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Tutorial Review

Tuning Interionic Interaction for Highly Selective In Vivo Analysis

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Development of highly selective methodologies to enable in vivo recording chemical signals is of great importance for studying brain functions and brain activity map. However, the complexity of cerebral systems presents a great challenge to the development of chem/(bio)sensors that are capable of directly and selectively recording bioactive molecules involved in brain functions. As one of the most important and popular interactions in nature, interionic interaction constitutes chemical essence of high specificity in natural system, which inspires us to develop highly selective chem/(bio)sensors for in vivo analysis by precisely engineering interionic interaction into the in vivo sensing system. In this tutorial review, we focus on the recent progress of tuning interionic interaction to improve the selectivity of biosensors for in vivo analysis. The type and property of interionic interaction is first introduced. And several strategies to improve the selectivity of the biosensors, including enzyme-based electrochemical biosensors, aptamer-based electrochemical biosensors, and the strategies to recruite recongnition molecules are reviewed. We also overview the potential applications of the biosensors for in vivo analysis and thereby for physiological invesitgations. Finally, we present the major challenges and opportunities regarding the highly selective of in vivo analysis based on tuning interionic interaction. We believe that this tutorial review provides critical insights for highly selective in vivo analysis and offers new concepts and strategies to understand brain chemistry.

Key Learning Points

1)The type and property of interionic interactions and their applications in biosensor designing will be addressed.

2)The principle and strategy for highly selective in vivo analysis by tuning interionic interaction will be discussed.

3)The challenges and the opportunities inherent in the future development of this research field will be outlooked.

1. Introduction

Development of new methodologies and technologies to enable in vivo recording chemical signals is of great importance for studying brain functions and brain activity map.[1-2] In this case, development of chem/(bio)sensors with a high selectivity towards one specific bioactive molecule are particularly attractive to accomplish this pursuit.[3-7] However, the complexity of the cerebral systems presents a great challenge to this solution. So far, two common strategies have been developed to in vivo sensor design, one is to take use of the physiochemical properties inherent in the bioactive molecules themselves to enable the chem/(bio)sensors to selectively recognize and sense the target.[8-12] In spite of its theoretical feasibility, this strategy remains practically difficult and necessitates both strong chemical background on the mechanistic design and the state-of-the-art systems to perform the analysis, due to the coexistence of the molecules with the similar physiochemical

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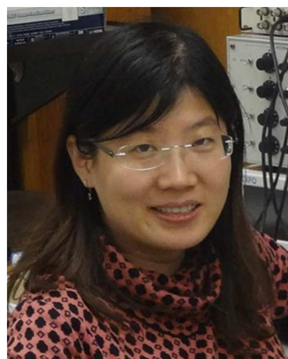

Ping Yu

Ping Yu received her B.Sc. and M. Sc. in Chemistry from Yantai Normal University (2001) and Xiangtan University (2004), respectively. She then joined Institute of Chemistry, the Chinese Academy of Sciences (ICCAS) and received her Ph.D. in 2007. She is currently an associate professor in Key Laboratory of Analytical Chemistry for Living Biosystems at ICCAS. Her ongoing work focuses on interionic interaction and chem/(bio)sensors.

Xiulan He

Xiulan He received her B.Sc. and M. Sc. in Chemistry from Xiangtan University in 2011 and 2014, respectively. She then joined ICCAS to pursue her PhD degree under the guidance of Lanqun Mao and Ping Yu. Her current research focuses on chem/(bio)sensors.

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properties with the target in the cerebral system. The other strategy is to efficiently couple biochemical reaction mechanisms in natural biosystems with reliable signal readout configuration to form chem/(bio)sensing platform that can be in turn used for selective in vivo sensing. The biochemical reactions that can be coupled into sensing scheme include those with enzymes, antibodies, receptors, nucleic acids, and saccharides. Motivated by the mechanism of these biochemical reactions, researchers have developed various technologies such as molecular imprinting,[13-14] SELEX technique,[15] host-guest recognition[16-18] for the development of chem/(bio)sensing with these artificial synthetic recognition units. While both strategies have been demonstrated to be useful, especially for in vitro analysis, the complexity of the cerebral system renders a great challenge to these methods due to the simple uses of the physiochemical properties inherent in the bioactive molecules themselves or the natural/artificial recognition units do not well meet the requirement of selective in vivo neurochemical sensing. Therefore, it is highly imperative to develop new principles to further improve the selectivity of the chem/(bio)sensors to advance the brain research project through developing innovative neurotechnologies to in vivo record the chemical signals involved in the brain functions.

