Journal Club – Optics

09/06/2020

RETURN TO ARTICLES ASAP | < PREV **PERSPECTIVE** NEXT >

Self-Healing Dyes—Keeping the Promise?

Michael Isselstein, Lei Zhang, Viktorija Glembockyte, Oliver Brix, Gonzalo Cosa, Philip Tinnefeld, and Thorben Cordes*

Cite this: *J. Phys. Chem. Lett.* 2020, 11, XXX, 4462–4480
Publication Date: May 13, 2020
https://doi.org/10.1021/acs.jpclett.9b03833

https://doi.org/10.1021/acs.jpclett.9b03833

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

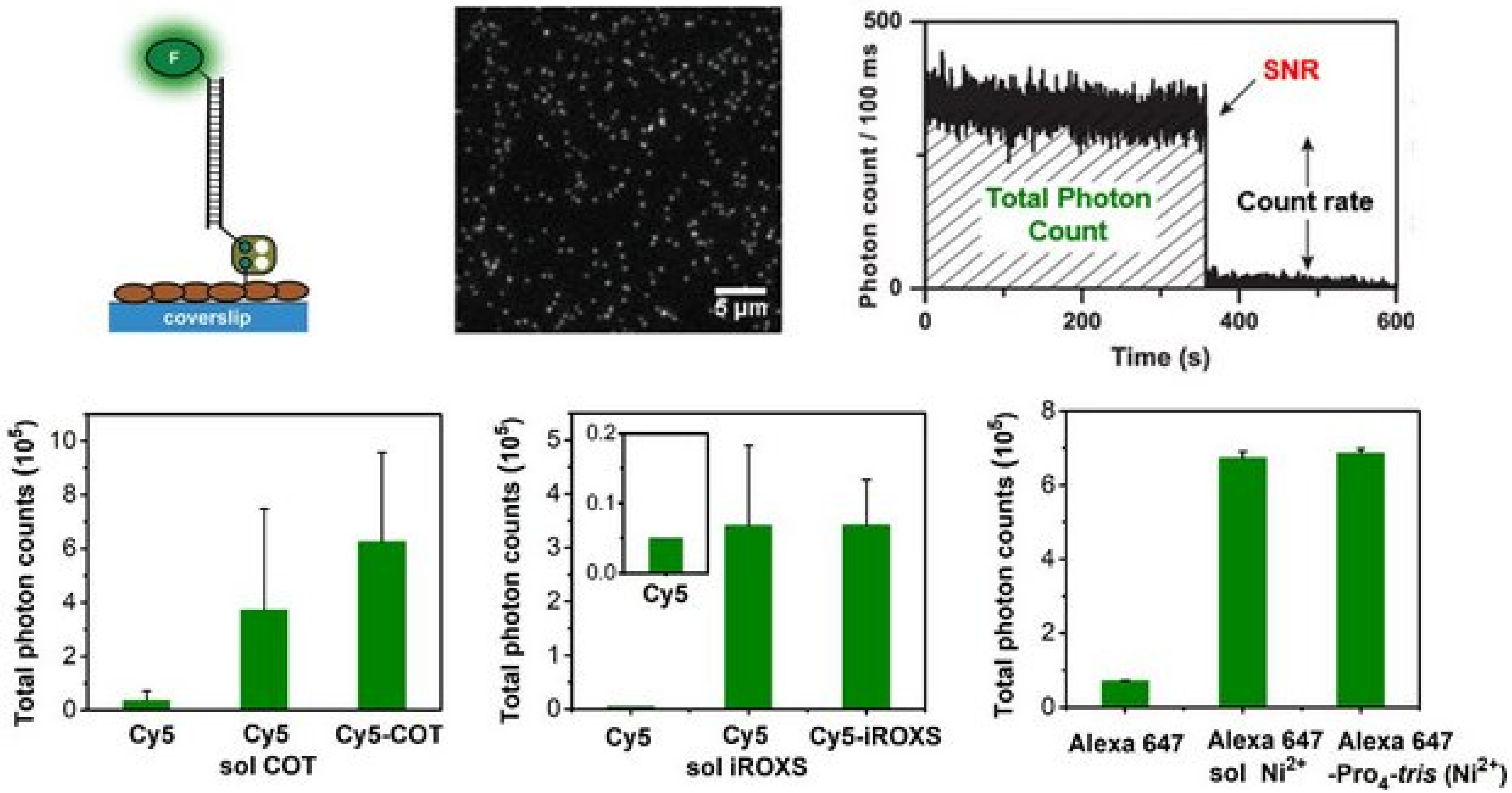
- No need for complex buffer systems
- Works when dyes are inaccessible to solution-based stabilizers
- Triplet-state quenching in the early 1980s for dye lasers
- Blanchard in 2012, Cordes in 2013, and others

