

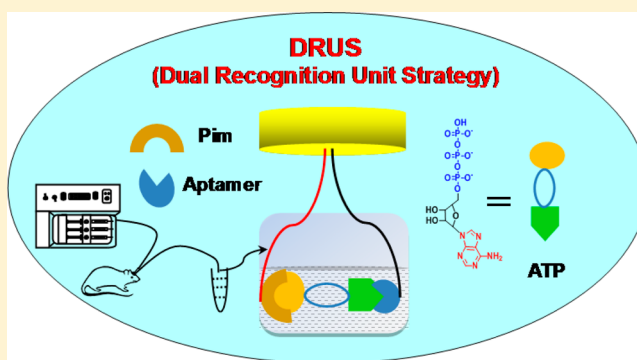
Dual Recognition Unit Strategy Improves the Specificity of the Adenosine Triphosphate (ATP) Aptamer Biosensor for Cerebral ATP Assay

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S Supporting Information

ABSTRACT: Adenosine triphosphate (ATP) aptamer has been widely used as a recognition unit for biosensor development; however, its relatively poor specificity toward ATP against adenosine-5'-diphosphate (ADP) and adenosine-5'-monophosphate (AMP) essentially limits the application of the biosensors in real systems, especially in the complex cerebral system. In this study, for the first time, we demonstrate a dual recognition unit strategy (DRUS) to construct a highly selective and sensitive ATP biosensor by combining the recognition ability of aptamer toward A nucleobase and of polyimidazolium toward phosphate. The biosensors are constructed by first confining the polyimidazolium onto a gold surface by surface-initiated atom transfer radical polymerization (SI-ATRP), and then the aptamer onto electrode surface by electrostatic self-assembly to form dual-recognition-unit-functionalized electrodes. The constructed biosensor based on DRUS not only shows an ultrahigh sensitivity toward ATP with a detection limit down to the subattomole level but also an ultrahigh selectivity toward ATP without interference from ADP and AMP. The constructed biosensor is used for selective and sensitive sensing of the extracellular ATP in the cerebral system by combining *in vivo* microdialysis and can be used as a promising neurotechnology to probing cerebral ATP concentration.



The development of new strategies and methodologies to directly, selectively, and sensitively record chemical signals of neurons involved in brain functions has drawn much attention, because recording of the dynamic change of chemical signals affords a platform for understanding the chemical essence in physiological and pathological events, for example, neurotransmission and the diagnosis and therapy of diseases.^{1–4} Adenosine triphosphate (ATP) is one of the most important chemical signaling agents, which plays a central role not only in energy metabolism but also in signal transduction.^{5–9} Recent studies have also indicated that ATP is one type of neurotransmitter that is related to the sense of taste.^{10,11} Although some elegant methods, including liquid chromatography, fluorescence, chemiluminescence, bioluminescence, and amperometric biosensors, have been employed for ATP sensing,^{12–17} the complexity of cerebral system presents a great challenge to the existing methods for direct, selective and sensitive determination of ATP in rat brain, especially for the basal level of ATP, because of its relatively low concentration.¹⁸

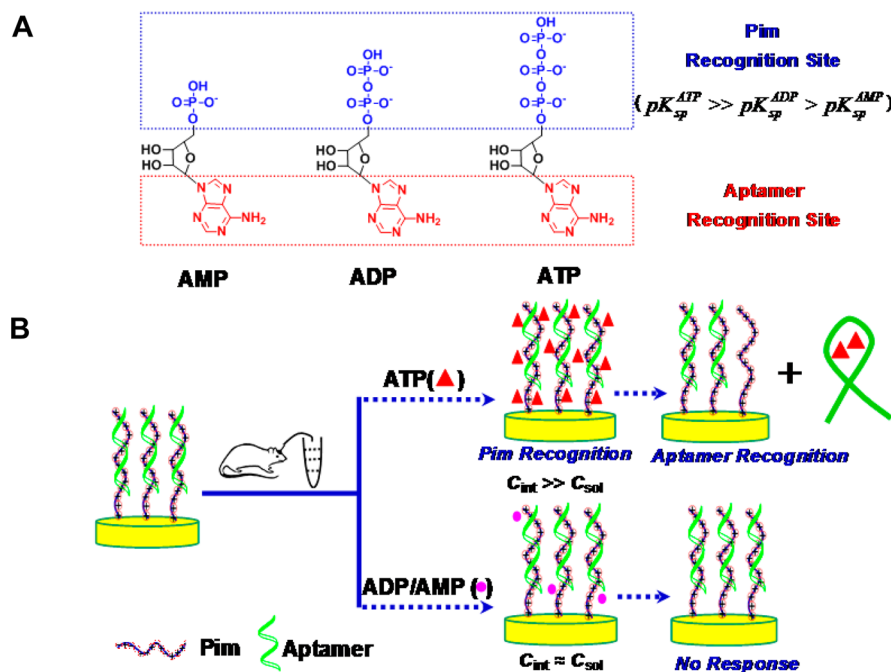
In this study, for the first time, we demonstrate a dual recognition unit strategy (DRUS) to improve selectivity and sensitivity of the ATP aptamer-based electrochemical biosensors and further validate the biosensors to sense ATP down to the subattomole level without interference from adenosine-

