Biosensors and sensors for dopamine detection

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Abstract
Dopamine is a key catecholamine neurotransmitter and it has critical roles in the function of the human central nervous system. Abnormal release of dopamine is related to neurological diseases and depression. Therefore, it is necessary to monitor dopamine levels in vivo and in real time to understand its physiological roles. In this review, we discuss dopamine detection focusing on the molecular recognition methods including enzymes, antibodies, and aptamers, as well as new advances based on nanomaterials and molecularly imprinted polymers (MIPs). A large fraction of these sensors rely on electrochemical detection to fulfill the requirement of fast, in situ, and in vivo detection with a high spatial and temporal resolution. These methods need to overcome interferences from molecules with a similar redox potential. In addition, fluorescent and colorimetric sensors based on aptamers are also quite popular, and care needs to be taken to validate specific dopamine binding. Combining aptamers or MIPs with electrochemistry promises to achieve rapid detection and increased selectivity. In this article, we pay more attention to the molecular recognition mechanism and critically review the sensor designs. In the end, some future directions are discussed.

KEYWORDS
aptamers, electrochemistry, enzymes, fluorescence, neurotransmitters

1 INTRODUCTION

Dopamine is one of the key neurotransmitters (NTs). This function was first discovered in 1958 by Carlsson and co-workers at the Laboratory for Chemical Pharmacology of the National Heart Institute of Sweden.1,2 For this contribution, Carlsson was awarded the Nobel Prize in Physiology or Medicine in 2000. NTs are functionally divided into an excitatory class and an inhibitory class to either activate receptors on the postsynaptic membrane or reverse it.3 Dopamine can function in both classes and plays vital roles in the central nervous, renal, cardiovascular, and hormonal systems.4,5 A high dopamine level indicates cardiotoxicity leading to rapid heart rates, hypertension, heart failure, and drug addiction.6 However, a low dopamine level may cause stress, Parkinson’s disease,7 schizophrenia,8 Alzheimer’s disease,9 and depression.10 It is obvious that dopamine measurements are required for understanding its biological functions and related biological processes and mechanisms. Mustafa et al11 developed an ultrahigh performance liquid chromatography-electrospray ionization-tandem mass spectrometric
method (UHPLC/ESI-Q-TOF-MS) for the analysis of dopamine level in Wistar rat brains. The method was successfully applied for in vivo profiling in rodents. Wei et al. used HPLC-MS/MS to measure dopamine in rat brain. Although these instrumentation methods for measuring dopamine are available, for most researchers, it remains difficult for in situ monitoring of dopamine with a high specificity and high temporal and spatial resolution, which are critical for the detection of dopamine.

For rapid, in situ detection of dopamine, specific sensors and biosensors have been developed. A typical biosensor contains a biological molecule for target recognition such as enzymes, antibodies or aptamers. Other recognition mechanisms using molecularly imprinted polymers or nanomaterials have also been explored. The biomolecule is then coupled to a signal transduction mechanism to indicate the specific binding event. Given the need for in situ measurement, electrochemical sensing is preferred. While other reviews on dopamine detection are available, they mainly focused on the application of nanomaterials or specific detection methods. Herein, we critically review the main molecular recognition approaches for the detection of dopamine, paying particular attention to binding mechanisms. Methods like Raman spectroscopy without a ligand for specific binding of dopamine are not included.

2 CHEMICAL PROPERTIES OF DOPAMINE

Dopamine has many analogues (Figure 1A), and some potential interferents (Figure 1B) to complicate its detection. At pH 7.0, dopamine (pK_a 8.9) is positively charged. Since dopamine can be oxidized easily, many electrochemical methods have been developed. Under alkaline conditions, polydopamine can be prepared by self-oxidation and polymerization, causing its color changing to brown. Also, peroxidase/H_2O_2 and peroxidase mimics were used as biomimetic oxidizing agents to examine the early stages of dopamine oxidation. The UV-Vis absorption peak of dopamine is at 300 nm, and a new peak at around 350 nm emerged when dopamine was oxidized. The fluorescence of dopamine was studied from pH 2.0 to 12.0, and stronger emission was observed when pH was below 6-7. The fluorescence emission was monitored at 279 nm at the optimal pH of 3.6. Recently, multiphoton microscopy was used to image dopamine with excitation at 540 nm. Under physiological conditions in nervous and bodily fluids, the normal concentration of dopamine is between 10 and 1000 nM. In addition, uric acid (UA) and ascorbic acid (AA) usually interfered with the detection of dopamine due to their susceptibility to oxidation. Therefore, specific ligands are needed to better distinguish dopamine from its interferents and analogues.

