A brilliant monomeric red fluorescent protein combining high brightness and fast maturation

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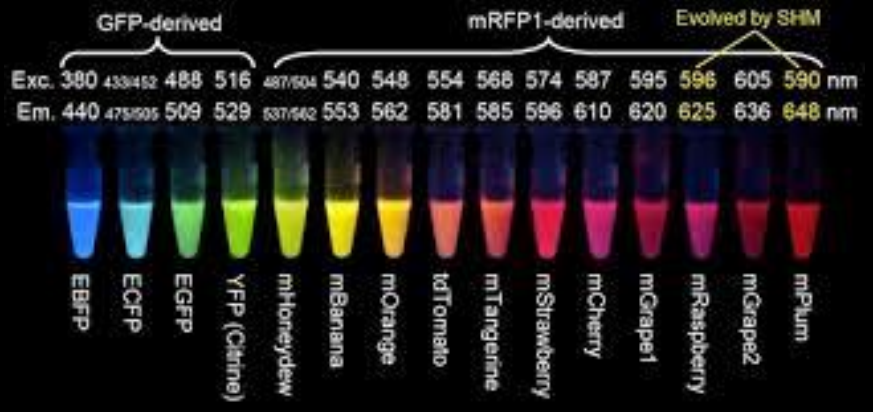
Introduction

- **MScarlet3:** a red fluorescent protein that can be used for biological imaging applications
- **Fluorescent proteins:** proteins that emit light when they are exposed to certain wavelengths of light
- Have revolutionized biological imaging by allowing researchers to visualize specific structures and processes in living cells and organism

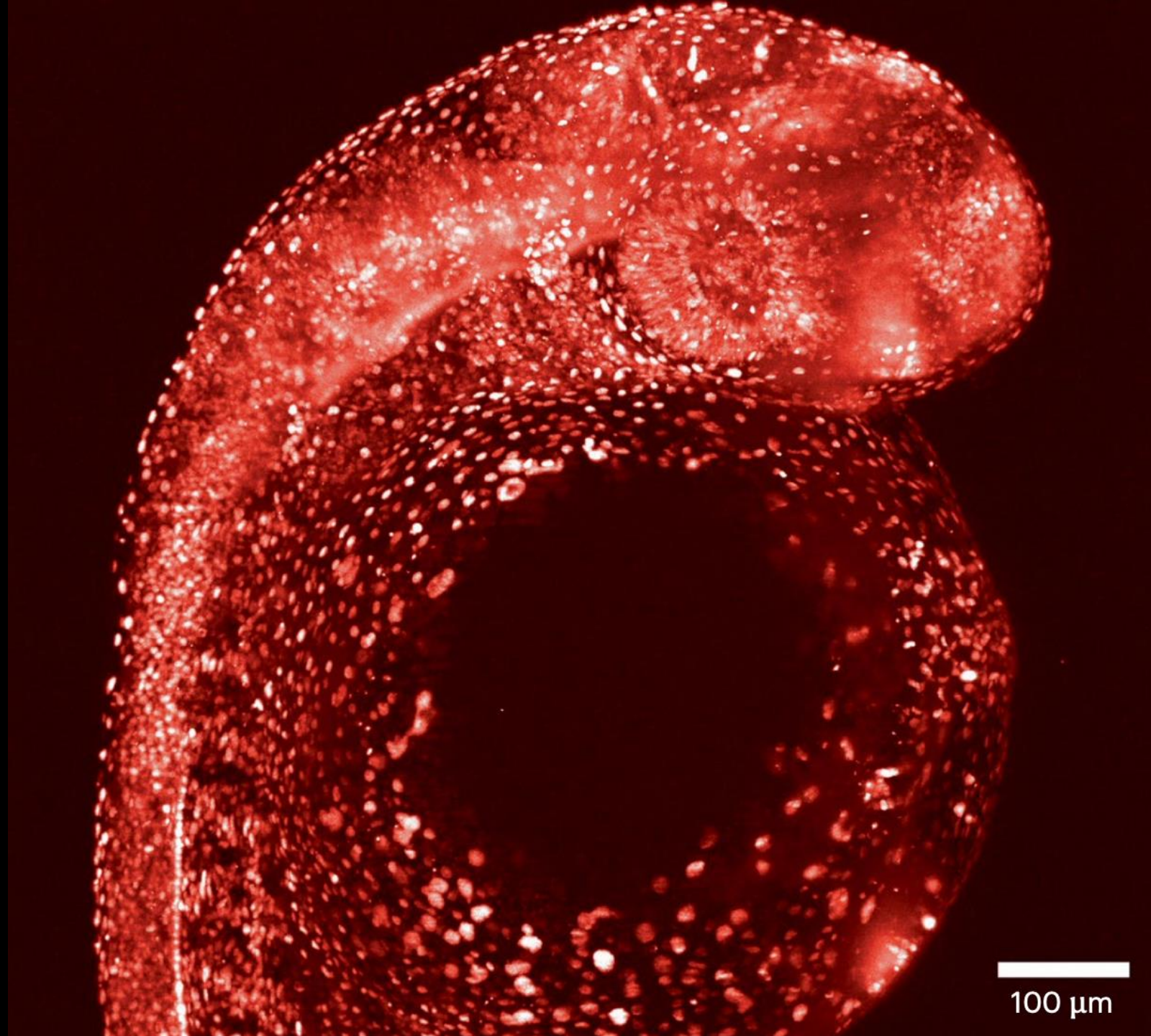
- **Current Limitations:** low brightness and slow maturation time, which can hinder their utility for some applications



