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Designing Recognition Molecules and Tailoring Functional Surfaces for In Vivo Monitoring of Small Molecules in the Brain

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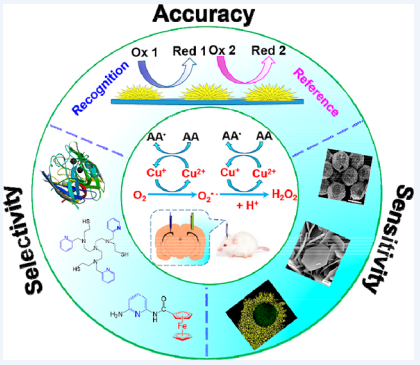
Supporting Information

CONSPECTUS: The in vivo analysis of chemical signals in brain extracellular fluid (ECF) using implanted electrochemical biosensors is a vital way to study brain functions and brain activity mapping. This approach offers excellent spatial (10−200 μm) and temporal (approximately second) resolution and the major advantage of long-term stability. By implantation of a microelectrode in a specific brain region, changes in the concentration of a variety of ECF chemical species can be monitored through applying a suitable electrical signal and, typically, recording the resulting Faradaic current. However, the high performance requirements for in vivo biosensors greatly limit our understanding of the roles that biomolecules play in the brain. Since a large number of biological species, including reactive oxygen species (ROS), metal ions, amino acids, and proteins, coexist in the brain and interact with each other, developing in vivo biosensors with high selectivity is a great challenge. Meanwhile, it is difficult to quantitatively determine target molecules in the brain because of the variation in the distinct environments for monitoring biomolecules in vitro and in vivo. Thus, there are large errors in the quantification of concentrations in the brain using calibration curves obtained in artificial cerebrospinal fluid (aCSF). More importantly, to gain a full understanding of the physiological and pathological processes in the brain, the development of novel approaches for the simultaneous determination of multiple species in vivo is urgently needed.



This Account provides insight into the basic design principles and criteria required to convert chemical/electrochemical reactions into electric signals, while satisfying the increasing requirements, including high selectivity, sensitivity, and accuracy, for the in vivo analysis of biomolecules in the brain. Recent developments in designing various functional surfaces, such as self-assembled monolayers, gold nanostructures, and nanostructured semiconductors for facilitating electron transfer from specific enzymes, including superoxide dismutase (SOD), and further application to an $O_2^{\bullet-}$ biosensor are summarized. This Account also aims to highlight the design principles for the selective biosensing of Cu^{2+} and pH in the brain through the rational design and synthesis of specific recognition molecules. Additionally, electrochemical ratiometric biosensors with current signal output have been constructed to correct the effect of distinct environments in a timely manner, thus greatly improving the accuracy of the determination of Cu^{2+} in the live brain. This method of using a built-in element has been extended to biosensors with the potential signal output for in vivo pH analysis. More importantly, the new concept of both current and potential signal outputs provides an avenue to simultaneously determine dual species in the brain.

The extension of the design principles and developed strategy demonstrated in this Account to other biomolecules, which may be closely correlated to the biological processes of brain events, is promising. The final section of this Account outlines potential future directions in tailoring functional surfaces and designing recognition molecules based on recent advances in molecular science, nanoscience and nanotechnology, and biological chemistry for the design of advanced devices with multiple target species to map the molecular imaging of the brain. There are still opportunities to engineer surfaces that improve on this approach by constructing implantable, multifunctional nanodevices that promise to combine the benefits of multiple sensing and therapeutic modules.

INTRODUCTION

The in vivo analysis of chemical signals is an essential way to investigate brain function and brain activity mapping.[1] The major obstacle to understanding the roles that biomolecules play in the brain is a lack of reliable and efficient methods for in vivo analysis. By implantation of a microelectrode in a specific brain region, changes in the concentration of a variety of ECF chemical species can be monitored with excellent spatial and temporal resolution.[2] However, it is challenging to develop directly implanted biosensors with high analytical performance for the in vivo determination of biological species in the brain. The first challenge is the improvement of the selectivity of in vivo biosensors to fulfill the requirements for real-time monitoring in the brain, because a large variety of biomolecules coexist and also interact with each other. To this end, fast-scan

