Calcium-regulated photoproteins and their "color" mutants as bioluminescent reporters

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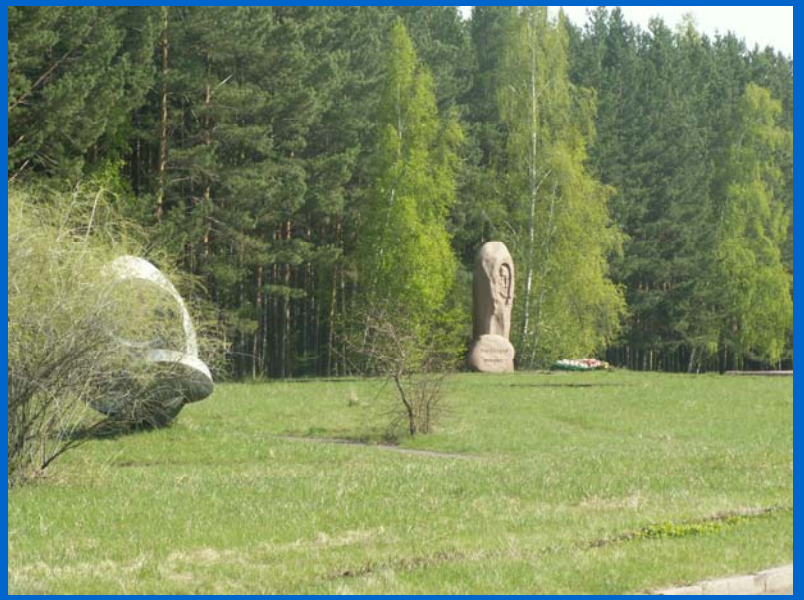


