

Supporting information for the manuscript:

**Mechanisms and advancement of antifading agents for fluorescence microscopy and
single-molecule spectroscopy**

Thorben Cordes^{1,2}, Andreas Maiser³, Christian Steinhauer¹, Lothar Schermelleh^{3*}, and Philip
Tinnefeld^{1,4*}*

¹ Applied Physics – Biophysics & Center for NanoScience (CeNS), Ludwig Maximilian University of
Munich, Amalienstr. 54, 80799 Munich, Germany

² Biological Physics Research Group, Department of Physics, University of Oxford, Clarendon
Laboratory, Parks Road, Oxford OX1 3PU, United Kingdom

³ LMU Biocenter, Department of Biology, Ludwig Maximilian University of Munich, Grosshaderner
Str. 2, 82152 Planegg-Martinsried, Germany

⁴ NanoBioSciences, Institute of Physical and Theoretical Chemistry, TU Braunschweig, Hans-Sommer-
Str. 10, 38106 Braunschweig, Germany

*corresponding authors: p.tinnefeld@tu-braunschweig.de, lothar.schermelleh@lmu.de,
thorben.cordes@physik.lmu.de, Fax: +49 531 391 5334

ADDITIONAL EXPERIMENTAL DATA AND RESULTS

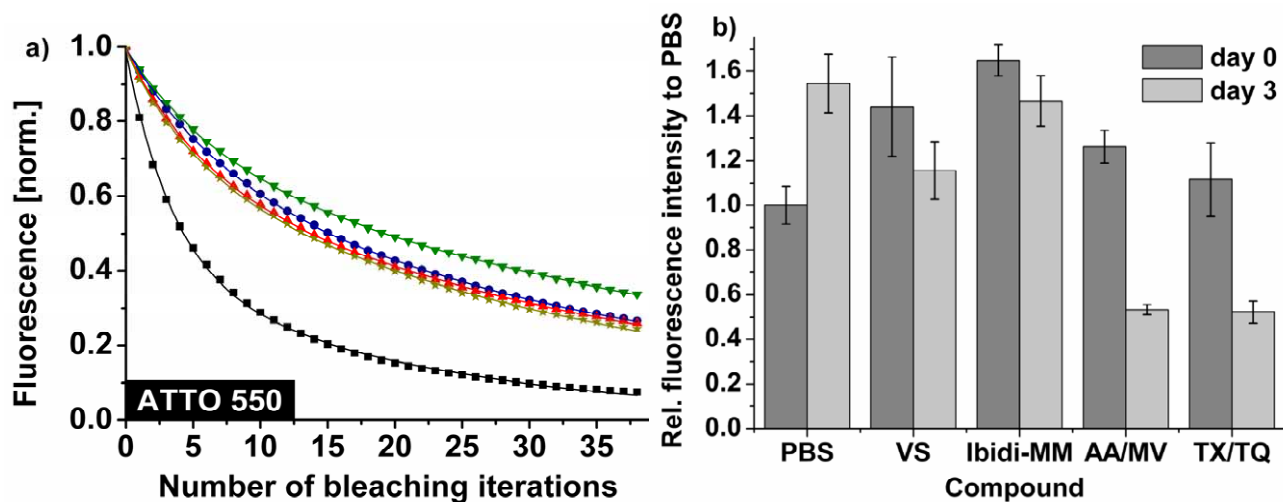


Figure S1 Results from photobleaching experiments of the dye ATTO 550. a) Time-course of the normalized fluorescence intensity over ≈ 35 bleaching cycles of different buffer conditions: PBS, squares; VS, circles; Ibidi-MM, stars; ROXS (AA/MV), triangles; ROXS (TX/TQ), inverted triangles. Bi-exponential fit curves are shown as solid lines in the color of the respective data set. Fit results were summarized in Table S3. b) Relative fluorescence intensity for the different buffer conditions before photobleaching and for aged samples (day 0 = fresh sample; day 3 = sample aged for three days).

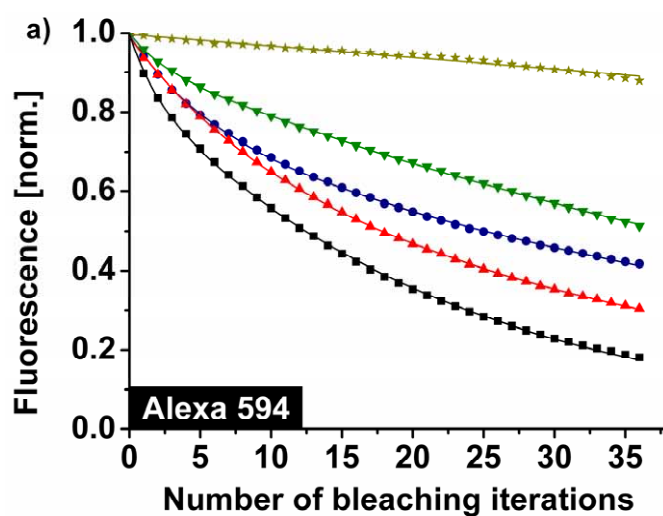


Figure S2 Results from photobleaching experiments of the dye Alexa 594. a) Time-course of the normalized fluorescence intensity over ≈ 35 bleaching cycles of different buffer conditions: PBS, squares; VS, circles; Ibidi-MM, stars; ROXS (AA/MV), triangles; ROXS (TX/TQ), inverted triangles. Bi-exponential fit curves are shown as solid lines in the color of the respective data set. Fit results were summarized in Table S1.

