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Single-molecule studies of multi-protein machines

ANTOINE VAN OIJEN, Harvard Medical School

Advances in optical imaging and molecular manipulation techniques have made it possible to observe individual enzymes and record molecular movies that provide new insight into their dynamics and reaction mechanisms. In a biological context, most of these enzymes function in concert with other enzymes in multi-protein complexes, so an important future direction will be the utilization of single-molecule techniques to unravel the orchestration of large macromolecular assemblies. Our group is developing the single-molecule tools that will make it possible to study biochemical pathways of arbitrary complexity at the single-molecule level. I will discuss results of single-molecule experiments on the replisome, the molecular machinery that is responsible for replication of DNA. We stretch individual DNA molecules and use their elastic properties to obtain dynamic information on the proteins that unwind the double helix and copy its genetic information. Furthermore, we visualize fluorescently labeled components of the replisome and thus obtain information on stochiometry and exchange kinetics. This simultaneous observation of catalytic activity and composition allows us to gain deeper insight into the structure-function relationship of the replisome.