Title

Electrochemical Aptamer-Based Sensors for Improved Therapeutic Drug Monitoring and High-Precision, Feedback-Controlled Drug Delivery

Journal

ACS Sensors, 4(10)

ISSN

2379-3694

Authors

Dauphin-Ducharme, Philippe
Yang, Kyungae
Arroyo-Currás, Netzahualcóyotl
et al.

Publication Date

2019-10-25

DOI

10.1021/acssensors.9b01616

Peer reviewed

HHS Public Access

Author manuscript

ACS Sens. Author manuscript; available in PMC 2020 April 25.

Published in final edited form as:

ACS Sens. 2019 October 25; 4(10): 2832–2837. doi:10.1021/acssensors.9b01616.

Electrochemical Aptamer-Based Sensors for Improved Therapeutic Drug Monitoring and High-Precision, Feedback-Controlled Drug Delivery

Philippe Dauphin-Ducharme[†,‡], **Kyungae Yang**[#], **Netzahualcóyotl Arroyo-Currás**[○], **Kyle L. Ploense**[†,‡], **Yameng Zhang**[#], **Julian Gerson**[§,‖,⊥], **Martin Kurnik**[†,‡], **Tod E. Kippin**[§,‖,⊥], **Milan N. Stojanovic**[#,▽], **Kevin W. Plaxco**[*,†,‡]

[†]Department of Chemistry and Biochemistry, University of California, Santa Barbara, Santa Barbara, California 93106, United States

[‡]Center for Bioengineering, University of California, Santa Barbara, Santa Barbara, California 93106, United States

[§]Department of Psychological and Brain Sciences, University of California, Santa Barbara, Santa Barbara, California 93106, United States

[‖]Department of Molecular, Cellular and Developmental Biology, University of California, Santa Barbara, Santa Barbara, California 93106, United States

[⊥]Neuroscience Research Institute, University of California, Santa Barbara, Santa Barbara, California 93106, United States

[#]Center for Innovative Diagnostic and Therapeutic Approaches, Department of Medicine, Columbia University, New York, New York 10032, United States

[▽]Department of Biomedical Engineering and Systems Biology, Columbia University, New York, New York 10032, United States

[○]Department of Pharmacology and Molecular Sciences, Johns Hopkins School of Medicine, Baltimore, Maryland 21205, United States

Abstract

The electrochemical aptamer-based (E-AB) sensing platform appears to be a convenient (rapid, single-step, and calibration-free) and modular approach to measure concentrations of specific molecules (irrespective of their chemical reactivity) directly in blood and even in situ in the living

[*]**Corresponding Author** kwp@ucsb.edu. Phone: (805) 893-5558.

ASSOCIATED CONTENT

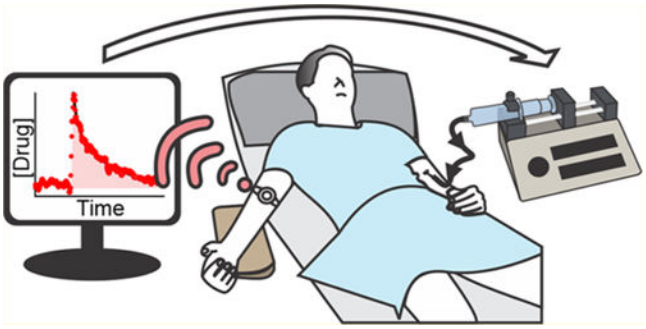
Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssensors.9b01616. Materials, chemicals, and experimental methods for the development of the vancomycin-binding E-AB sensors, including the selection of the aptamer, electrochemical characterization of the sensor with the calibration-free parameters, sensor's response time, and PID control of lower vancomycin doses (PDF)

The authors declare the following competing financial interest(s): K.W.P. discloses service on the scientific advisory boards of Diagnostic Biochips Inc. and Eccrine Systems, both of which are developing applications related to this work. P.D.-D., N.A.-C. and K.W.P. have filed provisional patents regarding intellectual property related to the work presented in this paper. M.N.S. discloses shares of company that licenses intellectual property related to the work presented in this paper.

body. Given these attributes, the platform may thus provide significant opportunities to render therapeutic drug monitoring (the clinical practice in which dosing is adjusted in response to plasma drug measurements) as frequent and convenient as the measurement of blood sugar has become for diabetics. The ability to measure arbitrary molecules in the body in real time could even enable closed-loop feedback control over plasma drug levels in a manner analogous to the recently commercialized controlled blood sugar systems. As initial exploration of this, we describe here the selection of an aptamer against vancomycin, a narrow therapeutic window antibiotic for which therapeutic monitoring is a critical part of the standard of care, and its adaptation into an electrochemical aptamer-based (E-AB) sensor. Using this sensor, we then demonstrate: (i) rapid (seconds) and convenient (single-step and calibration-free) measurement of plasma vancomycin in finger-prick-scale samples of whole blood, (ii) high-precision measurement of subject-specific vancomycin pharmacokinetics (in a rat animal model), and (iii) high-precision, closed-loop feedback control over plasma levels of the drug (in a rat animal model). The ability to not only track (with continuous-glucose-monitor-like measurement frequency and convenience) but also actively control plasma drug levels provides an unprecedented route toward improving therapeutic drug monitoring and, more generally, the personalized, high-precision delivery of pharmacological interventions.

