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Detecting Cas9-sgRNA Complex Interactions with DNA via Fluorescent Microscopy: Computer Simulations of Experimental Designs

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At the center of the modern CRISPR/Cas genome editing revolution is the engineered ribonucleoprotein complex of the Cas9 protein with sgRNA that is able to bind and cleave DNA at specific loci. The targeted activity is conferred by the ability of the complex to bind to the DNA regions which are complementary to the parts of the sequence encoded by the sgRNA. Apart from DNA cleavage activity the selective binding activity of CRISPR/Cas complexes alone have been exploited in many technological applications to target certain compounds to specific DNA loci (e.g. for detecting certain DNA sequences, marking up certain portions of the genome with fluorescent labels, altering epigenetic state of the genetic loci, etc.). The most common version of the CRISPR/Cas editing system is currently based on the spCas9 protein obtained from S. pyogenes. However, search for other systems which are more effective and have less off-target activity is under way. Particularly, Cas proteins from other species including alternative CRISPR systems (e.g. recently characterized CasX system) combined by artificially engineered mutations can be attempted[1,2]. Hence, an effective in vitro assay to characterize the binding affinity of the Cas-based ribonucleoprotein complex to its target DNA sequence is of high methodological importance. An effective way of measuring the biomolecular complex affinity can be based on using the FRET microscopy by monitoring the increase of the FRET signal as the two molecules labeled with a corresponding pair of fluorescent dyes bind together [3,4]. In this work by employing atomistic molecular simulations we show that such measurements are possible to study the binding of Cas9-sgRNA complex to its target DNA by attaching Cy3 and Cy5 labels to specific sites at DNA and RNA molecules. We simulate various attachment possibilities for optimal experimental designs.

To take into account the flexibility of linkers between labels and DNA, MD simulations of Cy3 and Cy5 attached to short dsDNA we carried out. Parametrization of labels was performed with SwissParam server [5]. Simulations were done in Gromacs [6]. All DNA atoms (including labeled thymidine atoms) were fixed in place using harmonic restraints. The overall calculation procedure was similar to that described elsewhere [7]. Simulations were performed for 10 ns. Positions of the centers of mass of chromophore groups with respect to the labeled thymidine nucleotide were then derived from each frame. Thus the ensemble of potential positions of Cy3 and Cy5 with respect to their attachment sites on DNA was extracted.

To find the optimal locations of the dyes we superposed the obtained ensembles of potential dye locations on sgRNA tetraloop (nucleotides 24-48), target DNA strand (nucleotides 1-18) and non-target DNA strand (nucleotides 41-56) of Cas9 complex (PDB ID 5Y36 [8]). The results of the screening are shown in figure 1A, we found that the most optimal locations for fluorescent labels are 37 on sgRNA, 11 on target DNA strand and 49 on non-target DNA strand. The latter are shown on the figure 1B, and correspond to short interdye distances of around 20 Å which will cause very high FRET efficiency[9].

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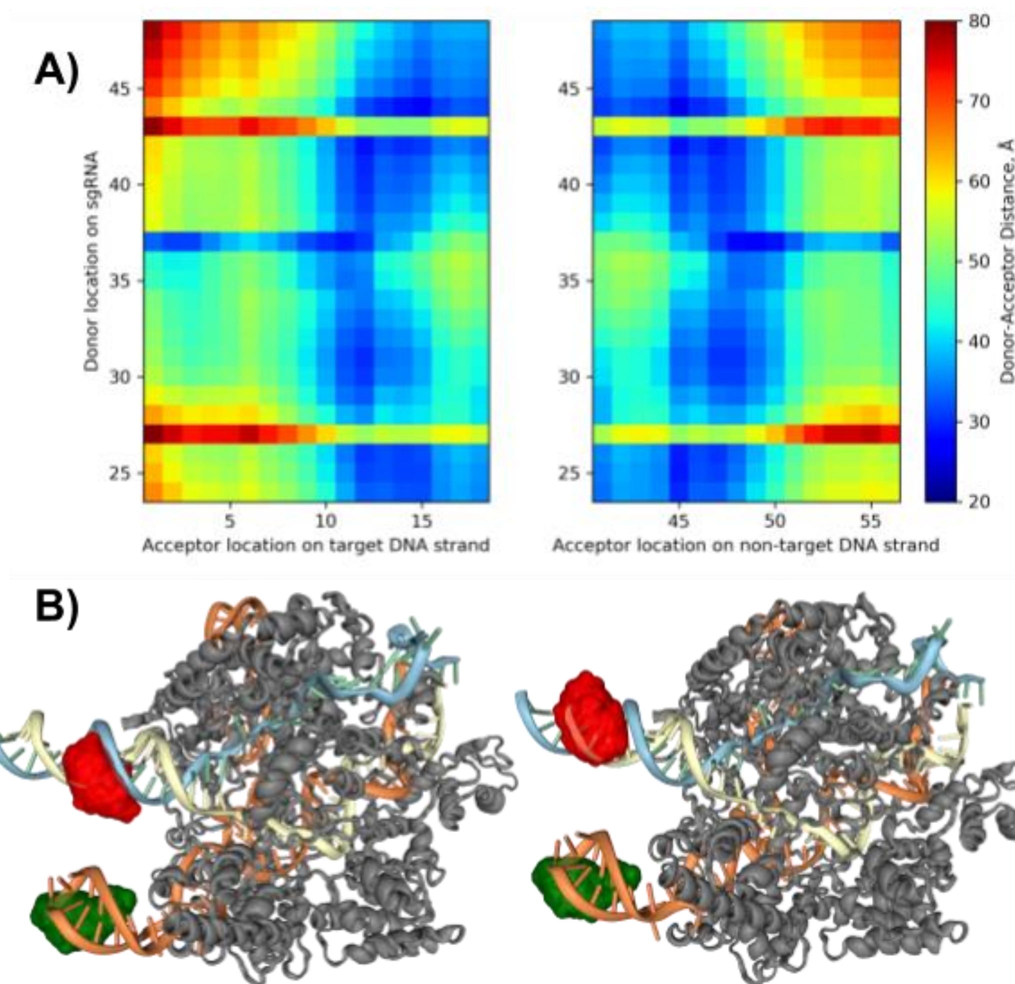

Figure 1. A) Screening of an interdye distances in Cas9-sgRNA complex. Short distances will correspond to high FRET and will likely be detected. The most optimal location on sgRNA is 37, on DNA chain C - 11, chain D - 49 B) Molecular models of Cas9-sgRNA complexes with fluorescent labels attached in locations which are expected to have high energy transfer.

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