Fluorescence Stopped-Flow Test Reaction:

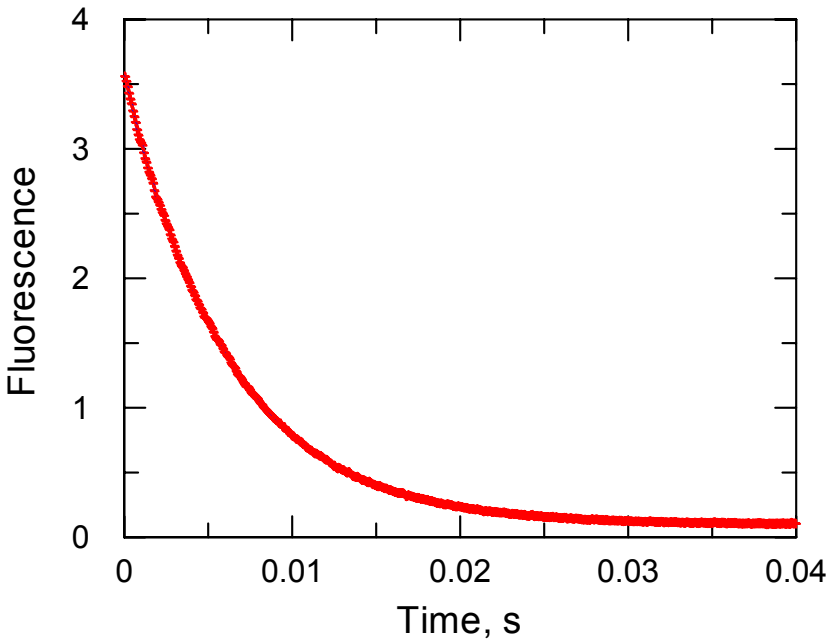
The performance of the stopped-flow can be evaluated based upon the method of Peterman published in Analytical Biochemistry 93, 442-444 (1979).

The signal is due to the fluorescence of N-acetyl-tryptophanamide which is quenched by reaction with N-bromosuccinamide (NBS). Fluorescence of N-acetyl-tryptophanamide is excited at 280 nm and observed at 340 nm. The signal is large and can be used to look for mixing artifacts and to measure the dead time of the instrument. The fluorescence signal should cleanly fit a single exponential with very little noise as illustrated below.

Stock Solutions:

1 mM N-acetyl-tryptophanamide in 100 mM phosphate buffer, pH 7.5 (24.5 mg/100ml). This solution can be made up in advance and stored for up to a year in the refrigerator. Dilute 100-fold with water to form the working solution at a concentration of 0.01 mM.

0.4 mM N-bromosuccinamide - make fresh daily by dissolving 7 mg in 100 ml of water.



Fuorescence Test Reaction. This figure shows the time dependence of the fluorescence change following the mixing of 0.01 mM N-acetyl-tryptophanamide with approximately 0.4 mM N-bromosuccinamide. The data and the fitted line are superimposed defining a rate of 160 s^{-1}. The observed rate will vary with the actual concentration of NBS and it is not necessary to make this solution accurately. Mixing artifacts will be revealed by deviation of the data from a single exponential. Errors in timing of the start of data collection will lead to an apparent lag.