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A Generalizable and Noncovalent Strategy of Interfacing Aptamers with Microelectrode for Selective Sensing of Neurotransmitter In Vivo

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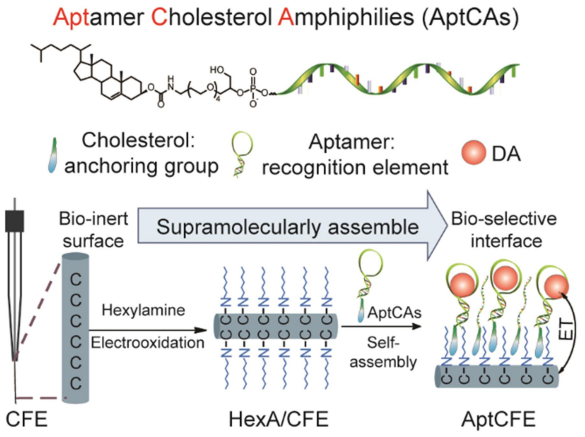
Abstract: Selective probing of neurochemicals is essential for understanding the chemical basis of brain function and pathology. To this aim, interfacing excellent recognition features of aptamers with in vivo compatible carbon fiber microelectrode (CFE)-based electroanalytical system offers a plausible means, however, is significantly challenged in terms of coupling chemistry, stability, and versatility. Here, we present a new interfacial functionalization strategy created through the assembly of aptamer cholesterol amphiphiles (aptCAs) onto the alkyl chain-functionalized CFE. We show that the noncovalent cholesterol-alkyl chain interactions are capable of effectively immobilizing aptamers onto CFE surface. Moreover, the strategy is generalizable, facile, and applicable of synergizing the merits of microelectrode and aptamers, allowing the generation of a highly selective bioelectronic system for probing neurochemical dynamics in living systems. To our knowledge, this is the first demonstration of using noncovalent cholesterol-based anchoring of aptamers to carbon-based electrodes, opening up a vast array of new opportunities for designing in vivo sensors to exploring brain chemistry.

Neurotransmitters are essential molecules to maintaining the homeostasis of brain activity and function, their relative and basal level in brain is directly related to specific disease states. For example, the controlled concentration of dopamine (DA), a representative monoamine neurotransmitter, plays fundamental roles in establishing “memory” and “reward” system in the brain, while the abnormal DA level can lead to neurological disorders such as Parkinson’s disease.[1] The selective and rapid probing of neurotransmitter dynamics in the central nervous system of living animal is an important tool for understanding the molecular basis of brain development and disease diagnosis.[2] Currently, microelectrode-based electrochemical platforms featuring excellent temporal and spatial resolution have been the main approaches employed for in vivo detection of neurotransmitter.[3] For instance, CFE being coupled with the fast scan cyclic voltammetry technique, has demonstrated to be an effective means to achieve in vivo detection of neurotransmitters.[4] Alternatively, chemical modification of electrode surface with target recognition ligand presents an appealing strategy for endowing the sensing system with excellent selectivity, in which designing new chemistries that modulate the interfacial recognition capabilities are the key challenges.[5]

Aptamers, are short, synthetic single-stranded nucleic acids that specifically recognize a wide variety of targets with high affinity.[6] These features make aptamers broadly applicable as analytical and diagnostic tools. For example, a DNA-based aptamer targeting DA (K_d=150 nM) has recently been reported and excellently employed in designing electronic sensing platform.[7] Particularly, integrating the molecular recognition properties of aptamers with implantable electrochemical platforms has presented exciting outcomes for highly selective molecular sensing, in which designing thiol-conjugation chemistry on gold electrodes is mainly employed to couple the specificity of aptamer-target recognition to a bioelectronic interface.[8] However, in comparison with its wide applications in in vitro diagnosis, few aptamer-gold electrodes have been explored for in vivo detection of neurotransmitters.[9] This is likely resulted from the potential competing interactions where the surface-bound oligonucleotide could be exchanged by the thiol-containing biomolecules presented in biological system. In this regard, CFE featuring excellent bio-stability, is an ideal electrode candidate for in vivo neurochemical measurements.[10] However, the integration of aptamers with the chemically inert surfaces of CFE presents a major challenge. Though pre-treating the electrode surface with positively charged coating likewise provides an effective strategy to immobilize aptamers onto the CFE surface through the electrostatic interaction, such binding is prone to be disrupted by ionic strength, showing low stability in physiological fluids. Moreover, the direct interaction with the phosphate backbone of aptamers likely decreases the conformational flexibility of aptamers, compromising its targeting property. Recently, a chemically engineered aptamer amphiphiles, consisting of a single-stranded hydrophilic oligonucleotide head and a hydrophobic lipid tail, have shown



