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

**Primary contact**

Genevieve Hughes, CDT Sensor Technologies and Applications (Sensor CDT), Department of Chemical Engineering and Biotechnology, University of Cambridge, [gh436@cam.ac.uk](mailto:gh436@cam.ac.uk)

**Summary**

Arsenic contamination of drinking water is a global issue affecting in the region of 150 million people. Currently, drinking water is predominantly tested via chemical sensors, which require toxic reagents, technical expertise and only produces qualitative results. Developments in synthetic biology have shown that biological components can be engineered for heavy metal detection, but methods are limited by GM contamination risks and only provide qualitative readouts. As such, an opportunity has been identified to develop a first-of -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 as follows:

Synthetic Biology

Elise Siouve (es677) – Biotechnologist

Carolina Orozco (co381) – Biotechnologist

Sina Schack (ss242) – Biochemist

Electrochemistry and Platform

Lisa Hecker (lh551) – Biophysicist

Alexandru Grigoroiu (ag745) – Biomedical Engineer

Sammy Mahdi (sm2205) – Electrical Engineer

James Vereycken (jev33) – Organic Chemist

Modelling

Francesco Tonolini (ft291) – Physicist

Electronic hardware

David-Benjamin Grys (dbg27) – Electrical Engineer

Software

Tess Skyrme (ts676) – Aerospace Engineer

Project Leads

Genevieve Hughes (gh436) – Earth Scientist

Ralf Mouthaan (rpm40) - Physicist

By working closely together, the various members of the team will have the opportunity to learn new skills and develop their subject knowledge beyond their specific work stream area.

**Proposal**

**Problem**

Arsenic contamination of groundwater occurs naturally in different parts of the world. Contamination typically arises from 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 temporally and spatially, potentially becoming a useful tool for informing policy.

**Biological 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 using such a genetic circuit for the detection of heavy metals will be the first of its kind.

**Hardware**

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 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.

**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. 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 and will also be made available to the wider public through outreach initiatives such as the EPSRC CamBridgeSens Sensors Day.

**Budget**

The proposed project is ongoing, and currently has £5000 funding provided by the Sensor CDT. Of this budget, it is anticipated that roughly £2,400 will be spent on biological reagents, £1,200 on developing the platform, and £400 on the electronic unit, all with a 25% contingency budget. The contribution from the Biomaker challenge will be used for purchase of commercial electrode inks and screen printed electrodes for reference and validation of our own printed electrodes.