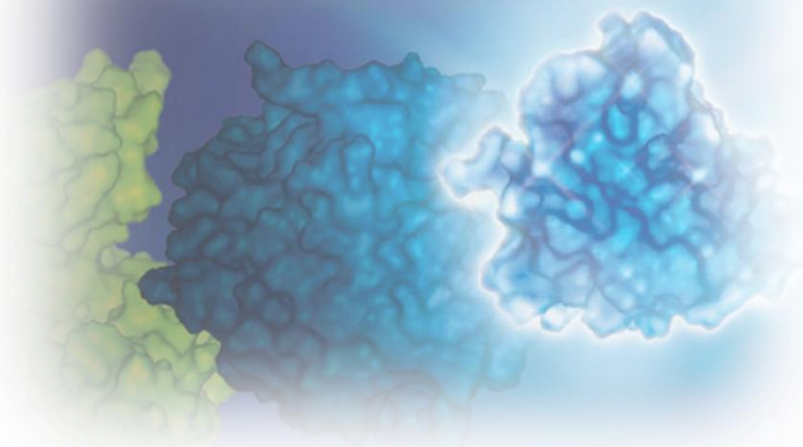


Light Up Your Research – The Power of Bioluminescence


Mgr. Lukáš Išler




About East Port

About us

- Over **25 years** supporting life science research in the Czech Republic
- More than **15 partnerships** with leading biotechnology suppliers
- Team with scientific background

 Headquarters

 Customers



Company Overview

We Provide

Innovative biological reagents and instrumentation



Scientific application support



Technical service

Covered Product Areas

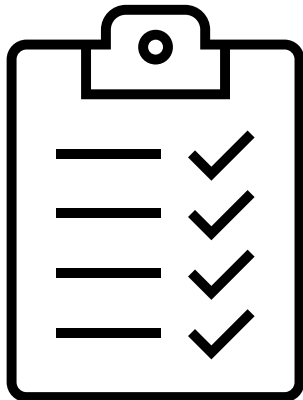
Molecular and cell biology

Instruments

Consumables

In vitro fertilization

Program



About Luminescence – Where It Begins

Luciferase and Luciferase-Based Reporters

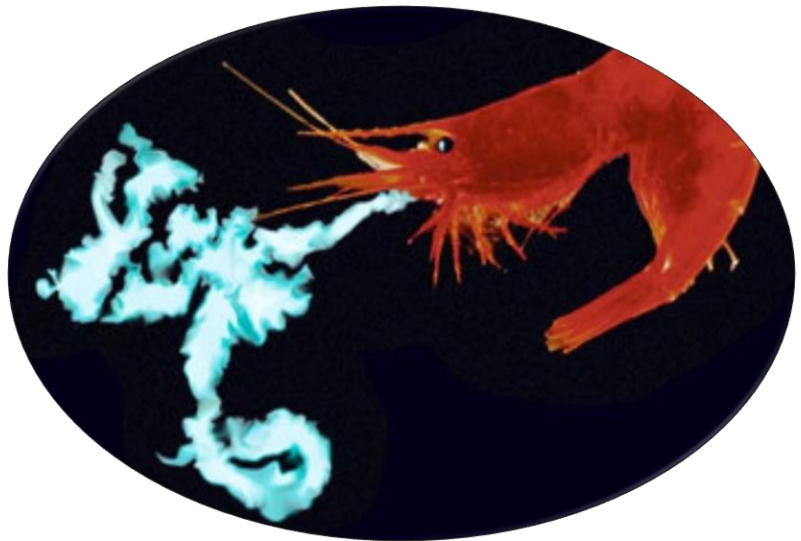
NanoLuc and Its Technologies

Reporter delivery and detection

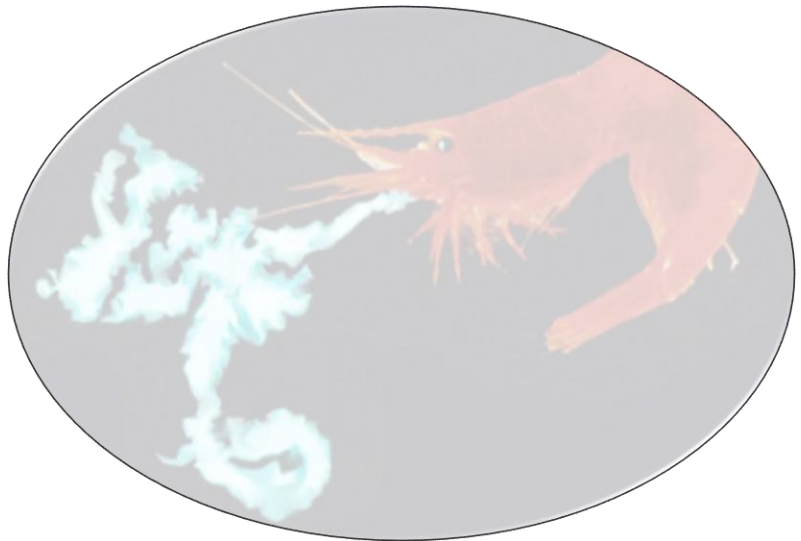
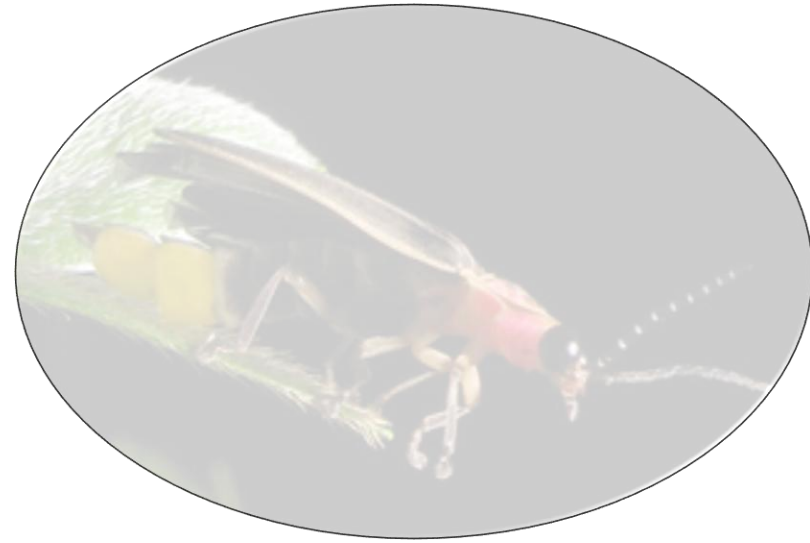
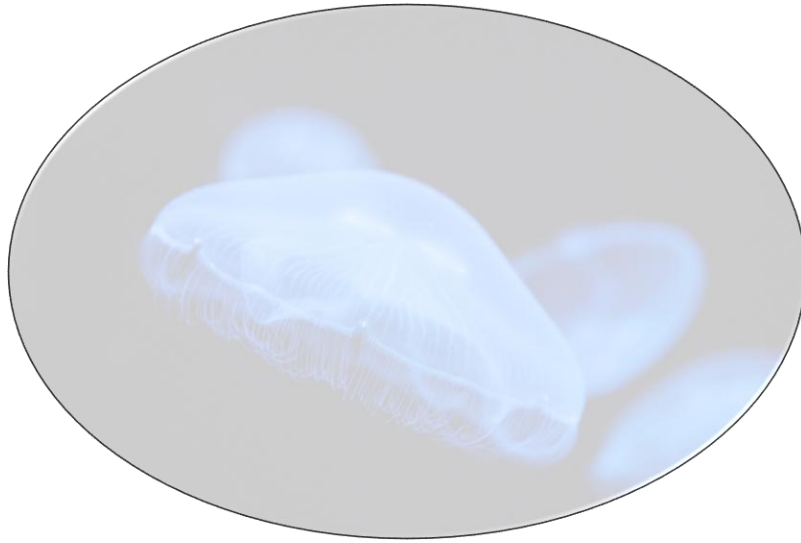
Luminescence in cell cultures

Final thoughts and discussion

Bioluminescence in Nature



Bioluminescence in Nature



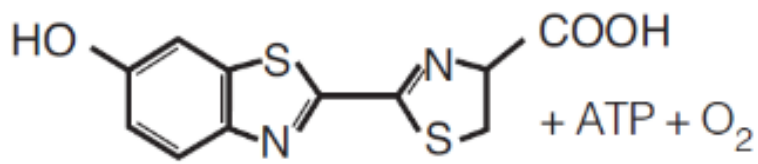
Bioluminescence – From Nature to Science

19th century - Raphaël Dubois

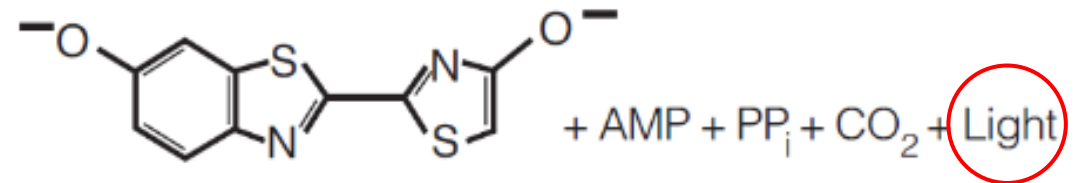
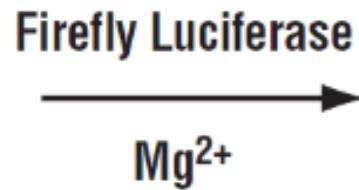
- Light production involves two components:
 - Organic molecule → **luciferin**
 - Enzyme → **luciferase**



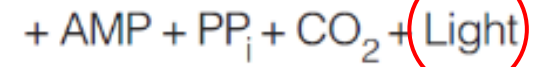
Bioluminescence as a product of chemical reaction



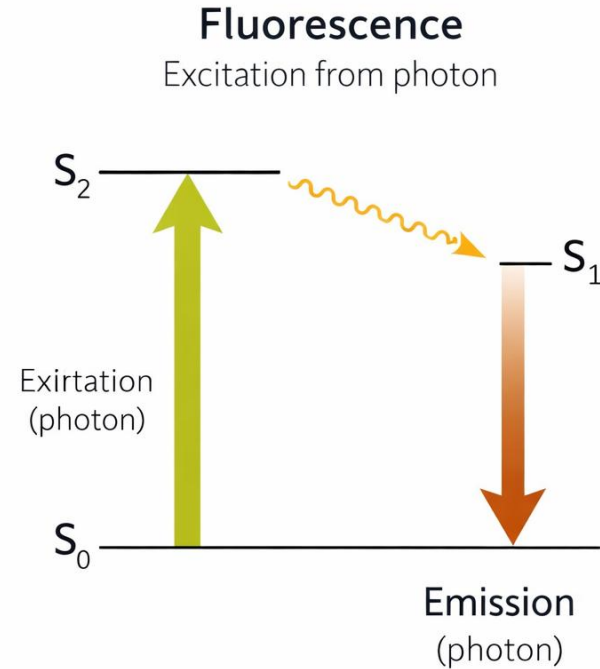
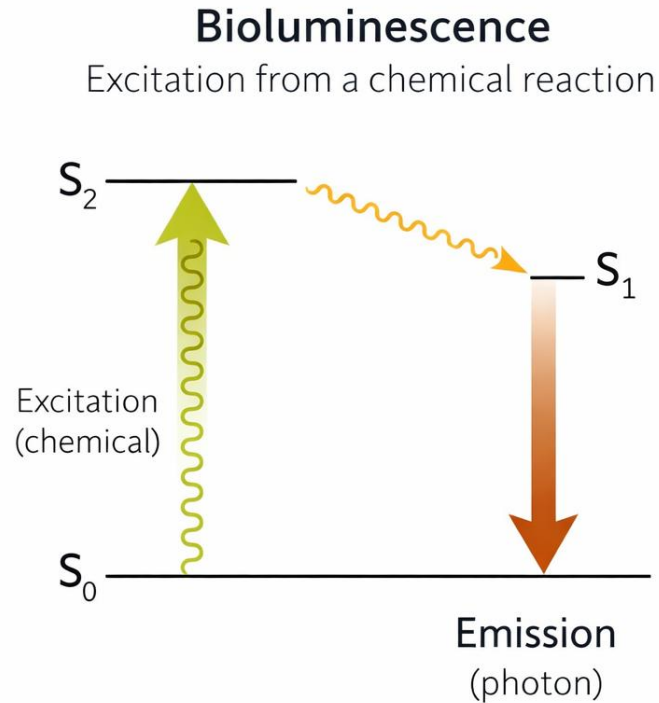
Beetle Luciferin



Oxyluciferin

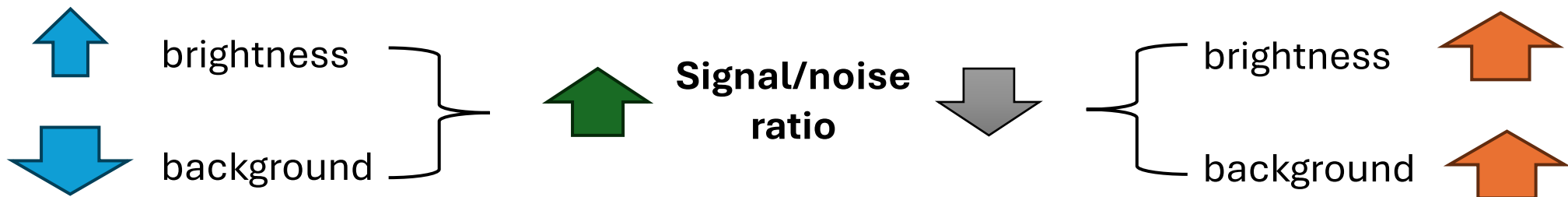


Bioluminescence as a product of chemical reaction

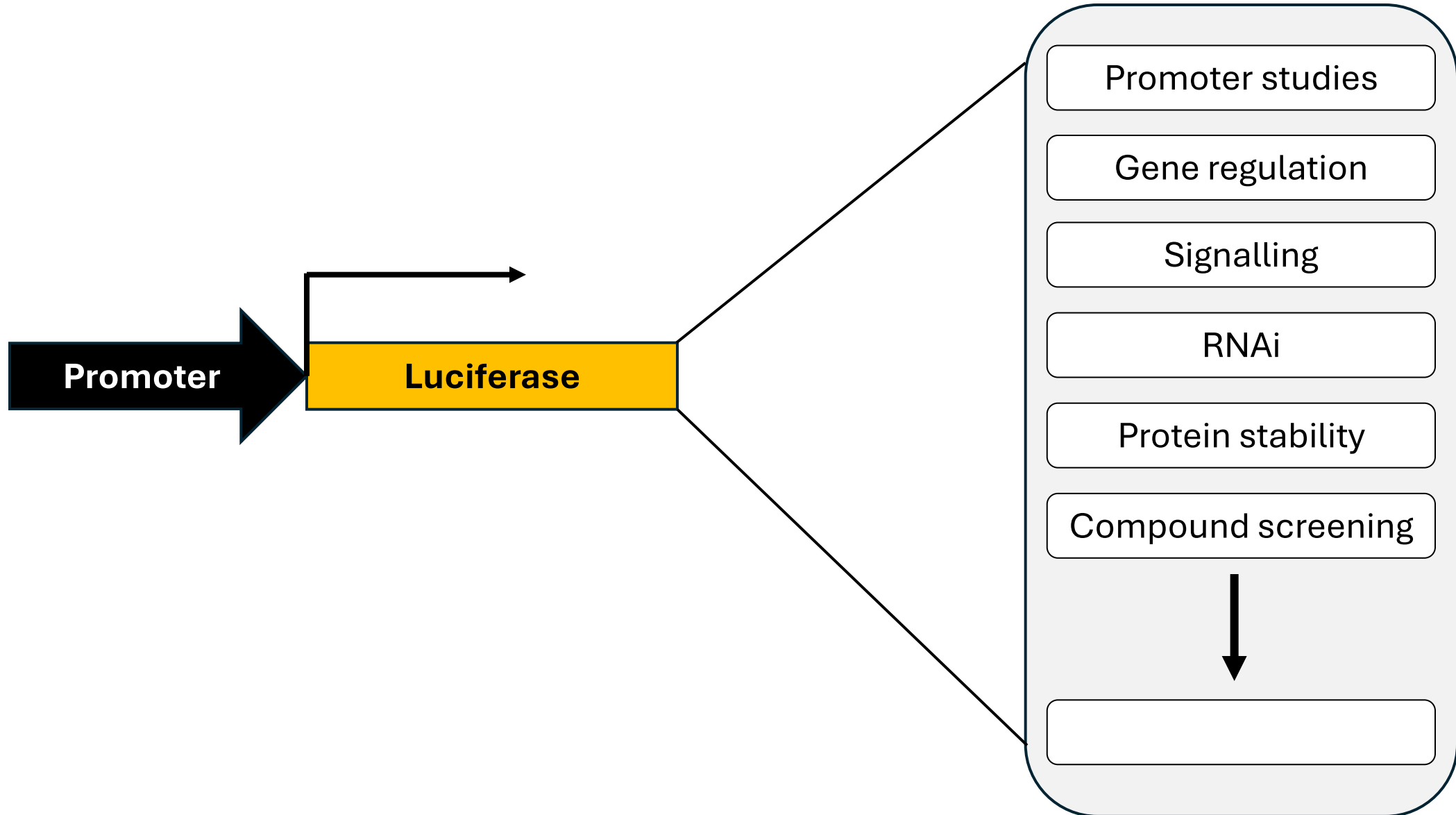


Excitation through chemical reaction

Excitation through light absorption

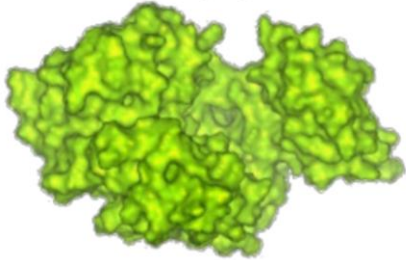


Luciferase-Based Reporters

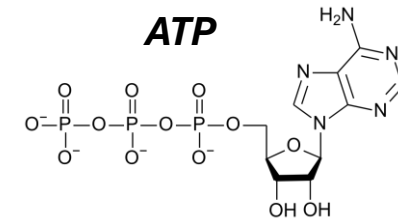
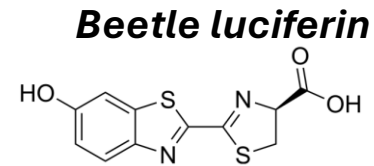


Luciferase-Based Reporters

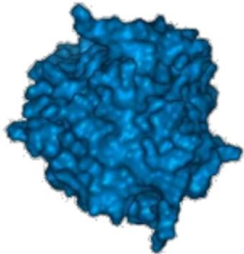
Firefly



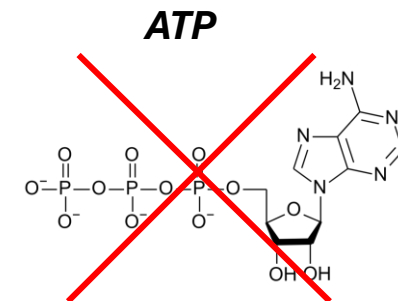
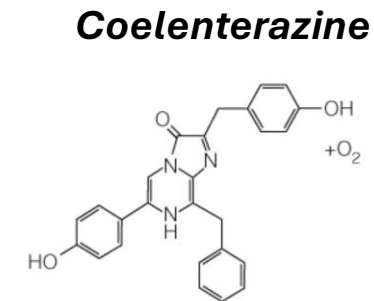
Photinus pyralis
(firefly)



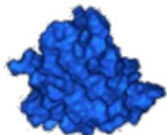
Renilla



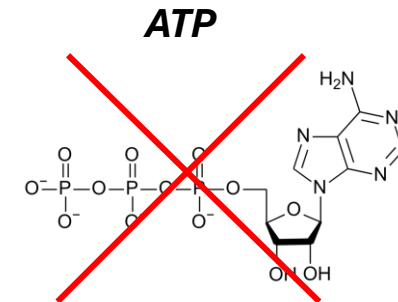
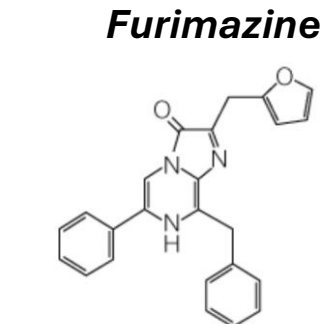
Renilla reniformis
(coral)



