Applications of a smaller, brighter, more versatile luciferase:

NanoLuc™ Luciferase Technology

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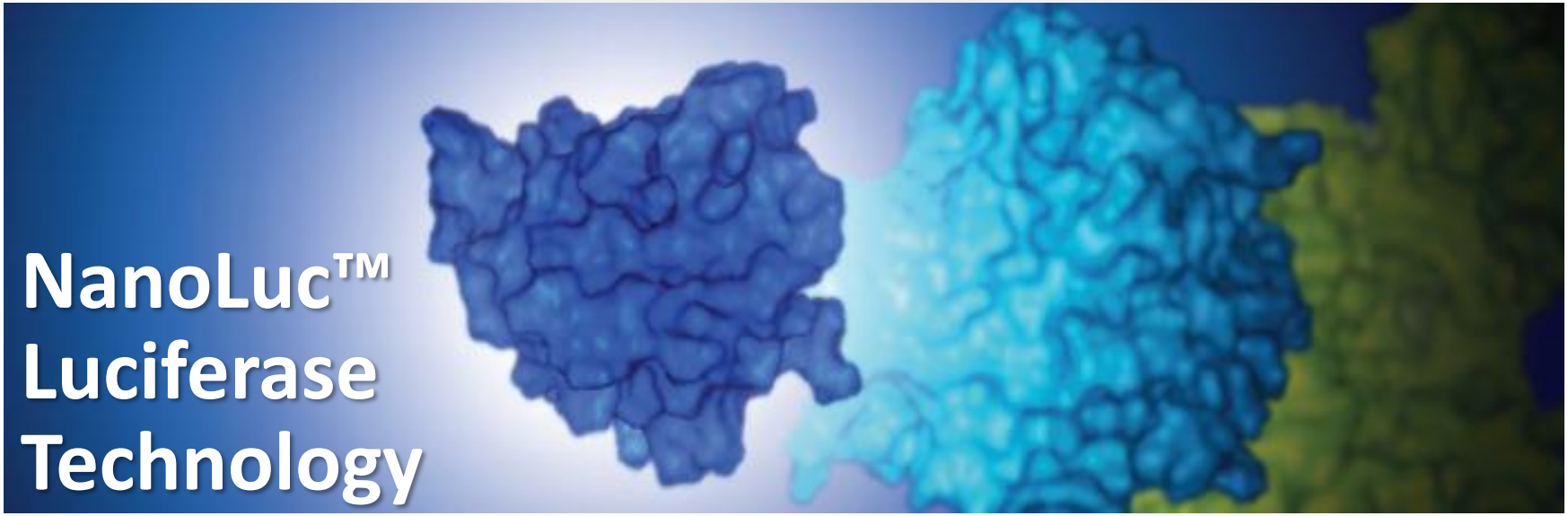
Fall 2012

Promega

Today, I will speak about…



Origins of NanoLuc Luciferase

NanoLuc Luciferase as a reporter

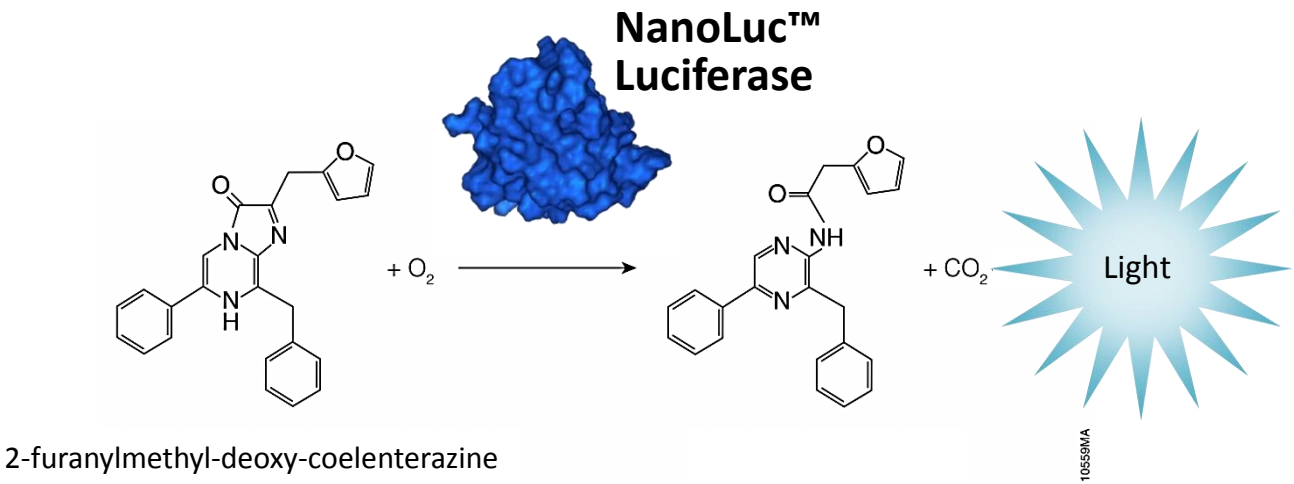
- Full-Length **Nluc**
- Destabilized, full-length **NlucP**
- Secreted, full-length **secNluc**

NanoLuc Luciferase as a fusion partner

- Protein Translocation
- Protein Stability
- Protein:Protein Interactions
- Receptor Interactions
- Biosensors

What is NanoLuc™ Luciferase?

NanoLuc™ (Nluc) is a 19.1 kDa, ATP-independent luciferase that utilizes a novel coelenterazine analog (furimazine) to produce high intensity, glow-type luminescence.

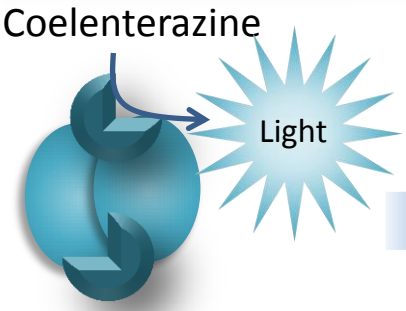


2-furanylmethyl-deoxy-coelenterazine

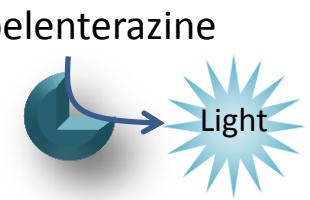
Furimazine **Furimamide**

Evolution of NanoLuc from ocean to lab bench

Oplophorus gracilirostris first cataloged in 1881



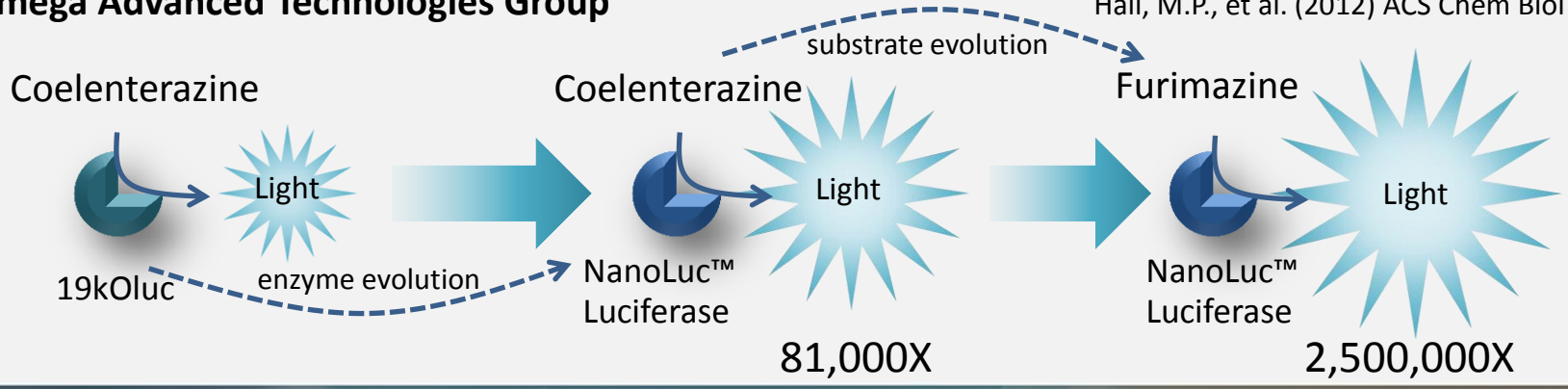
130kDa
Oplophorus luciferase
7X brighter than native
Renilla Luciferase
Shimomura, O., et al. (1978)



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

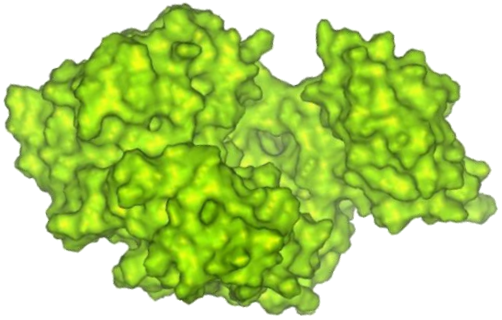
Promega Advanced Technologies Group

Hall, M.P., et al. (2012) ACS Chem Biol *in press*

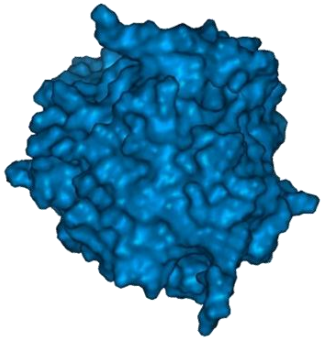


NanoLuc™ is very small

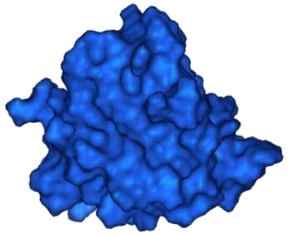
Firefly (Fluc)



Renilla (Rluc)

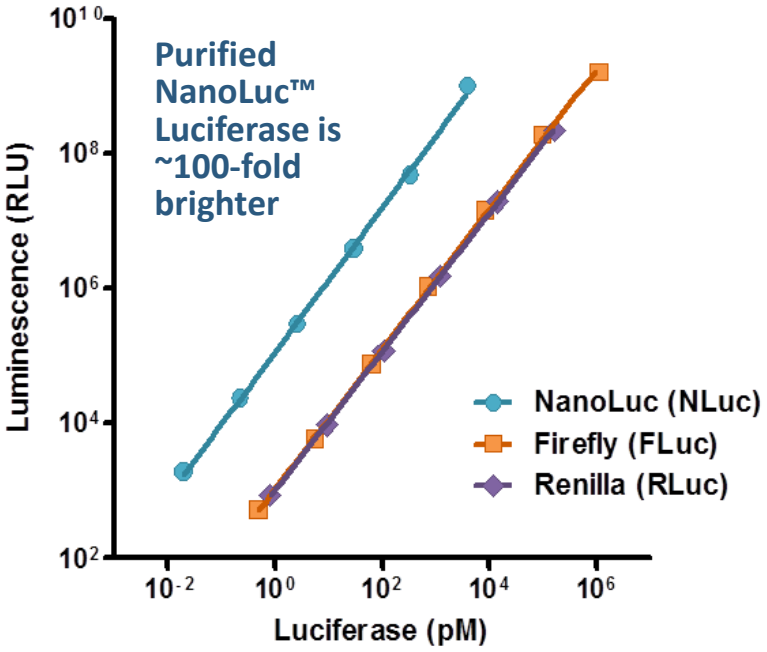


NanoLuc™ (Nluc)

