Non Invasive bioluminiscence imaging using caged luciferin

Eucaryotic luciferases: Different colors and chemistries

Purpose of Bioluminescence

- Find Food
- Attract Mates
- Defend against predators
- Camouflage

Bioluminescence Reaction

- Several different luciferases and substrates with different colors of emission
- But in all cases:

Energy + Oxygen + Substrate ⟶ Light

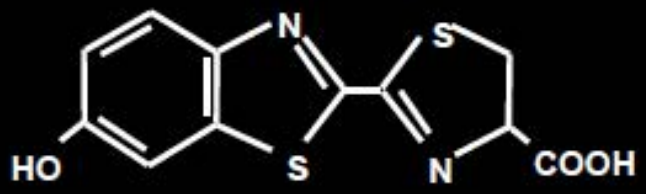
- May also require cofactors

Luciferases used *In vivo*

Firefly	Renilla	Bacterial
		
Luciferin + ATP + Mg^{2+} + O_2	Coelenterazine + O_2	Aldehyde + $FMNH_2$ + O_2
fluc	*hrLuc*	*luxAB*
Light	Light	Light
560 nm	475 nm	490 nm

Firefly Luciferin

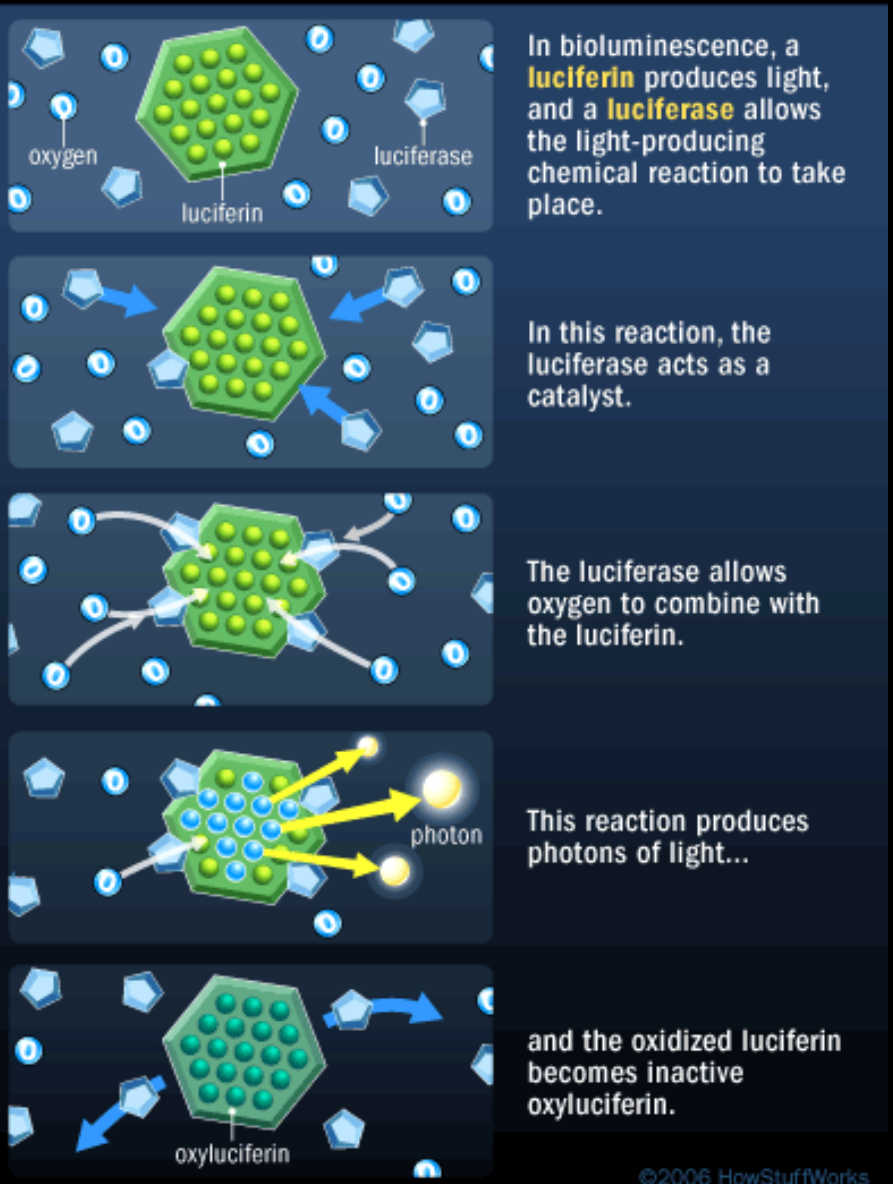
Firefly luciferin is used in a luciferin-luciferase system that requires ATP as a cofactor. Because of this, it can be used as a bio-indicator of the presence of energy or "life".



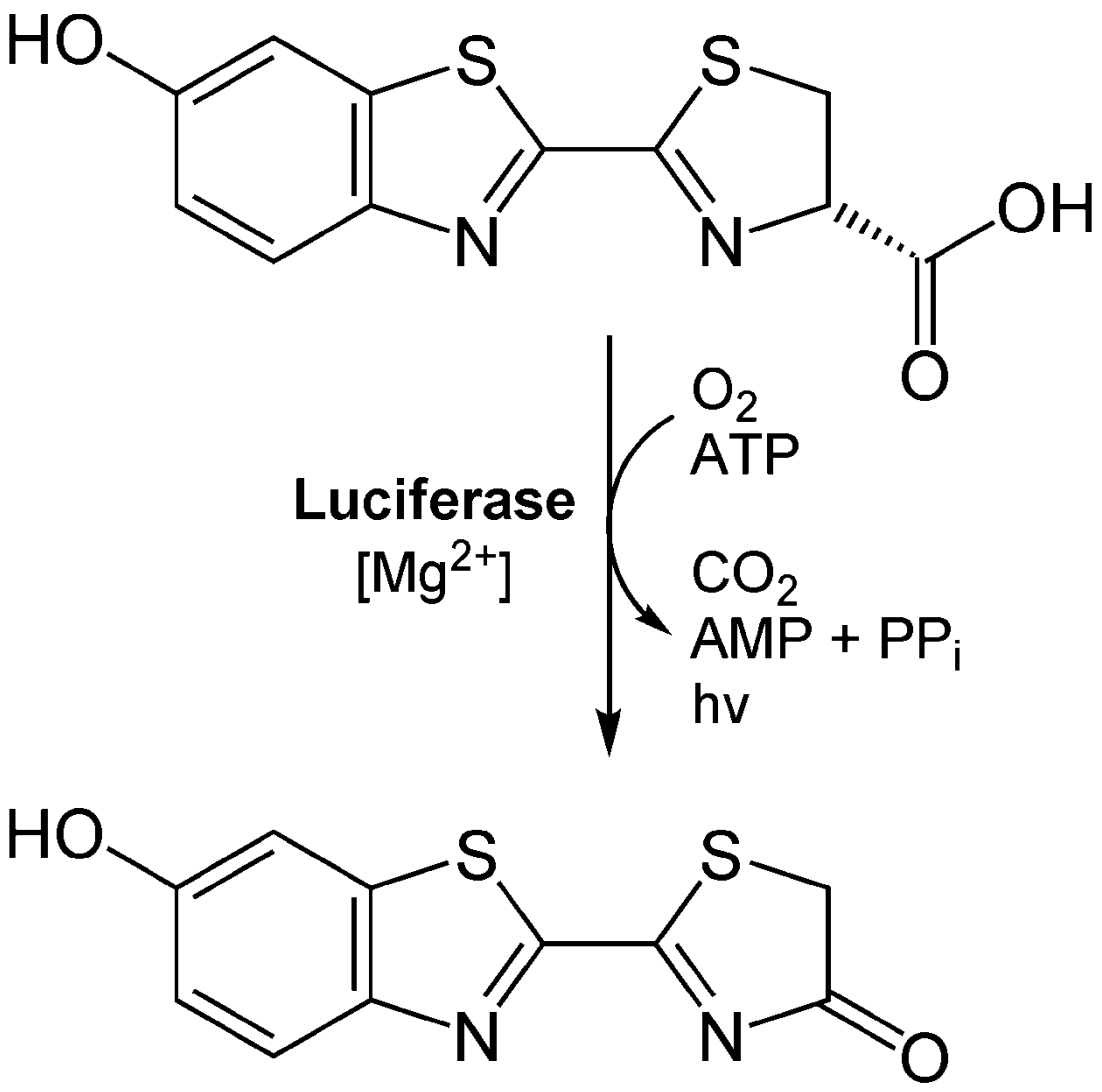
Benzothiazole

Considerations for *in vivo* applications: relatively long circulation time, crosses cell membrane, crosses blood brain barrier, crosses placental barrier, relatively non-toxic

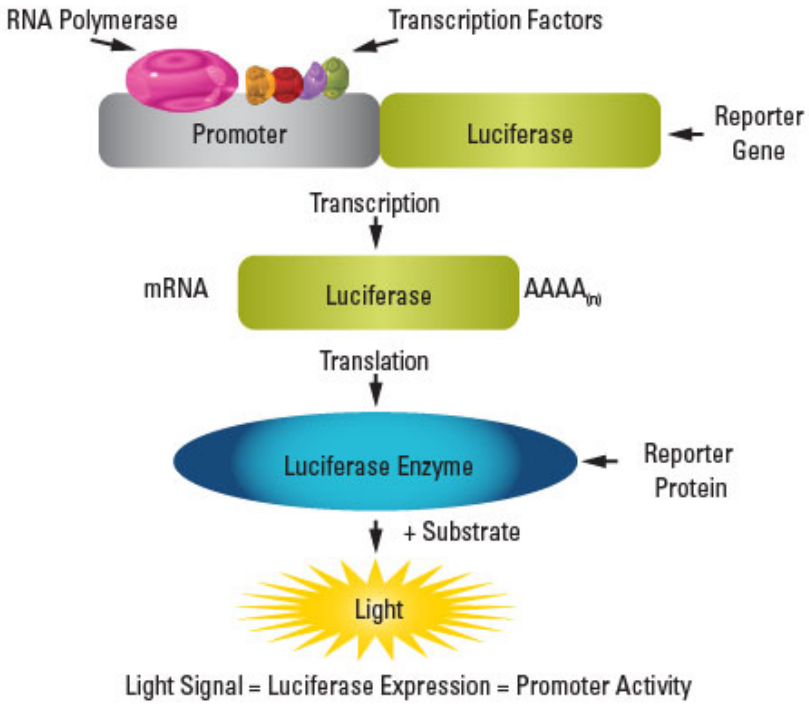
How Bioluminescence Works Luciferin and Luciferase

