Gene Cline
Mentor: Dr. Bill Miller III, Department of Chemistry, Truman State University

DNA polymerase is a vital enzyme involved in the replication of an organism's genome. A nucleotide and magnesium ion travel into the active site while the polymerase is in the open conformation. Catalysis occurs upon the transition to the closed conformation when the incoming nucleotide forms a Watson-crick base pair, with pyrophosphate (PPi) and a magnesium ion as a byproduct of the reaction. However, the detailed mechanism of byproduct release is currently unknown, thus this project details a method to determine the probable mechanism. There were are three possible mechanisms by which byproduct release could occur. First, byproduct release in the closed conformation causes conformational change from closed to open. Second, that conformational change from closed to open causes byproduct release. Third, that byproduct release and conformational change do not affect each other, in which byproduct release is caused through diffusional forces.

The structures of B. stearothermophilus DNA polymerase I in the open, closed, and ajar conformation were obtained from the Protein Data Bank and modified to simulate product release of post-catalytic state. Unrestricted molecular dynamics (MD) simulations starting from each of these conformations were performed using Amber 14. All trajectories were visually

analyzed using the VMD program and thermodynamic properties were calculated using MD analysis tools in Amber 14.

Of 18 systems simulated, the open system with no further modification to the byproducts had the byproducts leave the active site. There was no byproduct release in any of the other 17 systems. More importantly, there was a continual progression noted from closed to open confirmations. These early results indicate that the conformational change to open has to occur in order for byproduct release. So while byproduct release doesn't cause conformational change, it is unknown if confirmation change causes byproduct release or if it is just necessary for DNA polymerase to be in the open conformation to allow diffusional forces to occur for byproduct release. Further research has begun using steered molecular dynamics to determine further clarification on this mechanism.

Attached photo is from a research presentation at NCUR 2017, located in Memphis TN.