Electronic Supplementary Material (ESI) for Photochemical & Photobiological Sciences

Supporting Information

Photoinduced formation of reversible dye radicals and their impact on super-resolution imaging

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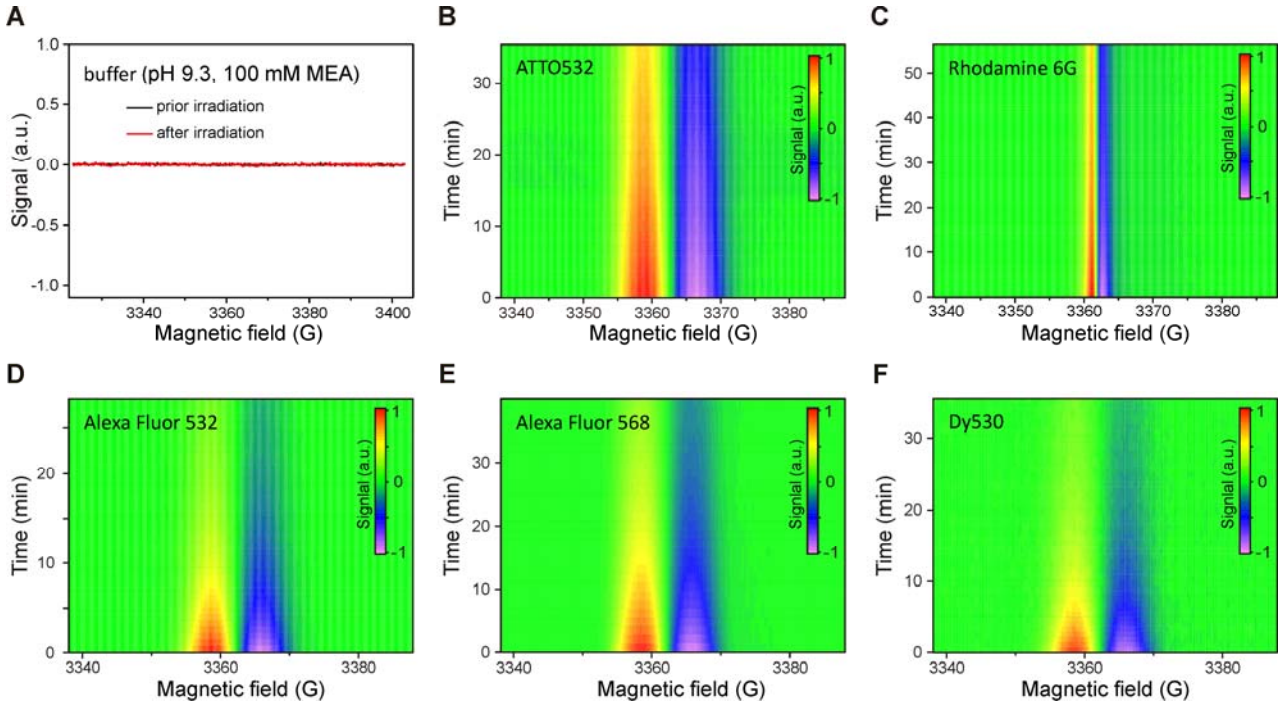

Figure S1. Time-dependent EPR signal of different rhodamine dye radicals. Prior to EPR measurements the non-sealed capillary tubes were irradiated with laser light of appropriate wavelength. (*A*) As control experiment the reaction buffer containing 100 mM MEA pH 9.3, was irradiated at 532 nm. Prior and after irradiation no signal is observed applying the same settings as in (*B*)**.** (*B*) EPR signal of the ATTO 532 radical recorded with time (100 mM MEA, pH 9.3), the lifetime was determined to 35.7 min. (*C*) EPR signal of rhodamine 6G, 200 mM MEA, pH 10.2; the lifetime was determined to 23.4 min. (*D*) Alexa Fluor 532, 200 mM MEA, pH 10.2, 8.7 min lifetime, (*E*) Alexa Fluor 568, 200 mM MEA, pH 10.2, 14.6 min lifetime, and (*F*) Dy 530, 100 mM MEA, pH 9.3, 9.6 min lifetime.

