

# Calcium-regulated photoproteins and their “color” mutants as bioluminescent reporters

N. Malikova and V. Borisova

Institute of Biophysics  
Russian Academy of Sciences  
Siberian Branch



# Bioluminescence - light emission of organisms

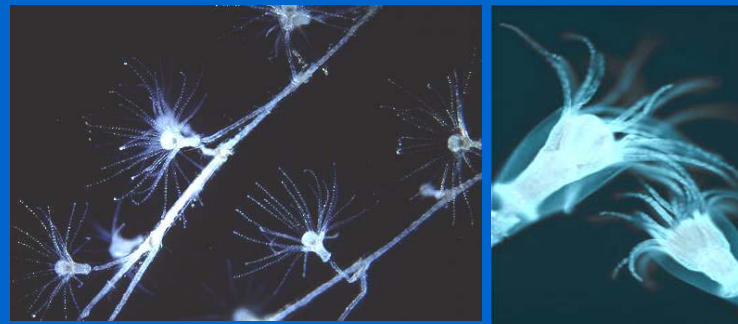
jellyfishes



fireflies



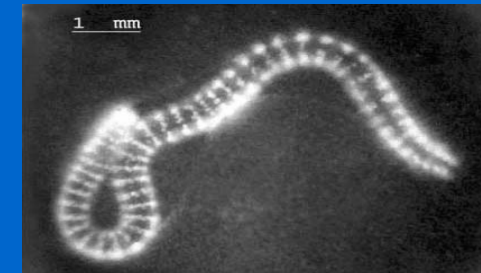
hydroid polyps



marine copepods



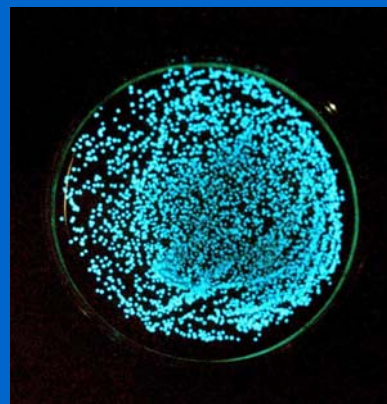
worms



sea pansy



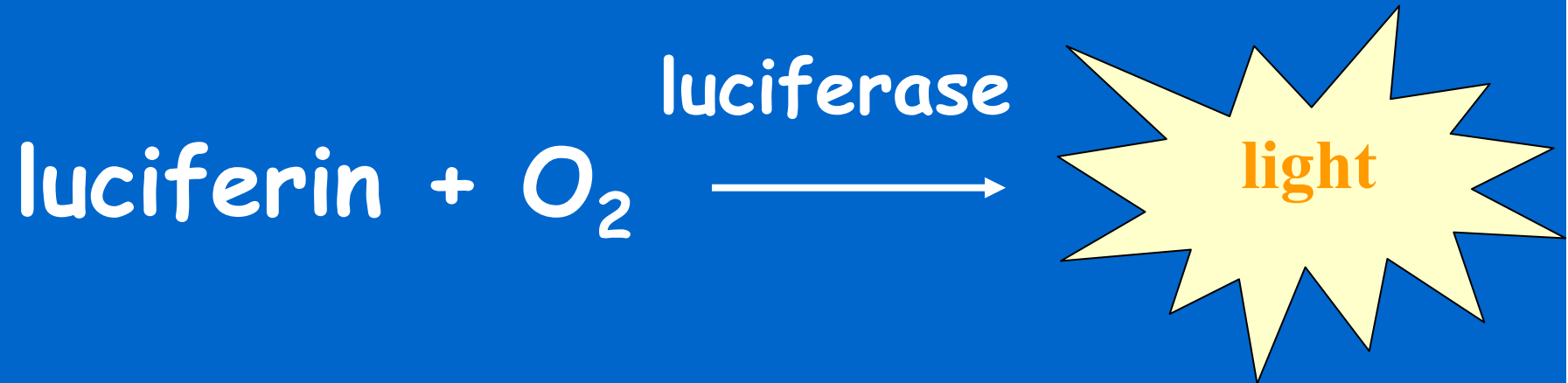
bacteria



ctenophores



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

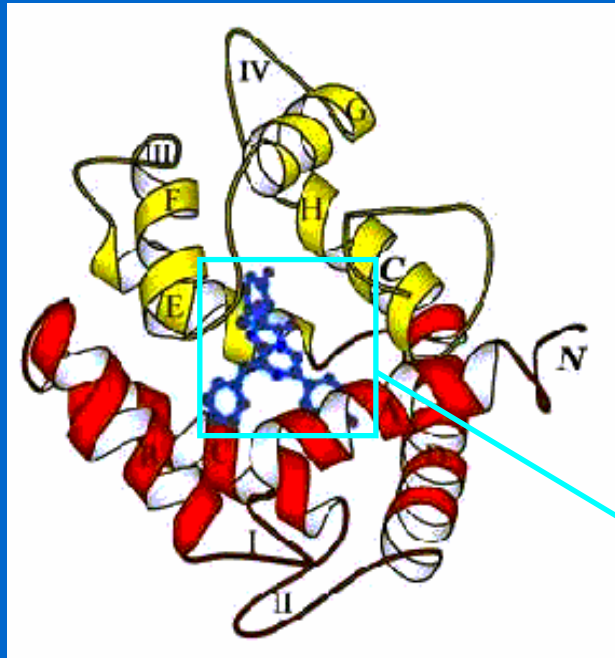




Obelin - stable "pre-charged" protein  
(single-chain, 22.2 kDa) triggered by calcium  
to emit light



