Supplementary Information for the manuscript:

Intramolecular photostabilization via triplet-state quenching: design principles to make organic fluorophores "self-healing"

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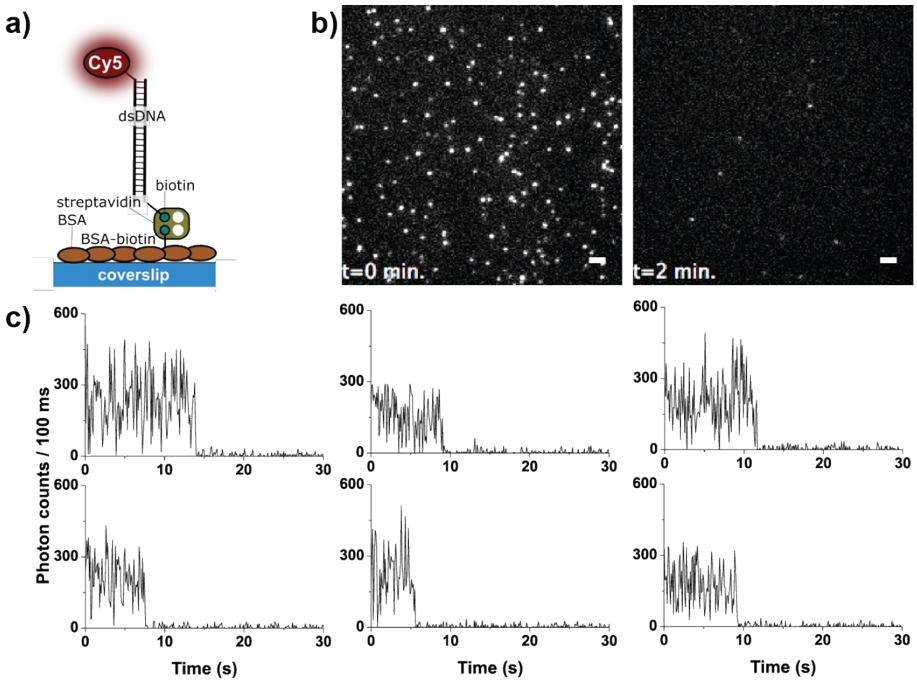
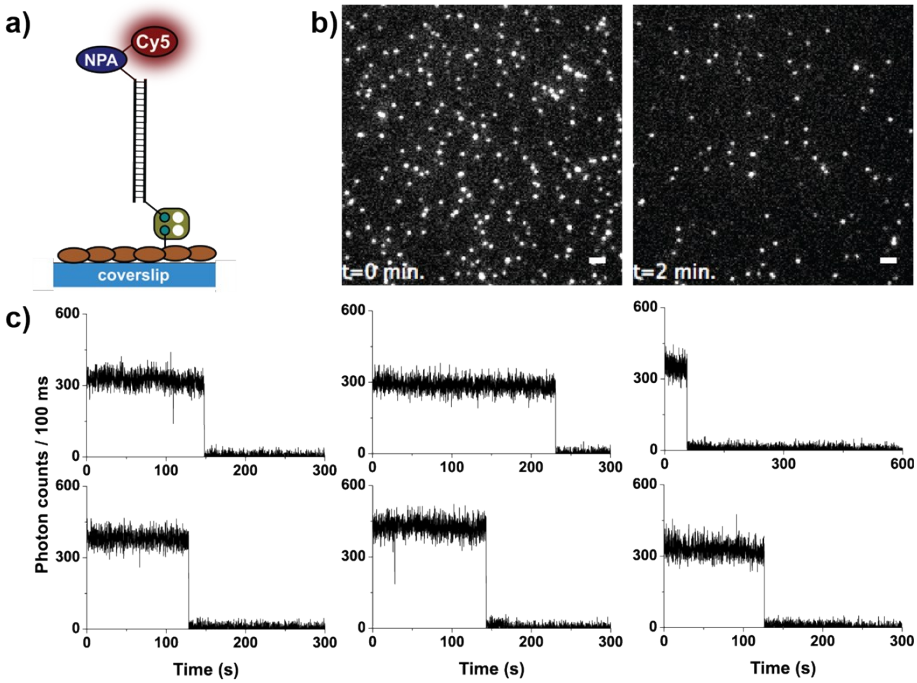
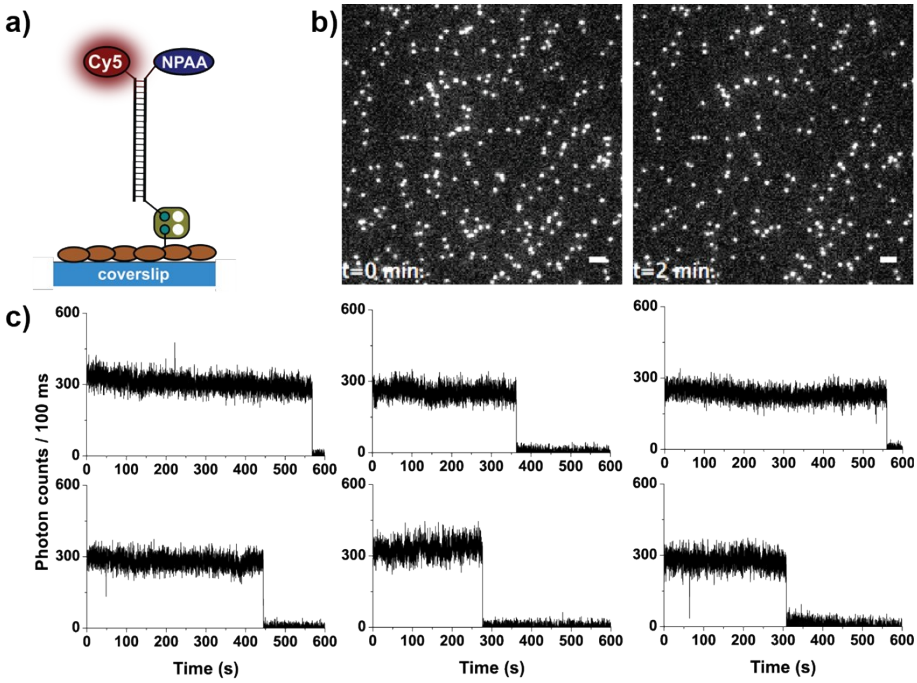