Chakravarthy, A. (2011, July 28). *Fluorescent Proteins in Imaging: Bright and Beautiful*. Exploreable; Exploreable. https://exploreable.wordpress.com/2011/07/28/fluorescent-proteins-in-imaging-bright-and-beautiful/

Introduction

- **MScarlet3** aims to improve on these limitations



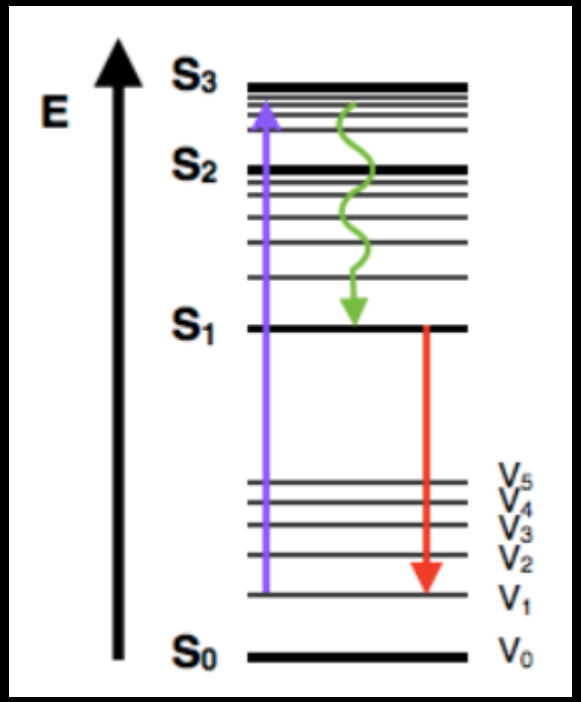
Introduction

- Paper presents methods used to optimize mScarlet3's properties, comparing them to other commonly used fluorescent proteins

- Ability to image a variety of biological structures and processes

Fluorescence principle and fluorescent protein

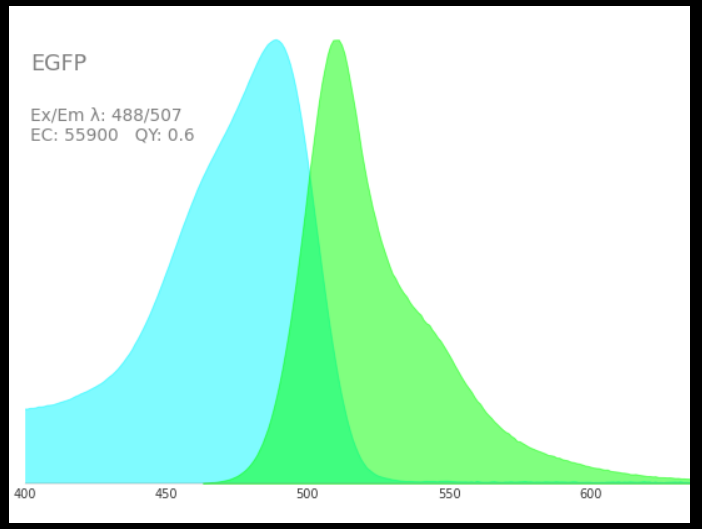
Principle: Jablonski diagram



chem.libretexts.org

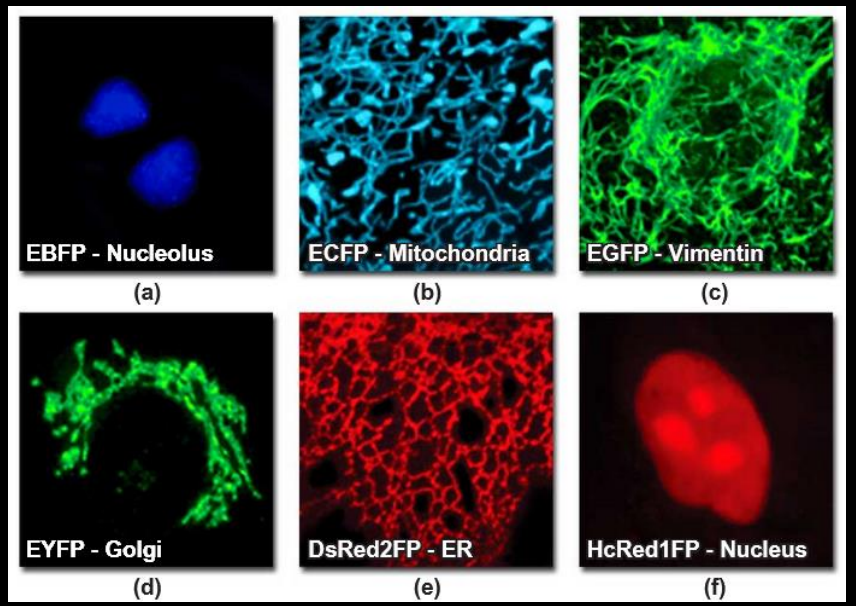
Absorption, *vibrational relaxation*, and *fluorescence*

Absorption/Emission spectrum



www.fpbase.org

Wide variety of molecules can be tagged in a cell



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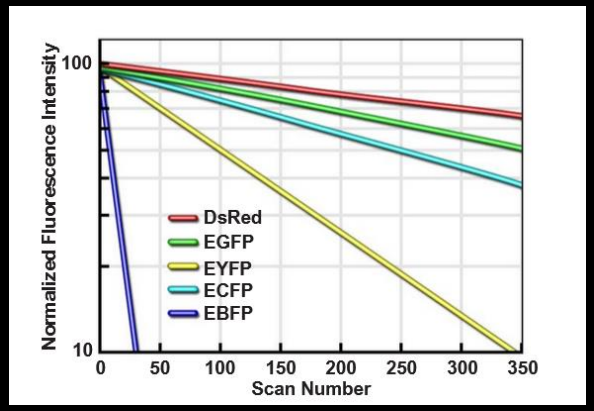
Fluorescent protein

