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Perspective

Levodopa: From Biological Significance to Continuous Monitoring

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Cite This: *ACS Sens.* 2024, 9, 3828−3839
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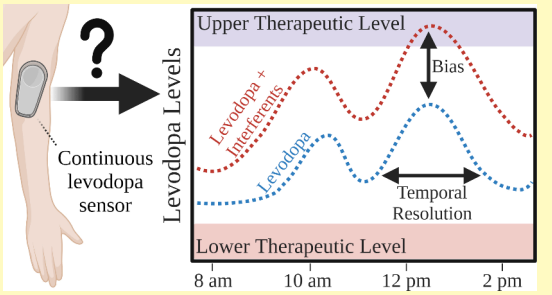
ABSTRACT: A continuous levodopa sensor can improve the quality of life for patients suffering with Parkinson's disease by enhancing levodopa titration and treatment effectiveness; however, its development is currently hindered by the absence of a specific levodopa molecular recognition element and limited insights into how real-time monitoring might affect clinical outcomes. This gap in research contributes to clinician uncertainty regarding the practical value of continuous levodopa monitoring data. This paper examines the current state of levodopa sensing and the inherent limitations in today's methods. Further, these challenges are described, including aspects such as interference from the metabolic pathway and adjunct medications, temporal resolution, and clinical questions, with a specific focus on a comprehensive selection of molecules, such as adjunct medications and structural isomers, as an interferent panel designed to assess and validate future levodopa sensors. We review insights and lessons from previously reported levodopa sensors and present a comparative analysis of potential molecular recognition elements, discussing their advantages and drawbacks.



KEYWORDS: *levodopa therapy, continuous levodopa monitoring, Parkinson's disease, biosensor development, molecular recognition elements, electrochemical detection, and sensor specificity*

Parkinson's disease (PD) is a neurodegenerative condition marked by the loss of dopaminergic neurons in the substantia nigra, leading to reduced dopamine production and symptoms like bradykinesia and tremors, collectively known as "parkinsonism".[1] In the US and Canada, PD affects approximately 500,000 individuals with an annual incidence of 86,000 and can progress at variable rates, typically over one to two decades before leading to severe disability.[2,3] Levodopa is the gold-standard treatment for PD, though not the only treatment available, with typical therapeutic concentrations ranging from approximately 400−1500 ng/mL (2−7.6 μM) in plasma to 150−250 ng/mL (0.76−1.26 μM) in cerebrospinal fluid.[4] Adjunctive medications such as catechol-O-methyl-transferase (COMT) and monoamine oxidase-B (MAO-B) inhibitors are generally used to enhance or prolong the effects of levodopa rather than as standalone therapies, while dopamine receptor agonists are less commonly used due to their lesser efficacy and greater side effects. Unlike dopamine, orally administered levodopa readily crosses the blood-brain barrier.[5] Once in the brain, levodopa is converted into dopamine by aromatic amino acid decarboxylase (AADC), where it can then be used normally in motor and nonmotor dopaminergic pathways and thus significantly improve quality of life by lessening PD symptoms, although it does not alter the progression of PD pathophysiology.[6] However, levodopa has a short *in vivo* serum half-life, ranging from 30 min to several hours, and is rapidly metabolized in the gut and peripheral tissues, reducing the amount that reaches the brain.[7] To increase its half-life, levodopa is often paired with peripheral AADC inhibitors such as carbidopa, which reduce side effects of levodopa while allowing increased central nervous system penetration of the drug.[8] A critical challenge of levodopa therapy is maintaining the correct dosage; too much can cause nausea, and dyskinesia, while too little can lead to reemergence of conventional PD symptoms, a state commonly referred to as "off-time". There is a tremendous need for real-time, continuous monitoring of levodopa in PD patients, both to improve current therapy and management of the disease, as well as to help develop future treatment options, such as the long-term goal of closed-loop levodopa therapy.[9] Continuous monitoring of levodopa will help patients and clinicians titrate therapy based on acute and chronic changes in levodopa metabolism, manage the narrowing of the therapeutic effective window, and understand interactions with other adjunct medications. Yet, despite such a need, there has been little progress on this front, mostly due to the lack of sensitive and specific molecular recognition elements and effective methods of transducing these interactions. This perspective will discusses these challenges by first delving into the biological importance of levodopa, exploring the critical metabolic pathways, and examining key adjunct medications. This

Received: March 14, 2024
Revised: June 21, 2024
Accepted: July 3, 2024
Published: July 24, 2024

https://doi.org/10.1021/acssensors.4c00602
ACS Sens. 2024, 9, 3828−3839

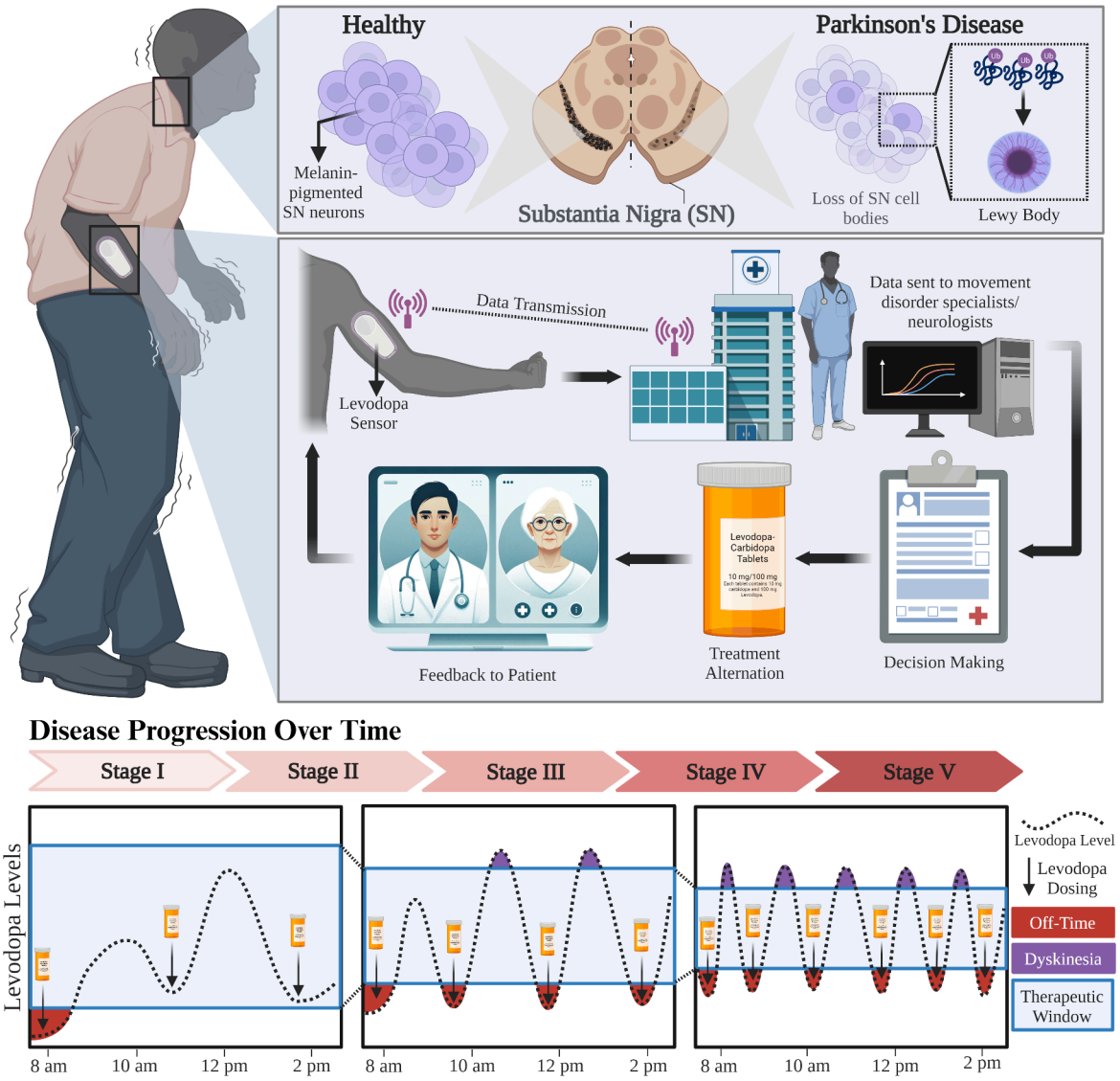


Figure 1. Proposed clinical pathway for a Parkinson's disease (PD) patient alongside a representation of PD progression across five stages. The top portion highlights the pathological progression of PD with the healthy brain showing melanin-pigmented substantia nigra (SN) neurons, while the PD-affected brain shows a loss of SN neurons and the presence of Lewy bodies. The middle portion shows a conceptual PD clinical management pathway employing a levodopa sensor which monitors and transmits levodopa levels to movement disorder specialists or neurologists who then analyze the data to inform treatment decisions and adjustments, with the goal of providing patient feedback. The lower portion shows the progression of PD from stages I to V, with the increase in "off-time" and levodopa-induced "dyskinesia" as PD progresses as the therapeutic window progressively narrows. Figure 1 was created with BioRender.com.

biological understanding is crucial for development and clinical translation a practical, continuous levodopa monitor. Thereafter, we discuss the current state of the art in levodopa sensing and what key challenges remain in the pathway of developing a continuous device. Finally, we close with some of the long-term challenges in the clinical adoption of a continuous sensor, including the gaps in compartment models, unknown pharmacokinetics between various tissues of interest, and temporal lag times between the metabolism of levodopa and clinically relevant symptoms/outcomes.