3 ENZYME-BASED BIOSENSORS

Enzymes are popular for sensing small molecular metabolites since have high catalytic efficiency and good specificity, and can quickly convert substrates into products. For the detection of dopamine, many enzymes have been used including polyphenol oxidase, tyramine oxidase, horseradish peroxidase (HRP), laccase, and tyrosinase.
TABLE 1  Enzyme-based biosensor used to detect dopamine

| Enzyme                          | Samples                        | LOD (nM) | Linear range (µM) | Reference |
|---------------------------------|--------------------------------|----------|-------------------|-----------|
| Polyphenol oxidase              | –                              | 50       | 0.050-0.08        | 27        |
| Tyramine oxidase, HRP           | PC12 cell                      | 10       | 0.001-1           | 28        |
| HRP                             | Dopamine injection sample      | 600      | 15-865            | 29        |
| Laccase                         | Human plasma, pharmaceutical samples | 29 | 0.5-13           | 30        |
| Laccase                         | Pharmaceutical injection       | 180      | 1.5-7.5           | 31        |
| Tyrosinase                      | –                              | 5 × 10³  | 5-50              | 32        |
| Tyrosinase                      | Rat brain in vivo              | 1.0      | 0.01-220          | 32        |
| Tyrosinase                      | Human urine                    | 30       | 0.01-1000         | 34        |
| Tyrosinase                      | Human serum                    | 60       | 0.1-6.0           | 35        |
| Tyrosinase                      | Human urine, blood serum       | 50 × 10³ | 50-1000           | 36        |
| Tyrosinase                      | Serum samples                  | 0.43 × 10³ | 2-30             | 37        |
| Tyrosinase                      |                              | 3.1 × 10⁻³ | 0.0075×10⁻³-0.15 × 10⁻³ | 38        |

FIGURE 2  Design principles of some enzyme-based biosensors. (A) A tyramine oxidase and HPR-based chemiluminescence biosensor as described in ref. 28. (B) Laccase-immobilized on magnetic particles for electrochemical detection as described in ref. 31. (C) Tyrosinase-based fluorescence-quenching biosensor as described in ref. 34. (D) Tyrosinase-based electrochemical biosensor as described in ref. 42.

The examples of enzyme-based biosensors used to detect dopamine are shown in Table 1.

Shinohara et al established a chemiluminescence enzyme-based biosensor, and the principle of detection is shown in Figure 2A.²⁸,³⁹ H₂O₂ was produced through the oxidation of dopamine by tyramine oxidase, and then luminol reacted with the produced H₂O₂ to generate chemiluminescence in the presence of HRP. A limit of detection (LOD) of 10 nM was achieved. This method was used for real-time monitoring of the dopamine released from PC12 cells, a model nerve cell line. This method possessed many advantages including high sensitivity, rapid measurement, and the homogeneous reaction condition.
Li et al used laccase (LAC) for detecting dopamine. The authors covalently immobilized LAC on silica-coated magnetic microspheres, which were attached onto a glassy carbon electrode (GCE). The working mechanism of this biosensor is shown in Figure 2B. Dopamine was oxidized by LAC (oxidized) to produce dopamine-o-quinone (DOQ), and then LAC (reduced) eliminated the electrons to form LAC (oxidized). Therefore, after a bioelectrocatalytic cycle, electrons were passed to the working electrode. This biosensor possessed good dopamine electrocatalytic activity with a linear range of 1.5-75 μM and a LOD of 0.177 μM. It showed strong anti-interference against AA, and was used to detect dopamine in pharmaceutical injections. Moreover, the covalent LAC immobilization yielded high stability, and the nanomaterials used were non-cytotoxic.

The most popular enzyme-based biosensors for dopamine were based on tyrosinase, which can function using fluorescent or electrochemical signaling mechanism. Li et al interfaced C₃N₄ with tyrosinase to form a bifunctional fluorescent probe, and the principle of this fluorescent sensor is shown in Figure 2C. In the detection system, dopamine was oxidized by tyrosinase to produce dopaminechrome, which significantly quenched the fluorescence of C₃N₄, allowing dopamine to be detected from 30 nM to 1 mM. This method was used for quantitative detection of human urine samples, and the results were comparable to those from a HPLC-based method. Tang et al developed a similar fluorescent probe using carbon dots (CDs) instead of C₃N₄. The fluorescence intensity of the CDs decreased linearly with dopamine concentration over the range of 0.1 to 6.0 μM, and this biosensor was employed for the detection of dopamine in spiked human serum. In addition, L-tyrosine (a substrate to tyrosinase) of the same concentration as dopamine was tested with this biosensor, but only a weak fluorescence quenching was observed, indicating this biosensor had excellent specificity for dopamine. In both examples, no covalent enzyme conjugation was required making this method readily achievable. However, both sensors showed quenched fluorescence in the presence of dopamine, and such “signal-off” sensors are analytically undesirable. In contrast, electrochemical sensors are more suitable for in vitro and in vivo measurements of dopamine with high spatial and temporal resolution. As such, tyrosinase electrode sensors were more widely used for the measurement of dopamine such as by immobilization on a nanotube (Figure 2D).