Example of limitations

- Photobleaching



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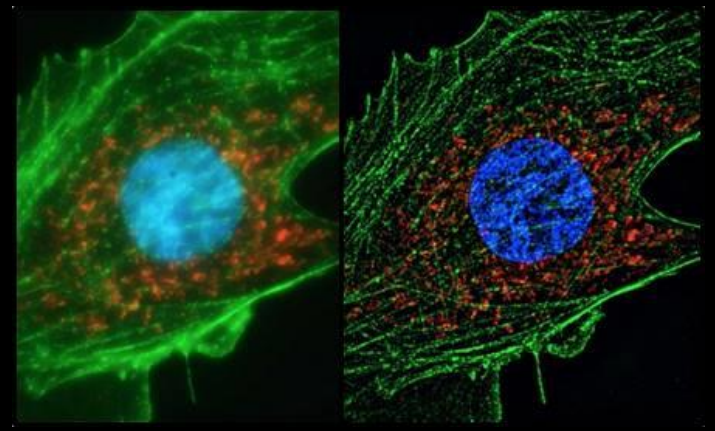
- Resolution



faes.org

- Distance measurement between 2 close proteins

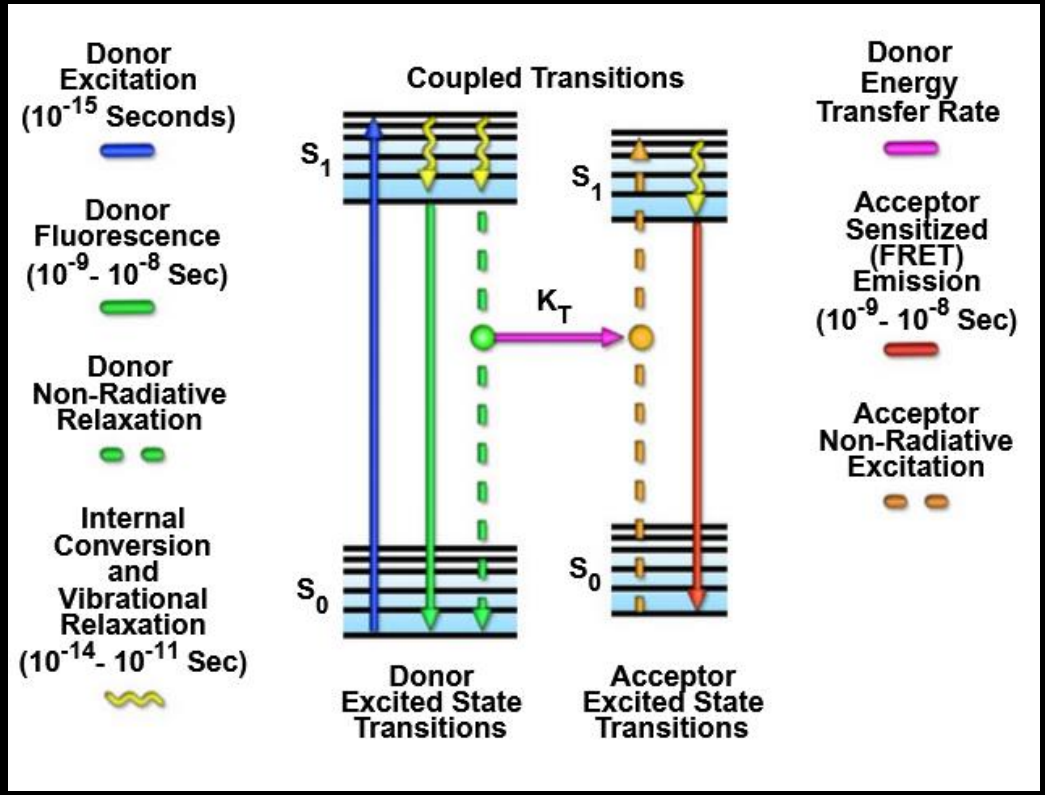
Fluorescence Resonance Energy Transfer (FRET)

Energy transfer between two fluorescent proteins

Donor fluorophore in an excited electronic state, which may transfer its excitation energy to a nearby acceptor fluorophore

FRET occurs between two appropriately positioned fluorophores only when the distance separating them is 8 to 10 nanometers or less.

Overcome limitations in determination of the spatial proximity of protein molecules



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Methodology

Gadella, T. W. J. Jr. *et al*., *Nat. Methods*, **2023**, *20*, 541-545.

Mutagenesis and brightness

- **Mutagenesis:** modifying the DNA of an organism to introduce a specific change
- For mScarlet3: creating variations of the original protein e.g. improved brightness
- **Brightness**: amount of light emitted by the protein when it is excited by a particular wavelength
- Higher brightness leads to a greater accuracy and sensitivity in detecting

Localisation and Oligomerisation

- **Localisation in cell biology:** Specific subcellular location of a protein or molecule within a cell
- Important for understanding functions and interactions within the cell
- **Oligomerization** refers to the process by which multiple copies of a protein ffrom a larger protein complex or "oligomer".
- Proteins can form various types of oligomers, including dimers, trimers, and higher-order complexes. Oligomerization is important for regulating protein activity and can affect protein stability, localization, and interactions with other molecules.

