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# Anti-CRISPR Based Platform for Rapid Detection and Quantification of Cas9-RNP

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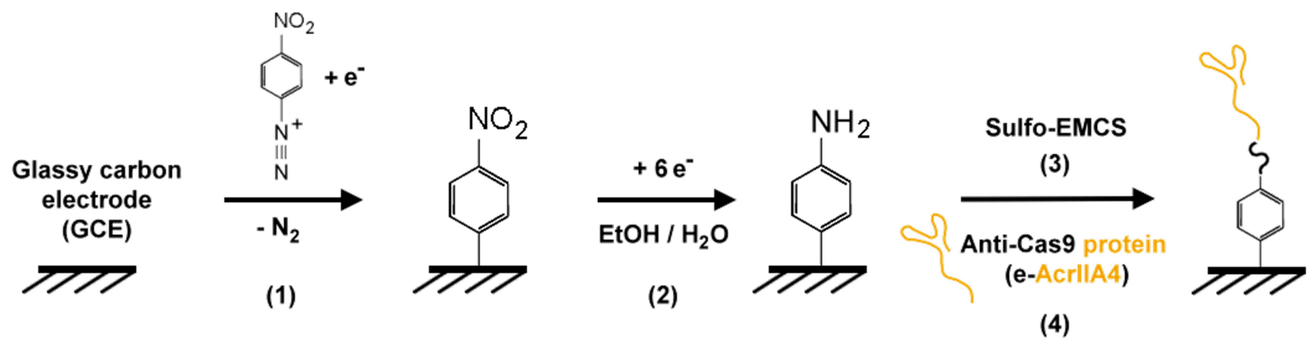
## Abstract

The demonstration of RNA-guided DNA editing using the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) system spawned a new era in biotechnology. The facile, programmable nature of gene editing afforded by CRISPR/Cas9 has led to a multitude of application in both the lab and the clinic. In these applications, detection of the CRISPR/Cas9 RNP within biological samples is critical for assessing gene-editing reagent delivery, retention, persistence, and distribution within living organisms to optimize editing while minimizing the potential for off target or other deleterious effects. Traditional biochemical-based detection methods (Western blot, ELISA), while effective, are either time consuming, expensive, or are only semi-quantitative, necessitating development of more rapid and precise analytical methods for detection of CRISPR components. Herein, we present development of a rapid bioassay platform for quantitative Cas9-RNP detection using the anti-CRISPR protein, AcrIIA4 as a novel affinity reagent. Glassy carbon electrode surfaces were functionalized via electrodeposition of aryl nitrodiazonium salts. Post functionalization, cysteine modified AcrIIA4 was attached to electrode surfaces via a heterobifunctional crosslinker. Using this platform, we demonstrate detection CRISPR/Cas9 RNP by, fluorescent, colorimetric, and electrochemical methods in less than 3 hours, achieving detection limits of 280 pM RNP in reaction buffer and 8 nM RNP in biologically representative conditions via electrochemical measurements. Our results demonstrate the ability of anti-CRISPR proteins to serve as robust, specific, flexible, and economical CRISPR/Cas9 recognition elements for developing precise, rapid, and economical biosensing devices for CRISPR/Cas9 RNP detection.

## Strategy and Approach

- Quantification of CRISPR/Cas 9 components needed for optimal gene editing activity, with minimal off target or other deleterious effects, is of great importance in achieving intended gene editing for both basic science and clinical approaches. Traditional antibody based detection methods (Western blot, ELISA), while effective, are either time consuming or are only semi quantitative. Further, development of effective capture ligands (e.g. antibodies, peptides) against these new targets is laborious, costly and time consuming
- Electrochemical biosensors have shown highly robust and selective detection capability of a variety of analytes, with detection limits in the picomolar and femtomolar range[1, 2]
- Natural Anti-CRISPR (Acr) proteins have great potential as novel capture ligands for incorporation into electrochemical biosensors to generate highly specific and economical capture ligands [3&4].
- We utilize a cysteine-modified SpyCas9 AntiCRISPR, AcrIIA4 (e-AcrIIA4) to develop an biosensing platform for colorimetric, fluorescent and electrochemical detection of Cas9-RNP with picomolar detection limits, in whole cell lysates, and a total assay time of less than three hours

