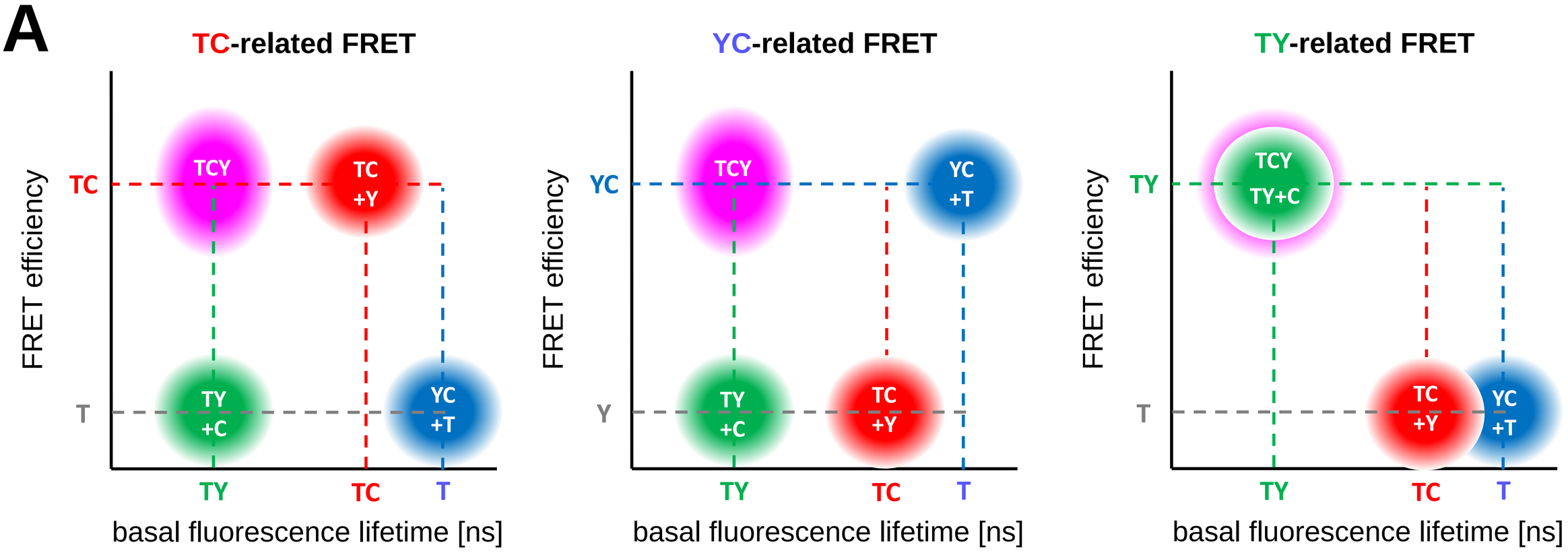
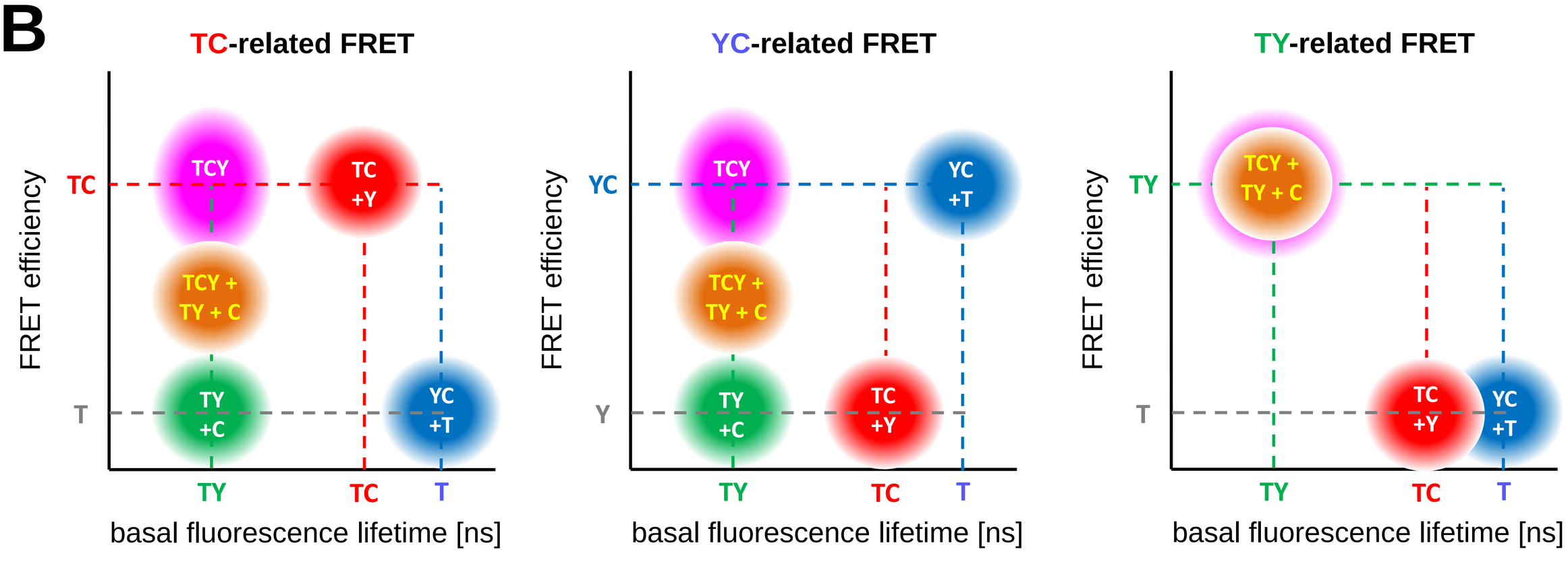
