Systematic evaluation of fluorescence correlation spectroscopy data analysis on the nanosecond time scale

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Supporting Information

This material contains six figures S1-S6 with data as described in the figure captions and discussed in more detail within the main text.

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Figure S1:

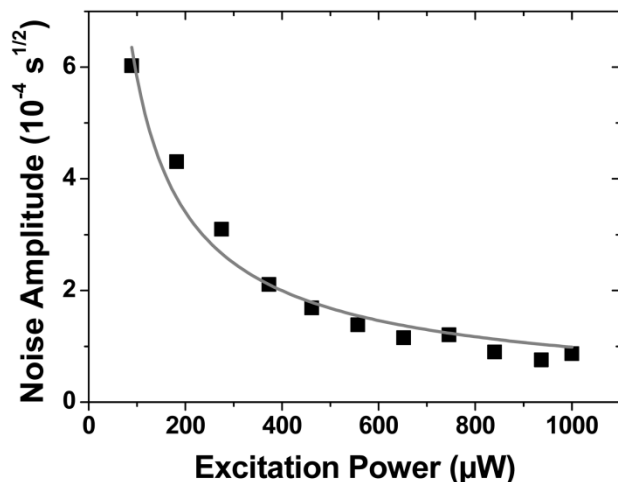**Fig. S1:** Noise amplitudes c(p) determined from a standard FCS sample as function of excitation power p. The data was recorded at 20°C for 15 minutes per data curve. The grey line is a data fit of the function $c(p)=a*p^m$ with a=(0.005±0.001) $s^{1/2}$ and m=-0.77±0.05.

Figure S2:

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Fig. S2: Fit parameter distributions for the fitting results on simulated data sets as described in the main text. (A) Exemplary data curve with noise of $c=6 \times 10^{-6}$ $s^{1/2}$ overlaid with a function fitted by the LM algorithm as implemented in Mathematica. (B) Fit parameter distributions showing correlations between the two amplitudes A and R (closed squares) as generated from fitting 100 simulated FCS curves in each data set. Four data sets are displayed for data simulated with a noise amplitude of $c=6 \times 10^{-6}$ $s^{1/2}$ or $c=15 \times 10^{-6}$ $s^{1/2}$, A=-2, R=1, k_{AB}=0.4, and k_{rot}=0.1 (or 0.05 or 0.025). No correlation appears between A_{AB} and corresponding χ^2 values (open circles). N_{false} indicates the number of unreasonable data fits with $A_{AB}<-3$ (from a total of 100 data fits).

