

Materials and Methods

FtsK_{50C}Δγ construct design.

PredictProtein¹ was used to create a multiple sequence alignment, which predicted conserved regions, secondary structure elements, and unstructured regions. 3D-PSSM threading² was used to predict tertiary folds adopted by the γ-domain.

FtsK_{50C}Δγ construct cloning.

Plasmid pTM12³, which contained the gene for FtsK_{50C} was inverse PCR amplified using AccuTaq LA (Sigma) and the primers (1) 5-Phos-TGATGAGGTGCGGGTGGTTTCGATGGCGCT and (2) 5-Phos-ACCTTCGCTTTCGCTGTCGGA, to insert a UGA stop codon after residue 487 in the FtsK_{50C} gene (which corresponds to residue 1254 of full-length FtsK from *E. coli*). PCR products were blunt-ended with Vent DNA Polymerase (NEB), gel purified, and circularized using T4 DNA ligase (NEB). Clones were propagated in *E. coli* strain DH5α and verified by sequencing.

FtsK_{50C} and FtsK_{50C}Δγ expression and purification.

FtsK_{50C} and FtsK_{50C}Δγ were expressed and purified as described³. The purest fractions from each purification (>95% pure), as determined by SDS-PAGE and coomassie blue staining, were pooled and quantified using the Bradford Method with BSA (NEB) as a standard. Stocks were stored at 4°C without detectable loss of activity over the course of the experiments. FtsK_{50C}Δγ displayed comparable DNA-dependent ATP hydrolysis characteristics to FtsK_{50C} (data not shown).

Triplex Displacement Assays.

Triplex substrates were prepared as described⁴. Triplex displacement assays were carried out as described⁴ with 235nM FtsK_{50C}, 470 nM FtsK_{50C}Δγ, or dialysis buffer. Background was subtracted as described⁴.

Optical Tweezers Substrates Preparation.

λ DNA single molecule substrates were prepared as described³. Briefly, biotin- and digoxigenin-modified PCR products were digested with KpnI or XbaI (NEB), to create linker molecules. Lambda DNA (NEB) was circularized using T4 DNA ligase (NEB), digested with KpnI and XbaI, and the 41 kbp fragment was gel purified and ligated to biotin- and digoxigenin-modified linker molecules to create DNA tethers.

Optical Tweezers Assay.

FtsK_{50C} and FtsK_{50C}Δγ were used at 150nM. At these concentrations, actively translocating particles of aggregated enzyme were observed and used to track the position of a single active FtsK⁴. This optical tweezers particle assay has been used extensively used to detect FtsK directionality (Refs. 1, 4). We believe that the FtsK particle motion observed in these experiments is due to a single active complex within the particle, as we previously showed (Ref. 4). The reasons are: *(i)* We never observe DNA reeling in from both sides of the FtsK particle. If multiple motors were active due to stochastic loading of the motors on the DNA, then we would expect to see some examples of the DNA being reeled in from both directions. *(ii)* We measured similar velocities at low FtsK concentrations where we see no aggregation and at concentrations >100 nM which promote aggregation. *(iii)* The change in direction of movement at non-FRS sequences is extremely rapid. The transition between the

forward and backward movements by the FtsK particle occurs within a 30th of a second, the temporal resolution of our assay. If multiple motors were active at the same time, then we would expect a slower transition. (iv) If there were multiple motors binding stochastically, there should occasionally be more than one motor acting on a DNA. However, the standard deviation of the rate is relatively small (4.0 ± 1 kb/sec). (v) Translocation rates and other properties of the FtsK motor remained the same independently of whether we observed FtsK particles in the optical tweezers or single FtsK complexes in the magnetic tweezers.

