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# Real-time, In-vivo Molecular Monitoring Using Electrochemical Aptamer Based Sensors: Opportunities and Challenges

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## Abstract

The continuous, real-time measurement of specific molecules in situ in the body would greatly improve our ability to understand, diagnose, and treat disease. The vast majority of continuous molecular sensing technologies, however, either (1) rely on the chemical or enzymatic reactivity of their targets, sharply limiting their scope, or (2) have never been shown (and likely will never be shown) to operate in the complex environments found in vivo. Against this background, here we review electrochemical aptamer-based (EAB) sensors, an electrochemical approach to real-time molecular monitoring that has now seen 15 years of academic development. The strengths of the EAB platform are significant: to date it is the only molecular measurement technology that (1) functions independently of the chemical reactivity of its targets, and is thus general, and (2) supports in vivo measurements. Specifically, using EAB sensors we, and others, have already reported the real-time, seconds-resolved measurements of multiple, unrelated drugs and metabolites in situ in the veins and solid tissues of live animals. Against these strengths, we detail the platform's remaining weaknesses, which include still limited measurement duration (hours, rather than the more desirable days) and the difficulty in obtaining sufficiently high-performance aptamers against new targets, before then detailing promising approaches overcoming these hurdles. Finally, we close by exploring the opportunities we believe this potentially revolutionary technology (as well as a few, possibly competing technologies) will create for both researchers and clinicians.

## Graphical Abstract

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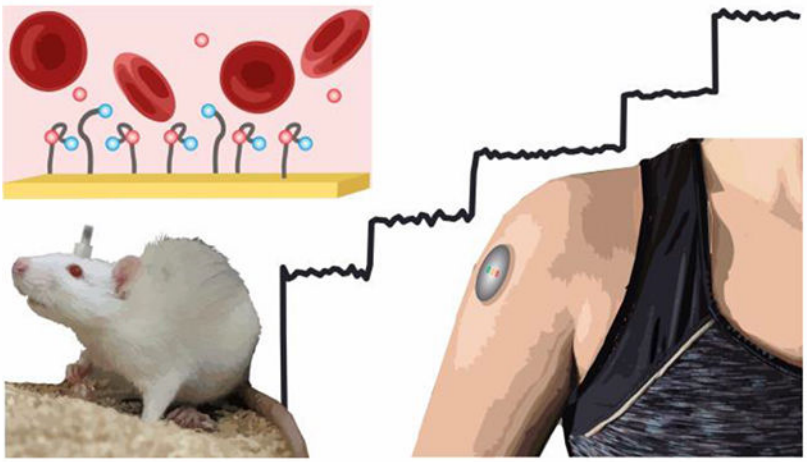

**Keywords**

In-vivo sensing; electrochemical aptamer sensors; EAB sensors; E-AB sensors; electrochemical biosensing; biosensors

## Motivation

Diseases are dynamic processes. Given this, the ability to track the concentrations of specific molecules in the body in real time would significantly improve our ability to study, monitor, and treat them. The ability to monitor blood glucose in real time, for example, has greatly advanced the treatment of diabetes. Likewise, pulse oximeters supporting real-time measurements of blood oxygenation have grown into ubiquitous tools both for surgery and routine health monitoring. Unfortunately, however, at present the continuous glucometer and the pulse oximeter are the sole commercially available sensors supporting high-frequency, in-vivo molecular measurements. Given the promise of time resolved in-vivo molecular sensing, why do we not have sensors for the many other molecules indicative of health, disease, and treatment status?

The answer to this query has been that, historically, the few sensors that support in vivo molecular monitoring are not generalizable, and the few generalizable sensing platforms fail when deployed in vivo. For example, although the continuous glucose monitor is a wildly successful example of in vivo molecular sensing, it relies critically on the enzymatic conversion of glucose into an easily detectable electroactive product[1]. Thus, it is difficult to generalize this approach to new targets. As it relies on a spectroscopic change produced by the covalent binding of oxygen to hemoglobin[2], the pulse oximeter is likewise difficult to adapt to new targets. Many other sensing approaches, in contrast, do not depend on the target's specific chemical reactivity, rendering them generalizable. Other platforms may detect binding of target to a receptor coated surface via changes in mass[3–5] or electrical response[6–8]. Unfortunately, while these approaches are general, they fail when challenged in bodily fluids due to the non-specific adsorption of interferents. This occurs because fouling generates a signal that is often indistinguishable from that produced by target binding.

A potential solution to the above-described challenges has been achieved with the invention electrochemical aptamer-based (EAB) sensors. First reported some 15 years ago, EAB sensors are the only technology described to date that both (1) functions independently of the reactivity of its targets and (2) is selective enough to work in situ in the living body. Comprised of an aptamer (a nucleic acid selected in vitro to bind to a specific molecular target) modified with a redox reporter and attached to an electrode via a self-assembled monolayer, EAB sensors rely on binding-induced changes in the conformation of this aptamer to generate a signal. Specifically, this binding-induced conformational change alters the rate of electron transfer from the reporter. This, in turn, produces an easily measured change in electrochemical signal when the sensor is interrogated using any of a range of electrochemical techniques, including square wave voltammetry[9], chrono-amperometry[10], cyclic voltammetry[11], and electrochemical impedance spectroscopy[12].