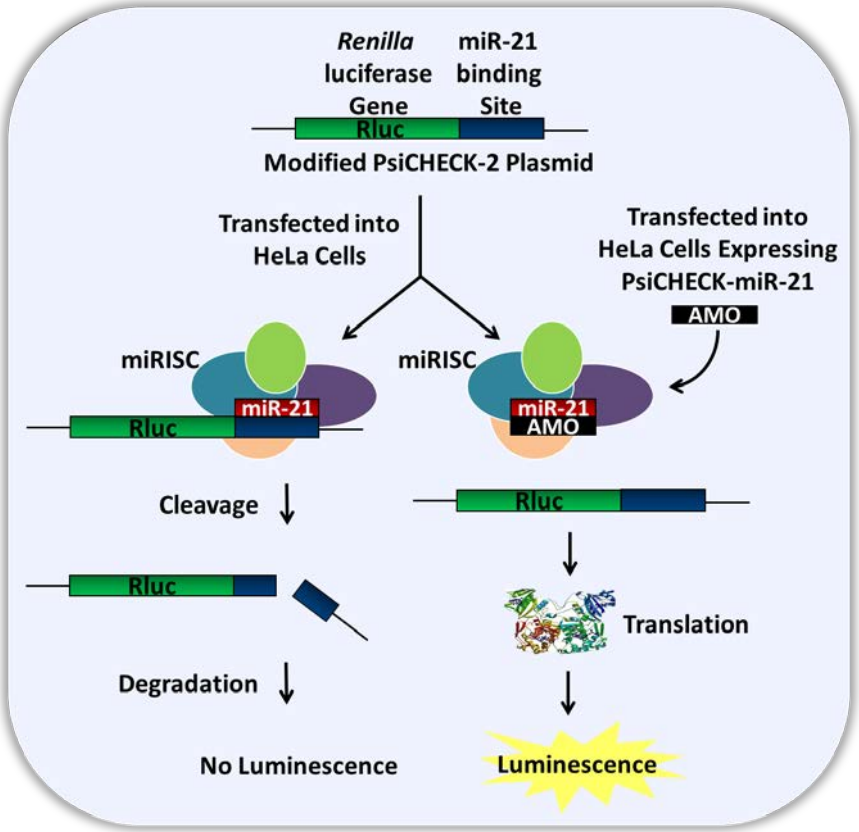


Luciferin + *Oxygen* —(*luciferase*)→ *Oxyluciferin* + *Light*

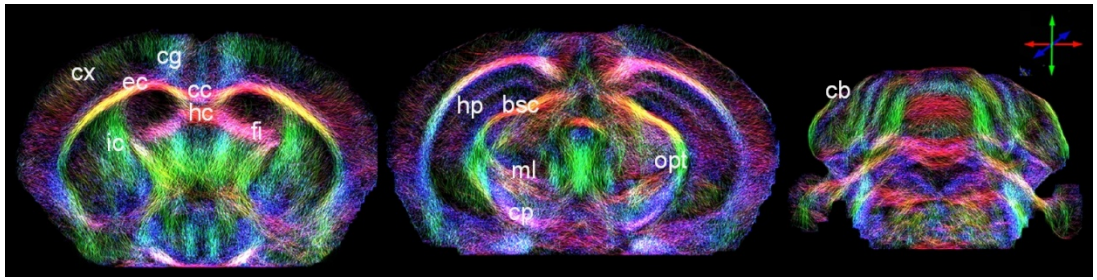

HO
S
S
N
N
OH
O
O2
ATP
Luciferase
[Mg2+]
CO2
AMP + PPi
hv
HO
S
S
N
N
O

Read out assays

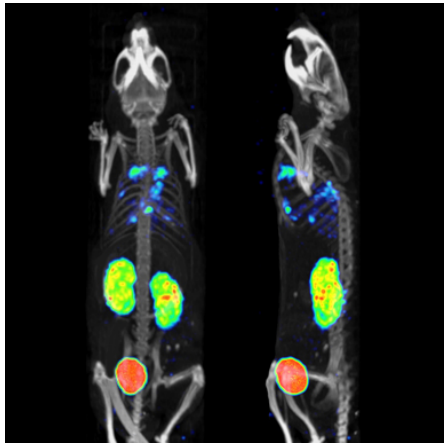




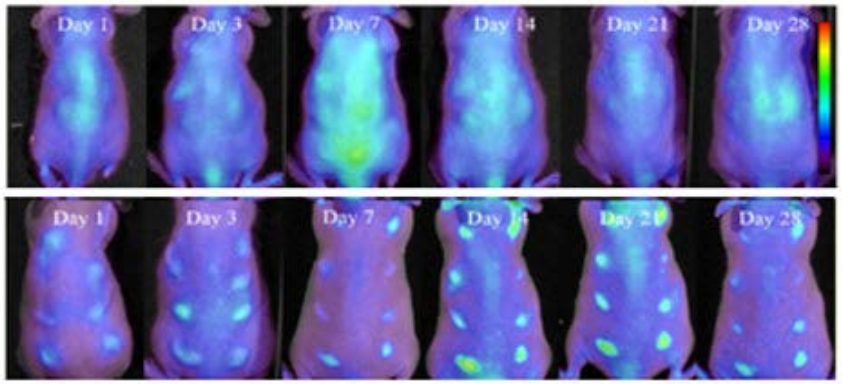
Different Imaging Techniques



MRI



SPECT



FLI

Advantages of BLI

. High sensitivity and low signal-to-noise ratio

• Quantitative correlation between signal strength and cell numbers

• Low background in animal tissues

• Variations of firefly luciferase (stabilized and red-shifted) and click beetle luciferases (red and green) are available

• Different colors allow multi-component monitoring

nature COMMUNICATIONS

ARTICLE

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Visualizing influenza virus infection in living mice

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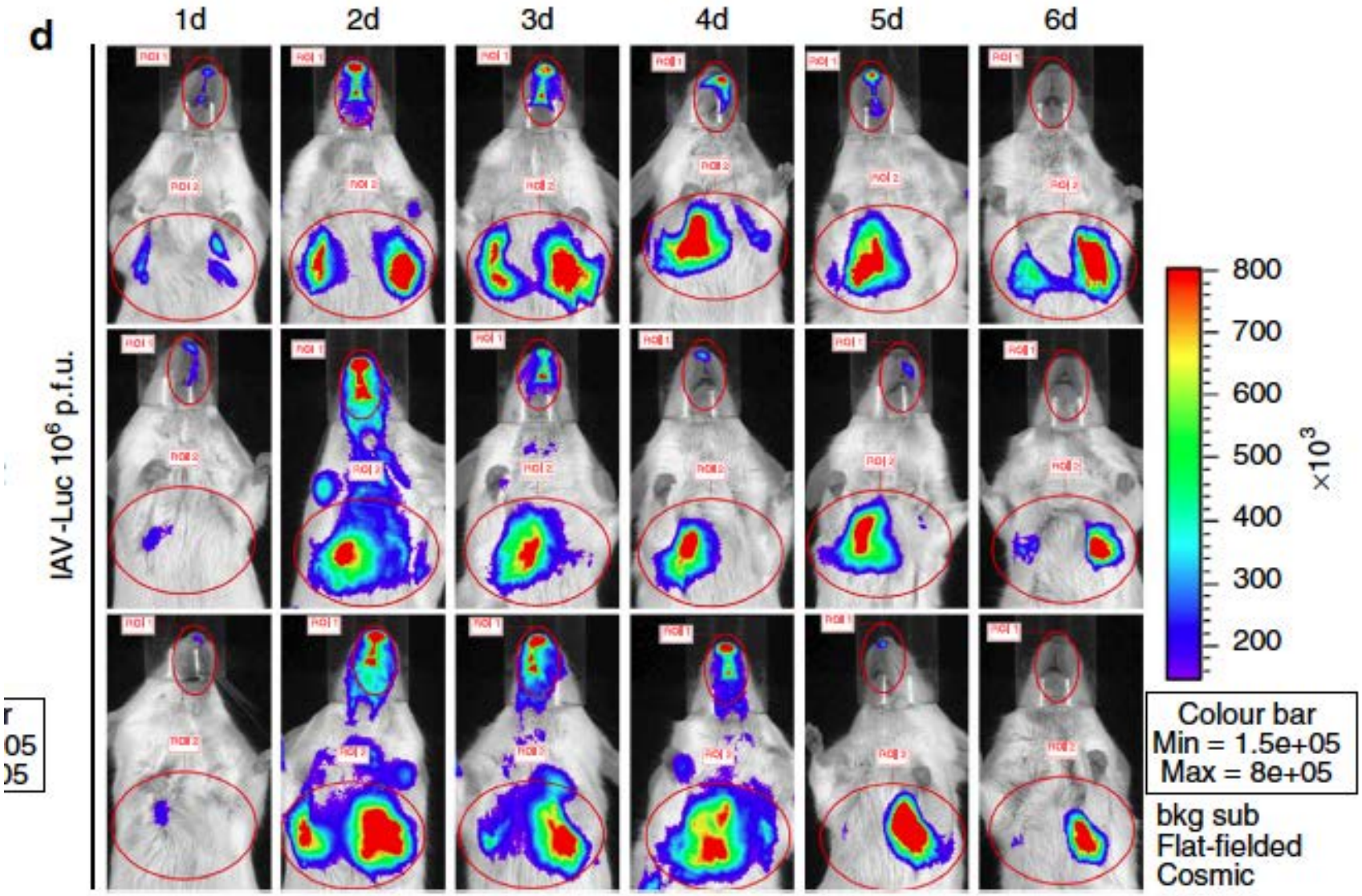
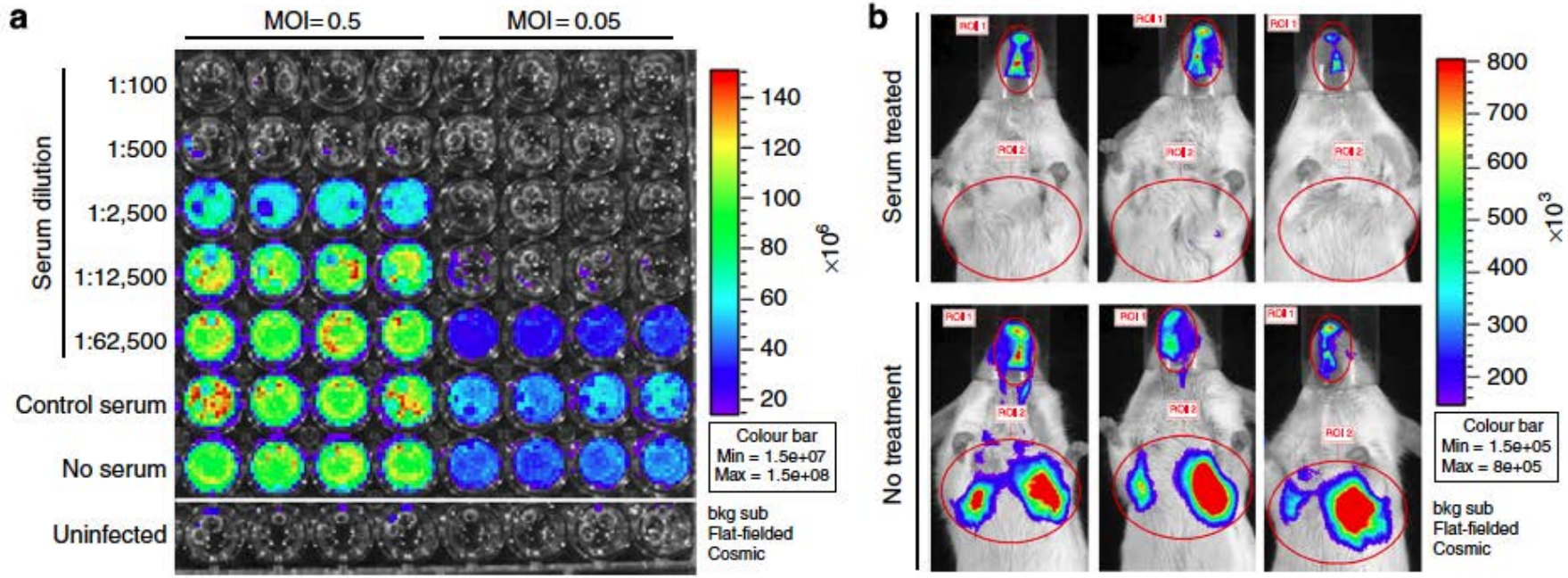
Influenza virus uptake




