Establishment of a multi input whole cell biosensor based on botulinum A light chain enzyme

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Microbial biosensors are used to detect the presence of compounds provided externally or produced internally. Synthetic biology based on engineering modularity serves as a new tool that could be used to engineer microbes to acquire desired functions through artificial design and precise regulation. In this study, we designed artificial genetic circuits in *Escherichia coli* that is based on the activity of botulinum light chain on its substrate SNAP-25. The sensor is triggered by two inputs. The first trigger leads to the expression of botulinum light chain enzyme; while the second (the "reporter") leads to the expression of a detector (GFP) connected to SNAP-25 and a degron. Without the first trigger, the degron will degrade the reporter and no visible color will appear. Only with the addition of the second input, the botulinum light chain enzyme will be expressed and cleave SNAP-25. The latter will result in separation between the degron and the rest of the reporter and GFP will be detected. Our preliminary results demonstrate the ability of botulinum light chain enzyme to separate efficiently the degron from the rest of the reporter and to facilitate light formation. The usage of the very effective botulinum light chain enzyme in this biosensor design is unique and permit tight control. With the use of the modular cloning kit (EcoFlex), different elements will be tested to optimize the sensor. The use of two parallel inputs in this biosensor promise its specificity. Establishment of this tool with multi-input processing will be implemented in future biosensors.