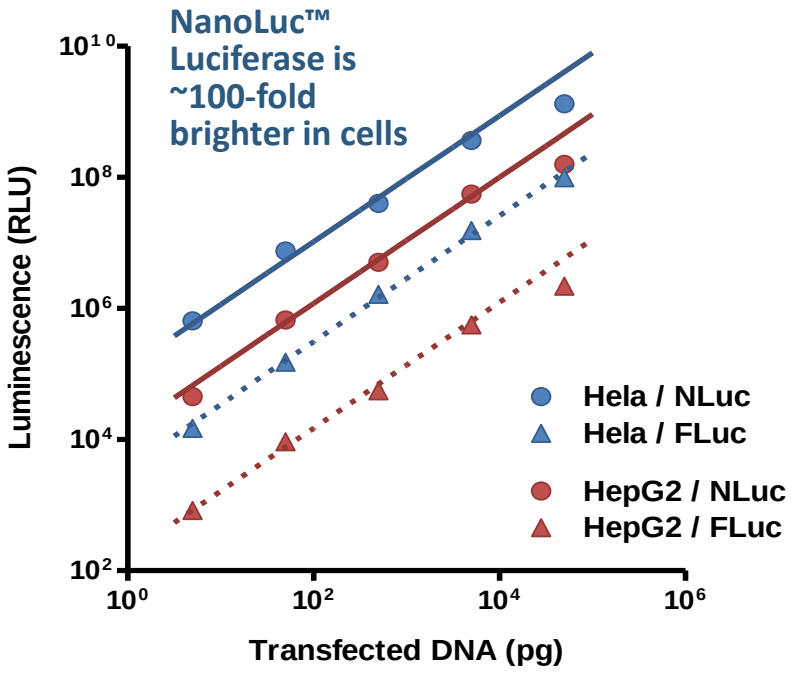


	Amino acids	M.W.	Mol. Vol. Å^3
Nluc	171	19.1	14
Rluc	312	36.0	32
Fluc	550	60.6	44

NanoLuc is bright



Recombinant NLuc/Nano-Glo™ Assay
Recombinant FLuc/ONE-Glo™ Assay
Recombinant RLuc/*Renilla*-Glo™ Assay



CMV-driven NLuc/Nano-Glo™ Assay
CMV-driven FLuc/ONE-Glo™ Assay

NanoLuc™ has excellent physical properties

Thermal stable enzyme

- Retains activity following 30 min incubation at 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

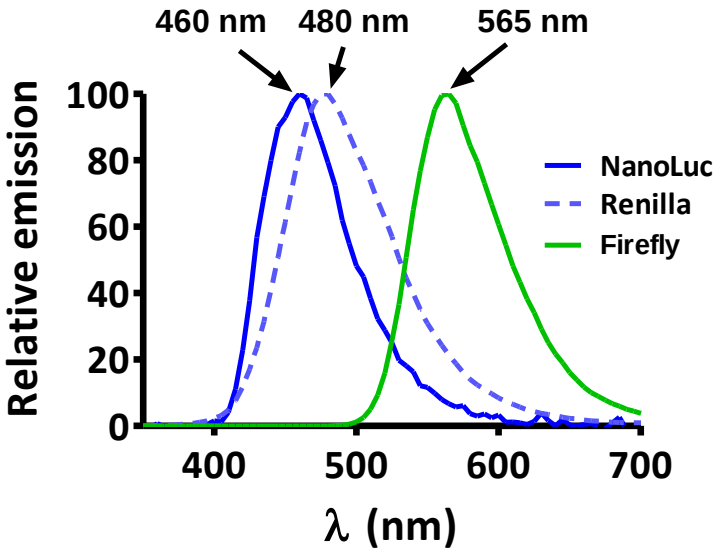
No post-translational modifications detected in mammalian cells

No disulfide bonds

- Supports high levels of activity inside living cells

Uniform distribution in cells

- No apparent compartmental bias in the absence of targeting sequences



unfused NLuc
Immunofluorescence

Nano-Glo™ Luciferase Assay Reagent

Nano-Glo™ Luciferase Assay Reagent:

Furimazine

- Provides maximal brightness

Glow kinetics (no flash reaction)

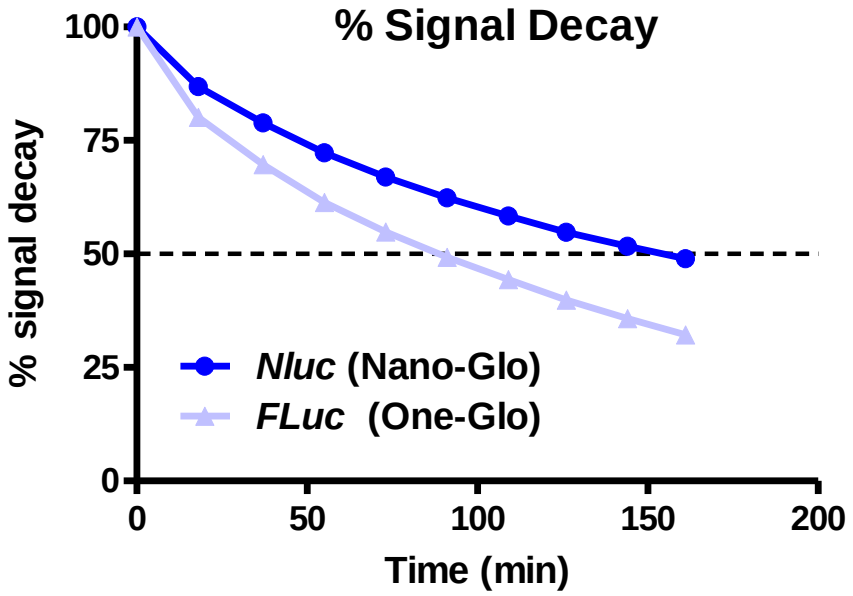
- Half-life routinely >2 hour at room temperature

Low autoluminescence background

- Enhances assay sensitivity

Stable reconstituted reagent:

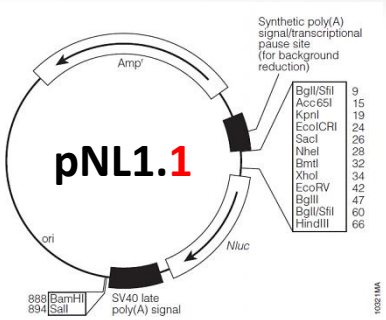
- ~10% decrease in activity over 8 hrs at RT

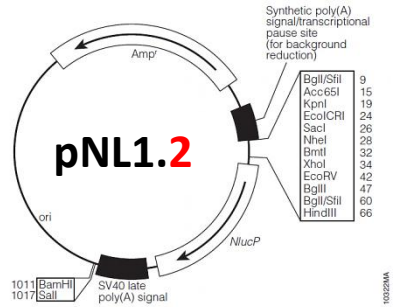


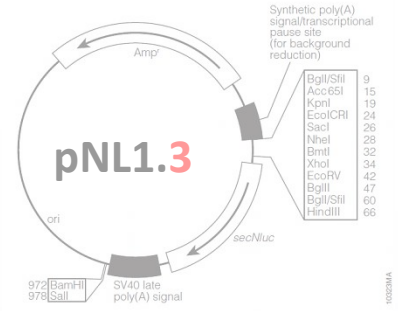
Add-Mix-Measure format like

- ONE-Glo™ Luciferase Assay System
- Bright-Glo™ Luciferase Assay System
- Steady-Glo® Luciferase Assay System
- *Renilla*-Glo™ Luciferase Assay System

3 Varieties of NanoLuc™ Luciferase for you







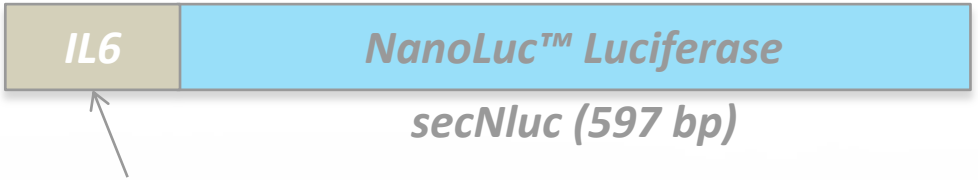
Intracellular Formats

Nluc (513 bp)

Protein destabilization domain

NlucP (636 bp)

Secretion Format

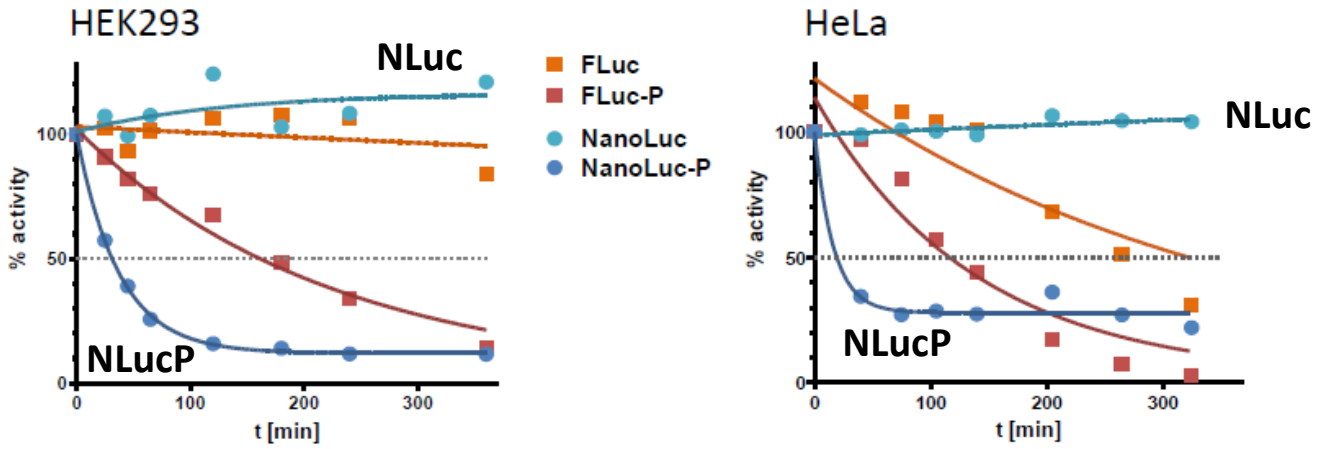


secNluc (597 bp)