In the early stages of PD, levodopa is particularly effective due to a broad therapeutic window and a long duration of therapeutic effects, lasting far beyond its expected serum half-life. This effect has been described as a "buffering capacity", where the brain has a large capacity to store converted dopamine, using it as needed over time.[10,11] This buffering effect diminishes with progression of PD, leading to a narrowed therapeutic window and more frequent occurrences of both dyskinesia and off-time (Figure 1). These "motor fluctuations" necessitate adjustments to dopaminergic therapy including higher doses of levodopa, increased frequency of levodopa administration, and addition of adjunctive medications designed to prolong the effects of levodopa, slow the breakdown of levodopa and dopamine, and manage dopaminergic side effects.[12] Despite optimal dosing and use of adjunctive medications, levodopa use in advanced PD is frequently complicated by rapid onset of off-time, dose failures and partial failures, unpredictable off-time, peak-dose and diphasic dyskinesia and dystonia, as well as various neuropsychiatric side effects.[12] These challenges have precipitated

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ACS Sens. 2024, 9, 3828−3839

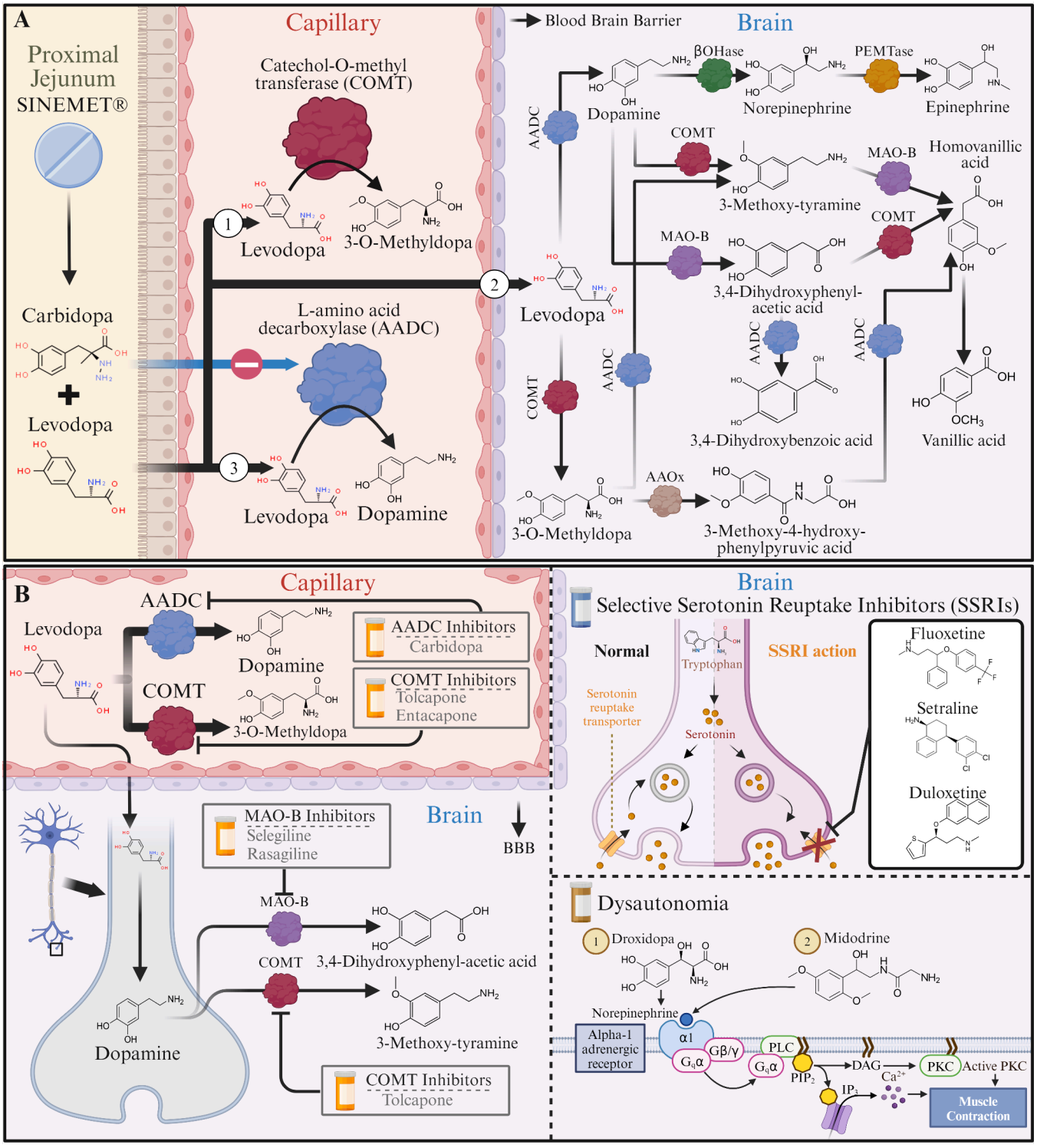

Figure 2. (A) Levodopa metabolic pathway and commonly administered (B) adjunct medication for PD treatment. Panel (A) outlines the absorption and metabolic conversion of orally administered Sinemet, a combined levodopa and carbidopa therapy, in the proximal jejunum, illustrating the metabolism of levodopa and its derivatives, detailing their transport across the jejunum, capillaries, and the blood-brain barrier. Panel (B) demonstrates the regulation of levodopa's metabolic pathway by AADC inhibitors like carbidopa and COMT inhibitors such as entacapone and tolcapone, both peripherally and within the brain. It also highlights the mechanism of action of selective serotonin reuptake inhibitors and medications for dysautonomia, conditions frequently associated with PD. Adapted from refs 16−22, 30, and 32−37. Figure 2 was created with BioRender.com.

the development of both intestinal and subcutaneous levodopa delivery systems, although the management of these systems may also be challenging in advanced PD patients with complex therapeutic needs.[13,14] This complexity of management options highlights the potential value of continuous levodopa monitoring. Analogous to continuous glucose monitoring (CGM) in diabetes mellitus, monitoring of levodopa levels in PD may potentially help clinicians more accurately titrate

https://doi.org/10.1021/acssensors.4c00602
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levodopa infusion therapies, adjust oral therapies, and accurately identify side effects related to levodopa peaks and troughs, as illustrated in Figure 1. However, no Food and Drug Administration (FDA)-approved biosensor for levodopa exists, mainly due to the lack of a stable, reversible biorecognition element, such as enzymes, aptamers, or antibodies, that can specifically bind to levodopa. Monitoring levodopa is inherently different from other continuous monitors such as CGM's, where glucose undergoes metabolic pathways to produce several metabolites that are not related to the structure being recognized by the biosensing molecule, thereby not causing any interferent signal. For example, compounds that could interfere with glucose measurement, such as maltose or xylose, are not found endogenously. Levodopa on the other hand, undergoes a vast metabolic pathway that liberates various metabolites structurally resembling levodopa, thereby potentially interfering with the sensor signal. Understanding these pathways and metabolites, as well as which exogenous adjunct medications may accompany levodopa is critical for both the development and commercial translation of a continuous levodopa monitor.

This perspective will discuss current levodopa detection methods, their limitations, and challenges in developing a continuous sensor, focusing on electrochemical detection, while excluding optical or mechanical detection, as the most viable for patient-centric applications. For an extensive review of academic research on levodopa detection, we refer readers to Crapnell et al.'s comprehensive analysis of the subject.[15]

BIOLOGICAL SIGNIFICANCE OF LEVODOPA

Levodopa Metabolic Pathway. Understanding levodopa's metabolic pathway and structure is key to identifying potential interfering molecules that may cause signal artifacts. As shown in Figure 2A, upon administration, levodopa can react with COMT, forming 3-O-methyldopa (1), or alternatively, can be decarboxylated by AADC to produce dopamine (3).[16] To minimize peripheral metabolism and increase levodopa's availability for central nervous system uptake, it is routinely coadministered with peripheral AADC inhibitors such as carbidopa.[8] As shown in Figure 2B, the further adjunctive use of COMT inhibitors, such as tolcapone and entacapone, and more recently, opicapone,[17] can reduce the breakdown of levodopa into 3- and 4-O-methyldopa in peripheral tissues.[18] Upon crossing the blood-brain barrier (2), levodopa is metabolized to 3-O-methyldopa by COMT or to dopamine by AADC.[18] After being converted to dopamine, it can then be transformed into norepinephrine by β-hydroxylase,[19] which in turn is converted to epinephrine by phenylethanolamine *N*-methyltransferase.[20] Dopamine can also be converted into 3-methoxytyramine by COMT or into 3,4-dihydroxyphenylacetic acid (DOPAC) by MAO-B.[21] Both metabolites may be further processed into homovanillic acid by MAO-B and COMT, with DOPAC also being converted into 3,4-dihydroxybenzoic acid.[30] Levodopa may also be converted into 3-O-methyldopa by COMT, which can then be converted into 3-methoxytyramine via AADC and eventually into homovanillic acid, or into 3-methoxy-4-hydroxyphenylpyruvic acid by amino acid oxidase, which is later reacted into homovanillic acid by additional enzymatic reactions.[22] The presence of 3-O-methyldopa and 4-O-methyldopa, which exhibit higher plasma concentrations ranging from 10 to 25 μg/mL—exceeding the 1 to 5 μg/mL concentration of levodopa—and possess a half-life of 15 h, may interfere with levodopa detection methods that are based on the direct oxidation of the 3,4-dihydroxyphenyl groups.[23] To the best of the authors' knowledge, the oxidation of 3-O-methyldopa by tyrosinase has not been evaluated, yet its structural resemblance to levodopa raises concerns over potential signal artifacts. As indicated in Supplementary Table 1, the effect of 3-O-methyldopa on detection methodologies has yet to be assessed in the literature.

