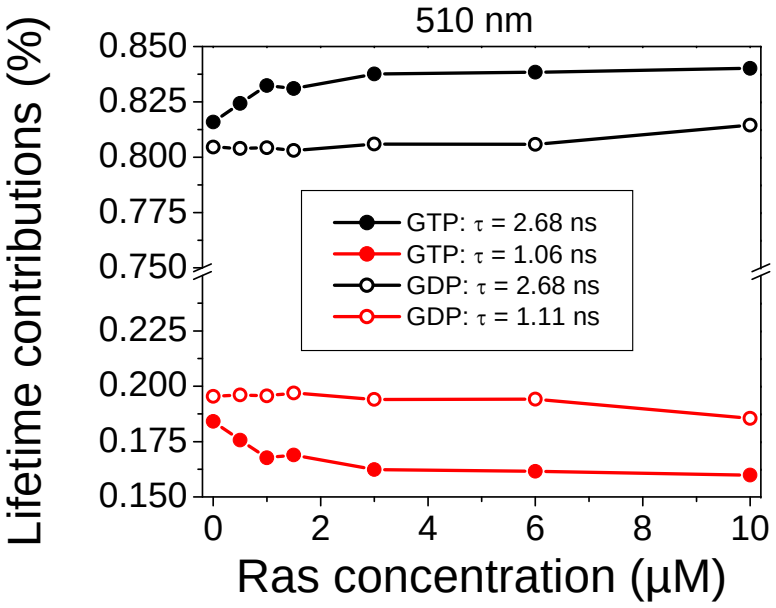
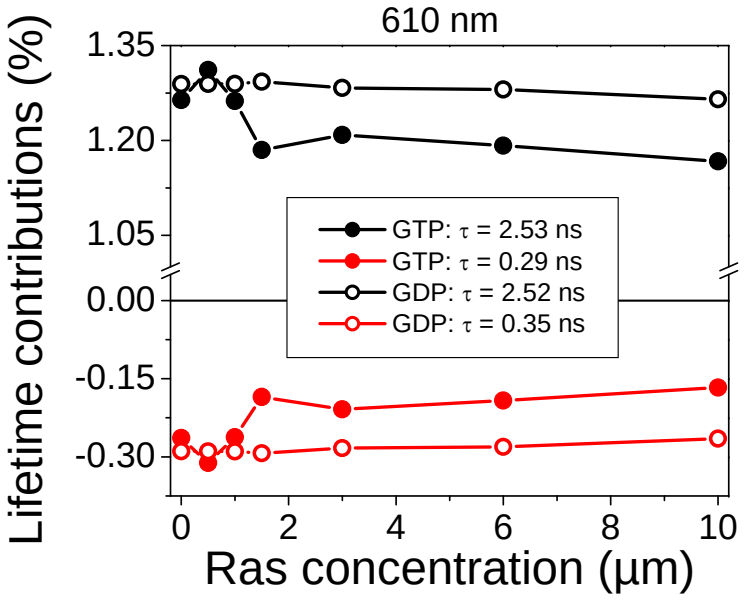
PLCε GFP/mRFP and Ras

Donor (GFP)



Acceptor (mRFP)

