

# mGold spec sheet

## mGold sequence

mGold is mVenus(L46F;T63S); FPbase ID = UNETX

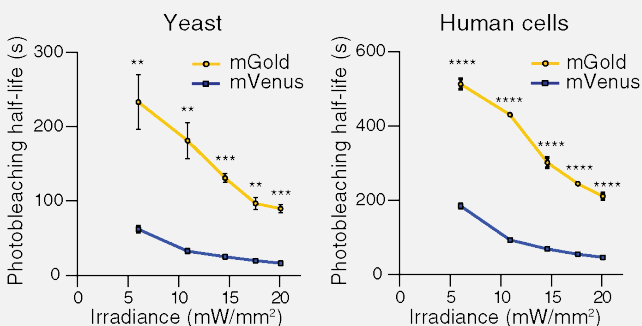
MVSKGEEFLTGVVPIVVELDGDVNGHKFSVSGEGEGDATYGKLT~~L~~**F**ICTTGKLPVWP~~TL~~**V**SLGYGLQC  
 FARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGDIFKEDGNILGHK  
 LEYNYNSHNVYITADKQKNGIKANFKIRHNIEDGGVQLADHYQQNTPIGDGPVLLPDNHYLSYQSKLSKD  
 PNEKRDMVLLFVTAAGITLGMDELYK

## in vitro characteristics

Protein	$\lambda_{exc}$	$\lambda_{em}$	$\epsilon$	$\Psi$	Molecular Brightness	pKa	$K_d$ for Cl <sup>-</sup> (M)	Photobleaching Half-Life (s)
mGold	515	531	107 ± 6	0.64	68	5.9	> 1	29.8 ± 0.1
mVenus	515	532	110 ± 6	0.65	72	5.9	> 1	10.1 ± 0.2

- $\lambda_{exc}$  = Excitation maximum (in nm).
- $\lambda_{em}$  = Emission maximum (in nm).
- $\epsilon$  = Extinction coefficient, in  $\text{mM}^{-1} \text{cm}^{-1}$ ; SEM is shown ( $n = 3$  technical replicates).
- $\Psi$  = Quantum yield of fluorescence.
- Molecular brightness was calculated as a product of  $\epsilon$  and  $\Psi$ .
- pKa was calculated as the pH at which the in vitro fluorescence intensity is half of its maximal value (SEM for  $n = 3$  technical replicates was < 0.1).
- $K_d$  for Cl<sup>-</sup> was calculated as the concentration of Cl<sup>-</sup> which fluorescence intensity to reach half its initial value. Both mGold and mVenus retained more than half their initial fluorescences at 1 M of Cl<sup>-</sup>.
- Photobleaching half-life was determined as the time taken for fluorescence intensity to reach half of its initial value with continuous widefield illumination with 510/25-nm light at 62.5  $\text{mW}/\text{mm}^2$ . Mean  $\pm$  SEM is shown.  $n = 7$  technical replicates.

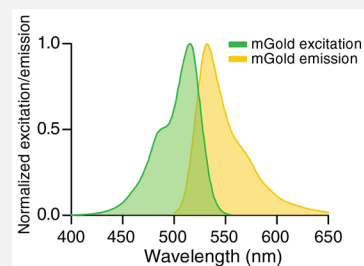
## in cellulo photostability at different irradiances



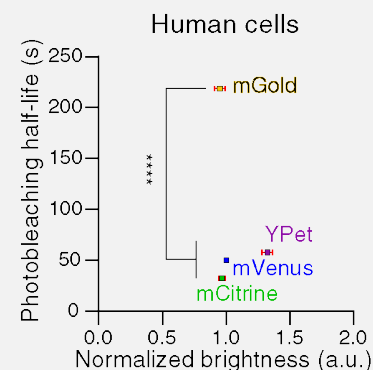
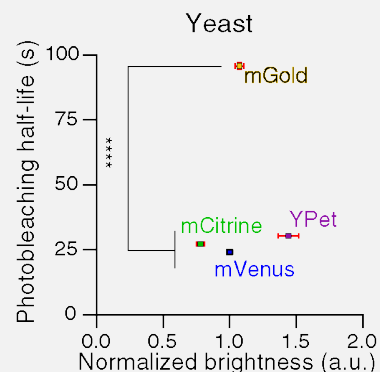
Photobleaching setup: continuous wide-field illumination of 510/25-nm LED light.  $n = 3$  colonies for yeast or 3 transfections for HEK293A cells. Error bars show SEM.

See DOI: 10.1126/sciadv.abb7438 for statistics and more information.

## Excitation and emission spectra



## mGold is more photostable than other commonly used YFPs



Photobleaching setup: continuous wide-field illumination of 510/25-nm LED light at 20  $\text{mW}/\text{mm}^2$ .  $n = 6$  colonies for yeast or  $n = 6$  transfections for HEK293A cells. Error bars show SEM.

See DOI: 10.1126/sciadv.abb7438 for statistics and more information.