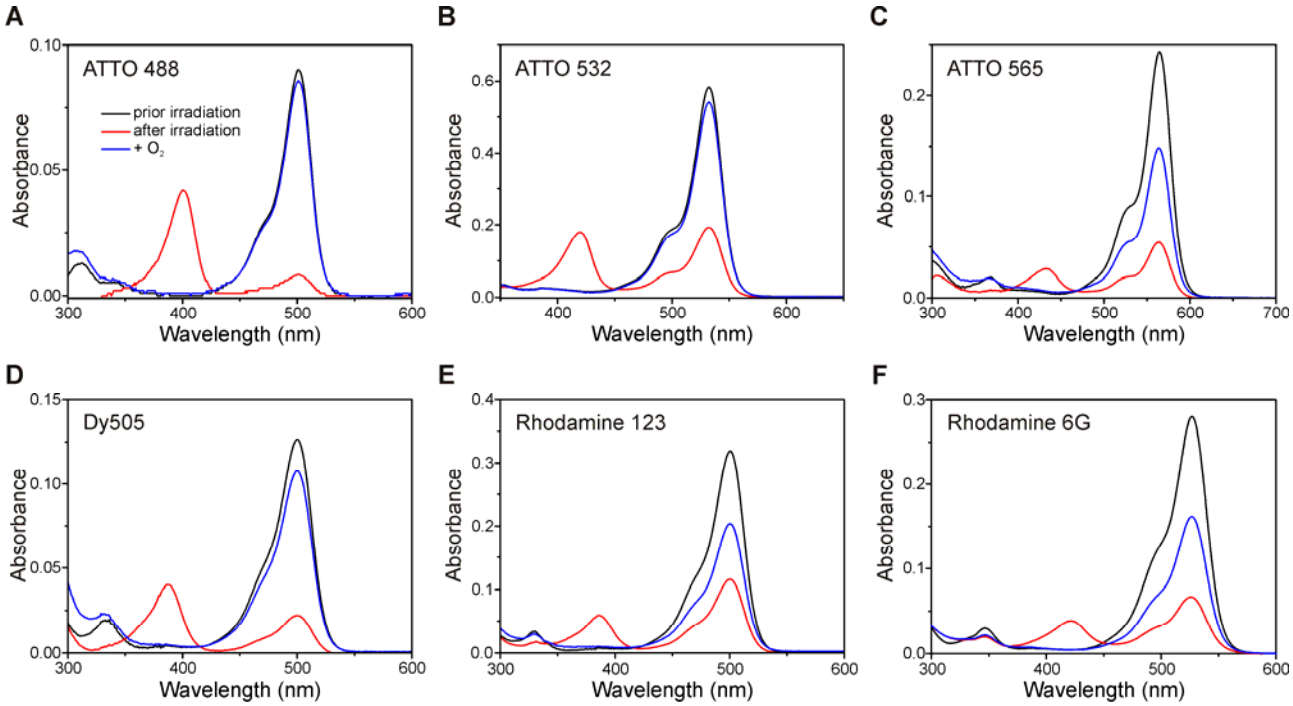

Figure S2. Absorbance spectra of different rhodamine dyes and their radical forms after irradiation. Radical anions of rhodamine dyes show absorbance maxima between 380 and 430 nm. Upon shaking the cuvette, the radical is oxidized accompanied by a change in the absorbance spectrum. The extinction coefficients of the radical anions of most rhodamines investigated were determined from the spectra shown and range between 30.000 – 50.000 L mol^{-1} cm^{-1} (Table 1). (*A*) ATTO 488, 100 mM MEA, pH 9.3; (*B*) ATTO 532, 100 mM MEA, pH 9.3; (*C*) ATTO 565, 200 mM MEA, pH 12.1; (*D*) Dy 505, 100 mM MEA, pH 9.3; (*E*) rhodamine 123, 100 mM MEA, pH 9.3; (*F*) rhodamine 6G, 200 mM MEA, pH 10.2. It has to be pointed out that for some rhodamine dyes photoswitching is not completely reversible most probably due to irreversible follow-up reactions.

