INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
PhD-17 SHORT ABSTRACT OF THESIS

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Thesis Title: **Structure-based thermodynamics of PAM Recognition by CRISPR/Cas9: Insight from computer simulations**

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SHORT ABSTRACT

The CRISPR/Cas9 system derived from *Streptococcus pyogenes* (*Sp*Cas9) has revolutionized molecular biology by allowing precise and programmable editing of DNA sequences in living cells. *Sp*Cas9 is a multi-domain RNA-guided DNA endonuclease that uses a single guide RNA (sgRNA) to bind and cut DNA at locations adjacent to a protospacer adjacent motif (PAM), which consists of the three-nucleotide canonical sequence 5'-NGG-3' (where N can be any nucleotide). The stringent PAM requirement (5'-NGG-3') limits the range of genomic sites accessible for editing. Therefore, understanding the molecular and energetic basis of PAM recognition is crucial for the rational engineering of Cas9 variants with broadened or altered PAM specificities. Mutations in SpCas9 enhance PAM recognition; however, the relationship between these mutations, PAM recognition energetics, and atomic structure remains unclear. This thesis employs molecular simulations using precatalytic *Sp*Cas9:sgRNA:dsDNA as a template to clarify the structure-based free energy landscape related to PAM selectivity in *Sp*Cas9 and its engineered variants. Using alchemical free energy calculations, the research examines how amino acid mutations in *Sp*Cas9 affect DNA binding. Moreover, the work also explores how the PAM binding affinity of *Sp*Cas9 changes in response to mutations in the canonical 5'-NGG sequence. Results indicated that the PAM recognition by *Sp*Cas9 is influenced by the local hydrophobicity and flexibility of its binding cleft. The flexibility of protein residues facilitates new interactions, while hydrophobicity enhances these interactions in non-canonical PAM sequences, thereby broadening PAM readability. The study establishes a direct connection between the estimated energetics and the molecular structures, providing an explanation for the experimentally observed cleavage activity of *Sp*Cas9. This work establishes a clear framework for understanding PAM recognition in *Sp*Cas9, laying the groundwork for designing new CRISPR-based genome editing tools.