*Obelia longissima*



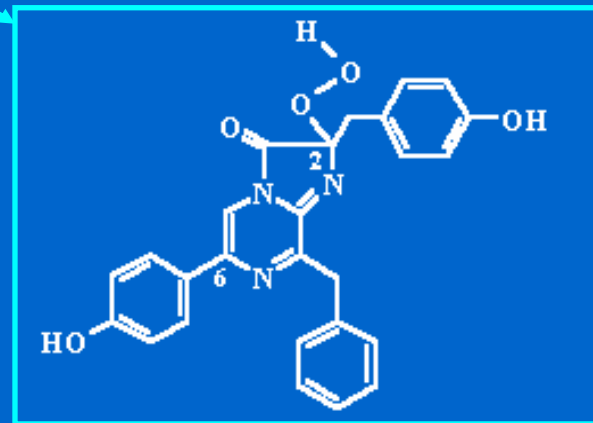
obelin molecule

$\text{Ca}^{2+}$



light

$\lambda_{\text{max}} = 485 \text{ nm}$



substrate: 2-hydroperoxycoelenterazine

# Application of photoproteins:

✓ *in vivo*

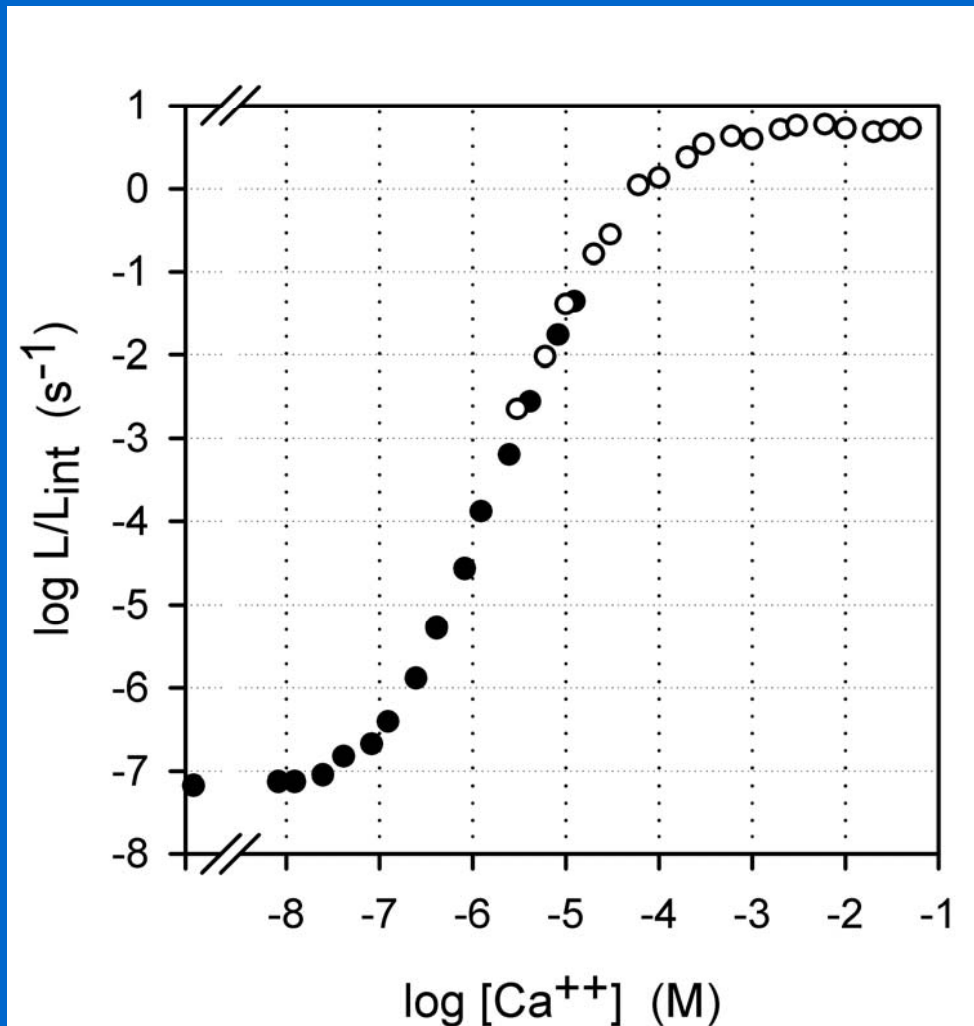
✓ *in vitro*

# Application of photoproteins:

✓ *in vivo*

✓ *in vitro*

# $\text{Ca}^{2+}$ concentration-effect curve for WT obelin

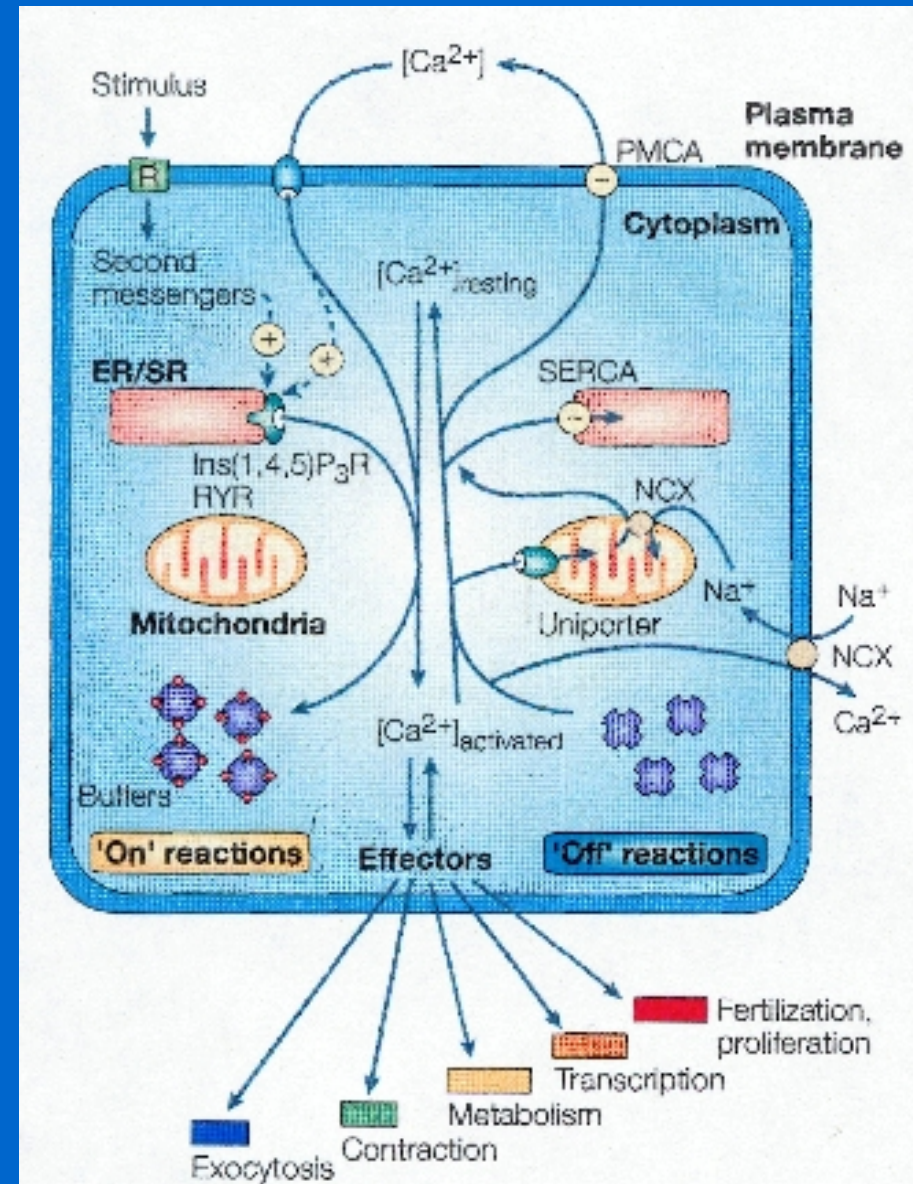


$[\text{Ca}^{2+}]$ :

