

## Structural Changes in Mutant Firefly Luciferases

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Luciferase is a bioluminescent enzyme composed of two terminals which undergo extensive domain movements. Upon the binding of luciferin, the C-terminal rotates 95 degrees toward the N terminal, and then catalyzes the next step with a 140 degree rotation around an axis passing through the C-terminal lengthwise.

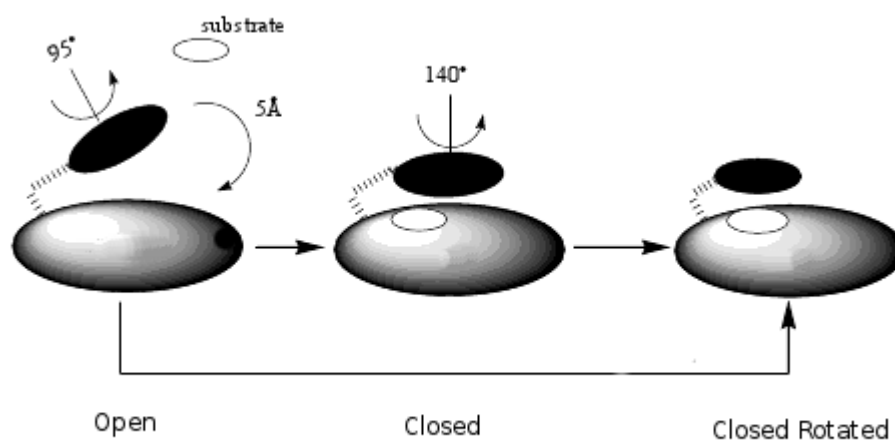


Figure 1: The three conformers of luciferase, open, closed, and closed-rotated

Our research focuses on two very different mutant luciferases. The first has 5 point mutations in the C-terminal which significantly increases luminescence. We are using molecular mechanics to try and determine how these changes affect the luminescence, even though they are far from the active site. Prior members in our lab used homology modeling to create a structure of luciferase in the closed form, as no crystal structures of this form were available. The model was used to find two residues that, if mutated with cystines, would allow 1,2-bis(maleimido)ethane to form a bridge between the two terminals in the closed rotated form. This link traps luciferase in the closed rotated form and allowed the first crystal structure of this conformation to be solved. We are now using computational methods to determine what perturbations the bridge has imposed on the closed rotated structure. This was done by graphically removing the bridge from the bridged crystal structure and finding the low energy conformations luciferase adopts when the structure relaxes.