EAB sensors are modular, such that the development of sensors against new targets requires only the discovery of the appropriate aptamer and its adaptation into the platform. Aptamer selection is performed in-vitro via a process termed Systemic Evolution of Ligand Exponential Enrichment, or "SELEX" (Figure 1A)[13, 14]. To adapt the resulting aptamer sequences into EAB sensors, they are first modified such that they undergo a binding-induced conformational change, typically via the introduction of destabilizing mutations that lead to binding-induced folding[15–17]. To convert this binding-induced conformational change into a measurable shift in electron transfer rate, the sequence is modified with an alkane thiol and a redox reporter capable of reversibly transferring electrons. Of the redox reporters explored, methylene blue has proven the most popular due to its stability under repeated electrochemical interrogation[18]. And while the electron transfer kinetics of this reporter depend upon pH[19], limiting its application to media with stable pH, new redox reporters are being explored that do not display such dependence[20]. Finally, the sensor is fabricated by attaching the redox-reporter-modified aptamer to a gold electrode via the formation of a thiol-on-gold bond followed by "backfilling" with an alkane thiol to form a stable, self-assembled monolayer (Figure 1B).

Any new sensors we create are validated in vitro prior to in vivo applications. Titrations in a simple media, such as phosphate buffered saline, provide an indication of whether the sensor produces a sufficiently large change to support in vivo sensing. If response is insufficient to achieve good signal-to-noise, the sensor may require modifications, including changes to the aptamer sequence (e.g., truncations) or the position of the redox reporter[17]. After achieving sufficient performance in simple media, additional titrations are used to assess performance in the biological fluid relevant to the in vivo application, such as whole blood (Figure 1D). If the sensors demonstrate sufficient signal in body temperature biological fluid, they warrant application in live animal models (to date in rats, Figure 1E).

Previous reviews of EAB sensors have explored the applicability of the platform for in vivo measurements[21], and detailed their fabrication, interrogation, and optimization[22]. In this perspective, in contrast, we explore the strengths and weaknesses of the EAB sensor platform, placing particular focus on their application in performing real-time measurements in the living body. We then discuss what we view as the opportunities and threats to be faced

in their translation from an academic technique to commercialized devices suitable for in vivo molecular monitoring.

## Strengths

We believe EAB sensors exhibit a number of strengths, positioning them as the first generalizable molecular sensing platform that can be deployed in situ in the living body.

### Generalizability

Because aptamers themselves function independently of the electrochemical or enzymatic reactivity of their targets, EAB sensors are generalizable to the measurement of a wide range of molecules. Consistent with this, EAB sensors have been described to date against a wide range of therapeutic drugs and drugs of abuse[17, 23, 24], proteins[1, 25–28],metabolites[29, 30], and toxins[31–34], with many more aptamer sequences having been reported but not yet adapted to the platform.

### High-frequency, real-time molecular measurements

EAB sensors monitor the concentration of their molecular target in real time[1, 36] and with seconds or even sub-second time resolution. This impressive time resolution arises from a number of attributes. First, the EAB sensor signal transduction mechanism avoids the need for reagent additions, washing steps, or other sensor regeneration, all of which would introduce significant time lags[37–39]. Second, for low molecular weight targets, target binding and the resulting aptamer conformational change are rapidly reversible, often rendering the electrochemical interrogation of the device the time-resolution-limiting step. Using square wave voltammetry, for example, typically yields time resolution of a few seconds to a few tens of seconds[23, 29, 40, 41]. In contrast, interrogation using chronoamperometry[10], intermittent pulse amperometry[42], and electrochemical impedance spectroscopy[12] can achieve sub-second time resolution. Such time resolution is faster than most physiological processes, making EAB sensors well-suited to monitoring patient health and treatment status.

### Performance in Undiluted Biological Media, and the Living Body

EAB sensors resist fouling sufficiently to support measurements in complex sample matrixes, including undiluted serum[1, 24, 43] and whole blood[30, 44] (Figure 3). This is not to say that EAB sensors are entirely unaffected by adsorption to the interface. Indeed, when EAB sensors are placed in the complex environments found within the body, fouling and other sensor-degradation phenomena contribute to an often significant decline in signal[45]. Fortunately, however, this drift can be corrected using a variety of approaches[10, 46, 47]. The “kinetic differential measurement” (KDM) technique, for example, leverages square wave frequency pairs that, when interrogated sequentially, drift in concert[46, 48]. Subtracting the normalized peak signals and dividing by their average thus corrects for sensor signal loss over time. Using such techniques has enabled the measurement of a number of targets with clinically-relevant accuracy and precision[17, 23, 25, 29, 35, 48, 49].