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cyclic voltammetry (FSCV) employing carbon fiber microelectrodes (CFME), which was pioneered by Wightman and co-workers, has been reported to achieve selective detection through time resolution, benefiting from a high scan rate (>100 V s^{-1}).[2a,3] Several electroactive neurotransmitters, such as dopamine, ascorbic acid (AA), and 5-hydroxytryptamine, have been selectively determined by FSCV.[2a,3] In addition, the selectivity has usually been improved under low overpotentials due to nanomaterials with high electrocatalytic activity. For example, the selective determination of AA has been achieved using carbon nanotube-based electrodes.[1b,2e] Moreover, enzyme-based sensors have been widely developed for the selective determination of various small molecules, such as the superoxide anion ($O_2^{\bullet-}$), H_2O_2, glucose, and lactate,[1b,4] but the number of sensors with demonstrated capabilities for in vivo operation is limited. The efficient integration of the functional surface provides a key bridge to connect in vivo monitoring of related important species in the live brain with high analytical performance.[1,5] Careful tailoring of the functionalized surface to facilitate direct electron transfer from enzymes or proteins on the electrode surface, such as superoxide dismutase (SOD) and cytochrome *c* (Cyt c), has allowed us to establish new approaches for the in vivo monitoring of $O_2^{\bullet-}$ and H_2O_2.[4,6] Unfortunately, there are limited natural biocatalysts and recognition molecules available. Therefore, the design and synthesis of new molecules (or biomolecules) is highly desirable to develop biosensors with high selectivity for in vivo analysis. In recent years, Cu-free derivatives of bovine erythrocyte SOD (E_2Zn_2SOD) and protoporphyrin IX have been rationally prepared for the specific recognition of Cu^{2+} and Cd^{2+}, respectively.[7] Meanwhile, a series of organic molecules including *N*-(2-aminoethyl)-*N*,*N*′,*N*′-tris(pyridine-2-yl-methyl)ethane-1,2-diamine (AE-TPEA), 2,2′,2″-(2,2′,2″-nitrilotris(ethane-2,1-diyl)-tris((pyridin-2-ylmethyl)azanediyl)-triethanethiol (TPAASH), and *N*,*N*-di(2-picolyl)-ethylenediamine (DPEA) have been synthesized for the selective recognition of Cu^{2+},[8] and *N*-(6-aminopyridin-2-yl)ferrocene (Fc-Py) has been designed for H^+.[9] By integrating these specific molecules with nanostructured surfaces, a variety of biosensors have been developed for the in vivo determination of metal ions and pH in the brain with high selectivity.[7−9]

Furthermore, another challenge in the elaboration of electrochemical devices for in vivo analysis is how to quantitatively determine target molecules in the brain because of the variation in distinct environments between in vitro and in vivo experiments. Several factors, such as fluctuations in ionic strength, the large solution resistance, and the nonspecific adsorption of biomolecules at the electrode surface, may all give rise to determination errors. These uncertainties lead to inaccurate signal outputs (current and the potential) in the brain due to the potential shifting of the reference electrode and the large IR drop. Thus, the calibration curves determined in vitro are hard to use to quantify concentrations in vivo. To solve this problem, mathematical analysis using information from the background charging current has been used to exactly calibrate the data obtained by FSCV for in vivo measurements.[10] More recently, principal component analysis in tandem with inverse least-squares regression has been introduced for resolving and quantify overlapping compounds in FSCV data.[10b] We initially created a two-channel ratiometric electrochemical biosensor for the determination of Cu^{2+} in rat brains with a stable built-in correction to avoid the error between in vitro and in vivo environments.[7a] However, this biosensor still suffered from inaccurate detection due to two different working electrodes. Next, a single ratiometric biosensor was designed and developed to measure Cu^{2+} and L-cysteine (CySH) with high selectivity and accuracy, and it was successfully applied for the real-time evaluation of the levels of Cu^{2+} and CySH in live rat brains with Alzheimer's disease (AD).[8c] More recently, this ratiometric strategy was extended to monitor pH and chloride (Cl^-) in the brain using inner reference molecules with stable potential outputs.[9] In this case, the half-wave potential difference ($\Delta E_{1/2}$) between the recognition and the reference molecules was used as a measurable signal, remarkably enhancing the accuracy of potential-dependent biosensors.[9]

More importantly, monitoring the variation in a single biomolecule is not enough to comprehensively elucidate physiological and pathological phenomena. For instance, previous studies have suggested that an increase in the Cu^{2+} concentration causes the overproduction of ROS, resulting in a decrease in the pH.[11] Meanwhile, the acidification of the brain environment would lead to more serious oxidative stress, which might result in brain diseases.[12] Consequently, the development of approaches for the simultaneous detection of multiple species with high selectivity, sensitivity, and accuracy is urgently needed. Based on our previous work, we further developed ratiometric biosensors with both current and potential signal outputs to simultaneously detect two species, such as Cu^+ and pH, pH and O_2, or glucose and pH.[13] This concept of dual signal outputs established a flexible and promising approach for the further analysis of multiple species.

This Account focuses on the main achievements in tailoring functional surfaces, as well as designing specific recognition molecules, which have led to improvement of the selectivity, accuracy, and simultaneous detection of two species. A brief overview of high analytical performance based on surface engineering strategies and molecule design describes the design principles guiding the development of biosensors for in vivo monitoring in the live brain and the multifunctional nanodevices that combine the benefits of multiple sensing capacities and the incorporation of therapeutic modules. The last section outlines potential future directions for synthesizing recognition molecules and tailoring functional surfaces from the development of single-component biosensors toward the design of advanced devices with multiple targets.

SELECTIVE DETECTION OF $O_2^{\bullet-}$ IN THE LIVE BRAIN BASED ON DIRECT ELECTRON TRANSFER FROM SOD

The superoxide anion, the primary species of ROS, is generated in significant quantities as a result of normal intracellular metabolism.[14] The difficulties in elucidating the mechanism of $O_2^{\bullet-}$-induced oxidative stress arise from the lack of selective and reliable analytical methods for the real-time determination of $O_2^{\bullet-}$ because of the unique properties of $O_2^{\bullet-}$, including its relatively high reactivity, short lifetime, and broad, variable concentration range. The typical method for $O_2^{\bullet-}$ determination is via direct electron transfer to an enzyme or protein. The two main biocatalysts are Cyt c and SOD. Taniguchi et al. first reported the direct redox reaction with Cyt c, promoted by monolayers of electrode modifiers.[15] Since then, the direct electrochemistry of Cyt c has been extensively studied, and much effort has been put into the design of a third-generation biosensor (Supporting Information (SI)) for $O_2^{\bullet-}$. Recently, a

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Cyt c-based biosensor via coimmobilization with a lipid biosensor was developed to measure the extracellular $O_2^{\bullet-}$ levels produced by injections of cocaine in a live rat brain.[16] In fact, Cyt c is not a specific biocatalyst for $O_2^{\bullet-}$. Many reductants present in the brain, such as ascorbate, glutathione, and H_2O_2 in particular, can reduce Cyt c. Therefore, the inherent properties of Cyt c cause the sensor to suffer from interference, which greatly limits the application of Cyt c-based $O_2^{\bullet-}$ sensors in living systems.