NanoLuc



Oplophorus gracilirostris
(shrimp)



Luciferase-Based Reporters

Basic rules for choosing a luciferase reporter

- Is one readout enough? Choose **single reporter**
- Do you need normalization? Choose **dual reporter**
 - Normalize for **transfection efficiency** or **cell number**
 - **Fluc + Rluc**
 - **Fluc + NanoLuc** → low background, higher sensitivity

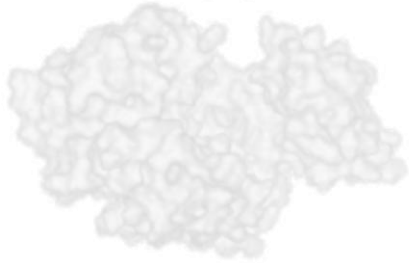
Luciferase-Based Reporters

Basic rules for choosing a luciferase reporter

- **Signal intensity vs signal duration**
 - Brighter signals → faster decay
 - Longer-lived signals → ideal for plate-based workflows
- Reporter **stability**
 - Ideal reporter reflects transcriptional dynamics
 - Responds specifically, low intrinsic stability
 - Very stable reporters → lags behind rapid biological changes

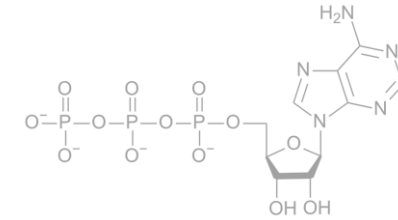
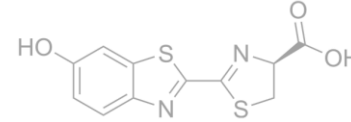
Luciferase-Based Reporters

Firefly



Photinus pyralis
(firefly)

Beetle luciferin

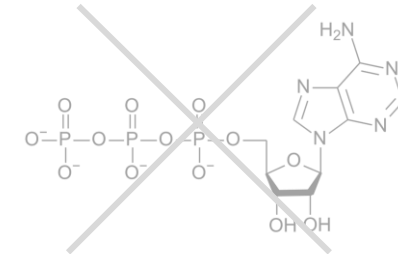
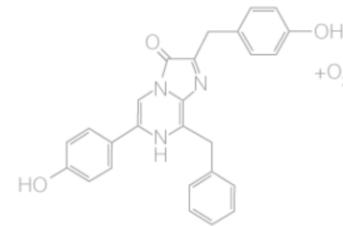


Renilla

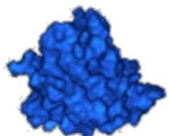


Renilla reniformis
(coral)

Coelenterazine

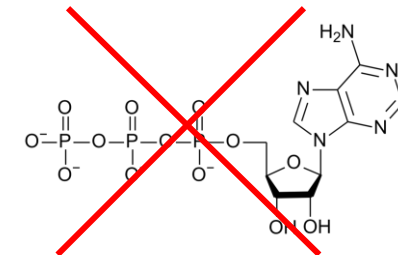
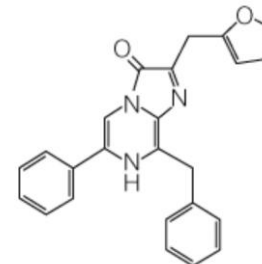


NanoLuc



Oplophorus gracilirostris
(shrimp)

Furimazine

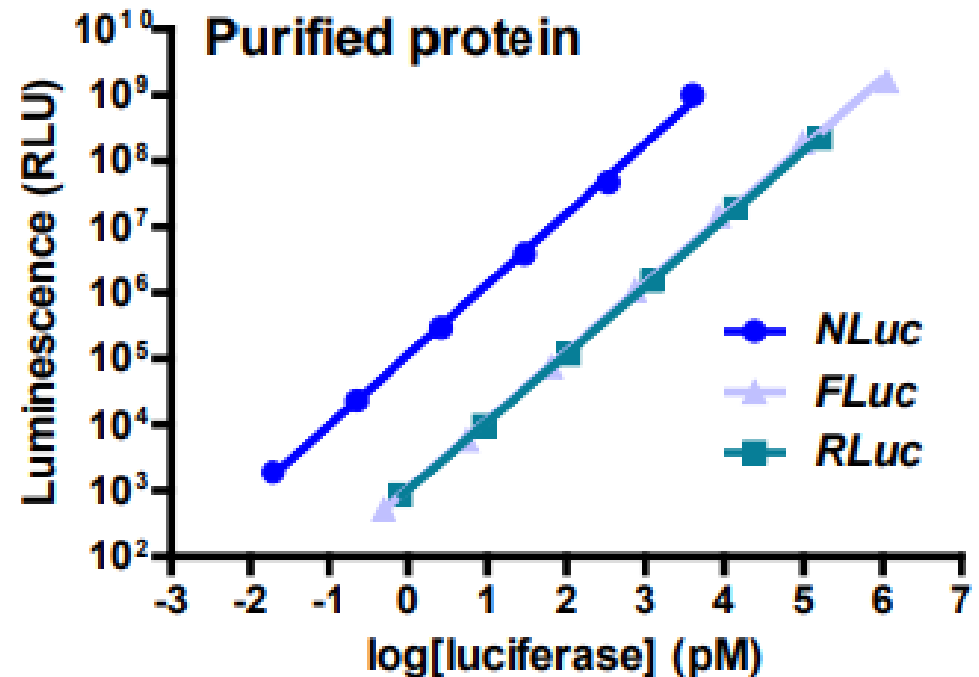
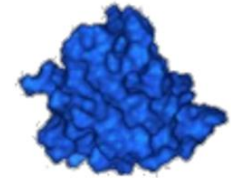


NanoLuc

NanoLuc Key Features

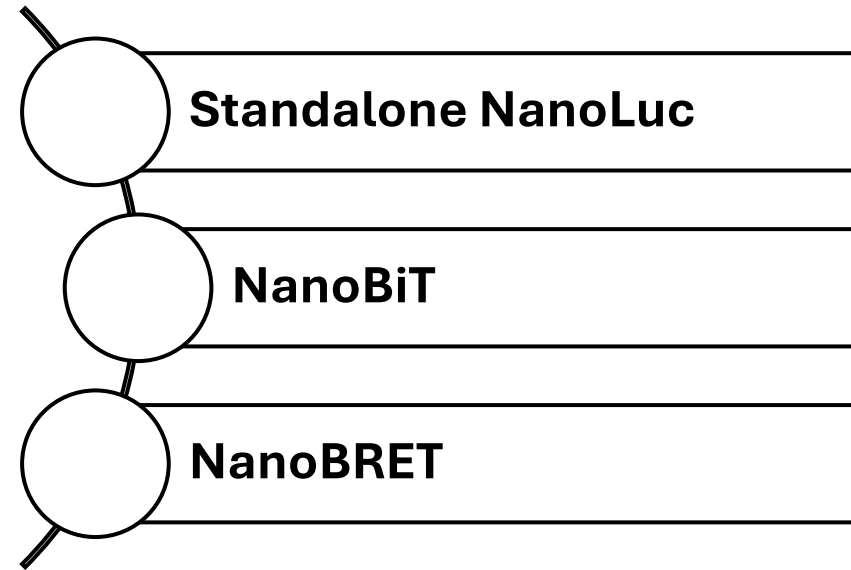
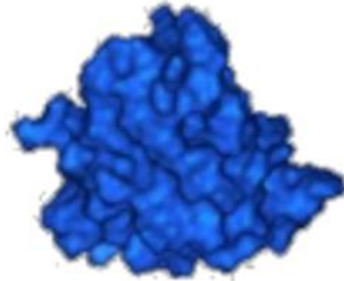
- **Small** → 19 kDa
- Very **bright** → over 100-fold then Fluc, RLuc
- Long **glow-type** signal → **2-hour half-life**
- **ATP-independent**
- Improved physical and pH stability

NanoLuc



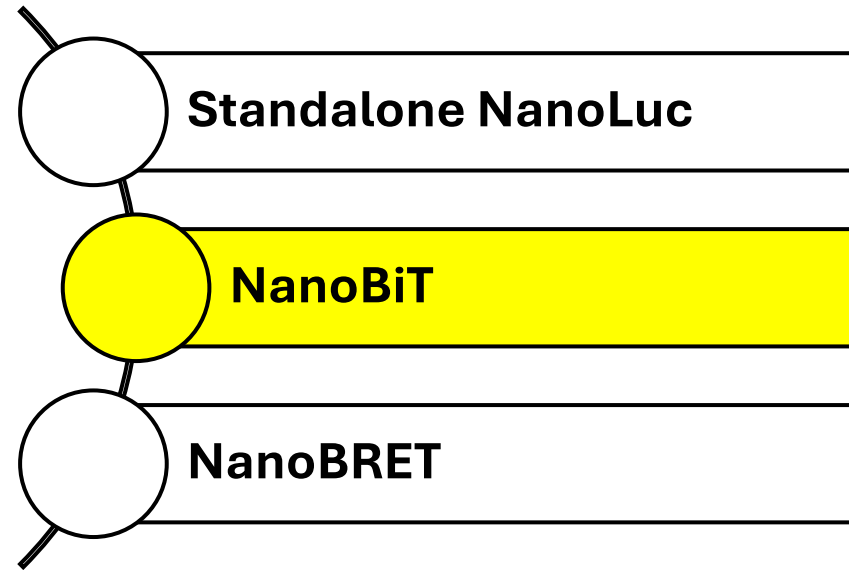
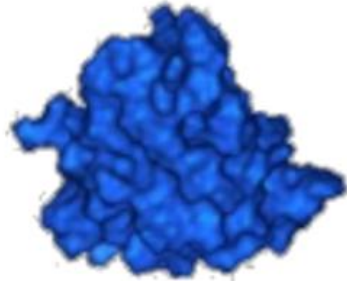
NanoLuc

