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# Citation Classic

# The mFruit Collection of Monomeric Fluorescent Proteins

Nathan C. Shaner[1*]

**Featured Article:** Shaner NC, Campbell RE, Steinbach PA, Giepmans BN, Palmer AE, Tsien RY. Improved monomeric red, orange and yellow fluorescent proteins derived from *Discosoma* sp. red fluorescent protein. Nat Biotechnol 2004:22;1567–72.[2]

Fluorescent proteins, starting with the green fluorescent protein (GFP)[3] from *Aequorea victoria*, have revolutionized our ability to noninvasively study living systems *(1)*. Practically every cell biologist has used fluorescent proteins to tag a favorite protein and watch its dynamic localization in living cells. For almost 10 years after the gene encoding GFP was cloned, researchers were limited to using only about half of the visible spectrum with these probes and were able to image only 2 different colors simultaneously, at best. In 1999, Mikhail Matz and colleagues at the Russian Academy of Science discovered the first known red fluorescent protein, in *Discosoma* sp. coral; that protein would become known as DsRed *(2)*. Hopes were high that the wavelength barrier had been broken, and many researchers jumped at the chance to try this new color. Unfortunately, it soon become apparent that although this protein was a homolog of GFP, it existed as a very tight tetramer and thus had very limited usefulness as a protein-localization tag.

By 2002, Robert Campbell, working in Roger Tsien's laboratory at the University of California, San Diego, had developed a monomeric form of DsRed by subjecting it to an arduous process of structure-guided directed evolution *(3)*. This protein, mRFP1 (monomeric red fluorescent protein 1), was the first generally useful red fluorescent fusion tag, and it gained very quick and widespread adoption within the biological research community. Despite its widespread use, however, mRFP1 had several limitations, including incomplete chromophore maturation, relatively low brightness, and fast photobleaching during imaging experiments. Work continued on improving mRFP1 in the Tsien laboratory, and I took up the project when I entered the laboratory later in 2002 as a graduate student.

In 2004, we published the first results of this work on the mRFP1 lineage in the *Nature Biotechnology* article featured here, which led to a series of mutants that have become collectively known as the "mFruits." Most notable among the variants described in this work were mCherry, an improved version of mRFP1 with more efficient maturation and much higher photostability; mOrange, a very bright orange variant; and tdTomato (technically a tandem dimer), which remains one of the brightest red fluorescent tags yet described. This set of mutants is among the most diverse groups of fluorescent proteins derived from a single ancestor. Along with collaborators, we would later go on to produce additional variants that expanded the wavelength diversity even further, as well as a set of mutants with enhanced photostability *(4, 5)*. Collaborators, including S. James Remington's laboratory, solved the crystal structures for several of the mFruits [e.g., *(6)*], which provided important insights into the structure–function relationship between the chromophore and peak wavelengths of these proteins. Other groups also have engineered various photoswitchable variants of mCherry and mOrange, as well as numerous fluorescent biosensors based on the original mFruit proteins.

Many biologists secretly relish the opportunity to invent clever and amusing acronyms for the proteins they discover. In the field of fluorescent protein engineering, there has always been something of a "space race" for naming new variants. Of all the efforts put into engineering the mFruits, perhaps the most contentious task among the members of our research team was the decision on how to name these variants. Since gemstone names had already been used for naming *Aequorea* GFP variants, we needed to think of another category of memorable names with easily recognizable colors. At first, we considered naming the proteins simply by color name or wavelength, but we ultimately decided that such designations would be too boring. After many weeks of discussion (and an in-depth study of crayon color names), we finally settled on fruits as a suitable naming category for our new fluorescent proteins. Although these names did not receive a universally positive reception, they have now become ubiquitous.

[1] Department of Photobiology and Bioimaging, The Scintillon Institute, San Diego, CA.
* Address correspondence to the author at: Department of Photobiology and Bioimaging, The Scintillon Institute, 6404 Nancy Ridge Dr., San Diego, CA 92121. Fax 858-587-6692; e-mail nathanshaner@scintillon.org.
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[2] This article has been cited more than 1500 times since publication.
[3] Nonstandard abbreviations: GFP, green fluorescent protein; mRFP1, monomeric red fluorescent protein 1.

One major question remains: How will we name the next set of novel fluorescent proteins?

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## References

1. Tsien RY. The green fluorescent protein. Ann Rev Biochem 1998;67:509–44.
2. Matz MV, Fradkov AF, Labas YA, Savitsky AP, Zaraisky AG, Markelov ML, et al. Fluorescent proteins from nonbioluminescent Anthozoa species. Nat Biotechnol 1999;17:969–73.
3. Campbell RE, Tour O, Palmer AE, Steinbach PA, Baird GS, Zacharias DA, et al. A monomeric red fluorescent protein. Proc Natl Acad Sci U S A 2002;99:7877–82.
4. Shaner NC, Lin MZ, McKeown MR, Steinbach PA, Hazelwood KL, Davidson MW, et al. Improving the photostability of bright monomeric orange and red fluorescent proteins. Nat Methods 2008;5:545–51.
5. Lin MZ, McKeown MR, Ng H-L, Aguilera TA, Shaner NC, Campbell RE, et al. Autofluorescent proteins with excitation in the optical window for intravital imaging in mammals. Chem Biol 2009;16:1169–79.
6. Shu X, Shaner NC, Yarbrough CA, Tsien RY, Remington SJ. Novel chromophores and buried charges control color in mFruits. Biochemistry 2006;45:9639–47.