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

Physiological concentrations  
of calcium in cells

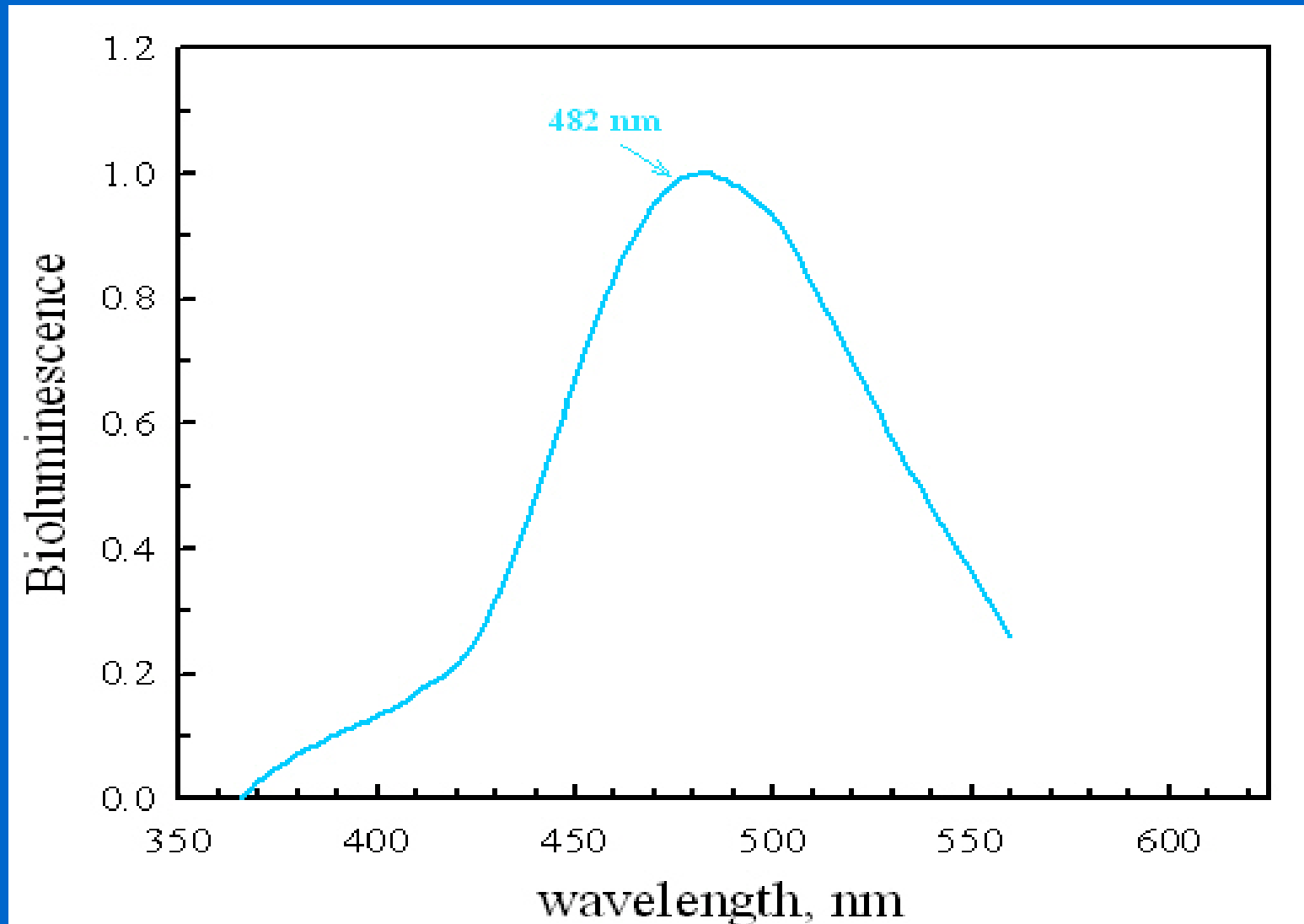
# Calcium-signalling dynamics and homeostasis