Bioluminescence - light emission of organisms

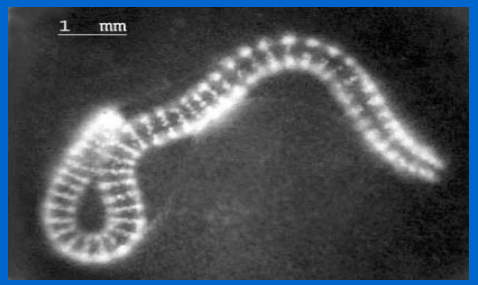
jellyfishes

fireflies

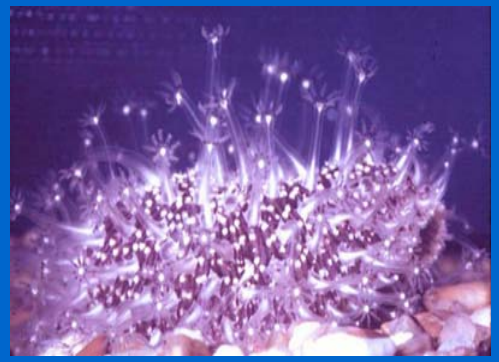
hydroid polyps

marine copepods

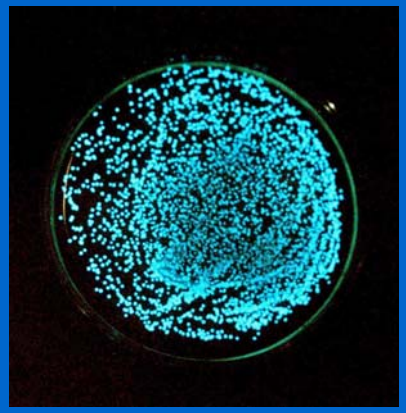
worms



sea pansy

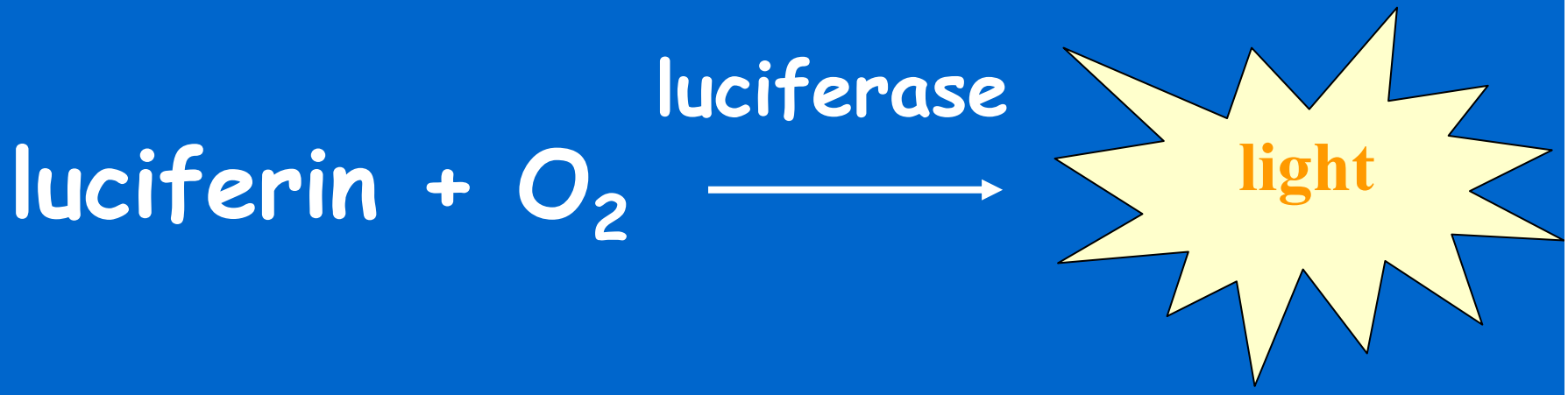


bacteria



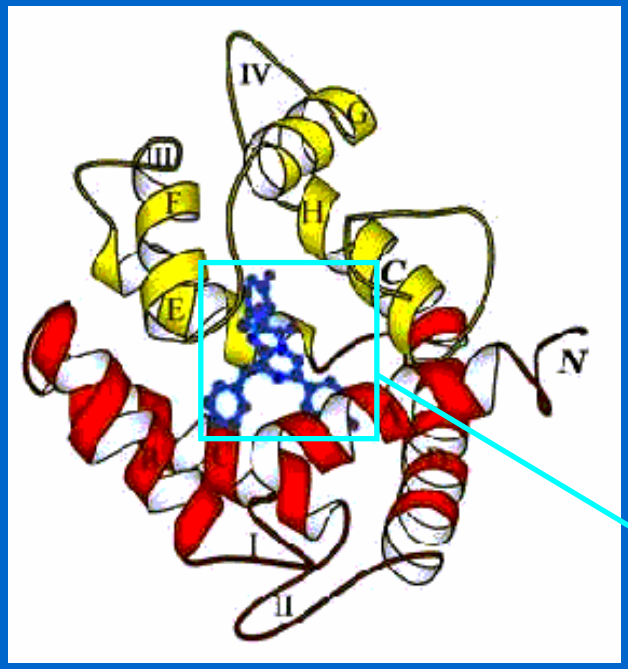
ctenophores

Bioluminescence is a chemiluminescent reaction in which **luciferin** (substrate) is oxidized by **luciferase** (specific enzyme)



Obelin - stable "pre-charged" protein (single-chain, 22.2 kDa) triggered by calcium to emit light

Obelia longissima

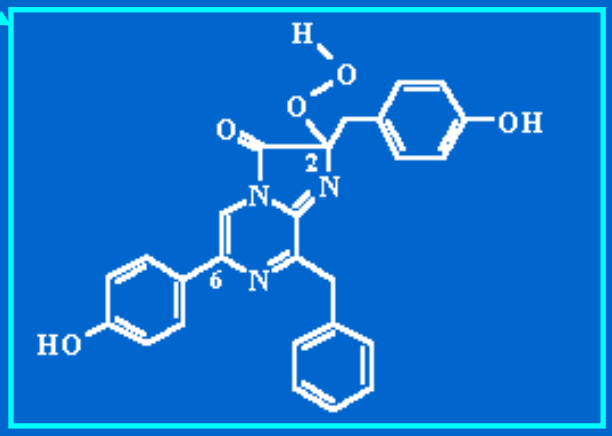


obelin molecule

Ca^{2+} →

light

λ_{max} = 485 nm



substrate: 2-hydroperoxycoelenterazine

Application of photoproteins:

- ✓ *in vivo*
- ✓ *in vitro*

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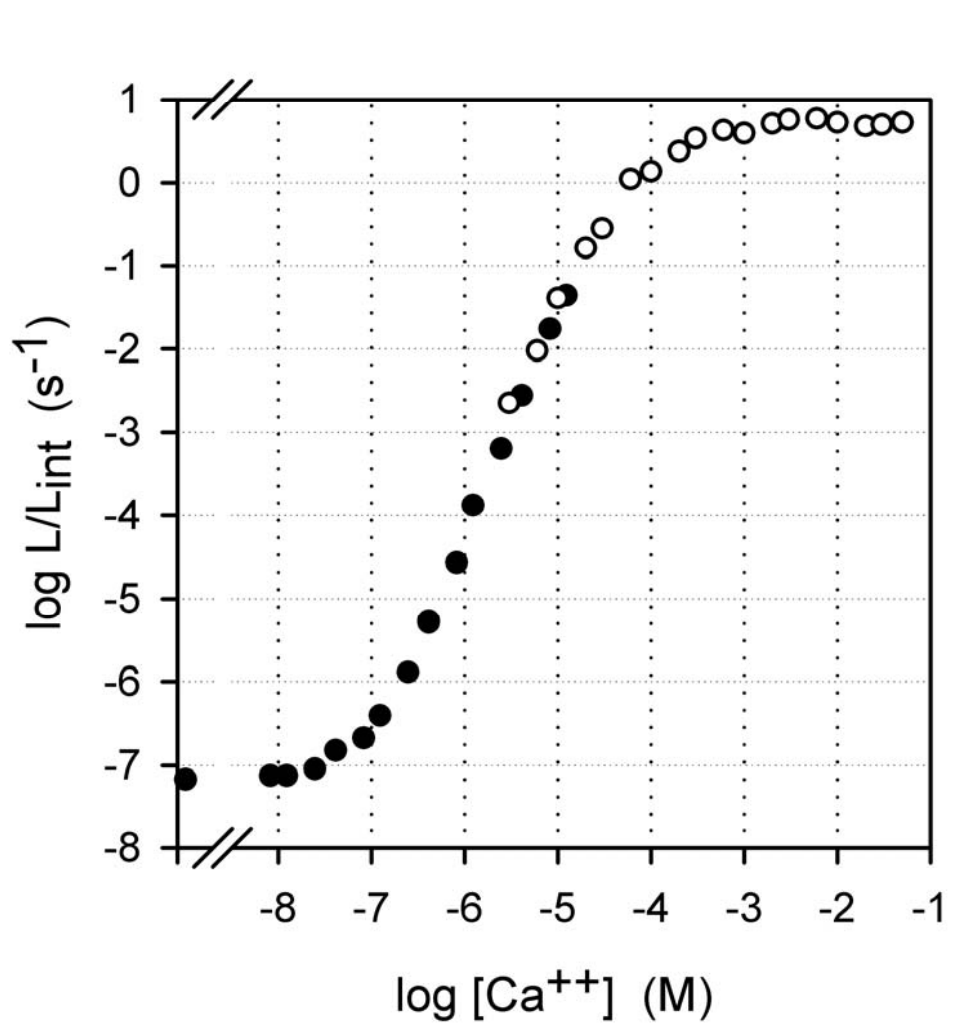
Ca^{2+} concentration-effect curve for WT obelin

