Targeting multiple brain cell types through engineered viral capsids

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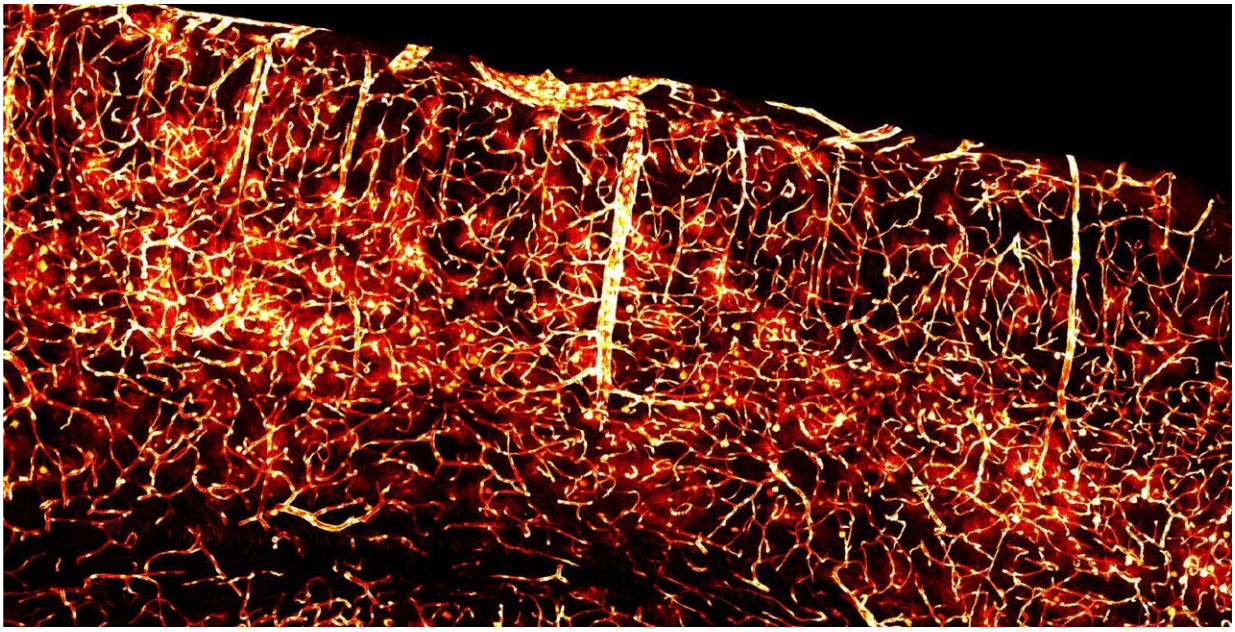


Credit: Sripriya Ravindra Kumar in the Gradinaru laboratory at the California Institute of Technology

Viruses are nature's Trojan horses: They gain entrance to cells, smuggle in their genetic material, and use the cell's own machinery to replicate. For decades, scientists have studied how to minimize their deleterious effects and even repurpose these invaders to deliver not their own viral genome, but therapeutics for treating disease and tools for studying cells. To be effective in these new roles however, each virus must safely,

efficiently, and selectively act on those cells in which its genetic cargo is desired. This is a task for which the natural repertoire of viruses is ill-equipped.

Caltech researchers have now developed a method to rapidly and efficiently identify designer adeno-associated virus (AAV) variants that can deliver to or "transduce" specific types of cells in mice, enabling scientists to choose a virus based on research or clinical needs. Natural AAVs are a particularly promising starting point for this work as the virus has no known disease association despite its wide prevalence in human populations, does not elicit a strong immune response, and cannot reproduce on its own. Caltech graduate student Sripriya Ravindra Kumar is the first author of a paper about the research appearing in the May issue of the journal *Nature Methods*.

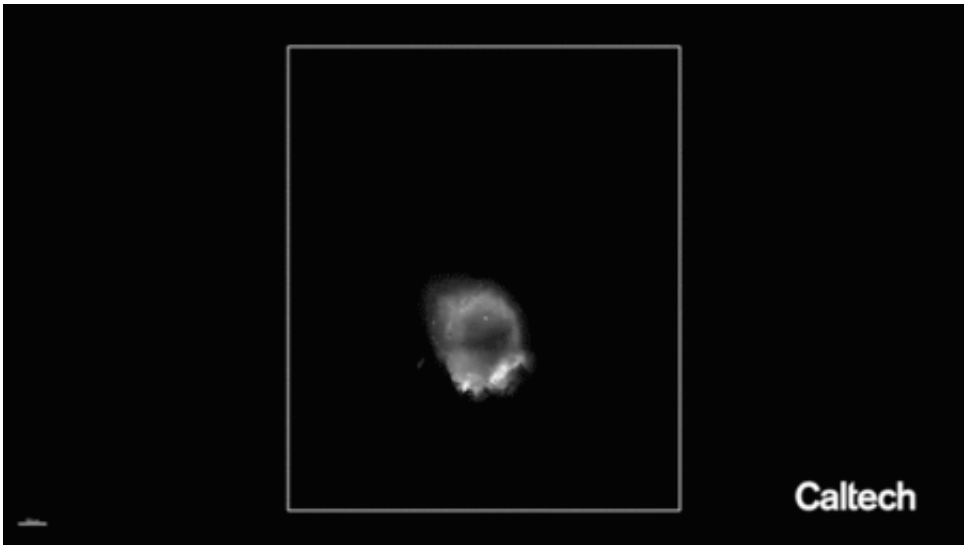
The work was conducted in the laboratory of Viviana Gradinaru (BS '05), professor of neuroscience and biological engineering, Heritage Medical Research Institute Investigator, and director of the Center for Molecular and Cellular Neuroscience at the Tianqiao and Chrissy Chen Institute for Neuroscience at Caltech.

