

5<sup>th</sup> International Conference on

# GLYCOBIOLOGY & GLYCOPROTEOMICS

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3<sup>rd</sup> International Conference on

# MOLECULAR BIOLOGY & NUCLEIC ACIDS

August 27-28, 2018 | Toronto, Canada

## Kinetic basis for DNA target specificity of CRISPR-Cas12a

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Class 2 CRISPR-Cas nucleases have become the gold standard for genome editing, as genetic manipulation can be achieved by a relatively simple and easily adaptable system<sup>1</sup>. The common class 2 CRISPR-Cas nucleases, Cas9 and Cas12a (previously known as Cpf1), are programmable via a guide RNA that uses complementarity with DNA to identify the correct target. Still, cleavage at off-target sites resembling the intended sequence remains a pervasive problem<sup>2</sup>. DNA targeting by Cas12a is more specific than Cas9, but the mechanistic basis for this increased specificity is not understood. To dissect the reaction steps of DNA targeting by *Acidaminococcus* sp Cas12a, we used a quantitative kinetics approach, measuring rate constants for binding, dissociation, and DNA strand cleavage. We show that Cas12a binds DNA in two kinetically-separable steps; protospacer-adjacent motif (PAM) recognition is followed by R-loop formation. Once it is bound, Cas12a cuts the non-target strand repeatedly, trimming it back towards the PAM, and then it cleaves the target strand imprecisely. Because R-loop formation is rate-limiting for cleavage, Cas12a binding inevitably leads to target DNA cleavage. Even when targeted to DNA with single mismatches, Cas12a binding remains rate-limiting for DNA cleavage. Thus, the specificity of Cas12a for the matched target is defined by the kinetics of binding rather than by affinity. Nevertheless, in contrast to simple expectations for R-loop formation, we show that Cas12a retains substantial specificity against mismatches throughout the R-loop. This strong discrimination indicates a late transition state for binding, implying that R-loop propagation is reversible up until the R-loop has almost entirely formed. Our results provide a mechanistic foundation for the DNA cleavage patterns of Cas12a measured in vivo<sup>3-5</sup> and lead to a model for the higher specificity of Cas12a than Cas9.

### Biography

Isabel Strohkendl has a strong curiosity for understanding the mechanisms by which proteins with diverse structures and functions act on DNA. Under the supervision of Dr Rick Russell at the University of Texas at Austin, she has been studying protein-nucleic acid interactions and using quantitative biophysical approaches to elucidate the mechanisms of DNA-binding enzymes. Currently, her work focuses on understanding how both nucleic acid and nucleic acid-protein interactions dictate substrate preference during Cas12a-crRNA assembly and contribute to Cas12a specificity during DNA targeting.

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