"Bioluminescent Cell-Based Assay European Seminar Tour 2014"

Multicolor Bioluminescence imaging: expanding the potentials of cell-based assays and in vivo imaging

Dr. Laura Mezzanotte

Dept. Of Radiology,Experimental Molecular Imaging
Leiden University Medical Center
The Netherlands

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Outline of the presentation

- Bioluminescence and analytical use of luciferases

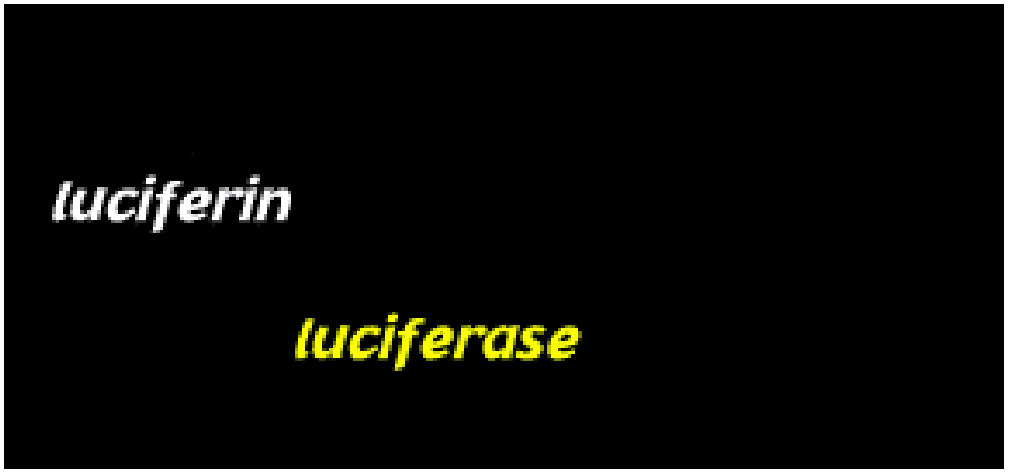
- Color tuning foundamentals and dual color imaging

- Application of multicolor bioluminescence in cell based assay

- In vivo applications: challanges and limitations

Bioluminescence

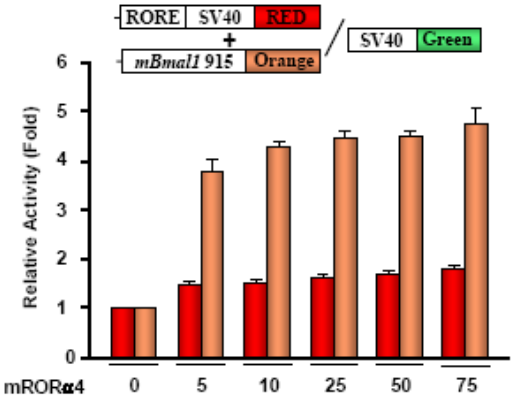
Bioluminescence (BL) is the production and emission of visible light by a living organism as a result of a chemical reaction (it's a kind of Chemiluminescence (CL)). It is generated by an enzyme catalyzed reaction wherein a luciferin substrate is oxydised by a luciferase.
Quantum efficency of BL systems are generally high: in the case of the firefly luciferase/luciferin it is about 0.44.



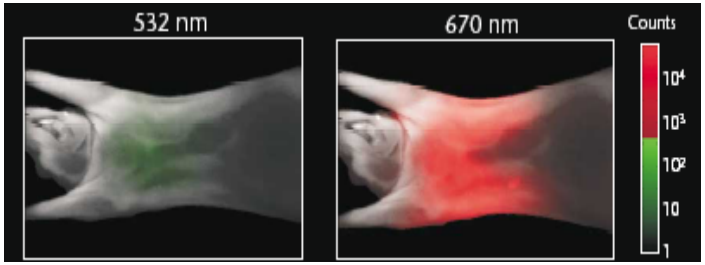
Haddock, S.H.D.; McDougall, C.M.; Case, J.F. "The Bioluminescence Web Page",http://lifesci.ucsb.edu/~biolum/ (created 1997; updated 2011)

Bioanalytical applications of luciferases

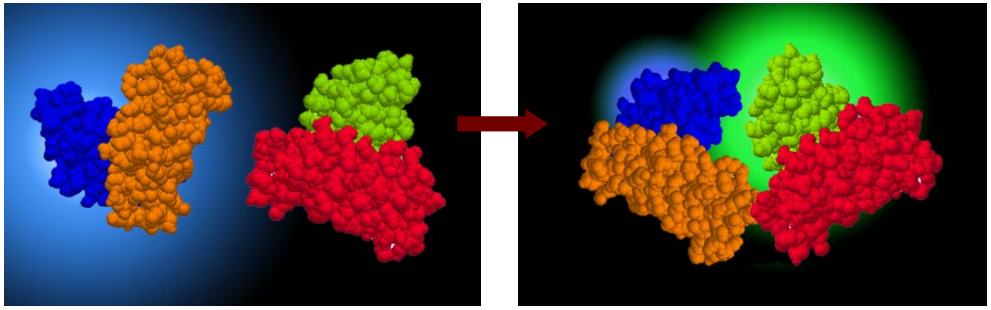
Reporter cell lines and biosensors for multiplex analisys



