



A field-reprogrammable toxin detection platform

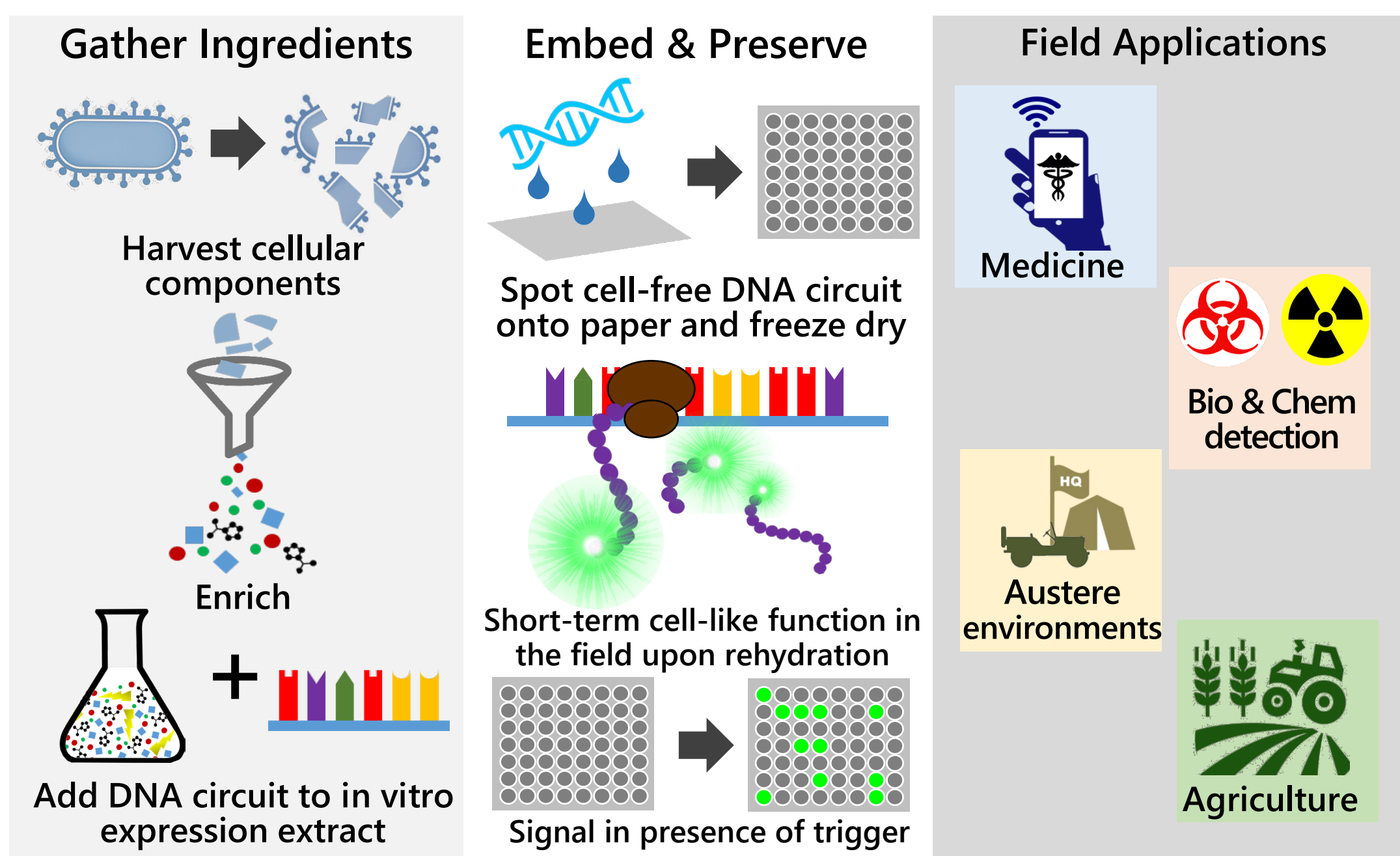
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