|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | | | | | **Gaussian fraction** | | | | | **Discrete fraction** | | |
| **Fluorophore configuration** | **Sample** | **Target DNA mismatch** | **DMSO** | **χ2** | **Tau (ns)** | **± error** | **FWHM (ns)** | **± error** | **α** | **Tau (ns)** | **± error** | **α** |
| DO | DNA only | No | No | 1.41 | 1.66 | 0.1 | 1.09 | 0.3 | 2.04 | 4.68 | 0.007 | 20.6 |
| DA | DNA only | No | No | 1.35 | 1.51 | 0.1 | 0.666 | 0.4 | 2.25 | 4.54 | 0.006 | 21.2 |
| DO | DNA only | No | Yes | 1.42 | 1.74 | 0.2 | 1.23 | 0.3 | 2.19 | 4.5 | 0.007 | 21.3 |
| DA | DNA only | No | Yes | 1.37 | 1.73 | 0.2 | 1.17 | 0.3 | 2.37 | 4.36 | 0.007 | 21.9 |
| DO | DNA only | Yes | Yes | 1.44 | 1.30 | 0.1 | 0.87 | 0.3 | 1.59 | 4.45 | 0.005 | 22.0 |
| DA | DNA only | Yes | Yes | 1.35 | 1.73 | 0.2 | 1.50 | 0.3 | 1.96 | 4.34 | 0.007 | 22.3 |
| DO | DNA only, 60C | Yes | Yes | 1.29 | 1.66 | 0.2 | 0.674 | 0.7 | 1.86 | 4.34 | 0.008 | 22.3 |
| DA | DNA only, 60C | Yes | Yes | 1.22 | 2.23 | 0.4 | 2.1 | 0.5 | 2.38 | 4.26 | 0.01 | 22.2 |
| DO | WT TCC | No | No | 1.36 | 1.71 | 0.1 | 0.979 | 0.3 | 2.23 | 4.7 | 0.007 | 20.4 |
| DA | WT TCC | No | No | 1.35 | 3.31 | 0.2 | 2.06 | 0.2 | 11.2 | 4.54 | n/a | 13.5 |
| DO | WT TCC | No | Yes | 1.40 | 1.91 | 0.2 | 1.68 | 0.3 | 2.30 | 4.51 | 0.008 | 21.1 |
| DA | WT TCC | No | Yes | 1.50 | 3.30 | 0.3 | 1.72 | 0.3 | 16.8 | 4.36 | n/a | 9.76 |
| DO | WT TCC | Yes | Yes | 1.33 | 2.30 | 0.3 | 1.97 | 0.5 | 2.25 | 4.53 | 0.01 | 20.9 |
| DA | WT TCC | Yes | Yes | 1.58 | 3.34 | 0.2 | 1.57 | 0.2 | 24.2 | 4.33 | n/a | 4.28 |
| DO | WT STC | No | No | 1.41 | 1.58 | 0.2 | 0.723 | 0.5 | 1.95 | 4.7 | 0.007 | 20.5 |
| DA | WT STC | No | No | 1.41 | 3.2 | 0.3 | 2.22 | 0.3 | 7.98 | 4.54 | n/a | 15.9 |
| DO | WT STC | Yes | Yes | 1.31 | 2.00 | 0.2 | 1.78 | 0.4 | 2.16 | 4.51 | 0.009 | 20.9 |
| DA | WT STC | Yes | Yes | 1.58 | 3.36 | 0.2 | 1.57 | 0.2 | 23.5 | 4.33 | n/a | 4.55 |
| DO | Δ domain III TCC | Yes | Yes | 1.28 | 2.12 | 0.4 | 2.25 | 0.6 | 1.81 | 4.49 | 0.01 | 21.2 |
| DA | Δ domain III TCC | Yes | Yes | 1.31 | 2.85 | 0.2 | 2.32 | 0.3 | 5.14 | 4.33 | n/a | 19.5 |
| DO | Δ domain III STC | Yes | Yes | 1.36 | 2.51 | 0.4 | 2.38 | 0.5 | 2.46 | 4.53 | 0.01 | 20.7 |
| DA | Δ domain III STC | Yes | Yes | 1.63 | 3.25 | 0.1 | 1.75 | 0.1 | 27.0 | 4.33 | n/a | 2.67 |
| DO | WT STC, 60C | Yes | Yes | 1.46 | 1.91 | 0.3 | 2.95 | 0.8 | 3.59 | 4.42 | 0.01 | 16.5 |
| DA | WT STC, 60C | Yes | Yes | 2.13 | 2.99 | 0.1 | 2.20 | 0.1 | 14.2 | 4.26 | n/a | 7.76 |
| DO | Δ domain III STC, 60C | Yes | Yes | 1.51 | 1.73 | 0.1 | 2.13 | 0.3 | 4.10 | 4.38 | 0.009 | 17.4 |
| DA | Δ domain III STC, 60C | Yes | Yes | 1.78 | 2.71 | 0.07 | 2.57 | 0.09 | 16.7 | 4.26 | n/a | 7.63 |

**Figure 6 – figure supplement 1**

Fluorescence lifetime fits across all samples. Each sample was fit as the sum of one gaussian and one discrete lifetime. A donor only fluorophore configuration indicates that the Atto647N FRET acceptor fluorophore was omitted from the target DNA. In the case of DNA only and Donor only samples, no variables were held constant. In samples with transpososomes present, the discrete lifetime from the matching DNA-only sample was held constant (and thus has no associated error) in order to account for an unbound/unbent/singly-labeled fraction. FWHM: Full width at half maximum. TCC: target capture complex, CDCs lacking the 3’OH strand transfer nucleophile were used. STC: strand transfer complex, CDCs fully capable of strand transfer were used. α refers to the pre-exponential factor for the decay component. DO, Donor fluorophore only; DA, Donor and Acceptor fluorophores present.