# Reusable, label-free electrochemical aptasensor for sensitive detection of small molecules†

**Bingling Li,[ab] Yan Du,[ab] Hui Wei[ab] and Shaojun Dong*[ab]**

*Received (in Cambridge, UK) 10th May 2007, Accepted 27th June 2007*
*First published as an Advance Article on the web 9th July 2007*
**DOI: 10.1039/b707057h**



**We report a sensitive electrochemical aptasensor for adenosine based on electrochemical impedance spectroscopy measurement, which gives not only a label-free but also a reusable platform to make the detection of small molecules simple and convenient.**

Since screened through the systematic evolution of ligands by exponential enrichment (SELEX) process from random RNA or DNA libraries,[1] aptamers have been widely used in analytical assays and shown their enormous potential in recognizing and detecting various targets[2–18] due to their generally impressive selectivity, affinity and their multifarious advantages over the traditional recognition elements.[19] As it was especially difficult to find a specific recognizing element for small molecules, aptamers from *in vitro* SELEX did possess considerable advantages in analytical assays for small molecules. Up to now, many aptamer-based methods have been used for small molecules,[10–18] such as fluorescence,[9–11] colorimetry[12] and electrochemistry.[14–18] Though all the methods developed could realize the small molecule detection successfully, some common disadvantages could not always be avoided. First, most of the existing methods required the step of labeling the probes onto the ssDNA molecules or the targets. Such a labeling process would not only make experiments relatively complex and expensive, but also affect the binding affinity between the targets and their aptamers to a greater or lesser degree. Secondly, many of the sensing systems rely on the conformational change of the aptamers after binding with their targets, therefore the application was obviously limited. Furthermore, many of the sensing systems were not reusable after detection, which increased the complication of operation.

Electrochemical impedance spectroscopy (EIS) is a rapidly developing electrochemical technique and has been imported to design the aptasensing systems for its advantages such as sensitivity, label-free nature, low-cost and convenience.[17] However, most of the EIS aptasensors focused on protein detection[17] but not of small molecules. Recently AMP[18] was detected based on a reagentless aptamer/complementary strand-aptamer/target sensing system using EIS and ISFET for measurement, which made the detection simpler. However, the sensing process based on the displacement of part of the complementary strands to the targets, could only be applied to the targets that did not lead to obvious impedance increase when complementary strands were displaced. Meanwhile, the sensing system was relatively difficult to reuse after one detection.

Herein a reusable and label-free electrochemical aptasensor for sensitive detection of small molecules has been developed based on EIS using adenosine[20] as a model molecule. Through the change from an HS-part complementary strand-aptamer/target to an HS-part complementary/aptamer-target system, as shown in Fig. 1, adenosine was easily detected by the decrease of the impedance. The relatively longer strand of aptamer was taken off from the electrode (but not the shorter complementary one), which could not only increase the detection sensitivity, but also provide a promising advantage of reuse of the sensing system.

According to previous work of Barton[21] and Willner,[22] a sensing interface was fabricated by functionalizing the gold electrode with part duplex hybridized by 5′-thiolated part complementary strand (PCS) and adenosine-binding aptamer strand (ABA) (the sequences[12] are listed in the ESI†). The formation of the self-assembly layer was then measured using both cyclic voltammetry and electrochemical impedance spectroscopy. For the Au/PCS-ABA electrode, the negatively charged interface repelled its negatively charged probe, $[Fe(CN)_6]^{4-/3-}$ anions, which retarded the interfacial electron-transfer kinetics of the redox probes.[17] As shown in Fig. 2 (inset), the electrochemical reaction of the redox couple was significantly inhibited and the