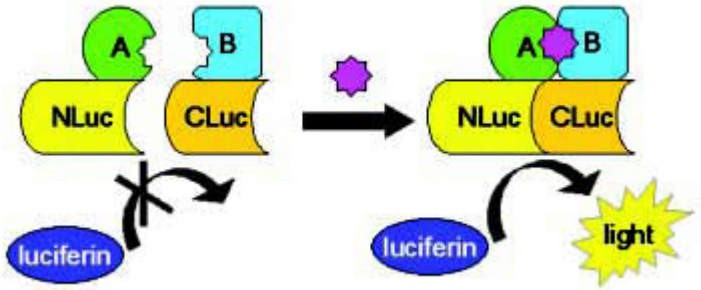
in vivo Imaging



BRET Systems



SPLIT Technology



Luciferases available as reporter genes

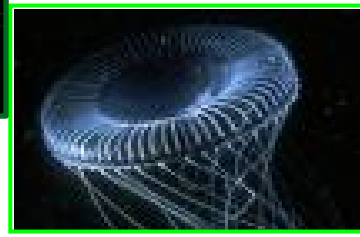
Click beetle luciferase

Gaussia luciferase

Metridia luciferase

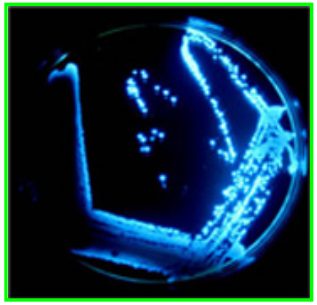
Oplophorus gracilirostris

Renilla luciferase



Click beetle larvae luciferase

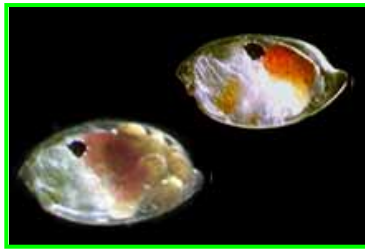
Bacterial luciferase



Photinus pyralis firefly luciferase

Luciola cruciata /Luciola Italica firefly luciferase

Cypridina sea firefly luciferase



Luciferase used in vivo

Firefly-

Click beetle

Luciferin +ATP+ Mg^{2+} + O_2

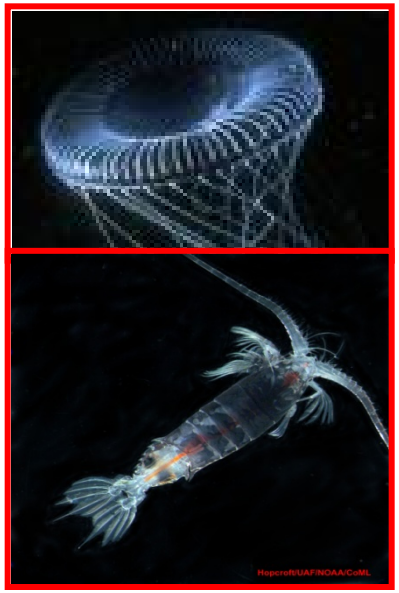
Fluc

CBluc

540-560nm

Renilla Gaussia Cypridina

Oplophorus



Coelenterazine/Vargulin/Furimazine

+O_2

hRluc

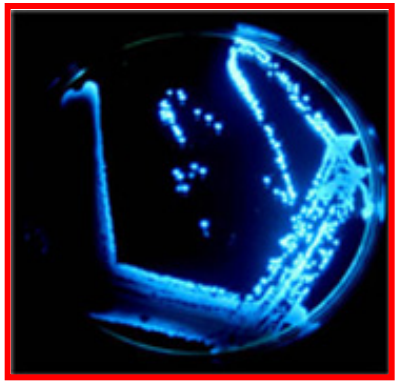
Gluc

CyLuc

NanoLuc

460-480nm

Bacterial



Aldehyde + $FMNH_2$ + O_2

luxAB

490nm

Smart reporter for bioluminescence imaging

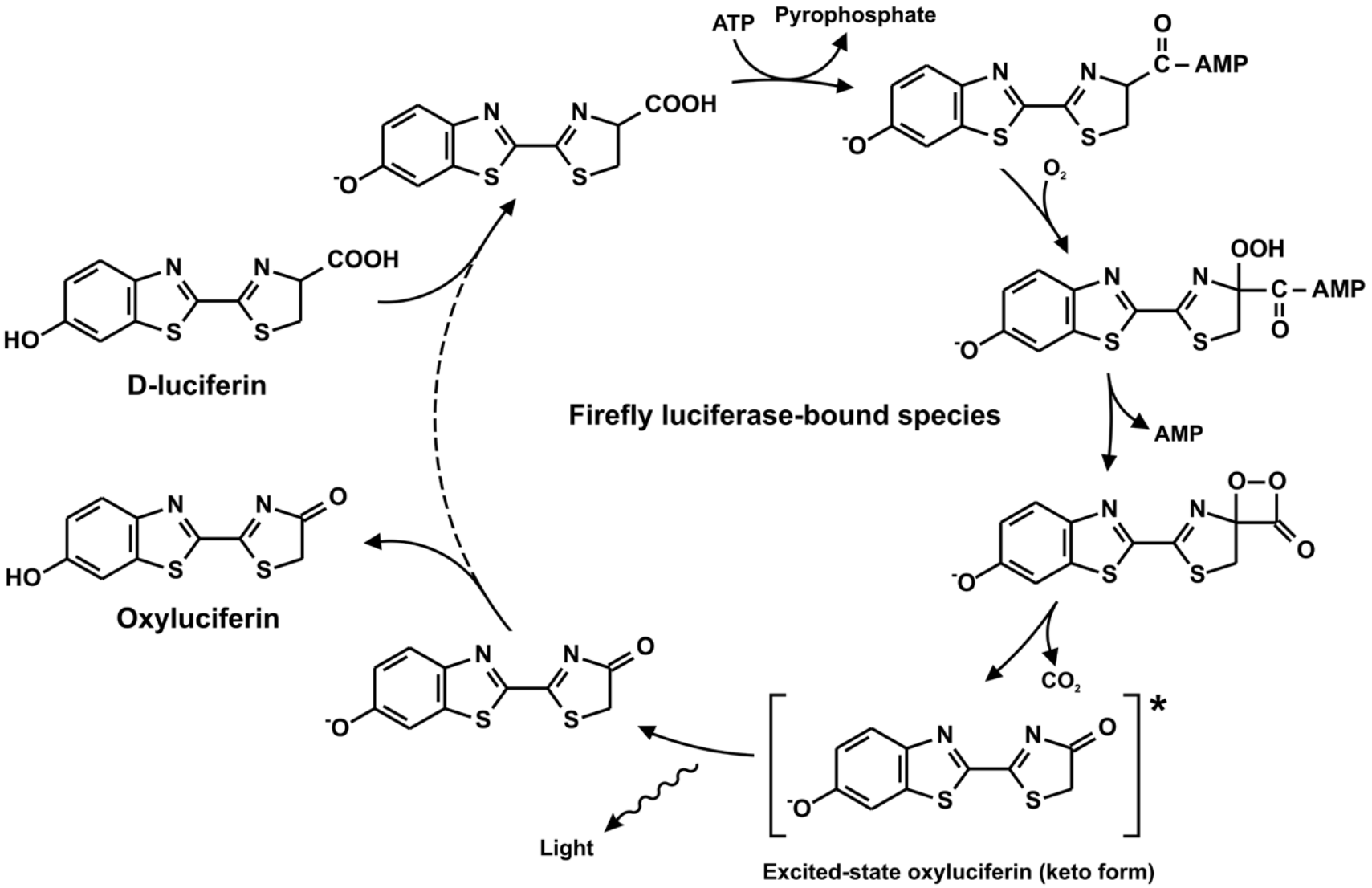
- High quantum yield of BL system
- High signal to background ratio
- Ph-insensitivity
- Thermostability
- No toxicity
- Narrow spectrum and isolated peak of emission for signal separation in multicolour applications
- Rapid turnover of substrate
- Biodistribution of substrate
- No immunogenicity

Sensitivity of detection?

Sensitivity of detection in vivo is determined by:

- Quantum Yield of Luciferase/Luciferin system
- Reporter Expression system
- Cell type
- Wavelenght of emission
- Quantum efficency and noise of camera
- Collection optics
- Depth within tissues
- Skin and fur color
- Background emission of live animals

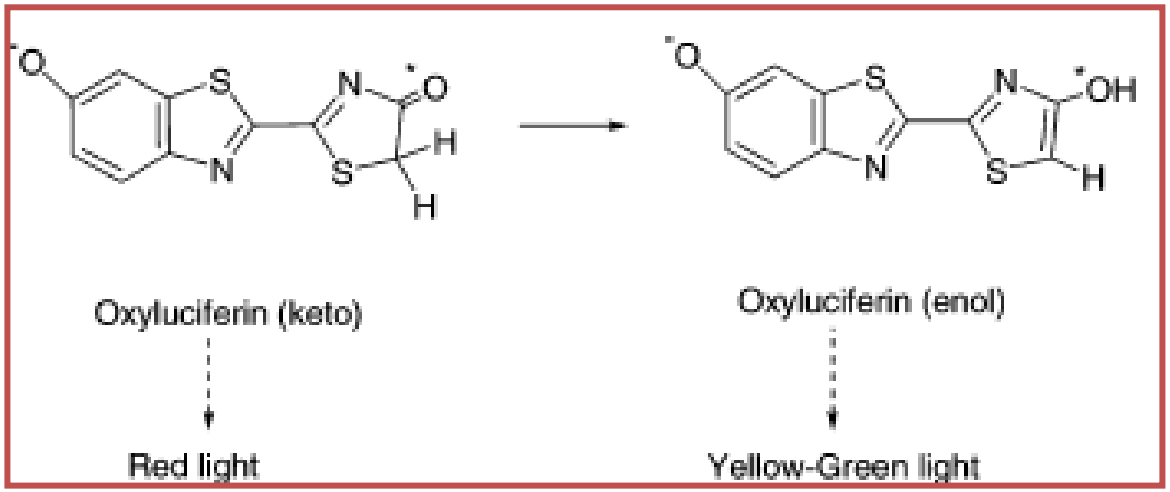
Luciferase-luciferin mechanism



Bioluminescence in analytical chemistry and in vivo imaging

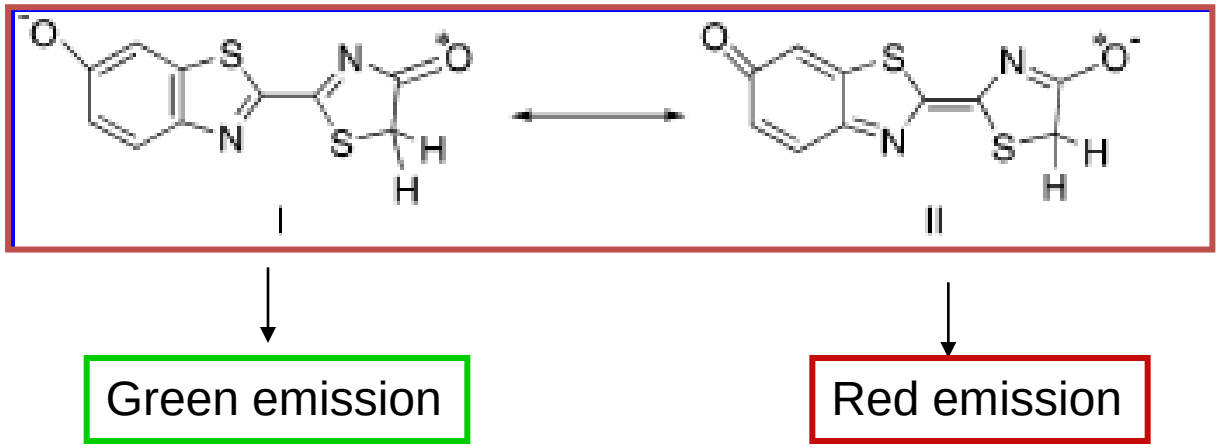
Roda A et al.*TrAC Trends in Analytical Chemistry, Volume 28, Issue 3, March 2009, Pages 307-322*

Theories on color tuning mechanism



1971 White et al.

Red light is ascribed to the keto form of excited state oxyluciferin and green light to the corresponding enol form of the emitter..



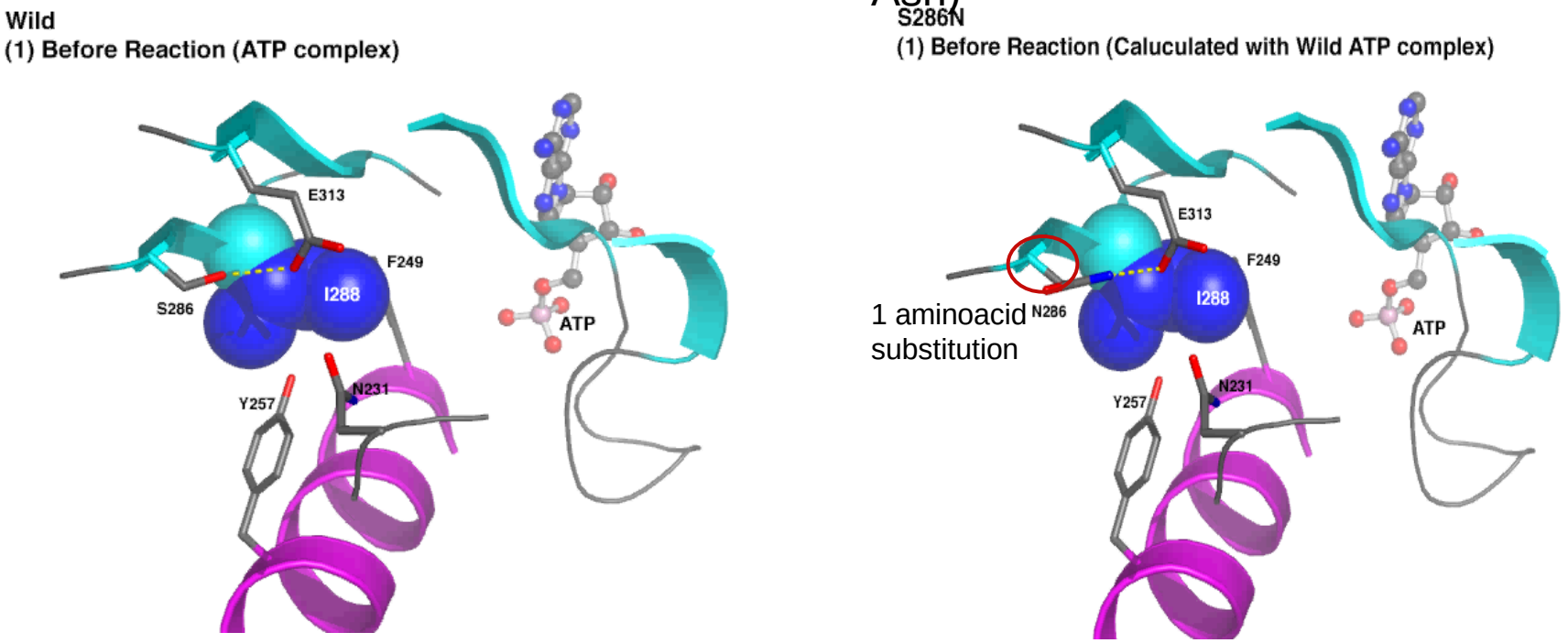
2004 Branchini et al.

A single emitter in the keto form could account for the range of bioluminescence color observed in nature. Resonance-based charge delocalization of the anionic keto form of the oxyluciferin.

