SUPPLEMENTARY FIGURES

Supplementary Figure 1. smFRET time-course traces showing CST binding to non-telomeric junction substrate.

(A) Selection of real-time traces showing change in FRET (bottom) and individual Cy3 and Cy5 signals (top) in the absence of CST (top left) or after CST binding to non-telomeric ss-dsDNA junction substrate with 18 nt overhang. **(B)** Control experiment to show loss of FRET after CST addition reflects protein binding and not photobleaching. The slide was illuminated with 532 nm light (green laser) for ~25 sec to demonstrate loss of FRET after CST binding. The green laser was then turned off and the red laser (640 nm light) turned on to demonstrate that the Cy5 could still be excited by direct illumination. The switch in illumination was repeated several times to demonstrate that each dye remained active.

Supplementary Figure 2. smFRET controls

(A) FRET histograms demonstrating the sensitivity of G4-forming substrate to salt concentration. Anchored $_3$G4-Cy3 was incubated with the indicated concentrations of NaCl (left) or LiCl (right) prior to data acquisition. Histograms were generated from FRET measurements of >4,000 individual molecules. **(B)** FRET histogram showing that BSA cannot disrupt G4 DNA; top, $_3$G4 alone; bottom, $_3$G4 + 0.1 mg/ml BSA.

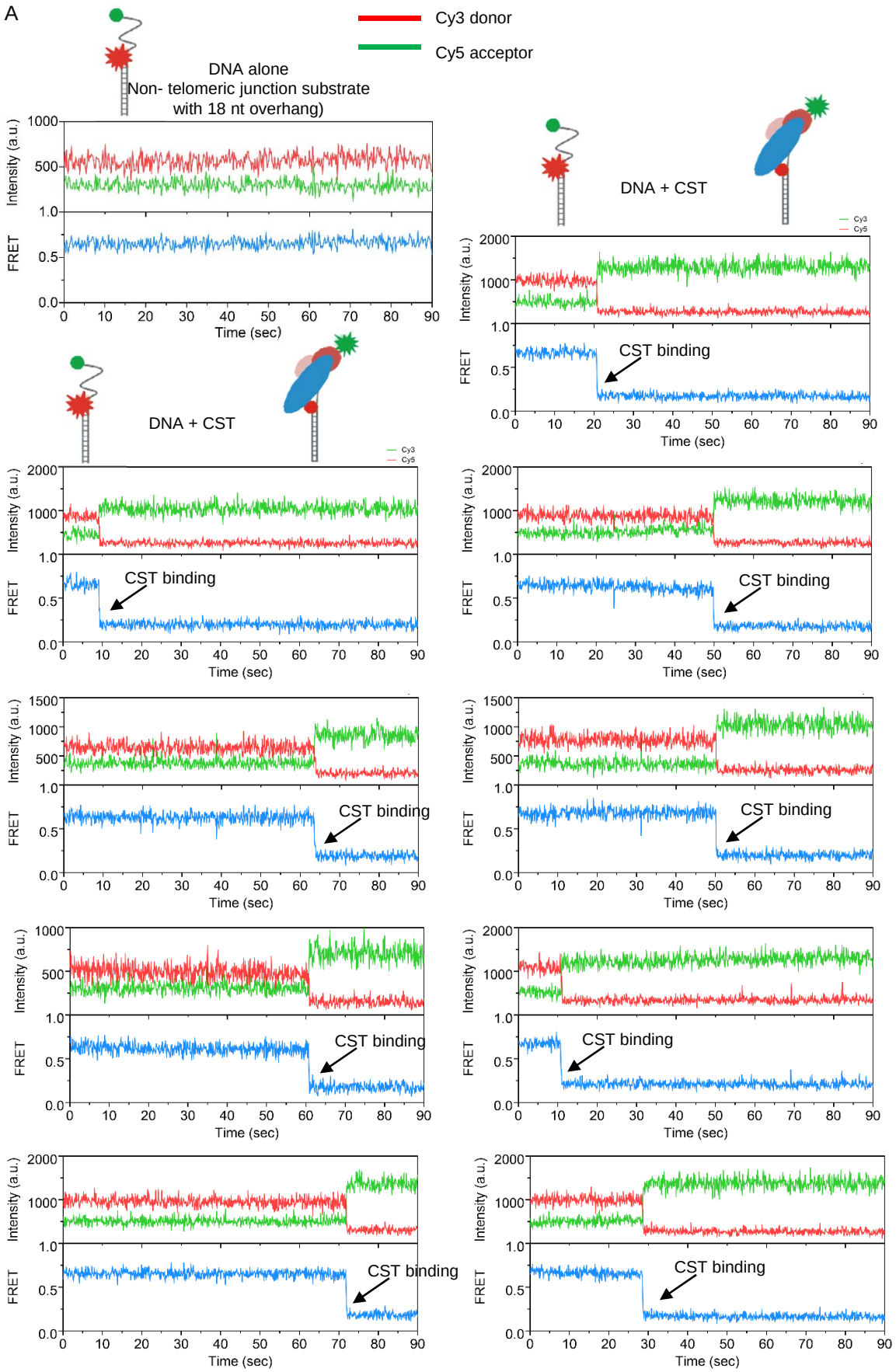
Supplementary Figure 3. smFRET time-course traces showing CST can unfold G4 DNA.