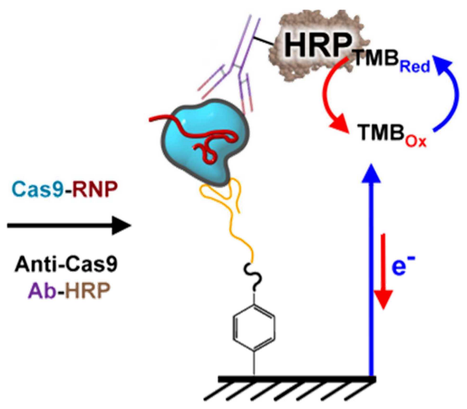
*Aryl diazonium chemistry allows for 1) controlled surface conjugation, 2) well defined amino group density, and 3) provides a robust and stable covalent bond between the capture ligand and electrode surface [2]*



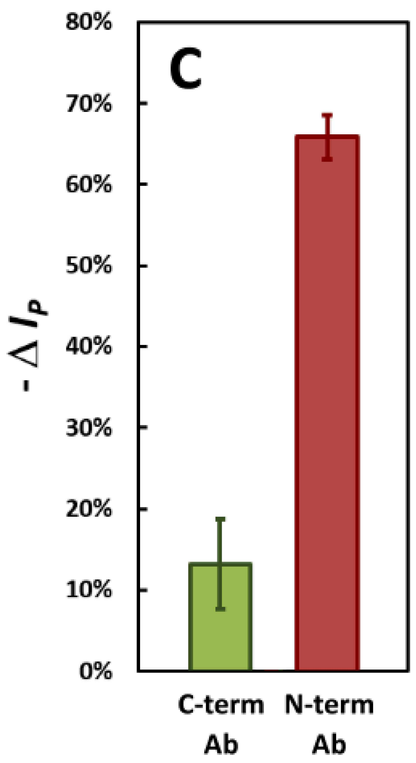

[1] Huang et. al.. *Sensors*. **2017** *10,* 2375
[2] Harper et al., *Electroanalysis* **2007** *19*, 1268; Polsky, Harper et al., *Electroanalysis* **2008**, *20*, 671.
[3] Bondy-Denomy., *ACS Chemical Biology* **2018** *13* 417-423
[4] Johnston et al *Biosensors and Bioelectronics* accepted

## Results

### AcrIIA4 is an Effective Cas9-RNP Capture Ligand

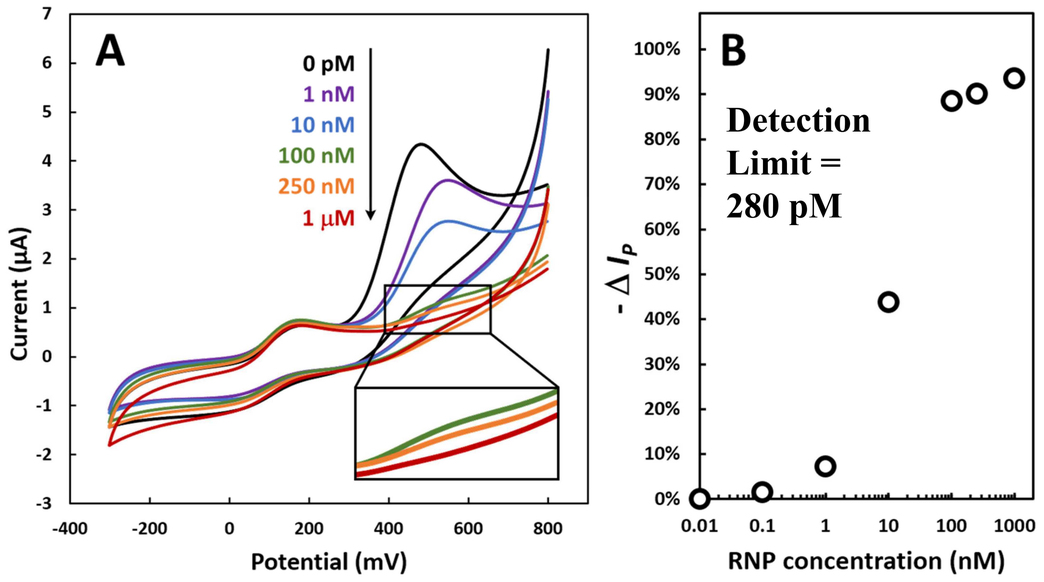



Capture of RNP and detection *via* an electroactive label (HRP) resulting in an electrocatalytic increase in TMB mediator reduction current, and a decrease in TMB oxidation current