Secretion signal

Intracellular stability of NanoLuc™ & Firefly

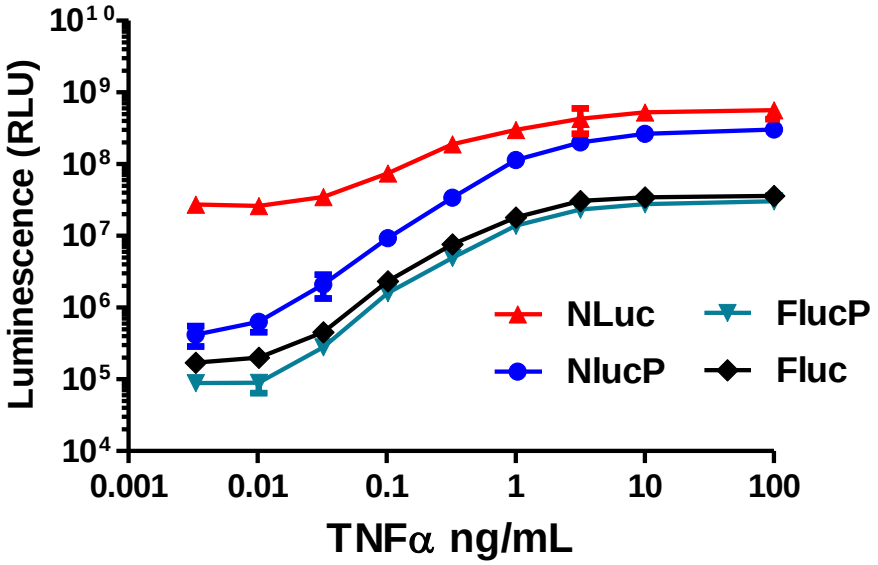
New protein synthesis blocked by addition of cycloheximide



Cell line	FLuc	FLucP	NLuc	NLucP
HEK-293	>6 h	2.0 ± 0.4 h	**>6 h**	**18 ± 11 min**
HeLa	3.8 ± 1.3 h	1.4 ± 0.2 h	**>6 h**	**20 ± 6 min**
U2OS (n=1)	>6 h	2.8 h	**>6 h**	**36 min**

Relative protein stability in cells: NlucP < FlucP < Fluc < Nluc

NlucP gives the greatest dynamic response



Experimental details: transient transfection of HEK293 cells with NF-κB inducible constructs. rhTNFα treatment for 5 hours.

Brightness

Nluc > NlucP > Fluc > FlucP

(18 experiments)

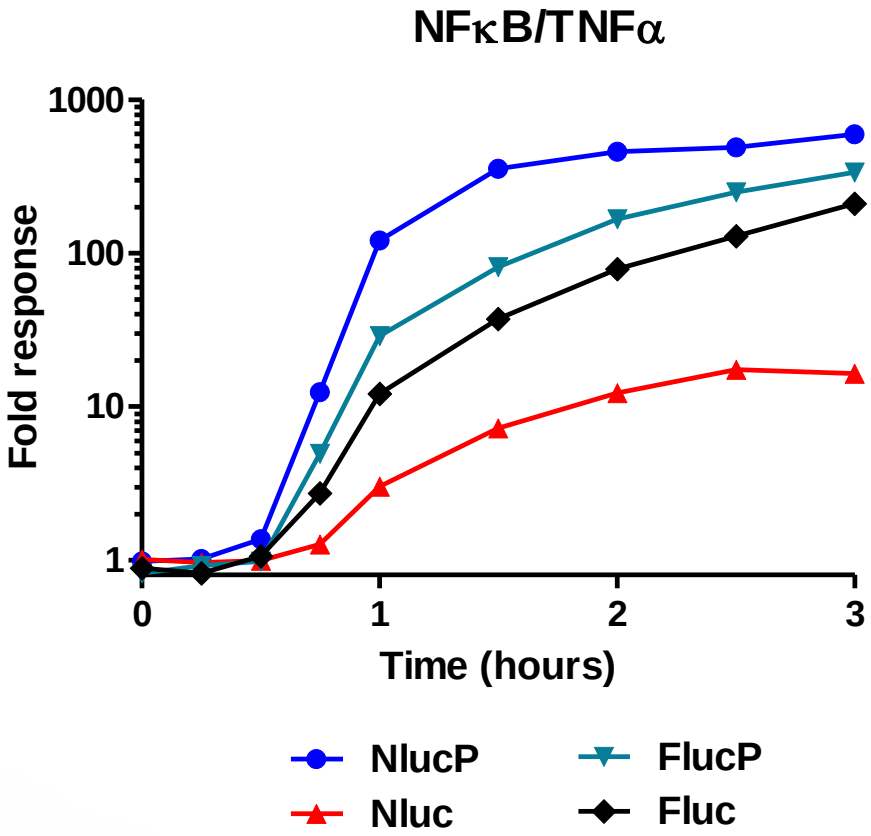
NLuc 13-236 fold brighter than Fluc (79 fold avg.)

NlucP 2-27 fold brighter than FLucP (10 fold avg.)

Nluc 10-78 fold brighter than NlucP (34 fold avg.)

→Very similar pharmacology/EC50s

NlucP responds earliest to stimuli

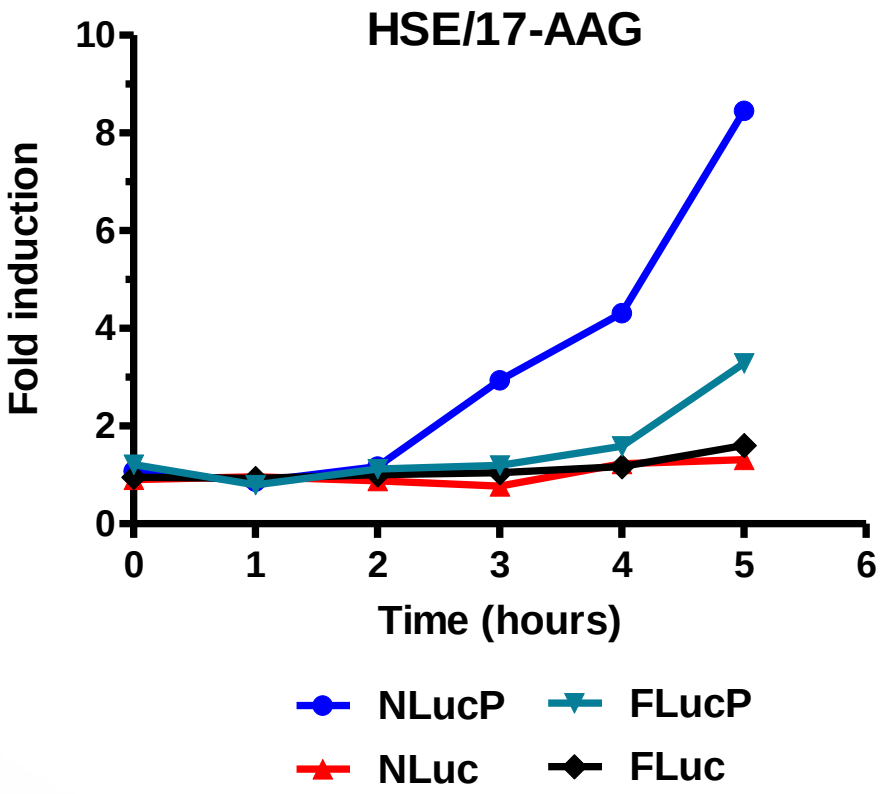


Relative Response

NlucP > FlucP > Fluc > Nluc

Experimental details: transient transfection of HEK293 cells with NFκB inducible constructs; addition of 100 ng/ml rhTNFα at time zero.

NLucP allows study of weakly induced responses



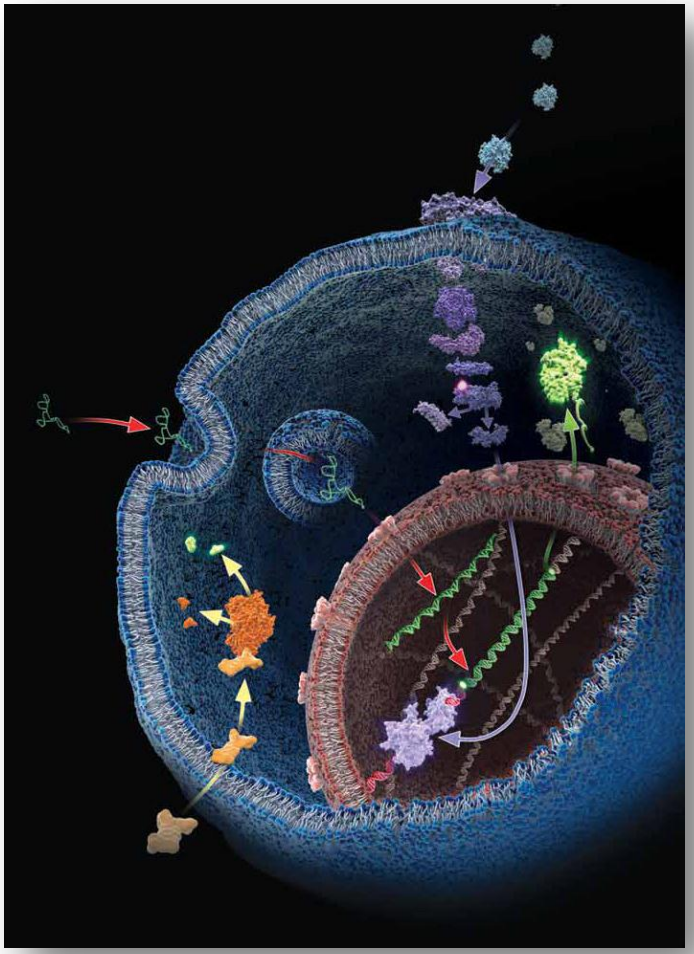
Relative Response

NlucP > FlucP > Fluc,Nluc

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

NanoLuc Luciferase as an intracellular reporter

- ✓ NlucP for a faster response
- ✓ NlucP for greatest dynamic range
- ✓ NlucP for measuring weak responses
- ✓ Nluc where maximum brightness is needed.



Should I switch from Firefly to NanoLuc™ Luciferase?