a
MOI = 0.5
MOI = 0.05
Serum dilution
1:100
1:500
1:2,500
1:12,500
1:62,500
Control serum
No serum
Uninfected
140
120
100
80
60
40
20
×10^6
Colour bar
Min = 1.5e+07
Max = 1.5e+08
bkg sub
Flat-fielded
Cosmic
b
Serum treated
No treatment
ROI 1
ROI 2
800
700
600
500
400
300
200
×10^3
Colour bar
Min = 1.5e+05
Max = 8e+05
bkg sub
Flat-fielded
Cosmic

ACS chemical biology

Articles

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Real-Time Noninvasive Imaging of Fatty Acid Uptake *in Vivo*

Amy H. Henkin,[†,¶] Allison S. Cohen,[‡,¶] Elena A. Dubikovskaya,[‡,⊥,¶] Hyo Min Park,[†] Gennady F. Nikitin,[⊥] Mathieu G. Auzias,[⊥] Melissa Kazantzis,[†] Carolyn R. Bertozzi,[‡,§,∥] and Andreas Stahl*[,†]

Departments of [†]Nutritional Science and Toxicology, [‡]Chemistry, and [§]Molecular and Cell Biology and [∥]Howard Hughes Medical Institute, University of California Berkeley, Berkeley, California 94720, United States

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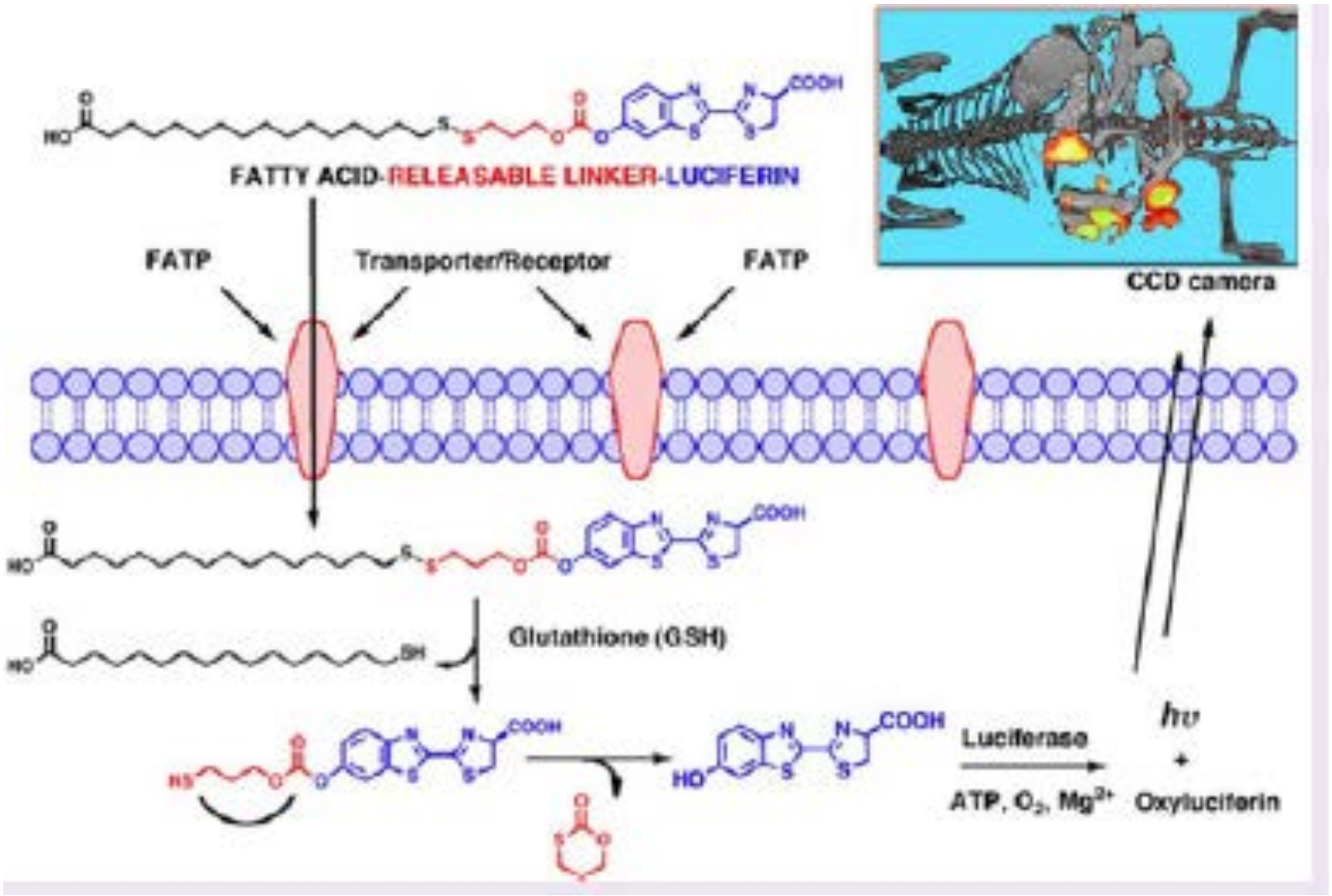
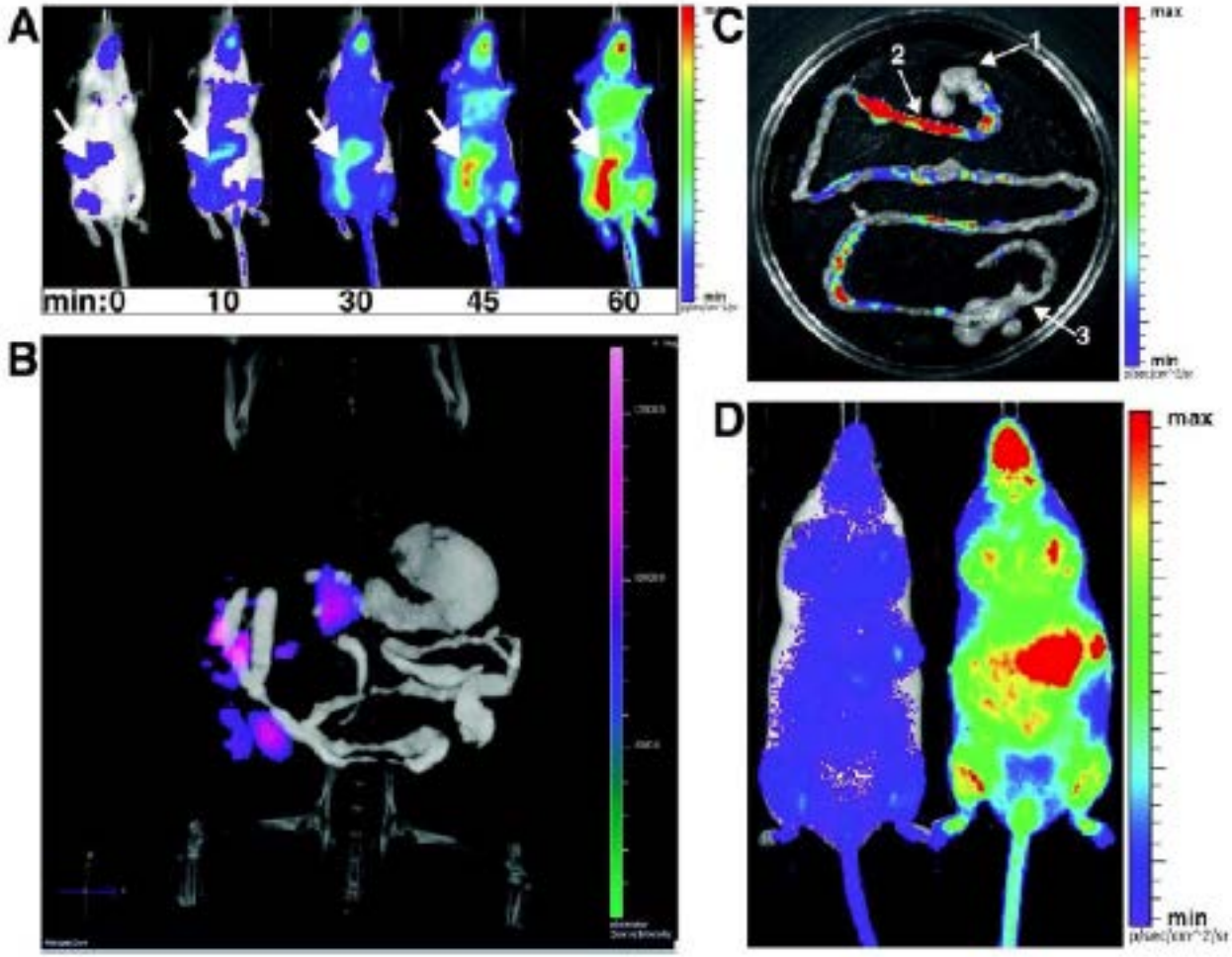
Supporting Information