Building on the above attributes, the EAB platform is the only technology demonstrated to achieved hours-long, real-time molecular measurements in the body without relying on the target's intrinsic reactivity[55], spectroscopic properties[56], or enzymatic activity[57]. Specifically, the platform has been used to perform real-time measurements of multiple drugs (including antibiotics[17, 48], chemotherapeutics[23, 48, 54], and drugs of abuse[52]) and metabolites (including ATP and phenylalinine[29, 30, 53]) in situ in the bodies of live rats (Table 2). The resulting measurements achieve unprecedented, seconds-resolved measurement of drug pharmacokinetics and metabolic return to homeostasis. EAB sensors' real-time measurements have even enabled feedback-controlled drug delivery, achieving exceptional precision and accuracy in the delivery of otherwise difficult-to-safely-administer antibiotics[17, 49].

### Miniaturizable

EAB sensors can be made small enough to implant easily into the body. For example, the in-vein EAB sensors reported to date typically utilize a thin (<300 μm) bundle of wires consisting of a sensor, reference electrode, and counter electrode inserted into a catheter housing[17]. In the future, performing measurements in other bodily locations, or targeting specific regions of the brain will require even smaller sensors. Sensor size represents a tradeoff between magnitude of electrochemical signal (which influences noise level in measurements) and macro-scale device dimensions. However, EAB sensors may be miniaturized further by increasing the microscopic surface area of the gold working electrode. Increasing the surface area with nanostructured surface morphologies may easily enable further sensor miniaturization. In recent work, for example, we applied nanoporous gold to reduce the dimensions of in-vein sensor size[58].

## Weaknesses

Our enthusiasm for EAB sensors aside, the technology is, of course, not without limitations.

### Adaptation of New Aptamer Sequences to the Platform

The selection and subsequent adaptation of aptamers into the EAB platform can present bottlenecks to producing sensors against new targets. SELEX identifies aptamers that bind to a target – these sequences are typically then characterized free in solution using either optical or calorimetric methods[59, 60]. When adapting "as-selected" aptamers into EAB sensors, however, their signal gain (the relative change in electrochemical response between the absence of target and saturating target) is often small. Fortunately, this can usually be rectified using a number of rational or semi-rational approaches[17, 40] to reengineer the aptamer to undergo a large-scale, binding-induced conformational change. Most often we achieve this via truncation, which destabilizes the aptamer such that it equilibrates between an unfolded conformation and the bound, folded complex[17, 40]. Alternative approaches include the introduction of long, flexible loops, such that target binding must close the loop[61], or complementary strands, such that the aptamer equilibrates between a double-helix and the target-binding conformation[62]. Recently, spectroscopic approaches have been described to guide the modification of aptamer sensors to generate the needed binding-induced conformational change, further improving the future success of adapting new

aptamers into the platform[63]. The existence of multiple techniques for modifying aptamers to support EAB signaling, however, does not guarantee an aptamer will produce a sensor with sufficient signal response over the desired target concentration range.

### Aptamer performance

EAB sensors are often sufficiently selective to monitor their targets in vivo. That is, nothing naturally present in blood or other bodily fluids produces a significant signal response. Nevertheless, EAB sensors cannot be any more specific than the aptamer they employ and, thus, specificity is an inherent concern. Specifically, because aptamers tend to bind a subset of the chemical groups on a target[64], and such groups can occur in multiple targets, cross-reactivity is seen in some aptamers[65] and in their resulting EAB sensors. For example, cross-reactivity has been observed in the aminoglycoside sensor, which binds to structurally-similar antibiotics, including tobramycin, gentamycin, and kanamycin[48]. Likewise, an aptamer selected against the drug of abuse, cocaine, also responds to procaine, quinine, and hydroxychloroquine[66]. Such cross-reactivity poses a challenge to in vivo monitoring if the interfering compound might also exist in the body at concentrations that produce a signal response. Application of EAB sensors to in vivo monitoring, then, requires an understanding of reactivity of the aptamer with any structurally similar compounds that may be present. Fortunately, however, negative aptamer selections provide a potential solution to this challenge. That is, because aptamers are generated via in vitro evolution (unlike, for example, antibodies), negative selective pressure can help remove aptamers with unwanted cross reactivity[67].

### In vivo measurement Duration

The measurement durations that can be achieved using EAB sensors in vivo remains uncertain. Almost all of the in-vivo EAB measurements reported to date have been collected for less than 6 h. This is primarily due to animal welfare guidelines that dictate that experiments under anesthesia do not exceed this duration. When challenged in vitro in undiluted, body temperature whole blood, however, EAB sensors can achieve durations in excess of 24 h, suggesting that longer duration in vivo measurements are within reach[45]. That said, it is clear that, eventually, signal loss will pose a challenge to increased measurement duration. A recent review has detailed efforts to address such signal loss in support of longer duration measurements[68].