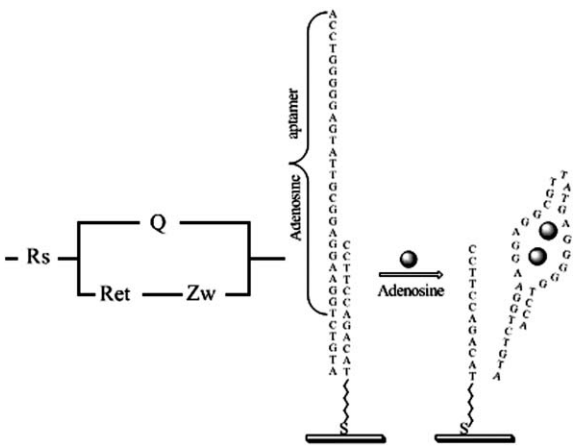


**Fig. 1** A circuit for the EIS (left) and a schematic of the method (right). Part of the duplex was first immobilized on the gold surface, followed by binding with adenosine to decrease the amount of the aptamer on the electrode surface.

[a]*State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin, 130022, P. R. China. E-mail: dongsj@ciac.jl.cn.; Fax: (+86) 431-85689711; Tel: (+86) 431-85262101*
[b]*Graduate School of the Chinese Academy of Sciences, Beijing, 100039, P. R. China*

† Electronic supplementary information (ESI) available: Details of the experimetal materials, instrumentation, experimental process and sensing ability of regenerated electrode. See DOI: 10.1039/b707057h

 

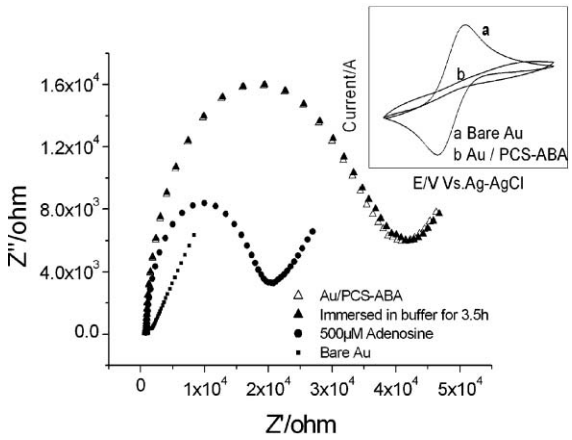


**Fig. 2** Nyquist plots of the control experiment for testing the stability of the Au/PCS-ABA sensing interface. Inset: The CV curve before (a) and after (b) functionalizing the electrode with PCS-ABA.

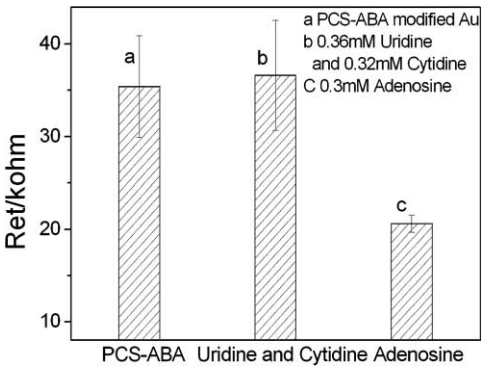


**Fig. 3** Control experiment of a mixed solution of 0.36 mM uridine and 0.32 mM cytidine. The error bars represent the standard deviation of two measurements.

peak-to-peak separation increased. This illustrated that the PCS-ABA self-assembly layer was compact enough for blocking the redox probes from effective electron-transfer at the sensing interface. A consistent result was also provided in the impedance spectra. Fig. 1 (left) shows the circuit that includes the commonly present electrolyte resistance (Rs), constant phase element (Q), Warburg impedance (Zw) and the electron-transfer resistance (Ret/Rct). The bare Au electrode showed a very small semicircle domain, which represented the Ret (Fig. 2), while for the Au/PCS-ABA system, the response of the equimolar $[Fe(CN)_6]^{4-/3-}$ anions was reduced and led to an obviously decreased electron-transfer efficiency. Both CV and EIS measurement demonstrated that the PCS-ABA functionalized electrode was very useful for adenosine sensing. To get a satisfactory sensing efficiency, comparison among Au/PCS-ABA, Au/PCS-ABA/MCH (6-mercatohexanol) and Au/PCS-ABA/MCE (2-mercatoethanol) was carried out. It was found that the usually used MCH and MCE [15–17] could not show a good function in this system, so we chose the Au/PCS-ABA as the sensing system, which has been proven to be applicable previously.[21,22]