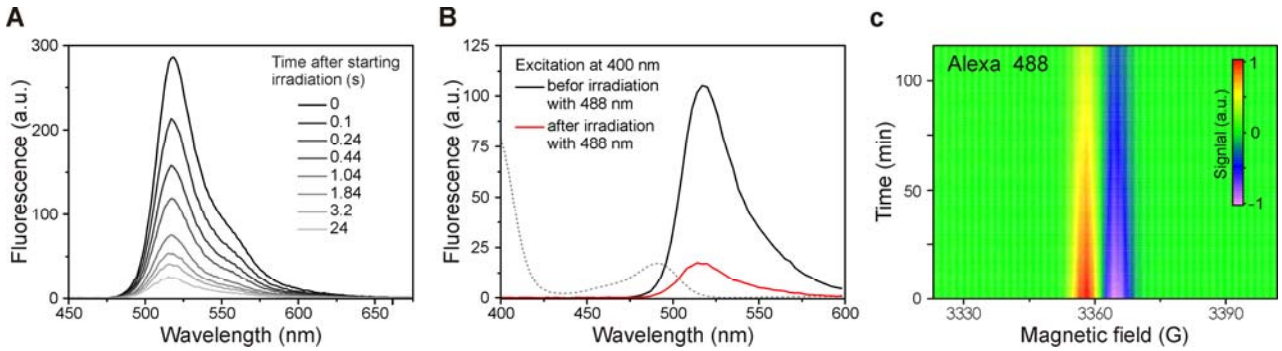

Figure S3. Photoswitching of Alexa Fluor 488. (*A*) Fluorescence spectra recorded in 100 mM MEA, pH 9.3 with proceeding irradiation at 488 nm. (*B*) Absorption and emission spectra. The dark state, i.e. the radical anion, does not show any fluorescence upon excitation at 400 nm. The absorption spectrum after irradiation is shown in dashed lines. (*C*) Time-dependent EPR signal of Alexa Fluor 488 dye radical of a 10^{-4} M aqueous solution in the presence of 100 mM MEA, pH 9.3 after irradiation at 488 nm.