FRET-FLIM

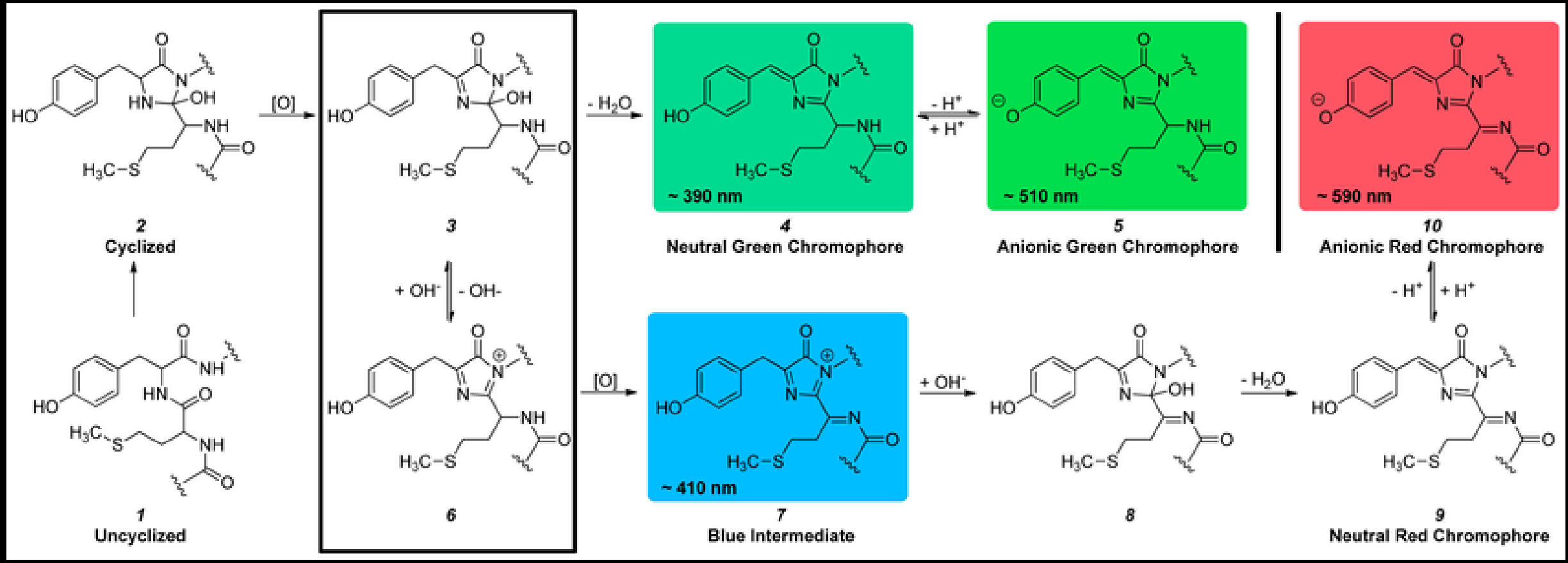
- **Fluorescence lifetime imaging microscopy (FLIM)**
- measures the lifetime of a fluorophore, which can provide information about its environment and interactions with other molecules
- Used to study protein-protein interactions and molecular dynamics
- Combining both gives insigts into the proximity and interactions of fluorescently labeled proteins in living cells

Maturation of FPs

- Faster maturation time is desired, because thus makes the radiation of color more rapid and particles can be detected sooner
- The extent of the maturation can be compared by measuring the intrinsic brightness and the corrected cellular brightness of the FPs
- After 5h of the transfection of mammalian cells ROI-sensors recorded the cyan and red emissions, further every 15 minutes the film was recorded for 24h
- When measuring the accumulation of red FP and cyan FP fluorescence of cells with discontinuous time traces were discarded

