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Rapid Electrochemical Detection and Quantification of CRISPR/Cas9 Components

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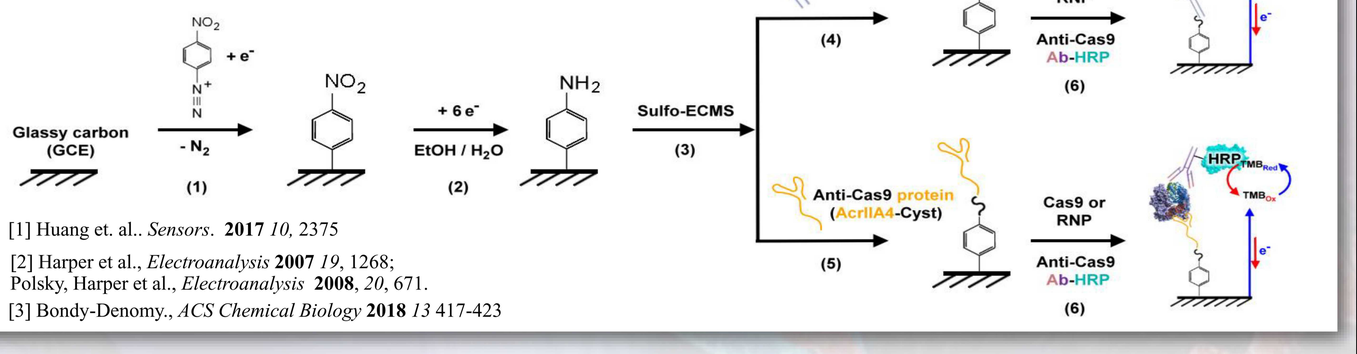
Abstract

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 *in-vitro* and *in-vivo* applications with basic science and clinical applications. Within these applications, quantification of the individual CRISPR/Cas9 components needed for optimal gene editing activity is important in order 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 or are only semi-quantitative, necessitating development of more rapid and precise analytical methods for detection of CRISPR components. We will present a rapid electrochemical bioassay platform for Cas9 protein that utilizes commercial Cas9 antibodies and the anti-CRISPR protein, AcrIIA4, for precise detection of Cas9 ribonucleoprotein (RNP). To develop this platform, gold or carbon electrode surfaces were functionalized via electrodeposition of aryl nitrodiazonium salts. Post functionalization, thiolated antibodies to the Cas9 C terminus or cysteine modified AcrIIA4 were attached to electrode surfaces via a heterobifunctional crosslinker. Using a horseradish peroxidase (HRP) conjugated detection antibody, and cyclic voltammetry, we show quantifiable detection of Cas9 protein and Cas9 RNP with high picomolar detection limits in whole cell lysates with a total assay time of less than three hours.

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].
- We utilize both commercial Cas9 Ab and a cysteine modified SpyCas9 AntiCRISPR, AcrIIA4 (AcrIIA4–Cyst) to develop an electrochemical biosensing platform for quantifiable electrochemical detection of Cas9 protein and 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]



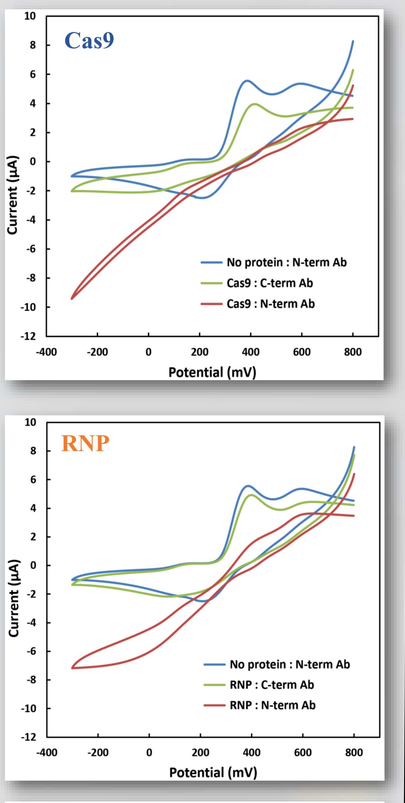
[1] Huang et. al.. *Sensors*. **2017** *10,* 2375

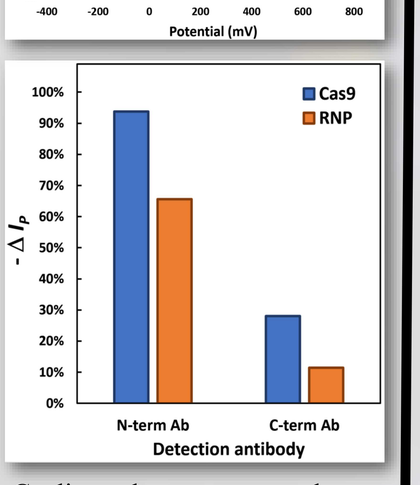
[2] Harper et al., *Electroanalysis* **2007** *19*, 1268; Polsky, Harper et al., *Electroanalysis* **2008**, *20*, 671.

