A cell-free sensor platform for the quantification of arsenic concentrations in drinking water.

Primary contact

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Summary

Arsenic contamination of drinking water is a global issue affecting more than 150 million people. Currently, drinking water is predominantly tested via chemical sensors, which require toxic reagents, technical expertise and only produce qualitative results. Developments in synthetic biology have demonstrated that biological components can be engineered for heavy metal detection, but methods are limited by GM contamination risks and only provide semi-quantitative readouts [Arsenic Project]. As such, an opportunity has been identified to develop a first of its kind cell-free quantitative heavy metal sensor which uses the synthesis of an electroactive metabolic enzyme for amperometric quantification of arsenic concentrations.

The system proposed will use a genetic circuit consisting of a promoter responsive to arsenic and a downstream reporter which produces an electroactive enzyme. The enzyme concentration will then be measured using amperometric detection methods allowing a quantitative measurement of arsenic concentration to be determined.

Team

This ambitious project requires a technically diverse cohort. The team consists of the 12 members of the MRes year students of the Sensor CDT programme, assigned to 5 main work streams according to subject expertise.

Team member	Background	CRSid	Contribution
Synthetic Biology			
Elise Siouve	Biotechnology	es677	Elise will be working on genetic engineering using her background in biotechnology, microbiology and chemistry.
Carolina Orozco	Biotechnology	co381	Carolina has experience of genetic engineering with modern tools such as CRISPR-Cas9 which makes her perfectly suited for working on the plasmid cloning and genetic circuit.
Sina Schack	Biochemistry	ss242	Sina has experience in creating genetic constructs; expertise she will apply in the designing and making of the genetic circuit. Her background of chemistry diversifying into biology also allows her to interconnect the biological and chemical aspects of the project.
Electrochemistry and Platform			
Lisa Hecker	Biophysics	lh551	Lisa will apply her interdisciplinary biophysics background to study the electrochemical and biological interactions.
Alexandru Grigoroiu	Biomedical Engineering	ag745	Alex will be using his combination of biological and engineering skills to focus on bringing together all aspects of the device onto a single disposable platform.
Sammy Mahdi	Electrical Engineer	sm2205	Sammy will be experimenting with different manufacturing methods for the milli-fluidic platform and fabrication of the electrodes. This is an area in which he has a strong interest and previous experience.
James Vereycken	Organic Chemistry	jev33	James' contribution will be in the development and experimentation of protocols for electrode inks and the choice of mediators using his strong chemical background.

Modelling			
Francesco Tonolini	Physics	ft291	Francesco has extensive experience of mathematical modelling. He will develop a computational model of the system to give a better understanding of the ongoing biological and electrochemical interactions
Electronic Hardware			
David-Benjamin Grys	Electrical Engineering	dbg27	David-Benjamin is an electrical engineer by training with many years of experience in hardware development. He will lead the design and manufacture of the reusable electrical unit that provides gives the arsenic concentration readout.
Software			
Tess Skyrme	Aerospace Engineering	ts676	Tess will be developing the Arduino-based data communication unit and user interface, drawing on her expertise in user focused design.
Project Leads			
Genevieve Hughes	Earth Science	gh436	Genevieve's background in earth science allows her to adopt a holistic view of the project which in addition to her technical understanding of the project problem and communication skills make her ideally suited to management.
Ralf Mouthann	Physics	rpm40	Ralf has previously delivered large- and small-scale projects for the UK's National Physical Laboratory. This has given him insight into managerial processes.

Proposal

The problem

Arsenic contamination of groundwater occurs naturally in different parts of the world. Contamination typically arises from the dissolution of arsenic-bearing minerals and is amplified in areas with major river basins or alluvial planes. As groundwater from shallow tube wells is the main drinking water source in Nepal and Bangladesh health issues are particularly prevalent, with long-term arsenic exposure leading to lesions, metabolic frailty, and fatalities. There is also a social stigmatisation of those suffering from the effects of chronic arsenic poisoning.

Current approaches to testing use toxic substances, require expert operators or involve sending samples to a central location for testing. The cell-free platform proposed will provide the proof of concept for a low-cost, rapid, safe and portable alternative. Such a sensor will facilitate the in-situ testing of wells and will allow the arsenic contamination levels to be mapped in time and space, potentially becoming a useful tool for informing policy.

