# *Bioluminescent Cellular Reporter Technologies Featuring NanoLuc Luciferase*

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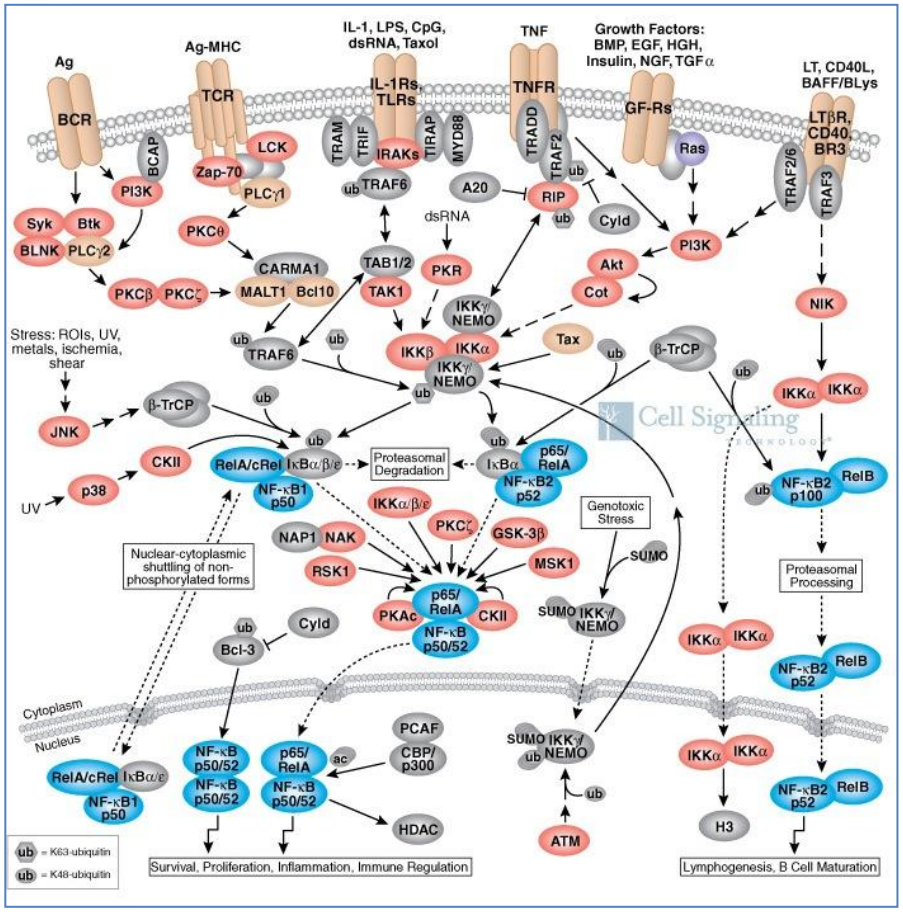
**Director of Research**

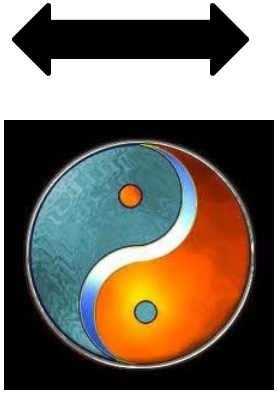
**March, 2014**

# *Outline*

- Bioluminescence
- NanoLuc luciferase
- Genetic reporter
  - Dual luciferase assays
  - Animal imaging
- Protein reporter
  - Location
  - Stability
  - Interactions (NanoBRET, NanoBiT)

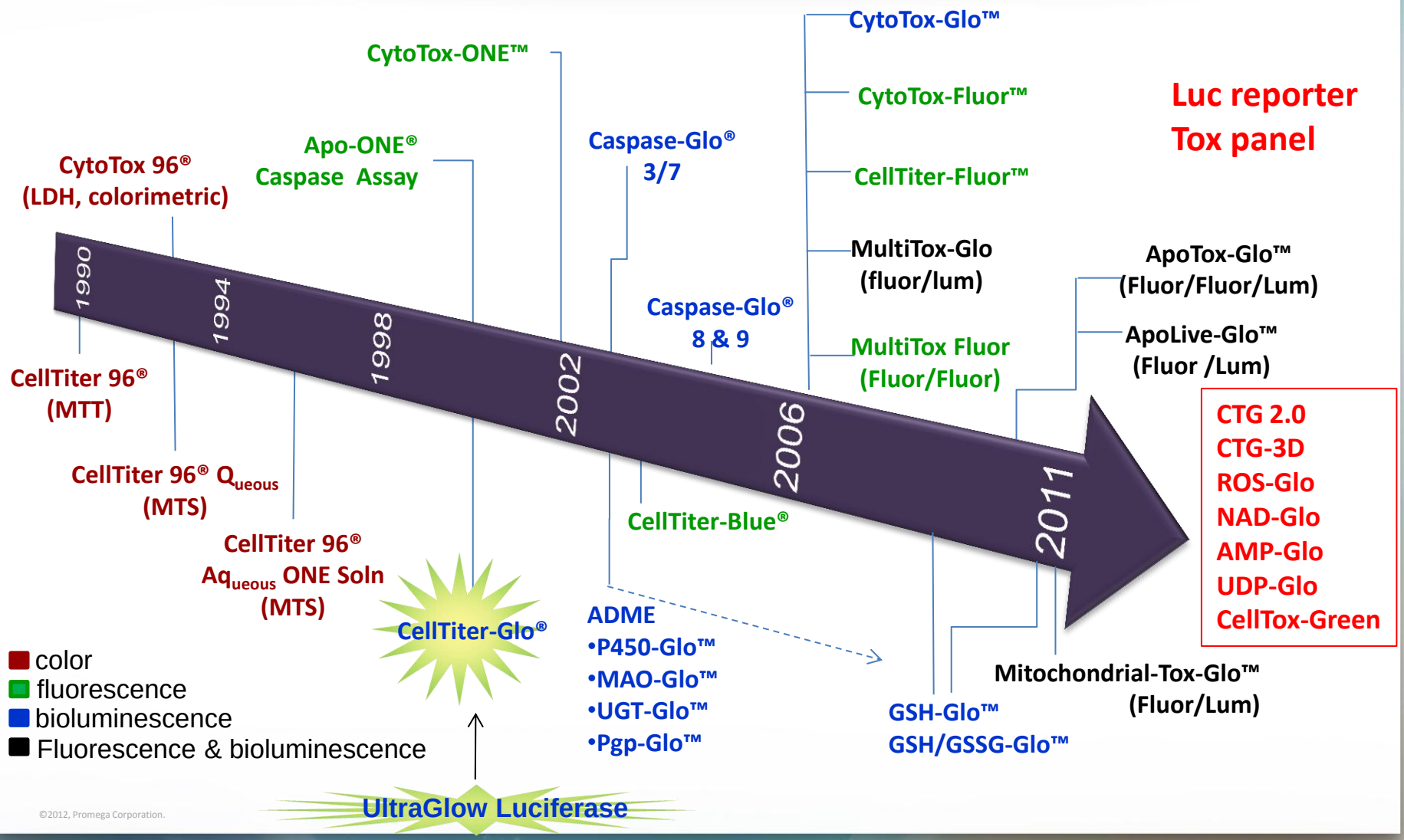
# *Complicated Biology ---- Simple Assay*



## Add-Mix-Read

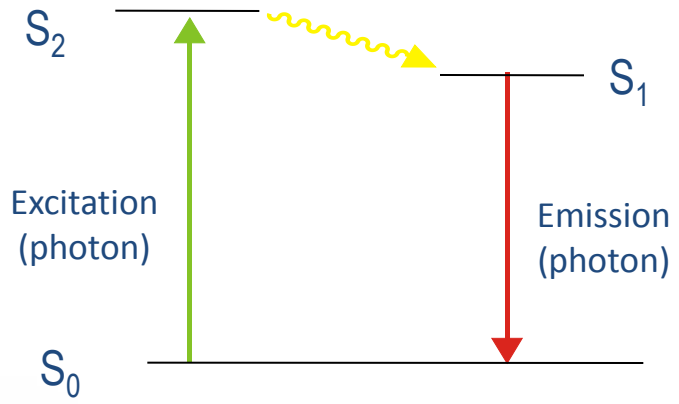
# *Evolution of assay design*



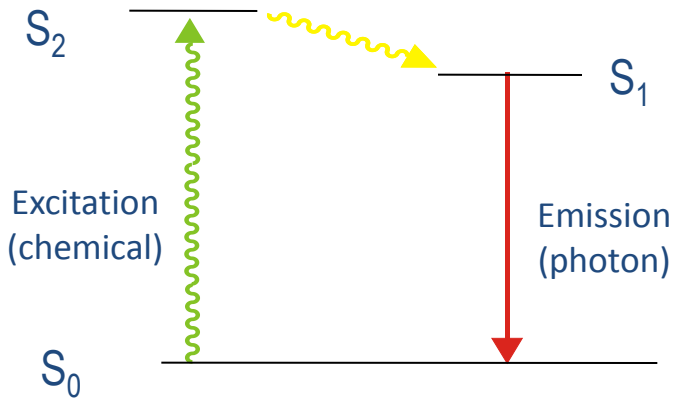
# *Fluorescence vs Luminescence*

- Fluorescence and luminescence differ primarily by the method for creating the excited-state of the photon emitter.
- The difference in how the excited state is created dictates practical differences in how these technologies perform.

