

Journal Club – Optics

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Self-Healing Dyes—Keeping the Promise?

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What made me pick this paper

- Getting a summary of current state of self-healing dyes
 - “Perspective”: not exactly a review, but also not much new data
- Getting some (probably biased) opinion on the potential

Scope/Claim of the paper

- Latest applications
- Remaining limitations
 - lower photostabilization of most self-healing dyes when compared to solution additives
 - limited mechanistic understanding on the influence of the biochemical environment and molecular oxygen
 - the lack of cheap and facile bioconjugation strategies
- Future advances
 - new data on redox blinking caused by double-stranded DNA
 - forthcoming work on intramolecular photostabilization of fluorescent proteins

Self-healing dyes

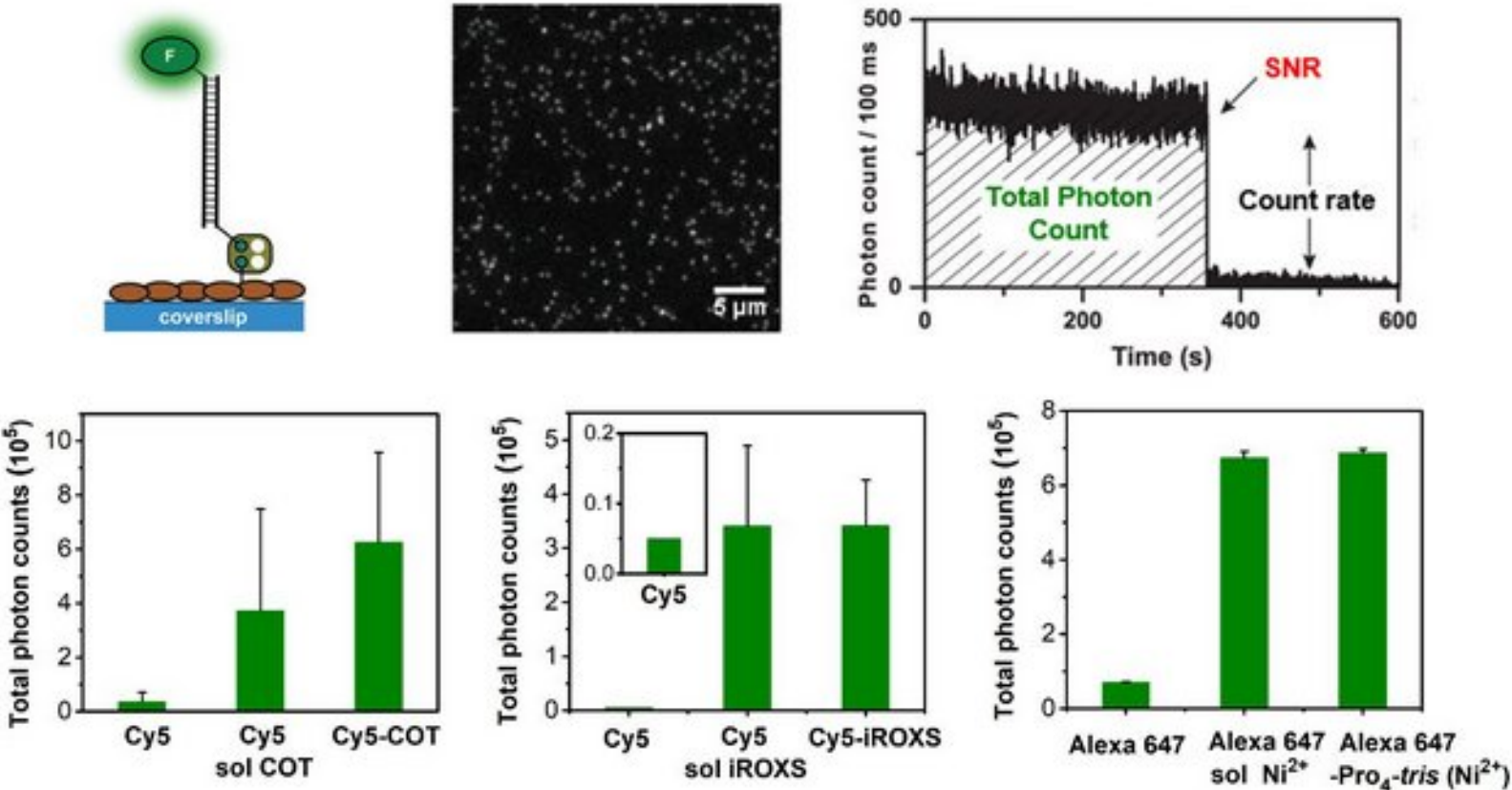
- No need for complex buffer systems
- Works when dyes are inaccessible to solution-based stabilizers
- Triplet-state quenching in the early 1980s for dye lasers
- Blanchard in 2012, Cordes in 2013, and others

