Supplemental FCS studies of dark transient states of p-FTAA

Power series measurement

p-FTAA was diluted in pH 10 buffer to a final concentration of 0.2 µM and placed in a sealed beaker. The excitation irradiance was varied from 25 kW/cm^2 up to 300 kW/cm^2. Initially, the model described in Eq. 1 in the main text was fit to the correlation curves. τ_D was calculated to 98 ± 3 µs, which, given the larger detection volume of this setup, agrees well with the earlier measurements. This model, however, did not fit the correlation function well in the triplet time regime. Therefore, a model assuming two independent exponential processes was applied, as described by Eq. 4 in the main text.

This generated significantly better fits. The amplitudes (*T* and *U*) and the relaxation times (τ_T and τ_U) of the processes, as defined in Eq. 4, are shown in Figure S1. Note that both processes contribute equally to the model function (Eq. 4). Hence, distinction and assignment was made after fitting based on the characteristic times. For interpretation of the data, *T* and τ_T may be interchanged with *U* and τ_U for any excitation power.

The process whose amplitude we denote *T* in Eq. 4 displayed an excitation power dependence typical for a triplet state. The fraction (*T*) of fluorophores in the triplet state increases with increasing power, because an increased population in the excited singlet state increases the probability of a fluorophore to enter the triplet state via intersystem crossing. The increased excitation rate also shortens the turnover time τ_T. The second component (split in U_1 and U_2 in Figure S1a, b) exhibited a more complex behaviour. The quality of the data is not sufficient for us to provide a fully reliable interpretation of what this component could represent. At excitation powers below 105 kW/cm^2 (420 µW), it described a very fast process (U_1), with relaxation times of a few hundred nanoseconds. For higher powers the model fit placed this process in the diffusion regime around 60 µs (U_2), and presented a behaviour similar to that expected from a photo induced isomerisation – decreasing turnover time (τ_{U2}) with increasing excitation irradiance, and essentially unaffected fraction U_2. It can not be excluded that the U_2 component is an artefact, caused by the fitting algorithm trying

to improve the fit to the diffusion part of the auto-correlation function, since the applied model does not include the aforementioned saturation effect. Nevertheless, it seems like a two-component system is not enough to completely describe the behaviour of the system over the whole excitation irradiance.

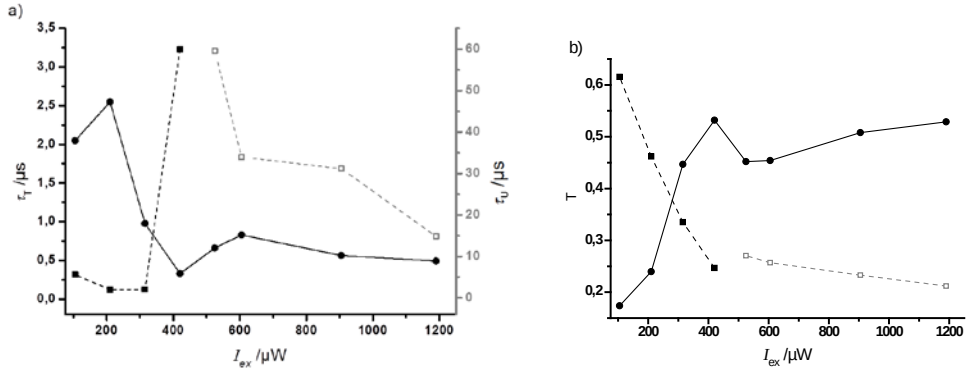

Figure S1a) Turnover time, (τ_T, τ_U) and b) relative fluctuation amplitudes (T, U) of exponential processes depending on the excitation power (I_{ex}) applying a two-component model, T ((■), solid), U_1 ((●), dashed) and U_2 ((□) dashed grey). Please note that the relaxation time of U_2 is plotted against the right axis.

Potassium iodide (KI) is well known to facilitate intersystem crossing (ISC),[1] thus affecting the fraction of triplets and their turnover rate.[2] KI was added to alkaline p-FTAA solution (p-FTAA 0.3 µM in pH 10) in a series of concentrations: 1, 5 and 10 mM. The presence of KI did not affect the absorption or fluorescence emission spectra (data not shown), but in the FCS correlation function a small difference was observed (Figure S2). Increasing KI concentration slightly increased the fraction of dark states and the turnover rate, further confirming the presence of triplets (Table S1).

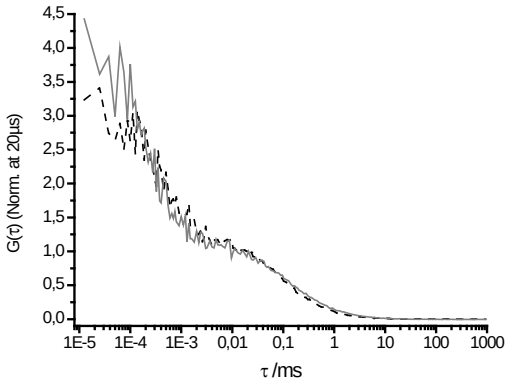

Figure S2. FCS correlation function, p-FTAA alone in pH 10 (dashed black), p-FTAA after addition of 10mM KI (solid grey) normalized against the diffusion component (20 µs).

