

Creating safer gene editing tools through protein engineering focused on a conserved bridge helix of CRISPR Cas

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The CRISPR-Cas system, a Nobel Prize-winning genome-editing tool, has transformative applications in agriculture, molecular diagnostics, and gene therapy. Cas9 and Cas12a, the two widely used CRISPR-Cas proteins, bind to cognate CRISPR-RNAs (crRNAs) to target and cleave double-stranded DNA with sequence specificity. However, a significant challenge in translational medicine is the off-target DNA cleavage, where sequences partially complementary to the designed crRNA are inadvertently cut, potentially leading to adverse outcomes. To address this, the Rajan lab has engineered variants of CRISPR-Cas proteins by focusing on the bridge helix (BH), a critical component that undergoes structural changes during DNA targeting. Specific alterations to the BH achieved reduction in off-target effects in biochemical assays (*in vitro*) for both Cas12a and Cas9. To understand this improved precision, we are using cryo-electron microscopy to capture the Cas variant in action. Given the conservation of the BH across CRISPR and various RNA-binding proteins, this approach holds potential for engineering new, precise protein variants for diverse biotechnological applications.