Intro

- Photophysical triplet-state quenching
 - energy transfer between the fluorescent dye (donor) and the quencher (acceptor)
 - cyclooctatetraene (COT), diphenylhexatriene, or Ni^{2+} ions
- Photochemical triplet-state quenching
 - combination of redox-active agents
 - Trolox (TX), ascorbic acid (AA), ferrocene, nitrobenzylalcohol (NBA), methylviologen (MV), Trolox-quinone, ...

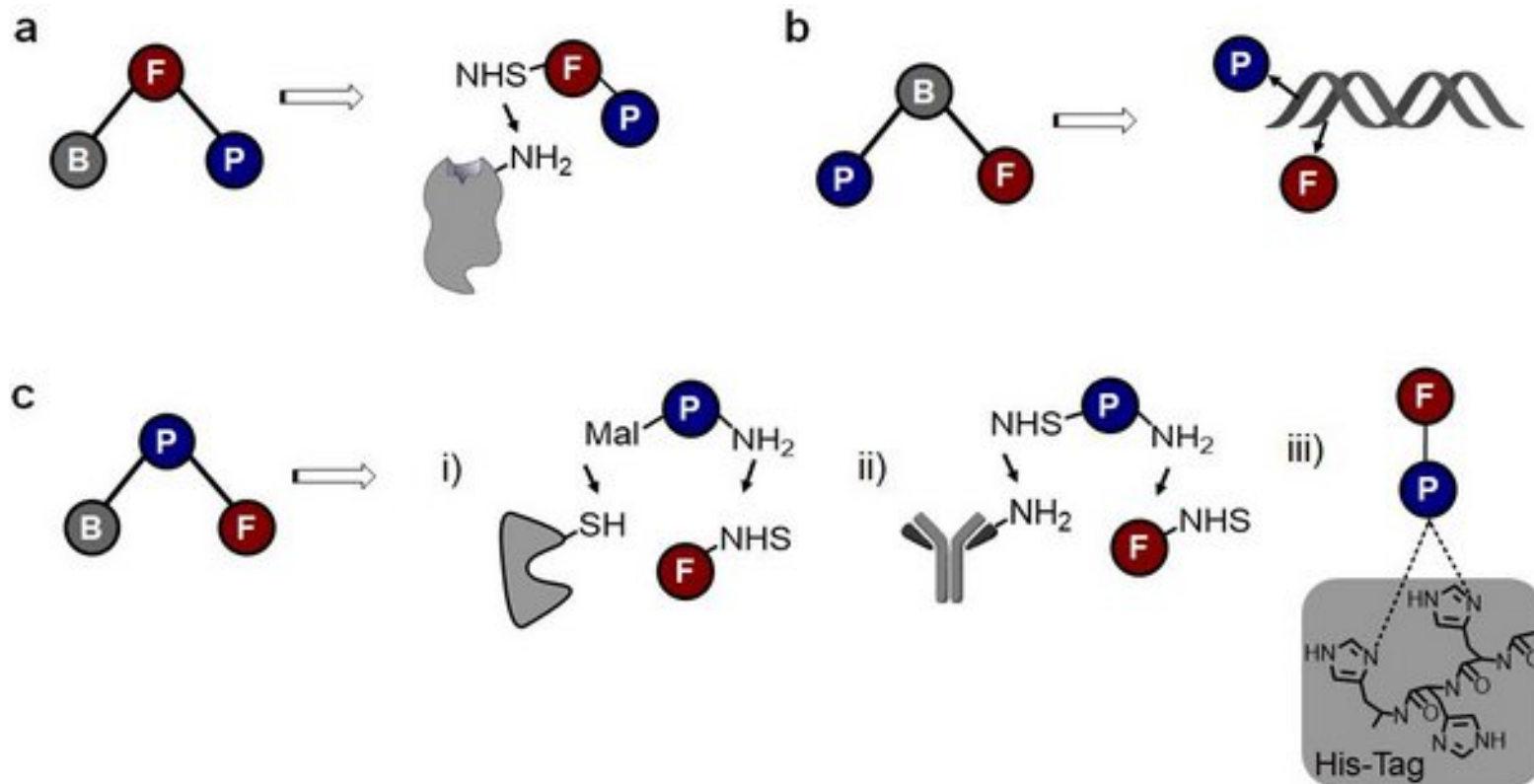
Performance of dye-stabilization

- Works with a variety of dyes
- Cy5-COT “is rather the exception than what is commonly seen with most self-healing dyes”



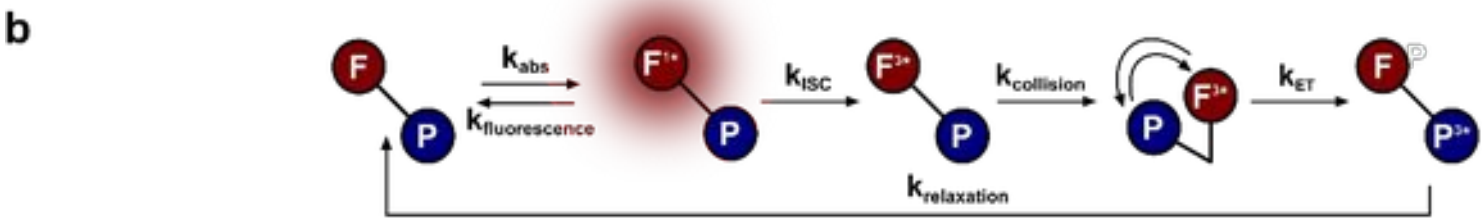
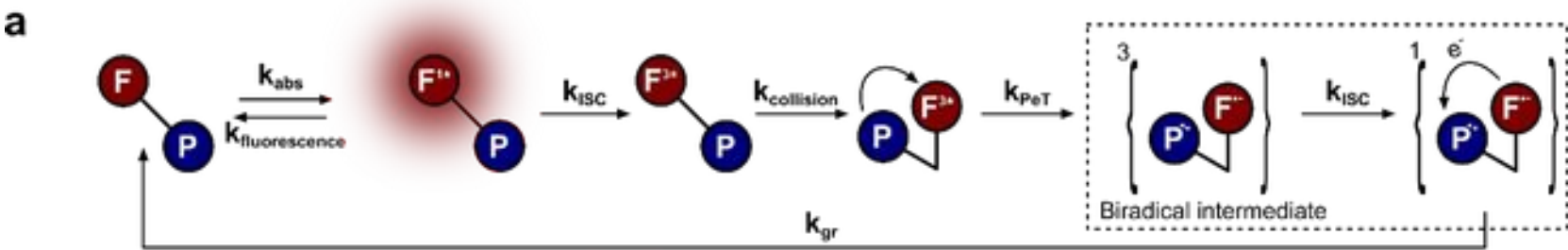
Conjugation approaches

- “direct” or “proximal-linkage” (panel b)