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

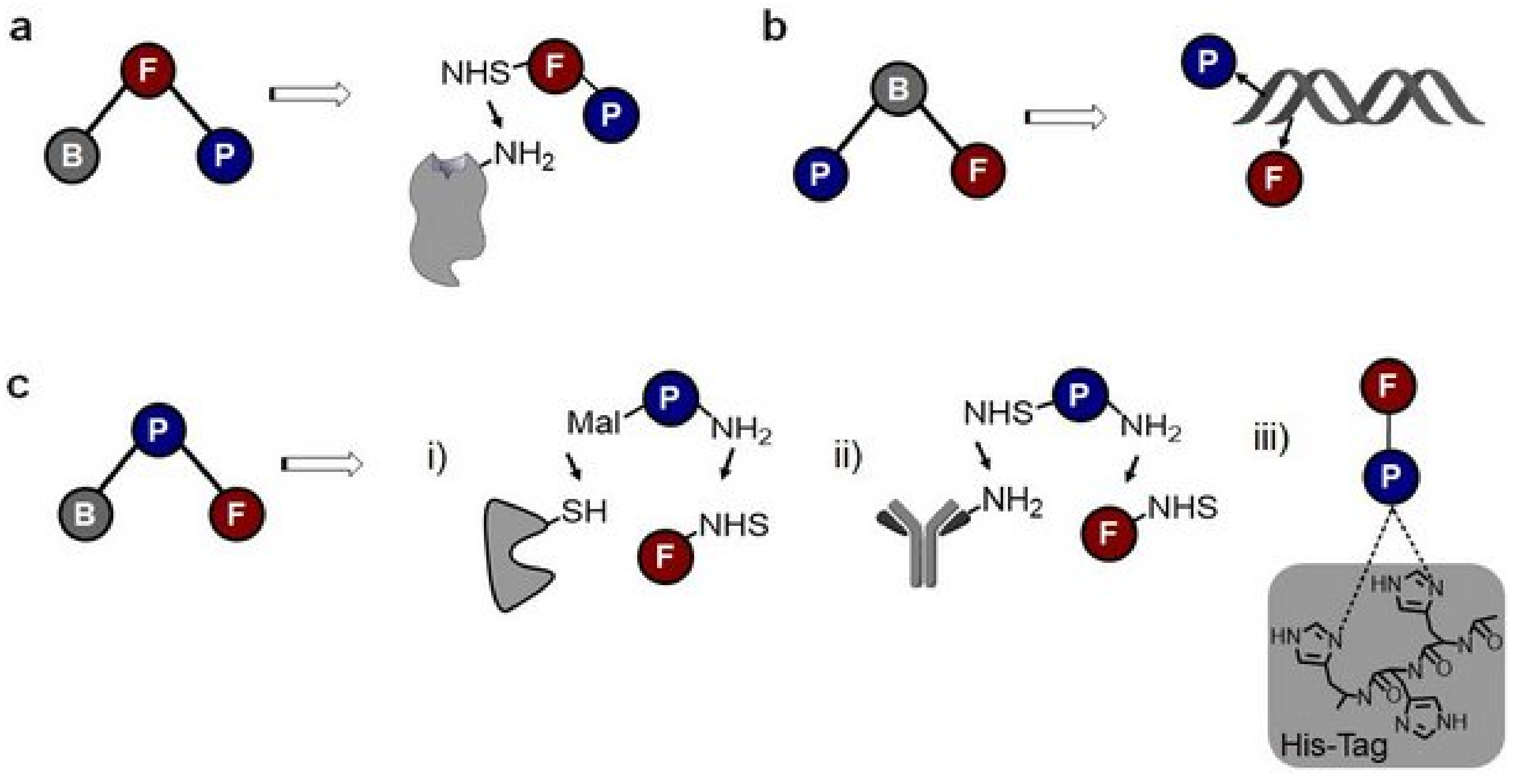
- Works with a variety of dyes



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

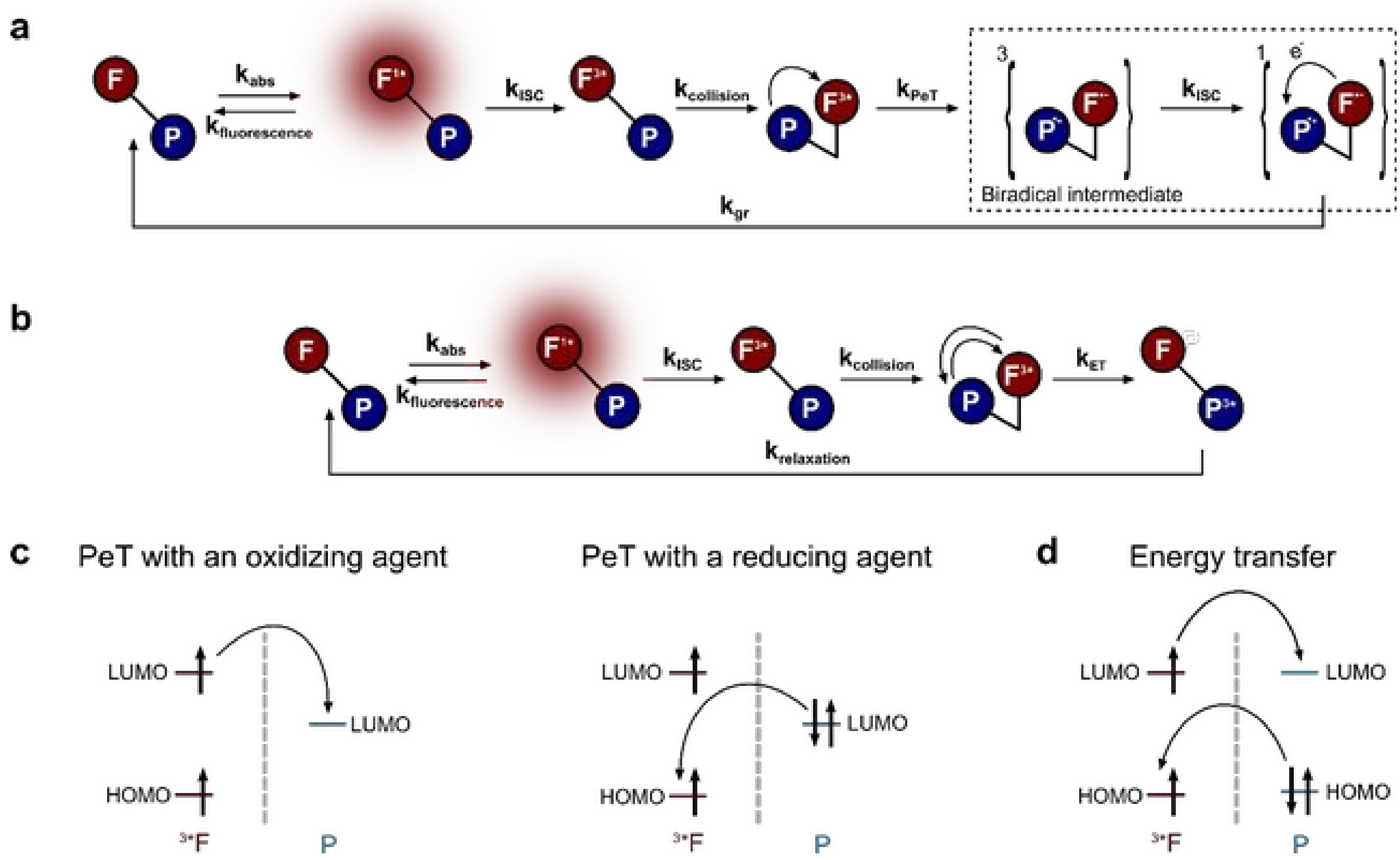
Conjugation approaches

