collected using the chip and X-Y plate goniometer, for optimization of crystallization conditions for both membrane proteins and macromolecular complexes. Complete data have also been collected from crystals obtained from initial screening experiments, enabling structure determination with < 3 ul sample.

Keywords: microfluidic, *in-situ* diffraction, free-interface diffusion

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Molecular mechanisms of self-assembly and motion of the bacterial flagellum

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The bacterial flagellum is made of a rotary motor and a long helical propeller by means of which bacteria swim. The motor rotates at around 300 Hz and drives the rapid rotation of each flagellum to propel cell movements. The propeller is a relatively rigid, supercoiled filament that switches between left- and right-handed helical forms in response to reversal of the motor rotation, allowing bacteria to alternate their swimming pattern between run and tumble for taxis. A short, highly curved segment called hook connects the motor and the helical propeller, and its bending flexibility makes it work as a nano-scale universal joint. The axial structure of the flagellum is constructed by self-assembly of proteins translocated from the cytoplasm to the distal end of the growing structure through its narrow central channel, where a cap complex helps efficient self-assembly. The flagellar type III protein export apparatus, which consists of six membrane proteins that form the export gate and three cytoplasmic proteins including an ATPase, FliI, drives the export process. It had long been thought that ATP hydrolysis by FliI provides energy for the export process, but our recent finding revealed that successive unfolding and translocation of export substrate proteins is driven by proton motive force and that ATP hydrolysis is used to disassemble the FliI hexamer that binds and inserts the N-terminal chain of export substrates from the export gate and substrate proteins to facilitate the export process. We combine electron cryomicroscopy and X-ray crystallography to visualize flagellar structures at nearly atomic resolution to understand molecular mechanisms of protein export, self-assembly, and rotation.

Keywords: bacterial falgellum, self-assembly, protein export

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Mechanism of DNA packaging in bacteriophage T4

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The tailed bacteriophage T4 packages about 171 kb DNA inside the virus capsid. Its packaging motor is composed of an empty prohead and an olgomeric terminase enzyme assembled at the special dodecameric portal vertex. The T4 terminase complex consists of the 70 kDa large terminase protein, gp17 and the 18 kDa small terminase protein, gp16. The large terminase protein gp17 is the key catalytic component of the motor. It has two domains, an N-terminal ATPase domain that powers DNA translocation and a C-terminal translocation domain that pushes DNA as well as cuts it after the head is full. Mutagenesis, bioinformatics, and biochemical studies have defined various functional motifs of each domain. Single molecule studies in collaboration with Dr. Doug Smith at UCSD showed that the phage T4 packaging motor is the fastest and the most powerful reported to date. The X-ray structures of the gp17 domains as well as the full-length protein, and the cryo-EM structure of the whole packaging motor were determined in collaboration with Dr. Michael Rossmann's lab at Purdue. These studies lead to the establishment of the DNA translocation mechanism in which the gp17 large terminase protein not only supplies the ATP hydrolysis energy but also acts as a force generating motor translocating DNA into the prohead. An electrostatic inchworm translocation mechanism is proposed, which may be common to the packaging motors of tailed bacteriophages and DNA viruses.

Keywords: virus assembly, DNA packaging, molecular motor

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DNA translocation by hexameric FtsK

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FtsK is a DNA translocase that coordinates chromosome segregation and cell division in bacteria. In addition to its role as activator of XerCD site-specific recombination, FtsK can translocate double-stranded DNA rapidly and directionally, and reverse direction. We present crystal structures of the FtsK motor domain monomer, showing it has a RecA-like core, and the Ftsk hexamer, showing it is a ring with a large central annulus. Electron microscopy demonstrates the DNA-dependent existence of hexamers in solution and shows that duplex DNA passes through the middle of each ring. Comparison of FtsK monomer structures from two different crystal forms highlights a conformational change that we propose is the structural basis for a rotary inchworm mechanism of DNA translocation. We then show how the C-terminal gamma-domains recognise specific DNA sequences to reverse the direction of the motor domain's movement on DNA: three gamma domains bind to the KOPS sequence in a dircetional manner and act by loading the motor complex in one orientation only.

Keywords: DNA translocation, bacterial cell division, FtsK