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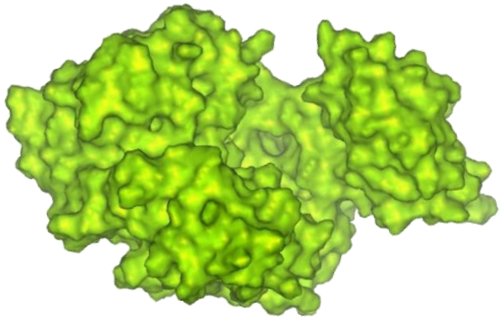
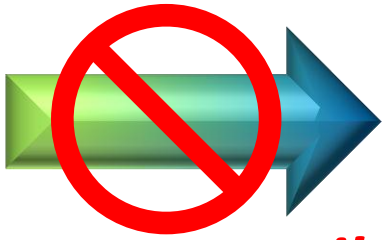
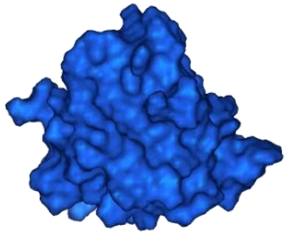
Firefly (Fluc)





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

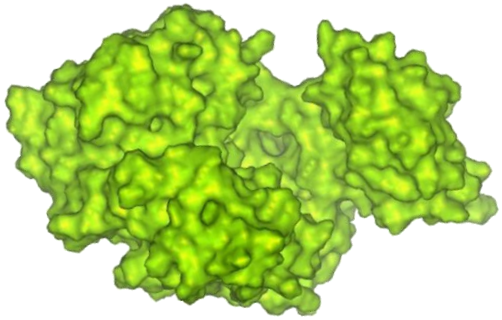
We just released new response element signaling pathway detection pGL4 vectors:

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

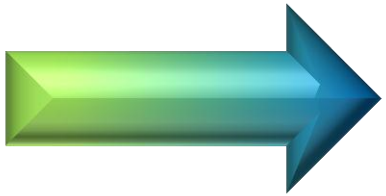
Should I switch from Firefly to NanoLuc™ Luciferase?

Promega

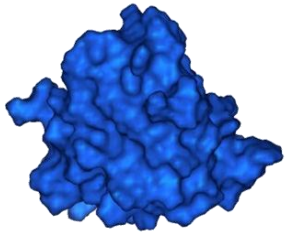
Firefly (Fluc)



Yes, if …



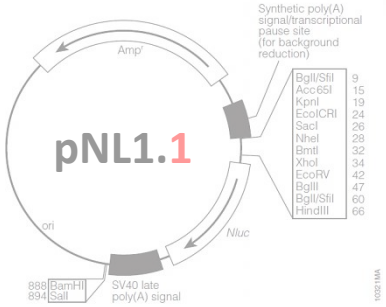
NanoLuc™ (Nluc)

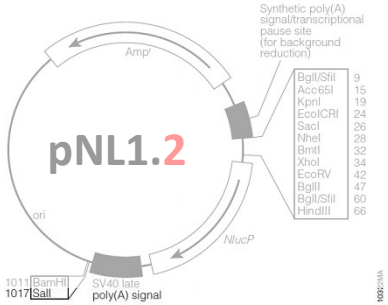


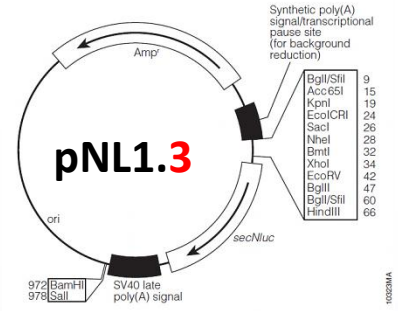
- ✓ **Transfection efficiency limits you to easy-to-transfect cell lines**
- ✓ **Signals are too weak to move to 96-well plates**
- ✓ **FLuc is just too big**

- 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

3 Varieties of NanoLuc™ Luciferase for you







Intracellular Formats

NanoLuc™ Luciferase

Nluc (513 bp)

Protein destabilization domain

NanoLuc™ Luciferase | PEST

NlucP (636 bp)

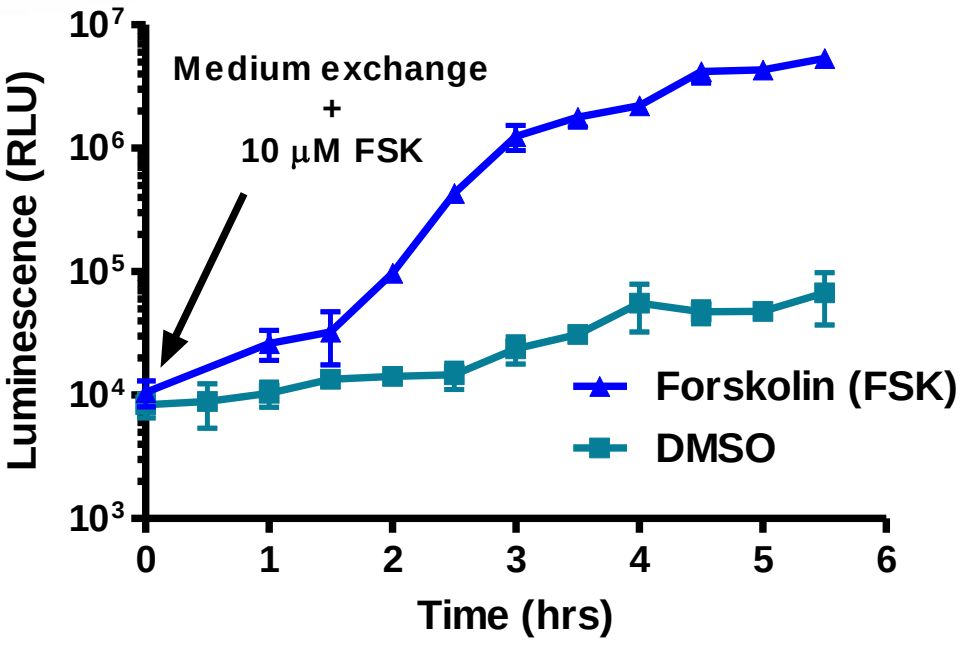
Secretion Format

IL6 | NanoLuc™ Luciferase

secNluc (597 bp)

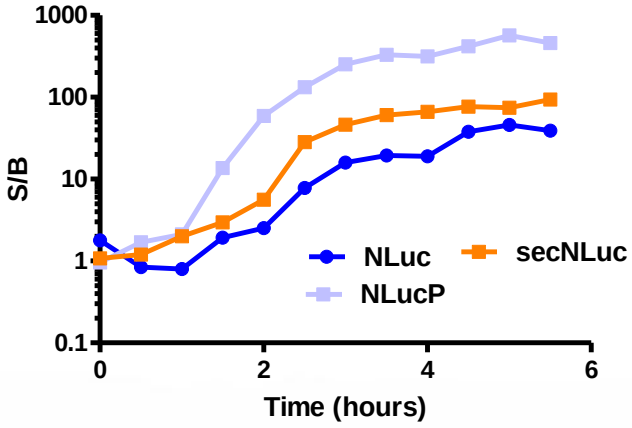
Secretion signal

Secretion based format using secNluc

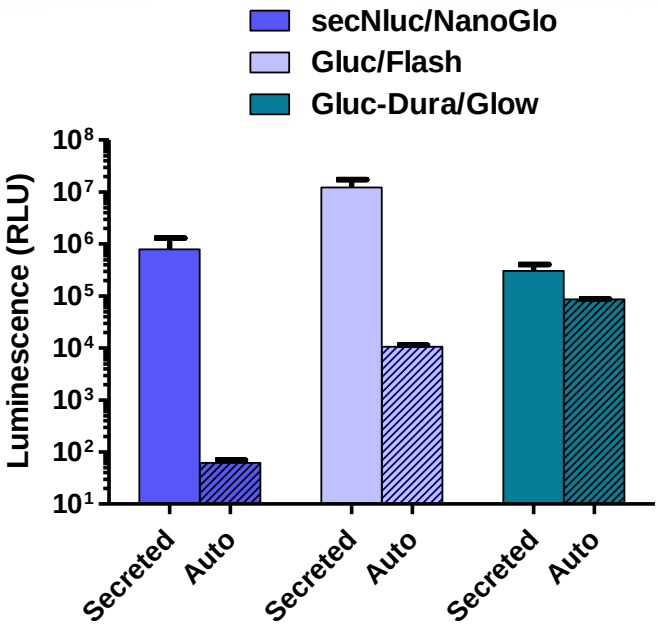


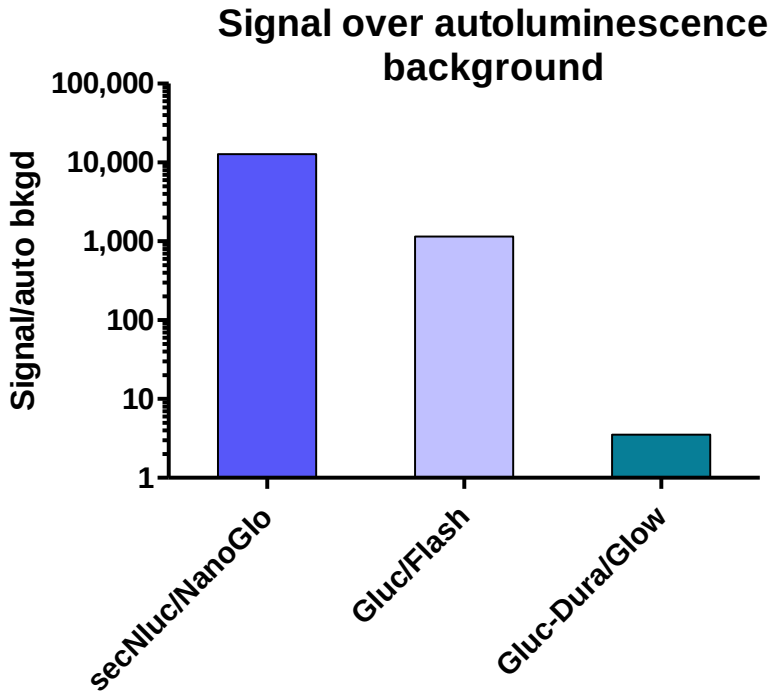
Experimental details: transient transfection of HEK293 cells with CREB inducible construct; addition of 10 µM forskolin at time zero.

- Sample medium at multiple time points without cell lysis
  - Kinetic studies from the same set of wells
- Half-life of secNluc protein > 4 days at 37°C in medium
- Response dynamics similar to unfused Nluc
- Similar pharmacology vs. Nluc/NlucP



Gluc kits: bright, but high autoluminescence background





Gluc kits: high background limits sensitivity & dynamic range

Experimental details: HepG2 cells (DMEM +10% FBS) transiently transfected w/CMV promoter constructs; removal of aliquots after 22 hrs; n = 12 per treatment.

