Real time monitoring of drugs in the body with potential for feedback controlled drug delivery

Jennifer L. Hoover, Chief Scientist, Antibacterial Discovery Performance Unit, GSK, Collegeville, PA

**Kevin W. Plaxco, Director of the Center for Bioengineering, University of California, Santa Barbara, CA. [*Address correspondence to kwp@chem.ucsb.edu]*

The Plaxco group at the University of California, Santa Barbara is developing a novel and potentially transformational technology, termed Electrochemical Aptamer-Based (E-AB) sensors [1], supporting the real-time, seconds-resolved measurement of specific molecules in situ in the living body. Monitoring has been demonstrated both in vitro in undiluted whole blood and in situ in the jugular of awake, freely moving rats by means of a sensor small enough to insert via a 21-gauge catheter. It presumably can also be performed in situ in tissues, although this has not yet been tested. The system is based upon binding of the target molecule to a custom-designed aptamer (a short nucleic acid generated via in-vitro selection to bind to a specific molecular target) immobilized onto an interrogating electrode and modified with a redox reporter (typically methylene blue). Upon binding, the aptamer undergoes a reversible conformational change that, in turn, produces an electrochemical signal (see Figure 1 for schematic). The signal is detected by a hand-held device and transformed into unbound concentrations of the target molecule. Measurements are made as frequently as every 3 seconds and achieve clinically relevant precision and specificity. A feedback control loop can also be incorporated to adjust dosing in real time and maintain a desired concentration or recreate a specific concentration-time profile in the subject.

