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A Biophysical Model of CRISPR/Cas9 on- and off-Target Binding for Rational Design of Guide RNAs

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Abstract : The CRISPR/Cas9 system has revolutionized genome engineering by enabling site-directed and high-throughput genome editing, genome insertion, and gene knockdowns in several species, including bacteria, yeast, flies, worms, and human cell lines. This technology has the potential to enable human gene therapy to treat genetic diseases and cancer at the molecular level; however, the current CRISPR/Cas9 system suffers from seemingly sporadic off-target genome mutagenesis that prevents its use in gene therapy. A comprehensive mechanistic model that explains how the CRISPR/Cas9 functions would enable the rational design of the guide-RNAs responsible for target site selection while minimizing unexpected genome mutagenesis. Here, we present the first quantitative model of the CRISPR/Cas9 genome mutagenesis system that predicts how guide-RNA sequences (crRNAs) control target site selection and cleavage activity. We used statistical thermodynamics and law of mass action to develop a five-step biophysical model of cas9 cleavage, and examined it in vivo and in vitro. To predict a crRNA's binding specificities and cleavage rates, we then compiled a nearest neighbor (NN) energy model that accounts for all possible base pairings and mismatches between the crRNA and the possible genomic DNA sites. These calculations correctly predicted crRNA specificity across 5518 sites. Our analysis reveals that cas9 activity and specificity are anti-correlated, and, the trade-off between them is the determining factor in performing an RNA-mediated cleavage with minimal off-targets. To find an optimal solution, we first created a scheme of safe-design criteria for Cas9 target selection by systematic analysis of available high throughput measurements. We then used our biophysical model to determine the optimal Cas9 expression levels and timing that maximizes on-target cleavage and minimizes off-target activity. We successfully applied this approach in bacterial and mammalian cell lines to reduce off-target activity to near background mutagenesis level while maintaining high on-target cleavage rate.

Keywords : biophysical model, CRISPR, Cas9, genome editing
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