Rahman et al designed a tyrosinase/multi-walled carbon nanotube (MWCNT)-modified GCE to detect dopamine with high sensitivity and selectivity. It possessed good long-term stability attributable to the immobilization of tyrosinase on MWNT. Moreover, the major interfering substance, AA, was negligible when monitoring 1 mM dopamine. Palomar et al immobilized tyrosinase on the WS₂ nanotubes functionalized with carboxyl groups to fabricate an electrochemical biosensor toward dopamine. Through the accumulation of dopamine due to interactions between the amine in dopamine and the carboxyl groups of the nanotubes, the sensitivity was enhanced. The calibration curve showed two linear ranges of 0.5-10 μM and 10-40 μM, respectively, and the latter range had a lower sensitivity. Overall, the enzyme-based biosensors can benefit from immobilization, and it is also important to use nontoxic nanomaterials and eliminate interfering effects in real samples.

4 | ANTIBODY-BASED BIOSENSORS

Antibodies are extremely popular for biosensor development, which offer many advantages such as high binding affinity, greater specificity, and mature detection platforms such as ELISA and lateral flow devices. However, antibodies suffer from stability and batch-to-batch variation since antibody production often requires animals. To our knowledge, only three reports used antibodies for dopamine detection. Choi et al decorated a monoclonal antibody (MAb) fragment specific for dopamine on gold (Au) nanorods. The antibody was reduced to cleave the disulfide bond linkages and generate free thiols for nanorods attachment. They used the localized surface plasmon resonance (LSPR) property of the nanorods for producing the signal (Figure 3, bottom part). By comparing pure Au nanorods with multiblock Au–Ag–Au nanorods, they found the magnitude of the quadrupole mode peak-shifting was more sensitive by using the multiblock ones. By using a five-block nanorods, the 1127 nm peak red shifted up to 47 nm upon addition of dopamine. However, the authors did not test dopamine analogs to show selectivity. Direct adsorption of dopamine on these nanorods cannot be ruled out, since dopamine is known to adsorb on both gold and silver surfaces. Choi et al immobilized a dopamine antibody on gold nanoparticles produced on an electrode surface, and the change of the LSPR peak was related to the concentration of the dopamine (Figure 3, top part). Their focused on the peak intensity at ~575 nm, which increased only around 0.001 OD when the dopamine concentration increased from 1 nM to 1 μM. However, this method was not yet used to detect dopamine in real samples.

Namkung et al established an indirect competitive immunoassay to detect dopamine. This method was effective for the detection of dopamine in urine, but was not sensitive enough at low concentrations (<0.1 μM). Therefore, this method was not used for serum samples.
FIGURE 3 The designs of antibody-based biosensor. The top part is based on the immobilization of the full antibody on gold nanoparticles and the LSPR peak height was monitored based on ref. 45. The lower part is immobilized antibody fragments and the red shift of the LSPR peak was monitored based on ref. 43.

due to the expected low concentration of dopamine in serum. The lack of immunoassays for dopamine may be explained by the following reasons. First, it is difficult to achieve fast, in situ detection using an immunoassay, which is critical for dopamine analysis. Second, there are many NTs analogues in the samples that may interfere with the detection. Third, the sensitivity is not high enough for real samples. Due to these shortcomings, it is desirable to develop another recognition molecule to overcome these challenges of antibodies.

5 | APTAMER-BASED BIOSENSORS

Aptamers are single-stranded nucleic acids that can bind target molecules with high affinity and specificity. Unlike large antibodies, aptamers are usually much lower in molecular weight. In addition, aptamer binding is often accompanied with a conformational change, which can be harnessed for developing specific biosensors with a fast response. Nucleic acids have programmable and predictable secondary structures, allowing versatile biosensor designs. Aptamers are particularly attractive for binding small molecules, whereas antibodies have more difficulty for these targets. Many naturally occurring aptamers found in the untranslated regions of mRNA (called riboswitches) bind small molecule metabolites. Unsurprisingly, efforts have been made to isolate aptamers for dopamine.

5.1 | Aptamers for dopamine

The first dopamine aptamer was an RNA isolated by Mannironi et al in 1997. The authors immobilized dopamine on a column via its primary amine group and exposed it to a large library containing $3.4 \times 10^{14}$ random RNA sequences. After nine rounds of selection, nearly 60% of the library could be eluted by 0.1 M dopamine. The final optimal sequence named dopa2 (Figure 4A) had a dissociation constant ($K_d$) of 1.6 μM, measured by the equilibrium filtration method. The predicted secondary structure has two hairpins linked by a few nucleotides.

An interesting attempt was made to directly convert the RNA aptamer to the same sequenced DNA in 2009 (Figure 4B). It was found that the DNA sequence bound dopamine even tighter with a $K_d$ of 0.7 μM measured using fluorescence anisotropy. This RNA-converted DNA aptamer has since been used by many other groups to develop biosensors. Kim and Paeng compared the DNA and RNA aptamers by a competitive enzyme-linked aptamer assay for dopamine. The authors immobilized the biotinylated aptamers, and added free dopamine and a dopamine-HRP conjugate for competitive binding. A dose-response curve was constructed, and the LOD was 63 nM using the RNA aptamer, while the LOD was 3.2 pM using the homolog DNA aptamer. Norepinephrine was still the next most active molecule for binding among the tested dopamine analogs. The authors concluded that the DNA aptamer can maintain the binding site and recogni-
tion activity. Based on the reported $K_d$ values, it appeared unreasonable to us that the DNA aptamer sensor can be more than four orders of magnitude more sensitive than the RNA one. In 2017, Alvarez-Martos and Ferapontova argued that this DNA sequence was not an aptamer. Using electrochemical measurements, the authors compared the specificity between the DNA and RNA for dopamine and norepinephrine, concluding that the DNA sequence was unable to specifically bind dopamine, while the RNA sequence was specific.