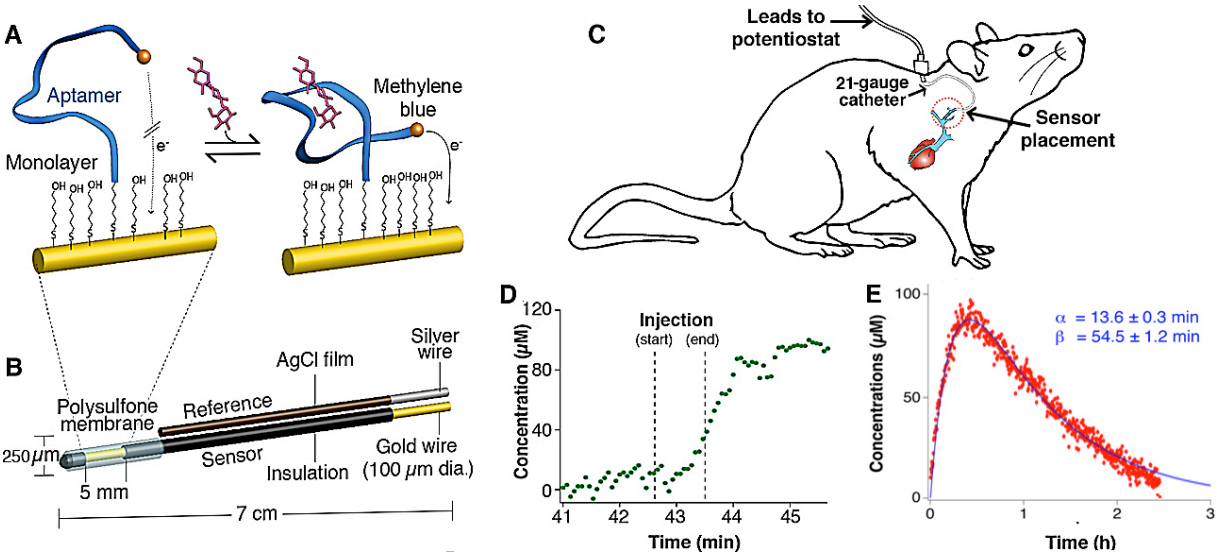

FIGURE 1 – The Plaxco group has achieved the continuous, real-time, multi-hour measurement of specific molecules in situ in the blood of awake, freely moving rats [Arroyo-Curras et al., 2017]. Innovations enabling this advance include: (**A**) Reagentless, reversible, E-AB sensors, in which an aptamer re-engineered to undergo binding-induced folding is modified with a redox reporter and attached (via a self-assembled monolayer) to a gold electrode. (**B**) This is then placed within a microporous (0.2 um) poly-ethersulfone membrane to prevent coagulation on its surface. (**C**) The resultant sensor is small and flexible and can be emplaced intravenously in a rat using a 21-gauge guide catheter. (**D**) Using square wave voltammetry to interrogate the device and measure the concentration of its target, the 3 s time resolution of the resultant in-vivo measurements enables the monitoring of even such rapid pharmacological events, as illustrated here, the few seconds duration *mixing phase* during which a 20 mg/kg IV bolus of tobramycin homogenizes in the blood after IV injection. (**E**) Their hours-long duration, in turn, enables the characterization of slower pharmacokinetic events, such as, shown here, the absorption of the same drug after IM injection and its subsequent excretion. The resultant data define the drug's two pharmacokinetic phases *in real time* and with *unprecedented precision*. Figure adapted from [2].

The team developing this exciting technology is a group of interdisciplinary researchers; as evidenced by their publication record, they have built strong expertise in protein biophysics and bioanalytical sensors. Capitalizing on the speed and specificity of biopolymer folding, the team aims to develop novel but broadly applicable biosensors for detection of a range of drugs or other biological molecules that can be used in real-time and real-world environments.

Description of the technology:

Although technologies already exist for the continuous measurement of a small number of metabolites (e.g., glucose, lactate, and oxygen) and neurotransmitters (e.g., dopamine, serotonin, glutamate, acetylcholine) in vivo, all are reliant on the specific chemical reactivities of their targets (e.g., the oxidation of glucose by glucose oxidase or dopamine's intrinsic redox chemistry) and thus are not generalizable to the detection of, for example, specific drugs [3]. In contrast, the aptamer-based technology developed by the Plaxco group is capable of detecting specific small molecules irrespective of their chemical reactivity, and it does so in a continuous, reversible manner that is selective enough to deploy directly in situ in live, mobile subjects. To date the Plaxco group and others have described more than a dozen such aptamer-based sensors, with the platform appearing to be broadly generalizable to new targets by leveraging the vast diversity of potential novel, specific aptamers [1]. Four of these have been adapted to continuous in-vivo monitoring in animals. Specifically, the Plaxco group has demonstrated the continuous measurement of the aminoglycoside antibiotics kanamycin, tobramycin and gentamicin; the drug of abuse cocaine; the chemotherapeutic doxorubicin; and the cytokine PDGF in real time in vivo in their rat live animal model.

