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Development of a Fast Folder and Super Fast Folder Blue Fluorescent Protein

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Fluorescent molecules are molecules that are photo luminescent from the molecule absorbing a specific excitation frequency while releasing a defined emission frequency. In order for a fluorescent protein to fluoresce, it must be properly folded. Expression of green fluorescent protein (GFP) does not efficiently fold in bacterial cells as determined by the high variability of the fluorescence intensity from cell to cell. The recent development of fast folder and super fast folder GFP have increased the number of cells with an high level of fluorescence. Our lab has recently developed a fast folder yeast enhanced GFP that can be expressed in *Candida albicans* and *E. coli*. There is a library of fluorescent proteins based on GFP including; blue fluorescent protein (BFP), enhanced blue fluorescent protein (EBFP), yellow fluorescent protein (YFP) and red fluorescent protein (RFP). Conversion of GFP to other fluorescent proteins is dependent on specific amino acid changes. My project is to develop a fast folder BFP (ffBFP) and a fast folder enhanced BFP (ffEBFP). A single amino acid substitution at position 66 (Y66H) converts GFP to BFP and has different excitation and emission spectrums (485 nm/525 nm vs 382 nm/459 nm). These differences are easily detectable but BFP is one of the weakest fluorescing proteins, so one of the aspects of this project is to determine if a ffBFP has an increase in fluorescence. In addition I will be introducing substitutions to convert ffBFP to ffEBFP (Y66H, F64L, S65T and Y145F). Once these are completed I will test these using a fluorescence plate reader at 37°C and various temperatures to test the stability of the fast folder proteins as one of the properties of fast folder fluorescent proteins is thermal stability. Once the super fast folder GFP has been completed in our lab I will produce sfBFP and sfEBFP.