Development of a paper-based cell-free expression device for biological sensing

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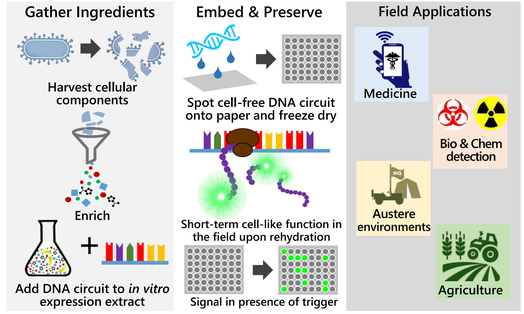
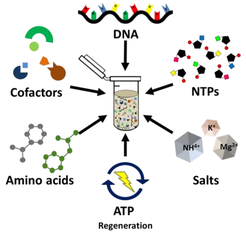
Abstract

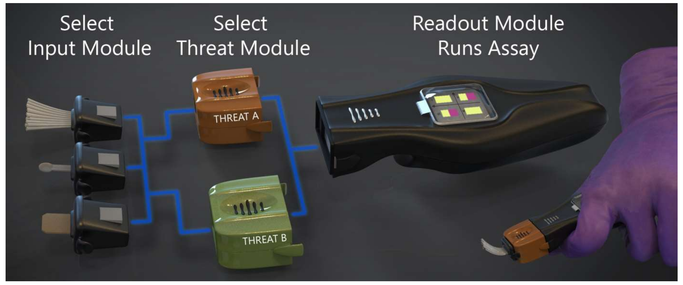
Most biological detection technologies have limited utility in the field because they require specialized laboratory equipment and training. Thus the current state of the art for unpowered on-site biological assays remains the lateral flow immunoassay (LFI), despite its reliance on cold chain storage and the long time required to develop against a new target. Exploiting cell-free expression systems (CFEs) for detection alleviates several of the disadvantages of LFIs, adds a tremendous amount of flexibility, and maintains the crucial features of being unpowered, small, light, and easy to use.



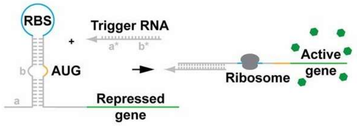
The use of CFE allows most natural genetic regulation mechanisms to be exploited as elements in a biological-sensing scheme, meaning that nucleic acids, proteins, and small molecules are all candidates for detection. Herein we explore the integration of CFE into a paper-based device for biological detection. In our system, a new sensing scheme is deployed as DNA, not a new device, and as such deploying a newly developed sensor carries a relatively low burden. Our work has included the design and testing of novel sensors against biological agents, reducing the time-to-detect, exploring several colorimetric outputs, and integration of multiple assay components into a single paper-based device.



Concept

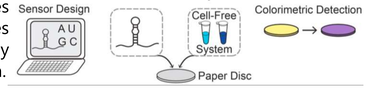


This project aims to integrate advancements in cell-free biology, synthetic biological detection circuits, 3D printing, and 'paperfluidics' to produce a means to perform next-generation biological (and eventually chemical) detection and identification in the field with minimal user burden.



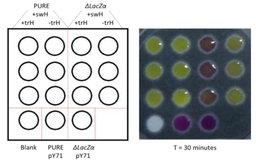
Our first prototypes make use of RNA toehold switches of the type first pioneered by Pardee and Green. These switches prevent a reporter gene (in our case LacZ) from being expressed until a trigger sequence is present to remove a designed hairpin structure.

The design workflow involves computational screening of switches against a target of interest followed by microplate-based screening for function.



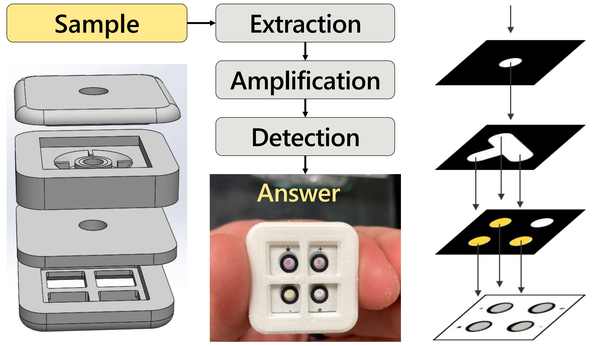
To date we have developed or adapted switches against pathogenic bacterial species, SARS-CoV-2, innocuous "simulant" bacterial species, and arbitrary synthetic sequences.

After switches are validated on standard microwell plates using fairly conventional laboratory methods, they are moved into a medium-throughput screen on paper tickets, using the same lyophilized cell-free lysates that are used in our prototype devices.



Prototyping

Validated switches are run on a prototype cartridge containing a multi-layer "3D paperfluidic" stack. The paper layers are wax-impregnated to define flow channels, and a combination of vertical and horizontal flow allows a single introduced sample to be split to pick up different reagents for (currently) multiple control reactions and (eventually) multiplexed identification reactions.



The "Dial a Threat" Assay: The design of our prototype is highly modular, with the main conserved element being high-performance lyophilized cell-free lysate. By swapping out modules the user can perform additional amplification reactions, and by putting different DNA sequences in the sample buffer, entirely different threat identification assays can be performed on the same device.

More Information

More information on what's "under the hood" is available on other DEVCOM CBC posters, which are available online.

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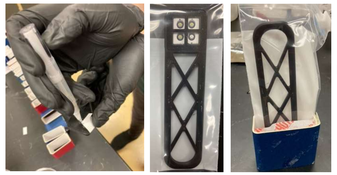
https://cbc.devcom.army.mil/cbdst-conference/

Poster 433: "Alternative reporters for paper-based cell-free biosensing"

Poster 439: "Optimizing cell-free reaction composition to accelerate toehold switch sensor response time"

Assessment

100 prototype assays were taken to the 2022 CBOA event for use and assessment by service members. One of our main objectives, low burden, was met as the devices were easily used with minimal training. Feedback about how to further improve the devices was received at the event as well.



Left; Sample brush or dropper is used to load input node, then device is sealed in a clear bag.

Middle; Ticket spots change from white to yellow, indicating reaction has started.

Right; Hand warmer is wrapped around device and incubated for 20-30 minutes.

Our approach to deploying synthetic biology for detection and identification in the field has demonstrated to work against multiple targets and has worked in simulated field conditions with non-expert users.

The ability to "reprogram" a stockpiled assay could provide a tremendous advantage in mitigating the impact of a novel or emerging threat.

Acknowledgments

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