Theories on color tuning mechanism

L. cruciata luciferase WILD TYPE

RED MUTANT (single mutation: Ser 286 Asn)



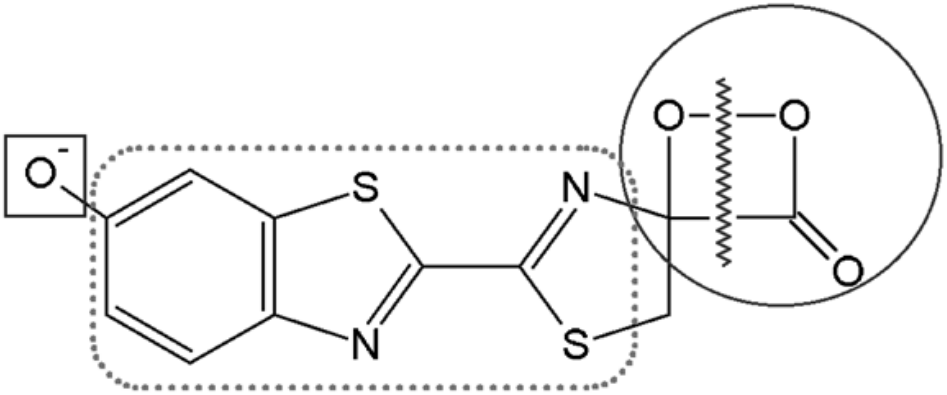
A conformational change in Ile 288 occurs in WT luc (in complex with AMP and LH_2) but not in the red mutant, shifting the emission wavelength.

The degree of molecular rigidity of the excited state → Shift in colour of BL emission

2006 Nakatsu et al. Nature

Theories on color tuning mechanism

CT (charge transfer) controlling group



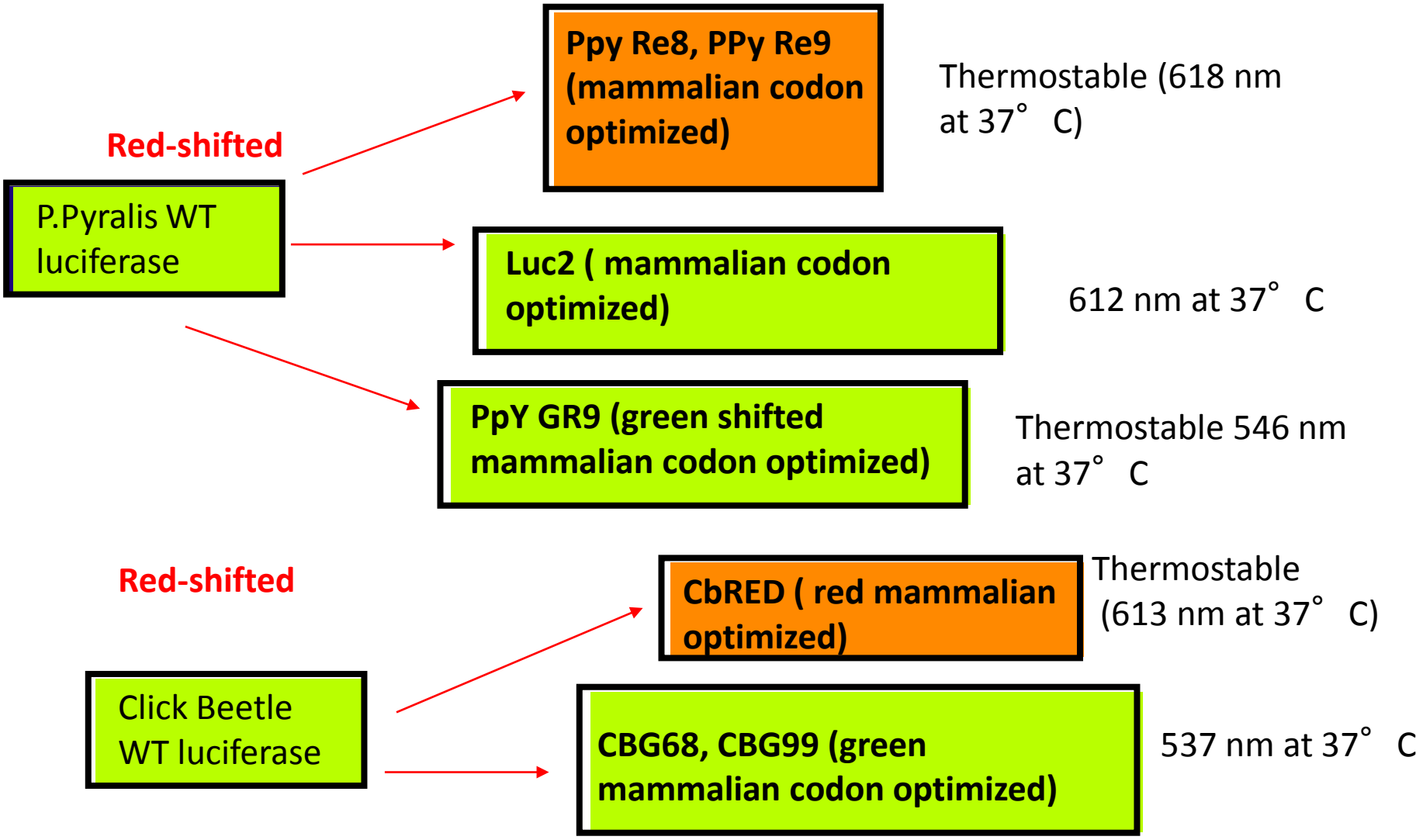
The chemiluminophore

The electron donating fragment

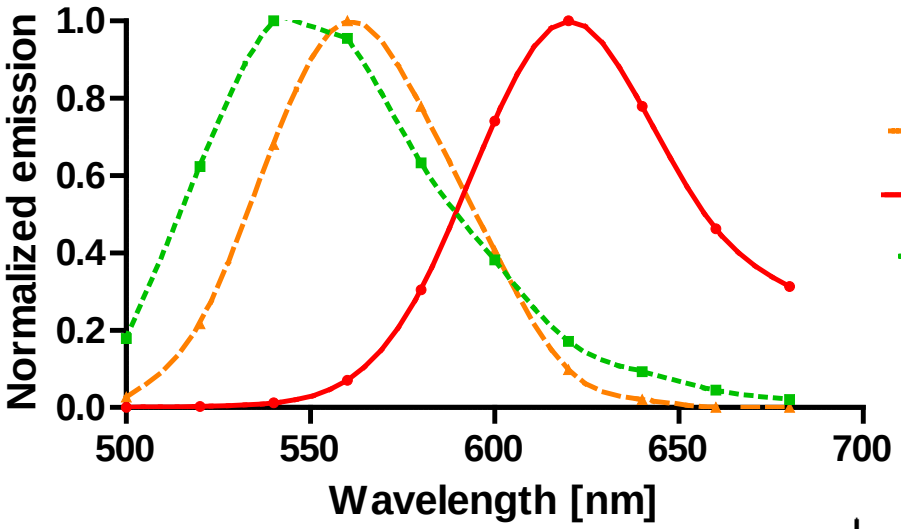
- The chemiluminophore opens the path to the excited-state surface
- The electron donating fragment lowers the activation energy of the reaction by means of a CT mechanism
- The CT controllin group turns the CT mechanism on or off and modulates the color emission, depending on the interactions between this moiety and the protein

The chemistry of bioluminescence: an analysis of chemical functionalities.
Navizet I et al. Chemphyschem. 2011 Dec 9; 12(17):3064-76.

Multicolor luciferases available

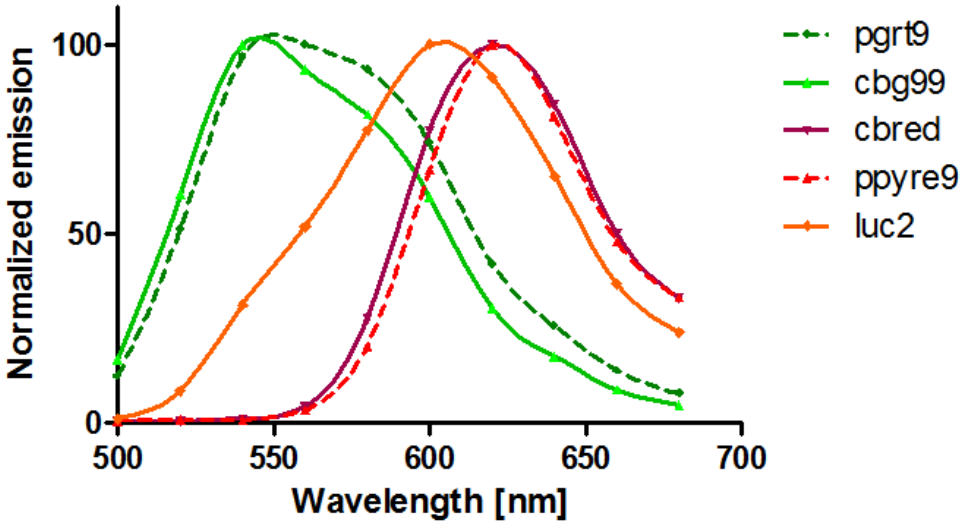


Multicolor luciferases: spectra of emission

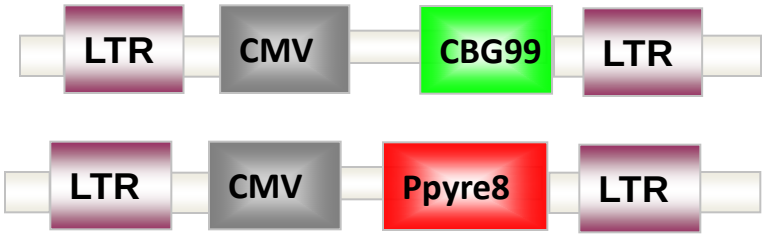


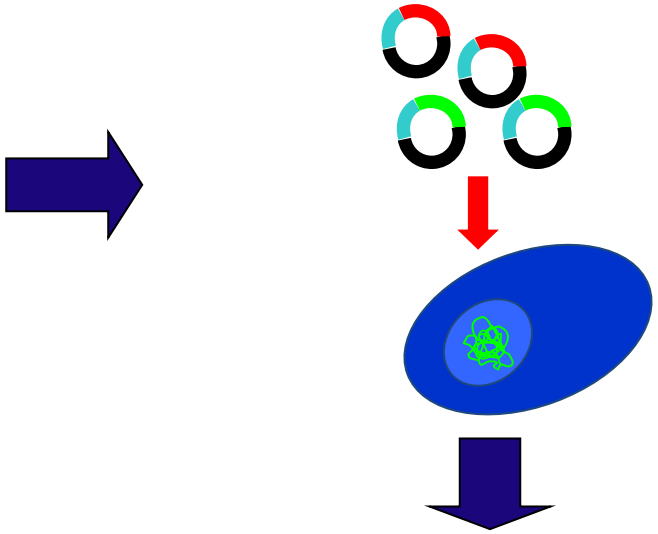
Normalized Emission spectra of multicolor luciferases at 25° C ph 7.8 on lysed cells using commercial luciferase substrate for assays

