

Computational biophysical studies on CRISPR/Cas9

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Class 2 CRISPR (clustered regularly interspaced short palindromic repeats) systems offer a unique protocol for genome editing in eukaryotic cells and has already been validated in many organisms [1]. In genome editing through CRISPR, the target DNA is first recognized by a complementary RNA bound to an endonuclease. The RNA-guided DNA unwinding leads to formation of a RNA-DNA hybrid duplex and a displaced DNA strand (**Figure 1**). Thereafter, the nuclease portion of the Cas9 protein closest to the DNA cleavage site attains a conformation essential for concerted DNA cutting.

The nuclease activity of Cas9 can be also get triggered when there is imperfect complementarity between the RNA guide sequence and an off-target genomic site, which is a major limitation of the CRISPR technique [2] for therapeutic applications. Our goal is to understand the binding mechanisms in CRISPR/Cas9 for predicting ways to increase its specificity.

We have performed a cumulative of micro-sec long classical MD simulations of CRISPR/Cas9 ternary complexes with the wild type Cas9 [4] and three mutations: K855A, H982A and the combination K855A+H982A, selected from the results of experimental work [3]. Our initial results reveal significant structural impact of the mutations, with possible implications for specificity.

References.

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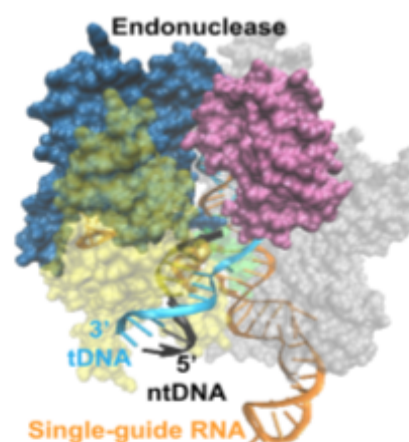


Figure 1. Representation of the crystal structure of a ternary CRISPR/Cas9 complex (PDB ID 4UN3). The cyan and black DNA strands represent the target and non-target DNA strands respectively. The target DNA strand is involved in RNA-DNA hybrid formation with a 20 base long segment of RNA (orange). The protein is represented with surfaces.