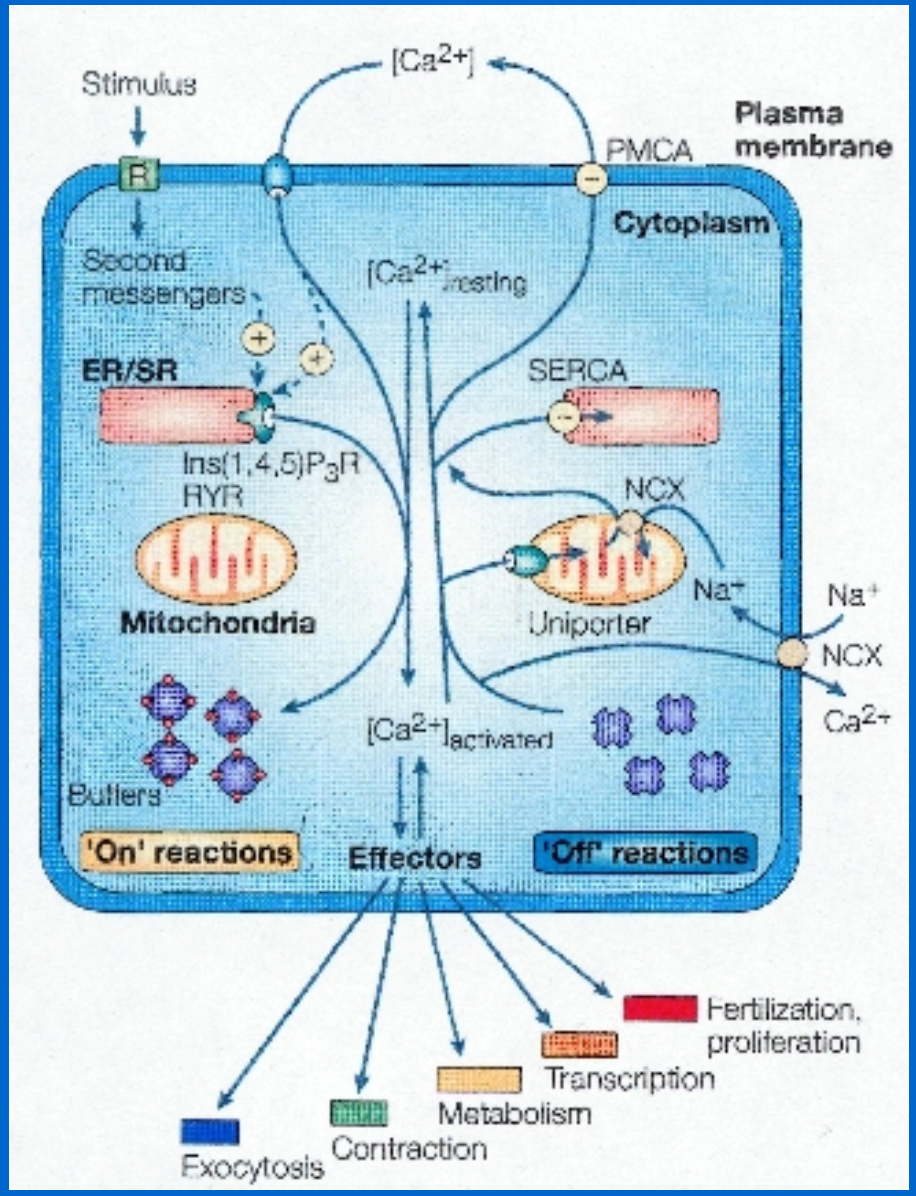


$[Ca^{2+}]$:

$10^{-7}M$ - $10^{-4}M$

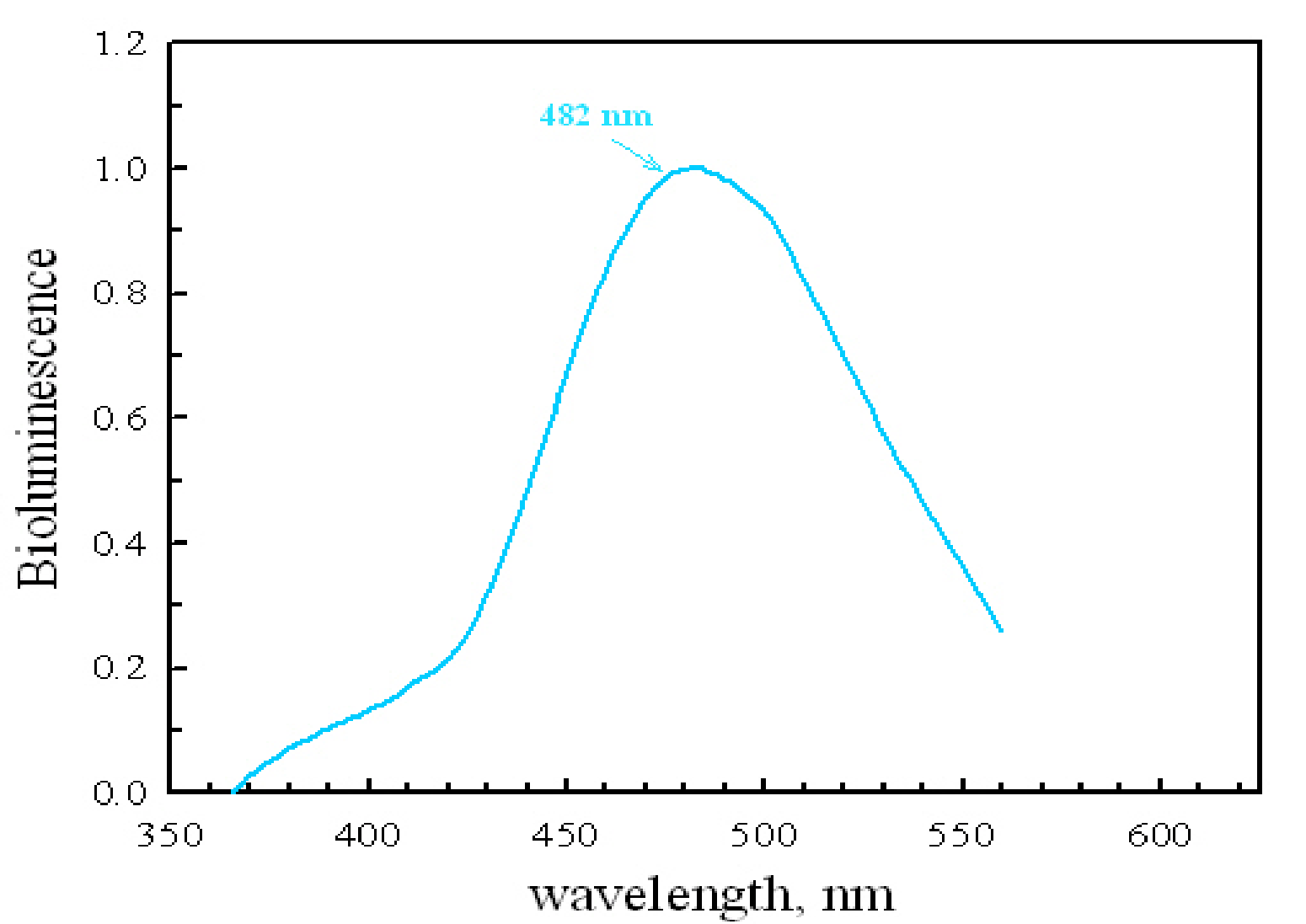
Physiological concentrations of calcium in cells

