Supporting information

Bioluminescence goes dark: boosting the performance of bioluminescent sensor proteins using complementation inhibitors

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Supplementary Materials and Methods

Materials

Peptides with acetylated N-terminus and amidated C-terminus were purchased from GenScript (SB86: VSGWRLFKKIS, ≥75% purity; DarkBiT 101: SVTGYALFEKESG, ≥75% purity). Terminal S and G were added to the DarkBiT peptide to account for linker amino acids in fusion proteins. NanoGlo substrate was purchased from Promega. Unless stated otherwise, chemicals were purchased from Sigma.

Cloning

DNA oligonucleotides were purchased from IDT. To obtain dark-LUMABS variants, existing pET28a(+) NB-LUMABS were mutated via Ligase Cycling reaction (1, 2) or annealed oligo cloning using Spe1 and Bsa1 restriction sites present in the NB-LUMABS plasmids. Successful cloning was confirmed via Sanger Sequencing (BaseClear).

Protein purification

For purification of dark-LUMABS variants and calibrator luciferase, E. coli BL21 (DE3) cells were transformed with corresponding pET28a(+) plasmids. Cells were grown in LB medium supplemented with 50 µg/ml kanamycin at 37 °C at 180 rpm in a shaking incubator. Large cultures (750 ml) in 2 l baffled flasks were inoculated with corresponding overnight cultures and induced with IPTG at OD₆₀₀ 0.6-0.8. Proteins were expressed overnight at 18 °C. Harvested cells were lysed with BugBuster reagent (Novagen) supplemented with Benzonase (Merck), and proteins were purified with Ni-NTA chromatography followed by Strep-Tactin XT (iba) using gravity flow columns. Protein purity was confirmed by reducing SDS-PAGE and concentrations calculated using A₂₈₀ nanodrop measurements and the corresponding extinction coefficients (based on protein sequence). Proteins in Strep-Tactin XT elution buffer (150 mM NaCl, 100 mM Tris-Cl pH 8, 1 mM EDTA, 50 mM D-biotin) were aliquoted, flash frozen in liquid N₂ and stored at -70 °C. NB-LUMABS-HIV6 was expressed and prepared as described in (3), 3E2H.37-LUMABS was expressed and purified as described in (4).

Thermodynamic equilibrium scheme

The equilibrium scheme that was devised in the main text Figure 5A is adapted from previous works on the LUMABS and RAPPID platforms (5, 6). The equilibrium constants are defined as follows: K₁, intramolecular release of the DarkBiT from LgBiT; K₂, monovalent antibody binding based on the 4 possible binding combinations and the monovalent affinity of the epitope K_DAb; K₃, intramolecular binding of SmBiT to LgBiT; K₄, formation of the bivalent complex. The equilibrium constants take effective concentrations (C_eff) into account that result from intramolecular linkage (for DarkBiT and SmBiT) or intermolecular recruitment (for the bivalent complex formation). The overall apparent dissociation constant K_D,app is the product of the inversed equilibrium constants K₁ to K₄ (5) and can be written as

\[ K_{D,app} = \frac{1}{2} \times \left( \frac{K_D^{Ab}}{K_D^{DarkBiT}} \right)^2 \times \frac{C_{eff}^{DarkBiT}}{C_{eff}^{Ab}} \times \frac{K_D^{SmBiT}}{C_{eff}^{SmBiT}} \]

With all other parameters known, C_eff^{Ab} for each sensor could be calculated from this equation.
**Calculation of effective concentrations**

The term effective concentration $C_{\text{eff}}$ connects an intramolecular affinity to an intermolecular affinity (7) and is defined as

$$C_{\text{eff}} = \frac{K_D^{\text{inter}}}{K_D^{\text{intra}}}$$

$C_{\text{effDarkBiT}}$ was derived from competition experiments as 1.73 mM (see main text). As the SmBiT is connected to the LgBiT with a well-understood, flexible GGS linker, the $C_{\text{effSmBiT}}$ was determined with the effective concentration calculator app (8), considering a persistence length of 3.7 Å (9) and distance of 30 Å between the N-terminus of LgBiT and the binding site of SmBiT measured using Chimera and the PDB file 5IBO. The app gave a $C_{\text{effSmBiT}}$ of 4.66 mM.