NanoLuc Technologies

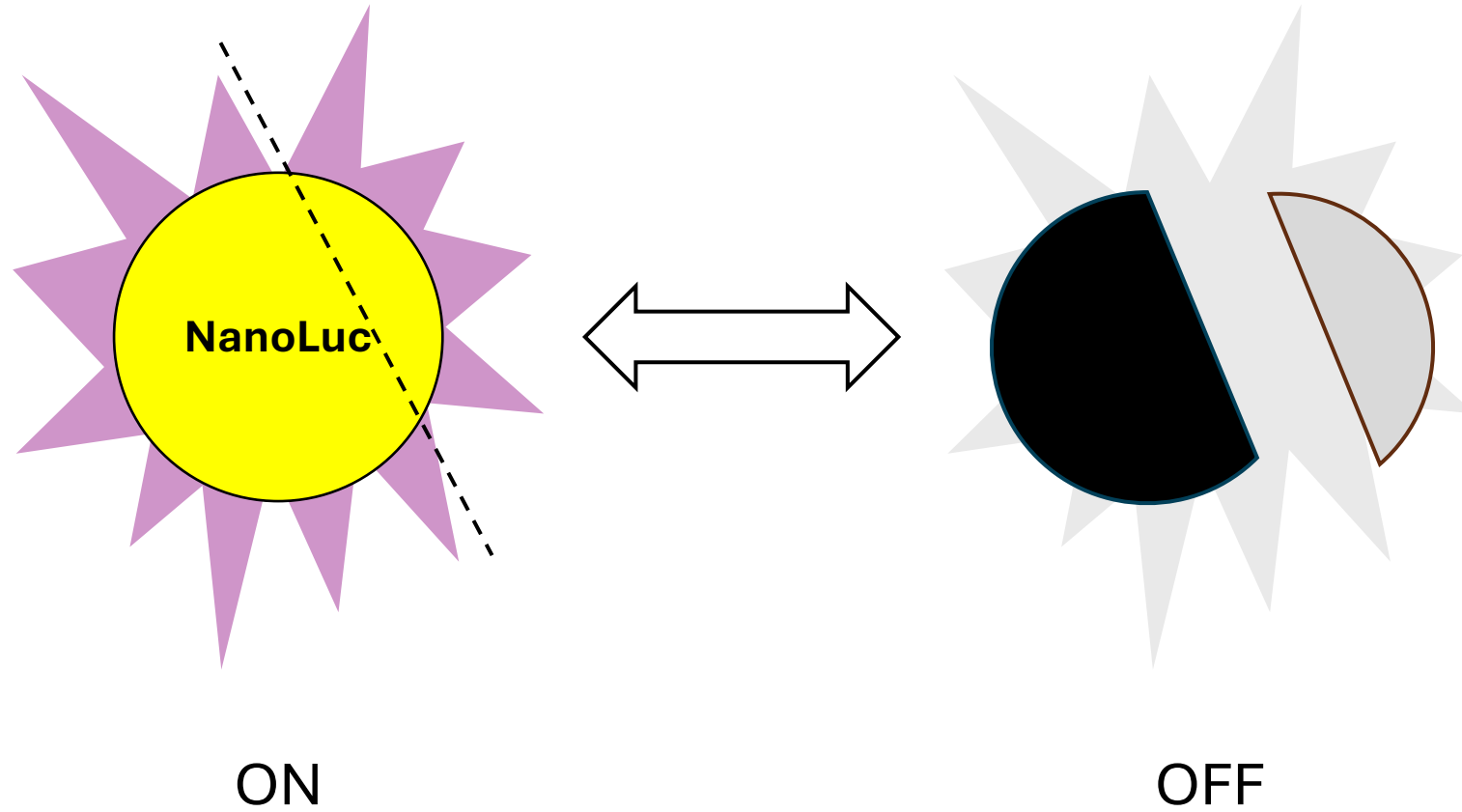


NanoLuc

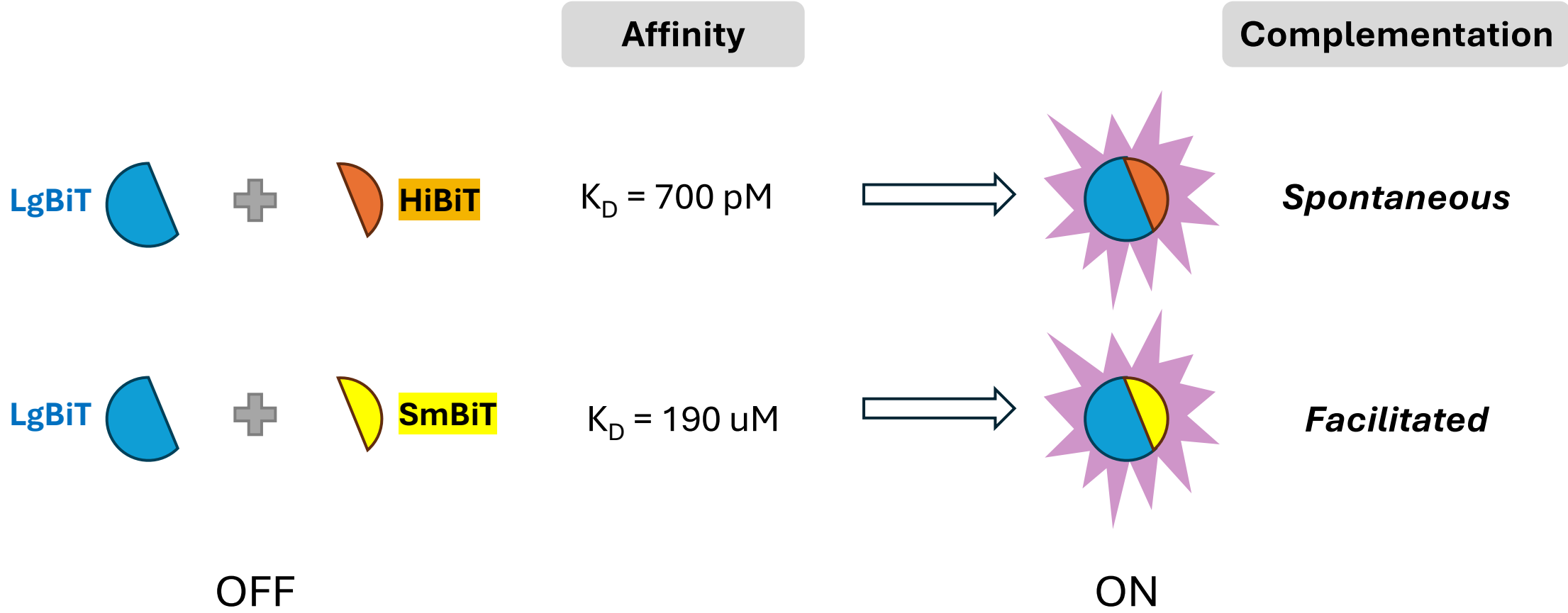
NanoLuc Technologies



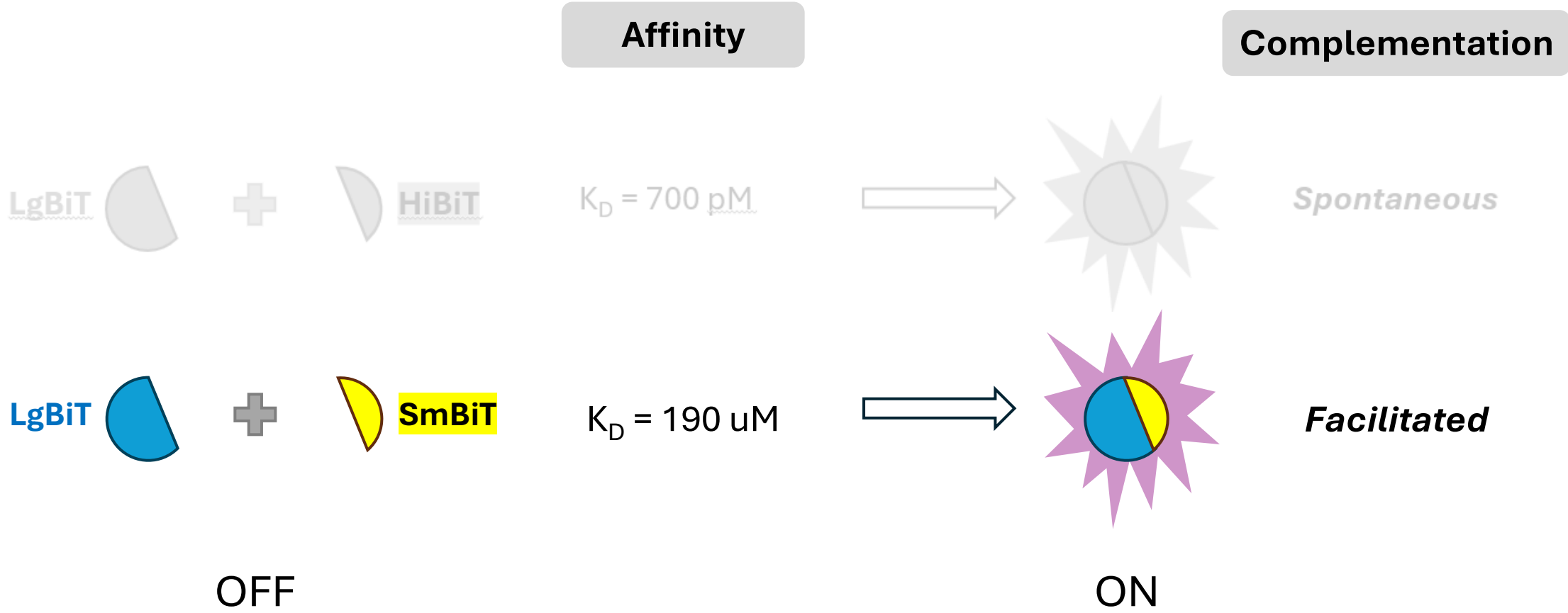
NanoLuc Binary Technology



NanoLuc Binary Technology



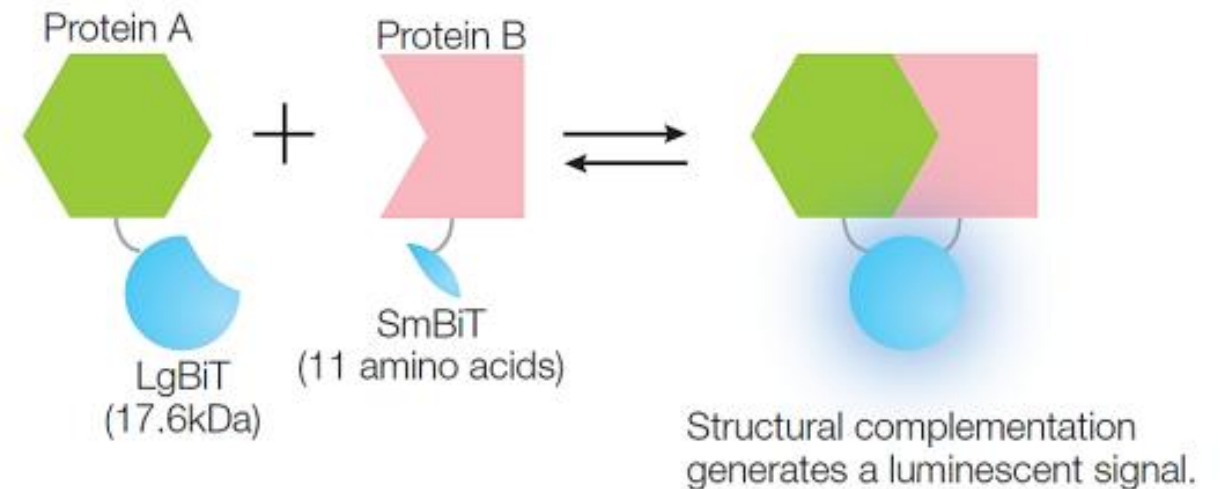
NanoLuc Binary Technology



NanoBiT Complementation Assay

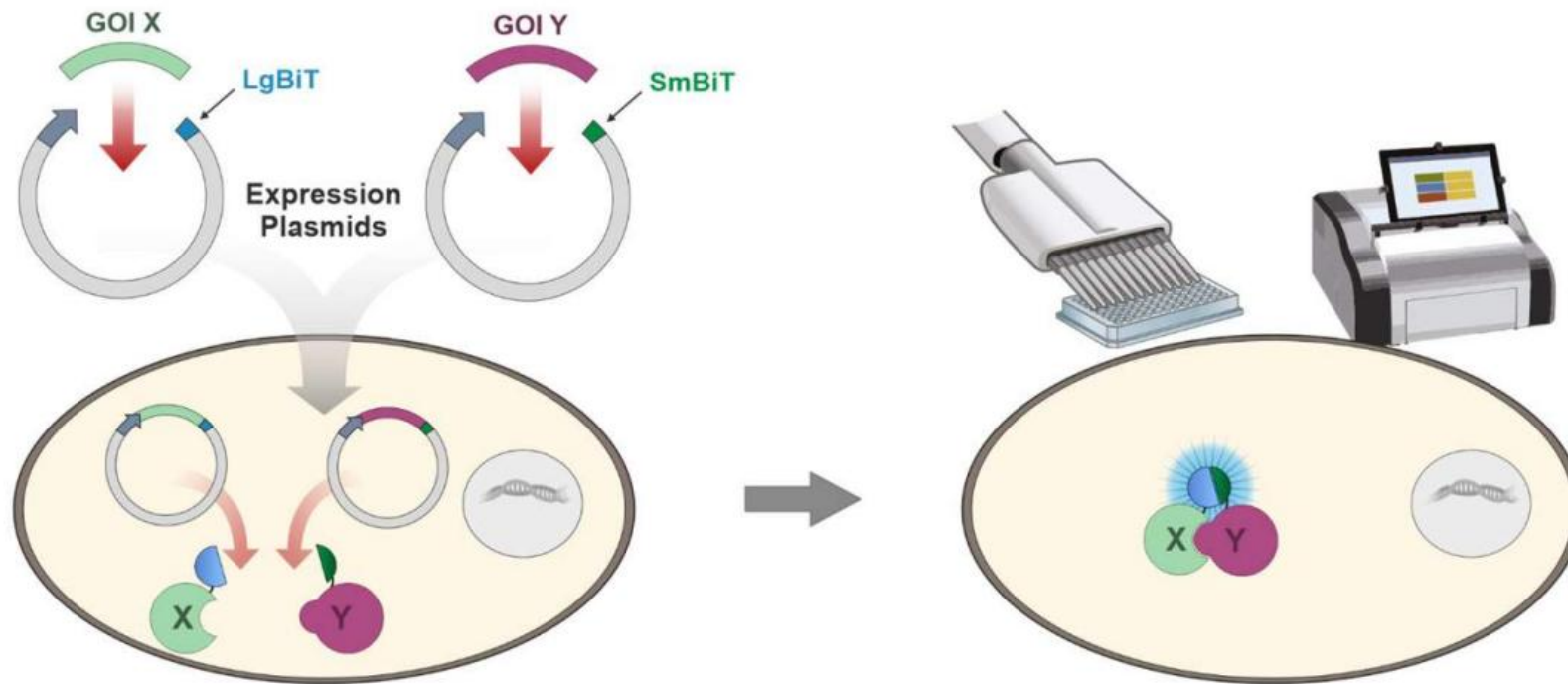
NanoBiT Protein-Protein (PPI) Interaction Assay

- Investigate PPI **dynamics** in live cells
- Screen for **inhibitors** of PPI
- **Small** tag size → minimize risk of steric hindrance



NanoBiT Complementation Assay

NanoBiT Protein-Protein (PPI) Interaction Assay



NanoBiT Complementation Assay

Application

Real-time PPI measurement

PPI modulator screening

Posttranslational modifications

Protein trafficking

NanoBiT Complementation Assay

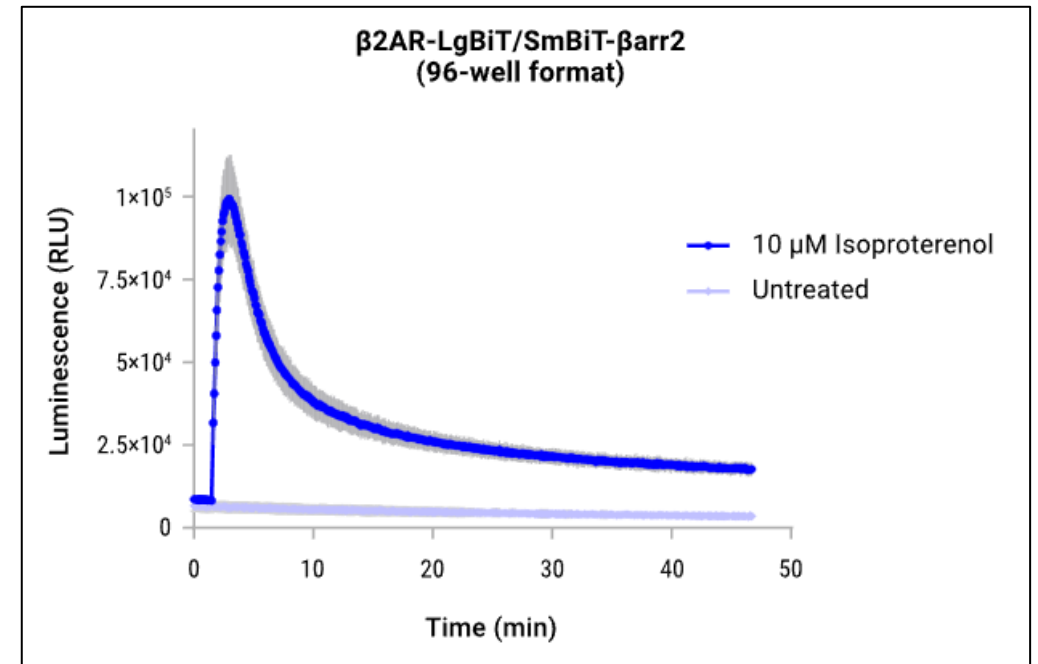
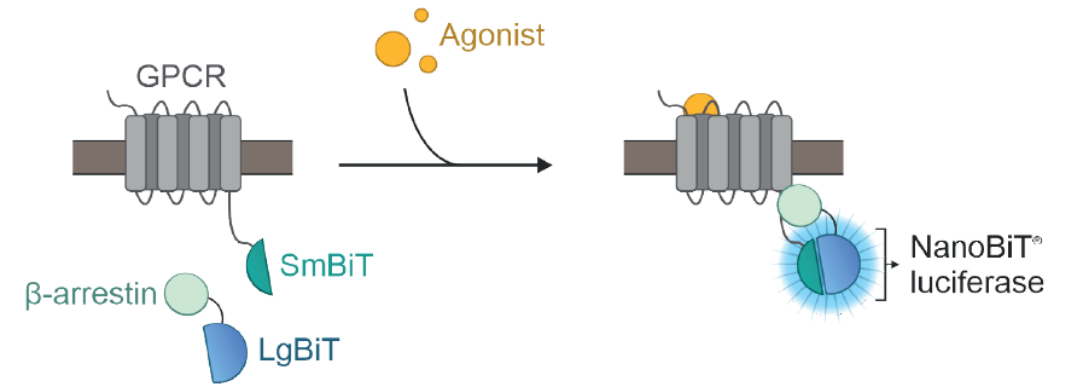
Application

Real-time PPI measurement

PPI modulator screening

Posttranslational modifications

Protein trafficking



NanoBiT Complementmentation Assay

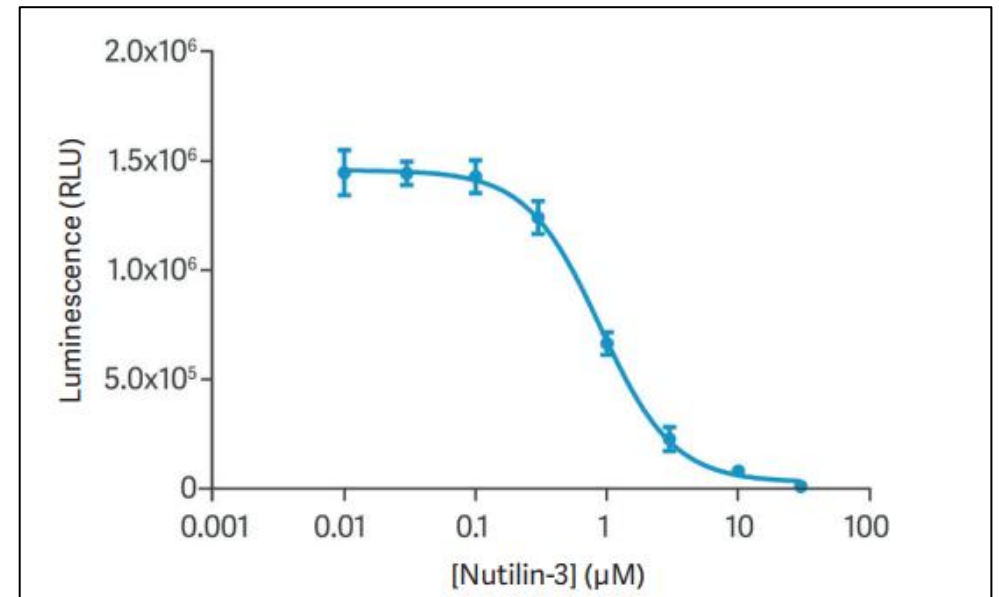
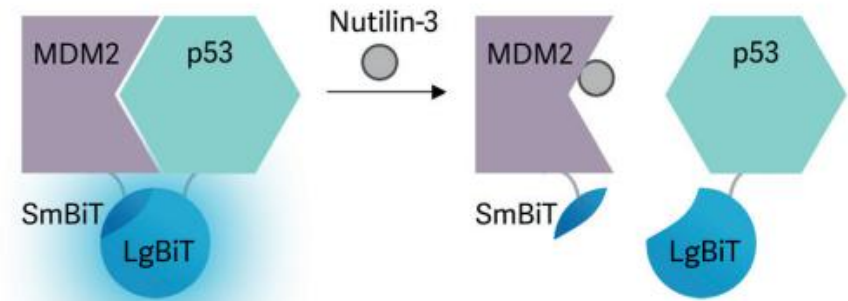
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PPI modulator screening

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Protein trafficking



NanoBiT Complementmentation Assay

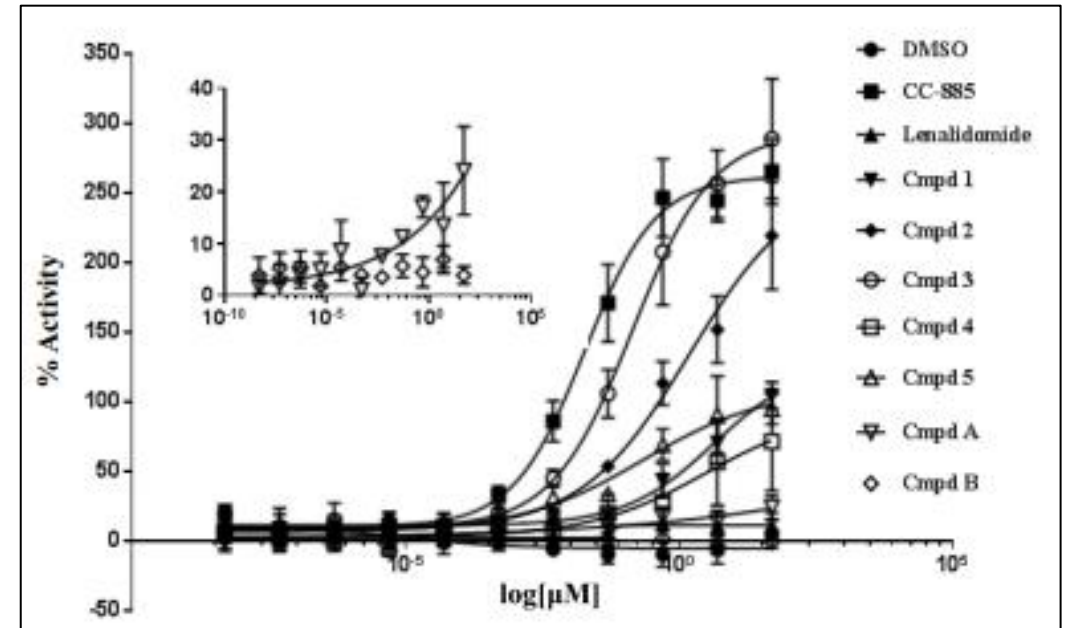
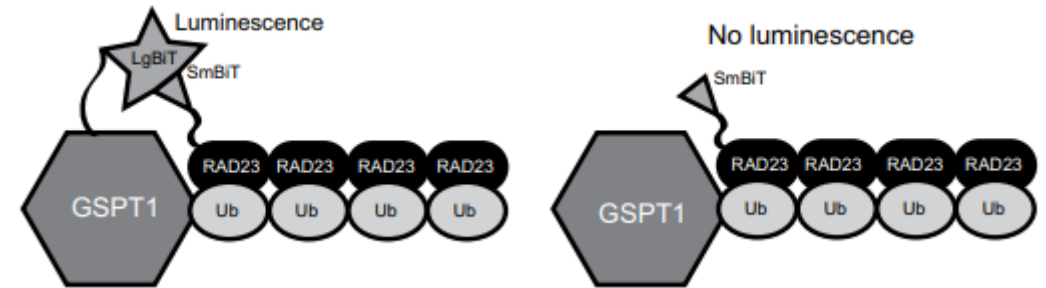
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Real-time PPI measurement

PPI modulator screening

Posttranslational modifications

Protein trafficking



<https://doi.org/10.1177/2472555219901261>

NanoBiT Complementation Assay

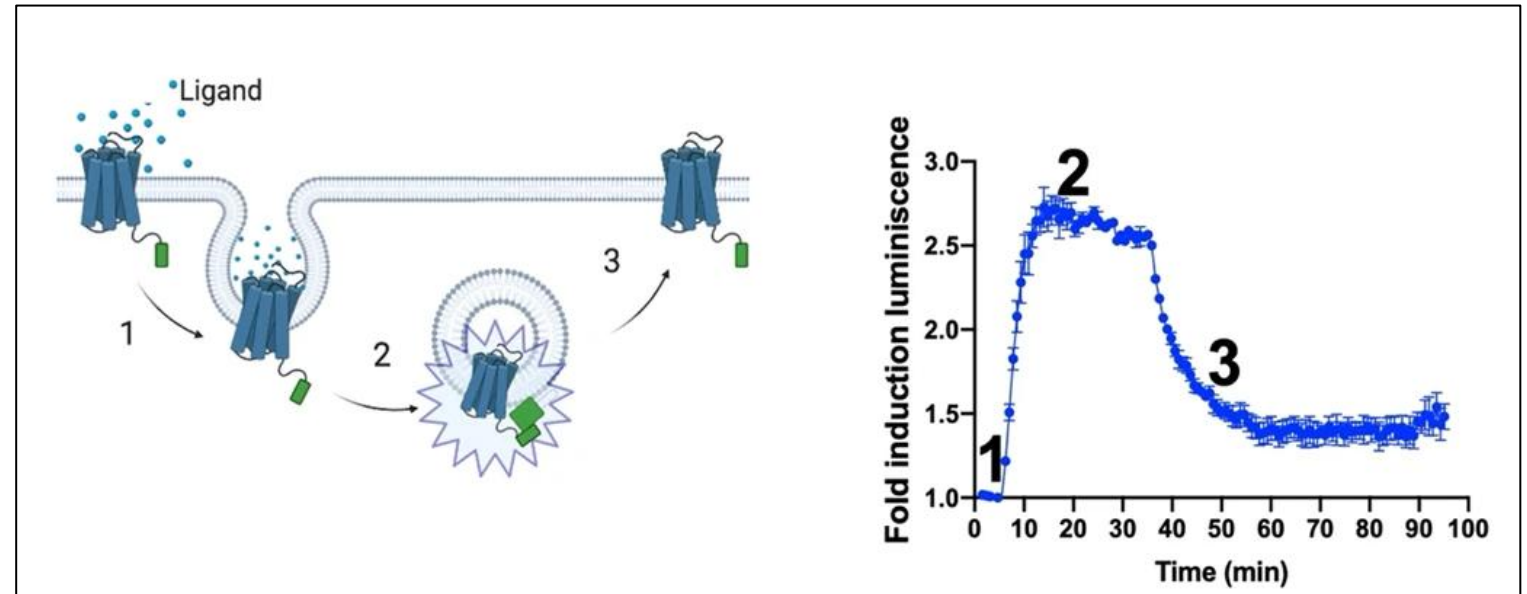
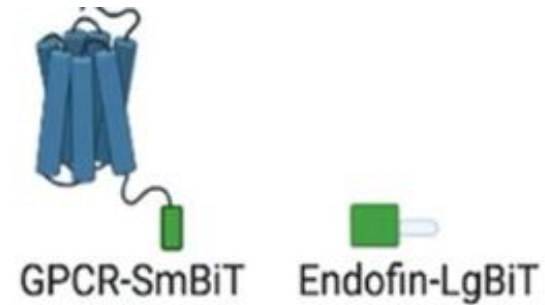
Application