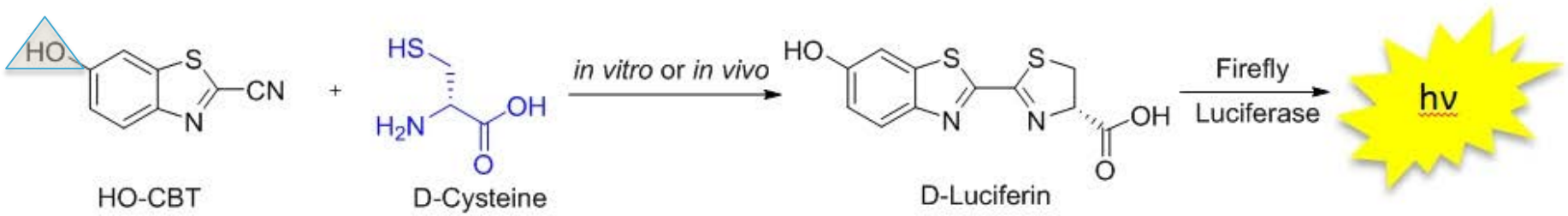

FATTY ACID-RELEASABLE LINKER-LUCIFERIN
FATP
Transporter/Receptor
FATP
CCD camera
Glutathione (GSH)
Luciferase
ATP, O_2, Mg^{2+}
hν
Oxyluciferin


A
min:0
10
30
45
60
B
C
1
2
3
max
min
D
max
min

Diadvantages of Luciferin

- Luciferin has low membrane permeability.
- High Background
- Pharmacokinetics

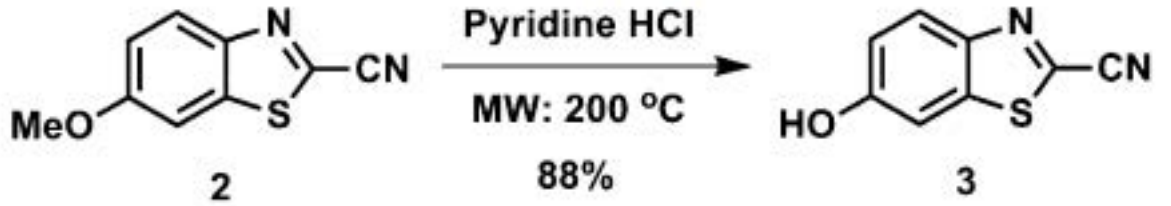
- Caged Luciferins

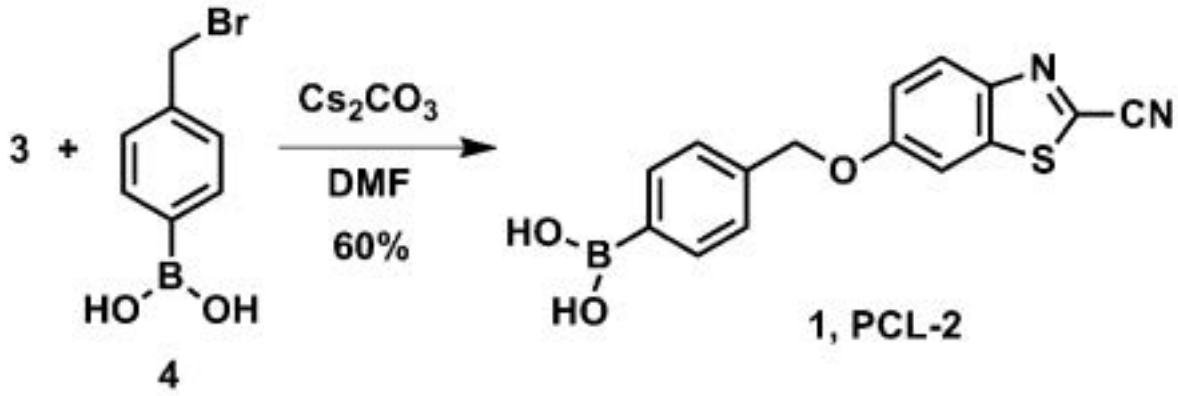


Cyanobenothiazole

Caged Luciferins

Scheme 1. Synthesis of Peroxy Caged Luciferin-2





ACS chemical biology

Articles

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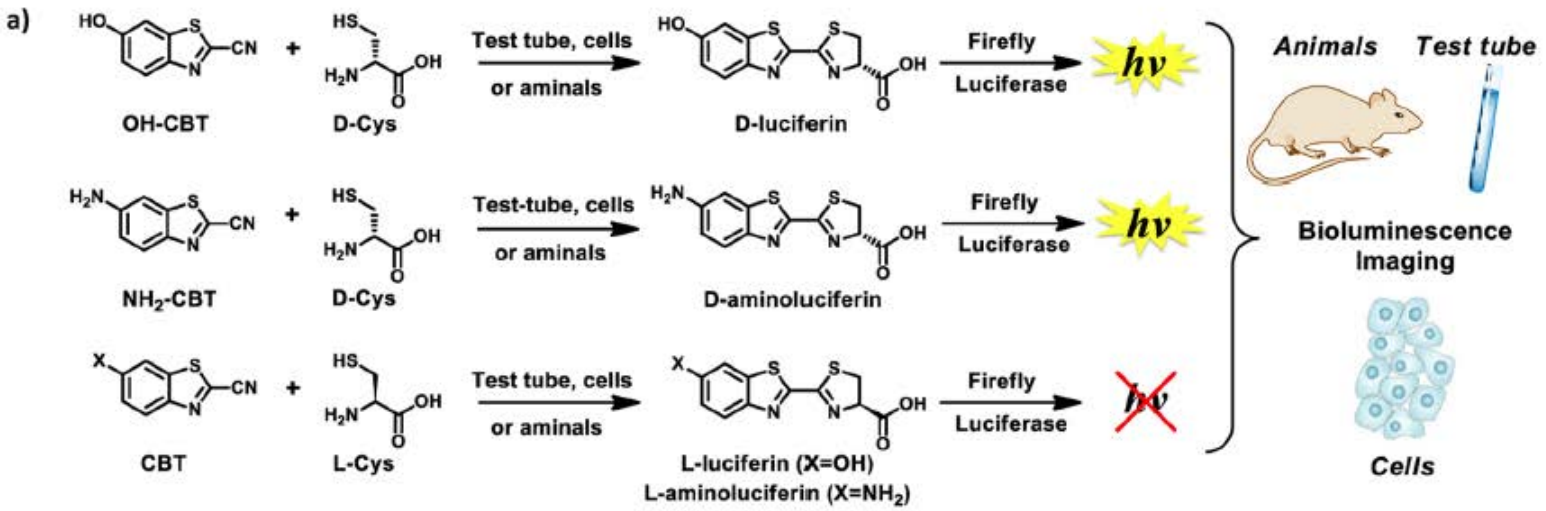
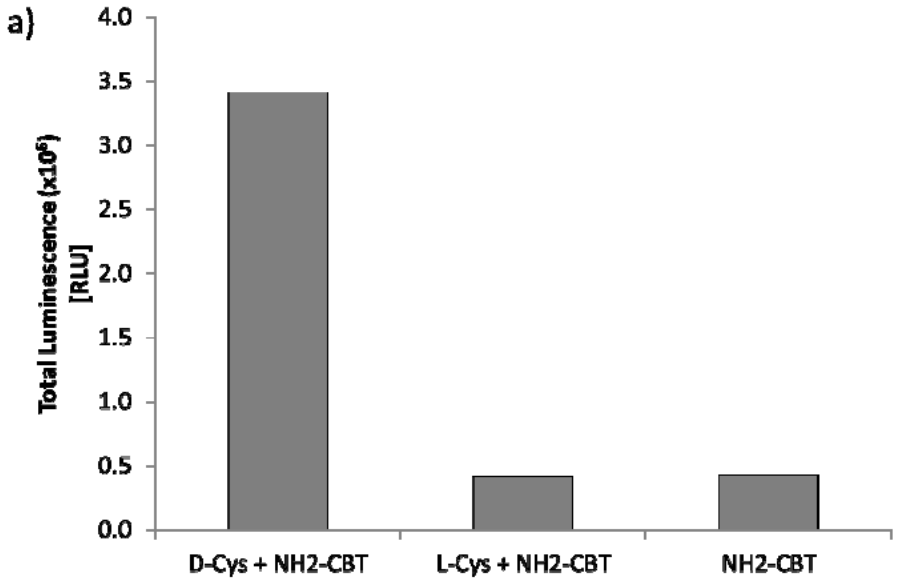
A Biocompatible *in Vivo* Ligation Reaction and Its Application for Noninvasive Bioluminescent Imaging of Protease Activity in Living Mice