**Thermodynamic equilibrium simulations using the expanded model**

To understand how the hook effect is affected by changes in the sensors intrinsic properties, simulations were performed with a general python model framework developed by Geertjens et al. (10) in the Spyder environment. The framework is freely available under DOI: 10.5281/zenodo.5531622. The mass balance equations used to build the model derive from the expanded thermodynamic equilibrium scheme shown in Figure S3, and were written in python as:

```
Sensor_o = Sensor_d; K_DD_BiT/M_effic_BiT
Sensor_o = Sensor_a; K_D_sm_BiT/M_effic_sm_BiT
Sensor_o + A1 = A1_Sensor_o; Kab/4
Sensor_d + A1 = A1_Sensor_d; Kab/4
Sensor_a + A1 = A1_Sensor_a; Kab/4
A1_Sensor_o + A1 = A1_A1_Sensor_o; Kab/2
A1_Sensor_a + A1 = A1_A1_Sensor_a; Kab/2
A1_Sensor_d + A1 = A1_A1_Sensor_d; Kab/2
A1_Sensor_o = A1_Sensor_oo; (2*Kab)/M_effic_ab
A1_Sensor_a = A1_Sensor_aa; (2*Kab)/M_effic_ab
```

Labeled specie:

```
data_mode: custom
custom input: (A1_Sensor_ao+A1_A1_Sensor_o+A1_Sensor_ao+Sensor_o)*constant
```

o/a/d suffixes relate to LgBiT in an open state, active state (bound to SmBiT) or dark state (bound to DarkBiT). The hook effect is represented by the equations in green font color. To simulate changes, all involved terms ($K_D$s, $C_{\text{eff}}$s) were kept constant except the term of interest.
Titrations

Unless specified otherwise, all titrations were performed in 1xPBS (10 mM phosphate buffer, 2.7 mM KCl and 137 mM NaCl, pH 7.4) supplemented with 1 mg/ml BSA to avoid unspecific adsorption of proteins to surfaces. As additive, BSA worked better and gave higher signal intensities than Tween20 in all experiments except titrations with SmBiT peptides. Assay components were diluted in the same buffer before measurements. Unless stated otherwise, the sensor proteins were incubated with different concentrations of target antibody for 16 h at 4 °C to allow equilibration, before NanoGlo substrate (furimazine) was added from a freshly prepared 10x stock directly before the measurement to obtain the final volume and concentrations. Unless stated otherwise, the final NanoGlo dilution during the measurements was 1:2000. Binding assays were performed in white 364-well plates (flat bottom, Nunc, ThermoFisher) and a total volume of 20 µl with n=3 technical replicates. Unless stated otherwise, bioluminescence at 458 nm was determined from bioluminescence spectra (398-653 nm, integration time 100 ms) measured at 20 °C in a TECAN Spark 10M. Resulting binding curves were fitted to the standard binding model \(\frac{P \cdot x}{K_D + x} + c\). Dynamic range (DR) was calculated via

\[
DR = \frac{\text{Max} - \text{Min}}{\text{Min}}
\]

With Max being the signal at saturated sensor (mean of n=3) and Min the signal in absence of the target (mean of n=3). DR uncertainties were propagated from the standard deviation of Max and Min (s(Max) and s(Min), respectively) using (11)

\[
\frac{1}{\text{Min}^2} \sqrt{(\text{Max} \cdot s(\text{Min}))^2 + (\text{Min} \cdot s(\text{Max}))^2}
\]

DarkBiT101 affinity

For data evaluation of competitive binding based on Motulsky and Neubig (12), DarkBiT101 concentrations were converted to logarithmic values and the bioluminescence signals were fitted to

\[
y = \text{background} + \frac{\text{maximum signal} - \text{background}}{1 + 10^{x - \log IC_{50}}}
\]

The background was constrained to positive values. Fitting was performed in Origin 2020. The \(K_i\) was then determined from

\[
K_i = \frac{IC_{50}}{[SmBiT86]} \frac{1 + \frac{1}{K_D}}{K_D}
\]

with the concentration of active SmBiT86 [SmBiT86] and its \(K_D\) for LgBiT binding that was determined in a parallel binding experiment (main text Figure 2A).
**Fitting of single exponential binding processes**

