23RSS

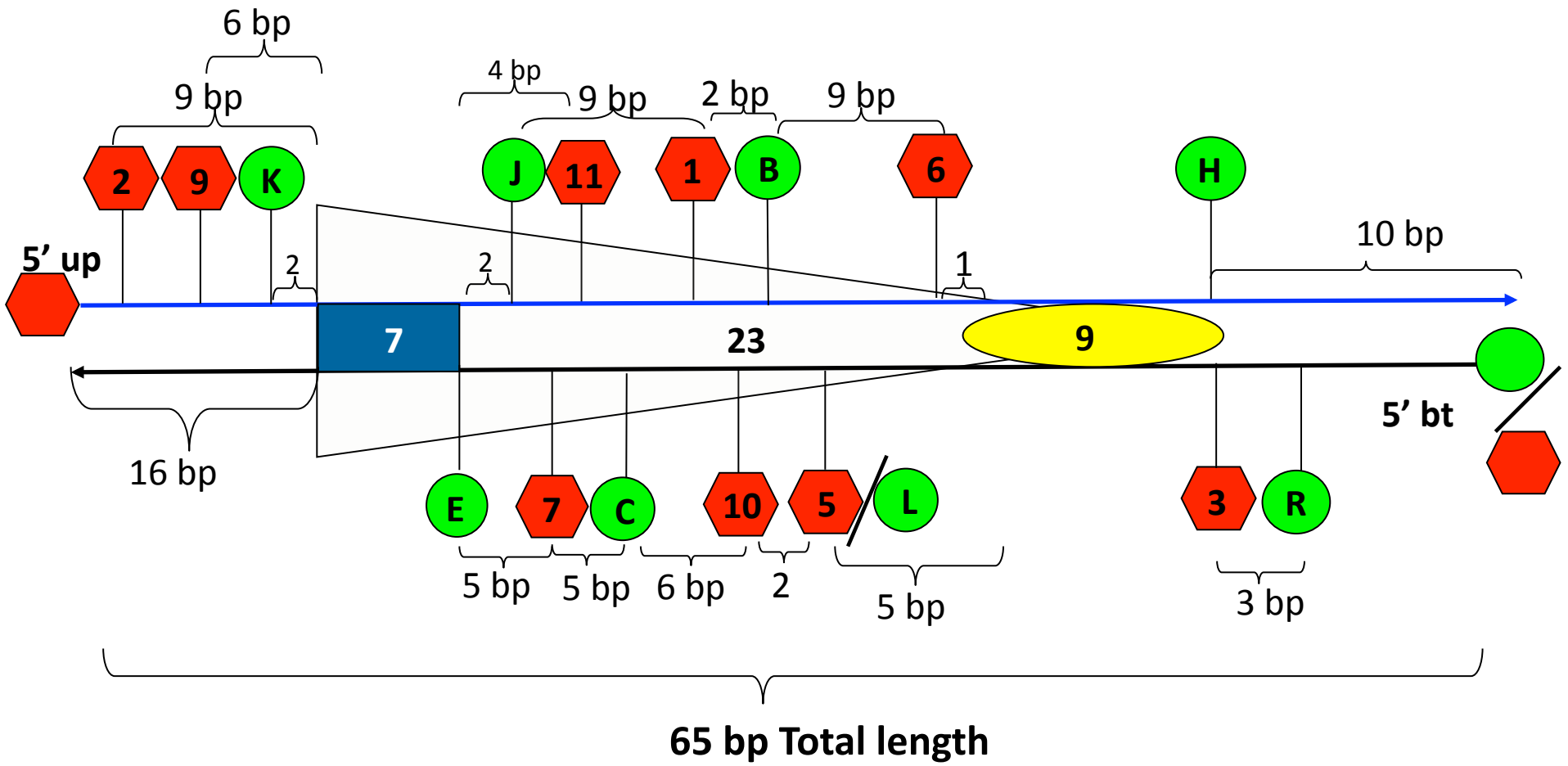
6 bp
9 bp
4 bp
9 bp
2 bp
9 bp
2
9
K
J
11
1
B
6
H
5' up
2
2
1
10 bp
7
23
9
5' bt
16 bp
E
7
C
10
5
L
3
R
5 bp
5 bp
6 bp
2
5 bp
3 bp
65 bp Total length
= FAM
= TAMRA

Fig. S1

Figure S1. Location of the fluorophore-labeling positions used in this study. Donor labeling positions (green) are designated with a letter, and acceptor labeling positions (red) are designated with a number, while labels at the 5' ends of the two DNA strands are indicated as 5' up for the top strand and 5' bt for the bottom strand.