Maturation of FPs

Maturation is a two-step process: folding and remolding the covalent bonds

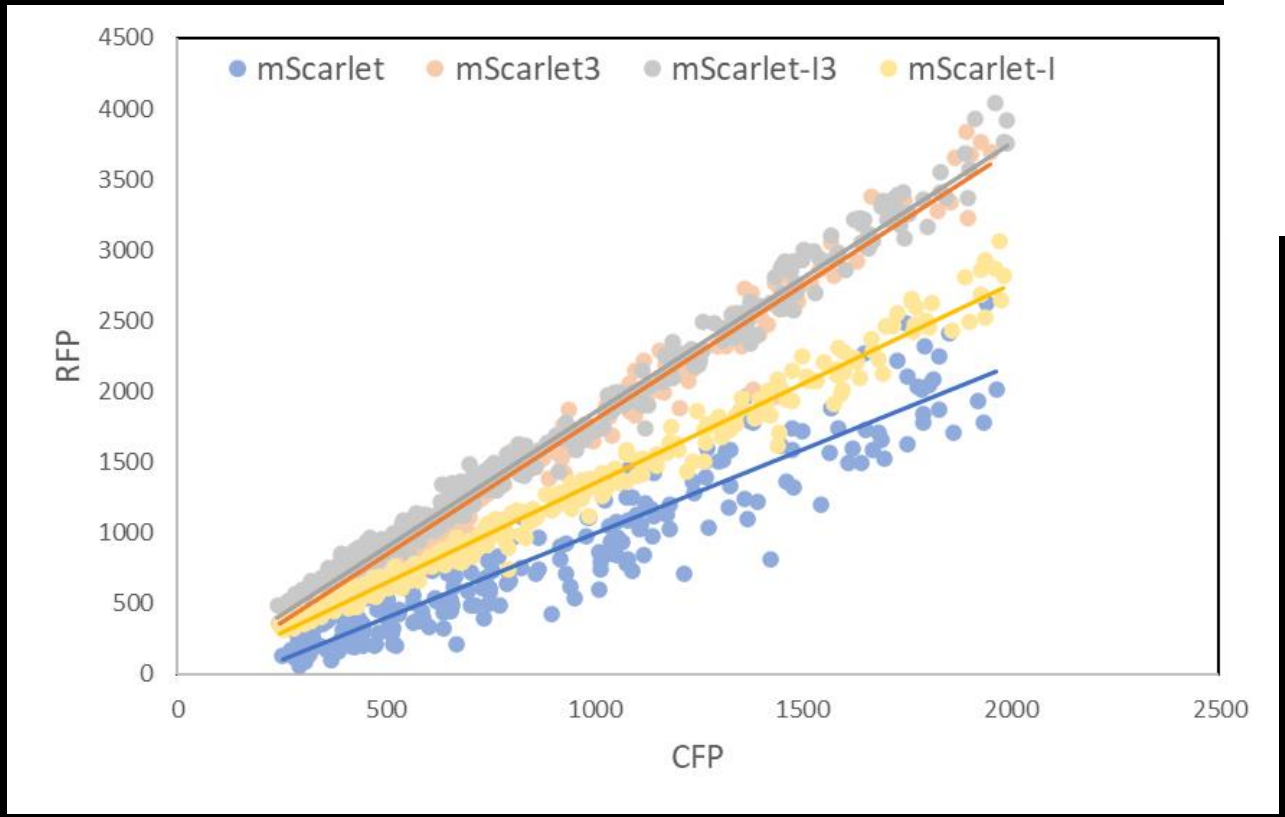


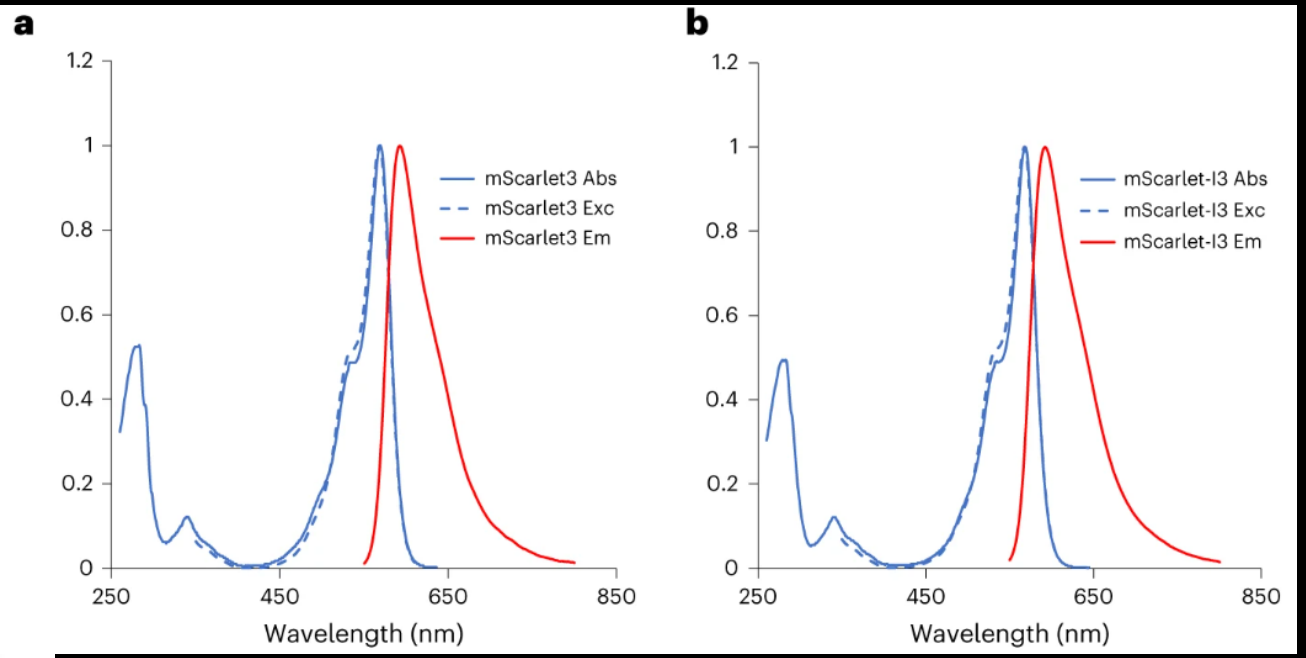
Results

Gadella, T. W. J. Jr. *et al*., *Nat. Methods*, **2023**, *20*, 541-545.

