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Photoactivatable Fluorescent Protein Development for Single Molecule Imaging

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Optical highlighter proteins or photoactivatable fluorescent proteins (PA-FPs) were initially developed as markers to monitor protein, organelle, and cell movement. Localization techniques, such as photoactivated localization microscopy (PALM)[1], fluorescence-PALM (F-PALM)[2], stochastic optical reconstruction microscopy (STORM)[3], and many other techniques, have extended the uses of these molecules into super-resolution imaging. By imaging molecules individually, determining their centers of fluorescent emission via a statistical fit of their point-spread-function and plotting their positions on a more highly resolved image, these methods are capable of optically resolving photoactivated subsets of proteins at mean separations of <50 nanometers. (F)PALM, STORM, etc. require photoactivatable, photoconvertible, or photoswitchable probes which can be turned on or turned off to maintain a density of fluorescing single molecules low enough to distinguish individual molecules. A better understanding of their fluorescence characteristics and highlighting mechanisms will be beneficial in improving upon their properties. The molecules are often deficient in the number of photons emitted before photobleaching, number of photons emitted before blinking into dark states, or background fluorescence in the absence of activation. As a result, many of our current studies are centered on further characterizing and developing these proteins for single molecule localization techniques. Here, work on developing red fluorescent PA-FPs[4] as well as our efforts to improve the green PA-FPs will be presented with particular attention to PAGFP[5]. Current studies include systematic mutagenesis to elucidate the role of numerous amino acid positions in the photoactivation mechanism.

In addition, we are developing an instrument for imaging and localizing optical highlighter molecules in three dimensions throughout the cell.[6] Molecules are imaged by collection of the fluorescence emission through a cylindrical lens to skew the point spread functions in a manner dependent on the position of the molecule in the z-axis. Coupling this approach with two-photon photoactivation to maintain a lower background of photoactivated molecules, we are applying PALM to the study of molecular localization throughout intracellular organelles.

References

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