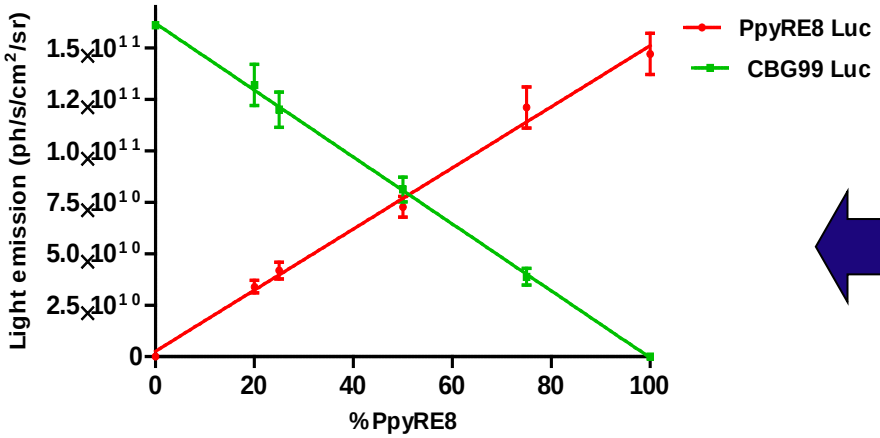
Normalized Emission spectra of multicolor luciferases at 37° C on live cells with 1mM luciferin

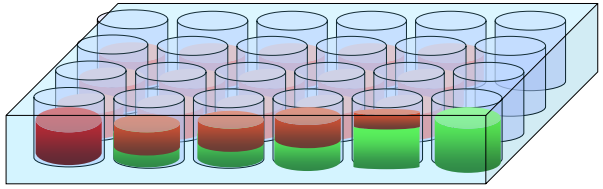


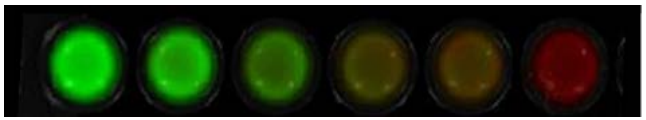
Spectral unmixing of bioluminescence





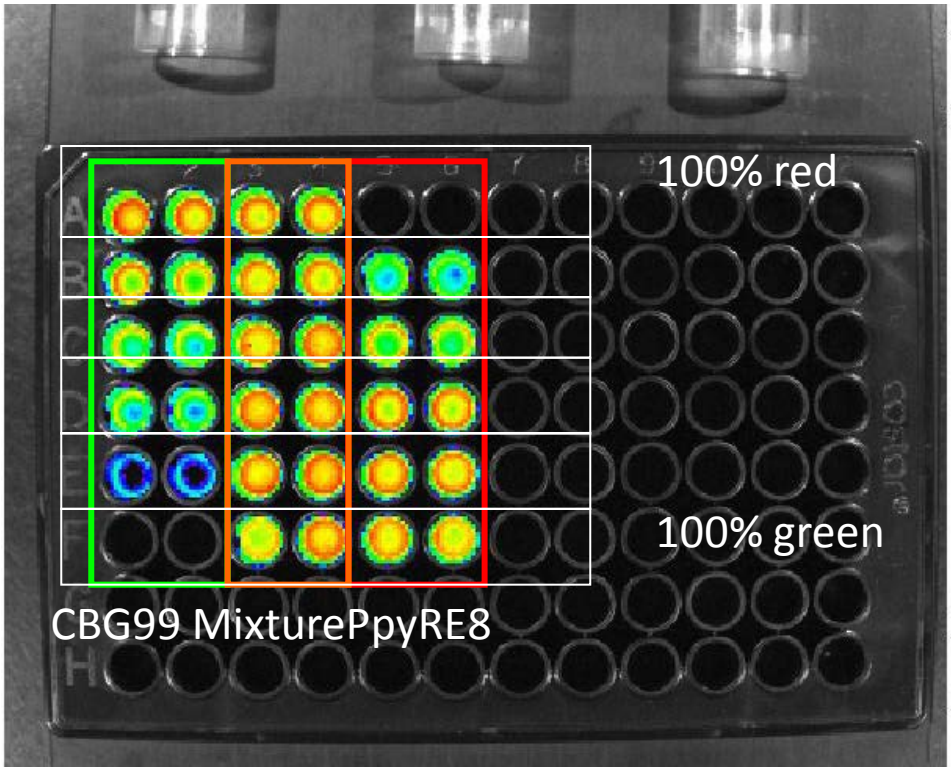


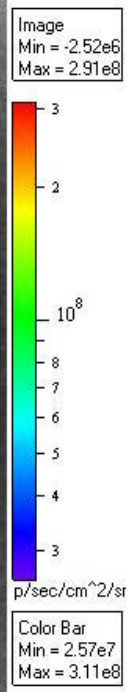




Mixing lysates of red and green luciferase expressing cells in different proportions

Spectral unmixing of bioluminescence





Unmixed1

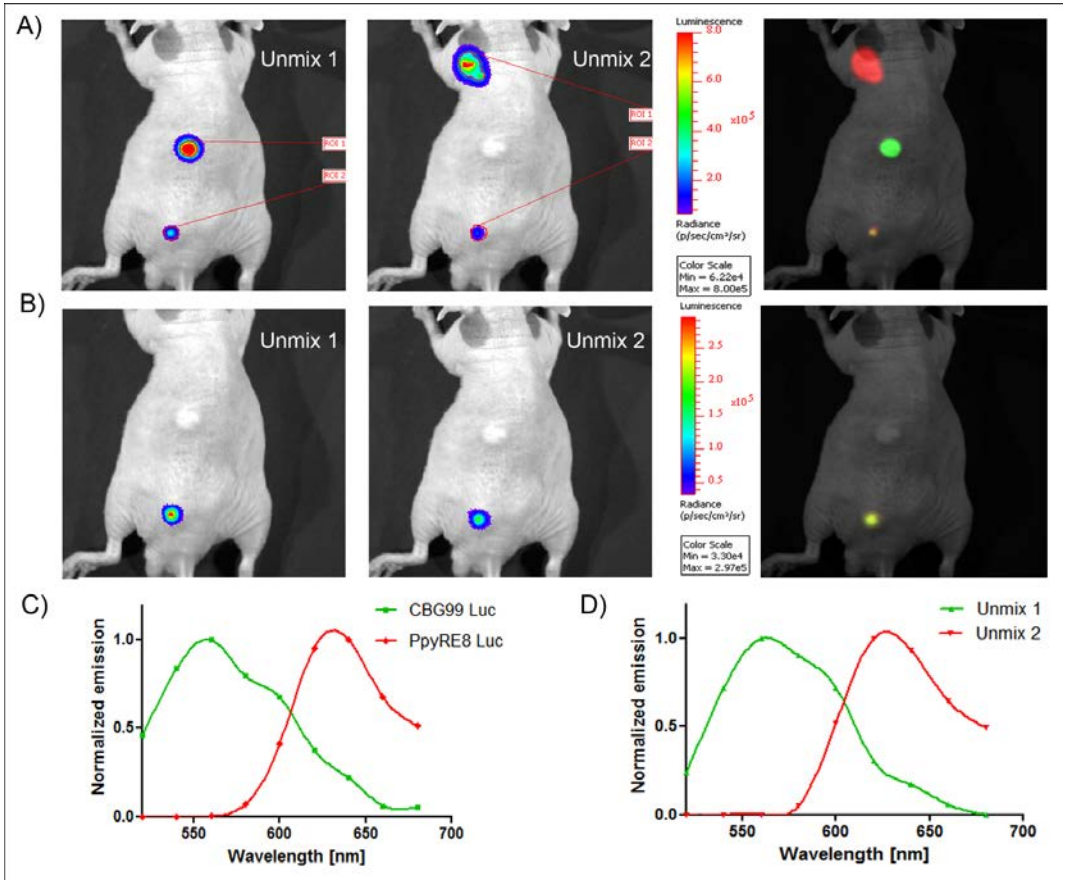
Unmixed2

Composite image

Spectral unmixing generated by Living image 4 software

Dual luciferase *in vivo* bioluminescence imaging

In vivo experiments by injecting Hek cells expressing PpyRE8 and CBG99 luc and a mixture and visualized after i.p. injection of D-luciferin



Mezzanotte L, Que I, Kaijzel E, Branchini B, Roda A, Lowik C Plos ONE 2011 Apr 22;6(4):e19277.

Animal model: which mouse is better?