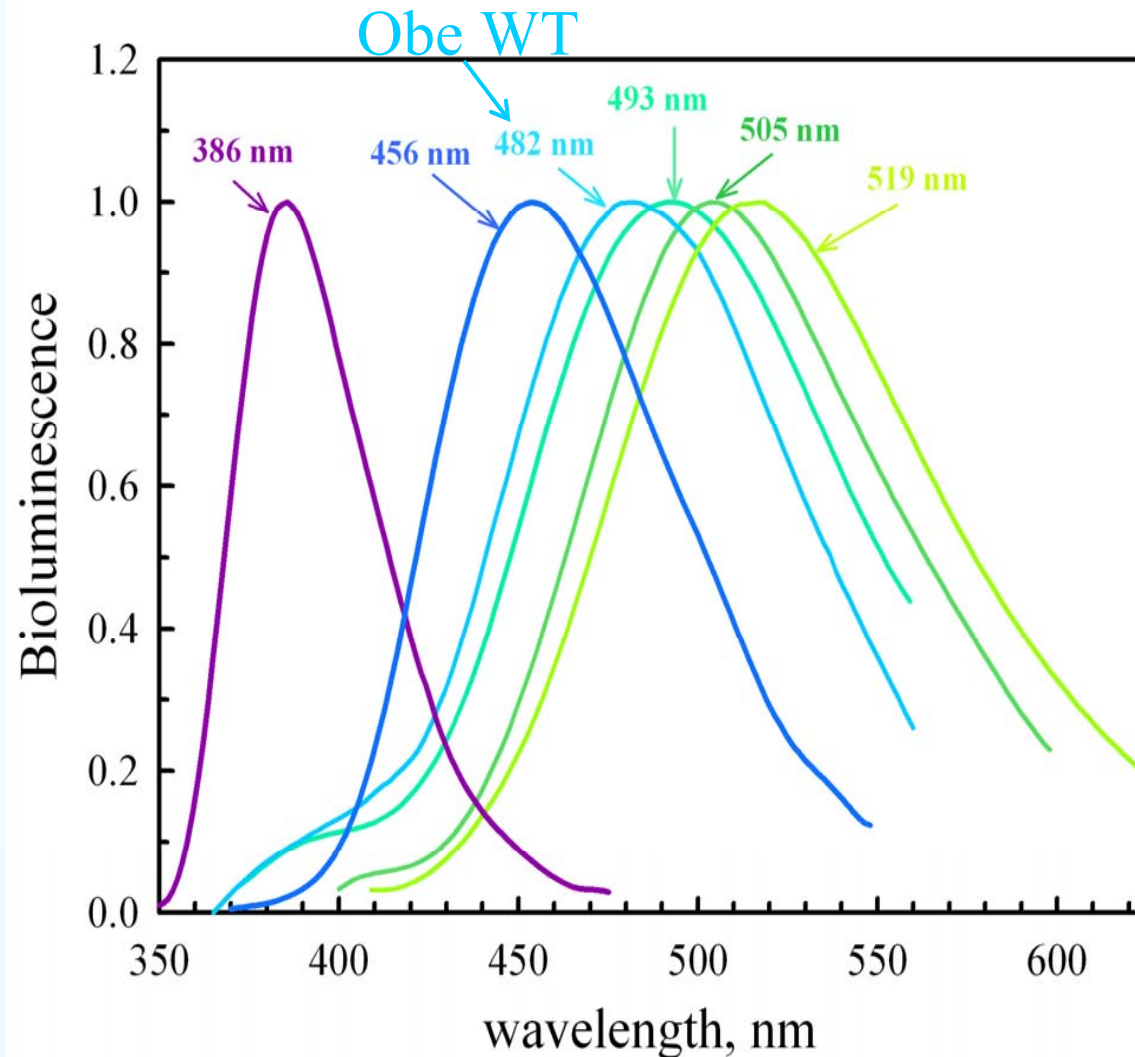
Nature Reviews/ Molecular Cell Biology,  
2003, V. 4, 517-529.