Proposed system

A genetic circuit is proposed consisting of a promoter deinhibited by arsenic i.e. pArs with an electroactive reporter enzyme e.g. glucose oxidase (redox enzyme).

The mechanism works in three phases (Figure 1):

1. If present, arsenic binds to ArsR, a protein inhibiting the promoter pArs.
2. The binding changes the conformation of ArsR, which releases the promoter
3. The genetic circuit transcribes and translates glucose oxidase (redox enzyme)

The use of this mechanism is the one area of innovation in this project, as this cell-free sensor is the first to use a genetic circuit for the detection of heavy metals.

The genetic circuit developed will be deposited onto a disposable platform along with cell-free extract and glucose. Electrodes will be screen printed onto the substrate. The consumption of glucose by the glucose oxidase will generate free electrons that can be transported to the electrodes by a mediator. This generates a current at the electrodes which can be related back to the arsenic concentrations. A reusable electronic unit will be developed to monitor the current generated during sensing. Different fabrication methods will be investigated for the disposable platform, including paper, 3D printing and laser cut acrylic.

The system will transmit recorded data to a central database via an open source Arduino GPRS link. A user interface will be developed to map the spatial and temporal distribution of arsenic concentrations. Given sufficient data collection this approach could be used to inform relevant policy.

Implementation

The five identified work streams will be overseen by the management team. Management will also be responsible for overall finances, purchasing and organising weekly progress report meetings. Implementation will occur over 15 weeks with all team members working full time. Gantt charts have been produced to identify delivery milestones that are critical to different work streams. Researchers will work on tasks in pairs with clear task ownership and responsibilities. It is anticipated that significant collaboration will be required to deliver the proposed work, which will be facilitated by sharing a workspace, by using a central DropBox repository for files and by using the Slack communication service.

Proposed Outcomes and Benefits

The aim of the project is to create a proof of concept prototype of a cell-free quantitative heavy metal sensor platform, with publishable results. This prototype will be the first to demonstrate a cell-free biosensor for heavy metal sensing and for producing quantitative readouts. With further development, such a sensor could be implemented to help provide clean drinking water to the 150 million population affected by arsenic contamination. By altering the genetic circuit, the sensor could be adapted to detect other heavy metals such as lead or mercury. It may also be adapted to quantify the concentration of other biomolecules of interest.

The outcomes of the project will be: published as a journal paper to be available on the University of Cambridge open access repository, documented on git hub and presented and shared to the wider academic field on the EPSRC CamBridgeSens Sensors Day.

Funding

The proposed project is ongoing, and currently has £5000 funding provided by the Sensor CDT. Of this budget, it is anticipated that roughly £2,700 will be spent on biological reagents, £1,350 on developing the platform, and £260 on the electronic unit, all with a 15% contingency budget.

The contribution from the Open Plant Fund will be used for more thorough investigation of the more costly, but critical, aspects of the project and additional final device testing. This would include purchase of: commercial electrode inks and screen printed electrodes for reference and validation of our own printed electrodes; additional cell free extract for further dilution tests & electrochemical tests; a science grade screen printing kit for manufacture of the electrodes and additional biological supplies.

Item	Cost
Novacentrix Screen Kit & Ink	£570
Mediator DropSens Electrodes	£360
Mycrocell Cell-Free Extract (2x 96 reactions)	£922
Rodeostat: Open Source Potentiostat + Accessories	£250
Adafruit FONA 808 Shield - Mini Cellular GSM + GPS for Arduino	£50
Membrane for screen-printed electrodes (100/pk)	£100
SDS-page Gels	~£200
Synthesis of GOx gene	~£800
3x NEB Q5 High-Fidelity DNA Polymerase	£240
Total	£3,491

Sponsor for the research and cost centre

Dr Oliver Hadeler, Centre for Doctoral Training in Sensor Technologies and Applications, Department of Chemical Engineering and Biotechnology, oh209@cam.ac.uk

I confirm that I have the full support of the sponsor listed above and that they can be added to the OpenPlant Fund mailing list to receive project updates (to which they can unsubscribe at any time).