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Developing biolight-based molecular technologies for simultaneous mapping, imaging, and controlling neural activity

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Abstract: We developed bioluminescent probes to image neural activity and harness biological light to serve as a multifunctional optogenetic actuator. This platform technology will enable noninvasive interrogation of activated circuits underlying behavior.

1. Genetically encoded activity dependent light emitters: expanding bioluminescent driven optogenetics

Bioluminescence is light emitted by a luciferase enzyme oxidizing its substrate. We previously have demonstrated that such "biological" light can activate optogenetic elements resulting in activation or silencing of neurons *in vitro* and *in vivo* altering behavior [1-3] . An all-biological system for the delivery and sensing of light for imaging and controlling a broad range of cellular processes in the brain would open opportunities for noninvasive circuit-based intervention of various psychiatric disorders. Therefore, we developed rationally engineered calcium dependent luciferases in order to harness bioluminescence beyond cellular imaging and applied these light producing enzymes for functionally mapping and manipulating activated circuits. We explored whether bioluminescent light production can be coupled to neural activity to effectively modulate this activity. Moreover, we applied our engineered luciferases not only to ion-moving photoreceptors but to the larger array of optogenetic tools as optical switches for controlling transcription.

2. LumiCaMPsin (LMC) reports cortical activity in vivo and enables real-time modulation of optogenetic tools

Here we take advantage of the unique possibility to make light emission itself activity dependent by using a Ca^{2+} dependent split luciferase. Intracellular increase of Ca^{2+} and the presence of a luciferase substrate, i.e. coelenterazine (CTZ), allows for the split luciferase to reconstitute and emit blue light (Figure 1A). We expressed a calcium sensing luciferase, LMC (Figure 1B), and a calcium insensitive variant (GLuc) in the barrel cortex in mice and imaged calcium dependent bioluminescence with a EMCCD camera. Pharmacologically increasing cortical activity with N-Methyl-D-aspartic acid (NMDA) leads to a 200% increase of light emission from LMC (red trace) as compared to the calcium insensitive luciferase (GLuc, blue trace).

Fig.1. Calcium dependent luciferases can report cortical neural activity *in vivo*

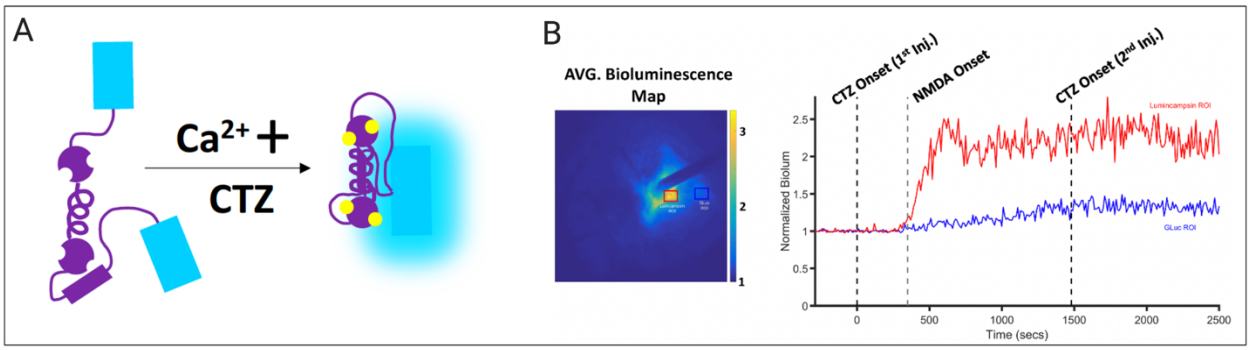
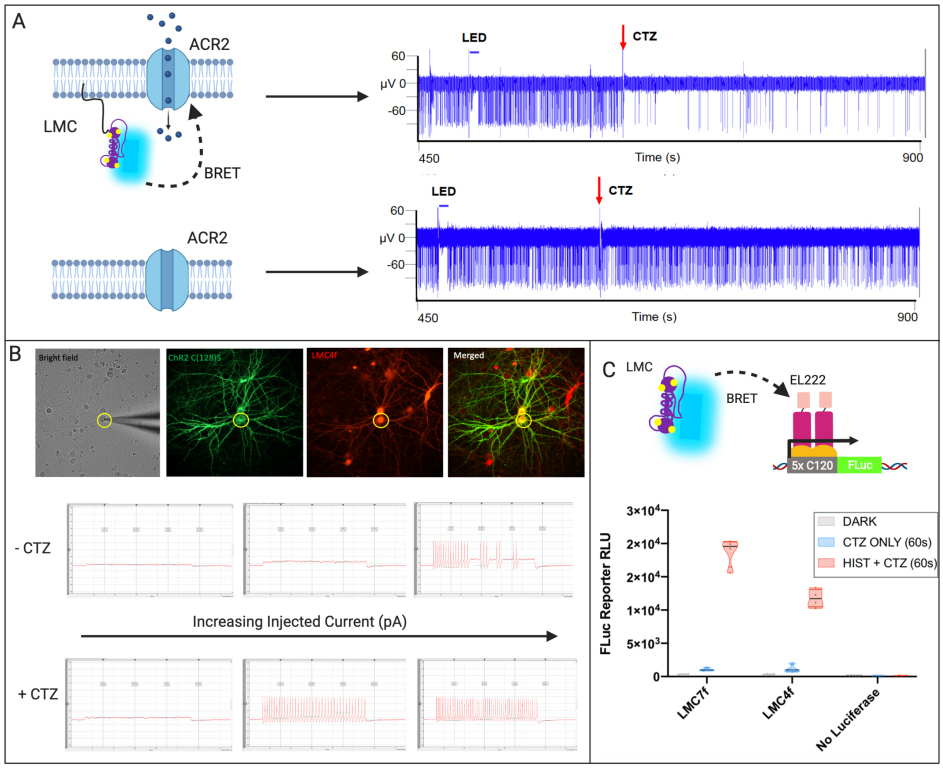


Fig. 2. Calcium dependent light emission from LumiCaMPsin (LMC) can control neural activity through modulation of opsins and transcription



We employed one of our Ca^{2+} dependent luciferases as a light source to control optogenetic elements in cortical neurons on multielectrode arrays. In this case (Figure 2A), a neuron expresses the Ca^{2+} dependent luciferase (LMC) and an inhibitory chloride conducting channelrhodopsin, ACR2. In the presence of the luciferin, CTZ, light emission will be dependent on the activity status of the neuron – only active neurons will reconstitute the luciferase that in turn activates the opsin through bioluminescence resonance energy transfer (BRET) and silence neural activity in LMC expressing neurons (Figure 2B). To determine if calcium dependent light emission of LMC can increase neural activity we examined the firing properties of cortical neurons co-expressing LMC and the excitatory step-function opsin ChR2-C(128)S (Figure 2B). In response to depolarizing square current injections of increasing magnitude, co-expressing cortical neurons treated with the luciferin, CTZ, displayed an increase in firing frequency as compared to the vehicle (-CTZ) (Figure 2B). These results suggest that in the presence of CTZ, the substrate for LMC, bioluminescence can activate the excitatory step-function ChR2-C(128)S and subsequently drive neural activity in an activity dependent manner. We expanded this calcium dependent light emission to the blue light photosensory protein, EL222 [4] in order to drive transcription in HeLa cells expressing LMC and EL222. We demonstrate that the LMC variant, LMC7 can lead to a 17x fold change in calcium dependent transcription. Collectively, neural activity dependent bioluminescence will enable *in vivo* applications that benefit from non-invasive light sources and engagement of spatially distributed cells.

3. References

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