- “direct” (panel a & c) or “proximal-linkage” (panel b)



Mechanisms

- Energy levels have to match



Mechanisms

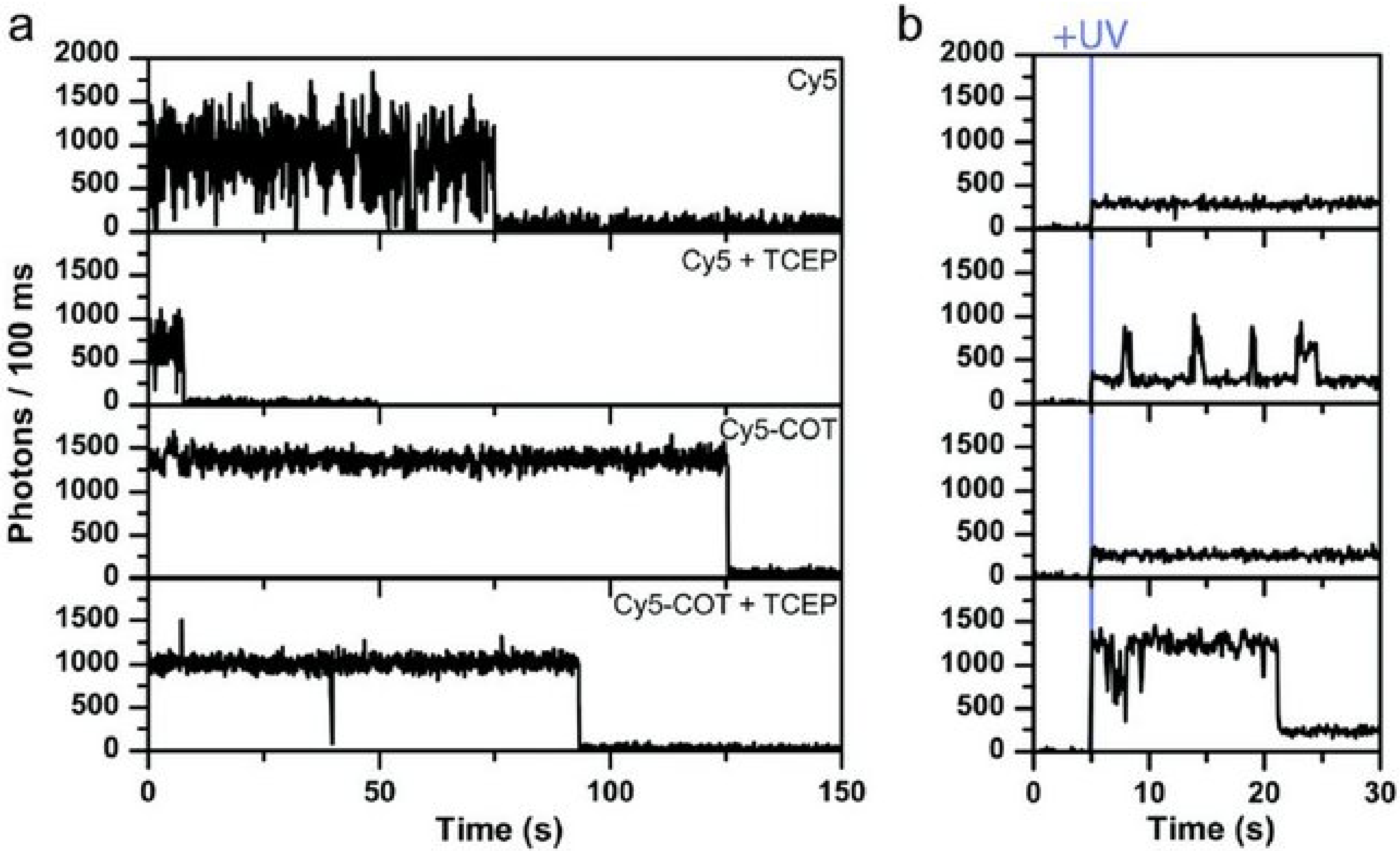
- resulting sensitized photostabilizer triplet state should be short-lived for efficient self-healing
  - should be available as a triplet-acceptor as soon as possible after energy transfer
  - COT, the triplet-state lifetime is on the order of ~100 μs
- high (local) concentrations of photostabilizers 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