Mechanistic studies of EAB sensor drift suggest approaches by which long duration in vivo measurements can be achieved. When EAB sensors are challenged in body temperature whole blood, their drift manifests as two distinct phases: an exponential signal decrease followed by a linear, sloping decrease[45] (Figure 4). Recent work suggests that the more rapid exponential phase arises due to fouling, which decreases the electron transfer rate from the redox reporter to the electrode surface[45]. Consistent with this, the exponential phase does not occur when sensors are interrogated in simple buffered solutions. Thus, improved monolayer selection may provide an important route to improving in vivo measurement duration. Thicker monolayers (longer alkane chains), for example, are more stable[69]. Unfortunately, however, electron transfer slows as the monolayer becomes thicker, limited the extent to which we can employ the thickest, most stable monolayers[70, 71]. The chemistry

of monolayer end groups or can also be modulated to improve measurement duration by, for example, reducing monolayer desorption[72] and fouling[47, 73]. Finally, the use of surface treatments and coatings[48, 74], or increasing the monolayer packing densities[44], could reduce fouling-derived drift by excluding access of proteins of a given size, or by "pre-fouling" the surface such that any further drift is reduced or eliminated.

In contrast to the biofouling-linked exponential phase of signal loss, the linear drift phase is dominated by the electrochemical effects of sensor interrogation[45]. When using square wave voltammetry, for example, the rate of the linear drift phase depends strongly on the width of the potential window employed[45]. When scanning further toward negative potentials, for example, the linear phase accelerates[45], presumably due to reductive desorption of the monolayer[75]. Likewise, applying wider potential windows in the positive direction, which likely increases oxidative desorption of the monolayer, also contributes to the linear loss phase[45]. Given this, narrowing the potential window used to interrogate EAB sensors reduces this linear drift phase[45]. In this light, we believe that the use of electrochemical techniques that scan rather narrow potential windows, such as electrochemical impedance spectroscopy, may prove an important route towards reduced signal drift and greater in vivo measurement duration.

## Opportunities

Not surprisingly, we believe the future is promising for in vivo application of EAB sensors, which appear well-suited for application to problems in both biomedical research and clinical care.

### Research Applications

As research tools, EAB sensors could significantly advance our understanding of metabolism, endocrinology, pharmacokinetics, and neurochemistry. Specifically, EAB sensors will enable better resolved, more quantitative measurements of such phenomena as drug delivery and clearance and the maintenance of metabolic homeostasis. With their ability to support feedback control, EAB sensors will similarly provide unprecedented opportunities to define the relationship between, for example, plasma drug levels and the resulting clinical or behavioral response. The ability of EAB sensors to perform simultaneous measurements in multiple locations throughout the body will enable a greater understanding of drug and metabolite transport through and between bodily compartments. Finally, in addition to in-body measurements, we believe EAB sensors could also prove useful in the real-time monitoring of cell culture applications ranging from small scale (e.g., "organ on a chip") to industrial scale (e.g., monitoring industrial bioreactors). In this application space, they have already demonstrated applications in monitoring ATP release in astrocytes[76, 77], and detecting serotonin in cell culture using glass nanopipettes[77].

### Clinical Applications

EAB sensors enable unprecedented opportunities to monitor molecules in the challenging in-vivo environment, and could produce innovations across clinical practice. We envision, for example, the adaptation of the EAB sensing platform into a wearable device which,

analogous to the continuous glucose monitor, can measure drugs and biomarkers indicative of health and disease in real-time. (To this end, the application of EAB sensors in the interstitial region of the skin are a development worth watching[51].) For patients suspected of having sepsis, for example, monitoring infection biomarkers, such a C-reactive protein, could provide a life-saving indicator of disease prognosis and severity[78]. Similarly, given that specific biomarkers, such as troponin, accompany heart attack onset[79], a convenient, wearable device could aid in early detection of heart attacks for individuals with high cardiac risk factors. Given that EAB sensors are the only technology capable of measuring picomolar concentrations of specific (non-enzyme) proteins in real time in complex sample matrices, the platform appears uniquely well-suited for such monitoring[25–27].

In addition to disease detection and monitoring, EAB sensors could also enable high-precision, highly-personalized drug dosing. Today, the bulk of pharmaceutical dosing is performed based on assumptions of how an average person absorbs and responds to a drug. The therapeutic windows of some drugs, however, are too narrow (relative to patient-to-patient or, even intra-patient variability) for this approach to work. For these, dosage is presently guided by (slow inconvenient, infrequent) blood draws and laboratory-based analysis, or by waiting for harmful side effects to appear. This can lead to dire consequences arising from either underdosing or overdosing. By providing a convenient, real-time window into plasma drug levels, applying EAB sensors to the problem of performing therapeutic drug monitoring could thus greatly improve the safety and efficacy of pharmacological treatments.

## Threats

Here, we consider competing technologies that might achieve the goal of continuous, long duration molecular monitoring in the living body.

