SUSTAINABLE SYNBIO SOLUTIONS FOR DETECTING WATER CONTAMINANTS

Whole-cell biosensors (WCBs) are cells that can sense and report a target or condition of interest. They are cheap and easy to produce and they are recyclable since they are purely biological materials. Therefore, they are sustainable analytical devices for detecting various environmental contaminants. However, they often have performance issues such as high limit of detection (LOD), high leakage (high output when no targets), or low output for real applications. To this end, a SynBio solution is created here using Synthetic Biology tools and concepts to improve WCBs sequentially.

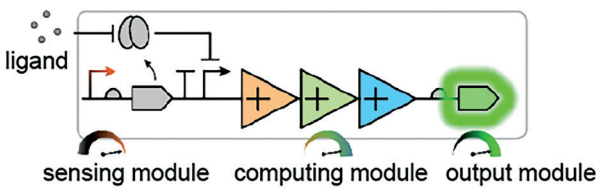

Fig. 1 | Architecture of a whole-cell biosensor (WCB) and the step-by-step optimization strategy. A WCB is composed of a sensing module, a computing module, and an output module. The optimization strategy presented in this work is to improve these three modules sequentially.

Universal SynBio tools for rapid development of WCBs

Synthetic Biology aims to engineer new biological systems that do not exist in nature or to redesign existing systems from scratch. It enables fast development of cheap, selective, sensitive, and easy-to-manufacture WCBs.

From the view of a synthetic biologist, every single cell can be considered as a mini-computer. All the biological parts in every WCB can be classified into three parts (Fig. 1): a part that can sense an input target (named as a sensing module or input module, like a keyboard), a part that can report when there is a target (named as an output module, just like a computer screen), and a part that processes the signals when sensing a target (we name it a computing module). All these parts are based on proteins, nucleic acids, and biochemistry reactions among them. The aforementioned Synbio solution is to make changes in these three parts to improve WCBs step-by-step, and this solution can be applied to many other WCBs. Particularly, this work has used this Synbio solution to improve an exemplary WCB detecting arsenic.

Case study – arsenic WCBs design & optimization

To simplify, this work developed a non-pathogenic *Escherichia coli*-based WCB that could produce green fluorescent protein (GFP) when detecting arsenic in water and applied above SynBio solution to improve the biosensor's LOD, output readout, and leakage step-by-step (Fig. 2).

The first step was to change the ArsR protein quantity in the sensing module. ArsR protein is an arsenic receptor protein that can interact with arsenic directly. It forms the sensing