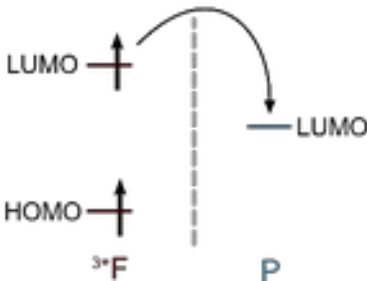


Mechanisms

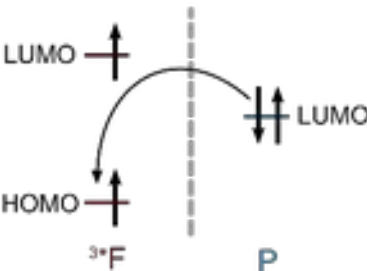
- Energy levels have to match



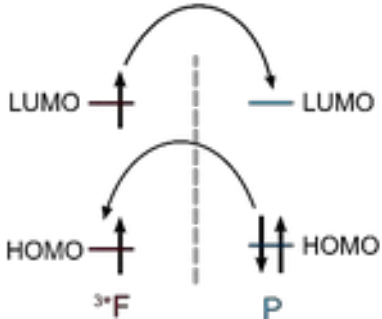
c PeT with an oxidizing agent



PeT with a reducing agent



d Energy transfer



Mechanisms

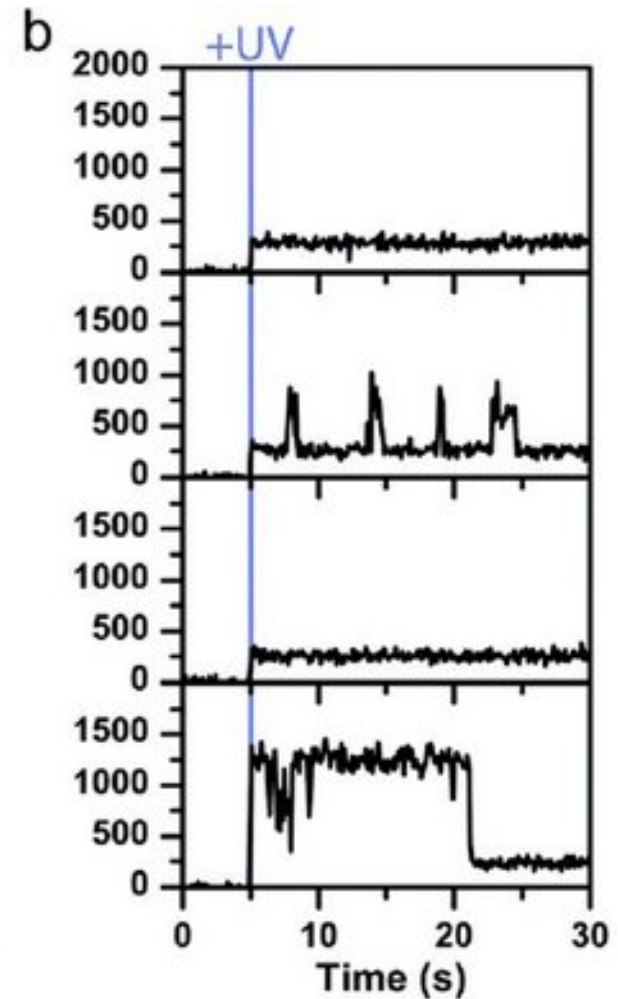
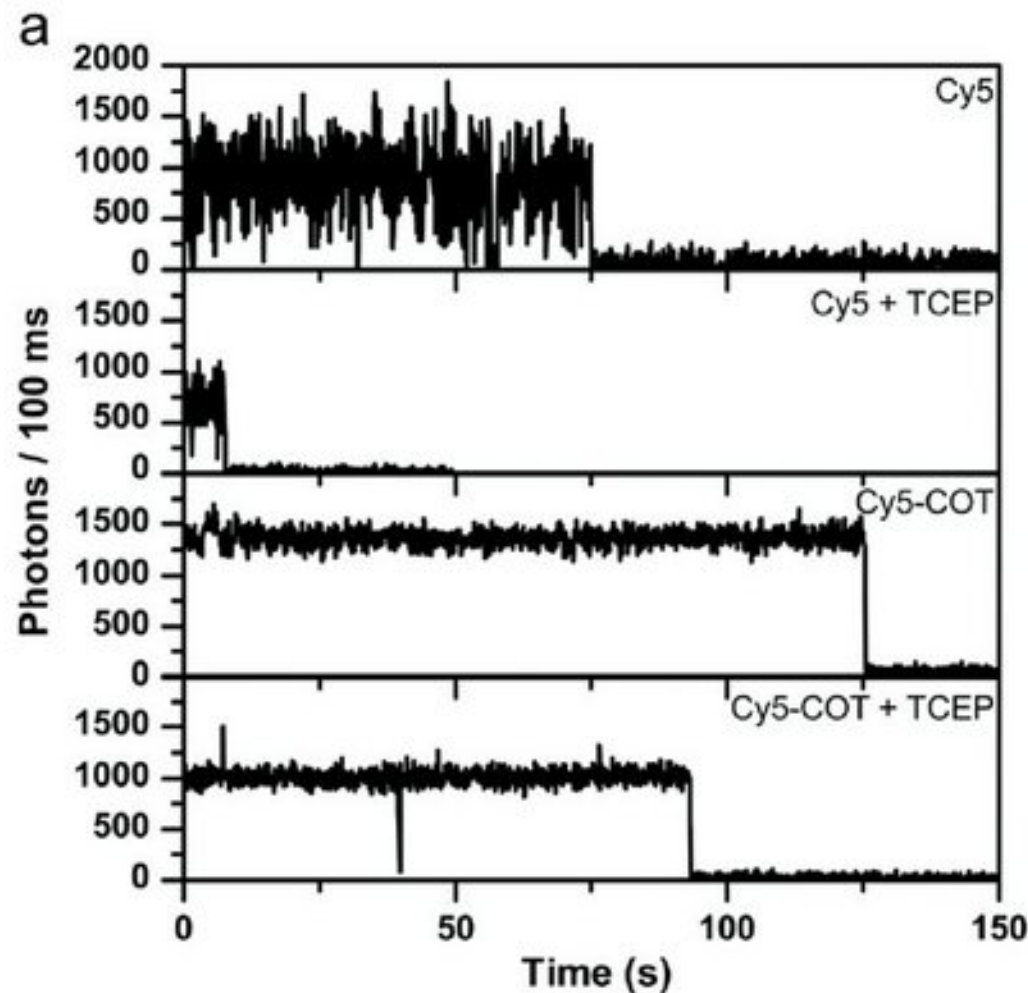
- resulting sensitized photostabilizer triplet state should be short-lived for efficient self-healing
 - should be available as a triplet-acceptor as soon as possible after energy transfer
 - COT, the triplet-state lifetime is on the order of $\sim 100 \mu\text{s}$
- high (local) concentrations of photostabilizers can cause singlet quenching decreasing the fluorescence quantum yield
 - linker design to optimize the collision rate and geometrical orientation

Optimization strategies

- “In many cases, the photostability of self-healing dyes was found to be lower than that of the pristine dye in photostabilizing buffer.”
- linker length and linking chemistry
 - Not one general rule: cases where “the closer the better”, but also optimal linker length at short distances
 - “at very short bond lengths of less than three carbon atoms, a through-bond energy transfer was detected”
- Additional photostabilizers in solution seem to have little to no effect

Photostabilization vs. Photoswitching

- Competition with reductive agents (again dye-specific)