### Direct Electrochemical Sensing

Certain molecular targets of interest are electroactive at potentials that can be safely applied within the body. These include the neurotransmitters dopamine, norepinephrine, epinephrine, histamine, serotonin, and adenosine[55, 80]. Other physiologically relevant, electroactive compounds include oxygen, nitric oxide, hydrogen peroxide and ascorbate[81, 82]. The oxidation or reduction of these species using an in vivo electrode allows for their direct detection, typically via chronoamperometry, differential pulse voltammetry, or fast scan cyclic voltammetry[55, 80, 83]. In the neurosciences, for example, direct electrochemistry has seen extensive application for the in vivo measurement of dopamine and serotonin[84, 85,86]. In vivo measurements of these compounds, however, often suffer from poor sensitivity and specificity. For example, overlapping redox potentials cause substances, such as ascorbic acid, to interfere with neurotransmitter detection[80]. This overlap also renders it difficult to differentiate between related molecules, such as the neurotransmitters dopamine and serotonin[87]. Likewise, many electrochemical techniques are not sensitive enough to detect biologically relevant levels of molecules when deployed in vivo[87]. These challenges, combined with the limited number of molecules of interest that are appropriately electroactive, limits the scope of in vivo measurements using direct electrochemistry[88].

### Enzymatic Sensors

As we noted above, enzymatic sensors, which instead rely on the enzymatic reactivity of their targets, are among the few commercially available molecular monitoring technologies capable of performing directly in the body. In addition to the glucose oxidase-based continuous glucose monitor[89], similar in vivo enzymatic sensors have also been described for lactate[90], acetylcholine[91, 92], and glutamate[81, 82, 93]. The further development of such sensors, however, has been limited by (1) a lack of suitable enzymes, (2) instability or toxicity of the mediator species used to enable electron transfer to the electrode[88], (3) poor enzyme stability[94], and (4) interference arising from endogenous electroactive active species[95].

### Optical Methods

A number of optical approaches have been reported for monitoring specific molecules in the body. As noted above, the pulse oximeter, which measures blood oxygen by directing infrared light through the skin, is a widespread optical sensor used in routine medical monitoring. In addition to the commercial pulse oximeter, other optical technologies relying on fluorescence[96–98] and photoacoustics[99] can track biomarkers in the living body. Injectable optical sensors, for example, have been reported that use target binding to an optical reporter to change either fluorescent or photoacoustic signal. These sensors require injection of photo-reactive, target-binding chemicals into the dermis, and have demonstrated in vivo detection of sodium[98], lithium[99], and histamine[100, 101]. And in contrast to electrochemical approaches, which often provide measurements at a single targeted location, optical methods can sometimes be used to provide spatially resolved measurements. Like enzymatic sensors, however, these approaches are not generalizable due to their reliance on the specific chemical reactivities of theirs target. Finally, optical probes inserted into the body often diffuse away from the site of injection, which can complicate quantification and impact measurement duration[101].

### Field Effect Transistors

Researchers have recently adapted field effect transistor based “aptasensors” from in vitro[60, 102] to in vivo measurements[103]. Instead of using a redox reporter modified aptamer probe on an electrode, these sensors utilize an aptamer (with no redox reporter attached) deposited on the gate region of a transistor. Target binding shifts the drain-source current, presumably due to changes in electric field associated with the distance between the negatively-charged DNA and the gate. Such sensors retain the generalizability of EAB sensors, and are reported to achieve significantly improved limits of detection. Uncertainties remain, however, regarding the applicability of this newly reported approach in vivo. For example, the only reported in vivo measurements presented to date (serotonin in the mouse brain before and after electrical stimulation) presented only ~10-20 data points collected over just a few minutes. Due to the comparatively short duration of these measurements, we cannot yet judge this platform’s applicability to long duration measurements in the living body. A second concern is that the response curves (concentration versus signal change) of sensors in this class, which often span many orders of magnitude of target concentration[60, 102, 103], are quite shallow[103]. Large changes in target concentration are

thus required to produce statistically significant changes in sensor output, greatly reducing measurement precision. For these reasons it appears that this approach has some significant ground to cover before it reaches the level of validation already achieved by in vivo EAB sensors.

## Conclusions

In vivo EAB sensors are still in their infancy, having yet to transition to either market-ready products (like the continuous glucose meter) or to widely applied research tools (like the direct electrochemical detection of neurotransmitters). And yet, EAB sensors are already a “class in their own” platform in terms of their ability to perform quantitative in vivo molecular measurements. This generalizable platform hosts a wealth of benefits, such as excellent time resolution, miniaturizability, and applicability to a range of device formats. And while the platform faces challenges along the path from aptamer discovery to EAB sensor development, this has not notably impeded the introduction of new, useful EAB sensors each year. For this field to mature and yield reliable analytical tools, however, we need to actively seek a greater understanding of interfacial stability, and pursue applications to reduce sensor drift. This is no surprise, however. The existing glucose meters rely on the use of selective membranes to mediate the effects of fouling in blood or the dermal space. Thus, we believe that, with the application of both electrochemical and interfacial methods to alleviate sensor drift, EAB sensors could support long-duration, time resolved measurements of a multitude of scientifically and clinically important molecules in situ in the living body.