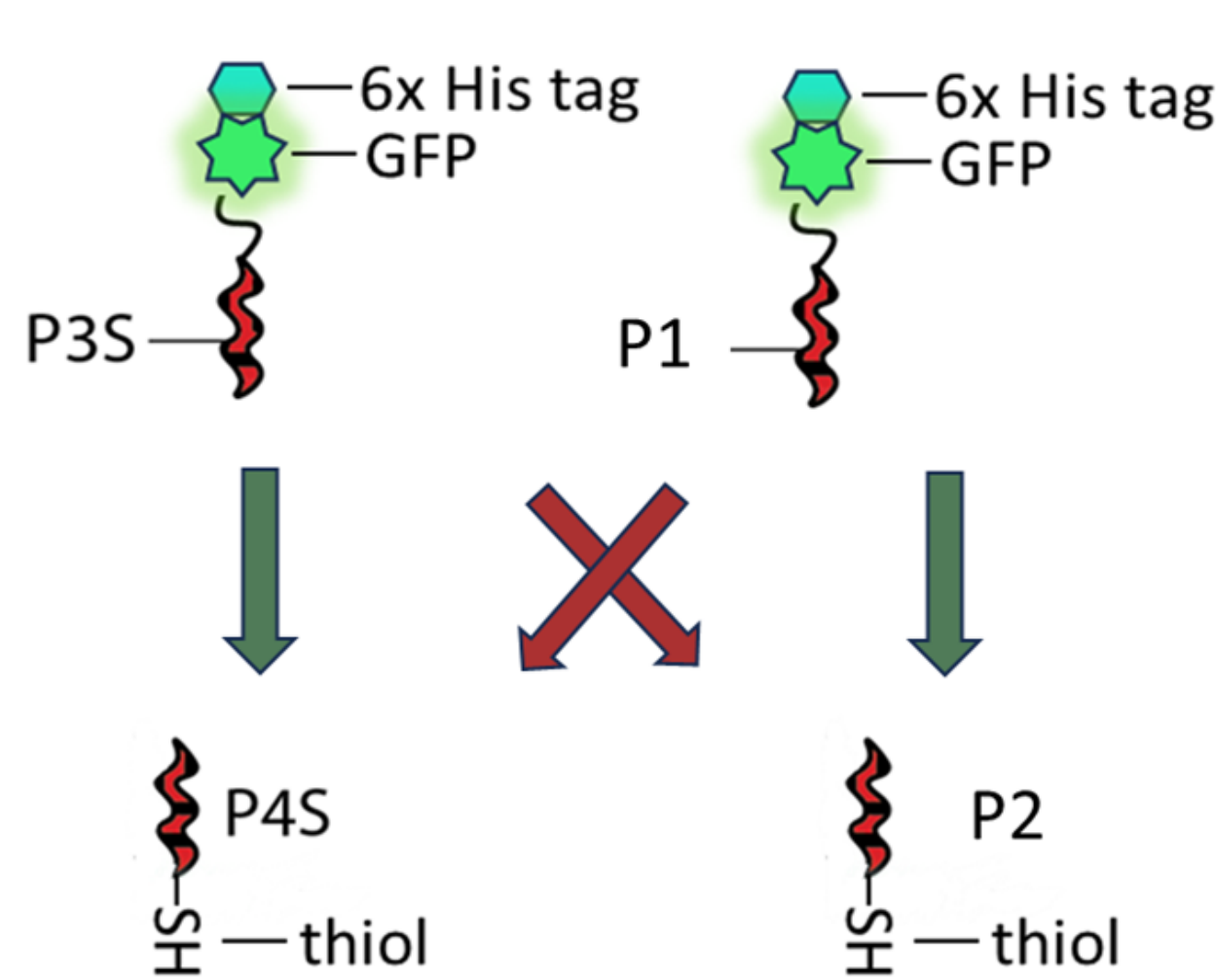
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Abstract

