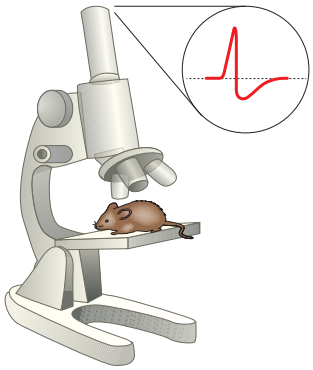
In vivo voltage sensors

Genetically encoded voltage indicators are on the brink of allowing neuronal activity to be directly imaged *in vivo*.

Visualizing the activity of neurons in living and behaving animals can shape our understanding of neuronal function in the context of an intact network. Genetically encoded voltage indicators report fluctuations in cellular membrane potential and therefore sense neuronal activity more directly than calcium indicators. In contrast to traditional electrophysiological techniques, these sensors allow for analysis of neuronal activity in many defined neurons at the same time. Despite recent progress in their development, fluorescent voltage indicators that work well in *in vivo* applications, especially in mammals, are still missing.

Ideally, these sensors would combine fast kinetics with high sensitivity while requiring low laser excitation power to minimize phototoxicity in living tissue. Sensors with these properties would enable the recording of single action potentials *in vivo* without the need for averaging signals from many trials. Furthermore, a good signal-to-noise ratio would be useful for monitoring subthreshold events such as excitatory or inhibitory postsynaptic potentials, which are less prominent than action potentials.



Katie Vicari/Nature Publishing Group

Imaging membrane potential in living animals.

In recent years, notable improvements to voltage sensors have made them suitable for many *ex vivo* applications. Reports of using voltage sensors in living animals, on the other hand, are few: these include ArcLight in *Drosophila melanogaster* (*Cell* **154**, 904–913, 2013) and Archer1 in *Caenorhabditis elegans* (*Nat. Commun.* **5**, 4894, 2014). In *Drosophila*, ArcLight could report individual action potentials even in single trials. Although the recently developed sensor MacQ-mCitrine has improved characteristics compared to ArcLight, sensing single action potentials in live mice is still at the edge of its capabilities (*Nat. Commun.* **5**, 3674, 2014).

We expect to see further improvements in the near future that will make voltage sensors suitable for robust *in vivo* imaging, in challenging scenarios similar to those for which calcium sensors are being used. These developments might include tweaks to existing sensor classes or involve completely new designs with improved kinetics, sensitivity and signal-to-noise ratio. Advances in image-based voltage sensing will bring within reach the answers to many important questions about neuronal activity in living

Next-generation CRISPRs

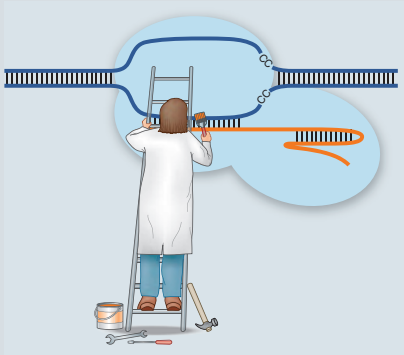
As the CRISPR-Cas system matures, specificity, efficacy and maybe even a eukaryotic nuclease are being considered.

When people think about enabling technologies, inventions such as the printing press and discoveries such as anesthetics come to mind. For scientists, the CRISPR-Cas9 system falls into a similar category. This bacterial immune system confers resistance to viruses by incorporating short repeats of the viral DNA into the bacterial genome. When a bacterium (or one of its descendants) is infected a second time, transcripts of these repeats target a nuclease to the invading, complementary DNA and destroy it. Several groups showed in 2013 that the system can also be used to edit eukaryotic genes, and CRISPRs have subsequently seen a meteoric rise in applications, from generating deletions and insertions in the genomes of many and diverse species to activating or repressing gene transcription. But as the system's popularity has risen, questions about its specificity and efficacy have emerged as well—and are only beginning to be addressed.

Reports of how to increase specificity of the guide RNA (gRNA) that brings the Cas9 nuclease to its genomic locus give seemingly contradictory answers. One group suggested truncated gRNAs (*Nat. Biotechnol.* **32**, 279–284, 2014) to reduce off-targets without affecting on-target activity; another recommended slightly longer gRNAs (*Genome Res.* **24**, 132–141, 2014).

The question of how to best screen for off-targets, and how much they really matter, will also have to be addressed systematically. Approaches that test only candidate sites with a few mismatches to the gRNAs may miss potential cleavage sites, and whole-genome sequencing is not efficient. A sensitive and unbiased method is needed that labels Cas9 target sites and allows them to be identified genome wide.

Other efforts to make the system more specific have included replacing the Cas9 nuclease with paired nickases that each cleave only one strand, or with a Cas9 mutant fused to the Fok1 nuclease that needs to dimerize for activity. Using Cas9 from different organisms will also add flexibility in targeting multiple loci. Further improving the delivery of the Cas9-gRNA complex to cells will increase efficiency. Despite its potential, Cas9 remains a bacterial protein, and for some applications—clinical ones in particular—it may be advantageous to replace Cas9 with a eukaryotic nuclease. In plants, some Argonaute nucleases are directed to DNA via small RNAs, an observation that could potentially be exploited for genome editing.



Debbie Maizels/Nature Publishing Group

CRISPR tools under construction.

Nicole Rusk