Enlisting E. Scherichia Holmes
A modular whole-cell biosensor for the detection of environmental pollutants

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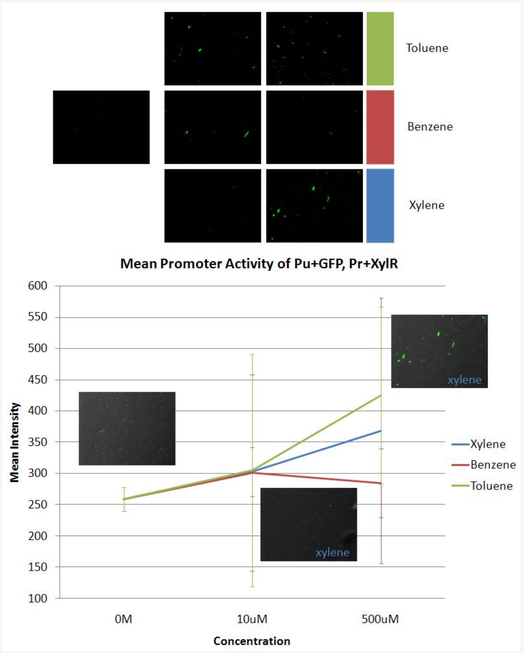
Our project was to design E. coli biosensors for the detection of water pollutants. Oil spills, such as the recent Deepwater Horizon spill, deposit long-lived organic compounds over a huge area. Fertilizer run-off, found in bodies of water near farmland, can cause algal blooms which deplete the oxygen in the water ("eutrophication"), hurting wildlife in surrounding areas. We focused on pollutants caused by oil spills and eutrophication: alkanes, aromatics, nitrates, and nitrites. We found parts already in the registry for the detection of aromatics, nitrates, and nitrites. We characterized the Biobrick Pu+GFP for the detection of aromatics. For nitrates and nitrites, we added different reporter genes to the PyeaR promoter Biobrick, characterized both resulting parts, and submitted these parts as Biobricks. We designed a part for the detection of alkanes from genomic data and attempted to design a novel Biobrick part.

All images were taken with Olympus IX81 automated inverted microscope specially equipped for live cell imaging. All experiments were performed with DH5α E.coli cells. The filter sets we used are: 545/30x (excitation) and 620/60m (emission) filters for DsRed, 470/40x (excitation) and 525/50m (emission) for GFP. Data collection and processing was performed by the SlideBook software.
The data was taken using cells that were grown overnight in 2mL of LB broth that had 50ug/mL of the appropriate antibiotic at 37C and 220rpm. This was used in a 1:50 dilution. Each sample had 40uL from the broth grown overnight and 2mL of the LB broth with the appropriate antibiotic. These were allowed to grow until a predetermined OD. Then the contaminants were added and the cells were allowed to grow for another 2 hours, before the images were taken.

Pu+GFP (BBa_K270003)

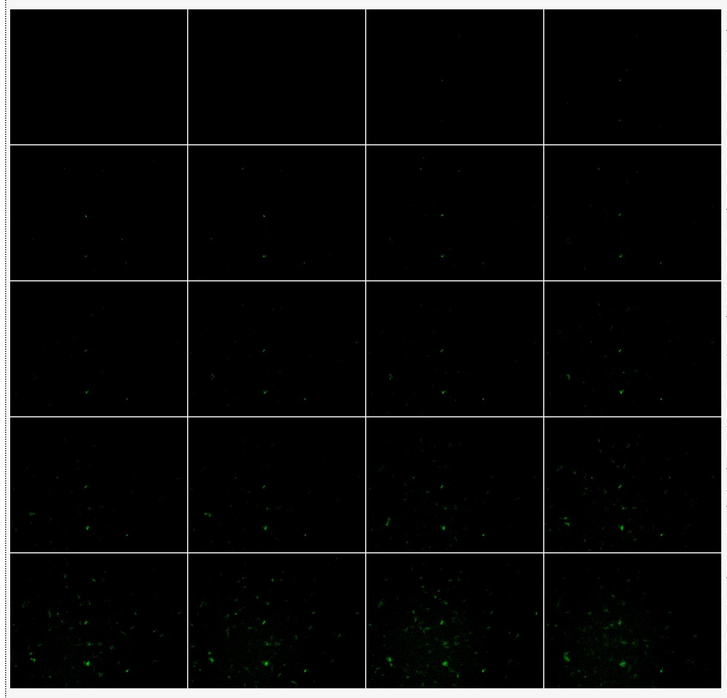


The data was taken using E. coli DH5α that was co-transformed with Pu+GFP and Pr+XylR.
The leftmost image is the negative control. The middle three images are the 10uM and the rightmost are the 500uM concentrations of toluene, benzene, and xylene.
The graph is of the average of all the maximum intensities of the cells in the image vs. the concentration of the aromatic added to the sample.
We also took data from 10mM and 100mM concentrations of aromatics, but most of the cells died at these higher concentrations.

This part, Pu+GFP, was made by Michigan 2009. We decided to characterize this part because it is an aromatic inducible promoter, if there is XylR in the cell. Aromatics binding to XylR alters its shape causing it to be able to then bind to Pu, inducing transcription. The Pr+XylR (BBa_I723032) is from Glasgow 2007. Pr is a constitutive promoter and XylR is therefore actively made in the cell.

