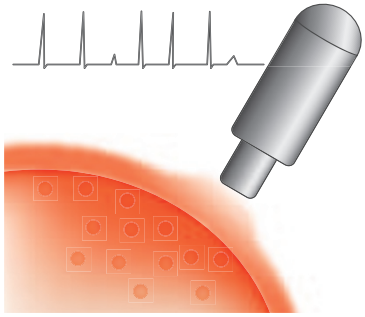
Light-based electrophysiology

Genetically encoded voltage sensors are finally measuring up.

Neuroscientists know well how technically challenging it is to poke a pipette into a living cell to measure its electrical properties. Skilled researchers with a gift for this meticulous methodology and the patience required to perform it are highly sought after in many laboratories, but they are becoming a rare species.

Voltage-sensitive fluorescent dyes have become an alternative to electrical cell recordings using pipettes. But although dyes are fast and sensitive enough to detect single action potentials in spiking neurons, phototoxicity and challenges in delivery have prevented their widespread use.

A fast and sensitive genetically encoded voltage indicator is high in the list of most-wanted tools by neuroscientists. Initial versions were substantially slower than dyes and not sensitive enough to reveal single action potentials in cells. Major efforts to develop better protein-based voltage sensors have come from Thomas Knöpfel at RIKEN, who uses fluorescence resonance energy transfer and a voltage-sensing phosphatase from a sea squirt as the basis for the design of new voltage-sensitive fluorescent proteins. These sensors have been used to detect action potentials in mammalian neurons *in vivo,* but their sensitivity is still not high enough to detect single action potentials from single trials (*Nat. Methods* **7**, 643–649; 2010).



Fast and sensitive detection of action potentials with a genetically encoded voltage indicator.

The group of Adam Cohen at Harvard University has recently used an entirely different class of proteins to develop fast and sensitive voltage indicators: microbial rhodopsins. The first of such voltage indicators, proteorhodopsin optical proton sensor (PROPS), is based on the endogenous fluorescence of a rhodopsin from marine bacteria, but its use is limited to prokaryotes (*Science* **333**, 345–348; 2011). In this issue, Cohen and colleagues describe the use of Archaerhodopsin 3 (Arch)—a light-driven proton pump better known for its capacity to silence neurons in optogenetic experiments—as a new class of voltage sensor for mammalian neurons (*Nat. Methods* **9**, 90-95; 2012).

Arch and its nonpumping mutant, Arch(D95N), could resolve individual action potentials in cultured mammalian neurons with high signal-to-noise ratio and low phototoxicity. But there is still much room for improvements that will one day lead to high-quality all-optical electrophysiology both *in vitro* and *in vivo*. **Erika Pastrana**

RNA structures

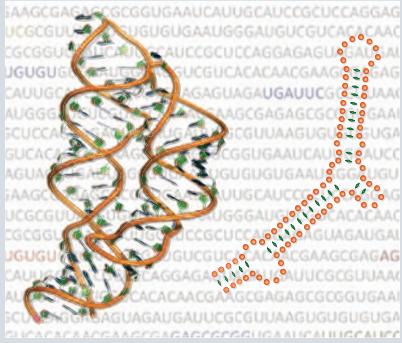
Accurate methods for RNA-structure determination are being developed.

Although synthesized as linear polymers, RNA transcripts fold into intricate structures crucial for cellular physiology. It is now evident that RNA functions extend far beyond the established roles of messenger, transfer and ribosomal RNAs, with examples in RNA splicing and editing, telomere maintenance, protein secretion, small-molecule sensing and reaction catalysis, to name just a few. How RNA achieves its arsenal of functions with a limited assortment of building blocks is a question of great interest, and the answer often lies in deciphering RNA structure at its different levels of complexity.

RNA-structure determination, however, is far from trivial. Many RNAs are poorly conserved, and their function cannot be inferred by simple homology searches based solely on primary structure. Covariation analyses of secondary structure conservation are therefore often preferred, and they can even guide the computational prediction of functional three-dimensional RNA modules.

To decipher RNA secondary structure in the first place, several high-throughput experimental approaches have been developed. Recent advances in chemical and enzymatic RNA footprinting have demonstrated the possibility for high-throughput secondary-structure mapping with single-nucleotide resolution. With careful design, such techniques can even provide information on the native three-dimensional fold of RNA transcripts.

An even higher-resolution picture of RNA tertiary structure can be obtained by classical structural biology methods. These, however, face limitations as throughput is usually low, nuclear magnetic resonance remains limited to fairly small molecules, and crystallization of large, negatively charged RNAs is far from trivial. Computational prediction methods have provided a valuable alternative, but they are often limited to conserved RNA folds, require substantial computer resources and cannot account for the full complexity of environment-dependent and intramolecular interactions influencing RNA structure.



Going from sequence to tertiary RNA structure (structural information is from *Structure* **19**, 1413–1423; 2011).

With single-molecule diffraction deemed theoretically possible and the ongoing development of X-ray free electron laser technology, a high-resolution picture of the 'RNA structurome' seems plausible. Concerted efforts can increase data collection and analysis throughput, and results can greatly facilitate prediction and high-fidelity modeling of additional transcripts. Although it could take some time for this to happen, we will certainly be watching for reliable new methods for RNA-structure determination. **Petya V Krasteva**