Graphical Abstract:



Keywords

vancomycin; therapeutic drug monitoring; controlled drug delivery; square-wave voltammetry; DNA aptamer; electrochemical DNA biosensor

INTRODUCTION

The therapeutic windows (range of plasma concentrations over which a molecule is therapeutically effective without causing significant adverse effects) of most drugs are wide relative to typical metabolic variability, and thus indirect predictors of patient metabolism, such as age, body mass, or genotype, are sufficient to ensure effective dosing.[1–3] The therapeutic windows of many drugs, however, are narrow relative to interpatient metabolic variability, and the accuracy of indirect pharmacokinetic predictors is insufficient to ensure safe dose determination and drug delivery.[1,4,5] Under these circumstances, therapeutic drug monitoring (TDM), the clinical practice in which plasma drug concentrations are measured to adjust dosing, remains the standard of care.[6–8] This is particularly true for the most

grievously ill patients as this is the population most likely to exhibit altered metabolism and the population for which the margin for therapeutic error is narrowest.[9–11]

The need for and problems associated with TDM are compellingly illustrated by the difficulty in properly delivering vancomycin, a “key access antibiotic”. Because plasma levels below the drug’s therapeutic window can lead to dangerously poor efficacy (e.g., death due to sepsis) and those above the window are associated with permanent hearing loss and nephrotoxicity, TDM is a recommended element of the standard of care in vancomycin treatment.[12–15] Current methods of monitoring plasma vancomycin levels, however, rely on venous blood draws that are analyzed using immunoassays[16,17] or HPLC-MS[16–19] and are thus slow, cumbersome processes requiring fully equipped laboratories and returning an answer only hours to days after sample collection (Figure 1A). As a result, TDM of vancomycin is performed more rarely than is optimal[2,12,13,15] and, even when performed, provides only a low resolution (a few data points) “snapshot” that may not accurately measure a patient’s metabolism[20–23] (Figure 1A). More generally, the slow, cumbersome nature of current drug measurement approaches remains a major hurdle hindering the wider adoption of TDM.[20,24,25] Against this background, our goal is to develop a platform that renders plasma drug level measurements as easy and convenient as current blood sugar measurements under the argument that at-home finger-prick measurements of plasma drug levels (Figure 1B) or, ultimately, continuous, real-time measurements performed via a wearable device (Figure 1C) would vastly improve our ability to accurately dose and properly deliver this and many other pharmacological therapies (Figure 1D).

In response to the need for improved methods of performing TDM, we have developed electrochemical aptamer-based (E-AB) sensors, a reagentless, single-step sensing platform for the rapid, and even real-time, measurement of plasma drug levels in whole blood and even directly in the body. E-AB sensors are composed of an electrode-bound redox-reporter-modified aptamer sequence that undergoes a binding-induced change in electron transfer kinetics easily monitored using, for example, square-wave voltammetry.[28–30] In this paper, we explore the potential of the E-AB platform as a means of performing TDM by selecting the first reported aptamer against vancomycin and adapting it into an E-AB sensor. We then applied this new sensor to (i) the rapid, calibration-free quantification of vancomycin ex vivo in unprocessed, finger-prick-scale volumes of whole blood; (ii) achieving seconds-resolved, high-precision vancomycin pharmacokinetic measurements; and (iii) performing high-precision feedback control over plasma levels of this historically difficult-to-dose drug.

RESULTS AND DISCUSSION

E-AB sensor development requires the availability of an aptamer that achieves clinically relevant affinity and specificity. To identify such an aptamer, we performed a solution-phase selection based on a stem-loop closing scheme where the details of which can be found in the Supporting Information and in prior works.[31,32] This selection starts with a random DNA library of ~6×10^{14} unique sequences in which each member is composed of a 45-base random element flanked by two partially complementary primers (Figure 2A). As previously reported,[33,34] the library members are attached to an agarose–streptavidin solid support via hybridization to a biotinylated anchor strand complementary to one of the two primers,

which also prevents the primers from hybridizing with one another to form a double-stranded stem.[31,32,35] Sequences that undergo a binding-induced conformational change leading to detachment (presumably due to stabilization of the stem) are then collected, amplified, and regenerated. Repeating this cycle 14 times, we produced an aptamer against vancomycin with a dissociation constant (K_D) of ~0.1 μM when measured free in a bulk solution using a previously described[32] optical assay (Figure S1).

To support E-AB sensing, an aptamer must undergo a binding-induced conformational change that, in turn, produces a significant change in electron transfer kinetics (Figure 2B). The full-length ("parent") aptamer does not exhibit this property (Figure S3A), presumably due to the stability of the folded structure. Circular dichroism studies suggest, however, that the removal of four base pairs from the stem produces the necessary binding-induced conformational change (Figure S3B). Consistent with this, when adapted into the E-AB platform, the truncated aptamer supports good E-AB signaling, exhibiting a relative signal change upon the addition of a saturating target of ~120% and a K_D of 45 μM when challenged in undiluted flowing whole blood (Figure 2C). These attributes support vancomycin measurements across the entire 6–35 μM clinical range of the antibiotic.[36] The sensor is also rapid: when challenged with 10 μM vancomycin, it completely equilibrates in the time (9 s) it takes to acquire a pair of square-wave voltammograms (Figure S4).