5'-diphosphate (ADP) or adenosine-5'-monophosphate (AMP). Although various aptamer-based ATP biosensors have been developed since the invention of *in vitro* selection techniques for aptamer screening, the poor selectivity of ATP aptamer toward ADP and AMP essentially limits the application of these biosensors for real sample analysis. This is because, although ATP aptamer shows a relatively high recognition ability toward A nucleobase, it shows fewer and less significant interactions with the sugar and especially the triphosphate moiety,^{19–21} resulting in almost the same affinity toward ATP, ADP, and AMP. These features unfortunately invalidate the ATP aptamer-based biosensors developed so far for cerebral ATP sensing, since ATP and ADP exist in the cerebral system with the concentrations almost in the same order.²² To solve the selectivity problem, Szostak and co-workers have demonstrated a strategy through optimizing the structure of the aptamer to enable its interaction with triphosphate moiety. However, the selected RNA aptamer shows a relatively low specificity toward the nucleobase.²³

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Scheme 1. (A) Illustration of Recognition Sites of Pim and Aptamer toward ATP, ADP, and AMP; (B) Schematic Illustration of DRUS for Cerebral ATP Assay with ATP Aptamer-Based Biosensor



It occurs to us whether the high specificity toward ATP could be realized by introducing a second recognition unit that is capable of interacting with triphosphate moiety in ATP molecule. Keeping this in mind, we interestingly find here that a polyimidazolium (Pim) brush could serve as the second recognition unit to discriminate ATP from ADP and AMP by recognizing the triphosphate moieties in these molecules (Scheme 1A). By combining the strong recognition ability of ATP aptamer (i.e., first recognition unit) toward A nucleobase, we thus demonstrate here a new ATP aptamer-based biosensor with DRUS (Scheme 1B). When the biosensors based on the ATP aptamer/Pim-modified electrodes are treated by ATP, the strong affinity between surface-confined Pim and the triphosphate moiety of ATP leads to a high interfacial concentration (C_{int}) of ATP and, consequently, results in highly sensitive recognition of aptamer toward interface-concentrated ATP, even when the solution concentration of ATP (C_{sol}) is relatively low ($C_{sol} \ll C_{int}$). In contrast, the relatively weak affinity of Pim toward the diphosphate moiety of ADP and the monophosphate of AMP does not result in the sensitive recognition of ATP aptamer toward ADP and AMP, because of the relatively low C_{int} of ADP and AMP ($C_{int} \approx C_{sol}$). This difference largely enables the selective and sensitive determination of ATP against ADP and AMP, and the resulting assay exhibits greatly enhanced selectivity toward ATP against ADP and AMP (ca. 2–3 orders) and could be used for selectively sensing ATP in the cerebral systems. To the best of our knowledge, this is the first example by using aptamer-based biosensors to sense cerebral species, which would open a new avenue to understanding brain chemistry.

EXPERIMENTAL SECTION

Reagents and Solution. Potassium ferricyanide ($K_3Fe(CN)_6$) was obtained from Beijing Chemical Co. (Beijing, China). *N*-hydroxysuccinimide (NHS), 2-bromoisobutyl bromide, copper(I) bromide ($CuBr$, 98%), and

N,N,N',N',N''-pentamethyldiethylenetriamine (PMDETA, 99%) were purchased from J&K Chemical Company (Tianjin, China). 1-Vinyl-3-butylimidazolium chloride ($[Vbim][Cl]$) and NHS-Br active ester initiator were synthesized according to the previous report.^{24,25} The 30-mer ATP-binding aptamer (5'-ACCTGGGGGAGTATTGCGGAGGAAGGTTT-3') was synthesized and purified by Invitrogen Biotech Co. Ltd. (Shanghai, China). Cysteamine, ATPase (EC 3.6.1.3), ATP, ADP, AMP, cytidine-5'-triphosphate (CTP), uridine-5'-triphosphate (UTP), guanosine-5'-triphosphate (GTP), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), uric acid (UA), ascorbate acid (AA), and 5-hydroxytryptamine (5-HT) were purchased from Sigma–Aldrich (Shanghai, China). Artificial cerebrospinal fluid (aCSF) used as the perfusion solution for *in vivo* microdialysis was prepared by mixing NaCl (126 mM), KCl (2.4 mM), KH_2PO_4 (0.5 mM), $MgCl_2$ (0.85 mM), $NaHCO_3$ (27.5 mM), Na_2SO_4 (0.5 mM) and $CaCl_2$ (1.1 mM) into water. Other chemicals were of at least analytical-grade reagents and were used as received. All the solutions were prepared with Milli-Q water.

Apparatus and Electrochemical Measurements. Electrochemical measurements were performed with a computer-controlled electrochemical analyzer (Model CHI 832, CHI Instruments, Shanghai, China) in a conventional three-electrode electrochemical cell with the as-prepared Au electrode as the working electrode, a platinum wire as the counter electrode, and Ag/AgCl electrode (KCl-saturated) as the reference electrode. Cyclic voltammetry (CV) was performed at a scan rate of 100 mV/s, and chronocoulometry (CC) was carried out at a pulse period of 250 ms and pulse width of 400 mV (from 0.4 to 0 V). All electrochemical experiments were performed at room temperature. Ultraviolet–visible (UV–vis) spectra were recorded on a Model TU-1900 spectrometer (Beijing, China). X-ray photoelectron spectroscopy (XPS) was performed on an ESCALab220i-XL electron spectrometer from VG Scientific, using 300 W Al $K\alpha$ radiation.

