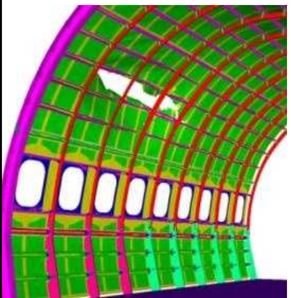
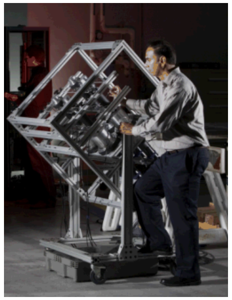
SAND2017-7004C

Single-Step, One-Pot, DNA Amplification & Electrochemical Detection via Loop-Mediated Isothermal Amplification (LAMP)

Jason C. Harper[1] and Robert J. Meagher[2]

[1]Bioenergy & Biodefense Technologies; [2]Biotechnology & Bioengineering, Sandia National Laboratories

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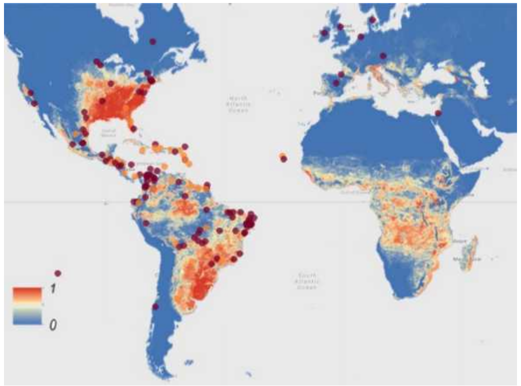
"Deadly diseases like Ebola, Marburg, and Anthrax are prevalent in Africa. These pathogens can be made into horrible weapons aimed at our troops, our friends and allies, and even the American public. This is a threat we cannot ignore." -Senator Lugar, stated during his 2010 trip to Africa

Ebola Outbreaks 2014

Zika Outbreaks 2015-2017



Anthrax Outbreaks 2011

Between 2005-2012 there were 3057 Anthrax outbreaks reported

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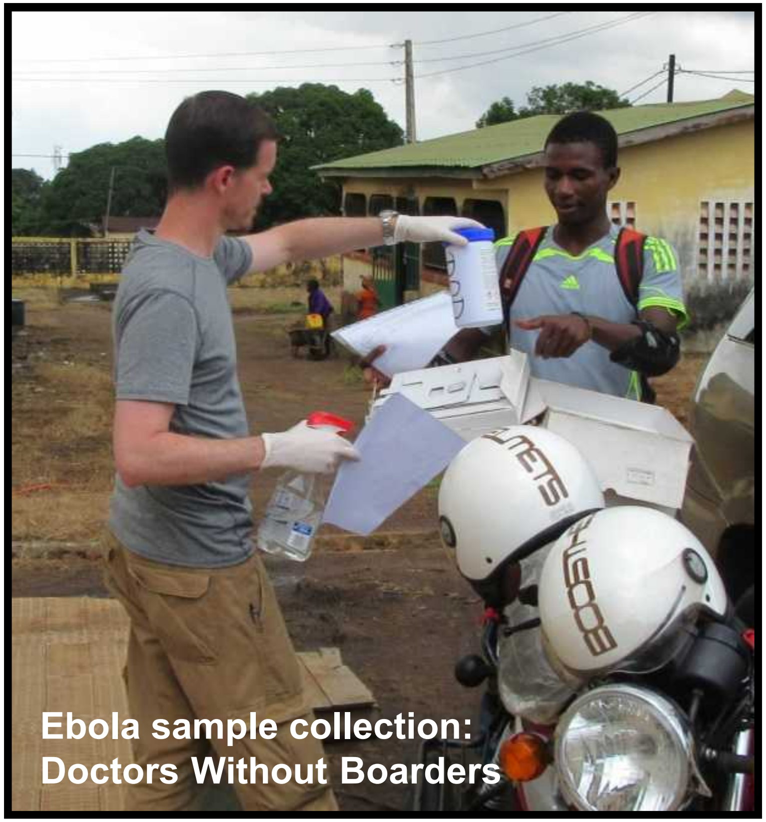
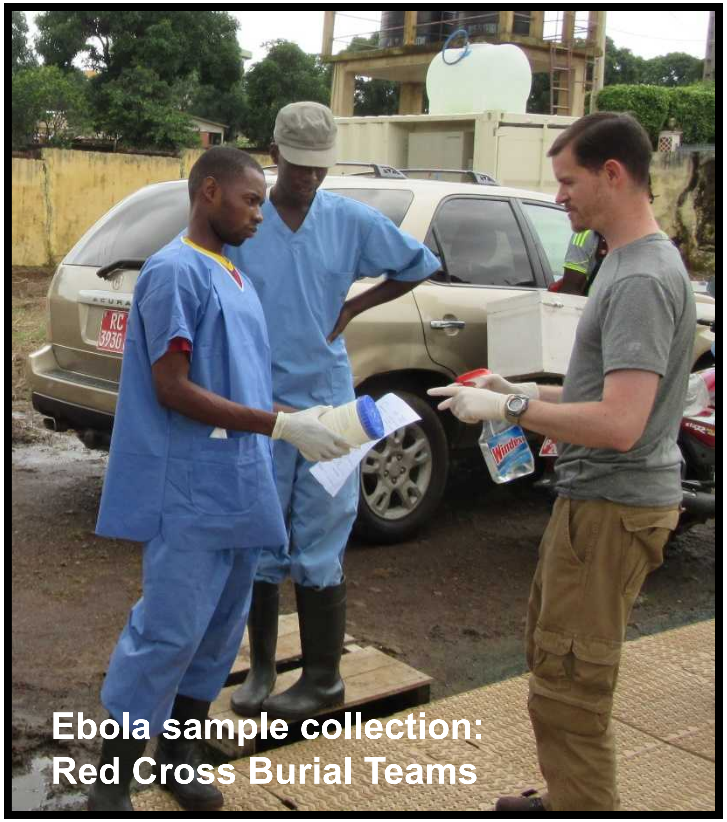
Ebola Outbreak Response Support
Conakry, Guinea



Ebola sample collection:
Doctors Without Boarders



Ebola sample collection:
Red Cross Burial Teams

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Ratoma Ebola Diagnostic Center (REDxC)