Scheme 1. Schematic Illustration of Aptamer Cholesterol Amphiphilies and Its Supramolecular Assembling on CFE for Bio-selective Electrochemical Interface.

exciting applications both in immobilization on hydrophobic membranes through noncovalent lipid-alkyl chains reactions, and excellent selectivity in molecular recognition.[11] Inspired by these studies, we hypothesized that the multifunctional features of aptCAs would afford incisive tools to address important surface functionalization challenges on CFE and advance aptamer-based molecular recognition in neuroscience.

Here, we demonstrate a new surface functionalization strategy by assembling aptamer cholesterol amphiphiles onto alkyl chain-functionalized CFE (aptCFE, Scheme 1). We show that the noncovalent cholesterol-alkyl chain interactions are capable of effectively immobilizing aptamers onto CFE surface, offering the bioelectrochemical interface with tailor-made selectivity. Moreover, the strategy is readily generalizable to any oligonucleotide sequences and applicable of synergizing the merits of microelectrode and aptamers. Using dopamine as a model target, we successfully monitored the DA dynamics in living rat brain with aptCFE. Our results demonstrate a great promise for designing selective sensors in exploring the frontiers of neuroscience.

We first designed oligonucleotide cholesterol amphiphilies (OCAs) featuring a 20-mer DNA homoA (A20) sequence covalently linked with an inert tri(ethyleneglycol) linkage and a cholesterol terminal. For the chemical engineering of CFE surface with alkyl chains, we adopted an electrochemical strategy where aliphatic amine can be electrooxidized, forming a hydrophobic alkyl chain monolayer on the surface of glassy carbon electrode (GCE) in a controlled manner.[12] Considering the similar chemical nature of CFE and GCE, we hypothesized that the alkyl chains modified CFE could be obtained by the same approach. Specifically, hexylamine (HexA) was selected to provide both hydrophobicity and minimized distance for electron transfer (ET). Briefly, we carried out 5 consecutive electrochemical scans (from -0.2 V to +1.6 V) with a freshly polished CFE (7 μm in diameter) in an ethanol solution containing 0.10 M $LiClO_4$ and 5 mM hexylamine at a scan rate of 10 mV s^{-1}. The formation of carbon-nitrogen bond at CFE surface was monitored and confirmed by cyclic voltammetry. As shown in Figure S1A, a single irreversible oxidation peak-current voltage at +1.40 V was observed, suggesting the successful oxidation of the amine group of n-hexylamine to its corresponding cationic radical. Moreover, we used XPS to measure the change of relative nitrogen content at CFE surface (Figure S1B), the position of the peak maximum (399.8 eV) is consistent with the formation of a carbon-nitrogen bond between the primary amine cation radical and an aromatic moiety of the CFE surface. We then set out to study the self-assembly of OCAs on electrode by immersing the HexA/CFE in OCAs (1 μM). It is of note that the introduction of negatively charged DNA onto the electrode would dramatically change the surface hydrophobicity, providing physical parameters that can be easily probed by contact angle measurements. For a brief demonstration, we employed GCE for its compatibility with the contact angle measurements system. As shown in Figure S1C, the contact angle of bare GCE and HexA/GCE is 64° and 84°, respectively, consistent with the presence of moderate hydrophobic 6-carbon alkyl chain on GCE after the electrooxidation. Importantly, the OCA/GCE shows a significantly decreased contact angle (19°), suggesting a highly hydrophilic surface, implying the successful immobilization of OCAs.