By analogy to the impact that the home glucose monitor has had on diabetes care, the E-AB platform could significantly improve on current TDM methods by moving it from laboratory-based tests to at-home "self-testing" (Figure 1B). To explore this possibility, we fabricated vancomycin-detecting sensors using wire electrodes small enough to immerse in sub-100 μL volumes (Figure 3A) and then deployed them in a calibration-free manner.[29] Briefly, this relies on the strong square-wave frequency dependence of E-AB signaling (Figure 2C) to produce a "nonresponsive" current that is independent of the target concentration used to correct for sensor-to-sensor variation.[28–30] Using this approach to measure vancomycin spiked into 100 μL samples of whole bovine blood returns concentration estimates within ±20% of the known concentration of the drug over the entire clinically relevant range (Figure 3B), suggesting that E-AB sensors support convenient, calibration-free, and finger-prick-style self-testing closely analogous to the home-glucose-meter testing employed by many diabetics.

Although self-testing of plasma vancomycin levels using finger-prick samples could improve the convenience of TDM, such measurements still only provide poorly time-resolved "snapshots" of metabolism. In contrast, the ability of indwelling E-AB sensors to achieve subsecond-resolved measurements directly in the living body[37–39] would provide an unprecedented route toward not only measuring plasma drug levels but also measuring patient-specific pharmacokinetics. To explore this, we placed E-AB sensors in the veins of live Sprague–Dawley rats via a 22 gauge catheter (Figure 4A). To remove the drift seen in in vivo deployments, we used a previously described drift correction approach: kinetic differential measurements (KDM).[38–40] This takes the difference between the sensor's response measured at two square-wave frequencies–one in which the sensor is "signal-on" and the other at which the sensor is "signal-off"–but that drift in concert.[40] Using KDM-corrected sensors can measure 9 s-resolved plasma vancomycin levels in real time after, for

example, the intravenous injection of human-equivalent, 30 mg kg^{-1} doses (Figure 4B). The resultant pharmacokinetic curves obtained from this experiment produces peak concentrations and plasma half-lives consistent with values reported in the literature.[41] The typical ~15 min time resolution of these prior (research, not clinical) measurements, however, is poorly matched to the metabolic timescale of the drug, and thus the precision with which they define its distribution (α) and elimination (β) time constants is limited.[26] In contrast, high-frequency E-AB measurements return parameter estimates with precision (defined as 95% confidence intervals) of better than 20%, which is more than sufficient to identify statistically significant pharmacokinetic differences between individual animals (Figure 4B).

The goal of TDM is to increase the time during which plasma drug levels remain within a drug's therapeutic window.[25] The ultimate realization of this would be to measure these levels in real time and then use this information to optimize dosing on a timescale faster than the metabolic timescale of the drug. The seconds-resolved, real-time measurements provided by indwelling E-AB sensors support just such feedback-controlled delivery.[38] To illustrate this, we placed an indwelling vancomycin sensor into the right jugular vein of a rat and a drug-delivery catheter into the left jugular vein that was attached to a syringe pump (Figure 5A). We then employed the sensor's output to drive a proportional–integral–derivative (PID) controller that, in turn, adjusted the flow rate of the syringe pump every 9 s. Using this system, we rapidly achieve (~30 min) and accurately maintain (± 2 μM) plasma vancomycin concentrations over the course of hours (Figure 5B and Figure S4) even in the face of several-fold variations in the rate with which the drug is eliminated from the body during this same period (Figure 5C). Note that the limited duration of these experiments is due to animal welfare concerns (which preclude keeping rats under anesthesia for more than 6 h) and not by loss of sensor performance.

Here, we have used a rat animal model to explore the application of E-AB sensors to the problem of performing TDM and, as the ultimate goal in TDM, feedback-controlled, high-precision drug delivery. To do so, we have selected a vancomycin-binding aptamer via a solution-phase selection scheme followed by its reengineering and adaptation into an E-AB sensor. Using this sensor, we then performed (i) the rapid (seconds), convenient (single-step, calibration-free), and accurate (± 20%) determination of vancomycin levels in finger-prick-scale volumes of unprocessed whole blood; (ii) the high-precision (± 20%) measurement of subject-specific vancomycin pharmacokinetics; and (iii) the high-precision (± 20%) feedback control of plasma vancomycin levels in the face of significant, hour-to-hour changes in drug metabolism.

TDM was introduced into clinical practice to counter the relatively narrow therapeutic windows of some drugs and, in doing so, to improve the safety and efficacy of treatments. TDM has been shown, for example, to significantly improve patient outcomes upon treatment by vancomycin and the aminoglycoside antibiotics.[25,42] TDM can likewise improve patient compliance (less of a critical concern for vancomycin considering that it is administered/monitored in a hospital setting but is a serious health issue for many other drugs[43,44]) and the cost-effectiveness of treatments.[25] Unfortunately, however, studies have repeatedly shown that clinicians often fail to achieve these goals,[12] suggesting that

improvements in our ability to perform TDM are a critical component of the move toward high-precision, personalized medicine.[14,20] Specifically, we believe that the development of a platform technology for convenient measurements of plasma drug levels would represent a paradigm shift in how TDM is performed and could significantly improve outcomes, reduce complications, and reduce health costs. Next steps are currently being taken in order to translate E-AB sensors for longer term in vivo measurements so that they can be more easily applied in clinical settings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

This work was supported partially by a grant from the National Institutes of Health (grant R01EB022015) and by a grant from the W. M. Keck Foundation. P.D.-D. was supported in part by Fonds de recherche du Québec - Nature et Technologies and the Natural Sciences and Engineering Research Council of Canada with postdoctoral fellowships.