Impact and value:

Application of this technology to antibiotics has the potential to transform the current dosing paradigm for treating bacterial infections. The following outline of the current antibacterial development pathway and the importance of pharmacokinetic/pharmacodynamic (PK/PD) principles in guiding antibiotic treatment regimens illustrates the potential impact of this technology. It is a widely accepted fact that effective antibiotic treatment depends on achieving target drug concentrations in the body, but it is challenging to ensure that this occurs in any given patient. Recommended dosing regimens, as indicated in drug labels and approved by regulatory agencies, are carefully selected by modeling and simulation to provide near-maximal probability of success; however, accumulating data suggests that this "one dose fits all" paradigm may not be applicable across all patient populations, particularly the critically ill. PK variability can be especially large in this population and, due to the challenges associated with conducting clinical trials in critically ill patients, sufficient PK data is not always collected from these patients to guide dosing adjustment. The current strategy is to inflate the PK variability observed in other populations by an arbitrary value (i.e., 40% to 50%) to account for increased variability in the critically ill; however, this precaution does not guarantee that adequate treatment is being provided to any given patient. Even for non-critically ill patients, drug levels may vary from day to day or as therapy progresses and may require dosing adjustment. An additional consideration is that some patients will be grossly overdosed using this strategy, which increases risk of adverse effects and tolerability issues. This collateral problem is accepted for most drugs as an unfortunate but necessary part of the current dosing paradigm. For some antibiotics (e.g., aminoglycosides), the window between efficacy and toxicity is known, and drug monitoring can be conducted to provide treatment within the ideal therapeutic window. This requires drawing blood and performing ex vivo assays to determine drug concentrations. The process may require specialized equipment and expertise (e.g., LC/MS/MS), assays must be conducted in a laboratory setting, there is a delay in obtaining the data, and it only provides a "snapshot" at the time(s) blood samples are taken. Given this background, the real-time drug monitoring technology described here could greatly impact both pharmacological research and clinical practice.