Reactions were conducted at room temperature in 50mM Tris, pH7.5/ 5mM $MgCl_2$ / 3mM ATP/ 50mM NaCl/ 1mM DTT/ 0.1mg/ml BSA. DNA tethers were bound between 2.8 μ m diameter anti-digoxigenin bead held in an optical trap and 2.2 μ m streptavidin-coated bead immobilized on a micropipette. Data was captured on digital video at 30Hz using a SONY Digital8 video walkman and analyzed with NIH Image and Matlab. Particles were tracked by following the movement of the centroid of FtsK particles in the direction parallel to the DNA tether as a function of time. A **translocation event** is defined as the whole period of activity in which a single FtsK particle moves for at least 0.5 μ m at a minimum speed of 0.25 μ m/sec.

These data were employed to calculate the number of FtsK particles traveling in the permissive $n_+(d)$ or non-permissive $n_-(d)$ orientation as a function of their position d on λ DNA (Fig. 1C). The probability of FtsK traveling in the permissive (non-permissive) direction was calculated by integrating $n_+(d)$ ($n_-(d)$) for all values of d in the interval [0, 44] kbp and normalizing by the total number of events. **The net distance translocated** by an FtsK particle was measured by subtracting the initial position of the FtsK particle on λ DNA from that at the end of the translocation event.

A **burst** was defined as the region of a translocation event in which the FtsK particle moves in one direction without pausing or turning around. The **burst size** was thus defined as the total distance translocated in a burst. Bursts were identified using a burst-finding algorithm with data averaged using a four point rolling average with a lower threshold velocity of 0.1 $\mu\text{m/s}$. The sign of the burst size was defined as positive when the particle traveled in the permissive orientation and negative when it traveled in the non-permissive one. The distribution of burst sizes was calculated from a total of 441 bursts for FtsK_{50C} and 262 bursts for FtsK_{50C} $\Delta\gamma$.

Optical tweezers were operated under constant feedback mode, and the tension on the DNA was always >20 pN. Although FtsK-induced loop formation could have occurred at the lowest forces used (20 pN), loop sizes and frequency were minimized by the immediate increase in force generated when looping displaced the bead from the optical trap. At forces >30 pN, FtsK is unable to efficiently loop DNA as we have previously shown (Refs. 1, 4). FtsK translocation velocities were measured by tracking the position of the centroid of FtsK particles as a function of time. Looping of DNA would not have changed the position of FtsK particles but rather the distance between the beads. Therefore, our velocity measurements directly reflect on the translocation of FtsK. The mean velocities measured with this assay (4 ± 1.5 kbp/s) were similar to those reported in Ref. 2 (~ 5.5 kbp/s at 3 mM ATP concentration), our previous optical tweezers experiments (5 ± 1 kbp/s, Ref. 4), and our magnetic tweezers experiments (~ 4.5 kbp/s).

Magnetic Tweezers Substrates Preparation.

A 12.2 kbp region of the *E. coli* K-12 genome (nucleotides 3178400-3190613) devoid of any FRS or RAG family motifs^{4,5} was PCR amplified using

AccuTaq LA DNA polymerase and primers
AGTAGTCTAGAGCGTGGAATCCAGGGCGCA and
GTGCAACCGGTACCTCTTCTCGTTC. PCR products were digested with XbaI,
KpnI, and DpnI and gel purified. Products were ligated into XbaI and KpnI sites of
pBS-SK(+). Clones were propagated in *E. coli* strain DH5 α and verified by
restriction digestion. To insert FRS sequences into these clones in the desired
location, inverse PCR with AccuTaq LA DNA polymerase and the primers 1)
5'ATGATCCATGGGCAGGGCAGGGCAGGGCCCGTCCATATTCAACGGCT
and 2) 5'ATGATCCATGGTGGATAGCGCACGCGCCCTT to introduce the FRS
sequences and a unique NcoI site for circularization. PCR products were digested
with NcoI and DpnI (NEB) and gel purified. T4 DNA ligase (NEB) was used to
circularize products. Clones were propagated as described above and verified by
sequencing. To create DNA tether molecules, the plasmid described above was
digested with either XbaI and KpnI or XbaI and SalI to create inserts of 12.2 kbp or
8.8 kbp with the FRS repeat in the center or 2.7 kbp from one end, respectively.
These molecules were ligated to biotin- and digoxigenin-modified PCR products to
create tether molecules.