Intermolecular interaction is the chemical essence inherent in the high specificity in nature, where biological macromolecules (i.e., natural recognition units) play essential roles in many biological activities mainly by weak bonding. Most of these weak bonding (e.g., hydrophobic, hydrogen bonding, and electrostatic interactions) have been clarified and well-known nowadays.[19-21] Recently, it is found that interionic interaction (e.g., cation-π,[22] and ionic-hydrogen bonding[23]) also plays key roles in natural molecular recognition possibly because most of the biomolecules are charged in biological systems. The interionic interaction demonstrated here refers to the interaction between ions and their counterparts, which is not only composed of electrostatic attraction between oppositely charged species but also other kinds of weak interactions. For example, ionic hydrogen bonding not only contains the contribution from both hydrogen bonding and electrostatic attractions. The involvement of other kinds of weak interactions makes the interionic interaction stronger than the single kind of weak interactions. It thus occurs to us if we could develop sensors by rationally engineering interionic interaction to refine the performance of the sensors for in vivo sensing applications, although such strategy has previously been used for in vitro fluorescent and electrochemical sensing.[24-28] Inspired by this idea, we have focused on developing in vivo chem/(bio)sensors by rationally tuning the interionic interaction to improve the selectivity of sensors over the last several years. Besides, we have found that it is also a good way to prepare new ionic materials with a high chemical sensing ability also through smartly engineering interionic interactions. This tutorial review summaries several examples of the strategies demonstrated on engineering interionic interaction for in vivo chem/(bio)sensors mainly with electrochemical and colorimetric mechanisms. For this purpose, we first demonstrate the strategies to improve the selectivity of enzyme-based biosensors by engineering the ionic hydrogen bonding and cation-π interactions. The strategy to improving the selectivity of aptamer-based biosensors was also demonstrated by using ATP aptamer as model. We then describe the strategies to recruit recognition molecules by tuning interionic interaction. Finally, we proof-of-concept demonstrate a strategy to synthesize the new sensing materials by interionic self-assembly.

Lanqun Mao

Lanqun Mao is currently a professor of Key Laboratory of Analytical Chemistry for Living Biosystems at ICCAS. He has been working on the interface between electrochemistry and neurosciences, with emphasis on the development of new neurotechnologies to understand the chemical signals involved in some physiological processes. He obtained his Ph.D. in East China Normal University in 1998 and then worked in BAS Inc. Japan as a research scientist (1998-2000) and pursued his post-doctoral studies at Department of Electronic Chemistry at Tokyo Institute of Technology (2000-2002). He was a recipient of the "Hundred Distinguished Young Scholars" from the Chinese Academy of Sciences (2002) and the "National Distinguished Young Scholars" from National Natural Science Foundation of China (2006).

2. Improving the Selectivity of the Existing Recognition Units-based Biosensors

Up to now, many kinds of recognition units have been developed, and various biosensors have been developed based on these recognition units. Although these existing recognition units, including naturally recognition units (e.g., enzyme) and artificial selected recognition units (e.g., aptamer) provides a great chance to construct the highly selective biosensors, the complexity of the cerebral system renders a great challenge to these methods. This part review several strategies to improve the selectivity of recognition units (e.g., oxidase, dehydrogenase and aptamer)-based biosensors for in vivo analysis by tuning the interionic interaction. The strategy to construct the highly selective biosensors by using of the different kinds of interionic interaction (e.g., Ionic hydrogen bonding, cation-π) was mainly discussed.

2.1 Ionic Hydrogen Bonding Interaction for Oxidase-Based In Vivo Electrochemical Biosensors.

Ionic hydrogen bonding is formed between ions and molecules with bonding strength of 5-35 kcal/mol, up to a third of the strength of covalent bonds. This interaction is important in bioenergetics including protein folding, membrane formation, proton transport, and biomolecular recognition.[23] Among all ionic hydrogen bonding-based receptors, imidazolium group has attracted much attention

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since they could form a strong interaction with anions through the formation of $(C\text{-}H)^{+}\ldots X^{-}$ type ionic hydrogen bonding.[24-27] We found that ionic hydrogen bonding interaction exists between polyimidazolium and redox mediator (i.e., $Fe(CN)_6^{3-/4-}$) frequently employed for oxidases. By using this interaction, we were able to tune the potential of the redox mediator in a negative direction, and thus achieved the selectivity for the oxidase-based electrochemical biosensors for in vivo applications,[29] as demonstrated below.

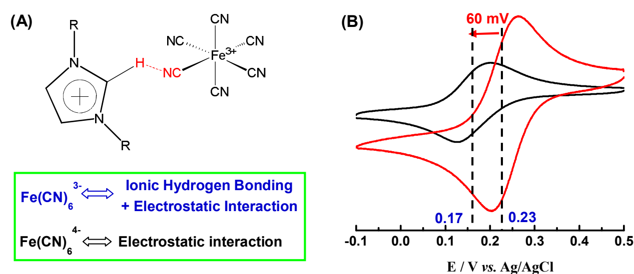

Fig. 1 (A) Schematic illustration of the interionic interaction between $Fe(CN)_6^{3-}$ and imidazolium. (B) Typical cyclic voltammograms of solution-phased (red curve) and surface-confined (black curve) $Fe(CN)_6^{3-}$. Scan rate, 100 mV s^{-1}. Reproduced with permission from ref. 29. Copyright 2012 American Chemical Society.

Generally, two kinds of enzymes (i.e., oxidase and dehydrogenase) are employed to construct electrochemical biosensors with different sensing mechanisms, of which the use of oxidases as the biorecognition elements normally necessitates an efficient electronic communication between oxidases and electrode, which are generally realized with the use of artificial redox mediators to shuttle the electron transfer of oxidases. In this case, the selectivity of the biosensors is also dominated by the potential of the redox mediators. While ferricyanide ($Fe(CN)_6^{3-}$) has been widely used as the redox mediator for most kinds of oxidases, it cannot be simply used to form an oxidase-based biosensor for in vivo sensing because of the high formal potential of $Fe(CN)_6^{3-/4-}$ in solution phase (i.e., +0.23 V vs. Ag/AgCl), which overlaps with those for the oxidation of electrochemically active neurochemicals endogenously existing in the cerebral systems such as dopamine, uric acid, catecholamines, and their metabolites (i.e., ca. +0.20 V vs. Ag/AgCl). From fundamental electrochemistry point of view, the formal potential of surface-confined species could be regulated by carefully controlling the adsorption ability of reactants and products onto electrode surface, as first reported by Wopschall and Shain.[30] Inspired by this, we designed and synthesized imidazolium-based polymer (Pim) due to its good anion recognition ability through ionic hydrogen bonding interaction, as described previously.[24-25] We interestingly found that Pim interacts with $Fe(CN)_6^{3-}$ through a strong ionic hydrogen bond interaction, but with the reduction product of $Fe(CN)_6^{3-}$ (i.e., $Fe(CN)_6^{4-}$) through only electrostatic interaction (Fig. 1A). This property not only enables the stable adsorption of both species onto electrode surface but also leads to a negative shift of the redox potential of $Fe(CN)_6^{3-/4-}$ from +0.23 V to +0.17 V (Fig. 1B), which well eliminated the interference from most

