Improving bacterial bioluminescence for single-cell imaging

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Abstract

The usability of bioluminescence for imaging of single cells is complicated by its low light levels compared to fluorescence measurements and the requirement of exogenous luciferin supply for most luciferases. Bacterial bioluminescence uses reduced flavin mononucleotide as a luciferin that is abundant in all cells. Therefore, this system is purely genetically encodable by the *lux* operon, which contains the bacterial luciferase and enzymes for substrate recycling. However, the brightness of bacterial bioluminescence is relatively low in comparison to other luciferases. We have generated an improved *lux* operon named *ilux* with an approximately sevenfold increased brightness when expressed in *E. coli* cells. The *ilux* operon consists of the *luxCDABE* genes and an additional FMN reductase, which were optimized for enhanced brightness by mutagenesis. *ilux* allows for imaging of single *E. coli* cells over timespans up to several days with enhanced spatiotemporal resolution. Since metabolic energy is required to generate auto-bioluminescence light, *ilux* can be used to assay cellular viability on the single-cell level, as demonstrated by application of different antibiotics.

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