REFERENCES

(1). de Lastours V Faut-il proposer une adaptation posologique lors d'une antibiothérapie ? Intern. Med. J 2018, 39, 171–177.

(2). Falagas ME; Karageorgopoulos DE Adjustment of dosing of antimicrobial agents for bodyweight in adults. Lancet 2010, 375, 248–251. [PubMed: 19875163]

(3). Bielicki JA; Barker CIS; Saxena S; Wong ICK; Long PF; Sharland M Not too little, not too much: problems of selecting oral antibiotic dose for children. BMJ 2015, 351, h5447. [PubMed: 26537515]

(4). Payne KD; Hall RG II Dosing of antibacterial agents in obese adults: does one size fit all? Expert Rev. Anti-Infect. Ther 2014, 12, 829–854. [PubMed: 24809811]

(5). Hall RG II; Payne KD; Bain AM; Rahman AP; Nguyen ST; Eaton SA; Busti AJ; Vu SL; Bedimo R Multicenter Evaluation of Vancomycin Dosing: Emphasis on Obesity. Am. J. Med 2008, 121, 515–518. [PubMed: 18501233]

(6). Papamichael K; Cheifetz AS Therapeutic Drug Monitoring in IBD: The New Standard-of-Care for Anti-TNF Therapy. Am. J. Gastroenterol 2017, 112, 673–676. [PubMed: 28220781]

(7). Fournier A; Eggimann P; Pantet O; Pagani JL; Dupuis-Lozeron E; Pannatier A; Sadeghipour F; Voirol P; Que Y-A Impact of Real-Time Therapeutic Drug Monitoring on the Prescription of Antibiotics in Burn Patients Requiring Admission to the Intensive Care Unit. Antimicrob. Agents Chemother 2018, 62, e01818–17. [PubMed: 29263079]

(8). Stott KE; Hope WW Therapeutic drug monitoring for invasive mould infections and disease: pharmacokinetic and pharmacodynamic considerations. J. Antimicrob. Chemother 2017, 72, i12–i18. [PubMed: 28355463]

(9). Felici A; Verweij J; Sparreboom A Dosing strategies for anticancer drugs: the good, the bad and body-surface area. Eur. J. Cancer 2002, 38, 1677–1684. [PubMed: 12175683]

(10). Franken LG; Mathot RAA; Masman AD; Baar FPM; Tibboel D; van Gelder T; Koch BCP; de Winter BCM Population pharmacokinetics of haloperidol in terminally ill adult patients. Eur. J. Clin. Pharmacol 2017, 73, 1271–1277. [PubMed: 28681176]

(11). Power BM; Forbes AM; van Heerden PV; Ilett KF Pharmacokinetics of Drugs Used in Critically Ill Adults. Clin. Pharmacokinet 1998, 34, 25–56. [PubMed: 9474472]

(12). Elbarbry F Vancomycin Dosing and Monitoring: Critical Evaluation of the Current Practice. Eur. J. Drug Metab. Pharmacokinet 2018, 43, 259–268. [PubMed: 29260505]

(13). Ye Z-K; Li C; Zhai S-D Guidelines for therapeutic drug monitoring of vancomycin: a systematic review. PLoS One 2014, 9, e99044–e99044. [PubMed: 24932495]

(14). Roberts JA; Norris R; Paterson DL; Martin JH Therapeutic drug monitoring of antimicrobials. Br. J. Clin. Pharmacol 2012, 73, 27–36. [PubMed: 21831196]

(15). Ye Z-K; Tang H-L; Zhai S-D Benefits of Therapeutic Drug Monitoring of Vancomycin: A Systematic Review and Meta-Analysis. PLoS One 2013, 8, No. e77169.

(16). Farin D; Piva GA; Gozlan I; Kitzes-Cohen R A modified HPLC method for the determination of vancomycin in plasma and tissues and comparison to FPIA (TDX). J. Pharm. Biomed. Anal 1998, 18, 367–372. [PubMed: 10096831]

(17). Oyaert M; Peersman N; Kieffer D; Deiteren K; Smits A; Allegaert K; Spriet I; Van Eldere J; Verhaegen J; Vermeersch P; Pauwels S Novel LC–MS/MS method for plasma vancomycin: Comparison with immunoassays and clinical impact. Clin. Chim. Acta 2015, 441, 63–70. [PubMed: 25523192]

(18). Javorska L; Krcmova LK; Solichova D; Solich P; Kaska M Modern methods for vancomycin determination in biological fluids by methods based on high-performance liquid chromatography – A review. J. Sep. Sci 2016, 39, 6–20. [PubMed: 26351070]

(19). de Jesús Valle MJ; López FG; Navarro AS Development and validation of an HPLC method for vancomycin and its application to a pharmacokinetic study. J. Pharm. Biomed. Anal 2008, 48, 835–839. [PubMed: 18639406]

(20). Brandhorst G; Oellerich M; Maine G; Taylor P; Veen G; Wallemacq P Liquid Chromatography–Tandem Mass Spectrometry or Automated Immunoassays: What Are the Future Trends in Therapeutic Drug Monitoring? Clin. Chem 2012, 58, 821–825. [PubMed: 22252324]

(21). Walson PD Practical problems of therapeutic drug monitoring of anticonvulsants. Brain Dev 1985, 7, 116–121. [PubMed: 4014608]

(22). Dasgupta A Limitations of immunoassays used for therapeutic drug monitoring of immunosuppressants In Personalized Immunosuppression in Transplantation; Elsevier, 2016; pp 29–56.