Real-time PPI measurement

PPI modulator screening

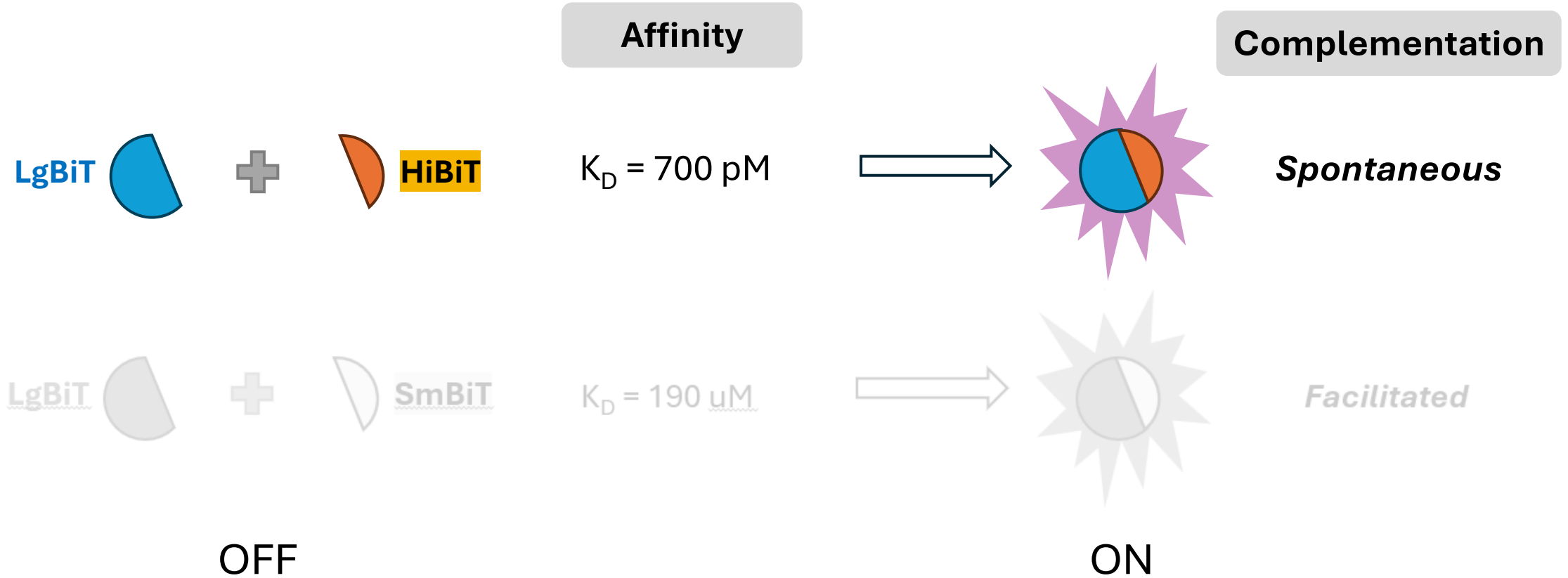
Posttranslational modifications

Protein trafficking/localization

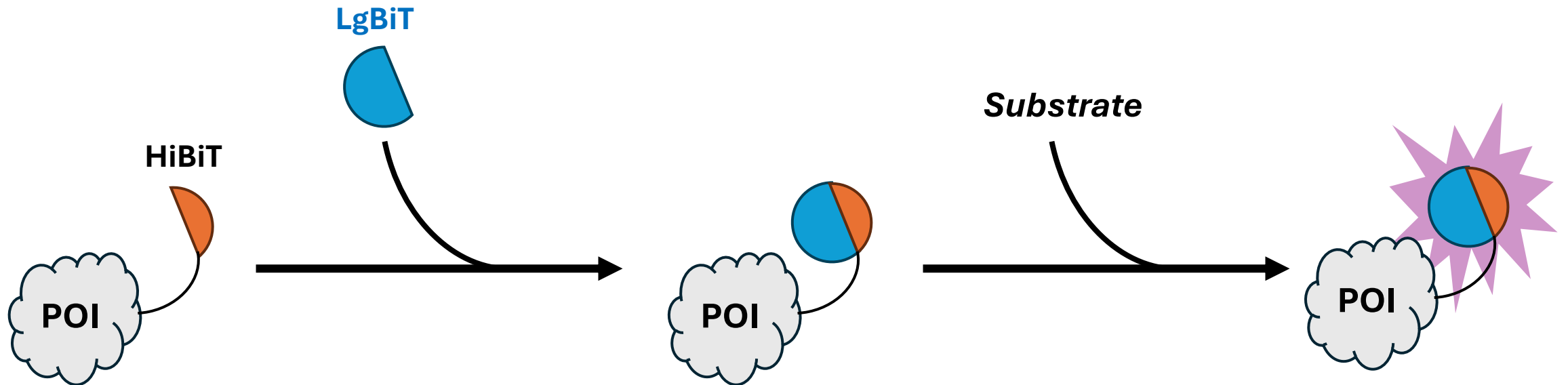


<https://doi.org/10.1038/s42003-022-03163-9>

HiBiT Tagging System – small by size, big by potential

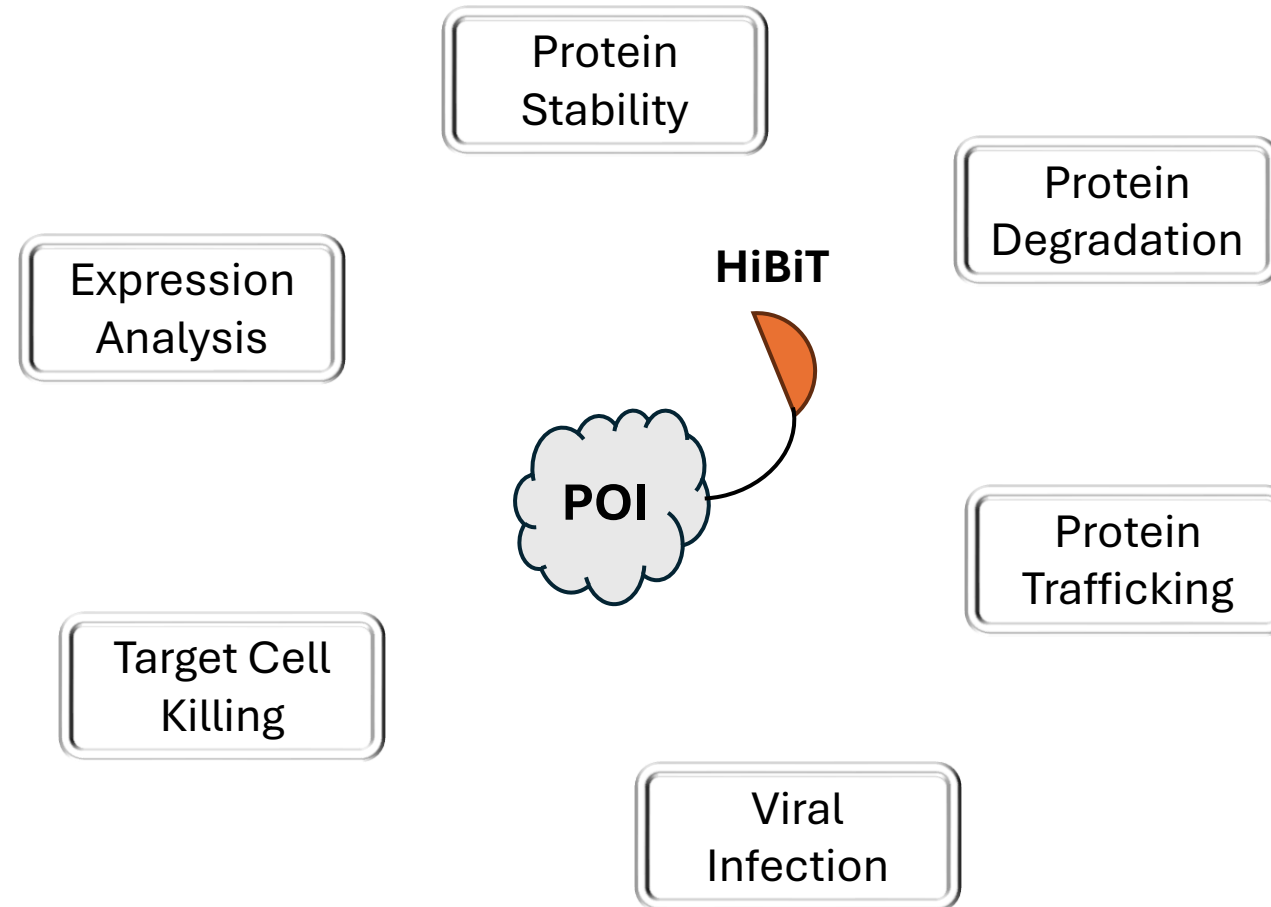


HiBiT Tagging System – small by size, big by potential



POI = protein of interest

HiBiT Tagging System – small by size, big by potential

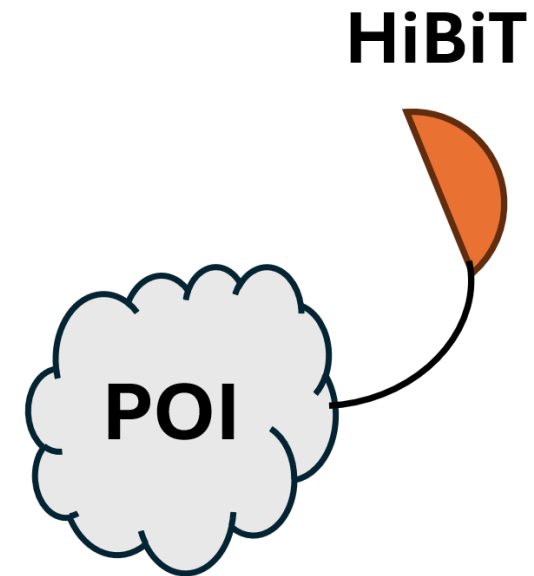


POI = protein of interest

HiBiT Tagging System – small by size, big by potential

Key features

- **Small** – reduced risk on POI function
- Vector or CRISPR/Cas delivery
- Simple, homogenous detection assay → **add-and-read**
- High sensitivity – ideal for **low abundant** proteins
- **HTS** compatibility



POI = protein of interest

HiBiT Tagging System – small by size, big by potential

Lytic Detection System

Protein Expression

Protein Degradation

Extracellular Detection System

Receptor Trafficking

Protein Secretion

Cytokine Secretion

Live Cell Assay System

Protein Degradation

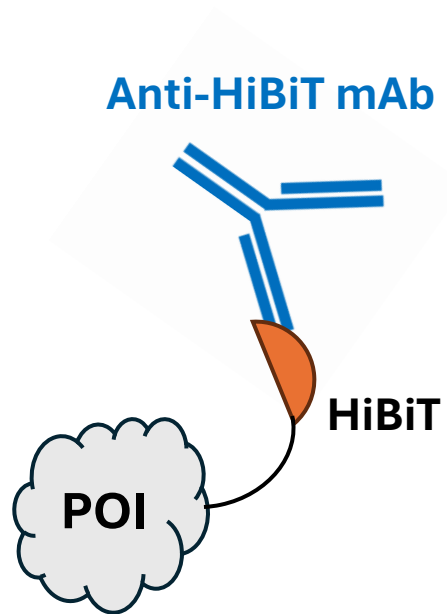
Protein Expression

Viral Infection

Target Cell Killing

HiBiT Tagging System – small by size, big by potential

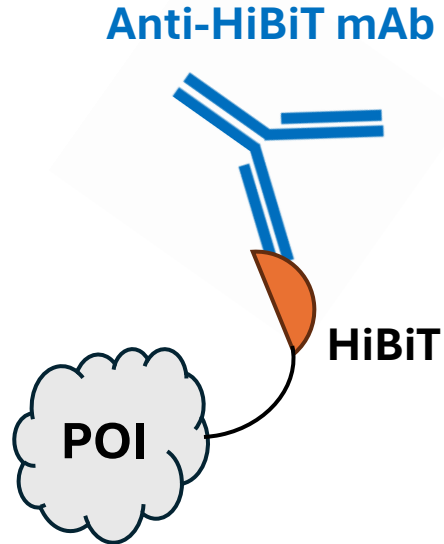
Other HiBiT application



POI = protein of interest

HiBiT Tagging System – small by size, big by potential

Other HiBiT application

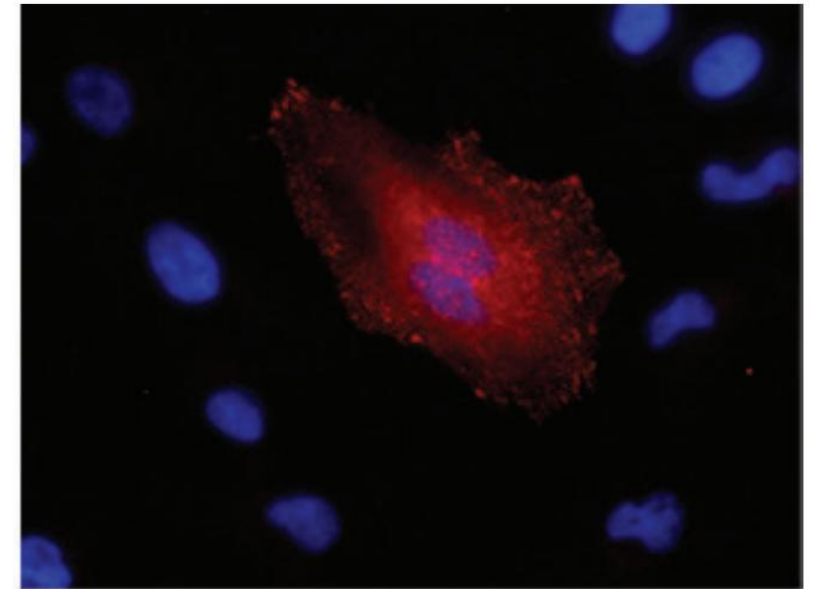


Immunofluorescence

Immunoblotting

Immunoprecipitation

FACS analysis

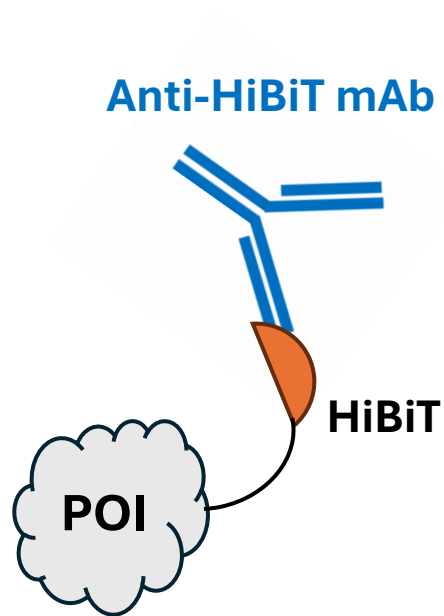


Vinculin
mxHiBiT; gxm AF-647; Hoechst

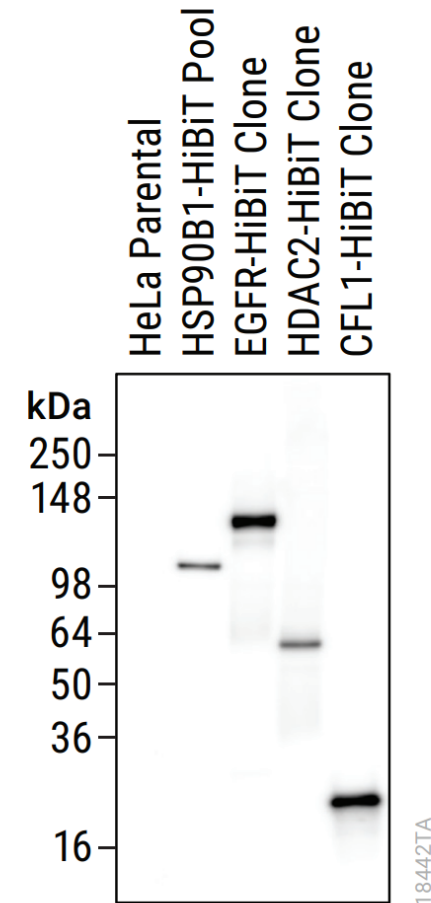
POI = protein of interest, mAb = monoclonal antibody; m = mouse; g = goat

HiBiT Tagging System – small by size, big by potential

Other HiBiT application



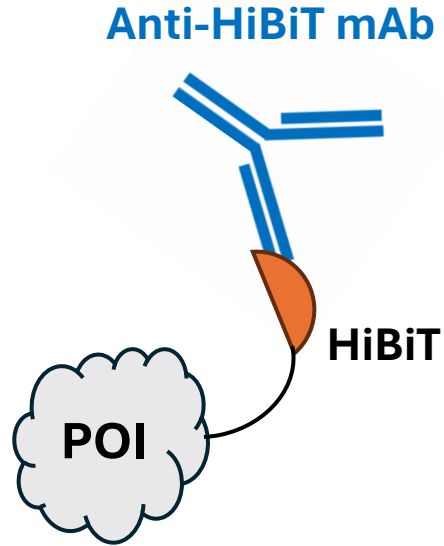
- Immunofluorescence
- Immunoblotting
- Immunoprecipitation
- FACS analysis



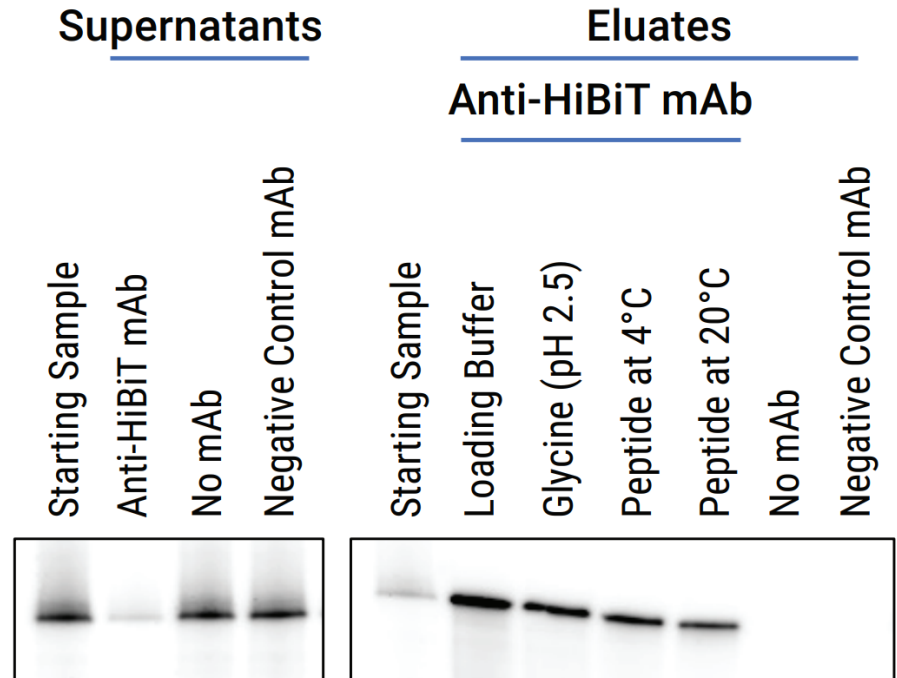
POI = protein of interest; mAb = monoclonal antibody