In non-clinical research settings, the proposed measurement technology could refine the conduct of experimental animal models. In larger species, PK data could be collected continuously and in real-time by insertion of the sensor into a pre-implanted catheter, providing ultra-high precision PK measurements with relative ease and convenience. In smaller species, blood samples could be collected and analyzed ex vivo by placing the sensor into the sample, again with far greater ease and cost-effectiveness than is the case with current approaches (e.g. LC/MS/MS). This would allow collection of more extensive PK/PD data, which would ultimately contribute to the advancement of clinical dosing. It would also simplify studies involving biothreat pathogens, as the samples could be evaluated directly within the restricted laboratory without requiring sterilization and shipment to off-site facilities for analysis.

The approach's real-time data supports feedback-controlled drug delivery, an advance that, for pre-clinical studies, would enable the accurate simulation of human PK in animal models. This is of special importance for studies designed to fulfill the FDA's Animal Rule (e.g. in vivo studies with biothreat pathogens) where accurate drug delivery and PK measurement in the animals is of utmost importance. Using feedback controlled dosing would also greatly simplify the process of recreating a human PK profile in animals. Controlling individual animals' PK profiles would reduce experimental variability, which is expected to translate to better results and has the potential to reduce the number of animals required for experimentation. These concepts of experimental refinement and reduction in animal use are two of the guiding principles for humane use of animals in research (i.e., the "Three Rs").

The potential clinical applications of the technology are also multi-fold. By allowing clinicians to monitor drug levels in each patient individually, the technology would remove one of the great hurdles for drug developers: how to select the single "right" dose. Currently, companies must employ techniques such as Monte Carlo (MC) simulation to determine one dosing regimen which will achieve the PK/PD target in at least 90% of all patients. Typically, as described above, this includes increasing PK variability by an arbitrary value to account for the more challenging populations. The logistical result of this practice is inflation of the dose, often at least doubling the original estimate. As the dose increases, so too does the likelihood of adverse events and poor tolerability. Likewise, increasing dosage leads to increased cost of goods (lowering the return on investment) and greater difficulty achieving an acceptable pharmaceutical product (due to pill size, solubility limitations, etc.). The inability of drug developers to reconcile all of these factors has led to termination of potentially useful drugs in the past, and it will likely continue to do so in the future. This is not a desirable outcome given our current desperate need for novel-acting antibiotics.

Finally, the feedback control enabled by a technology that provides continuous, real-time in-vivo drug measurements could also revolutionize drug delivery. The technology described here is easily transportable and has been demonstrated in animal studies to work on fully ambulatory subjects. Therefore, this system could be used either within medical facilities or outside of the hospital setting, such as treatment of wounded military personnel in the field. It would allow rapid adjustment of treatment to ensure that adequate but not excessive therapeutic concentrations are achieved at all times. Inclusion of autonomous real-time feedback control would optimize delivery of the ideal PK profile, reduce the burden on staff providing treatment, and improve adherence to frequent or more complex dosing regimens. Providing physicians with the means to individualize and control treatment in this way would transform medical practice for bacterial infections as well as other diseases.

