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Development of Rapid Diagnostic Tools for Detection and Quantification of Cas9 Presence and Activity

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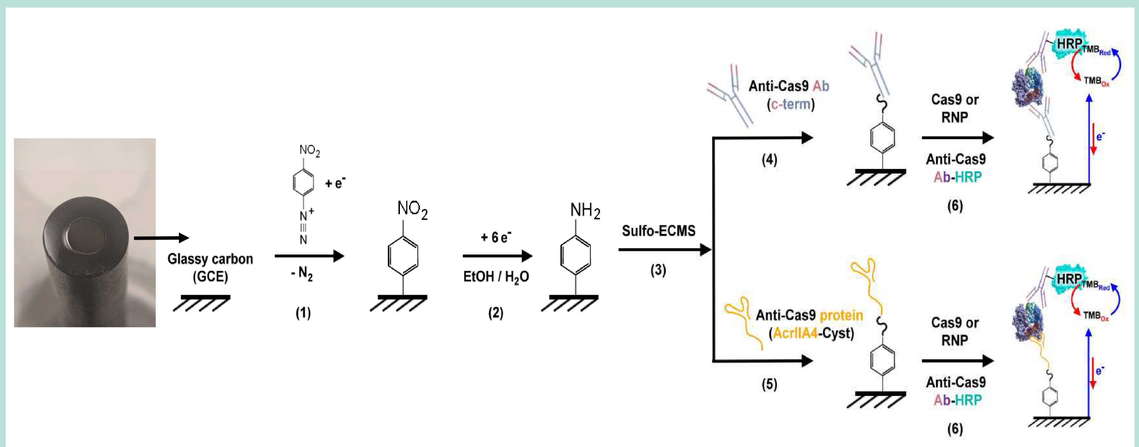
Introduction and Motivation

The facile, programmable nature of genome editing afforded by CRISPR/Cas9 has led to a multitude of *in-vitro* and *in-vivo* applications with basic science and clinical applications. Quantification of the Cas9 protein levels and activity is needed for 1) optimizing genome editing methods, while minimizing the potential for off target or other deleterious effects and 2) assessing successful clearing of editor components for clinical applications. Traditional biochemical-based detection methods (Western blot, ELISA), while effective, are time-consuming and are only semi-quantitative, giving rise to the need for improved rapid and precise analytical methods for detection of Cas9.

Assay Design Criteria

- Clinically relevant detection limits (fM- pM)
- Compatibility with complex sample matrices
- Inexpensive
- Potential for multiplexed detection
- Flexibility: different species and orthologs of Cas9

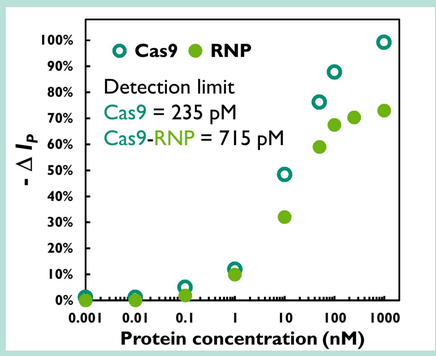
Electrochemical-based Detection of Cas9 Protein & RNP



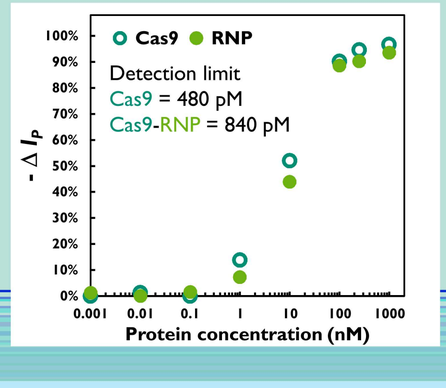
Platform Description

- Glassy Carbon Electrodes (GCE) functionalized with Cas9 antibody or Anti-CRISPR AcrIIA4 (Rausch, 2017) for Cas9/Cas9-RNP capture
- HRP conjugated detection antibody facilitates quantitative electrochemical analysis
- Electrochemical output measured via cyclic voltammetry (Harper, 2012)

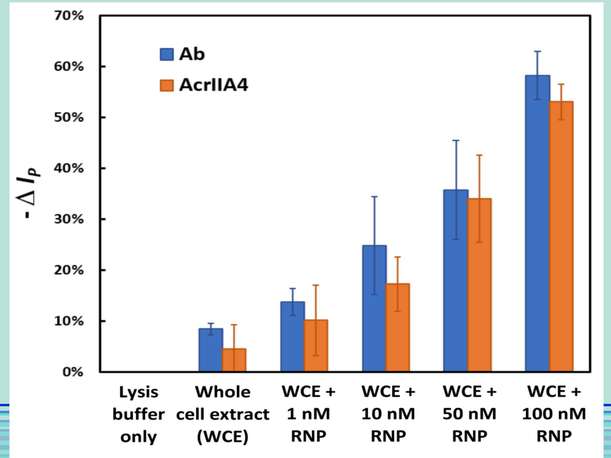
Antibody Capture Surface Detection Limits in Buffer



