



Aptamer-modified biosensors to visualize neurotransmitter flux

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ABSTRACT

Chemical biosensors with the capacity to continuously monitor various neurotransmitter dynamics can be powerful tools to understand complex signaling pathways in the brain. However, *in vivo* detection of neurochemicals is challenging for many reasons such as the rapid release and clearance of neurotransmitters in the extracellular space, or the low target analyte concentrations in a sea of interfering biomolecules. Biosensing platforms with adequate spatiotemporal resolution coupled to specific and selective receptors termed aptamers, demonstrate high potential to tackle such challenges. Herein, we review existing literature in this field. We first discuss nanoparticle-based systems, which have a simple *in vitro* implementation and easily interpretable results. We then examine methods employing near-infrared detection for deeper tissue imaging, hence easier translation to *in vivo* implementation. We conclude by reviewing live cell imaging of neurotransmitter release *via* aptamer-modified platforms. For each of these sensors, we discuss the associated challenges for translation to real-time *in vivo* neurochemical imaging. Realization of *in vivo* biosensors for neurotransmitters will drive future development of early prevention strategies, treatments, and therapeutics for psychiatric and neurodegenerative diseases.

1. Introduction

Real-time imaging of neurotransmitters in animal models performing behavioral tasks would enhance our fundamental understanding of brain function and facilitate connections between physiological processes, pharmacokinetics, and toxicology (Ceccarini et al., 2020). This knowledge is critical to advance the fields of preventive and personalized treatments for neurodegenerative diseases. However, continuous *in vivo* tracking of small molecules such as neurochemicals encounters numerous challenges. First, detection platforms must remain stable and retain sufficient sensitivity with minimal biofouling upon prolonged exposure to complex environments with high amounts of nonspecific biomolecules (Frutiger et al., 2021). Second, the dynamic range of the sensor must match the concentration, time scale, and frequency of neurotransmitter release rates in various brain regions. Third, neurochemical detection *in vivo* necessitates biorecognition elements that have high selectivity to differentiate similarly structured metabolites that co-exist in the same brain region (Nakatsuka and Andrews, 2017).

Selectivity of bioreceptors is critical for neurotransmitter detection in the complex brain milieu. The majority of interneuronal

communication in the central nervous system is mediated by glutamate and γ -aminobutyric acid (GABA), two highly abundant neurotransmitters. Glutamate is the precursor to GABA in the biochemical pathway and differs in structure by a single carboxyl group. Similarly, while less abundant, catecholamine neurotransmitters such as dopamine, norepinephrine, and epinephrine are sequentially synthesized from the amino acid precursor, tyrosine. Thus, each catecholamine neurotransmitter has overlapping chemical signatures that are challenging to distinguish. Further, as small molecules, neurotransmitters have few functional groups available for molecular recognition (Cao et al., 2018).

To address detection of structurally similar neurotransmitters, optical sensors such as cell-based neurotransmitter fluorescent engineered reporters (CNiFERS) have been developed (Muller et al., 2014). More recently, a novel suite of genetically encoded dopamine sensors termed dLight1 with improved temporal resolution compared to CNiFERS were reported that couple the conformational changes of endogenous dopamine receptors to changes in fluorescence intensity of a fluorescent protein (Patriarchi et al., 2018). Similarly, genetically encoded G-protein coupled receptor-activation-based serotonin sensors have been developed to report endogenous serotonergic activity in freely moving

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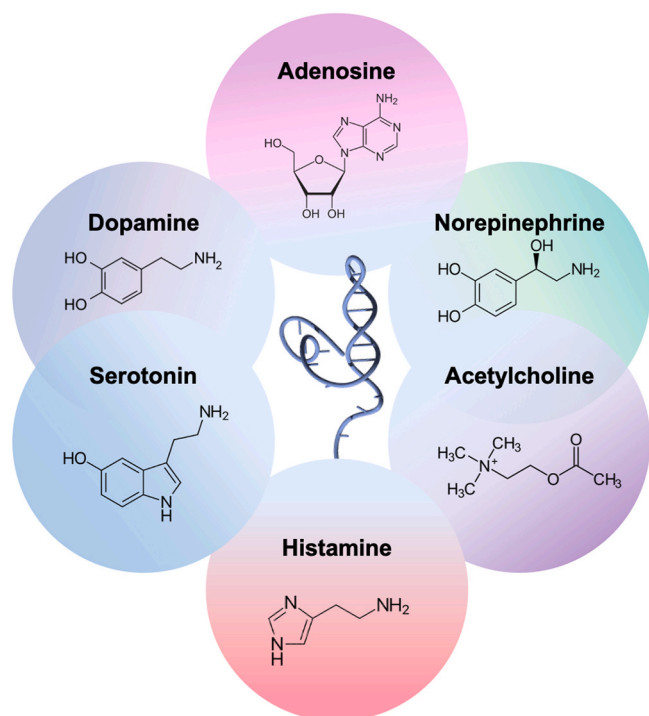


Fig. 1. Reported RNA and DNA aptamers targeting various neurotransmitters.

mice (Wang et al., 2021). However, despite the advantages of these optical sensors, the design and development of each sensor for a specific target takes significant time with challenges in tuning the binding affinity, selectivity, and sensitivity. Thus, alternative selective receptors that can be isolated in a high-throughput manner with tunable affinities and binding kinetics for diverse small-molecule neurotransmitters, would be advantageous.

Aptamers that recognize small-molecule neurotransmitters with high specificity and selectivity have emerged to tackle this challenge. Aptamers are single-stranded, short oligonucleotides (typically < 100 nucleotides) that are artificially designed and synthesized *via* a high-throughput *in vitro* selection method termed systematic evolution of ligands by exponential enrichment (SELEX) (Ellington and Szostak, 1990; Tuerk and Gold, 1990). Compared to conventional bioreceptors such as antibodies that often suffer from cross-reactivity and large batch-to-batch variations, aptamers have tuneable selectivities and stabilities *via* counterselection techniques (Yang et al., 2017, 2014, 2012) and chemical modifications (Ni et al., 2020) respectively. Further, optimization of specificity windows where aptamers have dynamic responses to concentration changes can be modulated (Ricci et al., 2016; Porchetta et al., 2012; Bissonnette et al., 2020).

To date, aptamers that recognize various neurotransmitters have been reported (Fig. 1, Table 1). One of the earliest reports was an RNA aptamer targeting adenosine, a putative neurotransmitter (Sassanfar and Szostak, 1993). This RNA adenosine aptamer was isolated in 1993 by the group of Szostak, one of the pioneers of SELEX. The binding affinity of receptors to their target of interest is typically quantified by the equilibrium dissociation constant (K_d). A K_d ranging from 0.7 to 8 μM was reported for the RNA adenosine aptamer depending on the specific salt concentration. Two years later, the same group discovered a DNA aptamer for adenosine, with an entirely different structure than that of the original RNA sequence, with a K_d of $\sim 10 \mu\text{M}$ (Huizenga and Szostak, 1995).

In 1997, Mannironi et al. isolated an RNA aptamer that recognized dopamine in solution with a K_d of 1.6 μM (Mannironi et al., 1997). This RNA dopamine aptamer was integrated into various biosensing platforms such as colorimetric and electrochemical sensors that will be

discussed in more detail in later sections. Then in 2009, Walsh and DeRosa reported the conservation of function for a DNA homolog of the original RNA dopamine aptamer with a ten-fold improvement in the affinity with a solution K_d of 0.7 μM (Walsh and DeRosa, 2009).

This DNA dopamine aptamer was subsequently used in an *in vivo* study to investigate the ability of DNA aptamers to retain their binding properties in complex environments (Holahan et al., 2011). While results suggested that aptamer injection in the brain does not have major consequences on behavioral characteristics, the actual regulatory function of the DNA aptamer was questionable as the aptamer cross-reacted to norepinephrine with a comparable binding affinity (Nakatsuka et al., 2018a; Kim et al., 2015). The nonspecific interactions of the DNA homolog of the original RNA dopamine aptamer with structurally related neurotransmitters has led to the claim that this sequence cannot be called an aptamer (Álvarez-Martos and Ferapontova, 2017). Incidentally, in one of the earliest reports on SELEX, all DNA versions of RNA aptamers were found to be inactive and *vice versa* (Ellington and Szostak, 1990).

In 2018, a highly specific and selective DNA dopamine aptamer ($K_d = 150 \text{ nM}$) was reported alongside a novel DNA serotonin aptamer ($K_d = 30 \text{ nM}$) (Nakatsuka et al., 2018b). The selectivity of the novel dopamine and serotonin DNA aptamers vs. similarly structured neurochemicals was validated in buffers mimicking the brain ionic milieu *via* multiple techniques such as fluorescence-based methods, circular dichroism, surface-enhanced Raman spectroscopy, and electronic transistor-based measurements. Further, the retention of serotonin aptamer function was demonstrated *ex vivo* in brain tissue. An alternative DNA aptamer targeting serotonin was published in 2017, however, the sequence was not reported due to purchase from a company, Base Pair Biotechnologies (Chávez et al., 2017).

Further, DNA aptamers targeting acetylcholine were developed (Bruno et al., 2008) with the goal of counteracting the effect of organophosphorus nerve agents that increase the concentration of acetylcholine at synapses, leading to overstimulation of postsynaptic cells and possible death (Curtin et al., 2006). Aptamers were designed to scavenge excess acetylcholine by binding to the specific target. However, the binding affinity was not reported, and the sequence demonstrated cross-reactivity towards small molecules with overlapping chemical signatures such as acetic acid, acetylchloride, and choline. Likely due to this selectivity issue, this acetylcholine aptamer has not been deployed as recognition elements for biosensing applications. Similarly, an aptamer targeting norepinephrine with a K_d of $\sim 200 \text{ nM}$ has also been isolated, (Kammer et al., 2014) but translation to monitoring the neurotransmitter in clinically relevant settings has not been forthcoming.