Molecular brightness

intensity of the sensitized emission

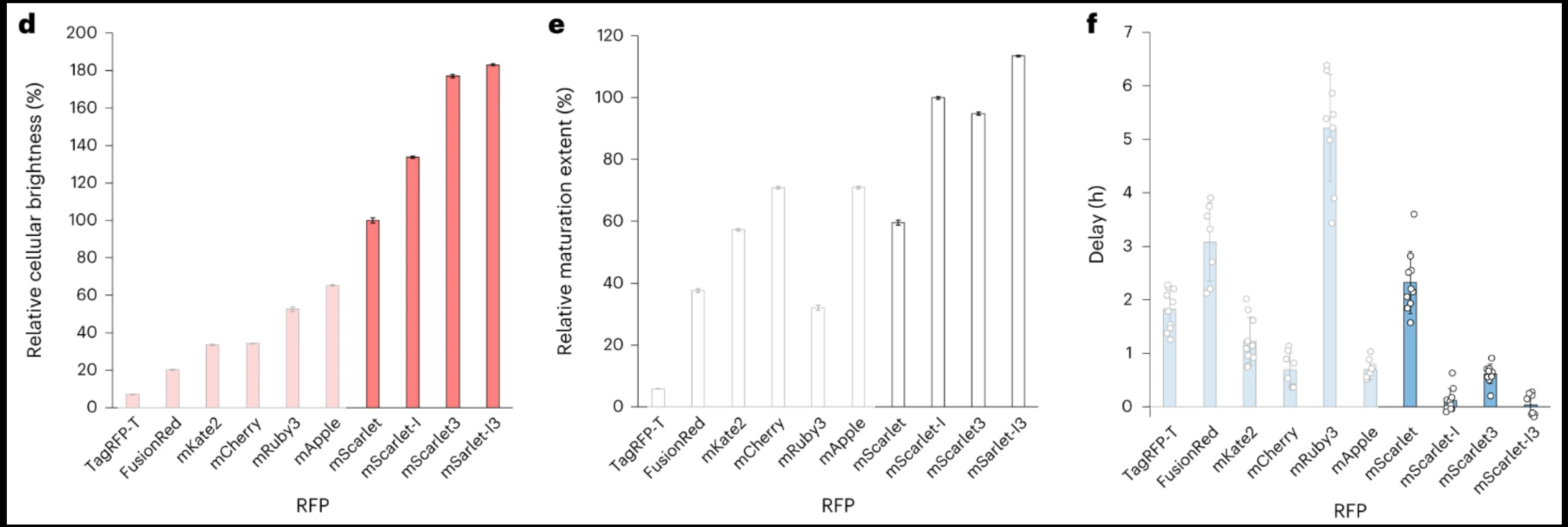




Quantum Yield
mScarlet3 75 %
mScarlet-I3 65%

BUT
intrinsic intensity
≠
cellular intensity

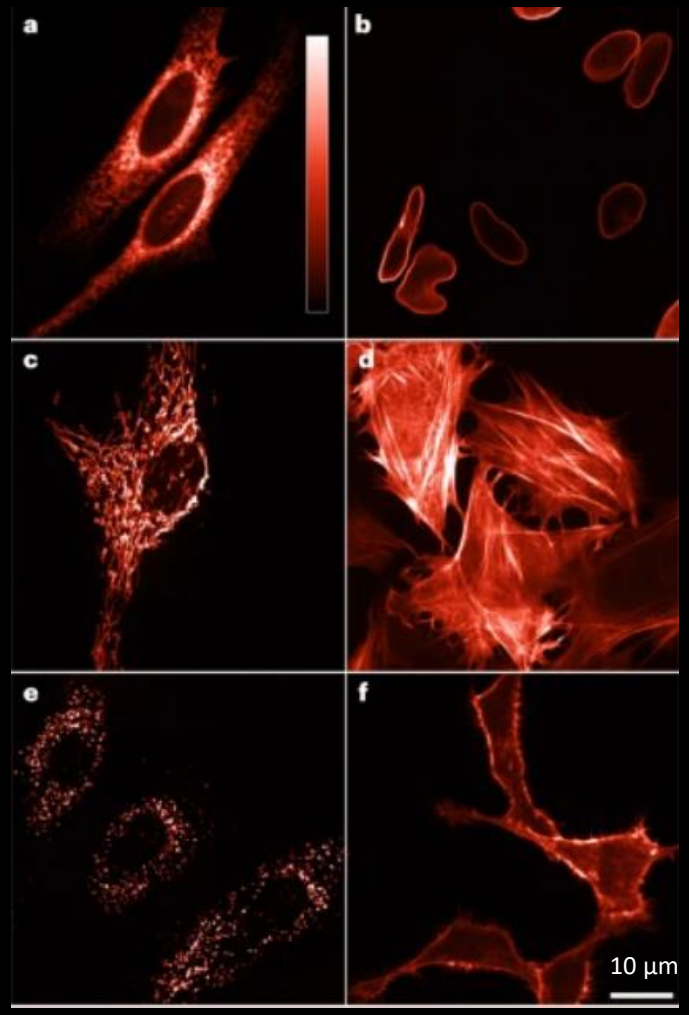
Cellular brightness and maturation



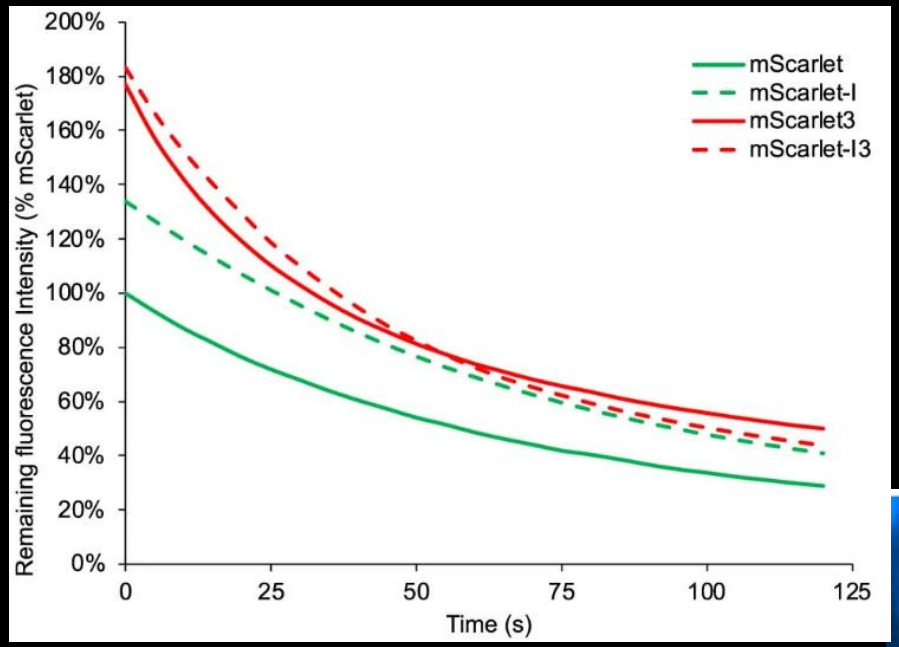
Data obtained 24 h after transfection into HeLa cells – human cell

$$\text{Maturation} = \frac{\text{cellular brightness}}{\text{intrinsic brightness}}$$