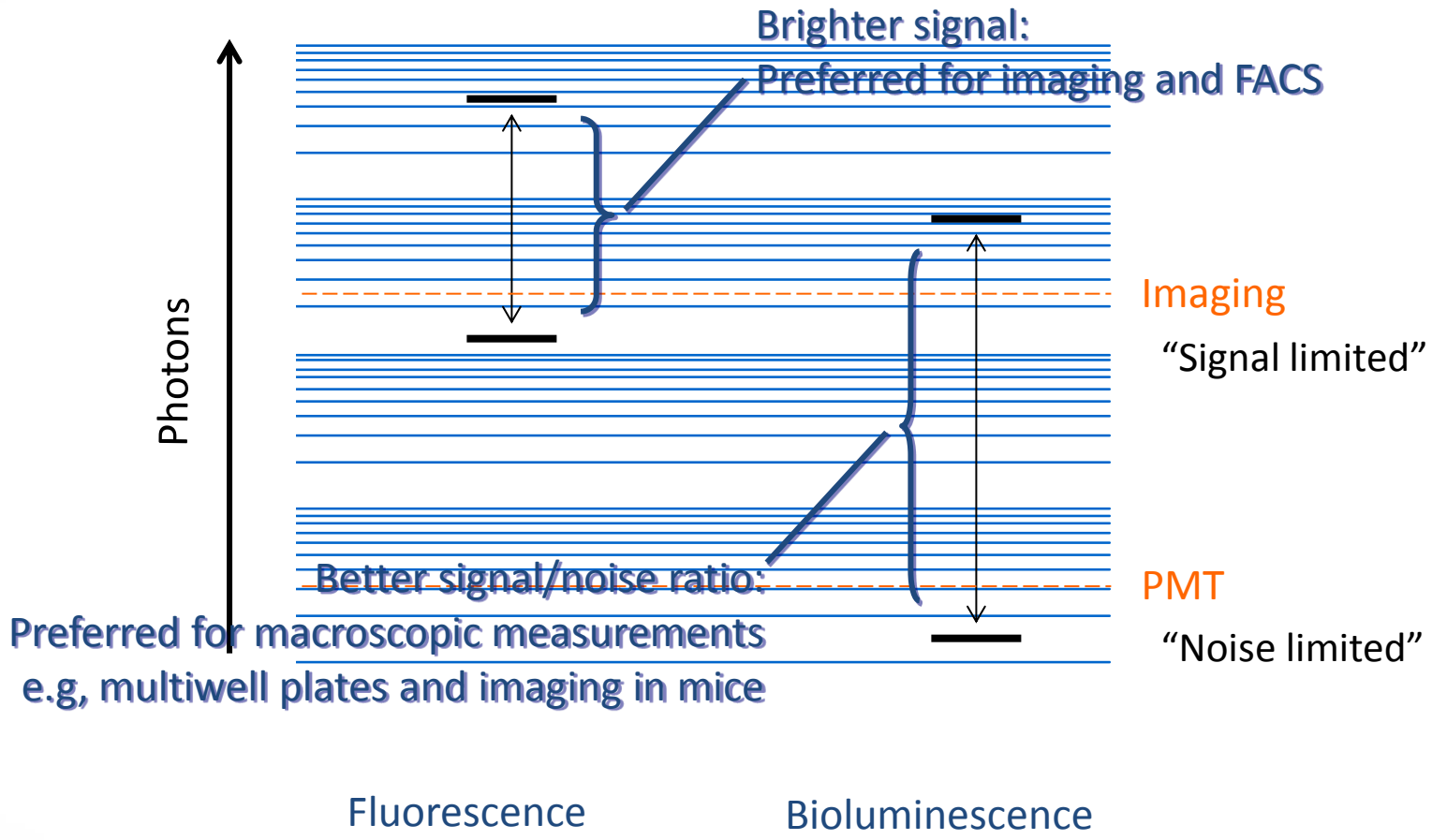
Fluorescence



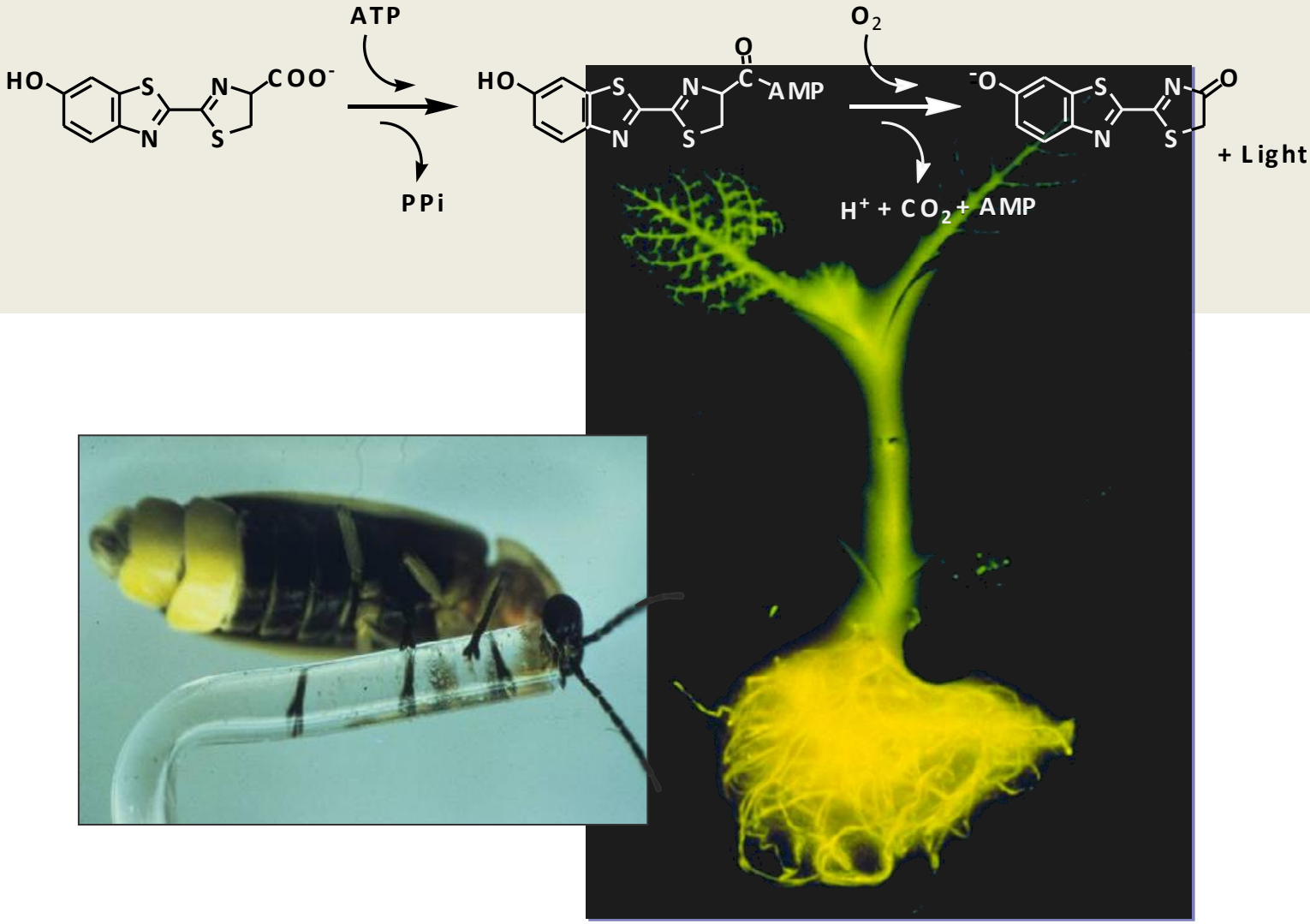

Luminescence



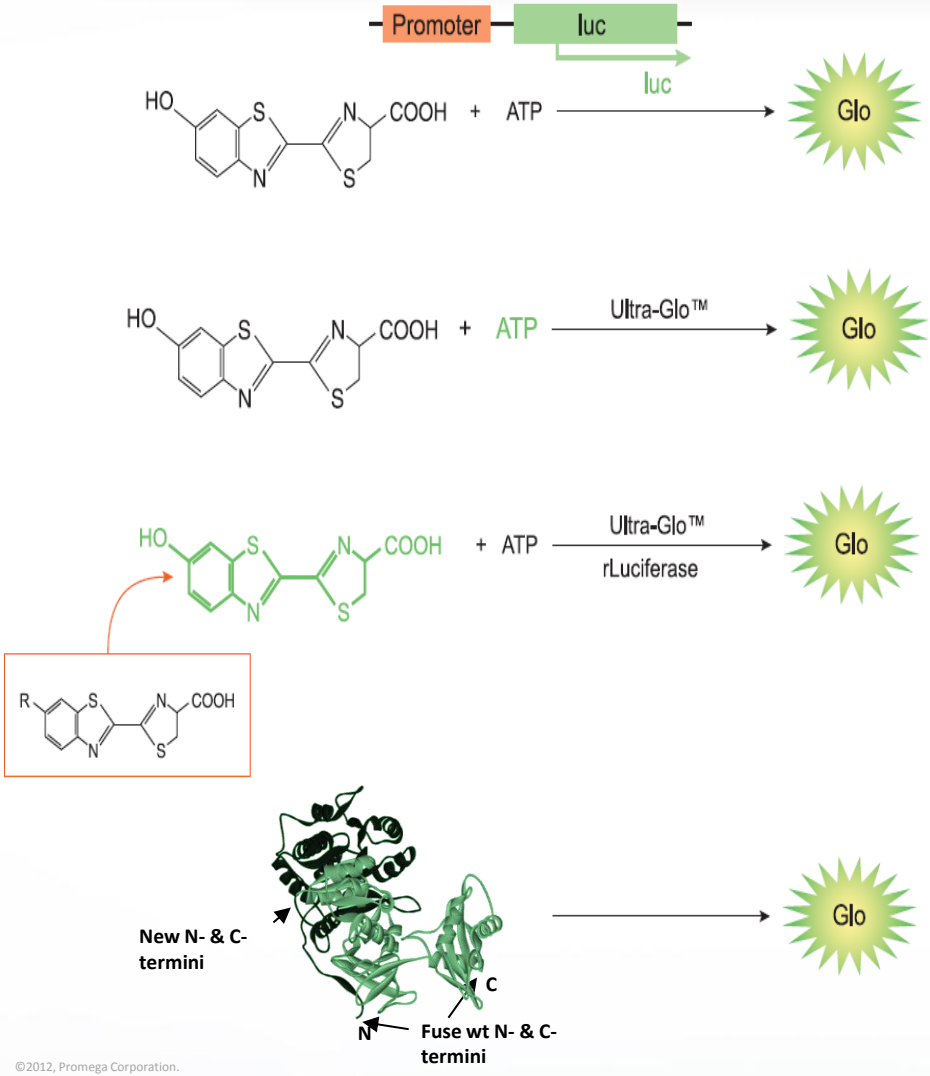
# Luminescent assay has superior sensitivity and dynamic range




Great for HTS, why?


ATP
PPi
HO
S
N
COO-
AMP
O2
H+ + CO2 + AMP
-O
+ Light

# *Bioluminescent Assays Developed From Firefly Luciferase Chemistry*



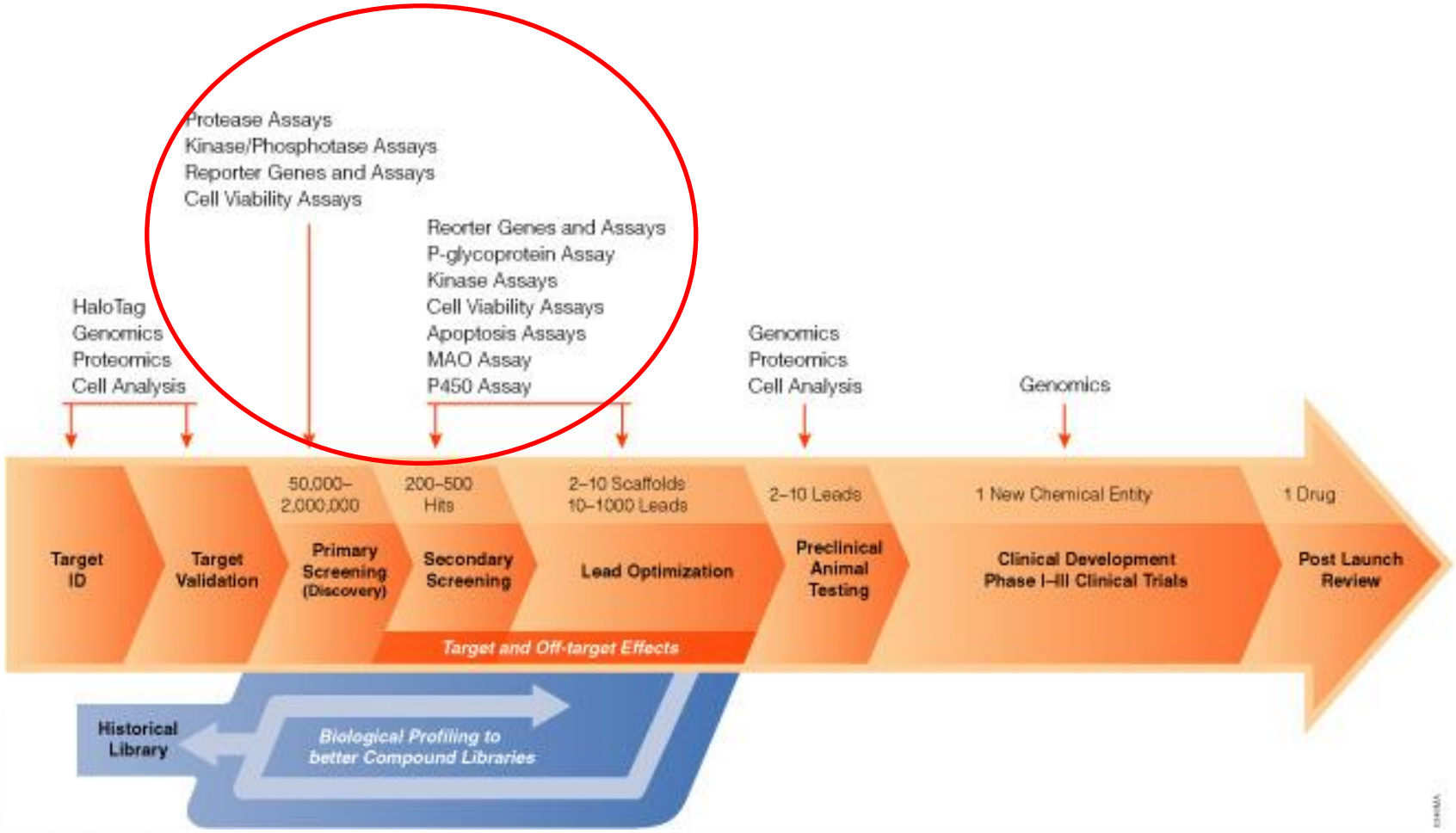

- Reporter gene assays
  GPCR & Nuclear Receptor

- Cell Viability
- Kinase assays
- cAMP & PDE assays
- P-glycoprotein assay

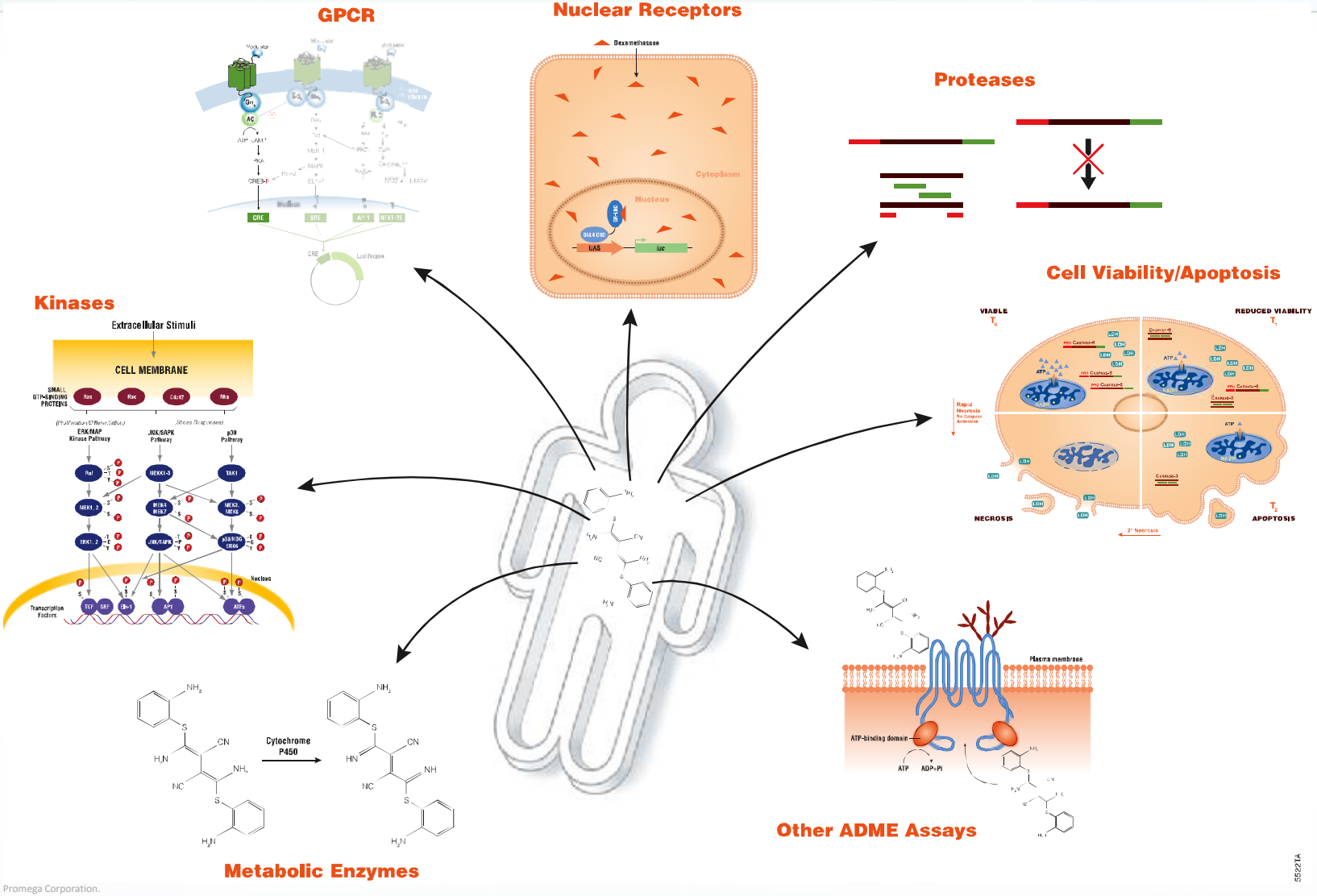
- Caspases/proteases
- CYP450 assays
- MAO assays
- GSH assays
- HDAC assays