Superoxide dismutase is known to be the specific enzyme that catalyzes the dismutation of $O_2^{\bullet-}$ to O_2 and H_2O_2, with a high rate constant up to the order of 10^9 M^{-1} s^{-1}. The unique properties of SOD motivated us to investigate the direct electron transfer to SODs, and we further developed SOD-based third-generation biosensors for the selective determination of $O_2^{\bullet-}$. The direct electrochemistry of bovine erythrocyte Cu,Zn-SOD was first achieved using a cysteine-modified electrode, and the promoted direct electron transfer was considered to be associated with the redox reaction of the copper site, which is also responsible for the dismutase activity.[17a] Next, the electron transfer of SODs was well-promoted on mercaptobenzoic acid (MPA)-modified Au electrodes.[17b] Moreover, these SOD-based biosensors showed bifunctional electrocatalysis toward $O_2^{\bullet-}$ with high selectivity. Then, with the development of nanomaterials, the electron transfer of SOD was facilitated on differently shaped gold nanostructures, as well as on TiO_2 nanoneedles.[18] In particular, rapid electron transfer with Cu,Zn-SOD was realized using highly conductive ZnO nanodisks (Figure 1A) with a formal redox potential ($E^{0\prime}$) located between the $E^{0\prime}$ values of the redox couples $O_2/O_2^{\bullet-}$ and $O_2^{\bullet-}/H_2O_2$ (Figure 1B).[19] The bidirectional electromediation observed on ZnO nanodisks is essentially based on the inherent specificity of SODs for the dismutation of $O_2^{\bullet-}$ (Figure 1C). This ZnO/SOD electrode is capable of sensing $O_2^{\bullet-}$ cathodically at 0 mV (vs Ag/AgCl), without interfering species from living systems, which exhibited higher selectivity and sensitivity and a wider dynamic range for the detection of $O_2^{\bullet-}$ than those using TiO_2 and gold nanomaterials. Finally, this ZnO/SOD microelectrode was successfully applied for the determination of $O_2^{\bullet-}$ in bean sprouts grown in O_2 hyperoxia.

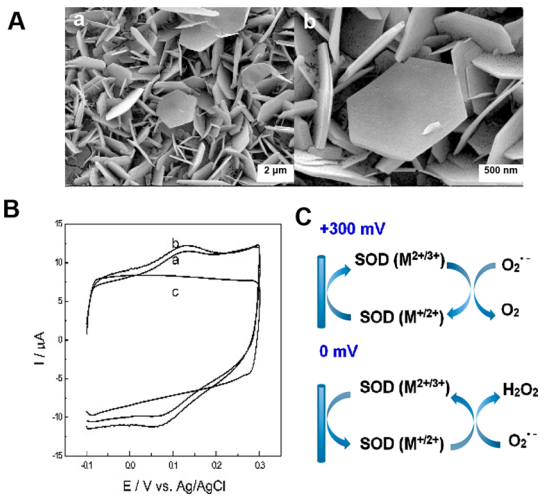

Figure 1. (A) (a) SEM image of an electrodeposited ZnO nanodisk film and (b) the enlarged SEM image of the film. (B) Cyclic voltammograms (CVs) obtained (a, b) using a Cu,Zn-SOD-modified nanostructured ZnO electrode (ZnO/SOD) (a) in the absence and (b) in the presence of 30 μM $O_2^{\bullet-}$ in 25 mM of phosphate buffer (PBS) (pH 7.2) and (c) using a bare ZnO nanodisk electrode in the presence of 30 μM $O_2^{\bullet-}$ in 25 mM PBS (pH 7.2). (C) Mechanism for the bidirectional catalysis of $O_2^{\bullet-}$ dismutation by SOD using the ZnO nanodisks film. Adapted with permission from ref 19. Copyright 2008 American Chemical Society.

However, this type of ZnO nanodisk electrode is still difficult to miniaturize to fulfill the requirements for the in vivo analysis of $O_2^{\bullet-}$ in the brain. Meanwhile, stabilizing SOD on the electrode is also a challenge. Recently, we decreased the size down to ~10 μm through employing CFME to develop a new $O_2^{\bullet-}$ biosensor using a nitrilotriacetic acid (NTA)/histidine tag (HT) technology to anchor the histidine residues in SOD through metal−chelate affinity (Figure 2A,B).[6b] This NTA-modified electrode provided a more stable model to assemble SOD on the CFME, and the direct electron transfer to SOD was greatly enhanced with a high rate constant (k_s) of 24 ± 1.1 s^{-1}. The optimized $O_2^{\bullet-}$ biosensor exhibited a broad dynamic range from 10^{-7} to 10^{-4} M and a low detection limit of 21 nM, with high selectivity and long-term stability. This durable and reliable biosensor was successfully applied to the monitoring of $O_2^{\bullet-}$ in the rat brain during ischemia and reperfusion (Figure 2C). The obvious increased current suggests a burst of $O_2^{\bullet-}$ during both the ischemia and reperfusion processes.