Histamine-specific RNA aptamers have been generated with a binding affinity ranging from 3 to 34 nM validated using four independent assays. However, during the selection procedure, the histamine was immobilized *via* the amino group, limiting the number of available functional groups for aptamer recognition. Studies have previously shown that mimicking free molecules in solution at screening surfaces is necessary to optimize aptamer functionalities (Nakatsuka et al., 2018a; Cao et al., 2018). To this end, alternative RNA histamine aptamers were isolated *via* immobilization of *L*-histidine to screening surfaces, to render both the imidazole and amine groups available for aptamer interactions (Dwidar et al., 2019). This RNA aptamer bound histamine with a K_d of 370 nM and demonstrated selectivity vs. similarly structured molecules and was integrated into a fluorescence aptamer-based sensor (aptasensor) to detect histamine as an indicator of spoilage in food samples (Dwidar and Yokobayashi, 2019).

Herein, we review works in which the aforementioned aptamers have been deployed specifically for imaging neurotransmitter release. Information on specific aptamers used for various optical platforms are summarized in Table 1. Advances in optical technologies offer the possibility for detailed observation of neurochemical dynamics with unprecedented spatial and temporal resolution. For each different

Table 1

List of neurotransmitter aptamers integrated on different optical platforms mentioned in this review in chronological section order.

Target	Aptamer reference	Probe	Mode	LOD	Linear range	Test media	Reaction time	Optical platform reference
Dopamine	Walsh & DeRosa (Walsh and DeRosa, 2009)	AuNPs	Colorimetry	36 μ M	5.4–54 μ M	PBS	5 min	(Zheng et al., 2011)
Dopamine	Walsh & DeRosa (Walsh and DeRosa, 2009)	AuNPs	Colorimetry	0.14 μ M	0.17–4.0 μ M	PBS, spiked deproteinized human plasma	6 min	(Zhang et al., 2016)
			Fluorometry	0.079 μ M	0.083–2.0 μ M			
Dopamine	Walsh & DeRosa (Walsh and DeRosa, 2009)	AuNPs	Colorimetry	0.2 nM	0.2–20 nM & 0.1–2 mM	PBS	–	(Cao and McDermott, 2018)
Dopamine	Nakatsuka (Nakatsuka et al., 2018b)	AuNPs	Colorimetry	0.065 μ M	0.065–3.3 μ M	Artificial urine	15 min	(Dalirirad and Steckl, 2020)
Serotonin	Base Pair Technologies	AuNPs	Colorimetry	0.3 μ M	0.75–2.5 μ M	PBS, treated FBS	15 min	(Chávez et al., 2017)
Adenosine	Huizenga (Huizenga and Szostak, 1995)	AuNPs	Colorimetry	10 nM	20–100 nM	Filtered and diluted urine	30 min	(Chen et al., 2008)
Adenosine	Genscript	AuNPs	Colorimetry	0.17 μ M	5.0–60.0 μ M	Water	20 min	(Zhou et al., 2020)
				0.48 μ M	5.0–50.0 μ M	Artificial urine		
Adenosine	Shanghai Sangon	AuNRs	Colorimetry	3.3 pM	10 pM - 5 nM	Ultrapure water	40 min	(Zhang et al., 2019)
				3.6 pM		Filtered serum		
Adenosine	SBS Genetech Co.	AuNRs	Colorimetry	2.0 nM	4.0–80.0 nM	Water, SD rats brain samples	–	(Wang et al., 2010a)
Histamine	Lerga (Lerga et al., 2019)	AuNPs	Colorimetry	8 nM	10 – 100 nM	Water, fish samples	–	(Lerga et al., 2020)
Dopamine	Walsh & DeRosa (Walsh and DeRosa, 2009)	Carbone QDs	Fluorometry	55 pM	0.10–5.00 nM	Spiked 60x diluted urine	60 min	(Zhu et al., 2016)
Dopamine	Walsh & DeRosa (Walsh and DeRosa, 2009)	MoS ₂ QDs	Fluorometry	45 pM	0.1–1000 nM	Spiked 100x diluted human serum	20 min	(Chen et al., 2019)
Dopamine	Nakatsuka (Nakatsuka et al., 2018b) (truncated)	Fluorophores	Fluorometry	0.9 μ M	0–15 μ M	1% FBS	< 1 min	(Liu et al., 2020b)
Dopamine	Nakatsuka (Nakatsuka et al., 2018b) (truncated)	Fluorophores	Fluorometry	0.12 μ M	0–8 μ M	PBS	–	(Liu et al., 2020b)
Serotonin	Nakatsuka (Nakatsuka et al., 2018b)	SWCNTs	Fluorescent	–	0.1–1 μ M	PBS, platelet cells	55 s	(Dinarvand et al., 2019)
Serotonin	Jeong (Jeong et al., 2019)	SWCNTs	Fluorescent	–	0.1 – 50 μ M	PBS, brain tissues	9 min	(Jeong et al., 2019)
Dopamine	Random ssDNA	SWCNTs	Fluorescent	0.1 – 4000 nM	–	PBS	60 s	(Mann et al., 2017)
ATP	–	SWCNTs	Fluorescent	4.5 nM	0.01–0.8 μ M	Tris-HCl, lysed cells	–	(Zhang et al., 2010)
ATP	Toc-fApt	Membrane anchored	Fluorescent	30 μ M	50–100 μ M	Selection buffer (Jhaveri et al., 2000) and HeLa cell culture medium (Tokunaga et al., 2012)	Ms range	(Jhaveri et al., 2000; Tokunaga et al., 2012)
Dopamine	DNA Nanoprisms	Membrane anchored	Fluorescent	0.45 nM	0–100 nM	Cell culture medium supplemented with 10% FBS	Ms range	(Zeng et al., 2020)
L-DOPA	Scaffolded RNA aptamer	Intracellularly expressed	Fluorescent	–	–	Lysogeny broth medium	Ms range	(Porter et al., 2017)
5-HTP	Scaffolded RNA aptamer	Intracellularly expressed	Fluorescent	–	–	Lysogeny broth medium	Ms range	(Porter et al., 2017)
Dopamine	Walsh & DeRosa (Walsh and DeRosa, 2009)	Raman dye-labeled	Raman spectroscopy	–	1–100 μ M	PBS + 5 mM KCl for dopamine secretion	> 1 h	(Choi et al., 2020)

aptamer-modified optical platform, we discuss the advantages and challenges for translation to live continuous imaging in animal models. We begin by discussing aptamer-modified nanoparticle-based systems due to their simplicity and ease of implementation *in vitro*. We then move onto methods that use near-infrared detection rather than visible light for deeper tissue penetration with higher implications for *in vivo* sensing. Finally, we conclude with aptamers incorporated into live cells to monitor neurotransmitter flux.

2. Colorimetric aptasensors

Gold nanoparticles (AuNPs) have emerged as promising imaging agents as a result of their surface chemistry, biocompatibility, and tunable absorption and emission properties (Nune et al., 2009). In particular, AuNPs have been integrated into colorimetric biosensors due to their ability to change color after a variation in the plasmon resonance frequency, which is dependent on the separation distance between AuNPs. The response of such biosensors can be perceived by eye and quantified by ultraviolet visible spectroscopy, removing the need for

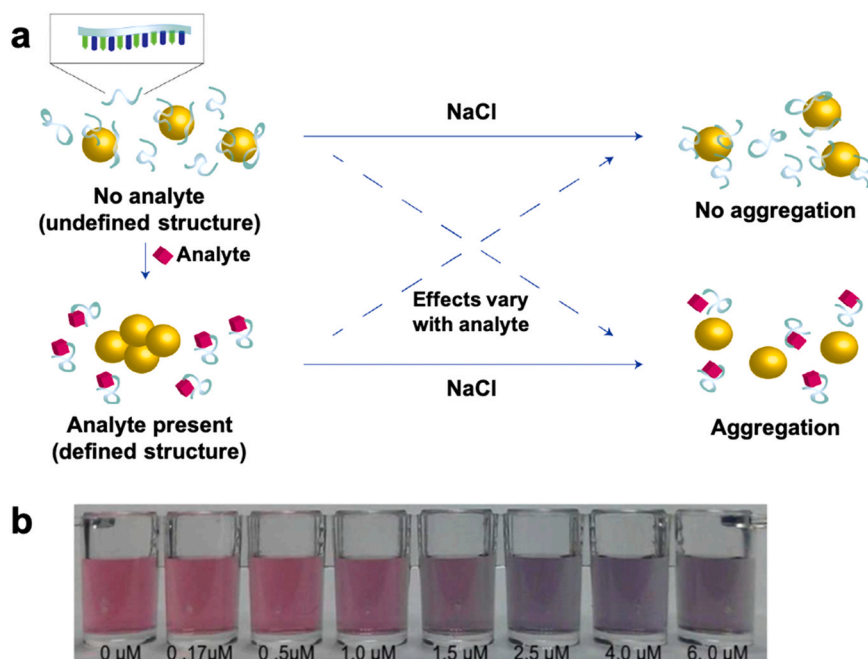


Fig. 2. Sensing mechanism of most gold nanoparticle (AuNP)-aptamer systems. (a) Schematic of the sensing mechanism. In the presence of the analyte of interest, aptamers undergo structural rearrangements. Depending on the analyte and the specific DNA conformations, the AuNPs can either be shielded or exposed to salt aggregation. Adapted with permission from Ref. (Beyene et al., 2019) Copyright 2019 American Vacuum Society. (b) Photographic images of the AuNP solutions in the presence of sodium chloride salt and dopamine-binding aptamers under various concentrations of dopamine (0–6 μM), with visible colorimetric change from pink to purple with increasing dopamine concentration. Reproduced with permission from Ref. (Zheng et al., 2011). Copyright 2016 Elsevier.

microscopy approaches (Beyene et al., 2019). Aptamers functionalized directly on the surface of AuNPs transduce measurable color change upon neurochemical recognition by varying the separation distance between particles.