- GloSensor cAMP
- GloSensor caspase

# *Bioluminescent assays for chemical drug discovery*



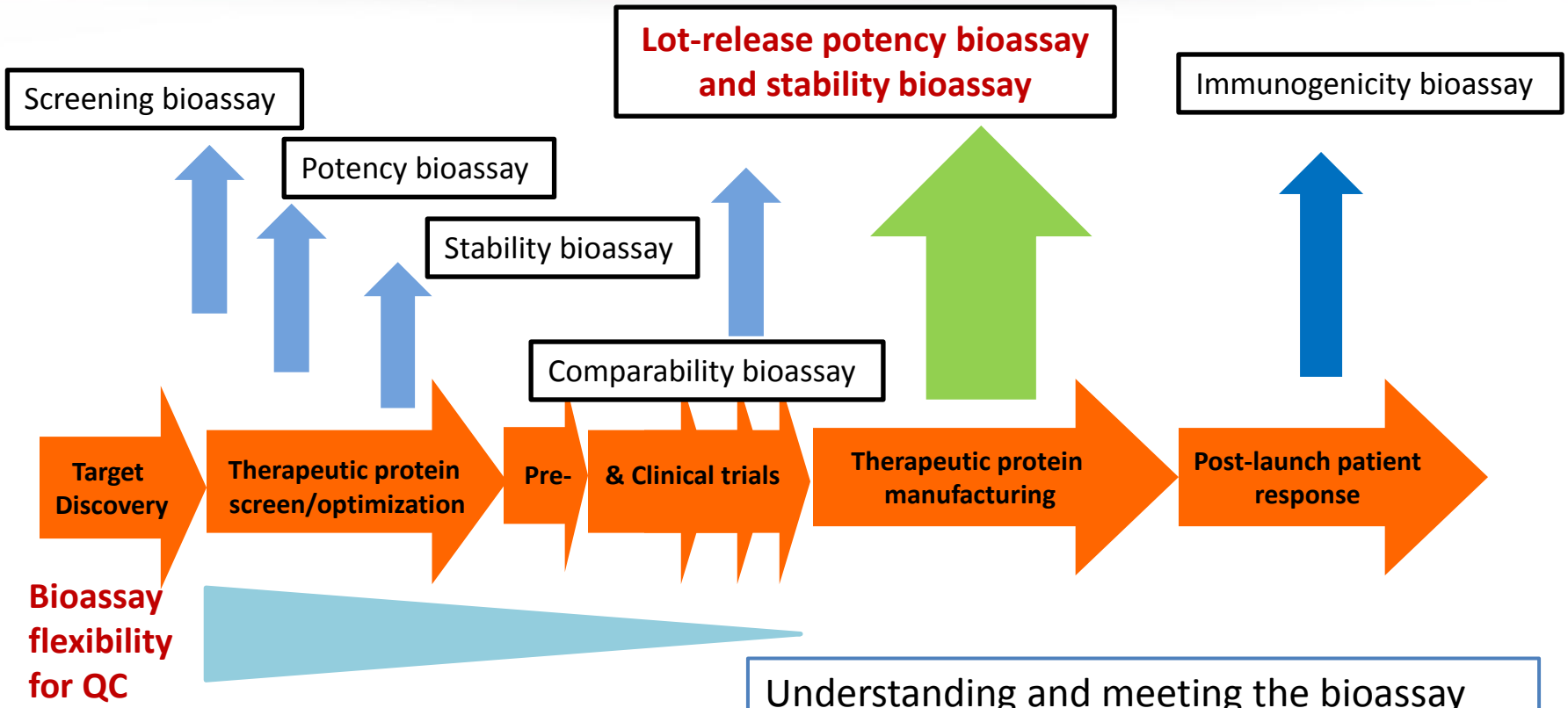
# *Bioluminescent assays for chemical drug discovery*






5522TA

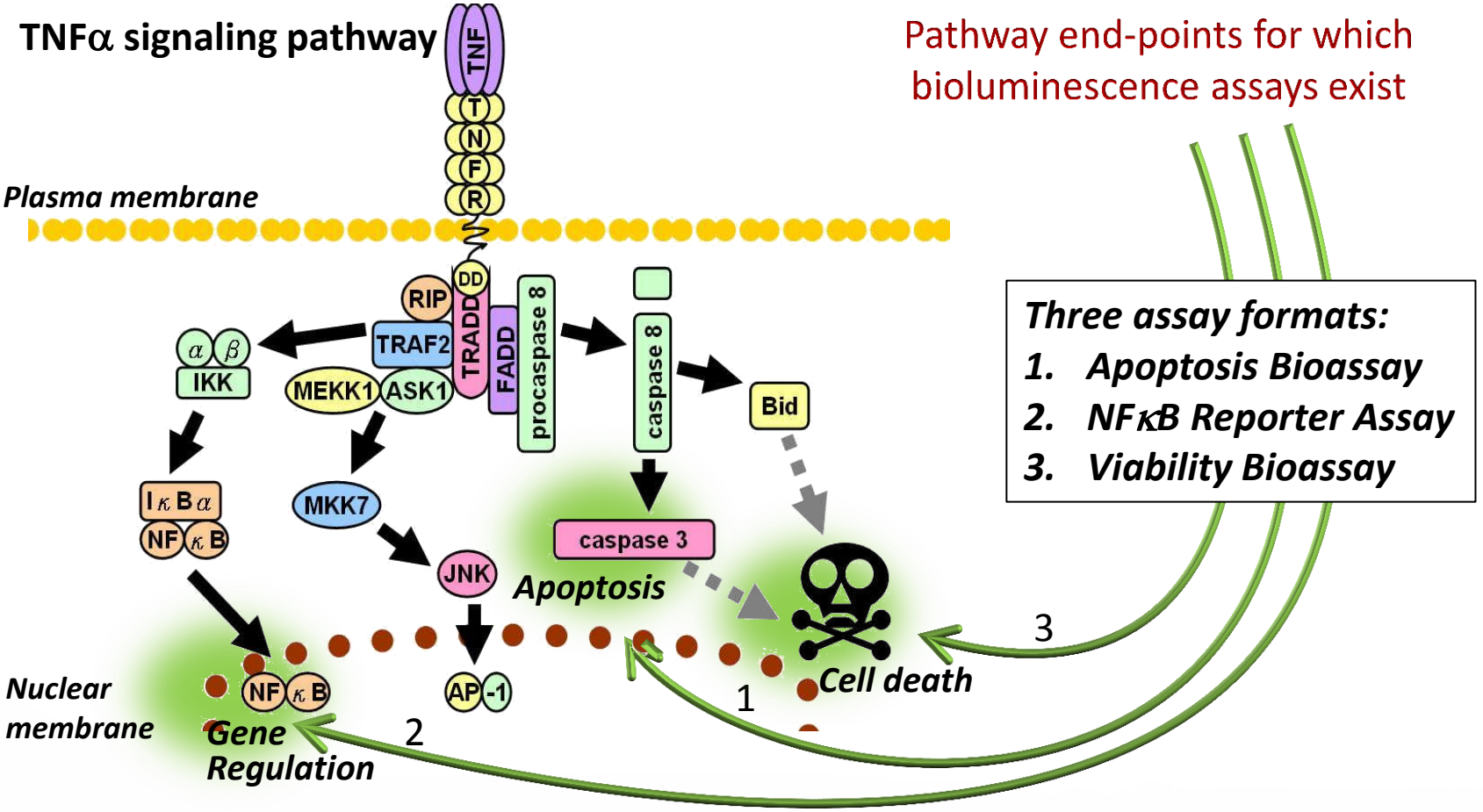
# *Bioluminescent bioassays for antibody drug development*

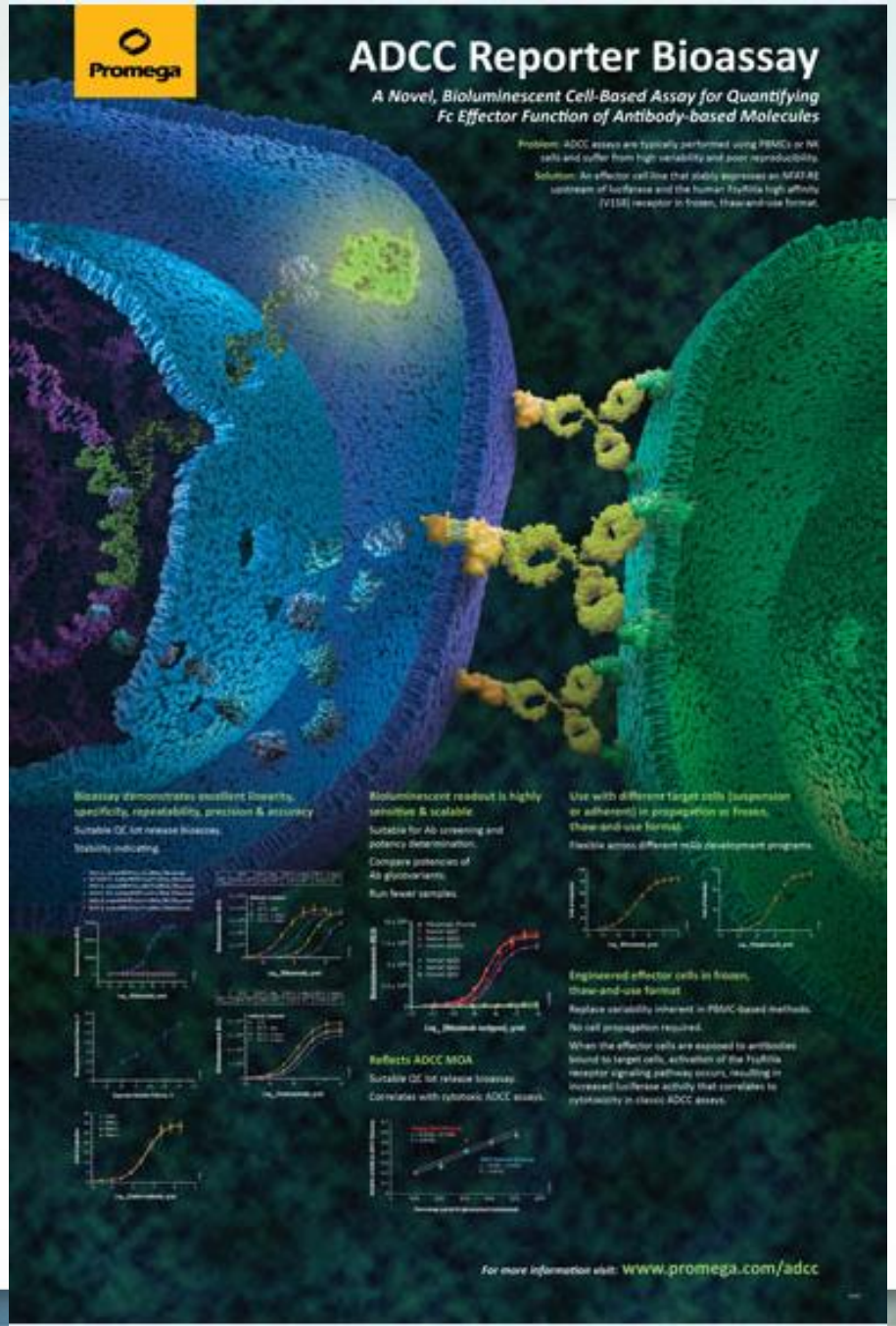



Understanding and meeting the bioassay needs of different areas during biologic drug development and manufacture