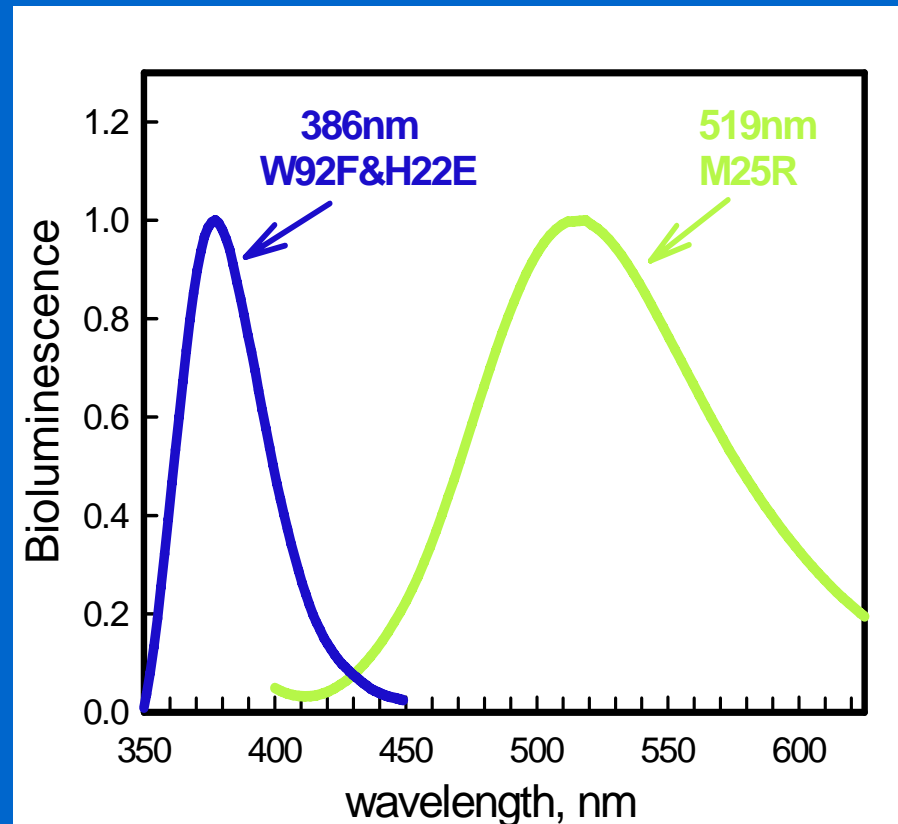
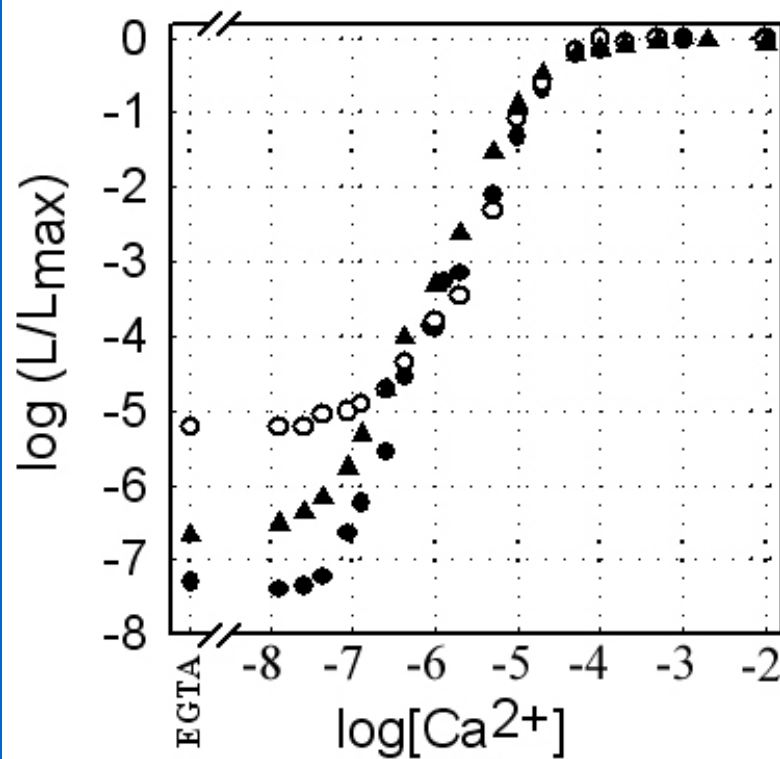
# Bioluminescence spectrum of WT obelin