Fig. S2

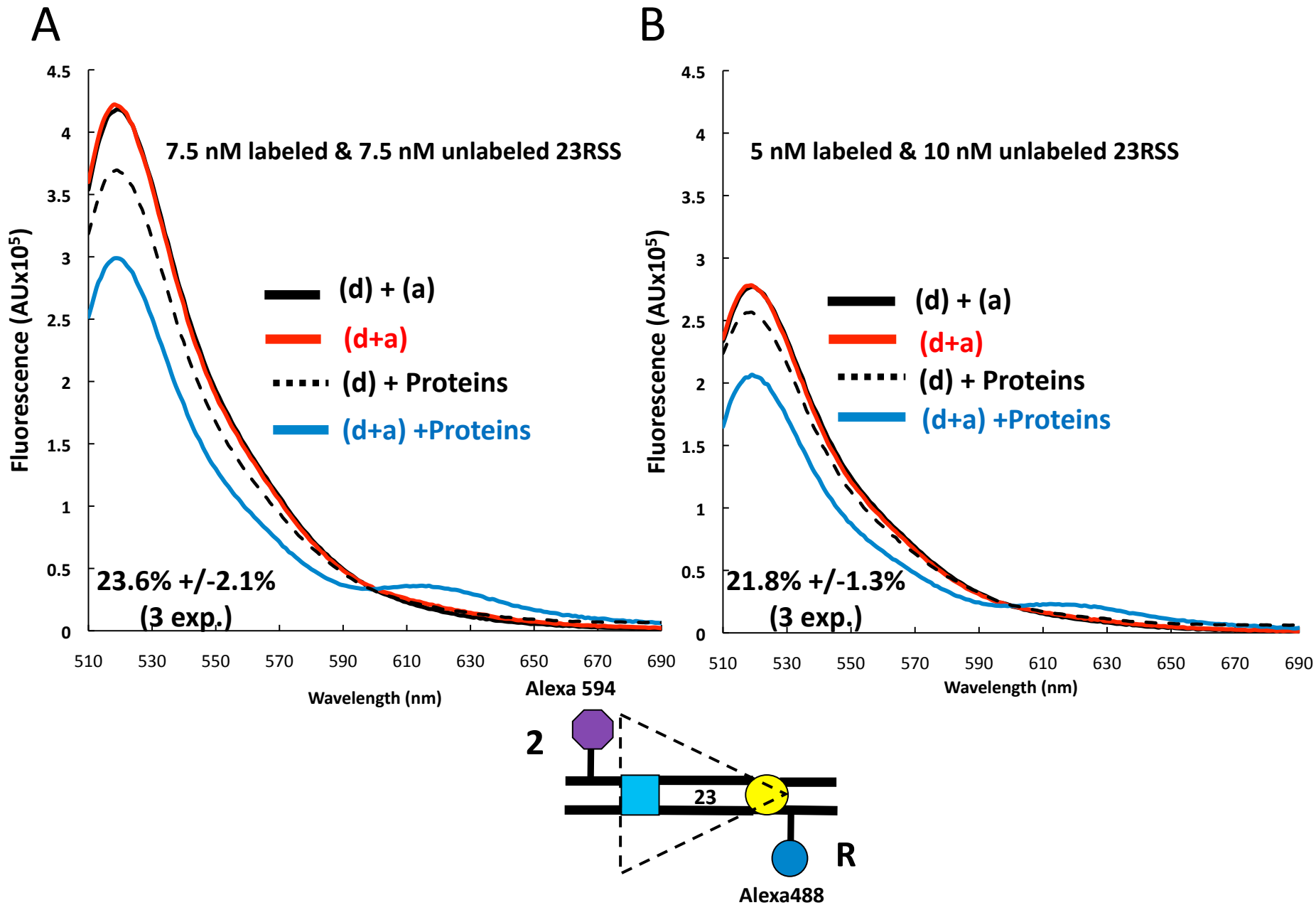
A
B
7.5 nM labeled & 7.5 nM unlabeled 23RSS
5 nM labeled & 10 nM unlabeled 23RSS
(d) + (a)
(d+a)
(d) + Proteins
(d+a) +Proteins
Fluorescence (AUx10⁵)
Wavelength (nm)
23.6% +/-2.1%
(3 exp.)
21.8% +/-1.3%
(3 exp.)
Alexa 594
2
23
R
Alexa488

Figure S2. Analysis of energy transfer when the $23RSS_dR2_a$ substrate is diluted with unlabeled 23RSS. FRET experiments were performed as in Fig. 2E (co-expressed MBP-RAG1c and MBP-RAG2c proteins) but instead of 15 nM $23RSS_dR2_a$ substrate as in Fig. 2E, 7.5 nM $23RSS_dR2_a$ substrate and 7.5 nM unlabeled 23RSS (A), or 5 nM $23RSS_dR2_a$ substrate and 10 nM unlabeled 23RSS (B) were used. Data are displayed as in Fig. 2E. Robust energy transfer is detected even after dilution of the labeled substrate, strongly arguing against a major contribution of energy transfer between different 23RSS molecules (in *trans*).

