Electrochemistry advances pharmokinetics

Improved electrochemical aptamer-based sensors, or E-ABs, allow real-time, *in vivo* measurements of molecular targets in the bloodstream.

Assessing the pharmokinetics and safety of a drug involves measuring the concentrations of different molecules in the bloodstream, including the drug itself and any resulting metabolites formed as the body processes it. Traditional methods are limited to snapshots, taken from blood samples drawn at different time points. If you're a rat, that means multiple handlings—and the inherent stress that comes along with those—and possibly anesthesia, which could influence how "normally" a drug is metabolized and limits translational relevance. Kevin Plaxco and his lab at the University of California Santa Barbara see an improvement to this process using customizable electrochemical aptamer-based sensors, or E-ABs.

"An aptamer," explains analytical chemist and postdoctoral fellow Netzahualcóyotl Arroyo, "is a synthetic piece of DNA that possesses very high affinity for molecular targets." Creating an aptamer involves an *in vitro* selection process, during which different DNA sequences are exposed to a desired target—a drug, or a protein or metabolite, for example—to see which strand binds best to it. To construct the sensor, the chosen aptamer is then modified to produce an electrical current whenever it binds its target, allowing the concentration of that molecule to be recorded.

E-ABs aren't new; the Plaxco lab has been studying them for over a decade, and they have been used for *ex vivo* sample measurements in the past. The goal in Santa Barbara was to develop an E-AB sensor capable of continuous, real-time molecular monitoring in an awake, freely moving animal; their proof-of-concept can be found in *The Proceedings of the National Academy of Sciences USA* (**114**, 645-650; 2017).

Moving into an animal meant the team had to overcome two main limitations from previous iterations: fouling and baseline signal drift. The circulatory system can be a harsh environment, and direct exposure to blood cells can quickly damage external devices. A commercially available, biocompatible polysulfone membrane—basically a hemodialysis membrane, Arroyo explains—was incorporated into the design to prevent direct interactions between blood cells and the surface of the sensor. To compensate for baseline signal drift, essential if the sensors were to be deployed *in vivo*, Arroyo took advantage of a previously published correction technique known as "Kinetic Differential Measurements," which corrects drift in real-time by use of electrical current recordings made at different sampling frequencies.

With improved sensors ready, the team first confirmed their functionality in anesthetized animals. The E-ABs, tuned to one of several different drugs including the anticancer agent doxorubicin and three antibiotics, were implanted consecutively into the jugular vein of Sprague-Dawley rats and left to measure the drug concentrations over several hours and multiple injections. The resulting concentration curves agreed with benchmarks established by traditional methods. The devices proved equally efficient in a second cohort of rats that were recorded while freely moving about a small cage, lasting for a full twelve hours and revealing details about inter- as well as intra-animal variability in concentrations over time.

The team plans to continue improving the sensors and explore where they can be successfully implanted—in rodents, and ultimately in people. "We want to achieve multi-compartment analysis, trying to measure different concentrations of drugs across the body in different places." This includes the brain, where Arroyo hopes that shrinking the sensors could lead to a breakthrough in the ability to monitor drugs crossing the blood-brain barrier. The lab is also interested in how the real-time monitoring the sensors provide could be used to improve therapeutic dosing based on patient-specific drug responses. Adding a chip capable of communicating via bluetooth would make the sensors wireless and the recorded data easily available to any paired device.

Arroyo hopes E-ABs sensors make it into the hands of other researchers as well. "The main limitation," he says, "is finding the right aptamer for the right molecule." The devices are built from commercially available materials, and the Plaxco lab continues to publish protocols and videos for others to follow. "We definitely are interested in teaching people how to use this technology so we can push hard into a future where aptamers can be used for real-time monitoring of a great variety of molecules….We are putting in the effort to make these as functional and robust as possible."

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