Recently, a new selection was performed by the Stojanović group. Instead of immobilizing dopamine, they immobilized a biotinylated DNA library on beads via biotin/streptavidin interactions. The binding sequences were eluted from the beads, and a high-quality aptamer was obtained (Figure 4C). Using a fluorescence-based competitive assay, the $K_d$ was determined to be 150 nM for dopamine by the authors. We measured its binding to dopamine and a few analogs using isothermal titration calorimetry (ITC, Figure 5). Dopamine had a $K_d$ of 1.9 $\mu$M, its affinity was ninefold lower for norepinephrine, while tyramine had no binding at all. Compared to the previous aptamer (57-mer), this new aptamer is slightly shorter (44-mer). In addition, this new aptamer was selected using the structure-switching mechanism, making it easy for sensor design.

5.2 Aptamer-based fluorescent biosensors

Various aptamer-based sensor designs have been reported, indicating versatility of aptamers. We first introduce the optical biosensors. Fluorescent biosensors are attractive for their rapid response, convenient operation, and high sensitivity. Many fluorescent aptamer-based biosensors were reported for dopamine using not only traditional fluorophores but also CDs and quantum dots (QDs). In terms of signal transduction, chemiluminescence, fluorescence resonance energy transfer, fluorescence anisotropy, fluorescent probe, and fluorescence dye staining have been reported as summarized in Table 2.

Most of the optical biosensors used the RNA-derived DNA aptamer, and many of them had a similar principle (Figure 6A). First, the aptamer was modified with a fluorescent label. A nanomaterial (eg, graphene oxide (GO) or MoS$_2$ nanosheets) was used as a quenching surface. The fluorophore-labeled aptamer was adsorbed on the quenching nanomaterials, which led to the fluorescence quenching. When dopamine was present, enhanced fluorescence was observed, which was attributed to aptamer binding. However, the fluorescence change was very moderate in this case. Chen et al. reported a biosensor based on aptamer-functionalized fluorescent MoS$_2$ QDs and MoS$_2$ nanosheet quencher. This system achieved excellent quenching efficiency, leading to over 10-fold of fluorescence increase upon addition of dopamine. This biosensor showed a high sensitivity with an LOD of 45 pM dopamine and excellent optical properties, good reproducibility, low cytotoxicity, and was used for detection in serum.

Wang et al. reported a label-free fluorescence aptasensor based on dopamine-triggered exonuclease III-assisted template DNA recycling. A specially designed duplex DNA containing an aptamer was used. When dopamine...
FIGURE 5  ITC analysis of the aptamer selected by the Stojanović group by titrating (A) dopamine, (B) norepinephrine, and (C) tyramine. Reprinted with permission from ref. 44. Copyright 2020 American Chemical Society

TABLE 2  Aptamer-based fluorescent or chemiluminescent biosensors for dopamine. All these sensors used the RNA-derived DNA aptamer

| Biosensor                                      | Interferents | Samples                 | LOD (nM)     | Linear range (µM) | Reference |
|------------------------------------------------|--------------|-------------------------|--------------|-------------------|-----------|
| Chemiluminescence                             | –            | –                       | 0.9 x 10^{-3} | 10^{-6}-10^{-3}   | 60        |
| Fluorescence stained by SYBR Green I          | –            | Mouse brain tissues     | 8 x 10^{-5}  | 10^{-4}-10^{-2}   | 64        |
| MoS₂ QDs as fluorophore and MoS₂ nanosheets as quencher | –            | Human serum             | 45 x 10^{-3} | 0.1 x 10^{-3}-1   | 63        |
| Fluorescent label-free detection with Ru complex and QDs | –            | Fetal bovine serum      | 19           | 0.03-0.21         | 59        |
| Fluorescent sensor with CDs and nano-graphite | UA, urea     | Human urine             | 0.55         | 0.1 x 10^{-5}-5 x 10^{-3} | 58        |
| Fluorescent sensor based on polymers/GO       | Epinephrine, AA | Human plasma, Human serum | 1.0         | 0-0.1             | 61        |

was present, it triggered the exonuclease III restriction digestion to release SYBR Green I, which resulted in decreased fluorescence signal. The overall change of fluorescence signal was quite small (<30%) and the authors only tested up to 10 nM of dopamine with a LOD of 0.08 nM. The sensor was applied to detect dopamine in mouse brain tissues. Cui and co-workers developed biosensors taking advantage of target-enhanced chemiluminescence produced by the reaction of an N-(4-aminobutyl)-N-ethylisoluminol (ABEI)-functionalized gold nanoparticles (ABEI-Au) with H₂O₂. When the aptamer bound dopamine, the chemiluminescence increased compared to that in the absence of dopamine. An apparent $K_d$ of 0.7 µM dopamine was obtained.