Adjunct Medication. Another source of nonspecific interactions or direct oxidation of interfering compounds is the broad range of adjunct medications commonly prescribed to PD patients with levodopa (Figure 2B). Levodopa alone has relatively poor accessibility from blood to brain due to the high rate of peripheral tissue metabolism, as evidenced by studies showing that without adjuncts like carbidopa, only about 30% of an oral levodopa dose reaches the systemic circulation intact.[24] Carbidopa, structurally similar to levodopa except for an additional carbon and amine group, is almost always coadministered with levodopa to inhibit AADC, thereby increasing the available concentration of levodopa to cross the blood-brain barrier.[25] The inclusion of carbidopa led to a 50% reduction in the infusion rate necessary for levodopa to elicit a therapeutic response, effectively doubling the bioavailability of orally administered levodopa.[26] However, tyrosinase, being nonspecific, will interact with both levodopa and carbidopa, potentially resulting in artificially high signal readings. Furthermore, since carbidopa does not cross the blood-brain barrier, its presence could skew the perceived correlation between interstitial fluid (ISF)/plasma levodopa concentration and that in the cerebral spinal fluid. It should be noted, though, that carbidopa is typically dosed at much lower concentrations than levodopa, generally in ratios ranging from 4:1 to 10:1 (levodopa: carbidopa), which suggests that the expected signal artifact might be minimal.[27] Following oral medication, carbidopa levels in plasma vary from 0 to 80 ng/mL[28] and levodopa from 400 to 1500 ng/mL;[4] these levels can be higher following intestinal gel infusion, with carbidopa ranging from 0.1−0.4 μg/mL and levodopa from 1−5 μg/mL.[23] This scenario differs with other inhibitors like entacapone, which may be dosed at higher concentrations than levodopa, reaching plasma levels of 4−50 μg/mL.[29] Entacapone also shares structural similarities with levodopa, having a 3,4-dihydroxy phenalenamide structure, has been shown to increase levodopa bioavailability by 30−40% when administered alongside carbidopa across varying doses.[30]

PD often accompanies various comorbid states that require additional therapies. Depression and anxiety are prevalent in PD patients and are typically treated with selective serotonin reuptake inhibitors or serotonin-norepinephrine reuptake inhibitors such as fluoxetine, sertraline, and duloxetine.[31,32] Although these medications do not share a structural similarity with levodopa, they alter the concentrations of endogenous molecules like norepinephrine. Norepinephrine, bearing a structure like levodopa, may undergo oxidation by tyrosinase or direct oxidation, potentially impacting sensor readings. Dysautonomia is another common comorbidity in PD, often manifesting as autonomic nervous system degeneration and leading to symptoms like orthostatic hypotension, anhidrosis, and constipation.[33−35] Common treatments for dysautonomia include midodrine, an alpha-1-agonist, and droxidopa, a norepinephrine prodrug, both acting as sympathomimetics.[36,37] Droxidopa closely resembles levodopa in structure, with plasma concentrations reaching up to 4 μg/mL, like

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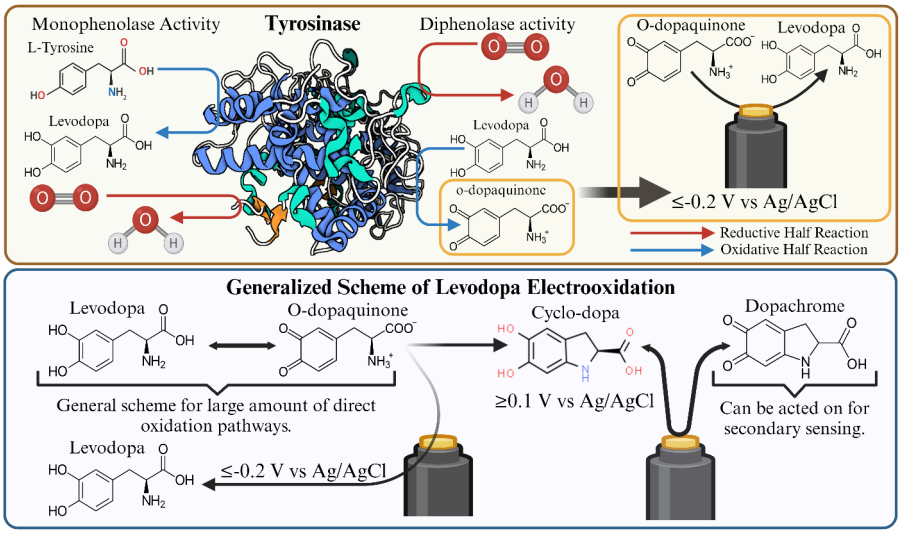

Figure 3. Mechanisms of levodopa sensing via tyrosinase-catalyzed and direct levodopa electrooxidation reactions. The top portion displays the mechanism of levodopa detection via tyrosinase, which exhibits monophenolase and diphenolase activities. Tyrosinase can catalyze the hydroxylation of L-tyrosine into levodopa which is paired with the reduction of oxygen to water. Subsequently, tyrosinase can then facilitate the oxidation of levodopa to o-dopaquinone, again reducing oxygen to water, which is acted on for secondary sensing. The bottom portion shows the generalized scheme for the direct electrooxidation of levodopa which can be oxidized to o-dopaquinone, which is then again reduced back to levodopa at potentials below −0.2 V vs a silver/silver chloride (Ag/AgCl) reference electrode. Alternatively, *o*-dopaquinone can form cyclo-dopa which at potentials greater than 0.1 V vs Ag/AgCl can be converted into dopachrome which can be acted on for secondary sensing. Figure 3 was created with BioRender.com.

levodopa. Given this structural similarity, particularly the 3,4-dihydroxyphenyl group, we anticipate signal artifacts in both tyrosinase-based and direct oxidation levodopa sensing methods.[37] These adjunct medications are summarized in Supplemental Table 2, including the physiological ranges in which they are commonly found.

CURRENT STATE AND LIMITATIONS OF CONTINUOUS MONITORING OF LEVODOPA

Currently, no clinically translatable levodopa sensors have been proposed that adequately meet the needs for PD patients and therapy. This shortfall primarily stems from three key challenges. First, there is a lack of specific molecular recognition elements specific to levodopa. Second, our understanding of levodopa's pharmacokinetics across various bodily compartments, such as ISF, plasma, and cerebrospinal fluid, remains limited. Finally, even with potential advancements in understanding this pharmacokinetics, there is an uncertainty regarding the clinical value of this data to enhance therapeutic outcomes for patients. To address these questions, and uncertainties, there must first be a reliable, continuous levodopa sensor available for clinician and patient use. To date, there currently are two pathways for the detection of levodopa using electrochemistry.

Detection Methods for Levodopa Sensing. Electrochemistry has been the primary approach by many groups for detecting levodopa, due to its straightforward design, potential for electronic miniaturization, and capability to enable various detection modalities. Common electrochemical techniques include chronoamperometry, cyclic voltammetry (CV), differential pulse voltammetry (DPV), square wave voltammetry (SWV), and electrochemical impedance spectroscopy (EIS). Chronoamperometry involves applying a constant voltage to the working electrode relative to a standard reference electrode, commonly silver/silver chloride (Ag/AgCl), where the resultant current measurement is directly dependent on the substrate, or some secondary byproduct.[38] This approach is commonly used for detection of levodopa by either direct oxidation, resulting in an anodic current, or by reducing dopaquinone, resulting in a cathodic current. Voltametric methods, such as in CV, involve sweeping a voltage between two potentials at a specific scan rate, measuring the resulting current over each sweep.[39] CV adds greater specificity by being able to measure multiple voltages in a single sweep. Other voltametric methods include DPV and SWV where the voltage is swept over a defined range, but each step alternates between anodic versus cathodic driven current. Generally, the Δ (difference) between anodic and cathodic current is taken, helping mitigate nonfaradaic or capacitive current from the signal, leading to greater sensitivity than CV or chronoamperometry.[40] During EIS, an alternating voltage is applied to the working electrode at a specific potential with an overlaid sinusoidal voltage. This sinusoidal potential will be swept over a broad range of frequencies, measuring the impedance and capacitance of the system. Commonly analyzed using a Randles circuit, which models the electrode as a resistor, in series with a parallel capacitive element and a resistor.[41,42] While EIS is incredibly sensitive, and produces large swaths of data, there are challenges in deciphering the signal from the noise, leading many groups to focus on techniques such as CV, SWV, or chronoamperometry when trying to translate a sensor from benchtop to practice. These approaches are dependent on the molecular recognition element used, with enzymes for example tending to fit well with chronoamperometry, whereas aptamers are commonly used in conjunction with SWV.