3901 samples tested†
63 samples were positive

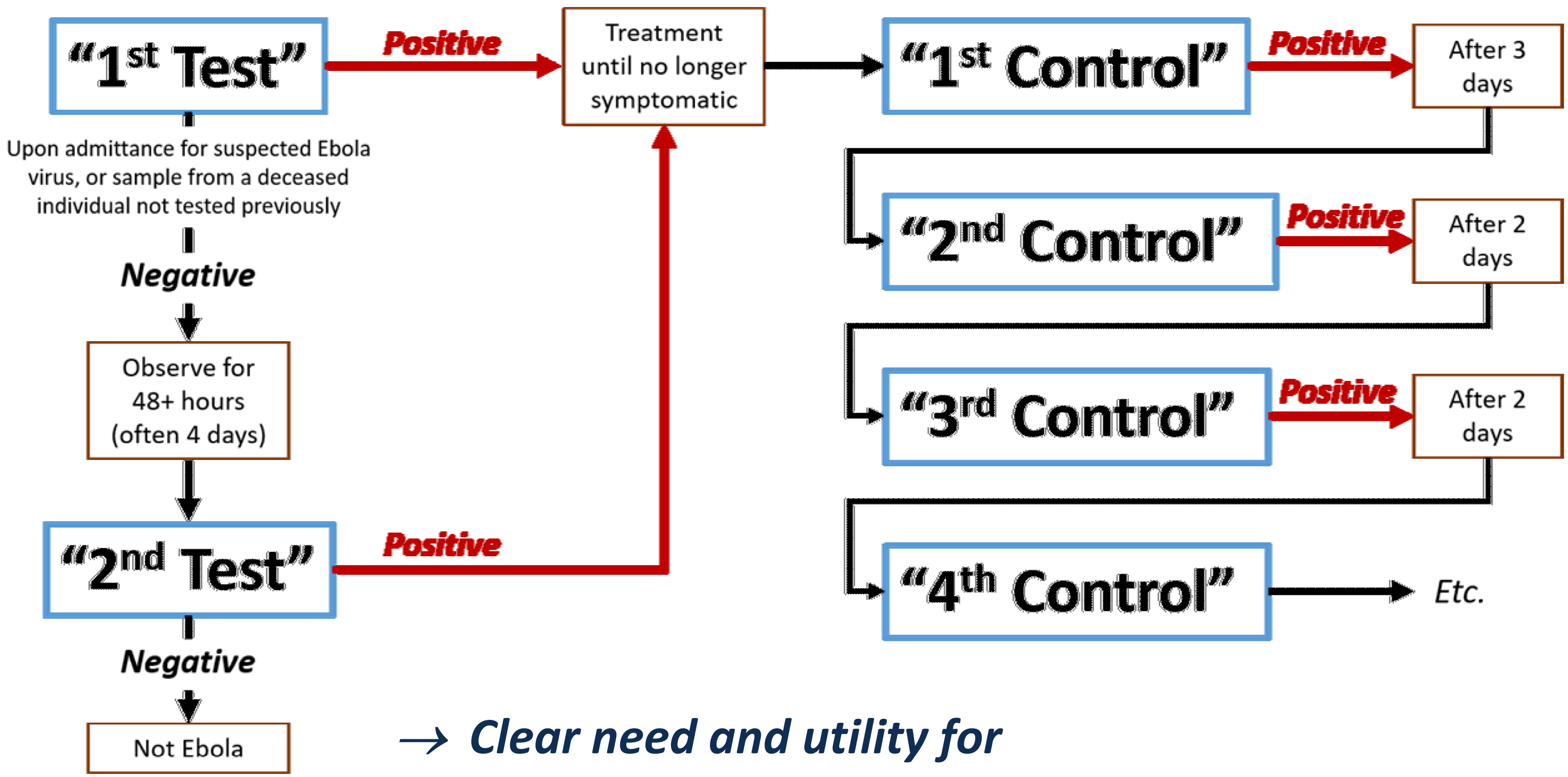
† As of March 27, 2016

Ebola Identification Assay and Test Flow

rRT-PCR assay designed by US Naval Medical Research Center

- Trizol inactivation; BioRad RNA extraction; Applied Biosystems (ABI) 7500 Fast Dx
- Not as sensitive as GenExpert assay
- Other systems in Guinea: BioFire, Genie III, LightCycler Nano, Rotor-Gene Q

Ebola Test Flow

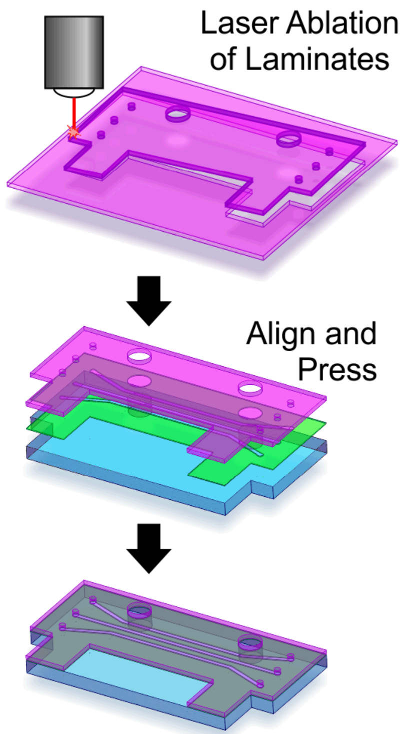


→ ***Clear need and utility for point-of-care diagnostic testing***

Towards Portable Diagnostic Devices for Use in Low Resource Environments†

Laser Machined Plastic Laminates

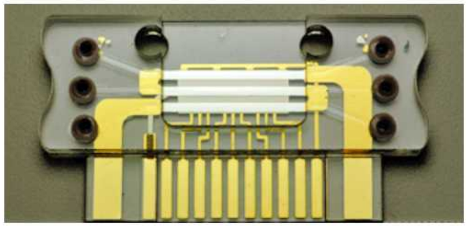
Simple 2D and 3D Fluidic Structures ⇨ **Integrated Materials and Processes** ⇨ **Added Functionality**

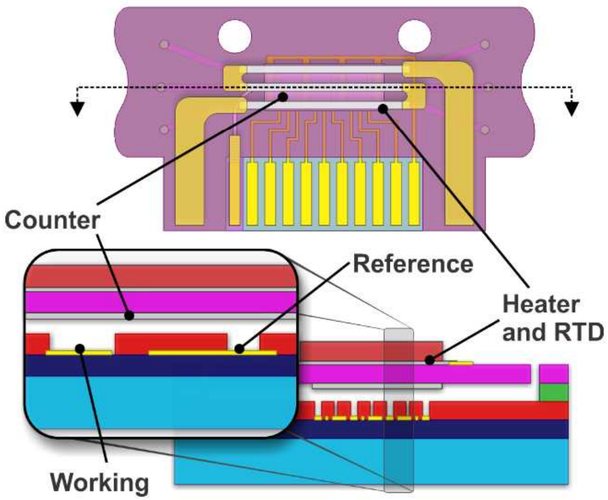


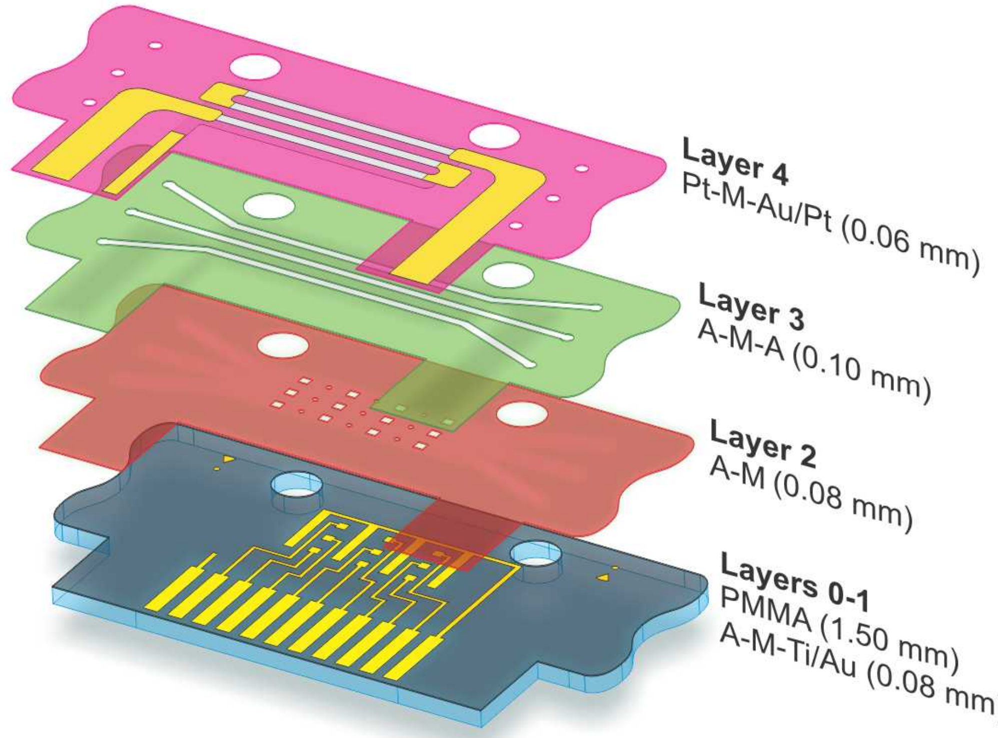
Integrated Materials and Processes		Added Functionality
ELECTRODES Gold Platinum ITO COTS parts	→ Individually Addressable Electrode Arrays →	Electrochemistry Electrochemiluminecense Electro-deposited nanoparticles Selective/catalytic thin films
THERMAL Resistive and conductive mat'ls	→ Heaters RTDs Thermocouples →	Temperature control to denature and anneal double-stranded DNA
	→ Fluid Channels to manage temp. →	
		On-device reagent storage
MAGNETICS NdFeB	→ Fluidic Valves →	Integrated, low-cost, simple fluidic distribution
LASER WELDING of Plastic Laminates	→ Chambers →	Sample capture and storage
MATRICES Silica Gels	→ Stabilize Eukaryote and Prokaryote living cells →	Cell-based biosensing

