

Topological Characterization of Single DNA Molecules by Tethered Fluorophore Motion

Riccardo Zirardo, Ryan P Compton, Andreas Hanke, Stephen D Levene

We present a method to determine the topology of single circular DNA molecules as a function of knot type, K , and linking number, Lk . Plasmid DNAs bearing fluorophores conjugated at specific sequence positions are immobilized to glass coverslips and analyzed using total-internal-reflection fluorescence (TIRF) microscopy. Using topoisomer pools and specific DNA-knot types, respectively generated by topoisomerase-I relaxation and Cre site-specific recombination, we are determining the characteristic fluorophore-emission image distributions belonging to particular DNA topologies. Comparing observed spatial fluorophore distributions with those obtained by Monte Carlo simulation, we seek to show that variations in plasmid topology lead to measurable, single-molecule-level differences in the spatial footprint of conjugated fluorophores.