Several works have already reported dopamine colorimetric sensors based on dopamine-binding aptamers as molecular recognition elements and unmodified AuNPs as reporting probes (Zheng et al., 2011; Zhang et al., 2016). In the presence of dopamine, Zheng et al. hypothesized that the conformational change of dopamine aptamers upon target binding facilitates salt-induced aggregation of the AuNPs (Zheng et al., 2011). In this work, the AuNP solution is stabilized by citrate anions, whose repulsion prevent the nanoparticles from aggregating. Adding salt neutralizes the negative charge of citrate and leads to aggregation. In the absence of analytes, the aptamers adopt a structure that protects the AuNP surfaces from the salt present in the surrounding media. However, upon dopamine exposure, the aptamers undergo a conformational change that reduces salt shielding, which leads to AuNP aggregation (Fig. 2a) (Beyene et al., 2019). Such aggregation changes the AuNPs plasmon resonance frequency and consequently induces a colorimetric

response that scales with the dopamine concentration (Fig. 2b) (Zheng et al., 2011).

Cao and co-workers were able to improve the sensitivity of dopamine AuNP aptasensors to the femtomolar range by additionally immobilizing a complementary single-stranded DNA (cDNA) probe that can hybridize to the dopamine aptamer and generate large surface plasmon signals (Cao and McDermott, 2018). In the absence of dopamine, the aptamers hybridize with their conjugate cDNA, which generates large plasmon signals. Upon dopamine titration, the aptamers bind the target, and de-hybridize from the cDNA. The resulting difference in the surface plasmon resonance signal is therefore enhanced by this competitive assay where dopamine off-competes cDNA. Additionally, by adjusting the concentration of cDNA, Cao et al. were able to generate calibration curves with different dynamic ranges (0.2–2 mM and 0.2–20 μM) (Cao and McDermott, 2018).

Alternatively, Zhang et al. introduced a rapid dual-mode dopamine sensing system with both colorimetric and fluorometric assays (Zhang et al., 2016). The sensing mechanism relies on color change induced by target binding as proposed by Zheng et al., (Zheng et al., 2011) and on

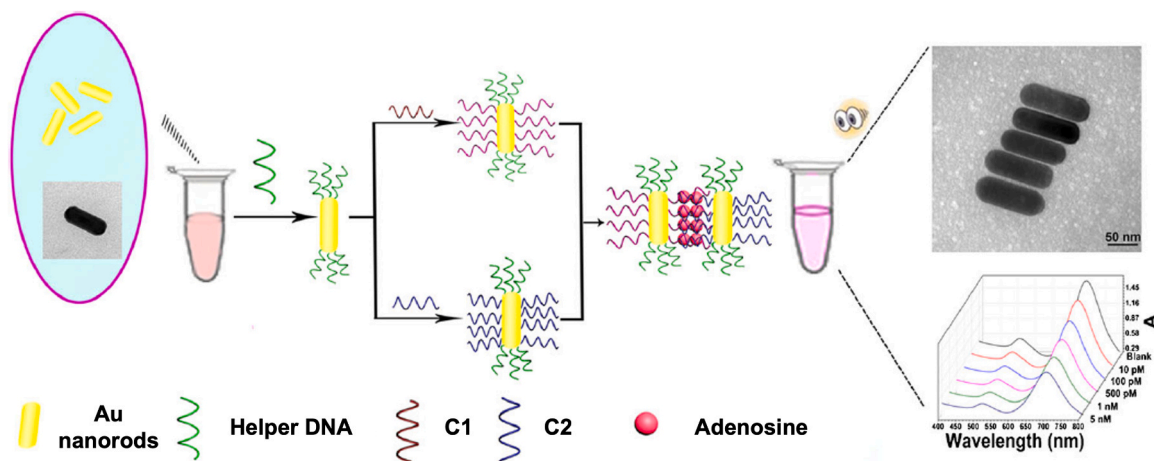


Fig. 3. Sensing mechanism of the gold nanorod-DNA aptamer systems for adenosine detection. Adapted with permission from Ref. (Zhang et al., 2019). Copyright 2019 Springer Nature.

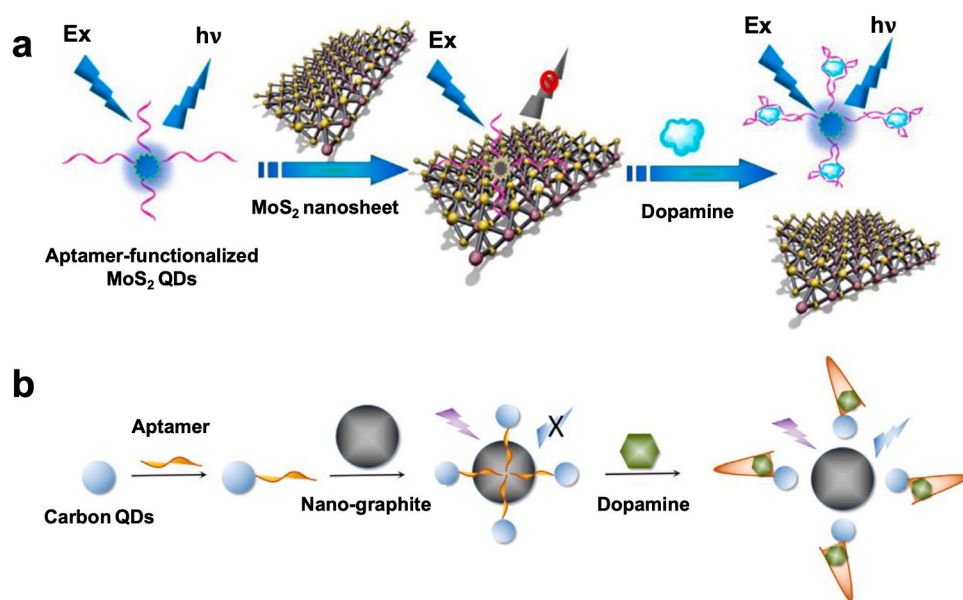


Fig. 4. Sensing mechanism of fluorometric aptamer-functionalized sensors with quantum dots (QDs). (a) Functionalized molybdenum disulfide (MoS₂) QDs adsorb on the MoS₂ nanosheet and cause fluorescence quenching. In the presence of dopamine, the aptamers bind the target and subsequently result in QD detachment from the nanosheet, which leads to fluorescence recovery. Adapted with permission from Ref. (Chen et al., 2019). Copyright 2019 Springer Nature. (b) Similar sensing mechanism based on carbon QDs and nano-graphite. Adapted with permission from Ref. (Zhu et al., 2016). Copyright 2016 Elsevier.

fluorescence readouts from dopamine-binding aptamers labeled with fluorophores (Zhang et al., 2016). More recently, an aptamer-based lateral flow assay for dopamine detection in urine was reported (Dalirirad and Steckl, 2020). Similarly to the work of Cao and co-workers, (Cao and McDermott, 2018) the aptamer hybridizes to a partially complementary DNA strand immobilized on the AuNPs. A higher dopamine concentration leads to an increased dissociation of the aptamer-DNA duplex, allowing the DNA to hybridize with its complementary strand on the test line of the assay (Dalirirad and Steckl, 2020).

Alternative neurotransmitters to dopamine have been detected using colorimetric AuNP-based assays such as adenosine (Chen et al., 2008; Zhou et al., 2020) and histamine (Lerga et al., 2019; Lerga et al., 2020). The sensing mechanism for these targets is similar to what was reported by Zheng et al., (Zheng et al., 2011) where the AuNPs undergo salt-induced aggregation as the analyte is added and binds to AuNP-immobilized aptamers. Adenosine was also detected colorimetrically using gold nanorods instead of AuNPs (Zhang et al., 2019; Wang et al., 2010a). In the presence of adenosine, the aptamer-functionalized nanorods capture adenosine, self-assemble into a sandwich structure, and connect to each other to form side-by-side structures, as illustrated in Fig. 3. As the adenosine concentration increases, larger quantities of nanorods assemble, which leads to an enhanced plasmon resonance light scattering, and a subsequent decrease in nanorod absorbance that scales with the adenosine concentration (Zhang et al., 2019; Wang et al., 2010a).

Also, a colorimetric biosensor for serotonin detection was introduced by Chavez et al., but unlike most other aptamer-AuNP assays, the extent of AuNP aggregation *decreases* in the presence of serotonin (Chávez et al., 2017). The authors hypothesized that the sensing mechanism is dependent on specific conformational changes induced by serotonin recognition, wherein the serotonin aptamer-target complex shields the AuNP surface against salt aggregation, thereby improving stability.