Selection of real-time traces showing change in FRET (bottom) and individual Cy3 and Cy5 signals (top) in the absence of CST (top left) or after CST binding to $_3$G4 substrate.

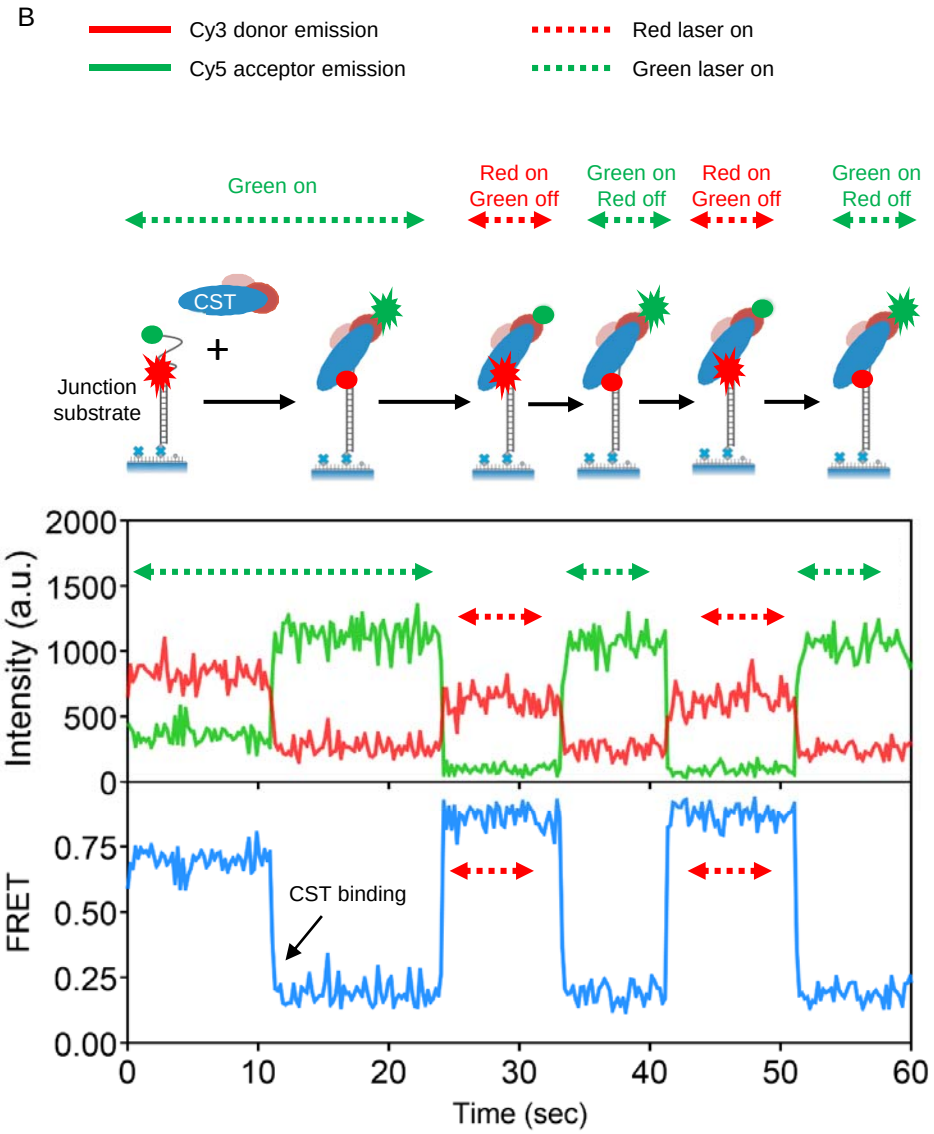
Cy3 and Cy5 signals show complementary transition upon CST binding. Measurement was performed in the presence of 2 nM CST (no washout).