RATIOMETRIC STRATEGY WITH SPECIFIC RECOGNITION FOR SELECTIVE AND ACCURATE DETERMINATION IN LIVE RAT BRAINS

As one of the essential components utilized in all domains of life, copper (Cu) can induce the overproduction of ROS, which generates oxidative damage to lipids, proteins, and nucleic acids.[11] High amounts of Cu^{2+} ions are found within the senile plaques of AD patients, directly bound to the amyloid-β peptide and are linked to neurotoxicity and oxidative stress.[11] Relying on the inherent redox signal of Cu^{2+}, a series of electrochemical methods for the measurement of Cu^{2+} have been established by our group through integrating specific recognition with efficient nanomaterials to form organic−inorganic hybrid systems. We first designed a two-channel ratiometric electrochemical strategy for the accurate detection of Cu^{2+} in rat brains.[6a] A Cu-free derivative of bovine erythrocyte E_2Zn_2SOD was employed for its specific recognition environmental effect, leading to the detection of Cu^{2+} with dual-channel signal outputs. The native SOD contains two metal centers, Zn^{2+} and Cu^{2+}. When Cu^{2+} was removed from SOD, E_2Zn_2SOD retained a specific recognition ability for Cu^{2+}, resulting in the selective determination of Cu^{2+}. Meanwhile, 6-(ferrocenyl)hexanethiol (FcHT) was used as reference element and modified at another electrode to provide a stable built-in correction (Figure 3A).

In addition, gold truncated octahedral microcages full of nanograins were synthesized to enhance the sensitivity ~7-fold, due to their large surface area and high electrocatalytic activity (Figure 3B). This biosensor shows a high sensitivity of 30.2 mA M^{-1} cm^{-2} and a low detection limit of 3 nM. Combined with the microelectrode technique, this biosensor was directly implanted into a normal rat brain and a rat brain following cerebral ischemia. The basal level of Cu^{2+} in the brain dialysates was estimated to be 1.67 ± 0.56 μM, while the Cu^{2+} increased ~5-fold after global cerebral ischemia (Figure 3C). However, the two independent working electrodes give rise to not only a more complicated operation and instrumentation but also more serious injury to the animal brain. More importantly, there is

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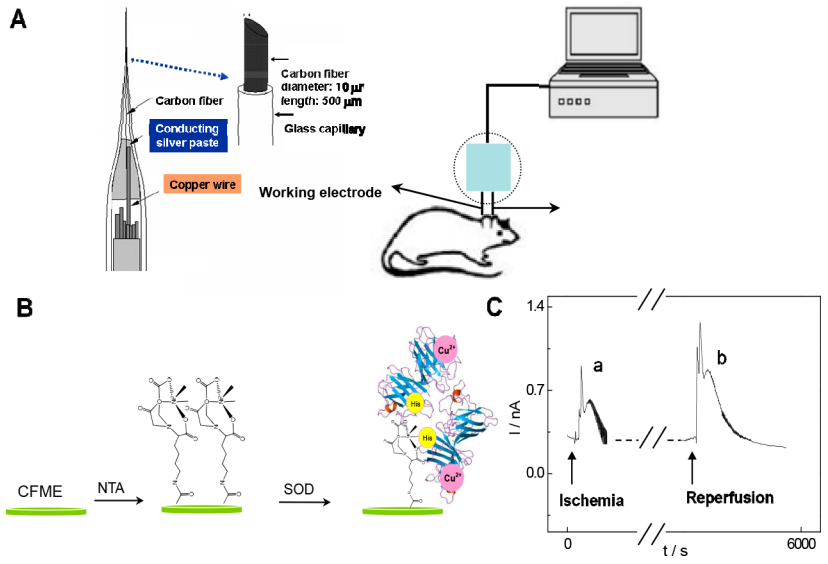

Figure 2. (A) Schematic diagram of an electroanalytical system for the in vivo monitoring of $O_2^{\bullet-}$ in a rat brain with CFME. (B) Schematic illustration of the modification processes of NTA and SOD. (C) Amperometric responses obtained at a SOD-immobilized CFME electrode implanted in the rat brain upon (a) ischemia and (b) reperfusion. Adapted with permission from ref 6b. Copyright 2013 Elsevier.