To further demonstrate the essential role of HexA and cholesterol interfacing for the assembly of oligonucleotides, we probed the assembling process in the absence of either HexA or cholesterol using fluorescent and electrochemical approaches. In an initial experiment, bare CFE and HexA/CFE were immersed in a green fluorescent dye (FITC) dual-labeled A20 OCAs, then the surface immobilization was examined by fluorescent microscopy. As shown in Figure 1, bare CFE showed neglectable fluorescence. In contrast, HexA/CFE showed an intense and evenly distributed green fluorescence, highlighting the critical role of HexA in facilitating the self-assembly of A20 OCAs. Moreover, when HexA/CFE was incubated with a red fluorescent dye (TMR) labeled T20 without cholesterol conjugation (TMR-A20), no fluorescence from CFE was observed, verifying the necessity of cholesterol for the assembly. As proof-of-concept for studying the selective recognition, we tested Chol-A20/HexA/CFE as a recognition element and T20-TMR as the target. We observed a strong red fluorescence from Chol-A20/HexA/CFE after immersing into T20-TMR, indicating an efficient and selective DNA hybridization and recognition. We further designed an electrochemical approach to verify this assembly and selective recognition events. To this end, Chol-A20 modified electrode was immersed in redox-labeled T20 (methylene blue, T20-MB) solution,

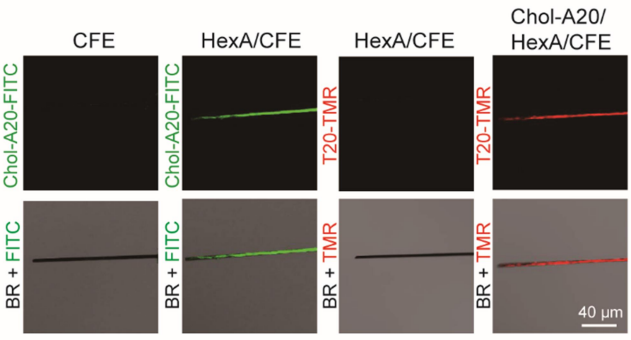


Figure 1. Evaluation of interfacial self-assembly and selective recognition. Fluorescent images of indicated electrodes after incubation with Chol-A20-FITC (green) or T20-TMR (red). BR, bright field. Scale bar, 40 μm.

followed by an electrochemical measurement in a blank solution. By doing this, only the electrode with successful T20-MB hybridization could generate corresponding current response (Figure S2). These observations are consistent with our fluorescent study, demonstrating that the supramolecular interaction between cholesterol and HexA not only dictates the interfacial assembly of oligonucleotide, but also preserves the high selectivity towards their targets, offering an effective approach to engineering CFE systems with programmed selectivity.

We then studied the capability of aptamer-assembled CFE (aptCFE) in neurochemical sensing. We expect that the selective recognition between an aptamer and DA on CFE would bring DA close to the electrode surface, producing a significant increase of the electrochemical signal due to its electrochemical oxidation. As typically shown in Figure S3, no obvious voltammetric responses were recorded for 10 μM of DA in artificial cerebrospinal fluid (aCSF) at HexA/CFE. Such inhibited electron transfer probably resulted from the repelling effect of the hydrophobic HexA layer to DA in solution. In sharp contrast, the modification of aptamers on HexA/CFE, leads to a significant increase in the current for the DA oxidation, demonstrating the key role of aptamers in capturing of DA, and achieving its selective sensing. Moreover, the current response is linearly correlated to the concentration of DA from 0.5 to 2 μM (Figure S4), ensuring the excellent sensitivity for DA sensing in physiologically relevant conditions. To further test the selectivity of aptCFE for DA detection, a variety of physiologically relevant substances, including ascorbic acid (AA), norepinephrine (NE), levodopa (L-DOPA), and 3,4-dihydroxyphenylacetic acid (DOPAC) were sequentially spiked into the system, and their responses were recorded accordingly. As shown in Figure 2A, these interferes generate no obvious current response, demonstrating an excellent selectivity of aptCEF for DA sensing. Moreover, by systematic comparison of the electrochemical responses between bare CFE and aptCFE in the presence of different concentrations of interferes, we observed that aptCEF exhibits a 4- and 3-fold increased selectivity towards NE and L-DOPA, respectively (Figure 2B, 2C, and Figure S5). Together, these results highlight the great advantages of designing aptCFE for selective neurotransmitter sensing in physiological system.