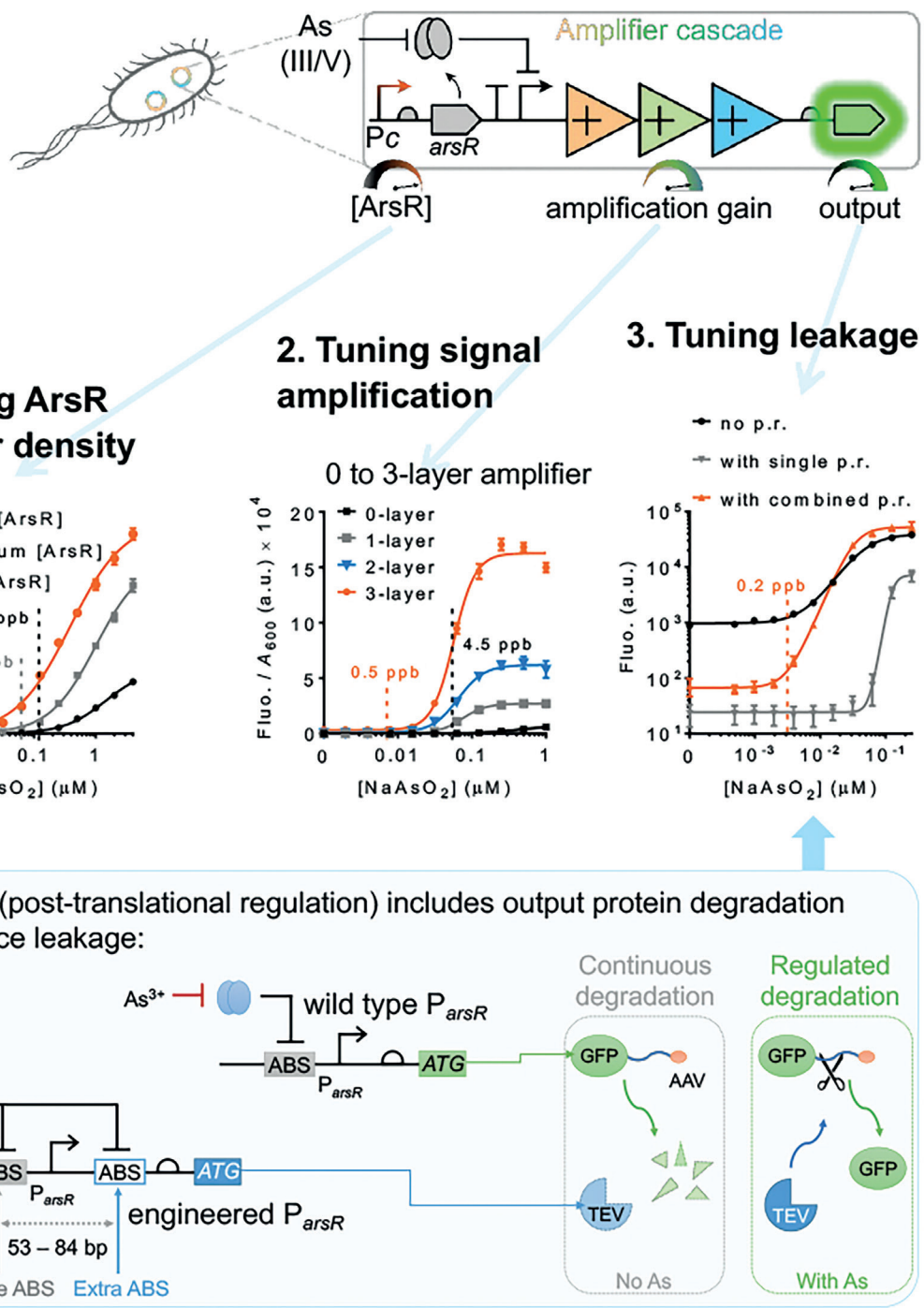

Fig. 2 | Step-by-step optimization of an arsenic WCB.

module of the arsenic WCB with its cognate promoter P_{arsR}. ArsR is expressed under a constitutive promoter (a piece of DNA in charge of continuous *arsR* gene expression). Different constitutive promoters of varying strengths to express ArsR were compared here. As a result, the weaker the promoter, the lower the ArsR intracellular concentration, and the lower the LOD and higher the output dynamic range of the sensor. Therefore the first step by lowering the ArsR intracellular quantity improved the sensor's LOD and output readout.

The second step was to introduce a transcriptional amplifier or cascaded amplifiers between the sensor module and the output module to amplify the output level. This work developed three single-layered transcriptional amplifiers using HrpRS, RinA, and ECF proteins-based activation systems respectively, and cascaded those amplifiers to make two and three-layered amplifiers. These amplifiers were new synthetic biology tools created in this work, and they increased the sensor's output by a few hundred folds.

The last step was to reduce the leakage without sacrificing the LOD and output readout overmuch. Some arsenic sensors with above optimization were leaky. The leaky sensors produced green light when there was no arsenic, therefore could cause false positive results. To this end, this work developed a post-translational regulation method. In this method, a protein degradation tag (AAV) was first fused to the GFP reporter protein to reduce the output basal expression, then a TEV protease-based reporter protein degradation control system was incorporated into the sensor to remove the AAV tag when there was arsenic induction. The TEV protease was under the control of an engineered P_{arsR} containing double arsenic binding sites to eliminate the leaky expression of TEV protease which could cleave a linker between the expressed GFP and its AAV tag only when there was arsenic induction. By doing this, the sensor's output GFP can be continuously degraded when there was no arsenic but will be protected from degradation when there was arsenic. Therefore the post-translational regulation method reduced the leakage of the arsenic sensor while maintaining low LOD and high output readout.

Overall, above 3-step optimization procedure improved the arsenic sensor's LOD by 50-fold and output level by 750-fold. The sensor's LOD was also much lower than WHO's guideline (i.e., 10 ppb) for drinking water, meeting the requirements for arsenic detection.

Sensor array design & validation using groundwater from Bangladesh

Bangladesh has been severely contaminated with arsenic in the drinking water and the arsenic poisoning is causing more than 40,000 annual death. Besides, the arsenic contamination may change from time to time, so the contamination has to be monitored more regularly than people usually expected. Therefore a cheap and easy-to-use device is urgently needed.