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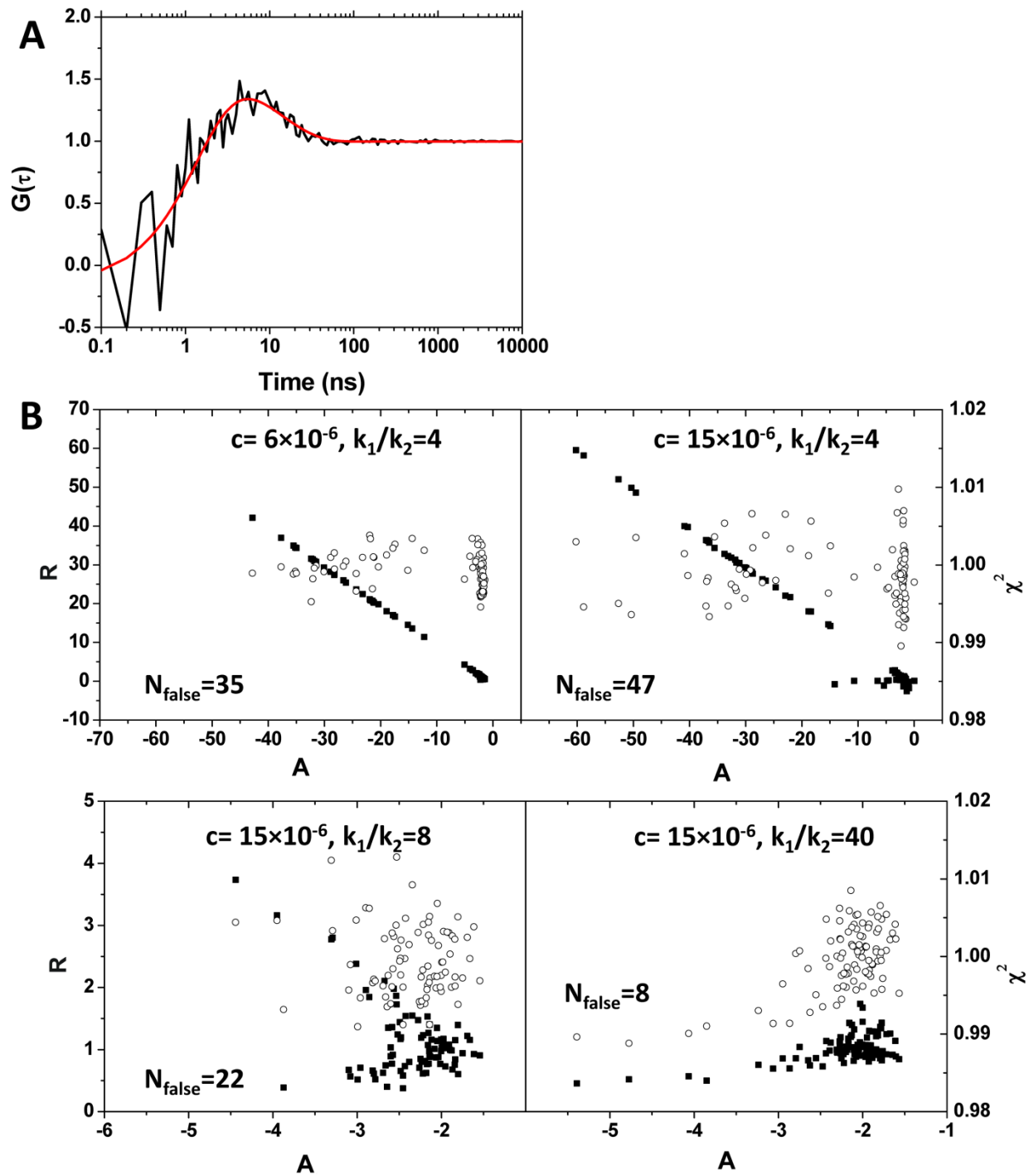
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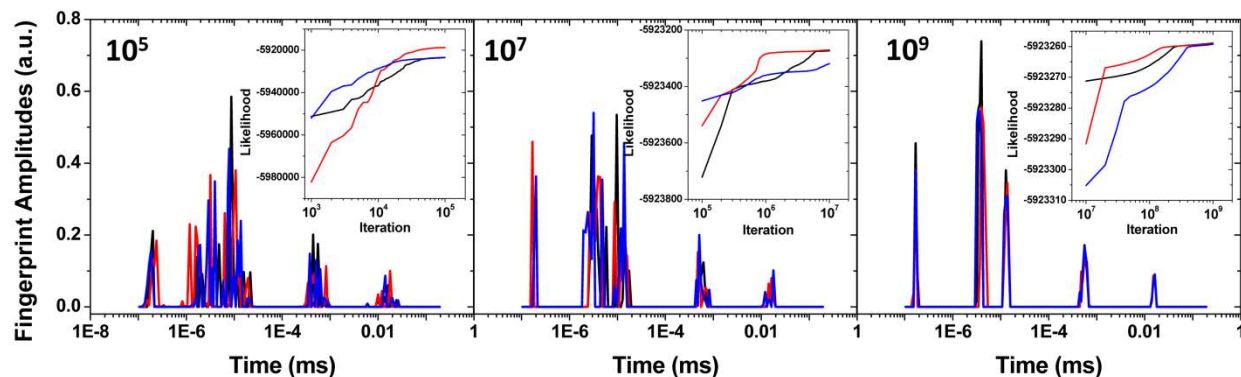
Figure S3:

Fig. S3: Dynamical fingerprints determined for experimental FCS data of a mixture of freely diffusing fluorophore MR121 (~1 nM) and Trp (10 mM) in PBS buffer with 60% sucrose as presented in Fig. 1. Dynamical fingerprints were determined three times from an identical FCS curve with 10^5, 10^7, or 10^9 iterations (from left to right). The insets show the likelihood for the corresponding series of iteration steps.
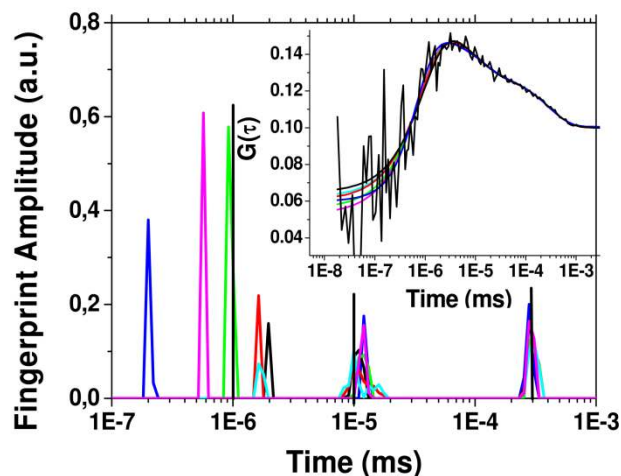
Figure S4:

Fig. S4: Dynamical fingerprints generated for simulated FCS data. Data was simulated using the model function Eq. 1 overlaid with noise following Eq. 2 with a noise amplitude $c=9 \times 10^{-6}$ $s^{1/2}$. Here the starting value for the antibunching time constant was varied (10^{-6} ms for black, 9×10^{-7} ms for cyan, 8×10^{-7} ms for green, 7×10^{-7} ms for red, 6×10^{-7} ms for pink, 5×10^{-7} ms for blue) while the relaxation time constants were kept constant in all simulations at $\tau_{ab}=10^{-6}$ ms, $\tau_{rel}=10^{-5}$ ms, $\tau_{isc}=3 \times 10^{-4}$ ms (indicated by black lines in the graph). The inset shows all fitted and the simulated data curve.