To determine the observed rate constants of the association reactions, $k_{\text{obs}}$, in kinetic experiments, the traces were fit to a single exponential binding process using (13)

$$Y = -Y_{eq} \cdot e^{-k_{\text{obs}} \cdot t} + Y_{eq}$$

Where $Y$ is the observed blue-to-green ratio, $Y_{eq}$ is the final equilibrium value of the blue-to-green ratio, $k_{\text{obs}}$ is the observed rate constant of the respective association reaction, and $t$ is the time in minutes.
**Supplementary Tables and Figures**

Table S1 Properties of dark-LUMABS-HIV variants tested in this study. The titration curves in Figure 5B (main text) were fitted until the start of the hook effect with a 1:1 binding model to obtain \( K_{D,\text{app}} \) values presented here.

| #  | Affinity level | \( K_{D,\text{app}} \)     | Dynamic Range (fold change) |
|----|----------------|----------------------------|-----------------------------|
| 1  | SmBiT          | 2.5 µM                     | 58 ± 14 pM                  | 4 ± 0                       |
|    | DarkBiT        | 2.5 µM                     | 31 ± 5 pM                   | 10 ± 1                      |
| 2  | SmBiT          | 2.5 µM                     | 21 ± 4 pM                   | 48 ± 8                      |
| 3  | SmBiT          | 190 µM                     | 94 ± 21 pM                  | 4 ± 1                       |
| 4  | DarkBiT        | 0.18 µM                    |                             |                            |
Figure S1 BSA affects the intensity of the luminescent signal and decreases the apparent affinity in titration experiments of SmBiT peptide binding to LgBiT. Further experimental conditions: 1xPBS plus indicated supplement, 15 pM LgBiT, 1:1000 diluted NanoGlo, 1h incubation at room temperature (22 °C).

Figure S2 Time-course, ratiometric experiment of the SmBiT86 peptide binding to the intramolecularly blocked LgBiT-DarkBiT101 fusion protein. A) Legend indicates the final concentrations of SmBiT86, dashed line indicates addition of SmBiT86 (20 µl) to preincubated sensor, calibrator and substrate (20 µl). The kinetic traces after SmBiT86 addition were fitted to $Y = -Y_{eq} \cdot e^{(-k_{obs} \cdot t)} + Y_{eq}$ and $k_{obs}$ values were plotted as a function of [SmBiT86] (B). Further experimental condition: 1xPBS +0.02% Tween20, final concentrations: 120 pM of LgBiT-DarkBiT101, 10 pM calibrator (GeNL), 1:1000 diluted NanoGlo, 22 °C, total volume after SmBiT86 addition: 40 µl.
Figure S3 Overview of Limit of Detection (LoD) determination. The shown graphs are zoom-ins of Fig 6 A, C and E in the main text. By applying the 3σ rule (mean of blank plus 3 times its standard deviation), the limit of detection for each antibody sensor was calculated and the corresponding bioluminescent signal is shown as red, dotted line in the figure. The reported LoD is then the first value with an intensity above this line. A) anti-HIV1-p17 dark-LUMABS, B) CTX-dark-LUMABS, C) 101F-dark-LUMABS. The values are listed in table 1 in the main text. The experimental conditions, as mentioned in Figure 6 in the main text, are as follows: A) 20 pM dark-LUMABS-HIV #3, B) 200 pM dark-LUMABS-CTX; C) 50 pM dark-LUMABS-101F. All experiments were performed in 1x PBS +1mg/ml BSA and a final NanoGlo dilution of 1:1000. The sensor proteins were incubated with different concentrations of target antibody for 16 h at 4 °C to allow equilibration, before NanoGlo substrate (furimazine) was added from a freshly prepared 10x stock directly before the measurement to obtain the final volume (20 µl) and concentrations. Error bars represent standard deviation based on 3 technical replicates.
Figure S4 Signal intensity comparison between full-length NanoLuc and dark-LUMABS-HIV #3 in presence of target antibody. The bioluminescent signal at 458 nm of the activated split luciferase (NanoBiT) in the dark-LUMABS switch is 4.4 times lower compared to the signal of full-length NanoLuc at the same protein concentration (200 pM). Experimental conditions: 1x PBS +1 mg/ml BSA, 1 nM anti-HIV1-p17, 200 pM full-length NanoLuc or dark-LUMABS-HIV #3, 1:1000 NanoGlo substrate. 1 h incubation time at room temperature (22 °C) before addition of NanoGlo and subsequent measurement at 22 °C.