The sensing interface was then used for response to the small molecule adenosine, which is an endogenous nucleoside with potent vasodilator and antiarhythmic activities.[17] Two control experiments were also carried out. First, to decrease the non-specific DNA absorption onto the electrode as much as possible, before use, the Au/PCS-ABA was dipped in the washing buffer for about 3 h with shaking. As shown in Fig. 2, the Ret of such a treated electrode stayed the same after 3.5 h immersion in the binding buffer (B-buffer used for adenosine and its aptamer reaction), which showed good stabilization of the well-washed electrode. By contrast, after 3 h immersion in 500 μM adenosine (dissolved in binding buffer), the Ret decreased significantly. This reflected that adenosine has effectively interacted with its aptamer and the loss of the ABA strand due to the formation of an adenosine–ABA complex. The other control experiment between adenosine and a uridine–cytidine mixed solution was also carried out. As shown in Fig. 3, after 3 h immersion in mixed uridine (0.36 mM) and cytidine (0.32 mM), the Au/PCS-ABA showed no response of Ret decreasing. The small increase of Ret may result from the non-specific absorption of small molecules onto the Au or DNA strands.

Because the sensor designed here was based on the adenosine competing reaction between adenosine and part complementary strand, the relatively longer ssDNA strand of the aptamer on the electrode would be decreased. This is different from the usual mode in which PCS is lost from the electrode. This could not only provide high sensitivity of detection but also an easily reusable sensing platform. Using the method here, the detection limit could reach $\sim 10^{-7}$ M, as shown in Fig. 4, as low as the most sensitive methods reported.[16,18] Meanwhile, the detection range could extend up to $\sim 10^{-2}$ M, with the most sensitive response range between $10^{-6}$ and $10^{-4}$ M. However, since we did not treat the electrode with small thiolated complexes as usually done, the array of the DNA duplex on the Au surface might not be very well ordered. Meanwhile, the adenosine needed to compete with PCS to bind with ABA, which might be relatively harder than the condition of only adenosine–ABA interaction. These factors could lead to relatively slow response to the target compared with some other sensors.[17] Work focusing on resolving this and other problems is now in progress through adjusting different factors such as ionic strength, temperature and the length or sequence of the part-duplex.

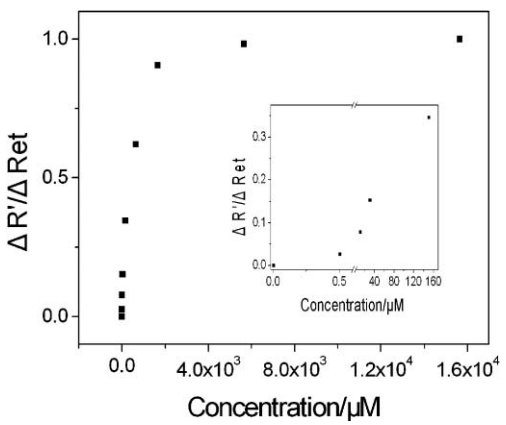


**Fig. 4** Response of the sensor to different concentrations of adenosine. Inset: the response to adenosine from 0 to 160 μM. ΔRet = Ret (max.) − Ret (min.); ΔR′ = Ret (max.) − Ret.

