Using biolight to drive transcription

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Bioluminescent Control of NON-Ion-Moving Photoreceptors

Bioluminescence, light emitted by a luciferase enzyme oxidizing a small molecule substrate, a luciferin, has been used in vitro and in vivo to activate light-gated ion channels and pumps in neurons. This bioluminescent optogenetics (BL-OG) approach is not limited for use in neuroscience. Rather, bioluminescence can be harnessed to activate any photosensory protein, thus enabling manipulation of a multitude of light-mediated functions in cells. A variety of luciferase-luciferin pairs can be matched with photosensory proteins requiring different wavelengths of light and light intensities. Depending on the specific application, efficient light delivery can be obtained by either luciferase-photoreceptor fusion proteins or by simple co-transfection. Photosensory proteins based on light-dependent dimerization or conformational changes can be driven by bioluminescence to effect cellular processes from protein localization, regulation of intracellular signaling pathways, to transcription.

Recently, several publications reported the use of bioluminescence as light source for activation of a variety of photoreceptors including LOV-domains, BLUF-domains and cryptochromes (Table below). Applications for bioluminescence driven activation of optical switches targeted intracellular processes from reactive oxygen species induced cell death, cAMP synthesis, protein recruitment and dissociation, to genomic recombination and induction of transcription.

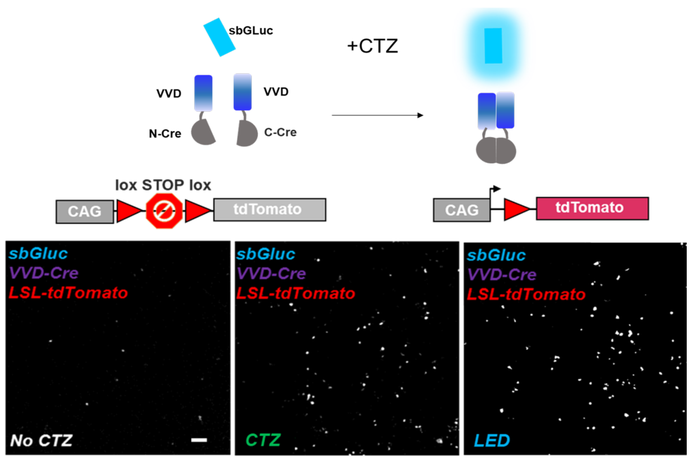
Light Emitter	Light Sensor	Arrangement of Moieties	Intra-Cellular Effects
	miniSOG bPAC LOVTRAP SPARK iLID		
NanoLuc Gaussia luciferase Renilla luciferase FRET constructs	FKF1/GI pMagnet dCas9 KillerRed FLiCRE CRY2/CIB GAVPO FLARE iCreV	Co-transfection Fusion proteins	Cell death cAMP synthesis Protein dissociation Protein recruitment Transcription Recombination

References in: Crespo et al., J Vis Exp 2021

Using luciferases as an alternative light source to activate light-sensing domains has several advantages. In contrast to optical fiber light activation, bioluminescence reaches every light sensing domain expressed in the target cell population as the light source is genetically encoded. Using bioluminescence alleviates concerns over tissue and cell damage by fiber optics and extended physical light exposure. The light is turned on with application of the luciferase substrate. The onset is immediate in vitro as well as in vivo depending on the route of administration and lasts for ~15-30 minutes; longer presence or phasic stimulation of light can be achieved with different luciferins and with additional or repeated applications of substrate. Lastly, bioluminescence emission can be tuned by varying the concentration of the luciferin.

Bioluminescent Control of Recombination

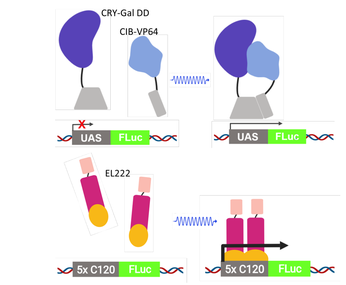
Photoactivatable recombinases provide a versatile tool for optogenomic manipulations. We tested bioluminescence activation of a photosensitive split Cre recombinase based on the Vivid LOV protein, iCreV (Yao et al., Nature Methods 2020). HEK293 cells were lipofected with plasmids, then kept in the dark. Twenty-four hours later cells were treated for 30 minutes with just medium (no CTZ) or with CTZ (100 µM final concentration) or with LED (duty cycle 25%, 5 s on/15 s off for 5 minutes; 14.81 mW light power, 20 mW/cm² irradiance) as a positive control. Microscope images of tdTomato fluorescence are shown using conditions as indicated.



