5'-pentachlorobiphenyl (PCB-101), and chlorobenzene, were obtained from Sigma–Aldrich. All other chemicals were of analytical grade and used as received.

All DNA sequences were synthesized and subjected to HPLC purification by Sangon Co., Ltd. (Shanghai, China). The sequences of the synthetic DNA are shown in Table S2. The oligonucleotides were dissolved in 10 mM HEPES (pH 7.4, containing 500 mM NaCl and 50 mM $MgCl_2$) to the desired concentrations and stored at −20°C.

Apparatus. CHI 660B electrochemical workstation (Shanghai Chenhua Instruments Co., Shanghai, China) was employed to accomplish the electrochemical experiments. A conventional three-electrode configuration was used, with a bare or modified Au electrode (AuE), a saturated calomel electrode (SCE) as a reference electrode, and a platinum wire as an auxiliary electrode. Scanning electron microscopy (SEM, Hitachi S4800) observations for the morphological measurements of $CaCO_3$. All pH measurements were measured with a Model pHs-3c meter (Shanghai, China).

Preparation of MB-loaded $CaCO_3$ microparticles. $CaCO_3$ particles were prepared by a precipitation reaction between equal amounts of $CaCl_2$ and Na_2CO_3 under magnetic stirring at room temperature, as described previously. $CaCO_3$ particles loaded with MB, were obtained through coprecipitation by mixing of $CaCl_2$ (300 μL, 0.33 M) and Na_2CO_3 (300 μL, 0.33 M) solution, in the presence of MB (30 μL, 8.0 mg/mL). Deionized water was added to obtain a total volume of 1020 μL. After magnetic stirring for 110 s, the suspension was left for 70 s at room temperature to settle down. The particles were centrifuged at 900 rpm for 20 s, followed by the removal of the supernatant solution, and the subsequent

resuspension of the particles in water. This washing procedure was repeated for three times, to remove byproducts resulting from the precipitation reaction.

Assembly of stepwise preparation of the PCB-72 aptamer-cross-linked DNA microcapsules. $CaCO_3$ microparticles (6 mg) were suspended in 300 mL of a 1 mg mL^{-1} solution of PAH (in 10 mM HEPES, pH 7.4, containing 500 mM NaCl and 50 mM $MgCl_2$) under continuously shaking. After an adsorption time-interval of 20 min, the particles were washed twice with 10 mM HEPES buffer (pH 7.4, containing 500 mM NaCl) followed by centrifuging at 900 rpm for 20 s. Subsequently, the obtained PAH-modified $CaCO_3$ microparticles were coated by sequential incubation of the particles in DNA solutions (S1 promoter nucleic acids was 10 mM) and kept under continuous shaking for an adsorption time interval of 30 min. After this adsorption step, once washing step was performed to remove the non-adsorbed nucleic acids. And then use the S2 (2 μM) repeat the above operation .After the assembly of the DNA shells, the template cores were dissolved by adding 0.5 M EDTA (pH 6.0) to yield a solution with the final concentration of 0.1 M EDTA for 1 h under gentle rotation. After the suspension became clear, the supernatant EDTA solution was removed through slow centrifugation to avoid aggregation of the DNA capsules. The capsules were washed three times with 10 mM HEPES buffer (pH 7.4, containing 500 mM NaCl) by centrifuging at 500 rpm for 20 min. The DNA assembled capsules was stored at 4 °C in HEPES buffer for further use (100 μL).

Pretreatment of the GE. Before the preparation of the aptasensor, the gold working electrode was first immersed in piranha solution at 90 °C for 5 min for chemical pretreatment, followed by adequate washing with ultrapure water. Subsequently, the electrode was polished sequentially with 1.0, 0.3 and 0.05 μm alumina and sonicated in ethanol and ultrapure water for 3 min each. Afterwards, the electrode was voltammetrically cycled in 0.1 M H_2SO_4 with the potential between -0.2 and 1.5 V at 0.1 V s^{-1} until a representative steady-state cyclic voltammogram was obtained. After being dried with pure nitrogen gas, the electrode was immediately used for the DNA immobilization.

Preparation of DNA dendrimers for NHCR reaction nanoprobe. All oligo DNA were dissolved in 10 mM HEPES (pH 7.4, containing 500 mM NaCl and 50 mM $MgCl_2$). Preparation of the sensor includes the following steps: at first, the immobilization of DNA onto the pre-cleaned electrode was performed as follows: 100 μL 0.1 μM of the S3 in immobilization buffer was heated to 90 °C for 5 min and then gradually cooled to room temperature. Then, the pre-cleaned electrode was incubated in the above resulting S3 solution for 10 h at room temperature. After that, the AuE was rinsed with ultrapure

