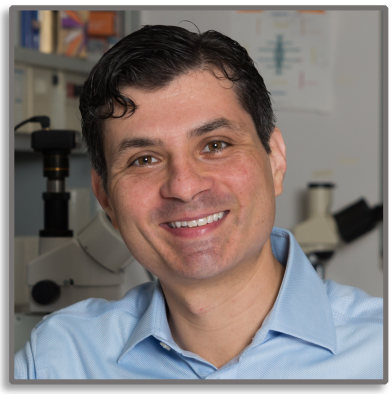

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Unraveling the Specificity and Inhibition of CRISPR-Cas Nucleases

Dr. Ilya Finkelstein is an Associate Professor of Molecular Biosciences at the University of Texas at Austin. Prior to his position at UT-Austin, Dr. Finkelstein received a B.S. from the University of California, Berkeley, a Ph.D. in Chemistry from Stanford University, and completed postdoctoral training at Columbia University Medical Center. Dr. Finkelstein's lab is investigating the molecular mechanisms of genome maintenance, CRISPR biology, and epigenetic inheritance. His group addresses these questions by combining biophysics, advanced microscopy and micro-/nano-scale engineering approaches. He ultimately hopes to apply these insights to understand the molecular underpinnings of and potential therapeutic avenues for malignancies and other diseases.

Abstract: CRISPR-Cas nucleases cleave genomic sites that are complementary to their CRISPR RNA (crRNA). However, all Cas nucleases bind and cleave near-cognate "off-target" sites, leading to adverse gene editing outcomes. I will describe several biophysical platforms for benchmarking diverse Cas nucleases. NucleaSeq, the nuclease sequencing pipeline, exhaustively measures cleavage kinetics and captures the time-resolved identities of cleaved products for a large library of partially crRNA-matched DNAs. The same DNA library is used to measure the binding specificity of each enzyme on repurposed next-generation DNA sequencing chips. This integrated workflow allowed us to benchmark the cleavage and binding specificities of Cas12a and five Cas9 variants for >105 DNAs containing mismatches, insertions, and deletions. I will also describe how Cas12a is inhibited by nucleosomes. Nucleosome unwrapping determines the extent to which both steps of Cas12a binding–PAM recognition and R-loop formation–are inhibited by the nucleosome. Nucleosomes inhibit Cas12a binding to DNA targets that extend beyond the canonical ~146 basepair core particle. Relaxing DNA wrapping within the nucleosome by reducing DNA bendability, adding histone modifications, or introducing a target-proximal nuclease-inactive Cas9 enhances DNA cleavage rates over 10-fold. Surprisingly, Cas12a readily cleaves DNA within chromatin-like phase separated nucleosome arrays. Taken together, these results highlight the importance of biophysical studies for understanding off-target and chromatin cleavage activities of Cas nucleases.

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