

## ***Using Mass Spectrometry to Elucidate the Structure of Bacteriophage T4 Helicase Dda***

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**Background and Objective:** Helicases are proteins that unwind double-stranded nucleic acids. Dda helicase from bacteriophage T4 has served as an excellent model for understanding the molecular mechanism of this class of enzymes. Also, Dda has been shown to interact with the T4 single stranded DNA binding protein, gp32. We have developed a homology model of Dda that we are using to guide further studies that will examine the structural and functional significance of the interaction between Dda and gp32 as well as the interaction between Dda and DNA, and how these interactions impact translocation on DNA.

**Methods:** We have tested the structural model by examining the protein surface using three common methods for mapping protein domains and for examining protein surfaces; chemical footprinting, formaldehyde crosslinking and limited proteolysis. All three methods rely on protein mass spectrometry.

**Results:** Our mass spectrometric studies allowed us to amend portions of our current homology model, and to select truncations for further structural studies. They also indicate the protein surfaces responsible for interaction between Dda and gp32 and Dda and DNA.

**Discussion and Conclusions:** Study of the structure of Dda may reveal why some helicases translocate in a 5'-to-3' direction on DNA, while others translocate in a 3'-to-5' direction.

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