Aurélien Godinat,[†] Hyo Min Park,[‡] Stephen C. Miller,[§] Ke Cheng,[∥] Douglas Hanahan,[∥] Laura E. Sanman,[⊥] Matthew Bogyo,[¶,#] Allen Yu,[‡] Gennady F. Nikitin,[†] Andreas Stahl,[‡] and Elena A. Dubikovskaya*[,†]

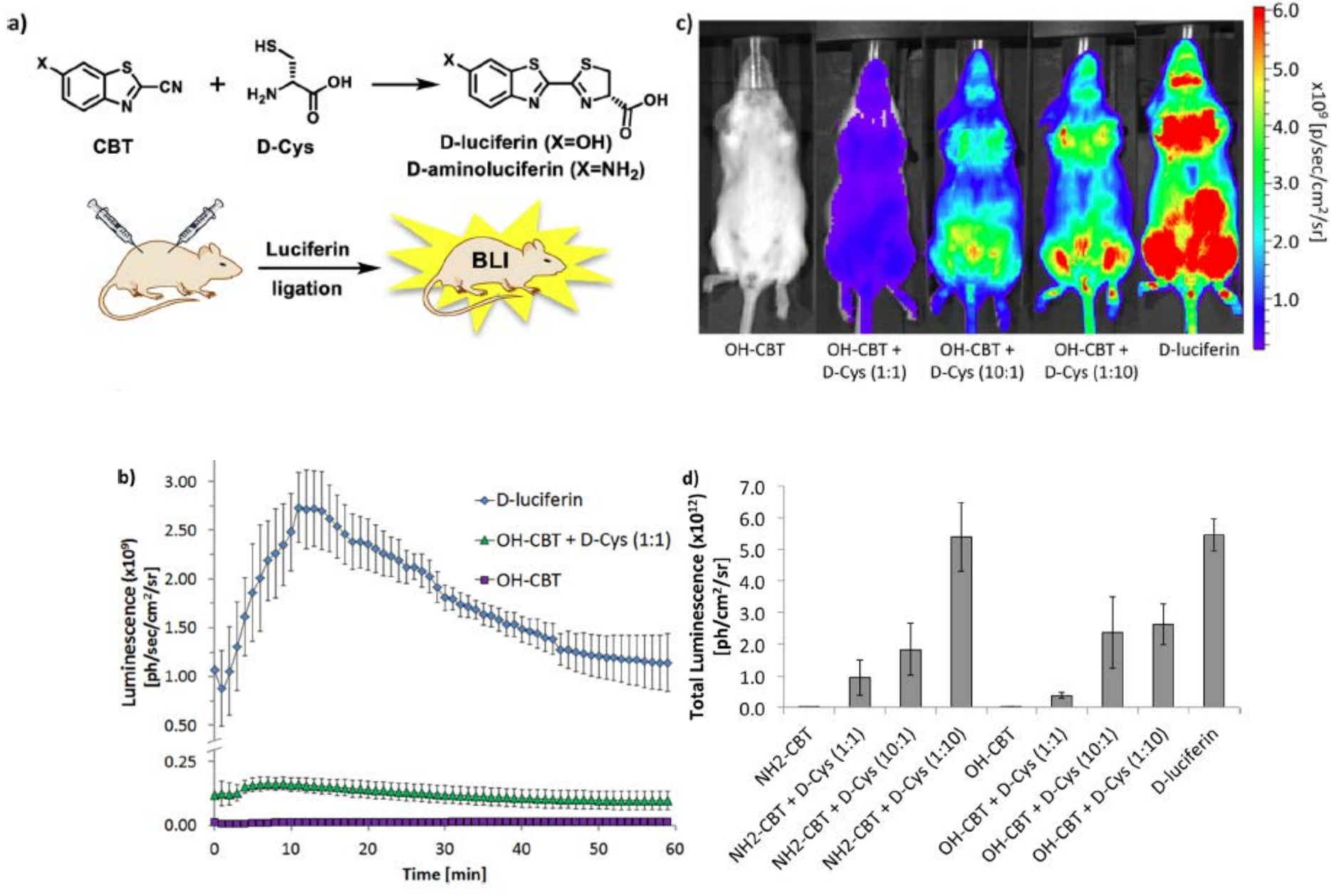
Protocol

1) Generation of the substrates
2) To be tested in the test tube
3) To be tested in the invitro cell culture
4) Tested in the live animals