water and incubated in 2 mM 6-mercaptohexanol (MCH) in HEPES buffer for 30 min to remove nonspecific DNA adsorption on the AuE surface. After being thoroughly rinsed with ultrapure water and dried under N_2 stream, the S3 probe modified AuE (S3/AuE) was stored at 4 °C in HEPES buffer for further use. the S3/AuE was transferred into the DNA assembled capsules solution for 30 min at 50 °C. Next, A different concentration of PCB-72 standard solution dissolved in a solution of H_2O and dimethylformamide (V:V, 4:1) was pipetted into the solution and stirred for 5 min to provide sufficient time for the aptamer to bind to PCB-72, then the DNA microcapsules is open and the released S2 is complementary with S3 to obtain the double-stranded substrate modified electrode (S2/S3/GCE). The S4 solution was mixed with S5 solution at molar concentration ratio of 1:1 for 30 min with two steps (0.1 μM). Then the double-stranded substrate modified electrode was incubated with 50 μL mixing solution containing S4 and S5 in hybridization solution for 20 min. Afterward, equal amounts of S6 and S7 (0.5 μM) were incubated in hybridization solution at 50 °C for 90 mins to produce the carrier of DNA dendrimer via self-assembly.

All the electrochemical experiments were carried out at room temperature. DPV was performed within the potential range from 0 to − 0.7 V using scan rate 100 mV s^{-1}. Different concentrations of the PCB-72 were investigated under the same conditions. The EIS measurements were carried out in a background solution of 20 mM $[Fe(CN)_6]^{3-/4-}$ containing 0.1 M KCl with a frequency range from 0.1 Hz to 100 kHz. The amplitude of the applied sine wave potential was 5 mV and the formal potential of the system was set at +0.22 V.

The electrochemical experiments of DPV towards the signal of MB for 5 min without applying any potential in 3 mL 25 mM HEPES (pH 7.4, containing 100 mM NaCl and 10 mM $MgCl_2$). After that, the electrode was washed with MB-free 25 mM HEPES pH 7.4 for 5 min to remove the physically adsorbed molecules.

Selectivity study and stability of DNA dendrimers. The DPV signal was obtained in the presence of 10 ng/L PCB-72, three structural analogues of PCB-72 (PCB-28, PCB-52, and PCB-101) and one functional analogues of PCB-72 (chlorobenzene) under the same procedure. In addition, the obtained electrode was sealed and stored at 4 °C. DPV of the electrode was tested once per day and tested for 7 days.

2. Optimization of Experimental Conditions

In order to achieve high assay sensitivity, a number of experimental conditions were optimized in this paper. The optimization of the variables of the system is shown in Fig. S1, including the concentration of MB, pH value in the process of DNA dendrimer, the incubation temperature and time of DNA dendrimer formation. The effect of the concentration of MB was investigated due to the concentration of MB was a crucial parameter influencing the current intensity of MB DNA dendrimers. As shown in Fig. S2A, with the increasing concentration of MB, the current increases rapidly at first and then tend to be stable when the concentration of MB is higher than 8 mg/mL. The result is maybe attributed to the fact that MB wrapped in DNA capsule was a certain value because of that the concentration of $CaCO_3$ was a certain value. In this system, the sensitivity can also be influenced by pH value of the buffer solution. Thus, the value of pH has a vital effect on the efficiency of the assay. Fig. S2B shows the effect of pH value on the response of the DNA dendrimer modified electrode in HEPES solution over the pH range from 4.0 to 10.0. Obviously, the current increased followed by the pH value increased from 4.0 to 7.4. However, when the pH was higher than 7.4, the current decreased rapidly. The effect of incubation temperature in the NHCR system was studied at varying temperatures ranging from 5 to 70 °C as shown in Fig. S2C. One observes that the currents response increased with the incubation temperature rising from 5 to 50 °C but then decreased gradually with the incubation temperature increasing from 50 to 70 °C. The effect of NHCR incubation time was investigated for different time periods ranging from 10 to 120 min. As we can see from Fig. S2D, the electrochemical response intensified as the incubation time increased and remained constant to a saturation value after about 90 min, indicating that 90 min incubation time was efficient for the assay.