5.3  Aptamer-based colorimetric biosensors

Aside from fluorescent sensors, colorimetric biosensors for dopamine have also been reported.65-67 Many sensors used gold nanoparticles (AuNPs) to produce color
taking advantage of its strong extinction coefficient and distance-dependent color. Zheng et al developed a colorimetric biosensor using unmodified AuNPs. The authors believed that dopamine binding can fold the RNA-derived DNA aptamer and prevent aptamer adsorption on the AuNPs. The unprotected AuNPs then readily aggregated and changed color to blue upon addition of salt. The linear range of dopamine was from 0.54 to 5.4 μM, and the corresponding LOD was 0.36 μM. Some common interferents such as epinephrine and AA showed little cross-reactivity, although this biosensor was not used for detection in real samples. Subsequently, Zhang et al established a dual-mode sensing system based on the FAM-labeled aptamer and AuNPs for rapid detection of dopamine. For the colorimetric model, the linear range of dopamine was 0.17 to 4.0 μM and the LOD was 0.14 μM, which was similar with Zheng’s report. We recently studied this system in detail by using non-aptamer sequences, and proposed an alternative mechanism indicating that the observed color change was due to adsorption of dopamine on AuNPs leading to its aggregation. The selectivity for dopamine was because of its stronger adsorption and easier to cause aggregation of the AuNPs. Essentially, the same color responses were observed regardless of the DNA sequence used.

Recently, a lateral flow based sensor strip was reported using the newly selected DNA aptamer. The aptamer was hybridized to a DNA immobilized on AuNPs. In the presence of dopamine, the aptamer was released from the duplex and bound dopamine, allowing the DNA on the AuNPs to hybridize with its complementary strand on the capture region of the strip. Using ImageJ software, the LOD was 65.2 nM dopamine, while visual detection can be achieved at ~330 nM. We expect more work to use this new aptamer in the near future due to its excellent binding properties.

5.4 | Other aptamer-based biosensors

Besides the fluorescent and colorimetric biosensors, many other aptamer-based biosensors have also been reported based on personal glucose meters, surface enhanced Raman spectroscopy, and surface plasmon resonance. Electrochemical biosensors will be discussed in a later section since aptamers were used mainly for target enrichment in those cases, while identification of dopamine was based on its electrochemical oxidation. Personal glucose meters are a simple yet promising detection method, which can be easily used for point-of-care testing. For dopamine detection, aptamers were immobilized on a solid-phase surface (eg, 96-well plate). An invertase (converting sucrose to glucose) modified complementary DNA (cDNA) was hybridized to the aptamer. When dopamine was added, it released the DNA-invertase complex that detached from the surface and reacted with sucrose to produce glucose. Finally, the produced glucose was measured with personal glucose meter. Using this method, Hun et al achieved a linear range from 0.08 to 100 μM and an LOD of 0.03 μM. The biosensor did not show a response to interferents such as AA, UA, glucose, or epinephrine, and was applied to detect dopamine in human blood serum.

6 | MOLECULARLY IMPRINTED POLYMER-BASED SENSORS

Molecular imprinted polymers (MIPs) are a useful materials to selective binding, and it is prepared using the following method. A template (target) molecule, one or a few types of monomers, and a cross-linker are dissolved in an appropriate solvent and form a highly crosslinked polymer. After polymerization, the template molecule was removed,
creating nano-sized cavities corresponding to the shape, size, orientation, and chemical interactions of the template molecule. Compared to antibodies and aptamers, MIPs are much lower in cost and higher in stability, although the binding affinity and specificity of MIPs is lower. For dopamine detection, Zaidi reviewed MIP-based strategies from 2010 to 2018. Therefore, we herein reviewed the new progress since then.\textsuperscript{79}

As a pretreatment absorbent,\textsuperscript{80} MIPs were used to extract dopamine from real samples, and then it was detected use in an instrumental method (Figure 7A). For example, Hou et al synthesized boronate-modified hollow dummy template imprinted polymers for selective pre-extraction of dopamine from urine samples, and then used HPLC-based detection with a UV detector.\textsuperscript{81} After optimization of the conditions, the LOD for dopamine was in the range from 82 to 257 nM. This method was used in the detection of real samples with good recoveries. Bouri et al described a magnetic MIP (MMIP) as an absorbent.\textsuperscript{82} After dopamine was extracted from the sample matrix by using the MMIP and separated by a magnet, it was then analyzed by capillary electrophoresis (CE), and the LOD was in the range of 40-60 nM. The proposed method was convenient for separating dopamine from real samples and was successfully applied to detecting dopamine in human urine.

7 | ELECTOCHEMICAL BIOSENSORS BASED ON OXIDATION OF DOPAMINE

Dopamine is an electroactive molecule that can be easily oxidized without an enzyme. Thus, it can be effectively measured using electrochemistry. The oxidation peak of dopamine occurs at 150 mV (vs Ag/AgCl). The sensitivity and selectivity for dopamine can be enhanced by modification of the electrode with nanomaterials and affinity ligands.