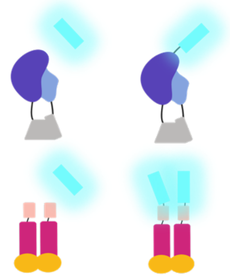
Bioluminescent Control of Transcription

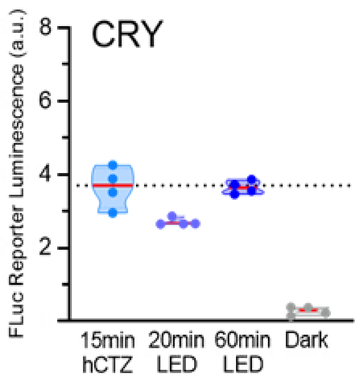
We employed NanoLuc for bioluminescent regulation of transcription through dimerization of CRY/CIB and the photosensitive transcription factor EL222. Bioluminescence, induced by adding hCTZ to HEK293 cells expressing the constructs and removing it after 15 minutes was more efficient in driving reporter transcription than 20 minutes of LED light exposure for both CRY/CIB and EL222. There were no significant differences in transcription efficacy between the two systems when co-transfected. For both systems fusion proteins led to significantly higher levels of transcription compared to co-transfected components, and fusion proteins of CRY/CIB were more efficient than those of EL222.

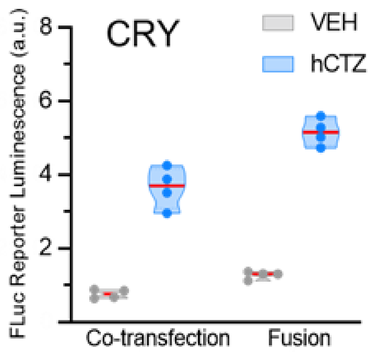
Bioluminescence vs LED

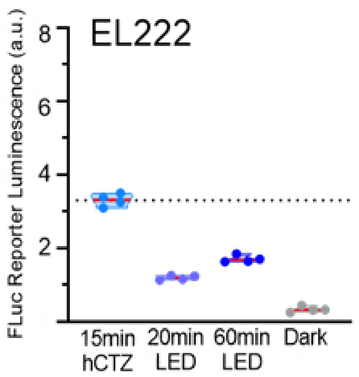


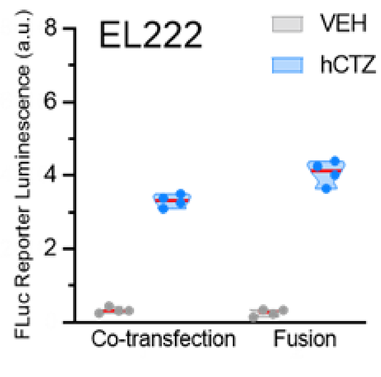
Co-Transfection vs Fusion



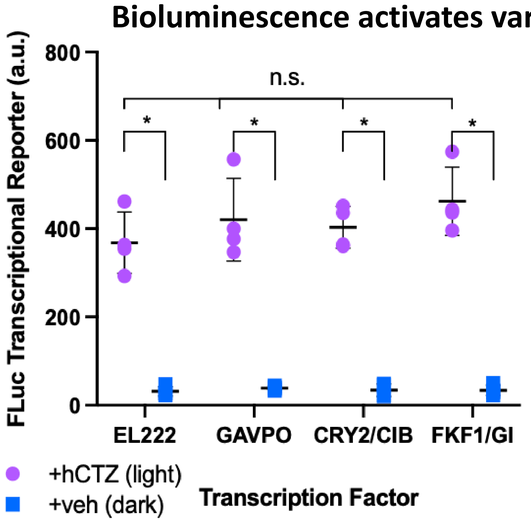








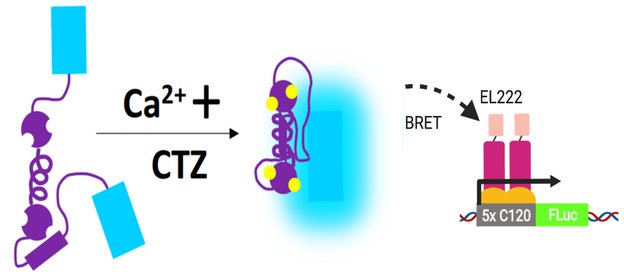
Bioluminescence activates various systems