\[
\begin{align*}
K_1 &= K_0^\text{DarkBiT} / C_{\text{eff}}^\text{DarkBiT} \\
K_2 &= 4 / K_0^\text{Ab} \\
K_3 &= C_{\text{eff}}^\text{SmBiT} / K_0^\text{SmBiT} \\
K_4 &= C_{\text{eff}}^\text{Ab} / 2K_0^\text{Ab} \\
K_{\text{hook}} &= 2 / K_0^\text{Ab}
\end{align*}
\]

Figure S5 Expanded thermodynamic equilibrium model including the hook effect (species in dashed-line rectangles). The hook effect in black dashed lines has the strongest effect. \(K_{\text{hook}}\) considers that a second antibody can bind in 2 ways to the sensor (hook effect) unless both epitope regions are already bound to one antibody.
Figure S6 Simulations using the expanded thermodynamic equilibrium model confirm stronger hook effect for higher DarkBiT affinities (lower $K_{D_{\text{DarkBiT}}}$) in Panel A. Sensor concentration was set at 20 pM. All other parameters (in M) were left as follows: $C_{\text{effDarkBiT}}$:0.00173, $K_{D_{\text{Ab}}}$:4.2E-8, $C_{\text{effSmBiT}}$:4.66E-3, $C_{\text{effAb}}$:1.14E-3.

1 h incubation 22 °C $- K_{D_{\text{app}}} = 1.8$ nM

16 h incubation 4 °C $- K_{D_{\text{app}}} = 52$ pM

Figure S7 Longer incubation times are necessary to reach binding equilibrium and determine $K_{D_{\text{app}}}$. Experimental conditions: 1xPBS +1mg/ml BSA, 20 pM dark-LUMABS-HIV #1, 1:2000 diluted NanoGlo, temperature and incubation time as written above the graphs.
Figure S8 Kinetic measurements and $k_{\text{obs}}$ plots of dark-LUMABS-HIV #3 (A&B), dark-LUMABS-CTX (C&D), and dark-LUMABS-101F (E&F) to investigate if the sensors activation is dependent on the target concentration. The time on the x-axis in A, C, and E refers to the time after target antibody addition. For the 101F measurement, the fitting was constrained to 21 min of the kinetic traces to achieve fits with $R^2 > 0.95$, as the blue/green ratio started decreasing afterwards. Experimental conditions: 1xPBS + 1mg/ml BSA, 1:1000 NanoGlo substrate, 22 °C. n=3 technical replicates per concentration. A) 0.2 nM of dark-LUMABS-HIV #3 sensor protein, 0.8 pM of calibrator (GeNL); C) 10 nM of dark-LUMABS-CTX sensor protein, 5 pM calibrator (GeNL); E) 1 nM of dark-LUMABS-101F sensor protein, 0.8 pM calibrator (GeNL). The kinetic traces were fitted to a single exponential binding process using $Y = Y_{eq} \cdot e^{(-k_{\text{obs}} \cdot t)} + Y_{eq}$ according to (13). The $k_{\text{obs}}$ values of the fitting results were plotted as a function of antibody concentrations to check for concentration dependency.
Figure S9 Kinetic behavior of anti-HIV1-p17 sensors. Legends indicate the concentration of anti-HIV1-p17 used, dashed line indicates addition of antibody to preincubated sensor, calibrator (in A) and substrate. A) dark-LUMABS-HIV #3 at 400 pM sensor and 2 pM calibrator luciferase. Zoom-in shows increase in blue/green ratio against background for 10 pM of target, detectable in a microwell platereader. Dynamic range between 0 and 1 nM target (t = 50 min): 1373 %. B) NB-LUMABS-HIV6 at 400 pM sensor. Zoom-in shows increase in blue/red ratio against background for lower target concentrations. Dynamic range between 0 and 1 nM target (t = 50 min): 141 %. Further experimental conditions: 1xPBS +1mg/ml BSA with 1:1000 NanoGlo substrate, 22 °C. n=3 technical replicates per concentration.
Figure S10 Kinetic behavior of 101F sensors. Legends indicates the concentration of target antibody used, dashed line indicates timepoint of antibody addition. A) dark-LUMABS-101F. 10 nM sensor and 2 pM calibrator were used in a final assay volume of 20 µl. Dynamic range between 0 and 2 nM target at t=50 min: 163%. B) 3E2H.37-LUMABS. Sensor concentration 400 pM, calibrator concentration 1 pM. Dynamic range between 0 and 2 nM target at t=50 min: 25 %. Further experimental conditions: 1xPBS + 1mg/ml BSA, 1:1000 NanoGlo substrate, 22 °C. n=3 technical replicates per concentration.

