

Cell-Free Water Quality Sensor for Detecting Toxic Heavy Metal Contamination in the Field

AFRL

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Background and Relevance

Assessing the safety of drinking water in remote and resource-limited environments is critical to maintaining the health of our warfighters

Army Guidelines

Brigade combat teams should be able to operate seven days without resupply¹
Require carrying 105 L (~235 lbs.) of water per person



Logistics

Increased susceptibility and logistical burden
Requires collection of indigenous water sources
Risk of Contamination: High levels of arsenic are common in S/SE Asia

Risks

Arsenic → partial paralysis and diarrhea; Mercury → kidney and nervous system damage; Cadmium → severe coughing and nausea
Detection of heavy metals is critical due to their severe toxicological effects and ability to persist in the environment

Limits

Current water evaluation can take 48 hours up to 8 weeks
Field samples often need to be shipped to a lab, assessment kits contain hazardous chemicals, and cost ~\$100 per test

There are both advantages and challenges with biosensors

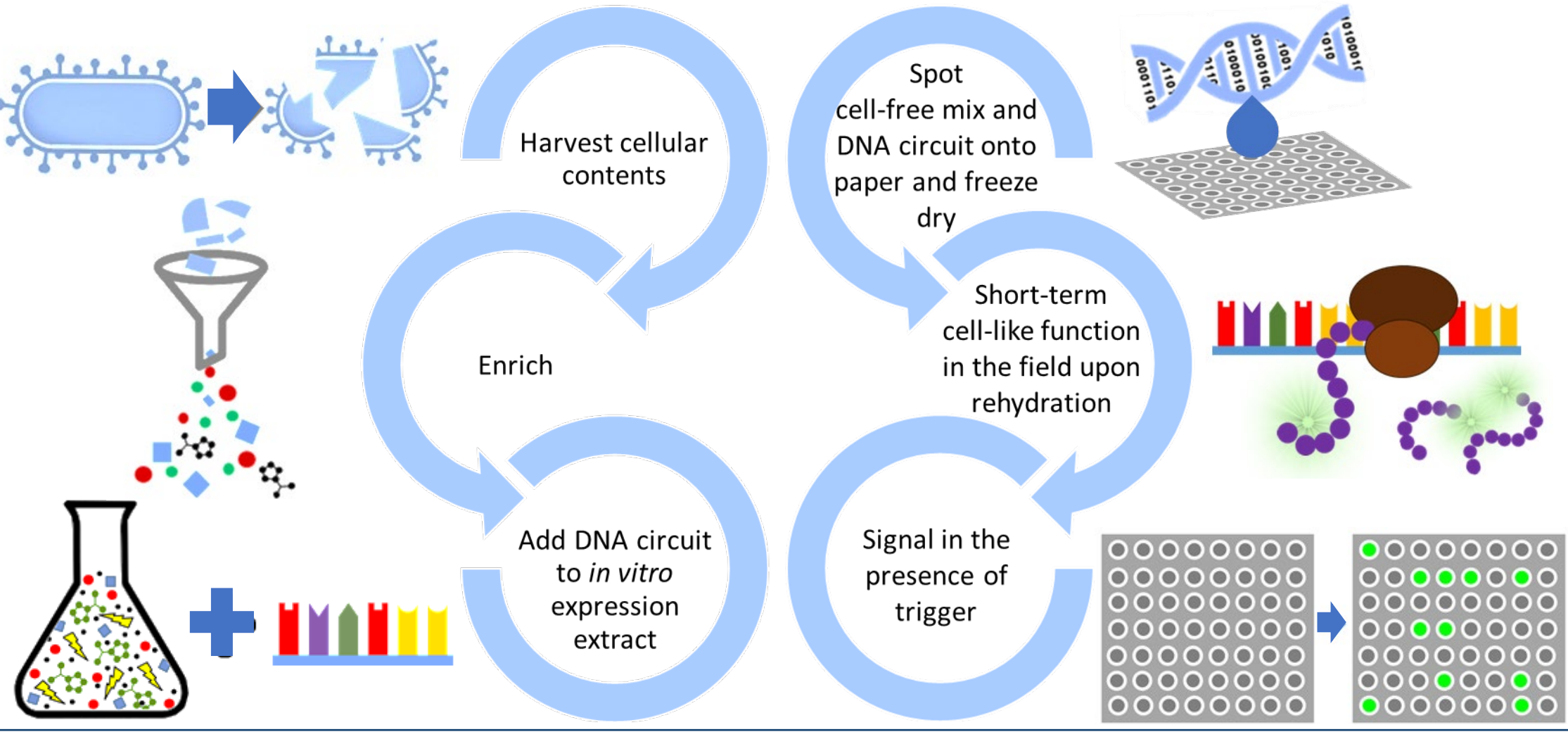
Bacteria routinely detect and dispose of toxic chemicals
Bacterial sensing elements can be highly sensitive, specific, and rapid