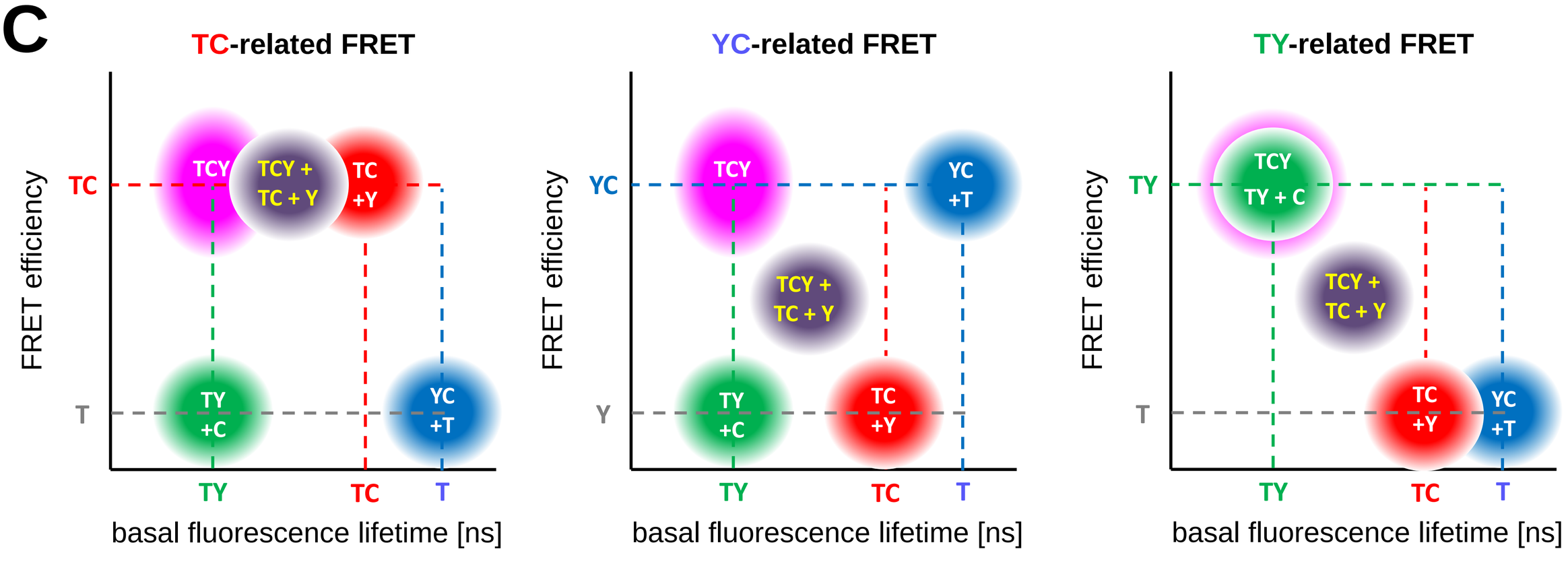
A