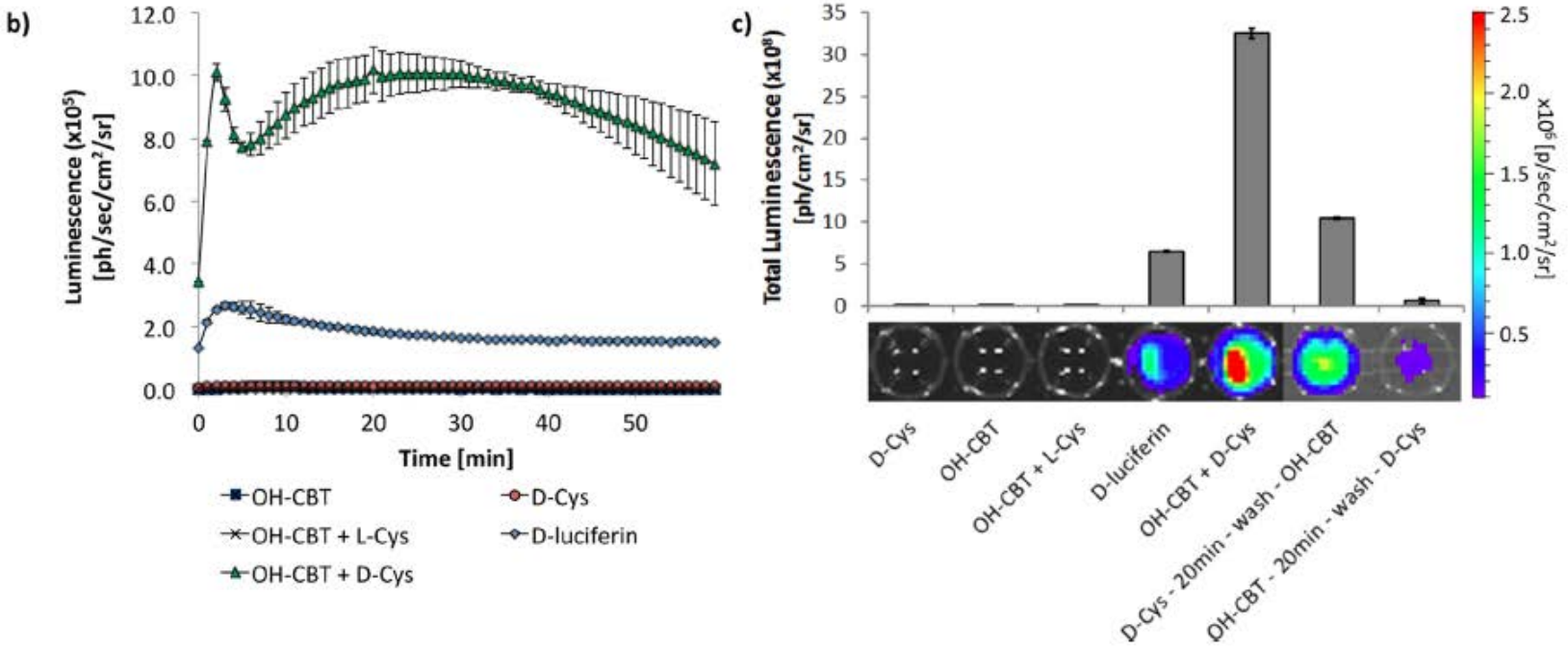




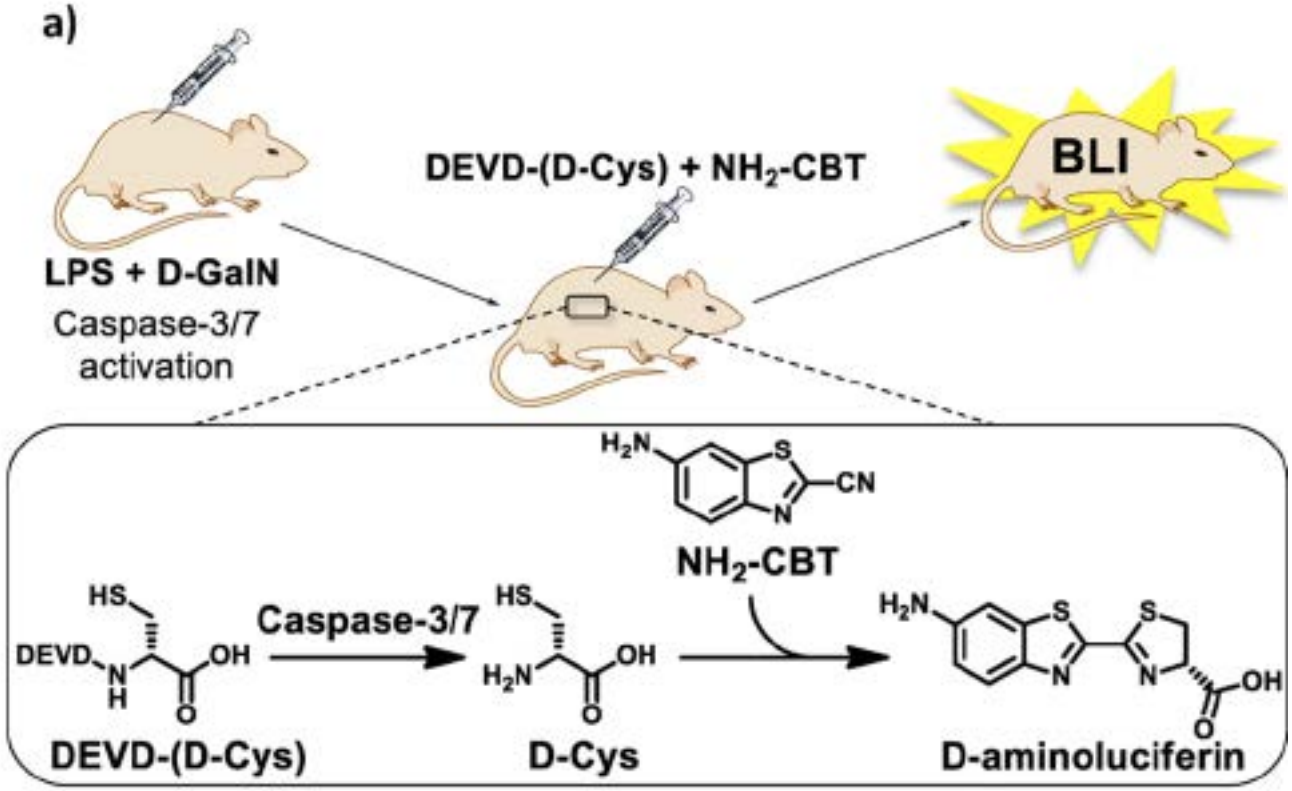
Test Tube analysis

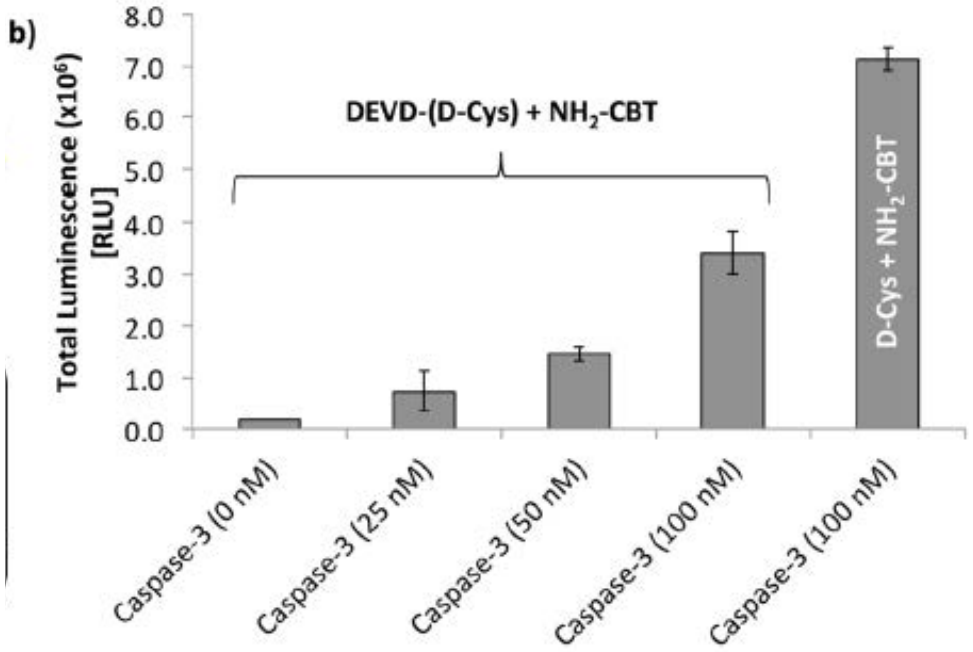

a)
CBT
D-Cys
D-luciferin (X=OH)
D-aminoluciferin (X=NH2)
Luciferin
ligation
BLI
c)
OH-CBT
OH-CBT + D-Cys (1:1)
OH-CBT + D-Cys (10:1)
OH-CBT + D-Cys (1:10)
D-luciferin
x10⁹ [p/sec/cm²/sr]
b)
Luminescence (x10⁹) [ph/sec/cm²/sr]
Time [min]
D-luciferin
OH-CBT + D-Cys (1:1)
OH-CBT
d)
Total Luminescence (x10¹²) [ph/cm²/sr]
NH2-CBT
NH2-CBT + D-Cys (1:1)
NH2-CBT + D-Cys (10:1)
NH2-CBT + D-Cys (1:10)
OH-CBT
OH-CBT + D-Cys (1:1)
OH-CBT + D-Cys (10:1)
OH-CBT + D-Cys (1:10)
D-luciferin

BLI in Live cells

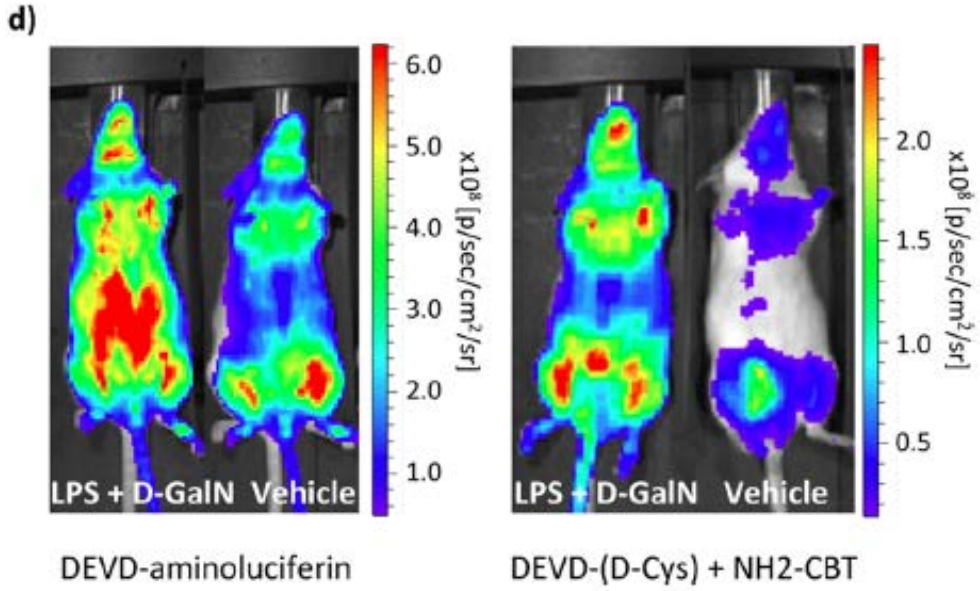


Protease activity in mice





In vitro assay shows linearity



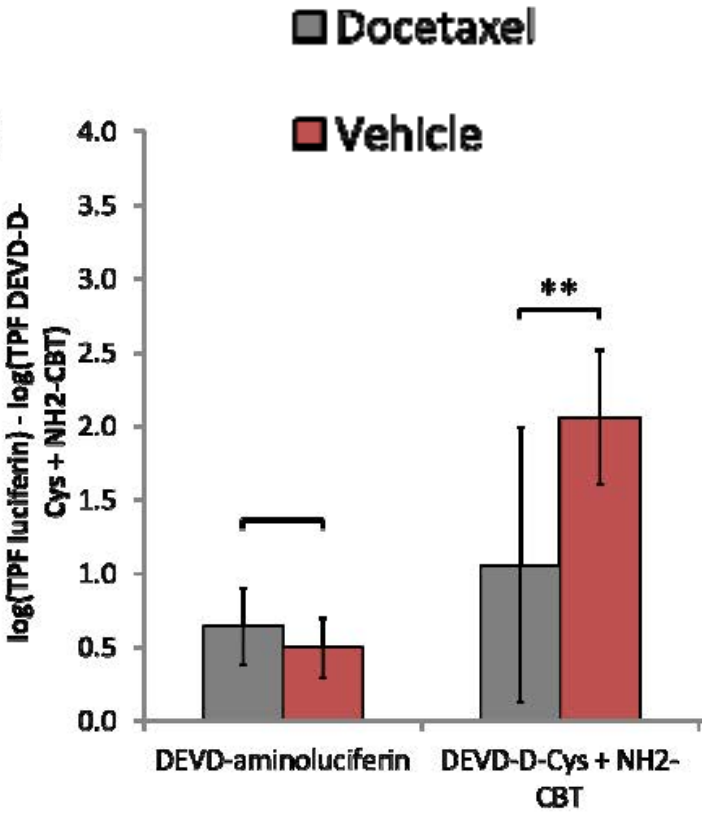
Decreased background
Compared to just using aminoluciferin

Animal model

a)