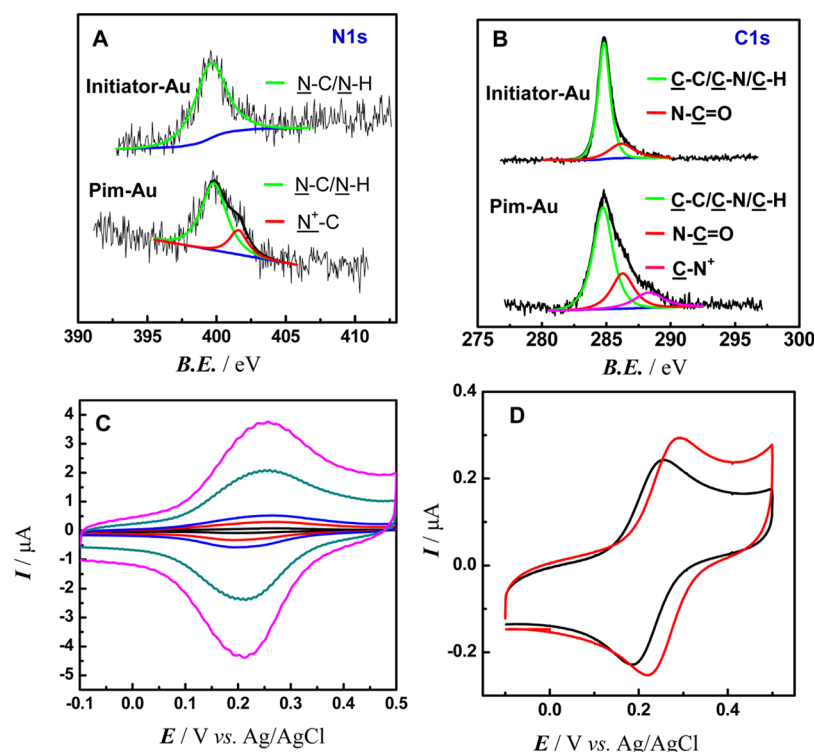


Figure 1. (A, B) X-ray photoelectron spectroscopy (XPS) results of N 1s (A) and C 1s (B) core-level spectra of an initiator-modified Au slice (initiator-Au) and Pim-modified Au slice (Pim-Au). (C) Typical cyclic voltammograms (CVs) obtained at the $\text{Fe}(\text{CN})_6^{3-}$ /Pim-modified Au electrode in phosphate buffer (0.10 M, pH 7.0) at different scan rates of 0.01, 0.05, 0.1, 0.5, and 1 V/s (from inner to outer). (D) CVs obtained at the Pim-modified Au electrode in phosphate buffer (25 mM, pH 7.0) containing $10 \mu\text{M}$ $\text{K}_3\text{Fe}(\text{CN})_6$ in the presence of 25 mM NaCl (black curve) or 500 mM NaCl (red curve). Scan rate = 0.1 V/s.

Preparation of a Polyimidazolium (Pim) Brush on Gold Electrodes and ATP Aptamer-Based Biosensors.

The formation of a Pim brush on the gold surface was carried out by surface-initiated atom transfer radical polymerization (SI-ATRP) with an NHS-Br active ester as the functional initiator, 1-vinyl-3-butylimidazolium chloride ($[\text{Vbim}][\text{Cl}]$) as the monomer, and $\text{CuBr}/\text{PMEDTA}$ as the catalyst (for details, see section S1 (Scheme S1) in the Supporting Information).²⁶ ATP aptamer-based biosensors were prepared by immersing the Pim-modified electrodes in $2 \mu\text{M}$ ATP aptamer in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) at room temperature for 2 h. After that, the electrodes were taken out of the solution and rinsed with water and used for ATP sensing.

In Vivo Microdialysis and Cerebral ATP Sensing. *In vivo* microdialysis was carried out with procedures reported in our earlier works.^{27,28} Briefly, adult male Sprague–Dawley rats (250–300 g) purchased from Health Science Center, Peking University were housed on a 12:12 h light-dark schedule with food and water *ad libitum*. A microdialysis guide cannula (BAS/MD-2250, BAS) was implanted into the brain cortex (AP = 0 mm, L = 3 mm from bregma, V = 2 mm from dura) using standard stereotaxic procedures.²⁹ The guide cannula was kept in place with three skull screws and dental cement. Stainless steel dummy blockers were inserted into the guide cannula and fixed until the insertion of the microdialysis probe. Throughout the surgery, the body temperature of the animals was maintained at 37°C with a heating pad. After the rats were allowed to recover for at least 24 h, a microdialysis probe (BAS: dialysis length, 4 mm; diameter, 0.24 mm) was first implanted into the brain cortex. The microdialysate was collected for analysis after the probe was continuously perfused with aCSF at

$1 \mu\text{L}/\text{min}$ for at least 90 min for equilibration. To sense ATP in brain cortex microdialysates, the biosensor was first immersed into phosphate buffer solution (PBS, 25 mM and 25 mM NaCl, pH 7.0) containing the microdialysates for 10 min and then the measurements were conducted with chronocoulometry.

RESULTS AND DISCUSSION

Formation and Electrochemistry of Polyimidazolium (Pim)-Modified Au Electrode.

