SENSORS AND PROBES

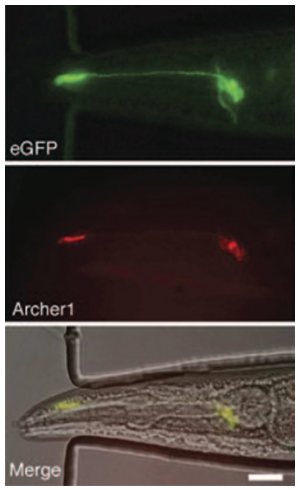
Voltage sensors revisited

Imaging of electrical activity *in vivo* in *Caenorhabditis elegans* is possible with the improved genetically encoded voltage sensors Archer1 and Archer2.

This past year has seen the publication of a number of new and improved voltage sensors, including ASAP1, MacQ, QuasAr1 and QuasAr2. The group of Viviana Gradinaru at the California Institute of Technology has now developed the most recent additions to the arsenal: Archer1 and Archer2.

Voltage sensors are the most direct sensors of neuronal activity. Ideally, they should possess high sensitivity, fast kinetics and high baseline fluorescence to allow monitoring of fast action potential trains and subthreshold events, which are synaptic events that do not trigger action potentials.

Archer1 and Archer2 possess these qualities. Both sensors are derived from archaerhodopsin-3 (Arch) and carry two or three mutations that were first identified in a mutagenesis screen of a related rhodopsin. In Archer1 and Archer2, these mutations lead to 3- to 5-times-higher baseline fluorescence, which allows imaging of these sensors at lower light intensities than that needed for wild-type Arch. They respond to artificial voltage changes with large fluorescence changes (85% and 60% $\Delta F/F$ per 100 mV for Archer1 and Archer2, respectively) that are in the range of the QuasArs' response and higher than the



Archer1-EGFP expression in *C. elegans* AWC neurons. Figure from Flytzanis *et al.*, Nature Publishing Group.

PROTEOMICS

PROBING THE PROTEIN STRUCTURE-OME

Protein structure changes can be charted on a global scale with a method that couples limited proteolysis with mass spectrometry–based proteomics.

The interior of the cell is like a city sidewalk at rush hour, crowded with dynamic entities bumping elbows and occasionally stopping to interact. A perturbation, such as an encounter with a ligand or a change in the environment, can cause structural changes to proteins. Although a number of methods exist to probe protein structural changes on the individual level, broader approaches that allow researchers to follow changes on a global scale upon perturbation have been lacking.

Paola Picotti of ETH Zurich and her team now provide such a method, which combines limited proteolysis—an established structural technique that has been used to probe individual proteins—with the large-scale power of proteomics. Picotti used limited proteolysis in her PhD work and honed her mass spectrometry skills as a postdoc. "The idea of coupling limited proteolysis to high-throughput proteomics tools was always in the back of my mind," she says. "And when I started my own lab, we gave it a try."

The method relies on applying broad-specificity proteases for short times to a protein sample—just enough to cleave proteins at flexible regions such as loops—thereby generating large protein fragments. The sample is then switched to denaturing conditions, and trypsin is applied to digest the fragments into peptides suitable for mass spectrometry analysis. Finding appropriate conditions to couple the limited proteolysis step to tryptic digestion was not exactly straightforward, however. "You need to find conditions that quench the activity of the broad-specificity protease but where trypsin is still active," notes Picotti. "This is where we struggled a bit."

By using a sample digested only with trypsin as a control, the team was able to

changes observed in ASAP1 and MacQ. Actual action potentials generate about half as much fluorescence change as the artificial situation.

Furthermore, Archer1 can track fast voltage changes (up to at least 150 Hz). This ability to track action potentials at high frequency places the Archer sensors in between ASAP1 and MacQ, whereas the QuasArs are best suited for neurons with lower action potential frequencies.

Finally, the improved Archer sensors display about 50- to 100-times-lower photocurrents than the original Arch when illuminated with red light. This property is useful because the activity of the monitored neurons is not influenced during the recording. On the other hand, Archer1 can serve as an optogenetic inhibitor when illuminated with green light. Under these conditions, Archer1 functions as an ion pump and can generate inhibitory currents. Thus, Archer1 can be used in a dual role: as a sensor and as an optogenetic inhibitor.

All of the mentioned voltage sensors have been shown to perform well in brain slices, but imaging neuronal activity *in vivo* is still difficult. Gradinaru and colleagues demonstrated the suitability of Archer1 for *in vivo* voltage monitoring in *C. elegans*. They expressed Archer1 in an olfactory neuron called AWC-ON and recorded the activity of this neuron in response to a suitable olfactory stimulus. Upon odor withdrawal, the researchers observed a slow increase in fluorescence that persisted for about 10 seconds. This response is consistent with previously reported measurements of calcium activity in these neurons and confirms Archer1 as a useful sensor *in vivo*, at least in neurons with slow electrical activity.

Researchers interested in visualizing voltage changes now have a variety of tools to choose from. Depending on the applications, some sensors may be better suited than others, but this will have to be tested empirically. In the case of the Archer sensors, it will be interesting to find out whether they can perform as well in the mammalian brain as in the *C. elegans* brain.

Nina Vogt

RESEARCH PAPERS

Flytzanis, N.C. *et al.* Archaerhodopsin variants with enhanced voltage-sensitive fluorescence in mammalian and *Caenorhabditis elegans* neurons. *Nat. Commun.* **5**, 4894 (2014).

compare two different sample conditions, each subjected to limited proteolysis, by mass spectrometry. Depending on whether one is interested in following specific proteins or the whole proteome, a targeted technique called selected reaction monitoring–mass spectrometry or a standard 'shotgun' proteomics approach, respectively, can be used. From the mass spectra, the researchers identified 'conformotypic' peptides: those that were specifically observed under a given condition. Such conformotypic peptides can also be mapped to an existing protein structure or homology model to gain insights about structural changes that occur following a perturbation.

As an example of a targeted application, the team compared unfolded and amyloid-like conformations of the Parkinson's disease–related α-synuclein protein spiked into a complex cell-extract background. They also were able to detect more subtle conformational differences, as between the holo and apo forms of myoglobin. Using a discovery-based approach, they followed over 1,000 yeast proteins upon a switch from glucose- to ethanol-based growth, which is known to result in metabolic remodeling. In total they identified 283 proteins with substantial structural changes, most of which were known to be involved in catalysis or metabolic processes.

"The method is applicable to many more questions," says Picotti. She suggests that this powerful combination of structure probing with mass spectrometry–based proteomics could be used to identify novel targets of drugs, to discover conformational biomarkers of disease and to assess protein stability on a global level. The method is currently limited to soluble proteins, but her team is also hard at work to extend it to membrane proteins.

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RESEARCH PAPERS

Feng, Y. *et al.* Global analysis of protein structural changes in complex proteomes. *Nat. Biotechnol.* **32**, 1036–1044 (2014).