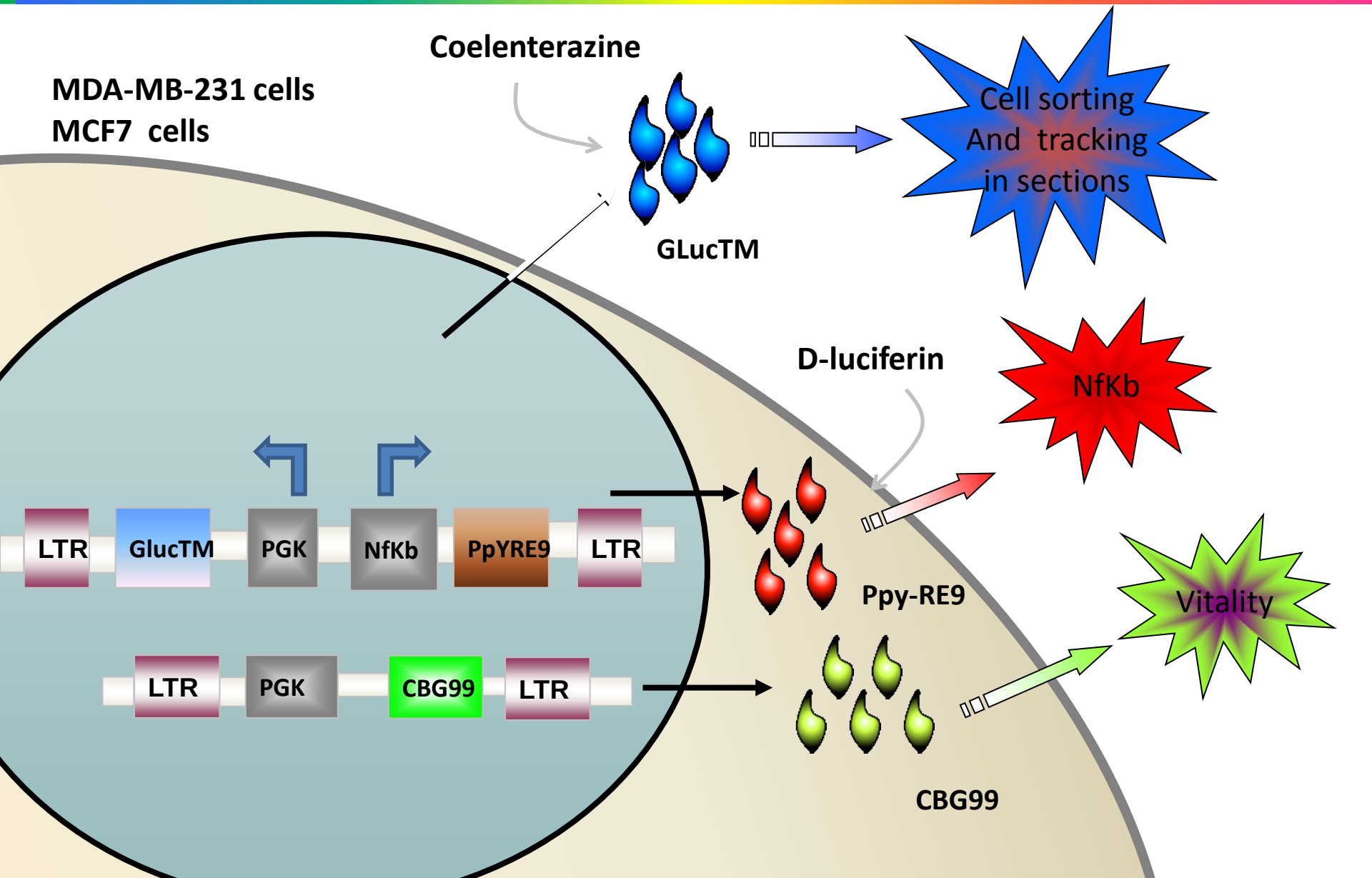
CD1 Nude mice (e.g. for tumor biology to obtain enhanced light transmission and reduced scattering)
Balb/c nude
NOD SCID nude

BALB/c
B6(Cg)-*Tyr*$^{c-2J}$/J (Albino B6)
NOD SCID

PGP Mouse P-glycoprotein Deficient (PGP-deficient blood brain barrier model, CNS, transport/excretion for neurobiology

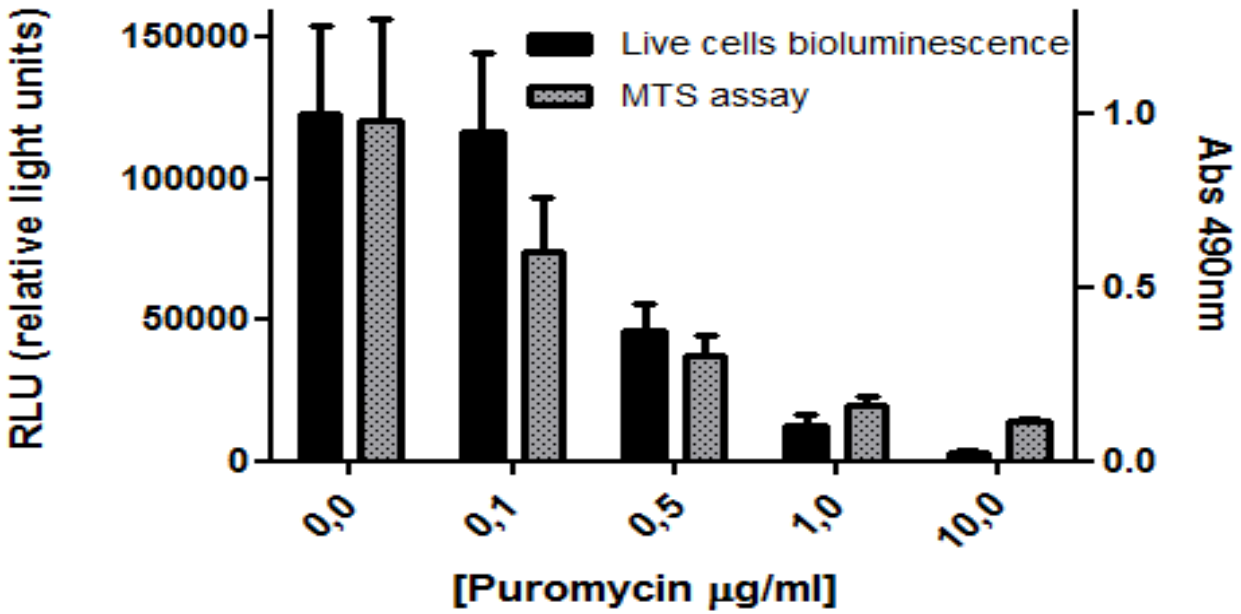
POUND MOUSE (for obesity, hyperinsulinemia, insulin resistance, dyslipidemia, metabolic syndrome)
C57BL/6

Triple color Breast cancer cell line for monitoring NfKb pathway in vitro and in vivo

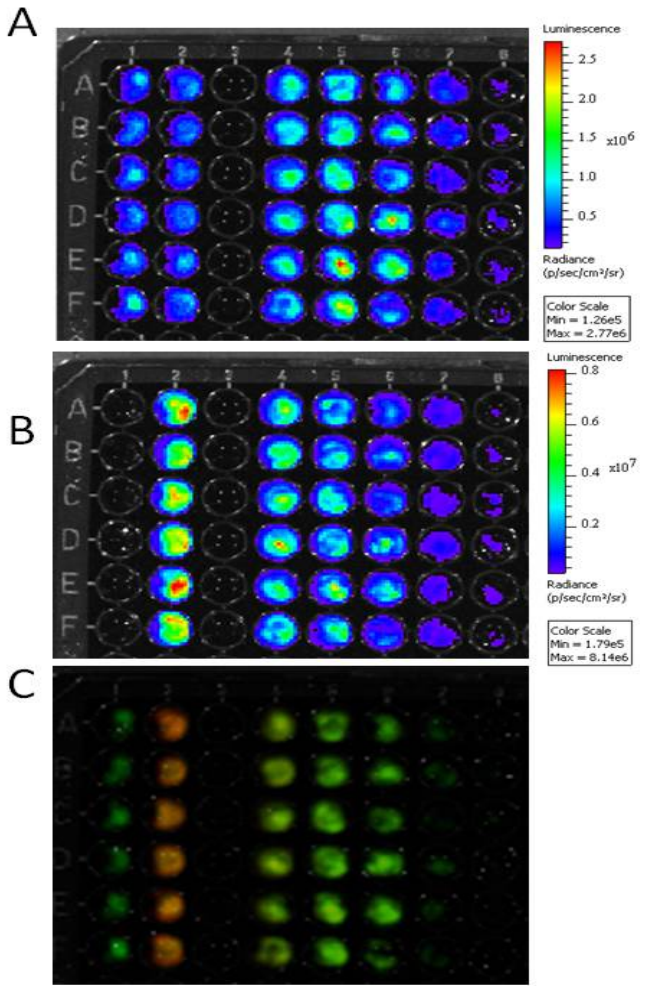


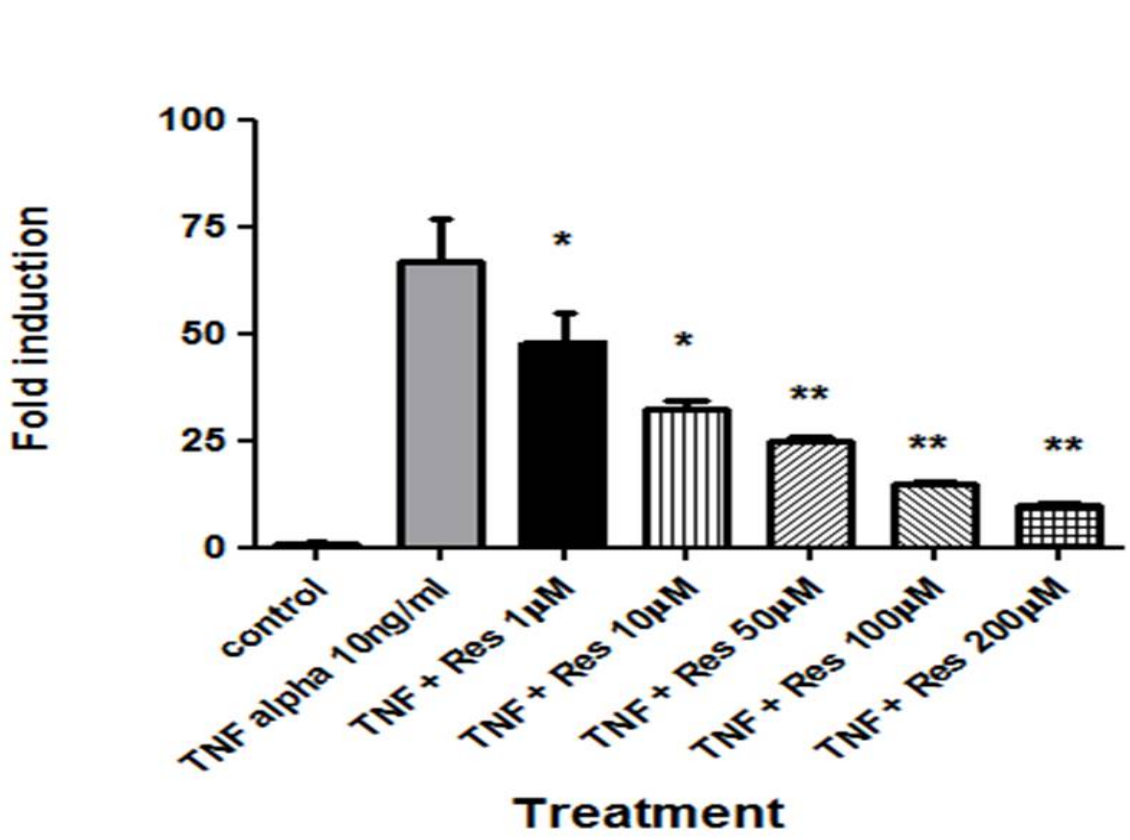
Validation of dual and triple color cell line