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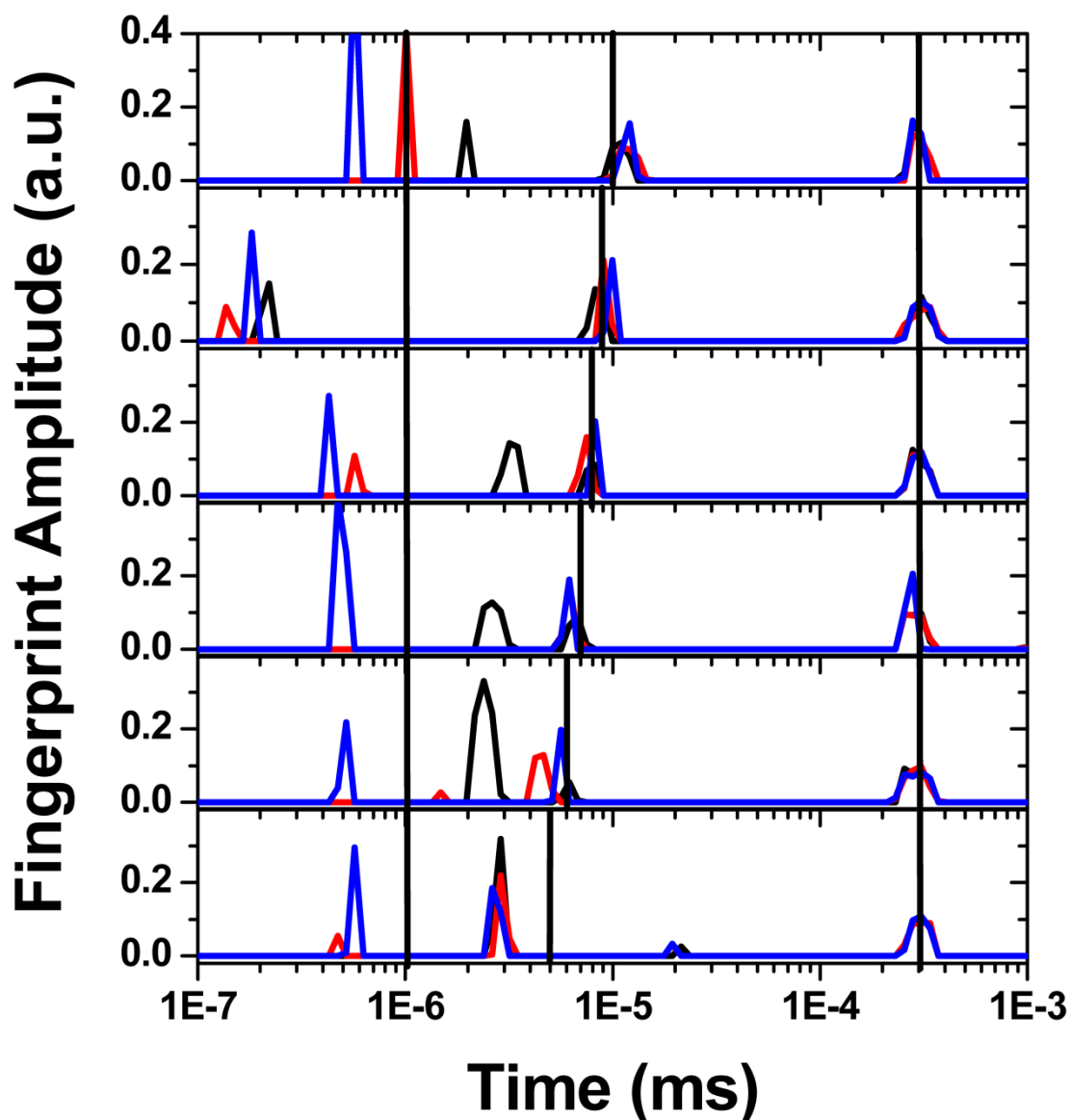
Figure S5:

Fig. S5: Dynamical fingerprints generated for simulated FCS data with a rotational correlation time constant approaching the antibunching time scale. Data was simulated using the model function Eq. 1 overlaid with noise following Eq. 2 with a noise amplitude $c=9\times10^{-6}$ $s^{1/2}$. The relaxation time of the rotational diffusion correlation decay was varied from 10^{-5} (top) to 5×10^{-6} (bottom) while the relaxation time constants for the antibunching signature and for the decay on larger times was kept constant at 10^{-6} ms and 3×10^{-4} ms, respectively (all indicated by vertical lines in the graphs). The three fingerprints were determined with a slight variation of the antibunching relaxation time starting value (1×10^{-6} for black; 8×10^{-7} for red, 6×10^{-7} for blue).