The formation of Pim onto Au electrode surface was characterized by X-ray photoelectron spectroscopy (XPS), as shown in Figure 1. Compared with the initiator-modified Au slice, the appearance of $\text{N}^+\text{C}-\text{N}$ signal on the N 1s (Figure 1A, red curve) and C 1s (Figure 1B, red curve) core-level spectra of Pim-Au revealed that Pim brushes were successfully grafted onto the gold surface.³⁰ Moreover, the change of interface capacitance also indicates the formation of Pim on the gold surface (section S1 (Figure S1) in the Supporting Information).

To investigate the electrochemical behavior of Pim brushes confined onto Au electrodes, $\text{Fe}(\text{CN})_6^{3-}$ was chosen as an electrochemical probe since $\text{Fe}(\text{CN})_6^{3-}$ bears a strong interaction with Pim by ionic hydrogen bonding interaction, as reported in our previous study.³¹ As shown in Figure 1C, after the Pim-modified Au electrode was immersed in the aqueous solution of $\text{Fe}(\text{CN})_6^{3-}$ ($100 \mu\text{M}$) for 10 min, taken out of the solution, rinsed with water, and then recycled in the pure phosphate buffer (100 mM, pH 7.0), a pair of well-defined redox peak was observed around +0.20 V with a full width at half-maximum (fwhm) of ca. 90 mV at the scan rate of 0.1 V/s (Figure 1C). These results demonstrated that $\text{Fe}(\text{CN})_6^{3-}$ was entrapped onto the Pim-modified electrode surface. Moreover,

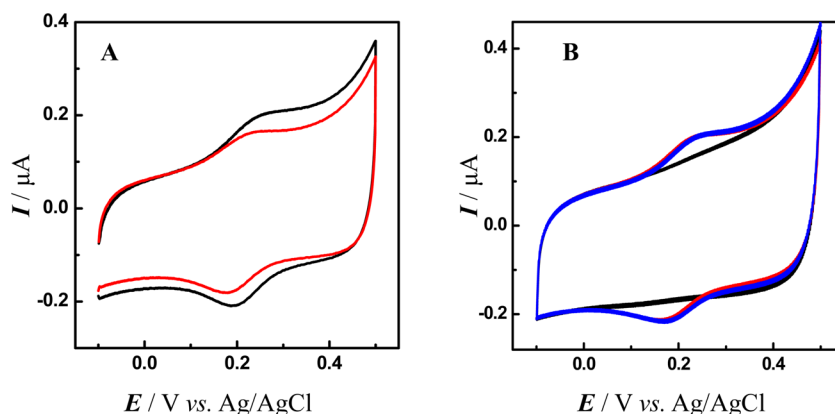


Figure 2. (A) CVs obtained at the Pim-modified Au electrode (black curve) and the aptamer/Pim-modified Au electrode (red curve) in 25 mM phosphate buffer (pH 7.0) containing 25 mM NaCl and $10 \mu\text{M}$ $\text{K}_3\text{Fe}(\text{CN})_6$. (B) CVs obtained at the aptamer/Pim-modified Au electrode in 25 mM phosphate buffer containing 25 mM NaCl in the absence (black curve) and presence (red curve and blue curve) of $10 \mu\text{M}$ $\text{K}_3\text{Fe}(\text{CN})_6$. The blue curve was obtained after the aptamer/Pim-modified Au electrode was immersed in $10 \mu\text{M}$ $\text{K}_3\text{Fe}(\text{CN})_6$ for 30 min. Scan rate = 100 mV s^{-1} .