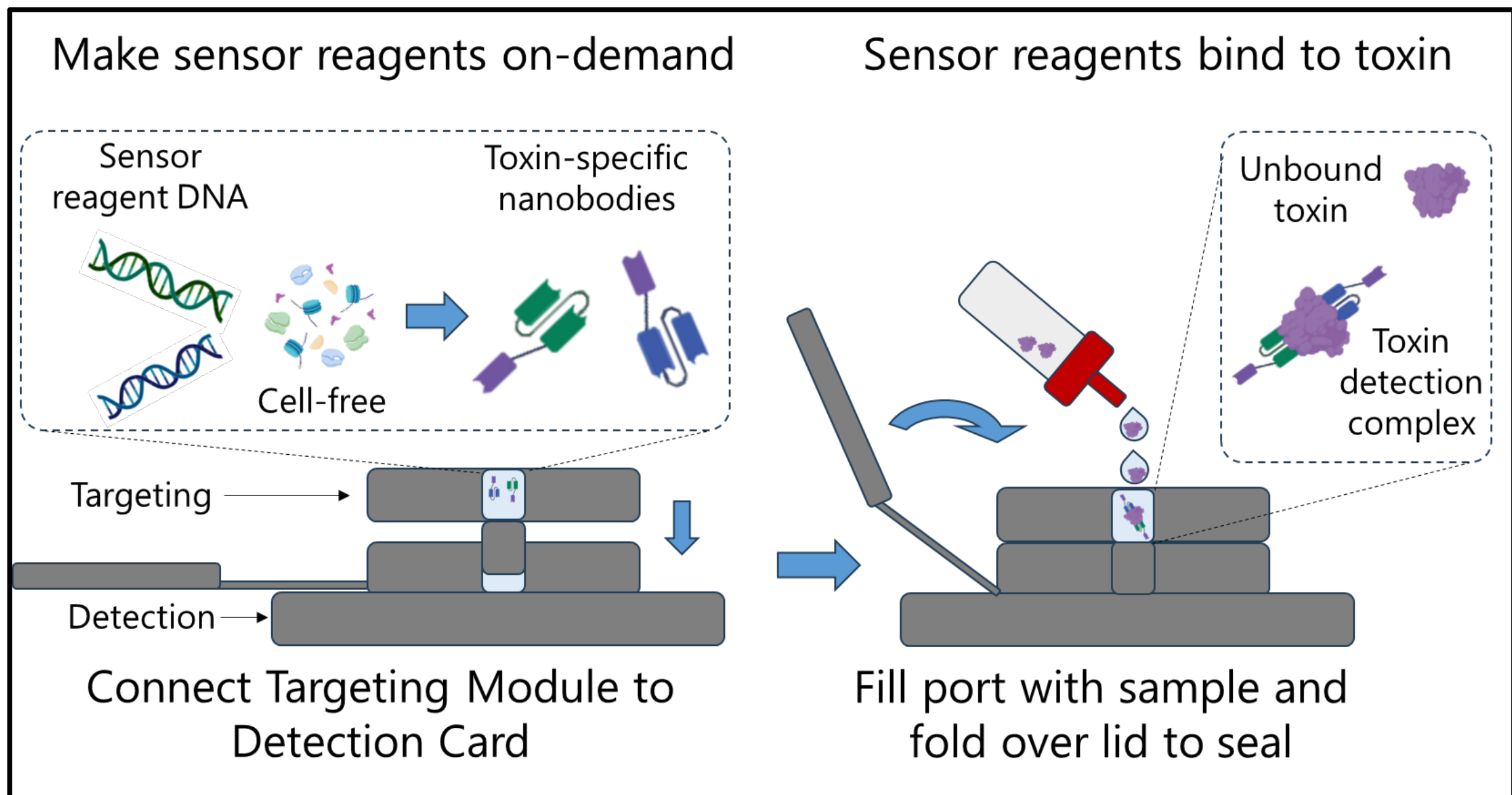
Lateral flow immunoassays (LFIs) enable rapid detection and diagnostic capabilities with relatively little training required. Antibody-based affinity capture reagents are the current gold standard for LFIs, but traditional LFIs have a shelf life dependent upon the date of manufacture, aren't reprogrammable, and frequently only target one antigen per test. By exploiting cell-free expression systems (CFEs) to produce our affinity reagents for a bioidentification device on demand, we can remove these potential disadvantages and bolster device designs for ease of use by the Joint Force. Within the Dial-a-Threat: Antigen device, lyophilized CFE lysates are rehydrated with nucleic acids encoding the individual components of a detection assay, allowing a stockpiled "blank" device to be functionalized at echelon. This strategy could facilitate detection capabilities during a distributed operation by lowering the logistical burden of manufacturing, storing, and shipping a new sensor or diagnostic device. Here, we demonstrate proof-of-concept by using this system to detect a non-mammalian protein toxoid, but the device could be readily reprogrammed to detect additional threats or diagnostic targets by changing the input nucleic acid "instructions".