- Less variability
- Higher reproducibility

# Bioluminescent Cell-based Bioassays

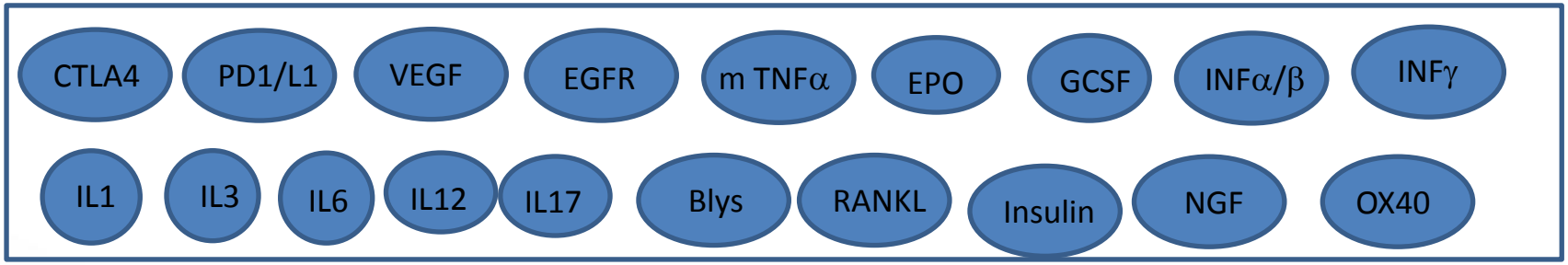




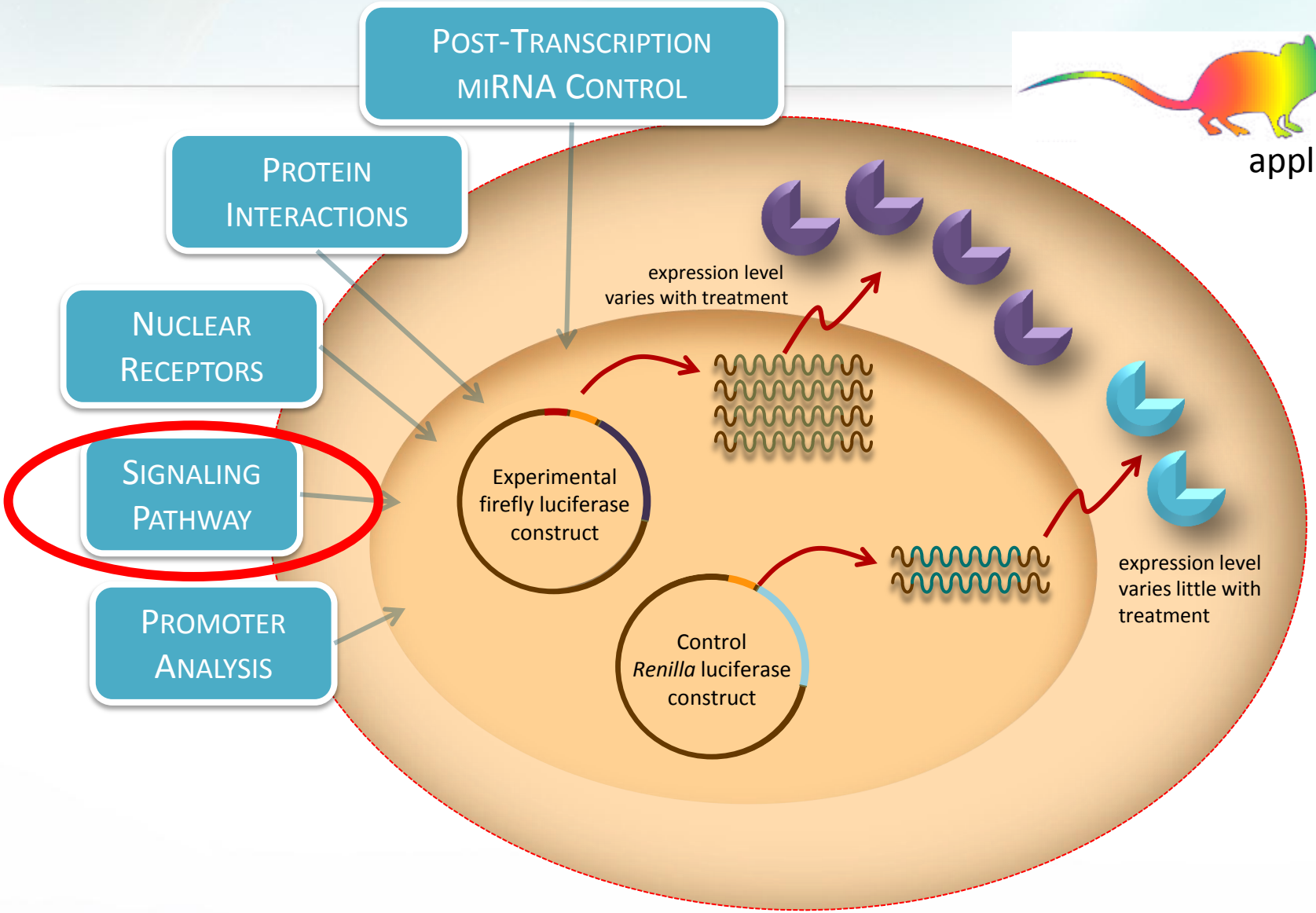

**Top10 Innovations**
**2013**
**The Scientist Magazine**

# *Bioluminescent bioassays*

- ADCC Reporter Assay F version
- Mouse ADCC Assay
- ADCP Reporter Assay
- T cell Activation Assay



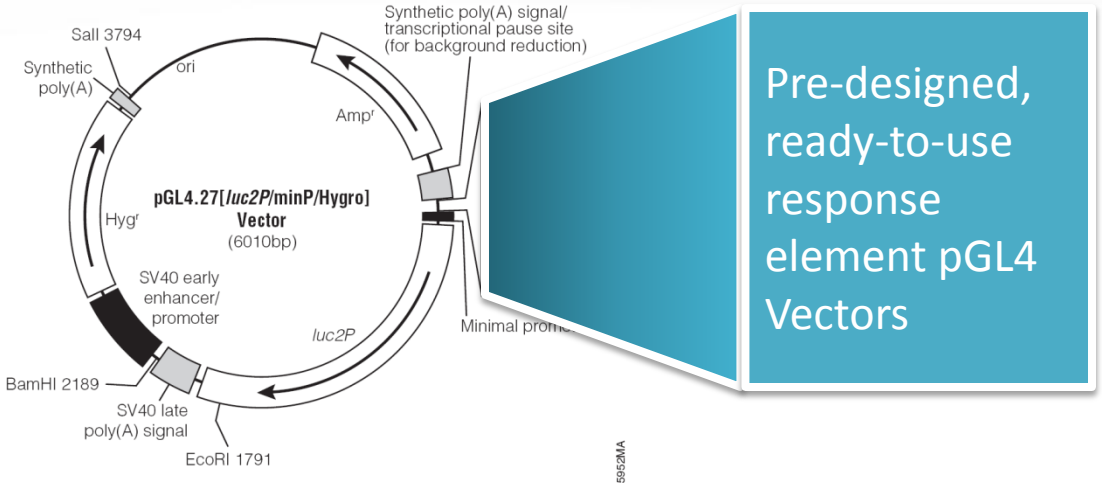
# Application Overview






In vivo applications

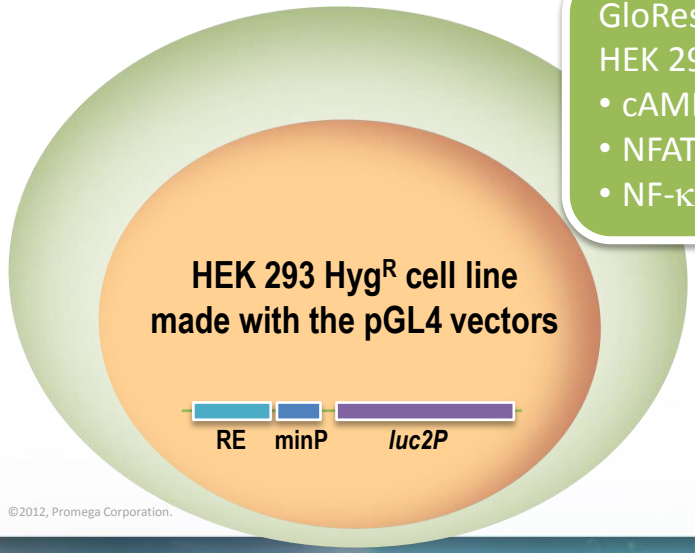
# *Promega offers complete solutions*



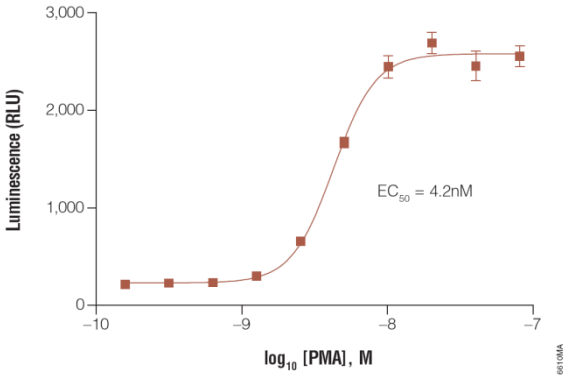

Pre-designed, ready-to-use response element pGL4 Vectors

- **Genes**
- **Vectors**
- **Cell lines**
- **Complete assays**

- **Catalog**
- **CAS**
- **CAM**

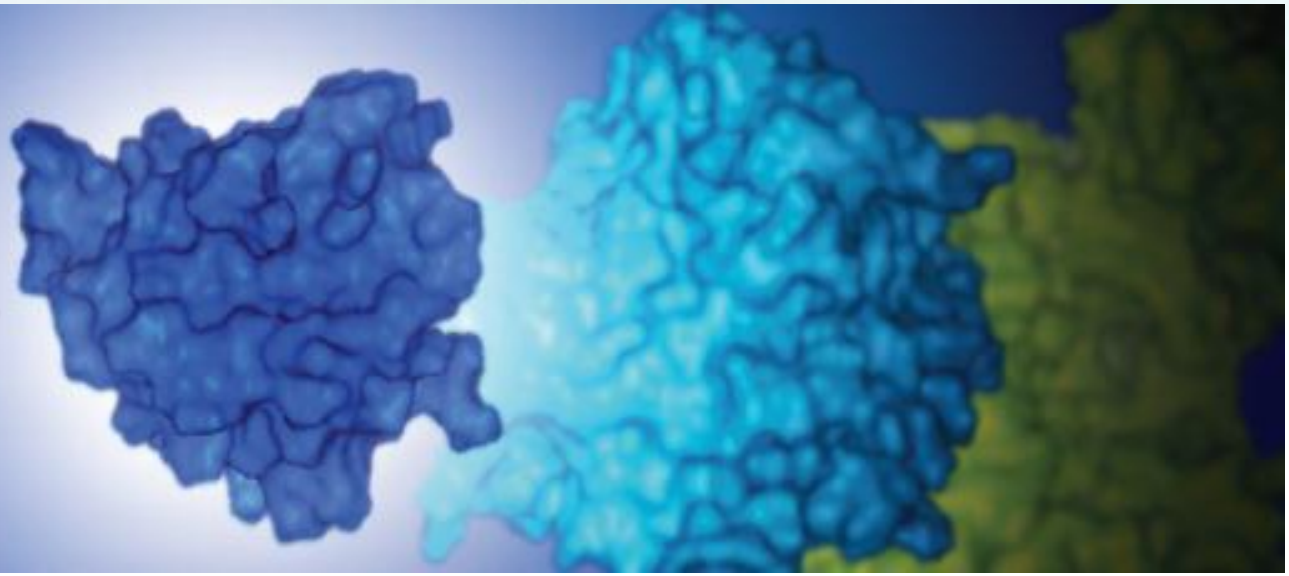



GloResponse™
HEK 293 cell lines:
- cAMP RE
- NFAT RE
- NF-κB RE




Dose response curve
GloResponse NFAT-RE-*luc2P*
HEK293 Cell Line

# NanoLuc™ Luciferase Technology



NanoLuc Luciferase as a genetic reporter

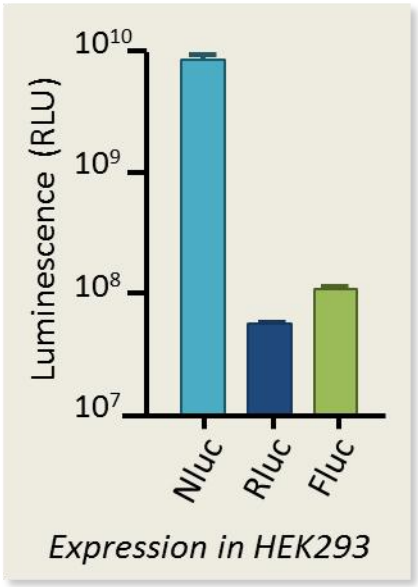
- Brighter, more responsive
- Smaller
- More versatile

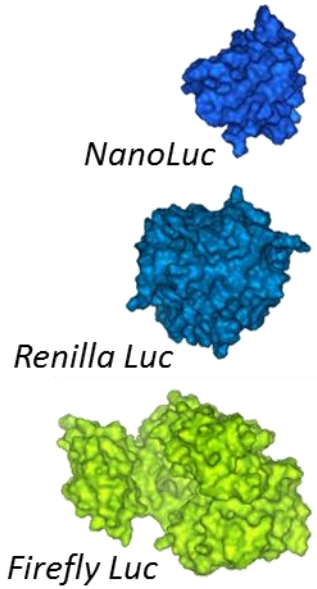
NanoLuc Luciferase as a protein function reporter