Calcium-signalling dynamics and homeostasis

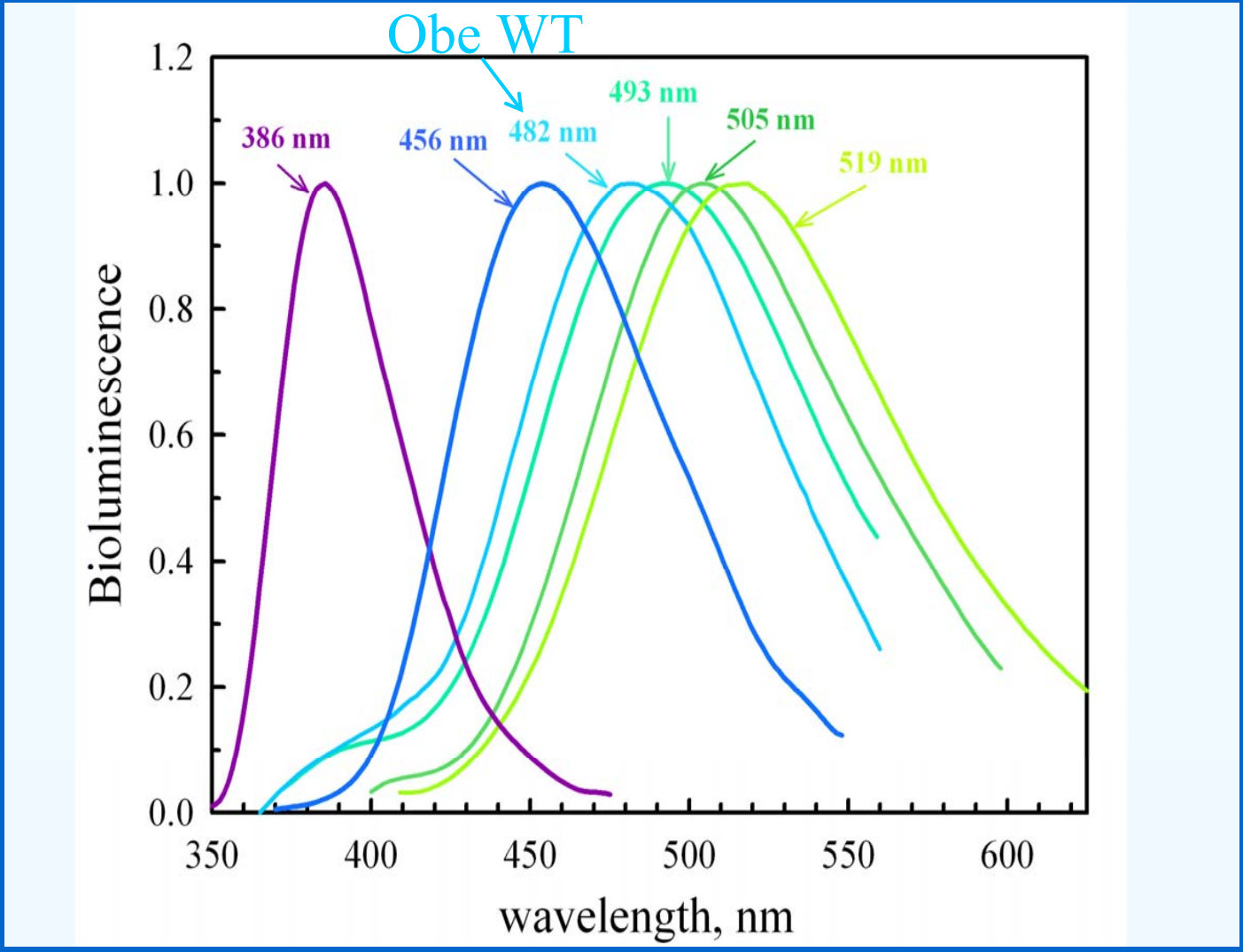


Nature Reviews/ Molecular Cell Biology, 2003, V. 4, 517-529.