Collectively, the advantages of these colorimetric aptasensing systems include their simplicity, rapid analysis, and easily interpretable results visible by eye. Additionally, the previously presented aptamer-based biosensors show minimal cross-reactivity to common interferent neurotransmitters and molecules, demonstrating sufficient selectivity to their respective target. Importantly, some colorimetric aptasensors have been tested for functionality in biological environments.

For example, the dual sensors from Zhang et al. detected dopamine spiked into deproteinized human plasma at concentrations as low as 500 nM (Zhang et al., 2016). Further, other studies have examined

dopamine (Dalirirad and Steckl, 2020) and adenosine (Zhang et al., 2019; Wang et al., 2010a) sensing in urine samples. The serotonin aptasensors were also deployed in pre-treated spiked fetal bovine serum (Chávez et al., 2017). Histamine AuNP assays were tested in various fish samples, whose histamine content had previously been measured by independent laboratories, and a good degree of correlation was obtained (Lerga et al., 2020). Finally, the nanorod-based aptasensors were able to quantify adenosine in pre-filtered human serum samples at concentrations as low as 10 pM, (Zhang et al., 2019) as well as in rat brain samples (Wang et al., 2010a). While these results are encouraging, the tested biofluids are often pre-treated (*i.e.*, filtered, deproteinized, *etc.*) and thus not representative of physiologically relevant conditions.

The initial tests of serotonin aptamer-modified AuNPs in serum indicated that the presence of proteins abundant *in vivo*, interfered with AuNP aggregation due to nonspecific electrostatic binding, which shielded the serotonin binding effect (Chávez et al., 2017). Accordingly, increasing the salt concentration significantly (~700 mM) was necessary to obtain an assay response in serum vs. buffers (Chávez et al., 2017). Notably, aptamers are sensitive to ionic content, and such high salt may alter their target capture capability (Nakatsuka et al., 2021a). Further, while competitive assays enable lower detection limits, (Cao and McDermott, 2018) they are impractical for *in vivo* platforms. Such an assay necessitates an aptamer-cDNA hybridized system as the starting point, which may not remain stable depending on parameters such as temperature, pH, or ionic conditions.

The majority of reported colorimetric aptasensors have only been tested in proof-of-concept aqueous solutions or buffers, and their performance in realistic biological environments remains unexplored. This limited testing represents a crucial challenge for their deployment *in vivo*, and currently such platforms remain best suited for *ex vivo* studies, where biological samples can be pre-treated to ensure optimal sensor performance. Additionally, potential nonspecific interactions between target molecules and AuNP surfaces are often not considered, which may result in misinterpreted analyses (Liu et al., 2020a).

This issue was demonstrated for the dopamine aptamer-AuNP system where similar color responses were observed regardless of the DNA sequence used, indicative of nonspecific adsorption of dopamine to the AuNP surfaces instead of specific binding to the surface-bound aptamers (Liu et al., 2020a). Different target molecules have different nonspecific adsorption characteristics to AuNPs, which necessitates a case-by-case study of the mechanism with highly stringent controls. Due to such challenges, colorimetric aptamer-based sensing systems are suited for *ex*

in vivo or *in vitro* studies. In the following chapters, we will explore alternative aptamer-based optical technologies more promising for *in vivo* imaging of neurotransmitter release.

3. Fluorometric aptasensors

Quantum dots (QDs) are semiconductor nanoparticles of a few nanometers in size. The small size of QDs introduces quantum confinement of electrons and holes, resulting in electronic and optical properties that differ from the bulk materials. Among other advantages, QDs have a long fluorescence lifetime, strong resistance to photobleaching, and high quantum yield (Park et al., 2017). Further, their optical properties are tunable by modifying their material, size, shape, and surface chemistry (Wang et al., 2013). Particularly, the emission spectrum can be adjusted from visible to near-infrared wavelengths. Such properties make QDs a great substitute for conventional dyes and fluorescent proteins in bioimaging or biosensing applications. Moreover, QDs have a broad absorption spectrum with a narrow emission spectrum, which makes them excellent donors for Förster resonance energy transfer (FRET)-based sensors (Wang et al., 2013).

The FRET phenomenon consists of a nonradiative energy transfer between a chromophore in an excited state (donor) to another chromophore (acceptor). This mechanism is dependent on the overlap between the donor emission and the acceptor absorption spectrum, the distance between the chromophores, and their relative orientation. The narrow emission bandwidths of QDs enable a good separation between the different pairs of QDs and acceptor dyes, reducing cross-activation between the dyes (Wang et al., 2013). Additionally, the broad absorption spectrums allow simultaneous excitation at specific wavelengths while avoiding direct excitation of the acceptor dyes. Such properties facilitate multiplexing of QD-based FRET sensors.

Sensing platforms based on FRET that employ aptamer-functionalized QDs as donors have been reported for the detection of dopamine (Chen et al., 2019; Zhu et al., 2016). Chen et al. developed a fluorometric dopamine assay based on the quenching of molybdenum disulfide (MoS₂) QDs by MoS₂ nanosheets (Fig. 4a) (Chen et al., 2019). The assay uses the ability of single-stranded DNAs to spontaneously adsorb on MoS₂ nanosheets through Van der Waals interactions with the nucleobases. However, rigid double-stranded DNAs do not interact with the nanosheets because the nucleobases are buried between the densely packed DNA backbone (Zhu et al., 2013). In the absence of dopamine, the aptamers would present a linear structure and adsorb onto the MoS₂ nanosheets. The proximity between the MoS₂ QDs and nanosheets combined with the overlap of their respective emission and absorption spectra would result in FRET and therefore quench the fluorescence of the QDs. Upon dopamine capture, the aptamers detach from the MoS₂ nanosheets. Consequently, the aptamer functionalized QDs would recover their fluorescence.

Based on a similar strategy, Zhu et al. reported a dopamine aptasensor using carbon QDs and nano-graphite (Fig. 4b) (Zhu et al., 2016). In the absence of dopamine, the carbon QDs are adsorbed on the nano-graphite. The strong π - π interactions between the nano-graphite and the carbon QDs were hypothesized to obstruct excited electrons from the conduction to the valence band in the carbon QDs, and, consequently, reduce the fluorescence of the nanoparticles.

The advantages of aptamer-modified QD-based methods consist in their low limit of detection (45 pM and 55 pM for MoS₂ QDs and carbon QDs respectively) as well as high sensitivity and selectivity of dopamine in the presence of interferent neurotransmitters and metabolites (Chen et al., 2019; Zhu et al., 2016). The selectivity reported vs. norepinephrine for these sensors was unexpected, considering the cross-reactivity previously shown by the dopamine aptamer isolated in 2009 (Walsh and DeRosa, 2009; Álvarez-Martos and Ferapontova, 2017). While detection of dopamine in human serum and urine for MoS₂ QDs and carbon QDs respectively, demonstrated the potential of both techniques for biological environments, the measurements were conducted in

diluted samples spiked with dopamine. Thus, like AuNP-based sensors, the applicability of fluorometric systems for *in vivo* sensing must be assessed in relevant undiluted samples.

For *in vivo* applications, the toxicity of QDs is a concern due to the potential intrinsic toxicity of the semiconductor components. Indeed, the degradation of the QDs would release toxic ions such as Cd²⁺, Hg²⁺, Pb²⁺, and As³⁻ (Wang et al., 2013). Several studies reported biocompatibility of MoS₂ and carbon QDs with minimal toxicity both *in vitro* and *in vivo* (Guo and Li, 2020; Luo et al., 2013; Mansuriya and Altintas, 2020; Shi et al., 2019). However, long-term toxicity *in vivo* of such QDs should be further assessed (Zhu et al., 2016; Guo and Li, 2020). The toxicity problem of QDs is complex because numerous factors such as the size, shape, concentration, and surface modifications play an important role in determining the biocompatibility. Therefore, these parameters should be comprehensively studied for each specific system. Selection of an appropriate surface coating may increase the biocompatibility of QDs in physiological conditions. For example, polymer coatings, such as polyethylene glycol (PEG), have been reported to successfully passivate the surface and increase the chemical stability of QDs, reducing their degradation (Wang et al., 2013).

Another important aspect to consider, when working with nanoparticles in complex environments, is the nonspecific interaction with proteins. Biological fluids contain a variety of proteins, which may adsorb on the surface of nanoparticles and create a layer called the protein corona (Ashraf et al., 2016; Motevalian et al., 2020; Nakatsuka et al., 2018; Perng et al., 2019; Salvati et al., 2013; Song et al., 2020). Such protein coronas may trigger the aggregation of the nanoparticles, which consequently impairs their functionality and/or significantly reduces their retention time in blood (Salvati et al., 2013). The chemical nature of the surface coating of nanoparticles have been reported as key factors in the formation of the protein corona (Ashraf et al., 2016; Perng et al., 2019). For instance, while PEG coatings in general reduce the protein adsorption on QDs, amine-terminated PEG still induces protein corona formation, while carboxyl-decorated PEG significantly reduces protein adsorption (Perng et al., 2019).

However, in some cases, protein corona formation may have beneficial effects on the properties of QDs (Frutiger et al., 2021). For example, the protein corona on thioglycolic acid-coated CdTe QDs increased their fluorescence by inducing the rearrangement of the surface coating and subsequently reducing surface defects (Motevalian et al., 2020). In addition, carefully regulated protein coronas has been harnessed to cloak nanoparticles from rapid clearance by the immune system for targeted drug delivery (Rampado et al., 2020; Oh et al., 2018). Further, human serum albumin protein corona formation has been shown to mitigate the toxicity of carbon QDs (Song et al., 2020). These findings highlight the possibility to exploit a highly controlled protein corona to increase the biocompatibility of QDs and other nanoparticles.