7.1 | Nanomaterial-enhanced sensors

Many nanomaterials possess excellent conductivity, sensitivity to ligand binding, biological compatibility,\textsuperscript{1} and even enzyme-mimicking activity. They were widely used to modify electrodes to directly detect dopamine. The carbon nanotube/nanoceria-poly (3,4-ethylenedioxythiophene) (CeO\textsubscript{2}-PEDOT) modified glassy carbon electrode (GCE) was fabricated for the detection of dopamine in the presence of interferents (UA and AA; Figure 8A).\textsuperscript{84} Under optimum conditions, the differential pulse voltammetry (DPV) curves are shown in Figure 8B, and the corresponding linear ranges of this sensor were 0.10-10 \( \mu \)M and 40-400 \( \mu \)M (Figure 8C). In addition, dopamine in
pharmaceutical samples was successfully analyzed using this new platform. Many similar nanomaterial-enhanced sensors were constructed and the commonly used nanomaterials include CNTs, graphene, CDs, QDs, AuNPs, silver nanoparticles, metal oxides, and metal-organic frameworks (MOFs). Fast-scan cyclic voltammetry (FSCV) is a popular method for real-time detection of NTs in vivo because of its fast detection speed. Compared with the traditional CV, the scan rate is improved by 1000-fold, allowing measurement of fast changes of NTs in the brain. FSCV is almost always performed with traditionally carbon-fiber microelectrodes containing negatively charged surface oxide functional groups, which are ideal for detection of positively charged dopamine. Recent studies have developed carbon nanomaterials as microelectrodes.

Yang et al. reported a cavity carbon-nanopipette electrodes (CNPE) with FSCV for dopamine detection with increased sensitivity compared to typical nanoelectrodes, and the detailed dopamine-oxidation–reduction is shown in Figure 9A, and a modeled dopamine concentration change in the cavity is shown in Figure 9B. The CNPEs have high selectivity for dopamine even in the presence of interfering AA. The cavity CNPEs detected exogenously used dopamine in mouse-brain slices, indicating they did not clog in the tissue. Thus, cavity CNPEs are applicable as neurochemical sensors that provide spatial resolution on the scale of hundreds of nanometers, which is useful for real-time monitoring in small model organisms or specific cells. FSCV is a useful method to detect dopamine in the brain, but the FSCV theory is complex. Venton et al. summarized fundamentals of FSCV for dopamine detection. Overall, these nanomaterial-based sensors allowed fast detection. The drawback of this sensor was low selectivity because of the similar oxidation signal from the interferents of dopamine analogs in biological samples or tissues.

7.2 | Aptamer-based electrochemical biosensors

The above nanomaterial-functionalized electrodes can increase the surface area, while an affinity ligand is needed to increase selectivity and enrich dopamine to the electrode surface. For this purpose, aptamer-based electrochemical biosensors are most popular. Most aptamer-based electrochemical biosensors are made up of three parts consisting of a DNA aptamer for dopamine, a gold or GCE working electrode, and a nanomaterial or nanocomposite to enhance sensitivity and conductivity. The role of aptamers is to selectively recruit dopamine to the electrode surface, while the signal was still from the redox chemistry of dopamine. Since 2012, many papers on this...
FIGURE 9 Dopamine-oxidation-reduction with the cavity CNPEs. (A) Dopamine-oxidation scheme. (B) Modeled concentrations of dopamine inside the CNPE. At 1.3 V, dopamine was fully oxidized, and subsequent decrease of the potential led to regeneration of dopamine. By -0.4 V, all of the dopamine was recycled back from dopamine-α-quinone. Reprinted with permission from ref. 104. Copyright 2019 American Chemical Society

topic have been published (Table 3).116-118 We reviewed some representative work with a good promise for application.

Wang et al described an ultrasensitive and selective voltammetric aptasensor for dopamine based on a poly(3,4-ethylenedioxythiophene) conducting polymer nanocomposite doped with reduced GO.119 The nanocomposite was then covalently modified with the RNA-derived DNA aptamer, and the signal change of DPV was used to monitor the concentration of dopamine. The calibration curve displayed a linear response in the range from 1 pM to 160 nM with an LOD of 78 fM. This sensor was successfully applied to spiked serum samples showing good recoveries. Talemi et al designed a sensitive and selective dopamine aptasensor based on gold nanostar-modified pencil graphite as a novel substrate.120 The thiolated DNA aptamer for dopamine was immobilized on the gold nanostars coated with positively charged cetyltrimethylammonium bromide (CTAB) surfactant. When dopamine was present, the charge transfer resistance (R-CT) on the electrode surface increased with the increase of the dopamine concentration attributed to the specific interaction between the aptamer and dopamine, which inhibited the electron-transfer. The biosensor showed a high sensitivity with a wide linearity in the range from 5 to 500 pM and an LOD of 1.59 pM. Finally, the sensor was applied for the determination of dopamine in human plasma and urine samples.