[3] Bondy-Denomy., *ACS Chemical Biology* **2018** *13* 417-423

Results

AcrIIA4 is an Effective Cas9 & Cas9-RNP Capture Ligand





Cyclic voltammograms show less significant reduction in electrocatalytic peak using an anti c-term detection Ab.

A recent report [4] showed that AcrIIA4 binds to the Cas9/Cas9-RNP c-term, hindering access.

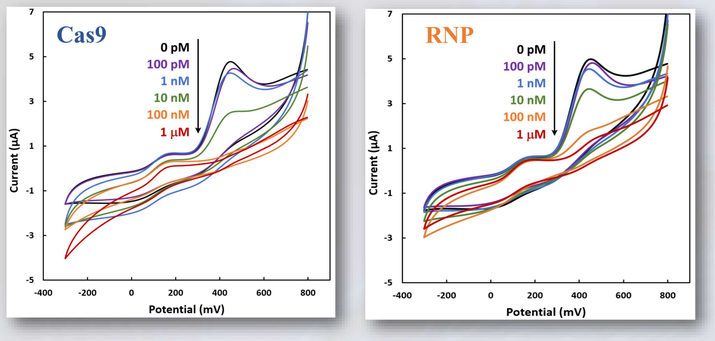
Detection assays subsequently used anti n-term detection Abs.

[4] Kim et al., *Sci. Rep.* **2018,** *8,* 3883

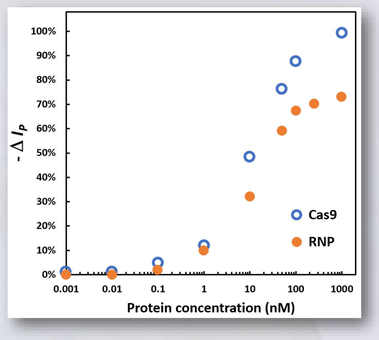
Detection of Cas9 and Cas9-RNP with Antibody and AcrIIA4 capture ligand

Antibody Capture Ligand

Electrochemical Detection



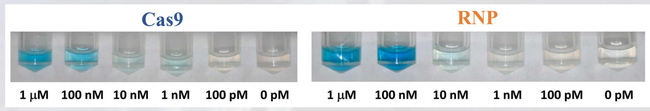
Signal vs. Concentration



Cyclic voltammograms show classic electrocatalytic behavior – decrease in enzyme substrate (TMB) oxidization wave – with increasing protein concentration.

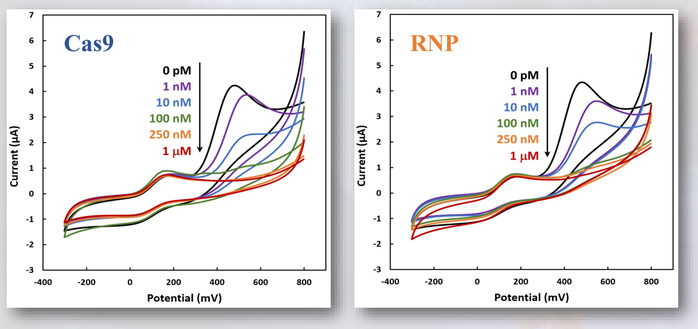
Detection limit (3s above background) for Cas9 = 235 pM, and Cas9-RNP = 715 pM.

TMB turnover results in visible product, providing an orthogonal colorimetric detection method

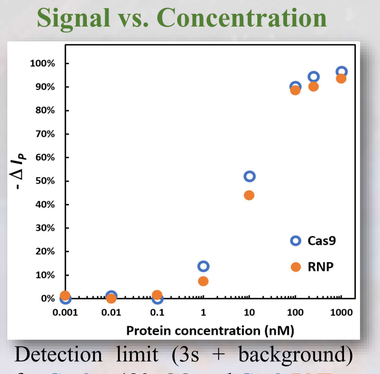


AcrIIA4 Capture Ligand

Electrochemical Detection



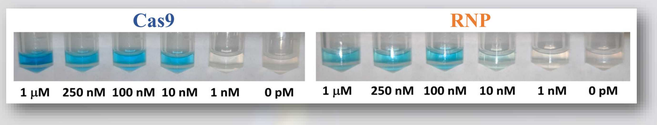
Signal vs. Concentration



Cyclic voltammograms show electrocatalytic behavior similar to Ab capture-based detection indicating AcrIIA4 can serve as an capture/binding ligand alternative to antibodies.