Further challenges for translation *in vivo* include the emission of MoS₂ and carbon QDs in the visible range. Visible light is greatly scattered and absorbed by biological tissues, which reduces the penetration depth to 0.5–2.5 mm (Xu et al., 2016; Cai et al., 2019; Jelínková, 2013). Moreover, visible light suffers from a considerable autofluorescence background from biological tissue, resulting in low signal-to-noise ratios of the sensor. Nanomaterials that emit in the second near infrared region (NIR-II; 1000–1450 nm), penetrate deeper in tissue (up to 3 cm) and show significantly improved signal-to-noise ratios (Cai et al., 2019). Recently, novel heavy metal-free QDs, which are characterized by low toxicity and emission in the NIR-II regions, have been developed and present improved potential for *in vivo* biosensing (Xu et al., 2016).

Such fluorometric sensing platforms enable dopamine imaging *in vitro* in diluted biological samples. However, translation to *in vivo* neurochemical imaging is limited by the absorption of the sensor signal by biological tissue and the possible material toxicity. Finally, the *in vivo* implementation of this type of competitive assay is very challenging because the QDs must remain mobile, but at the same time they should

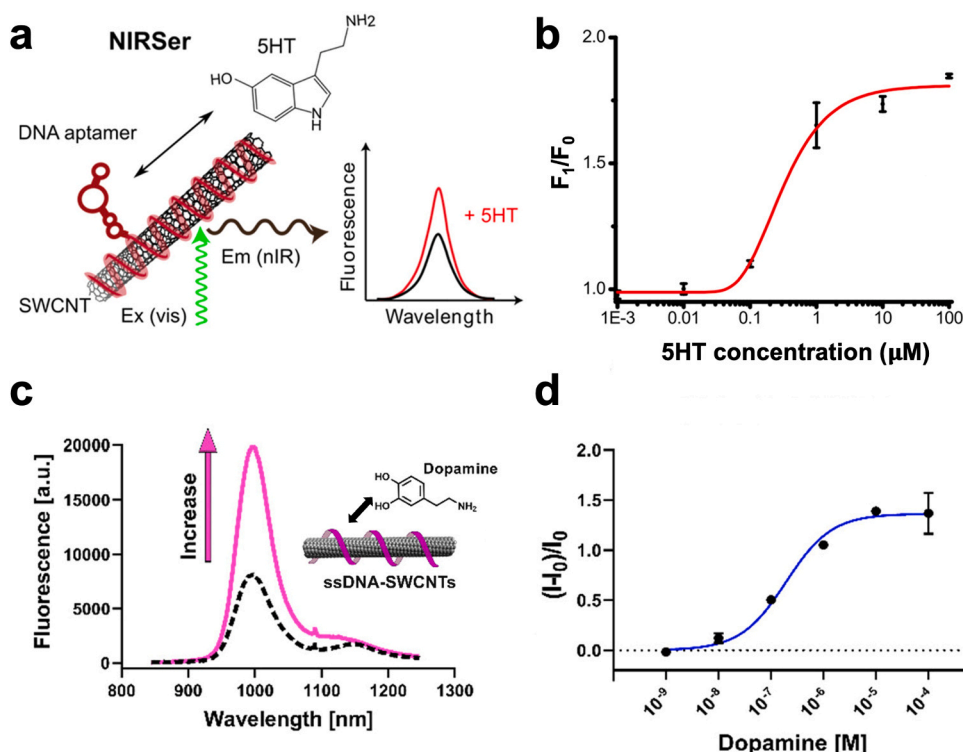


Fig. 5. Near-infrared (NIR) fluorescent sensors for neurotransmitters. (a) Working principle of NIR fluorescent sensor based on semi-conducting single-walled carbon nanotubes (SWCNTs) and specific DNA aptamers for serotonin detection. Serotonin target binding to the aptamers functionalized to SWCNTs leads to a conformational change of the DNA backbone and consequently an increase in the fluorescence of the SWCNTs is observed. (b) Calibration curve presents the fluorescence intensity ratios before (F_0) and after (F_1) serotonin addition at the 995 nm emission peak. The binding affinity was determined to be ~ 300 nM ($N = 3$, mean \pm standard error of the mean). Figures (a) and (b) were adapted with permission from Ref. (Dinarvand et al., 2019). Copyright 2019 American Chemical Society. (c) Fluorescence increases upon dopamine addition due to interactions with single-stranded DNA (ssDNA) modified on the surfaces of monochiral SWCNTs. (d) Dopamine-specific monochiral sensor response showing a maximum of $\sim 140\%$ fluorescence increase with a dissociation constant of ~ 100 nM ($N = 3$, mean \pm standard error of the mean). Figures (c) and (d) were adapted with permission from Ref. (Nišbler et al., 2021). Copyright 2021 American Chemical Society.

be retained near the surface of nanosheets. While the immobilization of the nanosheet on a substrate seems feasible, the QDs should be free in solution. The compartmentalization of the QD solution is difficult to integrate into a sensing platform.

An alternative FRET-based sensor using fluorophore-labeled aptamers instead of QDs has been presented by Liu et al. for the detection of dopamine (Liu et al., 2020b). Fluorescein was tethered to the 5'-end and a dark quencher was tethered to the 3'-end of the aptamer. The capture of dopamine induced a major conformational change of the DNA backbone and brought the two termini closer. Consequently, the fluorescence emission of the fluorescein is quenched. In a second design, the dark quencher was replaced by tetramethylrhodamine and the emission of the fluorophore was measured. This new configuration lowered the limit of detection marginally from 0.9 μM to 0.1 μM .

This FRET sensor based on aptamer folding dynamics is biocompatible, does not require complex synthesis steps, and has a fast response time. In general, the sensor showed adequate selectivity against dopamine analogs. However, the response of the sensor to norepinephrine was only three-fold lower than to dopamine. Such cross-reactivity is a concern for detection *in vivo*. To design aptamers with improved selectivity, methods such as counter-SELEX, where structurally similar molecules are incubated with selected aptamers in an additional step to discriminate nonspecific sequences, should be employed (Yang et al., 2017, 2016). The novel dopamine aptamer reported in 2018 used such stringent counter selection techniques, which was evidenced by the significantly improved selectivity vs. norepinephrine (Nakatsuka et al., 2018b). Further challenges for the FRET-based sensor include the necessity to immobilize the aptamer on the surface of implantable devices, which may affect the initial conformation of the aptamer and target-specific folding dynamics, reducing the sensitivity or even hindering the FRET mechanism. In addition, similarly to previously discussed QD-based fluorometric sensors, the emission in the visible range limits sensor functionality *in vivo*.

Alternative aptamer-based optical technologies can overcome the limitation of penetration depth of visible light by targeting the near-infrared (NIR) range instead. Improved visualization in deeper tissues

is more suitable for *in vivo* monitoring of biomolecules. In the following chapter, we will discuss aptasensors using carbon nanotubes (CNTs) that are able to emit in the NIR range.

4. Near-infrared fluorescent aptasensors

Among different nanomaterials employed for neurochemical sensing or imaging, one-dimensional single-walled carbon nanotubes (SWCNTs) are often used due to their advantageous structural and optical characteristics (Hendler-Neumark and Bisker, 2019; Antonucci et al., 2017). These SWCNTs can be visualized as a graphite sheet rolled into a tube, resulting in different nanotube chiralities depending on the orientation and the diameter of the tubular structures (Yang et al., 2020). Semi-conducting SWCNTs have a bandgap that leads to NIR fluorescence, in the spectral range from 900 to 1600 nm. The emission wavelength depends on the chirality of the nanotubes (Nišbler et al., 2021). This photostable fluorescence range is important as biological samples such as blood, tissues, and cells are relatively transparent, (Hendler-Neumark and Bisker, 2019) hence making possible both *in vivo* and *in vitro* applications (Beyene et al., 2019).

With a diameter that varies from 0.4 to 100 nm and a length up to tens of micrometers, SWCNTs have high aspect-ratios and subsequently high surface areas that can be functionalized for biosensing (Saifuddin et al., 2013). To preserve the optoelectronic properties of the SWCNTs, the sp^2 bonds must remain stable. Thus, noncovalent functionalization approaches are needed to reach the desired specificity towards targeted biomolecules (Antonucci et al., 2017). Furthermore, semiconducting SWCNTs do not photobleach (Hendler-Neumark and Bisker, 2019). A combination of these favorable optoelectronic properties of SWCNTs combined with selective aptamers, facilitates sensitive aptasensors for detection and imaging of neurochemicals (Yan et al., 2014; Landry et al., 2017; Cha et al., 2011; Lee et al., 2018; Taghdisi et al., 2014; Dinarvand et al., 2019; Zhang et al., 2010).