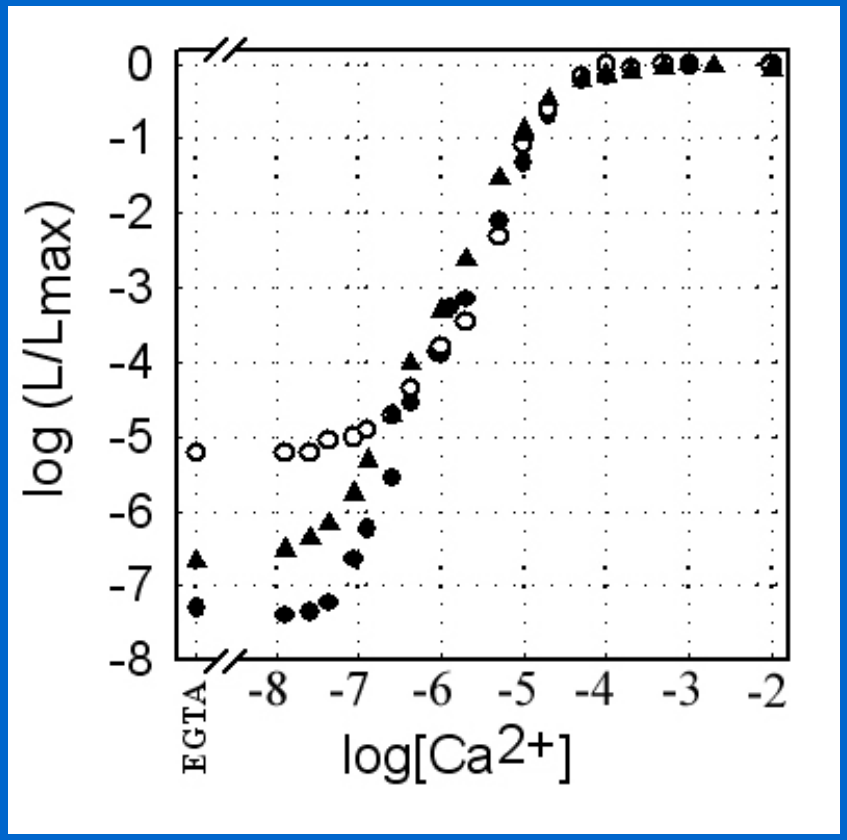
Bioluminescence spectrum of WT obelin

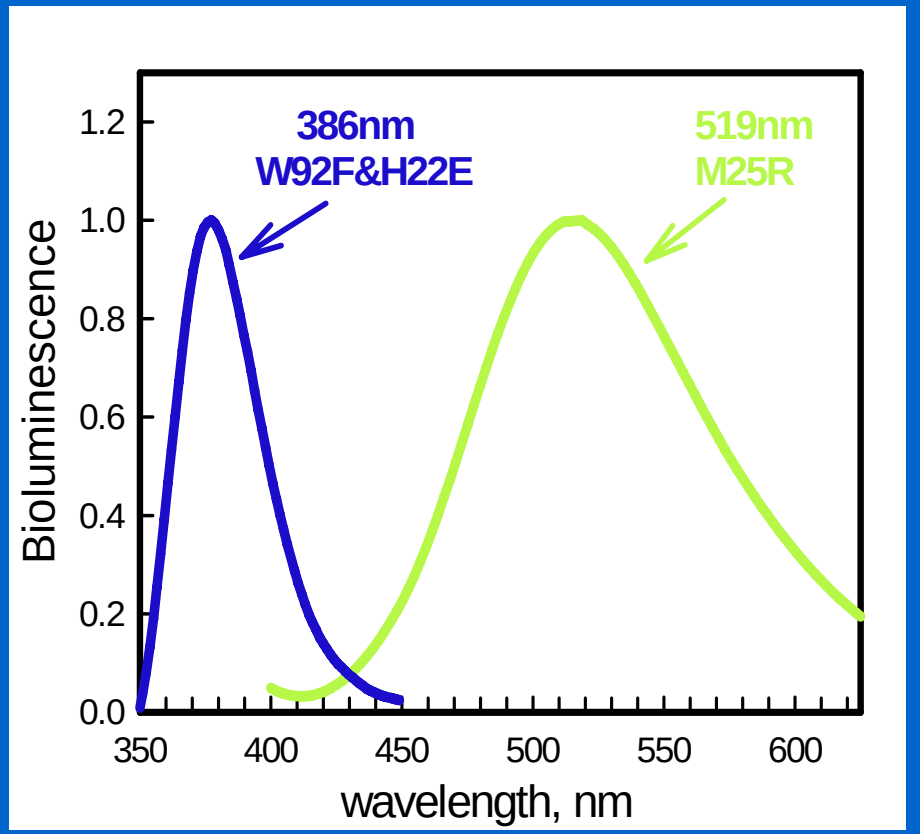


Site-directed mutagenesis of amino acids in obelin active site changes bioluminescence spectrum



Ca^{2+} concentration-effect curves for WT Obelin (●) W92F&H22E (▲) and M25R (○)

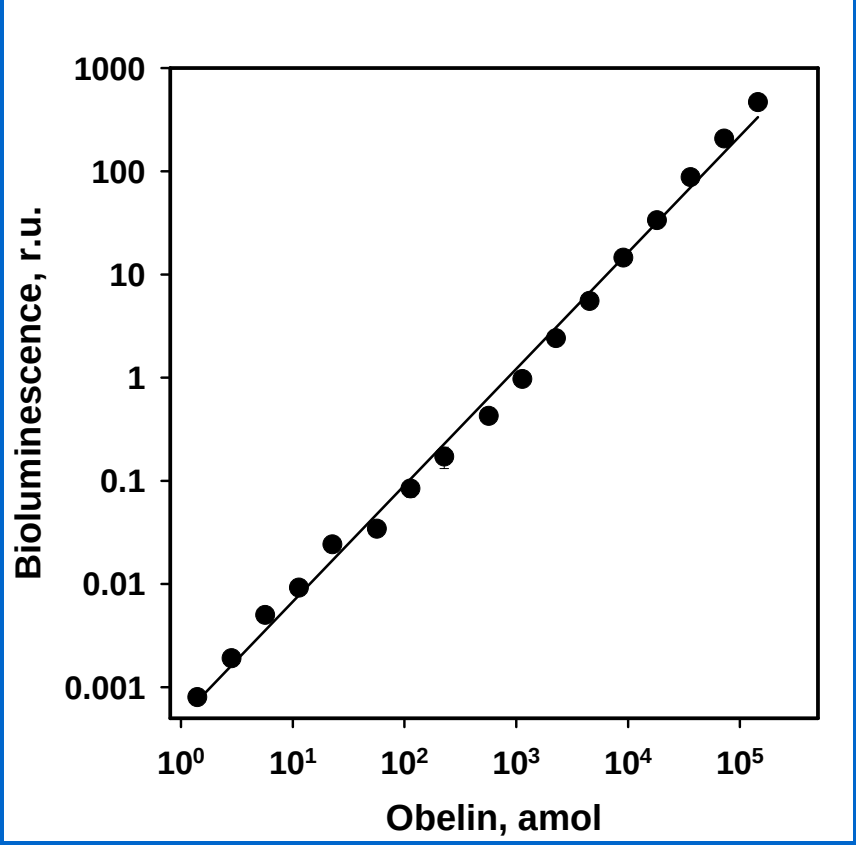




Application of photoproteins:

