Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Information

Quantitative Mechanistic Model for

Ultrasmall Nanoparticle–Protein Interactions

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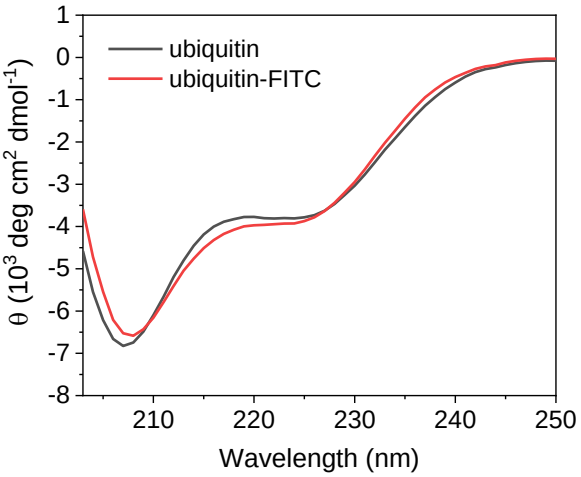

Figure S1. Comparison between the CD spectra of unlabeled ubiquitin and FITC-labeled ubiquitin. Data obtained in phosphate-citrate buffer solution containing 5 mM NaCl.

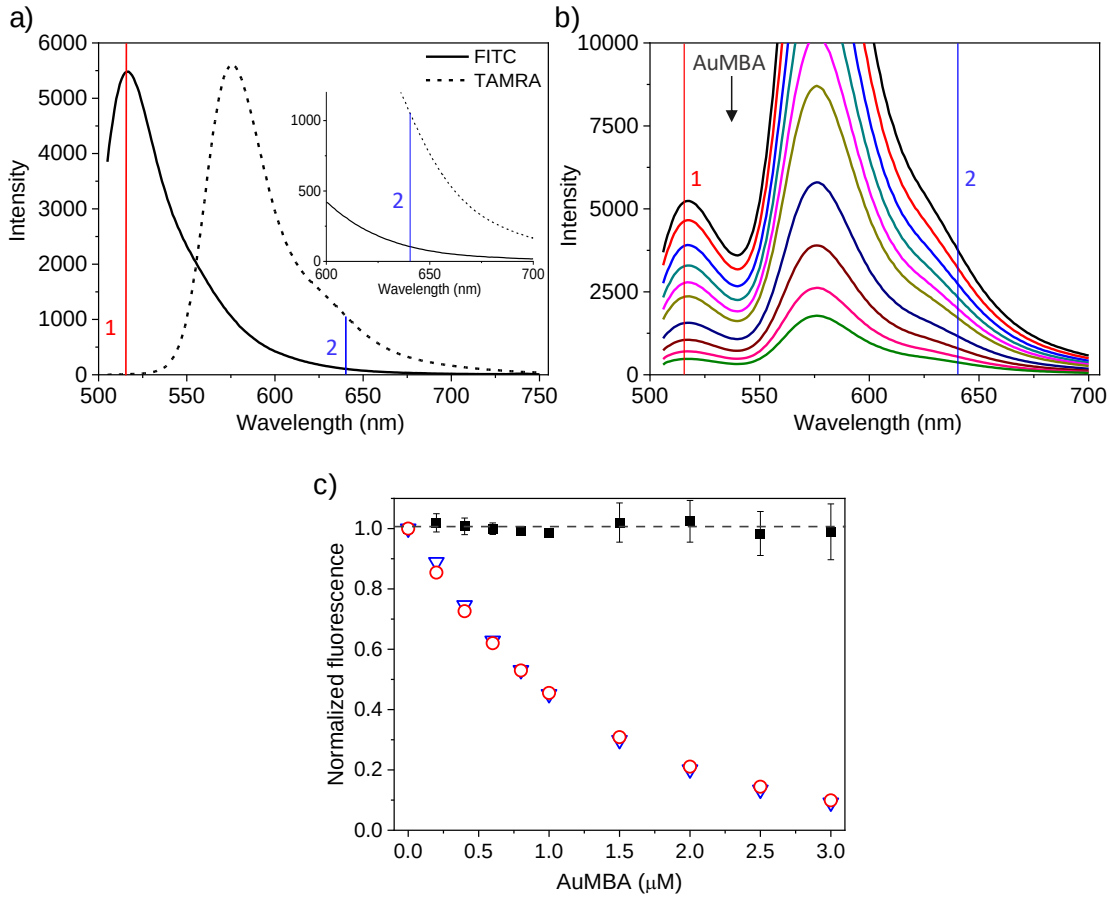

Figure S2. Correction of inner-filter effect. (a) Emission spectra of FITC and TAMRA obtained with excitation wavelength of 495 nm. Line 1 shows that TAMRA does not contribute any measurable signal at the wavelength of 515 nm. Line 2 at 640 nm shows that the long tail of the FITC signal extends somewhat within the TAMRA emission region. In (a), the FITC emission signal at 640 nm was 9% of the TAMRA signal at the same wavelength. (b) Titration of a mixture of FITC + TAMRA with AuMBA. The excitation wavelength was 495 nm. (c) Emission intensities recorded at 515 nm (red circles) and 640 nm (blue triangles) obtained from (b) plotted as a function of NP concentration. A corrected FITC titration curve (F_{corr}^{515}; black squares) was obtained by application of the equation: $F_{corr,j}^{515} = (F_j^{515}/F_0^{515})/(F_j^{640}/F_0^{640})$, where F_j refers to the uncorrected fluorescence signals for titration j and F_0 are the initial fluorescence signals. The average of three independent measurements yielded a horizontal line near 1, therefore confirming the accuracy of the inner-filter correction procedure under our experimental conditions.

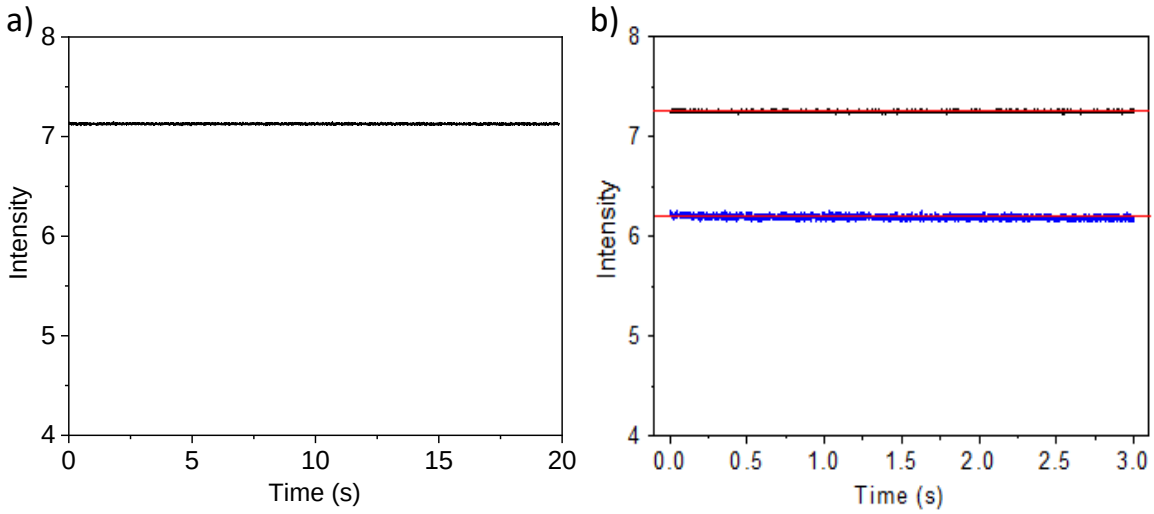

Figure S3. (a) Stopped-flow time trace of FITC mixed with buffer showing a high signal stability and lack of fluorescence bleaching up to 20 s. (b) Stopped-flow time traces of FITC (black) and FITC-labeled ubiquitin (blue) following rapid mixing with AuMBA and AuMBA pre-incubated with excess unlabeled ubiquitin, respectively. Red horizontal lines are a guide to the eye. FTIC and FITC-ubiquitin, 30 nM; AuMBA, 1 μM; unlabeled ubiquitin, 40 μM.