Dinarvand et al. presented a NIR fluorescent sensor based on semi-conducting SWCNTs and specific DNA aptamers for the detection and imaging of serotonin (Dinarvand et al., 2019). The authors employed a

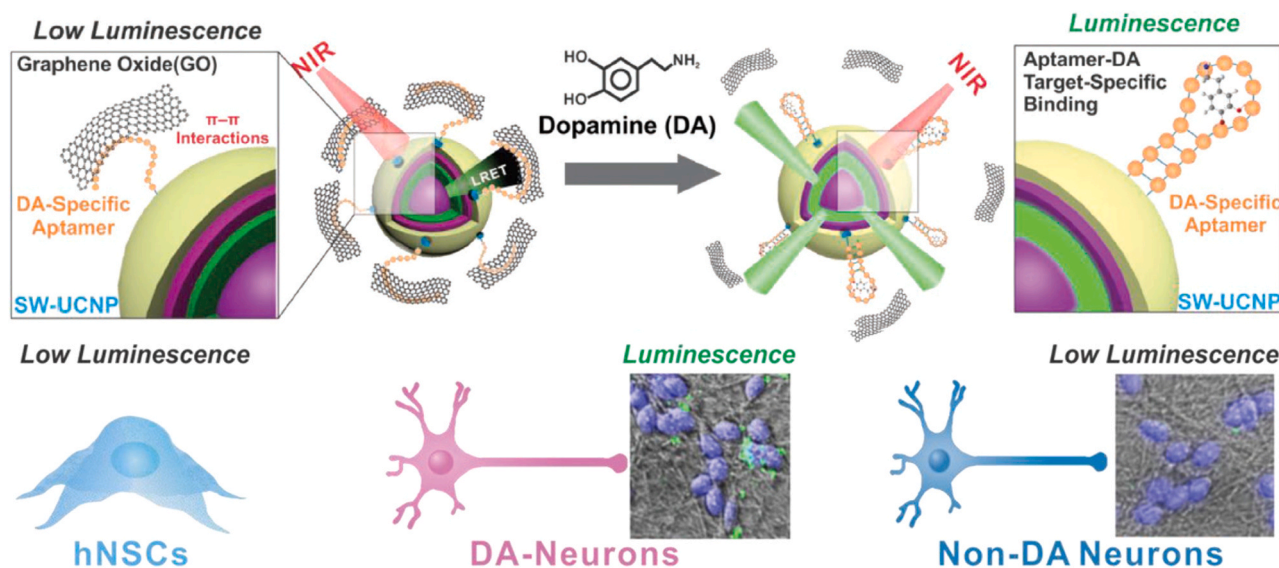


Fig. 6. Schematic diagram of the constructed near infrared (NIR)-based sensor for dopamine (DA) detection using the “sandwich” upconversion nanoparticles (UCNPs). Low luminescence is observed due to quenching by graphene-oxide (GO). Exposure to dopamine leads to aptamer conformational change, which leads to the release of GO and subsequent recovery of luminescence. The application of these DA UCNPs sensors was demonstrated by tracking the differentiation of human neural stem cells (hNSCs) into DA-neurons in real time and differentiating DA vs. non-DA neurons. Adapted with permission from Ref. (Rabie et al., 2019). Copyright 2019 John Wiley and Sons.

noncovalent approach for SWCNT functionalization with the serotonin aptamer *via* tip sonication (Fig. 5a). This serotonin aptamer has a linear nucleotide tail that does not participate in the three-dimensional structure for target recognition, but wraps around SWCNTs, producing a corona phase, which disperses the highly hydrophobic nanotubes in aqueous solution. The functional part of the aptamer remains free to bind to the serotonin and the consequent DNA conformational change leads to an increase in fluorescence. The hypothesized mechanism based on molecular dynamics simulations suggests that rearrangements of phosphate groups at the SWCNT surface, removes quenching sites and changes the local potential, and subsequently alters the SWCNT fluorescence quantum yield (Kruss et al., 2014, 2017).

The serotonin sensor showed a dynamic detection range for serotonin from approximately 100 nM to 1 μ M (Fig. 5b) and showed selectivity vs. glucose, tyrosine, tryptophan, and histamine. However, cross-reactivity to dopamine was observed, which was hypothesized to arise due to the hydroxyl groups of catecholamines that interact nonspecifically with ssDNA, which leads to a change in fluorescence intensity (Mann et al., 2017; Polo and Kruss, 2016). These sensors were then deployed to visualize serotonin release from platelet cells isolated from human blood at a single-cell level. The SWCNT-aptamer dispersions were coated on glass slides together with fibrinogen that facilitates platelet cell adhesion, and the image sequences were captured using a NIR fluorescence microscope. The obtained results are promising for pinpointing serotonin release at specific locations around cell membranes with high spatiotemporal resolution.

Similarly, Jeong et al. presented a NIR fluorescent sensor based on ssDNA specifically isolated for serotonin detection at the surface of SWCNTs (Jeong et al., 2019). In this approach, SELEX is implemented on SWCNT surfaces in a process coined “SELEC” to identify unique polynucleotide sequences with serotonin specificity when immobilized on SWCNTs. The sensor is reversible and produces approximately 200% fluorescence increase upon serotonin binding. The serotonin sensor was integrated into a brain imaging probe, showing a dynamic range from ~1–20 μ M with a K_d of ~6 μ M, which is likely insufficient for serotonin sensing *in vivo* that falls into a sensing regime typically in the high pM to nM range.

Discrimination of catecholamine neurotransmitters such as dopamine, epinephrine, and norepinephrine, is a difficult task to be achieved

due to overlapping chemical functionalities of the molecules. In this regard, Mann et al. presented a study on NIR fluorescent SWCNTs functionalized with ten different oligonucleotides with the main goal to discriminate these catecholamines (Mann et al., 2017). The study revealed that most DNA-functionalized SWCNTs respond to catecholamines, indicative of nonspecific interactions rather than sequence specificity. However, the K_d values (ranging from ~2 nM to 10 μ M) and detection limits were highly dependent on the ssDNA sequence. Interestingly, the “library” of ten different DNA sequences were chosen randomly, with no rationale behind the preselection. Such a study suggests that false positive signals due to nonspecific interactions with ssDNA are possible for SWCNT-based detection schemes, and necessitates robust characterization of specific aptamer-target interactions using alternative methods (Nakatsuka et al., 2018b).

As mentioned at the beginning of this section, the emission wavelength depends on the nanotube chirality. Most of the studies found in the literature use a mixture of SWCNT chiralities. Nißler et al. suggested that such heterogeneity would have a negative effect on the sensitivity and selectivity of the sensor, since different chiralities show different adsorption affinities toward oligonucleotides, hence varied sensor responses (Nißler et al., 2021). To test this hypothesis, pure chirality semiconducting SWCNTs were isolated and functionalized for specific detection of several analytes, including dopamine. Purified monochiral SWCNT sensors demonstrated fluorescence intensities up to 10 times higher in comparison to sensors composed of mixed SWCNT chiralities. The highest fluorescence enhancement achieved for dopamine sensors was 140% (Fig. 5c) with a K_d of ~100 nM (Fig. 5d). While the sensor response can be improved by using monochiral SWCNTs, obtaining pure chirality SWCNTs is a highly complicated procedure leading to a significant increase in costs and complications of sensor fabrication.

Alternatively, Zhang et al. reported a sensitive fluorescent sensor for adenosine triphosphate (ATP) based on the dye-labeled ATP aptamer and SWCNTs (Zhang et al., 2010). A stable dye-aptamer/SWCNT complex was dispersed in buffer, after which ATP was added and the fluorescence intensities were measured. In the absence of ATP, the dye labeled on the aptamer is in close proximity to the SWCNT surface, which leads to fluorescence quenching. Upon ATP addition, the dye moves away from the SWCNT surface, due to the DNA adopting a quadruplex upon ATP recognition, which results in detectable

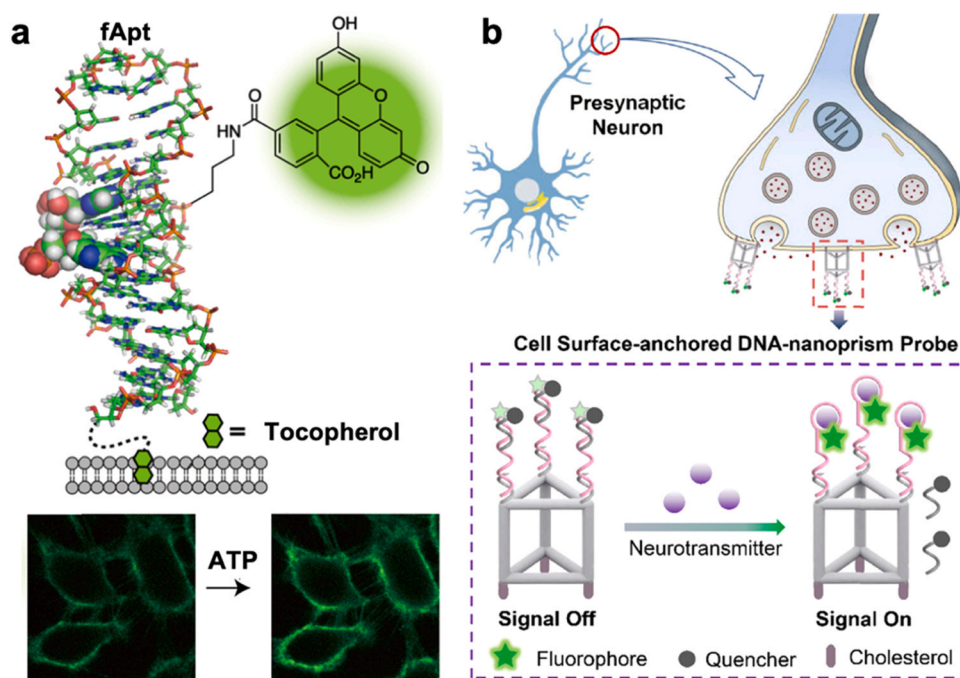


Fig. 7. Membrane-anchored fluorescent aptamer systems. (a) Tocopherol-anchored fluorescent aptamers (fApt) were directly anchored on cell surfaces. Confocal fluorescence images of fApt-integrated HeLa cells in the absence (left) and presence (right) of 200 μM adenosine triphosphate (ATP) is shown below. Adapted with permission from Ref. (Tokunaga et al., 2012). Copyright 2012 American Chemical Society. (b) DNA-nanoprism constructs are anchored in the cell membrane via cholesterol tags and enable dopamine detection via fluorophore- and quencher-labeled aptamer sequences. Reproduced with permission from Ref. (Zeng et al., 2020). Copyright 2020 American Chemical Society.

fluorescence. The hypothesized detection mechanism was supported by circular dichroism spectroscopy that can track aptamer secondary structural rearrangements (Kypr et al., 2009). A linear detection range from 10 nM to 0.8 μM was reported for the detection of ATP in lysed cells. However, it should be noted that normal cellular ATP concentrations are in the range of 1–10 mM, which should be taken into account to enable detection in physiologically relevant sensing regimes (Zimmerman et al., 2011).