- Protein Translocation
- Protein Stability
- Protein:Protein Interactions

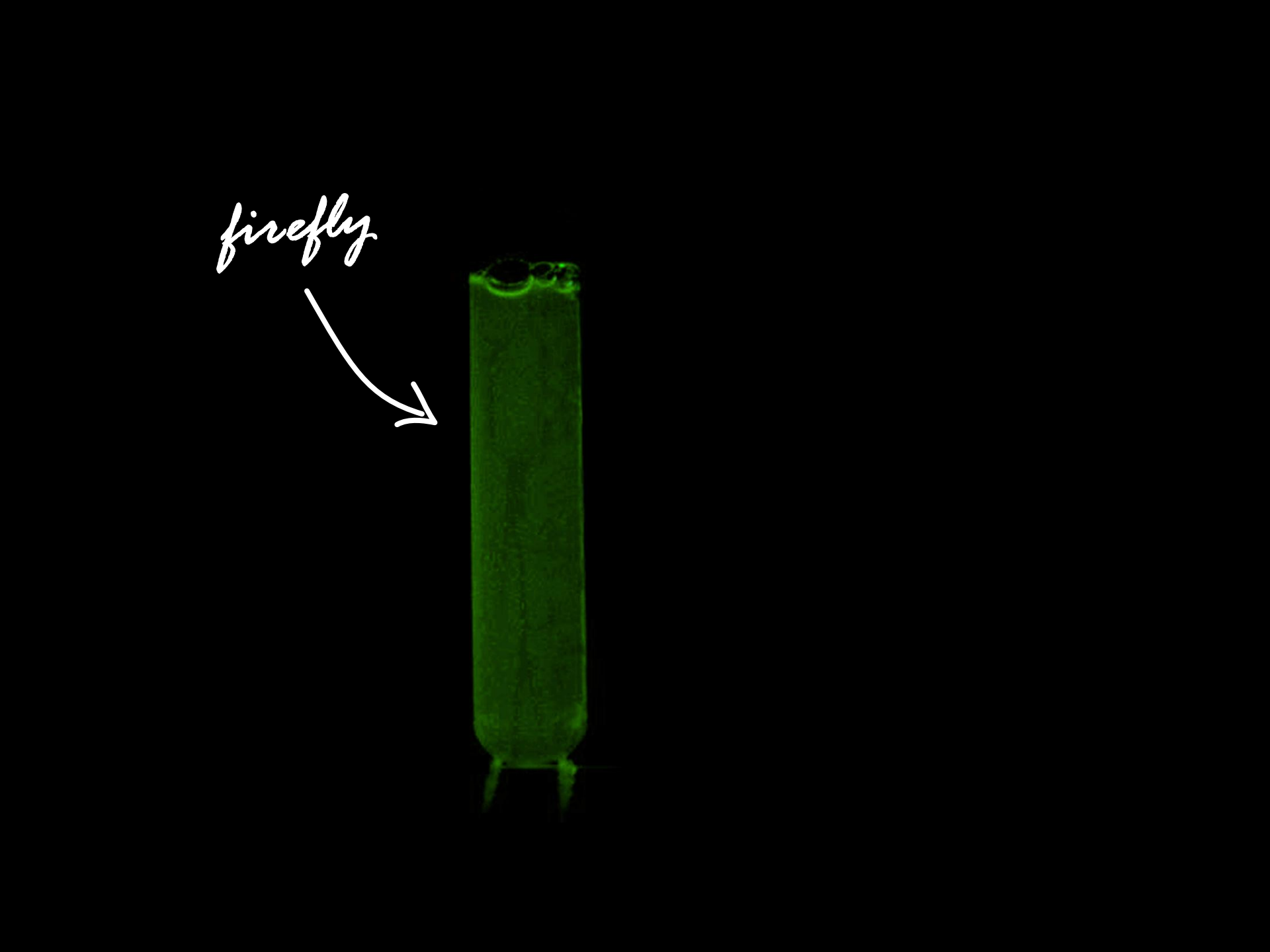
# *NanoLuc: a new generation of luciferase technology*

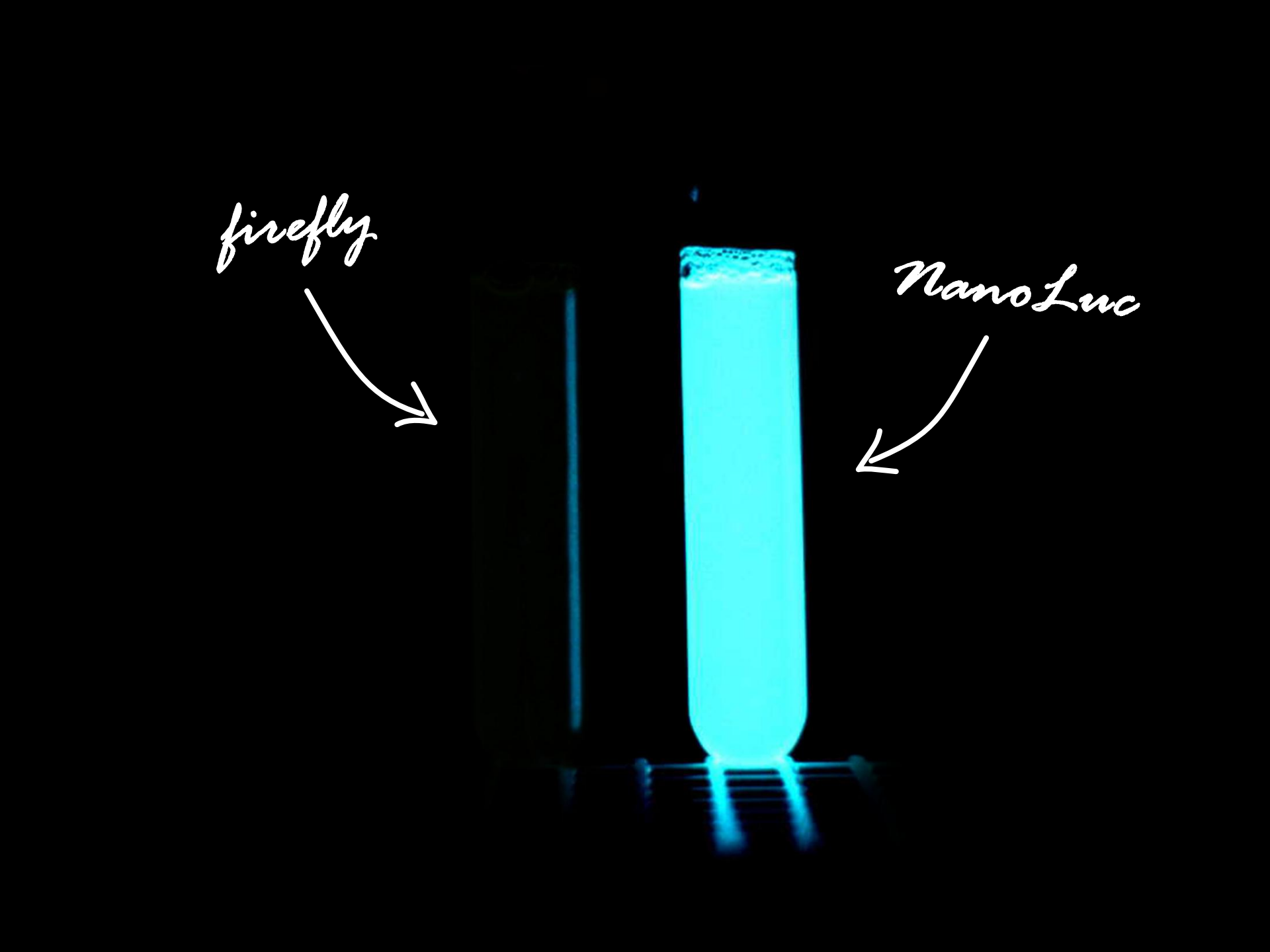
- Extremely bright
  - 100-fold greater light intensity
  - Much brighter in mammalian cells
  - Glow kinetics
- Very small (19kD)
  - Much smaller than Fluc or Rluc
  - Smaller than GFP
  - Ideal as fusion tag
- High physical stability
  - Compatible with fusion partners
  - Robust to experimental conditions
- Narrow emission spectrum
  - Less overlap with energy acceptor
  - Greater detection sensitivity



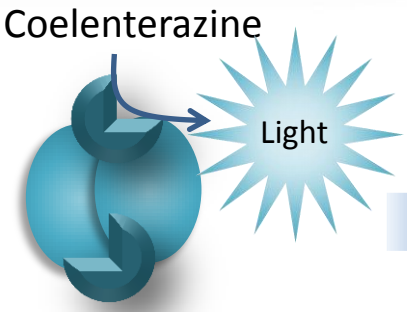

*Deep sea shrimp secretes extremely bright cloud of luminescence as a survival mechanism*


firefly

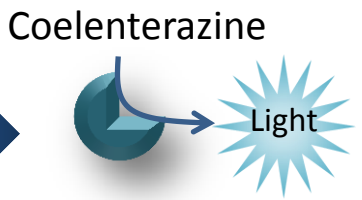

firefly
NanoLuc

# *Building a Better Luciferase*
## *NanoLuc® Evolution: From Ocean to Lab Bench*

*Oplophorus gracilirostris* first cataloged in 1881



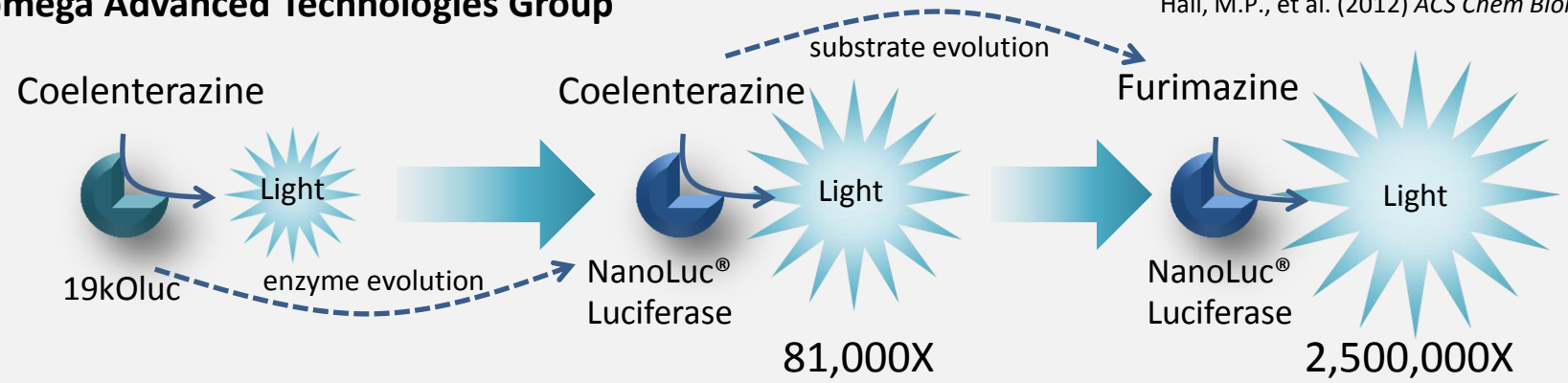

130kDa
*Oplophorus* luciferase
7X brighter than native
*Renilla* Luciferase
Shimomura, O., et al. (1978)




19kOluc
19kDa subunit is catalytic.
Light output & stability compromised.
Inouye, S., et al. (2000)

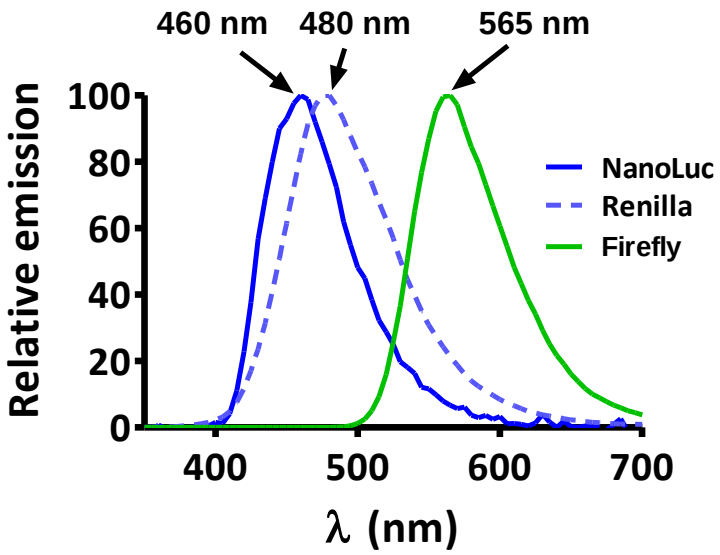
**Promega Advanced Technologies Group**

Hall, M.P., et al. (2012) *ACS Chem Biol* **7**:1848

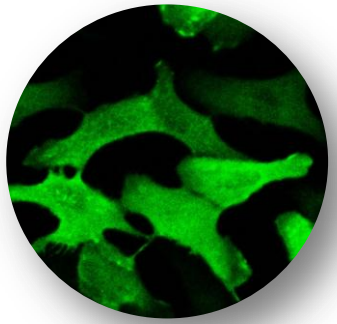


# *Excellent Physical Properties Make NanoLuc® a Powerful Research Tool*

- ✓ **Thermal stable**
  - Retains activity after 30 min @ 55°C
    - Melting temps: Nluc, 58°C; Fluc, 31°C
- ✓ **Active over broad pH range**
  - Fully active between pH 7-9
  - Retains significant activity at pH 5-7
  - Fluc: sharp decrease in activity below pH 8
- ✓ **Monomeric enzyme**
  - Facilitates use as transcriptional reporter or fusion partner
- ✓ **No mammalian post-translational modifications detected**
- ✓ **No disulfide bonds**
  - Supports high levels of activity in living cells
- ✓ **Uniform distribution in cells**
  - No apparent compartmental bias in the absence of targeting sequences

