The anatomy and action of molecular machines that replicate and segregate DNA

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By using a combination of *in vivo* biochemistry, cell biology, and *in vitro* analysis, we have dissected the action of the *in vivo* *Escherichia coli* replisome and the FtsK DNA translocase, which coordinates the late stages of cell division and chromosome segregation. Using a novel microscopic method, ‘slimfield’ illumination, we can visualize in living cells up to 3 different single protein molecules simultaneously with a temporal resolution of 3 ms and spatial precision of ~5 nm. This has allowed us to determine the *in vivo* stoichiometry of replisome components and their spatial distribution.

FtsK translocates DNA directionally at ~5 kb.s⁻¹, and acts in chromosome unlinking by activating XerCD site-specific recombination at *dif* located in the replication termination region of the *Escherichia coli* chromosome. In attempts to understand how FtsK translocates and how this translocation is controlled, we have characterized covalent multimers of FtsK that have defined mutations in the different hexameric subunits. We will also provide evidence that translocation and activation of unlinking are coupled, with FtsK capturing a XerCD-*dif* complex *in trans* and then transporting this nucleoprotein complex to a sister XerCD-bound *dif* site, thereby ensuring that the products of recombination are topologically unlinked.