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Other HiBiT application



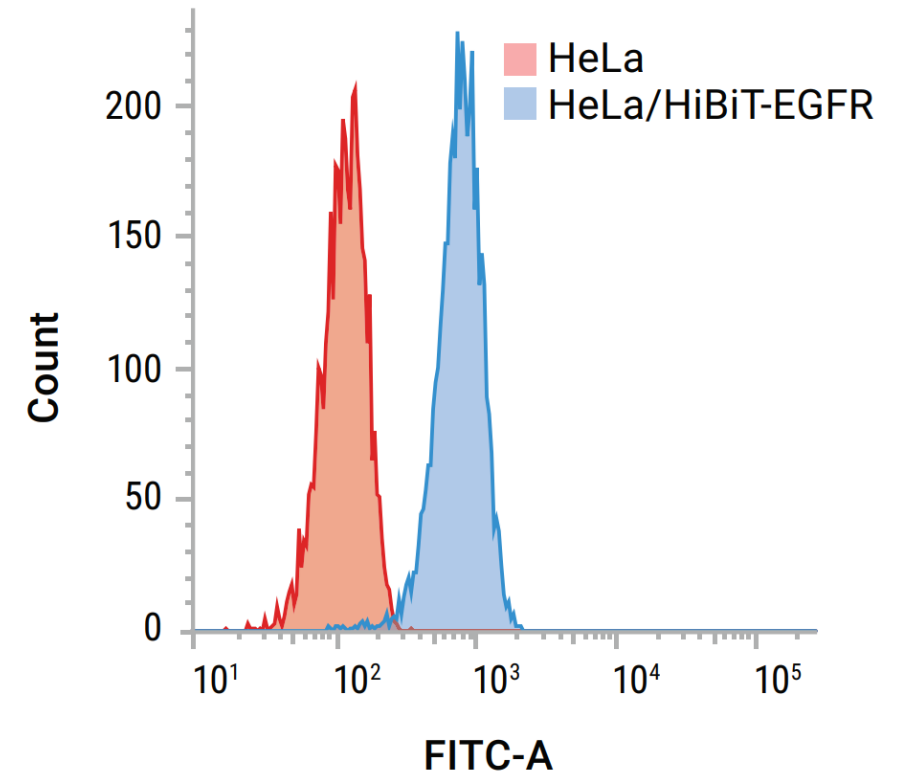
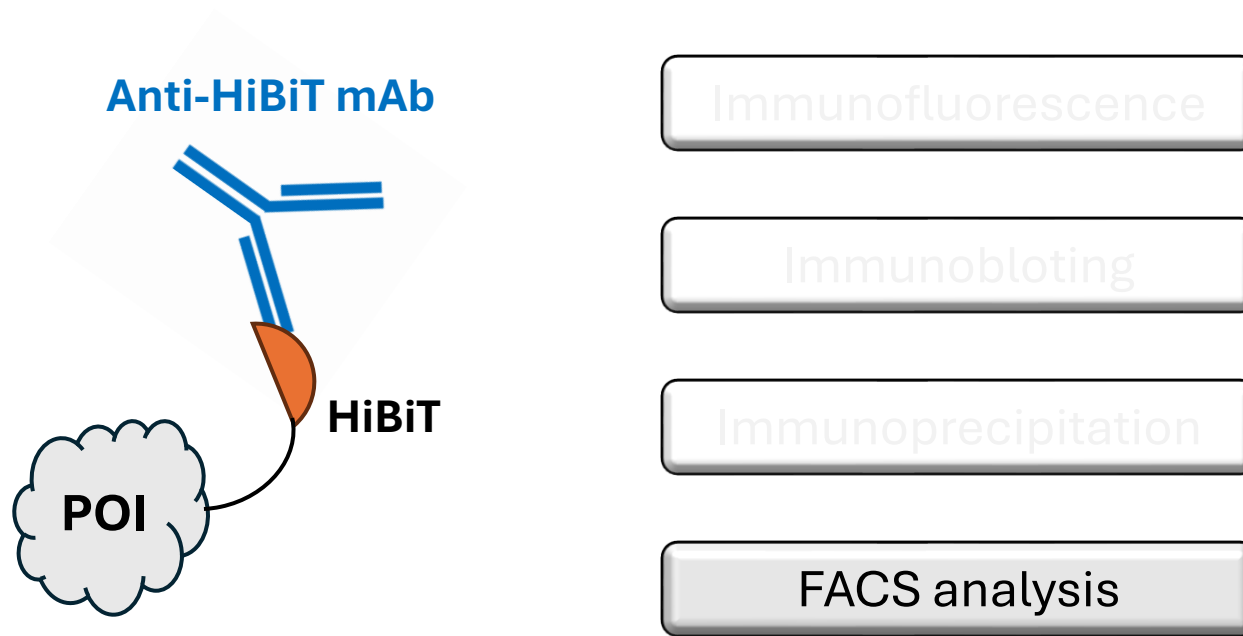
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POI = protein of interest; mAb = monoclonal antibody

HiBiT Tagging System – small by size, big by potential

Other HiBiT application



POI = protein of interest; mAb = monoclonal antibody

HiBiT Tagging System – small by size, big by potential

Application

Protein degradation

Membrane receptor trafficking

mRNA translation efficiency

Target Cell Killing

Viral release

POI = protein of interest

HiBiT Tagging System – small by size, big by potential

Application

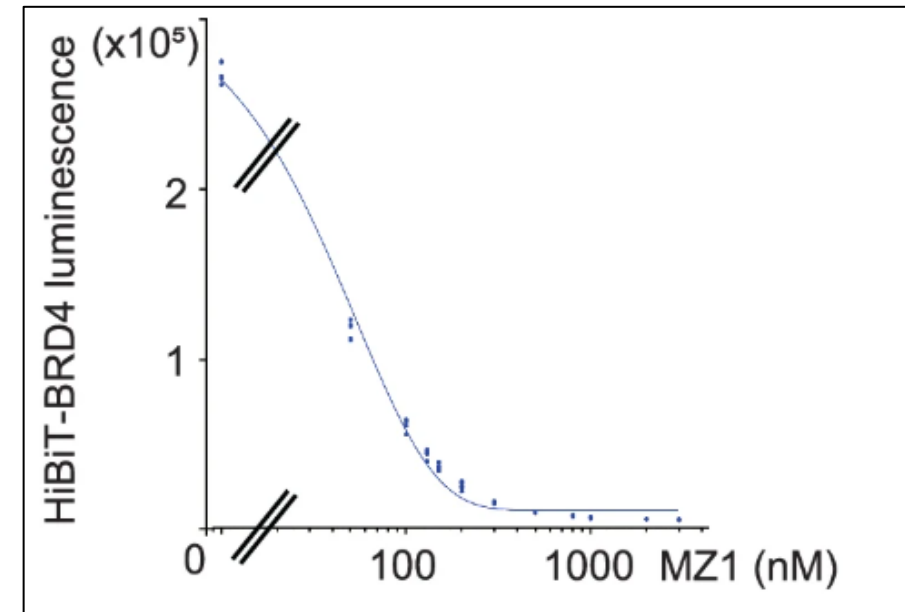
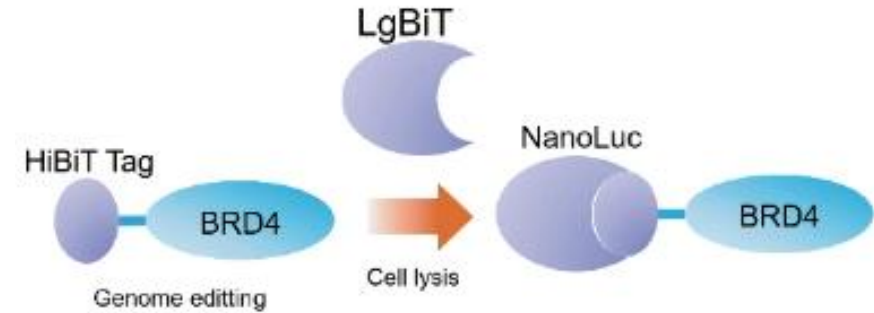
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<https://doi.org/10.1038/s41467-024-49519-z>

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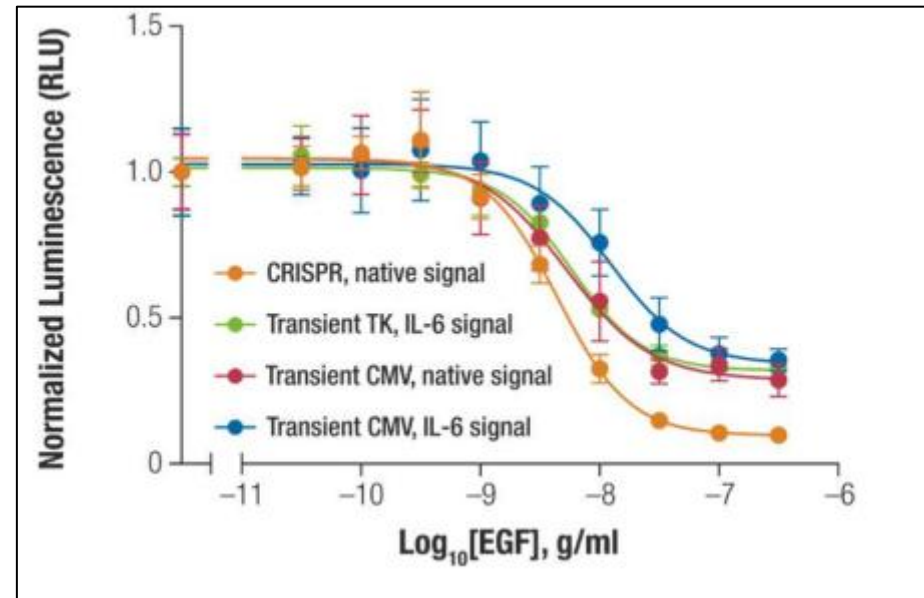
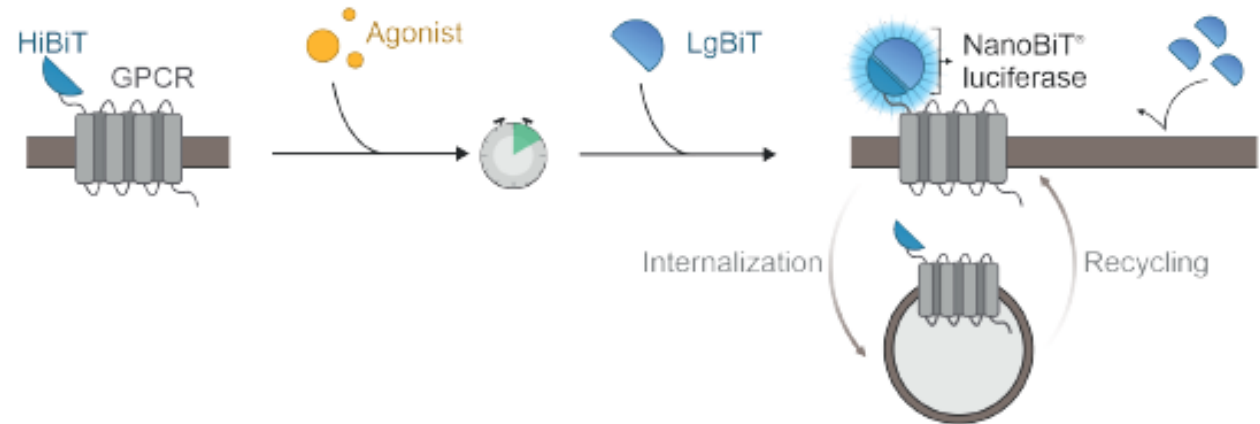
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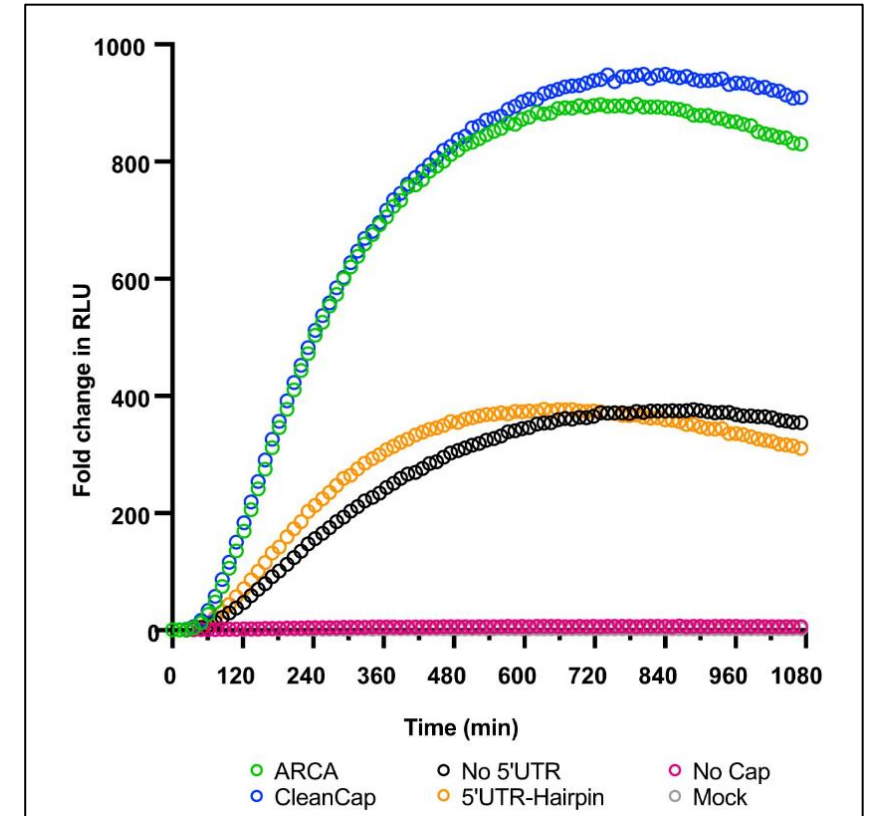
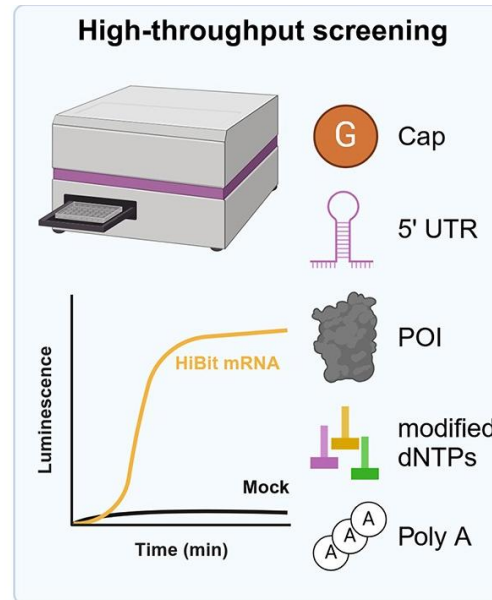
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<https://doi.org/10.1093/nar/gkaf496>

POI = protein of interest

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Application

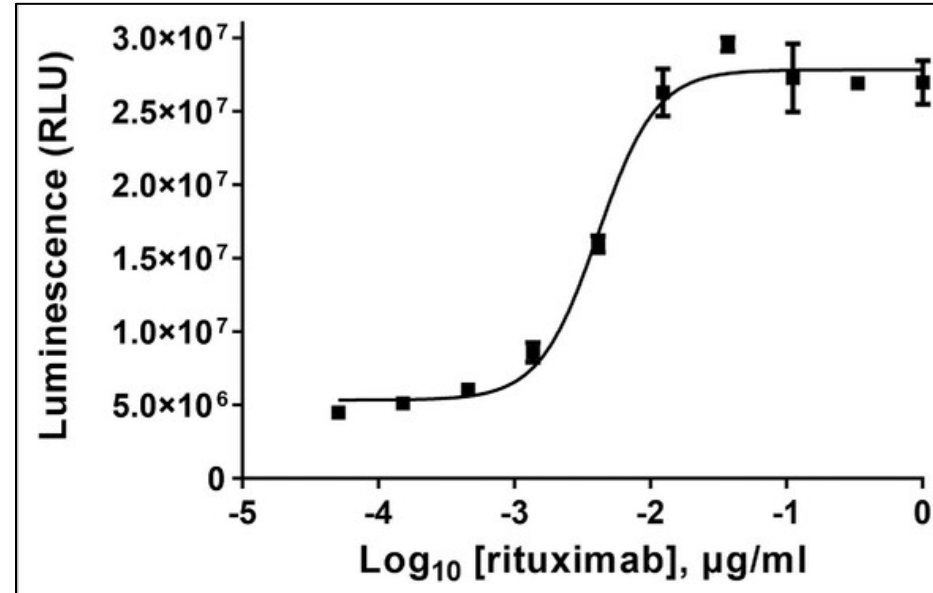
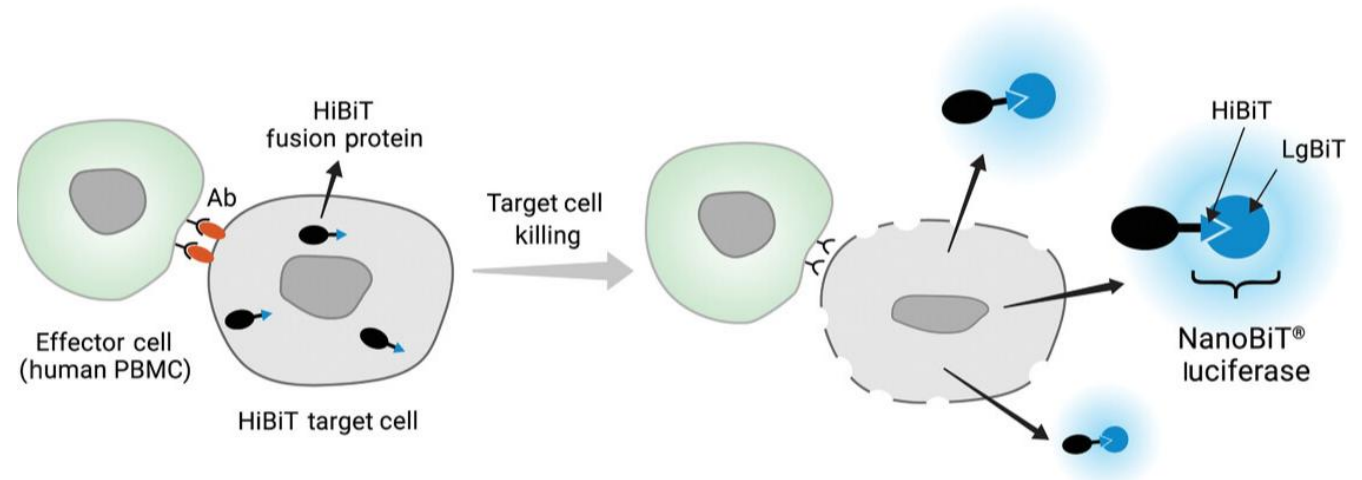
Protein degradation

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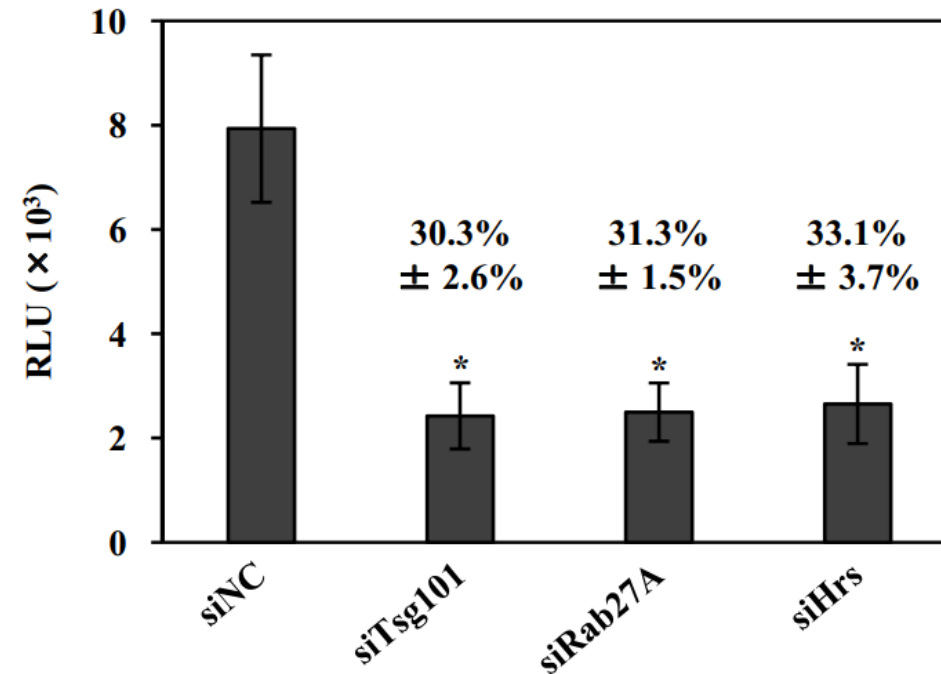
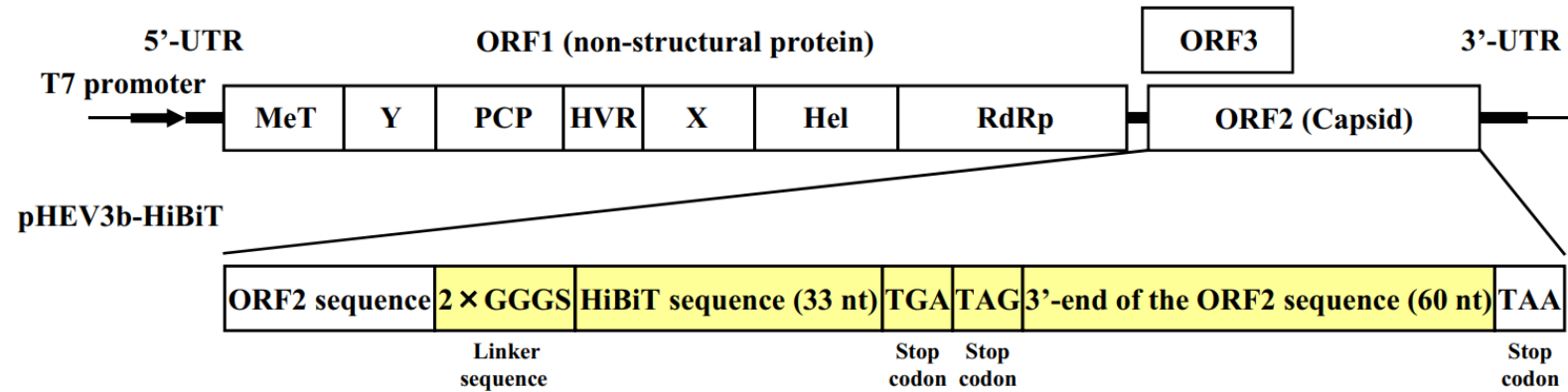
<https://doi.org/10.1002/cpz1.296>

