Transient Anomalous Subdiffusion: Effects of Specific and Non-specific Probe Binding with Actin Gels

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Supplemental Material: Photophysical effects on the temporal diffusion

Photophysical effects such as photobleaching and triplet kinetics are well documented to influence the autocorrelation function (ACF) [1-4]. These photophysical events can be incorporated into equations describing the ACF using the same formalism presented in the theory section in the main paper. This supplemental material is intended to show how the ACF modified by photophysical events would impact the $D_{app}(t)$ after the inversion methodology proposed in the paper is performed. To start, we first need to include these phenomena in the ACF. To do so, one can consider these effects as isomerization reactions.. In this case, the eigenvalues and eigenvectors of the matrix

$$M = \begin{pmatrix} -\left(D_A q^2 + k_{AB}\right) & k_{BA} \\ k_{AB} & -\left(D_B q^2 + k_{BA}\right) \end{pmatrix} \quad (A1)$$

are modified to account for exchanging between brightness states and not diffusion rates. Using these constraints, Eq. 8 from the main text becomes

$$G(\tau) = \frac{(2\pi)^{-3}}{\left(\overline{C}_A + \overline{C}_B\right)} \int d^3\vec{q}\, \exp\left(-\frac{\omega_{xy}^2}{8n}\left(q_x^2 + q_y^2\right)\right) \exp\left(-\frac{\omega_z^2}{8n}q_z^2\right) \exp\left(-D\tau\left(q_x^2 + q_y^2 + q_z^2\right)\right) \left. 1 + K\left(\frac{Q'-1}{Q'+K}\right)^2 \exp(-R\tau) \right|$$

(A2)

where $Q' = \dfrac{Q_A}{Q_B}$. Eq. A2 can be solved and simplified to

$$G(\tau) = \left(\frac{1}{\overline{N}_A + \overline{N}_B}\right)\left(1 + K\left(\frac{Q'-1}{Q'+K}\right)^2 \exp(-R\tau)\right)\left(\frac{1}{1+\tau/\tau_D}\right)\left(\frac{1}{1+\frac{1}{\kappa^2}\tau/\tau_D}\right)^{1/2}.$$

(A3)

The fundamental principle underlying bleaching or triplet kinetics is a change in the molecular brightness. This is maximized when the molecules enter state B which is

defined as non-emitting or $Q_B=0$, as in the case for bleaching and triplet kinetics. However, photobleaching occurs at longer timescales than triplet kinetics. Blinking is another mechanism that could be described in the same fashion and has similar kinetics as photobleaching.

We tested how bleaching, blinking and triplet kinetics, could affect the recovered $D_{app}(t)$ using

$$
NG(\tau) = \left(\frac{1}{1+\dfrac{4\langle r^2(\tau)\rangle}{3\omega_{xy}^2}} \cdot \frac{1}{1+\dfrac{1}{\kappa^2}\dfrac{4\langle r^2(\tau)\rangle}{3\omega_{xy}^2}} \right)^{1/2}
$$

(A4)

that corresponds to Eq. 24 in the main manuscript

We used Monte Carlo simulations identical to those described in the accompanying manuscript in which molecules in the simulation volume were allowed to exchange between two emitting states. State A emitted at 10 kcpmps while state B did not emit. These photophysical events do not perturb diffusion thus $D_A = D_B$. Panel A of the figure below shows the autocorrelation from simulating 100 molecules with no kinetics or with two different reaction rates. One at a rate $R = 10^5$ s^{-1} which simulates photobleaching and blinking [5], and the other one at $R = 10^6$ s^{-1} which represents the rate of transition to the triplet state [5]. Including a triplet fraction decreased the amplitude, $G(\tau)$, as expected. Both triplet and photobleaching produced a shift in the apparent diffusion towards faster timescales. The FCS community has extensively studied both of these effects [5-7].

When the FCS data presented in the supplemental figure panel A are reanalyzed to extract the $D_{app}(t)$, as done in the manuscript, one can observe at which time regimes these photophysical effects predominate. If one compares the dashed and solid lines in panel B) it is clear that the triplet effect occurs on time scales less than 0.05 ms and produces a significant increase in the $D_{app}(t)$. The solid line was produced by taking the same simulation data and using a form of Eq. A4 that accounts for a triplet fraction or

$$N_T G(\tau) = \left(1 + f \exp(-R\tau)\right) \left(\cfrac{1}{1 + \cfrac{4\langle r^2(\tau)\rangle}{3\omega_{xy}^2}}\right) \left(\cfrac{1}{1 + \cfrac{1}{\kappa^2}\cfrac{4\langle r^2(\tau)\rangle}{3\omega_{xy}^2}}\right)^{1/2} , \qquad (A5)$$

where N_T is the total number of molecules in the focal volume, and f is the fraction showing this effect. The dashed line did not consider the first factor on Eq. A5 to do the inversion. The same procedure was done for the case of photobleaching (compare dashed and solid lines in panel C). The photobleaching (or blinking) effect is apparent at less than 0.5 ms and produces a distinct valley in the trace, but the remainder of the curve is unaffected. It is clear that in both instances $D_{app}(t)$ appears flat over the entire temporal spectra if prior knowledge of the photophysical events are accounted for in the inversion process. When these factors are not taken into account the recovered D_{app} shows regions which deviate from the true $D_{app}(t)$. Thus, these photophysical effects would show up distinctively on the spectra but they are different from those found by generating ACF with one or two components of diffusion or the anomalous diffusion model.

