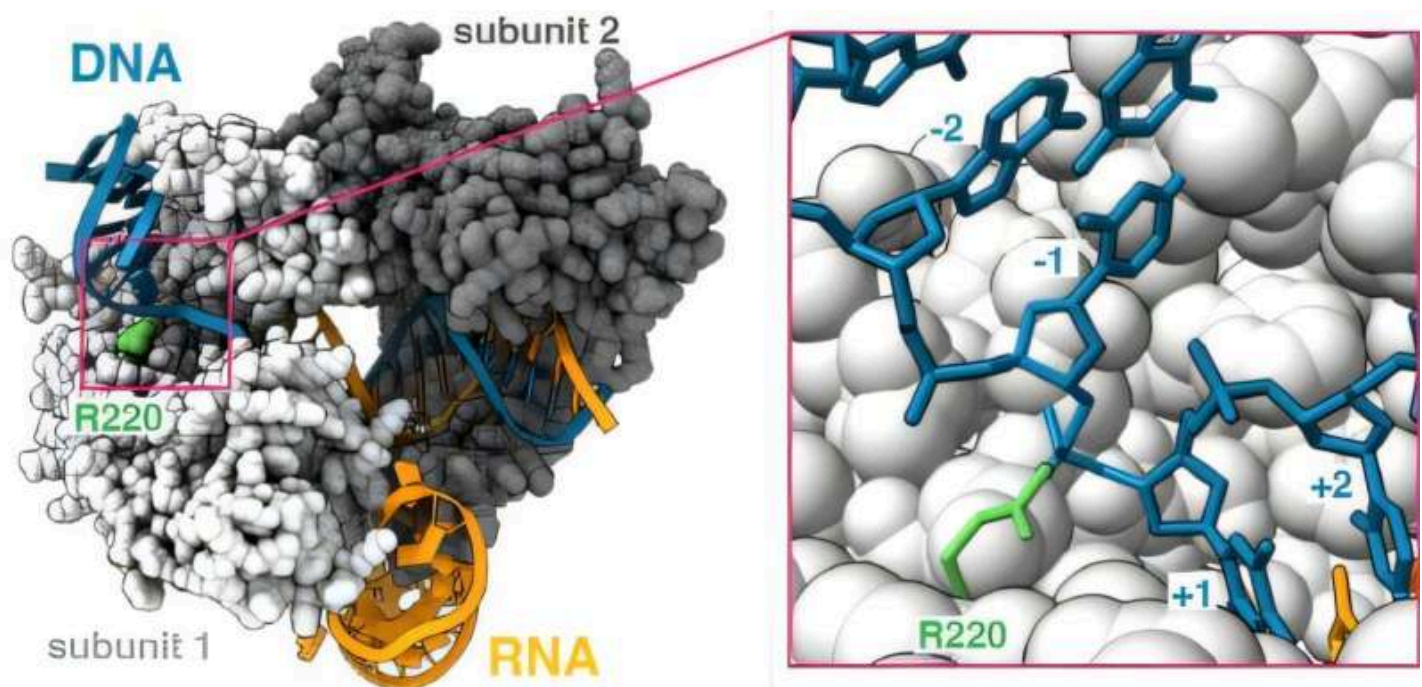


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NanoCas, a smaller version of CRISPR tested with a single AAV, delivers on-target results

by Justin Jackson, Phys.org



Engineering of an enhanced NanoCas genome editor. Structural model of NanoCas D220R-guide RNA-target DNA complex. Credit: *bioRxiv* (2025). DOI: 10.1101/2025.01.29.635576

Mammoth Biosciences researchers have developed NanoCas, an ultracompact CRISPR nuclease, demonstrating its ability to perform gene editing in non-liver tissues, including skeletal muscle, using a single adeno-associated virus (AAV) vector. Experiments in non-human primates (NHPs) resulted in editing efficiencies exceeding 30% in muscle tissues.

CRISPR gene editing has revolutionized genetics, but delivery challenges have restricted its clinical applications primarily to ex vivo and liver-directed therapies. Conventional CRISPR nucleases, including Cas9 and Cas12a, exceed the packaging limits of a single AAV vector, necessitating dual-AAV strategies that reduce efficiency.

Smaller CRISPR systems such as Cas12i and CasX have been identified, but they remain too large or exhibit low editing efficiency. Existing compact systems like Cas14 and IscB have not demonstrated robust efficacy in large animal models.

In the study, "Single-AAV CRISPR editing of skeletal muscle in non-human primates with NanoCas, an ultracompact nuclease," [published](#) on the *bioRxiv* preprint server, researchers performed a comprehensive metagenomic screening that analyzed 21,980 metagenomes to identify ultracompact CRISPR systems.

Researchers selected 176 candidate nucleases under 600 amino acids, evaluating them through computational RNA structure prediction, PAM sequence identification, and mammalian cell chromosomal editing assays.

The most efficient candidate, dubbed NanoCas, was further optimized through protein engineering. A variant with an arginine substitution at position 220 (D220R) improved DNA binding and editing efficiency. Editing performance was assessed in human embryonic kidney cells (HEK293T), T-cells, and CD34⁺ hematopoietic stem and progenitor cells.

For in vivo testing, NanoCas was delivered via AAV8 in mice to target Pcsk9, a cholesterol-regulating gene, achieving ~60% editing efficiency in the liver and reducing serum PCSK9 levels. Additional studies targeted exon splice sites in dystrophin to investigate potential applications for Duchenne muscular dystrophy.

Non-human primate experiments used AAV9-4A for muscle delivery. Cynomolgus macaques received systemic AAV9-NanoCas injections, with skeletal muscle biopsies taken at four and eight weeks and additional tissues analyzed at 12 weeks post-treatment.

NanoCas editing efficiencies in human cell lines averaged 20%, with 60% of tested guide RNAs achieving detectable activity. The D220R variant increased editing efficiency across different tissues. In vivo, NanoCas-mediated Pcsk9 editing in mice resulted in a 60% indel frequency, matching SaCas9 while benefiting from a smaller gene payload.

Duchenne muscular dystrophy (DMD) is a debilitating muscle weakness disease that is caused by genetic mutations and is a high-value target for potential gene editing therapies. DMD-targeting experiments in a humanized mouse model demonstrated 10–40% editing in quadriceps, calf, and heart tissues.

In non-human primates, NanoCas achieved up to 30% editing in skeletal muscles, with progressive editing levels increasing over time. Cardiac editing reached 15%, and off-target editing in the liver remained below 2%.

NanoCas represents the first single-AAV CRISPR system to demonstrate efficient in vivo muscle editing in non-human primates. The implications of such a compact nuclease are wide-reaching, enabling broader tissue targeting, more efficient gene therapies and precision editing applications, including applications in base editing and epigenetic modifications.

More information: Benjamin J. Rauch et al, Single-AAV CRISPR editing of skeletal muscle in non-human primates with NanoCas, an ultracompact nuclease, *bioRxiv* (2025). DOI: [10.1101/2025.01.29.635576](https://doi.org/10.1101/2025.01.29.635576)

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