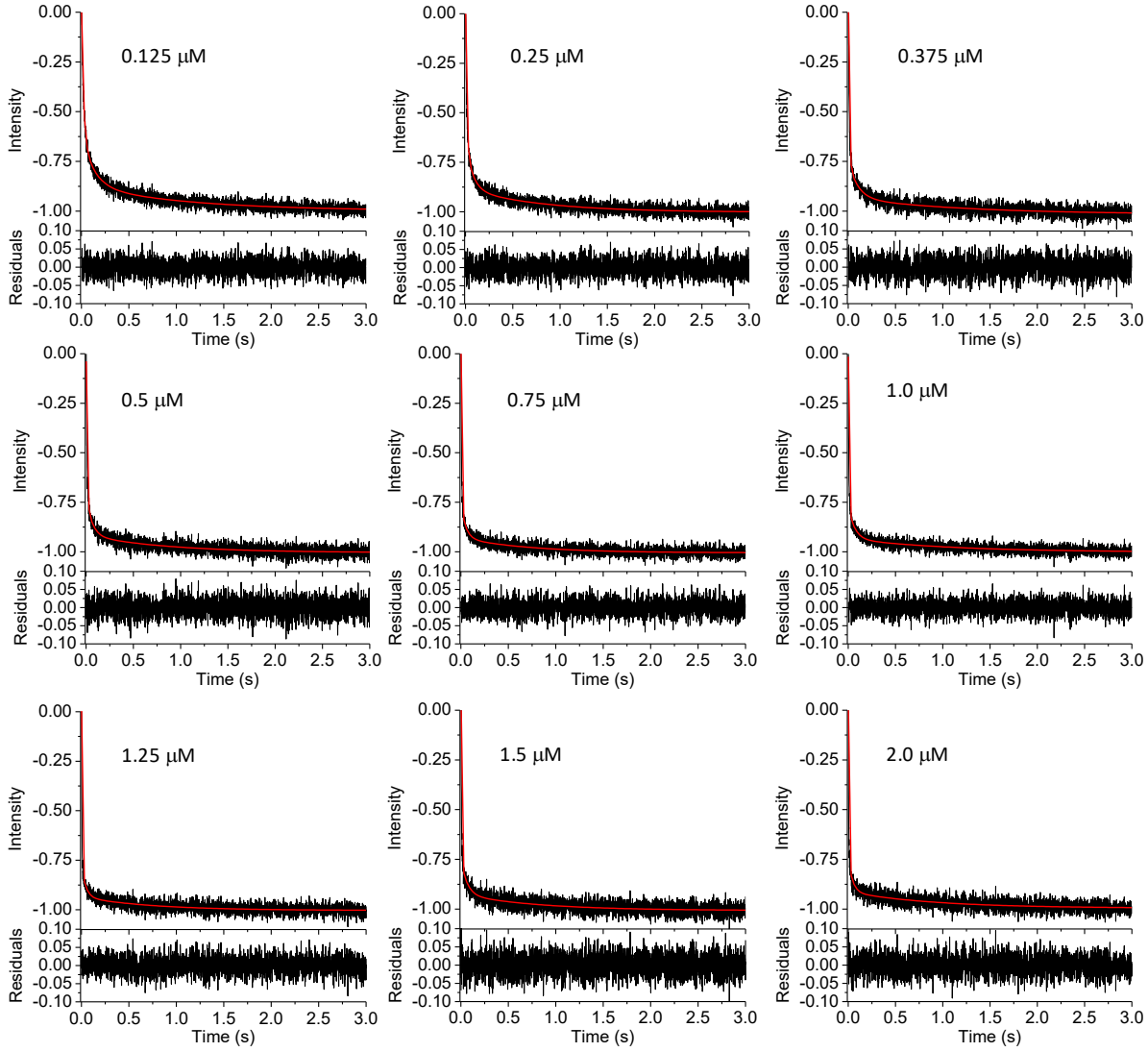

Figure S4. Characterization of AuMBA-ubiquitin association reactions. Illustration of stopped-flow time courses fitted to a triple exponential function in the range from 2 to 3000 ms. Fitted lines are shown in red. The different AuMBA concentrations are annotated in each plot. Data recorded in phosphate-citrate buffer solution containing 5 mM NaCl; FITC-ubiquitin, 30 nM.