Cells need to be maintained and have resources to live
Analytes must get through the cell wall
Releasing GMOs has PR risks, especially for DOW

Advantages

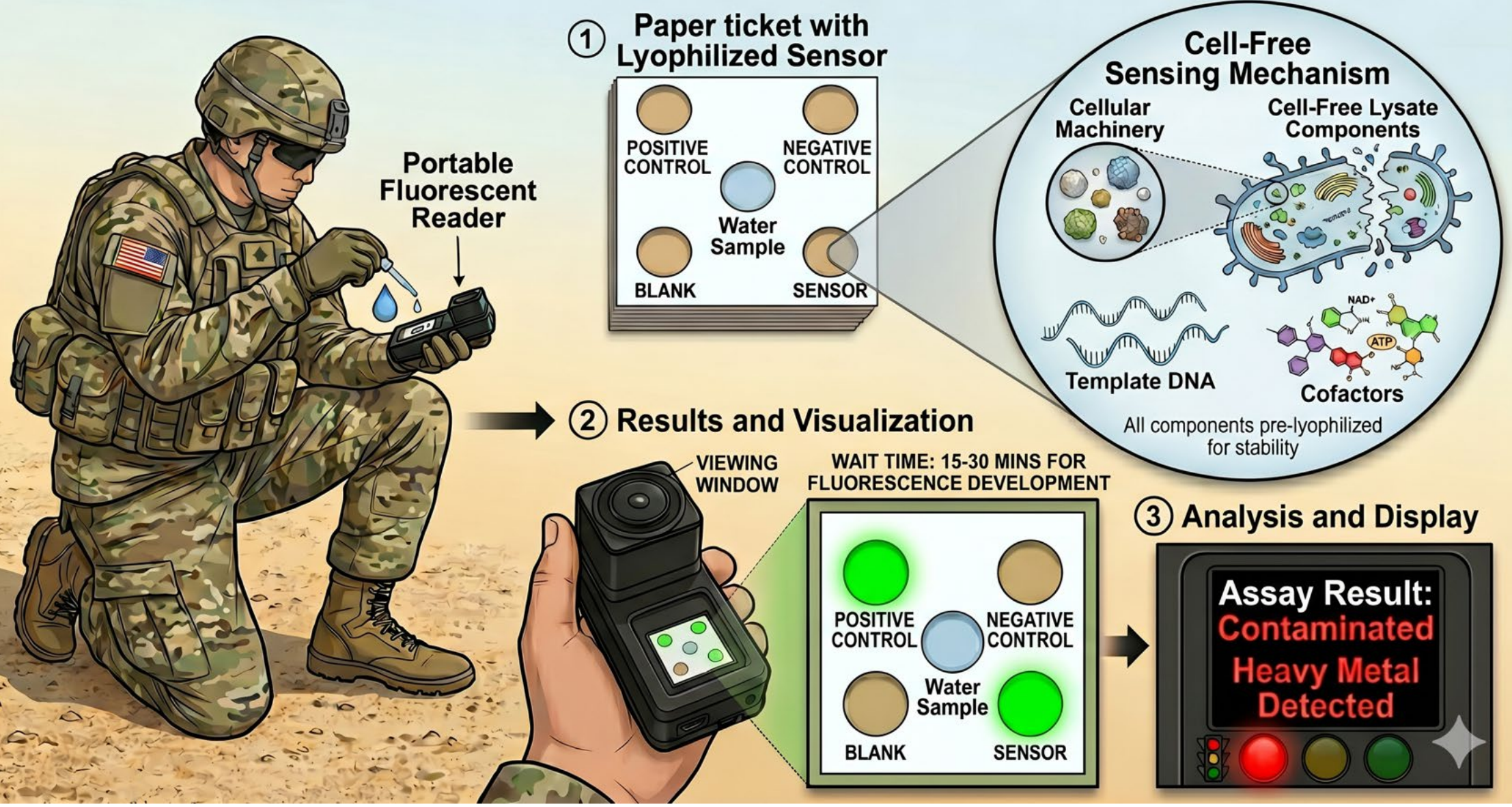
Challenges

Cell-free biosensors could provide a light-weight, cheap, deployable water quality test to help address these challenges



Technical Objective

Develop a field-portable, cell-free system to detect toxic heavy metals in drinking water at, or below, military exposure guidelines (MEG) in under 60 minutes.