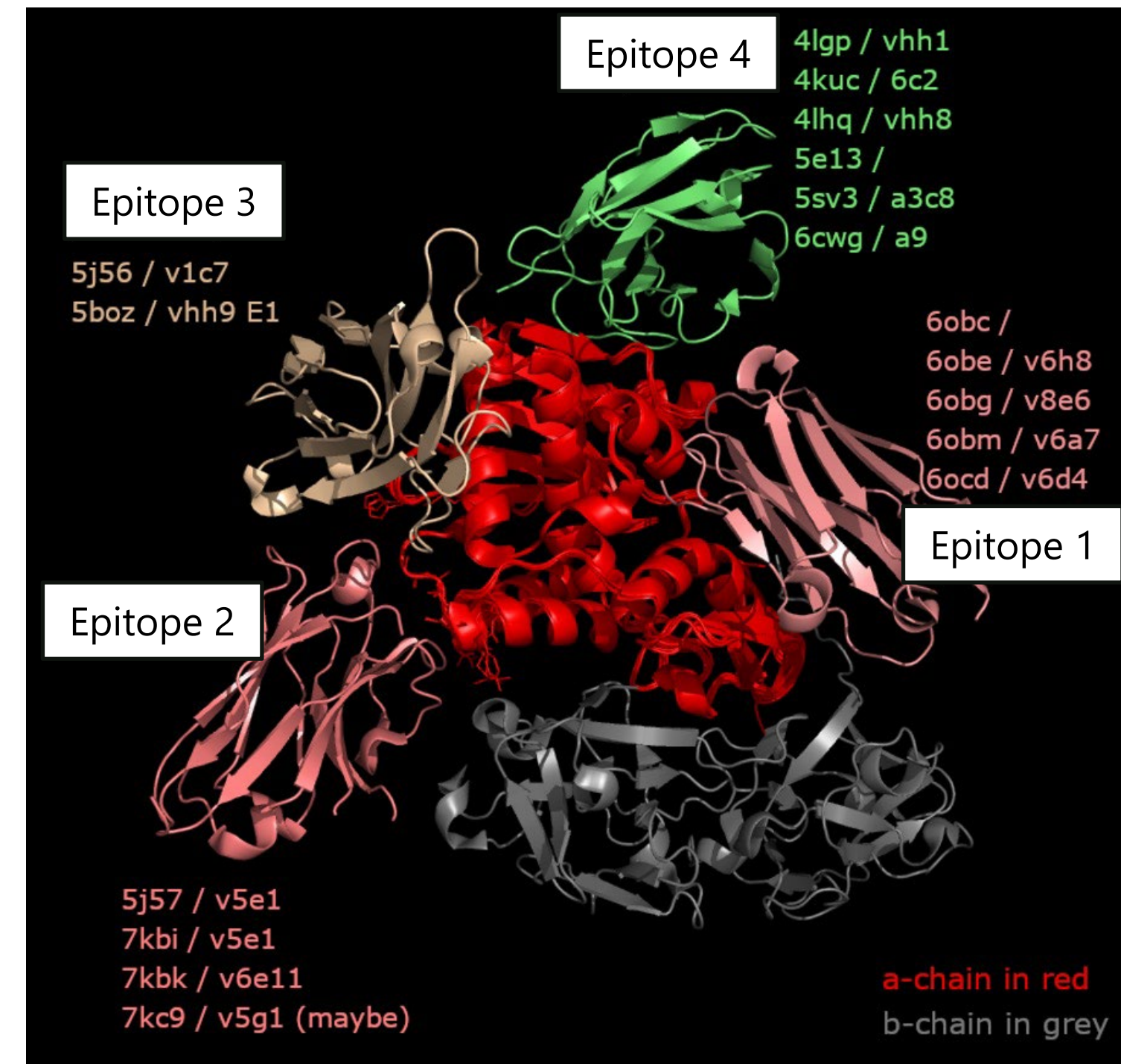
We incorporated a system of orthogonal helical peptide coil fusions to enable localization of sensor components (right). Here, only the green fluorescent protein (GFP)-tagged P3S coil should bind to the immobilized P4S coil, and the GFP-tagged P1 coil should only bind to the immobilized P2 coil.



