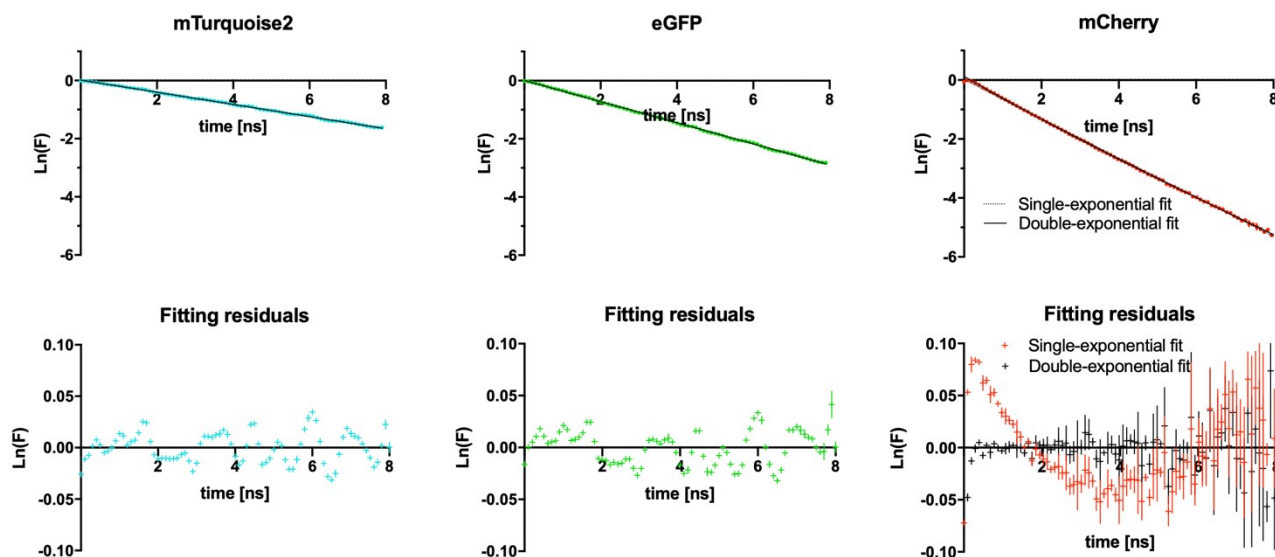
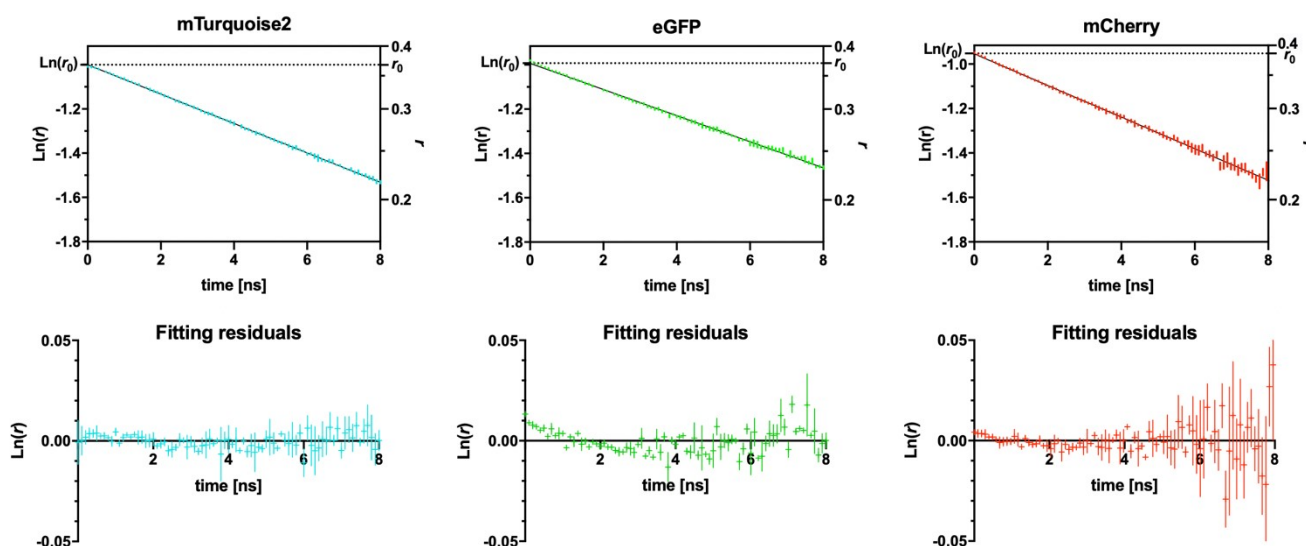


