Supporting Information for the article titled "Biotin-avidin Binding Kinetics Measured by Single Molecule Imaging"

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Contents: This Supporting Information contains photobleaching kinetics measured for immobilized tetramethylrhodamine. In addition, the binding kinetics step for the neutravidine unbinding experiment is shown, together with a fit to determine the binding rate constant.

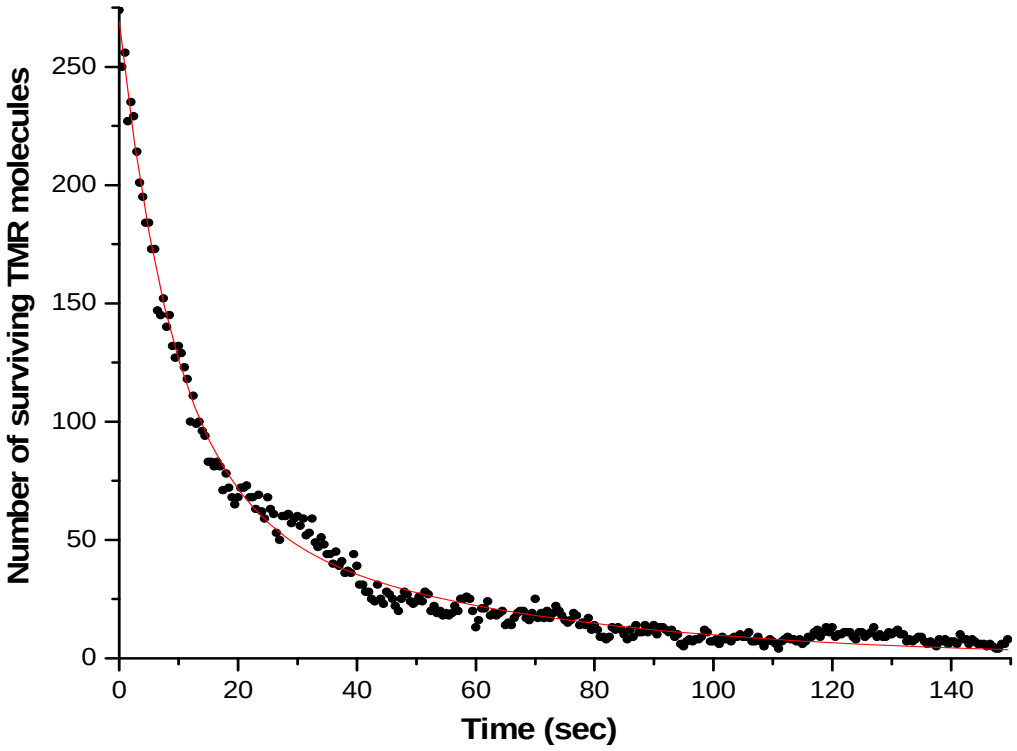

Figure 1. Photobleaching kinetics of immobilized tetramethylrhodamine. The rate of photo-bleaching of tetramethylrhodamine is measured by counting single molecules immobilized as TMR-succinimidyl ester onto propylamine-silane sites on a cyanoethylsilane surface. Under continuous illumination conditions at the same average power used for imaging TMR-labeled neutravidin, the photobleaching kinetics of tetramethylrhodamine were biexponential with a shorter-lived population, τ_1 = 9.2 (±0.3) s, and a longer-lived population, τ_2 = 50 (± 2) s. The population-weighted average photobleaching lifetime is τ_{pb} = 20.3 s.

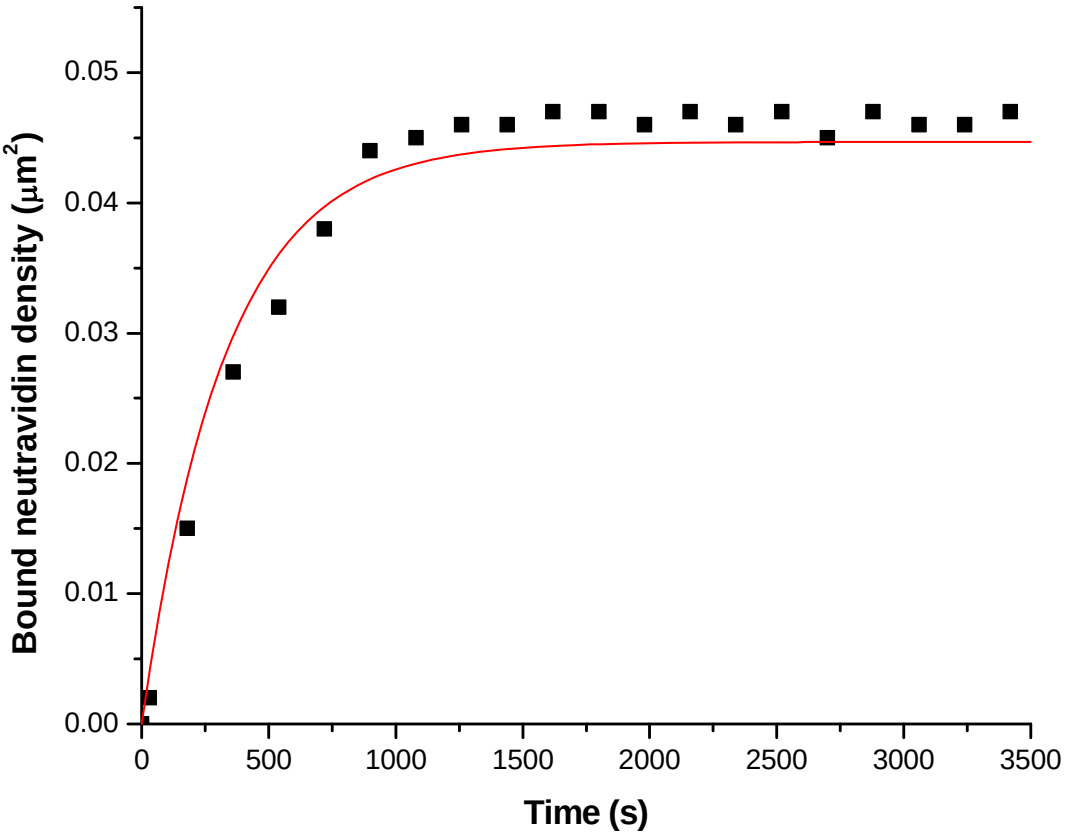

Figure 2. The neutravidin accumulation kinetics monitored prior to the unbinding kinetics experiment shown in Figure 5. The neutravidin solution concentration was 13.3 pM, matching the conditions of the top curve in Figure 4. The data are fit to Equation 1, and the binding rate constant is k_{bind} = 2.0 (±0.2) x 10^8 M^{-1} s^{-1}, consistent with the results found in Figures 4 and 6.