To explore design principles for optimal fluorescent nanosensors that can resolve fast concentration changes such as neurotransmitter release from cells, theoretical models have been developed. Meyer et al. presented stochastic Monte Carlo simulations by exposing SWCNT-based sensors to a concentration gradient of dopamine (Meyer et al., 2017). Then, binding/unbinding events to specific binding sites for the molecule of interest and the respective fluorescence change were simulated. The simulation data revealed that to resolve neurotransmitter release via imaging requires rate constants of $k_{\text{on}} = 10^6 \text{ M}^{-1} \text{ s}^{-1}$ and $k_{\text{off}} = 10^2 \text{ s}^{-1}$, which corresponds to high dissociation constants of $K_d > 100 \mu\text{M}$. Thus, to achieve high temporal resolution, the size, shape, geometry, and density of SWCNT-nanosensor arrays must be optimized. To take advantage of the advantageous optoelectronic properties of SWCNTs for practical use for *in vivo* detection and imaging, increased effort is needed to fine-tune the sensor configuration. Another issue that remains is the biocompatibility of nanotubes for *in vivo* applications (Barbarino and Giordano, 2021).

An alternative NIR-based method detected dopamine release from live stem cell-derived dopaminergic neurons (Rabie et al., 2019). Upconversion nanoparticles (UCNPs) were used due to their high photostability, low autofluorescent background, and deep tissue penetration. To achieve selective dopamine detection, the UCNPs were coated with a silica shell, followed by dopamine aptamer immobilization. To complete the sensor fabrication, graphene oxide (GO), which quenches the UCNP luminescence, was adsorbed to the dopamine aptamers via π - π stacking. Upon addition of dopamine, the aptamers undergo conformational changes that lead to release of GO and subsequent recovery of luminescence (Fig. 6).

The dopamine aptamer-UCNP sensor was able to detect dopamine in the range of 1–10 pM, a necessary detection range for monitoring *in situ* production of dopamine in the cytoplasm of neurons. However, for

translation *in vivo*, several limitations must still be assessed such as increasing upconversion quantum yields for improved brightness, a full assessment of potential toxicity in animal models, and strategies to improve delivery to target tissues (Del Rosal and Jaque, 2019). Following this work, we will next review the literature focused on aptamer-based platforms for live-cell imaging.

5. Aptasensors for live cell imaging neurotransmitter release

For visualization of neurotransmitter flux, fluorescent aptasensors are attractive systems since the signal readout can be recorded in real time and *in situ* with sufficient spatiotemporal resolution to resolve neuronal dynamics. While aptamer-fluorophore hybrids have been used to detect a variety of targets including metal ions and small molecules, (Zhou and Zhang, 2021) practical cellular applications have been limited to few cases with the goal of intracellular analysis of target biomolecules (Wang et al., 2010b). We now review reports wherein fluorescent aptasensors are integrated into live cells to enable real-time imaging of neurotransmitter dynamics at the single cell level.

In 2012, Tokunaga et al. reported an aptamer-based fluorescent system that can detect adenine compounds in real time (Tokunaga et al., 2012). Specifically, this aptamer was used to image ATP dynamics in brain astrocytes, where ATP can act as a neurotransmitter. The aptamer was integrated into the cell membrane via a tocopherol-anchor (Fig. 7a) (Tokunaga et al., 2012). By coupling the aptamer to such an anchor, an amphiphilic molecule was generated. When incubating the astrocytes with media containing the molecule, the anchor inserts into the cell membrane without introducing functional losses or cell toxicity. The mechanical stimulation of astrocytes has been shown to provoke the release of ATP and was therefore used to trigger ATP release for imaging (Newman, 2001).

After mechanical stimulation of neurons, ATP release and recognition led to aptamer structural switching, leading to a fluorescence enhancement from the fluorophore that could be used to visualize the neurotransmitter release in real time. So far, this method only allowed for relative quantification. Absolute quantification could be achieved via sensor calibration or having a ratiometric sensor with a second control probe that enables differential fluorescence recordings (Tokunaga et al., 2012).

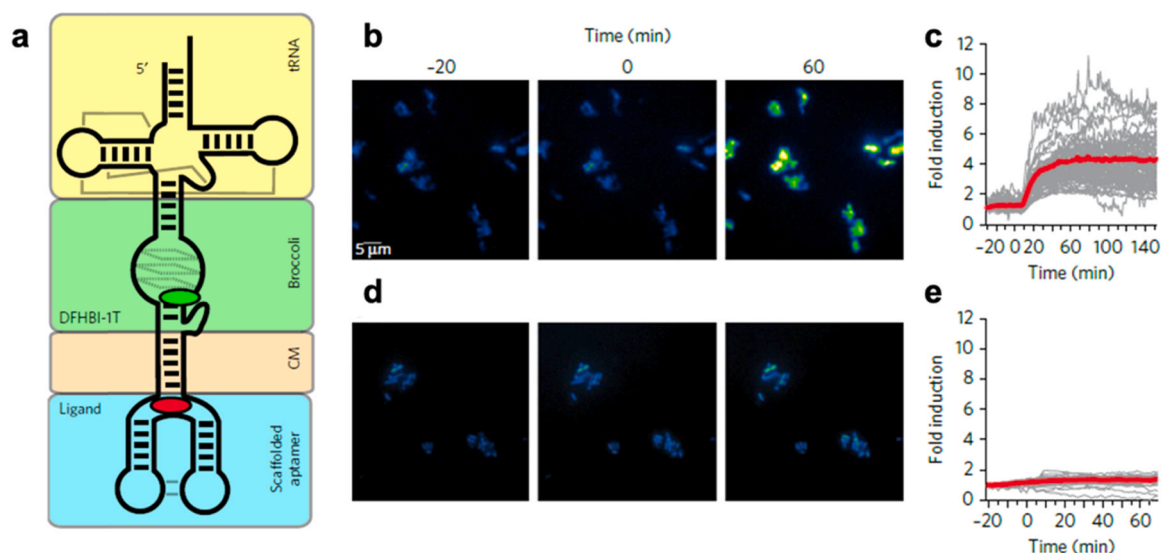


Fig. 8. Scaffolding aptamer constructs for real-time neurochemical monitoring in bacterial cells. (a) The target-binding aptamer domain is coupled to the fluorescent reporter (broccoli) sequence via a communication module (CM), which in turn is coupled to a tRNA sequence for stability. (b) Broccoli fluorescence signal in live *Escherichia coli* cells upon addition of 2 mM of 5-hydroxytryptophan (5-HTP) to the medium at minute 0. (c) Fold change in fluorescence intensity upon 5-HTP addition to the medium of the cells. (d) A mutated sequence (single point mutation) of the same aptamer disabled target recognition. (e) Negligible changes in fluorescence induction were observed. Reproduced with permission from Ref. (Porter et al., 2017). Copyright 2017 Springer Nature.

More recently, Zeng et al. developed a similar system by also anchoring fluorescent aptamers within the cell membrane for live-cell sensing of dopamine release (Zeng et al., 2020). Self-assembling DNA constructs termed nanoprisms, serve as the central building block, where cholesterol tags on one side enable membrane anchoring and fluorophore-labeled aptamer tags on the other side facilitate dopamine sensing (Fig. 7b). The system relies on aptamer conformational change upon target binding, which spatially separates the quencher and fluorophore, therefore inducing a fluorescent signal upon dopamine recognition (Zeng et al., 2020).

Porter et al. developed an RNA aptamer-based sensor that functions robustly in cellular environments (Porter et al., 2017). By reprogramming natural aptamer sequences (*i.e.* ribozymes) to elicit binding affinities towards selected target molecules, RNA aptamers for the neurotransmitter precursors, 3,4-dihydroxy-*L*-phenylalanine (*L*-DOPA) and 5-hydroxytryptophan (5-HTP) were developed. The sequence was translated into DNA and expanded with the sequence for the fluorogenic aptamer Broccoli (Filonov et al., 2014) as well as with the DNA complement of a tRNA sequence (Fig. 8a). The full sequence therefore has target-specific binding through the aptamer domain and fluorescent activity through the Broccoli domain. The tRNA scaffold disguises the recombinant RNA as natural RNA, escaping degradation by cellular RNases and conferring stability (Ponchon and Dardel, 2007). To express the sensor, *Escherichia coli* were transfected with this scaffolding aptamer construct. Target recognition specifically activated the fluorescence of the Broccoli reporter, and the real-time uptake of 5-HTP and *L*-DOPA were monitored in the bacterial cells (Fig. 8b) (Porter et al., 2017).