POI = protein of interest

HiBiT Tagging System – small by size, big by potential

Application

- Protein degradation
- Membrane receptor trafficking
- mRNA translation efficiency
- Target Cell Killing
- Viral release**

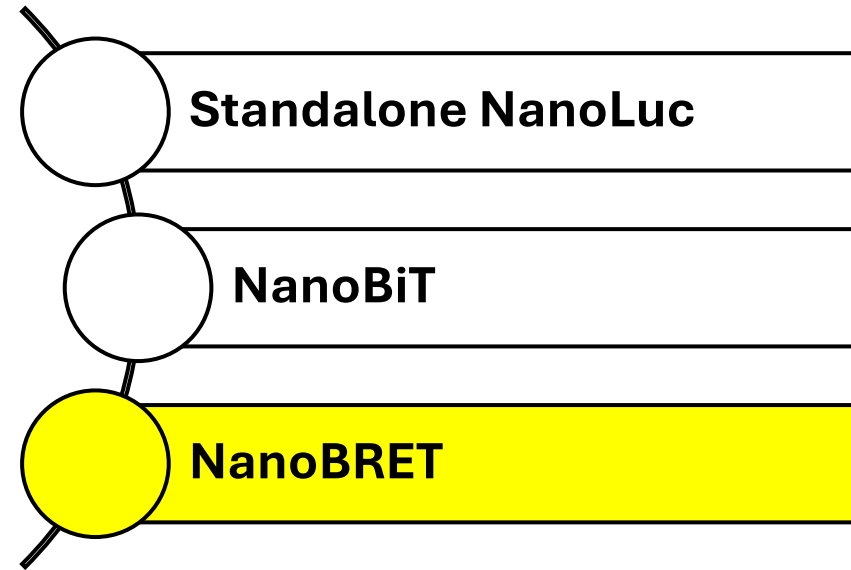
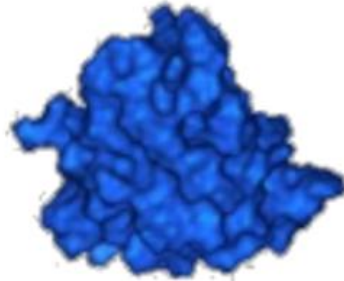


POI = protein of interest

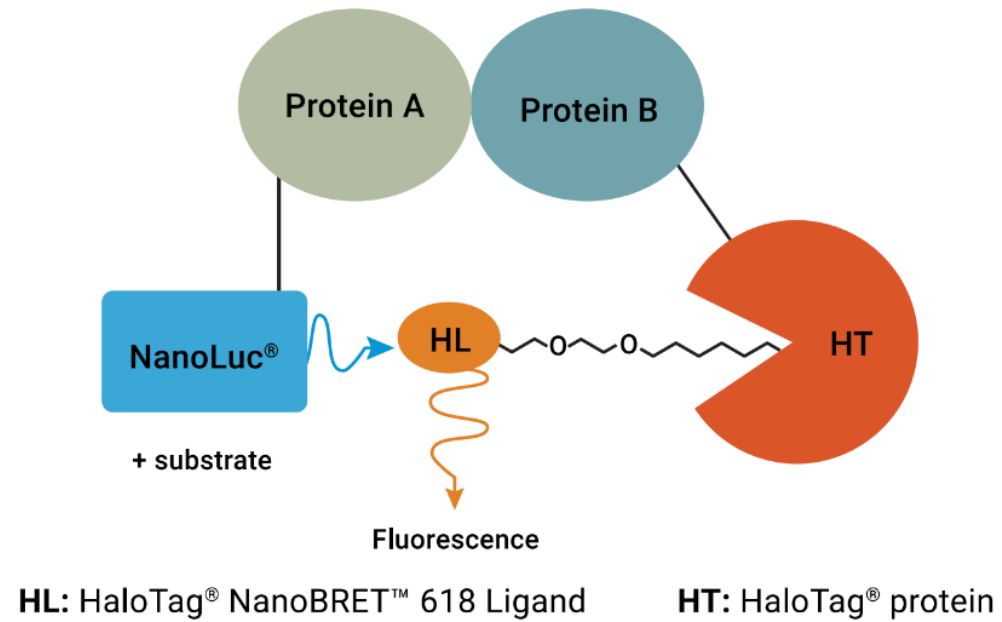
doi: 10.1128/jvi.00508-23

NanoLuc

NanoLuc Technologies



NanoBRET Protein-Protein Interaction Assay

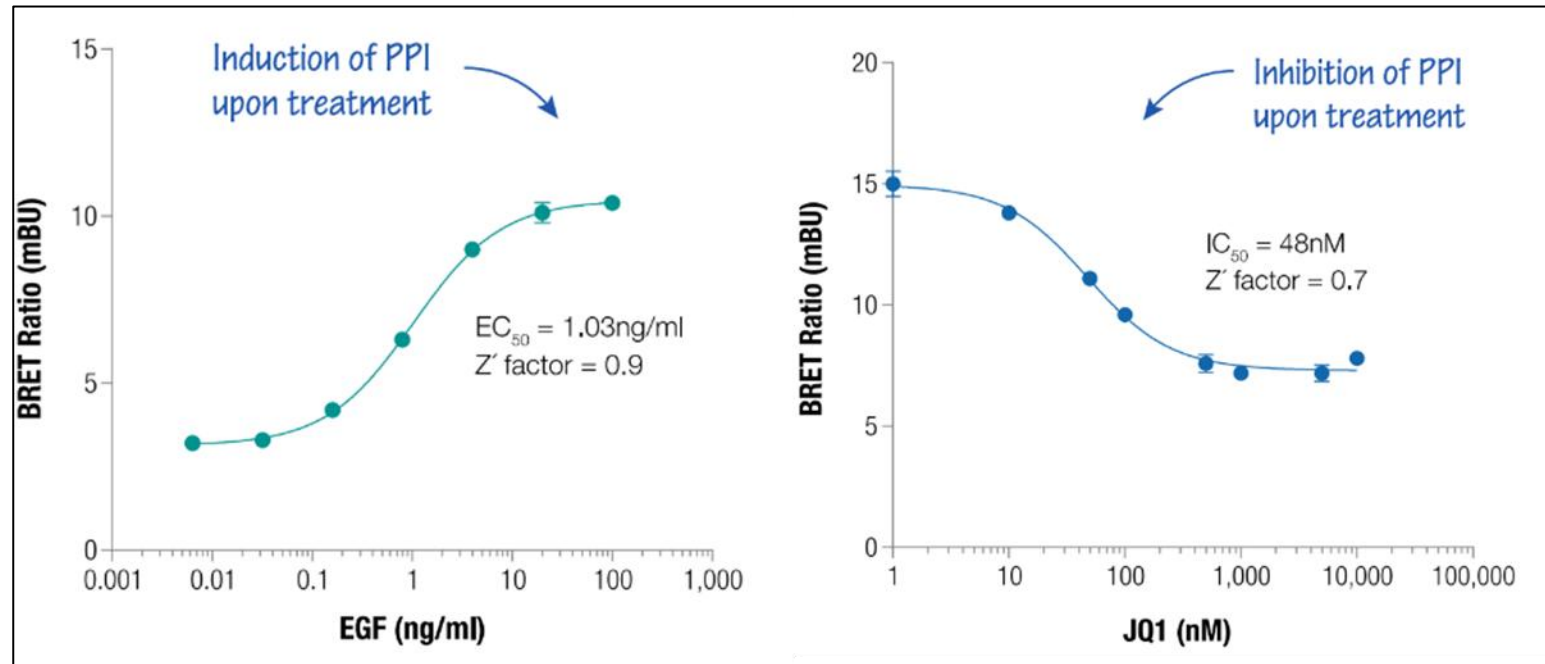


NanoBRET Protein-Protein Interaction Assay

Applications

Protein interaction dynamics

Target Engagement

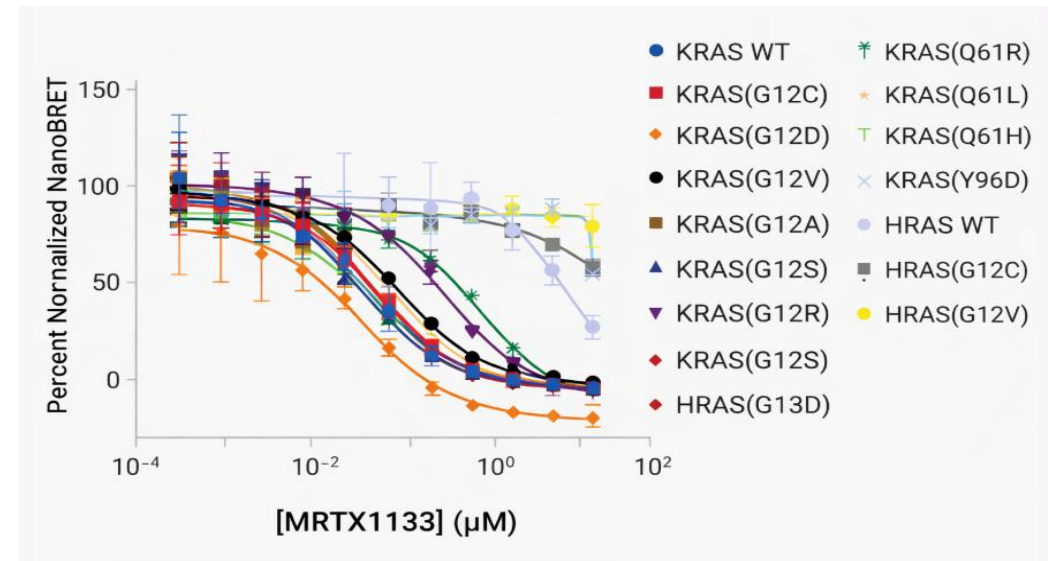
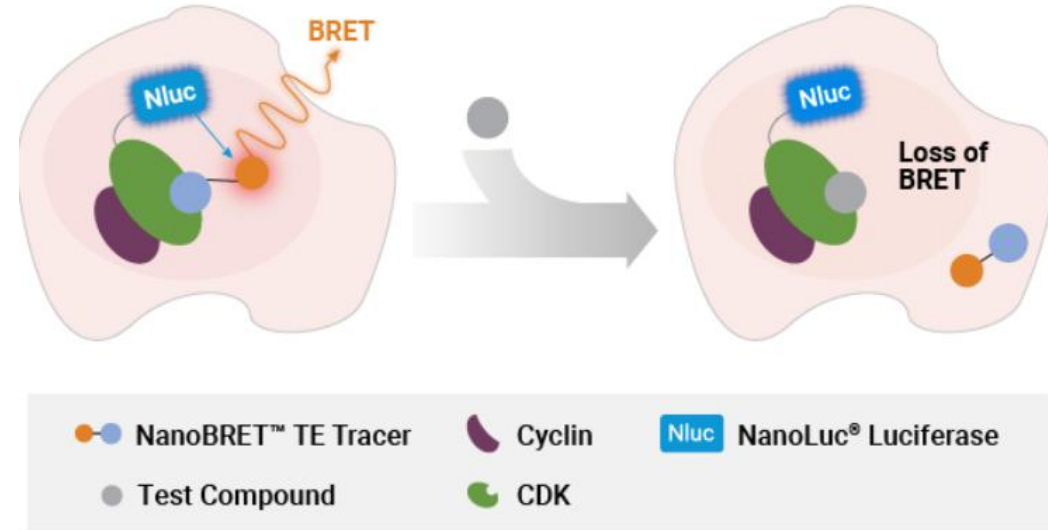


NanoBRET Protein-Protein Interaction Assay

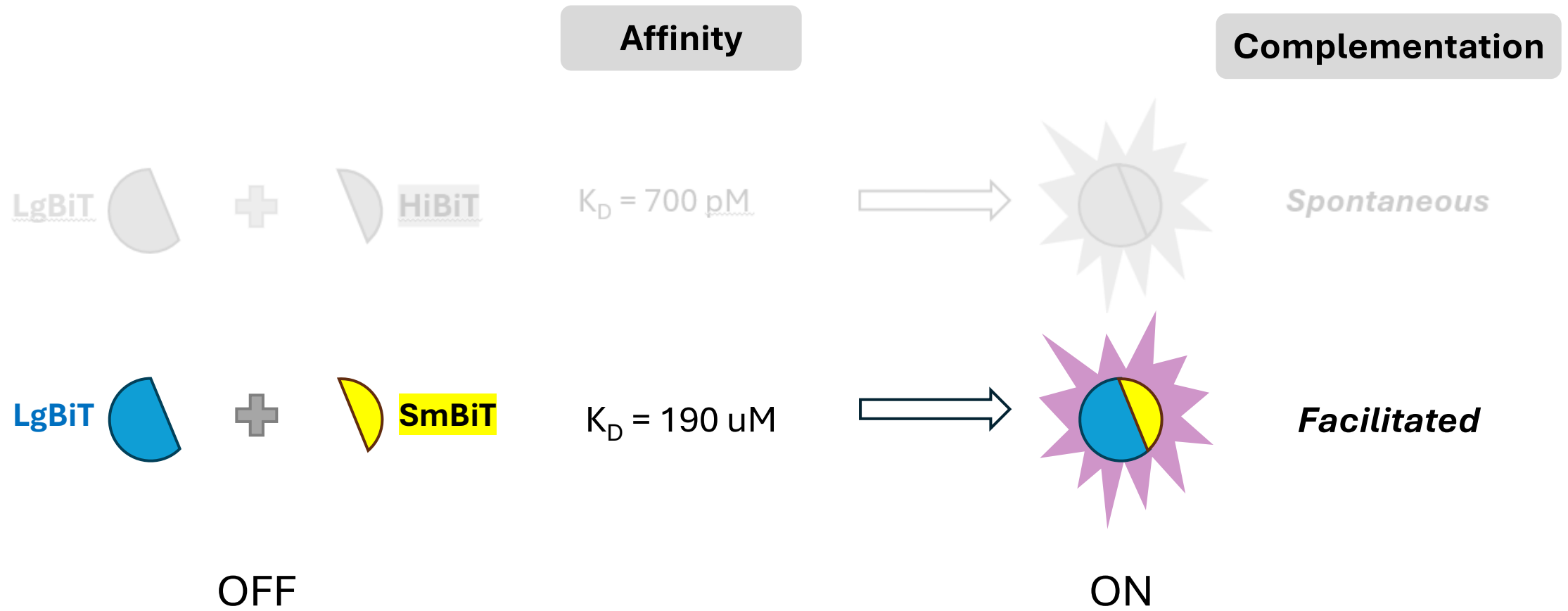
Applications

Protein interaction dynamics

Target Engagement

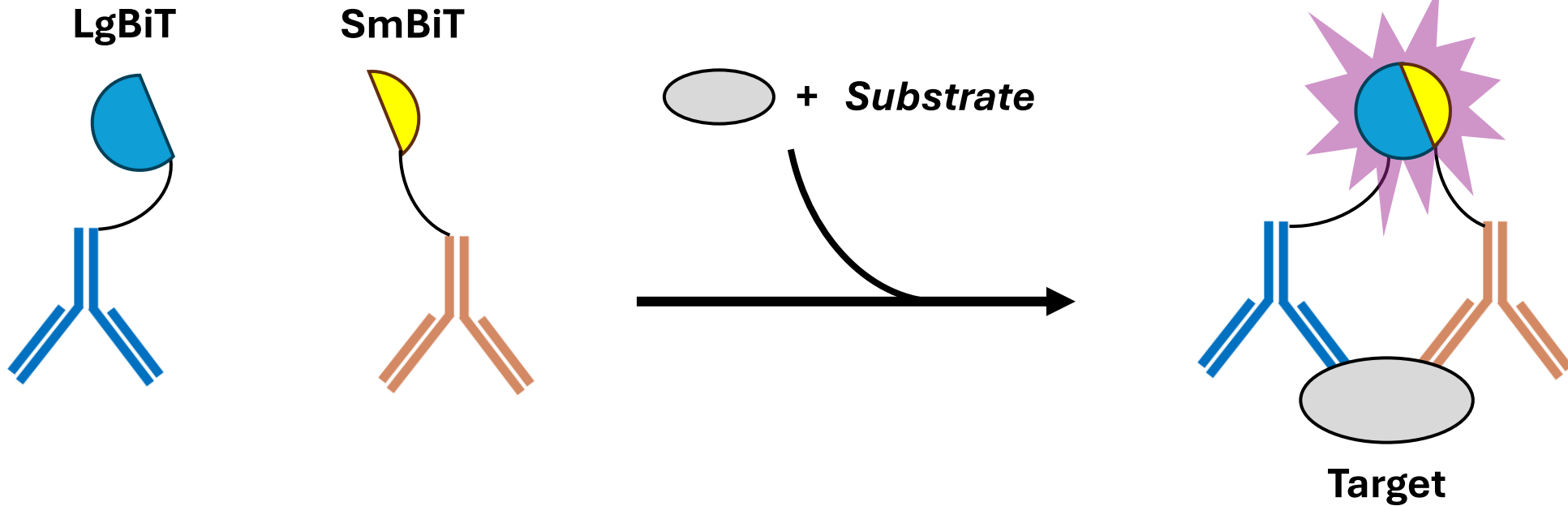


Lumit Immunoassays



Lumit Immunoassays

Lumit – alternative to Western blot and ELISA



Lumit Immunoassays

ELISA workflow



- Heterogeneous
- Multisteps
- Long incubation times

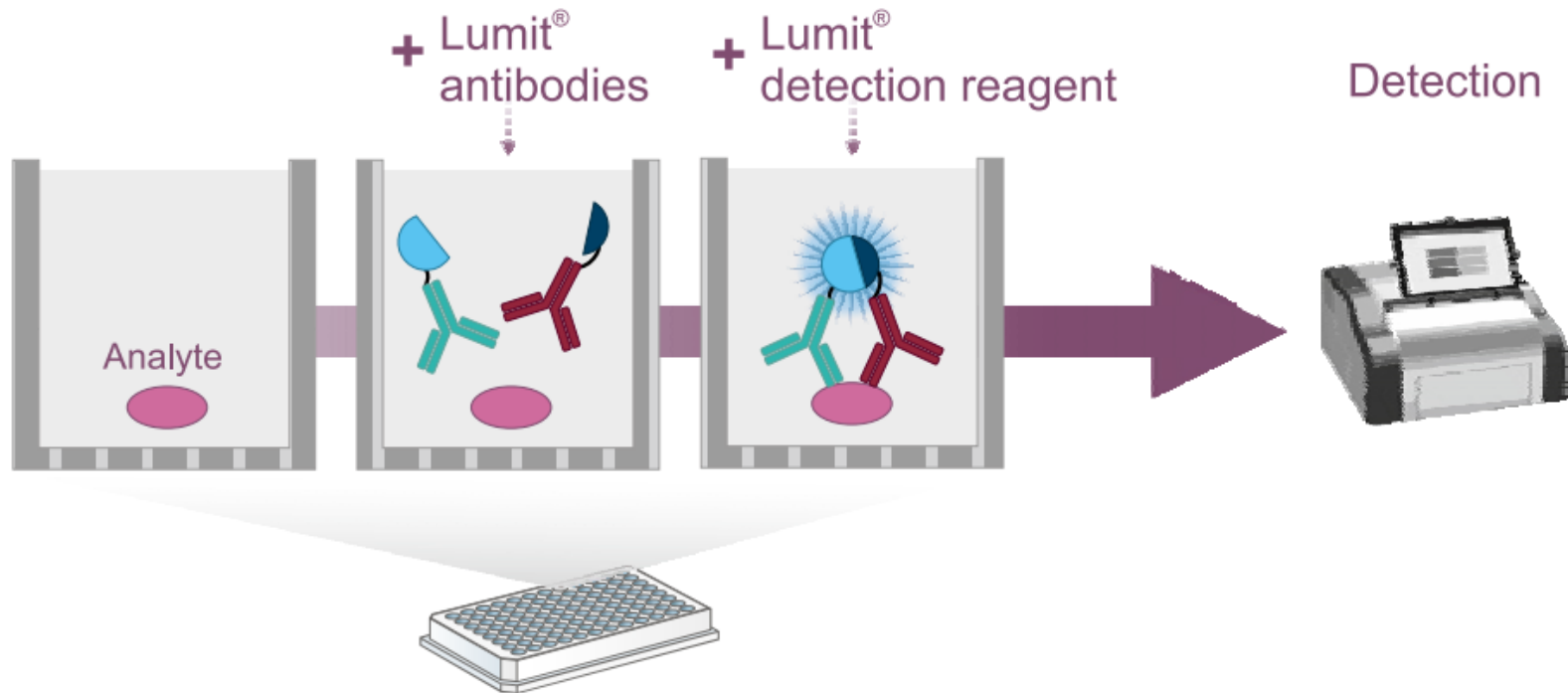
Lumit workflow



- Easy and fast (**30-120 min**)
- Homogeneous
- No immobilization or blocking steps needed