† Harper *et al. Electroanalysis* **2015**, *27*, 2503-2512

A Parallel Microfluidic Channel Fixture for E-chem & Chemiluminescent Detection of DNA †





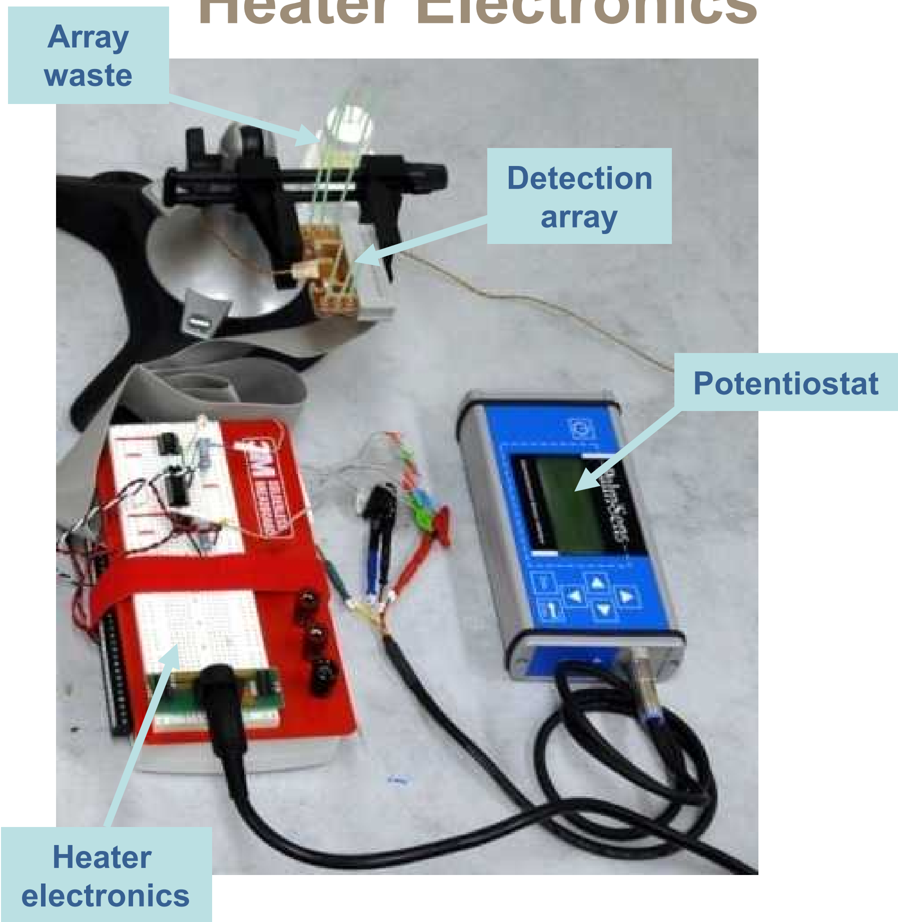


- *Three microfluidic channels*
- *Three working electrodes per channel*
- *Integrated resistive element heater*
- *Versatile electrode materials: carbon, ITO, Au, Pt*

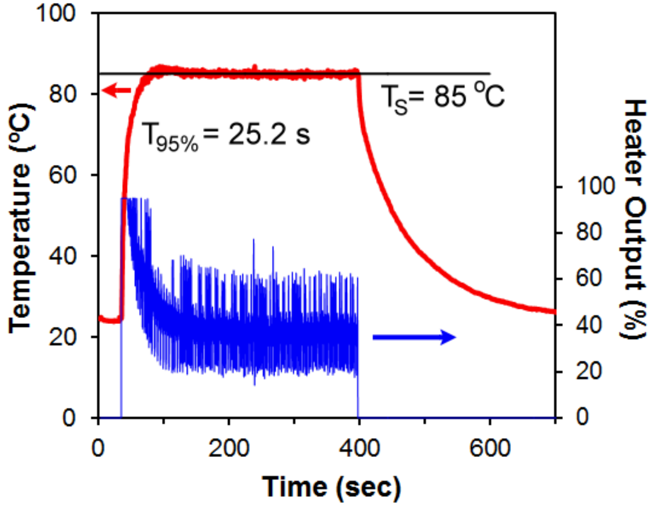
† Edwards, Harper *et al. Biomicrofluidics* **2011**, *5,* 044115