Handling the bright signal

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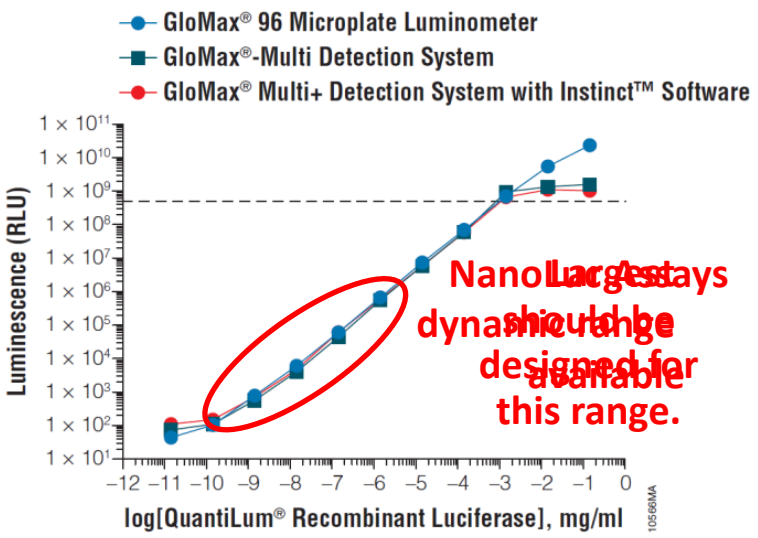
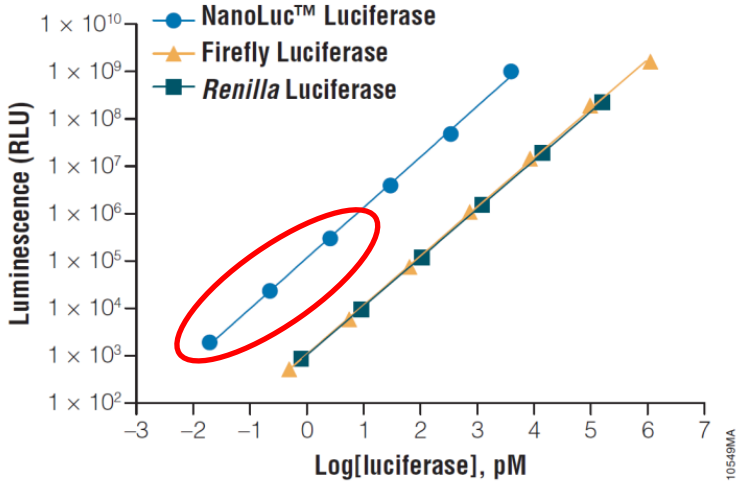
R&D used GloMax® Instruments for NanoLuc™ Development





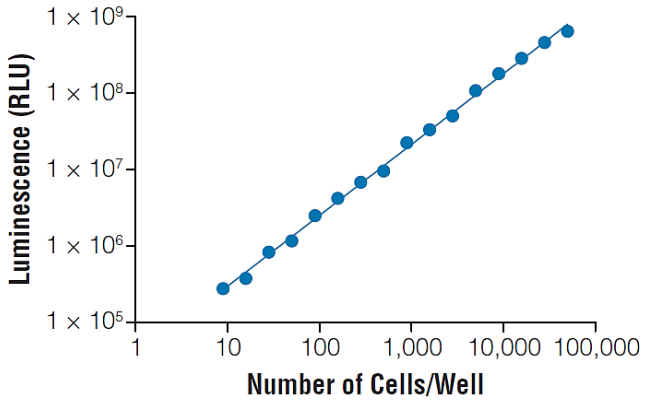
GloMax®-Multi+
Microplate Multimode Reader

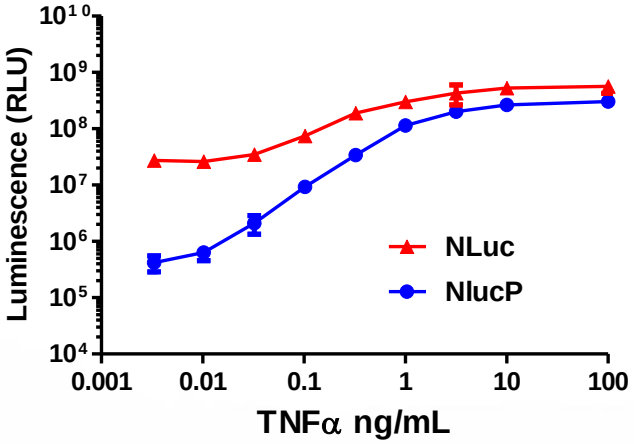
GloMax®-Multi
Microplate Multimode Reader

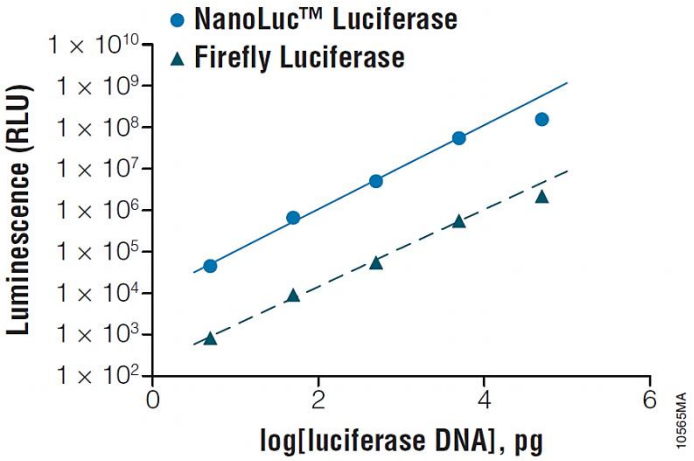
GloMax® 96
Microplate Luminometer

Getting NanoLuc™ Luciferase Signals in range

- ✓ Transfect fewer cells
- ✓ Transfect less DNA
- ✓ Switch to NlucP
- ✓ Use a weaker constitutive promoter







More details on NanoLuc™ Development

Webinar recording by lead R&D scientist for development of the NanoLuc™ Vectors and Nano-Glo™ Assay System

Brock F. Binkowski, Ph.D.
Sr. Research Scientist II

Broadcast from June 2012
~30 minutes long

www.promega.com/webinars

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NanoLuc™ Luciferase as a protein function probe

Applications of full-length NanoLuc™ Luciferase.

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

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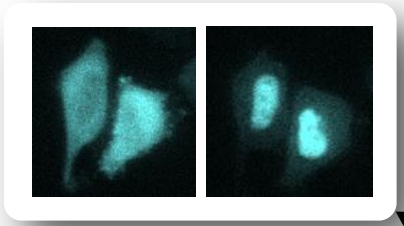
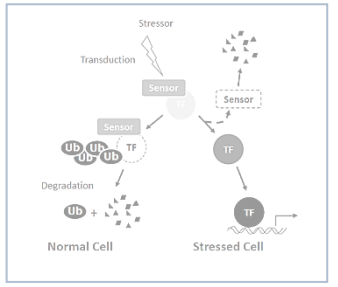
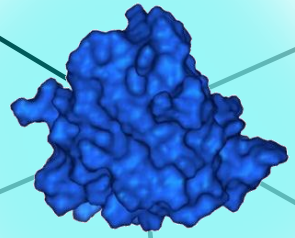
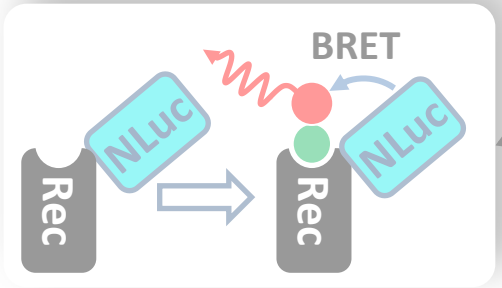


Protein Translocation

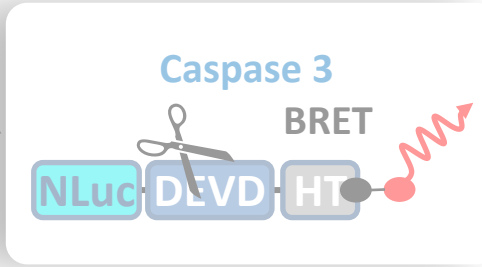
Protein Stability



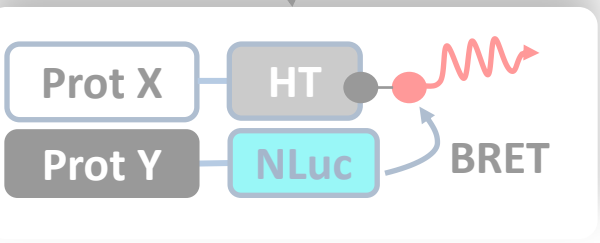




Receptor Interactions



Biosensors



Protein-Protein Interactions

NanoLuc™ Luciferase excels in bioluminescent imaging applications

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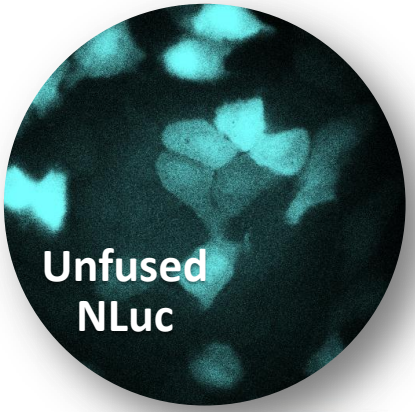
Nluc brightness leads to short exposure times:

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



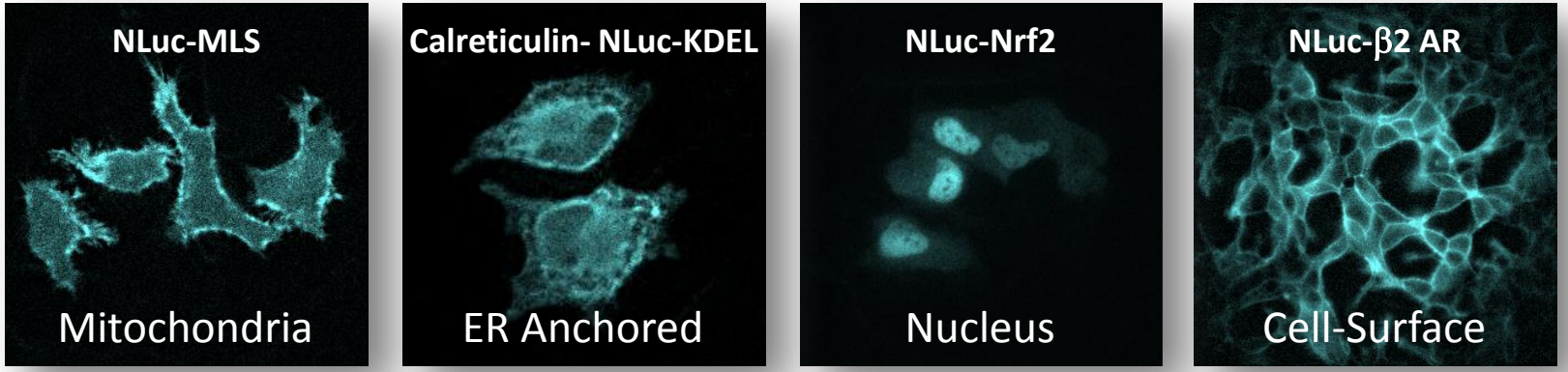
NanoLuc & LV200 featured @ASCB 2012 Olympus Product Showcase