Oxidation of Levodopa Using Tyrosinase. Tyrosinase (EC 1.14.18.1), also known as polyphenoloxidase, a copper containing enzyme, being a mushroom-derived tyrosinase from *Agaricus bisporus*, is the most used enzyme for levodopa detection due its cost effectiveness and high homology to human variants.[43−46] This enzyme has two copper binding

https://doi.org/10.1021/acssensors.4c00602
ACS Sens. 2024, 9, 3828−3839

sites that facilitates two reactions, with the first reaction being the hydroxylation of monophenols into o-diphenols (e.g., L-tyrosine to levodopa) and the second reaction being the oxidation of o-diphenols to o-quinones (e.g., levodopa to o-dopaquinone).[47] Both subsequent reactions involve oxygen as an electron acceptor, which is then converted into water. Figure 3 highlights the typical reaction associated with levodopa sensors via tyrosinase. Numerous groups have reported the development of levodopa sensors that measure o-dopaquinone, the product of the reaction between levodopa and tyrosinase, at potentials of 0.1 V vs Ag/AgCl or higher.[44] For example, Goud et. al utilized a multimodal microneedle platform to measure levodopa in an artificial ISF solution with a detection limit of 0.25 μM; however, the sensor was not tested with any molecules from the levodopa metabolic pathway or with adjunct medications other than carbidopa (see Supplemental Table 1).[45] Similarly, Moon et. al developed a levodopa sensor capable of detecting sweat levodopa concentrations with a detection limit of 300 nM, and although it was tested against various interferents, only dopamine from the levodopa metabolic pathway was included, which is present in physiologically low picomolar concentration (refer to Supplemental Table 1 and 2).[44]

Direct Oxidation of Levodopa. Levodopa can undergo a quasi-reversible electrochemical reaction, being oxidized into dopaquinone at a potential of 0.34 V and then reduced back to levodopa at 0.19 V versus Ag/AgCl using a graphite carbon electrode as shown in Figure 3.[48] In neutral buffers, a slower secondary reaction occurs, converting dopaquinone into cyclodopa, which can then be further oxidized and reduced at potentials of 0.09 V and −0.12 V versus Ag/AgCl, respectively.[48] Many groups have developed levodopa sensors based on direct oxidation, using a variety of electrode surfaces.[49−56] Direct oxidation methods show a key advantage in terms of sensor stability, as they are not limited by using biological materials such as enzymes or antibodies, allowing for long-term stability with repeatable measurements over time. While this advantage is enticing, there is an inherent weakness: the lack of specificity. Solid-state surfaces, such as graphite or single-walled carbon nanotubes, lack features that control the specificity of the signal. This limitation has prevented the translation of these sensors from benchtop experiments to practical *in vivo* use. For example, Mazloum-Ardakani et al. utilized hydroquinone and carbon nanotubes to simultaneously measure levodopa, carbidopa, and tryptophan with a detection limit of 0.094 μM (see Supplemental Table 1).[56] Despite testing a broad panel of 39 interferents, only dopamine, epinephrine, and norepinephrine from the levodopa metabolic pathway were included, all of which have physiological concentrations significantly lower than that of levodopa (see Supplemental Table 2). Similarly, Leite et al. employed a modified pyrolytic graphite electrode for levodopa detection with a detection limit of 0.86 μM.[55] Although this study tested additional adjunct medications such as carbidopa and benserazide, it did not account for other key levodopa metabolites like 3-O-methyldopa which is present at higher physiological concentrations (refer to Supplemental Tables 1 and 2).

Levodopa Detection Across Various Bodily Compartments. There are various fluid compartments in the body where continuous sensors are used. This includes sweat, saliva, ISF, plasma, and tear fluid. Currently, most groups engaged in levodopa sensing either use sweat, plasma, or ISF as the sampling matrix. The major challenges with continuous monitoring through sweat include the confounding interaction between current and past samples and understanding the compartmental relationships between sweat and plasma. Sweat sensing is advantageous in being inherently noninvasive and painless for patients.[57] ISF is the most common compartment used to interrogate analytes as demonstrated by CGM's on the market today. ISF has a key advantage in that the relationship between ISF and blood plasma is simple and direct.[58] In addition, changes in systemic blood plasma tend to reflect quickly in the ISF, usually within 10 min, as seen in CGMs.[59] One challenge with ISF is the requirements of breaking skin, which can lead to a low probability of infection. In addition, the sensor must remain stable for 10−14-day use, which is the current standard following CGM system, requiring large amounts of optimization prior to being translatable. Levodopa has undefined partitioning, time lags, and pharmacokinetics between ISF, sweat, and blood plasma. This adds additional work to be done in the development of a continuous levodopa sensor in either ISF or sweat as a biofluid.

DETECTION STRATEGIES OF LEVODOPA

An Ideal Molecular Recognition Element for the Monitoring of Levodopa. The development of a continuous or single use disposable type levodopa sensor has garnered significant interest, leading many groups to recently publish novel methods for levodopa quantification (Supplemental Table 1). Despite substantial progress, most approaches rely on either tyrosinase or direct oxidation, both of which have inherent specificity limitations. To address this, the creation of levodopa-specific recognition elements should be pursued. In engineering an 'ideal' recognition element for levodopa, certain parameters, such as reversibility, specificity, and stability of the element, must be considered. Crucial is achieving specificity to levodopa while minimizing interactions with isostructural chemicals. To date, no enzyme has demonstrated sufficient specificity toward levodopa for *in vivo* use. Therefore, several groups have developed innovative solutions to mitigate this nonspecific signal. For example, Goud et al. employed both chronoamperometric and square wave voltammetry in a dual system to account for the nonspecific interaction between tyrosinase and carbidopa. While this approach showed promise, it is nonideal method for the detection of levodopa due to the complexity of analysis, and the fact that both square wave voltammetry for direct oxidation and tyrosinase have limited specificity.[45]

In addition, for the sensor to function continuously, the recognition element should be reversible, capable of quantifying both increases and decreases in levodopa concentration. Levodopa has a therapeutic half-life of 2−3 h when dosed adjunctively with carbidopa.[60] Achieving reversibility is straightforward with enzymes, which are inherently regenerated, either through direct oxidation via oxygen or by applying an overpotential across a working electrode which reoxidizes the enzyme.[61,62] However, other molecular recognition elements such as aptamers, antibodies, or molecularly imprinted polymers have limited reversibility. This limitation may impact their ability to measure rapid changes, as their binding to the substrate is based on the equilibrium between levodopa concentration and the number of available binding sites. Several groups have developed continuous sensors for small molecules such as vancomycin and glucose using both aptamers and molecularly imprinted polymers.[63−68] Despite

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ACS Sens. 2024, 9, 3828−3839

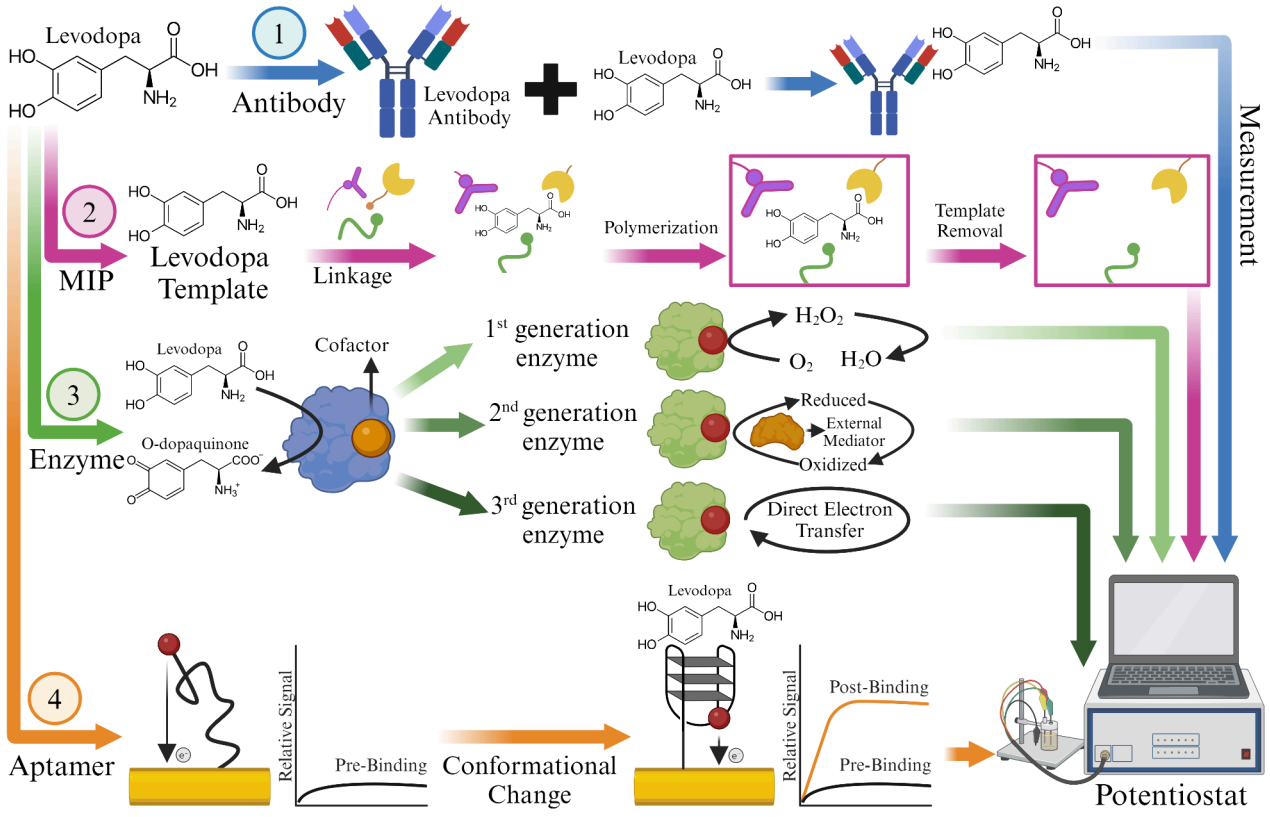

Figure 4. Representative pathways for levodopa detection via (1) antibody, (2) molecularly imprinted polymer (MIP), (3) enzymatic, and (4) aptamer-based mechanisms. The first section demonstrates how levodopa interacts with the variable region of a levodopa antibody through noncovalent bonds, allowing for detection. The second section depicts the MIP approach where linkers polymerize around a levodopa template; following template removal, the imprint can be used for subsequent detection. The third section outlines the enzymatic detection strategy where the enzyme produces o-dopaquinone, which can be sensed through three distinct mechanisms. The fourth section highlights how an aptamer would bind with levodopa, with the conformational change allowing for an increase in relative signal compared to the prebinding signal. Figure 4 was created with BioRender.com.

