

ABSTRACT

Title of Dissertation: EFFECT OF MISMATCHED BASE PAIRS
ON DNA PLECTONEMES

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Base pair mismatches in DNA occur during replication and can result in mutations and certain types of cancer. The exact mechanism by which mismatch repair proteins recognize mismatches is still not well understood. Structures of mismatch recognition proteins bound to a mismatch indicate that the process involves introducing a sharp bend in the DNA and flipping out the mismatched base. Under external torsional stress, an elastic rod with a defect would buckle at the defect, provided the defect reduces the local bending stiffness. *In vivo*, if the same energetic scenario prevails, it could localize (or pin) the mismatch at the plectoneme end loop (plectoneme refers to a structure formed by the DNA when it buckles, and its helical axis wraps or writhes around itself in the presence of a critical torsional stress) and make the mismatched base pair more accessible to the mismatch repair protein. In genomic DNA, however, the entropic cost associated with plectoneme localization could make pinning unfavorable. Magnetic-tweezers-based studies of DNA supercoiling, performed at high salt concentrations, have shown that in DNA harboring a single mismatch, the plectoneme will always localize at the mismatch. Theoretical studies

have predicted that under physiological salt concentrations, plectoneme localization becomes probabilistic, i.e., the plectoneme does not always localize at the mismatch. Plectoneme localization under physiological salt conditions is dependent on the number of mismatches and tension applied to the DNA. However, both experimental and theoretical approaches are currently limited to positively supercoiled DNA. In the current dissertation, we aim to study plectoneme localization, in physiologically relevant conditions, using state-of-the-art molecular dynamics (MD) simulations and single molecule magnetic tweezers-based experiments.

In order to simulate plectoneme localization we first develop a framework using the widely available sequence and salt dependent OxDNA2 model. We verify that the OxDNA2 model can quantitatively reproduce a reduction in bending rigidity due to the presence of the mismatch(es), similar to all-atom MD simulations. We then verify that the current framework can reproduce the experimentally observed plectoneme pinning (at the location of the mismatches). Next, we simulate plectoneme pinning under physiologically relevant conditions. We find that the plectoneme pinning (at the location of the mismatches) becomes probabilistic and this probability of plectoneme pinning increases with an increase in the number of mismatches. We also simulate a longer 1010 base pair long DNA to study the influence of entropic effects on plectoneme pinning.

Next, we extend the simulation framework to simulate a negatively supercoiled, i.e., under-wound, DNA molecule. *In vivo*, DNA is maintained in a negatively supercoiled state. Negative supercoiling can result in local melting at the mismatched base pairs: this local melting would further reduce the local bending rigidity at the

mismatched base pairs and could enhance plectoneme pinning. We find that negative supercoiling significantly enhances plectoneme pinning in comparison with equivalent levels of positive supercoiling. We also find that the mismatched base pairs are locally melted and the plectoneme end loop is bent significantly more as compared to the positive supercoiling case. Additionally, we simulate the 1010 base pair long DNA under two different negative super-helical densities, i.e., two different degrees of unwinding. We find that the super helical density does not affect the plectoneme pinning probabilities. We also conduct simulations of DNA under different stretching forces (0.3 pN, 0.4 pN and 0.6 pN). Negatively supercoiled DNA under relatively high stretching force (~ 0.6 pN) absorbs torsional stress by locally melting instead of supercoiling. Simulations of DNA under different forces allow us to study the effect of mismatches on the competition between supercoiling and local melting in a negatively supercoiled DNA. We find that higher stretching forces, up to a maximum set by the onset of melting, increase plectoneme pinning at the location of mismatch.

Finally, we propose and develop a single molecule assay to validate the simulations results presented in the previous chapters. Previous single-molecule magnetic tweezers measurements of mismatch DNA buckling and pinning were limited to the high force (~ 2 pN) – high salt (>0.5 M NaCl) regime. We propose to overcome this limitation by attaching a small gold nano-bead via a di-thiol group close to the mismatched base pairs, which permits direct observation of transient DNA buckling at the mismatch. We generate a DNA substrate that can be used to directly observe plectoneme pinning at the mismatch. We perform single-molecule magnetic tweezers

measurements to verify that the presence of the di-thiol group does not result in anomalous pinning in an intact DNA molecule.