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# Filament Formation Induces a Shape Change and Activation of the Nuclease SgrAI

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## Abstract

SgrAI is allosteric and is both activated as well as undergoes a change in substrate specificity (cleaving a secondary class of DNA sequences) upon binding to its primary recognition sequence in DNA. We have investigated the allosteric activation of SgrAI and this unusual expansion of DNA sequence specificity using structural, biophysical, and enzyme kinetic methods. 1– 2

A variety of biophysical techniques (native gel electrophoresis, analytical ultracentrifugation, ion mobility native mass spectrometry, x-ray crystallography and electron microscopy) to investigate complexes formed by SgrAI. Single turnover DNA cleavage assays were used to investigate the reaction mechanism of SgrAI, and global modeling to create a computation model of the full reaction pathway including rate constants for each important step. The biophysical characterizations revealed that SgrAI forms run-on oligomers or filaments when activated, and that these filaments are induced when SgrAI binds to its primary recognition sequence. SgrAI binds tightly to secondary site sequences, which differ from primary by a single base pair, but fails to cleave them and does not induce filament formation. However, SgrAI bound to the secondary site sequences will join filaments formed from SgrAI bound to primary site sequences. Protein-protein and protein-DNA contacts between SgrAI/DNA complexes in a filament stabilize a different conformation of SgrAI, hypothesized to be activated due to a reorganization of critical residues in the active site. DNA cleavage kinetics reveal activation of DNA cleavage activity by SgrAI of 200–1000 fold for primary and secondary site sequences upon filament formation. Full global kinetic analysis reveals a slow second order association step of SgrAI addition to a filament, followed by rapid DNA cleavage, then dissociation of SgrAI from the filament, and finally rapid product (*i.e.* cleaved DNA) release. The slow association step controls SgrAI activity to form filaments preferentially on DNA contiguous with primary site sequences, and incorporation of secondary site sequences into the filament increases the number of DNA cleavages on the

contiguous DNA. Hence, this system allows for rapid cleavage of invading phage DNA (with unprotected primary sites).

The SgrAI system uses formation of a filamentous form to stabilize an activated conformation. DNA sequence and slow second order association kinetics control where and when this filament is formed in a way that is tuned for optimal performance of its biological role.

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