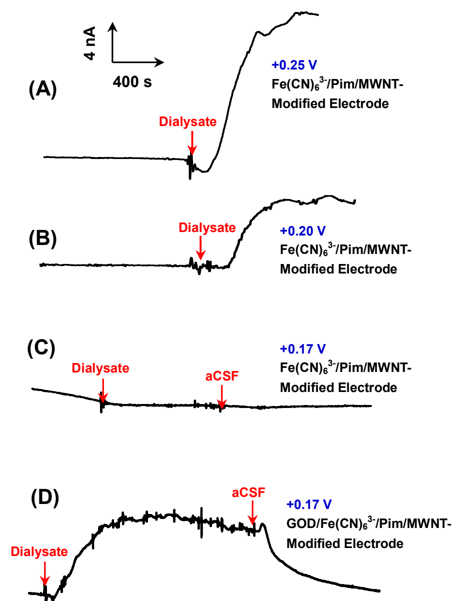

Fig. 2 Typical current-time responses obtained for the microdialysates continuously sampled from the striatum of guinea pig with the $Fe(CN)_6^{3-}$/Pim/MWNT (A, B, C)-modified electrode and glucose biosensor (D) fixed into the thin-layer electrochemical flow cell as the detector in the online detecting system with aCSF as the perfusion solution. Flow rate, 2 μL/min. Potential applied, +0.25 V (A), +0.20 V (B), +0.17 V (C), and +0.17 V (D). Reproduced with permission from ref. 29 Copyright 2012 American Chemical Society.

of electroactive neurochemicals endogenously existing in the cerebral systems. To investigate the validity of the biosensor prepared by engineering different interionic interactions between Pim and $Fe(CN)_6^{3-/4-}$ for in vivo sensing application, we developed an online detection system by directly coupling a thin-layer electrochemical flow cell sensor to in vivo microdialysis by using guinea pigs as the model animal.[3] As shown in Fig. 2, when the electrode was polarized at +0.25 V (Fig. 2A) and +0.20 V (Fig. 2B), an obvious current response was observed in both cases although the recognition unit is absent, indicating that some kinds of electroactive neurochemicals were oxidized at these potentials. In contrast, when the electrode was polarized at a lower potential of +0.17 V, almost no current increase was observed when biorecognition unit is absent (Fig. 2C), suggesting that in vivo sensing at this potential was virtually interference free. While, at the same low potential, the electrode showed good response toward glucose in the brain microdialysate after glucose oxidase was cross-linked onto the electrode (Fig. 2D). These results demonstrate that the rational design and careful construction of the interionic interaction between Pim and $Fe(CN)_6^{3-}$ redox mediator negatively shifts the formation potential of the $Fe(CN)_6^{3-/4-}$ and, as a result, greatly improves the selectivity of the biosensor. The concept demonstrated here could be further developed into a platform for in vivo sensing of different biological molecules by combining with different oxidases. The strategy may offer a bridge to fill the gap between in vitro and in vivo electrochemical

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biosensors by engineering the interionic interactions among the sensing units and their counterparts (e.g., Pim in this case).

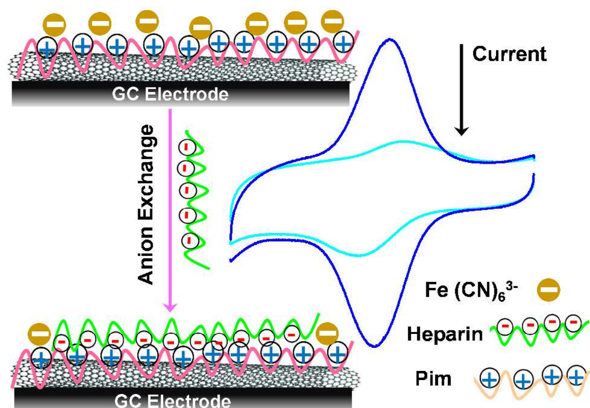

Fig. 3 Schematic illustration for amperometric heparin sensing based on different affinity between of the Pim toward electrochemically active $Fe(CN)_6^{3-}$ and electrochemically inactive heparin. Reproduced with permission from ref. 31 Copyright 2013 American Chemical Society.

In another study, we found that the strategy by engineering the ionic hydrogen bonding interaction between Pim and $Fe(CN)_6^{3-}$ could also be extended to a chemical sensing system with artificial synthetic receptor (i.e., Pim) as a recognition unit.[31] For example, by using the difference in the interaction strength (i.e., binding affinity) of the Pim receptor toward $Fe(CN)_6^{3-}$ and heparin, we developed a selective sensor for electrochemically inactive heparin (Fig. 3). The sensor was effectively used to sense heparin in the serum sample collected after intraperitoneally dosing heparin in rat. We believe that the strategy demonstrated here could be potentially expended to design other kinds of chem/(bio)sensors by combing different recognition units, such as aptamers and molecular imprinted polymers.

