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Single protein molecule reaction by two-photon excited fluorescence.

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Abstract

2-Photon fluorescence excitation occurs in a small volume (~0.1 fl), it has excellent background rejection, and it can detect single molecules. The study of protein reactions at the single molecule/protein level is a promising tool to address many fundamental issues regarding protein conformation and dynamics. In particular, we are interested in the observation of proteins in different conformational substates. For example, by analyzing the kinetics of single protein ligand binding, information on the statistical distribution of conformational substates at the single protein level may be obtained. Two major technical problems confront research in this area: 1) immobilization of proteins in a matrix in which native conformations occur and the background fluorescence is sufficiently low for single molecule detection, and 2) heat induced denaturation or heat induced substates changes upon excitation of the single fluorescing protein. Progress to overcome these obstacles by suitable choice of host environment will be discussed. Supported by NIH grant RRO3 155.