In summary, given the ability to easily and effectively monitor drug concentrations in individual patients on a continuous basis, we could:

1. Ease the burden on drug developers that is created by artificial dose inflation and thus increase the chances of success for development of new antibiotics.
2. Advance the Personalized Medicine movement by providing a means for high-precision, patient-specific dosing.
3. Provide optimal treatment to each individual by ensuring adequate therapy is maintained while reducing side effects by eliminating gross overdosing.

Progress and current status:

To date the Plaxco group has made and successfully tested sensors detecting multiple aminoglycosides (kanamycin, tobramycin and gentamicin), doxorubicin, and cocaine in their live animal rat model (Figure 2). Given the precision of the resultant measurements, the technology can easily measure inter-individual variability in systemic exposure of these drugs (Figure 3). The aptamer that has been developed to date

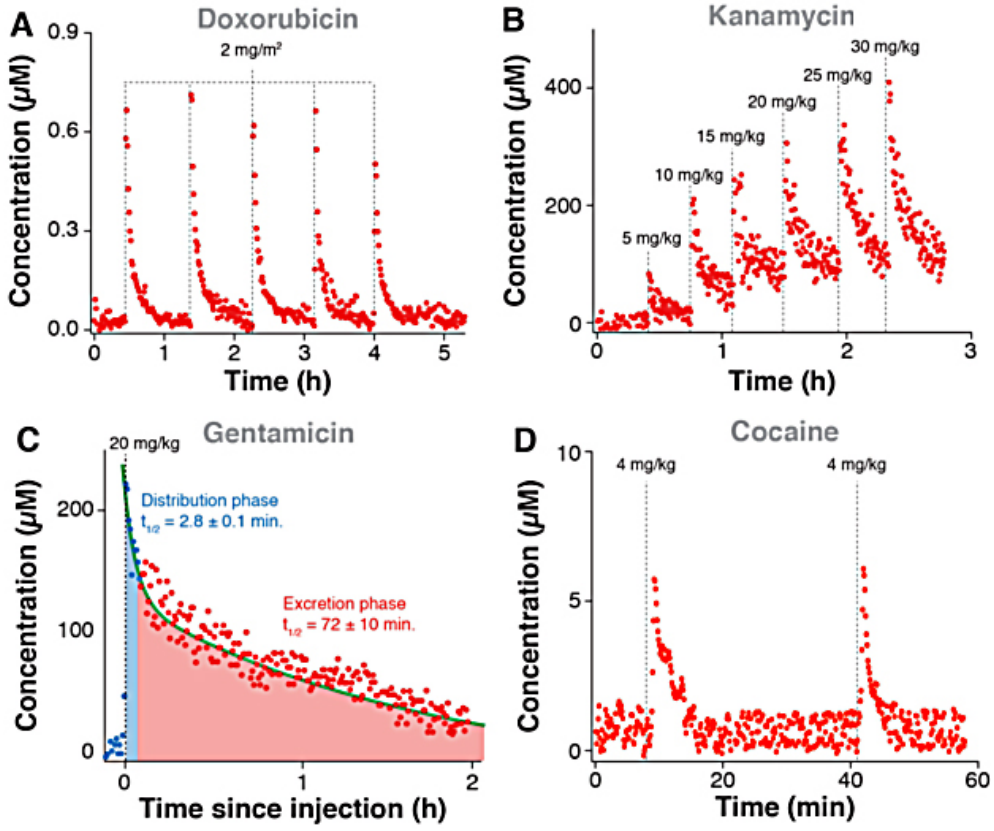

Figure 2. Continuous molecular measurements performed in situ in the living body. The Plaxco group has successfully used three different indwelling E-AB drug sensors to measure the plasma levels of five different drugs in situ in live rats, here using anesthetized animals. **(A)** Shown are five IV injections of the cancer chemotherapeutic doxorubicin at a dosage well below those typically used in humans. Using an aminoglycoside sensor the technology can likewise monitor in-vivo levels of the antibiotics (**B**) kanamycin, (**C**) gentamicin following one or more IV injections. The measured kanamycin doses span the 10-30 mg/kg therapeutic range used in humans. For gentamicin the data again illustrate the unprecedented resolution with which such results define pharmacokinetic phases. (**D**) Finally, a cocaine-detecting E-AB sensors has achieved the real-time in-vivo measurement of plasma cocaine levels, again in situ in the blood of a live rat after IV injection. In every case the kinetics and peak concentrations are consistent with values derived using established, if cumbersome, ex-vivo methods. Data from panels A, B, C: [Arroyo-Curras et al., 2017]; panel D: unpublished.