2.2 Cation-π Interaction for Dehydrogenase-based Electrochemical Biosensors

Cation-π interaction is one kind of noncovalent intermolecular interaction between the face of an electron-rich π system and an adjacent cation.[22] Similar to other kinds of weak interactions, cation-π interaction also plays an important role in nature and is used in supramolecular self-assembly. Demonstrated below is an example on how to use this interaction to prepare electrochemical biosensors with dehydrogenase as the sensing unit.[32]

Dehydrogenase is another kind of redox enzymes that can be used for electrochemical biosensing, which has been demonstrated to be more suitable for constructing in vivo biosensors compared with oxidase due to its O_2-independent nature.[33] However, the requirement of a coenzyme (i.e., NAD) in the dehydrogenase-based catalytic reactions makes this kind of biosensors more technically complicated than the oxidase-based ones, which unavoidably leads to a poor reproducibility in the sensor fabrication and thus limits their application for in vivo physiological studies. In this case, a gel that integrates all sensing elements including the cofactor, and electrocatalyst and, in the meantime, possesses a high electronic and ionic conductivity is highly demanded. By taking all accounts of these requirements into consideration, we believed that the uses of carbon nanostructures (e.g., carbon nanotubes, CNTs) and ionic liquids (ILs) to form a gel would be an effective solution to the pursuit mentioned above. This idea was initially inspired by the properties of both components; CNTs possess good electronic conductivity, and ILs exhibit a high ionic conductivity. More importantly, the cation-π interaction between both, on one hand, allows the formation of a gel (i.e., IL/CNT gel)[34] and, on the other hand, enables the formation of molecular films of water-miscible imidazolium-based ILs onto glassy carbon (GC) electrode, as demonstrated in our previous work.[35] To investigate whether the IL/CNT gel could be used as a gel to accommodate the biosensing elements, as our initial try, we studied the feasibility of the gel to accommodate the electrocatalysts for the reduction of oxygen. We found that the IL/CNT gel could be used as a "reservoir" to synthesize, dissolve, and accommodate the dual tailor-made electrocatalysts sequentially for 2e-reduction of O_2 into H_2O_2 and for the 2e-reduction of H_2O_2 into H_2O to form structurally homogeneous and electrochemically active materials (Fig. 4).[36]

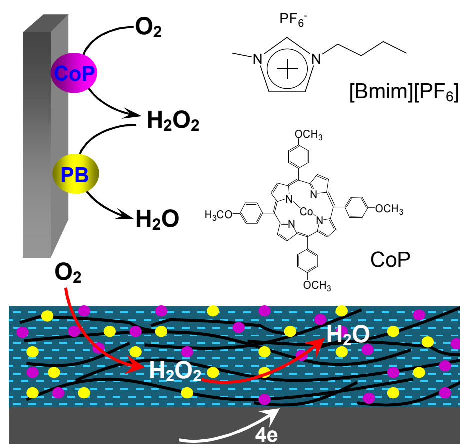

Fig. 4 Schematic illustration for electrocatalytic 4e-reduction of O_2 with IL/CNT gel as a "reservoir" to accommodate dual catalysts (i.e., Prussian blue, PB; and cobalt porphyrin, CoP). Reproduced with permission from ref. 36 Copyright 2008 American Chemical Society.

Furthermore, we have also systematically studied the interaction between CNTs and electrochemically active organic dye cation (e.g., methylene blue) and found that there was cation-π interaction between them, besides the hydrophobic and charge transfer interactions.[37] These interactions enabled not only a negative shift of the redox potential of methylene blue but also the stable attachment of the dye onto CNTs, both are very essential for the development of in vivo biosensors, as demonstrated in our study.[38] These earlier attempts, on one hand, demonstrate that the smart engineering and careful using of interionic interaction could form a IL/CNT gel that could be used as a "reservoir" to hold tailor-made functional elements for various applications and, on the other hand,

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provide the primary inspiration for us to use this gel to prepare dehydrogenase-based electrochemical biosensors, as demonstrated below.

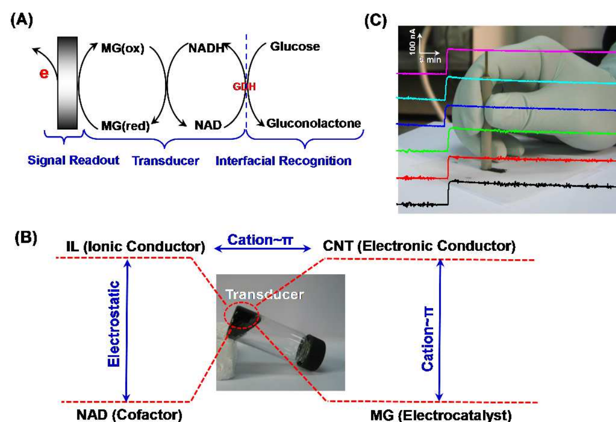

Fig. 5 (A) Schematic illustration of the reaction schemes involved in the dehydrogenase-based biosensor. (B) The digital picture, components, and the intrinsic interionic interaction of the multifunctional gel transducer. (C) Digital picture of surface confinement of the gel transducer by simply polishing the electrode on the gel and typical amperometric responses of glucose obtained at the six different multifunctional gel based biosensors prepared by the same method. The electrodes were poised at +0. 20 V. Reproduced with permission from ref. 32 Copyright 2011 American Chemical Society.