Concept

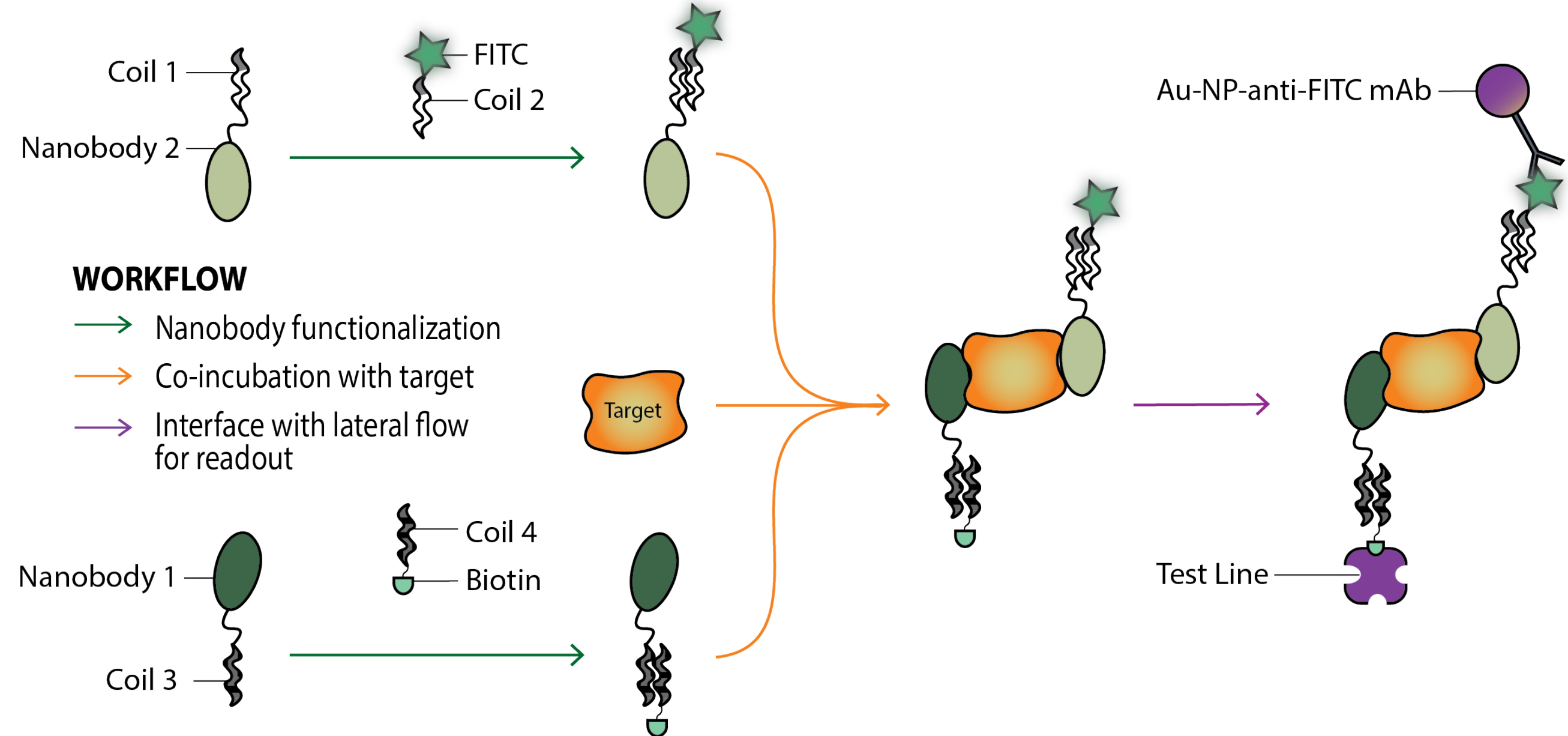


This project aims to integrate advancements in cell-free biology, synthetic biological detection circuits, 3D printing, and 'paperfluidics' to produce a means to perform next-generation biological detection and identification in the field with minimal user burden.