Molecular oxygen

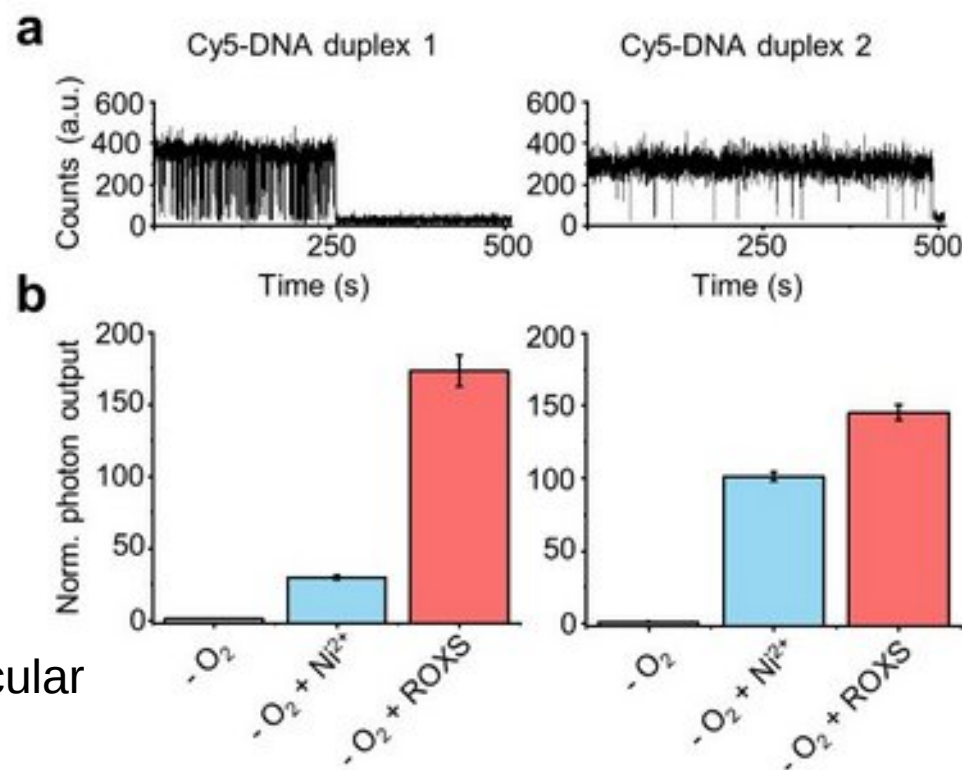
- “So far the self-healing dyes were shown to be rather ineffective in the presence of molecular oxygen with quite moderate photophysical properties”
- diffusion-limited rate constant of quenching by molecular oxygen $\sim 10^{10} \text{ M}^{-1} \text{ s}^{-1}$, concentration in solution 0.5 mM, quenching rates of $\sim 10^6 \text{ s}^{-1}$
- Quenching rates for 1 mM intermolecular COT: between $10^4 - 10^6 \text{ s}^{-1}$
- “we are still missing key pieces in our understanding”

Impact of biological structures

- DNA
 - Guanine is electron donor
 - Stacking of dye with bases at end of duplex as well as binding to major and minor grooves
- Protein
 - It's a mess

That's the new data!