Based on the attempts mentioned above,[35-38] we have recently developed a new strategy to simplify the biosensor fabrication and thus minimize the biosensor-to-biosensor deviation by using the multifunctional IL/CNT gel as the electronic transducer (Fig. 5).[32] This transducer integrated all the elements necessitated for efficiently transducing the biorecognition events to signal readout (Fig. 5A and B). (1) CNTs were used as the electronic conductor and ILs were used as the ionic conductor, both of which were very essential to an excellent transducer; (2) the electrocatalyst methylene green (MG) was integrated into the gel since it has a strong interaction with CNTs by cation-π and hydrophobic interactions; and (3) a new kind of IL with the cofactor (i.e., oxidized form of nicotinamide adenine dinucleotide, NAD) as the anion was the synthesized and used to form the gel with CNTs, which realized the integration of the cofactor into the biosensor. It is worthy to note, although the synthetic $Bmim^+NAD$ are water-soluble, the prepared biosensor shows a good stability on electrode surface, which may be benefited from the synergic interactions among the synthetic $Bmim^+NAD$, MG, and SWNTs in the as-prepared multifunctional gel. With such kind of rationally designed and one-step-formed multifunctional gel prepared by fully engineering the interionic interaction as the electronic transducer, the dehydrogenase-based electrochemical biosensors were able to be simply fabricated by polishing the electrodes onto the gel followed by enzyme immobilization (Fig. 5C). This capability greatly simplified the biosensor fabrication, prolonged the stability of the biosensors and, more remarkably, minimized the biosensor-to-biosensor deviation (Fig. 5C). All these excellent properties of the biosensors made them particularly attractive for in vivo biosensing applications.

2.3 Dual Recognition Unit Strategy for ATP Aptamer-based Electrochemical Biosensor

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. 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.[39] However, the selected RNA aptamer shows a relatively low specificity toward the nucleobase. We recently demonstrated a dual recognition unit strategy to improve the selectivity of ATP aptamer-based electrochemical biosensors by rationally tuning the interionic interaction and further validate the biosensors to cerebral ATP determination.[40]

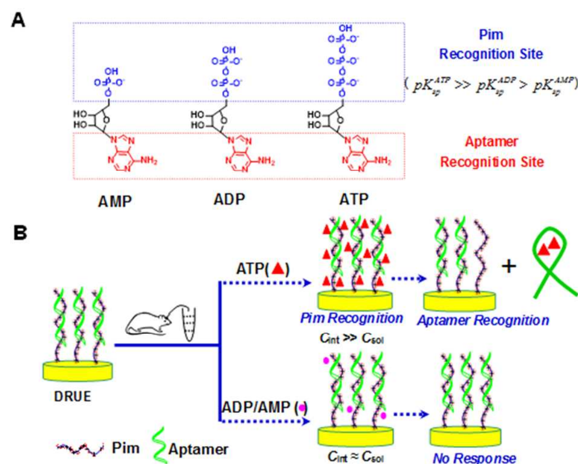

Fig. 6 A) Recognition Sites of Pim and Aptamer towards ATP, ADP, and AMP. B) Schematic Illustration of dual recognition unit strategy for Cerebral ATP Assay with ATP Aptamer-Based Biosensor. Reproduced with permission from ref. 40 Copyright 2015 American Chemical Society.

By systematic study the interaction between polyimidazolium (Pim) and different anions (i.e., $Fe(CN)_6^{3-}$, ATP, ADP and AMP), we interestingly found that Pim bears different affinity towards these anions and thus could be used as the second recognition units to improve the selectivity of aptamer-based ATP biosensors (Fig. 6A). 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 relative smaller K_{sp} ($pK_{sp} \approx 9.20$). Differently, the complexes formed by Pim with both ADP and AMP show the large solubility in water, which was similar to that of Pim-Cl. The almost 1000-fold difference in the K_{sp} values between Pim-$Fe(CN)_6^{3-}$ and Pim-ATP enables the electrochemical signal readout while the difference in affinity of Pim toward ATP, ADP, and AMP enables the selective accumulation of ATP, resulting

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in a much higher C_{int} for ATP than ADP and AMP, which were further recognized by the ATP aptamer (Fig. 6B). By combining the strong recognition ability of ATP aptamer toward A nucleobase, a highly selective sensor were constructed for cerebral ATP determination as shown in Fig. 6B. 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. With the biosensor developed here and combined with in vivo microdialysis technique, the basal level of ATP in the brain cortex microdialysate was determined to be 10.16 ± 3.57 nM (n = 3).[40] As described above, although the structures of ATP, ADP and ADP are quite similar, the difference in charges essentially offers us a good chance to differentiate them by tuning interionic interactions as demonstrate above. This study provides an effective strategy to direct selective sensing of ATP in the cerebral system.

3. Recruiting Recognition Molecules Based on Interionic Interaction for In Vivo Analysis

Although using the existing recognition units is an excellent way to construct the highly selective biosensors, the lack of recognition units for some molecules essentially limits its selective determination for in vivo analysis. Rationally tuning the interionic interaction essentially gives a good solution to construct highly selective biosensors towards these molecules. In this part, we demonstrate several examples to recruiting recognition molecules according to the structure of a specific molecule and further construct the highly selective biosensors for in vivo analysis. The concept demonstrated here could be applied for other kinds of molecules and may open a new way to design the highly selective biosensors for in vivo analysis.