(23). Johnson-Davis KL; McMillin GA Chapter 3 – Application of liquid chromatography combined with mass spectrometry or tandem mass spectrometry for therapeutic drug monitoring of immunosuppressants In Personalized Immunosuppression in Transplantation; Elsevier, 2016; pp 57–81.

(24). Enara M Therapeutic Drug Monitoring (TDM) Pitfalls and Limitations. MOJ Toxicol 2014, 9, 9–10.

(25). McCudden CR Quality, origins and limitations of common therapeutic drug reference intervals. Diagnosis 2018, 5, 47–61. [PubMed: 29794249]

(26). Rhodes NJ; Prozialeck WC; Lodise TP; Venkatesan N; O'Donnell JN; Pais G; Cluff C; Lamar PC; Neely MN; Gulati A; Scheetz MH Evaluation of Vancomycin Exposures Associated with Elevations in Novel Urinary Biomarkers of Acute Kidney Injury in Vancomycin-Treated Rats. Antimicrob. Agents Chemother 2016, 60, 5742–5751. [PubMed: 27431226]

(27). Bracco D; Landry C; Dubois M-J; Eggimann P Pharmacokinetic variability of extended interval tobramycin in burn patients. Burns 2008, 34, 791–796. [PubMed: 18395988]

(28). Dauphin-Ducharme P; Plaxco KW Maximizing the Signal Gain of Electrochemical-DNA Sensors. Anal. Chem 2016, 88, 11654–11662. [PubMed: 27805364]

(29). Li H; Dauphin-Ducharme P; Ortega G; Plaxco KW Calibration-Free Electrochemical Biosensors Supporting Accurate Molecular Measurements Directly in Undiluted Whole Blood. J. Am. Chem. Soc 2017, 139, 11207–11213. [PubMed: 28712286]

(30). White RJ; Phares N; Lubin AA; Xiao Y; Plaxco KW Optimization of Electrochemical Aptamer-Based Sensors via Optimization of Probe Packing Density and Surface Chemistry. Langmuir 2008, 24, 10513–10518. [PubMed: 18690727]

(31). Yang K-A; Barbu M; Halim M; Pallavi P; Kim B; Kolpashchikov DM; Pecic S; Taylor S; Worgall TS; Stojanovic MN Recognition and sensing of low-epitope targets via ternary complexes with oligonucleotides and synthetic receptors. Nat. Chem 2014, 6, 1003–1008. [PubMed: 25343606]

(32). Yang K-A; Pei R; Stojanovic MN In vitro selection and amplification protocols for isolation of aptameric sensors for small molecules. Methods 2016, 106, 58–65. [PubMed: 27155227]

(33). Nutiu R; Li Y In Vitro Selection of Structure-Switching Signaling Aptamers. Angew. Chem., Int. Ed 2005, 44, 1061–1065.

(34). Rajendran M; Ellington AD Selection of fluorescent aptamer beacons that light up in the presence of zinc. Anal. Bioanal. Chem 2008, 390, 1067–1075. [PubMed: 18049815]

(35). Nakatsuka N; Yang K-A; Abendroth JM; Cheung KM; Xu X; Yang H; Zhao C; Zhu B; Rim YS; Yang Y; Weiss PS; Stojanović MN; Andrews AM Aptamer–field-effect transistors overcome Debye length limitations for small-molecule sensing. Science 2018, 362, 319–324. [PubMed: 30190311]

(36). Martin JH; Norris R; Barras M; Roberts J; Morris R; Doogue M; Jones GR Therapeutic monitoring of vancomycin in adult patients: a consensus review of the American Society of Health-System Pharmacists, the Infectious Diseases Society of America, and the Society Of Infectious Diseases Pharmacists. The Clinical biochemist. Reviews 2010, 31, 21–24.

(37). Arroyo-Currás N; Dauphin-Ducharme P; Ortega G; Ploense KL; Kippin TE; Plaxco KW Subsecond-Resolved Molecular Measurements in the Living Body Using Chronoamperometrically Interrogated Aptamer-Based Sensors. ACS Sensors 2018, 3, 360–366. [PubMed: 29124939]

(38). Arroyo-Currás N; Ortega G; Copp DA; Ploense KL; Plaxco ZA; Kippin TE; Hespanha JP; Plaxco KW High-Precision Control of Plasma Drug Levels Using Feedback-Controlled Dosing. ACS Pharmacol. Transl. Sci 2018, 1, 110–118.

(39). Arroyo-Currás N; Somerson J; Vieira PA; Ploense KL; Kippin TE; Plaxco KW Real-time measurement of small molecules directly in awake, ambulatory animals. Proc. Natl. Acad. Sci. U. S. A 2017, 114, 645–650. [PubMed: 28069939]

(40). Ferguson BS; Hoggarth DA; Maliniak D; Ploense K; White RJ; Woodward N; Hsieh K; Bonham AJ; Eisenstein M; Kippin TE; Plaxco KW; Soh HT Real-Time, Aptamer-Based Tracking of Circulating Therapeutic Agents in Living Animals. Sci. Transl. Med 2013, 5, 213ra165.

