SENSORS AND PROBES

A sensor that makes sense

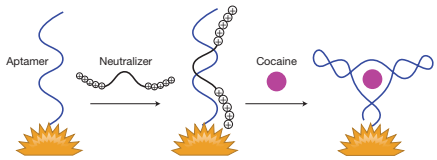
A 'neutralizer displacement assay' provides a general platform for electrochemistry-based sensing of any class of analyte molecule.

The features required in a molecular sensor are fairly plain. It should be highly sensitive and specific. It should be flexible and multiplexable. And, especially for clinical or field applications, it should be cheap, be simple to use and provide a rapid readout. Though the desirable features can be clearly defined, it has been a real challenge to achieve them in a single platform.

Shana Kelley and her team at the University of Toronto have been pursing an electrochemistry-based sensing assay that fits all these criteria. "We're very focused on electrochemical readout as a practical solution for biomolecular detection because it's a very easy type of readout to implement," she says. "One can use very cheap instruments to measure electrochemical signals...; for clinical diagnostics, that is key."

Kelley's team recently reported a technique they call the 'neutralizer displacement assay' (NDA). In a traditional electrochemistry-based sensor, a probe molecule such as an aptamer is attached to an electrode surface, to which the analyte molecule directly binds and triggers a change in the current. By contrast, the NDA is based on competitive displacement of a 'neutralizer,' a conjugate of a peptide nucleic acid (a synthetic DNA mimic) and cationic amino acids, which binds to a surface-immobilized anionic DNA probe and neutralizes its charge. Mismatches are designed into the probe-neutralizer complex such that binding of the analyte is favored over the neutralizer, resulting in neutralizer displacement. As the cationic neutralizer is released, this triggers a large measurable change in the surface charge.

The NDA expands the flexibility of traditional electrochemical sensors to detect any type of molecule with a single platform. "Usually different types of detection assays are geared towards one type of analyte," Kelley notes. "We thought it would be very interesting to come up with something that could really cover all of the major classes of molecules." NDA probe-neutralizer pairs can be designed to detect small molecules such as ATP, cocaine, DNA or RNA, or proteins such as thrombin, as the researchers showed in their recent report.



In the NDA, cocaine binds to an aptamer probe on an electrode surface, displacing the neutralizer and causing a large measurable change in surface charge. Reprinted from *Nature Chemistry*.

Additionally, NDA offers higher sensitivity than traditional electrochemical assays: displacement of neutralizer results in a dramatic change in the current, no matter the analyte charge, whereas in traditional sensors, the change in current depends on the charge of the analyte, which may be small or nonexistent. A side benefit of using a charge neutralizer is that the potentially high background signal (if the probe itself is charged) can be suppressed. The platform also offers specificity, as the team demonstrated detection of specific DNA in undiluted human serum.

The assay is also likely to be readily multiplexable, by endowing a sensor chip with multiple probes targeted to bind specific analytes. Although in a proof-of-principle experiment the researchers simultaneously detected just two small molecules, cocaine and ATP, they expect that multiplexed assays for up to 100 analytes could probably be achieved. This will be especially useful for clinical applications such as metabolite profiling, for example.

Additional developments, of course, are needed before NDA will become a useful clinical technique. The technology has been licensed to a spin-off company, so it may not be too long until it is available as a diagnostic tool. "The key thing that needs to be done for a commercial product is to automate the work flow," explains Kelley. "You have to give people a fully baked work flow so that it works for them every single time."

Allison Doerr

RESEARCH PAPERS

Das, J. *et al.* An ultrasensitive universal detector based on neutralizer displacement. *Nat. Chem.* **4**, 642–648 (2012).