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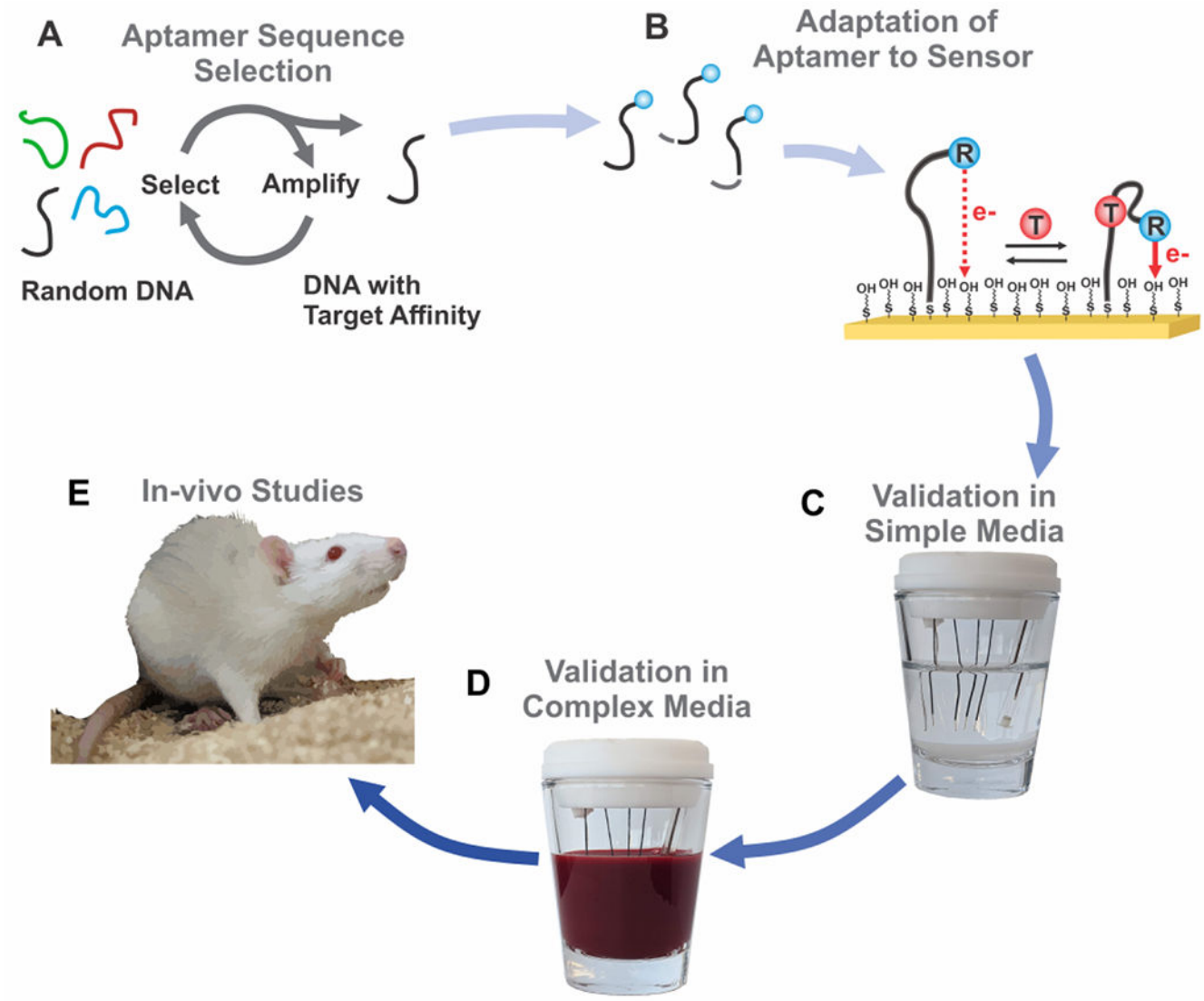


**Figure 1:**

**(A)** To develop an in-vivo EAB sensor, first an aptamer is selected for a given target using a procedure called Selective Evolution of Ligand Exponential Enrichment ("SELEX"). **(B)** Modifications to the selected aptamer, such as surface linkers, redox reporter tags, and truncations of aptamer stem length allow adaptation of a given sequence into an EAB sensor. **(C)** Titrations in simple media, such as phosphate buffered saline, assess aptamer response to target. **(D)** Pending success in simple media, aptamer response is measured in a media that more closely reflects the in-vivo environment (commonly, bovine blood for venous measurements, or cerebral spinal fluid for in-brain measurements). **(E)** If the sensor response aligns with the expected physiological target range, it may be applied for measurements in the living body.