Supplementary Figure 4. smFRET time course traces showing CST binding and disassociation.

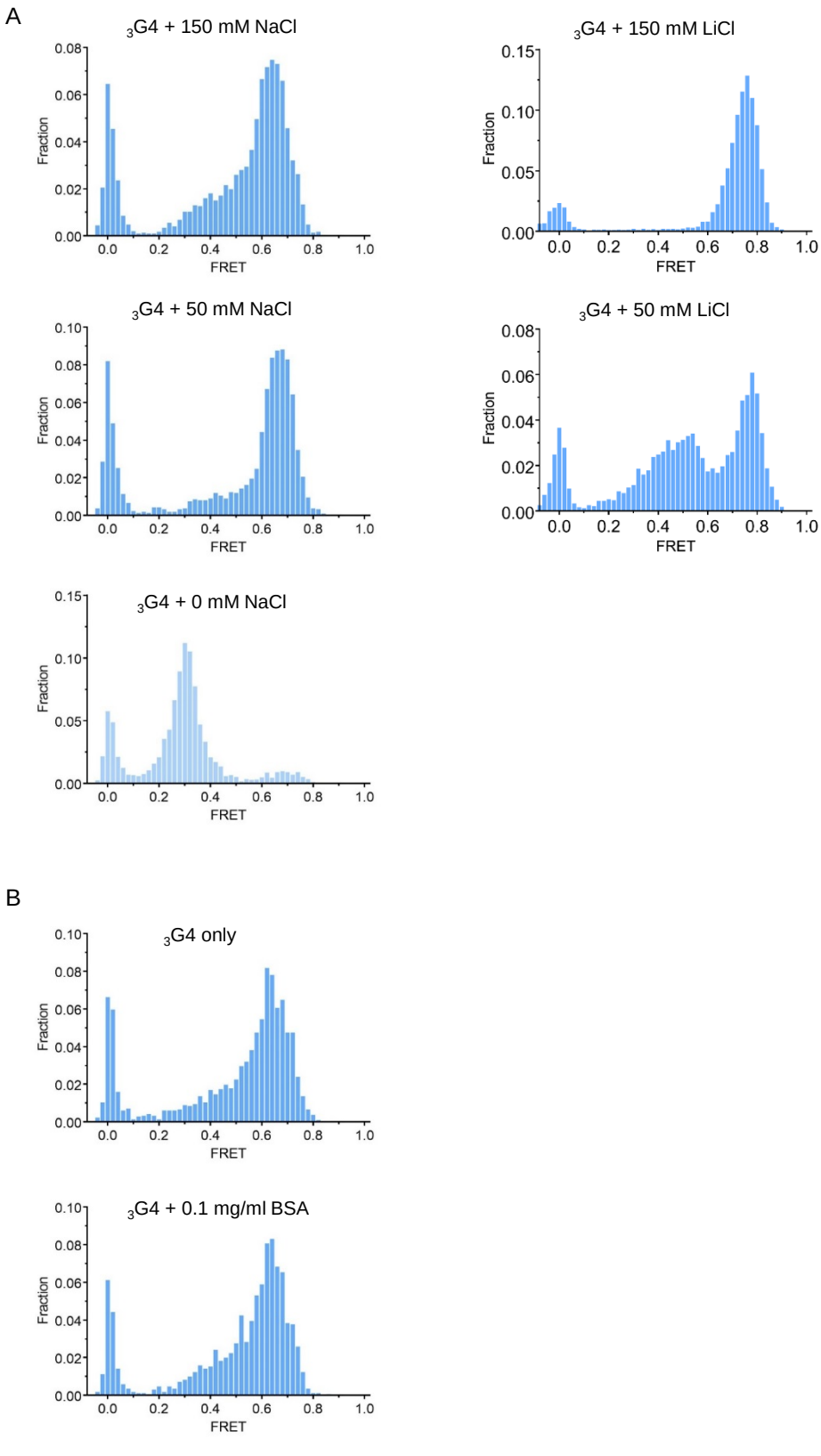
(A) Selection of real-time traces showing CST binding and dissociation from the non-telomeric junction substrate with 18 nt overhang. Top panels show individual Cy3 and Cy5 signals, bottom panels show change in FRET. **(B)** Selection of real-time traces showing CST binding and dissociation from the $_3$G4 substrate. **(C)** Analysis showing statistical significance for the change in CST dissociation frequency with increasing CST concentration.