Attempts have also been made to detect dopamine in situ such as in living cells or brain. Li et al developed a nano electronic biosensor for dopamine by modifying DNA aptamers on a multiple-parallel-connected (MPC) silicon nanowire field-effect transistor (referred to as MPC aptamer/SiNW-FET; Figure 10A).121 FETs work by measuring the current passing through a nanofabricated transistor. In this case, the current was modulated by aptamer binding. The linear working range and LOD of this biosensor were 0.01-10 nM and <10 pM, respectively (Figure 10B). It possessed good specificity and was able to distinguish dopamine from other interferents, such as AA, tyrosine, epinephrine, and norepinephrine. This MPC aptamer/SiNW-FET was also applied to real-time monitoring dopamine released under hypoxic stimulation from living PC12 cells.

Of note, since DNA is highly negatively charged and dopamine is positively charged under the physiological condition, DNA (non-aptamer)-modified electrodes have been used for detecting dopamine taking advantage of such nonspecific binding.122,123 Therefore, careful control experiments such as using mutated aptamers are needed to ensure specific binding of aptamers. Although RNA is less stable, the use of RNA aptamers was still explored for use in biosensors. Farjami et al. reported an electrochemical RNA aptamer-based biosensor resistant to interference from other NTs.124 The RNA aptamer was immobilized on a cysteamine-modified gold electrode to help the further electrochemical oxidation of dopamine. The amperometric signal was related with concentration of dopamine between 100 nM and 5 μM. The aptamer was immobilized by electrostatic interactions between the positively charged cysteamine and the negatively charged aptamer. However, the LOD was not low enough and the analysis was affected by nonspecific adsorption in biological fluids. Alvarez-Martos et al proposed a strategy to solve this problem by flowing serum samples in flow-injection wall-jet polycarbonate cell followed by washing steps in
### Table 3  Aptamer-based electrochemical biosensors for dopamine

| Biosensor                          | Interferents                        | Sample              | LOD (nM)       | Linear range (µM)     | Reference |
|-----------------------------------|-------------------------------------|---------------------|----------------|-----------------------|-----------|
| DNA aptamer-PEDOT/rGO             | –                                   | Human serum         | $78 \times 10^{-6}$ | $1 \times 10^{-6} - 0.16$ | 119       |
| DNA aptamer-Gold nanostars/pencil graphite | –                                   | Urine, Plasma samples | 0.002          | $0.005 \times 10^{-3} - 0.5 \times 10^{-3}$ | 120       |
| DNA aptamer-Graphene/polyaniline nanocomposites film | Tyramine, AA, L-3-hydroxytyrosine | Human serum         | 0.002          | $0.007 \times 10^{-3} - 0.90 \times 10^{-3}$ | 126       |
| DNA aptamer-Nanowire transistor biosensor | Epinephrine, Norepinephrine         | Living PC12 cells   | 0.01           | $10^{-5} - 10^{-2}$    | 121       |
| DNA aptamer-AuNPs/Prussian blue/CNTs/ | –                                   | Human serum         | 0.2            | 0.0005 - 0.05         | 111       |
| DNA aptamer-CNTs/GCE              | –                                   | Human blood         | 0.22           | $1 \times 10^{-3} - 30 \times 10^{-3}$   | 127       |
| DNA aptamer-AgNP/CNTs/GO composite | –                                   | Human serum         | 0.7            | 0.003 - 0.11          | 110       |
| DNA aptamer-collagen/GO composite | Dihydroxyphenylalanine, AA          | Blood serum         | 0.75           | $10^{-3} - 1$         | 128       |
| DNA aptamer-GO/nile blue/GCE      | –                                   | Human serum         | 1              | 0.01 - 200            | 115       |
| DNA aptamer-electrochemical chemical redox cycling | Norepinephrine, Epinephrine      | Serum sample        | 1.8            | 0.005 - 0.5           | 113       |
| DNA aptamer-AuNPs/ CNTs           | –                                   | Human serum         | 2.1            | $5 \times 10^{-3} - 300 \times 10^{-3}$ | 109       |
| RNA Aptamer-Au electrode          | –                                   | Vivo                | –              | 0.1 - 5               | 124       |
| DNA aptamer-carbon nanoparticles/AuNPs | –                                   | Human urine         | 10             | 0.03 - 3              | 107       |
| RNA aptamer tethered to Au electrodes | –                                   | Plasma, serum       | 62             | 0.01 - 1              | 129       |
| RNA aptamer/cysteamine modified electrode | –                                   | Undiluted serum     | 114±8          | 0.1 - 2               | 125       |
| RNA aptamer-rGO/AuNPs/ /GCE       | –                                   | Human serum         | 130            | 0.5 - 20              | 118       |

**Figure 10**  Ultrasensitive nanowire-transistor biosensor. (A) Illustration of the experimental setup of a DNA-aptamer modified MPC SiNW-FET device for detecting exocytotic dopamine under hypoxic stimulation from living PC12 cells. (B) A semi-log plot of response as a function of dopamine concentration. Reprinted with permission from ref. 121. Copyright 2013 American Chemical Society
a phosphate buffer. This way, the affinity binding by the aptamer enabled retention of dopamine and the LOD improved to 114 ± 8 nM.

