

Table 5. Steady state Anisotropy values for free dyes and doubly labeled FRET mutant

Anisotropy	Free dye	Doubly labeled protein w/donor excitation
Donor	0.202 ± 0.002	0.252 ± 0.019
Acceptor	0.128 ± 0.006	0.047 ± 0.021

Free dye was directly excited at 500 or 600 nM for donor or acceptor respectively. Protein sample was excited at 500 nM and anisotropy values were calculated for emission from either dye.