MICROFLUIDICS

Hands-off protein crystallography

A new microfluidic protein crystallization device allows users to screen 'kinetic' rather than chemical conditions to grow high-quality crystals that can be diffracted *in situ*.

Protein crystallization has always been a bit more of an art than a science. It is nearly impossible to predict exactly which conditions will cause a protein to form a large, high-quality crystal, so crystallographers typically often screen thousands of different conditions in an empirical fashion. "Although we have gotten much better at collecting data and solving crystal structures (as beamlines and computation have improved), actually growing the crystals has remained an obstinate bottleneck," says University of British Columbia professor Carl Hansen.

Hansen is a former member of Stephen Quake's group at Stanford University, undoubtedly a leader in developing microfluidic methods to address the needs of protein crystallographers. The Quake laboratory quite successfully has been able to miniaturize and automate the crystallization screening process, greatly reducing the quantity of protein required and allowing many more screening conditions to be tested in parallel. These innovations have been widely embraced by those with large structural pipelines (such as pharmaceutical companies), who use the commercial TOPAZ System (Fluidigm) invented by the Quake lab.

As recently reported in *Journal of the American Chemical Society*, the Quake lab has now put forth a slightly unconventional alternative to the crystallization screening problem, by constructing a free interface diffusion (FID)-based microfluidic device that uses 'kinetic optimization' instead of varying chemical conditions. "On this device the chemistry is completely fixed; instead we control the mixing and dehydration rates to screen different trajectories through chemical space," explains Hansen. Each FID crystallization chamber on the chip is accessed by fluid channels of different lengths, which control the mixing rates of the protein and precipitant. The chambers are separated from a fluid reservoir by a permeable poly(dimethylsiloxane) (PDMS) membrane, which regulates the hydration.

In addition, this device provides a very practical benefit to protein crystallographers. Crystals grown inside the PDMS membranes in the chip can be punched out of the device and mounted directly for diffraction studies, such that direct handling is never needed. High-resolution structures could be solved from the *in situ* diffraction data. "In all other crystallization formats this is really problematic, to the point where you almost always cannot tell whether the crystal was a good crystal that was damaged during harvesting, or if it was a poor crystal right from the start," says Hansen. This valuable feature could save protein crystallographers a lot of time, not to mention headaches.

Allison Doerr

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Hansen, C.L. *et al.* A microfluidic device for kinetic optimization of protein crystallization and *in situ* structure determination. *J. Am. Chem. Soc.*; **128**, 3142–3143 (2006).

BIOSENSORS

Coke and chips—a winning combination?

An aptamer-based electronic sensor for the detection of cocaine demonstrates the capabilities of a sensitive and generalizable new platform for small-molecule recognition.

Unlike that of proteins and nucleic acids, the rapid detection of small-molecule targets remains a considerable challenge—particularly for work with biological samples. "If a doctor wants to look for a drug or drug metabolite in blood serum, it's got to be sent off to some lab where some tech does gas chromatography and mass spectrometry on it, and sends the results back days later," says Kevin Plaxco, a chemist at the University of California at Santa Barbara.

Plaxco's team has amassed considerable experience in developing biological detection systems based on aptamers, small DNA molecules capable of binding to target molecules with high affinity and specificity. Last year, they described an electronic aptamer-based (E-AB) protein sensor, wherein a gold electrode was conjugated with thrombin-binding aptamers linked with methylene blue, a compound capable of mediating electron transfer to the electrode; the thrombin-aptamer complex assumes a conformation that inhibits electron transfer, and the resulting change in signal reveals the presence of thrombin in biological samples (Xiao *et al.*, 2005).

In new work, they describe a similar system for the detection of a small-molecule target—cocaine. Their E-AB sensor proved capable of detecting micromolar quantities of cocaine diluted into complex biological fluids, like human saliva, and could even recognize the drug when it had been 'cut' with commonly used substances such as baking soda (Baker *et al.*, 2006). Current detection limits are still an order of magnitude too poor to detect cocaine use in patients, but the level of sensitivity that Plaxco's team has obtained with other E-AB sensors—into the picomolar range—offers encouragement that sensitivity could be enhanced with the use of improved aptamers.

Combining simple construction—according to Plaxco, many of the sensors were assembled by high school students working in the lab—with broad flexibility, this system offers exciting possibilities for new diagnostic tools, and Plaxco's group is now looking to build E-AB sensors for the simultaneous detection of multiple compounds, and for the detection of disease biomarkers. "We've got a small molecule–sensing platform that's as generalizable as aptamers themselves are—and the literature suggests they're pretty generalizable," he says. "Using these it should be easy to make a palm-top device that can detect a wide range of small molecules in seconds. That is really unprecedented; that's what I'm most excited about."

Michael Eisenstein

RESEARCH PAPERS

Baker, B.R. *et al.* An electronic, aptamer-based small-molecule sensor for the rapid, label-free detection of cocaine in adulterated samples and biological fluids. *J. Am. Chem. Soc.*; published online 18 February 2006.

Xiao, Y. *et al.* Label-free electronic detection of thrombin in blood serum by using an aptamer-based sensor. *Angew. Chem. Int. Ed.* **44**, 5456–5459 (2005).

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