Single-molecule tools to probe how transitional kinetics between "open" and "closed" enzyme structures can be tuned by salt through intermediate states

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DNA helicases undergo conformational changes; however, their structural dynamics are poorly understood. Here, I report findings on the superfamily 1A DNA helicase Rep, a model enzyme for studying catalytically functional structural dynamics, which undergoes conformational transitions during bacterial DNA replication, repair and recombination. We use time-correlated single-photon counting (TCSPC), fluorescence correlation spectroscopy (FCS), rapid single-molecule Förster resonance energy transfer (smFRET), Anti-Brownian ELectrokinetic (ABEL) trapping and molecular dynamics simulations (MDS) to provide unparalleled temporal and spatial resolution of Rep's domain movements. We detect four states revealing two hitherto hidden intermediates (S2, S3), between the open (S1) and closed (S4) structures, whose stability is salt dependent. Rep's open-to-closed switch involves multiple changes to all four subdomains 1A, 1B, 2A and 2B along the S1→S2→S3→S4 transitional pathway comprising an initial truncated swing of 2B which then rolls across the 1B surface, following by combined rotations of 1B, 2A and 2B. High forward and reverse rates for S1→S2 suggest that 1B may act to frustrate 2B movement to prevent premature Rep closure in the absence of DNA. These observations support a more general binding model for accessory DNA helicases that utilises conformational plasticity to explore a multiplicity of structures whose landscape can be tuned by salt prior to locking-in upon DNA binding