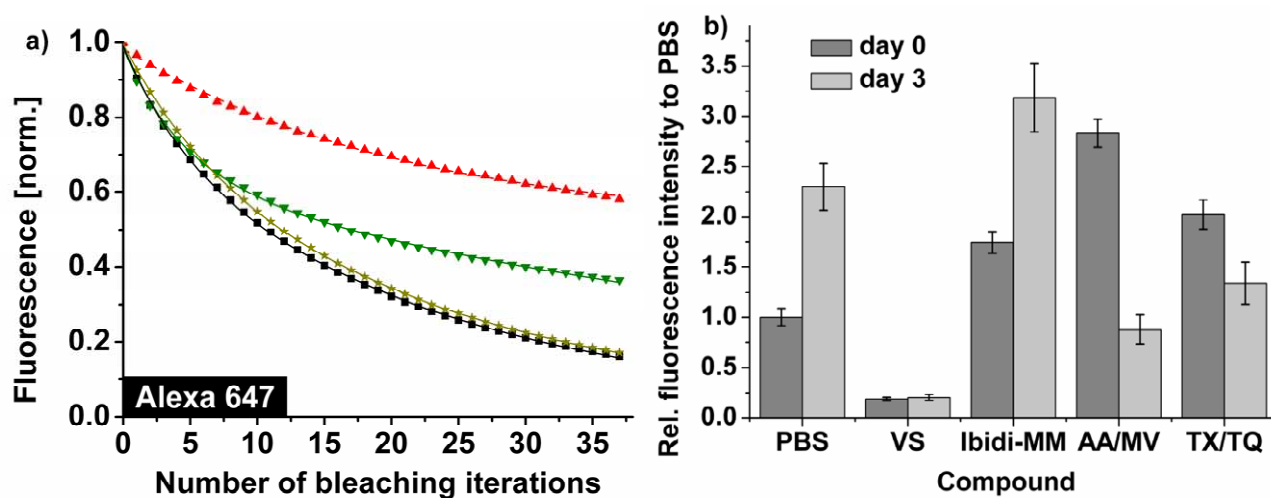


Figure S3 Results from photobleaching experiments of the dye Alexa 647. a) Time-course of the normalized fluorescence intensity over ≈ 35 bleaching cycles for the different buffer conditions: PBS, squares; Ibidi-MM, stars; ROXS (AA/MV), triangles; ROXS (TX/TQ), inverted triangles. Bi-exponential fits are shown as solid lines in the color of the respective data set. Fit results were summarized in Table S1. b) Relative fluorescence intensity for the different buffer conditions before photobleaching and for aged samples (day 0 = fresh sample; day 3 = sample aged for three days). Error bars indicate the standard error of mean intensities.

Relative " τ_m "	PBS	VS	Ibidi-MM	AA/MV	TX/TQ
Alexa 488	1.0 ± 0.4	2.9 ± 0.4	1.3 ± 0.2	1.1 ± 0.1	1.8 ± 0.2
Cy3B	1.0 ± 0.1	1.9 ± 0.4	1.8 ± 0.1	1.6 ± 0.1	2.1 ± 0.6
ATTO 550	1.0 ± 0.1	2.5 ± 0.3	1.2 ± 0.1	2.6 ± 0.1	3.2 ± 0.4
Alexa 594	1.0 ± 0.2	2.5 ± 0.3	n.a.	1.5 ± 0.1	2.9 ± 0.1
Alexa 647	1.0 ± 0.3	n.a.	1.1 ± 0.1	2.9 ± 1.3	3.0 ± 1.1
ATTO 647N	1.0 ± 0.1	1.3 ± 0.6	2.3 ± 0.2	2.9 ± 0.5	3.1 ± 0.5

Table 1 Relative bleaching behavior of the different dyes obtained by fitting the data with bi-exponential decay function (details see *Materials and Methods*). The bleaching was characterized by a mean bleaching constant $\tau_m = A_1 \times \tau_1 + A_2 \times \tau_2$. Relative values with respect to PBS are given in the table. Absolute values for PBS in number of bleaching cycles are given in the main text. n.a. = non applicable due to a non-exponential behavior.