Correlation between MTS proliferation assay and green signal



Validation of the triple color cell line: resveratrol



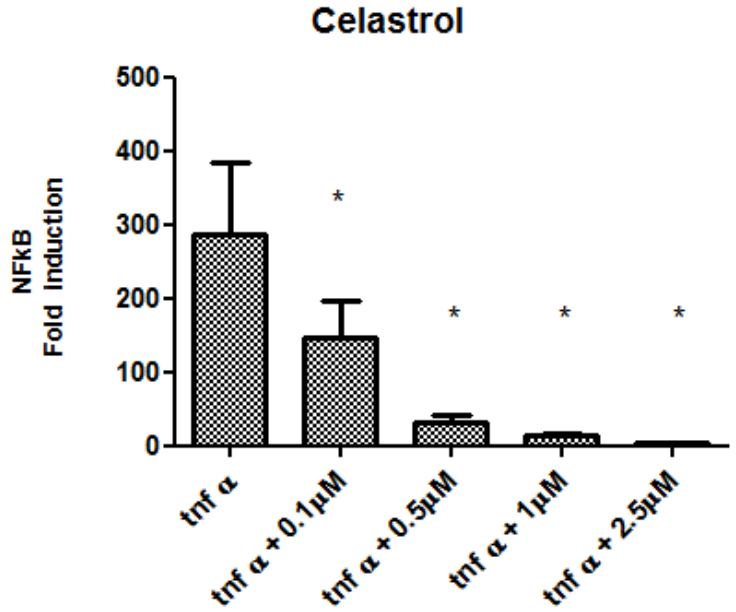


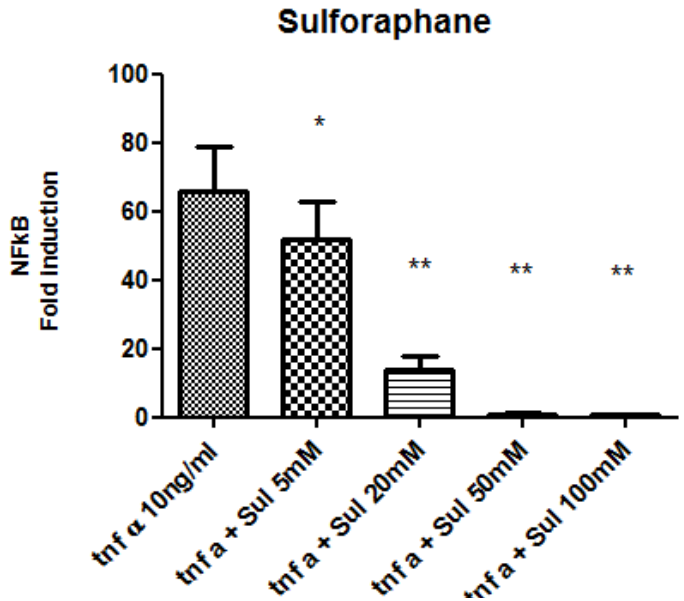
Intra assay variability 6%

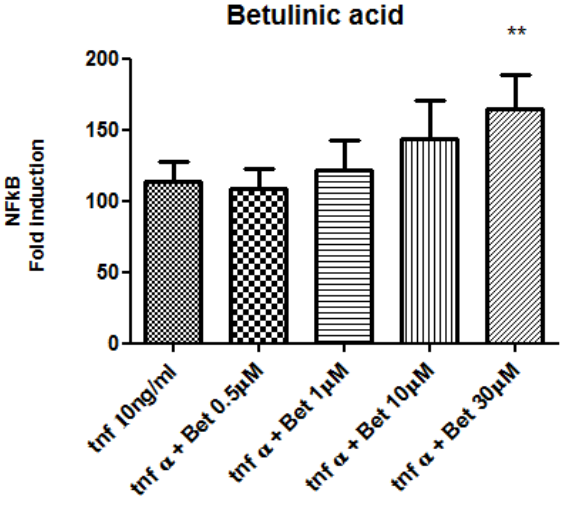
Inter assay variability 6.9% (within plate variability)

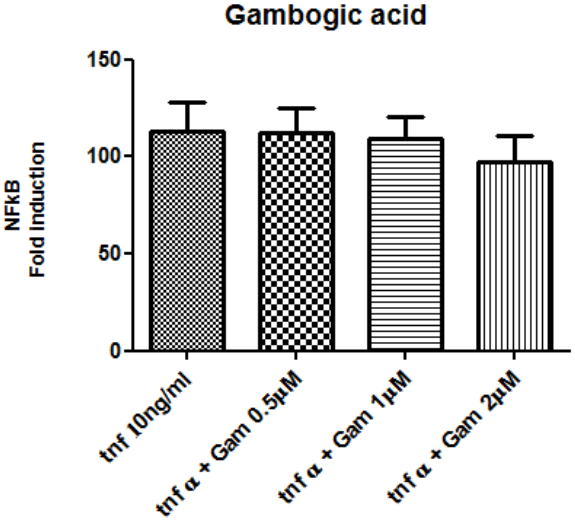
Inter day variability 34%

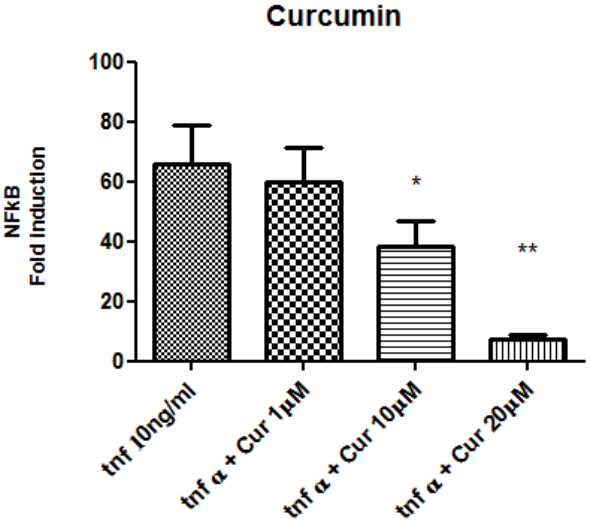
Testing chemopreventive compounds acting on NFkB signalling