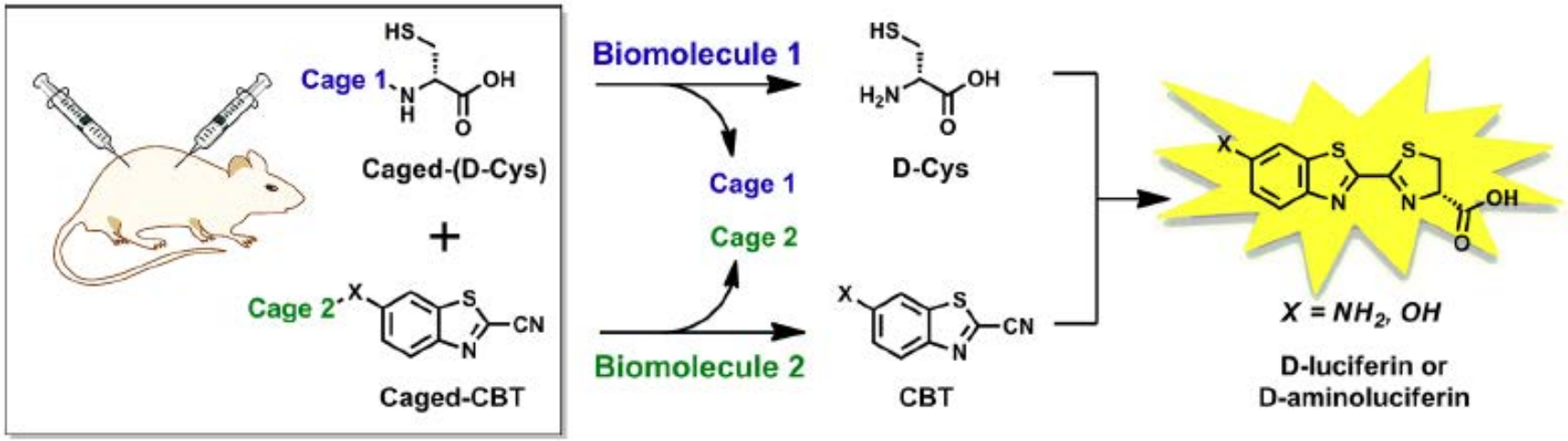
	Mice number	Docetaxel (60mg/kg)	Caspase-3/7 Probes
Group 1	9	+	DEVD-D-Cys + NH2-CBT
Group 2	9	-	DEVD-D-Cys + NH2-CBT
Group 3	4	+	DEVD-aminoluciferin
Group 4	4	-	DEVD-aminoluciferin
Group 5	4	+	NH2-CBT

b)