these few successful examples, a continuous sensor employing a nonenzymatic, affinity-based biosensing molecule has yet to be achieved. To the best of the authors' knowledge, there have been no reported instances of levodopa sensing using aptamers or antibodies. While levodopa-specific antibodies or ELISA are commercially available, their direct application for continuous levodopa sensors has not yet been achieved. Considering the superb features of aptamers, particularly their structural change upon target recognition, the development of aptamers specifically recognizing levodopa presents an exciting direction for future research. Although a commercial antibody for levodopa is available, the authors are not aware of any use for biosensing applications. Therefore, an enzyme-based approach would be more ideal for the proposed sensor when compared to other conventional recognition elements as described in Figure 4. There are three general enzymatic mechanisms described for electrochemical sensors, termed first, second, and third generation. These categories are based on the need and type of electron transfer pathway between the enzyme and electrode surface. First-generation enzymes require oxygen to facilitate electron transport to the electrode. Second-generation enzymes use synthetic electron acceptors (or mediators) for electron transport to the electrode. Third-generation enzymes are inherently capable of communicating with the electrode surface, eliminating the need for either oxygen or any mediator. Each of these different generation type enzymes has trade-offs, such as first-generation enzymes being influenced by oxygen concentrations, which may fluctuate during *in vivo* settings. Due to these various limitations, either second-generation or third-generation type enzymes are more ideal for stable, reversible, and specific *in vivo* monitoring of levodopa.[69−71] Currently, there is no enzyme that exhibits high specificity to levodopa, which is a significant limitation in the progress of developing a real-time monitor.

What Interference Should Be Considered When Evaluating a Levodopa Sensor? One of the most critical metrics for the successful translation of a biosensor from benchtop to *in vivo* application is the various ways in which the signal of interest can be skewed by artifacts found in the testing environment. These signal artifacts can be categorized in several ways and may vary depending on the sensor type, its application, and the environment where the sensor is employed. To define and categorize the types of artifacts that may impact a molecular recognition element for real-time, *in vivo* levodopa monitoring, it is advised to use the Clinical Laboratory Standards Institute (CLSI) EP07 'Interference Testing in Clinical Chemistry'. CLSI EP07 outlines various possible types of artifacts, including 1) Chemical artifacts, 2) Detection artifacts, 3) Physical artifacts, 4) Enzyme inhibition artifacts, 5) Nonselectivity or Cross-reactivity, 6) Water displacement, and 7) Additive artifacts. Each of these categories can introduce noise or reduce signal fidelity, posing

https://doi.org/10.1021/acssensors.4c00602
ACS Sens. 2024, 9, 3828−3839

Table 1. EP07 Guidelines Defining the Various Type of Interfering Mechanisms Which May Convolute Bias in the Measured Signal

Interference	Definition
Chemical artifacts	The interferent may suppress the reaction by competing for reagents, inhibiting indicator reactions, or altering the form of the measurand through complexation or precipitation.
Detection artifacts	The interferent might have properties similar to the measurand that are detected and measured, such as fluorescence, color, light scattering, elution potion or electrode response.
Physical artifacts	The interferent might alter a physical property of the specimen matrix, such as viscosity, surface tension, turbidity, or ionic strength, causing an apparent change in the concentration of the measurand.
Enzyme inhibition artifacts	The interferent might alter the activity of an enzyme (measurand or reagent) by sequestering metal activators, binding to the catalytic site, or oxidizing essential sulfhydryl groups. The interferent may also compete for a key substrate in an enzyme-based measurement procedure.
Nonselectivity or Cross-reactivity	The interferent might react in the same manner as the measurand. Although some experts differentiate nonselective from interference, although the practical effects are the same to the laboratory. An interferent structurally similar to an antigen may "cross-react" with the antibody in an immunochemical measurement procedure, which is a form of nonselectivity.
Water displacement	Nonaqueous substances affect activity-based measurements by displacing aqueous plasma volume.
Additive artifacts	An interferent or additional measurand might come from cells or from intravenous fluids.

challenges for accurate sensor readings.[72] Table 1 describes each of the interfering methods as described by the EP07 guidelines:

The development of an *in vivo*, real-time levodopa monitoring system requires an understanding of how various artifacts affect signal fidelity. Among the seven artifact types defined by the CLSI EP07 guidelines (Table 1), non-selectivity/cross-reactivity, detection artifacts, and physical artifacts are particularly relevant for *in vivo* electrochemical sensors. Given their potential to influence sensor readings, a clear understanding of endogenous and exogenous interfering compounds is essential to ensure signal reliability. This interference can be categorized into two general types: 1) substrates that nonspecifically or cross-react with a molecular recognition element, like tyrosinase or glucose oxidase, thereby biasing the signal, and 2) electroactive substrates that bypass the recognition element and directly interact with the working electrode, being either oxidized or reduced. Additionally, physical artifacts such as fibrous encapsulation can affect the diffusion of key molecules such as oxygen which can alter the measured electrode signal. If using chronoamperometric sensors, which relies on the diffusion limited current to quantify a substrate, this encapsulation process can impact both the absolute signal, as well as the time lag between the sensor and physiological changes.[62]

Detection artifacts are those which may cause signal artifact by sharing similar properties to the substrate which is transduced at the electrode surface. A common example is acetaminophen, which may be oxidized by gold at potentials greater than 0.4 V, leading to signal artifacts in many chronoamperometric sensors. These interactions can either amplify or diminish the apparent signal, depending on their reaction direction (oxidation vs reduction). For certain sensors, such as continuous glucose monitors and blood glucose monitors, the FDA mandates testing against a defined set of interfering compounds present in the clinically relevant samples found during regular use of the device.[73] However, there is no equivalent standard for levodopa. As indicated in Supplemental Table 1, a broad range of interfering compounds have been evaluated for levodopa sensors. These tested compounds fit several categories of interest described in the CLSI EP07 guidelines, including carbidopa (structurally similar to levodopa and commonly coadministered), which may be categorized in the nonselective and/or cross-reactivity category, and ascorbic acid, which is a detection artifact. Although a relatively large array of interfering compounds has been investigated, several practical interfering substrates have not been tested. Additionally, some compounds that are not expected to interfere, or are not present *in vivo* in appreciable concentrations, have been tested (Supplemental Table 1). There are many common electroactive substrates either found endogenously in the body or taken exogenously, which will affect electrochemical sensors. Probably the most frequently found and tested for is the impact of acetaminophen (the primary drug found in Tylenol), which can be oxidized at potentials ranging from 0.15−0.65 V vs Ag/AgCl depending on the pH of the buffer used.[74] Supplemental Table 2 gives an initial list of interfering substrates and their physiological concentrations that should be tested when developing a levodopa sensor based on a novel biomolecular recognition element while using an electrochemical transduction method. This table includes common endogenous and exogenous substrates found in the levodopa metabolic pathway, adjunct medication, and several general electroactive interferences that may cause detection artifacts in continuous sensors.

Additionally, large gaps exist in understanding the dynamics between various adjunct medications and their impact on levodopa pharmacokinetics in real-time. Real-time measurement of these pharmaceuticals face similar challenges to those of levodopa, including the lack of a molecular recognition element that displays adequate sensitivity and specificity, yet is still capable of producing signals within a temporally significant time frame. However, adjunct medications do not provide direct antiparkinsonian effects but rather modify the metabolism, absorption, and or enhance the tolerability of levodopa. Therefore, while the potential benefits and of measuring both levodopa and its respective adjunct medications using multiparametric sensors remain uncertain, the ability to concurrently measure levodopa alongside key adjunct medications could significantly enhance patient-centric therapy or precision medication that is tailored to individual patient requirements.

Device Form Factor. There are many suitable form factors that a sensor can take, ranging from wearable microneedle arrays to full implants, each having unique challenges and limitations. In PD, the most important information regarding levodopa concentrations resides in the brain. While it would be ideal to measure the real-time levodopa within the basal ganglia of the brain, there are several practical hindrances. First, intracranial brain surgery is significantly more invasive

https://doi.org/10.1021/acssensors.4c00602
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than measuring in ISF or sweat, and while these procedures are already performed for treatments such as deep brain stimulation, these devices are designed to last for 15 years or more, minimizing the risk of repeated surgeries.[75] Ideally, an equivalent biosensor could be inserted with the system, but no recognition element has yet demonstrated such stability. Due to this, we have focused on form factors that are being demonstrated in today's market, including microneedle arrays, subcutaneous sensors, and implants within the ISF region.