We next evaluated the applicability of aptCFE for DA sensing in living rat brain. It has been acknowledged that a dense and highly oriented arrangement of nucleic acids are resistant to protein absorption in vivo, leading to a hypothesis that aptCFE features similar structural properties would exhibit excellent stability for in vivo sensing. To study this, the amperometric responses of aptCFE toward DA before and after its implantation into a living rat brain were recorded. As a comparison, the responses of bare CFE toward DA under similar experimental conditions were included. It was found that CFE showed a 3-fold decrease of responses, while aptCFE displayed nearly identical responses (Figure 3A, 3B). Such unchanged sensitivity likely resulted from the minimal adsorption of biomacromolecules, mostly proteins, on the surface of aptCFE, which usually poses substantial challenges for CFE based in vivo measurements. Taken together, these results highlight the 3-fold function of aptCAs: assembly, selective recognition, and potential anti-fouling property, ultimately contributing to a sensitive, selective and stable in vivo sensor.

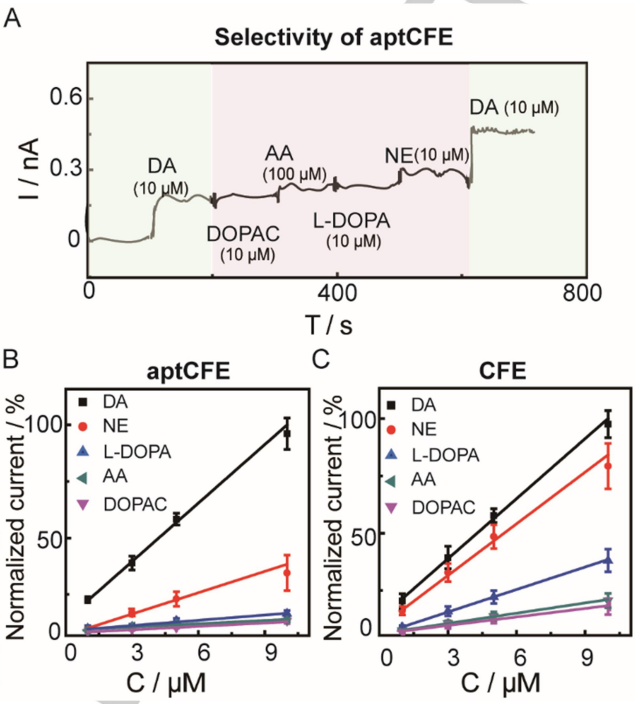


Figure 2. Selectivity study of aptCFE. (A) Typical amperometric responses of aptCFE toward the successive addition of DA, DOPAC, AA, L-DOPA, NE and DA. The current responses of (B) aptCFE, and (C) CFE to the indicated species from 1 to 10 μM in aCSF. Normalized current % = $I/I_{10\ \mu M\ DA}$ × 100% (n = 3, mean ± SD). Potential: +0.30 V *vs.* Ag/AgCl.

