AN4: NATA quenching by NBS.

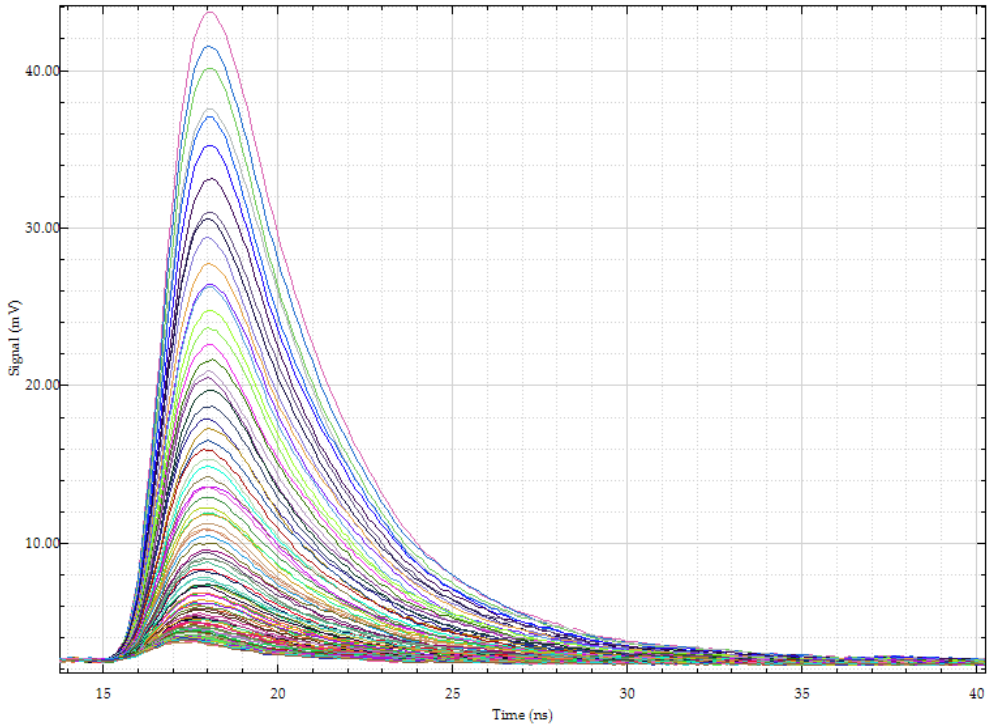
A quenching of tryptophan analog N-acetyltryptophanamide (NATA) by N-bromo-succinamide (NBS) was used for testing of instrument performance and mixer dead time determination. This system is routinely used to determine the dead time of rapid mixing devices [1, 2, 3].

Experiments were performed at T = 24C, in 20 mM phosphate buffer, pH 7. The solution of 20 uM NATA was mixed with solution containing 200 uM, 300 uM, 400 uM, 500 uM of NBS. Concentration of NBS was at least 10 times that of NATA to maintain pseudo-first-order reaction conditions.

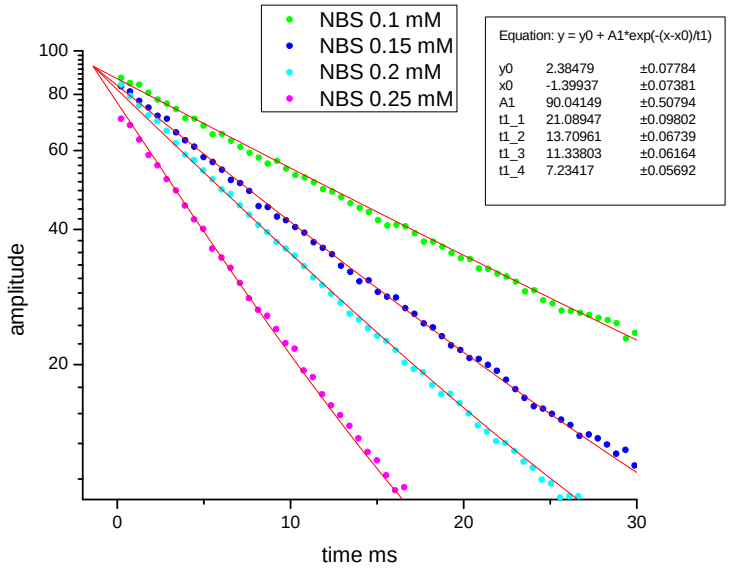
4^{th} harmonic of YAG laser (266 nm) was used for fluorescence excitation. PMT HV was adjusted to keep maximum of signal in range 60-100 mV. Emission was collected through sheet polarizer oriented at magic angle, no cut-off filter was used. Because PMT is not sensitive to 266 nm IRF was calculated using waveform of Rhodamine 6G luminescence excited by 4^{th} harmonic of the laser using lifetime value 3.9 ns. For each stopped-flow mix 500 signals were measured and then averaged in groups of 5 signals what reduced time resolution to 0.5 ms. All experimental data were averaged over 20 stopped-flow mixes.

The example of double-kinetic data for single NBS concentration is shown in Pic. 1 All of 100 waveforms for each NBS concentration were analyzed globally using single-exponent model with linked lifetime. Recovered lifetime was in range 2.6-2.7 ns what is in good agreement with published value [4]. Shown in Pic. 2 is a semi-logarithmic plot of obtained amplitudes for different NBS concentrations. Single exponent global fit with amplitude, time shift, and background parameters linked for all concentrations provides good agreement with data. Extrapolations of all fitting curves have a common point near time - 1.4 ns, the expected initial NATA fluorescence. This delay from common point of fittings to start of data acquisition is an estimation of mixer dead time - time what mixed solution needs to travel from mixer to observation point. This value is in good agreement with 2 ms value obtained from dead time estimation using information about dead volume and solution flow rate. Calculated first-order rate constants linearly increase with NBS concentration and corresponding second order rate constant 503 $mM^{-1}s^{-1}$ is in acceptable agreement with literature value 730 $mM^{-1}s^{-1}$ [2].

1. Bilsel, O., et al., *A microchannel solution mixer for studying microsecond protein folding reactions.* Review of Scientific Instruments, 2005. **76**(1).
2. Peterman, B.F., *Measurement of the Dead Time of a Fluorescence Stopped-Flow Instrument.* Analytical Biochemistry, 1979. **93**(2): p. 442-444.
3. Shastry, M.C.R., S.D. Luck, and H. Roder, *A continuous-flow capillary mixing method to monitor reactions on the microsecond time scale.* Biophysical Journal, 1998. **74**(5): p. 2714-2721.
4. Hart, L.P. and M. Daniels, *Lifetime Analysis of Weak Emissions and Time-Resolved Spectral Measurements with a Subnanosecond Dye-Laser and Gated Analog Detection.* Applied Spectroscopy, 1992. **46**(2): p. 191-205.



Pic. 1 The example of double-kinetic data of NATA quenching by NBS for single NBS concentration



Pic. 2 Semi-logarithmic plots of amplitudes obtained from global analysis of experimental data for different NBS concentrations are fitted by exponential decay function.