Microneedles pierce the upper layer of the dermis, allowing for sampling of ISF with minimal pain and supporting multimodal detection capabilities through the integration of several electrodes.[45] While there may be challenges in scaling up manufacturing for widespread clinical use, microneedles are well-poised for future biosensing applications due to their versatility and minimal invasiveness.[63,76] Current limitations in the practical translation of microneedles include ensuring consistent adhesion and pressure over the entire wear and mitigating the influence from motion artifacts. Most commercially available CGM systems use a subcutaneous formfactor to sample ISF by penetrating beyond the upper dermis.[62] This design allows for extended usage over several days with minimal discomfort, making it an extremely viable model to adapt for levodopa detection if levodopa ISF is a clinically significant biofluid for monitoring levodopa levels. The final form factor is implantable sensors, which are placed deeper into the dermis and are suited for long-term monitoring. Currently there is a CGM (Eversense, Senseonics) that is implanted for up to 6 months in the upper arm of patients.[77] The implantation process is generally associated with minimal side effects and requires less maintenance over extended periods, making this approach particularly suitable for patients who prefer reduced treatment maintenance and monitoring. Therefore, each of these proposed form factors can be effectively utilized for continuous detection, with the choice largely depending on the biofluid being sampled and the specific needs and circumstances of the patient.

Challenges in Practical Application of Levodopa Sensing. Real-time, continuous monitoring of levodopa has been hypothesized to lead to better patient outcomes and improved quality of life by enabling tighter control of dosing, thereby mitigating symptoms.[9] While this hypothesis is exciting, it has not yet been tested or validated due to the absence of any real-time levodopa sensor available for patient use, and several unknowns must be evaluated to validate this hypothesis. The relationship between ISF levodopa and plasma levodopa remains unknown, as does the correlation between ISF levodopa levels and the concentration of levodopa in the substantia nigra and putamen, which are critical areas for dopaminergic activity in PD. In a similar vein, CGMs measure ISF glucose levels, which correlate well with the physiologically relevant plasma glucose concentrations, benefiting from noninvasive monitoring. In contrast, for PD, the physiologically relevant concentration is CSF levodopa. Yet, the relationship between CSF, plasma, and ISF levodopa levels remains unknown due to the absence of a continuous levodopa sensor, complicating our understanding of levodopa's real-time pharmacokinetics. If future findings reveal significant time lags between CSF and plasma or ISF levodopa levels, such delays could significantly alter the utility and interpretative value of continuous levodopa monitoring data. However, this potential issue will remain speculative until a continuous levodopa sensor is developed. Understanding these pharmacokinetics and compartmental distribution is critical for the successful development of a clinically relevant levodopa sensor. Factors such as time lags or partition coefficients between bodily compartments will significantly influence the calibration and application of these proposed sensors. An additional challenge in applying a levodopa monitor is the neurodegenerative nature of PD. Over time, the efficacy of levodopa diminishes due to the reduced capacity to store and buffer converted dopamine, necessitating more frequent dosing. This change will affect the relationship between ISF levodopa and bioavailable levodopa in the substantia nigra for conversion into dopamine, potentially altering calibration requirements or the time lag of the sensor.

CONCLUSION

Real-time, continuous monitoring of levodopa is anticipated to enhance patient therapy by enabling precision medicine through dosing based on quantitative feedback. This, coupled with advancements in levodopa therapy, such as infusion pumps and water-soluble levodopa formulations, is progressively improving the treatment landscape for Parkinson's disease. These challenges, however, open the door to numerous opportunities in the innovative and creative development of molecular recognition elements that can be utilized for continuous levodopa sensing.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssensors.4c00602.

> Summary tables of (1) levodopa sensor approaches, including enzymatic versus direct oxidation mechanisms, measurement techniques, test environments, and interference evaluations; and (2) list of interfering substrates with their respective physiological plasma concentration and half-life (PDF)

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https://doi.org/10.1021/acssensors.4c00602
ACS Sens. 2024, 9, 3828−3839

Author Contributions

Conceptualization: K.B., D.P., J.Y., and K.S. Investigation: K.B., D.P., J.Y., and K.S. Visualization: K.B., D.P., J.Y., and K.S. Supervision: K.S. Writing-original draft: K.B., D.P., J.Y., and K.S. Writing-review and editing: K.B., D.P., J.Y., and K.S.

Notes

The authors declare the following competing financial interest(s): K.B., D.P., and K.S. are inventors of three patents related to this work filed by the University of North Carolina and Chapel Hill. (1) U.S. Provisional Patent Application No. 63/505,267, filed May 31, 2023, (2) U.S. Provisional Patent Application No. 63/505,267, filed May 31, 2023, and (3) U.S. Provisional Patent Application No. 63/505,259, filed May 31, 2023. The authors declare no other competing interests.

■ ACKNOWLEDGMENTS

The Joint Program of Biomedical Engineering at University of North Carolina and Chapel Hill supported this work. Additionally, Figures 1–4 and the table of contents graphic were created with BioRender.com. DALL-E was utilized for a portion of Figure 1.

■ ABBREVIATIONS

PD, Parkinson's disease; FDA, U.S. Food and Drug Administration; SN, Substantia nigra; Ag/AgCl, Silver/silver chloride reference electrode; AADC, Aromatic amino acid decarboxylase; PEMTase, Phenylethanolamine *N*-methyltransferase; CLSI, Clinical Laboratory Standards Institute; AAOx, Amino acid oxidase; COMT, Catechol-*O*-methyltransferase; βOHase, β-hydroxylase; MAO-B, Monoamine oxidase-B; DOPAC, 3,4-Dihydroxyphenylacetic acid; ISF, Interstitial fluid; CGMs, Continuous glucose monitors; CV, Cyclic voltammetry; DPV, Differential pulse voltammetry; SWV, Square wave voltammetry; EIS, Electrical impedance spectroscopy

■ REFERENCES

(1) Damier, P.; Hirsch, E. C.; Agid, Y.; Graybiel, A. M. The Substantia Nigra of the Human Brain. II. Patterns of Loss of Dopamine-Containing Neurons in Parkinson's Disease. *Brain J. Neurol.* **1999**, *122* (Pt 8), 1437−1448.

(2) Samii, A.; Nutt, J. G.; Ransom, B. R. Parkinson's Disease. *Lancet London Engl.* **2004**, *363* (9423), 1783−1793.

(3) Hawkes, C. H.; Del Tredici, K.; Braak, H. A Timeline for Parkinson's Disease. *Parkinsonism Relat. Disord.* **2010**, *16* (2), 79−84.

(4) Olanow, C. W.; Gauger, L. L.; Cedarbaum, J. M. Temporal Relationships between Plasma and Cerebrospinal Fluid Pharmacokinetics of Levodopa and Clinical Effect in Parkinson's Disease. *Ann. Neurol.* **1991**, *29* (5), 556−559.

(5) Wade, L. A.; Katzman, R. SYNTHETIC AMINO ACIDS AND THE NATURE OF L-DOPA TRANSPORT AT THE BLOOD-BRAIN BARRIER. *J. Neurochem.* **1975**, *25* (6), 837−842.

(6) LeWitt, P. A. Levodopa Therapy for Parkinson's Disease: Pharmacokinetics and Pharmacodynamics. *Mov. Disord. Off. J. Mov. Disord. Soc.* **2015**, *30* (1), 64−72.

(7) Dunner, D. L.; Keith, H.; Brodie, H.; Goodwin, F. K. Plasma DOPA Response to Levodopa Administration in Man: Effects of a Peripheral Decarboxylase Inhibitor. *Clin. Pharmacol. Ther.* **1971**, *12* (2), 212−217.

(8) Contin, M.; Martinelli, P. Pharmacokinetics of Levodopa. *J. Neurol.* **2010**, *257* (Suppl2), 253−261.

(9) Teymourian, H.; Tehrani, F.; Longardner, K.; Mahato, K.; Podhajny, T.; Moon, J.-M.; Kotagiri, Y. G.; Sempionatto, J. R.; Litvan, I.; Wang, J. Closing the Loop for Patients with Parkinson Disease: Where Are We? *Nat. Rev. Neurol.* **2022**, *18* (8), 497−507.

(10) Marsden, C. D.; Parkes, J. D. On-off" Effects in Patients with Parkinson's Disease on Chronic Levodopa Therapy. *Lancet London Engl.* **1976**, *307* (7954), 292−296.

(11) Black, K. J.; Acevedo, H. K.; Koller, J. M. Dopamine Buffering Capacity Imaging: A Pharmacodynamic fMRI Method for Staging Parkinson Disease. *Front. Neurol.* **2020**, *11*, 370.

(12) Fox, S. H.; Katzenschlager, R.; Lim, S.-Y.; Barton, B.; de Bie, R. M. A.; Seppi, K.; Coelho, M.; Sampaio, C. Movement Disorder Society Evidence-Based Medicine Committee. International Parkinson and Movement Disorder Society Evidence-Based Medicine Review: Update on Treatments for the Motor Symptoms of Parkinson's Disease. *Mov. Disord. Off. J. Mov. Disord. Soc.* **2018**, *33* (8), 1248−1266.

(13) Tsunemi, T.; Oyama, G.; Saiki, S.; Hatano, T.; Fukae, J.; Shimo, Y.; Hattori, N. Intrajejunal Infusion of Levodopa/Carbidopa for Advanced Parkinson's Disease: A Systematic Review. *Mov. Disord. Off. J. Mov. Disord. Soc.* **2021**, *36* (8), 1759−1771.

(14) Rosebraugh, M.; Liu, W.; Neenan, M.; Facheris, M. F. Foslevodopa/Foscarbidopa Is Well Tolerated and Maintains Stable Levodopa and Carbidopa Exposure Following Subcutaneous Infusion. *J. Park. Dis.* **2021**, *11* (4), 1695−1702.

(15) Crapnell, R. D.; Banks, C. E. Electroanalytical Overview: The Determination of Levodopa (L-DOPA). *ACS Meas. Sci. Au* **2023**, *3* (2), 84−97.