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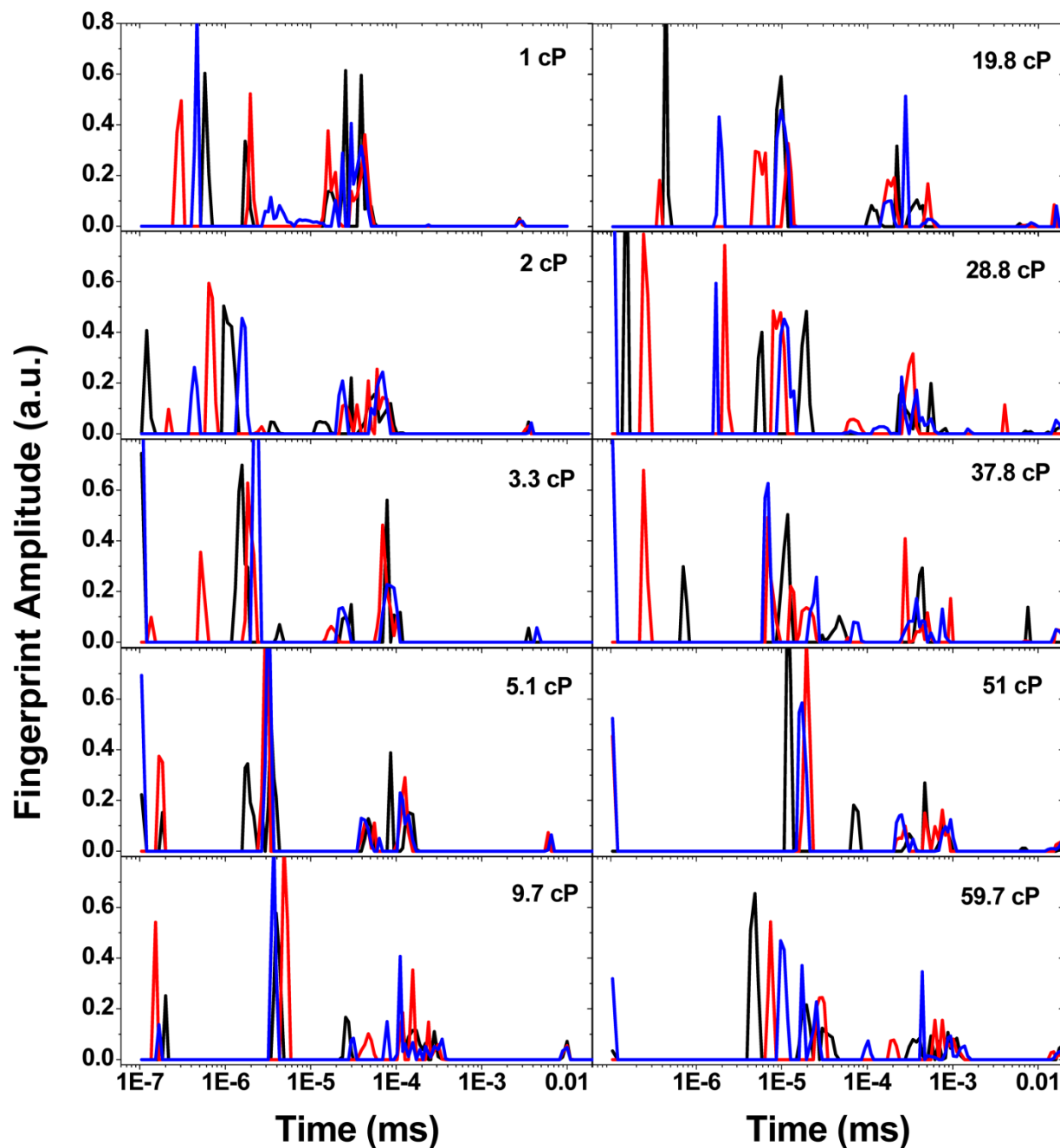
Figure S6:

Fig. S6: Dynamical fingerprints determined for experimental FCS data that was recorded for mixtures of freely diffusing fluorophore ATTO655 (~1 nM) and Trp (10 mM) in PBS buffer with various concentrations of sucrose (as indicated in the figure) at 20°C. The fingerprints reveal the same components as shown in Fig. 4 for MR121/Trp with a small additional decay component that mixes with the previous decays and effectively broadens the estimated fingerprint peaks.