

Biochemistry CUME April 2017
Topic: Protein Structure Analysis

This review article by Jiang and Doudna presents the current mechanistic understanding of Cas9-mediated RNA-guided DNA targeting and cleavage, based on the structural analysis of Cas9 apoenzyme, Cas9-sgRNA complex and Cas9-sgRNA-DNA complex.

1. **(25 points)** Cas9 is a multidomain and multifunctional DNA endonuclease that recognizes and cleaves double-stranded DNA substrates. Describe the overall architecture and major structural features of Cas9 that are responsible for its substrate specificity and endonuclease activities.
2. **(25 points)** Cas9 must be first assembled with guide RNA to form an active conformation that is competent for site-specific DNA recognition and cleavage. Based on the comparison between the apo-Cas9 and sgRNA-bound Cas9 structures, what major structural rearrangements occur in Cas9 upon binding of the guide RNA? What unique structural features are involved in binding and/or stabilization of sgRNA?
3. **(25 points)** A C-terminal domain (CTD) segment of Cas9 appears to be prepositioned in the Cas9-sgRNA complex for direct interactions with the PAM sequence in the non-target DNA strand. What amino acids are most likely involved in such direct protein-DNA interactions? What role(s) would these specific interactions play in facilitating site-specific DNA cleavages at the endonuclease active sites of Cas9?
4. **(25 points)** In the proposed mechanism for the CRISPR-Cas9 action, which aspects or steps need further clarification in your opinion? What additional structural studies would you suggest to address these issues?