Application in microscopy

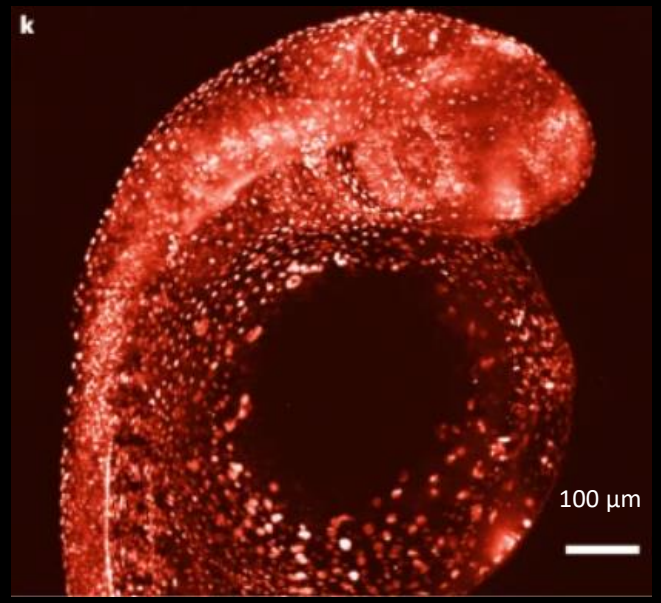


Localization of different organelles in HeLa

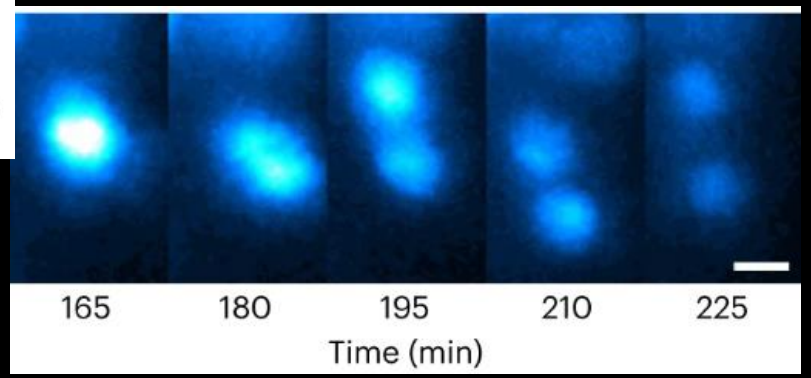


Less photobleaching
→ up to 1,000 confocal scans possible without noticable bleaching

