NEW YORK – Researchers at the New York Genome Center, Synthego, and New England Biolabs have developed chemically modified guide RNAs (gRNAs) for CRISPR-Cas13 that enhance the nuclease’s gene knockdown efficiency in human cells two- to fivefold over editing activity achieved with unmodified guides.

In a paper published on Monday in Cell Chemical Biology, the researchers noted that Cas13 has emerged as an important platform for transient modulation of gene expression due to its RNA-targeting properties. However, protein and CRISPR RNA (crRNA) delivery in human cells can be challenging and knockdown can be transient due to rapid crRNA degradation.

In their study, the researchers compared several chemical RNA modifications at different positions to identify synthetic crRNAs that improved RNA targeting efficiency and their half-life in human cells. They showed that codelivery of modified crRNAs and recombinant Cas13 enzyme in ribonucleoprotein (RNP) complexes enabled transient gene expression modulation in primary CD4-positive and CD8-positive T cells.

"Although genome editing has rightly been in the spotlight with many exciting new therapies in clinical trials, there is an emerging field of RNA-based therapeutics. We are very excited about CRISPR-Cas13 as a platform for RNA modification but were frustrated to find very little work in improving delivery into human cells, including primary immune cells like T cells," corresponding author Neville Sanjana said in an email.

In their study, he explained, he and his colleagues explored RNA modifications similar to those found in the mRNA COVID-19 vaccines in order to extend the half-life of the Cas13 gRNAs. They identified various RNA modifications, as well as precise positions in the gRNA that were most effective at boosting Cas13 gene knockdown.

"Given the tremendous diversity of Cas13 applications that are now emerging, such as modifying splicing or editing transcripts without editing the genome, we think that these chemically-modified Cas13 guide RNAs will be useful for a broad group of biomedical scientists interested in different kinds of transcriptome engineering," Sanjana added. "In the long run, we hope to see chemically-modified guide RNAs as an enabling technology for RNA knockdown and editing therapies in vivo."

For their study, the researchers first assessed the degree of target RNA knockdown efficiency upon exogenous delivery of unmodified and chemically modified crRNAs, and synthesized crRNAs with different chemical modifications at different nucleotides. These modifications included methylation, phosphorothioate linkage, a combination of the two, and an inverted thymidine.

They tested these modifications, several of which have been reported before to improve RNA stability and evade secondary immune responses, and then assessed target knockdown efficiency of three...
broadly expressed cell surface proteins (CD46, CD55, and CD71) that can be efficiently targeted with the Cas13 ortholog RfxCas13d.

They found that protein knockdown with unmodified crRNAs for each of the three target transcripts was barely detectable relative to non-targeting crRNAs, suggesting that unmodified crRNAs get rapidly cleared in human cells and cannot yield lasting knockdown effects. All of the chemically modified crRNAs, however, improved target knockdown, though they did so to varying degrees.

The researchers also sought to assess the temporal dynamics of Cas13 activity with synthetic crRNAs by comparing knockdown of CD46 over time. All three targeting crRNAs, including unmodified crRNAs, yielded almost complete CD46 knockdown at 24 hours after nucleofection, with around 95 percent protein loss for modified crRNAs. But while CD46 expression quickly recovered in cells targeted with unmodified crRNAs, modified crRNAs led to pronounced knockdown at 48 hours after crRNA delivery, and resulted in 40 percent knockdown even four days after nucleofection.

The team then conducted an experiment to test if synthetic crRNAs can degrade SARS-CoV-2. Indeed, similar to their experiments with endogenous human transcripts, they found that modified crRNAs targeting SARS-CoV-2 suppressed reporter protein expression, despite targeting an untranslated sequence.

"These results suggest that Cas13 together with chemically modified crRNAs may represent an efficient and programmable therapeutic approach to target universal SARS-CoV-2 sequences," the authors wrote. "Our study highlights the utility of optimized, chemically modified crRNAs for efficient transcriptome engineering. We anticipate that chemically modified crRNAs for RNA-targeting CRISPRs will be useful for both in vitro RNA diagnostics and in vivo where DNA editing is not feasible or desirable."