intermolecular

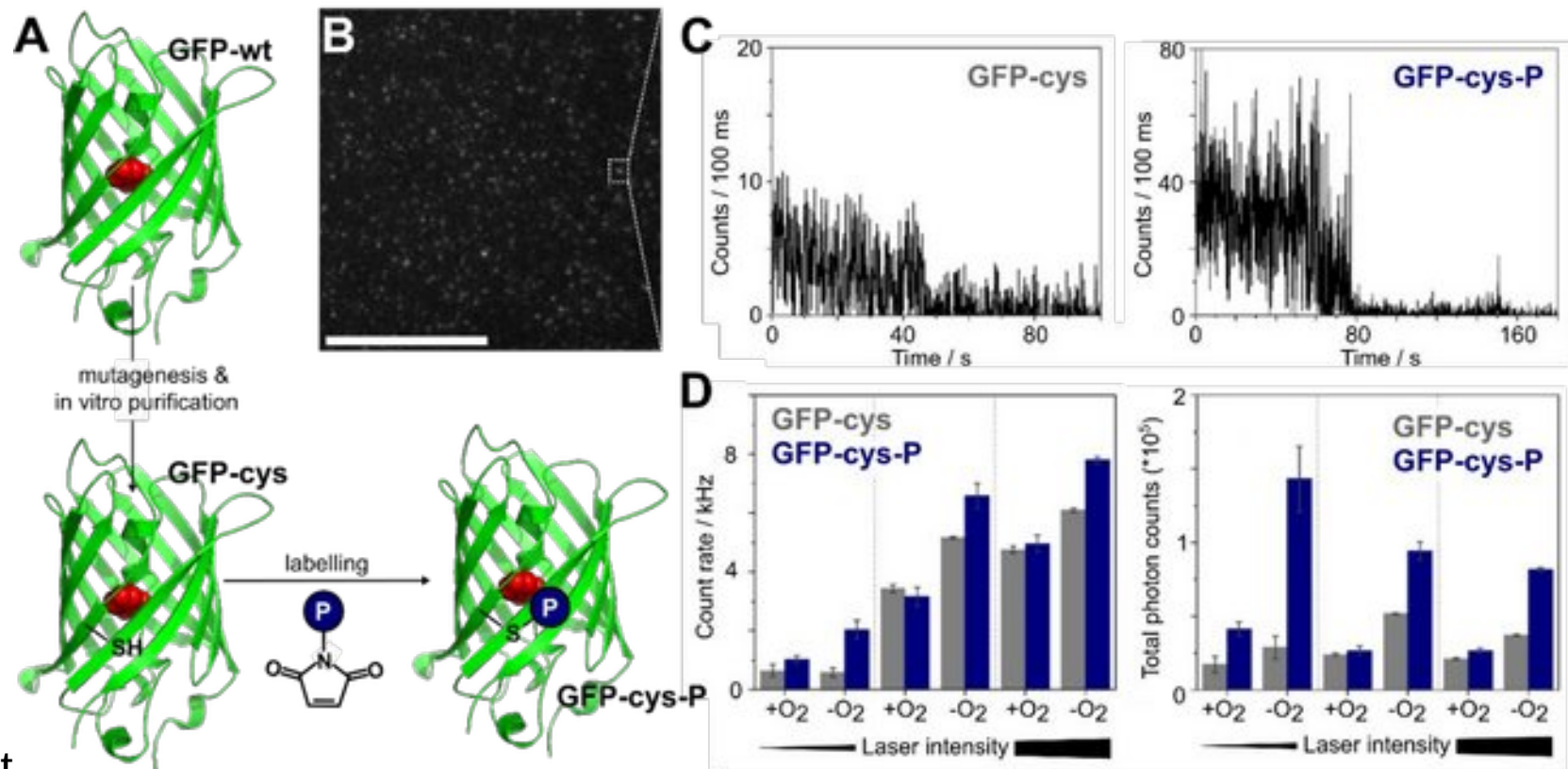


Recent applications

- Cellular imaging and high-resolution microscopy
 - potential for STED-type microscopy
 - to be shown for Minflux and others
 - SMLM → “complicated” (thiol derivatives for photoswitching)
- SmFRET
 - only a few examples so far
 - “increase in photostability compared to the parent fluorophore in absence of stabilizer”, but “the photostability for this dye pair did not meet the level of solution-based stabilization conditions”
 - potential for multi-color FRET where solution-based would not meet the requirements of all dyes

Toward a next generation

- Alternative fluorophores
 - fluorescent proteins → in which experimental situation would this be useful?



Cordes lab
GFP double
cysteine mutant

Toward a next generation

- New stabilizers
 - Potentially very hydrophobic
- Alternative conjugation strategies
 - Not much commercially available

Conclusion

- Paper is clear and understandable
- Self-Healing Dyes – Keeping The Promise?
 - A lot of mechanistic unknowns
 - Tool to understand photostability of dyes in general
 - Might be helpful in specific situations but rather not as a general replacement of more traditional dyes