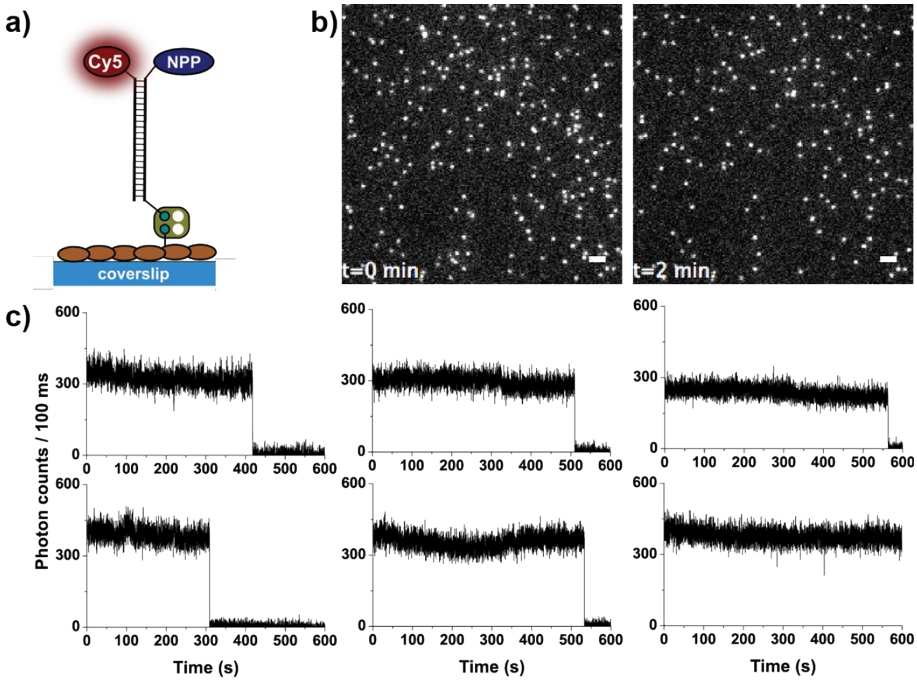
Supplementary Figure S1. Details of photophysical characterization of Cy5. (**a**) Schematic view of the photostabilizer-dye conjugate on DNA and the experimental strategy for surface immobilization. (**b**) Representative TIRF images of immobilized Cy5 molecules at two different time points (0 and 2 min, as given) after starting illumination and recording (scale bar 2 µm). (**c**) Representative fluorescence time traces of single emitters from TIRF recordings, Excitation intensities were 50 W/cm^2 (excitation at 640 nm, detection with ET700/75). Brightness and contrast settings were 3800 (low) to 9800 (high).