Portable system; dsDNA Melting & Detection

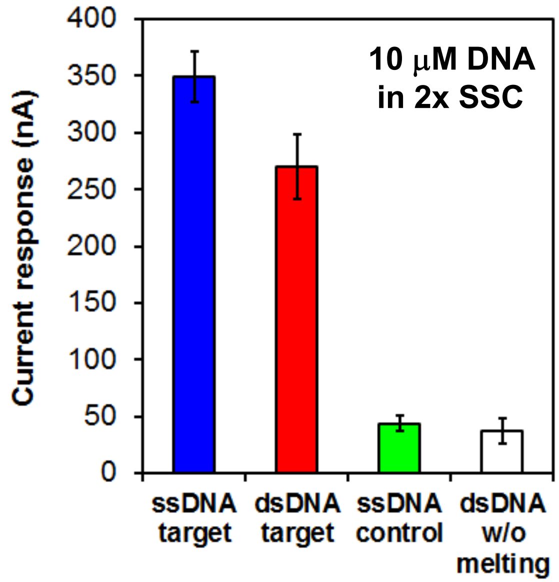
Detection and Heater Electronics



dsDNA Melting



dsDNA Detection



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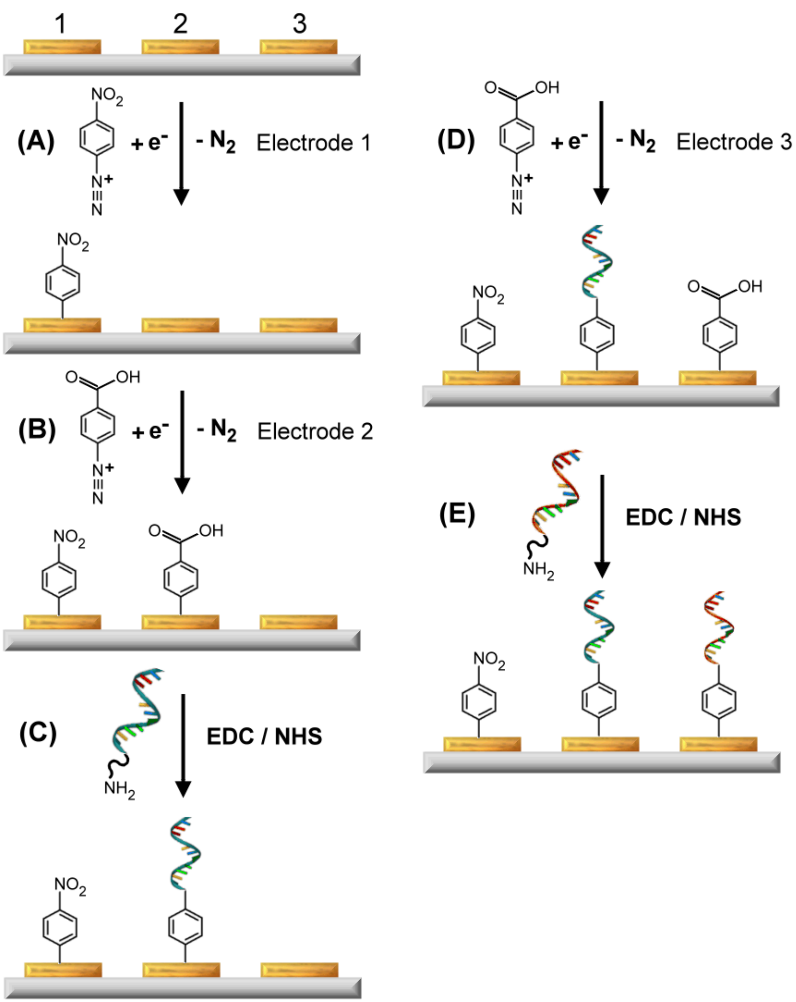
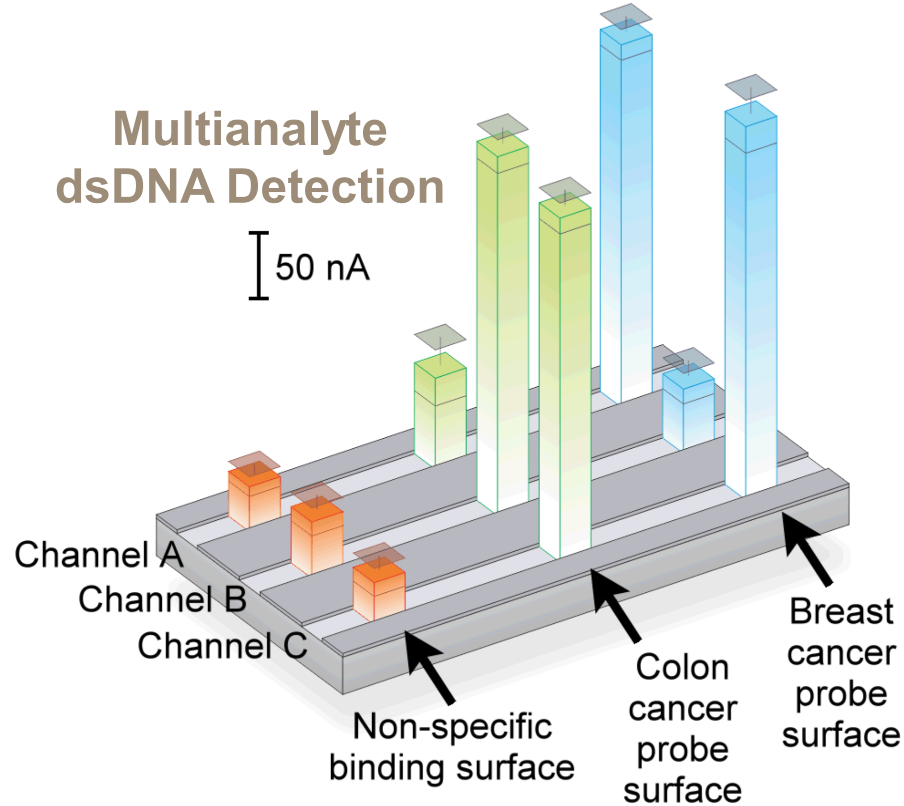
Selective Electrode Functionalization and Multianalyte dsDNA Detection

Selective Functionalization of 3 Element Au Array



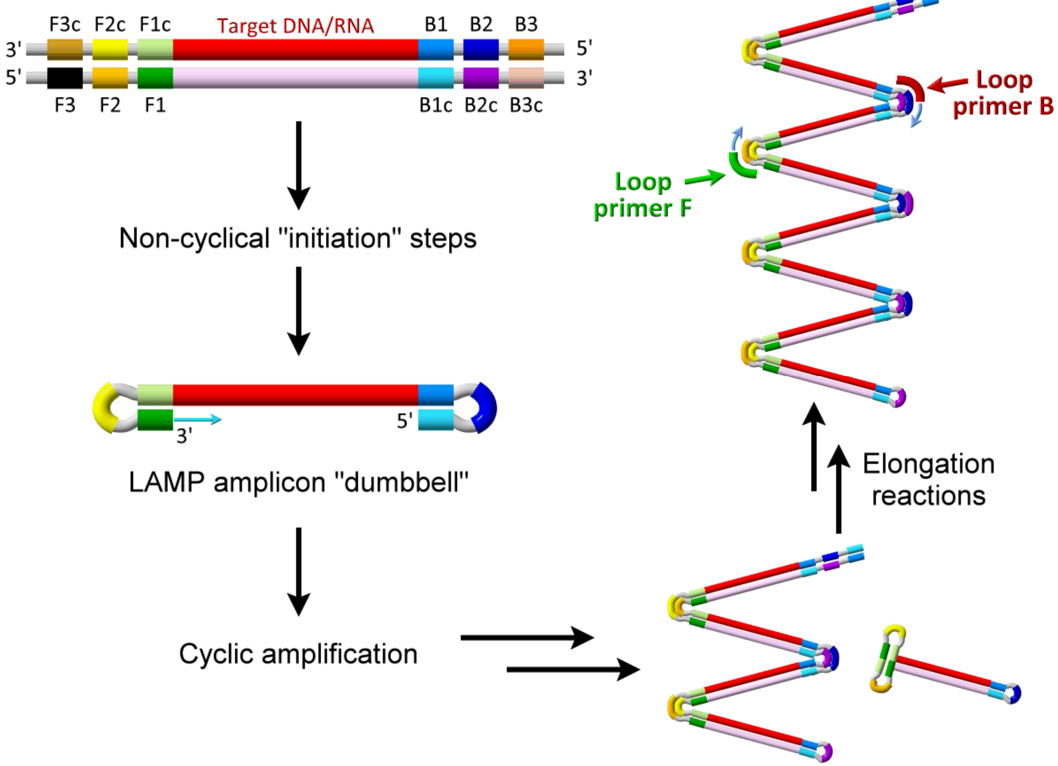
Multianalyte dsDNA Detection



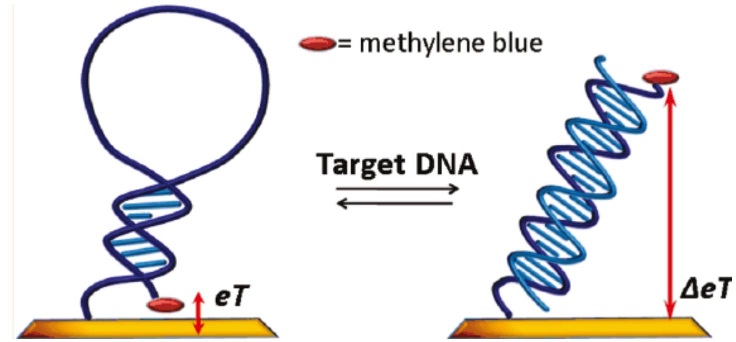
- → *High detection limit (~10 nM)*
- → *Multiple steps (washing, labeling, addition of reagent)*
- → *Not readily adaptable for RNA*

Single-Step, One-Pot, DNA & RNA Amplification & E-chemical Detection

Loop-Mediated Isothermal Amplification (LAMP)



