Examples of Assignment of Diagonal *versus* Adjacent to LRET Distances

**a.** Position L40C (top of S1)

With *fluorescein*, $R_o=45\text{Å}$, the LRET measurement for L40C was $46\text{Å}$.

1) Assuming that $46\text{Å}$ is the diagonal distance, then the calculated adjacent distance would be $32.5\text{Å} \left( \frac{46}{\sqrt{2}} \right)$.

Using *ATTO-465*, $R_o=27\text{Å}$, to identify a short distance comparable to the calculated $32.5\text{Å}$ a distance of $28.3\text{Å}$ was detected.

2) Alternatively, assuming that $46\text{Å}$ is the adjacent distance, then the calculated diagonal distance is $65\text{Å} \left( 46 \times \sqrt{2} \right)$.

With *Rhodamine*, $R_o=60\text{Å}$, only a distance $< 65\text{Å}$ was obtained, $45.8\text{Å}$, that in addition confirmed the *fluorescein* measurement.

Since a distance $> 46\text{Å}$ was not obtained with *Rhodamine* and a shorter distance that coincides with the calculated distance under the first assumption was obtained with *ATTO-465*, then it was concluded that the distance measured, $46\text{Å}$, is the diagonal distance.

**Figure S4a.** Correa