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Joachim D Müller, Osman Akcakir, Don C Lamb, J W Carlson, Weiming Yu, A Schulte, Stephen G Sligar, Enrico Gratton, and G Ulrich Nienhaus.

Reaction dynamics of single protein molecules.

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Abstract

Studies of protein reactions at the single-molecule level allow us to observe the heterogeneous behavior of individual proteins rather than entire ensembles. This additional information can provide insight into the fundamental ideas of protein dynamics and function. Here, we focus on the kinetics of ligand binding to single apomyoglobin molecules. Two-photon fluorescence excitation spectroscopy combines excellent background rejection with small probe volume selection, both of which are essential for the detection of single molecules. We observe the reaction of freely diffusing ANS with an immobilized protein in the excitation volume. The change in quantum yield of the fluorescent ligand upon binding to the protein by more than two orders of magnitude allows us to distinguish between the bound and unbound state of the protein. The rate coefficients of the binding reaction are directly extracted from the time sequence of photon counts. Our method demonstrates the use of two-photon spectroscopy for studying reactions of single protein molecules. Supported by NSF PHY95-13217 and NIH RR03155.