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FLUORESCENCE LIFETIME RESOLVED STOPPED-FLOW FLUOROMETER

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Fluorescence lifetime resolved stopped-flow fluorimeter.

36th Annual Meeting of the Biophysical Society, Houston, Texas, 9-13 February 1992.

Biophys J. 1992; 61(2 Pt 2): A177, 1023.

Abstract

Protein folding reactions generally occur on the millisecond to second time scales and are monitored by various spectroscopic methods, especially fluorescence. To better understand the mechanisms of these complex reactions it is informative to measure fluorescence lifetime changes associated with the slower folding process. Since the fluorescence lifetime (nanoseconds) is characteristic of the fluorophore's environment, it is possible to detect intermediates for each macroscopic slice of the folding time scale. We have constructed a portable, T-format, phase resolved, stopped-flow fluorimeter capable of measuring time dependent changes in the millisecond region for steady-state fluorescence intensity or polarization and fluorescence lifetimes by phase and modulation detection at variable frequencies. Using fused silica fiber optics, the excitation source (either a xenon arc lamp with a monochromator or a laser) is coupled to a Biologic stopped-flow apparatus. A Glan Thompson polarizer is placed in the excitation beam and polarized sheets are in the emission channels. The mixing chamber is connected to the photomultiplier tube (PMT) detectors via fiber optics. The PMTs are electronically coupled to a computer for data collection and processing. We use standard LFD software for data acquisition and triggering of the stopped-flow TTL logic inputs provided by Biologic. Global Analysis is used to analyze both steady-state and timeresolved fluorescence decay data. The data collection uses a variable time base with multiple scan averaging. Coupling the modulated source to cross correlation electronics allows for precise determination of fluorescence lifetimes. Examples of protein folding will be discussed. Grant support: NIH RR03155; AHA,IL; NIH ... [truncated at 250 words]