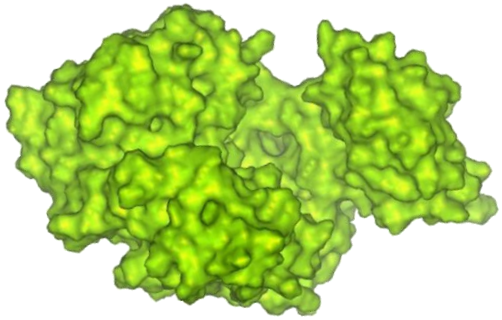


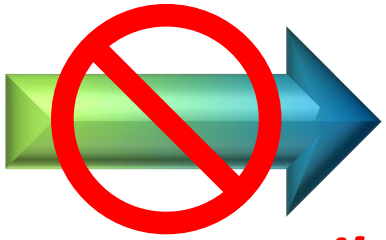

**Unfused Nluc Immunofluorescence**



# *Should I switch from Firefly to NanoLuc™ Luciferase?*

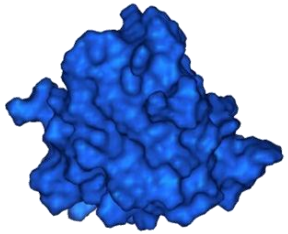
***Firefly (Fluc)***





***Not necessarily***

***NanoLuc™ (Nluc)***



**Does it allow you to do your work?**
**Do you plan to do work in vivo?**

**Firefly is a great reporter**

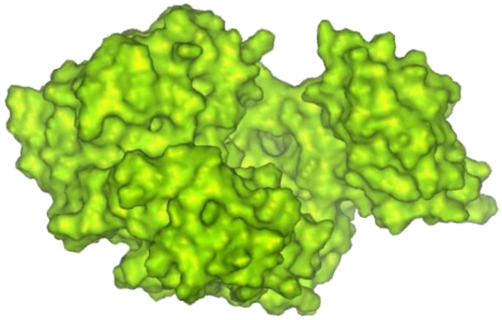
- ✓ Excellent signal:background
- ✓ Excellent dynamic range

We just released new response element signaling pathway detection pGL4 vectors:

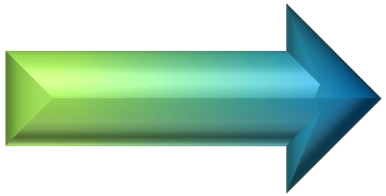
| | | | | |
|---|---|---|---|---|
| ARE | HSE | ISRE | STAT5 | SRE |
| p53 | HRE | SIE | NFAT | SRF |
| ATF6 | XRE | SBE | CRE | |
| MRE | AP1 | TCF-LEF | NF-κB | |

# *Should I switch from Firefly to NanoLuc™ Luciferase?*

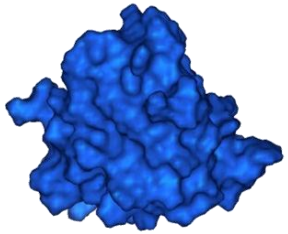
***Firefly (Fluc)***



***Yes, if …***



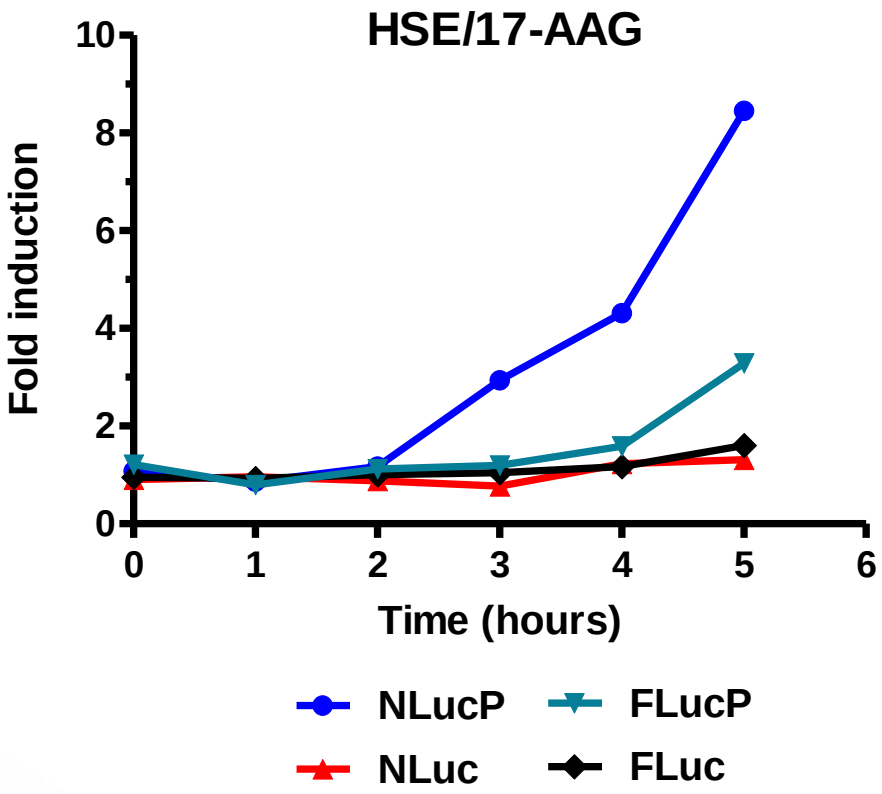
***NanoLuc™ (Nluc)***



- ✓ **Transfection efficiency limits you to easy-to-transfect cell lines**
- ✓ **Signals are too weak to move to 96-well plates**
- ✓ **FLuc is just too big**

- The increased brightness could allow a subtle signal become a reliable signal.
- The small size could allow gene replacement with minimal impact, especially in viral constructs

# *Relative response of reporter genes*



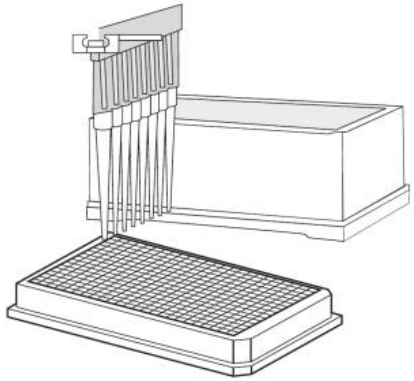

## Relative Response

**NlucP > FlucP > Fluc,Nluc**

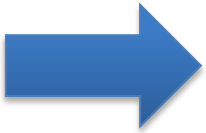
Experimental details: transient transfection of Hela cells w/ Hsf1 inducible constructs; addition of 500 nM 17-AAG at time zero.

# *NanoDLR: Fluc then Nluc multiplex -in development*

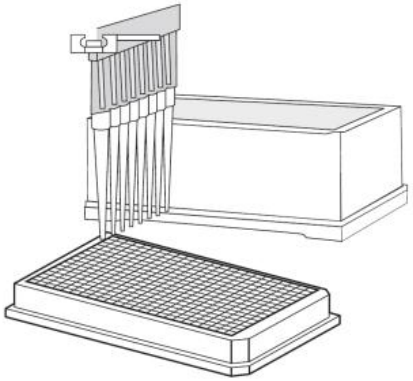
*Read #1: Fluc*



+ Fluc inhibitor
+ Furimazine



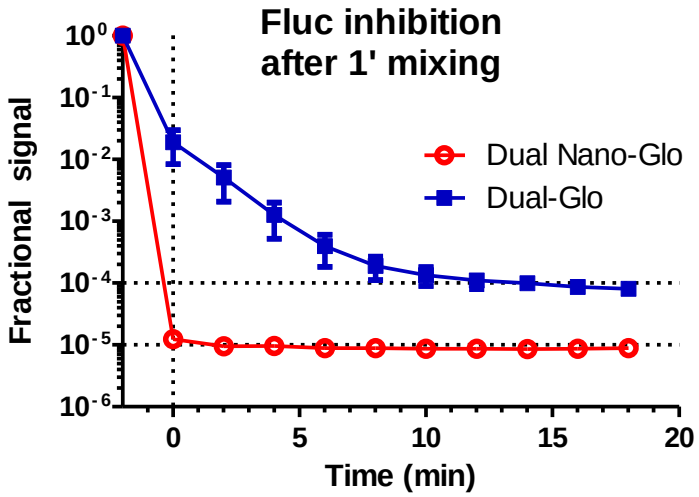
*Read #2: Nluc*

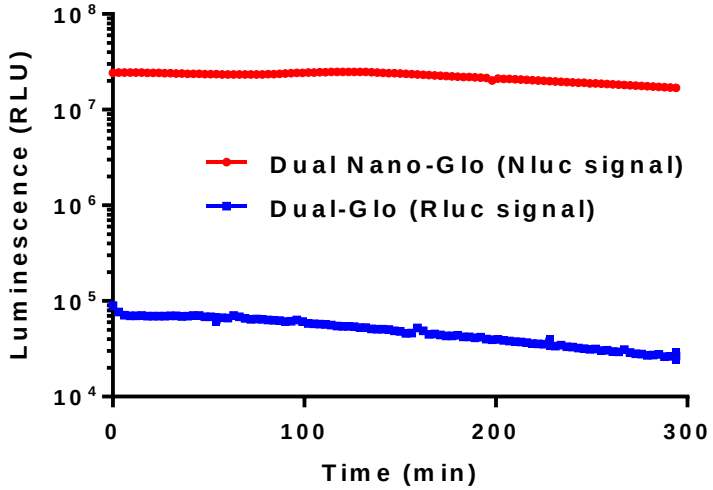


Possible formats (+/- PEST in all cases):

- Fluc dynamic/Nluc control
- Fluc control/Nluc dynamic
- Fluc dynamic/Nluc dynamic

# *NanoDLR Stop & Glo reagent*



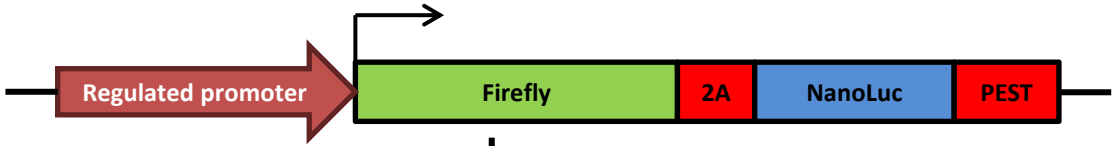




- Provides faster and more potent Fluc inhibition
  - Quickly take both reads for a single plate to facilitate HTS work-flow
  - Minimizes background for Nluc detection
- Large increase in sensitivity for detection of Nluc vs. Rluc
  - Lower residual Fluc signal
  - Nluc **much** brighter than Rluc

# *NanoLuc and firefly Luc in HTS*

- Compounds that inhibit/stabilize luciferase give too many false positives
  - Huge resource commitment to find the real hits
- Nanoluc less susceptible than other luciferases, but NlucP more susceptible than Nluc

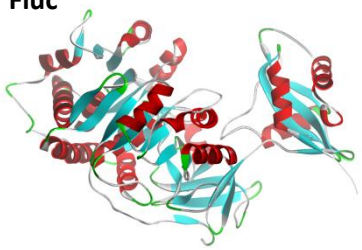
Co-incidence reporter developed at NIH NCGC*



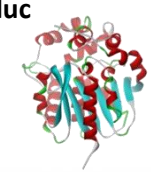

Transcription

Translation

Fluc



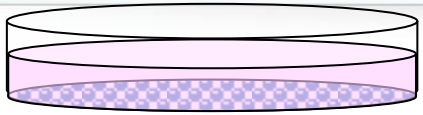
Nluc



- 2A promotes 'ribosome skip'
- Separate Luc enzymes w/ own inhibitor profiles
- True hits show induction of both
- False positives show induction of one, not both

* Cheng & Inglese *Nature Methods* vol. 9, p. 937.