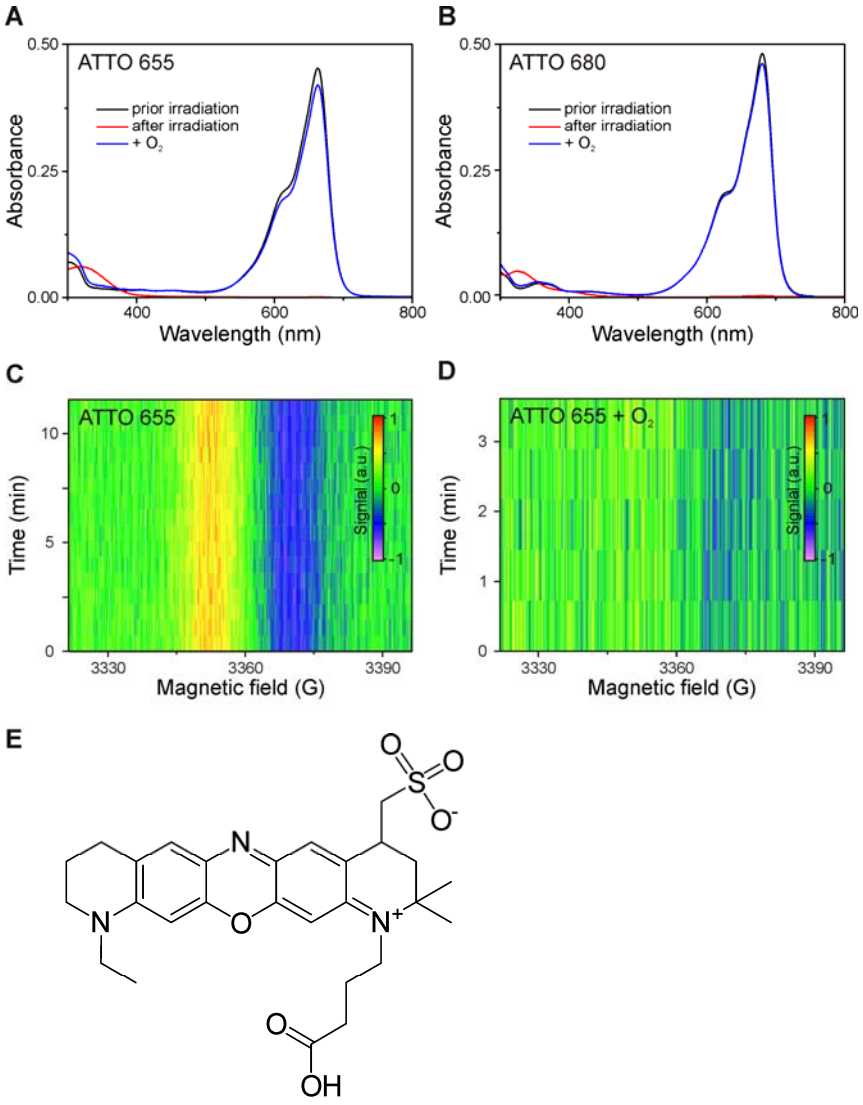

Figure S4. Photoreduction of oxazine dyes by thiols. (*A*) ATTO 655 and (*B*) ATTO 680 can be switched off very efficiently upon irradiation at 647 nm (100 mM MEA, pH 9.3). Upon agitating fresh oxygen is dissolved from the headspace of the cuvette and restores the colored form. Similar to MB, both oxazines do not form a stable radical anion absorbing around 400 nm. (*C*) An EPR signal of the radical anion of ATTO 655 can be measured when the concentration ratio [MEA]/[ATTO 655] is adjusted to 10 using 5 mM MEA, pH 7.4 and 0.5 mM ATTO 655. In these experiments the thiol concentration is too low to efficiently reduce the dye to the leuco form. (*D*) Upon purging the solution with air, the radical signal disappears. (*E*) Molecular structure of ATTO 655 adopted from Sigma-Aldrich. Due to the high dye concentration used to generate a sufficient radical anion concentration the absorption spectroscopic characterization of the radical anion was abandoned.