"If you can re-engineer a virus to deliver genetic messages that can translate to a protein of therapeutic interest, you can treat a disease at the cellular source," says Ravindra Kumar. "A lot of work remains to understand diseases at the cellular level though. The good news is that these viruses can be put to use by the larger scientific community to study cells and disease associations."

All viruses have the same fundamental design: they are pieces of genetic material, either RNA or DNA, protected by a shell called a capsid. An AAV capsid's surface chemistry can influence its ability to latch onto a cellular membrane protein, thereby enabling entry to a cell. In natural viruses, capsids have been evolved to latch onto proteins that are present

on the surfaces of many different tissues or cell populations. However, to make them useful to deliver therapeutics to only the cells that need them, bioengineers work toward designing capsids that enable entry into specific cell types. This is possible if the designer capsids can latch onto membrane proteins that are found uniquely in one cell type and not others.

To this end, the Gradinaru laboratory has been working toward assembling a catalogue of viral vectors for different cell types and uses by recruiting the power of directed evolution to survey millions of AAV capsid variants in a single host. In the new work, Ravindra Kumar and her collaborators developed a method called M-CREATE (Multiplexed Cre recombination-based AAV targeted evolution). This method rapidly screens large libraries of AAV capsids, each uniquely decorated with a new surface loop, to gain interactions with different cellular proteins, and thereby enter specific cell types in mice.



An engineered viral capsid, containing genes for fluorescent molecules, was delivered into the bloodstream of an adult mouse that was genetically modified to express a protein called Cre in its endothelial cells. Fluorescent gene expression was made dependent on expression of Cre so that only endothelial

cells fluoresce. One brain hemisphere was then cleared and labeled in order to visualize intact vasculature in large tissue volumes. Credit: Dr. Alon Greenbaum and the Gradinaru laboratory at the California Institute of Technology

"We now show by M-CREATE that AAVs can be engineered for cell-type specificity by the capsid alone, removing the need for gene regulatory elements to achieve specificity and therefore freeing up crucial space within the small AAV capsid for needed genetic material," says Gradinaru.

The work builds upon an earlier method developed in the Gradinaru laboratory. In 2016, the group determined how to engineer an AAV to pass through the blood–brain barrier (BBB), a tightly packed layer of cells that normally prevents circulating viruses and other pathogens from entering the brain and spinal cord. The modified viral vectors can now be administered through a simple intravenous systemic injection, allowing researchers to avoid invasive injections into the brain, and have been widely adopted as research tools. However, the previous screening process was time- and labor-intensive, identified only a handful of capsids, and could not accommodate screens across multiple targets necessary to uncover capsid specificity for multiple tissues and then the diverse cell types within those tissues.

Crucially, M-CREATE provides not only a "winner" capsid for the competition to transduce a cell type but a kind of finisher's podium, ranking the fitness of thousands of other capsid variants at the same time. This improvement allowed the team to identify new capsids that, upon delivery into the bloodstream, preferentially transduce either neurons in the brain or the vascular cells that form the blood-brain barrier. "Engineering gene-delivery tools that specifically target neuronal and non-neuronal brain cells relevant to disease, such as the brain

endothelial cells comprising the vasculature and the BBB, can enable paradigm-shifting research in, for example, neurodegeneration or stroke," says Gradinaru. "As an impaired BBB can allow pathological factors into the brain that initiate and/or accelerate neurodegeneration, functionally targeting BBB permeability via engineered AAVs can affect body-to-brain access through circulation. This will allow us to repair a weakened barrier in disease or, conversely, to temporarily permeabilize the healthy BBB if necessary for the purpose of delivering therapies to the brain through the bloodstream."

Ravindra Kumar and collaborators also identified many AAV capsids that traverse the blood-brain barrier with similar efficiency despite having highly distinct sequences engineered into the capsid. Unlike the previously engineered AAVs that displayed some strain specificity, the new capsids work equally well across tested strains of mice with distinct BBB profiles. "This suggests that the new engineered capsids can cross the blood-brain barrier through interactions with unique surface proteins. Identifying diverse mechanisms of action to access the same tissue may prove crucial to the translation of these tools from mice to other model organisms including humans," says Timothy F. Miles, scientific director of the Beckman Institute's CLOVER Center and second author of the study.

"What is exciting about M-CREATE is its potential to identify diverse capsids with unique surface structures that solve the same problem, such as crossing the blood-brain barrier, in different ways. The potential applications are numerous, from delivering therapeutic genes to animal models of disease to learning more about differences in cell surface receptors and the composition of the BBB across strains and species." says Gradinaru.

The paper is titled "Multiplexed Cre-dependent selection yields systemic AAVs for targeting distinct brain cell types."

More information: Sripriya Ravindra Kumar et al. Multiplexed Cre-dependent selection yields systemic AAVs for targeting distinct brain cell types, *Nature Methods* (2020). DOI: 10.1038/s41592-020-0799-7