Figure S11 Simulations using the thermodynamic equilibrium model show that the fraction of active bivalent complex is strongly dependent on the monovalent affinity of the epitopes, $K_{DAb}$. For lower $K_{DAb}$, more antibody is required to achieve an active, bivalent complex. For lower $C_{effAb}$, the equilibrium shifts to the dark, monovalent complex, which in turn leads to a stronger hook effect. Sensor concentration was set at 20 pM. All other parameters (in M) were left as follows: $C_{effDarkBiT}$=0.00173, $K_{DDarkBiT}$=2.6E-6, $K_{DSmBiT}$=1.9E-4, $C_{effSmBiT}$=4.66E-3.
Peptide & Protein sequences
In the following, SmBiTs are highlighted in yellow, DarkBiTs are highlighted in purple, epitopes are highlighted with red font, LgBiT or NanoLuc in light blue, mNeongreen in green.

DarkBiT101 – N-terminal acetylated, C-terminal amidated. Additional small amino acids were added to resemble the adjacent linker amino-acids in the switch construct

SVTGyalFKEKESG

SmBiT86 – N-terminal acetylated, C-terminal amidated

VSGWRLFKKIS

LgBiT

MVFTLEDVGDEQTAAYNLQVLEQQGVSSLLQNLAVSVTVPIQRIVRSGENALKIDHVIIIPYEGLSAD
QMAQIEEVPFVQVPDDHDFKIVLPGYTLPIDGVTPNMLNYESGRPYEGIAVFDGKKTIVTVTGLWLWNGNKLIDERLITPDDMSLFRVTINGTTSASWHPQFEK*

Two component model system – receptor: LgBiT-DarkBiT101 (precisely, LgBiT-epitope-linker-epitope-DarkBiT101)

MGSSHHHHHHSSGLVPRGSHMVFTLEDVGDEQTAAYNLQVLEQQGVSSLLQNLAVSVTVPIQRIVEGS
GENALKIDHVIIIPYEGLSADQMAQIEEVPFVQVPDDHDFKIVLPGYTLPIDGVTPNMLNYESGRPYEGIA
VFDGKKTIVTVTGLWLWNGNKLIDERLITPDDMSLFRVTINGTTSASWHPQFEK*

Two component model system – reporter: mNeongreen-SmBiT99 (the flexible C-terminus of mNeongreen was removed)

MGSSHHHHHHSSGLVPRGSHMVSGKEEDNAMASPHELTHIFGSGNGVDFMVGQTGPNPDGYEE
NLKSTKGDLYSPWILYHIFGQYLPVYPDGYPPQAMVDGSQYJHRTMQFEDGASLTQVNTYRTEGSHIKG
AQQVKTGF PADGPVMTNSLTADQCRSKKTYPESQTDKISTFKNWSYTTNGKRYRSTART
TYTFAKMPAAANYLKNQPMYFRKTELHKSKELENFKQWEKAPFTGFSGSVTGYRALFEKISGTTSASWHP
QFEK*

dark-LUMABS-HIV #1 (derived from HIV-NB-LUMABS-6)