TC-related FRET
YC-related FRET
TY-related FRET
FRET efficiency
TC
T
YC
Y
TY
TCY
TC +Y
TY +C
YC +T
TCY TY+C
basal fluorescence lifetime [ns]

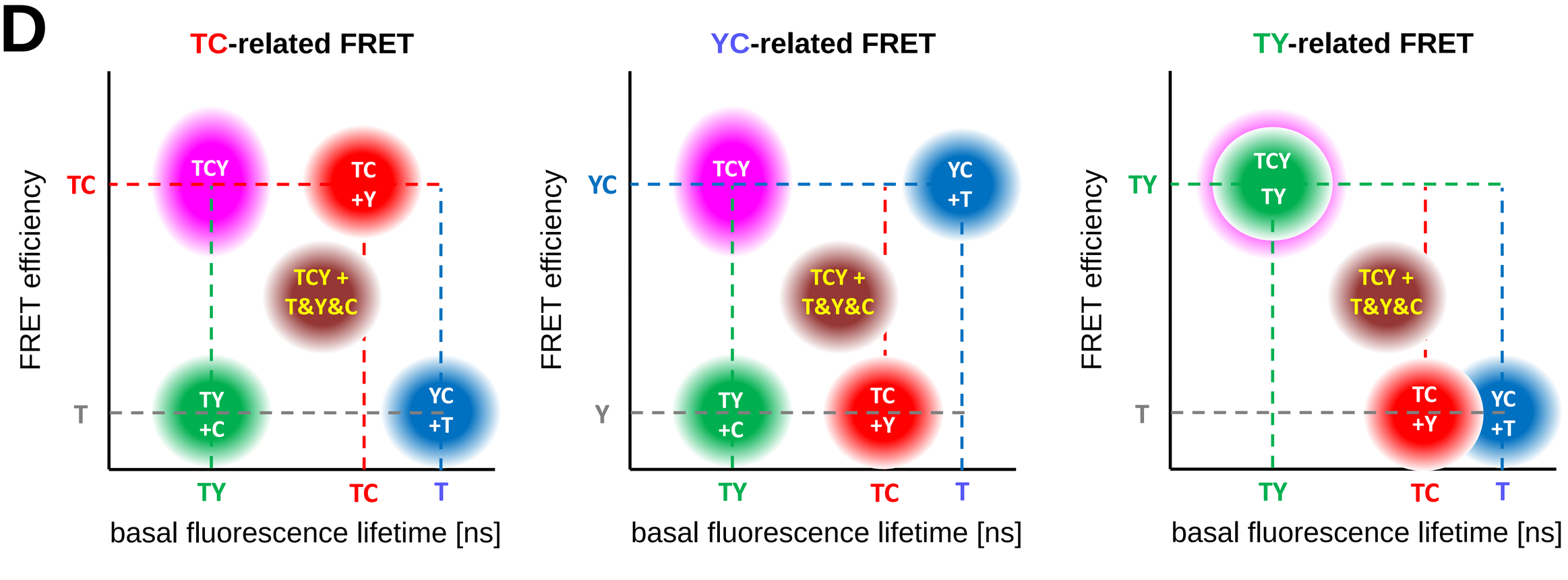
B


TC-related FRET
YC-related FRET
TY-related FRET
FRET efficiency
TC
T
YC
Y
TY
TCY
TC +Y
TCY + TY + C
TY +C
YC +T
basal fluorescence lifetime [ns]

C


TC-related FRET
YC-related FRET
TY-related FRET
FRET efficiency
TC
T
YC
Y
TY
TCY
TCY + TC + Y
TC +Y
TY +C
YC +T
TCY TY + C
basal fluorescence lifetime [ns]

D


TC-related FRET
YC-related FRET
TY-related FRET
FRET efficiency
TC
T
YC
Y
TY
TCY
TC +Y
TCY + T&Y&C
TY +C
YC +T
TCY TY
basal fluorescence lifetime [ns]

Simulation of correlation plot results assuming uniform ratios for trimeric and dimeric plus monomeric protein states. The characteristic lifetimes (TY, TC, T alone, segmented lines x-axis) and FRET efficiencies (interacting states (TC,YC,TY) and non-interacting states (T,Y), segmented lines y-axis) schematically shown in **A)** define the range of possible positions of the different combinations considered in this analysis. For our simulations, we assumed even ratios (e.g., 1:1:1) for trimeric and dimeric plus monomeric states, which allowed us to predict that FRET efficiencies and lifetimes of fluorescent-protein combinations could be expressed as averages of the respective experimentally determined FRET efficiencies and lifetimes, respectively. **B)** shows the predicted localization of TCY + TY + C in the two-dimensional FRET efficiency versus lifetime plot. Here, donor lifetime and TY-related FRET should be similar to the TY+C values, since TCY and TY+C exhibit similar TY-FRET. However, the TC- and TY-related FRET are assumed to adopt values that are, on average, intermediate between those observed in noninteracting and interacting states, since only the TCY, and not the TY+C combination, allows for TC- and YC-related FRET. **C)** shows the predicted localization of TCY + TC + Y in the two-dimensional FRET efficiency versus lifetime plot. Here, due to the co-occurrence of trimeric and dimeric plus monomeric states, the donor lifetime is assumed to adopt values that are on average between the TY and TC values. The TC-related FRET should be similar to the TC+Y values, since TCY and TC+Y exhibit similar TC-FRET. In contrast, the YC- and TY-related FRET are predicted to adopt values that, on average, lie in between the FRET values of the noninteracting and interacting states, respectively. **D)** shows the localization of TCY + T&Y&C in the two-dimensional FRET efficiency versus lifetime plot. The lifetime is assumed to reach values that lie between the lifetime values of TY and T. In addition, TC-, TY- and YC-related FRET are predicted to adopt values that, on average, are in between the FRET values of the noninteracting and interacting states, respectively.