Cell-free Design and Performance

Beabout, Bernhards, Thakur, et al. 2021. ACS Synthetic Biology.	Water Quality Standard	WHO Guideline	MEG (7 days, 5L/Day)	MEG (1 Year, 5L/Day)	Cell-Free Sensor LOD	Time to Detection (min)
Elemental Arsenic As ³⁺	μM	0.133	4	0.8	0.125	20
Elemental Cadmium Cd ²⁺	μM	0.027	0.5	0.062	0.5	25
Mercury Chloride Hg ²⁺	μM	0.022	-	0.155	0.011	25

Elemental Arsenic As³⁺

SENSE: P_{70a} → riboJ → 5' UTR → arsR → ArsR → ArsR-As³⁺ complex → Repressor linear construct.

RESPONSE: P_{ars} → riboJ → 5' UTR → sfGFP → Reporter linear construct.

Arsenic (DEVCOM CBC)

Elemental Cadmium Cd²⁺

SENSE: P_{cadC} → cadC → Cd²⁺ → Repressor / reporter plasmid construct.

RESPONSE: P_{cadC} → cadC → Cd²⁺ → sfGFP → MGapt.

Cadmium (NRL)

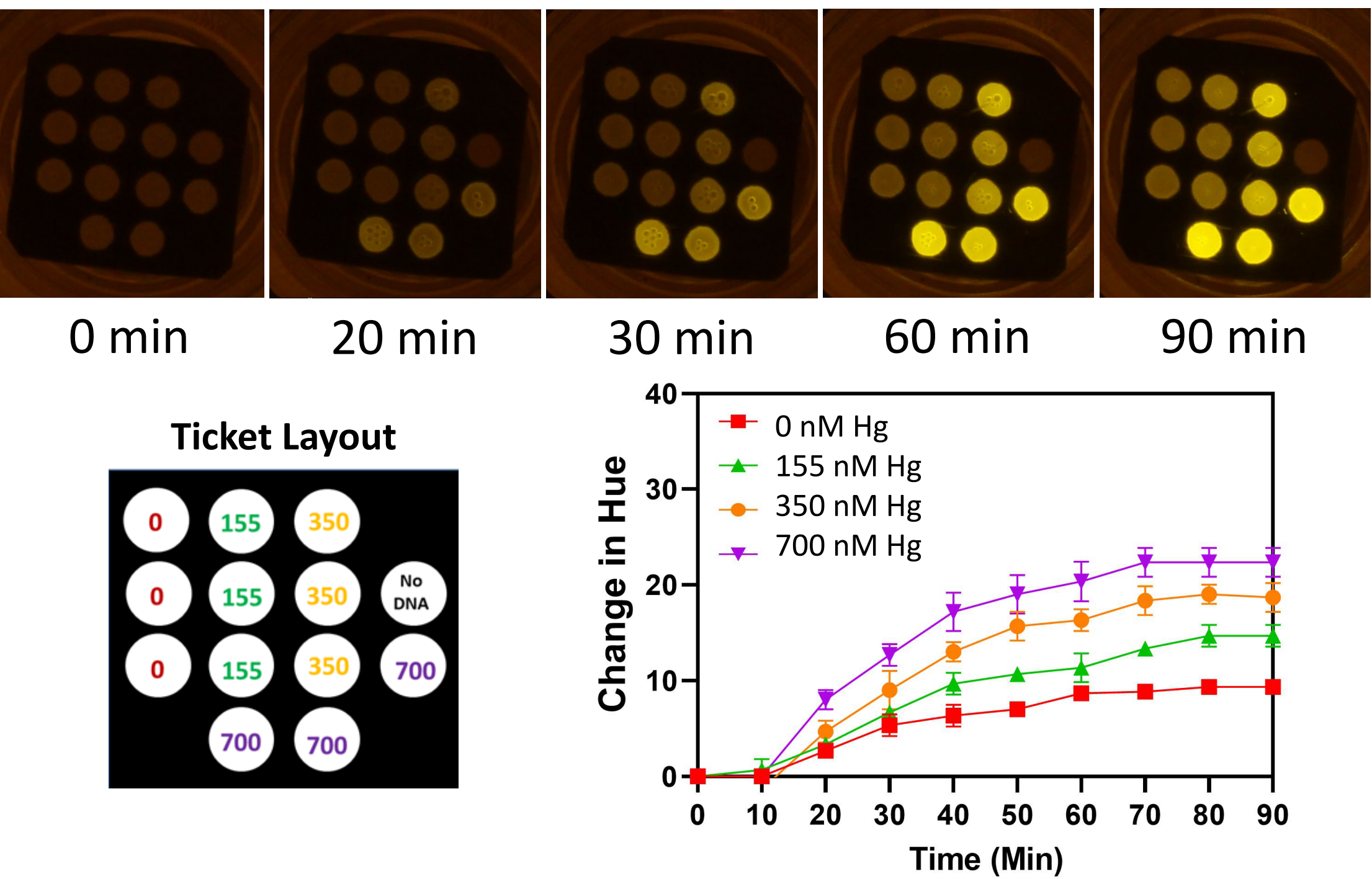
Mercury Chloride Hg²⁺

SENSE: P_{BBa_J23104} → merR → Hg²⁺ → merR → Activator linear construct.