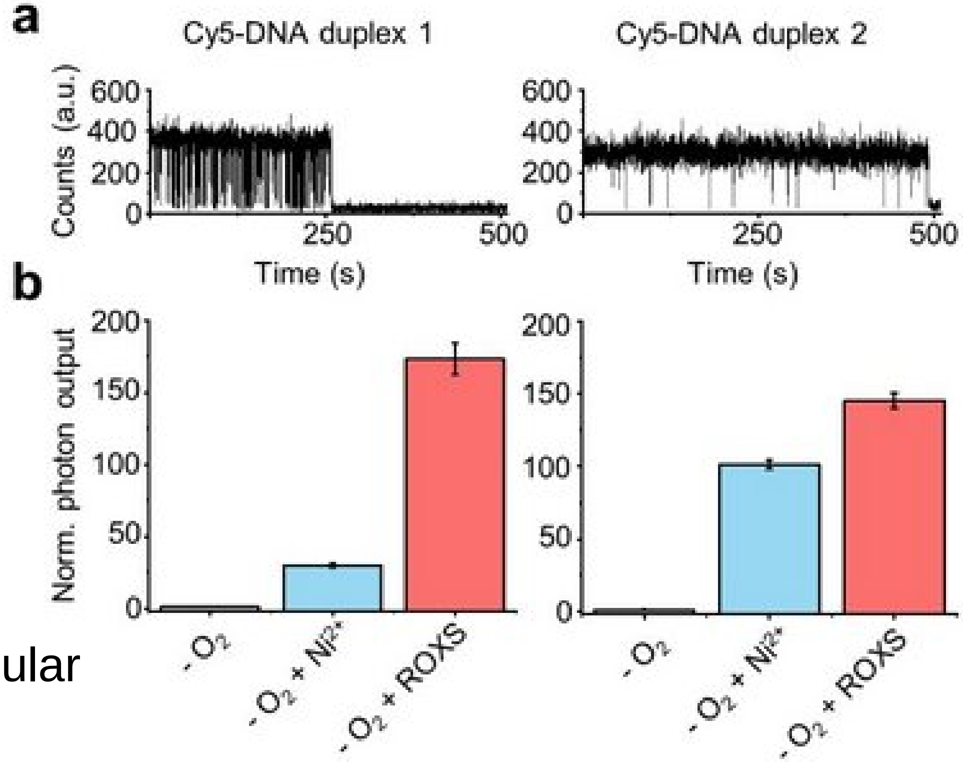
- “So far the self-healing dyes were shown to be rather ineffective in the presence of molecular oxygen with quite moderate photophysical properties”
- diffusion-limited rate constant of quenching by molecular oxygen ~10^{10} M^{-1} s^{-1} , concentration in solution 0.5 mM, quenching rates of ~10^{6} s^{-1}
- Quenching rates for 1 mM <u>inter</u>molecular COT: between 10^{4} - 10^{6} 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