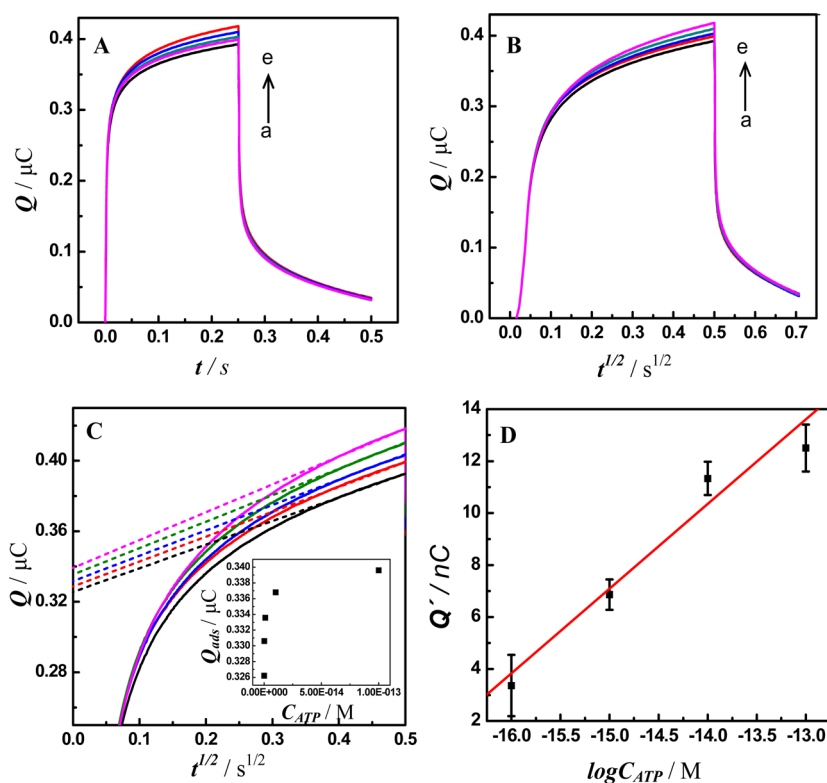


Figure 3. Typical chronocoulometric curves (A) and Anson Plot (B) obtained at the aptamer/Pim-modified Au electrode treated by ATP with different concentrations of (a) 0 M, (b) 10^{-16} M, (c) 10^{-15} M, (d) 10^{-14} M, and (e) 10^{-13} M. (C) Enlarged Anson plot showing the intercepts at $t = 0$, which represent the adsorbed charges of $\text{K}_3\text{Fe}(\text{CN})_6$ (Q_{ads}). Inset in panel C shows a plot of the intercepts in Anson plot versus the ATP concentration. (D) Plot of the increased charge ($Q' = Q_{\text{ads}} - Q_0$) versus the logarithmic concentration of ATP. Error bars show the standard deviation of the measurements taken from three independent experiments. Pulse period, 250 ms; pulse width, 400 mV; electrolyte, 25 mM phosphate buffer (pH 7.0) containing 25 mM NaCl and $10 \mu\text{M}$ $\text{K}_3\text{Fe}(\text{CN})_6$. Q_0 is the adsorbed charge of $\text{K}_3\text{Fe}(\text{CN})_6$ without ATP treatment ($C_{\text{ATP}} = 0$).

the CVs remained unchanged upon consecutive potential cycling, indicating the strong interaction between Pim and $\text{Fe}(\text{CN})_6^{3-}$.³¹ The electron transfer kinetics of the surface-adsorbed $\text{Fe}(\text{CN})_6^{3-}$ in Pim film was determined to be 8.24 s^{-1} with Laviron's equation^{32–34} (for details, see section S2 (Figure S2) in the Supporting Information). Both the strong adsorption and fast electron transfer process of $\text{Fe}(\text{CN})_6^{3-}$ in the Pim film enable $\text{Fe}(\text{CN})_6^{3-}$ as a good electrochemical probe for the development of ATP biosensors, as demonstrated below.

Different from $\text{Fe}(\text{CN})_6^{3-}$ entrapped into Pim film, $\text{Fe}(\text{CN})_6^{3-}$ in the solution phase shows a relatively large fwhm (ca. 100 mV) and a peak-to-peak separation of ca. 80 mV (0.1 V s^{-1}) in 25 mM phosphate buffer containing 25 mM NaCl and $10 \mu\text{M}$ $\text{K}_3\text{Fe}(\text{CN})_6$ at the Pim-modified Au electrode, as shown in Figure 1D (black curve). Moreover, because of the low concentration of $\text{K}_3\text{Fe}(\text{CN})_6$ (i.e., $10 \mu\text{M}$) in bulk solution, only one pair of redox wave was observed, which was partially ascribed to the electrochemical process of the surface-

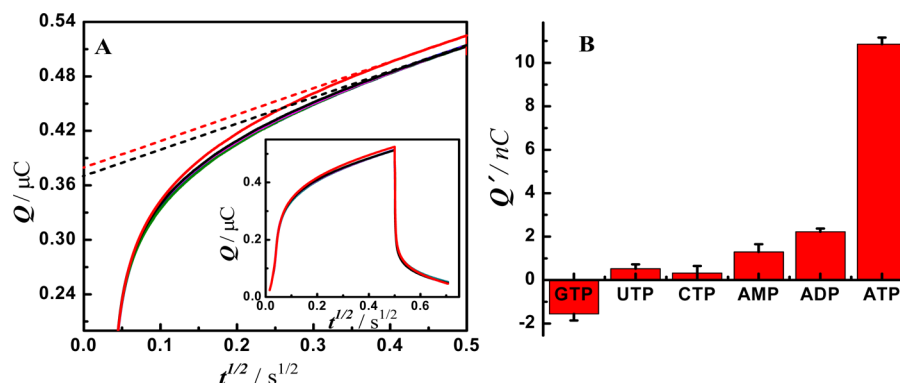


Figure 4. (A) Typical Anson plots obtained at the aptamer/Pim-modified Au electrode treated with blank, GTP (10^{-10} M), UTP (10^{-10} M), CTP (10^{-10} M), ADP (10^{-10} M), AMP (10^{-10} M), ATP (10^{-14} M). (B) Histogram of signal intensity for different of targets in panel A. Q' was calculated as demonstrated in Figure 3. Other conditions were the same as those described in Figure 3.