Lumit Immunoassays

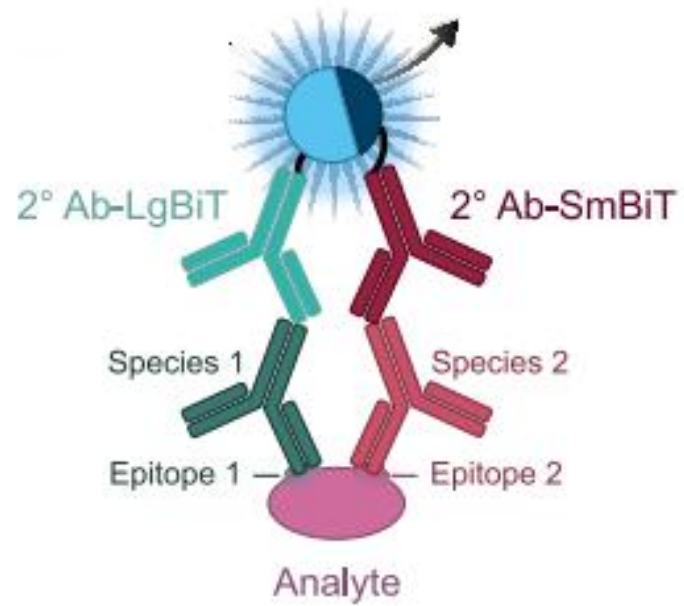
No washing steps



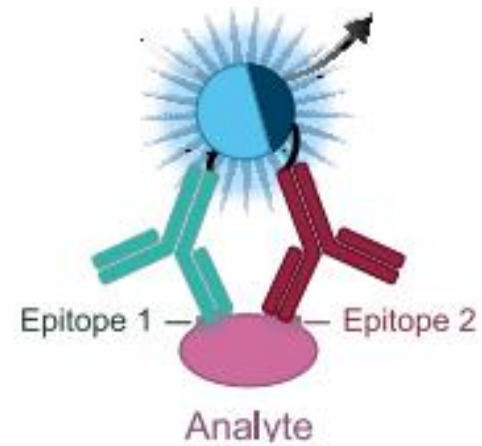
Lumit Immunoassays

Lumit formats

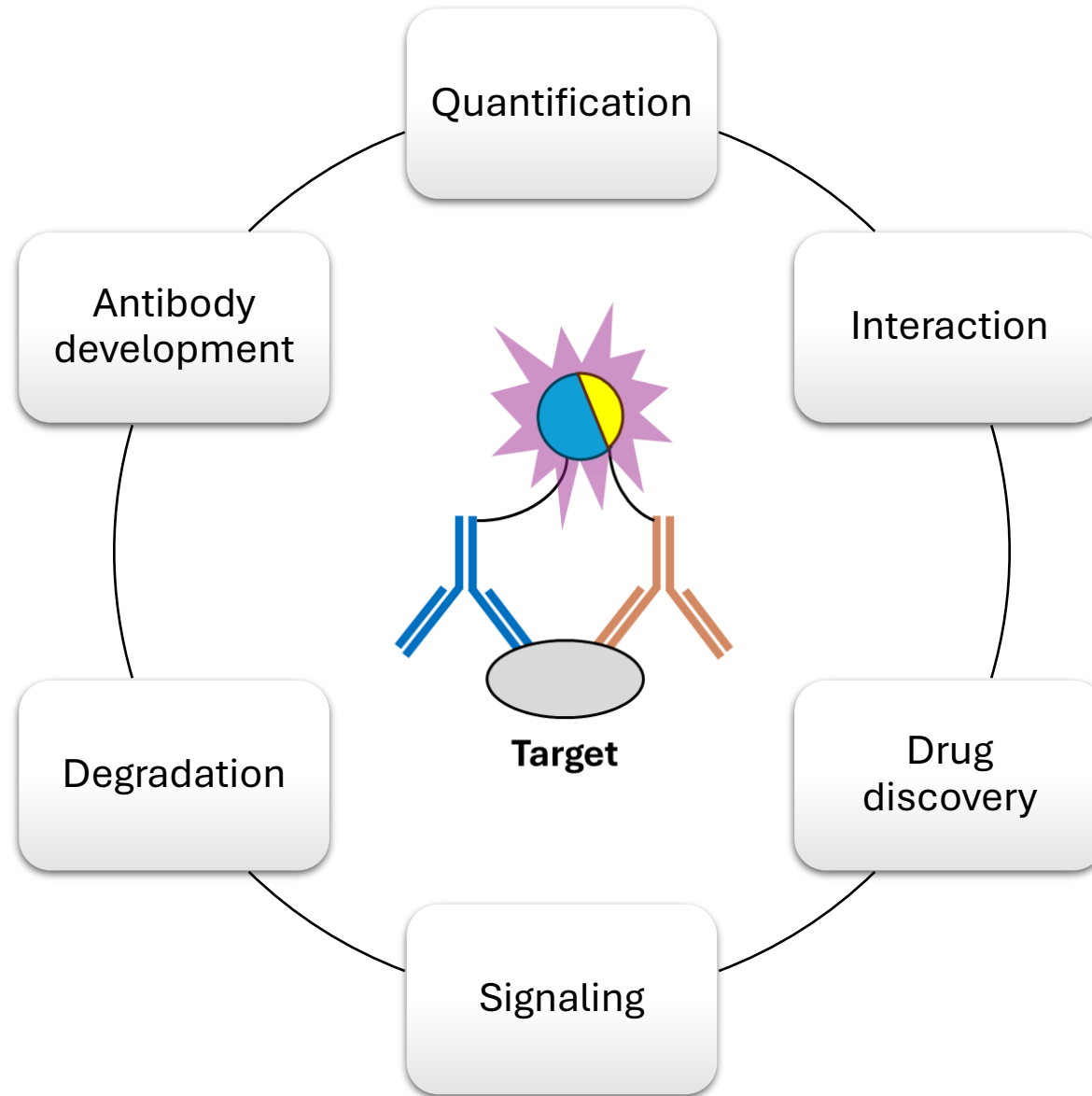
Indirect



Direct



Lumit Immunoassays



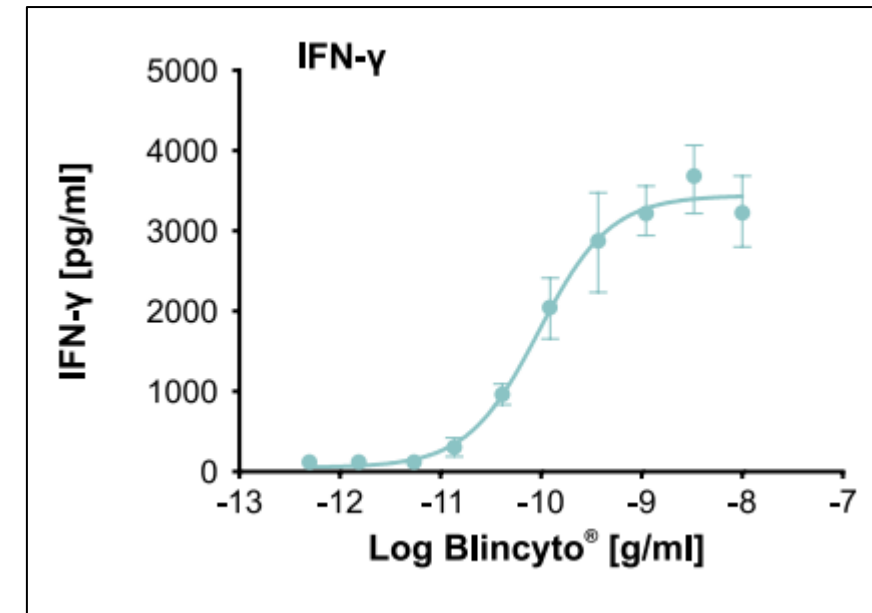
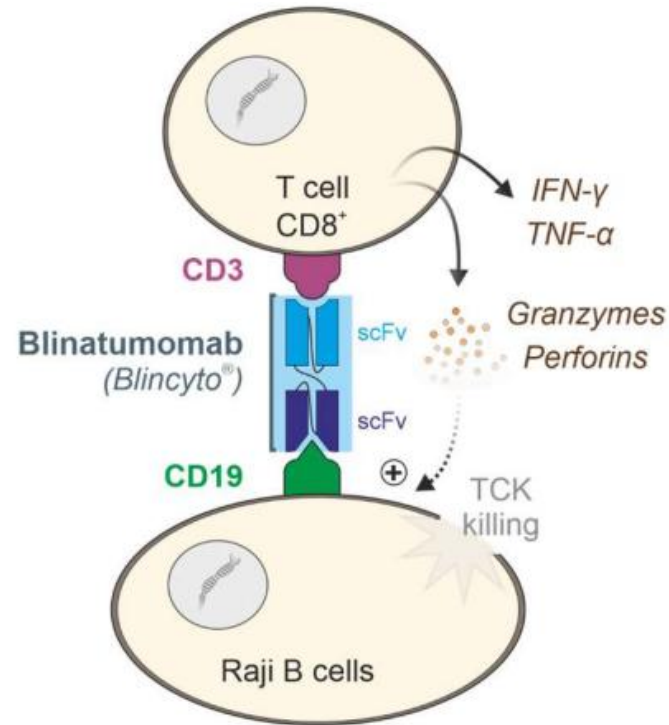
Lumit Immunoassays

Applications

Cytokine detection

Signaling

Protein-protein interaction



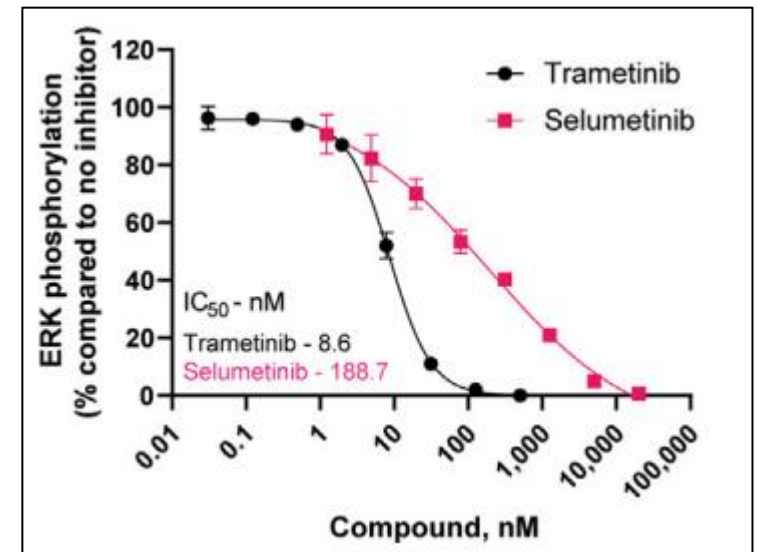
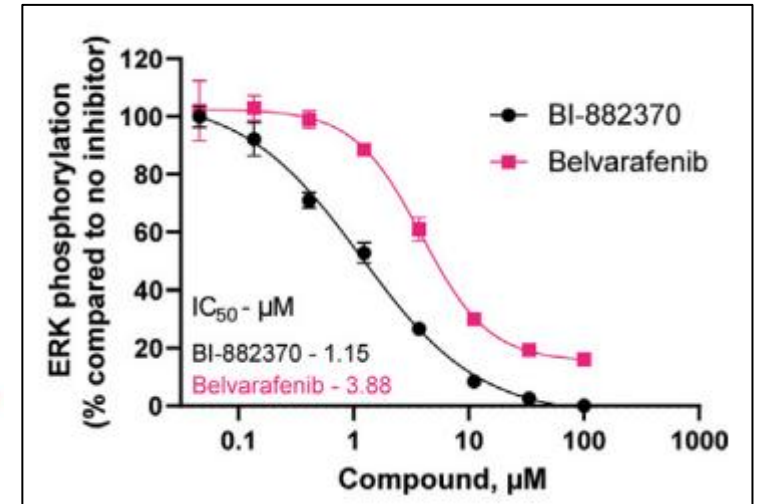
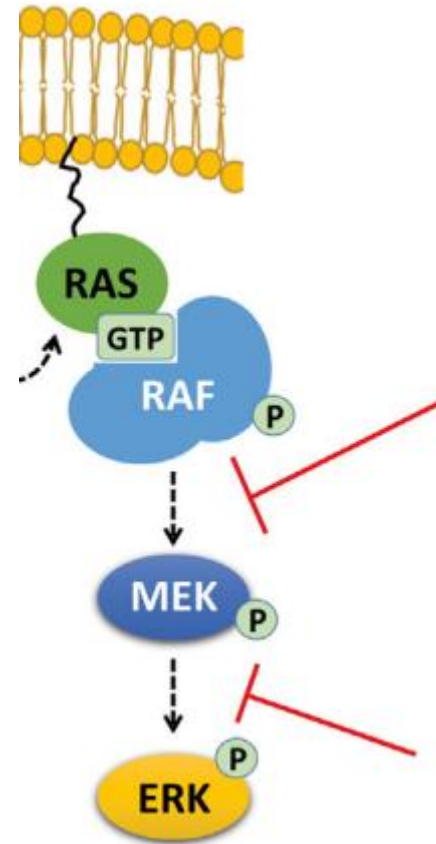
Lumit Immunoassays

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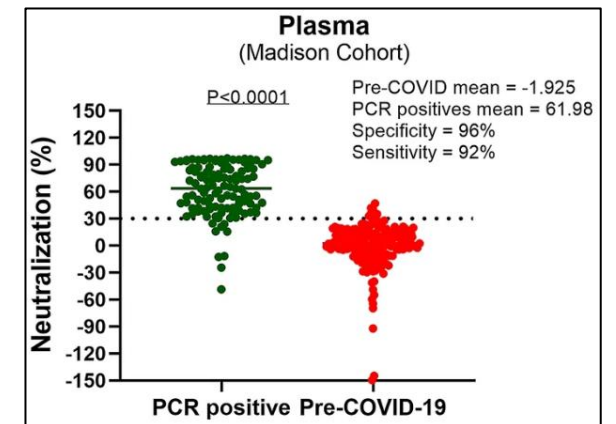
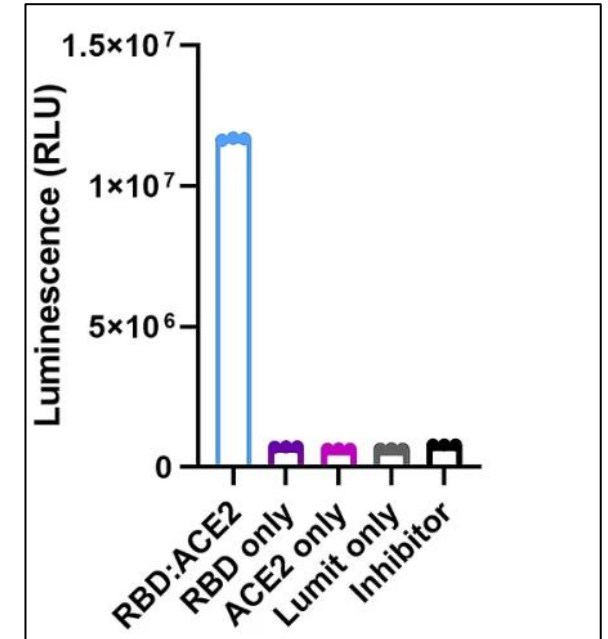
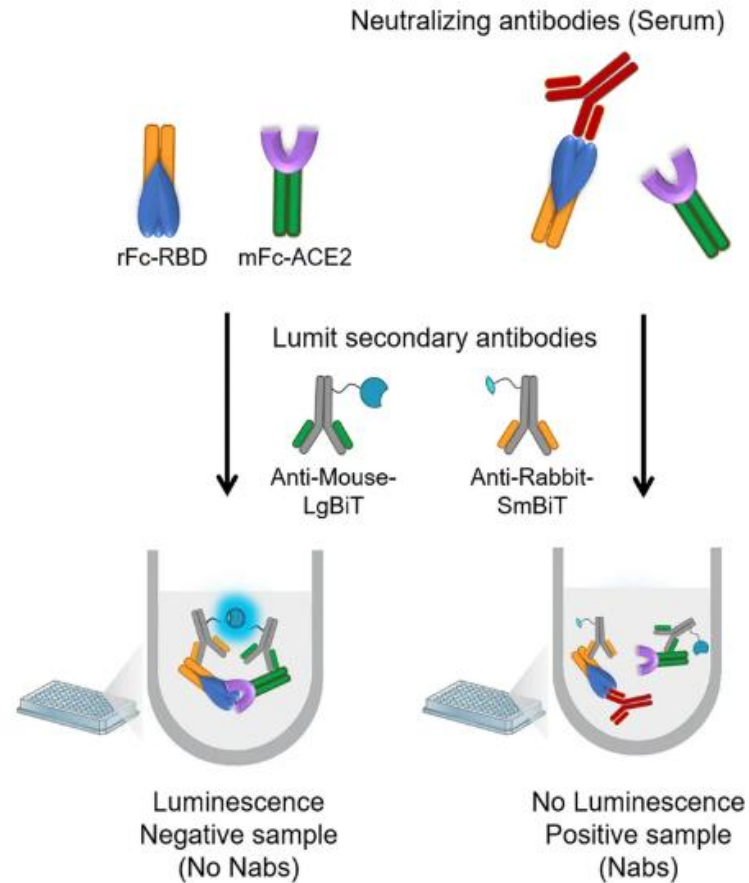
Lumit Immunoassays

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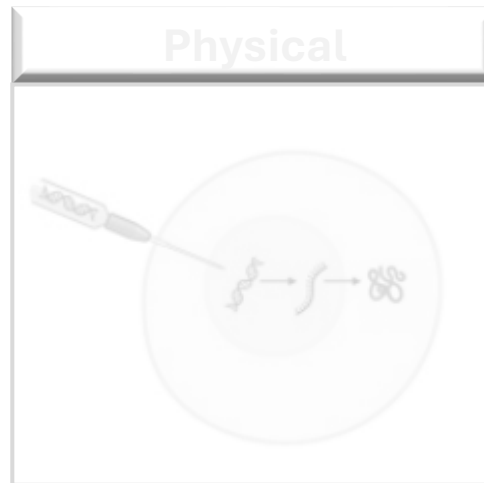
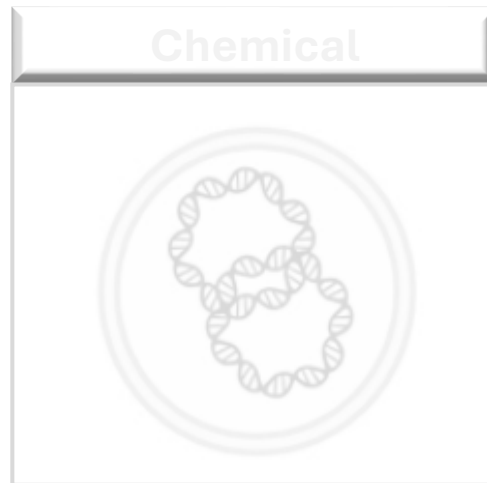
Reporter Vector Delivery

