Molecular Dynamics Simulations of 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.

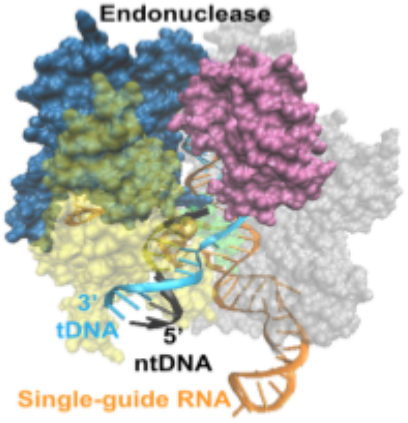

Figure 1. Representation of the crystal structure of a ternary CRISPR/Cas9 complex (PDB ID 4UN3). The cyan and black DNA strands represent the target and non-target DNA strands respectively. The target DNA strand is involved in RNA-DNA hybrid formation with a 20 base long segment of RNA (orange). The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

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 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. We find that the unwound part of non-target DNA strand shows highest structural changes for the combination mutation. The distance between the HNH domain of Cas9 and the unwound non-target DNA strand is significantly affected by different mutations, with possible implications for specificity.

References.

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