entrapped $\text{Fe}(\text{CN})_6^{3-}$ in the Pim film. The full width at half maximum (fwhm) was ca. 100 mV, which was slightly larger than the theoretical width of 90 mV expected for a reversible reaction of noninteraction, surface-confined species. This broadening in fwhm was predominantly attributed to concurrent reduction of solution phased $\text{Fe}(\text{CN})_6^{3-}$, which occurs close to 0.24 V. When the concentration of $\text{Fe}(\text{CN})_6^{3-}$ was increased to 500 μM , the reduction of both surface-confined and solution-phased species could be discerned.³¹ Such bimodal waves were previously observed for $\text{Ru}(\text{NH}_3)_6^{3+}$ in DNA films by Tarlov and co-workers.³⁵ Moreover, the peak position was strongly related to the ionic strength, as shown in Figure 1D (black and red curve); when the concentration of NaCl in electrolyte was increased from 50 mM to 500 mM, the peak position was positively shifted ca. 35 mV, which may have originated from the transport of counterion (i.e., Cl^-) from the solution during the reduction of $\text{Fe}(\text{CN})_6^{3-}$. When $\text{Fe}(\text{CN})_6^{3-}$ was reduced in the Pim film, a local deficit of positive charge was created. The deficit triggers an inflow of additional anions in order to keep electroneutrality of the near-surface region that contains the immobilized, positively charged Pim. Similar effects have been observed with a DNA brush and redox probe (e.g., $\text{Ru}(\text{NH}_3)_6^{3+}$ and MB^+).³⁶ Therefore, the biosensors constructed below was conducted in the solution containing 10 μM $\text{Fe}(\text{CN})_6^{3-}$ to prevent the leakage of the surface-entrapped $\text{Fe}(\text{CN})_6^{3-}$ and obtain the stable electrochemical response.

Before investigation on the biosensor applications, the electrochemical behavior and stability of the aptamer/Pim-modified Au electrodes were studied. As shown in Figure 2A, after the Pim-functionalized Au electrodes were further modified with aptamer, the redox current of $\text{Fe}(\text{CN})_6^{3-/4-}$ was decreased, obviously because of the displacement of negatively charged $\text{Fe}(\text{CN})_6^{3-}$ with aptamer. Based on this, the stability of the electrodes in the $\text{Fe}(\text{CN})_6^{3-}$ was investigated, which remains essential for developing ATP biosensor. As shown in Figure 2B (red and blue curve), no significant current change was observed after the electrode was immersed in the $\text{Fe}(\text{CN})_6^{3-}$ solution for 30 min, indicating that the interaction between Pim and aptamer was stronger than that of Pim and $\text{Fe}(\text{CN})_6^{3-}$ and further confirming the stability of the present biosensor in the absence of ATP. This strong interaction was also observed for other types of polyanions (e.g., heparin),³⁷ mainly originated from the multicharge factor of the aptamer. The adsorption of $\text{Fe}(\text{CN})_6^{3-}$ in Pim film, fast electron transfer rate of the surface-confined $\text{Fe}(\text{CN})_6^{3-}$, and the displacement

of surface-confined $\text{Fe}(\text{CN})_6^{3-}$ by ATP aptamer substantially validate the ATP aptamer/Pim-modified electrodes as the electrochemical aptamer biosensors for ATP sensing.

Linearity, Sensitivity, and Selectivity. The response mechanism of the ATP biosensor based on DRUS was essentially based on the change of surface charge by using $\text{Fe}(\text{CN})_6^{3-}$ as the signal readout (see section S3 (Scheme S2) in the Supporting Information). To quantitatively determine ATP concentration more sensitively and accurately, we employed chronocoulometry (CC) to record the change of redox charges of $\text{Fe}(\text{CN})_6^{3-/4-}$ on the electrode surface. This is because, on one hand, chronocoulometry could eliminate the interference from the solution-phased $\text{Fe}(\text{CN})_6^{3-}$, which may interfere with the determination when other electrochemical techniques, such as cyclic voltammetry and differential pulse voltammetry, are used.³⁸ On the other hand, almost all the electrochemical molecules are electroactive in the “static” chronocoulometric measurements, compared to the “dynamic” voltammetric scans, which was preferable to a highly sensitive determination.^{39,40} As shown in Figure 3, we observed that redox charges of $\text{Fe}(\text{CN})_6^{3-}$ on the biosensor increased upon treatment with ATP, which could be used for ATP sensing. As a consequence, we found that the increase in the signal intensity was linear with the logarithmic concentration of ATP within a concentration range from 10^{-16} M to 10^{-13} M ($Q'/nC = 5.59 + 3.26 \log C_{\text{ATP}}/\text{M}$ ($R^2 = 0.967$)). Moreover, the biosensor was quite reproducible, with less sensor-to-sensor variation (relative standard deviation (RSD) ranging from 4.3% to 8.9% for different ATP concentrations). Compared with the covalent DNA-assembly technique used in existing electrochemical aptasensors,^{41–45} the biosensor developed here with DRUS is sensitive, label-free, relatively convenient, and time-saving.

The selectivity of the biosensor is of great importance for cerebral analysis, because of the complexity of the cerebral system. Similar to the existing ATP aptamer biosensors,^{46–50} there was no obvious change after the biosensor developed here treated by a series of species coexisting in the cerebral system, such as UA, DA, AA, DOPAC, 5-HT, etc. (see section S4 (Figure S3) in the Supporting Information). Moreover, there was also no obvious change after the biosensor was treated with guanosine-5'-triphosphate (GTP), uridine-5'-triphosphate (UTP), or cytidine-5'-triphosphate (CTP), which was originated from the high affinity of the aptamer with ATP. More importantly, there was no significant change when the biosensor was separately treated with adenosine-5'-diphosphate