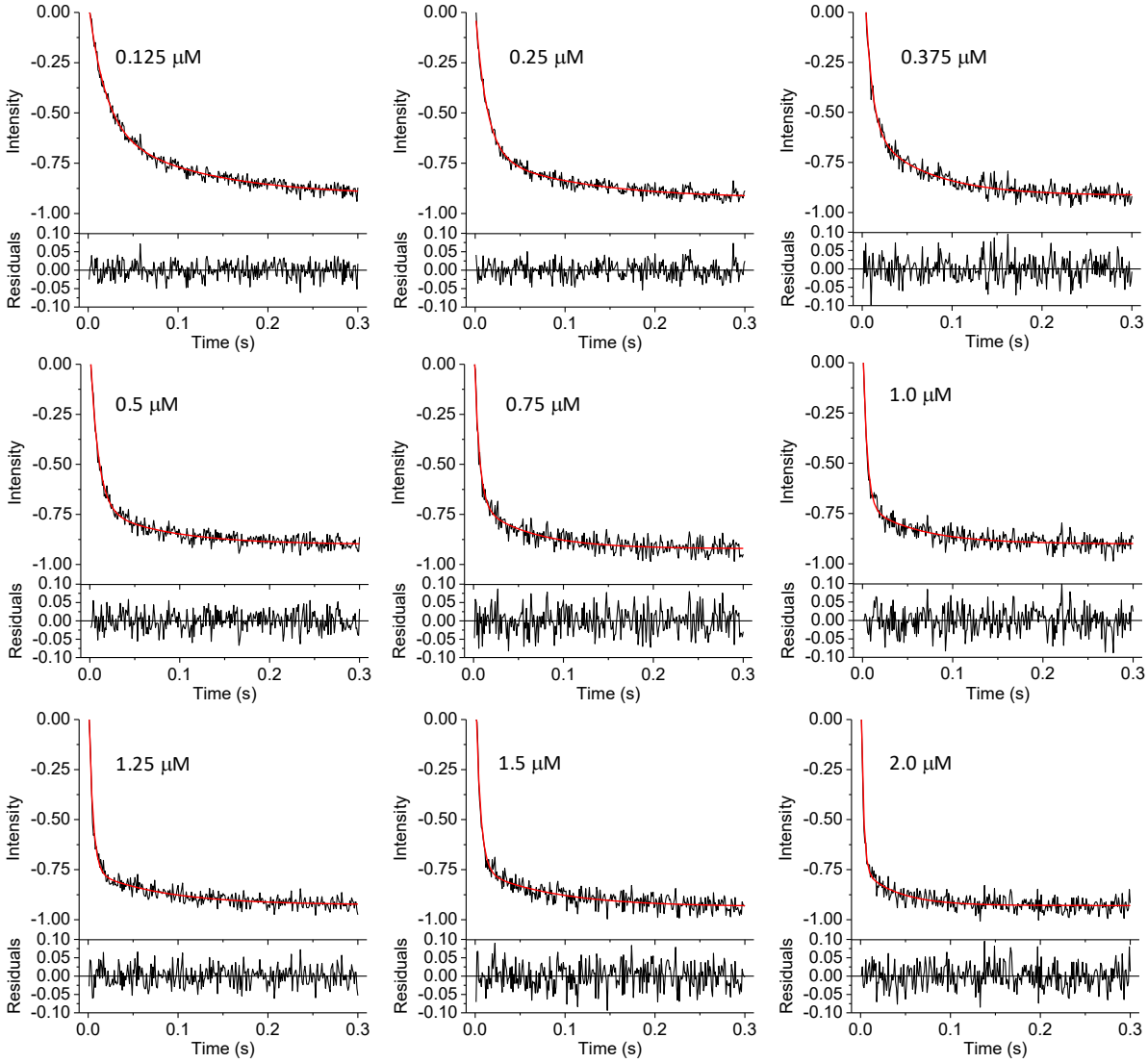

Figure S5. Characterization of AuMBA-ubiquitin association reactions. Same as in Fig. S4 but showing the fitting up to 300 ms only.