3.1 Ion Pair Interaction for Cysteine Sensing in Rat Brain

Ion pair is a pair of oppositely charged ions held together by Coulomb attraction without formation of a covalent bond.[41] It is mainly a Coulombic interaction, and its strength is directly related to the molecular structure and the charged groups. Compared with the simple Coulombic interaction, ion pair interaction can be directional, especially when structured organic ions are involved, which can be employed to develop highly selective chemical sensors through rationally tailoring the strength of the interactions.

As one kind of thiol-containing amino acids, cysteine plays important roles in the cerebral system, which is related with the pathogenesis of several neurological disorders such as Parkinson's and Alzheimer's disease.[42-43] However, selective sensing of cerebral cysteine still remains a challenge because, on one hand, the coexistence of cysteine analogues including thiol-containing species and other kinds of amino acids in the cerebral system invalidates existing methods for selective sensing of cerebral cysteine. On the other hand, the mechanisms employed so far for electrochemically and optically sensing of brain chemistry cannot be explored for cysteine sensing either since there are neither enzymes nor functional nanostructures that can be used to achieve the selectivity for cysteine sensing.

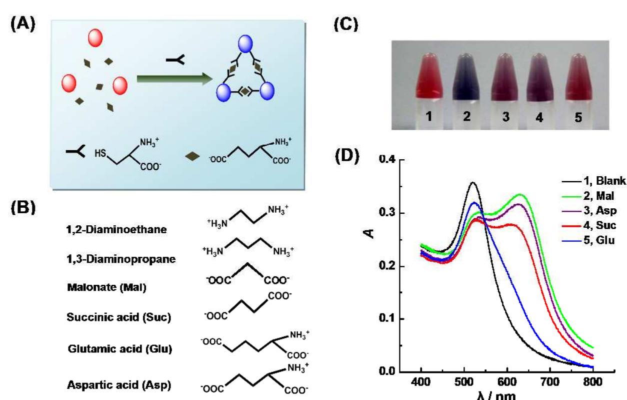

Fig. 7 (A) Schematic illustration of the mechanism for cysteine *sensing with aspartic* acid as the crosslinker based on ion pair interaction. (B) Molecular structures of the compounds investigated in this study in terms of their capability as the crosslinkers to trigger the aggregation of Au-NPs. (C) Photographs and (D) UV-Vis spectra of aqueous dispersions of Au-NPs containing different compounds with the addition of cysteine. Reproduced with permission from ref. 44 Copyright 2013 American Chemical Society.

By rationally tuning the ion pair interaction, we recently demonstrated a novel strategy for highly selective sensing of cerebral cysteine based on its intrinsic structure (Fig. 7A).[44] On one hand, cysteine bears a thiol group and could readily interact with gold nanoparticles (Au-NPs), validating the use of aggregation-induced changes in the color of the dispersion of Au-NPs as the signal readout. On the other hand, cysteine bears a negatively charged carboxyl group (pK_a = 1.71) and a positively charged amino group (pK_a = 10.78) in the physiological pH. This intrinsic structural property provides an opportunity to achieve the selectivity for cysteine sensing through rationally tuning the ion pair interaction with other charged species. To accomplish selective colorimetric sensing of cysteine based on ion pair interaction, various kinds of compounds (structure shown in Fig. 7B) with at least one charged moiety at each end were used to investigate their capability to act as cross-linkers to selectively trigger the aggregation of Au-NPs caused by subsequent addition of cysteine (Fig. 7C and D). We interestingly found that only the crosslinkers both with the amino group and the terminal carboxyl group at one end of the molecules, such as aspartic acid and glutamic acid, could enable the aggregation of Au-NPs to be triggered only by cysteine. This property originally comes from the ion pair interactions between cysteine self-assembled onto the surface of Au-NPs and the cross-

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linkers employed. Moreover, aspartic acid bears higher sensitivity than glutamic acid due to shorter carbon chain in its structure. By using aspartic acid as the cross-linker, the concentration of cysteine was visualized with the naked eye and determined by UV-vis spectroscopy. The strategy based on ion pair interaction was highly selective and was free from the interference of other natural amino acids and other thiol-containing species as well as the species commonly existing in the brain such as lactate, ascorbic acid, and glucose. Moreover, the signal output shows a linear relationship for cysteine within a concentration range from 0.166 to 1.67 μM, with a detection limit of 100 nM, which could be used for assay the concentration of cysteine in dialysate. These properties enabled the sensor to be useful for direct sensing of cysteine in rat brain upon the combination of in vivo microdialysis (Fig. 8). This study again demonstrates that the strategy described here by tuning the interionic interaction could pave a new avenue to in vivo chem/(bio)sensing.