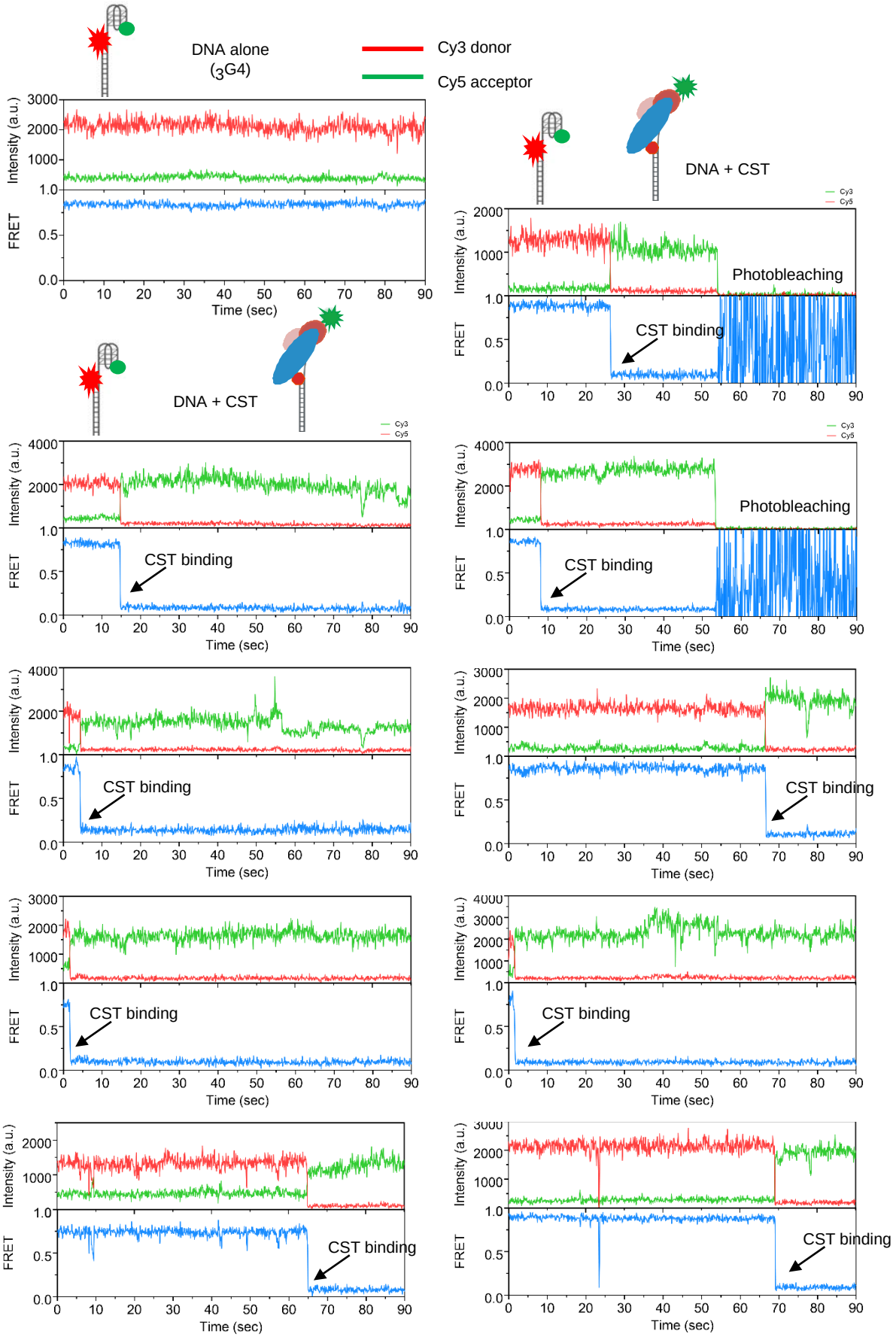
Bhattacharjee et al. Supplemental Figure 1A



