**Figure 5 - Figure supplement 2A.** **Fit parameters from Global Analysis of Filtered Fluorescence Correlation Spectroscopy (fFCS).** Cross-correlation decay times were fixed for all samples, and their amplitudes were set as global parameters when fitting the cross-correlation curves. The average and log-space average, diffusion time (*tdiff*.) and geometric factor *s* are also global fit parameters as defined in *Materials and Methods*. Details of variants can be found in Figure 1- and Table 1. Decay timescales correspond to and amplitudes to in *Materials and Methods.*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| FL Variant | 0.01 ms Amplitude | 0.10 ms Amplitude | 1.00 ms Amplitude | Average | *tdiff*. (ms) | S |
| P2-G6 | 0.02 | 0.37 | 0.61 | 0.65 | 8.64 | 4.70 |
| P3-G4 | 0.53 | 0.31 | 0.16 | 0.20 | 10.19 | 4.70 |
| P2-G1 | 0.54 | 0.01 | 0.45 | 0.46 | 2.27 | 4.42 |
| P3-G5 | 0.00 | 0.50 | 0.50 | 0.55 | 4.47 | 4.42 |
| P1-G3 | 0.23 | 0.17 | 0.60 | 0.62 | 0.91 | 4.42 |
| P1-G4 | 0.53 | 0.00 | 0.47 | 0.47 | 1.08 | 4.42 |
| P1-G2 | 0.47 | 0.13 | 0.39 | 0.41 | 2.05 | 4.42 |
| P3-S2 | 0.27 | 0.10 | 0.62 | 0.64 | 0.91 | 4.42 |
| P2-S2 | 0.03 | 0.41 | 0.56 | 0.60 | 7.03 | 4.70 |
| S2-G6 | 0.01 | 0.28 | 0.71 | 0.74 | 9.74 | 4.70 |
| S3-G1 | 0.63 | 0.00 | 0.37 | 0.37 | 1.21 | 4.70 |
| S1-G1 | 0.71 | 0.03 | 0.26 | 0.27 | 2.10 | 4.70 |
| PSG Variant | 0.01 ms Amplitude | 0.10 ms Amplitude | 1.00 ms Amplitude | Average | *tdiff*. (ms) | S |
| P2-G1 | 0.39 | 0.03 | 0.58 | 0.59 | 1.04 | 4.42 |
| P3-G5 | 0.29 | 0.18 | 0.53 | 0.55 | 0.79 | 4.42 |
| P1-G3 | 0.51 | 0.10 | 0.39 | 0.41 | 1.54 | 4.42 |
| P1-G4 | 0.58 | 0.00 | 0.42 | 0.43 | 0.80 | 4.42 |
| P1-G2 | 0.39 | 0.46 | 0.15 | 0.20 | 3.12 | 4.42 |
| P3-S2 | 0.01 | 0.31 | 0.68 | 0.72 | 0.96 | 4.42 |

**Figure 5 - Figure supplement 2B. Variant-specific Fit Parameters for Analysis of filtered Fluorescence Correlation Spectroscopy (fFCS).** The baseline/no-correlation parameter B, average number of bright molecules in the confocal volume *N*, and long-timescale decay amplitude (ATl for = 5.00 ms, accounts for long-timescale photophysical effects). Parameters correspond to low-FRET to high-FRET cross-correlation (LH), high-FRET to low-FRET cross-correlation (HL), low-FRET autocorrelation (LL), or high-FRET autocorrelation (HH). Details of variants can be found in Figure 1- and Table 1. Parameter definitions can be found in *Materials and Methods*.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| FL Variant | bLH | NB,LH | ATl,LH | bHL | NB,HL | ATl,HL | bLL | NB,LL | ATl,LL | bHH | NB,HH | ATl,HH |
| P2-G6 | 1.00 | 0.03 | 0.00 | 1.00 | 0.04 | 0.00 | 1.00 | 0.03 | 0.00 | 1.00 | 0.01 | 0.00 |
| P3-G4 | 1.02 | 37.23 | 0.59 | 1.02 | 4.85 | 0.01 | 1.02 | 0.06 | 0.78 | 1.02 | 0.03 | 0.89 |
| P2-G1 | 1.14 | 0.57 | 0.00 | 1.14 | 0.34 | 0.41 | 1.24 | 0.05 | 0.19 | 1.09 | 0.15 | 0.03 |
| P3-G5 | 1.03 | 1.10 | 0.12 | 1.03 | 0.96 | 0.23 | 1.01 | 0.06 | 0.27 | 1.00 | 0.21 | 0.20 |
| P1-G3 | 1.00 | 0.12 | 0.02 | 1.00 | 0.11 | 0.00 | 1.00 | 0.05 | 0.00 | 1.00 | 0.02 | 0.00 |
| P1-G4 | 1.24 | 0.20 | 0.06 | 1.24 | 0.26 | 0.00 | 1.32 | 0.06 | 0.00 | 1.20 | 0.01 | 0.00 |
| P1-G2 | 1.05 | 0.16 | 0.12 | 1.05 | 0.18 | 0.00 | 1.28 | 0.02 | 0.12 | 1.05 | 0.03 | 0.00 |
| P3-S2 | 1.35 | 0.14 | 0.06 | 1.35 | 0.10 | 0.29 | 1.45 | 0.03 | 0.00 | 1.50 | 0.05 | 0.00 |
| P2-S2 | 1.00 | 0.32 | 0.01 | 1.00 | 0.78 | 0.00 | 1.00 | 0.02 | 0.00 | 1.00 | 0.16 | 0.00 |
| S2-G6 | 1.00 | 0.82 | 0.00 | 1.00 | 0.95 | 0.00 | 1.00 | 0.43 | 0.00 | 1.00 | 0.51 | 0.00 |
| S3-G1 | 1.00 | 1.49 | 0.00 | 1.00 | 0.90 | 0.00 | 1.00 | 0.06 | 0.00 | 1.00 | 0.05 | 0.00 |
| S1-G1 | 1.00 | 1.99 | 0.00 | 1.00 | 1.20 | 0.21 | 1.00 | 0.05 | 0.04 | 1.00 | 0.05 | 0.04 |
| PSG Variant | BLH | NLH | ATl,LH | BHL | NHL | ATl,HL | BLL | NLL | ATl,LL | BHH | NHH | ATl,HH |
| P2-G1 | 1.05 | 0.09 | 0.57 | 1.05 | 0.12 | 0.00 | 1.11 | 0.01 | 0.00 | 1.03 | 0.04 | 0.00 |
| P3-G5 | 1.25 | 0.19 | 0.31 | 1.25 | 0.16 | 0.37 | 1.85 | 0.01 | 0.01 | 1.15 | 0.04 | 0.00 |
| P1-G3 | 1.18 | 0.43 | 0.00 | 1.18 | 0.48 | 0.00 | 1.20 | 0.08 | 0.00 | 1.17 | 0.02 | 0.00 |
| P1-G4 | 1.10 | 0.23 | 0.00 | 1.10 | 0.24 | 0.00 | 1.10 | 0.05 | 0.00 | 1.10 | 0.01 | 0.00 |
| P1-G2 | 1.17 | 0.08 | 0.04 | 1.17 | 0.07 | 0.19 | 1.34 | 0.01 | 0.23 | 1.10 | 0.03 | 0.05 |
| P3-S2 | 1.16 | 0.20 | 0.00 | 1.16 | 0.16 | 0.24 | 1.40 | 0.01 | 0.00 | 1.07 | 0.07 | 0.00 |