Thesis Title: Mechanistic Insights into RNA-guided Genome Editing Nucleases

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Abstract

Efficient manipulation of genes in organisms has propelled research in understanding mechanisms that govern life. New techniques that allow editing in single-celled organisms as well as complex life forms such as animals and plants are gaining traction. At the heart of such advances are custom-designed nucleases like zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and the CRISPR/Cas9 system.

A naturally occurring adaptive defence mechanism found in prokaryotes, the CRISPR/Cas9 system has been repurposed as an RNA-guided DNA targeting machinery. This transformative technology has shown great promise for biology, genetics and medicine. However, it’s widespread application for safe and effective genome editing and transcription modulation has been curbed by its inadequate specificity. In this thesis, the key factors involved in determining the efficacy of CRISPR/Cas9 have been studied that can be incorporated in guide RNA design tools to improve their predictions. Further, the study of the mechanism of on-target activity by Gaussian accelerated molecular dynamics solution has been carried out to determine sub-molecular interactions essential for driving Cas9 activity.