- ✓ *in vivo*
- ✓ ***in vitro***

Dependence of bioluminescence on photoprotein concentration



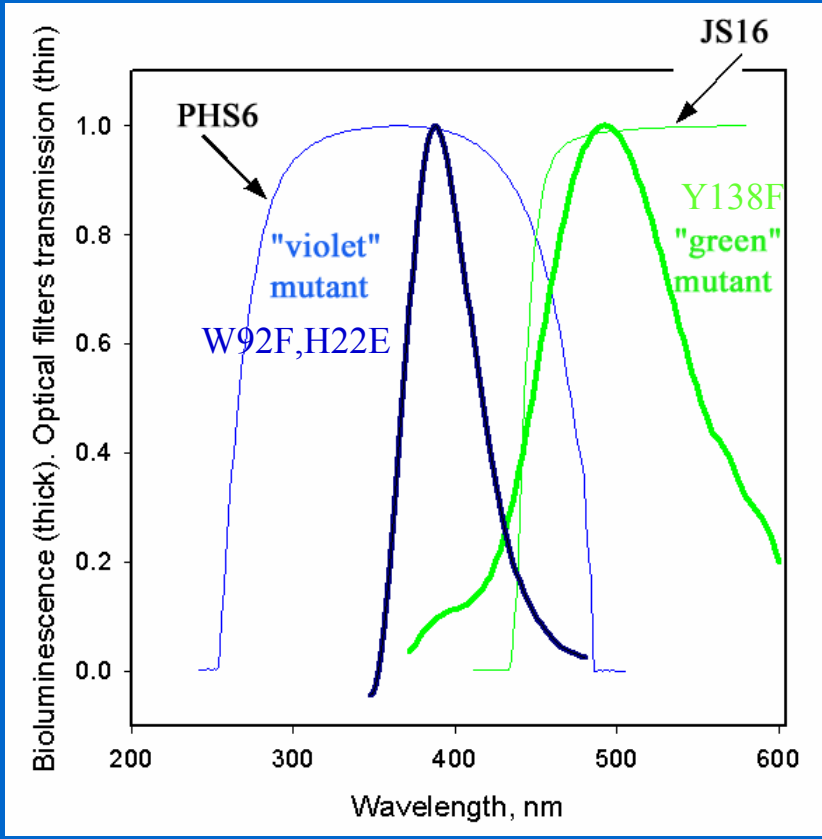
— in immunoassay

— in hybridization assay

Advantages of obelin label in assays *in vitro*

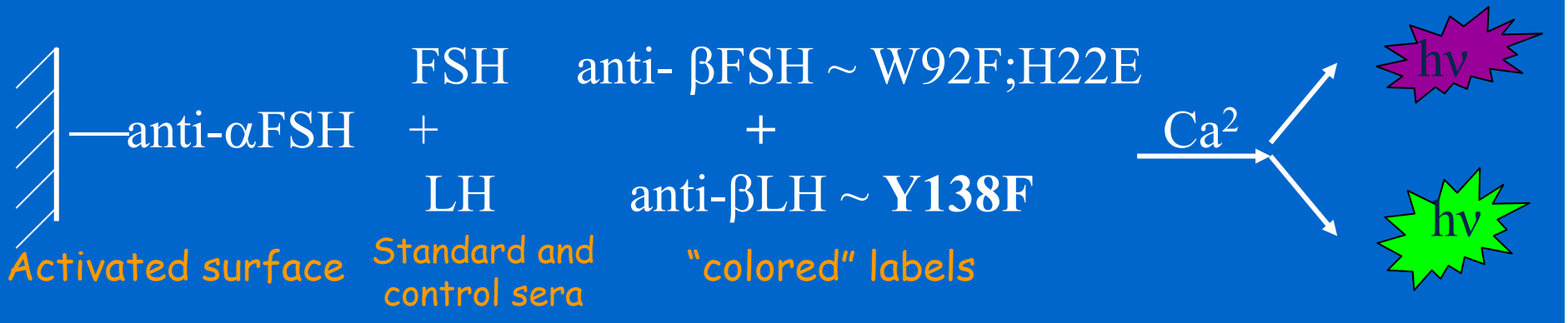
- Availability of recombinant protein and highly sensitive modern photometers
- High sensitivity
- Unlimited linear range to detect bioluminescent signal
- No background
- Simplicity of reaction triggerring
- Stability when stored in soluble and lyophilized state and under modifications
- No radiation hazard and wastes