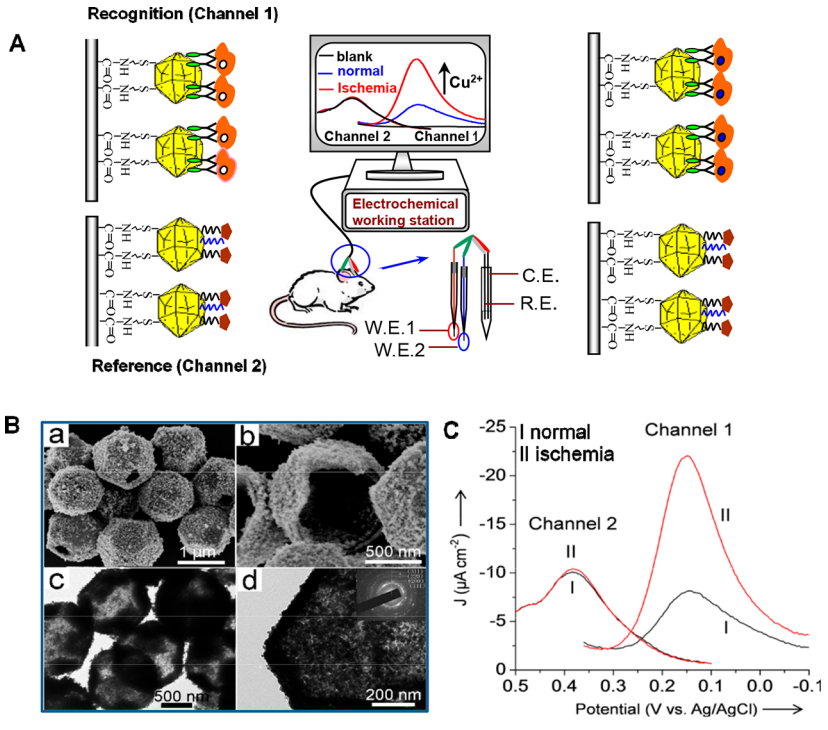

Figure 3. (A) The present two-channel biosensor for in vivo monitoring of Cu^{2+} in a rat brain. (B) SEM (a, b) and TEM (c, d) images of the Au microcages. (C) Differential pulse voltammetry (DPV) responses obtained using the two-channel ratiometric biosensor for the determination of Cu^{2+} in (I) a normal rat brain and (II) a rat brain following cerebral ischemia. Reproduced from ref 7a. Copyright 2013 WILEY-VCH Verlag GmbH&Co. KGaA, Weinheim.

still some error in the quantitative determination between the two electrodes. To this end, we further synthesized a new molecule, DPEA, and developed a single biosensor for the determination of Cu^{2+} followed by CySH (Figure 4A). The electrochemical reduction peak of Cu^{2+} increased with increasing concentrations of Cu^{2+} (Figure 4B). More interestingly, this peak current decreased after the addition of CySH, which could be attributed to the release of Cu^{2+} from the DPEA–Cu^{2+} complex when CySH bound to the Cu^{2+} center (Figure 4C). Meanwhile, 5′-MB-GGCGCGA$(T)_{13}$-SH-3′ (HS-DNA-MB, MB = methylene blue) with a separated peak potential was designed as an internal standard to avoid complicated interference in the brain. This ratiometric biosensor with a single electrode was successfully applied to evaluate the levels of Cu^{2+} and CySH in a live rat brain with AD. We found that the concentration of Cu^{2+} increased ~5-fold and that of CySH decreased ~3-fold in rat brains with AD compared with the concentrations in a normal rat brain.

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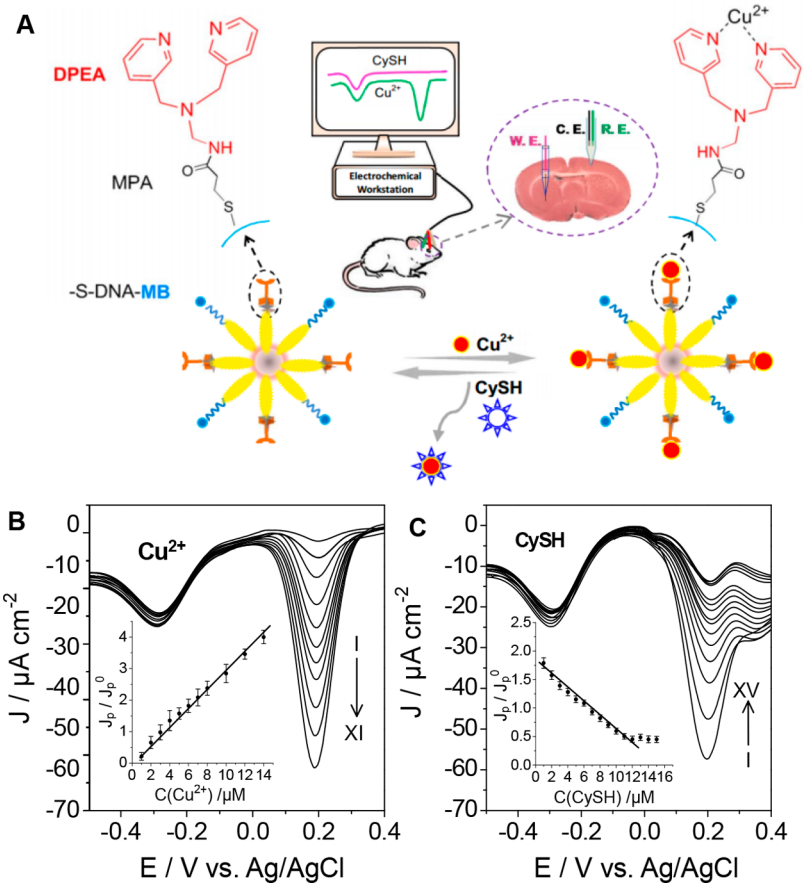

Figure 4. (A) Illustration of the single electrochemical biosensor for the in vivo ratiometric monitoring of Cu^{2+} and CySH in live rat brains with AD. (B) DPVs obtained at a microelectrode immobilized by DPEA and MB (DPEA+MB/Au/CFME) in aCSF (pH 7.4) with different concentrations of Cu^{2+}. Inset, linear relationship between the peak current density ratio obtained at 195 mV and −290 mV (J_p/J_p^0) and the Cu^{2+} concentration. (C) DPVs obtained at DPEA+MB/Au/CFME after reaction with Cu^{2+} in aCSF. Inset, linear relationship between J_p/J_p^0 and the CySH concentration. Adapted with permission from ref 8c. Copyright 2015 WILEY-VCH Verlag GmbH&Co. KGaA, Weinheim.