Electroactive Molecular Beacon†

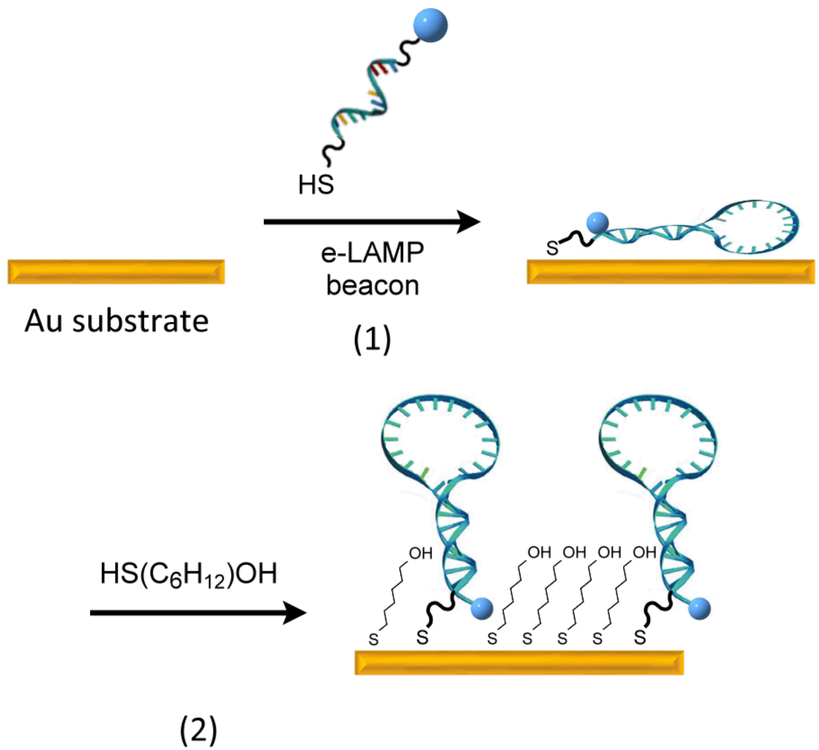


- *One-step (no washing, labeling)*
- *No addition of reagent*
- *Can be used for DNA & RNA detection*
- *Immobilization on electrode surface*
  - Simple multiplexing on individually addressable electrodes
  - Long-term stability
  - May be capable of reuse
  - May be capable of *in-situ* detection

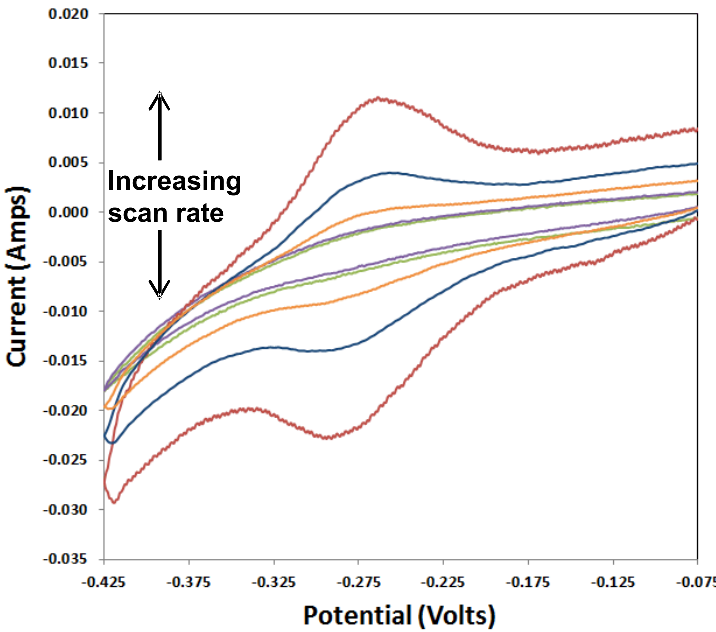
† Farjami et al. *Anal. Chem.* **2011**, *83*, 1594.

Assembly and Characterization of E-chem Molecular Beacon Thiol-Au SAM Surface †

Thiol-Au Self-Assembling Monolayer



Electrochemical Response



Cyclic voltamogram in 1x DPBS, pH 7.1, v = 10, 25, 50, 100, and 200 mV/s; Ag/AgCl reference.

- Direct electron transfer obtained between electrode and MB-molecular beacon
- Linear peak current vs. voltage scan rate (v) confirms surface immobilized
- Quasi-Nernstian redox response

† Harper and Meagher, *in preparation*

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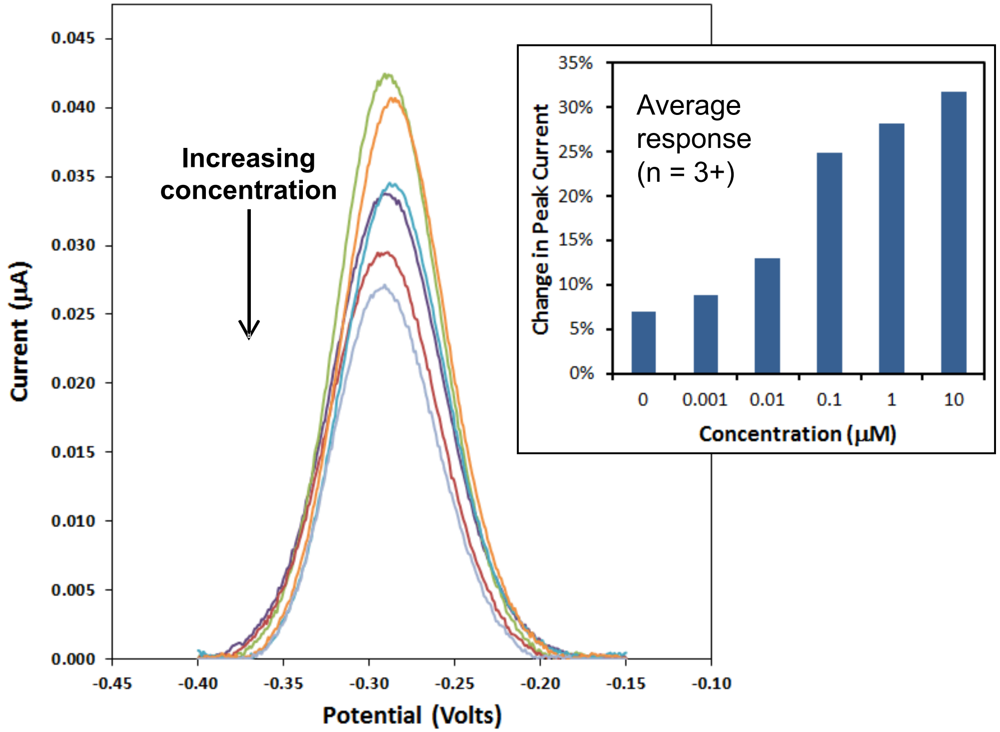
Label-Free and Reagent-less Electrochemical DNA Detection

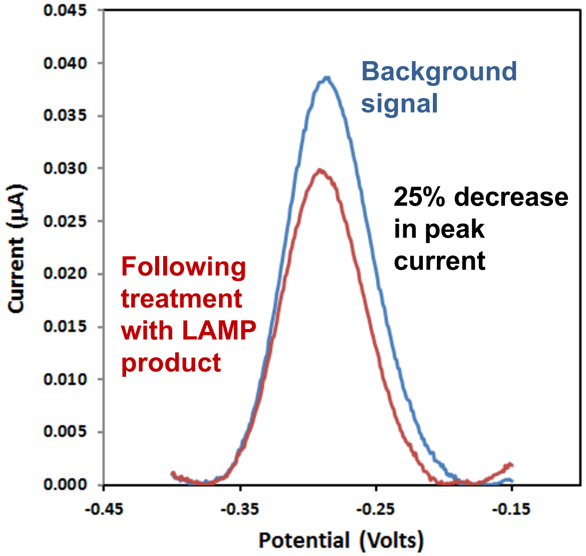
Electrochemical Detection of Loop F Complement (Synthetic)



Square wave voltammograms in 1x DPBS, pH 7.1, 25 mV pulse amplitude, 1 mV step, 10 Hz; Target DNA concentrations: 0, 1, 10, 100, 1000, 10000 nM; Ag/AgCl reference.