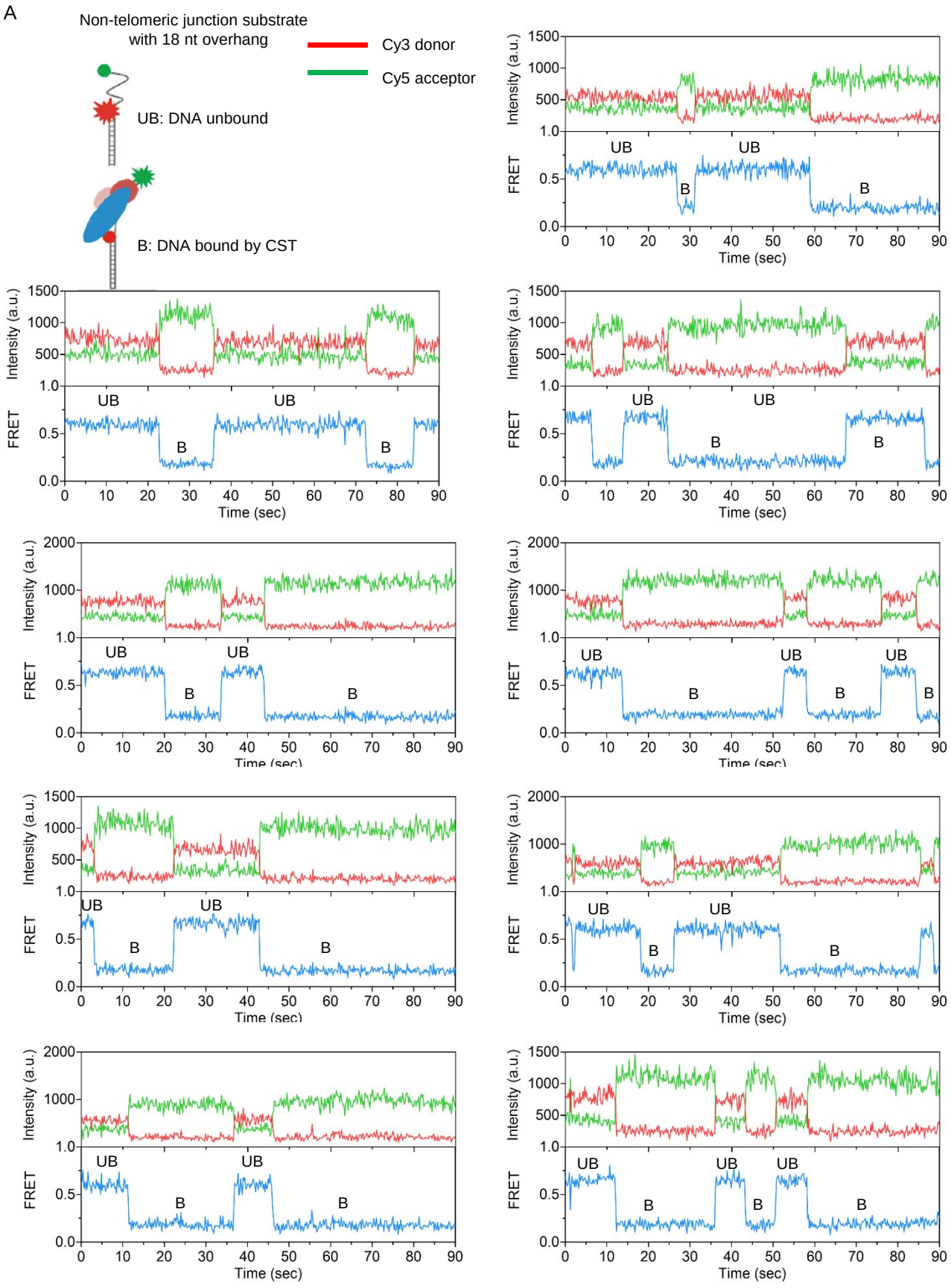
Bhattacharjee et al. Supplemental Figure 1B