POTENTIAL-DEPENDENT BIOSENSOR WITH AN INTERNAL REFERENCE FOR DETERMINING pH

The local pH of the brain microenvironment plays a significant role in signal transduction, enzymatic activity, and ion transport.[20] The production of ROS leads to a decrease in pH, which is thought to be related to many chronic degenerative diseases, such as AD and ischemia.[21] The most popular pH determination strategy is based on the Nernst equation, which yields a well-defined linear correlation between the potential and the pH, for example, using ion-sensitive microelectrodes (ISMEs).[22] Unfortunately, ISMEs made from glass are difficult to miniaturize for measurements in the brain. More importantly, in contrast to the small solution resistance in a 0.1 M dilute electrolyte solution (∼100 Ω), in the complicated brain environment the presence of many coexisting proteins and other biological species increases the solution resistance in cerebrospinal fluid up to 3000−5000 Ω.[23] As a result, the IR drop for a microelectrode with a current of ∼1 μA will increase to ∼3−5 mV in the brain. This value is comparable to the change in potential induced by a pH change of 0.1 pH units for an electrochemical process with a linear variation of 59 mV/pH unit. However, a pH variation as small as 0.2−0.5 pH units in living organisms has been attributed to a variety of severe cellular problems and diseases. Thus, pH determination approaches that rely on potential variations without built-in corrections lead to a determination error of ∼20−50% for pH in the brain. As a result, the real-time detection of pH in a live rat brain is still a great challenge due to not only selectivity but also accuracy.

To solve the problems demonstrated above, we recently developed a two-channel electrochemical pH biosensor with an internal reference for the real-time monitoring of pH in different regions of rat brains upon ischemia (Figure 5).[8] In one channel, Fc-Py was synthesized as an electrochemical probe for the selective recognition of pH (Figure 5A), in which the ferrocene group is electrochemically redox active and the pyridine group responds to protons. On the other channel, FcHT with a well-separated redox potential, inert to pH, was optimized as an internal reference to improve the accuracy. The pH calibration curve was obtained from the half-wave potential ($E_{1/2}$) difference between the response and the reference signal ($\Delta E_{1/2}$). The $\Delta E_{1/2}$ value is less dependent on the potential of the outer reference electrode and the potential shift caused by the IR drop, thus greatly improving the accuracy of the pH determination in vivo. This type of two-channel biosensor was directly implanted into brain regions including the striatum, the hippocampus, and the cortex and was successfully used for the real-time monitoring of pH values in these regions of brain after global cerebral ischemia. However, the sensitivity of this two-channel pH meter is 0.13 pH units. A more sensitive pH meter is necessary because the pH fluctuations in living system are generally small. Thus, we further developed a novel pH

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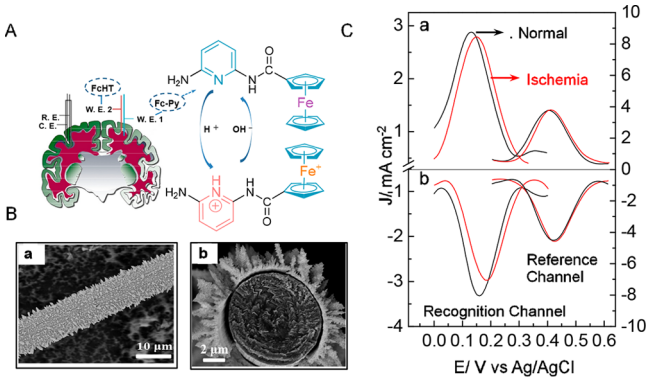

Figure 5. (A) Illustration of the two-channel electrochemical biosensor for the in vivo determination of pH in a live rat brain. (B) SEM images of CFME/Au: (a) top view and (b) cross section. (C) DPV responses for (a) oxidation and (b) reduction processes obtained at the two-channel ratiometric biosensor (recognition channel, *N*-(6-aminopyridin-2-yl)ferrocene (Fc-Py)-modified CFME/Au; response channel, 6-(ferrocenyl) hexanethiol (FcHT)-modified CFME/Au). Adapted with permission from ref 9a. Copyright 2016 Royal Society of Chemistry.

biosensor by coimmobilizing the specific recognition of 1,2-naphthoquinone (1,2-NQ), which has good electrochemical redox behavior, and FcHT at one electrode (CFME/Au/1,2-NQ+FcHT). This establishes a $2H^+/2e^-$ approach over a wide pH range from 5.8 to 8.0,[9] decreasing the limit of detection to 0.07 pH units (Figure 6A,B). The in vivo results demonstrated that the pH values were 7.21 ± 0.05 in the striatum, 7.13 ± 0.09 in the hippocampus, and 7.27 ± 0.06 in the cortex in the normal rat brains. However, the pH decreased to 6.75 ± 0.07 and 6.52 ± 0.03 in the striatum and the hippocampus, respectively, upon global cerebral ischemia, with a negligible pH change in the cortex (Figure 6C,D). These results indicate that ischemia can lead to variations in the acidity of the brain microenvironment. To the best of our knowledge, this is the first report of accurate pH values in the different regions of normal rat brains and those after global cerebral ischemia.