MGSSHHHHHHSSGLVPRGSHMVGYRLFEKESGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGS
VFTLEDVGDEQTAAYNLQVLEQQGVSSLLQNLAVSVTVPIQRIVRSGENALKIDHVIIIPYEGLSADQMAQIE
EVFKQVQVPDDHDFKIVLPGYTLPIDGVTPNMLNYESGRPYEGIAVFDGKKTIVTVTGLWLWNGNKLIDERLIT
PDGSMLFRVTINGTSGGSCGGGSVTGYRALFEKISGTTSASWHPQFEK*
dark-LUMABS-HIV #2 (derived from HIV-NB-LUMABS-7)
MGSSHHHHHHSSGLVPRGSHMVTGYRLEFESGSGSGLSGSGGSGGSGLSGGSGSGSGGSGGSGGSVFTL
EDFVGDEWEQTAAAYNLDQVLEQGVSLSLLQNVLAVSPTQIRGSRGENALKIDHIIPYELSGADQMAQIE
EVFKVVYPVDPDHHKVILPYGLTDVIPNMLNYGFRPEGYIAVFDBKKTIVTGTWNGKNIERLIT
PDGSMFLRVTINSGGGTELDRWEKIRLRPGGSGGSGGSGGGSGGGSGGAEAAAKEAAAKEAAAKEAAA
KEAAAKEAAAKAGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSSTL
EDFVGDWEQTVGGSSGLSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGGSVFTL
EDFVGDEWEQTAAAYNLDQVLEQGVSLSLLQNVLAVSPTQIRGSRGENALKIDHIIPYELSGADQMAQIE
EVFKVVYPVDPDHHKVILPYGLTDVIPNMLNYGFRPEGYIAVFDBKKTIVTGTWNGKNIERLIT
PDGSMFLRVTINSGGGTELDRWEKIRLRPGGSGGSGGSGGSGGSGGSGGSGGSGGAEAAAKEAAAKEAAAKEAAA
KEAAAKEAAAKAGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSSTL
EDFVGDWEQTVGGSSGLSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGS

dark-LUMABS-HIV #3 (derived from HIV-NB-LUMABS-1)
MGSSHHHHHHSSGLVPRGSHMVTGYRLEFEILGSGSGGGSGSGGSGSGSGGSGGSGGSGGSGSGGSGGSGGSGG
VFTL EDFVGDWEQTAAYNLDQVLEQGVSLSLLQNVLAVSPTQIRGSRGENALKIDHIIPYELSGADQMAQIE
EVFKVVYPVDPDHHKVILPYGLTDVIPNMLNYGFRPEGYIAVFDBKKTIVTGTWNGKNIERLIT
PDGSMFLRVTINSGGGTELDRWEKIRLRPGGSGGSGGSGGSGGSGGSGGSGGSGGAEAAAKEAAAKEAAAKEAAA
KEAAAKEAAAKAGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGS

dark-LUMABS-HIV #4 (derived from HIV-NB-LUMABS-4)
MGSSHHHHHHSSGLVPRGSHMVTGYRLEFEILGSGSGGGSGSGGSGSGGSGGSGGSGGSGGSGGSGGSGGSGG
VFTL EDFVGDWEQTAAYNLDQVLEQGVSLSLLQNVLAVSPTQIRGSRGENALKIDHIIPYELSGADQMAQIE
EVFKVVYPVDPDHHKVILPYGLTDVIPNMLNYGFRPEGYIAVFDBKKTIVTGTWNGKNIERLIT
PDGSMFLRVTINSGGGTELDRWEKIRLRPGGSGGSGGSGGSGGSGGSGGSGGSGGAEAAAKEAAAKEAAAKEAAA
KEAAAKEAAAKAGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGS

dark-LUMABS-CTX, without additional cysteine that was used in the NB-LUMABS system
MGSSHHHHHHSSGLVPRGSHMVTGYRLEFEILGSGSGGGSGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGG
VFTL EDFVGDWEQTAAYNLDQVLEQGVSLSLLQNVLAVSPTQIRGSRGENALKIDHIIPYELSGADQMAQIE
EVFKVVYPVDPDHHKVILPYGLTDVIPNMLNYGFRPEGYIAVFDBKKTIVTGTWNGKNIERLIT
PDGSMFLRVTINSGGGTELDRWEKIRLRPGGSGGSGGSGGSGGSGGSGGSGGSGGAEAAAKEAAAKEAAAKEAAA
KEAAAKEAAAKAGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGS

dark-LUMABS-101F
MGSSHHHHHHSSGLVPRGSHMVTGYRLEFEILGSGSGGGSGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGG
VFTL EDFVGDWEQTAAYNLDQVLEQGVSLSLLQNVLAVSPTQIRGSRGENALKIDHIIPYELSGADQMAQIE
EVFKVVYPVDPDHHKVILPYGLTDVIPNMLNYGFRPEGYIAVFDBKKTIVTGTWNGKNIERLIT
PDGSMFLRVTINSGGGTELDRWEKIRLRPGGSGGSGGSGGSGGSGGSGGSGGSGGAEAAAKEAAAKEAAAKEAAA
KEAAAKEAAAKAGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGSGS

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Green enhanced NanoLantern (GeNL); Calibrator luciferase (mNeongreen-NanoLuc, with shortened C and N-termini, respectively)
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