- Detection limit = 3 nM (5σ signal to noise)

Electrochemical Detection of LAMP Product (t_0 = 4 pM)

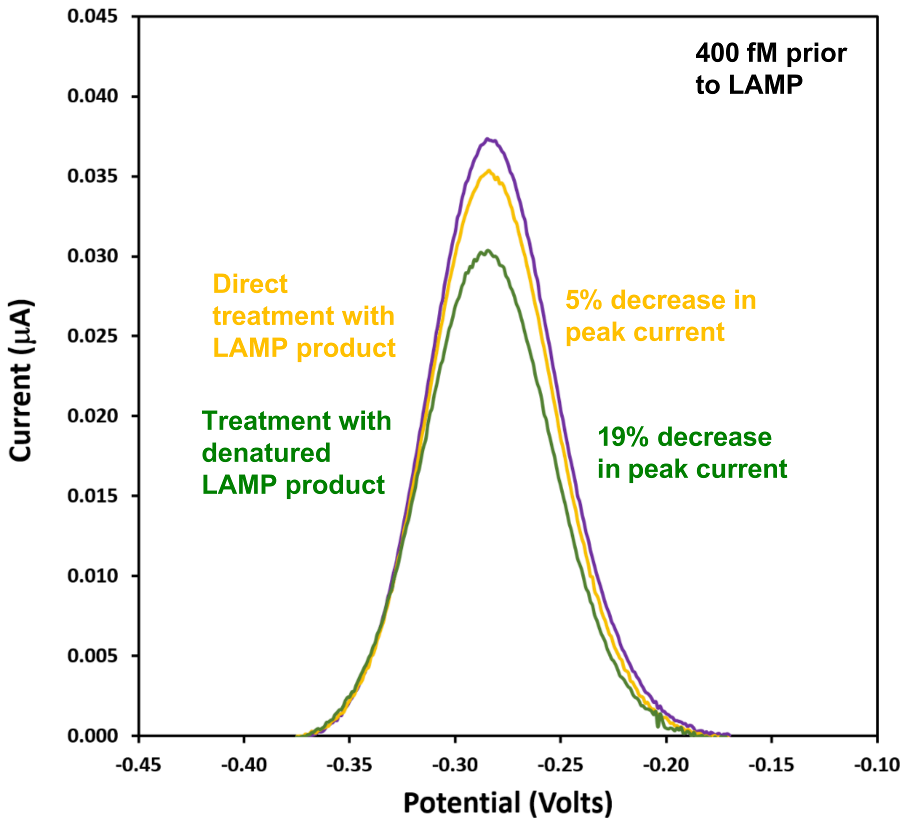


SWV in 1x DPBS, pH 7.1, 25 mV pulse amplitude, 1 mV step, 10 Hz; Ag/AgCl reference; MS2 LAMP product.

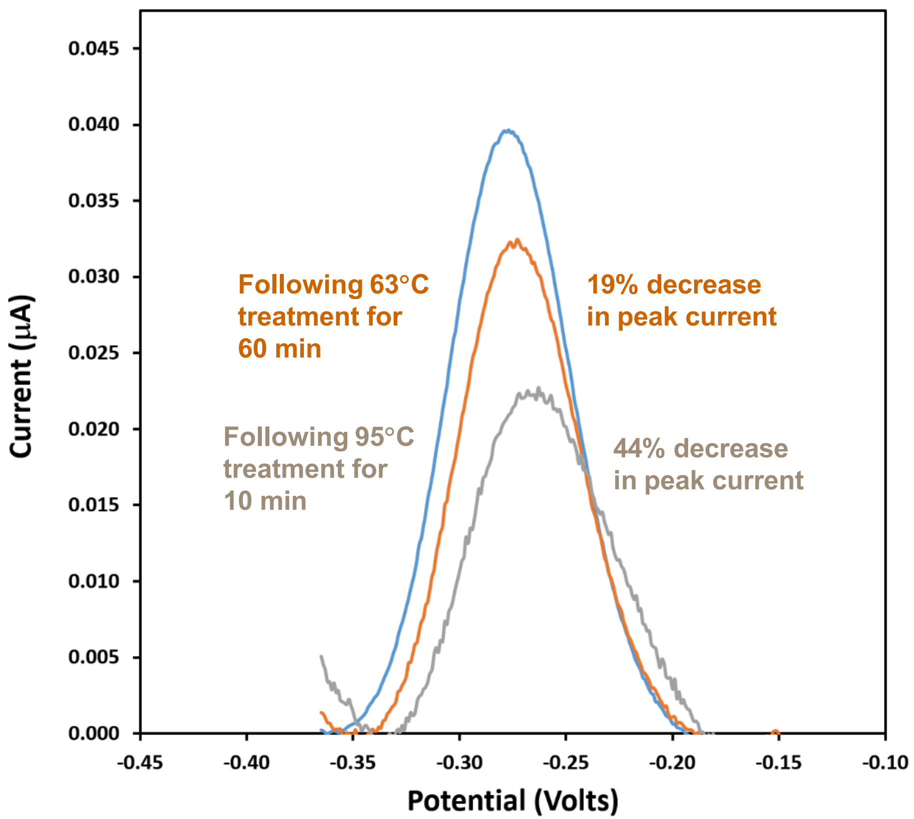
All measurements performed with hand-held potentiostat and smart phone/tablet app

In-Situ Detection of LAMP Product Requires dsDNA Melting → Disrupts Thiol-Au SAM

Direct Detection of LAMP Product vs. Denatured (95 °C) LAMP Product

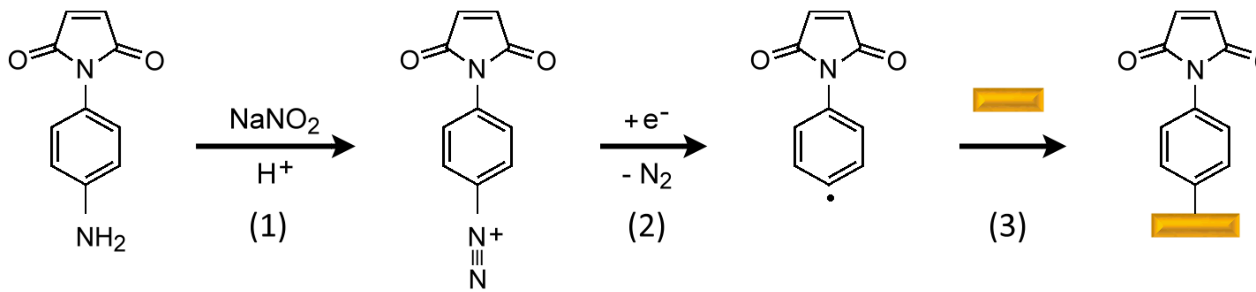


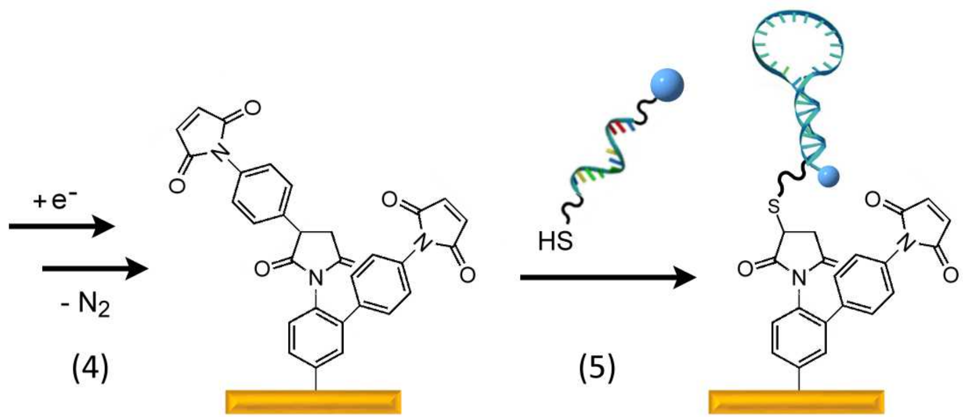
Thiol-Au SAM Stability to High Temperature Incubations