Magnetic Tweezers Experiments

Magnetic tweezers experiments were performed by using an FtsK
concentration ranging from 10 to 15 nM. At these concentrations, we have not
observed FtsK aggregates. In this assay, loop formation by FtsK was the signal used
to monitor FtsK activity. These experiments were conducted on a high-power
magnetic tweezers instrument described elsewhere^{6 7} (Hong S.C, Stone M.D,
Humphries D., N.M., C.B., and N.R.C., manuscript in preparation). Briefly, the

optical setup is a home-made inverted microscope, equipped with high power hybrid permanent magnets that can be translated to control precisely the degree of tension applied to single DNA molecules. The magnets enabled the application of stretching forces up to ~ 10 pN using 1 micron-diameter paramagnetic beads. Dynamic changes in DNA extension were measured in real time at 10-30 Hz by comparing the bead diffraction ring pattern with a previously calibrated set of images taken at known focal displacements. To ensure accuracy of our extension measurements, each experimental setup was individually calibrated by an automated routine. Activity assays were conducted in FtsK reaction buffer containing: 50 mM Tris-HCl pH 7.5, 3mM ATP, 10-15nM FtsK_{50C} or FtsK_{50C} $\Delta\gamma$, and 0.1 mg/ml BSA. The distance between the magnets and the sample was held constant throughout each experiment, resulting in constant force ($\sim 5-8$ pN) during measurements. Modified DNA molecules possessing multiply-labeled biotinylated and digoxigenated ends were oriented between an α -digoxigenin antibody coated glass surface (Roche) and a 1 μm diameter streptavidin bead (Dynal, My One Beads). FtsK activity was observed as a change in the DNA extension resulting from the formation or release of a DNA loop. In this assay, we have observed both spontaneous reverse translocation and abrupt loop release by the motor, as previously described in Ref. 2. In the range of forces used, however, we mostly observe translocation reversals.

In measuring translocation rates, waiting times prior to the onset of activity were required to be five times longer than the total burst time. The translocation rate was then measured in each burst by using a piecewise linear fitting algorithm. For the determination of mean translocation rates, 142 events on 6 tethers were considered for FtsK_{50C} and 186 events on 7 tethers for FtsK_{50C} $\Delta\gamma$. Occupancy time distributions for FtsK_{50C} and FtsK_{50C} $\Delta\gamma$ were measured by calculating the histogram of DNA

extensions during periods of activity and pauses for nine experiments on three and five tethers, respectively. Extensions corresponding to zero or the full length of the tether were not considered for clarity. The occupancy time distribution for FtsK_{50C} in Fig. 2D (blue) is higher at higher DNA extensions (after passing through the FRS repeat). Before translocation begins, the DNA tether has full extension and FtsK translocation by looping shortens the DNA extension. As shown in Fig. 2B, the recognition of FRS by FtsK leads to a translocation reversal, which implies that most of the time the DNA tether is longer than the position of FRS in the tether. Only in a few cases FtsK fails to recognize FRS and thus translocates the full length of the DNA tether. For this reason, most of the time the DNA length is higher than that corresponding to the position of FRS, and so the distribution for FtsK_{50C} is asymmetric with respect to this position.

The occupancy time distribution for FtsK_{50C} $\Delta\gamma$ (Fig. 2d, red) is, on the other hand, homogeneous. We have shown in the optical tweezers experiments that the mean processivity of the FtsK motor (FtsK_{50C} $\Delta\gamma$) is ~ 9 kbp. The tether used in the magnetic tweezers experiments was 12 kbp long, and so most often the activity of FtsK led to the full tether translocation (see Fig. 2C). For this reason, this distribution is homogeneous.

Supplementary References

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