Rather than integrating sensors into cell membranes, Choi et al. coated a GO surface with Raman-dye labeled dopamine aptamers (Choi et al., 2020). The aptamers adsorbed to the cell surface by π - π stacking interactions. Dopamine, released from neurons cultured on such substrates, was captured by the aptamers, which led to the release of the aptamer from the substrate surface. Aptamer detachment led to a signal decrease due to the Raman dyes, that significantly enhance Raman signals, leaving the GO surface at specific locations, enabling *in situ* dopamine detection (Choi et al., 2020). However, rather than tracking neurochemical release from neurons themselves, this approach would monitor neurochemicals that interact with aptamers adsorbed to substrate surfaces, which renders the system diffusion limited with lower

spatiotemporal resolution.

The aforementioned approaches have the advantage of tracking specific target molecules in real time. Since the conformational change of the aptamers are fast (stopped-flow fluorescence studies for the f-Apt-ATP interactions were reported to have $k_{on} = 7 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ and $k_{off} = 7.3 \times 10 \text{ s}^{-1}$), (Tokunaga et al., 2012) a rapid signal readout can be generated. In addition to this temporal resolution, immobilization of aptamers at the target release sites also provides high spatial resolution. If fully explored, such a system would have great potential to image various neurochemicals, since aptamers can be isolated for diverse classes of molecules.

However, to date, none of these aptasensors have been applied *in vivo*. To implement such a technology into a living organism, some major challenges would need to be overcome. While the aptamers need to be delivered to specific brain regions, the blood brain barrier shields certain biomolecules from crossing into the extracellular fluid of the central nervous system from peripheral circulating blood. Several groups have developed aptamers that specifically target the blood brain barrier but further investigations are necessary due to structural complexity (Bukari et al., 2020). Alternatively, injections directly into the brain tissue of interest might be a possibility, since the aptamers self-integrate into the cell membrane by being amphiphilic. However, such invasive techniques may alter the brain activity that is the subject of studying and therefore yield a confounding effect. Thus, a critical challenge will be to assemble the aptamers in precise locations *in vivo* without altering the experimental output by interfering with normal brain activity.

A promising study demonstrated that there is minimal cytotoxic effect to cells incubated with an aptamer-containing medium (Tokunaga et al., 2012). Expression of an aptamer construct by the cells themselves would be optimal. However, DNA aptamers cannot be expressed via the regular transcription and translation machinery of the host cell. A cell-expressed RNA aptamer sensor has been shown to be feasible, (Porter et al., 2017) but the task remains to position the aptamers in the right place: the membranes at the synaptic cleft. This challenge necessitates a targeting mechanism to deliver the aptamers to the cell membrane, which is certainly a non-trivial task.

6. Conclusions and outlook

We have presented an overview of aptamer-integrated platforms developed for neurotransmitter quantification *via* imaging methodologies. We summarize the advantages, challenges, and outlook of the aptasensors reviewed herein, in Table 2. Aptasensors developed and interrogated *in vitro* are often not amenable for investigating biological processes in living organisms. To evaluate the viability of neurochemical sensing platforms *in vivo*, several metrics must be taken into consideration such as selectivity, sensitivity, limit of detection, and spatiotemporal resolution in complex environments. Further, different neurotransmitters have dynamic concentration flux in different brain regions, which necessitates sensors that exhibit high sensitivity with a wide, physiologically relevant measurement range. To further the complexity of *in vivo* sensor design, the brain microenvironment has a significantly higher concentration of interfering compounds compared to the analytes of interest. Thus, in addition to the inherent selectivity of the bioreceptors themselves, nonspecific binding to the sensor surfaces must be carefully considered to minimize false signals and biofouling (Frutiger et al., 2021).

For *in vivo* deployment, sensor stability and biocompatibility must be investigated in depth, and many tests are required before being able to define a medical device as biocompatible. Evaluating biomedical implants in humans should be performed according to well-defined criteria established by the International Organization for Standardization (International Organization for Standardization, 2018). When considering insertion into animal models, the sensor must be small enough to cause minimal tissue damage while retaining mechanical robustness. To perform *in situ* measurements, the biosensors should also be capable of functioning reliably for long periods of time in complex biological medium, and then be recovered from the body or safely biodegraded once their sensing task is completed (Wilson and Gifford, 2005).

To target the correct sensing area, the sensors could be injected in the

circulatory system, enabling access to the entire body (Nelson et al., 2010). Yet, important challenges are brought by *in vivo* physiological characteristics such as varying vessel dimensions, pulsating fluid flow, (Nelson et al., 2010) and also the presence of other circulating molecules and cells (Masson et al., 2006). Additionally, the blood-brain barrier hinders certain molecular access from circulating blood into the brain milieu (Bukari et al., 2020).

Additionally, it must be ensured that the aptamer-based sensors do not alter cell physiology and neurological pathways in an undesired manner. While neurotransmitters captured by aptamers are released at a certain rate of dissociation based on the K_d , it is possible that *in vivo*, scavenged molecules affect neurotransmitter release. For example, DNA aptamers for acetylcholine were developed with the goal of scavenging excess acetylcholine that are increasingly released at synapses by organophosphorous nerve agents (Curtin et al., 2006). Most reported methodologies for neurochemical measurements are conducted far from synapses where highly confined release events occur, thus the capture of diffused neurotransmitters likely has minimal impact on local neurotransmitter release. However, for future sensing platforms that integrate high affinity aptamers at high densities localized near nanoscale synapses, this aspect should be considered.

Tackling the many dimensions that render continuous *in vivo* bio-sensing highly challenging necessitates collaborations between sensor engineers and clinical users, to identify the technical barriers for implementation. Sensor design should be application driven, to tune signal responses to clinically relevant sensing regimes in physiological environments. Aptamers have the potential to revolutionize neuro-technologies (Nakatsuka et al., 2021b, 2021c) that enable real-time imaging of neurotransmitter flux in the brain milieu due to their tunable properties such as binding affinities, kinetics, selectivities, and stability in complex environments. The future of aptasensors for imaging neurochemicals in animal models relies on harnessing such advantages.

Table 2

Aptasensors discussed throughout this review with their advantages, challenges, and proposed outlook.

Aptasensor	Advantages	Challenges	Proposed Outlook
Colorimetric Aptasensors	<ul style="list-style-type: none"> • Simplicity, rapid analysis, and easily interpretable results visible by eye • Minimal cross-reactivity to interferent neurotransmitters and molecules • Tested for functionality in pre-treated biological environments 	<ul style="list-style-type: none"> • Nonspecific binding with <i>in vivo</i> proteins interferes with AuNP aggregation • Nonspecific interactions between the target molecules and the AuNP surfaces • Not yet tested in realistic biological environments 	<ul style="list-style-type: none"> • Best suited for <i>ex vivo</i> and <i>in vitro</i> studies
Fluorometric QD-Based Aptasensors	<ul style="list-style-type: none"> • Low limit of detection • High sensitivity and selectivity of dopamine in the presence of interferent neurotransmitters and metabolites 	<ul style="list-style-type: none"> • Long-term toxicity <i>in vivo</i> of QDs not yet assessed • Nonspecific interaction with proteins and formation of protein coronas in biological environments • Absorption of the sensor signal by biological tissue (visible range) 	<ul style="list-style-type: none"> • Select appropriate surface coatings such as polyethylene glycol (PEG) to increase the biocompatibility of QDs • Exploit highly controlled protein coronas to increase the biocompatibility of QDs • Novel metal-free QDs characterized by low toxicity and emission in the NIR-II regions show improved potential for <i>in vivo</i> sensing • Employ methods such as counter-SELEX for improved bioreceptor selectivity
Fluorometric FRET-Based Aptasensors	<ul style="list-style-type: none"> • Biocompatible, simple synthesis, fast response time 	<ul style="list-style-type: none"> • Cross-reactivity with target analogs • Absorption of the sensor signal by biological tissue (visible range) 	<ul style="list-style-type: none"> • Use monochiral SWCNTs to improve sensor response. Yet, the process to acquire pure SWCNTs is difficult and costly • Further optimize the sensor configuration
Near-Infrared Fluorescent SWCNTs-Based Aptasensors	<ul style="list-style-type: none"> • Advantageous structural and optical characteristics • Photostable fluorescence range making possible both <i>in vivo</i> and <i>in vitro</i> applications 	<ul style="list-style-type: none"> • Nonspecific interactions rather than sequence specificity observed • Biocompatibility not yet addressed 	<ul style="list-style-type: none"> • Develop strategies for improved delivery to target tissues
Near-Infrared Fluorescent UCNPs-Based Aptasensors	<ul style="list-style-type: none"> • High photostability, low autofluorescent background, and deep tissue penetration 	<ul style="list-style-type: none"> • Long-term toxicity still not addressed • Necessity to increase upconversion quantum yields for improved brightness 	<ul style="list-style-type: none"> • Use a cell-expressed, self-assembling RNA aptamer for improved biocompatibility
Fluorescent Aptasensors for Live Cell Imaging Neurotransmitter Release	<ul style="list-style-type: none"> • Real-time tracking of specific target molecules • High spatial resolution • Minimal cytotoxic effect to cells incubated with an aptamer-containing medium 	<ul style="list-style-type: none"> • <i>In vivo</i> applications require either crossing the blood-brain-barrier or inserting the aptasensor <i>via</i> invasive methods • Biocompatibility still not optimal 	

Declaration of Competing Interest

The authors declare that they have no competing interests that could have appeared to influence the work reported in this paper.

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