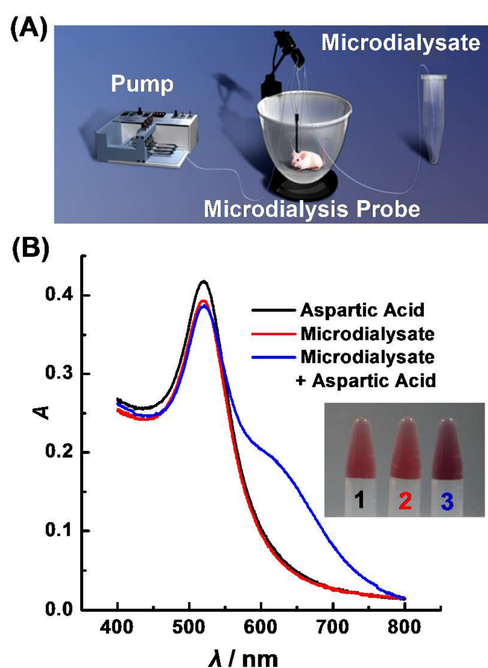

Fig. 8 (A) Schematic illustration of in vivo microdialysis. (B) Photographs (insert) and UV-Vis spectra of Au-NPs dispersions with the additions of different solutions. Black curve and vial 1: with sole addition of 20 μL aspartic acid solution (20 mM); Red curve and vial 2: with sole addition of 20 μL 2-fold diluted brain microdialysate; Blue curve and vial 3: with addition of 20 μL aspartic acid solution (20 mM) and 20 μL 2-fold diluted brain microdialysate. After being incubated for 10 min, each of the resulting mixture was diluted to 600 μL with Milli-Q water for UV/Vis detection. Reproduced with permission from ref. 44 Copyright 2013 American Chemical Society.

3.2 Dual Molecular Recognition for Dopamine Determination in Rat Brain

Another excellent example to construct the highly selective in vivo biosensors was developed by Tian's group. [45] They have successfully established a colorimetric method for selectively monitoring dopamine in rat brain based on double molecular recognition with 4-mercaptophenylboronic acid (MBA) and dithiobis (succinimidylpropionate) (DSP) (Fig. 9). In this case, Au-NPs were functionalized with both MBA and DSP and these two molecules not only act as stabilizers for Au-NPs but also interact with the hydroxyl and amino groups in dopamine to doubly recognize dopamine with a high specificity. With this method, the authors successfully detected DA in the brain microdialysate to be approximately 10 nM. This work essentially further validates the idea to construct the highly selective biosensors for in vivo analysis according to the structure of a specific molecule.

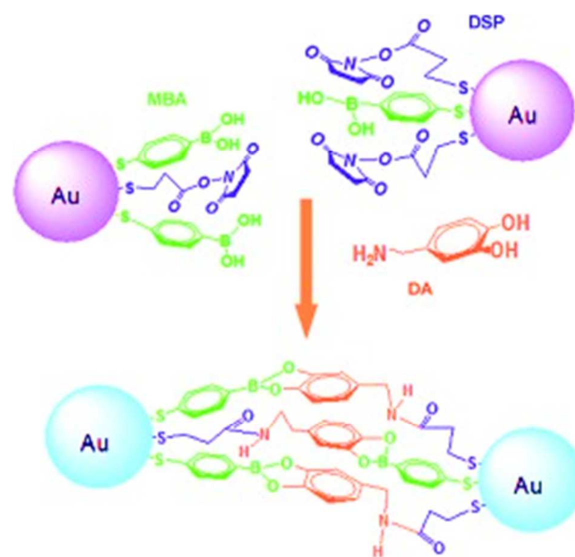

Fig. 9 Colorimetric Detection of dopamine based on double molecular recognition strategy. Reproduced with permission from ref. 45 Copyright 2011 Wiley-VCH.

4. Interionic Interaction for Sensing Materials Preparation

In addition to the attempts paid on the development of in vivo chem/(bio)sensors based on rationally engineering the interionic interactions, we were also interested in extension of the strategies demonstrated above to prepare new materials with an excellent sensing property based on interionic interactions. Very recently, we have prepared a new kind of supramolecular ionic materials (SIMs) that were formed from the aqueous solutions of imidazolium-based dication and dianionic dye (i.e., 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid, ABTS) through ionic self-assembly (Fig. 10A).[46-47] The formed SIMs not only show a good thermostability and unique optical/electrochemical properties that are raised from the precursors of the SIM, but also exhibits good water-stability, salt-stability, and adaptive encapsulation properties toward some heterocyclic cationic dye molecules. These unique properties essentially made the materials particularly attractive for chem/(bio)sensor development. For example, by encapsulating electroactive molecules (e.g., methylene green, MG) into the materials through the interionic interaction between MG and anionic building blocker (i.e., ABTS), an electrochemical sensor for NADH was developed at a low potential of -0.07 V (*vs.* Ag/AgCl).[46]

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Again, by encapsulating optically active rhodamine 6G into the materials, a visualization assay was successfully established for on-spot and fast sensing of alcohol with thin-layer chromatography.[48] As shown in Fig, 10A the rationale for the visualization assay was essentially based on the solvent-tunable interionic interaction; the addition of ethanol into a stable aqueous dispersion of Rh6G-encapsulated SIM (Rh6G-SIM) destructs the structure of Rh6G-SIM, resulting in the release of Rh6G from SIM into the solvent. Based on this, alcohol content was visualized with the naked eyes and quantified by measuring the fluorescence line of Rh6G released from Rh6G-SIM on a thin layer chromatography (Fig. 10B). More importantly, the accurately controlled interionic interaction was also used to differentiate methanol in adulterated spirits (Fig. 10C); the addition of methanol (1 µL/mL, i.e., 0.79 mg/ mL) in the ethanol/water mixture (v/v, 38%) results in an obvious increase of fluorescence line, demonstrating the high selectivity of the sensor developed with the new material based on the interionic interaction. We believe that this kind of new materials would be very useful for the development of in vivo chem/(bio)sensors by, for example, integrating excellent recognition units and further tuning the interionic interactions in a tailor-made mode.

