Investigating the interactions between DNA and a type IV pilin with molecular simulation

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One of the mechanisms by which bacteria can acquire genetic information from their external environment is through the use of type IV pilus filaments. Type IV pilus filaments are long biopolymers that emanate from the bacterial cell surface and are composed of a monomer protein subunit generally referred to as pilin. In Type IV pili, the "major" pilin is the protein that makes up the largest fraction of the filament monomers, however, other "minor" pilin proteins with specific functions can also be incorporated into these systems. One such "minor" pilin is the protein ComP that allows a type IV pilus to recognize and bind to double stranded helical DNA. A recent computational model of the ComP/DNA complex from *Neisseria subflava* has provided insights about which amino acids of ComP might be most important for stabilizing the protein/DNA interface, however the static docked model does not provide any insight into the dynamic motions of the ComP/DNA complex.

In this work, we run all-atom molecular dynamics simulations using the AMBER16 software on GPU computers to analyze the interactions between ComP and DNA in this complex. Specifically, we are interested in exploring the adhesive interactions between ComP and DNA. We have simulated both the wild-type system as well as several ComP mutants (K30A, K56A, K94A, R107A). The initial sites for mutation simulations were chosen based on a combination of previous NMR results as well as the calculation of electrostatic and van der Waals interactions between ComP and DNA from our wild-type simulations. This analysis narrowed down our choice of possible mutations to several basic protein residues. The effects of mutating the protein on the free energy of binding were calculated using the MM-PBSA method. We also monitored the stability of the protein/DNA interface by measuring distances between protein amino acids and DNA nucleotide base. Other structural properties of the protein and DNA were evaluated using backbone root mean square deviations, amino acid and nucleotide root mean square fluctuations, DNA sugar puckering angles, base pair distances, and orientation changes of the DNA strands on the protein-binding site. Interestingly, we have identified that there are multiple binding orientations available for the DNA on the protein in the wild-type state, and that the ComP protein can still remain effectively bound to DNA in the case of single amino acid point mutations over the simulated time scales. In the future, we plan on running further trials (e.g., double mutants, changes of the DNA nucleotide base sequence, etc.) and analyses to assess the overall binding and stability of the complex and to identify the ComP residues most important for adhesion.

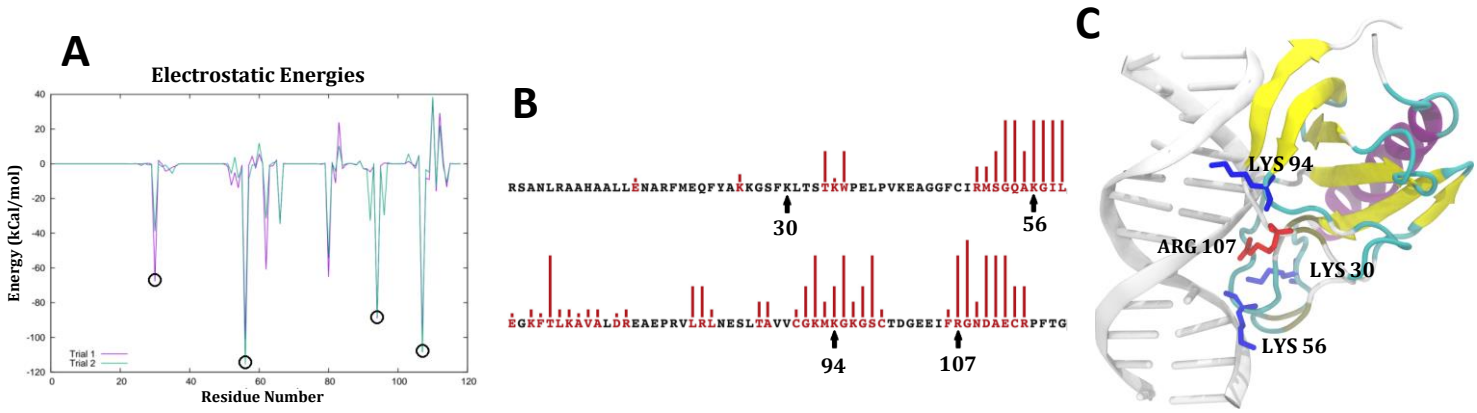

Figure 1. (A) Electrostatic energies between the residues in ComP and DNA for Trial 1 (purple) and Trial 2 (green) of the wild-type simulations. Peaks of greater magnitude are circled as sites for mutations. (B) Chemical shift perturbation analysis showing the relative magnitude of interactions between ComP and DNA (from Berry, et al., Structure. 2016; 24: 926-934). Residues corresponding to circled peaks in (A) are labeled. (C) Wild-type ComP/DNA complex with residues identified for mutation shown in blue (Lys 30, 56, 94) and red (Arg 107).