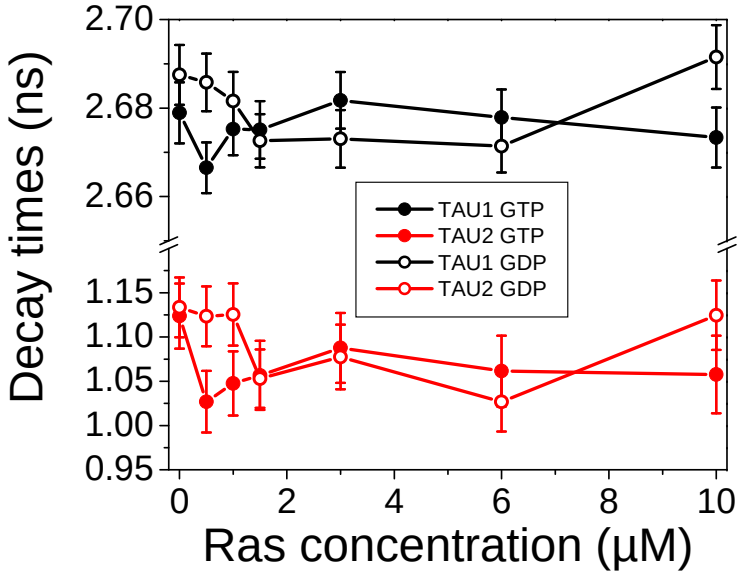


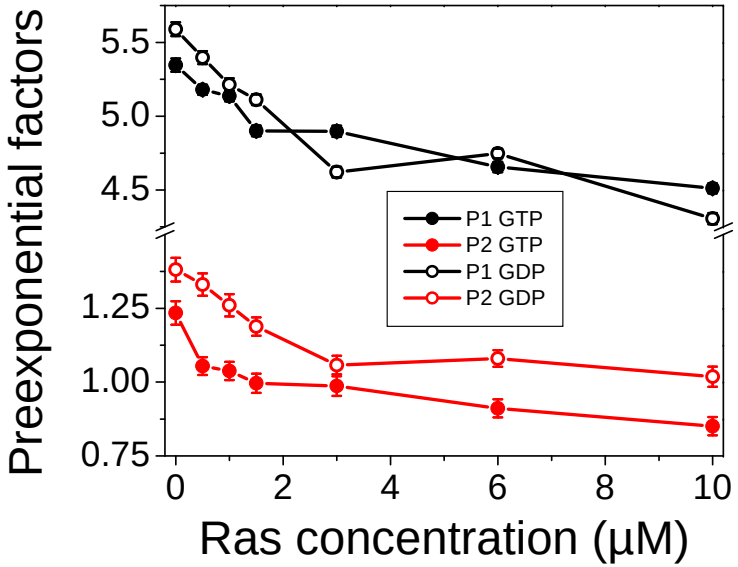
•The GFP lifetime was analyzed with 2 exponential decay times: 2.68 ns (not quenched) and 1.06 ns (component showing FRET). Approx. 18% of molecules show intramolecular FRET before interaction with Ras GTP. The percentage decreases to ≈ 16% after adding 10 µM Ras GTP. This reduction is an indication that the distance between the donor and the acceptor increases after Ras binding in the presence of GTP.

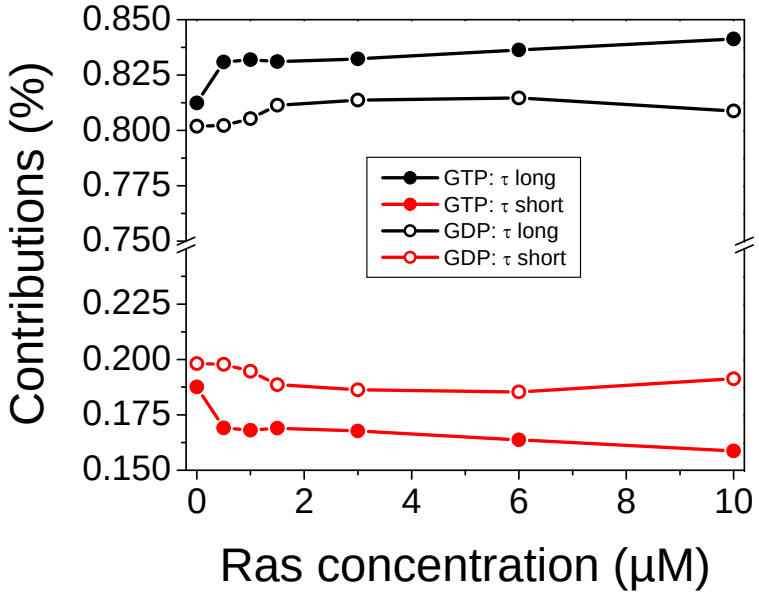
•In the presence of GDP, the percentage of molecules undergoing FRET remains constant, with a very small decrease only at high Ras concentrations (10 µM).

•The acceptor decay shows a long component (probably an intermediate lifetime between mRFP and the GFP leaking in the acceptor channel) and a short component with negative contribution, which is indicative for FRET. The same trend is observed as for the donor: no significant changes of the negative component in the presence of GDP, but a reduced contribution of the negative component in the presence of GTP. Less negative component means less FRET, so the distance between the donor and the acceptor increases.

Anlysis with all parameters free







Comparison average lifetime GFP tau linked/free