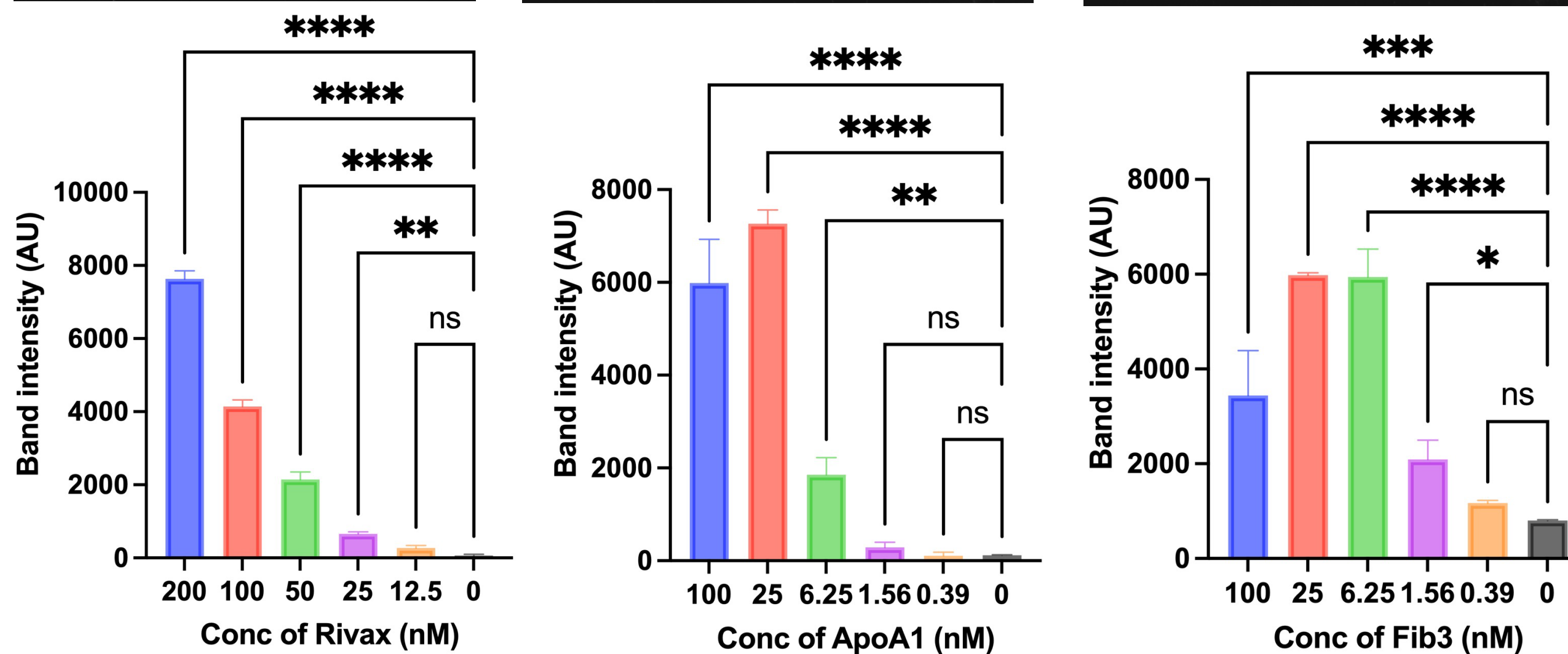
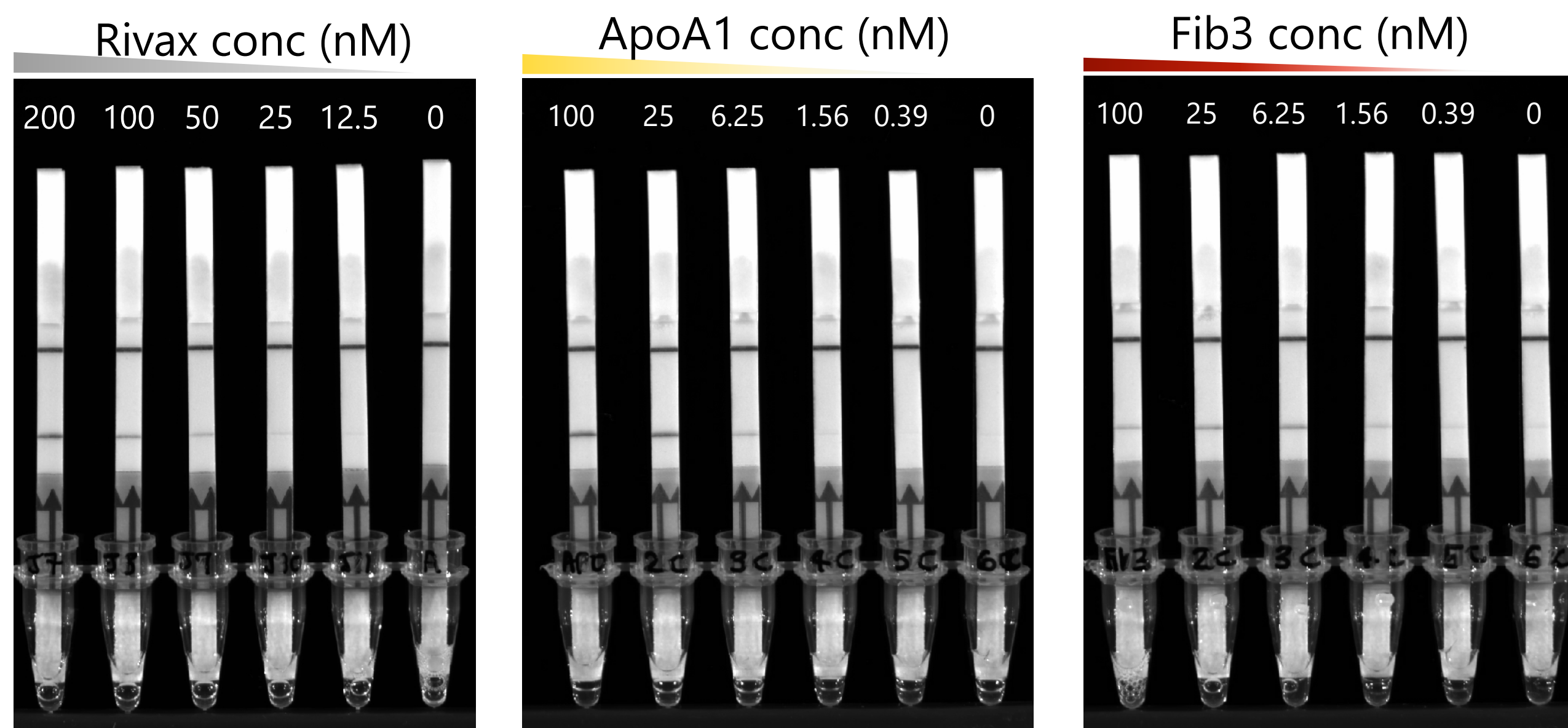