All the above aptamer-based biosensors were constructed with the RNA or its same sequenced DNA aptamer. The new aptamer isolated by Stojanović and co-workers has been confirmed to have excellent binding specificity and affinity. Nakatsuka et al detected dopamine under physiologically high-ionic conditions by modifying printed ultrathin metal-oxide FET arrays with this new aptamer. Target-induced conformational changes of the negatively charged aptamer in close proximity to semiconductor channels regulated the conductance in physiological buffers, resulting in highly sensitive detection (below 0.1 nM). Many analogues, including norepinephrine, serotonin, L-3,4-dihydroxyphenylalanine, and 3,4-dihydroxyphenylacetic acid, did not interfere with the detection of dopamine. This biosensor achieved real-time detect dopamine in artificial cerebrospinal fluid.

7.3 MIP-based electrochemical sensors

MIP is also a popular affinity ligand for electrochemical dopamine sensing, especially when combined with nanomaterials such as graphene, CNTs, QDs, and NPG. An electrochemical sensor displaying improved selectivity and sensitivity by Yang and co-workers is shown in Figure 7B. NPG was selected to enhance the output signal by providing a large surface for immobilization of polythionine (pThi) and the MIPs. This sensor had superior anti-interfering ability when interferents were present at high concentration in artificial cerebrospinal fluids attributable to the selective binding by the MIP.

Dual MIPs can recognize two molecules that coexist in the same sample. Fatma et al developed a dual imprinted GO/carbon black composite polymer by using a “surface-grafting from” approach on screen printed carbon electrodes for the electrochemical sensing of dopamine and epinephrine. At 200 mV, the oxidation peak potentials of the two targets were separated, indicating they can be analyzed simultaneously in real samples without cross reactivity, interferences, or false-positives. The LOD for dopamine was 0.1, 0.1, 0.2, and 0.3 nM in aqueous, blood serum, urine, and pharmaceutical samples, respectively. Lu and co-workers described a dual template MIP electrochemical sensor to simultaneously detect dopamine and chlorpromazine. For dopamine, the DPV response showed two linear ranges from 0.05 to 8 μM and from 8 to 40 μM, and the LOD was 2.8 nM. This sensor had good reproducibility and high selectivity for dopamine. Furthermore, the sensor was used in the analysis of human serum, urine and pharmaceutical samples.

8 CONCLUSION AND FUTURE PERSPECTIVES

Dopamine is an important NT, and it is desirable to monitor dopamine in vitro and in vivo for biochemical and physiological studies. In this review, we focused on the molecular recognition aspect of biosensors and sensors for dopamine detection. Enzyme-based biosensors have good specificity, but enzymes are difficult to purify, less stable, and have complex immobilization processes. Antibody-based biosensors are rarely developed for the detection of dopamine because it is difficult to obtain the fast and sensitive detection needed for in situ assays. Compared to antibodies, DNA aptamers possess many advantages including versatile signal transduction mechanism, fast response time, high stability, low cost and easy modification. Different kinds of aptamer-based biosensors were reported, and the most common types were optical and electrochemical biosensors. Nanomaterial-based sensors without a dopamine-specific binding ligand usually relied on the specific oxidation potential of dopamine for detection, although they often simultaneously detected multiple NTs and other redox molecules since they have similar oxidation signals. MIPs are synthetic polymers with good specificity, low cost, high stability, and they could be used to pre-treat samples or detect dopamine by combining with electrodes and nanomaterials to improve the sensitivity and specificity.

Given the many methods developed for the detection of dopamine, a side-by-side comparison of sensor performance would be useful. We have discussed several examples comparing DNA and RNA aptamers, but the comparison of different DNA aptamers, aptamers to antibodies, and aptamers to MIPs would all be interesting. We noted that the reported LODs ranged from 0.078 pM to 50 μM, spanning over 8 orders of magnitude. It is unlikely that using similar sensing molecules can result in such a big difference. Having some standard reference methods for a side-by-side comparison can also make the reported values more reliable.

Given that dopamine measurements in biological samples are essential and most sensors are immobilized on a surface, especially on electrodes, it is critical to understand the interface chemistry to ensure an optimal and stable immobilization. We have discussed our findings on dopamine adsorption on AuNPs, where if such an effect were to be ignored, it could lead to misinterpretation of the label-free aptamer sensing result. Such adsorption may also happen to many electrochemical sensors, although it was not discussed much in the literature. Given the vast number of different nanomaterials used, we expect more future work to be focused on the surface chemistry, especially for the more promising sensor designs.
The ultimate goal is to understand the biochemical and physiological roles of dopamine under various stimuli. Biosensors, especially electrochemical sensors, have the potential to achieve this goal. Aptamer-based sensors are particularly promising, especially with the development of aptamers with higher affinity and specificity. The complex in vivo environment has more demand on the specificity of sensors. Using multiple aptamers responding to various analogs and potential interfering molecules to form a sensor array might be a powerful method to not only increase selectivity for dopamine but also to obtain correlated molecular information. For example, the ability to monitor dopamine metabolites or molecules on its reaction pathways is particularly useful. This would require the isolation of more aptamers and their integration with signal and data processing methods.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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