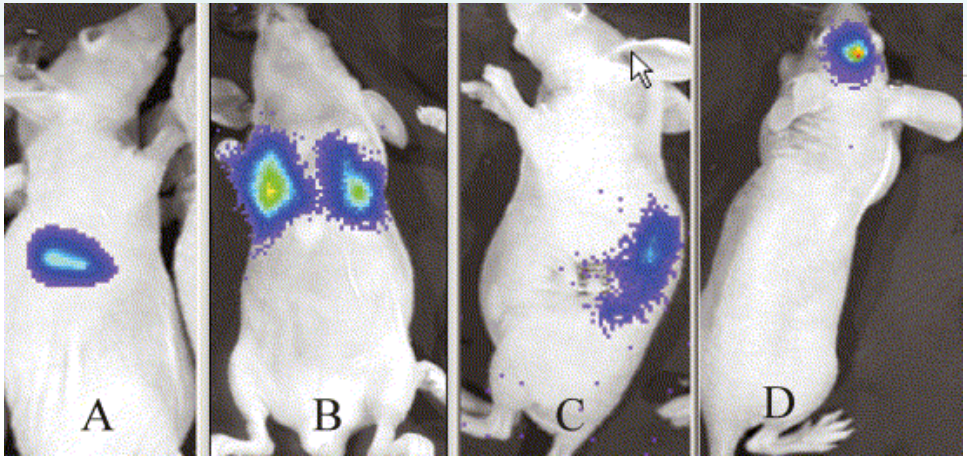
# *Insert a luciferase biomarker in the test cells*



tumor cells

stably transfect with luciferase construct

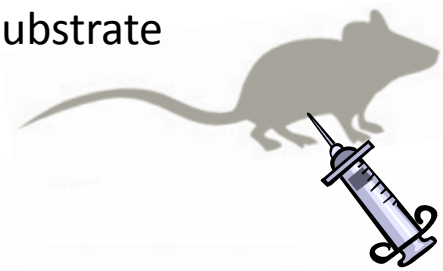
Inject cells




Bioluminescent imaging with CCD device (overlaid on mouse photos)

treat

Inject Luciferase substrate



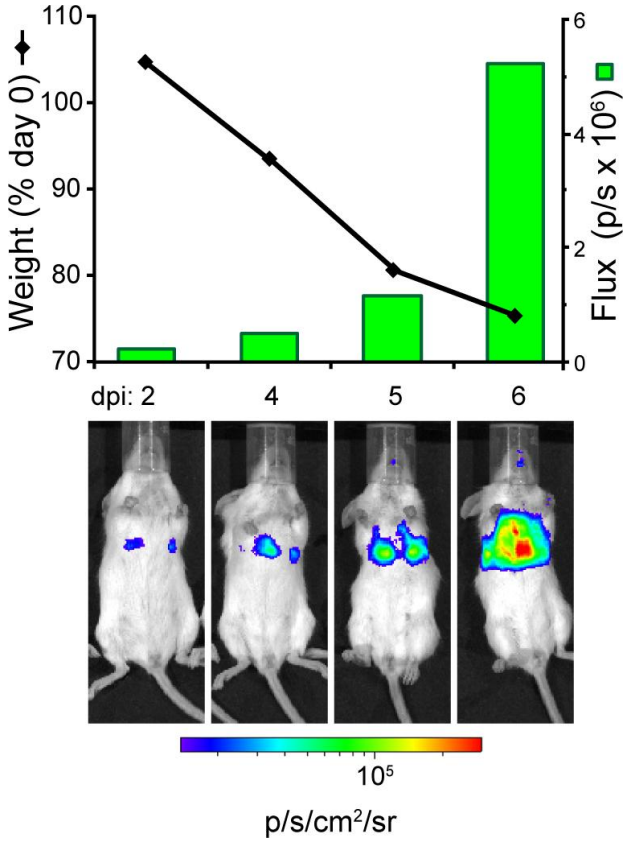
In this case, luciferase is acting as a viability marker.
This model can be used to evaluate treatments in vivo.

# *NanoLuc® for Viral Applications*
## *Small Size is Highly Advantageous*

- Data presented is from **Prof. Andrew Mehle's Lab** at the University of Wisconsin

- Historically, it's been difficult to stably integrate reporter genes into influenza
- Created influenza recombinant virus stably expressing NanoLuc®
  - No details on construction provided here
  - If you're interested in working with Andy or would like more info, please contact him directly: amehle@wisc.edu

# *Influenza - NanoLuc® Reporter Virus*
## *Real-time In Vivo Imaging of Infection*

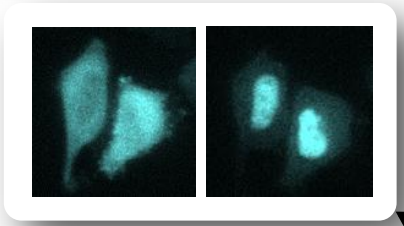



Data presented is from Dr. Andrew Mehle's Lab at the University of Wisconsin

# 20687

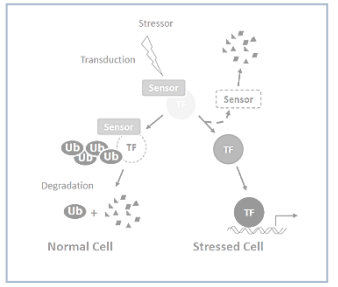
http://en.wikipedia.org/wiki/ENCODE_(ENCyclopedia_Of_DNA_Elements)

# *NanoLuc™ Luciferase as a fusion partner: Proof of concept experiments*

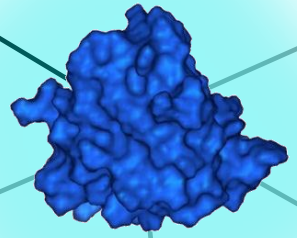


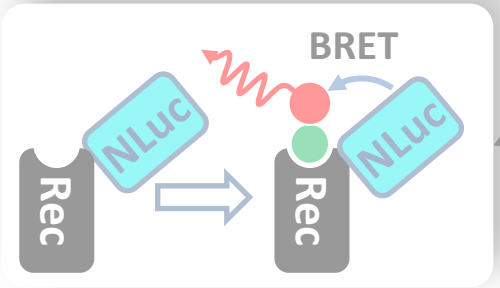
**Protein Translocation**

**Protein Stability**

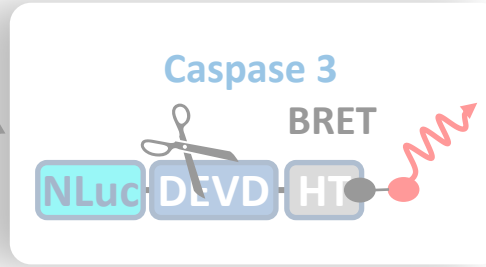




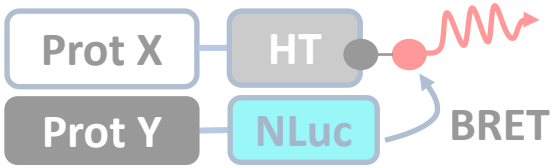





**Receptor Interactions**




**Biosensors**

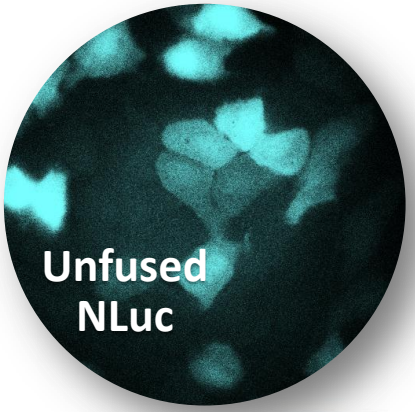



**Protein-Protein Interactions**

# *NanoLuc™ Luciferase excels in bioluminescent imaging applications*

Nluc brightness leads to short exposure times:

- Fluc/Rluc: 1-5min/exposure
- Nluc: 1-5sec/exposure



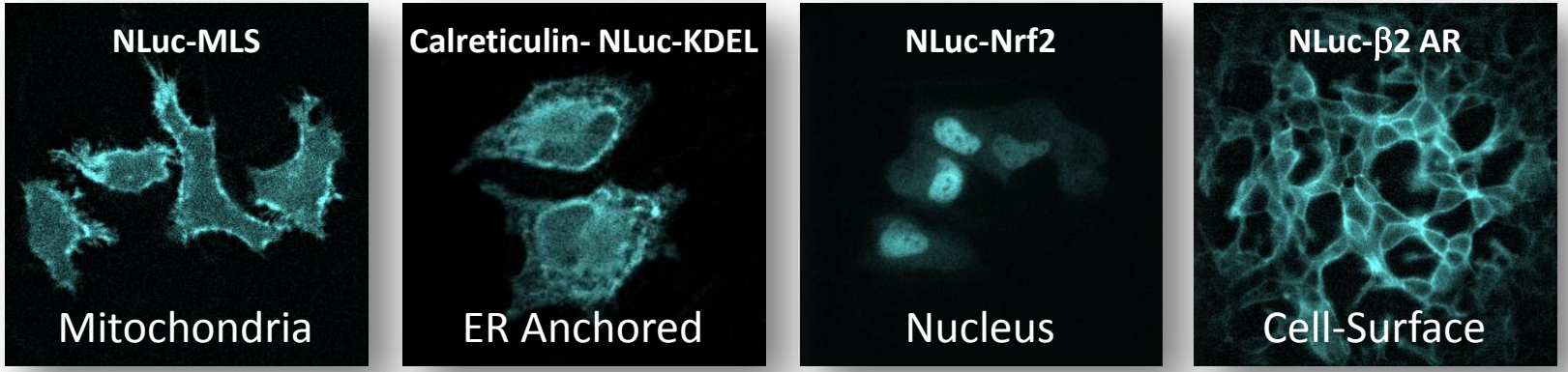

NanoLuc & LV200 featured @ASCB 2012 Olympus Product Showcase

Why bother? Fluorescence works.

- Fluors are susceptible to photobleaching.
- Excitation can cause autofluorescence of other fluors
- Luciferases will generate light as long as substrate is available

Olympus LV200
Bioluminescence Imager

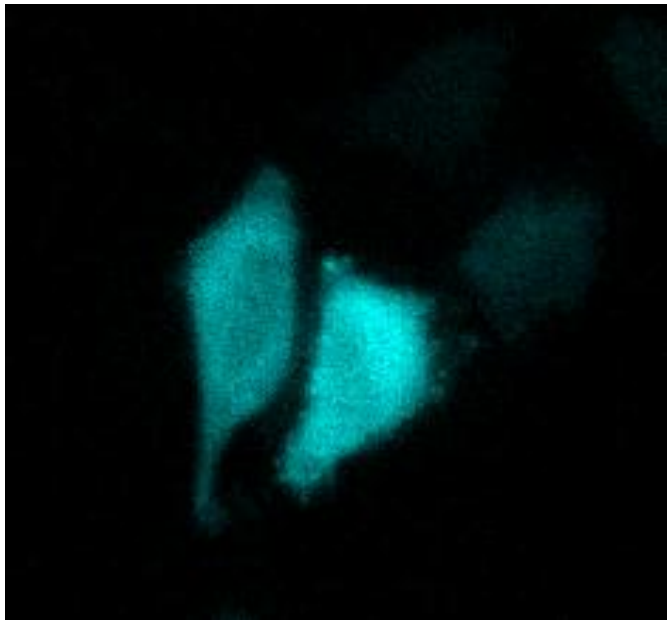
# *NanoLuc™ Fusions can go anywhere…*




NanoLuc™ Luciferase fusions could be a useful tool to investigate cell biology

# *Bioluminescence imaging of NanoLuc fusion proteins*

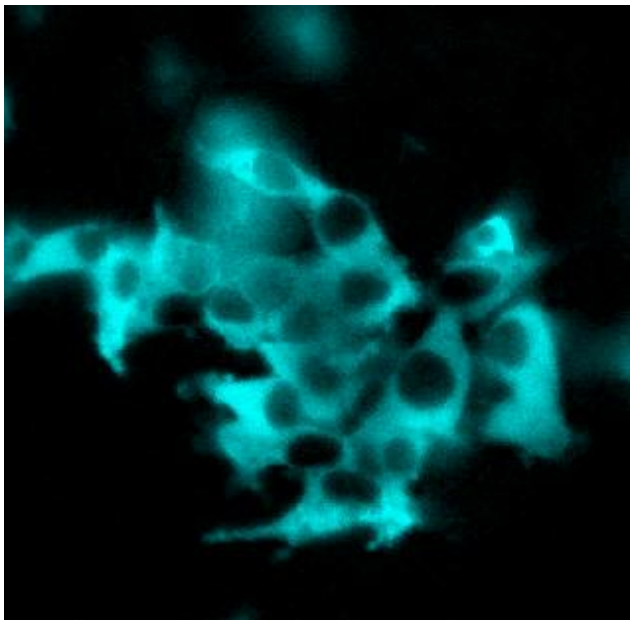
Glucocorticoid receptor (GR)



*Time lapse: 13 minutes*

Upon treatment with dexamethasone, the receptor dissociates from the Hsp90 complex and translocates to the nucleus.

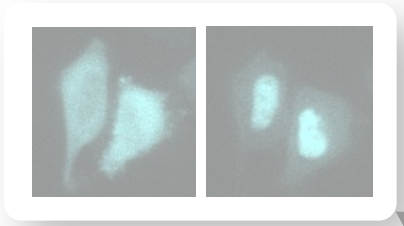
Protein Kinase C alpha (PKCα)



*Time lapse: 20 minutes*

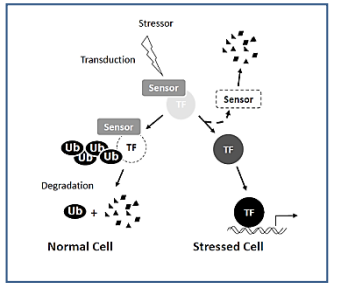
Upon treatment with phorbol esters, PKCα is recruited to the membrane.