The system uses toxin-specific nanobodies to form an ELISA-style "sandwich" on the target. The nanobodies shown here in pink, purple, yellow, and green bind to the different epitope sites on the protein-based toxin of interest (red and grey). Nanobodies from two different epitopes are fused to the orthogonal coils for targeting, reporter localization, and sensor reprogramming.

Breadboard

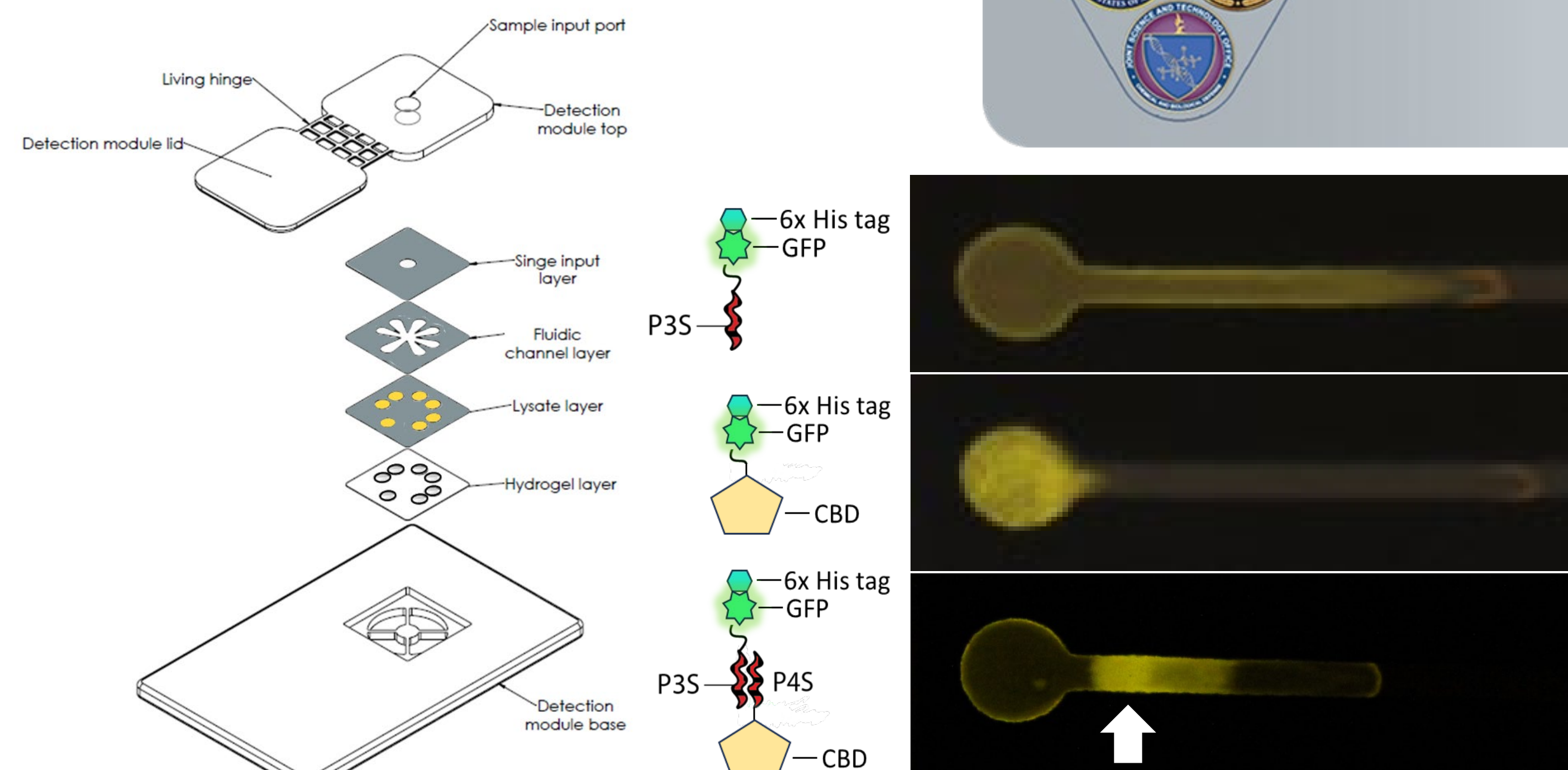


The nanobody coil fusions and their corresponding anchoring or reporter coil partners assemble the detection complex in the presence of the target. By swapping out the nanobody coils, we can reprogram the same stockpiled assay to identify a different target.