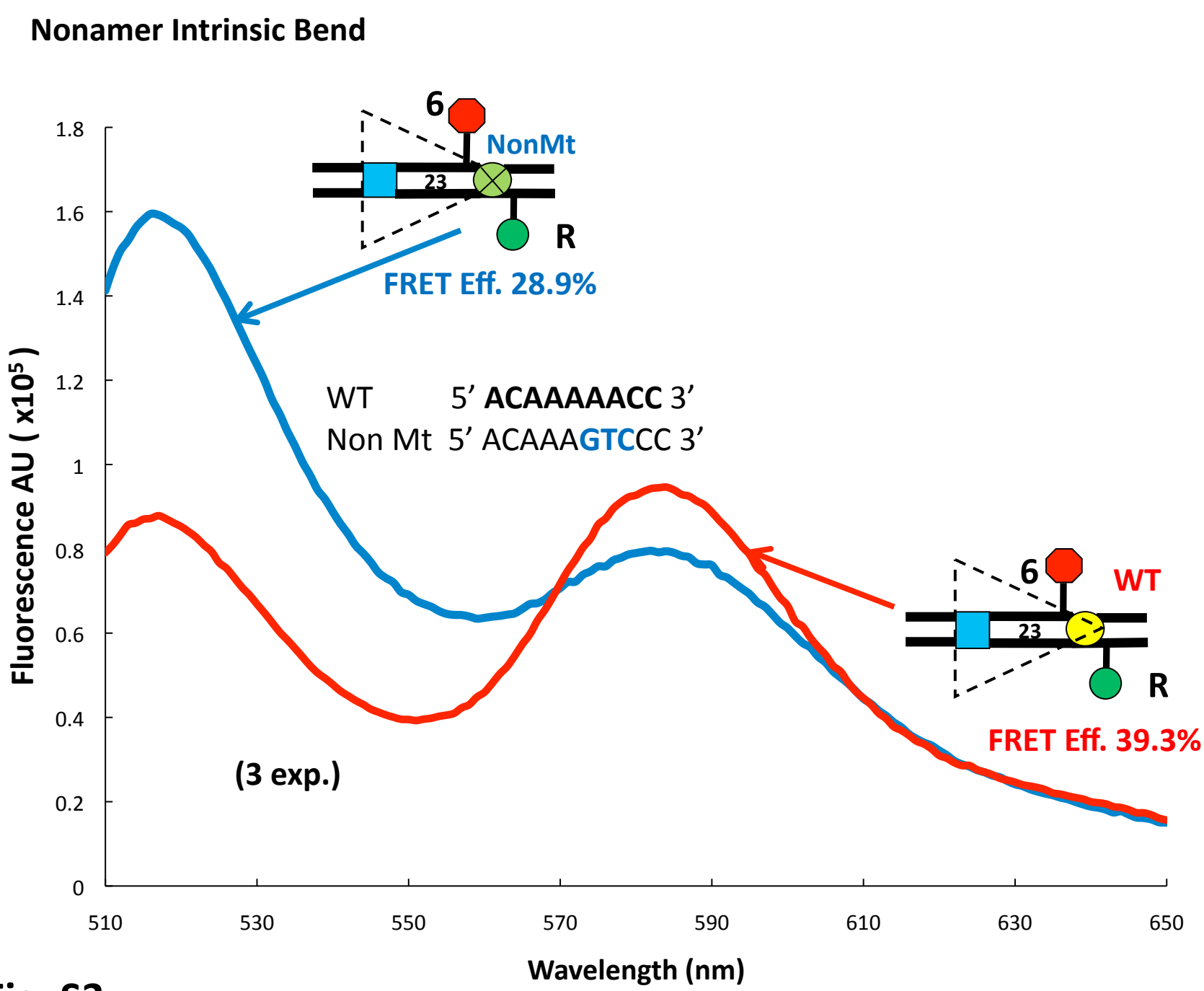
Nonamer Intrinsic Bend
6
NonMt
23
R
FRET Eff. 28.9%
WT 5' ACAAAAACC 3'
Non Mt 5' ACAAAGTCCC 3'
6
WT
23
R
FRET Eff. 39.3%
(3 exp.)
Fluorescence AU (x10^5)
Wavelength (nm)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
510
530
550
570
590
610
630
650

Fig. S3

Figure S3. Evidence for an intrinsic bend in the 23RSS nonamer. Emission spectra are shown for the $23RSS_dR6_a$ substrate (red) and the $23RSS_dR6_a$ NonMt substrate (blue) in which three bp in the A_5 tract of the nonamer have been mutated, as indicated. The fluorophores in this substrate are positioned so that the predicted intrinsic bend associated with an A_5 tract (36) would bring them closer together. The decrease in E-FRET observed in the $23RSS_dR6_a$ NonMt substrate as compared to the $23RSS_dR6_a$ substrate, while not statistically significant (P=0.25), is consistent with a loss of this intrinsic bend as a consequence of disrupting the A_5 tract.