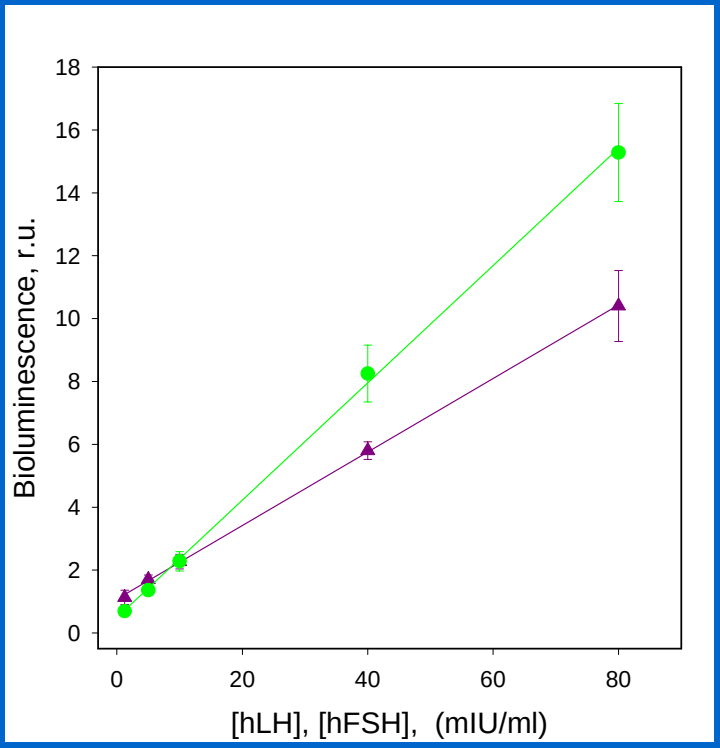
"Color" mutants of obelin in immunoassay

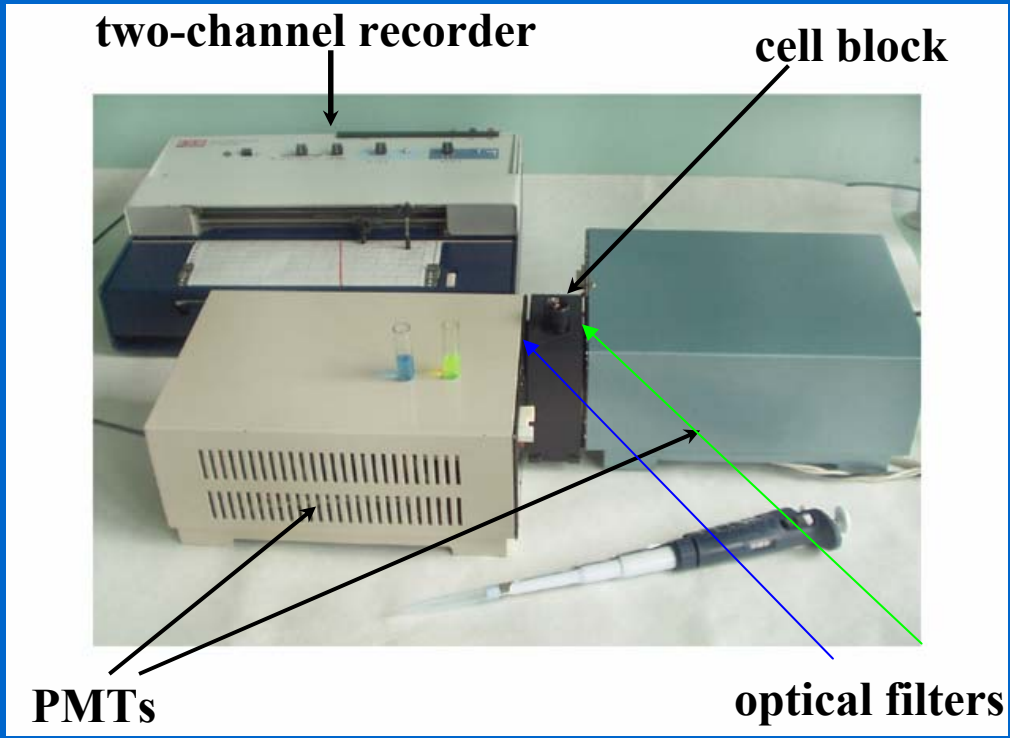


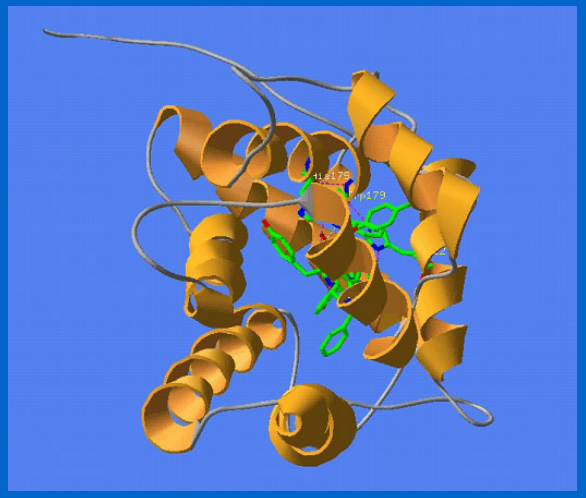
	λ_{max}	Activity%	$I_{Cafree}/I_{ca} \times 10^{-8}$
WT	**485**	**100**	**8.6**
W92F&H22E	**387**	**10**	**30**
Y138F	**493**	**67**	**25**

Simultaneous bioluminescent immunoassay of LH and FSH









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Thank you for attention!