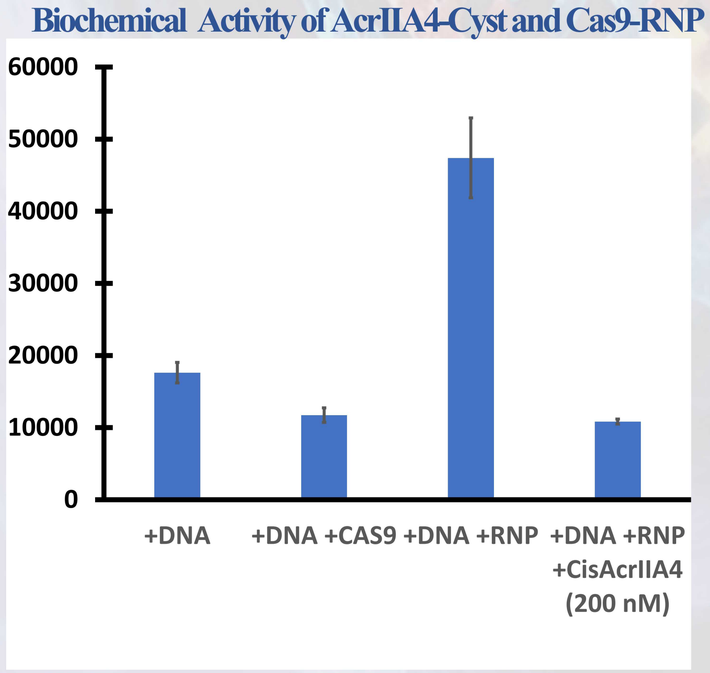
Detection limit (3s + background) for Cas9 = 480 pM, and Cas9-RNP = 840 pM. These higher detection limits are indicative of the higher K_D for AcrIIA4 [2] vs. IgG.

TMB turnover results in visible product\



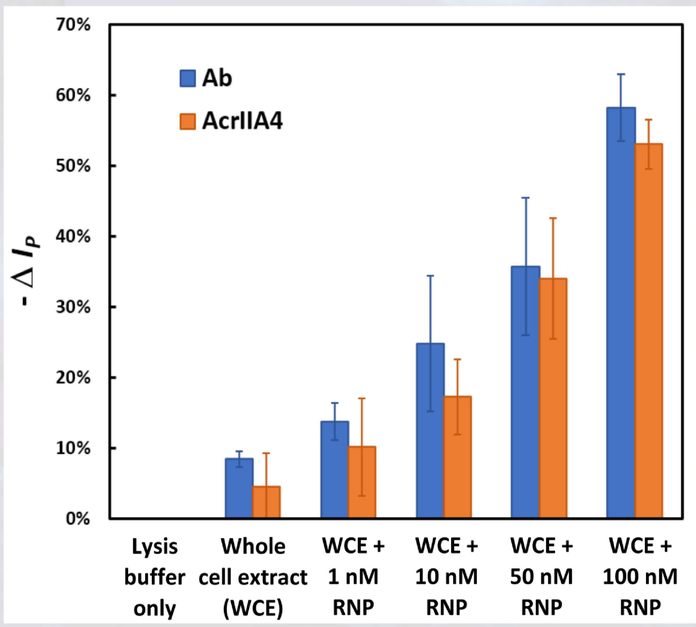
Detection of Cas9 and Biochemically Active Cas9-RNP in a Complex Sample Matrix

Biochemical Activity of AcrIIA4-Cyst and Cas9-RNP



Biochemical activity of CisAcrIIA4 and RNP reagents used in whole cell detection experiments was verified using a FRET based reporter assay [5]

AcrIIA4 vs. Ab Capture of Cas9-RNP in Whole-Cell Extracts



Both Ab-based and AcrIIA4-based capture surfaces show small (~5%) non-specific binding signals after treatment with whole-cell extracts (WCE). Both surfaces also show concentration dependent Cas9-RNP detection in the complex sample matrix.

[5] Seamon et. al. *Analytical Chemistry* **2018,** *90,* 6913–6921.

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