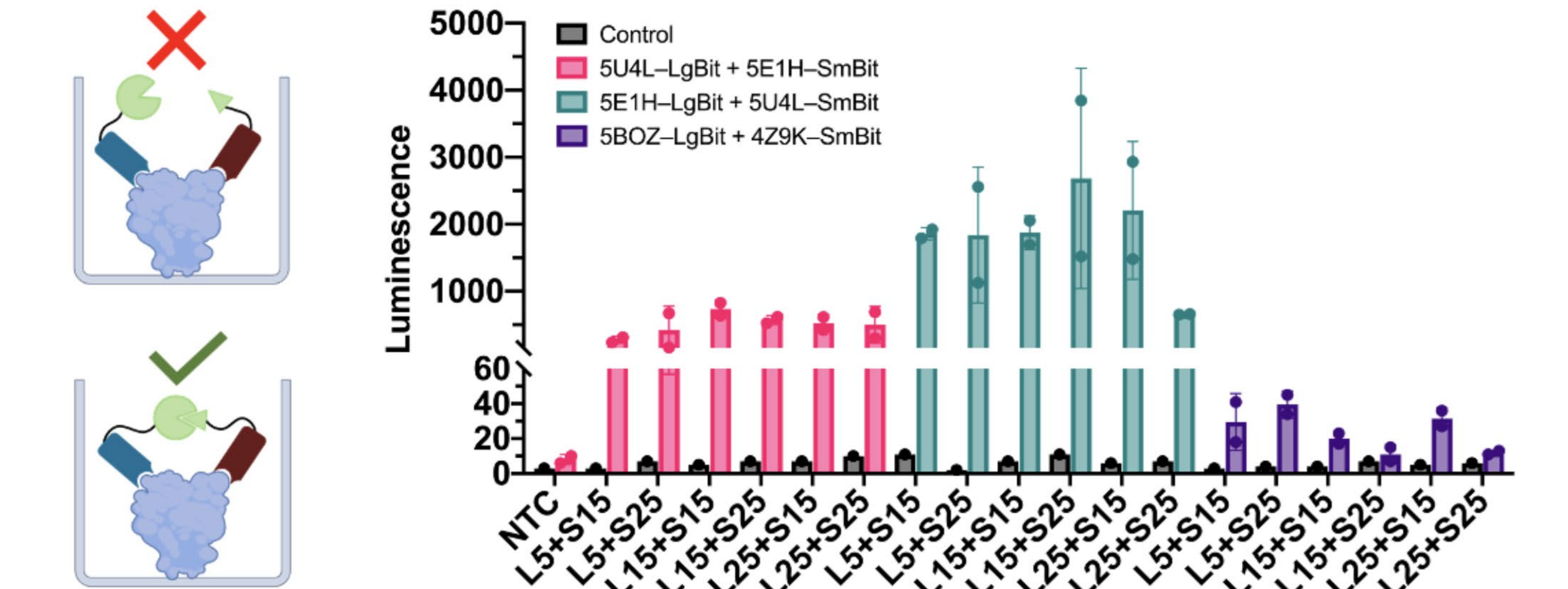


The localization and reporter coils were tagged with nanobodies targeting the vaccine candidate Rivax (left), a risk marker for cardiac disease Apolipoprotein A1 (middle), and the clotting factor Fibrinogen 3 (right). The same type of test strip was used for each assay to demonstrate the modularity of this system.

Prototype Integration



To incorporate this system into a paperfluidic Dial-a-Threat device (left), modifications to the anchoring mechanisms are needed. Here, a cellulose binding domain (CBD) coil fusion is used in lieu of biotinylation to localize the GFP-tagged coil to a specific region of the wax-printed paper channel (white arrow, right).



We are investigating alternative reporter strategies for our device prototypes. The split enzyme reporter shown here is only functional when both parts are brought together through binding of each nanobody fusion to different epitopes on the same toxin (left). When Rivax is present, the night vision optics-compatible split luciferase reporter activates, producing luminescence (right).



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