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

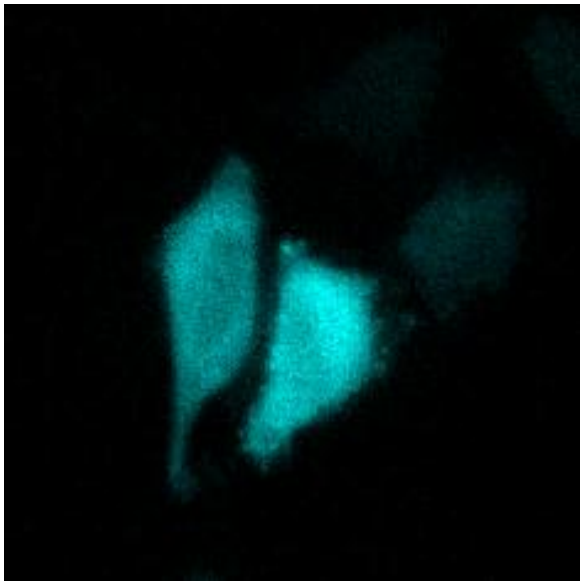
NanoLuc™ Fusions can go anywhere…



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

Bioluminescence imaging of protein translocation

cytoplasm → nucleus



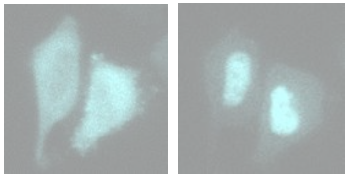
Time lapse: 13 minutes

NanoLuc fusion to
Glucocorticoid Receptor

HeLa cells; 500nM dexamethasone treatment

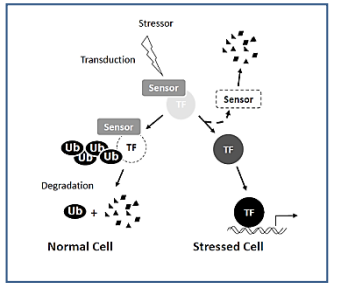
NanoLuc™ Luciferase as a fusion partner: Proof of concept experiments

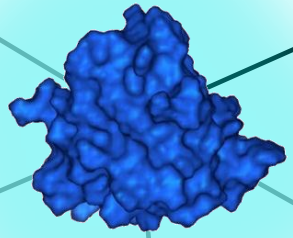
Promega

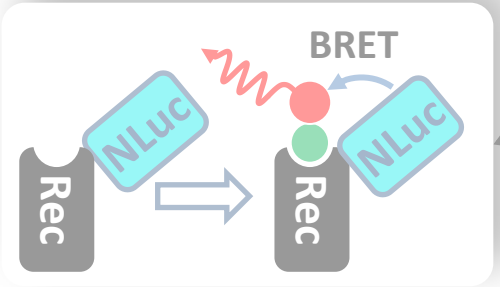


Protein Translocation

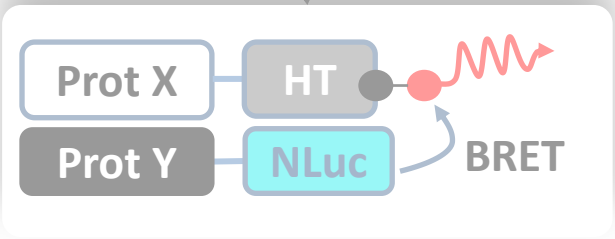
Protein Stability



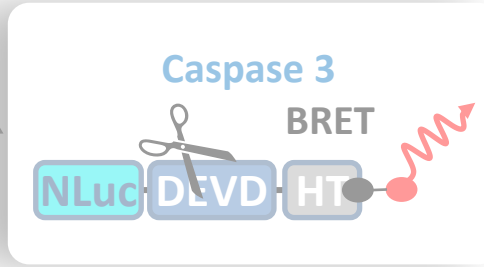




Receptor Interactions



Protein-Protein Interactions

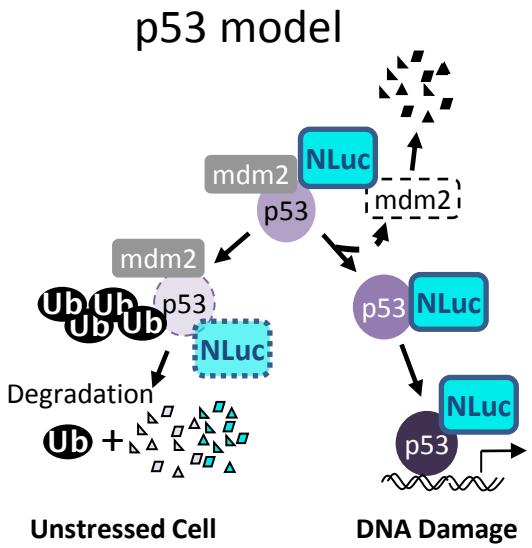


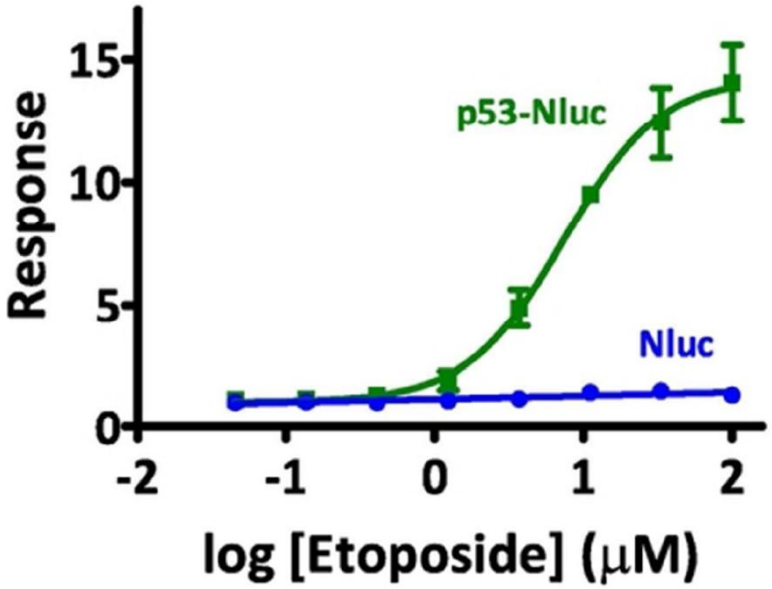
Biosensors

Monitoring Protein Stability with NanoLuc™ Luciferase

Promega

Can NanoLuc™ Luciferase be added to a protein as a probe for protein stability?

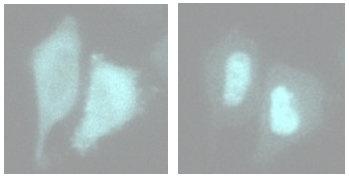




The fusion can be used as a probe of stability

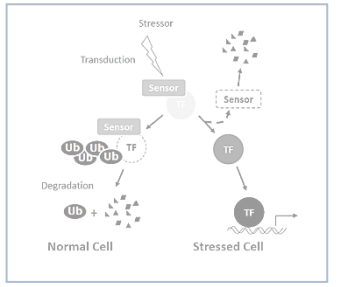
NanoLuc™ Luciferase as a fusion partner: Proof of concept experiments

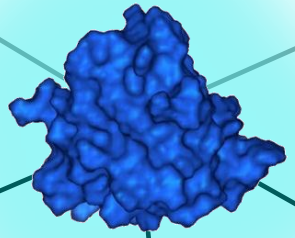
Promega

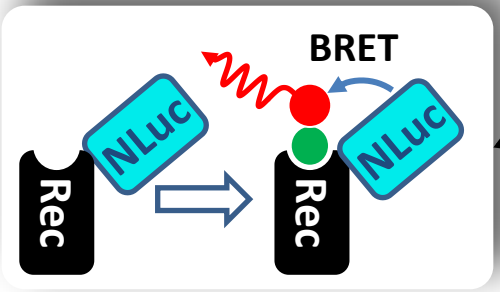


Protein Translocation

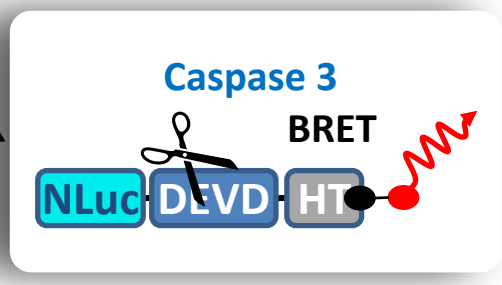
Protein Stability



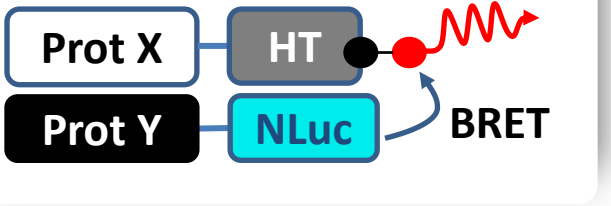




Receptor Interactions

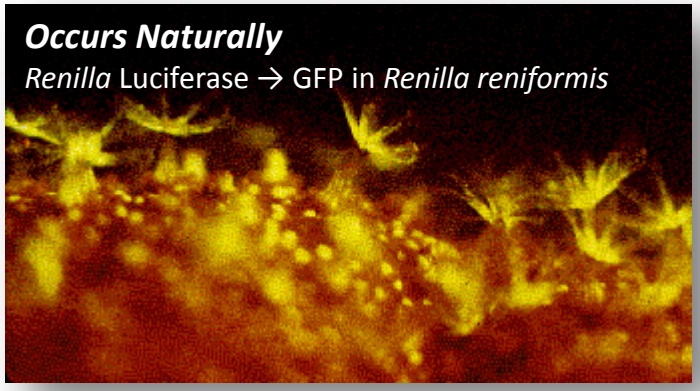


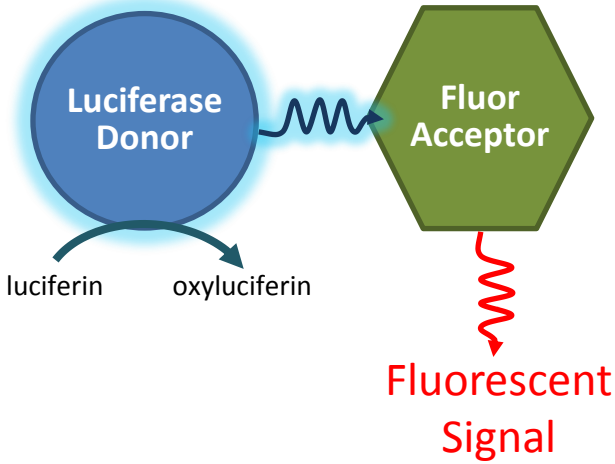
Biosensors



Protein-Protein Interactions

Bioluminescence Resonance Energy Transfer (BRET)

