Supplemental Table:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Fluorescence lifetime (ns)$$τ$$ | Correlation time 1 (ns)$$ϕ\_{1}$$ | Fraction correlation time 1 $χ\_{1}$ | Correlation time 2 (ns)$$ϕ\_{2}$$ |
| mCit-PKCα (EGTA) TCSPC | 3.43[3.42 to 3.45] | -- | -- | 32.6[30.0 to 35.9] |
| mCit-PKCα (Ca2+) TCSPC | 3.43[3.41 to 3.45] | -- | -- | 36.0[32.6 to 40.1] |
| PKCα-mCit (EGTA) TCSPC | 3.44[3.42 to 3.46] | 0.753[0.343 to 1.529] | 0.070[0.048 to 0.114] | 30.5[25.9 to 41.4] |
| PKCα-mCit (Ca2+) TCSPC | 3.45[3.42 to 3.46] | 0.753[0.0 to 0.93] | 0.065[0.055 to 0.076] | 32.1[29.0 to 35.9] |
| PKCα-mCit + liposome (EGTA) TCSPC | 3.46[3.44 to 3.48] | -- | -- | 31.2[28.5 to 36.0] |
| PKCα-mCit + liposome (Ca2+) TCSPC | 3.28[3.26 to 3.29] | 0.761[0.372 to 1.34] | 0.182[0.127 to 0.283] | 12.1[9.84 to 17.4] |
| PKCα-mCit (EGTA) DWR | 3.41[3.39 to 3.43] | 0.431[0.0 to 0.692] | 0.088[0.065 to 0.097] | 40.8[31.8 to 50.7] |
| PKCα-mCit (Ca2+) DWR | 3.41[3.39 to 3.43] | 0.349[0.0 to 0.577] | 0.093[0.071 to 0.102] | 41.5[32.1 to 51.1] |
| PKCα-mCit + liposome(EGTA) DWR | 3.42[3.40 to 3.44] | 0.540[0.217 to 0.760] | 0.094[0.0711 to 0.109] | 43.5[34.0 to 53.6] |
| PKCα-mCit + liposome (EGTA + Ca2+) DWR | 3.30[3.28 to 3.32] | 0.554[0.428 to 0.685] | 0.230[0.208 to 0.254] | 30.9[25.5 to 40.4] |
| PKCα-mCit + liposome (EGTA + Ca2+ + EGTA) DWR | 3.34[3.32 to 3.36] | 0.525[0.342 to 0.746] | 0.152[0.131 to 0.177] | 40.8[32.6 to 52.2] |
| C1a-SPASM-C2 (EGTA) DWR | 3.30[3.28 to 3.33] | 0.356[0.178 to 0.563] | 0.150[0.130 to 0.175] | 61.2[43.4 to 110] |
| C1a-SPASM-C2 (Ca2+) DWR | 3.18[3.15 to 3.20] | 0.225[0.063 to 0.349] | 0.236[0.208 to 0.259] | 129.7[62.2 to >1000] |

STable 1: **Values from TCSPC and DWR analysis.** Fluorescence lifetime of the sample obtained at the magic angle was fit by a single exponential function. The lifetime value was constrained as the anisotropy profile was fit to a 2 exponential function where the two correlation time parameters and the fractional contribution of the faster correlation time were co-estimated. Initial and final anisotropies were constrained to 0.4 and 0 respectively. For the conditions with only one reported correlation time, only a single correlation time was resolved by the fit (i.e., the fraction of the faster component was zero). Steady-state anisotropy was calculated as the fluorescence weighted average of the anisotropy decay given by the best-fit parameters. Standard errors of the fit (67% confidence interval) are listed in brackets.