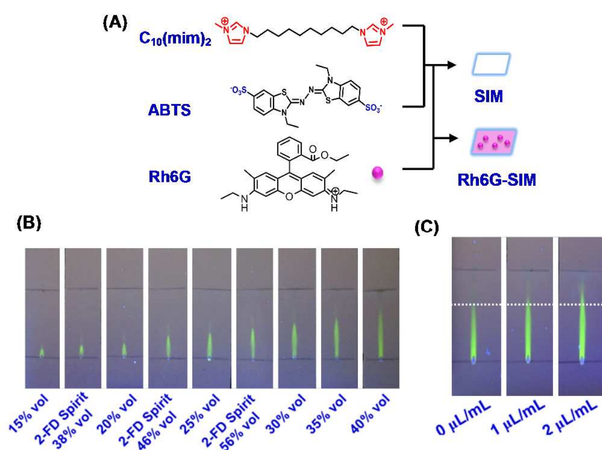

Fig. 10 (A) Formation of SIM and encapsulation of rhodamine 6G in SIM. (B) Photographs of fluorescence line of Rh6G for both real Chinese spirits and standard ethanol/water mixtures, as indicated in the figure. The alcohol contents of the three kinds of Chinese spirits were 38%, 46% and 56% and were 2-fold diluted (2-FD) by water before measurements. (C) Typical photographs of fluorescence line of Rh6G obtained on the Rh6G-SIM confined plates in response to the ethanol/water mixtures (v/v, 38%) in the absence and presence of different contents of methanol, as indicated in the figure. Reproduced with permission from ref. 48 Copyright 2014 American Chemical Society.

5. Conclusions and Outlook

Development of highly selective chem/(bio)sensors for in vivo neurochemical analysis is becoming an even hot topic in the interface between chemistry and life sciences, neuroscience in particular, because of its great roles in understanding of the molecular basis of brain functions. Our attempts over last decade have demonstrated the utility of rationally tuned interionic interaction could accomplish the purpose for the development of highly selective chem/(bio)sensors for in vivo applications. Based on this protocol, we have developed a series of new chem/(bio)sensors that could be used for in vivo neurochemical sensing by rationally tuning the different kinds of interionic interactions. Generally speaking, in the sensing system, processes for both electronic transducing and target recognition are always accompanied by various interactions. Thus, how to rationally tune and smartly use the main interionic interactions in a tailor-made mode in these processes becomes the key point to achieve a high selectivity (even other properties) for the chem/(bio)sensors. To realize the purpose for in vivo analysis, the design and rational tuning of the interionic interaction should be closely based on the unique physiochemical properties of the neurochemicals in the cerebral system. While this review is not a comprehensive review, we have demonstrated that the strategy described here is powerful and could be further developed into a general platform for developing new mechanisms and new sensing systems for in vivo recording chemical signals in the physiological processes.

On the other hand, we have to say that there are still numerous challenges and opportunities in this research field. First, we only demonstrate here the examples on how to tune and use the interionic interactions to improve the selectivity of biosensors with the recognition units of oxidase, dehydrogenase and aptamers. Other kinds of artificial recognition units, such as molecule imprinting polymer, peptide, and so on, could also be used to constitute new biosensors for in vivo applications provided strategies are made to further improve the analytical properties. It would become an interesting area if the strategy described in this review by carefully engineering the interionic interactions among all sensing elements could be extended for the development of in vivo biosensors with these artificial recognition units in the future. Second, as demonstrated here, the designing and engineering of interionic interaction to recruit recognition molecules according to the intrinsic molecular structure of the target is a powerful strategy to achieve a high selectivity for chem/(bio)sensors, especially for the target without any existing recognition units or easily usable optical/electrochemical property. The main challenge underlying in this strategy is the designing and construction of the interionic interactions and thus accurate engineering of them to be task-specific. Future studies should be conducted along with this line. Third, although the preparation of new materials by using interionic interactions hold a great promise for developing new chem/(bio)sensors, the large size of the materials prepared with interionic interactions yet limits their applications for in vivo biosensing. Accurate controlling of the size of the ionic materials would greatly promote their future applications in chem/(bio)sensing even in living animals. Finally, the strategy summarized in this tutorial review by tuning interionic interaction could also be further developed to improve other performances of the chem/(bio)sensors (e.g., sensitivity, stability, and anti-fouling ability). Although in vivo chemical signal recording is a long-standing challenge for the researchers, we have reasons to believe that the present strategy would well promote this research field and make a contribution to better understand molecular basis for brain functions and brain activities.

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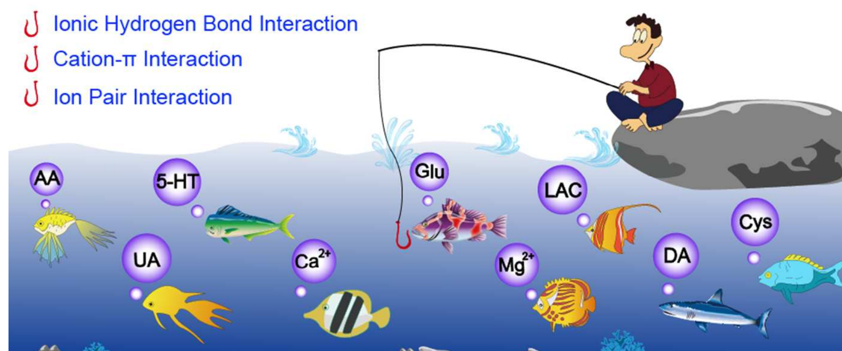
The interionic interaction demonstrated here refers to the interaction between ions and their counterparts, which is not only composed of electrostatic attraction between oppositely charged species but also other kinds of weak interactions. This review focuses on the recent progress of tuning interionic interaction to improve the selectivity of biosensors for in vivo analysis.



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