Table S1. Calculated values of the number of emitting diffusing units in focus (*N*), diffusion time (τ_D), turnover time of the two dark transient state (τ_T, τ_U), fraction of molecules in the two dark transient states (*T, U*)) and the absolute intensity from the sample (*I*). The table data is based on measurements performed on the upright FCS setup

sample	*N*	τ_D / µs	τ_T / µs	τ_U / µs	*T*	*U*	*I* / kHz	CPM / kHz
FTAA	102.0	120.8	0.67	52.2	0.59	0.15	58.0	1.13
KI 1mM	215	177.2	0.45	37.1	0.64	0.25	78.5	0.73
KI 5mM	168.3	176.0	0.38	28.5	0.68	0.20	81.0	0.96
KI 10mM	174.5	218.9	0.36	53.3	0.71	0.26	82.0	0.94

The role of oxygen on p-FTAA was further investigated by fluorescence lifetime measurements of p-FTAA in $NaCO_3$ buffer (pH 10). The fluorescence lifetime was unaffected upon removal of oxygen from the solution (Fig. S3).

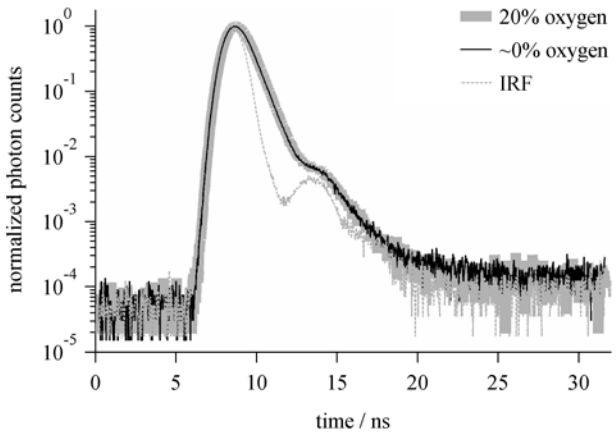

Figure S3, Normalized photon counts of 2 µM p-FTAA in $NaCO_3$ buffer (pH 10), with (▬)and without (—) oxygen in the buffer solution and the corresponding instrument response function IRF (┈). The fluorescence lifetimes of p-FTAA in the presence and absence of oxygen are identical within the resolution of the instrument.

Two lifetime components were used to fit the data, $\tau_1 \sim 0.7$ ns and $\tau_1 \sim 0.01$ ns. The fast component might originate from scattered laser light, which could not be avoided due to the weak fluorescence signal of p-FTAA at the low concentration used. Excitation at 495/30 nm, emission collected at 565/18 nm.

The normalized emission spectrum of p-FTAA in $NaCO_3$ buffer did also not change upon oxygen removal (Fig. S4). Only a slight increase (~30%) in the total fluorescence intensity was detected.

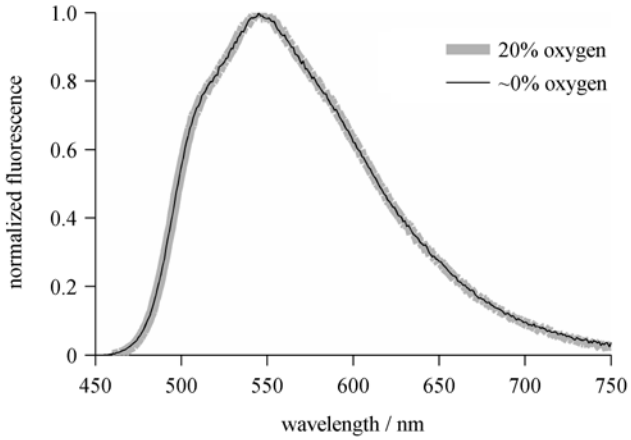

Figure S4, Normalized emission spectra of 2 µM p-FTAA in $NaCO_3$ buffer (pH 10), with (▬)and without (—) oxygen in the buffer solution. The spectra are identical. Excitation at 440/5 nm.

1. Kasha, M., Collisional perturbation of spin-orbital coupling and the mechanism if fluorescence quenching - a visual demonstration of the perturbation. *J. Am. Chem. Soc.* **1952,** *20* (71), 1952.

2. Widengren, J.; Schwille, P., Characterization of photoinduced isomerization and back-isomerization of the cyanine dye Cy5 by fluorescence correlation spectroscopy. *Journal of Physical Chemistry A* **2000,** *104* (27), 6416-6428.