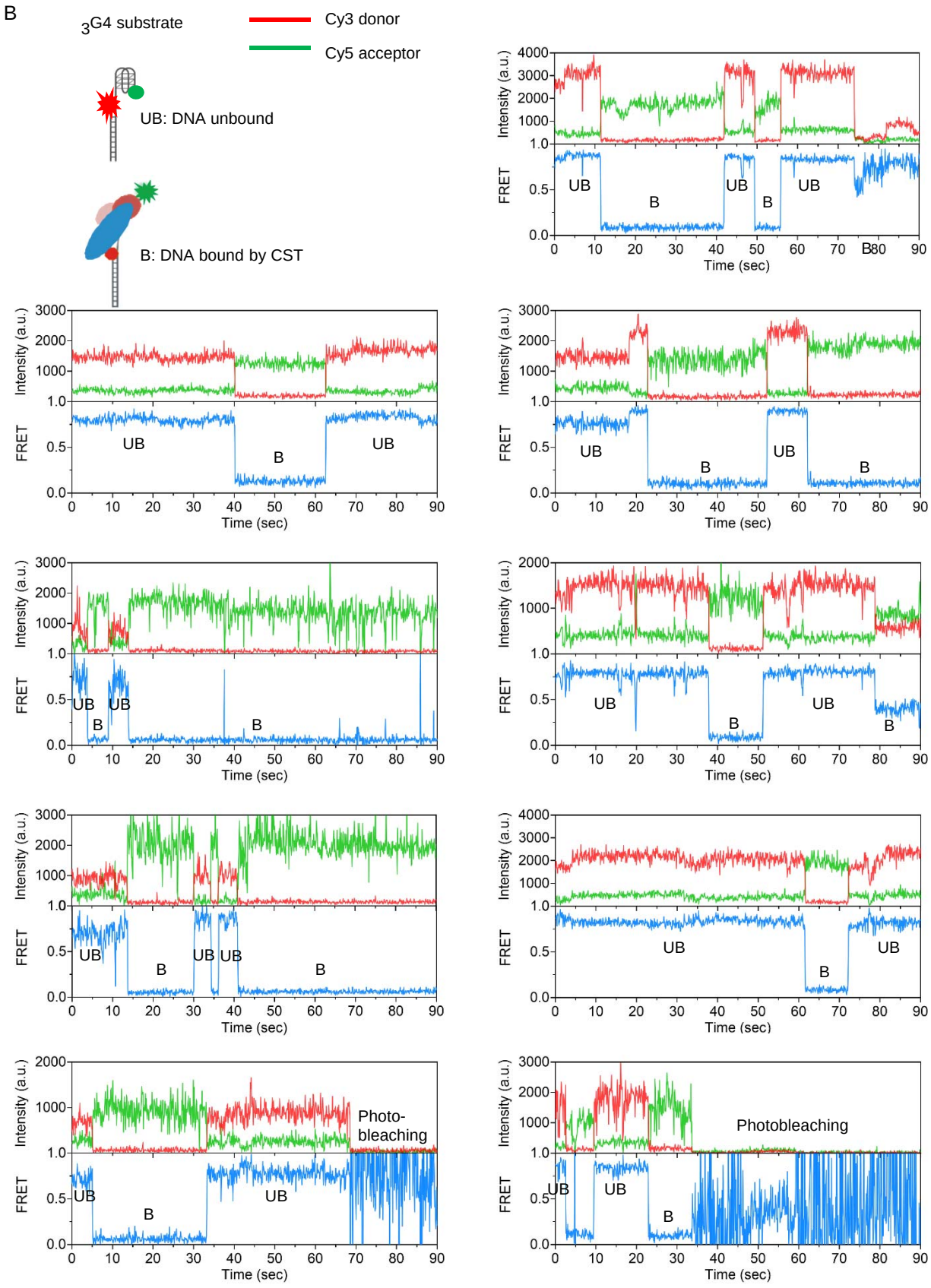
Bhattacharjee et al. Supplemental Figure 2



Bhattacharjee et al. Supplemental Figure 3



Bhattacharjee et al. Supplemental Figure 4A



Bhattacharjee et al. Supplemental Figure 4B

C

Ordinary one-way ANOVA

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary
Junction 0 nM vs. Junction 2 nM	-0.1003	-0.1745 to -0.02609	Yes	**
Junction 0 nM vs. Junction 5 nM	-0.3257	-0.3999 to -0.2516	Yes	****
Junction 2 nM vs. Junction 5 nM	-0.2255	-0.2997 to -0.1513	Yes	****
G4 0 nM vs. G4 2 nM	-0.1484	-0.2226 to -0.07426	Yes	***
G4 0 nM vs. G4 5 nM	-0.367	-0.4412 to -0.2928	Yes	****
G4 2 nM vs. G4 5 nM	-0.2186	-0.2928 to -0.1444	Yes	****
Junction 0 nM vs. G4 0 nM	0.06627	-0.007917 to 0.1404	No	ns
Junction 2 nM vs. G4 2 nM	0.01809	-0.05609 to 0.09227	No	ns
Junction 5 nM vs. G4 5 nM	0.02499	-0.04919 to 0.09917	No	ns