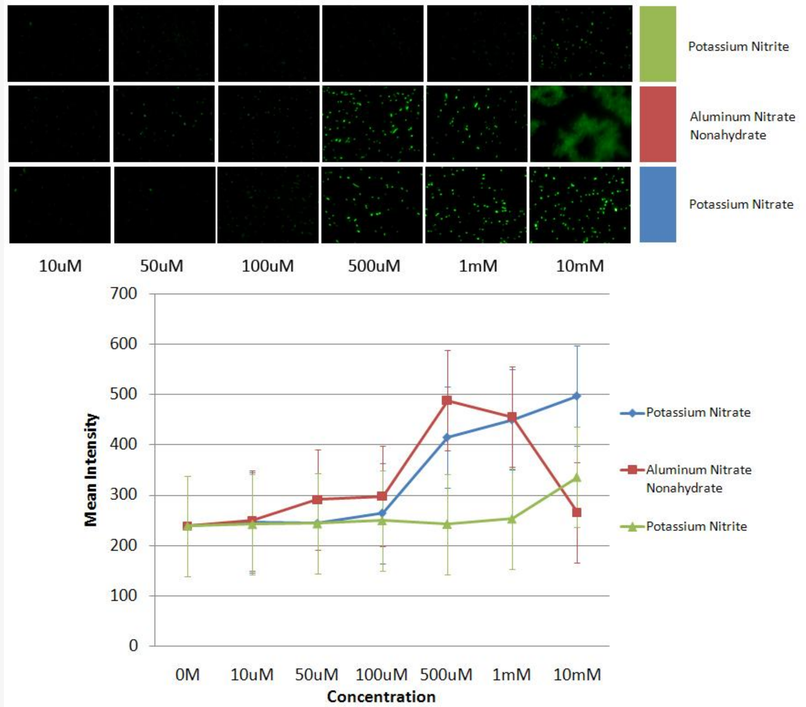
PyeaR+GFP (BBa_K412000)

PyeaR is a nitrate/nitrite inducible promoter, that was made by Edinburgh 2009, that we cloned to a green fluorescent protein coding site and a red fluorescent protein coding site to get biosensors.

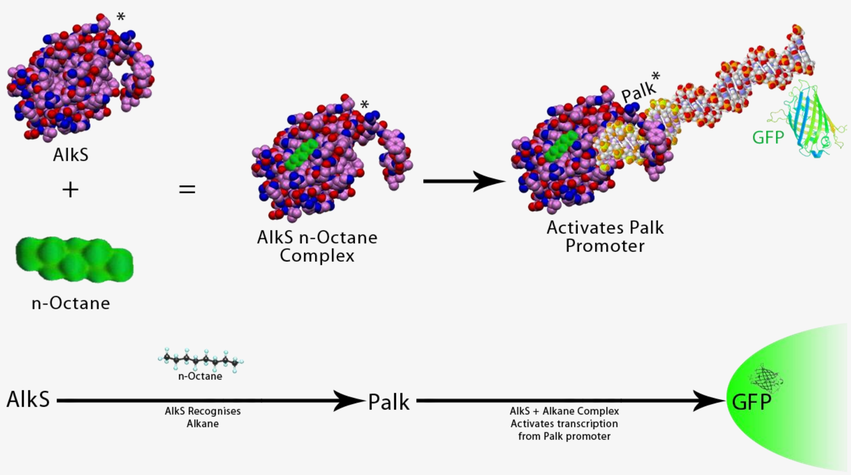


In the figure to the right:
The images at the top are the ones used to take the data.
From left to right the image is the 10uM to a 10mM concentration of the respective contaminants: potassium nitrite, aluminum nitrate nonahydrate, and potassium nitrate.
The graph shows the average of all the maximum intensities of the cells in the image vs. the concentration of nitrate/nitrite added to the sample.
The data shows that PyeaR+GFP responds better to nitrates than nitrites.
It also shows that the cell is more sensitive to aluminum nitrate nonahydrate, and therefore starts dying at a lower concentration.

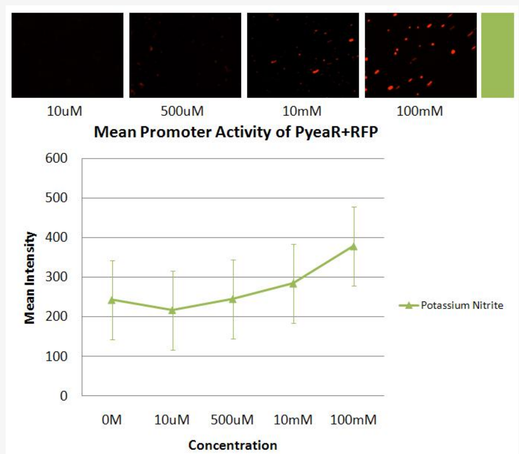
The images to the left are time-lapse images.
The images were taken every ten minutes after 10mM potassium nitrate was added to the sample.
The images show that some of the cells start getting brighter after the first ten minutes, but most of the cells are active after two hours, and are even brighter after three hours.



Alkanes or parafins make up a large portion of oil. So we designed a biological sensor for detecting trace levels of alkane. This is based on the work done by Dr. Jan Roelof van der Meer of the University of Lausanne. The transcription factor AlkS binds to an alkane and the complex positively regulates transcription from the Palk promoter. We were not successful in producing a Biobrick for this part. This is under planning and construction.



PyeaR+RFP (BBa_K412001)



The images and the graph are from PyeaR+RFP incubated with potassium nitrite. The graph is of the average of all the maximum intensities of the cells in the image vs. the concentration of nitrite added to the sample.

Acknowledgements

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References

Sticher, Patrick; Jaspers, Marco C.M.; Stemmler, Konrad; Harms, Hauke; Zehnder, Alexander J.B.; and van der Meer, Jan Roelof."Development and Characterization of a Whole-Cell Bioluminescent Sensor for Bioavailable Middle-Chain Alkanes in Contaminated Groundwater Samples" *Applied and Environmental Microbiology*, 63 (Fall 1997): 4053–4060.