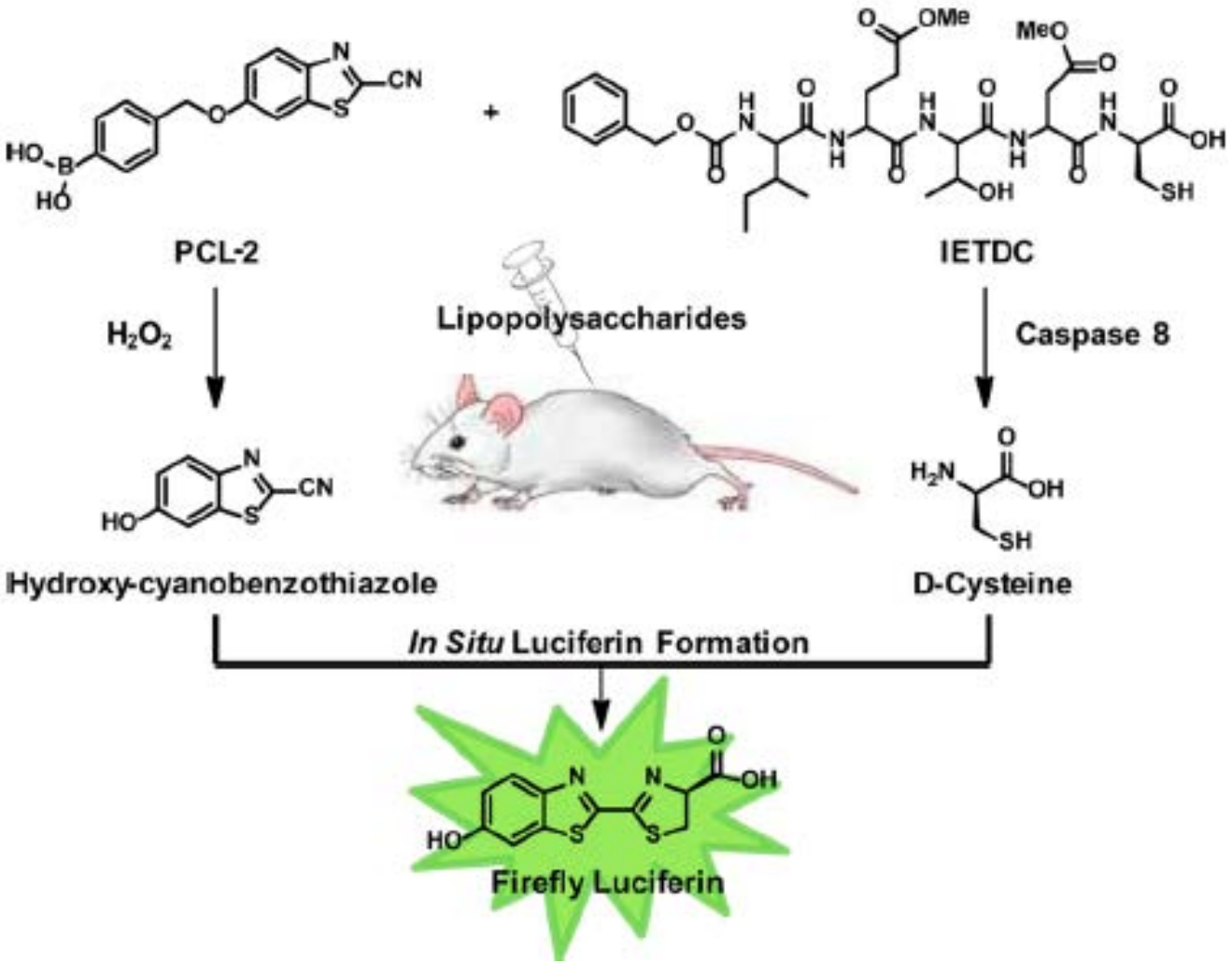


Tumor cells were transplanted into the ude mice

Double caging

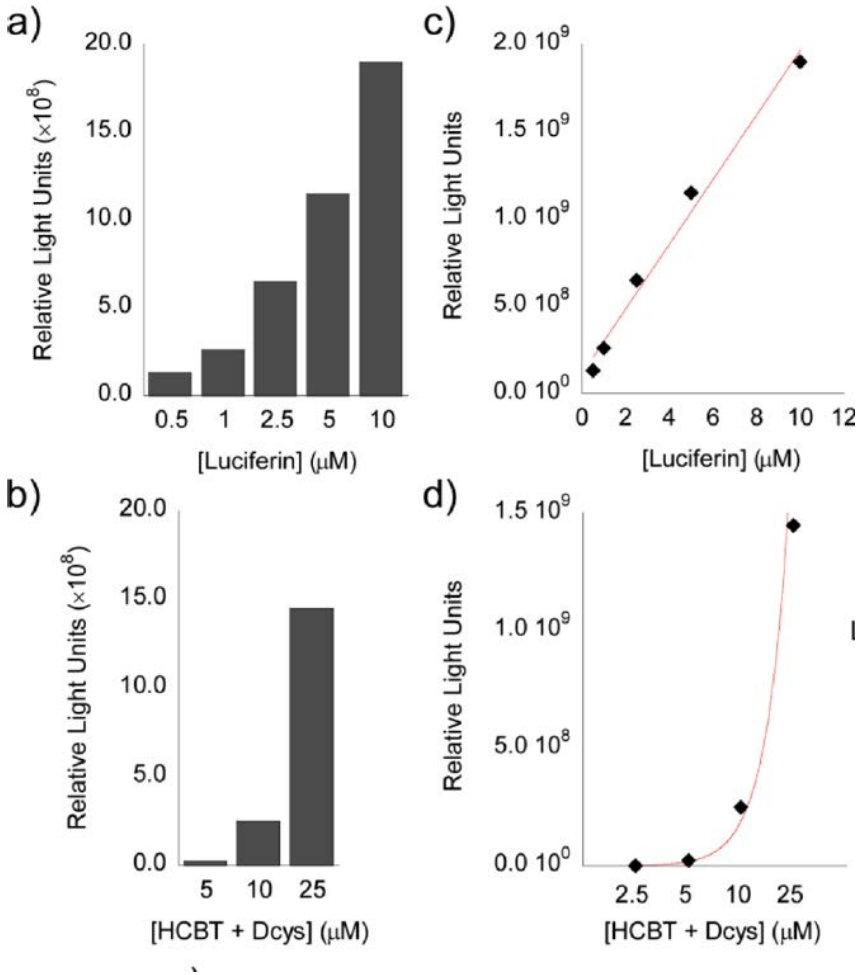


Design strategy for double cageing

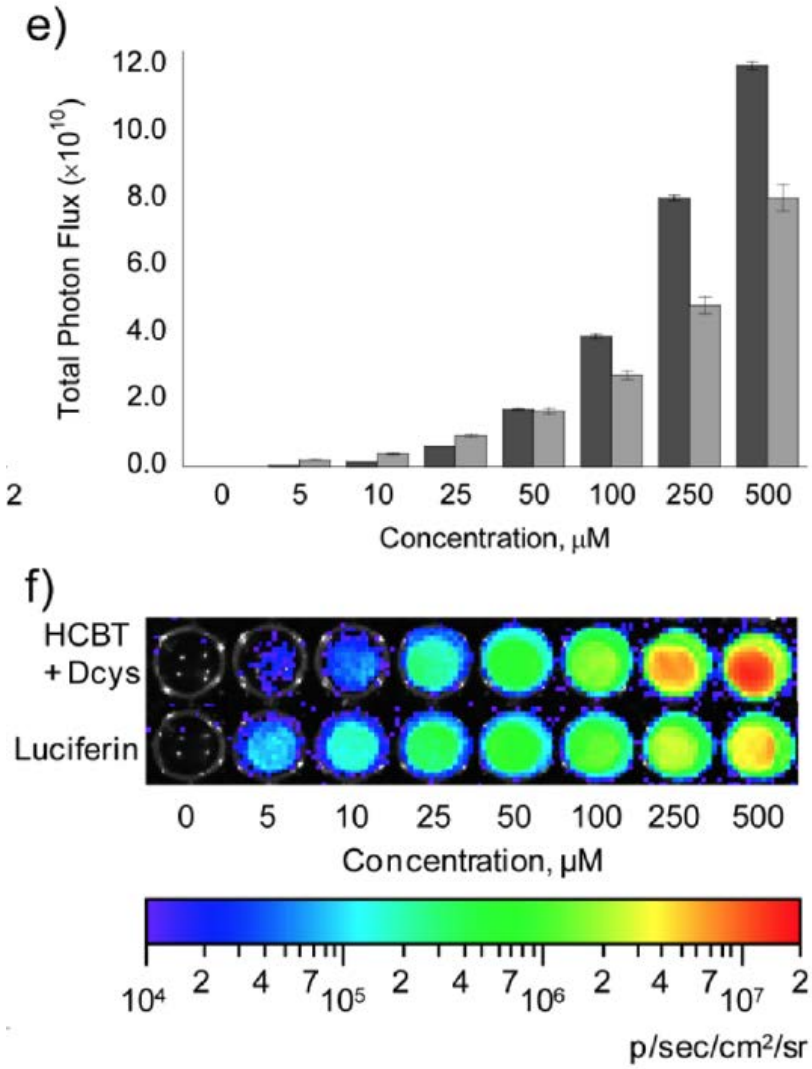


dx.doi.org/10.1021/ja309078t | J. Am. Chem. Soc. 2013, 135, 1783−1795

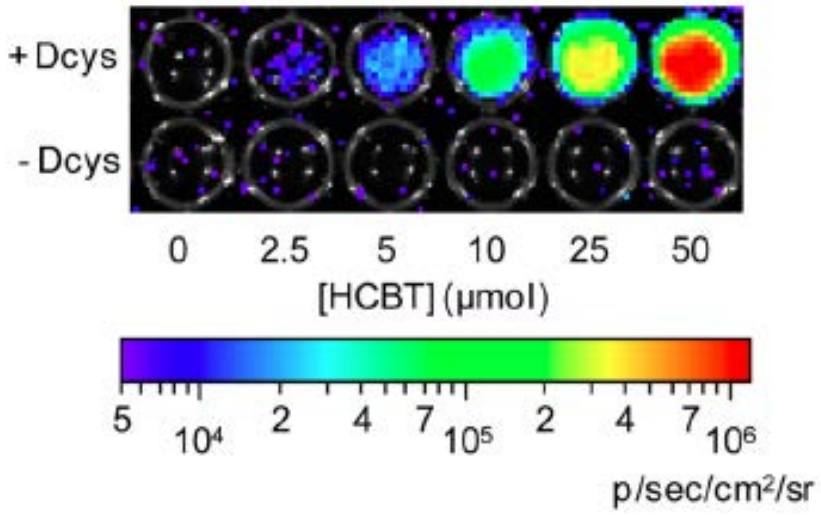
Test Tube Analysis

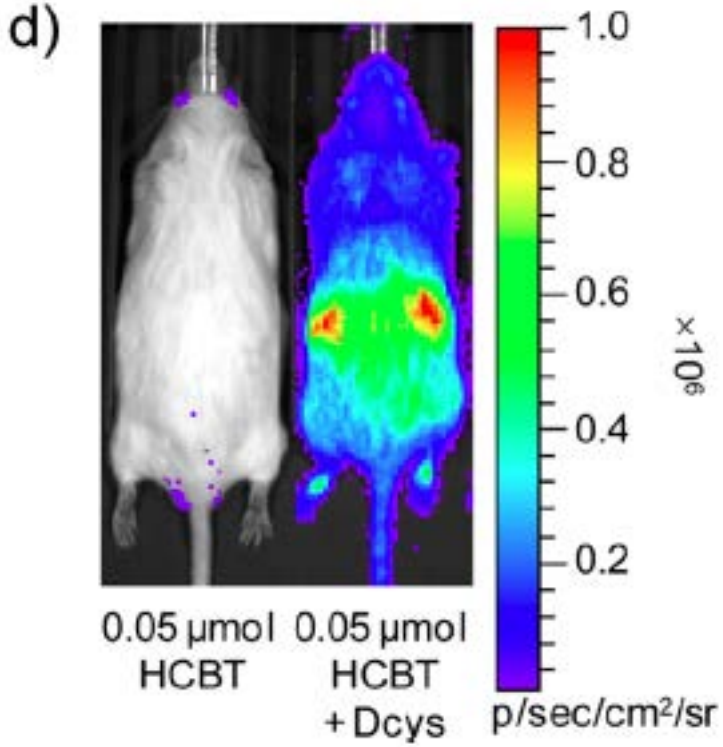


Analysis in Cell lines

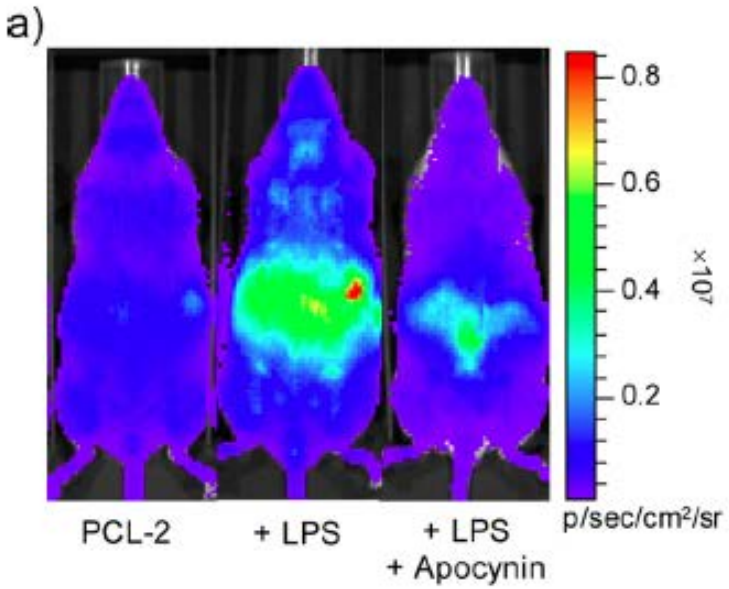


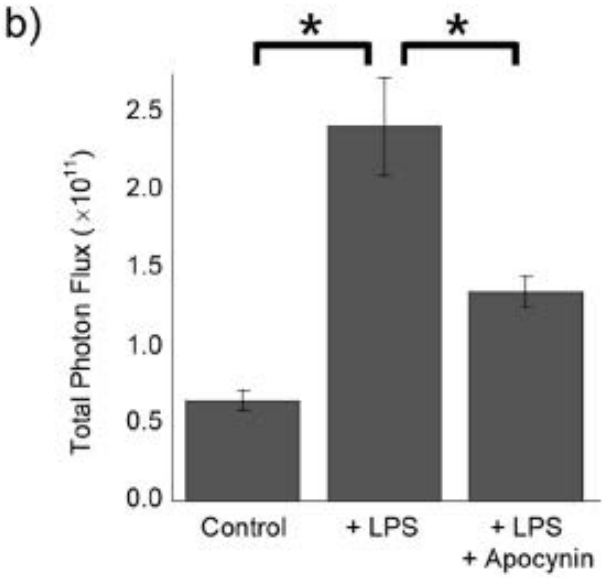
Background Luminiscence

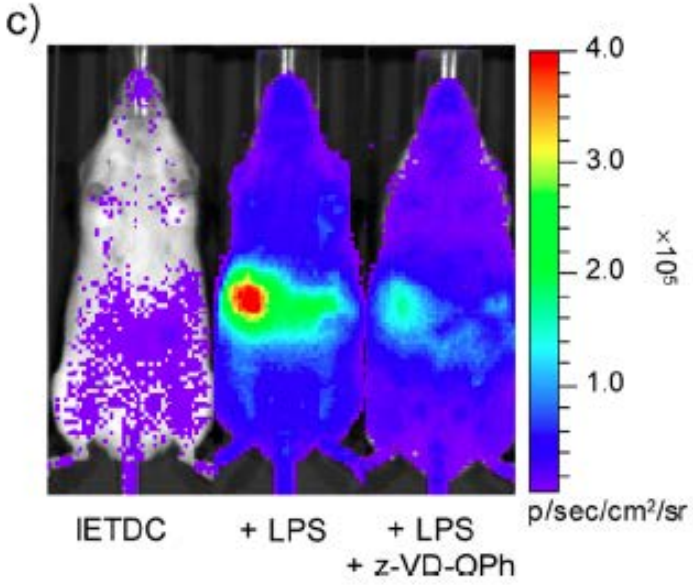


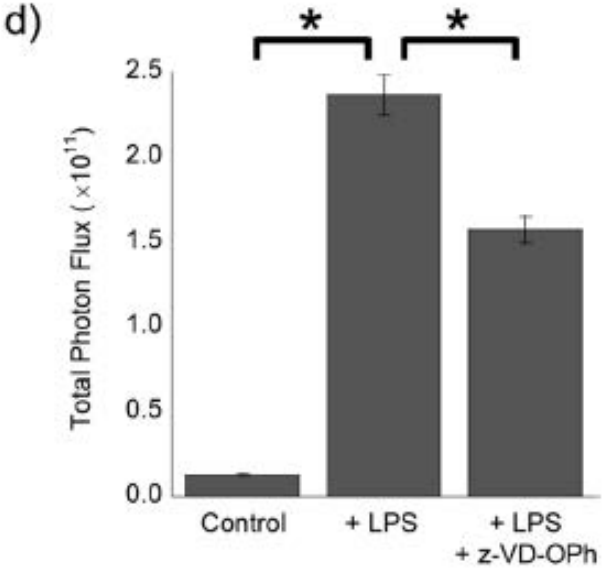


Activity of individual caged moities









Double caging Assay