AcrIIA4 Capture Surface Detection Limits in Buffer

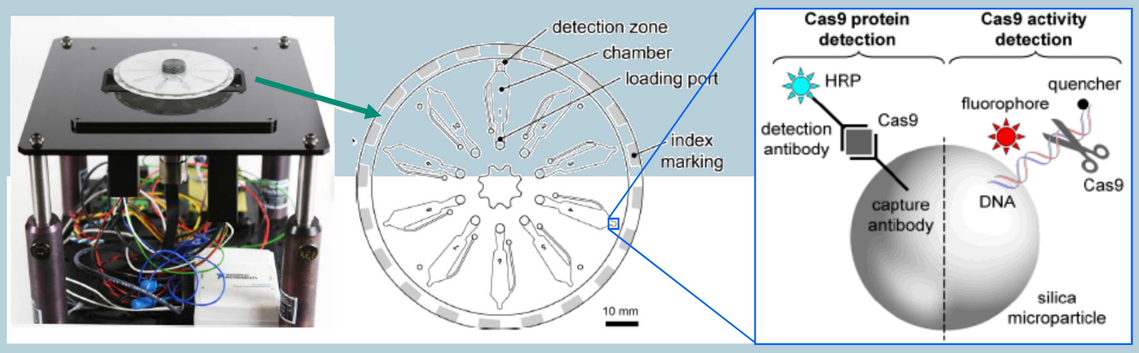


Antibody & AcrIIA4 Capture Surface RNP Detection in Whole Cell Extracts



Error bars represent standard deviation of three replicate experiments.

Centrifugal, Microfluidics Platform for Detection of Cas9 Activity

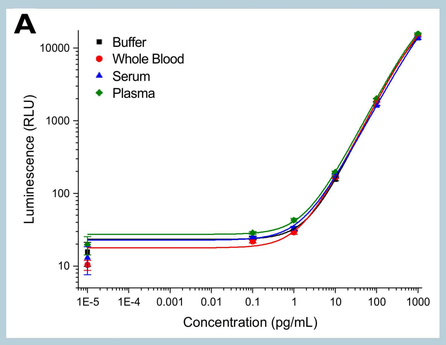


Platform Description

- 3-layer disc, cast acrylic sheets flanking a pressure sensitive adhesive layer of channels
- Centrifugal force transports microparticles enriched in signaling molecules from center to detection zone
- Optical detection: chemiluminescence (protein) or fluorescence (635 nm excitation, activity)

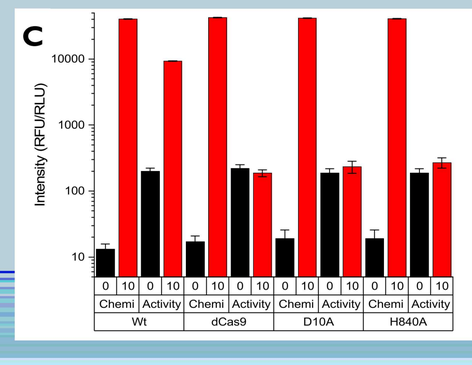
Detection Zone

- Protein detection accomplished with commercial antibodies
- Activity assay based on modification of a recently published solution-phase activity assay (Seamon 2018)
- Requires ~5 µL of sample @ 30 min assay time



Development of Cas9 Protein and Activity Detection Assays

A. Detection of spCas9 protein by capture and detection antibodies is relatively unchanged in different sample matrices. **B.** Microparticle-immobilized fluorescence-based detection of *S. pyogenes* Cas9 nuclease activity in the presence of the substrate-specific AAVS1 guide RNA. Error bars represent standard deviation of four replicates **C.** Simultaneous Cas9 protein and nuclease activity detection for wild type, the D10A and H840A nickase mutants with one active site disrupted, and D10A/H840A dead Cas9 (dCas9).



Summary

- Demonstrated sensitive detection of Cas9 protein, Cas9-RNP complex, and Cas9 activity
- Clinically relevant detection limits
- Realistic sample matrices

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Future Directions

- Multiplexed detection: Both platforms can accommodate multiplexed detection
  - Multiple channels on each disc
  - Electrically addressable arrays
- Extend to additional species and orthologs

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