

First-come first-serve – Competition for triple-state interactions in organic fluorophore and consequences for photostabilization

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Through recent developments in fluorescence microscopy both the achievable temporal and spatial resolution are now mostly limited by the employed fluorophores rather than the technical specifications of the microscope itself. An optimization of fluorescent output is traditionally achieved through the addition of empirically derived buffer cocktails. This ‘intermolecular photostabilization’ is still the most effective approach to reduce photobleaching of organic fluorophores. The approach uses collision-mediated quenching of reactive fluorophore states (e.g., the triplet state or radicals) to prevent photobleaching. In recent publications from us and others [1,2], intramolecular photostabilization has been revived as an alternative approach to impart synthetic organic fluorophores with ‘self-healing’ properties. Although this method does not suffer from the intrinsic drawbacks of buffer additives, the resulting photostabilization efficiency is not fully competitive with established solution-based methods.

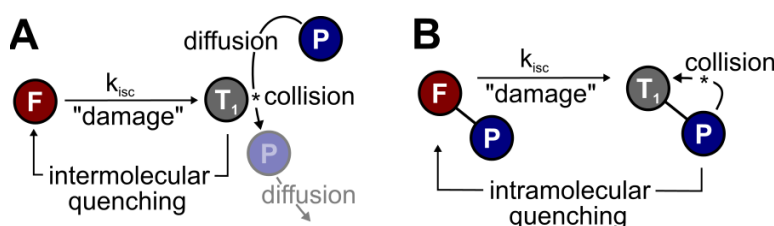


Figure 1. Methods to create collision between photostabilizer and reactive fluorophore state (here triplet T_1) using intermolecular diffusional quenching (A) and intramolecular processes (B). In both cases the long-lived reactive triplet state collides with the photostabilizer and the fluorophore is restored in its singlet ground state.

In this contribution, we seek to answer the question whether the photostabilization efficiency can be improved by a combination of both approaches. We find, however, that inter- and intramolecular processes compete with each other rather than being additive. In most cases intramolecular processes outcompete the effects of solution additives, except for solution-based quenching of fluorophores via molecular oxygen. In a “first-come first-serve” manner, only one of two components turns out to be relevant for photostabilization. This

principle is demonstrated for combinations of covalently-linked and solution-based photostabilizers (NPA, TX, COT), a covalently linked photostabilizer and a solution-based darkening agent (TCEP, MEA) or two covalently linked photostabilizers.

Our studies reveal the relevance of direct competition of triplet-state quenchers for interactions with the fluorophore. These make clear that photostabilizer-dye conjugates have only limited usability for super-resolution microscopy and are actually incompatible with STORM-type imaging. The results also suggest that photostabilizer-dye conjugates strongly suppress reversible triplet-based photochemistry via TCEP or MEA (“caging”), which often leads to an overestimation of photostabilization effects especially in cyanine dyes. Finally, our findings allow to speculate that the biochemical environment, e.g., proximity of reduction-competent amino-acids or nucleic acids to fluorophores, might reduce the photostabilization efficiency of buffer cocktails via similar means.

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References:

- [1] R.B. Altman, D.S. Therry, Z. Zhou et al, *Nature Methods* 9, 68–71 (2012)
- [2] J.H.M. van der Velde, J. Oelerich, J. Huang et al, *Nature Communications*, 7, 10144 (2016)