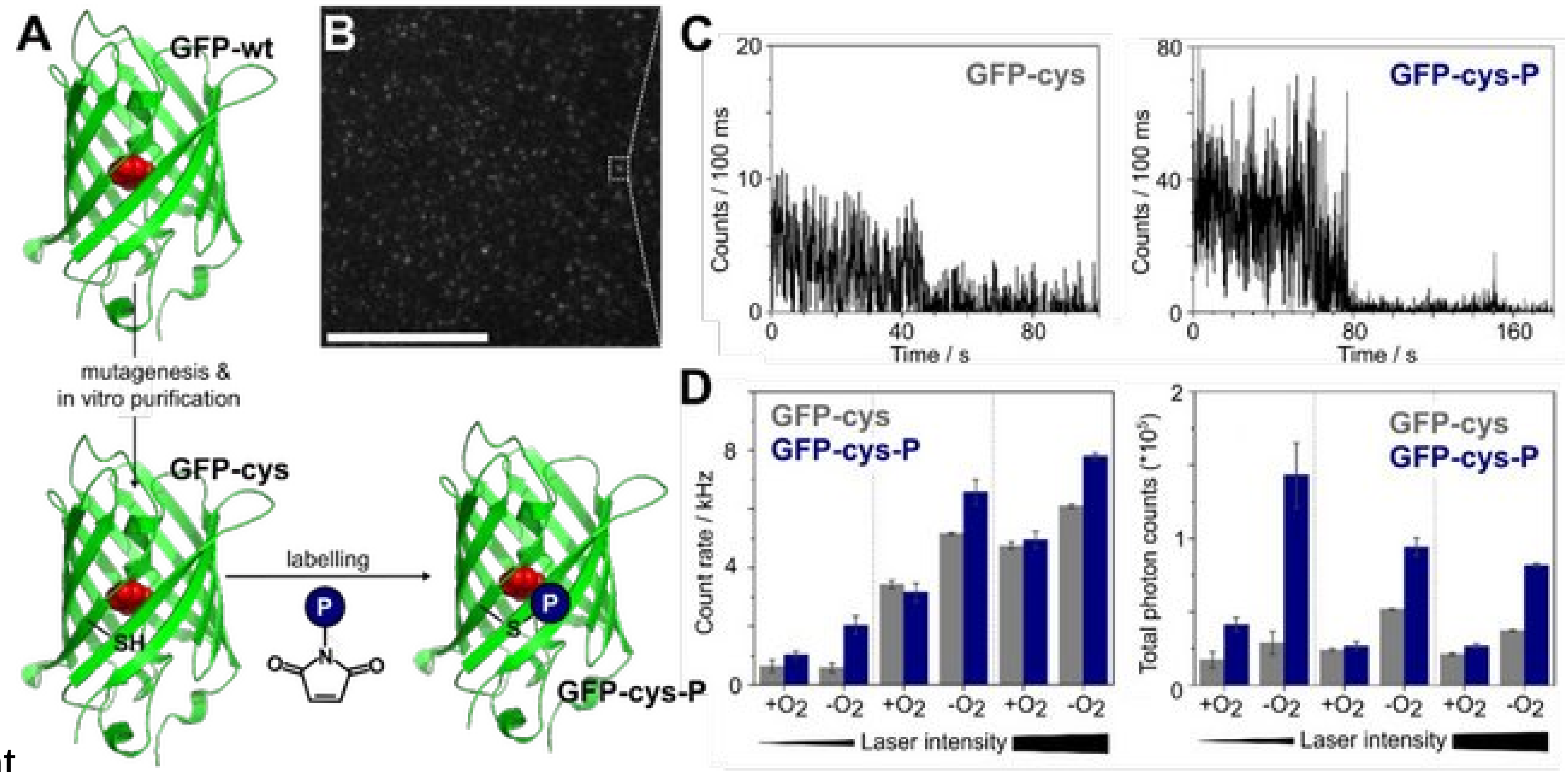


Recent applications

- Cellular imaging and high-resolution microscopy
  - potential for STED-type microscopy
  - to be shown for Minflux and others
  - SMLM → “complicated” (thiol derivatives for photoswtiching)
- 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