Atomic force microscopy shows that TubR bends the DNA forming a loop at tubC

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Most partition systems responsible for plasmid segregation and maintenance are composed by three components: a DNA centromere sequence, a centromere-binding protein (CBP), and a motor protein. According to the nature of the motor protein these systems can be classified into type I (Walker-A ATPase), type II (actin-like ATPase) and type III (tubulin-like GTPase). Clostridium botulinum phage c-st encode a type III partition system that likely function during the phage lysogenic phase and makes this organism a prototype model for the study of DNA segregation. Here, the motor protein (TubZ) moves the phage DNA through its interaction with the partitioning complex (centromere sequence -tubC- and the CBP –TubR-).
We use Atomic Force Microscopy (AFM) to image the interaction of TubR with tubC that expands along 500 bp. We found that TubR binding induces the deformation of DNA, bending it into one or two loops at saturated protein concentrations. This interaction is specific, as control experiments on random DNA did not show any deformation. Additionally, at TubR saturating conditions we did not observe any DNA aggregation or further DNA spreading. AFM volumetric analysis suggests the protein is a monomer. The typical size of closed loops is 15-20 nm in diameter, providing an estimation of 20 monomers per loop. The size of the loop is compatible with published models of TubZ filaments suggesting that this may be the structure recognized by TubZ. Preliminary AFM experiments with TubZ and TubR-DNA showed clustering of TubZ around the loop structures. All together, our data suggest a model where TubR binds and spreads along tubC while inducing a bend in the DNA.