(41). Fukushima K; Okada A; Hayashi Y; Ichikawa H; Nishimura A; Shibata N; Sugioka N Enhanced oral bioavailability of vancomycin in rats treated with long-term parenteral nutrition. SpringerPlus 2015, 4, 442–450. [PubMed: 26312207]

(42). Kang J-S; Lee M-H Overview of Therapeutic Drug Monitoring. Korean J. Intern. Med 2009, 24, 1–10. [PubMed: 19270474]

(43). Czobor P; Skolnick P The secrets of a successful clinical trial: compliance, compliance, and compliance. Mol. Interventions 2011, 11, 107–110.

(44). Ryan TP; Morrison RD; Sutherland JJ; Milne SB; Ryan KA; Daniels JS; Misra-Hebert A; Hicks JK; Vogan E; Teng K; Daly TM Medication adherence, medical record accuracy, and medication exposure in real-world patients using comprehensive medication monitoring. PLoS One 2017, 12, No. e0185471.

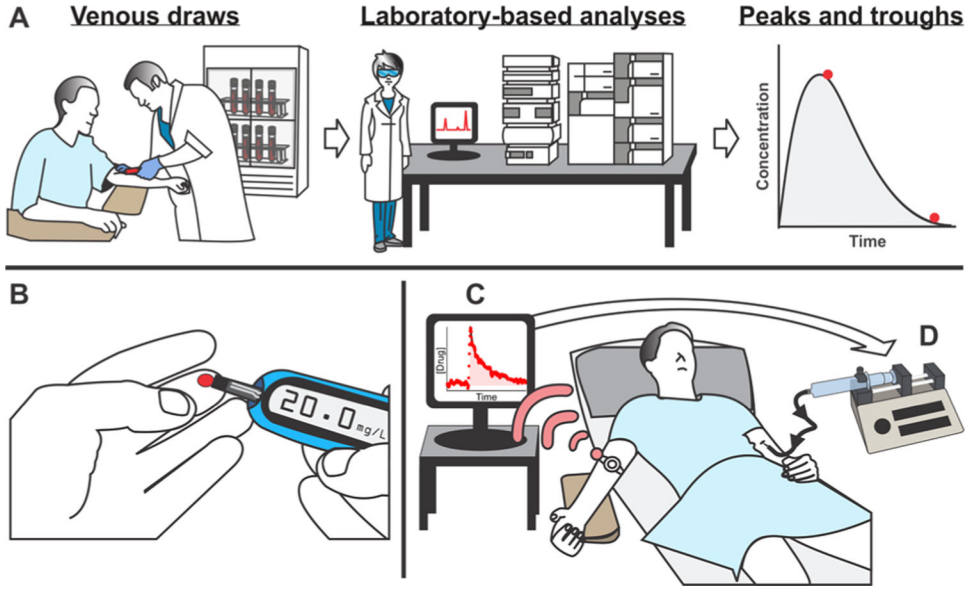


Figure 1.

(A) Current approaches to therapeutic drug monitoring (TDM) rely on venous blood draws and subsequent analysis in centralized laboratories, which are slow and cumbersome and provide only a few points for monitoring drug metabolism. Specifically, vancomycin's standard of care requires a "peaks and troughs" measurement (i.e., the highest and lowest concentration of the drug) in which often complex, multiphase pharmacokinetics are fitted.[26,27] Here, in contrast, we explore the ability of electrochemical aptamer-based (E-AB) sensors to improve on the convenience and accuracy of TDM. (B) For example, the ability of E-AB sensors to rapidly measure plasma drug levels using finger-prick volumes of unprocessed whole blood would enable self-testing and thus more frequently informed dosing. (C) Indwelling E-AB sensors supporting the high-frequency measurement of plasma drug levels could be used to determine patient-specific pharmacokinetics with unprecedented precision to (D) ultimately be used to perform feedback-controlled delivery in which dosing is optimized every minute in response to metabolic fluctuations.