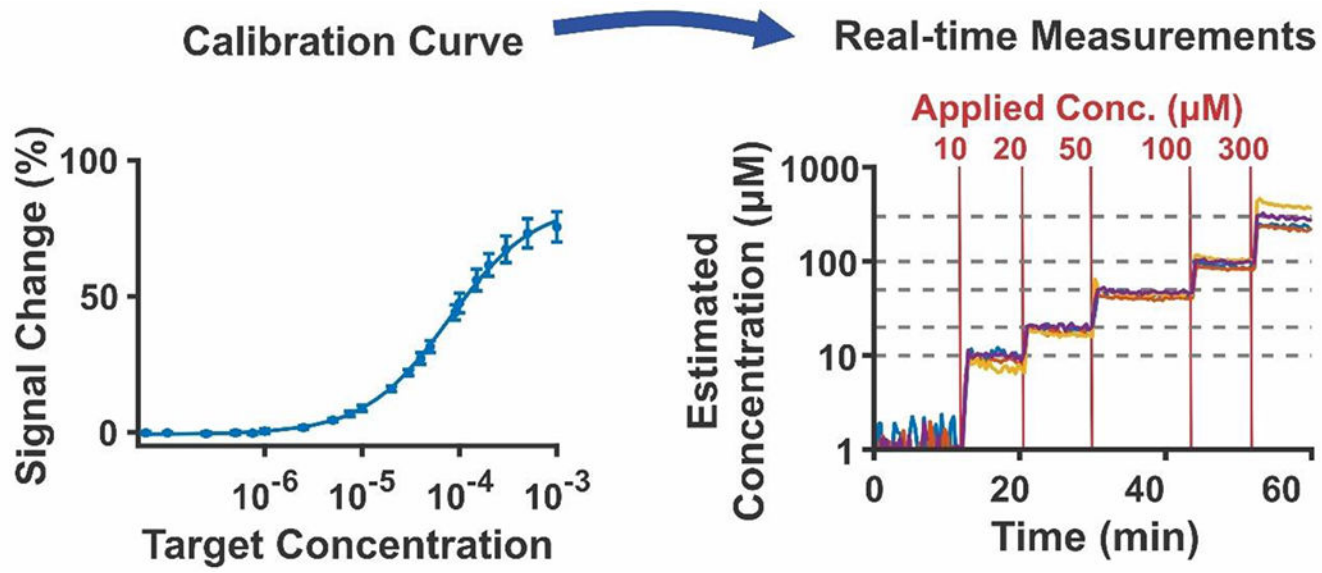


**Figure 2:**

EAB sensors are quantitative. **(A)** To achieve this, we first generate a calibration curve, in which we measure the electrochemical signal change in response to the addition of graduated concentrations of target. The resulting data is fit to a Hill-Langmuir isotherm, yielding parameters that enable **(B)** quantification of data inputted into the Hill-Langmuir equation. (Reproduced with permission from Downs, A.; Gerson, J.; Leung, K.; Honeywell, K.; Kippin, T.; Plaxco, K. Improved calibration of electrochemical aptamer-based sensors. *Scientific Reports* 2022[35]. )

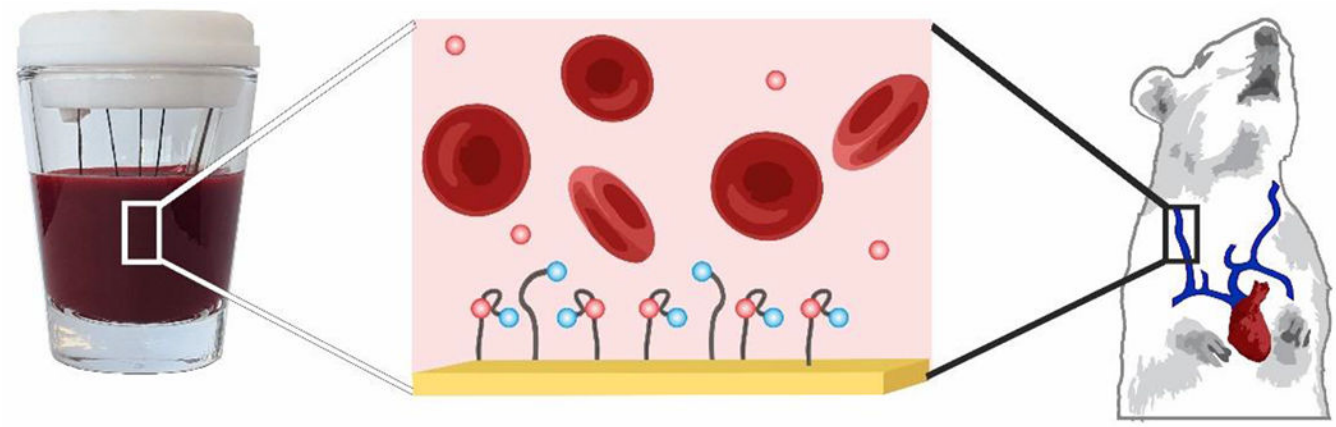

**Figure 3:**
EAB sensors maintain signaling even in undiluted whole blood, or the living body. In vivo, this has enabled measurements of pharmaceutical delivery and clearance.