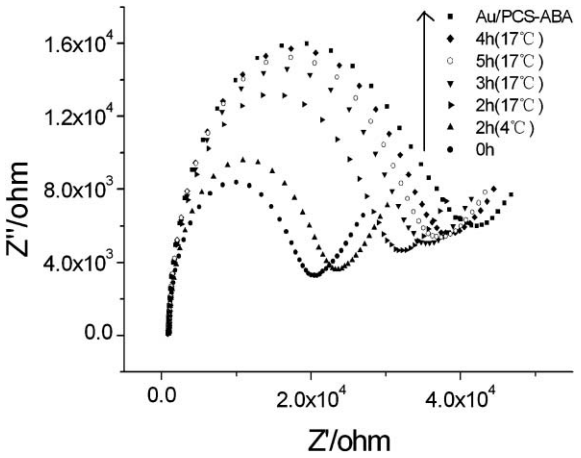


**Fig. 5** Nyquist plots of regenerating process of the sensing interface by treating the electrode with ABA solution after one time detection of 500 μM adenosine (squares).

Another advantage of the designed aptasensor was the regeneration ability of the aptasensor. Fig. 5 shows the regeneration process of the sensing interface after interacting with 500 μM adenosine (*i.e.* after one time use/detection). The electrode was first covered with 15 μL 2.8 μM ABA at 4 °C for 2 h, which could not lead to very effective hybridization as shown. Then the electrode was treated with the same method above at ~17–18 °C. The hybridizing process was obviously accelerated. After 4 h hybridization, the sensing interface was almost completely regenerated (~90%) and could be used again. The sensing ability of the regenerated electrode is provided in the ESI.†

In conclusion, a sensitive electrochemical aptasensor for adenosine has been developed using electrochemical impedance spectroscopy measurement. The EIS method provided a very simple detection means for small molecules based on a part complementary aptamer strand sensing platform. Through this method, relatively complicated and expensive steps such as labelling probes on the DNA or targets were avoided. Meanwhile, the relatively longer aptamer strand loss (but not the shorter complementary one) could not only make the detection sensitive, but also provide a regeneration ability of the sensing interface. Finally, the method proposed did not rely on the molecule size or the conformational change of the aptamer, so it might possess the potential of wider application for further targets, even proteins.

This work was supported by the National Natural Science Foundation of China (No. 20427003 and 20575064).

