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EBNet Environmental Biotechnology

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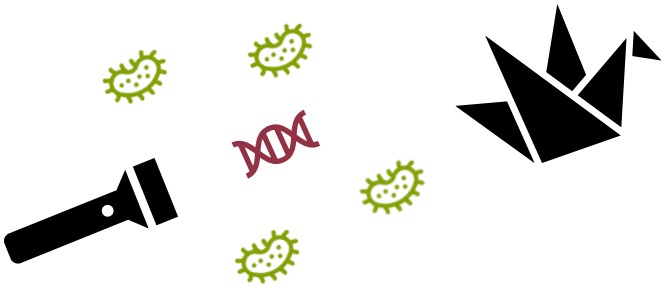
CRISPR/Cas-enabled paper-based sensors for rapid monitoring of antimicrobial resistance

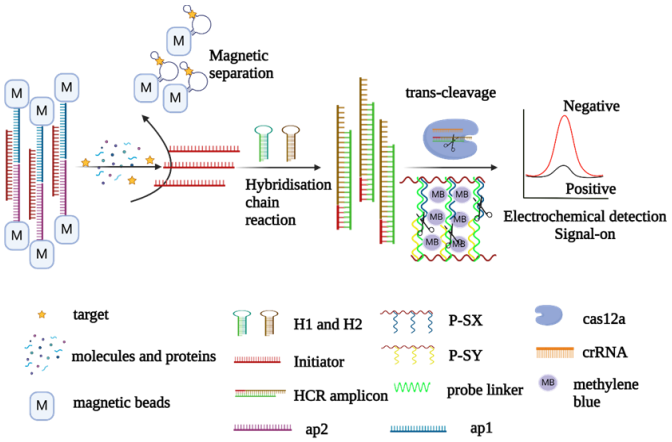
Aim: Environmental contamination with pathogenic bacteria and chemical pollutants is a global issue. However, current analytical methods for environmental samples are challenged by the complexity and heterogeneity of the matrices, as well as the ultra-low concentrations of the analytes.

Recently, beyond its extraordinary genome editing ability, clustered regularly interspaced short palindromic repeats and CRISPR-associated systems (CRISPR/Cas) have initiated a new era of biosensing applications due to their high base resolution and isothermal signal amplification, providing ultrasensitive, single-molecule level and highly specific sensing.

This capability can significantly improve the detection of low-level contaminants (such as antibiotics, including β-lactams etc.) in wastewater samples and aid in interpreting the detection with the engineering method onto devices for point-of-need monitoring (e.g., paper-microfluidic analytical devices).

This project aims to develop a novel, ultra-sensitive, and low-cost sensing platform to identify chemical pollutants in the environment which will leverage the CRISPR/Cas platform to offer a range of next generation of environmental sensors for rapid and on-site monitoring of chemical contaminants.





Scheme of antibiotics detection platform of hydrogel-based CRISPR/Cas assay

Outcome: a hydrogel-based HCR-CRISPR/Cas bioassay was designed for kanamycin detection. More specifically, we designed a signal amplification strategy to enhance the signal of antibiotics using an aptamer to specifically target kanamycin. The hydrogels wrapping methylene blue particles were synthesised and characterised through square wave voltammetry (SQW), which can be used as redox marker for electrochemical detection of antibiotics. We spent a long-time understanding the binding between the selected aptamer and antibiotics using simulation, and we found it's important to identify the high affinity aptamer against the antibiotics.

We will need more time to optimise the CRISPR/Cas system for signal amplification, and further evaluate the limit of detection, and use the optimised assay to detect antibiotics in real samples.

Further reading:
Review of paper-based microfluidic analytical devices for in-field testing of pathogens
By: Li W, Ma X, Yong YC, Liu G & Yang Z. In: Analytica Chimica Acta, 1278 (October) Article No. 341614.