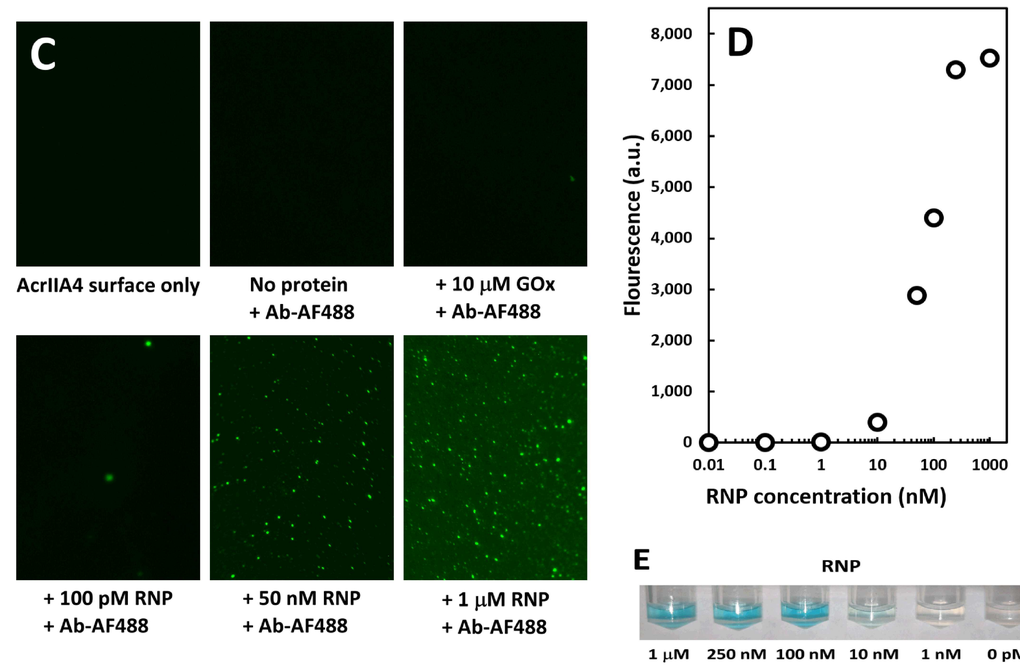



Plot of the decrease in TMB oxidation current peak height (-D$I_p$) for e-AcrIIA4 modified electrodes probed with anti-Cas9 C-term Abs (green) or N-term Abs (red) *vs.* the no-protein control from panel. Error bars are the standard deviation of three replicates. N-term Ab is a much better elcctroactive probe to interrogate Cas9-RNP binding by AcrIIA4

### Electrochemical , Fluorescent & Colorimetric Detection of Cas9-RNP using AcrIIA4 Capture Surfaces




Electrochemical detection of RNP using immobilized e-AcrIIA4 as an affinity reagent. (A) Cyclic voltammograms (CVs) from e-AcrIIA4 modified GCEs exposed to varying concentrations of RNP and (B) resultant impact of RNP concentration on current response (decease in TMB oxidation peak, y-axis).




Fluorescence microscopy images of e-AcrIIA4 immobilized GCE surfaces treated with no protein, Glucose Oxidase (Gox) or RNP, followed by incubation with AF488-conjugated Abs (C). All images were captured under identical magnification and camera settings, and are false colored. Scale = 20 µm. (D) Quantification of fluorescence intensity following treatment with varying RNP concentrations and (E) qualitative colorimetric detection of varying concentrations of Cas9-RNP.