## Notes and references


1 A. D. Ellington and J. W. Szostak, *Nature*, 1990, **346**, 818; C. Tuerk and L. Gold, *Science*, 1990, **249**, 505; M. P. Robertson and G. F. Joyce, *Nature*, 1990, **344**, 467.
2 T. G. McCauley, N. Hamaguchi and M. Stanton, *Anal. Biochem.*, 2003, **319**, 244.
3 R. Polsky, R. Gill, L. Kaganovsky and I. Willner, *Anal. Chem.*, 2006, **78**, 2268; V. Pavlov, B. Shlyahovsky and I. Willner, *J. Am. Chem. Soc.*, 2005, **127**, 6522.
4 Y. Xiao, A. A. Lubin, A. J. Heeger and K. W. Plaxco, *Angew. Chem., Int. Ed.*, 2005, **44**, 5456; Y. Xiao, B. D. Piorek, K. W. Plaxco and A. J. Heeger, *J. Am. Chem. Soc.*, 2005, **121**, 17990.
5 J. J. Li and W. H. Tan, *Nano Lett.*, 2002, **2**, 315; C. J. Yang, S. Jockusch, M. Vicens, N. J. Turroand and W. H. Tan, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 17278; L. Wang, K. M. Wang, S. Santra, X. J. Zhao, L. R. Hilliard, Z. E. Smith, Y. R. Wu and W. H. Tan, *Anal. Chem.*, 2006, **3**, 646.
6 Y. J. Jiang, X. H. Fang and C. L. Bai, *Anal. Chem.*, 2004, **76**, 5230; B. L. Li, H. Wei and S. J. Dong, *Chem. Commun.*, 2007, 73.
7 J. A. Hansen, J. Wang, A. N. Kawde, Y. Xiang, K. V. Gothelf and G. Collins, *J. Am. Chem. Soc.*, 2006, **128**, 2228; M. Liss, B. Petersen, H. Wolf and E. Prohaska, *Anal. Chem.*, 2002, **74**, 4488.
8 H. Zhang, Z. Wang, X. F. Li and X. C. Le, *Angew. Chem., Int. Ed.*, 2006, **45**, 1576; H. Fu, J. Guthrie and X. C. Le, *Electrophoresis*, 2006, **27**, 433.
9 J. Wang, Y. J. Jiang, C. S. Zhou and X. H. Fang, *Anal. Chem.*, 2005, **77**, 3542.
10 M. N. Stojanovic and D. W. Landry, *J. Am. Chem. Soc.*, 2002, **124**, 9678.
11 R. Nutiu, J. M. Y. Yu and Y. F. Li, *ChemBioChem*, 2004, **5**, 1139; R. Nutiu and Y. F. Li, *J. Am. Chem. Soc.*, 2003, **125**, 4771; R. Nutiu and Y. F. Li, *Angew. Chem., Int. Ed.*, 2005, **44**, 1061.
12 J. W. Liu and Y. Lu, *Nat. Protocols*, 2006, **1**, 246; J. W. Liu, D. Mazumdar and Y. Lu, *Angew. Chem., Int. Ed.*, 2006, **45**, 7955.
13 T. Li, B. L. Li and S. J. Dong, *Chem.–Eur. J.*, DOI: 10.1002/chem.200700068.
14 B. R. Baker, R. Y. Lai, M. S. Wood, E. H. Doctor, A. J. Heeger and K. W. Plaxco, *J. Am. Chem. Soc.*, 2006, **128**, 3138.
15 X. L. Zou, S. P. Song, J. Zhang, D. Pan, L. H. Wang and C. H. Fan, *J. Am. Chem. Soc.*, 2007, **129**, 1042.
16 Z. S. Wu, M. M. Guo, S. B. Zhang, C. R. Chen, J. H. Jiang, G. L. Shen and R. Q. Yu, *Anal. Chem.*, 2007, **79**, 2933; L. Shen, Z. Chen, Y. H. Li, P. Jing, S. B. Xie, S. L. He and Y. H. Shao, *Chem. Commun.*, 2007, 2169.
17 M. C. Rodriguez, A. N. Kawde and J. Wang, *Chem. Commun.*, 2005, 4267; D. K. Xu, D. W. Xu, X. B. Yu, Z. H. Liu, W. He and Z. Q. Ma, *Anal. Chem.*, 2005, **77**, 5107; D. K. Xu, H. H. Han, W. He, Z. H. Liu, D. K. Xu and X. J. Liu, *Electroanalysis*, 2006, **18**, 1815; A. E. Radi, J. L. A. Sanchez, E. Baldrich and C. K. O'Sullivan, *Anal. Chem.*, 2005, **77**, 6320; H. Cai, T. M. H. Lee and I. M. Hsing, *Sens. Actuators, B*, 2006, **114**, 433; M. Lohndorf, U. Schlecht, T. M. A. Gronewold, A. Malave and M. Tewes, *Appl. Phys. Lett.*, 2005, **87**, 243902.
18 M. Zayats, Y. Huang, R. Gill, C. A. Ma and I. Willner, *J. Am. Chem. Soc.*, 2006, **128**, 13666.
19 S. E. Osborne and A. D. Ellington, *Chem. Rev.*, 1997, **97**, 349.
20 D. E. Huizenga and J. W. Szostak, *Biochemistry*, 1995, **34**, 656.
21 E. M. Boon, "Electrochemical Sensors Based on DNA-Mediated Charge Transport Chemistry", ch. 3, PhD Dissertation, California Institute of Technology, Pasadena, CA, 2002.
22 F. Patolsky, A. Lichtenstein and I. Willner, *Chem.–Eur. J.*, 2003, **9**, 1137.


 