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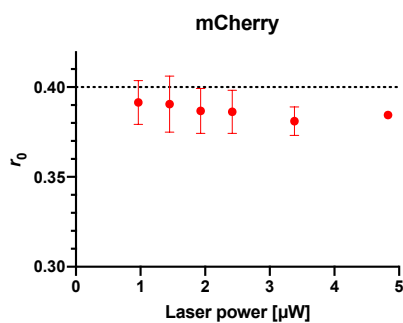
Supplementary information



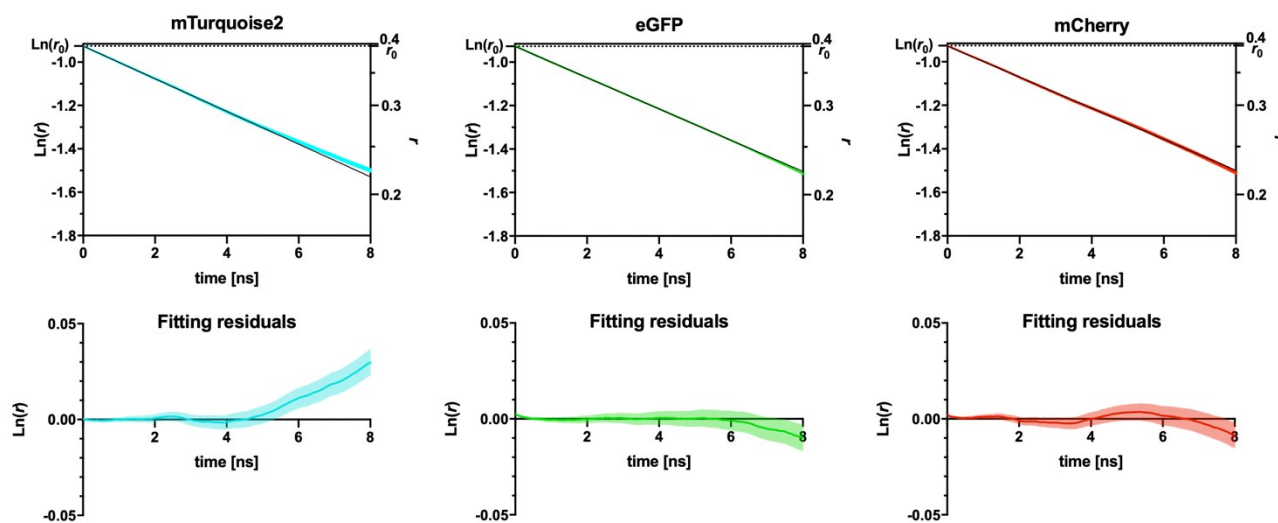
Supplementary Figure 1: Results of fitting time-resolved fluorescence intensity data. Linear fitting of logarithms of fluorescence intensities produced good fits for mTurquoise2 and eGFP but not for mCherry, as seen by the fitting residuals. For mCherry, a markedly better fit was obtained by using two exponential functions. Mean values and 95 % confidence intervals are shown in all plots.



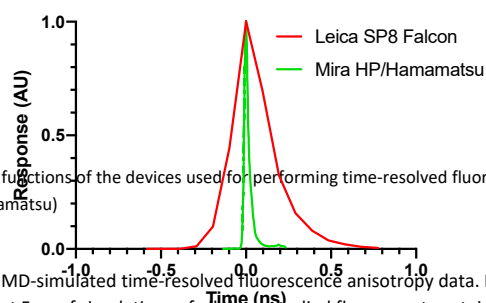
Supplementary Figure 2: Results of fitting time-resolved fluorescence anisotropy data. Linear fitting of logarithms of fluorescence anisotropy produced good fits for all three studied fluorescent proteins, as evidenced by the shown residuals. Mean values and 95 % confidence intervals are shown in all plots.



Supplementary Figure 3: Effects of laser power on determinations of initial fluorescence anisotropy (r_0) in mCherry. Laser power 5 μW yields the most precise results that do not statistically significantly deviate from results obtained with lower illumination intensities. Mean values and 95% confidence intervals are shown.



Instrument response functions



Supplementary Figure 5: Instrument response functions of the devices used for performing time-resolved fluorescence measurements of mTurquoise2 and eGFP (LeicaSP8 Falcon) and mCherry (Mira HP/Hamamatsu)

Supplementary Figure 4: Results of fitting of MD-simulated time-resolved fluorescence anisotropy data. Linear fitting of logarithms of fluorescence anisotropy (r) produced good fits for the first 5 ns of simulations of all three studied fluorescent proteins, as evidenced by the shown residuals. Mean values and 95 % confidence intervals are shown in all plots.