3. Supporting Figures and Tables

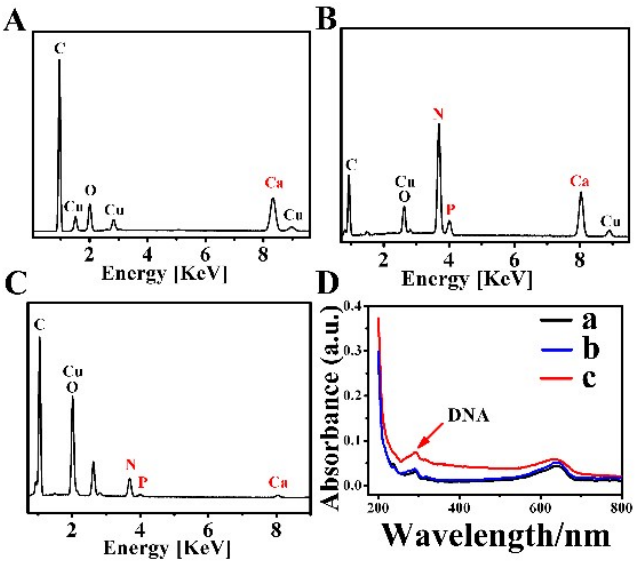

Fig. S1 EDS corresponding to uncoated $CaCO_3$ microparticles (A), DNA-coated $CaCO_3$ microparticles (B) and DNA microcapsules after dissolution of the $CaCO_3$ core (C), and (D) UV-vis for $CaCO_3$ microparticles(a), DNA-coated $CaCO_3$ microparticles(b), and DNA microcapsules(c).

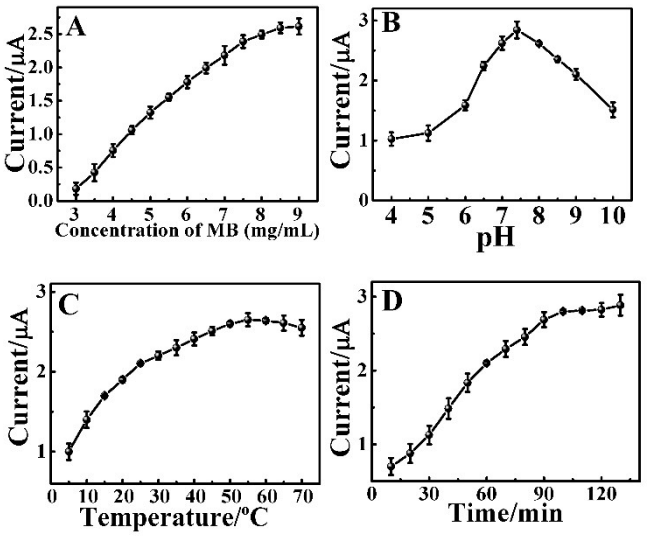

Fig. S2 Effect of MB concentration (A), pH value of HEPES (B), incubation temperature of NHCR (C), NHCR incubation time (D) on the sensor system. The error bars were the standard deviation of three repetitive measurements.

161 ***Table S1.*** List of other electrochemical signal and application.

Category	***electrochemical signal***	***Method***	***Target***	***Reference***
Label	*Alkaline phosphatase (ALP)*	*CV* [a]	*Influenza virus H5N1*	(Diba et al. 2015)[1]
	Ag NPS	*DPSV*[b]	*PDGF-BB*	(Song et al. 2014)[2]
	Cu-MOFs	*DPV* [c]	*Lipopolysaccharide*	(Shen et al. 2015)[3]
	Fc-SiNPs	*CV*	*Cardiac troponin I*	(Jo et al. 2015)[4]
	MB	*CV*	*Ochratoxin A*	(Tan et al. 2015)[5]
Label-free	*FGO@Fe−MOG*	*EIS* [d]	*Thrombin*	(Urbanova et al. 2018)[6]
	$[Ru(NH_3)_6]^{3+}$	*CC* [e]	*Adenosine*	(Shen et al. 2017)[7]
	Graphene	*DPV*	*17β-estradiol*	(Liu et al. 2019)[8]
	Fe_3O_4@Au	*EIS*	*Aflatoxin B1*	(Wang et al. 2018)[9]
	rGO/NB/AuNPs	*CV*	*Dopamine*	(Jin et al. 2018)[10]

162

163 [a] Cyclic voltammetry

164 [b] Differential pulse stripping voltammetric

165 [c] Differential pulse voltammetric

166 [d] Electrochemical impedance spectroscopy

167 [e] Chronocoulometry

168

169

170

171

172

173

174

175 ***Table S2.*** Oligonucletides sequence used in this work. DNA drawn in the same color are either identical or
176 complementary. Underline is the DNA aptamer.

Oligonucleotide	Sequence (5'-3')
Aptamer(S1)	[a]TTT-TTC-ACT-CGG-ACC-CCA-TTC-TCC-TTC-CAT-CCC-TCA-TCC-GTC-CAC-CAT-CAA-CTA-GTT
sDNA 2(S2)	AAC-TAG-TTG-ATG-AAG-CTG-GAC-ATAA-TAG-GCA-CAC-GAC-ATAA-TAG-GCA-CAC-
sDNA 3(S3)	GTG-TGC-CTA-TTA-TGT-CTC-CTC-CTG-TGT-GCC-TAT-TAT-GTC-TCC-TCC-TCA-GCT-TCA-TCA-ACT-AGT-TCG-TCA-$(CH_2)_6$-SH
Trigger(S4)	TGA-CGA-ACT-AGT-TGA-TGA-AGC-TG
sDNA 5(S5)	GTGCCTATTATGTCGTGTGCCTATTATGTCCAGCTT
sDNA 6(S6)	AGGAGGAGACATAATAGGCACACTGACGAACTAGTTGATGAAGCTG
sDNA 7(S7)	GTG-TGC-CTA-TTA-TGT-CTC-CTC-CTG-TGT-GCC-TAT-TAT-GTC-TCC-TCC-TCA-GCT-TCA-TCA-ACT-AGT-TCG-TCA