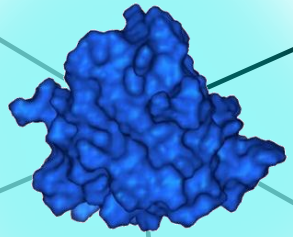
# *NanoLuc™ Luciferase as a fusion partner: Proof of concept experiments*

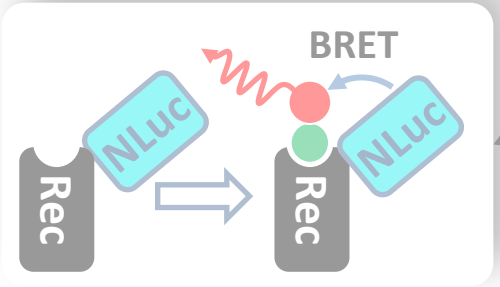


Protein Translocation

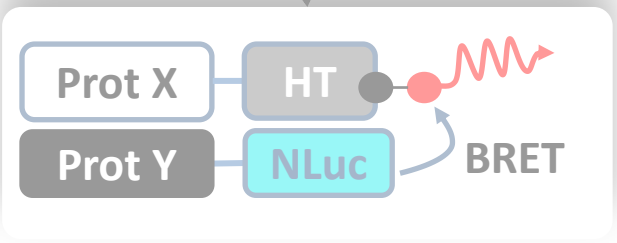
Protein Stability



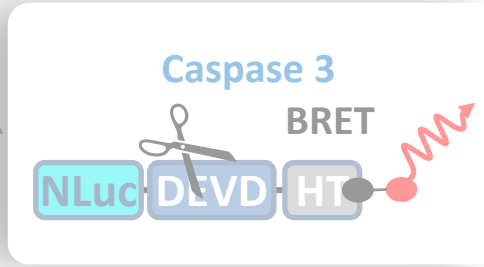






Receptor Interactions

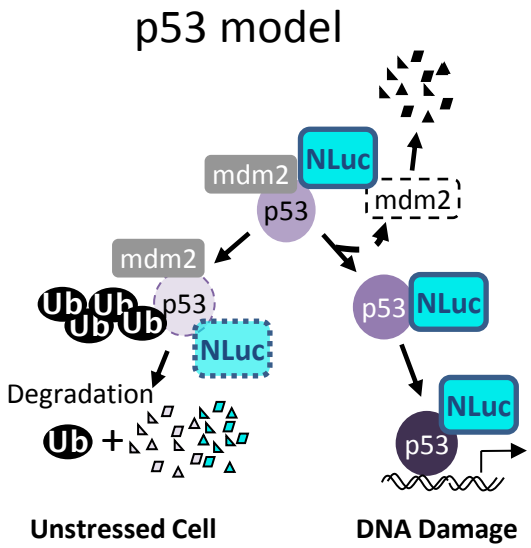


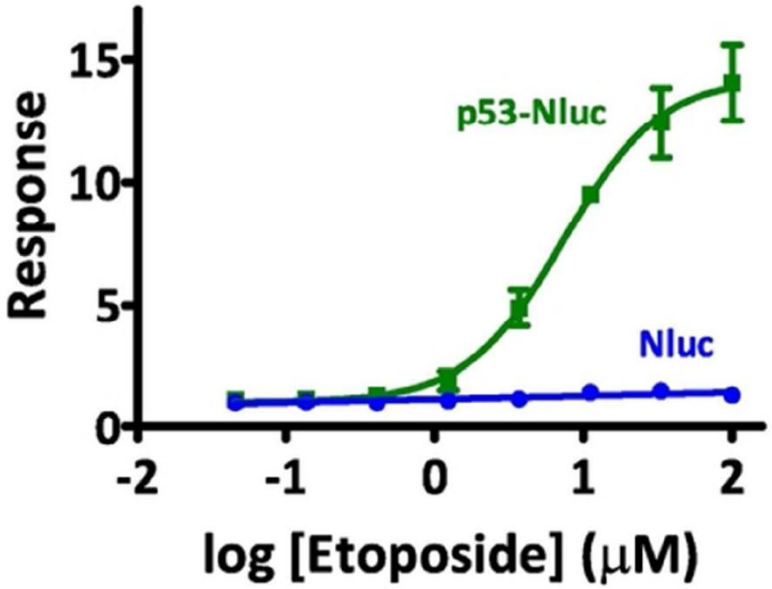

Protein-Protein Interactions




Biosensors

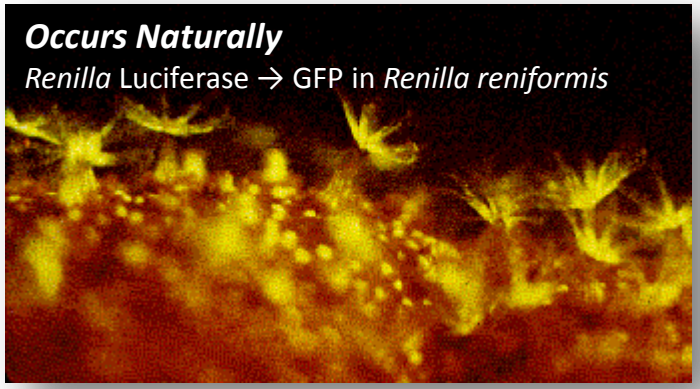
# *Monitoring Protein Stability with NanoLuc™ Luciferase*

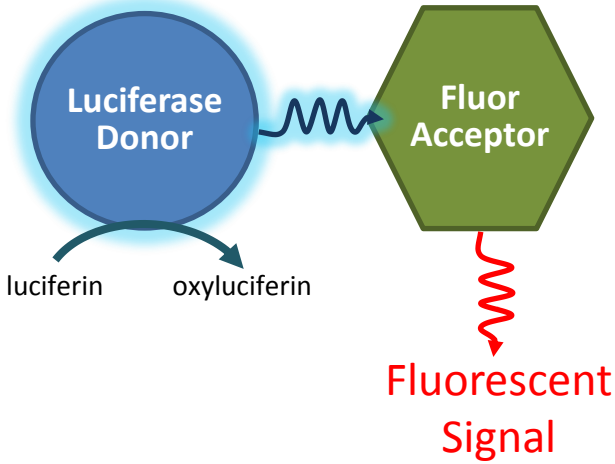



The fusion can be used as a probe of stability

# *Bioluminescence Resonance Energy Transfer (BRET)*

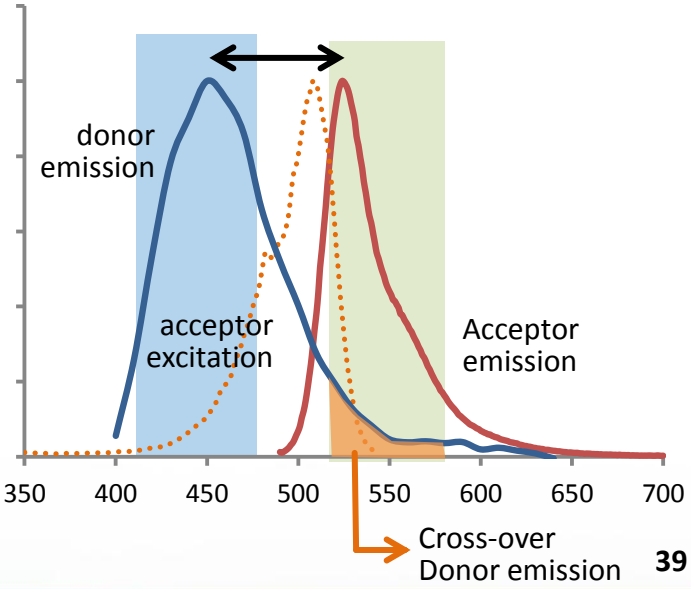


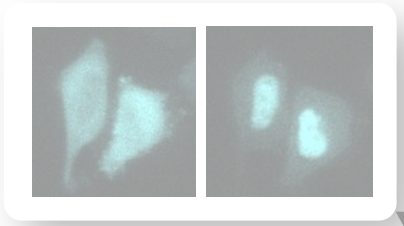

Important characteristics for BRET applications in research:

- Donor emission must overlap with acceptor excitation spectra
- Donor & Acceptor must be close (<10nm)
- Acceptor emission must be discernable from Donor emission
- Output intensity is dependent upon donor intensity

*Based on publications from S.S. Gambhir at Stanford*

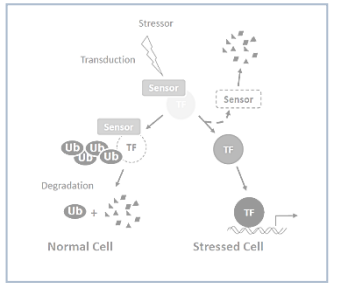


# *NanoLuc™ Luciferase as a fusion partner: Proof of concept experiments*

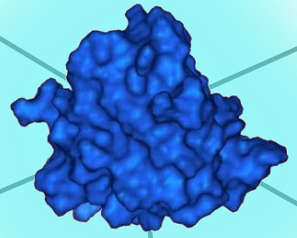


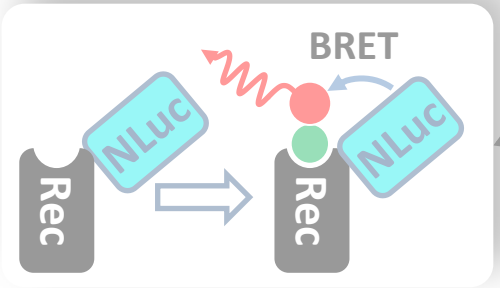
Protein Translocation

Protein Stability

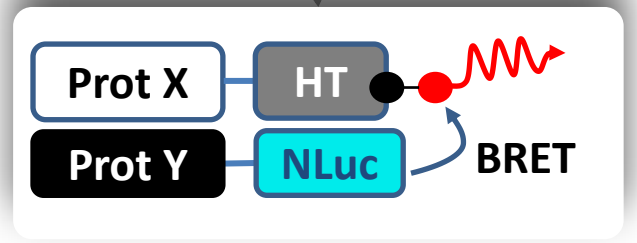




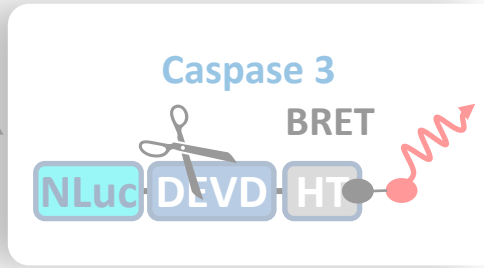





Receptor Interactions

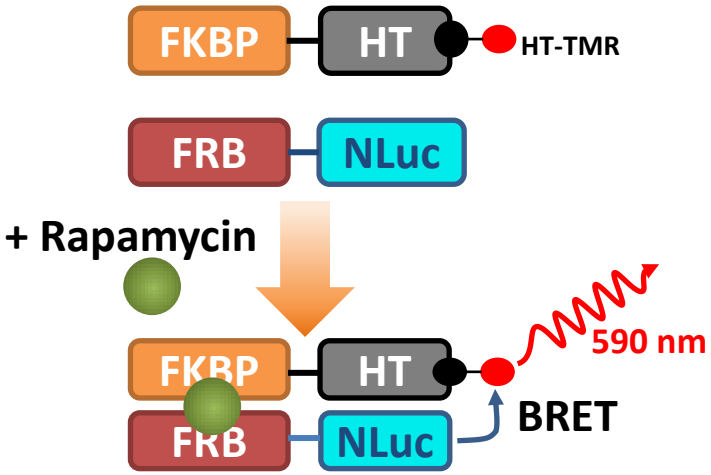


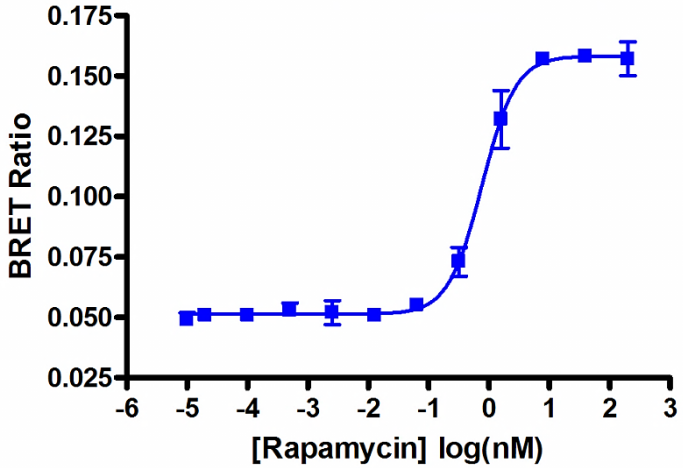

Protein-Protein Interactions




Biosensors

# *Can NLuc:HT Pair be used for Protein-Protein BRET?*

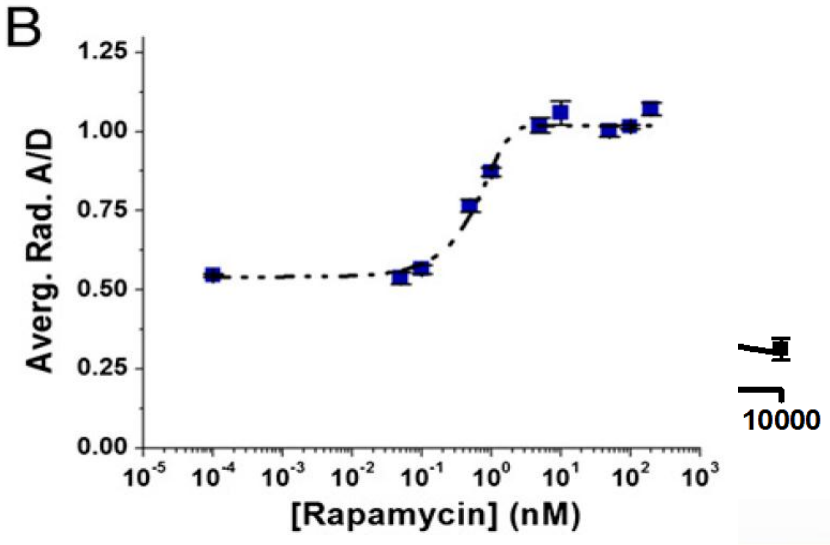



Same model system used with BRET 6 System

**RLuc8.6** → **TurboFP**

Dragulescu-Andrasi, A., et al (2011) *PNAS* **108**, 12060-5.



# *Summary*

- Bioluminescence
- NanoLuc luciferase
- Genetic reporter
  - Dual luciferase assays
  - Animal imaging
- Protein reporter
  - Location
  - Stability
  - Interactions (NanoBRET, NanoBiT)