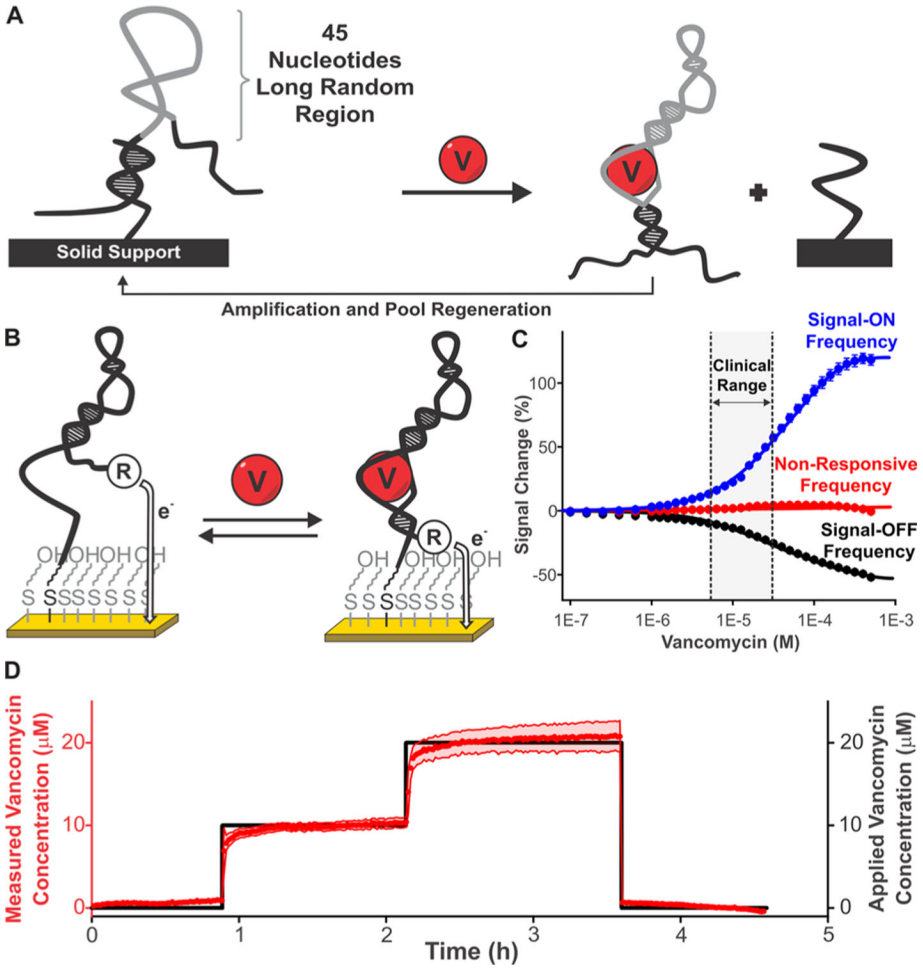


Figure 2.

We demonstrate here the fabrication of a new, vancomycin-detecting E-AB sensor. (A) To achieve this, we first performed a solution-phase aptamer selection scheme that utilizes a key stem-loop closing step. Briefly, this starts with a single-stranded, surface-attached sequence partially complimentary to a fixed region in a library pool of ~10^{14} distinct sequences flanked with two amplification regions and a random region of 45 nucleotides (in gray). Sequences that respond to the presence of target by detaching from their complement (presumably due to an intramolecular binding-induced conformational change) are then recovered, amplified, reconverted into a single-stranded library, and subjected to more rounds of selection. To measure the affinity of the resulting aptamer, we modified its sequence with a fluorophore and hybridized it using the partially complementary quencher-modified sequence (Figure S1). Titrating this with vancomycin further releases the complement sequence, thus increasing the measured fluorescence producing a dissociation constant (K_D) of ~0.1 μM. (B) We adapted the aptamer to the E-AB platform, which relies on a binding-induced change in the electron transfer kinetics of a redox reporter-modified (i.e., represented as “R”, which stands for methylene blue), surface-attached aptamer to produce a change in electron transfer rate that can be monitored using square-wave voltammetry. (C) Due to the dependence of electron transfer kinetics on the presence of target, the relative response of the vancomycin sensor is a strong function of square-wave

frequency, producing “signal-off,” “signal-on,” or “nonresponsive” behaviors[28–30] that cover the clinically relevant range concentrations[36] in undiluted, flowing whole blood. (D) Using the output at the nonresponsive frequency and the constants α (the ratio of the current at the nonresponsive frequency and at the responsive frequency at zero target concentration), K_D (the dissociation constant of the aptamer), and γ (signal gain) for vancomycin-detecting E-AB sensors (as a class), we can determine the concentration of vancomycin without the need for calibrating each individual device (see Table S1 for more details).[29] Doing so returns measured vancomycin concentrations within 20% of the applied concentration. The “error bars” shown here and those reported or illustrated elsewhere in this paper reflect 95% confidence intervals estimated from replicate measurements conducted on five independently fabricated sensors.

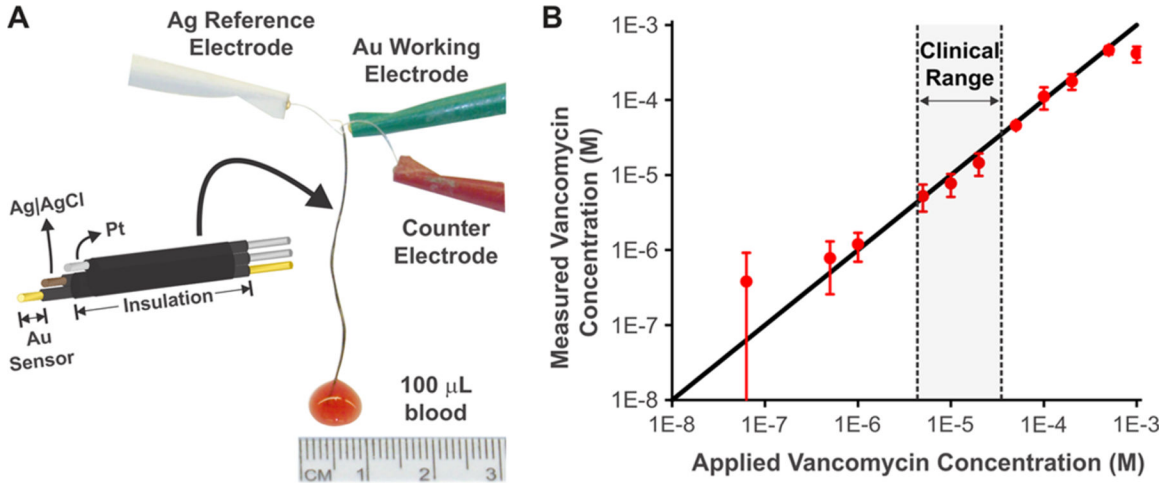


Figure 3.

