

Supporting Information

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SI Materials and Methods: Single-Molecule FRET Experiments

Single molecule FRET experiments were carried out on a home built laser confocal microscope system, using an Axiovert 200 microscope (Zeiss). Excitation was achieved by focusing the 488 nm line of a 543-AP-A01 tunable argon-ion laser (Melles Griot) inside the sample solution, 30 μm from the glass cover-slip surface, using a water immersion objective (1.2 NA, 63 \times ; Zeiss). The fluorescence emission was collected using the same objective, separated from the excitation light, using a dichroic mirror (Q495LP; Chroma Tech), spatially filtered using a 100 μm

pinhole, then separated into donor and acceptor components using a second dichroic mirror (560 DCXR; Chroma). The donor and acceptor signals (I_D and I_A) were further filtered using an HQ 525/50M band-pass filter (donor; Chroma) and a 590 LPV2 long-pass filter (acceptor; Chroma), then detected using SPCM-AQR-14 avalanche photodiode (APD) photon counting modules (Perkin-Elmer Optoelectronics). Photon counts were recorded using a photon counting card (PCI 6602, National Instruments) interfaced with a computer. Data analysis was performed as described in *Materials and Methods*.

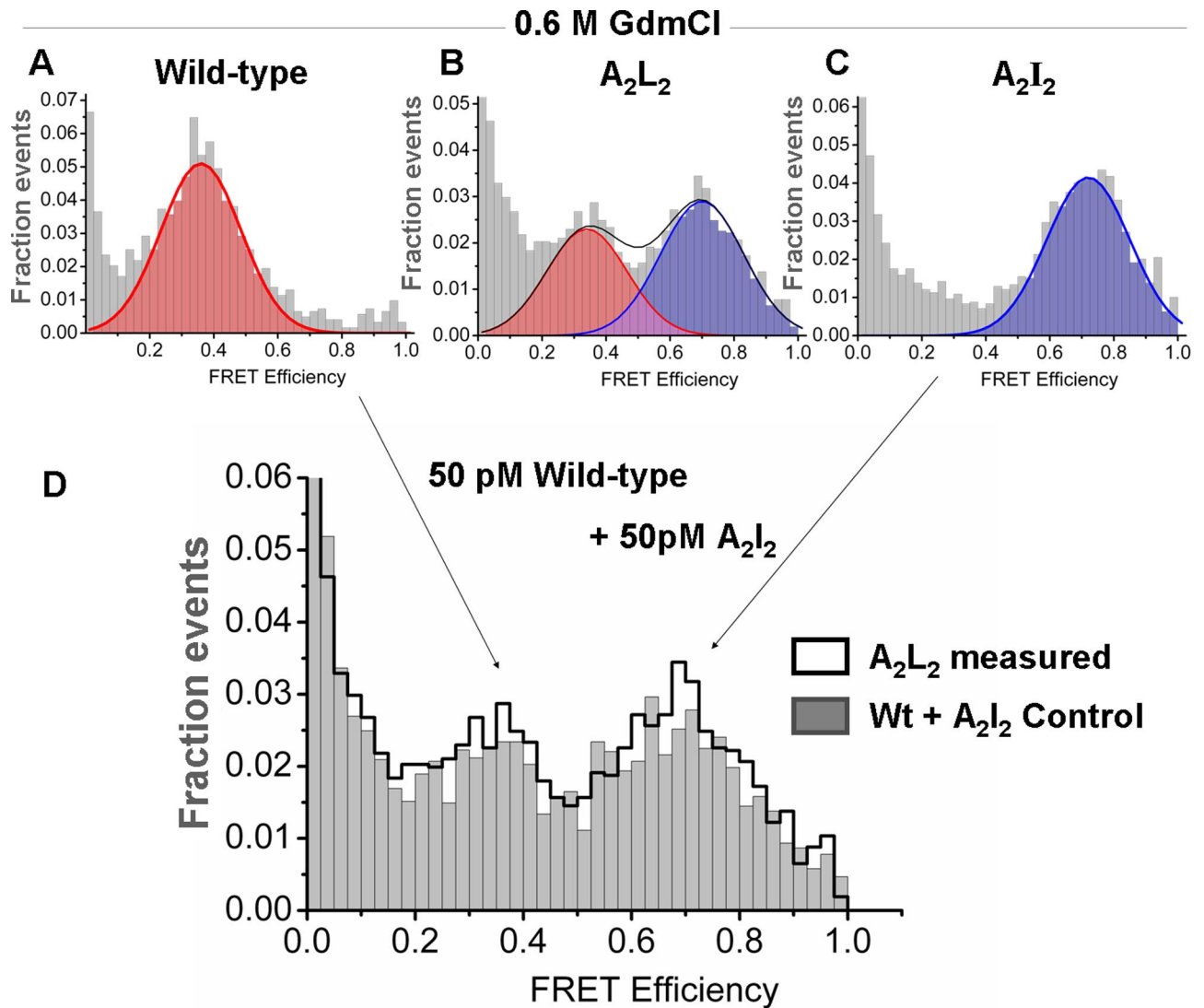


Fig. S4. Experimental control. A_2L_2 populates both *anti* and *syn* conformations under mild denaturation. (A–C) smFRET histograms obtained at 0.6 M GdmCl for WT, A_2L_2 and A_2I_2 . (D) Overlay of the data observed for A_2L_2 (black line) and of a mixture of 50 pM WT + 50 pM A_2I_2 (gray bars). The two dimers were preformed in native buffer, measured separately then mixed in 0.6 M GdmCl, leading to a histogram that matches the one obtained for A_2L_2 .

