A novel fluoride sensor based on a riboswitch construct

We have developed a fluoride sensor based on a riboswitch construct, which allows free entry of fluoride ions into the cell membrane and allows the cell to become more sensitive compared to inducible promoters. Our construct can be used to quantify the levels of fluoride in the environment.

- We have found that riboswitches can be used to control gene expression, which can help in the development of sensors. Our construct includes the WH1 guidelines for safety, while the riboswitch allows the cell to respond to changes in fluoride concentration.
- We chose to work with riboswitches as they are an underused resource by the iGEM community, and our construct is easy to manipulate and allows for improved response times compared to inducible promoters.
- We hoped our construct would function in both E. coli and B. subtilis, which has been directly reported, as well as many of the health problems associated with fluoride contamination.

We tested our construct in non-porous B. subtilis, and showed that this results in an additional emission at 540 nm, which significantly, but is unstable above 18°C. We wanted to use the bacterial lux operon for our light output, but it is not an adequate control for special cases. However, it is not an adequate control for special cases, and we do not intend to use it as a reference.

The principle of ratiometric reporters is the normalisation of an inducible signal to a constitutive one, to take variations in culture density, size and productivity into account internally. We decided to investigate the potential of ratiometric reporter constructs for accurate and reproducible characterisation and quantification. We designed fluorescence and luminescence-based constructs that would function in both E. coli and B. subtilis.

- We fused mOrange to the LuxA subunit as described by Dachuan Ke and Shiao-Chun Tu (photochem + photobio, 2011). They showed that this results in an additional emission at 540 nm.
- We designed a synthetic reporter construct including this second channel, which was generously synthesised by DNA 2.0 and under-optimised for Bacillus. Characterisation has been obstructed by toxicity and stability issues. The construct glows. Overlaying filters used in photospectroscopy were co-added to obtain an accurate representation of the signal.
- The tube of bacteria simply sits on the user's shelf until needed and overexpression of certain B. subtilis operons (we used spoV A) reduces the germination rate. When required, a simple protocol and germination medium supplied in the kit is applied, after a short incubation period the sensor can be loaded into the analytical device.

Using a facility suitable above a low angle, the following changes should be made:
- Indicators for presence of toxic substances should be included in the field of view, which may not be visible under normal dosing or analyses.
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We conducted a study into the suitability of our system for use in the field, which included a comparison of global geogenic arsenic contamination in groundwater. Environmental Science and Technology 42, 3669–3675.