Single molecule spectroscopic approach to study DNA replication machinery

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Single-molecule imaging techniques have become important elements of the biologist's toolkit to gain mechanistic insights into biological processes. By removing ensemble averaging, single-molecule methods provide unique access to the dynamic behavior of biomolecules. T4 bacteriophage is one of the major model systems for acquiring information on DNA replication and repair processes. The T4 bacteriophage helicase loader (gp59) is one of the main eight proteins which play an active role in the replisome. It binds preferentially to forked DNA and interacts directly with the T4 helicase (gp41), single-stranded DNA binding protein (gp32) and polymerase (gp43). However the stoichiometry and structure of the functional form of gp59 is not very well understood. In this present study, we employed single-molecule photobleaching (smPB) experiments to elucidate the stoichiometry of gp59 on a forked DNA and investigate its interaction with other proteins forming the primosome complex.

References

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