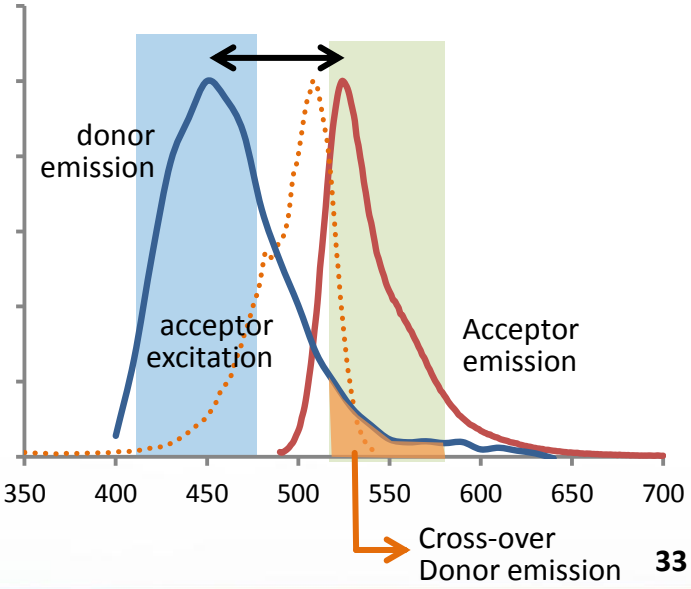




Important characteristics for BRET applications in research:

- 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

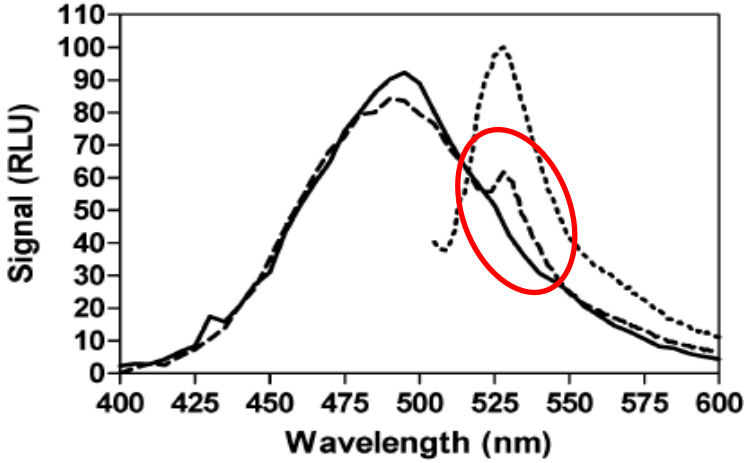


Could NanoLuc™ Luciferase work better as the luminescent donor?

Promega

RLuc → GFP

Donor brightness is a key limiter to current BRET technologies.



More spectral overlap needed to get sufficient signal

BRET-beneficial aspects of NanoLuc Luciferase:

~100-fold brighter than Rluc

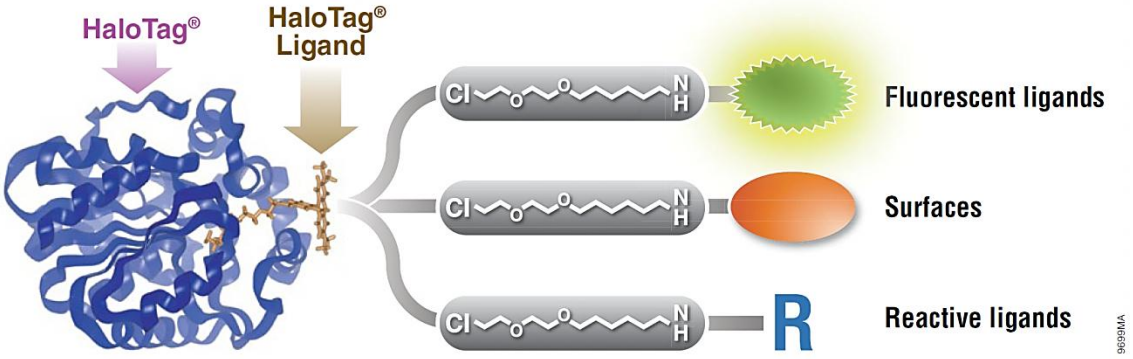
- ✓ need less spectral overlap with fluor
- ✓ gain greater spectral separation

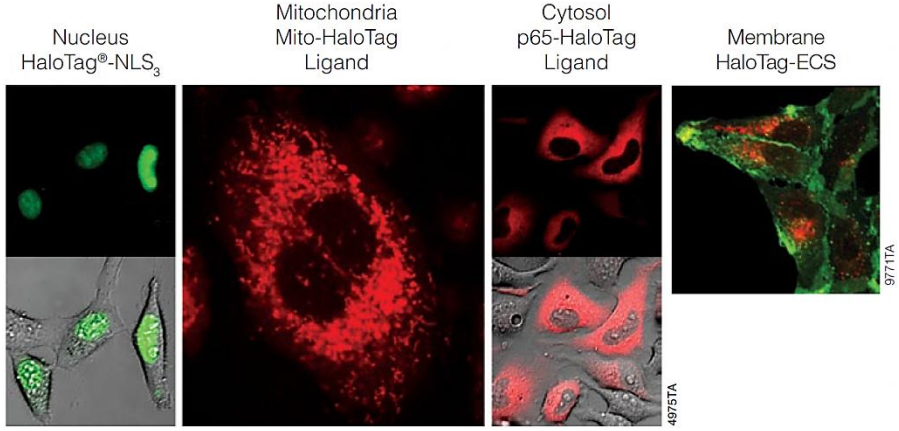
We have a potential acceptor fusion protein:

HaloTag® Fusion Protein

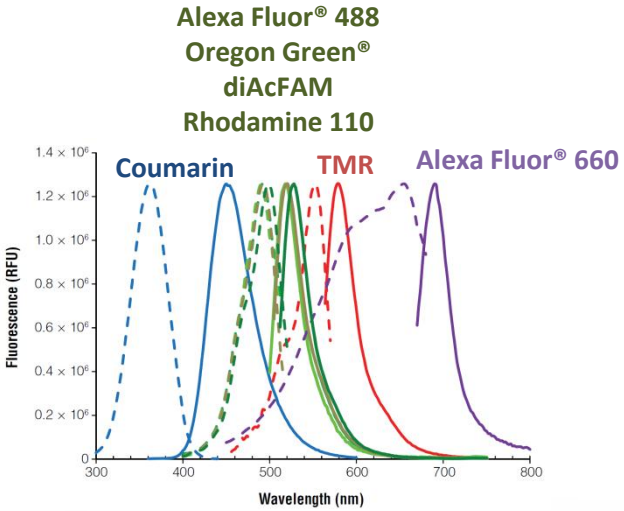
34.1kDa protein engineered from halophilic bacterial hydrolase.

- Engineered to lock into enzyme: substrate intermediate for **covalent attachment** .
- No homolog in mammalian cells.



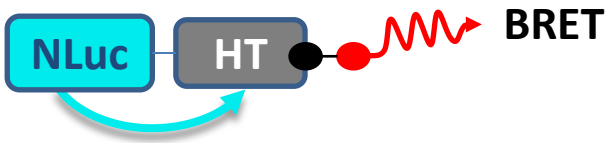


Goes anywhere in the cell

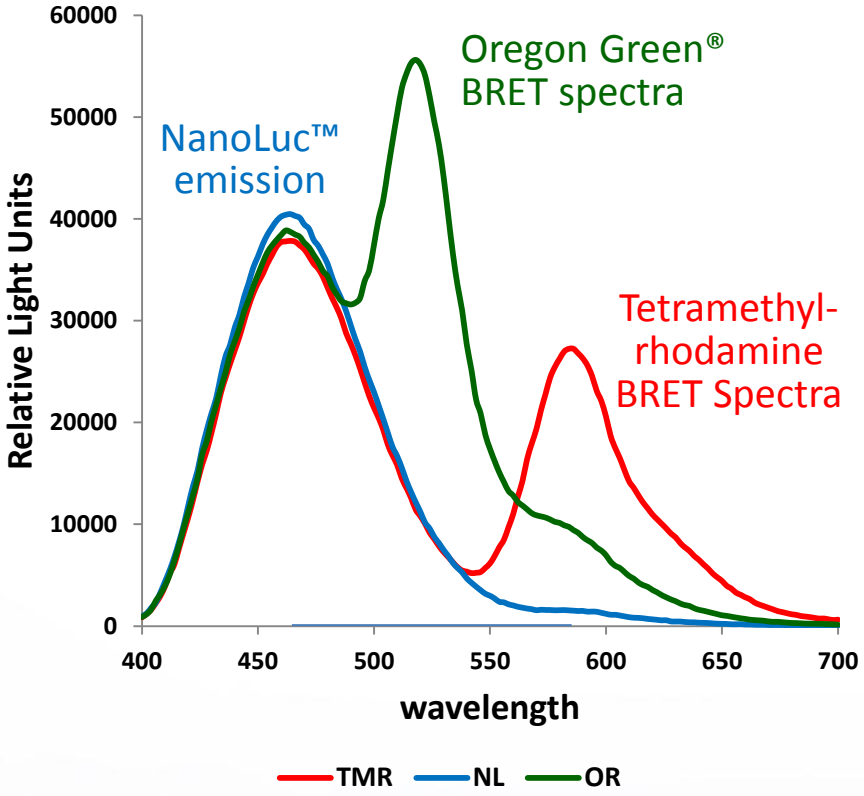


Variety of fluors ready-to-use

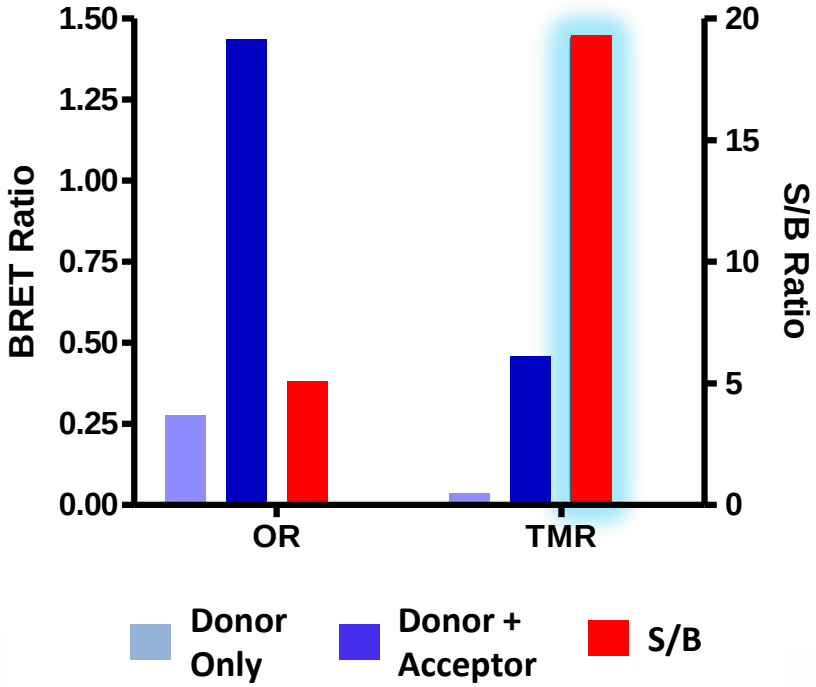
Testing the concept: NanoLuc™ & HaloTag® Fusion can perform BRET