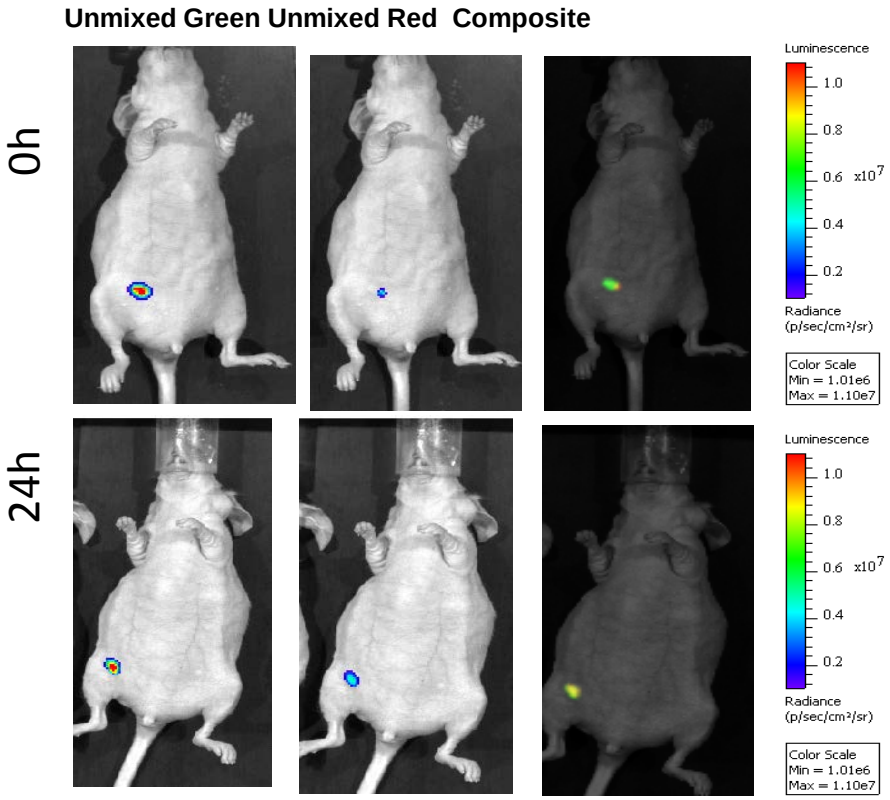


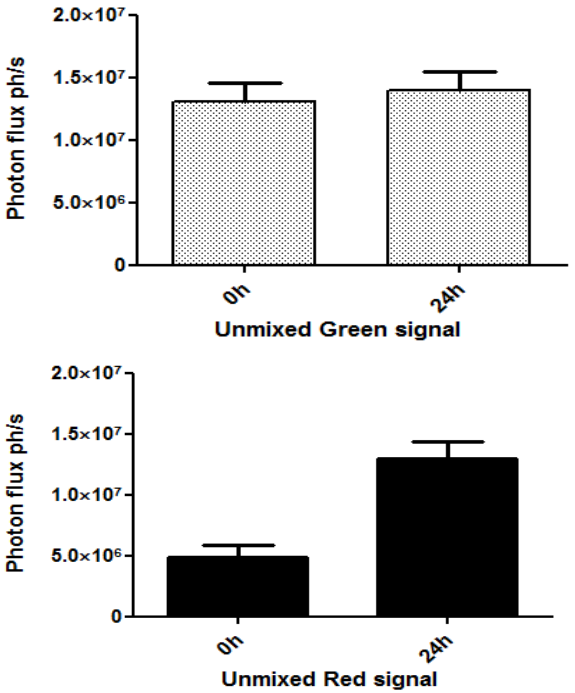


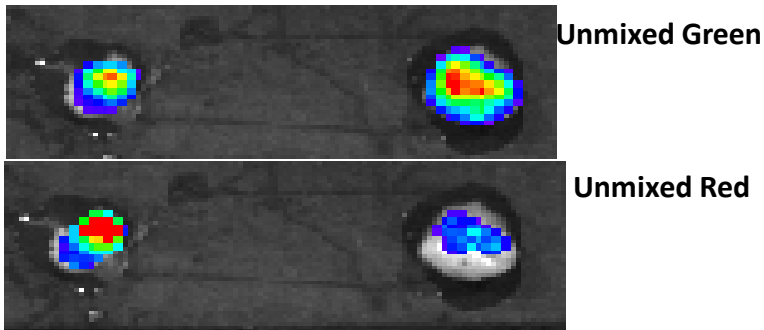


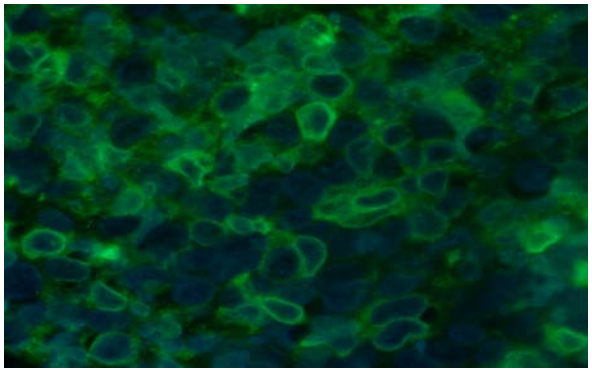


Validating the dual color luciferase system in vivo

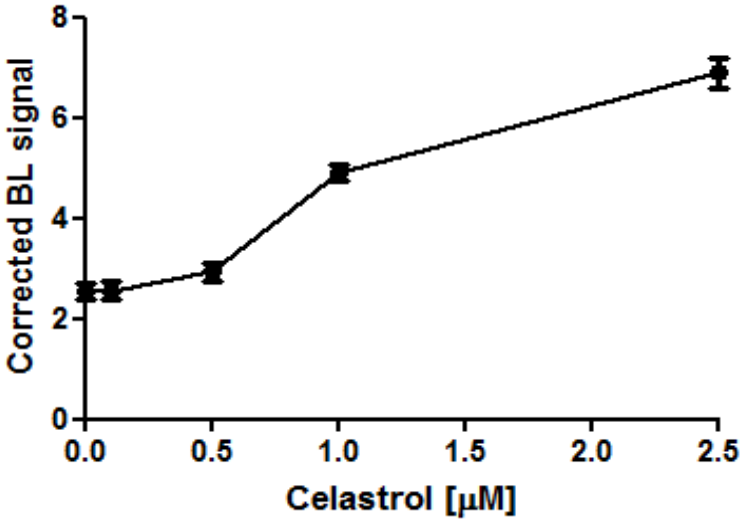




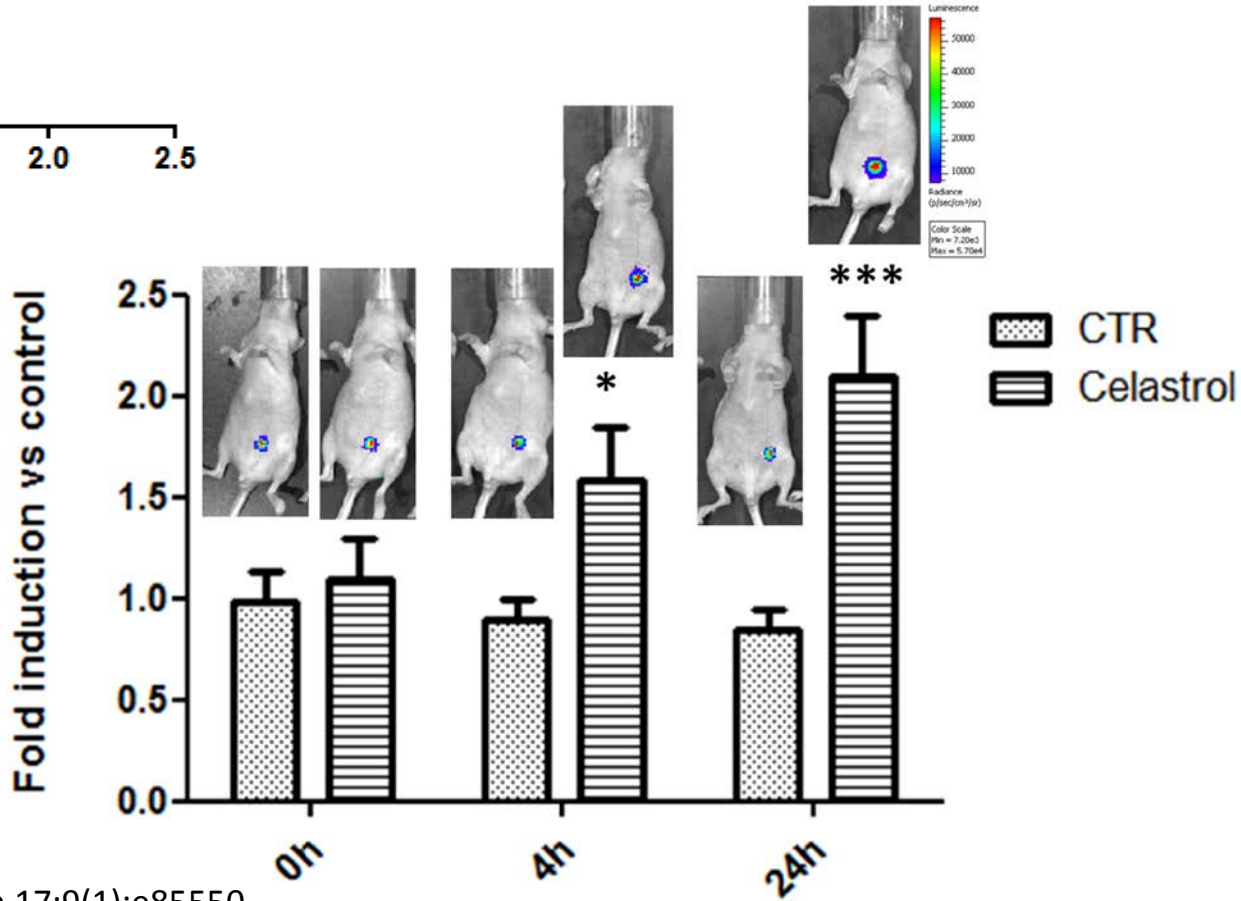




Detection of Apoptosis in vitro and in vivo

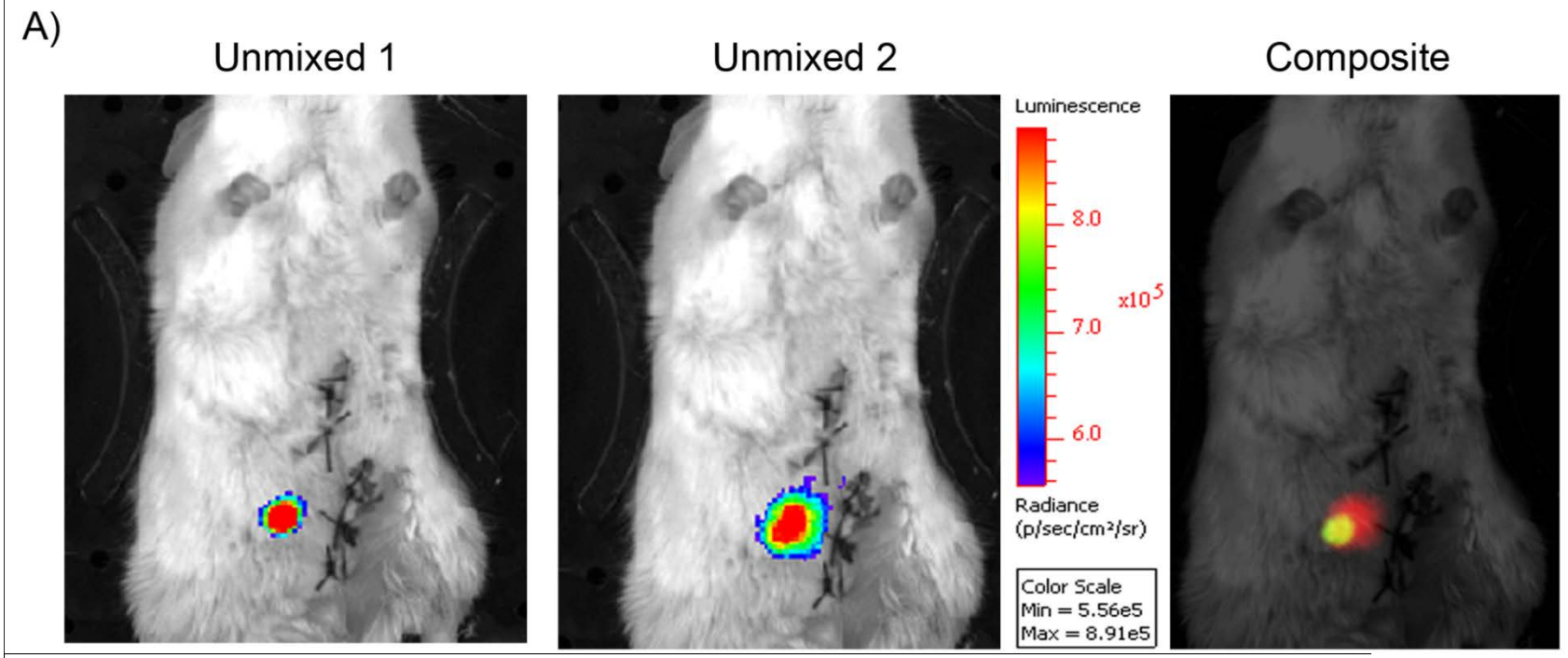


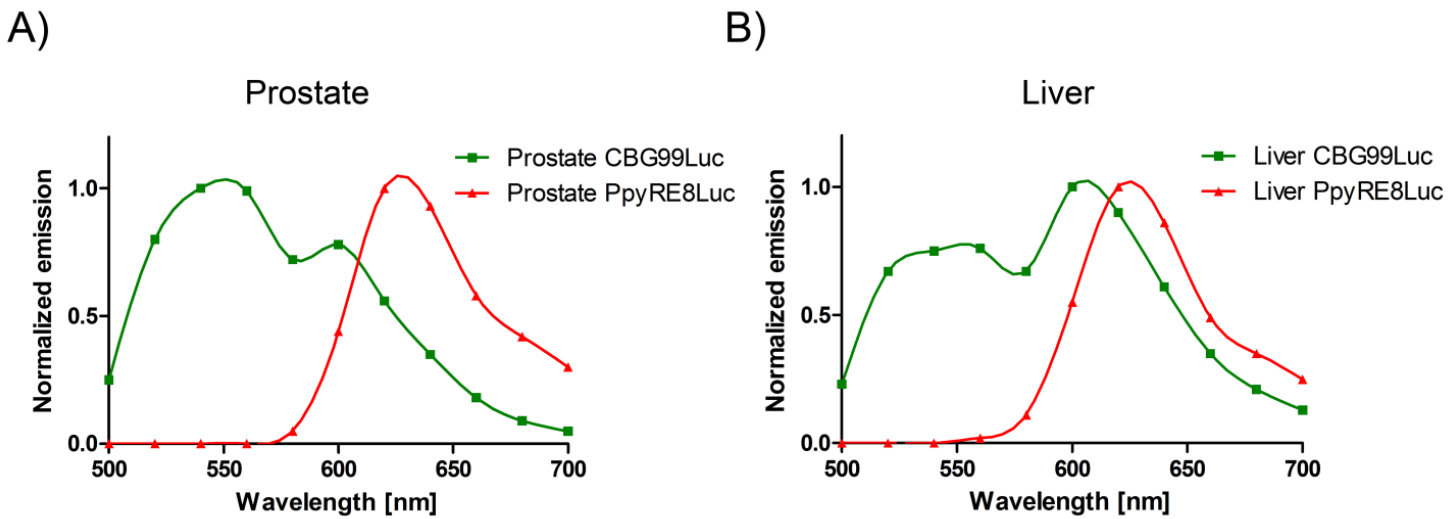
Caspase3/7 Glo and in vivo Caspase 3/7 substrate.



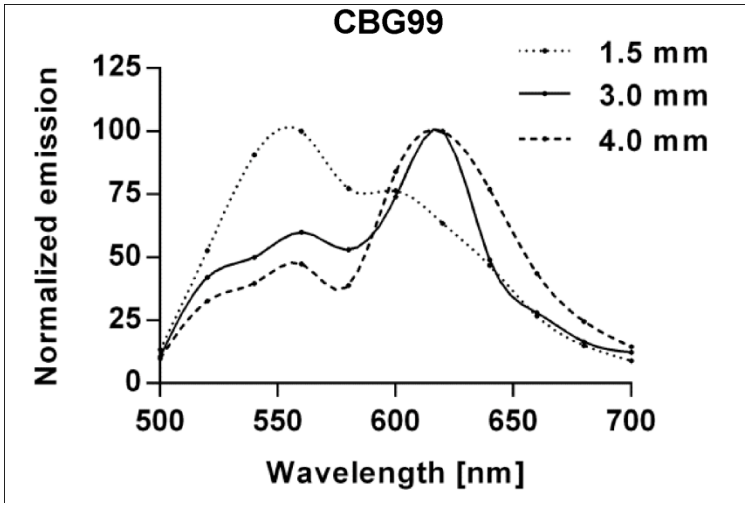
Mezzanotte el al. PLoS One. 2014 Jan 17;9(1):e85550.

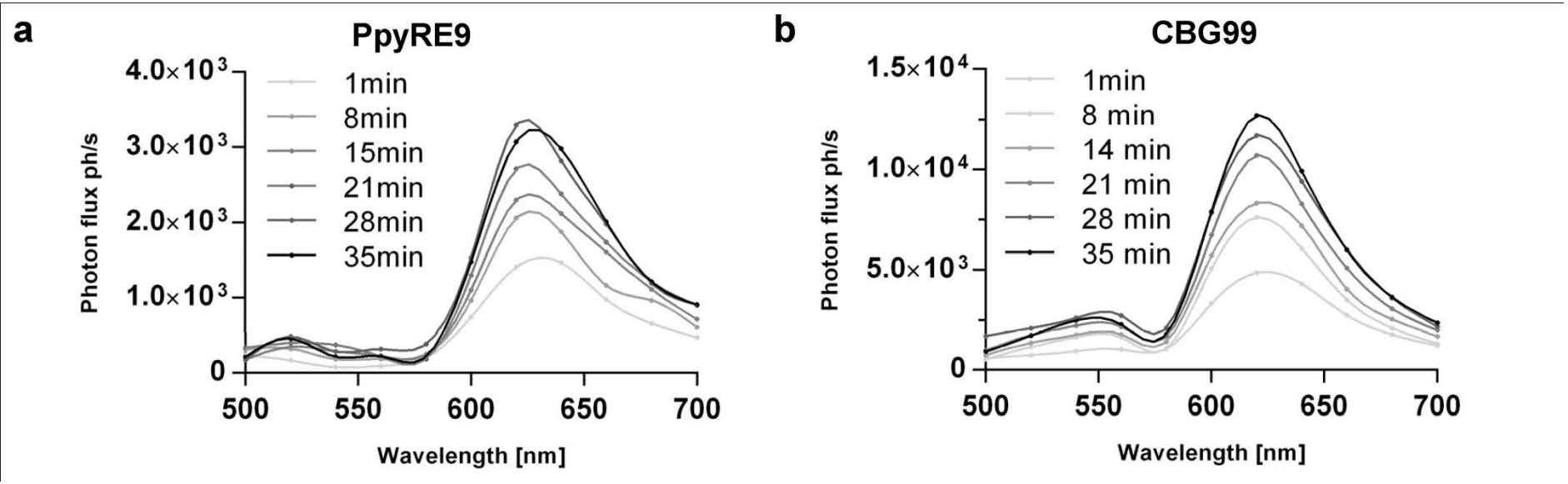
