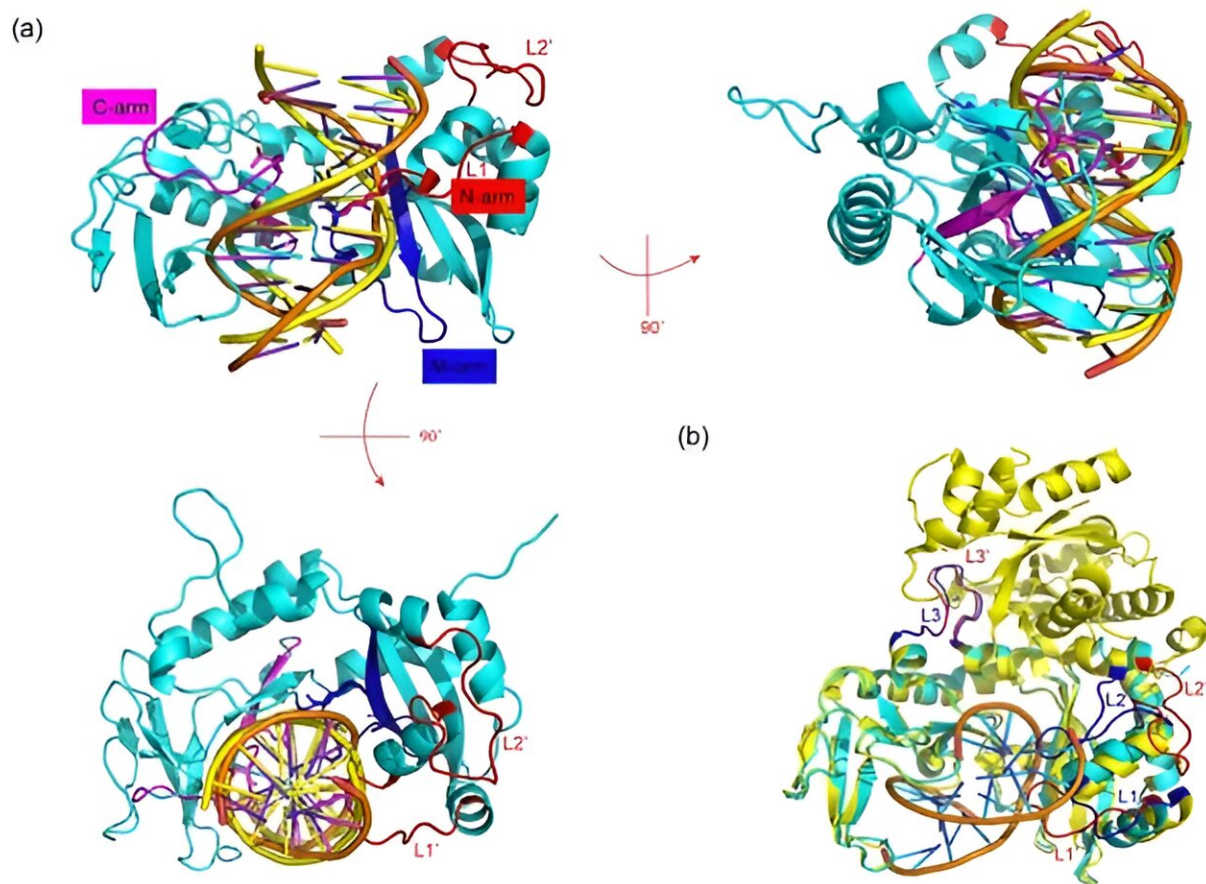


# Researchers determine how type II restriction endonuclease Sau3AI cleaves DNA

August 31 2023, by Li Yuan



The structure of Sau3AI C-terminal and DNA complex. Credit: SARI

Sau3AI is a type II restriction enzyme widely used for genetic manipulation, such as genome library construction. Sau3AI consists of

two domains, the N-terminal domain (Sau3AI-N) and the C-terminal domain (Sau3AI-C). How these two domains work together to cut DNA remains unclear.

Recently, a research team led by Prof. Yu Feng from the Shanghai Advanced Research Institute (SARI) of the Chinese Academy of Sciences and Prof. He Jianhua from Wuhan University reported a self-activating mechanism in which the Sau3AI C-terminal domain opens the N-terminal catalytic domain through allosteric effects to achieve cleavage of DNA-specific sites. The results were published in *Structure* on Aug. 30.

Due to DNA cleavage activity, Sau3AI cannot be expressed in *Escherichia coli*, and thus, a catalytic site mutant, Sau3AI-E64A, was used for exogenous expression and structural research. The crystal structure of the Sau3AI-E64A mutant reveals that when DNA is not bound, a loop region (333–342) of the C-terminal domain hangs over DNA binding site of N-terminal domain, preventing N-terminal domain from binding to DNA.

Analysis of the Sau3AI C-terminal domain and DNA complex structure indicated that the loop region (261–268 and 280–295) of C-terminal domain was significantly altered after binding to DNA, implying that Sau3AI may undergo [conformational changes](#) after C-terminal domain binds to DNA.

Furthermore, the researchers conducted gel shift experiment on the DNA binding key amino acid residue mutants (K257A, S424A, and T435A) in C-terminal domain to confirm that C-terminal binding DNA plays a crucial role in activating the catalytic activity of N-terminal domain. These results suggest that C-terminal domain activates the enzymatic activity of N-terminal domain through allosteric effects.

The researchers suggested that Sau3AI is a type IIE restriction enzyme, but unlike other type IIE restriction enzymes, it is monomeric rather than homodimer. Two [different domains](#) in Sau3AI play a similar role to homodimers in other type IIE restriction enzymes, suggesting that Sau3AI represents a new subclass of type IIE restriction enzymes.

**More information:** Yahui Liu et al, The crystal structures of Sau3AI with and without bound DNA suggest a self-activation-based DNA cleavage mechanism, *Structure* (2023). [DOI: 10.1016/j.str.2023.08.005](https://doi.org/10.1016/j.str.2023.08.005)

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