**Figure 8 - Figure supplement 3A.** **Attachment Atom Indices for Accessible Volume Simulations.** Fluorophores were simulated in the FPS software package using a three-sphere model (Radii R1, R2, R3), with flexible linker dimensions given by LL (length) and WL (width). Docking simulations were performed with Alexa-488 C5-maleimide attached to PDZ3 sites and Alexa-647 C2-maleimide attached to SH3-GuK. Parameters for accessible volume simulations used in rigid body docking and simulations were as follows: For Alexa-488 C5-maleimide, radii values of 5.0 Å, 4.5 Å, and 1.5 Å were used with linker length of 20.5 Å and width 4.5 Å. For Alexa-674 C2-maleimide, radii values of 7.15 Å, 4.5 Å, and 1.5 Å were used with linker length of 21.0 Å and width 4.5 Å. Details of variants can be found in Figure 1 and Table 1.

|  |  |
| --- | --- |
| Labeling Site | Atom Index |
| **PDZ3** |  |
| P1 | 14 |
| P2 | 554 |
| P3 | 778 |
| **SH3-GuK** |  |
| S1 | 519 |
| S2 | 664 |
| S3 | 870 |
| G1 | 1680 |
| G2 | 1830 |
| G3 | 1948 |
| G4 | 1972 |
| G5 | 2157 |
| G6 | 2477 |

**Figure 8 - Figure supplement 3B. Distance Bounds from FRET Network Robustness Analysis.** Mean distances from re-analysis of sub-sampled seTCSPC data are reported for the two limiting states from each variant (Figure 6-Supplementary File 2). These distances, along with AV simulations, were used for screening all snapshot structures from DMD simulations to estimate FRET-distances for each snapshot structure. Details of variants can be found in Figure 1 and Table 1. For inclusion in the basin ensembles from screening, simulated structures were rejected if the percent error of each distance was greater than the average σ%error from the corresponding basin (7.3% and 8.7% for limiting states A and B, respectively). Description of FRET Network Robustness Analysis is found in *Materials and methods*.

|  |  |  |
| --- | --- | --- |
| Sample | ± σ%error A (Å) | ± σ%error B (Å) |
| P2-G6 | 68.1 ± 5.0 | 37.7 ± 3.3 |
| P3-G4 | 66.3 ± 4.8 | 35.6 ± 3.1 |
| P2-G1 | 81.6 ± 6.0 | 34.5 ± 3.0 |
| P3-G5 | 64.3 ± 4.7 | 41.0 ± 3.6 |
| P1-G3 | 85.8 ± 6.3 | 41.4 ± 3.6 |
| P1-G4 | 75.4 ± 5.5 | 34.1 ± 3.0 |
| P1-G2 | 77.3 ± 5.6 | 27.2 ± 2.4 |
| P3-S2 | 28.3 ± 2.1 | 52.0 ± 4.5 |
| P2-S2 | 36.9 ± 2.7 | 59.1 ± 5.1 |
| S2-G6 | 33.4 ± 2.4 | 53.5 ± 4.7 |
| S3-G1 | 38.8 ± 2.8 | 73.2 ± 6.4 |
| S1-G1 | 65.9 ± 4.8 | 33.7 ± 2.9 |