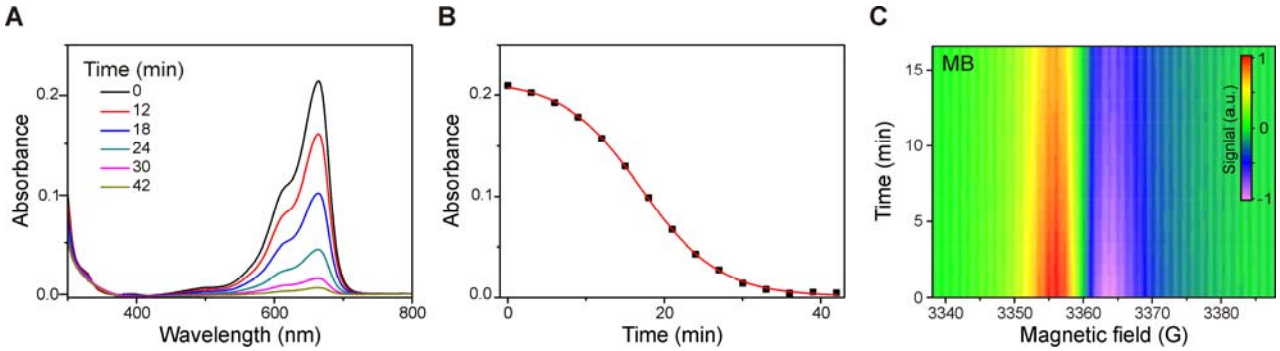

Figure S5. Photoreduction of thiazine dyes by thiols. (*A*) Absorbance spectrum of MB recorded with time (30 mM MEA, pH 9.3). As irradiation source the readout light of the absorbance spectrometer was used. As expected no radical anion around 400 nm was formed due to the formation of the leuco-dye (*B*) Time response of photoreduction as measured in the absorption spectrometer. (*C*) EPR time trace of the radical form of MB; Decreasing the thiol concentration to 5 mM MEA, pH 7.4 and increasing the dye concentration to 30 mM MB, the dye is trapped in its radical form and can be detected using EPR. Due to the high dye concentration used to generate a sufficient radical anion concentration the absorption spectroscopic characterization of the radical anion was abandoned.

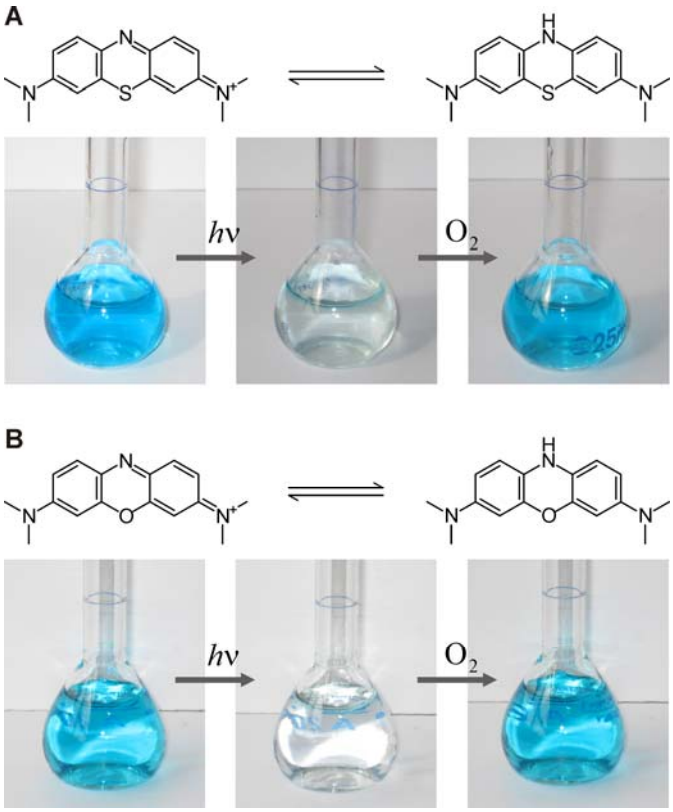

Figure S6. The photoinduced blue-bottle experiment. Thiazine (*A*) and oxazine dyes (*B*), e.g. MB and ATTO 655 show reversible photoswitching in aqueous solvent in the presence of 10 mM thiols at pH 7.0, and 100 mM thiols at pH 9.3, respectively, under sunlight (for the oxazine dyes only the basic molecular structure is given to point out similarities). The non-colored leuco-dye is formed upon illumination, and the colored form can be recovered efficiently by oxygen from the headspace after shaking of the reaction chamber. The reaction mechanism is highly reversible and the rate constant for production of the leuco-dye depends on the excitation intensity, the thiol concentration, and the pH of the solvent. Since most thiols (RSH) have a $pK_{a,SH}$ of 8-9 and the reducing species is the thiolate anion (RS^-), the reduction efficiency of compounds carrying one thiol group increases linearly with pH and saturates at pH >9 with all relevant functional groups ionized. Due to the slightly lower electron affinity of ATTO 655 as compared to MB, the pH value of the solvent has to be increased to 9.3 to enable photoswitching under sunlight.