DUAL SIGNAL OUTPUTS FOR THE IN VIVO ANALYSIS OF TWO SPECIES IN THE BRAIN

Both O_2 and pH are critically important species correlated with brain ischemia. During ischemia, when O_2 supply is limited, the electron transport chain of the linear mitochondrial membrane becomes high reduced. This reduced state may result in the production of ROS. Then, when respiration is inhibited but glycolysis persists, protons and lactate may be generated, leading to a decrease in the pH.[25] Over the past few decades, many elegant approaches have been developed for monitoring either O_2 or the pH.[24] However, the simultaneous recognition and accurate determination of O_2 and pH in the live brain is still challenging.

To accomplish the simultaneous determination of O_2 and pH, an organic molecule, hemin-aminoferrocene (hemin-Fc) was designed and synthesized (Figure 7A).[13a] Hemin-Fc has three functions. The cathodic current of the hemin group was employed as the specific response to determine the O_2 concentration, taking advantage of the four-electron catalytic activity of hemin for O_2. Meanwhile, the $E_{1/2}$ of the redox peaks was used to determine the pH, since the redox process of the porphyrin ring is pH-dependent. In addition, the Fc group is inert to both O_2 and pH and so provides a built-in correction

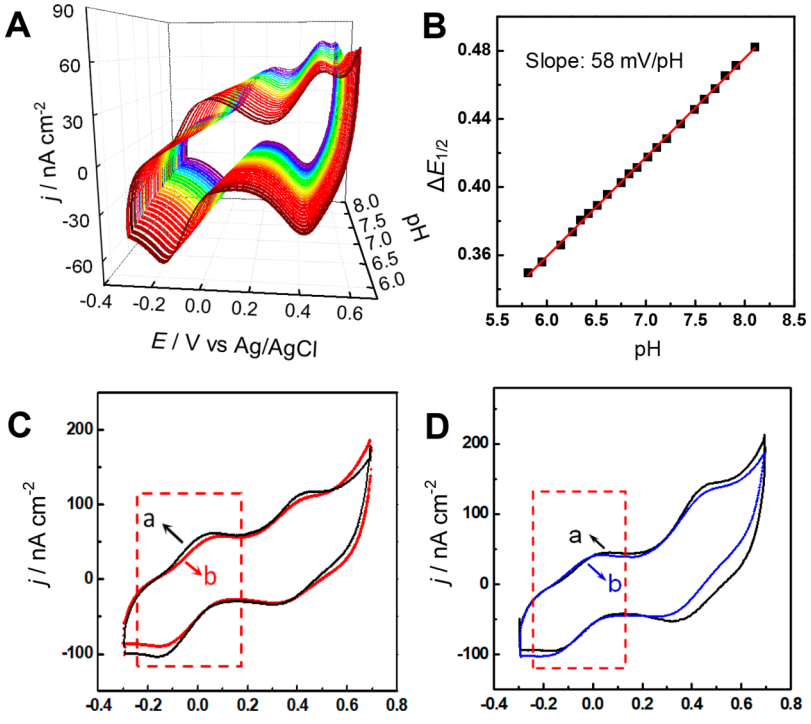

Figure 6. (A) CVs obtained at CFME/Au/1,2-NQ+FcHT in 10 mM of PBS with pH changes from 5.8 to 8.0. (B) Calibration plot of the half-wave potential difference between the 1,2-NQ and FcTH redox peaks ($\Delta E_{1/2}$) versus pH. (C) CVs obtained at a ratiometric CFME/Au/1,2-NQ+FcHT electrode for the determination of pH in the striatum of (a) a normal rat brain and (b) a rat brain following cerebral ischemia for 5 min and (D) (a) a rat brain upon ischemia for 5 min and (b) further microinjection of a 100-μM Na_2CO_3 solution. The enlarged pH shift, indicated by the red dashed rectangles, is shown in Figure S1 (SI). Adapted with permission from ref 9b. Copyright 2016 American Chemical Society.

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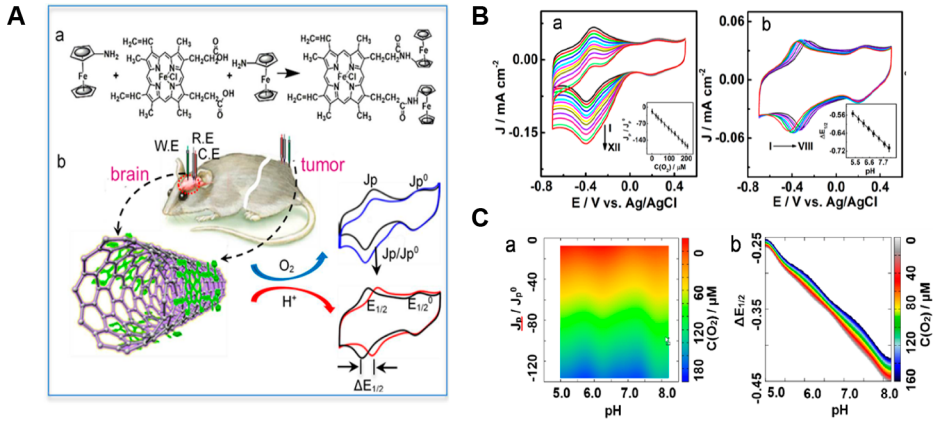

