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| **Figure 8–figure supplement 1. Responsiveness of Individual Variants to the Underlying Conformational Distribution.** Shown are the simulated interdye distances from accessible volume (AV) simulations using DMD structures.The 20871 snapshot structures from DMD were used to calculate the accessible volumes for the dyes at each labeling position, which were then used to calculate average interdye distances for each snapshot structure. These interdye distances for each variant are plotted against the distance between centers of mass (CoM) for the relevant domains. These 2D plots provide a qualitative analysis of how each FRET pair reflects changes in the underlying conformation. The limiting state distances for each variant are shown as vertical lines for state A (orange) and state B (purple). Overlaid are contours corresponding to DMD structures residing in state A (orange) and state B (purple), which are within the distance uncertainty from sub-sampling analysis of TCSPC fluorescence decays and the AV simulation distances. For most variants, limiting state distances qualitatively agree with the locations of local maxima in the simulated interdye distance distributions. However, some variants show both maxima along the same vertical line indicating that the conformational dynamics are not fully captured by that FRET pair. Further, presence of FRET pairs spanning SH3-GuK show the most difference between simulated and measured distances, likely owing to the limited dynamics of these domains *in silico*. |