→ SAM instability at high temperatures makes in-situ LAMP product detection more challenging

Phenylmaleimide Surface Formed from *In-Situ* Diazotization and Electrochemical Grafting



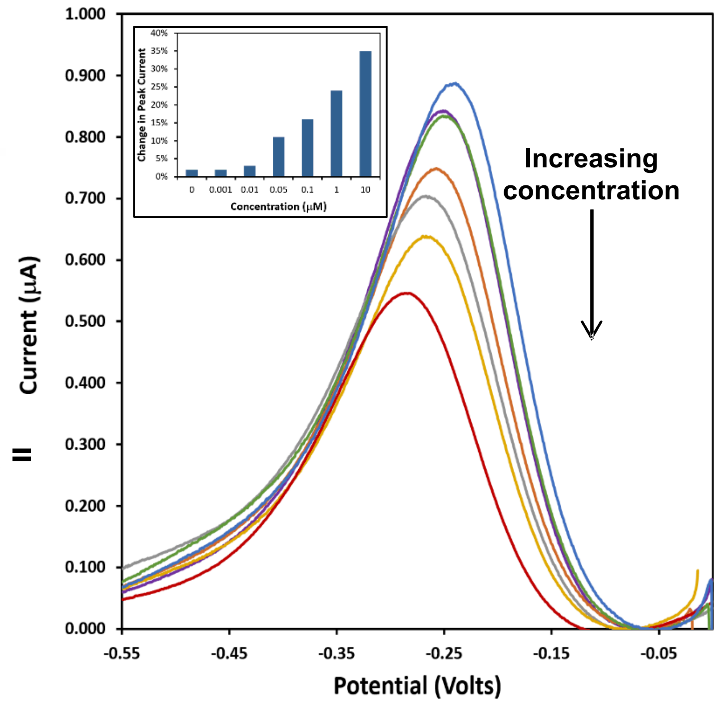


Deposition method	Film thickness (angstoms)	Equivalent monolayer†
25 sec CA	28.4 ± 5.3	3.1
80 sec CA	34.9 ± 3.9	3.8
300 sec CA	42.2 ± 2.5	4.6
1 CV	27.0 ± 5.9	3.0
2 CVs	32.1 ± 4.4	3.5
5 CVs	34.9 ± 4.1	3.8

Detection limit = 26 nM **(5σ STN)**

† Harper *at al. Langmuir* **2008**, *24*, 2206-2211

Electrochemical Detection of Loop F Complement (Synthetic)



SWV in 1x DPBS, pH 7.1, 25 mV pulse amplitude, 1 mV step, 10 Hz; Target DNA concentrations: 0, 1, 10, 50, 100, 1000, 10000 nM; Ag/AgCl reference.

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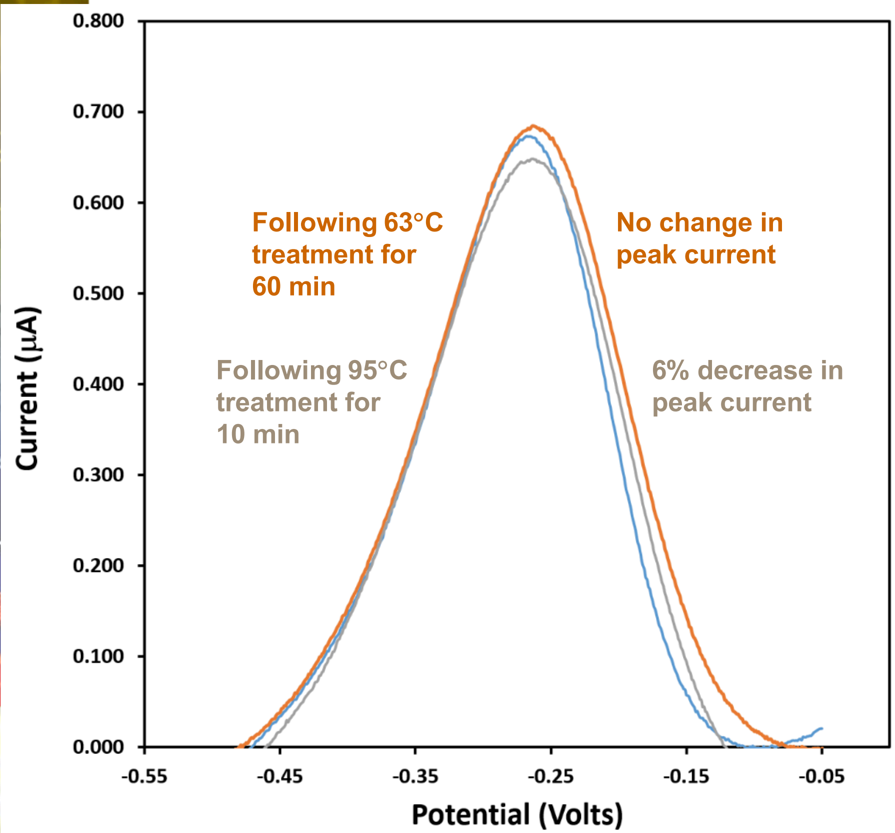
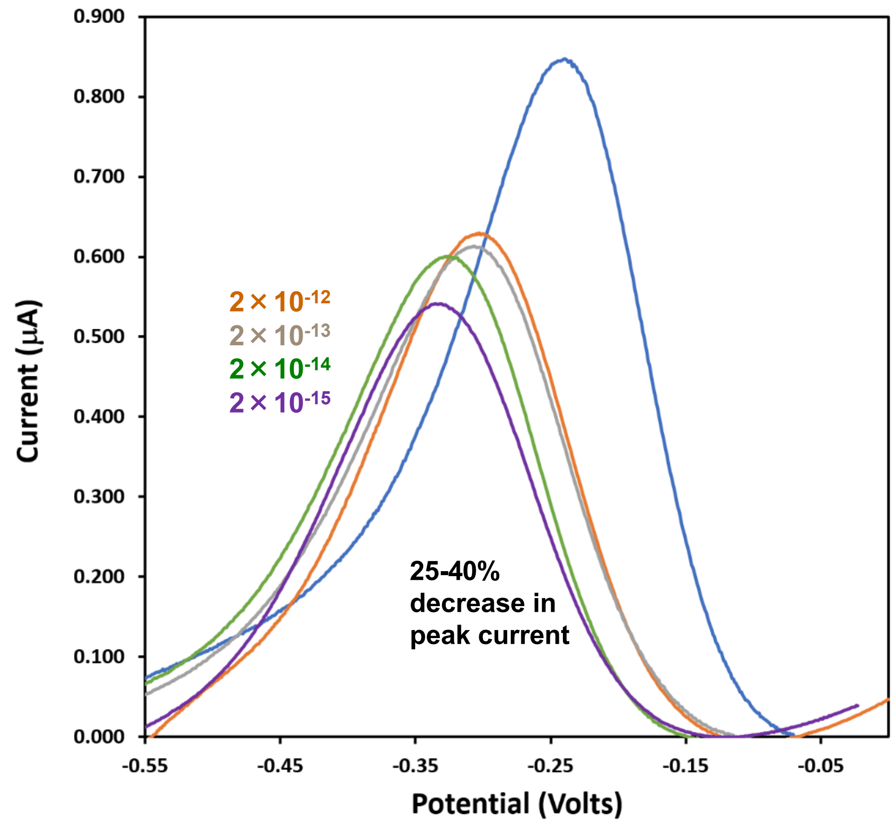
Single-Step, One-Pot, DNA Amplification & Electrochemical Detection

Phenylmaleimide Stability to High Temperature Incubations



Direct Detection of Denatured (95 °C, 10 min) LAMP Product



→ *Stability of phenylmaleimide e-chem hairpin surface may allow for in-situ detection of LAMP products*