Spectral unmixing in different organs



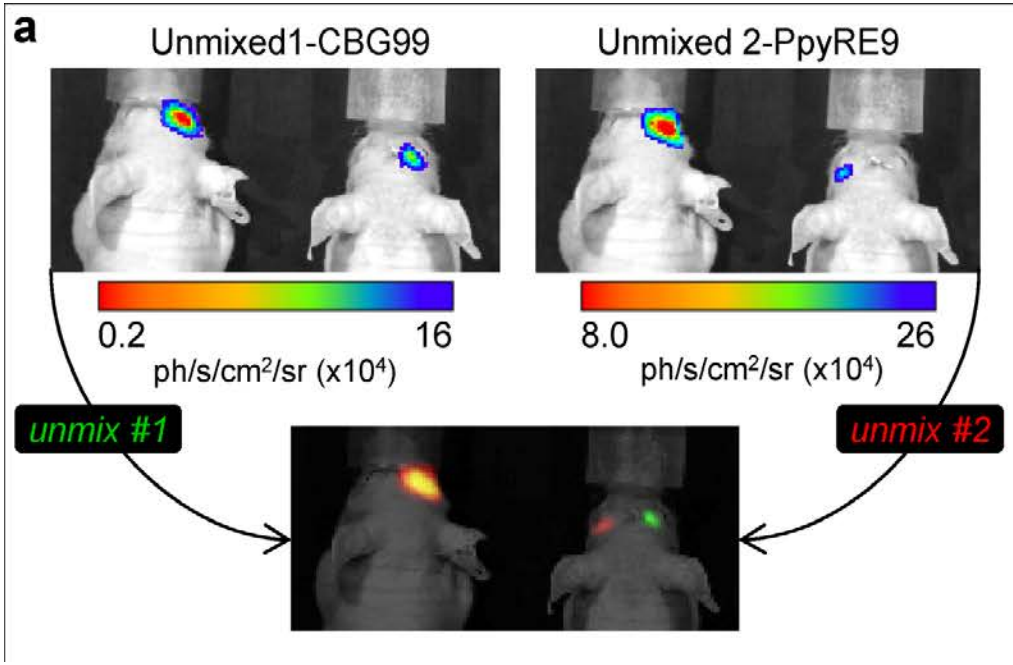


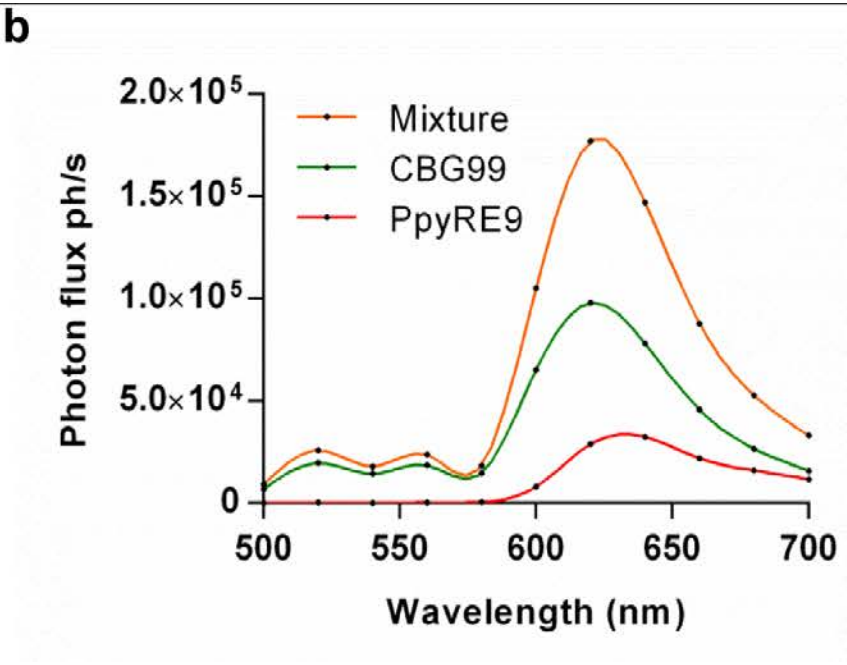
Spectral unmixing in brain





Spectral unmixing in brain





Mezzanotte L, Aswendt M et al. Evaluating reporter genes of different luciferases for optimized in vivo bioluminescence imaging of transplanted neural stem cells in the brain. Contrast Media Mol Imaging. 2013 Nov-Dec;8(6):505-13.

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Leiden University Medical Center,

Prof. Rob Hoeben

Dept. of Pharmaceutical Sciences,

University of Bologna

Prof. Aldo Roda

Dr. Elisa Michelini