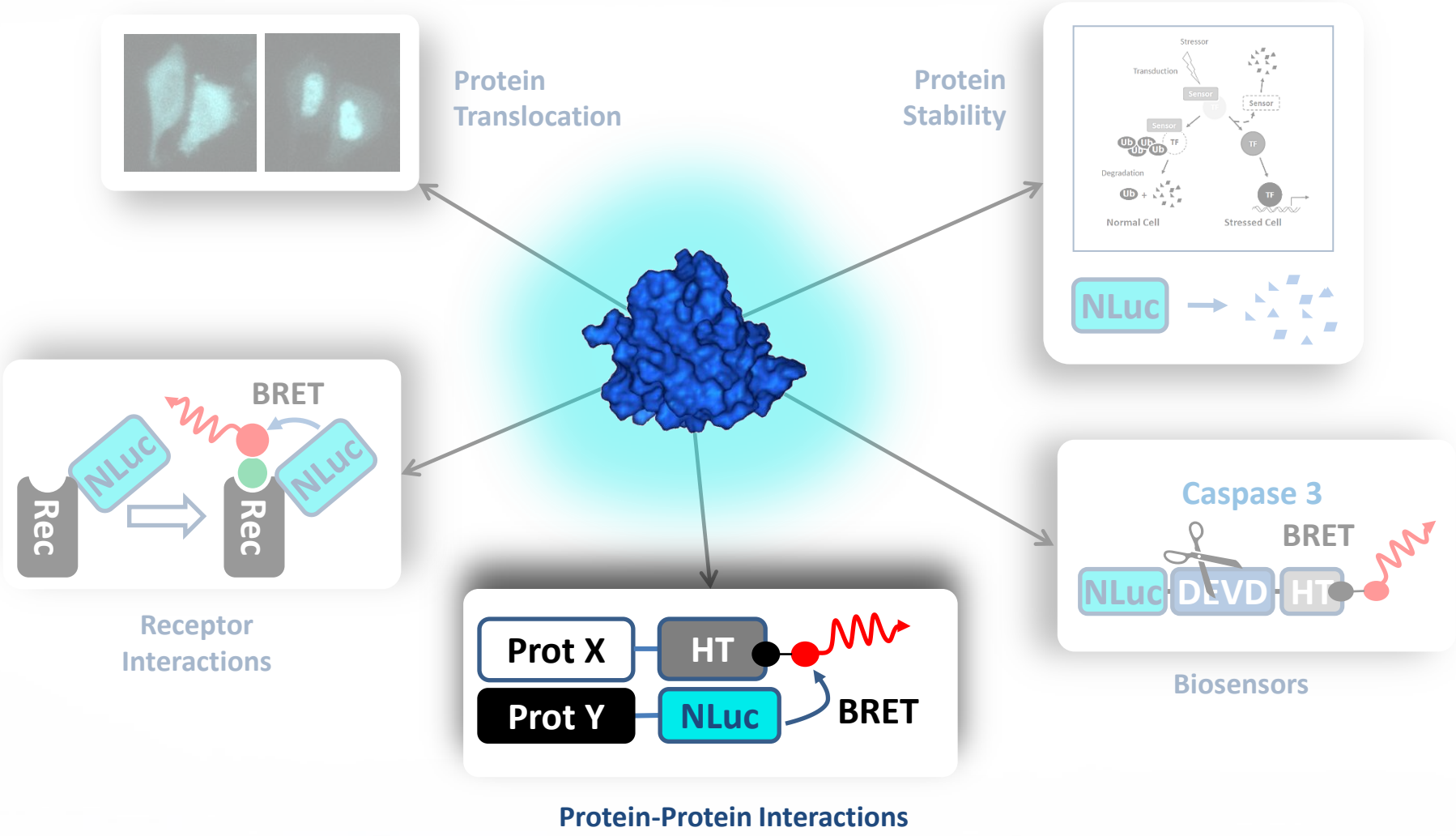
BRET Emission spectrum



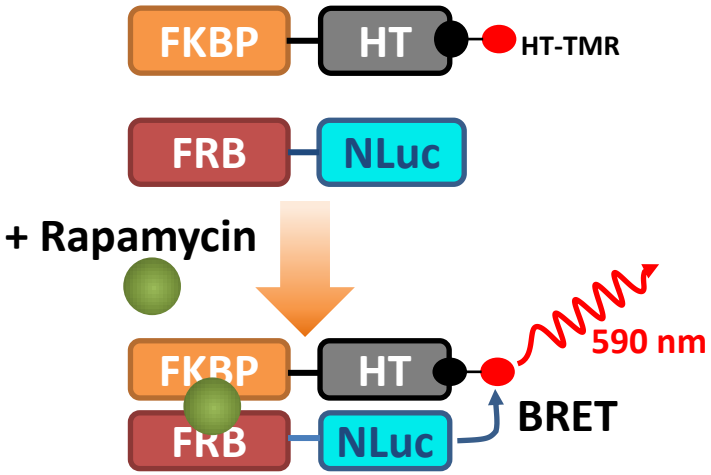
BRET Ratio (= Em_{acc}/Em_{don})

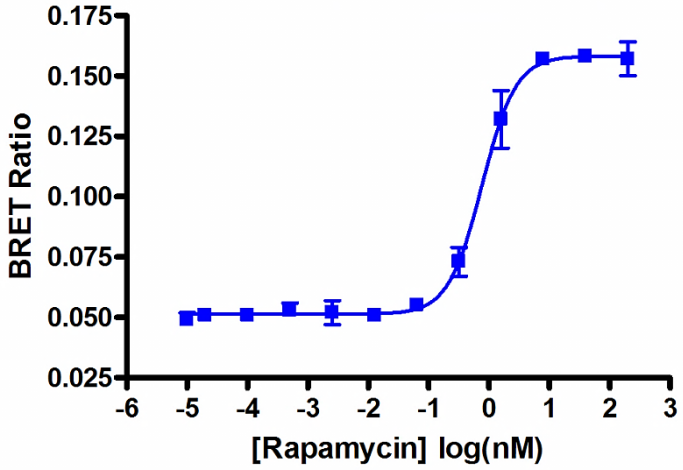


NanoLuc™ Luciferase as a fusion partner: Proof of concept experiments



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

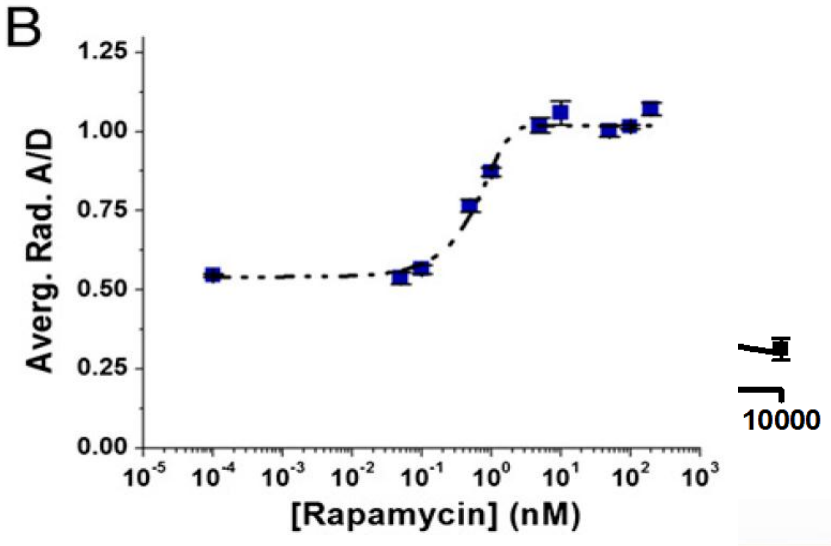




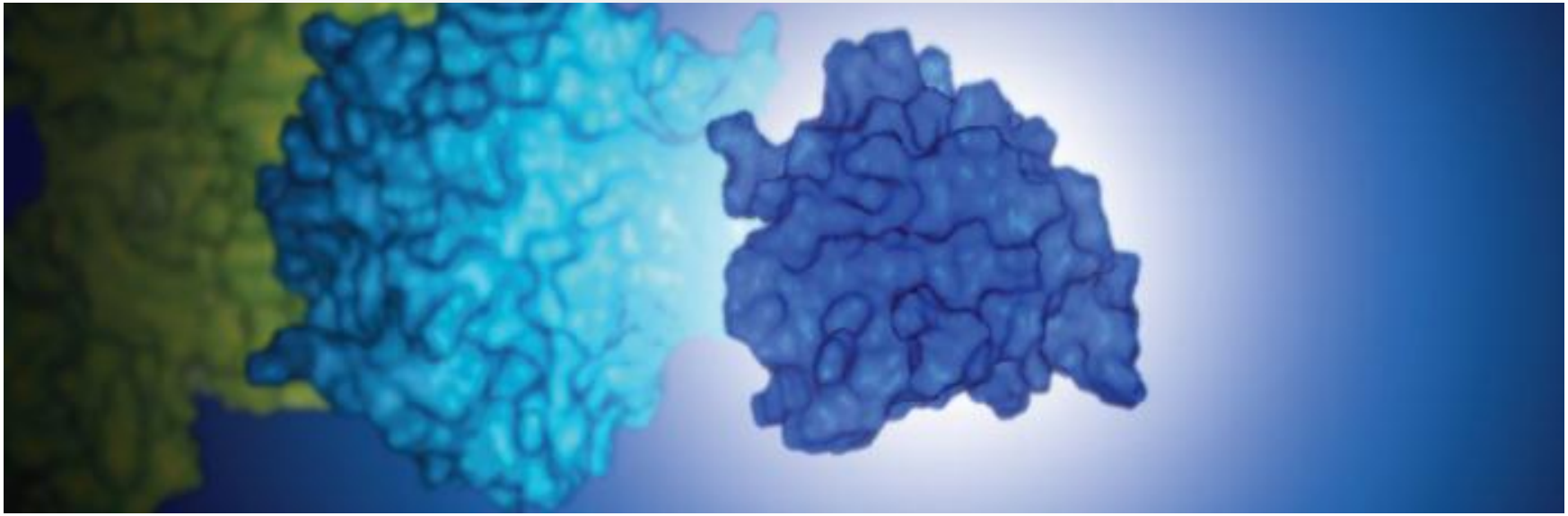
Same model system used with BRET 6 System

RLuc8.6 → **TurboFP**

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



Bright Future for NanoLuc™ Luciferase fusions



Brightness improves bioluminescent imaging

Versatility to go anywhere in cell

Versatility to allow stability measurements

Brightness allows BRET with HaloTag® Fusions

- Biosensors
- Protein:Protein Interactions

Brightness allows BRET with fluorescent ligands

- Ligand binding assays

Get a FREE Sample of the NanoLuc™ Vectors.

Ask for the < Source Code >

pNL 1.1 (*NLuc*)

pNL 1.2 (*NLucP*)

pNL 1.3 (*secNLuc*)

www.promega.com/nanoluc

Plus more information about all the pNL vectors

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Questions?

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