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



# Ca<sup>2+</sup> concentration-effect curves for WT Obelin (●) W92F&H22E (▲) and M25R (○)

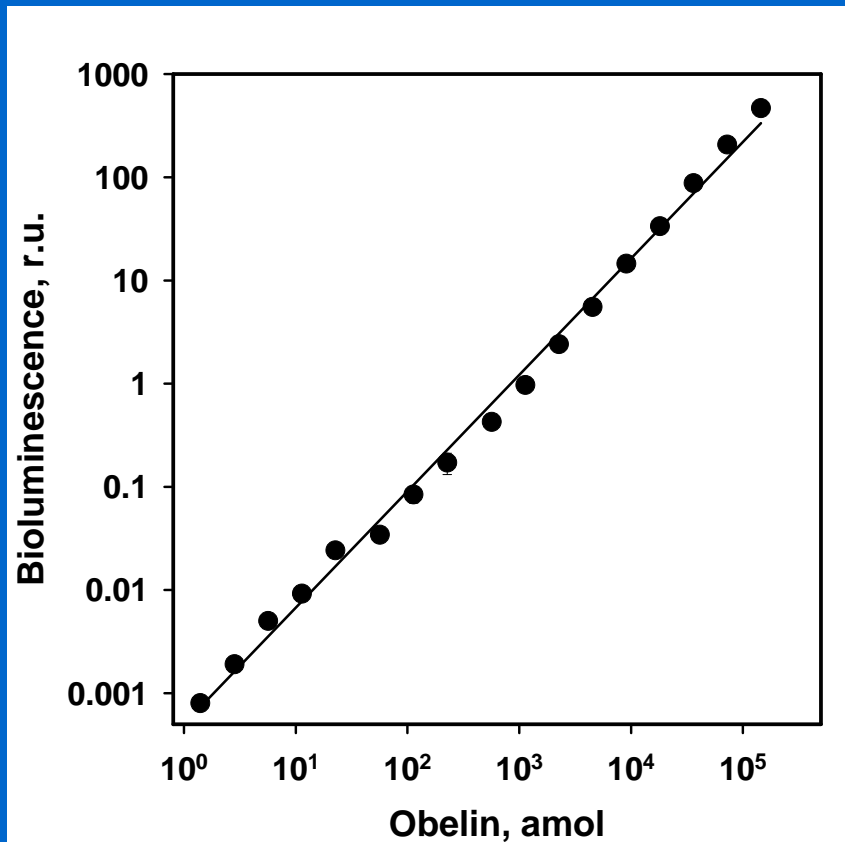


# Application of photoproteins:

✓ *in vivo*

✓ *in vitro*

# Dependence of bioluminescence on photoprotein concentration



— in immunoassay

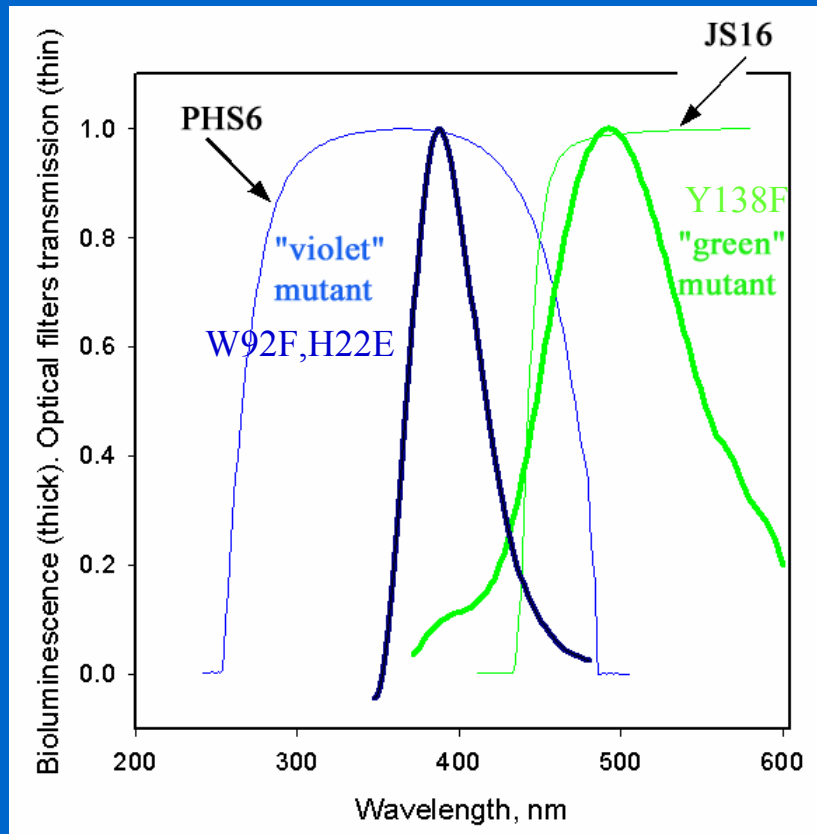
— in hybridization assay



# Advantages of obelin label *in assays in vitro*

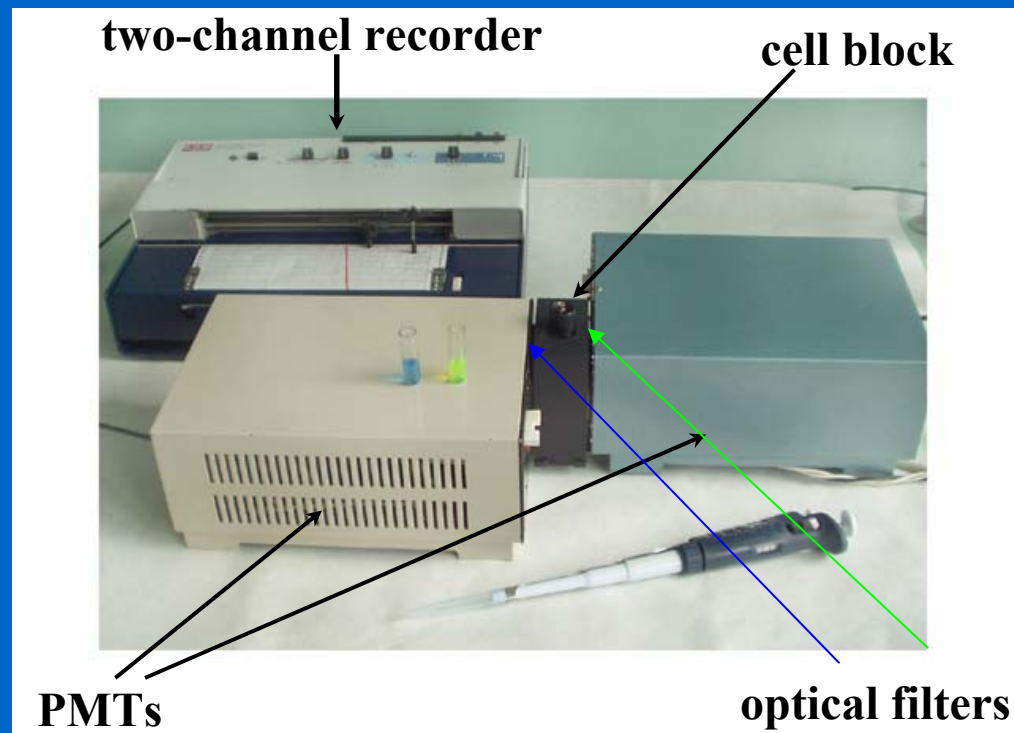
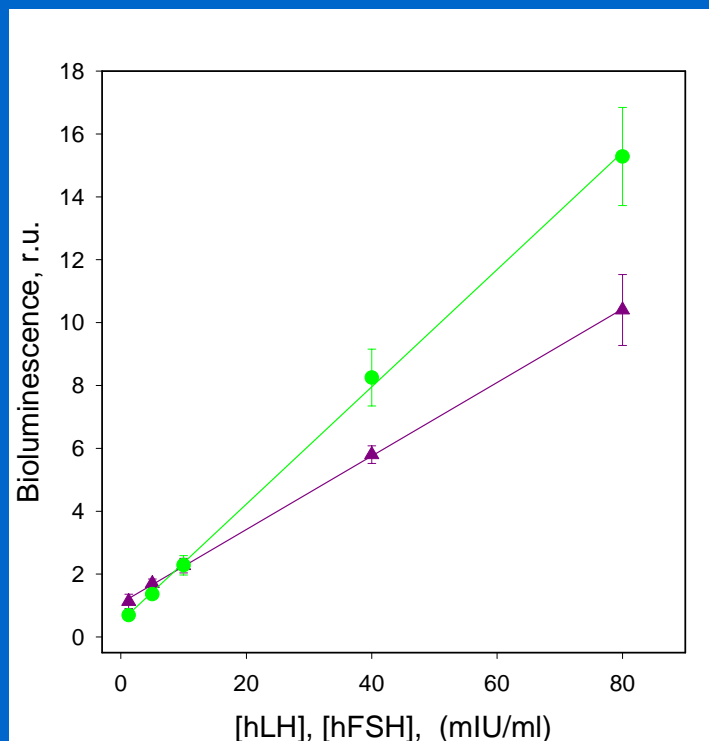
- Availability of recombinant protein and highly sensitive modern photometers
- High sensitivity
- Unlimited linear range to detect bioluminescent signal
- No background
- Simplicity of reaction triggering