Figure 7. (A) (a) Scheme for the formation of the conjugate hemin-Fc from hemin and Fc. (b) Illustration of the working mechanism of the present biosensor for the simultaneous determination of O_2 and pH. (B) CVs obtained with a hemin-Fc-modified carbon nanotube fiber (hemin-Fc/CNF) microelectrode (a) in 0.1 M PBS bubbled with different concentrations of pure O_2 and (b) different pH values. (C) (a) Relationship among the reduction peak current density ratio of hemin and Fc (J_p/J_p^0), the concentration of O_2, and the pH; (b) relationship among the half-wave potential difference between the hemin and Fc redox peaks ($\Delta E_{1/2}$), the concentration of O_2, and the pH. Reproduced from ref 13a. Copyright 2017 WILEY-VCH Verlag GmbH&Co. KGaA, Weinheim.

for the quantification of O_2 and pH. To improve the stability and sensitivity of the electrode performance, a carbon nanotube fiber was fabricated as a stable and biocompatible substrate to immobilize hemin-Fc via $\pi-\pi$ stacking. Notably, cubic spline interpolation was used to smooth the experimental measurements. Relationship images were employed to quantify the levels of O_2 and pH in live systems, as shown in Figure 7B,C. This straightforward bifunctional biosensor was first used for the simultaneous determination of O_2 and pH in a live brain with two ischemic animal models: one with middle cerebral artery occlusion (MCAO) and the other with carotid artery ligation (CAI). The pH value was estimated to be remarkably decreased by 0.34 units and 0.47 units in the cortex and the striatum of mouse brain, respectively, after a MCAO time of 1.5 h. The initial concentration of O_2 was estimated to be 61.0 ± 6.8 and 51.0 ± 5.9 μM in the cortex and the striatum, respectively, and gradually decreased to 17.0 ± 2.8 and 11.0 ± 2.5 μM, respectively, after a MCAO duration time of 1.5 h. After MCAO for 2 h, the longer time ischemia induced neuronal death. The present study provided direct evidence that the severity of neuronal death strongly depends on the increasing duration of brain acidity. From the results of the CAI ischemic model, we can see that the pH values in the striatum and the hippocampus remarkably decreased to 6.72 ± 0.03 and 6.58 ± 0.04, respectively, after a short ischemia time of 21 min.

This methodology employing dual signal outputs not only provides design criteria for artificial organic molecules but has also opened a route to developing biosensors for the determination of two targets in the live brain. Therefore, we further employed glucose oxidase as a specific recognition element for the simultaneous quantification of glucose and pH in a diabetic rat brain, because the active center (FAD) undergoes a $2H^+/2e^-$ process.[13b] The experimental data in vivo demonstrated that the basal pH level decreased to 6.9 ± 0.1 and 7.1 ± 0.1 in the striatum and the cortex of rat brains of a diabetic model, respectively. In contrast, the glucose concentrations were found to be 2.22 ± 0.18 mM and 1.44 ± 0.12 mM in the striatum and the cortex of normal rat brains and increased to 4.53 ± 0.40 mM and 6.65 ± 0.31 mM, respectively, in the rat brains of the diabetic model. Detailed information about the levels of pH and glucose in these rat brains may help us understand damage to brain function in diabetics. The simplicity of operation and instrumentation of the biosensor should promote its use in a broad range of biochemical applications.

CONCLUSIONS AND OUTLOOK

The development of in vivo monitoring strategies for biologically important species in brain chemistry is challenging but required for progress in understanding the roles that biomolecules play in pathological and physiological processes. Rationally tailoring functional surfaces, as well as designing specific molecules, has greatly improved the sensitivity and selectivity of biosensors for distinguishing between biomolecules and fulfilling the demanding requirements for in vivo measurements. The electrochemical ratiometric strategy has established fascinating approaches for the in vivo quantification of Cu^{2+}, pH, O_2, and glucose with high accuracy. As a result, the development of biosensors with remarkable analytical performance has allowed for the provision of accurate and reliable information regarding single molecules and/or multiple species in live brains; this information should be very beneficial for evaluating brain processes at the molecular level.

The design principles guiding the development of biosensors for biomolecules in the brain presented in this study show the potential to be extended to other small biomolecules, which may be closely correlated with biological processes and brain events. Moreover, recent advances in molecular science, nanoscience and nanoengineering, and biological chemistry should result in a shift in the focus of the surface engineering and functionality away from the synthesis of single-component biosensors toward the design of advanced devices that may even target multiple species to obtain more information about the brain. The possibility of designing and tailoring surfaces to construct implantable multifunctional nanodevices that promise to combine the benefits of multiple sensing capacities and incorporate therapeutic modules can be realized.

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■ ASSOCIATED CONTENT

Supporting Information

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Brief introduction about the third-generation biosensor and enlarged CV waves for redox peaks of 1,2-NQ (PDF)

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