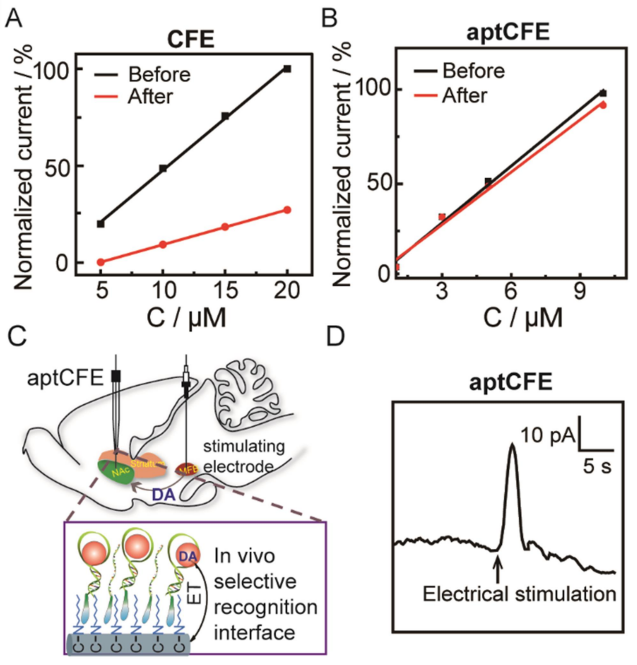


Figure 3. Stability and in vivo applicability of aptCFE. The current responses of (A) bare CFE (Normalized current % = $I/I_{20\ \mu M\ DA}$ × 100%) and (B) aptCFE

(Normalized current % = $I/I_{10\ \mu M\ DA}$ × 100%) to DA in aCSF before (black) and after (red) 2 h implantation in the striatum of rat brain. Potential: +0.30 V vs. Ag/AgCl. (C) Schematic illustration of aptCFE for in vivo DA sensing. (D) The current responses of aptCFE in the rat NAc upon electrical stimulation of rat MFB (3 s at 60 Hz, 300 μA, 2 ms per phase). Potential: +0.30 V *vs*. Ag/AgCl.

Finally, we demonstrated the efficacy of aptCFE for in vivo DA sensing. To this end, an aptCFE and a stimulation electrode were implanted into the rat nucleus accumbens (NAc) and medial forebrain bundle (MFB) region, respectively, and amperometry was used to monitor DA level changes during electrical stimulation. As typically shown in Figure 3C, upon electrical stimulation, the current signal ascribed to DA release rose rapidly, reaching its maximal value within few seconds. Afterward, it was quickly decreased to the basal level. This observation of DA release (nearly to 2 μM) is in good agreement with previous studies, demonstrating the reliability of aptCFE for in vivo DA sensing with sufficient spatio-temporal resolution.[13]

In summary, we have presented a versatile strategy to modify carbon-based electrode with programmed selectivity, and further demonstrated its practical application in highly selective detection of neurotransmitter in vivo. The sensor takes advantage of a multi-functional aptamer cholesterol amphiphiles, and its non-covalent interaction with alkyl chain functionalized CFE. The strategy is very facile, efficient, and most importantly, it synergizes the excellent attributes from both microelectrode-based in vivo systems and aptamers, generating the sensor with excellent spatio-temporal resolution, and greatly enhanced selectivity in the detection of dopamine in living rat brain. Given the feasibility of the assembly chemistry, and also the versatility of the recognition motif, aptCFE may be readily adapted to detect a wide variety of neurochemicals in vivo.

Acknowledgements

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Keywords: aptamer • in vivo electrochemistry • neurochemicals • selective sensing • surface chemistry

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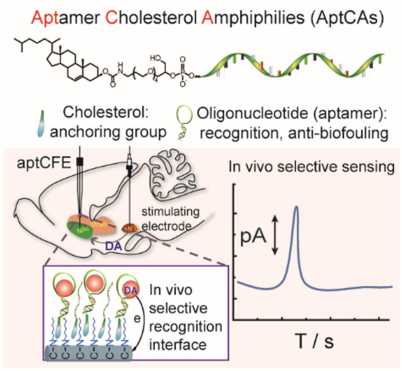

A versatile strategy to modify carbon-based microelectrode with programmed selectivity was demonstrated. The strategy is very facile, efficient, and most importantly, applicable of synergizing the merits of microelectrode and aptamers, allowing the generation of a highly selective bioelectronic system for probing neurochemical dynamics in living systems.