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'Self-healing' dyes: intramolecular stabilization of organic fluorophores

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Response to NMETH-C14399:

A battery of new mechanistic investigations will be required to provide a full explanation of the observation that individual, small-molecule compounds, referred to here as 'protective agents', have the capacity to promote dramatic enhancements to overall fluorophore performance¹. As Tinnefeld and Cordes suggest, cycles of reduction (red) and oxidation (ox) - and/or the reverse - may confer a 'self-healing' property to the fluorogenic center. This could indeed be achieved by a single neighboring molecule, like Trolox, facilitating the fluorophore's rapid return to the ground state from relatively long-lived, non-fluorescent, radical intermediates through 'ping-pong' red-ox chemistry. However, this model requires that the rates of red-ox cycling be properly matched for benefits to be achieved. Further insights into the prevalence and lifetimes of charged intermediates for each molecule will need to be quantified to validate this model.

Alternative mechanisms for photostabilization, which may play a significant or even dominant role, will also need to be carefully considered and quantified. Previous investigations, largely motivated by the once vigorous dye-laser industry, suggest that meaningful contributions to fluorophore performance may arise from changes in the rates of internal conversion and/or the introduction of mechanisms for triplet-triplet energy transfer and exciplex-type relaxation pathways². Triplet-triplet energy transfer, a mechanism that would also fall into the 'self-healing' category, could provide substantial benefits to fluorophore performance by reducing the lifetime of non-fluorescent triplet states from which photobleaching and/or radical states can occur. Given our estimates of the triplet energy and red-ox potential of cyclooctatetraene (unpublished data), this mechanism may potentially be the dominant pathway of cyanine fluorophore photostabilization. Triplet-triplet energy transfer can be differentiated from red-ox-type chemistries by quantifying changes in triplet state lifetimes and charged, intermediate species. Other pathways may also exist that are distinct from the 'self-healing' variety. For example, a 'self-protecting' mechanism that reduces the probability of transitions into "damaged" states in the first place would also give rise to enhancements in fluorophore performance.

Single-molecule imaging efforts can play an important role in the characterization of fluorophore performance by giving direct access to the frequency and duration of fluorescent and non-fluorescent states as well as a fluorophore's total photon output prior to blinking or photobleaching. However, a quantitative understanding of the distinct mechanisms for enhancing fluorophore performance can only be delineated with confidence

when married with bulk electrochemical and spectroscopic investigations. These combined approaches, while requiring large quantities of material, could provide grounded insights into the impact and relative weighting of 'self-healing' and 'self-protecting' mechanisms for distinct fluorophore types. Progress on this front will enable both further improvements in the performances of known protective agents and the search for new compounds with similar or improved properties. Developments of this kind may ultimately facilitate the design and synthesis of new classes of next-generation fluorophores spanning the visible spectrum that exhibit enhancements in performance even greater than those observed for the cyanine class (see Correspondence by Altman et al) and tailored properties for distinct experimental demands.

REFERENCES

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2. Turro, NJ.; Ramamurthy, V.; Scaiano, JC. *Modern Molecular Photochemistry of Organic Molecules*. University Science Books; 2010.