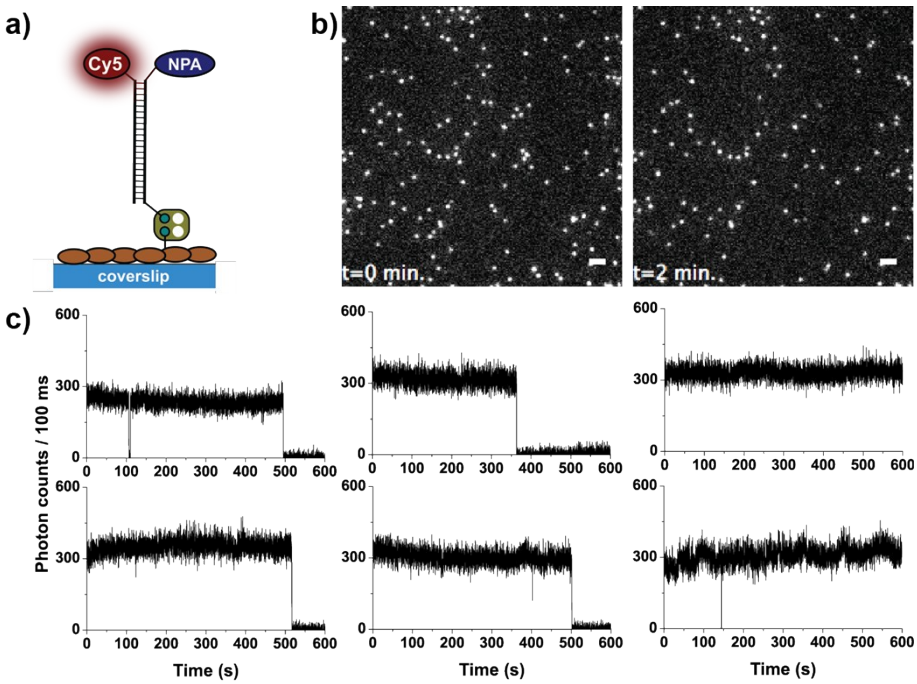
Supplementary Figure S2. Details of photophysical characterization of NPA-Cy5. (**a**) Schematic view of the photostabilizer-dye conjugate on DNA and the experimental strategy for surface immobilization. (**b**) Representative TIRF images of immobilized NPA-Cy5 molecules at two different time points (0 and 2 min, as given) after starting illumination and recording (scale bar 2 µm). (**c**) Representative fluorescence time traces of single emitters from TIRF recordings, Excitation intensities were 50 W/cm^2 (excitation at 640 nm, detection with ET700/75). Brightness and contrast settings were 3800 (low) to 9800 (high).



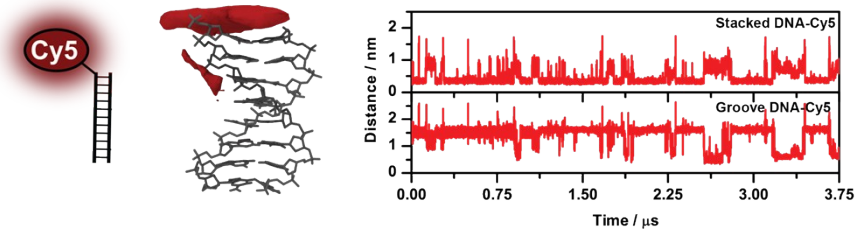
Supplementary Figure S3. Details of photophysical characterization of Cy5-NPAA. (**a**) Schematic view of the photostabilizer-dye conjugate on DNA and the experimental strategy for surface immobilization. (**b**) Representative TIRF images of immobilized Cy5-NPAA molecules at two different time points (0 and 2 min, as given) after starting illumination and recording (scale bar 2 µm). (**c**) Representative fluorescence time traces of single emitters from TIRF recordings, Excitation intensities were 50 W/cm^2 (excitation at 640 nm, detection with ET700/75). Brightness and contrast settings were3800 (low) to 9800 (high).



Supplementary Figure S4. Details of photophysical characterization of Cy5-NPP. (**a**) Schematic view of the photostabilizer-dye conjugate on DNA and the experimental strategy for surface immobilization. (**b**) Representative TIRF images of immobilized Cy5-NPP molecules at two different time points (0 and 2 min, as given) after starting illumination and recording (scale bar 2 µm). (**c**) Representative fluorescence time traces of single emitters from TIRF recordings, Excitation intensities were 50 W/cm^2 (excitation at 640 nm, detection with ET700/75). Brightness and contrast settings were 3800 (low) to 9800 (high).



Supplementary Figure S5. Details of photophysical characterization of Cy5-NPA. (**a**) Schematic view of the photostabilizer-dye conjugate on DNA and the experimental strategy for surface immobilization. (**b**) Representative TIRF images of immobilized Cy5-NPA molecules at two different time points (0 and 2 min, as given) after starting illumination and recording (scale bar 2 µm). (**c**) Representative fluorescence time traces of single emitters from TIRF recordings, Excitation intensities were 50 W/cm^2 (excitation at 640 nm, detection with ET700/75). Brightness and contrast settings were 3800 (low) to 9800 (high).



Supplementary Figure S6. Additional results from MD simulations showing the terminal attachment of Cy5 (left), its most probable location on DNA (middle) and the distance of the fluorophore to the top and groove of the DNA.

	Cy5-NPAA	**NPA-Cy5**	**NPA-4PEG-Cy5**
Collision frequency (ns^{-1})	0.09 ± 0.01	0.02 ± 0.01	0.10 ± 0.04
Bound/unbound ratio	1.4 ± 0.3	12 ± 11	5 ± 4
Mean contact time (ns)	6 ± 1	40 ± 20	11 ± 8
Median contact time (ns)	2.3 ± 0.2	2 ± 1	0.8 ± 0.1

Supplementary Table S1. Interactions of Cy5 and the photostabilizer during simulations for Cy5-NPAA, NPA-Cy5 and NPA-PEG4-Cy5: collision frequencies, bound/unbound ratio, the mean and median contact times for each system.