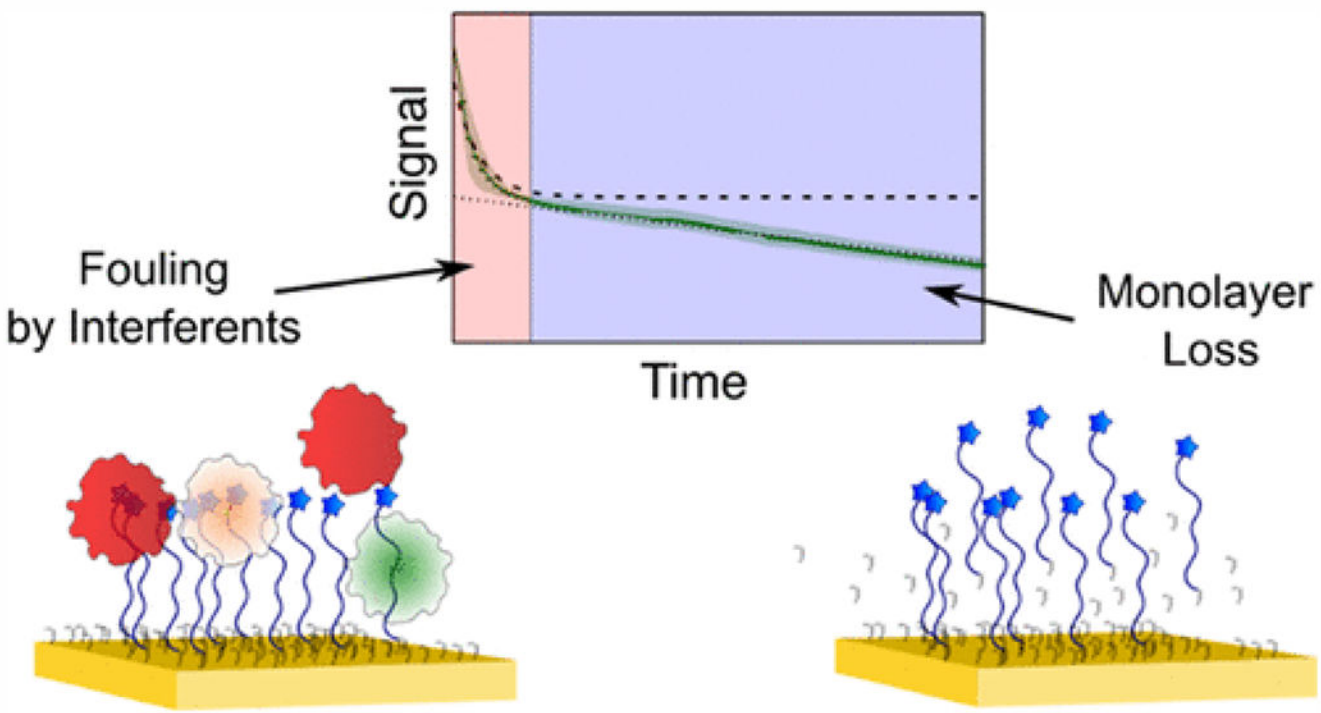


**Figure 4:**
When placed in undiluted whole blood at 37°C, EAB sensors fabricated using mercaptohexanol monolayers exhibit an exponential and linear phase of signal loss. The former seems to arise due to fouling, and the latter due to redox-driven loss of the DNA-modified monolayer. (Reproduced from Leung, K. K.; Downs, A. M.; Ortega, G.; Kurnik, M.; Plaxco, K. W., Elucidating the mechanisms underlying the signal drift of electrochemical aptamer-based sensors in whole blood. *ACS Sensors* 2021[45]. Copyright 2021 American Chemical Society.)

**Table 1:**

Summary of respective strengths, weaknesses, opportunities and threats for the EAB sensing platform in translation to in vivo measurements.

| **Strengths** | **Weaknesses** | **Opportunities** | **Threats** |
|---|---|---|---|
| High-frequency, real-time measurements<br><br>Generalizable to many targets<br><br>Selective enough to deploy directly in-vivo<br><br>Miniaturizable | The efficiency of adapting new aptamer sequences into EAB sensors<br><br>Aptamer performance<br><br>Measurement duration | Applications in biomedical research<br><br>Clinical applications | Other in-vivo molecular sensing strategies:<br>- Enzymatic<br>- Direct Electrochemistry<br>- Optical methods<br>- Field effect transistors |

**Table 2:**

In vivo EAB sensors reported to date.

| Target | Target class | In-vivo application | Duration |
|---|---|---|---|
| Vancomycin[17] | Antibiotic | Measurement of plasma pharmacokinetics, feedback-controlled delivery | 5 h |
| Tobramycin[10, 48–51] | Antibiotic | Measurement of plasma and interstitial fluid pharmacokinetics, feedback-controlled delivery | 4 - 12 h |
| Cocaine[52] | Drug of Abuse | Measurement of pharmacokinetics in the brain | 4.5 h |
| Kanamycin[48, 53] | Antibiotic | Measurement of plasma pharmacokinetics | 3 h |
| Phenylalinine[29] | Amino acid | Measurement of plasma kinetics | 1 h |
| Doxorubicin[54] | Chemotherapeutic | Measurement of plasma pharmacokinetics | 3 h |
| Adenine Triphosphate[53] | Metabolite | Measurement of plasma pharmacokinetics | 3 h |
| Irinotecan[23] | Chemotherapeutic | Measurement of plasma pharmacokinetics | 2 h |
| Gentamicin[48] | Antibiotic | Measurement of plasma pharmacokinetics | 4 h |
| Doxorubicin[48] | Chemotherapeutic | Measurement of plasma pharmacokinetics | 5 h |