Novel genome editing CRISPR-Cas nuclease mining and characterization for genetic disease treatments

'DNA surgery' – correction of disease-causing mutations in relevant organs – may provide once-and-done curative solutions to diseases with unmet medical needs. Precision genome editing such as based on the CRISPR-Cas system is a promising platform for developing programmable drugs for DNA surgery. The first step of CRISPR-Cas nucleases to engage with genome targets, however, is often restricted by protospacer adjacent motifs (PAMs). While protein engineering to relax the PAM restriction has been very useful in research settings, its consequential larger off-target effects limit therapeutic applications. We therefore adopt a specialized-PAM approach. However, characterizing the PAM requirements of different Cas enzymes is a bottleneck in the discovery of novel Cas proteins. To enable scalable characterization of PAM recognitions, we developed a simple technology, GenomePAM, for direct PAM characterization in mammalian cells. We rapidly identified from nature a dozen novel Cas nucleases with distinct PAM recognitions. One bears an 'NNNACT' PAM that allows allelic-specific DNA editing on a toxic protein-encoding allele, leaving the normal allele intact, to treat an autosomal-dominant disease. Beyond PAM characterization, GenomePAM allows for simultaneous comparison of activities and fidelities of Cas nucleases on tens of thousands of matched and mismatched sites across the human genome using a single guide RNA. GenomePAM also provides insights into the genome-wide chromatin accessibility profiles in different cell types.