RESPONSE: P_{T7} → merT → Hg²⁺ → sfGFP → Reporter plasmid construct.

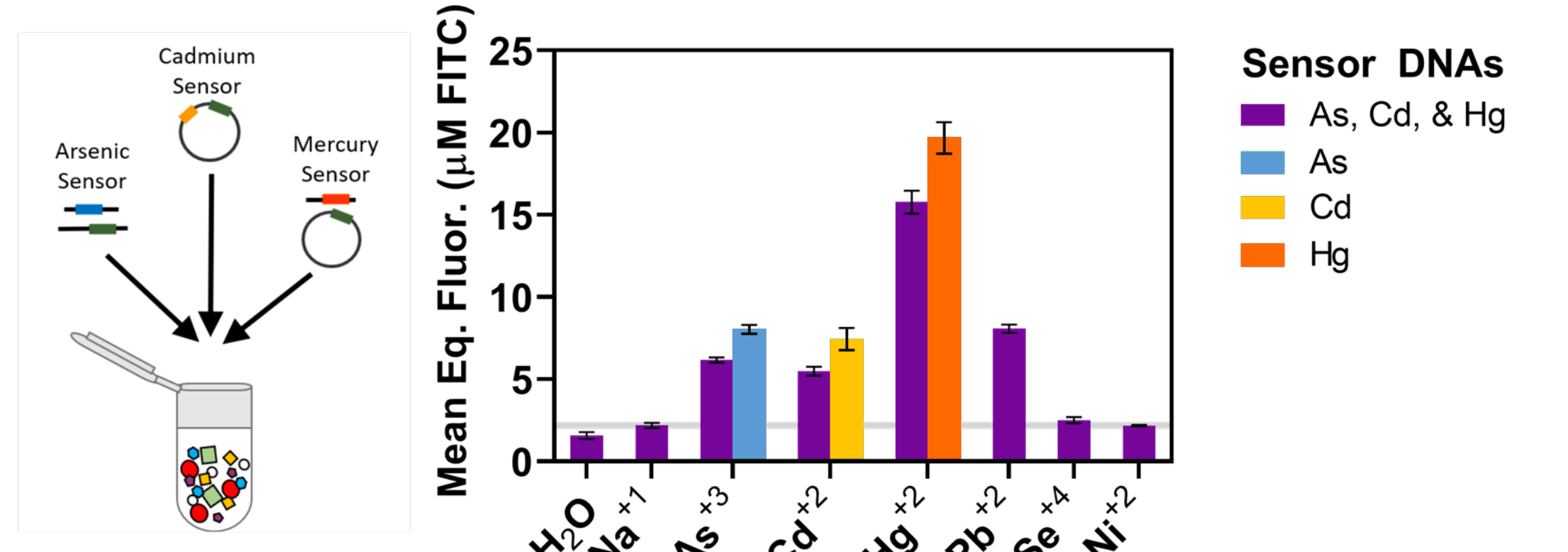
Mercury (AFRL)

Paper Ticket Performance – Mercury Sensor



Multi-Metal Assay

Combined DNA for all three sensors into one reaction



Ongoing work

- Identifying additional heavy metal and water contaminate sensors
 - Shelf-life stability testing of lyophilized reactions
 - Optimizing the multi-metal assay
 - Upscaling and manufacturability
 - Integrated heating element
- Planned Transitions:**
- Short-term: Replacement for existing platoon-level arsenic test kit
 - Future: Individual level multi-metal capability
 - Industry: At home or municipal water testing

Tri-Service Team:
DEVCOM Solider Center
DEVCOM Chemical Biological Center
NRL
AFRL

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References

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