177 [a] The underlined part is the aptamer recognition sequence of PCB-72

178

179

180

181

182

183

184

185 ***Table S3.*** Comparison between the proposed assay and other reported methods for the aptasensing of PCB

Method	***Linear range (ng/mL)***	***Detection Limit (ng/mL)***	***Reference***
GC-MS	0.02-250	0.03	(Ozcan, et al. 2009)[11]
Flurescence aptasensor	0.1–100	1.0	(Xu, et al. 2012)[12]
PFC-based self-powered sensor	10 - 1000	4.5	(Yan, et al. 2018)[13]
Ultra-performance liquid chromatography	10-3000	10	(Ramanujam, et al. 2017)[14]
Colorimetry aptasensor	0.5–900 nM	0.05 nM	(Cheng, et al. 2018)[15]
SERS aptasensor	10 nM–10 M	3.3×10^{-8} M	(Sun, et al. 2016)[16]
Electrochemical immunosensors	10-10000	10	(Serena, et al. 2000)[17]
UCNPs-HCR Flurescence aptasensor	0.004-800	0.0035	(Wang, et al. 2018)[18]
$CaCO_3$-nHCR Electrochemical aptasensor	0.001-1000	0.001	This work

186

187

188

189

190

191

192

193

REFERENCES

1. F. S. Diba, S. Kim and H. J. Lee, *Biosens Bioelectron*, 2015, **72**, 355-361.
2. W. Song, H. Li, H. Liang, W. Qiang and D. Xu, *Anal Chem*, 2014, **86**, 2775-2783.
3. W. J. Shen, Y. Zhuo, Y. Q. Chai and R. Yuan, *Anal. Chem.*, 2015, **87**, 11345-11352.
4. H. Jo, H. Gu, W. Jeon, H. Youn, J. Her, S. K. Kim, J. Lee, J. H. Shin and C. Ban, *Anal. Chem.*, 2015, **87**, 9869-9875.
5. Y. Tan, X. Wei, Y. Zhang, P. Wang, B. Qiu, L. Guo, Z. Lin and H. H. Yang, *Anal. Chem.*, 2015, **87**, 11826-11831.
6. V. Urbanova, K. Jayaramulu, A. Schneemann, S. Kment, R. A. Fischer and R. Zboril, *ACS Appl. Mater. Interfaces.*, 2018, DOI: 10.1021/acsami.8b14344.
7. J. Nie, L. Yuan, K. Jin, X. Han, Y. Tian and N. Zhou, *Biosens. Bioelectron.*, 2018, **122**, 254-262.
8. M. Liu, H. Ke, C. Sun, G. Wang, Y. Wang and G. Zhao, *Talanta*, 2019, **194**, 266-272.
9. C. Wang, J. Qian, K. An, C. Ren, X. Lu, N. Hao, Q. Liu, H. Li, X. Huang and K. Wang, *Biosens. Bioelectron.*, 2018, **108**, 69-75.
10. H. Jin, C. Zhao, R. Gui, X. Gao and Z. Wang, *Anal. Chim. Acta.*, 2018, **1025**, 154-162.
11. S. Ozcan, A. Tor and M. E. Aydin, *Anal. Chim. Acta.*, 2009, **647**, 182-188.
12. S. Xu, H. Yuan, S. Chen, A. Xu, J. Wang and L. Wu, *Anal. Biochem.*, 2012, **423**, 195-201.
13. K. Yan, Y. Zhu, W. Ji, F. Chen and J. Zhang, *Anal. Chem.*, 2018, **90**, 9662-9666.
14. N. Ramanujam, M. Sivaselvakumar and S. Ramalingam, *Biomed Chromatogr*, 2017, **31**.
15. R. Cheng, S. Liu, H. Shi and G. Zhao, *J. Hazard. Mater.*, 2018, **341**, 373-380.
16. K. Sun, Q. Huang, G. Meng and Y. Lu, *ACS Appl. Mater. Interfaces.*, 2016, **8**, 5723-5728.
17. L. Serena, F. Milan, M. Marco, Electroanalysis 2000, 1040-0397.
18. Y. Wang, J. Bai, B. Huo, S. Yuan, M. Zhang, X. Sun, Y. Peng, S. Li, J. Wang, B. Ning and Z.Gao, *Anal. Chem.*, 2018, **90**, 9936-9942.