Researchers demonstrate machine-guided engineering of AAV capsids

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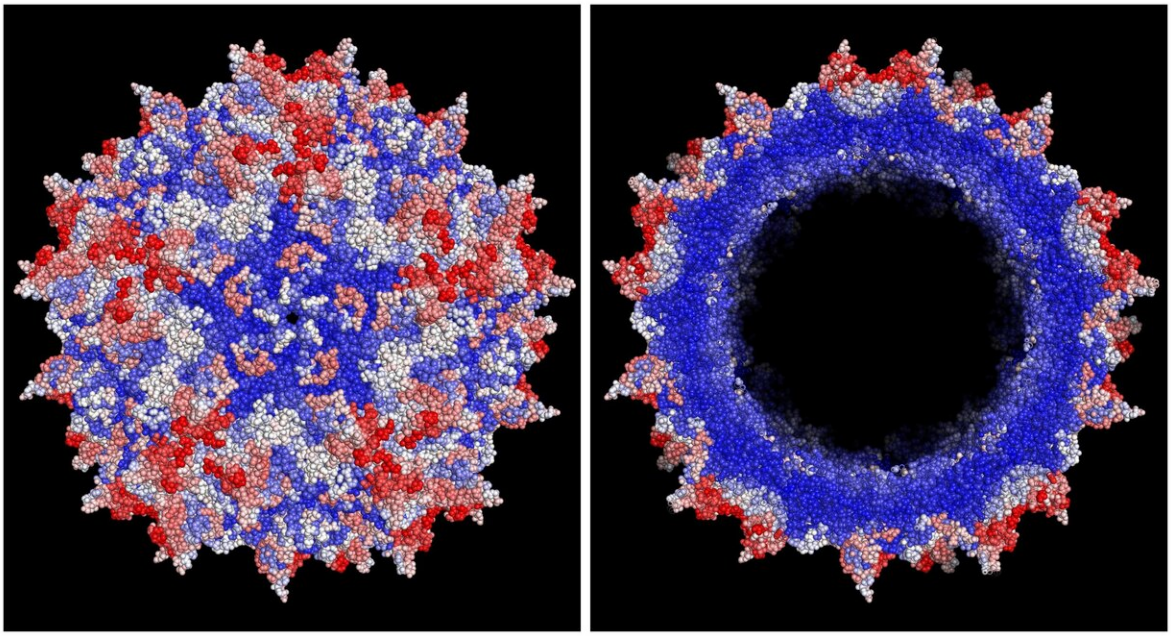

Improved AAV vector capsid for gene therapy engineered with a new machine-guided approach shows, in red, improvements in efficiency of viral production based on the average effect of insertions at all possible amino acid positions, with white showing neutral and blue showing deleterious positions. (Left: capsid viewed from outside, Right: cut-out to reveal inner positions). Credit: Eric Kelsic, Dyno Therapeutics

Adeno-associated viruses (AAVs) have become the go-to vehicle for

delivering therapeutic gene cargo to target tissues for the recent wave of gene therapies that are in development in academic and biotechnology laboratories. However, natural AAVs do not specifically target diseased cells and tissues, and they can be recognized by the immune system in ways that limit therapeutic success. To improve AAVs, synthetic biologists have been taking a "directed evolution" approach in which they randomly mutate specific amino acid building blocks of the capsid proteins that form the shell of the virus and directly contact target cells. By evaluating which changes can route the virus to target tissues and successively layering mutations on top of each other in an arduous iterative process, they aim to improve desirable AAV traits.

Now scientists at Harvard's Wyss Institute for Biologically Inspired Engineering and Harvard Medical School (HMS) report an approach to speed up the process of making such enhanced AAV capsids, and to develop even better viruses.

Taking a different, more systematic approach to the capsid protein-engineering problem, the team mutated one by one each of the 735 amino acids within the AAV2 capsid, the best-known member of the AAV family, including all possible codon substitutions, insertions and deletions at each position. They generated a virus library containing about 200,000 variants and identified capsid changes that both maintained AAV2's viability and improved its "homing" potential (tropism) to specific organs in mice. Unexpectedly, the team also discovered a new accessory protein hidden within the capsid-encoding DNA sequence that binds to the membrane of target cells. Their findings are reported in *Science*.

The team led by Wyss Core Faculty member George Church, Ph.D., and his former postdoctoral fellow Eric Kelsic, Ph.D., deployed an advanced synthetic biology armamentarium including next-generation DNA-synthesis, barcoding, and DNA sequencing capabilities for constructing

one of the most comprehensive AAV capsid libraries to date. "With the information generated by this library, we were also able to design capsids with more mutations than previous natural or synthetic variants, and furthermore with efficiencies of generating viable capsids that far exceed those of AAV created by random mutagenesis approaches" said Church, who is a Lead of the Wyss Institute's Synthetic Biology platform, and also the Robert Winthrop Professor of Genetics at HMS and Professor of Health Sciences and Technology at Harvard University and the Massachusetts Institute of Technology (MIT).

"These high-throughput technologies paired with machine-guided design lay the foundation for engineering superior and highly tailored AAV variants for future gene therapies", said co-first author Eric Kelsic, Ph.D., who is now CEO of Dyno Therapeutics. "Past approaches such as rational design or random mutagenesis each had their drawbacks, either being limited in the library size or being low in quality, respectively. Machine-guided design is a data-driven approach to protein engineering. Here we show that even a simple mathematical model, powered by enough data, can successfully generate viable synthetic capsids. This iterative and empirical approach to protein engineering enables us to get the best of both worlds and generate large numbers of high quality capsid variants."

In this photo Sam Sinai, George Church, Eric Kelsic, and Pierce Ogden are holden small models of the AVVs capsid in their hands. Credit: Wyss Institute at Harvard University

"Unexpectedly, the high-resolution data we generated enabled us to spot a new protein encoded by a different reading frame within the capsid's DNA sequence—which had escaped notice despite decades of intense research on the virus," said co-first author Pierce Ogden, Ph.D., a former graduate student and now postdoctoral fellow working with Church. "Membrane-associated accessory protein (MAAP), as we named it, exists in all of the most popular AAV serotypes and we believe that it plays a role in the virus' natural life cycle. Studying how MAAP

functions will be an exciting area for future research and could potentially lead to a better understanding of how to better produce and engineer AAV gene therapies."

According to co-author Sam Sinai, Ph.D., a former graduate student of Church, now a Machine Learning Scientist at Dyno Therapeutics, "This reveals the promise of data-driven protein engineering, in particular for proteins like the AAV capsid that are difficult to model with current computational approaches. Our results are highly encouraging but also only a first step. Using this data and those from future experiments, we will be building machine learning models to optimize capsids and address a wide variety of gene therapy challenges."

Kelsic, Sinai and Church are co-founders of Dyno Therapeutics Inc., and all hold equity in the company.

"This study is a landmark in the Wyss Institute's Synthetic Biology platform's effort to advance AAV technology to the next level. This work is also a great example of how we are beginning to integrate machine learning and artificial intelligence approaches into our therapeutics pipeline," said Wyss Institute Founding Director Donald Ingber, M.D., Ph.D., who is also the Judah Folkman Professor of Vascular Biology at HMS, the Vascular Biology Program at Boston Children's Hospital, and Professor of Bioengineering at Harvard's John A. Paulson School of Engineering and Applied Sciences (SEAS).

More information: "Comprehensive fitness landscape of AAV capsid reveals a viral gene and enables machine-guided design" *Science* (2019). science.sciencemag.org/cgi/doi … 1126/science.aaw2900

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