HEK293 cells were cotransfected with different light sensitive transcription factors and NanoLuc. 24 hrs later the luciferin (hCTZ) or vehicle were added for 5 min. 6-8 hrs later bioluminescence driven transcription of the reporter Fluc was measured. Bioluminescent light induced transcription at a significantly higher level compared to control conditions (ANOVA; $p<0.05$), while levels of transcription were comparable between the different systems (ns, not significant).

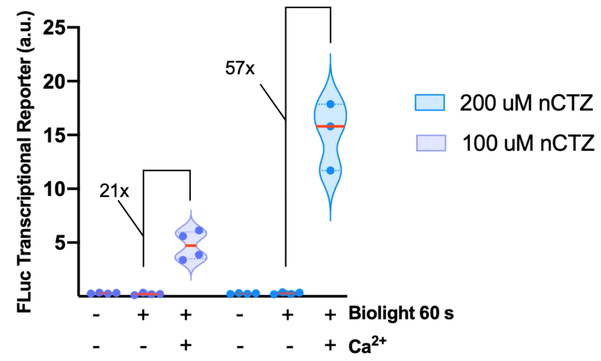
Integration with Intracellular Messengers

Bioluminescent light emission can be made dependent on intracellular processes, using photons for molecular integration. By using luciferases split by calmodulin-M13 sequences from GCaMPs, bioluminescence driven transcription depends on influx of calcium ions for functional reconstitution and light emission in the presence of the luciferin.



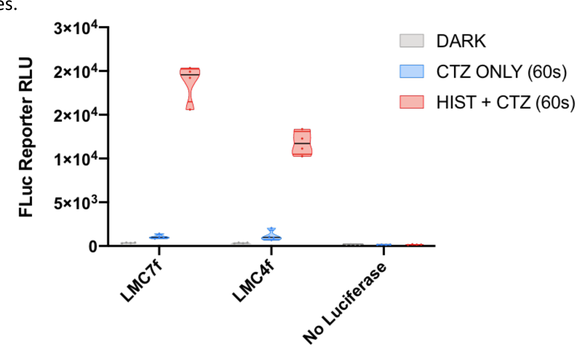
LuMiCampsin 4 (LMC4)

LMC4 is slow burn Gaussia luciferase (sbGLuc) split by the CaM-M13 sequence from GCaMP6f. HEK293 cells co-transfected with LMC4, EL222 and its reporter construct (FLuc) were exposed to the luciferin (nCTZ, biolight) in the presence or absence of ionomycin (Ca^{2+} influx). Robust transcription is achieved in the presence of both calcium and luciferin.

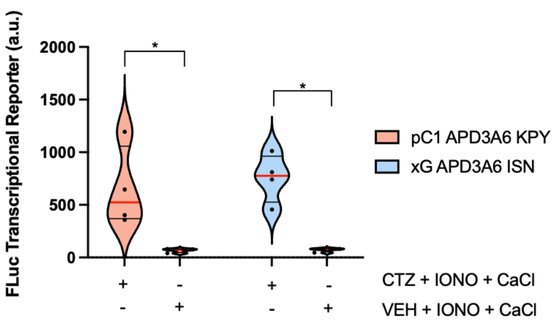


Improved Constructs

Exchanging the calcium binding region of LMC4 with that from Ca^{2+}-NanoLantern (LMC7) led to improvements in the ability to drive Ca^{2+}-dependent transcription. Here constructs were transfected into HeLa cells and the addition of histamine triggered release of Ca^{2+} from internal stores.



Recent split luciferases from the Shaner Lab show significant driving of transcription in HEK293 cells when exposed to calcium, ionomycin, and hCTZ (ANOVA, $p<0.05$).



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