Interactions between gRNA and CRISPR Nuclease preface in vitro cleavage activities

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Abstract

While genetic editing presents tremendous opportunities to address unmet needs, its therapeutic discovery and development may be hindered by the lack of structural-activity understanding, including the underlying interactions between nuclease and therapeutic guide RNAs (gRNAs). Here, we present the development of a novel tool to measure the binding affinity between gRNAs to CRISPR-Cas nuclease, by tracking the changes in size of RNAs in the presence of increasing concentration of nuclease using Flow-Induced Dispersion Analysis (FIDA). Furthermore, we designed a high-throughput competition assay to evaluate the affinities of numerous gRNAs with CRISPR-Cas nuclease. We uncovered the effect of different gRNA modifications on the binding strength to our nuclease, and that this gRNA-Cas interaction correlates with the editing potential of the RNP downstream. Together, we provided evidence that mechanistic understanding of RNA-guided CRISPR systems facilitates more rational designs of therapeutic guide RNAs.

Analytics for CRISPR-guided cleavage events

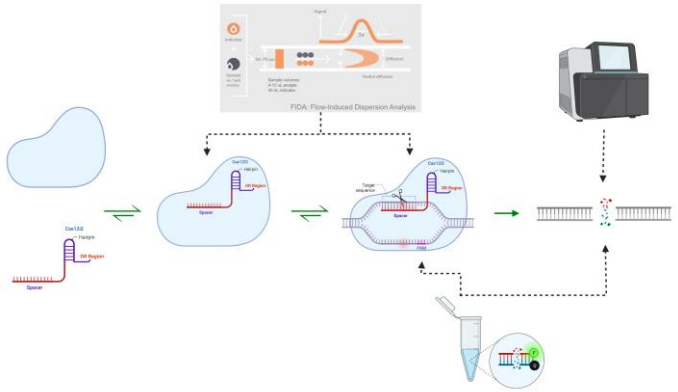

Figure 1: Step-wise Mechanism of CRISPR-mediated cleavage and proposed tools to characterize each stage. CRISPR nuclease and synthetic gRNA interact and form functional ribonucleoprotein complex (RNP), which recognizes target dsDNA and generates double-stranded break. FIDA-enabled binding assay can characterize the thermodynamic and kinetic of interaction between gRNA-Cas and RNP-dsDNA

Assay Design

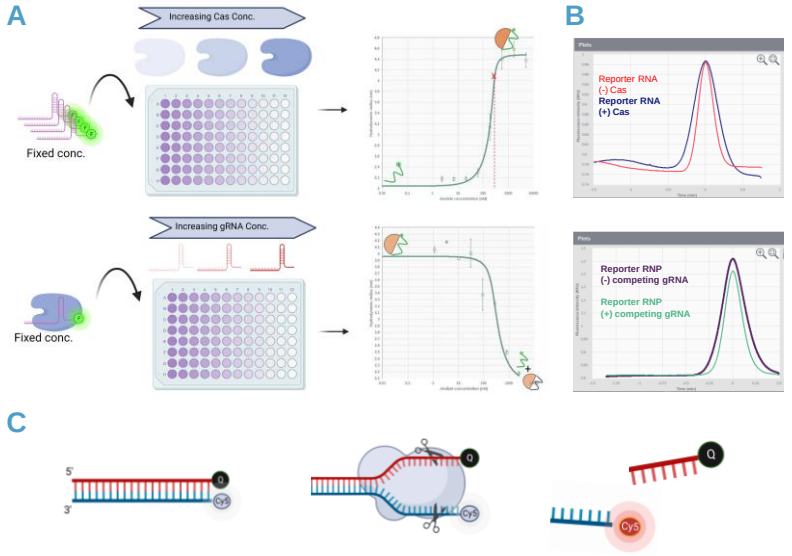

Figure 2: Schematics of (A) FIDA Relative binding assay. A reporter gRNA with canonical PAM designed to identify size of RNP and saturation point (top), whose reporter RNP construct serve as starting point to competition assay (bottom); (B) Raw FIDA data obtained during forward binding (top) and reverse titration (bottom); (C) Schematics of in-vitro fluorescent cleavage assay

Conclusion

In this work, we demonstrated an assay that can depict gRNA – nuclease binding, the first stage of the CRISPR-mediated genetic modifications. By measuring changes in size differences between RNA molecules and RNP complexes, relative binding affinity can be characterized. These structural properties can impact the downstream activities of CRISPR-Cas constructs, and thus understanding them can help design more rational gRNAs or nucleases

Modified binding correlates with RNP activity

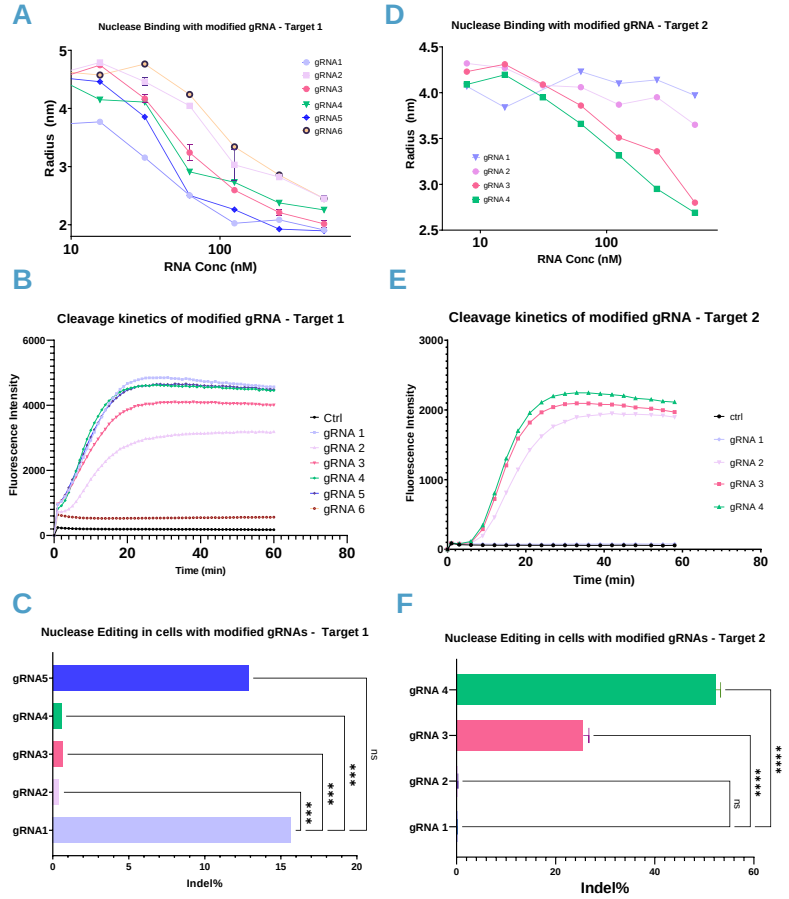

Figure 3: Correlation between binding and activity of modified gRNAs. Relative binding affinities of modified gRNAs targeting locus 1 (A) and 2 (B), with corresponding cleavage activities assessed by Fluorescent Cleavage Assay (C, D) and editing activities in hepatocytes cell lines at target 1 (E) and target 2 (F)