Binding Sites for DNA on a DNA-Unwinding Protein, Probed by Hydrogen/ Deuterium Exchange and Ion Cyclotron Resonance Mass Spectrometry

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Funding Grants: G.S. Boebinger (NSF DMR-1157490); M. A. Trakselis (American Cancer Society RSG-11-049-01-DMC)

For DNA to reproduce, its double-helix must be made to unwind so that new double-helices can be synthesized from each of the two strands. The archaebacterial MCM A "helicase" protein from *Sulfolobus* solfactaricus is a model for understanding this DNA unwinding. *Although interactions of the DNA double-strand portion within the central channel of the helicase are well known, interactions with the excluded unwound single strand on the exterior surface of the helicase protein had remained largely unexplored.*

By "spray-painting" the DNA:helicase complex (by exposing it to D_2O), researchers map the DNA:helicase contact regions as those regions that are protected against hydrogen/deuterium (H/D) exchange relative to the free protein. Each H replaced by D increases the protein mass by 1 mass unit.

To determine the location of the hydrogen-to-deuterium replacements, researchers enzymatically cut the protein into small segments and weigh them in the MagLab's 14.5 tesla Fourier Transform Ion Cyclotron Resonance Mass Spectrometer. That instrument readily resolves and identifies dozens of segments to provide a detailed map of the contact surface(s). *In this way, researchers find direct evidence for binding of the unwound single-strand portions of the DNA to specific regions of the helicase during the unwinding process.*

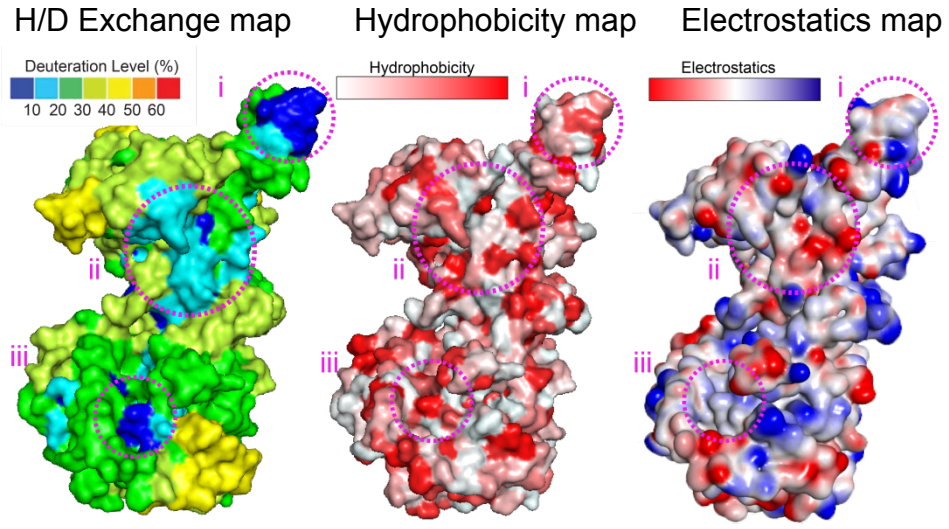

Figure: Maps of the surface of a helicase protein (a DNA-unwinding protein), based on H/D exchange (left), hydrophobicity (center), and electrostatics (right). Regions i, ii, and iii are solvent-exposed in the free helicase protein, but are protected against H/D exchange when the DNA and helicase form a complex. Comparison of the three maps helps to show which protein properties determine the sites of the interaction between the single-strand DNA and the helicase protein.

Facilities: Ion Cyclotron Resonance Facility, On-Line Liquid Chromatography Electrospray Ionization 14.5 T FT-ICR Mass Spectrometer

Citation: Graham, B. W.; Tao, Y.; Dodge, K. L.; Thaxton, C. T.; Olaso, D.; Young, N. L.; Marshall, A. G. Trakselis, M. A. "DNA Interactions Probed by Hydrogen Deuterium Exchange (HDX) Fourier Transform Ion Cyclotron Resonance Mass Spectrometry Confirm External Binding Sites on the Minichromosomal Maintenance (MCM) Helicase,"*J. Biol. Chem* **2016**, *291*, 12467-12480.