Using ~200 μm-diameter, 3 mm-long (active sensing area) E-AB sensors, we have performed the calibration-free measurement of vancomycin in finger-prick-scale volumes (100 μL) of whole blood. (A) To achieve this, we employed sensors fabricated using 75 μm-diameter gold, silver, and platinum wires that can easily be immersed in small volumes. (B) Using the nonresponsive[29] square-wave frequency (Figure 2B), we achieve clinically relevant (± 20%) precision in the measurement of vancomycin over its entire clinical concentration range via a rapid (seconds) and single step. The asymmetry of the error bars is due to the use of a logarithmic *y* axis.

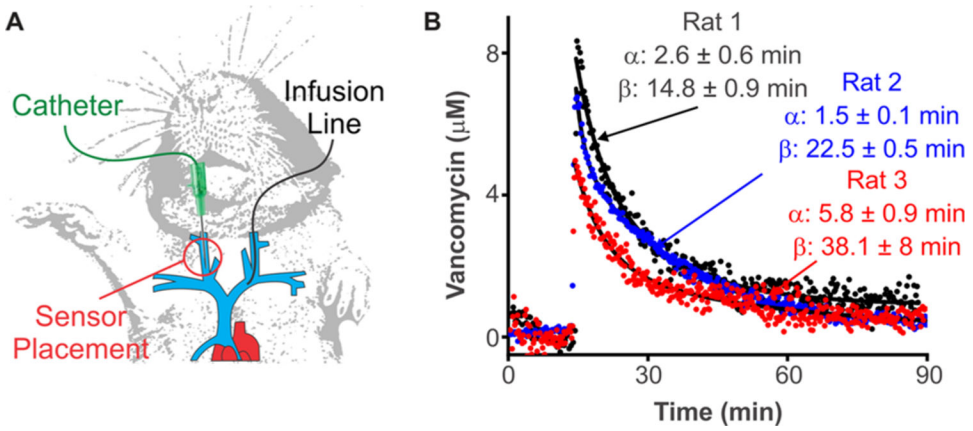


Figure 4.

Vancomycin E-AB sensor supports the high-precision measurement of patient-specific pharmacokinetics. (A) We show this via the insertion of a fine-wire sensor into the right jugular vein (via a 22G catheter) of a rat. We then insert an infusion line in the left jugular vein through which we administer vancomycin intravenously. (B) Intravenous dose of 30 mg kg^{-1} produces biphasic kinetics with elimination (β) time constants resolved to a precision of better than 20% (which represents the error at 95% confidence interval of the fitted trace).

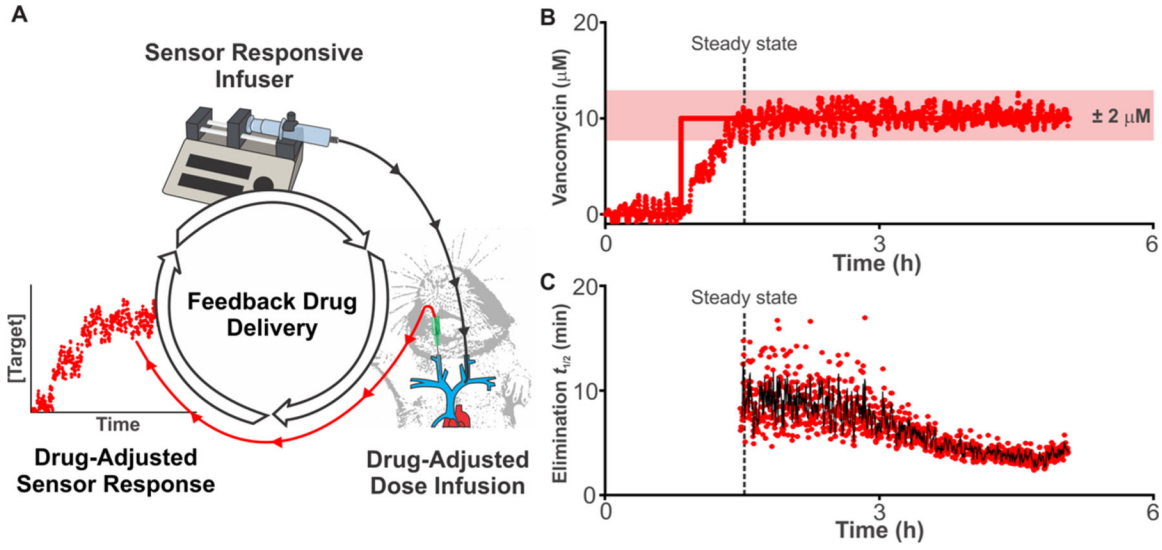


Figure 5.

E-AB measurements support feedback-controlled drug delivery, supporting in turn high-precision control over plasma drug levels. (A) For this, we use seconds-resolved E-AB measurements as the input to a proportional–integral–derivative (PID) controller that computes the dosing rate required to best reach the desired plasma concentration administered via a PID-controlled drug-delivery infuser. (B) Using this approach, we can maintain a plasma vancomycin concentration within 2 μM of, as shown here, a 10 μM set point for more than 5 h, a duration that is limited by animal welfare concerns rather than by sensor performance. (C) We maintain this concentration in the face of ~3-fold variation in the drug's elimination rate (calculated using the instantaneous infusion rate estimated by the PID); the black trace represents a 27 s (i.e., 3-point) rolling average.