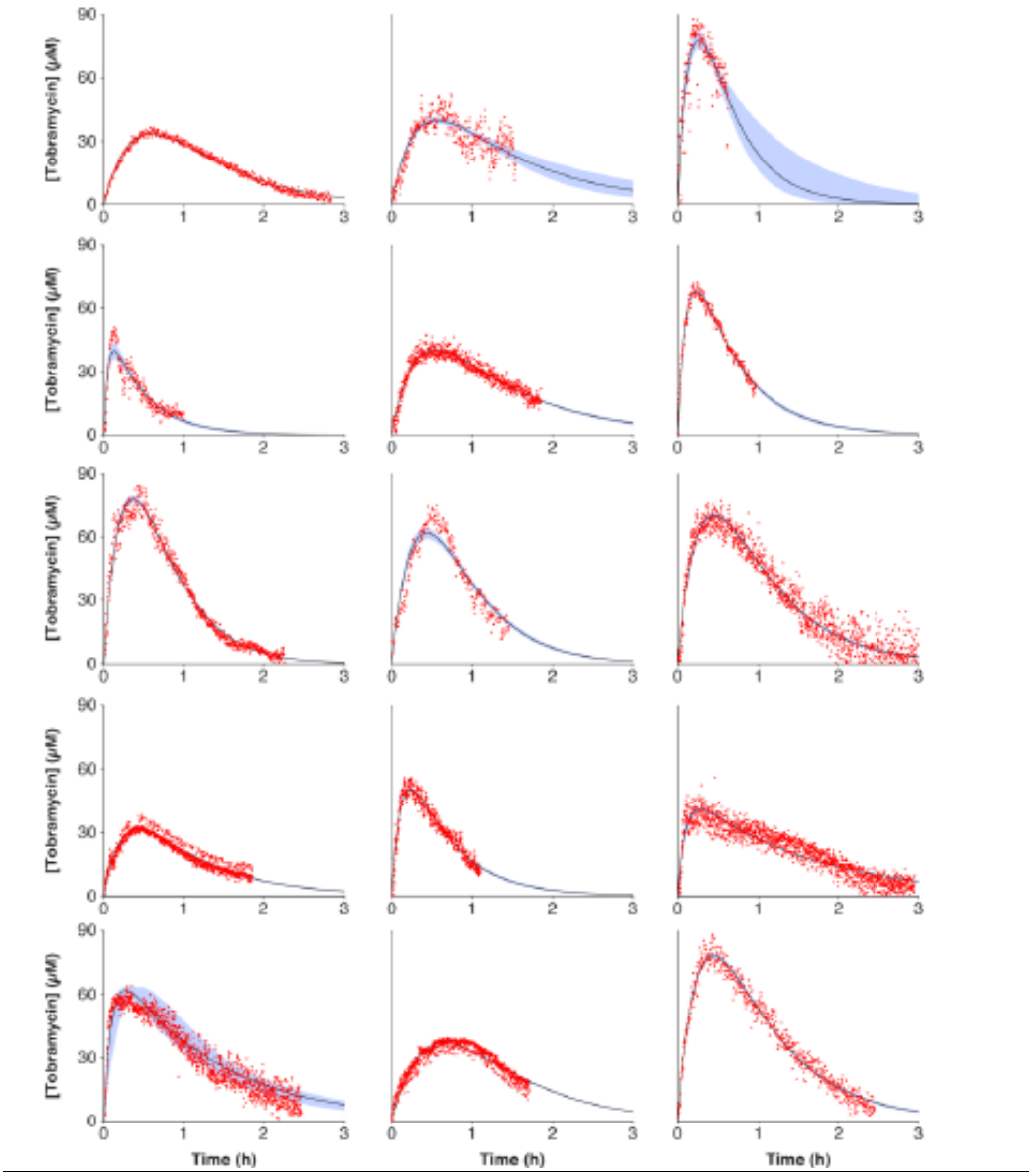

FIGURE 3 – Real time concentration-time profiles for tobramycin administered intramuscularly to different rats demonstrates the ease with which such high-precision PK data can be used to monitor inter-individual variability. Unpublished data.

for the aminoglycosides is generalizable to all aminoglycosides; in addition, aptamers specific to several individual aminoglycosides have recently been created. Aptamers have not yet been made for other antibiotics.

In initial in-vivo tests, signal "drift" was observed. To address this issue, the team modified the sensor to include a membrane covering and has developed a number of drift-correction [4, 5, 6] and drift avoidance [2, 4, 7] approaches that have been successfully applied.

The original sensor in rat studies fit an 18-gauge catheter [2]. This has now been reduced to 22-gauge, which requires a 75 um-diameter wire sensor. The physical properties of the sensor become limiting at that point. While it may be possible to further reduce the size, it would require microfabrication.

Frequency of signal detection has continued to increase as development of the technology progresses. The signal was detected approximately every 3 seconds in the initial publication [2], a measurement frequency that allows measurement of even the distribution phase of an IV bolus dose into the bloodstream (e.g., Figure 1d). More recently the measurement time has been pushed down to 300 ms.

Monitoring has been highly successful over short periods of time, i.e., up to 6 hours, in anesthetized animals. Tests to evaluate a full 24 hours of monitoring in awake animals have not yet been completed. Additional work is needed to explore monitoring duration. The current limitations are believed to be pragmatic challenges associated with experimental conditions (i.e., use of small lab animals) and not an inherent limitation of the technology.

Initial tests with feedback controlled dosing have been successfully completed, focusing on the feedback controlled dosing of tobramy cin in rats (Figure 4). Drug delivery was controlled to maintain the concentration-time profile at a fixed level midway between the toxicity threshold at the upper limit and the MIC_{90} at the lower limit (Figure 4c). Additional studies have demonstrated the feasibility of using feedback control to generate highly reproducible PK profiles from rat-to-rat (irrespective of variations in individual metabolism) and to produce PK profiles in rats that mimic known human PK profiles (Figure 4d).