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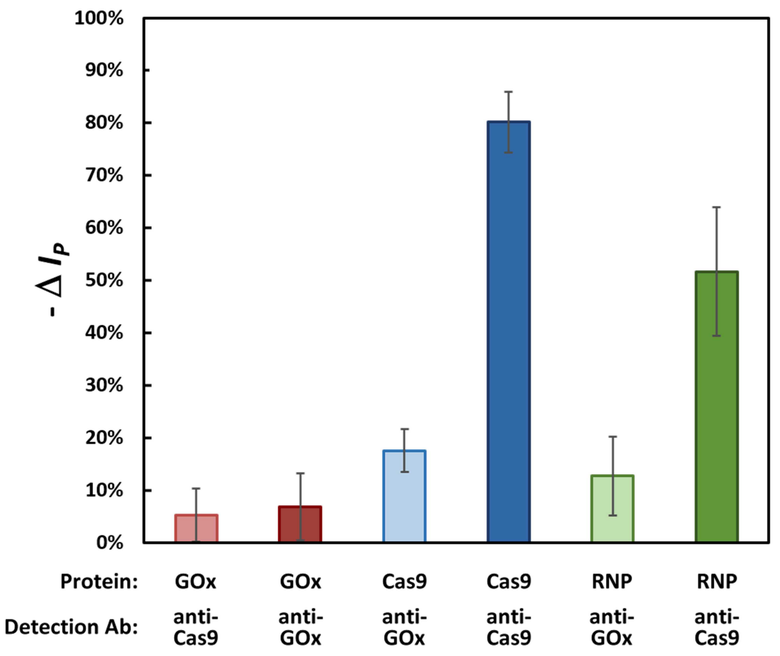
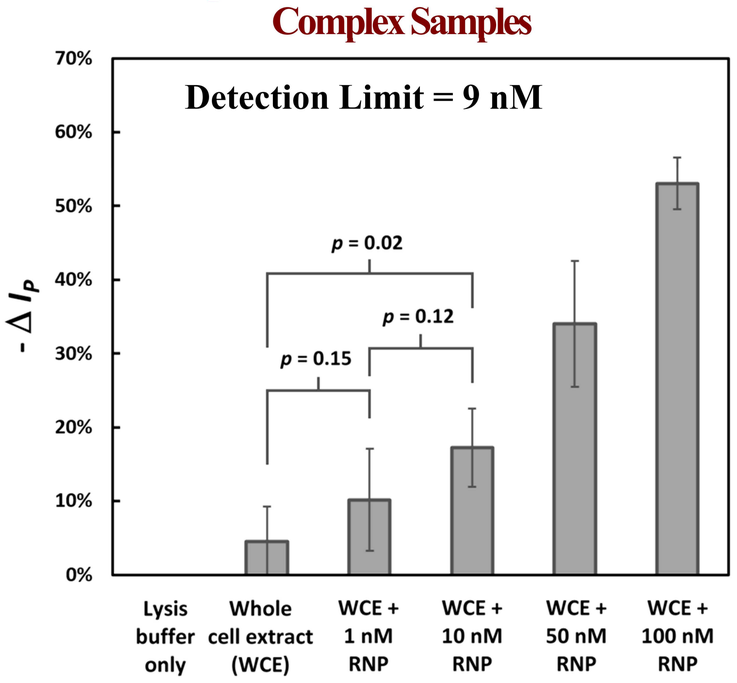
### AcrIIA4 Capture Surfaces are Specific for Cas9/Cas9 RNP




Change in TMB oxidation current response (-Δ*Ip*) upon exposure to 50 nM GOx, Cas9 (without sgRNA), or RNP and probed with either HRP-conjugated anti-GOx or anti-Cas9 Abs. Greatest signal change comes from e-AcrIIA4 modified electrodes exposed to Cas9/Cas9-RNP and probed with Ca9 N-term Ab.

### AcrIIA4 Capture Surfaces can Detect Cas9-RNP in Complex Samples




Change in TMB oxidation current response for e-AcrIIA4-modified electrodes exposed to lysis buffer, RAW264.7 whole cell extract (WCE), or WCE containing varying concentrations of RNP. Error bars indicate the standard deviation of three replicate assays

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