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Creating a Fast-Folder ROS-sensitive GFP and a Super-Fast ROS-Sensitive GFP

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Our lab has developed a reactive oxygen species sensitive green fluorescent protein (royGFP). This biosensor can be utilized to detect the effectiveness and biochemical pathways of novel antibiotics and antifungals. However, royGFP suffers from oligomerization within some of the major organelles, due to two wildtype cysteines forming disulfide bridges with other GFP proteins. By researching scientific articles, it became apparent how Fast-Folder GFP and Super-Fast Folder GFP (sfGFP) may bypass these problematic residues. Therefore, my goal for this project will be to develop fast folder and super fast folder and ROS variants of these two GFPs. We began our process by using yeast enhanced GFP (yEGFP) as our initial template. By inserting five mutations, F99S, M153T, V163A, F64L, and G65T via Quikchange, we created Fast Folder GFP (ffGFP). We conducted minipreps in between each mutation and after completing this set of five mutations, we then began synthesizing Super-Fast folder GFP, this was done by adding S30R, Y39N, N105T, Y145F, I171V, and A206V to our ffGFP. By adding the S147C and Q204C to both ffGFP and sfGFP, we synthesized two new ROS-sensitive GFPs. Our results show fast-folder GFP to have increase intensity, due to the uniform intensity and brightness in comparison to yEGFP and royGFP. By creating these two versions, it may be possible to accurately quantify the production and damage of ROS being produced by antibiotics and antifungals.