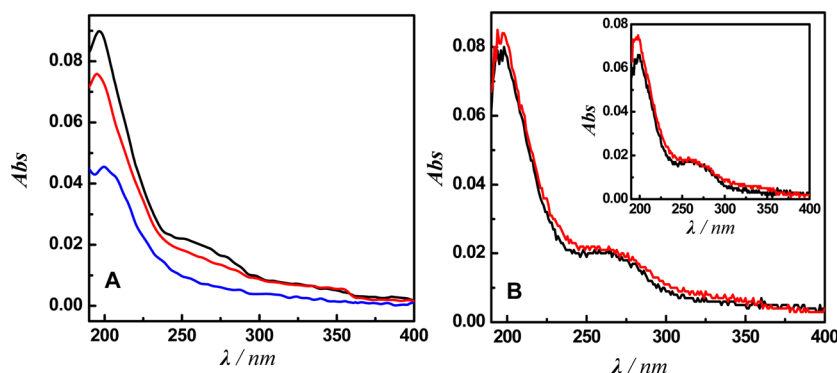


Figure 5. (A) Ultraviolet–visible (UV–vis) spectra of a Pim-modified SiO₂ slice (blue curve), an aptamer/Pim-modified SiO₂ slice before (black curve) and after treatment with 10^{−12} M ATP (red curve). (B) UV–vis spectra of aptamer/Pim-modified SiO₂ slice before (black curve) and after treatment with 10^{−10} M ADP (red curve). Inset in panel B shows UV–vis spectra of aptamer/Pim-modified SiO₂ slice before (black curve) and after treatment with 10^{−10} M AMP (red curve).

(ADP) and adenosine-5′-monophosphate (AMP), as shown in Figure 4, although the ATP aptamer shows the same order affinity toward AMP and ADP, as demonstrated previously.²²

To further study the Pim-based selectivity of the biosensor toward ATP against ADP and AMP, ultraviolet–visible (UV–vis) spectroscopy was used to monitor the change in the interface *in situ*, using a glass slide as the substrate. As shown in Figure 5, after the Pim-modified slice was treated by ATP aptamer, an obvious absorption peak at 260 nm was observed (Figure 5A, black curve), which was ascribed to the absorption of the aptamer, indicating the confinement of ATP aptamer onto the surface. After the slide was treated by 10^{−12} M ATP for 10 min, the absorption peak at 260 nm shows an obvious decrease (Figure 5A, red curve), demonstrating the dissociation of ATP aptamer from the surface. This result also indicates that the complex formed by ATP and its aptamer would dissolve into the solution. In contrast, when the slice was treated by 10^{−10} M ADP and AMP (Figure 5B), there was almost no change of absorption peak intensity at 260 nm (black and red curves), demonstrating that the complexes of ADP (or AMP) and aptamer could not be formed at this concentration and thus no response was obtained with the electrochemical biosensor (Figure 4). These results not only demonstrate that the different affinity of the biosensor toward ATP, ADP and AMP but also enables the use of CC method for highly sensitive ATP sensing, as described in Figure 3.

The high selectivity of the biosensor was considered to originate from the three combined effects: (i) the recognition ability of imidazolium toward different anions, (ii) the concentrated effect of polyelectrolyte (i.e., Pim) toward counterions, and (iii) the multiple charges of Pim. First, with regard to the recognition ability of imidazolium toward different anions, as reported previously, imidazolium can form strong (C–H)⁺⋯X[−] ionic hydrogen bonds with various anions.^{51–54} In this study, the different affinity of imidazolium toward Fe(CN)₆^{3−}, ATP, ADP, and AMP remains a keypoint for the selective sensing of ATP. Second, with regard to the concentrated effect of polyelectrolyte (i.e., Pim) toward counterions,^{55,56} this effect results in different interfacial concentrations of ATP, ADP, and AMP, even when the solution concentrations of each species were the same. Third, the multiple charges of Pim. This feature enables stronger affinity of Pim toward negatively charged aptamer than the small anions (i.e., Fe(CN)₆^{3−}, ATP, ADP, and AMP).

To quantitatively describe the affinity of Pim toward ATP, ADP, and AMP, the solubility constant (K_{sp}) of the complexes formed with Pim and ATP, or ADP, or AMP was determined (see section S5 (Table S1) in the Supporting Information). The complex of Pim–Fe(CN)₆^{3−} shows the smallest K_{sp} ($pK_{sp} \approx 12.05$), suggesting that Pim show a high affinity toward Fe(CN)₆^{3−} in water. The complex of Pim–ATP also shows a smaller K_{sp} ($pK_{sp} \approx 9.20$). The almost 1000-fold difference in the K_{sp} values of Pim–Fe(CN)₆^{3−} and Pim–ATP enables the total displacement of ATP by Fe(CN)₆^{3−} in the electrochemical experiments. Differently, the complexes formed by Pim with both ADP and AMP show the large solubility in water, which was almost similar to Pim–Cl. This difference in affinity of Pim toward ATP, ADP, and AMP enables the selective accumulation of ATP, resulting in a much higher C_{int} for ATP than ADP and AMP, which were further recognized by the ATP aptamer, as shown in Scheme 1B.