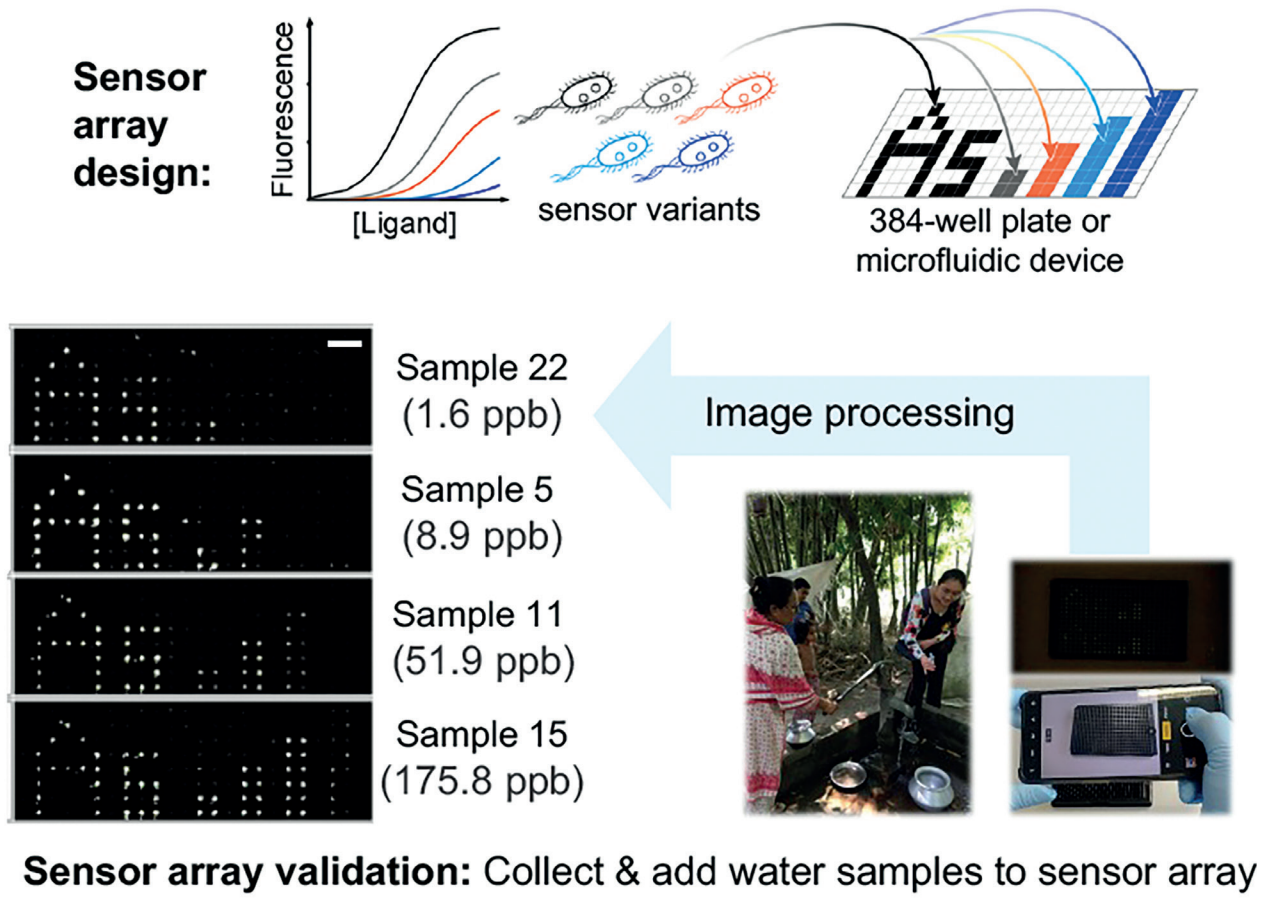

Fig. 3 | Arsenic sensor array design and its test with groundwater samples from Bangladesh.

In this work, a visible, user friendly and cost effective arsenic sensor cell array was developed for semi-quantification of arsenic in drinking water. The arsenic cell array was spotted with five different arsenic WCBs (As0–3 and As5) in the pattern of the arsenic element symbol "As" and volume bars. These five sensors have different LODs (As0 > 1 > 2 > 3 > 5) and were generated from the aforementioned optimization process. This sensor array prototype was tested using groundwater from Bangladesh and it could sense as low as 1.6 ppb of arsenic while distinguishing different concentrations of arsenic from the groundwater samples. More importantly, these results were visible and could be captured by a mobile phone. This means this arsenic sensor array could be used for the semi-quantification of arsenic without complicated data processing. It also means, in the future, people could just take a photo of the sensor cell array, and the mobile phone could analyze the results and upload them automatically to a global database so that the global environment could be easily monitored in a more cost-effective and sustainable way.

In summary, by using Synthetic Biology tools, this work developed a step-by-step optimization methodology to improve WCBs' LOD, output readout and leakage, and a microbial sensor array as a portable, low-cost environment monitoring tool. These will be widely applicable to many other WCBs, paving the way for their real-world applications.

References:

Wan, X., Volpetti, F., Petrova, E. et al. Cascaded amplifying circuits enable ultrasensitive cellular sensors for toxic metals. Nat Chem Biol 15, 540–548 (2019). https://doi.org/10.1038/s41589-019-0244-3

Wan, X., Ho, T.Y.H., Wang, B. (2019). Engineering Prokaryote Synthetic Biology Biosensors. In: Thouand, G. (eds) Handbook of Cell Biosensors. Springer, Cham. https://doi.org/10.1007/978-3-319-47405-2_131-1

Author Contact Details

Xinyi Wan • School of Life Sciences, Hainan University, Haikou, 570228, China • Email: xwan@hainanu.edu.cn

Qingyu Wang • School of Life Sciences, Hainan University, Haikou, 570228, China

• Email: qingyu.wang@hainanu.edu.cn

Shunqing Xu • School of Life Sciences, Hainan University, Haikou, 570228, China • Email: xus@hainanu.edu.cn

Baojun Wang • College of Chemical and Biological Engineering, Zhejiang University, Hangzhou, 310058, China
• Email: baojun.wang@zju.edu.cn

Xinyi Wan *Qingyu Wang* *Shunqing Xu* *Baojun Wang*