Summary

Loop-Mediated Isothermal Amplification

→ One-step, one-pot

→ Detection shown from 2 fM amplified to approx. 1 μM

→ Amplify DNA and RNA

Electroactive Molecular Beacon

→ One-step, one-pot (no washing)

→ Reagent-less

→ Pseudo label-free

→ Requires melting of dsDNA LAMP product

→ Need on-device heater ✓

→ Need background scan ✓

→ Signal 'off' mechanism

Thiol-Au Self Assembling Monolayer Surface

→ Detection limit = 3 nM

→ The Au-thiol bond was not stable at 63 °C or 95 °C

→ Can detect LAMP product if heating done in separate chamber

In-situ Diazotization Phenylmaleimide Surface

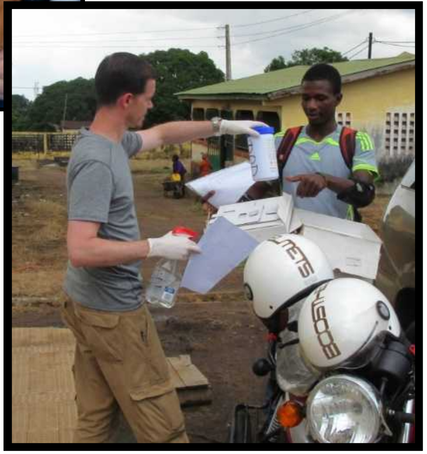
→ Detection limit = 26 nM

→ The phenyl-gold bond was stable at 63 °C and 95 °C

→ May allow of *in-situ* detection of LAMP product

→ May allow for multi-analyte detection (bias-assisted selective immobilization)

Acknowledgements



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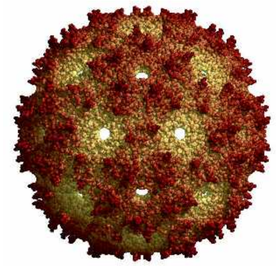
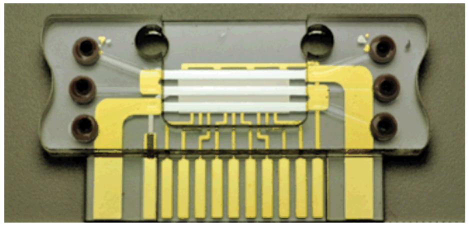
Ronen Polsky
Susan Brozik





Funding

Sandia National Labs LDRD
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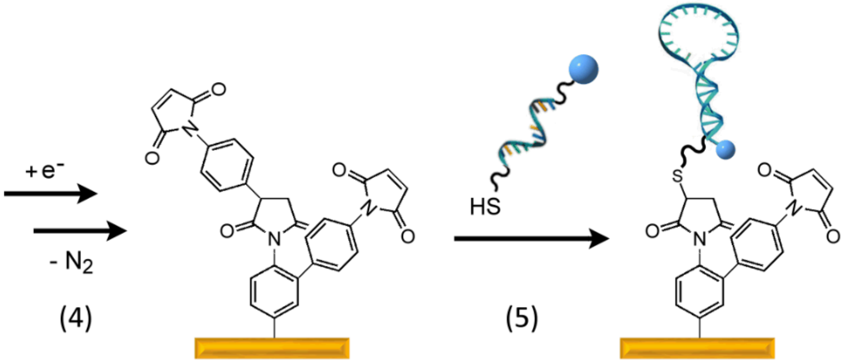

LDRD
LABORATORY DIRECTED RESEARCH & DEVELOPMENT

AQUILA

BIOSCIENCE RESEARCH FOUNDATION

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E-Chem Molecular Beacon Design; Au-Thiol Self-Assembling Monolayer (SAM)

Electroactive Molecular Beacon Design

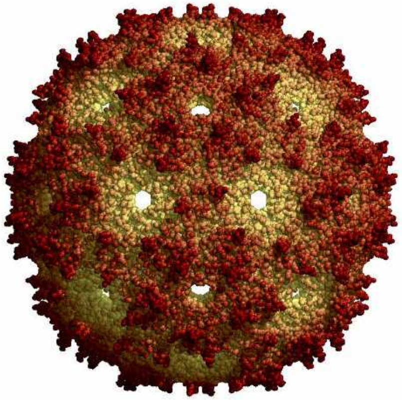
5' – Thiol (C6) GCGAG GATTCCGTAGTGTGAGCG CTCGC (C7) MB – 3'

Only one stable secondary structure (hair-pin)
Hair-pin melts at 49.6 °C; Loops melts at 56.3 °C

Thiol: Forms SAM on gold electrodes; handle for conjugation
Stem sequence: 5 bases with high G content
Loop sequence: LAMP primer Loop F sequence (MS2, 18 bases)
Methylene blue: Most stable commercial redox active modification; high redox activity in aqueous medium
Carbon spacers: Improve access of analyte DNA to immobilized beacon; improve mobility required for MB electron transfer

MS2 RNA Virus

- 3569 nucleotides of single-stranded, positive-sense RNA
- 27 nm diameter sphere
- One copy of the maturation protein
- 180 copies of the coat protein (organized as 90 dimers)

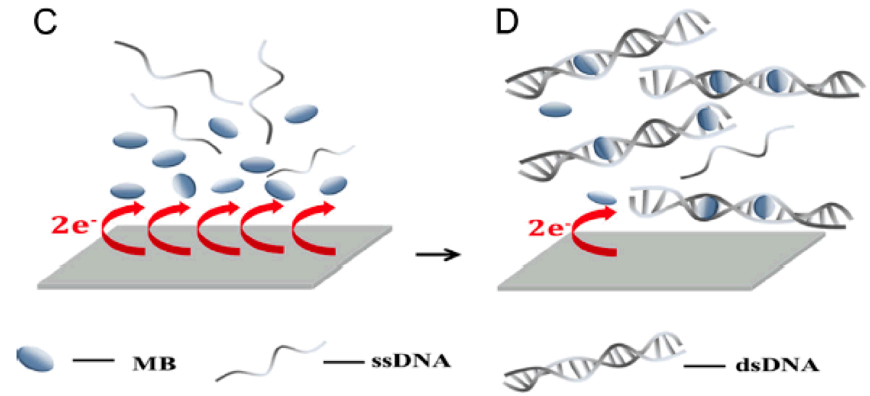


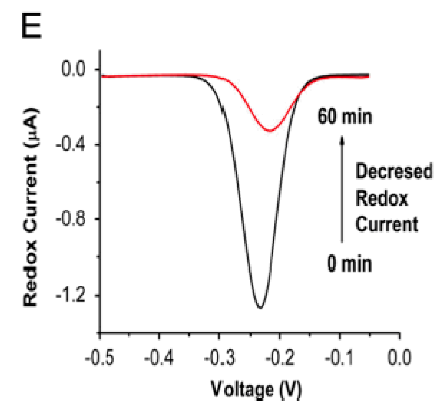
Electrochemical-Based Detection of LAMP Products

Electrochemical-based assays allow for simple and inexpensive biodetection with deployable microanalytical systems:

- Leverages integrated circuit technology producing tightly arrayed electrodes at very low cost
- Electrodes are readily functionizable with stable and addressable surface chemistry (diazonium, alkane-thiol)
- Electrochemical measurement is already in the format required for signal transduction and processing (no optics, photodiodes, etc. required)
- Simple multianalyte detection via selective functionalization of individually addressable electrode arrays and/or redox probes with differing formal potential
- Detection limits are comparable or lower than fluorescent-based detection

E-chem Detection of LAMP Products†, §





Both reports use redox active intercalators

- Allows for real-time detection
- Requires addition of reagent
- Signal "off" mechanism
- More challenging to multiplex

† Lou et al. *Biosens. Bioelectron.* **2014**, *60*, 84.

§ Safavieh al., *Biosens. Bioelectron.* **2014**, *58*, 101.