# BIOCHEMICAL APPLICATIONS OF DSRED-MONOMER UTILIZING FLUORESCENCE AND METAL-BINDING AFFINITY

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## Abstract:

The discovery and isolation of naturally occurring fluorescent proteins, FPs, have provided much needed tools for molecular and cellular level studies. Specifically the cloning of green fluorescent protein, GFP, revolutionized the field of biotechnology and biochemical research. Recently, a red fluorescent protein, DsRed, isolated from the Discosoma coral has further expanded the pallet of available fluorescent tools. DsRed shares only 23 % amino acid sequence homology with GFP, however the X-ray crystal structures of the two proteins are nearly identical. DsRed has been subjected to a number of mutagenesis studies, which have been found to offer improved physical and spectral

characteristics. One such mutant, DsRed-Monomer, with a total of 45 amino acid substitutions in native DsRed, has shown improved fluorescence characteristics without the toxic oligomerization seen for the native protein. In our laboratory, we have demonstrated that DsRed proteins have a unique and selective copper-binding affinity, which results in fluorescence quenching. This copper-binding property was utilized in the purification of DsRed proteins using copper-bound affinity columns. The work presented here has explored the mechanism of copper-binding by DsRed-Monomer using binding studies, molecular biology, and other biochemical techniques. Another focus of this thesis work was to demonstrate the applications of DsRed-Monomer in biochemical studies based on the copper-binding affinity and fluorescence properties of the protein. To achieve this, we have focused on genetic fusions of DsRed-Monomer with peptides and proteins. The work with these fusions have demonstrated the feasibility of using DsRed-Monomer as a dual functional tag, as both an affinity tag and as a label in the development of a fluorescence assay to detect a ligand of interest. Further, a complex between DsRed-Monomer-bait peptide/protein fusion and an interacting protein has been isolated taking advantage of the copper-binding affinity of DsRed-Monomer. We have also demonstrated the use of non-natural amino acid analogues, incorporated into the fluorophore of DsRed-Monomer, as a tool for varying the spectral properties of the protein. These mutations demonstrated not only shifted fluorescence emission compared to the native protein, but also improved extinction coefficients and quantum yields.

## Description:

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