- Stability when stored in soluble and lyophilized state and under modifications
- No radiation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

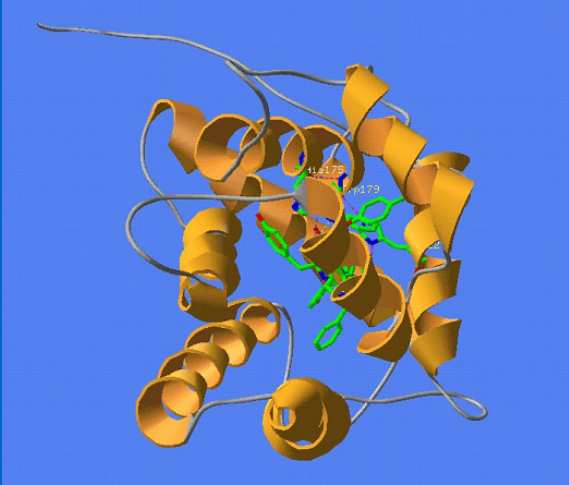
# "Color" mutants of obelin in immunoassay



	$\lambda_{\text{max}}$	Activity%	$I_{\text{Ca free}}/I_{\text{Ca}} \times 10^{-8}$
<i>WT</i>	485	100	8.6
<i>W92F&amp;H22E</i>	387	10	30
<i>Y138F</i>	493	67	25

# Simultaneous bioluminescent immunoassay of LH and FSH





## Acknowledgements:

The work was supported by the Grant of the President of Russian Federation (MK-1963.2005.4) and by the Lavrentiev grant for Young Scientists of the Siberian Branch of the Russian Academy of Sciences

# Thank you for attention!