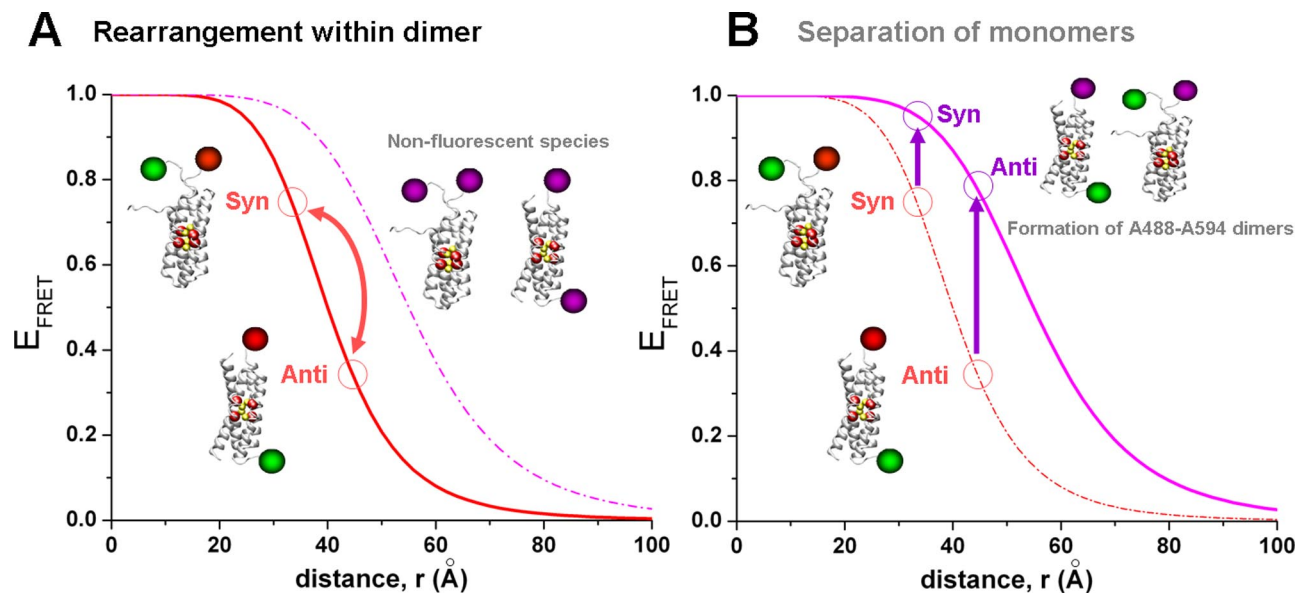


Fig. S5. Competition by monomers labeled with a second acceptor tests for dimer dissociation during conformational transitions. The 3-color experiments conducted in this work exploit the differences of distance-dependence of the FRET efficiencies between various FRET pairs. Especially, the A488-A647 and A488-A594 dye pairs have specific and well-separated Förster distances (R_0), which for a given donor-acceptor pair is the inter-dye distance for which $E_{FRET} = 0.5$. The red and purple lines in Fig. S5 shows the FRET efficiency as a function of inter-dye distance r ,

$$E_{FRET} = \frac{1}{1 + \left(\frac{r}{R_0}\right)^6}$$

for the A488-A647 and A488-A594 dye pairs, calculated with R_0 values of 40 Å and 55 Å respectively. As described in A, the slightly denaturing conditions trigger interconversion between *syn* and *anti* folding states for the A₂L₂ mutant. These two conformations can be separated thanks to the adequate R_0 value of the A488-A647 FRET pair. If monomers labeled with A488 were to separate and refold with the A594-monomers present in excess in the solution, novel FRET species would appear (B). As the Förster distance R_0 is greater for the A488-A594 pair, both the *syn* and *anti* states would have higher FRET efficiencies. Using the same two detection channels, these two conformations cannot be distinguished (as observed in Fig. 4C), but the resulting FRET peak with $E_{FRET} = 0.85$ is clearly separated from the original peaks obtained with the A488-A647 pair. Our experiment demonstrates simply that little exchange of monomers occur during the structural switch between *syn* and *anti* structures.