The origin of high sensitivity was considered in two different ways. One is the concentrated effect of polyelectrolyte (i.e., Pim), which has been widely studied both from theory and experiments.^{57–61} Moreover, the combination of other types of interaction (i.e., hydrogen bonding interaction) in the system enables our system with large accumulation interaction. For example, the interfacial concentration of Fe(CN)₆^{3−}, which was estimated from the surface charges, was calculated to be 66 mM when the solution concentration was only 10 μM (for calculation, see section S6 in the Supporting Information). The extraction coefficient ($K = C_{int}/C_{sol}$) was almost 2 orders of magnitude than the normal salt counterion (i.e., for Cl[−], $K = 500$).⁶² The large extraction coefficient results in the highly sensitive determination for ATP. The other effect was considered to arise from the low background of the Pim-based electrochemical aptamer biosensor since the charge neutralization between Pim and the aptamer leads to a low background, as illustrated by Kelley and co-workers.⁴¹ These two effects enable a highly sensitive determination of ATP.

ATP Assay in Rat Brain. To demonstrate the application of the as-constructed Pim-based aptamer biosensor for selectively sensing the cerebral ATP, the microdialysate from cortex of rat brain was first sampled by *in vivo* microdialysis. The microdialysates were then 10⁶-fold diluted by phosphate buffer (25 mM and containing 25 mM NaCl, pH 7.0) prior to the measurements. After the biosensor was directly treated by the diluted brain microdialysates for 10 min, it was measured by chronocoulometry in the phosphate buffer containing 25 mM

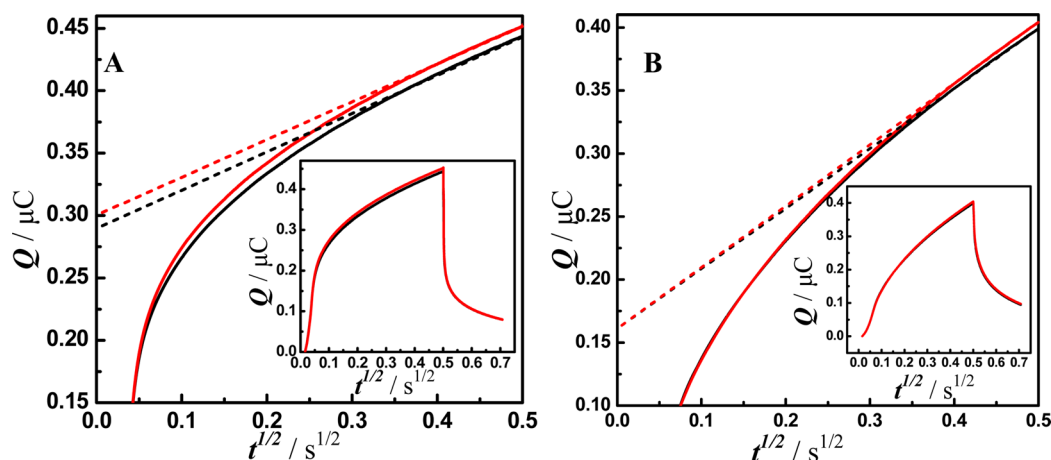


Figure 6. (A) Anson plot obtained at the biosensor before (black curve) and after the aptamer/Pim-modified gold electrode was treated with the diluted brain microdialysates in vivo sampled from the brain cortex of rats (red curve). (B) Anson plot obtained at the biosensor before (black curve) and after (red curve) the aptamer/Pim-modified Au electrode was treated with brain microdialysate pretreated by ATPase. Other conditions were the same as those described in Figure 3.

NaCl and $10\ \mu\text{M}$ $\text{K}_3\text{Fe}(\text{CN})_6$. As shown in Figure 6A, an obvious increase in charge was observed after the biosensor was treated by the diluted microdialysates, essentially demonstrating its validity for sensing ATP in the brain microdialysate. Considering the complexity of the brain system, we further verified the specificity of the biosensor by using ATPase, a class of enzymes that specially catalyze the decomposition of ATP into ADP and a free phosphate ion. Figure 6B shows the chronocoulometric curve of the biosensor in phosphate buffer (black curve) and after the biosensor was treated by the brain microdialysate, which was pretreated by ATPase (red curve). As shown, we did not observe any change after the brain microdialysate was treated by ATPase, indicating the excellent specificity toward ATP. This specificity substantially validates our aptamer-based biosensor toward the cerebral ATP sensing.

With the biosensor developed here, the basal level of ATP in the brain cortex microdialysate was determined to be $10.16 \pm 3.57\ \text{nM}$ ($n = 3$), which was almost consistent with the reported values.⁶³ These properties demonstrate that the DRUS offers an effective strategy to direct selective sensing of ATP in the cerebral system and this study paves a promising route to probing brain chemistry.

CONCLUSIONS

In summary, we have demonstrated that the dual recognition unit strategy is an efficient strategy for constructing highly selective and sensitive ATP aptamer-based biosensor. The constructed biosensor could be used for selective and sensitive determination of extracellular ATP concentration without the interference from ADP and AMP and other types of physiologically important species. Compared with the traditional methods by using one type of recognition unit, the present strategy by introducing the second recognition unit not only improves the selectivity but also the sensitivity. Moreover, the DRUS could also be extended for other targets by combining different aptamers. The concepts demonstrated here by rationally tuning the interfacial supramolecular ionic interaction could also be used to construct other types of biosensors without aptamers as the recognition units, such as enzymes and artificial receptors. More remarkably, the constructed biosensor could be used for selectively and sensitively sensing the extracellular ATP in rat brain, which is

envisaged to be of great importance in understanding the molecular basis of physiological and pathological events.

ASSOCIATED CONTENT

Supporting Information

Additional information as noted in the text. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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