NEUROSCIENCE

A nontoxic rabies virus for neural circuit mapping

A double-deletion rabies virus variant overcomes the cytotoxicity that has previously limited long-term applications for retrograde labeling of projection neurons.

Recombinant rabies viruses (RVs) can be used to infect neurons at axonal projections, at which point they are transported to the soma. The infection can either spread to neurons presynaptic to the starter neurons or simply lead to marker gene expression in the targeted projection neurons, both of which are important applications in neural circuit mapping. RVs known as ΔG viruses are attenuated owing to the deletion of the viral envelope glycoprotein, but even they cause cytotoxicity that can impair the structure and function of infected neurons.

Attempts to reduce RV-associated toxicity have included the use of a less toxic RV strain or a self-inactivation strategy, but these approaches do not entirely overcome the problem. "We just wanted to make a completely benign rabies virus," says Ian Wickersham from the Massachusetts Institute of Technology. In collaboration with scientists from the Allen Institute for Brain Science, Wickersham and his team pursued a double-deletion strategy. "The idea was to remove almost entirely [the virus's] ability to express any genes from its genome," he explains. The commonly used ΔG virus is impaired in the final stages of the life cycle, but it can still replicate, which dooms the infected cells. To avoid viral replication, the researchers generated ΔGL viruses by deleting the viral polymerase gene, "which is absolutely required for expression of genes from the viral genome," says Wickersham.

Abolishing essentially all expression from the viral genome means that the expression of engineered cargo, such as fluorescent proteins, also will be affected, which can be a problem. However, each viral particle "comes with a few polymerase proteins packaged in the virion," says Wickersham. These polymerases lead to a "tiny amount" of transcription, which, although

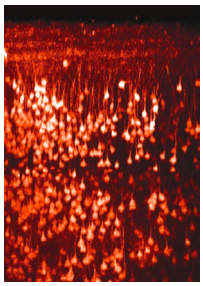
IMAGING

CORRELATION ANALYSIS IN SINGLE-MOLECULE LOCALIZATION MICROSCOPY

A coordinate-based framework quantifies the correlation and interaction of biomolecules in images acquired with super-resolution microscopy techniques.

Correlation analysis is an image-processing method routinely used to measure spatiotemporal relationships within images and structures. This information can be used for measurements of colocalization and clustering, and to align images of individual structures. When acquired with conventional fluorescence microscopy, images consist of pixels (2D) or voxels (3D), and there are many methods available to analyze and quantify correlations in such data.

In localization-based super-resolution microscopy, however, the situation is different. "Over the years, when we were trying to apply super-resolution microscopy to answering various biological questions, we kept running into problems of quantitatively analyzing and interpreting our images," writes Bo Huang from the University of California, San Francisco. Because super-resolution microscopy images are a collection of 2D or 3D coordinates that are additionally associated with localization uncertainty, "existing image analysis packages do not work at all for localization-based super-resolution images," and this, says Huang, is an underlying reason for the difficulties encountered by his team. One way to address this problem is to bin the coordinates on a pixel grid so that a correlation function can be computed. However, this comes with the drawback of lost localization information. Another approach researchers have resorted to is to calculate the distribution of point distances. Huang and his colleagues have now shown "that these two routes are actually mathematically equivalent" and have developed a software based on this conceptual framework with the advantage that it can also model the effect of localization uncertainty. The user-friendly software enables analysis of common scenarios where correlation is of interest.



Neurons remain healthy after long-term infection with ΔGL RV. Adapted with permission from Chatterjee *et al.* (2018).

insufficient for reporter gene expression, can generate enough Cre or Flp recombinase to induce Cre- or Flp-dependent reporter gene expression.

The researchers verified that the ΔGL virus is benign by monitoring the health of infected neurons over four months. They found that neither the structure nor the response properties of infected neurons changed over time.

In addition to the complete absence of cytotoxicity, the tropism of the ΔGL virus, meaning its preference for infecting particular cell types, distinguishes this RV from alternative retrograde tracing tools such as CAV2 and rAAV-retro. For corticocortical projections, "the rabies virus had broader tropism than either of those two species," says Wickersham. This property makes the ΔGL RV more broadly applicable.

The researchers demonstrated the utility of the nontoxic RV for labeling projection neurons in different brain regions in mice and rats. However, a major application for RVs is monosynaptic tracing. For this, the ΔG RV must pick up G-glycoproteins that are supplied in starter neurons, so that the virus can complete its life cycle and infect neurons presynaptic to the starter cells. Wickersham and his team are currently working to establish monosynaptic tracing for their ΔGL virus by providing both G and L proteins in the starter cells. "It will lead to toxicity in the starting cells," concedes Wickersham, but they are planning to express the proteins at low levels and to use the previously described less toxic CVS N2c strain. Such a system would enable long-term functional studies with, for example, optogenetic tools.

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Chatterjee, S. *et al*. Nontoxic, double-deletion-mutant rabies viral vectors for retrograde targeting of projection neurons. *Nat. Neurosci.* **21**, 638–646 (2018).

The researchers first illustrate how their framework aligns and averages multiple super-resolution images. This strategy increases the signal-to-noise ratio and improves the effective resolution of an image. Previously, this process relied mostly on either binning of the coordinates on a pixel grid or manual image stacking and use of a predefined structural model, which can lead to artifacts if the wrong initial model is chosen. To demonstrate the workflow of their algorithm, the researchers used noisy DNA-PAINT images of a DNA origami structure and integrated their framework into a cryo-EM reconstruction strategy. First, the images are rotated and translated to create overlapping centers of mass. The sum of all these images serves as the new reference image. Then, the software calculates the rotational cross-correlation of each image with the reference and finds the correlation maxima. Subsequently, each image is rotated and translated accordingly to maximize its correlation with the reference image. The authors repeated this process several times until the alignment was satisfactory. In their example, the researchers achieved high alignment precision, as indicated by the agreement of the mean localization precision and the peak width of the correlation function.

The other two applications enabled by the software are the characterization of fast molecule diffusion on the plasma membrane and quantification of colocalization in STORM images.

Although the framework has been applied only to 2D data so far, it should be applicable to 3D data as well. Huang also stresses that the framework is versatile and "should enable power users to design more specific implementations for a wide range of applications not covered in our paper, such as measurement of image resolution and cluster analysis."

Christian Schnell

RESEARCH PAPERS
Schnitzbauer, J. *et al.* Correlation analysis framework for localization-based superresolution microscopy. *Proc. Natl. Acad. Sci. USA* **115**, 3219–3224 (2018).