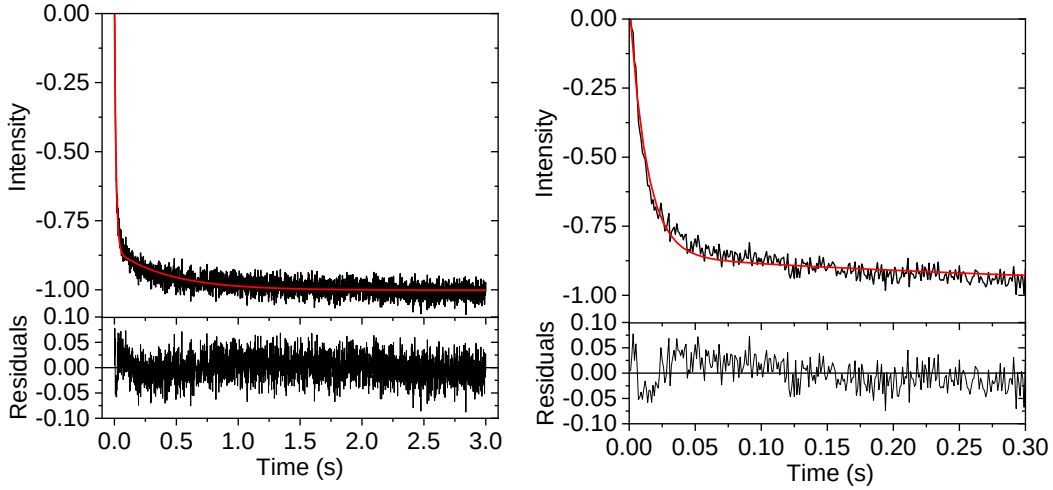

Figure S6. Example of bad quality fitting of stopped-flow time traces obtained with a double exponential function. Fitted line is shown in red. Data illustrated for [AuMBA] = 0.75 μM.