No photochromic effect

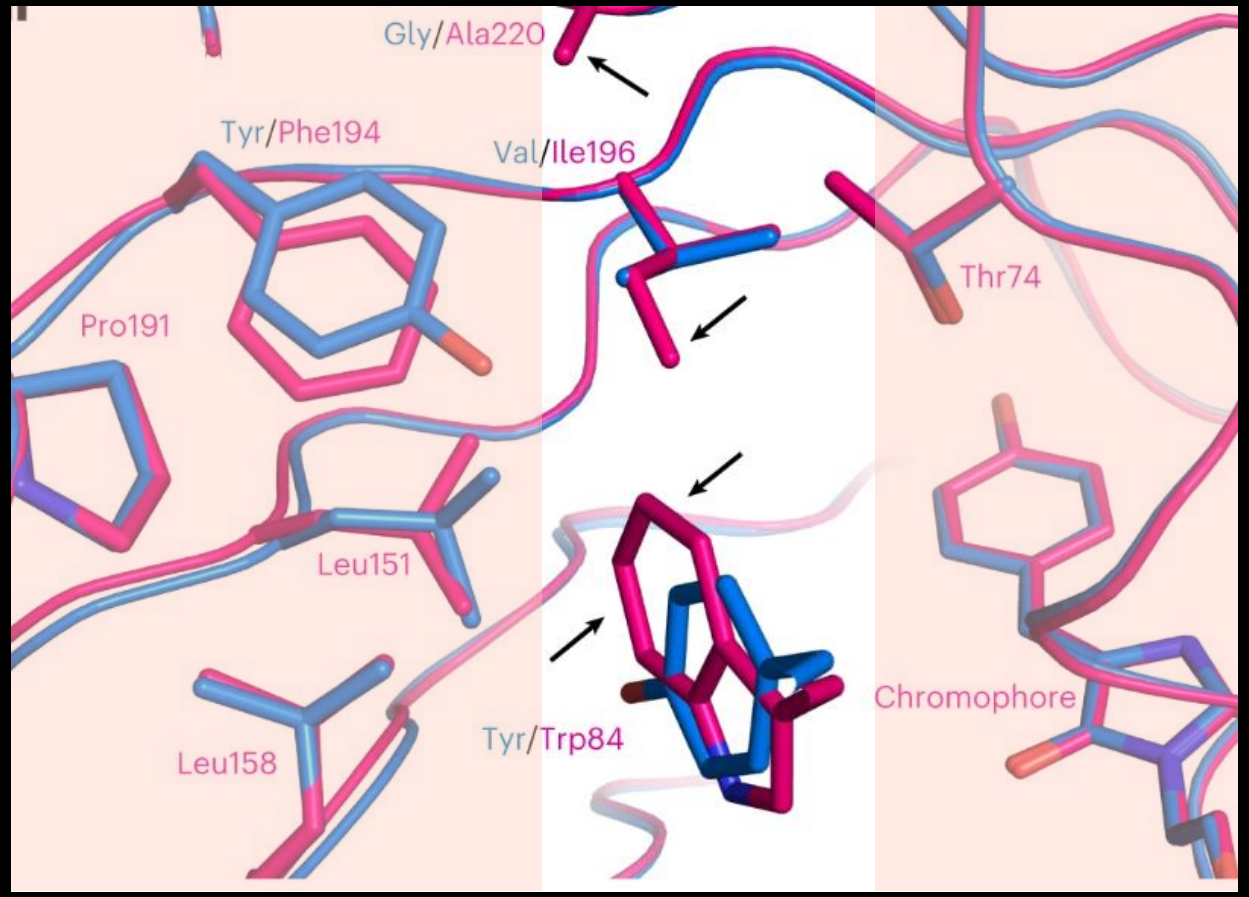


Cell nuclei in zebrafish



Retention of light intensity druring cell division in zebrafish

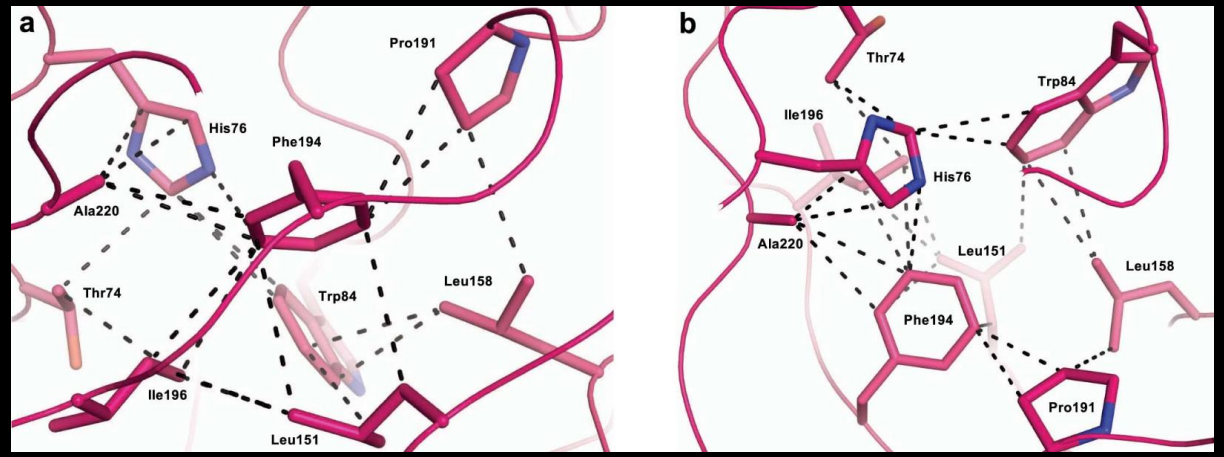
Structural analysis after mutagenesis



Blue – mScarlet
Pink – mScarlet3

Hypothesis

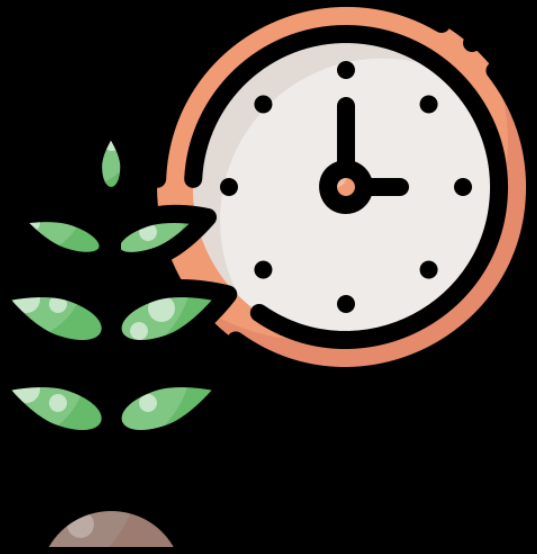
Structural change on marked sites responsible for improved properties



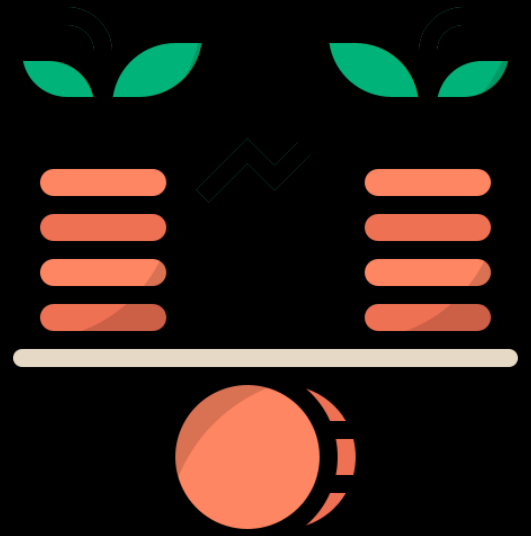
vdW interactions in mScarlet3

Conclusion

Higher brightness



Faster and more complete maturation



Photostability

Conclusion

- mScarlet3 is a valuable addition to the toolkit of fluorescent protein-based imaging techniques