

Supporting material

Single-molecule characterization Cy3.5 - Cy5.5 dye pair for FRET studies of nucleic acids and nucleosomes

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Sequences of ssDNA oligonucleotides used to prepare Cy3.5-Cy5.5 doubly labeled dsDNA constructs:

[A] For dsDNA constructs with 4-, 13-, 16-, 19-, 25-, & 33- bp separations between Cy3.5 and Cy5.5,

Biotinylated – **Cy5.5** labeled ssDNA sequence:

5'- /5BiotinTEG/AG GGA GCA CAC CGG T/iAmMC6T+**Cy5.5**/T GAA TAA ATG GAA ATA TAA AGT
GAA ATG TCG TAT GAG GCG

Complementary **Cy3.5** labeled ssDNA sequences:

4 bp separation

5'- CGC CTC ATA CGA CAT TTC ACT TTA TAT TTC CAT TTA /iAmMC6T+**Cy3.5**/TC AAA CCG G

13 bp separation

5'- CGC CTC ATA CGA CAT TTC ACT TTA TAT /iAmMC6T+**Cy3.5**/TC CAT TTA TTC AAA CCG G

16 bp separation

5'- CGC CTC ATA CGA CAT TTC ACT TTA /iAmMC6T+ **Cy3.5**/AT TTC CAT TTA TTC AAA CCG G

19 bp separation

5'- CGC CTC ATA CGA CAT TTC ACT /iAmMC6T+ **Cy3.5**/TA TAT TTC CAT TTA TTC AAA CCG G

25 bp separation

5'- CGC CTC ATA CGA CAT /iAmMC6T+**Cy3.5**/TC ACT TTA TAT TTC CAT TTA TTC AAA CCG G

33 bp separation

5'- CGC CTC A/iAmMC6T+**Cy3.5**/A CGA CAT TTC ACT TTA TAT TTC CAT TTA TTC AAA CCG G

[B] For dsDNA construct with 0-bp separation between Cy3.5 and Cy5.5,

Biotinylated – **Cy5.5** labeled ssDNA sequence

5'- /5BiotinTEG/ AGG GAG CAC ACA TCG TCT GAT ACA TGG CTG TAA AGT ATG GAG ATT CTC
TG/3AmMC6T+**Cy5.5**/

Complementary **Cy3.5** labeled ssDNA sequence

5'- /5AmMC6+**Cy3.5**/ CAG AGA ATC TCC ATA CTT TAC AGC CAT GTA TCA GAC GAT G

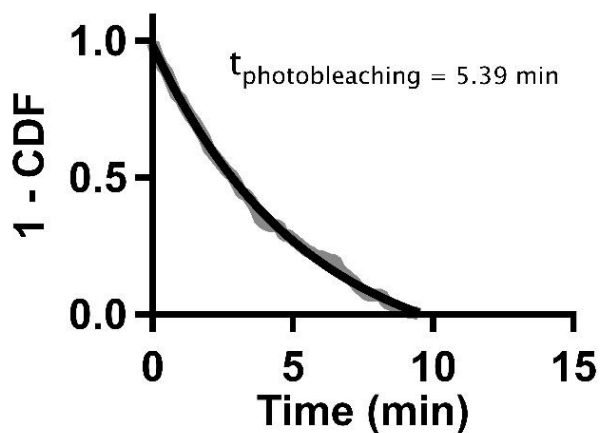


Figure S1:

1 - CDF (or 1 - cumulative distribution function), gray line, of photobleaching time of Cy3.5 alone (N = 142) fit to a single exponential (black line).