SPECIFIC AIMS: Double-stranded DNA (dsDNA) viruses utilize highly processive, virally encoded molecular motors to package their genomes into pre-assembled protein capsids. These multimeric NTPase ring motors convert chemical energy derived from ATP binding and hydrolysis into mechanical translocation of DNA. Packaging ATPases generate extremely high forces to compact DNA to near crystalline density within capsids and are among the most powerful biological motors known. Thus, understanding the mechano-chemistry of these motors provides a unique window into how maximal forces can be derived from ATPase activity. Further, viral DNA packaging motors belong to a large class of ASCE ring NTPases that drive many basic cellular functions and thus characterizing their mechanochemistry will illuminate the general principles of this ancient and ubiquitous class of molecular motors. Understanding this similarities and differences between disparate ASCE ring motors provides an opportunity to develop novel drug therapies that selectively target pathogen-specific ASCE ring motors as well as dysregulated ATPases that contribute to other human disease states.

Fundamental remaining questions regarding how virally encoded ASCE motors drive DNA packaging are: 1) how do changes in the tertiary and quaternary structure of motor complexes drive DNA translocation; 2) what is the molecular basis of force generation that drives these changes; and 3) how does coordinated nucleotide binding, hydrolysis, and exchange regulate force generation. The DNA packaging system developed for bacteriophage φ29 is especially well suited to address these questions as a highly efficient, easily manipulated *in vitro* packaging system, along with a battery of functional assays, has made it possible to experimentally probe all aspects of packaging. Additionally, our previous high-resolution single-molecule studies have revealed essential aspects of motor operation and coordination. Similarly, our advanced structural and computational analyses of the motor allowed us to visualize structural changes that drive packaging and propose a comprehensive mechanism of DNA translocation. While this work provided important new insights regarding the different conformational states that the motor cycles through to translocate DNA, several critical questions remain, such as how these transitions drive packaging, and how these transitions are regulated in the motor. In this grant, we will build on our prior success advancing our understanding of viral DNA packaging via single molecule and structural approaches to address these critical questions.

Most recently, our structural analysis of packaging motors indicated that the ATPase component of the motor can adopt both ring- and helix-like configurations. Based on these results and the prior single molecules studies, we proposed that DNA translocation is powered by stepwise helical-to-planar motor transitions that are tightly coordinated by ATP binding, hydrolysis, and product release. To test and fully develop this model, we will: 1) use single particle analysis (SPA) cryo-EM image processing to reconstruct mechanistically critical intermediates as well determine how energy landscapes of the motor in different nucleotide-bound states determine its conformational trajectory during packaging; and 2) use combined single-molecule fluorescence and force measurement approaches (“fleezers”) to monitor conformational changes in the motor that accompany DNA translocation and characterize the effect of substituting mechanistically critical motor residues on motor function. While these two approaches address common mechanistic questions, two specific aims are organized around different experimental approaches that are tied together by complementary biological analyses woven across the whole project. The two specific aims are to:

1) **Aim 1: Characterize the structure, dynamics, and energy landscape of DNA packaging motors.** In **Aim 1.1**, we will use standard cryoEM SPA and nested highly focused reconstructions to test structural predictions of the helical to planar model. In **Aim 1.2:** we will utilize advanced image processing approaches to characterize the dynamics and energy landscape of packaging motors.

2) **Aim 2: Single-molecule studies of ring motor mechanism.** In **Aim 2.1** we will use single molecule optical laser tweezers to dissect how key residues participate in critical functions during DNA translocation. Further, we will utilize combined single molecule imaging and force measurements to characterize DNA rotation during DNA packaging (**Aim 2.2**) and to correlate changes in motor conformation with DNA translocation (**Aim 2.3**).

Completion of these Aims will provide a comprehensive description of the molecular basis of force generation and the resulting coordinated conformational changes that drive viral DNA packaging. Therefore, our results have the potential to provide new targets for anti-viral therapeutics and inform the rational design of molecular motors. Lastly, this work will provide fundamental insight into molecular recognition, the basis of coordinated protein-protein and protein-nucleic acid interactions, the mechanism of nucleic acid translocation and compaction, and the conversion of chemical energy into mechanical motion in biological systems.