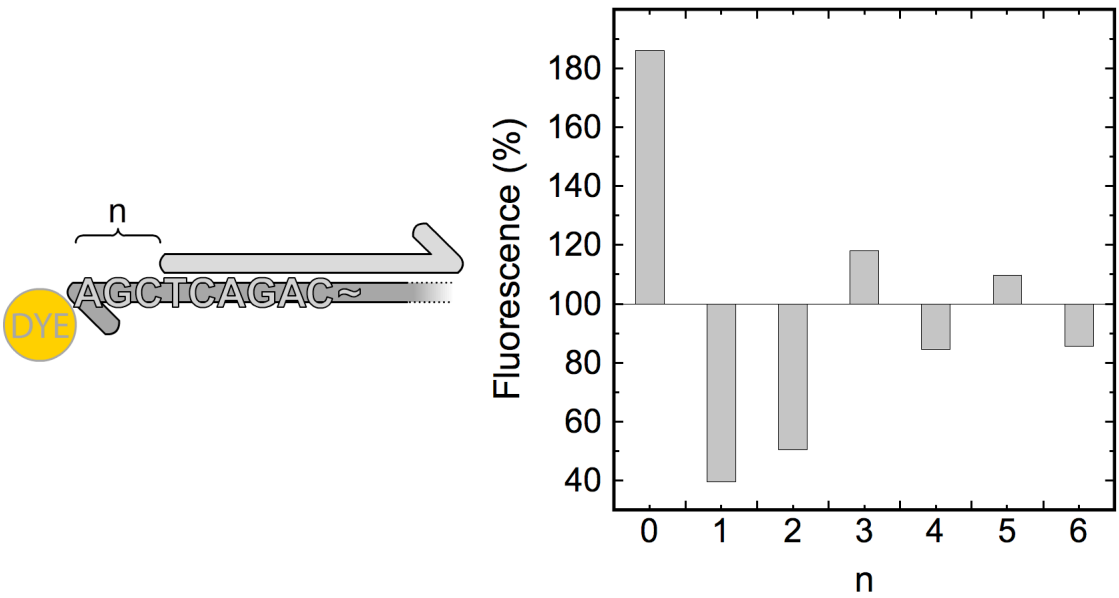
Supplementary Figure S1

On the experiment of Figure 3, we observed a pattern of alternating negative and positive changes of fluorescence as the signal molecule was shifted away from the template's 3' fluorophore. This pattern is consistent with the trend observed for signal oligonucleotides hybridizing next to the fluorophore (blunt end): decrease of fluorescence for a terminal C-G and increase of fluorescence for a terminal A-T base-pair. Therefore we may conclude that the unpaired bases in-between the dye and the closest base-pair have only a secondary effect on the quenching.

However, to check unambiguously this result, we performed another experiment with a different sequence. This was done using the same assay as the experiment of Figure 3, but with another signal oligonucleotide whose sequence displayed a different alternation of A-T and C-G bases. Supplementary Figure S1 shows the results of this experiment: the direction of the fluorescence intensity shifts is not regularly alternated anymore, but follows the pattern of A or T versus G or C in the sequence. As in Figure 3, the shift intensity gradually decreases as the distance increases.

This confirms that as the signal molecule is shifted away from the fluorophore, the fluorescence change upon hybridization still depends on the nature of the fluorophore's nearest base-pair: decrease of fluorescence for a terminal C-G and increase of fluorescence for a terminal A-T.



Supplementary Figure S1. Fluorescence intensity shift upon hybridization of a signal oligonucleotide moved from n=0 to n=6 bases away from the template 3'-terminal dye. Fluorescence intensity is expressed as a percentage of the fluorescence of the TAMRA-labeled template put alone in solution. The full sequence of the labeled template is 5'-TTACTCAGCCAAGACAACAGACTCGA-3', with a 3'-terminal TAMRA NHS ester modification.