Key consideration

- **Cell Type Specificity** → different reagents for specific cell types
- **Cell Viability** → balance between high efficiency and low cytotoxicity
- **Stable vs Transient** Transfection
- **Safety**

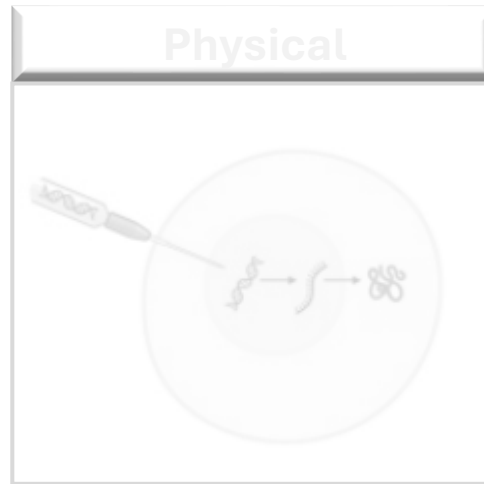
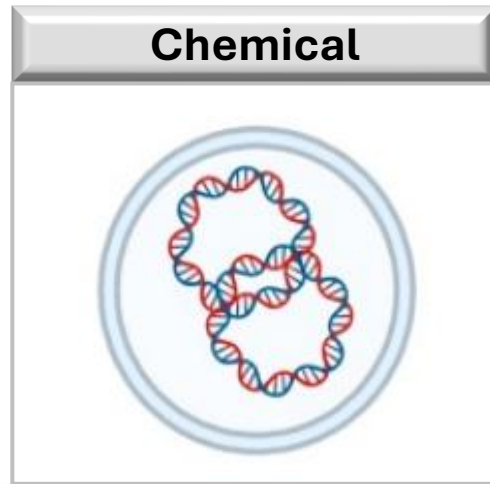
Reporter Vector Delivery

General methods for cell transfection



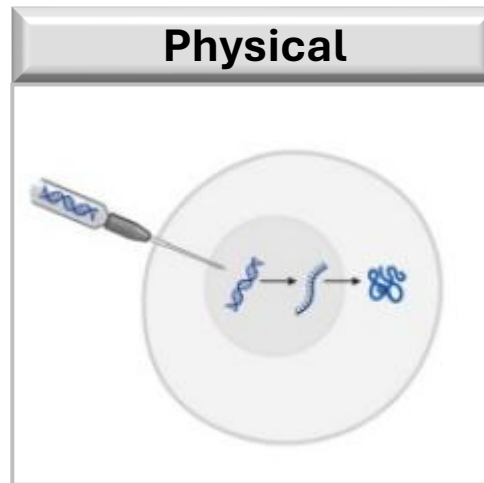
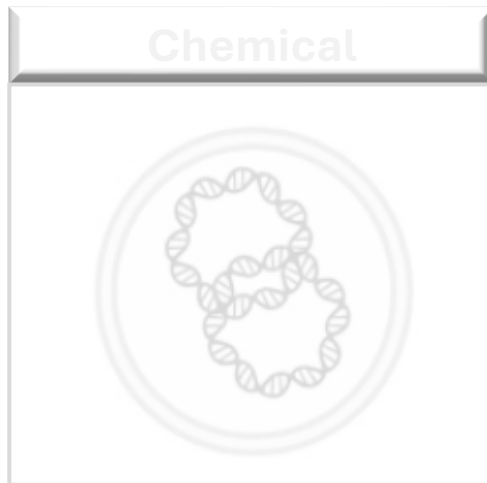
Reporter Vector Delivery

General methods for cell transfection



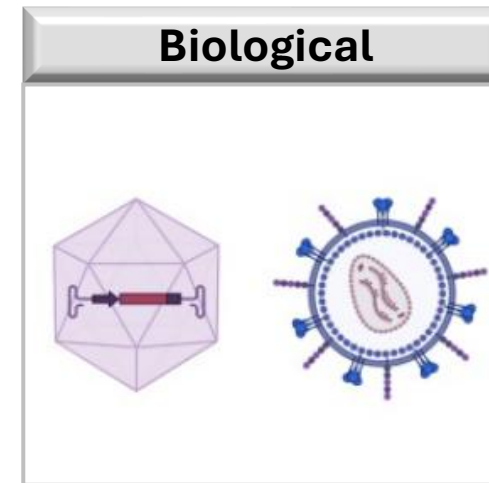
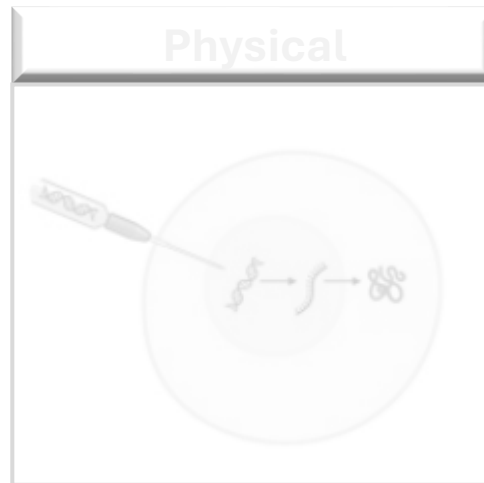
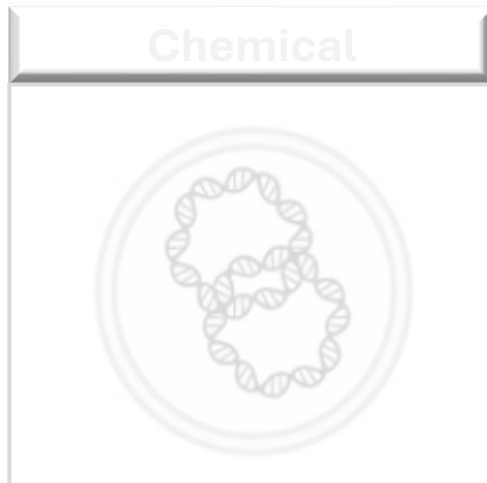
Reporter Vector Delivery

General methods for cell transfection



Reporter Vector Delivery

General methods for cell transfection



Reporter Vector Delivery

Chemical transfection

- Delivery of nucleic acids chemically
- Lipids, polymers
- Simple and widely used method
- Suitable mainly for in vitro studies

Our solution

FuGENE Reagent

- *FuGENE 6*
 - ✓ U20S, COS7 etc.
- *FuGENE HD*
 - ✓ Jurkat, HT-29 etc.
- *FuGENE 4K*
 - ✓ HEK293, HCT116 etc.

Reporter Vector Delivery

Lonza

Physical transfection

- Delivery through membrane disruption
- Includes electroporation and microinjection
- Useful for difficult to transfect cells
- Can achieve higher delivery efficiency



patricia.holubova@eastport.cz

Our solution

4D Nucleofector Lonza



Luminescence Detection

GloMax Plate Readers



GloMax® Navigator

96-well

- ✓ Luminescence



GloMax® Explorer

6-, 12-, 24-, 48-, 96- and 384-well

- ✓ Heating
- ✓ Shaking
- ✓ Luminescence
- ✓ Fluorescence

Available Upgrades

- ✓ Vis Absorbance
- ✓ UV/Vis Absorbance
- ✓ BRET / FRET



GloMax® Discover

6-, 12-, 24-, 48-, 96- and 384-well

- ✓ Heating
- ✓ Shaking
- ✓ Luminescence
- ✓ Fluorescence
- ✓ UV/Vis Absorbance
- ✓ BRET / FRET

Luminescence Detection

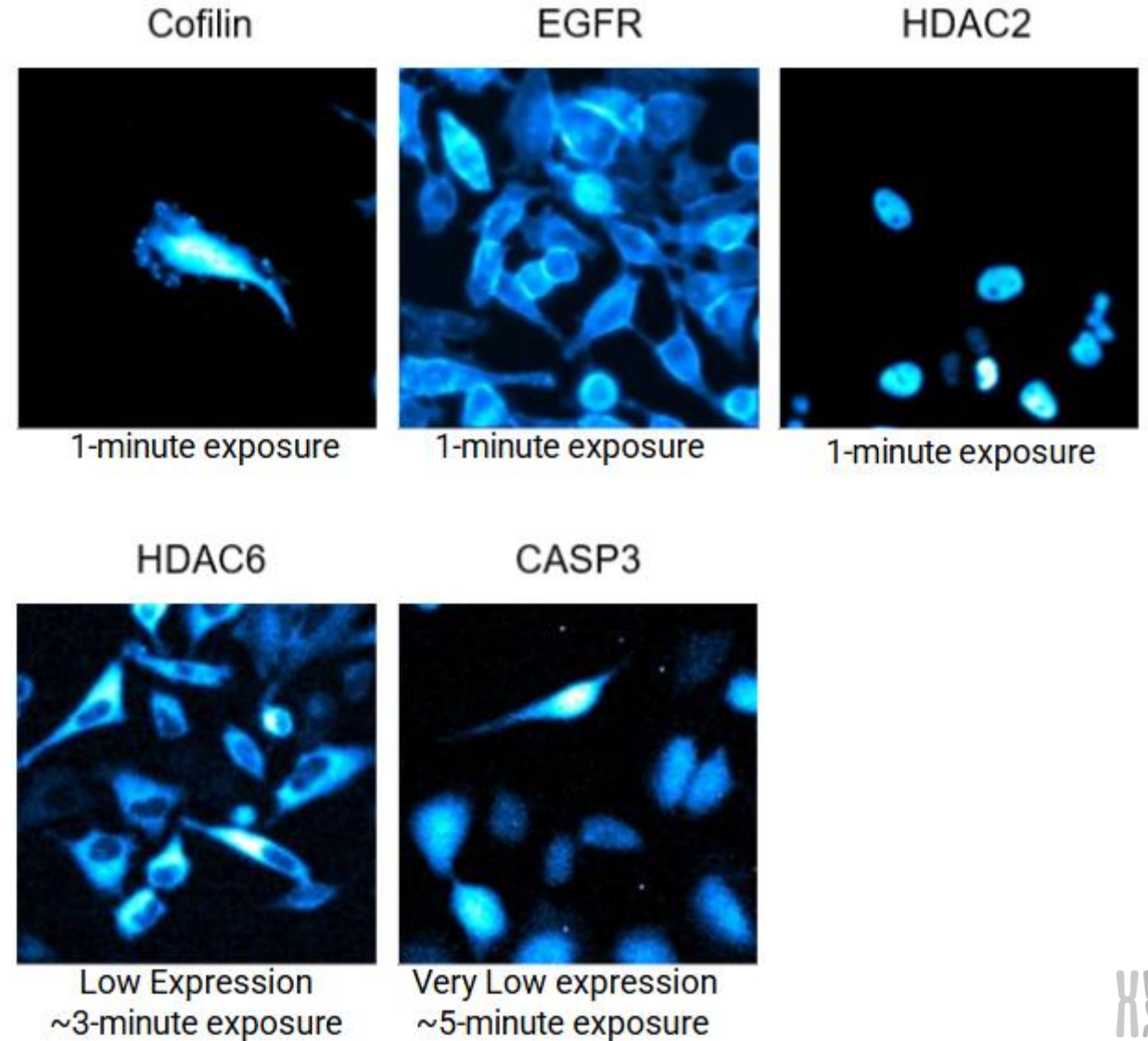
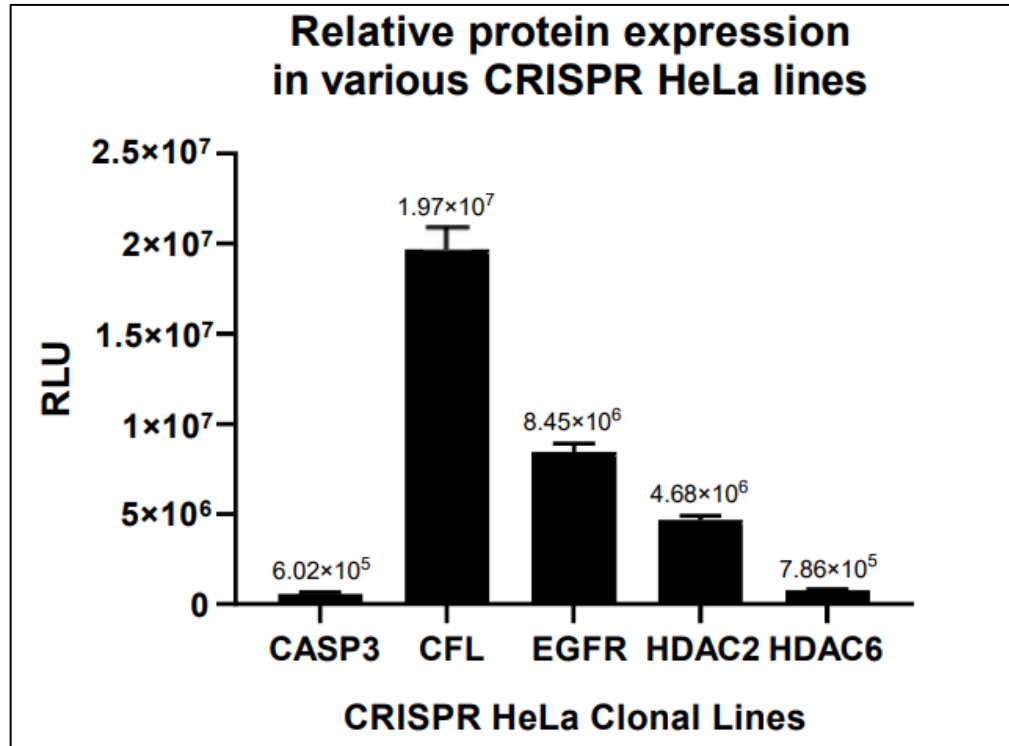
GloMax Galaxy

- Bioluminescence imager system
- Image NanoLuc Technologies – **NanoBiT, HiBiT, NanoBRET**
- Living & fixed cells
- Study Protein Dynamics in real-time
- Luminescence, fluorescence, brightfield



Luminescence Detection

Glomax Galaxy



Luminescence Detection



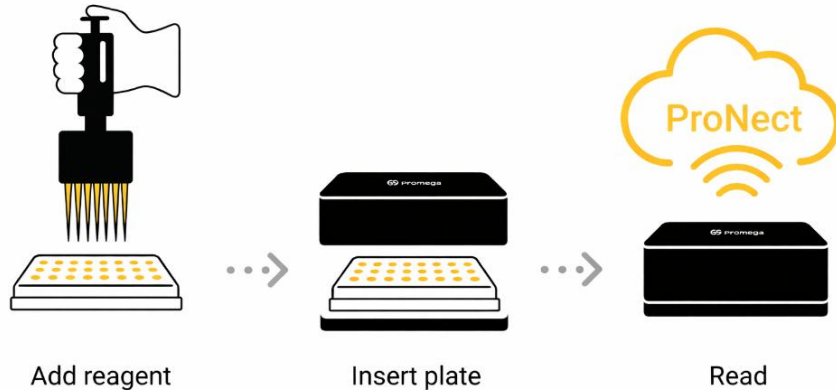
MyGlo Reagent Reader

- Compact and portable **96-well** plate reader
- Works with **wide range** of luminescent assays
- Perfect addition to **CellTiter-Glo Viability Assay**



Easy Workflow

- Measurement setup and analysis via **ProNect App**
- Results in less than 30 s (full 96-well, 1s integration time)
- Cloud accesible via any device (laptop, PC, smartphone)



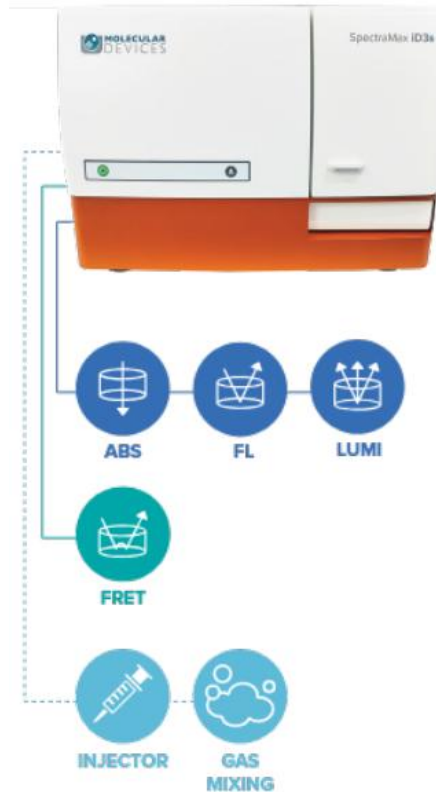
Luminescence Detection

SpectraMax Plate Readers

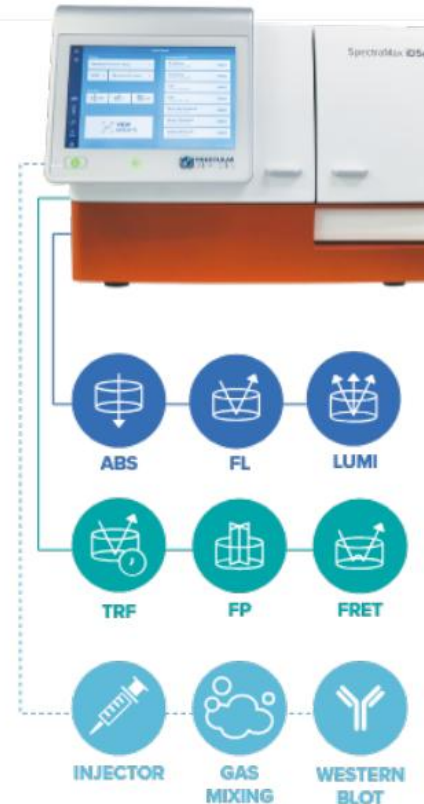


vojtech.andrle@eastport.cz

SpectraMax® iD3s



SpectraMax® iD5e



SpectraMax® i3x



Bioluminescence in Cell Cultures

Use luminescence method to assess

Viability

Cytotoxicity

Apoptosis

Metabolism

Oxidative Stress

Signalling

Bioluminescence in Cell Cultures

Use luminescence method to assess

Viability

CellTiter-Glo, RealTime-Glo MT

Cytotoxicity

Apoptosis

Metabolism

Oxidative Stress

Signalling

Bioluminescence in Cell Cultures

Use luminescence method to assess

Viability

Cytotoxicity

Apoptosis

Metabolism

Oxidative Stress

Signalling

LDH-Glo, CytoTox-Glo

Bioluminescence in Cell Cultures

Use luminescence method to assess

Viability

Cytotoxicity

Apoptosis

Metabolism

Oxidative Stress

Signalling

RealTime-Glo™ Annexin V Apoptosis, Caspase-Glo® 3/7 Assay

Bioluminescence in Cell Cultures

Use luminescence method to assess

Viability

Cytotoxicity

Apoptosis

Metabolism

Oxidative Stress

Signalling

NAD⁺ /NADH-Glo™ Assay, Glucose-Glo™ Assay

Bioluminescence in Cell Cultures

Use luminescence method to assess

Viability

Cytotoxicity

Apoptosis

Metabolism

Oxidative Stress

Signalling

GSH/GSSG-Glo™ Assay, ROS-Glo™ H2O2 Assay

Bioluminescence in Cell Cultures

Use luminescence method to assess

Viability

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Apoptosis

Metabolism

Oxidative Stress

Signalling

cAMP-Glo™ Max Assay, ADP-Glo™ Kinase Assay

Bioluminescence in Cell Cultures

Use luminescence method to assess

Viability

Cytotoxicity

Apoptosis

Metabolism

Oxidative Stress

Signalling

„How Healthy Are Your Cells?“

April 21-23

Prague, Brno, Olomouc

Registration soon

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LinkedIn



Thank you for your attention!

Technologies We Represent

