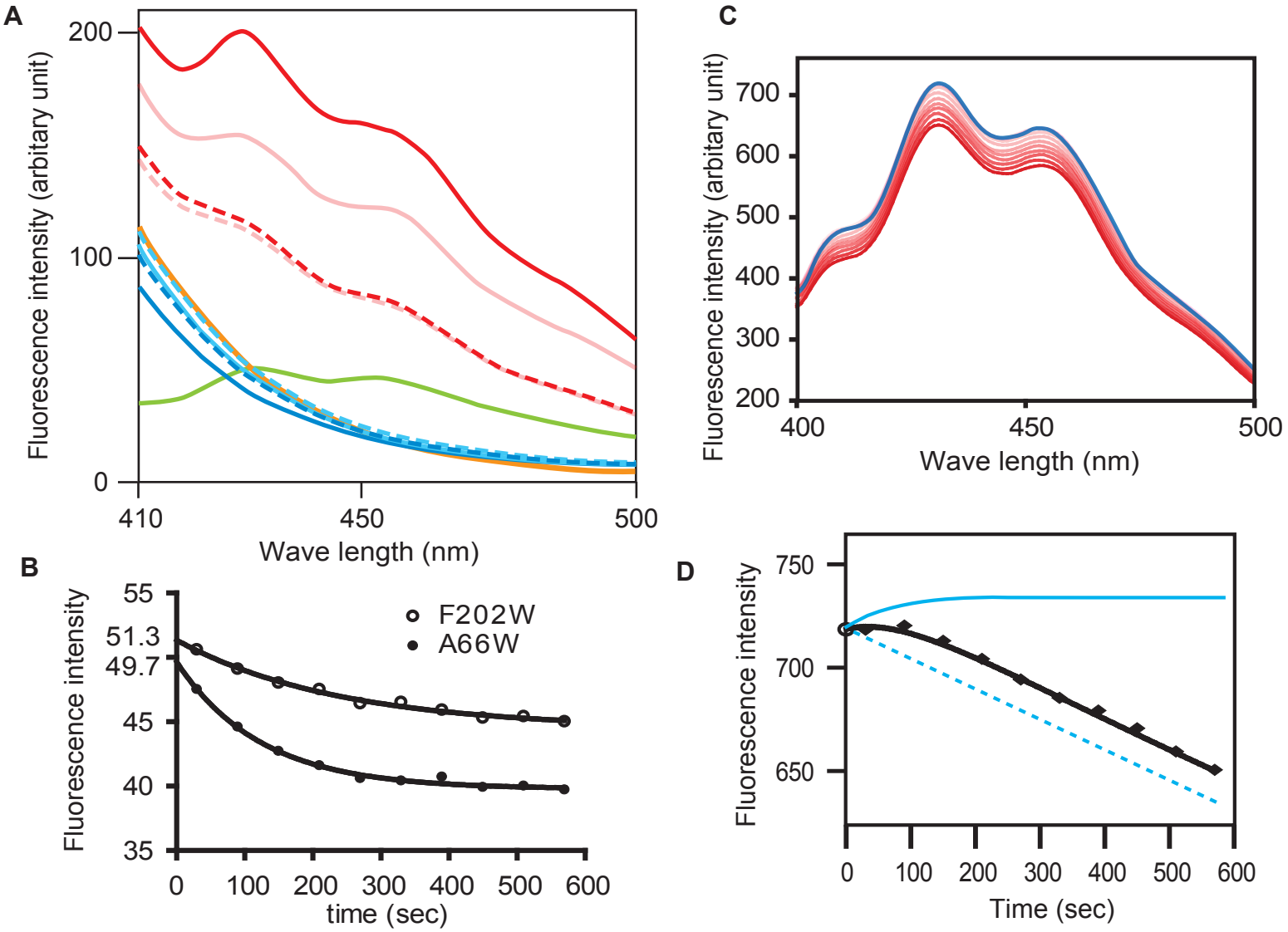
Supplementary Figure 6



Supplementary Figure 6. DPH-FRET assays. Othwise indicated, protein (100 µg/ml)/DPH-liposome (200 µg/m, DPH:lipid=1:500) mixture were excited at 280 nm. (A) Fluorescence spectra of A66W/DPH-liposome mixture (red solid curves), F202W/DPH-liposome mixture (red hatched curves), A66W/control-liposome mixture (blue solid curves), F202W/control-liposome mixture (blue hatched curves) and DPH-liposome alone (green). The pale lines are scans at 30 sec after mixing and the dark lines are those at 570 sec. Only the A66W/DPH-liposome combination shows a substantial FRET. No FRET is seen when DPH-liposome supernatant recovered after ultracentrifugation is used instead of the liposomes (orange). This is an indication of negligible leak of DPH from liposomes and ensures that the FRET is induced by the protein/liposome interaction. (**B**) Time courses of the 430-nm fluorescence of protein/control-liposome mixtures shown in A. The time 0 values are estimated by single exponential fit. Since the 430-nm intensity of the control liposomes alone excited at 280 nm is negligible, the time 0 value of protein/DPH-liposome mixture can be estimated as the sum of the 430-nm intensity of the DPH-liposome alone and that of the control liposome/protein mixture at time 0. (C) Fluorescence intensity changes of A66W/DPH-liposome mixture excited at the DPH's excitation maximum of 360 nm. DPH-liposomes alone, blue; pale to dark red, the mixture at 30 sec to 570 sec. (D) Time course of the 430-nm peak fluorescence shown in C. Since the fluorescence of the A66W mutant alone excited at 360 nm is negligibly small, the value of DPH-liposome alone can be a good estimate of the time 0 value. The DPH fluorescence decreases steadily with an initial lag of 100 sec. Even if this decrease would be entirely caused by decay or loss of DPH probe (hatched blue line), the maximal fluorescence increase induced by protein-liposome interactions (blue line) would be no more than 15 units. This experiment excludes the possibility that the increased fluorescence intensity in the FRET condition is caused by a change in the DPH fluorescence efficiency induced by membrane deformation.