(16) van Kessel, S. P.; El Aidy, S. Contributions of Gut Bacteria and Diet to Drug Pharmacokinetics in the Treatment of Parkinson's Disease. *Front. Neurol.* **2019**, *10*, 1087.

(17) Ferreira, J. J.; Poewe, W.; Rascol, O.; Stocchi, F.; Antonini, A.; Moreira, J.; Guimarães, B.; Rocha, J.-F.; Soares-da-Silva, P. Effect of Opicapone on Levodopa Pharmacokinetics in Patients with Fluctuating Parkinson's Disease. *Mov. Disord. Off. J. Mov. Disord. Soc.* **2022**, *37* (11), 2272−2283.

(18) Rivest, J.; Barclay, C. L.; Suchowersky, O. COMT Inhibitors in Parkinson's Disease. *Can. J. Neurol. Sci. J. Can. Sci. Neurol.* **1999**, *26* (Suppl 2), S34−38.

(19) Rahman, Md. K.; Rahman, F.; Rahman, T.; Kato, T. Dopamine-β-Hydroxylase (DBH), Its Cofactors and Other Biochemical Parameters in the Serum of Neurological Patients in Bangladesh. *Int. J. Biomed. Sci. IJBS* **2009**, *5* (4), 395−401.

(20) Daubner, S. C.; Le, T.; Wang, S. Tyrosine Hydroxylase and Regulation of Dopamine Synthesis. *Arch. Biochem. Biophys.* **2011**, *508* (1), 1−12.

(21) Muñoz, P.; Huenchuguala, S.; Paris, I.; Segura-Aguilar, J. Dopamine Oxidation and Autophagy. *Park. Dis.* **2012**, *2012*, 920953.

(22) Deleu, D.; Northway, M. G.; Hanssens, Y. Clinical Pharmacokinetic and Pharmacodynamic Properties of Drugs Used in the Treatment of Parkinson??S Disease. *Clin. Pharmacokinet.* **2002**, *41* (4), 261−309.

(23) Nyholm, D.; Odin, P.; Johansson, A.; Chatamra, K.; Locke, C.; Dutta, S.; Othman, A. A. Pharmacokinetics of Levodopa, Carbidopa, and 3-O-Methyldopa Following 16-h Jejunal Infusion of Levodopa-Carbidopa Intestinal Gel in Advanced Parkinson's Disease Patients. *AAPS J.* **2013**, *15* (2), 316−323.

(24) Khor, S.-P.; Hsu, A. The Pharmacokinetics and Pharmacodynamics of Levodopa in the Treatment of Parkinson's Disease. *Curr. Clin. Pharmacol.* **2007**, *2* (3), 234−243.

(25) Durso, R.; Evans, J. E.; Josephs, E.; Szabo, G.; Evans, B.; Fernandez, H. H.; Browne, T. R. Variable Absorption of Carbidopa Affects Both Peripheral and Central Levodopa Metabolism. *J. Clin. Pharmacol.* **2000**, *40* (8), 854−860.

(26) Nutt, J. G.; Woodward, W. R.; Anderson, J. L. The Effect of Carbidopa on the Pharmacokinetics of Intravenously Administered Levodopa: The Mechanism of Action in the Treatment of Parkinsonism. *Ann. Neurol.* **1985**, *18* (5), 537−543.

(27) Kaakkola, S.; Männistö, P. T.; Nissinen, E.; Vuorela, A.; Mäntylä, R. The Effect of an Increased Ratio of Carbidopa to Levodopa on the Pharmacokinetics of Levodopa. *Acta Neurol. Scand.* **1985**, *72* (4), 385−391.

https://doi.org/10.1021/acssensors.4c00602
ACS Sens. 2024, 9, 3828−3839

(28) Benetello, P.; Furlanut, M.; Zara, G.; Baraldo, M.; Hassan, E. Plasma Levels of Levodopa and Its Main Metabolites in Parkinsonian Patients after Conventional and Controlled-Release Levodopa-Carbidopa Associations. *Eur. Neurol.* **2004**, *33* (1), 69−73.
(29) Habet, S. Clinical Pharmacology of Entacapone (Comtan) From the FDA Reviewer. *Int. J. Neuropsychopharmacol.* **2022**, *25* (7), 567−575.
(30) Heikkinen, H.; Varhe, A.; Laine, T.; Puttonen, J.; Kela, M.; Kaakkola, S.; Reinikainen, K. Entacapone Improves the Availability of L-Dopa in Plasma by Decreasing Its Peripheral Metabolism Independent of l-Dopa/Carbidopa Dose. *Br. J. Clin. Pharmacol.* **2002**, *54* (4), 363−371.
(31) Takahashi, M.; Tabu, H.; Ozaki, A.; Hamano, T.; Takeshima, T. REBORN study group. Antidepressants for Depression, Apathy, and Gait Instability in Parkinson's Disease: A Multicenter Randomized Study. *Int. Med. Tokyo Jpn.* **2019**, *58* (3), 361−368.
(32) Marino, S.; Sessa, E.; Di Lorenzo, G.; Digangi, G.; Alagna, A.; Bramanti, P.; Di Bella, P. Sertraline in the Treatment of Depressive Disorders in Patients with Parkinson's Disease. *Neurol. Sci. Off. J. Ital. Neurol. Soc. Ital. Soc. Clin. Neurophysiol.* **2008**, *29* (6), 391−395.
(33) Benarroch, E. E. The Clinical Approach to Autonomic Failure in Neurological Disorders. *Nat. Rev. Neurol.* **2014**, *10* (7), 396−407.
(34) Goldstein, D. S. Dysautonomia in Parkinson's Disease: Neurocardiological Abnormalities. *Lancet Neurol.* **2003**, *2* (11), 669−676.
(35) Yoo, S.-W.; Kim, J.-S.; Yoo, J.-Y.; Yun, E.; Yoon, U.; Shin, N.-Y.; Lee, K.-S. Delayed Orthostatic Hypotension in Parkinson's Disease. *Npj Park. Dis.* **2021**, *7* (1), 37.
(36) Sánchez-Ferro, Á.; Benito-León, J.; Gómez-Esteban, J. C. The Management of Orthostatic Hypotension in Parkinson's Disease. *Front. Neurol.* **2013**, *4*, 64.
(37) Hauser, R. A.; Heritier, S.; Rowse, G. J.; Hewitt, L. A.; Isaacson, S. H. Droxidopa and Reduced Falls in a Trial of Parkinson Disease Patients With Neurogenic Orthostatic Hypotension. *Clin. Neuropharmacol.* **2016**, *39* (5), 220−226.
(38) Bard, A. J.; Faulkner, L. R. *Electrochemical Methods: Fundamentals and Applications*, 2nd ed.; Wiley: New York, 2001.
(39) Elgrishi, N.; Rountree, K. J.; McCarthy, B. D.; Rountree, E. S.; Eisenhart, T. T.; Dempsey, J. L. A Practical Beginner's Guide to Cyclic Voltammetry. *J. Chem. Educ.* **2018**, *95* (2), 197−206.
(40) Pellitero, M. A.; Shaver, A.; Arroyo-Currás, N. Critical Review—Approaches for the Electrochemical Interrogation of DNA-Based Sensors: A Critical Review. *J. Electrochem. Soc.* **2020**, *167* (3), 037529.
(41) Lehr, J.; Fernandes, F. C. B.; Bueno, P. R.; Davis, J. J. Label-free Capacitive Diagnostics: Exploiting Local Redox Probe State Occupancy. *Anal. Chem.* **2014**, *86*, 2559.
(42) Xu, Q.; Davis, J. J. The Diagnostic Utility of Electrochemical Impedance. *Electroanalysis* **2014**, *26* (6), 1249−1258.
(43) Xiao, J.; Fan, C.; Xu, T.; Su, L.; Zhang, X. An Electrochemical Wearable Sensor for Levodopa Quantification in Sweat Based on a Metal-Organic Framework/Graphene Oxide Composite with Integrated Enzymes. *Sens. Actuators B Chem.* **2022**, *359*, 131586.
(44) Moon, J.-M.; Teymourian, H.; De la Paz, E.; Sempionatto, J. R.; Mahato, K.; Sonsa-ard, T.; Huang, N.; Longardner, K.; Litvan, I.; Wang, J. Non-Invasive Sweat-Based Tracking of L-Dopa Pharmacokinetic Profiles Following an Oral Tablet Administration. *Angew. Chem., Int. Ed. Engl.* **2021**, *60* (35), 19074−19078.
(45) Goud, K. Y.; Moonla, C.; Mishra, R. K.; Yu, C.; Narayan, R.; Litvan, I.; Wang, J. Wearable Electrochemical Microneedle Sensor for Continuous Monitoring of Levodopa: Toward Parkinson Management. *ACS Sens.* **2019**, *4* (8), 2196−2204.
(46) Brunetti, B.; Valdés-Ramírez, G.; Litvan, I.; Wang, J. A Disposable Electrochemical Biosensor for L-DOPA Determination in Undiluted Human Serum. *Electrochem. Commun.* **2014**, *48*, 28−31.
(47) Solomon, E. I.; Sundaram, U. M.; Machonkin, T. E. Multicopper Oxidases and Oxygenases. *Chem. Rev.* **1996**, *96* (7), 2563−2606.
(48) Dăscălescu, D.; Apetrei, C. Voltammetric Determination of Levodopa Using Mesoporous Carbon—Modified Screen-Printed Carbon Sensors. *Sensors* **2021**, *21* (18), 6301.
(49) Bergamini, M. F.; Santos, A. L.; Stradiotto, N. R.; Zanoni, M. V. B. A Disposable Electrochemical Sensor for the Rapid Determination of Levodopa. *J. Pharm. Biomed. Anal.* **2005**, *39* (1), 54−59.
(50) Aslanoglu, M.; Kutluay, A.; Goktas, S.; Karabulut, S. Voltammetric Behaviour of Levodopa and Its Quantification in Pharmaceuticals Using a β-Cyclodextrine Doped Poly (2,5-Diaminobenzenesulfonic Acid) Modified Electrode. *J. Chem. Sci.* **2009**, *121* (2), 209−215.
(51) Yue, H. Y.; Wang, B.; Huang, S.; Gao, X.; Song, S. S.; Guan, E. H.; Zhang, H. J.; Wu, P. F.; Guo, X. R. Synthesis of Graphene/ZnO Nanoflowers and Electrochemical Determination of Levodopa in the Presence of Uric Acid. *J. Mater. Sci. Mater. Electron.* **2018**, *29* (17), 14918−14926.
(52) Hu, G.; Chen, L.; Guo, Y.; Wang, X.; Shao, S. Selective Determination of L-Dopa in the Presence of Uric Acid and Ascorbic Acid at a Gold Nanoparticle Self-Assembled Carbon Nanotube-Modified Pyrolytic Graphite Electrode. *Electrochim. Acta* **2010**, *55* (16), 4711−4716.
(53) Shahrokhian, S.; Asadian, E. Electrochemical Determination of L-Dopa in the Presence of Ascorbic Acid on the Surface of the Glassy Carbon Electrode Modified by a Bilayer of Multi-Walled Carbon Nanotube and Poly-Pyrrole Doped with Tiron. *J. Electroanal. Chem.* **2009**, *636* (1), 40−46.
(54) Mathiyarasu, J.; Nyholm, L. Voltammetric Determination of L-Dopa on Poly(3,4-Ethylenedioxythiophene)-Single-Walled Carbon Nanotube Composite Modified Microelectrodes. *Electroanalysis* **2010**, *22* (4), 449−454.
(55) Leite, F. R. F.; Maroneze, C. M.; de Oliveira, A. B.; dos Santos, W. T. P.; Damos, F. S.; Luz, R. de C. S. Development of a Sensor for L-Dopa Based on Co(DMG)2ClPy/Multi-Walled Carbon Nanotubes Composite Immobilized on Basal Plane Pyrolytic Graphite Electrode. *Bioelectrochemistry* **2012**, *86*, 22−29.
(56) Mazloum-Ardakani, M.; Ganjipour, B.; Beitollahi, H.; Amini, M. K.; Mirkhalaf, F.; Naeimi, H.; Nejati-Barzoki, M. Simultaneous Determination of Levodopa, Carbidopa and Tryptophan Using Nanostructured Electrochemical Sensor Based on Novel Hydroquinone and Carbon Nanotubes: Application to the Analysis of Some Real Samples. *Electrochim. Acta* **2011**, *56* (25), 9113−9120.
(57) Min, J.; Tu, J.; Xu, C.; Lukas, H.; Shin, S.; Yang, Y.; Solomon, S. A.; Mukasa, D.; Gao, W. Skin-Interfaced Wearable Sweat Sensors for Precision Medicine. *Chem. Rev.* **2023**, *123* (8), 5049−5138.
(58) Heikenfeld, J.; Jajack, A.; Feldman, B.; Granger, S. W.; Gaitonde, S.; Begtrup, G.; Katchman, B. A. Accessing Analytes in Biofluids for Peripheral Biochemical Monitoring. *Nat. Biotechnol.* **2019**, *37* (4), 407−419.
(59) Bailey, T.; Bode, B. W.; Christiansen, M. P.; Klaff, L. J.; Alva, S. The Performance and Usability of a Factory-Calibrated Flash Glucose Monitoring System. *Diabetes Technol. Ther.* **2015**, *17* (11), 787−794.
(60) Nutt, J. G. Pharmacokinetics and Pharmacodynamics of Levodopa. *Mov. Disord.* **2008**, *23* (S3), S580−S584.
(61) Fourmond, V.; Plumeré, N.; Léger, C. Reversible Catalysis. *Nat. Rev. Chem.* **2021**, *5* (5), 348−360.
(62) Lee, I.; Probst, D.; Klonoff, D.; Sode, K. Continuous Glucose Monitoring Systems - Current Status and Future Perspectives of the Flagship Technologies in Biosensor Research -. *Biosens. Bioelectron.* **2021**, *181*, 113054.
(63) Siavashi, R.; Beitollahi, H. Molecularly Imprinted Polymer Based Sensor for Measuring of Levodopa: Evaluation as a Modifier for Glassy Carbon Electrode in Electrochemically Detection. *Russ. J. Electrochem.* **2023**, *59* (1), 70−78.
(64) Omidvar, A. H.; Amanati Shahri, A.; Serrano, A. L. C.; Gruber, J.; Pamplona Rehder, G. A Highly Sensitive Molecularly Imprinted Polymer (MIP)-Coated Microwave Glucose Sensor. *Sensors* **2022**, *22* (22), 8648.
(65) Dauphin-Ducharme, P.; Yang, K.; Arroyo-Currás, N.; Ploense, K. L.; Zhang, Y.; Gerson, J.; Kurnik, M.; Kippin, T. E.; Stojanovic, M.

