

**GRADUATE SCHOOL OF BIOMEDICAL SCIENCES**

**BIOCHEMISTRY AND MOLECULAR PHARMACOLOGY**

**Ph.D. THESIS DEFENSE**

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MENTOR: Brian Kelch, PhD

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**The Mechanism and Regulation of Bacteriophage DNA Packaging Motors**

Many double-stranded DNA viruses use a packaging motor during maturation to recognize and transport genetic material into the capsid. In terminase motors, the TerS complex recognizes DNA, while the TerL motor packages the DNA into the capsid shell. Although there are several models for DNA recognition and translocation, how the motor components assemble and power DNA translocation is unknown.

Using the thermophilic P74-26 bacteriophage model system, we discover that TerL uses a *trans*-activated ATP hydrolysis mechanism. Additionally, we identify critical residues for TerL ATP hydrolysis and DNA binding. With a combination of x-ray crystallography, SAXS, and molecular docking, we build a structural model for TerL pentamer assembly. Apo and ATP analog-bound TerL ATPase domain crystal structures show ligand-dependent conformational changes, which we propose power DNA translocation. Together, we assimilate these findings to build models for both motor assembly and DNA translocation.

Additionally, with the P76-26 system, we identify the TerS protein as gp83. I find that P74-26 TerS is a nonameric ring that stimulates TerL ATPase activity while inhibiting TerL nuclease activity. Using cryoEM, I solve 3.8 Å and 4.8 Å resolution symmetric and asymmetric reconstructions of the TerS ring. I observe in P74-26 TerS, the conserved C-terminal beta-barrel is absent, and instead the region is flexible or unstructured. Furthermore, the helix-turn-helix motifs of P74-26 TerS are positioned differently than those of known TerS structures, suggesting P74-26 uses an alternative mechanism to recognize DNA.

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