A

B Triplet
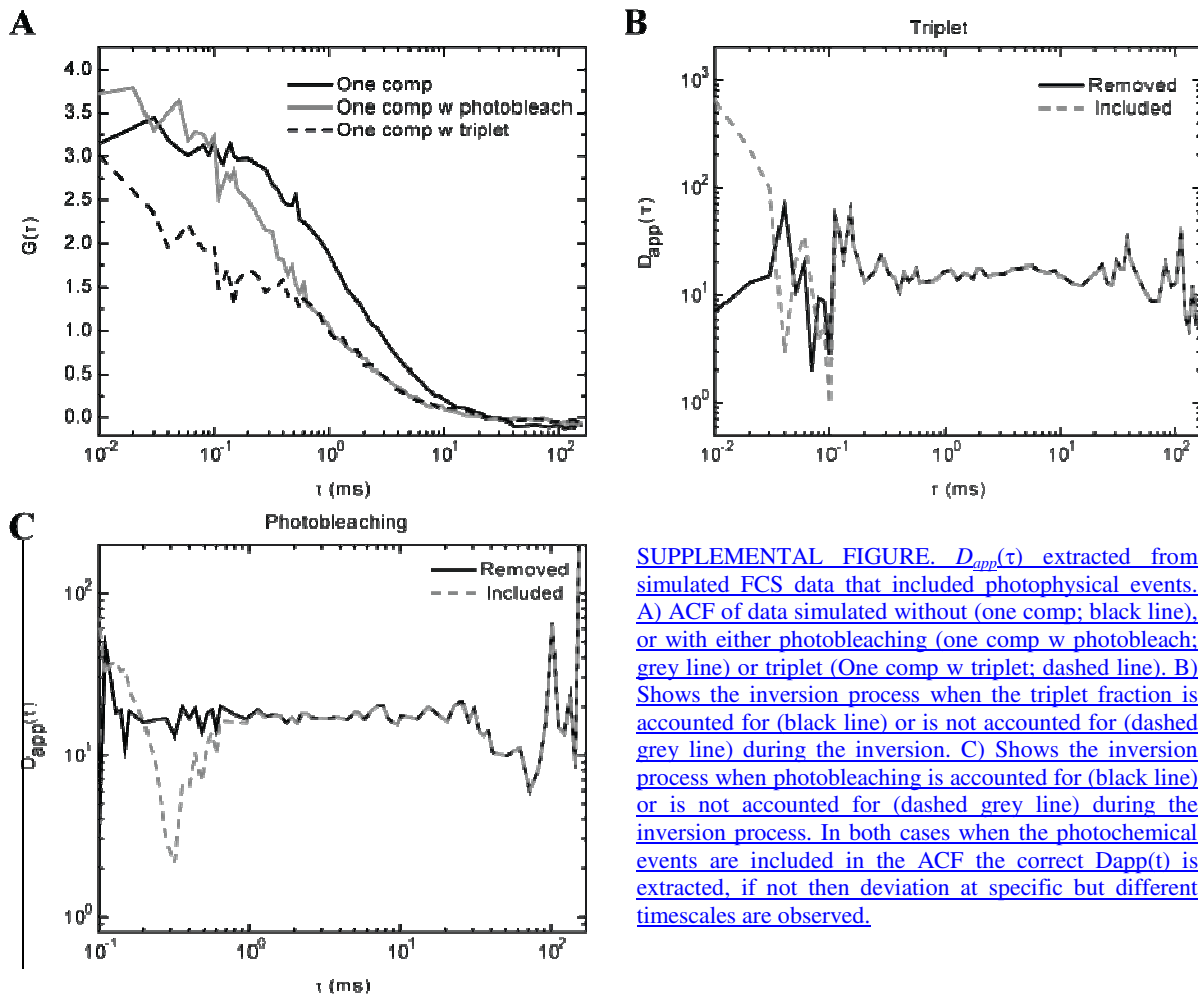
C Photobleaching

SUPPLEMENTAL FIGURE. $D_{app}(\tau)$ extracted from simulated FCS data that included photophysical events. A) ACF of data simulated without (one comp; black line), or with either photobleaching (one comp w photobleach; grey line) or triplet (One comp w triplet; dashed line). B) Shows the inversion process when the triplet fraction is accounted for (black line) or is not accounted for (dashed grey line) during the inversion. C) Shows the inversion process when photobleaching is accounted for (black line) or is not accounted for (dashed grey line) during the inversion process. In both cases when the photochemical events are included in the ACF the correct Dapp(t) is extracted, if not then deviation at specific but different timescales are observed.

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