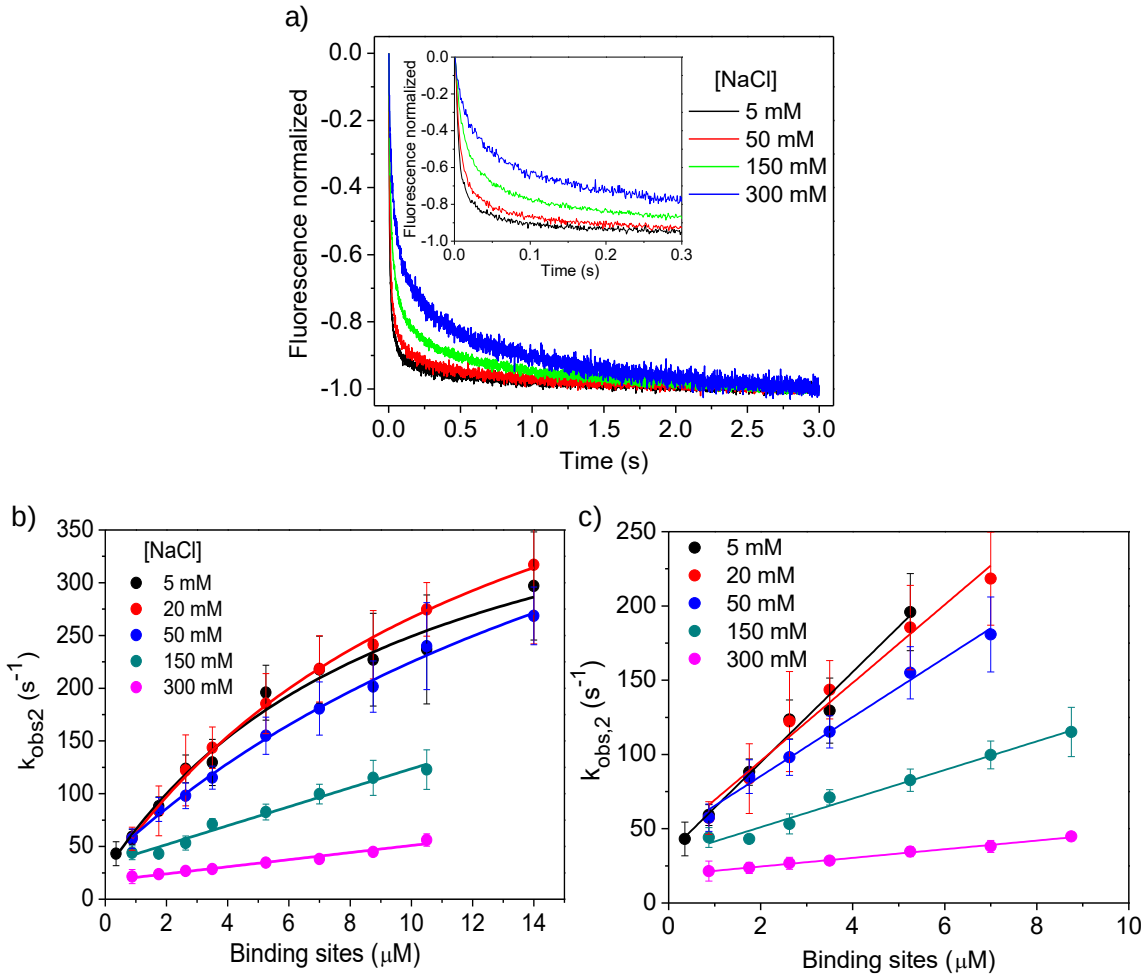

Figure S7. Characterization of AuMBA-ubiquitin association reactions under different NaCl concentrations. (a) Normalized time traces of fluorescence quenching following rapid mixing of FITC-labeled ubiquitin with AuMBA. Experiments performed in phosphate-citrate buffer solution containing from 5 to 300 mM NaCl. Data illustrated for [AuMBA] = 0.75 μM. (b) Dependence of k_{obs2} *vs*. [NP] on the NaCl concentration. (c) Same as (b), but showing only the linear portions of the k_{obs2} plots, in which case [NP] << K_{D1}. From Eq. 5 in the main text, it follows that $k_{obs2} = (k_2/K_{D1})$x[NP] + k_{-2} when [NP] << K_{D1}. This approximation allows estimating K_{D1} for [NaCl] = 150 and 300 mM from the corresponding slopes (k_2/K_{D1}) of the linear plots and assuming a fixed k_2 of ~ 600 s^{-1} (the k_2 obtained at 50 mM; Table 2). The calculations yielded K_{D1} ~ 60 μM and K_{D1} ~ 200 μM at the NaCl concentrations of 150 and 300 mM NaCl, respectively (Table 2).

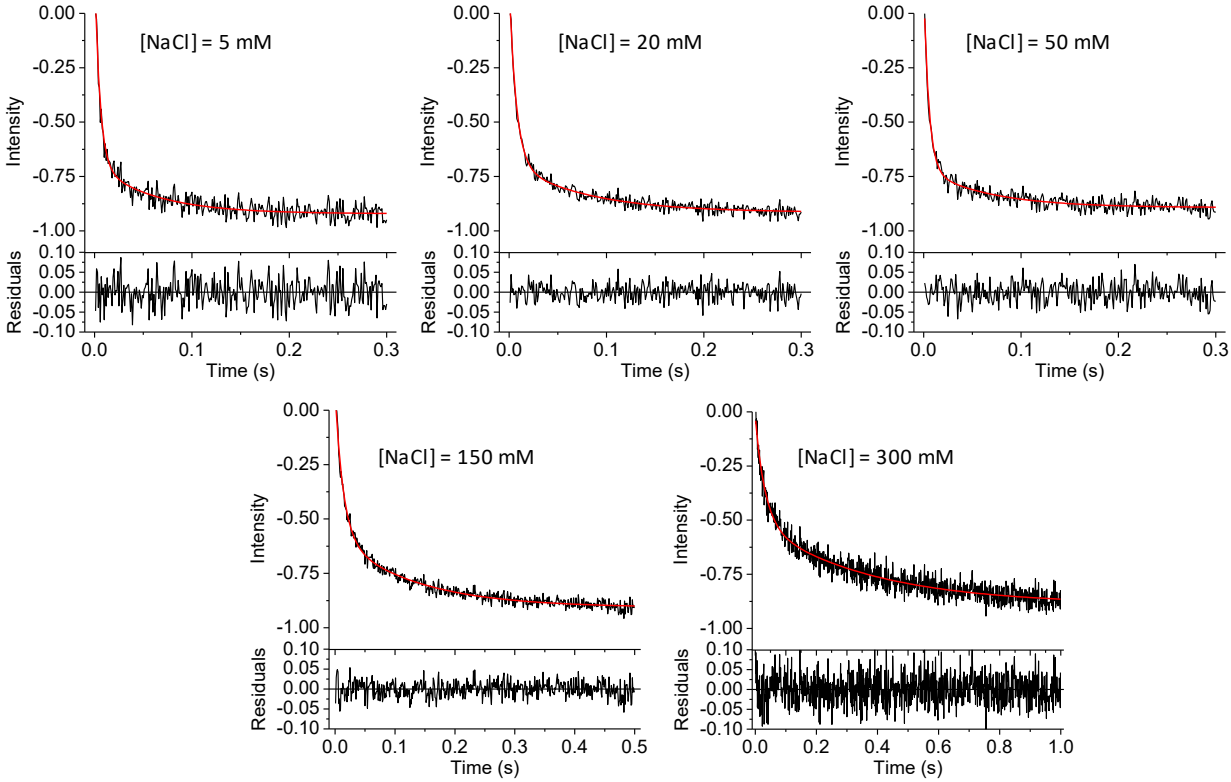

Figure S8. Fitting of stopped-flow time courses recorded under different NaCl concentrations. Fitted lines are shown in red. Data sets illustrated for [AuMBA] = 0.75 μM.