Developing an Optimal Fluorescent Protein Tag

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In order to study and understand biological processes, it is necessary to be able to track intracellular interactions between molecules. Fluorescent proteins are often fused onto a protein of interest since this makes the fused protein able to be detected optically, making many experiments easier, such as the detection of protein-protein interactions. mRuby2, a red fluorescent protein (RFP), is a convenient tag to work with since it is highly expressed in bacteria and is detectable to the naked eye. It also remains stable at temperature and pH extremes and has potential for deep tissue imaging. Unfortunately, mRuby2 has the property of binding other proteins in a non-specific manner, which has impeded its use for detecting protein-protein interaction. The goal in this experiment was to develop a version of the mRuby2 tag which was monomeric while retaining its useful properties. In order to create this new monomeric Ruby (seRuby), it was necessary to determine the cause of mRuby2's aggregation. A structural model of the protein was created to identify the sites mRuby2 was most likely to interact with itself and form an oligomer. Once these sites have been identified, mutations will be made to the gene in the future to form seRuby which will hopefully have specific binding properties. These properties will be tested side by side to determine if the new Ruby is a better one. A new version of Ruby will be extremely useful for those seeking to use it as a probe to understand biological processes.