https://doi.org/10.1021/acssensors.4c00602
ACS Sens. 2024, 9, 3828−3839

N.; Plaxco, K. W. Electrochemical Aptamer-Based Sensors for Improved Therapeutic Drug Monitoring and High-Precision, Feedback-Controlled Drug Delivery. *ACS Sens.* **2019**, *4* (10), 2832−2837.
(66) Wilson, E.; Probst, D.; Sode, K. *In Vivo* Continuous Monitoring of Peptides and Proteins: Challenges and Opportunities. *Appl. Phys. Rev.* **2023**, *10* (4), 041309.
(67) Arroyo-Currás, N.; Somerson, J.; Vieira, P. A.; Ploense, K. L.; Kippin, T. E.; Plaxco, K. W. Real-Time Measurement of Small Molecules Directly in Awake, Ambulatory Animals. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114* (4), 645−650.
(68) Idili, A.; Gerson, J.; Kippin, T.; Plaxco, K. W. Seconds-Resolved, In Situ Measurements of Plasma Phenylalanine Disposition Kinetics in Living Rats. *Anal. Chem.* **2021**, *93* (8), 4023−4032.
(69) Bollella, P.; Katz, E. Enzyme-Based Biosensors: Tackling Electron Transfer Issues. *Sensors* **2020**, *20* (12), 3517.
(70) Das, P.; Das, M.; Chinnadayyala, S. R.; Singha, I. M.; Goswami, P. Recent Advances on Developing 3rd Generation Enzyme Electrode for Biosensor Applications. *Biosens. Bioelectron.* **2016**, *79*, 386−397.
(71) Ferri, S.; Kojima, K.; Sode, K. Review of Glucose Oxidases and Glucose Dehydrogenases: A Bird's Eye View of Glucose Sensing Enzymes. *J. Diabetes Sci. Technol.* **2011**, *5* (5), 1068−1076.
(72) McEnroe, R.; Dimeski, G.; Durham, P.; Miller, J.; Miller, G.; Petrides, V.; Rheinheimer, D.; Smith, M.; Warren, K.; Zaharik, M.; Sonntag, O. *EP07 Interference Testing in Clinical Chemistry A Guideline for Global Application Developed through the Clinical and Laboratory Standards Institute Consensus Process*; **2018**.
(73) Center for Devices and Radiological Health. *Self-Monitoring Blood Glucose Test Systems for Over-the-Counter Use*. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/self-monitoring-blood-glucose-test-systems-over-counter-use (accessed 2024−01−03).
(74) Nematollahi, D.; Shayani-Jam, H.; Alimoradi, M.; Niroomand, S. Electrochemical Oxidation of Acetaminophen in Aqueous Solutions: Kinetic Evaluation of Hydrolysis, Hydroxylation and Dimerization Processes. *Electrochim. Acta* **2009**, *54* (28), 7407−7415.
(75) Medtronic. *How To Use Your Patient Recharger | Deep Brain Stimulation for Parkinson's Disease*. https://www.medtronic.com/uk-en/patients/treatments-therapies/deep-brain-stimulation-parkinsons-disease/living-with-dbs/how-to-use-your-patient-recharger.html (accessed 2024−04−30).
(76) Oliveira, D.; Correia, B. P.; Sharma, S.; Moreira, F. T. C. Molecular Imprinted Polymers on Microneedle Arrays for Point of Care Transdermal Sampling and Sensing of Inflammatory Biomarkers. *ACS Omega* **2022**, *7* (43), 39039−39044.
(77) Irace, C.; Cutruzzolà, A.; Nuzzi, A.; Assaloni, R.; Brunato, B.; Pitocco, D.; Tartaglione, L.; Di Molfetta, S.; Cignarelli, A.; Laviola, L.; Citro, G.; Lovati, E.; Gnasso, A.; Tweden, K. S.; Kaufman, F. R. Clinical Use of a 180-Day Implantable Glucose Sensor Improves Glycated Haemoglobin and Time in Range in Patients with Type 1 Diabetes. *Diabetes Obes. Metab.* **2020**, *22* (7), 1056−1061.

https://doi.org/10.1021/acssensors.4c00602
ACS Sens. 2024, 9, 3828−3839