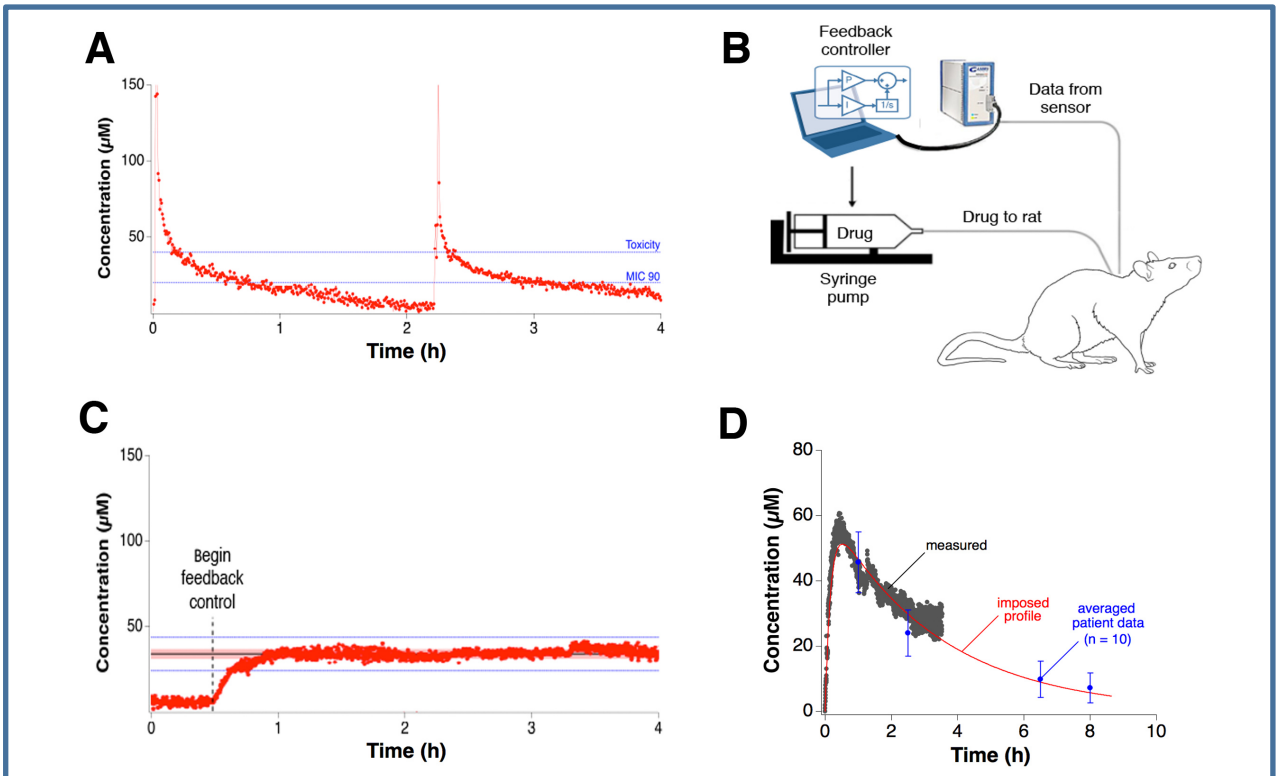

FIGURE 4 – A) Sequential intravenous injections (here in rats) leads to highly variable plasma drug levels. B) The ability to determine in-vivo drug concentrations in real time, however, supports closed-loop feedback control. C) This control, in turn, provides an unprecedented ability to maintain plasma drug concentrations near a set point midway within the ideal therapeutic window (i.e., between MIC_{90} and toxicity threshold). D) Using a dynamic, time-varying set point the technology can also be used to mimic, for example, human PK profiles in, as shown, a rat animal model. (See figure 3 for typical rat PK profiles . Unpublished data.

Requirements for further development:

In order to expedite further development of this technology, there are two key immediate requirements: funding and scientific input. There is also a longer term requirement for production/commercialization, even small scale, to support necessary testing and validation.

Current sources of funding for the research include the Army Research Laboratory and the Keck Foundation. Both, however, are ending soon and are tied to specific goals that diverge from antibiotic treatment as described in the Impact and Value section. To address antibiotic-specific goals, additional sources of funding will be necessary.

From a scientific perspective, the Plaxco group has renowned expertise in many aspects of this technology (e.g., biomolecular physics, electrochemistry, biosensors). However, their team would benefit from the expertise of infectious disease physicians, drug developers, and public health agencies. Furthermore, creation of aptamers for a broad range of antibiotics is not a trivial task. Additional scientific expertise and/or resource for aptamer selection may be required.

Finally, as the technology progresses into more advanced stages of testing, sources of production and commercialization will be needed. Even for small-scale production, the team will need partners with expertise in production of medical devices to make and assemble the various pieces of the sensor and potentiostat. Diagnostic Biochips, a current collaborator and licensee, may be able to fulfill this requirement. However, additional funding and/or support may be necessary.

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