mScarlet3: a brilliant and fast maturing red fluorescent protein for cellular imaging

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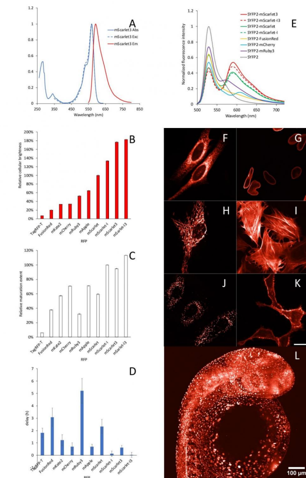
Abstract

We report the evolution of mScarlet3, a cysteine-free monomeric red fluorescent protein (RFP) with fast and complete maturation, as well as record brightness, quantum yield (75%) and fluorescence lifetime (4.0 ns) [1].

In 2017 two seriously improved red fluorescent proteins: mScarlet and mScarlet-I were generated from a synthetic consensus RFP template mRed7 [2]. While mScarlet displayed highest intrinsic brightness (multiplication of extinction coefficient and quantum yield), mScarlet-I was brighter in cells owing to a substantially faster and more complete maturation, compensating for its decreased intrinsic brightness. Obviously, a variant that combines both maximal intrinsic brightness and fast, complete maturation is highly desirable. To this end we set out to further mutagenize mScarlet and mScarlet-I by screening for variants with increased cellular brightness early after transfection in mammalian cells (which is a hallmark of fast and more complete maturation) and high fluorescence lifetime (which is a hallmark of high intrinsic brightness or quantum yield) using quantitative ratiometric imaging and fluorescence lifetime imaging microscopy, respectively [3].

We finally selected two variants with seriously enhanced properties: mScarlet3 and mScarlet-I3, both being ~ 80% brighter in cells than mScarlet. The maturation speed for mScarlet3 was four times enhanced as compared to mScarlet.

We solved the mScarlet3 crystal structure which revealed that all 4 mutated internal residues form a large hydrophobic patch at one head of the beta-barrel. mScarlet3 performs very well as FRET acceptor of YFP. Its enhanced maturation causes increased FRET and donor quenching, while it's enhanced quantum yield increases sensitized red emission, both contributing to increased ratio contrast. mScarlet3 behaves well as fusion tag, displays no apparent cytotoxicity. Unlike other red fluorescent proteins like mRuby, FusionRed, TagRFP-T and mApple, both mScarlet3 and mScarlet-I3 show no apparent photochromicity in microscopy experiments where blue and green excitation light is alternated.



A: Spectra of purified mScarlet3, B: Cellular brightness of RFPs in mammalian cells (relative to mScarlet), C: Maturation efficiency of RFPs in mammalian cells (relative to mScarlet-I), D: Maturation speed of RFPs in mammalian cells (relative to mTurquoise 2), E: corrected FRET spectra of YFP-RFP fusion proteins in living mammalian cells, F-K: Confocal imaging of mScarlet3 as fusion tag in living mammalian cells labelling endoplasmic reticulum (F), nuclear envelope (G), mitochondria (H), actin filaments (I), peroxisomes (J) and plasma membrane, bar is 10 µm. L : Maximum projection of a z-stack from a live zebrafish embryo in which all nuclei are labeled by mScarlet3-H2B fusion protein, obtained by light sheet microscopy (bar is 100 µm).

References

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2. Bindels DS, Haarbosch L, van Weeren L, Postma M, Wiese KE, Mastop M, Aumonier S, Gotthard G Royan A, Hink MA and Gadella TWJ (2017). mScarlet: A bright monomeric red fluorescent protein for cellular imaging. Nat Methods 14, 53–56.
3. Bindels DS, Postma M, Haarbosch L, van Weeren L and Gadella TWJ (2020) Multiparameter screening method for developing optimized red-fluorescent proteins. Nat Protoc. 15, 450-478.