

Unraveling the Photophysical Properties of 6-azaindole

Eric R. Bonfield, Dr. Susan Twine, Dr. Arthur Szabo

Wilfrid Laurier University

University of Victoria

The fluorescent properties of Tryptophan (Trp) have been widely exploited as natural intrinsic probes of protein structure and dynamics. The use of Trp fluorescence becomes more complex when attempting to resolve emission from multiple Trp residues in protein partners. This problem may be circumvented by the use of biosynthetically incorporated Trp analogues, such as 7-azatryptophan and 5-hydroxytryptophan. Such analogues have an absorbance spectrum that is red shifted relative to that of natural Trp. This allows for selective excitation of analogue containing proteins in the presence of proteins containing natural Tryptophan. Another analogue, 6-azatryptophan (6AW), has an intense absorbance maximum at 325 nanometers at pH 7. The photophysical properties of 6AW and the parent indole moiety have been characterized previously. The photophysics of 6AI have proven to be complex, involving a series of ground and excited state equilibria. In this study we have used N6-methyl-6-azaindole to further dissect the complex nature of the photophysical process. 6-azatryptophan was incorporated into a single Trp mutant of Calmodulin (Y99W) and Parvalbumin (F102W). Photophysical properties of these two analogue-containing proteins will be investigated and the data will be compared to that obtained from the free amino acids.